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Optimization of an LC-MS/MS Method for the Determination of Xenobiotics in Biological Matrices

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Kurzfassung

Der Mensch ist durch seine unmittelbare Umgebung ständig einer Vielzahl von chemischen Einflüssen ausgesetzt. Dazu gehören einerseits künstlich erzeugte Umweltschadstoffe oder Bestandteile von Produkten die wir im täglichen Leben verwenden. Beides ist eine direkte Folge unseres modernen industrialisierten Lebensstils. Andererseits gibt es natürlich vorkommende Substanzen, welchen wir seit Anbeginn der Zeit ausgesetzt sind.

Diese Verbindungen kontaminieren die Luft die wir atmen, das Wasser das wir trinken, die Lebensmittel die wir essen und in einigen Fällen schlicht die Dinge die wir berühren. Einige von ihnen können biologische Aktivität mit akuten als auch chronischen negativen Folgen auf die menschliche Gesundheit aufweisen sobald sie in unseren Körper gelangt sind. Sie können das empfindliche Gleichgewicht verschiedener biologischer Prozesse, wie z.B. des Hormon-, Nerven- oder Immunsystems, stören oder direkt wesentliche Vorgänge des Atmungs- oder Kreislaufsystems unterbrechen. Einige können sogar die Grundlage allen Lebens, den genetischen Code, beeinflussen.

Human Biomonitoring (HBM) befasst sich mit der direkten Identifizierung und Quantifizierung dieser Substanzen und ihrer Metabolite um geeignete Biomarker der Exposition, sowie Marker für nachfolgende gesundheitliche Auswirkungen, beim Menschen zu bestimmen. Durch die Analyse der effektiven Gesamtbelastung des Körpers durch chemische Giftstoffe und die Kombination mit epidemiologischen und mechanistischen Erkenntnissen über die Krankheitsentstehung will das Biomonitoring den Beitrag dieser Substanzen zur Pathogenese aufklären um damit eine zuverlässigere Risikoabschätzung zu ermöglichen, als es anhand reiner Umweltanalytik oder Lebensmittelanalytik möglich wäre. Nichtsdestotrotz beschäftigt HBM nicht nur die Untersuchung der Exposition auf individueller-, sondern auch auf Bevölkerungsebene. Somit ist es nicht nur ein wissenschaftliches Instrument für den medizinischen Gebrauch, sondern dient auch der Gesetzgebung um besonders gefährdete Gruppen vor schädlichen Expositionen zu schützen.

Das Ziel dieses Projekts war die Erweiterung einer umfassenden Flüssigchromatographie-Tandem-Massenspektrometrie Methode zum ganzheitlicheren Human-Biomonitoring einer Vielzahl toxischer Xenobiotika. Eine intern etablierte LC-MS/MS Methode für die Bestimmung von Xeno- und endogenen Östrogenen in menschlichem Urin, Serum und Muttermilch wurde auf ein neues LC-MS-System (Agilent Infinity II gekoppelt an eine Sciex QTrap 6500^{+}) übertragen. Um bestmögliche Sensitivitäten zu erreichen wurden zahlreiche MS und MS/MS-Parameter optimiert. Die LC-Methode und die Probenvorbereitungsprotokolle wurden beibehalten. Die Entwicklung der Methode umfasste insgesamt 95 Substanzen. Davon waren 75 bereits in der zuvor etablierten Methode inkludiert, während 20 neue toxische Xenobiotika mit der aktuellen Methode hinzugefügt wurden. Sensitivitäten wurden im Mittel um den die Faktoren 20, 17 und 25 in Urin, Serum und Muttermilch verbessert. Die mittleren Wiederfindungsraten reichten von 93% in Urin und 87% in Serum bis 54% in Muttermilch.

Intern wurden 39 Substanzen erfolgreich in Urin-, 50 in Serum und 15 in Muttermilch anhand aller gestellten Kriterien validiert. Die vollständige Validierung war für 30 Substanzen in Urin, 16 in Serum und 21 in Muttermilch, aufgrund von geringer Wiederholbarkeit und/oder weil die Extraktionsexperimente des niedrigen Konzentrationslevels nicht erfolgreich waren, nicht möglich. In den Fällen dieser Xenobiotika stellt dies keine Beeinträchtigung der Anwendbarkeit der Methode zur Analyse unbekannter biologischer Proben dar. Die Validierung der restlichen Substanzen war aufgrund von mehreren Kriterien nicht erfolgreich. Als Proof Of Concept wurden bei der Analyse von unbekannten Urin-, Serum- und Muttermilchproben jeweils 42, 31 und 29 Substanzen identifiziert und teilweise quantifiziert. Hierbei wurden erstmals toxische Pyrrolizidin- und Tropanalkaloide in menschlicher Brustmilch detektiert.

Abstract

Humans are constantly exposed to a variety of chemicals through food and environment. On one hand, this includes synthetic substances such as environmental pollutants or components of personal care products. Both are a direct result of our modern industrialised lifestyle. On the other hand, there are naturally occurring substances we have been exposed to since the dawn of humanity.

These compounds are contaminating the air we breathe, the water we drink, the foods we eat and in some cases simply the things we touch. Once they have found their way into our body, some of them exhibit biological activity which may lead to acute or chronic effects on health. They might disturb the delicate balances of various biological processes, such as the hormone-, nervous- or immune system, or directly disrupt essential processes of the respiratory or circulatory system. Some may even directly interfere influence our genetic code.

Human Biomonitoring (HBM) deals with the direct investigation of these substances and their metabolites by identifying appropriate biomarkers of exposure, as well as markers of subsequent health effects in humans. By analysing the effective total burden of chemical toxicants on the body and combining it with epidemiological and mechanistic knowledge of disease development, biomonitoring intends to elucidate their contribution to pathogenesis, thus allowing for more reliable risk assessment than mere environmental- or food monitoring. However, HBM not only aims to study exposure at an individual, but also at a population level. Hence, it is not only a scientific tool for environmental health research, but also for informed policy making to help protect particularly vulnerable groups from adverse exposures.

The general aim of this project was the extension of a comprehensive liquid chromatography tandem mass spectrometry method, originally developed for xenoestrogens, towards a multiclass assay covering various toxic xenobiotics. The in-house established LC-MS/MS method for the determination of xeno- and endogenous estrogens in human urine, serum and breast milk was transferred onto a newly installed LC-MS system (Agilent Infinity II coupled to a Sciex QTrap 6500^{+}). MS and MS/MS parameters were optimised to achieve the best achievable sensitivity, while the LC method remained as originally developed. Method development included 95 substances in total. Among them, 75 had been used in the method by Preindl et al. (278), while 20 new toxic xenobiotics were added. Median improvements in sensitivity through the new LC-MS/MS system were 20-fold in urine, 17-fold in serum and 25-fold in breast milk. Median extraction recoveries ranged from 93% in urine and 87% in serum to 54% in breast milk. Of 95 analytes included in method validation, 39 analytes met all in-house validation criteria in urine, 50 in serum and 15 in breast milk. Validation parameters were not fully met either because of low repeatability and/or validation issues at the low concentration level for 30 analytes in urine, 16 in serum and 21 in breast milk. This does not detract from the methods ability to quantify or screen for these xenobiotics biological samples. Multiple validation parameters were not met for the remaining compounds. The applicability value of the method was clearly demonstrated by

the analysis of unknown urine, serum and breast milk samples. Many analytes (42, 31 and 29) were identified, partly for the first time in the respective biological matrix, such as toxic pyrrolizidine- and tropane alkaloids in breast milk.

List of abbreviations

Abbreviation	Definition
3-OH-BaP	3-hydroxy-benzo[a]pyrene
8-PN	8-prenylnaringenin
16EpiE3	16-epiestriol
16OHE1	16-α-hydroxyestrone
17EpiE3	17-epiestriol
2MEOE1	2-methoxyestrone
2MeOE2	2-methoxyestradiol
2OHE2	2-hydroxyestradiol
4MeOE1	4-methoxyestrone
4MeOE2	4-methoxyestradiol
4OHE1	4-hydroxyestrone
AAs	aristolochic acids
ACN	acetonitrile
ADME	absorption, distribution, metabolism, excretion
AL	aristolactam
AME	alternariol monomethyl ether
APCI	atmospheric pressure ionisation
B[a]P	benzo[a]pyrene
BC	benzylidencamphor
BP	butylphenol
BPA	bisphenol A
BPAF	bisphenol AF
BPB	bisphenol B
BPC	bisphenol C
BPF	bisphenol F
BPS	bisphenol S
CAD	collisionally-activated dissociation gas
CE	collision energy
conc.	concentration
cps	counts per seconds
CUR	curtain gas
CXP	collision cell exit potential
CYPs	cytochromes P450
DBPs	disinfection by-products
DC	direct current
DNA	deoxyribonucleic acid
DP	declustering potential
E2	estradiol
E3	estriol
EDCs	endocrine disrupting chemicals
EI	electron ionisation
EMS	Enhanced MS Scan
EPI	Enhanced Product Ion Scan
ESI	electrospray ionisation

Abbreviation	Definition
Eq.	equation
FIA	flow injection analysis
GC	gas chromatography
GlcA	glucuronide
GS1	sheath gas
GS2	drying gas
HAAs	heterocyclic aromatic amines
HBCDDs	hexabromocyclododecanes
HBM4EU	Human Biomonitoring for Europe
HFPO-DA	hexafluoropropylene oxide dimer acid
HILIC	hydrophilic interaction chromatography
HL	high level (spike)
HMF	5-hydroxymethylfurfural
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
IDA	Information Dependent Acquisition
IS	ion spray voltage
LC	liquid chromatography
LC-MS/MS	liquid chromatography tandem mass spectrometry
LL	low level (spike)
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
m/z	mass to charge ratio
MBC	methylbenzylidencamphor
MBP	mono-*n*-butyl phthalate
MCA	multi-channel averaging
MEHP	mono-2-ethylhexyl phthalate
MeOH	methanol
MRM	multiple reaction monitoring
MS	mass spectrometry
NAT	N-acetyltransferase
NSSP	new substance spiked (standard)
NDEA	N-nitrosodiethylamine
NDMA	N-nitrosodimethylamine
OMC	octyl methoxycinnamate
OP	octylphenol
PAHs	polycyclic aromatic hydrocarbons
PAPS	3'-phosphoadenosine-5'-phosphosulfate
PAs	pyrrolizidine alkaloids
PCBs	polychlorinated biphenyl esters
PDBEs	polybrominated diphenyl esters
PFAs	perfluorinated alkylated substances
PFAS	perfluorooctanesulfonic acid
PFOA	perfluorooctanoic acid
PhIP	2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine
*p*OHBA	para-hydroxybenzoic acid

Abbreviation	Definition
ppb	parts per billion
ppm	parts per million
PVC	polyvinyl chloride
Q	quadrupole
QqQ	triple quadrupole
QuEChERS	Quick Easy Cheap Effective Rugged and Safe
R_E	extraction recovery
RF	radio frequency
RP	reversed-phase
RSD_R	Intermediate Precision
RSD_r	Repeatability
RT	retention time
s	second(s)
S/N	signal to noise ratio
SPE	solid-phase extraction
SRM	single/selected reaction monitoring
SSE	signal suppression/enhancement
Std	standard
SULT	sulfotransferase
TAs	tropane alkaloids
TBPA	tetrabromobisphenol A
TEM	temperature
TIC	total ion chromatogram
TOF	time of flight
UDP	uridine diphosphate
UGT	UDP-glucuronosyltransferases
UHPLC	ultra-high-performance liquid chromatography
ZAL	zearalanol
ZAN	zearalanone
ZEL	zearalenol
ZEN	zearalenone

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1.Theoretical background

1.1.The Exposome

In 2005, the cancer epidemiologist Dr. Christopher Wild was the first to put forth the concept of the "exposome" as the complementary environmental component to the genome for determining the risk of disease development [1]. He defined it as the multitude of exposures an individual experiences throughout one's lifetime (Figure 1). This includes environmental-, diet- and lifestyle influences, endogenous processes as well as their associated biological responses [2, 3]. One of the ideas of the exposome is to complement genomic research by taking into account the importance of gene-environment interactions for specific pathogeneses [4, 5]. Similar to genome-wide association studies, exposure-wide association studies may provide the missing link to help improve the determination if any particular variant is associated with specific traits or risks [6, 7]. However, there are large challenges that hinder the successful analysis of multiple external and internal exposures from different sources continuously over a lifetime.

Advancing research efforts in this most recently emerging field may lay a new foundation to control adverse environmental factors to provide and sustain healthier living with improved management and prediction of disease. This holistic approach may result in a great tool to estimate the total environmental burden on the individual, eventually opening the possibility of more precise medical approaches against disease development, coining the term "personalised exposome medicine" [8].

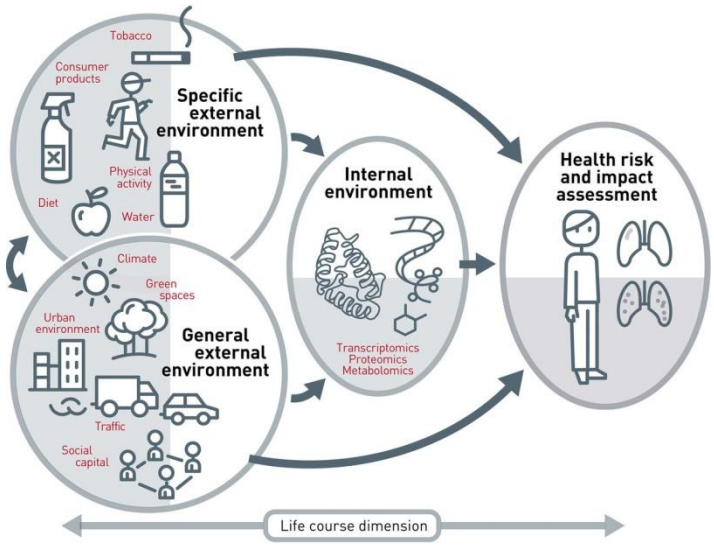

Figure 1: The concept of the exposome [9]

The exposome may be subdivided in different categories [9]. The external exposome includes the general external environment with factors such as climate conditions, the urban environment, air pollution and various social factors. These influences are largely assessed on a geographical and/or community level [10]. Meanwhile the specific external exposome includes factors that need to be investigated on an individual level, such as diet and chemical contamination [9]. Lastly, the internal

exposome accounts for biological contributions such as metabolic factors, the gut microflora, oxidative stress or age [9].

Wild hypothesized that all different components of the exposome leave their specific fingerprints in our internal environment [11]. This is where the high-throughput "omics-"methodologies come into play. They enable the analysis of complete sets of biological molecules, starting with gene expression (transcriptomics and epigenomics) to the proteome (proteomics) and eventually smaller molecules (metabolomics) or even reactive electrophilic species (adductomics [12]). Hence, they may be used to create molecular profiles and help find relationships between external exposure and the internal domain.

In 1936, one of the first studies that could be attributable to this field drew an association between the industrial exposure to benzene with differences in gene transcription and translation in peripheral blood cells [13]. Among many other examples, more recent research drew associations between arsenic poisoning or air pollution and epigenetic changes [14, 15], induction of specific DNA mutation patterns with different carcinogens [16] and changes in lipidomic fingerprinting with exposure to oxidative environments [17].

It may be possible to characterise pathological conditions by their transcriptome, proteome and metabolome, but the exposomics approach may be the future key to bridging the gap between the knowledge of underlying disease mechanisms and the epidemiology of public health [18].

As an example, cancer is known to be a multifactorial disease. Combined effects of genetic and environmental factors may act concurrently or sequentially to promote pathogenesis. Overwhelming evidence in cancer etiology suggests that the influence of external, partly environmental factors is truly the predominant risk for cancer development and therefore potentially avoidable [19]. A persuasive example for the importance of environmental factors compared to mere genetic factors is reflected in studies of migrant populations in their new host countries who generally show a shift in cancer risk towards the one prevailing in their new environment [20], or that cancer risk in twins is not in accordance between the individuals [21, 22].

Lastly, interactions between drug and dietary components as well as drug-drug interactions are well known [23, 24]. One substance may affect the activity of another, resulting in an increased, decreased or entirely new effect which would not be expected from each substance on its own [24]. Efforts towards an expansion of this concept towards the totality of potentially interacting influences are being made [25]. The elucidation of these drug-exposome interactions could help improve existing knowledge about the complexities behind different responses to medication and help define effectiveness and safety on an individual basis.

1.2. Human biomonitoring

Humans as well as other living organisms are constantly exposed to a variety of different classes of chemicals found in our environment. Since the last century, with the expansion of industry and agriculture, this chemical burden has been ever increasing [26]. Among many others, sources include food contaminants, phyto- or mycotoxins and naturally occurring or synthetic industrial pollutants originating from modern anthropogenic activities [27].

Some of these substances may accumulate in the organism before unfolding their biological activity with potentially disruptive consequences (Figure 2). While genetic influences on human health have been a focus of medicinal research for decades, we are only starting to understand the extent and complexity of the impact that environmental chemicals have on disease development [28].

Human biomonitoring is a scientific discipline that targets the determination of chemicals of interest, including their metabolites or reaction products in different human matrices. The subfield "biological monitoring of exposure" allows for the assessment of whether and to what extent these environmental substances enter our bodies, while "biological effect monitoring" is concerned with the determination of potentially adverse biological effects of long term exposure on the population as well as on an individual [29].

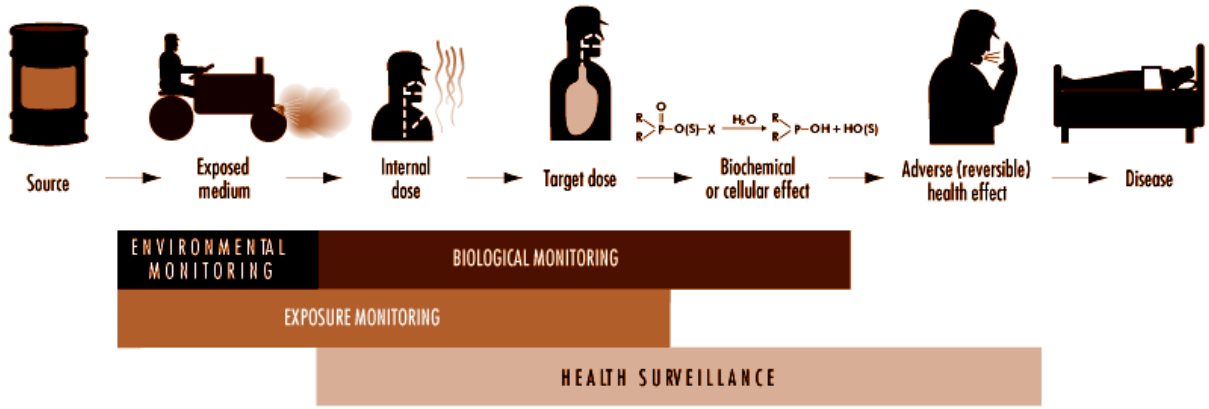

Figure 2: Exposure and biological monitoring bridging the gap between environmental influences and health effects [29]

The actually absorbed quantity of a substance also depends on individual factors other than the degree of exposure itself, including factors that influence the toxicokinetic profile/fate of the chemical like age, sex, genetic features or the current functional state of the whole organism [30]. Moreover, various routes of absorption need to be considered, including inhalation, ingestion and dermal absorption [30]. Compared to mere environmental monitoring, biological monitoring offers several advantages as it takes all of these additional aspects into account. It assesses the overall exposure and absorption from different sources and their associated effects over extended periods of time.

Biomonitoring is not supposed to completely replace environmental monitoring, but to complement it, as it is still necessary to identify and reduce sources of emission, if biological monitoring suggests that detected levels of exposure are actually of concern. As an example, schools in Germany were decontaminated because of the detection of toxic polychlorinated biphenyls, which were used in sealants, in ambient air. However, biomonitoring showed that the actual internal dose in children attending such schools was not significantly higher than background levels in the general population [31]. Of course, if avoidable, it is always advisable to minimize external exposure to toxic substances. Nonetheless, while biomonitoring can identify concerning levels of new chemical exposure, it can also relativize outcomes of environmental monitoring.

The sampling and analytical demands of biological monitoring are generally of far greater challenge than those associated with the measurement of environmental matrices [30]. In addition, most human biomonitoring studies are restricted to selected groups of compounds measured in a targeted analysis at only one point in time [32]. Definitions of priority lists of chemicals to be investigated are not always clearly established and the selection of the ideal biological matrices requires considerable diligence as the presence and concentration of different biomarkers may depend on the matrix and time of sampling [33].

Nonetheless, biomonitoring data is often the most relevant measure for health impact assessments, especially for bioaccumulating, persistent chemicals that are stored in the body for longer periods of time. The objectives of analysing human health indicators include the uncovering of temporal trends in exposure, including differences among geographic- and population related subgroups as well as contributing cultural and lifestyle factors, thus leading to more informed policy-making on a national and ideally international scale [34].

A major challenge is the identification and attribution of these environmental influences to certain kinds of pathogeneses. Traditional public health epidemiology cannot isolate the specific effects of individual exposures as multiple of them usually occur together and often, studies rely on using surveys of people's recall of past exposures. Accurate measurement of not merely external exposure, but also the actual internal exposure levels in human matrices helps to alleviate this problem and increases accuracy of epidemiological studies on disease risk assessment [35].

In the past, human biomonitoring methods have primarily been developed for the detection of chemicals associated with workplace exposure. More recently, methods for heavy metals, biocides and industrial chemicals relevant for the whole population have been established [36]. Today, we also know that various environmental pollutants, food contaminants, phyto- and mycochemicals outline a massive pool of xenobiotics with potentially harmful biological properties.

Each year, many national and international panels of experts of scientific agencies put forth lists of chemical of interests relevant for human biomonitoring. Selection criteria include toxicological

relevance, human bioavailability and likelihood of exposure [37]. The Human Biomonitoring for Europe (HBM4EU) project is an international joint effort funded in 2017. Its main aim is the coordination and advancement of human biomonitoring in Europe to provide better understanding of the chemical exposure and health effects on a population basis to improve policy making [38]. It has previously defined chemicals of high concern, among them various classes of substances such as plasticizers, plastic components and their substitutes, per- and polyfluorinated alkylated substances (PFAs), flame retardants, heavy metals, polycyclic aromatic hydrocarbons (PAHs), mycotoxins, phytotoxins, pesticides, personal care product ingredients, including cosmetics and fragrances, by-products of food processing and many more.

1.2.1. Matrices and toxicokinetics

Among the very first examples of chemical analysis of human matrices related to environmental exposure is the direct determination of lead in human blood [39] or benzene metabolites in urine [40] in the 1930s. Biological matrices should be easily and ideally non-invasively in sufficient amounts accessible for routine analysis. For both of these reasons, blood and urine are the most approved and commonly used matrices for biomonitoring purposes [41].

Many of the chemicals measured in blood have also been confirmed in breast milk [42] as well as adipose tissue [43]. The former does not only provide data on the baby's exposure through ingestion, but also on maternal exposures. Less common possibilities include the sampling of hair, teeth, nails or even pulmonary air for human biomonitoring purposes. However, problems such as interindividual variability and availability, the current lack of standard operating procedures, quality assessment schemes and missing knowledge of representative metabolites and biomarkers in these emerging matrices that still hamper their application for routine analysis [41]. Nonetheless, non-invasive matrices like hair, saliva and nails are becoming increasingly relevant in human biomonitoring [44-46].

The four main pharmacokinetic steps of absorption, distribution, metabolism and excretion (ADME) describe the processes taking place following the organism's exposure to the chemical and play a major role in matrix selection. In order to properly assess exposure, it is important to monitor the most suitable step in a substance's pharmacokinetic profile [47].

Substance uptake occurs by the main routes of dermal absorption, inhalation and ingestion and leads to an internal concentration of the chemical distributed in various tissues. The final bodily burden that is being exerted on the organism depends not only on the physical and chemical properties of the substance, but also on the physiological characteristics of the individual [44]. Compounds are excreted without metabolisation, metabolised and excreted or stored and only slowly excreted. Excretion can take place via matrices such as urine, saliva, faeces or breast milk. However, some substances are long-term stored in adipose- or even bone tissue. These processes are subject to individual variability [47].

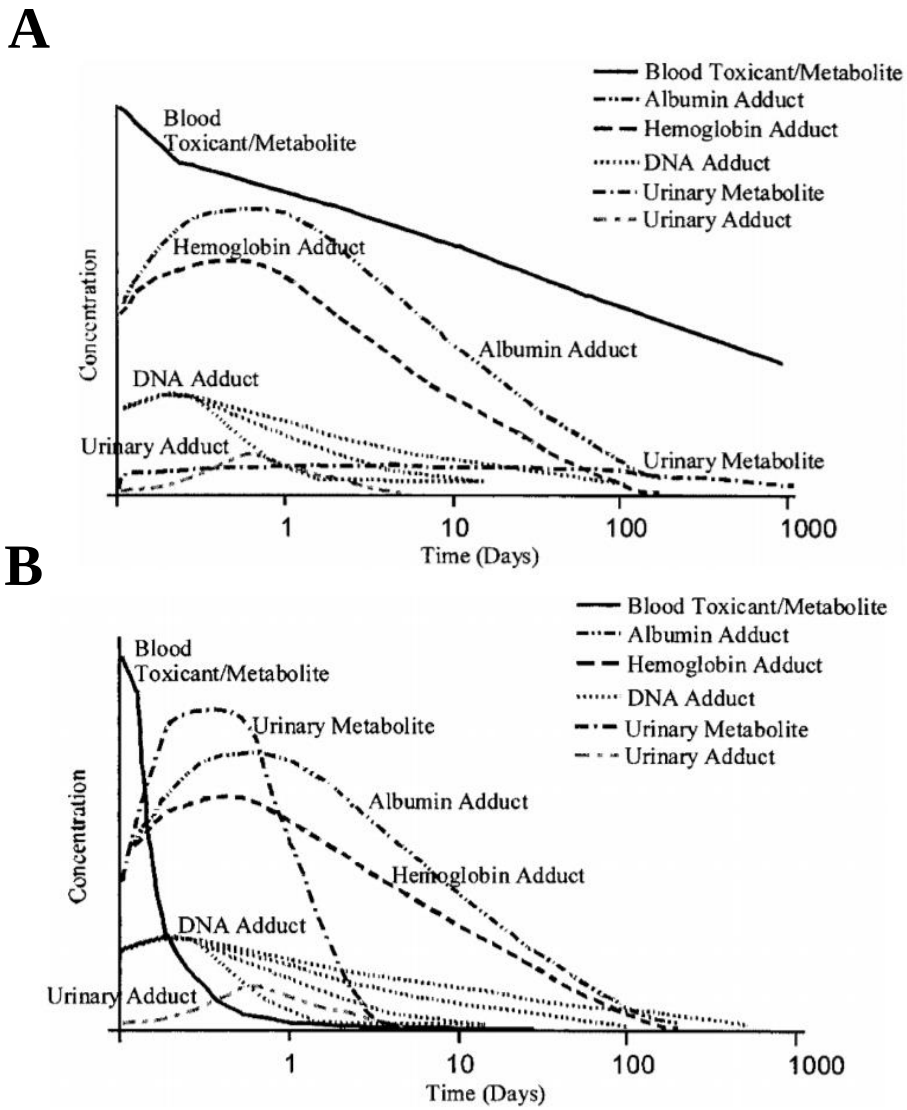

Figure 3: Post-exposure fate of a persistent- (A) and nonpersistent (B) toxicant in blood and urine [48]

Some compounds are readily absorbed and stored, hence monitoring of bodily tissues, mostly blood, is the matrix of choice (Figure 3A). However, other nonpersistent compounds may be metabolised and excreted more readily, thus monitoring of urine may be the more suitable choice (Figure 3B). Persistent chemicals feature very long half-lives in the body. In some cases they stay months [49] or

even years in the organism [50, 51]. Blood may be regarded as the ideal matrix for monitoring persistent chemicals, as it is in direct, steady state contact with most tissues where substances are eventually deposited. Hence the determination of blood chemistry may allow for a judgement of the whole organism's level of exposure to the examined chemical [44].

The time interval between last exposure and sample collection is crucial for the choice of matrix as well. Even nonpersistent toxicants may be measured in blood if the sample is collected soon after exposure or if the analytical method of choice allows for sensitive determination [48].

Failing to confirm the presence of a substance of interest in a certain matrix does not necessarily indicate that exposure has not taken place as matrix selection and time of sampling are crucial for discovering markers of exposure. Therefore, it is advisable to monitor circulatory as well as excretory matrices concurrently for exposure evaluation as the chemical's concentration in the former (i.e. blood) may be several orders of magnitude lower than in the latter (i.e. urine) [52].

1.2.2. Xenobiotic metabolism

Once lipophilic compounds have been absorbed, our body typically deals with them by transforming them into more water-soluble substances, thus increasing their rate of excretion via urine. This process of biotransformation usually takes place in two steps of phase I and phase II metabolism (Figure 4) [53].

Enzymes involved in phase I metabolism include different kinds of hydrolases, monooxygenases, oxidases, cyclooxygenases, alcohol or aldehyde dehydrogenases and oxidoreductases [53]. The reductive, oxidative or hydrolytic reactions in phase I metabolism introduce or unmask polar functional groups such as amines, hydroxyls, thiols or carboxyl groups, thus functionalizing the substrate. The superfamily of polymorphic haem-containing P450 monooxygenases (cytochromes P450 – CYPs) is notable for its diverse reactivity involving various chemically different substrates upon which they act on [54]. Hence, they are of utmost importance to the metabolism of xenobiotic substrates, as they account for 75% of total metabolism [55].

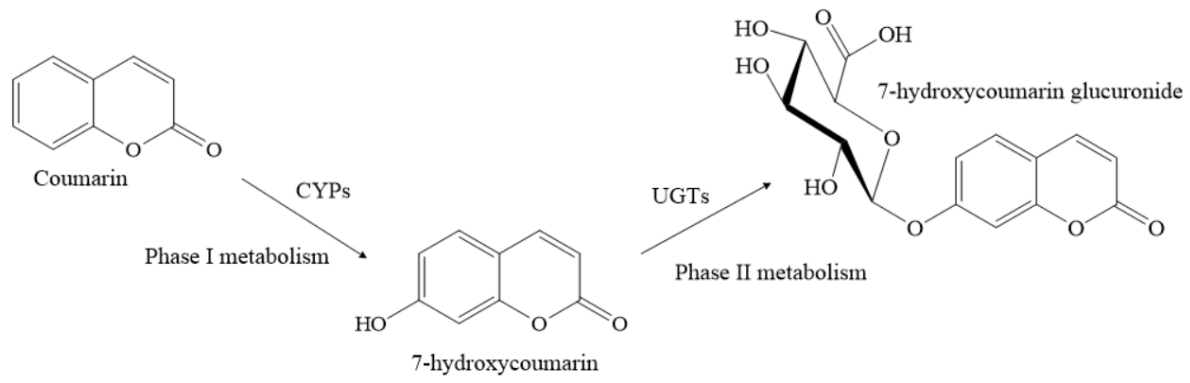

Figure 4: Phase I and phase II metabolism of the phytochemical coumarin [adapted from 56]

Phase II enzymes then conjugate functionalized phase I metabolites to more polar, inactive, water soluble metabolites, ultimately increasing the hydrophilicity for excretion. Glutathione S-transferases form conjugates with the tripeptide glutathione, UDP-glucuronosyltransferases (UGTs) catalyse the transfer of glucuronic acid from uridine diphosphate-glucuronic acid while sulfotransferases and N-acetyltransferases enable the linkage to sulfate- and acetyl groups respectively [53].

The functionalisation during phase I metabolism is essential for the detoxification of many xenobiotics. Without functional activation, phase II metabolism could not easily take place and in many cases, phase I metabolism already results in polar compounds ready for excretion. However, in many other cases phase I metabolism is the cause of a substance's disruptive biological properties (Figure 5). CYP enzymes may generate intermediates that are of higher toxicity than the original substrate and if the detoxification during phase II metabolism is insufficient, these reactive metabolites may be allowed to unfold e.g. their cytotoxicity by binding to proteins and/or nucleic acids [55]. Albeit not as frequent, toxic phase II metabolites have also been described [57-59].

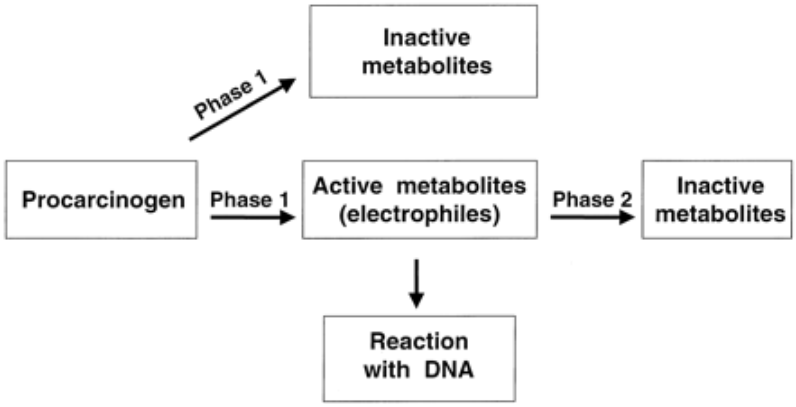

Figure 5: Activation and inactivation during the metabolism of a procarcinogen [60]

Interindividual variability is an important factor when it comes to the judgement of potentially adverse chemical exposures (Figure 6). Early pharmacogenetic research has already revealed the possibility of polymorphisms in drug-metabolizing enzymes, meaning that multiple, slightly differing genetic variants of a given enzyme may be spread among certain populations or between individuals [61]. Very recent research suggests that the reason for the differences in lung cancer risk between African American- and white smokers may be explainable by differences in xenobiotic metabolism [62]. As another example, the so called "slow acetylator" phenotype describes the underlying polymorphic differences of drug N-acetylation. Slow acetylators feature either a lack of N-acetyltransferase or a decrease in its activity [63]. This often leads to increased toxicity of amine-based drugs such as isoniazid, hydralazine, procainamide as well as different kinds of sulfonamides that are usually metabolised by N-acetylation, while fast acetylators may show no response at all. This consideration has shown to be of utmost importance concerning individual drug dosing and precision medicine, as for instance approximately 50 percent of Caucasians are known to be slow acetylators [64].

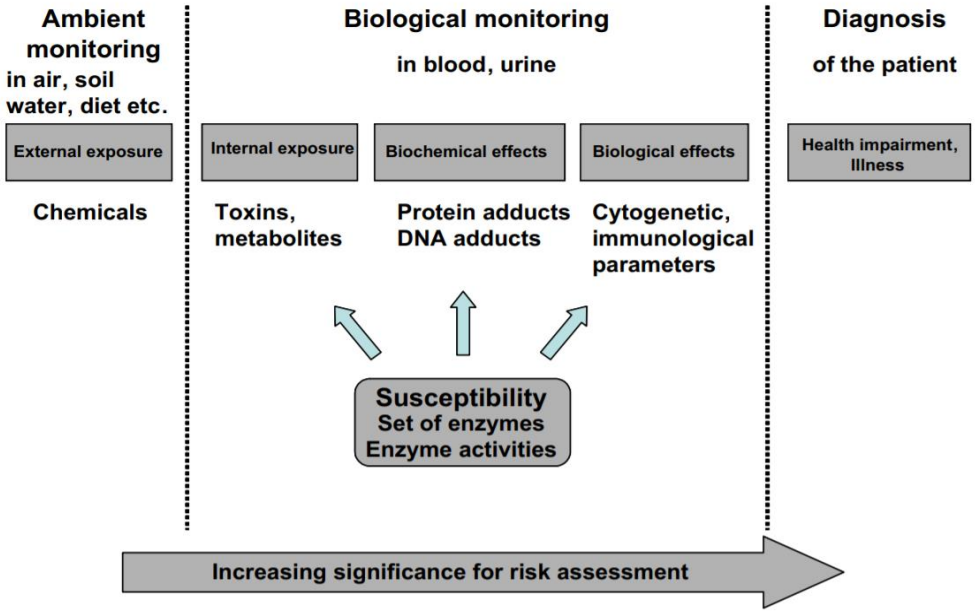

Figure 6: Individual susceptibilities influencing disease development resulting from ambient exposure [65]

The genetic polymorphisms of CYPs is another well documented feature that affects therapeutic responsiveness to drugs and also influences the biological impact of environmental pollutants. Nonetheless, it is still controversial whether they are relevant to the risk assessment of occupational ingestion concerning low general levels of environmental exposures in comparison to the relatively high levels of therapeutics ingested [61].

The proper choice of analysis varies depending on the question that is to be answered. The determination of urinary metabolites may be a possibility to assess exposure, but it may not be suitable at all to investigate its biological impact. While the measurement of the internal dose of a toxicant or/and its metabolites in tissues or bodily fluids might be associated with ambient exposures, it does not necessarily provide accurate data about interactions of the compound with critical cellular targets or with an established surrogate marker [66]. In many cases, the relationship between the levels of toxicant in the body and the toxic response is rather complex and difficult to predict since it depends on several genetic and toxicokinetic factors mentioned before. Figure 3 already indicates that a variety of adducts between the toxicant and cellular macromolecules may form. In this case, monitoring of DNA-adducts would be the more mechanistically relevant biomarker than merely the internal dose of the toxin, because it takes into account differences in individual metabolic responses (activation, detoxification). However, if only the determination of the internal dose is of primary concern, a basic notion of the substance's most relevant metabolites needs to be available to choose the ideal candidates to maximise analytical sensitivity.

1.2.3. LC-MS for human biomonitoring

The coupling of liquid chromatography (LC) and tandem mass spectrometry (MS/MS) combines the separation capabilities of LC with the high sensitivity, specificity and accuracy of MS. With the development of electrospray ionisation (ESI) in the 1980s, soft electrochemical ionisation of large biomolecules was finally a possibility [67]. Ever since then, the usage of LC-MS/MS for bioanalysis grew tremendously with it today being widely used in pharmaceutical-, environmental- and clinical laboratories. It is thus regarded as one of the most important analytical tools in studies of drug metabolism, pharmacokinetics and biochemical toxicology.

1.2.3.1. Applicability of LC-MS and comparison to GC-MS

LC-MS and gas chromatography (GC) MS are the two workhorses for the analysis of organic compounds in complex matrices. GC-MS is generally accepted to be the more robust methodology with better separation capabilities. It can mainly be used for nonpolar, heat resistant, volatile substances often involving an additional derivatisation step during sample preparation. Furthermore, sample throughput is smaller with GC-MS, as GC-gradients are generally longer. While GC-MS is still the preferred method of analysis for certain groups of organic compounds such as free fatty acids, steroids, oils, polysaccharides, esters and terpenes [68, 69], one of the advantages of LC-MS is that it significantly expands the number of substances accessible to mass spectrometry. Polar, charged, thermally unstable and non-volatile compounds can all be analysed using LC-MS, along with molecules of higher molecular weights. Depending on the analytes, LC gradients and eluents can be adjusted in a versatile manner to achieve separation. In the case of ESI, different eluent additives may even allow for efficient ionisation of nonpolar compounds that are usually measured using GC-MS, such as polycyclic aromatic hydrocarbons [70, 71] or steroid-like molecules [73]. Considering the wide range of structurally and chemically different environmental pollutants, food chemicals, phyto- or mycotoxins and all corresponding metabolites which are of interest to human biomonitoring, LC-ESI-MS is the tool of choice as it enables concurrent analysis of multiple classes of xenobiotics with relatively simple sample preparation in a single run [74-75].

In the case of substances that are not polar enough to guarantee sensible ESI ionisation, atmospheric pressure chemical ionisation (APCI) is an alternative soft ionisation technique for LC-MS. Figure 7 illustrates the expansion of the compound library open to LC-MS with the additional option of APCI and compares it to the applicability of GC-MS. With APCI, ionisation is initiated in the gas phase using a corona discharge current which is more suitable for nonpolar compounds.

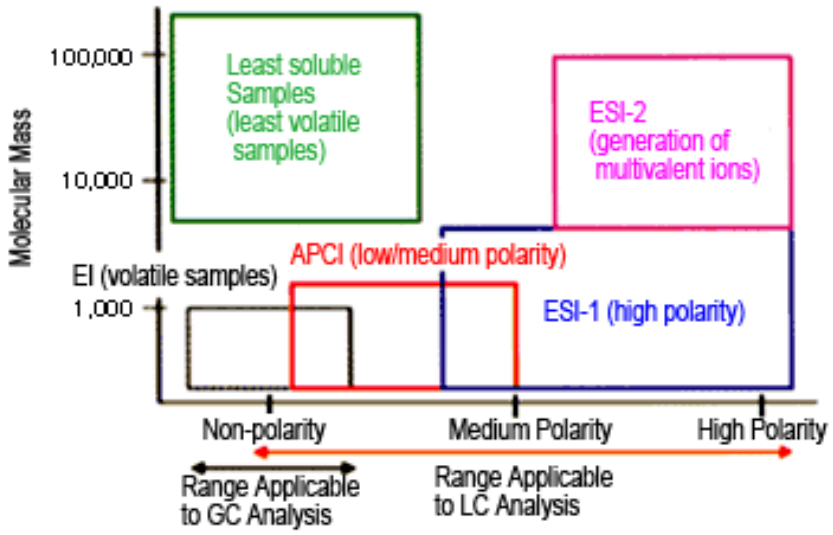

Figure 7: Comparison of applicable compounds for LC and GC analysis whereby ESI-2 stands for multiply charged ions formed by ESI of molecules with higher molecular weights and EI for electron ionisation, the ionisation technique used with GC [76]

A disadvantage of ESI is its susceptibility to ion suppression or enhancement, which depends on both the compound, the matrix and varies depending on the ionization mode as well as eluent composition [77]. For this reason matrix matched calibration, usage of isotopically labelled internal standards and standard addition is employed to avoid faulty analyses. APCI is less dependent on eluent composition and less susceptible to matrix effects that may cause ion suppression, which is very relevant to human biomonitoring. However, similar to GC, analytes need to be thermally stable to guarantee effective transfer into the gas phase before ionisation occurs with APCI. Additionally, APCI might have difficulty with representative ionisation of complex mixtures and as a result only capture the most stable ionisation products while excluding less stable ones [78]. As most mass spectrometers are sold with ESI alongside APCI sources, which are often interchangeable, they are complementary techniques, each with advantages and disadvantages, yielding comparable results for many compound classes [79].

1.2.3.2. Sample preparation

The employment of appropriate sample preparation and extraction methods for biological matrices is essential to achieve sensitive and representative results. While direct analysis of samples with minimal preparatory time investment would be ideal, the inherent complexity of biological matrices like blood, urine or (breast-) milk usually demands more elaborate approaches compared to environmental monitoring. Biofluids are rich in proteins, fats, carbohydrates, essential vitamins and minerals. These constituents create interferences and ion suppression, hence samples need more intensive clean-up prior to analysis. Additionally, comprehensive analysis probably includes analytes of different polarities, thus sophisticated methods to ensure quantitative extraction need to be employed. Ideally, a structurally closely related or isotopically labelled internal standard is added to account for losses

during extraction. Besides the reduction of ion suppression, precipitation and denaturation of proteins also increases extraction efficacy of lipophilic substances [45]. However, more steps during sample preparation also means more risks to loose analytes, which is problematic since often only small sample amounts are available for biomonitoring, especially in the case of invasive matrices.

Basic methods for sample preparation are liquid-liquid extraction (LLE) and solid-phase extraction (SPE). LLE uses water-immiscible organic solvents to extract analytes from the aqueous phase. Methanol may be used for the extraction of polar-, acetonitrile for varied polarities and hexane for the most nonpolar compounds [45]. LLE has been extensively applied as a routine technique for human biomonitoring, but it is a time consuming, labour intensive and solvent demanding procedure [45]. The technique has thus been replaced by SPE in various applications, which uses solid sorbents to retain analytes while washing steps remove matrix interferences. Except of usually being faster, less solvent demanding and containing an intrinsic clean-up step, a big advantage of SPE is that it can be automated and employed for on-line analyses [80]. Depending on the chemical properties of the analytes, different sorbents, elution solvents and an appropriate pH need to be selected [45].

Currently, QuEChERS ("Quick Easy Cheap Effective Rugged and Safe") approaches have been applied beyond its original field, which is the analysis of pesticides in food and environmental matrices, for analysis of biological fluids [81, 82]. It employs an initial LLE using acetonitrile, followed by a SPE with a salt mixture. Then a second, dispersive-SPE clean-up of the organic phase using a polymeric sorbent and salt mixture is utilized. The use of such generic extraction methods with relatively fast, yet effective procedures would not only enable higher sample throughput, but also expand the possibilities of biomonitoring.

Lastly, additional deconjugation steps might be employed before sample extraction. This treatment is mostly done for glucuronide- and sulfate metabolites, using β-glucoronidase and sulfatase respectively. In some cases, simple dilution followed by acidic incubation may suffice to achieve quantitative deconjugation [45]. This treatment reverts conjugated forms of the targeted biomarker back to the original compound structure, which is useful for multiple reasons. Firstly, it enables a more comprehensive analysis of internal exposure if no standards of the conjugated metabolites are available for calibration and method development. Furthermore it potentially increases sensitivity if direct monitoring of the metabolite is affected by stronger matrix effects or interferences, or if, depending on the method used, worse extraction efficiency is caused by the metabolite's higher polarity. However, the matrix might contain both the conjugated metabolite and the parent compound, which means they can no longer be independently quantified following deconjugation. In such a case, measurements with and without enzymatic pre-treatment may need to be carried out if independent quantification is of particular concern. In addition, results will change with deconjugation efficiency, which may vary depending on the matrix or enzyme used. Matrix components might bind

glucuronides or sulfates and thus decrease accessibility for the enzyme [83]. Therefore it is important to also monitor deconjugation efficiency.

1.2.3.3. Liquid chromatography

The majority of current LC-MS approaches use silica-based reversed phase (RP) C18 columns. Alternative RP columns incorporate phenyl groups and may be used if higher retention capabilities for polar compounds are required [84-86]. Otherwise, hydrophobic interaction liquid chromatography (HILIC) is used for small, highly polar compounds that cannot be sufficiently retained on regular RP columns [87].

Methanol and acetonitrile are the standard organic eluents for RP chromatography. Properties like pH, ion-pairing reagents and ion strength influence retention times and ionization efficiencies. Thus, different eluent compositions are used to improve peak separation and facilitate ionisation, depending on the chemical properties of the analytes. Acetic acid, formic acid and their ammonium salts, proton donators and acceptors respectively, are the most commonly used solvent modifiers [88].

1.2.3.4. Mass spectrometry and the Sciex QTrap 6500^{+}

Tandem mass spectrometry (MS/MS) combines two mass analysers in one instrument (Figure 8). In general, a triple quadrupole (QqQ) setup is used. The first analyser (Q1) filters out the precursor mass to charge ratio (m/z) of interest, while the second analyser (Q3) filters product ions of the precursor that were formed in a collision cell (Q2) by collisions with neutral gas ions between Q1 and Q3. This increases sensitivity through noise reduction and enhances compound specificity by assessing characteristic fragmentation patterns.

Figure 8: Basic components of an MS/MS setup [89]

Targeted applications in environmental- or biological monitoring typically use either simple quadrupoles, 3D quadrupole ion traps or 2D linear quadrupole ion traps (Figure 9B-D), with the latter being of highest sensitivity and dynamic range. Hybrid instruments using time of flight-type analysers (Figure 9A) offer up to ten-fold higher mass resolution compared to conventional quadrupoles [90] and the highest mass range and scan rate of all instrumentations. While of broad application range, they are mostly used for screening purposes in non-targeted analysis [91]. Lastly, high resolution hybrid instruments using 3D ion traps such as orbitraps or ion cyclotron resonance analysers (Figure 9E, F) are used if mass resolution for structural identification is the single most important aspect [92].

Conventional targeted MS/MS approaches, typically in a triple quadrupole configuration, are methods for the quantitative determination of a list of selected analytes. The availability of standard is required. This usually poses no problem for environmental monitoring, as pesticides, pollutants and all leading compounds are readily available. However, in biomonitoring, not only the leading substances are of interest, but more importantly a whole range of metabolic transformation products and biomarkers. In many such cases, only standards of a few metabolites are available for analytical method development. Thus it is impossible to cover all compounds of interest using a targeted approach and only a limited number of analytes is actively monitored. Nonetheless, higher confidence in compound identification and higher sensitivity important for quantification of low concentrated contaminants compared to untargeted approaches are reached.

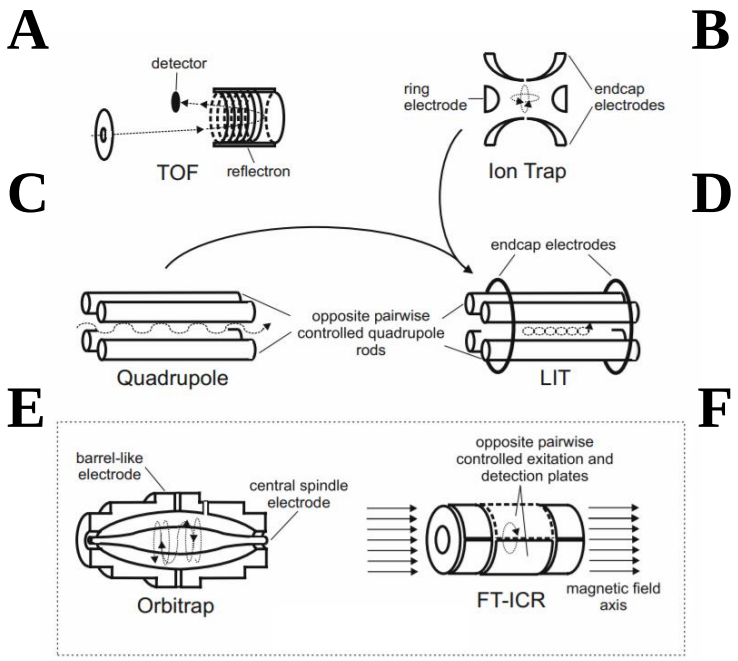

Figure 9: Types of mass analyzers used in tandem MS [90] are time of flight (A), 3D quadrupole ion trap (B), quadrupole (C), linear ion trap (D), orbitrap (E) and ion cyclotron resonance (F) spectrometers

Modern target-compound screening is operated in selected- or multiple reaction monitoring mode (SRM or MRM) covering hundreds of mass transitions, with MRM being the application of SRM to multiple product ions formed by one or multiple precursor ion masses. Using scheduled MRM, transitions are only measured within a retention-time window, which maximises the number of transitions that can be monitored without loss of sensitivity. The time spent acquiring data for each transition is optimised, ensuring sufficient data points across each chromatographic peak [93]. Typically two tandem-mass transitions are monitored for each compound, one to quantify the compound (quantifier) and an additional one to confirm its identity (qualifier). Such methods rely on appropriate chromatographic compound separation, regular check up on peak drifts and optimisations of the acquisition method to maximise performance.

Untargeted, full scan high resolution tandem MS (HRMS/MS) allows the post-run detection of known and unknown compounds suspected of being contaminants without standard reference material or pre-programmed mass acquisition. This is possible by relying on high-mass resolving power with accuracies in the sub 5 ppm range [94] and established compound databases. Albeit usually of lower sensitivity, HRMS is theoretically unlimited in the number of compounds that can be analysed and is able to screen for unknowns, which is an attractive possibility if standards are not commercially available. However, non-targeted HRMS strongly depends on appropriate, time consuming and complicated data evaluation and is in need of much higher computing power. The chance of false positives and false negatives increases with complex matrices. Its usage for biomonitoring purposes is still in its infancy and has not yet been comprehensively established [45, 94, 95].

In this work, a new ultra-sensitive MS/MS system by Sciex was used (Figure 10 A).

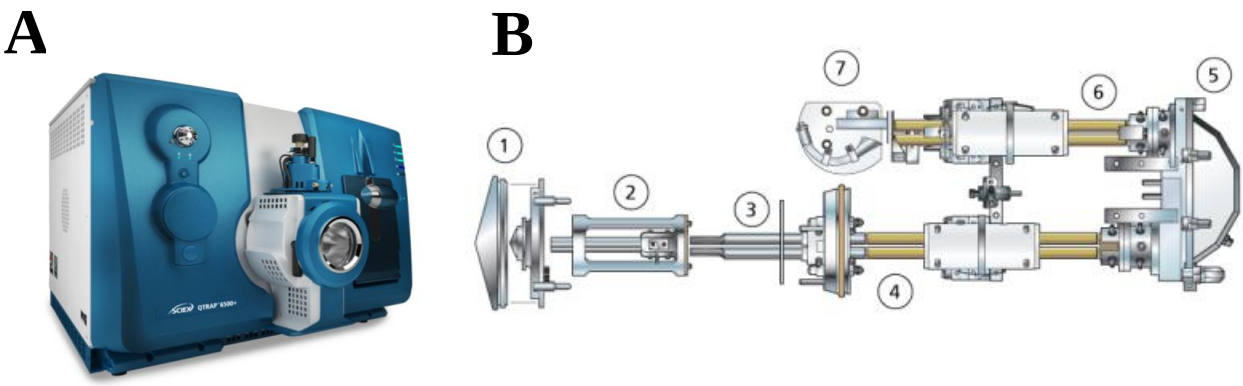

Figure 10: The SciexQTrap 6500$^+$ system (A) and its components (B) [96]
Orifice plate (1), QJet ion guide (2), Q0 region (3), quadrupole Q1 (4), collision cell Q2 (5), linear ion trap Q3 (6), detector (7)

The QTrap 6500$^+$ uses a state of the art 2D linear ion trap as final mass analyser (Q3) of the triple quadrupole setup. Ion traps generally are of higher sensitivity than traditional quadrupoles, because a full mass spectrum can be generated for each pulse of ions that has been admitted into the trap. In addition, 2D linear ion traps are capable of higher ion storage- and trapping efficiency, since there is no quadrupole field along the z-axis. A bigger dynamic range and less space charge effects due to increase storage volume compared to traditional 3D quadrupole ion traps [97] are observed. Figure 10 B displays a schematic of the internal construction of the system. It consists of a series of quadrupole filters transmitting ions of certain mass to charge (m/z) ratios until they reach the terminal detector.

Following ionisation using an ESI source, compounds enter the system through the orifice plate in a relatively wide ion flux. To increase sensitivity and m/z selectivity, this flow is further focused in the first two quadrupoles, the Qjet ion guide and the Q0. Q1 is the first filtering quadrupole which, when operated in multiple-reaction monitoring or product ion mode, selects a specific precursor ion m/z and guides it towards the high pressure, U-shaped LINAC collision cell Q2. In this non-filtering quadrupole, selected ions are axially accelerated in a radio frequency (RF)-only field and undergo

collision induced dissociation by colliding with neutral nitrogen molecules. Formed fragments are then guided into the linear ion trap Q3. The curved shape helps eliminate neutrals [96].

In MRM mode, the ion trap is also set to the specific m/z ratio of a fragment of interest. Using an RF voltage on the quadrupoles, ions are confined along the radial axis (x- and y axis), while a repulsive direct current (DC) voltage on the exit lens prevents ions from leaving the quadrupole, thus collecting and trapping ions inside the linear ion trap [98]. Once the predetermined fill time has elapsed, a repulsive DC barrier is placed on the entrance lens which stops further ions from accumulating inside the trap, while the collected ions are confined along the z axis by the DC voltage barriers on the entrance and exit lenses.

Then, during scan out, the DC voltages on the exit lens and the main RF voltage are ramped up simultaneously, while an additional single frequency dipolar auxiliary alternating current (AC) is applied to the quadrupole rods of the ion trap.

Once the main RF voltage reaches sufficiently high amplitudes, ions of specific m/z are brought into resonance with the auxiliary AC frequency and acquire enough axial velocity to overcome the exit lens DC barrier. They are then axially ejected towards the detector. To simplify, ions are excited radially to eject them axially. Spectra are acquired by rapidly scanning the main RF voltage.

Radial excitation resulting in axial acceleration is explainable through the inherent creation of deformed RF fringing fields near the quadrupole exits (Figure 11). Normally considered detrimental artefacts of electrode geometry, these fields are exploited to eject ions axially. In these fringing fields, the RF induced radial ion motion is coupled to axial motion once the frequency of ion motion along the radial axis corresponds to the frequency of the applied auxiliary AC field. This allows the ions to gain axial kinetic energy, until they eventually are able to overcome the repulsive DC barrier on the exit lens. Depending on the RF amplitude and the ion m/z, the fringing field near the end lenses accelerates the radially oscillated ions into the axial direction. Thus, selective ion ejection is realized by changing the RF potential so that different m/z ratios are brought to the boundary of their stability conditions. This effect is called mass selective resonance excitation and results in mass selective axial ejection. Ions can be axially ejected in a mass-selective way by ramping of the RF amplitude, which brings ions of increasingly higher m/z ratios into resonance with the single frequency auxiliary AC voltage. These ions gain additional radial amplitude until they are ejected axially [97, 100, 101].

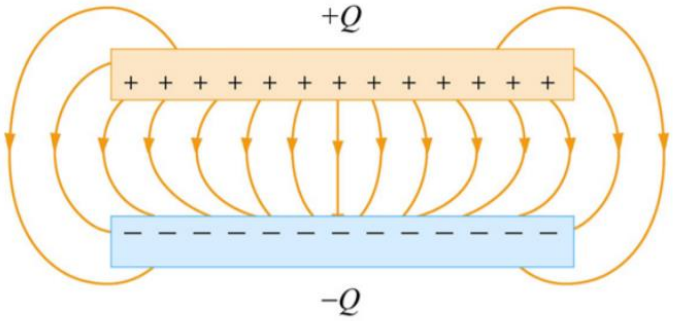

Figure 11: Non-direct fringing field lines towards the ends of two rod-like electrodes [99]

As the RF fringing fields are short-ranged, only about 20% of trapped ions are ejected towards the detector [103]. In general, radial ejection is another possibility for linear ion traps (Figure 12). Here, the RF amplitude is ramped until the ions are able to overcome the radial voltage barrier, eventually reaching one of two detectors. An AC auxiliary voltage is applied between the two opposing exit rods and enables ion ejection perpendicular to the central axis once resonance between the ion amplitudes and the AC voltage is reached. The problem with this setup is that the ions have to leave through holes in the quadrupole electrodes. These holes may cause significant RF field imperfections that could lead to ejection at points which were not predicted by the ion stability calculations [97, 103]. Still, while radial ejection may be of higher sensitivity than axial ejection, linear ion traps using axial ejection can be switched to function as a simple quadrupole, which enables them to perform all selective scan modes, such as product ion scan, neutral loss or MRM scan of a triple quadrupole instrument with linear ion trap sensitivity and scan rate [104].

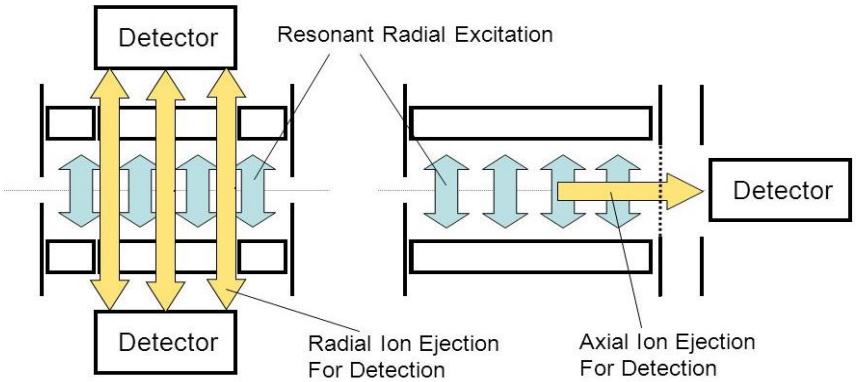

Figure 12: Comparison between radial- and axial ejection in linear ion traps [102]

After ejection out of the ion trap, an electron multiplier is used to detect incoming ions by converting the ion current into a voltage pulse through secondary electron emission in a continuous dynode arrangement. The registered intensities of these voltage pulses are directly proportional to the number of ions entering the detector.

The first step of targeted MS method development is the determination of precursor masses and their respective MS/MS fragments, followed by optimization of ion optic parameters. Each (product-) ion features different intrinsic molecular dynamics. By adjusting ion dependent parameters, ion transmission and fragmentation yields can be optimized. Afterwards, the most stable product ion, meaning it features highest detector intensities, of each precursor compound is selected for quantitative evaluation (quantifier). Along with the quantifier ion and the LC retention time, the second most stable fragment is selected for additional qualitative evaluation (qualifier) in the MRM method.

Figure 13 displays parameters of the QTrap 6500^{+} that mainly concern lens elements and ion paths which need to be optimized for each compound individually.

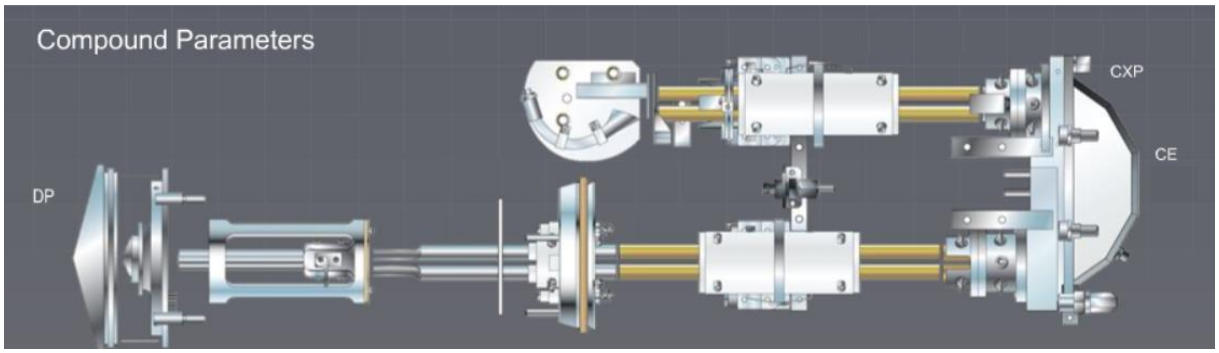

Figure 13: Compound dependent MS/MS parameters [96]

Parameters that are to be optimised are the following:

- DP: declustering potential – this voltage is applied between the orifice plate, the entry point of the MS. It prevents analyte ions from clustering together or from forming adducts with other charged- (mainly sodium or ammonium ions) or neutral species. The higher the DP, the higher the energy transfer onto the ions on their way to the QJet ion guide. Too high voltages may lead to unwanted fragmentation. This parameter is specific for each Q1 precursor ions mass.

- CE: collision energy – controls the potential between the Q0 region and the Q2 collision cell. The higher CE, the higher the acceleration precursor ions experience as they are sent into the collision cell to collide with neutral nitrogen molecules. This parameter is specifically optimized to achieve maximum yield of each fragment of the precursor ion created in the collision cell. A standard collision energy spread (CES) of 5V around the CE is applied.

- CXP: collision cell exit potential – focuses and accelerates the fragment ions out of the collision cell on towards the ion trap. Again, this parameter is optimized for each fragment ion mass to enhance ion transmission.

Once MRM parameters are set, optimisation of ion source parameters is the second step to achieve improved sensitivity in targeted MS/MS method development. In the case of the Turbo-V™ ESI source used during this work, parameters include the vertical position of the probe as well as different gas flows, the source temperature and the ion spray voltage.

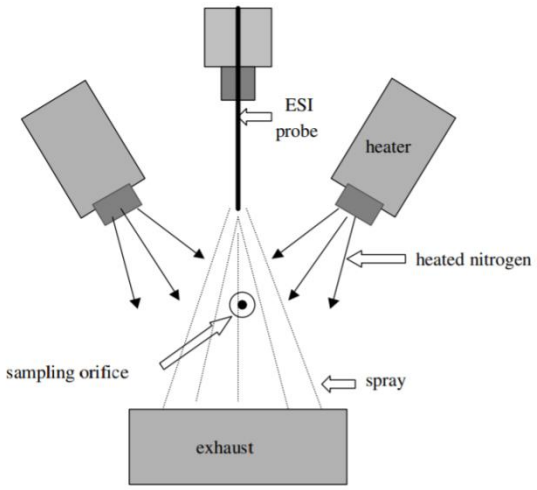

Figure 14: Schematic of the ESI geometry [105]

In general, the higher the flow rate, the farther away the ideal needle position from the orifice to minimise background noise [96]. However, this also affects analyte transmission. For this reason, determination of an ideal distance between the electrode tip and the aperture (Figure 14) is important as it affects the signal to noise ratio.

Figure 15 displays gas and source parameters which can be optimised once an ideal vertical probe positions has been determined.

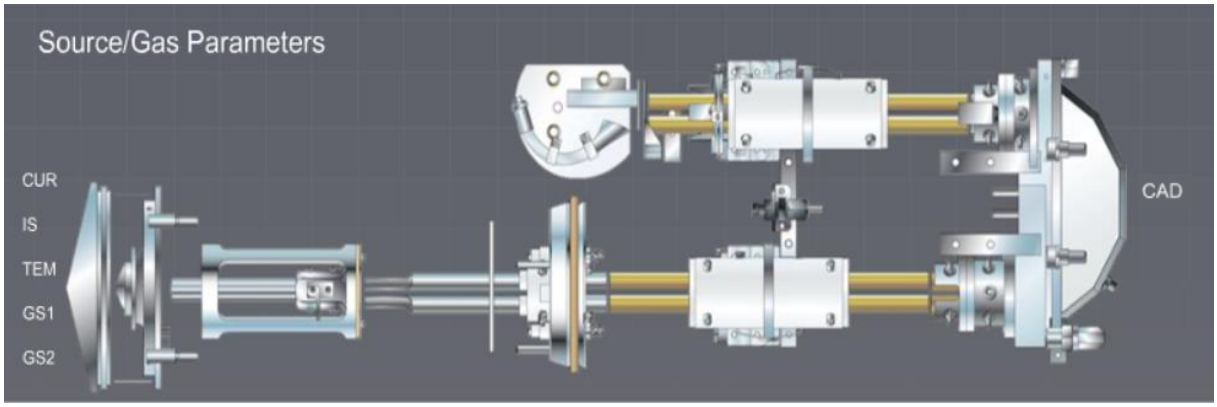

Figure 15: Compound dependent source- and gas parameters [96]

- CUR: Curtain gas flow – This counter-flow of nitrogen gas is used as an insulator between the ion source and the vacuum chamber. It helps with ion declustering, ideally leading to complete separation of the analyte ions and solvent molecules. It also prevents any of the vapours, solvent droplets, solid and neutral particles stemming from the sample, nebulizer and heat gas from entering the orifice and contaminating the optics. CUR should be as high as possible as long as it does not result in a loss of ion transmission.

- GS1: Ion source gas 1 – This is the ESI nebulizer- or sheath gas flow emanating coaxially surrounding the probe. It shears droplets off the liquid sample stream from the tip of the needle and facilitates evaporation of solvent molecules. It directly affects flow stability and sensitivity. Generally, higher sample flow rates demand higher nebulizer gas flows.

- GS2: Ion source gas 2 – This is the heated gas flow that accelerates solvent evaporation. Optimal sensitivity is achieved once temperature and GS2 flow bring solvent droplets to almost complete evaporation. Again, the higher the liquid flow rate, the higher the GS2 flow to achieve desolvation. However, too high temperatures, along with too high GS2 flows and a vertical probe setting that places the needle too low, may also lead to premature and complete vaporization of the solvent which could cause an increased background noise and unstable signals.

- TEM: Temperature – The temperature that is applied to the sample electrospray through the GS2 flow. While too high temperatures may cause premature evaporation, compound instability at high temperatures may also cause losses in sensitivity.

- IS: Ion spray voltage – The voltage that is applied to the needle to ionize the sample in the electrospray. It also impacts the ion spray stability and sensitivity and needs to be optimized for both polarities.

- CAD: Collisionally-activated dissociation gas – This last gas parameter does not affect ion transmission in the ESI source, but describes the extent of nitrogen gas pressure in the collision cell Q2. In MRM it acts as the target to fragment precursor ions.

1.3.Xenobiotics

A xenobiotic is any chemical that would normally not be found in a living organism or be expected to be produced by it [106]. Despite originating from different sources such as food, water, ambient air, consumer products or contaminated soil, biological activity has been established for many of these structurally often extremely diverse compounds. The range of potentially detrimental xenobiotics, of natural or synthetic origin, we are exposed to in our daily lives is tremendous. In line with the context of this work, endocrine disrupting- and (geno-) toxic xenobiotics shall be highlighted.

1.3.1. Endocrine disrupting xenobiotics

Endocrine disrupting chemicals (EDCs) are defined as substances that interfere with endogenous hormone biosynthesis, metabolism or action. The term xenoestrogens is used to specifically describe chemicals with similar structure as endogenous estrogens, allowing them to potentially interfere or mimic the actions of estrogen [107].

The idea that xenobiotic chemicals may be able to modulate the endocrine systems of exposed organisms has been established for nearly 30 years [109]. However, whether the concentrations at which these effects can be observed are of any relevance considering the doses encountered *in vivo* has often been a controversy between academic experts, non-governmental organizations and industry [110-112]. Nonetheless, alarming global trends are being associated with EDCs due to their biological influences on our modernised world. This includes observations in wildlife [113, 114], humans [115-117] and plants [118]. A challenge in the field of endocrine disruption has been the structural diversity when it comes to fast risk assessment, for example when new industrial alternatives are developed [119]. Although there are plenty of *in vitro* and *in vivo* studies demonstrating and describing the actual toxic effects and concentration ranges of certain chemicals, extrapolating these findings to our natural environment and the general human population has been difficult.

EDCs can either act as agonists or antagonists of the hormone systems by a variety of mechanisms. The most direct way is the interaction of an EDC with a hormone receptor, leading either to stimulation or competitive inhibition of downstream signalling pathways [120]. Moreover, EDCs may indirectly interact with endogenous signalling by affecting the synthesis of endogenous hormones, leading either to their overexpression or degradation [121].

Recent evidence even points towards the possibility that in addition to altering nuclear signalling, EDCs are also capable of acting through non-steroid receptor pathways, as transcriptional activators, in metabolism and other mechanisms influencing the endocrine and reproductive system [122, 123]. In some cases, U-shape dose-response effects, where lower doses of an EDC elicited a higher effect than higher doses, have been observed [124, 125]. This might be attributable to the tremendous intrinsic complexities of endocrine regulation. It is proven that EDCs generally exhibit lower affinity towards receptor binding than endogenous hormones [124]. However they also feature lower specificity, which

often results in their incorporations in several different pathways as they are able to interact with numerous partners to variable degrees [124]. Agonistic effects at one site combined with antagonistic effects at another site may explain these non-linear U-shape profiles. This complexity combined with the high number of EDCs in our environment renders the task of accurate endocrine-disrupting risk assessment an enormous challenge.

EDCs from different sources may have organisational effects on the development of structures and functions of the body during critical times of life such as intrauterine, perinatal, juvenile or puberty periods when we are more sensitive to hormonal disruption [125]. These organisational effects of hormonal action lead to permanent changes as they may alter sexually-dimorphic behaviours and phenotypes as was previously observed in animals [113, 114]. Furthermore, impaired development of the central nervous system, the limbs, the skeleton, the reproductive-, cardiovascular- and immune system as well as a link to childhood cancer as a result of exposure during fetal and embryonic stages has been proposed [126]. During this time, a sequential activation of genes providing numerous targets for environmental chemicals might explain this vulnerability [127].

In recent years, attention has been shifted more and more towards the possibility that combinatory effects of numerous different EDCs may result in stronger effects than which would have been expected based on a single component's individual bioactivity (synergistic or additive effect) [128-130]. This underlines the importance of the inclusion of holistic biomonitoring approaches for proper risk assessment, since these combinatory cocktail effects may explain dissonances between individual laboratory-established endocrine activities of substances and epidemiological observations. Similarly to other environmental contaminants, sources of EDCs are diverse and widespread. They may be of natural origin or synthetic chemicals used in consumer products or industrial applications.

1.3.1.1. Synthetic EDCs

Synthetic endocrine disrupting contaminants in our environment being produced in high volumes and found in nearly every household originate from modern anthropogenic activities. Thousands of man-made compounds on the market have been classified as EDCs when their ability to mimic and interfere with hormonal action came to light [125]. This resulted in their ban or removal from production, while others are still in use to this day. Industry still needs to quench the demand of consumers, but as long as there is no data on their environmental persistence and possible risks for humans, each new attempt to find alternatives with yet similar physical-chemical properties is a leap into the unknown.

An example is the former use of polychlorinated biphenyls (PCBs) in a broad range of applications from dielectrics, coolants, plasticizers, surface coatings to flame retardants and many more. More recent research revealed that among many other properties, PCBs may be able to disrupt follicular steroidogenesis, alter hormone synthesis and disrupt enzymes involved in hormone secretion [131].

Their production has been banned worldwide in 1977 ever since exposure was associated with increased risks of various cancers [132] and the occurrence of a condition called chloracne following direct contact with skin [133]. However, PCBs still remain widely distributed in the environment caused by their persistent nature. Human exposure continues, mainly through the food chain, indoor dust and air, resulting in PCB incorporation in fatty tissues and thus contributing to the xenobiotic cocktail. Fortunately, human blood PCB levels are decreasing each year in the western world [134].

Bisphenol A (BPA) is used as a plasticizer in polycarbonate plastics, epoxy resins and in thermal paper used by cash registers to print receipts. Its widespread use has led to environmental contamination due to its migration out of materials. Over the past decade, concerns of the health effects of BPA have forced food and beverage companies to abandon its use and look for alternatives. Thus, substitutes like Bisphenol B (BPB), Bisphenol C (BPC), Bisphenol S (BPS), Bisphenol F (BPF) and Bisphenol AF (BPAF) have been developed. However, similar properties concerning estrogenic activity were observed *in vitro* and *in vivo* [135-137]. This illustrates the problematic nature of the reformulation processes to replace EDCs and other harmful industrial substances.

Phthalates are another family of synthetic compounds manufactured to be used as plasticizers, solvents and in hundreds of industrial products such as vinyl flooring, adhesives, detergents, lubricating oils and personal care products like soaps, shampoos and hair sprays. While underlying mechanisms are still not fully elucidated, some phthalates exhibit endocrine disrupting activity and are associated with reproductive toxicity [138]. Similar to bisphenols, phthalates are not covalently bound to plastics and migrate out of polyvinyl chloride (PVC) containing items into food, air, water and soils [139].

Per- and polyfluorinated alkylated substances (PFAS) are used as oil- and water repellent in coatings. Due to their excellent chemical stability, they are of high environmental persistence and feature bioaccumulative potential [140]. Exposure to PFAS is suggested to cause adverse health effects such as infertility, abnormal maturation, endocrine disruption and even cancer [141-143]. The historically most commonly used PFAS, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), are already targets of regulation and phasing-out, while many alternatives remain largely unregulated and are substances of growing concern [144, 145]. An emerging substitute is hexafluoropropylene oxide dimer acid (HFPOA-DA, also known as *GenX*), a compound used in polymerization following the restrictions on PFOA [146]. It has already been detected in the river waters downstream of fluorochemical plants in Europe, China [147] and the US [148]. New toxicological research suggests that it may be of similar if not stronger endocrine activity [149, 150].

Flame retardants, another group of interest, are used in electronics, clothing and furniture to reduce flammability. Primary compounds of interest are polybrominated diphenyl esters (PDBEs) and hexabromocyclododecanes (HBCDDs), like PCBs, they show high environmental persistence [151] and bioaccumulative properties. Both groups of compounds have been associated with neurotoxic,

carcinogenic and endocrine disrupting effects [152-154]. Tetrabromobisphenol A (TBPA) is the most produced brominated flame retardant worldwide [155]. Unlike PDBEs, HBCDDs and PCBs, it is covalently bound to the product it is incorporated in, reducing its ability to migrate and accumulate. Hence, it still is believed that bioavailability and toxicity of TBPA is low [156]. However, it has been found in the environment and human tissue as well [157]. Toxicological studies also have shown hazardous properties including endocrine disruption [156, 158], raising even more concern in recent years. In a similar manner to plasticisers and PFAs, replacement flame retardants may feature similar health concerns [159-161].

Para-substituted alkylphenols and esters of 4-hydroxybenzoic acid (parabens) represent other large groups of prominently known potential endocrine disruptors. Their xenoestrogenicity is caused by the structural similarity to endogenous estrogens. Generally, higher estrogenicity seems to be observed with longer side chain lengths [162, 163]. Their environmental accumulation is caused by their use in detergents, paints, herbicides, pesticides, plastic polymers, food preservatives and personal care products.

Another group of EDCs are UV Filters which are used in cosmetics, plastics and adhesives. They too feature high bioaccumulative properties due to their lipophilicity and stability. Toxicological research on the main structural groups of benzophenone-, camphor- as well as cinnamate-derivatives has established estrogenic, anti-androgenic and thyroid disrupting activity [164-168]. With residues having been detected in environmental matrices such as wastewater, rivers, soil, human breast milk and even the placenta [169-171], they are a class of contaminants of emerging concern.

Lastly, environmental contamination with stable pharmaceutical estrogens such as diethylstilbestrol and ethinylestradiol pose a considerable problem. The former is still permitted as veterinary drug after it has been banned for human use since 1970 as it promotes adverse outcomes in pregnant women as well as increased cancer risk [172]. The latter is part of the formulation of hormonal contraceptives and has stronger estrogen receptor affinity than the endogenous ligand itself [173].

1.3.1.2. Naturally occurring EDCs

Some natural substances influence endocrine activity as well. Phytoestrogens are secondary metabolites of plants fulfilling various internal functions, or are compounds used for protection against pathogens and herbivores. Consequently they are found in a wide variety of food, most notably soy and hops. The flavonoids are among the most well-known and characterised groups of phytochemicals. While being known for their anti-inflammatory properties [174], several compounds, especially if they lack conjugation to any sugar residues, show biological activity at steroid- as well as thyroid stimulating hormone receptor sites [175-177]. Genistein is a major phytoestrogenic flavonoid originating from soy plants. Coumestans and lignans are other classes of phytoestrogens that show

relatively strong endocrine disrupting properties, with coumestrol and enterodiol or enterolactone being the most relevant compounds of each class respectively [178].

Compared to plants, fungi as sources of EDCs have not been as thoroughly examined. The most relevant and one of the most common mycotoxins is zearalenone (ZEN). Produced by the filamentous fungus genus *Fusarium*, it is present in numerous agricultural products, most notably corn, oats, wheat and rice, leading to the contamination of livestock and food. Among many other adverse health effects such as genotoxicity, ZEN binds competitively to estrogen receptors [179] and is thereby associated with increasing incidences of breast cancer [180]. It has been shown that ZEN is absorbed quickly into the body and then transformed into metabolites of even higher toxicity [181]. The presence of ZEN in human and animal blood indicates a problem on an international scale [182]. Most recently it has been confirmed to be able to cross the human placental barrier [183], thus potentially causing adverse effects during foetal development [184].

Alternariol is a toxic metabolite of phytopathogenic fungi of the genus *Alternaria* which shows its estrogenic activities mainly through nonsteroidal anti-androgenicity [185, 186]. It is also of widespread occurrence in food and plant products [187].

1.3.2. Toxic and carcinogenic xenobiotics

In general, toxicity of xenobiotics can be the result of different mechanisms. Damage to- or inhibition of enzyme systems, disruption of protein synthesis or signalling pathways and general alteration of physiological mechanisms are a few manifestations of toxicity. Xenobiotics may induce their adverse effects directly or indirectly following activation by biotransformation. It is believed, that a great majority of toxic effects are caused by indirect-acting chemicals [188]. In these cases, the key role in toxicity is the formation of electrophilic properties that enable interactions with cellular macromolecules such as proteins, lipids and DNA [188]. Moreover, different organs or tissues may be targeted to different extents, which often also depend on the type of exposure [189]. For example, acute alcohol exposure could impair the nervous system, while chronic exposure primarily affects the liver [190].

Increased risk of cancer development as a result of chronic environmental exposure to certain chemicals has been well documented and is a main subject of concern when it comes to xenobiotic risk assessment [19].While many of the EDCs mentioned before may cause cancer indirectly through the inherent association of increased estrogen exposure with cancer development [191], other carcinogenic xenobiotics do so through a variety of different mechanisms, including the activation of oncogenic signalling pathways, the inhibition of tumour- growth suppressing pathways or direct induction of mutations. In some cases it is likely that the carcinogenic effects of a xenobiotic might even depend on simultaneous activation of multiple cancer hallmark mechanisms [192].

1.3.2.1. Environmental and food-processing xenobiotics

Food-processing is one of the major sources of exposure to toxic xenobiotics. High temperatures during frying, grilling and roasting are responsible for the formation of harmful contaminants. Heterocyclic aromatic amines (HAAs) and N-nitrosamines are formed during heating of foods rich in protein, while acrylamide and 5-hydroxymethylfurfural (HMF) are products of carbohydrate heating [193-195].

Ambient air and drinking water are other sources of potential xenobiotic exposure. Outdoor air pollution is caused mainly by the combustion of petroleum products or coal by industrial engines and power plants [196]. Polycyclic aromatic hydrocarbons (PAHs) are well known toxic products of incomplete organic combustion processes. While disinfection of drinking water represents a historic milestone in the reduction of epidemics caused by micro-organisms, such processes may generate disinfection by-products (DBPs) of relatively recent health concern [197].

HAAs have been extensively studied and accumulated the strongest scientific evidence to be a colorectal cancer risk factor among contaminants caused by food-processing. It is generally accepted that their main mode of action is the induction of mutations by forming DNA-adducts [198]. 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is the most abundant HAA formed during cooking of meat and fish and may account for up to 75% of the genotoxic material isolated from the crust of the cooked product [199]. CYP metabolism generates activated N-hydroxy-PhIP which can be detoxified by glucuronidation to form the major urinary metabolite. However, alternatively N-hydroxy-PhIP may be further activated by sulfation or acetylation which results in the generation of highly reactive electrophilic esters that are capable of forming DNA adducts (Figure 16) [58]. Moreover, PhIP has been shown to be able to cross the blood brain barrier and besides their high reactivity, its hydroxylated phase I metabolites share structural features of dopaminergic neuro toxicants [200].

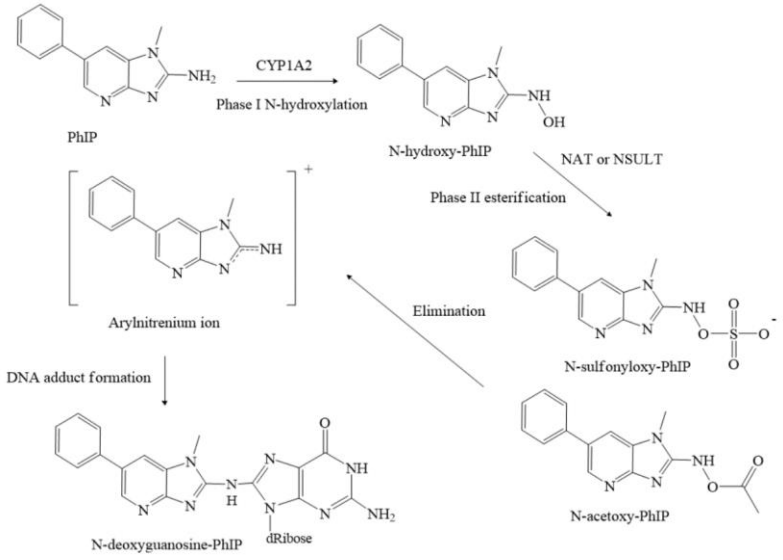

Figure 16: Metabolic activation of PhIP and DNA adduct formation [adapted from 58]
NAT: N-acetyltransferase, NSULT: N-sulfotransferase

N-nitrosamines are natural by-products of food processing whose formation is amplified by the addition of nitrites as food preservatives. They are a product of the reaction of nitrogen oxides with organic molecules, mainly secondary amines and have been confirmed in tobacco smoke as well [201].

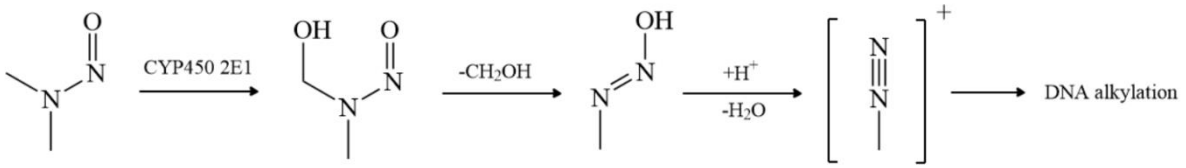

Figure 17: Metabolic activation of NDMA generates alkylating diazonium ions [adapted from 204]

This ubiquitous class of environmental contaminants is comprised of many structurally different compounds, with the two most relevant being N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), both of which are classified as definitely carcinogenic [202]. Their main contribution to carcinogenesis is the CYP mediated formation of highly reactive diazonium ions that are capable of DNA alkylation (Figure 17) [203].

Industrial usage of acrylamide is mainly limited to its use as non-toxic polyacrylamide for laboratory work, formulation of cosmetics, body care products or the textile industry. While, some of the monomer is still released into the environment [205], acrylamide in food products is of much greater concern for the general population. Frying of carbohydrate-rich foods results in concentrations up to the mg/kg range in produce such as potato chips and French fries [206]. Once absorbed, acrylamide is either directly detoxified by conjugation to glutathione, or oxidised by CYPs to create glycidamide. While both glycidamide- as well as acrylamide-protein adducts have been confirmed in human blood, only glycidamide has been established to form DNA-adducts, thus suggesting its function as the main genotoxic metabolite (Figure 18) [207].

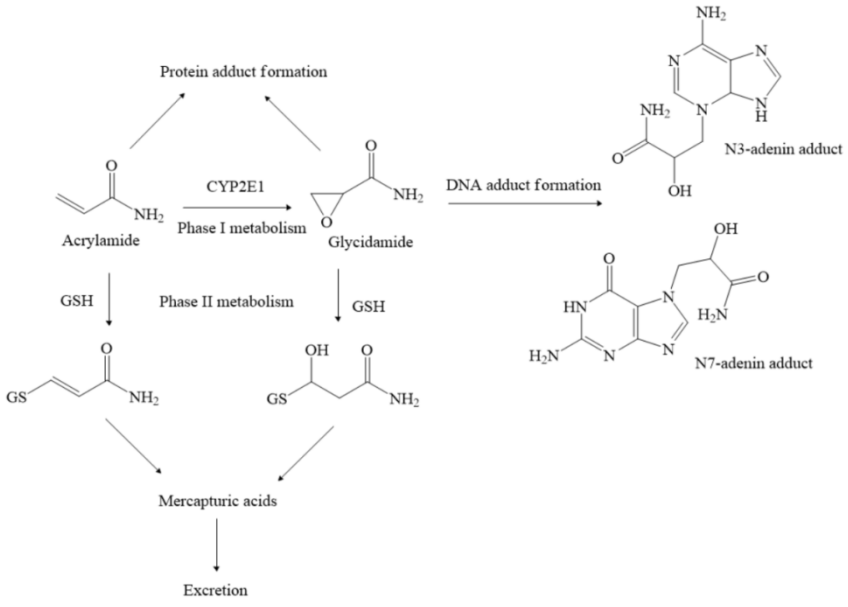

Figure 18: Metabolism of acrylamide [adapted from 208]

Furfural and HMF are food contaminants of relatively recent interest. They are both intermediates of the Maillard reaction [209] and, at low pH, dehydration products of pentoses and hexoses respectively [210]. *In vivo*, phase II metabolism of HMF yields the sulfoconjugate 5-sulfoxymethylfurfural (SMF), which is theorized to be able to form a strong electrophile by elimination of its sulfate group, thus enabling interactions with nucleic acids and proteins (Figure 19) [57]. For a long time, SMF has only been confirmed in animal models, but recent detection in human plasma as a result of average dietary HMF intake indicates the potential need for biomonitoring efforts [211].

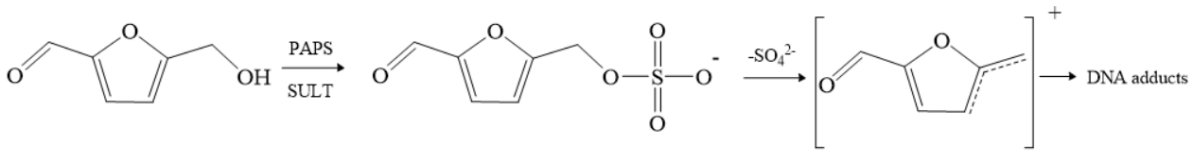

Figure 19: Metabolic activation of HMF [adapted from 212]
PAPS: 3'-Phosphoadenosine-5'-phosphosulfate, SULT: Sulfotransferase

Furfural is used as industrial feedstock and as a solvent for oil refining [213]. While being an irritant, suspected genotoxicity [214] has not yet been established for humans [215]. Still, as an industrial organic solvent, environmental exposure to furfural has been of concern for far longer than exposure to HMF. Concerning its ubiquitous occurrence as a food contaminant, it has been used as a marker of thermal treatment of food and thus as a potential biomarker for exposure to pyrolytic products such as the ones mentioned before [216, 217].

Among many other aryl hydrocarbons like dioxins, which are environmentally persistent by-products of the production of organochlorides, PAHs are able to bind to aryl hydrocarbon receptors, whose activation and signalling cascade is strongly correlated with the toxicity of these compounds. In addition, various hydroxylated phase I metabolites of PAHs may directly cause mutations by DNA-adduct formation (Figure 20) [218] and mono hydroxylated metabolites of PAHs do appear slightly estrogenic [219, 220].

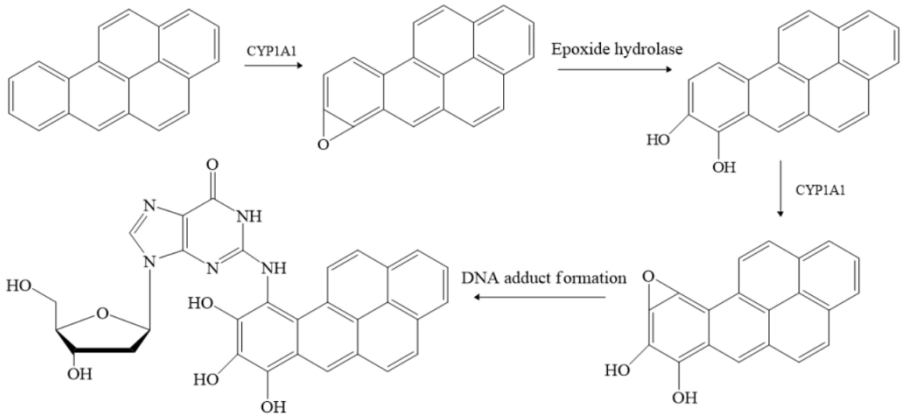

Figure 20: Metabolic activation of benzo[a]pyrene, the most potent PAH, and guanine adduct formation [adapted from 218]

They are a group of several hundred ubiquitous compounds of varying toxicity, where only a limited number are targets of regulations, regular analysis and data reporting [221]. Exposure is associated with heightened risk of cancer, inflammation and cardiovascular disease [222]. Examples for sources of PAHs are smoking, ingestion of burnt foods and occupational inhalation of vehicle exhaust fumes [223, 224].

DBPs form during treatment of drinking water when the oxidizing reagents (chlorine, ozone, chlorine dioxide or chloramines) used react with organic matter and industrial (halogenated-) contaminants (Figure 21). While over 800 DBPs have been identified, only 11 are currently under regulation, among them mainly the most prevalent classes of trihalomethanes and haloacetic acids. It is estimated that over 50% of halogenated by-products might be yet unknown and poorly characterised [225].

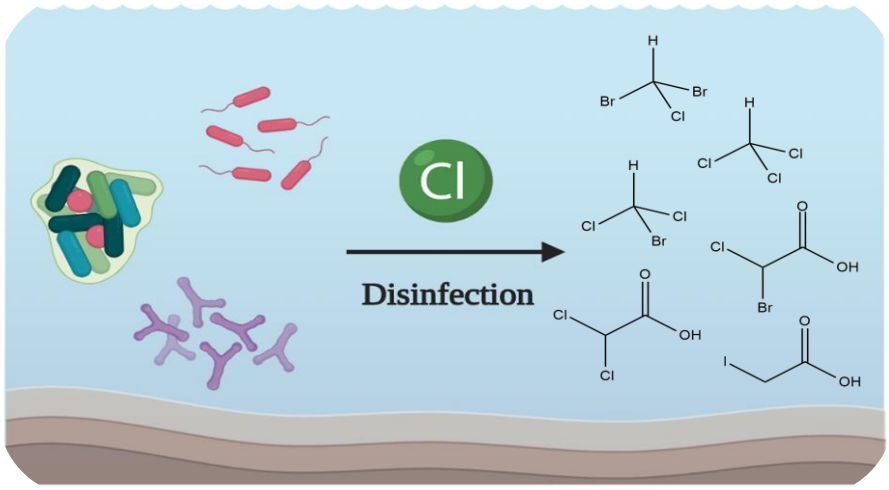

Figure 21: Chlorination of organic matter leading to DBPs during water treatment (created with *Biorender*)

Toxicological research associates DBPs with potential cytotoxicity, carcinogenicity [226, 227] and in recent years endocrine disruption [228], with iodo-DBPs having generally been confirmed to be of considerably higher toxicity compared to chloro- and bromo-DBPs [229, 230]. Epidemiological data consistently finds greater risk for the development of urinary bladder cancer as a result of exposure to DBPs via drinking water. Limited mechanistic studies suggest adverse modulation of several signalling pathways involving CYP2E1, glutathione s-transferase GSTZ1 and GSTT1 by DBPs as the putative mutagenic mode of action [225, 231, 232].

1.3.2.2. Phyto- and mycotoxins

While exposure to many classes of synthetic chemicals and environmental pollutants mentioned before are of concern and should be avoided if possible, it is important not to exclude the hazard of naturally occurring toxicants that have always been part of human life. Natural toxins such as myco- and phytotoxins have been studied in food and feed. It has been estimated before, that the vast majority of human dietary intake of toxic pesticides is attributable to naturally occurring toxic metabolites produced by plants, fungi or microorganisms as defence mechanism [160], with most toxic natural compounds exceeding synthetic chemicals in their overall risk to human health and environmental safety [234].

Mycotoxins are produced by certain types of moulds that grow on various foodstuffs. Besides ingestion of contaminated foods such as grains, fruits and nuts, a main threat is the indirect ingestion caused by a carry-over of mycotoxins and metabolites into animal tissue by feeding with contaminated feed crops [235]. Aflatoxins, ochratoxins and fumonisins are amongst the most poisonous known mycotoxins, with the former being the most common class [236]. A striking example of aflatoxin toxicity is the death of about 100.000 turkeys in England in the 1960s, which was eventually attributed to the ingestion of animal feedstock contaminated with aflatoxins in imported groundnuts [237]. This sparked extensive characterisation and monitoring efforts concerning this class of mycotoxins. Besides acute teratogenicity, hepatotoxicity and immunosuppressive effects, chronic exposure to aflatoxins is strongly carcinogenic. Aflatoxin B1, the most prevalent and most potent compound, has been tested for carcinogenicity in many animal species and found to produce tumours mainly in the liver, colon and kidneys [238]. It is thus being considered a class 1 carcinogen [239]. CYP mediated phase I metabolism yields highly reactive genotoxic intermediates that bind to DNA in liver cells [240].

Similar to mycotoxins, secondary plant metabolites may be produced to defend the host organism and help compete with other species, hence often classified as bioherbicides. While environmental exposures and effects of mycotoxins is receiving more attention in recent years, phytotoxins have not been as focused yet [241]. Different types of food, seasoning and herbs that have been consumed by humans for centuries, partly as supplements, contain phytochemicals with potentially carcinogenic properties [242].

Some plants used as herbal medicine contain pyrrolizidine alkaloids (PAs), a class of carcinogenic phytotoxins of concern, as they are frequently consumed in quantities up to 100 times the suggested daily intake by health authorities [243]. There are several principle metabolic pathways for PAs. Hydrolysis results in in necines and necic acid and is the primary route of detoxification and excretion. N-oxidation enables conjugation and excretion, but N-oxides may also be converted back to their parent compounds, which are able to undergo CYP-mediated oxidation to form mutagenic dehydropyrrolizidine alkaloids (Figure 22) [244]. These activated metabolites are able to crosslink DNA and proteins with their main targets being hepatocytes. Acute and chronic poisoning of humans has been confirmed. Several studies have suggested using PA-DNA or protein adducts as biomarkers of liver tumour formation [245, 246].

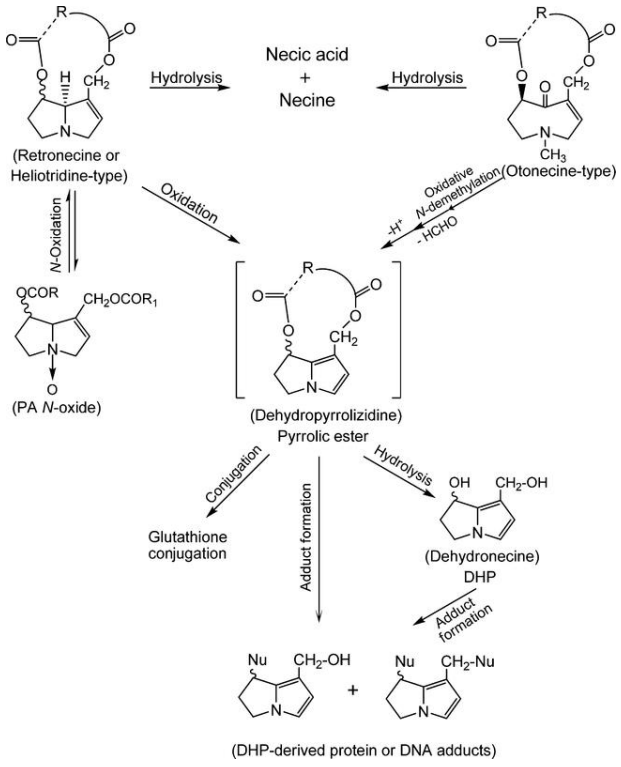

Figure 22: Metabolism of pyrrolizidine alkaloids [244]

Recently a number of studies performed risk assessment of PAs in herbal tea mixtures [247-249]. As more than 6000 plant species are known to produce several of the 600 PAs that have been identified so far, different herbal teas contain a large variety of structurally different compounds of, then again, different toxicities. For this reason, comprehensive trace analysis and quantifications of total PA burden is a challenge as only a fraction of known naturally occurring PAs are commercially available [250].

Aristolochic acids (AAs) are among the most potent naturally occurring renal toxicants and upper urinary tract carcinogens [251]. Balkan endemic nephropathy is a slowly progressing chronic renal disease resulting in kidney failure and is associated with high incidences of urothelial cancer. It was first described with highest prevalence in rural areas of Balkan states [252]. While multiple aetiological hypotheses have been made, the only conclusive evidence points towards the intake of AAs as a main culprit. AAs are metabolites of the endemic weed *Aristolochia clematitis*, whose seeds may contaminate and thus commingle with local wheat during harvesting [253]. Furthermore, in 1990 a Belgian clinic prescribed slimming pills containing Chinese herbal remedies, resulting in end-stage renal disease in hundreds of young women, originally prescribed as Chinese herbs neuropathy. This appeared to be a consequence of the ingestion of the root extract of *Aristolochia fangchi*, another plant rich in AAs, which was accidentally substituted for another herb with a similar name in Chinese language during the formulation process [254].

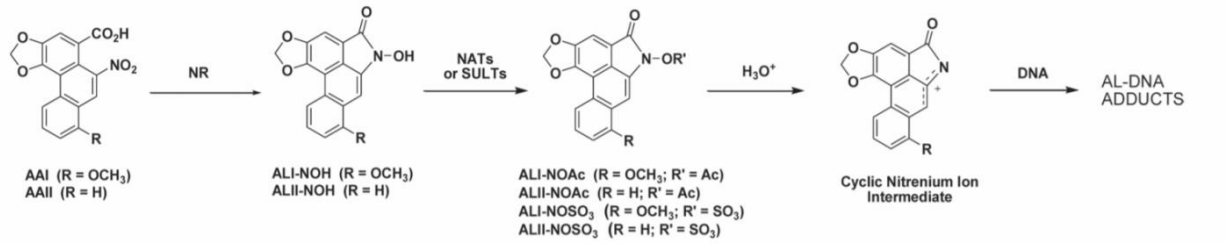

Figure 23: Metabolic activation of aristolochic acids [59]
AL: Aristolactam, NAT: N-acetyltransferase, SULT: Sulfotransferase

Both major components, AAI and AAII, are enzymatically metabolised by CYP reductases resulting in aristolactams which are then conjugated to glucuronide- or sulfate residues during phase II metabolism and ideally excreted. However, reactive intermediates generated after deconjugation of phase II metabolites are able to form DNA-AA adducts, while hydroxylated phase I metabolites display little to no activity in this regard (Figure 23) [59, 255]. With suspected month-long half-lives [256], these adducts are of remarkably high persistence and have been used for biological effect biomonitoring before [251].

Lastly, tropane alkaloids (TAs) are another class of secondary plant metabolites used as a defence mechanism against predators. Occurring mainly in plants of the *Solanaceae* family, so far more than 200 compounds have been identified with the most relevant ones being atropine, scopolamine, anisodamine, and the most prominent one being cocaine. They are anticholinergic reagents, meaning they prevent binding of acetylcholine to its receptor (Figure 24), that are used as premedication of anaesthesia, as analgesics, muscle relaxants and in traditional Chinese herbal medicine [257].

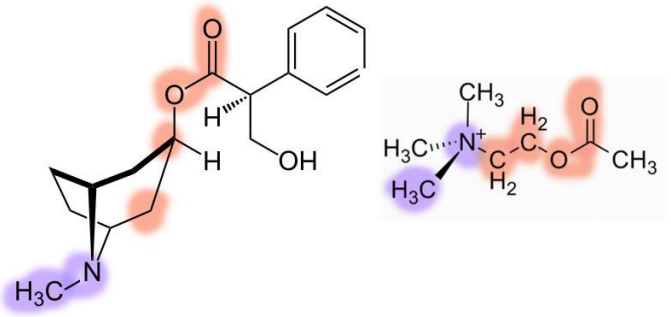

Figure 24: Structural similarities between atropine and acetylcholine [258]

Human poisoning may be the result of the ingestion of contaminated foods, intentional ingestion of various plant species for their psychoactive properties or the consequence of improper use of *Solanaceae* plants as herbal tea remedies [259]. Symptoms include confusion, agitation, anxiety, hallucinations, seizures, coma and numerous peripheral effects like dry mucous membranes, thirst, reduced respiration and flushed skin [257]. Several methods have been established for the detection of TAs in the environment and biological matrices obtained from poisoned individuals [260, 261].

1.3.3. Multi-analyte methods for biomonitoring of xenobiotics

Numerous methods for the determination of compounds of aforementioned classes of xenobiotics in human matrices have been developed before. However, most research merely focuses on single substance classes of specific health concern and only in a few matrices. Few published analyses include synthetic and naturally occurring EDCs, carcinogens or otherwise toxic compounds in the same method. Table 1 summarises recent multi-analyte methods for multiple classes of xenobiotics and their metabolites including analysed compound classes, biological matrices and type of analytical method used.

Table 1: Multiclass-methods for the determination of xenobiotics in human matrices

Method	Matrix	Substances	Reference
LC-MS/MS	(rodent-) tissue	2 DNA adducts of nitrosamines, 1 DNA adduct each of PhIP, 4-aminobiphenyl, AA, B[a]P	Guo et al. 2016 [262]
GC-MS	hair	4 phthalates, 2 parabens, 2 flame retardants, 2 pesticides	Martin et al. 2015 [263]
LC-MS/MS	hair	3 endogenous estrogens, BPA	Lee et al. 2017 [264]
LC-MS/MS	hair	6 PFAs, 6 benzophenones, 5 bisphenols, 4 parabens,	Rodriguez-Gomez et al. 2017 [265]
LC-MS/MS	plasma	5 parabens, 4 bisphenols, 3 endogenous estrogens	Koloratova et al. 2017 [266]
LC-MS/MS	plasma	15 bromophenols, 10 PBDEs, 4 endogenous estrogens, BPA	Chang et al. 2010 [267]
LC-MS/MS	plasma, amniotic fluid	5 parabens and metabolites, 2 alkylphenols, BPA, triclosan	Shekhar et al. 2017 [268]
LC-MS/MS	prostate tissue	3 DNA adducts of HAAs, 1 DNA adduct each of B[a]P, 4-aminobiphenyl	Xiao et al. 2016 [269]
LC-Fluorescence detection	saliva, serum	7 bisphenols, 2 phthalates, 4-nonylphenol, triclosan	Russo et al. 2018 [270]
GC-MS/MS	urine	6 benzophenones, 6 parabens, 2 bisphenols	Vela-Soria et al. 2014 [271]
LC-MS/MS	urine	11 phthalates, 5 bisphenols	Heffernan et al. 2016 [272]
LC-MS/MS	urine	7 bisphenols, 5 phthalates, 5 benzophenones, 2 antimicrobials	Rocha et al. 2018 [273]
LC-MS/MS	urine	8 bisphenols, 5 benzophenones; 10 parabens, 2 antimicrobials,	Asimkapopoulos et al. 2016 [274]
LC-MS/MS	urine	7 parabens, 5 BPA ethers, 5 benzophenones, 2 pesticides,	Asimakopoulos et al. 2014 [275]
LC-MS/MS	urine, serum	3 phytoestrogens, 3 mycoestrogens	Fleck et al. 2016 [276]
GC-MS	urine, serum, breast milk	7 parabens, 2 alkylphenols, 2 phenylphenols, BPA, triclosan	Azzouz et al. 2016 [277]
LC-MS/MS	urine, serum, breast milk	13 phytoestrogens and metabolites, 12 endogenous estrogens and metabolites, 12 mycoestrogens and metabolites, 6 bisphenols, 6 parabens, 2 phthalate metabolites, 2 PFAs, 2 pesticides, 2 benzophenones, 2-naphthol, ethinylestradiol	Preindl et al. 2019 [278]

To the best of our knowledge, most reported multi-methods only incorporate different classes of synthetic industrial and consumer products and pesticides. These are fairly related substance groups considering their endocrine disruptive capabilities and often rather similar structures. Biological effect monitoring may include DNA adducts originating from few different classes of environmental carcinogens, however no method that incorporates the parent compounds in the same analysis has yet been established. It is apparent that it is difficult to create extraction and chromatographic separation procedures that are effective for a wide range of structurally and chemically very different substances. Nonetheless, no approaches have been described when it comes to the simultaneous determination of acutely or chronically toxic, carcinogenic and endocrine disruptive xenobiotics of synthetic or natural origin as of yet.

2. Aims and objectives

The aims of this thesis were:

1. **Transfer of an existing targeted LC-MS/MS method** for the determination of endogenous estrogens and endocrine disrupting chemicals to a new state-of-the-art mass spectrometer.

2. **Expansion of the method with new substances** including air pollutants, disinfection by-products, food processing by-products, phytotoxins and metabolites as representatives for different classes to constitute a broad multi-xenobiotic HBM assay.

3. **In-house validation** evaluating linearity, selectivity, matrix effects, extraction efficiency, repeatability, intermediate precision, limits of quantification and detection of the method and comparison of the parameters to the previously published method by Preindl et al. 2019.

4. **Application of the method to biological samples** in first proof of concept experiments including structural confirmation of newly identified biomarkers of exposure.

3. Materials

3.1. Chemicals and reagents

CAS numbers and supplier information of all reagents and standard compounds used in this work are displayed in Table 24 and 25 (see Appendix). New compounds that were introduced to the method that was originally developed by Preindl et al. [278] are depicted in Figures 49-51 (see Appendix).

3.2. Instrumentation

Table 2 summarises all devices and instruments used during this work.

Table 2: Devices and supplier information

Sample preparation	Name	Supplier
Centrifuge	Mikro 220R	Hettich
Ultrasonic bath	Ultrasonic Cleaner	VWR
Vacuum concentrator	CentriVap	Labonco
LC-MS System		
HPLC System	Agilent 1290 Infinity II	Agilent
Column	Acquity HSS T3 (1.8 µm, 2.1 mm × 100 mm)	Waters
Pre-column	VanGuard (1.8 µm)	Waters
ESI Source	Turbo-V™	Sciex
Mass Spectrometer	QTrap®6500+	Sciex

3.2.1. LC system

A 1290 Infinity II HPLC system (Agilent) was used for the measurements. A VanGuard (1.8 µm) pre-column and an Acquity HSS T3 (1.8 µm, 2.1 mm × 100 mm) reverse phase column (both Waters) were used.

3.2.2. Mass spectrometer

Coupled to the LC-system was a QTrap®6500+ (Sciex) mass spectrometer equipped with a Turbo-V™ ESI source. The measurements were performed in scheduled MRM (sMRM) mode using fast polarity switching. Conformational MS/MS experiments were performed in the Enhanced Product Ion Scan mode (EPI).

3.2.3. Software

The LC-MS system was operated using Analyst® Software (Sciex). Targeted data analysis was carried out using Sciex OS software (Sciex). Final calculations and statistical evaluations were done using Microsoft Excel (Microsoft).

4. Methods

4.1. Liquid chromatography

The HPLC method and gradient were taken from a previously published method by Preindl et al. [278] for xenoestrogens. The method uses LC-MS grade water with 0.3 mM ammonium fluoride as aqueous eluent A and 100% ACN as organic eluent B. Fluoride ions are the solvent additive of choice to enhance ionisation of steroid hormone-like compounds [72, 73]. Table 3 summarises the chromatographic setup.

Table 3: Setup of the HPLC system

Parameter	Value
Temperature of the column department	40°C
Temperature of the autosampler	7°C
Sample injection volume	5 µL
Flow rate	0.4 mL/min

The gradient elution starts with 5% B from 0-1 min; rises to 18% until 1.8 min; rises then to 35% B until 4.2 min; followed by a rise to 48% B until 13 min; rises finally to 90% B until 14 min, followed by flushing with 98% B from 15.8 to 17.6 min and final re-equilibration with 5% B from 17.7 to 20 min. Figure 25 displays the programmed gradient.

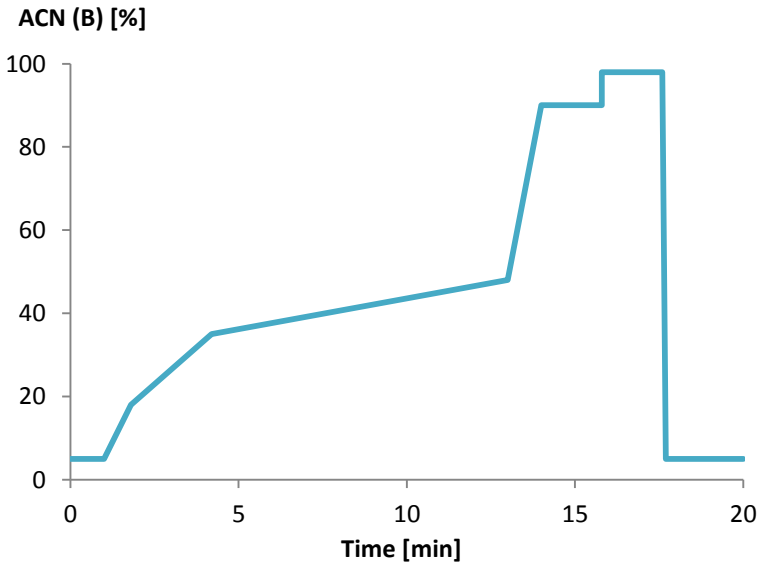

Figure 25: Eluent composition of the LC gradient

At the beginning of each measurement, the LC system was thoroughly purged by flushing the solvent lines with 100% B at a flow rate of 5 mL/min, followed by 0% B for again 5 minutes at the same flow to remove contamination and air bubbles from the pump and tubing.

Equilibration of the column was performed with the LC method's starting eluent composition of 5% B. The initial flow of 0.1 mL/min was held until the pressure was constant. Then, the flow rate was slowly increased until a constant pressure at the final flow rate of 0.4 mL/min was achieved. The column was usually equilibrated for at least 20 minutes. Once the pressure was constant, the measurements were started.

4.2. Optimisation of LC-MS/MS parameters

The method transfer started with the optimization of compound dependent parameters of the mass spectrometer to achieve the best possible sensitivity for each compound. These include the precursor ion masses and the ion optic parameters declustering potential (DP), collision energy (CE) and cell exit potential (CXP). Most selected mass transitions and ion optic parameters of the final method originate from the tuning process described in section 4.2.1. However, some compounds were not tuned within the scope of this work. In these cases, transitions and corresponding parameters were either adopted from in-house tuning that had been done before, or from literature that had used the same instrument with similar eluents.

Then, the vertical ESI probe position and ion source parameters (curtain gas flow (CUR), ion source gas 1 and 2 (GS1/2), source temperature (TEM), ion spray voltages (IS) and collisionally-activated dissociation gas (CAD)) were optimised for a selected group of structurally representative compounds as they are kept constant throughout the LC run.

4.2.1. Compound optimisation by infusion injection

To optimize compound dependent parameters, single standard solutions (ranging from 50 ng/mL to 5 µg/mL, depending on the compound, in 10% ACN) of the analytes were injected into the ESI source.

For most of the compounds that were manually tuned, a concentration of 50 ng/mL was sufficient. If no precursor mass was detected in both polarities or the optimisation was cancelled due to low ion intensity, the process was repeated with a 500 ng/mL solution. Xanthohumol, octyl methoxycinnamate (OMC) and 3-hydroxy-benzo[a]pyrene (3-OH-BaP) required a concentration of 5 µg/mL to achieve sufficient ionisation to enable the tuning process.

The sample inlet capillary was disconnected from the diverter valve and connected to the grounding unit. A syringe that had been cleaned with MeOH was filled with ~0.4 mL of analyte solution, connected to the grounding and placed into the syringe pump system right of the ESI source (Figure 26).

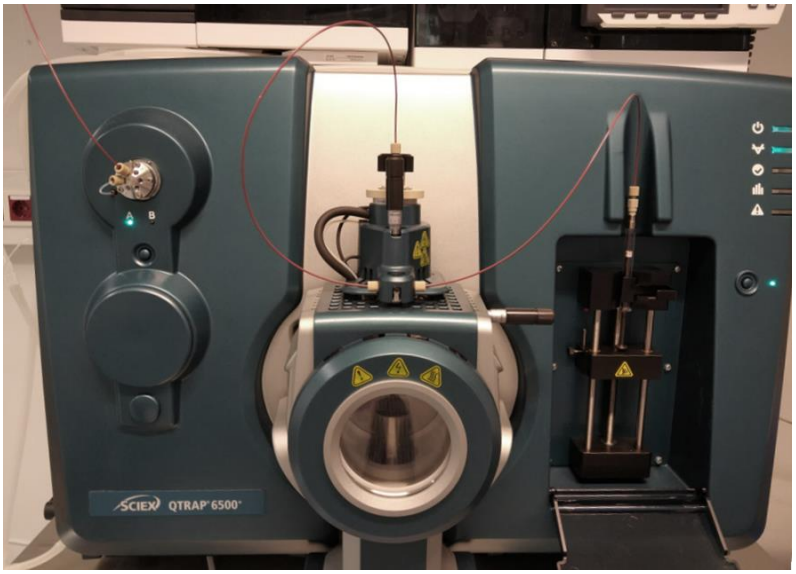

Figure 26: The direct infusion setup with the syringe system connected to the ESI source

The system was flushed with the standard solution by introducing a flow of 50 µL/min for ~30 seconds. Then, the flow rate of was adjusted to 10 µL/min and a Q1 full scan monitoring a 100 Da window around the anticipated precursor ion mass (scan rate 200 Da/s) was started. Once the total ion chromatogram (TIC) remained stable, a product ion scan of the selected precursor ion mass was initiated. The fragment spectrum was observed and the presence of the correct precursor ion was confirmed by comparison of the detected fragments with known anticipated fragmentation products of the compound according to Preindl et al. [278] or literature and database research to discriminate between true compound peaks and isobaric interferences or background noise in case of low signal intensities.

A final 10 second Q1 scan using multi-channel averaging (MCA) was carried out to assess the signal to noise ratio (S/N) of the precursor ion mass. Both the negative as well as the positive ionisation mode were monitored. The polarity that yielded the highest S/N ratio of the respective precursor mass was then chosen for further optimisation steps. Once an average intensity of about 10^6 counts was achieved, an automatic compound optimization tool included in the Analyst software was used to determine optimal DP, CE and CXP for each injected single compound.

After manually selecting the precursor ion mass of the analyte, the applied tool uses unit resolution to isolate and detect the precursor mass in a Q1 only scan while scanning the DP through a 300V range (in negative mode: -300V to -1V, in positive mode: 1V to 300V) in 5 V steps. The voltage that yields highest ion intensities is then designated as the ideal DP for the specific precursor mass. The system then switches to full product ion scan mode and uses default parameters to induce collision induced fragmentation of the precursor mass in the collision cell. For most compounds, product ions within 19 Da of the precursor mass, and product ions lighter than 60 Da were excluded for further considerations. If literature research or the method by Preindl et al. [278] indicated that the most stable

and characteristic product ions might be within 19 Da of the precursor mass or smaller than 60 Da, the selection criteria were adjusted accordingly, i.e. a tolerance window of +/- 10 Da of the precursor mass and a minimum mass of 40 Da. This was the case for 2-tert-BP, coumestrol, methylparaben, triclosan, chloroacetic acid, dichloroacetic acid, acrylamide, glycidamide, HMF, HMFA, NDMA and NDEA.

Depending on the compound, the top 5 to 12 fragments were monitored. For each fragment the CE was varied from +/-180V to +/-5V in 2 V steps, determining the optimal CE value. Then, the same was done for the CXP in a range from +/-55V to 0V in 2 V steps.

Transitions for BPA, BPF, BPS, α-ZAL, β-ZAL and β-ZEL were adopted form in-house tuning results and were not tuned in the scope of this work. Moreover, transitions for E1, E2, ethinylestradiol, alternariol, AME, α-ZEL, ZEN and ZAN were adopted from literature [279-283].

Isotopically labelled internal standards were not separately tuned either. Their purpose is to control for extraction and ionisation yields. Ideally, they share similar chemical and optical properties as the non-labelled counterpart. Thus, for internal standards, product ion masses were adopted from Preindl et al. (278) and ion optic parameters were taken from the non-isotopically labelled standard. In the case of $^{13}C_{12}$ BPA and $^{13}C_{18}$ ZEN, parameters were taken from in-house methods that had already been validated and therefore differ from the non-labelled standards.

4.2.2. Pre-experiments for the determination of retention times and matrix effects

LC-MS/MS experiments were performed in sMRM mode with fast polarity switching. Measurements using the MRM parameters (precursor masses, DP, CXP and CE) resulting from infusion injection described in section 4.2.1, literature research and already established in-house methods were evaluated to determine the most sensitive transitions for each compound in the investigated matrices and respective retention times.

4.2.2.1. Xenoestrogens

The following default ion source parameters were adopted for the measurements: vertical ion probe position at 2 mm, CUR of 30 psi, IS of -4500V and 4500V for negative and positive mode respectively, TEM of 450°C, GS1- and GS2 gas flow of 50 psi and 60 psi respectively, CAD medium. The chromatographic method described in section 4.1 was applied.

Approximate retention times for the analytes were taken from Preindl et al. [278]. Matrix matched calibration standards and extracted (matrix-) blanks used for validation measurements for Preindl et al. [278] were used as samples. Tables 26 and 27 (see Appendix) display the individual compound concentrations of the matrix matched standards dissolved in 10% ACN. Matrix matched standards 1, 10, 30 and 100 in urine, serum and breast milk were chosen for the pre-experiment to determine exact retention times and the most intensive transitions considering matrix effects for each compound in all three human matrices. To support correct peak identification, solvent standards and matrix blanks were measured as well.

4.2.2.2. Additional compounds

To determine retention times for the newly added analytes, seven multi component standards in 10% ACN with concentrations for each of the 24 analytes from 0.001 ng/mL to 1000 ng/mL were prepared and measured using the liquid chromatography method described in section 4.1 and the final optimised ESI parameters described in section 5.2.3.2.

Once solvent retention times and approximate limits of quantification of the new compounds had been established, 80 µL of matrix matched standards 3, 10 and 30 from previous experiments as mentioned above (Table 26 see Appendix) were spiked with 20 µL of the new multi component standards (10% ACN) to create the spiked matrix matched standards (NSSP 1, NSSP 10 and NSSP 100 respectively) with concentrations of the new compounds near their approximate LOQs (Table 28 see Appendix). These standards as well as matrix blanks were measured again using the same LC and MS parameters that had been used with the solvent standards to determine matrix retention times and the most intensive transitions.

4.2.3. Optimisation of ESI parameters

Once analyte specific parameters had been selected, the settings of the electrospray ionisation source were optimised. Among them are the vertical position of the probe, the gas flows CUR, GS1, GS2, the IS voltage, the source temperature and the CAD pressure.

4.2.3.1. Vertical probe position

Before the determination of optimal gas and source parameters, the position of the ESI probe itself had to be determined. The ESI system user guide suggests a vertical probe micrometre setting between 2 mm and 5 mm for a flow rate between 200 µL/min and 1 ml/min [284]. A setting of 2 mm places the needle farther away from the orifice than the setting of 5 mm.

Vertical probe positions of 2 mm and 5 mm were compared. The same LC-MS/MS method as used in section 4.2.2.1 was used to take into account not only correct eluent composition, but especially matrix effects and contamination. Since it was considered the most relevant matrix, the urine matrix matched standards 1, 10 and 30 from the validation by Preindl et al. [278] were measured twice in the same sequence with manual adjustment of the vertical probe position from 5 mm to 2 mm during a blank injection in between the standard batches.

Twelve compounds (BPA, butylparaben, enterolactone, equol, estradiol, genistein, MBP, PFOS, triclosan, ZEN, ZEN-14-GlcA, ZEN-14-sulfate) of particular relevance for biomonitoring, or with representative substructures of the different substance classes that are included in the method, were chosen for monitoring the influence of the probe positions on the signal to noise ratio (S/N) of the selected analytes' most abundant product ion (quantifier). A representative value for the detector noise was estimated by averaging the immediate baseline intensity before the compound peak as depicted in Figure 27. The S/N ratios of quantifier ions were calculated as described in Equation 1.

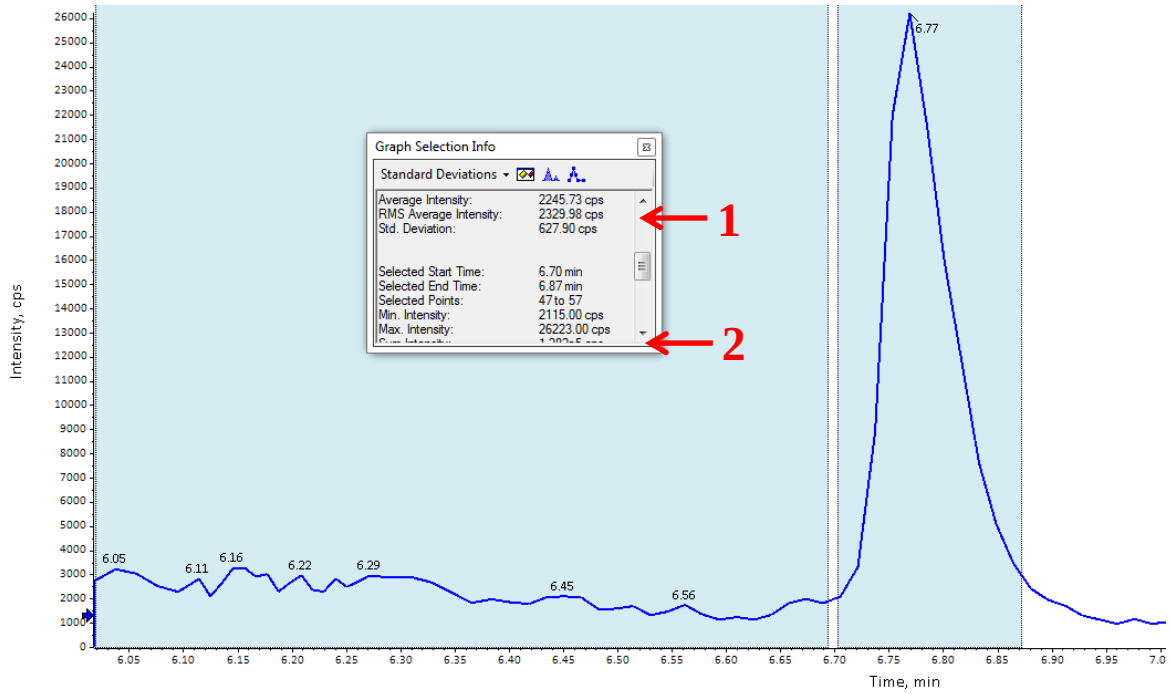

Figure 27: Determination of the peak height and the average baseline noise intensity of enterolactone in the urine matrix matched standard at a concentration of 1 ng/mL using SCIEX OS Analytics with 1 depicting the average baseline intensity before the analyte peak and 2 depicting the analyte peak height

$$S/N = \frac{\text{peak height [cps]} - \text{average baseline intensity [cps]}}{\text{average baseline [cps]}} \qquad \text{Eq. 1}$$

Measurements of the lowest standard concentration where the analyte had still been detected were used to determine S/N ratios of both vertical probe positions.

4.2.3.2. Source and gas parameters

The last step of parameter optimization involved ion source parameters that control the ionisation and ion transmission into the MS orifice, as well as all nitrogen gas flows that enter the system. In contrast to the ion optic parameters, the source and gas parameters are constant throughout the LC-MS/MS runs, with exception of the ion spray voltage that can be rapidly switched between a negative and positive value. For this reason, the final method cannot use the most optimal parameters for each of the 99 compounds, since they prefer different temperatures, spray voltages and gas flows. A reasonable compromise that enhances sensitivity for the majority of the analytes compared to the default parameters needed to be accepted.

4.2.3.3. Flow injection analysis

The Analyst software offers another tool to automatically optimize source and gas parameters. However, this time the LC system needs to be engaged as well. A flow injection analysis (FIA) setup was used to achieve this. Here, the LC column was replaced by a connecting piece and the sample was injected into the MS via the autosampler. Hence, no chromatographic separation was achieved, but the run time of 1 min allowed the testing of multiple source parameter combinations. The MS was able to monitor up to nine transitions at a time. During FIA, the system changes ion source- and gas parameters between each injection while using default values for the remaining parameters. Once one parameter is finished, variation of the next one starts.

As most compounds are monitored in negative mode, gas flows and the temperature were optimised using nine structurally representative compounds that are measured in negative polarity. Once this was achieved, nine other compounds were monitored to determine the optimal positive spray voltage. Only quantifier ion transitions were considered.

A multi component standard (with the 18 model substances mentioned above) in ACN, with compound concentrations similar to those that were used for infusion injection in section 4.2.1, was used for FIA analysis. Ideally, the same eluent composition at the respective retention time during a full LC-MS run would be used for each compound. As this was not possible using the FIA setup, 50% eluent B was chosen as a compromise between compounds that elute early and those that elute late. Table 4 summarises concentrations of the nine compounds used for optimisation of CUR, GS1, GS2, TEM and IS in negative mode and the nine compounds used for optimisation of the IS in positive mode. While an important compound, BPA was omitted as FIA tuning in-house had already been done and optimal parameters were known.

Table 4: Compounds and concentrations in the multi component mix used for FIA

Negative mode	c [ng/mL]	Positive mode	c [ng/mL]
Enterolactone	30	3-BC	1500
Equol	30	4-MBC	600
Estradiol	60	Benzyl butyl phthalate	150
Genistein	15	Fenarimol	1.5
MBP	60	Glycitein	15
PFOS	90	Isoxanthohumol	1.5
Triclosan	30	Methiocarb	90
ZEN	30	OMC	1500
ZEN-14-GlcA	30	Prochloraz	3

Table 5 summarises all the parameter values that were monitored in the FIA cycles. For most parameter values, three injections were conducted. For CAD and IS^+, only two injections were feasible to save standard solution. However, this was deemed sufficient to find the optimal parameter as the variance between the repeated injections was small.

Table 5: Parameter ranges monitored during FIA

Parameter	Values
CUR	30; 35; 40; 50
GS1	40; 60; 65; 70; 75; 80; 85; 90
GS2	40; 60; 65; 70; 75; 80; 85; 90
TEM	300; 350; 400; 450; 500; 550; 600
IS	**neg.:** -4500; -4000; -3500 **pos**.: 3500; 4000; 4500; 5000; 5250; 5500
CAD	Medium; High

Ion counts for each monitored parameter value were averaged and normalised relative to the default parameter values that had been used in previous measurements described in section 4.2.2.1 up until this point.

4.2.3.4. Confirmation of ideal ion source parameters

4.2.3.4.1. Comparison of default source parameters and optimised parameters

To confirm the overall improvements in sensitivity, LC-MS/MS measurements identical to those in section 4.2.2.1 using the new optimised parameters were performed. The same matrix matched standards described in Table 26 (see Appendix) were measured again and signal to noise ratios were calculated for all compounds as described in section 4.2.3.1. This way, sensitivities that were achieved using default parameters were compared to those resulting from the optimised parameters.

4.2.3.4.2. Determination of ideal GS2 and CAD values

To test whether the high GS2 flow and CAD pressure caused lower S/N ratios for some compounds when matrix components were included, matrix matched standards 1 and 0.1 of all matrices were measured again with variation of GS2 and CAD between the default values and the ideal values according to the ion source parameter optimisation. Matrix matched standard 0.1 was created for a more reliable estimation of the LOQs later (see section 4.3) by a tenfold dilution of standard 1 using the according matrix matched blanks, so as not to dilute matrix components as well.

All samples (urine matrix matched standards 1/0.1, serum matrix matched standards 1/0.1 and breast matrix matched standards 1/0.1) were measured with each of the four different acquisition methods described in Table 6. Peak intensities, baseline noises and signal to noise ratios of the same 12 representative compounds considered in section 4.2.3.1 were described for all four different methods.

Table 6: Acquisition methods with varying GS2 and CAD values

Parameter	Method ①	Method ②	Method ③	Method ④
CUR	30 psi			
GS1	80 psi			
GS2	**90 psi**	**60 psi**	**90 psi**	**60 psi**
TEMP	500°C			
IS	neg.: -4500 V pos.: 5500 V			
CAD	**High**	**High**	**Medium**	**Medium**

4.3. Approximation of limits of quantification and matrix effects

In order to design a multi-analyte standard mix and calibration standards for method validation, limits of quantification (LOQ) had to be estimated. The LOQ is the lowest amount of analyte in a sample that can still be quantitatively determined with suitable precision and accuracy. Most international bodies define the LOQ as the concentration that yields a S/N ratio of 10 [285].

For LOQ determination the transferred compounds, the dilution series [standard 1 – standard 100] described in Table 26 (see Appendix) which was measured in section 4.2.2.1 resulted in S/N ratios of above 100 for many compounds. For a more accurate estimation using the final ion source parameters, standards yielding lower S/N ratios closer to the LOQ would be more suitable. For this reason, matrix matched standard 0.1 of the different matrices had been created as described in section 4.2.3.4.2. The results of the measurements of standard 1 and standard 0.1 using the optimised compound dependent MS parameters (Table 7 in section 5.1), the standard LC method (see section 4.1) and the final ion source parameters as described in Table 10 in section 5.2.3.2 were used for measurements estimating the LOQs for of all xenoestrogens.

For the newly added compounds, the measurements of the spiked matrix matched standards NSSP 1, NSSP 10 and NSSP 100 (Table 28 see Appendix), as described in section 4.2.2.2, using the same standard LC method and the final ion source parameters were used to estimate LOQs. While the matrix content of the matrix matched standards NSSP 1, 10 and 100 was diluted by 20% (20 µL of solvent standard in 10% ACN added to 80 µL of extracted matrix in 10% ACN), this was deemed acceptable to estimate LOQs.

The standards that yielded S/N ratios closest to 10 were chosen to estimate LOQs for each respective compound (Equation 2).

$$\text{LOQ [ng/mL]} = \frac{\text{10 x standard concentration [ng/mL]}}{\text{S/N resulting from the measured standard}} \qquad \text{Eq. 2}$$

Several compounds occurred naturally in the different biological matrices. In addition, n-butylbenzenesulfonamide, benzyl butyl phthalate, dibutyl phthalate, 4-tert-octylphenol, nonylphenol and an isobaric interference to scopolamine were also detected in solvent blanks. They were most likely introduced during sample preparation or present in the LC system as contaminants. The isobaric interference to scopolamine was also detected in solvent blanks. This was suspected to be due to sample carryover after measurements of the highly concentrated solvent standards described in section 4.2.2.2. This hypothesis was confirmed after the preparation of a new solvent blank resolved this issue for later measurements. In either case, this did not allow for a direct estimation of the LOQ, as the actual analyte concentrations that were measured depended not only on the amounts that had been added by fortification, but also on the amounts already present in the matrix. Since the latter was unknown, the extent of contamination had to be determined before LOQs were calculated.

To do this, results of the measurements of matrix blanks and matched standards 1, 10, 30 described in section 4.2.2.1 and results of the measurements of matrix matched spiked standards NSSP 1, NSSP 10 and NSSP 100 as described in section 4.2.2.2 were used for standard addition. The absolute value of the x-intercept describes the compound contamination in the matrix (Figure 28). Equation 3 was used to estimate matrix contamination.

$$\text{matrix contamination [ng/mL]} = \frac{\text{y-intercept [cps]}}{\text{slope [cps/ng/mL]}} \qquad \text{Eq. 3}$$

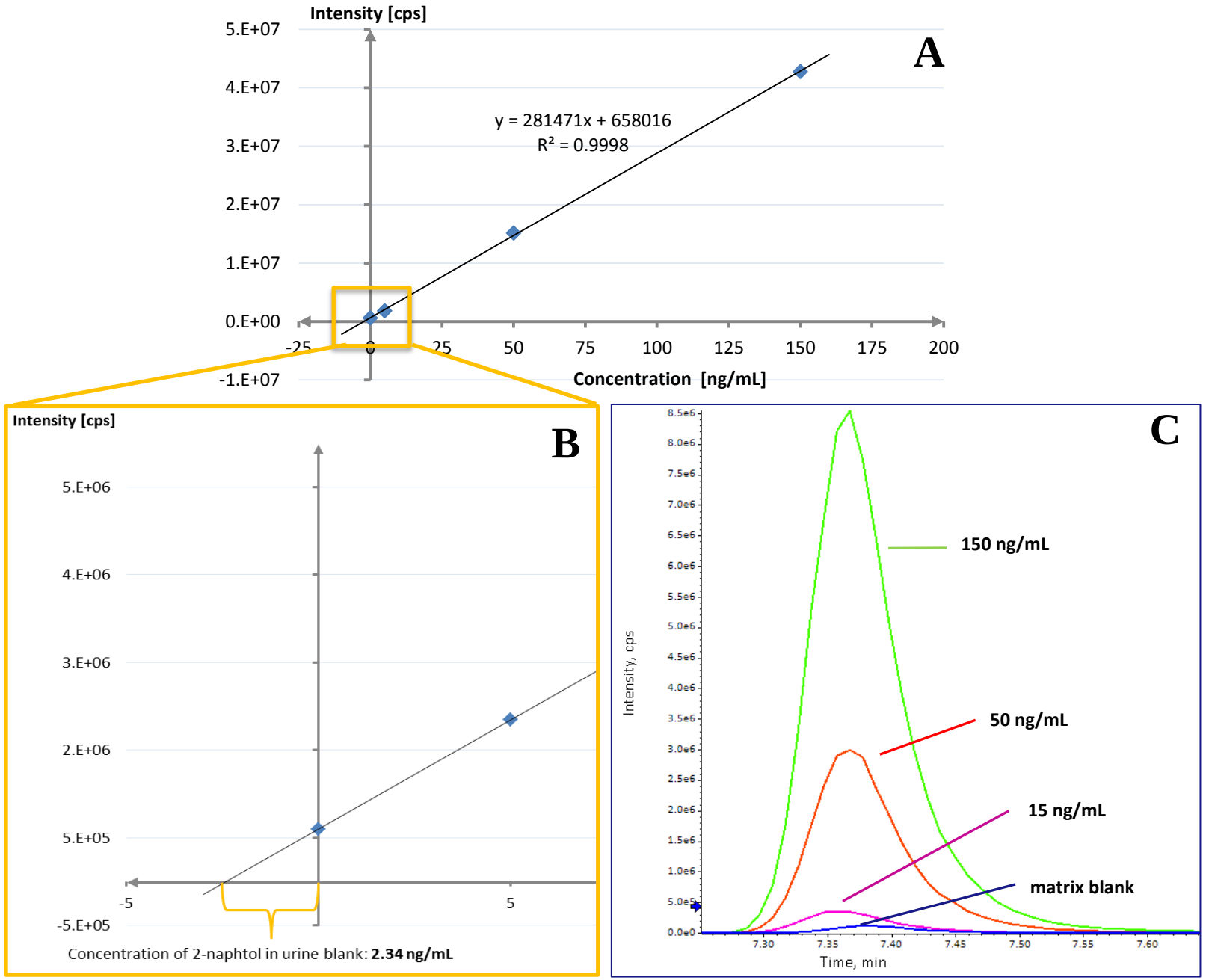

Figure 28: Determination of urine matrix contamination with 2-naphthol by standard addition (A, B), chromatograms of 2-naphthol depicting quantifier transitions in the urine standards and the urine matrix blank used for standard addition (C)

The estimated contamination was then used to correct the compound concentrations in the matrix matched standards. Equation 4 calculates the approximate LOQs for each compound with matrix contamination.

$$\text{LOQ [ppb]} = \frac{10 \text{ x (standard concentration [ng/mL] + matrix contamination [ng/mL])}}{\text{S/N resulting from the measured standard}} \qquad \text{Eq. 4}$$

4.4. Method validation

4.4.1. Calibration standards

For method validation, a standard dilution series ranging from 100x the LOQ down to the limit of detection (LOD: 0.3x the LOQ) was designed according to the estimated LOQs. The LOD was defined as the concentration that yields a S/N ratio of three, thus three tenth of the LOQ. For each compound, the calibration range was based on the LOQ of the most sensitive matrix, since the establishment of a generally highly sensitive method had been prioritised.

Table 29 (see Appendix) displays the compound concentrations in the prepared multi-analyte mix and the standard dilution series in 10% ACN. Table 30 (see Appendix) displays concentrations in the multi-internal standard mix and an additional ^{13}C-ZEN solution that had to be prepared (see section 5.4).

4.4.2. Sample preparation

For matrix matched calibration, commercially available pooled male AB plasma derived serum, human urine from a female volunteer who avoided consuming foods/cosmetics stored in plastic containers and foods rich in phytoestrogens for two days before sample collection to reduce xenoestrogen levels, and breast milk provided by the Semmelweis Women's Clinic in Vienna were used. Matrices, standards and samples were all stored at -20°C until analysis. Sample preparation was carried out on ice to minimise compound degradation.

For urine and serum samples, 200 µL aliquots were spiked with 10 µL of the internal standard mix (10% ACN) and 10 µL of a ^{13}C-ZEN solution (10% ACN). Either 20 µL spiking standard (Std 30 and Std 300) in 10% ACN or 20 µL 10% ACN were then added, followed by 770 µL ACN/MeOH (1:1, v:v). For extraction the samples were vortexed and subsequently sonicated for 10 min in an ice bath. To precipitate proteins, samples were stored at -20°C for at least 2 hours and centrifuged for 10 min at 18.000g at 4°C. The supernatant was dried in a speed vacuum concentrator at 4°C overnight. The residue was reconstituted in either 200 µL of calibration standard (10% ACN) or 200 µL 10% ACN for process blanks. Afterwards they were again vortexed and centrifuged for 10 min at 18.000g at 4°C. The supernatant was finally transferred to amber glass vials for LC-MS/MS measurement.

For breast milk samples, 250 µL aliquots were spiked with 12.5 µL of internal standard mix (10% ACN) and 12.5 of the ^{13}C-ZEN solution (10% ACN), followed by addition of either 25 µL spiking standard (Std 30 and Std 300) or 25 µL 10% ACN. To denature proteins, 250 µL 1% formic acid in ACN was added and the samples were vortexed for 3 min. The solution was added to 100 mg anhydrous $MgSO_4$ and 25 mg NaCl and vortexed for 3 min, followed by centrifugation at 18000 g for 10 min at 4°C. The upper organic phase was stored at -20°C for at least 2 hours and again centrifuged at 18.000g for 10 min at 4°C. The supernatant was then dried using the speed vacuum concentrator at temperatures between 4°C and 15°C until dryness was achieved. For most samples, this took 48-72 hours with several short intervals of vortexing do break up lipid layers that kept the solution from evaporating. The residue was reconstituted in 250 µL calibration standard or 250 µL 10% ACN for process blanks and again vortexed and centrifuged at 18.000g for 10 min at 4°C. The supernatant was transferred to amber glass vials for LC-MS/MS measurements.

In addition to the standards and extraction experiments used for method validation, biological samples from ongoing HBM projects of the department were prepared for measurements following method validation. Overall, 16 urine samples of unknown individuals were prepared in duplicates, while 86 breast milk samples of one person were prepared in one biological replicate. Two adult serum samples and serum samples of 21 extremely premature infants had already been extracted by a colleague without the addition of the internal standards.

4.4.3. Validation measurements

Method validation was carried out according to the European Commission decision No. 657/2002 of 12.08.2002 [286]. To satisfy the requirements, three independent experiments were carried out with of least one week between each of the measurements.

Validation sequences for urine, serum and breast milk were measured. These included three system- and matrix blanks, six spiked samples and the matrix matched- and solvent calibration series, which were measured in the beginning and in the end of each matrix batch. Before each matrix batch, matrix blanks and a matrix matched standard were injected to equilibrate the column for the corresponding matrix. Internal quality control samples were included in the beginning and in the end of a measured sequence to monitor deviations in system performance during the sequence (Table 31 see Appendix).

Solvent- and matrix matched standards were independently prepared for each experiment from the multi-analyte mix according to Table 29 (see Appendix) and the sample preparation protocol described in section 4.4.2 In addition, three matrix blanks and three system solvent blanks (using water instead of the matrix) were extracted as well according to section 4.4.2. Lastly, triplicate spiking experiments at two levels aimed at the compound concentrations of approximately 3x LOQ and 30x LOQ were carried out to determine extraction recoveries in all matrices.

The final LC method used for the validation measurements is described in section 4.1, while technical MS parameters are summarised in Table 10 in section 5.2.3.2 with quantifier/qualifier transitions and retention times of compounds being described in Table 7 in section 5.1.

4.4.4. Data evaluation

Data evaluation was carried out using Sciex OS Analytics. Linear regressions for all analytes in the matrices were created using the corresponding matrix matched standard series which were measured twice. In the case of BPA, ZEN, MEHP, MBP, estradiol, 4-*tert*-OP, butyl-, ethyl-, methyl-, propylparaben, pOHBA, PFOS, PFOA and genistein, internal standard matrix regressions and correction of extractions were applied if feasible. Solvent linear regressions for all analytes were created using the solvent series that were also measured twice for each matrix batch.

Selectivity, linearity, matrix effects, extraction recoveries, intermediate precision, repeatability and LOQs were evaluated according to the European Commission decision No. 657/2002 of 12.08.2002 [286].

Extraction recoveries (R_E) were calculated as the ratio between the spiked concentration and the measured concentration of the spiked sample as described in Equation 5.

$$R_E\,[\%]\ (n=9) = \frac{\text{conc. determined by matrix calibration [ng/mL]}}{\text{spiked conc. [ng/mL]}} \text{ x } 100 \qquad \text{Eq. 5}$$

Linearity of calibration was described by the matrix regression coefficient R^2 of the matrix matched standard series. Selectivity was evaluated by determination of potential interferences in the matrix blanks.

Matrix effects were evaluated by calculation of the signal suppression or enhancement (SSE) using the ratio of the slope of the matrix matched calibration regression and the solvent calibration regression as described in Equation 6.

$$\text{SSE}\,[\%]\ (n=3) = \frac{\text{slope of matrix matched calibration regression [cps/ng/mL]}}{\text{slope of solvent calibration regression [cps/ng/mL]}} \text{ x } 100 \qquad \text{Eq. 6}$$

LOQs were calculated, as described in section 4.3 as a S/N ratio of 10, once in all matrices for each validation sequence.

Intermediate Precision (RSD_R) was determined by the evaluation of the samples spiked at two levels in three independent experiments (nine measurements per spiking level) under reproducibility conditions and calculated as described in Equation 7.

$$RSD_R\,[\%] = \frac{\text{standard deviation of conc. determined in spiked samples (n=9) [ng/mL]}}{\text{average conc. determined in spiked samples (n=9) [ng/mL]}} \text{ x } 100 \qquad \text{Eq. 7}$$

If a single suspected outlier with considerably different extraction recoveries was observed, it was subjected to Dixon's Q- and Grubbs's testing (Equations 9-11 see Appendix). If the value was characterized as an outlier by both tests, it was rejected and data evaluation using the remaining 8 measurements was done.

Repeatability (RSD_r) was determined by repeated measurement of one validation batch on the same day (six measurements per spiking level) under repeatability conditions and calculated as described in Equation 8.

$$RSD_r\,[\%] = \frac{\text{standard deviation of conc. determined spiking experiments (n=6) [ng/mL]}}{\text{average conc. determined in spiking experiments (n=6) [ng/mL]}} \text{ x } 100 \qquad \text{Eq. 8}$$

4.5. Measurements of real-life biological samples

As a proof of concept, the method was applied to the unknown biological samples. The 32 urine samples, 21 serum samples from extremely prematurely born infants and two adult serum control samples and 86 breast milk samples from a longitudinal study of one woman were prepared as described in section 4.4.2, measured and quantified using the respective matrix matched standard series of the last validation, including system and matrix blanks. As the serum samples had already been prepared by a colleague for a different experimental setup, internal standard calibration and correction was not realised since internal standards had not been added. Some breast milk samples were injected a second time using a higher injection volume (20 µL) to confirm suspected low-level phytotoxin contamination near the LOD.

Analyte concentrations in unknown samples were corrected with the respective extraction efficiencies if detected concentrations were above the LOQ. To precisely quantify compounds that are natural contaminants of the biological matrices used for calibration, the y-intercept of the calibration curve (corresponds to the peak area of the matrix blank) was added to the detected peak areas in the unknown samples to enable quantification. For analytes found in system blanks, the y-intercept of the calibration curve that was added to the peaks in unknown samples was corrected by subtraction of the peak area of the system blank, which was corrected by the SSE of the analyte according to the results of method validation.

Enhanced Product Ion Scans (EPI) were conducted to confirm the presence of selected analytes. MS2 spectra of standards at concentrations similar to the detected concentrations in the unknown samples were compared to the MS2 spectra of the unknown samples. BPA, PFOS, 2MeOE1, methylparaben, HMFA and HMF were monitored in specific serum samples. TBPA, 8-prenylnaringenin and prochloraz were investigated in specific urine samples as well as alternariol, prochloraz, PhIP, anisodamine, jacobine-N-oxide, riddelliin-N-oxide and scopolamine in individual breast milk samples.

Two EPI approaches were utilised. In the first method, EPI scans were programmed to be dependent on the compound's MRM transition. Once a pre-determined threshold in intensity was achieved, the EPI scan would commence (IDA - "Information Dependent Acquisition"). The threshold for each compound was adjusted according to the expected peak heights. With this method, one EPI spectrum consisted of three full MS2 scans from 50 Da up to the compounds precursor mass utilising a CE spread of +/- 15V around the ideal CE values of the quantifier and qualifier transitions of the MRM method. This means that a programmed CE value of -50V sums scans at -35V, -50V and -65V. In addition, the system conducted additional survey scans to perform dynamic background subtraction on each EPI scan, meaning fragment masses that were present throughout the whole chromatography were not included in the final MS2 spectra. A scan rate of 10 000 Da/s was utilised.

The second approach utilised non-dependent EPI scans throughout the whole run. MS2 spectra were then manually selected according to the compound's retention time. With this method, no CE spread was programmed and MS2 spectra were recorded at a single CE voltage. During preliminary experiments, this CE parameter was adjusted to enable thorough precursor fragmentation while still leaving remnants of the precursor mass for most compounds, ideally at least 10% of the most abundant MS2 fragment. A scan rate of 1000 Da/s was utilised.

If needed, 10-20 µL of sample volume were injected to enhance the precursor yield of low concentrated compounds during the EPI scans (2MeOE1 in serum, phytotoxins and PhIP in breast milk). Moreover, an Enhanced MS Scan (EMS) of one serum sample to monitor the isotope pattern of the precursor ion of triclosan was conducted to help with identification as only one product ion was established.

5. Results

5.1. Retention times, optimal transitions and compound dependent parameters

Table 7 summarises retention times of the compounds in all matrices, final precursor ion masses, most intense quantifier/qualifier transitions and analyte specific MS/MS parameters (collision energy (CE), declustering potential (DP), cell exit potential (CXP)).

Table 7: Analyte specific LC-MS/MS parameters. Most compounds were tuned using a 50 ng/mL solution. Compounds that were tuned using a 500 ng/mL solution are marked with (*) and compounds that were tuned using a 5 µg/mL solution are marked with (**). U … urine, S … serum, M … breast milk

Compound	MS/MS parameters						Retention time [min]
	Polarity	Q1 mass [Da]	DP [V]	Q3 mass [Da]	CE [V]	CXP [V]	U/S/M
Plasticizer/plastic components							
Bisphenol A (BPA)	neg	227.0	-140	133.0 116.9	-32 -56	-17 -21	8.2
Bisphenol AF (BPAF)	neg	335.0	-80	265.1 176.8	-32 -58	-19 -29	12.0
Bisphenol B (BPB)	neg	241.1	-45	212.1 210.9	-26 -40	-11 -15	10.0
Bisphenol C (BPC) *	neg	255.0	-70	240.2 147.0	-26 -34	-29 -9	11.8
Bisphenol F (BPF)	neg	198.6	-120	105.0 106.0	-28 -28	-11 -11	6.3
Bisphenol S (BPS)	neg	249.1	-150	108.0 155.9	-36 -28	-13 -13	4.8
Mono-n-butyl phthalate (MBP)	neg	221.0	-5	77.2 71.1	-24 -20	-5 -11	4.2/3.4-3.6/ 4.2
Mono-2-ethylhexyl phthalate (MEHP)	neg	277.0	-40	134.0 127.1	-20 -20	-9 -11	8.3-8.8/8.0-8.8/ 8.3-8.8
N-butylbenzenesulfonamide	neg	211.9	-30	140.9 64.8	-30 -24	-9 -29	9.0
Benzyl butyl phthalate	pos	313.0	36	91.0 149.0	45 19	10 12	15.1
Dibutyl phthalate	pos	279.0	11	148.9 204.8	21 11	16 20	15.2
Tetrabromobisphenol A (TBPA)*	neg	542.6	-110	445.8 419.8 417.9	-45 -53 -56	-28 -23 -33	15.0
Perfluorinated alkylated substances							
Perfluorooctanoic acid (PFOA)	neg	412.9	-20	368.9 168.9	-18 -22	-19 -13	7.5/7.4/7.5
Perfluorooctanesulfonic acid (PFOS)	neg	498.8	-80	80.0 98.9	-126 -92	-37 -3	10.8-12.0 (isomers)
Industrial side products and pesticides							
2-Naphthol *	neg	143.0	-20	115.0 114.0	-34 -42	-13 -17	7.4
Methiocarb	pos	226.0	31	168.7 121.1	13 31	18 16	10.8
Prochloraz	pos	375.9	21	307.8 265.7	17 23	42 16	14.6
2-tert-Butylphenol (2-tert-BP) *	neg	149.0	-85	132.9 93.0	-32 -30	-7 -11	13.3
4-Octylphenol (4-OP) *	neg	205.1	-15	105.9 118.9	-28 -44	-19 -1	15.5
4-tert-Octylphenol (4-tert-OP) *	neg	205.1	-15	134.0 133.0	-24 -36	-11 -19	15.1
Fenarimol	pos	330.9	121	267.9 189.1	31 59	18 12	11.9
Nonylphenol	neg	219.1	-20	133.0 134.0	-42 -24	-11 -9	15.4
Endogenous estrogens							
Estrone (E1)	neg	269.4	-140	144.8 143.0	-50 -50	-2 -2	10.5
Estradiol (E2)	neg	271.1	-50	144.9 183.0	-52 -52	-15 -2	8.9

Compound	Polarity	MS/MS parameters					Retention time [min]
		Q1 mass [Da]	DP [V]	Q3 mass [Da]	CE [V]	CXP [V]	U/S/M
Estradiol-17-glucuronide (E2-17-GlcA)*	neg	447.1	-55	113.0 271.0	-30 -36	-9 -21	4.0/3.8/4.0
Estradiol-3-sulfate (E2-3-sulfate) *	neg	351.1	-170	271.1 80.0	-44 -62	-17 -11	4.8-5.2/4.6-5.0/4.8-5.2
Estriol (E3)	neg	287.0	-140	171.0 145.0	-48 -52	-27 -9	5.0
16-Epiestriol (16EpiE3) *	neg	287.1	-95	145.0 171.1	-50 -52	-11 -11	6.0
16-α-Hydroxyestrone (16OHE1) *	neg	285.1	-160	145.0 143.0	-46 -73	-11 -19	6.1
17-Epiestriol (17EpiE3) *	neg	287.1	-125	145.0 143.1	-52 -64	-13 -9	6.3
2-Methoxy estrone (2MeOE1) *	neg	299.1	-30	284.2 159.9	-32 -54	-21 -9	11.6
2-Methoxy estradiol(2MeOE2) *	neg	301.1	-45	286.1 285.2	-34 -48	-17 -21	10.0
4-Methoxy estrone (4MeOE1)	neg	299.1	-55	284.1 283.2	-28 -42	-23 -19	11.1
4-Methoxy estradiol (4MeOE2) *	neg	301.1	-40	286.1 284.9	-28 -46	-17 -23	9.3
2-Hydroxy estradiol (2OHE2) *	neg	287.0	-155	147.0 161.1	-58 -54	-15 -13	7.0/7.2/7.0
4-Hydroxy estrone (4OHE1)	neg	285.2	-130	161.1 159.1	-50 -66	-15 -9	7.9-8.9
Phytoestrogens and metabolites							
8-Prenylnaringenin	neg	339.0	-15	219.0 119.0	-26 -38	-19 -15	11.6
Coumestrol	neg	267.0	-50	265.9 211.0	-38 -38	-15 -11	6.4
Daidzein	neg	253.0	-10	224.0 207.9	-36 -40	-17 -13	5.2
Enterodiol	neg	301.1	-90	253.1 271.1	-32 -32	-15 -19	5.2
Enterolactone	neg	297.1	-105	253.1 107.0	-28 -32	-43 -11	6.8
Equol	neg	241.1	-45	120.9 118.9	-18 -28	-13 -5	6.5
Formononetin	neg	267.0	-90	252.0 223.0	-28 -42	-17 -17	7.9
Genistein	neg	269.0	-5	133.1 132	-38 -54	-11 -21	6.4
Glycitein *	pos	285.0	166	270.0 241.9	33 43	22 14	5.4
Isoxanthohumol	pos	355.1	31	179.0 298.9	35 21	12 18	8.4
Matairesinol *	neg	269.0	-65	83.0 137.1	-26 -30	-11 -11	6.5
Resveratrol	neg	227.0	-45	185.0 142.9	-26 -30	-11 -17	5.0
Xanthohumol **	pos	355.0	76	179.0 299.0	29 17	10 20	14.7
Mycoestrogens and metabolites							
Alternariol	neg	257.0	-110	213 215	-32 -34	-13 -13	6.8
Alternariol monomethyl ether	neg	271.1	-95	256 228	-32 -39	-13 -20	11.4
α-Zearalanol (α-ZAL)	neg	321.1	-120	277.1 303.1	-30 -30	-18 -20	8.8
β-Zearalanol (β-ZAL)	neg	321.1	-120	277.1 303.1	-30 -30	-18 -20	7.5
α-Zearalenol (α-ZEL)	neg	319.1	-80	160 174	-44 -37	-13 -9	9.2
β-Zearalenol (β-ZEL)	neg	319.1	-115	275.2 160.0	-30 -44	-15 -13	7.7
α-Zearalenol-14-glucuronide (α-ZEL-14-GlcA)	neg	495.0	-20	319.2 113.0	-38 -26	-23 -13	4.1/3.9/4.0
β-Zearalenol-14-glucuronide (β-ZEL-14-GlcA)	neg	495.1	-25	319.2 112.9	-38 -28	-23 -55	3.7/3.5/3.7
Zearalanone (ZAN)	neg	319.2	-75	275.1 161.0	-35 -38	-20 -15	11.5
Zearalenone (ZEN)	neg	317.1	-75	175.0 131.0	-34 -40	-9 -9	11.7
Zearalenone-14-glucuronide (ZEN-14-GlcA)	neg	493.0	-35	317.0 112.9	-36 -26	-25 -13	4.4/4.2/4.4

Compound	Polarity	MS/MS parameters					Retention time [min]
		Q1 mass [Da]	DP [V]	Q3 mass [Da]	CE [V]	CXP [V]	U/S/M
Zearalenone-14-sulfate (ZEN-14-sulfate)	neg	397.1	-80	316.9 175.0	-28 -46	-23 -15	5.5/5.3/5.5
Personal care product ingredients, pharmaceuticals and metabolites							
Benzophenone 1	neg	213.0	-60	91 134.9	-34 -28	-11 -17	9.0
Benzophenone 2	neg	245.1	-40	135 109	-22 -30	-23 -15	5.5
Benzylparaben	neg	227.0	-20	92.1 136	-30 -20	-13 -13	10.5
Butylparaben	neg	193.1	-25	92 137	-32 -22	-7 -21	10.1
Ethylparaben	neg	164.9	-30	92.1 136.9	-30 -20	-9 -15	6.1
Isobutylparaben	neg	193.0	-55	92 135.9	-32 -24	-7 -11	9.9
Methylparaben	neg	151.0	-35	92.0 135.9	-26 -20	-13 -15	4.9
Propylparaben	neg	179.0	-55	92.0 93.0	-30 -26	-9 -11	7.8
Ethinylestradiol	neg	295.1	-155	145 159	-48 -48	-13 -17	10.2
3-Benzylidencamphor (3-BC)	pos	241.1	76	91.0 165.1	51 51	10 14	15.2
4-methylbenzylidencamphor (4-MBC) *	pos	255.1	81	104.8 141.2	37 59	12 12	15.5
Octyl methoxycinnamate (OMC) **	pos	291.1	156	179.0 161.1	13 25	10 18	10.2/10.2/not det.
p-Hydroxybenzoic acid (pOHBA)	neg	136.9	-5	92.9 65.0	-18 -36	-17 -9	1.0-2.9/0.7-2.2/2.1
Triclosan *	neg	286.8	-5	35.0	-38	-15	14.9
Phytotoxins							
Anisodamine	pos	306.1	106	140.1 122.1	33 35	10 10	3.5/3.8/3.5
Aristolochic acid I	pos	358.9	26	298 296	15 15	18 8	5.3/4.7/5.2
Aristolactam I	pos	293.9	191	279 250.9	37 47	18 22	10.5
Jacobine	pos	352.0	136	155 119.9	37 39	18 14	3.5/4.1/3.5
Jacobine-N-oxide	pos	368.0	116	296.0 120.1	33 43	18 8	3.1
Riddelliin	pos	350.0	86	120.0 138.0	37 39	14 16	3.3/3.8/3.3
Riddelliin-N-oxide	pos	366.0	126	120.0 118.0	41 39	14 12	3.1
Scopolamine	pos	304.0	41	156.2 138.1	23 25	10 16	3.5/4.2/3.5
Disinfection by-products							
Bromoacetic acid *	neg	136.9 139.0 (isotope)	-10	78.9 81.0	-13 -13	-26 -26	0.8/0.7/0.7-0.9
Dibromoacetic acid *	neg	216.8	-5	172.8 80.9	-14 -32	-27 -9	1.1/0.9/1.2
Chloroacetic acid *	neg	92.9	-25	35.0 49.0	-18 -24	-5 -11	0.6
Dichloroacetic acid *	neg	126.9	-10	83.0 35.0	-32 -12	-5 -15	0.9/0.8/0.8-1
Food processing by-products							
Acrylamide *	pos	72.0	26	44.0 55.0	49 41	14 10	1
Glycidamide *	pos	87.9	56	44.0 45.0 71.0	25 13 11	6 12 10	0.9
5-Hydroxymethylfurfural (HMF)	pos	126.9	21	109 81.0	15 23	14 10	2.4
5-Hydroxymethyl-2-furanoic acid (HMFA) *	neg	140.9	-5	97.1 69.0	-10 -18	-15 -9	0.7/0.7/0.7-1
N-Nitosodimethylamine (NDMA) *	pos	74.9	26	43.1 58.0 44.0	21 17 17	20 8 6	1.4
PhIP	pos	225.1	51	210.1 140.1	41 69	10 14	5.3

Compound	MS/MS parameters					Retention time [min]
	Polarity	Q1 mass [Da]	DP [V]	Q3 mass [Da]	CE [V]	CXP [V]	U/S/M
Air pollutants							
N-Nitrosodiethylamine *	pos	102.9	1	75.0 47.0	15 13	8 12	3.7
Cotinine	pos	176.9	86	80.0 98.0	31 27	10 18	3.1
Trans-3-hydroxy cotinine	pos	193.0	86	80.1 134	33 27	10 14	2.6
1-Hydroxy pyrene *	neg	217.0	-115	189.0 188.0	-46 -48	-13 -25	14.3
3-Hydroxy phenanthrene*	neg	193.0	-135	165.0 164.2	-40 -46	-13 -13	11.5
3-hydroxy benzo[a]pyrene (3-OH-BaP) **	pos	269.0	141	240.0 239.0 252.1	51 75 45	46 24 54	15
Internal Standards							
$^{13}C_{12}$ Bisphenol A (BPA)	neg	239.0	-140	139	-38	-21	8.2
$^{13}C_{18}$ Zearalenone (ZEN)	neg	335.2	-110	185.1	-34	-13	11.7
$^{13}C_2$ mono-2-ethylhexyl phthalate (MEHP)	neg	281.1	-40	136.9	-20	-9	8.3-8.8/8.0-8.8/8.3-8.8
$^{13}C_2$ mono-*n*-butyl phthalate (MBP)	neg	225.0	-5	79.0	-24	-5	4.2/3.4-3.6/4.2
$^{13}C_3$ Estradiol (E2)	neg	274.1	-50	186.0	-52	-2	8.9
$^{13}C_6$ 4-tert-Octylphenol (4-tert-OP)	neg	211.1	-15	139.0	-36	-19	15.1
$^{13}C_6$ Butylparaben	neg	199.0	-25	98.0	-32	-7	10.1
$^{13}C_6$ Ethylparaben	neg	171.0	-30	97.9	-30	-9	6.1
$^{13}C_6$ Methylparaben	neg	157.0	-35	97.9	-26	-13	4.9
$^{13}C_6$ p-Hydroxybenzoic acid (pOHBA)	neg	142.9	-5	98.9	-18	-17	1.0-2.9/0.7-2.2/2.1
$^{13}C_6$ Propylparaben	neg	185.0	-55	97.9	-30	-9	7.8
$^{13}C_8$ Perfluorooctanesulfonic acid (PFOS)	neg	506.8	-80	79.9	-126	-37	11.8
$^{13}C_8$ Perfluorooctanoic acid (PFOA)	neg	420.9	-20	375.9	-18	-19	7.5/7.4/7.5
D_4 Genistein	neg	272.9	-5	136.9	-54	-21	6.4

Overall, 28 compounds are monitored in positive- and 71 in negative polarity. The best ionisation mode for the analytes included in the work of Preindl et al. [278] was already known beforehand. Precursor intensities clearly confirmed these ionisation modes as ideal for the QTrap 6500^{+} since the majority of precursor ion masses could only be detected in one polarity during the direct injection experiments.

With the exception of aristolochic acid I, 3-hydroxy phenanthrene and 3-OH-BaP, only one polarity exhibited acceptable ionisation yields above the baseline noise during the tuning process. To determine the most sensitive polarity for these three analytes, they were monitored in both ionisation modes during the pre-experiments using the solvent standards described in section 4.2.2.2. Ultimately, positive mode transitions showed higher sensitivities for aristolochic acid I and 3-OH-BaP, while the negative mode was the better choice for 3-hydroxy phenanthrene.

For the transferred compounds, retention times generally shifted by -30s for most analytes compared to the published method [278], with the majority yielding identical, stable retention times in all matrices. In a few cases, serum samples eluted slightly faster (10-30s) compared to the same analytes in urine and breast milk. Baseline separation was achieved for all analytes but two anisodamine isomers and isobutylparaben and butylparaben, which was also observed with the published method [278]. In the case of PFOS, the detection of three distinct peaks in the given elution time window points towards the presence of three different isomers, with the most abundant ionisation product eluting at 11.8 min, identical to the pure internal standard which is of linear structure. In urine and serum, *p*-OHBA could not be effectively retained on the reverse-phase column and eluted in multiple peaks in the given time window (0.5 min to 3 min). In the case of TBPA, the most intensive transition changed depending on the matrix, hence three product ions were adopted. Similar to Preindl et al. [278], only one product ion was successfully detected for triclosan across all matrices, while OMC could not be detected in breast milk.

The polar disinfection by-products as well as HMFA and glycidamide were hardly retained on the reverse phase column, as they eluted before one minute and in some cases even at the column dead time of around 0.6 minutes. No secondary transition could be determined for bromoacetic acid. Hence, the ^{81}Br isotope precursor mass and transition was included to support peak identification. Furthermore, background noise and interferences required the incorporation of three transitions for glycidamide, NDEA and 3-OH-BaP to enable compound identification. Anisodamine eluted in two peaks as the commercial standard used was a mixture of two diastereomers. While the naturally occurring 6S, 2'S enantiomer features the highest anticholinergic activity, synthetic anisodamine, a racemic mixture of the two diastereomers (6S, 2'S/6R 2'R, and 6S, 2'R/6R, 2'S) is marketed as an ingredient of traditional Chinese medicine [287]. Thus, the incorporation of both diastereomers has its merits when it comes to distinguishing natural exposure from synthetic exposure [287].

5.2. Optimisation of ESI parameters

5.2.1. Vertical probe position

Table 8 summarises the S/N ratios of the selected representative compounds in the matrix matched urine standards depending on the vertical probe position.

Table 8: Comparison of signal to noise ratios for key analytes dependent on vertical probe position

Compound	Concentration [ng/mL]	S/N	
		5 mm	2 mm
BPA	1	117	89
Butylparaben	0.5	233	285
Enterolactone	1	9.4	11
Equol	1	177	215
Estradiol	2	121	153
Genistein	0.5	105	127
MBP	2	18	20
PFOS	3	30	37
Triclosan	1	710	772
ZEN	1	362	377
ZEN-14-GlcA	10	6.7	7.3
ZEN-14-sulfate	1	32	32

As expected, the ESI probe being placed closer to the orifice (5 mm position) resulted in higher peak intensities and higher baseline noise than the probe being placed farther away (2 mm position). This trend was noticeable among all standard concentrations measured. However, while of lower peak height and peak area, all analytes with the exception of BPA yielded better S/N ratios with the probe at position 2 mm. Thus, the 2 mm vertical probe position was chosen for further method development.

5.2.2. Flow injection analysis

Figure 29 summarises plots depicting improvements or deterioration of signal intensity depending on the chosen settings. The values were normalised to the default parameters (CUR: 30 psi, GS1: 60 psi, GS2: 60 psi, TEM: 450°C, $IS^{+/-}$: -4500/4500V, CAD: Medium).

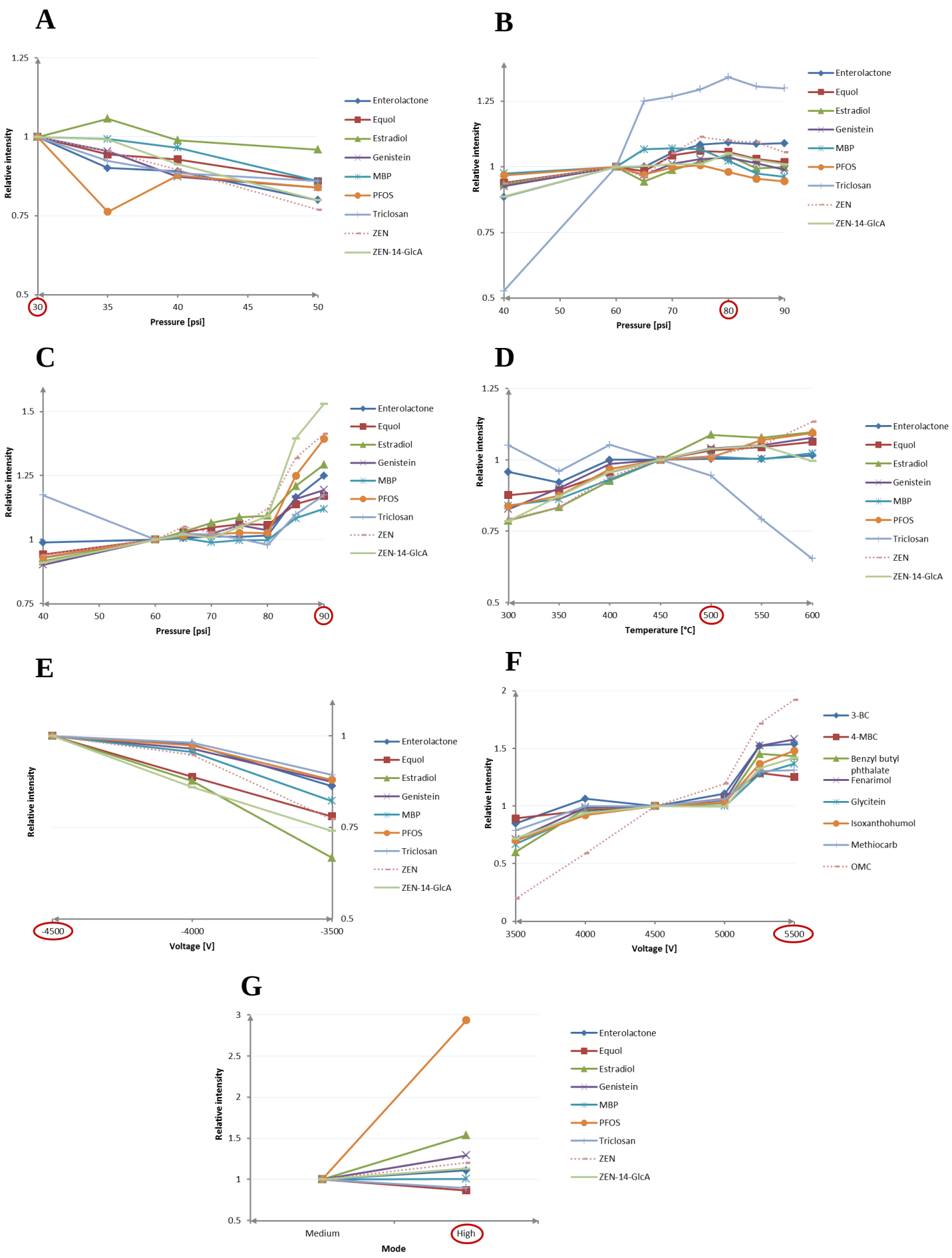

Figure 29: FIA of the CUR flow (A), GS1 flow (B), GS2 flow (C), temperature (D), ion spray voltage for the negative mode (E), ion spray voltage for the positive mode (F) and CAD pressure (G) for structurally representative compounds. Optimal chosen values are marked on the x-axis.

Ion source values that were selected according to FIA are summarised in Table 9 and compared to the values that had been used during the pre-experiments.

Table 9: Values chosen after FIA compared to the default value

Parameter	Default value	Selected optimal value
CUR	30 psi	30 psi
GS1	50 psi	80 psi
GS2	60 psi	90 psi
TEM	450°C	500°C
IS	**neg.:** -4500V **pos.:** 4500V	**neg.:** -4500V **pos.:** 5500V
CAD	Medium	High

The Turbo-V Ion source operator guide recommends not using CUR pressures below 30 psi to prevent contamination of the ion optics. FIA showed that CUR above 30 psi yields losses in ion transmission. Hence an optimal CUR parameter of 30 was adopted. Increase of the GS1 flow from 60 psi towards 80 psi resulted in, albeit small, signal improvement among analytes. A further increase to 90 psi did not yield any significant improvements, thus 80 psi was adopted as the ideal GS1 pressure. The increase of GS2 from 60 to 80 psi resulted in similar results, however a further increase to 90 psi yielded sudden and comparatively high signal improvements across all compounds. Thus, a GS2 pressure of 90 psi was adopted. A higher temperature seemed to improve ion transmission for most compounds. Nonetheless, severe losses in transmission were monitored for triclosan with raising temperatures. It was assumed that more of the 99 compounds included in the MRM method could potentially feature similar temperature sensitivity. If signal improvements were monitored between 500°C and 600°C, they were relatively small in comparison. For this reason, a temperature of 500°C was adopted.

Negative as well as positive ion spray voltages showed clear signal improvements for all compounds with stronger voltages. The maximum voltages of 5500V and -4500V for positive and negative ion mode respectively were adopted. Lastly, an increase in CAD pressure resulted in signal improvements among most compounds, with PFOS featuring a three-fold improvement. The CAD mode “High” was therefore selected.

5.2.3. Confirmation of ideal ion source parameters

5.2.3.1. Comparison of default source parameters and FIA optimised parameters

With 75 transferred compounds in 3 matrices, a maximum of 225 compound identifications were possible. Looking at the default method and the new parameters after FIA (Table 9 see section 5.2.2), 223 compounds were detected in both cases. Of these, 141 transitions yielded a better S/N ratio with the new method. However in many cases, the baseline noise increased by a factor of up to 5. This resulted in 42 transitions sustaining worse S/N ratios by a factor smaller than 0.5.

It was suspected that the relatively high increase in ion transmissions with a GS2 flow of 90 psi and the CAD mode "High" had caused a comparatively bigger increase in background noise during full LC-MS/MS measurements when matrix components were included. As mentioned before, the ion source system user guide also addresses the possibility of decreased sensitivity with GS2 flows that are too high [284].

5.2.3.2. Determination of ideal GS2 and CAD values

The method using the highest GS2 and CAD pressures resulted in the highest peak intensities and baseline noise by far. Generally, reducing only GS2 to 60 resulted in reduced baseline noise for most compounds, however reduction of only CAD to "Medium" yielded an even greater baseline noise reduction for a wider range of compounds. Reducing both parameters resulted in the lowest baseline noise for most compounds in most matrices. An evaluation of the signal to noise ratios for all four methods showed the highest sensitivity for the method using a GS2 flow of 60 psi and the CAD mode "Medium". Therefore, signal to noise ratios of measurements using these parameters and the default parameters used in the pre-experiments described in Table 9 in section 5.2.2 were compared for all detected compounds. Of 223 detected transitions, 149 yielded improved S/N ratios and merely 19 sustained a loss in sensitivity by a factor of less than 0.5. Due to these results, a GS2 flow of 60 psi and the CAD mode "Medium" were selected as final parameters.

Taking the results of FIA and the additional experiments described in section 4.2.3.4 into account, Table 10 summarises the final ion source parameters that were used for method validation.

Table 10: Final ESI parameters that were adopted for method validation

Source parameters	Value
Vertical probe position	2 mm
CUR	30 psi
GS1	80 psi
GS2	60 psi
TEM	500°C
IS	-4500/+5500 V
CAD	Medium

In summary, due to the structural multitude and high number of substances included in this method, it is not possible to determine technical parameters that result in a maximised sensitivity for all compounds. However, the final method yielded overall the best S/N ratios and was deemed a good compromise.

5.3. Estimation of limits of quantification and potential matrix contamination

The LOQ was estimated for all compounds in the three matrices except for OMC in breast milk, chloroacetic acid in all matrices, HMFA in urine and cotinine and trans-3-hydroxy cotinine in serum. OMC could not be detected in breast milk. No LOQs were calculated for chloroacetic acid in all matrices because the compound was not retained on the column and thus eluted early with intensive interferences which did not allow for linear regression to estimate matrix contamination. LOQs for HMFA in urine, cotinine and trans-3-hydroxy cotinine in serum were not estimated either because no linear increase in signal intensity was detected with higher standard concentrations as matrix contamination was too high during the pre-experiments.

In the case of matrix contamination in the blank matrix, standard addition was performed to estimate the level of contamination. Linear regression yielded acceptable R^2 values above 0.95 for all compounds except for dibutyl phthalate and nonylphenol in breast milk (R^2: 0.70 and 0.67, respectively). Isobaric interferences were detected in the case of bromoacetic acid, dibromoacetic acid, scopolamine and triclosan.

For most compounds, with the exception of contaminants of matrix blanks, the LOQ was extrapolated from standards with a S/N ratio between 3 and 100. Estimated LOQs and matrix contaminations that were used to correct the compound concentration are summarised in Table 11. LOQs could not be determined for some compounds in some matrices as mentioned above.

Table 11: Estimated LOQs and matrix contaminations. LOQs or the matrix contamination could not be determined (n.d.) for all analytes in all matrices.

Compound	LOQ [ng/mL]			Matrix contamination [ng/mL]		
	Urine	Serum	Milk	Urine	Serum	Milk
Plasticizer/plastic components						
Bisphenol A (BPA)	0.089	0.11	0.091			
Bisphenol AF (BPAF)	0.097	0.064	0.072			
Bisphenol B (BPB)	0.014	0.027	0.017			
Bisphenol C (BPC)	0.21	0.3	0.4			
Bisphenol F (BPF)	0.059	0.093	0.048			
Bisphenol S (BPS)	0.014	0.0028	0.0022			
Mono-n-butyl phthalate (MBP)	0.85	0.52	0.45	1.9	1.9	2.4
Mono-2-ethylhexyl phthalate (MEHP)	0.13	0.35	0.86	0.23	18	0.81
N-butylbenzenesulfonamide	1.4	1.2	1.9	1.3	1.2	1.9
Benzyl butyl phthalate	0.25	1.8	15	2.2	9.3	65
Dibutyl phthalate	6.9	13	60*	70	93	360*
Tetrabromobisphenol A (TBPA)	0.1	0.11	4.5			
Perfluorinated alkylated substances						
Perfluorooctanoic acid (PFOA)	0.043	0.13	0.015		0.32	
Perfluorooctanesulfonic acid (PFOS)	0.87	0.14	0.013		1.2	
Industrial side products and pesticides						
2-Naphthol	0.28	0.053	0.032	2.3	7.5	8.1
Methiocarb	0.0042	0.037	0.01			
Prochloraz	0.00043	0.00085	0.0012			
2-tert-Butylphenol (2-tert-BP)	68	120	180		14	
4-Octylphenol (4-OP)	14	9.3	53		1.3	
4-tert-Octylphenol (4-tert-OP)	1.7	1.3	31	0.21	3.5	11
Fenarimol	0.0035	0.0027	0.02			
Nonylphenol	40	2.6	150*	95	59	490*
Endogenous estrogens						
Estrone (E1)	0.0037	0.0062	0.015			
Estradiol (E2)	0.038	0.14	0.073			
Estradiol-17-glucuronide (E2-17-GlcA)	0.93	0.16	0.06			
Estradiol-3-sulfate (E2-3-sulfate)	0.17	0.032	0.013			
Estriol (E3)	0.22	0.028	0.16			
16-Epiestriol (16EpiE3)	0.1	0.13	0.19			
16-α-Hydroxyestrone (16OHE1)	0.064	0.016	0.058			
17-Epiestriol (17EpiE3)	0.13	0.11	0.27			
2-Methoxy estrone (2MeOE1)	0.023	0.068	0.033			
2-Methoxy estradiol(2MeOE2)	0.02	0.024	0.037			
4-Methoxy estrone (4MeOE1)	0.0053	0.011	0.0064			
4-Methoxy estradiol (4MeOE2)	0.011	0.015	0.013			
2-Hydroxy estradiol (2OHE2)	0.1	140	21			
4-Hydroxy estrone (4OHE1)	0.0067	52	11			
Phytoestrogens and metabolites						
8-Prenylnaringenin	0.042	0.032	0.16			
Coumestrol	0.0087	0.0035	0.0055			
Daidzein	0.0081	0.0083	0.0041			
Enterodiol	0.034	0.0052	0.0051			
Enterolactone	0.85	0.2	0.6	0.78		0.88
Equol	0.019	0.062	0.024			
Formononetin	0.0037	0.0035	0.0023			
Genistein (GEN)	0.011	0.012	0.0036			
Glycitein	0.13	0.026	0.0054			
Isoxanthohumol	0.015	0.00087	0.013			
Matairesinol	0.28	0.048	0.16			
Resveratrol	1.5	3	2.2			
Xanthohumol	0.59	0.082	2.9			
Mycoestrogens and metabolites						
Alternariol	0.24	0.15	0.094			
Alternariol monomethyl ether	0.0047	0.005	0.015			
α-zearalanol (α-ZAL)	0.063	0.1	0.11			
β-zearalanol (β-ZAL)	0.1	0.061	0.075			
α-zearalenol (α-ZEL)	0.0022	0.011	0.068			
β-zearalenol (β-ZEL)	0.16	0.14	0.12			
α-zearalenol-14-glucuronide (α-ZEL-14-GlcA)	17	0.46	0.014			
β-zearalenol-14-glucuronide (β-ZEL-14-GlcA)	42	0.79	0.016			
Zearalanone (ZAN)	0.029	0.18	0.16			
Zearalenone (ZEN)	0.034	0.11	0.19			
Zearalenone-14-glucuronide (ZEN-14-GlcA)	9.3	0.37	0.05			
Zearalenone-14-sulfate (ZEN-14-sulfate)	0.11	0.073	0.014			

* Matrix contaminations and LOQs of dibutyl phthalate and nonylphenol in breast milk are based on unsatisfying regression coefficients (0.70 and 0.67 respectively)

Compound	LOQ [ng/mL]			Matrix contamination [ng/mL]		
	Urine	Serum	Milk	Urine	Serum	Milk
Personal care product ingredients, pharmaceuticals and metabolites						
Benzophenone 1	0.017	0.017	0.067	0.035	0.053	0.067
Benzophenone 2	0.016	0.025	0.021			
Benzylparaben	0.0014	0.0021	0.0044			
Butylparaben (BP)	0.01	0.011	0.013			
Ethylparaben (EP)	0.012	0.026	0.033		0.13	0.27
Isobutylparaben	0.0099	0.01	0.013			
Methylparaben (MP)	0.099	0.025	0.045	0.14	0.28	0.38
Propylparaben (PP)	0.027	0.022	0.04	0.12	0.14	0.34
Ethinylestradiol	0.096	0.21	0.26			
3-Benzylidencamphor (3-BC)	14	17	32	76	21	
4-methylbenzylidencamphor (4-MBC)	1.7	4.7	61		26	
Octyl methoxycinnamate (OMC)	600	20	n.d.			
p-Hydroxybenzoic acid (pOHBA)	48	8	4	1800	13	19
Triclosan	0.12	0.14	3.5	0.24	0.5	1
Phytotoxins						
Anisodamine	0.16	0.006	0.0049			
Aristolochic acid I	0.21	0.15	0.12			
Aristolactam I	0.076	0.049	0.054			
Jacobine	0.43	0.069	0.024			
Jacobine-N-oxide	0.034	0.01	0.0042			
Riddelliin	0.98	0.35	0.033			
Riddelliin-N-oxide	1.7	0.071	0.019			
Scopolamine	0.012	0.0034	0.0017		0.008	0.008
Disinfection by-products						
Bromoacetic acid	155	11	1.8		41	<LOQ
Dibromoacetic acid	26	1.4	3.5	4		
Chloroacetic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dichloroacetic acid	37	7.5	6.9			
Food processing by-products						
Acrylamide	31	9.5	12			
Glycidamide	1845	3218	1131			
5-Hydroxymethylfurfural (HMF)	28	3.7	4.5			
5-Hydroxymethyl-2-furanoic acid (HMFA)	n.d.	813	19	n.d.		
N-Nitosodimethylamine (NDMA)	33	52	61			
PhIP	0.015	0.017	0.0093			
Air pollutants						
N-Nitrosodiethylamine	2208	6220	379			
Cotinine	0.18	n.d.	0.23	0.17	n.d.	1.2
Trans-3-hydroxy cotinine	1.5	n.d.	0.037		n.d.	
1-Hydroxy pyrene	0.23	0.38	0.21			
3-Hydroxy phenanthrene	0.025	0.016	0.013			
3-hydroxy benzo[a]pyrene (3-OH-BaP)	142	10482	324			

Glycidamide, 3-OH-BaP and NDEA were excluded from method validation following the estimation of the LOQs. The blank matrixes were contaminated with high amounts of HMFA in urine, cotinine and trans-3-hydroxy cotinine in serum and chloroacetic acid interferences in all matrices causing insufficient linear regression unsuitable for standard addition and preventing the estimation of accurate LOQs. Much higher spiking levels would have been needed for chloroacetic acid to distinguish the hardly retained chromatographic peak from the interferences and/or any matrix contaminations. Thus, it was excluded from method validation as well.

5.4. Method validation

The method was evaluated according to the Commission Decision No. 657/2002 [286]. Table 12 summarises perquisites that needed to be met for successful method validation. As no specific guidelines for spiking concentrations below 100 ng/mL are described, the RSD_R and RSD_r limits for compounds with a spiking concentration below 100 ng/mL were set to 25% according to the validation by Preindl et al. [278].

Table 12: Requirements for validation according to European Commission Decision No. 657/2002 [286]

Parameter	Requirement
R_E	50-120% for conc. < 1 ng/mL 70-110% for conc. 1-10 ng/mL 80-110% for conc. > 10 ng/mL
RSD_R	< 25% for conc. < 100 ng/mL < 23% for conc. 100-1000 ng/mL < 16% for conc. > 1000 ng/mL
RSD_r	≤ RSD_R
Retention	RT ≥ 2x column dead time (≥ 1.1 min); max. 2.5% deviation from standard

4-*tert*-OP was not evaluated using internal calibration in urine, while MEHP was not evaluated using internal calibration in breast milk. In both cases, IS recoveries were too low and thus too variable to enable linear regression. In some samples they were not detected at all. Furthermore, ZEN was not evaluated using internal calibration in the first validation run for urine and serum, because the IS that had originally been added to the IS mix had a lower concentration than expected. Hence, ^{13}C-ZEN was not detected and a higher concentrated solution had to be prepared and added during subsequent sample preparations.

2OHE2 was not detected in any sample, including the highest solvent standards. Measurement of the parent solution of 2OHE2 that had been added to the standard mix exhibited lower sensitivity than expected based on the pre-experiments. This indicated that the compound might have no longer been present or dissolved at the listed concentration.

Table 13 summarises extraction recoveries, intermediate precision and repeatability of the analytes in all matrices in the three validation experiments. A retention time shift outside the MRM window was observed for MEHP, PFOS and AAI in the last breast milk validation. Consequently, method repeatability could not be evaluated and the displayed data for these three compounds stems from the evaluation of only two validation experiments. Extraction results of individual validations are summarised in Tables 32-34 (see Appendix).

Table 13: Extraction recovery (R_E), intermediate precision (RSD_R) and repeatability (RSD_r) of the investigated analytes in three matrices after evaluation of three validation experiments at the low spiking level (LL) and the high spiking level (HL). Parameters that could not be determined are displayed as (n.d.).

Compound	Urine			Serum			Breast milk		
	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL [%]	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL[%]	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL [%]
Plasticizer/plastic components									
Bisphenol A (BPA)	116±23	94±9	13/7	104±20	89±21	13/7	82±15	81±20	11/10
Bisphenol AF (BPAF)	97±5	88±5	4/4	96±10	94±16	2/5	47±22	52±16	13/8
Bisphenol B (BPB)	91±15	91±5	16/9	76±17	89±16	8/11	53±47	60±21	55/11
Bisphenol C (BPC)	95±6	92±5	9/4	82±11	88±12	8/5	50±22	56±19	14/8
Bisphenol F (BPF)	100±7	98±5	21/5	73±20	81±6	20/6	72±18	81±9	8/4
Bisphenol S (BPS)	87±29	90±11	30/7	109±42	90±17	32/7	95±16	83±10	12/9
Mono-n-butyl phthalate (MBP)	95±13	101±12	11/5	138±26	143±28	6/3	78±17	75±13	16/5
Mono-2-ethylhexyl phthalate (MEHP)*	n.d.	99±10	n.d./5	n.d.	n.d.	n.d.	47±31	39±14	n.d.
N-butylbenzenesulfonamide	96±7	92±4	9/2	87±12	88±8	5/4	71±61	69±15	80/5
Benzyl butyl phthalate	80±15	68±10	22/13	78±25	65±13	13/9	n.d.	n.d.	n.d.
Dibutyl phthalate	36±102	35±56	12/73	95±28	50±14	20/13	n.d.	n.d.	n.d.
Tetrabromobisphenol A (TBPA)	91±20	81±19	18/10	56±49	49±20	31/8	n.d.	n.d.	n.d.
Perfluorinated alkylated substances									
Perfluorooctanoic acid (PFOA)	119±25	90±9	9/8	84±16	82±15	6/8	75±25	59±19	16/5
Perfluorooctanesulfonic acid (PFOS)*	100±47	84±21	4/2	n.d.	56±25	6/5	95±18	66±11	n.d.
Industrial side products and pesticides									
2-Naphthol	95±19	85±10	7/13	96±18	81±4	18/4	76±71	52±19	32/8
Methiocarb	n.d.	83±5	7/5	n.d.	85±7	3/7	38±25	52±16	27/12
Prochloraz	n.d.	83±15	11/9	n.d.	85±12	30/8	n.d.	n.d.	n.d.
2-tert-Butylphenol (2-tert-BP)	n.d.	n.d.	n.d.	5±73	6±35	15/12	20±56	24±49	44/67
4-Octylphenol (4-OP)	43±82	n.d.	0.27/n.d.	106±34	101±32	14/10	n.d.	n.d.	n.d.
4-tert-Octylphenol (4-tert-OP)	n.d.	6±159	12/73	80±11	75±15	6/5	n.d.	26±18	57/21
Fenarimol	88±13	85±6	3/6	95±5	92±10	9/4	n.d.	37±24	n.d./13
Nonylphenol	37±92	n.d.	n.d.	121±58	89±21	24/11	n.d.	n.d.	n.d.
Endogenous estrogens									
Estrone (E1)	129±14	94±13	37/12	82±20	85±14	20/13	n.d.	44±56	n.d./42
Estradiol (E2)	107±23	102±12	9/7	76±28	86±13	21/8	n.d.	50±21	n.d./13
Estradiol-17-glucuronide (E2-17-GlcA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	77±28	73±9	31/8
Estradiol-3-sulfate (E2-3-sulfate)	n.d.	91±9	45/10	77±21	90±8	12/5	56±41	51±20	36/16
Estriol (E3)	95±21	97±7	31/7	104±23	86±7	20/8	60±33	66±12	16/11
16-Epiestriol (16EpiE3)	91±13	95±4	13/4	93±11	83±8	11/6	57±25	60±18	16/9
16-α-Hydroxyestrone (16OHE1)	67±20	88±9	27/8	82±18	81±8	18/6	74±30	70±14	11/6
17-Epiestriol (17EpiE3)	94±19	96±8	16/3	86±9	85±7	8/3	57±24	64±14	24/8
2-Methoxy estrone (2MeOE1)	97±12	94±5	17/5	97±8	84±10	11/4	43±53	49±17	51/15
2-Methoxy estradiol(2MeOE2)	99±12	96±5	8/4	90±11	86±6	8/2	n.d.	46±18	n.d./14
4-Methoxy estrone (4MeOE1)	96±16	93±5	13/3	83±12	88±10	10/3	n.d.	53±20	n.d./8
4-Methoxy estradiol (4MeOE2)	95±17	98±5	11/4	91±17	88±6	6/3	27±54	50±19	69/9
4-Hydroxy estrone (4OHE1)	79±21	82±18	20/6	n.d.	n.d.	n.d.	n.d.	40±24	47/7

* Data for MEHP and PFOS in breast milk stem from two evaluated validations only

Compound	Urine			Serum			Breast milk		
	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL [%]	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL[%]	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL [%]
Phytoestrogens and metabolites									
8-Prenylnaringenin	88±11	90±6	12/6	87±7	91±11	2/2	39±22	41±25	14/12
Coumestrol	96±11	92±5	10/4	94±20	85±8	14/7	58±24	68±19	16/7
Daidzein	98±21	96±5	23/10	84±28	81±11	13/5	n.d.	93±13	42/12
Enterodiol	89±15	104±11	12/8	92±13	82±6	4/6	43±64	42±34	46/27
Enterolactone	101±6	97±5	9/5	90±7	88±7	3/2	77±33	83±10	8/5
Equol	97±5	98±3	6/3	97±6	85±3	5/1	60±22	62±22	18/10
Formononetin	100±6	94±5	5/4	94±11	91±10	9/2	58±19	66±19	11/8
Genistein	107±18	96±13	10/7	71±22	82±15	21/10	184±73	80±18	110/17
Glycitein	n.d.	89±17	21/4	107±15	85±5	14/6	72±24	79±10	13/10
Isoxanthohumol	91±17	89±5	7/4	83±23	92±13	9/4	n.d.	57±20	n.d./17
Matairesinol	78±13	96±7	19/4	83±14	84±7	11/3	83±19	80±8	12/5
Resveratrol	93±17	92±17	4/1	72±8	77±13	8/7	10±39	10±61	37/31
Xanthohumol	91±17	89±5	7/4	83±23	92±13	9/4	n.d.	57±20	n.d./17
Mycoestrogens and metabolites									
Alternariol	94±8	94±4	5/4	91±11	89±10	4/3	61±20	67±17	10/6
Alternariol monomethyl ether	94±13	92±5	8/5	66±24	88±15	15/6	n.d.	51±19	n.d./15.4
α-Zearalanol (α-ZAL)	97±4	94±5	4/3	96±12	88±9	4/3	44±20	54±20	15/12
β-Zearalanol (β-ZAL)	102±4	96±4	7/3	91±6	86±5	6/2	54±22	63±22	13/9
α-Zearalenol (α-ZEL)	n.d.	89±8	14/7	n.d./7	86±14	n.d./8	n.d.	56±30	n.d./15
β-Zearalenol (β-ZEL)	100±6	95±6	16/3	94±5	87±6	5/2	51±21	59±21	12/10
α-Zearalenol-14-glucuronide (α-ZEL-14-GlcA)	n.d.	n.d.	n.d.	n.d.	83±12	n.d./7	61±22	59±23	16/15
β-Zearalenol-14-glucuronide (β-ZEL-14-GlcA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	64±23	54±13	21/9
Zearalanone (ZAN)	98±9	92±4	10/5	88±17	92±9	8/4	n.d.	50±24	16/10
Zearalenone (ZEN)	98±11	94±7	7/6	86±8	87±11	6/8	n.d.	57±17	8/10
Zearalenone-14-glucuronide (ZEN-14-GlcA)	n.d.	n.d.	n.d.	n.d.	83±5	n.d./6	68±24	76±13	23/12
Zearalenone-14-sulfate (ZEN-14-sulfate)	99±9	97±4	9/5	91±7	89±9	5/3	46±43	46±33	22/12
Personal care product ingredients, pharmaceuticals and metabolites									
Benzophenone 1	98±5	93±4	3/3	96±15	97±13	25/8	58±16	61±20	12/7
Benzophenone 2	97±8	95±7	5/5	89±8	88±20	9/23	75±13	80±6	9/6
Benzylparaben	97±7	92±5	7/5	92±6	90±10	5/3	51±19	57±22	23/8
Butylparaben	87±5	90±8	4/6	85±7	86±14	4/1	47±28	52±22	18/5
Ethylparaben	93±10	94±10	5/8	81±15	87±13	14/5	103±63	71±17	36/4
Isobutylparaben	87±5	86±3	3/3	91±9	89±9	4/2	45±23	57±24	26/10
Methylparaben	95±13	98±10	8/8	74±20	89±15	18/4	85±33	68±23	26/4
Propylparaben	93±7	93±8	5/5	80±11	86±12	6/2	78±36	68±19	29/3
Ethinylestradiol	97±13	94±6	11/6	93±11	88±12	14/5	n.d.	51±18	n.d./7
3-Benzylidencamphor (3-BC)	n.d.	n.d.	n.d.	34±16	26±19	10/13	n.d.	27±31	7/5
4-methylbenzylidencamphor (4-MBC)	n.d.	18±83	13/77	64±16	45±15	9/13	n.d.	n.d.	n.d./7
Octyl methoxycinnamate (OMC)	n.d.	103±32	n.d./22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
p-Hydroxybenzoic acid (pOHBA)	n.d.	n.d.	n.d.	76±43	88±9	86/5	74±18	78±8	7/3
Triclosan	72±12	59±16	13/25	100±14	75±17	10/6	n.d.	25±39	n.d./8

Compound	Urine			Serum			Breast milk		
	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL [%]	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL[%]	$R_E \pm RSD_R$ (LL) [%]	$R_E \pm RSD_R$ (HL) [%]	RSD_r LL/HL [%]
Phytotoxins									
Anisodamine	n.d.	87±14	n.d./7	106±23	88±8	10/3	43±26	57±12	97/19
Aristolochic acid I*	78±23	88±16	23/7	73±39	91±18	6/7	n.d.	46±13	n.d.
Aristolactam I	93±10	86±6	8/4	84±11	84±15	3/4	n.d.	33±19	n.d./14
Jacobine	n.d.	71±24	n.d./5	90±11	89±7	10/3	45±29	42±27	25/12
Jacobine-N-oxide	n.d.	82±14	n.d./13	102±24	83±15	13/6	29±72	24±44	53/19
Riddelliin	n.d.	76±23	n.d./9	88±13	91±8	10/3	43±25	53±22	11/27
Riddelliin-N-oxide	n.d.	76±23	n.d./10	101±13	91±9	10/6	39±44	39±31	29/29
Scopolamine	102±17	90±13	16/6	96±4	92±7	2/1	56±23	65±10	16/14
Disinfection by-products									
Bromoacetic acid	n.d.	n.d.	n.d.	n.d.	53±61	n.d./8	n.d.	n.d.	n.d.
Dibromoacetic acid	n.d.	83±10	11/8	83±10	82±11	3/3	37±39	44±31	34/30
Dichloroacetic acid	n.d.	98±7	16/6	101±11	87±11	8/2	29±49	31±42	41/62
Food processing by-products									
Acrylamide	n.d.	86±23	n.d./6	64±13	64±10	6/8	28±33	34±16	26/59
5-Hydroxymethylfurfural (HMF)	n.d.	27±21	84/26	75±12	74±9	4/5	n.d.	19±87	n.d./54
5-Hydroxymethyl-2-furanoic acid (HMFA)	n.d.	n.d.	n.d.	86±10	91±5	11/5	50±17	52±14	5/18
N-Nitosodimethylamine (NDMA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PhIP	96±13	99±9	8/4	93±4	86±10	3/1	55±22	68±19	11/14
Air pollutants									
Cotinine	86±16	96±7	13/3	n.d.	n.d.	n.d.	n.d.	n.d.	68/57
Trans-3-hydroxy cotinine	n.d.	91±16	45/5	n.d.	n.d.	74/5	n.d.	n.d.	136/46
1-Hydroxy pyrene	91±25	93±16	15/8	62±20	75±17	12/8	104±48	35±29	27/15
3-Hydroxy phenanthrene	92±8	88±6	7/4	87±12	86±15	10/5	n.d.	50±32	n.d./15

* Data for aristolochic acid I in breast milk stem from two evaluated validations only

Table 14 summarises the average regression coefficient, calibrated concentration range, LOQ, SSE, ion ratios and retention times of all compounds in all matrices. The second validation in urine/serum and the first validation of breast milk were measured in the same sequence. This measurement exhibited noticeably higher baseline noise for BPF, methiocarb, prochloraz, OP, prochloraz, 8-prenylnaringenin, HMF and NDMA. This temporary issue was resolved with following measurements, but consequently, standard deviations of the LOQs are comparatively high and the average LOQ may be above the methods actual capabilities.

Table 14: Average R^2, LOQs, SSE, ion ratios and retention times of the investigated analytes in urine (U), serum (S) and breast milk (M). Parameters that could not be determined are displayed as (n.d.). Data for MEHP, PFOS and aristolochic acid I in breast milk stem from two validations only (*)

Compound	Regression coefficient R^2	Calibration range [ng/mL]	LOQ ± s [ng/mL]			SSE ± s [%]			Ion ratio	Retention time [min]
	U/S/M	U/S/M	U	S	M	U	S	M	U/S/M	U/S/M
Plasticizer/plastic components										
Bisphenol A (BPA)	0.992/0.985/0.991	0.03-10/0.1-10/0.1-10	0.21±0.0045	0.57±0.07	0.4±0.11	103±6	58±5	88±7	6/5/5	8.2
Bisphenol AF (BPAF)	0.993/0.995/0.989	0.015-5	0.038±0.0079	0.075±0.039	0.041±0.0059	87±7	48±6	32±1	6/6/6	12
Bisphenol B (BPB)	0.999/0.993/0.993	0.03-1	0.022±0.0016	0.043±0.02	0.028±0.011	100±6	52±3	68±5	49/47/46	10
Bisphenol C (BPC)	0.999/0.998/0.993	0.06-20/0.2-20/0.2-20	0.22±0.042	0.85±0.57	0.24±0.081	99±6	50±3	51±3	97/99/95	11.8
Bisphenol F (BPF)	0.996/0.996/0.996	0.05-5	0.064±0.037	0.068±0.048	0.028±0.036	119±18	80±12	114±16	8/7/8	6.3
Bisphenol S (BPS)	0.99/0.994/0.988	0.002-0.2	0.036±0.008	0.0086 ±0.0031	0.0078 ±0.0017	57±15	87±6	96±17	30/28/28	4.8
Mono-n-butyl phthalate (MBP)	0.998/0.998/0.987	0.15-50	0.34±0.075	0.2±0.062	0.16±0.034	103±10	96±15	88±3	48/50/51	4.0-4.3/3.6-4.1/4.1-4.9
Mono-2-ethylhexyl phthalate (MEHP)*	0.996/n.d./0.992	0.045-15/n.d./0.045-4.5	0.047±0.0053	n.d.	3.8±0.022	100±8	n.d.	11±4	52/51/51	8.4-9/8.2-8.5/8.2- after 9.5*
N-butylbenzenesulfonamide	0.998/0.997/0.992	0.3-100	1.2±0.043	1±0.079	1.5±0.26	100±3	95±3	82±2	94/95/94	9
Benzyl butyl phthalate	0.98/0.949/n.d.	0.075-25/ 0.075-25/n.d.	0.18±0.062	0.66±0.17	n.d.	43±7	11±3	n.d.	75/72/70	15.1
Dibutyl phthalate	0.934/0.983/n.d.	1.5-500/1.5-500/n.d.	3.1±0.7	7.6±2.2	n.d.	56±11	17±7	n.d.	53/53/57	15.2
Tetrabromobisphenol A (TBPA)	0.988/0.986/n.d.	0.03-10/0.3-10/n.d.	0.021±0.0028	0.33±0.2	n.d.	44±4	70±44	n.d.	111/99/106; 97/86/77**	15
Perfluorinated alkylated substances										
Perfluorooctanoic acid (PFOA)	0.994/0.992/0.98	0.015-1.5/0.0045-1.5/0.0045-0.45	0.22±0.0096	0.18±0.033	0.092±0.036	131±13	73±12	110±14	43/50/41	6.8-7.1/6.7-6.9/7-7.7
Perfluorooctanesulfonic acid (PFOS)*	0.993/0.991/0.997	0.045-1.5/0.015-1.5/0.015-1.5	0.049±0.035	0.14±0.054	0.016±0.011	108±10	42±3	86±6	17/17/17	11-11.4/10.7-11/11- after 12.3*
Industrial side products and pesticides										
2-Naphthol	0.998/0.994/0.991	0.009-3/0.01-3/0.009-3	0.019±0.0074	0.029±0.011	0.021±0.0035	130±12	85±9	103±14	2/2/1	7.4
Methiocarb	0.992/0.992/0.991	0.05-0.5/0.015-0.5/0.015-0.5	0.017±0.022	0.036±0.054	0.015±0.012	72±3	67±9	35±5	69/70/68	10.8
Prochloraz	0.992/0.992/0.955	0.0005-0.05/0.0015-0.05/0.015-0.05	0.0003 ±0.0002	0.00076 ±0.00075	0.05±0.083	58±1	35±5	11±10	13/15/16	14.6
2-tert-Butylphenol (2-tert-BP)	0.998/0.998/0.995	15-5000	34±1.6	63±7.1	98±13	97±2	60±1	37±6	88/88/87	13.3
4-Octylphenol (4-OP)	0.991/0.967/0.928	3-300/3-1000/30-1000	1.4±0.98	2.3±2.6	24±6.8	27±12	42±15	1.3±0.4	1/1/2	15.5
4-tert-Octylphenol (4-tert-OP)	0.991/0.993/0.992	0.45-150/0.45-150/15-45	0.14±0.006	3.3±3.5	40±6.1	77±7	18±1	4±1	99/99/97	15.1
Fenarimol	0.982/0.997/0.993	0.003-0.3/0.003-3/0.009-0.3	0.0028 ±0.0016	0.0055 ±0.0027	0.0077 ±0.0038	48±12	56±3	13±0.3	30/27/30	12

* Data for MEHP and PFOS in breast milk stem from two evaluated validations only as a retention time shift outside the programmed sMRM window was observed in the last validation run

** A second qualifier transition was included for TBPA

Compound	Regression coefficient R^2	Calibration range [ng/mL]	LOQ ± s [ng/mL]			SSE ± s [%]			Ion ratio	Retention time [min]
	U/S/M	U/S/M	U	S	M	U	S	M	U/S/M	U/S/M
Nonylphenol	0.989/0.951/n.d.	0.75-250/7.5-250/n.d.	3.2±2.1	1.6±1.1	n.d.	33±18	53±14	n.d.	13/13/14	15.4
Endogenous estrogens										
Estrone (E1)	0.993/0.993/0.989	0.003-0.3/0.009-0.3/0.009-0.3	0.0059±0.0016	0.0085±0.0026	0.0069±0.0014	111±15	69±11	41±2	29/28/26	10.5
Estradiol (E2)	0.996/0.995/0.98	0.09-3	0.051±0.019	0.076±0.015	0.093±0.034	104±6	79±7	46±5	85/80/79	8.9/8.6-8.9/8.9
Estradiol-17-glucuronide (E2-17-GlcA)	n.d./0.983/0.996	n.d./1.5-5/0.15-5	n.d.	4.9±0.93	0.91±0.39	n.d.	232±77	198±77	n.d./103/74	n.d./3.8-3.9/3.9-4.2
Estradiol-3-sulfate (E2-3-sulfate)	0.996/0.994/0.997	0.045-1.5/0.015-1.5/0.0045-1.5	0.2±0.035	0.012±0.0015	0.012± 0.00098	103±18	96±5	91±4	12/4/5	4.7/4.5-4.7/4.7-4.9
Estriol (E3)	0.995/0.996/0.995	0.09-3/0.03-3/0.09-3	0.12±0.042	0.058±0.028	0.11±0.033	122±23	117±17	110±17	76/75/74	5
16-Epiestriol (16EpiE3)	0.995/0.995/0.997	0.3-10	0.23±0.049	0.13±0.042	0.19±0.05	129±19	103±27	98±15	92/91/93	6
16-α-Hydroxyestrone (16OHE1)	0.996/0.995/0.996	0.045-1.5	0.096±0.044	0.034±0.0065	0.037±0.0098	122±16	104±21	98±13	35/38/39	6.1
17-Epiestriol (17EpiE3)	0.998/0.997/0.998	0.03-10/0.1-10/0.1-10	0.12±0.013	0.087±0.042	0.11±0.059	128±19	103±22	92±11	56/57/57	6.3
2-Methoxy estrone (2MeOE1)	0.999/0.998/0.989	0.025-2.5/0.075-2.5/0.075-2.5	0.046±0.01	0.05±0.011	0.045±0.021	134±31	94±24	43±2	4/4/4	11.6
2-Methoxy estradiol(2MeOE2)	0.998/0.998/0.986	0.006-2/0.02-2/0.06-2	0.018±0.0044	0.027±0.011	0.032±0.023	134±36	100±30	42±5	2/2/2	10
4-Methoxy estrone (4MeOE1)	0.998/0.995/0.988	0.005-0.5/0.015-0.5/0.015-0.5	0.016±0.0056	0.011±0.0052	0.009±0.0047	106±10	75±6	44±3	58/60/59	11.1
4-Methoxy estradiol (4MeOE2)	0.999/0.998/0.99	0.01-1/0.03-1/0.03-1	0.024±0.0054	0.015±0.0038	0.013±0.0038	107±13	87±8	44±2	46/44/46	9.3
4-Hydroxy estrone (4OHE1)	0.997/n.d./0.991	0.005-0.5/0.15-5/0.015-0.5	0.013±0.0037	n.d.	0.0073±0.003	162±29	126±13	134±33	15/x/16	8.3
Phytoestrogens and metabolites										
8-Prenylnaringenin	0.99/0.997/0.993	0.009-3/0.009-3/0.03-3	0.01±0.009	0.023±0.024	0.015±0.0045	87±7	62±25	23±4	59/58/60	11.6-11.7
Coumestrol	0.997/0.996/0.996	0.005-0.5/0.015-0.5/0.015-0.5	0.01±0.0026	0.0061 ±0.0057	0.0048 ±0.0019	193±30	103±13	137±6	43/41/42	6.4
Daidzein	0.99/0.986/0.982	0.005-0.5/0.005-0.5/0.0015-0.5	0.025±0.0079	0.015±0.0052	0.0083±0.002	142±27	118±18	139±34	101/102/103	5.2
Enterodiol	0.993/0.997/0.996	0.015-0.5/0.005-0.5/0.0015-0.5	0.18±0.048	0.0045 ±0.0019	0.0014 ±0.000099	112±5	91±8	105±13	20/27/26	5.2
Enterolactone	0.999/0.999/0.998	0.06-20	1.1±0.11	0.17±0.038	0.17±0.11	114±9	84±13	115±12	77/77/78	6.8
Equol	0.999/0.998/0.999	0.006-2/0.02-2/0.02-2	0.0076 ±0.0024	0.028±0.012	0.014±0.0054	132±16	85±20	114±13	65/68/67	6.5
Formononetin	0.9995/0.998/0.998	0.00075-0.25	0.0028 ±0.00052	0.001 ±0.00037	0.00067 ±0.0005	109±9	84±20	76±4	27/27/27	7.9
Genistein	0.992/0.989/0.985	0.005-0.5/0.015-0.5/0.005-0.5	0.011±0.0033	0.019±0.011	0.019±0.0019	154±26	90±17	129±20	40/43/42	6.4
Glycitein	0.992/0.994/0.998	0.015-5/0.05-5/0.0015-0.5	0.13±0.0068	0.057±0.012	0.0023 ±0.0003	55±24	162±55	162±70	51/54/54	5.4
Isoxanthohumol	0.992/0.993/0.996	0.001-0.1/0.003-0.1/0.001-0.1	0.0072 ±0.00036	0.011±0.0032	0.0095 ±0.0036	48±8	50±21	55±7	42/41/42	8.4
Matairesinol	0.997/0.996/0.999	0.05-5/0.05-5/0.15-5	0.65±0.096	0.11±0.012	0.13±0.045	162±41	145±51	149±29	68/68/68	6.5
Resveratrol	0.995/0.991/0.998	1.5-150/0.45-45/0.45-150	2.2±0.48	0.92±0.38	0.3±0.053	201±78	116±85	381±224	70/74/71	5
Xanthohumol	0.941/0.895/0.98	0.03-10/0.1-10/0.1-3	0.052±0.02	0.14±0.024	0.22±0.21	16±9	34±14	6±3	81/78/82	14.7
Mycoestrogens and metabolites										
Alternariol	0.999/0.999/0.999	0.1-10	0.16±0.029	0.14±0.093	0.076±0.051	138±19	81±21	102±13	73/72/71	6.8
Alternariol monomethyl ether	0.997/0.997/0.983	0.015-0.5	0.0077±0.003	0.011±0.0022	0.019±0.0088	97±6	57±16	20±1	24/25/23	11.4
α-Zearalanol (α-ZAL)	0.9995/0.999/ 0.993	0.05-5/0.015-5/0.05-5	0.1±0.038	0.068±0.031	0.092±0.019	106±11	88±12	54±4	28/28/28	8.8-8.9
β-Zearalanol (β-ZAL)	0.999/0.999/0.998	0.015-5/0.05-5/0.05-5	0.13±0.052	0.06±0.025	0.053±0.024	113±11	98±5	84±3	27/28/28	7.5
α-Zearalenol (α-ZEL)	0.995/0.993/0.99	0.02-0.2/0.02-0.2/0.06-0.2	0.012±0.0025	0.0084 ±0.0037	0.063±0.034	104±6	71±18	41±7	94/99/84	9.2
β-Zearalenol (β-ZEL)	0.999/0.999/0.997	0.1-10	0.4±0.08	0.24±0.11	0.16±0.053	114±10	90±7	77±4	45/45/45	7.7
α-Zearalenol-14-glucuronide (α-ZEL-14-GlcA)	n.d./0.997/0.997	n.d./0.45-1.5/0.045-1.5	n.d.	0.66±0.16	0.02±0.0025	n.d.	140±23	132±21	n.d./89/90	n.d./3.8-3.9/3.9-4.2
β-Zearalenol-14-glucuronide (β-ZEL-14-GlcA)	n.d./n.d./0.997	n.d./n.d./0.045-1.5	n.d.	0.42±0.29	0.04±0.015	n.d.	73±67	169±46	n.d./72/91	n.d./3.5-3.6/3.6-3.8

Compound	Regression coefficient R^2	Calibration range [ng/mL]	LOQ ± s [ng/mL]			SSE ± s [%]			Ion ratio	Retention time [min]
	U/S/M	U/S/M	U	S	M	U	S	M	U/S/M	U/S/M
Zearalanone (ZAN)	0.999/0.996/0.992	0.09-3	0.08±0.045	0.2±0.1	0.12±0.075	98±6	71±19	37±5	69/68/72	11.5
Zearalenone (ZEN)	0.998/0.996/0.989	0.009-3/0.03-3/0.09-3	0.025±0.006	0.032 ±0.00025	0.086±0.025	98±7	53±7	35±6	76/77/76	11.7
Zearalenone-14-glucuronide (ZEN-14-GlcA)	n.d./0.996/0.996	n.d./0.5-5/0.15-5	n.d.	1.2±0.38	0.093±0.031	n.d.	155±12	143±33	n.d./83/83	n.d./4.1-4.2/4.3-4.5
Zearalenone-14-sulfate (ZEN-14-sulfate)	0.999/0.999/0.998	0.015-1.5/0.015-1.5/0.0045-1.5	0.15±0.047	0.021±0.0037	0.015±0.0046	156±20	122±11	102±6	14/14/14	5.3/5.1-5.3/5.3-5.6
Personal care product ingredients, pharmaceuticals and metabolites										
Benzophenone 1	0.999/0.997/0.994	0.006-2	0.0054±0.001	0.017±0.0095	0.012±0.003	106±3	79±28	65±2	99/98/99	9
Benzophenone 2	0.999/0.995/0.999	0.015-1.5/0.0045-1.5/0.0045-1.5	0.018 ±0.00069	0.027±0.022	0.0096 ±0.0065	149±48	108±7	152±53	59/61/62	5.5
Benzylparaben	0.999/0.998/0.993	0.0045-0.15	0.00055 ±0.0003	0.0011 ±0.00055	0.00058 ±0.00024	101±4	70±20	46±1	75/75/73	10.5
Butylparaben	0.999/0.999/0.987	0.003-1/0.01-1/0.003-1	0.0072 ±0.00065	0.0096 ±0.0021	0.0099 ±0.0055	104±1	64±0	60±1	30/30/30	10.1
Ethylparaben	0.998/0.996/0.994	0.003-1/0.003-1/0.01-1	0.0039 ±0.00086	0.019±0.0026	0.041±0.012	128±4	108±6	110±3	65/66/67	6.1
Isobutylparaben	0.999/0.998/0.996	0.01-1/0.01-1/0.003-1	0.0083 ±0.0014	0.0073 ±0.0019	0.0086 ±0.0041	103±3	76±18	63±1	37/36/37	9.9
Methylparaben	0.998/0.994/0.992	0.0075-2.5	0.048±0.0083	0.035±0.0031	0.028±0.01	137±5	121±7	117±7	38/39/40	4.9
Propylparaben	0.999/0.997/0.992	0.006-2	0.011±0.003	0.0081 ±0.0069	0.01±0.0011	111±2	88±4	92±3	34/34/33	7.8
Ethinylestradiol	0.999/0.999/0.993	0.1-10/0.1-10/0.3-10	0.04±0.018	0.12±0.09	0.21±0.1	102±6	65±3	42±3	64/66/64	10.2
3-Benzylidencamphor (3-BC)	0.983/0.967/0.985	4.5-450/4.5-1500/ 4.5-450	0.3±0.1	2±0.95	6.1±1.4	61±6	17±3	4±1	69/73/73	15.2
4-methylbenzylidencamphor (4-MBC)	0.987/0.936/0.979	0.45-150/0.45-150/1.5-150	0.43±0.21	0.41±0.13	14±2.2	49±6	37±6	4±4	71/73/71	15.5
Octyl methoxycinnamate (OMC)	0.965/0.972/n.d.	200-600/60-200; 200-600; 600-2000/n.d.	160±120	260±240	730±270	9±1	90±79	n.d.	18/28/0	16.1
p-Hydroxybenzoic acid (pOHBA)	n.d./0.988/0.995	1.5-500/5-500/1.5-500	n.d.	9.8±11	7.6±12	n.d.	531±109	403±283	4/4/5	0.7-2.4/0.7-1.4/0.9-2.7
Triclosan	0.997/0.97/0.985	0.03-10/0.03-10/0.3-10	0.035±0.028	0.048±0.042	1.0±0.61	64±7	24±6	2±0.3	no qualifier	14.9
Phytotoxins										
Anisodamine	0.993/0.997/0.996	0.05-0.5/0.005-0.5/0.0015-0.5	0.066±0.012	0.008±0.0042	0.001 ±0.00033	35±11	98±6	95±5	15/12/12	3.6/3.6-3.8/3.2-3.5
Aristolochic acid I [*]	0.984/0.994/0.993	0.3-10/0.3-10/0.1-10	0.33±0.1	0.24±0.18	0.44±0.031	37±13	75±20	24±7	44/43/45	5.3-5.6/4.7-5.2/5.3- after 6*
Aristolactam I	0.997/0.994/0.987	0.015-5/0.05-5/0.15-5	0.016±0.002	0.019±0.0065	0.2±0.016	46±8	44±7	3±0.3	41/41/41	10.5
Jacobine	0.972/0.995/0.998	0.075-2.5/0.075-2.5/0.025-2.5	0.4±0.055	0.042±0.024	0.035±0.0086	28±10	100±8	94±8	102/91/92	3.5-3.7/3.6-4.4/3.3-3.6
Jacobine-N-oxide	0.994/0.99/0.997	0.05-0.5/0.015-0.5/0.0015-0.5	0.061±0.004	0.0086 ±0.0035	0.002 ±0.00039	16±5	75±17	87±2	43/42/40	3.1
Riddelliin	0.989/0.996/0.998	0.3-3/0.09-3/0.03-3	1.2±0.18	0.18±0.057	0.12±0.047	30±13	104±13	95±7	74/76/77	3.4/3.4-3.8/3.1-3.3
Riddelliin-N-oxide	0.995/0.997/0.996	0.6-2/0.06-2/0.006-2	1±0.13	0.04±0.0013	0.021±0.0045	17±6	84±10	86±4	92/88/87	3.1
Scopolamine	0.992/0.998/0.999	0.00045-0.15	0.0025 ±0.00012	0.00042 ±0.00032	0.00014 ±0.000062	32±10	101±7	98±4	85/92/90	3.6-3.7/3.7-4.2/3.4-3.6
Disinfection by-products										
Bromoacetic acid	n.d./0.985/n.d.	n.d./2-200/n.d.	n.d.	79±36	n.d.	n.d.	2±1.6	n.d.	no qualifier	n.d./0.7-0.9/0.7
Dibromoacetic acid	0.993/0.998/0.998	4.5-150/0.45-150/0.45-150	32±4.5	1.8±0.95	2.7±1.4	35±6	80±23	93±18	15/18/17	0.9/0.9-1/0.9-1.2
Dichloroacetic acid	0.996/0.996/0.992	22.5-750/2.25-750/7.5-750	60±3.6	10±5.2	6.6±4.4	29±3	32±4	74±9	63/67/57	0.8/0.8/0.8-1

[*] Data for aristolochic acid I in breast milk stem from two evaluated validations only as a retention time shift outside the programmed sMRM window was observed in the last validation run

Compound	Regression coefficient R^2	Calibration range [ng/mL]	LOQ ± s [ng/mL]			SSE ± s [%]			Ion ratio	Retention time [min]
	U/S/M	U/S/M	U	S	M	U	S	M	U/S/M	U/S/M
Food processing by-products										
Acrylamide	0.987/0.998/0.995	30-1000/10-1000/10-1000	92±34	5.3±1.5	22±12	23±3	56±5	73±11	57/60/57	0.9-1
5-Hydroxymethylfurfural (HMF)	0.991/0.992/0.991	3.5-350/1.05-350/1.05-350	29±18	16±23	5.6±3.2	23±11	91±13	84±17	34/34/33	2.4
5-Hydroxymethyl-2-furanoic acid (HMFA)	n.d./0.993/0.994	n.d./60-2000/6-2000	n.d.	150±20	22±16	n.d.	2±0.4	57±29	27/36/26	0.7/0.7/0.7-1.1
N-Nitosodimethylamine (NDMA)	0.996/0.999/0.999	30-3000/90-3000/ 30-3000	240±280	230±210	170±140	76±4	103±4	105±2	31/33/35; 8/8/9**	1.4
PhIP	0.991/0.994/0.999	0.003-1/0.01-1/0.003-1	0.011±0.004	0.01±0.00034	0.0042±0.0013	136±6	140±32	190±60	15/16/15	5.3/5.3/5.1-5.3
Air pollutants										
Cotinine	0.989/0.765/0.994	0.045-15/0.45-15/0.045-15	0.89±0.41	0.054±0.043	0.11±0.079	20±8	n.d.	96±3	32/32/32	3.1
Trans-3-hydroxy cotinine	0.987/0.979/0.993	0.15-5/0.05-5/0.015-5	2.3±0.19	0.065±0.033	0.025±0.011	20±9	66±27	95±14	95/89/90	2.7/2.7/2.2-2.7
1-Hydroxy pyrene	0.989/0.996/0.991	0.06-20/0.2-20/0.2-6	0.19±0.059	0.22±0.077	1.3±0.69	128±42	48±22	10±7	12/13/11	14.2
3-Hydroxy phenanthrene	0.997/0.997/0.992	0.015-1.5/0.045-1.5/0.045-1.5	0.021±0.011	0.032±0.0055	0.053±0.015	111±6	56±7	22±4	2/2/2	11.4-11.5

** A second qualifier transition was included for NDMA

Outcomes of method validation for all investigated analytes in the three matrices, including parameters outside validation limits, are displayed in Table 15.

Table 15: Summary of validation outcomes. Listed parameters did not meet the set validation criteria. LL and HL describe parameters of the low- and high level spiking experiment respectively. "Calibration" is listed as a parameter outside validation limits if calibration was not possible using at least two concentrations levels. Successfully validated compounds are marked with (✓).

Compound	Validation outcomes and parameters outside validation limits			Comment
	Urine	Serum	Breast milk	
Plasticizer/plastic components				
Bisphenol A (BPA)	✓	✓	✓	
Bisphenol AF (BPAF)	✓	✓	R_E LL/HL	
Bisphenol B (BPB)	RSD_r LL/HL	✓	RSD_R LL, RSD_r LL	
Bisphenol C (BPC)	RSD_r LL	✓	R_E HL	
Bisphenol F (BPF)	RSD_r LL	✓	✓	
Bisphenol S (BPS)	RSD_R LL, RSD_r LL	RSD_R LL	✓	
Mono-n-butyl phthalate (MBP)	✓	R_E LL/HL, RSD_r LL/HL	R_E HL	
Mono-2-ethylhexyl phthalate (MEHP)	R_E LL	R^2	(R_E LL/HL, RSD_R LL)*	Retention time shift out of MRM window in 2 LL samples in urine; high matrix contamination did not allow for linear regression in serum; retention time shift out of MRM window of all samples and standards in third breast milk validation
N-butylbenzenesulfonamide	RSD_r LL	✓	RSD_R LL/HL, RSD_r LL	
Benzyl butyl phthalate	R_E HL, RSD_r LL/HL	R_E HL	R^2	High matrix contamination did not allow for linear regression in breast milk
Dibutyl phthalate	R_E LL/HL, RSD_R LL/HL, RSD_r HL	R_E HL, RSD_R LL	R^2	Could not differentiate LL spike from matrix contamination in urine; high matrix contamination did not allow for linear regression in breast milk
Tetrabromobisphenol A (TBPA)	✓	R_E HL, RSD_R LL/HL	Calibration	Sensitivity issues hindered calibration in breast milk
Perfluorinated alkylated substances				
Perfluorooctanoic acid (PFOA)	✓	✓	✓	
Perfluorooctanesulfonic acid (PFOS)	RSD_R LL	R_E LL	(✓)*	Could not differentiate LL spike from matrix contamination in serum; retention time shift outside of MRM window in third breastmilk validation
Industrial side products and pesticides				
2-Naphthol	RSD_r HL	✓	RSD_R LL	
Methiocarb	R_E LL	R_E LL	R_E LL, RSD_r LL	High baseline noise during second validation in all matrices: LL could not be detected
Prochloraz	R_E LL	R_E LL	Calibration	High baseline noise during second validation in all matrices: LL could not be detected in urine/serum, LL/HL could not be detected in breast milk
2-tert-Butylphenol (2-tert-BP)	R_E LL/HL	R_E LL/HL, RSD_R LL/HL	R_E LL/HL, RSD_R LL/HL, RSD_r HL	
4-Octylphenol (4-OP)	R_E LL/HL	RSD_R LL/HL	R^2	

* Only two validation sequences were considered for MEHP and PFOS in breast milk

Compound	Validation outcomes and parameters outside validation limits			Comment
	Urine	Serum	Breast milk	
4-tert-Octylphenol (4-tert-OP)	R_E LL/HL	R_E HL	Calibration	
Fenarimol	✓	RSD_r LL	R_E LL/HL	
Nonylphenol	R_E LL/HL	R_E LL, RSD_R LL	R^2	Could not differentiate HL spike from matrix contamination in urine; high matrix contamination did not allow for linear regression in breast milk
Endogenous estrogens				
Estrone (E1)	R_E LL, RSD_r LL	✓	R_E LL/HL RSD_R HL	
Estradiol (E2)	✓	RSD_R LL	R_E LL	
Estradiol-17-glucuronide (E2-17-GlcA)	Calibration	Calibration	RSD_R LL, RSD_r LL	Sensitivity only allowed calibration in breast milk
Estradiol-3-sulfate (E2-3-sulfate)	R_E LL, RSD_r HL	✓	RSD_R LL	
Estriol (E3)	RSD_r LL	RSD_r HL	RSD_R LL	
16-Epiestriol (16EpiE3)	RSD_r HL	✓	R_E HL	
16-α-Hydroxyestrone (16OHE1)	RSD_r LL	✓	RSD_r LL	
17-Epiestriol (17EpiE3)	✓	✓	R_E HL	
2-Methoxy estrone (2MeOE1)	RSD_r LL	RSD_r LL	R_E LL/HL, RSD_R LL	
2-Methoxy estradiol(2MeOE2)	✓	✓	R_E LL/HL	
4-Methoxy estrone (4MeOE1)	✓	✓	R_E LL	
4-Methoxy estradiol (4MeOE2)	✓	✓	R_E LL, RSD_R LL, RSD_r LL	
4-Hydroxy estrone (4OHE1)	✓	Calibration	R_E LL/HL	Sensitivity issues hindered calibration in serum
Phytoestrogens and metabolites				
8-Prenylnaringenin	RSD_r LL	✓	R_E LL/HL	
Coumestrol	✓	✓	✓	
Daidzein	RSD_r LL/HL	RSD_R LL	RE LL	Could not differentiate LL spike from matrix contamination in breast milk
Enterodiol	✓	✓	R_E LL/HL, RSD_r LL/HL	
Enterolactone	RSD_r LL	✓	RSD_R LL	
Equol	RSD_r LL	✓	✓	
Formononetin	✓	✓	✓	
Genistein	✓	✓	R_E LL, RSD_R LL, RSD_r LL	
Glycitein	R_E LL	RSD_r HL	✓	
Isoxanthohumol	✓	✓	R_E LL	
Matairesinol	RSD_r LL	✓	✓	
Resveratrol	✓	R_E HL	R_E LL/HL RSD_R LL/HL	
Xanthohumol	✓	✓	R_E LL/HL	
Mycoestrogens and metabolites				
Alternariol	✓	✓	R_E HL	
Alternariol monomethyl ether	✓	✓	R_E LL	Could not differentiate LL spike from matrix contamination in breast milk
α-Zearalanol (α-ZAL)	✓	✓	R_E LL/HL	
β-Zearalanol (β-ZAL)	RSD_r LL	✓	R_E HL	
α-Zearalenol (α-ZEL)	R_E LL	R_E LL	R_E LL/HL, RSD_R HL	
β-Zearalenol (β-ZEL)	RSD_r LL	✓	R_E HL	

Compound	Validation outcomes and parameters outside validation limits			Comment
	Urine	Serum	Breast milk	
α-Zearalenol-14-glucuronide (α-ZEL-14-GlcA)	Calibration	R_E LL	✓	Sensitivity issues hindered calibration in urine
β-Zearalenol-14-glucuronide (β-ZEL-14-GlcA)	Calibration	Calibration	✓	Sensitivity issues hindered calibration in breast milk
Zearalanone (ZAN)	RSD_r LL/HL	✓	R_E LL	
Zearalenone (ZEN)	✓	✓	R_E LL	
Zearalenone-14-glucuronide (ZEN-14-GlcA)	Calibration	R_E LL, RSD_r HL	✓	Sensitivity issues hindered calibration in urine
Zearalenone-14-sulfate (ZEN-14-sulfate)	RSD_r HL	✓	R_E LL/HL, RSD_R LL/HL	
Personal care product ingredients, pharmaceuticals and metabolites				
Benzophenone 1	✓	RSD_r LL	✓	
Benzophenone 2	✓	RSD_r LL/HL	✓	
Benzylparaben	✓	✓	RSD_r LL	
Butylparaben	✓	✓	R_E LL, RSD_R LL	
Ethylparaben	✓	✓	RSD_R LL	
Isobutylparaben	✓	✓	R_E LL, RSD_r LL	
Methylparaben	✓	✓	RSD_R LL	
Propylparaben	✓	✓	RSD_R LL	
Ethinylestradiol	✓	RSD_r LL	R_E LL, R_E HL	
3-Benzylidencamphor (3-BC)	R_E LL/HL	R_E LL/HL	R_E LL, R_E HL	Could not differentiate LL/HL spikes from matrix contamination in urine/breast milk
4-methylbenzylidencamphor (4-MBC)	R_E LL/HL	R_E LL/HL	R_E LL/HL, RSD_R HL	Could not differentiate LL/HL spikes from matrix contamination in urine/breast milk
Octyl methoxycinnamate (OMC)	R^2	R^2	R^2	No linear regression using three concentration levels possible in any matrix
p-Hydroxybenzoic acid (pOHBA)	R^2	R_E LL, RSD_r LL, RSD_r LL, RT	R_E LL/HL, RT	No linear regression in one validation in urine possible due to high matrix contamination
Triclosan	R_E HL, RSD_r LL/HL	Selectivity	R_E LL/HL, RSD_R HL	1 transition only
Phytotoxins				
Anisodamine	R_E LL	✓	R_E LL, RSD_R LL, RSD_r LL/HL	
Aristolochic acid I	✓	RSD_R LL	(R_E LL/HL)*	Retention time shift out of MRM window in third breast milk validation
Aristolactam I	✓	✓	R_E LL, RSD_R HL	
Jacobine	R_E LL	✓	R_E LL/HL, RSD_R LL/HL	
Jacobine-N-oxide	R_E LL	✓	R_E LL/HL, RSD_R LL/HL	
Riddelliin	R_E LL	✓	R_E LL, RSD_r HL	
Riddelliin-N-oxide	R_E LL	✓	R_E LL/HL, RSD_R LL/HL, RSD_r HL	
Scopolamine	✓	✓	RSD_r HL	

* Only two validation sequences were considered for aristolochic acid I in breast milk

Compound	Validation outcomes and parameters outside validation limits			Comment
	Urine	Serum	Breast milk	
Disinfection by-products				
Bromoacetic acid	Calibration	R_E LL, R_E HL, RSD_r HL, RT	Calibration	Early elution did not allow selective peak annotation in urine/breast milk
Dibromoacetic acid	R_E LL, RT	RT	R_E LL/HL, RSD_R LL/HL, RT	
Dichloroacetic acid	R_E LL, RT	RT	R_E LL/HL, RSD_R LL/HL, RSD_r HL, RT	
Food processing by-products				
Acrylamide	R_E LL, RT	R_E LL/HL, RT	R_E LL/HL, RSD_R LL/HL, RSD_r HL, RT	No linear regression in one validation in urine possible due to high matrix contamination
5-Hydroxymethylfurfural (HMF)	R_E LL/HL, RSD_r HL	R_E LL/HL	R_E LL/HL, RSD_R HL	
5-Hydroxymethyl-2-furanoic acid (HMFA)	R^2	RSD_r LL, RT	R_E LL/HL, RSD_r HL, RT	
N-Nitosodimethylamine (NDMA)	R_E LL/HL	R_E LL/HL	R_E LL/HL	
PhIP	✓	✓	✓	
Air pollutants				
Cotinine	✓	R^2	R_E LL/HL	Could not differentiate LL/HL spike from matrix contamination in breast milk; high matrix contamination did not allow for linear regression in serum
Trans-3-hydroxy cotinine	R_E LL	R_E LL/HL	R_E LL/HL	Could not differentiate LL spike in urine-, LL/HL spike in serum-, LL/HL spike in breast milk from matrix contamination
1-Hydroxy pyrene	✓	✓	R_E HL, RSD_R LL/HL	
3-Hydroxy phenanthrene	✓	✓	R_E LL/HL, RSD_R HL	

5.5. Unknown biological samples

Analytes detected and quantified in the urine samples are summarised in Tables 16 and 17. HMFA was likely detected in all samples, but certainty cannot be assured since its low retention did not allow for accurate peak identification and separation from any interferences in the samples. Moreover, it was not quantifiable as matrix contamination of the urine used for method development did not allow for linear calibration. In addition to its high contamination not allowing for linear regression, high variance in retention times and high matrix interference hindered peak identification in the case of *p*OHBA. MEHP was not detected in the urine samples as its retention time shifted outside of the programmed MRM detection window for this sequence of measurements (see discussion). The presence of triclosan was merely determined with its single available transition.

Table 16: Analysis of unknown urine samples in two biological replicates (Table I). Concentrations are given in ng/mL. Analytes that were detected below the LOQ are displayed as (<LOQ).

Compound [ng/mL]	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2	E_1	E_2	F_1	F_2	G_1	G_2	H_1	H_1
Plasticizer/plastic components																
Bisphenol A (BPA)			0.26									<LOQ	<LOQ			
Bisphenol S (BPS)					<LOQ	<LOQ										
Mono-n-butyl phthalate (MBP)							0.80	0.89	<LOQ	<LOQ	<LOQ	<LOQ	0.50	0.53		
N-butylbenzenesulfonamide		<LOQ	2.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ			<LOQ	<LOQ				
Benzyl butyl phthalate	0.39	0.25	1.30	0.48	0.32	0.25	0.39	0.28	<LOQ	<LOQ	<LOQ	<LOQ				
Dibutyl phthalate	21	14	17	32	16	11	18	11	11	12	10	<LOQ				
Tetrabrombisphenol A (TBPA)																
Perfluorinated alkylated substances																
Perfluorooctanoic acid (PFOA)							<LOQ	<LOQ								
Perfluorooctanesulfonic acid (PFOS)			<LOQ								<LOQ	<LOQ				
Industrial side products and pesticides																
2-naphthol	<LOQ	0.031	0.15	0.031	0.053	0.065	0.025	0.044	0.069	0.073	0.042	0.055	0.70	0.71	0.040	0.041
Prochloraz																
Nonylphenol	20	31	25	27	31	32	25	22	21	25	21	18	16	20	17	17
Endogenous estrogens																
Estrone (E1)					0.027	0.0056		<LOQ			0.013	0.018	0.036	0.066		
Estradiol (E2)																
Estriol (E3)																
16-epiestriol (16EpiE3)																
16-α-hydroxyestrone (16OHE1)																
17-epiestriol (17EpiE3)																
2-methoxy estrone (2MeOE1)																
2-methoxy estradiol(2MeOE2)																
4-methoxy estrone (4MeOE1)																
4-hydroxy estrone (4OHE1)									0.029	0.031			0.20	0.19		

Compound [ng/mL]	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2	E_1	E_2	F_1	F_2	G_1	G_2	H_1	H_1
Phytoestrogens and metabolites																
8-prenylnaringenin													<LOQ	<LOQ	<LOQ	<LOQ
Coumestrol	0.025	0.024			0.36	0.77	0.012	0.021	0.012	0.016	<LOQ	<LOQ	0.17	0.061	<LOQ	0.019
Daidzein	2.6	2.8	0.29	0.35	73	76	0.095	0.093	0.11	0.13	0.35	0.36	3.3	2.8	0.16	0.18
Enterodiol	0.82	0.80	0.31	0.43		<LOQ	0.22	0.25			<LOQ	<LOQ	3.7	3.6	0.34	0.34
Enterolactone			<LOQ	<LOQ			2.1	2.3			1.5	1.5	2.7	2.7	2.7	2.5
Equol											0.014	0.015	0.18	0.17	0.013	0.0092
Formononetin	0.080	0.087			0.044	0.054	0.0035	0.0041	0.0039	0.0037	<LOQ		0.41	0.35	0.028	0.026
Genistein (GEN)	0.83	1.4	0.25	0.32	10	9.1	0.15	0.11	0.21	0.23	0.048	0.051	7.3	7.0	0.035	0.044
Glycitein			<LOQ	<LOQ	6.5	8.1							0.16	<LOQ		
Isoxanthohumol											0.023	0.020		<LOQ	0.061	0.060
Resveratrol																
Mycoestrogens and metabolites																
Alternariol						<LOQ							0.21	<LOQ	<LOQ	<LOQ
Alternariol monomethyl ether					<LOQ	0.021			<LOQ				0.036	0.012	<LOQ	0.012
Personal care product ingredients, pharmaceuticals and metabolites																
Benzophenone 1		<LOQ	0.010	0.010	0.011	0.011	0.015	0.011	0.048	0.053	0.35	0.37	0.29	0.27	0.020	0.013
Benzylparaben					<LOQ						0.0010	0.0013		<LOQ		
Ethylparaben (EP)																
Methylparaben (MP)					0.15	0.15	<LOQ	<LOQ	0.051	0.052	0.76	0.73	0.20	0.23	0.079	0.085
Propylparaben (PP)	0.014	<LOQ	0.020	0.013	0.028	0.022	0.026	0.027	0.015	0.015	0.13	0.13	0.019	0.025	0.020	0.020
Triclosan	<LOQ	<LOQ	<LOQ	<LOQ			<LOQ	<LOQ	<LOQ	.			0.070	0.065		
Air pollutants																
Cotinine	<LOQ	<LOQ														

Table 17: Analysis of unknown urine samples in two biological replicates (Table II). Concentrations are displayed in ng/mL. Analytes that were detected below the LOQ are displayed as (<LOQ).

Compound {ng/mL]	I_1	I_2	J_1	J_2	K_1	K_2	L_1	L_2	M_1	M_2	N_1	N_2	O_1	O_2	Z_1	Z_2
Plasticizer/plastic components																
Bisphenol A (BPA)	<LOQ		<LOQ			<LOQ		<LOQ	0.48	<LOQ		<LOQ			<LOQ	
Bisphenol S (BPS)			<LOQ	<LOQ	0.13	0.14			<LOQ	<LOQ					0.055	0.050
Mono-n-butyl phthalate (MBP)					0.35	<LOQ					0.69	0.89				
N-butylbenzenesulfonamide	<LOQ	<LOQ	<LOQ	1.3			<LOQ	<LOQ	3.8	3.4	<LOQ	<LOQ	<LOQ			
Benzyl butyl phthalate	0.59	0.47	0.28		0.29	0.28	0.29	0.33	0.49	0.65	0.39	0.28	0.35	0.36	0.27	<LOQ
Dibutyl phthalate	21	23	16	<LOQ	20	11	12	25	27	35	22	11	17	13	<LOQ	<LOQ
Tetrabrombisphenol A(TBPA)			3.6													
Perfluorinated alkylated substances																
Perfluorooctanoic acid (PFOA)															<LOQ	<LOQ
Perfluorooctanesulfonic acid (PFOS)	<LOQ	<LOQ		<LOQ									<LOQ			
Industrial side products and pesticides																
2-naphthol	0.066	0.070	0.086	0.072	0.097	0.12	0.055	0.064	0.088	0.064	0.066	0.063	0.079	0.084	0.047	0.045
Prochloraz			<LOQ	0.0014			<LOQ	<LOQ			0.00065					
Nonylphenol	19	22	35	22	11	11	29	21	21	22	20	17	17	14	20	17
Endogenous estrogens																
Estrone (E1)	<LOQ	0.0092	0.19	0.10	0.11	0.10					<LOQ	<LOQ	0.038	0.044	8.5	7.9
Estradiol (E2)			0.067	0.14	0.10	0.068									2.3	2.4
Estriol (E3)					<LOQ	<LOQ									37	35
16-epiestriol (16EpiE3)															3.6	3.3
16-α-hydroxyestrone (16OHE1)															12	11
17-epiestriol (17EpiE3)															1.2	1.2
2-methoxy estrone (2MeOE1)															0.42	0.39
2-methoxy estradiol(2MeOE2)															0.034	0.035
4-methoxy estrone (4MeOE1)															<LOQ	<LOQ
4-hydroxy estrone (4OHE1)			0.32	0.27	0.10	0.10					0.031	0.028	0.052	0.052	1.8	1.8
Phytoestrogens and metabolites																
8-prenylnaringenin			0.058	<LOQ	<LOQ	<LOQ	4.50									
Coumestrol			0.10	<LOQ			0.024	0.011			0.76	1.3	1.7	2.3	0.018	0.032
Daidzein	0.19	0.12	181	151	0.62	0.63	12	11	0.75	0.71	4.1	4.3	12	13	148	152
Enterodiol			2.6	2.6	0.48	0.50	0.35	0.27	<LOQ	<LOQ	0.26	0.25	1.00	0.91	0.27	0.24
Enterolactone	<LOQ	<LOQ	63	53	11	11	3.0	2.6	<LOQ	<LOQ	1.4	1.4	<LOQ	<LOQ	14	13
Equol			0.15	0.10	0.93	0.91			0.019	0.019	0.28	0.26	0.055	0.049	0.025	0.022
Formononetin			0.041	0.016	0.0035	0.0033					0.0071	0.0091	0.0097	0.0085	0.014	0.013
Genistein (GEN)			89	88	0.22	0.16	0.92	0.83	0.11	0.10	0.84	0.89	2.5	2.4	82	87
Glycitein			31	17			0.92	0.82	<LOQ	<LOQ	0.28	0.31	0.58	0.65	5.6	5.8
Isoxanthohumol							<LOQ	<LOQ								
Resveratrol													6.00	8.00		
Mycoestrogens and metabolites																
Alternariol			0.27	<LOQ							<LOQ	<LOQ	<LOQ	<LOQ		<LOQ
Alternariol monomethyl ether			0.060								<LOQ	0.017	<LOQ	<LOQ	0.016	0.022

Compound {ng/mL]	I_1	I_2	J_1	J_2	K_1	K_2	L_1	L_2	M_1	M_2	N_1	N_2	O_1	O_2	Z_1	Z_2
Personal care product ingredients, pharmaceuticals and metabolites																
Benzophenone 1	0.011	0.0076	0.080	0.064	0.054	0.050	0.039	0.038	0.0065	<LOQ	0.017	0.019	0.025	0.016	0.086	0.078
Benzylparaben			0.0033	0.0017												
Ethylparaben (EP)					1.0	1.0			0.015	0.022						
Methylparaben (MP)			0.55	0.54	0.087	0.071	<LOQ	<LOQ	0.11	0.13	0.10	0.097	0.11	0.098	0.39	0.38
Propylparaben (PP)	<LOQ	<LOQ	0.094	0.085	0.030	0.019	<LOQ	<LOQ	0.026	0.038	0.032	0.039	0.021	0.019	0.013	0.015
Triclosan			0.068	<LOQ	0.073	<LOQ					<LOQ	<LOQ	<LOQ	<LOQ	0.075	0.081
Air pollutants																
Cotinine									<LOQ	<LOQ						

Analytes detected and quantified in the baby serum samples and two adult control samples are summarised in Table 18. MEHP and cotinine were detected, however they were not quantifiable as matrix contamination of the serum used for method development did not allow for linear calibration. High variance in retention times and high matrix interference hindered peak identification in the case of *p*OHBA. Because of its lacking retention, the presence of HMFA had to be confirmed with an additional Enhanced Product Ion Scan (EPI). For MPB, peaks at the matching RT were only observed for three samples. However, interferences in the qualifier transition were observed for two of the three samples with peak shapes differing from the standard in all three quantified samples. Thus the presence of MBP was questionable. Unfortunately, additional EPI scans to confirm its presence were not possible due to insufficient sample volume. Hence it was decided against declaring the identification of MBP. Similarly, no sample volume was available to further confirm the presence of TBPA in sample PB 57. Attempts to confirm the presence of triclosan via its isotope pattern in additional enhanced Q1 scans (EMS) were not successful as the expected isotope pattern was not observed in a higher concentrated matrix matched standard.

Table 18: Analysis of baby serum and two adult control samples. Concentrations are given in ng/mL.
Analytes that were detected below the LOQ are displayed as (<LOQ). Analytes that were detected but could not be quantified because of lacking linearity are displayed as (det.).

Compound [ng/mL]	Ad 5	Ad 6	PB 24	PB 26	PB 27	PB 29	PB 30	PB 31	PB 33	PB 34	PB 36	PB 37	PB 41	PB 42	PB 44	PB 45	PB 49	PB 50	PB 52	PB 53	PB 54	PB 56	PB 57
Plasticizer/plastic components																							
Bisphenol A (BPA)	0.79	<LOQ	11	1.9	1.8	0.84	2.5	4.4	0.81	3.1	1.4	0.76	15	4.3	3.3	2.8	13	0.72	11	3.3	1.6	9.5	8.3
Bisphenol AF (BPAF)	<LOQ	<LOQ	<LOQ		<LOQ		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		<LOQ	<LOQ
Bisphenol F (BPF)									0.091									0.39					
Mono-2-ethylhexyl phthalate (MEHP)*			det*	det*	det*	det*	det*	det*	det*	det*	det*		det*	det*	det*	det*	det*	det*	det*	det*	det*	det*	det*
N-butylbenzenesulfonamide	23	12	46	20	12	4.7	19	39	38	52	39	13	40	38	26	11	44	30	17	36	2.2	28.	53
Benzyl butyl phthalate	1.2	1.5	1.2	1.9	1.3	2.1	1.7	1.3	2.0	2.7	1.7	2.5	1.1	<LOQ	1.1	0.95	1.6	1.3	<LOQ	1.1	<LOQ	<LOQ	1.1
Dibutyl phthalate	12	33	25	89	37	100	71	35	47	77	75	96	31	22	54	26	52	28	<LOQ	<LOQ	<LOQ	11	23
Tetrabrombisphenol A (TBPA)																							<LOQ
Perfluorinated alkylated substances																							
Perfluorooctanoic acid (PFOA)	2.6	5.4	12	0.68	6.0	0.35	2.6	0.92	3.7	2.3	0.96	1.5	8.0	8.7	1.6	2.4	1.7	1.3	2.3	0.87	0.50	1.8	2.1
Perfluorooctanesulfonic acid (PFOS)	16	49	11	0.61	4.8	<LOQ	1.1	2.5	2.2	2.4	0.92	1.2	8.1	9.3	1.7	2.4	8.3	1.6	2.2	2.9	0.34	3.7	4.0
Industrial side products and pesticides																							
2-naphthol	6.1	3.6	17	3.8	4.2	1.2	2.8	10	9.6	13	9.5	3.3	16	14	6.8	4.8	21	11	6.7	12	0.95	10	15
4-tert-octylphenol (4-tert-OP)	8.2	6.2	9.4	<LOQ	4.5		<LOQ	4.7	6.4	6.1	4.7	7.9	5.3	4.9	11	6.9	22	6.7	7.9	5.6	<LOQ	5.8	5.6
Nonylphenol	39	27	56	18	26	5.0	15	27	32	39	33	14	54	61	20	24	69	34	35	38	8.3	33	39
Endogenous estrogens																							
Estrone (E1)	0.040	0.073											0.058				0.016			0.010			
Estradiol (E2)	<LOQ	0.18																					

* No linear calibration possible for MEHP because of high matrix contamination

Compound [ng/mL]	Ad 5	Ad 6	PB 24	PB 26	PB 27	PB 29	PB 30	PB 31	PB 33	PB 34	PB 36	PB 37	PB 41	PB 42	PB 44	PB 45	PB 49	PB 50	PB 52	PB 53	PB 54	PB 56	PB 57
2-methoxy estrone (2MeOE1)		0.054																					
Phytoestrogens and metabolites																							
Coumestrol	0.0081	<LOQ	0.012					0.0092	0.015	0.0080	0.021		0.020	0.0084		0.013		0.0087	0.051		0.042		
Enterodiol	<LOQ	0.0067																					
Matairesinol						<LOQ		0.26						0.26								<LOQ	
Mycoestrogens and metabolites																							
Alternariol monomethyl ether		<LOQ	0.026					<LOQ				<LOQ	0.016	0.041	0.26	<LOQ	0.020	<LOQ				<LOQ	0.030
Personal care product ingredients, pharmaceuticals and metabolites																							
Benzophenone 1	<LOQ	<LOQ	<LOQ		0.024		0.024	0.022	<LOQ	<LOQ	<LOQ	<LOQ	0.017	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.031		<LOQ	<LOQ
Benzylparaben			<LOQ											<LOQ									
Butylparaben (BP)	<LOQ	<LOQ	<LOQ	0.33	<LOQ				0.013	<LOQ	<LOQ		0.10	<LOQ	0.016		<LOQ	<LOQ	<LOQ	<LOQ		<LOQ	<LOQ
Ethylparaben (EP)	0.069	0.034	0.13	0.058	0.049	0.024	0.057	0.11	0.19	0.13	0.13	0.025	0.12	0.10	0.17	0.040	0.12	0.062	0.26	0.10	0.11	0.090	0.17
Isobutylparaben	<LOQ	<LOQ	<LOQ	0.30						<LOQ				<LOQ		<LOQ	<LOQ					<LOQ	
Methylparaben (MP)	0.13	0.077	15	3.2	2.0	0.061	1.90	0.70	5.20	2.70	1.80	0.77	1.70	3.80	1.20	0.22	0.22	0.30	0.095	0.82	1.50	0.58	2.30
Propylparaben (PP)	0.042	0.017	2.90	0.096	0.096	0.016	0.060	0.12	0.065	0.12	0.069	0.020	0.23	0.25	0.094	0.026	0.29	0.065	0.084	0.095	0.049	0.13	0.14
Triclosan			<LOQ					<LOQ		<LOQ	<LOQ		<LOQ	<LOQ			<LOQ	<LOQ		<LOQ		<LOQ	<LOQ
Food processing by-products																							
5-Hydroxymethylfurfural (HMF)													4.4										
5-Hydroxymethyl-2-furanoic acid (HMFA)				232		<LOQ	230						2555	464		426	1186	211		186		240	195
Air pollutants																							
Cotinine*	det*	det*	det*	det*	det*	det*	det*	det*	det*	det*	det*		det*	det*	det*	det*	det*		det*	det*	det*	det*	det*

* No linear calibration possible for cotinine because of high matrix contamination

Analytes detected and quantified in the breast milk samples are summarised in Tables 19-23. Results of the 10 µL injections to confirm certain analytes are summarised in Table 35 (see Appendix). Contrary to method validation, BPA was not internally calibrated as IS recovery was too low to allow for sensible quantitation. High variance in retention times and high matrix interference hindered peak identification in the case of *p*OHBA. Prochloraz was quantified without additional extraction efficiency correction, since it could not be detected in the extraction experiments during method validation. Xanthohumol was detected but could not be quantified as no linear calibration using more than two standards was possible. The presence of prochloraz, PhIP and the phytotoxins was examined using further EPI scans.

Table 19: Analysis of breast milk samples of one individual spanning a period of 209 days (Table I). Concentrations are given in [ng/mL]. Analytes that were detected below the LOQ are described as (<LOQ) and analytes that were only detected in the 10 µL injections are described with (*).

Compound [ng/mL]	Day																
	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18
Plasticizer/plastic components																	
Bisphenol A (BPA)		<LOQ*															
Bisphenol F (BPF)																	
Bisphenol S (BPS)		<LOQ	<LOQ	<LOQ	<LOQ*	<LOQ			<LOQ		0.051		<LOQ	<LOQ			8
Mono-n-butyl phthalate (MBP)		0.47	<LOQ	0.28	0.3	1.4	0.98	<LOQ	0.3	<LOQ	0.41		2.4	0.24	0.34		<LOQ
N-butylbenzenesulfonamide	4.3	6	6.3	13	9.3	9.6	13	6.0	10	7.3	9.6	6.0	4.4	7.7	3.7	11	11
Perfluorinated alkylated substances																	
Perfluorooctanoic acid (PFOA)		<LOQ		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		<LOQ		<LOQ	<LOQ
Perfluorooctanesulfonic acid (PFOS)		0.027		0.025	<LOQ*	0.038	<LOQ	0.026	0.025	<LOQ	<LOQ*	<LOQ		0.038	<LOQ	0.026	<LOQ
Industrial side products and pesticides																	
2-naphthol	0.33	0.36	0.70	0.34	0.4	0.36	1.0	0.37	0.46	0.36	0.36	0.33	0.23	0.31	0.27	0.43	0.51
Prochloraz																	
Phytoestrogens and metabolites																	
8-prenylnaringenin	0.054			<LOQ		0.020*					0.022						<LOQ
Daidzein		<LOQ	0.032	0.020	<LOQ	<LOQ	<LOQ	0.022	0.10	0.048	0.021						0.046
Enterodiol							0.0083				0.018	0.011					
Enterolactone		<LOQ*									<LOQ*						
Glycitein	0.0030	<LOQ	0.0054	0.0054	0.0020*				0.0092	0.0078	<LOQ					<LOQ	0.0048
Isoxanthohumol		<LOQ*				<LOQ*		0.078			0.018			<LOQ*			0.027
Resveratrol						<LOQ											
Xanthohumol																	
Mycoestrogens and metabolites																	
Alternariol																	
Personal care product ingredients, pharmaceuticals and metabolites																	
Benzophenone 1	0.029	0.033	<LOQ	<LOQ	<LOQ	0.036	0.038	<LOQ	0.035	<LOQ	0.027	0.022	0.021	0.021	<LOQ	0.023	<LOQ
Benzophenone 2																	
Butylparaben (BP)						0.025*					0.036						
Ethylparaben (EP)	0.063	<LOQ	<LOQ	0.12	0.083	0.072	0.11	<LOQ	0.082	0.06	0.092	0.059	0.057	0.056	<LOQ	0.063	0.052
Methylparaben (MP)	0.069	0.075	0.098	0.33	1.1	0.35	0.24	0.12	0.19	0.17	0.22	0.13	0.15	0.12	0.099	0.17	0.14
Propylparaben (PP)	0.037	0.041	0.043	0.13	0.99	0.14	0.10	0.046	0.094	0.065	0.12	0.048	0.071	0.050	0.049	0.090	0.045
Phytotoxins																	
Anisodamine		0.031			0.0044						0.0083			0.0052			
Jacobine-N-oxide						<LOQ											
Riddelliin-N-oxide																	
Scopolamine											<LOQ						<LOQ
Food processing by-products																	
PhIP																	

Table 20: Analysis of breast milk samples of one individual spanning a period of 209 days (Table II). Concentrations are given in [ng/mL]. Analytes that were detected below the LOQ are described as (<LOQ) and analytes that were only detected in the 10 µL injections are described with (*). Analytes that were detected but could not be quantified because of lacking linearity are displayed as (det.).

Compound [ng/mL]	Day																
	19	20	21	23	24	25	27	29	30	31	32	33	35	36	37	38	40
Plasticizer/plastic components																	
Bisphenol A (BPA)						<LOQ											
Bisphenol F (BPF)																	
Bisphenol S (BPS)	<LOQ			<LOQ	0.012	<LOQ*	0.0091	<LOQ	0.0097		<LOQ	<LOQ	<LOQ	<LOQ		<LOQ	<LOQ
Mono-n-butyl phthalate (MBP)	<LOQ	0.25		0.23		0.36	1.1	1.4	6.4	0.21	0.35	<LOQ	0.25	<LOQ	0.30	0.54	0.25
N-butylbenzenesulfonamide	6.1	8.6	9.6	5.5	5.3	4.3	12	8.1	9.5	9.1	11	8.6	8.5	6.6	3.4	8.9	11
Perfluorinated alkylated substances																	
Perfluorooctanoic acid (PFOA)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Perfluorooctanesulfonic acid (PFOS)	0.03.	<LOQ	0.026	0.027	0.025	0.032	0.048		0.027	<LOQ	0.033		<LOQ	0.025	0.035	0.042	0.027
Industrial side products and pesticides																	
2-naphthol	0.31	0.49	0.75	0.34	0.32	0.21	0.52	0.43	0.49	0.29	0.43	0.96	0.39	0.76	0.24	0.40	1.1
Prochloraz						0.082											
Phytoestrogens and metabolites																	
8-prenylnaringenin				0.042		<LOQ*											
Daidzein	0.014	0.14	0.014	0.077			0.013				<LOQ		<LOQ	<LOQ		0.026	<LOQ
Enterodiol											0.011	0.0082					0.0079
Enterolactone											<LOQ						<LOQ
Glycitein		0.011		0.0046							<LOQ		0.0053			<LOQ	<LOQ
Isoxanthohumol						<LOQ		<LOQ			<LOQ						
Resveratrol																	
Xanthohumol**									det.**								det.**
Mycoestrogens and metabolites																	
Alternariol																	
Personal care product ingredients, pharmaceuticals and metabolites																	
Benzophenone 1	0.024	<LOQ	0.026	0.021	<LOQ	<LOQ	0.021	0.022	<LOQ	0.020	0.023	<LOQ	<LOQ	<LOQ	<LOQ	0.021	0.021
Benzophenone 2			<LOQ												0.020		
Butylparaben (BP)	0.033								0.021*								.
Ethylparaben (EP)	0.050	0.072	0.072	0.05	<LOQ	<LOQ	0.09	0.10	0.091	0.071	0.077	0.055	0.067	<LOQ	<LOQ	0.082	0.065
Methylparaben (MP)	0.11	0.18	0.11	0.13	0.096	0.12	0.28	0.26	0.72	0.24	0.34	0.19	0.19	0.11	0.12	0.21	0.17
Propylparaben (PP)	0.079	0.056	0.056	0.049	0.033	0.041	0.16	0.088	0.82	0.19	0.22	0.14	0.13	0.078	0.077	0.12	0.071
Phytotoxins																	
Anisodamine				0.0062													
Jacobine-N-oxide																0.11	
Riddelliin-N-oxide																	
Scopolamine				0.00040													
Food processing by-products																	
PhIP								<LOQ	<LOQ*								

** No linear calibration using more than two concentration levels possible for xanthohumol

Table 21: Analysis of breast milk samples of one individual spanning a period of 209 days (Table III). Concentrations are given in [ng/mL]. Analytes that were detected below the LOQ are described as (<LOQ) and analytes that were only detected in the 10 µL injections are described with (*). Analytes that were detected but could not be quantified because of lacking linearity are displayed as (det.).

Compound [ng/mL]	Day																
	41	42	43	46	47	49	52	53	55	57	58	59	63	67	78	85	87
Plasticizer/plastic components																	
Bisphenol A (BPA)																	
Bisphenol F (BPF)																	
Bisphenol S (BPS)	<LOQ*	<LOQ	0.0099	0.0093		<LOQ*	<LOQ	0.036	<LOQ	<LOQ				<LOQ		<LOQ	0.0088
Mono-n-butyl phthalate (MBP)	0.69	0.28		0.32	0.39	0.51	0.21	0.77	<LOQ	<LOQ	0.35	<LOQ	0.38	0.24	<LOQ	5.6	1.1
N-butylbenzenesulfonamide	8.9	9.3	7.8	10	11	5.5	7.8	5.0	6.6	8.9	9.1	7.3	9.6	12	9.6	6.0	4.5
Perfluorinated alkylated substances																	
Perfluorooctanoic acid (PFOA)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Perfluorooctanesulfonic acid (PFOS)	<LOQ	0.025	<LOQ	0.04	0.028	0.026	<LOQ	0.04	<LOQ	0.033	<LOQ	0.03	<LOQ	0.034	<LOQ	0.037	<LOQ
Industrial side products and pesticides																	
2-naphthol	0.94	0.73	0.91	0.33	0.37	0.32	0.34	0.25	0.26	0.90	0.38	0.95	0.45	0.37	1.0	0.28	0.23
Prochloraz																	
Phytoestrogens and metabolites																	
8-prenylnaringenin					<LOQ			<LOQ			<LOQ	0.23					
Daidzein							<LOQ		0.018	0.017	0.013	0.018	0.019	<LOQ	0.048		0.019
Enterodiol															0.016		
Enterolactone																	
Glycitein													<LOQ		<LOQ		0.050
Isoxanthohumol	<LOQ*		<LOQ*	<LOQ*					<LOQ	<LOQ				<LOQ			
Resveratrol																	
Xanthohumol**			det.**														
Mycoestrogens and metabolites																	
Alternariol																	
Personal care product ingredients, pharmaceuticals and metabolites																	
Benzophenone 1	<LOQ	<LOQ	0.022	0.022	0.029	0.022	0.028	<LOQ	<LOQ	<LOQ	0.028	<LOQ	<LOQ	0.027	<LOQ	<LOQ	0.033
Benzophenone 2																	
Butylparaben (BP)																	0.024
Ethylparaben (EP)	0.068	0.064	0.062	0.13	0.11	0.077	0.05	0.058	0.054	0.057	0.068	0.069	0.10	0.10	0.071	0.13	0.059
Methylparaben (MP)	0.12	0.14	0.11	0.23	0.23	0.17	0.14	0.16	0.39	0.33	0.57	0.17	0.19	0.23	0.13	0.42	0.17
Propylparaben (PP)	0.061	0.076	0.057	0.099	0.097	0.065	0.051	0.089	0.26	0.24	0.49	0.11	0.083	0.11	0.059	0.095	0.058
Phytotoxins																	
Anisodamine			<LOQ			0.024								0.0032			
Jacobine-N-oxide				0.0043*		0.031											
Riddelliin-N-oxide																	
Scopolamine																	
Food processing by-products																	
PhIP																	

** No linear calibration using more than two concentration levels possible for xanthohumol

Table 22: Analysis of breast milk samples of one individual spanning a period of 209 days (Table IV). Concentrations are given in [ng/mL]. Analytes that were detected below the LOQ are described as (<LOQ) and analytes that were only detected in the 10 µL injections are described with (*)

Compound [ng/mL]	Day 110	111	120	121	124	131	134	136	138	139	140	143	146	150	152	153	154
Plasticizer/plastic components																	
Bisphenol A (BPA)																	
Bisphenol F (BPF)			<LOQ										<LOQ*				
Bisphenol S (BPS)		<LOQ		<LOQ	<LOQ	<LOQ		0.0060*		<LOQ	<LOQ	<LOQ	<LOQ	0.015	<LOQ	<LOQ	0.0098
Mono-n-butyl phthalate (MBP)	0.44	0.28		<LOQ	0.58	0.73	0.79	1.5	0.94	0.44	0.81	0.60	0.36	0.31	0.53	2.1	2.1
N-butylbenzenesulfonamide	4.4	10	8.8	11	4.9	7.2	7.6	8.6	7.0	14	4.6	11	6.0	11	5.6	7.2	6.5
Perfluorinated alkylated substances																	
Perfluorooctanoic acid (PFOA)		<LOQ		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Perfluorooctanesulfonic acid (PFOS)	0.026	<LOQ	0.031	0.027	0.036	0.038	0.032	0.028	0.031	<LOQ	0.031	0.034	0.030	0.027	0.044	0.034	
Industrial side products and pesticides																	
2-naphthol	0.33	1.1	0.37	0.76	0.23	0.34	0.34	0.29	0.56	0.39	0.22	0.84	0.85	1.1	0.28	0.91	0.31
Prochloraz																	
Phytoestrogens and metabolites																	
8-prenylnaringenin			0.081					0.042			<LOQ	0.099	<LOQ				<LOQ
Daidzein			<LOQ			0.010	<LOQ			<LOQ*			0.063	0.0098			0.018
Enterodiol										0.01			0.025	0.007*			
Enterolactone										<LOQ*							
Glycitein													0.0057				
Isoxanthohumol			0.018				0.026	<LOQ*					<LOQ*			<LOQ*	
Resveratrol																	
Xanthohumol																	
Mycoestrogens and metabolites																	
Alternariol													0.51				
Personal care product ingredients, pharmaceuticals and metabolites																	
Benzophenone 1	<LOQ	0.028	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.026	0.034	<LOQ	0.023	<LOQ	<LOQ	0.039	<LOQ	0.028	0.034
Benzophenone 2																	
Butylparaben (BP)								<LOQ*				<LOQ*	<LOQ*	<LOQ*		<LOQ*	
Ethylparaben (EP)	0.055	0.081	0.068	0.079	<LOQ	<LOQ	0.089	0.094	0.06	0.11	<LOQ	0.079	0.06	0.082	0.056	0.072	0.052
Methylparaben (MP)	0.13	0.15	0.16	0.15	0.12	0.12	0.34	0.20	0.12	0.21	0.15	0.38	0.15	0.20	0.42	0.35	0.24
Propylparaben (PP)	0.067	0.074	0.065	0.071	0.069	0.043	0.091	0.089	0.072	0.11	0.074	0.25	0.13	0.11	0.39	0.24	0.14
Phytotoxins																	
Anisodamine														0.0030*			
Jacobine-N-oxide																	
Riddelliin-N-oxide					<LOQ												
Scopolamine																<LOQ	
Food Processing by-products																	
PhIP																	

Table 23: Analysis of breast milk samples of one individual spanning a period of 209 days (Table V). Concentrations are given in [ng/mL]. Analytes that were detected below the LOQ are described as (<LOQ) and analytes that were only detected in the 10 µL injections are described with (*). No date of sampling was recorded for two samples.

Compound [ng/mL]	Day																	
	155	156	157	158	159	162	163	164	165	168	169	171	176	179	181	209	Not known	Not known
Plasticizer/plastic components																		
Bisphenol A (BPA)																		
Bisphenol F (BPF)																		
Bisphenol S (BPS)	<LOQ	<LOQ	<LOQ		0.0093	<LOQ	<LOQ	<LOQ		0.012	<LOQ	<LOQ	<LOQ	0.015	<LOQ			<LOQ
Mono-n-butyl phthalate (MBP)	2.0	1.1	1.1	<LOQ	1.2	1.1	0.36	0.99	2.8	0.83	0.54	0.87	1.1	0.88	1.0	0.25	0.41	0.94
N-butylbenzenesulfonamide	8.9	7.6	7.2	9.1	6.5	7.8	9.3	6.6	12	11	9.8	7.5	12	12	6.4	6.5	9.2	9.8
Perfluorinated alkylated substances																		
Perfluorooctanoic acid (PFOA)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Perfluorooctanesulfonic acid (PFOS)	0.027	0.030	0.043	0.030	0.026	0.035*	<LOQ	0.032	0.032	0.028*	0.026	0.029	0.028	0.043	<LOQ		0.027	0.028
Industrial side products and pesticides																		
2-naphthol	0.23	0.30	0.23	0.49	0.29	0.31	1.0	0.38	0.34	1.1	0.63	0.24	0.39	0.80	0.30	0.25	0.77	0.70
Prochloraz																		
Phytoestrogens and metabolites																		
8-prenylnaringenin			<LOQ		<LOQ	<LOQ*				0.13		<LOQ						
Daidzein			0.040		0.018	0.014	0.029	0.017	0.020				0.043				0.12	
Enterodiol				0.016			0.0097										0.012	
Enterolactone													<LOQ					
Glycitein			0.0057		<LOQ								0.0049				0.017	
Isoxanthohumol	0.018			<LOQ					<LOQ	<LOQ*								
Resveratrol																		
Xanthohumol																		
Mycoestrogens and metabolites																		
Alternariol																		
Personal care product ingredients, pharmaceuticals and metabolites																		
Benzophenone 1	<LOQ	<LOQ	<LOQ	0.031	<LOQ	0.023	<LOQ	<LOQ	0.025	0.027	0.021	0.021	0.024	0.023	<LOQ	<LOQ	<LOQ	<LOQ
Benzophenone 2																		
Butylparaben (BP)										<LOQ*		0.030	.	0.031			0.065	
Ethylparaben (EP)	0.075	0.073	0.058	0.076	0.05	0.12	0.07	0.091	0.11	0.075	0.064	0.068	0.084	0.12	0.062	0.096	0.14	0.075
Methylparaben (MP)	0.32	0.27	0.27	0.17	0.16	0.29	0.32	0.34	0.55	0.24	0.23	0.24	0.27	0.28	0.25	23	0.37	0.16
Propylparaben (PP)	0.28	0.18	0.20	0.062	0.12	0.18	0.30	0.23	0.47	0.16	0.17	0.21	0.18	0.17	0.12	16	0.11	0.075
Phytotoxins																		
Anisodamine										<LOQ								
Jacobine-N-oxide																		
Riddelliin-N-oxide																		
Scopolamine																		
Food processing by-products																		
PhIP																		

5.5.1. Confirmational MS/MS experiments (EPI scans)

Centroid MS2 spectra were created from the EPI scans acquired in profile mode described in section 4.5. In some cases the IDA EPI method yielded better results, in other cases the full scan EPI method was preferred. Spectra shown below depict the quantifier ion chromatogram and the MS2 spectra generated by the EPI scans.

5.5.1.1. Urine samples

MS2 spectra of the precursor mass of TBPA (542.5 Da) in one replicate of sample J (3.1 ng/mL before correcting by extraction efficiency) and the matrix matched standard (1 ng/mL) are compared in Figure 30. The IDA EPI scan was conducted at the peak maximum in both cases (see discussion). The spectra of the unknown sample are magnified since the high intensities of the precursor mass ($3e^4$ cps in the sample, $1.4e^4$ in the sample) make it difficult to discern the remaining fragments. Quantifier and qualifier fragments used in the MRM method are the masses of 445.8 Da, 419.8 Da and 417.9 Da respectively.

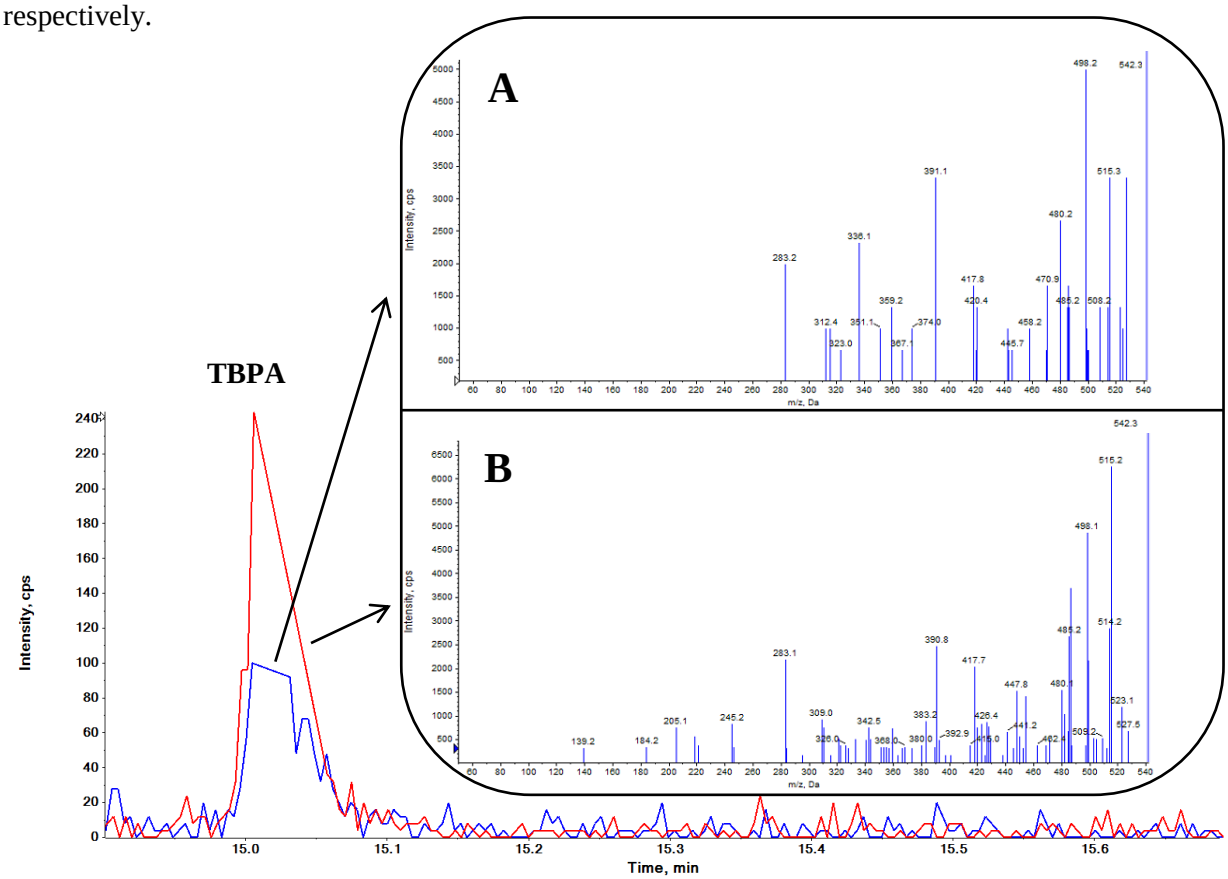

Figure 30: Comparison of the MS2 spectra of TBPA in the urine standard at 1 ng/mL and in sample J at 3.1 ng/mL (B) using a CE at -50 +/-15V. Both spectra are magnified to facilitate the recognition of fragments in contrast to the abundant precursor mass ($3e^4$ cps in A, $1.4e^4$ in B)

MS2 spectra of the precursor mass of 8-prenylnaringenin (339 Da) in one replicate of sample L (4 ng/mL) and the matrix matched standard (3 ng/mL) are compared in Figure 31. Quantifier and qualifier fragments used in the MRM method are the masses of 219 and 119 Da respectively. While the IDA EPI scan was not triggered at the peak maximum, the high precursor intensity still yielded characteristic MS2 spectra.

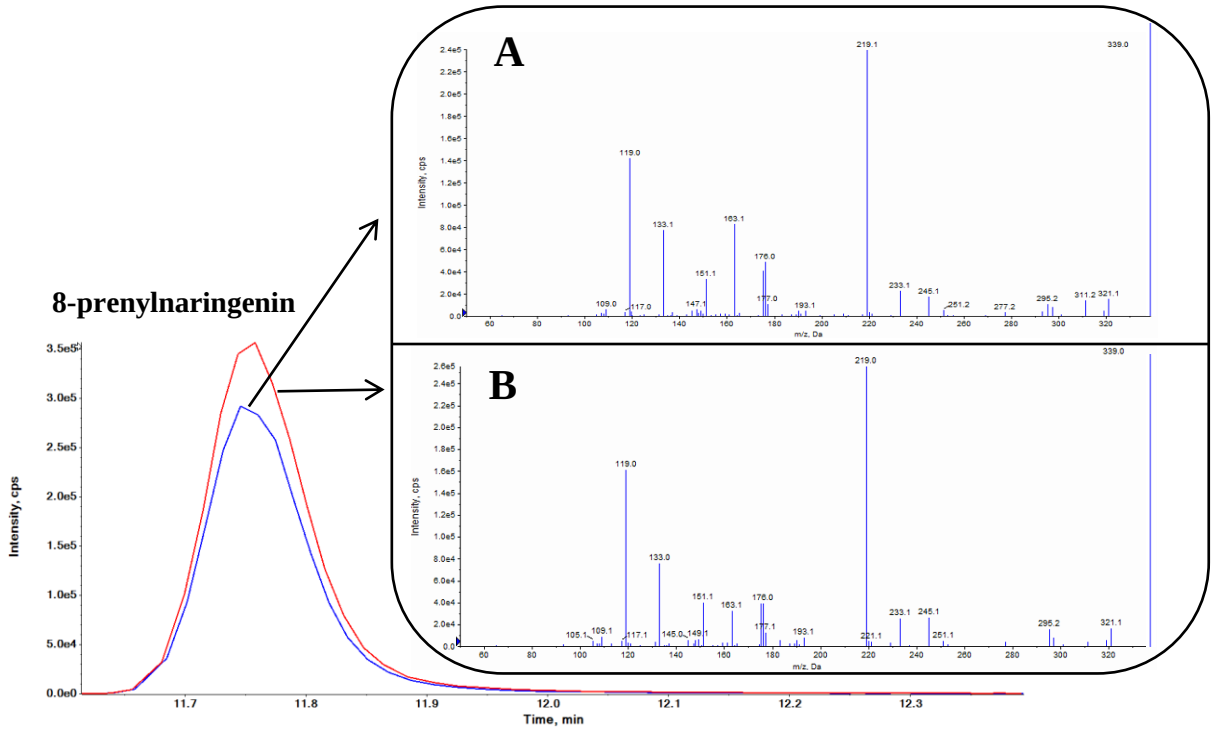

Figure 31: Comparison of the MS2 spectra of 8-prenylnaringenin in the urine standard at 3 ng/mL (A) and in sample L at 4 ng/mL(B) using a CE at -30 +/-15V.

MS2 spectra of the precursor mass of prochloraz (376 Da) in one replicate of the sample N (0.00054 ng/mL) and the matrix matched standard (0.0005 ng/mL) are compared in Figure 32. The IDA EPI scan was conducted at the peak maximum in both cases. Quantifier and qualifier fragments used in the MRM method are the masses of 307.8 Da and 265.7 Da respectively. Neither was detected in the standard or the sample.

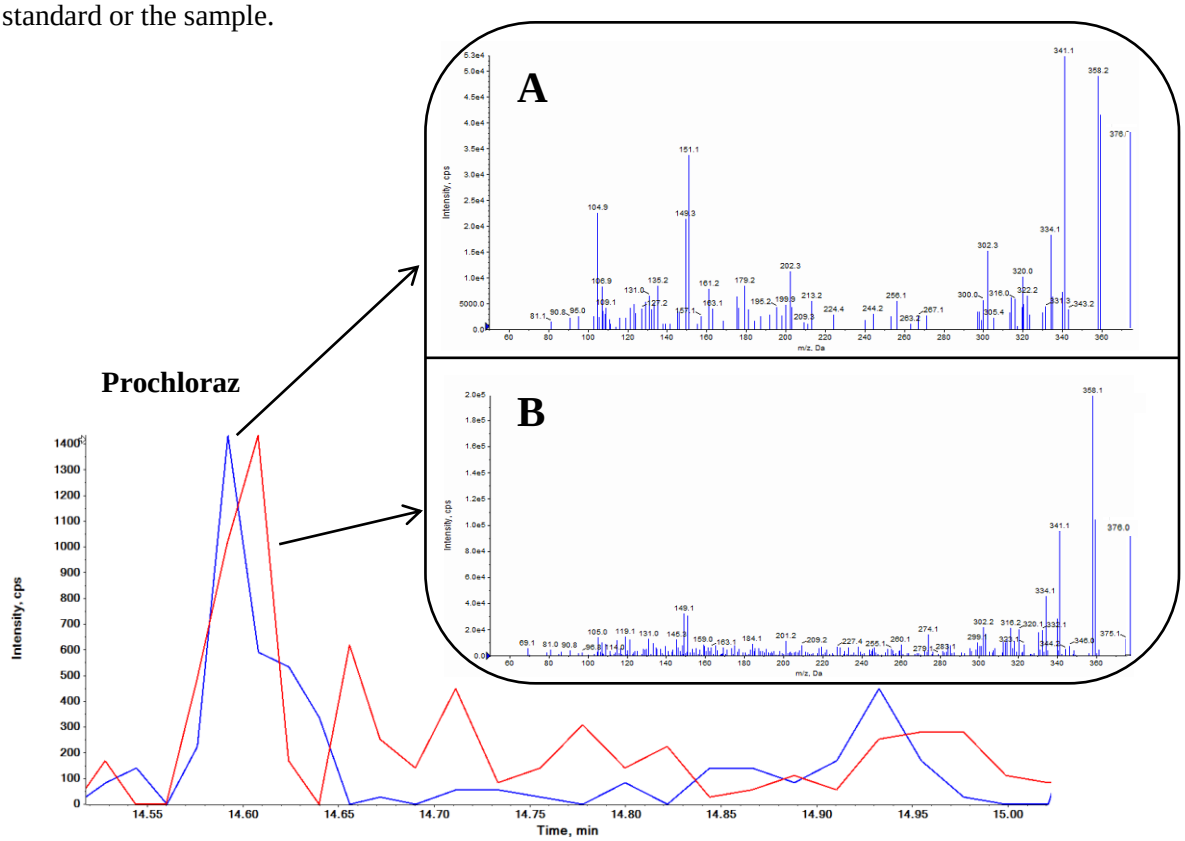

Figure 32: Comparison of the MS2 spectra of prochloraz in the urine standard at 0.0005 ng/mL (A) and in sample N at 0.00054 ng/mL(B) using a CE of 20 +/-15V

5.5.1.2. Serum samples

MS2 spectra of the precursor mass of 2MeOE1 (299 Da) in the serum sample of adult 6 (0.049 ng/mL) and the matrix matched standard (0.025 ng/mL) are compared in Figure 33. To enhance precursor yield for the EPI scans, 10 µL of the sample as well as the standard were injected. The IDA EPI scan was conducted at the peak maximum in both cases. An interfering mass of 185 Da was observed throughout the whole measurement in previous scans (even though dynamic background subtraction was enabled) occupying most of the instruments trapping capability and thus distorting the MS2 spectra (no precursor mass detectable any longer, no quantifier transition and only few other fragments observable). As this fragment mass is not characteristic for 2MeOE1, it was manually excluded in this scan and only a mass range from 50 Da to 184 Da and 186 Da to 299 Da was observed. Quantifier and qualifier fragments used in the MRM method are the masses of 284.1 Da and 159.9 Da respectively. The spectra are magnified since the high intensities of the precursor mass ($2.5e^4$ cps both the standard and the sample) make it difficult to discern the remaining fragments.

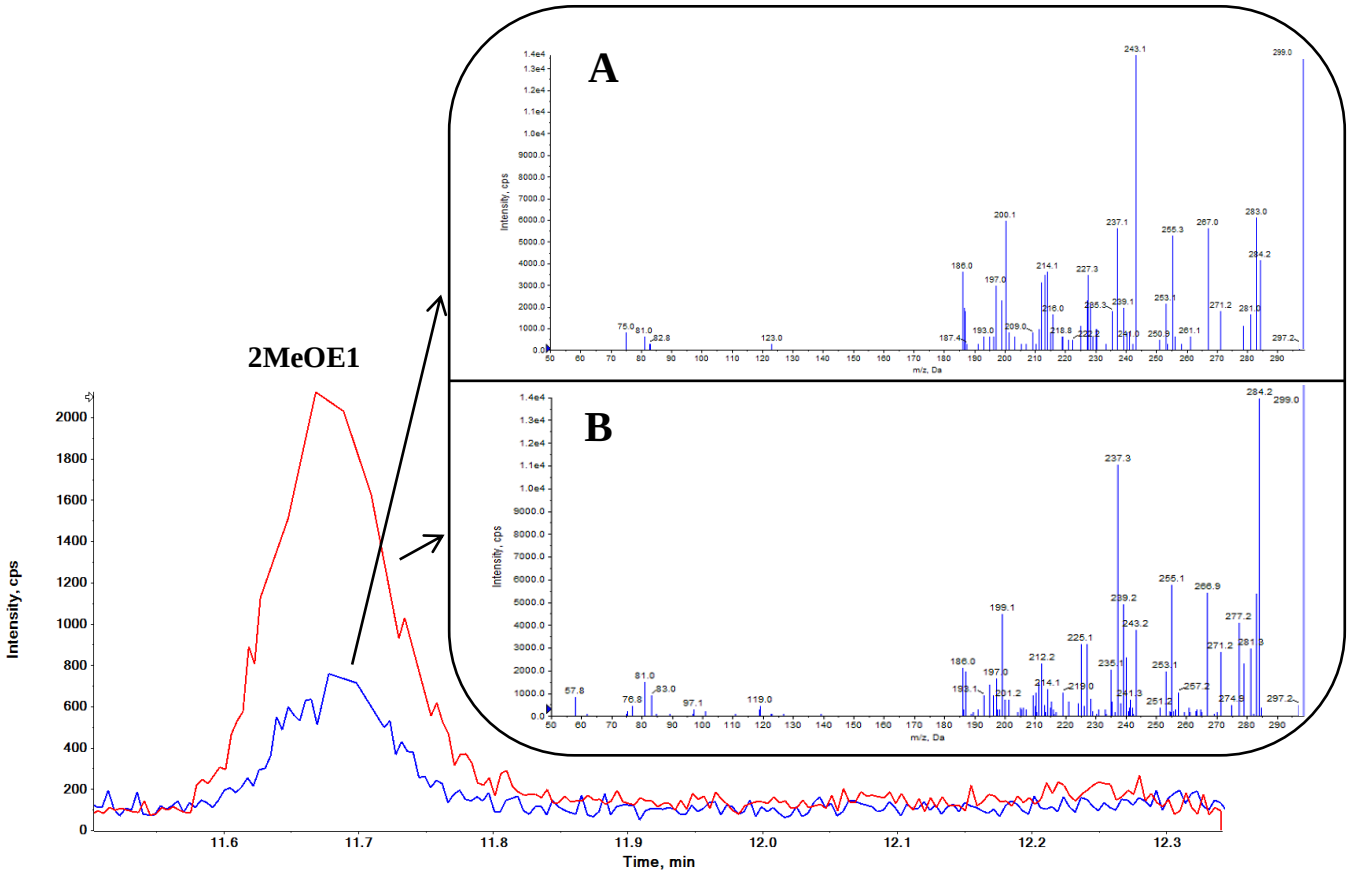

Figure 33: Comparison of the MS2 spectra of 2MeOE1 in the serum standard at 0.025 ng/mL (A) and in the serum sample of adult 6 at 0.049 ng/ml (B) using a CE of -45 +/- 15V. Peak heights of the precursor mass 299 Da were $2.5e^4$ cps in both the standard and the sample.

MS2 spectra of the precursor mass of HMF (127 Da) in the baby sample PB 41 (3.9 ng/mL) and the matrix matched standard (3.5 ng/mL) are compared in Figure 34. Quantifier and qualifier fragments used in the MRM method are the masses of 109 Da and 81 Da respectively. The non-IDA full-scan EPI method was used here.

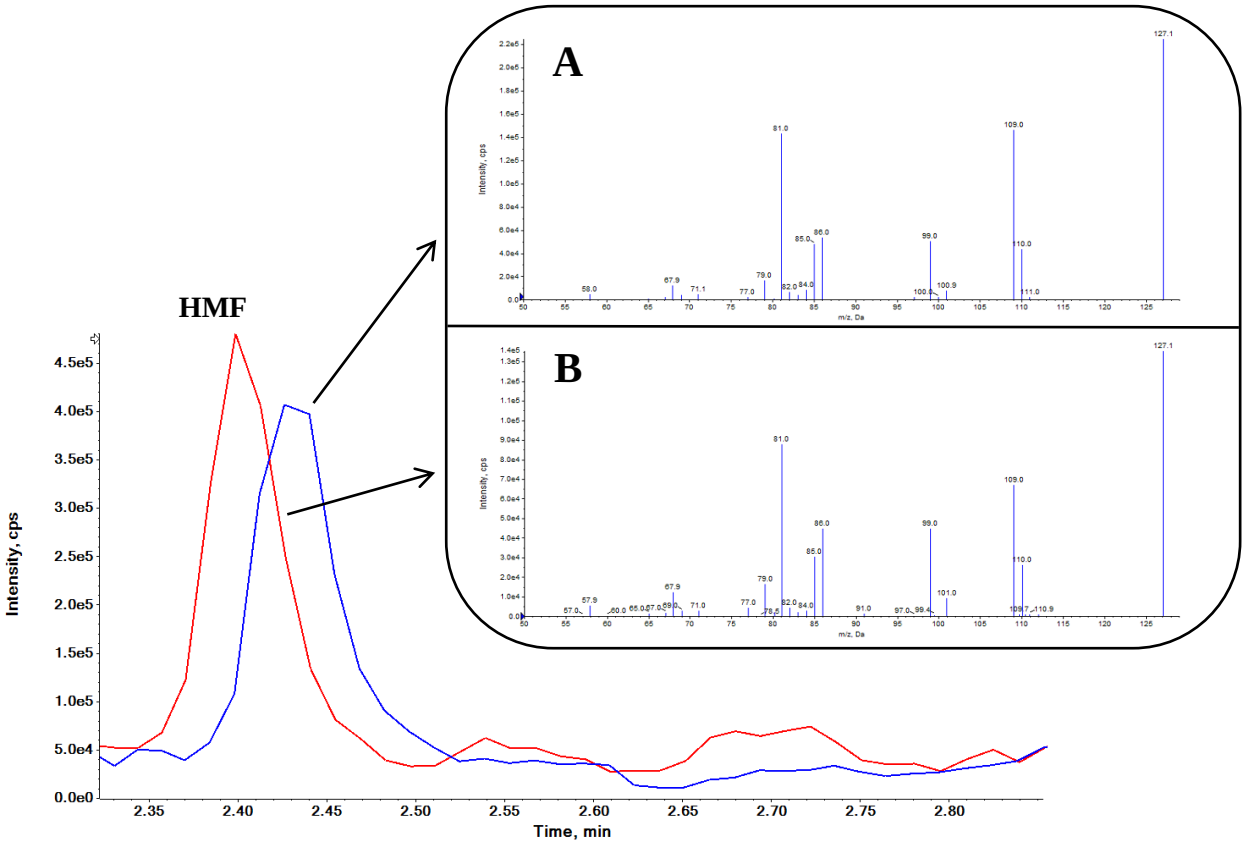

Figure 34: Comparison of the MS2 spectra of HMF in the serum standard at 3.5 ng/mL (A) and in the baby sample PB 41 at 3.9 ng/mL (B) using a CE of 20V

MS2 spectra of the precursor mass of HMFA (140.9 Da) in the baby sample PB 41 (2266 ng/mL) and the matrix matched standard (2000 ng/mL) are compared in Figure 35. While the IDA EPI scan was not triggered at the peak maximum, the high precursor intensity still yielded characteristic MS2 spectra. Quantifier and qualifier fragments used in the MRM method are the masses of 97.1 Da and 69 Da respectively.

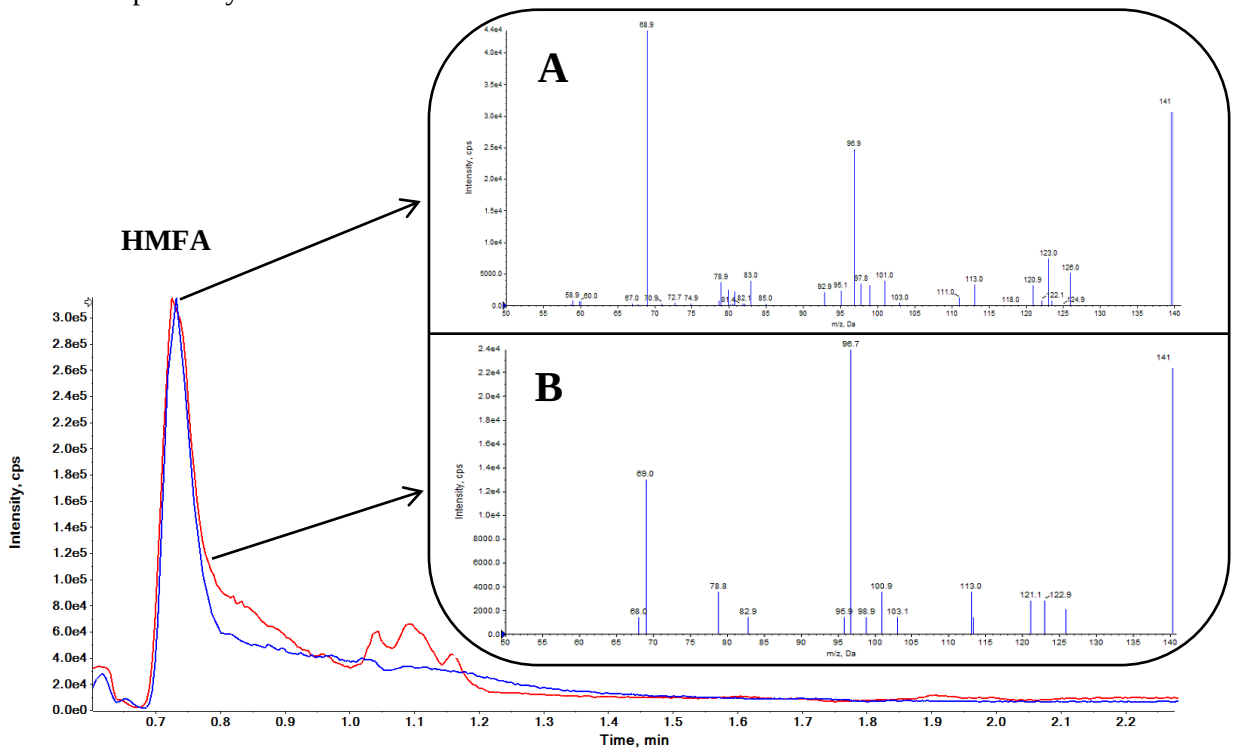

Figure 35: Comparison of the MS2 spectra of HMFA in the serum standard at 2000 ng/mL (A) and in the baby sample PB 41 at 2266 ng/mL(B) using a CE of -20 +/-15V.

MS2 spectra of the precursor masses of BPA (227 Da), PFOS (498.8 Da) and methylparaben (151 Da) in the matrix matched standard (10 ng/ml, 1.5 ng/mL, 2.5 ng/mL) and the respective baby samples PB 24 (11 ng/mL), PB 53 (1.6 ng/mL) and PB 37 (0.63 ng/mL) are compared in Figure 36. Quantifier and qualifier fragments used in the MRM method are the masses of 133 Da and 117 Da (BPA), 80 Da and 98.9 Da (PFOS) and 91.9 Da and 136 Da (methylparaben) respectively. The non-IDA full scan EPI method was used for BPA and PFOS, while the IDA method yielded acceptable spectra for methylparaben.

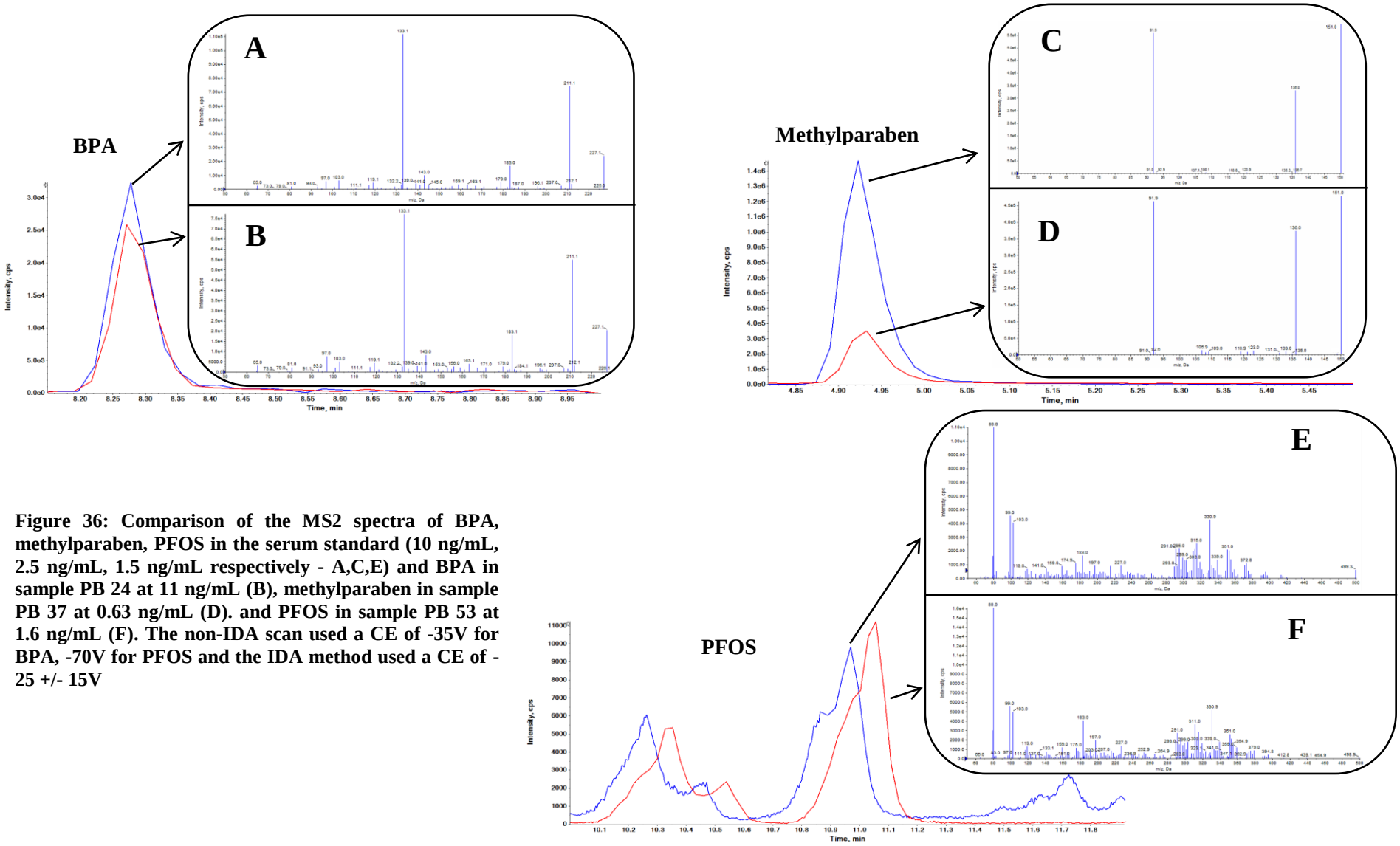

Figure 36: Comparison of the MS2 spectra of BPA, methylparaben, PFOS in the serum standard (10 ng/mL, 2.5 ng/mL, 1.5 ng/mL respectively - A,C,E) and BPA in sample PB 24 at 11 ng/mL (B), methylparaben in sample PB 37 at 0.63 ng/mL (D). and PFOS in sample PB 53 at 1.6 ng/mL (F). The non-IDA scan used a CE of -35V for BPA, -70V for PFOS and the IDA method used a CE of -25 +/- 15V

5.5.1.3. Breast milk samples

Breast milk samples were exclusively measured using the full scan EPI method. To enhance precursor yield, 20 µL were injected for all samples except the samples containing prochloraz, alternariol and jacobine-N-oxide.

MS2 spectra of the precursor mass of scopolamine (304 Da) in the breast milk sample 11 (<LOQ) and the matrix matched standard (0.0015 ng/mL) are compared in Figure 37. Quantifier and qualifier fragments used in the MRM method are the masses of 138 Da and 156 Da respectively.

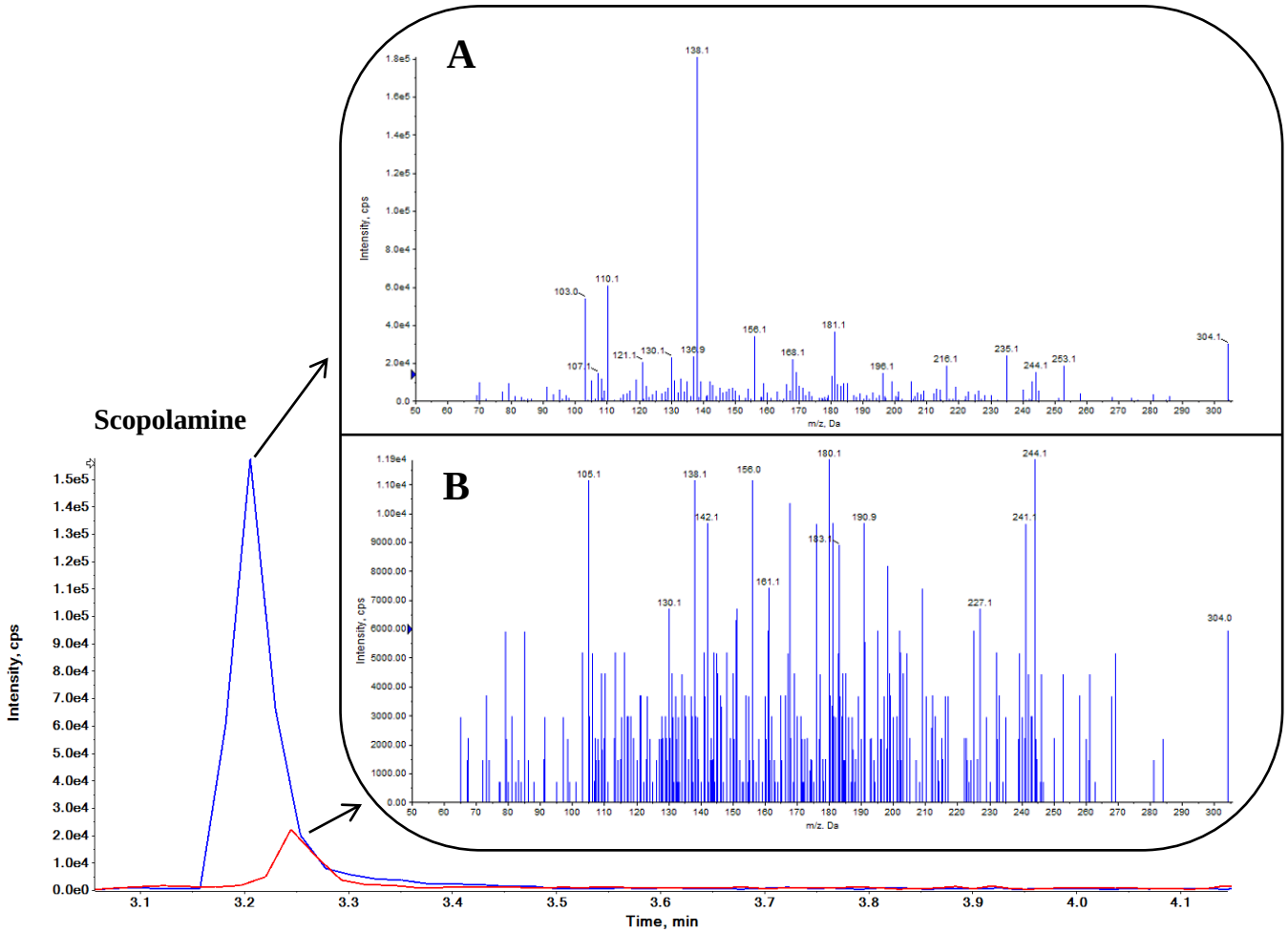

Figure 37: Comparison of the MS2 spectra of scopolamine in the breast milk standard at 0.0015 ng/mL (A) and in sample 11 below the LOQ (B) using a CE of 35V.

MS2 spectra of the precursor mass of jacobine-N-oxide (368.1 Da) in the breast milk sample 38 (0.029 ng/mL) and the matrix matched standard (0.015 ng/mL) are compared in Figure 38. Quantifier and qualifier fragments used in the MRM method are the masses of 296 Da and 120 Da respectively.

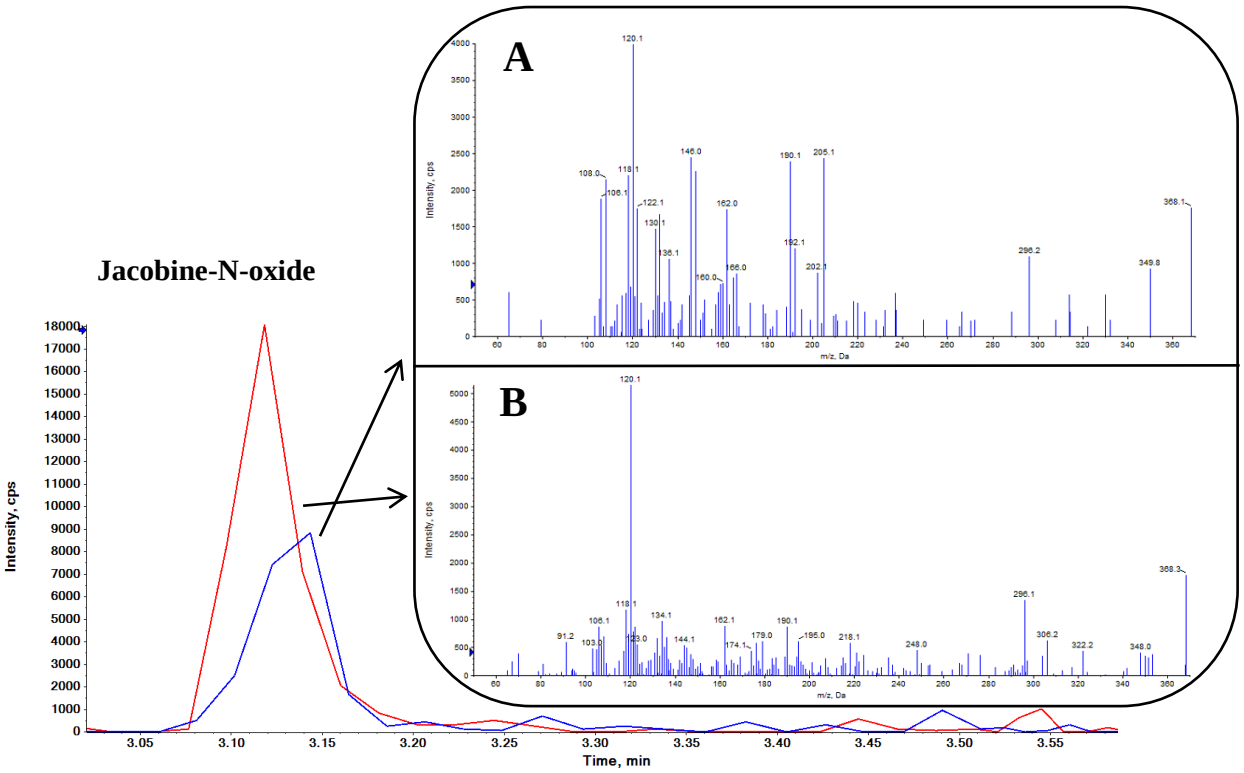

Figure 38: Comparison of the EPI MS2 spectra of jacobine-N-oxide in the breast milk standard at 0.015 ng/mL (A) and in sample 38 at 0.029 ng/mL (B) using a CE of 45V

MS2 spectra of the precursor mass of riddelliin-N-oxide (366.1 Da) in the breast milk sample 124 (<LOQ) and the matrix matched standard (0.06 ng/mL) are compared in Figure 39. Quantifier and qualifier fragments used in the MRM method are the masses of 120 Da and 118 Da respectively.

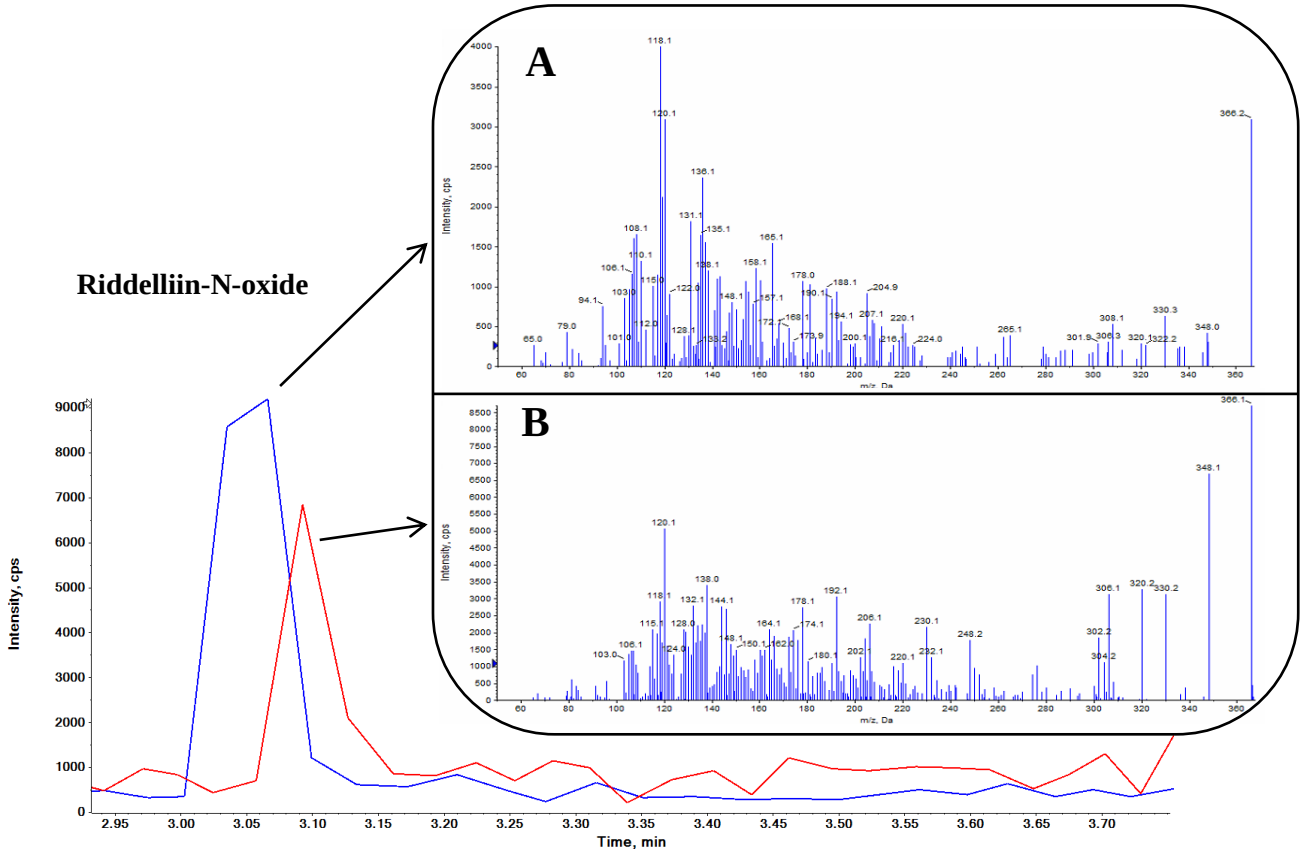

Figure 39: Comparison of the MS2 spectra of riddelliin-N-oxide in the breast milk standard at 0.06 ng/mL (A) and in sample 124 below the LOQ (B) using a CE of 45V.

MS2 spectra of the precursor mass of anisodamine (306.1 Da) are displayed in Figure 40. Since there are two eluting isomers, separate EPI scans for each are illustrated. Concentrations describe the total sum of both isomers. A and C display the first eluting isomer and the second eluting isomer in the matrix matched standard (0.015 ng/mL in total) respectively, while B displays the first isomer in the breast milk sample 49 (0.048 ng/mL in total) and D displays the second isomer in breast milk sample 11 (0.017 ng/mL in total). Quantifier and qualifier fragments used in the MRM method are the masses of 140.1 Da and 122.1 Da respectively.

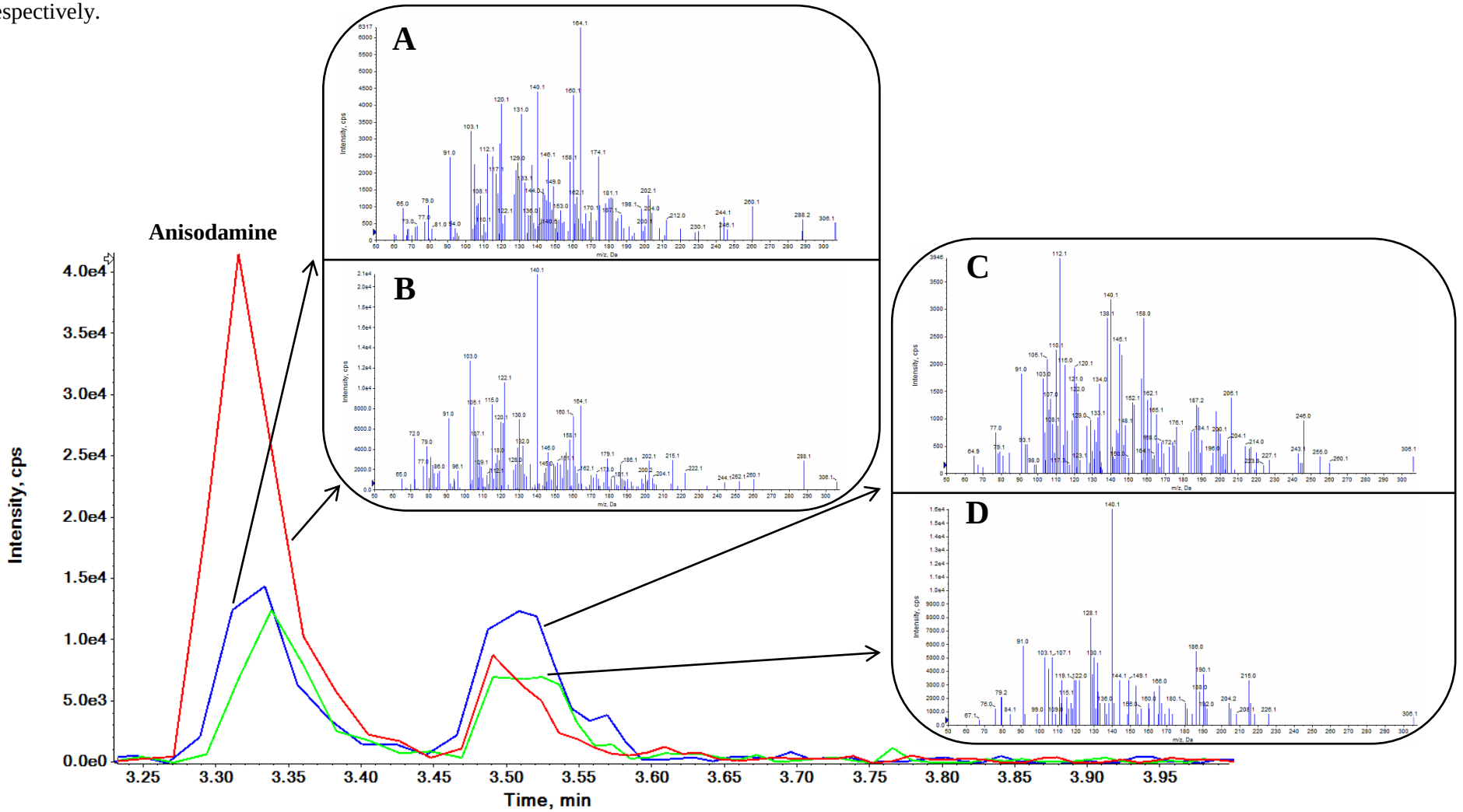

Figure 40: MS2 spectra of the first and second eluting isomer of anisodamine in the breast milk standard at 0.015 ng/mL in total (A, C) compared to the MS2 spectra of the first and second eluting isomer in samples 49 at 0.048 ng/mL in total and 11at 0.017 ng/mL in total respectively (B, D) using a CE of 45V.

MS2 spectra of the precursor mass of PhIP (225.1 Da) in the breast milk sample 29 (<LOQ) and the matrix matched standard (0.03 ng/mL) are compared in Figure 41. Quantifier and qualifier fragments used in the MRM method are the masses of 210.1 Da and 140.1 Da respectively.

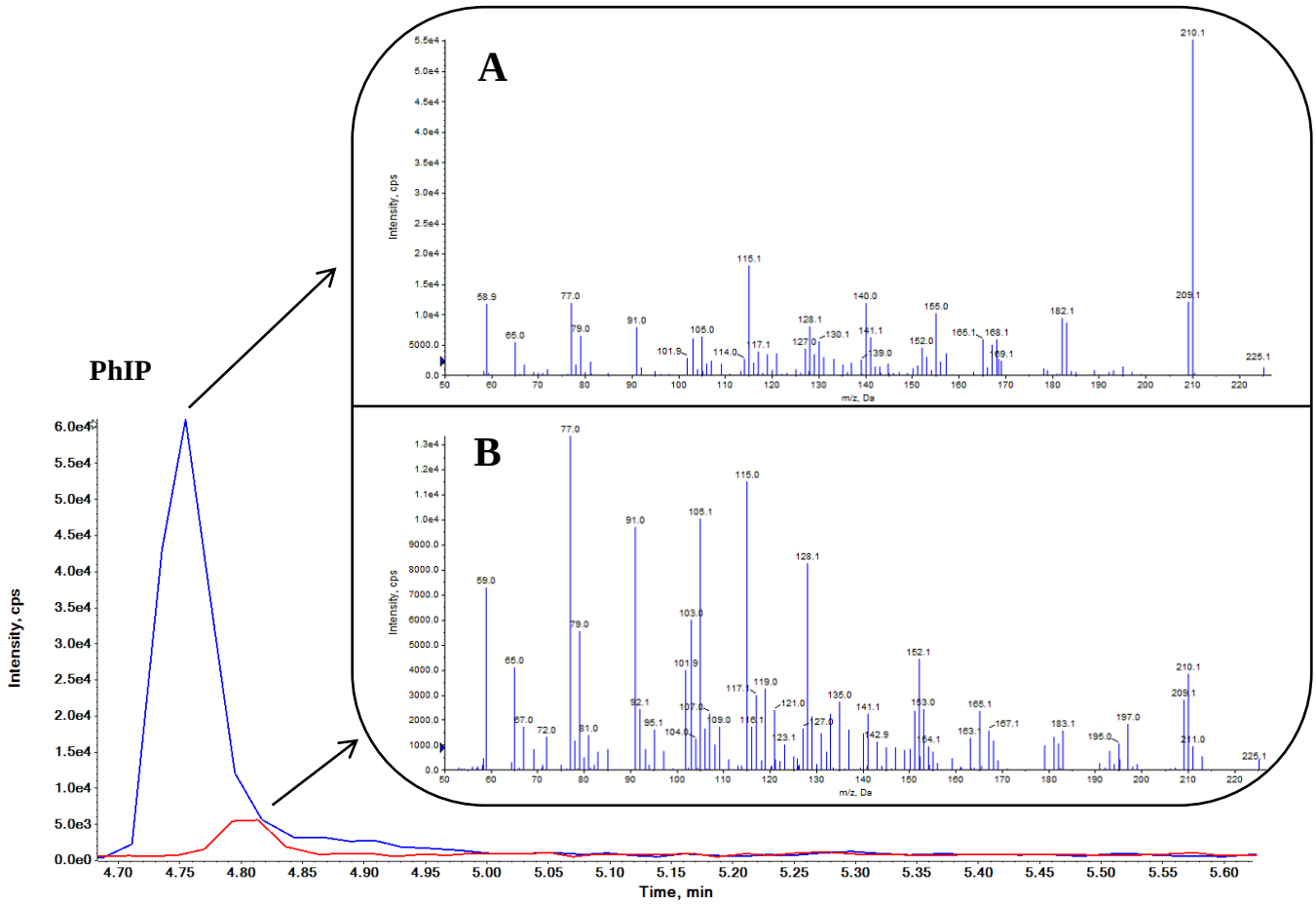

Figure 41: Comparison of the MS2 spectra of PhIP in the breast milk standard at 0.03 ng/mL (A) and in sample 29 below the LOQ (B) using a CE of 50V.

MS2 spectra of the precursor mass of alternariol (255.0 Da) in the breast milk sample 146 (0.51 ng/mL) and the matrix matched standard (10 ng/mL) are compared in Figure 42. The spectrum of the unknown sample (B) is magnified since the high intensities of the interfering masses of 119 Da and 183 Da (both above $5e^4$ cps) makes it difficult to discern the remaining fragments. Quantifier and qualifier fragments used in the MRM method are the masses of 213 Da and 215 Da respectively.

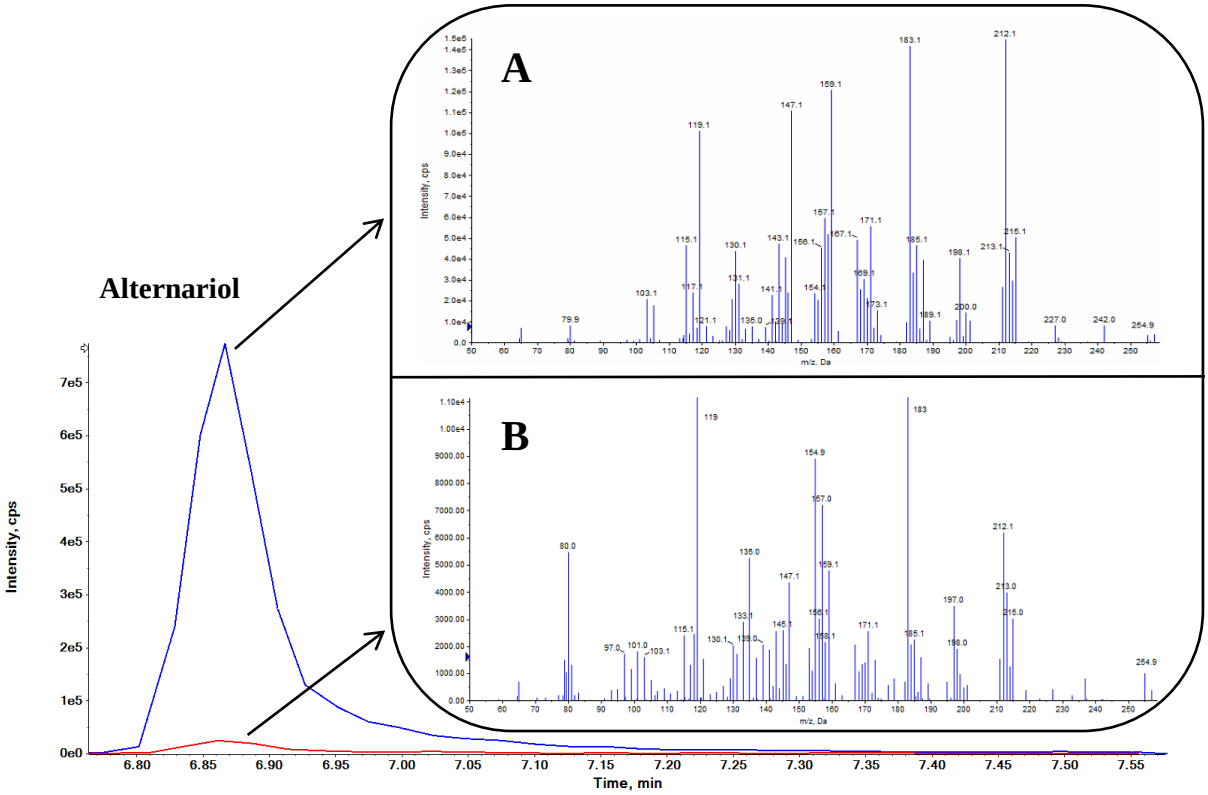

Figure 42: Comparison of the MS2 spectra of alternariol in the breast milk standard at 10 ng/mL (A) and in sample 146 at 0.51 ng/mL (B) using a CE of -50V. Spectrum B is magnified to facilitate the recognition of fragments in contrast to the abundant interfering masses of 119 and 183 Da (both above $5e^4$ cps).

MS2 spectra of the precursor mass of prochloraz (376.1 Da) in the breast milk sample 25 (0.082 ng/mL) and the matrix matched standard (0.05 ng/mL) are compared in Figure 43. Quantifier and qualifier fragments used in the MRM method are the masses of 307.8 Da and 265.7 Da respectively. Neither was detected in the standard or the sample.

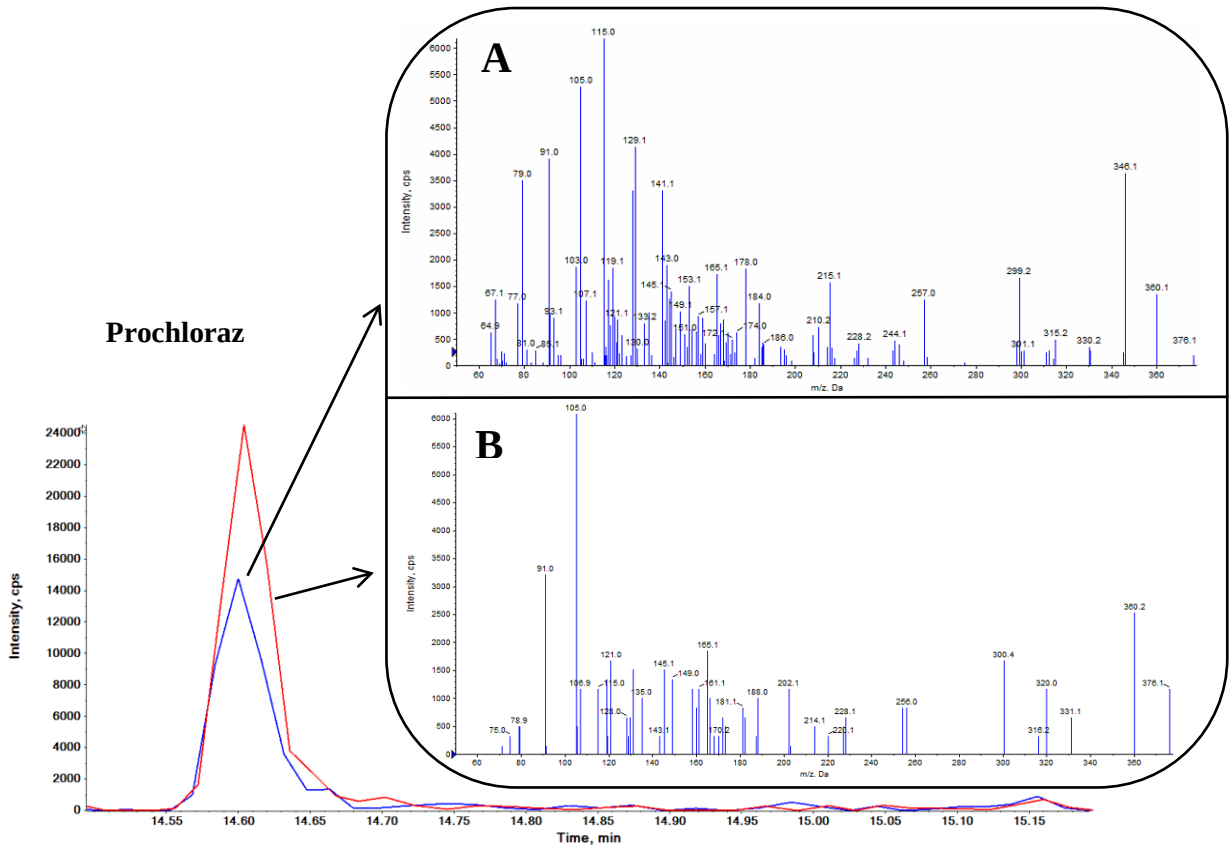

Figure 43: Comparison of the MS2 spectra of prochloraz in the breast milk standard at 0.05 ng/mL (A) and in sample 25 at 0.082 ng/mL (B) using a CE of 55V.

6. Discussion

6.1. Method validation

Of the 95 compounds that were included for method validation, 39 fully met all the criteria laid out by the Commission Decision No. 657/2002 [286] in urine, 50 in serum and 15 in breast milk. Not meeting the minimum extraction recovery (50-120% for concentrations >1 ng/mL, 80-110% for concentrations ranging 1-10 ng/mL, 80-110% for concentrations >10 ng/mL) at one or both levels was the main reason for unsuccessful validation of most compounds in breast milk, while validation failure in urine and serum was mostly only due to lacking intermediate precision and/or repeatability.

In general, extraction recoveries for breast milk were significantly worse compared to urine and serum (Figure 44). Breast milk was considered to be the most demanding matrix owing to its high fat content/non-polarity and protein content, hence the different extraction protocol. The use of inorganic salts to induce phase separation is more time consuming and more prone to variation, i.e. even with fast inversion, bigger or smaller salt pellets clumped at the bottom of the reaction tube. This may have resulted in different salt concentrations in the solution during subsequent vortexing. The loss of analytes that potentially remained in the aqueous phase also needs to be considered. Additionally, depending on the aliquot, vacuum evaporation took up to 72 hours even with increasing temperatures because of lipid layer formation on top of the sample. Consequently, evaporation then resulted in small lipid droplets which were not dissolvable in 10% ACN before transfer of the final solution into the LC vials. This might have contributed to the reduced extraction recoveries as well. Thus, lower extraction efficiencies (median of 54% compared to 93% in urine and 87% in serum) and intermediate precisions (RSD_R mostly above 10%, even at the HL) compared to urine and serum are explainable to some extent.

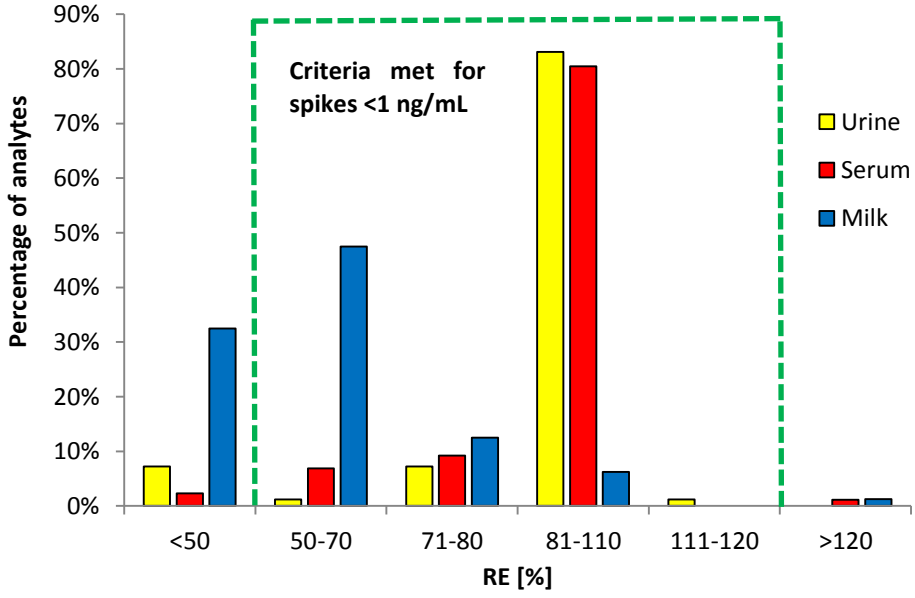

Figure 44: Extraction recoveries of analytes in each biological matrix. The criteria set by the EC Decision No. 657/2002 [286] for spiked concentrations <1 ng/mL is indicated. Unless analytes were only successfully extracted at the HL, extraction recoveries for this illustration were calculated as the average of the LL and HL recoveries.

Moreover, it needs to be mentioned that spiking concentrations for most compounds during method validation (median spike of 0.09 ng/mL at the LL, see Table 29 Appendix) were far below the lowest concentrations used by the guideline [286] to define minimum extractions efficiencies (>50% for substances <1 ng/mL) and maximum RSD_R (<23% for substances 1000-100 ng/mL, adjusted to <25% for substances <100 ng/mL as defined by Preindl et al. (278)). For instance, validation of daidzein in serum failed merely because of a LL RSD_R of 28.4% with a spiked concentration of 0.015 ng/mL. Similarly, validations of riddelliin and isobutylparaben in breast milk failed because of R_E of 44% and 45%, with spiked concentrations of 0.03 ng/mL and 0.009 ng/mL respectively. One may argue that an adjustment of extraction criteria towards even smaller minima and higher maxima for lower concentration levels would be appropriate. According to the commission decision itself, the Horwitz Equation that is used to determine maximum RSD_R is not suited for calculations below 100 ng/mL [286].

Xanthohumol did not exhibit reliable linearity in the given concentration ranges in urine and serum (average R^2 of 0.941 and 0.895 respectively). In spite of this, it still met all validation criteria in these two matrices since there are no set guidelines on accuracy of linear regression. The lacking linearity needs to be kept in mind nonetheless when considering this particular validation outcome.

For some compounds, a big disparity in sensitivity between matrices was already apparent during method development. This explains why the glucuronides were not calibrated in urine, why 4OHE1 was not calibrated in serum and why TBPA was not calibrated in breast milk. This however was expected, since the premise of method development was to take the matrix with highest sensitivity as basis of method validation. Therefore, the design of three individual calibration series in the future to account for the differences in sensitivities between the matrices would definitely enable the successful validation of more compounds in each matrix. However, additional time and resources wold be needed.

In many cases, a lower repeatability than intermediate precision ($RSD_r > RSD_R$) was the only parameter that did not meet validation criteria. It should be noted that the method repeatability was merely evaluated by one repeated measurement of one validation run, while intermediate precisions were evaluated by three independent validation runs. Repeated measurements of all three independent sample preparations would have provided a higher statistical validity to assess repeatability. Furthermore, 16EpiE3, ZAN, ZEN-14-sulfate in urine and glycitein in serum are examples of compounds that yielded low RSD_R of below 5% with slightly higher RSD_r. Intermediate precision and repeatability are relatively high, yet according to the guidelines, validation still failed as repeatability was a bit lower. In these cases, the largest part of the method's variance, be it with independent experiments or under repeatability conditions, is most likely mainly caused by random errors as a result of natural fluctuations in system performance or other uncontrollable variables. Overall, 18 compounds met all validation criteria but the RSD_r limit in urine, 7 in serum and 3 in breast milk.

Thus, outright discarding the method to measure aforementioned substances would be misguided. For other substances, criteria could not be met for the low spiking level, while the high spiking experiments were successful. 12 analytes failed validation because of low R_E and/or high RSD_R at the low level in urine, 9 in serum and 19 in breast milk. This means that the method may still suitable to be applied for qualitative determinations for biological samples as well as quantifications if contaminations higher or equal to the HL experiments are detected. Simple semi-quantitative measurements may still be feasible as well.

In cases such as PFOS in serum and AME in breast milk, validation failed in the LL spike because the spiked amount in one or more samples was way smaller than the matrix contamination itself. As a result, the variance of natural matrix contamination following the measurements was too high to determine the additionally spiked amount. The spiked concentration was not distinguishable from the natural matrix contamination. This meant that one or more spiking experiments yielded recoveries below zero. One can conclude that the method's sensitivity reaches beyond levels of natural contamination. Thus, since the HL spiking experiments met all criteria, application of the method to measure PFOS in serum and AME in breast milk may be reasonable nonetheless.

In addition, validation for benzyl butyl phthalate, resveratrol, 4-*tert*-OP in serum, BPC, MBP, 16EpiE3, 17EpiE3, alternariol, β-ZAL and β-ZEL in breast milk only failed because extraction requirements changed from the LL to the HL spike (>50% for concentrations <1 ng/mL, > 70% for concentrations >1 ng/mL) and thus were not met in the latter. The method should not be considered inappropriate for these substances in the given matrix only due to the fact that extraction recoveries did not increase substantially with higher spiking concentrations. Furthermore it can be argued, that the LL spiking experiment is more important to judge the method's applicability for trace level analysis, or conversely, that constant extraction recoveries over larger ranges in concentration implicates less variance in precision between the analyses of high and low concentrated samples.

Baseline noise for methiocarb and prochloraz was tenfold higher during one validation sequence (second validation for urine and serum, first validation for breast milk) compared to the remaining two validations in all matrices. Without changing any of the properties of the LC system or any additional thorough washings of the column, this issue was resolved with subsequent measurements and the baseline noise during the remaining validations remained at the expected levels. A temporary system contamination with isobaric impurities could be an explanation. For this reason however, LL spikes were not detected in urine and serum for prochloraz and methiocarb, while prochloraz could not be calibrated in breast milk during the validation run mentioned above. Consequently, both substances were not validated at the LL in urine and serum. Judging by the robust LL extraction recoveries from the other two validations for both compounds in urine and serum, validation at the LL would have most likely been successful without this issue. However, the sensitivity to successfully detect the spiked concentrations of prochloraz in breast milk would have been too low in any case.

Similarly, a retention time shift for MEHP, PFOS and AAI during the last validation sequence for breast milk just outside the programmed MRM window prevented successful validation. MEHP was already known to be prone to shifts in retention times, since two LL spikes during one urine validation also shifted slightly outside the MRM window. This issue however resolved in subsequent measurements of unknown breast milk samples using the same matrix matched standards that had been used for validation three. Only considering the remaining two validation sequences, PFOS would have been on track towards successful validation in breast milk, keeping in mind however, that there is no available data on repeatability in breast milk, since the repeated measurement too was affected by this temporary change in elution properties.

It was not possible at all to calibrate *p*OHBA, HMFA in urine, MEHP, cotinine in serum and benzyl butyl phthalate, dibutyl phthalate, nonylphenol in breast milk since matrix contamination was too high to differentiate between matrix matched standards. In addition, LL and HL spikes of cotinine in breast milk and LL spikes of trans-3-hydroxy cotinine in urine as well as LL and HL spikes of trans-3-hydroxy cotinine in serum and breast milk could not be differentiated from natural matrix contamination. Higher matrix matched standard concentrations would have been needed to calibrate these compounds. This demonstrates that an ultra-sensitive approach down to the system's LOQ/LOD for these compounds in these matrices is unnecessary.

Lastly, triclosan met all validation criteria in serum with the exception of the limit of at least four identification points (one precursor mass and two transition products), since only one transition was established. Thus, certain qualitative identification of triclosan in serum is not feasible using this method, but quantification if contamination is known is still possible. However, the main application of this method is supposed to be the detection of trace levels in biological matrices, which comes with the need of certain identification.

6.2. Comparison of transferred compounds using the QTrap 6500$^+$ and the TSQ Vantage

Median improvements in sensitivity for all detectable xenoestrogens were around 20 fold in urine, 17 fold in serum and 25 fold in breast milk. Worse LOQs by factors of 0.3-0.9 were determined for 2-*tert*-butylphenol in serum/breast milk, enterolactone in urine, isoxanthohumol in serum/breast milk and OMC in serum. Glucuronides were not detected in urine as mentioned before, while 4OHE1 was barely detected in validation run two in serum (~LOD). While detectable after method transfer, the system used by Preindl et al. [278] was not able to detect 2-*tert*-butylphenol in urine, OMC and triclosan in breast milk at all.

TBPA, fenarimol, 4OHE1 and xanthohumol are compounds that were not successfully validated in any matrix by Preindl et al. [278], while validation following method transfer succeeded in at least one matrix. However, MEHP, PFOS, methiocarb, prochloraz, E2-17-GlcA, E3, 2MeOE1, daidzein and α-

ZEL were successfully validated in at least one matrix by Preindl et al. [278]. This was not the case following method transfer for different reasons that were discussed above.

On the former system 55, 53 and 31 out of 75 analytes were in-house validated in urine, serum and breast milk respectively. Of the same 75 compounds, merely 32, 40 and 14 were successfully validated at both spiking levels after method transfer. As mentioned before, it was expected that certain compounds would most likely only be able to be validated in one matrix or only at one spiking level in the other matrices, since the gap in sensitivities between the matrices increased with the new instrumentation and calibration was designed with the most sensitive matrix in mind. Therefore, a simple comparison of the amount of successfully validated analytes does not accurately describe whether method transfer was or was not successful. The exact outcomes for each substance need to be considered individually. However, an overall gain in sensitivity was achieved for most compounds.

Extraction recoveries between 81-110% were achieved for ~80% of compounds in urine/serum and ~45% in breast milk on the former system. Similar extraction efficiencies were achieved for urine and serum, while only ~6% of analytes could be recovered at 81-110% efficiency, ~48% between 50-70% and 33% below 50% in breast milk after method transfer (Figure 44). Similar problems were encountered by Preindl et al. [278] using the same sample preparation protocol for breast milk, yet extraction recoveries were significantly higher. Therefore, lower compound concentrations or individual practical execution are possible reasons for this significant difference in extraction efficiency.

6.3. Ion ratio, retention time and peak shape

The small polar compounds acrylamide, HMFA and the disinfection by-products all eluted before or at one minute. Chloroacetic acid was not included in method validation as it was not discernible from interfering peaks in any matrix. For bromoacetic acid, this was at least possible in serum using the heavy isotope as a quantifier transition, albeit still difficult to differentiate from interfering peaks. It was expected that it would be a challenge to include aforementioned polar compounds with non-polar xenoestrogens using a reversed-phase LC method that was optimised for the latter.

Similarly to the method on the former system [286], *p*OHBA elutes in several broad peaks among many interferences in a large retention time window starting from the dead time up until around three minutes in some cases in all matrices. Figure 53 C (see Appendix) illustrates the difficulty of correct peak selection for *p*OHBA as even the IS elutes in several wide peaks. Variations in this behaviour between validations were observed as well. Accurate peak annotation to enable regression was only possible by using the internal standard and the changes in peak height with different standard levels as reference. Even if validation was a success, accurate peak identification would be practically impossible for unknown samples without standard addition.

Baseline separation for butylparaben and isobutylparaben was not achieved either, however separate linear calibration was easily possible for both isomers individually as stable retention times and two clearly distinct peaks were observed (Figure 52 D see Appendix). Complete baseline separation for the isomers of anisodamine was not achieved either, however both peaks were integrated collectively since no qualitative information on the isomers was available in any case (Figure 53 A see Appendix). PFOS isomers (Figure 53 B see Appendix) were not quantified separately for the same reason.

Peak tailing was observed for AL, 4OHE1 and AME in all matrices and the solvent calibration (Figure 52 A-C see Appendix). Peak widths were between 0.5 and 1 minute and thus relatively long compared to all other compounds. All of these observations stayed constant for the mentioned substances during all validation measurements for high and low standard concentrations. This indicates that they were not caused by newly acquired impurities on the column as a result of repeated measurements, column overload or general issues with column voids, the injection itself or the pump system, as this would have affected all compounds. Column pressure after equilibration also stayed similar between validations. The column had been used for other projects to analyse complex biological matrices beforehand, but was still far from being considered old or excessively used. Eluents were freshly prepared for all measurements. This points towards issues with retention itself for these compounds, meaning that the chemical properties of the column or the eluent composition might have not been ideal.

As mentioned before, the only notable problem with LC retention itself occurred with the third breast milk validation sequence, where several compounds displayed a shift towards longer retention times compared to the previous measurements. PFOA and AAI shifted by 0.7 minutes, MEHP and PFOS by more than one minute and a single peak of *p*OHBA was observed for the first time during method validation at 2.5 minutes instead of being spread in multiple peaks starting from 0.9 minutes. Moreover, isomers of anisodamine were not baseline separated in the highest concentrated standard for the first time during said measurement. The unknown urine samples were measured as well following the validation sequence. While solvents were freshly prepared for each validation sequence, contamination of one or both eluents with traces of formic- or acetic acid when exchanging eluents is the only possible explanation that comes to mind, as this issue was resolved with later measurements with new eluents. The resulting reduced pH may be the reason for the different retention properties of pH-sensitive compounds. The fact that all influenced analytes, apart from anisodamine, are acids fortifies this assumption. The low pH may have led to the creation of neutral species which then retained longer on the reversed phase column.

Ion ratios of the quantifier and qualifier transitions of 2-naphthol, OP, E2-3-sulfate (only in serum, breast milk), 2MeOE1, 2MEOE2, *p*OHBA and 3-hydroxy phenanthrene were equal or below 5%. This is problematic when it comes to the analysis of unknown trace amounts in real samples if the

quantifier transition features several interfering peaks nearby, but it did not pose any problems for method validation as the presence of the analytes was expected.

As mentioned before, no second transition for triclosan was established, while no ion ratio between bromoacetic acid and its isotope could be calculated in the biological matrices because of early eluting interferences. This demonstrated the selectivity problem concerning the analysis of small organic acids that do not feature several collision products in tandem MS.

Ion ratios stayed constant between matrices for all compounds except E2-3-sulfate in urine, which features twice the relative area of the qualifier transition and thus a lower ion ratio compared to serum and breast milk. A potential co-elution of an impurity may be an explanation as interferences were most commonly observed with conjugated compounds in urine (glucuronides or sulfates). However, peak shapes of the qualifier transition did not differ in urine from the other matrices or the solvent standards, which does not support this hypothesis unless the impurities were non-separated isomers of E2-3-sulfate.

Contrary to the pre-experiments, ion ratios of TBPA did not change depending on the matrix. Thus, while the incorporation of the third transition may have not been necessary, its high ion ratio remains an aid to peak identification.

6.4. Sensitivity and applicability to biological samples

SSE seems to be more compound dependent than matrix dependent. Different compounds showed either high signal suppression or enhancements in different matrices, hence distinct trends are not discernible. In general, SSEs were not as extreme in serum compared to urine and breast milk as the majority of compounds displayed SSEs between 50 and 120% (Figure 45).

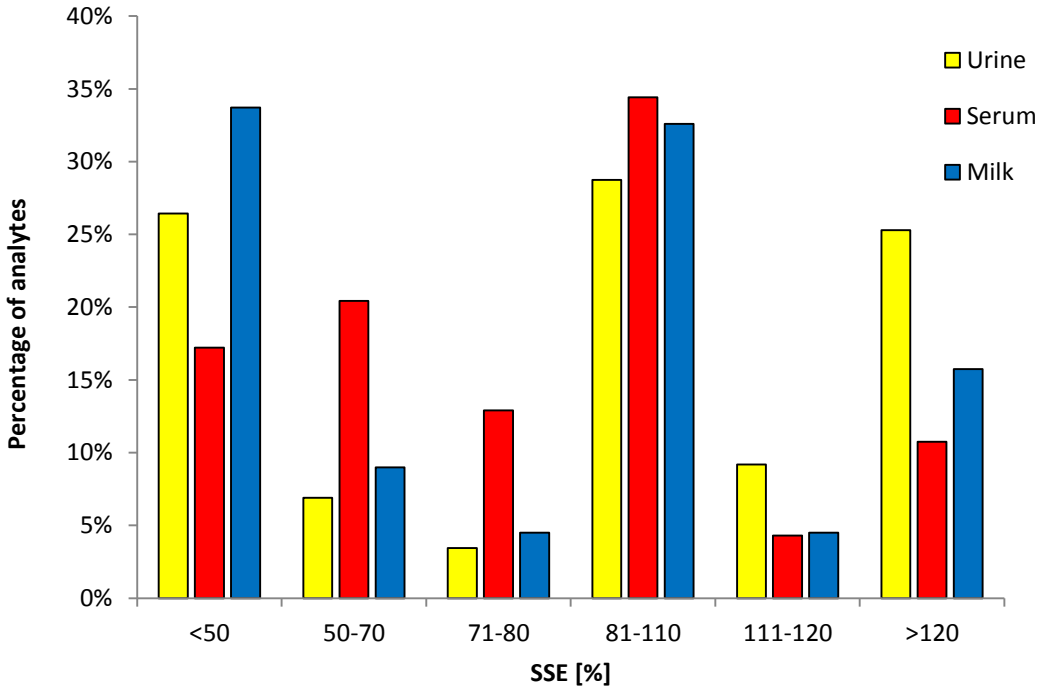

Figure 45: Signal suppression or enhancement of analytes the three biological matrices

However, Figure 45 displays average SSEs of all three validations. SSEs for some compounds were stable, while others showed high variance between validation experiments (see SSE standard deviations in Table 14 in section 5.4). The best example is resveratrol, whose SSE ranged from 260% to 110% in urine, 210% to 50% in serum and 570 to 130% in breast milk. Interestingly, these large differences in SSE for some compounds were without exception caused by variance in the slope of the solvent calibration, while the matrix calibration remained stable in comparison.

Improvements of LOQs of E1 and E2 towards 0.009 ng/mL and 0.08 ng/mL respectively are noteworthy as detection and quantification of physiological levels of E1 and E2 in men and women alongside xenoestrogens may now be possible using this LC-MS/MS approach [288].

It was expected that the additional hydroxyl group would result in superior ionisation of PAHs in negative mode. This was the case for 1-hydroxy pyrene and 3-hydroxy phenanthrene, however not for 3-OH-BaP. It appears that the single hydroxyl group did not provide enough polarity to enable sensible negative ionisation yields of the aromatic 5-ring system of B[a]P. Thus, the positive polarity remained the better choice. Sensitivities for 1-hydroxy pyrene and 3-hydroxy phenanthrene reach levels that were detected before in human urine using gas chromatography [289]. As both have

successfully been validated in urine and serum, the method now may be used to assess smoke exposure. On the other hand, sensitivities for cotinine and trans-3-hydroxy-cotinine are far beyond needed sensitivities to detect usual biological concentrations as mentioned before. The very high LOQs of 3-OH-BaP (~14 ng/mL in urine, ~10500 ng/mL in serum) and NDEA (~2200 ng/mL in urine and ~6200 ng/mL in serum) indicated that detection of physiologically relevant concentrations of benzo[a]pyrene (<1 ng/mL in serum following smoke exposure [290]) or 3-OH-BaP (<0.001 ng/mL in urine of smokers [291]) and NDEA (~0.1 ng/mL in smoker urine [292]) would not be possible using this method.

The major phase-two metabolite of glycidamide *N*-(*R*/*S*)-Acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine has only been detected below concentrations of ~50 ng/mL in urine so far [293]. Thus, it was approximated that the LOD of glycidamide in this method (~330 ng/mL in breast milk) would be too high to detect any trace amounts. The lack of an acidic additive (i.e. formic acid) is suspected to be a reason of the low ionisation efficiencies of glycidamide and NDEA, as much lower LOQs (10 ng/mL glycidamide in plasma [294] and 0.04 ng/mL NDEA in urine [292]) have been achieved before using LC-ESI-MS/MS. Similarly, even if NDMA was successfully validated, the LOQs of ~200 ng/mL would be far off any biological concentrations as well [292].

Interestingly, with the exception of NDMA and bromoacetic acid, the small and rather polar compounds acrylamide, HMF, HMFA and the dihaloacetic acids exhibited extraction recoveries (>65%) comparable to some xenoestrogens in serum, although the sample preparation protocol was developed to extract mostly non-polar compounds.

Sensitivities for the tropane alkaloids anisodamine and scopolamine in blood and serum are beyond the needed sensitivities to detect acute poisoning [295], hence this method may be suitable to assess long term exposure as well. So far, exposure to aristolochic acids has been assessed via aristolactam-DNA adducts or other biomarkers [296]. While direct measurement of the precursor compounds in biological matrices has not yet been documented in humans to the best of our knowledge, lower LOQs in urine have been documented before using acidic LC additives [297].Similarly, pyrrolizidine alkaloid-derived DNA adducts have been detected in biological matrices of animals before [298], while data concerning long term exposure and biological concentrations in humans remains missing. However, sensitivities below 0.05 ng/mL for jacobine, jacobine-N-oxide and riddelliin-N-oxide in serum and slightly lower in breast milk are promising and have not yet been reported before. Comparable sensitivities for PhIP in urine and serum samples have been documented, however no physiological concentrations of PhIP were detected without additional acidic or enzymatic hydrolysis [299].

The fundamental feasibility of analysis of disinfection by-products in biological matrices using this LC-MS/MS approach has been demonstrated, but sensitivities above 1 ng/mL in biological matrices are still rather low, considering that drinking-water concentrations have only been detected in a similar concentration range [300] and that data on metabolisation is still lacking.

The system used in this work outperforms some similar, more specialised methods for the determination of far smaller numbers of xenoestrogens that are mentioned in section 1.3.3. Sensitivities for parabens in serum are 10- to 100 fold better than the sensitivities reported by Sosvorova et al. [266], while sensitivities for bisphenols and endogenous estrogens are comparable (between 10 fold and 0.1 fold). Fleck et al. [276] report sensitivities for the isoflavones genistein, daidzein and equol in the 2-20 ng/mL range in urine, while this method achieves sensitivities ranging 0.01-0.02 ng/mL for the same analytes in urine. While Rocha et al. [273] achieved similar sensitivities for bisphenols and triclosan in urine, sensitivities for parabens and benzophenone 1 were lower by a factor of 10 to 100. While not all analytes were successfully validated in this work, it demonstrates nonetheless that it is possible to monitor multiple classes of structurally different xenobiotics in the same method with better or similar sensitivities compared to more exclusive LC-MS/MS methods.

6.5. Application to biological samples

Of the 95 compounds included, 42 were detected and quantified in the urine samples, 31 in the serum samples and 29 in the breast milk samples. These results confirm the general applicability of the method to different biological matrices. Keeping in mind that not all analytes were successfully validated at both the LL and HL concentrations, a semi quantitative analysis is still a possibility in these cases.

6.5.1. Urine

The detection of multiple endogenous estrogens in some urine samples (high levels in sample Z) and the differences among phytoestrogen concentrations between samples (i.e. high contents in sample J) are notable. Sample Z was most likely spiked by the corporation partner who sent the urine samples, while no data on the habits of the urine donors was known. Furthermore, only one replicate of sample J yielded high concentrations of TBPA. This is unexpected since TBPA showed high extraction recoveries, no large interferences and was successfully validated in urine during method validation. Moreover, the sample was not injected after a highly concentrated standard, thus carryover from the previous injection cannot explain this either. Similarly, high contaminations with prochloraz in sample N and 8-prenylnaringenin in sample L were detected in one replicate, yet not in the second. All three of these observations were confirmed with further EPI scans.

6.5.2. Serum

Endogenous estrogens were successfully quantified in a few serum samples. The samples of prematurely born babies showed high variability in content of different xenoestrogens. For instance BPA and PFAs concentrations ranged from >10ng/mL to <1 ng/mL, in some cases even higher than in the adult samples. High amounts of BPA, methyl- and propylparaben were detected in baby sample PB 24.

TBPA was detected in sample PB 57. Unfortunately, the latter's presence could not be further examined using additional EPI scans as no more sample volume was available. Since TBPA was also detected in one urine replicate, but not in the second, as mentioned above, contamination during sample preparation may be an explanation.

Lastly, HMF was detected in sample PB 41, which also featured the highest concentration of HMFA, thus being a first proof of principle for the simultaneous monitoring of a food processing-by product and its metabolite.

BPA, N-butylbenzenesulfonamide, benzyl butyl phthalate, dibutyl phthalate, PFOA, PFOS, 2-naphthol, nonylphenol, ethylparaben, methylparaben, propylparaben were detected in all serum samples. Figure 46 compares the number of identifications of all compounds and illustrates which classes of compounds are the most prevalent. According to these results, synthetic compounds constitute the majority of the xenoestrogenic burden which is measurable using this method, while nutrition-associated xenobiotics are far less represented. In addition, cotinine was detected in 19 of the 21 baby samples, which points towards the possibility of systemic passive smoke exposure. Combining all of these findings with the individual medical histories of the babies may result in interesting conclusions following further data evaluation.

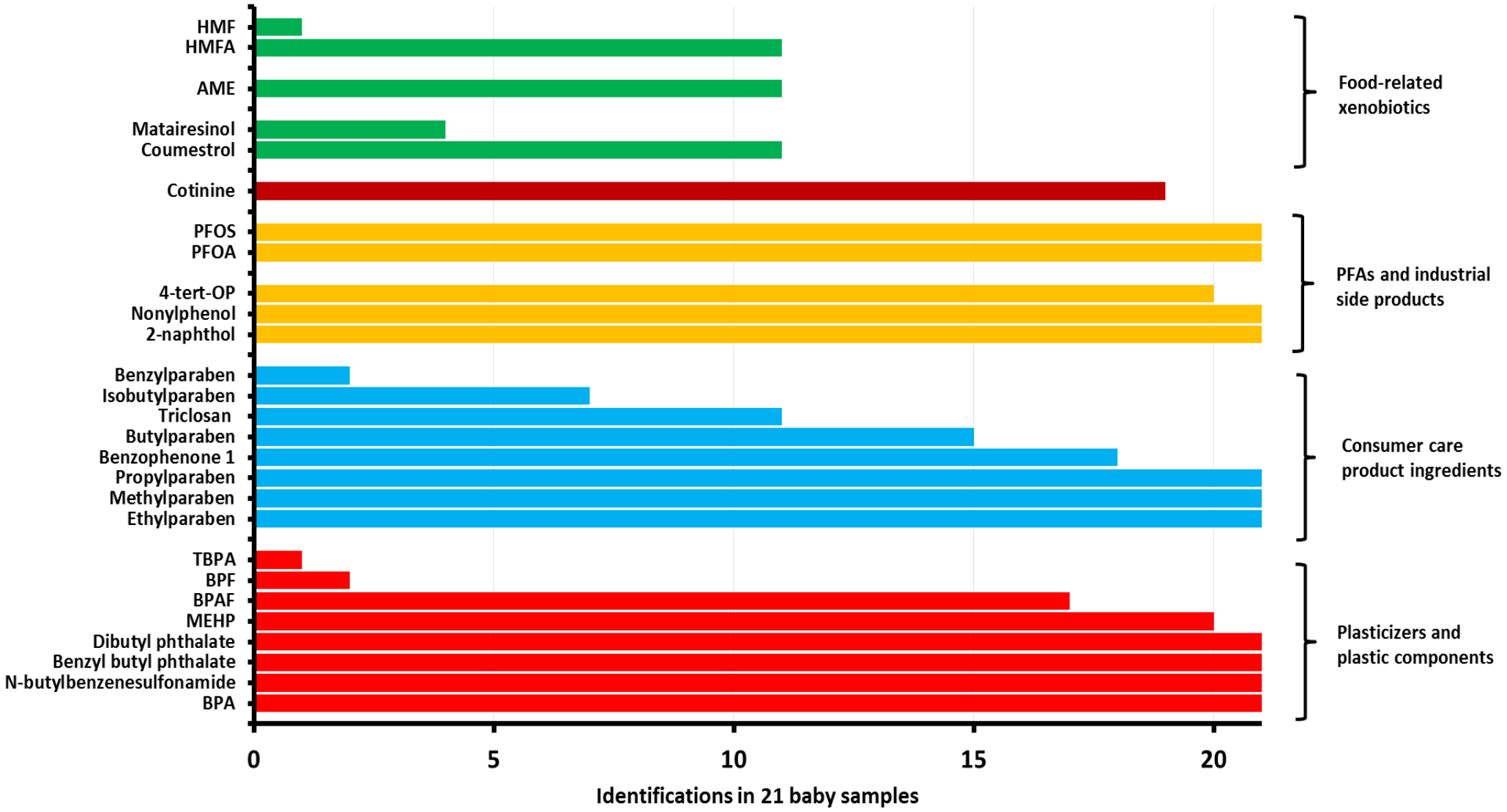

Figure 46: Number of identifications of each xenobiotic among the 21 baby serum samples.

6.5.3. Breast milk

To the best of our knowledge, the detection of the pyrrolizidine alkaloid metabolites jacobine- and riddelliin-N-oxide as well the tropane alkaloids anisodamine and scopolamine in the breast milk samples have not yet been reported before. It was known that the individual regularly consumed herbal tea mixtures during the sampling period, which are known sources of these phytotoxins. In addition, PhIP was detected below the LOQ in samples of two successive days and high contamination of prochloraz in only one sample. The presence of these compounds were confirmed by second injections with volumes of 10 µL to see if the detected peaks increased in size and were therefore no system-interference, and eventually further EPI scans.

Of note are high concentrations of methylparaben and propylparaben (~100 fold higher compared to the remaining samples) detected for day nr. 209. Both of these substances were not detected in the system blank and are therefore no contaminants of the LC-system. A major lifestyle change or the utilisation of a new consumer product during the 28 days after the previous sample was taken could have created this large increase in paraben contamination. Rises and decreases in methylparaben and propylparaben concentrations correlate throughout the whole period of sampling.

While phytoestrogens show very dynamic changes in detection levels over the sampling period, paraben concentrations are less dynamic and PFA levels remained even more stable in comparison to the remaining analytes. These observations may be associated with the compounds' routes of exposure and chemical properties. Phytoestrogen exposure is mostly dependent on nutritional intake, which may change completely between days, while paraben exposure is associated with the usage of personal care and consumer products, which may not be as variable as nutrition. Exposure to PFAs is widespread from private to industrial and environmental settings. Their long half-life may be a contributing factor to the comparatively constant rate of detection during the 209 day sampling period. To illustrate this behaviour, Figure 47 dynamically displays the concentrations of PFOS, ethylparaben and daidzein as selected analytes.

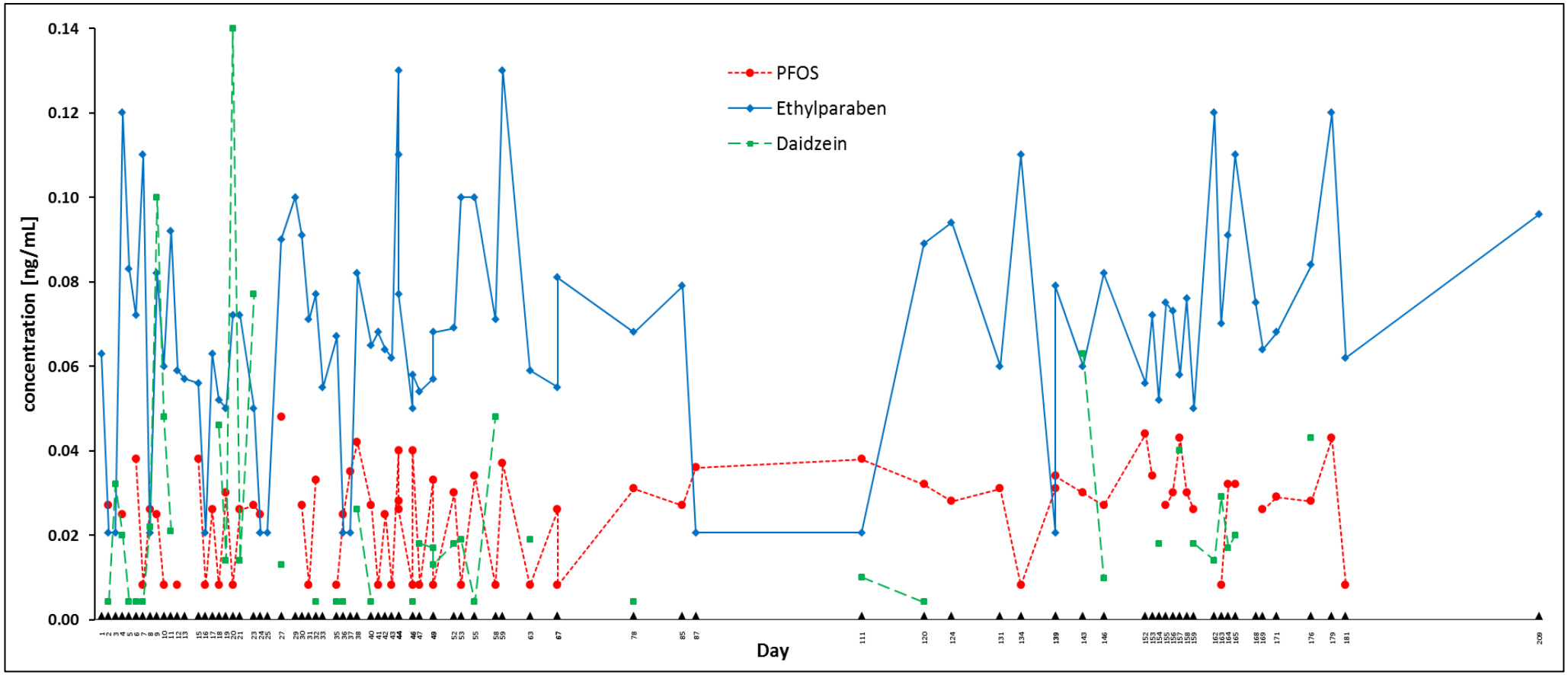

Figure 47: Exposure over time of selected compounds in the breast milk samples. Days of sampling are annotated on the x-axis. Points connected by lines depict detections on consecutive days of sampling. Analytes detected below the LOQ are depicted at concentrations of half their respective LOQ

6.5.4. Confirmational MS2 scans

It was expected that the IDA EPI method would conduct EPI scans as long as the MRM threshold was surpassed, i.e. scanning the whole MRM peak of the compound. However, scans were only done at the single point in time after the threshold was surpassed (Figure 48).

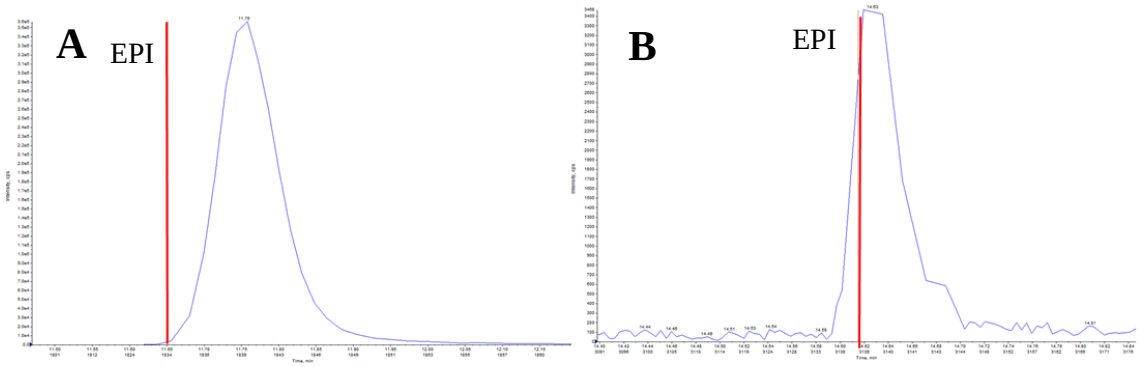

Figure 48: Point in time of the single IDA EPI scan along the compound MRM peak. 8-prenylnaringenin in urine sample L (A) and prochloraz in urine sample N (B)

For highly concentrated samples (8-prenylnaringenin in urine, methylparaben and HMFA in serum) this still yielded characteristic spectra, but it is not ideal for the application to lowly concentrated samples. In some cases (TBPA and prochloraz in urine, 2MeOE1 in serum) the EPI scans were conducted close to or at the peak maximum, but these were only exceptions and not the rule in numerous pre-experiments and setups of the method. The exact peak heights would need to be known to catch the ideal time to trigger the scan, however this would not have been feasible as peak heights may change with different measurement. Thus, a full scan approach needed to be designed.

However, a big advantage of the IDA EPI method was the dynamic background subtraction, which is useful to acquire high quality spectra for low concentrated compounds. Unfortunately, no dynamic background subtraction was possible with the full scan EPI method with optimised single CE values. Manually excluding interfering masses would be a big additional effort and not feasible if only low sample volumes were left. The system uses a dynamic fill time depending on the most abundant fragment masses to enhance their yield. Consequently, spectra of 2MeOE1 in serum yielded only interfering fragments with no precursor or quantifier/qualifier masses. For this reason, the results of the IDA method were preferable in this case.

The IDA EPI method was used for the confirmation of TBPA in the first replicate of the urine sample J. The measured concentration was low, but the EPI scan was triggered at the peak's maximum for both the standard and the sample. The precursor ion mass of 542.5 Da was still identified, however the qualifier transition (445.8 Da) and both secondary transitions (419.8 Da and 417.9 Da), while still present, are not intense enough to be easily discernible from interfering fragments. However, MS2

scans of both the sample and the standard yielded a similar peak pattern. As the precursor ion was present at a high intensity, a higher CE voltage could have resulted in more intense fragments.

Similarly, higher CE voltages could have produced more characteristic MS2 spectra for prochloraz in the urine sample or the urine standard using the IDA method, as the precursor intensities in Figure 33 are still comparatively high, while quantifier (307.8 Da) and qualifier (265.7 Da) fragments have not been detected. However, higher CE voltages were used with the full scan EPI method in the breast milk sample and breast milk standard 100 (Figure 44), yet no quantifier or qualifier masses were detected either. Characteristic fragments were either lost among interferences, or they further dissociated into smaller fragments which are not known. In both cases however, MS2 spectra are similar between standards and samples in urine and breast milk.

The EPI scans of 8-prenylnaringenin in urine, HMFA and methylparaben in serum clearly confirmed their presence even though the IDA methods was not triggered at the peak maximum. While precursor intensities are still rather high in the MS2 spectra, the high concentrations still yielded enough MS2 fragments for a feasible comparison between the standard scans and the unknown samples. The EPI scans of BPA and PFOS in serum, PhIP, jacobine-N-oxide, riddelliin-N-oxide, anisodamine and alternariol in the breast milk samples showed very similar MS2 spectra including both the quantifier and the qualifier transition in comparison to the respective standards. Spectra for 2MeOE1 in serum are very similar as well, however only the quantifier transition was detected.

The low concentration of scopolamine in the monitored sample (below the LOQ of 0.00014 ng/mL) yielded only very low intensities of the expected fragments, but both were present and the remaining most abundant peaks are similar between the standard and the sample. Perfect accordance cannot be expected with such low concentrations and the different sample matrix may also influence the presence and ratios of certain fragments in the MS2 spectra in general. Achieving high quality MS2 spectra for low concentrated substances is difficult as the fragments originating from the precursor may have a low intensity and may be barely distinguishable from matrix interferences.

A lot of trial and error went into the creation of demonstrative EPI scans to illustrate the presence of certain analytes. Too high CE voltages fragmented all of the precursor mass, while low CE voltages produced few fragments and thus low qualifier/quantifier intensities. The background subtraction of the IDA method was advantageous to discern interferences from fragment masses, but it was difficult to produce spectra at the compounds MRM peak maximum. In some cases, using EPI scans to confirm the presence of trace level contaminations below the LOQ was shown to be problematic (TBPA, 2MeOE1) without background subtraction. The presence of high amounts of interferences resulted in skewed MS2 spectra where characteristic fragments are hardly discernible. Even if EPI scans of standard and sample yield similar MS2 spectra, confirming the presence of a compound using its precursor fragmentation pattern is only really plausible if known characteristic MS2 fragments are

detected. The usage of Q1 resolution higher than unit resolution in the EPI scan could potentially reduce interfering precursor masses and thus reduce MS2 interferences, however this would also reduce the actual compound of interest's precursor yield.

6.5.5. Problems and challenges during the analysis of unknown samples

The trace analysis of analytes that contaminate the LC-system or are introduced during the extraction (most notably BPA, BPS, butyl-/dibutyl phthalate, nonylphenol and benzophenone 1) can be subject to comparatively high imprecision, as in addition to variable extraction efficiencies, the variance in system contamination between samples may be higher than the natural contamination of the sample itself. Even though a system blank correction was done, this may partly explain the large disparities between some biological replicates in the urine samples for butyl-/dibutyl phthalate and nonylphenol. The larger the system contamination compared to the actual sample concentration, the larger the impact of this effect on quantification.

MBP, PFOA, PFOS, BP, EP, MP and PP were quantified using internal standard correction in the breast milk samples. Hence, samples which were injected a second time with double the volume were corrected by double the IS standard concentration. Consequently the second injection was expected to yield similar concentrations as the first injection for these IS-calibrated analytes. All other compounds were expected to yield a concentration twice as high, as no IS correction took place. This assumes that the higher injection volume would not result in stronger matrix effects. The second injection was primarily done to confirm the presence of phytotoxins, however it also pointed out this issue of variance in system blank contamination concerning the quantification of other analytes such as benzophenone 1. Here, the calculated concentrations areas did not double in many cases. The reason for this may be that benzophenone might not have been present at all in these breast milk samples, and that all the detected peaks may only stem from system contamination that was higher than the contamination of the system blank sample which was used to correct for system contamination. No difference between the 5 µL and 10 µL injections means that the detected signal originates most likely from system contaminations and not from the sample itself, since true sample contamination can never be irrespective of the injected volume.

The highest matrix matched standards for daidzein, enterodiol, enterolactone, genistein, glycitein, E1, E2 and E3 were lower than the contents detected in several urine samples. Similarly, 2-naphthol, PFOA and PFOS concentrations in the serum samples exceeded the highest concentrated standards. This is why the quantitative data for highly contaminated samples may potentially be inaccurate. However, no de-linearization of the calibration towards higher concentrations was observed for these analytes during method validation. Moreover, the method was primarily developed to conduct trace level analysis. If higher concentrations are to be quantified, the addition of further standard solutions is always possible in any case. Alternatively, highly concentrated samples may be diluted and measured again.

Lastly, the feasibility of an accurate determination of LOQs/LODs needs to be discussed. For method validation, they were determined for the respective matrix that was used for method development. If the calculated concentration in an unknown sample was below the pre-determined LOQ but above the LOD, the compound was merely declared as "detected". However, the baseline noise for some analytes in some unknown samples may be higher or lower than the noise in the calibration matrix and peak shapes may differ as well. Standard matrices and sample matrices will never be identical. This means that, if not inspected manually, some compounds may likely be declared as "detected below the LOQ" even though the S/N ratio of the peak might have been above 10 in the unknown sample. On the other hand, some analytes would have still been quantified in the unknown samples, even though S/N of below 10 were clearly the case. The inherent variation in sensitivity between different measurements (see standard deviation of LOQs in Table 14 in section 5.4.) is another factor that adds to this issue. The calculated concentration of HMF in the baby serum sample PB 41 of 3.9 ng/mL (before accounting for the extraction efficiency) featured a S/N of above 10, yet the LOQ following method validation in serum is declared at 16 ng/mL as one validation measurement featured a much higher baseline noise. It does make sense to approximate the sensitivities of a method, however the application to a different sample matrix than the one used for method development and calibration may differ from these estimates to some extent. Ideally, when measuring unknown samples, the declaration of method sensitivity should be based on its performance on that day, thus taking noise disparities between the sample- and matrix standards and variations in system baseline noise into account. However, this comes with additional effort as thorough manual inspection of peak shapes and S/N ratios would be needed.

7. Conclusion and outlook

A unique multi-analyte LC-MS/MS method involving 75 xenoestrogen was transferred onto a new MS system with the addition of 20 new xenobiotics. Improvements in sensitivities were achieved when compared to the former method, however validation did not succeed for as many compounds as it did for Preindl et al. [278]. Disparities in sensitivities between matrices, the generally lower spiking concentrations and the naturally occurring contaminations of the spiked matrices are main reasons for this outcome. Most compounds that failed full validation at either only the low spiking level or merely because of lacking repeatability compared to the intermediate precision. This should not necessarily detract from the method's general applicability for a very high number of xenobiotics. In these cases, the method may be used for semi-quantitative screening at extremely low concentrations rather than absolute quantification.

Breast milk remains the most demanding matrix concerning quantitative extraction and its variance by far. Improvements of the sample preparation protocol are always a possibility. Nonetheless, this should not come in the form of a completely new, highly complicated and sophisticated approach, since the underlying purpose of this method is supposed to be a quick and easy, yet comprehensive monitoring approach for a multiclass assessment of environmental and food-related contaminations.

The analysis of unknown samples yielded a wide range of detected analytes, among them the PFAs in all matrices, high amounts of phytoestrogens in urine, first time detections of phytotoxins in breast milk and the improved sensitivities of endogenous estrogens even allowed their quantification in serum samples. Few analytes were detected in concentrations far above the calibration range. The incorporation of additional standards is a possibility if accurate quantification of highly contaminated samples is needed. However, the primary purpose of this method is the detection and quantification of trace levels of xenobiotics.

In summary, the presented results clearly demonstrate the method's applicability and its potential to be applied in HBM and epidemiological studies for correlating xenobiotic exposure and disease development in the future.

8. Appendix

Equations 9 and 10 display Dixon's Q–tests for small- and large outliers respectively (values arranged in ascending order: X_1, X_2, … X_n). Assuming a confidence interval of 95%, a critical value Q of above 0.493 (n = 9) reveals a statistically relevant outlier [301].

$$Q = \frac{X_2 - X_1}{X_n - X_1} \qquad \text{Eq. 9 for small outliers} \qquad Q = \frac{X_n - X_{n-1}}{X_n - X_1} \qquad \text{Eq. 10 for large outliers}$$

Equation 11 describes the Grubbs's test with the data set's mean $\overline{X}$, standard deviation σ and a suspected outlier X_o. Here, a critical value Z of above 2.21 reveals a statistically relevant outlier [301].

$$Z = \left| \frac{\overline{X} - X_o}{\sigma} \right| \qquad \text{Eq. 11}$$

Table 24: Reagents included in this work

Reagent	CAS	Catalogue number	Supplier	Lot number
Ammonium fluoride	12125-01-8	52481-50g	Honeywell Fluka	H0790
LC-MS grade water		83645.320	VWR chemicals	various
LC-MS grade acetonitrile (ACN)	75-05-8	34967	Honeywell Riedel-del Haen	various
LC-MS grade methanol (MeOH)	67-56-1	34966	Honeywell Riedel-del Haen	various
Magnesium sulfate (anhydrous)	7487-88-9	413485000	Acros organics	A0379632
Sodium chloride	7647-14-5	9268.1	Roth	406250348
Human serum		H4522	Sigma Aldrich	SLBW8068

Table 25: Compounds included in this work and supplier information

Compound	CAS	Catalogue number	Supplier	Lot number
$^{13}C_{12}$-Bisphenol A (BPA)	263261-85-0	720186-5.00mg	Sigma Aldrich	MBBB1923V
$^{13}C_{18}$-Zearalenone (ZEN)			Romer Labs	
$^{13}C_{2}$-Mono-*n*-butyl phthalate (MBP)		CLM-4590-MT-1.2	Cambridge Isotope Laboratories	SDIC-008
$^{13}C_{2}$-mono-2-ethylhexyl phthalate (MEHP)		CLM-4584-MT-1.2	Cambridge Isotope Laboratories	SDHC-027
$^{13}C_{3}$-Estradiol (E2)	1261254-48-1	719552-1mg	Sigma Aldrich	MBBC4575
$^{13}C_{6}$-4-*tert*-octylphenol (4-*tert*-OP)	1173020-24-0	33565	Sigma Aldrich	BCBW5639
$^{13}C_{6}$-Butylparaben		32125	Sigma Aldrich	BCBT7363
$^{13}C_{6}$-Ethylparaben		32125	Sigma Aldrich	BCBT7363
$^{13}C_{6}$-*p*-hydroxybenzoic acid (*p*OHBA)	267399-29-5	587869-10.0mg	Sigma Aldrich	MBBB9644V
$^{13}C_{6}$-Propylparaben		32125	Sigma Aldrich	BCBT7363
$^{13}C_{8}$-Perfluorooctanoic acid (PFOA)		ES-5571	Cambridge Isotope Laboratories	PR-28266
$^{13}C_{8}$-Perfluorooctanesulfonic acid (PFOS)		ES-5571	Cambridge Isotope Laboratories	PR-28266
16-Epiestriol (16EpiE3)	547-81-9	E586500	TorontoResearchChemicals	29-AZC-158-2
16-α-Hydroxyestrone (16OHE1)	566-76-7	H941900	TorontoResearchChemicals	3 CGF-676
17-Epiestriol (17EpiE3)	1228-72-4	E586510	TorontoResearchChemicals	20-THT-39-2
1-Hydroxypyrene	5315-79-7	H952700	TorontoResearchChemicals	8-BHW-120-2
2-*tert*-Butylphenol (2-*tert*-BP)	88-18-6	B99405-50mL	Sigma Aldrich	SHBD7123V
2-Hydroxyestradiol (2OHE2)	362-05-0	H941890	TorontoResearchChemicals	5-SBT 161-4
2-Methoxyestradiol (2MeOE2)	362-07-2	89242-1mg	Sigma Aldrich	BCBT7859
2-Methoxyestrone (2MeOE1)	362-08-3	73532-25mg	Sigma Aldrich	BCBS2816V
2-Naphthol	135-19-3	185507-5g	Sigma Aldrich	BCBV0179
3-Benzylidencamphor (3-BC)	15087-24-8	91556-25mg	Sigma Aldrich	BCBT9983
3-Hydroxybenzo[a]pyrene (3-OH-BaP)	13345-21-6	H829400	TorontoResearchChemicals	6-MRS-68-3
3-Hydroxyphenanthrene	605-87-8	P295010	TorontoResearchChemicals	3-GRS-168-1
4-Hydroxyestrone (4OHE1)	3131-23-5	H941950	TorontoResearchChemicals	5KH 128-1
4-Methoxyestradiol (4MeOE2)	26788-23-8	M262630	TorontoResearchChemicals	4-YKZ-7-1
4-Methoxyestrone (MeOE1)	58562-33-7	M226135	TorontoResearchChemicals	1SRE-175-1
4-Methylbenzyliden camphor (4-MBC)	36861-47-9	61547-50mg	Sigma Aldrich	BCBS7570V
4-Octylphenol (4-OP)	1806-26-4	384445-1g	Sigma Aldrich	MKBW6803V
4-*tert*-Octylphenol (4-*tert*-OP)	140-66-9	442858	Sigma Aldrich	LRAB8559
5-Hydroxymethylfurfural (HMF)	67-47-0	W501808	Sigma Aldrich	STBJ7088
5-Hydroxymethyl-2-furanoic acid (HMFA)	6338-41-6	H947020	TorontoResearchChemicals	2-JMO-50-1
8-Prenylnaringenin (8-Pn)	53846-50-7	75119	Sigma Aldrich	BCBR2514V
α-Zearalanol (α-ZAL)	26538-44-3		Sigma Aldrich	
α-Zearalenol (α-ZEL)	36455-72-8	S0242	RomerLabs	L14424Z
α-Zearalenol-14-glucuronide (α-ZEL-GlcA)	Synthesized at the Technical University of Vienna and kindly provided by Dr. Mikula			
Alternariol	641-38-3	A575760	Sigma Aldrich	
Alternariol monomethyl ether (AME)	26894-49-5	A575770	TorontoResearchChemicals	10-CGS-13-1
Acrylamide	79-06-1	A191300	TorontoResearchChemicals	8-ABY-165-1
Anisodamine	55869-99-3	SML0252	Sigma Aldrich	0000049069
Aristolactam I	13395-02-3	A771200	TorontoResearchChemicals	1-AKS-87-1

Compound	CAS	Catalogue number	Supplier	Lot number
Aristolochic acid I	313-67-7	A771300	TorontoResearchChemicals	1-JLW-39-1
Benzophenone 1	131-56-6	126217-100G	Sigma Aldrich	BCBN3480V
Benzophenone 2	131-55-5	T16403-25g	Sigma Aldrich	MKBN6515V
Benzyl butyl phthalate	85-68-7	442503	Sigma Aldrich	LC26422V
Benzylparaben	94-18-8	07389-100mg	Sigma Aldrich	BCBR26688V
β-Zearalanol (β-ZAL)	42422-68-4		Sigma Aldrich	
β-Zearalenol (β-ZEL)	71030-11-0	S0243	RomerLabs	L15241D
β-Zearalenol-14-glucuronide (β-ZEL-GlcA)	Synthesized at the Technical University of Vienna and kindly provided by Dr. Mikula			
Bisphenol A (BPA)	80-05-07	239658	Sigma Aldrich	MKBS0991V
Bisphenol AF (BPAF)	1478-61-1	90477-100mg	Sigma Aldrich	BCBV2156
Bisphenol B (BPB)	77-40-7	50877-100mg	Sigma Aldrich	BCBS0967
Bisphenol C (BPC)	79-97-0	68118-100mg	Sigma Aldrich	BCBV2464
Bisphenol F (BPF)	620-92-8	51453-100mg	Sigma Aldrich	BCBT7018
Bisphenol S (BPS)	80-09-1	43034-100mg	Sigma Aldrich	BCBV2462
Bromoacetic acid	79-08-3	B679075	TorontoResearchChemicals	1-CAL-99-1
Butylparaben	94-26-8	PHR1022-1g	Sigma Aldrich	LRAB3701
Chloroacetic acid	79-11-8	36544	Sigma Aldrich	BCCC8186
Cotinine	486-56-6	74003	Sigma Aldrich	BCCC4550
Coumestrol	479-13-0	BML-S180-0005	Enzo Life sciences	11071708
d4-Genistein	187960-08-3	D-6282	CDN Isotopes	k-457
Daidzein	486-66-8	D7802	Sigma Aldrich	013M4027V
Dibromoacetic acid	631-64-1	442551	Sigma Aldrich	LRAC3539
Dibutyl phthalate	84-74-2	18281-50mg	Sigma Aldrich	BCBV9940
Dichloroacetic acid	79-43-6	36545	Sigma Aldrich	BCBW5201
Estradiol-17-glucuronide (E2-17-GlcA)	15087-02-2	E1127	Sigma Aldrich	4856
Enterodiol	80226-00-2	45198-1MG-F	Sigma Aldrich	BCBT3858
Enterolactone	78473-71-9	45199-1mg-F	Sigma Aldrich	BCBT6193
Equol	94105-90-5	13184	Cayman Chemical Company	0433202-6
Estradiol (E2)	50-28-2	E8875	Sigma Aldrich	SLBP6339V
Estradiol-3-sulfate (E2-3-sulfate)	4999-79-5	E9505-25mg	Sigma Aldrich	067M4032V
Estriol (E3)	50-27-1	E1253	Sigma Aldrich	115H0256
Estrone (E1)	53-16-7	46573-250mg	Sigma Aldrich	SZBE205XV
Ethinylestradiol	57-63-6	E685100	TorontoResearchChemicals	1-TIM-118-1
Ethylparaben	120-47-8	PHR1011-1g	Sigma Aldrich	LRAB3628
Fenarimol	60168-88-9	45484-250mg	Sigma Aldrich	BCBW4607
Formononetin	485-72-3	94334-50mg	Sigma Aldrich	BCBT8620
Genistein	446-72-0	1097	Extrasynthese	Batch 24 ID 0421/0
Glycidamide	5694-00-8	G615250	TorontoResearchChemicals	12-YMK-158-4
Glycitein	40957-83-3	G635400	TorontoResearchChemicals	4-GRS-54-1
Isobutylparaben	4247-02-3	715077-25g	Sigma Aldrich	23283
Isoxanthohumol	70872-29-6	1367S	Extrasynthese	Batch 01 ID: 1020/0
Jacobine	6870-67-3	6219.88	PhytoPlan	18101105
Jacobine-N-oxide	38710-25-7	6222.96	PhytoPlan	19010306
Matairesinol	580-72-3	40043-5MG-F	Sigma Aldrich	BCBW3093
Methiocarb	2032-65-7	36152-100mg	Sigma Aldrich	SZBF106XV
Methylparaben	99-76-3	47889	Sigma Aldrich	LRAB6911
Mono-2-ethylhexyl phthalate (MEHP)	4376-20-9	796832-500mg	Sigma Aldrich	MKCD4270
Mono-*n*-butyl phthalate (MBP)	131-70-4	30751-100mg	Sigma Aldrich	BCBT4836
N-butylbenzenesulfonamide	3622-84-2	B90653-250mL	Sigma Aldrich	MKCD8065
N-nitrosodiethylamine (NDEA)	55-18-5	N525465	TorontoResearchChemicals	50-GHZ-66-1
N-nitrosodimethylamine (NDMA)	62-75-9	CRM40059	Sigma Aldrich	LRAC5069
Nonylphenol	84852-15-3	46018-1g	Sigma Aldrich	BCBT5112
Octyl methoxycinnamate (OMC)	5466-77-3	55529-100mg	Sigma Aldrich	BCBQ5953V
Perfluorooctanoic acid (PFOA)	335-67-1	171468-5g	Sigma Aldrich	MKCC6736
Perfluorooctanesulfonic acid (PFOS)	1763-23-1	33607	Sigma Aldrich	BCBW0899
PhIP	105650-23-5	A617000	TorontoResearchChemicals	7-RCD-119-3
p-Hydroxybenzoic acid (*p*OHBA)	99-96-7	H5376	Sigma Aldrich	090K8911
Prochloraz	67747-09-5	45631-250mg	Sigma Aldrich	BCBW4694
Propylparaben	94-13-3	PHR1010-1G	Sigma Aldrich	LRAB2246
Resveratrol	501-36-0	R5010	Sigma Aldrich	SLBC6832V

Compound	CAS	Catalogue number	Supplier	Lot number
Riddelliin	23246-96-0	6312.98	PhytoPlan	17100501
Riddelliin-N-oxide	75056-11-0	6313.97	PhytoPlan	17100601
Scopolamine	51-34-3	S200005	TorontoResearchChemicals	11-DPM-182-2
Tetrabromobisphenol A (TBPA)	79-94-7	11223-100mg	Sigma Aldrich	BCBW5493
Trans-3-hydroxy-cotinine	34834-67-8	H924500	TorontoResearchChemicals	2-WHH-123-9
Triclosan	3380-34-5	93453-100mg	Sigma Aldrich	BCBS1844
Xanthohumol	569-83-5	13496S	Extrasynthese	Batch03 ID 0524/0
Zearalanone (ZAN)	5975-78-0		Sigma Aldrich	
Zearalenone (ZEN)	17924-92-4	001009	RomerLabs	S13114Z
Zearalenone-14-glucuronide (ZEN-14-GlcA)	Synthesized at the Technical University of Vienna and kindly provided by Dr. Mikula			
Zearalenone-14-sulfate (ZEN-14-sulfate)	Gift from Prof. Berthiller from the University of Natural Resources and Life Sciences Vienna			

Table 26: Compound concentrations in matrix matched standards used in pre-experiments to determine retention times and optimal transitions of xenoestrogens. Analytes marked with (*) were not successfully validated in any matrix by Preindl et al. [278].

Compound	Matrix matched standards				
	100	30	10	3	1
	Concentration [ng/mL]				
Plasticizer/plastic components					
Bisphenol A (BPA)	100	30	10	3	1
Bisphenol AF (BPAF)	50	15	5	1.5	0.5
Bisphenol B (BPB)	100	30	10	3	1
Bisphenol C (BPC)	200	60	20	6	2
Bisphenol F (BPF)	200	60	20	6	2
Bisphenol S (BPS)	10	3	1	0.3	0.1
Mono-n-butyl phthalate (MBP)	200	60	20	6	2
Mono-2-ethylhexyl phthalate (MEHP)	200	60	20	6	2
N-butylbenzenesulfonamide	200	60	20	6	2
Benzyl butyl phthalate *	500	150	50	15	5
Dibutyl phthalate*	1000	300	100	30	10
Tetrabromobisphenol A (TBPA) *	200	60	20	6	2
Perfluorinated alkylated substances					
Perfluorooctanoic acid (PFOA)	50	15	5	1.5	0.5
Perfluorooctanesulfonic acid (PFOS)	300	90	30	9	3
Industrial side products and pesticides					
2-Naphthol	500	150	50	15	5
Methiocarb	300	90	30	9	3
Prochloraz	10	3	1	0.3	0.1
2-tert-Butylphenol (2-tert-BP) *	2000	600	200	60	20
4-Octylphenol (OP) *	1000	300	100	30	10
4-tert-Octylphenol (4-tert-OP) *	1000	300	100	30	10
Fenarimol *	5	1.5	0.5	0.15	0.05
Nonylphenol *	500	150	50	15	5
Endogenous estrogens					
Estrone (E1)	100	30	10	3	1
Estradiol (E2)	200	60	20	6	2
Estradiol-17-glucuronide (E2-17-GlcA)	500	150	50	15	5
Estradiol-3-sulfate (E2-3-sulfate)	200	60	20	6	2
Estriol (E3)	200	60	20	6	2
16-Epiestriol (16EpiE3)	500	150	50	15	5
16-α-Hydroxyestrone (16OHE1)	100	30	10	3	1
17-Epiestriol (17EpiE3)	500	150	50	15	5
2-Methoxy estrone (2MeOE1)	300	90	30	9	3
2-Methoxy estradiol(2MeOE2)	300	90	30	9	3
4-Methoxy estrone (4MeOE1)	50	15	5	1.5	0.5
4-Methoxy estradiol (4MeOE2)	50	15	5	1.5	0.5
2-Hydroxy estradiol (2OHE2) *	1000	300	100	30	10
4-Hydroxy estrone (4OHE1) *	500	150	50	15	5
Phytoestrogens and metabolites					
8-Prenylnaringenin	10	3	1	0.3	0.1
Coumestrol	100	30	10	3	1
Daidzein	50	15	5	1.5	0.5
Enterodiol	50	15	5	1.5	0.5
Enterolactone	100	30	10	3	1
Equol	100	30	10	3	1
Formononetin	50	15	5	1.5	0.5
Genistein (GEN)	50	15	5	1.5	0.5
Glycitein	50	15	5	1.5	0.5
Isoxanthohumol	5	1.5	0.5	0.15	0.05
Matairesinol	200	60	20	6	2
Resveratrol	500	150	50	15	5
Xanthohumol *	50	15	5	1.5	0.5
Mycoestrogens and metabolites					
Alternariol	50	15	5	1.5	0.5
Alternariol monomethyl ether	50	15	5	1.5	0.5
α-Zearalanol (α-ZAL)	100	30	10	3	1
β-Zearalanol (β-ZAL)	100	30	10	3	1
α-Zearalenol (α-ZEL)	100	30	10	3	1
β-Zearalenol (β-ZEL)	100	30	10	3	1
α-Zearalenol-14-glucuronide (α-ZEL-14-GlcA)	100	30	10	3	1
β-Zearalenol-14-glucuronide (β-ZEL-14-GlcA)	100	30	10	3	1
Zearalanone (ZAN)	100	30	10	3	1
Zearalenone (ZEN)	100	30	10	3	1
Zearalenone-14-glucuronide (ZEN-14-GlcA)	100	30	10	3	1
Zearalenone-14-sulfate (ZEN-14-sulfate)	100	30	10	3	1
Personal care product ingredients, pharmaceuticals and metabolites					
Benzophenone 1	10	3	1	0.3	0.1
Benzophenone 2	50	15	5	1.5	0.5
Benzylparaben	5	1.5	0.5	0.15	0.05
Butylparaben (BP)	50	15	5	1.5	0.5

Compound	Matrix matched standards				
	100	30	10	3	1
	Concentration [ng/mL]				
Ethylparaben (EP)	50	15	5	1.5	0.5
Isobutylparaben	50	15	5	1.5	0.5
Methylparaben (MP)	100	30	10	3	1
Propylparaben (PP)	50	15	5	1.5	0.5
Ethinylestradiol	500	150	50	15	5
3-Benzylidencamphor (3-BC) *	5000	1500	500	150	50
4-methylbenzylidencamphor (4-MBC) *	2000	600	200	60	20
Octyl methoxycinnamate (OMC) *	5000	1500	500	150	50
p-Hydroxybenzoic acid (*p*OHBA) *	3000	900	300	90	30
Triclosan *	100	30	10	3	1

Table 27: Internal standard concentrations in the samples used for pre-experiments

Internal Standards	Concentration in standards [ng/mL]
$^{13}C_{12}$ Bisphenol A (BPA)	5
$^{13}C_{18}$ Zearalenone (ZEN)	10
$^{13}C_{2}$ mono-2-ethylhexyl phthalate (MEHP)	5
$^{13}C_{2}$ mono-*n*-butyl phthalate (MBP)	5
$^{13}C_{3}$ Estradiol (E2)	10
$^{13}C_{6}$ 4-tert-Octylphenol (4-tert-OP)	50
$^{13}C_{6}$ Butylparaben	5
$^{13}C_{6}$ Ethylparaben	5
$^{13}C_{6}$ Methylparaben	5
$^{13}C_{6}$ p-Hydroxybenzoic acid (pOHBA)	300
$^{13}C_{6}$ Propylparaben	5
$^{13}C_{8}$ Perfluorooctanesulfonic acid (PFOS)	10
$^{13}C_{8}$ Perfluorooctanoic acid (PFOA)	10
D_{4} Genistein	5

Table 28: Compound concentrations in the matrix matched standards spiked with new substances used in pre-experiments to determine retention times and optimal transitions

Compound	Matrix matched standards		
	NSSP 100	NSSP 10	NSSP 1
	Concentration [ng/mL]		
Phytotoxins			
Anisodamine	5	0.5	0.05
Aristolochic acid I	10	1	0.1
Aristolactam I	5	0.5	0.05
Jacobine	1	0.1	0.01
Jacobine-N-oxide	0.5	0.05	0.005
Riddelliin	10	1	0.1
Riddelliin-N-oxide	1	0.1	0.01
Scopolamine	0.5	0.05	0.005
Disinfection by-products	100	30	10
Bromoacetic acid	500	50	5
Dibromoacetic acid	100	10	1
Chloroacetic acid	100	10	1
Dichloroacetic acid	500	50	5
Food processing by-products	100	30	10
Acrylamide	1000	100	10
Glycidamide	1000	100	10
5-Hydroxymethylfurfural (HMF)	250	25	2.5
5-Hydroxymethyl-2-furanoic acid (HMFA)	500	50	5
N-Nitosodimethylamine (NDMA)	5000	500	50
PhIP	10	1	0.1
Air pollutants			
N-Nitrosodiethylamine (NDEA)	10000	1000	100
Cotinine	5	0.5	0.05
Trans-3-hydroxy cotinine	10	1	0.1
1-Hydroxy pyrene	500	50	5
3-Hydroxy phenanthrene	5	0.5	0.05
3-hydroxy benzo[a]pyrene (3-OH-BaP)	10000	1000	100

Table 29: Compound concentrations in validation standards and the mastermix

Compound	Mastermix	Standards for validation						
		300	100	30	10	3	1 (LOQ)	0.3 (LOD)
	Concentration [ng/mL]							
Plasticizer/plastic components								
Bisphenol A (BPA)	200	30	10	3	1	0.3	0.1	0.03
Bisphenol AF (BPAF)	100	15	5	1.5	0.5	0.15	0.05	0.015
Bisphenol B (BPB)	20	3	1	0.3	0.1	0.03	0.01	0.003
Bisphenol C (BPC)	400	60	20	6	2	0.6	0.2	0.06
Bisphenol F (BPF)	100	15	5	1.5	0.5	0.15	0.05	0.015
Bisphenol S (BPS)	4	0.6	0.2	0.06	0.02	0.01	0.002	0.0006
Mono-n-butyl phthalate (MBP)	1000	150	50	15	5	1.5	0.5	0.15
Mono-2-ethylhexyl phthalate (MEHP)	300	45	15	4.5	1.5	0.45	0.15	0.045
N-butylbenzenesulfonamide	2000	300	100	30	10	3	1	0.3
Benzyl butyl phthalate	500	75	25	7.5	2.5	0.75	0.25	0.075
Dibutyl phthalate	10000	1500	500	150	50	15	5	1.5
Tetrabromobisphenol A (TBPA)	200	30	10	3	1	0.3	0.1	0.03
Perfluorinated alkylated substances								
Perfluorooctanoic acid (PFOA)	30	4.5	1.5	0.45	0.15	0.05	0.015	0.0045
Perfluorooctanesulfonic acid (PFOS)	30	4.5	1.5	0.45	0.15	0.05	0.015	0.0045
Industrial side products and pesticides								
2-Naphthol	60	9	3	0.9	0.3	0.09	0.03	0.009
Methiocarb	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
Prochloraz	1	0.15	0.05	0.02	0.005	0	0.0005	0.00015
2-tert-Butylphenol (2-tert-BP)	100000	15000	5000	1500	500	150	50	15
4-Octylphenol (4-OP)	20000	3000	1000	300	100	30	10	3
4-tert-Octylphenol (4-tert-OP)	2000	300	100	30	10	3	1	0.3
Fenarimol	6	0.9	0.3	0.09	0.03	0.01	0.003	0.0009
Nonylphenol	5000	750	250	75	25	7.5	2.5	0.75
Endogenous estrogens								
Estrone (E1)	6	0.9	0.3	0.09	0.03	0.01	0.003	0.0009
Estradiol (E2)	60	9	3	0.9	0.3	0.09	0.03	0.009
Estradiol-17-glucuronide (E2-17-GlcA)	100	15	5	1.5	0.5	0.15	0.05	0.015
Estradiol-3-sulfate (E2-3-sulfate)	30	4.5	1.5	0.45	0.15	0.05	0.015	0.0045
Estriol (E3)	60	9	3	0.9	0.3	0.09	0.03	0.009
16-Epiestriol (16EpiE3)	200	30	10	3	1	0.3	0.1	0.03
16-α-Hydroxyestrone (16OHE1)	30	4.5	1.5	0.45	0.15	0.05	0.015	0.0045
17-Epiestriol (17EpiE3)	200	30	10	3	1	0.3	0.1	0.03
2-Methoxy estrone (2MeOE1)	50	7.5	2.5	0.75	0.25	0.08	0.025	0.0075
2-Methoxy estradiol(2MeOE2)	400	60	20	6	2	0.6	0.2	0.06
4-Methoxy estrone (4MeOE1)	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
4-Methoxy estradiol (4MeOE2)	20	3	1	0.3	0.1	0.03	0.01	0.003
2-Hydroxy estradiol (2OHE2)	200	30	10	3	1	0.3	0.1	0.03
4-Hydroxy estrone (4OHE1)	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
Phytoestrogens and metabolites								
8-Prenylnaringenin	60	9	3	0.9	0.3	0.09	0.03	0.009
Coumestrol	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
Daidzein	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
Enterodiol	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
Enterolactone	400	60	20	6	2	0.6	0.2	0.06
Equol	40	6	2	0.6	0.2	0.06	0.02	0.006
Formononetin	5	0.75	0.25	0.08	0.025	0.01	0.0025	0.00075
Genistein (GEN)	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
Glycitein	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
Isoxanthohumol	2	0.3	0.1	0.03	0.01	0	0.001	0.0003
Matairesinol	100	15	5	1.5	0.5	0.15	0.05	0.015
Resveratrol	3000	450	150	45	15	4.5	1.5	0.45
Xanthohumol	200	30	10	3	1	0.3	0.1	0.03
Mycoestrogens and metabolites								
Alternariol	200	30	10	3	1	0.3	0.1	0.03
Alternariol monomethyl ether	10	1.5	0.5	0.15	0.05	0.02	0.005	0.0015
α-zearalanol (α-ZAL)	100	15	5	1.5	0.5	0.15	0.05	0.015
β-zearalanol (β-ZAL)	100	15	5	1.5	0.5	0.15	0.05	0.015
α-zearalenol (α-ZEL)	4	0.6	0.2	0.06	0.02	0.01	0.002	0.0006
β-zearalenol (β-ZEL)	200	30	10	3	1	0.3	0.1	0.03
α-zearalenol-14-glucuronide (α-ZEL-14-GlcA)	30	4.5	1.5	0.45	0.15	0.05	0.015	0.0045
β-zearalenol-14-glucuronide (β-ZEL-14-GlcA)	30	4.5	1.5	0.45	0.15	0.05	0.015	0.0045
Zearalanone (ZAN)	60	9	3	0.9	0.3	0.09	0.03	0.009
Zearalenone (ZEN)	60	9	3	0.9	0.3	0.09	0.03	0.009
Zearalenone-14-glucuronide (ZEN-14-GlcA)	100	15	5	1.5	0.5	0.15	0.05	0.015
Zearalenone-14-sulfate (ZEN-14-sulfate)	30	4.5	1.5	0.45	0.15	0.05	0.015	0.0045

Compound	Mastermix	Standards for validation						
		300	100	30	10	3	1 (LOQ)	0.3 (LOD)
	Concentration [ng/mL]							
Personal care product ingredients, pharmaceuticals and metabolites								
Benzophenone 1	40	6	2	0.6	0.2	0.06	0.02	0.006
Benzophenone 2	30	4.5	1.5	0.45	0.15	0.05	0.015	0.0045
Benzylparaben	3	0.45	0.15	0.05	0.015	0	0.0015	0.00045
Butylparaben	20	3	1	0.3	0.1	0.03	0.01	0.003
Ethylparaben	20	3	1	0.3	0.1	0.03	0.01	0.003
Isobutylparaben	20	3	1	0.3	0.1	0.03	0.01	0.003
Methylparaben	50	7.5	2.5	0.75	0.25	0.08	0.025	0.0075
Propylparaben	40	6	2	0.6	0.2	0.06	0.02	0.006
Ethinylestradiol	200	30	10	3	1	0.3	0.1	0.03
3-Benzylidencamphor (3-BC)	30000	4500	1500	450	150	45	15	4.5
4-methylbenzylidencamphor (4-MBC)	3000	450	150	45	15	4.5	1.5	0.45
Octyl methoxycinnamate (OMC)	40000	6000	2000	600	200	60	20	6
p-Hydroxybenzoic acid (pOHBA)	10000	1500	500	150	50	15	5	1.5
Triclosan	200	30	10	3	1	0.3	0.1	0.03
Phytotoxins								
Anisodamine	100	1.5	0.5	0.15	0.05	0.015	0.005	0.0015
Aristolochic acid I	200	30	10	3	1	0.3	0.1	0.03
Aristolactam I	1000	15	5	1.5	0.5	0.15	0.05	0.015
Jacobine	150	7.5	2.5	0.75	0.25	0.075	0.025	0.0075
Jacobine-N-oxide	10	1.5	0.5	0.15	0.05	0.015	0.005	0.0015
Riddelliin	60	9	3	0.9	0.3	0.09	0.03	0.009
Riddelliin-N-oxide	40	6	2	0.6	0.2	0.06	0.02	0.006
Scopolamine	3	0.45	0.15	0.045	0.015	0.0045	0.0015	0.00045
Disinfection by-products								
Bromoacetic acid	4000	600	200	60	20	6	2	0.6
Dibromoacetic acid	3000	450	150	45	15	4.5	1.5	0.45
Dichloroacetic acid	15000	2250	750	225	75	22.5	7.5	2.25
Food processing by-products								
Acrylamide	20000	3000	1000	300	100	30	10	3
5-Hydroxymethylfurfural (HMF)	7000	1050	350	105	35	10.5	3.5	1.05
5-Hydroxymethyl-2-furanoic acid (HMFA)	40000	6000	2000	600	200	60	20	6
N-Nitosodimethylamine (NDMA)	60000	9000	3000	900	300	90	30	9
PhIP	20	3	1	0.3	0.1	0.03	0.01	0.003
Air pollutants								
Cotinine	300	45	15	4.5	1.5	0.45	0.15	0.045
Trans-3-hydroxy cotinine	60	9	3	0.9	0.3	0.09	0.03	0.009
1-Hydroxy pyrene	400	60	20	6	2	0.6	0.2	0.06
3-Hydroxy phenanthrene	30	4.5	1.5	0.45	0.15	0.045	0.015	0.0045

Table 30: Internal standard multi-analyte mix and a separate ^{13}C-ZEN solution used for method validation

Internal Standard in the multimix	Concentration [ng/mL]
$^{13}C_{12}$ Bisphenol A (BPA)	20
$^{13}C_{2}$ mono-2-ethylhexyl phthalate (MEHP)	40
$^{13}C_{2}$ mono-*n*-butyl phthalate (MBP)	40
$^{13}C_{3}$ Estradiol (E2)	20
$^{13}C_{6}$ 4-tert-Octylphenol (4-tert-OP)	200
$^{13}C_{6}$ Butylparaben	10
$^{13}C_{6}$ Ethylparaben	10
$^{13}C_{6}$ Methylparaben	10
$^{13}C_{6}$ p-Hydroxybenzoic acid (pOHBA)	600
$^{13}C_{6}$ Propylparaben	10
$^{13}C_{8}$ Perfluorooctanesulfonic acid (PFOS)	20
$^{13}C_{8}$ Perfluorooctanoic acid (PFOA)	20
D_{4} Genistein	10
Separate ^{13}C-ZEN solution	
$^{13}C_{18}$ Zearalenone (ZEN)	20

Table 31: Representative sequence of validation measurements describing sample succession and their purpose for method evaluation

Urine batch			Serum batch			Breast milk batch		
Sample Nr.	Sample	Purpose	Sample Nr.	Sample	Purpose	Sample Nr.	Sample	Purpose
1	Solvent blank	Column rinse	48	Solvent blank	Column rinse	90	Solvent blank	Column rinse
2	QC	System quality control	49	Serum blank_1		91	Milk blank_1	
3	QC		50	Serum Std30	Equilibration	92	Milk Std30	Equilibration
4	QC		51	Serum blank_1		93	Milk blank_1	
5	Solvent blank	Column rinse	52	Solvent blank	Column rinse	94	Solvent blank	Column rinse
6	Solvent blank	Column rinse	53	Solvent Std0.3		95	Solvent Std0.3	
7	Urine blank_1		54	Solvent Std1		96	Solvent Std1	
8	Urine Std30	Equilibration	55	Solvent Std3	SSE	97	Solvent Std3	SSE
9	Urine blank_1		56	Solvent Std10		98	Solvent Std10	
10	Solvent blank	Column rinse	57	Solvent Std30		99	Solvent Std30	
11	Solvent Std0.3		58	Solvent Std100		100	Solvent Std100	
12	Solvent Std1		59	Solvent blank	Column rinse	101	Solvent blank	Column rinse
13	Solvent Std3	SSE	60	Serum Std0.3		102	Milk Std0.3	
14	Solvent Std10		61	Serum Std1		103	Milk Std1	
15	Solvent Std30		62	Serum Std3	SSE and R_E	104	Milk Std3	SSE and R_E
16	Solvent Std100		63	Serum Std10		105	Milk Std10	
17	Solvent blank	Column rinse	64	Serum Std30		106	Milk Std30	
18	Urine Std0.3		65	Serum Std100		107	Milk Std100	
19	Urine Std1		66	Solvent blank	Column rinse	108	Solvent blank	Column rinse
20	Urine Std3	SSE and R_E	67	System blank_1	Evaluation of selectivity	109	System blank_1	Evaluation of selectivity
21	Urine Std10		68	System blank_2		110	System blank_2	
22	Urine Std30		69	System blank_3		111	System blank_3	
23	Urine Std100		70	Solvent blank	Column rinse	112	Solvent blank	Column rinse
24	Solvent Blank	Column rinse	71	Serum blank_1	Evaluation of selectivity	113	Milk blank_1	Evaluation of selectivity
25	System blank_1	Evaluation of selectivity	72	Serum blank_2		114	Milk blank_2	
26	System blank_2		73	Serum blank_3		115	Milk blank_3	
27	System blank_3		74	Solvent Blank	Column rinse	116	Solvent Blank	Column rinse
28	Solvent Blank	Column rinse	75	Serum Spike3_1		117	Milk Spike3_1	
29	Urine blank_1	Evaluation of selectivity	76	Serum Spike3_2	R_E	118	Milk Spike3_2	R_E
30	Urine blank_2		77	Serum Spike3_3		119	Milk Spike3_3	
31	Urine blank_3		78	Solvent Blank	Column rinse	120	Solvent Blank	Column rinse
32	Solvent blank	Column rinse	79	Serum Spike30_1	R_E	121	Milk Spike30_1	
33	Urine Spike3_1		80	Serum Spike30_2		122	Milk Spike30_2	R_E
34	Urine Spike3_2	R_E	81	Serum Spike30_3		123	Milk Spike30_3	
35	Urine Spike3_3		82	Solvent blank	Column rinse	124	Solvent blank	Column rinse
36	Solvent Blank	Column rinse	83	Serum Std0.3		125	Milk Std0.3	
37	Urine Spike30_1	R_E	84	Serum Std1		126	Milk Std1	
38	Urine Spike30_2		85	Serum Std3	SSE and R_E	127	Milk Std3	SSE and R_E
39	Urine Spike30_3		86	Serum Std10		128	Milk Std10	
40	Solvent blank	Column rinse	87	Serum Std30		129	Milk Std30	
41	Urine Std0.3		88	Serum Std100		130	Milk Std100	
42	Urine Std1		89	Solvent blank	Column rinse	131	Solvent blank	Column rinse
43	Urine Std3	SSE and R_E				132	Solvent Std0.3	
44	Urine Std10					133	Solvent Std1	
45	Urine Std30					134	Solvent Std3	SSE
46	Urine Std100					135	Solvent Std10	
47	Solvent blank	Column rinse				136	Solvent Std30	
						137	Solvent Std100	
						138	Solvent blank	Column rinse
						139	Solvent blank	Column rinse
						140	QC	System quality control
						141	QC	
						142	QC	
						143	Solvent blank	Column rinse

Table 32: Extraction recoveries in urine. LL/HL displays the low level and high level spiking experiment. In some cases, the spiked amount could not be differentiated from the natural matrix contamination (marked with "<0"). Values determined as outliers by Dixon's Q- and Grubbs's testing are marked with (*).

Compound	Extraction recoveries in urine [ng/mL]																							
	Validation 1 LL			Validation 2 LL			Validation 3 LL			Validation 3 LL - repeatability			Validation 1 HL			Validation 2 HL			Validation 3 HL			Validation 3 HL - repeatability		
Plasticizer/plastic components																								
Bisphenol A (BPA)	0.30	0.25	0.30	0.33	0.48	0.46	0.36	0.37	0.27	0.34	0.28	0.36	2.7	2.8	3.0	2.7	2.8	3.4	2.8	2.8	2.5	2.7	2.8	2.4
Bisphenol AF (BPAF)	0.16	0.15	0.14	0.14	0.15	0.13	0.15	0.15	0.14	0.14	0.15	0.13	1.3	1.3	1.3	1.3	1.5	1.4	1.3	1.3	1.3	1.4	1.3	1.2
Bisphenol B (BPB)	0.033	0.029	0.031	0.022	0.025	0.027	0.027	0.03	0.021	0.029	0.021	0.022	0.27	0.26	0.27	0.28	0.28	0.28	0.26	0.3	0.26	0.28	0.32	0.26
Bisphenol C (BPC)	0.54	0.58	0.54	0.60	0.61	0.58	0.60	0.58	0.51	0.62	0.56	0.49	5.2	5.2	5.2	5.9	5.6	5.8	5.6	5.6	5.5	6.0	5.7	5.3
Bisphenol F (BPF)	0.15	0.14	0.14	0.14	0.16	0.16	0.16	0.16	0.15	0.11	0.11	0.097	1.4	1.4	1.4	1.6	1.5	1.4	1.5	1.4	1.5	1.3	1.4	1.4
Bisphenol S (BPS)	0.0084	0.0037	0.0047	0.0040	0.0050	0.0064	0.0056	0.0038	0.0056	0.0054	0.0066	0.0025	0.063	0.052	0.048	0.055	0.052	0.046	0.059	0.061	0.052	0.058	0.064	0.059
Mono-n-butyl phthalate (MBP)	1.3	1.3	1.3	1.7	1.7	1.5	1.2	1.3	1.5	1.3	1.3	1.6	15	16	15	15	17	18	14	14	13	14.0	14.0	13.0
Mono-2-ethylhexyl phthalate (MEHP)	1.5	1.6	1.4	0.51	0.64	0.42	n.d.	n.d.	0.4	0.43	0.4	0.43	4.2	4.8	4.5	4.1	4.9	5.2	4.3	4.2	3.8	4.4	4.2	3.9
N-butyl benzenesulfonamide	3.1	2.9	2.8	3.1	3.0	2.8	3.0	2.8	2.5	3.1	2.6	2.6	26	27	26	29	28	28	29	28	28	29	29	28
Benzyl butyl phthalate	0.56	0.64	0.44	0.51	0.67	0.62	0.54	0.68	0.7	0.55	0.8	0.43	5.1	4.5	5.2	5.8	5.0	4.5	5.8	5.6	4.6	5.9	4.4	4.6
Dibutyl phthalate	7.9	7.6	5.6	<0	<0	<0	9.1	12.0	11.0	9.9	12	11	56	46	29	57	66	28	102	85	7.0	79	75	2.8
Tetrabromobisphenol A (TBPA)	0.31	0.26	0.22	0.3	0.39	0.29	0.23	0.27	0.21	0.22	0.33	0.27	1.9	2.2	2.4	2.7	3.2	3.0	2.4	2.1	1.9	2.5	2.2	2.1
Perfluorinated alkylated substances																								
Perfluorooctanoic acid (PFOA)	0.044	0.043	0.031	0.048	0.068	0.067	0.069	0.057	0.058	0.059	0.067	0.057	0.39	0.44	0.45	0.38	0.41	0.37	0.44	0.41	0.35	0.44	0.44	0.44
Perfluorooctanesulfonic acid (PFOS)	0.062	0.065	0.071	0.047	0.055	0.048	0.019	0.018	0.019	0.019	0.018	0.017	0.36	0.45	0.34	0.28	0.29	0.31	0.46	0.47	0.45	0.47	0.46	0.45
Industrial side products and pesticides																								
2-Naphthol	0.074	0.066	0.069	0.12	0.098	0.096	0.079	0.077	0.092	0.081	0.088	0.08	0.71	0.71	0.67	0.84	0.79	0.85	0.81	0.82	0.64	0.82	0.8	0.61
Methiocarb	0.012	0.013	0.012	n.d.	n.d.	n.d.	0.014	0.014	0.013	0.013	0.015	0.015	0.13	0.13	0.12	0.12	0.12	0.12	0.13	0.13	0.12	0.14	0.14	0.14
Prochloraz	0.0014	0.0019	0.0017	n.d.	n.d.	n.d.	0.0017	0.0016	0.0018	0.0013	0.0017	0.0014	0.014	0.014	0.013	0.012	0.0098	0.0096	0.014	0.013	0.012	0.015	0.015	0.013
2-tert-Butylphenol (2-tert-BP)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Octylphenol (4-OP)	9.5	12	7.0	2.0	3.1	4.4	21	31	26	16	22	24	33.0	63.0	5.3	76.0	74.0	21.0	350.0	270.0	n.d.	296	233	n.d.
4-tert-Octylphenol (4-tert-OP)	0.97	1.0	0.33	n.d.	n.d.	n.d.	1.1	1.4	1.4	1.0	1.2	1.2	0.61	1.1	0.25	0.66	0.5	0.25	10	11	0.49	9.9	9.5	0.28
Fenarimol	0.0093	0.0081	0.0085	0.0064	0.0062	0.0075	0.0080	0.0086	0.0083	0.0078	0.008	0.0082	0.081	0.081	0.079	0.074	0.075	0.067	0.082	0.078	0.073	0.087	0.083	0.083
Nonylphenol	2.9	3.2	1.0	0.11	0.099	0.38	4.6	6.7	5.8	3.7	4.9	5.3	4.1	7.2	1.0	5.3	4.2	0.95	77.0	69.0	<0	66	59	n.d.
Endogenous estrogens																								
Estrone (E1)	0.011	0.011	0.013	0.0091	0.013	0.013	0.0096	0.013	0.0026*	0.0054	0.0058	0.0088	0.071	0.070	0.084	0.079	0.10	0.092	0.078	0.093	0.089	0.075	0.10	0.095
Estradiol (E2)	0.11	0.087	0.14	0.085	0.11	0.096	0.076	0.076	0.084	0.095	0.089	0.088	0.92	0.96	0.80	1.0	1.1	0.97	0.84	0.85	0.79	0.89	0.93	0.79
Estradiol-17-glucuronide (E2-17-GlcA)	No linear calibration																							
Estradiol-3-sulfate (E2-3-sulfate)	n.d.	n.d.	n.d.	0.025	0.075	0.056	0.039	0.053	0.057	0.021	0.024	0.022	0.39	0.40	0.40	0.39	0.43	0.36	0.44	0.49	0.40	0.46	0.42	0.36
Estriol (E3)	0.12	0.073	0.017*	0.11	0.090	0.089	0.063	0.072	0.077	0.068	0.069	0.024	0.83	0.95	0.82	0.89	0.88	0.86	0.94	0.88	0.77	0.86	0.8	0.85
16-Epiestriol (16EpiE3)	0.32	0.28	0.29	0.25	0.28	0.30	0.21	0.23	0.29	0.26	0.24	0.21	2.8	2.9	2.7	2.8	2.9	2.9	3.0	2.9	2.8	3.0	2.8	2.7
16-α-Hydroxyestrone (16OHE1)	0.034	0.027	0.027	0.039	0.024	0.039	0.024	0.03	0.026	0.045	0.044	0.042	0.37	0.39	0.33	0.45	0.38	0.39	0.43	0.41	0.42	0.49	0.40	0.41
17-Epiestriol (17EpiE3)	0.22	0.23	0.24	0.34	0.32	0.37	0.25	0.24	0.3	0.33	0.36	0.27	2.7	2.6	2.6	3.2	3.0	3.1	2.9	3.1	2.9	2.9	2.9	2.9
2-Methoxy estrone (2MeOE1)	0.069	0.069	0.084	0.078	0.076	0.073	0.081	0.064	0.057	0.055	0.059	0.052	0.66	0.69	0.68	0.78	0.68	0.73	0.72	0.72	0.71	0.68	0.73	0.64
2-Methoxy estradiol(2MeOE2)	0.053	0.055	0.048	0.071	0.061	0.057	0.06	0.068	0.063	0.066	0.061	0.054	0.52	0.55	0.54	0.59	0.58	0.59	0.61	0.61	0.57	0.59	0.57	0.55
4-Methoxy estrone (4MeOE1)	0.013	0.012	0.014	0.017	0.018	0.013	0.016	0.014	0.012	0.016	0.014	0.013	0.13	0.13	0.13	0.14	0.15	0.14	0.14	0.14	0.14	0.15	0.14	0.14
4-Methoxy estradiol (4MeOE2)	0.03	0.022	0.022	0.026	0.026	0.029	0.035	0.033	0.033	0.027	0.033	0.028	0.29	0.27	0.27	0.29	0.3	0.29	0.32	0.3	0.31	0.31	0.29	0.28
4-Hydroxy estrone (4OHE1)	0.0097	0.0081	0.0096	0.015	0.014	0.014	0.013	0.011	0.012	0.015	0.017	0.018	0.093	0.093	0.11	0.15	0.11	0.15	0.13	0.12	0.13	0.12	0.11	0.12
Phytoestrogens and metabolites																								
8-Prenylnaringenin	0.083	0.082	0.082	0.083	0.088	0.088	0.071	0.075	0.061	0.081	0.087	0.075	0.75	0.82	0.78	0.85	0.88	0.85	0.85	0.8	0.74	0.84	0.80	0.75
Coumestrol	0.018	0.013	0.015	0.015	0.013	0.016	0.014	0.014	0.013	0.011	0.014	0.012	0.13	0.12	0.14	0.14	0.14	0.15	0.14	0.14	0.14	0.15	0.13	0.13
Daidzein	0.019	0.014	0.016	0.016	0.016	0.017	0.013	0.009	0.012	0.016	0.017	0.017	0.15	0.14	0.14	0.13	0.15	0.14	0.14	0.15	0.15	0.14	0.12	0.12
Enterodiol	0.015	0.01	0.011	0.012	0.014	0.015	0.016	0.013	0.015	0.011	0.013	0.013	0.17	0.13	0.13	0.17	0.14	0.16	0.18	0.17	0.15	0.16	0.15	0.15
Enterolactone	0.59	0.54	0.58	0.61	0.65	0.63	0.67	0.62	0.59	0.53	0.53	0.58	5.6	5.6	5.3	6.2	6.0	5.7	6.1	6.1	5.6	5.8	5.8	5.4
Equol	0.06	0.056	0.058	0.064	0.060	0.057	0.061	0.056	0.054	0.052	0.052	0.058	0.57	0.56	0.55	0.61	0.61	0.59	0.6	0.59	0.58	0.6	0.59	0.56
Formononetin	0.0082	0.0076	0.0071	0.0077	0.0069	0.0076	0.0076	0.0078	0.0071	0.0076	0.0069	0.0071	0.066	0.069	0.065	0.073	0.073	0.069	0.075	0.072	0.07	0.075	0.073	0.069
Genistein	0.015	0.018	0.012	0.02	0.02	0.016	0.017	0.013	0.014	0.015	0.015	0.013	0.14	0.15	0.14	0.14	0.17	0.18	0.14	0.13	0.12	0.14	0.14	0.14
Glycitein	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.019	0.014	0.015	0.0097	0.014	0.016	0.13	0.13	0.13	0.11	0.097	0.12	0.15	0.16	0.16	0.15	0.16	0.17
Isoxanthohumol	0.003	0.0031	0.0028	0.0023	0.0022	0.002	0.0032	0.0032	0.0028	0.0029	0.0028	0.0028	0.027	0.026	0.025	0.026	0.026	0.027	0.029	0.027	0.026	0.028	0.028	0.028
Matairesinol	0.11	0.11	0.11	0.11	0.15	0.11	0.13	0.12	0.10	0.14	0.17	0.17	1.4	1.4	1.3	1.5	1.5	1.5	1.6	1.5	1.5	1.6	1.5	1.4
Resveratrol	3.8	3.7	3.6	5.1	5.6	4.4	3.9	3.8	4.0	3.8	3.6	3.6	34	35	41	45	42	57	38	39	39	39	38	38
Xanthohumol	0.26	0.27	0.26	0.19	0.19	0.19	0.30	0.35	0.40	0.26	0.38	0.45	1.8	3.8	3.2	2.6	3.0	2.9	4.1	3.1	4.1	4.0	3.1	4.1
Mycoestrogens and metabolites																								
Alternariol	0.30	0.27	0.28	0.31	0.30	0.30	0.26	0.25	0.27	0.27	0.25	0.28	2.7	2.7	2.7	2.9	2.9	2.9	3.0	2.8	2.9	2.9	2.7	2.7
Alternariol monomethyl ether	0.016	0.014	0.013	0.012	0.016	0.017	0.013	0.015	0.012	0.015	0.013	0.015	0.13	0.13	0.13	0.15	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.14
α-Zearalanol (α-ZAL)	0.14	0.14	0.15	0.14	0.15	0.13	0.16	0.15	0.15	0.16	0.15	0.15	1.3	1.4	1.3	1.4	1.4	1.4	1.5	1.4	1.4	1.5	1.5	1.4
β-Zearalanol (β-ZAL)	0.15	0.16	0.15	0.15	0.15	0.14	0.16	0.16	0.15	0.16	0.14	0.14	1.3	1.4	1.3	1.5	1.5	1.5	1.5	1.5	1.4	1.5	1.5	1.4
α-Zearalenol (α-ZEL)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0048	0.0045	0.0046	0.0042	0.0032	0.0038	0.06	0.049	0.049	0.05	0.06	0.059	0.052	0.051	0.052	0.057	0.059	0.049
β-Zearalenol (β-ZEL)	0.32	0.29	0.29	0.31	0.31	0.32	0.31	0.27	0.29	0.32	0.20	0.28	2.6	2.7	2.6	3.0	2.8	3.0	3.1	2.9	2.9	3.1	2.9	2.8
α-Zearalenol-14-glucuronide (α-ZEL-14-GlcA)	No linear calibration																							
β-Zearalenol-14-glucuronide (β-ZEL-14-GlcA)	No linear calibration																							

Compound	Extraction recoveries in urine [ng/mL]																							
	Validation 1 LL			Validation 2 LL			Validation 3 LL			Validation 3 LL - repeatability			Validation 1 HL			Validation 2 HL			Validation 3 HL			Validation 3 HL - repeatability		
Zearalanone (ZAN)	0.092	0.089	0.098	0.077	0.094	0.082	0.094	0.093	0.075	0.098	0.089	0.081	0.76	0.82	0.81	0.86	0.80	0.86	0.83	0.84	0.82	0.93	0.90	0.84
Zearalenone (ZEN)	0.10	0.088	0.083	0.085	0.073	0.079	0.10	0.094	0.089	0.098	0.086	0.10	0.80	0.82	0.78	0.82	0.81	0.83	0.90	0.86	0.97	0.89	0.84	0.98
Zearalenone-14-glucuronide (ZEN-14-GlcA)	No linear calibration																							
Zearalenone-14-sulfate (ZEN-14-sulfate)	0.049	0.049	0.045	0.04	0.044	0.043	0.038	0.047	0.048	0.04	0.042	0.044	0.43	0.43	0.4	0.45	0.45	0.43	0.46	0.45	0.41	0.45	0.44	0.41
Personal care product ingredients, pharmaceuticals and metabolites																								
Benzophenone 1	0.06	0.056	0.055	0.057	0.057	0.058	0.062	0.064	0.06	0.064	0.061	0.064	0.51	0.54	0.53	0.58	0.58	0.56	0.56	0.57	0.56	0.59	0.58	0.55
Benzophenone 2	0.044	0.041	0.039	0.046	0.043	0.051	0.042	0.045	0.043	0.041	0.043	0.039	0.44	0.41	0.41	0.46	0.46	0.45	0.39	0.43	0.38	0.41	0.42	0.43
Benzylparaben	0.0043	0.0045	0.004	0.0049	0.0048	0.0043	0.0044	0.0041	0.004	0.0047	0.0047	0.0041	0.04	0.04	0.039	0.044	0.043	0.045	0.042	0.042	0.04	0.044	0.045	0.042
Butylparaben	0.027	0.026	0.027	0.026	0.028	0.026	0.024	0.024	0.026	0.025	0.024	0.027	0.27	0.29	0.28	0.26	0.29	0.31	0.26	0.25	0.23	0.26	0.25	0.23
Ethylparaben	0.031	0.028	0.028	0.026	0.033	0.025	0.026	0.028	0.026	0.027	0.025	0.029	0.27	0.3	0.3	0.27	0.31	0.33	0.29	0.25	0.24	0.28	0.25	0.25
Isobutylparaben	0.027	0.026	0.025	0.027	0.028	0.027	0.026	0.025	0.024	0.027	0.026	0.026	0.26	0.24	0.25	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.27	0.25
Methylparaben	0.077	0.064	0.065	0.075	0.091	0.067	0.075	0.059	0.069	0.068	0.064	0.071	0.72	0.78	0.74	0.71	0.82	0.83	0.73	0.66	0.6	0.74	0.66	0.64
Propylparaben	0.06	0.052	0.051	0.058	0.063	0.053	0.059	0.054	0.054	0.056	0.052	0.056	0.52	0.58	0.55	0.55	0.6	0.65	0.56	0.52	0.49	0.55	0.53	0.5
Ethinylestradiol	0.25	0.26	0.25	0.3	0.33	0.29	0.35	0.31	0.26	0.28	0.28	0.28	2.5	2.8	2.7	3.0	2.9	3.0	2.7	3.0	2.9	3.1	2.7	2.7
3-Benzylidencamphor (3-BC)	5.5	6.7	<0	<0	<0	<0	7.0	10.0	10.0	6.9	9.1	9.0	48	37	n.d.	43	25	7	120	110	<0	112	102	n.d.
4-methyl benzylidencamphor (4-MBC)	1.6	1.7	0.58	<0	<0	<0	1.7	2.1	2.0	1.5	2.2	1.9	7.7	8.3	0.84	9.0	7.6	2.4	19.0	18.0	0.096	19	17	0.083
Octyl methoxycinnamate (OMC)	No linear calibration																							
p-Hydroxybenzoic acid (pOHBA)	No linear calibration																							
Triclosan	0.22	0.24	0.18	0.21	0.21	0.23	0.19	0.26	0.22	0.19	0.22	0.21	1.5	1.8	1.6	1.8	1.9	1.9	2.2	1.9	1.2	2.1	1.8	1.1
Phytotoxins																								
Anisodamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14	0.14	0.11	0.12	0.12	0.1	0.14	0.16	0.13	0.13	0.15	0.15
Aristolochic acid I	0.27	0.30	0.27	0.22	0.2	0.27	0.28	0.15	0.16	0.21	0.21	0.19	2.5	2.9	2.3	2.3	2.3	2.0	3.1	3.0	3.1	2.7	2.8	2.8
Aristolactam I	0.14	0.13	0.13	0.14	0.13	0.12	0.17	0.14	0.15	0.15	0.14	0.14	1.3	1.3	1.3	1.2	1.2	1.2	1.4	1.3	1.3	1.4	1.4	1.4
Jacobine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.53	0.53	0.39	0.47	0.41	0.39	0.72	0.67	0.65	0.74	0.73	0.71
Jacobine-N-oxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.13	0.11	0.15	0.14	0.14	0.11	0.12	0.1	0.14	0.11	0.1
Riddelliin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.56	0.75	0.6	0.62	0.54	0.52	0.92	0.94	0.74	1.0	0.95	0.91
Riddelliin-N-oxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.46	0.5	0.34	0.64	0.53	0.52	0.31	0.38	0.41	0.33	0.33	0.33
Scopolamine	0.0052	0.0059	0.0041	0.0042	0.0045	0.0043	0.0054	0.0039	0.0036	0.0049	0.0041	0.0039	0.044	0.044	0.036	0.038	0.037	0.031	0.041	0.048	0.045	0.041	0.044	0.046
Disinfection by-products																								
Bromoacetic acid	No linear calibration																							
Dibromoacetic acid	4.6	5.6	5.3	1.5	n.d.	n.d.	3.5	2.9	3.7	3.7	3.2	3.9	36	34	37	34	36	6.2*	38	44	41	39	39	35
Dichloroacetic acid	13	15	10	n.d.	n.d.	14	21	18	14	22	16	19	230	239	237	229	217	194	206	222	204	207	215	237
Food processing by-products																								
Acrylamide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	225	313	245	239	229	139	294	322	305	268	298	313
5-Hydroxymethylfurfural (HMF)	4.7	4.1	4.1	n.d.	n.d.	n.d.	2.1	2.1	2.3	18	15	14	28	28	37	27	23	34	21	21	33	31	30	40
5-Hydroxymethyl-2-furanoic acid (HMFA)	No linear calibration																							
N-Nitosodimethylamine (NDMA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PhIP	0.025	0.025	0.028	0.036	0.033	0.026	0.032	0.028	0.027	0.032	0.026	0.029	0.27	0.27	0.33	0.28	0.28	0.34	0.3	0.29	0.32	0.30	0.29	0.30
Air pollutants																								
Cotinine	0.43	0.36	0.44	0.3	0.33	0.46	0.47	0.33	0.36	0.36	0.35	0.37	4.1	4.2	4.0	5.0	4.3	4.1	4.4	4.5	4.5	4.3	4.4	4.1
Trans-3-hydroxy cotinine	0.13	0.089	0.11	<0	<0	0.044	0.013	0.084	0.11	0.13	0.11	0.10	1.1	1.1	1.6	1.2	1.4	1.8	1.3	1.4	1.4	1.3	1.3	1.5
1-Hydroxy pyrene	0.58	0.65	0.54	0.71	0.70	0.58	0.43	0.32	0.40	0.31	0.31	0.33	4.7	4.7	6.7	6.0	5.8	7.2	5.0	4.8	5.4	4.6	4.2	4.7
3-Hydroxy phenanthrene	0.037	0.039	0.044	0.046	0.042	0.045	0.042	0.037	0.04	0.042	0.038	0.035	0.36	0.39	0.38	0.43	0.41	0.43	0.41	0.38	0.40	0.42	0.42	0.38

Table 33: Extraction recoveries in serum. In some cases, the spiked amount could not be differentiated from the natural matrix contamination (marked with "<0"). Values determined as outliers by Dixon's Q- and Grubbs's testing are marked with (*).

Compound	Extraction recoveries in serum [ng/mL]																							
	Validation 1 LL			Validation 2 LL			Validation 3 LL			Validation 3 LL - repeatability			Validation 1 HL			Validation 2 HL			Validation 3 HL			Validation 3 HL - repeatability		
Plasticizer/plastic components																								
Bisphenol A (BPA)	0.24	0.28	0.32	0.29	0.45	0.35	0.31	0.24	0.31	0.26	0.29	0.24	2.5	2.0	2.1	2.8	3.6	3.6	2.2	2.7	2.7	2.6	2.5	2.6
Bisphenol AF (BPAF)	0.14	0.13	0.14	0.14	0.18	0.15	0.13	0.13	0.14	0.14	0.14	0.14	1.1	1.4	1.6	1.8	1.5	1.5	1.3	1.1	1.3	1.3	1.2	1.3
Bisphenol B (BPB)	0.021	0.017	0.02	0.02	0.023	0.029	0.026	0.025	0.026	0.024	0.021	0.026	0.21	0.3	0.3	0.32	0.27	0.32	0.26	0.2	0.23	0.25	0.21	0.27
Bisphenol C (BPC)	0.47	0.52	0.41	0.48	0.53	0.45	0.55	0.58	0.45	0.52	0.5	0.52	4.5	5.2	5.9	6.5	5.4	5.8	5.0	4.6	4.9	5.1	4.5	5.1
Bisphenol F (BPF)	0.081	0.097	0.1	0.14	0.1	0.11	0.089	0.15	0.11	0.12	0.15	0.11	1.3	1.2	1.2	1.4	1.2	1.1	1.2	1.3	1.1	1.3	1.2	1.3
Bisphenol S (BPS)	0.0084	0.0077	0.008	0.012	0.0071	0.0043	0.0026	0.0044	0.005	0.0023	0.0052	0.0037	0.076	0.051	0.046	0.055	0.046	0.054	0.051	0.053	0.053	0.057	0.055	0.062
Mono-n-butyl phthalate (MBP)	1.6	1.2	1.8	2.9	2.1	2.8	2.1	2.1	1.8	2.0	2.0	2.0	15	14	15	27	31	27	22	21	20	22	21	21
Mono-2-ethylhexyl phthalate (MEHP)	No linear calibration																							
N-butyl benzenesulfonamide	2.5	2.0	2.3	3.0	2.8429	2.7	2.9	2.8	2.5	2.6	2.5	2.6	23	24	26	29	27	29	27	26	28	26	26	29
Benzyl butyl phthalate	0.61	0.65	0.44	0.83	0.50	0.36	0.64	0.71	0.55	0.75	0.76	0.61	5.0	3.9	5.1	5.6	5.2	3.7	4.7	5.5	4.9	5.0	5.9	4.9
Dibutyl phthalate	12	16	7.1	22	16	13	14	14	15	22	20	18	62	66	67	82	92	87	68	84	70	78	97	76
Tetrabromobisphenol A (TBPA)	0.089	0.091	0.26	0.17	0.083	0.08	0.26	0.23	0.25	0.21	0.15	0.1	1.5	1.6	1.1	2.1	1.4	1.3	1.4	1.5	1.3	1.3	1.5	1.6
Perfluorinated alkylated substances																								
Perfluorooctanoic acid (PFOA)	0.044	0.030	0.050	0.034	0.035	0.033	0.039	0.037	0.037	0.040	0.035	0.034	0.37	0.40	0.35	0.27	0.40	0.30	0.38	0.45	0.39	0.42	0.38	0.35
Perfluorooctanesulfonic acid (PFOS)	0.017	>0	0.012	0.018	0.081	0.024	0.031	0.029	0.033	0.030	0.028	0.032	0.17	0.23	0.17	0.23	0.36	0.26	0.28	0.30	0.28	0.28	0.29	0.26
Industrial side products and pesticides																								
2-Naphthol	0.059	0.07	0.08	0.088	0.10	0.078	0.10	0.099	0.096	0.086	0.086	0.059	0.72	0.72	0.74	0.78	0.75	0.75	0.70	0.72	0.71	0.77	0.72	0.73
Methiocarb	0.014	0.014	0.012	n.d.	n.d.	n.d.	0.013	0.013	0.013	0.014	0.013	0.013	0.11	0.12	0.13	0.13	0.13	0.14	0.13	0.13	0.14	0.12	0.12	0.14
Prochloraz	0.0018	0.0014	0.0015	n.d.	n.d.	n.d.	0.0015	0.0010	0.0011	0.00068	0.0015	0.00090	0.012	0.011	0.011	0.015	0.014	0.015	0.012	0.011	0.014	0.013	0.013	0.012
2-tert-Butylphenol (2-tert-BP)	2.2	4.1	4.0	4.4	5.3	5.5	18	14	14	18	12	14	54.0	60.0	63.0	83.0	110.0	69.0	120.0	110.0	140.0	124	113	149
4-Octylphenol (4-OP)	23.0	24	24.0	26.0	28.0	25.0	51	42	44	42	35	38	171	219	239	309	254	266	430	401	429	360	333	367
4-tert-Octylphenol (4-tert-OP)	3.3	3.4	3.3	4.1	3.2	3.4	3.8	4.0	4.1	4.4	3.9	3.7	28.0	28.0	27.0	34.0	41.0	38.0	34.0	38.0	36.0	35.0	39.0	35.0
Fenarimol	0.0089	0.0083	0.0089	0.0087	0.0086	0.009	0.0085	0.0076	0.0088	0.0070	0.0072	0.0078	0.071	0.074	0.079	0.098	0.084	0.088	0.089	0.079	0.082	0.081	0.082	0.080
Nonylphenol	6.6	2.9	9.0	3.9	21.0	13.0	10.0	7.1	8.5	8.7	5.7	5.5	42	64	69	95	70	75	62	56	67	59	48	62
Endogenous estrogens																								
Estrone (E1)	0.009	0.0091	0.0094	0.0008*	0.0061	0.0057	0.0070	0.0068	0.0062	0.0092	0.010	0.0072	0.066	0.071	0.083	0.096	0.085	0.083	0.069	0.062	0.075	0.07	0.068	0.09
Estradiol (E2)	0.043	0.068	0.087	0.087	0.094	0.075	0.043	0.052	0.067	0.063	0.077	0.051	0.67	0.8	0.68	0.74	0.98	0.82	0.82	0.72	0.71	0.73	0.86	0.80
Estradiol-17-glucuronide (E2-17-GlcA)	No linear calibration																							
Estradiol-3-sulfate (E2-3-sulfate)	0.029	0.028	0.046	0.028	0.04	0.025	0.041	0.036	0.038	0.047	0.048	0.045	0.35	0.38	0.41	0.42	0.39	0.46	0.4	0.41	0.42	0.37	0.40	0.43
Estriol (E3)	0.088	0.08	0.079	0.11	0.12	0.12	0.072	0.069	0.091	0.079	0.076	0.11	0.76	0.72	0.7	0.81	0.73	0.84	0.73	0.83	0.82	0.82	0.71	0.89
16-Epiestriol (16EpiE3)	0.26	0.26	0.34	0.24	0.29	0.26	0.27	0.27	0.32	0.28	0.28	0.22	2.4	2.3	2.4	2.3	2.4	2.6	2.6	2.7	2.8	2.6	2.4	2.6
16-α-Hydroxyestrone (16OHE1)	0.031	0.03	0.032	0.041	0.046	0.041	0.027	0.041	0.042	0.034	0.041	0.046	0.35	0.3	0.39	0.36	0.34	0.39	0.38	0.39	0.37	0.37	0.42	0.42
17-Epiestriol (17EpiE3)	0.28	0.28	0.26	0.28	0.24	0.23	0.27	0.24	0.23	0.26	0.26	0.29	2.5	2.5	2.6	2.5	2.3	2.4	2.8	2.8	2.6	2.6	2.7	2.7
2-Methoxy estrone (2MeOE1)	0.07	0.074	0.073	0.068	0.069	0.071	0.085	0.066	0.078	0.073	0.063	0.07	0.54	0.58	0.67	0.75	0.66	0.66	0.61	0.59	0.6	0.64	0.61	0.66
2-Methoxy estradiol(2MeOE2)	0.051	0.059	0.052	0.042	0.059	0.051	0.06	0.055	0.057	0.053	0.05	0.048	0.49	0.47	0.54	0.56	0.53	0.51	0.51	0.51	0.52	0.51	0.52	0.54
4-Methoxy estrone (4MeOE1)	0.011	0.012	0.012	0.012	0.015	0.014	0.012	0.011	0.012	0.015	0.014	0.013	0.11	0.12	0.14	0.15	0.13	0.15	0.13	0.13	0.13	0.12	0.13	0.13
4-Methoxy estradiol (4MeOE2)	0.021	0.028	0.022	0.034	0.027	0.035	0.026	0.026	0.027	0.028	0.024	0.028	0.24	0.24	0.27	0.29	0.26	0.27	0.27	0.27	0.26	0.25	0.26	0.26
4-Hydroxy estrone (4OHE1)	No linear calibration																							
Phytoestrogens and metabolites																								
8-Prenylnaringenin	0.074	0.077	0.085	0.071	0.086	0.082	0.077	0.073	0.077	0.077	0.078	0.075	0.67	0.82	0.93	0.97	0.78	0.85	0.79	0.79	0.75	0.78	0.80	0.77
Coumestrol	0.0087	0.013	0.012	0.018	0.016	0.016	0.014	0.015	0.015	0.014	0.013	0.01	0.11	0.12	0.13	0.14	0.12	0.12	0.13	0.13	0.14	0.13	0.14	0.12
Daidzein	0.011	0.011	0.017	0.017	0.013	0.0056	0.014	0.013	0.012	0.013	0.015	0.01	0.13	0.11	0.11	0.11	0.11	0.13	0.12	0.14	0.14	0.13	0.14	0.13
Enterodiol	0.012	0.015	0.015	0.017	0.013	0.011	0.014	0.014	0.014	0.015	0.014	0.015	0.11	0.12	0.13	0.11	0.13	0.12	0.14	0.13	0.12	0.11	0.13	0.13
Enterolactone	0.56	0.54	0.51	0.58	0.57	0.60	0.50	0.50	0.50	0.52	0.54	0.50	4.6	4.9	5.3	5.8	5.3	5.6	5.2	5.4	5.1	5.2	5.1	5.0
Equol	0.056	0.061	0.059	0.059	0.061	0.063	0.055	0.052	0.056	0.054	0.052	0.049	0.48	0.5	0.52	0.53	0.5	0.52	0.51	0.52	0.51	0.52	0.5	0.52
Formononetin	0.0064	0.0063	0.0064	0.0075	0.0083	0.0075	0.0063	0.0077	0.0074	0.0069	0.0064	0.0064	0.055	0.061	0.067	0.078	0.071	0.073	0.07	0.07	0.069	0.066	0.069	0.068
Genistein	0.0092	0.0091	0.015	0.011	0.0099	0.011	0.013	0.0095	0.0073	0.0089	0.011	0.0082	0.12	0.14	0.10	0.10	0.15	0.15	0.11	0.11	0.11	0.13	0.14	0.13
Glycitein	0.015	0.017	0.014	0.018	0.013	0.021	0.014	0.018	0.014	0.015	0.013	0.012	0.14	0.12	0.12	0.12	0.13	0.13	0.14	0.13	0.13	0.12	0.14	0.14
Isoxanthohumol	0.0026	0.0028	0.0022	0.0018	0.0021	0.0017	0.0032	0.0032	0.0029	0.0027	0.0026	0.0027	0.023	0.025	0.029	0.035	0.029	0.03	0.026	0.026	0.026	0.029	0.028	0.027
Matairesinol	0.11	0.14	0.14	0.12	0.13	0.094	0.13	0.11	0.14	0.15	0.13	0.14	1.1	1.1	1.2	1.2	1.3	1.3	1.3	1.3	1.4	1.3	1.4	1.4
Resveratrol	3.1	3.5	3.0	3.7	3.0	3.1	2.9	3.3	3.6	3.2	3.3	3.7	38	31	26	35	33	36	34	40	37	35	41	38
Xanthohumol	0.28	0.32	0.28	0.25	0.21	0.19	0.31	0.28	0.25	0.26	0.23	0.19	1.9	2.4	1.9	2.5	2.3	1.6	1.6	1.7	1.5	1.6	1.8	1.6
Mycoestrogens and metabolites																								
Alternariol	0.22	0.29	0.24	0.27	0.32	0.30	0.27	0.27	0.27	0.26	0.26	0.25	2.2	2.5	2.7	3.2	2.7	2.7	2.7	2.7	2.6	2.6	2.7	2.6
Alternariol monomethyl ether	0.011	0.0096	0.0096	0.0065	0.0074	0.008	0.010	0.013	0.013	0.0092	0.012	0.013	0.11	0.14	0.15	0.16	0.14	0.14	0.12	0.11	0.11	0.13	0.12	0.11
α-Zearalanol (α-ZAL)	0.13	0.13	0.13	0.16	0.16	0.18	0.13	0.14	0.14	0.13	0.14	0.13	1.1	1.2	1.3	1.5	1.4	1.5	1.3	1.3	1.3	1.3	1.4	1.3
β-Zearalanol (β-ZAL)	0.13	0.13	0.13	0.15	0.14	0.14	0.13	0.14	0.14	0.13	0.13	0.15	1.2	1.2	1.3	1.3	1.3	1.4	1.3	1.3	1.3	1.4	1.4	1.3
α-Zearalenol (α-ZEL)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0063	n.d.	n.d.	0.041	0.043	0.045	0.052	0.052	0.06	0.057	0.062	0.053	0.05	0.06	0.054
β-Zearalenol (β-ZEL)	0.28	0.28	0.31	0.3	0.26	0.27	0.27	0.27	0.29	0.26	0.25	0.27	2.3	2.4	2.8	2.8	2.6	2.7	2.7	2.6	2.6	2.7	2.8	2.7
α-Zearalenol-14-glucuronide (α-ZEL-14-GlcA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.29	0.32	0.37	0.39	0.37	0.44	0.39	0.4	0.41	0.34	0.35	0.39
β-Zearalenol-14-glucuronide (β-ZEL-14-GlcA)	No linear calibration																							
Zearalanone (ZAN)	0.084	0.060	0.060	0.10	0.071	0.074	0.085	0.084	0.091	0.085	0.072	0.092	0.69	0.81	0.83	0.91	0.9	0.92	0.8	0.81	0.74	0.82	0.83	0.79
Zearalenone (ZEN)	0.078	0.071	0.070	0.072	0.091	0.075	0.079	0.079	0.078	0.081	0.086	0.073	0.67	0.77	0.85	0.92	0.8	0.71	0.73	0.85	0.71	0.72	0.80	0.72
Zearalenone-14-glucuronide (ZEN-14-GlcA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.2	1.3	1.3	1.1	1.2	1.2	1.3	1.2	1.3	1.2	1.4	1.3
Zearalenone-14-sulfate (ZEN-14-sulfate)	0.041	0.036	0.038	0.043	0.043	0.046	0.039	0.039	0.043	0.041	0.039	0.038	0.34	0.34	0.40	0.43	0.41	0.42	0.42	0.42	0.43	0.4	0.42	0.43

Compound	Extraction recoveries in serum [ng/mL]																							
	Validation 1 LL			Validation 2 LL			Validation 3 LL			Validation 3 LL - repeatability			Validation 1 HL			Validation 2 HL			Validation 3 HL			Validation 3 HL - repeatability		
Personal care product ingredients, pharmaceuticals and metabolites																								
Benzophenone 1	0.049	0.047	0.048	0.054	0.069	0.06	0.058	0.067	0.068	0.035	0.045	0.046	0.45	0.50	0.56	0.67	0.58	0.59	0.59	0.67	0.62	0.56	0.68	0.61
Benzophenone 2	0.037	0.042	0.037	0.038	0.043	0.041	0.035	0.042	0.044	0.045	0.04	0.04	0.37	0.38	0.36	0.36	0.34	0.36	0.39	0.6	0.41	0.40	0.62	0.42
Benzylparaben	0.004	0.0041	0.0038	0.0044	0.0045	0.0045	0.004	0.0041	0.004	0.0039	0.0038	0.0036	0.033	0.039	0.045	0.048	0.041	0.042	0.041	0.039	0.038	0.039	0.038	0.038
Butylparaben	0.024	0.024	0.028	0.027	0.026	0.026	0.024	0.025	0.022	0.023	0.024	0.023	0.22	0.22	0.23	0.25	0.34	0.28	0.26	0.26	0.26	0.25	0.26	0.26
Ethylparaben	0.021	0.019	0.025	0.018	0.028	0.025	0.028	0.028	0.025	0.026	0.021	0.02	0.22	0.23	0.23	0.27	0.32	0.27	0.26	0.28	0.27	0.28	0.3	0.27
Isobutylparaben	0.029	0.028	0.023	0.029	0.03	0.03	0.025	0.026	0.027	0.025	0.026	0.027	0.23	0.25	0.27	0.31	0.28	0.28	0.27	0.27	0.26	0.26	0.27	0.25
Methylparaben	0.045	0.04	0.058	0.046	0.068	0.051	0.073	0.064	0.054	0.077	0.049	0.054	0.52	0.59	0.57	0.63	0.86	0.71	0.67	0.75	0.71	0.68	0.73	0.71
Propylparaben	0.043	0.039	0.044	0.052	0.056	0.048	0.049	0.05	0.048	0.046	0.054	0.045	0.44	0.46	0.46	0.50	0.64	0.55	0.52	0.56	0.54	0.53	0.55	0.53
Ethinylestradiol	0.27	0.29	0.27	0.23	0.26	0.33	0.24	0.3	0.31	0.27	0.22	0.3	2.2	2.3	2.9	3.3	2.6	2.8	2.5	2.7	2.6	2.8	2.6	2.8
3-Benzylidencamphor (3-BC)	14	13	13	15	14	14	20	18	16	20	18	16	88	100	95	134	152	108	107	140	112	108	137	109
4-methyl benzylidencamphor (4-MBC)	2.7	2.9	2.3	2.8	3.8	2.4	3.2	2.9	2.9	3.2	2.9	2.5	19	21	20	26	23	19	17	22	16	17	20	16
Octyl methoxycinnamate (OMC)	No linear calibration																							
p-Hydroxybenzoic acid (pOHBA)	12	12	13	14	19	14.0	1	12	7	2	5	2	119	118	118	131	155	135	133	140	134	126	138	144
Triclosan	0.30	0.34	0.30	0.36	0.33	0.30	0.28	0.27	0.22	0.28	0.25	0.23	2.0	2.2	2.2	3.0	2.6	2.3	1.9	2.1	1.9	2.1	2.0	1.8
Phytotoxins																								
Anisodamine	0.013	0.013	0.012	0.021	0.018	0.022	0.014	0.015	0.015	0.012	0.012	0.013	0.11	0.12	0.13	0.14	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Aristolochic acid I	0.16	0.14	0.08	0.22	0.21	0.22	0.31	0.32	0.32	0.33	0.29	0.28	1.7	2.1	2.8	3.2	3.0	3.1	2.9	2.8	2.8	3.2	3.2	3.3
Aristolactam I	0.12	0.11	0.10	0.14	0.15	0.13	0.13	0.13	0.13	0.12	0.12	0.12	1.0	1.2	1.4	1.6	1.3	1.3	1.2	1.1	1.2	1.1	1.1	1.2
Jacobine	0.072	0.064	0.065	0.078	0.067	0.078	0.063	0.06	0.059	0.07	0.076	0.064	0.6	0.64	0.61	0.72	0.64	0.72	0.67	0.69	0.72	0.72	0.71	0.72
Jacobine-N-oxide	0.019	0.022	0.016	0.015	0.0089	0.013	0.014	0.012	0.016	0.012	0.015	0.012	0.14	0.10	0.10	0.10	0.12	0.13	0.13	0.15	0.14	0.15	0.15	0.13
Riddelliin	0.074	0.065	0.081	0.076	0.089	0.072	0.1	0.077	0.079	0.08	0.082	0.081	0.73	0.68	0.84	0.84	0.82	0.88	0.87	0.85	0.85	0.83	0.85	0.8
Riddelliin-N-oxide	0.063	0.052	0.063	0.065	0.071	0.069	0.059	0.056	0.047	0.054	0.045	0.054	0.47	0.51	0.48	0.56	0.59	0.53	0.62	0.58	0.58	0.63	0.55	0.54
Scopolamine	0.0044	0.0042	0.0041	0.0042	0.0043	0.0046	0.0044	0.0043	0.0044	0.0042	0.0042	0.0044	0.037	0.038	0.038	0.044	0.042	0.044	0.043	0.043	0.043	0.044	0.044	0.043
Disinfection by-products																								
Bromoacetic acid	2.4	1.3	8.3	2.2	7.6	1.6	n.d.	5.3	n.d.	1.3	n.d.	n.d.	27	20	15	39	13	7.7	52	55	59.0	50	55	62
Dibromoacetic acid	3.8	4.0	3.5	3.9	3.0	3.4	4.1	3.9	3.9	3.8	3.7	3.8	43	34	34	30	34	36.	41	41	39	39	42	40
Dichloroacetic acid	26	25	24	21	19	21	20	24	23	20	22	23	188	183	202	163	172	186	216	223	219	219	229	217
Food processing by-products																								
Acrylamide	18	17	17	23	19	24	19	19	18	18	20	17	200	160	176	220	180	200	180	189	211	175	196	211
5-Hydroxymethylfurfural (HMF)	8.6	8.4	7.9	7.0	6.6	6.7	8.3	8.7	9.0	9	9	8	80	71	79	81	64	75	86	84	83	94	89	81
5-Hydroxymethyl-2-furanoic acid (HMFA)	53	58	52	59	49	54	45	45	51	36	44	42	571	527	497	537	581	547	582	542	537	601	593	585
N-Nitosodimethylamine (NDMA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16.0	29.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PhIP	0.028	0.028	0.027	0.029	0.029	0.029	0.027	0.027	0.026	0.029	0.028	0.028	0.29	0.23	0.23	0.24	0.23	0.24	0.28	0.28	0.28	0.28	0.28	0.28
Air pollutants																								
Cotinine	No linear calibration																							
Trans-3-hydroxy cotinine	<0	<0	<0	<0	0.26	0.22	0.25	0.14	0.20	0.051	0.044	0.045	<0	1.3	1.5	1.6	1.3	1.1	1.5	1.6	1.7	1.5	1.6	1.7
1-Hydroxy pyrene	0.50	0.48	0.39	0.33	0.29	0.36	0.33	0.35	0.31	0.31	0.28	0.25	4.4	4.9	5.1	5.9	4.2	4.7	4.1	3.3	3.9	3.9	3.6	4.1
3-Hydroxy phenanthrene	0.044	0.047	0.039	0.033	0.038	0.033	0.041	0.039	0.038	0.037	0.034	0.03	0.33	0.4	0.44	0.49	0.40	0.42	0.34	0.32	0.35	0.32	0.33	0.36

Table 34: Extraction recoveries in breast milk. In some cases, the spiked amount could not be differentiated from the natural matrix contamination (marked with "<0"). Values determined as outliers by Dixon's Q- and Grubbs's testing are marked with (*).

Compound	Extraction recoveries in breast milk [ng/mL]																							
	Validation 1 LL			Validation 2 LL			Validation 3 LL			Validation 3 LL - repeatability			Validation 1 HL			Validation 2 HL			Validation 3 HL			Validation 3 HL - repeatability		
Plasticizer/plastic components																								
Bisphenol A (BPA)	0.22	0.21	0.22	0.31	0.28	0.25	0.2	0.26	0.26	0.21	0.25	0.24	2.5	2.2	2.0	3.5	2.6	2.8	2.2	1.9	2.2	2.0	2.6	2.1
Bisphenol AF (BPAF)	0.057	0.081	0.053	0.067	0.091	0.094	0.054	0.061	0.075	0.068	0.059	0.074	0.67	1.0	0.84	0.55	0.73	0.86	0.79	0.76	0.85	0.75	0.77	0.92
Bisphenol B (BPB)	0.016	0.02	0.014	0.024	0.026	0.022	0.0089	0.0054	0.0071	0.019	0.017	0.024	0.14	0.26	0.18	0.13	0.17	0.19	0.18	0.17	0.19	0.17	0.17	0.22
Bisphenol C (BPC)	0.20	0.29	0.19	0.32	0.37	0.38	0.3	0.3	0.35	0.24	0.24	0.29	2.6	4.4	3.4	2.2	3.3	3.7	3.5	3.4	3.8	3.2	3.2	3.8
Bisphenol F (BPF)	0.098	0.14	0.085	0.086	0.10	0.10	0.12	0.11	0.13	0.12	0.10	0.12	1.2	1.4	1.3	1.0	1.2	1.2	1.3	1.2	1.2	1.1	1.1	1.2
Bisphenol S (BPS)	0.0058	0.0057	0.0061	0.0054	0.0063	0.0075	0.0057	0.0041	0.0051	0.0046	0.0051	0.0044	0.053	0.053	0.046	0.05	0.059	0.044	0.046	0.047	0.05	0.038	0.044	0.047
Mono-n-butyl phthalate (MBP)	0.97	1.1	1.3	1.5	1.2	1.2	1.0	1.4	0.97	1.1	1.3	0.9	10	9	10	12	12	13	12	11	11	11	10	11
Mono-2-ethylhexyl phthalate (MEHP)	0.21	0.2	0.35	0.17	0.18	0.17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.1	1.9	1.5	1.8	1.6	1.6	n.d.	n.d.	n.d.	n.d.	n.d.	3.5
N-butyl benzenesulfonamide	1.7	2.5	0.76	3.9	1.3	0.98	1.7	4.6	1.8	0.42	3.4	0.45	18	23	20	15	26	20	22	21	21	20	20	22
Benzyl butyl phthalate	No linear calibration																							
Dibutyl phthalate	No linear calibration																							
Tetrabromobisphenol A	No linear calibration																							
Perfluorinated alkylated substances																								
Perfluorooctanoic acid (PFOA)	0.023	0.036	0.025	0.044	0.043	0.042	0.024	0.033	0.034	0.027	0.035	0.026	0.26	0.24	0.20	0.30	0.35	0.33	0.25	0.22	0.23	0.25	0.25	0.25
Perfluorooctanesulfonic acid (PFOS)	0.049	0.047	0.052	0.035	0.035	0.037	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.28	0.33	0.24	0.30	0.31	0.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Industrial side products and pesticides																								
2-Naphthol	0.053	0.063	0.0054	0.084	0.0058	0.041	0.13	0.091	0.15	0.12	0.061	0.16	0.33	0.49	0.55	0.34	0.54	0.41	0.48	0.54	0.55	0.49	0.53	0.60
Methiocarb	0.0044	0.0066	0.0062	0.0071	0.0069	0.0062	0.0043	0.0031	0.0063	0.0045	0.0059	0.0038	0.070	0.090	0.077	0.053	0.077	0.087	0.078	0.074	0.095	0.067	0.080	0.086
Prochloraz	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.00048	n.d.	0.00014	0.00031	n.d.	n.d.	n.d.	0.0029	0.004	0.0080	0.00008	0.0046	0.0013	0.0012	0.0066	0.0016
2-tert-Butylphenol (2-tert-BP)	19	15	14	40	60	48	33	10	33	31	12	32	272	374	574	392	327	646	30	327	321	28	323	321
4-Octylphenol (4-OP)	n.d.	n.d.	n.d.	9.6	14.0	13.0	n.d.	n.d.	n.d.	n.d.	n.d.	2	130.0	n.d.	n.d.	97.0	68.0	230.0	43.0	32.0	34.0	44	33	33
4-tert-Octylphenol(4-tert-OP)	n.d.	n.d.	n.d.	3.3	2.5	2.5	2.0	1.4	0.59	0.23	1.5	1.1	9.7	13	9.2	14	13	12	14	11	8.5	8.9	8.1	11.0
Fenarimol	0.0025	0.0032	0.0026	0.0066	0.006	0.0072	n.d.	n.d.	n.d.	n.d.	n.d.	0.0019	0.030	0.044	0.043	0.032	0.038	0.039	0.021	0.025	0.028	0.022	0.027	0.029
Nonylphenol	No linear calibration																							
Endogenous estrogens																								
Estrone (E1)	n.d.	n.d.	n.d.	0.0070	0.0033	0.0044	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.028	0.087	0.057	0.013	0.034	0.047	0.030	0.019	0.045	0.045	0.047	0.073
Estradiol (E2)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.47	0.43	0.45	0.57	0.53	0.58	0.37	0.31	0.36	0.43	0.33	0.31
Estradiol-17-glucuronide (E2-17-GlcA)	0.093	0.092	0.072	0.14	0.13	0.15	0.091	0.11	0.18	0.091	0.12	0.084	1.0	1.3	1.2	1.1	1.1	1.1	1.1	1.0	0.97	0.85	1.0	1.0
Estradiol-3-sulfate (E2-3-sulfate)	0.016	0.021	0.019	0.018	0.023	0.049	0.022	0.028	0.033	0.0091	0.020	0.027	0.20	0.26	0.15	0.26	0.19	0.28	0.25	0.2	0.27	0.19	0.20	0.27
Estriol (E3)	0.038	0.058	0.043	0.079	0.081	0.063	0.034	0.049	0.038	0.034	0.032	0.038	0.62	0.72	0.61	0.53	0.48	0.61	0.61	0.53	0.66	0.52	0.51	0.65
16-Epiestriol (16EpiE3)	0.21	0.17	0.11	0.14	0.21	0.24	0.17	0.15	0.14	0.17	0.17	0.22	1.5	2.1	1.7	1.2	1.7	2.2	1.8	1.9	2.1	1.8	1.7	2.1
16-α-Hydroxyestrone (16OHE1)	0.029	0.053	0.033	0.017	0.025	0.04	0.038	0.030	0.036	0.037	0.036	0.029	0.27	0.40	0.29	0.26	0.33	0.31	0.34	0.33	0.30	0.30	0.30	0.31
17-Epiestriol (17EpiE3)	0.13	0.16	0.13	0.21	0.21	0.23	0.18	0.11	0.17	0.14	0.13	0.22	1.6	2.4	1.9	1.5	1.8	2.1	2.0	1.8	2.0	1.8	1.7	2.0
2-Methoxy estrone (2MeOE1)	0.023	0.049	0.04	0.042	0.046	0.048	0.00023	0.019	0.021	0.025	0.023	0.017	0.32	0.49	0.36	0.27	0.39	0.41	0.35	0.31	0.39	0.36	0.38	0.47
2-Methoxy estradiol (2MeOE2)	n.d.	n.d.	n.d.	0.024	0.023	0.024	n.d.	n.d.	n.d.	0.015	0.012	0.014	0.21	0.35	0.27	0.24	0.30	0.35	0.24	0.23	0.28	0.29	0.29	0.34
4-Methoxy estrone (4MeOE1)	n.d.	n.d.	n.d.	0.0049	0.0076	0.0061	n.d.	n.d.	n.d.	0.0046	0.0031	0.0074	0.064	0.10	0.072	0.05	0.089	0.098	0.079	0.073	0.083	0.083	0.076	0.091
4-Methoxy estradiol (4MeOE2)	0.0058	0.0062	0.0094	0.011	0.014	0.014	0.0036	0.004	0.0035	0.0078	0.0016	0.012	0.11	0.20	0.15	0.11	0.15	0.18	0.14	0.15	0.16	0.14	0.13	0.16
4-Hydroxy estrone (4OHE1)	0.0094	n.d.	n.d.	0.0078	0.0066	0.0053	0.0070	0.0030	0.0060	0.0087	0.0024	0.0040	0.045	0.084	0.060	0.048	0.064	0.039	0.060	0.067	0.074	0.067	0.070	0.073
Phytoestrogens and metabolites																								
8-Prenylnaringenin	0.036	0.042	0.026	0.032	0.046	0.045	0.027	0.031	0.032	0.023	0.024	0.025	0.30	0.51	0.43	0.30	0.36	0.49	0.30	0.26	0.34	0.29	0.26	0.35
Coumestrol	0.0065	0.0076	0.0054	0.0079	0.0094	0.011	0.01	0.0083	0.012	0.0089	0.0095	0.012	0.079	0.13	0.11	0.071	0.097	0.12	0.099	0.11	0.11	0.098	0.10	0.12
Daidzein	0.0061	0.018	<0	0.032	0.022	0.014	0.027	0.018	0.015	0.034	0.010	0.019	0.14	0.15	0.16	0.13	0.17	0.11	0.12	0.14	0.13	0.097	0.12	0.13
Enterodiol	0.0033	0.014	0.0065	0.0013	0.0051	0.0051	0.0038	0.012	0.0069	0.0044	0.011	0.012	0.043	0.10	0.047	0.07	0.041	0.082	0.06	0.044	0.079	0.057	0.043	0.079
Enterolactone	0.22	0.31	0.35	0.63	0.68	0.49	0.52	0.45	0.52	0.48	0.43	0.52	4.6	5.8	5.2	4.0	5.0	4.8	5.2	5.1	5.3	4.6	4.8	5.2
Equol	0.032	0.037	0.024	0.028	0.035	0.042	0.033	0.043	0.050	0.030	0.039	0.040	0.23	0.48	0.37	0.27	0.33	0.42	0.40	0.37	0.46	0.38	0.35	0.45
Formononetin	0.0037	0.0047	0.003	0.0036	0.0044	0.0055	0.0049	0.0043	0.0055	0.0044	0.004	0.0051	0.039	0.064	0.048	0.033	0.048	0.052	0.05	0.054	0.056	0.046	0.049	0.056
Genistein	0.030	0.025	0.050	0.064	0.028	0.014	0.032	0.00005	0.0061	0.024	0.00046	0.0093	0.10	0.11	0.14	0.15	0.14	0.10	0.097	0.13	0.10	0.12	0.11	0.15
Glycitein	0.0086	0.0093	0.0067	0.014	0.010	0.011	0.015	0.012	0.011	0.013	0.010	0.012	0.12	0.13	0.12	0.12	0.14	0.12	0.12	0.095	0.12	0.11	0.097	0.12
Isoxanthohumol	0.0012	0.0012	0.0011	0.0019	0.0016	0.002	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.014	0.022	0.018	0.013	0.018	0.021	0.013	0.015	0.019	0.014	0.019	0.02
Matairesinol	0.093	0.14	0.084	0.15	0.13	0.14	0.12	0.12	0.13	0.13	0.16	0.12	1.1	1.4	1.2	1.0	1.3	1.2	1.2	1.2	1.2	1.1	1.2	1.3
Resveratrol	0.28	0.77	0.43	0.37	0.37	1.3	0.24	0.57	0.41	0.22	0.50	0.36	3.5	10	0.97	3.5	3.2	8.6	3.2	3.1	5.4	3.0	3.0	5.3
Xanthohumol	n.d.	0.012	0.17	0.16	0.18	0.18	0.043	0.02	0.071	0.034	0.078	0.093	1.6	0.74	0.94	1.4	1.2	2.2	0.92	0.76	0.99	0.89	0.79	0.81

Compound	Extraction recoveries in breast milk [ng/mL]																							
	Validation 1 LL			Validation 2 LL			Validation 3 LL			Validation 3 LL - repeatability			Validation 1 HL			Validation 2 HL			Validation 3 HL			Validation 3 HL - repeatability		
Mycoestrogens and metabolites																								
Alternariol	0.13	0.16	0.12	0.20	0.20	0.23	0.20	0.18	0.22	0.18	0.17	0.20	1.7	2.5	2.1	1.3	1.9	2.1	2.0	2.1	2.2	2.0	2.0	2.2
Alternariol monomethyl ether	n.d.	n.d.	n.d.	0.0074	0.013	0.011	<0	<0	<0	n.d.	n.d.	n.d.	0.065	0.097	0.095	0.054	0.072	0.09	0.071	0.07	0.075	0.056	0.052	0.076
α-Zearalanol (α-ZAL)	0.048	0.065	0.058	0.060	0.077	0.084	0.051	0.068	0.081	0.061	0.073	0.071	0.60	1.1	0.80	0.58	0.74	0.92	0.79	0.77	0.94	0.75	0.81	1.0
β-Zearalanol (β-ZAL)	0.057	0.081	0.058	0.073	0.088	0.10	0.083	0.084	0.11	0.099	0.089	0.11	0.72	1.3	0.87	0.58	0.89	1.0	1.0	0.98	1.1	0.9	0.93	1.1
α-Zearalenol (α-ZEL)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.030	0.050	0.038	0.029	0.036	0.044	0.023	0.018	0.033	0.014	0.013	0.027
β-Zearalenol (β-ZEL)	0.093	0.14	0.12	0.15	0.17	0.20	0.16	0.16	0.19	0.16	0.17	0.21	1.3	2.4	1.7	1.2	1.7	2.0	1.8	1.8	2.1	1.7	1.8	2.1
α-Zearalenol-14-glucuronide (α-ZEL-14-GlcA)	0.018	0.029	0.019	0.024	0.033	0.033	0.031	0.031	0.028	0.024	0.020	0.027	0.24	0.41	0.27	0.25	0.21	0.30	0.24	0.21	0.25	0.19	0.19	0.27
β-Zearalenol-14-glucuronide (β-ZEL-14-GlcA)	0.032	0.032	0.035	0.030	0.016	0.035	0.022	0.033	0.023	0.018	0.027	0.026	0.21	0.30	0.22	0.28	0.24	0.27	0.21	0.22	0.24	0.18	0.23	0.21
Zearalanone (ZAN)	n.d.	n.d.	n.d.	0.043	0.053	0.053	0.022	0.029	0.029	0.037	0.029	0.032	0.43	0.71	0.49	0.33	0.39	0.51	0.39	0.37	0.45	0.34	0.38	0.42
Zearalenone (ZEN)	0.055	0.077	0.060	0.077	n.d.	0.068	0.041	0.040	0.043	0.038	0.047	0.045	0.48	0.53	0.56	0.60	0.66	0.51	0.38	0.47	0.41	0.35	0.41	0.39
Zearalenone-14-glucuronide (ZEN-14-GlcA)	0.11	0.11	0.13	0.13	0.053	0.087	0.12	0.084	0.094	0.098	0.073	0.14	1.2	1.3	1.4	0.99	1.0	1.1	1.0	1.0	1.1	0.85	1.2	0.94
Zearalenone-14-sulfate (ZEN-14-sulfate)	0.0059	0.013	0.015	0.018	0.029	0.033	0.019	0.027	0.029	0.017	0.020	0.027	0.21	0.20	0.087	0.19	0.14	0.32	0.26	0.20	0.26	0.23	0.20	0.25
Personal care product ingredients pharmaceuticals and metabolites																								
Benzophenone 1	0.029	0.043	0.033	0.028	0.034	0.042	0.028	0.035	0.038	0.028	0.034	0.033	0.30	0.50	0.40	0.24	0.34	0.40	0.35	0.38	0.40	0.35	0.36	0.41
Benzophenone 2	0.032	0.030	0.028	0.030	0.033	0.039	0.038	0.032	0.041	0.037	0.036	0.034	0.36	0.41	0.36	0.33	0.37	0.35	0.37	0.34	0.37	0.34	0.36	0.4
Benzylparaben	0.0019	0.0026	0.0018	0.0024	0.0026	0.0027	0.0018	0.0018	0.0029	0.0019	0.0019	0.0028	0.020	0.036	0.027	0.017	0.022	0.028	0.025	0.027	0.028	0.024	0.028	0.03
Butylparaben	0.013	0.012	0.014	0.017	0.021	0.018	0.0092	0.011	0.011	0.012	0.015	0.011	0.14	0.13	0.14	0.18	0.20	0.22	0.14	0.13	0.13	0.13	0.14	0.14
Ethylparaben	0.032	0.026	0.023	0.079	0.025	0.012	0.029	0.036	0.014	0.033	0.032	0.015	0.17	0.17	0.20	0.24	0.28	0.26	0.20	0.20	0.19	0.19	0.20	0.19
Isobutylparaben	0.011	0.015	0.0096	0.012	0.014	0.016	0.014	0.011	0.020	0.012	0.011	0.017	0.13	0.23	0.18	0.10	0.15	0.18	0.17	0.20	0.20	0.16	0.20	0.20
Methylparaben	0.086	0.091	0.051	0.20*	0.067	0.027	0.076	0.065	0.050	0.079	0.058	0.038	0.45	0.44	0.55	0.59	0.74	0.57	0.40	0.43	0.39	0.40	0.42	0.40
Propylparaben	0.045	0.044	0.038	0.082	0.042	0.022	0.057	0.055	0.037	0.047	0.045	0.023	0.31	0.30	0.34	0.46	0.52	0.5	0.42	0.41	0.42	0.40	0.42	0.40
Ethinylestradiol	n.d.	n.d.	n.d.	0.11	0.11	0.16	n.d.	n.d.	n.d.	0.018	0.15	0.22	1.4	2.2	1.5	1.2	1.4	1.6	1.5	1.4	1.5	1.5	1.4	1.7
3-Benzylidencamphor (3-BC)	<0	6.4	32	14	18	17	7.1	7.3	8.2	7.0	7.9	8.1	180	112	136	115	111	176	89	80	85	81	91	83
4-methyl benzylidencamphor (4-MBC)	n.d.	n.d.	<0	n.d.	n.d.	n.d.	0.11	0.13	n.d.	n.d.	0.43	0.84	19.0	n.d.	n.d.	14	13	21	8.8	9.4	8.5	9	10	8.8
Octyl methoxycinnamate (OMC)	No linear calibration																							
p-Hydroxybenzoic acid (pOHBA)	9.2	11	6.8	13	12	12	11	13	12	11	13	11	108	115	124	118	129	132	110	110	108	115	112	117
Triclosan	n.d.	n.d.	n.d.	0.13	0.12	0.15	n.d.	n.d.	n.d.	0.12	0.14	0.13	1.1	0.42	0.39	0.93	0.93	1.2	0.57	0.59	0.70	0.68	0.65	0.64
Phytotoxins																								
Anisodamine	0.0033	0.0075	0.0047	0.0076	0.0070	0.0078	0.0062	0.0079	0.036	0.0027	0.0058	0.029	0.084	0.11	0.094	0.084	0.089	0.083	0.071	0.09	0.072	0.052	0.085	0.068
Aristolochic acid I	0.081	0.16	0.11	n.d.	n.d.	0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.3	1.5	1.5	1.2	1.2	1.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Aristolactam I	n.d.	n.d.	0.020	0.098	0.10	0.099	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.45	0.41	0.56	0.47	0.49	0.72	0.41	0.47	0.55	0.38	0.52	0.49
Jacobine	0.023	0.045	0.028	0.047	0.027	0.049	0.029	0.032	0.029	0.016	0.021	0.023	0.32	0.34	0.34	0.38	0.46	0.33	0.23	0.21	0.2	0.16	0.21	0.18
Jacobine-N-oxide	0.0026	0.012	0.0027	0.0029	0.002	0.0061	0.0015	0.0046	0.0048	0.0024	0.0036	0.0077	0.023	0.055	0.033	0.056	0.044	0.051	0.020	0.018	0.022	0.012	0.019	0.022
Riddelliin	0.022	0.048	0.038	0.052	0.049	0.043	0.032	0.037	0.031	0.029	0.036	0.029	0.57	0.59	0.58	0.46	0.55	0.42	0.36	0.48	0.30	0.25	0.50	0.32
Riddelliin-N-oxide	0.014	0.021	0.010	0.037	0.023	0.041	0.017	0.020	0.027	0.013	0.013	0.018	0.18	0.36	0.24	0.29	0.27	0.29	0.13	0.22	0.15	0.091	0.19	0.18
Scopolamine	0.0015	0.0027	0.0018	0.0028	0.0029	0.003	0.0023	0.0031	0.0027	0.0021	0.0027	0.0023	0.030	0.035	0.032	0.028	0.029	0.028	0.027	0.03	0.026	0.02	0.031	0.027
Disinfection by-products																								
Bromoacetic acid	No linear calibration																							
Dibromoacetic acid	1.5	1.8	1.5	3.0	1.6	2.1	1.8	0.54	1.2	1.4	1.1	1.3	23	17	26	25	29	19.	10	18	12	10	19	13
Dichloroacetic acid	8.1	6.7	7.9	13	6.8	6.8	3.7	3.0	2.3	5.3	4.4	1.5	89	40	101	92	101	69	14	76	50	11	78	54
Food processing by-products																								
Acrylamide	8.5	6.6	9.5	14	7.8	10	8.4	5.3	5.3	7.0	4.4	4.9	101	72	111	109	119	102	21*	116	83	18	106	85
5-Hydroxymethylfurfural (HMF)	0.79	2.7	1.4	0.92	3.2	5.5	n.d.	n.d.	2.9	n.d.	0.42	3.3	2.9	25	23	45	7.5	49	6.7	5.2	15	6.1	5.9	16
5-Hydroxymethyl-2-furanoic acid (HMFA)	29	32	25	37	7.9*	38	26	26	28	26	29	28	305	277	374	94*	290	357	300	355	255	220	345	280
N-Nitosodimethylamine (NDMA)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	25	15	88	123	105	n.d.	54	n.d.	n.d.	50	n.d.
PhIP	0.013	0.014	0.011	0.019	0.018	0.022	0.018	0.015	0.019	0.018	0.016	0.020	0.15	0.28	0.23	0.17	0.21	0.22	0.17	0.21	0.18	0.14	0.21	0.17
Air pollutants																								
Cotinine	<0	<0	<0	<0	<0	0.19	0.14	0.11	0.35	0.14	0.071	0.39	0.52	1.8	0.93	<0	<0	0.71	0.6	1.4	0.59	0.34	1.4	0.57
Trans-3-hydroxy cotinine	<0	<0	<0	<0	<0	<0	0.0072	0.0054	0.13	0.0089	0.0013	0.12	0.025	0.32	0.064	<0	<0	<0	0.068	0.16	0.081	0.048	0.16	0.094
1-Hydroxy pyrene	1.0	0.98	0.91	0.58	0.73	0.52	0.20	0.42	0.29	0.38	0.41	0.26	3.0	1.9	2.4	2.3	1.5	3.0	1.5	1.6	2.0	1.5	1.3	1.6
3-Hydroxy phenanthrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0093	0.012	0.012	0.20	0.35	0.32	0.15	0.18	0.28	0.17	0.19	0.18	0.12	0.17	0.15

Table 35: Results of 10 µL injections of breast milk samples. Concentrations are given in [ng/mL]. Analytes that were detected below the LOQ are described as (<LOQ). Analytes that were detected but could not be quantified because of lacking linearity are displayed as (det.*).

Compound [ng/mL]	Day																							
	2	5	6	11	15	18	23	25	30	38	41	43	46	49	67	124	136	139	143	146	150	153	162	168
Plasticizer/plastic components																								
Bisphenol A (BPA)	<LOQ							0.93																
Bisphenol F (BPF)																				<LOQ				
Bisphenol S (BPS)	0.016	0.0097	0.016	0.099	<LOQ	0.017	0.013	<LOQ	0.025	0.0091	<LOQ	0.018	0.023	<LOQ	0.011	0.0088	0.012	0.011	0.014	0.012	0.039	13	0.012	0.024
Mono-n-butyl phthalate (MBP)	0.47	<LOQ	1.3	0.27	<LOQ	<LOQ	<LOQ	<LOQ	6.2	0.42	0.61		<LOQ	0.44	<LOQ	0.44	1.2	0.32	0.51	0.28	<LOQ	2.0	0.94	0.67
N-butylbenzenesulfonamide	11	19	19	21	15	22	11	9.2	19	19	18	16	21	12	24	11	18	27	24	12	23	16	17	22
Perfluorinated alkylated substances																								
Perfluorooctanoic acid (PFOA)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
Perfluorooctanesulfonic acid (PFOS)	<LOQ	<LOQ	<LOQ	<LOQ	0.029	0.025	0.028	0.032	<LOQ	0.037	<LOQ	<LOQ	0.031	<LOQ	0.027	0.029	0.029	0.028	0.03	0.038	0.026	0.034	0.035	0.028
Industrial side products and pesticides																								
2-naphthol	0.62	0.84	0.72	0.78	0.67	0.98	0.66	0.46	0.96	0.87	1.9	1.8	0.74	0.71	0.87	0.44	0.61	0.77	1.7	1.5	2.3	2.0	0.66	2.3
Prochloraz								0.16																
Phytoestrogens and metabolites																								
8-prenylnaringenin			0.040	0.043		0.053	0.043	<LOQ									0.085		0.18	0.08			<LOQ	0.23
Daidzein	0.0095	0.014	0.011	0.042		0.083	0.15			0.042					0.011			<LOQ		0.14	0.015		0.034	
Enterodiol				0.038														0.020		0.032	0.014			
Enterolactone	<LOQ			<LOQ														<LOQ						
Glycitein	0.0064	0.0039		0.0078		0.0087	0.0072			0.0043										0.0085				
Isoxanthohumol	<LOQ		<LOQ	0.037	<LOQ	0.05		<LOQ			<LOQ	<LOQ	<LOQ		0.024		<LOQ			<LOQ		<LOQ		<LOQ
Resveratrol			4.2																					
Xanthohumol									det.*			det.*												
Mycoestrogens and metabolites																								
Alternariol																				1.0				
Personal care product ingredients, pharmaceuticals and metabolites																								
Benzophenone 1	0.044	0.023	0.056	0.043	0.023	0.029	0.026	0.034	0.025	0.031	0.023	0.037	0.03	0.029	0.029	0.025	0.032	0.026	<LOQ	0.021	0.072	0.041	0.029	0.030
Benzophenone 2																								
Butylparaben (BP)			0.025	0.025					0.021								<LOQ		<LOQ	<LOQ	<LOQ	<LOQ		<LOQ
Ethylparaben (EP)	<LOQ	0.084	0.087	0.088	0.056	0.053	0.050	0.048	0.094	0.086	0.062	0.059	0.12	0.081	0.084	<LOQ	0.091	0.11	0.077	0.053	0.083	0.074	0.12	0.072
Methylparaben (MP)	0.081	1.1	0.33	0.22	0.12	0.13	0.13	0.11	0.73	0.21	0.12	0.12	0.23	0.16	0.22	0.12	0.20	0.21	0.37	0.15	0.20	0.35	0.30	0.23
Propylparaben (PP)	0.043	0.90	0.14	0.11	0.061	0.046	0.047	0.046	0.82	0.12	0.056	0.059	0.11	0.072	0.11	0.069	0.083	0.099	0.25	0.13	0.12	0.25	0.20	0.16
Phytotoxins																								
Anisodamine	0.058	0.011		0.019	0.011		0.014					0.0043		0.050	0.0098						0.0059			0.0073
Jacobine-N-oxide			0.0097							0.24			0.0085	0.064										
Riddelliin-N-oxide																<LOQ								
Scopolamine				<LOQ		<LOQ	0.0009															<LOQ		
Food processing by-products																								
PhIP									<LOQ															

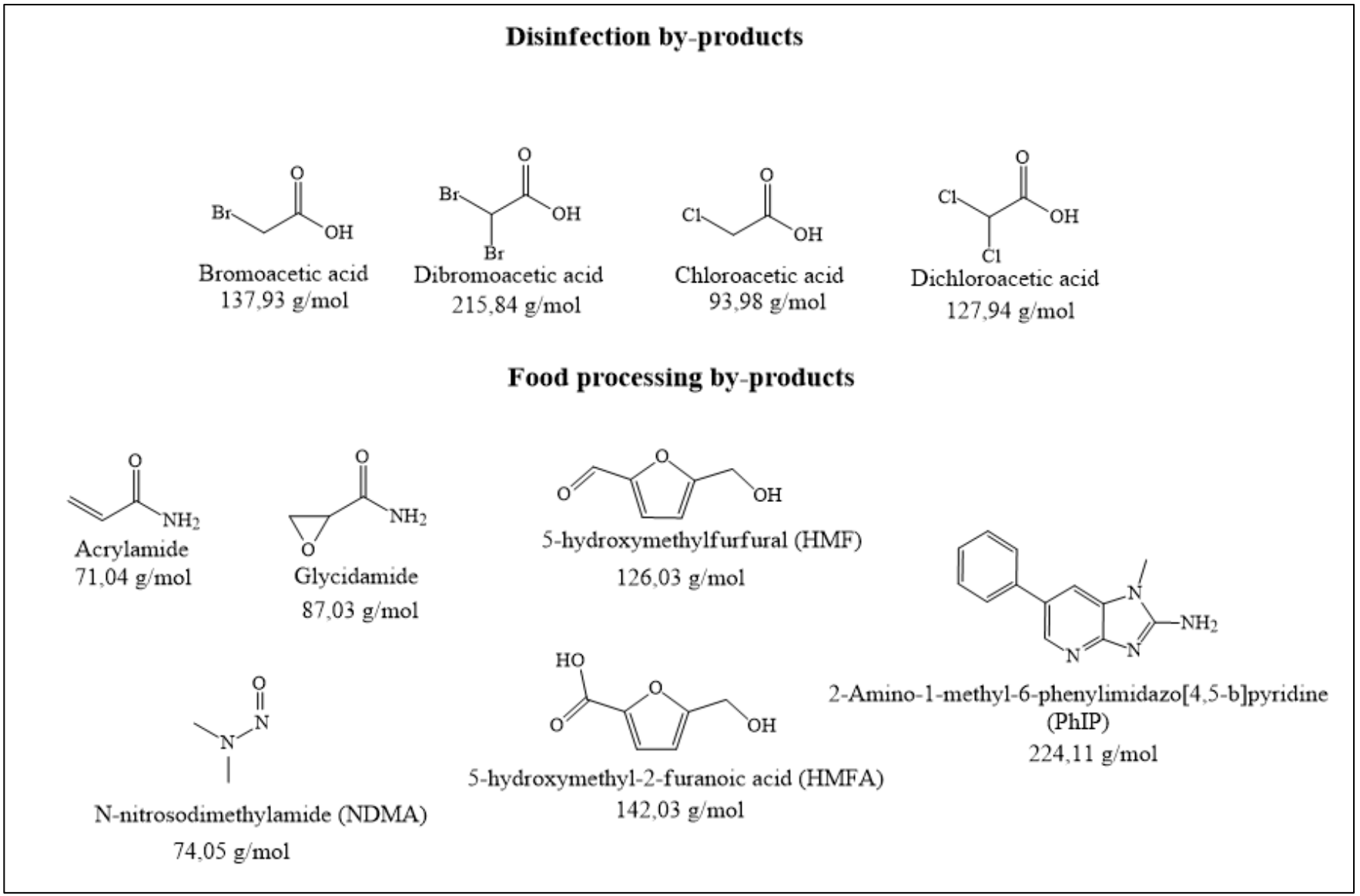

Figure 49: Structures of disinfection by-products and food processing by-products included in method development

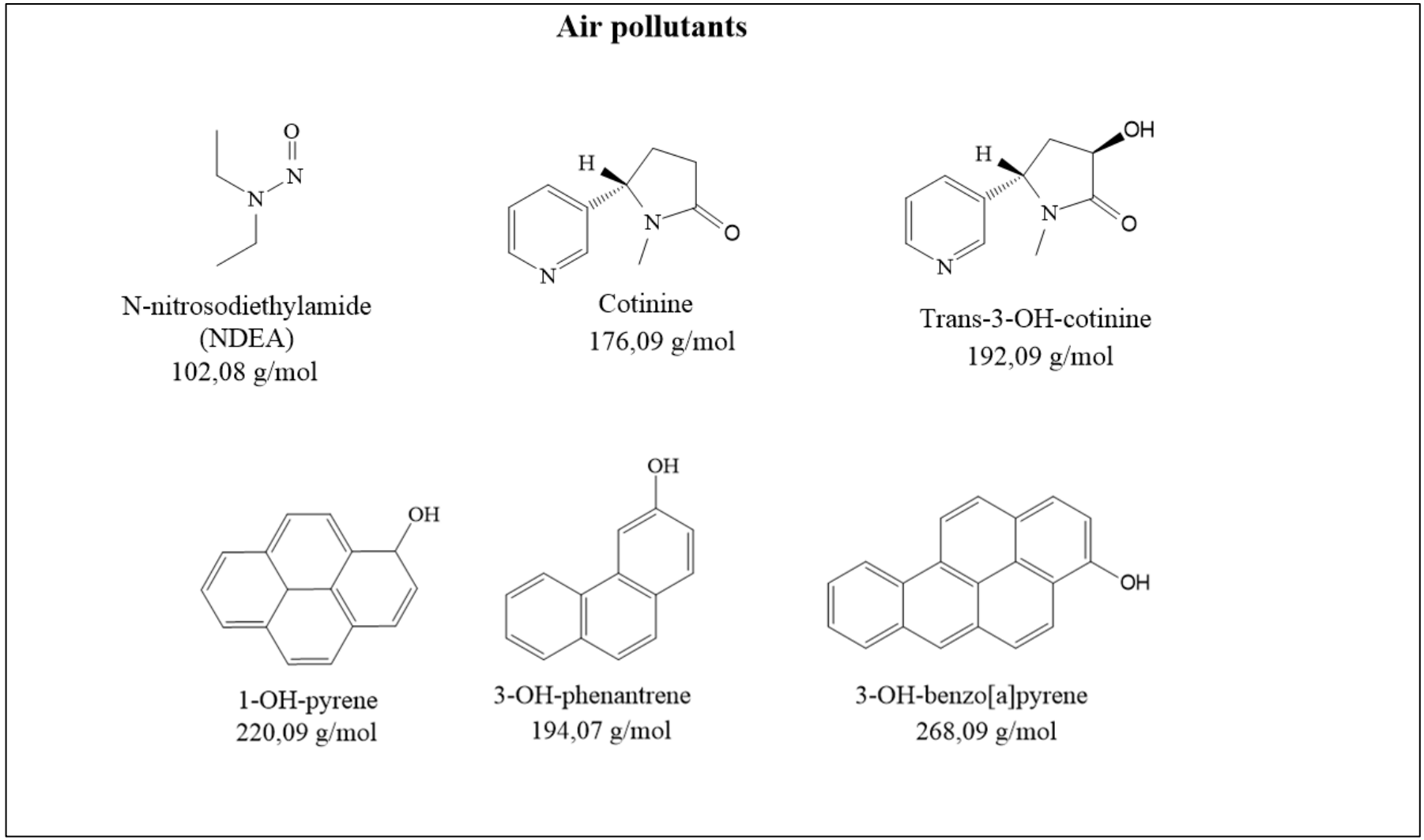

Figure 50: Structures of air pollutants and markers of smoke exposure included in method development

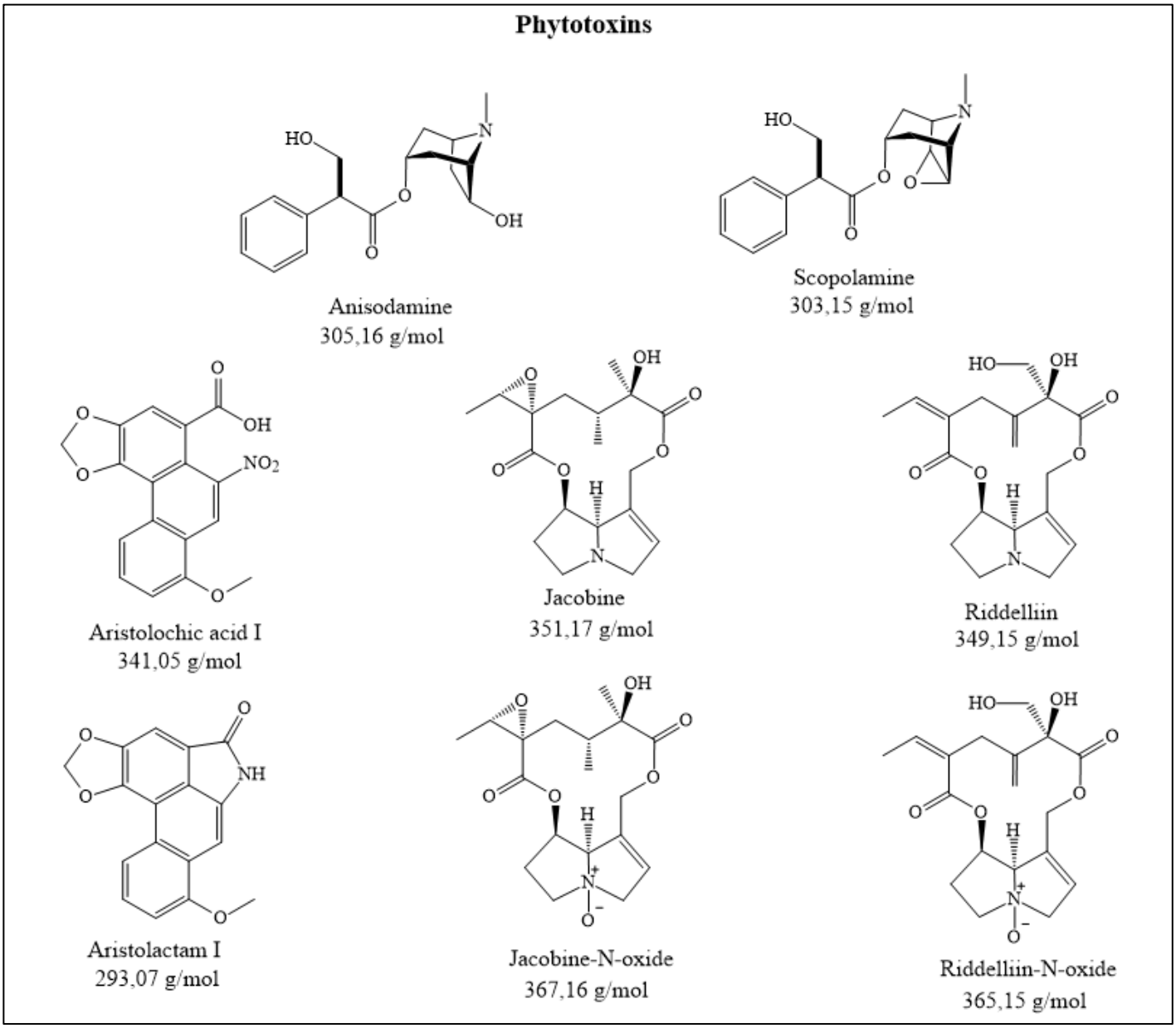

Figure 51: Structures of phytotoxins included in method development

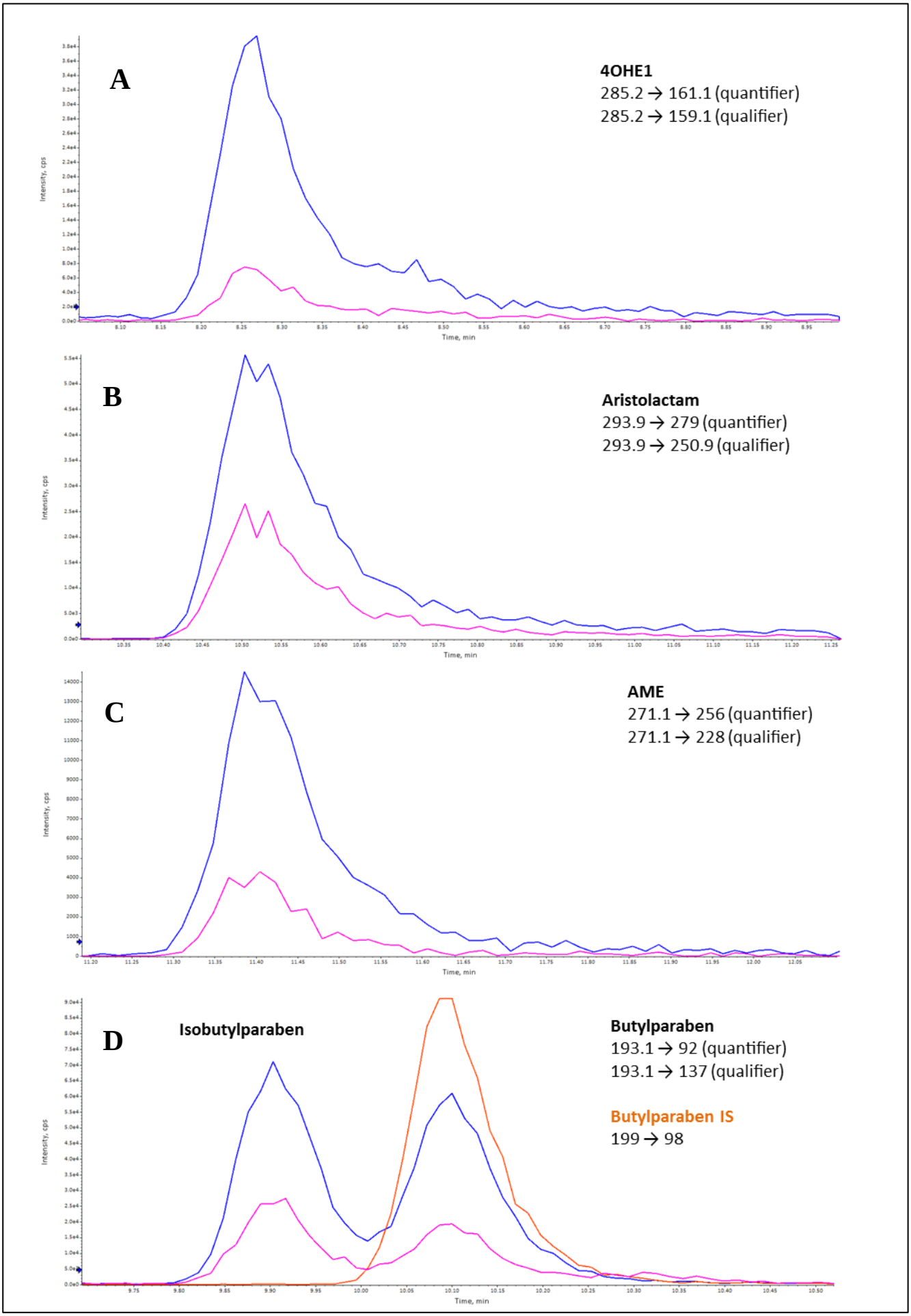

Figure 52: Chromatograms of 0.5 ng/mL 4OHE1 in urine (A), 0.5 ng/mL aristolactam in solvent (B), 0.15 ng/mL AME in solvent (C) and 0.3 ng/mL butylparaben in urine (D) with respective mass transitions given in [Da]

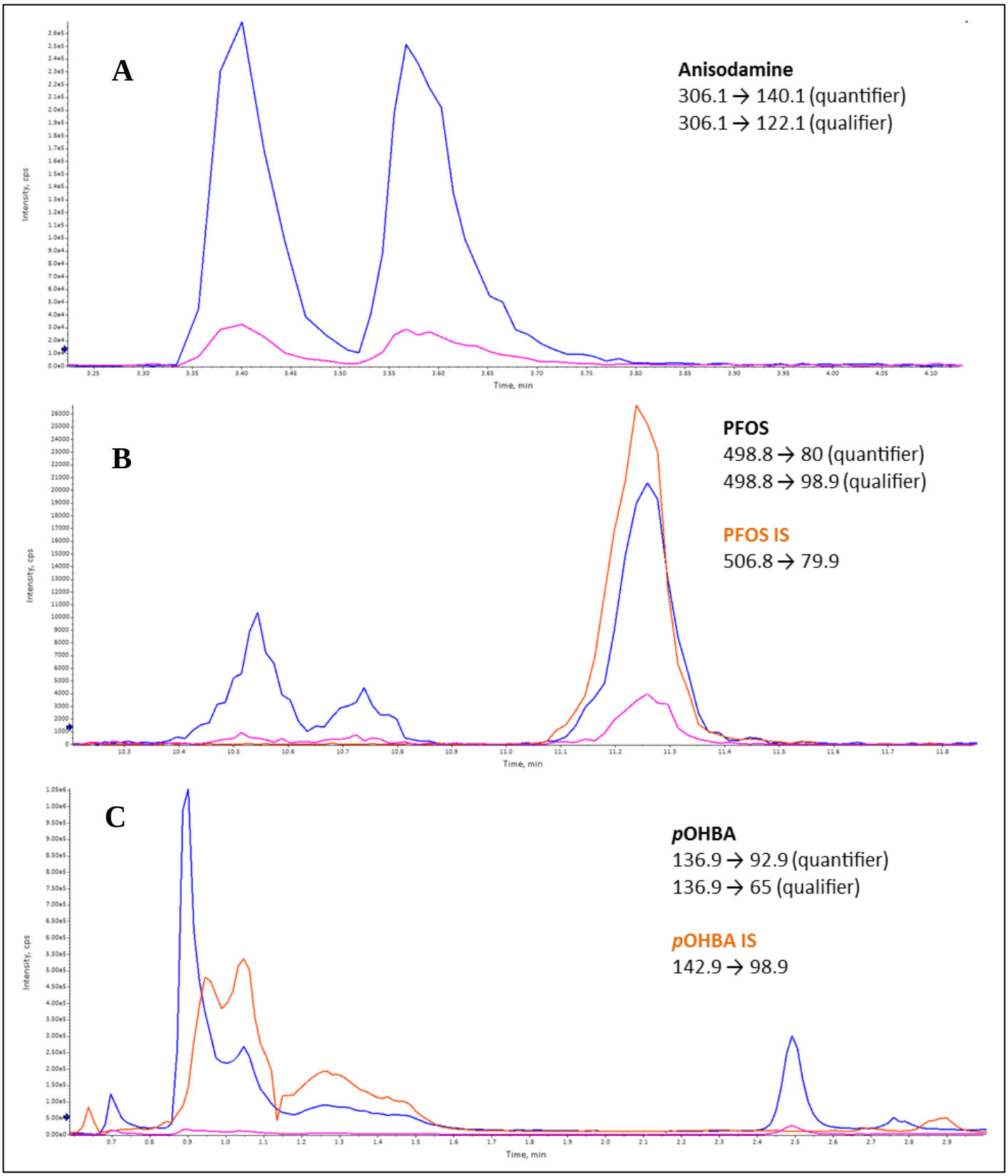

Figure 53: Chromatograms of 0.5 ng/mL anisodamine in solvent (A), 1.5 ng/mL PFOS in urine (B) and 1.5 ng/mL pOHBA in breast milk (C) with respective mass transitions given in [Da]

9. List of figures

10. List of Tables

11. References

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Summary of LC-MS/MS Sequences

Batch name	Purpose of experiment	LC-MS/MS Method	Data storage (D:\Analyst Data\Projects\2020\XenoQTrap\Data\-)
200115_XenOQTrap_v1_Multimix_Solv_Matrix	Determination of retention times and most intensive transitions of xenoestrogens	200113_XenoQTrap_v1	200115_XenOQTrap_v1_Multimix_Solv_Matrix
200131_XenoQTrap_v2_Multimix_Solv_Matrix	Determination of retention times and most intensive transitions of xenoestrogens	200131_XenoQTrap_v2_Top3Transition	200131_XenoQTrap_v2_Multimix_Solv_Matrix
200204_XenoQTrap_v2.1_Multimix_Solv_Matrix_MatrixBlanks	Determination of retention times and most intensive transitions of xenoestrogens	200204_XenoQTrap_v2.1_MissingAnalytes	200204_XenoQTrap_v2.1_Multimix_Solv_Matrix_MatrixBlanks
200218_XenoQTrap_v4+GasTransitions_Multimix_Matrix_MatrixBlanks	S/N ratios depending on needle position (2 mm vs 5 mm); determination of retention times and most intensive transitions of xenoestrogens	200218_XenoQTrap_v4; GasTransitions+missinganalytes	200218_XenoQTrap_v4+GasTransitions_Multimix_Matrix_MatrixBlanks
200310_XenoQTrap_v5_Multimix_Matrix_MatrixBlanks_Seq1	Estimation of LOQs of xenoestrogens	200310_XenoQTrap_v5	200310_XenoQTrap_v5_Multimix_Matrix_MatrixBlanks_Seq1
200313_XenoQTrap_v6_Multimix_Matrix_MatrixBlanks_Seq2	Estimation of LOQs of xenoestrogens; comparison of S/N ratios depending on ESI parameters (GS2 90/60, CAD Medium/High)	200313_XenoQTrap_v6_1;_2;_3;_4	200313_XenoQTrap_v6_Multimix_Matrix_MatrixBlanks_Seq2
200522_NS_v1_NS_SolventStandards	Determination of retention times and most intensive transitions of new compounds	200522_NS_v1; 20522_Aas+Jacobines	200522_XenoQTrap_v1_NewSubstances
200525_NS1+2_v2_NS_SolventStandards	Determination of retention times and most intensive transitions of new compounds	200525_NS_v2	200525_XenoQTrap_v2_NewSubstances
200527_XenoQTrap_v8_NS_MatrixSpikes	Estimation of LOQs of new compounds	200527_XenoQTrap_v8	200527_XenoQTrap_v8_NS_MatrixSpikes
200610_XenoQTrap_v9_Val1	Validation sequence 1	200610_XenoQTrap_v9	200610_XenoQTrap_v9_Val1
200626_XenoQTrap_v10_Val2	Validation sequence 2	200623_XenoQTrap_v10	200626_XenoQTrap_v10_Val2
200709_XenoQTrap_v10_Val3	Validation sequence 3 and baby serum samples	200623_XenoQTrap_v10	200709_XenoQTrap_v10_Val3
200722_XenoQTrap_v10_Val4	Validation sequence 4 (breast milk only)	200623_XenoQTrap_v10	200722_XenoQTrap_v10_Val4
200724_XenoQTrap_Milk_LongE	Xenobiotics in breast milk over time (samples 101-165)	200623_XenoQTrap_v10	200724_XenoQTrap_Milk_LongE
200730_XenoQTrap_Milk_LongE(166-189)+Urine_SiliconValley	Xenobiotics in breast milk over time (samples 166-189) and urine samples from silicon valley	200623_XenoQTrap_v10	200730_XenoQTrap_Milk_LongE(166-189)+Urine_SiliconValley
200804_XenoQTrap_Milk_LongE(adjusted RT)	Xenobiotics in breast milk over time (samples 101-189) with adjusted retention times	200803_XenoQTrap_v11 (RT PFOA, PFOS, AAI, MEHP, MEHP longer)	200804_XenoQTrap_Milk_LongE(adjusted RT)
200903_XenoQTrap_EPI_MS2_Standards	EPI and MS2 scans of standards	200903_XenoQTrap_EPI_MS2_Standards	200903_XenoQTrap_EPI_MS2_Standards
200904_XenoQTrap_MRM_EPI_IDA_Standards_Urine_Milk	IDA-EPI scans of serum standards and urine/breast milk samples	200904_XenoQTrap_MRM_EPI_IDA_"Standard Name " "Analyte Name"	200904_XenoQTrap_MRM_EPI_IDA_Standards_Urine_Milk
200906_XenoQTrap_MRM_EPI_IDA_RemainingUrineSamples	IDA-EPI scans of remaining urine samples	200906_XenoQTrap_MRM_EPI_IDA_"Standard Name " "Analyte Name"	200906_XenoQTrap_MRM_EPI_IDA_RemainingUrineSamples
200906_XenoQTrap_MRM_EPI_IDA_BabySerum	IDA-EPI scans of serum standards and baby serum samples	200906_XenoQTrap_MRM_EPI_IDA_"Sample Name" "Analyte Name"	200906_XenoQTrap_MRM_EPI_IDA_BabySerum
200910_XenoQTrap_EPI_full scan 1000Da per sec	Full scan EPI of serum and breast milk standards	200910_XenoQTrap_EPI_"AnalyteName"	200910_XenoQTrap_EPI_Stds+adult6_full scan 1000Da per sec
200915_XenoQTrap_EPI_serum_milk_full scan 1000Da per sec	Full scan EPI of baby serum and breast milk samples	200915_XenoQTrap_EPI_"AnalyteName"	200915_XenoQTrap_EPI_serum_milk_full scan 1000Da per sec