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Sensitive and scalable quantification of the chemical exposome by solid-phase extraction and LC-MS

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Abstract

The exposome is the measure of total environmental exposures and has a significant impact on human health outcomes. Exposomics research typically examines complex biological samples and assesses thousands of small molecules associated with chemical exposure events. To achieve comprehensive data, LC-MS/MS used for targeted analysis, and/or LC-HRMS for nontargeted analysis (NTA) have emerged as the preferred analytical tool in the field. Targeted assays focus on quantifying pre-defined compounds with high accuracy and precision while NTA can identify unknown chemicals. Even with these techniques, sensitivity and reproducibility still plague exposomic studies due to matrix effects and interferences associated with complex biological samples along with the challenge of typically very low abundance analytes. This highlights the critical need for advanced sample preparation and targeted LC-MS/MS analysis. Yet, proper methods, balancing sensitivity, broad analyte coverage, method robustness, and throughput, remain a major bottleneck in exposomics. To address these challenges, a well-balanced solid-phase extraction (SPE) protocol in 96-well format was developed for a panel of 94 highly diverse exposures in human urine and plasma using targeted LC-MS/MS. The established SPE method was shown to be approximately 10× faster than a routinely used protein precipitation approach when comparing the estimated total processing time of sample preparation. The method's applicability for NTA was tested and compared to the protein precipitation protocol using NIST standard reference materials for urine (SRM 3672) and plasma (SRM 1950) using LC-HRMS. Favorable performance was shown for the protein precipitation method while the SPE protocol demonstrated promising results. As a next step, a scalable workflow was developed and in-house validated for analyzing >230 biomarkers in human urine, plasma, and serum using targeted LC-MS/MS, leveraging the developed SPE method for sample clean-up. Method sensitivity and robustness were demonstrated for most of the analytes across the investigated matrices. 200 urine samples from pregnant women in a longitudinal pregnancy cohort were analyzed. More than 100 analytes were detected, several for the first time in a U.S. urinary biomonitoring study. This advanced workflow balances analyte coverage, sensitivity, robustness, and sample throughput, thereby meeting the stringent performance requirements for future exposome-scale research in public and personalized prevention.

Kurzfassung

Das Exposom ist das Maß der gesamten Umwelteinflüsse und hat einen signifikanten Einfluss auf die menschliche Gesundheit. Die Exposomforschung untersucht typischerweise komplexe biologische Proben und bewertet tausenden kleinen Molekülen, die mit chemischen Expositionsereignissen in Verbindung stehen. Um umfassende Daten zu erhalten, haben sich LC-MS/MS für die gerichtete Analyse und/oder LC-HRMS für die nichtgerichtete Analyse (NTA) als bevorzugte analytische Werkzeuge in diesem Bereich etabliert. Gerichtete Assays konzentrieren sich auf die Quantifizierung vordefinierter Verbindungen mit hoher Genauigkeit und Präzision, während NTA unbekannte Chemikalien identifizieren kann. Selbst mit diesen Techniken leiden Exposom Studien weiterhin unter Problemen mit Sensitivität und Reproduzierbarkeit, aufgrund von Matrixeffekten und Interferenzen in komplexen biologischen Proben sowie der Herausforderung von typischerweise sehr geringen Analytmengen. Dies unterstreicht den kritischen Bedarf an fortschrittlicher Probenvorbereitung und gerichteter LC-MS/MS-Analyse. Dennoch bleiben geeignete Methoden, die Empfindlichkeit, breite Analytenabdeckung, Methodenrobustheit und hohen Probendurchsatz ausbalancieren, weiterhin ein wasentlicher Engpass in der Exposomforschung. Um diesen Herausforderungen zu begegnen, wurde ein Festphasenextraktionsprotokoll (SPE) im 96-Well-Plattenformat für eine Gruppe von 94 hochdiversen Expositionsverbindungen in menschlichem Urin und Plasma unter Verwendung gerichteter LC-MS/MS entwickelt. Die etablierte SPE-Methode erwies sich im Vergleich zu einem routinemäßig verwendeten Proteinpräzipitationsansatz als etwa 10-mal schneller, wenn die geschätzte Gesamtbearbeitungszeit der Probenvorbereitung verglichen wurde. Die Anwendbarkeit der Methode für NTA wurde mittels LC-HRMS getestet und mit dem Proteinpräzipitationsprotokoll, unter Verwendung von NIST-Standardreferenzmaterialien für Urin (SRM 3672) und Plasma (SRM 1950), verglichen. Die Proteinpräzipitationsmethode zeigte eine vorteilhafte Leistung, während das SPE-Protokoll vielversprechende Ergebnisse lieferte. Als nächsten Schritt wurde ein skalierbarer Workflow entwickelt und intern validiert, um >230 Biomarker in menschlichem Urin, Plasma und Serum mittels gerichteter LC-MS/MS zu analysieren, wobei die entwickelte SPE-Methode zur Probenaufreinigung genutzt wurde. Die Empfindlichkeit und Robustheit der Methode wurden für die meisten Analyten in den untersuchten Matrizen demonstriert. 200 Urinproben von schwangeren Frauen einer longitudinalen Schwangerschaftskohorte wurden analysiert. Mehr als 100 Analyten wurden detektiert, einige davon zum ersten Mal in einer US-amerikanischen Biomonitoring-Studie für Urin. Dieser fortschrittliche Workflow gleicht Analytenabdeckung, Empfindlichkeit, Robustheit und Probendurchsatz aus und erfüllt somit die strengen Leistungsanforderungen für zukünftige Exposom-Forschung im Bereich der öffentlichen und personalisierten Prävention.

Contents

1. Structure of the thesis

This thesis is written as a cumulative dissertation made up of four parts described below.

First, the introduction outlines and describes the background of exposome research, classification of key chemical exposures, sample preparation strategies for omic-scale measurements, analytical instrumental methods in exposomics, and method validation for exposome-scale analytical workflows.

The second part details the methodologies employed in this thesis, including a theoretical overview of solid-phase extraction (SPE) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The principles of method validation and quality control process are also discussed. Furthermore, current challenges hampering the field are introduced.

The third part contains a peer-reviewed review article, and two original research works that have been published by peer-reviewed journals.
The final section summarizes the main conclusion and major breakthroughs from the research presented in this dissertation. The remaining questions that should trigger future research in this field are outlined.

2. Scope and aims

The "exposome" is the totality of environmental exposures throughout an individual's lifetime, which links to many human diseases, like chronic diseases or cancer [1]. Exposomic assessment connects environmental exposures to chemical exposures in humans for further exposome-wide association studies (ExWASs) [2]. Liquid chromatography-mass spectrometry (LC-MS) is currently the most relevant technique for targeted and nontargeted analysis (NTA) in exposomics [3, 4]. Despite these advances, these LC-MS protocols face persistent hurdles, including maintaining consistent sensitivity and reproducibility which arise from matrix effects and interferences in complex biological samples, and the ultra-low abundance of many analytes (e.g., pg/mL levels) [5].

Efficient sample preparation can reduce matrix effects, interferences, and/or concentrate analytes in diverse matrices, and targeted LC-MS/MS methods are favored for quantifying various chemicals due to their high sensitivity and inherent robustness [6]. However, developing an exposome-scale targeted LC-MS/MS protocol still presents significant methodological limitations. This is because achieving high sensitivity and throughput, broad analytical coverage, and robust method performance simultaneously often introduces considerable workflow complexity and comprehensiveness. Beyond this, rigorous method validation is crucial to ensure the workflow's robustness, particularly concerning accuracy, repeatability, and consistency. Ultimately, the method's applicability must be demonstrated through its successful implementation in epidemiological cohort studies.

The specific aims of this thesis were as follows:

1. To review current sample preparation strategies and identify a promising approach for the further development of an exposome-scale analytical workflow.

2. To optimize a high-throughput sample preparation method using SPE 96-well plate format for the analysis of > 90 diverse analytes in human urine and plasma samples for both targeted and nontargeted exposomics.

3. To develop and in-house validate a well-balanced LC-MS/MS workflow for over 200 key biomarkers in human urine, plasma, and serum, utilizing the established SPE clean-up method.

4. To analyze urine samples from a longitudinal pregnancy cohort to test the workflow's applicability in a cohort study and investigate exposure patterns in U.S. women.

3. Key outcomes

The key outcomes of this thesis include the following points:

- After reviewing commonly used sample preparation methods for small molecules in omic studies, the SPE approach was identified as having the most promising potential to balance broad chemical exposure coverage with high measurement sensitivity and robustness for exposome-scale analysis.

- A well-balanced SPE method was developed for the targeted analysis of >90 highly diverse exposures in human urine and plasma. It was subsequently applied to nontargeted exposomics, yielding promising results, particularly in urine samples. This SPE protocol was estimated to handle over 1000 samples with <20 hours of lab work, demonstrating improved efficiency.

- A sensitive quantitative LC-MS/MS workflow was developed and in-house validated, covering >200 chemical exposures and incorporating the developed SPE clean-up for human urine, plasma, and serum. A supplementary conceptual validation framework was proposed for exposome-scale LC-MS methods, drawing upon empirical validation data.

- In the cohort application, >100 analytes were detected in 200 urine samples from 50 pregnant participants across four gestation weeks (12th, 20th, 28th, and 36th weeks). Several were detected for the first time in a U.S. urinary biomonitoring study. 16 compounds showed significant trends ($p < 0.05$) throughout the four weeks.

4. Introduction

4.1. The exposome

The concept of the "exposome", introduced by Wild in 2005, encompasses the complete spectrum of environmental exposures an individual encounters over their entire lifespan [7]. Exposomics aims to thoroughly evaluate chemical exposures and uncover potential connections to negative health outcomes, such as chronic diseases or cancer [8, 9]. A comprehensive analysis of these chemical exposures in human samples necessitates the use of analytical techniques that are sensitive, specific, and robust, such as liquid chromatography-mass spectrometry (LC-MS). While establishing unbiased and systematic links presents a challenge, it can be addressed through an exposome-wide association study (ExWAS) approach. It allows researchers to investigate the source, pathway, and temporal fluctuations of environmental exposures [10].

4.2. Classification of key chemical exposures

Exposomics research characteristically investigates a plethora of varied chemical exposures linked to specific chemical exposure events. The predominant exposures can generally be categorized into several classes, as depicted in the following Figure 1.

Personal Care Products (PCPs) comprise a wide array of chemicals present in cosmetics, pharmaceuticals, food items, and industrial goods [11]. Although the human body is generally capable of degrading most of these compounds, their widespread application in contemporary society results in ongoing low-level exposure [12]. A notable concern arises from the endocrine-disrupting characteristics of many PCPs, which carry potential implications for the reproductive health of both human and animal populations [13]. A remarkable example within PCPs is parabens, which are not only prevalent in the environment but have also been identified in diverse human biological samples, including urine, blood, and breast milk [14, 15, 16]. Studies indicate a potential association between paraben levels in human samples and detrimental health outcomes such as oxidative stress, DNA damage, perturbation of thyroid hormones, and an elevated risk of breast cancer development [17].

Plastic-related chemicals constitute a heterogeneous group of compounds deliberately added to plastic products to improve the quality and performance. Human exposure to these chemicals is extensive, occurring via multiple pathways such as occupational environments, food and beverage packaging, compromised water systems, and indoor air [18]. A primary concern stems from the endocrine-disrupting properties of many plastic-

related compounds, which present substantial health risks to humans. Phthalates, for instance, are a thoroughly investigated class of plasticizers. Moderate evidence indicates a correlation between phthalate exposure and adverse health effects, including reduced birth weight, endometriosis, diminished testosterone levels, neurodevelopmental disorder, type 2 diabetes, and an elevated incidence of breast and uterine cancers [19].

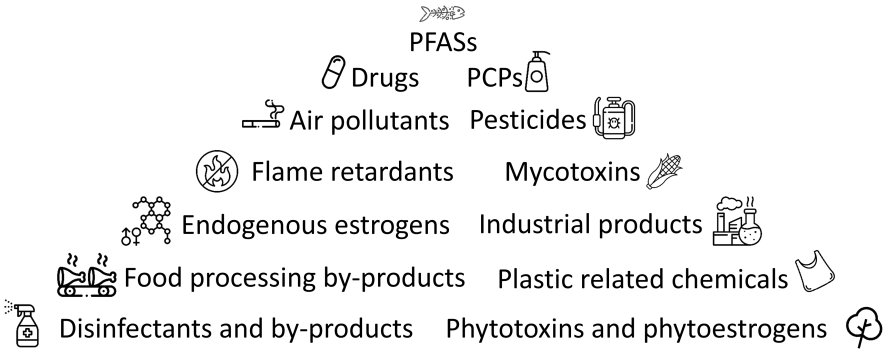

Figure 1. Classification of key chemical exposures from natural and synthetic sources, based on diverse criteria such as environmental/biological origin, chemical structure, and primary usage. Reprinted with permission from Yunyun Gu, Max L. Feuerstein, Lloyd T. Dillon; Patel J. Chirag; Caroline H. Johnson, Benedikt Warth. *Environmental Science & Technology*. 2025. DOI: 10.1021/acs.est.5c04458. Copyright 2025 American Chemistry Society [20].

Mycotoxins are toxic secondary metabolites produced by various fungal species that frequently contaminate agricultural crops both before and after harvest. This widespread contamination causes significant economic losses and presents serious health risks [21]. Chronic dietary exposure to mycotoxins is linked to severe long-term adverse health effects in humans, including cancer and even death [22]. Numerous studies have confirmed the presence of mycotoxins in human biological samples such as urine, blood, and breast milk [6]. This ubiquitous exposure raises considerable concern, especially regarding early life exposure for infants, even at low concentrations [5].

Per- and polyfluoroalkyl substances (PFASs) represent a class of highly persistent pollutants, widely employed in diverse industrial and commercial applications since the 1950s, notably in the manufacture of fluorinated polymers and surfactants [23, 24]. PFAS compounds tend to bioaccumulate within the body owing to their inherent stability and lipophilicity [25]. Investigations in animal models have correlated PFAS exposure with a spectrum of deleterious effects, including hepatotoxicity, tumor induction, developmental toxicity, immunotoxicity, neurotoxicity, and endocrine disruption [26]. The ubiquitous nature of PFAS is further substantiated by human studies. For example, Nielsen et al. (2024) [27] identified four distinct PFAS compounds, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), in the majority of human tissue samples

analyzed, such as liver, kidneys, lungs, spleen, brain cortex, and blood, at mean concentrations of 0.02-6.3 ng/g wet weight. This underscores the extensive human exposure to these persistent environmental contaminants.

Other key exposome biomarkers include pesticides, drugs, phytotoxins, phytoestrogens, flame retardants, air pollutants, industrial byproducts, and endogenous estrogens [28, 29]. These are particularly significant either because of their detrimental health impacts or their established connection to lifestyle and dietary risk factors for various diseases, such as cancer [30, 31].

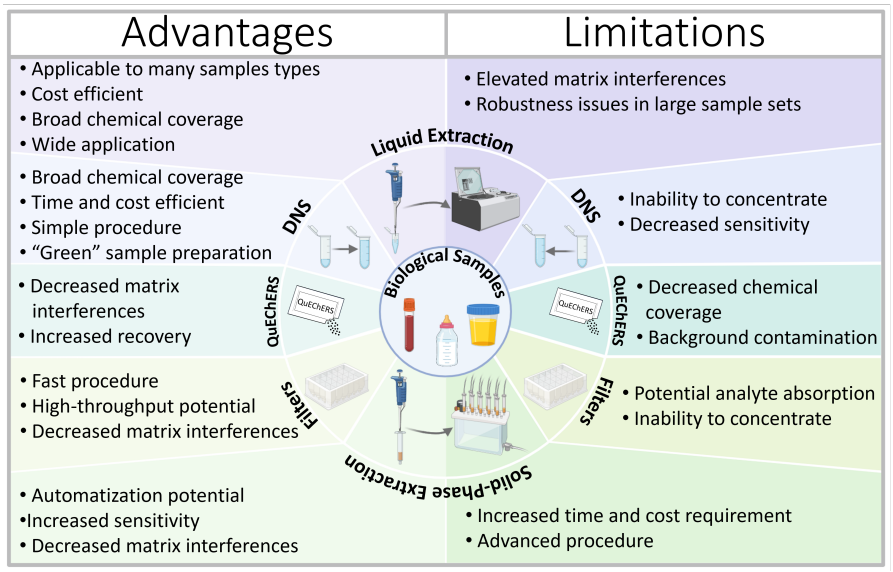

Figure 2. Overview and comparison of prevalent sample preparation methods for biological samples in exposome research: liquid extraction, dilute and shoot (DNS), filtration, QuEChERS, and solid-phase extraction (SPE). Reprinted with permission from Yunyun Gu, Jesse T. Peach, Benedikt Warth. *TrAc Trends in Analytical Chemistry*. 2023, Volume 166. Copyright 2023 Elsevier [32].

4.3. Sample pretreatment strategies in exposomics

Sample preparation protocols, applied before instrumental analysis, can effectively minimize interferences while also separating and concentrating analytes across diverse matrices [33]. Figure 1 provides illustrations of common sample preparation methods. Liquid extraction, usually including a protein precipitation (PPT) step, and dilute and shoot (DNS) are two of the most frequently utilized sample preparation methods for exposome-scale analysis. The popularity stems from their relatively broad chemical coverage, ease of use, and minimal analyte loss [34]. To further mitigate interference effects, liquid extraction

in exposome research can also incorporate the QuEChERS method, a fast and simple approach efficient at extracting pesticides and other exogenous chemicals [35]. Additional preparation options include the use of filters and solid-phase extraction (SPE). Lipid and/or protein removal filters have been increasingly adopted for multi-target xenobiotics analysis [36]. These filters are specifically designed to reduce phospholipids and diminish matrix interferences in biological samples. SPE has likewise found application in exposome research and human biomonitoring, facilitating the extraction of a broad array of analytes from various sample types, including urine [37], blood [38], and other biological matrices [39].

As depicted in Figure 2 and Figure 3A, each approach has advantages and limitations. Protocols based on liquid extraction, DNS and PPT provided broad analytical coverage. Yet, without extensive clean-up procedures, matrix effects and interferences can compromise reproducibility and decrease sensitivity with these methods. This poses a critical challenge for exposomics, given that some environmental chemicals exert significant health impacts even at extremely low concentrations, necessitating minimal matrix interference for reliable measurements [40]. Conversely, employing QuEChERS, filters, and SPE generally enhances sensitivity and reproducibility, but at the expense of reduced chemical coverage. This trade-off is crucial for both metabolome and exposome analysis, where comprehensive coverage is paramount for a holistic understanding of biological processes. Thus, the real challenge lies in striking the optimal balance (illustrated in Figure 3B) between mitigating matrix effects and retaining the broadest possible chemical coverage [41, 42].

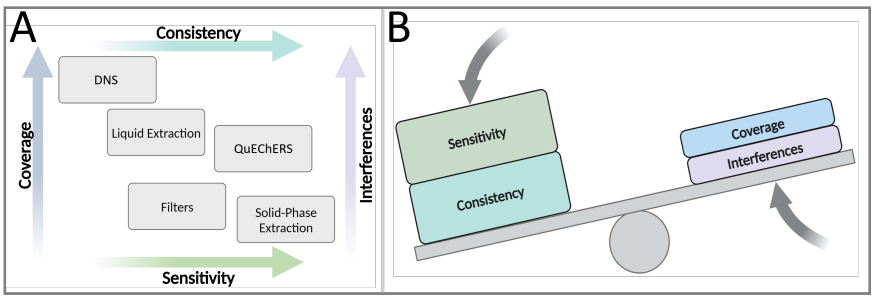

Figure 3. Balancing sample preparation for omic-scale chemical exposure assessment. (A) A comparison of sample preparation techniques in exposome research, considering chemical coverage, sensitivity, matrix interferences, and repeatability in large cohort studies. (B) Crafting an effective sample preparation protocol involves strategically allowing gaps and maintaining a pragmatic, yet proper, balance among four key metrics: coverage, sensitivity, interferences, and consistency. Reprinted with permission from Yunyun Gu, Jesse T. Peach, Benedikt Warth. *TrAc Trends in Analytical Chemistry*. 2023, Volume 166. Copyright 2023 Elsevier [32].

4.4. Analytical instrumental approaches in exposomics

Many analytical techniques, like enzyme-linked immunosorbent assay (ELISA) [43], chromatographic techniques, particularly LC and gas chromatography (GC) coupled with ultraviolet (UV) or MS [44], have been widely applied in human biomonitoring of multiple exposures. While ELISA is a rapid and user-friendly method, numerous studies have frequently reported false positive reactions, leading to poor selectivity in the overall ELISA-based workflow [45, 46]. Methods based on LC-UV and GC-UV are robust and known for their consistency [47]. Nevertheless, reaching the picomolar detected levels and high throughput commonly required in exposomics remains challenging [48].

MS-based methods, particularly LC-MS approaches, have gained increasing favor in current exposome research due to their capacity to improve throughput, sensitivity, and selectivity. These methods can be created using either a targeted or nontargeted strategy. Targeted analysis relies on measuring a defined set of previously identified small molecules while nontargeted analysis adopts a comprehensive approach, measuring all resolvable features with a specific method, which can number in the thousands or even tens of thousands [49]. These methods thus employ opposite analysis pathways with targeted analysis utilizing reference standards to first make identifications and then determine the statistical importance of compounds (bottom-up) whereas nontargeted analysis typically begins with statistical analysis before determining fragmentation patterns, collision cross-section, and mass values, among other physical characteristics, to annotate features (bottom-down) [41]. Exposomic studies can also incorporate suspect screening, a largely nontargeted method which includes a list of annotated target components previously defined in the MS method [50]. Regardless of the targeted and nontargeted analysis, the successful application of LC-MS methods in exposomics necessitates careful development. This involves generating pragmatic workflows that directly address pertinent biological and epidemiological questions, rather than solely focusing on maximizing chemical coverage or achieving exceptional analytical figures of merit. Crucially, these LC-MS workflows must strike an optimal balance among throughput, sensitivity, selectivity, and robustness to effectively meet the complex demands of exposome analysis.

4.5. Method validation for exposome-scale analytical workflows

Rigorous method validation is essential for ensuring high-quality data in analytical chemistry. Methods should be meticulously evaluated over several days, adhering to the official guidelines. Key validation parameters include linearity, selectivity, matrix effects (ME), sensitivity (limit of detection and quantification, LOD and LOQ), trueness (extraction recovery, RE), intermediate precision (inter-day relative standard deviation, RSDR), and repeatability (intra-day RSD, RSDr) [51, 52, 53].

To quantify a compound in samples, a series of calibrators, working solutions ranging from low to high concentrations, is prepared using a verified reference in appropriate

matrices (e.g., solvent or matrix-matched). This defines a linear range, spanning from the lower limit of quantification (LLOQ) to the higher limit of quantification (HLOQ) [54]. Linearity is crucial because it ensures the analytical response accurately reflects the authentic analyte concentration across the entire calibration range [53].
S electivity refers to the method's ability to differentiate the target analyte from other similar compounds in various matrices [55]. These potential interferences may originate from various sources, including the biological sample matrices, sample preparation, or the other environment [56].

ME is a common challenge in mass spectrometry, particularly with soft ionization techniques like electrospray ionization [57]. Interferences present in biological samples, such as phospholipids in human blood, can either suppress or enhance the mass spectrometry signals (signal suppression/enhancement, SSE) [58].

The LOD represents the lowest concentration of a compound that can be reliably detected, either by the instrument alone (instrument LOD) or through the entire analytical workflow (method LOD) [53]. A related term is LOQ, which is the lowest concentration that can be accurately and precisely quantified. Many laboratories consider LOQ and LLOQ to be synonymous in their routine analytical workflows.

Trueness, intermediate precision, and repeatability are vital for establishing the robustness of an analytical method [51, 53]. Trueness indicates how closely measured concentrations align with the theoretical concentrations of a compound spiked into a pure solvent or other biological sample matrices. RE during sample preparation is a common metric used to assess trueness. Repeatability (intra-day RSD) and intermediate precision (inter-day RSD) are determined by the coefficient of variation of measured concentrations from duplicate samples analyzed within the same day and across different days, respectively. Many prominent guidelines such as European Commission (EC) No.2021/808 provide systematic criteria for trueness, intermediate precision and repeatability, but these often fall short when dealing with the high number of diverse analytes and ultra-low concentrations typical in exposomic studies.

Unless otherwise specified by additional references, the information presented in this Chapter 4 (Introduction), especially Section 4.3 (Sample pretreatment strategies in exposomics), is based on the research detailed in my prior works, Gu et al. (2023) [32], Gu et al. (2025a)[20] and Gu et al. (2025b) [59].

5. Methodology

5.1. Solid-phase extraction

Solid-phase extraction (SPE), developed in the 1970s, offers a powerful alternative to liquid extraction [60]. This technique operates on the same fundamental principle as affinity-based separation methods like liquid chromatography (LC), relying on the differential distribution of analytes between a solid stationary phase and a liquid mobile phase [61]. Currently, SPE has become a cornerstone in various analytical workflows, particularly in targeted analysis, due to its effectiveness in isolating and retaining analytes from diverse sample matrices [62, 63]. Its widespread applicability stems from several key advantages. SPE efficiently enriches samples, minimizes the use of organic solvents compared to liquid extraction, is relatively simple to execute, possesses significant automation potential [64], and effectively mitigates matrix effects [65].

Recent human exposome research highlights the significant benefits of SPE, particularly when utilizing hydrophilic-lipophilic balance (HLB) sorbents. A comprehensive publication by Jagani et al. (2022) [66] reported encouraging results using Oasis HLB 96-well plates. This research achieved low LODs ranging from 0.010 to 1.0 ng/mL and RSDs below 20% for 50 multi-class exposures spiked into urine at two concentration levels (1 ng/mL and 10 ng/mL). These findings underscore the strong potential of SPE in exposome analysis, though further validation and analysis are still required.

5.2. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) workflow

Figure 4 provides an overview of standard liquid chromatography-tandem mass spectrometry workflow (LC-MS/MS) system. The LC component focuses on differentiating chemicals based on their varying interactions with sorbents within one or more LC columns. In this process, a sample containing various compounds dissolved in a reconstitution solvent (typically a mixture of water, methanol, and/or acetonitrile, sometimes with volatile acid additives) is injected into the auto sampler. The compounds then go through the LC column eluted by liquid mobile phases.Figure 4 provides an overview of standard liquid chromatography-tandem mass spectrometry workflow (LC-MS/MS) system. The LC component focuses on differentiating chemicals based on their varying interactions with sorbents within one or more LC columns. In this process, a sample containing various compounds dissolved in a reconstitution solvent (typically a mixture of water, methanol, and/or acetonitrile, sometimes with volatile acid additives) is injected

into the auto sampler. The compounds then go through the LC column eluted by liquid mobile phases.

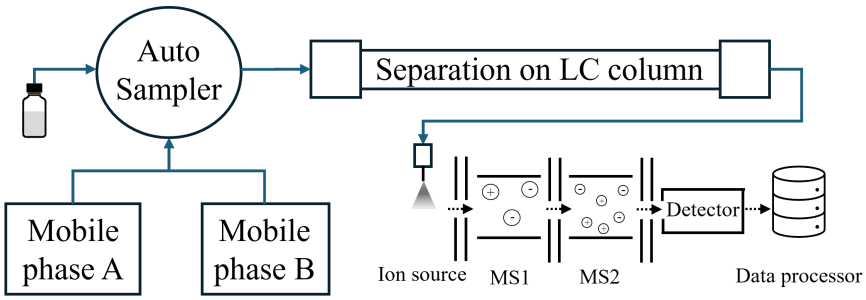

Figure 4. Overview of an LC-MS/MS-based analytical workflow.

A mass spectrometer fundamentally comprises an ion source, one or more mass analyzers, a detector, and a data processing system. In the ion source with either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), compounds are ionized in either positive or negative mode depending on the chemical nature of the analytes, particularly their ability to accept or donate a proton [67, 68]. APCI is generally well-suited for the ionization of relatively volatile and non-polar chemicals, whereas ESI is typically preferred for more polar and non-volatile compounds, which encompass many important biomarkers in exposomics such as pesticides [69, 70]. After the ionization, these generated ions (precursor/parent ions) then proceed to the first mass analyzer, where they are separated according to their mass-to-charge ratio (m/z). Following this separation, the selected precursor ions undergo fragmentation typically in a collision cell. The resulting fragments (product/daughter ions) then enter a second mass analyzer, where they are again separated based on their m/z. Finally, the detector measures the abundance of these product ions, converting this information into electrical signals. These signals are then processed and transmitted by the data processing system [71].

5.3. Method validation

Method validation is carried out usually over three days. These parameters typically include linearity, selectivity, matrix effects (signal suppression/enhancement, SSE), sensitivity (limit of detection and quantification, LOD and LOQ), trueness (extraction recovery, RE), intermediate precision (inter-day relative standard deviation, RSDR), and repeatability (intra-day RSD, RSDr). Sample preparation, measurements, and data analysis are regularly conducted individually for each batch.

Method linearity is assessed using either external or matrix-matched calibrations, while selectivity is verified by inspecting diverse pooled biological samples for interferences. Matrix-matched calibration standards are usually prepared by spiking extracts from

pooled biological matrices at different concentration levels. Matrix effect (ME) is quantified as SSE in many studies [72]. While commonly evaluated by comparing an analyte's response (e.g. concentration or peak area) in diverse matrices against its response in a pure solvent [73, 74], a primary concern for ME calculation especially in quantitative bioanalysis is the ratio of the slopes of matrix-matched and external calibration curves due to the robustness and closeness to routine measurement [75, 76]. LODs and LOQs are usually determined by analyzing replicates of low concentration spiked biological samples based on either signal to noise ratio or standard deviation of measured concentrations [53].

Many authoritative guidelines recommend the RE to express trueness and the coefficient of variation (CV) of repeated analysis to intermediate precision (inter-day RSD, RSDR) and repeatability (intra-day RSD, RSDr) [53, 51, 52]. Acceptable ranges of RE, RSDr and RSDR in an analytical method vary based on analyte levels and the purpose of the analysis, as indicated in Table 1. Take the European Commission Decision (EC, No. 2021/808) as an example. For concentrations exceeding 10 µg/kg, RE should be between 80% and 120%. For concentrations between 1 µg/kg and 10 µg/kg, the acceptable RE range is 70%-120%. For concentrations below 1 µg/kg, acceptable REs fall within the 50%-120% range. Similarly, acceptable values for RSDR and RSDr (calculated as the CV of RE) are also concentration-dependent. The guideline mandates CVs <16% for concentrations above 1000 µg/kg, and CVs <22% for concentrations between 120 µg/kg and 1000 µg/kg. For concentrations below 120 µg/kg, the guideline recommends minimizing CVs for both RSDr and RSDR to the lowest technically achievable level.

Table 1. Criteria for trueness (RE), intermediate precision (RSDR), and repeatability (RSDr) of a compound across different levels, as specified by four prominent guidelines for various application scenarios. NA, not applicable mandatory criteria.

Levels	RE	RSD_r	RSD_R	Application Scenarios	Guideline
LLOQ	80-120%	≤20%	≤20%	Drug related applications, and other related applications	FDA 2018
Other levels	85-115%	≤15%	≤15%		
LLOQ, ULOQ	75-125%	≤25%	≤25%	Drug related applications, and other related applications	EMA 2019
Other levels	80-120%	≤20%	≤20%		
< 1 µg/kg	50% - 120%	NA	NA	Residues of pharmacologically active substances in live food-producing animals, their body parts and fluids, excrements, tissues, products of animal origin, animal by-products, feed and water	European Commission 2021
1-10 µg/kg	70% - 120%	NA	NA		
10-120 µg/kg	80% - 120%	NA	NA		
120-1000 µg/kg	80% - 120%	<22%	<22%		
>1000 µg/kg	80% - 120%	<16%	<16%		
Not mentioned	70% - 120%	≤20%	≤20%	The official control of pesticide residues in food and feed across the European Union (EU)	SANTE 2021
	30% -140%	≤20%	≤20%		

5.4. Data processing and quality control

To obtain comprehensive data, robust quality control (QC) strategies are essential during LC-MS/MS data processing, such as the adoption of systematic stability test (SST), blank samples including solvent blank and process blank, internal standards, and in-house and external QC samples [77]. SST is carried out prior to and/or following each analytical sequence to ensure consistent instrumental performance. Process blanks are designed to identify possible contamination originating from the instrument, pure solvent, and particularly any additional contamination introduced throughout the sample preparation process. For a more specific assessment of instrument or solvent-derived contamination, solvent blanks are typically utilized. Internal standards, particularly isotope-labelled ones, and the QC samples are generally employed to either monitor MS signal fluctuations or correct for biased results, especially in large-scale quantitative MS analysis.

5.5. Current methodological challenges

Significant challenges arise when attending to measure the chemical exposures in complex human biological samples due to poor repeatability and consistency across large exposome sample sets, particularly with low-abundance analytes in the pg/mL levels or lower [34].

Addressing these challenges requires sophisticated optimization of an entire analytical workflow, including sample preparation protocols. Liquid extraction and protein precipitation are widely used extraction methods for exposomics due to their comparably wide chemical coverage [3]. However, these techniques are prone to preserve matrix interferences which can hamper overall method performance by compromising sample cleanliness. Recently, SPE got more attention as an option for sample preparation in exposomics, owing to its capability to reduce ME, enhance sensitivity, and its potential of improving consistency in high-throughput studies. Nonetheless, the creation of SPE protocols offering broad analyte coverage, essential for thorough exposomic research, is still a notable hurdle. Similarly, protocols compatible with nontargeted data acquisition strategies are largely uncommon. Furthermore, the practical considerations of processing time for SPE protocols applied to large sample sets have seldom been evaluated.

Targeted LC-MS methods are often preferred for quantifying various exposures in multi-class human biomonitoring studies owing to their high sensitivity and inherent robustness [6]. While a good workflow in targeted exposomics aims to quantify hundreds of analytes in a single run, this ambition highlights a crucial limitation. The workflow's sensitivity and robustness can be compromised by the limited duty cycles of a single MS scan when numerous analytes are included, ultimately leading to reduced data quality [78]. Consequently, developing an exposome-scale targeted LC-MS/MS protocol necessitates a delicate balance between extensive analytical coverage and robust method performance.

Furthermore, rigorous method validation is crucial for estimating an analytical workflow's performance. Although many guidelines offer systematic validation criteria (e.g., trueness, precision, and repeatability), their direct applicability to exposome-scale targeted

approaches can be constrained. These criteria may be insufficient for studies involving a high number of diverse analytes and low concentrations. For instance, clear criteria for assessing precision for analytes below 120 µg/kg are not yet established in the EC criteria. More fit-for-purpose validation criteria should be established for exposome-scale analytical protocols. In the end, applicability for cohort studies should be tested to showcase the methods' robustness, especially for susceptible populations with chronic low-level exposure scenarios.

Unless otherwise specified by additional references, the information presented in this Chapter 5 (Methodology), especially Section 5.1 (Solid-phase extraction) and Section 5.3 (Method validation), is based on the research detailed in my prior works, Gu et al. (2023) [32] and Gu et al. (2025b) [59].

6. Peer-reviewed articles

6.1. Overview of the review and original research articles included in this thesis

Publication #1 (Review): Sample Preparation Strategies for Mass Spectrometry Analysis in Human Exposome Research: Current Status and Future Perspectives

Yunyun Gu, Jesse T. Peach, Benedikt Warth. "Sample Preparation Strategies for Mass Spectrometry Analysis in Human Exposome Research: Current Status and Future Perspectives", *TrAC Trends in Analytical Chemistry*, **2023**, Vol. 166:117151, DOI: 10.1016/j.trac.2023.117151

Publication #2 (Original Research): High-Throughput Solid Phase Extraction for Targeted and Nontargeted Exposomics

Yunyun Gu, Max L. Feuerstein, Benedikt Warth. "High-Throughput Solid Phase Extraction for Targeted and Nontargeted Exposomics", *Analytical Chemistry*, **2025**, Vol. 97(11): 6075-6082, DOI: 10.1021/acs.analchem.4c06177

Publication #3 (Original Research): Quantitative exposomics targeting over 200 toxicants and key biomarkers at the picomolar level

Yunyun Gu, Max L. Feuerstein, Lloyd T. Dillon, Patel J. Chirag, Caroline H. Johnson, Benedikt Warth. "Quantitative exposomics targeting over 200 toxicants and key biomarkers at the picomolar level", *Environmental Science & Technology*, **2025**, online first, DOI: 10.1021/acs.est.5c04458

6.2. Publication #1: Gu et al. (2023)

Status	Published
Title	**Sample Preparation Strategies for Mass Spectrometry Analysis in Human Exposome Research: Current Status and Future Perspectives**
Authors	Yunyun Gu[1,2], Jesse T. Peach[1], Benedikt Warth[1,3]
Affiliations	[1]University of Vienna, Faculty of Chemistry, Department of Food Chemistry and Toxicology, Währinger Straße 38, 1090 Vienna, Austria [2]University of Vienna, Vienna Doctoral School of Chemistry, Währinger Straße 42, 1090, Vienna, Austria [3]Exposome Austria, Research Infrastructure and National EIRENE Node, 1090, Vienna, Austria
Year	2023
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Contribution	Yunyun Gu performed literature research, took the lead in discussion and summarizing the ideas presented in the work, and wrote the initial version of manuscript draft.

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Sample preparation strategies for mass spectrometry analysis in human exposome research: Current status and future perspectives

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ABSTRACT

The exposome is the measure of total environmental exposures and has a significant impact on human health outcomes. Exposomics research typically examines complex biological samples and assesses 100s to 1,000s of small molecules associated with chemical exposure events. To acquire robust and comprehensive datasets encompassing a broad range of multi-class biomarkers of chemical exposures, high-resolution and tandem mass spectrometry have emerged as the preferred analytical tools in the field. Due to matrix effects and interferences associated with complex biological samples together with the presence of very low abundance analytes, sensitivity and reproducibility have plagued exposomic studies. This is especially true for large sample sets where adequate robustness is frequently lacking. A possible solution to this problem is the development of non-discriminatory sample preparation methods which can reduce interferences while increasing sensitivity and reproducibility. However, sample preparation techniques intrinsically result in a discrete loss of analytes leading to the outstanding analytical challenge of finding a proper balance. Therefore, approaches which decrease matrix interferences while preserving broad chemical coverage need to be developed. In this review, sample preparation procedures currently applied in exposome research and related fields are summarized and critically evaluated. This includes a comparison of advantages and limitations between different techniques such as solid-phase extraction, liquid extraction, QuEChERS, filters and dilute and shoot with a liquid chromatography-mass spectrometry analysis focus. Based on this evaluation, recommendations promoting balanced strategies for sample preparation in future exposome research applications are provided.

1. Introduction

In 2005, Wild proposed the concept of the "exposome" as the totality of environmental exposures throughout an individual's lifetime [1]. The exposome has been shown to play a pivotal role in many human diseases and can be partially assessed through a series of environmental and biological measurements [1,2]. Exposomic assessment relies on linking environmental exposures to exogenous and/or endogenous chemicals in humans. Providing links in an unbiased, systematic way is challenging, yet can be accomplished using an exposome-wide association study (EWAS) approach to investigate the source, pathway, and temporal variation of environmental exposures [3,4].

Generally, chemical exposures are determined by measuring a predefined number of known toxic and/or bio-active chemicals. Methods for these analyses can be optimized to improve accuracy and sensitivity [5,6]. However, exposome research relies upon a large-scale investigation of exogenous and endogenous small molecules involved in diverse exposure scenarios. Yet, caution should also be exercised in method development and boldness is required to generate pragmatic workflows that answer biological and epidemiological questions and do not simply reach for high chemical coverage or superb analytical figures of merit alone. In the context of having the courage of leaving gaps, methods can be cleverly created using either a targeted or non-targeted approach.

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Abbreviation	Definition
ACN	Acetonitrile
BrPs	Brominated phenols
DNS	Dilute and shoot
EHTBB	2-ethyl-hexyl tetrabromobenzoate
FRs	Flame retardants
GC	Gas chromatography
H_2O	Water
HBCD	Hexabromocyclododecane
HLB	Hydrophilic-lipophilic-balanced
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
MeOH	Methanol
$MgSO_4$	Magnesium sulfate
MS	Mass spectrometry
Na_2SO_4	Sodium sulfate
NaCl	Sodium chloride
NBFRs	Novel brominated flame retardants
NFRs	Novel flame retardants
NH_4FA	Ammonium formate
OCPs	Organochlorine pesticides
OH-PAHs	Hydroxyl polycyclic aromatic hydrocarbons
OH-PBDEs	Hydroxyl polybrominated diphenyl ethers
OPFRs	Organophosphate flame retardants
OPs	Organophosphorus compounds
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzodioxins and dibenzofurans
PCPs	Personal care products
PFASs	Per- and polyfluoroalkyl substances
POPs	Persistent organic pollutants
PPCPs	Pharmaceuticals and personal care products
QACs	Quaternary ammonium compounds
QE Focus Orbitrap	Q Exactive Focus Orbitrap
QqQ	Triple quadrupole
QTOF	Quadrupole time-of-flight
TBBPA	Tetrabromobisphenol A
RSD	Relative standard deviation
UPLC	Ultrahigh-performance liquid chromatography
VOCs	Volatile organic compounds
WAC	White analytical chemistry

Targeted analysis relies on measuring a defined set of previously identified small molecules using authentic reference standards while non-targeted analysis takes a comprehensive approach and measures all the features resolved using a specific method, which can number in the thousands or tens of thousands [7–9]. These methods therefore incorporate opposite analysis pathways with targeted analysis utilizing reference standards to first make identifications and then determine the statistical importance of compounds while the statistical analysis generally occurs first in untargeted analysis after which fragmentation patterns and collision cross section and mass values, among other physical characteristics, can be determined to annotate features [10,11]. Exposomic studies can also incorporate suspect screening, a largely non-targeted method which includes a list of targets previously defined [12,13]. Regardless of the analytical pipeline, both targeted and non-targeted exposomics have significant challenges, including sensitivity of low-abundance analytes (typically pg/mL level or less) [14–16] and serious interferences from complex biological samples [2] as well as issues with repeatability and consistency when measuring large sample sets.

Possible solutions to these critical obstacles in current exposome research are smartly optimized sample pretreatment protocols. Treatment prior to instrumental analysis can reduce interferences, and separate and concentrate analytes in various matrices [17,18]. Liquid extraction (LE), often including a protein precipitation (PPT) step [19], and dilute and shoot (DNS) are two of the most commonly employed sample pretreatment methods for exposome-scale analysis due to their comparatively wide chemical coverage, simplicity and minimal analyte loss [20,21]. In order to reduce the effects of interferences, LE in exposome research can also include the QuEChERS method, a fast and simple approach efficient at extracting pesticides and other exogenous chemicals [22–25]. Other pretreatment methods include the use of filters and solid-phase extraction (SPE). Lipid and/or protein removal filters have been increasingly adopted for multi-target xenobiotics analysis [26,27]. These filters are designed to reduce phospholipids and reduce matrix interferences in biological samples. SPE has also been employed in exposome research and human biomonitoring to extract a wide range of analytes in various sample types including urine [28], blood [24], and several other biological matrices [29,30].

As illustrated in Fig. 1A, each approach has advantages and limitations. Protocols based on LE, DNS and PPT provided broad analytical coverage. Yet, without extensive clean-up procedures, matrix effects and interferences limit reproducibility and decrease sensitivity using these methods. This is an issue critical to exposomics as some environmental chemicals can have major health impacts at extremely low levels and may require minimal matrix impact to enable robust measurements [14,16,31]. The use of QuEChERS, filters and SPE typically results in increased sensitivity and reproducibility at the cost of decreased chemical coverage. This is vital to both metabolome and exposome analysis, where broad coverage is of uttermost importance and allows for a comprehensive view of biological processes. The challenge therefore becomes to find the optimal balance between decreasing matrix effects and preserving as much chemical coverage as possible [32–34] (Fig. 1B).

This review aims to summarize and critically evaluate the existing literature focused on sample preparation procedures used for both, targeted and non-targeted exposome-related research (Fig. 2). Although both gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry methods are included in the review, an emphasis is placed on LC-MS approaches. We discuss currently applied sample preparation protocols and analyze their advantages and limitations. We then recommend potential balanced strategies for sample pretreatment in future exposomic applications to address this emerging analytical challenge.

2. Currently used approaches for sample preparation in exposome research

Sample pretreatment methods applied in recent targeted (Table 1) and non-targeted (Table 2) exposome-type studies that analyzed human samples were compiled and summarized. Since few human studies focused on the advanced evaluation and reporting of sample preparation protocols, other biological species and uncommon biological matrices have been included for a more comprehensive overall picture of analytical strategies. One of the most frequently used techniques, LE, is often performed in conjunction with PPT and DNS. This approach is widely applied to LC-MS “omics” research and multi-class analyses [35,36,37]. However, the development of other sample pretreatment methods, such as LE with QuEChERS, SPE and the use of filters, have become increasingly prevalent in the research landscape [38,39,40,41].

In this section, brief descriptions of LE, DNS, filters, LE with QuEChERS and SPE are introduced and their applications are reviewed to multi-class (more than a single group of xenobiotics) or omics-scale (intended to capture all xenobiotics the analytical approach allows for) exposure assessment. Moreover, advantages and shortcomings of these techniques are discussed in terms of matrix effects (MEs) to reflect matrix interferences, limits of detection (LOD) and/or limit of quantification (LOQ) to evaluate

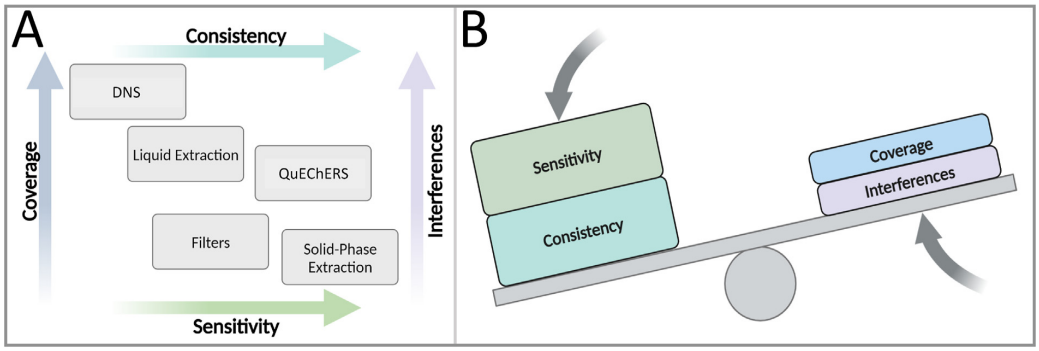

Fig. 1. Finding balance in sample preparation for the omic-scale assessment of chemical exposure. (A) Comparison of sample pretreatment techniques applied in exposome research considering the topics of chemical coverage, sensitivity, matrix interferences and repeatability in large cohort studies; (B) Developing an effective sample pretreatment protocol requires the courage of leaving gaps and maintaining a pragmatic, though proper balance between four metrics: coverage, sensitivity, interferences and consistency.

sensitivity and chemical compound coverage. To compare data from different articles, the values of MEs from different studies were normalized such that 100% indicates no ME.

2.1. Liquid extraction

Liquid extraction (LE) is a classic, widely used isolation technique well-suited for multi-residue analysis in various biological specimens, including liquid, semisolid and solid [15,58,59]. LE is characterized by the addition of a liquid extraction solution, generally including an organic solvent, accompanied by the transfer or separation of analytes from samples to the extraction solution liquid phase [60,61]. There are several LE variations, including Bligh and Dyer, PPT, cold temperature LE, acidic LE and salting-out assisted liquid-liquid extraction (SALLE).

The Bligh and Dyer technique is a commonly used LE method in metabolomic and lipidomic research that allows for biphasic separation and the isolation of lipids in an organic phase. This technique extracts lipids by using MeOH, chloroform and H_2O, and has been employed for the extraction of polar chemicals and lipids from human samples for both exogenous and endogenous small molecule measurement. Cano-Sancho et al. used a modified Bligh and Dyer method to extract and isolate non-polar lipids and polar metabolites from human breast milk samples [14]. In this study, an aqueous solution containing NaCl was first added, followed by the addition of MeOH, and finally with an addition of chloroform to isolate lipids in the chloroform phase and other metabolites in the aqueous phase. PPT is another classic and fast sample preparation technique that has been widely used in bioanalytical methods which has also been frequently reported in exposome study methods [16,44,45,35,62]. PPT with miscible solvents should be categorized as an LE method since it is characterized by the same extraction principles [59]. PPT involves denaturation of proteins (loss of tertiary and secondary structures) present in biomatrix through the application of an external stress, most commonly in the form of an organic solvent such as ACN or MeOH [63].

LE extraction efficiency has been improved by modulating extraction conditions such as temperature and pH and by combining it with other techniques including sonication [53,64–66]. Another LE optimization technique is the SALLE method which is used to increase the extraction efficiency as well as to remove water from organic solvents by using miscible organic solvents and salts to cause phase and analyte separation in liquid samples [67]. These LE methods have been shown to have wide and synergistic applications in an array of exposome applications. Decreasing temperature has been reported to assist PPT in the removal of lipids from samples and to prevent the degradation of unstable chemicals [16,53,68]. Ref. [62] successfully extracted fish plasma by deproteinized samples with 80% ice-cold MeOH prior to a non-targeted exposomic analysis. Analysis of urine, including a sonication pretreatment step, allowed for the isolation of almost 50 xenobiotics [53]. More than 1000 chemicals were characterized using LE for both human urine and plasma by Ref. [45]. In this study, acidified water was used to dilute urine and plasma was treated with cold ACN containing formic acid and NH_4FA in a Sirocco Protein Precipitation plate followed by cold storage (10 min, -20 °C) to promote protein precipitation. Preindl et al. [16] adopted a cold ACN/MeOH (1/1, v/v) extraction to isolate 61 xenoestrogens and 14 endogenous estrogens in human urine and serum samples. Breast milk samples investigated in this study were treated with acidified ACN, followed by SALLE with $MgSO_4$ and NaCl, and a final freeze-out step (2 h, −20 °C) further increasing the efficiency of PPT. Finally, whole blood samples of green sea turtles were extracted via ACN/H_2O (3/1, v/v) in the presence of anhydrous $MgSO_4$ and NaCl (SALLE) resulting in the characterization of 26 chemicals by Ref. [48].

LE offers a simple procedure that can minimize the loss of analytes and enable a wide coverage of different chemical classes. These streamlined processes are also cost-efficient. However, drawbacks including severe matrix effects and interferences are frequently reported in high-throughput measurements for both targeted and non-targeted measurements utilizing electrospray ionization, often resulting in retention time shifts, peak area variation and significant MEs, which could be the underlying cause of inconsistent results with high relative standard deviations (RSD, %) [16,35,69], especially when characterizing chemicals present at very low levels (pg/mL or lower) [70–72]. Without sufficient sample clean-up preparation, serious interferences can accumulate in the analytical system as the number of injections increase which may bias results and/or require additional time-consuming cleaning efforts. The use of internal standards, normalization with quality control samples and dilution techniques can be adopted to address and partly solve these problems in LE analysis [31].

2.2. Dilute and shoot

Empowered by the ever-increasing sensitivity of state-of-the-art mass spectrometers, particularly in the tandem MS configuration, dilute and shoot (DNS) has become a popular option over the last two decades in omics, food safety and human biomonitoring

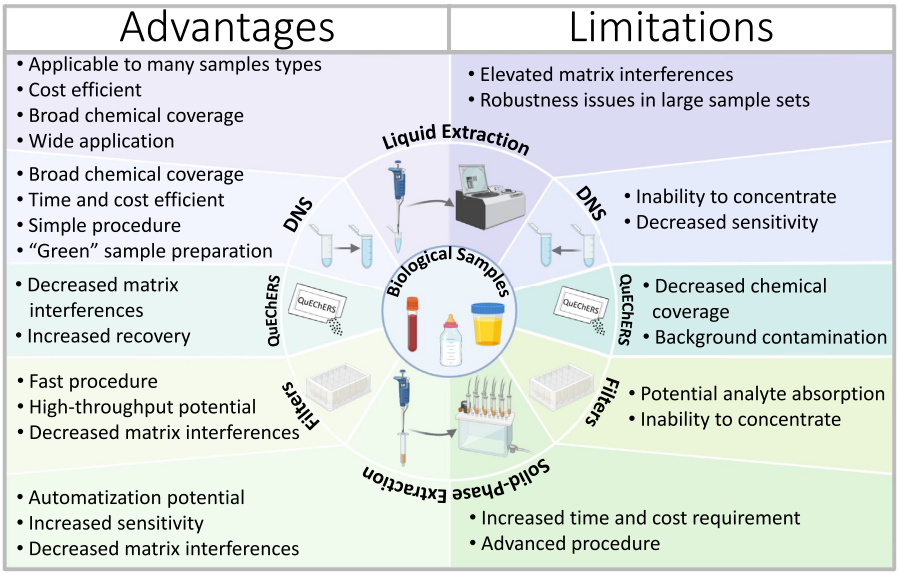

Fig. 2. Comparison of the most common sample pretreatment methods including liquid extraction, dilute and shoot (DNS), filters, QuEChERS and solid-phase extraction for biological samples in exposome research.

research due to its simple process, minimal loss of analytes and high throughput potential [73–75]. This straightforward technique is composed of two steps: the dilution of samples or sample extracted liquids and directly analyzing (i.e. "shooting") the diluted sample via an analytical instrument, usually a mass spectrometer [76]. For some liquid samples, like urine or oral fluids, samples can be diluted and directly analyzed. However, solid samples or more complex samples like serum or plasma usually require the addition of PPT, LE or QuEChERS prior to DNS [45,77,78].

The dilution step of DNS reduces matrix influences and is particularly useful when samples have high analyte concentrations, generally at the ng-μg/mL level or higher [79,80]. With DNS as the final preparation step in a study investigating polyphenols in urine, the mean MEs were around 100% for five chemical groups, indicating minimal matrix effects [81]. However, the DNS process decreases sensitivity, especially for low-abundance analytes, presenting a delicate balance for exposomic applications. Moreover, since DNS is commonly applied in urine, it faces many issues from the frequently applied deconjugation procedures, such as high background from deconjugation of glucuronide and sulfate conjugates in urine [82], due to the lack of clean-up procedures [83].

2.3. QuEChERS

Combining LE with QuEChERS is also a simple and straightforward extraction technique with a certain degree of clean-up. It includes the use of SALLE, usually with the addition of $MgSO_4$ and NaCl for extraction, as well as a clean-up step using dispersive solid-phase extraction (d-SPE). This clean-up step is typically completed with primary secondary amine (PSA), octadecyl silica (C18) and graphitized carbon black (GCB).

The LE with QuEChERS approach was originally developed for recovering pesticide residues from fruits and vegetables, but it rapidly gained popularity in the comprehensive isolation of analytes from different matrices due to its fast and easy procedure. Recently, it has also been applied in the sample pretreatment of several exposome-type studies [47,49,54]. Rial-Berriel et al. [39] introduced a fast, one-step QuEChERS-based extraction for whole animal blood using acidified ACN. Results collected in this study are promising wherein 360 toxic chemicals were identified with low LODs (0.1–8.0 ng/mL), while 57% of the analytes had MEs in the range of 80%–120%. Another study extracted human serum by LE with QuEChERS and included a d-SPE step using C18 for simultaneous extraction and clean-up. This method was reported to remove lipids from the samples and isolate six NBFRs and eight common PBDEs from human serum [84]. Ref. [16] used acidified ACN with $MgSO_4$ and NaCl with the aid of cold storage to examine breast milk samples for a broad array of xenoestrogens. The results indicated that more than 55% of the analytes showed MEs in the range of 40%–138%, which the authors deemed satisfactory given the high diversity of analytes and the low concentrations used for realistic spiking experiments.

Studies comparing LE with QuEChERS against other extraction methods have indicated encouraging results in terms of recovery and MEs. Ref. [51] compared the clean-up effects of gel permeation chromatography (GPC), QuEChERS with PSA and QuEChERS without a clean-up step by spiking breast milk and pure solvent with 23 OCPs, 18 PCBs and 16 PAHs (20 ng/mL). QuEChERS with PSA and GPC exhibited similar performance in terms of recovery, ranging from 70% to 110% and from 75% to 110%, respectively. QuEChERS without a clean-up step generated lower recoveries ranging from 24% to 80% with an average recovery of approximately 50%. Ref. [47] applied both QuEChERS with PSA/C18 and GPC to human serum for the measurement of 103 analytes, including 16 PAHs, 20 OCPs, 56 PCBs, and eleven PBDEs, with similar performance reported for QuEChERS and GPC. Ref. [56] compared QuEChERS, lipid and/or protein filtration and DNS to prepare human urine samples for a non-targeted analysis and reported that QuEChERS and filters had similar results in terms of MEs and reproducibility.

Although QuEChERS is an attractive extraction option, the addition of QuEChERS sorbents also causes potential problems. The

Table 1
Literature examples of sample preparation methods for targeted exposome-type studies in human and other biological samples.

Number of Analytes	Molecular classes analyzed	Biological samples material	Sample Preparation	Instrument	LOD and/or LOQ	Analytes with Acceptable MEs[a]	References
50	PCPs, PAHs, OPFRs, pesticides, VOCs, tobacco alkaloids and drugs of abuse	Human urine	SPE	Exion UPLC and Qtrap 6500+ (both Sciex)	LOD: 0.010–1.0 ng/mL; LOQ:0.020–3.0 ng/mL	NA	[42]
81	Plasticizers, PFASs, industrial side products and pesticides, endogenous estrogens, phytoestrogens and metabolites, mycoestrogens and metabolites, PPCPs, pharmaceuticals and metabolites	Human urine, serum and human breast milk	PPT, QuEChERS (only for breast milk)	1290 UPLC (Agilent) coupled to Sciex Qtrap 6500+ (Sciex)	LOQ: 0.0003–240 ng/mL (urine), 0.0042–230 ng/mL(serum), 0.00014 –730 ng/mL (milk)	34% (urine); 37% (serum); 34% (milk)	[20]
36	PCBs, OCPs, PBDEs, HBCD and non-persistent NFRs	Human serum	PPT and SPE	7890 GC (Agilent) coupled to Xevo TQ-S MS (Waters), an 8890 GC coupled to a 7000D TQ MS (both Agilent), a 1290 UPLC (Agilent) coupled to a Qtrap 5500 (Sciex)	LOD: 0.36–66 pg/mL; LOD (EHTBB): 298 pg/mL	NA	[24]
121	Pesticides, plastic related products, parabens, UV filters, chlorophenols and exogenous metabolites	Human urine	SPE	Exion UPLC coupled to Qtrap 5500+ (both Sciex)	LOD (101 analytes): ≤0.10 ng/mL; LOD (18 analytes): 0.10–1.0 ng/mL	18%	[38]
245	Pesticides, veterinary and human pharmaceuticals, caffeine, parabens, PFASs, UV filters, phytoestrogen, corrosion inhibitors, photosensitizers, plastic related products, parabens, industrial antioxidants, food additives, other industrial products and exogenous metabolites	Commercial milk and human breast milk	PPT, QuEChERS and filter	Ultimate 3000 UPLC and QE Focus Orbitrap (both Thermo)	LOD: <0.47–61 ng/g, LOQ: <0.38–52 ng/g	NA	[43]
30	Halogenated compounds	Human breast milk	PPT and filter	Ultimate 3000 UPLC and Q-ExactiveMS (both Thermo)	Instrument LOD: 1.0 –100 ng/mL	63%	[44]
476	Food metabolites, arsenic metabolites, PAHs, pesticides, plastic related products, parabens, PFASs, UV filters, nicotine, veterinary and human pharmaceuticals, exogenous and endogenous metabolites	Human urine and plasma	PPT and filter (plasma); DNS (urine)	1290 UPLC (Agilent) coupled to QTrap 6500 MS (both Sciex)	LOQ: 0.10–839 ng/mL (plasma), LOQ: 0.10 –905 ng/mL (urine)	82% (urine), 75% (plasma)	[45]
180	Pesticides, veterinary and human pharmaceuticals, PFASs, food additive, other industrial products	Fish muscle	LE and filter	Ultimate 3000 UPLC coupled to QE Focus Orbitrap (both from Thermo)	LOD: 1.0–74 ng/g; LOQ: 10 –84 ng/g	86% of chemicals have MEs of 70 –130%	[27]
360	Pesticides, veterinary and human pharmaceuticals, plant growth regulator, agricultural preservatives and exogenous metabolites	Animal whole blood	QuEChERS	1290 UPLC tandem coupled to 6460 MS, 7890B GC tandem coupled to 7010 MS (all Agilent)	LOQ: 0.46–6.5 ng/mL	57%	[39]
35	OH-PAHs, BRPs, OH-PBDEs, triclosan and TBBPA	Human urine	SPE	1260 HPLC coupled to 6470 MS (both Agilent)	LOD: 0.0080–0.16 ng/mL	80%	[46]
36	Parabens, pesticides, UV filters and plastic related products	Human serum	PPT and SPE	Exion UPLC coupled to Qtrap 5500 (Sciex)	LOD: 0.0010–0.16 ng/mL, LOQ: 0.0020–0.53 ng/mL	90%	[6]
75	Endogeneous estrogens, mycoestrogens, phytoestrogens, PFASs, plastic related products, pesticides, parabens, UV filters, human pharmaceuticals, other industrial products, endogenous and exogenous metabolites	Human urine and serum	PPT	Ultimate 3000 UPLC coupled to TSQ Vantage MS (Thermo)	LOD: 0.0050–60 ng/mL (urine), 0.050–4.8 ng/mL (serum); LOQ: 0.015 –200 ng/mL (urine), 0.050 –16 ng/mL (serum)	75% (urine), 53% (serum)	[16]
		Human breast milk	PPT and QuEChERS		LOD: 0.0090–12 ng/mL; LOQ: 0.030–40 ng/mL	28%	
103	PAHs, OCPs, PCBs and PBDEs	Human serum	QuEChERS and SPE	7890B GC coupled with 7010B MS (both Agilent)	NA	NA	[47]
78	FRs, PFASs, OCPs and PCBs	Human serum	SPE and QuEChERS	7890A GC coupled to 7000B MS (both Agilent)	LOQ: 0.010–0.33 ng/mL, LOQ (some FRs): 0.050 –2.5 ng/g	NA	[5]
26	Sulfonic acids, insecticides and other industrial products	Whole blood of green sea turtles	PPT and QuEChERS	Nexera × 2 UPLC system (Shimadzu) coupled to TripleTOF 5600 System (SCIEX)	LOD: 0.14–2.6 ng/mL, LOQ: 0.46–6.5 ng/mL	46%	[48]
117	PCBs, PAHs, PBDEs and PCDD/Fs	Fish and mussell tissue	PPT, QuEChERS, GPC and SPE	7890 A GC (Agilent) coupled to HRMS Autospec Premier (Waters)	LOD: 0.25–682 ng/kg, LOQ: 0.82–2274 ng/kg	NA	[49]
64	Pesticides, cosmetic ingredients, FRs, parabens, PAHs, PCBs, PFASs, plastic related products, QACs, UV-filters and other industrial products	Pig blood	QuEChERS	A 1260 HPLC (Agilent) coupled to QTrap 6500 MS (Sciex), 6890 N GC coupled to 5973 MS (both Agilent)	LOQ (LC-MS): <2.0 - > 1000 ng/mL, LOQ (GC-MS): <2.0–10 ng/mL	NA	[50]
57	OCPs, PCBs and PAHs	Human breast milk	QuEChERS	Trace GC Ultra coupled to TSQ Quantum Max QqQ MS (both Thermo)	LOQ: 0.050–0.50 ng/mL	NA	[51]

PPT, protein precipitation; NA, data not available; MEs, matrix effects; LE, liquid extraction; QuEChERS, quick, easy, cheap, effective, rugged, and safe; GPC, gel permeation chromatography; SPE, solid phase extraction.

[a] There are several different functions to calculate the values of MEs, usually the ratio of signals in matrix and solvent. Here we uniformed the values of MEs from literature so that 100% ME means no matrix effects. The values of 80%–120% are used for acceptable MEs.

Table 2
Published sample preparation methods for non-targeted or suspected screening exposome-type studies in biological samples. L1 and L2 refer to the structural identification levels proposed by Ref. [52].

Number of Analytes[a]	Sample Matrices	Sample Preparation	Instrument	References
70 identified - L1	Human urine	LE	Vanquish Duo UPLC coupled to Q Exactive HF quadrupole-Orbitrap MS (both Thermo)	[53]
102 identified - L1	Human breast milk	QuEChERS	1200 HPLC (Agilent) coupled to Exactive Orbitrap MS (Thermo)	[14]
7 identified - L1	Human whole blood	QuEChERS	Ultimate 3000 UPLC coupled to Q Exactive HF hybrid Quadrupole-Orbitrap MS (both Thermo)	[54]
6 identified - L1	Human serum	SPE	1260 HPLC coupled to QTOF/MS 6550 (Both Agilent)	[40]
274 annotated - L2	Human hair wash	LE	1290 UPLC (Agilent) coupled to Impact II QTOF/MS (Bruker)	[55]
276 annotated - L2	Human hair extraction			
73 annotated - L2, 17 identified - L1	Human maternal and cord paired serum	PPT	1290 UPLC coupled to 6550 QTOF/MS (both Agilent)	[35]
21 annotated - L2, 2 identified - L1	Human urine	Filter	1290 UPLC coupled to 6560 QTOF/MS (both Agilent)	[56]
33 identified - L1	Plasma, stool and lung and thyroid tissue	QuEChERS	Q Exactive GC hybrid quadrupole Orbitrap MS (both Thermo Scientific)	[57]

[a] "Annotated" indicates molecules matched with fragmentation spectra from databases or libraries (Level 2 identification); "Identified" indicates molecules confirmed by retention time and *m/z* of authentic standards (Level 1 identification) [52].

sorbents have the potential to absorb some analytes and can contribute to background contamination. Ref. [57] added formic acid directly to plasma samples, followed by an extraction with hexane-ethyl acetate (2:1 v/v). The organic supernatant was treated by QuEChERS for non-targeted analysis, employing high-purity $MgSO_4$ to avoid contamination. Some compounds, such as phthalic acid, mono-methyl phthalate, mono-(3-carboxypropyl) phthalate, dinotefuran, and 2,4-dichlorophenoxyacetic acid, were lost in the extraction step. In another study, quantification of environmental phenols was affected by strong MEs even after purification by d-SPE (containing $MgSO_4$ and C18). Furthermore, the d-SPE step also increased the background contamination level of some target compounds in blanks (e.g., mono-(2-ethylhexyl) phthalate and BPA) [38]. A similar outcome was reported by Plassmann et al. [50]; where loss of perfluorinated carboxylates (logKoW, 2.14–8.83) and tetrabromobisphenol A (TBBPA, logKoW 7.2) was observed when QuEChERS clean-up steps were employed in the pretreatment process. Although extractions employing QuEChERS have been frequently reported in large-scale xenobiotic and non-targeted exposome research [20,43,47], there is a lack of conclusive data indicating that the positive results from the clean-up procedures based on QuEChERS overcome the associated negative side effects in exposome-type studies [51,85].

2.4. Filters

The use of commercial filters in the sample preparation for biological liquid samples in exposome research has, like DNS, become a common method due to its simple and efficient process [43–45,56]. These filters were initially designed to remove phospholipids, one of the major contributors to matrix effects in blood samples [86]. As filters have increased in popularity, a wide range of products have been marketed to remove a variety of molecule classes including lipids, protein, and other compounds contributing to matrix interferences. Filters generally come in cartridge or 96-well plate format and include products such as Captiva Enhanced Matrix Removal (EMR), Captiva ND and Captiva ND^{Lipids} from Agilent, LipiFiltr® from Ultra Clean Technology (UCT) and Sirocco Protein Precipitation plates from Waters. The use of multi-well plates greatly decreases the time and cost of the overall extraction procedure.

Recent studies have reported encouraging results indicating that filters decrease matrix interferences when compared to other sample preparation methods, such as DNS. Pourchet et al. [44] used a technique with a PPT step followed by filtering with Captiva EMR-Lipid cartridges (Agilent, USA) and reported MEs of 84%–126% for 19 halogenated compounds in human milk, while Ref. [16] reported MEs of 0–12% for 3 halogenated chemicals in the same matrix with PPT and QuEChERS. Ref. [27] extracted 180 xenobiotics in fish muscle and compared the results using mixed-mode SPE, normal-phase SPE and two filters, i.e., Captiva-EMR and Captiva-ND. When filters were used, 88% (Captiva EMR) and 79% (Captiva ND-Lipid) of the analytes showed MEs between 70% and 130% indicating minor matrix effects. In the case of mixed-mode SPE, only 45% of the target compounds had MEs in the range of 70%–130%. Regarding repeatability, excellent RSDs lower than 10% were obtained for 96% and 86% of the analytes with the CaptivaND-Lipid and Captiva EMR filters, respectively. These results were then compared to matrix effects for the same samples extracted using normal-phase SPE which exhibited severe MEs ~180% for most analytes.

Even though positive data concerning MEs was often reported with the use of filters in exposome studies, a limitation of filtration is the potential for filter material to adsorb analytes and create a concentration bias [87]. Recoveries were often biased with respect to specific functional groups, like carboxylic acids in the study by Chaker et al. [69]. In this publication, six of eleven compounds containing carboxylic acids showed low mean recoveries (<12%) which were calculated as the ratio of peak area of each compound in spiked serum before and after extraction of PL or PL Ultra filter plates (Merck, Germany). Moreover, the major shortcoming of filters is the lack of enrichment which limits its mainstream use due to the importance of capturing very low-abundance analytes in exposome research.

2.5. Solid-phase extraction (SPE)

Solid-phase extraction (SPE) was developed in the 1970s as an alternative to LE [18]. It is based on the same principle as affinity-based techniques, such as liquid chromatography [63], where analytes are separated between a solid and a liquid phase [18]. Currently, SPE is widely used for retaining and isolating analytes in a large number of sample matrices, especially in targeted workflows [17]. SPE is widely applicable as it effectively enriches samples, needs little organic solvent (compared to LE), is relatively easy to perform, has a strong automation potential [17], and effectively eliminates matrix effects [6,88].

The results from current human exposome research which included the use of SPE, especially with hydrophilic-lipophilic balance (HLB) sorbents, indicated removal of interferences and an overall increase in sensitivity. With the use of Oasis HLB 96-well plates, encouraging results were reported with low LODs (0.010–1.0 ng/mL) and RSDs (<20%) for spiked urine with two concentration levels (1 ng/mL and 10 ng/mL) in a comprehensive recent publication [42]. This study reported 50 chemicals including 12 PCPs, five PAHs, five OPFRs, 18 pesticides, five VOCs, four tobacco alkaloids, and one drug of abuse. Lin et al. [46] also used Oasis HLB cartridges (Waters, USA) to detect several pollutants, including OH-PAHs, BRPs, OH-PBDEs, triclosan and TBBPA in human urine with low LODs ranging from 0.008 to 0.16 ng/mL. Reported MEs from this study ranged from 82% to 163% and 80% of analytes had MEs in the range of 80%–120%. The Oasis HLB 96-well plate has performed well with reported LODs of 0.36–2983 pg/mL for 36 POPs, seven PCBs, 12 OCPs, ten PBDEs, HBCD and six non-persistent NFRs in human serum [24]. The application of SPE in non-targeted analysis has also been initially investigated. Wang et al. [40] analyzed 75 serum samples from pregnant women using HLB cartridges (10 mg, 1 mL, Waters) prior to LC-QTOF/MS analysis. Suspect screening was then conducted for 696 environmental organic acids. Using this method, 56 suspect organic acids were detected in each sample, and six were identified by standards. These results show the strong potential SPE analysis possesses, although further analysis and validation is needed.

Based on the described performance, including the reduction of MEs [89] and LODs, SPE methods may be able to improve sensitivity and consistency in large-scale cohort studies. However, SPE was applied less often than other techniques, such as LE and PPT, for exposome-scale analysis. This is likely a product of challenges associated with cost and extraction time. However, one study reporteddecreases in sensitivity and increased ME fluctuations with a large sample sets using SPE after the use of filters [46,69]. Ref. [43] isolated 245 diverse xenobiotics in milk and used Captiva ND-Lipids filters after LE with ACN in the presence of anhydrous Na_2SO_4 and NaCl. The results showed that the addition of SPE using Oasis HLB cartridges after the use of filters resulted in poorer MEs (81–112%) relative to MEs without SPE (92–119%). SPE also decreased sensitivity as lower LODs were seen for analytes without the addition of SPE. Additionally, for large sample sets the SPE process is time consuming and is only feasible with advanced robotic sample handling systems and/or 96 well plate formats. Although promising, data from studies investigating factors such as LOD, MEs, signal to noise ratios and mass accuracy fluctuations in SPE based non-targeted exposomics research needs to be completed [40,90,91].

2.6. Sustainability of sample preparation methods

Sustainability, particularly the reduced use of energy and harmful chemicals, has become an important metric to consider when designing an analytical workflow. This is especially true for sample extractions with large solvent inputs and large sample sizes. To determine the impact of a procedure, Nowak et al. developed a scoring system in 2021 which they coined white analytical chemistry (WAC) that determines the environmental impact of an analysis pipeline while also considering analytical and practical concerns [92]. Per this model, red, blue, and green are used to designate analytical efficiency, practical considerations such as time and cost and the environmental impact, respectively (Fig. 3A, Supplemental Table 1). Each analytical pipeline is given a score for each metric from 0 to 100, with 0 indicating a poor rating and 100 indicating an excellent rating for the target application. We evaluated and scored each of the five sample preparation methods using WAC principles for exposomics studies with LC-MS/MS analysis assuming the analysis of 200 analytes in 1000 plasma samples. As indicated by Fig. 3A, SPE had the highest red score while DNS had the highest blue and green score. However, SPE had the highest overall WAC score, indicating that SPE is the most appropriate method when balancing practicality, analytical confidence, and environmental impact (Fig. 3B).

3. Conclusions

Broad analyte coverage, ultimate trace-level sensitivity, reduction of interferences from complex biological matrices, and consistency/robustness during large-scale EWASs are the most essential considerations when designing sample preparation protocols in both targeted and non-targeted exposomics [16,31,93] (Fig. 1B). Many different sample preparation techniques have been optimized for specific sample types and analytical outcomes. For samples with high concentrations of targeted xenobiotics, dilute and shoot or LE have been shown to perform well. These preparation techniques can cover a wide range of analytes but have lowered sensitivity and matrix interference problems as well as method consistency issues due to the limited clean-up process. For samples with high matrix effects, both QuEChERS and filters are a viable option and can decrease matrix effects by providing a supplementary clean-up technique. Yet, the purification ability of QuEChERS was not strongly supported by the reported data and the addition of QuEChERS increases the risk of introducing background contamination while filters are limited by their inability to significantly increase sensitivity as well as potentially absorbing certain xenobiotics and biasing results.

To overcome the current challenges in assessing chemical exposure in human samples, pragmatic analytical approaches with a certain courage of leaving gaps are essential. While not always easy to accept for analytical chemists that have been trained mostly on targeted assays, omic-scale workflows that hold the potential to answer biological and epidemiological questions have to be established and harmonized rather than those delivering superb analytical figures of merit at the cost of chemical coverage and biological meaning.

The current literature points to the fact that SPE might be a preferred approach for future exposomics applications for broad coverage. SPE has shown encouraging results with respect to reducing matrix effects, analyte enrichment and overall sensitivity [6,38] and holds promise for improving consistency in sample preparation methods for high-throughput studies by cleaning samples including decreasing salt levels. However, only very few studies have evaluated the application of SPE-based methods in large-scale epidemiological EWAS studies or those investigating more than 100 xenobiotics simultaneously. Also, the so-far limited chemical coverage, the need for automatization and harmonization leading to high-throughput potential, and the lack of data in non-targeted applications need to be addressed. To further optimize and translate SPE application to exposome-scale analysis, new SPE

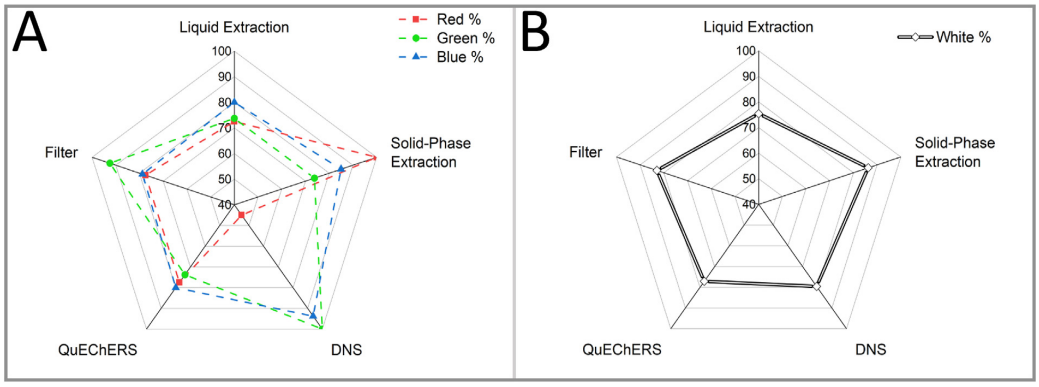

Fig. 3. Sustainability analysis for each sample preparation technique. To determine the white analytical chemistry (WAC) values for each technique, an exposome-scale application based on LC-MS/MS for 200 analytes in 1000 plasma samples was modeled. (A) Percentage values for each of the three main metrics in the WAC analysis. Red, green and blue correspond to analytical efficiency, environmental impact, and practical considerations like time and cost, respectively. (B) Percentage values for composite scores of each sample preparation technique in the WAC analysis.

methods should be developed to increase exposome-specific chemical coverage, high-throughput methods should also be developed and evaluated including the use of 96-well plates and the efficacy of SPE-based non-targeted exposomics methods needs to be extensively evaluated. Advances also should include different sorbent materials enabling research targeting specific classes of analytes as well as expanding the physical design of SPE technologies for challenging research questions [94,95]. Although each commonly used sample preparation method has individual merits that can be tailored to suit specific sample types and analyses, SPE holds a special promise for exposomics research and should be at centre stage of future of exposome exploration.

Declaration of competing interest

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Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trac.2023.117151.

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Supplementary Information

A supplementary data file (xlsx) is available online for this publication that contains two tables about sustainability analysis for the commonly used sample preparation techniques.

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To download the supplementary two tables, scan here.

6.3. Publication #2: Gu et al. (2025a)

Status	Published
Title	**High-Throughput Solid Phase Extraction for Targeted and Nontargeted Exposomics**
Authors	Yunyun Gu[1,2], Max L. Feuerstein[1,3], Benedikt Warth[1,2,3]
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Contribution	Yunyun Gu optimized the sample preparation method, evaluated and interpreted the results, and wrote the initial version of manuscript draft.

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High-Throughput Solid Phase Extraction for Targeted and Nontargeted Exposomics

Yunyun Gu, Max Lennart Feuerstein, and Benedikt Warth*

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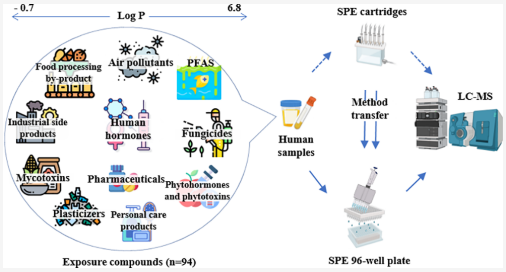
ABSTRACT: Characterizing the chemical exposome relies on advanced instrumentation including tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS), and nontargeted analysis (NTA) using high-resolution MS. However, proper sample pretreatment, balancing broad analyte coverage, method robustness, and throughput remain a major bottleneck in exposomics. Here, we developed a robust and scalable solid phase extraction (SPE) protocol for human urine and plasma and optimized it for a panel of 94 highly diverse environmental and food-related contaminants (LogP −0.7 to 6.8). Extraction recoveries (RE) and signal suppression and enhancement (SSE) were determined using targeted LC-MS/MS. Acceptable REs (60−140%) were achieved for >70% of analytes, and acceptable SSE values (60−140%) for 86% and 90% in urine and plasma, respectively. Subsequently, the method was transferred to 96-well plate format, significantly improving throughput to meet the capacity requirements needed for exposome-wide association studies (ExWAS). The established workflow is approximately 10× faster than routinely used metabolomics-based protein precipitation approaches when comparing the estimated total analysis time for 1000 samples. The method's applicability for NTA and suspect screening was tested and compared to a generic protein precipitation protocol using NIST standard reference materials for urine (SRM 3672) and plasma (SRM 1950). Favorable performance was shown for the protein precipitation workflow while the SPE protocol demonstrated promising results. The developed workflow is thus not only superior for future high-throughput targeted exposomics but also offers an option for NTA applications. The presented well-balanced approach is scalable and also applicable to research in the fields of pharmacology, food safety, and systems toxicology.



Humans are consistently exposed to a myriad of chemicals during their daily lives through various sources, including dietary intake and environmental pollutants.[1−3] Exposomics represents the comprehensive analysis of all environmental and food-related exposures and associated influences on various health outcomes.[4] To allow a full assessment of the totality of chemical exposures in human samples, sensitive, specific, and robust analytical techniques, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) are required. Multiclass or next-generation human biomonitoring (HBM) methods have recently been developed to monitor a wide range of environmental chemicals and are needed to address the full complexity of chemical exposure.[5,6] In contrast, nontargeted analysis (NTA) is an emerging technique for exposomics using high-resolution mass spectrometry (HRMS) and allows the exploration of unknown chemicals in human samples.[7,8] Furthermore, suspect screening methods use large chemical databases and spectral libraries to prioritize detected features and annotate compound identities.[9,10] Both targeted and nontargeted exposomic methods face significant challenges related to complex biological sample matrices,[11] and ensuring repeatability and consistency across large sample sets is mandatory, especially when dealing with low-abundance analytes (i.e., pg/mL levels or lower).[12−14] Addressing these challenges in exposomics requires sophisticated optimization of the entire analytical workflow, including sample preparation protocols. Efficient sample pretreatment before instrumental analysis can reduce interferences, separate, and concentrate analytes in diverse matrices[15,16] and various sample preparation techniques have been optimized to analyze the exposome. Liquid extraction (LE) and protein precipitation (PPT) are widely used extraction methods for exposomics due to their comparably wide chemical coverage.[6,17] However, these techniques are prone to preserve matrix interferences which can hamper overall method performance due to limited sample cleanup.

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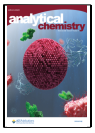

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Recently, solid phase extraction (SPE) received more attention as an option for sample preparation in exposomics and is discussed as a promising approach for future large-scale applications, owing to its capability to reduce matrix effects, enhance sensitivity, and improve consistency in high-throughput studies.[18,19] SPE has been frequently used for the purification and extraction of various analytes from diverse sample matrices for HBM including urine,[20] blood,[21] and various other biological matrices.[22,23] However, a systematic development of an exposome-scale SPE method compatible with both, targeted and nontargeted data acquisition strategies has rarely been performed. This challenge originates from the complexity of designing a balanced method with suitable method robustness and broad chemical space coverage. Most existing SPE methods focused on a limited selection of targeted analytes to obtain reliable protocols without assessing the potential coverage for NTA[24] due to the lack of certified reference standards, which restricts their utility in NTA.

In this study, a targeted high-throughput sample pretreatment method using SPE cartridges was optimized to ensure the applicability of the workflow for urine and plasma samples. The predicted octanol−water partition coefficient (logP) from PubChem (pubchem.ncbi.nlm.nih.gov) was utilized to evaluate the chemical coverage in terms of analyte lipophilicity of compounds in this study. Target analytes with a wide range of physicochemical properties, including polarity, presents a central challenge for SPE methods in exposomics, due to varying analyte-sorbent interactions. This issue is a key factor that needs to be addressed during method development aiming for a comprehensive chemical coverage. Here, 94 highly diverse analytes from multiple toxicant classes including perfluorinated substances (PFAS), plasticizers, personal care products, food processing byproducts, pesticides, industrial chemicals, air pollutants, disinfecting agents, mycotoxins, phytoestrogens and phytotoxins were used to optimize an SPE protocol for high-throughput applications. Optimization steps included selecting SPE sorbents and sample buffers, optimizing washing and elution steps, and improving efficiency. Subsequently, the protocol was transferred to 96-well plates, and analytical performance and throughput capacity were rigorously tested. Finally, the potential for NTA was explored using NIST standard reference materials (SRMs) for urine and plasma. The results indicate the feasibility of the approach for scaling and large-scale exposome-wide association studies.

EXPERIMENTAL SECTION

Biological Samples and Chemicals. Human pooled plasma was purchased from Innovative Research (Novi, MI). Pooled urine was obtained from a female volunteer who abstained from consuming food and beverages stored in plastic containers, phytoestrogens rich food, and cosmetics containing parabens for 2 days before sample collection.[14] Standard reference materials, including urine (SRM 3672) and plasma (SRM 1950) were obtained from the National Institute of Standards and Technology (NIST, Gaithersburg) for NTA. Detailed information on standards, including selection criteria and spiking levels can be found in Tables S1−S4. The selection of target analytes and analyte classes was intended to be as broad as technically feasible with differing structures, real-life exposure levels, and toxicological modes of action. It was based on our previous work on next-generation biomonitoring.[6]

Method Development and Optimization. The sample pretreatment approach was developed and optimized in several steps. Initially, two universal SPE sorbents, a commercial hydrophilic−lipophilic balanced sorbent (HLB) from Waters and an in-house prepared mixed-mode SPE sorbent, were compared. The mixed-mode sorbent was prepared from two SPE sorbents, primary second amine (PSA) and C18 (PSA+C18), by mixing equal weights before packing. Details of the preparation are described in the Supporting Information (SI). Packed SPE cartridges (HLB or PSA+C18) were then used to optimize the full processes including sample loading, washing, and elution. A mixture of 94 reference standards was spiked into urine, plasma, and water (H_2O) samples both before ("prespiked") and after ("postspiked") the SPE process. Analyte loss (%), extraction recovery (RE, %), and signal suppression and enhancement (SSE, %) were calculated based on the peak areas from these "prespiked" and "postspiked" samples.

Analyte loss was defined as the fraction of an analyte that was not retained by the SPE sorbent. It was calculated as the ratio of peak areas of the effluents collected during sample loading and washing from "prespiked" samples compared to peak areas of "postspiked" effluents. Analyte loss of 0% was achieved for complete analyte retention, whereas completely unretained analytes are characterized by a loss of 100%.

The term extraction recovery (RE) was used to describe the recovery of the full SPE process including loading, washing and elution. Thus, RE was calculated as the ratio of peak areas of a "prespiked" and "postspiked" sample. Full analyte recovery results in RE of 100%, whereas 0% indicates no analyte recovery of the SPE procedure. RE greater than 100% indicate contamination or carryover introduced during sample preparation or sample injection.

Matrix effects in terms of signal suppression and enhancement (SSE) depend on the influence of the sample matrix on the analyte's signal intensity. This was calculated as the ratio of peak areas for "postspiked" samples to a standard in pure solvent.[25] SSE values close to 100% indicate negligible effects of the sample matrix on the detector response, which is required for accurate analyte quantification.

To evaluate the method performance and provide a rationale for the individual optimization steps, the metrics described above were classified into three categories: "good", "acceptable", and "poor", as shown in Table S5. Noteworthy, they do not represent method validation criteria but are used for method optimization.

Subsequently, the SPE method was applied to pooled human urine and plasma samples to test applicability to analyze human samples. Sample pretreatment was further streamlined and transferred from SPE cartridges to 96-well plates to allow parallel sample extraction and maximize sample throughput. Furthermore, the applicability of the developed workflow for NTA and suspect screening was tested to further expand the coverage of the targeted biomonitoring assay.

SPE Sorbent Selection. SPE cartridges with two different sorbent types, HLB and PSA+C18, were considered and the stability and robustness of the material were tested. For this purpose, loading, washing, and elution procedures were individually evaluated. In this first evaluation, analytical standards in LC-MS grade H_2O were used as samples to minimize influences of the sample matrix. Analyte loss (%) for both SPE sorbents was tested using H_2O or 2% methanol ($MeOH/H_2O$, 2/98, v/v) for sample loading and washing. Subsequently, RE was assessed using various elution solvents including 3% formic acid in methanol (FA/MeOH, 3/97, v/v),

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Anal. Chem. 2025, 97, 6075−6082

3% ammonia in MeOH (NH_3/MeOH, 3/97, v/v), and pure MeOH. Details including conditioning of SPE sorbents, analyte spiking procedures and spiking levels, as well as calculations of the analyte loss and RE, are reported in the SI.

Sample Buffer Selection before Loading. Urine and plasma are commonly used sample matrices for HBM and, especially in the case of urine, varying concentrations of matrix components can influence analyte extraction. To ensure method reliability, commonly utilized buffers, PBS (pH 7.4, 200 mM) and NH_4AC (pH 6.0, 2.5 M), and pure H_2O were tested for sample dilution before the SPE cleanup. Based on the results of our previously published work, a final dilution ratio of 2 was used.[26]

Optimization of Washing, Elution, and Reconstitution Steps. Water was selected as the washing solvent and the required volume was optimized by comparing the SSE of either washing one or two times with 1 mL. MeOH was selected as the primary component of the elution solvent due to its widespread application in SPE processes.[27] Considering the strong anion-exchange interactions of PSA with commonly encountered acidic compounds in exposomics,[9,28] a basic MeOH solution (3% NH_3) was used as the elution solvent to compare the RE of the mixed-mode sorbent (PSA+C18). For HLB, both acidic (3% FA) and basic MeOH (3% NH_3) were evaluated due to the anion and cation exchange properties of HLB. Furthermore, MeOH and two commonly used solvents, acetonitrile (ACN) and isopropanol (ISO), and a mixture of MeOH/ACN/ISO (1/1/1, v/v/v), were evaluated as elution solvents. The solvent mixture was selected based on results obtained from urine samples. Elution volumes of 200 μL, 400 μL, and 800 μL were subsequently compared using pooled urine as a matrix.

A typical approach to improve detection limits is to evaporate raw extracts and reconstitute them in a defined volume. Despite its clear benefits for enhancing the detection of low-abundant analytes, this step is rather time-consuming, especially when dealing with a large number of samples. To further enhance sample throughput and assess the effect of evaporation and reconstitution, two workflows were tested and compared. One set of SPE extracts was dried using a CentriVap Vacuum concentrator (Labconco) and reconstituted with 50% MeOH (MEOH/H_2O, 1/1, v/v). In contrast, a second set of extracts was diluted with H_2O, resulting in a final mixture of 50% MeOH for comparison. The final concentrations in the extracts of both sets were the same.

Method Transfer to 96-Well Plates. After the optimization, pooled urine and plasma were used to test extraction and cleanup using the developed workflow for SPE cartridges. Then, the method was transferred to a 96-well plate SPE packed with HLB (Waters Corporation) and the following parameters: SPE plates were conditioned with 1 mL of MeOH, followed by 1 mL of H_2O. Then, 400 μL of urine or plasma sample were mixed with 400 μL of PBS. The mixture was loaded on the SPE plate and washed twice with 1 mL of H_2O each. Gravity flow was selected for sample loading, washing and analyte elution. Analytes were eluted using two times 200 μL of MeOH, resulting in a total volume of 400 μL. To ensure reproducible elution, residuals of the eluents were collected by applying negative pressure using a vacuum manifold. RE and SSE were calculated and compared to results determined for SPE cartridges to prove applicability for high-throughput applications.

Assessing the Potential of SPE for NTA and Suspect Screening. SPE is a promising approach to enhance method performance for targeted applications, but systematic testing for evaluating chemical coverage is a necessity before any potential application in nontargeted analysis. The coverage of other typically detected analytes in human samples and the effect on signal intensities were evaluated by comparing results of the novel 96-well plate-based SPE approach to results from a PPT method[29] as the reference method for nontargeted applications. SRM 1950 (plasma) and SRM 3672 (urine) were extracted with both workflows and were analyzed using LC-HRMS for NTA. Unknown compounds were annotated using publicly available spectral libraries and a set of 234 authentic analytical standards (Table S2) was spiked into samples for compound identification and to test the method for targeted screening. Results were reported based on established reporting schemes, with identified compounds reported with the highest confidence level ("level 1": identified based on authentic chemical standards). Therefore, standards were spiked into extracts from the SPE process. Spiking levels of these standards are reported in Table S4. Following annotation, the effect of the sample preparation method on relative feature intensities was compared. Results were further investigated as a function of retention times and classifications of the annotated features. Details are outlined in the SI.

Data Acquisition. *Targeted LC-MS/MS.* Front-end LC separation was performed using our previously published method.[6] Identical separation gradients were used for LC-MS/MS and LC-HRMS(/MS) using an Agilent 1290 Infinity II and a Thermo Scientific Vanquish Horizon, respectively. An Acquity HSS T3 column (1.8 μm, 2.1 × 100 mm, Waters) was used with gradient elution using a flow rate of 0.4 mL/min and 0.3 mM ammonium fluoride in H_2O as eluent A and ACN as eluent B. Details are reported in Table S6. A QTrap 6500+ mass spectrometer equipped with an electrospray ionization (ESI) source (Sciex) was operated using a previously published multiple reaction monitoring (MRM) methods with fast polarity switching.[6] Full method parameters including MRM transitions and ESI parameters are provided in the SI.

Nontargeted HRMS(/MS). An Orbitrap Exploris 480 mass spectrometer (Thermo Scientific) was used for HRMS(/MS) measurements. Samples were analyzed using full scan mode and AcquireX was used to generate fragment spectra using a mass resolution of 120,000 at m/z 200, with full method parameters reported in the SI.

Data Analysis. *Quality Control.* Several types of QC samples were used to ensure reliable results. A systems suitability test sample (SST) was measured before and after each analytical sequence to ensure favorable instrument performance (see SI and Table S7). In addition, a multianalyte reference standard mixture consisting of 94 highly diverse chemicals in pure solvent was injected. Solvent blanks were used to monitor instrumental background and carryover and process blanks were used to monitor background concentrations/contaminations.

Targeted Data Analysis. RE and SSE values were evaluated for the set of 94 analytes[6] using standards in neat solvent, pooled urine, and pooled plasma. Peak integration was carried out using Multiquant (v3.0.2, Sciex) and Skyline (v21.2.2.536, McCoss Lab).[30] Calculation of RE and SSE was performed using Microsoft Office 365. Graphics were created by Origin 2021b (v9.8; OriginLab Corporation).

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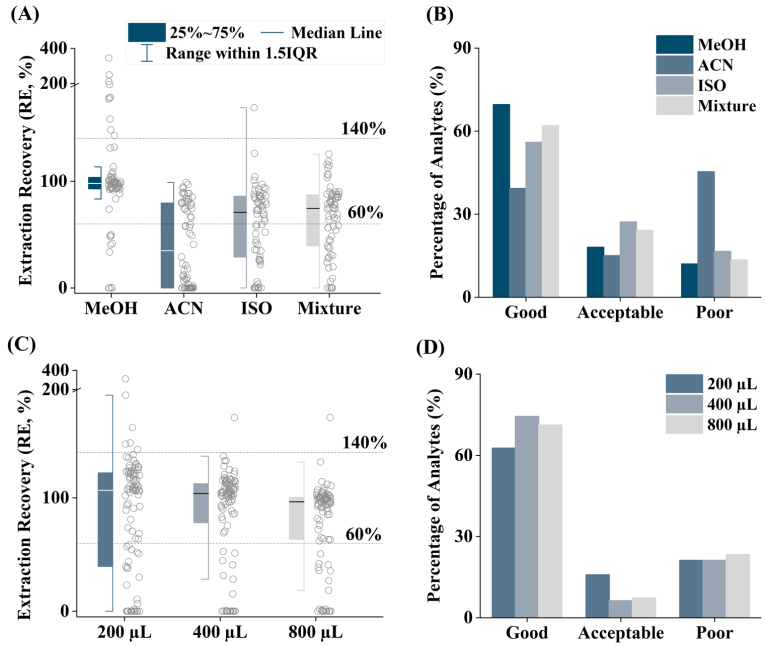

Figure 1. Extraction recovery (RE, %) for 94 analytes in pooled urine using HLB cartridges with different elution conditions (A) and classification of the performance into three categories (B) using methanol (MeOH), acetonitrile (ACN), isopropanol (ISO), and a mixture of ISO/MeOH/ACN (1/1/1, v/v/v). Panels (C) and (D) show results for the optimization of the elution volume for MeOH (200 μL, 400 μL, and 800 μL). The following classification was used in panels (B) and (D): "Good": RE 60−140%, "Acceptable": RE 20−60% or 140−180%, "Poor": RE <20% or RE >180%.

Nontargeted Data Analysis. Raw data files were analyzed using MSDIAL (v5.2).[31] Full data processing settings including data extraction, peak picking, compound identification, and alignment are reported in the SI. To evaluate the qualitative analyte coverage for NTA and suspect screening, MassBank of North America (MoNA, https://mona.fiehnlab.ucdavis.edu/) was used as a spectral library. Annotations were then classified based on the level of confidence scheme according to Schymanski et al.[32] using confidence levels 1 (confirmed by authentic standards), level 2 (confirmed by spectral library match), and level 3 (confirmed by spectral library match, but with isomeric structures). Additional data analysis and visualization was performed using Microsoft Office 365, Origin 2021b, MetaboAnalyst (v6.0), and Inkscape (version 1.2.2; Inkscape).

RESULTS AND DISCUSSION

SPE Sorbent Selection. Analyte loss was compared for both SPE sorbents and HLB and PSA+C18 showed comparable performance (see Figure S1). "Good" performance (analyte loss <10% during loading and washing) was achieved for 90% of all analytes, whereas "good" RE was achieved for 59% of all analytes using HLB compared to 39% using the mixed-mode sorbent PSA+C18.

Analyte loss >5% was observed for six analytes with LogP values between 1.7 and 3.2 when using the PSA+C18 for SPE, whereas analyte loss >5% was only observed for a single compound when using the HLB sorbent (see Figure S2). Most analytes showed sufficient RE (50−150%) using either of the two sorbents but RE <50% were observed for 15 analytes using the mixed-mode sorbent, whereas only six analytes with RE <50% were observed using the HLB sorbent. Consequently, the HLB was selected as the sorbent material for further workflow optimization.

Buffer Selection for Sample before Loading. Subsequently, the buffer system used for sample loading was optimized. As shown in Figure S3, >95% of all analytes showed analyte losses <10% after diluting samples with PBS buffer. This fraction dropped to 80% and 87% of the analytes when using NH_4Ac and H_2O as dilution solvents. Consequently, PBS was chosen as the sample buffer for further process optimization.

Washing, Elution, and Reconstitution Optimization. Thereafter, washing, elution and reconstitution steps were optimized. Matrix effects were investigated in terms of SSE (%) and results for the two tested washing protocols were largely comparable (Figure S4). However, using two times 1 mL of H_2O improved SSE by approximately 5% for most analytes, especially for those with RT between 1.0 and 7.0 min (Figure S5). Consequently, two washing cycles using 1 mL H_2O each were used in the final protocol.

The use of acidic MeOH solutions (3% FA) as elution solvent resulted in lower RE (<50% for most analytes), whereas better RE (50−150% for most analytes) was achieved using pure MeOH or basic MeOH (3% NH_3) for elution. Six nonpolar compounds (LogP, 4.0−5.5) had surprisingly high RE values >150% using the basic MeOH solution while only a

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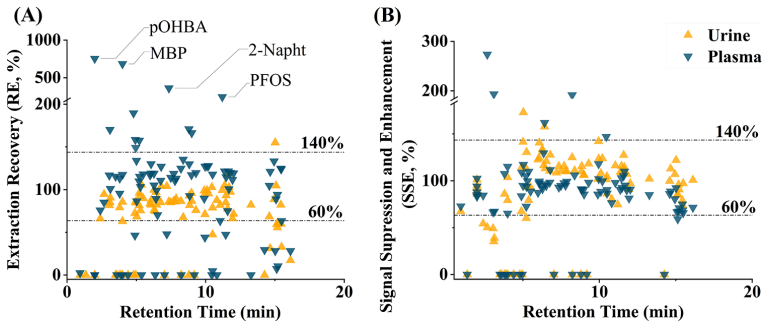

Figure 2. Extraction recoveries (A) and signal suppression and enhancement (SSE, %) (B) for 94 analytes in urine (yellow) and plasma (blue) using the optimized protocol based on SPE 96-well plate format. pOHBA, p-hydroxybenzoic acid; MBP, monobutyl phthalate; 2-Napht, 2-naphthol; PFOS, perfluorooctanesulfonic acid.

single compound had RE >150% using pure MeOH (Figure S2 (F)). Thus, pure MeOH was selected as the elution solvent for further optimization. In addition, it should be noted that basic solvents with high ammonia concentrations may cause issues including unstable RTs and potential damage to both the UPLC column sorbent and the UPLC system itself.[33] Different elution solvents (MeOH, ACN and ISO) were compared and elution volumes were optimized. "Good" REs were achieved for approximately 70% using MeOH for elution, whereas this reduced to 39−62% when ACN, ISO or a 1:1:1 mixture of all solvents was used (Figure 1).

Solvent volumes were optimized for MeOH and 200 μL, 400 μL, and 800 μL were tested for elution (see Figure 1C,D). Approximately 70% of compounds achieved "good" REs when using 400 μL or 800 μL of MeOH for elution, whereas this fraction dropped to 63% when eluting with 200 μL MeOH. The smaller solvent volumes (200 μL) resulted in inefficient elution for around 10% of all analytes and 400 μL (using 2 times 200 μL) was selected for further method optimization, as it represented a well-balanced compromise between good RE and low solvent consumption. Regardless of the elution conditions, certain highly polar compounds in pooled urine were poorly retained on the SPE cartridges. These compounds were primarily low-mass organic acids such as 5-hydroxymethyl-2-furanoic acid, dibromoacetic acid, dichloroacetic acid, bromoacetic acid, and p-hydroxybenzoic acid and were also only weakly retained by the reversed-phase (RP-)LC column with the applied method (i.e., RT <1 min).

The effect of drying the raw extracts followed by reconstitution was investigated (see Figure S6), and diluting the raw extracts resulted in improved recoveries, indicating the need to consider the optimization of reconstitution steps during method development. Approximately 6% of analytes with "good" REs using the original protocol (i.e., SPE followed by dilution), fell into the "acceptable" category after drying and reconstitution of the extracts. Potential causes include short vortexing, the absence of sonication steps, or the selection of the resuspension solvent containing 50% MeOH in H_2O (1/1, v/v). For example, the RE for 2-*tert*-butylphenol decreased significantly from 99.7% with dilution to 23% after drying and reconstitution. Furthermore, the study found an increased background signal for certain plastics related exposures including bisphenol F and S, when using drying and reconstitution. Consequently, a direct dilution step is clearly favorable in terms of simplicity and speed, which are both required for high-throughput applications.

Transfer from Cartridges to 96-Well Plate Format. For maximizing sample throughput, the SPE protocol was transferred to 96-well plates (plate-based SPE) and performance was compared to the initially optimized cartridge-based SPE protocol. In total, 72% of all analytes fulfilled the criteria for "good" RE when using the cartridge-based protocol for extracting urine or plasma samples, and 86% and 90% of the analytes were classified as "good" in terms of SSE. When using 96-well plates, 71% and 60% of the analytes achieved "good" REs while 76% and 78% of all analytes obtained "good" SSE in urine and plasma, respectively. Most analytes with "good" REs using the cartridge-based workflow were also categorized as "good" using the 96-well plate workflow for the extraction of urine, whereas the performance of the 96-well plate method was reduced for 12% of the analytes in plasma. Similarly, the cartridge-based protocol outperformed the plate-based SPE method in 10% of the cases in terms of matrix-induced SSE (see Figure S7).

In total, around 25% of the analytes were poorly extracted from plasma and 20% from urine using the plate-based protocol. The observed discrepancies might be explained by differences in the manufacturing process, including sorbent packing, or differences in sample handling when working with 96-well plates. In addition to weakly retained compounds (low-mass acids, see above), additional compounds in plasma, such as p-hydroxybenzoic acid (pOHBA), monobutyl phthalate (MBP), 2-naphthol (2-Napht) or perfluorooctanesulfonic acid (PFOS), were reported with "poor" RE. This was due to their background levels being 2 to 10 times higher than the spiked levels. In general, RE and SSE were only slightly better when using cartridges compared to 96-well plates (see Figure 2). Despite the slightly reduced performance in selected examples, the application of 96-well plates still holds promise for large-scale applications due to its comparable performance to the cartridges in combination with notable improvement in sample throughput, and handling. A schematic representation of the SPE workflow based on 96-well plates is presented in Figure 3 and more details are given in Table S8. To assess the full potential for high-throughput applications, the total analysis time of the 96-well plate-based SPE workflow was predicted for the analysis of 1000 samples, and time estimates were compared to our routinely used PPT protocol.[29] The new plate-based SPE protocol allows extracting 1000 biological

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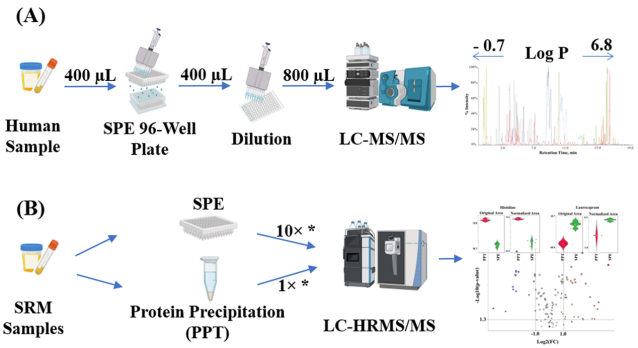

Figure 3. (A) SPE workflow for targeted analysis. (B) Illustration of the feasibility test of the established SPE method for nontargeted analysis (NTA). * The SPE protocol in 96-well plate format is approximately 10× faster than a state-of-the-art protein precipitation workflow when calculated for 1000 samples. The −0.7 to 6.8 logP range indicates the workflow's ability to cover chemicals with varying polarities.

samples within 3 days, whereas the same amount of samples would require 29 days using a routinely used PPT workflow as shown in Table S9.

Assessing the Potential of SPE for NTA and Suspect Screening. While systematic optimization and characterization of sample preparation methods for targeted (multianalyte) assays can be performed easily, full-performance comparison for nontargeted applications is less straightforward. We aimed to characterize the applicability of the developed SPE method for NTA and suspect screening, by comparing performance to a well-established protein precipitation method (PPT). Thus, extracts obtained with PPT and 96-well plate-based SPE were analyzed using LC-HRMS(/MS) (see Figure 3B), and results were compared on the feature level. Extracts of NIST SRM 1950 (plasma) and SRM 3672 (urine) were analyzed on an LC-HRMS instrument. Peak picking, feature alignment and compound annotation were performed using MSDIAL together with reference mass spectra from MassBank of North America (MoNA). Relative intensities were determined for annotated features as the area under curve (AUC), and results of the PPT and SPE workflows were compared for annotated and identified features. Values of fold change (FC >2) and significance (p-value <0.05) were analyzed using MetaboAnalyst.[34] Approximately 50% of the annotated features detected in SRM 1950 (plasma), showed significantly higher AUCs (FC >2, p-value <0.05) when using the PPT workflow (see Table S10). Yet, SPE extraction resulted in significantly higher signal intensities for 13% of the features, and 36% of the features were not significantly affected by the choice of sample preparation. For SRM 3672 (urine), we observed significantly higher AUCs using PPT in 32% of cases, whereas 10% were significantly enhanced using SPE, and 58% of annotated features were not significantly different (Table S11).

Figure 4A,B shows results of the comparison of relative feature intensities as fold changes of AUC as a function of the RT, whereas results are summarized on the compound class level in Figure 4C. For SRM 1950 (plasma), higher relative signal intensities in PPT extracts were observed for polar compounds, mainly amino acids and organic acids with RT <3 min, which is well in line with the results from the targeted assay. Additionally, several nonpolar features, mainly lipids with RT >10 min were higher in the PPT extracts. Similar results were observed for SRM 3672 urine, with polar compounds including amino acids and derivatives or organic acids with RT <3 min showing higher AUCs in PPT extracts compared to SPE. However, some key low-abundance biomarkers were observed with significantly higher AUCs using SPE, like nicotine in urine (SRM 3672) and cocaine in plasma (SRM 1950, see Figure S8). Interestingly, no significant differences between PPT and SPE were observed for major metabolites of nicotine and cocaine such as benzoylecgonine[35] for cocaine, and hydroxycotinine and cotinine for nicotine.

Despite these differences, comparable results were obtained for a large fraction of features and diverse compound classes including amino acids, carnitines, natural products, or synthetic compounds such as medical drugs and pesticides. For instance, 16 drugs were detected with comparable AUCs in PPT and SPE extracts of SRM 3672 urine. Similarly, 12 drug-related features were detected in the plasma with comparable AUCs using both methods.

In conclusion, the PPT workflow offers wider coverage of annotated features, but comparable results were obtained for many annotated compounds. In urine, the results were comparable for 58% of annotated features detected in extracts using both methods. However, PPT extraction provided broader feature coverage for the analysis of plasma samples, particularly endogenous polar metabolites, whereas SPE extraction improved the detection of low-abundance exogenous compounds at the cost of losing several polar metabolites.

LIMITATIONS

While the established workflow is effective for most analytes in targeted multiclass analysis of human samples and performs comparably well for a relative majority of analytes in NTA, several limitations remain. The analytes selected for method optimization were highly diverse and yet still represent only a limited subset of the known chemical exposome. Although analytes were selected with a special emphasis on covering a wide range of physicochemical properties, coverage needs to be further expanded for exposome-scale work. A broader selection of compounds and wider chemical coverage would be needed for a truly holistic representation of the exposome in future work. Moreover, certain analytes, especially polar acidic compounds, are partly lost during the SPE process due to weak retention by the sorbent, which was expected. Although

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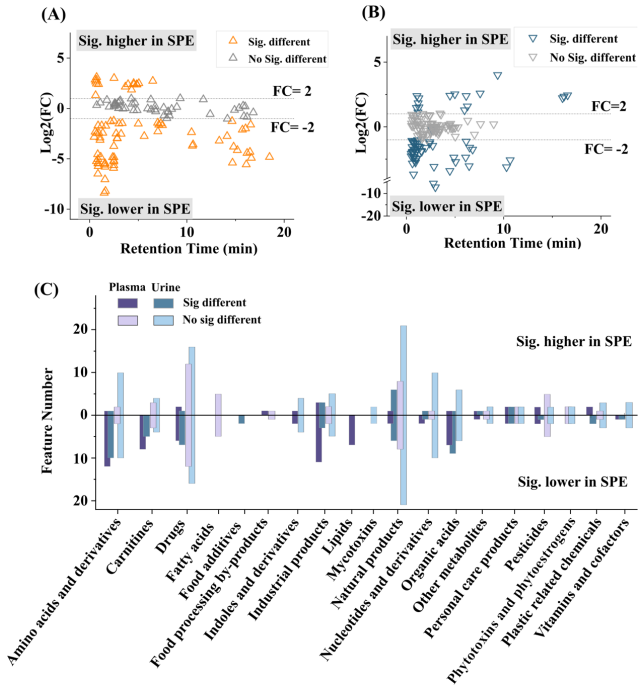

Figure 4. Evaluation of SPE performance compared to protein precipitation for nontargeted analysis (NTA). Comparison of peak areas of annotated features (confidence levels 1−3) in SRM 1950 plasma (A) and SRM 3672 urine (B) as a function of retention time as log2 fold change of the peak area, and summary of the results based on the compound class (C).

different workflows could address these specific shortcomings by targeting particular sets of analytes, conducting multiple measurements to gather sufficient information is time-consuming. Therefore, achieving favorable performance in terms of comprehensive analyte coverage, efficient cleanup, and minimal time consumption simultaneously is not always feasible and not always required.[36]

CONCLUSIONS AND OUTLOOK

In conclusion, we present the development of a high-throughput SPE protocol based on 96-well plate format for faster, cheaper, and more robust omic-scale exposure analysis. This workflow was optimized comprehensively considering a large scale of targeted analytes, and chemical coverage was further compared with a validated PPT method to ensure sufficient analyte coverage for targeted LC-MS/MS and nontargeted LC-HRMS applications. Despite reduced method performance for very polar compounds, the comparison of SPE and PPT workflows clearly indicated that the SPE method is a suitable candidate for large-scale NTA or suspect screening applications, especially due to the outstanding enhancement of sample throughput and instrumental robustness. This is underlined by the comparable overall performance of PPT and SPE workflows for a wide range of analytes, especially when RP-LC is used as a front-end separation technique. A pragmatic balance between reduced matrix complexity and chemical coverage was achieved, and due to the high sample throughput, the presented workflow can be regarded as a suitable candidate for exposome-wide association studies (ExWAS). Future work will include the expansion to even more toxicologically relevant chemicals and testing the capacity of the developed sample preparation workflow in large cohort studies to investigate the impact on instrumental robustness and downtimes.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.4c06177.

Methods and the results; Figures S1−S8; and references (PDF)

Tables S1−S11 (XLSX)

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Notes

The authors declare no competing financial interest.

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Supplementary Information

Supplementary data files are available online for this publication that contain various tables (xlsx), and supplementary figures and results (PDF).

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6.4. Publication #3: Gu et al. (2025b)

Status	Published
Title	**Quantitative exposomics targeting over 200 toxicants and key biomarkers at the picomolar level**
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Contribution	Yunyun Gu developed and validated the method, evaluated and interpreted the results, and wrote the initial version of manuscript draft.

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Article

Quantitative Exposomics Targeting over 200 Toxicants and Key Biomarkers at the Picomolar Level

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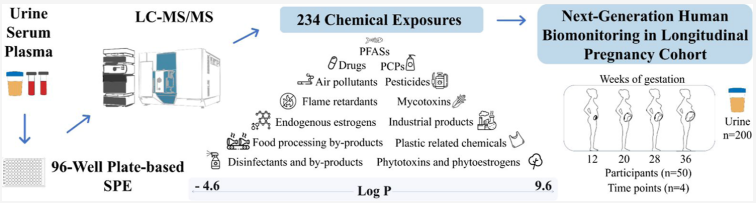

ABSTRACT: The exposome encompasses environmental exposures throughout life and significantly impacts health and disease. Exposure chemicals, present at trace levels, are frequently quantified using targeted LC−MS/MS. Many existing methods are limited to a narrow range of analyte classes or lack sufficient sensitivity for exposomic analyses, and applicability to large sample cohorts for exposome-wide association studies (ExWAS) remains to be demonstrated. Here, we present a scalable, fit-for-purpose next-generation human biomonitoring (HBM) workflow for analyzing >230 biomarkers in urine, plasma, and serum using solid-phase extraction in 96-well plates and LC−MS/MS. Moreover, a complementary conceptual framework for validation criteria of assays designed to analyze large panels of highly diverse compounds at trace levels is proposed. Method robustness was evaluated, demonstrating extraction recovery (60−130%), matrix effects (SSE, 60−130%), inter-/intraday precision (RSD <30%), and high sensitivity (limit of detection <0.1 ng/mL) for 59−80% of the analytes across the investigated biological matrices. To showcase the method's applicability in epidemiological studies, 200 urine samples from pregnant women in a longitudinal pregnancy cohort were analyzed. More than 100 analytes including PFAS, drugs, air pollutants, pesticides, flame retardants, mycotoxins, industrial products, food processing contaminants, plastics-related chemicals, and phytotoxins, were detected, several for the first time in a U.S. urinary biomonitoring study. With its broad analyte coverage, ultimate sensitivity, robustness, and high sample throughput, this method meets the performance requirements for future large-scale ExWAS applications in public and personalized prevention research.

KEYWORDS: *next-generation human biomonitoring, mass spectrometry, early life chemical exposure, exposome-wide association studies (ExWAS), public and environmental health*

INTRODUCTION

Individuals are exposed to a plethora of chemicals through dietary intake, inhalation, dermal contact, and other environmental sources. Exposomics comprehensively assesses these exposures and identifies potential links to adverse health effects, including chronic diseases or cancer.[1−3] Liquid chromatography coupled to mass spectrometry, encompassing low-resolution mass spectrometry (LC−MS/MS) and high-resolution MS (LC-HRMS), is currently the most relevant technique in exposome-wide association studies (ExWAS) for targeted and nontargeted analysis (NTA).[4−6] Targeted assays focus on quantifying predefined compounds with high sensitivity, selectivity, accuracy, and precision,[7,8] while NTA aims to identify unknown chemicals and discover novel or unexpected exposures.[9] Multianalyte targeted LC−MS/MS methods are ideal for quantifying various exposure chemicals at trace-levels due to favorable method sensitivity and robustness.[10,11]

Suitable sample preparation protocols are essential as sample matrix components can impact signal intensities, particularly for trace-level compounds.[12] Various extraction protocols, including liquid extraction, protein precipitation (PPT), and solid phase extraction (SPE), are commonly used for diverse human matrices. PPT is widely used across omics studies due to its broad analyte coverage,[7,13,14] whereas SPE is traditionally optimized for a smaller number of analytes classes. Broad

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analyte coverage is critical for multiclass analysis, but SPE protocols for diverse chemical classes remain rare due to varying analyte−sorbent interactions. Yet, SPE is increasingly popular in exposomics due to its efficient cleanup capabilities for complex samples.[15] Recently, we developed a generic SPE protocol for 94 exposure compounds with diverse physicochemical properties and highlighted its applicability to targeted and nontargeted exposomics.[16]

To ensure suitable data quality, rigorous method validation is essential. The European Commission guideline No. 2021/808 provides systematic validation criteria (referred to as "EC criteria") in terms of trueness, precision, and repeatability.[17] However, the EC criteria and those of other guidelines may have limited applicability for exposome-scale LC−MS/MS approaches. Such methods can target hundreds of analytes in a single run, and workflow complexity requires a delicate balance between analytical coverage and method performance, e.g., due to a limited duty cycle that hampers data quality when targeting hundreds of analytes in a single run. Additionally, concentrations of most exposures are very low in human matrices, typically with concentrations in the pico- and nanomolar range. The EC criteria do not account for a higher number of diverse analytes and low concentrations. For instance, clear criteria for assessing precision for analytes below 120 μg/kg have not yet been established. Therefore, tailored validation criteria based on systematically evaluated empirical data may be a helpful addition to extend existing validation schemes for fit-for-purpose exposomic assays. Finally, applicability for large-scale studies should be tested to showcase the methods' robustness, especially for populations with low-level exposures like pregnant women.[10,18−20]

In the presented work, an LC−MS/MS method was developed and validated for a high number of exposures, covering a wide range of physicochemical properties. For example, the analyte panel covered a log P range of −4.6 to 9.6. The method achieved detection limits down to the picomolar range and incorporated a 96-well plate-based SPE protocol for clean-up of human urine, plasma, and serum. Based on empirical data from relevant literature and generated within this study, a conceptual validation framework for exposome-scale methods is proposed as a potential complement to existing criteria. Fitness-for-purpose was assessed using the new approach and EC criteria for comparison purposes. The obtained validation results enabled the classification of analytes into fully quantitative, semiquantitative, or qualitative data. To assess the assay feasibility in a real-world exposure study, it was applied to 200 urine samples obtained from 50 pregnant US females during gestation (12th, 20th, 28th, and 36th week).

MATERIALS AND METHODS

Chemicals and Materials. The diverse analyte panel consisted of 234 compounds and was developed based on previous work.[7,12,21,22] Furthermore, 58 additional endocrine-disrupting chemicals (EDCs) were selected based on relevant databases, i.e., compounds listed by the U.S. Environmental Protection Agency (EPA), European Human Biomonitoring Initiative (HBM4 EU), Comparative Toxicogenomics Database (CTD), and selected human biomonitoring (HBM) studies (see Table S1). In total, this panel covered 13 classes of compounds including perfluorinated alkylated substances (PFAS), medical drugs, personal care products, air pollutants, pesticides, flame retardants, mycotoxins, industrial products, food processing byproducts, plastics-related chemicals, disinfectants and byproducts, phytotoxins/-estrogens, and endogenous estrogens. Working stock solutions of all analytes (referred to as "STD mix") were created by diluting individual stock solutions with acetonitrile.

The following isotope-labeled chemicals were used as internal standards: $^{13}C_{12}$-bisphenol A, $^{13}C_6$-butylparaben, $^{13}C_{15}$-deoxynivalenol, $^{2}H_3$-erythromycin, $^{13}C_6$-ethylparaben, $^{13}C_2$-monobutyl phthalate, $^{13}C_6$-methylparaben, $^{13}C_8$-perfluorooctanoic acid, $^{13}C_8$-perfluorooctanesulfonic acid, $^{13}C_6$-propylparaben, and $^{13}C_{18}$-zearalenone. To account for variations in the SPE process, $^{13}C_{18}$-zearalenone (100 ng/mL) was added before sample preparation, while the other internal standards (referred to as the "ISTD mix") were added after SPE to track instrumental performance and matrix effects. Respective concentrations are provided in Table S2 and product numbers and suppliers are listed in Table S3. Oasis PRiME HLB 96-well plates (30 mg, 2 mL) were from Waters (Vienna). Further details regarding the preparation of stocks, mixtures, solvents, and spiking process are provided in the Supporting Information.

Samples. Urine samples (n = 200) were collected from 50 pregnant women in Connecticut (U.S.) participating in the Yale Pregnancy Outcome Prediction Study (YPOPS). Participants self-collected samples at gestational weeks (GW) 12, 20, 28, and 36 during a single-day visit. These time points were selected since the 12th, 28th, and 36th GWs mark the end of each trimester and the 20th GW was when the routine fetal anatomy scan was performed. Urine was collected into sterile cups, kept on ice, and sent to a collection center, where 1.5 mL aliquots were transferred to cryovials, stored at −80 °C, and shipped on dry ice to the Global Exposomics and Biomonitoring Laboratory at the University of Vienna for analysis. Previously, these samples have been analyzed for biomarkers of mycotoxin exposure.[10] The study was approved by the Yale University Human Investigation Committee (#1601017004), and samples were banked by the Yale University Reproductive Sciences (YURS) Biobank (HIC #1309012696).

Pooled urine, plasma, and serum samples were used for the method development and validation. The pooled urine was collected from a female volunteer following a two-day period of abstaining from foodstuff and beverages stored in plastic containers, phytoestrogen-rich foods, and cosmetics containing parabens.[12] Pooled plasma was from Innovative Research (IPLAWBLIH-31982) (Novi, MI, USA), and serum was from Sigma-Aldrich (H4522, Sigma-Aldrich, Germany). Details of these commercial products are given in the Supporting Information. All samples were aliquoted and stored at −80 °C until analysis.

Sample Preparation. A recently optimized SPE protocol utilizing 96-well plates was applied.[16] In short, SPE plates were conditioned with methanol (MeOH) and water (H_2O). Subsequently, 400 μL of sample was mixed with 4 μL of $^{13}C_{18}$-zearalenone (100 ng/mL) and diluted with 396 μL of phosphate-buffered saline (PBS) before loading onto the SPE plates. Plates were washed with H_2O and eluted with 2 × 200 μL of MeOH. Four microliters of the ISTD mix were added, and extracts were diluted with 396 μL of H_2O, resulting in a total volume of 800 μL. Feasibility testing of the SPE protocol is visualized in Figure S1. In case of enzymatic hydrolysis tested during validation, a subset of urine samples was treated with β-glucuronidase and arylsulfatase (details provided in the

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Supporting Information section "Sample Preparation", Enzyme Hydrolysis for Urine Prior to SPE Process).

UHPLC−MS/MS. LC separation was performed using a generic reversed-phase method,[7,12] with parameters presented in the Supporting Information and Table S4. Briefly, an Agilent 1290 Infinity II UPLC system was equipped with an Acquity HSS T3 column (1.8 μm, 2.1 × 100 mm) and a VanGuard precolumn (1.8 μm), both purchased from Waters. Retention times (RTs) for single compounds and potential RT shifts were determined by injecting standard solutions of single compounds or compound mixtures using concentrations of 0.5, 1, and 3 ng/mL.

During the optimization of the multiple reaction monitoring (MRM) method, transitions for targeted analytes were tuned individually, including precursor ion (Q1) and product ion (Q3) selection, optimization of entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP). Values of Q1, Q3, EP, and CE were optimized on a QTRAP 7500 instrument (Sciex, Vienna) using method parameters of previously published methods as basis if available.[7,12,21,22] MRM parameters for all additional analytes were optimized using direct infusion of standard solutions (5−10 ng/mL) with a syringe pump at a flow rate of 7 μL/min. Analytes were introduced either as mixtures or as individual solutions (Table S5). Poorly ionizing compounds were reoptimized individually. Two fragment ions were selected per compound as quantifier and qualifier ions. To ensure optimum data quality, dwell times were optimized for all targets to ensure enough data points per chromatographic peak. Fast polarity switching was used in scheduled MRM mode. More details, including ion source parameters and MRM transitions, are listed in Table S6.

Quality Control, Quantification, and Statistics. A system suitability test was performed before and after each analytical sequence to track the instrument performance (Tables S7−S9). Multianalyte external calibration solutions were prepared in 50% MeOH in H_2O at nine concentration levels (Table S10). Linear range and coefficient of determination (R^2) are reported in Table S11. Water was used for preparation of process blanks, and 50% MeOH was used as solvent blank to control possible background contamination and carryover. Three types of quality control (QC) samples were used including pooled samples ("nonspiked QC") and pooled samples spiked after extraction ("postspiked QC") and before extraction ("prespiked QC"). Furthermore, 11 isotopically labeled internal standards were used to track method performance during the analysis of 200 urine samples (for details, see Supporting Information). Sciex OS (version 4.1, Sciex, Vienna) was used to integrate peaks using the MQ4 algorithm and to build calibration curves for quantification (Supporting Information, Tables S10 and S11). Further data evaluation and visualization was done in Microsoft Excel (v16.0; Microsoft Office), PowerPoint (v16.0; Microsoft Office), Origin 2021b (v 9.8; OriginLab Corporation), and Inkscape (version 1.2.2; Inkscape). Concentration trends with gestation weeks were analyzed using a Linear Mixed-Effects Model (R lmer function, version 4.5.0). Details are reported in Supporting Information.

In-House Validation. Full in-house validation was performed for urine and plasma matrices, while serum and hydrolyzed urine were subject to a more limited performance evaluation. Following the European Commission Decision (EC) No. 2021/808,[17] performance parameters were evaluated over 3 days, including linearity, selectivity, matrix effects (signal suppression/enhancement, SSE), sensitivity (LOD and LOQ), trueness (extraction recovery, RE), intermediate precision (interday RSD, RSD_R), and repeatability (intraday RSD, RSD_r). Sample preparation, measurements, and data analysis were performed for each batch individually. Method linearity was evaluated by standards in solvent, and selectivity was tested by inspection of pooled urine and plasma samples for interferences. Matrix-matched calibration standards were prepared by spiking urine and plasma extracts at seven levels (Table S12). SSE (%) was calculated as the ratio of slopes of the matrix-matched and solvent calibration and was calculated for three batches.[23] LODs and LOQs were determined by analyzing spiked urine and plasma samples at low concentrations as replicates (m = 9; see Table S13). Method-based LOD and LOQ were calculated based on signal-to-noise ratios of 1/3 and 1/10, respectively, if no background signal was present in the nonspiked matrix sample or, if a background signal was interfering, according to the EURACHEM guideline as 3 and 10 times the standard deviation of the measured concentrations divided by the square root of the number of replicates as described in the Supporting Information.[24]

RE was calculated based on determined concentrations in prespiked samples using matrix-matched calibration. RE was determined for two concentration levels, approximately 3 and 30 x LOQ (n = 3 each, see Table S14). Blank correction was performed by concentrations determined in nonspiked samples (n = 4) for RE calculation. RSD_R and RSD_r were determined as coefficient of variation (CV) of the RE within a single batch (n = 9) and within three validation batches.

To assess the method's applicability across different human sample matrices, native and spiked pooled serum samples were extracted for evaluating trueness, precision, and matrix effects. The influence of enzymatic hydrolysis of urine samples on method performance was investigated by performing deglucuronidation and desulfation prior to SPE extraction and evaluating the trueness, repeatability, matrix effects, and sensitivity. The full validation details are provided in the Supporting Information, Tables S14 and S15.

Validation Criteria for Trueness, Precision, and Repeatability Based on EC Guidelines. The EC criteria specify performance criteria for analytical method validation for a wide range of analyte concentrations. Acceptable ranges for trueness in terms of RE depend on the spiked concentrations. For concentrations >10 μg/kg, RE should be between 80% and 120%, 70−120% is acceptable for concentrations between 1 μg/kg and 10 μg/kg, whereas acceptable REs are in the range of 50−120% for concentrations <1 μg/kg. In analogy, acceptable values for RSD_R and RSD_r, determined as CV of RE, are also concentration-dependent. Following the guideline, CVs <16% are required for concentrations >1000 μg/kg, and CV <22% is required for concentrations between 120 μg/kg and 1000 μg/kg. For concentrations <120 μg/kg, the guideline recommends minimizing CVs for both RSD_r and RSD_R to the lowest technically feasible level. To improve the comparability of mentioned concentration ranges with our data, we converted μg/kg to ng/mL. This conversion was performed by applying a multiplication factor of 1.0 g/mL, which approximates the densities of human urine and plasma (details in Table S16).

Complementary Conceptual Framework for Validation Criteria in Exposome-Scale Studies. While current EC criteria provide the criteria for trueness, precision, and

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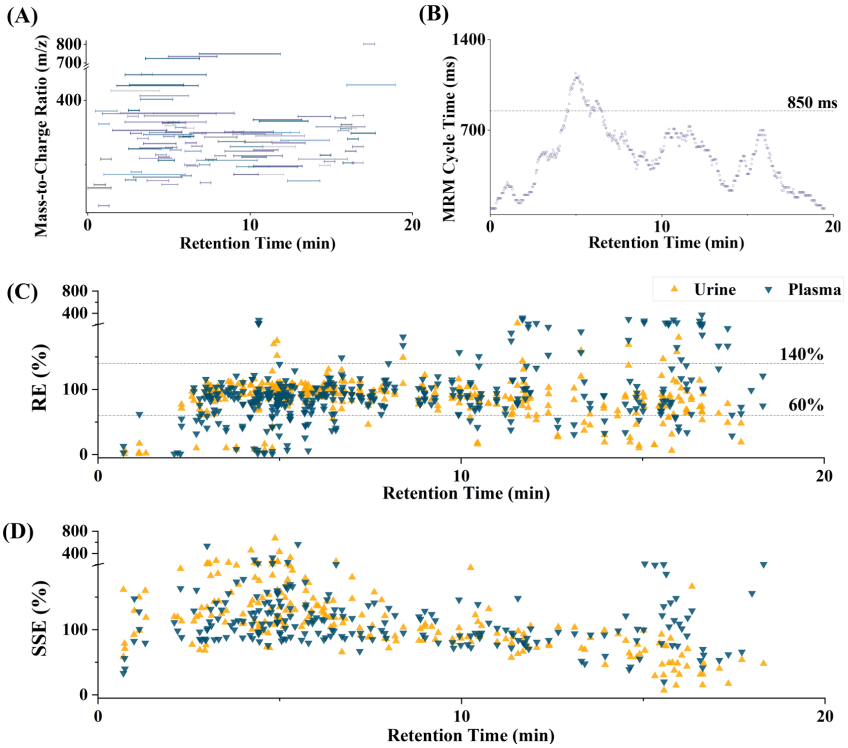

Figure 1. Scheduled Multiple Reaction Monitoring (MRM) windows (A) and MRM cycle time (ms, B) as a function of the chromatographic retention time (RT, min) of the optimized LC−MS/MS method. Extraction recovery RE (%, C) and signal suppression and enhancement SSE (%, D) in dependence of RTs for analytes in urine and plasma. Four analytes (2,5-dichlorophenol, xanthohumol, bisphenol M, and triclosan) are not shown in panel (C) due to high RE caused by interferences and/or matrix effects.

repeatability for analyte concentrations >120 ng/mL, a comprehensive framework for trace analysis in exposomic-scale investigations is currently missing. However, the high number of analytes per assay and the required ultimate method sensitivity in the pg-ng/mL range warrant tailored adjustment of analytical figures of merit. To address this challenge, we propose novel tailored validation criteria based on empirical data. Validation criteria for exposomics were developed using published validation data of exposomics assays for large analyte panels (i.e., >90 analytes) covering a wide range of physicochemical properties (i.e., covering a log *P* range of >5) and results from our experiments as a rational basis (see Table S17).[7,13] Subsequently, the expected ranges for key parameters including RE, RSD_R, and RSD_r values were derived as 5th and 95th percentiles of reported data and were tested as new criteria for assessment and comparison in exposomic-type LC−MS investigations. RSD_R and RSD_r values were derived as the 95th percentile.

RESULTS AND DISCUSSION

LC−MS Method Optimization. To ensure favorable method performance, RT windows and dwell times for all scheduled MRM transitions were optimized based on observed peak widths and RT shifts in biological matrices. Optimization of window width and dwell times is essential to balance the total cycle time, number of data points per peak, RT stability, and method sensitivity. Based on empirical observation of chromatographic peak widths, method development aimed for a total cycle time <850 ms, which could be achieved for the majority of all analytes (see Figure 1B). Due to unstable RTs in matrix samples, diethyl dithiophosphate, ibuprofen, perfluorodecanoic acid, and perfluorononanoic acid, as well as six phthalates were monitored during the entire runtime. After method optimization, the number of data points per peak was investigated in spiked samples (n = 3) to ensure sufficient data quality for quantification, with 91% of the analytes having >7 data points per peak (Table S18 and Figure S2), which can be regarded as sufficient for quantification.[25]

Development of Tailored Validation Criteria for Exposomics. Several different guidelines for analytical method validation provide valuable frameworks and criteria for method validation in diverse applications areas. The choice of the used guideline and criteria typically depends on the specific research area. Among them, the EC guidelines provide a comprehensive framework for method validation for various application fields, yet several key challenges inherent to exposome-scale LC−MS/MS analysis are not addressed. Particularly, the methodological complexity and sensitivity requirements necessary for detecting a large range of diverse analyte mixtures at trace levels in a single assay are not fully

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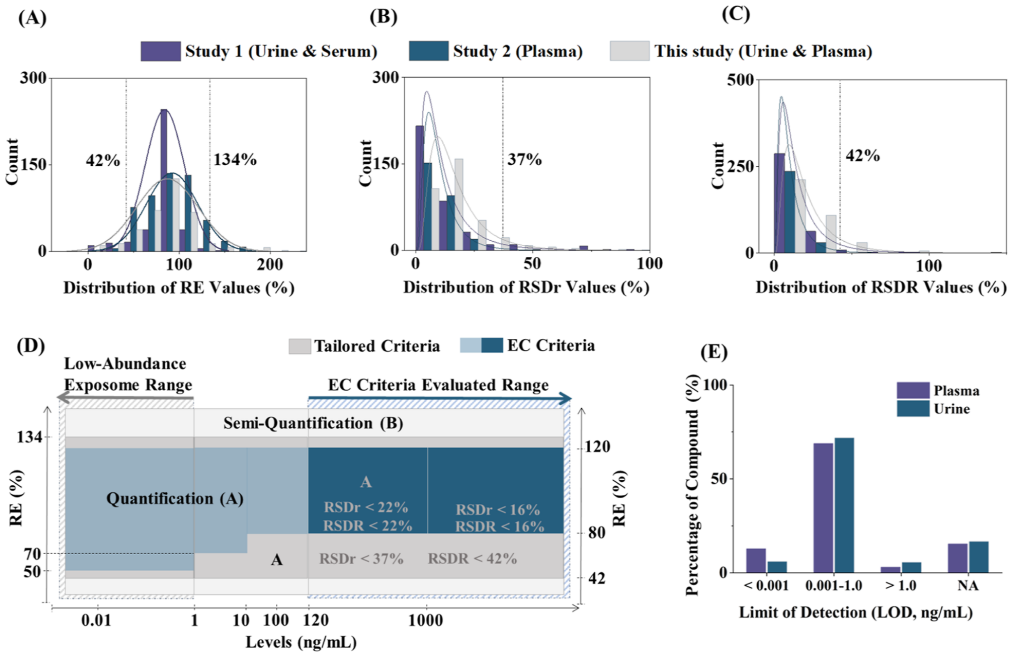

Figure 2. Distribution of extraction recovery RE (%, A), repeatability RSD_r (%, B), and precision RSD_R (%, C) reported in two published exposome-scale targeted LC−MS/MS studies[7,13] and data generated in this study. Empirical data was used to derive tailored validation criteria for exposome-type method validation to complement and extend EC criteria (EC No. 2021/808)[17] for low-abundance analyte concentrations in multianalyte assays (D). Panel (E) shows the distribution of the method-based LOD values in the present study. NA, not applicable with explanations reported in Table S20.

addressed. To fill this gap, we developed a tailored framework for validation criteria for exposomics based on published data sets of exposomics-scale LC−MS/MS methods and our own data set presented as part of this work. Therefore, we included methods quantifying >90 analytes from different analyte classes with a broad log *P* range >5 (Table S19). These proposed novel in-house validation criteria for exposomics-scale multianalyte assays were derived from empirical data using the 5th and 95th percentiles of the compiled data sets. Figure 2A−C show the distribution of RE and RSD reported in two published methods[7,13] and results from the study at hand, indicating largely comparable results between the included data sets. The 5th and 95th percentiles of RE were calculated as 42% and 134%, respectively, highlighting certain limitations in terms of analyte recovery for currently used LC−MS workflows in the case of sets of extremely diverse analytes at trace concentrations. These empirically determined limits were subsequently proposed as the acceptable range for RE in quantitative exposomics (Figure 2A), whereas the 95th percentile was used as the upper limit for assay repeatability (RSD_r <37%) and intermediate precision (RSD_R <42%) (Figure 2B,C). These custom validation criteria were then used to complement the EC criteria for the relevant concentration range in exposome research (i.e., <1 ng/mL) and were integrated into our proposed complementary method validation framework (see Figure 2D and Table S16). Figure 2 illustrates a schematic representation of the criteria provided in the EC guidelines and proposed new data-derived criteria for exposomics including relevant concentration ranges. Furthermore, a simple three-level scheme is suggested to categorize quantitation confidence for the targeted analytes based on validation results: quantitative data is reported as data quality category A, semiquantitative data as category B, and qualitative data as category C. Detailed definitions for A, B, and C are described below (section Classification of Data Quality in Quantitative Exposomics). We see this framework as a starting point to spark a discussion within the field concerning "fit-for-purpose" approaches rather than a finalized set of criteria. It is not intended to replace established standard guidelines but to complement them.

Results of the In-House Validation. Excellent method linearity was achieved, with 88% of compounds achieving R^2 values exceeding 0.99 in pure solvents (Table S11). Sensitivity was also excellent for the majority of analytes with LOD values <1 ng/mL observed for 73% of compounds in urine and 74% in plasma. Approximately 10% of the analytes showed exceptionally low LODs (<0.001 ng/mL) in both sample matrices (Figure 2E). For approximately 70% of the analytes, RE values were in the range of 42−134% in both sample matrices (Figure 3A), and 80% and 77% of the analytes showed RSD_r <37% in plasma and urine, respectively. Notably, 63% displayed RSD_r values below 20% (Figure 3B). RSD_R was determined, with 67% of all compounds showing RSD_R <37% and 38% and 57% of compounds showing RSD_R <20% in plasma and urine, respectively (Figure 3C). Matrix effects were evaluated, and effective sample cleanup with SPE was

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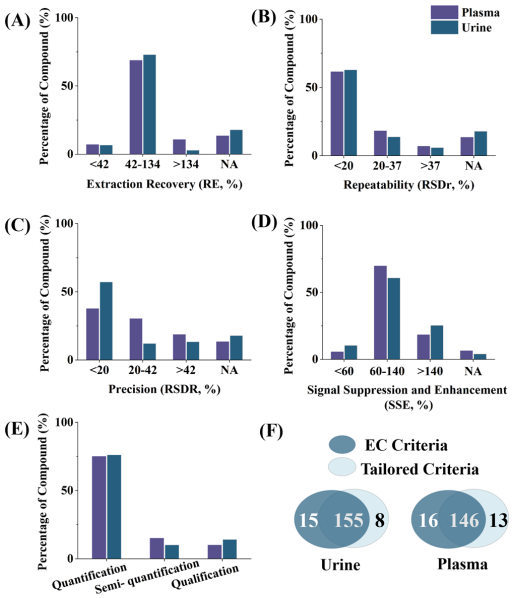

Figure 3. Results of the in-house method validation: analytes with RE (%, A), RSD_r (%, B), RSD_R (%, C), and SSE (%, D) within the range required for quantitative exposomics. Classified validation results following EC criteria[17] and proposed tailored criteria using three categories: quantification, semiquantification, and qualification (E). Number of analytes classified as "quantification" for at least one spiking level according to the EC criteria and/or the tailored criteria (F). NA, and applicable data with explanations reported in Tables S14 and S20.

demonstrated for many analytes, as previously reported for a smaller subset of the analyte panel.[16] In total, 61% of the analytes in urine and 70% in plasma showed SSE within 60−140% (Figure 3D). For compounds with SSE values outside the 60−140% range in both, urine and plasma, distinct trends emerged: some polar compounds (including specific drugs, mycotoxins, pesticides, and phytotoxins and phytoestrogens), typically with log *P* below 3.0, exhibited matrix enhancement (SSE > 140%), while some nonpolar chemicals (e.g., personal care products), primarily with log *P* above 3.0, showed matrix suppression (SSE < 60%). Full validation results are provided in Tables S14 and S20. For serum and hydrolyzed urine, validation results are presented in the Supporting Information (Figure S3 and Tables S14 and S20).

Classification of Data Quality in Quantitative Exposomics. To be in line with the EC criteria, analytes need to meet the following requirements for at least one spiking level to be reported as quantitative data (category A): (1) RE within 50−120% for concentrations ≤1 ng/mL, between 70% and 120% for concentrations between 1−10 ng/mL, and 80−120% for concentrations between 10 and 120 ng/mL or higher. (2) RSD_R and $RSD_r \leq 22\%$ are required for concentrations within 120−1000 ng/mL, RSD_R and $RSD_r \leq 16\%$ for concentrations >1000 ng/mL, whereas no clear limit is defined for RSD_R and RSD_r for compounds at trace and ultratrace levels (see Figure 2D). Using the proposed tailored criteria, data was reported as quantitative if the following requirements were met for at least one spiking level: (1) RE in the range of 42−134% and (2) $RSD_r \leq 37\%$ and $RSD_R \leq 42\%$ (Figure 2D). To implement a clear scheme for reporting quantitative, semiquantitative, and qualitative data in exposomics, two additional categories were introduced for the classification of the data quality: data was classified as semiquantitative (category B) if either RE or RSD values failed to meet quality criteria for quantitative data described above. Qualitative data (category C) was reported for compounds with stable RT, but accuracy and repeatability could not be evaluated due to poor sensitivity or high background. More details are listed in Table S14.

Figure 3F illustrates the number of quantitative analytes meeting the EC criteria and/or the tailored criteria. Using the EC criteria, 170 analytes were classified as "quantitative" in urine, whereas using the tailored criteria results in 163 analytes. In plasma, the EC criteria resulted in 162 quantifiable analytes, compared to 159 using our tailored criteria. Noteworthily, most analytes (155 in urine and 146 in plasma) met the quantification requirements of both validation schemes. A subset of compounds (15 in urine and 16 in plasma) met quantification category A under the EC criteria, yet were classified as category B when using the tailored criteria. This difference reflects the EC criteria's greater tolerance for poor precision and repeatability at extremely low analyte concentrations. In contrast, another subset of analytes (8 in urine and 13 in plasma) was classified as semiquantitative (category B) using EC criteria but as quantitative (category A) using the tailored classification scheme due to a higher tolerance in regards of RE. Here, the tailored criteria allow higher tolerance for trueness, due to the complexity of large-scale exposome-scale assay. This takes into consideration that correction for "trueness" (i.e., lower RE) is often possible if the method's precision is acceptable and a suitable correction approach (e.g., using internal standards or quality control samples) is used. Given the complementary nature of both approaches and the complex nature of large-scale multianalyte LC−MS/MS assays, compounds meeting the quality criteria of either of the validation schemes were considered as suitable for quantification. In total, 76% and 75% of all analytes met the requirements for quantification (category A) in urine and plasma, respectively. Another 10% and 15% of all analytes met the requirements for semiquantification (category B), whereas the remaining 14% and 10% can only be reported as qualitative data (i.e., detected and nondetected, category C) in urine and plasma, respectively (see Figure 3E).

Benchmarking Coverage, Sensitivity, and Throughput. The presented validated exposomics workflow allows analyzing diverse exposure chemicals covering a wide range of polarities and concentrations. Method performance and coverage were compared to three relevant published LC−MS/MS protocols for benchmarking (Table S17). Noteworthily, the presented method covers the widest range of analyte polarities and achieved comparable sensitivity for the majority of analytes (Figure 4). Especially, the fraction of extremely sensitive analytes (LOQ < 0.0033 ng/mL) was approximately two times higher than in previously published studies (see Figure 4B).[7,13,26]

While Huang et al. (2023)[13] presented a method with more analytes, the presented workflow (n = 234) offers the most diverse set in terms of analyte log *P*. This is a critical metric for demonstrating sufficient coverage and scalability potential for exposomics applications, highlighting its competitive edge among state-of-the-art LC−MS/MS-based exposomics work-

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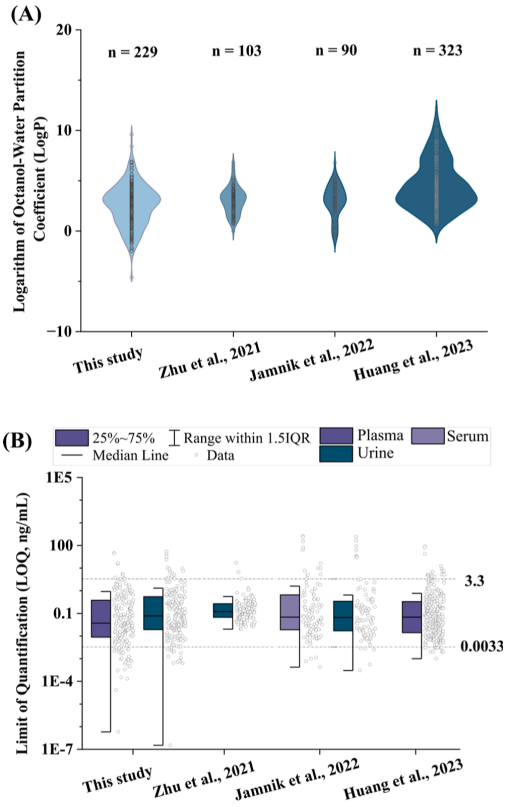

Figure 4. (A) Coverage of the chemical space of multianalyte exposomics assays in terms of logarithm of octanol−water partition coefficient (log *P*) values for analytes in this study (n = 229) and three published papers: Zhu et al. (2021; n = 103)[26], Jamnik et al. (2022; n = 90)[7], and Huang et al. (2023; n = 323) [13]. (B) Limit of quantification (LOQ, ng/mL) for analytes in plasma (n = 209) and urine (n = 206) in this study, in urine (n = 114) in Zhu et al. (2021)[26], serum (n = 92) and urine (n = 87) in Jamnik et al. (2022)[7], and plasma (n = 295) in Huang et al. (2023)[13]. Note: Due to the lack of reported LOQ values in Zhu et al. (2021)[26], we estimated LOQs as 3.33 x the reported LODs in (B). Log *P* values listed in PubChem (https://pubchem.ncbi.nlm.nih.gov) were used for panel (A). IQR, interquartile range.

flows (Figure 4A).[7,13,26] Assay sensitivity was benchmarked by comparing method LOQs against relevant literature, focusing on analytes with good sensitivity (LOQs < 3.3 ng/mL). While the overall number of such analytes was comparable to other assays, our method demonstrated outstanding sensitivity for 6−13% of compounds, achieving LOQs < 0.0033 ng/mL in both urine and plasma. In contrast, other published work reported comparable LOQs for only 4−7% of analytes (Figure 4B and Table S17).

The applicability of the developed method for high-throughput exposomics was assessed and compared to other protocols in terms of sample requirements, solvent consumption, and total analysis time (Table S21). Using the SPE method presented herein, 1.4 mL of solvents are required per sample, compared to 0.8 mL estimated for a previously published PPT protocol.[7] Similar calculations were performed for other published works, resulting in even higher solvent consumption of approximately 6 mL per sample calculated for the sample preparation protocol of Huang et al. (2023).[13] While these estimations include some uncertainties, this further highlights the high efficiency of our workflow, which would result in a theoretical reduction of 4.6 L solvents calculated for the extraction of 1000 samples. Finally, sample preparation in 96-well plates is time efficient. It was estimated that the total required laboratory time of ∼20 h for sample preparation 1000 samples including SPE cleanup is sufficient, whereas the same number of samples would need ∼175 h of laboratory work using a traditional PPT protocols in Eppendorf tubes.[7,16] This estimate intentionally excludes factors such as injection time and data processing, as these are highly variable and depend on the specific instruments and software used.

Real-Life Exposure Patterns in Pregnant Women from the YPOPS Cohort. To demonstrate favorable sensitivity and general performance of the new workflow and its applicability to real-life research questions, 200 urine samples of women (n = 50) from a U.S. pregnancy cohort were analyzed. Pregnant women present a highly vulnerable population group, and expected exposure levels are typically lower than in the average population. Yet, 109 out of 234 target analytes were detected, with 25 exposure compounds present with very high detection frequencies (DF) above 70% (Figure 5). The detected and quantified biomarkers of exposure included chemicals from various exposure routes, application fields, and compound classes including antibiotics, mycotoxins, medical drugs, pesticides, personal care products, phytotoxins, phytoestrogens, plasticizers, and PFAS chemicals. Examples for Selected Toxicants are Briefly Discussed Below. The full data set including the classification of the data quality based on the obtained validation data is presented in Table S22. This table provides an overview of all of the compounds. A more detailed breakdown of the results according to quantitation categories are reported in Tables S22a−c: S22a for quantified compounds; S22b for semiquantified analytes; and S22c for qualified compounds in cohort samples. The detailed results are listed in Table S23. For instance, urinary cotinine, a biomarker for active or passive cigarette smoke, was determined in 46% of the samples (median concentration < 0.15 ng/mL, range < 0.15 ng/mL − 23 ng/mL), demonstrating again the high sensitivity (LOD, 0.03 ng/mL). In analogy, *trans*-3-hydroxycotinine, a phase-I-metabolite of cotinine, was quantified in 85% of the samples at a lower median concentration (median 0.053 ng/mL, range < 0.018 ng/mL − 25 ng/mL). Importantly, these levels typically indicate environmental/passive exposure to cigarette smoke rather than active smoking.

Several PFAS chemicals were detected including perfluorooctanoic acid (PFOA; median 0.024 ng/mL, range < 0.0071 ng/mL − 0.12 ng/mL) in 66% of the samples. Ten out of 12 monitored bisphenol derivatives or metabolites were quantified in the sample set. As expected, the BPA phase-II metabolite, BPA-glucuronide, was the most frequently detected analyte of this class. However, data quality allows only qualitative assessment of this analyte in the presented data set (data quality category C—qualitative results). The parent molecule BPA was less frequently detected due to a higher LOD value.

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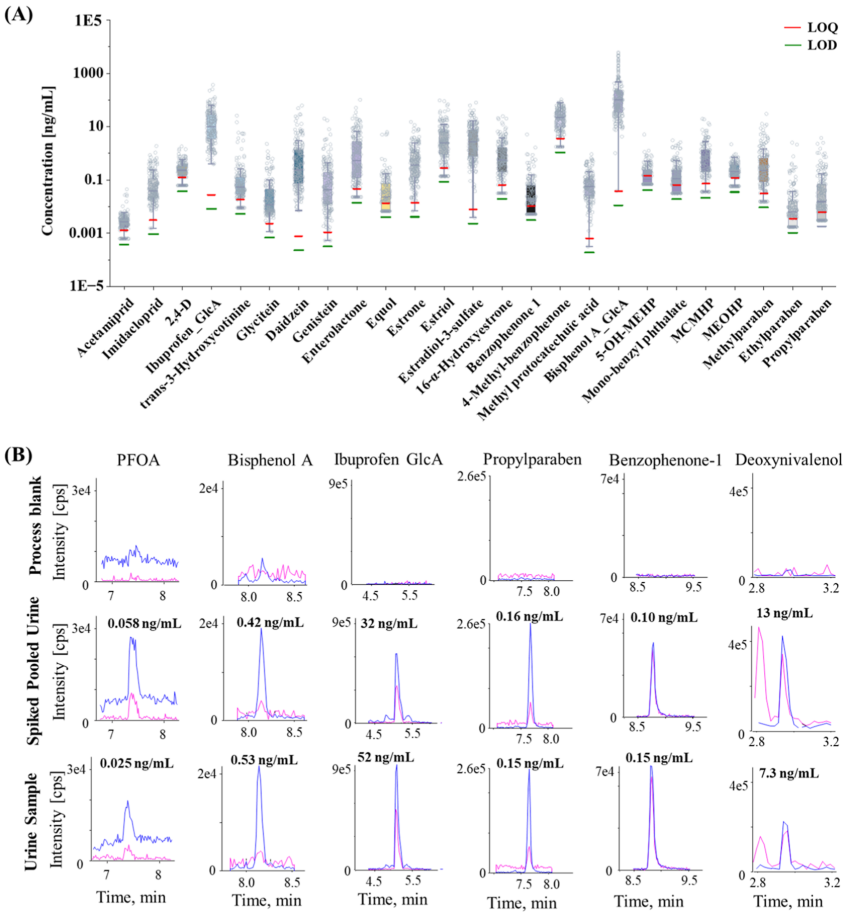

Figure 5. (A) Concentrations of 25 frequently detected compounds (DF > 70%) in urine samples from pregnant women in the YPOPS cohort. (B) Representative chromatograms of compounds in process blank, spiked pooled urine, and a detected sample from the cohort. Note: Concentrations below the limit of quantification (LOQ) were imputed with LOQ/2. 2,4-D, 2,4 dichlorphenoxyacetic acid; GlcA, glucuronide; 5-OH-MEHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MCMHP, mono-[(2-carboxymethyl) hexyl] phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate.

BPF was also frequently detected in 62% of the samples (median 0.44 ng/mL, range <0.12 ng/mL − 1.5 ng/mL).

The pesticides acetamiprid, imidacloprid (Figure 5), the pesticide metabolite 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), and 2,4-dichlorophenoxyacetic acid (2,4-D) were detected in most samples. The results were compared to other published data sets of pesticide exposure in pregnant women from Asia and Europe. For acetamiprid and imidacloprid, DFs of 89% and 98% were observed in our study, with concentration levels ranging between <0.0012 − 0.044 ng/mL and 0.0031 − 1.8 ng/mL, respectively. Lower DFs (18% and 26%) and comparable urinary concentrations (<0.0025 ng/mL − 1.3 ng/mL and <0.025 ng/mL − 0.95 ng/mL) were reported for 617 urine samples collected from 62 pregnant women in Japan.[27] Similarly, IMPY, which is a key metabolite of the organophosphate pesticide diazinon, was detected at concentration levels between <0.023 ng/mL and 2.1 ng/mL (DF = 25%) in the present study. In a study from Spain, IMPY was present at concentrations between <1.6 ng/mL and 744.2 ng/mL and was detected in 12% of urine samples from pregnant women (n = 573) in Valencia.[28] 2,4-D was detected with a concentration range of < 0.12 ng/mL − 1.8 ng/mL for most samples (DF = 99%) in our study. In data sets of the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2014, 2,4-D was reported at concentrations of 0.010 ng/mL − 18 ng/mL with lower DF (75%) for urine samples collected from pregnant women (n = 398) in the United States.[29]

The data obtained from this high-performance multianalyte assay also demonstrated proper performance when comparing it to a more tailored HBM assay covering multiple mycotoxins that was applied to the same set of sample before.[10] The most concentrated and prevalent fungal toxin, deoxynivalenol (DON) was determined in 42% of the samples with a median concentration of <3.5 ng/mL (Table S22), notably in its native form. The median in the tailored HBM assay that measured

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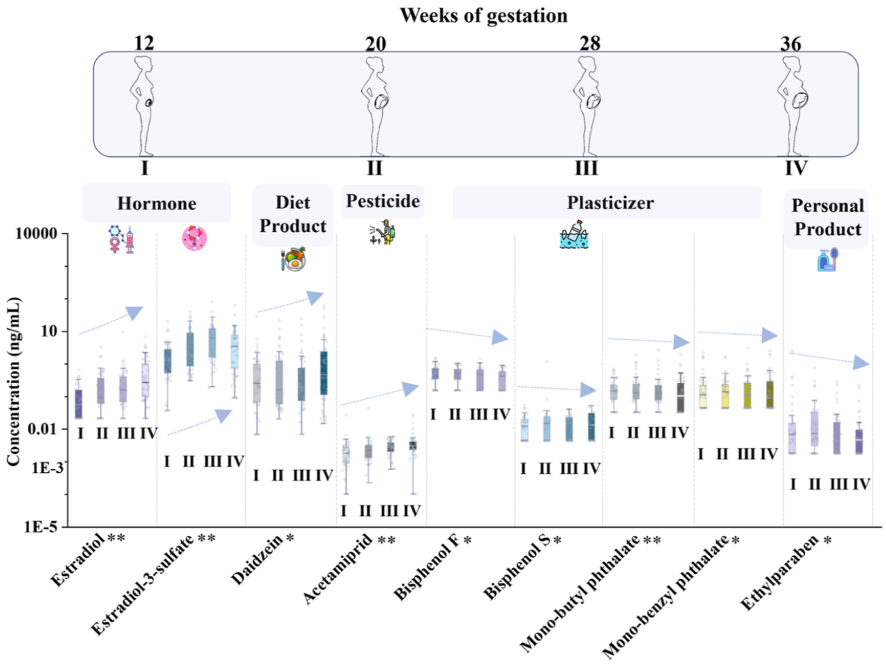

Figure 6. Longitudinal concentration profiles of frequently detected compounds (DF > 50%) in urine samples obtained from 50 pregnant females with significant trends ($p < 0.05$) across four gestational periods in the Yale Pregnancy Outcome Prediction Study (YPOPS). Here, concentrations below the limit of quantification (LOQ) were replaced with a value of LOQ/2, and concentrations below limit of detection (LOD) are not shown. *$p < 0.05$; **$p < 0.01$. The indicated trends need to be interpreted with caution, and it needs to be considered that other exposures were stable throughout pregnancy.

total deoxynivalenol after enzymatic deconjugation of glucuronide and sulfate conjugates was reported as 23 ng/mL (DF = 99%, 0.95 ng/mL – 436 ng/mL).[10] Given the fact that the vast majority of deoxynivalenol is present conjugated in human urine,[30,31] this demonstrated comparable performance of the new assay for the assessment of DON exposure. The same is true for the *Alternaria* toxin alternariol (DF = 14%), in line with the quantitative results in a cohort of primary school children in Austria (DF = 8%).[32] For citrinin, a nephrotoxic mycotoxin, the sensitivity of the presented assay was even enhanced so that a low number of samples indicated exposure while the previously published, tailored HBM assay was unable to detect this food contaminant in urine. The same was true of enniatin B, a Fusarium toxin, which was quantified in one sample (0.0064 ng/mL). Beauvericin was detected (DF = 2%) in human urine biomonitoring for the first time to the best of our knowledge (Table S22).

In addition, many other natural toxins and bioactives have been identified and quantified. Some of them are detected in the urine from U.S. citizens for the first time to the best of our knowledge, while others, especially those occurring at higher concentrations, have been reported before.[33,34] The first-time detection of compounds in urine from U.S. citizens includes aristolactam I, a nephrotoxic plant toxin (Table S23).

The exposome is defined as the totality of exposure to the (chemical) environment over the lifespan, and longitudinal studies are of special relevance. In the investigated sample cohort of the YPOPS study, 50 pregnant women were sampled at four time points during pregnancy. Table S24 reports statistically significant trends through gestation weeks. Eleven compounds, comprising nine endogenous hormones, the insecticide acetamiprid, and the phytoestrogen daidzein, showed increasing trends ($p < 0.05$). Conversely, ethylparaben and four plasticizers, including monobutyl phthalate, monobenzyl phthalate, bisphenol F, and bisphenol S, significantly decreased over the same period (Figure 6 and Table S24). However, due to the mild changes and the limited sample number, these results need to be interpreted with caution.

Limitations. While the robustness and sensitivity as well as time and cost-efficiency of the developed workflow were clearly demonstrated, several limitations remain. Although the presented analyte panel was well designed and aims for high diversity and representativeness, still only a limited subset of the chemical exposome can be covered in a single assay. For a comprehensive assessment of the human exposome, even wider chemical coverage, combinations of complementary methods, or more human matrices are required. Furthermore, several very polar compounds (8%) have been lost during the SPE process due to weak retention with the sorbent, and substantial matrix effects were observed for some other analytes (6%). Combinations of different orthogonal sample preparation and analyte separation approaches might address these limitations, yet this is time-consuming and costly. Clearly, broad coverage exposome-type assays need to find a well-balanced compromise between analyte coverage, efficient sample cleanup, assay sensitivity, and time requirements. The

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

overall performance of the method in the real-life case study yielded highly information-rich exposure patterns, indicating that the chosen pragmatic approach is fit-for-purpose. While the insights on longitudinal exposure patterns in pregnant U.S. women are of high novelty, it needs to be noted that the cohort may not be representative for other exposomic applications.

Traditionally used criteria for analytical method validation show limited applicability for exposome research, because of the required high sensitivity and method complexity. Our newly established validation criteria aim to propose a complementary, conceptionally novel, and evidence-based framework to systematically extend established guidelines for analytical method validation to meet the requirements of quantitative exposomics. This is relevant to ensure comparable reporting standards and method performance. Yet, our proposed tailored validation criteria rely on data derived from only a small number of published exposome-scale studies ($n = 3$) due to limited availability of fully validated exposome-scale LC−MS/MS assays. Further integration of additional data sets would be essential before providing a comprehensive framework for method validation in exposomics-scale HBM.

Implications. In summary, a high-performance workflow for exposome analysis characterized by broad chemical coverage and good method sensitivity across diverse human matrices was developed. The analytical pipeline was validated based on existing guidelines and using a conceptionally new and tailored framework of validation criteria designed for exposomics. This is necessary to face typically observed challenges in exposomics such as broad exposome-scale analyte coverage and compounds present at trace concentrations. Method performance was characterized and offers a robust foundation for quantifying numerous priority exposures in population-scale studies. The versatile assay extends analyte coverage beyond traditional food and environmental toxicants and includes biotransformation products, medical drugs, and microbiome-related compounds besides typically monitored toxins and contaminants of emerging concern. Laboratory sample throughput presents one of the critical bottlenecks in large-scale exposomics, and we reduced the required laboratory work by approximately a factor of 10 using SPE in 96-well plates. The scalability of the method was demonstrated and now offers the required capacity to perform large-scale population-based studies for exposome-wide association studies (ExWAS). Such large-scale studies are essential to enhancing our understanding of the chemical exposome, which is needed to improve disease prevention in public health and personalized medicine. Due to the high method sensitivity and sample throughput, the presented validated workflow demonstrated its fitness for purpose to increase our understanding of the chemical exposome and will be used in large-scale population studies. Such environmental health studies will be crucial for unraveling the relationships between (co)exposure to diverse exposure chemicals and human disease in the future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.5c04458.

Detailed descriptions on materials, sample preparation, LC−MS/MS analysis, in-house validation, and assessment for efficacy and environmental impact, results and discussion, and supplementary figures (PDF)

Detailed tables of standards, classifications, concentrations of standards, information on LC−MS/MS parameters, validation results, and detailed results from YPOPS urine samples (XLSX)

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Notes

The authors declare no competing financial interest.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

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Supplementary Information

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7. Conclusions and outlook

Broad analyte coverage, trace-level sensitivity, reduction of interferences from complex biological matrices, and consistency/robustness are the most essential considerations when designing an analytical protocol in exposomics [79, 80, 81]. Frist, we review commonly used sample preparation techniques optimized for specific sample types in omic studies. Although each technique has individual merits that can be tailored to suit specific sample types and analyses, SPE holds the most promise for exposomics research and should be the future of exposome exploration.

Then, we present the development of a high-throughput SPE protocol based on 96-well format for faster, and more robust omic-scale exposure analysis. This workflow was first optimized comprehensively considering diverse targeted 94 analytes using LC-MS/MS, and chemical coverage was further compared with a validated protein precipitation method in nontargeted coverage using LC-HRMS. Laboratory sample throughput, a critical bottleneck in large-scale exposomics, was dramatically improved by a factor of ten through the use of SPE in 96-well plates. Despite reduced method performance for very polar compounds, the comparison of SPE and the protein precipitation workflows clearly indicated that the SPE method is a suitable candidate for large-scale nontargeted or suspect screening applications. This is underlined by the comparable overall performance of the protein precipitation and SPE methods for a wide range of analytes, especially when reversed phase-LC is used as a front-end separation technique.

Leveraging the SPE method, we established a quantitative exposomics LC-MS/MS workflow capable of analyzing 234 compounds with high sensitivity across diverse human matrices. This assay uniquely broadens the scope of exposomic analysis by including biotransformation products, medical drugs, and microbiome-related compounds, extending far beyond traditional food and environmental toxicants. The analytical pipeline was rigorously validated, not only against existing guidelines but also through a novel, tailored framework specifically designed to meet the complex demands of exposomics, such as vast analyte coverage and trace-level detection. This workflow's good scalability, throughput, sensitivity, and robustness were conclusively proven in a US pregnancy cohort study, offering a robust foundation for quantifying numerous priority exposures in population-scale research.

This validated workflow is fit-for-purpose, significantly advancing our understanding of the chemical exposome. Its future application in large-scale population studies will be crucial for improving disease prevention in public health and facilitating personalized medicine.

8. Use of generative AI

In the preparation of this thesis, generative AI tools, specifically Google AI Studio, were utilized as an aid for getting generic information, refining language, correcting grammar and spelling errors, improving overall clarity and conciseness, and translating English abstract to German version. All AI-generated suggestions were meticulously reviewed, edited, and validated by the author to ensure factual accuracy, originality, and complete alignment with the intellectual content and arguments presented. The author retains full responsibility for all scholarly content, interpretations, and conclusions within this work.

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List of Figures

A. Appendix

A.1. List of additional contributions to peer-reviewed publication

Krausová, M., Ayeni, K. I., **Gu, Y.,** Borutzki, Y., O'Bryan, J., Perley, L., Silasi, M., Wisgrill, L., Johnson, C. H., Warth, B. (2024). Longitudinal biomonitoring of mycotoxin exposure during pregnancy in the Yale Pregnancy Outcome Prediction study. Environment International, 109081; doi.org/10.1016/j.envint.2024.109081

Hossain, Z. M., Feuerstein, M. L., **Gu, Y.**, Warth, B. (2024) Scaling up a targeted exposome LC-MS/MS biomonitoring method by incorporating veterinary drugs and pesticides. Analytical and Bioanalytical Chemistry; doi.org/10.1007/s00216-024-05374-x

A.2. List of oral presentations

	JunganalytikerInnen Forum 2023, Leoben, Austria
	Yunyun Gu, Benedikt Warth.
05/2023	*Optimization of A High-throughput SPE Clean-up for Urine and Plasma Exposomics**
	DoSChem Panel B Retreat 2024, Sidonia Castle Hotel, Hungary
	Yunyun Gu , Benedikt Warth.
04/2023	*Optimization of A High-throughput SPE Clean-up for Urine and Plasma Exposomics**
	DoSChem Panel B Retreat 2022, Linz, Austria
	Yunyun Gu, Benedikt Warth.
06/2022	*An Exposome-Scale Sample Clean-up Method Based on Solid Phase Extraction*

*The same abstract and poster were presented due to the near timeframe of these events.

A.3. List of poster presentations

09/2025 **Exposome Austria Retreat 2024**, Horn, Austria
Yunyun Gu, Maren Kirchner, Claudia Agnoli, Dillon T. Lloyd, Caroline H. Johnson, Sabina Sieri, Chirag J. Patel, Benedikt Warth.
*Broad chemical space sample preparation and sensitive LC-MS/MS for high-throughput exposomics and Exposome-Wide Association Study (ExWAS)**

06/2025 **ASMS Conference on Mass Spectrometry 2025**, Baltimore, USA
Yunyun Gu, Maren Kirchner, Claudia Agnoli, Dillon T. Lloyd, Caroline H. Johnson, Sabina Sieri, Chirag J. Patel, Benedikt Warth.
*Broad chemical space sample preparation and sensitive LC-MS/MS for high-throughput exposomics and Exposome-Wide Association Study (ExWAS)**

09/2024 **DoSChem Symposium 2024**, Vienna, Austria
Yunyun Gu, Benedikt Warth.
Broad chemical space sample preparation and sensitive LC-MS/MS for high-throughput exposomics

02/2024 **Austrian MS Forum 2024**, Vienna, Austria
Yunyun Gu, Benedikt Warth.
*Optimization of A High-throughput SPE Clean-up for Urine and Plasma Exposomics**

04/2023 **DoSChem Panel B Retreat 2023**, Fürstenfeld, Austria
Yunyun Gu, Benedikt Warth.
*Optimization of A High-throughput SPE Clean-up for Urine and Plasma Exposomics**

03/2023 **Exposome Austria Retreat 2023**, Retz, Austria
Yunyun Gu, Benedikt Warth.
*Exposome-Scale Sample Clean-up Based on Solid Phase Extraction**

09/2022 **DoSChem Symposium 2022**, Vienna, Austria
Yunyun Gu, Benedikt Warth.
*Exposome-Scale Sample Clean-up Based on Solid Phase Extraction**

07/2022 **MassSpec-Forum 2022**, Vienna, Austria
Yunyun Gu, Benedikt Warth.
*Exposome-Scale Sample Clean-up Based on Solid Phase Extraction**

05/2022 **JunganalytikerInnen Forum 2022**, Tulln, Austria
Yunyun Gu, Benedikt Warth.
*Exposome-Scale Sample Clean-up Based on Solid Phase Extraction**

*The same abstract and poster were presented due to the near timeframe of these events.

A.4. List of abbreviations

LC, liquid chromatography; MS, mass spectrometry; HRMS, high-resolution MS; SPE, solid-phase extraction; NTA, nontargeted analysis; NIST, National Institute of Standards; SRM, standard reference materials; ExWAS, exposome-wide association studies; EC, European Commission; LE, liquid extraction; PPT, protein precipitation; DNS, dilute and shoot; QuEChERS, quick, easy, cheap, effective, rugged and safe; PCPs, Personal care products; EDCs, endocrine-disrupting compounds; PFASs, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; RE, extraction recovery; RSD, relative standard deviation; LLOQ, low limit of quantification; HLOQ, high limit of quantification; ESI, electrospray ionization; GC-MS; gas chromatography coupled to mass spectrometry; EI, electron ionization; ME, matrix effect; LOD, limit of detection; LOQ, limit of quantification; QC, quality control; m/z, mass-to-charge ratio; SSE, signal suppression/enhancement; CV, coefficient of variation; YPOPS, Yale Pregnancy Outcome Prediction Study; GW, gestational weeks during pregnancy; ORDET, hORmones and Diet in the ETiology of breast cancer; SST, systems suitability testing; STD mix, mixture of standards; ISTD mix, mixture of internal standards.

A.5. Glossary

Term	**Definition**
Exposome	Totality of environmental exposures, including lifestyle factors from conception onwards
Exposomics	Systematic investigation of environmental exposures
ExWAS	An observational study typically involves a cohort of individuals sharing common characteristics, who are followed over time to measure outcomes at designated time points
Method Validation	Systematically evaluation of an analytical method in a series of parameters, like accuracy, precision, repeatability, selectivity, and etc.
Cohort study	An observational study involving individuals sharing some characteristics in a group, followed up over time, and outcomes measured at one or more time points
Exposome-scale	Refers to large numbers of either samples or exposures in exposome
Xenobiotics	Chemicals found but not produced in organisms or from the environment
QC	A systematic process of quality control for the stability of method performance during routine analysis

A.6. Summary of measured sequences and raw data storage

Batch Name	Purpose of Experiment	Methods	Raw Data Storage	Instrument
211115_YPOPS_Urine	Measurement of 32 mycotoxins in 200 urine samples from YPOPS	191213_MycoU_Method_32_VALIDATION	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2021\YPOPS_Urine\Data\YPOPS_Urine	Qtrap 6500+
220214_NiUr_TJ_Urine	Measurement of 96 chemicals in79 urine samples from Nigeria	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220214_NiUr_TJ_Urine	Qtrap 6500+
220317_HLB optimization_TJ	Optimization of SPE clean-up for 96 chemicals based on Jannik's method, comparing acidic, neutral, and basic loading and elution.	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220317_HLB optimization_TJ	Qtrap 6500+
220408_HLB optimization_TJ	Optimization of SPE clean-up for 96 chemicals based on Jannik's method, comparing two SPE sorbents, HLB and PSA+C18.	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220408_HLB optimization_TJ	Qtrap 6500+
220414_SPEopt3	Optimization of SPE clean-up for 96 chemicals based on Jannik's method, comparing two SPE sorbents, HLB and PSA+C18, and comparing acidic and neutral elution.	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220414_SPEopt_3	Qtrap 6500+
220427_SPEopt_4	Optimization of SPE clean-up for 96 chemicals based on Jannik's method, applying buffer to stabilize the pH of samples.	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220427_SPEopt_4	Qtrap 6500+
220630_SPEopt_5	Optimization of SPE clean-up for 96 chemicals based on Jannik's method, comparing three organic solvent and their mixture for elution of HLB	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220630_SPEopt_5	Qtrap 6500+
220708_SPEopt_6	Optimization of SPE clean-up for 96 chemicals based on Jannik's method. Test of two kinds of blank urine. Test of solvent effects.	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220708_SPEopt_6	Qtrap 6500+
220715_SPEopt_7	Optimization of SPE clean-up for 96 chemicals based on Jannik's method. 1, Optimization of volume of MeOH in Elution 2, Increasing the intensity of washing to reduce MEs 3, Dilute the sample solution after SPE with H2O to replace Speedvacuum	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220715_SPEopt_7	Qtrap 6500+
220801_SPEopt_8	Optimization of SPE clean-up for 96 chemicals based on Jannik's method. 1, Optimization of volume of MeOH in Elution, comparison of 200uL*2 MeOH and 100uL*2 MeOH 2, Application of plasma 3, Increasing the injection volume from 5 uL to 20 uL to increase the sensitivity	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220801_SPEopt_8	Qtrap 6500+
220906_SPEopt_9	Optimization of SPE clean-up for 96 chemicals based on Jannik's method. 1, 96-well plate application for both urine and plasma to estimate SSEs and recoveries. 2, Addition of internal standards after SPE to reestimate matrix effects and recoveries. 3, Addition of ISs before SPE to reestimate matrix effects and recoveries. 4, Test SSEs of measurement with 5uL injection and 20 uL injection (use the samples from 8th round).	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\220906_SPEopt_9	Qtrap 6500+
221209_SPEopt_10	Optimization of SPE clean-up for 96 chemicals based on Jannik's method. 1, test spiked plasma samples in 96-well plate SPE to find the reason of the low recoveries. 2, do not use the vacuum when loading and washing. 3, detect liquids from loading and washing steps... 4, use more methanol to elute.	220211_XenoQTrap_v12 (RT PFOA, PFOS, AAL MEHP longer)_elutenB_2%sA	Inch-40 MSC raw files\QTrap6500+Analyst Data\Projects\2022\XenoQTrap\221209_SPEopt_10	Qtrap 6500+
not available	Development LC-MS method for 234 chemicals. Tuning results saved in PDF for new added 58 endocrine disruptors	not available	Inch-40 MSC raw files\QTrap7500\DATA\2023_SCIEX OS Data\230720_SCIEX OS Data\2023 Xeno\Quantitation Results	Qtrap 7500
20230223_XQb test	Development LC-MS method for 234 chemicals. Check retention times for part of these compounds	LC-20230428_Xeno95_LC_V1_savedAgain; MS-20230506_XtremeXeno_23.05.05_V1&20230506_XtremeXeno_V2	Inch-40 MSC raw files\QTrap7500\DATA\2023_SCIEX OS Data\230720_SCIEX OS Data\2023 Xeno\Data\20230223_XQb test	Qtrap 7500
240307_SPE&NTA_2_YG	Non-targeted analysis/suspect screening (Compound Discoverer) Sum and compare the annotated features (Li1-5) in SRM samples from two different sample pretreatment methods.	20240226_Final_Full_Scan_JF_DCF_neg.meth; 20240226_Final_Full_Scan_JF_DCF_pos.meth; 20240307_RPLC_AcqX_MS2_neg_DCF_JF_YG_MLF.meth; 20240307_RPLC_AcqX_MS2_pos_DCF_JF_YG_MLF.meth; 240308 AcquireX.ppts (screenshot of details)	Inch-40 MSC raw files\Exploris 480\240307_SPE&NTA_2_YG	Exploris 480
20230308_IS mix test	Development LC-MS method for 234 chemicals. Check retention times for internal standards	20230217_Xeno95_LC_V1.lcm; 20230222_Xeno95_MS_optimized_V3.msm	Inch-40 MSC raw files\QTrap7500\DATA\2023_SCIEX OS Data\230720_SCIEX OS Data\2023 Xeno\Data\20230308_IS mix test	Qtrap 7500

Batch Name	Purpose of Experiment	Methods	Raw Data Storage	Instrument
20230330_XtremeXeno method test	Development LC-MS method for 234 chemicals. Check retention times for mycotoxins, pesticides, and antibiotics	20230217_Xeno95_LC_V1.lcm; 20230330_XtremeXeno_myco_test2.msm; 20230330_XtremeXeno_175_V1.msm; 20230330_issued VDs&myco_full scan_test4.msm; 20230330_XtremeXeno_VDs_IS_test2.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230330_XtremeXeno method test	Qtrap 7500
20230405_XtremeXeno myco test	Development LC-MS method for 234 chemicals. Check retention times for some mycotoxins can't find retention times in the previous experiment	20230217_Xeno95_LC_V1.lcm; 20230405_XtremeXeno_myco_test3.msm; 20230405_XtremeXeno_myco_sMRM_test4.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230405_XtremeXeno myco test	Qtrap 7500
20230406_XtremeXeno VDs test	Development LC-MS method for 234 chemicals. Check retention times for some pesticides and antibiotics can't find retention times in the previous experiment	20230217_Xeno95_LC_V1.lcm; 20230406_XtremeXeno_VDs_test5.msm; 20230406_XtremeXeno_VDs_test6.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230406_XtremeXeno VDs test	Qtrap 7500
20230414_XtremeXeno EDCs test	Development LC-MS method for 234 chemicals. Check retention times for newly added endocrine disruptors-1	20230217_Xeno95_LC_V1.lcm; 20230414_EDCs_full scan_test1.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230414_XtremeXeno EDCs test	Qtrap 7500
20230417_XtremeXeno EDCs test	Development LC-MS method for 234 chemicals. Check retention times for newly added endocrine disruptors-2	20230217_Xeno95_LC_V1.lcm; 20230417_EDCs_full scan_test2.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230417_XtremeXeno EDCs test	Qtrap 7500
20230428_XtremeXeno EDCs test	Development LC-MS method for 234 chemicals. Check retention times for newly added endocrine disruptors-3	20230428_LC_V1_savedAgain.lcm; 20230428_EDCs_full scan_test3.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230428_XtremeXeno EDCs test	Qtrap 7500
20230504_XtremeXeno EDCs test	Development LC-MS method for 234 chemicals. Check retention times for newly added endocrine disruptors-4	20230428_Xeno95_LC_V1_savedAgain.lcm; 20230504_8_questioned_EDCs_full scan_test4.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230504_XtremeXeno EDCs test	Qtrap 7500
20230505_XtremeXeno EDCs test	Development LC-MS method for 234 chemicals. Check retention times for newly added endocrine disruptors-5	20230428_Xeno95_LC_V1_savedAgain.lcm; 20230506_XtremeXeno_EDCs_23.05.05_V1.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230505_XtremeXeno EDCs test	Qtrap 7500
20230505_XtremeXeno EDCs test_Round2	Development LC-MS method for 234 chemicals. Check retention times for newly added endocrine disruptors-6	20230428_Xeno95_LC_V1_savedAgain.lcm; 20230506_XtremeXeno_V2.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2023_SCIEX OS Data:230720_SCIEX OS Data:2023 Xeno:Data:20230505_XtremeXeno EDCs test_Round2	Qtrap 7500
230524_XtremeXeno_6 retuning chemicals	Development LC-MS method for 234 chemicals. Check retention times for 6 mycotoxins, and linearity check	230523_XtremeXeno_Uniformed_V1.lcm; 20230524_XtremeXeno_6 returning chemicals.msm; 20230524_XtremeXeno_V3.msm; 20230524_XtremeXeno_V4.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2024 9 9_SCIEX OS Data:2023 Xeno:Data:230524_XtremeXeno_6 retuning chemicals	Qtrap 7500
230525_XtremeXeno_background&STC&BEA&OC	Development LC-MS method for 234 chemicals. Check retention times for 3 mycotoxins, and background check	230523_XtremeXeno_Uniformed_V1.lcm; 20230524_XtremeXeno_V4.msm	Inch:40 MSC raw files:QTrap:7500:DATA:2024 9 9_SCIEX OS Data:2023 Xeno:Data:230525_XtremeXeno_background&STC&BEA&OC	Qtrap 7500
230526_XtremeXeno_InstruLOD	Development LC-MS method for 234 chemicals. Estimate LOD of instrument	230523_XtremeXeno_Uniformed_V1.lcm; 20230526_XtremeXeno_V5.msm; 230526_XtremeXeno_V6.msm; 20230524_XtremeXeno_V4.msm; 230506_XtremeXeno_GroupName_V6.msm; 230526_XtremeXeno_window_V7.msm;	Inch:40 MSC raw files:QTrap:7500:DATA:2024 9 9_SCIEX OS Data:2023 Xeno:Data:230526_XtremeXeno_InstruLOD	Qtrap 7500
230606_SPE&NTA_1	1, apply SPE method for untargeted analysis, including DIA and DDA; 2, compare SPE method with PPT in identified and annotated features	211115_HSST3_20min_NH4F_v2	Inch:40 MSC raw files:Q_Exactive_HF:2023:230606_SPE&NTA_1	Q_Exactive_HF
230623_XtremeXeno_MRMinterfer	Development LC-MS method for 234 chemicals. Check if there is isomers, or some other similar compounds can't be separated by both LC and MS methods	230523_XtremeXeno_Uniformed_V1.lcm; 230622_XtremeXeno_V8.msm;	Inch:40 MSC raw files:QTrap:7500:DATA:2024 9 9_SCIEX OS Data:2023 Xeno:Data:230623_XtremeXeno_MRMinterfer	Qtrap 7500

Batch Name	Purpose of Experiment	Methods	Raw Data Storage	Instrument
230706_Calibration Test	Development LC-MS method for 234 chemicals. Check calibration linearity quickly.	230523_XtremeXeno_Uniformed_V1.lcm; 230622_XtremeXeno_V8.msm; 230706_XtremeXeno_V9.msm; 230706_8 analytes missing msm;	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\230706_Calibration Test	Qtrap 7500
230711_IS&SPE&Calibration check_Exp11	Development LC-MS method for 234 chemicals. Check calibration linearity and accuracy of spiked urine and plasma.	230523_XtremeXeno_Uniformed_V1.lcm; 230711_XtremeXeno_V11.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\230711_IS&SPE&Calibration check_Exp11	Qtrap 7500
230816_Check of IS&New MRM with Julia_Exp12	Development LC-MS method for 234 chemicals. 1. Select 12 internal standards (IS), adjust the spiking concentrations and make a new IS mixture. 2. Test intensity and stability of plasma samples with pre-spiking and post-spiking IS as well as calibrators. 3. Test intensity and linearity of new MRM transitions Vinicius offered.	230523_XtremeXeno_Uniformed_V1.lcm; 230810_XtremeXeno_V13.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\230816_Check of IS&New MRM with Julia_Exp12	Qtrap 7500
230901_Method Validation_Exp14	Development LC-MS method for 234 chemicals. 1. Select 2 MRM methods, test stability	230523_XtremeXeno_Uniformed_V1.lcm; 230901_Method Validation_Exp14.msm; 230831_XtremeXeno_V14_wide window.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\230901_Method Validation_Exp14	Qtrap 7500
230920_XtremeXeno MethValid_Day1_Exp15	Validation of LC-MS method for 234 chemicals - day 1	230523_XtremeXeno_Uniformed_V1.lcm; 230904_XtremeXeno_V15.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\230920_XtremeXeno MethValid_Day1_Exp15	Qtrap 7500
230928_XtremeXeno MethValid_Day2_Exp16	Validation of LC-MS method for 234 chemicals - day 2	230523_XtremeXeno_Uniformed_V1.lcm; 230904_XtremeXeno_V15.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\230928_XtremeXeno MethValid_Day2_Exp16	Qtrap 7500
231215_XtremeXeno MethValid_Day3_Exp19	Validation of LC-MS method for 234 chemicals - day 3	230523_XtremeXeno_Uniformed_V1.lcm; 230904_XtremeXeno.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\231215_XtremeXeno MethValid_Day3_Exp19	Qtrap 7500
231121_XtremeXeno_YPOPS U_Exp18	Application for the LC-MS method analyzing 234 chemicals	230523_XtremeXeno_Uniformed_V1.lcm; 230904_XtremeXeno_V15.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\231121_XtremeXeno_YPOPSU_Exp18	Qtrap 7500
231012_µSPEopt_1	Optimization of sample clean-up methods using µSPE (first round)-bachelor's thesis	230523_XtremeXeno_Uniformed_V1.lcm; 230904_XtremeXeno_V15.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\231012_µSPEopt_1	Qtrap 7500
231116_µSPE_2	Optimization of sample clean-up methods using µSPE (second round)-bachelor's thesis	230523_XtremeXeno_Uniformed_V1.lcm; 230904_XtremeXeno_V15.msm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2023 Xeno Data\231116_µSPE_2	Qtrap 7500
241119_ORDET_Urine_Batch 1_2	Measurement of 1079 urine samples using the high-throughput LC-MS workflow covering 234 chemicals - batch 1	240814_XtremeXeno.msm 241113_XtremeXeno_Uniformed_V3.lcm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2024 XtremXeno\ERC\Raw data	Qtrap 7500
241123_ORDET_Urine_Batch 3_4	Measurement of 1079 urine samples using the high-throughput LC-MS workflow covering 234 chemicals - batch 2	240814_XtremeXeno.msm 241113_XtremeXeno_Uniformed_V3.lcm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2024 XtremXeno\ERC\Raw data	Qtrap 7500
241127_ORDET_Urine_Batch 5_6	Measurement of 1079 urine samples using the high-throughput LC-MS workflow covering 234 chemicals - batch 3	240814_XtremeXeno.msm 241113_XtremeXeno_Uniformed_V3.lcm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2024 XtremXeno\ERC\Raw data	Qtrap 7500
241202_ORDET_Urine_Batch 7_8	Measurement of 1079 urine samples using the high-throughput LC-MS workflow covering 234 chemicals - batch4	240814_XtremeXeno.msm 241113_XtremeXeno_Uniformed_V3.lcm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2024 XtremXeno\ERC\Raw data	Qtrap 7500
241206_ORDET_Urine_Batch 9_10	Measurement of 1079 urine samples using the high-throughput LC-MS workflow covering 234 chemicals - batch 5	240814_XtremeXeno.msm 241113_XtremeXeno_Uniformed_V3.lcm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2024 XtremXeno\ERC\Raw data	Qtrap 7500
241210_ORDET_Urine_Batch 11_12	Measurement of 1079 urine samples using the high-throughput LC-MS workflow covering 234 chemicals - batch 6	240814_XtremeXeno.msm 241113_XtremeXeno_Uniformed_V3.lcm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2024 XtremXeno\ERC\Raw data	Qtrap 7500
241214_ORDET_Urine_Batch 13_14; 250116_ORDET_Urine_Batch 14_26samples	Measurement of 1079 urine samples using the high-throughput LC-MS workflow covering 234 chemicals - batch 7	240814_XtremeXeno.msm 241113_XtremeXeno_Uniformed_V3.lcm	Inrich 40 MSC raw files\QTrap 7500\DATA\2024 9 9_SCIEX OS Data\2024 XtremXeno\ERC\Raw data	Qtrap 7500

A.7. Standard Operating Procedures (SOP) of quantitative assay of 200+ chemical exposures

SOP #1 – Extraction of 234 biomarkers and toxins in human urine, serum, and plasma using 96-well plate-based SPE

Version 1

By Yunyun Gu

Oct. 17. 2025

0. This workflow is used for the two articles

Yunyun Gu, Max L. Feuerstein, Benedikt Warth. "High-Throughput Solid Phase Extraction for Targeted and Nontargeted Exposomics", *Analytical Chemistry*, 2025, Vol. 97(11): 6075-6082, DOI: 10.1021/acs.analchem.4c06177

Yunyun Gu, Max L. Feuerstein, Dillon T. Lloydd, Chirag J. Patel, Caroline H. Johnson, Benedikt Warth. "High-performance quantitative exposomics covering up to >230 toxicants and key biomarkers", *Environmental Science & Technology*, 2025, DOI: doi.org/10.1021/acs.est.5c04458

1. Sample preparation procedure

For the extraction and quantification of 234 biomarkers and toxins in human urine, serum, and plasma using a 96-well plate-based solid-phase extraction (SPE) workflow, several types of samples and solutions are required. Section 1 describes the preparation of all sample types for quality control (QC), including process blanks, pre-spiked and post-spiked QC samples, solvent calibration standards, and reference material. Section 2 details the preparation of all solutions required for the process, and Section 3 outlines the SPE workflow used during sample preparation.

1.1. Preparation of matrix samples

The SPE workflow for urine, serum, or plasma samples is described in detail in Section 3.

1.2. Preparation of process blank

This step is carried out before starting the SPE process. The blank sample consists of LC-MS grade water (H_2O) and is subjected to the entire sample pretreatment workflow in Section 3.

1.3. Preparation of non-spiked QC (non-spiked pooled sample, raw material)

This step is carried out before starting the SPE process. The non-spiked QC is produced by pooling 50 µL of raw samples from either randomly selected samples or all samples. Here, raw material means the samples without any sample preparation.

- Aliquots of pooled QC samples are spiked with the STD mix to prepare the pre-spiked QC samples. Ensure sufficient volumes of the pooled samples before starting the SPE process (see section 1.4.).
- Remaining non-spiked pooled QC samples are processed according to the SPE workflow described in section 3. Aliquots of the resulting extracts are spiked with the STD mix to prepare the post-spiked QC samples (see section 1.5.). Ensure sufficient volume of the extract of pooled samples before starting SPE process.

1.4. Preparation of pre-spiked QC (pre-spiked pooled sample)

This step should be carried out before starting the SPE process. Prepare the pre-spiked QC according to **Table 1**. Here, raw material means the samples before additional sample treatment. The volume for each pre-spiked QC is 400 µL. A pipetting scheme for preparing standard calibrations and spiking solutions is presented in **Table 4.** Adjust volumes according to your batch design to ensure sufficient volumes for the current experiment. The prepared pre-spiked QC samples are then processed according to the SPE process described in Section 3.

Table 1. Preparation of pre-spiked QC (from raw sample material)

STD mix (µL)	Non-spiked QC (raw material, µL)	Final Volume (µL)
3	997	1000

1.5. Preparation of post-spiked QC (post-spiked pooled sample)

This step should be carried out after the SPE process. First, pool equal volumes (500 µL) of the non-spiked QC extracts to create a pooled extract. (Note: Each individual extract has a total volume of 800 µL) and spike with the STD mix, see **Table 2**.

Table 2. Preparation of post-spiked QC (from SPE extracts)

STD mix (µL)	Extract of non-spiked QC (SPE extract, µL)	Final Volume (µL)
3	1997	2000

1.6. Preparation of solvent blank

This step can be carried out before or after the SPE process. Solvent blanks consist of 50% MeOH in water ($MeOH/H_2O$, 50/50, v/v). The volume per blank is 400 µL. Ensure sufficient volumes for instrumental analysis.

2. Preparation of required solutions for sample preparation

2.1. Preparation of internal standards

Part 1: Preparation of ISTD mix for post-spiking

A mixture of ten isotope-labeled internal standards ($^{13}C_{12}$-bisphenol A, $^{13}C_6$-butylparaben, $^{13}C_{15}$-deoxynivalenol, $^{2}H_3$-erythromycin, $^{13}C_6$-ethylparaben, $^{13}C_2$-mono-butyl phthalate, $^{13}C_6$-methylparaben, $^{13}C_8$-perfluorooctanoic acid, $^{13}C_8$-perfluorooctanesulfonic acid, and $^{13}C_6$-propylparaben) is prepared in acetonitrile, referred to as "ISTD mix".

Part 2: Preparation of $^{13}C_{18}$-Zearalenone ISTD for pre-spiking

$^{13}C_{18}$-Zearalenone ($^{13}C_{18}$-ZEN) is diluted in acetonitrile to reach 100 ng/mL. Concentrations of internal standards in samples are summarized in **Table 3** below. During sample preparation, both the ISTD mix and $^{13}C_{18}$-ZEN are used at a final dilution of 1:100 in samples, like urine, plasma and serum (see **Table 3**).

Table 3. Concentrations of isotope-labeled internal standards (ng/mL) in stock solutions and in samples, including urine, plasma, and serum.

Name	Concentration in stock solution (ng/mL)	Concentrations in samples (ng/mL)
ISTD mix (for post-spiking)		
$^{13}C_{15}$-Deoxynivalenol	750	7.50
$^{13}C_2$-Mono-n-butyl phthalate	625	6.25
$^{13}C_6$-Methylparaben	125	1.25
$^{13}C_6$-Ethylparaben	125	1.25
D_3-Erythromycin A hydrate	125	1.25
$^{13}C_6$-Propylparaben	125	1.25
$^{13}C_8$-Perfluorooctanoic acid	62	0.62
$^{13}C_{12}$-Bisphenol A	375	3.75
$^{13}C_6$-Butylparaben	125	1.25
$^{13}C_8$-Perfluorooctanesulfonic acid	62	0.62
For pre-spiking		
$^{13}C_{18}$-Zearalenone	100	1.00

2.2. Preparation of phosphate-buffered saline

Part 1: Prepare 10x phosphate-buffered saline (10xPBS) Stock Solution

1. Accurately weigh the following salts using an analytical balance:
   Sodium chloride (NaCl): 100 g
   Potassium chloride (KCl): 2.54 g
   Disodium phosphate (Na_2HPO_4): 14.2 g
   Potassium dihydrogen phosphate (KH_2PO_4): 2.45 g
2. Transfer weighed salt into a clean 1 L beaker. Add 800 mL of LC-MS grade H_2O, a magnetic stir bar and place the beaker on a stirring plate to ensur complete dissolvation of the salts.
3. Adjust pH: Once salts are dissolved, adjust the solution's pH to 7.4 using dropwise additions of concentrated HCl (to decrease pH) or concentrated NaOH (to increase pH). Use a calibrated pH meter to track the pH.
4. Quantitatively transfer the pH-adjusted solution to a 1 L volumetric flask and fill up with LC-MS grade H_2O until the 1 L mark is reached. Cap the flask and mix by inverting several times. The resulting solution is used as a 10x PBS stock solution.
5. Aliquot the 10x PBS stock solution and store the aliquots (4 mL each) at -20 °C.

Part 2: Prepare 200 mM PBS Working Solution

1. To prepare the 200 mM PBS (pH 7.4) working solution, dilute the 10x PBS stock solution by a factor of 10 with LC-MS grade H_2O.
2. Store the 200 mM PBS (pH 7.4) in 4 °C until use.

2.3. Preparation of standard mix (STD mix)

A mixture of 234 standards is prepared in acetonitrile, referred to as “STD mix”. Concentrations of analytes in the STD mix are presented in **Table 4**. The mixture is aliquoted and stored at -20°C until the day of the experiment.

Table 4. Concentrations (ng/mL) of 234 standards prepared in acetonitrile in STD mix and final concentrations in pre-/post-spiked pooled samples.

Compounds	Abbreviation	Concentration STD mix (ng/mL)	Concentration pre-/post-spiked pooled samples (ng/mL)
16-Epiestriol	16EpiE3	100	0.3
16-α-Hydroxyestrone	16OHE1	15	0.045
17-Epiestriol	17EpiE3	100	0.3
1-Hydroxypyrene	1OHPyrene	200	0.6
2,2',6,6'-Tetrachlorobisphenol A	TCBPA	200	0.6
2,4,5-Trichlorophenol	2,4,5-TCP	5000	15
2,4-Dichlorophenoxyacetic acid	2,4-D	60	0.18
2,5-Dichlorophenol	2,5-DCP	600	1.8
2-Hydroxy-4-methoxybenzophenone	BP-3	300	0.9
2-isopropyl-4-methyl-6-hydroxypyrimidine	IMPy	60	0.18
2-Methoxyestradiol	2MeOE2	20	0.06
2-Methoxyestrone	2MeOE1	25	0.075
2-Naphthol	2-Napht	30	0.09
2-Phenylphenol	2-PP	1200	3.6
2-tert-Butylphenol	2-tert-But	50000	150
3 Phenoxybenzoic acid	3PBA	60	0.18
3,5,6-Trichloro-2-pyridinol	TCPy	2000	6
3-Benzylidenecamphor	3-BC	15000	45
3-Hydroxyphenanthrene	3OHPhen	15	0.045
4-Hydroxybenzophenone	4-OH-BP	1000	3
4-Hydroxyestrone	4OHE1	5	0.015
4-Methoxy Estradiol	4MeOE2	10	0.03
4-Methoxy estrone	4MeOE1	5	0.015
4-Methyl-benzophenone	4-MBP	400	1.2
4-Methylbenzylidene camphor	4-MBC	1500	4.5
4-Octylphenol	4-OP	10000	30
4-Phenylphenol	4-PP	1200	3.6
4-tert-Octylphenol	4-tert-OP	1500	4.5
5-Hydroxymethyl-2-furanoic acid	HMFA	20000	60
5-Hydroxymethylfurfural	HMF	3500	10.5
8-Prenylnaringenin	8-Pn	30	0.09
Acetaminophen	APAP	5000	15
Acetaminophen_glucuronide	APAP_GlcA	100	0.3
Acetamiprid	ATMP	1	0.003
Acrylamide	Acrylamide	10000	30
Aflatoxicol	AFL	2000	6
Aflatoxin B1	AFB1	200	0.6
Aflatoxin B1-N7-guanine	AFB1_Gua	100	0.3

Compounds	Abbreviation	Concentration STD mix (ng/mL)	Concentration pre-/post-spiked pooled samples (ng/mL)
Aflatoxin B2	AFB2	400	1.2
Aflatoxin G1	AFG1	300	0.9
Aflatoxin G2	AFG2	400	1.2
Aflatoxin M1	AFM1	300	0.9
Aflatoxin M2	AFM2	1200	3.6
Aflatoxin P1	AFP1	1200	3.6
Aflatoxin Q1	AFQ1	1200	3.6
Alternariol	Alternariol	100	0.3
Alternariol monomethyl ether	AME	105	0.315
Amoxicillin	Amox	1200	3.6
Ampicillin	Ampi	20	0.06
Anisodamine	Anisodamine	5	0.015
Aristolactam I	AL	50	0.15
Aristolochic acid I	AAI	100	0.3
Atrazine	ATZ	60	0.18
Avobenzone	AVO	4000	12
Beauvericin	BEA	20	0.06
Benzophenone 1	BP-1	20	0.06
Benzophenone 2	BP-2	15	0.045
Benzophenone-6	BP-6	600	1.8
Benzyl Butyl Phthalate	BenzButPht	250	0.75
Benzylparaben	BenzPara	1.5	0.0045
Bis(1,3-dichloro-2-propyl) phosphate	BDCPP	4000	12
Bis(1-chloro-2-propyl) phosphate	BCIPP	2670	8.01
Bisphenol A	BPA	100	0.3
Bisphenol A_glucuronide	BPA_GlcA	100	0.3
Bisphenol AF	BPAF	50	0.15
Bisphenol AP	BPAP	400	1.2
Bisphenol B	BPB	10	0.03
Bisphenol C	BPC	200	0.6
Bisphenol E	BPE	1000	3
Bisphenol F	BPF	50	0.15
Bisphenol FL	BPFL	300	0.9
Bisphenol M	BPM	800	2.4
Bisphenol S	BPS	2	0.006
Bisphenol Z	BPZ	1000	3
Bromoacetic acid	Br.ac.ac	2000	6
Butylparaben	ButylPara	10	0.03
Cefaclor	Cefac	200	0.6
Chalcone	Chalcone	600	1.8

Compounds	Abbreviation	Concentration STD mix (ng/mL)	Concentration pre-/post-spiked pooled samples (ng/mL)
Chloramphenicol	Chloram	60	0.18
Chlorotetracycline	CTC	2000	6
Chlorpyrifos	CFs	600	1.8
Chlorpyrifos-methyl	CPM	60	0.18
Chrysin	Chrysin	2000	6
Ciprofloxacin	Cipro	2400	7.2
Citrinin	CIT	5000	15
Clarithromycin	Clari	180	0.54
Cotinine	Cotinine	150	0.45
Coumestrol	Coumestrol	5	0.015
Culmorin	CUL	500	1.5
Culmorin_11_glucuronide	CUL_11_GlcA	25	0.075
Daidzein	Daidzein	5	0.015
Daidzein_glucuronide	Daidzein_GlcA	50	0.15
Danofloxacin	Danof	400	1.2
Deepoxy-deoxynivalenol	DOM-1	400	1.2
Deoxynivalenol	DON	6000	18
Di-2-propylheptyl phthalate	DPHP	4000	12
Diazinon	DIZN	20	0.06
Dibromoacetic acid	Di.Br.Ac.ac	1500	4.5
Dibutyl phthalate	DiButPht	10000	30
Dichloroacetic acid	Di.Cl.Ac.ac	7500	22.5
Diethyl dithiophosphate	DEDTP	180	0.54
Diethyl phthalate	DEP	3000	9
Diethyl thiophosphate	DETP	60	0.18
Dihydrocitrinone	DHC	200	0.6
Dimethoate	DMT	0.5	0.0015
Dimethyl phosphate	VPM_DMP	60	0.18
Dimethyl phthalate	EDCs_DMP	800	2.4
Dioxybenzone	BP-8	900	2.7
Doxycycline	DOX	1200	3.6
Duloxetine	Duloxetine	100	0.3
Enniatin B	EnnB	20	0.06
Enniatin B1	EnnB1	20	0.06
Enrofloxacin	Enro	1000	3
Enterodiol	Enterodiol	5	0.015
Enterolactone	Enterolactone	200	0.6
Equol	Equol	20	0.06
Erythromycin	Ery	200	0.6
Estradiol	E2	30	0.09

Compounds	Abbreviation	Concentration STD mix (ng/mL)	Concentration pre-/post-spiked pooled samples (ng/mL)
Estradiol-17-glucuronide	E2_17_GlcA	50	0.15
Estradiol-3-sulfate	E2_3_SO4	15	0.045
Estriol	E3	30	0.09
Estrone	E1	3	0.009
Ethinylestradiol	EthinylE2	100	0.3
Ethyl protocatechuic acid	OH-EtP	100	0.3
Ethylparaben	EthylPara	10	0.03
Fenarimol	Fenarimol	3	0.009
Fipronil	FIPL	600	1.8
Florfenicol	Florf	1800	5.4
Fluconazole	Fluco	6	0.018
Formononetin	Formononetin	2.5	0.0075
Fumonisin B1	FB1	1000	3
Genistein	Genistein	5	0.015
Glycitein	Glycitein	5	0.015
Glyphosate	Glyphosate	500	1.5
Heptylparaben	HeP	14	0.042
Ibuprofen	Ibuprofen	4000	12
Ibuprofen_glucuronide	Ibuprofen_GlcA	100	0.3
Imidacloprid	IDC	20	0.06
Isobutylparaben	IsobutPara	10	0.03
Isoxanthohumol	Isoxanth	1	0.003
Ivermectin	Iverm	2	0.006
Jacobine	Jacobine	25	0.075
Jacobine-N-oxide	JacobineNox	5	0.015
Kaempferol	Kaempferol	5000	15
Lomefloxacin	Lomef	1200	3.6
Malathion	MATH	6	0.018
Malathion dicarboxylic acid	MDA	180	0.54
Matairesinol	Mataires	50	0.15
Methiocarb	Methiocarb	5	0.015
Methyl protocatechuic acid	OH-MeP	200	0.6
Methylparaben	MethylPara	25	0.075
Metribuzin	Metribuzin	400	1.2
Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	400	1.2
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	5-OH-MEHP	200	0.6
Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP	200	0.6
Mono-(3-carboxypropyl) phthalate	MCPP	4000	12
Mono-[(2-carboxymethyl) hexyl] phthalate	MCMHP	400	1.2
Mono-2-ethylhexyl phthalate	MEHP	150	0.45

Compounds	Abbreviation	Concentration STD mix (ng/mL)	Concentration pre-/post-spiked pooled samples (ng/mL)
Mono-benzyl phthalate	MBeP	200	0.6
Mono-butyl phthalate	MBP	500	1.5
Mono-cyclohexyl phthalate	MCHP	1200	3.6
Mono-hexyl phthalate	MHP	1200	3.6
Mono-isobutyl phthalate	MIBP	1200	3.6
Mono-isodecyl phthalate	MIDP	1000	3
Mono-iso-nonyl phthalate	MINP	4000	12
Mono-methyl phthalate	MMP	5000	15
Mono-n-pentyl phthalate	MnPeP	400	1.2
Mono-octyl phthalate	MOP	4000	12
Mycophenolic acid	MPA	4000	12
Mycophenolic acid_glucuronide	MPA_GlcA	100	0.3
N,N-dimethylbenzamide	DBM	6	0.018
N-butylbenzenesulfonamide	N-butylbenz	1000	3
Nivalenol	NIV	2000	6
N-nitrosodimethylamine	NDMA	30000	90
Nonylphenol	Nonylph	2500	7.5
Norfloxacin	Norf	2400	7.2
Ochratoxin A	OTA	100	0.3
Ochratoxin alpha	OTalpha	400	1.2
Ochratoxin B	OTB	100	0.3
Octyl methoxycinnamate	OMC	20000	60
Ofloxacin	Oflox	2	0.006
Oxytetracycline	OTC	600	1.8
Pefloxacin	Peflox	1200	3.6
Perfluorobutanesulfonic acid	PFBS	20	0.06
Perfluorodecanoic acid	PFDA	200	0.6
Perfluorohexanoic acid	PFHxA	100	0.3
Perfluorononanoic acid	PFNA	300	0.9
Perfluorooctanesulfonic acid	PFOS	15	0.045
Perfluorooctanoic acid	PFOA	15	0.045
Perfluoroundecanoic acid	PFUA	200	0.6
PhIP	PhIP	10	0.03
p-Hydroxybenzoic acid	pOHBA	5000	15
p-Nitrophenol	3PNP	540	1.62
Prochloraz	Prochloraz	0.5	0.0015
Propiconazole	Propiconazole	100	0.3
Propylparaben	PropylPara	20	0.06
Resveratrol	Resver	1500	4.5
Riddelline	Riddelliin	30	0.09

Compounds	Abbreviation	Concentration STD mix (ng/mL)	Concentration pre-/post-spiked pooled samples (ng/mL)
Riddelline-N-oxide	RiddelliinNox	20	0.06
Roxithromycin	Roxi	540	1.62
Scopolamine	Scopolamine	1.5	0.0045
SN38	SN38	100	0.3
SN38_glucuronide	SN38_GlcA	100	0.3
Sterigmatocystin	STC	20	0.06
Sulfadiazine	SulfDZ	6	0.018
Sulfadimethoxine	Sulf-DM	20	0.06
Sulfamethazine	Sulf-M	6	0.018
Sulfamethoxazole	Sulf-X	6	0.018
T-2 toxin	T-2	10000	30
Tebuconazole	Tebuconazole	200	0.6
Tetrabrombisphenol A	TBPA	100	0.3
Tetracycline	TC	1200	3.6
Tramadol	Tramadol	300	0.9
trans-3-Hydroxycotinine	t3OHCot	50	0.15
Triclocarban	Triclocarban	200	0.6
Triclosan	Triclosan	100	0.3
Trimethoprim	Trim	60	0.18
Tri-n-butyl phosphate	TBP	200	0.6
Triphenyl phosphate	TPhP	200	0.6
Tris(2-chloroethyl)phosphate	TCEP	5000	15
Tylosin	Tylo	600	1.8
Venlafaxine	Venlafaxine	300	0.9
Vinclozolin	Vinclozolin	5000	15
Xanthohumol	Xanth	100	0.3
Zearalanone	ZAN	30	0.09
Zearalenone	ZEN	30	0.09
Zearalenone-14-glucuronide	ZEN_14_GlcA	50	0.15
Zearalenone-14-sulfate	ZEN_14_SO4	15	0.045
α-Zearalanol	α-ZAL	50	0.15
α-Zearalenol	α-ZEL	2	0.006
α-Zearalenol-14-glucuronide	α-ZEL_14_GlcA	15	0.045
β-Zearalanol	β-ZAL	50	0.15
β-Zearalenol	β-ZEL	100	0.3
β-Zearalenol-14-glucuronide	β-ZEL_14_GlcA	15	0.045

2.4. Preparation of solvent calibration curve

Part 1: Preparation of dilution solvent

Dilution solvent is prepared by mixing 2970 µL of 50% MeOH (MeOH/H2O, 1/1, v/v), 15 µL of $^{13}C_{18}$-ZEN (100 ng/mL) and 15 µL of the ISTD mix (see **Table 5** below).

Table 5. Preparation of dilution solvent

Solvent or mix of internal standards	Volume (µL)
50% MeOH	2970
$^{13}C_{18}$-ZEN (100 ng/mL)	15
ISTD mix	15
Dilution solvent	**3000**

Part 2: Preparation of calibration standards

The highest calibration level (Level 9, L9) is prepared by mixing 20 µL of the STD mix with 360 µL of the dilution solution. Serial dilutions are used with the dilution solution to yield all other calibration levels (Level 8 to Level 1, L8-L1), details presented in **Table 6**. Concentrations of individual analytes at the nine calibration levels are shown in **Table 7**.

Table 6. Preparation of calibration levels (Level 1-9)

Calibration Level	Standard solution	Dilution solvent (µL)	Final Volume (µL)
L9	20 µL of STD mix	380	400
L8	90 µL of L9	210	300
L7	50 µL of L9	450	500
L6	90 µL of L7	210	300
L5	50 µL of L7	450	500
L4	90 µL of L5	210	300
L3	50 µL of L5	450	500
L2	90 µL of L3	210	300
L1	40 µL of L3	360	400

Table 7. Concentrations (ng/mL) of nine calibration levels prepared in 50% methanol in water (MeOH/H2O, 1/1, v/v).

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
16-Epiestriol	16EpiE3	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
16-α-Hydroxyestrone	16OHE1	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5
17-Epiestriol	17EpiE3	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
1-Hydroxypyrene	1OHPyrene	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
2,2',6,6'-Tetrachlorobisphenol A	TCBPA	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
2,4,5-Trichlorophenol	2,4,5-TCP	0.05	0.15	0.5	1.5	5	15	50	150	500
2,4-Dichlorophenoxyacetic acid	2,4-D	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6
2,5-Dichlorophenol	2,5-DCP	0.006	0.018	0.06	0.18	0.6	1.8	6	18	60
2-Hydroxy-4-methoxybenzophenone	BP-3	0.003	0.009	0.03	0.09	0.3	0.9	3	9	30
2-isopropyl-4-methyl-6-hydroxypyrimidine	IMPy	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6
2-Methoxyestradiol	2MeOE2	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
2-Methoxyestrone	2MeOE1	0.00025	0.00075	0.0025	0.0075	0.025	0.075	0.25	0.75	2.5
2-Naphthol	2-Napht	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3	0.9	3
2-Phenylphenol	2-PP	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
2-tert-Butylphenol	2-tert-But	0.5	1.5	5	15	50	150	500	1500	5000
3 Phenoxybenzoic acid	3PBA	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6
3,5,6-Trichloro-2-pyridinol	TCPy	0.02	0.06	0.2	0.6	2	6	20	60	200
3-Benzylidenecamphor	3-BC	0.15	0.45	1.5	4.5	15	45	150	450	1500
3-Hydroxyphenanthrene	3OHPhen	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5
4-Hydroxybenzophenone	4-OH-BP	0.01	0.03	0.1	0.3	1	3	10	30	100
4-Hydroxyestrone	4OHE1	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
4-Methoxy Estradiol	4MeOE2	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1
4-Methoxy estrone	4MeOE1	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
4-Methyl-benzophenone	4-MBP	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
4-Methylbenzylidene camphor	4-MBC	0.015	0.045	0.15	0.45	1.5	4.5	15	45	150

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
4-Octylphenol	4-OP	0.1	0.3	1	3	10	30	100	300	1000
4-Phenylphenol	4-PP	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
4-tert-Octylphenol	4-tert-OP	0.015	0.045	0.15	0.45	1.5	4.5	15	45	150
5-Hydroxymethyl-2-furanoic acid	HMFA	0.2	0.6	2	6	20	60	200	600	2000
5-Hydroxymethylfurfural	HMF	0.035	0.105	0.35	1.05	3.5	10.5	35	105	350
8-Prenylnaringenin	8-Pn	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3	0.9	3
Acetaminophen	APAP	0.05	0.15	0.5	1.5	5	15	50	150	500
Acetaminophen_glucuronide	APAP_GlcA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Acetamiprid	ATMP	0.00001	0.00003	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1
Acrylamide	Acrylamide	0.1	0.3	1	3	10	30	100	300	1000
Aflatoxicol	AFL	0.02	0.06	0.2	0.6	2	6	20	60	200
Aflatoxin B1	AFB1	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Aflatoxin B1-N7-guanine	AFB1_Gua	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Aflatoxin B2	AFB2	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Aflatoxin G1	AFG1	0.003	0.009	0.03	0.09	0.3	0.9	3	9	30
Aflatoxin G2	AFG2	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Aflatoxin M1	AFM1	0.003	0.009	0.03	0.09	0.3	0.9	3	9	30
Aflatoxin M2	AFM2	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Aflatoxin P1	AFP1	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Aflatoxin Q1	AFQ1	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Alternariol	Alternariol	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Alternariol monomethyl ether	AME	0.00105	0.00315	0.0105	0.0315	0.105	0.315	1.05	3.15	10.5
Amoxicillin	Amox	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Ampicillin	Ampi	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Anisodamine	Anisodamine	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
Aristolactam I	AL	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
Aristolochic acid I	AAI	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
Atrazine	ATZ	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6
Avobenzone	AVO	0.04	0.12	0.4	1.2	4	12	40	120	400
Beauvericin	BEA	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Benzophenone 1	BP-1	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Benzophenone 2	BP-2	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5
Benzophenone-6	BP-6	0.006	0.018	0.06	0.18	0.6	1.8	6	18	60
Benzyl Butyl Phthalate	BenzButPht	0.0025	0.0075	0.025	0.075	0.25	0.75	2.5	7.5	25
Benzylparaben	BenzPara	0.000015	0.000045	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15
Bis(1,3-dichloro-2-propyl) phosphate	BDCPP	0.04	0.12	0.4	1.2	4	12	40	120	400
Bis(1-chloro-2-propyl) phosphate	BCIPP	0.0267	0.0801	0.267	0.801	2.67	8.01	26.7	80.1	267
Bisphenol A	BPA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Bisphenol A_glucuronide	BPA_GlcA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Bisphenol AF	BPAF	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
Bisphenol AP	BPAP	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Bisphenol B	BPB	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1
Bisphenol C	BPC	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Bisphenol E	BPE	0.01	0.03	0.1	0.3	1	3	10	30	100
Bisphenol F	BPF	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
Bisphenol FL	BPFL	0.003	0.009	0.03	0.09	0.3	0.9	3	9	30
Bisphenol M	BPM	0.008	0.024	0.08	0.24	0.8	2.4	8	24	80
Bisphenol S	BPS	0.00002	0.00006	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2
Bisphenol Z	BPZ	0.01	0.03	0.1	0.3	1	3	10	30	100
Bromoacetic acid	Br.ac.ac	0.02	0.06	0.2	0.6	2	6	20	60	200
Butylparaben	ButylPara	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1
Cefaclor	Cefac	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Chalcone	Chalcone	0.006	0.018	0.06	0.18	0.6	1.8	6	18	60
Chloramphenicol	Chloram	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
Chlorotetracycline	CTC	0.02	0.06	0.2	0.6	2	6	20	60	200
Chlorpyrifos	CFs	0.006	0.018	0.06	0.18	0.6	1.8	6	18	60
Chlorpyrifos-methyl	CPM	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6
Chrysin	Chrysin	0.02	0.06	0.2	0.6	2	6	20	60	200
Ciprofloxacin	Cipro	0.024	0.072	0.24	0.72	2.4	7.2	24	72	240
Citrinin	CIT	0.05	0.15	0.5	1.5	5	15	50	150	500
Clarithromycin	Clari	0.0018	0.0054	0.018	0.054	0.18	0.54	1.8	5.4	18
Cotinine	Cotinine	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5	4.5	15
Coumestrol	Coumestrol	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
Culmorin	CUL	0.005	0.015	0.05	0.15	0.5	1.5	5	15	50
Culmorin_11_glucuronide	CUL_11_GlcA	0.00025	0.00075	0.0025	0.0075	0.025	0.075	0.25	0.75	2.5
Daidzein	Daidzein	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
Daidzein_glucuronide	Daidzein_GlcA	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
Danofloxacin	Danof	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Deepoxy-deoxynivalenol	DOM-1	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Deoxynivalenol	DON	0.06	0.18	0.6	1.8	6	18	60	180	600
Di-2-propylheptyl phthalate	DPHP	0.04	0.12	0.4	1.2	4	12	40	120	400
Diazinon	DIZN	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Dibromoacetic acid	Di.Br.Ac.ac	0.015	0.045	0.15	0.45	1.5	4.5	15	45	150
Dibutyl phthalate	DiButPht	0.1	0.3	1	3	10	30	100	300	1000
Dichloroacetic acid	Di.Cl.Ac.ac	0.075	0.225	0.75	2.25	7.5	22.5	75	225	750
Diethyl dithiophosphate	DEDTP	0.0018	0.0054	0.018	0.054	0.18	0.54	1.8	5.4	18
Diethyl phthalate	DEP	0.03	0.09	0.3	0.9	3	9	30	90	300
Diethyl thiophosphate	DETP	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6
Dihydrocitrinone	DHC	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Dimethoate	DMT	0.000005	0.000015	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05
Dimethyl phosphate	VPM_DMP	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
Dimethyl phthalate	EDCs_DMP	0.008	0.024	0.08	0.24	0.8	2.4	8	24	80
Dioxybenzone	BP-8	0.009	0.027	0.09	0.27	0.9	2.7	9	27	90
Doxycycline	DOX	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Duloxetine	Duloxetine	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Enniatin B	EnnB	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Enniatin B1	EnnB1	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Enrofloxacin	Enro	0.01	0.03	0.1	0.3	1	3	10	30	100
Enterodiol	Enterodiol	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
Enterolactone	Enterolactone	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Equol	Equol	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Erythromycin	Ery	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Estradiol	E2	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3	0.9	3
Estradiol-17-glucuronide	E2_17_GlcA	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
Estradiol-3-sulfate	E2_3_SO4	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5
Estriol	E3	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3	0.9	3
Estrone	E1	0.00003	0.00009	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3
Ethinylestradiol	EthinylE2	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Ethyl protocatechuic acid	OH-EtP	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Ethylparaben	EthylPara	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1
Fenarimol	Fenarimol	0.00003	0.00009	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3
Fipronil	FIPL	0.006	0.018	0.06	0.18	0.6	1.8	6	18	60
Florfenicol	Florf	0.018	0.054	0.18	0.54	1.8	5.4	18	54	180
Fluconazole	Fluco	0.00006	0.00018	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6
Formononetin	Formononetin	0.000025	0.000075	0.00025	0.00075	0.0025	0.0075	0.025	0.075	0.25
Fumonisin B1	FB1	0.01	0.03	0.1	0.3	1	3	10	30	100
Genistein	Genistein	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
Glycitein	Glycitein	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
Glyphosate	Glyphosate	0.005	0.015	0.05	0.15	0.5	1.5	5	15	50
Heptylparaben	HeP	0.00014	0.00042	0.0014	0.0042	0.014	0.042	0.14	0.42	1.4
Ibuprofen	Ibuprofen	0.04	0.12	0.4	1.2	4	12	40	120	400
Ibuprofen_glucuronide	Ibuprofen_GlcA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Imidacloprid	IDC	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Isobutylparaben	IsobutPara	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1
Isoxanthohumol	Isoxanth	0.00001	0.00003	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1
Ivermectin	Iverm	0.00002	0.00006	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2
Jacobine	Jacobine	0.00025	0.00075	0.0025	0.0075	0.025	0.075	0.25	0.75	2.5
Jacobine-N-oxide	JacobineNox	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
Kaempferol	Kaempferol	0.05	0.15	0.5	1.5	5	15	50	150	500
Lomefloxacin	Lomef	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Malathion	MATH	0.00006	0.00018	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6
Malathion dicarboxylic acid	MDA	0.0018	0.0054	0.018	0.054	0.18	0.54	1.8	5.4	18
Matairesinol	Mataires	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
Methiocarb	Methiocarb	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
Methyl protocatechuic acid	OH-MeP	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Methylparaben	MethylPara	0.00025	0.00075	0.0025	0.0075	0.025	0.075	0.25	0.75	2.5
Metribuzin	Metribuzin	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	5-OH-MEHP	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Mono-(3-carboxypropyl) phthalate	MCPP	0.04	0.12	0.4	1.2	4	12	40	120	400
Mono-[(2-carboxymethyl) hexyl] phthalate	MCMHP	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Mono-2-ethylhexyl phthalate	MEHP	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5	4.5	15

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
Mono-benzyl phthalate	MBeP	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Mono-butyl phthalate	MBP	0.005	0.015	0.05	0.15	0.5	1.5	5	15	50
Mono-cyclohexyl phthalate	MCHP	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Mono-hexyl phthalate	MHP	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Mono-isobutyl phthalate	MIBP	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Mono-isodecyl phthalate	MIDP	0.01	0.03	0.1	0.3	1	3	10	30	100
Mono-iso-nonyl phthalate	MINP	0.04	0.12	0.4	1.2	4	12	40	120	400
Mono-methyl phthalate	MMP	0.05	0.15	0.5	1.5	5	15	50	150	500
Mono-n-pentyl phthalate	MnPeP	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Mono-octyl phthalate	MOP	0.04	0.12	0.4	1.2	4	12	40	120	400
Mycophenolic acid	MPA	0.04	0.12	0.4	1.2	4	12	40	120	400
Mycophenolic acid_glucuronide	MPA_GlcA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
N,N-dimethylbenzamide	DBM	0.00006	0.00018	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6
N-butylbenzenesulfonamide	N-butylbenz	0.01	0.03	0.1	0.3	1	3	10	30	100
Nivalenol	NIV	0.02	0.06	0.2	0.6	2	6	20	60	200
N-nitrosodimethylamine	NDMA	0.3	0.9	3	9	30	90	300	900	3000
Nonylphenol	Nonylph	0.025	0.075	0.25	0.75	2.5	7.5	25	75	250
Norfloxacin	Norf	0.024	0.072	0.24	0.72	2.4	7.2	24	72	240
Ochratoxin A	OTA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Ochratoxin alpha	OTalpha	0.004	0.012	0.04	0.12	0.4	1.2	4	12	40
Ochratoxin B	OTB	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Octyl methoxycinnamate	OMC	0.2	0.6	2	6	20	60	200	600	2000
Ofloxacin	Oflox	0.00002	0.00006	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2
Oxytetracycline	OTC	0.006	0.018	0.06	0.18	0.6	1.8	6	18	60
Pefloxacin	Peflox	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120
Perfluorobutanesulfonic acid	PFBS	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Perfluorodecanoic acid	PFDA	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
Perfluorohexanoic acid	PFHxA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Perfluorononanoic acid	PFNA	0.003	0.009	0.03	0.09	0.3	0.9	3	9	30
Perfluorooctanesulfonic acid	PFOS	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5
Perfluorooctanoic acid	PFOA	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5
Perfluoroundecanoic acid	PFUA	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
PhIP	PhIP	0.0001	0.0003	0.001	0.003	0.01	0.03	0.1	0.3	1
p-Hydroxybenzoic acid	pOHBA	0.05	0.15	0.5	1.5	5	15	50	150	500
p-Nitrophenol	3PNP	0.0054	0.0162	0.054	0.162	0.54	1.62	5.4	16.2	54
Prochloraz	Prochloraz	0.000005	0.000015	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05
Propiconazole	Propiconazole	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Propylparaben	PropylPara	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Resveratrol	Resver	0.015	0.045	0.15	0.45	1.5	4.5	15	45	150
Riddelline	Riddelliin	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3	0.9	3
Riddelline-N-oxide	RiddelliinNox	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Roxithromycin	Roxi	0.0054	0.0162	0.054	0.162	0.54	1.62	5.4	16.2	54
Scopolamine	Scopolamine	0.000015	0.000045	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15
SN38	SN38	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
SN38_glucuronide	SN38_GlcA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Sterigmatocystin	STC	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Sulfadiazine	SulfDZ	0.00006	0.00018	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6
Sulfadimethoxine	Sulf-DM	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2	0.6	2
Sulfamethazine	Sulf-M	0.00006	0.00018	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6
Sulfamethoxazole	Sulf-X	0.00006	0.00018	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6
T-2 toxin	T-2	0.1	0.3	1	3	10	30	100	300	1000
Tebuconazole	Tebuconazole	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Tetrabrombisphenol A	TBPA	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Tetracycline	TC	0.012	0.036	0.12	0.36	1.2	3.6	12	36	120

Compounds	Abbreviation	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8	Level 9
Tramadol	Tramadol	0.003	0.009	0.03	0.09	0.3	0.9	3	9	30
trans-3-Hydroxycotinine	t3OHCot	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
Triclocarban	Triclocarban	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Triclosan	Triclosan	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Trimethoprim	Trim	0.0006	0.0018	0.006	0.018	0.06	0.18	0.6	1.8	6
Tri-n-butyl phosphate	TBP	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Triphenyl phosphate	TPhP	0.002	0.006	0.02	0.06	0.2	0.6	2	6	20
Tris(2-chloroethyl)phosphate	TCEP	0.05	0.15	0.5	1.5	5	15	50	150	500
Tylosin	Tylo	0.006	0.018	0.06	0.18	0.6	1.8	6	18	60
Venlafaxine	Venlafaxine	0.003	0.009	0.03	0.09	0.3	0.9	3	9	30
Vinclozolin	Vinclozolin	0.05	0.15	0.5	1.5	5	15	50	150	500
Xanthohumol	Xanth	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
Zearalanone	ZAN	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3	0.9	3
Zearalenone	ZEN	0.0003	0.0009	0.003	0.009	0.03	0.09	0.3	0.9	3
Zearalenone-14-glucuronide	ZEN_14_GlcA	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
Zearalenone-14-sulfate	ZEN_14_SO4	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5
α-Zearalanol	α-ZAL	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
α-Zearalenol	α-ZEL	0.00002	0.00006	0.0002	0.0006	0.002	0.006	0.02	0.06	0.2
α-Zearalenol-14-glucuronide	α-ZEL_14_GlcA	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5
β-Zearalanol	β-ZAL	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1.5	5
β-Zearalenol	β-ZEL	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
β-Zearalenol-14-glucuronide	β-ZEL_14_GlcA	0.00015	0.00045	0.0015	0.0045	0.015	0.045	0.15	0.45	1.5

3. SPE extraction in 96-well plates

Required before SPE process

1. Transfer 400 µL of urine, plasma, or serum into a clean empty 96-well plate (2 mL, WAT058958, Waters Corporation). Use water for the process blanks.
2. Add 4 µL of $^{13}C_{18}$-ZEN (100 ng/mL).
3. Add 396 µL of PBS (200 mM, pH 7.4), resulting in a final volume of 800 µL.
4. Close the plates carefully with a clean sealing mat (186002484, Waters Corporation) by pressing the mat on each well position.
5. Vortex the plate using the plate shaker at 800 rpm for 3 mins with temperature set to room temperature or below.

During SPE process

6. Condition each well of the SPE plate (Oasis PRiME HLB 96-well plates, 30 mg/2 mL, 186008054, Waters Corporation) with 1 mL of methanol followed by 1 mL of water. Wait until the solvent has completely passed through and plates are dried under gravity after each conditioning step.
7. Load the previously prepared 800 µL sample mixture (see step 4 above) on the SPE plate, dry under gravity.
8. Wash the SPE plate twice with 1 mL of water (total 2 mL) under gravity.
9. Dry the SPE plate by applying a negative vacuum of -30 kPa using a 96-well plate manifold for 5 minutes.
10. Elute the SPE plate twice with 200 µL of methanol and collect extracts (total 400 µL) with a fresh 96-well plate (1mL, WAT058957, Waters Corporation). Before removing the SPE plate, collect retained residues of methanol inside the SPE sorbent material using vacuum again for 10 mins (see step 9).
11. Add 4 µL of the ISTD mix to the extracts.
12. Dilute the extracts with 396 µL of water.
13. Close plates carefully with a clean sealing mat (WAT058959, Waters Corporation).
14. Vortex the plate using the plate shaker at 800 rpm for 3 mins with temperature set to room temperature or below.
15. Centrifuge the plate using a CentriVap Vacuum concentrator (Labconco) for 30 s before LC-MS/MS analysis.

SOP #2 – Quantitative targeted LC-MS/MS of 234 biomarkers and toxins in scheduled MRM mode on a SCIEX Qtrap 7500

Version 1

By Yunyun Gu

Oct. 17. 2025

0. This workflow is used for the article

Yunyun Gu, Max L. Feuerstein, Dillon T. Lloydd, Chirag J. Patel, Caroline H. Johnson, Benedikt Warth. "High-performance quantitative exposomics covering up to >230 toxicants and key biomarkers", *Environmental Science & Technology*, 2025, online first, DOI: doi.org/10.1021/acs.est.5c04458

1. LC-MS/MS Measurement

Once the samples are prepared, the following LC-MS/MS method should be followed:

Table 1. Information about LC-MS/MS method.

<table>
<tr><td>Instrument</td><td colspan="4">Agilent Infinity 1290 II UHPLC with SCIEX QTrap 7500</td></tr>
<tr><td>Acquisition method</td><td colspan="4">240814_XtremeXeno.msm (MS method)
241113_XtremeXeno_Uniformed_V3.lcm (LC method)
A copy is located at: lmch\ 40 MSC raw files\QTrap7500\DATA \2024.9.9_SCIEX OS Data\2024 XtremXeno\Acquisition Methods</td></tr>
<tr><td>Injection volume</td><td colspan="4">5 µL</td></tr>
<tr><td>Column temperature</td><td colspan="4">40 °C</td></tr>
<tr><td>Column</td><td colspan="4">Acquity UPLC HSS T3 (2.1 × 100 mm, 1.8 µm, Waters)
With a VanGuard precolumn (1.8 µm, Waters)</td></tr>
<tr><td>Flow rate</td><td colspan="4">0.4 mL/min</td></tr>
<tr><td>Needle wash</td><td colspan="4">Methanol/acetonitrile/isopropanol/water (1/1/1/1, v/v/v/v)</td></tr>
<tr><td>Eluent A</td><td colspan="4">0.3 mM of Ammonium fluoride</td></tr>
<tr><td>Eluent B</td><td colspan="4">Acetonitrile</td></tr>
<tr><td>Gradient</td><td colspan="4"><table><tr><th>Time (min)</th><th>Eluent B (%)</th><th>Flow rate (mL/min)</th></tr><tr><td>0.00</td><td>5.0</td><td>0.40</td></tr><tr><td>1.00</td><td>5.0</td><td>0.40</td></tr><tr><td>1.80</td><td>18.0</td><td>0.40</td></tr><tr><td>4.20</td><td>35.0</td><td>0.40</td></tr><tr><td>13.00</td><td>48.0</td><td>0.40</td></tr><tr><td>15.80</td><td>90.0</td><td>0.40</td></tr><tr><td>15.81</td><td>98.0</td><td>0.40</td></tr><tr><td>17.60</td><td>98.0</td><td>0.40</td></tr><tr><td>17.70</td><td>5.0</td><td>0.40</td></tr><tr><td>20.00</td><td>5.0</td><td>0.40</td></tr></table></td></tr>
<tr><td>Curtain gas</td><td colspan="4">45 arb</td></tr>
<tr><td>Ion source gas 1</td><td colspan="4">50 arb</td></tr>
<tr><td>Ion source gas 2</td><td colspan="4">50 arb</td></tr>
<tr><td>CAD gas</td><td colspan="4">9 arb</td></tr>
<tr><td>Ion spray voltage</td><td colspan="4">+2500V/-2500V</td></tr>
<tr><td>MRM transitions</td><td colspan="4">See Table 8 below</td></tr>
</table>

Table 2. Multiple Reaction Monitoring (MRM) transitions of 243 target analytes and eleven isotope-labeled internal standards, including names, abbreviations, retention times (RT), retention time windows (± s), dwell times, precursor ion m/z (Q1), product ion m/z (Q3), entrance potential (EP), collision energy (CE), collision cell exit potential (CXP), and polarity settings. For each analyte, one MRM transition was selected for quantification (denoted by "_1" at the end of the transition name) and a second for qualification (denoted by "_2").

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
1	16-Epiestriol	16EpiE3	16EpiE3_1	5.92	20	10	287.101	145	-10	-50	-13	Negative
			16EpiE3_2	5.92	20	3	287.101	171.1	-10	-52	-16	Negative
2	16-α-Hydroxyestrone	16OHE1	16OHE1_1	6.01	20	10	285.056	145	-10	-52	-13	Negative
			16OHE1_2	6.01	20	3	285.056	143	-10	-78	-16	Negative
3	17-Epiestriol	17EpiE3	17EpiE3_1	6.21	20	10	287.093	145	-10	-52	-10	Negative
			17EpiE3_2	6.21	20	3	287.093	143.1	-10	-70	-4	Negative
4	1-Hydroxypyrene	1OHPyrene	1OHPyrene_1	14.49	90	20	216.997	189	-10	-46	-7	Negative
			1OHPyrene_2	14.49	90	10	216.997	188	-10	-47	-13	Negative
5	2-Naphthol	2-Napht	2-Napht_1	7.26	20	10	143.01	115	-10	-32	-13	Negative
			2-Napht_2	7.26	20	3	143.01	114	-10	-44	-10	Negative
6	2-Phenylphenol	2-PP	2-PP_1	10.55	90	20	168.98	141.093	-10	-34	-11	Negative
			2-PP_2	10.55	90	5	168.98	115.05	-10	-42	-9	Negative
7	2-tert-Butylphenol	2-tert-But	2-tert-But_1	13.3	60	20	148.999	132.9	-10	-28	-16	Negative
			2-tert-But_2	13.3	60	10	148.999	93	-10	-31	-10	Negative
8	2,4-Dichlorophenoxyacetic acid	2,4-D	2,4-D_1	4.19	60	8	219	161	-10	-16	-19	Negative
			2,4-D_2	4.19	60	2	219	125	-10	-42	-33	Negative
9	2,4,5-Trichlorophenol	2,4,5-TCP	2,4,5-TCP_1	11.72	90	20	194.873	158.629	-10	-22	-11	Negative
10	2,5-Dichlorophenol	2,5-DCP	2,5-DCP_1	4.3	90	8	160.88	124.956	-10	-24	-16	Negative
11	2-Methoxyestrone	2MeOE1	2MeOE1_1	11.55	90	20	299.097	284.2	-10	-32	-7	Negative
			2MeOE1_2	11.55	90	5	299.097	159.9	-10	-50	-16	Negative
12	2-Methoxyestradiol	2MeOE2	2MeOE2_1	9.93	90	20	301.089	286.1	-10	-32	-25	Negative
			2MeOE2_2	9.93	90	5	301.089	285.2	-10	-46	-19	Negative

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
13	3-Benzylidenecamphor	3-BC	3-BC_1	16.2	20	20	241.088	91	10	53	12	Positive
			3-BC_2	16.2	20	10	241.088	165.1	10	59	9	Positive
14	3-Hydroxyphenanthrene	3OHPhen	3OHPhen_1	11.42	90	20	193.008	165	-10	-42	-11	Negative
			3OHPhen_2	11.42	90	5	193.008	164.2	-10	-46	-10	Negative
15	3 Phenoxybenzoic acid	3PBA	3PBA_1	4.84	30	8	213	93	-10	-26	-15	Negative
	p-Nitrophenol	3PNP	3PNP_1	5.05	30	8	138	108	-10	-22	-5	Negative
			3PNP_2	5.05	30	2	138	46	-10	-58	-19	Negative
17	4-Methylbenzylidene camphor	4-MBC	4-MBC_1	16.64	20	20	255.143	104.8	10	47	12	Positive
			4-MBC_2	16.64	20	10	255.143	141.2	10	62	12	Positive
18	4-Methyl-benzophenone	4-MBP	4-MBP_1	14.61	20	20	197.128	104.941	10	26	8	Positive
			4-MBP_2	14.61	20	10	197.128	76.958	10	43	10	Positive
19	4-Hydroxybenzophenone	4-OH-BP	4-OH-BP_1	7.2	20	10	199.194	121.025	10	24	16	Positive
			4-OH-BP_2	7.2	20	3	199.19	105	10	24	20	Positive
20	4-Octylphenol	4-OP	4-OP_1	16.63	20	20	205.093	105.9	-10	-27	-10	Negative
			4-OP_2	16.63	20	10	205.093	118.9	-10	-55	-10	Negative
21	4-Phenylphenol	4-PP	4-PP_1	9.75	45	20	168.945	92.972	-10	-44	-11	Negative
			4-PP_2	9.75	45	5	168.945	141.119	-10	-42	-13	Negative
22	4-tert-Octylphenol	4-tert-OP	4-tert-OP_1	16.06	20	20	205.078	133	-10	-36	-10	Negative
			4-tert-OP_2	16.06	20	10	205.078	134	-10	-25	-16	Negative
23	4-Methoxy estrone	4MeOE1	4MeOE1_1	10.98	90	20	299.008	284.1	-10	-30	-19	Negative
			4MeOE1_2	10.98	90	5	299.008	283.2	-10	-44	-21	Negative
24	4-Methoxy Estradiol	4MeOE2	4MeOE2_1	9.28	20	20	301.078	286.1	-10	-32	-22	Negative
			4MeOE2_2	9.28	20	5	301.078	284.9	-10	-50	-19	Negative
25	4-Hydroxyestrone	4OHE1	4OHE1_1	8.2	60	20	285.188	161.1	-10	-54	-11	Negative
			4OHE1_2	8.2	60	10	285.188	159.1	-10	-68	-13	Negative
26	Mono-(2-ethyl-5-hydroxyhexyl) phthalate	5-OH-MEHP	5-OH-MEHP_1	4.93	90	8	293.066	76.998	-10	-46	-4	Negative

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
			5-OH-MEHP_2	4.93	90	2	293.066	121.002	-10	-24	-13	Negative
27	8-Prenylnaringenin	8-Pn	8-Pn_1	11.69	90	20	339.023	219	-10	-30	-16	Negative
			8-Pn_2	11.69	90	5	339.023	119	-10	-38	-7	Negative
28	Aristolochic acid I	AAI	AAI_1	6.55	150	10	358.866	298	10	16	44	Positive
			AAI_2	6.55	150	3	358.866	296	10	26	44	Positive
29	Acrylamide	Acrylamide	Acrylamide_1	0.99	20	10	72	55	10	16	6	Positive
			Acrylamide_2	0.99	20	3	72	44	10	27	5	Positive
30	Aflatoxin B1	AFB1	AFB1_1	6.22	20	10	313	241	10	49	14	Positive
			AFB1_2	6.22	20	3	313	213	10	61	16	Positive
31	Aflatoxin B1-N7-guanine	AFB1_Gua	AFB1_Gua_1	3.66	20	8	480	152.1	10	23	10	Positive
			AFB1_Gua_2	3.66	20	2	480	135	10	60	14	Positive
32	Aflatoxin B2	AFB2	AFB2_1	5.72	20	10	315	243	10	53	16	Positive
			AFB2_2	5.72	20	3	315	203	10	49	12	Positive
33	Aflatoxin G1	AFG1	AFG1_1	5.71	20	10	329.1	243.1	10	39	14	Positive
			AFG1_2	5.71	20	3	329.1	200	10	59	12	Positive
34	Aflatoxin G2	AFG2	AFG2_1	5.29	20	8	331.1	313.2	10	35	18	Positive
			AFG2_2	5.29	20	2	331.1	245.2	10	43	14	Positive
35	Aflatoxicol	AFL	AFL_1	6.71	20	10	297	269.1	10	29	12	Positive
			AFL_2	6.71	20	3	297	115	10	83	14	Positive
36	Aflatoxin M1	AFM1	AFM1_1	4.94	20	8	329.1	273.2	10	35	16	Positive
			AFM1_2	4.94	20	2	329.1	229.1	10	59	12	Positive
37	Aflatoxin M2	AFM2	AFM2_1	4.94	20	8	331	259	10	33	16	Positive
			AFM2_2	4.94	20	2	331	241	10	57	14	Positive
38	Aflatoxin P1	AFP1	AFP1_1	4.8	60	8	299.1	270.7	10	35	18	Positive
			AFP1_2	4.8	60	2	299.1	215.1	10	38	11	Positive
39	Aflatoxin Q1	AFQ1	AFQ1_1	4.94	20	8	328.7	206	10	33	14	Positive

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
			AFQ1_2	4.94	20	2	328.7	177	10	47	12	Positive
40	Aristolactam I	AL	AL_1	10.45	90	20	293.928	279	10	43	39	Positive
			AL_2	10.45	90	5	293.928	250.9	10	46	24	Positive
41	Alternariol	Alternariol	Alternariol_1	6.74	20	10	257	213	-10	-32	-13	Negative
			Alternariol_2	6.74	20	3	257	215	-10	-36	-16	Negative
42	Alternariol monomethyl ether	AME	AME_1	11.38	60	20	271.1	256	-10	-30	-15	Negative
			AME_2	11.38	60	5	271.1	228	-10	-40	-17	Negative
43	Amoxicillin	Amox	Amox_1	1.14	40	10	366	349	10	13	20	Positive
			Amox_2	1.14	40	3	366	114	10	29	20	Positive
44	Ampicillin	Ampi	Ampi_1	2.8	20	10	350	106	10	23	14	Positive
			Ampi_2	2.8	20	3	350	192	10	25	24	Positive
45	Anisodamine	Anisodamine	Anisodamine_1	3.2	100	10	306.1	140.1	10	34	18	Positive
			Anisodamine_2	3.2	100	3	306.1	122.1	10	35	21	Positive
46	Acetaminophen	APAP	APAP_1	2.64	20	10	151.983	110.1	10	23	18	Positive
			APAP_2	2.64	20	3	151.983	93	10	29	14	Positive
47	Acetaminophen_glucuronide	APAP_GlcA	APAP_GlcA_1	1	20	10	325.899	150	-10	-34	-9	Negative
			APAP_GlcA_2	1	20	3	325.899	113	-10	-18	-21	Negative
48	Acetamiprid	ATMP	ATMP_1	4.7	20	8	223	126	10	29	10	Positive
			ATMP_2	4.7	20	2	223	99	10	53	12	Positive
49	Atrazine	ATZ	ATZ_1	7.6	20	20	216	174	10	25	14	Positive
			ATZ_2	7.6	20	10	216	96	10	33	12	Positive
50	Avobenzone	AVO	AVO_1	15.9	20	20	311.27	134.954	10	32	14	Positive
			AVO_2	15.9	20	10	311.27	161.005	10	32	18	Positive
51	Bis(1-chloro-2-propyl) phosphate	BCIPP	BCIPP_1	3.53	60	8	248.892	34.999	-10	-76	-18	Negative
52	Bis(1,3-dichloro-2-propyl) phosphate	BDCPP	BDCPP_1	15.57	90	20	317.119	35.003	-10	-44	-17	Negative
			BDCPP_2	15.57	90	10	318.905	35.003	-10	-52	-17	Negative

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
53	Beauvericin	BEA	BEA_1	17.34	20	20	801.5	244.2	10	42	14	Positive
			BEA_2	17.34	20	10	801.5	134	10	99	14	Positive
54	Benzyl Butyl Phthalate	BenzButPht	BenzButPht_1	16.22	20	20	313.044	91	10	38	12	Positive
			BenzButPht_2	16.22	20	10	313.044	149	10	18	16	Positive
55	Benzophenone 1	BP-1	BP-1_1	8.94	90	20	213.037	91	-10	-38	-10	Negative
			BP-1_2	8.94	90	5	213.037	134.9	-10	-28	-10	Negative
56	Benzophenone 2	BP-2	BP-2_2	5.42	21	2	245.063	135	-10	-22	-9	Negative
			BP-2_1	5.42	21	8	245.063	109	-10	-30	-9	Negative
	Benzylparaben	BenzPara	BenzPara_1	10.49	90	20	226.992	92.1	-10	-32	-7	Negative
			BenzPara_2	10.49	90	5	226.992	136	-10	-20	-11	Negative
58	2-Hydroxy-4-methoxybenzophenone	BP-3	BP-3_1	14.65	20	20	229.25	150.953	10	26	22	Positive
			BP-3_2	14.65	20	10	229.25	104.929	10	26	12	Positive
59	Benzophenone-6	BP-6	BP-6_1	13.39	90	20	275.193	150.987	10	24	8	Positive
			BP-6_2	13.39	90	10	275.193	108.06	10	62	10	Positive
60	Dioxybenzone	BP-8	BP-8_1	10.98	45	20	245.246	120.991	10	24	14	Positive
			BP-8_2	10.98	45	5	245	150.9	10	26	21	Positive
61	Bisphenol A	BPA	BPA_1	8.17	20	20	226.981	133	-10	-34	-9	Negative
			BPA_2	8.17	20	10	226.981	116.9	-10	-56	-11	Negative
62	Bisphenol A_glucuronide	BPA_GlcA	BPA_GlcA_1	4.25	60	8	402.849	227	-10	-40	-13	Negative
63	Bisphenol AF	BPAF	BPAF_1	12.07	90	20	334.945	265.1	-10	-30	-13	Negative
			BPAF_2	12.07	90	10	334.945	176.8	-10	-58	-10	Negative
64	Bisphenol AP	BPAP	BPAP_1	11.85	90	20	289.054	274.028	-10	-29	-15	Negative
			BPAP_2	11.85	90	5	289.054	210.997	-10	-39	-19	Negative
65	Bisphenol B	BPB	BPB_1	9.95	90	20	241.064	212.1	-10	-24	-15	Negative
			BPB_2	9.95	90	5	241.064	210.9	-10	-38	-11	Negative
66	Bisphenol C	BPC	BPC_1	11.8	60	20	255.044	240.2	-10	-26	-13	Negative

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
			BPC_2	11.8	60	5	255.044	147	-10	-38	-7	Negative
67	Bisphenol E	BPE	BPE_1	7.22	30	10	213.042	198.182	-10	-26	-21	Negative
			BPE_2	7.22	30	3	213.042	196.962	-10	-36	-11	Negative
68	Bisphenol F	BPF	BPF_1	6.3	20	10	198.64	105	-10	-30	-7	Negative
			BPF_2	6.3	20	3	198.64	106	-10	-28	-5	Negative
69	Bisphenol FL	BPFL	BPFL_1	13.95	60	20	348.992	255.992	-10	-39	-15	Negative
			BPM_2	15.56	20	10	345	133.1	-10	-50	-9	Negative
70	Bisphenol M	BPM	BPM_1	15.56	20	20	345	251	-10	-42	-35	Negative
	Bisphenol S	BPS	BPS_1	4.76	20	8	249.09	108	-10	-36	-7	Negative
			BPS_2	4.76	20	2	249.09	155.9	-10	-30	-11	Negative
72	Bisphenol Z	BPZ	BPZ_1	12.4	60	20	267	173	-10	-38	-23	Negative
			BPZ_2	12.4	60	10	267	145	-10	-50	-13	Negative
73	Bromoacetic acid	Br.ac.ac	Br.ac.ac_1	0.74	21	10	136.905	78.9	-10	-14	-10	Negative
74	Butylparaben	ButylPara	ButylPara_1	10.17	60	20	193.051	92	-10	-32	-11	Negative
			ButylPara_2	10.17	60	5	193.051	137	-10	-22	-13	Negative
75	Cefaclor	Cefac	Cefac_1	2.85	20	10	368	174	10	21	18	Positive
			Cefac_2	2.85	20	3	368	106	10	35	12	Positive
76	Chlorpyrifos	CFs	CFs_1	16.66	20	20	352	200	10	27	16	Positive
77	Chalcone	Chalcone	Chalcone_1	14.9	20	20	209.143	130.986	10	24	27	Positive
			Chalcone_2	14.9	20	10	209.143	102.973	10	38	18	Positive
78	Chloramphenicol	Chloram	Chloram_1	5.1	20	8	321	152	-10	-24	-35	Negative
			Chloram_2	5.1	20	2	321	194	-10	-18	-15	Negative
79	Chrysin	Chrysin	Chrysin_1	9.78	60	20	255.187	152.95	10	44	12	Positive
			Chrysin_2	9.78	60	5	255.187	102.975	10	46	12	Positive
80	Ciprofloxacin	Cipro	Cipro_1	4.8	150	8	332	288	10	29	16	Positive
			Cipro_2	4.8	100	2	332	245	10	25	18	Positive

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
81	Citrinin	CIT	CIT_1	4.21	60	8	251	233.1	10	23	14	Positive
			CIT_2	4.21	60	2	251	205.1	10	37	18	Positive
82	Clarithromycin	Clari	Clari_1	9.36	150	20	748	158	10	35	20	Positive
			Clari_2	9.36	150	5	748	590	10	27	38	Positive
83	Cotinine	Cotinine	Cotinine_1	3	20	10	176.9	80	10	32	9	Positive
			Cotinine_2	3	20	3	176.9	98	10	32	12	Positive
84	Coumestrol	Coumestrol	Coumestrol_1	6.35	20	10	267.003	265.9	-10	-40	-17	Negative
			Coumestrol_2	6.35	20	3	267.003	211	-10	-40	-13	Negative
85	Chlorpyrifos-methyl	CPM	CPM_1	15.94	20	20	324	125	10	25	10	Positive
			CPM_2	15.94	20	10	324	292	10	23	18	Positive
86	Chlorotetracycline	CTC	CTC_1	4.8	150	8	479	444	10	31	28	Positive
			CTC_2	4.8	100	2	479	461	10	23	32	Positive
87	Culmorin	CUL	CUL_1	16.97	45	20	297.4	59	-10	-35	-11	Negative
88	Culmorin_11_glucuronide	CUL_11_GlcA	CUL_11_GlcA_1	4.72	90	8	413.5	113.1	-10	-40	-11	Negative
			CUL_11_GlcA_2	4.72	90	2	413.5	175.1	-10	-35	-11	Negative
89	Daidzein	Daidzein	Daidzein_1	5.15	21	8	253.026	224	-10	-36	-17	Negative
			Daidzein_2	5.15	21	2	253.026	207.9	-10	-44	-11	Negative
90	Daidzein_glucuronide	Daidzein_GlcA	Daidzein_GlcA_1	2.94	90	10	429.14	113	-10	-22	-17	Negative
			Daidzein_GlcA_2	2.94	90	3	429.14	252.8	-10	-32	-23	Negative
91	Danofloxacin	Danof	Danof_1	4.41	210	8	358	340	10	31	22	Positive
			Danof_2	4.41	100	2	358	314	10	25	24	Positive
92	N,N-dimethylbenzamide	DBM	DBM_1	4.32	40	8	150	105	10	21	8	Positive
			DBM_2	4.32	40	2	150	77	10	37	6	Positive
93	Diethyl dithiophosphate	DEDTP	DEDTP_1	1.59-2.71	Full window	10	185	111	-10	-28	-17	Negative
			DEDTP_2	1.59-2.71	Full window	3	185	156	-10	-20	-11	Negative
94	Diethyl phthalate	DEP	DEP_1	10.25	45	20	223.184	148.929	10	28	8	Positive

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
			DEP_2	10.25	45	5	223.184	176.993	10	14	12	Positive
95	Diethyl thiophosphate	DETP	DETP_1	3.5	150	8	169	95	-10	-24	-17	Negative
			DETP_2	3.5	150	2	169	141	-10	-18	-23	Negative
96	Dihydrocitrinone	DHC	DHC_2	4.44	60	2	265	246.9	-10	-26	-15	Negative
			DHC_1	4.44	60	8	265	176.9	-10	-34	-13	Negative
97	Dibromoacetic acid	Di.Br.Ac.ac	Di.Br.Ac.ac_1	1.13	20	10	216.8	172.8	-10	-16	-10	Negative
			Di.Br.Ac.ac_2	1.13	20	3	216.8	80.9	-10	-37	-7	Negative
98	Dichloroacetic acid	Di.Cl.Ac.ac	Di.Cl.Ac.ac_1	0.73	43	10	126.9	83	-10	-14	-7	Negative
			Di.Cl.Ac.ac_2	0.73	43	3	126.9	35	-10	-30	-3	Negative
99	Dibutyl phthalate	DiButPht	DiButPht_1	16.34	20	20	279.043	148.9	10	20	12	Positive
			DiButPht_2	16.34	20	10	279.043	204.8	10	11	21	Positive
100	Diazinon	DIZN	DIZN_1	15.75	20	20	305	153	10	29	14	Positive
			DIZN_2	15.75	20	10	305	169	10	29	14	Positive
101	Dimethyl phthalate	EDCs_DMP	EDCs_DMP_1	6.44	20	10	195.163	162.978	10	16	20	Positive
			EDCs_DMP_2	6.44	20	3	195.163	76.984	10	40	12	Positive
102	Dimethyl phosphate	VPM_DMP	VPM_DMP_1	0.7	35	10	125	79	-10	-34	-7	Negative
			VPM_DMP_2	0.7	35	3	125	63	-10	-22	-11	Negative
103	Dimethoate	DMT	DMT_1	4.52	20	8	230	199	10	13	14	Positive
			DMT_2	4.52	20	2	230	125	10	29	8	Positive
104	Deepoxy-deoxynivalenol	DOM-1	DOM-1_1	3.4	20	8	280.9	109.1	10	21	12	Positive
			DOM-1_2	3.4	20	2	280.9	137.1	10	19	10	Positive
105	Deoxynivalenol	DON	DON_1	2.97	20	10	296.9	249.3	10	15	16	Positive
			DON_2	2.97	20	3	296.9	203	10	21	12	Positive
106	Doxycycline	DOX	DOX_1	4.3	150	8	445	428	10	25	38	Positive
			DOX_2	4.3	50	2	445	267	10	47	8	Positive
107	Di-2-propylheptyl phthalate	DPHP	DPHP_1	17.46	90	20	447.306	148.927	10	50	14	Positive

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108	Duloxetine	Duloxetine	Duloxetine_1	8	90	20	298.366	153.965	10	10	16	Positive
109	Estrone	E1	E1_1	10.5	50	20	269.4	144.8	-10	-49	-10	Negative
			E1_2	10.5	50	5	269.4	143	-10	-71	-10	Negative
110	Estradiol	E2	E2_1	8.87	50	20	271.1	144.9	-10	-52	-13	Negative
			E2_2	8.87	50	5	271.1	183	-10	-54	-13	Negative
111	Estradiol-17-glucuronide	E2_17_GlcA	E2_17_GlcA_1	4.24	100	8	447.105	113	-10	-34	-8	Negative
			E2_17_GlcA_2	4.24	100	2	447.105	271	-10	-38	-15	Negative
112	Estradiol-3-sulfate	E2_3_SO4	E2_3_SO4_1	5.15	100	8	351.046	271.1	-10	-50	-22	Negative
			E2_3_SO4_2	5.15	100	2	351.046	80	-10	-68	-7	Negative
113	Estriol	E3	E3_1	5.01	20	8	286.98	171	-10	-48	-16	Negative
			E3_2	5.01	20	2	286.98	145	-10	-54	-7	Negative
114	Enniatin B	EnnB	EnnB_1	17.71	60	20	657.5	196.3	10	45	18	Positive
			EnnB_2	17.71	60	10	657.5	214.1	10	47	18	Positive
115	Enniatin B1	EnnB1	EnnB1_1	18.31	60	20	671.4	196	10	43	12	Positive
			EnnB1_2	18.31	40	10	671.4	210	10	41	12	Positive
116	Enrofloxacin	Enro	Enro_1	5.5	210	8	360	316	10	27	20	Positive
			Enro_2	5.5	100	2	360	245	10	37	16	Positive
117	Enterodiol	Enterodiol	Enterodiol_1	5.3	30	8	301.085	253.1	-10	-32	-19	Negative
			Enterodiol_2	5.3	30	2	301.085	271.1	-10	-34	-17	Negative
118	Enterolactone	Enterolactone	Enterolactone_1	6.79	30	10	297.059	253.1	-10	-30	-19	Negative
			Enterolactone_2	6.79	30	3	297.059	107	-10	-34	-10	Negative
119	Equol	Equol	Equol_1	6.49	30	10	241.069	120.9	-10	-20	-11	Negative
			Equol_2	6.67	30	3	241.069	118.9	-10	-30	-7	Negative
120	Erythromycin	Ery	Ery_1	6.47	90	10	734	158	10	29	46	Positive
			Ery_2	6.47	90	3	734	576	10	35	6	Positive
121	Ethinylestradiol	EthinylE2	EthinylE2_1	10.26	40	20	295.1	145	-10	-48	-10	Negative

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
			EthinylE2_2	10.26	40	5	295.1	159	-10	-46	-10	Negative
122	Ethylparaben	EthylPara	EthylPara_1	6.06	20	10	164.943	92.1	-10	-34	-7	Negative
			EthylPara_2	6.06	20	3	164.943	136.9	-10	-18	-22	Negative
123	Fumonisin B1	FB1	FB1_1	5.22	100	8	722.5	334.4	10	57	4	Positive
			FB1_2	5.22	100	2	722.5	352.3	10	55	12	Positive
124	Fenarimol	Fenarimol	Fenarimol_1	11.9	60	20	330.943	267.9	10	31	34	Positive
			Fenarimol_2	11.9	60	5	330.943	189.1	10	63	32	Positive
125	Fipronil	FIPL	FIPL_1	15.44	20	20	454	437	10	25	14	Positive
			FIPL_2	15.44	20	10	454	368	10	39	14	Positive
126	Florfenicol	Florf	Florf_1	4.68	30	8	356	336	-10	-26	-55	Negative
			Florf_2	4.68	30	2	356	185	-10	-14	-17	Negative
127	Fluconazole	Fluco	Fluco_1	4	20	8	307	220	10	25	24	Positive
			Fluco_2	4	20	2	307	238	10	23	14	Positive
128	Formononetin	Formononetin	Formononetin_1	7.93	30	20	267.001	252	-10	-30	-7	Negative
			Formononetin_2	7.93	30	10	267.001	223	-10	-44	-13	Negative
129	Genistein	Genistein	Genistein_1	6.4	20	10	268.988	133.1	-10	-40	-11	Negative
			Genistein_2	6.4	20	3	268.988	132	-10	-58	-11	Negative
130	Glycitein	Glycitein	Glycitein_1	5.35	20	8	284.977	270	10	37	22	Positive
			Glycitein_2	5.35	20	2	284.977	241.9	10	45	18	Positive
131	Glyphosate	Glyphosate	Glyphosate_2	3.09	185	3	170.161	152.002	10	13	12	Positive
			Glyphosate_1	3.09	185	10	170.161	106.956	10	19	12	Positive
132	Heptylparaben	HeP	HeP_1	15.71	20	20	235.087	92.028	-10	-36	-7	Negative
			HeP_2	15.71	20	10	235.087	136.039	-10	-26	-15	Negative
133	5-Hydroxymethylfurfural	HMF	HMF_1	2.3	20	10	126.9	109	10	14	6	Positive
			HMF_2	2.3	20	3	126.9	81	10	23	9	Positive
134	5-Hydroxymethyl-2-furanoic acid	HMFA	HMFA_1	0.7	20	10	140.9	97.1	-10	-12	-7	Negative

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			HMFA_2	0.7	20	3	140.9	69	-10	-18	-7	Negative
135	Ibuprofen	Ibuprofen	Ibuprofen_1	9.70-10.56	Full window	20	205.072	161.12	-10	-12	-15	Negative
136	Ibuprofen_glucuronide	Ibuprofen_GlcA	Ibuprofen_GlcA_1	5.79	90	10	380.958	174.96	-10	-16	-9	Negative
			Ibuprofen_GlcA_2	5.79	90	3	380.958	161.102	-10	-24	-15	Negative
137	Imidacloprid	IDC	IDC_1	4.37	20	8	256	209	10	23	12	Positive
			IDC_2	4.37	20	2	256	175	10	27	16	Positive
138	2-isopropyl-4-methyl-6-hydroxypyrimidine	IMPy	IMPy_1	3.12	20	10	153	84	10	25	12	Positive
			IMPy_2	3.12	20	3	153	70	10	25	8	Positive
139	Isobutylparaben	IsobutPara	IsobutPara_1	9.98	60	20	192.995	92	-10	-32	-5	Negative
			IsobutPara_2	9.98	60	5	192.995	135.9	-10	-24	-13	Negative
140	Isoxanthohumol	Isoxanth	Isoxanth_1	8.4	40	20	355.063	179	10	33	12	Positive
			Isoxanth_2	8.4	40	10	355.063	298.9	10	17	28	Positive
141	Ivermectin	Iverm	Iverm_1	17.99	20	20	897	753	10	59	54	Positive
142	Jacobine	Jacobine	Jacobine_1	3.25	90	10	352	155	10	38	21	Positive
			Jacobine_2	3.25	90	3	352	119.9	10	42	18	Positive
143	Jacobine-N-oxide	JacobineNox	JacobineNox_1	3.1	20	10	368	296	10	34	30	Positive
			JacobineNox_2	3.1	20	3	368	120.1	10	44	14	Positive
144	Kaempferol	Kaempferol	Kaempferol_1	6.71	200	10	284.952	184.9	-10	-40	-15	Negative
			Kaempferol_2	6.71	200	3	284.952	117.1	-10	-54	-13	Negative
145	Lomefloxacin	Lomef	Lomef_1	4.8	210	8	352	308	10	33	16	Positive
			Lomef_2	4.8	100	2	352	265	10	23	22	Positive
146	Matairesinol	Mataires	Mataires_1	6.51	20	10	357.058	83	-10	-30	-7	Negative
			Mataires_2	6.51	20	3	357.058	137.1	-10	-30	-8	Negative
147	Malathion	MATH	MATH_2	14.52	20	10	331	127	10	17	12	Positive
			MATH_1	14.52	20	20	331	285	10	12	20	Positive

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
148	Mono-benzyl phthalate	MBeP	MBeP_1	5	90	8	255.135	77.025	-10	-24	-9	Negative
			MBeP_2	5	90	2	255.135	183.148	-10	-14	-13	Negative
149	Mono-butyl phthalate	MBP	MBP_1	4.43	200	8	220.994	77.2	-10	-26	-7	Negative
			MBP_2	4.43	200	2	220.994	71.1	-10	-18	-10	Negative
150	Mono-cyclohexyl phthalate	MCHP	MCHP_1	5.6	150	10	247.023	97.003	-10	-22	-13	Negative
			MCHP_2	5.6	150	3	247.023	77.012	-10	-36	-7	Negative
151	Mono-[(2-carboxymethyl) hexyl] phthalate	MCMHP	MCMHP_1	4.59	120	8	307.026	159.056	-10	-16	-15	Negative
			MCMHP_2	4.59	120	2	307.026	113.109	-10	-44	-7	Negative
152	Mono-(3-carboxypropyl) phthalate	MCPP	MCPP_1	2.1	90	10	250.977	102.986	-10	-12	-9	Negative
			MCPP_2	2.1	90	3	250.977	165.016	-10	-16	-15	Negative
153	Malathion dicarboxylic acid	MDA	MDA_1	2.27	100	10	273	141	-10	-14	-13	Negative
			MDA_2	2.27	100	3	273	157	-10	-35	-9	Negative
154	Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	MECPP_1	4.56	200	8	307.033	159.076	-10	-20	-10	Negative
			MECPP_2	4.56	200	2	307.033	113.067	-10	-40	-13	Negative
155	Mono-2-ethylhexyl phthalate	MEHP	MEHP_1	7.77-8.59	Full window	20	277.039	134	-10	-22	-16	Negative
			MEHP_2	7.77-8.59	Full window	10	277.039	127.1	-10	-14	-4	Negative
156	Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP	MEOHP_1	4.93	90	8	291.02	120.97	-10	-28	-3	Negative
			MEOHP_2	4.93	90	2	291.02	143.099	-10	-22	-17	Negative
157	Methiocarb	Methiocarb	Methiocarb_1	10.75	60	20	226.039	168.7	10	15	20	Positive
			Methiocarb_2	10.75	60	5	226.039	121.1	10	29	16	Positive
158	Methylparaben	MethylPara	MethylPara_1	4.93	30	8	151.009	92	-10	-28	-10	Negative
			MethylPara_2	4.93	30	2	151.009	135.9	-10	-20	-7	Negative
159	Metribuzin	Metribuzin	Metribuzin_2	6.26	20	3	215.204	145.018	10	23	10	Positive
			Metribuzin_1	6.26	20	10	215.204	187.032	10	25	22	Positive

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
160	Mono-hexyl phthalate	MHP	MHP_1	5.26-5.81	Full window	20	248.952	77.055	-10	-34	-17	Negative
			MHP_2	5.26-5.81	Full window	10	248.952	98.925	-10	-22	-5	Negative
161	Mono-isobutyl phthalate	MIBP	MIBP_2	4.42	90	2	220.964	77.002	-10	-24	-9	Negative
			MIBP_1	4.42	90	8	220.964	134.06	-10	-18	-7	Negative
162	Mono-isodecyl phthalate	MIDP	MIDP_1	12.26-13.00	Full window	20	305.065	155.125	-10	-22	-15	Negative
			MIDP_2	12.26-13.00	Full window	10	305.065	76.988	-10	-36	-13	Negative
163	Mono-iso-nonyl phthalate	MINP	MINP_1	9.95-10.64	Full window	20	291.087	141.029	-10	-24	-13	Negative
			MINP_2	9.95-10.64	Full window	10	291.087	76.954	-10	-34	-7	Negative
164	Mono-methyl phthalate	MMP	MMP_2	2.19	90	3	178.906	107.058	-10	-14	-13	Negative
			MMP_1	2.19	90	10	178.906	76.994	-10	-26	-9	Negative
165	Mono-n-pentyl phthalate	MnPeP	MnPeP_1	4.63-4.80	Full window	10	235.016	77.008	-10	-28	-17	Negative
			MnPeP_2	4.63-4.80	Full window	3	235.016	85.101	-10	-20	-15	Negative
166	Mono-octyl phthalate	MOP	MOP_1	8.20-9.15	Full window	20	276.972	127.032	-10	-24	-11	Negative
			MOP_2	8.20-9.15	Full window	5	276.972	76.98	-10	-34	-9	Negative
167	Mycophenolic acid	MPA	MPA_2	7.89	200	10	318.912	274.9	-10	-22	-19	Negative
			MPA_1	7.89	200	20	318.912	191	-10	-32	-39	Negative
168	Mycophenolic acid_glucuronide	MPA_GlcA	MPA_GlcA_1	3.75	100	8	494.883	318.9	-10	-28	-33	Negative
			MPA_GlcA_2	3.75	100	2	494.883	175	-10	-20	-19	Negative
169	N-butylbenzenesulfonamide	N-butylbenz	N-butylbenz_1	9	30	20	211.917	140.9	-10	-32	-10	Negative
			N-butylbenz_2	9	30	5	211.917	64.8	-10	-26	-11	Negative
170	N-nitrosodimethylamine	NDMA	NDMA_1	1.3	20	10	74.9	43.1	10	20	6	Positive
			NDMA_2	1.3	20	3	74.9	58	10	17	15	Positive
171	Nivalenol	NIV	NIV_1	2.8	60	10	371	281.1	-10	-20	-19	Negative
			NIV_2	2.8	60	3	371	59.1	-10	-40	-9	Negative

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
172	Nonylphenol	Nonylph	Nonylph_1	16.57	21	20	219.117	133	-10	-46	-10	Negative
			Nonylph_2	16.57	21	10	219.117	134	-10	-25	-10	Negative
173	Norfloxacin	Norf	Norf_1	4.65	204	8	320	276	10	25	18	Positive
			Norf_2	4.65	100	2	320	233	10	35	16	Positive
174	Ofloxacin	Oflox	Oflox_1	4.87	210	8	362	318	10	27	20	Positive
175	Ethyl protocatechuic acid	OH-EtP	OH-EtP_1	4.95	30	8	180.986	107.964	-10	-32	-11	Negative
			OH-EtP_2	4.95	30	2	180.986	152.954	-10	-20	-11	Negative
176	Methyl protocatechuic acid	OH-MeP	OH-MeP_1	3.99	30	8	166.938	107.963	-10	-26	-9	Negative
			OH-MeP_2	3.99	30	2	166.938	152.013	-10	-20	-11	Negative
177	Octyl methoxycinnamate	OMC	OMC_1	17.09	21	20	291.1	179	10	14	6	Positive
			OMC_2	17.09	21	10	291.1	161.1	10	29	12	Positive
178	Ochratoxin A	OTA	OTA_1	5.7	200	10	404	239	10	37	16	Positive
			OTA_2	5.7	200	3	404	102	10	105	14	Positive
179	Ochratoxin alpha	OTalpha	OTalpha_1	3.98	60	8	254.9	166.9	-10	-36	-11	Negative
			OTalpha_2	3.98	60	2	254.9	123	-10	-40	-17	Negative
180	Ochratoxin B	OTB	OTB_1	5.21	100	8	370.1	205	10	33	12	Positive
			OTB_2	5.13	100	2	370.1	103.1	10	77	16	Positive
181	Oxytetracycline	OTC	OTC_1	3.81	90	8	461	426	10	25	28	Positive
			OTC_2	3.81	90	2	461	443	10	17	28	Positive
182	Pefloxacin	Peflox	Peflox_1	4.99	210	8	334	290	10	25	22	Positive
183	Perfluorobutanesulfonic acid	PFBS	PFBS_1	5.87	90	10	298.854	79.921	-10	-68	-13	Negative
			PFBS_2	5.87	90	3	298.854	98.888	-10	-34	-13	Negative
184	Perfluorodecanoic acid	PFDA	PFDA_1	10.52-11.54	Full window	20	512.81	468.865	-10	-14	-21	Negative
			PFDA_2	10.52-11.54	Full window	10	512.81	218.912	-10	-24	-11	Negative
185	Perfluorohexanoic acid	PFHxA	PFHxA_2	5.69	90	3	312.95	118.8	-10	-28	-7	Negative

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
			PFHxA_1	5.69	90	10	312.868	268.937	-10	-14	-15	Negative
186	Perfluorononanoic acid	PFNA	PFNA_1	8.64-9.59	Full window	20	462.826	418.782	-10	-12	-11	Negative
			PFNA_2	8.64-9.59	Full window	5	462.826	218.883	-10	-28	-17	Negative
187	Perfluorooctanoic acid	PFOA	PFOA_1	6.97-7.71	Full window	20	412.922	368.9	-10	-14	-25	Negative
			PFOA_2	6.97-7.71	Full window	5	412.922	168.9	-10	-26	-13	Negative
188	Perfluorooctanesulfonic acid	PFOS	PFOS_1	11.17-12.33	Full window	20	498.782	80	-10	-110	-10	Negative
			PFOS_2	11.17-12.33	Full window	10	498.782	98.9	-10	-91	-10	Negative
189	Perfluoroundecanoic acid	PFUA	PFUA_1	14.82	150	20	562.836	518.898	-10	-17	-7	Negative
			PFUA_2	14.82	150	10	562.836	268.862	-10	-28	-13	Negative
190	PhIP	PhIP	PhIP_1	5.17	20	8	225.1	210.1	10	42	24	Positive
			PhIP_2	5.17	20	2	225.1	140.1	10	67	12	Positive
191	p-Hydroxybenzoic acid	pOHBA	pOHBA_1	1.32	60	10	136.919	92.9	-10	-19	-7	Negative
			pOHBA_2	1.32	60	3	136.919	65	-10	-42	-7	Negative
192	Prochloraz	Prochloraz	Prochloraz_1	15.04	20	20	375.874	307.8	10	18	26	Positive
			Prochloraz_2	15.04	20	10	375.874	265.7	10	23	26	Positive
193	Propiconazole	Propiconazole	Propiconazole_1	14.84	20	20	342.146	158.907	10	38	18	Positive
			Propiconazole_2	14.84	20	10	342.146	204.914	10	24	12	Positive
194	Propylparaben	PropylPara	PropylPara_1	7.55	30	10	178.955	92	-10	-30	-10	Negative
			PropylPara_2	7.55	30	3	178.955	93	-10	-24	-7	Negative
195	Resveratrol	Resver	Resver_1	5.04	30	8	227.015	185	-10	-26	-13	Negative
			Resver_2	5.04	30	2	227.015	142.9	-10	-36	-13	Negative
196	Riddelline	Riddelliin	Riddelliin_1	3.07	100	10	350	120	10	42	30	Positive
			Riddelliin_2	3.07	100	3	350	138	10	34	39	Positive
197	Riddelline-N-oxide	RiddelliinNox	RiddelliinNox_2	3.02	20	3	366	118	10	46	16	Positive
			RiddelliinNox_1	3.02	20	10	366	94.064	10	59	16	Positive

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
198	Roxithromycin	Roxi	Roxi_1	11.37	150	20	838	680	10	31	38	Positive
			Roxi_2	11.37	150	5	838	158	10	41	38	Positive
199	Scopolamine	Scopolamine	Scopolamine_1	3.3	100	8	304	138.1	10	23	21	Positive
			Scopolamine_2	3.3	100	2	304	156.2	10	23	6	Positive
200	SN38	SN38	SN38_1	5.29	20	8	392.867	348.9	10	37	28	Positive
			SN38_2	5.29	20	2	392.867	248.7	10	61	14	Positive
201	SN38_glucuronide	SN38_GlcA	SN38_GlcA_1	3.32	60	8	569.1	393.044	10	39	26	Positive
			SN38_GlcA_2	3.32	60	2	569.1	349.128	10	55	20	Positive
202	Sterigmatocystin	STC	STC_1	12.66	60	20	325.1	281.1	10	51	16	Positive
			STC_2	12.66	60	10	325.1	310.2	10	35	18	Positive
203	Sulfadimethoxine	Sulf-DM	Sulf-DM_1	5.29	30	8	311	156	10	29	12	Positive
			Sulf-DM_2	5.29	30	2	311	108	10	39	12	Positive
204	Sulfamethazine	Sulf-M	Sulf-M_1	3.86	20	8	279	186	10	25	22	Positive
			Sulf-M_2	3.86	20	2	279	124	10	25	16	Positive
205	Sulfamethoxazole	Sulf-X	Sulf-X_1	4.43	60	8	254	156	10	25	18	Positive
			Sulf-X_2	4.43	60	2	254	108	10	33	8	Positive
206	Sulfadiazine	SulfDZ	SulfDZ_1	3	60	10	251	156	10	21	16	Positive
			SulfDZ_2	3	60	3	251	108	10	31	18	Positive
207	T-2 toxin	T-2	T-2_1	10.38	60	20	467.3	215.2	10	29	18	Positive
			T-2_2	10.38	60	5	467.3	185.1	10	31	11	Positive
208	trans-3-Hydroxycotinine	t3OHCot	t3OHCot_1	2.59	20	10	192.981	80.1	10	45	39	Positive
			t3OHCot_2	2.59	20	3	192.981	134	10	29	21	Positive
209	Tri-n-butyl phosphate	TBP	TBP_1	15.56	40	20	267.225	98.902	10	36	10	Positive
			TBP_2	15.56	40	10	267.225	80.929	10	80	4	Positive
210	Tetrabrombisphenol A	TBPA	TBPA_1	15.99	20	20	542.545	445.8	-10	-44	-11	Negative
			TBPA_2	15.99	20	10	542.545	419.8	-10	-58	-27	Negative

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
211	Tetracycline	TC	TC_1	4.4	240	8	445	410	10	27	28	Positive
			TC_2	4.4	100	2	445	427	10	21	28	Positive
212	2,2',6,6'-Tetrachlorobisphenol A	TCBPA	TCBPA_1	15.52	45	20	363.012	311.883	-10	-36	-28	Negative
			TCBPA_2	15.52	45	10	363.012	200.916	-10	-38	-19	Negative
213	Tris(2-chloroethyl)phosphate	TCEP	TCEP_1	6.99	30	10	285.282	222.95	10	19	8	Positive
			TCEP_2	6.99	30	3	285.282	62.961	10	49	12	Positive
214	3,5,6-Trichloro-2-pyridinol	TCPy	TCPy_1	5.79	150	10	198	107	10	41	12	Positive
			TCPy_2	5.79	150	3	198	134	10	29	14	Positive
215	Tebuconazole	Tebuconazole	Tebuconazole_2	13.93	90	10	308	125	10	52	14	Positive
			Tebuconazole_1	13.93	90	20	308.283	69.986	10	70	8	Positive
216	Triphenyl phosphate	TPhP	TPhP_2	15.63	20	10	327.1	77.1	10	59	10	Positive
			TPhP_1	15.63	20	20	327.1	152.1	10	52	12	Positive
217	Tramadol	Tramadol	Tramadol_1	4.41	60	8	264.271	58.02	10	49	6	Positive
			Tramadol_2	4.41	60	2	264.271	246.155	10	16	30	Positive
218	Triclocarban	Triclocarban	Triclocarban_1	15.8	20	20	313	160	-10	-22	-9	Negative
219	Triclosan	Triclosan	Triclosan_1	15.85	30	20	286.819	35	-10	-38	-10	Negative
			Triclosan_2	15.85	30	20	288.9	36.9	-10	-38	-10	Negative
220	Trimethoprim	Trim	Trim_1	3.59	60	8	291	230	10	33	22	Positive
			Trim_2	3.59	60	2	291	123	10	27	8	Positive
221	Tylosin	Tylo	Tylo_1	7.44	150	10	916	174	10	49	12	Positive
			Tylo_2	7.44	150	3	916	772	10	41	54	Positive
222	Venlafaxine	Venlafaxine	Venlafaxine_1	5.17	90	8	278.313	58.034	10	62	30	Positive
			Venlafaxine_2	5.17	90	2	278.313	120.984	10	42	20	Positive
223	Vinclozolin	Vinclozolin	Vinclozolin_1	6.99	20	10	286.23	223.092	10	18	6	Positive
224	Xanthohumol	Xanth	Xanth_1	15.32	20	20	355.014	179	10	35	18	Positive
			Xanth_2	15.32	20	10	355.014	299	10	17	26	Positive

Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
225	Zearalanone	ZAN	ZAN_1	11.56	100	20	319.2	275.1	-10	-30	-19	Negative
			ZAN_2	11.56	100	5	319.2	161	-10	-38	-10	Negative
226	Zearalenone	ZEN	ZEN_1	11.77	100	20	317.1	175	-10	-34	-16	Negative
			ZEN_2	11.77	100	5	317.1	131	-10	-38	-7	Negative
227	Zearalenone-14-glucuronide	ZEN_14_GlcA	ZEN_14_GlcA_1	4.67	100	8	493.033	317	-10	-36	-21	Negative
			ZEN_14_GlcA_2	4.67	100	2	493.033	112.9	-10	-28	-10	Negative
228	Zearalenone-14-sulfate	ZEN_14_SO4	ZEN_14_SO4_1	6.16	100	10	397.067	316.9	-10	-34	-17	Negative
			ZEN_14_SO4_2	6.16	100	3	397.067	175	-10	-46	-12	Negative
229	α-Zearalanol	α-ZAL	α-ZAL_1	8.83	30	20	321.1	277.1	-10	-30	-19	Negative
			α-ZAL_2	8.83	30	5	321.1	303.1	-10	-30	-22	Negative
230	α-Zearalenol	α-ZEL	α-ZEL_1	9.18	150	20	319.1	160	-10	-42	-11	Negative
			α-ZEL_2	9.18	150	5	319.1	174	-10	-36	-17	Negative
231	α-Zearalenol-14-glucuronide	α-ZEL_14_GlcA	α-ZEL_14_GlcA_1	3.75	120	8	495.07	319.2	-10	-36	-16	Negative
			α-ZEL_14_GlcA_2	3.75	120	2	495.07	113	-10	-28	-9	Negative
232	β-Zearalanol	β-ZAL	β-ZAL_1	7.5	60	10	321.101	277.1	-10	-32	-19	Negative
			β-ZAL_2	7.5	60	3	321.101	303.1	-10	-30	-22	Negative
233	β-Zearalenol	β-ZEL	β-ZEL_1	7.89	60	20	319.101	160	-10	-40	-10	Negative
			β-ZEL_2	7.89	60	10	319.101	275.2	-10	-30	-7	Negative
234	β-Zearalenol-14-glucuronide	β-ZEL_14_GlcA	β-ZEL_14_GlcA_1	3.75	120	8	495.141	319.2	-10	-40	-9	Negative
			β-ZEL_14_GlcA_2	3.75	120	2	495.141	112.9	-10	-30	-8	Negative
Internal standards												
1	13C12-Bisphenol A	BPA_IS	BPA_1_IS	8.17	20	10	239	139	-10	-34	-9	Negative
2	13C6-Butylparaben	ButylPara_IS	ButylPara_1_IS	10.17	60	10	199	98	-10	-32	-11	Negative
3	13C15-Deoxynivalenol	DON_IS	DON_1_IS	2.97	20	10	312.2	263.3	10	17	39	Positive
			DON_2_IS	2.97	20	3	312.2	216	10	25	18	Positive

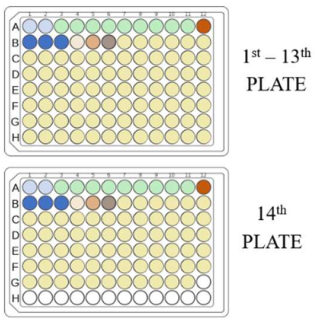
Nr	Compounds	Abbreviation	Transition Name	RT/min	RT window/ (+/- s)	Dwell time (ms)	Q1	Q3	EP/V	CE/V	CXP/V	Polarity
4	D3-Erythromycin	Ery_IS	Ery_1_IS	6.47	90	10	737	579	10	27	40	Positive
			Ery_2_IS	6.47	90	3	737	161	10	37	8	Positive
5	13C6-Ethylparaben	EthylPara_IS	EthylPara_1_IS	6.06	20	10	171	97.9	-10	-34	-7	Negative
6	13C2-Mono-butyl phthalate	MBP_IS	MBP_1_IS	4.43	200	10	225	79	-10	-26	-7	Negative
7	13C6-Methylparaben	MethylPara_IS	MethylPara_1_IS	4.93	30	10	157	97.9	-10	-28	-10	Negative
8	13C8-Perfluorooctanoic acid	PFOA_IS	PFOA_1_IS	11.17-12.33	Full window	10	420.9	375.9	-10	-14	-25	Negative
9	13C8-Perfluorooctanesulfonic acid	PFOS_IS	PFOS_1_IS	11.17-12.33	Full window	10	506.8	79.9	-10	-110	-10	Negative
10	13C6-Propylparaben	PropylPara_IS	PropylPara_1_IS	7.55	30	10	185	97.9	-10	-30	-10	Negative
11	13C18-Zearalenone	ZEN_IS	ZEN_1_IS	11.77	60	10	335.2	185.1	-10	-34	-16	Negative

2. 96-Well Plate and Sequence Design for 1079 Urine Samples

2.1. 96-Well plate design

All urine samples (n=1079) were randomly allocated within 96-well plates. As shown in **Figure 1(A),** each plate included 78 experimental samples (B7-H12), except for the last plate, which contained a lower number. Urine samples were distributed into 14 plates, with 65 samples in the 14th plate (B7-G11)

(A)



(B)

	Content	Replicates	Tech. replicates	Total
	Solvent blank	2	4	8
	Process blank	3	3	9
	External calibration	9 Levels	1 each	9
	Nonspiked pooled QC	1	9	9
	Prespiked pooled QC	1	3	3
	Postpiked pooled QC	1	3	3
	NIST SRM3672/1950	1	3	3
	Sample	78	1	78
			Sum	122

Figure 1. Sample distribution design in 96-well plates for measurement of 1079 human urine samples with 78 samples in each plate from the 1st to the 13th plate, and with 65 samples in the 14th plate. (A), Full plate design including external calibration, quality controls, and sample extracts; (B), Number of replicates and technical replicate injections.

2.2. Sequence design

Multiple QC samples were analyzed throughout the sequence and analytical batches. These include:

(1) Systems suitability testing (SST). The SST mix was injected three times before each analytical batch, and three times after maintenance of the LC-MS/MS system. Note: LC column and LC-MS acquiring method for SST are different from these described in the SOP #2 – Quantitative Targeted LC-MS/MS of 234 biomarkers and toxins in scheduled MRM mode on a SCIEX Qtrap 7500. Please check the SOP of SST for details.

(2) Instrument maintenance. Maintenance was performed after every two 96-well plates which were considered as one analytical batch. It included changing LC eluents, cleaning the curtain plate, exchanging the inline filter installed after the injector, and replacing the pre-column.

(3) Blanks. The first two wells (A1-A2) were filled with solvent (**Figure 1)**, which was MeOH/H_2O (1:1, v/v). Furthermore, triplicate process blanks were measured within each plate (B1-B3).

(4) Calibration. External calibrators occupied wells A3 to A11, ordered from lowest to highest levels.

(5) Non-spiked QC. Prepared by pooling 50 µL of raw samples from 156 randomly selected samples. The QC was distributed into well B3 of each plate prior to sample preparation.

(6) Pre-spiked QC. Prepared by spiking a mixture of 234 standards into pooled urine before the SPE process. The pooled urine was prepared by pooling 200 µL of urine from each of the 156 samples, which were also used to obtain the non-spiked QC after the sample preparation process.

(7) Post-spiked QC. Prepared by spiking the STD mix into extracts of the non-spiked QC. The post-spiked QC was then transferred to well B4 of each plate before analysis. Pre-spiked and post-spiked QCs account for analyte recovery during SPE, matrix effects, and fluctuation of the whole workflow. Spiked concentrations of these QC samples are presented in **"SOP #1 – Extraction of 234 biomarkers and toxins in human urine, serum, and plasma using 96-well plate-based SPE" – Table 4**.

(8) Standard reference materials SRM3672/SRM1950. Extracts of triplicate extractions, prepared using a separate 96-well plate, independent of the analytical batches described beforehand. Extracts were pooled and transferred into well A12 of each plate, see **Figure 1(A)**. This procedure was chosen to reduce the amount of SRM needed for the total analysis, to ensure comparability of the SRM extracts between all batches.

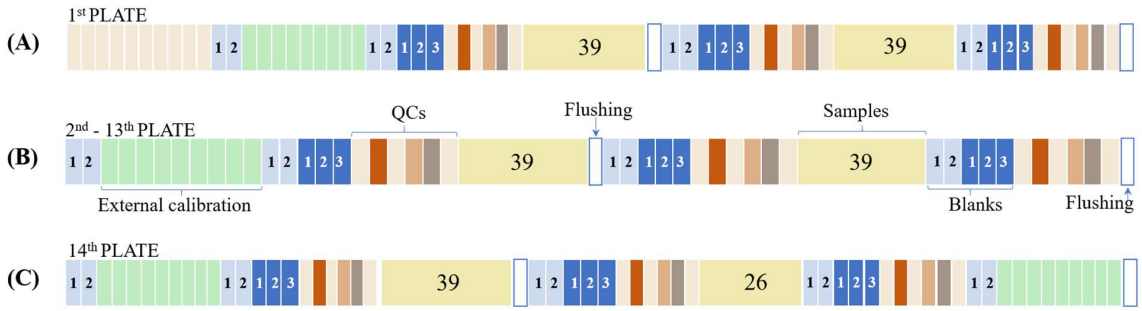


Figure 2. Sequence design for the first plate (A), the second to the 13th plates (B), and the 14th plate (C) with description in Part 1: 96-Well plate design

3. Data Analysis

3.1. Calibration curve

Calibration curves were created by analyzing standards in neat solvent. Concentrations of calibrators are presented in **"SOP #1 – Extraction of 234 biomarkers and toxins in human urine, serum, and plasma using 96-well plate-based SPE" – Table 7.** Sciex OS (version 4.1, Sciex, Vienna) was used to integrate peaks using the MQ4 algorithm and to build calibration curves using 1/x weighting for quantification. Peaks were only integrated if the signal-to-noise ratio was larger than three (S/N>3). For some compounds, a linear-through-zero model was applied to their calibration curves (**Table 10**). Quantitation methods can be found under lmch\40 MSC raw files\QTrap7500\DATA\2024.9.9_SCIEX OS Data\2024 XtremXeno\Quantitation Methods.

Table 1. Compounds with concentrations calibrated with a linear-through-zero model.

Component	Abbreviation
Benzyl Butyl Phthalate	BenzButPht
Cotinine	Cotinine
Dibutyl phthalate	DiButPht
Dimethyl phthalate	EDCs_DMP
Enniatin B	EnnB
Octyl methoxycinnamate	OMC
Tris(2-chloroethyl)phosphate	TCEP
Triphenyl phosphate	TPhP
Vinclozolin	Vinclozolin
2-Naphthol	2-Napht
2-Phenylphenol	2-PP
p-Nitrophenol	3PNP
4-Octylphenol	4-OP
4-Phenylphenol	4-PP
Bisphenol S	BPS
Dichloroacetic acid	Di.Cl.Ac.ac
Mono-methyl phthalate	MMP
Nonylphenol	Nonylph
Perfluorobutanesulfonic acid	PFBS
p-Hydroxybenzoic acid	pOHBA
Mono-2-ethylhexyl phthalate	MEHP

3.2. Quality control of peak integration

A signal-to-noise ratio (S/N) > 3 was used to filter peaks. Peaks in unknown samples were only accepted if the ion ratios between the quantifier and qualifier ions differs less than 20% (50% for level 1-2 calibrators) compared to the neat solvent calibrators, and pre-spiked and post-spiked QC samples. In addition, a retention time deviation of ± 0.1 min is allowed for most compounds. For certain compounds, a larger retention time deviation, ranging from ± 0.2 to ± 1.1 min, is permitted, see **Table 11**.

Table 2. Limits for retention time deviation (min) for 12 compounds.

Compounds	Abbreviation	Transition Name	Limits for retention time deviation/min
Diethyl dithiophosphate	DEDTP	DEDTP_1	1.1
		DEDTP_2	1.1
Ibuprofen	Ibuprofen	Ibuprofen_1	1.0
Mono-2-ethylhexyl phthalate	MEHP	MEHP_1	1.0
		MEHP_2	1.0
Mono-hexyl phthalate	MHP	MHP_1	0.6
		MHP_2	0.6
Mono-isodecyl phthalate	MIDP	MIDP_1	0.8
		MIDP_2	0.8
Mono-iso-nonyl phthalate	MINP	MINP_1	0.7
		MINP_2	0.7
Mono-n-pentyl phthalate	MnPeP	MnPeP_1	0.2
		MnPeP_2	0.2
Mono-octyl phthalate	MOP	MOP_1	0.9
		MOP_2	0.9
Perfluorodecanoic acid	PFDA	PFDA_1	1.0
		PFDA_2	1.0
Perfluorononanoic acid	PFNA	PFNA_1	1.0
		PFNA_2	1.0
Perfluorooctanoic acid	PFOA	PFOA_1	0.8
		PFOA_2	0.8
Perfluorooctanesulfonic acid	PFOS	PFOS_1	1.1
		PFOS_2	1.1

3.3. Correction of measured concentrations

As described in the *Sequence design*, process blank, non-spiked pooled samples, pre-spiked pooled samples, and post-spiked pooled samples must be prepared in each batch. Spiked concentrations in the pooled samples are described in **"SOP #1 – Extraction of 234 biomarkers and toxins in human urine, serum, and plasma using 96-well plate-based SPE" – Table 4**.

Non-spiked pooled samples and pre-spiked pooled samples are used to calculate the apparent recovery (*RE*, see Formula 1). This includes matrix effects (signal suppression and enhancement, SSE), and the extraction recovery. The post-spiked pooled samples are only used to assess MS signal drifts and retention time shifts during the sequence.

$$RE\ (\%) = \frac{C_P - C_n}{C_t} * 100\% \quad [1]$$

RE is the apparent extraction recovery; C_p is the mean concentration of a compound measured in pre-spiked samples; C_n is the mean concentration of the compound measured in non-spiked samples; C_t is the theoretical concentration of the compound.

Reported concentrations were derived as follows: First, the concentrations in the extracts (measured concentration, C_m) were calculated from peak areas using solvent calibration. Afterwards, C_m was corrected for the apparent recovery (*RE*) and average process blank concentration (C_b) was used for blank subtraction (see Formula 2).

$$C_r = \frac{C_m}{RE} - C_b \quad [2]$$

C_R is the reported concentration; C_m is the measured concentration; RE is the apparent extraction recovery; C_b is the mean process blank concentration.

Finally, all concentrations <LOD were reported as not detected (i.e., "<LOD") and all concentrations <LOQ were reported as not detected (i.e., "<LOQ"). The limits of detection and quantification (LODs and LOQs) for the 234 compounds are presented in **Table 12**.

Table 3. Summary of method sensitivity for the 234 analytes: Limits of detection (LODs) and quantification (LOQs) in ng/mL for urine and plasma. NA, not applicable. NA[1], Interfering peaks for specific analytes, exhibiting close retention times with the target analytes in pooled blank matrices, compromised validation results, especially for low-concentration measurements; NA[2], Interfering peaks for specific analytes, detected at identical retention times within pooled blank matrices, adversely affected validation results, particularly at the lower end of the concentration range; NA[3], This compound exhibited significant retention time, causing them to fall outside the scheduled MRM acquisition windows; NA[4], The excessively wide peak shape compromised method sensitivity and influenced validation results; NA[5], These compounds exhibited poor retention or premature elution during the SEP process.

Component	Abbreviation	LOD (ng/mL)		LOQ (ng/mL)	
		Urine	Plasma	Urine	Plasma
3-Benzylidenecamphor	3-BC	0.73	0.40	2.4	1.3
4-Methylbenzylidene camphor	4-MBC	0.18	0.11	0.60	0.38
4-Methyl-benzophenone	4-MBP	1.0	0.41	3.5	1.4
4-Hydroxybenzophenone	4-OH-BP	0.36	0.31	1.2	1.0
Aristolochic acid I	AAI	0.20	0.070	0.67	0.23
Acrylamide	Acrylamide	NA[1]	NA[1]	NA[1]	NA[1]
Aflatoxin B1	AFB1	0.0017	0.00064	0.0056	0.0021
Aflatoxin B1-N7-guanine	AFB1_Gua	0.081	0.024	0.27	0.078
Aflatoxin B2	AFB2	0.0040	0.0016	0.013	0.0053
Aflatoxin G1	AFG1	0.0031	0.0014	0.010	0.0045
Aflatoxin G2	AFG2	0.010	0.0044	0.035	0.015
Aflatoxicol	AFL	0.0023	0.0023	0.0077	0.0078
Aflatoxin M1	AFM1	0.00076	0.00090	0.0025	0.0030
Aflatoxin M2	AFM2	3.1	1.1	10	3.8
Aflatoxin P1	AFP1	0.083	0.023	0.28	0.077
Aflatoxin Q1	AFQ1	0.015	0.0064	0.050	0.021
Aristolactam I	AL	0.0065	0.0046	0.022	0.015
Amoxicillin	Amox	0.90	0.57	3.0	1.9
Ampicillin	Ampi	0.024	0.010	0.080	0.033
Anisodamine	Anisodamine	0.0068	0.0018	0.023	0.0061
Acetaminophen	APAP	10	0.049	32	0.16
Acetamiprid	ATMP	0.00037	0.000012	0.0012	0.000041
Atrazine	ATZ	0.0019	0.0016	0.0063	0.0054
Avobenzone	AVO	1.2	0.46	3.9	1.5
Beauvericin	BEA	0.0033	0.00090	0.011	0.0030
Benzyl Butyl Phthalate	BenzButPht	0.082	0.15	0.27	0.49
2-Hydroxy-4-methoxybenzophenone	BP-3	0.055	0.039	0.18	0.13
Benzophenone-6	BP-6	0.011	0.011	0.038	0.036
Dioxybenzone	BP-8	0.017	0.014	0.058	0.048
Cefaclor	Cefac	0.24	0.045	0.80	0.15
Chlorpyrifos	CFs	NA[2]	NA[2]	NA[2]	NA[2]
Chalcone	Chalcone	0.10	0.037	0.35	0.12
Chrysin	Chrysin	0.72	0.37	2.4	1.2
Ciprofloxacin	Cipro	2.7	0.55	8.9	1.8
Citrinin	CIT	0.073	0.77	0.24	2.6
Clarithromycin	Clari	0.081	0.056	0.27	0.19
Cotinine	Cotinine	0.046	NA[1]	0.15	NA[1]

Component	Abbreviation	LOD (ng/mL)		LOQ (ng/mL)	
		Urine	Plasma	Urine	Plasma
Chlorpyrifos-methyl	CPM	NA[3]	0.19	NA[3]	0.64
Chlorotetracycline	CTC	0.18	0.12	0.60	0.39
Danofloxacin	Danof	NA[4]	NA[4]	NA[4]	NA[4]
N,N-dimethylbenzamide	DBM	0.0024	0.000088	0.0080	0.00029
Diethyl phthalate	DEP	NA[2]	4.6	NA[2]	15
Dibutyl phthalate	DiButPht	2.0	4.9	6.6	16
Diazinon	DIZN	0.0030	0.0033	0.010	0.011
Dimethyl phthalate	EDCs_DMP	0.30	0.21	1.0	0.71
Dimethoate	DMT	0.000000045	0.00000018	0.00000015	0.00000058
Deepoxy-deoxynivalenol	DOM-1	0.20	0.024	0.67	0.080
Deoxynivalenol	DON	1.0	0.028	3.5	0.092
Doxycycline	DOX	0.073	0.050	0.24	0.17
Di-2-propylheptyl phthalate	DPHP	NA[2]	NA[2]	NA[2]	NA[2]
Duloxetine	Duloxetine	0.0073	0.0016	0.024	0.0054
Enniatin B	EnnB	0.0019	0.00068	0.0063	0.0023
Enniatin B1	EnnB1	NA[5]	0.013	NA[5]	0.043
Enrofloxacin	Enro	NA[1]	NA[1]	NA[1]	NA[1]
Erythromycin	Ery	0.091	0.064	0.30	0.21
Fumonisin B1	FB1	0.32	0.42	1.1	1.4
Fenarimol	Fenarimol	0.0025	0.0027	0.0085	0.0090
Fipronil	FIPL	NA[1]	NA[1]	NA[1]	NA[1]
Fluconazole	Fluco	0.0092	0.0018	0.031	0.0062
Glycitein	Glycitein	0.00068	0.0029	0.0023	0.010
Glyphosate	Glyphosate	NA[1]	NA[1]	NA[1]	NA[1]
5-Hydroxymethylfurfural	HMF	0.18	0.13	0.61	0.44
Imidacloprid	IDC	0.00092	0.00087	0.0031	0.0029
2-isopropyl-4-methyl-6-hydroxypyrimidine	IMPy	0.0069	0.0046	0.023	0.015
Isoxanthohumol	Isoxanth	0.0011	0.00056	0.0038	0.0019
Ivermectin	Iverm	NA[5]	NA[5]	NA[5]	NA[5]
Jacobine	Jacobine	0.066	0.018	0.22	0.058
Jacobine-N-oxide	JacobineNox	0.097	0.0023	0.32	0.0075
Lomefloxacin	Lomef	1.3	0.41	4.3	1.4
Malathion	MATH	0.0092	0.0034	0.031	0.011
Methiocarb	Methiocarb	0.0030	NA[5]	0.010	NA[5]
Metribuzin	Metribuzin	0.010	0.0045	0.034	0.015
N-nitrosodimethylamine	NDMA	NA[5]	NA[5]	NA[5]	NA[5]
Norfloxacin	Norf	NA[1]	0.56	NA[1]	1.9
Ofloxacin	Oflox	NA[1]	NA[1]	NA[1]	NA[1]
Octyl methoxycinnamate	OMC	1.7	1.0	5.7	3.3
Ochratoxin A	OTA	0.23	0.0077	0.76	0.026
Ochratoxin B	OTB	0.0065	0.0024	0.022	0.0081
Oxytetracycline	OTC	0.047	0.018	0.16	0.060
Pefloxacin	Peflox	NA[1]	1.0	NA[1]	3.3
PhIP	PhIP	0.0013	0.0024	0.0044	0.0079
Prochloraz	Prochloraz	0.00061	0.00028	0.0020	0.00094

Component	Abbreviation	LOD (ng/mL)		LOQ (ng/mL)	
		Urine	Plasma	Urine	Plasma
Propiconazole	Propiconazole	0.0063	0.00063	0.021	0.0021
Riddelline	Riddelliin	0.093	0.025	0.31	0.084
Riddelline-N-oxide	RiddelliinNox	0.15	0.0087	0.50	0.029
Roxithromycin	Roxi	0.33	0.19	1.1	0.63
Scopolamine	Scopolamine	0.00019	0.000044	0.00063	0.00015
SN38	SN38	0.0049	0.0011	0.016	0.0035
SN38_glucuronide	SN38_GlcA	NA[1]	0.047	NA[1]	0.16
Sterigmatocystin	STC	0.00062	0.00031	0.0021	0.0010
Sulfadimethoxine	Sulf-DM	0.0011	0.00051	0.0038	0.0017
Sulfamethazine	Sulf-M	0.0079	0.00074	0.026	0.0025
Sulfamethoxazole	Sulf-X	0.012	0.0012	0.041	0.0042
Sulfadiazine	SulfDZ	0.0087	0.0030	0.029	0.010
T-2 toxin	T-2	1.5	0.85	4.9	2.8
trans-3-Hydroxycotinine	t3OHCot	0.0054	NA[2]	0.018	NA[2]
Tri-n-butyl phosphate	TBP	0.20	0.15	0.68	0.50
Tetracycline	TC	0.055	0.13	0.18	0.43
Tris(2-chloroethyl)phosphate	TCEP	0.36	0.28	1.2	0.93
3,5,6-Trichloro-2-pyridinol	TCPy	6.8	1.6	23	5.3
Tebuconazole	Tebuconazole	0.0022	0.0019	0.0072	0.0065
Triphenyl phosphate	TPhP	0.27	0.088	0.90	0.29
Tramadol	Tramadol	0.016	0.010	0.054	0.034
Trimethoprim	Trim	0.024	0.0036	0.081	0.012
Tylosin	Tylo	0.083	0.015	0.28	0.050
Venlafaxine	Venlafaxine	0.0058	0.0075	0.019	0.025
Vinclozolin	Vinclozolin	13	1.5	43	5.1
Xanthohumol	Xanth	0.0028	0.00054	0.0093	0.0018
16-Epiestriol	16EpiE3	0.084	0.024	0.28	0.080
16-α-Hydroxyestrone	16OHE1	0.019	0.0079	0.063	0.026
17-Epiestriol	17EpiE3	0.25	0.026	0.83	0.086
1-Hydroxypyrene	1OHPyrene	NA[5]	NA[5]	NA[5]	NA[5]
2-Naphthol	2-Napht	0.023	0.027	0.077	0.090
2-Phenylphenol	2-PP	0.12	0.14	0.41	0.46
2-tert-Butylphenol	2-tert-But	16	14	53	46
2,4-Dichlorophenoxyacetic acid	2,4-D	0.037	0.0061	0.12	0.020
2,4,5-Trichlorophenol	2,4,5-TCP	0.37	0.24	1.2	0.81
2,5-Dichlorophenol	2,5-DCP	NA[1]	1.0	NA[1]	3.5
2-Methoxyestrone	2MeOE1	0.066	0.0093	0.22	0.031
2-Methoxyestradiol	2MeOE2	0.10	0.011	0.34	0.035
3-Hydroxyphenanthrene	3OHPhen	0.0060	0.0044	0.020	0.015
3 Phenoxybenzoic acid	3PBA	0.29	NA[1]	1.0	NA[1]
p-Nitrophenol	3PNP	0.012	0.0095	0.039	0.032
4-Octylphenol	4-OP	NA[2]	1.0	NA[2]	3.3
4-Phenylphenol	4-PP	0.11	0.075	0.36	0.25
4-tert-Octylphenol	4-tert-OP	0.31	0.16	1.0	0.55
4-Methoxy estrone	4MeOE1	0.0054	0.0032	0.018	0.011
4-Methoxy Estradiol	4MeOE2	0.0011	0.00010	0.0035	0.00033
4-Hydroxyestrone	4OHE1	NA[5]	NA[5]	NA[5]	NA[5]

Component	Abbreviation	LOD (ng/mL)		LOQ (ng/mL)	
		Urine	Plasma	Urine	Plasma
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	5-OH-MEHP	0.041	0.00018	0.14	0.00060
8-Prenylnaringenin	8-Pn	0.0090	0.0044	0.030	0.015
Alternariol	Alternariol	0.027	0.010	0.091	0.035
Alternariol monomethyl ether	AME	0.026	0.0074	0.087	0.025
Acetaminophen_glucuronide	APAP_GlcA	NA[1]	NA[1]	NA[1]	NA[1]
Bis(1-chloro-2-propyl) phosphate	BCIPP	0.49	0.30	1.6	1.0
Bis(1,3-dichloro-2-propyl) phosphate	BDCPP	NA[1]	13	NA[1]	44
Benzophenone 1	BP-1	0.0031	0.0018	0.010	0.0059
Benzophenone 2	BP-2	0.0025	0.0016	0.0085	0.0052
Benzylparaben	BenzPara	0.00021	0.00021	0.00071	0.00070
Bisphenol A	BPA	0.09	0.62	0.29	2.1
Bisphenol A_glucuronide	BPA_GlcA	0.011	0.15	0.037	0.50
Bisphenol AF	BPAF	0.0076	0.0049	0.025	0.016
Bisphenol AP	BPAP	0.011	0.0050	0.037	0.017
Bisphenol B	BPB	0.0097	0.0057	0.032	0.019
Bisphenol C	BPC	0.052	0.15	0.17	0.51
Bisphenol E	BPE	0.0087	0.0070	0.029	0.023
Bisphenol F	BPF	0.036	0.011	0.12	0.036
Bisphenol FL	BPFL	0.0058	0.0020	0.019	0.0066
Bisphenol M	BPM	0.026	0.010	0.087	0.035
Bisphenol S	BPS	0.0090	0.0052	0.030	0.017
Bisphenol Z	BPZ	0.023	0.020	0.076	0.067
Bromoacetic acid	Br.ac.ac	0.18	NA[5]	0.61	NA[5]
Butylparaben	ButylPara	0.0019	0.0012	0.0063	0.0040
Chloramphenicol	Chloram	0.0058	0.0017	0.019	0.0058
Coumestrol	Coumestrol	0.0010	0.000039	0.0033	0.00013
Culmorin	CUL	NA[1]	NA[1]	NA[1]	NA[1]
Culmorin_11_glucuronide	CUL_11_GlcA	NA[1]	NA[4]	NA[1]	NA[4]
Daidzein	Daidzein	0.00023	0.00028	0.00076	0.00092
Daidzein_glucuronide	Daidzein_GlcA	NA[1]	0.0053	NA[1]	0.018
Diethyl thiophosphate	DETP	NA[1]	NA[1]	NA[1]	NA[1]
Dihydrocitrinone	DHC	0.054	0.011	0.18	0.036
Dibromoacetic acid	Di.Br.Ac.ac	0.17	NA[5]	0.55	NA[5]
Dichloroacetic acid	Di.Cl.Ac.ac	0.18	NA[5]	0.60	NA[5]
Dimethyl phosphate	VPM_DMP	NA[2]	NA[2]	NA[2]	NA[2]
Estrone	E1	0.0041	0.00029	0.014	0.0010
Estradiol	E2	0.054	0.025	0.18	0.084
Estradiol-17-glucuronide	E2_17_GlcA	NA[1]	NA[1]	NA[1]	NA[1]
Estradiol-3-sulfate	E2_3_SO4	0.0023	0.013	0.0077	0.042
Estriol	E3	0.083	0.033	0.28	0.11
Enterodiol	Enterodiol	0.0021	0.00077	0.01	0.0026
Enterolactone	Enterolactone	0.014	0.011	0.046	0.038
Equol	Equol	0.0040	0.0054	0.013	0.018
Ethinylestradiol	EthinylE2	0.082	0.083	0.27	0.28

Component	Abbreviation	LOD (ng/mL)		LOQ (ng/mL)	
		Urine	Plasma	Urine	Plasma
Ethylparaben	EthylPara	0.0010	0.00079	0.0034	0.0026
Florfenicol	Florf	0.79	0.22	2.6	0.75
Formononetin	Formononetin	0.00031	0.00014	0.0010	0.00048
Genistein	Genistein	0.00032	0.00026	0.0011	0.00087
Heptylparaben	HeP	0.00052	0.00037	0.0017	0.0012
5-Hydroxymethyl-2-furanoic acid	HMFA	NA[5]	0.44	NA[5]	1.5
Ibuprofen_glucuronide	Ibuprofen_GlcA	0.0081	0.10	0.027	0.32
Isobutylparaben	IsobutPara	0.0020	0.0014	0.0068	0.0047
Kaempferol	Kaempferol	0.72	0.51	2.4	1.7
Matairesinol	Mataires	0.084	0.019	0.28	0.063
Mono-benzyl phthalate	MBeP	0.019	0.0066	0.063	0.022
Mono-butyl phthalate	MBP	0.16	0.34	0.54	1.1
Mono-cyclohexyl phthalate	MCHP	0.096	0.049	0.32	0.16
Mono-[(2-carboxymethyl) hexyl] phthalate	MCMHP	0.021	0.013	0.071	0.043
Mono-(3-carboxypropyl) phthalate	MCPP	NA[5]	0.42	NA[5]	1.4
Malathion dicarboxylic acid	MDA	NA[5]	NA[5]	NA[5]	NA[5]
Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	NA[5]	0.021	NA[5]	0.069
Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP	0.035	0.048	0.12	0.16
Methylparaben	MethylPara	0.0092	0.00014	0.031	0.00046
Mono-isobutyl phthalate	MIBP	0.39	0.039	1.3	0.13
Mono-methyl phthalate	MMP	NA[5]	0.29	NA[5]	1.0
Mycophenolic acid	MPA	0.12	0.050	0.41	0.17
Mycophenolic acid_glucuronide	MPA_GlcA	NA[1]	NA[1]	NA[1]	NA[1]
N-butylbenzenesulfonamide	N-butylbenz	0.33	0.049	1.1	0.16
Nivalenol	NIV	NA[5]	NA[5]	NA[5]	NA[5]
Nonylphenol	Nonylph	2.9	0.31	10	1.0
Ethyl protocatechuic acid	OH-EtP	0.0030	0.0031	0.010	0.010
Methyl protocatechuic acid	OH-MeP	0.00019	0.00024	0.00062	0.00081
Ochratoxin alpha	OTalpha	0.045	0.051	0.15	0.17
Perfluorobutanesulfonic acid	PFBS	0.041	0.013	0.14	0.042
Perfluorohexanoic acid	PFHxA	0.021	0.035	0.070	0.12
Perfluoroundecanoic acid	PFUA	0.023	0.011	0.077	0.035
p-Hydroxybenzoic acid	pOHBA	2.2	NA[5]	7.4	NA[5]
Propylparaben	PropylPara	0.0018	0.00023	0.0061	0.00077
Resveratrol	Resver	0.17	0.22	0.57	0.74
Tetrabrombisphenol A	TBPA	0.020	0.0074	0.065	0.025
2,2',6,6'-Tetrachlorobisphenol A	TCBPA	0.0095	0.0029	0.032	0.010
Triclocarban	Triclocarban	0.029	0.065	0.10	0.22
Triclosan	Triclosan	0.032	0.011	0.11	0.036
Zearalanone	ZAN	0.022	0.032	0.073	0.11
Zearalenone	ZEN	0.012	0.0085	0.040	0.028
Zearalenone-14-glucuronide	ZEN_14_GlcA	NA[1]	NA[1]	NA[1]	NA[1]
Zearalenone-14-sulfate	ZEN_14_SO4	0.0083	0.022	0.028	0.073

Component	Abbreviation	LOD (ng/mL)		LOQ (ng/mL)	
		Urine	Plasma	Urine	Plasma
α-Zearalanol	α-ZAL	0.014	0.0065	0.046	0.022
α-Zearalenol	α-ZEL	0.0091	NA[5]	0.030	NA[5]
α-Zearalenol-14-glucuronide	α-ZEL_14_GlcA	NA[1]	NA[1]	NA[1]	NA[1]
β-Zearalanol	β-ZAL	0.020	0.0059	0.067	0.020
β-Zearalenol	β-ZEL	0.014	0.0083	0.047	0.028
β-Zearalenol-14-glucuronide	β-ZEL_14_GlcA	NA[1]	NA[1]	NA[1]	NA[1]
Diethyl dithiophosphate	DEDTP	NA[5]	0.28	NA[5]	0.95
Ibuprofen	Ibuprofen	0.53	0.073	1.8	0.24
Mono-2-ethylhexyl phthalate	MEHP	0.016	NA[2]	0.053	NA[2]
Mono-hexyl phthalate	MHP	0.047	0.024	0.16	0.079
Mono-isodecyl phthalate	MIDP	0.15	0.095	0.51	0.32
Mono-iso-nonyl phthalate	MINP	0.27	0.20	0.89	0.67
Mono-n-pentyl phthalate	MnPeP	0.032	0.0033	0.11	0.011
Mono-octyl phthalate	MOP	0.79	NA[2]	2.6	NA[2]
Perfluorodecanoic acid	PFDA	0.011	0.0047	0.036	0.016
Perfluorononanoic acid	PFNA	0.013	0.0052	0.044	0.017
Perfluorooctanoic acid	PFOA	0.0051	0.11	0.0170	0.37
Perfluorooctanesulfonic acid	PFOS	0.0081	NA[2]	0.027	NA[2]