

응용 자료

Quantifying Primary Aromatic Amines in Polyamide Kitchenware Using the ACQUITY UPLC I-Class System and Xevo TQ-S micro

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Abstract

This application note demonstrates a sensitive LC-MS/MS method for the analysis of 23 common PAAs with very easy sample preparation. The Xevo TQ-S micro provides sensitive detection levels to satisfy the EU 2020/1245 regulations.

Benefits

- Single method for analysis of 23 PAAs
- No need for ion-pairing reagents, or the removal of acetic acid from the sample extract prior to analysis
- Sensitive detection at levels well below the EU guidelines with Xevo TQ-S micro Triple Quadrupole Mass Spectrometry

Introduction

Primary Aromatic Amines (PAAs) are a class of compounds of which the simplest form is aniline (Figure 1). PAAs are substances that are used, for example, in the production of certain colorants, so-called azo pigments, notably in the color range yellow – orange – red. Whereas a large number of PAAs are safe for human health, some PAAs are known human carcinogens. For kitchenware, paper napkins, baker's bags with colorful print, and other printed items that come in contact with food, some PAAs may pose a health risk, if they are transferred to the food.

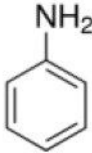
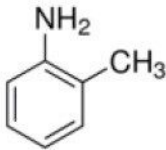
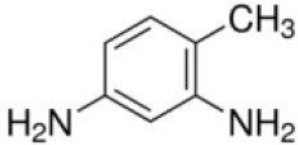
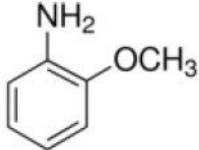
Compound	Mass	Structure
Aniline	93	
o-Toluidine	107	
2,4-Diaminotoluene	122	
o-Anisidine	123	

Figure 1. Chemical structures of some PAAs.

Because of the potential health risks, specific migration limits (SMLs) are put in place. The longstanding EU 10/2011 regulation¹ has recently been amended with EU 2020/1245.² Whereas previously, in relation to PAAs, 'Plastic materials and articles shall not release primary aromatic amines, excluding those appearing in Table 1 of Annex I, in a detectable quantity into food or food simulant. The detection limit is 0.01 mg of substance per kg of food or food simulant. The detection limit applies to the sum of primary aromatic amines released'. The recent EU 2020/1245 update has identified that PAAs listed in Annex I of 10/2011/EU (for example 1,3 phenylenediamine) must not be detectable above 0.002 mg/kg in food or food simulant and individual PAAs listed in entry 43 of Appendix 8 of 1907/2006/EC³ shall not be detectable above 0.002 mg/kg in food or food

simulant. Furthermore, the EU 2020/1245 amendment stipulates for other PAAs (not listed in Annex I of the regulation nor Appendix 8 of 1907/2006) should be assessed for risk, but to avoid potential combined toxicity, their total migration is set to a maximum of 0.01 mg/kg food or food simulant.

The provisions state that for primary aromatic amine migration from polyamide kitchenware, only one migration test will be carried out, if this first extract is compliant within limits. However, if this first simulant extract exceeds the permitted limits, then two subsequent migration studies are required.⁴ This PAAs migration testing is conducted with simulant B, 3% (w/v) acetic acid, as it has been demonstrated that this simulant represents the worst case for the migration of PAAs from polyamide kitchenware.⁵

PAAs are small, basic compounds, which are ionized with low pH. As a result of their basic properties and the 3% acetic acidic sample solvent, some PAAs don't focus well on the head of the column, resulting in poor peak shape and/or loss of retention. In order to improve chromatographic retention ion-pairing reagents are often used.⁴ Unfortunately these reagents have a negative impact on the electrospray sensitivity and are to be avoided where possible.

In this application note we describe a LC-MS/MS method for the analysis of 23 common PAAs in kitchenware after migration, using a Waters ACQUITY UPLC I-Class System coupled to a Xevo TQ-S micro Mass Spectrometer. The described method does not use an ion-pair reagent to improve chromatographic retention.

Experimental

UPLC Conditions

UPLC system:	ACQUITY UPLC I-Class
Sample manager:	Flow-Through Needle
Column:	ACQUITY UPLC HSS T3, 1.8 μ m, 2.1 x 100 mm
Mobile phase A:	Water
Mobile phase B:	Methanol

Column temp.:	45 °C
Sample temp.:	10 °C
Flow rate:	0.4 mL/min
Run time:	15 min
Injection volume:	20 µL

Gradient

0 min	5% B
10 min	100% B
12 min	5% B
12.01 min	5% B
15 min	5% B

MS Conditions

MS system:	Xevo TQ-S micro
Ionization mode:	ESI +
Capillary voltage:	2 kV
Desolvation temp.:	600 °C
Desolvation gas flow:	1200 L/hr
Source temp.:	150 °C

Acquisition:

Multiple Reaction Monitoring (MRM)

MS Methods and Data Acquisition

Two MRM transitions were used, unless otherwise stated. The dwell times were chosen automatically using the built-in points-per-peak calculator in the MS method. The data were acquired using MassLynx v4.1 Software, and processed using TargetLynx XS Application Manager. Table 1 summarizes all MRM transitions. Figure 2 shows the retention time windows of the MRM method.

Compound	Transitions	Cone voltage (V)	Collision energy (eV)
Aniline	93.8>77.0	40	15
o-Toluidine	107.8>91.0	40	15
	107.8>93.0	40	15
2,4-Diaminotoluene	122.8>106.2	40	15
	122.8>108.3	40	18
o-Anisidine	123.9>65.0	40	20
	123.9>109.0	40	15
4-Chloroaniline	127.8>93.1	40	18
	129.8>93.1	40	18
3-Chloro-o-toluidine	140.8>77.1	40	10
	140.8>95.1	40	10
2,4,5-Trimethyl aniline	135.9>91.0	40	20
	135.9>121.0	40	15
2-Methoxy-5-methylaniline	137.8>78.1	40	25
	137.8>123.1	40	15
4-Chloro-2-methylaniline	141.8>107.0	40	15
	141.8>125.0	40	18
2-Amino naphthalene	143.8>117.1	40	20
	143.8>127.0	40	20
2-Methyl-5-nitroaniline	152.8>107.0	40	15
	152.8>121.0	40	10
4-Aminobiphenyl	169.9>92.0	40	20
	169.9>152.1	40	25
2-Aminobiphenyl	169.9>92.0	40	20
	169.9>152.1	40	25
Benzidine	184.9>167.1	40	25
	184.9>168.1	40	18
4-Phenyl azoaniline	197.95>77.0	40	18
	197.95>105.0	40	12
4,4'-Diamino diphenylmethane	199.0>77.1	40	22
	199.0>106.0	40	22
4,4'-Oxydianiline	200.95>108.0	40	20
	200.95>184.1	40	20
3,3'-Dimethyl benzidine	213.0>180.0	40	30
	213.0>196.0	40	30
4,4'-Thiodianiline	216.95>124.0	40	20
o-Amino azotoluene	226.0>91.0	40	20
3,3'-Dimethyl-4,4'-diaminodiphenylmethane	227.0>120.2	40	20
3,3'-Dimethoxy benzidine	245.0>213.1	40	18
	245.0>230.1	40	18
3,3'-Dichloro benzidine	252.9>182.1	40	25
	252.9>217.0	40	20
4,4'-Methylene bis (2-chloroaniline)	266.9>140.1	40	25
	266.9>231.1	40	22

Table 1. Overview of MRM transitions for all 23 PAAs.

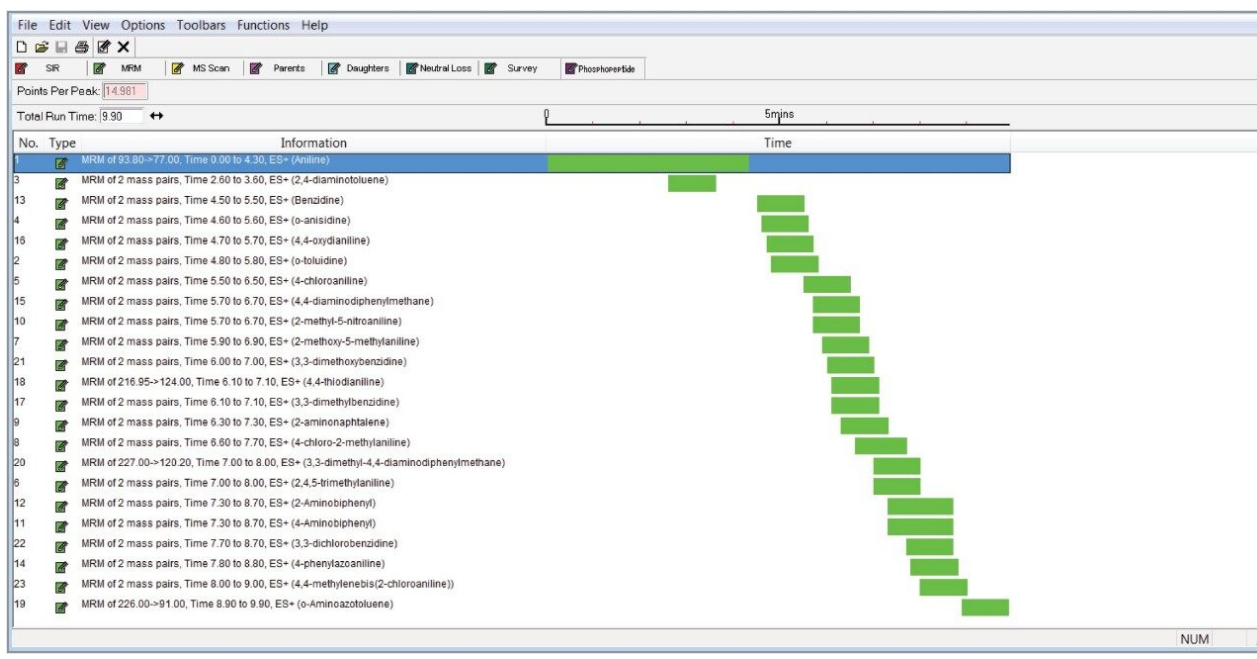


Figure 2. Retention time windows for the PAAs acquisition method.

Standards

A mixed standard solution containing all PAAs at a concentration of 100 µg/mL was used. The working standards were further diluted with the 3% acetic acid food stimulant solution. For the solvent calibration a dilution series starting at 100 ng/mL down to a level of 0.78 ng/mL was made.

Sample Preparation

Nine polyamide kitchenware utensils were extracted with a 3% acetic acid solution according to the procedure described in the EU 10/2011 guidelines.¹

Results and Discussion

UPLC Method Development

Because of the basic properties of PAAs, and the fact that acetic acid is used as a migration stimulant, some PAAs don't focus well on the head of the column, resulting in poor peak shape and/or loss of retention.

Aniline elutes early and is therefore prone to this effect. As a result, some literature references cite the use of

ion-pair reagents.⁴ Adding ammonium hydroxide to the 3% acetic acid samples prior to injection, the pH of the sample is increased and the polar and weakly basic PAAs such as aniline will be in their neutral form. A volume of 10 μL of a 25% NH_4OH solution was added to 1 mL of sample. This approach resulted in more robust results and is therefore preferred over the use of ion-pair reagent. Figure 3 shows a chromatogram of aniline with an unchanged pH (top) and adjusted pH (bottom). The neutralization of the pH drastically improves the peak shape of aniline, without the need for ion-pairing reagent.

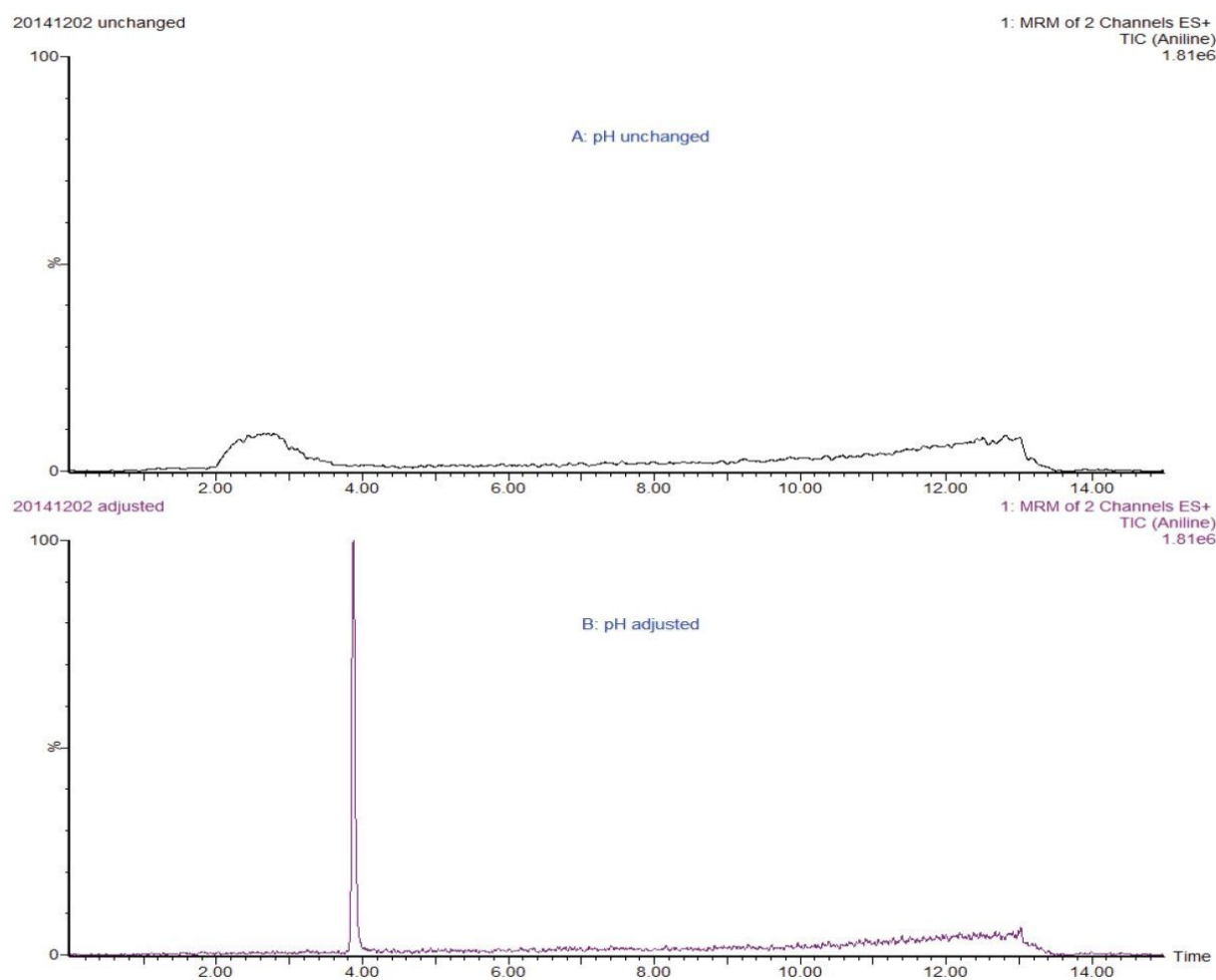


Figure 3. Chromatogram of aniline in 3% acetic acid food stimulant without (top) and with (bottom) pH adjustment.

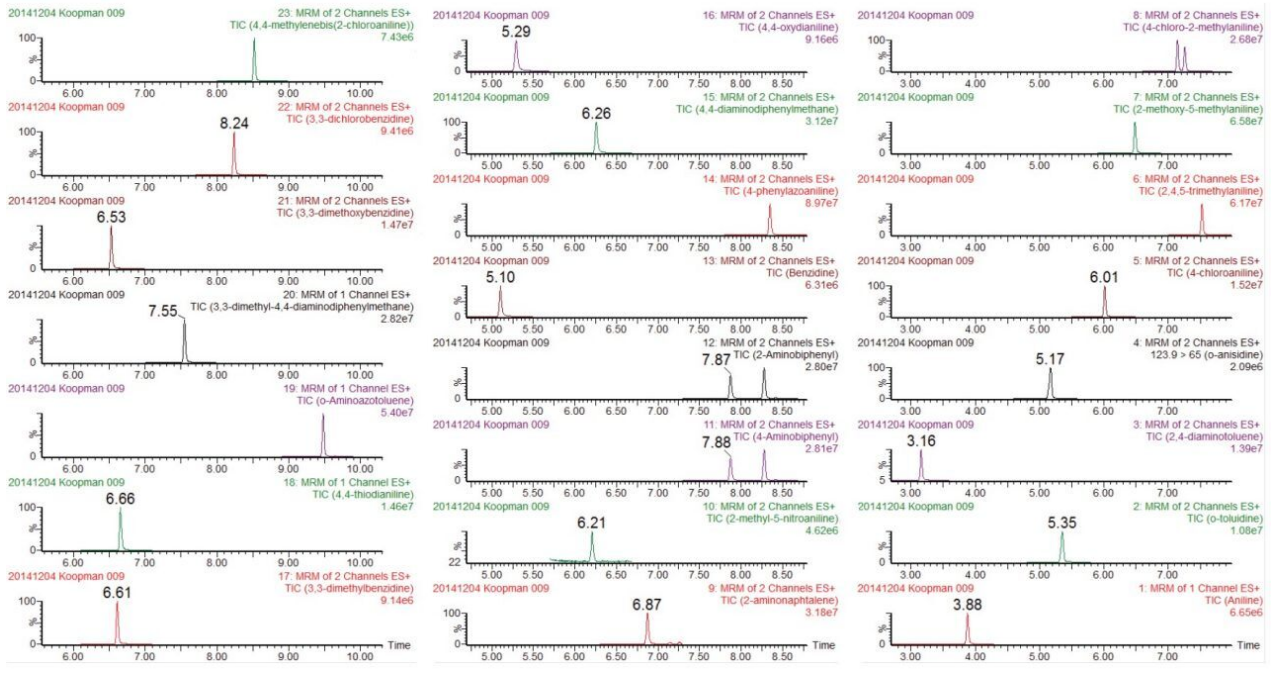


Figure 4. Chromatograms of all 23 PAAs.

Linearity

Calibration curves were prepared from 0.78 ng/mL to 100 ng/mL for all compounds. An example is given for aniline (Figure 5). For each calibration curve, a linear regression and a 1/X weighting was applied. All compounds show good linearity across the range of concentrations as well as excellent % residual values.

Compound name: Aniline
Correlation coefficient: $r = 0.999930$, $r^2 = 0.999859$
Calibration curve: $11380.5 * x + -898.041$
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

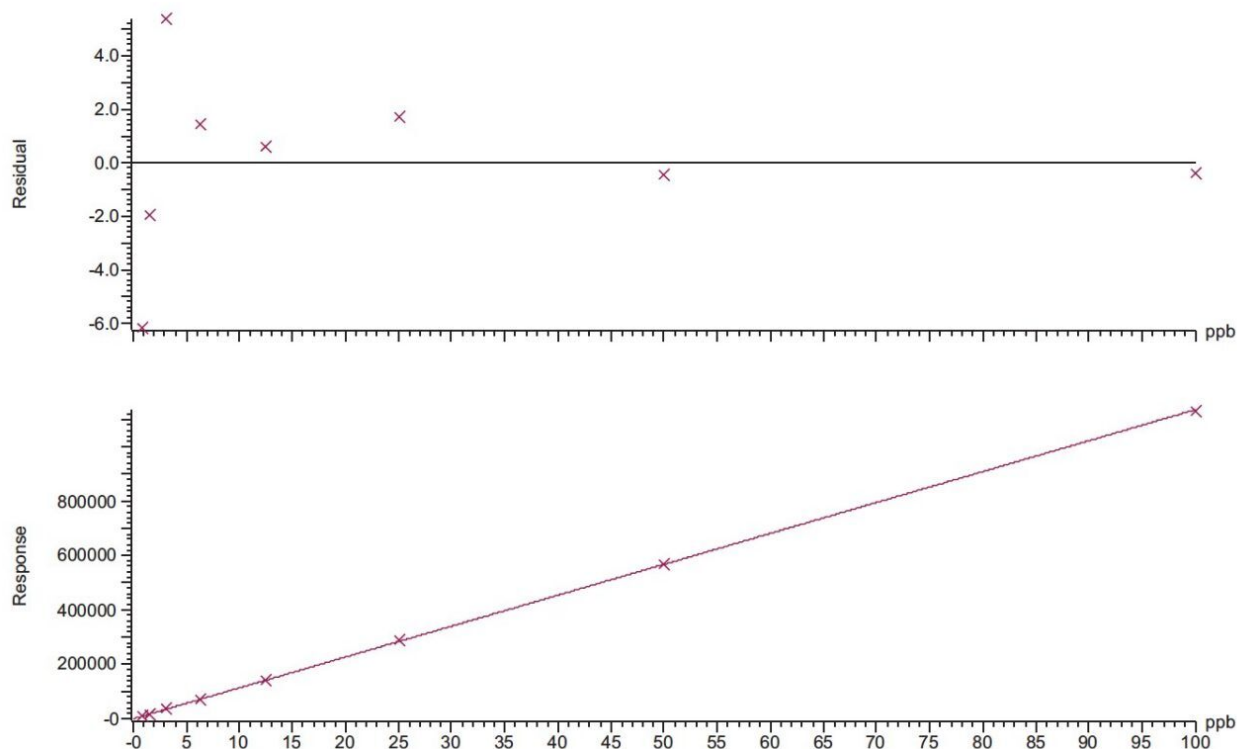


Figure 5. Calibration curve (bottom) and residuals plot (top) for aniline in the range 0.78 to 100 ng/mL.

Acidified mobile phases aid in the protonation of compounds and therefore improve the sensitivity in positive ion electrospray. As no acid was added to the mobile phases, we investigated whether a post-column addition (PCA) with formic acid would be beneficial. Using the Xevo TQ-S micro's built-in IntelliStart fluidics, a solution of 2% formic acid was infused at a constant flow rate of 20 $\mu\text{L}/\text{min}$ into the UPLC flow exiting the column. As such the formic acid solution was diluted 20-fold with the mobile phase, resulting in a final concentration of 0.1% of formic acid going into the ESI source. Figure 6 shows how this PCA was configured in the acquisition method, while Figure 7 shows the chromatograms for a selection of PAAs with (top trace) and without (bottom trace) this post-column addition. For better interpretation, the intensity axes have been linked. As can be seen from the chromatograms, the sensitivity is significantly improved when formic acid is added to the eluent.

Events			Initial Settings	
Time / Mins	Event	Action	Stop flow	No Change
0.00	Stop flow	On	Switch 2	No Change
0.50	Flow State	Combined	Switch 3	No Change
0.50	Refill	Refill	Switch 4	No Change
0.50	Reservoir	A	Infusion	No Change
0.50	Flow Rate	20	Flow State	LC
0.51	Infusion	Start	Flow Rate $\mu\text{l}/\text{min}$	20.0
			Reservoir	A
			Refill	Refill
			Volume μl	250
<input type="button" value="Add"/> <input type="button" value="Change"/> <input type="button" value="Delete"/> <input type="button" value="Clear All"/>			Solvent Delay Options	
			API Probe Temperature $^{\circ}\text{C}$	20
<input checked="" type="checkbox"/> Enable			<input type="button" value="OK"/> <input type="button" value="Cancel"/>	

Figure 6. Post-column addition in the MS acquisition method.

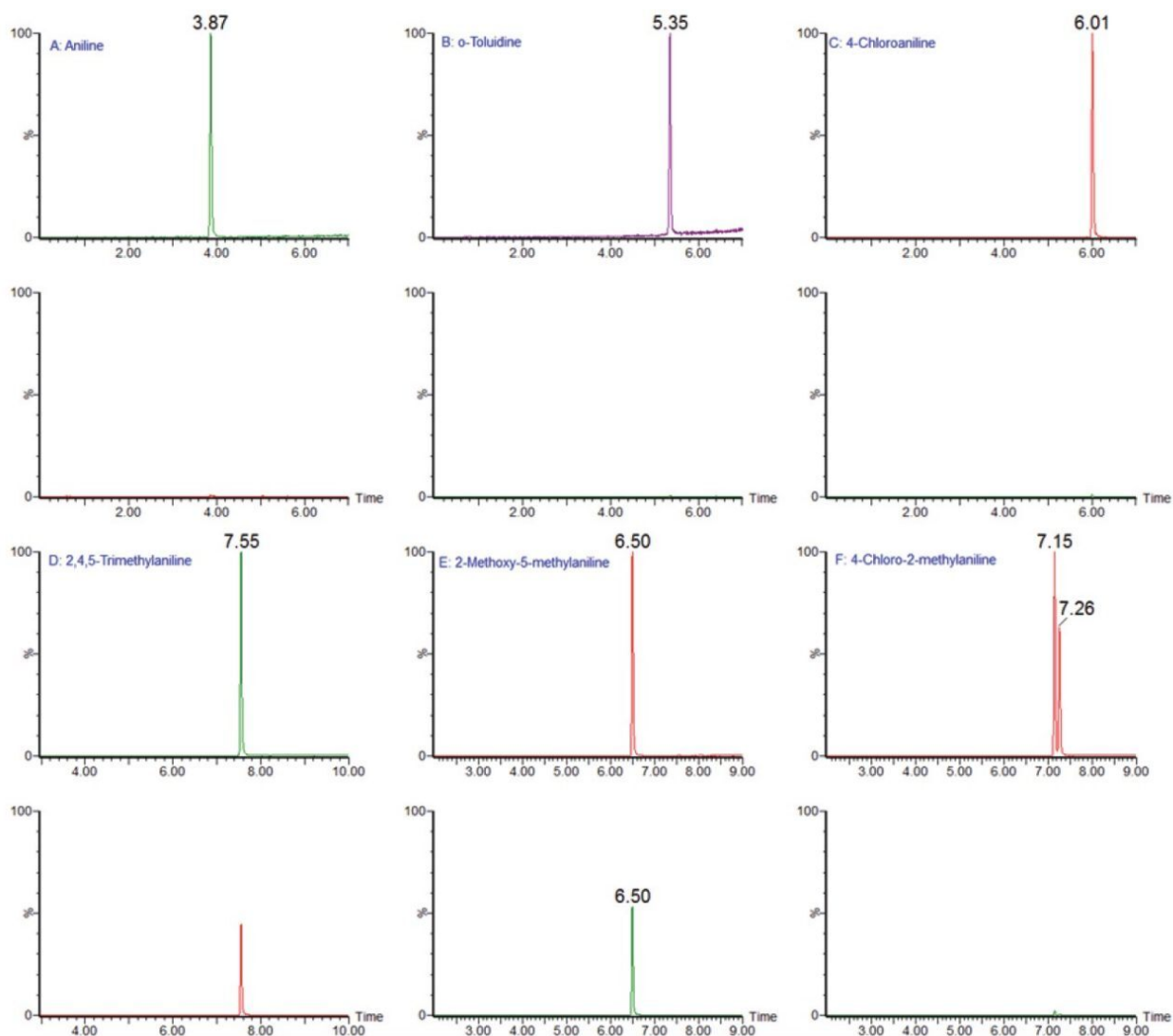


Figure 7. Increase in sensitivity with the use of a formic acid post-column addition (top), and without (bottom), illustrated for:

A. aniline,

B. *o*-Toluidine,

C. 4-Chloroaniline,

D. 2,4,5-Trimethylaniline,

E. 2-Methoxy-5-methylaniline, and

F. 4-Chloro-2-methylaniline.

Table 2 summarizes the quantitation limits (LOQ) for all compounds using this PCA approach. The LOQ is defined as the concentration giving rise to a signal-to-noise (S/N) value of 10:1. For the calculation of S/N, raw data was used and the peak-to-peak algorithm was applied. An extrapolation was made in most cases,

as the reported S/N values were still significantly high, even at the lowest reported standard level of 0.78 ng/mL. Calculated LOQs below 20 pg/mL are not mentioned specifically but are cut off at this level. The reported LOQ concentrations range between 20 pg/mL and 300 pg/mL.

Compound	S/N ratio	LOQ (ng/mL)
Aniline	377	0.02
o-Toluidine	768	<0.02
2,4-Diaminotoluene	52	0.15
o-Anisidine	89	0.09
4-Chloroaniline	323	0.03
2,4,5-Trimethyl aniline	693	<0.02
2-Methoxy-5-methylaniline	1444	<0.02
4-Chloro-2-methylaniline	3503	<0.02
2-Amino naphthalene	1858	<0.02
2-Methyl-5-nitroaniline	27	0.29
4-Aminobiphenyl	226	0.04
2-Aminobiphenyl	272	0.03
Benzidine	559	<0.02
4-Phenyl azoaniline	1931	<0.02
4,4'-Diamino diphenylmethane	1353	<0.02
4,4'-Oxydianiline	312	0.03
3,3'-Dimethyl benzidine	165	0.05
4,4'-Thiodianiline	2582	<0.02
o-Amino azotoluene	1746	<0.02
3,3'-Dimethyl-4,4'-diaminodiphenylmethane	1818	<0.02
3,3'-Dimethoxy benzidine	528	<0.02
3,3'-Dichloro benzidine	926	<0.02
4,4'-Methylene bis (2-chloroaniline)	1522	<0.02

Table 2. Calculated S/N values at 0.78 ng/mL and estimated LOQ values for all 23 PAAs investigated.

Matrix Effects

Internal standards were not used in this method. Therefore it was investigated whether the food simulant extract leads to ion suppression. One of the samples was spiked to a final concentration of 10 ppb and this sample was compared with a standard dissolved in the same food stimulant solution. All spike recoveries were within 90% to 107%, indicating that matrix effects were low to non-existing for the 23 compounds under investigation.

Kitchenware Samples

Using the external calibration curves, nine kitchenware samples were quantified. Except for aniline and 4,4'-diamino diphenylmethane found in all nine samples at levels between 0.4 to 1.1 ppb and 0.04 to 0.11 ppb,

respectively, no other PAAs were detected. Figure 8 shows the chromatograms of aniline in the sample containing 0.4 ppb and of 4,4'-diamino diphenylmethane in the sample containing 0.04 ppb. As can be seen sensitivity was excellent at these sub ppb level.

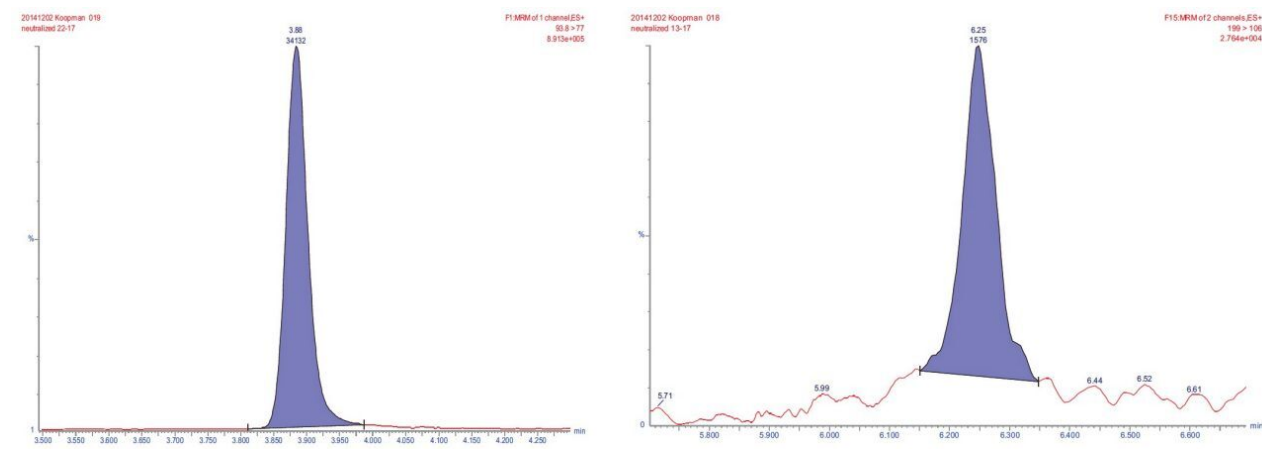


Figure 8. Chromatograms of aniline in kitchenware samples present at 0.4 ppb (left), and of 4,4'-Diamino diphenylmethane in the sample containing 0.04 ppb (right).

Conclusion

We have demonstrated a sensitive method for 23 PAAs with very easy sample preparation. The addition of ammonium hydroxide as neutralizing agent, and a post-column addition of formic acid into the Xevo TQ-S micro via IntelliStart's built-in fluidics – resulted in a very sensitive assay which could reach sub ppb levels. Linearity was observed over a large range and up to 100 ppb. The samples were all below detection limits except for aniline which was detected at 0.4 to 1.1 ppb, and 4,4'-diamino diphenylmethane which was detected at 0.04 to 0.11 ppb. The migration of all PAAs were compliant with the individual and summed SML (SML(T)) limits, as stipulated in Regulation EU 2020/1245.

References

1. Commission Regulation (EU) No 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to

Come into Contact with Food.

2. <https://op.europa.eu/en/publication-detail/-/publication/55eb5fee-edaf-11ea-991b-01aa75ed71a1/language-en> <<https://op.europa.eu/en/publication-detail/-/publication/55eb5fee-edaf-11ea-991b-01aa75ed71a1/language-en>>
3. <https://osha.europa.eu/en/legislation/directives/regulation-ec-no-1907-2006-of-the-european-parliament-and-of-the-council> <<https://osha.europa.eu/en/legislation/directives/regulation-ec-no-1907-2006-of-the-european-parliament-and-of-the-council>>
4. LB-NA-24815-EN-N, Technical Guidelines on Testing the Migration of Primary Aromatic Amines from Polyamide Kitchenware and of Formaldehyde from Melamine Kitchenware.
5. Analysis of Primary Aromatic Amines (PAA) in Black Nylon Kitchenware 2014. Selected Samples from the Norwegian Market.

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[ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>](https://www.waters.com/134613317)

[Xevo TQ-S micro Triple Quadrupole Mass Spectrometry <https://www.waters.com/134798856>](https://www.waters.com/134798856)

[MassLynx MS Software <https://www.waters.com/513662>](https://www.waters.com/513662)

[TargetLynx <https://www.waters.com/513791>](https://www.waters.com/513791)

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