For Research Use Only. Not for Use In Diagnostic Procedures.

Food and Environmental

SCIEX

Rapid Determination of 33 Primary Aromatic Amines in Kitchen Utensils, Textiles Dyes and Food Packaging Materials

Xiaojie Sun[1]，Haiyan Cheng[1], Lijun Li[1]，Jianru Stahl-Zeng[2], Ashley Sage[3] and Wenhai Jin[1]
[1.]SCIEX，Shanghai，CHINA；[2.]SCIEX，Darmstadt，DE; [3]SCIEX, UK

Introduction

Primary Aromatic Amines (PAAs) are a class of compound of which the simplest form is aniline. 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.

Because of the potential health risk, specific migration limits are put in place. According to the regulation on plastics EU/2011[1], plastic materials and articles shall not release PAAs in a detectable quantity into food or food simulant. The LOD of 0.01 mg of substance per kg of food or food simulant is applied to the sum of PAAs released. Recently, the Federal Institute for Risk Assessment in Germany released an opinion and suggested that the limit value of not detectable with an LOD of 0.01 mg kg^{-1} food for PAAs indicated in Annex Ⅱ of Regulation (EU) No 10/2011 should be applied to PAAs in kitchen utensils, textiles dyes and food packaging.

The Challenge of Small Basic Compounds

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 or loss of retention. 33 PAAs including 4 groups isomer which increase the difficulty of separation.
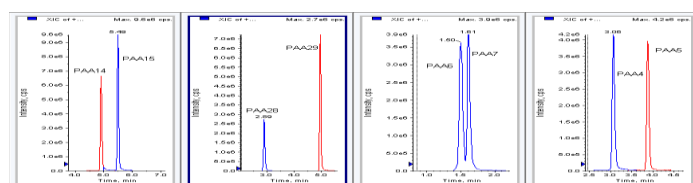

Figure 1. SCIEX Triple Quad^{TM} 5500 System

Key Advantage Presented

All compounds were well retained and had good shape. 4 group isomers have a good separation on the column of Kinetex F5.

The whole time of the separation method is 12 minutes which can improve the efficient of analysis.

Under the three concentrations of 0.1 ng/ml, 5 ng/ml, 20 ng/ml, the recoveries of the method were from 82.1% to 105.7%, the RSD% (n=6) of all PAAs were less than 9%.

Matrix effects were largely eliminated using two simulant solvents. There were almost no matrix effects for all PAAs when using the simulant C of 20% ethanol, but then over 50% matrix effects were observed for PAA 30 and PAA 32, and there were no matrix effects for the other compounds when using the simulant B of 3% acetic acidic. This may because of the formation of the ionic state of the two compounds with acetic acidic, which could cause the ionization efficiency becomes worse.
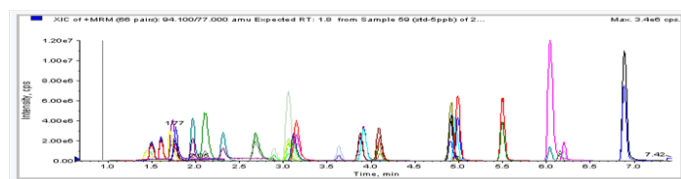

Figure 2. Isomers Separation on Kinetex F5 Column

Figure 3. XIC Chromatograph of 33 PAAs

Experimental Consideration

Sample Extraction

All samples were extracted with a 3% acetic acid solution and 20% ethanol solution according to the procedure described in the EU 10/2011 guidelines.

Briefly, kitchen utensils, textiles dyes and food packaging were cut into pieces approximately 1-2 cm^2 in size. A total of 10 g of samples were weighted into a 200 ml conical flask. 20% ethanol was used as simulated solvent in the food packaging migration experiment, which was immersed at 40°C for 7 days. 3% acetic acid was used as simulated solvent in the kitchen utensils, textiles dyes migration experiment, which was immersed at 100°C for 2 hours.

For the determination of the recovery, samples which on the basis of previous tests did not contain any PAAs, were spiked with three levels of a solution containing a mix of PAAs (0.1, 5, 20 ng/ml) in duplicate and the spiked samples were extracted and analysed as described above. The recovery was calculated for each PAA from the difference of results for the spiked samples and the simulants.

LC Separation and MS-MS Detection

LC: SCIEX ExionLC^{TM} AD

Column: Phenomenex, Kinetex 2.6 μm F5 100 Å, 100 X 3.0 mm

Mobile Phase: A 0.05% Formic Acid; B MeOH

Flow Rate: 0.3 ml/min

Column Temp: 40 ℃

Injection Volume: 5 μL

Run Time: 12 min

Separation Gradient is shown in Table 1

MS: SCIEX Triple Quad^{TM} 5500 System

Acquisition Modes: scheduled-MRM

Source parameters are shown in Table 2

LC Analysis

During verification of the method, the primary focus was on achieving stable peak shapes and retention times for all analytes. Initial conditions, gradient and pH of the mobile phase had very significant effects, so the final optimized method should be fixed, and fresh mobile phases prepared regularly. The content of formic acid, the content of initial methanol and the selection of the column should be optimized to improve the sensitivity and the separation for all PAAs. Compared with 0.0% formic acid, 0.01%formic acid, 0.05% formic acid, 0.1% formic acid, and 0.2% formic acid in water, the best sensitivity may be

Table 1. LC Separation Gradient

Time (min)	A (%)	B (%)
0	70	30
7	10	90
9	5	95
9.1	70	30
12	70	30

Table 2. Source parameters for the SCIEX 5500 System

Source Parameters	
Curtain Gas(CUR)	30pis
Collision Gas(CAD)	9pis
IonSpray Voltage(IS)	1200v
Temperature(TEM)	600°C
Ion Source Gas(GS1)	65pis
Ion Source Gas(GS2)	60pis

observed when added 0.05% formic acid. Also, we optimized the initial percent of methanol for separation and sensitivity. We found that the higher of the initial percent of methanol, the best of sensitivity for all PAAs, but the worse of separation for isomers, so when the initial percent of methanol is 30%, we got the best results between sensitivity and separation. Finally, we also compared with different columns, such as Phenomenex Omega; Phenomenex F5; Waters HSS T3, and finally we found that in order to get the best separation for the isomers, F5 column give us the best results, so F5 was chosen as the separation column.

MS-MS Analysis

Analyses were performed using a SCIEX Triple Quad^{TM} 5500 mass spectrometer in positive electrospray ionization mode. At least two MRM transitions were optimized for each analyte as outlined in Table 3. Data was acquired using Analyst® 1.6.3 .and processed for quantitation and confirmation using MultiQuant™ 3.0.2 software. Figure 4 shows the chromatograph of 25 PAAs.
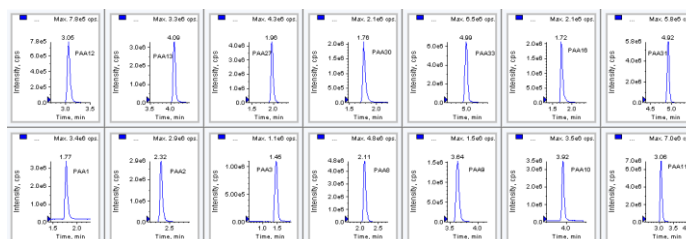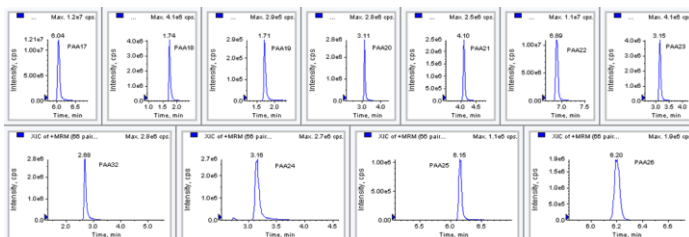
Figure 4. Extracted 25 PAAs excluding 4 groups of isomer

Source parameters were optimized for the best sensitivity. During the optimization, we found that the IonSpray Voltage should be set to 1200v for the best sensitivity of all PAAs. We can see the optimized result of IonSpray Voltage in Figure 5. Obviously, due to the addition of formic acid, each compound may reach the best sensitivity under the 1200v IonSpray Voltage.
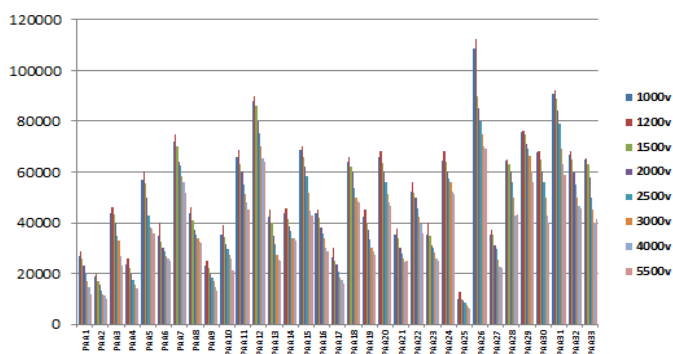

Figure 5. Optimized the IonSpray Voltage for each compound

Results and Discussion

Analytical performances

Repeatability of the analysis was calculated on 0.1 ng/ml, 5 ng/ml, 20 ng/ml injections of 3% acetic acid and 20% ethanol solutions spiked with 0.1, 5, 20 ng/ml of each PAA and it ranged from 1.9% to 8.9%. Within-laboratory reproducibility (intermediate precision) was also calculated by repeating the procedure described for repeatability at three different times and it ranged from 2.3% to 9.1%. Average recoveries were >85% for 30 PAAs and 60 - 85% for 3 PAAs, showed in Table 4.

The linearity of instrument response evaluated in a concentration range between 0.05 and 50 ng/ml showed very good regression coefficients for all the PAAs (0.992- 0.999), showed in Table4. LODs were in the range of 0.001-0.029 ng/ml for 33 PAAs. LODs were lower than the limit of total PAA of 10 ng/ml in the EU plastic FCM Regulation (EU) No 10/2011.

Sample analysis

The survey on the samples showed in the Table 5 from the market. Using the external calibration curves, three series samples were quantified.

Table 5. List of samples collected from the market

Sample Type		
kitchen utensils	plastic spoon	5
	plastic fork	5
	paper cup	2
textiles dyes	coloured paper napkin	5
food packaging	plastic package of cookie	5

Some of PAAs detected in samples, but total concentrations of PAAs in all samples were lower than 10 ng/ml, not exceeding the limit of total PAA in the EU plastic FCM Regulation (EU) No 10/2011. Figure 6 shows the chromatograms of some detected PAAs, we can see the detected results in Table 6.
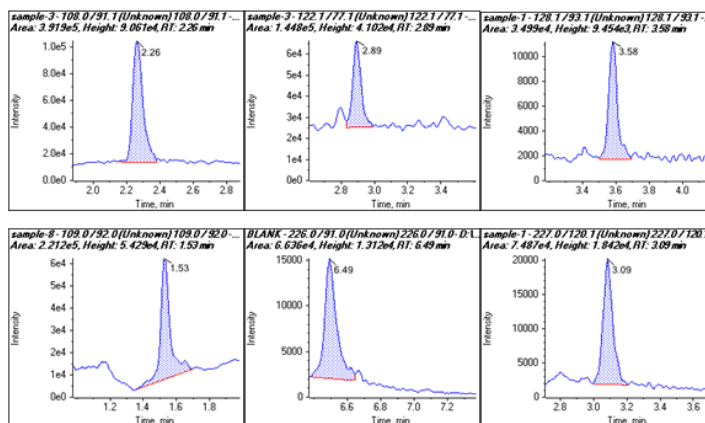

Figure 6. Chromatograph of some PAAs in samples

Conclusions

This study developed a multi-analyte method based on UHPLC-MS/MS quantification for the analysis of PAAs content in kitchen utensils, textiles dyes and food packaging from the market. We have demonstrated a sensitive method for 33 PAAs with very easy sample preparation. Linearity was observed over a large range and up to 50 ng/ml. The samples were all below detection limits. The total PAAs content for all samples was below the 0.01 mg/kg as stipulated in the regulations EU 10/2011.

Table 3. List of analytes with MRM transitions

Compound	MH+	Product ion(Q/C)ᵃ
Aniline (PAA 1)	94.1	77/51
o-Toluidine (PAA 2)	108	91.1/93
M-phenylenediamine (PAA 3)	109	92/65
2,4-dimethylaniline (PAA 4)	122	77.1/105.1
2,6-dimethylaniline (PAA 5)	122	77.1/105.1
2,4-diaminotoluene (PAA 6)	123.1	106.1/77.1
2,6-diaminotoluene (PAA 7)	123.1	106.1/77.1
o-Anisidine (PAA 8)	124.1	109.1/65
4-chloroaniline (PAA 9)	128.1	93.1/75
2,4,5-trimethylaniline (PAA 10)	136	91.1/121.1
P-cresidine (PAA 11)	138	123.1/78
4-methoxy-1,3'-phenylenediamine (PAA 12)	139	124.1/108.1
2-naphthylamine (PAA 13)	144	127/77.1
4-aminobiphenyl (PAA 14)	170	152/153.1
2-aminobiphenyl (PAA 15)	170	152/153.1
4,4-diaminobiphenyl (PAA 16)	185	168.1/167
4-aminoazobenzene (PAA 17)	198	77.1/105.1
Bis-(4-aminophenyl)methane (PAA 18)	199	106.1/77
4,4'-oxydianiline (PAA 19)	201	108.1/184
3,3'-dimethylbenzidine (PAA 20)	213	180/196.1
4,4'-diaminodiphenylsulfide (PAA 21)	217	124/200
o-Aminoazotoluene (PAA22)	226	91/121.1
4,4'-diamino-3,3'-dimethyliphenylmethane (PAA 23)	227	120.1
3,3'-dimethoxybenzidine (PAA 24)	245.1	230.1/187.1
3,3'-dichlorobenzidine (PAA 25)	253.1	217.1/182.1
4,4'-methylene-bis(2-chlorozniline) (PAA 26)	267	231.1/140.1
3-amino-4-methylbenzamide (PAA 27)	151	108.1/93
3-chloro-4-methoxyaniline (PAA 28)	158	123.1/80.1
5-chloro-2-methoxyaniline (PAA 29)	158	143/108.1
1,5-diaminonaphtalene (PAA 30)	159	115.1/143.1
4-chloro-2,5-dimethoxyaniline (PAA 31)	188	173.1/130
3-amino-p-anisanilide (PAA 32)	243	212.1/227
2-methoxy-4-nitroaniline (PAA 33)	169	152/122.1

Note: ᵃ (Quantitation Ion / Confirmation Ion)

Table 4. Main analytical parameters [a]   **Table 6. Some detected PAAs in samples**

PAA	Equation	R	LOD(ng/ml)	LOQ(ng/ml)	RSD(%)	Rec(%)
Aniline	y=1.905e6x+1.604e5	0.9962	0.029	0.09	8.9	94.2
o-Toluidine	y=2.12e6x+14137	0.9981	0.012	0.04	3.67	87.4
M-phenylenediamine	y=1.329e6x+10356	0.9998	0.02	0.062	2.22	106.1
2,4-dimethylaniline	y=1.7840e6x+8177	0.9977	0.01	0.041	2.08	103.4
2,6-dimethylaniline	y=1.6121e6x+25871	0.9975	0.009	0.033	1.9	105.9
2,4-diaminotoluene	y=1.444e6x+23110	0.9995	0.011	0.045	4.12	98.3
2,6-diaminotoluene	y=1.23246e6x+20386	0.9930	0.008	0.028	2.89	94.3
o-Anisidine	y=4.354e6x+3.0078e4	0.9945	0.0049	0.015	4.83	100.9
4-chloroaniline	y=1.1465e6x+5264	0.9987	0.0048	0.021	4.9	86.8
2,4,5-trimethylaniline	y=2.5363e6x+25932	0.9972	0.01	0.034	3.68	91.9
P-cresidine	y=6.667e6x+3.307e4	0.9966	0.001	0.0045	3.98	99.4
4-methoxy-1,3'-phenylenediamine	y=5.8050e6x+2242	0.9993	0.01	0.05	3.20	109.5
2-naphthylamine	y=2.5801e6x+3999.3	0.9991	0.005	0.016	2.96	105.5
4-aminobiphenyl	y=2.8274e6x+8232.5	0.9972	0.01	0.038	4.30	89.1
2-aminobiphenyl	y=3.8334e6x+4.6294e4	0.9899	0.01	0.030	4.16	87.9
4,4-diaminobiphenyl	y=1.5268e6x+13989	0.9984	0.005	0.019	3.09	93.1
4-aminoazobenzene	y=1.4826e6x+7.47e5	0.9958	0.001	0.0036	3.25	91.0
Bis-(4-aminophenyl)methane	y=3.0494e6x+3.23e4	0.9980	0.005	0.018	2.65	98.2
4,4'-oxydianiline	y=2.2383e6x+18163	0.9987	0.001	0.0033	2.08	95.5
3,3'-dimethylbenzidine	y=2.269e6x-8183	0.9990	0.01	0.045	2.77	107.3
4,4'-diaminodiphenylsulfide	y=1.769e6x+9944	0.9988	0.01	0.048	3.46	106.1
o-Aminoazotoluene	y=1.1079e7x+4.6817e5	0.9944	0.001	0.0041	4.03	86.0
4,4'-diamino-3,3'-dimethyliphenylmethane	y=3.4679e6x+29360	0.9955	0.005	0.015	3.49	85.8
3,3'-dimethoxybenzidine	y=2.6076e6x+5.7198e4	0.9955	0.05	0.13	3.37	100.7
3,3'-dichlorobenzidine	y=6.5351e6x+27514	0.9992	0.01	0.030	3.33	99.5
4,4'-methylene-bis(2-chlorozniline)	y=1.0702e6x+4.7568e4	0.9986	0.005	0.0178	3.54	101.1
3-amino-4-methylbenzamide	y=3.0257e6x+27540	0.9952	0.005	0.0164	3.66	109.4
3-chloro-4-methoxyaniline	y=1.0783e6x+3546	0.9997	0.01	0.0334	3.15	102.3
5-chloro-2-methoxyaniline	y=2.8078e6x+26640	0.9957	0.005	0.0152	3.45	85.2
1,5-diaminonaphtalene	y=1.4086e6x+2846	0.9992	0.02	0.068	2.59	64.2
4-chloro-2,5-dimethoxyaniline	y=3.681e6x+4.046e4	0.9923	0.005	0.0193	4.23	86.4
3-amino-p-anisanilide	y=2.269e6x+2.763	0.9962	0.05	0.163	3.17	69.1
2-methoxy-4-nitroaniline	y=4.946e6x+25359	0.9956	0.005	0.0152	2.69	88.3

Note: [a] Calibration curve equations, regression coefficients (R), limit of detection (LOD), limit of quantitation (LOQ), repeatability of three different concentrations, and mean recovery of 3 (0.1, 5, 20 ng/ml) spike levels.

PAA	Plastic Spoon	Plastic Fork	Paper cup	Napkin	Plastic packaging
o-Toluidine	0.05	0.04	N/A	0.07	0.04
M-phenylenediamine	0.04	N/A	N/A	0.04	N/A
2,4-dimethylaniline	N/A	N/A	N/A	0.13	0.038
2,6-diaminotoluene	0.048	0.078	0.057	0.088	N/A
4-chloroaniline	N/A	N/A	N/A	0.061	N/A
4,4-diaminobiphenyl	N/A	N/A	N/A	0.138	N/A
Bis-(4-aminophenyl)methane	0.023	0.031	N/A	0.23	N/A
4,4'-oxydianiline	0.047	0.038	N/A	0.028	N/A
4,4'-diaminodiphenylsulfide	N/A	N/A	N/A	0.025	N/A
o-Aminoazotoluene	N/A	N/A	0.02	0.027	0.021
4,4'-diamino-3,3'-dimethyliphenylmethane	N/A	N/A	0.065	0.022	N/A
3,3'-dichlorobenzidine	N/A	0.155	N/A	N/A	N/A
3-amino-4-methylbenzamide	N/A	N/A	N/A	0.136	N/A
3-chloro-4-methoxyaniline	0.32	N/A	N/A	N/A	N/A
5-chloro-2-methoxyaniline	N/A	0.197	N/A	0.222	0.049
3-amino-p-anisanilide	0.06	N/A	N/A	0.04	N/A

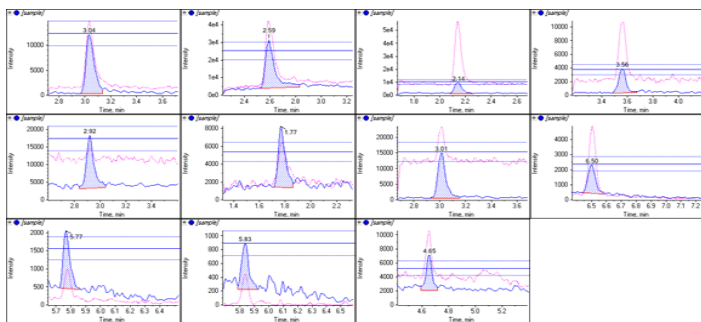Note: unit, ng/ml; N/A, not detected

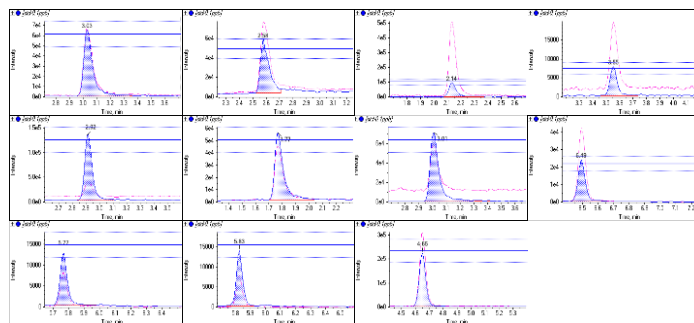**Figure 7. Chromatograph of some PAAs in samples with MRM Ratio Lines**

Figure 8. Chromatograph of Standard PAAs with MRM Ratio Lines

References

1. Commission regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Official Journal of the European Union L 12, 15.1.2011.
http://eur-lex.europa.eu/eli/reg/2011/10/oj

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices