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Analysis of Primary Aromatic Amines in Cosmetics and Personal Care Products Using the ACQUITY UPLC H-Class System with the ACQUITY QDa Detector and Empower 3 Software

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APPLICATION BENEFITS

ACQUITY® QDa® linked to the ACQUITY UPLC® H-Class System provides improved confidence in the identification and quantification of Primary Aromatic Amines (PAAs) in cosmetics and personal care products offering:

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

WATERS SOLUTIONS

ACQUITY UPLC H-Class System

ACQUITY QDa Detector

Empower® 3 Chromatography Data Software

KEY WORDS

Primary aromatic amines, PAAs, azo dyes, cosmetics, personal care products

INTRODUCTION

Primary aromatic amines (PAAs) have been broadly used in large amounts as a chemical feedstock within the chemical industry. Many PAAs have either a proven or suspected carcinogenic nature and are rated as highly toxic,[1,2,3] so there are a range of potential health risks, which have led to worldwide regulations. In the EU Cosmetic Regulations (EC) No 1223/2009,[4] many PAAs are prohibited for use in cosmetic products.

Despite the toxic and carcinogenic nature of PAAs, they are an important feedstock used in the production of many commodity products such as pharmaceuticals, pesticides, explosives, epoxy polymers, rubber, aromatic polyurethane products, and azo dyes. While not desirable in final products, the presence of PAAs may be due to incomplete reactions, impurities, by-products, or as degradation products. For example PAAs can be produced as by-products of azo dyes which are a diverse and extensively used group of organic dyes. Azo dyes are used in special paints, printing inks, varnishes and adhesives, and can be found in many products such as textiles, cosmetics, personal care products, plastics, and also in food contact material.

In order to ensure public safety and product efficacy, the cosmetics and personal care industry is highly legislated. Hence, manufacturers who use feedstock materials such as PAAs in the production of their products 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;[5,6]
- UPLC® analysis following a solid phase extraction (SPE) using cation-exchange cartridges;[7]
- reduction by liquid phase sorbent trapping followed by thermal desorption GC/MS analysis.[8]

However, many previously used methods for PAA analysis lack robustness, selectivity and sensitivity, and require lengthy, costly, and time-consuming pre-treatments (derivatization, SPE).

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC H-Class
Runtime:	10.00 min
Column:	ACQUITY BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Column temp.:	40 °C
Sample temp.:	10 °C
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Methanol + 0.1% formic acid
Flow rate:	0.4 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	0.400	95	5	–
2	1.00	0.400	95	5	6
3	3.10	0.400	75	25	6
4	6.10	0.400	59	41	6
5	8.00	0.400	0	100	6
6	9.00	0.400	0	100	6
7	9.01	0.400	95	5	6
8	10.00	0.400	95	5	6

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

MS conditions

Mass detector:	ACQUITY QDa
Ionization mode:	ESI +
Capillary voltage:	0.8 kV
Probe temp.:	450 °C
Acquisition:	Selected Ion Recording (SIR)
Cone voltage:	15 V

The list of PAAs, associated CAS number, *m/z*, and expected retention times, are detailed in Table 2.

An ideal solution for the cosmetic and personal care industry for the analysis of PAAs, would overcome the limitations of prior methodologies, while ensuring confidence and versatility in order to meet the regulatory requirement.

This application note describes an accurate, fast, and robust alternative method for the rapid analysis of PAAs in cosmetic and personal care products, using Waters® ACQUITY UPLC H-Class System coupled with the ACQUITY QDa Detector, and controlled by Empower 3 Software.

Instrument control, data acquisition, and result processing

Empower 3 Software was used to control the ACQUITY UPLC H-Class System and the ACQUITY QDa Detector, as well as for data acquisition and quantitation.

Sample preparation

Cosmetic and personal care product sample analysis (eyeshadow, blush, shampoo)

- 0.5 g (solid samples) or 0.5 mL (liquid samples), add 8 mL water and 2 mL methanol. Vortex mixture for 2 min (1600 rpm).
- Centerfuge approximately 1 mL extract for 5 min (10,000 rpm).
- Centrifuge extract diluted with methanol in LC vials ready for analysis (250 µL extract plus 750 µL methanol).

PAA number	Primary Aromatic Amines (PAAs)	CAS number	*m/z*	Retention time (min)
1	Aniline	62-53-3	94	0.47
2	o-Toluidine	95-53-4	108	0.96
3	1,3-Phenylenediamine	108-45-2	109	0.33
4	2,4-Dimethylaniline	95-68-1	122	2.55
5	2,6-Dimethylaniline	87-62-7	122	3.04
6	2,4-Toluenediamine	95-80-7	123	0.40
7	2,6-Toluenediamine	823-40-5	123	0.34
8	o-Anisidine	90-04-0	124	0.82
9	4-Chloroaniline	106-47-8	128	1.84
10	2-Methoxy-5-methylaniline	120-71-8	138	2.53
11	4-Methoxy-m-phenylenediamine	615-05-4	139	0.38
12	2-Naphtylamine	91-59-8	144	3.71
13	3-Amino-4-methylbenzamide	19406-86-1	151	0.71
14	3-Chloro-4-methoxyaniline	5345-54-0	158	1.45
15	5-Chloro-2-methoxyaniline	95-03-4	158	4.70
16	1,5-Diaminonaphtalene	2243-62-1	159	0.43
17	2-Methoxy-4-nitroaniline	97-52-9	169	4.62
18	4-Aminobiphenyl	92-67-1	170	5.62
19	2-Aminobiphenyl	90-41-5	170	6.83
20	Benzidine	92-87-5	185	0.42
21	4-Chloro-2,5-dimethoxyaniline	6358-64-1	188	4.76
22	4-Aminoazobenzol	60-09-3	198	8.14
23	4,4'-Methylenedianiline	101-77-9	199	0.67
24	3,3'-Dimethylbenzidine	119-93-7	213	2.37
25	4,4'-Thioaniline	139-65-1	217	3.98
26	o-Aminoazotoluene	97-56-3	226	8.62
27	4,4'-Diamino-3,3'-dimethylbiphenylmethane	838-88-0	227	3.32
28	3-Amino-p-anisanilide	120-35-4	243	5.10
29	o-Dianisidine	119-90-4	245	2.61
30	4,4'-Diamino-3,3'-dichlorobiphenylmethane	101-14-4	267	8.18

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

RESULTS AND DISCUSSION

Optimum UPLC and SIR conditions were developed, with the elution of all compounds occuring within a 10 minute run. The speed of method development was markedly improved using the ACQUITY QDa Detector instead of UV detection.

Typically during method development, different conditions/parameters are considered such as choice of columns, mobile phases, and gradients. These choices could potentially result in changes to the elution order of the compounds being considered. The peak tracking when using UV detection only would require the analysis of the individual authentic standards in order to confirm the elution order (Rt). However, with mass detection, the movement of chromatographic peaks can easily be followed, and the presence of co-eluting peaks can also be easily identified.

An illustration of the identification of the co-eluting peaks is shown in Figure 1 which shows two PAAs (4,4'-Methylene-Dianiline and 2-Methoxy-5-Methylaniline) that have similar optimum wavelengths.

Mixed calibration standards, over the range of 0.001 µg/mL to 1.0 µg/mL were prepared and analyzed for all the PAAs considered (equivalent range of 0.08 to 80 mg/Kg in the extracted sample, using the developed method, greater with extract dilution). The SIR chromatograms for each PAA are shown in Figure 2.

The SIR mass detection conditions detailed in Table 2 were used after appropriate sample preparation to screen for PAAs in cosmetic and personal care samples.

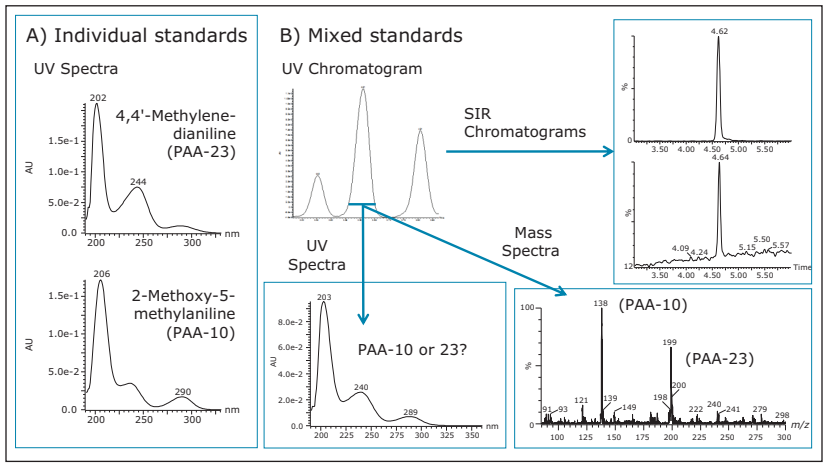

Figure 1. An illustration of the advantages of mass detection for the identification of co-eluting peaks during method development, considering two PAAs (4,4'-Methylene-dianiline and 2-Methoxy-5-methylaniline); a) UV spectra from individual standards, b) UV and mass spectra, and SIR chromatograms from mixed standards.

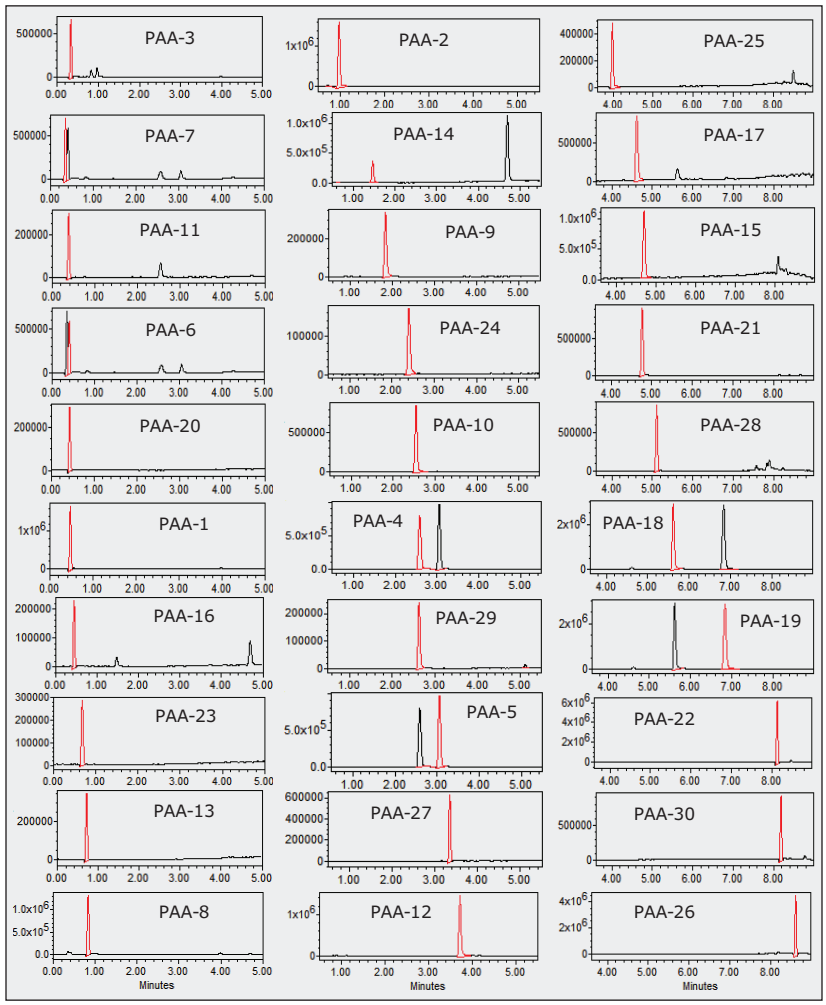

Figure 2. SIR chromatograms for 30 PAAs in a mixed 0.5 µg/mL calibration standard.

Cosmetic and personal care sample analysis

Samples were fortified at various levels with selected PAAs, then prepared for analysis as described in the Experimental section. The results obtained for shampoo, blush, and eyeshadow are detailed in Tables 3, 4, and 5, and a selection of SIR chromatograms achieved are shown in Figure 3.

Amine	Fortified mg/Kg	mg/Kg	Recovery (%)*
Aniline	0	0.012	N/A
	0.25	0.213	80.5%
	0.5	0.371	71.8%
	1.0	0.831	81.8%

*Table 3. Shampoo fortified at various levels with aniline. Results quantified against mixed calibration standards. *Blank corrected recovery data.*

Amine	Fortified mg/Kg	mg/Kg	Recovery (%)*
2,6-Dimethylaniline	0	0.018	N/A
	0.25	0.202	73.6
	0.5	0.417	84.0
	1.0	0.895	90.4
4-Chloroaniline	0	0.045	N/A
	0.25	0.222	70.8
	0.5	0.429	76.8
	1.0	0.785	74.0
2-Naphthylamine	0	ND	N/A
	0.25	0.254	101.6
	0.5	0.404	80.8
	1.0	0.865	86.5

*Table 4. Blush fortified with various levels of selected PAAs. Results quantified against mixed calibration standards. *Blank corrected recovery data.*

The recoveries obtained (ranging between 72% to 104%) demonstrated that minimal signal enhancement/ suppression was observed using UPLC chromatographic separation with ESI ionization for the analysis of PAAs in the cosmetic and personal care products considered.

Amine	Fortified mg/Kg	mg/ Kg	Recovery (%)*
2,6-Dimethylaniline	0	ND	N/A
	0.25	0.207	82.8
	0.5	0.353	70.6
	1.0	0.775	77.5
4-Chloroaniline	0	0.095	N/A
	0.25	0.354	103.6
	0.5	0.455	72.0
	1.0	0.857	76.2
5-Chloro-2-methyoxyaniline	0	0.069	N/A
	0.25	0.268	79.6
	0.5	0.510	88.2
	1.0	0.893	82.4

*Table 5. Eyeshadow fortified with various levels of selected Primary Aromatic Amines. Results quantified against mixed calibration standards. *Blank corrected recovery data.*

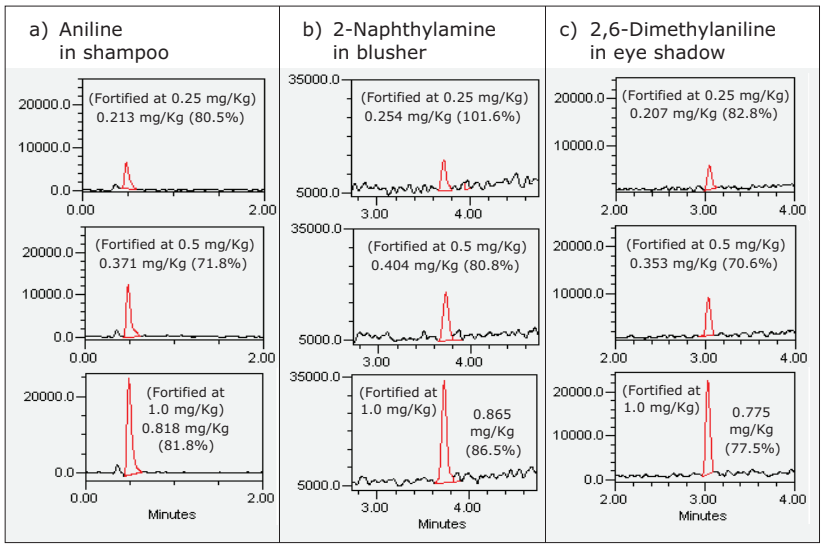

Figure 3. SIR chromatograms for selected PAAs in matrix: a) shampoo b) blush, and c) eyeshadow.

CONCLUSIONS

- A fast, robust, and sensitive method has been developed for the analysis of PAAs in cosmetic and personal care product samples.
- The ACQUITY QDa Detector provides more cost-effective and reliable mass confirmation, demonstrating improved experimental confidence over UV detection, during both method development and routine analysis.
- Combining the ACQUITY UPLC H-Class System with the ACQUITY QDa Detector offers accurate and reproducible quantification.
- Empower 3 Chromatography Data Software provides assurance in data management, data processing, and reporting.
- Business benefits compared to previous methodology include:
  - Increased sample throughput
  - Reduction of solvent usage due to no time-consuming derivatization or pre-concentration steps.
  - Reduced run times.
- The ACQUITY H-Class System, a quarternary system based on UPLC Technology, offers the best in chromatographic resolution and sensitivity.

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