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Applikationsbericht

The Analysis of Primary Aromatic Amines in Ink Using the ACQUITY UPLC H-Class System with the SQ Detector 2 and MassLynx Software

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Abstract

This application note describes the use of Waters ACQUITY UPLC H-Class coupled with the SQ Detector 2 for the rapid analysis of PAA in ink.

Benefits

This application provides improved confidence in the identification and quantification of Primary Aromatic Amines (PAAs) offering:

- Increased sample throughput and a reduction of solvent usage due to reduced run times.
- Improved sensitivity, selectivity, and robustness, compared with existing methodologies.
- The ultimate in chromatographic resolution and sensitivity.
- Cost-effective, reliable mass confirmation.

Introduction

PAAs are widely used in high amounts as a chemical feed stock within the chemical industry, and many of them are highly toxic to humans.[1,2,3] PAAs can be used to produce many commodities, such as pharmaceuticals, pesticides, explosives, epoxy polymers, rubber, aromatic polyurethane products, and azo-dyes. They can be found in final products due to incomplete reactions, as impurities, by-products, or as degradation products. PAAs can be produced as by-products of azo dyes, which are a diverse and widely used group of organic dyes. Azo dyes have a wide range of uses including special paints, printing inks, varnishes, and adhesives; and can be found in many products such as textiles, cosmetics, plastics, and also in food contact material.

The inks and dyes industry is highly legislated and manufacturers that use these materials must monitor and quantify various regulated parameters, such as the presence or absence of PAAs.

Previous example methodologies for the analysis of PAAs include: GC-MS analysis following ion-pair extraction with bis-2-ethyl phosphate followed by derivatization with isobutyl chloroformate;[4,5] UPLC analysis following a solid phase extraction (SPE) using cation-exchange cartridges;[6] and reduction by liquid phase sorbent trapping followed by thermal desorption GC-MS analysis.[7] Many previously used methods for

PAA analysis lack robustness, selectivity, and sensitivity, and require lengthy, costly and time-consuming pre-treatments (derivatization, SPE).

Many PAAs have either a proven or suspected carcinogenic nature and are highly toxic, so there are a range of potential health risks that have led to strict worldwide regulations. U.S. FDA regulations (21 CFR 74.705 and 21 CFR 74.706) restrict the use of azo dyes that could degrade to PAAs; whereas EU regulations (commission directive 2002/72/EC and the amendment 2007/19/EC) set legislative limits for the release of total PAAs from food contact material.

Analytical laboratories require accurate and robust techniques to ensure confidence and versatility in meeting these legislative requirements The SQ Detector 2 offers a flexible solution for the ink and dyes industry.

This application note describes the use of Waters ACQUITY UPLC H-Class coupled with the SQ Detector 2 for the rapid analysis of PAAs in ink.

Experimental

Sample preparation

Ink analysis

- Neat ink diluted 1:100 with 5% methanol/95% water.
- Diluted ink samples were transferred to LC vials for analysis.

Paper analysis

- For each experiment 10 cm x 10 cm pieces of paper were cut up and extracted with 100 mL of water for over 24 hours.
- Aliquots were transferred into LC vials for analysis.

LC conditions

LC system:	ACQUITY UPLC H-Class
Runtime:	10.00 min

Column:	ACQUITY UPLC BEH C_{18}, 1.7 mm, 2.1 x 50 mm
Column temp.:	40 °C
Mobile phase A:	10 mL of 1 M aqueous ammonium acetate solution and 990 mL water
Mobile phase B:	10 mL of 1 M aqueous ammonium acetate solution and 990 mL methanol
Flow rate:	0.5 mL/min
Injection volume:	10.0 µl

Mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.50	95	5	-
2	1.00	0.50	95	5	6
3	3.10	0.50	75	25	6
4	6.10	0.50	59	41	6
5	8.00	0.50	0	100	6
6	9.00	0.50	0	100	6
7	9.01	0.50	95	5	6
8	10.00	0.50	95	5	6

Table 1. ACQUITY UPLC H-Class mobile phase gradient.

MS conditions

MS system:	SQ Detector 2
Ionization mode:	ESI positive
Capillary voltage:	3.0 kV

Source temp.:	150 °C
Desolvation temp.:	350 °C
Desolvation gas:	650 L/hr
Cone gas:	20 L/hr
Acquisition:	Selected Ion Recording (SIR)

Variables such as cone voltages, desolvation gas (temperature and flow rate), and cone gas flow rate were optimized using solvent standards. The list of PAAs, associated CAS number, expected retention times, and cone voltages are detailed in Table 2. The established SIR MS method is illustrated in Figure 1.

PAA number	Primary Aromatic Amines (PAAs)	CAS Number	*m/z*	Retention time (minutes)	Cone Voltage (V)
1	Aniline	62-53-3	94	2.17	40
2	o-Toluidine	95-53-4	109	3.80	40
3	1,3-Phenylenediamine	108-45-2	109	0.62	40
4	1,4-Phenylenediamine	106-50-3	109	0.41	43
5	2,4-Dimethylaniline	95-68-1	122	5.58	43
6	2,6-Dimethylaniline	87-62-7	122	5.33	43
7	2,4-Toluenediamine	95-80-7	123	1.64	40
8	2,6-Toluenediamine	823-40-5	123	0.85	40
9	o-Anisidine	90-04-0	124	3.74	45
10	4-Chloroaniline	106-47-8	128	4.6	40
11	2,4,5-Trimethylaniline	137-17-7	136	7.06	40
12	2-Methoxy-5-methylaniline	120-71-8	138	5.36	40
13	4-Methoxy-m-phenylenediamine	615-05-4	139	1.51	36
14	2-Naphtylamine	91-59-8	144	6.18	40
15	3-Amino-4-methylbenzamide	19406-86-1	151	2.19	35
16	3-Chloro-4-methoxyaniline	5345-54-0	158	4.00	40
17	5-Chloro-2-methoxyaniline	95-03-4	158	6.06	40
18	1,5-Diaminonaphtalene	2243-62-1	159	2.52	40
19	2-Methoxy-4-nitroaniline	97-52-9	169	4.37	30
20	4-Aminobiphenyl	92-67-1	170	7.57	43
21	2-Aminobiphenyl	90-41-5	170	7.71	50
22	Benzidine	92-87-5	185	4.01	43
23	4-Chloro-2,5-dimethoxyaniline	6358-64-1	188	5.79	40
24	4-Aminoazobenzol	60-09-3	198	7.84	30
25	4,4'-Methylenedianiline	101-77-9	199	5.64	43
26	4,4'-Diaminodiphenylether	101-80-4	201	4.36	45
27	3,3'-Dimethylbenzidine	119-93-7	213	6.01	43
28	4,4'-Thioaniline	139-65-1	217	6.29	43
29	o-Aminoazotoluene	97-56-3	226	8.28	43
30	4,4'-Diamino-3,3'-dimethylbiphenylmethane	838-88-0	227	7.39	40
31	3-Amino-p-anisanilide	120-35-4	243	6.06	40
32	o-Dianisidine	119-90-4	245	6.00	45
33	3,3'-Dichlorobenzidine	91-94-1	253	7.76	45
34	4,4'-Diamino-3,3'-dichlorobiphenylmethane	101-14-4	267	7.90	60

Table 2. PAAs, associated CAS number, m/z, expected retention times, and cone voltages.

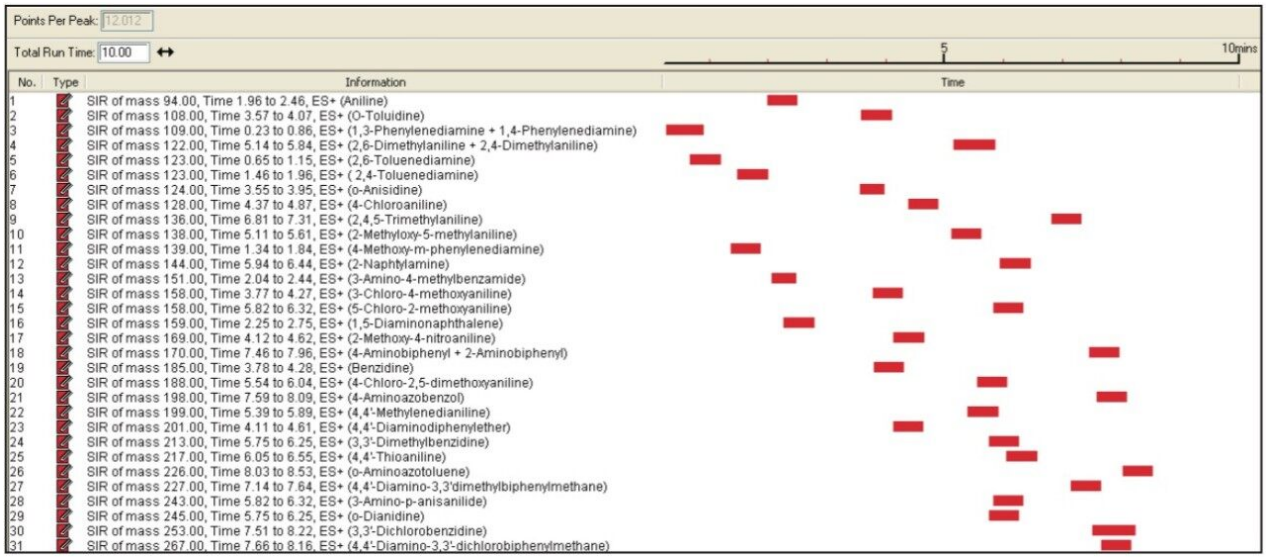


Figure 1. PAAs SIR method. 34 compounds covered over 31 channels (including 3 isomer pairs).

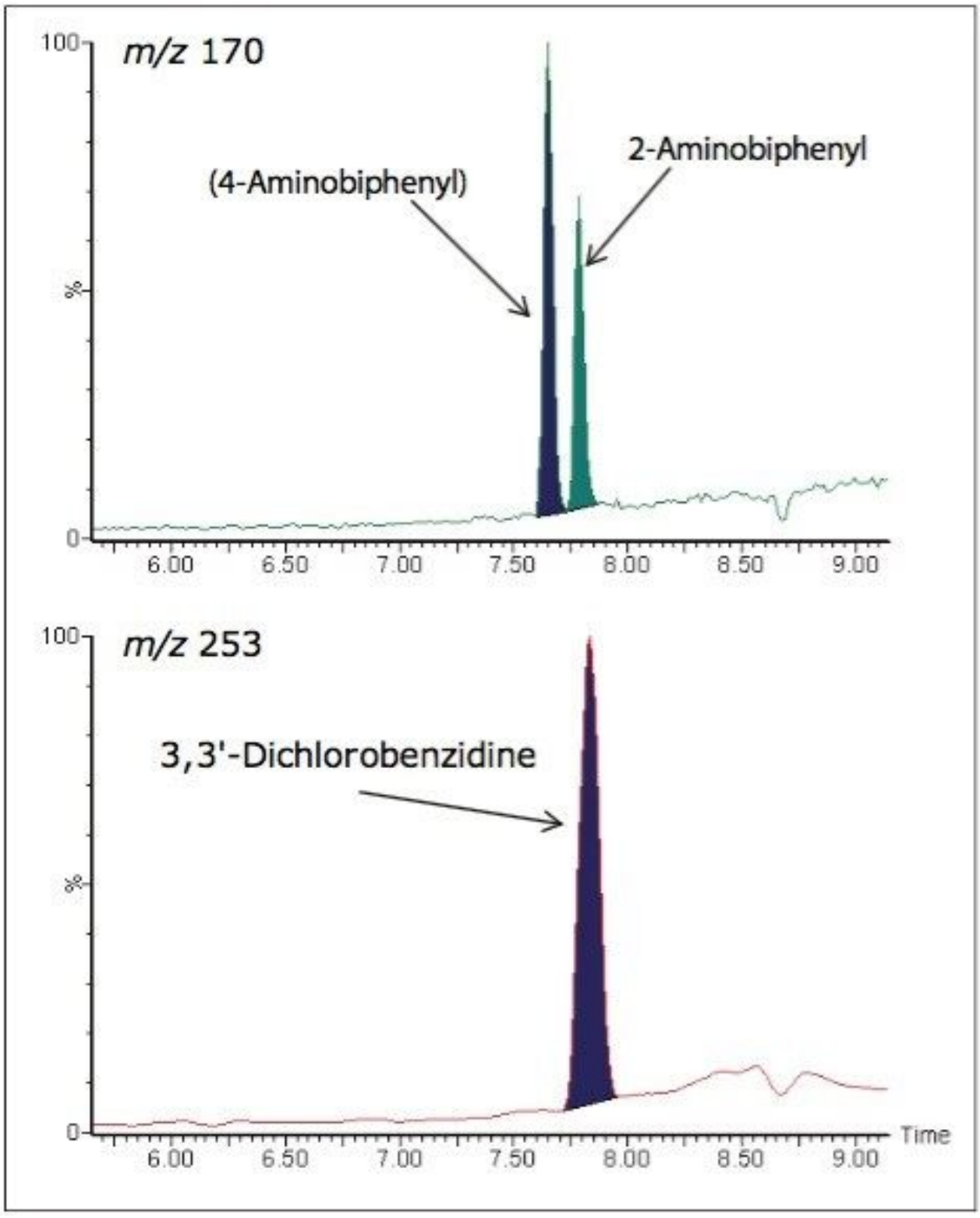
Instrument control, data acquisition, and result processing

MassLynx Software v.4.1 was used to control the ACQUITY UPLC H-Class and the SQ Detector 2 and also for data acquisition. Data quantitation was achieved using TargetLynx Application Manager.

The advantages of mass spectral detection over core detectors

Many gains can be accomplished using an ACQUITY UPLC System for chromatographic separation, due to the reduced column particle size (sub-2 µm), which results in improvements in speed and peak capacity, with superior sensitivity and resolution efficiently achievable over HPLC analysis.

During method development, considerations need to be given to the appropriate detector to use in order to meet the analytical requirements. The use of mass spectral detection over core detectors (e.g. UV or fluorescence) offers advantages in areas such as sensitivity and selectivity, especially where complex matrices are present. Matrix effects can be greatly reduced by using mass spectral detection over DAD (UV) detection and this can be demonstrated by considering many of the PAAs detailed within this application. Examples can be seen considering the PAAs, 2-Aminobiphenyl and 3,3'-Dichlorobenzidine. When using the current UPLC conditions the two compounds are not completely resolved giving retention times of 7.71 and 7.76 minutes respectively. Using mass spectral detection, the resulting efficient selectivity is illustrated in Figure 2.



In this example when using UV detection due to the UV absorbing nature of the solvents used, the ink matrix, and other PAAs present this level of selectivity is very hard to achieve. This reduced selectivity can be

demonstrated by again considering the PAAs, 2-Aminobiphenyl and 3,3'-Dichlorobenzidine in solvent standards. When considering individual solvent standards for 2-Aminobiphenyl and 3,3' Dichlorobenzidine, maximum UV absorbance can be found at 295 and 284 nm respectively. When comparing individual solvent standards against mixed solvent standards, the reduction in selectivity is demonstrated in Figures 3a and 3b, which could potentially lead to misidentification, poor integration, and false positive results.

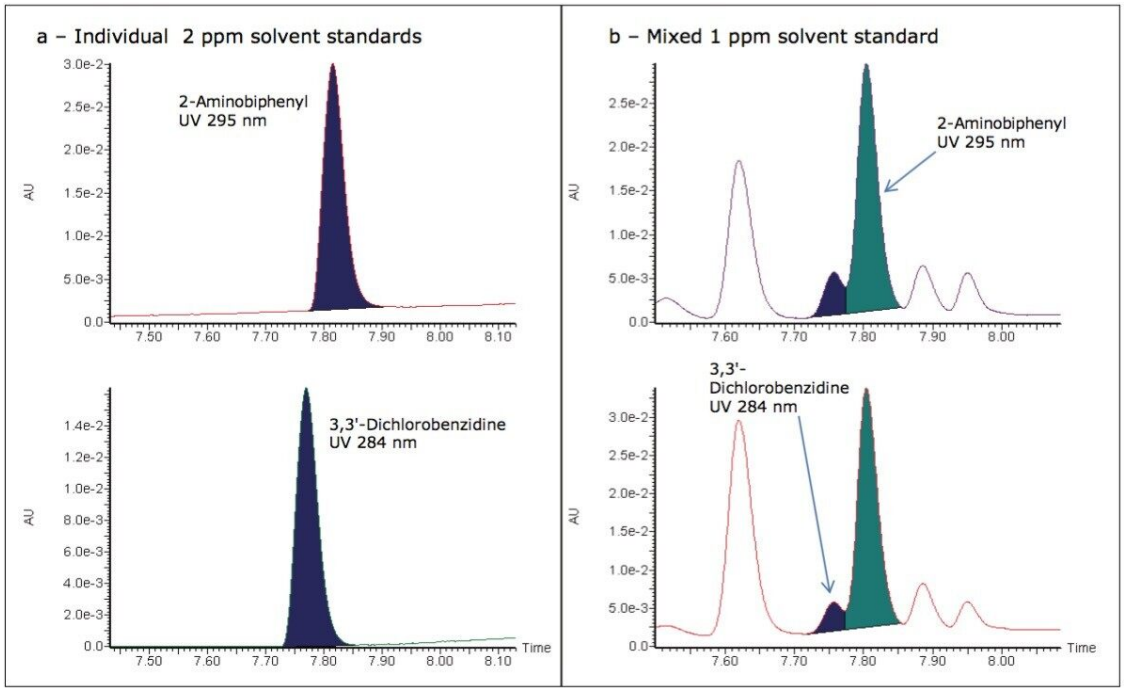


Figure 3. a) UV chromatograms for 2-Aminobiphenyl and 3,3' Dichlorobenzidine in individual solvent standards;

b) UV chromatograms for 2-Aminobiphenyl and 3,3'-Dichlorobenzidine in a mixed solvent standard.

Improvements in selectivity in this example could only be made by changing the chromatographic separation by altering the UPLC conditions to reducing the solvent gradient, which would result in longer run times and associated increases in solvent usage.

Results and Discussion

The analysis of 34 PAAs was achieved using Waters SQ Detector 2 with an electrospray ionization (ESI) source, coupled to an ACQUITY UPLC H-Class System in SIR mode.

Optimum UPLC and SIR conditions were developed, with the elution of all compounds within a 10-minute run.

Mixed calibration standards were prepared and analyzed for all the PAAs considered. The TargetLynx Quantify results for aniline are shown in Figure 4, and the SIR chromatograms for each PAA are shown in Figure 5.

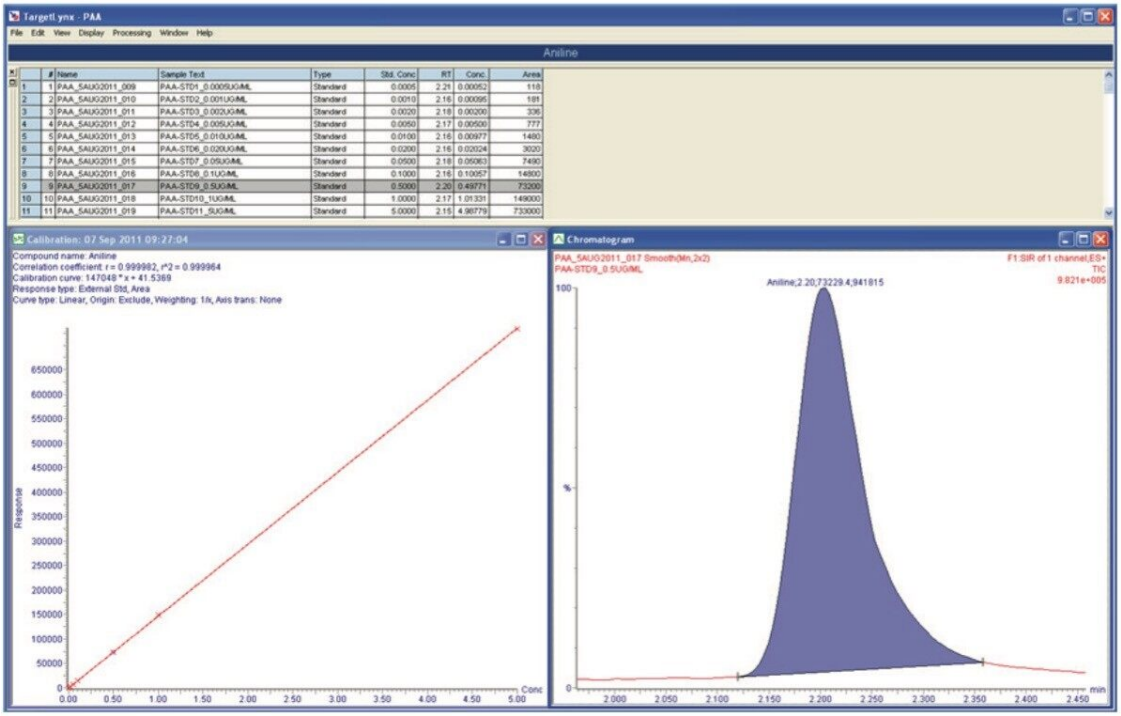


Figure 4. TargetLynx Quantify results browser showing the calibration quantitation results, calibration curve, and example SIR chromatogram for aniline.

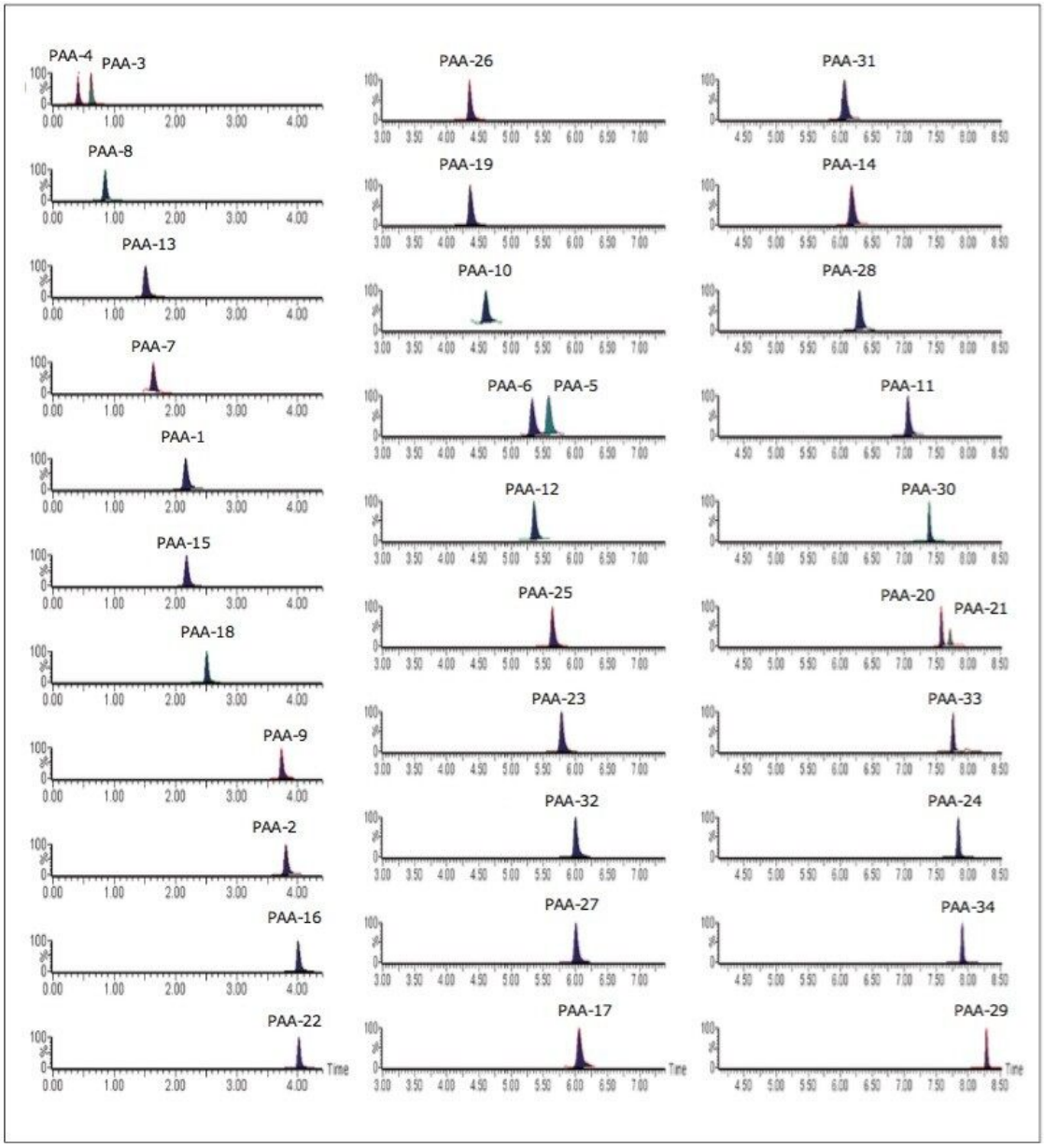


Figure 5. SIR chromatograms for 34 PAAs in a mixed 1 µg/mL calibration standard.

The SIR mass detection method detailed in Figure 1 was used after appropriate sample preparation to screen for PAAs in ink (containing PAAs) and paper (applied with ink containing PAAs).

Ink analysis

Neat ink diluted 1:100 with 5% methanol/95% water was fortified at various levels with selected PAAs, and analyzed without any further cleanup or concentration steps. The results obtained are detailed in Table 3.

Amine	Sample	Replicate injection results (µg/mL)			Average recovery (blank corrected) %	RSD (%)
		1	2	3		
Aniline	Ink blank	0.0031	–	–	–	–
	Ink 0.0220 µg/mL	0.0243	0.0229	0.0238	93.5	3.4
	Ink 0.135 µg/mL	0.139	0.132	0.131	97.1	3.4
	Ink 4.60 µg/mL	4.66	4.61	4.61	100.5	0.6
o-Toluidine	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0221	0.0223	0.0220	100.6	0.7
	Ink 0.135 µg/mL	0.137	0.140	0.136	102.0	1.5
	Ink 4.60 µg/mL	4.62	4.53	4.56	99.3	1.0
2,4-Dimethylaniline	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0184	0.0190	0.0184	84.5	1.9
	Ink 0.135 µg/mL	0.134	0.135	0.131	98.6	1.6
	Ink 4.60 µg/mL	4.60	4.58	4.60	99.8	0.3
2,6-Dimethylaniline	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0210	0.0174	0.0208	89.7	10.3
	Ink 0.135 µg/mL	0.134	0.128	0.136	98.1	3.3
	Ink 4.60 µg/mL	4.55	4.46	4.62	98.8	1.7
o-Anisidine	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0207	0.0198	0.0213	93.6	3.7
	Ink 0.135 µg/mL	0.144	0.140	0.139	104.4	1.7
	Ink 4.60 µg/mL	4.38	4.57	4.49	97.4	2.1
4-Chloroaniline	Ink blank	0.0074	–	–	–	–
	Ink 0.0220 µg/mL	0.0277	0.0243	0.0269	85.8	9.6
	Ink 0.135 µg/mL	0.137	0.134	0.134	94.4	1.4
	Ink 4.60 µg/mL	4.66	4.54	4.58	99.7	1.3
2,4,5-Trimethlaniline	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0204	0.0198	0.0192	90.0	3.0
	Ink 0.135 µg/mL	0.133	0.138	0.135	100.2	1.9
	Ink 4.60 µg/mL	4.70	4.84	4.74	103.5	1.9
3-Chloro-4- methoxyaniline	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0189	0.0184	0.0177	83.3	3.3
	Ink 0.135 µg/mL	0.127	0.135	0.131	96.8	2.9
	Ink 4.60 µg/mL	4.63	4.62	4.78	101.7	1.9
5-Chloro-2-methoxyaniline	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0227	0.0204	0.0188	93.8	9.5
	Ink 0.135 µg/mL	0.143	0.148	0.143	107.3	1.9
	Ink 4.60 µg/mL	4.67	4.55	4.57	99.9	1.5
2-Aminobiphenyl	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0269	0.0227	0.0218	108.2	11.4
	Ink 0.135 µg/mL	0.144	0.140	0.145	106.1	1.9
	Ink 4.60 µg/mL	4.57	4.52	4.54	98.8	0.6
4-Chloro-2,5-dimethoxyaniline	Ink blank	ND	–	–	–	–
	Ink 0.0220 µg/mL	0.0184	0.0197	0.0184	85.6	4.0
	Ink 0.135 µg/mL	0.122	0.124	0.122	90.9	1.2
	Ink 4.60 µg/mL	4.30	4.34	4.29	93.7	0.7

Table 3. Ink fortified with PAAs recovery data. Results quantified against mixed calibration standards.

The efficient recoveries obtained (ranging between 83% to 108%) demonstrated that minimal signal enhancement/suppression was observed using ESI ionization for the analysis of PAAs within an ink matrix.

Paper analysis

Within the food packaging industry great efforts are made to reduce food contamination in order to guarantee consumer safety and comply with regulations. The design of the packaging and the products used ideally afford minimal leaching and hence reduce potential contamination of the food product. Such packaging leachables have a large number of potential sources including PAAs from the ink used within the packaging.

In order to consider the EU regulations with regard to the release of total PAAs from food contact material, a cold water paper extraction based on the European standard (EN 646:1993) was used.

Three pieces of paper (10 cm x 10 cm) were taken, one kept as a blank and two applied with 100 µL ink previously fortified with selected PAAs. The paper was left to dry and then cut up and extracted in sealed containers with 100 mL of water and left for over 24 hours prior to analysis. The results obtained are detailed in Table 4.

Amine	Sample	Replicate injection results (µg/mL)			Average µg aniline equivalents /kg of food*	Leachability (%)	RSD (%)
		1	2	3			
Aniline	A	ND	–	–	–	–	–
	B	0.0898	–	–	–	89.8	–
	C	ND	–	–	–	–	–
	D	0.00863	0.00970	0.00983	2.8	9.4	7.0
	E	0.0129	0.0144	0.0149	4.2	28.1	7.6
o-Toluidine	A	ND	–	–	–	–	–
	B	0.0985	–	–	–	98.5	–
	C	ND	–	–	–	–	–
	D	0.0110	0.00965	0.0102	2.7	10.3	6.7
	E	0.00896	0.00925	0.00774	2.3	17.3	9.3
2,4-Dimethylaniline	A	ND	–	–	–	–	–
	B	0.0908	–	–	–	90.8	–
	C	ND	–	–	–	–	–
	D	0.0177	0.0189	0.0177	4.2	18.1	3.7
	E	0.0120	0.0139	0.0113	2.9	24.8	11.0
o-Anisidine	A	ND	–	–	–	–	–
	B	0.101	–	–	–	101.2	–
	C	ND	–	–	–	–	–
	D	0.0391	0.379	0.346	57.8	37.2	6.3
	E	0.217	0.197	0.190	45.6	40.2	7.0
3-Chloro-4-methoxyaniline	A	ND	–	–	–	–	–
	B	0.104	–	–	–	103.7	–
	C	ND	–	–	–	–	–
	D	0.0911	0.0884	0.0898	15.9	89.8	1.5
	E	0.0405	0.0398	0.0405	7.1	80.5	0.9
5-Chloro-2-methoxyaniline	A	ND	–	–	–	–	–
	B	0.103	–	–	–	103.2	–
	C	ND	–	–	–	–	–
	D	0.0851	0.0870	0.0888	15.4	87.0	2.2
	E	0.0414	0.0424	0.0438	7.5	85.1	2.9

Table 4. Leachability results for paper previously applied with ink containing selected PAAs.

*A = water blank, B = water containing 0.1 µg/mL PAAs, C = paper blank with no ink, D = paper applied with ink containing 10 µg PAAs, E = paper applied with ink containing 5 µg PAAs. *Calculated using a conventional surface area/volume conversion factor of 6 dm2/kg as established in the EU commission Directive 2007/19/EC.*

Sample A results demonstrate that there were no residual PAAs in the water used or as background within the system. Sample B shows the efficacy of the extraction method used, as demonstrated by the high leachability recovery values observed (90% to 104%) when PAAs were added to the water with no paper present. The results most relevant to the food packaging industry were obtained for Samples C and D, which revealed the different extents to which the selected PAAs were being absorbed and not leached from paper.

Conclusion

- A fast, robust, and sensitive method has been developed for the analysis of PAAs in ink.
- SQ Detector 2 linked to the ACQUITY UPLC H-Class System offers improved confidence in identification and quantification.
- Business benefits include increased sample throughput and a reduction of solvent usage with no time-consuming derivatization or pre-concentration stages and reduced run times.
- The ACQUITY UPLC H-Class System, a quarternary system based on UPLC, offers the best in chromatographic resolution and sensitivity.
- The SQ Detector 2 offers cost-effective, reliable mass confirmation.

References

1. Benigni R, Passerini L. Carcinogenicity of the aromatic amines: from structure-activity relationships to mechanisms of action and risk assessment. *Mutation Research*. 511: 191-206, 2002.
2. Anirban M P, Cote R J. Molecular Pathogenesis and Diagnosis of Bladder Cancer. *Annual Review of Pathology*. 4: 501-506, 2009.
3. Ward E, Carpenter A, Markowitz S, et al. Excess Cancers in Workers Exposed to Ortho-Toluidine and Aniline. *National Cancer Institute*. 83(7): 501-506, 1991.
4. Akyuz M, Ata S. Determination of aromatic amines in hair dye and henna samples by ion-pair extraction and gas chromatography-mass spectrometry. *J Pharm Biomed Anal.* 47: 68, 2008.
5. Ekladius L, King H K. A colorimetric method for the determination of aliphatic amines in the presence of ammonia. *Chromatography. A*. 1129(1), 2006. Epub 2006 Jul 14.
6. Aznar M, Canallas E, Nerin J. Quantitative determination of 22 primary aromatic amines by cation-exchange solid-phase extraction and liquid chromatography-mass spectrometry. J *Chromatography A.* 1216: 5176-5181, 2009.
7. Zhang Q, Wang C, et al. Determination of aromatic amines from azo dyes reduction by liquid-phase sorbent trapping and thermal desorption-gas chromatography-mass spectrometry. *J Sep Sci.* 32: 2434-2441, 2009.

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