Determination of 24 primary aromatic amines in aqueous food simulants by combining solid phase extraction and salting-out assisted liquid–liquid extraction with liquid chromatography tandem mass spectrometry

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ABSTRACT

Carcinogenic primary aromatic amines (PAAs) can be released from improperly manufactured food packaging materials. The limit for the sum of PAAs is set to 10 µg kg^{-1} in Commission Regulation No. 10/2011 (FCM Regulation). However, a lower individual limit, 2 µg kg^{-1} has been recently introduced for the carcinogenic PAAs in Commission Regulation No. 2020/1245. As the majority of the previously published methods are no longer compliant with the current regulation, a UHPLC-MS/MS method was developed to enable food packaging compliance testing for PAAs not only from 3% (w/v) acetic acid, but also from 10% (v/v) ethanol food simulant. Since the latest amendment of the FCM Regulation refers to the list of the 22 restricted PAAs of EU Regulation No. 1907/2006, these PAAs were selected as target compounds along with aniline and p-toluidine, the most common impurities of azo colorants and isocyanates. An enrichment factor of 20 could be achieved combining solid phase extraction with salting-out assisted liquid–liquid extraction. The method was successfully validated and applied on real samples. Limit of quantitation (LOQ) and limit of detection (LOD) values were 0.15 µg L^{-1} and 0.05 µg L^{-1} for both food simulants, respectively; except for 2,4-diaminotoluene, aniline and 4,4′-oxydianiline. However, even these compounds had lower LOD values than the new individual limit of 2 µg kg^{-1}. Cumulative LOD values for both food simulants (1.6 µg L^{-1} and 1.5 µg L^{-1} for 3% (w/v) acetic acid and 10% (v/v) ethanol, respectively) were lower than the 10 µg kg^{-1} specified in the FCM Regulation. Accuracy values were between 70 and 118% for both food simulants for the majority of PAAs. Both within-day and between-day precision values were below 20%. This method proved to be suitable for daily routine analysis enabling compliance testing of food packaging materials according to the latest regulations. The method was successfully applied for the analysis of plastic kitchenware samples.

1. Introduction

Articles coming into contact with food during production, packaging, shipping, storage and serving are classified as food contact materials (FCMs). A large proportion of FCMs are food packaging materials made of plastic. Food packaging is designed to preserve food quality and to increase the shelf-life. However, hazardous contaminants can migrate into food from packaging materials as they get in contact with each other. For this reason, specific migration limits were set for numerous chemicals - including primary aromatic amines (PAAs) - by Commission Regulation No. 10/2011 (FCM Regulation) [1].

PAAs are aniline (ANL) derivatives; hence, they are organic substances that have a primary amino group linked to an aromatic ring. They are used as building blocks and intermediates in the synthesis of pharmaceuticals, pesticides, antioxidants, polymers and dyes [2,3]. Consumers are mainly exposed to PAAs through azo dyes and polyurethane (PU) based adhesives [4–12]. The most frequent products of concern apart from dyed textiles and leathers [6,13] are food packaging materials [2,8–10,14] along with kitchenware made of plastic [15–20]. PAA migration was previously reported from paper napkins [21,22] food wrappers and bakery bags made of recycled paper and board [7,9] multilayered plastic laminates [2,9,23–26] black polyamide [15,17–20,26] and colored silicone [20] kitchen utensils.

A major health risk is posed due to the presence of PAAs in consumer goods. Many PAAs are known to have allergenic or genotoxic effects [3] others are listed as carcinogens by the International Agency for Research on Cancer (IARC) [27] EU Regulation No. 1272/2008 (CLP Regulation) [28] and the German MAK Commission [29]. Due to the health risk of PAAs, the use of those azo colorants in textile and leather products that can release the 22 listed PAAs is restricted by EU Regulation No. 1907/2006 (REACH Regulation) [30]. Also, the use of many PAAs is prohibited in the production of cosmetics by Regulation No. 1223/2009 [31]. In case of FCMs, a 10 μgkg^{-1} general migration limit was set for the sum of PAAs [1]. Moreover, Commission Regulation No. 2020/1245 [32] the latest amendment of the FCM Regulation, has recently introduced an individual 2 μgkg^{-1} limit for carcinogenic PAAs and refers to those that are listed in the REACH Regulation [30].

Strict legislation generates a need for analytical method development to enable compliance testing. A spectrophotometric method was developed first, based on diazotization followed by coupling with *N*-(1-naphthyl)ethylene-1,2-diamine (NEDA) producing an azo compound [2,10,17,33]. The main drawback of this method is its lack of selectivity. Other compounds such as primary aliphatic amines or sulfonamides can also react with NEDA, resulting in false positives [10]. Moreover, the total PAA content is given in ANL equivalent even though some PAAs give lower responses compared to ANL, which can lead to underestimation [34]. Consequently, the spectrophotometric method is suitable for screening purposes only [35]. For individual PAA determination, selective methods applying different separation techniques are required. There are few applications of capillary electrophoresis [13,14] with ultraviolet (UV) [14] or laser-induced fluorescence [13] detection. These methods are useful in case of limited sample volume but not suitable for separating large numbers of PAAs. In the majority of analytical techniques gas chromatography (GC) [6,23,36,37] or liquid chromatography (LC) [4,7–9,16,20,22,24,25,35,38–43] is applied. Analytical methods suitable for PAA determination from leather, textiles and toys are described in ISO 17234 [44,45] ISO 14362 [46,47] and EN 71 [48–50] standard series, respectively. GC separation and mass spectrometric (MS) detection without derivatization is suggested in these standard methods. However, to improve chromatographic peak shapes and to produce less polar and thermally more stable products, derivatization of PAAs is very common prior to GC–MS analysis. For this purpose, commonly different types of chloroformates [36] and acid anhydrides [23,37] are used. The greatest disadvantage of GC methods is their incompatibility with aqueous samples. Since this is the most frequent sample type, solvent exchange is often inevitable for GC analysis.

In the case of testing PAA migration from FCMs the majority of published methods are working with the 3% (w/v) acetic acid food simulant only, since the EU FCM guideline [35] claims that the 3% (w/v) acetic acid food simulant represents the worst-case scenario for PAA migration testing. However, based on the FCM Regulation [1] the general food simulant for aqueous food is 10% (v/v) ethanol; migration testing with 3% (w/v) acetic acid should be performed only if the article might come into contact with food of a pH lower than 4.5.

For determining PAAs with LC generally UV [4,35,43] or tandem MS (MS/MS) [7–9,16,20,22,24,25,35,38–42] detection is used. The latter has higher selectivity and sensitivity, thus it is more common. Both hydrophilic interaction (HILIC) [39] and reversed phase (RP) [4,7–9,16,20,22,24,25,35,38,40–43] LC techniques can be applied. Separation of PAAs with diverse polarity can, however, be a challenging task since HILIC is limited to highly polar compounds, whereas they have poor retention in RP. Although ion pairing eluent additives can improve the retention of polar PAAs [8,9,18,22,24,35] in RP mode, the use of them may result in serious ion suppression for others. Another difficulty is the difference in the base strength of PAAs, thus pH control can be necessary to avoid split peaks or tailing. Buffers are suitable for this purpose [4,35,40,43] but they may cause reduced sensitivity through ion suppression in the electrospray ionization (ESI) source. Nitrated and halogenated derivatives are relatively weak bases, thus some of them are poorly ionizable at pH levels compatible with the LC column. E.g. 2-methyl-5-nitroaniline (2M5NA) is frequently reported not to give a signal in ESI-MS [24,25,38]. To overcome limited ionization and achieve lower limit of detection (LOD), sample preconcentration can be necessary. For this purpose, usually liquid–liquid extraction (LLE) [7,43,51] and solid phase extraction (SPE) [7,10,23,25,35,38,43,51,52] techniques are used.

In LLE, solvents used for the extraction from aqueous medium are water-immiscible. To enhance phase separation or to improve analyte recovery, salting out is often applied [7,43]. In salting-out assisted liquid–liquid extraction (SALLE) the solvent used for extraction is water-miscible but it can be salted out. [53,54] This technique is common in bioanalysis [53,54] and it was successfully used for the extraction of biogenic amines [55,56] from beverages, Yet, SALLE is rarely applied in food simulant analysis. For PAA enrichment from aqueous samples, cation exchange (CATEX) SPE [10,25,35,43,52] is reported to be the most efficient method. Achieving sufficient enrichment factors can be challenging though. Conventional elution solvent compositions in CATEX SPE are incompatible with RP-LC-MS. Dilution of the SPE eluent or the eluate can be a solution, but both result in lower enrichment factor. Solvent exchange can be another solution. However, some PAAs are volatile, thus evaporation can lead to analyte loss [13,46,52]. As a result of inevitable compromise during sample preparation, some PAAs (e.g. 2,4-diaminoanisole (DAANI) and 2,4-diaminotoluene (DATOL)) are usually reported to have recoveries below 50% [7,20,22,38,44,46,51]. Even standardized methods [44–47] accept 20–50% recoveries for some PAAs. DAANI, DATOL and 2M5NA are included in the list of REACH Regulation [30] yet at least one of them is usually omitted in the majority of published methods due to the above-mentioned difficulties. However, the list of targeted PAAs in compliance testing should not be based on analytical compromises. Considering the 22 PAAs of the REACH Regulation [30] along with ANL and *p*-toluidine (PTOL), the most common impurities of azo colorants and isocyanates, gives a notably more legitimately established list.

Our work aimed to enable compliance testing of FCMs with regard to PAA release. This called for an analytical method that could target all the above mentioned 24 PAAs with appropriate accuracy and precision, including the analytically challenging DAANI, DATOL and 2M5NA as well. Furthermore, both the commonly used 3% (w/v) acetic acid and 10% (v/v) ethanol, the general food simulant, were included as sample types. Since a lower individual limit for carcinogenic PAAs has been introduced [32] the majority of the previously published methods are no

longer up-to-date. For long-term applicability of the method achieving both individual and cumulative LOD values lower than 2 µgkg^{-1} was crucial.

2. Materials and methods

2.1. Chemicals and equipment

2.1.1. Analytical standards

All analytical grade standards were purchased from Sigma-Aldrich (St. Louis, Missouri, United States of America) except for 2,4,5-trimethylaniline (TMA) and 3,3′-dichlorobenzidine (DCLB), which were ordered from LGC Standards (Manchester, New Hampshire, United States of America). The target compounds (24 PAAs) with their CAS registry number; structure; IARC [27] CLP [28] and MAK [29] carcinogenic category and their entries in regulations and standards are listed in Tables 1.A and 1.B. Analytical grade isotopically labeled internal standards (I.S.): *p*-toluidine-d$_3$ (methyl-d$_3$, 98.4% isotopic purity, PTOLD3) and aniline-2,3,4,5,6-d$_5$ (98% isotopic purity, ANLD5) were purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada) and Sigma-Aldrich, respectively.

2.1.2. Other chemicals and equipment

Methanol, ethanol, acetonitrile and ultrapure water (MQ water) were used as solvents. Methanol and acetonitrile (OPTIGRADE) were purchased from LGC Standards. Absolute ethanol (EMSURE) was purchased from Merck KGaA (Darmstadt, Germany). MQ water was produced by a Milli-Q Direct 8 water purification system (Merck KGaA).

EMSURE grade formic acid (98–100%) and trisodium-citrate-dihydrate were purchased from Merck KGaA. NORMAPUR grade acetic acid (96%) and ammonia (25%) were obtained from VWR International (Radnor, Pennsylvania, United States of America).

Individual 1 mgmL^{-1} stock solutions of each target compound and I. S. were prepared. The mixed working solutions contained 24 PAAs at either 1 µgmL^{-1} or 2.5 µgmL^{-1} level, whereas the I.S. working solution had 2 µgmL^{-1} of both I.S. All solutions were prepared in acetonitrile and stored at 4 °C in amber glass bottles. Food simulants of 3% (w/v) acetic acid and 10% (v/v) ethanol were prepared by diluting 96% acetic acid and absolute ethanol with MQ water. The SPE eluent contained 0.35 molL^{-1} trisodium-citrate-dihydrate in 25% (v/v) acetonitrile.

ISOLUTE SCX-2 (500 mg/3mL, Biotage AB, Uppsala, Sweden) cartridges, a Supelco vacuum manifold and empty 60 mL SPE tubes (Sigma-Aldrich), 10 mL syringes and 8 mL amber vials were used for the SPE procedure. Centrifuge tubes of 50 mL were used for centrifugation.

2.1.3. Plastic kitchenware samples

Ten colored spatulas, 5 made of polyamide and 5 made of silicone, were purchased from local retail stores in Budapest, Hungary. The removable handles of the silicone spatulas were removed. The spatulas were cut into 4 cuboid-like test specimen pieces with an approximate dimension of 35 × 20 × 2 mm. Since most of the test specimens had irregular shapes, overall surface areas were estimated separately. Each side of every single test specimen was approximated with an appropriate geometrical shape and the relevant dimensions were measured with a digital caliper. The colors and estimated surface area values of the test specimens are summarized in the supplementary material (Table S.1).

2.2. Instruments

An IKA vortex mixer (IKA-Werke, Staufen, Germany) and a Hermle Z206 A centrifuge (HERMLE Labortechnik GmbH, Wehingen, Germany) were used during sample preparation.

Samples were measured on an Agilent 1200 LC system coupled with an Agilent 6460 triple quadrupole MS instrument (Agilent Technologies, Santa Clara, California, United States). The LC system consisted of a degasser, a binary pump, an autosampler and a column thermostat

module with a column switching valve. The MS instrument was equipped with an Agilent Jet Stream (AJS) ESI source.

2.2.1. LC conditions

An Acquity UPLC HSS T3 1.8 µm column (C18, 2.1 × 100 mm, Waters Corporation, Milford, Massachusetts, United States of America) connected to an Acquity UPLC HSS T3 1.8 µm VanGuard pre-column (C18, 2.1 × 5 mm, Waters Corporation) was applied for chromatographic separation. The column thermostat was operated at 40 °C. Eluents were MQ water: methanol 4:1 (v/v) (A) and 0.1% (v/v) formic acid in methanol (B). An injector program was optimized to enable a larger injection volume from a less polar solvent composition (40% (v/v) acetonitrile). The autosampler was programmed to wash the needle 10 times in acetonitrile and 5 times in MQ water. Then it drew 7 µL from the sample vial and 5 µL MQ water from a separate vial. The autosampler repeated this cycle 4 times and the program ended with a needle wash cycle before injection. As a result, 28 µL of the sample could be injected without peak distortion. The eluent flow rate was 0.25 mLmin^{-1}. The initial composition of the gradient elution was 100% A. Within 4 min it decreased to 80%, then to 0% in 2 min. This composition was held for 4 min. The post-time consisted of 26 min: 0% eluent A composition was maintained for 6 min, while 6 × 48 µL acetonitrile were injected to eliminate run-to-run cross contamination. In the remaining 20 min, the column was equilibrated with 100% A for the next run. In the last 6 min of equilibration, the autosampler carried out the injector program for the next injection. Retention times of each compound are listed in the supplementary material (Table S.2).

2.2.2. MS settings

The ESI source was operated in positive mode. 5.0 grade nitrogen (Messer Hungarogáz Kft., Budapest, Hungary) was used as drying, nebulizer and sheath gas. Drying gas temperature and flow rate were set to 250 °C and 12 Lmin^{-1}. The nebulizer gas pressure was set to 25 psi. Sheath gas temperature and flow rate were set to 350 °C and 7 Lmin^{-1}. Capillary and nozzle voltages were set to 5500 and 1500 V, respectively. Multiple reaction monitoring (MRM) data acquisition mode was applied in 2 time segments. The 1st started at the time of injection and the 2nd started at 6 min 15 s. Cell accelerator voltage was set to 4 V. MRM transitions are listed in the supplementary material (Table S.2).

2.3. Sample preparation

50 mL of food simulants were spiked with I.S. at 3 µgL^{-1}. The spiked 3% (w/v) acetic acid samples were ready to load, but to the 10% (v/v) ethanol samples 1.5 mL formic acid was added prior to SPE.

2.3.1. SPE

Cartridges were conditioned with 3 mL methanol. Equilibration was carried out with 3 mL of either 3% (w/v) acetic acid or 3% (v/v) formic acid in 10% (v/v) ethanol according to the sample to be applied. Then, 50 mL of spiked samples were loaded with approximately 1.5–2 mLmin^{-1} loading rate. Cartridges were washed with 3 mL MQ water after loading, then dried by pumping through 3 × 10 mL air with a syringe. PAAs were eluted with 2 × 2 mL SPE eluent (0.35 molL^{-1} trisodium-citrate-dihydrate in 25% (v/v) acetonitrile) into 8 mL amber vials.

2.3.2. SALLE

The first step of SALLE was to homogenize the SPE eluates by vortexing. Then approximately 3 g trisodium-citrate-dihydrate was added to the eluates and the vials were vortexed again. Samples were then centrifuged for 2 min at 1260 relative centrifugal force (RCF). In the case of insufficient phase separation, vortex mixing and centrifugation were repeated.

In order to inject a large volume to an RP column without excessive peak broadening from acetonitrile, the supernatant was diluted. To get

Table 1A
List of target compounds, their carcinogenic categorization and records in regulations and standards.

No.	Name	Structure	CAS	IARC[1]	CLP[2]	MAK[3]	REACH[4]	EN-71[5]	ISO 14362[6] ISO 17234[6]
1.	2,4-diaminoanisole (DAANI)		615–05-4	2B	1B	2	+	–	+
2.	2,4-diaminotoluene (DATOL)		95–80-7	2B	1B	2	+	–	+
3.	aniline (ANL)		62–53-3	3	2	4	–	+	–
4.	*p*-toluidine (PTOL)		106–49-0	n.i.[7]	2	3B	–	–	–
5.	4,4′-oxydianiline (ODIA)		101–80-4	2B	1B	2	+	–	+
6.	benzidine (BNZ)		92–87-5	1	1A	1	+	+	+
7.	*o*-anisidine (OANI)		90–04-0	2B	1B	2	+	+	+
8.	4,4′-diaminodiphenylmethane (DDPM)		101–77-9	2B	1B	2	+	–	+
9.	*o*-toluidine (OTOL)		95–53-4	2A	1B	1	+	+	+
10.	*p*-cresidine (PCRES)		120–71-8	2B	1B	2	+	–	+
11.	3,3′-dimethoxybenzidine (DMXB)		119–90-4	2B	1B	2	+	+	+
12.	3,3′-dimethylbenzidine (DMTB)		119–93-7	2B	1B	2	+	+	+
13.	3,3′-dimethyl-4,4′-diaminodiphenylmethane (DMAPM)		838–88-0	2B	1B	2	+	–	+
14.	4-chloroaniline (4CLA)		106–47-8	2B	1B	2	+	+	+
15.	2,4,5-trimethylaniline (TMA)		137–17-7	3	1B	2	+	–	+
16.	4,4′-thiodianiline (TDIA)		139–65-1	2B	1B	2	+	–	+
17.			91–59-8	1	1A	1	+	+	+

(continued on next page)

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Table 1A (*continued*)
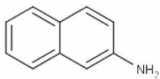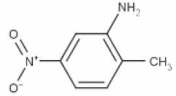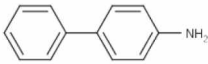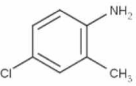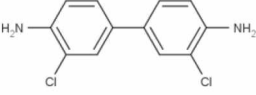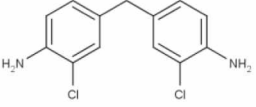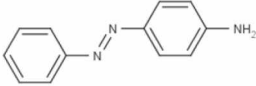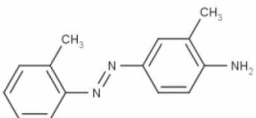
No.	Name	Structure	CAS	IARC[1]	CLP[2]	MAK[3]	REACH[4]	EN-71[5]	ISO 14362[6] ISO 17234[6]
	2-naphthylamine (NAP)								

Table 1B
List of target compounds, their carcinogenic categories and records in standards. (cont.)

No.	Name	Structure	CAS	IARC[1]	CLP[2]	MAK[3]	REACH[4]	EN-71[5]	ISO 14362[6] ISO 17234[6]
18.	2-methyl-5-nitroaniline (2M5NA)		99–55-8	3	2	2	+	–	–
19.	4-aminobiphenyl (4ABP)		92–67-1	1	1A	1	+	–	+
20.	4-chloro-*o*-toluidine (4CLOT)		95–69-2	2A	1B	1	+	–	+
21.	3,3′-dichlorobenzidine (DCLB)		91–94-1	2B	1B	2	+	+	+
22.	4,4′-diamino-3,3′-dichlorodiphenylmethane (MOCA)		101–14-4	1	1B	2	+	–	+
23.	4-aminoazobenzene (4AZB)		60–09-3	2B	1B	n.c.[8]	+	+	+
24.	*o*-aminoazotoluene (OAZT)		97–56-3	2B	1B	2	+	+	–

[1] Categorization of the International Agency for Research on Cancer (IARC). Group 1: carcinogenic to humans. Group 2A: probably carcinogenic to humans. Group 2B: possibly carcinogenic to humans. Group 3: not classifiable as to its carcinogenicity to humans [27].
[2] Categorization of the EU Regulation No. 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Category 1A: known human carcinogen based on human evidence. Category 1B: presumed human carcinogen based on animal evidence. Category 2: suspected human carcinogen [28].
[3] Categorization of the German MAK Commission. Category 1: substances that cause cancer in humans based on human epidemiological studies. Category 2: substances that are considered to be carcinogenic based on animal epidemiological studies. Category 3: substances with concern of being carcinogenic for humans without conclusive assessment due to lack of data. Category 4: non-genotoxic substances without expected contribution to human cancer risk. Category 5: genotoxic substances that contribute slightly to human cancer risk [29].
[4] EU Regulation No. 1904/2006 concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), Appendix 8: Entry 43 [30].
[5] EN-71 Toy Safety Standard series [48–50].
[6] Standard methods for the determination of primary aromatic amines derived from azo colorants in textile (ISO 14362) and leather (ISO 17234) products [44–47].
[7] Not included.
[8] Not categorized.

wider overall working range, two dilution procedures were applied according to the expected concentrations in the samples.

2.3.3. Dilution procedure No. 1

400 μL of supernatant was pipetted into an amber LC vial with 590 μL MQ water and 10 μL 25% ammonia solution. The sample was then homogenized. An enrichment factor of 20 was achieved with this dilution procedure.

2.3.4. Dilution procedure No. 2

80 μL of supernatant was pipetted into an amber LC vial with 320 μL acetonitrile, 590 μL MQ water and 10 μL 25% ammonia solution. The sample was then homogenized. This dilution procedure gives an enrichment factor of 4 considering the whole sample preparation process.

The sample preparation procedure is summarized in Fig. 1. The LC-MS/MS extracted ion chromatograms of each PAA are shown in Fig. 2.
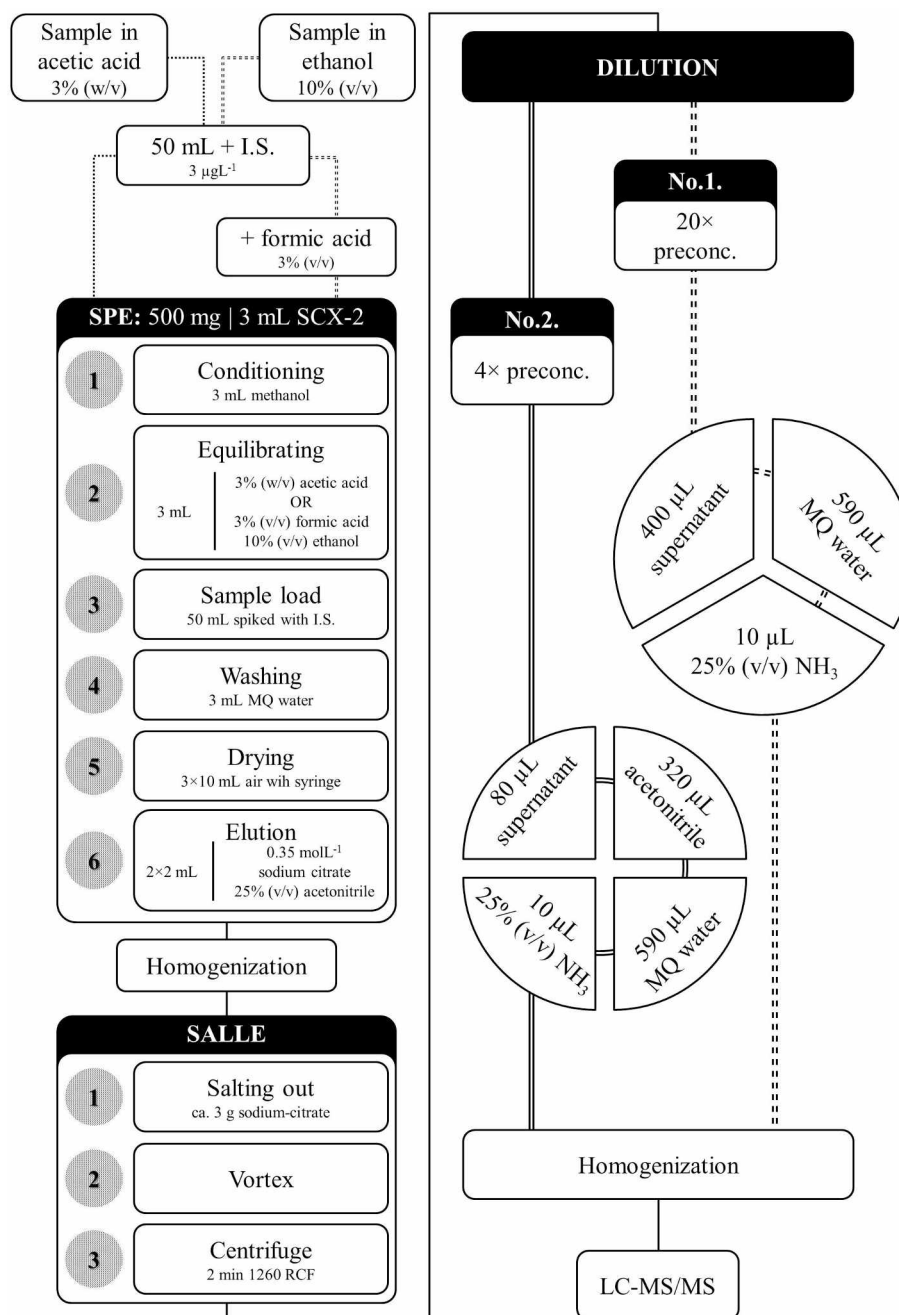
Fig. 1. Scheme of the sample preparation procedure.

2.4. Within-laboratory validation

A within-laboratory validation was performed based on an in-house protocol that suits the requirements of a daily routine analysis in a profit-oriented service laboratory. The number of replicate measurements was kept at the necessary minimum yet all possible pitfalls one might encounter were considered.

Freshly prepared 3% (w/v) acetic acid and 10% (v/v) ethanol food simulants were used as blanks and also spiked with PAAs at the concentration levels of 0.05, 0.15, 0.45, 2.5, 3.5, 10, 15 and 20 μgL^{-1}. Three replicate samples were prepared at each level including system blanks on 2 different days using SPE cartridges, acetic acid and absolute ethanol from 2 different batches. All samples were spiked with I.S. at 3 μgL^{-1} except for the system blanks. Dilution procedure No. 1 (see Section 2.3.3.) was applied for samples spiked between 0.05 and 3.5 μgL^{-1}. For

samples spiked between 3.5 and 20 μgL^{-1}, dilution procedure No. 2 (see Section 2.3.4.) was applied.

2.4.1. Selectivity

MRM detection usually provides enough selectivity, except for isobar compounds. There are 6 pairs among the target compounds with possible isobaric interferences in their MRM transitions. Since PAAs are ANL derivatives, they can produce similar fragment ions as ANL. Thus, gradient elution was optimized so that ANL would be separated with a baseline from other PAAs as well as the 6 isobar pairs from each other. To investigate how occasional peaks would influence PAA quantitation, system blanks of each food simulant and calibration blanks were compared with samples spiked at the level of LOD and the lowest calibration point, respectively. Six replicates were investigated for each type of sample.
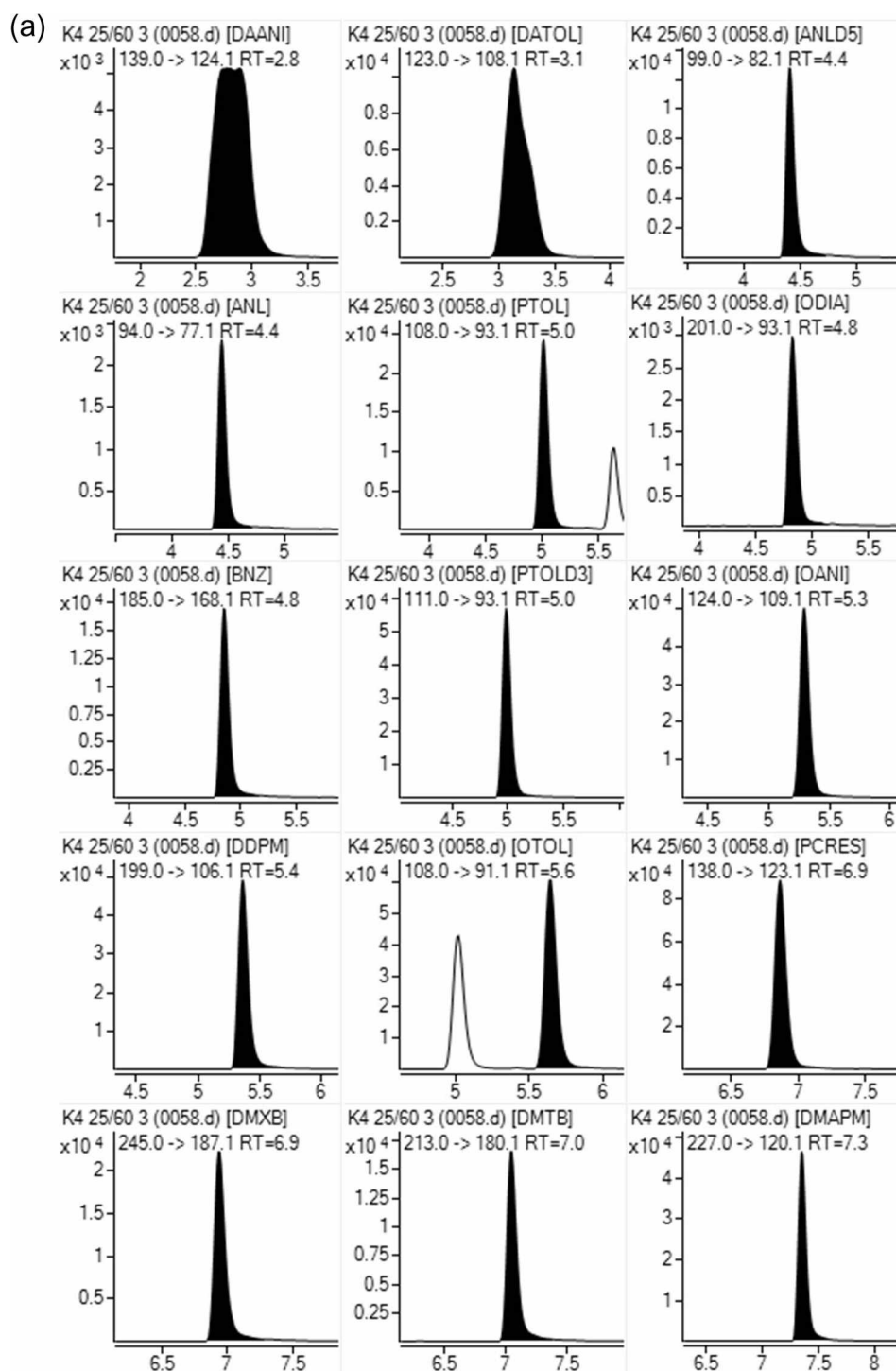
Fig. 2. (a and b) Extracted ion chromatograms of a 25 µgL^{-1} calibration solution containing 60 µgL^{-1} I.S. including quantifier MRM transitions and retention times (RT).

2.4.2. Calibration

Either a linear or a quadratic curve was fitted depending on the regression coefficient (R^2) and the accuracy of each calibration point. Calibration was divided into two ranges: one at 3–70 µgL^{-1} and the other at 10–100 µgL^{-1}, corresponding to dilution procedure No. 1 and No. 2 (in Sections 2.3.3 and 2.3.4), respectively. A set of 7 points was prepared in both ranges. The calibration points were 3, 6, 9, 25, 40, 55, 70 µgL^{-1} and 10, 25, 40, 60, 80, 100 µgL^{-1} in the lower and in the higher calibration range, respectively. I.S. calibration was applied with an I.S.

concentration of 60 and 12 µgL^{-1}. Calibration points had the same solvent composition as the prepared samples (40% (v/v) acetonitrile, 0.25% (v/v) ammonia). Each calibration point was measured in 3 replicates.

2.4.3. Accuracy

Although quality control material is available at FAPAS, it contains only 2 of the 24 PAAs. Therefore, accuracy was assessed by comparing the spiked concentration with the concentration calculated using the
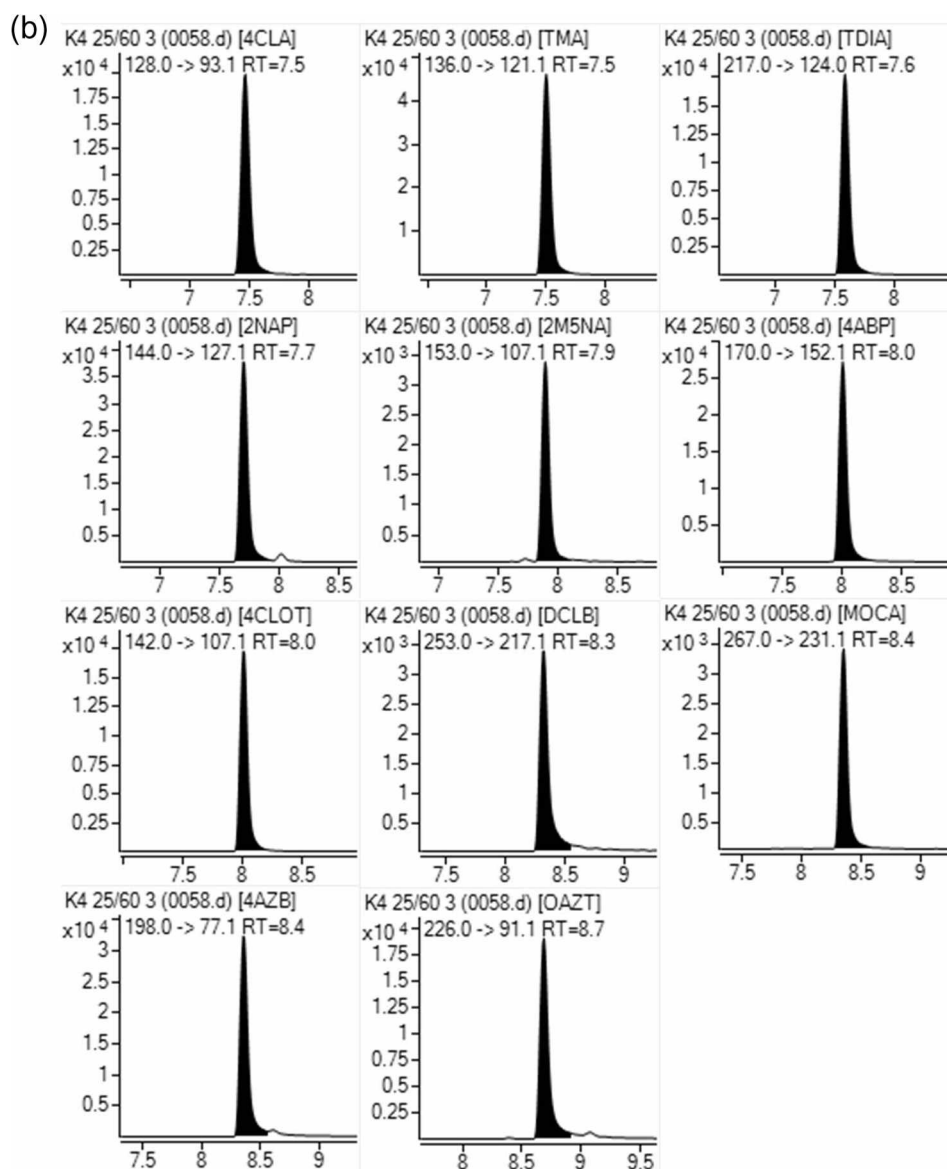
Fig. 2. (*continued*).

calibration curve. Data from 6 replicates were used for accuracy calculations. The total sample preparation recovery was also calculated from the same dataset using external standard calibration.

2.4.4. Precision

Within-day precision was determined as a measure of repeatability. Three replicate samples were prepared on the same day in the same laboratory by the same technician and measured on the same instrument by the same operator. This project is about a within-laboratory validation on a single instrument. Therefore, as a measure of reproducibility, between-day precision was determined with a total of 6 replicates. Thus, 3 further replicate samples were prepared on separate days in the same laboratory by the same technician using SPE cartridges, acetic acid and absolute ethanol of a different batch and measured on the same instrument by the same operator on separate days. Both within-day and between-day precision were expressed as the percentage of relative standard deviation (*RSD%*).

2.4.5. LOQ

The lowest level of spike was accepted as the LOQ where quantitation of samples could be performed with acceptable accuracy and precision.

2.4.6. LOD

Since LOD determination is just an estimation usually based on the calibration curve or signal to noise ratios, the 1/3 of LOQ was accepted as the LOD. In case of occasional interfering peaks, the lowest level of spike was accepted as the LOD where the analytical signal was surely caused by the presence of a target compound.

2.5. Migration test of kitchenware samples

A two-hour-long migration test was conducted at 70 °C according to the EU FCM guideline [35] with both 3% (w/v) acetic acid and 10% (v/v) ethanol. All the test specimens were rinsed with MQ water and dried before the migration test.

Pairs of test specimens of the same spatula were placed into 105 mL preheated portions of both food simulants measured into 250 mL glass bottles previously. The bottles were firmly sealed and were put in a water bath at 70 °C for 2 h. From the resulting extracts, 50 mL was prepared according to the procedure described in Section 2.3 applying dilution procedure No. 1 (see Section 2.3.3). Blank food simulants and

food simulants spiked at LOD were also prepared. The samples were analyzed in the lower calibration range first (Section 2.4.2). A calibration blank was also measured. Some of the prepared samples contained PAAs exceeding the lower calibration range. Those samples were analyzed again in the higher calibration range after a five times dilution with a diluting solvent containing 40% (v/v) acetonitrile and 0.25% (v/v) ammonia in MQ water.

For each pair of test specimens, a correction factor was calculated so that the results could be adjusted to the appropriate surface to volume ratio of 6 dm^2kg^{-1} food simulant required by the FCM Regulation [1]. These correction factors, specified as the quotient of the required and the applied surface to volume ratios, are listed in the supplementary material (Table S.1). Individual PAA concentrations were calculated in μgL^{-1} referring to the food simulants and considering the appropriate enrichment factors. The adjusted PAA concentrations in μgkg^{-1} were originated from the sum of PAAs multiplied by the correction factor and considering the densities of the food simulants as 1 kgL^{-1}.

3. Results

3.1. Selectivity

Several peaks appeared in the chromatograms of the system blanks of food simulants. Most of these peaks were negligible compared to samples spiked at the LOD, except for ANL and ODIA. Assuming that those peaks had a plastic origin, the sample preparation procedure was repeated using distilled water instead of MQ water and the plastic SPE cartridges were changed to glass. Still, the peaks appearing in the blanks could not be eliminated. The source of these peaks could be the colored plastic caps of the absolute ethanol and acetic acid bottles. ODIA peaks also appeared in calibration blanks but did not appear in MQ water or acetonitrile reagent blanks. Ammonia was suspected to be the source of ODIA peaks in calibration blanks but changing to LC-MS grade ammonia did not solve the issue. ODIA peak areas of the calibration blanks decreased dramatically during the first 3 injections and then became constant in every sequence. It is possible that some plastic accessories of the LC system are responsible for these peaks. Since these sources of interference could not be eliminated, it was concluded that method selectivity had to be evaluated in every single sequence as a part of quality assurance. To set passing criteria, the greatest peak area of the system blank was compared with the smallest peak area of the sample spiked at LOD level sequence by sequence. In the case of calibration blanks, the blank peak areas of 2 injections were compared individually with the lowest peak area of the lowest calibration point sequence by sequence. To check the appropriateness of this approach and to determine the acceptance limits, data were acquired in 4 separate sequences on 4 separate days. Peak area limits were then set arbitrarily based on the noise level of MRM transitions and the integrator algorithm of the data evaluation software. A sequence was accepted if peak areas of system blanks were lower than 15% of the peak areas of LOD samples. Due to the frequent occurrence of ANL, ODIA, 2M5NA, MOCA and OAZT in system blanks, the limit for these compounds was elevated to 65%. Similarly, the peak areas of the calibration blanks must be lower than 15% of the peak areas of the lowest calibration point. In the case of ODIA, calibration blank peak areas must be lower than 50%. During validation these quality assurance criteria proved to be adequate to avoid false positives at LOD level.

3.2. Calibration

ANLD5 and PTOLD3 were chosen as the I.S. of ANL and the rest of the PAAs, respectively. All calibration points were used for curve fitting for all PAAs, except for ODIA. The lower calibration range of ODIA was between 9 and 70 μgL^{-1}. A linear calibration curve was fitted without any weighting in both calibration ranges for most PAAs. A quadratic calibration curve was fitted only in the high calibration range for

DAANI, DCLB, MOCA and OAZT. A quadratic calibration curve was fitted in both calibration ranges for DATOL, ODIA, BNZ, PCRES and 2M5NA. All fitted curves had an R^2 value above 0.99. I.S. assignation, calibration curve types and R^2 values are summarized in the supplementary material (Table S.2). Accuracy values of all calibration points were between 90 and 110% except for the lowest calibration points. Those occasionally had accuracy values between 70 and 90% and 110–130%.

3.3. Accuracy and precision

For the 3% (w/v) acetic acid food simulant, accuracy values were between 80 and 118% for the majority of PAAs. However, the accuracy values of DAANI and DATOL were between 62 and 98% and 50–130%, respectively. For the 10% (v/v) ethanol food simulant, accuracy values were between 70 and 122% for the majority of PAAs. For DAANI and ANL, accuracy values were between 64 and 103% and 113–131%, respectively. Both within-day and between-day precision values were below 20% for all compounds in both food simulants. Accuracy and precision values are summarized in Tables 2.A and 2.B. The total sample preparation recovery values were generally higher than 100% with an average of 128%. This is not unexpected at all since both SALLE and the final dilution procedure can be a source of systematic volumetric errors. These volumetric errors are uncorrected in the case of an external standard calibration and easily lead to apparently high recoveries. Since the recoveries of I.S. followed the same pattern, the method was found to be accurate.

3.4. LOQ and LOD

The majority of PAAs from both food simulants had satisfactory accuracy and precision values at the level of 0.15 μgL^{-1}, therefore it was accepted as the LOQ. For these compounds, 0.05 μgL^{-1} was accepted as the LOD. The only exception in the case of the 3% (w/v) acetic acid food simulant was ODIA. Due to the peaks present in the blank samples, 0.45 μgL^{-1} was chosen as LOD for ODIA from the 3% (w/v) acetic acid food simulant to minimize the occurrence of false positive results. The LOQ for ODIA was accepted to be 2.5 μgL^{-1}. The 10% (v/v) ethanol food simulant had 3 exceptions: DATOL, ANL and ODIA had satisfactory accuracy and precision values at 0.45 μgL^{-1}, therefore 0.45 μgL^{-1} was accepted as their LOQ. For these compounds, 0.15 μgL^{-1} was accepted as the LOD.

3.5. Working range

This method is designed to determine whether a sample exceeds the limits either for the sum of PAAs or for any individual PAA. Since most samples are expected to be either negative or contain PAAs around LOQ and LOD, calibration in a wide range in every sequence is a waste of resources. Therefore, the working range of LOQ-25 μgL^{-1} was divided into 2 sections. The lower working range of LOQ-3.5 μgL^{-1} is sufficient for daily routine analysis. In this range, samples are prepared according to Dilution procedure No. 1 (Section 2.3.3.). This gives an overall enrichment factor of 20 and the calibration is prepared in the range of 3–70 μgL^{-1} with an I.S. concentration of 60 μgL^{-1}. In case any individual PAA content is found to exceed this range, it is not necessary to start the sample preparation procedure over again. Instead, these concentrated samples can be analyzed again after a 5 times dilution (as if Dilution procedure No. 2, according to Section 2.3.4, would have been applied). In this case, the overall enrichment factor is 4. Since the I.S. content of the sample is diluted, the calibration is prepared in the range of 10–100 μgL^{-1} with an I.S. concentration of 12 μgL^{-1}. Thus, the working range of the method can be easily extended for all PAAs above the first section's 3.5 μgL^{-1} upper limit without compromising the accuracy of the measurements at low concentrations.

Table 2A
Validation results of 3% (w/v) acetic acid food simulant.

Name	20×[a]														4×[a]												
	50 ngL⁻¹	0.15 µgL⁻¹ LOQ*			0.45 µgL⁻¹			2.5 µgL⁻¹			3.5 µgL⁻¹			3.5 µgL⁻¹			10 µgL⁻¹			15 µgL⁻¹			20 µgL⁻¹		
		A%[b]	RSD%		A%[b]	RSD%		A%[b]	RSD%		A%[b]	RSD%		A%[b]	RSD%		A%[b]	RSD%		A%[b]	RSD%		A%[b]	RSD%	
			WD[c]	BD[d]		WD[c]	BD[d]		WD[c]	BD[d]		WD[c]	BD[d]		WD[c]	BD[d]		WD[c]	BD[d]		WD[c]	BD[d]		WD[c]	BD[d]
DAANI	LOD	68	2.3	8.6	62	11.3	12.0	68	3.8	5.1	69	3.3	5.9	95	7.4	8.1	98	3.9	6.5	100	5.1	3.9	98	8.1	6.0
DATOL	LOD	51	2.2	12.7	50	9.0	12.1	57	4.4	7.1	61	4.6	5.5	84	3.5	3.6	110	7.6	5.6	118	4.9	8.1	130	3.7	16.2
ANL	LOD	106	2.3	9.5	98	1.2	2.9	99	1.0	0.8	96	2.3	2.5	105	1.9	2.0	104	2.3	2.8	107	1.3	3.4	109	2.7	2.3
PTOL	LOD	99	1.9	1.8	111	2.2	12.7	100	0.3	0.4	110	0.7	9.8	98	0.4	3.0	101	1.2	1.5	101	0.4	2.3	101	0.1	2.5
ODIA*	–	–	–	–	LOD			96	1.0	1.7	99	5.6	6.4	98	2.2	2.4	108	1.0	5.0	110	1.1	3.5	111	6.1	5.5
BNZ	LOD	86	0.5	6.1	86	2.1	3.8	95	3.4	3.4	94	6.1	5.5	101	1.7	5.3	106	0.5	2.9	108	1.3	2.4	111	5.0	3.8
OANI	LOD	85	2.3	4.0	102	0.8	2.0	100	1.3	1.6	101	1.7	1.2	94	0.7	2.8	103	0.9	4.4	102	0.4	5.0	100	2.2	4.6
DDPM	LOD	83	2.6	3.1	93	0.9	1.2	98	0.9	1.5	96	3.5	3.1	97	1.1	4.7	104	1.8	4.0	106	0.6	3.2	105	8.0	5.9
OTOL	LOD	90	0.9	8.4	93	1.0	2.7	97	2.6	2.6	97	7.0	4.6	93	1.3	4.0	101	1.5	7.2	99	0.7	7.9	97	1.7	8.2
PCRES	LOD	97	0.8	2.2	103	0.2	2.0	97	1.6	1.6	100	0.9	2.4	99	1.5	1.9	100	1.2	2.2	99	0.6	1.4	99	2.3	1.9
DMXB	LOD	84	6.1	7.6	89	1.6	3.2	100	4.5	6.1	96	7.0	5.5	107	1.9	9.6	102	1.5	5.9	105	3.1	5.4	106	9.0	7.9
DMTB	LOD	81	1.5	4.6	100	0.9	12.0	104	5.8	5.9	104	4.0	10.2	107	1.0	5.3	105	1.8	4.2	107	2.8	3.8	107	5.6	4.9
DMAPM	LOD	91	0.6	8.9	99	0.7	3.0	103	2.7	2.8	100	6.6	4.5	108	2.2	5.5	104	2.6	4.8	106	2.2	5.2	106	9.4	7.5
4CLA	LOD	92	1.2	7.4	109	0.7	5.5	100	2.1	1.5	106	1.6	5.9	97	2.1	1.7	101	2.2	2.1	100	0.4	1.5	100	2.8	2.5
TMA	LOD	80	0.7	5.6	97	0.7	4.4	101	1.0	0.9	96	1.4	4.6	101	1.7	2.1	103	2.1	2.8	102	1.0	2.1	101	3.8	3.2
TDIA	LOD	102	3.4	3.0	104	0.8	3.3	104	1.1	2.4	104	5.0	3.3	118	1.0	1.0	106	2.1	3.4	107	2.3	3.5	108	6.5	5.5
2NAP	LOD	104	0.6	4.3	101	0.3	3.5	101	1.7	1.3	100	1.0	3.6	108	1.3	1.2	104	2.3	2.2	104	0.5	1.9	104	3.5	2.7
2M5NA	LOD	106	8.5	6.7	106	6.8	6.3	106	3.7	3.3	105	2.3	6.6	112	3.4	4.3	109	1.9	2.3	107	1.7	2.9	105	3.9	3.1
4ABP	LOD	109	1.8	4.3	103	1.2	3.3	102	1.7	1.5	101	1.7	3.5	113	1.2	1.1	104	1.3	3.0	104	0.8	3.0	103	6.9	5.1
4CLOT	LOD	101	2.7	3.6	104	0.5	3.6	101	1.4	1.4	102	0.9	4.0	108	0.7	0.9	102	1.6	4.3	102	0.8	3.9	100	6.5	5.0
DCLB	LOD	90	3.7	5.5	90	1.6	4.1	95	2.3	1.7	93	2.4	3.3	113	4.2	5.5	101	2.1	8.5	102	0.3	6.1	97	15.4	11.3
MOCA	LOD	93	2.3	3.3	90	1.6	1.5	98	2.8	2.5	91	1.1	0.9	113	2.0	4.6	102	2.2	7.2	101	1.7	4.7	97	15.9	11.7
4AZB	LOD	102	1.9	2.8	100	0.4	8.6	97	1.7	1.4	100	1.1	8.6	107	2.0	4.0	96	0.5	7.6	96	0.8	6.5	94	13.0	10.8
OAZT	LOD	105	6.9	6.3	93	1.5	4.0	91	1.2	0.9	93	1.8	4.4	108	1.8	2.8	93	1.5	13.1	95	2.1	10.7	90	14.3	14.6

[a] Erichment factor.
[b] Method accuracy, calculated from 6 replicates.
[c] Within-day precision, calculated from 3 replicates.
[d] Between-day precision, calculated from 6 replicates.
* LOQ of ODIA is 2.5 µgL⁻¹.

Table 2B
Validation results of 10% (v/v) ethanol food simulant.

Name	20×ᵃ 50 ngL⁻¹	0.15 µgL⁻¹ LOQ*			0.45 µgL⁻¹			2.5 µgL⁻¹			3.5 µgL⁻¹			4×ᵃ 3.5 µgL⁻¹			10 µgL⁻¹			15 µgL⁻¹**			20 µgL⁻¹		
		A%[b]	RSD% WD[c]	BD[d]	A%[b]	RSD% WD[c]	BD[d]	A%[b]	RSD% WD[c]	BD[d]	A%[b]	RSD% WD[c]	BD[d]	A%[b]	RSD% WD[c]	BD[d]	A%[b]	RSD% WD[c]	BD[d]	A%[b]	RSD% WD[c]	BD[d]	A%[b]	RSD% WD[c]	BD[d]
DAANI	LOD	65	6.6	12.2	64	5.0	9.1	79	2.5	4.6	69	3.7	18.3	85	9.1	18.9	90	8.6	19.4	100	2.6	19.1	103	9.4	18.2
DATOL*	–	LOD			76	2.0	11.9	88	4.7	14.3	84	12.1	10.4	86	1.9	6.2	98	8.1	13.9	101	0.7	9.1	122	7.6	18.3
ANL*	–	LOD			131	2.7	9.3	123	1.8	10.7	129	4.9	11.0	118	4.4	9.6	115	1.8	10.1	113	2.1	13.2	125	1.5	14.1
PTOL	LOD	95	1.6	9.9	100	0.8	2.5	100	1.1	1.0	101	0.6	1.3	102	0.8	1.5	102	0.8	1.2	102	0.1	1.1	102	0.5	0.8
ODIA*	–	LOD			88	1.8	2.2	99	2.4	6.7	106	1.8	4.9	101	1.7	2.7	104	6.1	5.1	108	1.5	3.7	108	1.9	4.5
BNZ	LOD	91	4.6	5.1	87	0.8	6.9	95	1.5	7.6	103	2.1	4.9	99	0.5	6.6	99	6.8	8.6	104	0.8	7.8	101	1.2	8.9
OANI	LOD	86	1.7	1.1	104	0.6	0.5	103	0.1	0.9	102	0.6	1.3	98	0.6	3.0	105	1.7	2.7	103	0.3	4.3	102	0.9	3.4
DDPM	LOD	70	0.5	11.1	89	1.9	1.7	96	1.0	2.0	96	1.8	1.9	94	1.8	14.9	101	6.0	7.3	105	1.1	6.0	101	1.3	6.7
OTOL	LOD	98	1.7	10.0	103	2.5	1.7	103	1.1	2.7	104	2.3	4.7	98	1.5	7.6	105	6.2	6.5	100	0.1	10.9	103	1.0	9.9
PCRES	LOD	96	1.1	1.0	98	1.2	1.1	96	1.4	1.5	98	2.1	4.0	100	0.6	2.0	102	2.3	2.2	102	0.4	1.6	100	0.9	1.9
DMXB	LOD	92	5.2	6.3	85	1.2	2.3	92	0.7	2.3	94	2.2	3.1	99	3.3	14.7	98	7.2	11.5	105	0.9	13.5	102	0.9	14.2
DMTB	LOD	85	1.2	16.9	90	1.1	5.7	95	0.7	1.5	95	1.8	2.9	103	0.2	10.9	101	4.8	8.6	106	0.8	10.0	103	1.5	10.6
DMAPM	LOD	86	2.7	3.4	92	0.9	2.9	97	0.8	1.5	96	1.8	2.0	100	0.4	14.8	98	4.6	10.0	102	0.5	10.8	97	3.0	12.8
4CLA	LOD	94	1.3	12.6	102	1.6	3.3	100	1.0	0.9	100	2.1	3.5	105	0.3	2.2	104	1.8	2.2	106	0.5	1.4	103	1.2	1.7
TMA	LOD	83	2.7	12.3	99	0.9	2.1	98	1.5	1.5	98	0.5	2.7	101	1.2	3.5	104	2.4	3.4	106	0.3	2.8	103	1.1	3.0
TDIA	LOD	105	1.4	3.1	98	0.2	1.9	97	1.3	1.7	97	0.6	1.0	111	0.9	5.8	103	4.7	5.7	106	0.8	6.3	104	1.8	7.0
2NAP	LOD	101	2.8	4.6	99	1.8	1.9	97	0.8	2.1	100	1.4	2.7	109	1.0	2.9	105	1.6	3.9	108	0.5	3.6	107	1.0	4.8
2M5NA	LOD	102	3.0	6.9	107	2.2	1.6	103	2.9	2.7	106	0.3	4.7	106	4.3	3.8	110	3.5	4.2	112	1.0	5.5	110	3.4	5.7
4ABP	LOD	103	0.8	4.1	101	1.4	1.6	99	0.4	1.2	102	0.6	4.2	107	0.7	6.6	104	5.3	8.3	106	0.4	8.3	104	2.0	10.0
4CLOT	LOD	97	1.6	7.9	103	1.3	1.1	100	1.3	2.0	103	0.7	5.3	110	0.3	2.1	107	3.2	5.5	111	0.7	5.1	109	1.9	6.2
DCLB	LOD	104	3.8	7.0	95	5.4	6.2	94	2.7	2.0	97	0.9	4.1	105	2.4	2.1	107	3.9	9.5	108	1.5	9.2	104	2.5	11.1
MOCA	LOD	103	6.1	6.6	100	4.2	4.0	96	0.7	1.4	99	0.8	5.5	104	2.0	4.8	101	3.5	10.2	104	0.5	10.1	102	1.6	13.3
4AZB	LOD	97	2.2	2.2	90	2.6	2.4	90	0.3	1.5	93	0.2	4.1	104	0.6	2.7	97	4.8	5.5	100	1.7	5.9	99	1.4	6.5
OAZT	LOD	118	7.4	5.8	99	2.1	3.1	93	1.5	3.1	99	1.5	6.4	104	1.6	2.4	101	3.6	7.4	105	1.1	5.3	100	0.8	5.0

[a]Enrichment factor.
[b]Method accuracy, calculated from 6 replicates.
[c]Within-day precision, calculated from 3 replicates.
[d]Between-day precision, calculated from 6 replicates.
*LOQ of DATOL, ANL and ODIA is 0.45 µgL⁻¹.
**Accuracy and between-day precision calculated from 5 replicates.

3.6. Kitchenware migration test results

The migration test results of kitchenware samples are summarized in Table 3. All of the analyzed spatula samples were found to release some PAAs. ANL could be identified in all samples and DDPM was the second most common PAA to be found. DATOL, BNZ, PTOL, OTOL, 4CLA, 2NAP and 4CLOT could also be detected occasionally. The migration of these PAAs from polyamide and silicone kitchen utensils is well-known from previously published studies [15,18–20,26]. Three injected samples originating from black polyamide spatulas contained DATOL, ANL and DDPM in such large amounts that the lower calibration range was exceeded. Therefore, these samples were diluted 5 times and analyzed again.

3.7. Discussion

Our within laboratory validation and the testing of kitchenware samples have proven that the proposed method is appropriate for measuring 24 PAAs from food simulants 3% (w/v) acetic acid and 10% (v/v) ethanol. During method development, volumetric errors due to SALLE and the repeated occurrence of low level contamination in the blanks were identified as limitations of our method. This means that the use of I.S. is inevitable to ensure acceptable accuracy. Also, monitoring of blanks must be included in every batch as a part of the quality

assurance. These measures proved to be adequate to ensure acceptable accuracy and to avoid false positives.

The analytical features of our and previously published relevant methods are summarized in Table 4. Comparing these methods is not simple due to the different approaches applied to determine the analytical performance characteristics. The list of PAAs covered by a certain method is, however, always clear. Although some methods can determine more PAAs [22,38] only ours covers the 22 PAAs of the REACH Regulation [30] from food simulants. To achieve this, we had to overcome the challenge of determining DAANI, DATOL and 2M5NA together. 2M5NA was previously reported as not giving a signal in ESI-MS [24,25,38]. Our method could successfully determine it with 102–112% accuracy and a precision (both within-day and between-day) lower than 10% from both food simulants. The most probable reason for this is that we did not use ion pairing agent in our eluents. Furthermore, we also achieved improved accuracy for DAANI and DATOL compared to previously published methods [7,38]. Since these compounds are volatile, not only solvent evaporation but also vacuum drying of SPE cartridges was avoided during the preconcentration procedure. These compounds also lack stability at low pH; therefore, their contact time with acidic medium was minimized and amber vials were used to protect them from light.

Beside our method, two others are also capable of PAA determination from multiple food simulants [24,38] but Burch and Cooper determined

Table 3
Kitchenware migration test results of detectable PAAs.

Sample ID	3% (w/v) acetic acid			10% (v/v) ethanol		
	Individual PAAs [a]	$\sum$ PAAs [a] (μgL^{-1})	$\sum_{adj.}$ PAAs [b] (μgkg^{-1})	Individual PAAs [a]	$\sum$ PAAs [a] (μgL^{-1})	$\sum_{adj.}$ PAAs [b] (μgkg^{-1})
G-PA-SP-A	0.47 μgL^{-1} ANL 0.61 μgL^{-1} DDPM	1.09	2.03	<LOQ ANL 0.73 μgL^{-1} DDPM	0.73	1.39
G-PA-SP-B	0.42 μgL^{-1} ANL 0.76 μg^{-1} DDPM	1.19	2.24	<LOQ ANL 1.02 μgL^{-1} DDPM	1.02	1.84
B-PA-SP-C	0.43 μgL^{-1} ANL	0.43	0.70	<LOQ ANL 0.20 μgL^{-1} DDPM	0.20	0.33
B-PA-SP-D*	20.95 μgL^{-1} DATOL 25< μgL^{-1} ANL** 0.82 μgL^{-1} BNZ 0.65 μgL^{-1} PTOL 25< μgL^{-1} DDPM** 0.26 μgL^{-1} 4CLA 0.19 μgL^{-1} 2NAP < LOQ 4CLOT	22.87≪	37.02≪	<LOQ ANL 0.24 μgL^{-1} DDPM	0.24	0.39
B-PA-SP-E*	20.37 μgL^{-1} DATOL 25< μgL^{-1} ANL** 0.93 μgL^{-1} BNZ 0.65 μgL^{-1} PTOL 25< μgL^{-1} DDPM** 0.23 μgL^{-1} 4CLA 0.18 μgL^{-1} 2NAP < LOQ 4CLOT	22.36≪	35.01≪	18.47 μgL^{-1} DATOL 25< μgL^{-1} ANL** 0.67 μgL^{-1} BNZ 0.51 μgL^{-1} PTOL 25< μgL^{-1} DDPM** 0.22 μgL^{-1} OTOL 0.20 μgL^{-1} 4CLA 0.23 μgL^{-1} 2NAP 0.24 μgL^{-1} 4CLOT	20.54≪	34.00≪
B-SIL-SP-A	0.69 μgL^{-1} ANL < LOQ DDPM	0.69	1.01	0.69 μgL^{-1} ANL < LOQ DDPM	0.69	0.90
W-SIL-SP-B	1.34 μgL^{-1} ANL < LOQ DDPM	1.34	1.81	0.93 μgL^{-1} ANL	0.93	1.11
P-SIL-SP-C	1.54 μgL^{-1} ANL < LOQ PTOL <LOQ DDPM < LOQ OTOL	1.54	2.09	1.39 μgL^{-1} ANL < LOQ PTOL < LOQ DDPM < LOQ OTOL	1.39	1.68
R-SIL-SP-D	1.21 μgL^{-1} ANL 0.16 μgL^{-1} PTOL < LOQ DDPM 0.15 μgL^{-1} 4CLOT	1.52	1.66	0.64 μgL^{-1} ANL < LOQ PTOL < LOQ DDPM < LOQ 4CLOT	0.64	0.66
G-SIL-SP-E	1.09 μgL^{-1} ANL < LOQ DDPM	1.09	1.22	0.69 μgL^{-1} ANL	0.69	1.09

[a] Referring to the migrate.
[b] PAA content of the migrate adjusted to the 6 dm^2kg^{-1} surface to volume ratio by the correction factors from Table S.1. The densities of both food simulants were considered as 1 kgL^{-1}.
* Samples needed to be diluted to analyze again.
** Out of working range.

Table 4

Comparison of analytical features with previously pubished LC-MS/MS methods suitable for PAA determination from food simulants.

No. PAAs / REACH PAAs[a]	Food simulant	LOD[b] (µgL^{-1})	Σ LOD[c] (µgL^{-1})	LOQ[b] (µgL^{-1})	Accuracy or Trueness (%)	Repeatability (RSD%)[d]	Reproducibility (RSD%)[d]	Spike levels (µgL^{-1})	DAANI	DATOL	2M5NA	Reference
24/22	3% (w/v) acetic acid	0.05 (LOQ/3) 0.45 (blank)	1.6	0.15 and 2.5 (accuracy & precision)	50-130 (with I.S.)	0.1-15.9 (n=3)	0.4-16.2 (n=6)	0.05, 0.15, 0.45, 2.5, 3.5, 10, 15, 20	68% accuracy (2.5 µgL^{-1})	57% accuracy (2.5 µgL^{-1})	106% accuracy (2.5 µgL^{-1})	Our method
	10% (v/v) ethanol	0.05 (LOQ/3) 0.15 (blank)	1.5	0.15 and 0.45 (accuracy & precision)	64-131 (with I.S.)	0.1-12.1 (n=3)	0.8-19.4 (n=6)		79% accuracy (2.5 µgL^{-1})	88% accuracy (2.5 µgL^{-1})	103% accuracy (2.5 µgL^{-1})	
22/21	3% (w/v) acetic acid	0.06–5.27 (Eurachem)[e]	23.94	0.08–5.45 (Eurachem)[e]	71-131 (with I.S.)	≤10 (n=4)	≤17 (n=12)	0.75, 7, 120	n.a.[g]	71% trueness (7 µgL^{-1})	72% trueness (7 µgL^{-1})	[8]
22/21	3% (w/v) acetic acid	0.1–1 (S/N=3)[h]	6.3	0.1–3.6 (S/N=10)[h]	86.8-142.3 (with I.S.)	0.8-33.5 (n=3)	6.0-8.9* (n=3)	3.75, 8.75	86.8% trueness (3.75 µgL^{-1})	118.3% trueness (3.75 µgL^{-1})	n.a.[g]	[9]
18/13	3% (w/v) acetic acid	0.002-0.013 (n.i.[f])	0.109	0.007–0.042 (n.i.[f])	n.i.[f]	0.8-83.6 (n.i.[f])	n.i.[f]	n.i.[f]	n.i.[f]	n.i.[f]	n.a.[g]	[10]
36/16	cold water extract**	0.03–1.38 (IUPAC[i])	14.75	0.08–4.60 (IUPAC[i])	n.i.[f]	2.3-15.0 (n=10)	2.9-18.5 (n=30)	5, 10, 50	52.3% recovery (mean)	60.5% recovery (mean)	85.1% recovery (mean)	[22]
20/14	3% (w/v) acetic acid	0.28–3 (DANAK[j])	19.32	n.i.[f]	In terms of recovery and precision	3.9-19 (n=6)	12 (mean) (n=7)	2, 10, 25	ca. 86% recovery (2 µgL^{-1})	ca. 96% recovery (2 µgL^{-1})	n.a.[g]	[24]
	water***	0.27–1 (DANAK[j])	12.57						n.i.[f]	n.i.[f]		
22/15	3% (w/v) acetic acid	0.02–2.4 (n.i.[f])	6.61	n.i.[f]	n.i.[f]	n.i.[f]	4.5-13.4 (n=3)	30	87% recovery (30 µgL^{-1})	93% recovery (30 µgL^{-1})	n.a.[g]	[25]
30/20	water***	0.03–2.54[k] (3×RMS[l] noise)	11.99[k]	1–10 (lowest robust calibrant)	n.i.[f]	2.9-280 (n=8)	n.i.[f]	2, 10	24.3% recovery (2 µgL^{-1})	9.4% recovery (2 µgL^{-1})	n.a.[g]	[38]
	3% (w/v) acetic acid					3.8-282.2 (n=8)			38.9% recovery (2 µgL^{-1})	61.6% recovery (2 µgL^{-1})		
	10% (v/v) ethanol					3.9-145.2 (n=8)			4% recovery (2 µgL^{-1})	7.4% recovery (2 µgL^{-1})		
	olive oil					4.8-143.0 (n=8)			15.1% recovery (2 µgL^{-1})	28.5% recovery (2 µgL^{-1})		
8/4	3% (w/v) acetic acid	0.5–1.0 (S/N=3)[h]	4.5	n.i.[f]	In terms of recovery and precision	n.i.[f]	5.6-21.4 (n is obscure)	2, 5, 10, 20	n.a.[g]	obscure	n.a.[g]	[40]

[a]Number of determined PAAs / PAAs determined out of the 22 listed in REACH Regulation [30].

[b]Method of LOD and LOQ determination is given in parentheses.

[c]Cumulative LOD.

[d]Number of replicates is given in parentheses marked with *n*.

[e]Based on Eurachem validation guideline.

[f]No information.

[g]Not analyzed.

[h]Signal-to-noise ratio. [i] Based on the validation guideline of International Union of Pure and Applied Chemistry (IUPAC).

[i]Based on the guideline of the Danish national accreditation body (DANAK).

[k]Instrument LOD.

[l]Root-mean-square of the baseline.

* Determined only in case of 3 PAAs with FAPAS proficiency testing.

** Not a food simulant according to the FCM Regulation [1] but used in standards for migration testing from paper napkins.

*** Withdrawn food simulant included in previous versions of the FCM Regulation [1].

the LOD without the matrix [38] whereas in the other case one of the simulants is no longer relevant [24] due to changes in regulation.

The greatest advantage of our method is the extremely low cumulative LOD. Half of the compared methods [8,22,24,38] are not even compliant with the latest FCM regulation [1] anymore. One method with lower cumulative LOD was found [10] however this method is not completely characterized and covers fewer PAAs. Also, our method is compliant with the latest amendment [32] of the FCM Regulation [1] in terms of all individual LOD values. This could be achieved through an enrichment factor of 20, which is a result of changing the common CATEX SPE eluent composition of 5% ammonia in methanol [10,25,52] to 0.35 $molL^{-1}$ trisodium-citrate-dihydrate in 25% (v/v) acetonitrile. The previous eluent operates by deprotonating PAAs bound to the CATEX phase. Our SPE eluent provides not only alkaline medium but high concentration (1.05 $molL^{-1}$) of competitive sodium ions as well to promote elution. Moreover, its acetonitrile content not only makes it a stronger eluent for less polar PAAs, but also provides a possibility for further clean up by SALLE.

The within-laboratory validation process proved our method's suitability for daily routine analysis. It has been successfully applied for migration testing of kitchenware samples. Most samples (8 out of 10) were compliant with not only the current overall migration limit of 10 μgkg^{-1}, but with the new individual limit of 2 μgkg^{-1}, too. However, 2 samples released ten times more DATOL than the new individual migration limit. Additionally, neither ANL nor DDPM could be quantified in these samples as both of their amounts exceeded the upper limit of the working range. These results show that despite the strict legislation and continuous compliance testing, problematic products can still be found in the retail market within the EU.

Comparison of the adjusted migration test results of the same spatulas in different food simulants shows that slightly more PAAs were released into 3% (w/v) acetic acid than into 10% (v/v) ethanol. This could verify that the worst-case scenario in case of a two-hour-long migration test carried out at 70 °C is represented with 3% (w/v) acetic acid food simulant. However, these differences may also be the result of the inhomogeneity of the plastic.

4. Conclusions

The latest amendment [32] of the FCM Regulation [1] itself proves the undeniable importance of a method determining PAAs in food simulants with low LOD and LOQ values. The amendment [32] has recently introduced a 2 μgkg^{-1} limit for carcinogenic PAAs and refers to those that are listed in the REACH Regulation [30]. Since the limit for the sum of PAAs migrating from FCMs is 10 μgkg^{-1}, it is reasonable to extend the list of carcinogenic PAAs with ANL and PTOL, the most common impurities of azo colorants and isocyanates. But this list of PAAs poses challenges not only because the included PAAs cover a wide range of polarity but also because it includes DAANI, DATOL and 2M5NA together. To meet the resulting challenge, a UHPLC-MS/MS method was developed, avoiding the use of ion pairing reagents and buffers during chromatographic separation to reduce ion suppression in the ESI source. To achieve low LOD, SPE was combined with SALLE during sample preparation. Thus, an enrichment factor of 20 could be achieved. An injector program was also optimized to enable a larger injection volume. We proved the suitability of the resulting method for daily routine analysis of PAAs migrating from FCMs by a within-laboratory validation. The method was then successfully applied for the analysis of extracts originating from a migration test of polyamide and silicone kitchenware samples in both 3% (w/v) acetic acid and 10% (v/v) ethanol food simulants.

CRediT authorship contribution statement

Bálint Sámuel SZABÓ: Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Péter**

Pál JAKAB: Methodology, Investigation. **János HEGEDŰS:** Methodology, Investigation, Writing - review & editing. **Csaba KIRCHKESZNER:** Validation, Writing - review & editing. **Noémi PETROVICS:** Validation, Writing - review & editing. **Zoltán NYIRI:** Validation, Writing - review & editing. **Zsolt BODAI:** Supervision, Conceptualization, Methodology, Writing - review & editing. **Tamás RIKKER:** Resources, Funding acquisition, Project administration. **Zsuzsanna EKE:** Supervision, Conceptualization, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2021.105927.

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