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List of abbreviations

IS	Indicator substance
ISTD	Internal standard
no.	Number
GC	Gas chromatography
MSD	Mass selective detector
FTIR	Fourier-transform infrared spectrometer
SIM	Single Ion Monitoring
Rt	Retention time
m/z	Mass divided by charge number
R^2	Coefficient of determination
SY3	Solvent Yellow 3
R1	Disperse Red 1
CV	Coefficient of variation
SD	Standard deviation
ATR	Attenuated total reflection
NIR	Near infrared
HPLC	High pressure liquid chromatography
CE	Capillary electrophoresis
DAD	Diode-array-detector
TLC	Thin-layer chromatography
HPTLC	High Performance Thin Layer Chromatography
T	Transmission
FG	Force Gauge
C.I.	Color Index
IUPAC	International Union of Pure and Applied Chemistry

1. Introduction

Azo dyes are synthetic dyes with one or more azo groups,
they are synthesized by the diazotization of aromatic amines to form compounds and subsequent reaction with suitable coupling components.
In the textile-industry, azo dyes are used in a prohibited manner for cost reasons. The average concentration of colorants in our clothing is approx. 0.25% for light shades and 3.5% for dark shades.
(Fassold et al. 1999)

The subject of the Azofarbstoff-VO 2004 are azo dyes that are obtained by reductive cleavage of one or more azo groups of one or more aromatic amines in detectable concentrations (>30 ppm) in the product or in dyed parts of it, and in textile-articles which are in direct contact with the human skin or oral cavity directly and for a prolonged period (e.g. clothing, bed linen, towels, hairpieces, wigs, hats, diapers, sleeping bags, shoes, gloves, watch straps, upholstery fabrics, textile toys and toys with textile clothing) are used. The regulation also states that it is prohibited to place on the market articles in the manufacture of which azo dyes have been used. *(RIS 2013)*

After oral or percutaneous ingestion, the dyes enter the blood and accumulate in the liver, water-soluble direct dyes, due to their poor resorption, pass unchanged into the large intestine where they are splitted reductively by anaerobic bacteria and get excreted. The aromatic amines are absorbed and undergo further metabolic transformations in the liver, before being further metabolized in the kidney and bladder and subsequently excreted in the urine. The bioavailability is low, and therefore concentrations below the limit value (30 ppm) can probably be classified as harmless. *(Fassold et al. 1999)*

The released aromatic amines are considered to have a carcinogenic effect (in terms of bladder and liver tumors), especially in the case of dyes consisting of doubly diazotized benzidine and components derived from benzidine. *(Fassold et al. 1999)*

In connection with the negative effects and the metabolic cleavage of azo dyes on the skin by bacteria, there are mainly animal studies on rodents. The results from the studies are not transferable to humans, as our skin has a poorer permeability for the substances. In addition, the metabolism has not yet been fully clarified, which has the consequence that a non-detection of aromatic amines (biomarker) in the urine is not equivalent to a lack of metabolization of the azo dye to free aromatic amines in the skin or liver. *(Brüning et al. 2009)*

In addition, it is assumed that the released aromatic amines can lead to allergic reactions or asthma. The ingestion of azo dyes can be especially problematic for infants and young children if the textiles exceed the limits. The tendency is for goods from countries such as China, India, Korea, Taiwan, and Argentina. *(Fassold et al. 1999)*

In humans, toxic effects have been described exclusively after exposure to soluble azo dyes, but not to insoluble azo pigments. Information on acute toxicity from wearing textiles dyed with azo dyes does not exist. Nevertheless, there are risk groups, such as people with inflammatory bowel disease, where the absorption of azo dyes is increased, the same effect can occur by certain antibiotics. Occupational groups such as painters, varnishers, workers in the printing or textile industry and hairdressers are particularly affected by contamination. *(Brüning et al. 2009)*

For this reason, the research work is concerned with the validation of a GC-MS-based method for the detection of azo dyes in fibers (textiles). The ÖNORM EN14362-1 and EN14362-3 specify the methodology. For the implementation and validation of the methodology, real samples are spiked with analytes and parameters such as the limit of quantification, limit of detection, linearity, repeatability, precision, robustness etc. are determined. In addition, sample preparation techniques for the extraction of the dyes (extraction with Chem Elut S cartridges, extraction with Xylol, and separation of the amines with a diatomaceous earth column, liquid-liquid extraction without a diatomaceous earth column) are tested to determine the ideal method for the determination of azo dyes. *(Austrian Standards International & CEN 2017)*, *(Austrian Standards International & CEN 2 2017)*

Unlike other studies and validation attempts, the developed method directly examines the efficiency of extraction and reductive cleavage. In addition, 7 different disperse dyes and 1 solvent dye are investigated for their amine cleavage products. In similar studies, only different amounts of the amine stock solution were added with buffer and analyzed from the step of separation and restriction of amines.

FTIR is used to check the suitability of the fiber-type in advance.
This approach is new and can help to reduce the risk and exposure to azo dyes in textiles, and to determine different fiber types which are suitable for the analysis.

Since the analysis of azo dyes in textiles can produce numerous amines, the number of analytes is very high. This leads to the problem of matrix effects and the differentiation of isomers that differ in their retention time by only seconds. Aromatic amines in textiles were also analyzed by HPLC.

Liquid chromatography is an efficient method, for the analysis of azo dyes. With a LC triple Quad MS, it is also possible to distinguish isomers. Thereby, it is always necessary to perform two types of analysis to provide meaningful results. *(Giovanni et al. 2014)*

The aim of this work is to implement a new method for the fast discrimination of fiber types (FTIR), as well as to develop a method for a subsequent GC-MS measurement, based on the existing ISO Norm 14362. With accurate measurement parameters and the appropriate column, the analysis time in gas chromatography can also be drastically reduced. In addition, with a measurement in SIM-mode, the detector sensitivity can be increased and the lower limit of quantification and detection can be decreased. The use of SIM mode results in analytes generating unique and high m/z ions that exhibit high abundance. The amines produce high-intensity molecular ions under electron ionization in SIM mode. These molecular ions are unique to their corresponding components. Therefore, it is possible to obtain precise results with a GC-MS method. Additionally GC-MS is faster and very sensitive compared to LC-MS in literature.*(Stevens 2016, Giovanni et al. 2014)*

2. Literature review

Textile dyeing exists for more than 4000 years. Except for the last 150 years, dyes have been obtained exclusively from natural sources. The big change in dyes came after the discovery of mauveine by William Henry Perkin in 1856, when he tried to find a way to synthesize quinine. *(Benkhaya et al. 2020)*

Nowadays various organic dyes are used to color different textile products. Their chemical structures are diverse and include azo- and nitro-dyes, phthalocyanine- and diarylmethane-dyes with very different chemical and physical properties. Approximately 70 % of all the dyes used in industry are azo dyes. *(Benkhaya et al. 2020)*

Chemical compounds that have the property of coloring other materials are referred as “colorants”. According to DIN 55934, these are colorants that are soluble in their application medium. Insoluble colorants are called “pigments”. *(Moragues & Song 2022)*

If a compound absorbs a certain partial range of the visible spectrum from 400 - 800 nm, it is colored. The color effect is based on the presence of several conjugated chromophores, which impart color to a compound by selective light absorption. The human eye perceives the non-absorbed, scattered and reflected part of the spectrum. It corresponds to the complementary color of the absorbed light. However, the compound only becomes a colorant when it adheres to the carrier material. In the simplest case, this occurs with dyes through a functional group. *(Fassold et al.1999)*

2.1 Azo-dyes

2.1.1 Introduction and regulations for azo-dyes:

Azo colorants are the most important colorants for dyeing clothing-textiles. They are characterized by the azo group R - N = N – R and they are produced by coupling single or multiple diazotized arylamines and can be degraded to aromatic amines under reductive conditions. Additionally, they can be cleaved in metabolism to the corresponding aromatic amines. In the case of some of these dyes, carcinogenic amines are formed during cleavage. *(Platzek 1999)*

Azo dyes are not only used for dyeing textiles, also for food-coloring. If the dyes tartrazine (E102), yellow orange (E110), azorubine (E122), cochineal red A (E124), allura red (E129) or quinoline yellow (E104) are used in a food-product, the product must bear the label "may impair activity and attention in children". In addition, azo dyes are also used in fats, oils, wood,

paper, cosmetic products, leather, plastic and rubber products, pharmaceuticals, paints, varnishes and wood stains.
(McCann et al. 2007)

It should be mentioned that textiles are less regulated in the European Union (EU) and the United States (US) than other consumer products such as toys and cosmetics, which are subject to more stringent requirements. Nevertheless, it is critical that clothing textiles are evaluated for their safety in terms of exposure to potentially hazardous chemicals used in the manufacturing process. *(Keshava et al. 2023)*

Regulations:
In the EU, Regulation (EC) 1907/2006 provides the legal basis for the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). This regulation restricts the use of certain azo dyes, which can be cleaved to form one (or more) of the 22 listed aromatic amines (see azo dye regulation 2004), which have carcinogenic potential.

Since the possible cleavage products of all azo dyes on the market have not yet been researched and new types of dyes are constantly being developed, there are numerous other amines that can be produced by reductive cleavage which are not regulated yet.
(Keshava et al. 2023), *(REACH-VO 2021)*

There is also a voluntary eco-labelling called OEKO-TEX®, which further restricts the use of some allergenic dyes. *(Carlsson 2022)*

The basic limit of aromatic amines in textiles is 30mg/kg (ppm). In other regions of the world, such as China, these limits are even stricter at 20mg/kg. *(Fassold et al.1999)*

2.1.2 Structure, azo-dye types and occurrence of azo dyes:

Structure:
The structure of an azo dye is divided into 3 parts, as shown in the following figure 1. The chromophore, the auxochrome and the matrix. The chromophore consists of groups of atoms, such as nitro (–NO2), azo (–N=N–), nitroso (–N=O), thiocarbonyl (–C=S), carbonyl (–C=O) and alkenes (–C=C–). The absorption of electromagnetic waves by the chromophore is due to the excitation of electrons of a molecule. The chromogenic molecule can be colored by adding other atomic groups called "auxochromes." The auxochromic groups serve to fix the dyes and can change the color of the dye. They are acidic (COOH, SO3 and OH) or basic (NH2, NHR and NR2). The rest of the atoms of the molecule correspond to the matrix. *(Benkhaya et al. 2020)*

The distribution of dyes in water increased with the increase in the molecular weight of the azo dyes, in form of increased azo bonds, resulting in a decrease in the rate degradation of azo dyes. *(Benkhaya et al. 2 2020)*

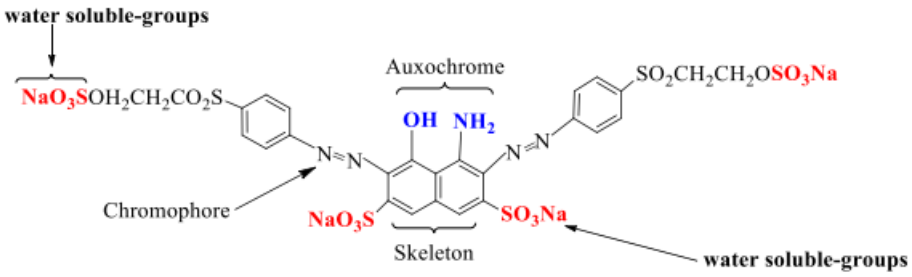


Figure 1: Structure of an azo reactive dye.

Azo-dye types:

Acid dyes (acidic or anionic dyes): They are water-soluble and contain one or more ionic substituents (mainly sulfone groups). They are mainly used for dyeing natural fibers of animal origin (wool) and polyamide fibers in the textile industry. (also for leather, paper, jute, straw, anodized aluminum, insecticides, fertilizers, plastics, wood stains, clear lacquers, glazes, cosmetics, food and beverages).

The name acid dye is derived from the dyeing process, as they are applied to the substrate in acidic solution. The dyeing effect is based on a salt formation of the basic groups of the protein of the carrier material with the acidic groups of the dyes. *(Fassold et al. 1999), (Brüning et al. 2009)*

Examples of acid dyes are Acid Red 26 (C.I. No. 16150), Acid Red 114 (C.I. No. 23635) and Acid Violet 49 (C.I. No. 42640). (DIN 54231:2022-09)

Basic dyes (basic or cationic dyes): contain amino groups which are not free but are included in the resonance. Basic dyes are mainly used for dyeing polyacrylonitrile fibers and anionically modified polyester fibers. (also for paper and leather dyeing, production of flexographic printing inks, ballpoint pen pastes and stamp inks). *(Brüning et al. 2009)*

Examples of basic dyes are Basic Yellow 2 (C.I. No. 41000), Basic Red 9 (C.I. No. 42500) and Basic Green 4 (C.I. No. 42000). (DIN 54231:2022-09)

Direct Dyes (substantive dyes): they are used for dyeing cotton, paper and leather. (as well as ink, watercolors, wood stains, some printing inks, synthetic resin, regenerated cellulose, cellophane, hair dyes and soap).
The water-soluble, elongated molecules accumulate in the cavities of the macromolecules of cellulose. There they aggregate and can no longer be washed out. *(Fassold et al. 1999)*

Examples of direct dyes are Direct Black 38 (C.I. No. 30235), Direct Blue 6 (C.I. No. 22610) and Direct Brown 95 (C.I. No. 30145). (DIN 54231:2022-09)

Disperse dyes: they contain no dissociating functions, but hydrophobic groups such as -OR, -NR2, -NO2 or -CN, which are often also hydrogen bond donors or acceptors. The dyes dissolve only slightly in water without being completely insoluble. Disperse dyes are suitable for dyeing hydrophobic fibers such as polyester or acetyl cellulose. They are absorbed by the fiber from an aqueous dispersion and adhere to it, probably as a result of hydrogen bonds, dipole-dipole and Van der Waals forces between the fiber and the dye. *(Fassold et al. 1999)*

Examples of disperse dyes are Disperse Blue 3 (C.I.-No. 64500), Disperse Orange 3 (C.I.-No. 11005) and Disperse Red 1 (C.I.-No. 11110). The disperse dyes represent the largest group of azo dyes with potential health hazards. (DIN 54231:2022-09)

Reactive Dyes: a very versatile and modern dyeing method for fabrics is reactive dyeing. In this process, the dye and fiber react to form a covalent bond. The reaction leading to the bond is either a nucleophilic substitution or a nucleophilic addition. Reactive dyes are particularly suitable for dyeing cellulose, wool and polyamide. *(Fassold et al. 1999)*

Examples of reactive dyes are Reactive Orange 107, Reactive Blue 2 and Reactive Red 227.

Solvent Dyes: are water-insoluble dyes that are soluble in various organic solvents such as alcohols, esters or hydrocarbons. Solvent dyes are used as a component of paints, for coloring petroleum products (Sudan dyes), wax, inks and various transparent plastics. Solvent dyeing is not effective for natural fiber dyeing. It is only suitable for dyeing man-made fibers mainly polyester, nylon and acetate. *(Meghmani 2022)*

Examples of solvent dyes are Solvent Yellow 3 (C.I. No. 11160) and Solvent Yellow 14 (C.I. No. 11160). (DIN 54231:2022-09)

***Digression* - nomenclature:**
The nomenclature of azo dyes is very complex, with many representatives, but also with different names for the same dye. IUPAC (International Union of Pure and Applied Chemistry) has approved trivial names for azo dyes, such as tatrazine. However, tatrazine is also known as Acid Yellow 23, Food Yellow 4, trisodium-5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-

sulfonatophenyl)diazen-1-yl]-1H-pyrazole-3-carboxylate, flavazine or E102. The structure of the dye can be seen in the following figure 2:

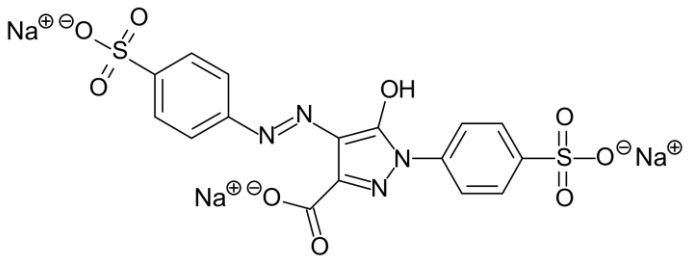


Figure 2: C.I. Acid Yellow 23.

Azo dyes are classified according to the number of their azo bonds into monoazo-, diazo-, triazo-, polyazo- and azoic-compounds. The Colour Index (CI) is a reference for all common colorants and dye base chemicals. In the CI-system, azo dyes are identified by numbers ranging from 11.000 to 39.999 according to their chemical structure. The color index number developed by the society of dyers and colorists is used to classify dyes. The correct designation according to Color Index would be C.I. Acid Yellow 23. The "Acid" indicates the application type, "Yellow" the color and "23" the identification number. *(Benkhaya et al. 2 2020)*

***Digression* - Isomers of aromatic amines:**

Isomers have the same molecular formula but a different structural formula. Azo dyes can become aromatic amines by reductive cleavage, these aromatic amines have different forms of isomers. What makes it very difficult to distinguish the individual structures analytically. As an example, if the isomers of toluidine are all incorrectly identified as *o*-toluidine, this can lead to incorrect rejection of the textile sample. *o*-toluidine is an important starting compound for the production of the dye Solvent Yellow 3. The isomer *p*-toluidine is used in the production of dyes (C.I. Basic Red 9), textile auxiliaries, pharmaceuticals, crop protection agents and vulcanization accelerators. *m*-Toluidine is present in aviation gasoline, developers in color photography and electrophotographic materials and is used in the manufacture of dyes (C.I. Direct Yellow 50), vulcanization accelerators, textile auxiliaries, pharmaceuticals and polyester resins. However, among the three isomers, only *o*-toluidine is regulated in the REACH-VO and may not be released by reductive cleavage of azo dyes in textiles or leathers. *(Benkhaya et al. 2 2020), (REACH-VO 2021)*

Occurrence of azo dyes:
The azo dyes and their coupling components most frequently used in Austria (not only in the textile industry) are listed in the following table 1:

Table 1: Most frequently used azo-dyes in Austria. (Fassold et al. 1999)

C.I.-name	coupling component
Direct Black 38	benzidine
Direct Orange 37	*p*-cresidine
Direct Orange 6	3,3-dimethylbenzidine
Acid Red 128	3,3-dimethoxybenzidine
Direct Green 26	aniline
Direct Orange 102	aniline
Direct Red 23	aniline

Azo dyes are mainly produced in China, India, Korea, Taiwan, and Argentina. The amount of textile dyes produced worldwide is more than 10.000 tons and about 100 tons of the produced dyes end up in wastewater.
It is estimated that a total of about 1000 azo dyes are in use worldwide and 500 of them have potential carcinogenic properties.
(Brüning et al. 2009), (Benkhaya et al. 2 2020)

Azo dyes are used for textile dyeing because they are cheap in production and can intensively dye textiles even in small quantities. Therefore, it is necessary to regularly analyze textiles for the use of azo dyes.
According to an investigation of the CVUA Stuttgart, costumes, underwear, sportswear, sleeping bags and carnival costumes were examined. The carnival costumes were conspicuous because they contained 1,4-phenylenediamine. *(Köhler et al. 2018)*

In another investigation by AGES in the framework of a focus action in 2017, 38 random textile-samples from all over Austria were tested for azo dyes and none of them were objected to. *(AGES 2017)*

In an investigation by LAVES, 26 different samples (textiles such as children's clothing, bed linen, socks, underwear, nightwear, a towel, thermal pants, leggings, a scarf, a leisure suit and T-shirts) were tested for azo dyes and only one T-shirt contained a forbidden amine (chloraniline), which was far below the maximum value of 30mg/kg (ppm). *(LAVES 2023)*

Another study was made on the Swedish market. Therefore, synthetic garments were analyzed with High-performance liquid chromatography/mass spectrometry (HPLC-MS), gas chromatography/mass spectrometry (GC-MS) and computerized data mining, on the occurrence of azo dyes.

82 low- to mid-price common textile garments were purchased from different suppliers, from different countries. The samples were skin-close garments, such as T-shirts and socks, made of more than 70% polyester fibers. *(Carlsson 2022)*

62 azo-dyes were detected, with Disperse Red 167:1 occurring in 67%, and 14 other dyes were found in >20% of the garments. The figure 3 below lists the textile articles with the highest concentrations of azo dyes. A blue t-shirt reached the highest value with a concentration of 596µg/g (= mg/kg) of disperse red.
(Carlsson 2022)

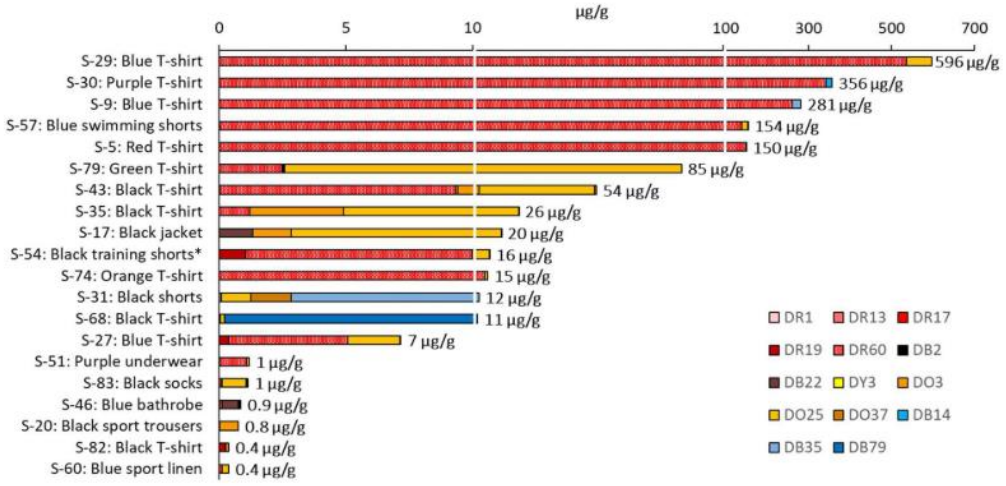


Figure 3: Textile samples and detected azo-dye amount [µg/g]. (Carlsson 2022)

The most frequently detected aromatic amines (arylamines) are listed in the following figure 4.

			Concentration (µg/g)			
Compound	Abbrev.	Frequency (%)	Min	Max	Mean	Median
Arylamines						
4-Nitroaniline	4-NA	44	0.013	200	15	3.0
6-Chloro-2,4-dinitroaniline	6-Cl-2,4-DNA	39	1.0	2900	440	39
2,6-Dichloro-4-nitroaniline	2,6-DCl-4-NA	37	0.12	94	14	2.2
2-Chloro-4-nitroaniline	2-Cl-4-NA	35	0.11	86	14	5.9
2-Bromo-4,6-dinitroaniline	2-Br-4,6-DNA	34	0.38	3000	290	110
2,4-Dinitroaniline	2,4-DNA	30	0.51	260	47	28
4-Chloro-2-nitroaniline	4-Cl-2-NA	22	0.15	600	49	1.7
4-Chloroaniline	4-Cl-A	11	0.073	6.9	1.4	0.48

Figure 4: Detected arylamines. (Carlsson 2022)

Based on the results, some of the detected aromatic amines are above the limit value of 30mg/kg. This is alarming due to the possible adverse effects (carcinogenic, sensitizing potential, etc.) of the amines. *(Carlsson 2022)*

Therefore, further investigations of textiles should take place continuously. Particularly for imported goods from non-EU countries and especially for carnival costumes.

2.1.3 Synthesis and degradation of azo dyes:

Synthesis of azo dyes:

Most azo dyes are synthesized by diazotization of an aromatic primary amine, followed by coupling with one or more electron-rich nucleophiles.
Other methods of synthesis of azo dyes are reduction of nitro-aromatic derivatives in alkaline medium, reduction of nitroso compounds, oxidation of primary amines by permanganate potassium or lead tetraacetate, condensation of hydrazines and quinones, condensation of primary amines with nitroso derivatives, etc. *(Benkhaya et al. 2 2020)*

The process of azo dye synthesis is described in the following chapter.

1.Diazotization:

Diazotization is the first step of the synthesis of azo dyes, it refers to the nitrosation of primary aromatic amines with nitrous acid, resulting in the formation of diazonium-cations, as seen in figure 5. Diazotization also occurs with primary aliphatic amines, but their diazonium-ions are generally unstable and they immediately convert to carbenium-ions with nitrogen elimination. Diazonium salts are important intermediates for organic syntheses. *(Moragues & Song 2022)*

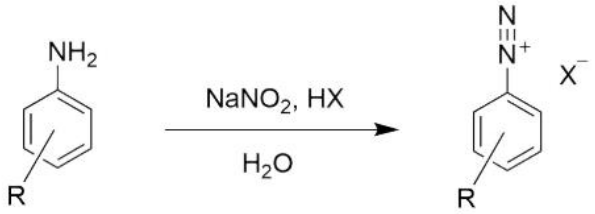


Figure 5: Formation of a diazonium salt from an aniline-derivative. (Moragues & Song 2022)

At the beginning of the synthesis a nitrosonium-ion is formed from the nitrite-ion, by adding an acid, showed in figure 6.

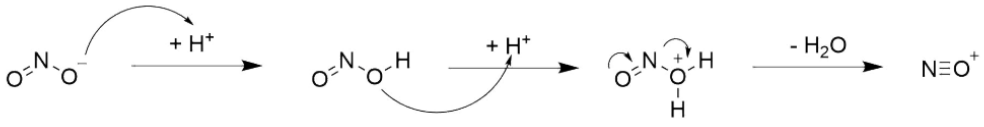


Figure 6: Formation of a nitrosonium-ion. (Moragues & Song 2022)

Then the aromatic amine, for example aniline can react with the nitrosonium ion. The reaction proceeds over several stages and at the end a diazonium compound is formed. *(Moragues & Song 2022)*

2. Azo-coupling:

Azo compounds are synthesized by the reaction of the diazonium salt with an electron-rich aromatic compound. In the following figure 7 Y stands for a donor substituent. *(Moragues & Song 2022)*

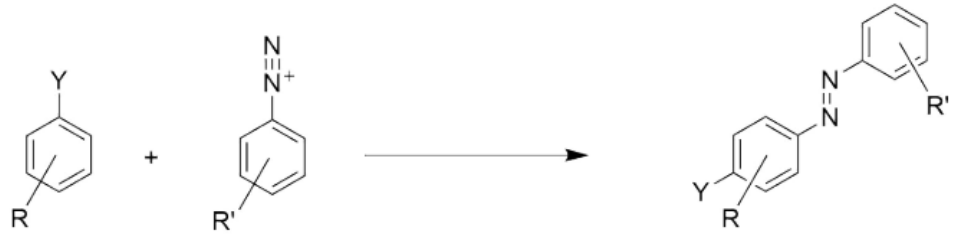


Figure 7: Azo-coupling of the diazonium-salt. (Moragues & Song 2022)

Aryldiazonium salts are weak electrophiles, they only enter into reactions with activated aromatic compounds. Only phenolates are sufficiently reactive for this. The azo coupling takes place selectively in the para position, unless a substituent is already present at this position (then ortho), since there are usually fewer steric problems in the para position. *(Moragues & Song 2022)*

The azo group may be bonded to benzene rings, naphthalenes, aromatic heterocycles or to enolizable aliphatic groups, these are essential to give the color of the dye, with their shades of different intensities. *(Benkhaya et al. 2 2020)*

Degradation of azo dyes:

The degradation of azo dyes can be achieved by reductive cleavage. This can be done with the aid of sodium dithionite in the analytical context. The human body has bacteria on the skin (in form of sweat) and in the intestine that are able to cleave azo dyes.

The aromatic amines formed during the cleavage of azo dyes are generally rather able to penetrate through the skin. In earlier investigations it could be shown that water-soluble direct dyes, are reductively cleaved by typical skin bacteria.

In an investigation by the bgvv, it was analyzed if lipophilic dyes can also be cleaved by these skin bacteria and if dyed textiles are also used by the bacteria as a substrate for azo cleavage. The azo dye disperse orange 3 (DO 3) was introduced into the experiments as a model substance. *(Platzek 1999)*

To analyze the cleavage products of DO 3, the dye was reductively cleaved with sodium dithionite. The cleavage products (as illustrated in the following figure 8) were detected by HPLC analysis. *(Platzek 1999)*

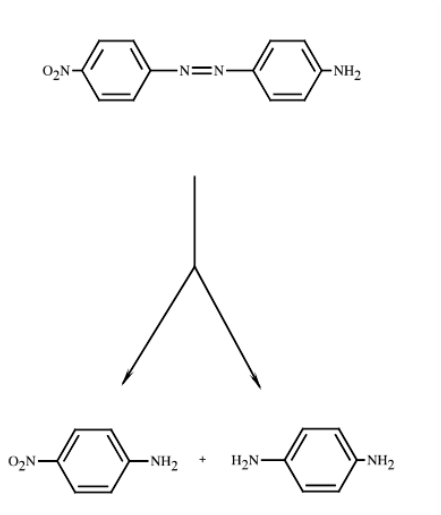


Figure 8: Reductive cleavage of DO 3 into 4-nitroaniline (left) and 1,4-diaminobenzene (right). (Platzek 1999)

This experiment was also performed with a sweat solution containing bacteria (Staphylococcus epidermidis). The dye DO 3 was incubated in the solution and the cleavage products were also determined via HPLC-MS. The same cleavage products were detected. This means that sweat in combination with bacteria can dissolve azo dyes from textiles and partially cleave them to aromatic amines. *(Platzek 1999)*

The tests were carried out with textiles of the fiber types polyester, polyamide and acetate. The color fastness (resistance of dyed textiles to external physical and chemical effects) of DO3 on these textiles was very weak. *(Platzek 1999)*

2.2 Amines

2.2.1 Metabolism and skin mobility of aromatic amines:

Metabolism:

Insoluble azo pigments can be absorbed by the body via the lungs and the gastrointestinal tract (GIT). Due to their insolubility, pigments cannot be cleaved into aromatic amines.

Soluble azo dyes can be absorbed through the lungs, the GIT and through the skin. Due to their solubility, aromatic amines can be released from them by reductive enzymatic cleavage on the skin or in the liver after absorption via the lungs, GIT and/or skin. *(Brüning et al. 2009)*

The uptake of azo dyes can occur by inhalation (dust or aerosol), dermal and oral routes. On the skin, sweat and friction cause elution of dyes from dyed textiles worn close to the body, which can be absorbed through the skin. *(Brüning et al. 2009)*

Absorption via the skin and GIT depends on the molecular mass and polarity of the azo dye. People with inflammatory bowel disease exhibit accelerated absorption of azo dyes. In animal studies, increased absorption of azo dyes was also observed after simultaneous ingestion of antibiotics such as neomycin or tetracycline. *(Brüning et al. 2009)*

Azo dyes are extensively metabolized and excreted in the urine. When absorption happened through the skin, the soluble azo dyes are partially reduced by microorganisms on the skin. The metabolites are absorbed and enter the liver where they are further metabolized. Fat-soluble azo dyes can be absorbed unchanged through the skin and they are only metabolized in the liver. After oral uptake of azo dyes, a similar cleavage and resorption of the metabolites or resorption of the unchanged azo dye takes place in the GIT. This happens especially for water-soluble azo dyes (food colors) they are excreted more rapidly due to their increased polarity. *(Brüning et al. 2009)*

After absorption in the body, azo dyes can be metabolized to aromatic amines. This occurs through various reductases in the GIT and in the liver. Hydrophilic substances are already partially metabolized in the GIT by the cytochrome P450 system and lipophilic substances are metabolized mainly microsomal in the liver. The reduction takes place in the GIT by bacteria of the intestinal flora. The bacteria of the physiological skin flora also possess reductase activity.
Two reductase systems were identified in bacteria, an anaerobic monomeric flavin-free reductase system and a polymeric flavin-dependent reductase system, which can reduce both aerobically and anaerobically. *(Brüning et al. 2009)*

After inhalation or dermal uptake, oxidation (phase-I reaction) of the azo dye takes place in the liver. Followed by the phase II reaction (esterification with glucuronic acid, formation of acetates and sulfates), which makes the molecule water-soluble. Now it can be excreted via the bile into the intestine or via the urinary bladder.
The ester groups can be split off to form the very reactive nitrenium-ion in the urinary bladder, which can react with a nucleophilic group of the genetic material DNA in the bladder epithelium (carcinogenic potential). *(Brüning et al. 2009)*

Skin mobility:
The structure of the human skin is shown schematically in the following figure 9. The skin is composed of the epidermis, dermis and subcutis. The cellular components are keratinocytes and melanocytes in the epidermis and fibroblasts, Langerhans cells, macrophages, lymphocytes and sebo-

cytes in the dermis. The skin forms a physical and biochemical barrier to foreign substances to prevent potentially dangerous absorption and penetration of hazardous substances. *(Brüning et al. 2009)*

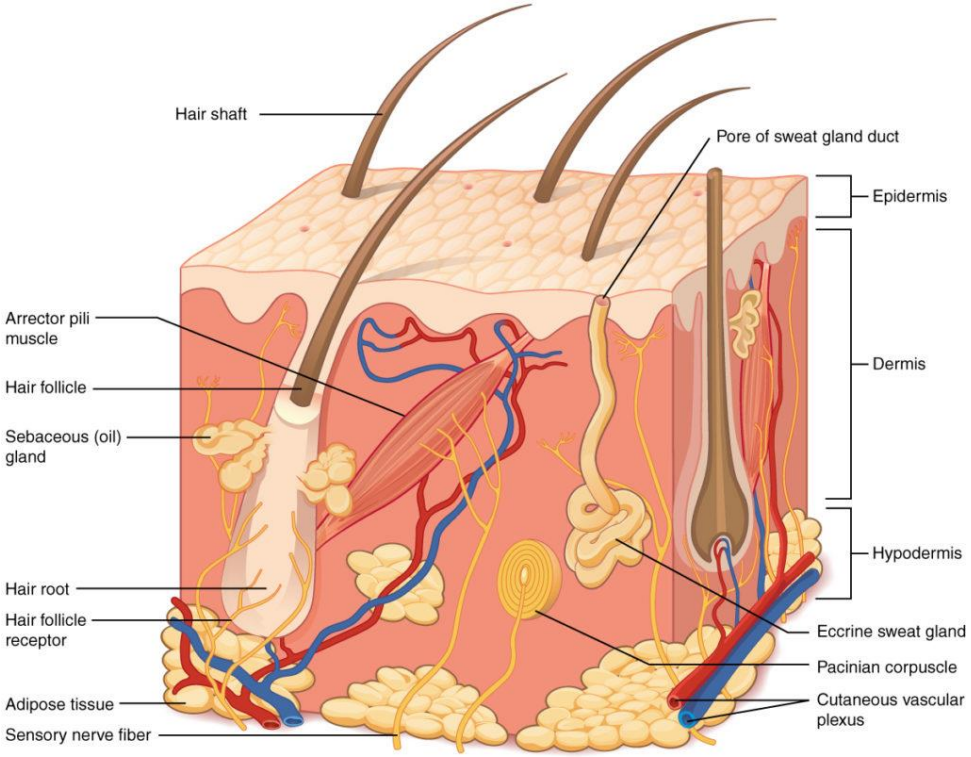


Figure 9: Structure of the human skin. (https://courses.lumenlearning.com/suny-wmopen-biology2/chapter/structure-and-function-of-skin/ cc by biology for mayors II.)

Several different proteins are expressed in the skin, which are involved in the metabolism of exogenous and endogenous low molecular weight substances (including cytochromes P450, CYP450) and proteins associated with the transport of foreign substances, including P-glycoproteins (PGPs), other multi-drug-resistance proteins (MRPs) and so-called lung-resistant proteins (LRPs). In the skin, the azo-reductase system is also located in the cytosol and in the endoplasmic reticulum of the cells. There the process of azo cleavage occurs very rapidly. Cytochromes P450 are responsible for oxidative degradation. PGPs are ATP-dependent membrane proteins, they are responsible for the accelerated removal of substances from the cell. MRPs are mainly responsible for transport (e.g. of glutathione-S conjugates). *(Brüning et al. 2009)*

When the substance penetrates the skin, it is oxidatively metabolized by CYP450, which leads to the formation of aromatic amines from azo dyes (= toxification). During the metabolism, acute contact dermatitis (ACD) may be the result of an allergic reaction to one of these metabolites. *(Brüning et al. 2009)*

Hydrophilic azo dyes can be reduced by bacteria of the physiological skin flora already within one hour incubation time. With a dermal exposure of 1-10 µg/kg, a human exposure of 1 mg/d is assumed. The washing-model used to simulate the uptake of dyes from repeatedly worn and cleaned clothing, showed a decrease in release with an increase in washing procedures. Consideration at the molar level showed that between 9 and 13% of the released amount of azo dyes is converted by the mixed flora of the skin. *(Platzek 1999)*

It is estimated that the quantity of amines released from azo dyes is 25 to 30%. The working group "Textiles" of the Federal Institute for Risk Assessment (BfR) assumes a release rate of 0.1% per day. *(Platzek 1999)*

In studies with radioactively labeled azo dyes, applied epicutaneously in vitro to the skin of hairless mice, hairless guinea pigs and human skin, the poorer permeability of human skin to these substances was found in contrast to rodents. *(Collier 1993)*

Furthermore, dermal exposure depends not only on the dye type but also on the textile material, the dye content and the fastness of the dyeing. *(Brüning et al. 2009)*

A study by Xuezhang et al. (2017) investigated the risk for skin exposure to azo dyes in textiles. For this purpose, 20 textile samples with an amine content of 20mg/kg, were examined by rubbing on a dermal exposure model. It was found that no exposure occurred by dry rubbing, only by wet rubbing in combination with artificial sweat (mainly benzidine was released).
The highest contamination level of benzidine was 0.318 ng/kg/day (chronic exposure), which is below the LOAEL but above the virtually safe dose (VSD) of 4.3 × 10-3 ng/kg-day. Through the study, it was shown that short-term dermal exposure to azo dye-containing textiles has no adverse effects on human health but long-term exposure can lead to exceeding the limit values. *(Xuezhang et al 2017)*

The following table 2 contains a list of amines with their corresponding azo dyes, which have been proven to be skin sensitizing. *(Brüschweiler et al. 2014)*

Table 2: List of skin sensitizing amines and corresponding azo dyes. (Brüschweiler et al. 2014)

aromatic amine	corresponding azo dye
1,3-phenylenediamine-4-sulfonic acid	C.I. Acid Brown 123
3,4-dichloroaniline	C.I. Disperse Yellow 241
N,N-dimethyl-1,4-phenylenediamine	C.I. Basic Blue 54, C.I. Disperse Red 41 etc.
4-aminodiphenylamine	C.I. Acid Yellow 36, C.I. Disperse Orange 1
p-phenylenediamine	C.I. Acid Brown 4, C.I. Acid Brown 237 etc.
sulfanilic acid	C.I. Acid Black 210, C.I. Acid Brown 106 etc.
4-aminophenol	C.I. Acid Orange 33, C.I. Disperse Orange 13 etc.
4-ethoxyaniline	C.I. Acid Yellow 38, C.I. Acid Yellow 159 etc.
4-(N,N-diethyl)-2-methyl-p-phenylenediamine monohydrochloride	C.I. Acid Red 397, C.I. Disperse Red Dye etc.
1,4-diamino-2-methoxybenzene	C.I. Acid Yellow 219, C.I. Disperse Orange 29
2,5-diaminotoluene	C.I. Solvent Red 25

2.2.2 The possible genotoxicity and carcinogenicity of aromatic amines released from azo dyes:

Azo dyes with a free p-amino group exhibit mutagenic properties more often than others, but their genotoxicity is generally considered to be low. A bacterial (Ames-test) and a non-bacterial test without and with metabolic activation are performed to determine genotoxicity. Subsequently, tests for carcinogenicity are carried out.

Carcinogenic aromatic amines are assigned to category 1 (corresponding to MAK III A1) if they have been proven to cause cancer in humans, category 2 (MAK III A2) if this property has been demonstrated in animal experiments. Cancer research in connection with azo dyes shows that especially dyes from double diazotized benzidine and benzidine-derived components play a role in carcinogenesis. Especially bladder cancer and liver tumor cases are known (animal experiments or workplace contamination). *(Fassold et al. 1999)*

A study by Keshava et al. (2023) summarized 187 relevant studies in humans and animals related to azo dyes and their sensitizing, genotoxic, and carcinogenic potential. The three dyes Acid Yellow 23 (Tatrazine), Food Yellow 3 and Acid Red 18 turned out to be harmful to health in the human studies, when administered orally (food). In addition, they can lead to hy-

persensitivity in children. In animal studies, Acid Yellow 23 was also found to be carcinogenic in rats. In addition, 8 of the 30 dyes studied were found to be genotoxic (e.g. Acid Black 172, Direct Black 19, Reactive Orange 72, Reactive Red 120 and 239). *(Keshava et al. 2023)*

Methneni et al. (2021) showed that administration of wastewater from a textile industry for 90 days negatively affected oxidative stress status in baby mice, leading to lesions of the kidney and liver such as leukocyte infiltration, vascular congestion of the central vein, and hepatic steatosis, illustrated in the following figure 10.
In an investigation of the wastewater by LC-MS/MS, it was found that especially the azo dye disperse yellow 3 was present in high concentration. *(Methneni et al. 2021)*

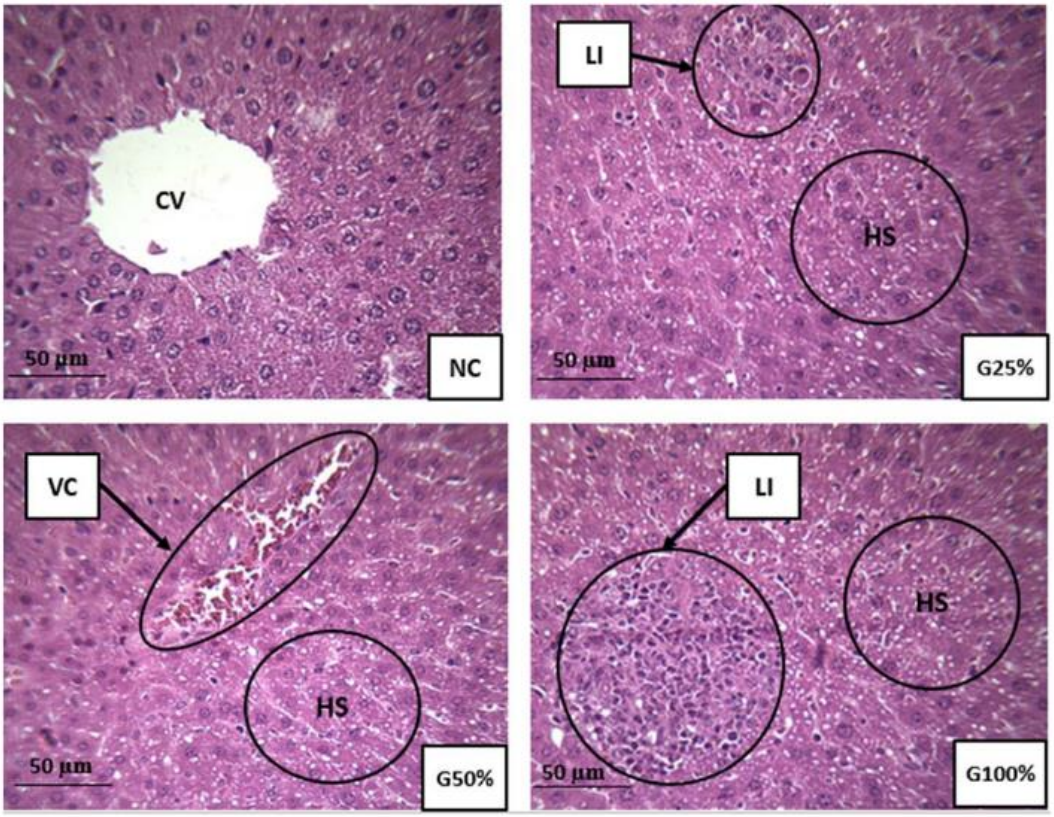


Figure 10: Histological sections of the liver from mice exposed to various dilutions of waste water. NC: negative control with a healthy architecture of hepatocytes. G25%: liver sections of mice receiving 25% diluted waste water. G50%: liver sections of mice receiving 50% diluted waste water. G100%: liver sections of mice receiving 100% waste water. CV = central vein, LI = leucocyte infiltration, VC = vascular congestion of the central vein, HS = hepatic steatosis. (Methneni et al. 2021)

Another study was performed by Leme et al. (2015). To determine dermal exposure to azo dyes, a 3D model of human skin was made to mimic tissue function and to test azo dyes for in vitro genotoxicity. Two azo dyes, Reactive Green 19 and Disperse Red 1, were tested and Reactive Green

19 was found to be genotoxic, but Disperse Red 1 was not. By using 2D models of the skin, Disperse Red 1 was shown to be genotoxic in human hepatoma cells (HepG2) and Reactive Green 19 was nongenotoxic for normal human dermal fibroblasts (NHDF).

These differences are probably caused by the different metabolic activity of the different cell types (organ-specific genotoxicity) and the test setup systems used in each study. Therefore, it is important to perform further studies and using in vitro models that more closely resemble in vivo tissue architecture. *(Leme et al. 2015)*

The following table 3 lists aromatic amines and their corresponding azo dyes with possible genotoxic or carcinogenic potential.

Table 3: List of possible genotoxic/cancerogenic amines with the corresponding azo dye. (Brüschweiler et al. 2014)

aromatic amine	corresponding azo dye
2,6-xylidine	C.I. Acid Orange 134
2,4-xylidine	C.I. Acid Red 8, C.I. Acid Red 26 etc.
3,4-dichloroaniline	C.I. Disperse Yellow 241
2-methoxy-4-nitroaniline	C.I. Disperse Red 41
2-amino-4-nitrophenol	C.I. Acid Brown 349, C.I. Acid Yellow 119 etc.
2-amino-4-nitroanisol	C.I. Pigment Red 23
4-nitroaniline	C.I. Acid Black 1, C.I. Acid Black 210 etc.
2-amino-5-nitrothiazol	C.I. Disperse Blue 102, C.I. Disperse Blue 106 etc.
2-amino-5-nitrophenol	C.I. Disperse Red 16
4-aminophenol	C.I. Acid Orange 33, C.I. Disperse Orange 13 etc.
2-amino-6-methoxybenzothiazol	C.I. Disperse Red 379
4-nitro-1,3-phenylenediamine	C.I. Acid Brown 83
2-amino-6-nitrobenzothiazol	C.I. Disperse Red 177, C.I. Disperse Red 179
3-amino-5-nitro-2,1-benzisothiazol	C.I. Disperse Blue 148
2,5-diaminotoluene	C.I. Solvent Red 25

Since it is difficult to perform human studies, the effect of azo dyes on the worm species *Girardia tigrina* was investigated. For this purpose, the animals were contaminated with waste water from the textile industry (with high concentrations of Disperse Red 1). Behavioral changes were noticed, as well as increased mucus production and temporary skin coloration. 8 of 10 animals died 96h after contamination (concentration of waste water = 200mg/L) others showed regenerative delays and changes in feeding behavior. The contamination had little effect on the fertility of the animals. An LD_{50} of 75-152mg/L was detected for Disperse Red 1. *(Ribeiro et al. 2014)*

The number of studies on the genotoxic and carcinogenic potential of azo dyes is not extensive. Human studies are very rare, so a classification of the negative effects that can be caused by azo dyes and their cleavage products cannot be clearly defined.

2.2.3 The allergic potential of aromatic amines released from azo dyes:

Patch tests are used to determine the allergic potential of azo dyes. The dyes are stuck to the skin with a patch and checked after various periods of time for redness, rashes, skin changes or generally for signs of allergic reactions. Allergic reactions to textiles do not always have to be related to the dye used, also the type of fiber (e.g. natural fiber of animal origin) can be decisive. In general textile-related contact allergies rarely occur. The reason is usually the poor quality of the dyeing of textiles, with disperse dyes. If these dyes are used in the wrong combination with fiber types such as polyester or polyamide, the textile exhibits insufficient color fastness and tends to "bleed". Therefore, textiles should always be washed before being worn. *(Bode 2007)*

In a review of 54 studies on contact allergy of disperse dyes in textiles, from 1990 to 2012, 26 disperse dyes were tested. On average, the probability of contact allergy was >1% for the dyes Disperse Blue 106, Disperse Blue 124 and Disperse Orange 3.
(Malinauskiene 2013)

The study situation for contact allergies, in connection with azo dyes in textiles is not very developed. More studies and data are available in the food and cosmetics sector. For example, a 22-year-old woman developed an itchy rash on the scalp after application of a permanent hair dye. Despite local corticosteroid therapy, the itching resolved only slowly.

Patch tests were performed with the ingredients of the hair dye, including p-phenylenediamine. The test substances were applied to the upper back for 2 days. The patient reacted positively to textile dye mixture and p-phenylenediamine. Afterwards single ingredient tests were performed, showing a positive reaction for Disperse Red 1, Disperse Orange 3 and Disperse Yellow 3 dyes. *(Mancuso 2020)*

2.3 Textiles and dye process

2.3.1 Fiber types and textile production:

Fiber types:
In fiber production, there is a fundamental difference between the production of natural fiber and synthetic fiber. Natural fibers are obtained from nature, i.e. an animal (e.g. wool or silk) or a plant (e.g. cotton, sisal, kapok). Wool belongs to the group of protein fibers (protein fibers), which consist mainly of a horn mass (keratin). Wool is mainly used for clothing (woven and knitted) and often in combination with synthetic man-made fibers. Cotton is the most important cellulosic fiber by volume. It consists of 80% to 90% cellulose and contains 4% to 5% hemicellulose and pectins. Cotton is used for underwear, outerwear and sportswear (for example jeans). *(Bode 2007)*

Synthetic fibers are produced from crude oil or natural gas and the required fibers are created via polymerization, polyaddition, polycondensation or by reaction on polymers. The viscose fiber is a cellulosic synthetic fiber (it consists of 90% cellulose). Polyester fiber is a synthetic fiber synthesized by polycondensation from bifunctional monomers. Polyester fibers are suitable for outdoor and functional clothing, and especially for suits, jackets and pants, for dresses, blouses and skirts. The polyester fiber has good moisture transport, therefore it dries quickly. Polyester is the most used type of fiber, followed by polyamide. The following steps are necessary to produce synthetic fibers: production of polymers, production of a spinning mass from polymers, spinning of a continuous filament, production of staple fibers, drawing, yarn production and texturing. *(Bode 2007)*

Textile production:
To produce a finished textile piece from a fiber, the fiber must first be transformed into a yarn. This is done by parallelizing, drawing and twisting together (spinning) the individual natural and man-made fibers or mixtures of the two. So that the fibers can be processed into yarns special chemical auxiliaries are required in the spinning mill. They are needed to solve physical problems in the spinning process (e.g. reducing friction) and to ensure quality of the product. *(Bode 2007)*

In the production of synthetic fibers, spinning-preparations regulate the sliding and adhesion behavior between fiber and fiber and between fiber and metal. They regulate moisture, reduce electrostatic charge and have a softening effect. These preparations consist, of non-ionic, anionic or cationic surfactants (for example, fatty alcohol or fatty amine ethoxylates) or sulfated vegetable oils, stearic soaps, ester and silicone oils. Other auxilia-

ry components in textile production are adhesives and lubricants. Sulfated vegetable oils are usually used as adhesives, while emulsified kerosene or white oils are used as lubricants. In addition, melting agents impart smoothness, suppleness and anti-electrostatic properties. *(Bode 2007)*

In the next processing step, the yarns are transformed into textile surfaces. To produce woven fabrics, textile threads are crossed at right angles, whereby they are laid under or over each other (weaving). Before weaving, the vertically running warp threads must be arranged in the required weaving width. To make the warp threads more resistant to mechanical-stress such as rubbing, pulling and bending, they are treated with starch, cellulose derivatives, polyvinyl alcohols or polyacrylates. Natural oils and fats (fish oil, beef tallow), natural and synthetic waxes and kerosene are used as smoothing products. *(Bode 2007)*

Apart from woven fabric, there is also knitted fabric, in which interlocking yarn stitches are produced, arranged horizontally next to each other as well as vertically above each other. There are also nonwoven fabrics, which are produced by mechanical, physical and chemical methods.

Fibers and textile fabrics such as woven, nonwoven and knitted fabrics, as well as the garments, home textiles or technical textiles made from them, are refined to give them the desired appearance, handle quality and functionality. This is done by pretreatment (washing, bleaching, mercerizing etc.), coloring and special treatments (crease resistance, easy ironing, water resistance, increased slip resistance). *(Bode 2007)*

2.3.2 Textile dyeing process and waste water in the textile industry:

Textile dyeing process:

For the dyeing of textiles, the type of fiber and the dye must be matched to each other to incorporate the textile dye as permanently and evenly as possible into the textiles. During dyeing, the fabric is treated with an aqueous dye solution.

The composition of the dye bath is important, it should have the correct ratio between textile and dye solution, but also temperature, treatment time, quality of the water (contamination, hardness level), electrolyte content and pH value must be considered, the textile auxiliaries must be correctly dosed. The dyeing of mixed fabrics is complex, because different materials are combined in these fabrics, it is sometimes difficult to achieve a result with the same color tone. *(Bode 2007)*

In the dyeing process, a distinction is made between exhaust processes and forced application processes. In the exhaust process, the dissolved or

dispersed dyestuffs from a dye bath are drawn onto the textile fiber. In the forced application process, a machine with a roller system is used. The textile is first drawn through the dye bath in an immersion trough. The fabric is then passed through two rollers, thus forcing the dye solution into the textile fabric by "squeezing". For the dye to bond permanently with the fiber, it must be fixed by drying and steaming. *(Bode 2007)*

The dyeing of the textiles must also be gentle on the fibers, temperature-resistant and abrasion-resistant. In addition, the aspects of economy, resource conservation and health protection must not be neglected. To meet all requirements, a variety of dyeing auxiliaries are needed in addition to high-quality textile dyes:

- Dye solvents and hydrotropic agents support the dissolution of dyes in the dye bath. (alcohols or esters)

- Dispersing agents form stabilize dispersions with dyes that are poorly soluble and insoluble in water. (sulfated fatty acid esters and amides, alkyl aryl sulfonates and fatty acid ethoxylates)

- Protective colloids coat dispersed particles and prevent flocculation of the dispersions. (lignosulfonates, polyacrylates)

- Wetting agents reduce the interfacial tension between the textile and the dye liquor and ensure rapid and uniform access of the dye to the fiber. (Alkyl sulfates, alkane sulfonates, salts of sulfosuccinic acid and phosphoric acid)

- Leveling agents have the task to achieve a uniform dyeing. (fatty acid esters and amides, alkylamines)

- Dyeing accelerators are used in the exhaust dyeing of polyester fabric with disperse dyes to the dye liquor so that the dyes diffuse more quickly into the fiber and the dye yield is increased. (hydrocarbons, phthalic acid imides)

- Post-treatment agents improve rub, wet or light fastness.

Another technique for coloring textile materials is fabric printing. It is mainly used for textile fabrics and knitted fabrics. *(Bode 2007)*

Waste water in the textile industry:
Textile dyeing requires a lot of water and it contributes to the pollution of water, soil and air. In non-EU countries, textile wastewater (15-50%) is often directly discharged into groundwater.
If azo dyes enter the soil via wastewater, they can interact with soil bacteria and got degraded very slowly by soil microbes. In addition, the gram-negative bacteria in the soil are reduced by the contamination. In the study by Imran et al. (2015), the dyes Direct Red 81, Reactive Black 5 and Acid Yellow 19 were introduced into the soil at a concentration of 160mg/kg and their concentrations were measured. The dyes were very stable, after 14 days concentrations of 17.3% to 87.5% were measured. *(Imran 2015)*

The following figure 11 shows the methods for the degradation of azo dyes. Biodegradation of azo dyes can happen by using different types of bacteria. Reductive cleavage of -N=N- is the primary step of the degradation process.

For practical and economic reasons, wastewater is primarily treated by the physico-chemical method. This includes techniques such as membrane filtration, coagulation/flocculation, precipitation, flotation, adsorption, ion exchange, ion pair extraction, ultrasonic mineralization, electrolysis, and advanced oxidation. *(Selvaraj 2021)*

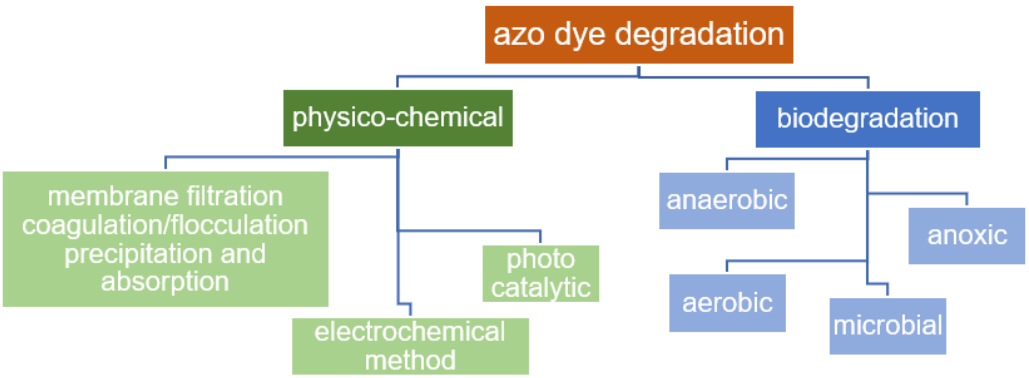


Figure 11: Waste degradation methods for azo dyes. (Selvaraj 2021)

2.4 Detection methods

Since the detection of azo dyes or their aromatic amines is very complex and the number of analytes is high. It is necessary to confirm an analysis with a second measurement method. The different methods range from simple qualitative screening methods like TLC to extremely precise methods like HPLC or GC, where amine concentrations in the ppm range can be detected. The different detection methods are presented in the follow-

ing section and the pre-determination of the fiber type with FTIR (or NIR) is explained.

2.4.1 FTIR and introduction of the alternative NIR-measurement:

FTIR is the abbreviation for Fourier Transform Infrared. This non-destructive method of vibrational spectroscopy is used to characterize materials. In infrared spectroscopy, radiation in the mid-infrared range - indicated by the wavenumber [cm^{-1}] - from 100 cm^{-1} to several 1.000 cm^{-1} is irradiated into a substance. Some wavelengths are reflected and other wavelengths are absorbed by the material because their energies excite the vibration of certain molecular groups. Absorption bands are found in the spectrum at these energies. The non-absorbed radiation is measured as transmission at the detector, as an electronic signal. *(Brucker 2023)*

In contrast to dispersive measurements, we first obtain an interferogram, which then yields an IR spectrum by means of Fourier transformation. This interferogram (a raw signal) represents the light intensity not as a function of wavelength, but as a function of the position of a mirror in the interferometer. Therefore, the signal must be Fourier transformed (FT) to produce the usual IR representation of light intensity as a function of wavenumber. This results in several advantages. Firstly, measuring FT-IR spectra is much faster than with dispersive spectrometers and the FT-IR spectra show a better signal-to-noise ratio. In addition, there is a much higher wavelength accuracy because the wavelength scale is very precisely calibrated with a laser. *(Brucker 2023)*

Like a fingerprint, the infrared spectrum is characteristic of the molecule under investigation and can be used, for example, to identify substances. *(Brucker 2023)*

The most common design is a Fourier transform (FT) IR spectrometer, which uses a Michelson interferometer (figure 12) to analyze the signal. Here, the center of the FTIR is an interferometer and the IR radiation is introduced into the system and passed through a beam splitter to a fixed mirror and a moving mirror. The beam is then recombined, creating interference, and directed to the sample. The spectra information of all wavelengths is recorded simultaneously, which is very time-saving. *(Brucker 2023)*

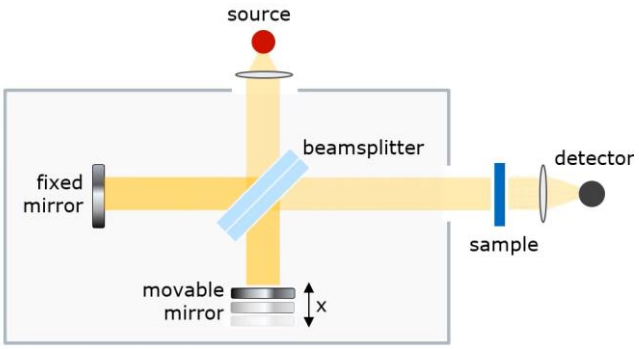


Figure 12: Michelson interferometer.

If a sample is now to be measured with FTIR, the baseline must be established. Therefore, a spectrum with the unloaded FTIR device and a background spectrum is recorded. This reference spectrum gets subtracted from the spectrum of the sample measurement and the result is the transmission spectrum of the sample. (Transmission [%] plotted against the wavenumber [cm^{-1}]) *(Brucker 2023)*

The "Attenuated Total Reflection" (ATR) method has become the standard technique for measuring FTIR spectra (figure 13), it allows simple sampling for solids and liquids. Here, infrared light passes through a crystal made of an IR-transparent material (diamond, ZnSe or germanium) and then interacts with a sample that has previously been pressed onto the crystal. The crystal must have a higher refractive index than the sample. The close contact is particularly important to obtain high-quality IR spectra. The IR beam hits the crystal and is reflected and then absorbed by the sample on the crystal. The reflected beam now carries the absorption information and is measured with the detector. *(Brucker 2023)*

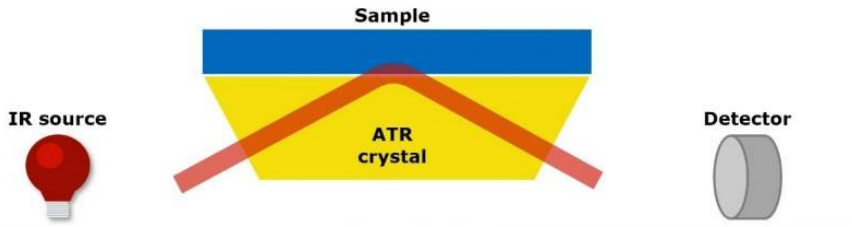


Figure 13: ATR-FTIR.

Since an FTIR-instrument is very expensive and is not portable, an alternative for the measurements of the fiber type was investigated. The determination of different fiber types in textile samples can also be carried out with NIR. Devices such as the microPhazir work with NIR-technology and can determine fiber types in seconds. For this purpose, a reference spectrum of the respective fiber is recorded in advance and stored in a database. If fabric samples are scanned with the microPhazir, it can indicate the fiber type of the textile. Up to two fiber types can be distinguished.

It is cheaper than an FTIR and still fulfills the distinction between natural and synthetic fibers. In addition, it is also possible to distinguish between synthetic fibers. The companies from which the fabric and fiber samples originate use equipment from thermofisher, such as the thermofisher scientific microphazirGP, for the fiber analyses. *(Thermo fisher Scientific 2011)*

Since extraction of samples is only necessary when a synthetic fiber is present because only these contain disperse dyes, which must be extracted before the rest of the experiment, determining the type of fiber can simultaneously determine the experimental procedure. Moreover, disperse dyes are mainly used in polyester and triacetate fibers. This means, if the FTIR or NIR analysis is polyester, we can assume that a disperse dye (or pigment) was used. *(Austrian Standards International & CEN 2017)*

2.4.2 GC-MS:

GS-MS is ideally suited for routine analysis because it works with short analysis times and provides high-resolution spectra of the detected amines. *(Stevens & English 1907)*

Even better results can be obtained by varying the analytical workflow prior to GC-MS analysis. For example, GCB-SPE cartridges have been used to achieve very efficient purification of textile extracts, which simplifies and improves the robustness of subsequent GC-MS analysis. For this purpose, after solvent extraction of the textile sample, the extract is passed through a GCB-SPE cartridge, which retains interfering compounds in the matrix, resulting in a clean extract suitable for GC-MS analysis. *(Luongo et al 2016)*

In addition, Chem Elut S cartridges can also be used for assisted liquid extraction instead of the diatomaceous earth columns described in standard 14362-1. With the cartridges a high recovery (87 to 119 %) and excellent reproducibility (< 9 % RSD) can be achieved. The Chem Elut S products use a synthetic medium optimized to overcome the problems associated with conventional diatomaceous earth sorbents. In contrast to the irregularly shaped diatomaceous earth particles, which exhibit considerable variability and contain fines, Chem Elut S particles have a narrow particle size distribution and contain no fines. These properties promote ideal flow characteristics. In addition, the Chem Elut S sorbent has a higher sample adsorption capacity than the diatomaceous earth sorbents, thus providing efficient sample adsorption and reducing the risk of sample breakdown. *(Lucas 2019)*

2.4.3 HPLC:

High performance liquid chromatography with electrospray ionization tandem mass spectrometry is usually used in the detection of aromatic amines by HPLC. With this measurement method, it is possible to detect not only the 22 listed amines that are considered carcinogens, but many other isomeric compounds that differ only slightly in their retention times. *(Kämpfer et al. 2019)*

In the study by Kämpfer et al. (2019), a processing method was also developed without the step of separating the amines with diatomaceous earth columns and tert-butyl methyl ether. In this case, the extraction with xylene of disperse dyes was maintained according to standard 14362-1. The amount of textile sample was reduced to 0.5 g, the citric acid-sodium hydroxide buffer to 8.5mL and the sodium dithionite solution to 1.5mL. After reductive cleavage, 20mL of methanol was added and the solution was treated with an ultra-sonic bath for 10 minutes. Afterwards the solution was filled up to 50mL with water, shaken and centrifuged. The supernatant was diluted with a mixture of buffer, methanol and water. This solution was analyzed by HPLC-MS/MS. The method was validated by adding the selected amines to the extracts of empty textiles. *(Kämpfer et al. 2019)*

With this method of sample preparation and the optimized column parameters, running medium changes and pH settings, it was possible to distinguish positional isomers from each other (figure 14). *(Kämpfer et al. 2019)*

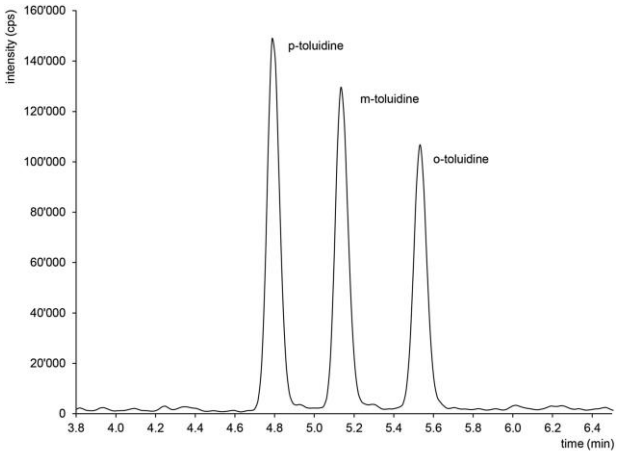


Figure 14: Detection of toluidine-isomers with HPLC. (Kämpfer et al. 2019)

2.4.4 TLC:

Also TLC can be used for the detection of aromatic amines. The classical TLC method uses silica gel plates with fluorescent indicator. Spray reagents and UV detection are used for the detection. *(Austrian Standards International & CEN 2017)*

A more powerful method of TLC is high-performance thin-layer chromatography (HPTLC). The study by Freeman (2013) examined 18 fabric samples (women's underwear) for their amine content. For this purpose, the samples were subjected to reversed-phase HPTLC. Fabric sample no. 1 consisted of nylon (blue), no. 2-5 of cotton (black), no. 6 of polyester (brown), no. 7 of cotton (purple), no. 8-11 of nylon (purple), no. 12 of polyester (purple), no. 13 and 14 of nylon (pink), no. 15 of cotton (red) and no. 16-18 of nylon (red). The bands of the analyzed fabric samples are shown in the figure 15 below. It is evident that especially the 6 samples with purple dyes have very different bands. The results of the 4 samples with black dyes were very similar. It was shown that 10 different amines could be detected from the 18 samples. The chemical identification of the bands was performed by MS-detection. The amines detected were then used to draw conclusions about the azo dyes used. Fabric samples from the same fiber material with the same colors but from different countries were dyed with the same azo dye. *(Freeman 2013)*

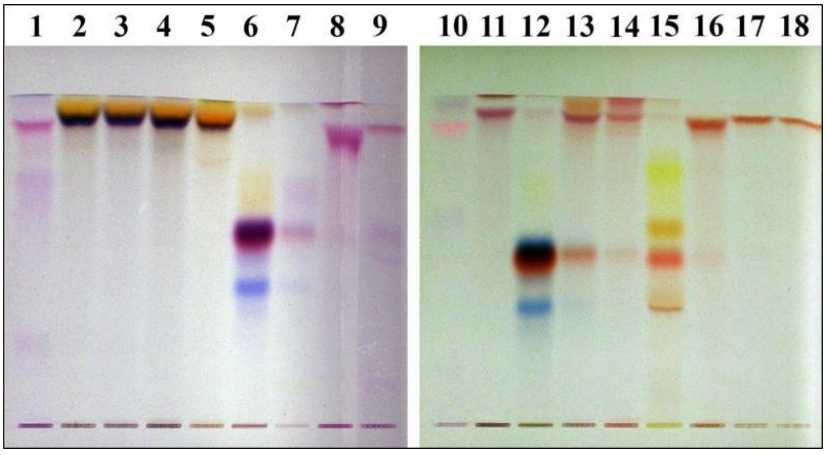


Figure 15: HPTLC of 18 textile samples.

2.5 Study aims and design

The first objective of the study was to develop a measurement method (FTIR) to distinguish textile fiber types and to create a database. To date, FTIR spectroscopy has been applied primarily in the pharmaceutical, chemical, food and polymer industry. It is used to understand the molecular structure of materials as well as the kinetics, mechanism and pathways in chemical reactions and catalytic cycles. Determining fiber type using an FTIR based method to create a database which can be used to classify samples into natural and synthetic fibers has never been done before. As a sample preparation step for the 14362-1 standard and possibly also for other analyses (e.g. plasticizers and flame retardants in textiles), FTIR can contribute to a considerable facilitation of the subsequent test performance. Subsequently, this preliminary analysis can be used to facilitate the second objective, the implementation and validation of a GC-MS based method.
For the GC-MS analysis, the standard method 14362-1 was implemented for AGES-Linz. Since this was a very time and material consuming method and no sufficiently good recovery of the amines could be achieved, an optimized method was created. Subsequently, tests on the storage stability of the amines and on the matrix dependence of the measurements were performed, and a validation plan was developed. (see figure 16)

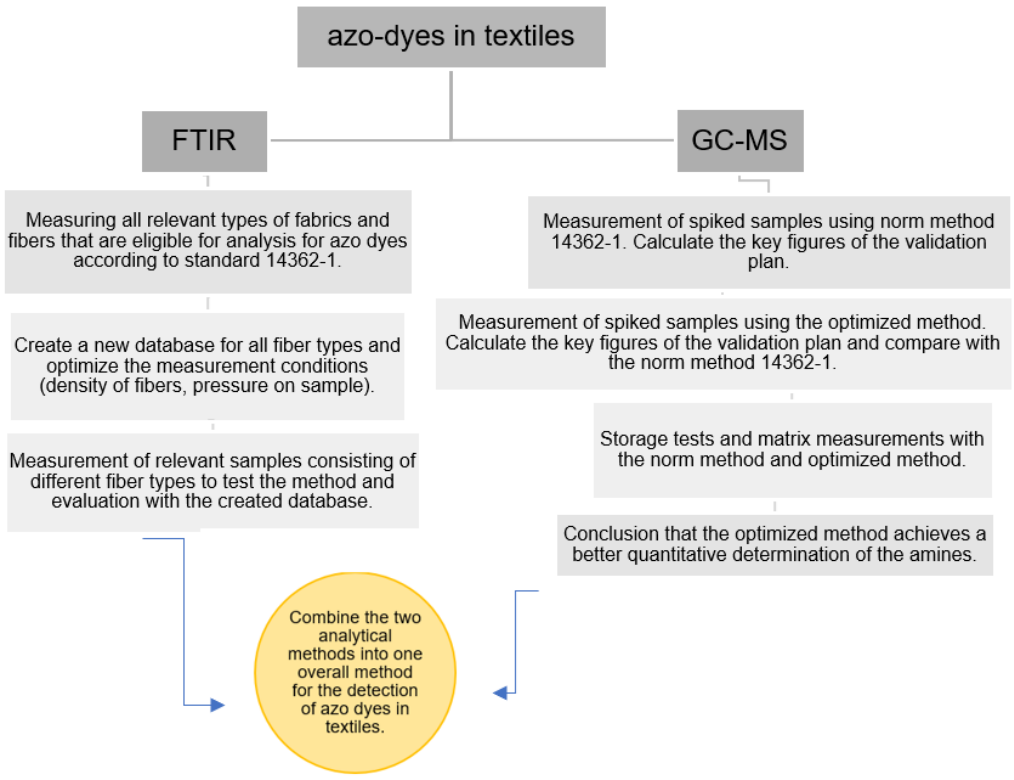


Figure 16: Organization-chart of the study.

3. Material and methods

In the following section, all chemicals, materials, equipment, methods, the software used and the sample-description are listed. (table 4-10)

3.1 Chemicals.

Table 4: List of chemicals for GC-MS.

chemicals	CAS no.	producer/type
xylene (isomer mixture)	1330-20-7	Supelco Item no. 1.08297.2500
acetonitrile	70-05-8	Supelco Item no. 1.00030.2500
methanol	67-56-1	Supelco Item no. 1.06035.2500
tert-butylmethylether	1634-04-4	Sigma-Aldrich Item no. 29,321-0
citric acid monohydrate for the citrate buffer (pH = 6, *c* = 0,06mol/L)	5949-29-1	ROTH Item no. 5110.1 weight in 12,6084g in 900mL ultra-pure water (quality 3) and adjust the pH with NaOH, fill up to 1000mL.
sodium dithionite for the aqueous sodium dithionite solution (ρ = 200mg/ml) Freshly made on the day of use.	7775-14-6	Supelco Item no. 1.06505.1000 weight in the required amount of sodium dithionite and fill up with ultra-pure water (quality 3) let the solution rest for 55min, afterwards fill the solution into a beaker and let it rest for another 5min. *(Austrian Standards International & CEN 2 2017)*
diatomaceous earth	-	Macherey-Nagel GmbH & Co KG Item no. 730595.1000 (XTR)
NaOH tablets for the 10% solution	1310-73-2	Supelco Item no. 1.06469.5000 Weight in 11,0964g in 100mL volumetric flask and fill up with ultra-pure water (quality 3)
water (quality 3) ISO 3696	-	-
acetone (for gas chromatography)	67-64-1	Supelco Item no. 1.00012.2500

Table 5: List of reference substances and azo dyes.

amine reference substances	CAS no.	producer/type
4-aminobiphenyle (1)	92-67-1	Sigma Aldrich Item no. A2898-5g
benzidine (2)	92-87-5	Sigma Aldrich Item no.12115-10g
4-chlor-o-toluidine (3)	95-69-2	Sigma Aldrich Item no. 590711-10g
2-naphthylamine (4)	91-59-8	Sigma Aldrich Item no. 31618-100mg
o-aminoazotuluol (5)	97-56-3	Sigma Aldrich Item no. 31629-250mg
5-nitro-o-toluidine (6)	99-55-8	Sigma Aldrich Item no. 45984
4-chloraniline (7)	106-47-8	Sigma Aldrich Item no. 477222-1g
4-methoxy-m-phenylendiamine (8)	615-05-4	Sigma Aldrich Item no. 32831-100mg
4,4-methylenedianiline (9)	101-77-9	Sigma Aldrich Item no. 32950-50g
3,3-dichlorbenzidine (10)	91-94-1	Sigma Aldrich Item no. 48029
3,3-dimethoxybenzidine o-dianisidine (11)	119-90-4	Sigma Aldrich Item no. D9143-5g
3,3-dimethylbenzidine (12)	119-93-7	Sigma Aldrich Item no. 31659-100mg
4,4-methylenedi-o-toluidine (13)	838-88-0	Sigma Aldrich Item no. 46106-100mg
6-methoxy-m-toluidine (14)	120-71-8	Sigma Aldrich Item no. 103284-100g
4,4-methylene-bis-(2-chloro-aniline) (15)	101-14-4	Sigma Aldrich Item no. 66681-1g
4,4-oxydianiline (16)	101-80-4	Sigma Aldrich Item no. 46117-250mg-R Storage at 6°C.
4,4-thiodianiline (17)	139-65-1	Sigma Aldrich Item no. 32955-1g
o-toluidine (18)	95-53-4	Sigma Aldrich Item no. 185426-5g
4-methyl-m-phenylenediamine (19)	95-80-7	Sigma Aldrich Item no. 101915-50g
2,4,5-trimethylaniline (20)	137-17-7	Apollo Scientific Item no. OR27602 Storage at 6°C.
o-anisidine (21)	90-04-0	Merck-Schuchardt Item no. 8.00461.0100
4-aminoazobenzol (22)	60-09-3	Supelco Item no. 46130
aniline (23)	62-53-3	Sigma Aldrich Item no. 242284-250ml
1,4-phenylenediamine (24)	106-50-3	Fluka

		Item no. 78429
Disperse Blue 1 (30% dye content) (B1)	2475-45-8	Sigma Aldrich Item no. 215643-5G
Disperse Blue 3 (dye content 20%) (B3)	2475-46-9	Sigma Aldrich Item no. 215651-50G
Disperse Blue 35 (dye content 100%) (B35)	12222-75-2	Fluka Analytical Item no. 17992-5G
Disperse Blue 106 (dye content 100%) (B106)	12223-01-7	Fluka Item no. 28241-5G
Disperse Orange 37 (dye content 100%) (O37)	13301-61-6	Fluka Item no. 21603-5G
Disperse Yellow 3 (dye content 30%) (Y3)	2832-40-8	Sigma Aldrich Item no. 215686-50G
Disperse Red 1 (dye content 95%) (R1)	2872-52-8	Sigma Aldrich Item no. 344206-5G
Solvent Yellow 3 (dye content 97%) (SY3)	97-56-3	Sigma Aldrich Item no. 121568-100G

Table 6: List of ISTD and IS.

ISTD/IS	CAS no.	producer/type
ISTD: naphthalene-d_8 (ρ = 1mg/ml)	1146-65-2	Sigma Aldrich Item no. 176044-1g
ISTD: 2,4,5-trichloraniline (ρ = 1mg/ml)	636-30-6	Sigma Aldrich Item no. 35828-100mg
ISTD: anthracene-d_{10} (ρ = 1mg/ml)	1719-06-8	Sigma Aldrich Item no. 176591-1g
IS: benzidine-d_8 (ρ = 0.5mg/ml)	92890-63-6	Sigma Aldrich Item no. 617490-100mg

3.2 Material

Table 7: Material for GC-MS.

material	producer/type
Agilent J&W GC Columns Length (m) 30 Diam. (mm) 0.250 narrow bore Film (µm) 0.25 Temperature Limits: from 50°C to 240°C (360C°)	Agilent Technologies Part no. 122-3832E Durabond DB-35MS
GC	Agilent Technologies 6890 Series
MSD	Agilent Technologies 5975B inert XL MSD
Ultra Inert Inlet Liners Splitless, SngI tpr, Wool 25pk	Agilent Technologies Item no. 5190-3167
Agilent Premium Inlet Septa Advanced Green Non-Stick 11mm Septa	Agilent Technologies Part no. 5183-4759-100
CEM EDGE	CEM GmbH EDGE 60 EX-001 + Q-Cups und Q-Disc Sandwich C9 + G1 + C9
analytical balance	SARTORIUS

	Item no. 701A839 D-K-19398-01-00
analytical balance	SARTORIUS Item no. 701A424 D-K-19398-01-00
water bath	LAUDA Eco Silver ECO E20
ultrasonic bath	Elma Transsonic TI-H-10
block heater with sample concentrator (nitrogen)	Stuard SBH130D/3 and SBHCONC/1
extraction apparatus: 	Reflux condenser with standard ground joint NS29/32. Twine for fixing the sample. 100ml round bottom flask with standard ground joint NS29/32. Electric heater. (Lap Heat)
reaction vessel (20-50ml)	Pyrex bottle 50mL.
column for separation of the amines (empty columns, for norm method)	Macherey-Nagel GmbH & Co. KG volume: 70ml polypropylene-columns Item no. 730158 Length = ~ 3cm Inner diameter = ~ 13cm Filled with 20g diatomaceous earth
column for separation of the amines (empty columns, for optimized method)	VARIAN Chem ELUT CE1005 length = 10cm inner diameter = 2cm filled with 6g diatomaceous earth
glass fiber filter	Pall Corporation ACRODISC 25mm with 1µm glass fiber Item no. 4523T
glass wool	-
vacuum rotary evaporator	Heidolph Hei-VAP- Value Digital and vacuubrand PC500 series CVC3000
pipettes	10-100µL M100 GILSON microman + pipette tip GILSON Item no. F148415 50-250µL MR-250 RAININ Pos.D + pipette tip RAININ Item no. 17008608 100-1000µL M1000 GILSON microman + pipette tip RAININ Item no. 17008609 GC syringe for small voluminas SGE

	Analytical Science Item no. 002987 10µL

Table 8: Material for FTIR.

material	producer/type
FTIR-Spectrometer	Perkin Elmer FTIR-Spectrometer Frontier Item no. 100643
hydraulic press	Perkin Elmer RANA/Presse1 Item no. 1000-136389-0
+ ethanol and isopropanol for cleaning.	

3.3 Software

Table 9: Software for GC-MS.

software	type
Agilent Mass Hunter for quantitative Analysis	product version: 6.0.388.0 file version: 6.0.388.0
Agilent Qualitative Analysis MSD Chem Stadion E.02.02.1431	product version: B06.00 file version: 6.0.633.10
GC-MSD Translator	product version: 6.0.0.0 file version: 1.0.0.0
GC-MSD Data Acquisition	product version: 6.0.0.0 file version: 1.0.0.0

Table 10: Software for FTIR.

software	type
Perkin Elmer Spectrum IR	Spectrum 10 software

3.4 GC-MS

3.4.1 ISO EN Norm Method 14362

Area of application:

The standard Method 14362 describes a method for the detection of azo dyes in textiles which are accessible to the reducing agent with or without prior extraction. Azo dyes which are accessible to the reducing agent without prior extraction are those used for dyeing with pigments or for dyeing cellulosic fibers (e.g. cotton, viscose), protein fiber materials (e.g. wool, silk) and synthetic fibers (e.g. polyamide, acrylic). *(Austrian Standards International & CEN 2017)*

Azo dyes accessible by prior extraction are those used to dye synthetic fibers with disperse dyes. The following synthetic fibers can be dyed with disperse dyes: Polyester, polyamide, acetate, triacetate, acrylic and chlorine fibers. The process is relevant for all colored textiles, e.g. dyed, printed and coated textiles. *(Austrian Standards International & CEN 2017)*

Brief description:

The selected textile sample is subjected to the dye extraction method for disperse dyes and/or the direct reduction method for the other dye classes (pigments and/or dyes). The application of one of the two methods is based on the type of fiber of the test sample (composed of the pure fiber or of fiber blends) and the color treatment (dyeing or printing method). *(Austrian Standards International & CEN 2017)*

In dye extraction for disperse dyes, the dye is first extracted from the fiber in the vapor space using xylene under reflux. The extract is concentrated and transferred with methanol to the reaction vessel for subsequent reduction with sodium dithionite in an aqueous citrate buffer solution (pH = 6) at 70 °C. If the textile sample was not completely decolorized after xylene extraction, a new test sample must be prepared and reprocessed using the procedure for non-extractable dye classes. When the procedure for non-extractable dye classes is carried out, the measurement sample is treated with sodium dithionite in an aqueous citrate buffer solution (pH = 6) at 70 °C in a closed vessel. *(Austrian Standards International & CEN 2017)*

After reduction, the amines released in the process are transferred to a tert-butyl methyl ether phase using liquid-liquid extraction with silica columns. The tert-butyl methyl ether extract is then concentrated, and the residue is taken up in acetonitrile. *(Austrian Standards International & CEN 2017)*

In addition, it is possible to carry out a screening method without diatomaceous earth columns (norm 14362-1, appendix E). Since the screening method is similarly time-consuming and material-intensive as the norm method with diatomaceous earth columns, and since the norm method must be performed anyway when amines are detected with the screening method, it is more efficient to perform the norm method immediately. *(Austrian Standards International & CEN 2017)*

The aromatic amines can also be detected directly from a dye (norm 14362-1, appendix F). In this case, the extraction step is omitted and the dye sample, dissolved in methanol, is subjected directly to reductive cleavage. This possibility of detecting amines in azo dyes was used to screen 8 different dyes for their amines. *(Austrian Standards International & CEN 2017)*

Sample preparation and fiber types:

The textile sample is prepared by cutting it into pieces (approx. 5mm x 5mm) or into strips to obtain a total mass of 1g. Samples subjected to dye extraction are cut into strips and tied together with twine. Fastening with a hook in the extraction apparatus (as suggested by the norm) was not possible. Samples that are directly subjected to reductive cleavage are cut into pieces. *(Austrian Standards International & CEN 2017)*

It is possible to examine up to 3 colors together. Each color should have approximately the same mass (total sample mass = 1g). If the analysis of the mixed sample shows a detection of 5-30mg/kg for one of the amines, a separate analysis of the colors is necessary. *(Austrian Standards International & CEN 2017)*

Whether extraction is necessary is determined by the type of fiber and the fiber blend (table 11):

Table 11: Application of dye extraction for disperse dyes depending on the fiber type.

fiber type	Use of disperse dyes?	type	Is an extraction necessary?
Natural fiber	No	A	No
Synthetic fiber	No	B	No
	Not specified	C	Yes
	Yes	D	Yes

No examination is necessary if the fiber is undyed/white.

Natural fiber means fibers of animal origin such as wool and silk, or those of plant origin such as sisal, ramie, kapok, hemp, jute, cotton and flax. For these fibers, basic and acid dyes, chrome and metal complex dyes, direct dyes, azoic/development dyes, sulfur or vat dyes, reactive dyes or pigments are used for dyeing. Synthetic fibers are defined as fibers such as polyester, polyamide, triacetate, acetate, acrylic, viscose and chlorofibers. For these fibers, disperse dyes can be used (except viscose) in addition to the dyes mentioned above. Disperse dyes are mainly used for dyeing polyester and triacetate. *(Austrian Standards International & CEN 2017)*

For fiber blends, dye extraction must be performed as soon as type C or D fibers are present in the blend. *(Austrian Standards International & CEN 2017)*

Implementation Norm 14362-1:

a. Extraction of disperse dyes with xylene:

The prepared sample is clamped in the extraction apparatus with the twine and held over boiling xylene (boiling point at 140°C) for 40min (or until the

sample is colorless). The obtained extract is cooled to room temperature before being removed from the apparatus. *(Austrian Standards International & CEN 2017)*

The extract is then concentrated to dryness at 55°C. The residue is quantitatively transferred to the reaction vessel with 2.5mL of methanol (2x each 1mL and 1x 0.5mL). If the final volume is greater, the methanol extract is concentrated again. An ultrasonic bath can be used to disperse the dye. The dye extract can be stored in the freezer (-18°C) until the next day, or the reductive cleavage can be carried out directly. *(Austrian Standards International & CEN 2017)*

If the textiles were dyed with pigments or dyes other than disperse dyes (type A and B fibers), the test sample is placed directly into the reaction vessel (in small 5x5mm pieces) and reductive cleavage is performed. If the textile sample was not completely decolorized during extraction, a second sample is prepared, placed directly into the reaction vessel for reductive cleavage.

b. Reductive cleavage:

Pipette 15mL of the buffer solution (pH = 6, c = 0.06mol/L) prewarmed to 70°C into the reaction vessel. The reaction vessel is tightly sealed and left in the water bath at 70 [+/- 2] °C for 30 [+/- 1] min. *(Austrian Standards International & CEN 2017)*

Subsequently, the azo groups are cleaved, by the addition of 3.0mL aqueous sodium dithionite solution (ρ= 200mg/mL). The solution is then shaken vigorously (at this point, decolorization can be observed for some dyes) and immediately left at 70 [+/- 2] °C for another 30 [+/- 1] min. The solution is then cooled to room temperature within 3 min using a cooled water bath (e.g. with ice or cold packs). *(Austrian Standards International & CEN 2017)*

c. Separation and constriction of the amines:

Add 0.2mL of 10% NaOH solution to the reaction solution and shake vigorously. The reaction solution is then added to the diatomaceous earth column and absorbed by the column for 15min. Pipette 10mL of tert-butylmethylether into the reaction vessel and shake. After the 15min, the contents of the reaction vessel are decanted onto the column. The eluate is collected in a 100mL round bottom flask. The reaction vessel is then refilled with 10mL tert-butylmethylether and the solution is decanted onto the column. Then 60mL of tert-butylmethylether is added directly to the column. To ensure a faster processing, the use of a Peleus ball to pump

the ether through the column is recommended, since the use of a vacuum pump is not possible due to the dimensions of the instrument. *(Austrian Standards International & CEN 2017)*

The tert-butylmethylether extract is concentrated at 40°C at about 200mbar to about 1mL. The extract is then transferred to eprouvettes and concentrated to dryness using a weak stream of inert gas. The residue is immediately taken up in acetonitrile and dissolved using an ultrasonic bath. From this extract, 980µL is taken and pipetted into a vial along with 10µL each of ISTD and IS and immediately measured by GC-MS (parameters table 12). The ISTD and IS are added just before injection, otherwise the substances would be lost through reductive cleavage. Further tests should be carried out to determine whether it would be possible to integrate the two substances earlier in the process. The remainder of the extract can be frozen at -18°C for later measurement. If complete analysis cannot be performed within 24h, the extract should be stored at -18°C. *(Austrian Standards International & CEN 2017)*

Table 12: GC-MS parameters for measurement.

GC-MS	
column	DB-35MS (J & W)®, length: 35 m, inner diameter 0.25 mm, Film thickness: 0.25µm
Injector system	split or splitless
Injector temperature	260°C
Carrier gas	Helium
Temperature program	100 °C (2 min), 100 °C to 310 °C (15 °C/min), 310 °C (2 min)
Injection volume	1.0µl, split 1:15
Detection	MS

d. Detection and quantitative determination of amines:

Verification of the process and evaluation with the daily calibration.

When measuring a processed sample series, a blank solution, a daily calibration solution and a check solution are included and measured each time:

Blank = 980µL acetonitrile and 10µL of IS and ISTD. The blank is used to check impurities in the instrument or the IS or ISTD solution, which can lead to false positive results.

Daily calibration = consists of the stock solution of amines and is diluted with acetonitrile to a concentration of 15µg/mL. In the course of the exper-

imental work, 2 stock solutions of the amines were prepared: *(Austrian Standards International & CEN 2017)*

- Amin-mix 1 (old) = amine mix of all amines apart from o-aminoazotoluene (97-56-3), 5-nitro-o-toluidine (99-55-8) and aminoazobenzene (60-09-3) plus aniline and 1,4-phenylenediamine. (c = 40µg/mL) Prepared from the amine single standards (c = 1mg/mL). For daily calibration, 375mL of this was pipetted into a vial, mixed with 10µL of IS and ISTD and 7.5µL of the dissolved standard of 3,3-dichlorobenzidine (91-94-1) in methanol (c = 2000ug/mL) and diluted to 1mL with 597.5µL acetonitrile.

- Amin mix 2 (new) = amine mix of all 22 amines plus aniline and 1,4-phenylenediamine (c = 1000µg/mL). Since the standard suggests a concentration of 300µg per amine per mL or greater for the stock solution. For the daily calibration, 15µL of this was pipetted into a vial with 10µL each of IS and ISTD and 7.5µL of the dissolved standard of 3,3-dichlorobenzidine (91-94-1) in methanol (c = 2000µg/mL) and diluted to 1mL with 957.5µL acetonitrile.

The daily calibration is used to quantitatively calculate the amine concentration in the sample solution and for recovery matching with the 30ppm check. If an amine is detected with the daily calibration with greater than 5mg/kg the quantification must be followed up with a calibration function of at least three points.

Check 30ppm = was prepared from 750µL amine mix old + 15µL standard of 3,3-dichlorobenzidine (91-94-1) or 30µL amine mix new and 15µL standard of 3,3-dichlorobenzidine (91-94-1). This was placed in a reaction tube and 15mL of the citrate buffer solution was added, then the check was cooled together with the samples and from the step of separation and restriction of the amines, the same procedure was followed as with the samples.

During the measurements it became apparent that it was not relevant which amine mix was used for the daily calibration. Both gave the same concentration of the amines when measured, even after longer storage, as the amines are stable in acetonitrile and under conditions below 8°C. For the 30ppm check, there was no difference in the evaluation either. However, if amines such as o-toluidine (95-53-4) or 4-methyl-m-phenylenediamine (95-80-7) are analyzed in routine analysis, it is advisable to prepare an amine mix without o-aminoazotoluene (97-56-3) and 5-nitro-o-toluidine (99-55-8), since these are reduced to 95-53-4 and 95-80-7

during the test and can give falsified results. In addition, 4-aminoazobenzene (60-09-3) is also omitted from the amine mix, since dyes that can form that amine produce aniline (62-53-3) and 1,4-phenylenediamine (106-50-3) during the procedure. If aniline is detected at a concentration greater than 5mg/kg during a test, the textile sample should be treated with the procedure according to 14362-3 (Detection of the use of certain azo dyes that can release 4-aminoazobenzene). *(Austrian Standards International & CEN 2017)*, *(Austrian Standards International & CEN 2 2017)*

Calculation with the daily calibration and the ISTD:

$$\rho_S = \rho_c \times \frac{A_s \times A_{ISC}}{A_c \times A_{ISS}} \times \frac{V_s}{V}$$

ρ_s … is the amine concentration in the sample solution, in µg/ml.
A_s … the peak area of the amine in the sample solution, in area units.
A_c … the peak area of the amine in the calibration solution, in area units.
A_{ISS} … the peak area of the Internal Standard in the sample solution, in area units.
A_{ISC} … the peak area of the Internal Standard in the calibration solution, in area units.
V … the final volume of the sample prepared according to 10.4, in ml. (2mL)
V_s … the volume of amine solution used to verify the procedure, in ml. (amin-mix old = 0,375mL; amin-mix new = 0,015mL)
ρ_c … the amine concentration in the calibration solution, in µg/ml. *(Austrian Standards International & CEN 2017)*

The amine content is calculated as the mass fraction *w*, in **mg/kg** of the sample using the following equation:

$$w = \frac{\rho_s \times V}{m_E}$$

ρ_s ... the interpolated amine concentration, in µg/ml.
V ... the final volume of the extract corresponding to 10.4, in ml. (2mL)
mE ... the mass of the textile sample, in g. *(Austrian Standards International & CEN 2017)*

Evaluation with a 10-point calibration series.

For the quantitative determination of the amines, a calibration series can be generated. Since the measurement accuracy of the GC-MS can change over the duration of use and due to contamination, it is necessary

to produce these freshly at least every week. The calibration series can then be entered into the processing method on the PC and the calculation of the concentrations in the sample in ug/mL is performed automatically by the software. For this purpose, a 10-point calibration in the range from 2µg to 50µg of each amine per mL was prepared in acetonitrile, from the amine mix new. 10µL of IS and ISTD per mL were added to each of the 10 concentrations.

As an example, for the final calibration series, the measured values of the 10-point calibration series for 1,4-phenylenediamine are given in the appendix, as well as the corresponding calibration function as a graph.

Work process of the Master thesis:

The amine monosubstances were prepared by weighing all 22 amines, as well as aniline and 1,4-phenylenediamine, and diluting them with acetonitrile to a concentration of 1mg/mL.

The individual standards were stored in pyrex-bottles in the refrigerator.

The amine 3,3-dichlorobenzidine (91-94-1) was insoluble in acetonitrile, so this was purchased diluted in methanol. In addition, an amine mix was also prepared from these individual standards.

Using the software, the SCAN mode was set and the individual substances, as well as the amine mix, were measured. For the single substances, the retention time, the m/z of the main peaks and the relevant qualifiers were then determined using a database and the signals from the measurement. A SIM method was created for quantitative analysis and the ions were divided into groups (table 13):

Table 13: Rt, main peak, qualifier, and ion-groups.

amine no.	Rt	m/z main peak	m/z qualifier		number of ions	Dwell
Group 1:						
aniline	2.89	93		66		
18	3.87	106	107			
21	5.11	108	80	123	9	35
7	5.47	127				
ISTD	4.97	136				
Group 2:						
14	6.06	122	94	137		
20	6.11	120		135	9	35
1,4-phenylenediamine	6.42	108	80			
3	6.45	106	141			

Group 3:						
19	7.64	121	122			
8	8.51	123		138		
4	9.13	143		115	9	35
6	9.62	152	122	77		
1	10.68	169				
Group 4:						
22	13.13	92	197			
9	13.62	198	197			
IS	13.65	192			8	40
2	13.67	184	92			
16	13.5	200	93			
Group 5:						
5	14.14	106				
13	14.57	226	211		5	60
12	14.75	212	106			
Group 6:						
17	15.38	216		184		
15	15.59	231		266	7	45
11	15.65	244		201		
10	15.61	252				

All Rt and m/z values for the respective amines are listed in the appendix, along with the database reference spectrum.

Afterwards, the calibration series could be measured in SIM mode with 6 different concentrations (c = 3-35µg/mL). The evaluation was initially performed using the peak areas and a calibration function was created for each amine. As an example, figure 17 shows the calibration curve for 1,4-phenylenediamine for the m/z = 108.
Based on this evaluation, a quadratic calibration function gave the best coefficient of determination (R^2 greater than 0.98). In addition, it could be determined that analyte concentrations far below the limit value (30ppm) can be measured with the method.

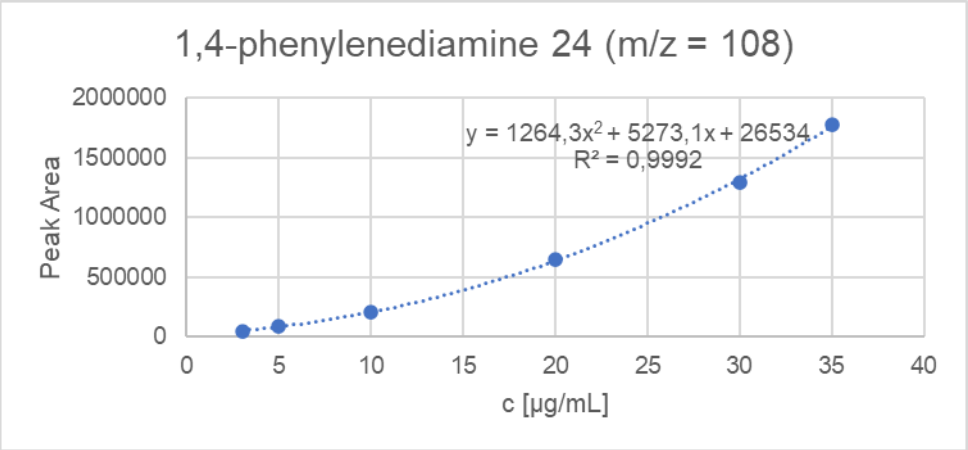


Figure 17: Calibration curve of 1,4-phenylenediamine.

To evaluate qualitatively with the software, a 10-point calibration series (c = 2-50µg/mL) was recreated from the amine mix and measured. Subsequently, the processing method was written and the calibration series was entered with the respective concentration levels.

The three possible ISTDs naphthalene-d_8 (1146-65-2), 2,4,5-trichloroaniline (636-30-6) and athracene-d_{10} (1719-06-8) were measured (first in SCAN mode to determine the m/z) and quantitatively evaluated using the SIM mode. For this purpose, 10uL ISTD was added to each vial of a 5-point calibration series of the amine mix (3-30 ug/mL) and the peak areas of the possible ISTDs were evaluated. Naphthalene-d_8 (Rt = 4,97) was chosen as ISTD as it gave the highest signals in the spectrum and had the best CV (illustrated in the following table 14) and there was still room for another ion in group 1 without reducing the dwell time too much.

Table 14: 3 ISTD's results and statistics.

ISTD	Rt [min]	m/z	peak area [$\bar{X}$±SD; CV]
naphthalene-d_8 (1146-65-2)	4.979	136	800742.62 ± 55748.63; 6.96
2,4,5-trichloroaniline (636-30-6)	10.846	188	249877.02 ± 61039.13; 24.43
athracene-d_{10} (1719-06-8)	8.721	195	97696.75 ± 26186.11; 26.80

Benzidine d_8 (92890-63-6) is used as IS, it shows interferences in the later part of the gas chromatogram and the recovery should be above 30%.

To validate the experimental procedure, fabric samples were spiked with azo dyes. The fabric samples were white fabrics made of polyester, polyamide, and viscose, since at AGES-Linz mainly carnival costumes and toys will be tested with the method and, according to experience and research, these mainly consist of the synthetic fiber types mentioned.

To find suitable azo dyes for spiking, 7 disperse dyes and one solvent dye (SY3) were investigated. A list of relevant azo dyes for textile dyeing was found in DIN 54231:2005-11.

Additionally in an investigation by the investigation offices for food monitoring and animal health in Baden-Württemberg, carnival costumes, sports underwear, sleeping bags and traditional costumes were tested for the content of azo dyes. Almost all samples were unremarkable, except for the carnival costumes, where 1,4-phenylenediamine was found in 12 of 12 samples. This report also lists disperse dyes that should no longer be used due to their sensitizing potential. (Opinion No. 041/2012 of the Federal Institute for Risk Assessment (BfR) of July 6, 2012). The dyes listed were dispersion-blue 1, dispersion-blue 35, dispersion-blue 106, dispersion-blue 124, dispersion-yellow 3, dispersion-orange 3, dispersion-orange 37 and dispersion-red 1. *(Köhler et al. 2018)*

The following dyes (table 15) were weighed into the reaction vessel (200mg) and diluted with 2mL methanol and subjected to reductive cleavage and subsequently measured and quantitatively evaluated for the detected amines. Since the dye samples were very strongly colored after the procedure, they were diluted again 1:2 with acetonitrile. Otherwise, impurities or carryover could occur during a direct measurement.

Table 15: Detected amines in 8 different azo-dyes.

amine	B1	B3	B35	B106	R1	Y3	O37	SY3	
1	2.0	2.0		2.0	2.3	2.0			µg/mL
2					3.9				µg/mL
3	2.4	2.4			13.7	2.5	2.4	2.7	µg/mL
5							2.8	548.0	µg/mL
7	2.3	2.3	2.3		3.6	4.3	2.6	2.3	µg/mL
8				2.5				2	µg/mL
14		2.3	2.2			2.31			µg/mL
18	2.1		2.2	2.2	2.1		2.1	343.4	µg/mL
19		2.6			13.2	2.8	40.8		µg/mL
21								2.5	µg/mL
aniline	2.1	2.1	2.1		4.9	3.1	2.3	2.2	µg/mL
1,4-phenylenediamine	2.1	2.9	2.1		208.8	14.9	3.1	2.5	µg/mL

Since Disperse Red 1 (R1) and Solvent Yellow 3 (SY3) provided very high concentrations of 1,4-phenylenediamine and o-toluidine, these dyes were selected for further studies.

A solution of the dyes in acetonitrile was prepared, which was used for spiking the textile samples. The samples were spiked with 0.3; 1.5; 2.5

and 3.5 mg R1 and 0.15; 0.8; 1.5 and 2 mg SY3 and processed according to standard 14362-1. Since R1 is a disperse dye, the spiked textile sample had run throught an extraction with xylene in advance. SY3 was added directly to the reaction vessel with the textile sample.

For the validation of the method, three measurement runs with each of the four concentration levels had to be processed.
At the end of the measurements, a storage test was carried out with the stored samples.

Difficulties and disadvantages of the norm method:

After performing the norm method for the detection of azo dyes in textiles several times, some difficulties and complications were encountered:

- The method is very time-consuming (e.g. filling the diatomaceous earth columns, which are not commercially available) and expensive, as large amounts of material and chemicals are consumed.

- The chemicals used are hazardous in their application (poisons).

- The amines are very unstable substances and can be degraded by incorrect storage, an incorrect solvent (e.g. amine 8 and 19 in methanol), during concentration, as well as delays in the workflow.

- Pigments may remain as insoluble residue in the reaction vessel after extraction with xylene and replacement of the solvent with methanol.

- False positive results are possible due to isomers, matrix components (polyurethane, crosslinking agents, etc. especially amine 9, 15, 16 and 19 are affected), high temperatures in the GC injector (especially amine 12, 18, 21, 9 and 19 are affected) and due to amines, that can be formed from other dyes (e.g. 1, 4 and 8). *(Austrian Standards International & CEN 2017)*

- Recovery of some amines (3, 7, 14, 18, 20, 21 and aniline) was not good enough after workup with the norm method.

- SY3 did not provide good results. Even after a direct weighing in of the dye into the reaction vessel, without the textile-matrix, no evaluable results were obtained. Therefore, it was not included in the following measurements.

3.4.2 Optimized Method

a. Altered extraction conditions:

In norm method 14362-1, the extraction of azo dyes is performed with a mixture of isomers of xylene. This is quite expensive to purchase and has the following hazard statements:

H226	Flammable liquid and vapor.
H304	May be lethal if swallowed and enters airways.
H312+H332	Harmful in contact with skin or if inhaled.
H315	Causes skin irritation.
H319	Causes severe eye irritation.
H335	May cause respiratory irritation.
H373	May cause damage to organs (central nervous system, liver, kidney) through prolonged or repeated exposure. *(ROTH 2016)*

Xylene is only slightly volatile compared to acetone or ether, but due to the time-consuming extraction (40min or longer) and cooling in the extraction apparatus, solvent losses do occur. In addition, the thread for fixing the textile sample is located between the reflux condenser and the round-bottom flask sections, which has disadvantages for the tightness of the apparatus.

Therefore, an alternative for the extraction method was invented. The solvent was changed to acetone and instead of the conventional extraction apparatus, the CEM EDGE (figure 18) was used for automated extraction. A filter sandwich of C9 + G1 + C9 was incorporated into the Q-Cup and the extraction method was determined (table 16):

Table 16: Parameters for CEM-extraction.

cycle	solvent	top volume [mL]	bottom volume [mL]	temperature [°C]	hold time [min]	rinse volume [mL]
1	Acetone	10	5	110	05:00	0
2	Acetone	10	0	110	05:00	0
3	Acetone	10	0	100	05:00	0
cleaning	Acetone	10		80	30sec	
cleaning	Acetone	10		80	30sec	

The sample could be weighed in and spiked directly in the Q-Cup and extracted immediately. One run took about 17min, which is two times faster than conventional extraction (40min). In addition, acetone has fewer health hazards and is cheaper to purchase. It was additionally found that the ex-

tracts after the optimized extraction method were more color intensive (dark red) than those after the conventional method (orange).

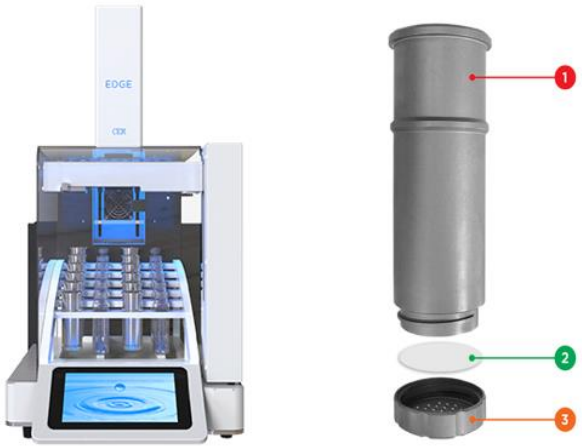


Figure 18: CEM EDGE extraction apparatus and Q-Cup (1), Filter (2) and threaded bottom (3). (C.E.M. Corporation 2017)

Work-flow of the CEM-EDGE:

1. The Q-Cups containing samples are loaded into the EDGE and a method is selected. Then hit “Start.”

2. The autosampler loads the Q-Cup automatically into the chamber. Then the pressure cap creates a pressurized seal on the top of the Q-Cup.

3. The solvent is added through the bottom to fill the gap between the chamber and Q-Cup, this aids in heat transfer. Afterwards the solvent is added through the top of the Q-Cup to wet the sample. When the chamber walls are heated, the pressure in the gap increases. This overcomes the pressure inside the Q-Cup, forcing the solvent to disperse into the sample. (figure 19)

4. When the sample reaches temperature, the solvent is dispensed through the Q-Disc, the cooling coil, and into a collection vial. *(C.E.M. Corporation 2017)*

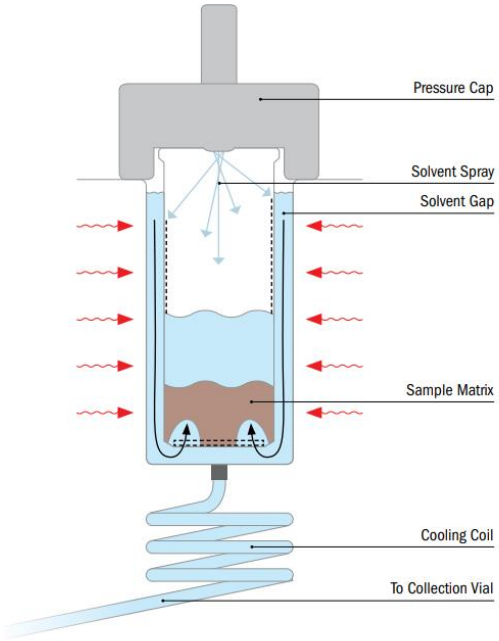


Figure 19: CEM extraction work-flow. (C.E.M. Corporation 2017)

The Q-Screen Kit (steel screen with matching tool for fixation) was used to obtain the sample tightly packed in the Q-Cup. The dense packing allows the solvent to penetrate more evenly and less solvent is needed for extraction.
To avoid the volatility of acetone, collection containers with a suitable septum should be used.

b. Altered material and chemicals:

Since the norm method involves the use of very large quantities of chemicals and materials, and this is associated with increased work effort, costs and potential hazards, the next step was to optimize this ratio. The following parameters were optimized by reworking the method several times:

- Diatomaceous earth columns: the columns were exchanged for smaller columns and filled with only 6g of diatomaceous earth.

- Filter: instead of the expensive glass fiber filters, a small amount of glass wool was added to the columns before filling with diatomaceous earth.

- *tert*-butylmethylether: the amount used was reduced to 40mL (10mL, 10mL and 20mL).

- Buffer: the amount used was reduced to 4.5mL.
- Sodium dithionite: the amount used was reduced to 0.9mL.
- NaOH 10%: the amount used was reduced to 60µL.

c. Advantages due to the optimized method:

- Improved extraction conditions (less time intensive for single samples, lower working temperature, clean working with Q-Cups, up to 12 samples per run possible) with more extraction yield and thus more accurate determination of the actual amount of amines in the sample.
- Use of less hazardous solvents. (Workplace safety)
- Faster and more efficient processing of the experiment and therefore less amine loss due to delays in the workflow. (separation of amines with diatomaceous earth columns possible without mechanical action by peleus-ball).
- Cost savings due to less material and chemicals used.
- Diatomaceous earth columns with 6g can also be purchased, in contrast to the 20g columns described in the norm.
- Better recovery of the amines.

3.5 FTIR

Any electromagnetic radiation has an influence on molecular bonds. When infrared radiation (0.8-500 µm) is absorbed, the bonds are excited to vibrations (by near, mid and far IR) and rotations (far IR). The molecular vibrations are measured by the FTIR instrument as absorption of the radiation in the infrared spectrum. The rate of attenuation of each frequency is recorded as an absorption band in the spectrum. Depending on the absorption, it can be determined what type of vibration it is. Since the vibrations are characteristic of molecular bonds, molecular bonds can be determined. *(Brunner 2015)*

The excitation of an oscillation can be illustrated as follows: the molecule passes under absorption of a light quantum from the oscillation state with the quantum number n into a higher one, e.g. with n + 1. The energy dif-

ference of the two states corresponds thereby to the energy of the light quantum (resonance condition). The transition from n = 0 to n = 1 is the fundamental oscillation, the transition from n = 0 to n = 2 is the first harmonic, which has approximately twice the frequency. The probability of these transitions and the intensity of the absorption bands strongly decreases with increasing size of the quantum jump.
Besides quantum conditions, the occurrence and intensity of absorption bands depend on the dipole moment of a molecule. Infrared radiation is absorbed only if the dipole moment interacts with the electric vector of the light. *(Brunner 2015)*

Depending on the oscillation form, one distinguishes between valence or deformation oscillations, and depending on the symmetry behavior, symmetrical, antisymmetrical and degenerate oscillations. Based on their characteristic vibrations, functional groups of a compound can be identified. In addition to the determination of functional groups, the position and intensity of the position and intensity of the bands, pure substances can also be clearly identified. Reference databases are used for this purpose. *(Brunner 2015)*

The aim of the study was to create a database using FTIR to distinguish textile samples by FTIR measurement on their fiber type and to determine by a fast and non-destructive measurement method whether it is a natural or synthetic fiber.

The difficulty of the measurement was to obtain reference samples as pure as possible and to measure on surfaces as smooth as possible. Sample preparation by pressing the samples reduced the air space between the fibers and enabled improved measurement. In addition, FTIR is a point-by-point measurement, so fiber samples must be measured at several points to guarantee sample homogeneity.

For the spectra of the reference-substances and the measured samples, the transmission [%] is plotted against the wave number [cm^{-1}]. It was measured in a range from (X-axis start value) 4000cm^{-1} to (X-axis end value) 650cm^{-1}.
An IR spectrum contains two major regions: Above 1500 cm^{-1} there are absorption bands, which can be assigned to individual functional groups, while the region below 1500 cm^{-1} contains many bands and characterizes the molecule. This region is called the "fingerprint" region. *(Brunner 2015)*

Measurement of fiber type with FTIR:

Area of application:

Qualitative identification of materials. ATR spectroscopy enables surface analyses of samples which can be brought into contact with a crystal with a high refractive index. Since the radiation is guided by total reflection at the interface of the ATR crystal, it only comes into contact with the surface of the sample. The sample interface is a diamond ATR crystal. Its mechanical and chemical resistance allow samples to be analyzed directly. The ATR (Attenuated Total Reflection) measurement technique is very fast and requires almost no sample preparation. *(Schwartz et al. 2021)*

The sample is simply brought into contact with the diamond ATR crystal to record an IR spectrum. After the measurement, the spectrum of the sample is identified by an automatic comparison with spectra from the spectra databases. A prerequisite for ATR measurements is good contact between the sample and the crystal (contact pressure). *(Schwartz et al. 2021)*

Sampling:

Not applicable.

Brief description:

The FT-IR measurement technique is based on the interaction between infrared radiation and matter. The infrared radiation excites functional groups of substances to different oscillations. The energy required for this is absorbed by the sample and thus appears as a band in the spectrum. Certain conditions must be fulfilled for a compound to be infrared active. This is true for most chemical compounds. Based on these physical principle's materials can be identified. *(Schwartz et al. 2021)*

Check and replace the desiccant:

To protect the beam splitter and sample chamber windows from moisture damage, replaceable packets of desiccant are provided in the top cover of the spectrometer. The top cover of the spectrometer contains a desiccant status indicator whose sectors change color successively with the consumption of the desiccant. The sectors of the indicator change color from blue to pink as the desiccant is used up. *(Schwartz et al. 2021)*

The numbers correspond approximately to the % relative humidity in the spectrometer. The desiccant in the spectrometer should be replaced when the sector marked 10 became pink, while sectors 15 and 20 are blue.
The desiccant packets are regenerated by baking them in a drying oven at 140°C for 6 hours. To cool down to room temperature, the desiccant packs are stored in a desiccator that is filled with silica gel (silica gel must be deep blue) as a desiccant. *(Schwartz et al. 2021)*

Implementation:

Sample preparation:

If the sample does not have a flat surface or cannot be clamped with the holder due to its geometry, a sample piece is produced with the aid of a suitable cutting tool. Care must be taken to ensure that the contact surface is as flat as possible. If the specimen shows surface contamination (e.g. grease residues), these should either be removed with an ethanol-soaked cleaning cloth for glasses or cleaned in an ethanol-filled beaker in an ultrasonic bath for approx. 5 minutes. After the ethanol has dried completely, the specimen is ready for measurement. If there is a large air space between the individual fibers of the sample, it was processed in advance with a press to minimize this air space and obtain more accurate measurement results.

Readiness Check:

The spectrometer is to be tested daily during initial start-up to ensure that it is functioning properly. For this purpose, the automatic readiness check of the Spectrum software is carried out. The readiness check consists of 4 parts, the abscissa-check (3060cm^{-1}, 1601cm^{-1}, 1028cm^{-1}), the noise check (0% T), the throughput check (100% T) and the contamination check (0.05 A). Once the readiness check has been passed, the unit is ready for use. The instrument ready check report is inserted in the appendix.

Subsurface spectrum:

Before the first sample measurement and after carrying out the readiness check a spectrum of the background is recorded. If a disturbing shift of the baseline is observed during later measurements, the spectrum of the subsurface must be recorded again. The recording of this spectrum works like a regular measurement, but without a sample. The spectrum is recorded four times in total.

Measurement:

The prepared sample is placed with the flat side down onto the ATR-crystal (figure 20). Since this measuring technique has a certain penetration depth, it may be necessary to fold very thin samples several times to create a suitable layer thickness and the corresponding absorption. Differentiating between several different layers based on a single measurement is not possible with this technique, since it basically provides a mixed spectrum over the entire measurement depth.

Figure 20: FTIR with sample clamped between crystal and fixation.

To obtain a meaningful spectrum, the following points must be observed:

- plane support of the sample
- complete coverage of the crystal by the sample
- dryness of the sample (after cleaning)
- sufficient contact pressure (pressure should not exceed 150 FG)

After the sample has been clamped, the order number and sample coding can be entered in the software. The spectra are then recorded in the range from 650 to 4000 wavenumbers.

Evaluation:

For evaluation, the spectra database created especially for this method is used, which is composed of spectra of previously verified materials.
The software is used to determine the correlation between the sample spectrum and the spectra of the database. If the correlation of a match with a spectrum of the database is at least 95% the material can be assumed as identified.

In addition, before the result is released, a plausibility check is carried out to determine whether the sample can be this material.
If the agreement is less than 95%, although it can be assumed that it is a material from the database, the possible cause must be determined. If there are problems with the spectrum recording (interfering bands due to contamination, insufficient sample contact, background, etc.), the measurement must be repeated. *(Schwartz et al. 2021)*

If the agreement in the repeated measurement is still below 95%, the spectrum can be assigned manually based on the material-specific vibration bands. In this case the result must be verified by a second trained person.
All measured reference substances for the database and samples are listed with their respective spectrum in the appendix.

3.6 Sample-description (table 17-21)

Table 17: Different matrices for GC-MS.

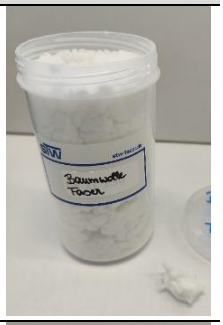
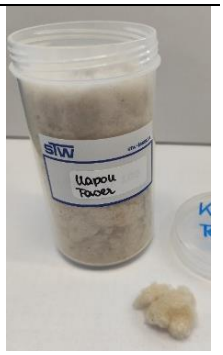
sample	appearance	texture	odor
Astronaut costume white (100% Polyester)	uniformly colored white. Slightly shiny.	lightweight fabric with finely woven fibers	No odor.
Bathing suite white (90% Polyamide, 10% spandex)	uniformly colored white. Matt.	elastic fabric with undetectable fiber structures, heavier than polyester matrix sample	No odor.
Blouse white (95% Viscose, 5% spandex)	uniformly colored white. Matt.	rough fiber structure, almost transparent, no fiber structures visible, heavier than polyester matrix sample	No odor.

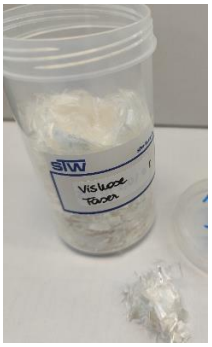
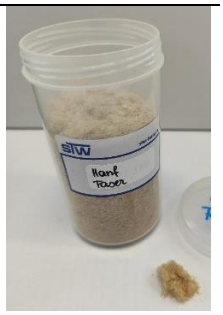
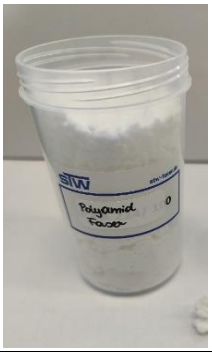
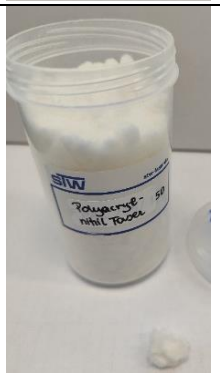
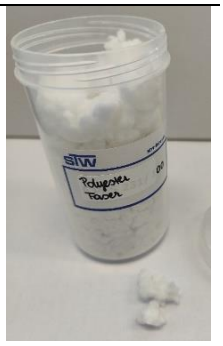
Table 18: Reference-sample-description FTIR. (samples from Stucken Melchers GmbH & Co. KG, 28195 Bremen, Am Wall 162/3)

sample	appearance	texture	odor	sample picture
kapok (fiber 1)	Light beige with dark brown and black particles. Slightly shiny.	Very light and fine, inhomogeneous as there are occasional darker particles in it. Fibers can be easily separated. Extremely soft and fine-pored.	Wood, fresh grass or hay.	
hemp (fiber 2)	Gray, beige and black fibers mixed. Individual fibers are recognizable. Matt surface.	Coarse texture, easy to separate. Rough and scratchy on the skin. Inhomogeneous structure due to the coarse fibers and color mixture.	Herbal, very discreet, a little like glue.	
silk (fiber)	Pure white and strongly shiny. Fine fibers, but recognizable structures.	Homogeneous mixture. Very soft consistency. Had to cut with scissors to separate the fibers.	Pure, something like glue.	
flax (fiber 2)	Very similar to hemp. Beige, gray and black fibers. Fiber texture recognizable. Matt surface.	Inhomogeneous mixture. Softer than hemp, but texture identical. Easy to separate, rough, scratchy.	Herbal and like hay.	

wool (fiber)	Beige/white with small darker particles. Matt and fibers are difficult to see.	Inhomogeneous due to the particles that are between the fibers. Soft and not scratchy, but heavier than kapok, for example. Easy to separate, light knots noticeable.	Neutral after wood and slightly animal note.	
ramie (fiber)	White but darker than silk, individual fibers recognizable. Matt surface.	Homogeneous, very stable fibers. Soft and not scratchy.	Neutral and sterile, a little like glue.	

Table 19: Reference-Sample description FTIR. (samples from STW Heinrich Kautzmann GmbH, 77773 Schenkenzell, Aue 3)

sample	appearance	texture	odor	sample picture
cotton (fiber)	White fiber and matt surface.	Like powder, very fine and homogeneous.	No odor.	
kapok (fiber 2)	Beige with dark particles. Matte surface.	Very fine and soft, like powder. Not homogeneous.	Quite discreetly like glue.	

viscose (fiber)	Single fiber pieces which are well recognizable, shiny and egg-shell-white color.	Homogeneous, very fine but pieces recognizable. Soft but not silky.	Plastic, chemical, like liquid glue, slight sulfur note.	
hemp (fiber 1)	Beige with lighter and darker very small particles. Matte surface.	Like coarse powder, not completely homogeneous. Texture reminiscent of wood chips.	Woody smell, natural, earthy.	
polyamide (fiber)	White fiber with matt surface.	Similar to cotton but coarser and not as soft. Homogeneous.	Smell of grains and cereals.	
polyacrylnitrile (fiber)	Eggshell-white fiber with matt surface.	Homogeneous and very fine. Like powder, not soft rather scratchy.	Almost no odor, subtle note of glue.	
polyester (fiber)	White with gray shimmer. Matte surface.	Homogeneous and very fine, like powder. Tends to accumulate in balls.	No odor.	

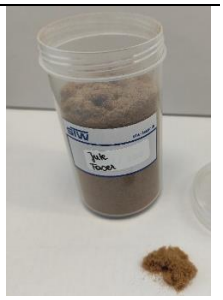
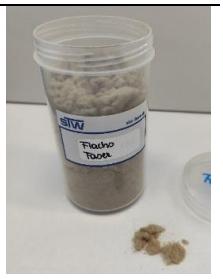
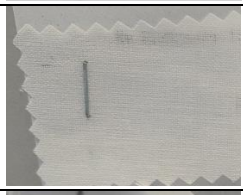
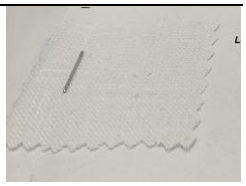
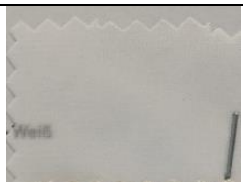
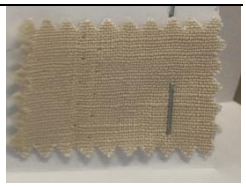
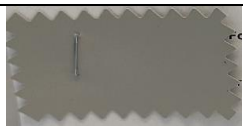
sisal (fiber)	Ocher with lighter and darker particles. Matt surface.	Not completely homogeneous, very fine like powder, but the particles are recognizable. Soft.	Smell of worn car tires or wicker baskets. Unpleasant and intense.	
jute (fiber)	Dark brown with lighter and darker particles. Matt surface.	Not completely homogeneous, very fine like powder. Particles recognizable.	Smell of glue and earth.	
flax (fiber 1)	Light brown. Matte surface.	Homogeneous, very fine like powder.	Smell of earth, wood and grain.	

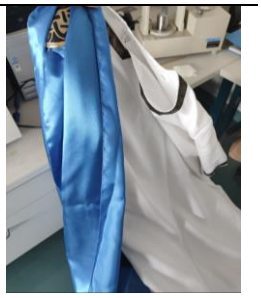
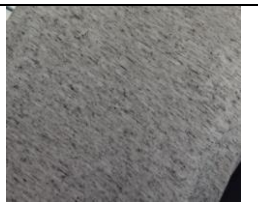
Table 20: Reference-sample description FTIR. (samples from living floor Gmbh 76879 Essingen, Spanierstraße 69 (sisal), fabfab GmbH, 22869 Schenefeld, Osterbrocksweg 35-45 (polyacrylic, wool, hemp, polyamide), Stoffmeile GmbH, 95213 Münchberg, Heinrich-Wirth-Str. 28 (cotton, polyester, acetate, flax, ramie), Stoff Palette GmbH, 95213 Münchberg, Hans-Hofmann-Straße 11 (PVC, jute, viscose, silk).

sample	appearance	texture	odor	sample picture
sisal (fabric)	Mustard yellow, fibers woven into carpet, uniform. Matt surface.	Rough and scratchy, with raised and deep areas.	Earthy, natural.	
polyacrylic (fabric)	Gray fabric, finely woven. Uniform. Matt surface.	Smooth surface, homogeneous structure.	No odor.	
wool (fabric)	Gray-white mottled, uneven texture. Matt surface.	Soft, smooth surface.	No odor.	

polyamide (fabric)	White, like wax paper. Uniform texture. Matt surface.	Smooth surface. Homogeneous.	No odor.	
hemp (fabric)	Beige, fibers clearly visible. Matt surface.	Smooth surface. Homogeneous.	No odor.	
cotton (fabric)	Pure white, fibers visible. Matt surface. Uniform structure.	Smooth surface. Homogeneous.	No odor.	
polyester (fabric)	Pure white, very fine, matt. Fibers not visible. Uniform structure.	Smooth surface. Homogeneous. Soft.	No odor.	
flax (fabric)	Washed, pure white, fibers recognizable. Matt surface. Uniform structure.	Smooth surface. Homogeneous.	No odor.	
acetate (fabric)	Pure white, glossy, very finely woven.	Smooth surface. Homogeneous. Soft.	No odor.	
ramie (fabric)	Beige. Structure recognizable. Matt surface. Uniform texture.	Smooth surface. Homogeneous. Coarse structure.	No odor.	
PVC coated foil (fabric)	Light gray, glossy, like foil, uniform structure.	Very smooth surface. Homogeneous.	No odor.	
silk (fabric)	Beige-gold, glossy. Fibers recognizable.	Smooth surface, thicker fibers perceptible. Homogeneous.	No odor.	
jute (fabric)	Eggshell yellow, coarse texture with fibers visible. Matt surface.	Smooth surface, thicker and thinner fibers, tactile. Homogeneous.	No odor.	

viscose (fabric)	Pure white with very fine texture, slightly glossy.	Smooth surface, homogeneous, soft.	No odor.	

Table 21: Sample-description FTIR.

sample	appearance	texture	odor	sample picture
tights	Uniform skin colored fabric, slightly transparent.	Lightweight and uniform fabric. Very elastic.	No odor.	
greek costume blue part	Blue and shiny fabric, finely woven fibers.	Uniform fabric, not elastic.	Artificial smell of plastic and varnish.	
greek costume white part	White fabric with matte surface, evenly woven fibers.	Uniform fabric, not elastic.	Artificial smell of plastic and varnish.	
wig	Dark brown curly synthetic hair, which was sewn on a hair net. Shiny surface.	Uniform structure, thick hair.	Artificial smell of plastic and varnish.	
silk-cloth	Colorful fabric in blue, green, orange, red, yellow and white. Finely woven, matte surface.	Uniform fabric. Soft and not elastic.	No odor.	
t-shirt	Gray, white and black fibers, unevenly woven. Matt surface. Individual fibers not recognizable.	Uniform, thin fabric. Soft and elastic.	Smell of freshly washed laundry.	

labor coat	Pure white fabric with matte surface. Fibers are densely woven.	Uniform, thick fabric, not elastic.	No odor.	
labor coat 2	Pure white fabric with matte surface. Fibers are densely woven.	Uniform, thick fabric, not elastic.	No odor.	No photo available.
t-shirt 2	Gray-beige thin fabric. Matt surface. Individual fibers no longer recognizable.	Uniform fabric, elastic and lightweight.	Smell of freshly washed laundry.	No photo available.
clove	Pure white fabric. Individual fibers no longer recognizable. Matt surface.	Uniform fabric, not elastic and lightweight.	No odor.	
clove 2	White and gray fabric with black inscription. Individual fibers no longer recognizable. Alternating glossy (imprint and protective coating) and matte surface.	Uneven, elastic and lightweight fabric with protective layer on the palm and print.	Artificial smell of plastic.	

4. Results and discussion

4.1 GC-MS

For the analysis with the norm method and the optimized method, fabric samples from white polyester fibers were spiked with the previously selected dyes SY3 (0.15-2mg) and R1 (0.3-3.5mg). For this purpose, 4 different concentrations were processed and measured 3 times each. The samples spiked with R1 were subjected to extraction, as it is a disperse dye. R1-spiked samples were subsequently analyzed for amine 24 and 7, as these gave relevant signals in the preliminary analysis of azo dyes. SY3-spiked samples were added directly to the reaction vessel and analyzed for amine 18.

In the following presentation of the results and discussion, only the spiked samples with R1 are considered, with the amine 1,4-phenylenediamine (24) as analyte. Since the additional specification of the evaluation with amine 7 would be too extensive. In addition, the spiking of the samples with SY3 and an evaluation with amine 18 did not provide stable measurement values. This may be due to the fact that amine 5 is also present in the dye, which may be reduced to unequal amounts to amine 18 during the experimental procedure. In addition, amine 18 has a poor recovery and is therefore not optimally suited for a comparative measurement. In addition, SY3 is a solvent dye, which means it is soluble in organic solvents and is mainly used to dye resins, inks and plastics. If SY3 is used for dyeing textiles is not clear. In addition, the spiked fabric samples were still colored yellow after reductive cleavage (spiked fabric sample was added directly to the reaction vessel). Even after separation of the amines with the diatomaceous earth column and the ether, the fibers were still yellowish and not white, which could indicate that there was still dye in the fibers and that it had not been completely converted.

In addition, polyamide and viscose samples were also spiked (with 1.5mg of R1) and measured using the norm and optimized methods.

After the measurements, the samples got examined for storage stability by measuring again after a period of time. All results are summarized in the following.

4.1.1 Results of the measurements with the norm method and the optimized method and their comparison:

Norm method 14362-1:

The concentrations [mg/kg] of the amines in the samples were calculated using the daily calibration and the weight of the textile sample (1g). In addition, the 10-point calibration function was also used for evaluation, which resulted in the same values. If a 10-point calibration series is used to calculate analyte concentrations, it must be prepared at least on a weekly basis. Since the measuring accuracy of the device can change over time (longer activation times lead to a more accurate measurement of the analytes). Additionally, impurities that have accumulated over the various measurements can also lead to altered signals. In routine analysis it is recommended to perform at least a 5-point calibration (e.g. 5, 10, 20, 30, 40mg/kg) and to evaluate it afterwards with the software.

For each standard, the mean ($\overline{X}$), standard deviation (SD), and coefficient of variation (CV) were determined.
The results for the preparation and measurement of the standard S1-4 for R1-spiking and evaluation with amine 24 (1,4phenylenediamine) can be seen in figure 21 and table 22.

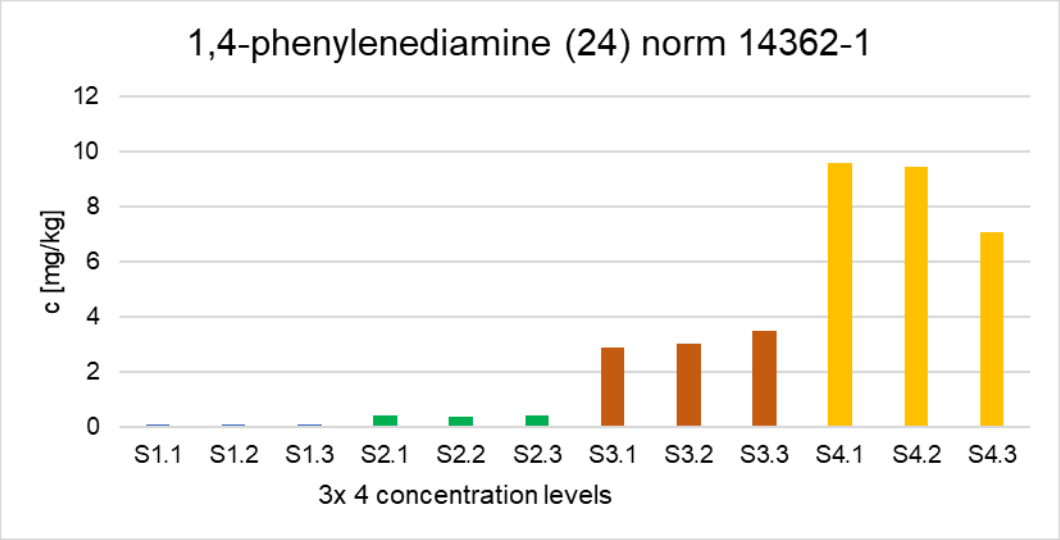


Figure 21: 1,4-phenylenediamine (24) norm method.

Table 22: $\bar{X}$, SD and VC for norm method.

standard	mg of spiked R1	$\bar{X}$ [mg/kg] amine 24	SD [mg/kg]	VC [%]
S1	0.3	0.078	0.007	8.725
S2	1.5	0.399	0.016	4.072
S3	2.5	3.128	0.255	8.145
S4	3.5	8.712	1.163	13.351

When measuring with the norm method with R1-spiking, only low analyte concentrations of amine 24 and amine 7 were detected. The highest concentration of amine 24 was found in standard 4 with 8.712 mg/kg, which is far below the limit value of 30 mg/kg. As the procedure to implement and validate a method by spiking different matrix samples with an azo dye was our own idea, no conclusions could be drawn how high the concentration of the amines should be after their reductive cleavage. The measurement results show a low standard deviation and an acceptable coefficient of variation and are therefore useful for evaluation.

Due to the low analyte concentrations that were analyzed, it was suspected that amine loss occurs either during extraction or the experimental procedure.

In the case of the extraction, the apparatus is not optimally suited, since no dense conditions can be guaranteed due to the twine between the round bottom flask and the reflux condenser. In addition, the extraction takes 40 minutes and the subsequent cooling down to room temperature takes several minutes. Xylene is a volatile compound and therefore not ideally suited for longer periods of sample exposure to air. The boiling point of xylene is 140°C, which could not be precisely regulated by the heating pads, as they only have two temperature programs (min and max) and no precise temperature specifications were found. The high temperatures can also lead to a loss of analytes. With the optimized method, the CEM EDGE works under constant and more controlled conditions. But it is not possible to extract several samples parallel.

Optimized Method:

In the optimized method, the same procedure was used to calculate the analyte concentration as in the norm method. The analysis was also performed with 1,4-phenylenediamine (24). (figure 22 and table 23)

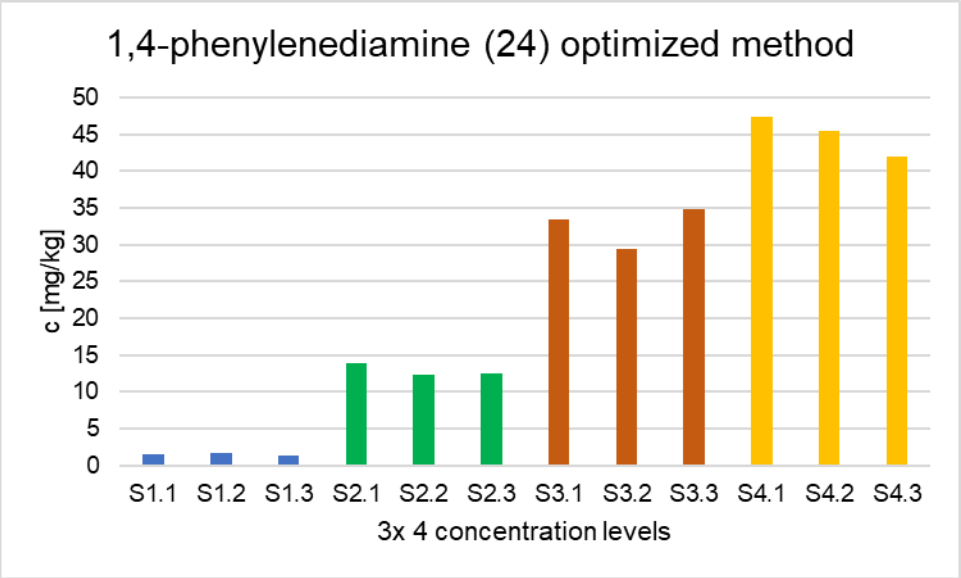


Figure 22: 1,4-phenylenediamine (24) optimized method.

Table 23: $\bar{X}$, SD and VC for optimized method.

standard	mg of spiked R1	$\bar{X}$ [mg/kg] amine 24	SD [mg/kg]	VC [%]
S1	0.3	1.520	0.160	10.545
S2	1.5	12.943	0.707	5.465
S3	2.5	32.612	2.259	6.926
S4	3.5	44.973	2.299	5.113

The optimized method detected higher analyte concentrations than the norm method. In addition, all measurements performed with the optimized method resulted in the same analyte concentrations. With the norm method, some tests had to be repeated because the method is more susceptible to interference than the optimized method. With the optimized method the acetone extracts could be concentrated immediately after extraction and there was almost no light or air contamination, the processing with the optimized method was faster and the diatomaceous earth columns did not have to be mechanically processed to ensure elution, the second concentration step was also carried out faster with the optimized method because less ether was used, so the finished amine extract was exposed to the water bath temperature for less time, which can also lead to amine losses.

Particularly in the extraction step according to the norm, it was not clear how good the quantitative yield was by the norm method, because the stock samples sometimes took different lengths of time to completely decolorize. In addition, the amine-loss during extraction could not be estimated. The optimized method and the extraction with the CEM EDGE result in constant test conditions and identical sample preparation, thus guaranteeing better repeatability. In addition, the extract after extraction

with acetone in the CEM EDGE was dark red, like the dye R1 used for spiking. Whereas the extract according to the norm method was only orange (figure 23). Which suggests that the extraction by the norm method is less efficient and not the entire amount of dye is in the extract.

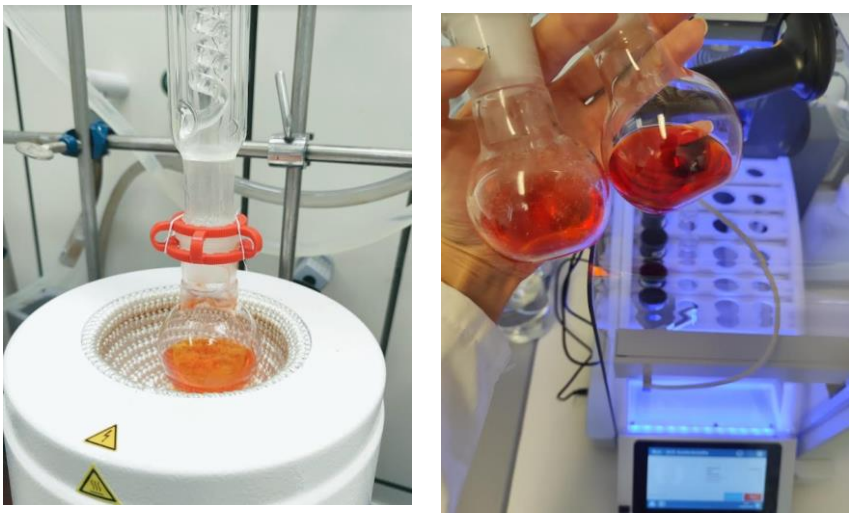


Figure 23: Different colors of extracts with norm method (left, orange) and optimized method (right, dark red).

In addition, the optimized method also uses lower extraction-temperature, which could contribute to less dye-loss. In addition, the sample extraction with the CEM EDGE requires 17min per sample and the standard extraction 40min per sample, which means the time factor also favors the optimized method.

Furthermore, the increased analyte concentrations cannot be related to matrix components or impurities, since the modified solvent acetone is rotated to dryness and the same chemicals are used in the further procedure after extraction as in the standard. Therefore, it can be assumed that the optimized method provides a more efficient analysis.

In addition, better recovery of the amines also speaks in favor of the optimized method. In the standard processing, amines 3, 7, 14, 18, 20, 21 and aniline did not meet the minimum recovery requirements. In the work-up according to the optimized method, only amine 18 and aniline are below the limit value (specified by norm 14362-1) as illustrated in the following table 24.

It is assumed that the two amines 18 and aniline degrade or transform during the experiment and that this is the reason for the poor recovery.

Aniline is strongly activated by the amino group (-NH_2) for electrophilic aromatic substitutions and is the starting compound for numerous reactions. The toluidine's are weak bases with base constants of the same order of magnitude as those of aniline. In addition, both amines are very small molecules and can act as precursor compounds for other amines.

According to standard 14362-1, amines 5 and 6 can be reduced to amines 18 and 19 during the experiment. However, this was disproved by performing a measurement where amines 5 and 6 were in the amine mix for daily calibration and for verification, and these provided the same results after evaluation.

Furthermore, according to standard 14362-1, amine 22 can be degraded to aniline and 1,4-phenylenediamine during the test procedure. However, by performing a test where amine 22 was also present in the amine mix, this resulted in a 100% recovery.

Table 24: Minimum recovery requirements for all amines. (optimized method)

amine	recovery [%]	minimum recovery-requirement [%]	meet requirements?
amine 1	109.11	70	yes
amine 2	103.37	70	yes
amine 3	88.42	70	yes
amine 4	103.25	70	yes
amine 5	No minimum requirements set by 14362-1.		
amine 6	No minimum requirements set by 14362-1.		
amine 7	80.80	70	yes
amine 8	91.84	20	yes
amine 9	106.41	70	yes
amine 10	98.21	70	yes
amine 11	103.10	70	yes
amine 12	105.77	70	yes
amine 13	108.54	70	yes
amine 14	90.27	70	yes
amine 15	109.91	70	yes
amine 16	109.94	70	yes
amine 17	121.78	70	yes
amine 18	35.80	50	**no**
amine 19	98.75	50	yes
amine 20	85.36	70	yes
amine 21	75.38	70	yes
amine 22	No minimum requirements set by 14362-1.		
aniline	12.97	70	**no**
1,4-phenylenediamine	No minimum requirements set by 14362-1.		

Matrix measurements:

For the applicability of the method, 3 different matrix samples (1g of each, polyester, polyamide and viscose) were spiked with R1 (1.5mg). The samples were processed on different days and evaluated with the respective daily calibration. The recovery in each matrix sample was compared to rule out a matrix effect.

With a total result of 12.422mg/kg ± 0.700mg/kg of amine 24 and a coefficient of variation of 5.631%, the applicability of the optimized method could be proven. The following figure 24 shows 2 different samples (processed with the optimized method) per fiber type with their final concentration [mg/kg].

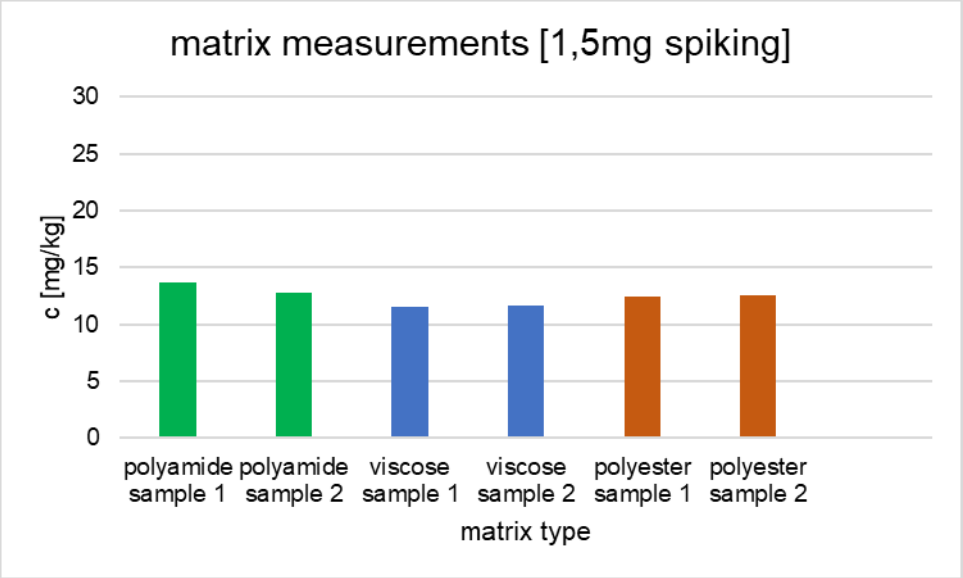


Figure 24: Matrix measurements of three different fiber types.

The same three matrix types were used for the processing with the norm method. The concentrations detected were very low and therefore poorly evaluable. The mean value of the measurements resulted in 0.61mg/kg ± 0.52mg/kg of amine 24 and a variation coefficient of 85.19%.

This result may be due to the fact that more densely woven fiber types are better able to retain a dye during extraction according to the norm and it is extracted later or not completely. In addition, as mentioned before, the extraction apperature according to the norm is not ideal, since the lack of a seal (due to the clamped twine between the reflux condenser and the round-bottom flask) can lead to sample losses and thus the extraction parameters are not always the same.

In addition, it should be noted that dyeing with disperse dyes is not normally used for viscose-fibers.

4.1.2 Study of storage stability of the amines:

In the storage experiment of the prepared samples, the samples were stored in the freezer at below -18°C and analyzed after 24-72h. No relevant changes in the concentration of the standards were measured. A slight increase in the analyte concentrations may be because the GC-MS measures more accurately the longer it runs, or may also lead to less precise measurements or impurities with so many samples (in the storage test all samples were measured in one run). In routine analysis, therefore, only a maximum of 10-15 samples/vials should be measured at one run, due to the stability of the amines over a longer period at room temperature. The following figure 25 shows a direct comparison of the sample concentrations of amine 24 (S1-4) before and after storage.

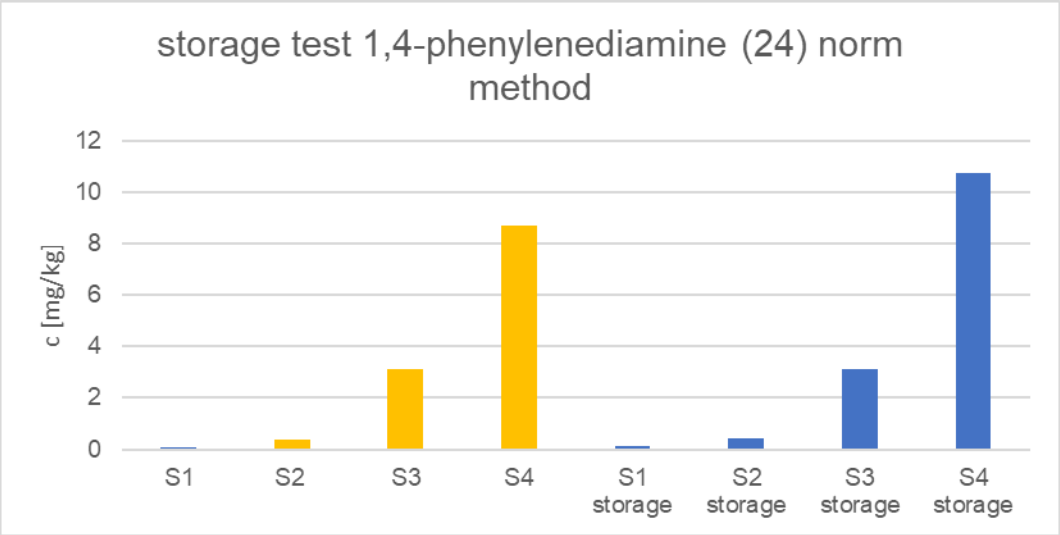


Figure 25: Storage test 1,4-phenylenediamine (24) norm method.

The storage tests with the optimized method behaved the same as the norm method. Which ensures a storage stability of the processed samples of 1-3 days or more, at temperatures below -18°C.
Increased storage studies should be made on the shelf life of the samples. Especially periods of more than one week and longer would be interesting for routine analytic. The single standards and the amine mix in acetonitrile showed a storage stability of several months, although they were only stored refrigerated at 6°C.

It is also possible to store the amines for a few hours at room temperature under the influence of light. To prove this statement, the 10-point calibration series was measured with GC-MS. After measurement the vials were

left at room temperature for 8h and were measured again. The following table 25 lists the concentrations of 1,4 -phenylenediamine (24) in the calibration standards before and after storage.

Table 25: Storage test of the calibration-standards.

c [µg/mL]	measurement c [µg/mL]	stability check c [µg/mL]
2µg/ml	2.11	2.01
3µg/ml	3.03	2.88
5µg/ml	5.02	4.86
7µg/ml	6.49	6.54
10µg/ml	9.77	9.62
20µg/ml	20.05	20.27
25µg/ml	25.28	26.07
30µg/ml	29.97	30.64
40µg/ml	41.57	41.01
50µg/ml	48.72	49.08

Another storage test was carried out with a time interval of 9 days and storage of the samples at room temperature (22°C) in white glass vials (light influence) and with perforated septum at the closure cap (air influence). A decrease of the analyte concentrations was detected. The following figure 26 shows the concentration of the S2 standard (1.5 mg doping) on a polyamide matrix, for measurement on the day of processing and 9 days later.

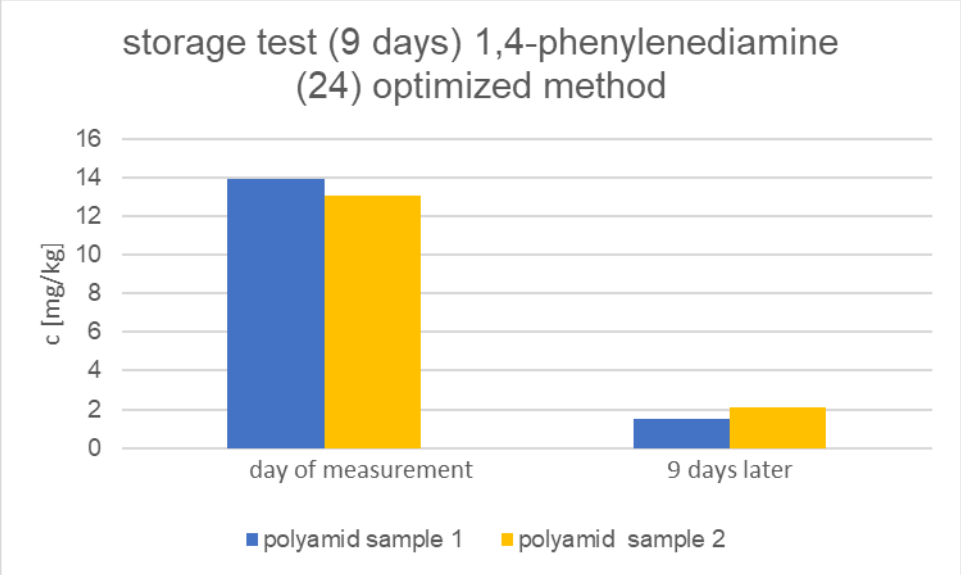


Figure 26: Storage test (9 days) 1,4-phenylenediamine optimized method.

To create ideal measurement conditions, the processing and measurement of the samples should happen as soon as possible. Apart from the intermediate storage step of the dye extracts after extraction, no further storage steps should be taken, if it is not necessary.

Some amines exhibit light sensitivity, so standards and amine mix should be stored in amber glass bottles or in the dark.
Further storage test studies should be conducted to assess the stability of the amines under the influence of time, light, air and temperature.

4.1.3 Current study situation:

There are animal studies for the detection of azo dyes in textiles *(Keshava 2023)*, as well as studies on environmental pollution from textile effluents *(Imran 2015)* and tests with skin models which are supposed to mimic human skin. *(Xuezhang 2017, Leme 2015)* Very few studies exist in connection with genotoxic and carcinogenic effects on humans. *(Keshava 2023)* Only minor contamination is assumed here, since azo dyes are largely banned in food, textiles and cosmetics.*(Camargo 2013)*

The biological activity differs between individual azo dyes, therefore the toxicological properties of one dye cannot be applied to all dyes. Acute toxicity of azo dyes, defined by the European Union criteria for classification of dangerous substances, is very low, and only a few of them have LD50 values below 250 mg/kg body weight. *(Camargo 2013)*
Occupational sensitivity to azo dyes has been demonstrated in the textile industry since 1930, e.g., for disperse dyes that have been associated with allergic reactions. *(Camargo 2013)*
Several studies show that the release of azo dyes into the environment due to the toxic, mutagenic and carcinogenic properties of these dyes and their biotransformation products are of concern, which can cause various damages to the organisms. *(Camargo 2013)*

The study by Amin et al. also evaluated the toxic effects of the two food dyes tatrazine and carmoisine when administered orally, in rats for 30 days. The two dyes caused damage to the liver, kidneys and increased the oxidative stress status of the animals and the formation of free radicals. *(Amin 2010)*

The Biswas study showed that the azo dye p-dimethyl aminobenzene has showed cytotoxic and genotoxic effects in experiments with rats. In addition, mutagenic effects were found for the textile dye azo red 120, in animal experiments with fish. *(Biswas 2005)*

For the dye Disperse Blue 291 a risk for genotoxicity and mutagenicity was found, based on the induction of fragmentation in DNA, formation of micronuclei in cells and increase of apoptosis index in mammalian cells (HepG2).

For C.I. Disperse Red 1 and C.I. Disperse Orange 1, mutagenic potential in human lymphocytes and mammalian cells (HepG2), was found. *(Camargo 2013)*

In addition to carcinogenic and teratogenic effects, azo dyes cause dysfunction in the reproductive organs of rodents. For example, prenatal exposure to Congo Red before birth decreased the number of germ cells in male and female rats and mice. *(Camargo 2013)*

Nevertheless, the dyeing process in the textile industry with azo dyes is inexpensive and some countries such as China, Brazil etc. continue to use azo dyes. Therefore, a mature detection method is necessary. Standard method 14362-1 describes such a method, but it is not an ideal method for routine analysis because much analyte is lost during sample preparation (extraction). The optimized method is more precise and can measure higher analyte concentrations, and it also has better recovery. Therefore, it can be assumed that the optimized method will be used in the future at the AGES-Linz site as an "in-house method" for the detection of azo dyes in textiles.

The international standard method 14362 is used worldwide for the determination of azo dyes in textiles. It is usually coupled with a GC-MS, but the study by Tölgyesi (2020) showed that comparable results can also be obtained with an LC-MS/MS. In addition, insufficient recovery was also obtained for amine 18 (o-toluidine), which was not due to losses during sample preparation but to ion suppression in the ion source. Therefore, a background correction was necessary. *(Tölgyesi 2020)*

In comparable studies with the application of a GC-MS based measurement, other GC instruments were used, but the same column. Therefore, the retention times of the analytes were not 100% identical to the own measurements. However, the m/z ratios determined agreed, as well as the temperature program used. *(Stevens 2016)*

In comparable studies, only the amines were used to spike the samples and no azo dyes were used. Therefore, a direct comparison with other studies is not possible, because in other studies the extraction procedure is disregarded.

4.2 FTIR

4.2.1 Interpretation of the results of the FTIR measurement of reference materials and samples and findings of the study:

The following figure 27 shows 4 different spectra of natural and synthetic fibers in comparison. The first spectrum of the figure shows the measurement of natural fibers of animal origin (wool). Characteristic signals are located at 3400cm^{-1}, 3000cm^{-1} and 1600cm^{-1} and in the fingerprint region at 1500cm^{-1} and 1250cm^{-1}.
The second spectrum of the figure shows the measurement of natural fibers of plant origin (cotton). Characteristic signals are located at 3400cm^{-1}, 2900cm^{-1} and 1600cm^{-1} and in the fingerprint region at 1400cm^{-1} and 1000cm^{-1}.
The third spectrum of the figure shows the measurement of synthetic fibers (polyester). Characteristic signals are located at 1750cm^{-1} and in the fingerprint region at 1250cm^{-1}, 1100cm^{-1} and 700cm^{-1}.
The fourth spectrum of the figure shows the measurement of synthetic fibers (viscose). Characteristic signals are in the fingerprint region at 1000 cm^{-1}.

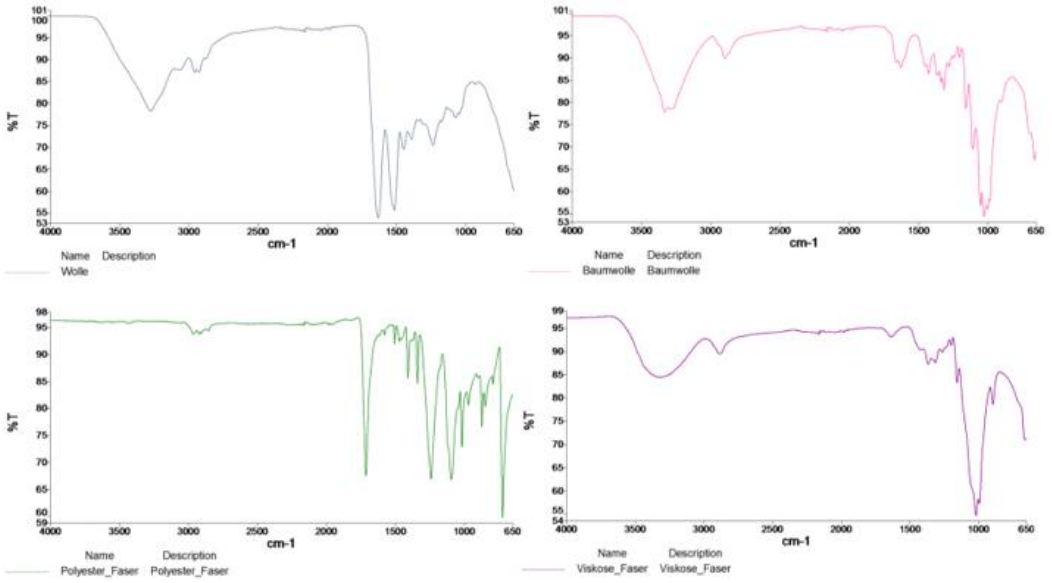


Figure 27: Spectrum of wool (grey), cotton (pink), polyester (green) and viscose (violet).

Due to the different spectra, it was possible to distinguish between natural fibers and synthetic fibers. In addition, natural fibers of animal and plant origin could be distinguished from each other and the synthetic fibers internally from each other. The samples brought along could be clearly assigned, e.g. a carnival costume sample, which consisted of 100% polyester, corresponded to the spectrum for polyester with a correlation factor of

0.98. Samples consisting of fiber mixtures, such as the sample "tights" (75% polyamide, 25% spandex) could also be assigned to the fiber type, with a correlation factor of 0.53 for polyamide. In addition, samples made of natural fibers of animal origin, such as silk, were also correctly assigned to the spectrum for silk, with a correlation factor of 0.98 (and not to the spectrum of wool), see apprendix "silk-cloth". All samples with their corresponding fiber type and correlation factor are listed in table 26.

Table 26: FTIR-results from different samples.

sample	fiber type	correlation factor	declaration according to label
tights	polyamide	0.53	75% polyamide, 25% spandex
greek costume blue part	polyester	0.98	100% polyester
greek costume white part	polyester	0.99	100% polyester
wig	polypropylene	0.99	100% polypropylene
silk-cloth	silk	0.92	100% silk
t-shirt	polyester	0.99	93% polyester, 7% flax
labor coat	polyester	0.91	65% polyester, 35% cotton
	cotton	0.27	
labor coat 2	polyester	0.96	not specified
t-shirt 2	polyester	0.82	not specified
clove	cotton (and other natural fiber types)	0.82	100% cotton
clove 2	polyethylene	0.86	polyethylene

Fiber blends of natural fibers and synthetic fibers could be correctly assigned (see spectrum "labor coat", blend of cotton and polyester).
There was only one discrepancy in the fiber assignments when measuring the "glove" sample, which was supposed to be made of cotton. Here, no clear assignment to the cotton spectrum was possible because other natural fiber types of plant origin also showed a correlation (flax, hemp and ramie).

During the measurement, it did not matter whether the fiber was natural or bleached (many fabric samples were bleached), the fiber types were determined with the same spectrum. Even fibers from different manufacturers had the same spectrum, as expected. Basically, the fabric samples were measured with a better transmission due to the more densely arranged fibers.

In addition, the PVC film brought along was only PVC coated and consisted mainly of polyester. Although the side of the coating was directly placed on the crystal, the software evaluated the sample as polyester fiber.

Therefore, for the reference spectrum of PVC, a foil from AGES-Vienna was used to complete the database.

4.2.3 Current study situation:

Since the determination of textile fiber species by FTIR is a new method, there are not that much studies or literature with the same topic. However, a study was conducted by Oudiani (2017), where agave fibers were investigated using an FTIR method. Agave fibers are also called "sisal". In this study, the agave fibers themselves were extracted and bundled and pressed into a KBr-pellet. A Nicolet 510 M spectrophotometer with a resolution of 2 cm^{-1} and 100 scans per sample was used. The infrared spectra were recorded in the range of 4000-500 cm^{-1} .The FTIR spectrum was plotted with wavenumber [cm^{-1}] against absorbance. The spectrum was split into two parts. The zone above 2000cm^{-1} includes fewer peaks. The CH-alkane stretching at about 2900cm^{-1} proves the presence of saturated carbons, while the bands above 3000 cm-1 indicate the presence of unsaturated carbons. The large band between 3200-3700 cm^{-1} can be attributed to hydrogen bonding. *(El Oudiani 2017)*

The second part of the spectrum (below 2000cm^{-1}) contains significantly more peaks. The absorption of double bond groups (carbonyl) can be seen by the presence of a strong peak around 1600 cm^{-1}. The single bond C-O is indicated by absorbances at 1100-1300 cm^{-1}.
The region 1800-2800 cm^{-1} was not investigated since there is no presence of alkyne or nitrile groups in the fiber structure. *(El Oudiani 2017)*

The results of the study are comparable to our own measurements of the sisal fiber. Since the tendency of fiber-type-use of textile manufacturers or consumer behavior is changing, it is relevant to correctly determine fiber types, due to quality parameters and falsification of products.

FTIR can be applied not only for sample preparation for azo dye analysis, but also for textile type determination of products in general. In addition, the fiber type may also be related to the use of plasticizers or flame retardants and can also serve as a sample pre-test for these methods.

4.3 Validation concept

For the validation and later introduction of the method, a validation plan was drawn up together. The necessary parameters for the validation were defined and the targets and limits for the individual parameters were determined. The original validation plan from AGES-Linz can be found in the appendix and describes the optimized method as a more suitable variant

to determine azo dyes in textiles with GC-MS. A more comprehensive version of the validation plan is presented in table 27.

Table 27: Validation concept and comparison of the methods.

parameter	process characteristics to be determined	identified process characteristics norm 14362-1	identified process characteristics optimized method	minimum requirements
Calibration	Determine correlation coefficient for all parameters in the workspace. A calibration curve was created for each amine and R^2 was calculated.	Correlation factors were (R^2) in the selected working range between 0.985 and 0.999 for all parameters.	Correlation factors were (R^2) in the selected working range between 0.985 and 0.999 for all parameters.	R^2 = 0.98 or better.
Recovery	Determination with doped matrix samples with 3 independent workups each at four different concentration levels. Additionally the recovery was calculated by comparison of the daily calibration and the verification of the method.	Matrices mostly 80-100% recovery between the different measurements and the different levels. (By evaluating amine 24) However, the recovery is worse than the minimum requirements when comparing the verification of the method and the daily calibration for amines 3, 7, 14, 18, 20, 21 and aniline.	Matrices mostly 90-100% recovery between the different measurements and the different levels. (by evaluating amine 24) Here only the recovery of amine 18 and aniline is worse than the minimum requirement, when comparing the daily calibration with the verification of the method.	Minimum recovery requirements are described for each amine individually and range from 20-70%. (see table 24)
Precision	Determination from the data of the overall procedure for the work area. Calculation of the coefficient of variation (CV = standard deviation / mean value * 100).	Worst CV during reprocessing with the norm method = 13.35%. Slightly better precision was obtained by intra-day analyses than by inter-day analyses.	Worst CV during reconditioning with the optimized method = 10.54%. Slightly better precision was obtained by intra-day analyses than by inter-day analyses.	CV should be ±15% or better. The greater the coefficient of variation, the more scattered are the measurements and the less precise is the method.
Correctness	Participation in a laboratory	Participation in an interlaboratory comparison is performed as soon		z-score max. 2

	comparison test/ring test.	as possible. It was not possible to carry this out during the master's thesis.		
Selectivity	Determination of the selectivity of the GC-MS method for all parameters in their measurement windows.	Selectivity factor 0.9999 for all parameters. The selectivity is given through the measurement by GC-MS: Rt, target ion T, qualifier ion Q, ratio T/Q.		>0.9
LOD	Determine the detection limit using the calibration curve method according to DIN 32645 or another method of determination.	LOD = 0mg/kg with blank method. (10 measurements)	LOD = 2.888mg/kg with residual variance method. LOD = 0mg/kg with blank method. (10 measurements)	not applicable
LOQ	Determine the limit of quantification using the calibration curve method according to DIN 32645 or another method of determination.	LOQ = 0mg/kg with blank method. (10 measurements)	LOQ = 9.626mg/kg with residual variance method. LOQ = 0mg/kg with blank method. (10 measurements)	10 mg/kg
Applicability	Verification with at least 3 representative matrices.	Measurement of the spiked matrix-samples (R1 1.5mg) using the norm method resulted in concentrations between 0.11mg/kg and 1.45mg/kg. Which can be attributed to insufficient extraction.	Matrix 1 (polyester), matrix 2 (polyamide) and matrix 3 (viscose) doped with azo dye (disperse red 1 1.5mg) gave the same concentrations of analytes (12.4mg/kg) with sample preparation using the optimized method. (Evaluation with amine 24)	Matrices polyester, polyamide, and viscose.
Robustness	Comparative measurement with another operator as well as variation of extraction parameters (e.g.: extraction volume, sample quantity).	The norm and optimized method was performed under the same conditions internally by another employee to compare the results. Both the standard method and the optimized method yielded comparable results.		Correlation of the results should be ± 10% or better.

Range	A 10-point calibration series with concentrations between 2-50mg/kg on each amine is established to determine the working range.	A quadratic function was chosen as the calibration function. With the calibration function it is possible to detect amine concentrations from 2mg/kg to 50mg/kg (or above the limit value 30mg/kg).	With the method, concentrations at and above the 30mg/kg (30ppm) limit should be detectable for each amine.
Matrix effect	Measurement of 10 blank samples, each with 1g polyester weight in, and addition of the ISTD and IS.	No amine was detected at a concentration of = or > 2mg/kg in any of the 10 blank samples. Therefore, the matrix does not cause false positive results.	No matrix effects should be detected since there are no azo dyes in white fabric samples.

For each of the 22 amines, 1,4-phenylenediamine and aniline, the 10-point calibration series was evaluated with a quadratic function and the coefficient of determination was determined. The coefficient of determination was above 0.98 for every single analyte, thus fulfilling the requirements for validation.

The recovery of the overall method (with extraction) was more complex to determine, since spiked samples were used and the amine concentration resulting from a spiked azo dye was unknown. Therefore, the recovery of the total method (with extraction) must be confirmed with another measurement method (e.g. HPLC). Or textiles are examined where the amine content is already known.
The recovery at the point of reductive cleavage could be determined with the verification of the method. The individual recoveries for each amine were calculated for the validation plan. Every amine apart from amine 18 and aniline showed excellent recovery with the optimized method.

The calculation of the validation-parameters precision, LOD, LOQ as well as applicability was performed using 1,4-phenylenediamine as an example.
The precision of the method was performed with the coefficient of variation of the 3 different measurements at 4 levels. The worst value of the standard method was 13.15% and of the optimized method was 10.54%. Both are in the range of the requirements for the method, therefore the norm method and the optimized method can be considered as precise.

The selectivity of the method was also guaranteed by the GC-MS measurement by assigning each analyte an Rt, an m/z of the target ion and relevant qualifiers. This made it possible to avoid false positive results based on isomers.
The limit of detection and the limit of quantification are below the limit value and show that the method is suitable for determining amine concentrations even below the limit value.
The applicability also shows that the method can be used with different matrix samples.

To complete the method validation, an interlaboratory comparison should be performed soon.

In addition, the developed FTIR method represents a significant improvement for routine analysis. With FTIR, textile samples can be determined for their fiber type in seconds, thus avoiding unnecessary additional extraction of the samples. In fact, extraction is only necessary for synthetic fibers, as only these can contain disperse dyes.

5. Conclusion

In conclusion, the developed FTIR method works successfully and the created database of natural and synthetic fibers can be used to evaluate the fiber type of laboratory samples. The spectra of fiber types were different between natural and synthetic fibers, which allows reliable differentiation between the fiber groups. In addition, natural fibers of animal origin can also be distinguished from natural fibers of plant origin, as well as the synthetic fibers among each other. It is not possible to distinguish natural fibers of plant origin among each other. In addition, mixed samples from different fiber types can be analyzed. A cost-effective and practical alternative for measurement with FTIR would be an NIR-instrument such as the thermofisher MicroPhazir.

By determining the fiber type of the textile sample, it is possible to save time and materials in the further analysis of azo dyes. Since pre-extraction of the sample is only necessary if dyeing was done with a disperse dye. These are only found in synthetic fibers (except viscose). Processing according to norm 14362-1 worked for the samples spiked with Disperse Red 1, but the concentrations of the analyte amine 1,4-phenylenediamine were very low, which was attributed to the extraction method. The extraction takes a long time and the extraction apparatus was not ideal due to insufficient tightness and very high temperatures (boiling point xylene = 140°C). In addition, further workup with the high amount of chemicals and the 20g diatomaceous earth columns caused delays in the workflow. The elution of the ether through the diatomaceous earth columns to separate the amines also required mechanical assistance.

The optimized method provided more stable analytical results, better recovery of the amines and higher analyte-amine concentrations. By extracting with the CEM EDGE, all the dye was extracted, with a less hazardous solvent (acetone) and at a low temperature (110°C), and the extraction happened in a sealed chamber under pressure, with uniform conditions throughout the process. The smaller diatomaceous earth columns (6g) in the optimized method, as well as the glass wool used instead of glass fiber filters and the reduced amount of ether led to an easier and faster elution.

With the following GC-MS measurement it is possible to measure far below the limit value and precise isomer separation is also possible (Rt, m/z target-ion and qualifier), therefore no false positive results are analyzed and high selectivity is achieved. LOD (2.888mg/kg) and LOQ (9.626mg/kg) are below the limit value of 30mg/kg. The method is also suitable for dif-

ferent matrix samples, the different fiber types polyester, polyamide and viscose gave the same results when analyzed after dye spiking.

In addition, the finished sample extracts can be stored (cooled and in the dark) for several days. At room temperature and light exposure, the amine concentration decreases continuously. It is possible to store the samples in methanol (frozen and in the dark) after extraction before further processing.

According to the analyses, a concentration of 44.97mg/kg of 1,4-phenylenediamine can already be released from a 1g textile-sample spiked with 3.5g of Disperse Red 1, which exceeds the limit of 30mg/kg. The LD_{50} value of 1,4-phenylenediamine is 80mg/kg via oral intake (animal test). *(Roth 2022)*
Since textile dyeing with azo dyes requires less dye compared to regular dyes, the contamination is probably rather low, mainly because friction and perspiration presumably do not release 100% of the dye. Exact data on the amount of azo dyes used for specific textile quantities could not be found.

6. Zusammenfassung

Die Verwendung von Azofarbstoffen zur Textilfärbung ist laut REACH-VO (EG) 1907/2006 untersagt, wenn sie eines oder mehrere der 22 gelisteten aromatischen Amine bilden können. Diese Amin Verbindungen stehen im Verdacht, genotoxisch, kanzerogen und sensibilisierend zu wirken, was zu allergischen Reaktionen führen kann. Besondere Aufmerksamkeit verdient die Möglichkeit, dass Farbstoffanteile durch Reibung und Schweiß aus Textilien freigesetzt werden. Aufgrund fehlender Humanstudien ist es von entscheidender Bedeutung, die Exposition gegenüber Azofarbstoffen durch geeignete Analyseverfahren abzuschätzen.

Das Hauptziel dieser Arbeit bestand darin, eine Methode zu entwickeln und zu optimieren, um Azofarbstoffe und ihre Amin Bestandteile in Textilien nachzuweisen. Bisher war eine solche Analytik basierend auf der Norm-Methode 14362-1 zeitaufwendig, materialintensiv und von schlechter Wiederfindung der Amine gekennzeichnet.

Die entwickelte Methode integrierte eine FTIR-Voruntersuchung zur Bestimmung der Faserart mit einer GC-MS-Analyse unter Verwendung dotierter Proben. Die neue und herkömmliche Methode wurde an verschiedenen Matrixproben getestet, und es wurden Lagerungsversuche durchgeführt.

Die Resultate zeigten, dass die optimierte Methode herausragende Leistungen in Bezug auf Reproduzierbarkeit, Amin-Wiederfindung, Selektivität und Präzision aufwies. In einer mit 3,5 mg R1 dotierten 1g Polyester-Probe (S4) wurde eine Freisetzung von 44,97 mg/kg 1,4-Phenylenediamin festgestellt, was einer Freisetzungsrate von 1,28% des eingesetzten Farbstoffs entspricht, unter der Annahme einer vollständigen Umsetzung des Azofarbstoffs. Diese Ergebnisse wurden mit der optimierten Methode analysiert. Bei der Durchführung der Norm Methode wurden lediglich 8,71 mg/kg an 1,4-Phenylenediamin analysiert, unter den gleichen Bedingungen. Dies lässt sich auf Farbstoffverluste durch die Extraktion laut Norm zurückführen.

Die Optimierung der Methode, verbunden mit der FTIR-Voruntersuchung, erwies sich als geeignet für die Routineanalytik von Textilproben. Dies ermöglicht eine präzise Abschätzung der Exposition gegenüber Azofarbstoffen, insbesondere in Bezug auf Faschingskostüme und Spielwaren, wo diese Stoffe am häufigsten anzutreffen sind.

7. Abstract

The use of azo dyes for textile dyeing is prohibited according to REACH Regulation (EC) 1907/2006 if they can form one or more of the 22 listed aromatic amines. These amine compounds are suspected to have genotoxic, carcinogenic, and sensitizing effects, potentially leading to allergic reactions. Of particular concern is the possibility that dye components may be released from textiles through friction and sweat. Due to a lack of human studies, it is crucial to estimate exposure to azo dyes through suitable analytical methods.

The primary goal of this study was to develop and optimize a method for detecting azo dyes and their amine components in textiles. Historically, such analysis based on the standard method 14362-1 has been time-consuming, material-intensive, and characterized by poor amine recovery.

The developed method integrated an FTIR preliminary analysis for fiber type determination with a GC-MS analysis using doped samples. Both the new and conventional methods were tested on various matrix samples, and storage stability experiments were conducted.

The results demonstrated that the optimized method excelled in terms of reproducibility, amine recovery, selectivity, and precision. In a 3,5 mg R1 spiked 1g polyester-sample (S4), a release of 44.97 mg/kg 1,4-phenylenediamine was observed, corresponding to a release rate of 1.28% of the applied dye, assuming complete conversion of the azo dye. These results were analyzed using the optimized method. Applying the norm method, only 8.71 mg/kg of 1,4-phenylenediamine was analyzed under the same conditions. This can be attributed to dye losses due to extraction according to the norm.

The method optimization, coupled with FTIR preliminary analysis, proved suitable for the routine analysis of textile samples. This allows for a precise estimation of exposure to azo dyes, particularly concerning costumes and toys, where these substances are most commonly found.

8. List of references

AGES (2017) *Faschingskostüme – Azofarbstoffe und Entflammbarkeit*. Endbericht der Schwerpunktaktion A-001-17. (access 11.10.2023) https://www.ages.at/download/sdl-eyJ0eXAiOiJKV1QiLCJhbGciOiJIUzI1NiJ9.eyJpYXQiOjE2MDk0NTkyMDAsImV4cCI6NDA3MDkwODgwMCwidXNlciI6MCwiZ3JvdXBzIjpbMCwtMV0sImZpbGUiOiJmaWxlY-WRtaW5cL0FHRVNfMjAyMlwvMl9NRU5TQ0hcL1NjaHdlcnB1bmt0ZVwvTGViZW5zbWl0dGVsa29udHJvbGxlXC9TcGllbHpldWdcLzIwMTdcL1NQQV8tX0EtMDAxLTE3X0Zhc2NoaW5nc2tvc3RcdTAwZmNtZV9cdTIwMTNfQXpvZmFyYnN0b2ZmZV91bmRfRW50ZmxhbW1iYXJrZWl0X0hvbWVwYWdlLnBkZiIsInBhZ2UiOjIyODN9.eBqB401qjkS_B01Vc23Lgfwtd54oOYZt9Z1d3fYyjQM/SPA_-_A-001-17_Faschingskost%C3%BCme_%E2%80%93_Azofarbstoffe_und_Entflammbarkeit_Homepage.pdf

Amin, H. Abdel Hameid, and A.H. Abd Elsttar, (2010) “Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats”, Food and Chemical Toxicology, vol. 48, pp.2994-2999.

Austrian Standards International und Europäisches Komitee für Normung CEN (2017) OENORM EN ISO 14362-1 2017.05.15. *Textilien – Verfahren für die Bestimmung gewisser aromatischer Amine aus Azofarbstoffen.* Teil 1: Nachweis der Verwendung gewisser Azofarbstoffe mit und ohne Extraktion der Fasern. ICS 59.060.01.

Austrian Standards International und Europäisches Komitee für Normung CEN 2 (2017) OENORM EN ISO 14362-3 2017.05.15. *Textilien - Verfahren für die Bestimmung gewisser aromatischer Amine aus Azofarbstoffen.* Teil 2: Nachweis der Verwendung gewisser Azofarbstoffe, die 4-Aminoazobenzol freisetzen können. ICS 59.060.01.

Benkhaya, S., M’ rabet, S., & El Harfi, A. (2020). A review on classifications, recent synthesis and applications of textile dyes. *Inorganic Chemistry Communications*, *115*(March), 107891. https://doi.org/10.1016/j.inoche.2020.107891

Benkhaya, S., M’rabet, S., & El Harfi, A. 2 (2020). Classifications, properties, recent synthesis and applications of azo dyes. *Heliyon*, *6*(1). https://doi.org/10.1016/j.heliyon.2020.e03271

Biswas, and A.R. Khuda-Bukhsh, (2005) “Cytotoxic and genotoxic effects of the azo-dye pdimethylaminoazobenzene in mice: a time-course study,” Mutation Research, vol. 587, pp. 1-8, 2005.

Bode, A. et al. (2007) *Informationsserie Textilchemie.* TEGEWA. FCI. (access 12.10.2023) https://www.vci.de/vci/downloads-vci/publikation/textilchemie-textheft.pdf

Brucker (2023) Grundlagen der FTIR-Spektroskopie. (access 05.10.23) https://www.bruker.com/de/products-and-solutions/infrared-and-raman/ft-ir-routine-spectrometer/what-is-ft-ir-spectroscopy.html

Brüning, T., Käfferlein, H. U., & Slowicki, A. (2009). Azofarbmittel und deren Hautgängigkeit beim Menschen. *BGFA – Forschungsinstitut Für Arbeitsmedizin Der Deutschen Gesetzlichen Unfallversicherung Institut Der Ruhr-Universtität Bochum*.

Brunner, S. Allscher, T., Emmerling E. (2015). *FTIR__in_Abgeschwaechter_Totalreflexion__ATR__und_Externer_Reflexion__ER_ _an_Kunststoffen_mit Spektr*.

Brüschweiler, B. J., Küng, S., Bürgi, D., Muralt, L., & Nyfeler, E. (2014). Identification of non-regulated aromatic amines of toxicological concern which can be cleaved from azo dyes used in clothing textiles. *Regulatory Toxicology and Pharmacology*, *69*(2), 263–272. https://doi.org/10.1016/j.yrtph.2014.04.011

C.E.M Corporation. (2017). *Automated Extraction System EDGE*. http://cem.com/edge/

Camargo, B. de C. V., & Morales, M. A. M. (2013). Azo Dyes : Characterization and Toxicity – A Review. *Textiles and Light Industrial Science and Technology, 2(2)*, *2*(2), 85–103.

Carlsson, J., Åström, T., Östman, C., & Nilsson, U. (2022). Disperse azo dyes, arylamines and halogenated dinitrobenzene compounds in synthetic garments on the Swedish market. *Contact Dermatitis*, *87*(4), 315–324. https://doi.org/10.1111/cod.14163

Collier (1993) SW at al., Reduction of azo dyes during in vitro percutaneous absorption, Toxicol. Appl. Pharmacol. 118: 73-79.

El Oudiani, A., Msahli, S., & Sakli, F. (2017). In-depth study of agave fiber structure using Fourier transform infrared spectroscopy. *Carbohydrate Polymers*, *164*, 242–248. https://doi.org/10.1016/j.carbpol.2017.01.091

Fassold, E., Häusler, G., & Hohenblum, P. (1999). *Azofarbstoffe in Leder und Textilien*. https://www.umweltbundesamt.at/fileadmin/site/publikationen/r159.pdf (access 30.09.23)

Freeman, H. S. (2013). Aromatic amines: Use in azo dye chemistry. *Frontiers in Bioscience*, *18*(1), 145–164. https://doi.org/10.2741/4093

Giovanni R., Giuseppe B. & Buzzi A. (2014) *Quantitative determination of 26 aromatic amines derived from banned azo dyes in textiles through the use of IC, tandem mS, and identification of some structural isomers.* https://www.agilent.com/cs/library/applications/5991-5169EN.pdf

Imran, M., Shaharoona, B., Crowley, D. E., Khalid, A., Hussain, S., & Arshad, M. (2015). The stability of textile azo dyes in soil and their impact on microbial phospholipid fatty acid profiles. *Ecotoxicology and Environmental Safety*, *120*, 163–168. https://doi.org/10.1016/j.ecoenv.2015.06.004

Kämpfer, P., Crettaz, S., Nussbaumer, S., Scherer, M., Krepich, S., & Deflorin, O. (2019). Quantitative determination of 58 aromatic amines and positional isomers in textiles by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Journal of Chromatography A*, *1592*, 71–81. https://doi.org/10.1016/j.chroma.2019.01.039

Keshava, C., Nicolai, S., Vulimiri, S. V., Cruz, F. A., Ghoreishi, N., Knueppel, S., Lenzner, A., Tarnow, P., Vanselow, J. T., Schulz, B., Persad, A., Baker, N., Thayer, K. A., Williams, A. J., & Pirow, R. (2023). Application of systematic evidence mapping to identify available data on the potential human health hazards of selected market-relevant azo dyes. *Environment International*, *176*(April), 107952. https://doi.org/10.1016/j.envint.2023.107952

Keshava, C., Nicolai, S., Vulimiri, S. V., Cruz, F. A., Ghoreishi, N., Knueppel, S., Lenzner, A., Tarnow, P., Vanselow, J. T., Schulz, B., Persad, A., Baker, N., Thayer, K. A., Williams, A. J., & Pirow, R. (2023). Application of systematic evidence mapping to identify available data on the potential human health hazards of selected market-relevant azo dyes. *Environment International*, *176*(April), 107952. https://doi.org/10.1016/j.envint.2023.107952

Köhler, M., Kaufmann, A., Perz R. (2018) *Bekleidung im Fokus – wie sicher sind die verwendeten Farbstoffe?* (access 07.10.2023) https://www.ua-bw.de/pub/beitrag.asp?subid=1&Thema_ID=3&ID=2852

LAVES (2023) *Verbotene Azofarbstoffe in Textilien?* LAVES UNTERSUCHT GEFÄRBTE BEKLEIDUNGSGEGENSTÄNDE. (access 11.10.2023) https://www.laves.niedersachsen.de/startseite/bedarfsgegenstande/bedarfsgegenstande_mit_korperkontakt/verbotene-azofarbstoffe-in-textilien-161798.html

Leme, D. M., Primo, F. L., Gobo, G. G., Da Costa, C. R. V., Tedesco, A. C., & De Oliveira, D. P. (2015). Genotoxicity Assessment of Reactive and Disperse Textile Dyes Using Human Dermal Equivalent (3D Cell Culture System). *Journal of Toxicology and Environmental Health - Part A: Current Issues*, *78*(7), 466–480. https://doi.org/10.1080/15287394.2014.999296

Lucas, D. (2019). *Bestimmung aromatischer Amine aus Azofarbstoffen mittels GC / MS unter Verwendung von Chem Elut S-Kartuschen für die unterstützte Flüssigextraktion*.

Luongo, G., Iadaresta, F., Moccia, E., Östman, C., & Crescenzi, C. (2016). Determination of aniline and quinoline compounds in textiles. *Journal of Chromatography A*, *1471*, 11–18. https://doi.org/10.1016/j.chroma.2016.09.068

Malinauskiene, L., Bruze, M., Ryberg, K., Zimerson, E., & Isaksson, M. (2013). Contact allergy from disperse dyes in textiles-a review. *Contact Dermatitis*, *68*(2), 65–75. https://doi.org/10.1111/cod.12001

Mancuso, G. (2020). Concomitant long-lasting allergic patch test reactions to p-phenylenediamine and textile azo dyes. *Contact Dermatitis*, *83*(6), 514–515. https://doi.org/10.1111/cod.13646

McCann D et al. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. The Lancet 370, 1560–1567.

Meghmani Group (2022) *WHAT IS SOLVENT DYE? ITS FEATURES & USES.* (access 11.10.2023) https://www.meghmaniglobal.com/what-is-solvent-dye-its-features-uses/

Methneni, N., Ezdini, K., Abdeljelil, N. Ben, Van Loco, J., Van den Houwe, K., Jabeur, R., Sallem, O. F., Jaziri, A., Fernandez-Serrano, M., Khdary, N. H., & Mansour, H. Ben. (2021). Occurrence of textile dyes and metals in tunisian textile dyeing effluent: Effects on oxidative stress status and histological changes in balb/c mice. *International Journal of Molecular Sciences*, *22*(22), 1–15. https://doi.org/10.3390/ijms222212568

Moragues, T. & Song, C. (2022) *Lebensmittelfarbstoffe*. Synthese von Azorubin - Nachweis mittels Dünnschichtchromatographie. (access 10.10.2023) https://ethz.ch/content/dam/ethz/special-interest/chab/chab-dept/research/documents/ICB/ICB-

Practi-
ca/Skripte_HEST_FS2022/Kapitel_4_Lebensmittelfarbstoffe_Chemiepraktikum_376-0010-00P_FS22.pdf

Platzek, T. (1999). Freisetzung aromatischer Amine aus Azofarbstoffen in Textilien durch Hautbakterien. *BgVV*, *8*(624).

REACH-VO (2021). *Verordnung (EG) Nr. 1907/2006 des eurpäischen Parlaments und des Rates*. 1–552.

Ribeiro, A. R., & Umbuzeiro, G. de A. (2014). Effects of a textile azo dye on mortality, regeneration, and reproductive performance of the planarian, Girardia tigrina. *Environmental Sciences Europe*, *26*(1), 1–8. https://doi.org/10.1186/s12302-014-0022-5

RIS (2013) Azofarbstoffverordnung - Verordnung der Bundesministerin für Gesundheit und Frauen über das Verbot der Verwendung bestimmter Azofarbstoffe bei Gebrauchsgegenständen (Azofarbstoffverordnung 2004) [CELEX-Nr.: 32002L0061, 32004L0021] StF: BGBl. II Nr. 320/2004 Fassung vom 08.02.2006 Azofarbstoffe, H., Nachweismethoden, A., En, N., En, N., Iso, N. C. E. N., Nr, I. I., Liste, A., & Stoffname, C. I. E. (2013). *Langtitel Änderung Präambel / Promulgationsklausel*. 3–5.

ROTH (2016). *ABSCHNITT 1 : Bezeichnung des Stoffs beziehungsweise des Gemischs und des Unternehmens ABSCHNITT 2 : Mögliche Gefahren ABSCHNITT 3 : Zusammensetzung / Angaben zu Bestandteilen ABSCHNITT 4 : Erste-Hilfe-Maßnahmen*. *2006*(1907), 1–12. https://www.carlroth.com/medias

Roth (2022) Sicherheitsdatenblatt gemäß Verordnung (EG) Nr. 1907/2006 (REACH) 1,4-Phenylendiamin ≥99 %, zur Synthese. (access 13.10.20) https://www.carlroth.com/medias/SDB-4499-DE-DE.pdf?context=bWFzdGVyfHNlY3VyaXR5RGF0YXNoZWV0c3wzNTM1MjJ8YXBwbGljYXRpb24vcGRmfHNlY3VyaXR5RGF0YXNoZWV0cy9oNWQvaDAwLzkwNTgwNjE2MTUxMzQucGRmfGJjZWQxOGYzYjRjNmI2NDg5OGM2Y2YwMTg1NTE5NTNlMzFmNzVkYzBkYzI3NzE1ZGRiOWE1NDgzZmZhNzQzNjg

Schwartz, T. et al. (2021) *FTIR Prüfvorschrift – Identifizierung von Materialien mittels FTIR-Spektrometrie.* Dok.Nr. PV 4791_03. AGES-Wien. Vorlage 669_5.

Selvaraj, V., Swarna Karthika, T., Mansiya, C., & Alagar, M. (2021). An over review on recently developed techniques, mechanisms and intermediate involved in the advanced azo dye degradation for industrial applications. *Journal of Molecular Structure*, *1224*, 129195. https://doi.org/10.1016/j.molstruc.2020.129195

Stevens, R., & English, C. (2016). *Azo Dyes and Products of Reductive Cleavage Excellent Resolution of EU-Regulated Azo Dye Aryl Amines by GC-MS on the Rxi-35Sil MS GC Column*. www.restek.com

Thermo fisher Scientific (2011) Infrared, N., Scientific, T. T., Purpose, G., & Nir, H. (n.d.). *Thermo Scientific microPHAZIR GP Thermo Scientific microPHAZIR GP*. https://unicam.hu/files/microphazir-gp-hordozhato-nir-spektrometer/ThermoScientific_microPHAZIR_GP_Spec_Sheet.pdf

Tölgyesi, Á., & Sharma, V. K. (2020). Quantification of aromatic amines derived from azo colorants in textile by ion-pairing liquid chromatography tandem mass spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life

Sciences, 1137(October 2019), 121957. https://doi.org/10.1016/j.jchromb.2019.121957

Xuezhang, C., Deng, Q., Lin, S., Du, C., Zhao, S., Hu, Y., Yang, Z., Lyu, Y., & Han, J. (2017). A new approach for risk assessment of aggregate dermal exposure to banned azo dyes in textiles. *Regulatory Toxicology and Pharmacology*, *91*(November), 173–178. https://doi.org/10.1016/j.yrtph.2017.10.022

9. Appendix

FTIR – Ready Check

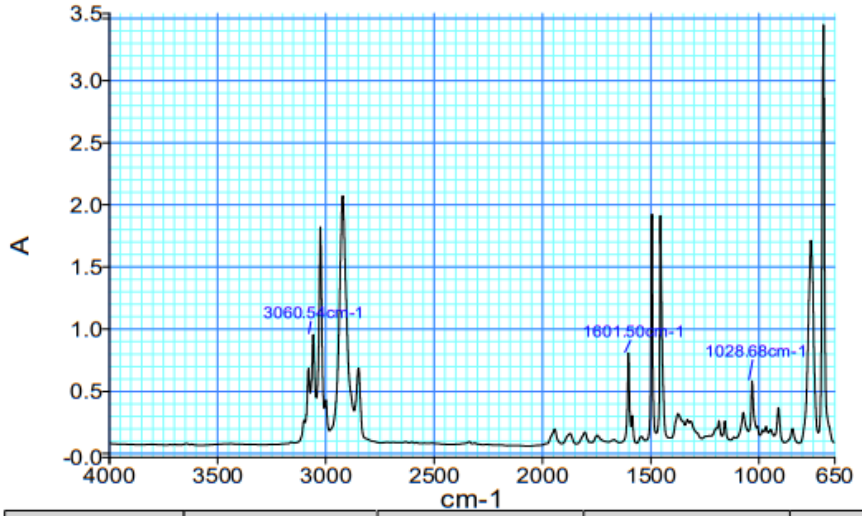
PerkinElmer Spectrum IR Version 10.6.2
Thursday, June 29, 2023 9:05 AM

Instrument Ready Checks Report

Analyst	Theresa Lindner
Date/Time	Thursday, June 29, 2023 9:03 AM
Software Product	PerkinElmer Spectrum IR Version 10.6.2
Report Name	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Ready_Checks_Log.rtf
Instrument Serial Number	C100600
Instrument Name	Frontier
Instrument Configuration	Diamond ATR
Accessory Serial Number	39336
Sampling	Internal APV

Abscissa Check PASS

Algorithm	Interpolated Peak
Resolution	4 cm-1
Accumulations	4
Accessory Background	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Abscissa_Background.sp
Abscissa Check Spectrum	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Abscissa.sp



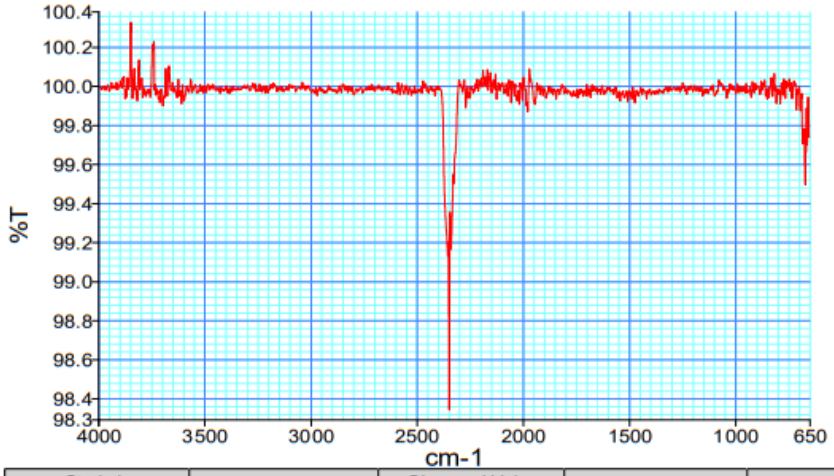
Nominal (cm-1)	Observed (cm-1)	Lower Limit (cm-1)	Upper Limit (cm-1)	Result
3060.0000	3060.5372	3058.5000	3061.5000	PASS
1601.0000	1601.4982	1600.0000	1602.0000	PASS
1028.0000	1028.6761	1027.0000	1029.0000	PASS

Noise Check PASS

Range	2600 to 2500 cm-1
Resolution	4 cm-1
Accumulations	4

Page 1

Accessory Background	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Noise_Background.sp
Noise Check Spectrum	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Noise.sp



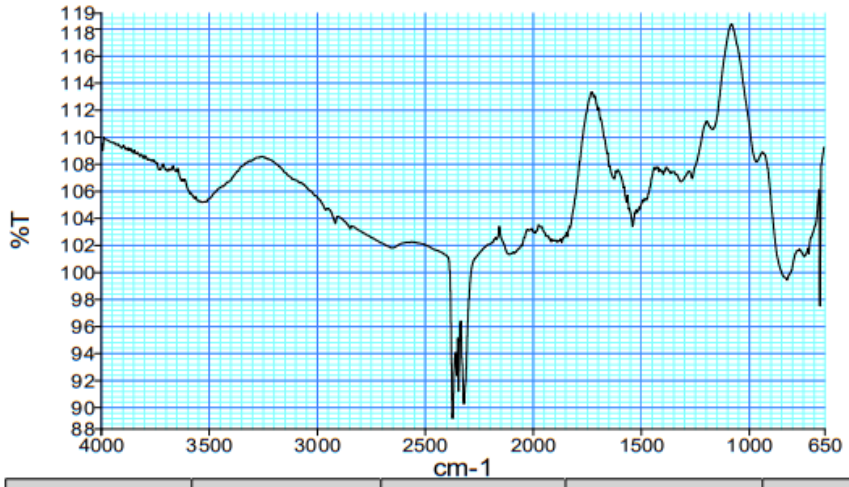
Statistic	Nominal	Observed Value	Limit	Result
RMS (%T)	0.0000	0.0146	1.0000	PASS
Peak to Peak (%T)	0.0000	0.0660	1.0000	PASS
Trend (%T/cm-1)	0.0000	0.0054	1.0000	PASS

Throughput Check PASS

Resolution	4 cm-1
Accumulations	4
Reference Background	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Throughput_RefBackground.sp
Accessory Background	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Throughput_Background.sp

Throughput Check Spectrum

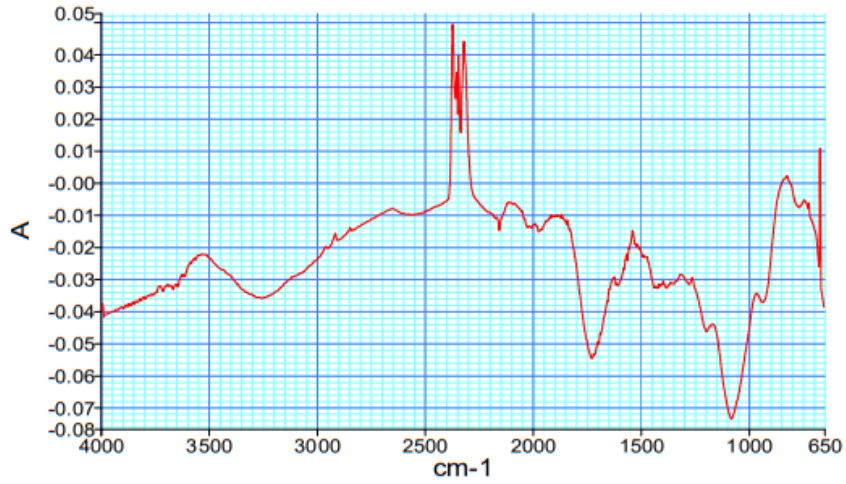
C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Throughput.sp



Position (cm-1)	Nominal (%T)	Observed (%T)	Lower Limit (%T)	Result
4000.00	100.0000	109.5725	80.0000	PASS
2600.00	100.0000	102.1896	80.0000	PASS
1000.00	100.0000	111.2431	80.0000	PASS

Contamination Check PASS

Resolution	4 cm-1
Accumulations	4
Reference Background	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Contamination_RefBackground.sp
Accessory Background	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Contamination_Background.sp
Contamination Check Spectrum	C:\pel_data\Ready Checks\2023_6_29\C100600[Frontier]_Check_Contamination.sp



Range (cm-1)	Nominal (A)	Observed (A)	Upper Limit (A)	Result
3100.00 - 2800.00	0.0500	-0.0130	0.1000	PASS
1800.00 - 1600.00	0.0500	-0.0256	0.1000	PASS
1400.00 - 1100.00	0.0500	-0.0284	0.1000	PASS

FTIR – natural fiber (animal source)

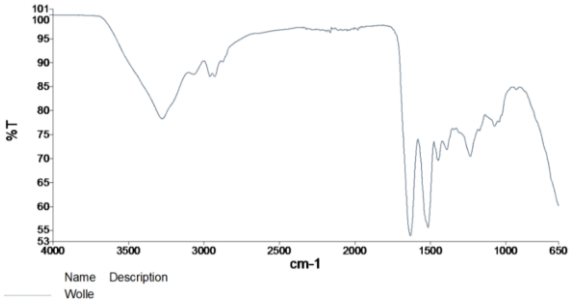


Figure 28: Spectrum wool (fabric sample).

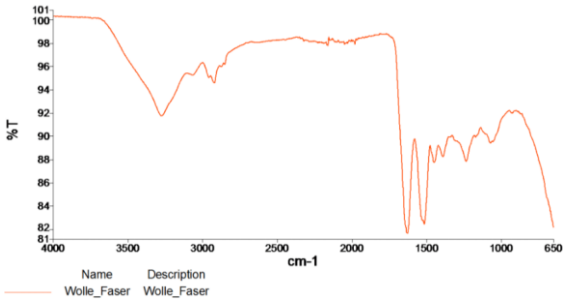


Figure 29: Spectrum wool (fiber sample).

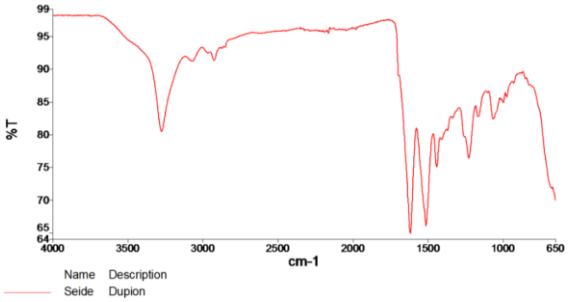


Figure 30: Spectrum silk "dupion" (fabric sample).

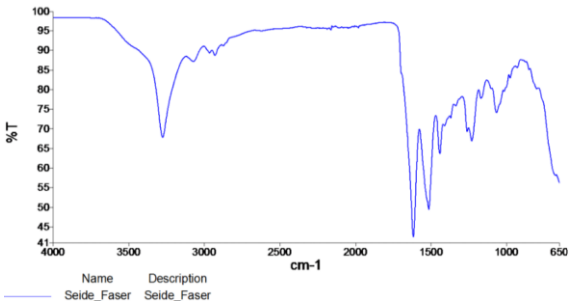


Figure 31: Spectrum silk (fiber sample).

FTIR – natural fiber (plant source)

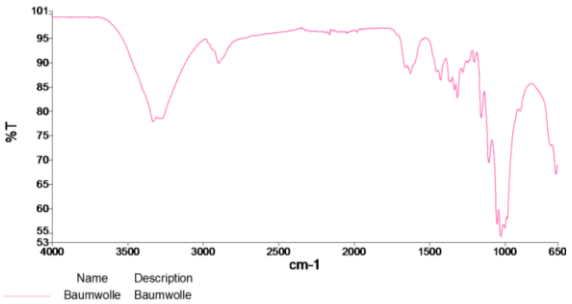


Figure 32: Spectrum cotton (fabric sample).

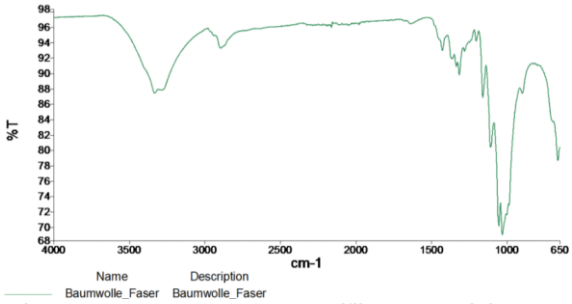


Figure 33: Spectrum cotton (fiber sample).

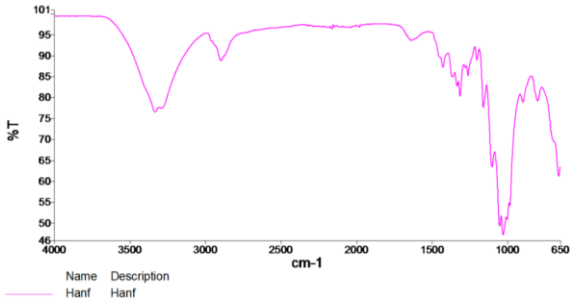


Figure 34: Spectrum hemp (fabric sample).

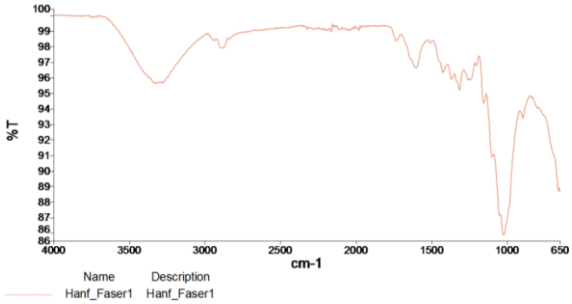


Figure 35: Spectrum hemp (fiber sample 1).

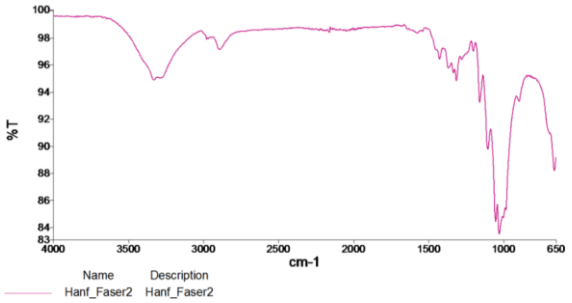


Figure 36: Spectrum hemp (fiber sample 2).

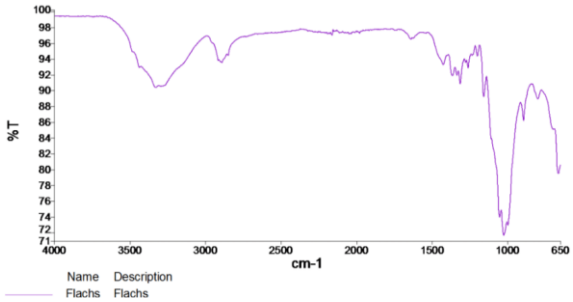


Figure 37: Spectrum flax (fabric sample).

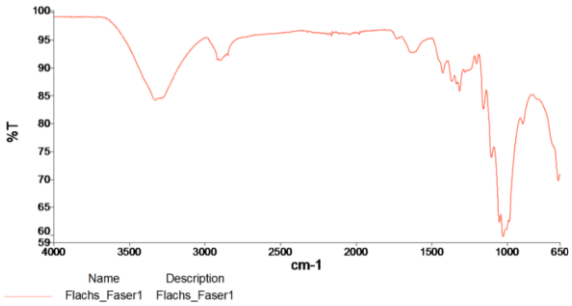


Figure 38: Spectrum flax (fiber sample 1).

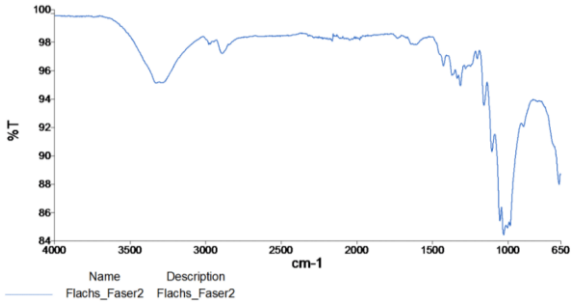


Figure 39: Spectrum flax (fiber sample 2).

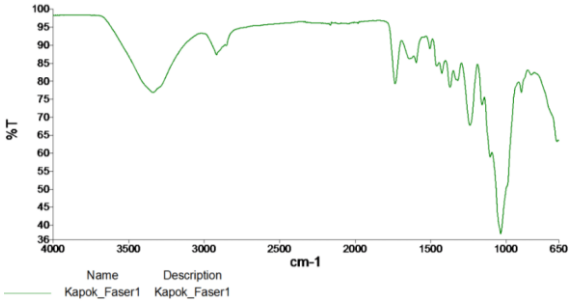


Figure 40: Spectrum kapok (fiber sample 1).

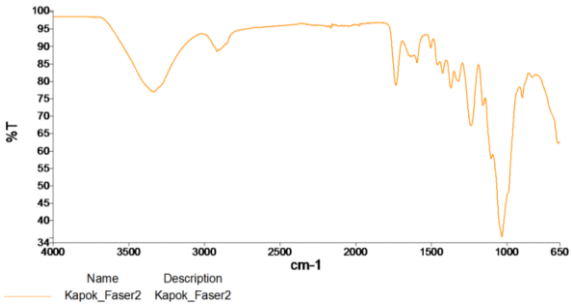


Figure 41: Spectrum kapok (fiber 2).

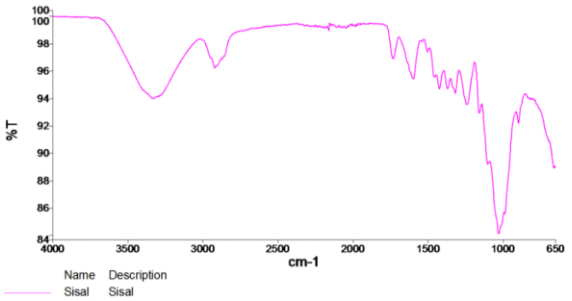


Figure 42: Spectrum sisal (fabric sample).

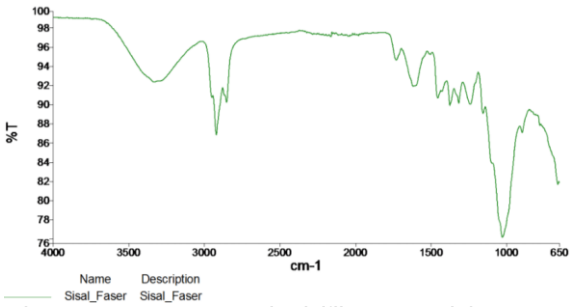


Figure 43: Spectrum sisal (fiber sample).

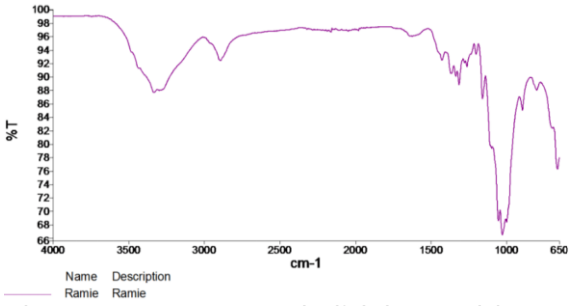


Figure 44: Spectrum ramie (fabric sample).

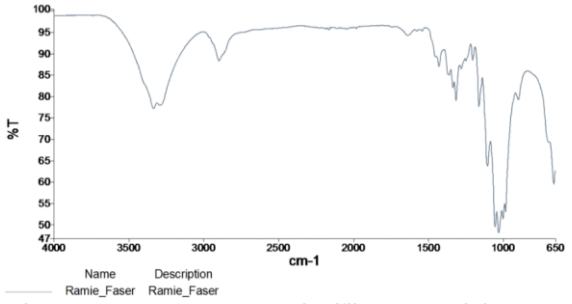


Figure 45: Spectrum ramie (fiber sample).

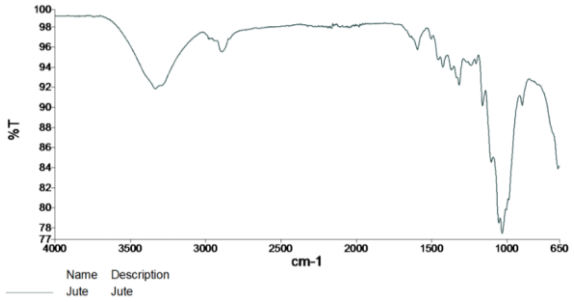


Figure 46: Spectrum jute (fabric sample).

Figure 47: Spectrum jute (fiber sample).

FTIR – synthetic fibers

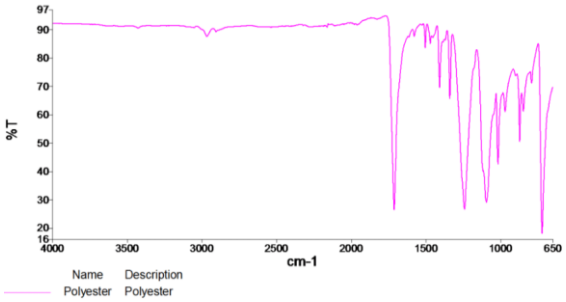


Figure 48: Spectrum polyester (fabric sample).

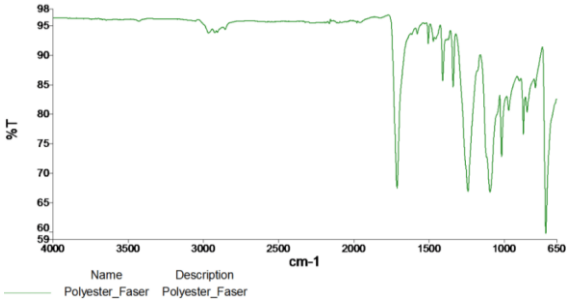


Figure 49: Spectrum polyester (fiber sample).

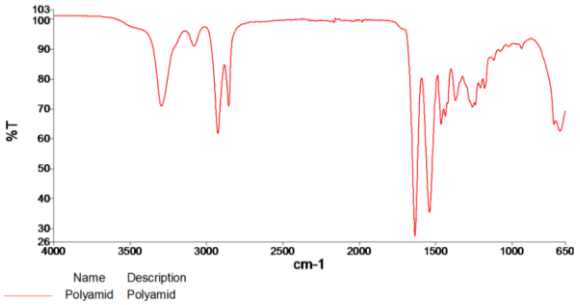


Figure 50: Spectrum polyamide (fabric sample).

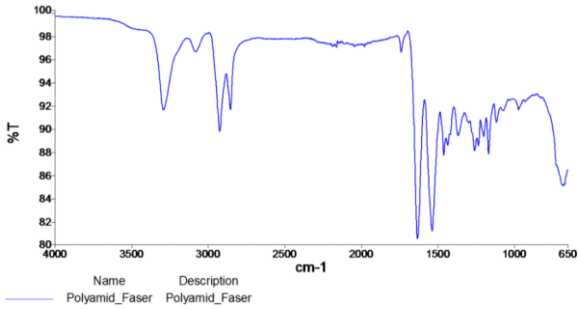


Figure 51: Spectrum polyamide (fiber sample).

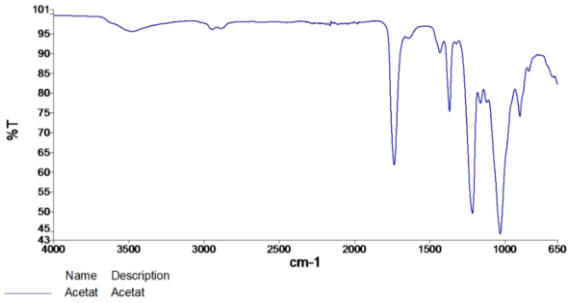


Figure 52: Spectrum acetate (fabric sample).

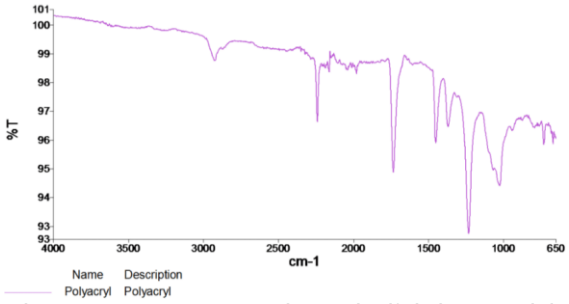


Figure 53: Spectrum polyacryle (fabric sample).

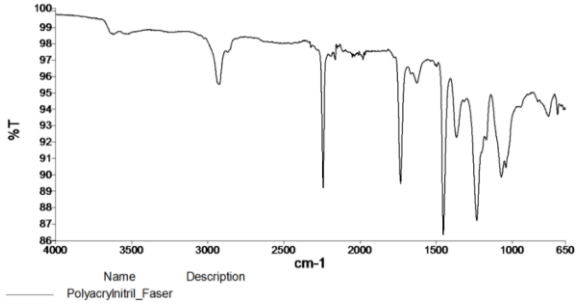


Figure 54: Spectrum polyacrylnitrile (fiber sample).

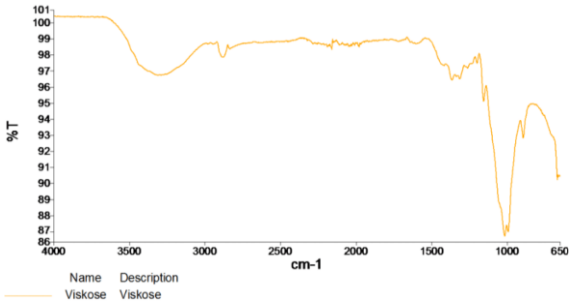


Figure 55: Spectrum viscose (fabric sample).

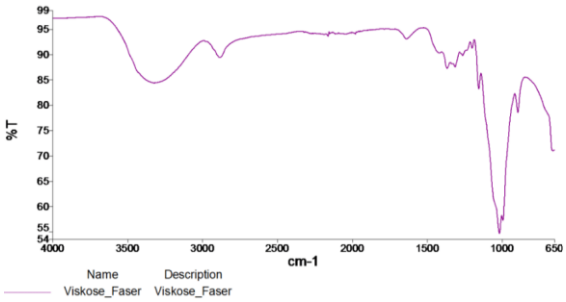


Figure 56: Spectrum viscose (fiber sample).

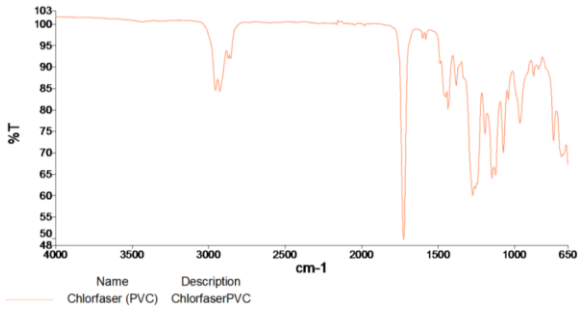


Figure 57: Spectrum PVC coated polyethylene (fabric sample).

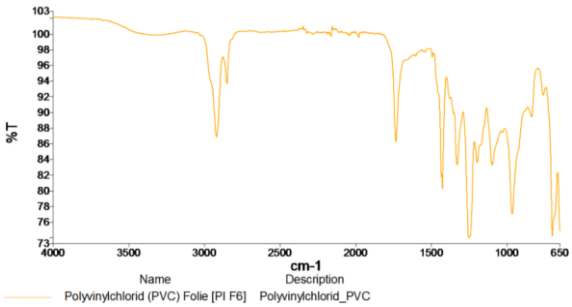


Figure 58: Spectrum PVC (foil from AGES-Wien).

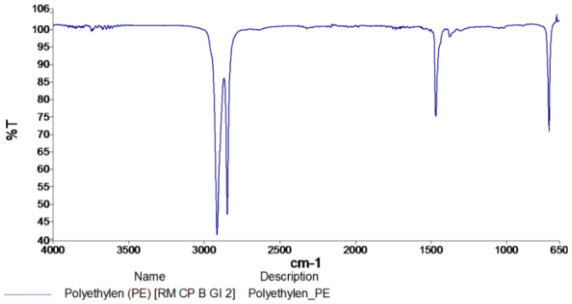


Figure 59: Spectrum polyethylene (AGES-Wien).

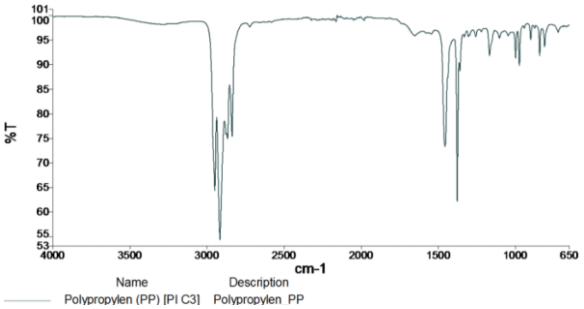


Figure 60: Spectrum polypropylene (AGES-Wien).

FTIR – samples with results.

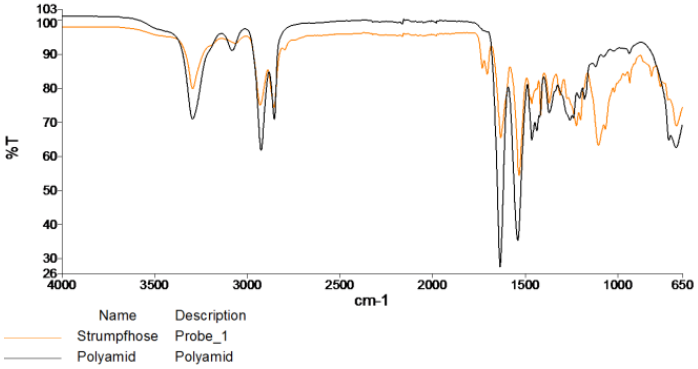


Figure 61: Spectrum tights (polyamide).

Table 28: Tights - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polyamide	0.534994	composition: 75% polyamide, 25% spandex
polyamide fiber	0.534503	

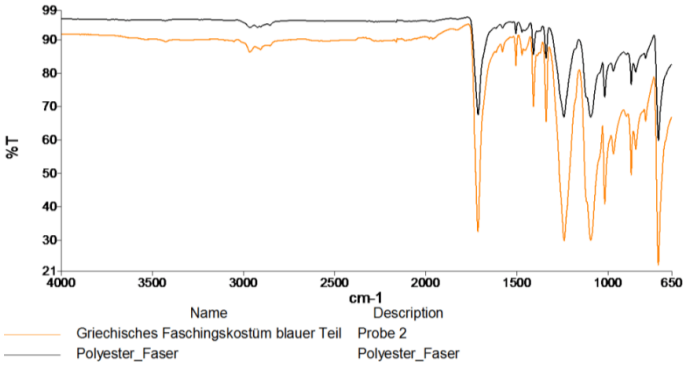


Figure 62: Spectrum greek costume blue part (polyester fiber).

Table 29: Greek-costume blue part - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polyester fiber	0.986508	composition: 100% polyester
polyester	0.983999	

Figure 63: Spectrum greek costume white part (polyester fiber).

Table 30: Greek-costume white part - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polyester fiber	0.995029	composition: 100% polyester
polyester	0.980843	

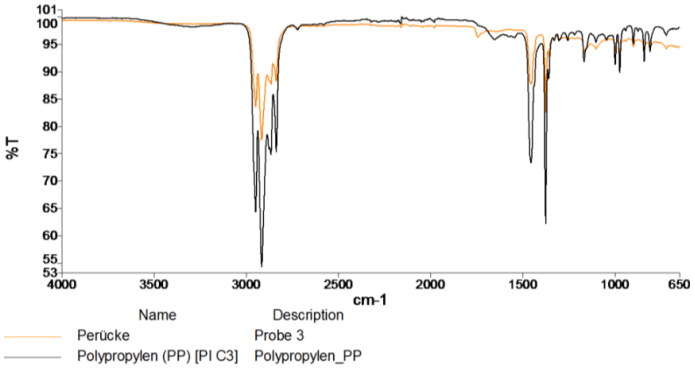


Figure 64: Spectrum wig (polypropylene).

Table 31: Wig - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polypropylene	0.991525	composition: 100% polypropylene

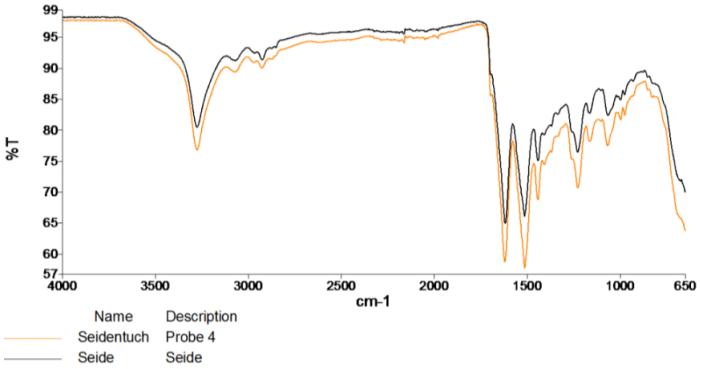


Figure 65: Spectrum silk-cloth (silk).

Table 32: Silk-cloth - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
silk	0.983008	composition: 100% silk
silk fiber	0.929705	

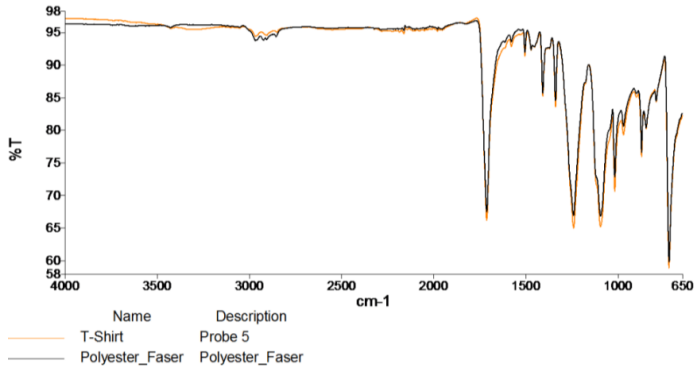


Figure 66: Spectrum t-Shirt (polyester fiber).

Table 33: T-Shirt - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polyester fiber	0.997191	composition: 93% polyester, 7% flax
polyester	0.965789	

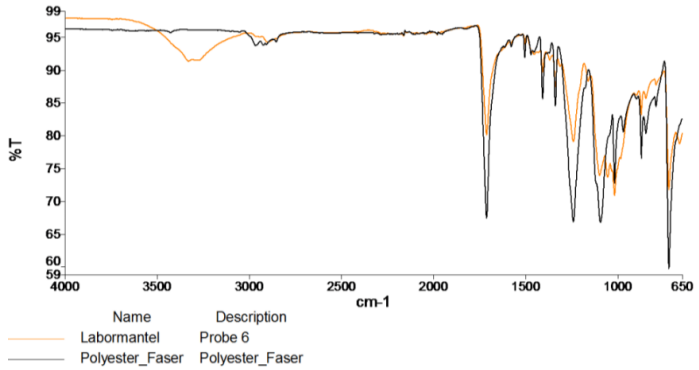


Figure 67: Spectrum labor coat (polyester fiber).

Table 34: Labor coat - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polyester fiber	0.911167	composition: 65% polyester, 35% cotton
polyester	0.891842	
cotton	0.275147	

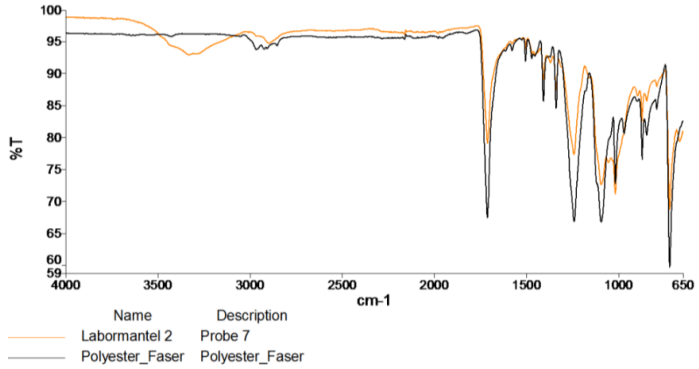


Figure 68: Spectrum labor coat 2 (polyester fiber).

Table 35: Labor coat - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polyester fiber	0.967292	composition: not specified
polyester	0.955361	

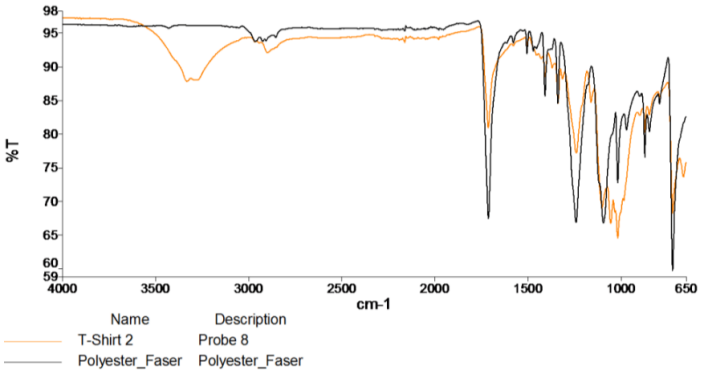


Figure 69: Spectrum t-shirt 2 (polyester fiber).

Table 36: T-shirt 2 – material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polyester fiber	0.825798	composition: not specified
polyester	0.799308	

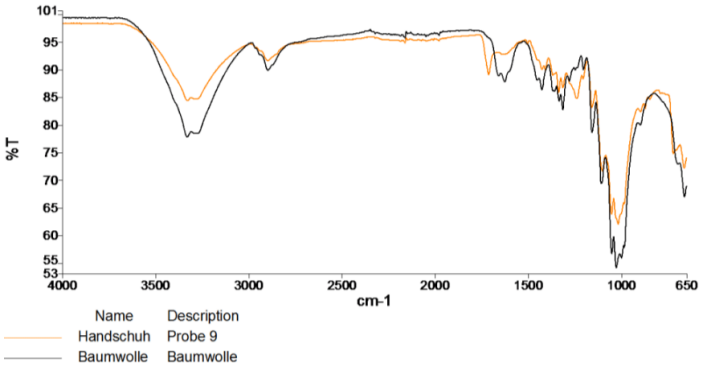


Figure 70: Spectrum glove (cotton).

Table 37: Clove (cotton) - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
flax fiber 1	0.83225	composition: 100% cotton
hemp fiber 2	0.832112	
cotton	0.830719	
cotton fiber	0.828871	
flax fiber 2	0.826353	
hemp	0.810167	
ramie fiber	0.809946	

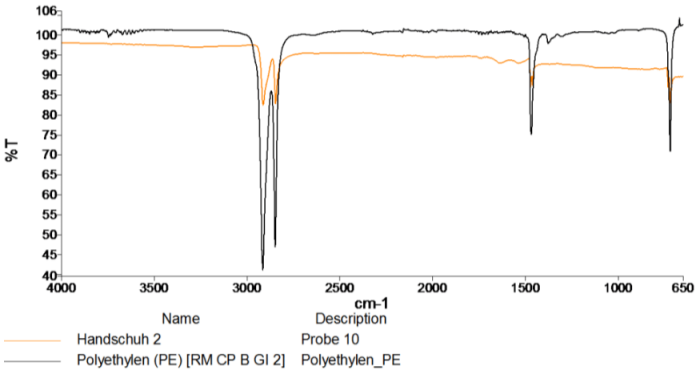


Figure 71: Spectrum glove 2 (polyethylene).

Table 38:Clove 2 (polyethylene) - material and correlation factor.

fabric/fiber-material	correlation factor	declaration according to label
polyethylene	0.864663	composition: dyneema (polyethylene)

GC-MS spectrums (reference substances), Rt and qualifier.

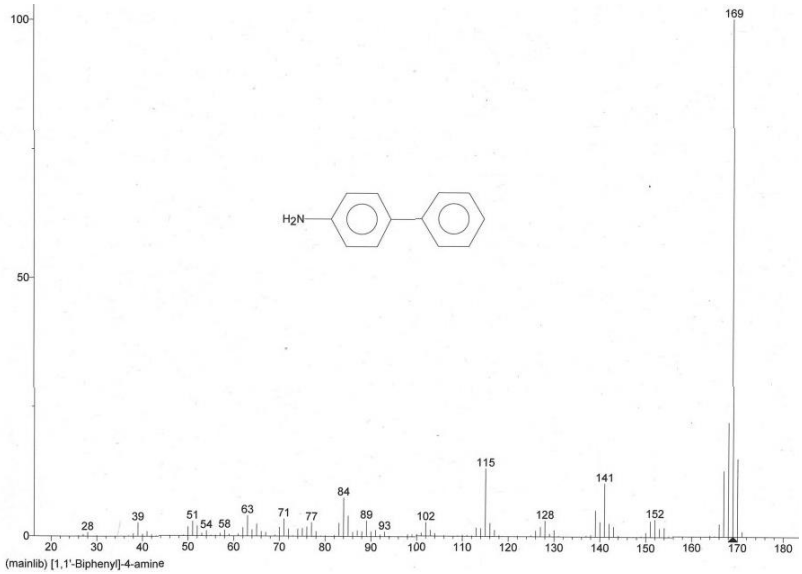


Figure 72: GC-MS spectrum 4-aminobiphenyl. (1)

Table 39: Rt, main-peak and qualifier 4-aminobiphenyl. (1)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
10.68	169		

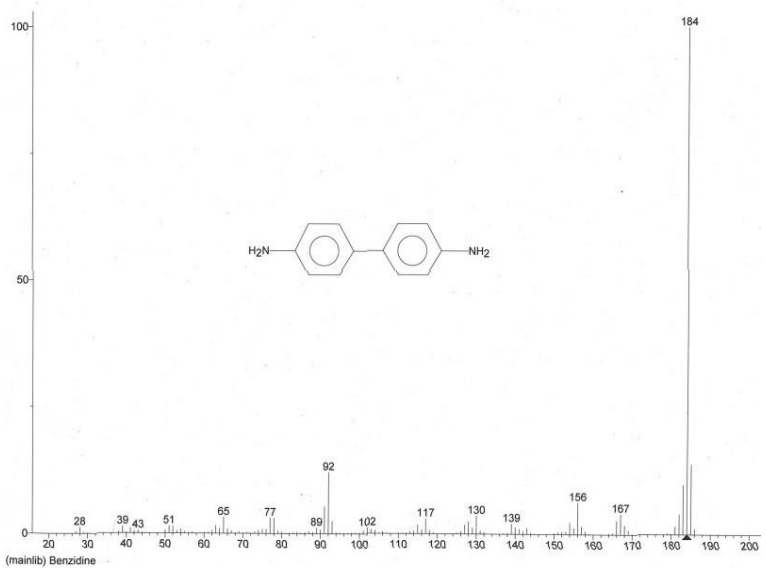


Figure 73: GC-MS spectrum benzidine. (2)

Table 40: Rt, main-peak and qualifier benzidine. (2)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
13.67	184	92	

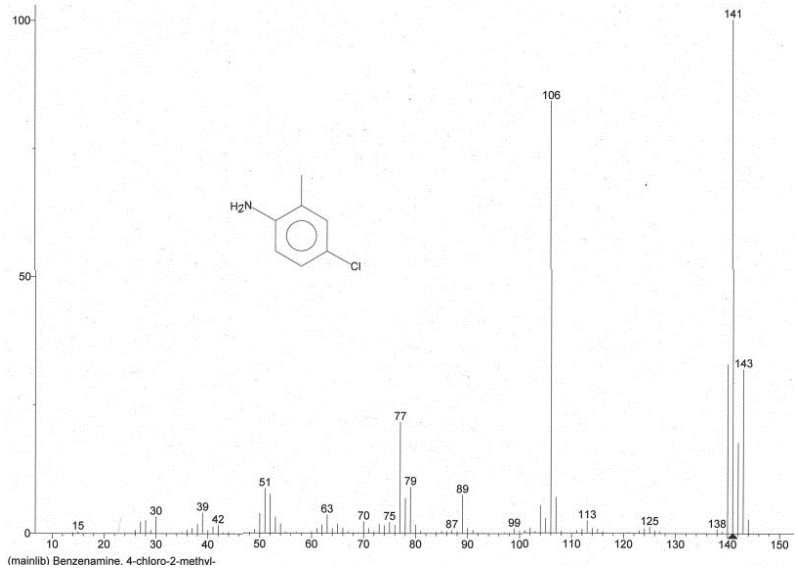


Figure 74: GC-MS spectrum 4-chlor-o-toluidine. (3)

Table 41: Rt, main-peak and qualifier 4-chlor-o-toluidine. (3)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
6.45	106	141	

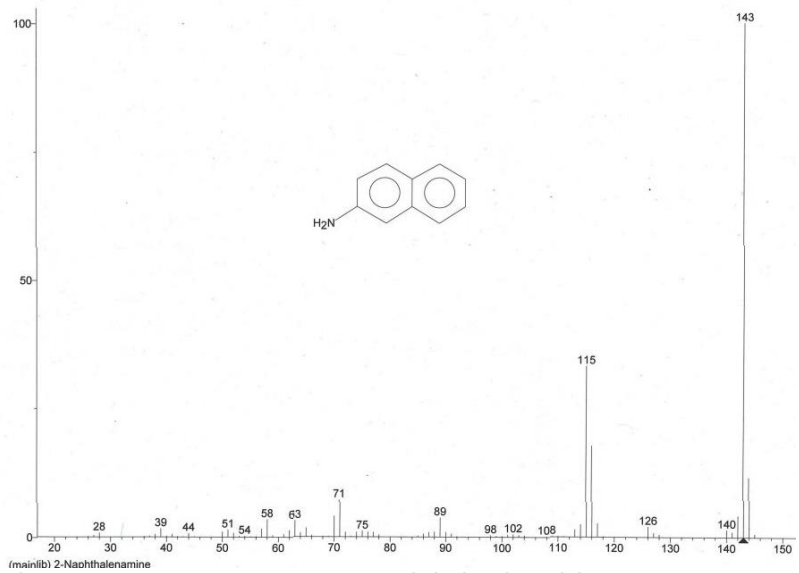


Figure 75: GC-MS spectrum 2-naphthylamine. (4)

Table 42: Rt, main-peak and qualifier 2-Naphthylamine. (4)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
9.13	143	115	

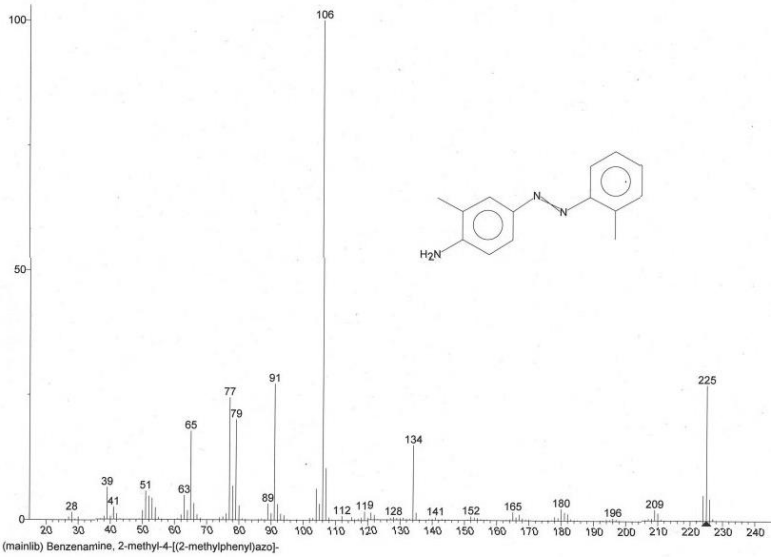


Figure 76: GC-MS spectrum o-aminoazotoluol. (5)

Table 43: Rt, main-peak and qualifier o-aminoazotoluol. (5)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
14.14	106		

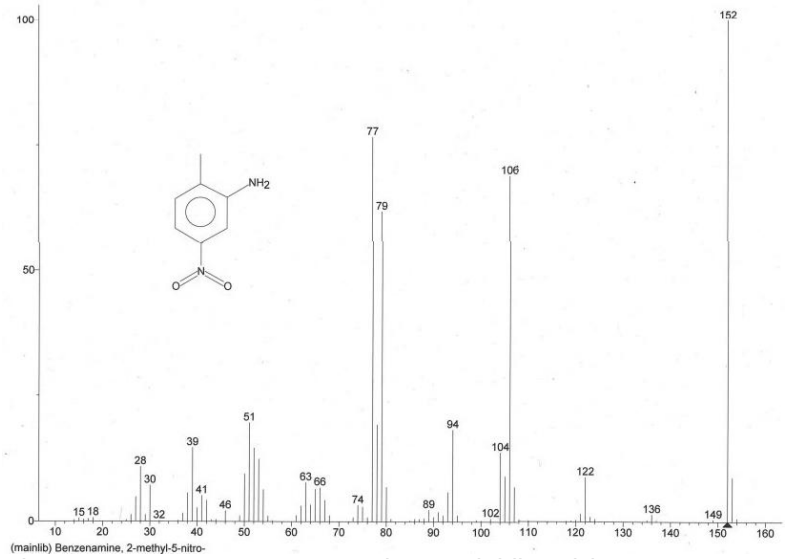


Figure 77: GC-MS spectrum 5-nitro-o-toluidine. (6)

Table 44: Rt, main-peak and qualifier 5-nitro-o-toluidine. (6)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
9.62	152	122	77

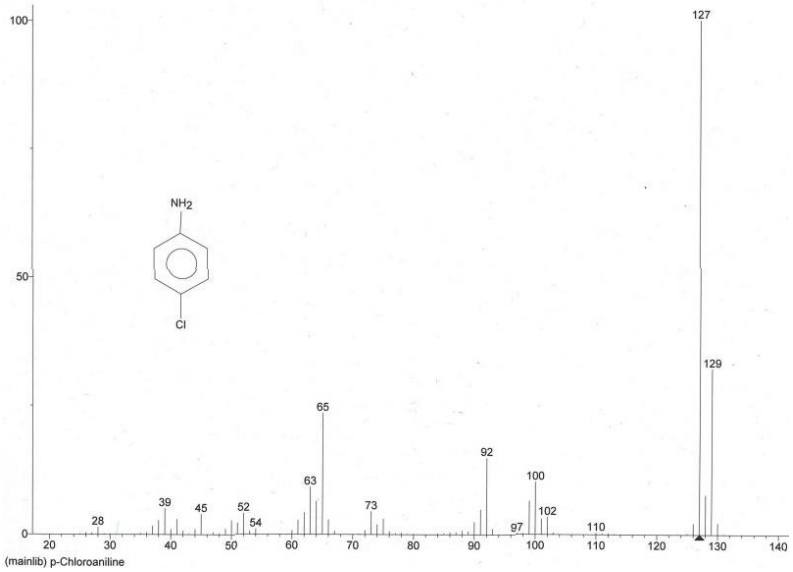


Figure 78: GC-MS spectrum 4-chloroaniline. (7)

Table 45: Rt, main-peak and qualifier 4-chloroaniline. (7)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
5.47	127		

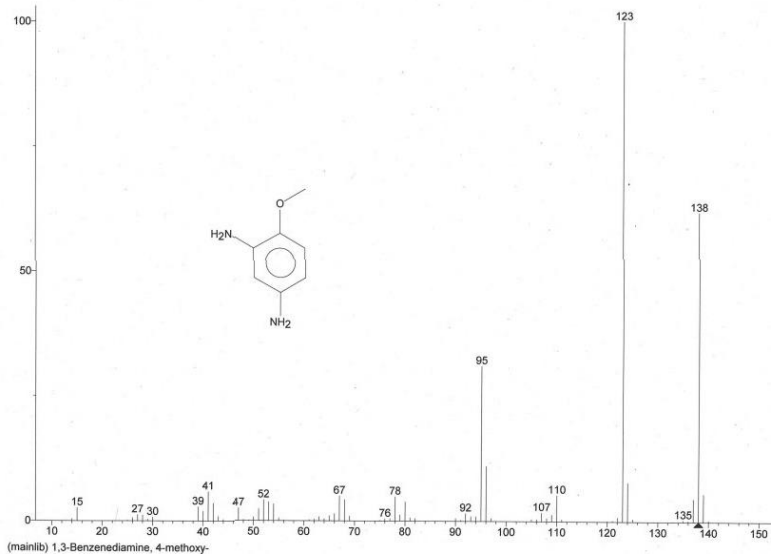


Figure 79: GC-MS spectrum 4-methoxy-m-phenylenediamine. (8)

Table 46: Rt, main-peak and qualifier 4-methoxy-m-phenylenediamine. (8)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
8.51	123	138	

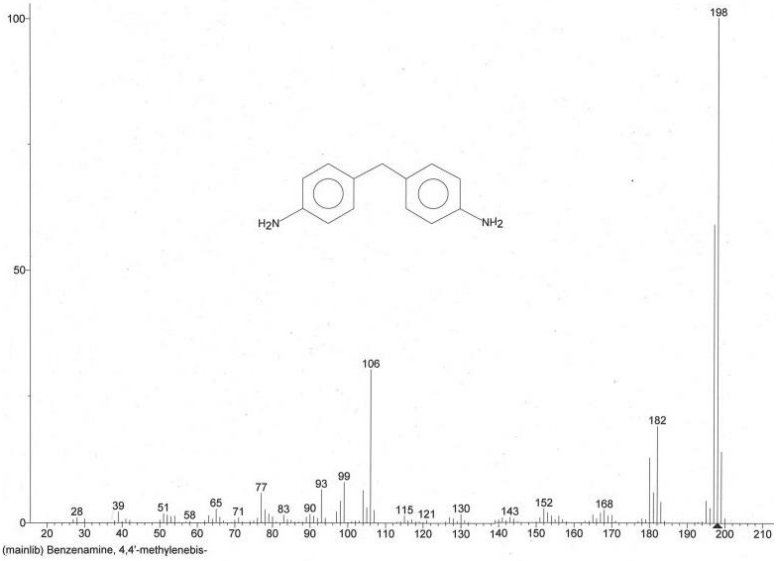


Figure 80: GC-MS spectrum 4,4-methylenedianiline. (9)

Table 47: Rt, main-peak and qualifier 4,4-methylenedianiline. (9)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
13.62	198	197	

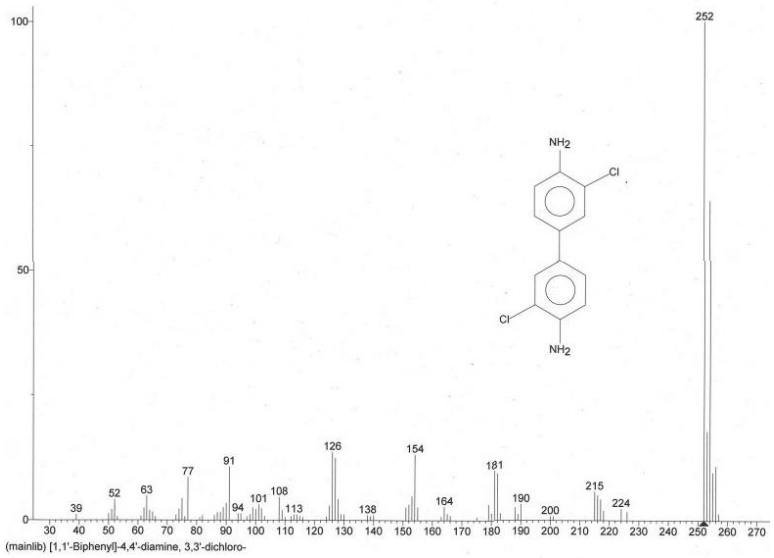


Figure 81: GC-MS spectrum 3,3-dichlorbenzidine. (10)

Table 48: Rt, main-peak and qualifier 3,3-dichlorbenzidine. (10)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
15.61	252		

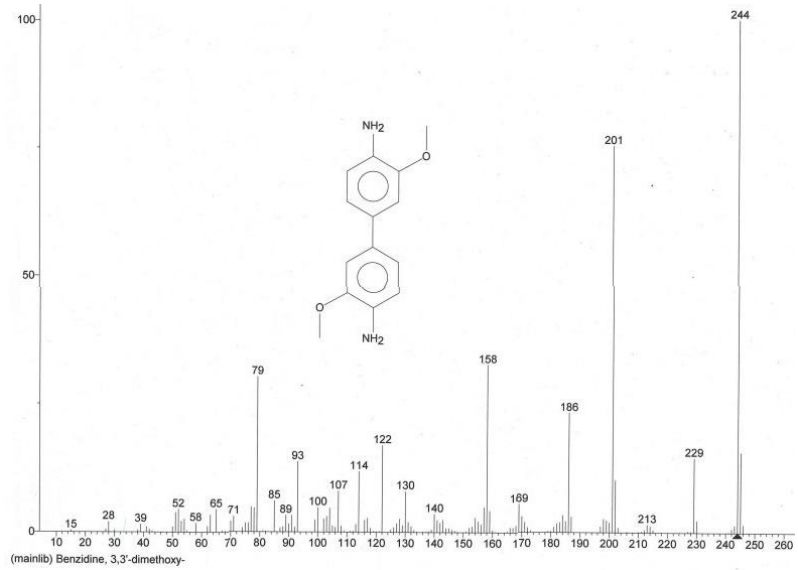


Figure 82: GC-MS spectrum 3,3-dimethoxybenzidine. (11)

Table 49: Rt, main-peak and qualifier 3,3-dimethoxybenzidine. (11)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
15.65	244	201	

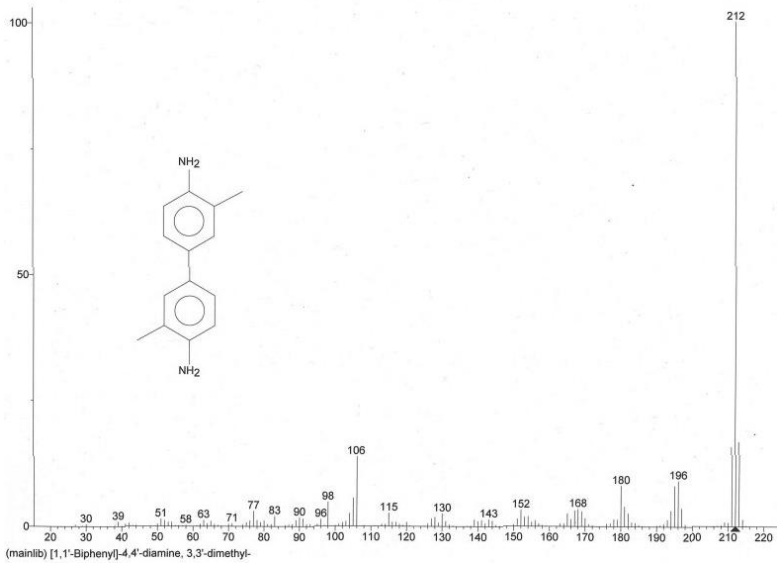


Figure 83: GC-MS spectrum 3,3-dimethylbenzidine. (12)

Table 50: Rt, main-peak and qualifier 3,3-dimethylbenzidine. (12)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
14.75	212	106	

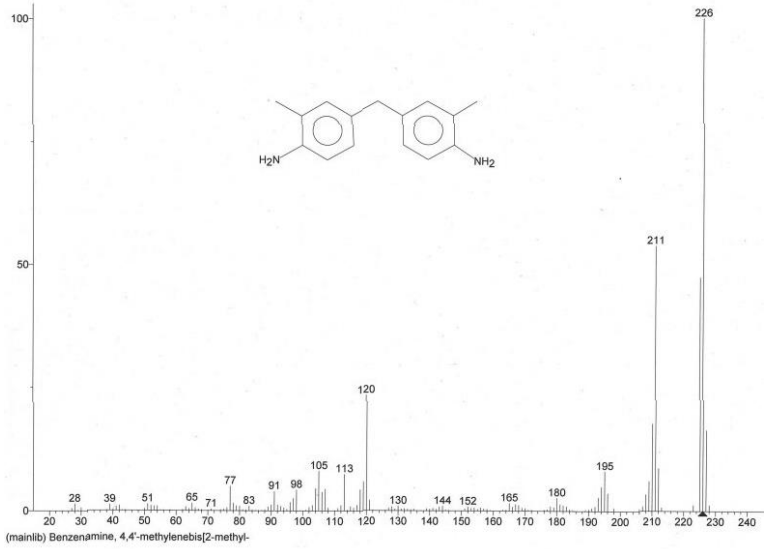


Figure 84: GC-MS spectrum 4,4-methylenedi-o-toluidine. (13)

Table 51: Rt, main-peak and qualifier 4,4-methylenedi-o-toluidine. (13)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
14.57	226	211	

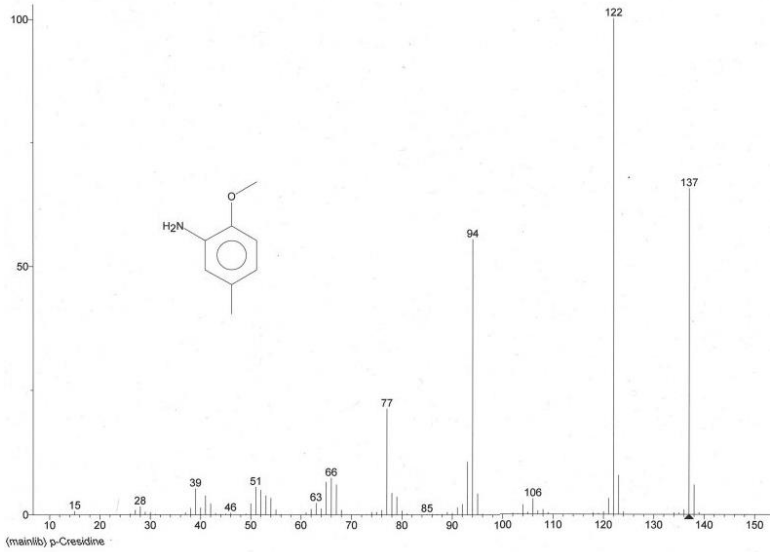


Figure 85: GC-MS spectrum 6-methoxy-m-toluidine. (14)

Table 52: Rt, main-peak and qualifier 6-methoxy-m-toluidine. (14)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
6.06	122	94	137

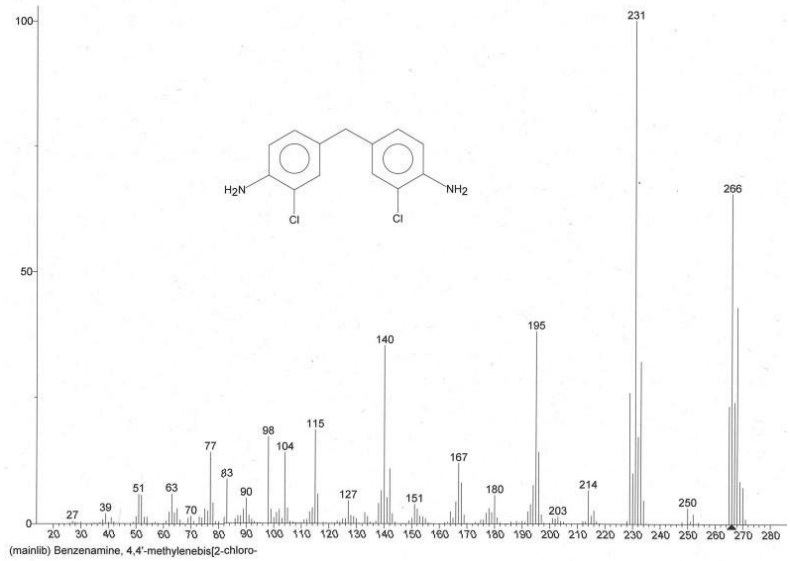


Figure 86: GC-MS spectrum 4,4-methylene-bis-(2-chloro-aniline). (15)

Table 53: Rt, main-peak and qualifier 4,4-methylene-bis-(2-chloro-aniline). (15)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
15.59	231	266	

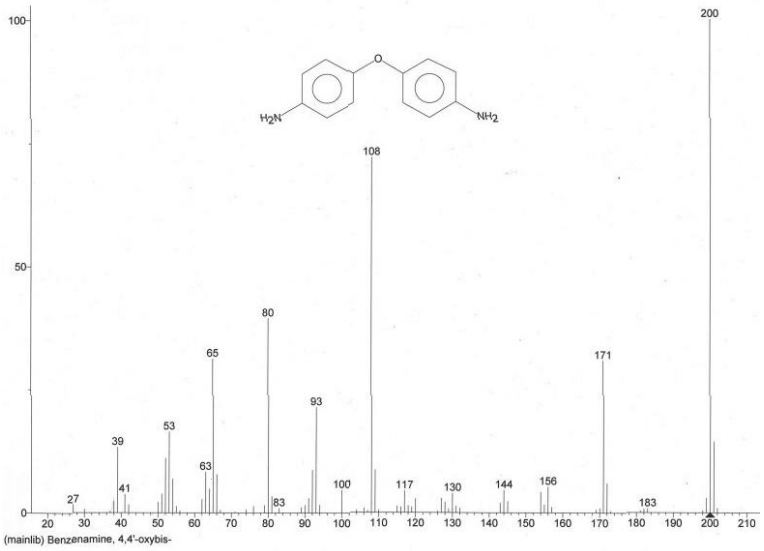


Figure 87: GC-MS spectrum 4,4-oxydianiline. (16)

Table 54: Rt, main-peak and qualifier 4,4-oydianiline. (16)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
13.50	200	93	

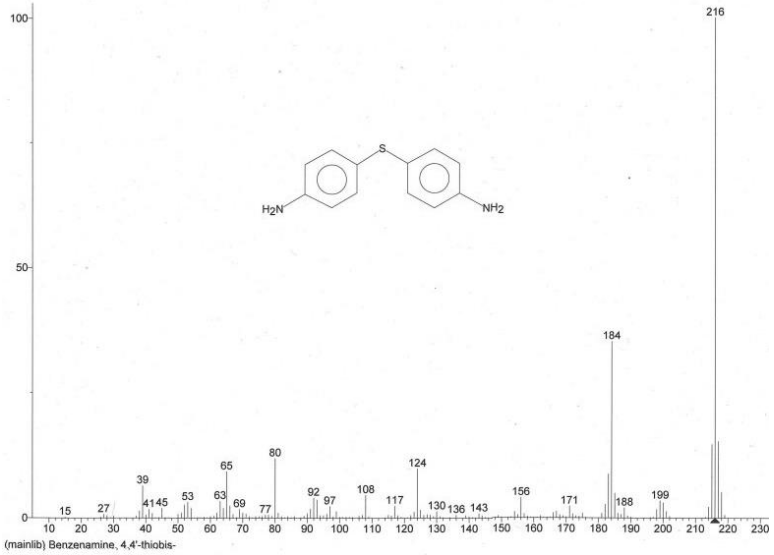


Figure 88: GC-MS spectrum 4,4-thiodianiline. (17)

Table 55: Rt, main-peak and qualifier 4,4-thiodianiline. (17)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
15.38	216	184	

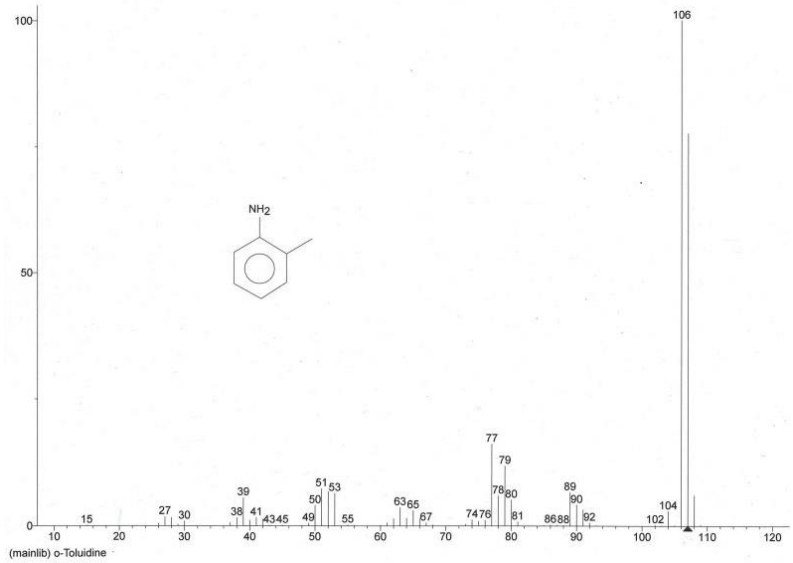


Figure 89: GC-MS spectrum o-toluidine. (18)

Table 56: Rt, main-peak and qualifier o-toluidine. (18)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
3.87	106	107	

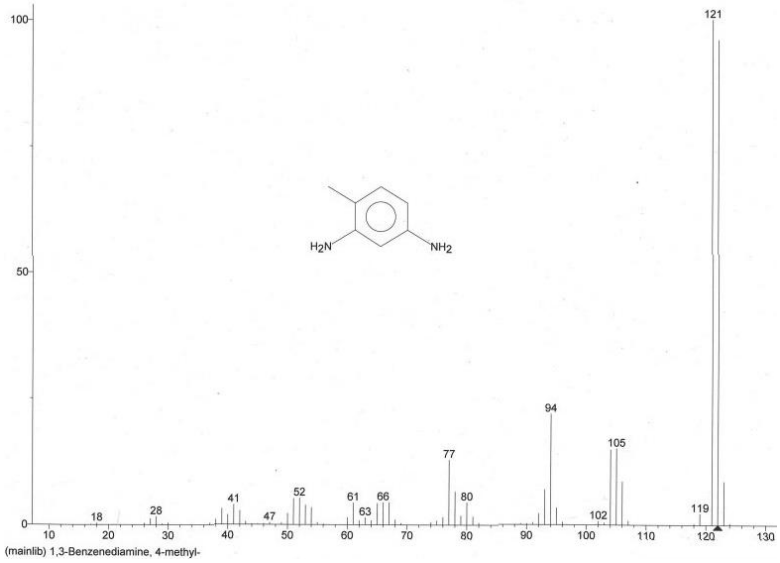


Figure 90: GC-MS spectrum 4-methyl-m-phenylenediamine. (19)

Table 57: Rt, main-peak and qualifier 4-methyl-m-phenylenediamine. (19)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
7.64	121	122	

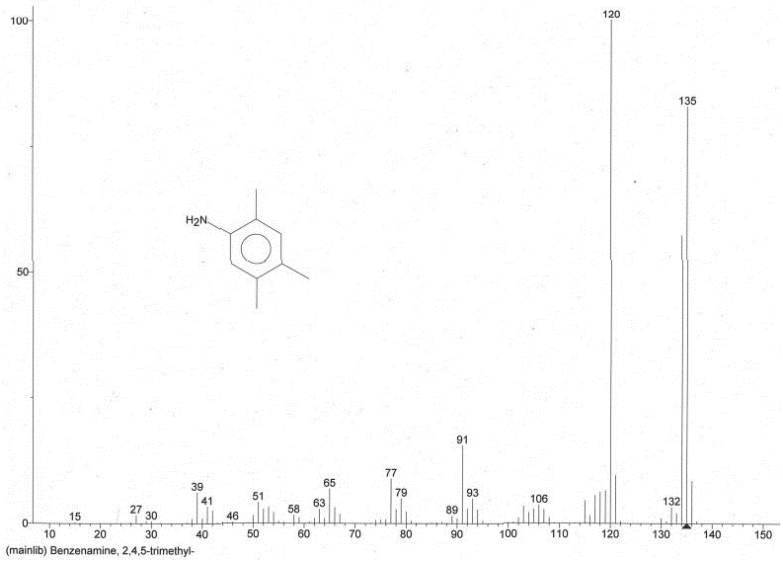


Figure 91: GC-MS spectrum 2,4,5-trimethylaniline. (20)

Table 58: Rt, main-peak and qualifier 2,4,5-trimethylaniline. (20)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
6.11	120	135	

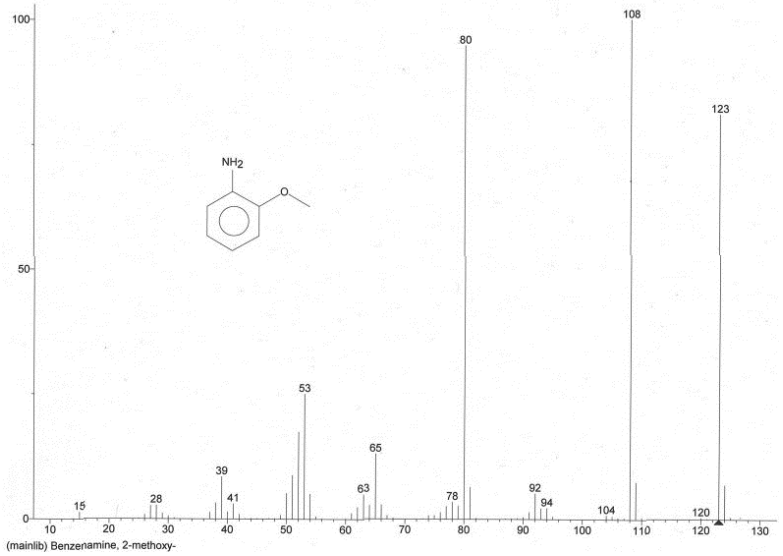


Figure 92: GC-MS spectrum o-anisidine. (21)

Table 59: Rt, main-peak and qualifier o-anisidine. (21)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
5.11	108	80	123

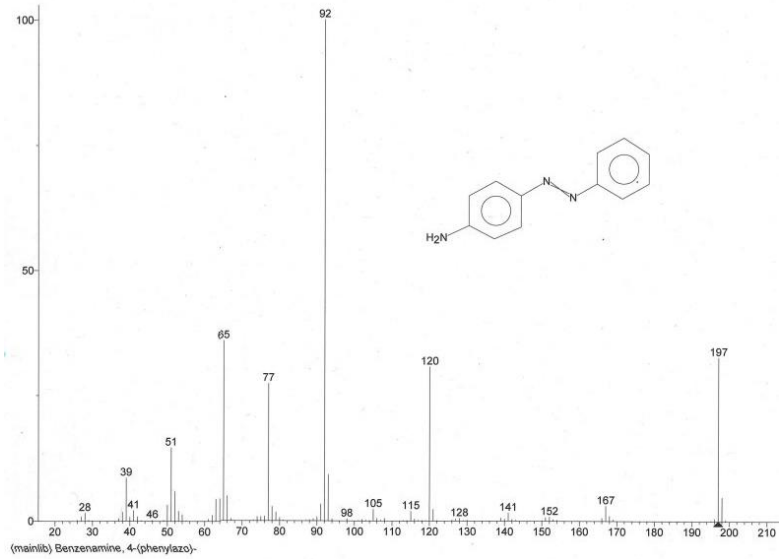


Figure 93: GC-MS spectrum 4-aminoazobenzol. (22)

Table 60: Rt, main-peak and qualifier 4-aminoazobenzol. (22)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
13.13	92	197	

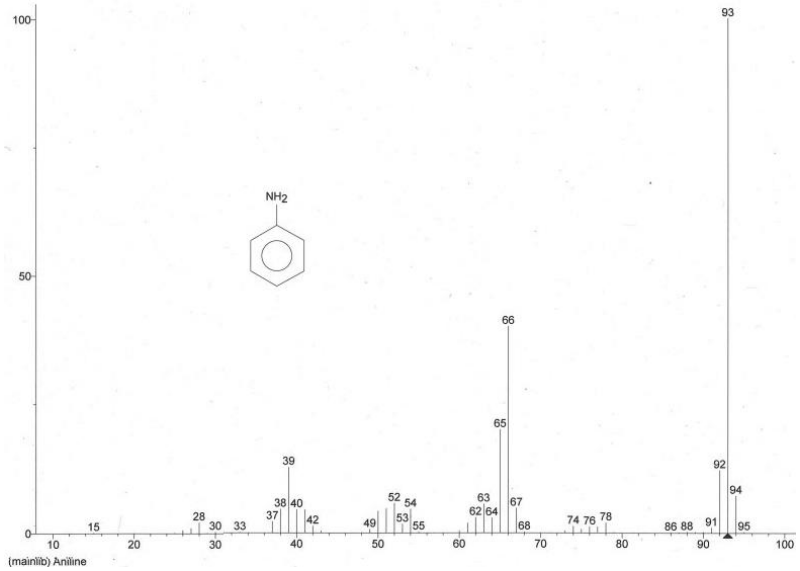


Figure 94: GC-MS spectrum aniline. (23)

Table 61: Rt, main-peak and qualifier aniline. (23)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
2.89	93	66	

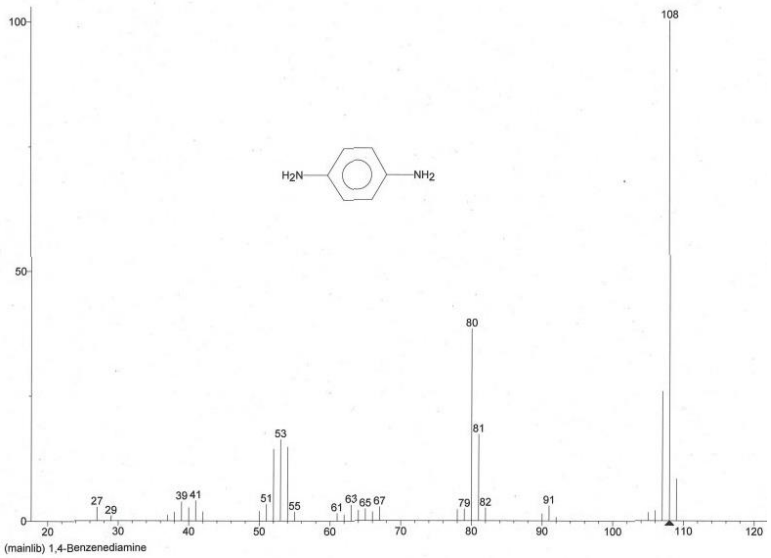


Figure 95: GC-MS spectrum 1,4-phenylenediamine. (24)

Table 62: Rt, main-peak and qualifier 1,4-phenylenediamine. (24)

Rt [min]	main-peak [m/z]	qualifier 1 [m/z]	qualifier 2 [m/z]
6.42	108	80	

GC-MS calibration curve for 1,4-phenylenediamine:

Table 63: Evaluation of 1,4-phenylenediamine with the calibration series.

calibration series		1,4-phenylenediamine results			naphthaline d8 (ISTD) results	benzidine d8 (IS) results
Name	Level	RT	Final Conc. [µg/mL]	Area	Area	Area
2ug/ml	1	6.4219	2.113095853	133170.55	1602867.8	232368.8702
3ug/ml	2	6.4218	3.027998163	198825.66	1466155.1	248505.992
5ug/ml	3	6.4219	5.022710478	342983.68	1371006.4	273244.659
7ug/ml	4	6.4217	6.485876589	463541.15	1386883.4	257516.0475
10ug/ml	5	6.422	9.770141773	793808.94	1517743.9	308683.768
20ug/ml	6	6.4219	20.04882871	1435668.7	1287913.3	270236.8877
25ug/ml	7	6.422	25.27606763	2032372.4	1435149.7	274774.217
30ug/ml	8	6.422	29.9684351	2148895.4	1273796.0	328529.732
40ug/ml	9	6.4218	41.56695781	3042729.4	1290558.8	280831.4982
50ug/ml	10	6.4218	48.71937852	3881280.6	1400121.7	298860.7545

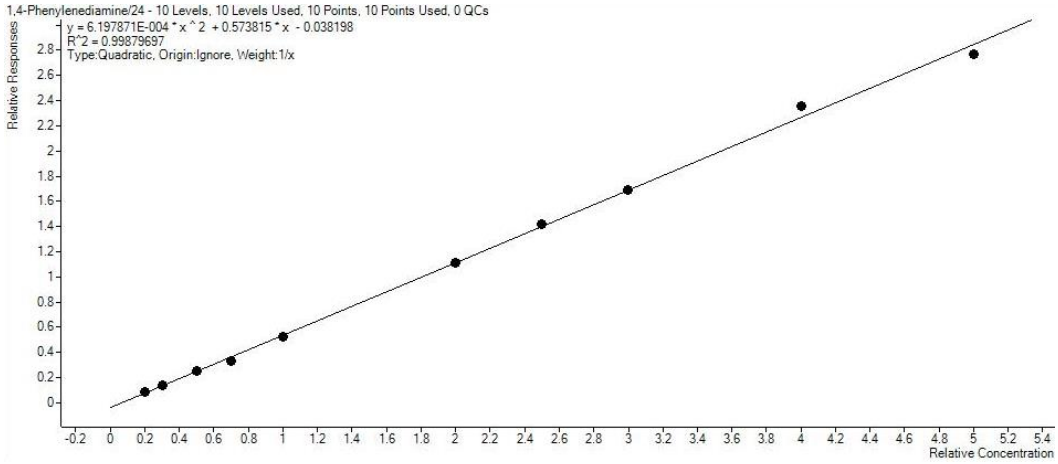


Figure 96: Calibration curve for 1,4-phenylenediamine.

Validation plan and evaluation of chemical-physical test methods

Test specification no:	Document no. according to Doxis	**Organizational Unit:**	Institute for Food Safety Linz (LSL), LILA
Method Title:	Determination of azo dyes in textiles according to EN 14362-1 by GC-MS		
Matrix:	Textiles	**Meter type:**	GC-MS, Agilent 6890, 5975 B
Study Objective:	Limit value monitoring according to the Azodyes Ordinance 2004 (BGBl. II No. 320/2004) of textiles in which contained azo dyes can release aromatic amines by reductive cleavage of one or more azo groups. The limit value for detectable concentrations is > 30 ppm.		

Procedure type:
☐ Trace analysis quant. ☐ Trace analysis qual./neg. ☐ Ingredient analytics ☐ Gravimetry/volumetry
☒ Convention procedure ☐ Physical measurements ☐ Screening procedure

Standard procedure: ☐ completely taken over ☒ Modified adopted ☐ not adopted ☐ No standard available

Referenced standard:	EN 14362-1 Textiles - Method for the determination of certain aromatic amines from azo dyes - Part 1: Detection of the use of certain azo dyes with and without extraction of the fibers.
Deviations from the norm:	Modified extraction method with different solvent, less chemical consumption and changes in the material used.
Reason for non-use:	Improved recovery, time and cost savings with the optimized method.

Validation procedure: NG/BG determined according to calibration curve method according to DIN 32645, measurement uncertainty according to EURACHEM/CITAC guide, accuracy and robustness determined by participation in a round robin test.

External specifications for validation:
☒ statutory (national/EU) Azo Dyes Ordinance 2004 (Federal Law Gazette II No. 320/2004)
☐ recognized guide

Validation status:
☒ Initial validation ☐ Revalidation Justification:
☐ Verification (e.g. for standard methods)

Table 64: Validation parameters.

	process characteristics to be determined	identified process characteristics	minimum requirements
Calibration	Determine correlation coefficient for all parameters in the workspace.	Correlation factors in the selected working range between 0.985 and 0.999 for all parameters.	0.98 or better
Recovery	Determination with doped matrix samples with 3 independent workups each at four different concentration levels. Because of azo dye spiking it is not possible to determine exactly how high the found amines will be concentrated, the evaluation of the recovery by comparison of the daily calibration and the verification of the method is more reasonable. Nevertheless, it can be claimed that with the optimized method higher concentrations of amines could be found in the same amounts of azo dye.	**Norm:** Matrices mostly 80-100% recovery between the different measurements and the different levels. (By evaluating amine 24) However, the recovery is worse than the minimum requirements when comparing the verification of the method and the daily calibration for amines 3, 7, 14, 18, 20, 21 and aniline. **Optimized method:** Matrices mostly 90-100% recovery between the different measurements and the different levels. (by evaluating amine 24) Here only the recovery of amine 18 and aniline is worse than the minimum requirement, when comparing the daily calibration with the verification of the method.	Minimum recovery requirements are described for each amine individually and range from 20-70%.
Precision	Determination from the data of the overall procedure for the work area.	Worst CV during reprocessing with the standard method = 13.35%. Worst CV during reconditioning with the optimized method = 10.54%.	±15% or better
Correctness	Participation in a laboratory comparison test/ring test.	Participation in an interlaboratory comparison is performed as soon as the method is registered in the validation program (Valex).	z-score max. 2
Selectivity	Determination of the selectivity of the GC-MS method for all parameters in their measurement windows.	Selectivity factor 0.9999 for all parameters. Measurement by GC-MS: Rt, target ion T, qualifier ion Q, ratio T/Q.	>0.9

LOD	Determine the detection limit using the calibration curve method according to DIN 32645 or another method of determination.	LOD = 2.888mg/kg with residual variance method. LOD = 0mg/kg with blank method.	not applicable
LOQ	Determine the limit of quantification using the calibration curve method according to DIN 32645 or another method of determination.	LOQ = 9.626mg/kg with residual variance method. LOQ = 0mg/kg with blank method.	10 mg/kg
Applicability	Verification with at least 3 representative matrices.	Matrix 1 (polyester), matrix 2 (polyamide) and matrix 3 (viscose) doped with azo dye (disperse red 1 1.5mg) gave the same concentrations of analytes (12.4mg/kg) with sample preparation using the optimized method. (Evaluation with amine 24)	Matrices polyester, polyamide, and viscose.
Robustness	Comparative measurement with another operator as well as variation of extraction parameters (e.g.: extraction volume, sample quantity).	The optimized method will be performed under the same conditions internally by another employee to compare the results.	± 10% or better
Uncertainty of measurement	Uncertainty of measurement determined according to EURACHEM/CITAC guide.	Meeting the requirements after calculation in the Valex program.	± 20% or better

Declaration under oath.

I declare in lieu of an oath that I have written this thesis independently, that I have not used any other aids than those indicated and that I have quoted all formulations and concepts taken from unprinted sources, printed literature or from the internet in wording or in essential content
in accordance with the guidelines for scientific papers, marked them with footnotes or indicated the exact source.

Linz, 2023