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Stability study of primary aromatic amines in aqueous food simulants under storage conditions of food contact material migration studies

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ABSTRACT

The stability of target compounds under migration conditions is scarcely investigated. To provide data for better regulations and guidelines, the stability of 24 primary aromatic amines (PAAs) was investigated under several storage conditions in all aqueous food simulants of Commission Regulation No. 10/2011. Eleven carcinogenic PAAs appeared to be less stable under at least one of the investigated conditions. PAAs appeared to be the least stable in 3% (*m/V*) acetic acid. This is highly problematic because this food simulant represents the worst-case scenario regarding PAA migration testing. Since 3 mmolL^{-1} HCl solution with similar pH showed that PAAs were more stable in this medium we suggest its consideration as an alternative food simulant. In ethanol containing food simulants, most PAAs proved to be stable. Decreased temperature improved PAA stability, whereas shortened storage time improved PAA recovery.

1. Introduction

Primary aromatic amines (PAAs) are used as basic building blocks in the chemical industry for the synthesis of adhesives, pharmaceuticals, pesticides, polymers, and colorants (Kolado & Balcerzak, 2008; Radomski, 1979). Some of the PAAs are proven to be carcinogenic (Radomski, 1979; Trakoli, 2012), while others are known allergens (Onder, 2003; Radomski, 1979). Still, these substances can be present in cosmetics (Hailong et al., 2013), textiles and leathers (Ahlström et al., 2005; Kawakami et al., 2010), indoor dust (Chinthakindi & Kannan, 2021), as well as in food contact materials (FCMs) such as food packaging (Aznar et al., 2009; Pezo et al., 2012; Ramey, 2020; Wang & Chen, 2009) and plastic kitchenware (Perez et al., 2021; Perez et al., 2019; Sanllorente et al., 2016; Szabó et al., 2021; Trier et al., 2010).

FCMs include all articles that come into contact with food during preparation, transport, storage and consumption (Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, 2004). Some of these FCMs can be a major source of PAA exposure. For example, polyamide based kitchen utensils are frequently used for cooking due to their heat resistance. Besides, dyes and pigments are used as colorants in

Abbreviations: 2H-70 C, migration/stability test for 2 hours at 70 °C; 2H-70C+10D-40C, migration/stability test for 2 hours at 70 °C followed by storage for 10 days at 40 °C; 2H-70C+10D-60C, migration/stability test for 2 hours at 70 °C followed by storage for 10 days at 60 °C; 2H-70C+24H-40C, migration/stability test for 2 hours at 70 °C followed by storage for 24 h at 40 °C; 2M5NA, 2-methyl-5-nitroaniline; 2NAP, 2-naphthylamine; 4ABP, 4-aminobiphenyl; 4AZB, 4-aminoazobenzene; 4CLA, 4-chloroaniline; 4CLOT, 4-chloro-*o*-toluidine; 4H-R, refluxing for 4 h; *A*%, accuracy; ANL, aniline; ANLD5, aniline-2,3,4,5,6-d_5; BNZ, benzidine; DAANI, 2,4-diaminoanisole; DATOL, 2,4-diaminotoluene; DCLB, 3,3′-dichlorobenzidine; DDPM, 4,4′-diaminodiphenylmethane; DMAPM, 3,3′-dimethyl-4,4′-diaminodiphenylmethane; DMTB, 3,3′-dimethylbenzidine; DMXB, 3,3′-dimethoxybenzidine; FCM, food contact material; ISTD, internal standard; LC, liquid chromatography; LD, limit of detection; LQ, limit of quantitation; MOCA, 4,4′-diamino-3,3′-dichlorodiphenylmethane; MS, mass spectrometry; MS/MS, tandem mass spectrometry; OANI, *o*-anisidine; OAZT, *o*-aminoazotoluene; ODIA, 4,4′-oxydianiline; OTOL, *o*-toluidine; PAA, primary aromatic amine; PCRES, *p*-cresidine; PTOL, *p*-toluidine; PTOLD3, *p*-toluidine-d_3 (methyl-d_3); *R*%, recovery; REACH Regulation, Regulation No. 1907/2006; *RSD*%, relative standard deviation; *SD*, standard deviation; SPE-SALLE, solid phase extraction and salting-out assisted liquid-liquid extraction; TDIA, 4,4′-thiodianiline; TMA, 2,4,5-trimethylaniline.

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all kinds of FCMs. Since PAAs are widely used in the manufacturing of polyamide and azo dyes, PAA residues can migrate into food from polyamide kitchen utensils (Brede & Skjevrak, 2004; McCall et al., 2012; Sanllorente et al., 2016; Tishkova et al., 2015; Trier et al., 2010) and colored FCMs (Perez et al., 2019; Yang et al., 2016; Yavuz et al., 2016). Printing inks used for labeling food packaging can also be a source of PAA exposure as the components can migrate through the package layers (Bundesinstitut Für Risikobewertung, 2014; Bundesinstitut Für Risikobewertung, 2017; Clemente et al., 2016; Sanchis et al., 2019). Composite food packaging materials have layered structures that are commonly held together by polyurethane based adhesives. PAAs can be released into food through the hydrolysis of isocyanate monomer residues from such adhesives (Aznar et al., 2009; Campanella et al., 2015; Clemente et al., 2016; Pezo et al., 2012; Ramey, 2020).

Due to the high risk of food contamination caused by migration from FCMs, the European Union implemented a legal act to ensure food quality and safety. Commission Regulation (EU) No 10/2011 (of 14 January 2011 on Plastic Materials and Articles Intended to Come into Contact with Food, 2011) lists those substances that can be used for manufacturing plastic FCMs. The regulation sets migration limits for both intentionally and non-intentionally added substances and specifies migration testing conditions for compliance testing. PAAs fall into the category of non-intentionally added substances and have one of the lowest migration limit values. According to the latest amendment (Commission Regulation (EU) 2020/1245), those 22 carcinogenic PAAs that are listed in Regulation (EC) No 1907/2006 (of the Council of 18 December 2006 Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)) have an individual migration limit of 2 $\mu g kg^{-1}$. Furthermore, the sum of all PAAs migrating from a plastic article shall not exceed 10 $\mu g kg^{-1}$ (Commission Regulation (EU) No 10/2011). Since analysis directly from various food would be extremely challenging, the Commission Regulation (EU) No 10/2011 introduces food simulants and includes a detailed guide of appropriate food simulant selection for migration testing. Out of the 4 aqueous food simulants, 3% (*m/V*) acetic acid, 10% and 20% (*V/V*) ethanol are assigned to hydrophilic food, whereas 50% (*V/V*) ethanol is assigned to food with lipophilic character. A further specification is that 3% (*m/V*) acetic acid should only be used in case of foreseeable contact with food having a pH value below 4.5.

The regulation also specifies conditions of migration testing and aims for the foreseeable worst-case scenario of contact (Commission Regulation (EU) No 10/2011). Over the past decade technical guidelines (Simoneau et al., 2011; Simoneau, 2009) suggested several types of migration tests focusing on realistic conditions. Due to the alkaline properties of PAAs, 3% (*m/V*) acetic acid food simulant is assigned for their migration testing as the worst-case scenario (Aznar et al., 2009; Simoneau et al., 2011). Considering contact time and temperature, various testing conditions are suggested for kitchenware. Refluxing for 4 h (4 H-R) is appointed as the general migration test in case of any food contact above 40 °C and for articles without a label or with unspecified purpose of use (Simoneau, 2009). A hot fill procedure and/or a less than 15 min foreseeable contact time with food between 70 − 100 °C is to be represented by a test of 2 h at 70 °C (2 H-70 C) (Simoneau et al., 2011; Simoneau, 2009). Hot fill and storage above 6 months at room temperature should be modeled by a migration test of 2 h at 70 °C followed by 10 days at 60 °C (2 H-70 C+10D-60 C) (Simoneau, 2009). In case of storage above 30 days in a fridge or a freezer, possibly after a hot fill, the appropriate circumstance for the testing is 2 h at 70 °C followed by 10 days at 40 °C (2 H-70 C+10D-40 C) (Simoneau, 2009). These guidelines had been under revision since 2019 and an updated version has been released recently (Beldi et al., 2021). According to the updated guideline, the hot fill combination with storage should be modeled with a test of 2 h at 70 °C followed by 24 h at 40 °C (2 H-70 C + 24 H-40 C) (Beldi et al., 2021). In case of long-term storage, 10D-60 C and 10D-40 C experiments are still suggested, but without the preceding 2 H-70 C (Beldi et al., 2021). In these ten-days-long migration experiments a new temperature, 50 °C is also included (Beldi et al., 2021). As for refluxing, the testing time may vary from 30 min up to 4 h depending on the foreseeable use of the product (Beldi et al., 2021).

Aniline and many of its derivatives are volatile (Hailong et al., 2013; Testud & Descotes, 1996) and they are prone to oxidation (Bhat & Gogate, 2021). Some PAAs are sensitive to light, moreover they may be converted to various polymerization products through photooxidation (Brill & Radomski, 1965; McKellar, 1965). Therefore, stock solutions are usually protected from light (Hailong et al., 2013; Mortensen et al., 2005; Sanllorente et al., 2016; Sendón et al., 2010; Shahrestani et al., 2018; Simoneau et al., 2011; Yang et al., 2016). It was reported, that stock solutions of several PAAs are prepared frequently due to possible degradation (Aznar et al., 2009; Mortensen et al., 2005). Some of the protocols for PAA determination in the annex of the guideline of Simoneau et al. (2011) suggest that the calibration solutions prepared in 3% (*m/V*) acetic acid should not be stored for more than a couple of days even if they are refrigerated. This could be explained with the conversion of PAAs in acetic acid, e.g., amide formation of benzidine (BNZ) in glacial acetic acid has been reported by Lakshmi et al. (2001). Only a few case studies are available investigating the stability of PAAs during migration testing conditions (Burch et al., 2008; Sendón et al., 2010). Sendón et al. (2010) investigated the stability of 8 PAAs in 3% (*m/V*) acetic acid after 2 H-70 C and 2 h at 100 °C contact conditions. One PAA (1,5-diaminonaphthalene) was reported exceedingly unstable under both conditions, having more decreased recovery in the case of higher temperature. A few other PAAs had significantly lower mean recovery in the test samples compared to the control samples in both cases. Another stability trial was carried out with 32 PAAs in water, 3% (*m/V*) acetic acid, 10% ethanol and olive oil after 2 h at 121 °C and 2 h at 121 °C followed by storage of 10 days at 40 °C. Possible degradation of 2,4-diaminoanisole and 3,3′-dimethoxybenzidine was reported and several other PAAs had lower recovery after heating and storage (Burch et al., 2008). These experiments highlight the stability issues of some of the PAAs.

Our main objective was to systematically study the stability of PAAs under migration testing conditions to fill in the gaps of knowledge targeting the REACH-listed 22 carcinogenic PAAs along with aniline and *p*-toluidine. Storage experiments were designed based on previous (Simoneau et al., 2011; Simoneau, 2009) and current (Beldi et al., 2021) guidelines. Due to the known stability issues of PAAs in acetic acid, we aimed to propose a possible alternative for this food simulant. Therefore, the stability of PAAs was investigated in a HCl solution with similar pH as 3% (*m/V*) acetic acid along with 10%, 20% and 50% (*V/V*) ethanol food simulants for a comprehensive study.

2. Materials and methods

2.1. Chemicals and equipment

2.1.1. Analytical standards

Analytical grade standards of 2,4-diaminoanisole (DAANI), 2,4-diaminotoluene (DATOL), aniline (ANL), *p*-toluidine (PTOL), 4,4′-oxydianiline (ODIA), benzidine (BNZ), *o*-anisidne (OANI), 4,4′-diaminodiphenylmethane (DDPM), *o*-toluidine (OTOL), *p*-cresidine (PCRES), 3,3′-dimethoxybenzidine (DMTB), 3,3′-dimethylbenzidine (DMTB), 3,3′-dimethyl-4,4′-diaminodiphenylmethane (DMAPM), 4-chloroaniline (4CLA), 4,4′-thiodianiline (TDIA), 2-naphthylamine (2NAP), 2-methyl-5-nitroaniline (2M5NA), 4-aminobiphenyl (4ABP), 4-chloro-*o*-toluidine (4CLOT), 4,4′-diamino-3,3′-dichlorodiphenylmethane (MOCA), 4-aminoazobenzene (4AZB) and *o*-aminoazotoluene (OAZT) were purchased from Sigma-Aldrich. Analytical grade standards of 2,4,5-trimethylaniline (TMA) and 3,3′-dichlorobenzidine (DCLB) were purchased from LGC Standards. Analytical grade deuterated standards of *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 used as internal standards (ISTDs) and purchased from C/D/N Isotopes Inc. and

Sigma-Aldrich, respectively.

2.1.2. Solvents and reagents

Methanol (Promochem®) and acetonitrile (Optigrade®) were purchased from LGC Standards. Absolute ethanol (Emsure®), hydrochloric acid (Suprapur®, 30%), formic acid (Emsure®, 98–100%) and trisodium citrate dihydrate (Emsure®) were ordered from Merck KGaA. Ultrapure water (MQ water) was produced by a Milli-Q Direct 8 Water Purification System (Merck KGaA). Acetic acid (Normapur®, 96%) and ammonia (Normapur®, 25%) were purchased from VWR Chemicals.

2.1.3. Solutions

Stock solutions of all analytical standards and ISTDs were prepared in methanol at 1 $mgmL^{-1}$. A working solution containing all 24 PAAs and a separate ISTD working solution containing both ISTDs at 1 μgmL^{-1} were prepared in acetonitrile. All stock and working solutions were stored at 4 °C in amber glass vials. The solid phase extraction (SPE) eluent consisted of 0.35 $molL^{-1}$ trisodium citrate dihydrate and 25% (*V/V*) acetonitrile in MQ water. The solutions of 3 $mmolL^{-1}$ HCl solution and food simulants of 3% (*m/V*) acetic acid, 10%, 20% and 50% (*V/V*) ethanol were prepared by diluting the appropriate solvent or reagent with MQ water.

2.1.4. Instrumentation and laboratory equipment

Samples were analyzed with an UltiMate 3000 RS LC system coupled with a TSQ Fortis Triple Quadrupole MS instrument equipped with an OptaMax NG Ion Source with a H-ESI probe (Thermo Fischer Scientific). Samples were stored at different temperatures in a Memmert WB 22 waterbath, a Memmert UNB 200 laboratory oven (Memmert GmbH+Co. KG) and a POL-EKO ST 2 Basic cooled incubator (POL-EKO-APARATURA Sp. J.) during the stability trial. Heating mantles, round bottom flasks and Allihn condensers were used for refluxing. For sample enrichment, ISOLUTE SCX-2 (500 mg, Biotage AB) SPE cartridges were used. Solutions were homogenized with an IKA vortex mixer (IKA-Werke). Samples were centrifuged on a Hermle Z206 A centrifuge (HERMLE Labortechnik GmbH) during sample preparation.

2.2. Analytical method

The analytical method applied during the stability trial was based on a previously published method (Szabó et al., 2021) using LC-MS/MS technique with solid phase extraction and salting-out assisted liquid-liquid extraction (SPE-SALLE) sample enrichment procedure. Some modifications were carried out during sample handling prior to sample load to the SPE cartridge to enable sample enrichment from 50% (*V/V*) ethanol food simulant (see Section 2.2.1.). Method efficiency in case of 20% and 50% (*V/V*) ethanol samples spiked at a level lower than the current migration limit was characterized for the first time in this work. Due to method transfer, minor modifications of the LC-MS/MS method were necessary (see Section 2.2.2. and 2.2.3.).

2.2.1. Sample preparation

Sample aliquots of 50 mL were spiked with the ISTD working solution of 2 ISTDs at 1.5 $ngmL^{-1}$. The SPE-SALLE sample enrichment procedure was carried out from samples of 3% (*m/V*) acetic acid and 10% (*V/V*) ethanol the same way as it had previously been published (Szabó et al., 2021) with the final dilution procedure that gives an enrichment factor of 20. Samples of 3 $mmolL^{-1}$ HCl solution and 20% (*V/V*) ethanol were prepared exactly the same way as samples of 3% (*m/V*) acetic acid and 10% (*V/V*) ethanol, respectively. In case of 50% (*V/V*) ethanol samples, 50 mL sample aliquots were spiked with ISTDs and then diluted with 50 mL MQ water. The diluted samples were then acidified with formic acid to 3% (*V/V*) before they were loaded to the cartridge. No further changes were applied in the sample preparation. A flowchart of the modified sample preparation procedure can be found in the supplementary material (Figure S.1.).

2.2.2. LC parameters

Chromatographic separation was carried out on an Acquity UPLC HSS T3 1.8 μm column (C18, 2.1 ×100 mm, Waters Corporation) equipped with an Acquity UPLC HSS T3 1.8 μm VanGuard pre-column (C18, 2.1 ×5 mm, Waters Corporation) and an ACQUITY Column In-Line Filter (Waters Corporation). MQ water: methanol 4:1 (*V/V*) mixture and 0.1% (*V/V*) formic acid in methanol were used as eluents 'A' and 'B', respectively. Gradient elution was applied with 250 $\mu Lmin^{-1}$ flow rate and an initial composition of 100% 'A'. Eluent composition was changed to 80% 'A' with a linear gradient within 5 min. Then, within 1 min, it changed to 10% 'A' which was held for 2 min. Then, the eluent composition was set back to 100% 'A' immediately and held for 17 min. The column oven was operated at 40 °C. An injector program was used for sample injection. The needle was washed with 500 μL wash liquid (same as eluent 'A') first, then 7 μL MQ water and 5 μL sample were drawn. This cycle was repeated four times and the program was finished with a needle wash followed by drawing a last portion of 7 μL MQ water. All together 20 μL sample was injected. To avoid sample-to-sample cross contamination, each sample vial had its own MQ vial. Due to the different structure of the autosampler and the use of stainless steel Viper™ capillaries (Thermo Fischer Scientific), no further post-time actions were required to avoid run-to-run cross contamination compared to the original method (Szabó et al., 2021). Therefore, the overall runtime of the analytical method including the injector program was 30 min.

2.2.3. MS parameters

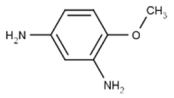
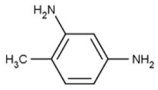
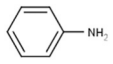
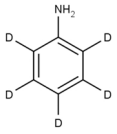
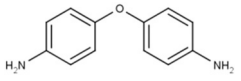
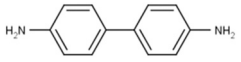
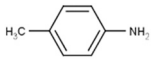
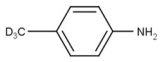
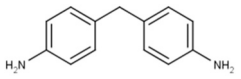
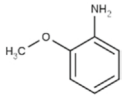
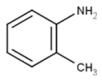
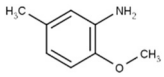
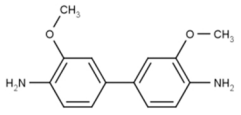
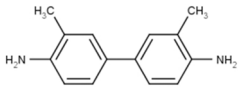
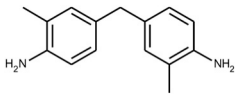
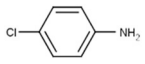
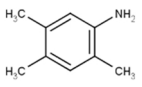
The spray voltage in static mode was set to 5500 V for positive ions. Nitrogen (5.0 purity, Messer Hungarogáz Kft.) was used as sheath, auxiliary and sweep gas. The gas parameters were set to 40, 18 and 10 (arbitrary units, corresponding to approximately 4.58, 11.28 and 8.21 $Lmin^{-1}$ flow rate), respectively. The temperatures of the ion transfer tube and vaporizer were set to 300 and 400 °C, respectively. Argon (4.6 purity, Messer Hungarogáz Kft) was used as collision gas with a parameter set to 2.5 mTorr. Scheduled single reaction monitoring (SRM) mode was applied with a dwell time of 20 ms for each transition to detect the target compounds. Names with abbreviations, structures, CAS registry numbers along with scan parameters and retention times of the target compounds are listed in Table 1.

2.3. Stability trial

Solutions of 3 $mmolL^{-1}$ HCl solution, 3% (*m/V*) acetic acid, 10%, 20% and 50% (*V/V*) ethanol were prepared the day before starting a set of stability trial and stored overnight in a water bath at 70 °C. The preheated solutions were spiked with the working solution of 24 PAAs at 1.5 $ngmL^{-1}$ on the day of start. The spiked solutions were divided into portions of approximately 260 mL. The sample preparation procedure with one portion was immediately initiated to serve as a reference. The other portions were stored under several different conditions. A water bath, a laboratory heating oven and an incubator were used to keep the samples at 70 °C for 2 h, followed by storage at 60 °C and 40 °C for 10 days, respectively. Samples were placed in the hot water bath and a timer was started for 2 h after the water temperature reached 70 °C again. The countdown for 24 h and ten days started when samples from the water bath were placed into the preheated oven or incubator. In case of refluxing, a timer was set for 4 h and it was started when the solutions started to boil in the flask.

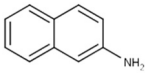
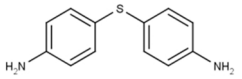
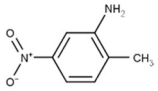
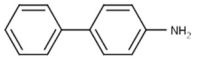
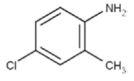
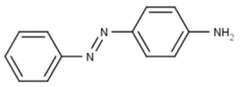
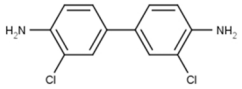
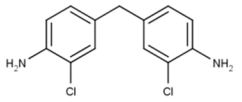
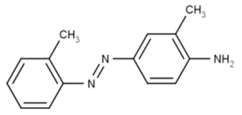
Storage experiments were carried out once while refluxing experiments were duplicated in acidic food simulants. Reference samples were prepared every time when a new set (*N*) of stability experiments was started. Within one set, multiple storage conditions were tested occasionally. Therefore, when a set included multiple storage conditions, their reference samples were the same. Samples were always prepared in 5 replicates (*n*). The spike level of PAAs and ISTDs in the analyzed samples following the enrichment procedure (see Section 2.2.1. and

Table 1
General information and single reaction monitoring scan parameters of target compounds and internal standards.

Compound	Name	CAS No.	Structure	RT[a] (min)	RTW[b] (min)	Precursor (*m/z*)	TL[c] (V)	SF[d] (V)	Product[e] (*m/z*)	CE[f] (V)
DAANI	2,4-diaminoanisole	615–05–4		2.7	2	139.1	40	10	124.1 108.1	15 17
DATOL	2,4-diaminotoluene	95–80–7		3.1	2	123.1	47	10	108.1 106.1	17 16
ANL	aniline	62–53–3		3.5	2	94.2	81	10	77.3 51.4	19 32
ANLD5 *	aniline-2,3,4,5,6-d_5	4165–61–1		3.5	2	99.1	56	10	82.3 54.4	20 32
ODIA	4,4′-oxydianiline	101–80–4		3.6	2	201.2	83	10	108.0 156.1	20 24
BNZ	benzidine	92–87–5		3.7	2	185.2	93	10	168.2 139.1	18 50
PTOL	*p*-toluidine	106–49–0		3.8	2	108.1	48	10	93.2 91.3	16 19
PTOLD3 *	*p*-toluidine-d_3 (methyl-d_3)	23346–25–0		3.8	2	111.1	52	10	93.3 94.2	17 20
DDPM	4,4′-diaminodiphenylmethane	101–77–9		4.0	2	199.2	68	10	106.1 77.3	24 49
OANI	*o*-anisidine	90–04–0		4.1	2	124.0	44	10	109.1 80.3	16 30
OTOL	*o*-toluidine	95–53–4		4.4	2	108.1	62	10	91.3 65.3	19 27
PCRES	*p*-cresidine	120–71–8		5.6	2	138.1	46	10	123.1 78.3	16 30
DMXB	3,3′-dimethoxybenzidine	119–90–4		5.6	2	245.2	99	10	230.2 187.1	18 33
DMTB	3,3′-dimethylbenzidine	119–93–7		5.8	2	213.2	65	10	180.2 198.3	35 21
DMAPM	3,3′-dimethyl-4,4′ -diaminodiphenylmethane	838–88–0		6.3	2	227.2	73	10	120.1 178.2	25 26
4CLA	4-chloroaniline	106–47–8		6.6	2	128.0	51	10	93.3 111.0	18 24
TMA	2,4,5-trimethylaniline	137–17–7		6.8	2	136.1	55	10	121.1 91.3	16 23
2NAP	2-naphthylamine	91–59–8		7.1	2	144.0	60	10	127.1 77.3	23 37

(*continued on next page*)

Table 1 (*continued*)

Compound	Name	CAS No.	Structure	RT[a] (min)	RTW[b] (min)	Precursor (*m/z*)	TL[c] (V)	SF[d] (V)	Product[e] (*m/z*)	CE[f] (V)
TDIA										
	4,4′-thiodianiline	139–65–1		7.1	2	217.1	66	10	124.1 200.1	20 18
2M5NA	2-methyl-5-nitroaniline	99–55–8		7.4	2	152.9	49	0	107.1 89.2	15 33
4ABP	4-aminobiphenyl	92–67–1		7.5	2	170.1	102	10	152.1 127.1	30 35
4CLOT	4-chloro-*o*-toluidine	95–69–2		7.5	2	142.1	56	10	107.1 125.0	17 21
4AZB	4-aminoazobenzene	60–09–3		7.8	2	198.1	46	10	77.3 51.5	20 48
DCLB	3,3′-dichlorobenzidine	91–94–1		7.8	2	253.1	100	10	217.1 182.2	20 28
MOCA	4,4′-diamino-3,3′ -dichlorodiphenylmethane	101–14–4		7.8	2	267.1	83	10	231.1 140.0	21 26
OAZT	*o*-aminoazotoluene	97–56–3		8.1	2	226.2	48	10	91.3 121.1	21 22

* Internal standard.
[a] Retention time.
[b] Retention time window for scheduled single reaction monitoring.
[c] Tube lens voltage.
[d] Source fragmentation voltage.
[e] The first fragment is the quantifier, the second is the qualifier.
[f] Collision energy.

Figure S.1.) was 30 ngmL^{-1}. A blank sample was prepared for each reference and storage condition in every set. An ISTD calibration was prepared freshly every time at 3, 5, 10, 15, 30, 50 and 70 ngmL^{-1} concentration levels. The level of ISTDs was 30 ngmL^{-1}. ANLD5 was assigned to ANL and PTOLD3 was assigned to the rest of the PAAs. (For more details, see Sections 3.2. and 3.3. in Szabó et al., 2021.) The calibration solutions contained 40% (*V/V*) acetonitrile and 0.25% (*V/V*) ammonia in MQ water. Accuracy values (*A*%) were determined (1) from the measured concentration of samples ($c_{i,\text{sample}}$) calculated from the equations of the ISTD calibration curves and the spike level ($c_{i,\text{spike}}$). For stability evaluation, average accuracy values ($\overline{A}_i$%) were calculated (2) with 95% confidence level (using the two-tailed $t_{\alpha,n-1}$ score when $\alpha = 0.05$). PAAs were considered stable if the accuracy intervals of the reference and the stored samples overlapped. Recovery values (*R*%) were also calculated (3) from the accuracy of stored samples ($A_{i,\text{stored}}$) referring to the average accuracy of reference samples ($\overline{A}_{i,\text{reference}}$).

$$A\% = \frac{c_{i,\text{sample}}}{c_{i,\text{spike}}} \bullet 100\% \quad (1)$$

$$\overline{A}_i\% = \overline{A}_{i,n}\% \pm \frac{t_{\alpha,n-1} \bullet SD}{\sqrt{n}} \quad (2)$$

$$R\% = \frac{A_{i,\text{stored}}}{\overline{A}_{i,\text{reference}}} \bullet 100\% \quad (3)$$

2.3.1. Trial of 3% (m/V) acetic acid food simulant

The stability of the target compounds was tested during the conditions of 2 H-70 C, 2 H-70 C+ 10D-60 C, 2 H-70 C+ 10D-40 C, 2 H-70 C + 24 H-40 C and 4 H-R. The refluxing experiment was carried out in duplicates. The experiments were carried out in 5 different sets (i.e. started on 5 different days). The trials of 2 H-70 C and 2 H-70 C+ 10D-60 C were started on the same day, therefore they had the same reference.

2.3.2. Trial of 3 mmolL^{-1} HCl solution

Target compounds were monitored after storage of 2 H-70 C, 2 H-70 C+ 10D-60 C, 2 H-70 C+ 10D-40 C, 2H70C+ 24 H-40 C and 4 H-R. The refluxing experiment was performed in duplicates. The experiments were carried out in 3 different sets. The trials of 2 H-70 C, 2 H-70 C+ 10D-60 C, 2 H-70 C+ 10D-40 C and the first 4 H-R were started on the same day, therefore they had the same reference.

2.3.3. Trials of 10%, 20% and 50% (V/V) ethanol food simulants

Storage experiments of 2 H-70 C, 2 H-70 C+ 10D-60 C, 2 H-

70 C+ 10D-40 C and 4 H-R were carried out in 3 different sets in case of 10% (*V/V*) ethanol. The trials of 2 H-70 C and 2 H-70 C+ 10D-60 C were started on the same day, therefore they had the same reference.

Conditions of 2 H-70 C, 2 H-70 C+ 10D-60 C and 4 H-R were investigated in 2 different sets in the case of 20% (*V/V*) ethanol. The trials of 2 H-70 C and 4 H-R were started on the same day, therefore they had the same reference.

As for 50% (*V/V*) ethanol, conditions of 2 H-70 C, 2 H-70 C+ 10D-60 C and 4 H-R were investigated in 2 different sets. The trials of 2 H-70 C and 2 H-70 C+ 10D-60 C were started on the same day, therefore they had the same reference.

3. Results

3.1. Reference samples

In case of 3% (*m/V*) acetic acid, 5 reference samples were prepared on different days, with 5 replicates on each day. The average accuracy, the within-day precision and the between-day precision ranged between 66 – 103%, 0.9 – 15.8% and 2.6 – 17.5%, respectively. From 3 mmolL^{-1} HCl solution, 3 reference samples were prepared on separate days, with 5 replicates on each day. The average accuracy, the within-day precision and the between-day precision ranged between 76% and 101%, 0.7 – 16.2% and 2.1 – 13.4%, respectively. Three different reference samples were prepared from 10% (*V/V*) ethanol on separate days, with 5 replicates on each day. The average accuracy, the within-day precision and the between-day precision ranged between 81 – 100%, 0.4 – 15.5% and 3.0 – 12.7%, respectively. As for 20% and 50% (*V/V*) ethanol, 2 reference samples were prepared for each, on different days, with 5 replicates on each day. The average accuracy, the within-day precision and the between-day precision from 20% (*V/V*) ethanol ranged between 67 – 103%, 1.0 – 15.4% and 2.1 – 10.7%, respectively. The average accuracy, the within-day precision and the between-day precision from 50% (*V/V*) ethanol ranged between 64 – 98%, 1.1 – 11.9% and 1.7 – 9.5%, respectively. Detailed results for each target compound can be found in the supplementary material (Table S.1.).

These results show that the accuracy of the applied analytical method is above 75% for all tested compounds in each tested food simulants, except for DAANI (64%) and DATOL (72%). Although 20% and 50% (*V/V*) ethanol were not investigated in the previous publication of Szabó et al. (2021), these results show the applicability of the analytical method for these food simulants as well.

3.2. Stability after 2 h at 70 °C

The range of accuracy values in the reference samples and the ones stored at 70 °C for 2 h overlapped for all PAAs in all solutions, except for DAANI in both acidic solutions and DCLB in 3% (*m/V*) acetic acid. Recovery values were in the range of 90 – 108% after storage for all PAAs in all solutions, except for DAANI, DATOL and DCLB. DAANI had a recovery of 20%, 62% and 83% in 3% (*m/V*) acetic acid, 3 mmolL^{-1} HCl solution, and 20% (*V/V*) ethanol, respectively. DATOL and DCLB had recovery values of 89% and 80% in 3% (*m/V*) acetic acid, respectively. Accuracy values of DAANI and DCLB in the reference and stored samples of 3% (*m/V*) acetic acid, 3 mmolL^{-1} HCl solution and 10% (*V/V*) ethanol are shown in Fig. 1.a–c, while the recovery values are shown in Fig. 1.d. The recovery of DAANI is approximately three times higher in 3 mmolL^{-1} HCl solution compared to that in 3% (*m/V*) acetic acid. Recovery and precision values for all PAAs in the stored samples can be found in the supplementary material (Table S.2.).

These results show that PAAs other than DAANI and DCLB were stable for 2 h at 70 °C in all investigated solutions. The recoveries of these two increased remarkably when acidity of the food simulant was set using HCl instead of acetic acid.

3.3. Stability after 2 h at 70 °C followed by 10 days at 60 °C

The result after exposure to 70 °C for 2 h followed by 60 °C for 10 days showed greatly varying stability of PAAs in 3% (*m/V*) acetic acid. DAANI, DATOL, DMXB and DMTB could not be detected (LD is 0.05 ngmL^{-1}, *R*% < 3.3%) and the amount of PCRES was below the limit of quantitation (LQ is 0.15 ngmL^{-1}, *R*% < 10%) in the stored sample. Recoveries of BNZ, OANI, DMAPM, TDIA, 2NAP and DCLB ranged between 11 – 80%. The range of accuracy values measured in the reference sample did not overlap with that of the stored sample in any case of the previously listed PAAs. The rest of the PAAs had recoveries in the range of 84 – 105%.

The stability of several PAAs improved in 3 mmolL^{-1} HCl solution. DAANI, DATOL and DMXB still could not be detected (*R*% < 3.3%), but DMTB and PCRES had recovery values of 46% and 67%, respectively. BNZ, OANI, DMAPM, TDIA, 2NAP and DCLB had recoveries between 83 – 99% with only DMAPM and TDIA having non-overlapping ranges of accuracy for the stored and the reference samples. The rest of the PAAs had recoveries between 95 – 112%. Fig. 2.a shows the improved stability of several PAAs in 3 mmolL^{-1} HCl solution compared to that in 3% (*m/V*) acetic acid.

Most of the PAAs were stable in 10%, 20% and 50% (*V/V*) ethanol with a range of recovery between 85 – 112%, except for DAANI and

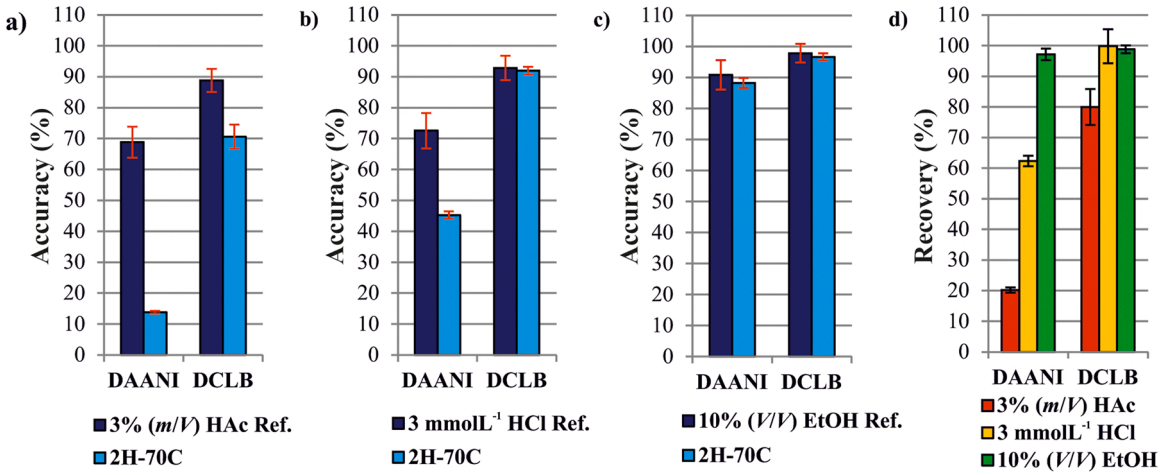


Fig. 1. Accuracy (a-c) and recovery (d) values of DAANI and DCLB after storage of 2 h at 70 °C in 3% (*m/V*) acetic acid (a), 3 mmolL^{-1} HCl solution (b) and 10% (*V/V*) ethanol (c). Samples were spiked at 1.5 ngmL^{-1} ($n = 5$). Error bars represent *SD*.

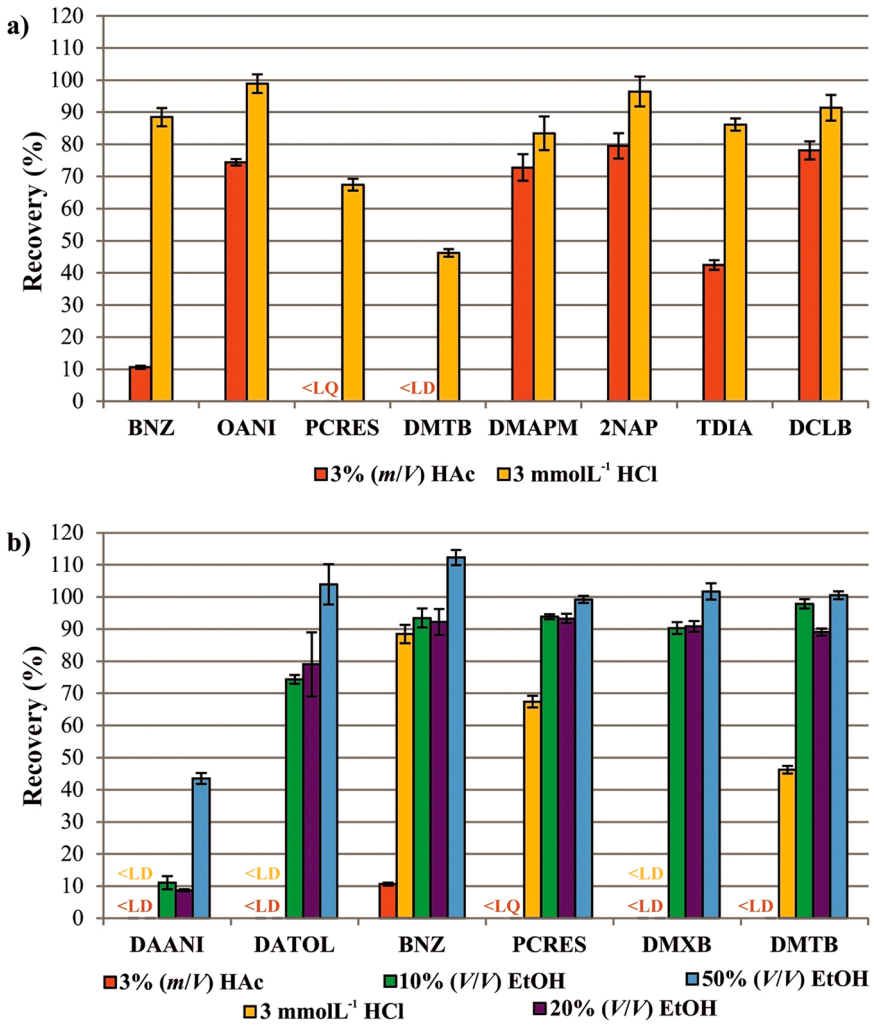


Fig. 2. Recoveries of several PAAs after storage of 2 h at 70 °C followed by a storage of 10 days at 60 °C in different acidic media (a) and in all of the investigated solutions (b). Samples were spiked at 1.5 ngmL^{-1} ($n = 5$). Error bars represent *SD*.

DATOL. DAANI had a recovery around 10% in both 10% and 20% (*V/V*) ethanol, but it increased four times in 50% (*V/V*) ethanol. DATOL had a recovery of 74%, 79% and 104% in 10%, 20% and 50% (*V/V*) ethanol, respectively. Those PAAs that are less stable in acidic medium, had their best recoveries in 50% (*V/V*) ethanol. Fig. 2.b compares the recovery of DAANI, DATOL, BNZ, PCRES, DMXB and DMTB in all tested solutions. Recovery and precision values for all PAAs in the stored samples can be found in the supplementary material (Table S.3.).

These results confirmed that some PAAs are less stable in 3% (*m/V*) acetic acid. This issue could be partially overcome with the use of 3 mmolL^{-1} HCl solution. Recovery could be further improved with ethanolic food simulants, of which 50% (*V/V*) ethanol provided the best results.

3.4. Stability after 2 h at 70 °C followed by 10 days at 40 °C

Stability of several PAAs can be increased by lowering the storage temperature. In the experiments where 2 h at 70 °C was followed by 40 °C for 10 days only 5 PAAs were less stable in 3% (*m/V*) acetic acid: DAANI and DMXB could not be detected (*R*% < 3.3%) in the stored sample; DATOL, PCRES and DMTB had recovery values of 13%, 68% and 70%, respectively. The rest of the PAAs had recovery values between 88 – 107% with overlapping accuracy ranges for the stored and the reference samples.

Stability improved in 3 mmolL^{-1} HCl solution. Only DAANI could not be detected (*R*% < 3.3%) in the stored sample, DATOL and DMXB had recovery values of 65% and 84%, respectively. The rest of the PAAs could be recovered in the range of 96 – 114% with overlapping accuracy ranges of the stored and the reference samples.

In 10% (*V/V*) ethanol only DAANI did not have overlapping accuracy range for the reference and the stored samples, and even this compound had a recovery of 71%. The rest of the PAAs had a range of recovery between 89 – 107%. These results along with the stability results of PAAs in 20% and 50% (*V/V*) ethanol during long-term storage at 60 °C indicated that most probably all investigated PAAs would be stable during a ten-days-long storage at 40 °C in both 20% and 50% (*V/V*) ethanol. Therefore, we did not test these storage conditions. All recovery and precision values of PAAs in the stored samples can be found in the supplementary material (Table S.4.).

These results show that the stability of PAAs can be improved in all tested media by lowering the storage temperature. Still, 3 mmolL^{-1} HCl solution proved to be better than 3% (*m/V*) acetic acid considering the recovery values of the less stable PAAs. Moreover, after 10 days at 40 °C all investigated PAAs are recoverable from each ethanolic food simulant, including the one with the lowest ethanol content (10%).

3.5. Stability after 2 h at 70 °C followed by 24 h at 40 °C

All PAAs could be detected and quantified from the solutions kept at 70 °C for 2 h followed by 40 °C for 24 h. The data demonstrate that

shorter time at lower temperature could help with PAAs' stability issues. DAANI, DATOL and DMXB had recovery values of 10%, 67%, 26% and 41%, 100%, 85% in 3% (*m/V*) acetic acid and in 3 mmolL^{-1} HCl solution, respectively. The rest of the PAAs had recovery values in the range of 85 − 112% with overlapping accuracy ranges, comparing the stored sample with the reference. The two least stable PAAs, DAANI and DMXB, had approximately four and three times higher recovery in 3 mmolL^{-1} HCl solution compared to that in 3% (*m/V*) acetic acid, respectively. All recovery and precision values of PAAs in the stored samples can be found in the supplementary material (Table S.5.).

Fig. 3. compares the recovery values of DAANI, DATOL, BNZ, PCRES, DMXB and DMTB in 3% (*m/V*) acetic acid (a), 3 mmolL^{-1} HCl solution (b) and 10% (*V/V*) ethanol (c) during the stability trials carried out at 60 °C and 40 °C. Since the recovery of all target compounds were above 71% after the ten-days-long experiment performed at 40 °C (Table S.4.), this shortened storage experiment in 10% (*V/V*) ethanol was not carried out.

These experiments proved that shorter storage time could prevent loss due to the lack of stability of PAAs so much, that only 3 target compounds had recoveries below 85% in 3% (*m/V*) acetic acid and only 1 in 3 mmolL^{-1} HCl solution (Table S.5.). This is obviously a considerable improvement compared to the long-term storage conditions, which highlights the applicability of acidic food simulants during short-term migration testing, especially in the case of 3 mmolL^{-1} HCl solution.

3.6. Stability after 4 h of refluxing

After refluxing for 4 h in 3% (*m/V*) acetic acid, DAANI could not be detected (*R*% < 3.3%) and the amount of DMXB was below the limit of quantitation (*R*% < 10%). DATOL, BNZ, DMTB, DMAPM and TDIA had 44%, 63%, 14%, 78% and 78% recovery, respectively. Recoveries for the rest of the PAAs ranged between 88 − 112%. In 3 mmolL^{-1} HCl solution only DAANI could not be detected (*R*% < 3.3%), DATOL, DMXB and DMTB had a recovery value of 55%, 21% and 56%, respectively. Recoveries for the rest of the PAAs ranged between 80 − 123%.

In 10%, 20% and 50% (*V/V*) ethanol recovery values for all PAAs were in the range of 90 − 110%, except for DAANI that had a recovery of 77% in 50% (*V/V*) ethanol. Fig. 4. shows recoveries of DAANI, DATOL, ANL, BNZ, DMXB, DMTB, DMAPM and TDIA in 3% (*m/V*) acetic acid, 3 mmolL^{-1} HCl solution and 50% (*V/V*) ethanol after 4 h of refluxing. All recovery and precision values of PAAs in the refluxed samples can be found in the supplementary material (Table S.6.).

Refluxing for 4 h seemed to have a similar effect on the stability of PAAs as the storage for 10 days at 60 °C. Therefore, migration testing of PAAs at reflux temperature in acidic media is not that feasible due to stability issues, whereas all PAAs were found to be stable in ethanol-water mixtures under such conditions.

4. Discussion

It is reassuring that almost all investigated PAAs seemed to be stable during one of the most commonly used conditions for PAA migration testing from FCMs: 2 h at 70 °C in 3% (*m/V*) acetic acid. But DAANI had 20% recovery in this storage experiment with a method accuracy of 66% from freshly spiked 3% (*m/V*) acetic acid. These results are in harmony with previous findings. Low recovery values have already been reported in many cases for DAANI (Burch et al., 2008; Ramey, 2020; Yang et al., 2016; Yavuz et al., 2016). The usual explanation is instability or volatility (Burch et al., 2008; Ramey, 2020; Yang et al., 2016). It is important to note that as a consequence of these issues, the current method for migration testing can easily give false negative results for DAANI. Nonetheless, dismissing this compound from the testing of PAAs is inadvisable. DAANI was mostly used to synthetize dyes (National Institute for Occupational Safety and Health (NIOSH), 1978) that might still be used illegally in printing inks on labels of FCM products, and components of printing inks have previously been reported to migrate

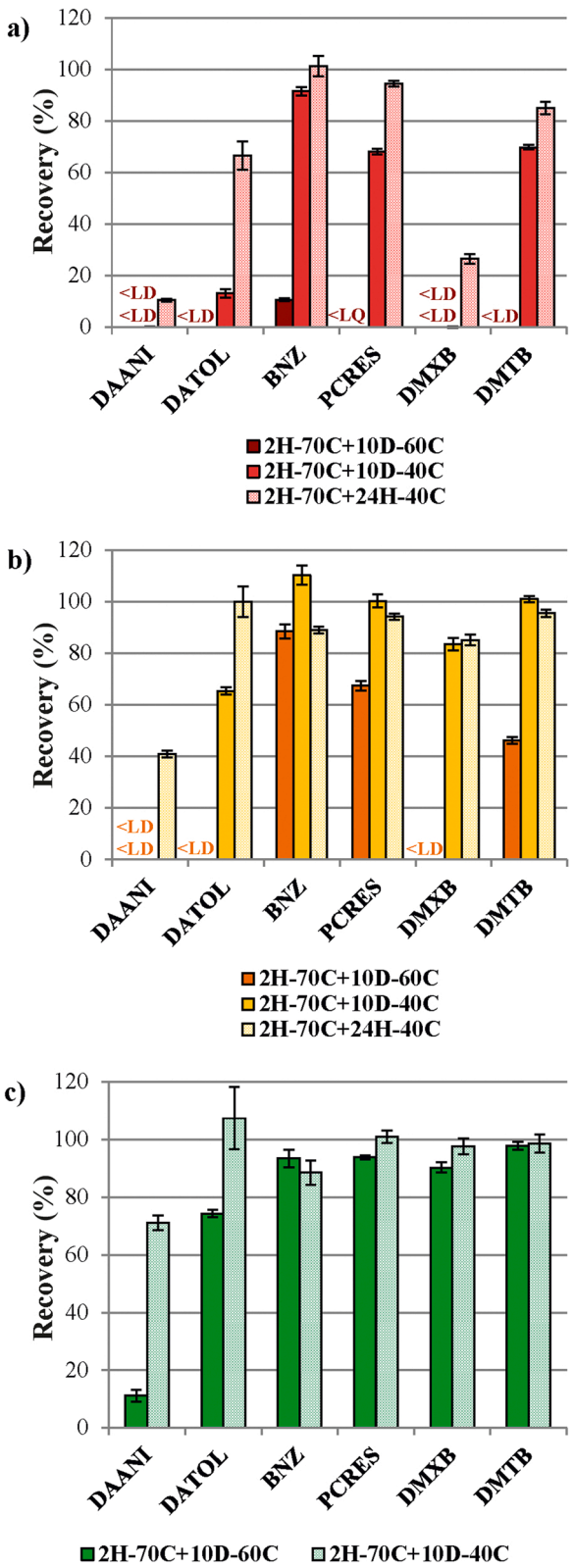


Fig. 3. Recoveries of several PAAs after storage of 2 h at 70 °C followed by storages of 10 days at 60 °C and 40 °C, and that of 24 h at 40 °C in 3% (*m/V*) acetic acid (a) and 3 mmolL^{-1} HCl solution (b). Recoveries of several PAAs after storage of 2 h at 70 °C followed by a storage of 10 days at 60 °C and 40 °C in 10% (*V/V*) ethanol (c). Samples were spiked at 1.5 ngmL^{-1} ($n = 5$). Error bars represent *SD*.

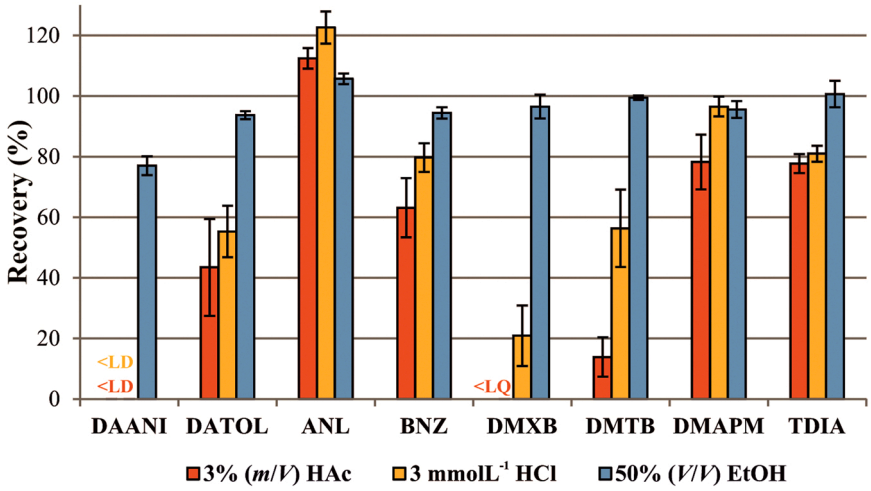


Fig. 4. Recoveries of several PAAs after refluxing for 4 h in 3% (*m/V*) acetic acid ($n = 10$), 3 mmolL^{-1} HCl solution ($n = 10$) and 50% (*V/V*) ethanol ($n = 5$). Samples were spiked at 1.5 ngmL^{-1}. Error bars represent *SD*.

through packaging (Clemente et al., 2016; Luo et al., 2022; Sanchis et al., 2019).

Our results showed that DAANI can be analyzed with similar method accuracy (Fig. 1a-b) but with a recovery approximately three times higher if 3 mmolL^{-1} HCl solution is used as food simulant (Fig. 1d). This solution has similar pH as the 3% (*m/V*) acetic acid food simulant, thus it could be an alternative solution for migration testing of PAAs from FCMs intended to come into contact with acidic food. On the other hand, due to the alkaline character of PAAs, 3% (*m/V*) acetic acid is the preferred food simulant for any FCMs, independently from the foreseeable use. But even, if the acidic medium is expected to facilitate the migration of PAAs best, the stability of target compounds must be considered in the design of migration experiments regarding both contact temperatures and contact times. With the least stable PAAs in mind even changing to non-acidic food simulants seems to be a viable idea to lower the chances of false negative results.

After keeping the PAAs in 3% (*m/V*) acetic acid at 70 °C for 2 h followed by 10 days at 60 °C (typical for modeling hot fill followed by long-term storage) seven of them (DAANI, DATOL, BNZ, PCRES, DMXB, DMTB and TDIA) could not be detected ($R\% < 3.3\%$) due to their instability. As Fig. 2.b shows, in case of DAANI, DATOL and DMXB the reason for this instability can be the acidic medium itself, as these PAAs could not be detected ($R\% < 3.3\%$) in neither acidic solution. However, Fig. 2a shows that the recovery, thus the stability of BNZ, PCRES, DMTB and TDIA could be increased just by changing 3% (*m/V*) acetic acid to 3 mmolL^{-1} HCl solution. This suggests that 3% (*m/V*) acetic acid itself could be the reason for their instability, which raises doubts about being an appropriate simulant in this case. Lakshmi et al. (2001) demonstrated that BNZ can be acetylated with glacial acetic acid. In their experiment, acetic acid was in approximately a thousand times excess and the reaction occurred in 40 mins at 110 °C. Due to the low PAA concentration levels, acetic acid was in more than a million times excess in our storage experiment, thus the assumption of a similar reaction in 10 days at 60 °C even in aqueous medium is plausible.

Fig. 3. shows that these stability issues can be mitigated by lowering the temperature during the migration study. Consequently, even though 10 days at 40 °C represents a shorter storage condition according to the regulation (Commission Regulation (EU) No 10/2011), it can improve the detection of PAAs. The stability of DATOL, BNZ, PCRES, DMXB and DMTB improved in acidic media by lowering the temperature from 60 °C to 40 °C (Fig. 3.a-b). But even after storage at this lower temperature DMXB could only be detected in 3 mmolL^{-1} HCl solution. Analogously, the other 4 of the previously listed 5 PAAs had higher recoveries in 3 mmolL^{-1} HCl solution compared to that in 3% (*m/V*) acetic acid (Fig. 3a-b). Recoveries of DAANI could not be improved in neither acidic medium by lowering the temperature only. To enable its detection at least either the experiment has to be stopped after 24 h at 40 °C (Fig. 3a-b) or the acidic media has to be changed (Fig. 3c). In 10% (*V/V*) ethanol not only DAANI's, but DATOL's recovery also improved considerably by lowering the temperature alone (Fig. 3c). The rest of the PAAs had acceptable recoveries in all investigated ethanol-water mixtures even at higher temperature, having slightly higher recovery values in 50% (*V/V*) ethanol (Fig. 2b).

The results of the two storage experiments conducted at 40 °C show that shortening the storage time to 24 h in 3% (*m/V*) acetic acid considerably improves the stability of DATOL, PCRES, DMXB and DMTB (Fig. 3a), yet DMXB still had a low recovery (26%). On the other hand, in 3 mmolL^{-1} HCl solution, 22 PAAs had at least 84% recovery even after the long-term storage. Still, reducing the storage time to 24 h could considerably improve the recoveries of DAANI and DATOL (Fig. 3a–b). Changing the 3% (*m/V*) acetic acid (Fig. 3a) to 3 mmolL^{-1} HCl solution (Fig. 3b) assures approximately four and three times increase in the recoveries of DAANI and DMXB (the two least stable PAAs) under the conditions of 2 h at 70 °C followed by 24 h at 40 °C storage, respectively.

Considering that several PAAs (DAANI, DATOL, BNZ, PCRES, DMXB, and DMTB) were more stable in 3 mmolL^{-1} HCl solution than in 3% (*m/V*) acetic acid during multiple storage conditions (2 H-70 C+10D-60 C, 2 H-70 C+10D-40 C, and 2 H-70 C + 24 H-40 C), it is strongly suggested that the use of 3% (*m/V*) acetic acid food simulant representing the worst-case scenario for migration testing of PAAs should be revised. However, this reconsideration requires further investigations on PAA stability in real food, as the goal is to choose the simulant that gives only slight overestimation of the expected PAA concentrations in real food. Furthermore, if PAAs show similar instability in real food, identification and toxicological evaluation of the possible decomposition or conversion products should be top priority.

Refluxing had similar effect on PAAs as storage for 10 days at 60 °C. Although fewer PAAs showed instability in acidic medium, in the case of DATOL, BNZ, DMXB and DMTB we experienced unusually high standard deviation (Fig. 4.). This could challenge the repeatability of migration testing with acidic medium. In case of DATOL, BNZ and DMAPM, refluxing in 3 mmolL^{-1} HCl solution provided better repeatability. It is interesting to note, that this is the only condition, in which ANL recoveries are above 110% from acidic medium. It could be explained by assuming BNZ degradation to ANL. Considering the 80% recovery of BNZ (Table S.6.) from 3 mmolL^{-1} HCl solution (thus 20% conversion, assuming no analyte loss) and that one BNZ (184 gmol^{-1}) molecule

yields two ANL (93 gmol^{-1}) molecules, assuming that all BNZ converted to ANL and that ANL remained stable (100% recovery of the spiked amount) during the 4 h of refluxing, the calculated recovery of ANL would be around 120%. Since BNZ had lower recovery in 3% (*m/V*) acetic acid (63% (Table S.6.) thus 37% conversion), following the same logic, ANL recovery would be around 137%. ANL recoveries are 123% and 112% in 3 mmolL^{-1} HCl solution and in 3% (*m/V*) acetic acid, respectively. These numbers may support the theory of BNZ to ANL conversion in 3 mmolL^{-1} HCl solution, but not in 3% (*m/V*) acetic acid. The measured lower recovery of ANL in 3% (*m/V*) acetic acid suggests that not all BNZ converts to ANL and it points towards a side reaction that might occur between BNZ and acetic acid (e.g. amide formation). However, this hypothesis needs further investigation. PAAs appeared to remain stable while refluxing in ethanol-water mixtures. Thus, 10%, 20% or 50% (*V/V*) ethanol might be a better selection of simulant when this general testing condition has to be applied.

The updated guideline (Beldi et al., 2021) specifies only 24 h storage at 40 °C after the hot-fill if the article is used for short-term storage. Our results show that this shortening of the contact time and temperature can help in detecting the migrating PAAs. However, modeling long-term storage – either with or without hot-fill – is no longer combined with hot-fill modeling (2 H-70 C), only10 days at 60 °C is recommended. But in our experiments most PAAs remained stable after 2 h at 70 °C. This means that stability issues probably arise during the ten-days-long storage. Thus, dismissing the hot fill part of the testing without lowering the temperature and/or shortening the contact time may not mean much help in preventing the loss of PAAs during the migration experiment. On the other hand, shortened contact time may lead to less migration. For this reason, the investigation of migration kinetics of PAAs from common polymers would be crucial to determine the optimal contact time, which belongs to a realistic worst-case scenario that enables the determination of the maximum concentration caused by the migration of PAAs.

5. Conclusions

The presented stability study showed that 2 h at 70 °C can be used without any concern for the testing of migration of PAAs from FCMs, as it is suggested in the updated guideline (Beldi et al., 2021). Besides, 12 out of the 24 investigated PAAs proved to be stable during the conditions of the four most commonly used migration tests: 2 h at 70 °C followed by 10 days at 60 °C, 2 h at 70 °C followed by 10 days at 40 °C, 2 h at 70 °C followed by 24 h at 40 °C and 4 h of refluxing. These PAAs are ANL, ODIA, PTOL, DDPM, OTOL, 4CLA, TMA, 2M5NA, 4ABP, 4CLOT, MOCA, 4AZB and OAZT. However, half of the 22 carcinogenic PAAs listed in the REACH Regulation appeared to be less stable during at least one of the investigated conditions. These PAAs are DAANI, DATOL, BNZ, OANI, PCRES, DMXB, DMTB, DMAPM, TDIA, 2NAP, and DCLB. Fig. 5. summarizes the recovery data of these 11 PAAs along with ANL from all tested solutions under all tested conditions. (For numerical data see Table S.7.).

Our data show that DAANI and DMXB are the least stable PAAs with the biggest number of occurrences of not being detectable ($R\% < 3.3\%$). Further major instability is shown in case of DATOL, BNZ, PCRES and DMTB. In most of the cases, PAA stability issues occur in acidic medium, especially when acetic acid is used to set the right pH. This is problematic, since 3% (*m/V*) acetic acid is generally considered the best solution to model the worst-case scenario for migration testing of PAAs due to their alkaline properties. However, PAA stability could be preserved by changing 3% (*m/V*) acetic acid to 3 mmolL^{-1} HCl solution and/or lowering the temperature of the migration test representing long-term storage. Shortening the storage time could also lead to improved recoveries. Considering these changes, the recovery of overall 16 PAAs could be improved by at least 15%.

Except for DAANI and DATOL, all investigated PAAs appeared to be stable in 10%, 20% and 50% (*V/V*) ethanol under all conditions. It seems there is not much difference between the three ethanol-water mixtures in terms of PAA stability. On the other hand, DAANI and DATOL had

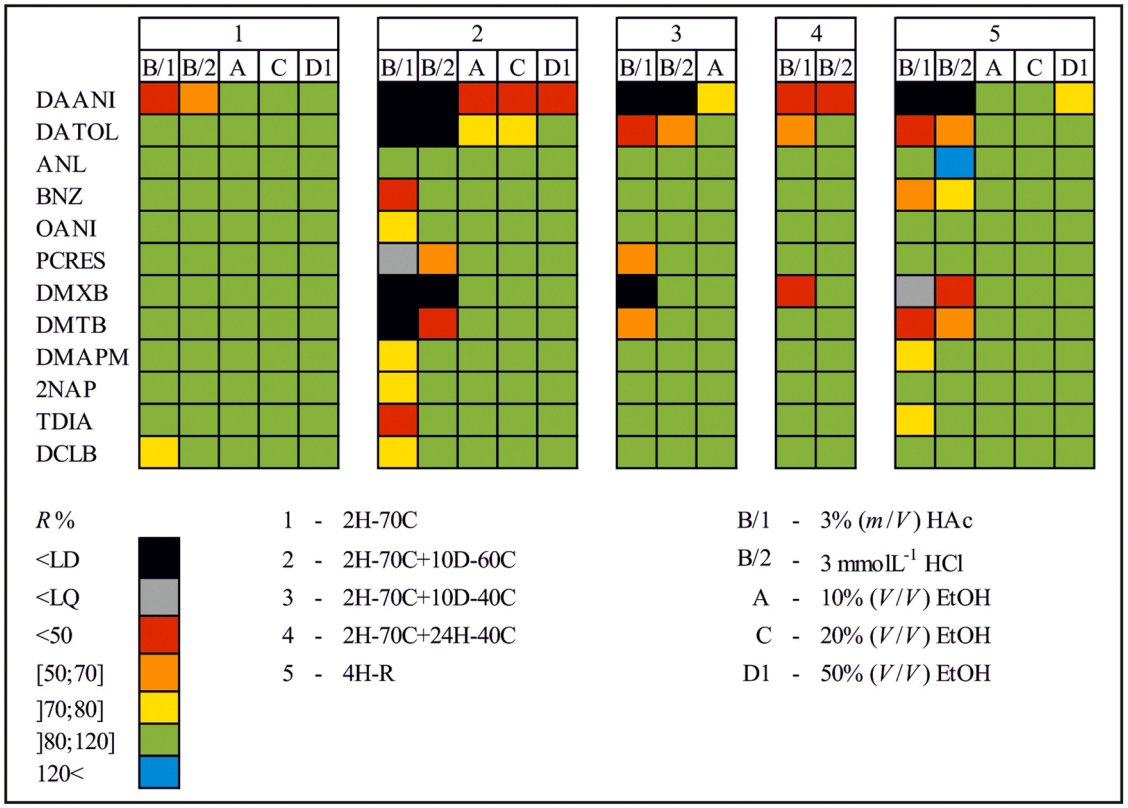


Fig. 5. Summary of recoveries of the 11 less stable PAAs and aniline in all investigated solutions under all storage conditions.

unquestionable stability issues in acidic media. For these compounds the best stability was observed in 50% (*V/V*) ethanol. This suggests that for long-term migration testing or migration testing at higher temperatures, ethanol containing food simulants could be more suitable in terms of avoiding false negatives due to stability issues.

Stability of PAAs is a serious issue during migration studies. Although the decrease in PAA concentrations during migration testing could be compensated using isotopically labeled ISTDs, but it would be rather expensive. Also, as the concentrations of less stable PAAs decrease below LD ($R\% < 3.3\%$), this procedure is not effective. Therefore, the ultimate solution for this problem is to assure the stability of PAAs during migration tests. Our results show that, due to the stability issues, in the case of long-term storage and storage combined with hot-fill modeling migration studies, the use of 3 $mmolL^{-1}$ HCl solution as an alternative acidic food simulant would be advantageous. However, the suggested modifications need to be verified by comparative migration studies using real food, the proposed alternative simulant and all aqueous food simulants specified by Commission Regulation (EU) No 10/2011. If PAAs also degrade or convert in real acidic food, the possible decomposition or conversion products should be investigated and in case of a major health concern they should be regulated also.

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CRediT authorship contribution statement

Bálint Sámuel Szabó: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Noémi Petrovics**: Methodology, Writing – review & editing. **Csaba Kirchkeszner**: Methodology, Writing – review & editing. **Zoltán Nyiri**: Writing – review & editing. **Zsolt Bodai**: Writing – review & editing. **Zsuzsanna Eke:** Supervision, Conceptualization, Project administration, Writing – review & editing.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2022.100909.

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