Consumer Products Testing Application Notebook

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Consumer Products Testing:

Reduce risk, ensure quality, and achieve compliance.

The analytical testing of consumer products is a critical step towards ensuring the safety of end users, maintaining product quality, and protecting your brand.

Manufacturers and their contract testing laboratories must have the ability to increase throughput, maximize uptime, and readily characterize a wide range of complex samples – all while meeting national and international standards for permissible levels of target chemical compounds.

Waters Corporation has a long history of helping manufacturers and contract testing organizations overcome scientific challenges and streamline operations.

With our continuous technological advancements in sample preparation and columns, chromatography, mass spectrometry, and data management software, you can reduce analyses times, consolidate methods, lower costs, and achieve regulatory and contractual compliance.

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BIOCIDES

Transferring Multiple Methods into a Single Analytical Solution Using Biocides in Consumer Products as a Model

Jane Cooper

GOAL

To illustrate the simplicity of optimizing multiple methods for diverse biocides used in consumer products.

The ACQUITY UPLC H-Class System enables a streamlined approach to multiple method analysis.

BACKGROUND

Waters® ACQUITY UPLC® H-Class System offers the flexibility of the quaternary solvent pump, with the simplicity of flow-through needle injections.

With a wide range of UPLC® column substrates and chemistries available, and the multi-solvent blending capabilities available with the ACQUITY UPLC H-Class System, additional scope both during method development and with routine analysis is now obtainable.

Biocides are chemical substances used in a wide range of products to either control or render safe harmful organisms. Various biocides are included in restricted substances lists (RSLs) utilized by many companies in order to protect their products for consumers, their workers, and also the community/environment.

This technology brief illustrates the flexibility users have with the ACQUITY UPLC H-Class System to combine biocide analytical suites of analyses and achieve high resolution, sensitivity, and throughput with sub-2-µm particle columns.

The scope to combine suites of analyses offers the chemical industry valuable time and cost savings, faster sample turnaround times, and an associated reduction in solvent usage.

Figure 1. ACQUITY UPLC H-Class System with Xevo TQD.

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THE SOLUTION

This investigation uses the Xevo® TQD with atmospheric pressure chemical ionization (APCI), coupled to an ACQUITY UPLC H-Class System, for the detection of a range of biocide compound groups that would typically be analyzed using different chromatographic conditions.

Optimum multiple reaction monitoring (MRM) or selected ion recording (SIR) conditions were developed, as shown in Table 1.

Chemical substance	MRM / SIR	APCI (+/-)	Cone voltage (V)	MRM transitions/ or SIR mass (*m/z*)	Collision energy
Triclosan	MRM	-	63	250.9 > 186.9	33
				250.9 > 215.0*	21
Tetrachlorophenol (3 isomers)	MRM	-	45	194.9 > 122.9	26
				194.9 > 158.7*	16
Pentachlorophenol	SIR	-	31	264.8	N/A
Tributyltin chloride	MRM	+	15	332.0 > 235.1	11
				332.0 > 291.1*	6
Triphenyltin chloride	MRM	+	17	392.0 > 169.9	35
				392.0 > 351.0*	12
2-Octyl-3-isothiazolinone	MRM	+	25	214.1 > 43.0	10
				214.1 > 101.9*	22
2-Methyl-3-isothiazolinone	MRM	+	45	392.0 > 70.9*	19
				392.0 > 100.9	21

*Table 1. Biocides, ionization mode, cone voltages, MRM transitions or SIR mass, and associated collision energy values (*refers to the quantification transition).*

In order to optimize chromatographic separations, there are many important factors to consider including: LC system, detection method, choice of LC column, mobile phase(s), and mobile phase buffers. The choices of mobile phase buffers depend upon the compounds to be analyzed and the associated ionization mode used for detection. Buffers affect both the pH and the ionic strength of the mobile phase, and influence such factors as selectivity and peak shape.

Effects caused by the choice of mobile phase buffer with regard to the analysis of biocides are illustrated in Figure 2. The three tetrachlorophenol isomers were resolved when the basic ammonium acetate (AA) buffer was used, but the peak shape of the tin compounds, shown in Figure 2a, was very poor. The three tetrachlorophenol isomers were not resolved, however, when the acidic formic acid (FA) buffer was used, while the peak shape of the tin compounds, shown in Figure 2b, was markedly improved.

Typically, to achieve improved resolution of the three tetrachlorophenol isomers, improved selectivity, and peak shape of the tin compounds, two separate LC methods would be required; one using an acidic buffer, and the other using a basic buffer. Using the quaternary pump of the ACQUITY UPLC H-Class System, this can all be achieved in one UPLC method, shown in Figure 2c, by swapping the choice of buffer during the run.

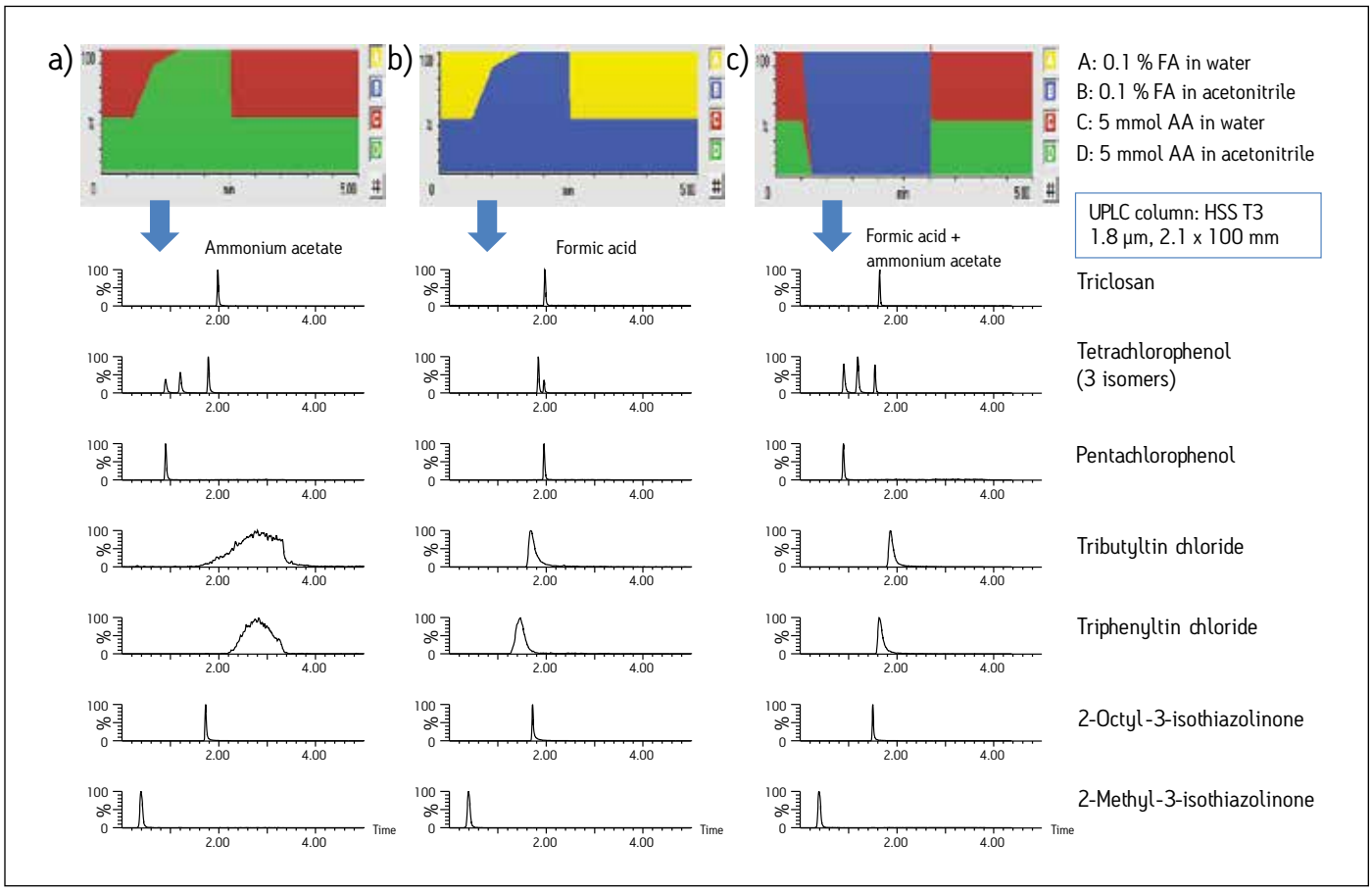

Figure 2. MRM / SIR chromatograms for select biocides compounds in a mixed solvent standard using different mobile phase buffer: 2a. ammonium acetate, 2b. formic acid; and 2c. a combination of ammonium acetate and formic acid.

Combining methods offer time and cost savings, enabling greater sample throughput and reduced solvent consumption. Also reducing the amount of LC methods required saves time spent on switching and equilibrating LC methods in between different analytical suites of analyses.

SUMMARY

The flexibility offered using the quaternary solvent pump of the ACQUITY UPLC H-Class System expands the benefits of UPLC technology. It provides improved scope for method development and multi-method analyses, while maintaining high resolution, sensitivity, and throughput offered by sub-2-µm particle columns.

The scope to combine suites of analyses offers the chemical industry valuable time and cost savings, faster sample turnaround times, and an associated reduction in solvent usage.

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ACQUITY UPLC PDA Analysis of Biocides (Part 1)

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APPLICATION BENEFITS

- Improves laboratory productivity by enabling the rapid and sensitive separation of six commonly used biocides.
- Library matching and quantification automated with Empower® 3 Software increases confidence in peak confirmation and helps ensure product quality.
- Suitable for cosmetic and personal care product development and quality control analytical testing.

WATERS SOLUTIONS

ACQUITY UPLC® System

ACQUITY UPLC PDA Detector

Empower 3 Chromatography Data Software

ACQUITY UPLC BEH C_{18} Column

KEY WORDS

Biocides, cosmetics, personal care products, consumer products, product development, quality control, regulatory compliance, library matching

INTRODUCTION

Unwanted micro-organisms such as bacteria, viruses, and molds can grow wherever there is a source of nutrition and moisture. This unwanted growth may negatively impact human health, interfere with manufacturing processes, damage building structures, and spoil consumer goods. The principal defense against deleterious micro-organisms is biocides, commonly classified as disinfectants, preservatives, antifouling products, and pest controls.

Biocides are used as additives in cosmetics and personal care, household, and industrial products. To protect the environment and human health, many countries regulate biocide use.[1] In the European Union, this is done through the Directive 98/8/EC (The Biocidal Products Directive) and Regulation (EU) No 528/2012 (The Biocidal Products Regulation). In the United States, regulatory control of biocides falls under the EPA and the biocides applications in cosmetics, food, and personal health care are regulated by the U.S. FDA. Regulations impact the registration, labeling, and composition of biocides.[2] Reliable and rapid methods are therefore essential to ensure effective product quality control. This application note describes a three-minute separation of six biocides using the Waters® ACQUITY UPLC PDA System with Empower 3 Software. With PDA library matching and the built-in advanced mathematical algorithms, each biocide in the mixture can be identified and quantified; the analysis is fast and reproducible. The ability to quickly and unambiguously analyze biocide content can facilitate workflow related to the quality control and regulatory compliance of biocide containing products. This methodology benefits new product development, product troubleshooting and biocide production.

EXPERIMENTAL

Sample preparation

Analytes are:

Kathon CG/ICP
[containing 0.4% of **2-methyl-4-isothiazolin-3-one** (1a), 1.2% of **5-chloro-2-methyl-4-isothiazolin-3-one** (1b)];

Carbendazim (2);

Benzisothiazol-3(2H)-one (3);

2-phenoxyethanol (4);

Benzoic Acid (5);

Methyl paraben (6).

LC conditions

LC system:	ACQUITY UPLC PDA
Software:	Empower 3
Weak wash:	95:5 Water: CH_3CN (600 µL)
Strong wash:	50:50 Water: CH_3CN (200 µL)
Seal wash:	90:10 Water: CH_3CN (5 min)
Column temp.:	30 °C
Flow rate:	1 mL/min
Injection:	5 µL
Detection:	PDA 210 to 500 nm
Sampling rate:	20 pts/s
Filter response:	0.1 s
Column:	ACQUITY UPLC BEH C_{18} 2.1x 50 mm
Mobile phase A:	0.05 v% Trifluoroacetic acid (TFA) in water
Mobile phase B:	0.05 v% TFA in CH_3CN
Linear gradient:	5% to 15% B in 2.9 min

Note: The column was equilibrated with 5% B for 2 minutes before injection, and was washed with 100% B for 2 minutes at the end of each run.

RESULTS AND DISCUSSION

Figure 1 shows the structures of the biocides (1a, 1b, 2-6); a 5 ppm mixture of 1–6 was separated using the Waters ACQUITY UPLC System with a three-minute linear gradient method. These compounds are frequently used in adhesives, paint and coatings, latex and sealants, inks, wood and paper products, textile and leather products, metalworking fluids, personal care products, cosmetics, laundry detergents, dishwashing liquids, and household and industrial cleaners. The acetonitrile/ water mobile phase with TFA modifier is compatible with mass spectrometry detectors, if needed.

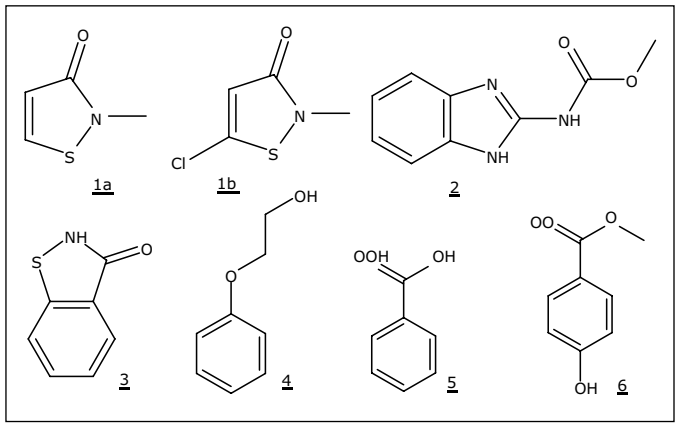

Figure 1. Chemical structures of biocides.

UV photodiode array (PDA) detection combined with Empower 3 Software enables a powerful range of detection and identity confirmation possibilities for chromatographic separations. PDA timed wavelength chromatograms can be plotted using the λmax of each analyte. This increases the detection limit when the analytes have very different λmax and aids quantification. Figure 2 is an overlay of nine replicate injections of PDA timed wavelength chromatograms, demonstrating that the overall reproducibilty is excellent. The three-minute linear gradient easily resolves the two active components of Kathon CG/ICP (1a and 1b) and the other five biocides. The retention time and peak area of each component observed in the above nine replicate injections are listed in Tables 1 and 2, with statistical analysis results generated using Empower 3 Software. The excellent % RSD results indicate the suitability of UPLC with BEH column chemistry for biocides.

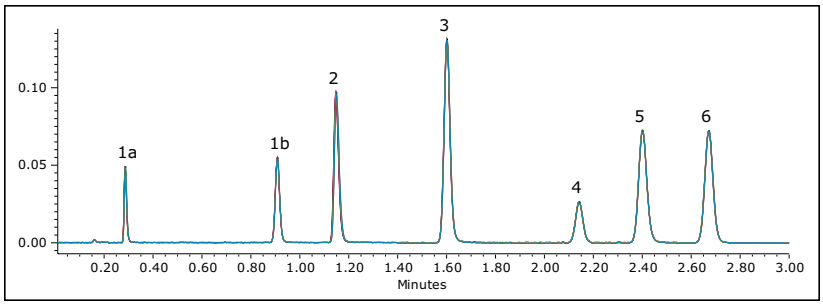

Figure 2. Overlay PDA timed wavelength chromatograms, retention time and peak area tables of 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

	1a (min)	1b (min)	2 (min)	3 (min)	4 (min)	5 (min)	6 (min)
1	0.286	0.908	1.147	1.600	2.141	2.400	2.671
2	0.287	0.908	1.147	1.601	2.141	2.400	2.671
3	0.286	0.908	1.147	1.601	2.141	2.401	2.672
4	0.286	0.908	1.148	1.602	2.142	2.401	2.672
5	0.286	0.908	1.148	1.601	2.141	2.400	2.671
6	0.286	0.908	1.149	1.602	2.142	2.401	2.672
7	0.287	0.908	1.148	1.601	2.141	2.400	2.671
8	0.286	0.908	1.150	1.602	2.142	2.401	2.672
9	0.287	0.909	1.150	1.602	2.142	2.401	2.672
Mean	0.286	0.908	1.148	1.601	2.142	2.401	2.672
Std. Dev.	0.001	0.000	0.001	0.001	0.001	0.001	0.001
% RSD	0.19	0.04	0.10	0.03	0.03	0.02	0.02

Table 1. Component summary for retention time for 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

	1a	1b	2	3	4	5	6
1	34745	68748	134846	227719	57857	172510	173458
2	34684	69423	134511	227840	57682	170783	172192
3	34730	68894	134698	228692	57882	173440	172053
4	34741	69168	135187	228238	57388	173125	172113
5	34761	68952	134533	228331	58008	170433	172156
6	34673	69132	134817	228461	57802	170579	171725
7	34753	68903	135014	228616	57863	172557	171723
8	34781	68736	135018	227710	57845	170954	171833
9	34782	69050	134694	228489	57809	172072	172143
Mean	34739	69001	134813	228233	57793	171828	172155
Std. Dev.	38	219	229	383	174	1157	523
% RSD	0.1	0.3	0.2	0.2	0.3	0.7	0.3

Table 2. Component summary for area for 9 replicate injections of sample containing 1.25 ppm of 1a, 3.75 ppm of 1b and 5 ppm of 2–6: (0.00 min, 275 nm; 1.40 min, 225 nm; 2.55 min, 255 nm).

Six levels of calibration standards (containing Kathon and 2–6 from 2.5 to 20 ppm) were analyzed. Empower 3 Software has built-in mathematical features and functions. Calibration curves were created from the standards and the quantity of analyte in each sample was calculated automatically. Figure 3 shows the calibration plots generated by Empower 3, using the peak areas vs the concentration. The linearity of the calibration curves is excellent with the R^2 values (residual sum of squares) above 0.9999, except one with 0.9998. Table 3 shows a typical results analysis table for peak identification and quantification using a biocides standard mixture mix as a sample. The last column shows that amounts match well with actual values (1.25 ppm for 1a, 3.75 ppm for 1b, and 5 ppm for 2–6). The data suggest that UPLC/PDA is well suited to meet the regulatory demands for quantitative analysis of biocides.

Empower 3 Software provides the capability of creating a PDA library from pure component peaks in user chromatograms. Afterwards, the library matching and peak purity features can be used with samples to confirm peak identities and to give added confidence that spectrally distinct peaks are not-coeluting. Empower 3 uses Spectral Contrast™ theory to quantitatively compare the shapes of UV spectra during library matching and Peak Purity analysis.[3-6] Figure 4 shows UV spectra, extracted from PDA chromatograms of standards (1a, 1b, 2-6); these spectra were used to create a library with names and retention times. Table 3 shows an example of a default Empower Report table with PDA library matching and Peak Purity results. The values of Match Angle are smaller than Match Threshold and the values of Purity Angle are smaller than Purity Threshold, indicating that the analytes were well separated and matched with PDA library of biocides.

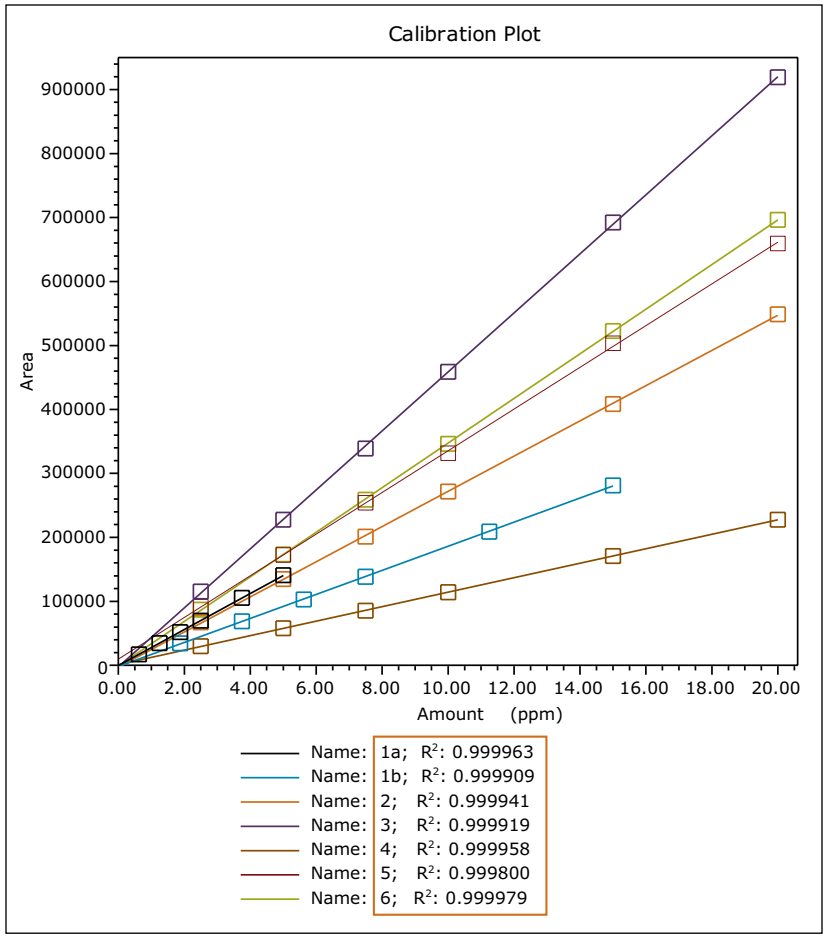

Figure 3. Calibration curves for (1a, 1b, 2-6).

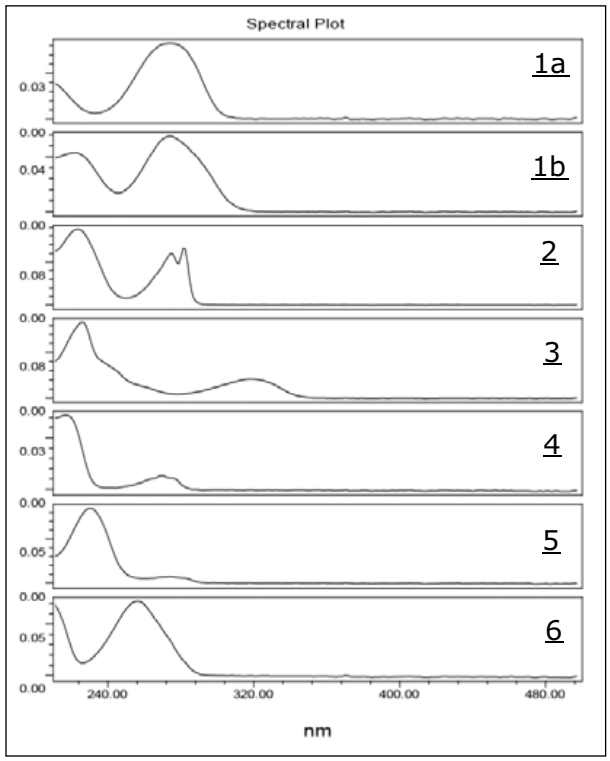

Figure 4. UV spectra of 1a, 1b, and 2-6 extracted from PDA data.

Component		Match1 Spect. Name	Match1 Angle	Match1 Threshold	Purity1 Angle	Purity1 Threshold	Amount (ppm)
1	1a	2-methy l-4-isothiazolin-3-one	0.312	1.598	0.792	1.086	1.25
2	1b	5-chloro-2-methy l-4-isothiazoline-3-one	0.401	1.474	0.676	0.863	3.78
3	2	carbendazim	0.184	1.244	0.337	0.554	5.01
4	3	1,2-benzisothiazol-3-one	0.174	1.282	0.368	0.628	5.01
5	4	2-phenoxyethanol	0.992	2.663	2.296	2.461	5.00
6	5	benzoic acid	0.322	1.399	0.560	0.760	4.99
7	6	methyl paraben	0.235	1.388	0.508	0.743	4.97

Table 3. Peak identification and quantification results shown on an Empower Report table, with additional PDA library matching and Peak Purity results.

CONCLUSIONS

Compliance with regulations that limit the type and concentration of biocides in a variety of applications necessitates analytical testing. This note illustrates that the Waters ACQUITY UPLC System with PDA detection enables rapid and sensitive separations of six commonly used biocides. With Empower 3 Software, library matching and quantification can be automated to add confidence in peak confirmation that is unavailabe with a single wavelength UV detector. This method is simple to use and suitable for quality control, new product development, and troubleshooting for both cosmetic and personal care manufacturers.

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ACQUITY UPLC PDA Analysis of Biocides (Part 2) Pass or Fail Custom Calculation

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APPLICATION BENEFITS

- Increases laboratory productivity by enabling the high resolution, high sensitivity separations of biocides in three minutes.
- Helps improve decision-making, and ensure product quality by rapidly extracting and delivering critical QC data based on user criteria.

WATERS SOLUTIONS

ACQUITY UPLC® System

ACQUITY UPLC PDA Detector

Empower® 3 Chromatography Software

KEY WORDS

Biocides, cosmetics, personal care, user set criteria, custom calculations, custom field, Quality Control, pass or fail

INTRODUCTION

Making more informed decisions in less time is essential in today's Cosmetics, Personal Care, and Food analytical laboratories. Whether you work in method development, quality assurance, new product development, or testing for biocides, you seek a productivity edge. Waters® ACQUITY UPLC System provides that edge, enabling high resolution, high sensitivity biocide separations in three minutes.[1] Better quality information on product biocide levels can now be generated far more rapidly than with traditional HPLC methods. UPLC® combined with Empower 3 Software can effectively run separations, analyze data, and report results automatically. With the custom calculation function of Empower 3, raw data can be converted into the required format and the critical results can be used quickly by decision-makers to further enhance productivity. This application note describes the benefits of using a simple custom calculation created to determine if the biocide concentration in a sample passes or fails user set criteria. This type of custom calculation eliminates the need for manual calculation and prevents potential human errors. The ability to deliver critical information with speed and accuracy can help manufacturers reduce failed products, avoid product recalls and liability litigations. An example detailing the creation of a custom calculation shown in the note is provided in the Experimental section.

EXPERIMENTAL

QC Criteria Example for Biocide

Kathon amount < 4.85 ppm: Fail

Kathon amount > 5.15 ppm: Fail

Kathon amount from 4.85 ppm to 5.15 ppm: Pass

Create a Custom Field Calculation Method with Empower 3

1. Click Configure System to open the Configuration Manager window, click Projects in the tree.
2. Select and highlight the working project, then right click the highlighted project.
3. Select Properties to open Project Properties window.
4. Click the Custom Fields tab, then click New to open New Custom Field Wizard: Data and Type Selection window.
5. Select the Field Type: Peak, and Data Type: Enum, then click Next to open Source Selection window.
6. Select Data Source: Calculated, Sample Type: Unknown, Peak Type: Group Only, then click Next to open Formula Entry window (Figure 1).
7. In the Operations list, double-click **ENUM(** and **LT(.**
8. In the Fields list, double-click Amount, in the formula workspace, enter **,4.85),**
9. In the Operations list, double-click **LTE(.**
10. In the Fields list, double-click Amount, in the formula workspace, enter **,5.15).**
11. In the Operations list, double-click **GT(.**
12. In the Fields list, double-click Amount, in the formula workspace, enter **,5.15)).**
13. Click Next to open Translation Definition Table.
14. Enter **Fail** next to 0, **Pass** next to 1, **Fail** next to 2, click Next to open Name Entry window. (Note: you can enter Pass/Fail in another language).
15. Enter a name for the custom field in the Field Name text box: Pass or Fail, click Finish.

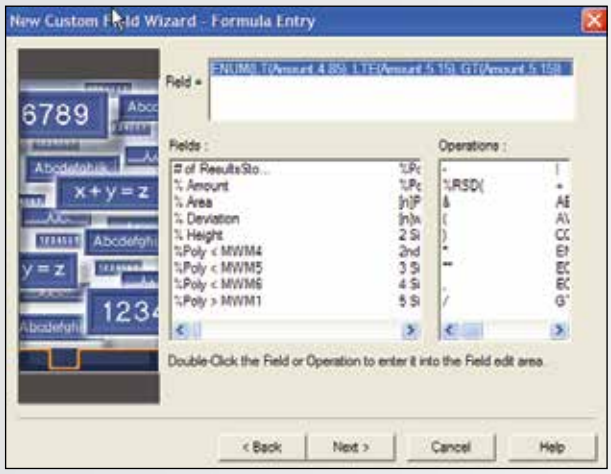

Figure 1. Formula Entry window.

Create Named Groups in the Processing Method

1. From Processing Method window, select the Named Groups tab.
2. Enter Kathon in the Name text box.
3. Select the option of Sum Peaks, Curve or Sum Peaks for Quantitation.
4. Drag 1a and 1b from Single Peak Components into the tree of Kathon as shown in Figure 2.

Sample preparation and UPLC Methods

The methods used are the same as described previously.[1] Analytes are Kathon CG/ICP [containing 0.4% 2-methyl-4-isothiazolin-3-one (1a), 1.2% 5-chloro-2-methyl-4-isothiazolin-3-one (1b)], Carbendazim (2), Benzisothiazol-3(2H)-one (3), 2-phenoxyethanol (4), Benzoic Acid (5), and Methylparaben (6).

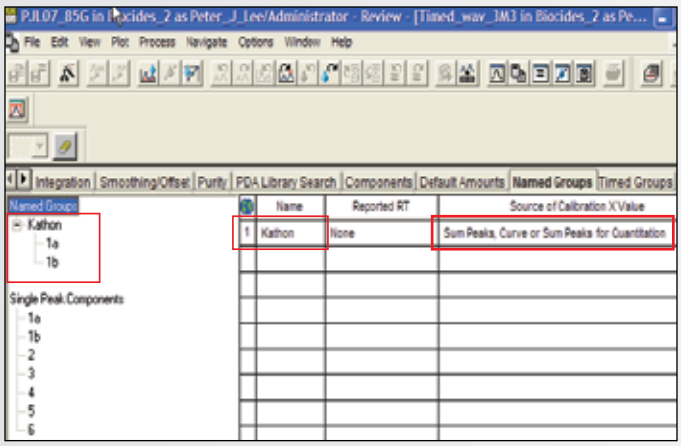

Figure 2. Processing method window.

RESULTS AND DISCUSSION

Figure 3 shows chromatograms of three biocide samples. There are seven well-resolved peaks in each sample. 1a and 1b are the two active ingredients of Kathon. The amount of 1a and 1b in each sample can be calculated automatically in Empower 3 Software from calibration curves as described previously.[1]

For this discussion, a passable QC level for the Kathon biocide is the range 4.85 ppm to 5.15 ppm. To determine Pass or Fail biocide status of each sample, the amount of total Kathon must be calculated, that is the sum of 1a and 1b, and the result must be compared with the QC criteria.

Figure 4 shows the Custom Field Formula described in the Experimental section. The QC Pass or Fail criteria is based on the Kathon biocide content (5 ppm ± 3%).

Using both the Named Groups and Custom Field calculation functions, Empower can be set up to automatically calculate and report the quantity of Kathon in each sample and determine if the sample met the QC criteria (Table 1). In an enterprise environment, the critical Pass or Fail results can be e-mailed to product and plant management. These advanced functions of Empower eliminate the need for manual calculations, which saves time and reduces errors.

	SampleName	Component	Amount (ppm)	Pass/Fail
1	PJL07_85G	Kathon	5.03	Pass
2	PJL07_85H_Low	Kathon	2.57	Fail
3	PJL07_85F_High	Kathon	7.43	Fail

Table 1. Biocides Test: Pass or Fail.

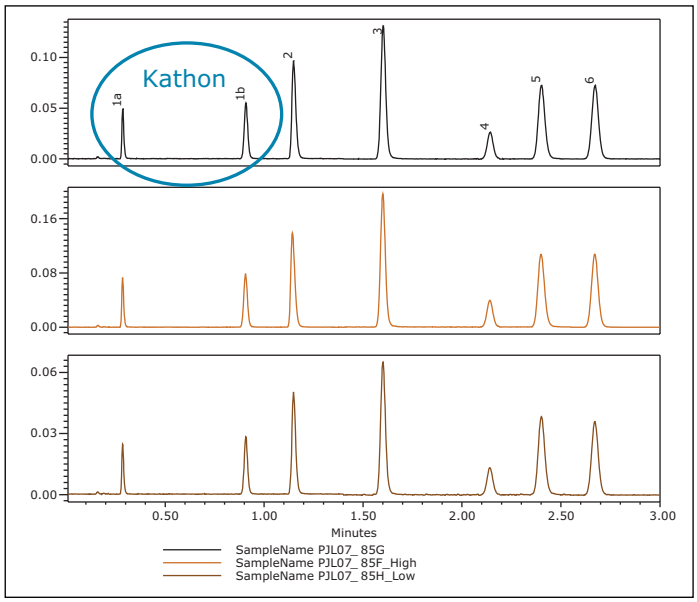

Figure 3. UPLC chromatograms of three biocide samples.

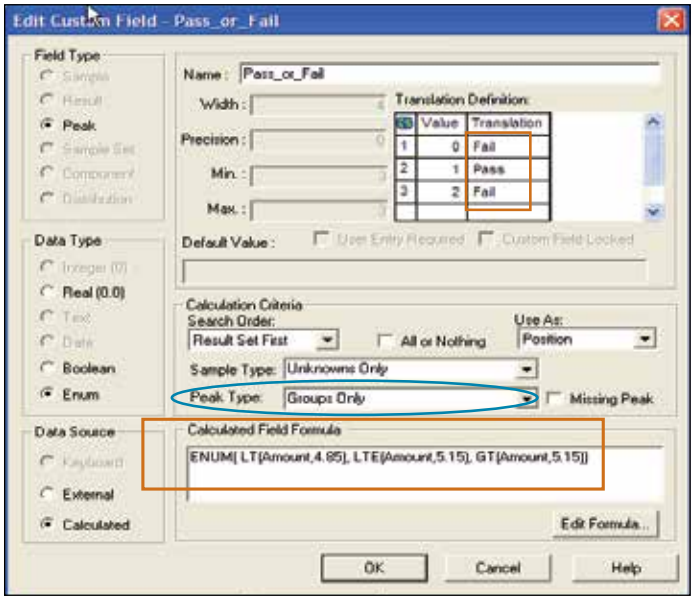

Figure 4. Custom Field formula.

CONCLUSIONS

Hundreds of biocide containing product samples can be automatically analyzed on a daily basis using the Waters ACQUITY UPLC PDA System with Empower 3 Software. Critical product QC information can be accurately extracted and rapidly delivered based on user set criteria. The method represents a significant productivity enhancement relative to the manual verification of QC biocide data. It can be very effective for food, cosmetics and personal care manufacturers, and formulators to have a report with a simple Pass or Fail for the sample displayed.

References

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UPLC Analysis of Benzalkonium Chloride (BAC) in Consumer Products using ACQUITY UPLC CSH C_{18}

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APPLICATION BENEFITS

- Accurate determination of benzalkonium chloride (BAC) content in consumer products
- Improved peak shape and stability relative to current USP method
- 95% reduction in solvents used relative to currently accepted methods
- 80% reduction in analysis time enables high throughput analysis

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

ACQUITY UPLC CSH™ C_{18} Column

XSelect® CSH C_{18} ***XP*** Column

ACQUITY UPLC Columns Calculator

KEY WORDS

Benzalkonium, quaternary ammonium, ophthalmic, disinfect, sanitize, fungicide, algaecide, antimicrobial, biocide, cationic surfactant, emulsifier, CSH, ***XP***

INTRODUCTION

Benzalkonium Chloride (BAC) refers to a series of quaternary ammonium chloride homologues with the structure shown in Figure 1. The pervasive use of BAC in consumer products results from its antiseptic and antifungal properties with widespread applications ranging from cleaning products and disinfectants to sanitizing wipes and ophthalmic solutions. Because of its extensive use, BAC has been the subject of numerous studies, including the evaluation of the reactivity of BAC with ocular tissue[1,2] and the study of worldwide municipal wastewater, which found BAC to be the most prevalent quaternary ammonium compound in wastewater, with concentrations ranging between 200 and 300 mg/L.[3,4]

The USP method for the quantitation of BAC utilizes a 10 µm particle size cyano column (L10) for the separation of the BAC homologues.[5] The isocratic method uses acetonitrile and 0.1M sodium acetate (pH 5.0) as mobile phases, resulting in a separation requiring between 15 and 30 minutes. In addition to long analysis times, these separations suffer from reproducibility issues due to the traditionally poor chemical and mechanical stability of the cyano stationary phases.[6] Here we present an alternative method employing a Charged Surface Hybrid (CSH) C_{18} stationary phase under UPLC® conditions, resulting in improved peak shapes with significant reductions in both analysis time and solvent consumption. Additionally, we include an example of this method that is transferred to conditions using the XSelect CSH C_{18} ***XP*** 2.5 µm stationary phase (UPLC and HPLC), demonstrating the ability to transfer methods across different instrument platforms.

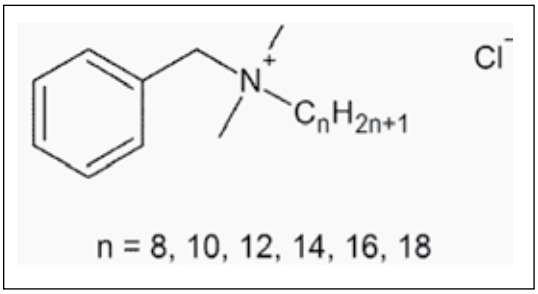

Figure 1. Structure of benzalkonium chloride (BAC). The C_{12}, C_{14}, and C_{16} homologues are the most common homologues found in consumer products.

EXPERIMENTAL

Sample preparation

Standard Solution: Prepared from a USP Reference Standard containing 10% (w/v) of BAC. Diluent was 50:50 acetonitrile/water. Standard solutions were prepared at concentrations of 800, 500, 200, 100, 75, and 50 ppm (µg/mL).

Samples

Consumer products were prepared to a final concentration of approximately 80 ppm (0.008% *w/v*), based on label claim, with 50:50 acetonitrile/water as diluent. Products in liquid form were diluted and analyzed without additional sample preparation or filtering.

UPLC conditions

System:	ACQUITY UPLC H-Class with PDA Detector
Column:	ACQUITY UPLC CSH C_{18}, 1.7 µm, 2.1 x 50 mm, part number 186005296
Mobile phase A:	100 mM ammonium acetate in water at pH 5.6 (adjusted with glacial acetic acid)
Mobile phase B:	100% methanol
Mobile phase C:	200 mM tetrabutyl ammonium hydrogen sulfate [TBAHS] in water for paired-ion chromatography (PIC)
Column temp.:	35 °C
Isocratic:	17% A / 78% B / 5% C (78% MeOH, 17 mM AmOAc, 10 mM TBAHS)
Flow rate:	0.6 mL/min
UV detection:	262 nm (40 pts/sec)
Injection volume:	8 µL
Needle wash:	50:50 acetonitrile/water
Purge:	50:50 acetonitrile/water
Seal wash:	50:50 acetonitrile/water

Note: After analysis, the chromatographic system and column were flushed with 50:50 acetonitrile/water followed by an additional flush with 100% acetonitrile to prevent any precipitation of the buffer or PIC reagent in the system or column.

RESULTS AND DISCUSSION

Methods for the analysis of BAC, based on the USP method, rely on cyano ligand bondings on silica base particles. Employing such methods using modern stationary phases based on high purity silica can result in a significant degradation of peak shape, with increased peak tailing and propensity for overloading. This is attributed to the lack of charged impurities in the highly pure silica base particles, resulting in an increase in the undesirable interactions between the charged analyte and the stationary phase. Paired Ion Chromatography [PIC] is a technique for separating charged analytes on reversed-phase columns by exploiting electrostatic interactions between the analyte and the charged PIC reagent. Paired-ion reagents added to the mobile phase are adsorbed onto the stationary phase, where they alter the interaction between the analyte and the stationary phase surface. For oppositely charged analytes, increased stationary phase interactions can result in analyte retention, whereas similarly charged analytes can exhibit decreased interactions and result in faster elution.[7] In this application, tetrabutyl ammonium hydrogen sulfate [TBAHS], a low UV absorbing PIC reagent, is used to reduce the unwanted interaction of the charged quaternary ammonium salt with the stationary phase, producing sharper peaks with reduced tailing. Figure 2 demonstrates the separation, based on the USP method, of BAC on a traditional Spherisorb Cyano column and on the high purity silica HSS Cyano column, with and without the PIC reagent. Increased interactions between the BAC homologues and the high purity silica result in severe peak tailing. The addition of a similarly charged PIC reagent to the mobile phase reduces this interaction resulting in a decrease in retention and a significant improvement in peak shape.

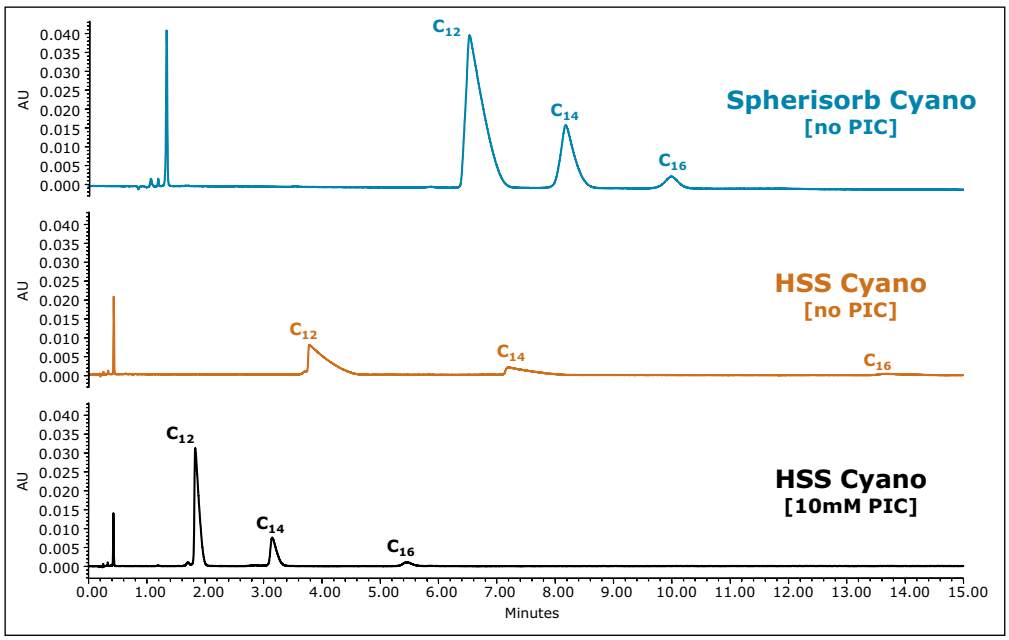

Figure 2. HPLC separations of the BAC reference standard on cyano columns: 5 µm, 4.6 x 150 mm Spherisorb Cyano (top), and on a 3.5 µm, 2.1 x 100 mm XSelect HSS Cyano, without PIC reagent (middle) and with the TBAHS PIC reagent (bottom). The isocratic separations, based on the USP method, used 45:55 acetonitrile/100 mM sodium acetate (pH=5.0) at flow rates of 1.8 mL/min for the 4.6 mm I.D. column and 1.2 mL/min for the 2.1 mm I.D. column.

Additional improvements in peak shape and analyte loadability are realized with the use of the Charged Surface Hybrid (CSH) C_{18} stationary phase, and the replacement of acetonitrile with methanol. Figure 3 demonstrates the improvement in peak shape and loading for the C_{12} BAC homologue on the CSH C_{18} column as a function of analyte concentration and PIC reagent concentration.

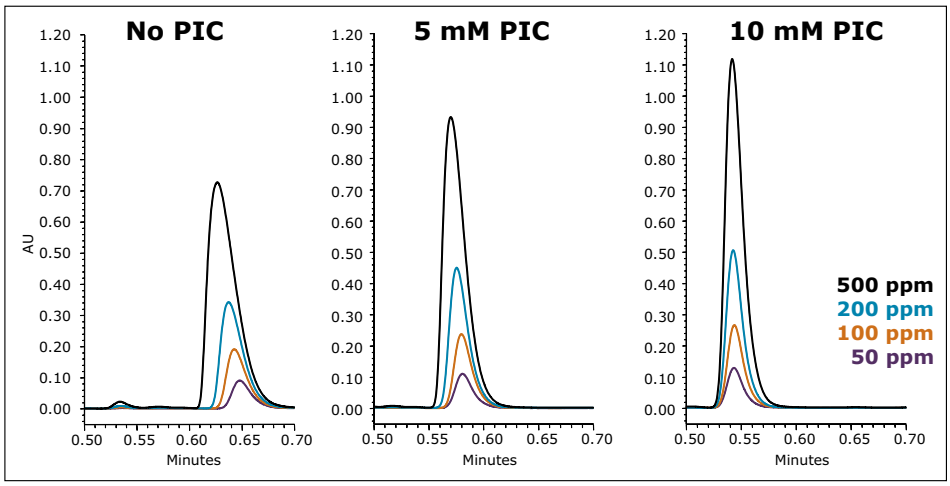

Figure 3. The UPLC separation of the C_{12} BAC homologue using a 1.7 µm, ACQUITY UPLC CSH C_{18} column (2.1 x 50 mm) under isocratic conditions with 80:20 methanol/100 mM ammonium acetate (pH 5.6). The BAC reference standard was prepared at four concentrations (500, 200, 100, and 50 ppm). Separations are shown using no PIC reagent (left), 5 mM PIC reagent (middle), and 10 mM PIC reagent (right).

Although there is an improvement in peak shape with the use of a PIC reagent, a decrease in analyte retention is also observed, as demonstrated in Figure 3. A simple adjustment in the organic concentration of the mobile phase, from 80% to 78% methanol, is all that is required to increase the retention factor, while still maintaining the improvement in peak shape. The resulting chromatography, shown in Figure 4 (bottom), under UPLC conditions, gives excellent peak shape and sensitivity for the USP reference standard for BAC, facilitating integration and quantitation. With the aid of the ACQUITY UPLC Columns Calculator, the method was also easily scaled to utilize the XSelect CSH C_{18} ***XP***, 2.5 µm column (3.0 x 75 mm) under UPLC (middle) and HPLC (top) conditions. The column dimension was chosen in order to maintain the same length to particle size ratio (L/d_p) as for the separation on the 1.7 µm particle size. When scaling methods between different column configurations, maintaining the L/d_p ratio, while scaling flow rates and injection volumes accordingly, results in similar chromatography, with different time scales.

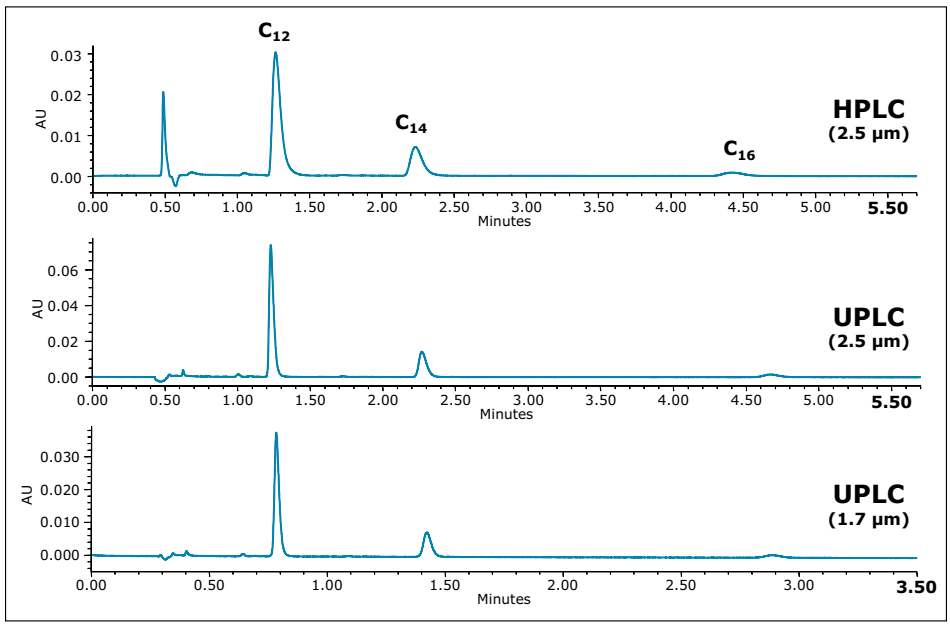

*Figure 4. The UPLC separation of BAC homologues using a 1.7 µm, 2.1 x 50 mm ACQUITY CSH C_{18} column (bottom). The UPLC isocratic separation was achieved using 78% methanol at a flow rate of 0.6 mL/min. The ACQUITY UPLC Columns Calculator was used to scale the method to utilize the 2.5 µm, 3.0 x 75 mm, XSelect CSH C_{18} **XP** column under UPLC (middle) and HPLC (top) conditions. The USP reference standard for BAC was prepared at a concentration of 100 ppm.*

Integration of UPLC chromatograms for the C_{12}, C_{14}, and C_{16} homologues in the BAC reference standard, prepared at various concentrations from 50 to 800 ppm (µg/mL), shows excellent linearity of detector response versus concentration with R^2 values greater than 0.999 (Figure 5).

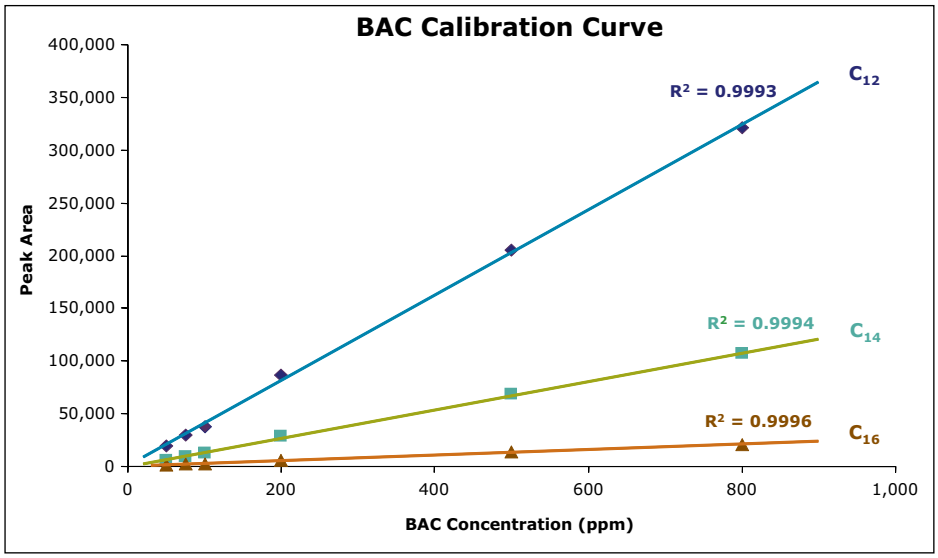

Figure 5. Calibration curve generated under UPLC conditions for the C_{12}, C_{14}, and C_{16} homologues in the USP BAC reference standard. Samples were prepared at concentrations of 800, 500, 200, 100, 75, and 50 ppm.

The UPLC method developed using the USP reference standard was applied to a variety of consumer products. Figure 6 shows a small sampling of products tested, each confirming the applicability of this method.

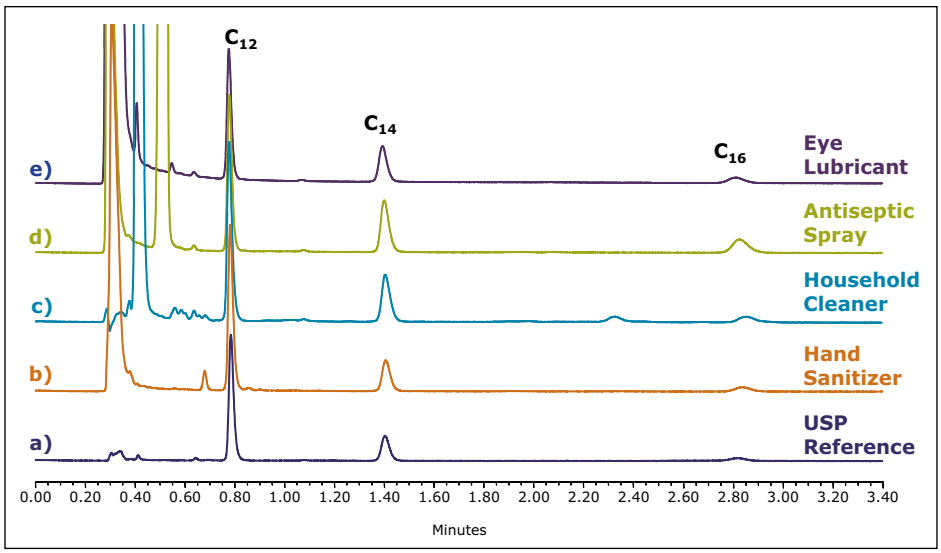

Figure 6. Application of the current method to various consumer products (from bottom to top), (a) USP reference standard-100 ppm, (b) hand sanitizer, (c) household cleaner, (d) antiseptic spray, and (e) eye lubricant. Chromatograms shown were collected on the 1.7 µm ACQUITY UPLC CSH C_{18} column (2.1 x 50 mm) using 78% methanol at a flow rate of 0.6 mL/min.

The BAC concentrations in each sample can be calculated by integration of the individual peak areas for the C_{12}, C_{14}, and C_{16} homologues, and comparing those values with the peak areas from the BAC reference standard using the following equations (results shown in Table 1):

$$Average\ Relative\ Molecular\ Mass\ (ARMM) = \sum_{i=12,14,16} W_i \text{ x } \frac{A_i}{A_T}$$

$$Percentage\ of\ each\ homologue, \%C_i = \frac{W_i \text{ x } A_i}{\sum_{i=12,14,16} W_i \text{ x } A_i} \text{ x } 100$$

$$Assay\ of\ BAC\ (\%w/v) = \frac{\sum_{i=12,14,16} W_i \text{ x } A_i}{\sum_{k=12,14,16} W_k \text{ x } A_k} \text{ x } Conc_{Std} \text{ x } DF$$

Where:

$W_{i,k}$ = Relative molecular mass for the given homologue: 340, 368, and 396 for the C_{12}, C_{14}, and C_{16} homologues, respectively.

A_i = Area of the peak due to the given homologue in the sample preparation.

A_k = Area of the peak due to the given homologue in the reference standard preparation.

A_T = Sum of the areas of the peaks due to all homologues in the sample preparation.

$Conc_{Std}$ = Concentration of BAC reference standard (100 ppm)

DF = Dilution Factor for sample preparation.

BAC Analysis	USP Tailing (C_{12})	USP Efficiency (C_{12})	ARMM	% C_{12}	% C_{14}	% C_{16}	Assay of BAC (% w/v)
Eye Lubricant	1.28	8,576	353.2	59.3%	30.9%	9.8%	0.011
Antiseptic Spray	1.30	7,135	357.2	50.9%	32.7%	16.4%	0.213
Household Cleaner	1.45	7,250	351.9	62.6%	29.4%	8.1%	0.178
Hand Sanitizer	1.39	7,485	349.4	70.3%	23.0%	6.7%	0.132
BAC Ref Std	1.34	7,521	349.4	69.5%	24.9%	5.5%	10.0

Table 1. Summary of BAC concentrations in consumer products.

CONCLUSIONS

Analysis of BAC homologues with the ACQUITY UPLC CSH C_{18} stationary phase offers a rapid, reproducible alternative to existing methods. The use of a PIC reagent reduces the undesirable interactions between the charged quaternary ammonium analyte and the stationary phase, resulting in significant improvements in peak shape. The improvement in peak shape, in combination with the excellent linearity of response, facilitates quantitation. The UPLC method reduces solvent consumption by 95% and analysis time by 80% relative to currently accepted methods, yielding significant cost savings while enabling high-throughput analyses. Additionally, scaling methods to utilize XSelect CSH C_{18} ***XP*** 2.5 µm columns results in lower system operating pressures compatible with HPLC, maximizing the number of LC instruments that can be used for these analyses and facilitating the transfer of methods between facilities with combinations of UHPLC and HPLC instrumentation.

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Part numbers

ACQUITY UPLC® CSH C_{18} 1.7 µm, 2.1 x 50 mm column [PN:186005296]

XSelect CSH C_{18} ***XP***, 2.5 µm, 3.0 x 75 mm column [PN:186006106]

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DISPERSE DYES

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Analysis of Disperse Dyes Using the ACQUITY Arc System with PDA and Mass Detection, and Empower Software

Marian Twohig,[1] Michael O'Leary,[1] and Jane Cooper[2]
[1]Waters Corporation, Milford, MA, USA
[2]Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Enhanced confidence in the profiling of impurities using PDA and mass detection.
- Ease of use with single point control via Empower® 3 Software.
- Dual-flow paths to emulate HPLC and UHPLC separations.

WATERS SOLUTIONS

ACQUITY® Arc™ System

2998 Photodiode Array (PDA) Detector

ACQUITY QDa® Detector

XBridge® C_{18} Column

Empower 3 CDS Software

KEY WORDS

Disperse dyes, consumer products, textile, impurity identification, mass detection

INTRODUCTION

Disperse dyes are low molecular weight synthetic dyes. The structure of the dyes can often contain azo or anthraquinone functional groups.[1] The primary application of disperse dyes is in consumer products such as textiles, paper, toys etc. Several of the dyes have been found to induce an allergic response as a result of prolonged exposure to the skin.[2] The presence of azo groups in the structure of some dyes provides the possibility for them to be converted to potential or known carcinogenic aromatic amines.[2]

The existence of these dyes in consumer products has led to increased awareness of the potential harmful effects to consumer health. Legislation controlling the use of several of these dyes was introduced in Germany in 1996. This led to the development of the DIN 54231 standard procedure which describes a method for the analysis of disperse dyes that employs high performance liquid chromatography (HPLC) or thin layer chromatography (TLC) with either ultraviolet (UV), mass spectrometry (MS), or densitometry detection for the analysis of the dyes.[3-5]

In this application note, we present the analysis of nine disperse dyes (Figure 1) using the standard DIN 54231 procedure with a combination of UV and mass detection, and a dual-flow path liquid chromatography system capable of emulating HPLC or UHPLC separations.[6] The inclusion of the mass detector allowed increased information to be derived from the analysis including confirmation of impurity peaks in specific dye samples. The detection limit when measured using the disperse blue 1 dye standard is specified as 0.7 mg/L in the DIN 54231 method. Using Waters® ACQUITY Arc System and the ACQUITY QDa Detector, the detection limit achieved significantly surpassed the specified detection limit for all compounds evaluated.

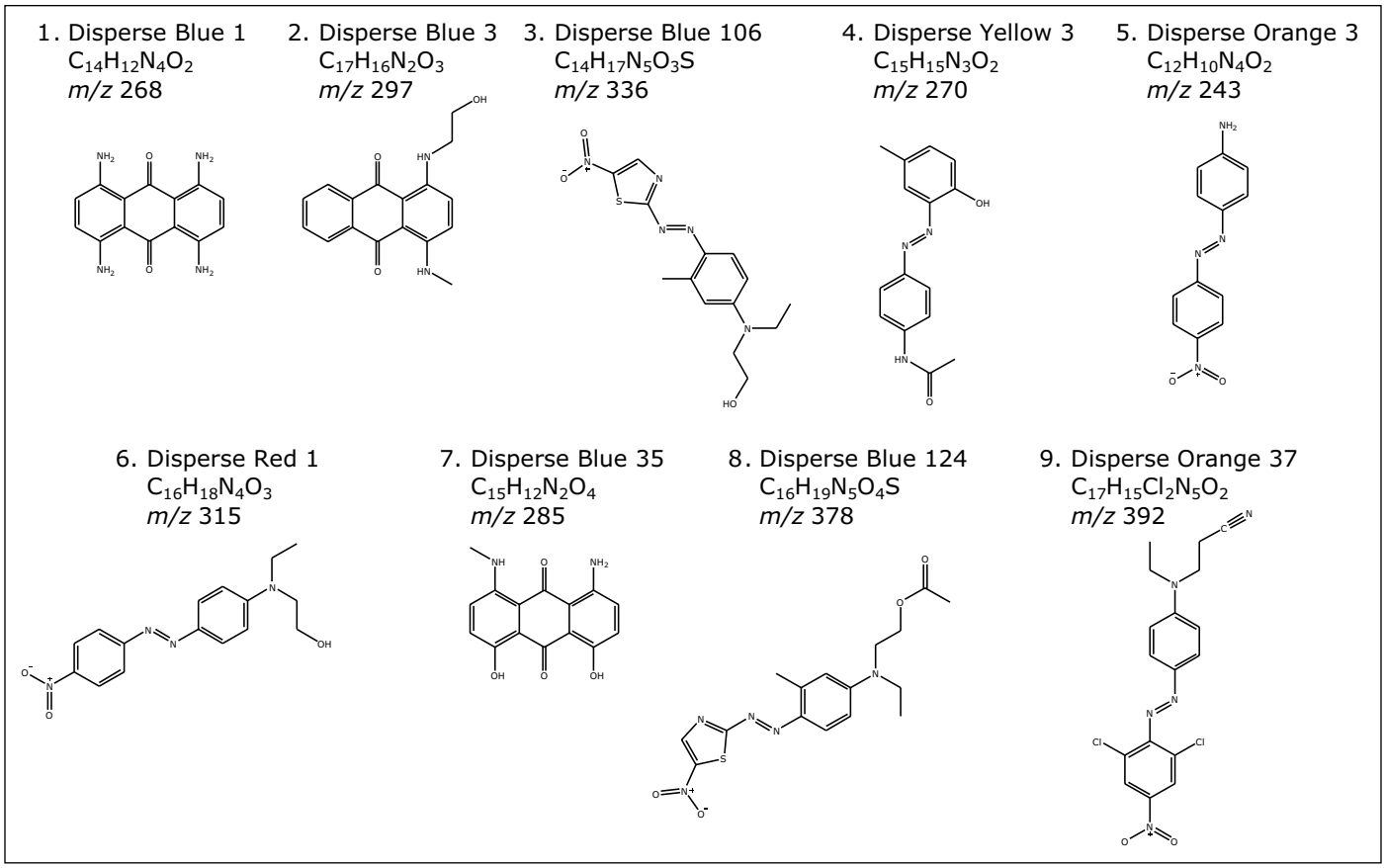

Figure 1. Empirical formulas, structures, and m/z for the disperse dyes used in this study.

EXPERIMENTAL

Instrumentation and software

All separations were performed on the ACQUITY Arc System equipped with a 2998 Photodiode Array (PDA) Detector and positive ion electrospray mass spectrometry (MS) using the ACQUITY QDa Detector. Empower 3 Software was used for data acquisition and processing.

Sample preparation

The dye standards were dissolved in methanol and sequentially diluted in preparation for sample analysis.

LC conditions

HPLC method (DIN 54231)

LC system:	ACQUITY Arc
Separation mode:	Gradient
Column:	XBridge C_{18}, 2.1 x 150 mm, 5 µm
Solvent A:	Ammonium acetate 10 mmol pH 3.6
Solvent B:	Acetonitrile
Flow rate:	0.30 mL/min
PDA detection:	210 to 800 nm
Column temp.:	30 °C
Injection volume:	5 µL
Analysis time:	30 min
Gradient conditions:	0 min 40% B, 7 min 60% B, 17 min 98% B, 24 min 98% B, return to initial conditions.

MS conditions

MS system	ACQUITY QDa
Ionization mode:	ESI +
Capillary voltage:	1.2 kV
Cone voltage:	10 V
Desolvation temp.:	600 °C
Source temp.:	150 °C
MS scan range:	100 to 600 *m/z* and Selected Ion Recording (SIR)
Sampling rate:	5 Hz

Figure 2 shows a PDA chromatogram at 240 nm resulting from the separation of a mixture of nine disperse dye standards (lower trace), and the superimposed SIR channels (top trace) obtained using a 2.1 x 150 mm, 5-µm XBridge C_{18} Column, (Part no. 186003110).

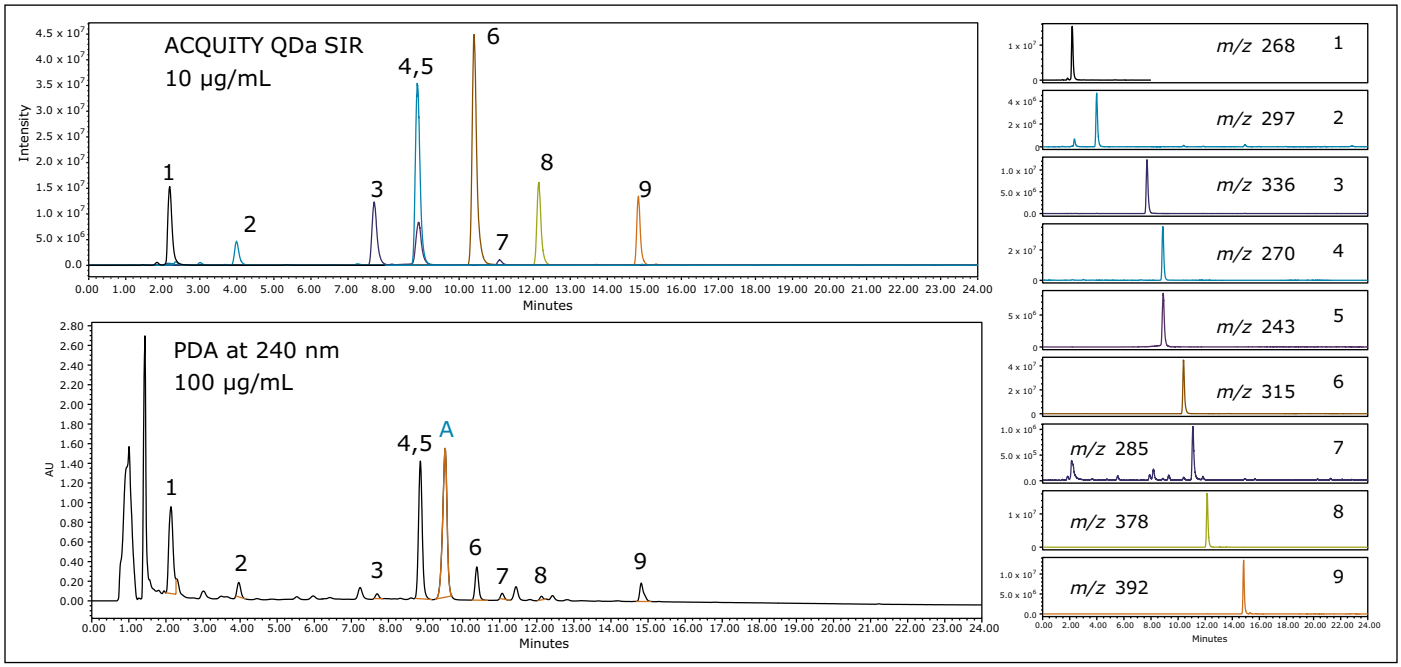

Figure 2. ACQUITY Arc chromatogram from the separation of nine disperse dye standards (100 µg/mL, 5 µL injection) at 240 nm using the DIN 54231 standard method and an XBridge C_{18}, 2.1 x 150 mm, 5.0-µm Column (lower). The superimposed (top) and the individual stacked (right) SIR channel chromatograms (10 µg/mL, 5 µL injection) are also shown.

Note that there is a coelution of the chromatographic peaks resulting from disperse yellow 3 (peak 4), and disperse orange 3 (peak 5) which makes accurate detection by UV alone challenging. Chromatographic separation of the components would be required for accurate detection if UV was to be used which would extend the method development time. The components have different *m/z* ratios, which enabled independent detection using the ACQUITY QDa despite the coelution, as can be seen from the stacked individual SIR chromatograms shown in Figure 2. Detection sensitivity was significantly improved using the mass detector.

Impurity analysis

A prominent peak (peak A) was detected in the PDA data at a retention time (t_R) of 9.5 minutes. This signal was absent from the SIR channels as the specific *m/z* for this unknown component was not monitored in the experimental method. An MS full scan experiment was performed simultaneously with the PDA detector making it possible to determine the mass spectra as well as the UV spectra for all components in the mixture (Figure 3).

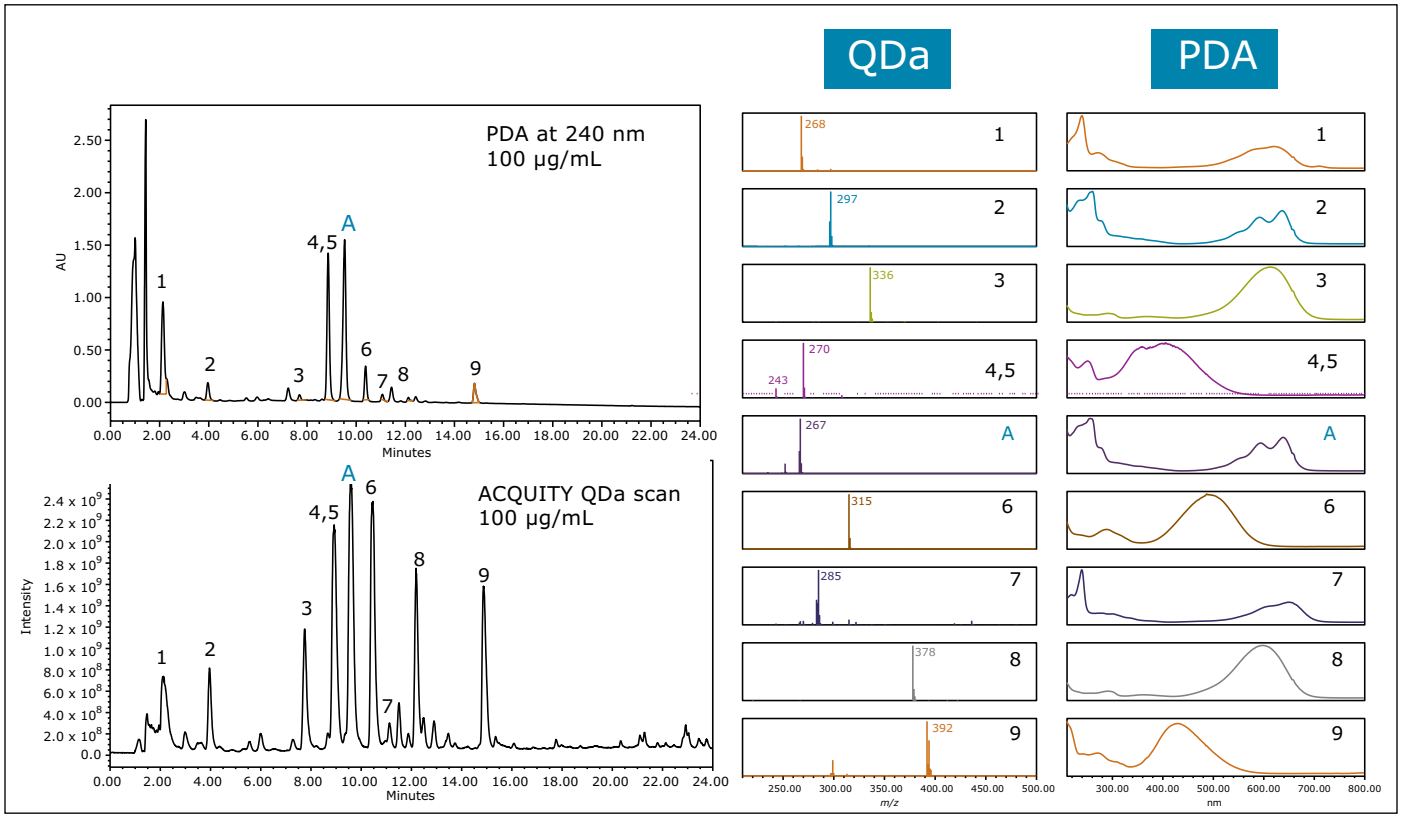

Figure 3. ACQUITY Arc chromatograms from the separation of nine disperse dye standards at 240 nm (top) (100 µg/mL, 5 µL injection) and QDa MS scan (100–600 m/z) (bottom) using the DIN 54231 standard method and an XBridge C_{18}, 2.1 x 150 mm, 5.0-µm Column. The MS and UV spectra are also shown.

The MS spectra for the unknown component A showed a large spectral peak with *m/z* 267. In addition the UV spectra of peak 2 which corresponds to disperse blue 3 and that of unknown peak A had similar features indicating that they may share common structural characteristics. A standard solution containing only disperse blue 3 which had a dye content of 20% was analyzed (Figure 4). The Mass Analysis window from Empower Software allowed rapid confirmation of the identity of disperse blue 3 (*m/z* 297) by displaying both the UV and mass spectra simultaneously. The mass spectrum for unknown peak A indicates that the base peak for this component is *m/z* 267 which matched the previous analysis of the mixture. In addition the t_R and the UV spectra were the same in both analyses. A second unknown component with a t_R of 11.4 minutes, labeled B, with an *m/z* 254 was also detected in the analysis of the disperse blue 3 dye standard. The ACQUITY QDa and PDA data provided complementary information which allowed us to conclude that the impurity A previously detected in the mixture of dyes originated from the disperse blue 3 standard.

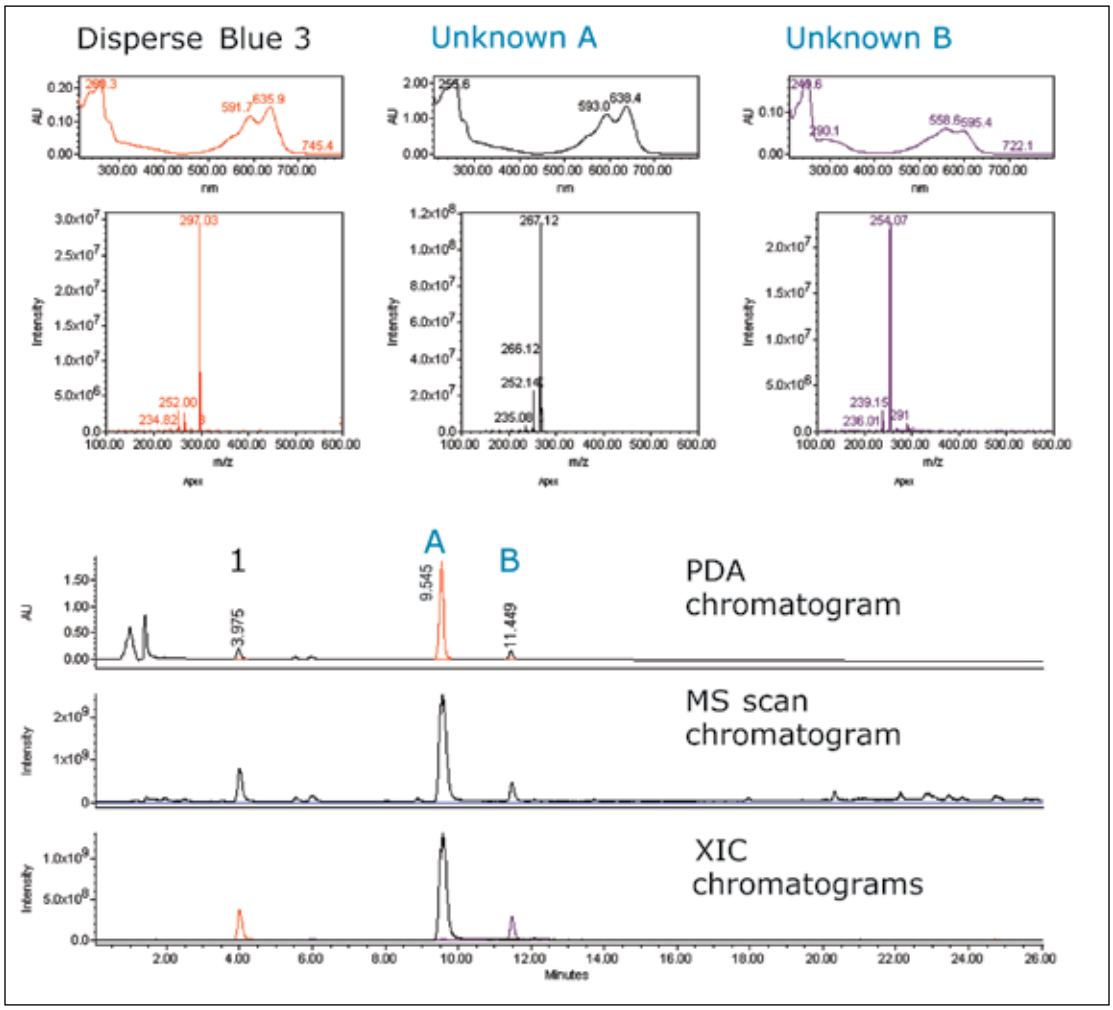

Figure 4. Empower Software's Mass Analysis window showing UV and MS spectra (top). ACQUITY Arc -, PDA, MS scan (100–600 m/z) and superimposed XIC chromatograms of a single standard of disperse blue 3 using the DIN 54231 standard method.

CONCLUSIONS

The addition of mass detection as a complementary analytical detection technique enhances confidence in compound detection and identification. Co-eluting components with different *m/z* ratios can be reliably analyzed using mass detection. The detection limits required for the DIN method can be surpassed for all compounds using the described analytical methodology. The presence of both PDA and mass detection helped confirm that an impurity detected during method development originated in the disperse blue 3 standard. Thus the addition of mass detection acts as a complementary technique for impurity analysis.

The ACQUITY Arc System provides increased flexibility for chromatographic separations and maximizes, productivity by accommodating 3.0 µm to 5 µm particles for HPLC methods, while also supporting rapid and efficient UHPLC separations using 2.5 to -2.7 µm particles.[6]

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Improving the Speed and Quantitative Performance for the Analysis of Allergenic and Carcinogenic Dyes in Industrial, Cosmetic, Personal Care, and Consumer Products

Jane Cooper[1] and Jérémy Marchand[2]
[1] Waters Corporation, Manchester, UK; [2]University of Nantes, France

APPLICATION BENEFITS

This application note illustrates increased sample throughput for the identification and quantification of allergenic and carcinogenic disperse, acid, direct, and basic dyes in consumer products offering:

- Reduced solvent usage due to reduced run times.
- Improved sensitivity, selectivity, and robustness, compared with existing methodologies.

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

Xevo® TQD

MassLynx® MS Software

ACQUITY UPLC BEH C_{18} Column

KEY WORDS

Disperse, acid, direct, basic dyes, consumer products, textile, cosmetics, restricted substances, personal care products

INTRODUCTION

Dyes are added to change or add color to a product, with the aim to add appeal and improve sales by making the product more authentically pleasing.

Dyes are used in many products, for example industrial products such adhesive glues and industrial cleaning products; agricultural products such as seed colorants; cosmetics products (for example lipstick and eye shadow); personal care products (for example soaps, hair dye, and wigs); consumer products (for example inks, candles, fabric, paper, and leather); automotive products (for example car washes and polishes).

Originally, all dyes were natural compounds, but gradually a wide range of synthetic dyes were developed that could be produced faster at a lower cost. Synthetic dyes are classified according to how they are used in the dyeing process. Lipophilic disperse dyes are used for dyeing many synthetic fibers, such as polyester, nylon, cellulose acetate, synthetic velvets, and PVC. Whereas, water-soluble dyes, such as anionic acid dyes, cationic basic dyes, and direct dyes have a wide variety of uses on both natural and synthetic fibers. For example, acid dyes can be used on silk, wool, nylon, and modified acrylic fibers; basic dyes can be used on acrylic fibers, wool, silk, and paper; and direct dyes can be used on cotton, paper, leather, wool, silk, and nylon.

Many companies, in order to fulfill their commitment to protect the consumers of their products, their workers, and the community/environment, develop restricted substances lists (RSL). RSL detail both legislated and non-legislated requirements to be upheld in every part of their product supply production chains to reduce or eliminate hazardous substances and processes. In doing so, they also add environmental sustainability value to their products, and ensure that their products are safe and legally compliant. Many potentially hazardous disperse, acid, direct, and basic dyes are detailed in many consumer product suppliers' RSL.

EXPERIMENTAL

Sample description

Textile

- Textile (0.5 g) was cut up and extracted with 20 mL of methanol for 15 min using an ultrasonic bath (50 °C).
- 100 µL of the extract was transferred in an LC vial and diluted with 900 µL of water.

LC conditions

LC system:	ACQUITY UPLC H-Class
Run time:	7 min
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm
Column temp.:	30 °C
Sample temp.:	10 °C
Mobile phase A:	Water (5 mmol/L ammonium acetate)
Mobile phase B:	Acetonitrile (5 mmol/L ammonium acetate)
Flow rate:	0.6 mL/min
Injection volume:	5 µL

The mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.60	90	10	–
2	0.50	0.60	90	10	6
3	3.00	0.60	5	95	6
4	5.00	0.60	5	95	6
5	5.01	0.60	90	10	6
6	7.00	0.60	90	10	6

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

MS conditions

MS system:	Xevo TQD
Ionization mode:	ESI+ and -
Capillary voltage:	0.7 kV
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas:	1000 L/h
Cone gas:	20 L/h
Acquisition:	Multiple Reaction Monitoring (MRM)

Examples of both legislated and non-legislated regulations and standards developed by various countries and international organizations with regard to dyes include the following: European Committee for Standardization with regard to toy safety standards (BS EN 71 part 9),[1] Sustainable Textile Production (STeP),[2] European Union Commission Decision (2009/567/EC),[3] the German Food and Commodities law (LFGB § 30), and Cosmetic Directive 1223/2009.[4] All detail many of the potentially sensitizing, carcinogenic, mutagenic, or toxic to reproduction dyes as prohibited.

The standard method for the analysis of disperse dyes in textile products and components is DIN54231,[5] using high performance liquid chromatography (HPLC) or thin layer chromatography (TLC) with either ultraviolet (UV), mass spectrometry (MS), or densitometry detection.

Other methodologies for the analysis of disperse dyes include: electrochromatography with electrospray ionization (ESI) and MS detection,[6] HPLC with: UV/VIS detection,[7] atmospheric pressure chemical ionization (APCI) and MS detection,[8] ESI and MS detection,[9,10] and ion-exchange high-performance liquid chromatography (HPIEC) with MS detection.[11]

This application note, using Waters® ACQUITY UPLC H-Class System coupled with the Xevo TQD, describes the advantages of analyzing disperse, acid, direct, and basic dyes compared to previous methodologies. The results show increased robustness, selectivity, and sensitivity, with reduced run times and associated savings in solvent usage.

MS conditions were optimized, as shown in Table 3, for the analysis of disperse, acid, direct, and basic dyes. CAS numbers, empirical formulas, and structures are displayed in Table 2. The established dyes MRM method, which utilizes fast polarity switching available on the Xevo TQD, is illustrated in Figure 1. This enables the analysis of positive and negative dyes within the same analytical analysis.

Disperse, acid, direct, and basic dyes

1. **Disperse Blue 3** CAS: 2475-46-9 $C_{17}H_{16}N_2O_3$	**2.** **Disperse Blue 7** CAS: 3179-90-6 $C_{18}H_{18}N_2O_6$	**3.** **Disperse Blue 35** CAS: 12222-75-2 $C_{20}H_{14}N_2O_5$	**4.** **Disperse Blue 102** CAS: 69766-79-6 $C_{15}H_{19}N_5O_4S$
5. **Disperse Blue 106** CAS: 68516-81-4 $C_{14}H_{17}N_5OS$	**6.** **Disperse Blue 124** CAS: 61951-51-7 $C_{16}H_{19}N_5O_4S$	**7.** **Disperse Brown 1** CAS: 23355-64-8 $C_{16}H_{15}Cl_3N_4O_4$	**8.** **Disperse Orange 1** CAS: 2581-69-3 $C_{18}H_{14}N_4O_2$
9. **Disperse Orange 3** CAS: 730-40-5 $C_{12}H_{10}N_4O_2$	**10.** **Disperse Orange 11** CAS: 82-28-0 $C_{15}H_{11}NO_2$	**11.** **Disperse Orange 37** CAS: 13301-61-6 $C_{17}H_{15}Cl_2N_5O_2$	**12.** **Disperse Orange 149** CAS: 85136-74-9 $C_{25}H_{26}N_6O_3$
13. **Disperse Red 1** CAS: 2872-52-8 $C_{16}H_{18}N_4O_3$	**14.** **Disperse Red 11** CAS: 2872-48-2 $C_{15}H_{12}N_2O_3$	**15.** **Disperse Red 17** CAS: 3179-89-3 $C_{17}H_{20}N_4O_4$	**16.** **Disperse Yellow 1** CAS: 119-15-3 $C_{12}H_9N_3O_5$
17. **Disperse Yellow 3** CAS: 2832-40-8 $C_{15}H_{15}N_3O_2$	**18.** **Disperse Yellow 23** CAS: 6250-23-3 $C_{18}H_{14}N_4O$	**19.** **Disperse Yellow 39** CAS: 12236-29-2 $C_{17}H_{16}N_2O$	**20.** **Disperse Yellow 49** CAS: 54824-37-2 $C_{21}H_{22}N_4O_2$
21. **Acid Red 26** CAS: 3761-53-3 $C_{18}H_{14}N_2Na_2O_7S_2$	**22.** **Basic Red 9** CAS: 569-61-9 $C_{19}H_{18}N_3Cl$	**23.** **Basic Violet 14** CAS: 632-99-5 $C_{20}H_{20}ClN_3$	**24.** **Direct Red 28** CAS: 573-58-0 $C_{32}H_{22}N_6Na_2O_6S_2$

Table 2. Disperse, acid, direct, and basic dyes, associated CAS numbers, empirical formulas, and structures.

No	Chemical substance	Retention time (min)	ESI (+/-)	Cone voltage (V)	Transition	Collision energy
1	Disperse Blue 3	2.41	+	45	297.0 > 235.1	33
					297.0 > 252.0 *	21
2	Disperse Blue 7	2.26	+	50	359.0 > 283.0 *	32
					359.0 > 314.0	20
3	Disperse Blue 35	2.97	+	36	285.0 > 185.0	12
					285.0 > 270.0*	28
4	Disperse Blue 102	2.53	+	42	366.0 > 147.0	31
					366.0 > 208.1*	18
5	Disperse Blue 106	2.71	+	42	336.0 > 147.0	35
					336.0 > 178.0*	17
6	Disperse Blue 124	3.04	+	39	378.1 > 160.1	23
					278.0 > 220.1*	16
7	Disperse Brown 1	2.84	+	53	433.0 > 197.1*	31
					433.0 > 357.0	37
8	Disperse Orange 1	3.36	+	49	319.0 > 122.0*	22
					319.0 > 169.0	26
9	Disperse Orange 3	2.77	+	45	243.0 > 92.0	22
					243.0 > 122.0*	18
10	Disperse Orange 11	2.80	+	53	238.0 > 165.0*	30
					238.0 > 223.0	25
11	Disperse Orange 37	3.27	+	50	392.0 > 133.0*	38
					392.0 > 350.9	22
12	Disperse Orange 149	3.60	-	69	457.1 > 121.0*	52
					457.1 > 266.0	33
13	Disperse Red 1	2.91	+	51	315.1 > 134.0*	25
					315.1 > 284.1	23
14	Disperse Red 11	2.40	+	51	268.0 > 225.0*	28
					268.0 > 253.0	21
15	Disperse Red 17	2.64	+	53	345.1 > 164.1*	26
					345.1 > 269.1	28
16	Disperse Yellow 1	2.57	-	32	274.0 > 166.0*	12
					274.0 > 226.0	15
17	Disperse Yellow 3	2.80	-	37	268.0 > 134.0*	18
					368.0 > 253.0	18
18	Disperse Yellow 23	3.37	+	46	303.1 > 105.0*	21
					303.1 > 181.0	17
19	Disperse Yellow 39	2.83	+	55	291.0 > 130.0*	29
					291.0 > 245.1	28
20	Disperse Yellow 49	3.02	-	22	373.1 > 168.0*	27
					373.1 > 209.1	21
21	Acid Red 26	1.80	+	47	437.0 > 121.1*	25
					437.0 > 355.1	19
22	Basic Red 9	2.01	+	60	288.2 > 195.1*	33
					288.2 > 271.1	35
23	Basic Violet 14	2.12	+	68	302.1 > 195.1	35
					302.1 > 209.1*	32
24	Direct Red 28	2.02	-	81	325.0 > 81.0	27
					325.0 > 152.0*	23

*Table 3. Disperse, acid, direct, and basic dyes, expected retention times, ionization mode, cone voltages, MRM transitions, and associated collision energy values (*refer to the quantification transition).*

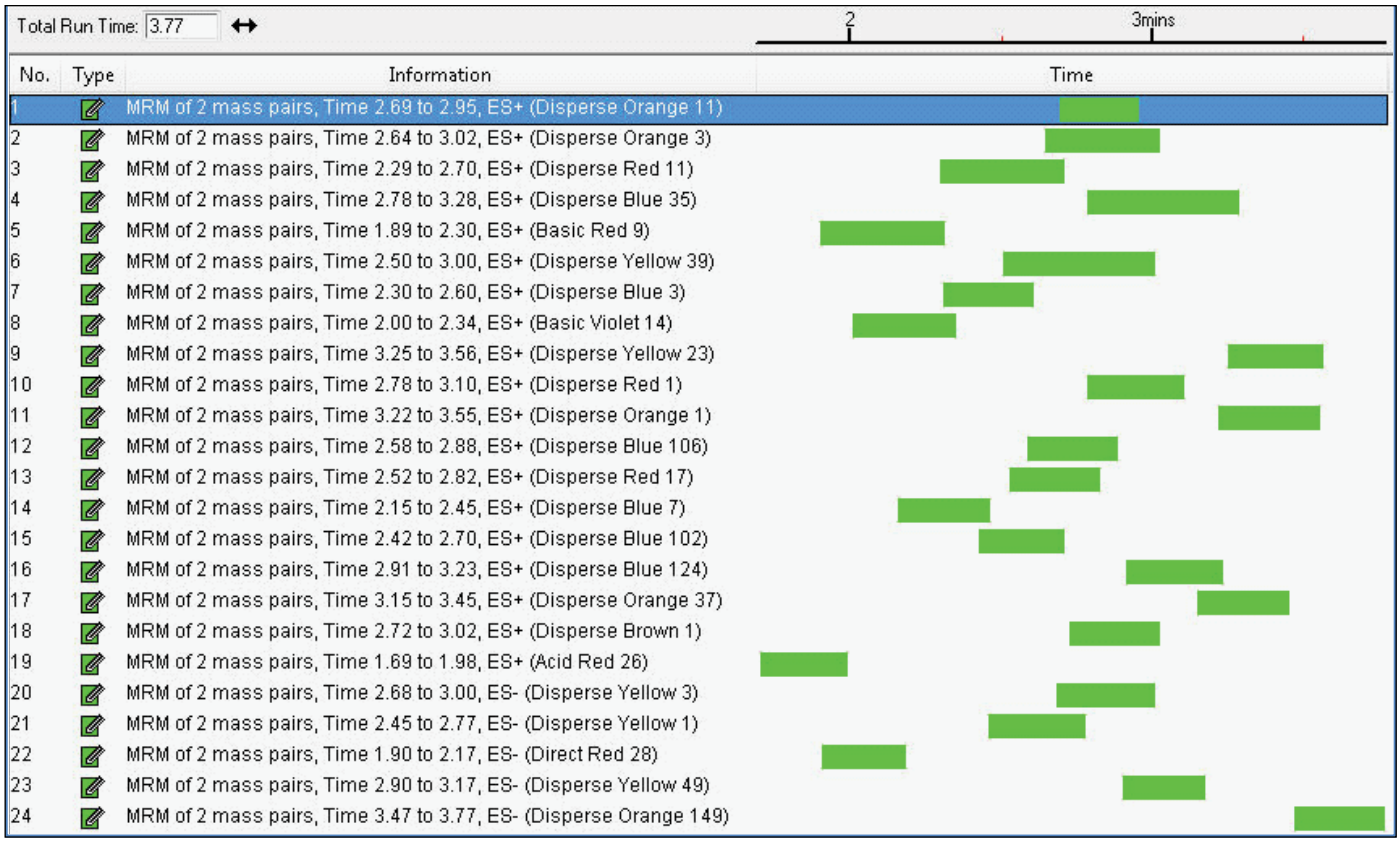


Figure 1. MRM method for 24 disperse, acid, direct, and basic dyes.

Instrument control, data acquisition, and results processing

MassLynx Software was used for data acquisition, and control of the ACQUITY UPLC H-Class System and the Xevo TQD. Data quantification was achieved using the TargetLynx™ Application Manager.

RESULTS AND DISCUSSION

The analysis of 24 disperse, acid, direct, and basic dyes was achieved using Waters' Xevo TQD in MRM mode with ESI ionization, coupled with the ACQUITY UPLC H-Class System.

Optimum MRM conditions were developed and, initially, HPLC conditions based on the work performed by Qiang *et al.*[7] (mobile phase, column, and gradient) were implemented. The method migration from HPLC to UPLC was aided by using tools developed by Waters including the following: the Waters Column Selectivity Chart[12-13] to aid the selection of a suitable UPLC column and the ACQUITY UPLC Column Calculator[13] to aid the development of UPLC gradient and flow. The optimized UPLC conditions resulted in the elution of all compounds within a seven minute run.

The fast cycle and polarity switching times of the Xevo TQD enable the UPLC narrow peaks to be efficiently resolved. A comparison between HPLC and UPLC chromatograms is shown in Figure 2, illustrating improvements in sensitivity and sample throughput.

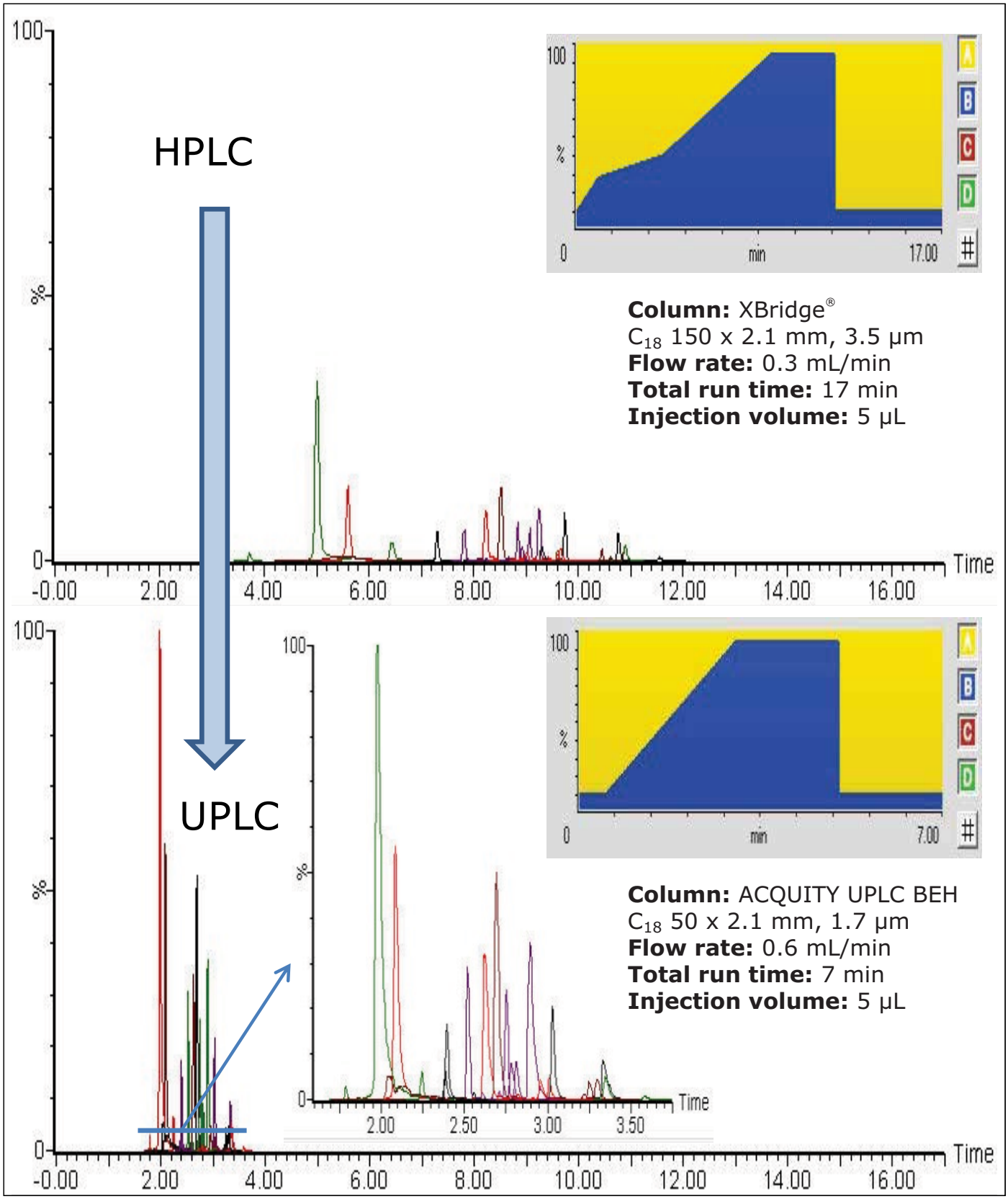
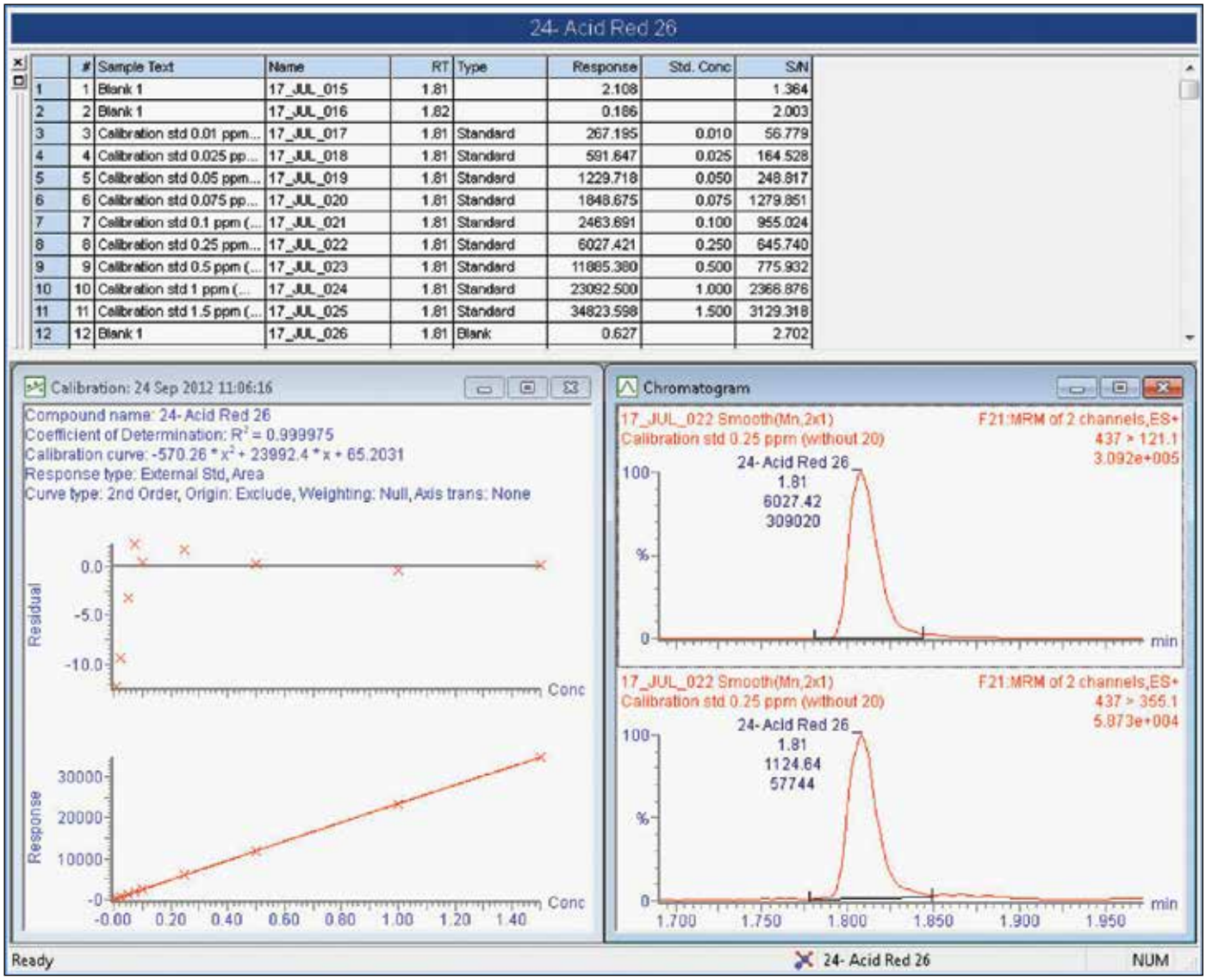


Figure 2. HPLC and UPLC overlaid 1 ppm chromatograms, mobile phase A: water (5 mmol/L ammonium acetate), and mobile phase B: acetonitrile (5 mmol/L ammonium acetate).

Mixed calibration standards, ranging from 0.01 to 1.5 µg/mL, were prepared and analyzed for all of the compounds considered (equivalent range of 4 to 600 µg/g in textile samples). The TargetLynx Quantify results for acid red 26 are shown in Figure 3, and the MRM chromatograms for each compound are shown in Figure 4.



	#	Sample Text	Name	RT	Type	Response	Std. Conc	S/N
1	1	Blank 1	17_JUL_015	1.81		2.108		1.364
2	2	Blank 1	17_JUL_016	1.82		0.186		2.003
3	3	Calibration std 0.01 ppm...	17_JUL_017	1.81	Standard	267.195	0.010	56.779
4	4	Calibration std 0.025 pp...	17_JUL_018	1.81	Standard	591.647	0.025	164.528
5	5	Calibration std 0.05 ppm...	17_JUL_019	1.81	Standard	1229.718	0.050	248.817
6	6	Calibration std 0.075 pp...	17_JUL_020	1.81	Standard	1848.675	0.075	1279.851
7	7	Calibration std 0.1 ppm (...	17_JUL_021	1.81	Standard	2463.691	0.100	955.024
8	8	Calibration std 0.25 ppm...	17_JUL_022	1.81	Standard	6027.421	0.250	645.740
9	9	Calibration std 0.5 ppm (...	17_JUL_023	1.81	Standard	11885.380	0.500	775.932
10	10	Calibration std 1 ppm (...	17_JUL_024	1.81	Standard	23092.500	1.000	2366.876
11	11	Calibration std 1.5 ppm (...	17_JUL_025	1.81	Standard	34823.598	1.500	3129.318
12	12	Blank 1	17_JUL_026	1.81	Blank	0.627		2.702

Figure 3. TargetLynx Quantify results browser showing the calibration quantification results, calibration curve, and example MRM chromatogram for acid red 26.

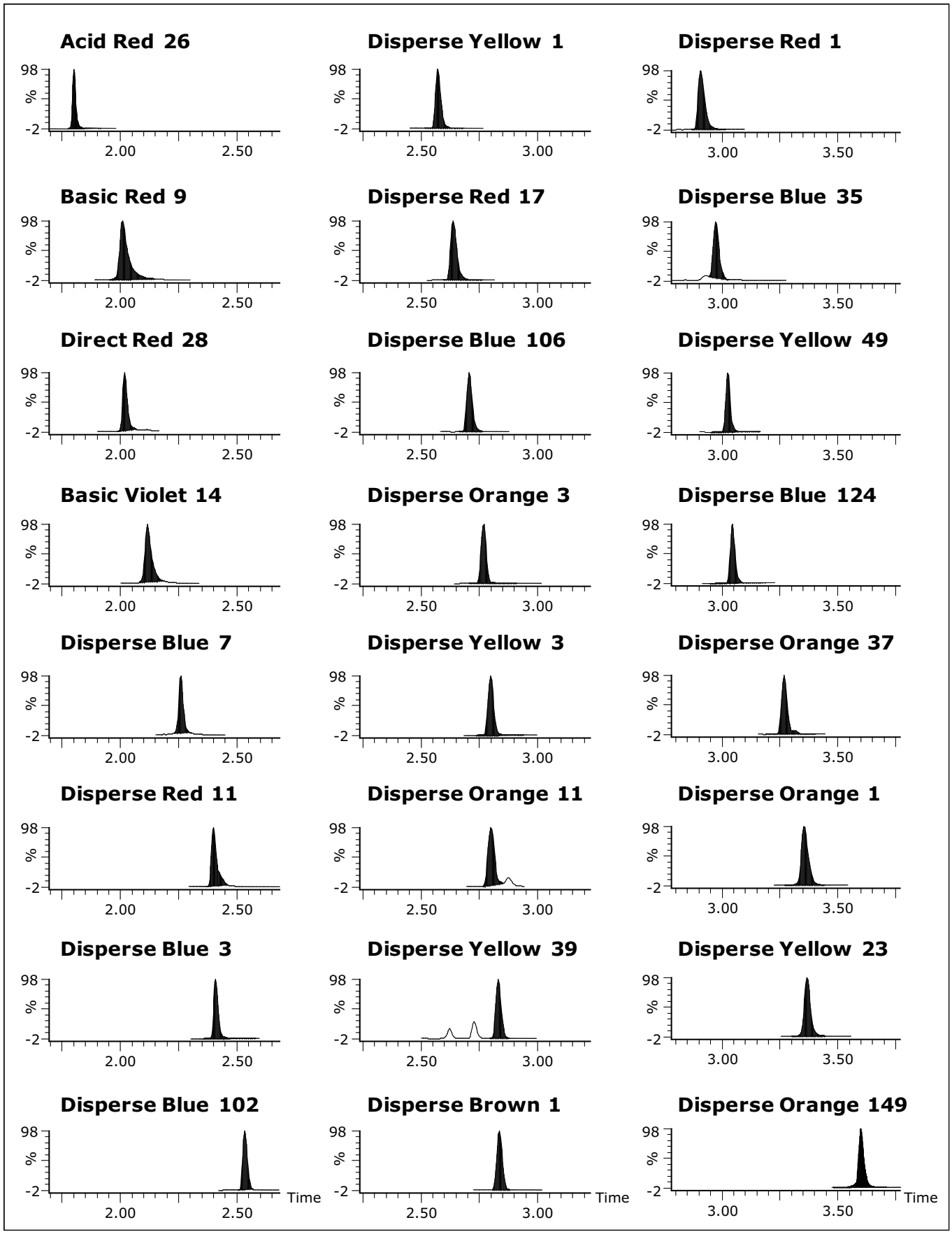


Figure 4. MRM chromatograms for disperse, acid, direct, and basic dyes in a mixed 0.5 µg/mL calibration standard (equivalent to 200 µg/g in textile samples).

Textile analysis

The MRM mass detection method, shown in Figure 1, was used after appropriate sample preparation to quantify for dyes.

Using the extraction protocol (based on DIN 54231)[5] and the instrument parameters as detailed, the results obtained for the analysis of synthetic textile samples spiked at 75 and 30 µg/g are shown in Table 4. Many laboratories that base their extraction protocol for disperse dyes on DIN 54231,[5] accept 75 µg/g as the practical detection limit. Recoveries were obtained by comparing extracted spiked textile samples with calibration standards.

Dye	Sample	Replicate injection results (µg/g)			Average recovery (blank corrected) %	RSD (%)
		1	2	3		
Disperse Brown 1	Blank	ND	ND	ND	-	-
	75 µg/g	67.7	71.6	74.8	95.1	5.0
	30 µg/g	27.7	27.2	27.2	91.2	1.1
Disperse Red 1	Blank	ND	ND	ND	-	-
	75 µg/g	75.3	75.0	78.8	102	2.8
	30 µg/g	33.2	31.8	33.7	110	3.3
Disperse Yellow 1	Blank	ND	ND	ND	-	-
	75 µg/g	77.1	80.9	82.2	107	3.3
	30 µg/g	28.0	30.4	29.5	97.7	4.1
Disperse Yellow 39	Blank	0.28	0.36	0.40	-	-
	75 µg/g	74.0	80.8	81.6	105	5.4
	30 µg/g	30.3	30.4	31.2	101	1.6
Disperse Yellow 49	Blank	ND	ND	ND	-	-
	75 µg/g	71.2	72.6	73.8	96.7	1.8
	30 µg/g	27.3	27.0	27.7	91.1	1.3

Table 4. Textile samples spiked with selected disperse dyes recovery data. Results obtained using mass spectrometric detection and quantified against mixed calibration standards. ND = not detected.

Efficient recoveries were obtained, ranging between 91% and 110% for the three replicates.

Additional benefits over previous methodology include improved selectivity and sensitivity for the analysis of dyes using the ACQUITY UPLC H-Class System coupled with the Xevo TQD with reduced run times, and associated savings in solvents.

CONCLUSIONS

By utilizing the ACQUITY UPLC H-Class System coupled with the Xevo TQD, a fast, selective, and sensitive method was developed for the analysis of disperse, acid, direct, and basic dyes.

Rapid polarity switching technologies, available on the Xevo TQD, enabled UPLC analysis of positive and negative dyes from a single injection.

The described approach offers the following benefits when compared with standard methodology:

- Business benefits of using UPLC analysis, when comparing HPLC/UV to UPLC/MS analysis, include a greater than five times increase in sample throughput and more than an 86% reduction in solvent usage.
- Enhanced sensitivity and selectivity resulting in improved confidence in the identification and quantification offered by the ACQUITY UPLC H-Class System coupled with the Xevo TQD.
- Fast method migration from HPLC to UPLC aided by the use of tools developed by Waters including the following: the Column Selectivity Chart used to aid the selection of a suitable UPLC column, and the ACQUITY UPLC Column Calculator used to aid the development of UPLC conditions.

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Rapid Screening of Consumer Products for Disperse Dyes

Jane Cooper and Jérémy Marchand

GOAL

To quickly screen for the presence of disperse dyes in consumer products without sample preparation or chromatographic separation.

Xevo TQD with ASAP provides a rapid and easy screening method for disperse dyes.

BACKGROUND

Disperse dyes are used for coloring many synthetic fibers used in consumer products, such as polyester, nylon, synthetic velvets, and PVC.

Disperse dyes are included in the restricted substances lists (RSL) utilized by many companies to fulfill their commitment to protect the consumers of their products, their workers, as well as the community/environment.

There are legislated and non-legislated regulations and standards that detail many of the potentially sensitizing, carcinogenic, mutagenic, or toxic to reproduction disperse dyes as prohibited including: European Committee for Standardization with regard to toy safety standards (BS EN 71 part 9),[1] Oeko-Tex Standard 1000,[2] European Union Commission Decision (2009/567/EC),[3] and the German Food and Commodities law (LFGB 30).

This study describes the simple and rapid screening of disperse dyes in consumer products without sample preparation or chromatographic separation.

Qualitative screening methods offer the chemical industry valuable time and cost savings, faster sample turnaround times, and reduced use and associated disposal of non-environmentally friendly solvents and chemicals.

Figure 1. Atmospheric Solids Analysis Probe (ASAP).

THE SOLUTION

Using the Waters® Atmospheric Solids Analysis Probe (ASAP) with the Xevo® TQD, this study demonstrates the rapid screening of consumer products for disperse dyes by direct analysis under atmospheric pressure ionization (API) conditions, with no sample preparation or chromatographic separation. ASAP can easily and quickly be fitted to the API source in less than 2 min by replacing the current probe and fitting a corona discharge pin. Optimum MRM conditions were developed, as shown in Table 1.

0.5 g of synthetic fabric was spiked with select disperse dyes at a level of 75 µg/g (many laboratories that base their extraction protocol for disperse dyes on standard method DIN 54231[4] accept 75 µg/g as the practical detection limit).

The ASAP assembly is in two parts, as seen in Figure 1, where the outer probe is continually attached to the source throughout usage; whereas, the inner probe can be removed to load the sample without compromising the source conditions. The sample was loaded by rubbing the end of a sealed glass melting point capillary tube over the fabric sample. The glass capillary, attached to the inner ASAP probe, was loaded directly into the source enclosure of the Xevo TQD. The sample was vaporized in a stream of heated nitrogen and ionized by gas phase ion/molecule reactions from a corona discharge needle. A vial of methanol was also placed in the source to act as a chemical modifier, influencing the ionization mechanism to favor the formation of protonated ions. The MRM chromatograms, revealing the desorption profiles generated from ASAP analysis on spiked fabric samples, are shown in Figure 2.

Chemical substance	Cone voltage (V)	Transitions	Collision energy
Disperse Blue 3	45	297.0 > 235.1*	33
		297.0 > 252.0	21
Disperse Blue 106	42	336.0 > 147.0*	35
		336.0 > 178.0	17
Disperse Brown 1	53	433.0 > 197.1	31
		433.0 > 357.0*	37
Disperse Orange 1	49	319.0 > 122.0	22
		319.0 > 169.0*	26
Disperse Orange 3	45	243.0 > 92.0*	22
		243.0 > 122.0	18

*Table 1. Disperse dyes, cone voltages, MRM transitions, and associated collision energy values (*refer to the confirmation transition).*

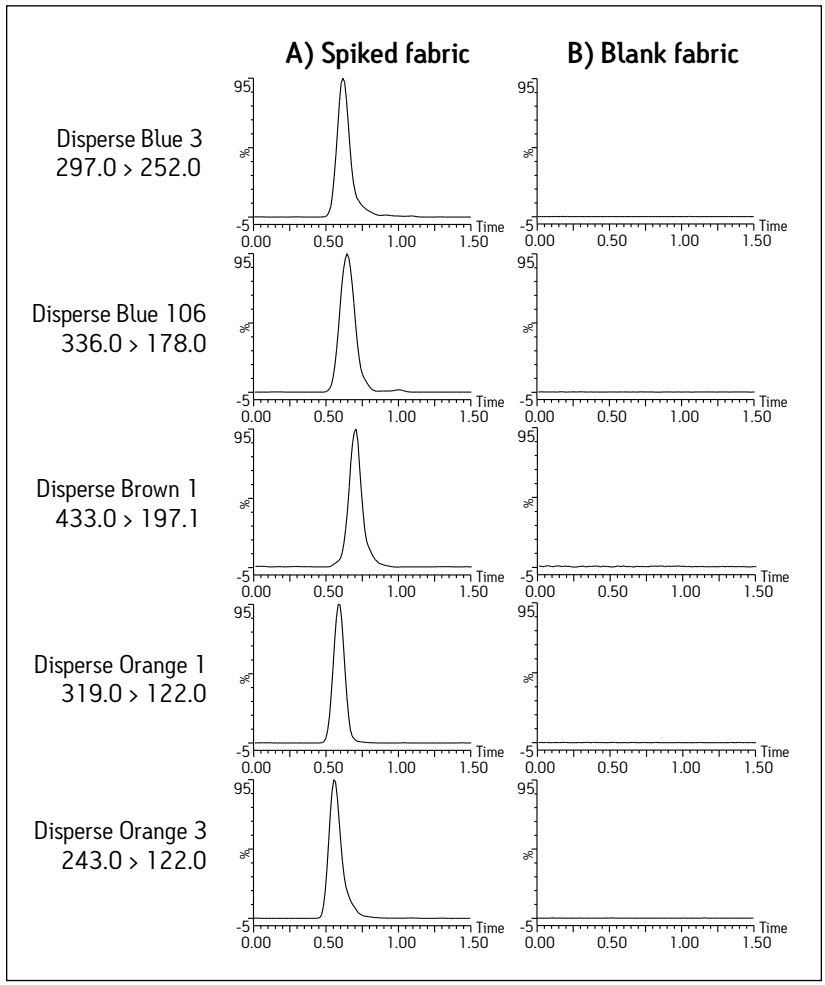

Figure 2. MRM chromatograms for selected disperse dyes showing the desorption profiles generated from ASAP analysis on A) a spiked fabric sample (equivalent to 75 µg/g), and B) a blank fabric sample.

SUMMARY

Waters Xevo TQD with ASAP can be used to provide a rapid, simple screening solution for disperse dyes in consumer products.

No sample preparation or chromatographic separation was required, resulting in a fast run time and reduced overall analysis time, offering businesses valuable productivity and consumables cost savings.

The Xevo TQD offers the ability to acquire data in MRM mode, providing improved selectivity and sensitivity to screen for disperse dyes. The system can also be rapidly switched to UPLC/MS/MS mode if quantification and confirmation of disperse dyes in consumer products is required.

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Rapid Screening of 36 Synthetic Dyes using the ACQUITY UPLC H-Class System with the ACQUITY QDa Detector

Melvin Gay, Jin Chang Huang, Qi Cai, and Qing Long Sun

GOAL

To selectively analyze 36 disperse, basic, acid, solvent and direct synthetic dyes to below EU legislative limits.

BACKGROUND

Synthetic dyes are classified according to how they are used in the dyeing process. For example, disperse dyes which are mostly azo- or anthraquinone compounds are generally used for dyeing synthetic textile materials such as polyester, nylon, and PVC. The type of bonds formed between the dyes and the fabric, determine the properties of the dyes. For example, disperse dyes are not chemically bonded to the fibers of the textile, thus they can easily migrate onto the skin of the person wearing the garment, especially if the textile fastness is poor.

A number of synthetic dyes are known to be allergenic when they come into contact with human skin or if they are are classified as potentially sensitizing, carcinogenic, mutagenic, or toxic to reproduction. 20 known allergenic dyes are listed by the Oeko-tek Standard 100 and the permitted limit is <50 mg/kg. Other legislation such as European Union (EU) 2009/567/EC has banned the use of these sensitizing dyes.

The standard method for the analysis of disperse dyes in textile products and components is DIN 54231-2005 using HPLC-UV-MS with an analysis time of 17 minutes.

Reduce analysis times from 17 min to <5 min.

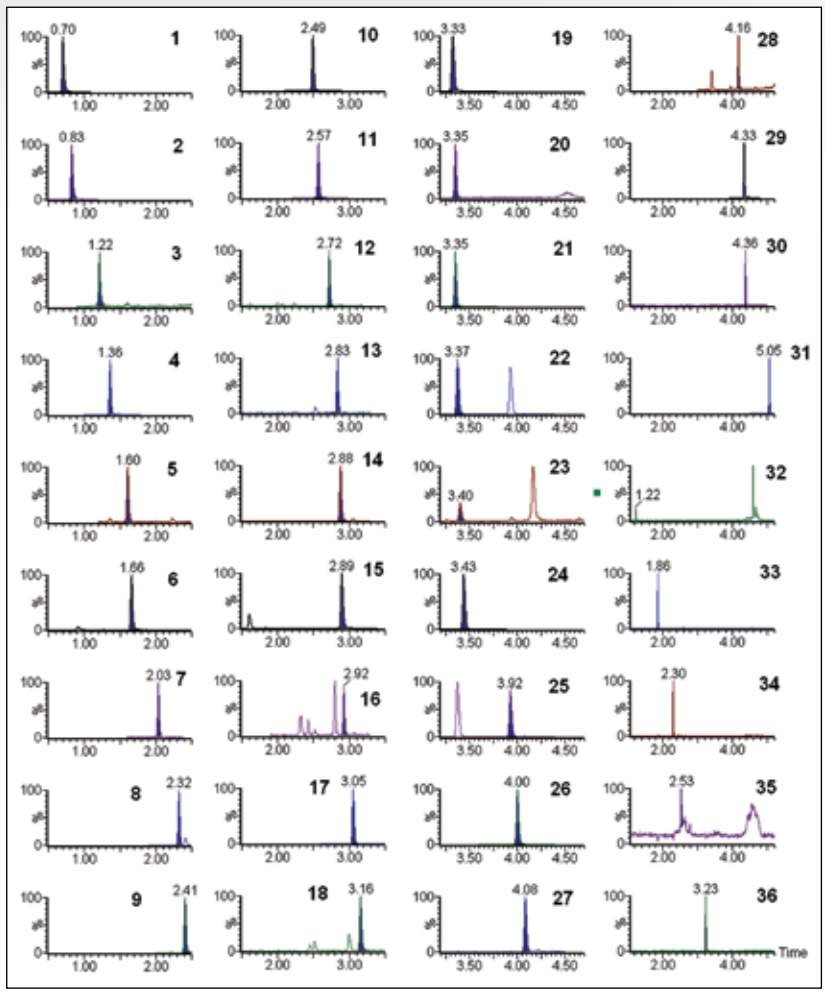

Figure 1. SIR chromatogram of 36 disperse, basic, acid, solvent and direct dye standards. SIR 1 to 31 were acquired in positive polarity, and 32 to 36 were acquired in negative polarity.

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THE SOLUTION

The Waters® ACQUITY UPLC® H-Class System with the ACQUITY® QDa® Detector was used to monitor a total of 36 disperse, basic, acid, solvent, and direct dyes including 28 dyes listed in the Oeko-Tex Standard 100 and 2009/567/EC. The list of the synthetic dyes considered are provided in Table 1.

The time required for method development was greatly reduced using the pre-optimized source parameters in the ACQUITY QDa Detector, where the required sensitivity were achieved in both polarities for both positive and negative ionizing dyes. The low system dispersion in the ACQUITY UPLC H-Class System and the use of sub-2-µm particle columns also greatly increased peak resolution and enhanced sensitivity. Here, two methods were developed for both positive and negative ionizing dyes with analysis time of 5 and 4 minutes respectively.

Dyes were monitored according to their respective retention time, ionizing polarity, and Single Ion Recording (SIR) mass-to-charge ratio (*m/z*), as described in Table 1. In the highly regulated inks and dyes industry, SIR provides more selectivity and sensitivity compared to HPLC-UV analysis. The SIR chromatograms, shown in Figure 1, indicate that synthetic dyes can be easily and confidently detected at low levels.

Current EU legislation prohibits the use of sensitizing dyes in textiles, while allowing 5 ppm limit on the other dyes. Quantitation was also carried out with concentrations ranging from 0.3 to 2.0 ppm, and linearity of >0.997 was achieved for all the dyes. The calibration curve of Disperse Blue 3 is shown in Figure 2.

Table 1. Retention times, SIR m/z, and polarity for 36 disperse, basic, acid, solvent, and direct dyes.

	Compounds	CAS no.	Retention time	SIR	Polarity
1	Basic Red 9	569-61-9	0.70	288	+
2	Basic Violet 14	632-99-5	0.83	302	+
3	Disperse Blue 7	3179-90-6	1.22	359	+
4	Acid Violet 49*	1694-09-3	1.36	712	+
5	Disperse Red 11	2872-48-2	1.60	269	+
6	Disperse Blue 3	2475-46-9	1.66	297	+
7	Disperse Blue 102	69766-79-6	2.03	366	+
8	Disperse Red 17	3179-89-3	2.32	345	+
9	Solvent Yellow 1*	60-09-3	2.41	198	+
10	Disperse Blue 106	68516-81-4	2.49	336	+
11	Disperse Orange 3	730-40-5	2.57	243	+
12	Disperse Yellow 3	2832-40-8	2.72	270	+
13	Disperse Yellow 39	12236-29-2	2.83	291	+
14	Basic Violet 1*	8004-87-3	2.88	358	+
15	Disperse Blue 1	2475-45-8	2.89	268	+
16	Disperse Brown 1	23355-64-8	2.92	433	+
17	Disperse Red 1	2872-52-8	3.05	315	+
18	Disperse Blue 35A	56524-77-7	3.16	285	+
19	Basic Violet 3*	548-62-9	3.33	372	+
20	Disperse Orange 11	82-28-0	3.35	238	+
21	Disperse Yellow 49	54824-37-2	3.35	375	+
22	Solvent Yellow 2*	60-11-7	3.37	226	+
23	Disperse Blue 26	3860-63-7	3.40	299	+
24	Disperse Blue 124	61951-51-7	3.43	378	+
25	Solvent Yellow 3*	97-56-3	3.92	226	+
26	Basic Blue 26*	2580-56-5	4.00	470	+
27	Disperse Orange 37/76	13301-61-6	4.08	392	+
28	Disperse Blue 35B	56524-76-6	4.16	299	+
29	Disperse Orange 1	2581-69-3	4.33	319	+
30	Disperse Yellow 23	6250-23-3	4.36	303	+
31	Disperse Orange 149	85136-74-9	5.05	459	+
32	Direct Blue 6	2602-46-2	1.22	421	-
33	Acid Red 26	3761-53-3	1.86	435	-
34	Direct Red 28	573-58-0	2.30	651	-
35	Direct Brown 95*	16071-86-6	2.53	357	-
36	Direct Black 38	1937-37-7	3.23	736	-

** Compounds not listed in the Oeko-tek Standard 100 and EU 2009/567/EC*

SUMMARY

The ACQUITY UPLC H-Class System with the ACQUITY QDa Detector provides a faster and more reliable analytical tool for the identification and quantification of synthetic dyes. Two methods of less than 5 minutes have been developed for both positive and negative ionizing dyes that provide increased throughput and reduced solvent usage.

The ACQUITY QDa Detector can also be easily integrated into current LC-UV analysis methods which allows unsurpassed sensitivity and selectivity, with limits of detection achieved well below the EU regulatory limit of 5 ppm.

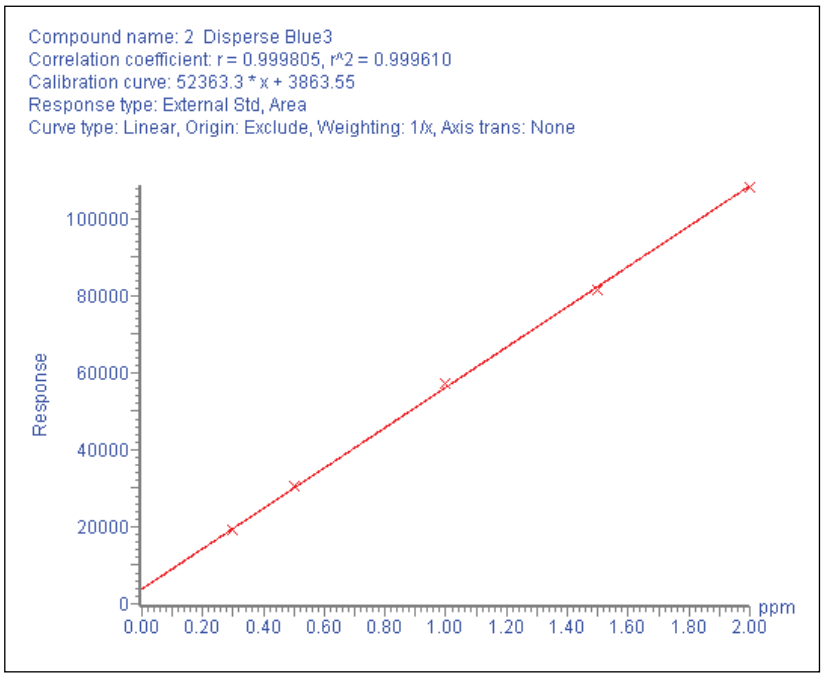

Figure 2. Calibration curve of Disperse Blue 3.

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FLAME RETARDANTS

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Utilizing the Speed and Resolution of UPLC to Enhance the MS/MS Detection of HBCD and TBBP-A Diastereomers

Keith Worrall,[1] Peter Hancock,[1] Alwyn Fernandes,[2] Malcolm Driffield,[2] Martin Rose[2]
[1]Waters Corporation, Manchester, UK; [2]Central Science Laboratory, York, UK

APPLICATION BENEFITS

- Enables significant improvement in chromatographic resolution and run time over and above current methods.
- All five HBCD diastereomers and TBBP-A could be determined rapidly
- Increases laboratory productivity by reducing run time and acquisition-to-report time.
- Reduces cost and environmental impact through lower solvent usage.
- Results compare favorably with fully validated (ISO 17025) method ensuring confidence in results.

WATERS SOLUTIONS

ACQUITY UPLC® System

Tandem (Triple) Quadrupole Mass Spectrometer

MassLynx® Mass Spectrometry Software

KEY WORDS

Tetrabromobisphenol-A, TBBP-A, Hexabromocyclodecane, HBCD, diastereomers, Brominated Flame Retardants, BFRs, Persistent Organic Pollutants, POPs

INTRODUCTION

Brominated Flame Retardants (BFRs) are chemicals commonly used in many domestic and industrial appliances, equipment and textiles to increase their resistance to fire. The use of BFRs has seen an exponential rise over the last few decades with HBCD and TBBP-A being two of the most common chemicals used in the highest levels.

HBCD (Figure 1) is used around the world as a flame retardant in thermal insulation foam for building and construction applications, as well as in upholstery textile coatings, to help prevent deaths and injuries from fire. The HBCD technical product is composed of a number of diasterioisomers of which the α, β, and γ forms predominate. During manufacture, γ-HBCD is the most dominant diastereoisomer formed, contributing approximately 80% of the technical formulation.

TBBP-A (Figure 2) is used to improve the fire safety of electrical and electronic equipment. It is the largest volume BFR for this application in production today.

Both HBCD and TBBP-A are currently marketed around the world without any legislative restrictions. However, as emerging contaminants or Persistent Organic Pollutants (POPs), the importance of continuous monitoring to quantify the impact of these chemicals on human health and the environment is paramount.

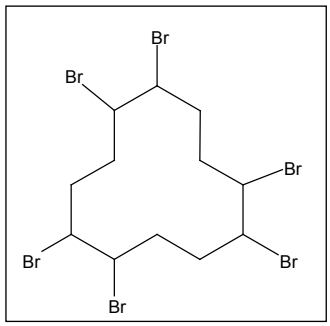

Figure 1. Hexabromocyclododecane.

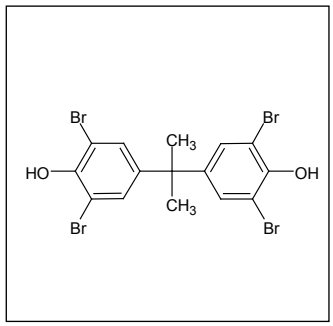

Figure 2. Tetrabromobisphenol-A.

There is current concern as to the persistent nature of these chemicals and consequently, the detrimental effects they may have. Various studies have shown a presence of HBCD and TBBP-A in the environment as well as in aquatic and human tissues. There are indications that the residue levels of HBCD have increased significantly between 1969 and 1997.[1,2]

At their Twenty-third Meeting in December 2006, the Advisory Committee on Hazardous Substances (ACHS) highlighted the following problems with HBCD[3]:

- The substance is extensively monitored and found in water, sediment, soil, and most importantly biota.
- Levels are rising rapidly.
- Levels are rising in top mammals such as whale, dolphin, porpoise, and in birds.
- Monitoring shows a different isomeric ratio to HBCD marketed, which suggests different metabolic behavior of different diastereomers.

There are robust methodologies reported in the literature[4,5] for the analysis of TBBP-A and HBCD diastereomers using HPLC-MS/MS. However, advantages can be gained by the use of Waters® UltraPerformance Liquid Chromatography (UPLC®) through enhanced chromatographic resolution and throughput of the analytical method.[6]

In this application note, we describe a UPLC-MS/MS analysis of all five diastereomers of HBCD and TBBP-A in samples of marine origin. The optimized separation used in this method resulted in a run time of 10 minutes, with the five HBCD diastereomers and TBBP-A analyzed being separated to <10% valley.

Comparability of the UPLC data for real samples to that run using a standard HPLC-MS/MS validated method is excellent. The run time reduction of up to 15 minutes using UPLC offers a throughput improvement of up to five times, combined with a superior separation of target analytes.

EXPERIMENTAL

Standards

Extracts of marine origin and calibration solutions containing BFRs and 13C-Labelled BFRs (internal standards) were provided by the Central Science Laboratory, (CSL) York, UK. The α, β, γ, δ, ε HBCD and TBBP-A single compound standards were supplied by Wellington Laboratories. The methodology used for sample preparation and HPLC-MS/MS analysis are described elsewhere.[8]

UPLC conditions

LC system:	ACQUITY UPLC	
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 150 mm, 1.7 µm	
Column temp.:	60 °C	
Flow rate:	500 µL min^{-1}	
Mobile phase A:	Water	
Mobile phase B:	Methanol	
Gradient:	Time	0.00 min 20% A
	Time	5.00 min 20% A
	Time	6.00 min 0% A
	Time	8.00 min 0% A
	Time	8.10 min 20% A
Total run time:	10 min	
Injection volume:	10 µL, Full loop injection	

MS conditions

MS system:	Quattro Premier™ XE
Ionization mode:	ESI
Capillary voltage:	2.5 kV
Desolvation gas:	Nitrogen, 1000 L/Hr, 400 °C
Cone gas:	Nitrogen, 20 L/Hr
Source temp.:	120 °C
Acquisition:	Multiple Reaction Monitoring (MRM)
Transitions:	See Table 1
Collision gas:	Argon at 3.5 x 10^{-3} mBar

Compound	Transition	Cone voltage (V)	Collision energy (eV)
TBBP-A	542.6>419.7	55	40
TBBP-A	542.6>447.6	55	35
^{13}C-TBBP-A	544.6>80.9	55	55
^{13}C-TBBP-A	554.6>430.6	55	40
HBCD	640.4>78.9	15	15
HBCD	640.4>80.9	15	15
^{13}C-HBCD	652.4>78.9	15	15
^{13}C-HBCD	652.4>80.9	15	15

Table 1. MRM transitions used for target analytes and their internal standards.

Acquisition and processing methods

The data were acquired using Waters MassLynx Software v. 4.1. The data was processed using TargetLynx™ Application Manager. This quantification package is capable of automating quality control checks such as calculating ion ratios, flagging analytical results above/below thresholds set by the user, and many other features.

RESULTS AND DISCUSSION

Current HPLC based methods are becoming more isomer specific[7,8] to enable more specific toxicological studies to be performed.

Until recently, published HBCD concentration data have been derived by Gas Chromatographic (GC) techniques.

However, GC analysis is currently limited as it is unable to chromatographically resolve the different diastereoisomers using standard GC parameters. The diastereomers are thermally labile, with degradation or interconversion observed at temperatures >160 °C. Thus, values have been reported as total HBCD.

During development, two UPLC methods were assessed with comparisons being made to the original HPLC based method. The initial evaluation was a direct transfer of the separation achieved with HPLC. The chromatogram for the four target compounds (TBBP-A, a, b and g HBCD) using HPLC separation is shown in Figure 3.

It was possible to achieve slightly improved chromatographic resolution, while reducing the total run time from 25 minutes to 5 minutes by using an ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 x 50 mm, part no. 186002350. The chromatograms for the optimized UPLC separation are shown in Figure 4. It can be seen that the UPLC separation would allow the laboratory to increase throughput from 2.4 samples per hour to 12 samples per hour.

After optimization of the rapid separation, a mixed standard containing the five HBCD diastereomers and TBBP-A was analyzed. To achieve the required separation, the UPLC BEH C_{18} 150 x 2.1 mm, 1.7 µm column was required, which resulted in a total run time of 10 minutes (6 samples per hour).

Acquisition of the five single component HBCD

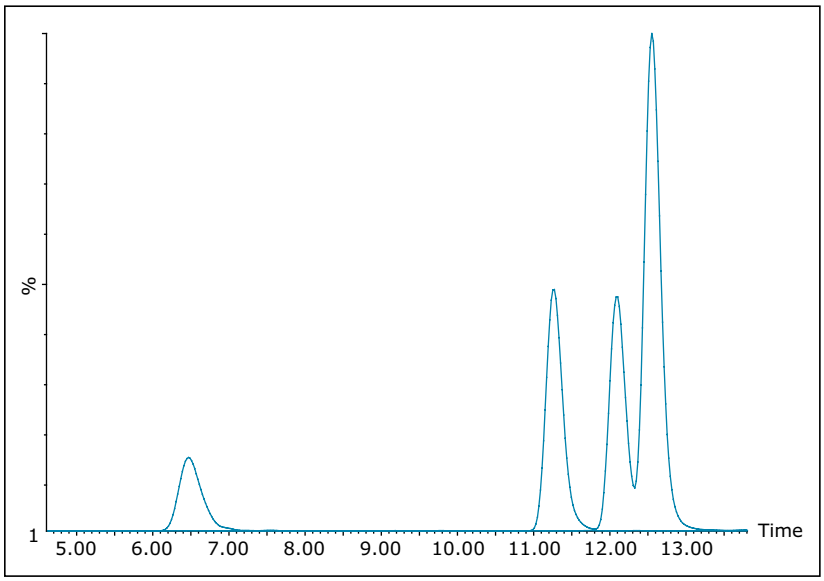

Figure 3. HPLC separation of TBBP-A (6.49 mins), α-HBCD (11.25 mins), β-HBCD (12.08 mins), and γ-HBCD (12.55 mins).

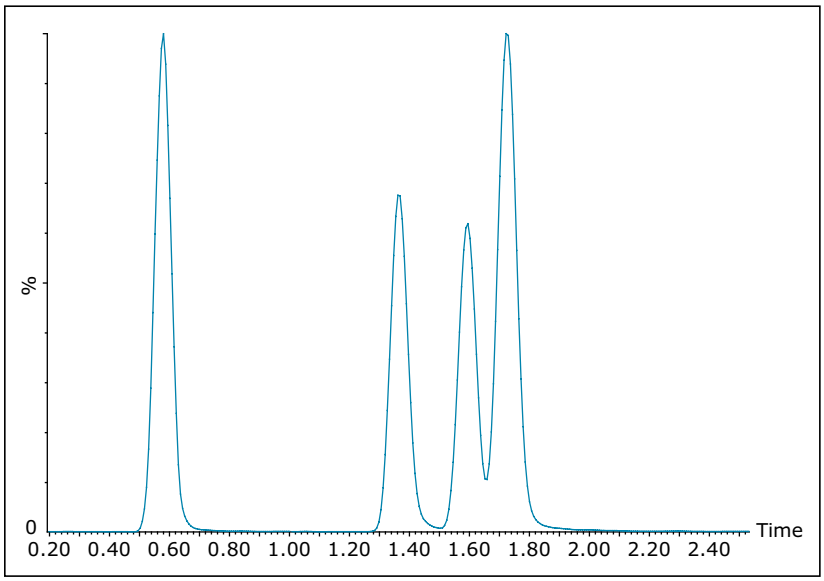

Figure 4. UPLC separation of TBBP-A (0.59 mins), α-HBCD (1.26 mins), β-HBCD (1.47 mins), and γ-HBCD (1.60 mins).

standards resulted in the elution order of α, β, γ, δ, and ε being deduced, with peak widths of 0.15 minutes. The chromatogram for the eluting peaks, including TBBP-A is presented in Figure 5, where the valley of <10% between δ and ε HBCD can be observed.

Following this analysis, a bracketed calibration curve was acquired with a number of sample extracts. This curve contained both native and ^{13}C-labelled α, β, γ-HBCDs and TBBP-A, with native concentrations from 5 ng mL^{-1} to 600 ng mL^{-1}.

The linearity of measurement over the calibration curve range was good for the four compounds determined quantitatively (TBBP-A, α, β, γ-HBCDs), with all coefficients of determination (r^2) being >0.999 for the un-weighted curves.

The reduction in peak widths achieved using UPLC separation resulted in significant sensitivity gains when compared with HPLC separation, with the TBBP-A peak width being reduced from 0.67 minutes to 0.15 minutes and the HBCDs peak widths being reduced from 0.53 minute to 0.16 minute. The chromatograms in Figure 6 show the comparative signals for the target compounds for a 10 ng mL^{-1} solvent standard.

TBBP-A was detected in all of the marine origin samples analyzed, with α, β, γ-HBCDs being detected in most extracts, δ and ε-HBCD were not detected in any. The α-enantiomer dominated in all the samples analyzed, as observed in other reports.[5,7] This profile is characteristic of marine biota and probably arises as a result of selective metabolism of the different enantiomers and/or biotransformation processes.

A typical marine origin extract with concentrations

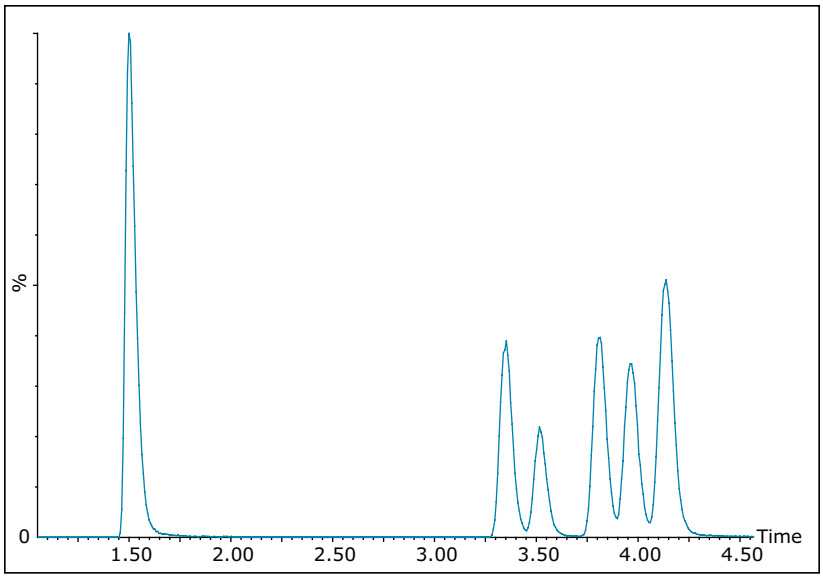

Figure 5. Optimized separation of TBBP-A (1.50 mins) and the five HBCD diastereomers, with the final peak eluting in less than 4.25 mins.

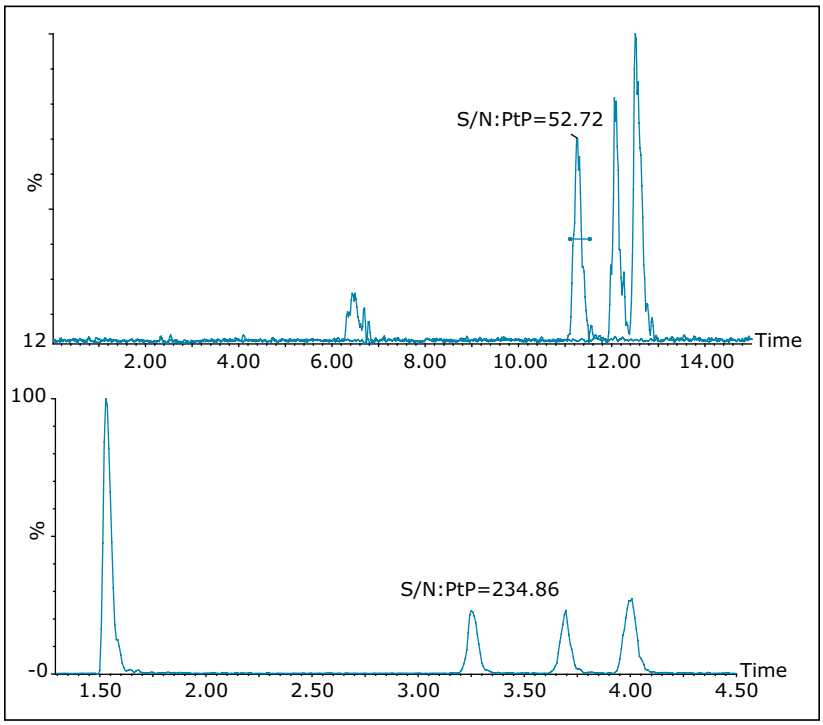

Figure 6. Comparison of HPLC and UPLC 10 ng/mL^{-1} solvent standard injections.

of 0.38, 0.056, and 0.032 ng g^{-1} for α, β, γ-HBCD respectively is given in Figure 7.

All results were compared between the two methods. The mean deviation between HPLC and UPLC was <20%. α-HBCD results across the batch are shown in Table 2, with all data shown with 15% error bars, conforming with a typical measurement uncertainty.

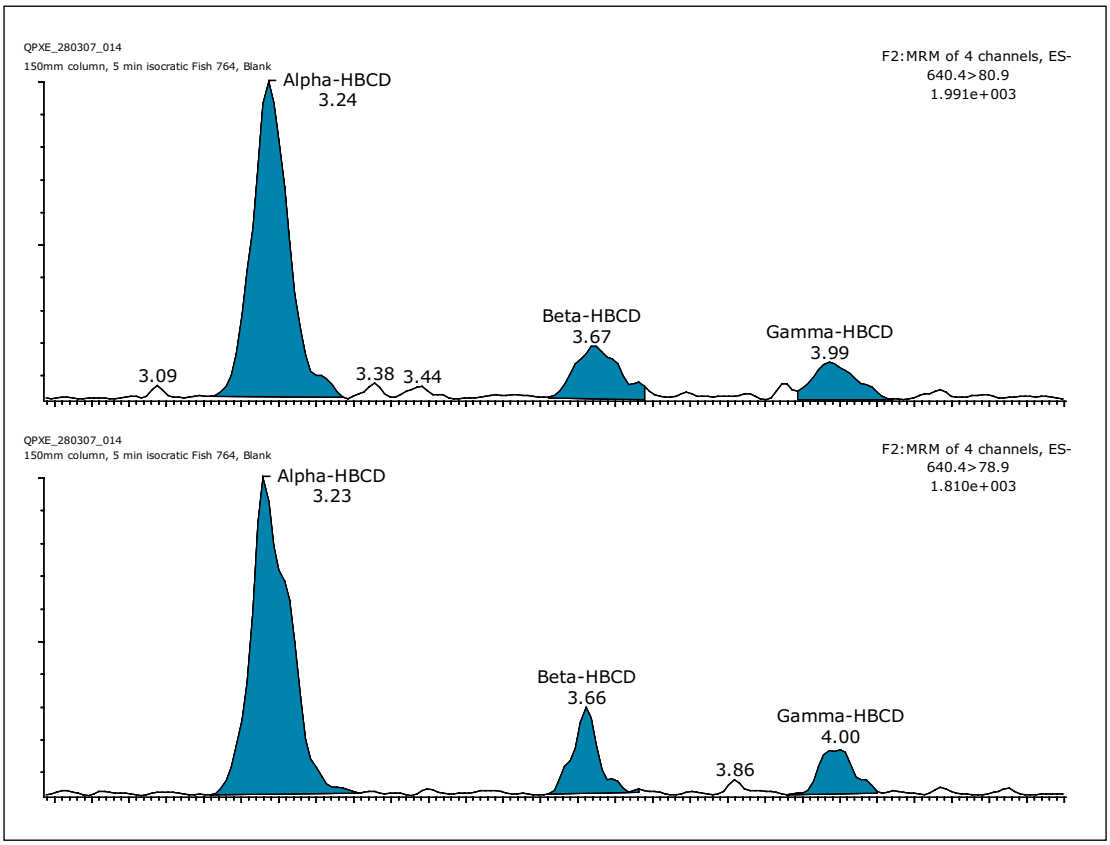

Figure 7. TargetLynx view of a typical extract of marine origin showing low levels of TBBP-A and HBCDs.

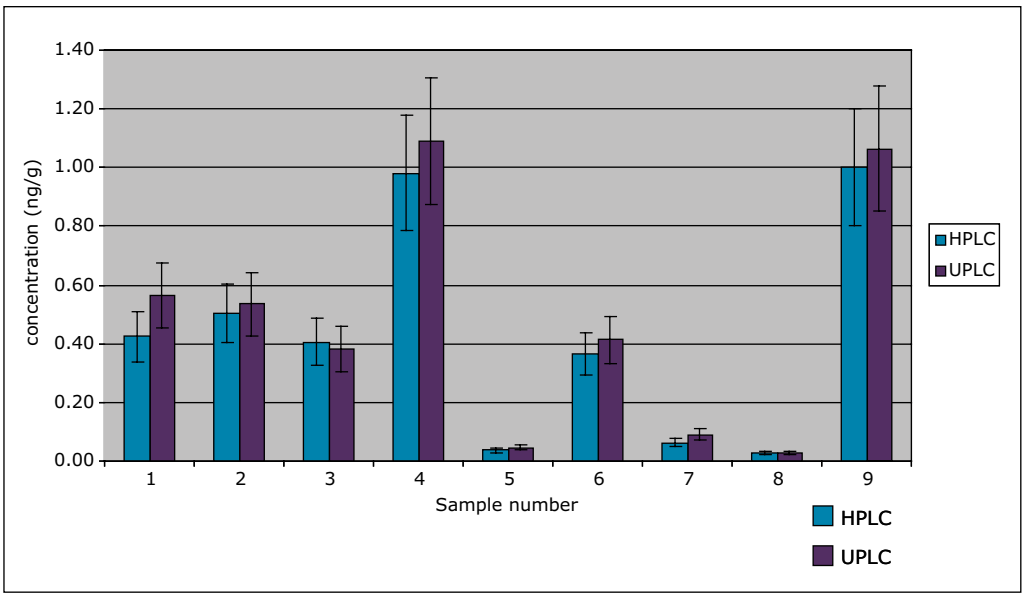

Table 2. Comparison of TargetLynx determined concentrations in samples of marine origin for α-HBCD using HPLC and UPLC.

CONCLUSIONS

The use of the ACQUITY UPLC System with tandem (triple) quadrupole mass spectrometry enabled a significant improvement in chromatographic resolution and a reduction of run time over current HPLC methods.

All five HBCD diastereomers and TBBP-A could be determined rapidly, with added confidence given through TargetLynx data processing.

This vastly increases a cost conscious laboratory's productivity by reducing both run time and acquisition-to-report time. Also, cost and environmental impact will be reduced through lower solvent usage required with UPLC.

Final results compare favorably with an established fully validated (ISO 17025) method ensuring confidence in results.

This methodology carried out on the Waters ACQUITY UPLC System with tandem (triple) quadrupole mass spectrometry can enable laboratories to achieve increased sample capacity, flexibility in workflow, and improved lab efficiency, leading to maximized asset utilization and a faster return on investment.

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Detection of Unexpected Contaminants During Consumer Protection Analysis in Support of Restriction of Hazardous Substances (RoHS) Legislation

GOAL

To demonstrate the advantages of collecting multiple reaction monitoring (MRM) and MS scan data in parallel (RADAR™ acquisition), in support of consumer protection legislation using the Xevo® TQ-S MS System with an APGC Source.

BACKGROUND

Brominated flame retardants (BFRs) are compounds used to decrease the likelihood and intensity of fires. These compounds are present in a wide variety of consumer products, including electronics, clothes, and furniture. Due to the Restriction of Hazardous Substances (RoHS) Directive (2002/95/EC), these compounds are banned in various types of electronic equipment. This is also closely linked with the Waste Electrical and Electronic Equipment Directive (WEEE, 2002/96/EU), which is a legislative initiative to solve the problem of large amounts of toxic e-waste.

BFRs are most commonly analyzed by targeted GC/MS methods using single ion recording (SIR) or MRM. The problem with a purely targeted approach is that it ignores other related compounds or matrix background. The Waters® Xevo TQ-S MS System allows collection of MRM and MS scan data in parallel in one acquisition using RADAR.

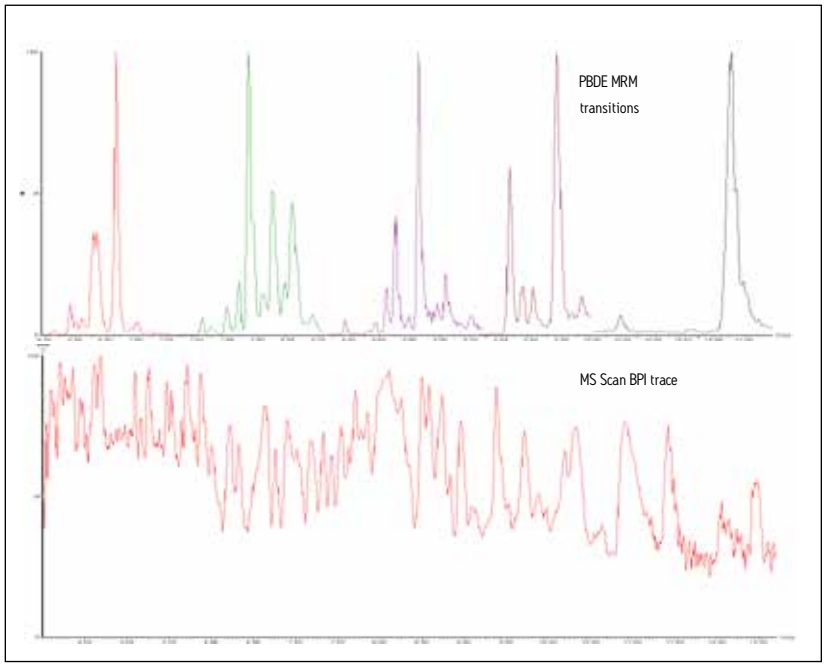


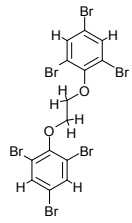
Figure 1. An overlaid trace of the MRM transitions (upper chromatogram) along with a BPI trace (lower chromatogram) of the MS scan data.

THE SOLUTION

The Xevo TQ-S MS System was coupled with a GC using an APGC Source and operated in RADAR mode. A suite of polybrominated diphenyl ethers (PBDEs) was analyzed with two MRM transitions chosen for each degree of bromination. An MS scan function with a mass range 50 to 1050 Da was simultaneously acquired. An extracted sample of a PC keyboard – produced before the July 2006 legislation banning BFRs – was analyzed for PBDEs.

The acquired MRM transitions were used for quantification of PBDEs in the sample. The MS Scan function was interrogated for related compounds or possible interferences. Figure 1 shows an overlaid trace of the MRM transitions (upper chromatogram), along with a BPI trace (lower chromatogram) of the MS scan data. The MS scan in Figure 1 is intense and very complex. The cluster/strip function within MassLynx® Software was used to extract pairs of ions with a separation of 2 Da, which highlighted spectra with halogenated isotope patterns. The NIST08 mass spectral library was used to find spectra of compounds suspected to be in a sample of this nature. Figure 2 shows the spectrum of the peak at 11.43 mins, with the molecular ion cluster magnified. Also, a comparison between the isotope cluster extracted from the suspect peak against the theoretical isotope model of $C_{14}H_8Br_6O_2$ is shown.

The proposed molecular formula is consistent with the compound tribromophenoxyethane, which is the active ingredient from a commercial product, FireMaster 680. Moreover, the fragment cluster (*m/z* 357 and 359) indicates dissociation (loss of $C_6H_2Br_3$+CH_3) of the molecular ion, which further supports this hypothesis. Further work is required to determine the identity of this compound with more confidence.



Molecular formula of the flame retardant Firemaster 680.

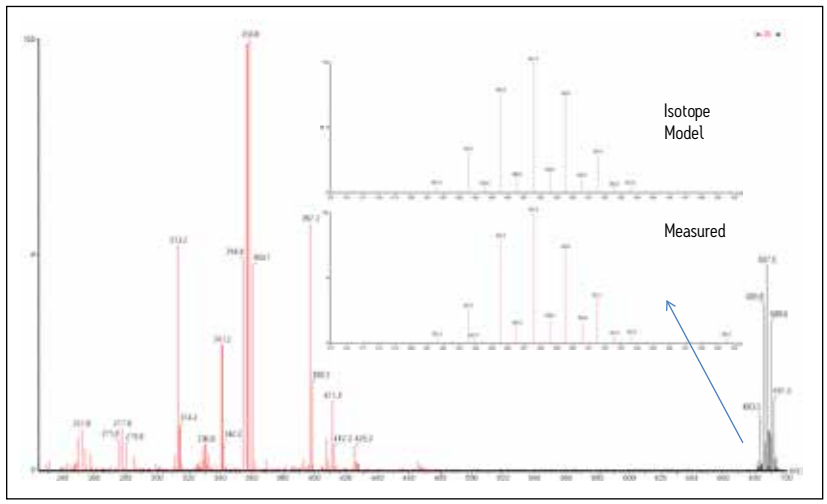


Figure 2. Mass spectra at peak Rt-11.43 (molecular ion cluster magnified), and a comparison between the measured and theoretical isotope patterns.

SUMMARY

- MRM and full scan data for the analysis of PBDEs in a PC keyboard sample were successfully acquired with the APGC and Xevo TQ-S using RADAR functionality.
- RADAR allows accurate quantification of target compounds, while at the same time, it acquires MS scan data to monitor matrix components or other compounds. This is accomplished with little or no impact on the MRM data.
- A flame-retardant compound not originally targeted was tentatively identified after interrogation of the MS Scan function.
- It is only possible to run using RADAR because of the instrument's ability to rapidly alternate between MS and MS/MS modes without compromising performance.

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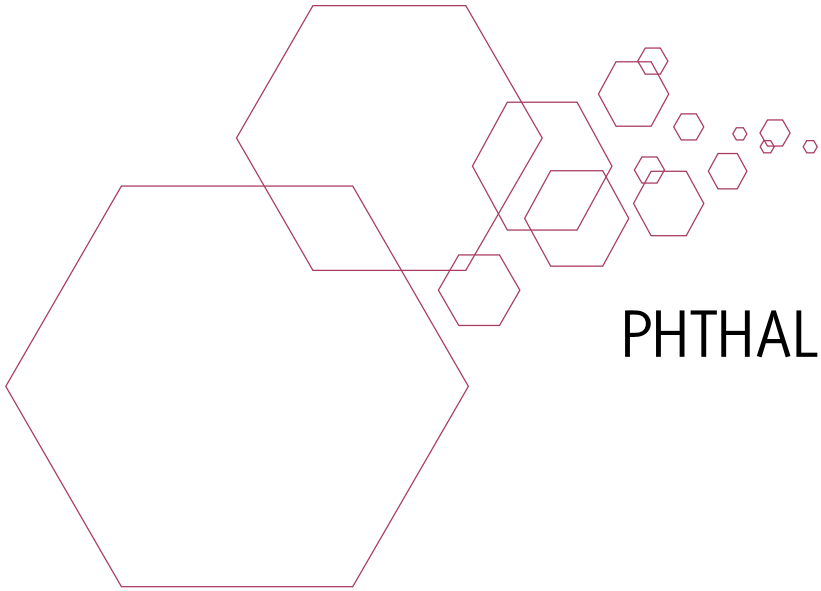

PHTHALATES

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High Throughput Analysis of Phthalates and Parabens in Cosmetics and Personal Care Products Using UPLC with Mass Detection and Empower 3 Software

Jane Cooper
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

The ACQUITY® QDa® Detector linked to the ACQUITY UPLC® H-Class System provides improved confidence in the identification and quantification of phthalates and parabens in cosmetics and personal care products offering:

- 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 UPLC BEH Column

ACQUITY QDa Detector

Empower® 3 CDS Software

KEY WORDS

Phthalates, parabens, triclocarban, consumer products, cosmetics, personal care products

INTRODUCTION

Phthalates are esters of phthalic acid that have extensively been used as plasticizers to increase flexibility, transparency, durability, and longevity in a wide variety of consumer and household products, such as children's toys, electronics, clothes, flooring, wallpaper, and paints. Phthalates are also used, as plasticizers, solubilizers, or denaturants in cosmetics and personal care products, such as perfumes, nail polishes, and hair sprays.

Parabens are esters of parahydroxybenzoic acid, which due to their low volatility, high stability, antibacterial and antifungal properties, have been used as preservatives in cosmetics, personal care, pharmaceutical, food, and industrial products.

Triclocarban is an antibacterial and antifungal agent that is used in many cosmetic and personal care products, including soap, toothpaste, deodorant, shampoo and shaving cream. Triclocarban is also used in several consumer products including kitchen cutting boards, shoes, towels, and clothing, as well as in medical disinfectants and medical products. But there are several health concerns related to the use of triclocarban, including potential hormone and endocrine disruption, and also its potential to contribute to the development of antibiotic resistance.

Many phthalates are classified as hazardous because of their effects on the reproductive system and their association with an increased risk of cancer. Parabens are associated with allergenic contact dermatitis and rosecea. Studies[1,2] have also suggested parabens may be carcinogenic and possess estrogenic disrupting activities. Due to these properties phthalates, parabens, and triclocarban are either banned or restricted, as regulated by the Cosmetic Directive 1223/2009.[3]

In order to accommodate consumer demands for higher standards, many manufacturers are developing, and labeling cosmetic and personal care products 'free from' phthalates and parabens.

Previous example methodologies for the analysis of phthalates include GC-MS,[4] and HPLC-UV[4]; GC-FID,[5] HPLC-UV,[4,6] HPLC-MS,[7] GC-MS,[4] and capillary electrophoresis[6] for the analysis of parabens; and HPLC-MS[8] for the analysis of triclocarban.

Accessible and intuitive as an optical detector, the ACQUITY QDa Detector has been designed for chromatographers with ease of use in mind. Mass detection can be used to achieve reliable analytical methods to unequivocally identify and quantify compounds such as phthalates, parabens, and triclocarban, during both method development stages, and during routine regulatory analysis.

This application note considers the method development, sample extraction, and mass spectral analysis of parabens, phthalates, and triclocarban using Waters® ACQUITY UPLC H-Class System, coupled to the ACQUITY QDa Detector.

EXPERIMENTAL

Sample preparation

Cosmetic and personal care sample analysis

- Add 2.5 mL water and 2.5 mL methanol to 0.2 g sample.
- Vortex mixture for 2 minutes (1600 rpm).
- Further extract mixture in an ultrasonic bath for 30 minutes.
- Centrifuge approximately 1 mL of extract for 5 min (10,000 rpm).
- Transfer centrifuge extract to LC vials for analysis.

LC conditions

LC system:	ACQUITY UPLC H-Class
Runtime:	5.00 min
Column:	ACQUITY UPLC 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:	0.6 mL/min
Injection volume:	5.0 µL

Mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.60	30	70	–
2	1.00	0.60	30	70	6
3	1.50	0.60	10	90	6
4	4.00	0.60	10	90	6
5	4.01	0.60	30	70	6
6	5.00	0.60	30	70	6

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

MS conditions

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

The list of compounds considered, including phthalates, parabens, and triclocarban, along with their expected retention times are detailed in Table 2.

	ESI ionization mode (-/+)	SIR (*m/z*)	Retention time (minutes)
Diethyl phthalate	+	223.1	0.37
Dipropyl phthalate	+	251.1	0.58
Dibutyl phthalate	+	279.2	1.12
Benzylbutyl phthalate	+	313.4	1.07
Bis(2-ethylhexyl) phthalate	+	391.3	2.92
Diisobutyl phthalate	+	279.2	1.04
Di-n-pentyl phthalate	+	307.2	2.10
Di-n-hexyl phthalate	+	335.2	2.44
Dicyclohexyl phthalate	+	331.2	2.09
Di-(2-methoxyethyl)-phthalate	+	283.1	0.28
Di-n-octyl phthalate	+	391.3	3.10
Methylparaben	-	151.1	0.27
Ethylparaben	-	165.0	0.30
Propylparaben	-	179.0	0.35
Butylparaben	-	193.1	0.44
4-Hydroxybenzoic acid	-	137.0	0.24
Benzylparaben	-	227.1	0.43
Triclocarban	-	315.0	1.07

Table 2. Phthalates, parabens, and triclocarban; ionization mode, SIR m/z, and expected retention times.

The empirical formulas and structures are detailed in Tables 3 and 4.

Instrument control, data acquisition, and result processing

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

Phthalates			
Diethyl phthalate CAS: 84-66-2 $C_{12}H_{14}O_4$	**Dipropyl phthalate** CAS: 131-16-8 $C_{14}H_{18}O_4$	**Dibutyl phthalate** CAS: 84-74-2 $C_{16}H_{22}O_4$	**Benzyl butyl phthalate** CAS: 85-68-7 $C_{19}H_{20}O_4$
Bis(2-ethylhexyl) phthalate CAS: 117-81-7 $C_{24}H_{38}O_4$	**Diisobutyl phthalate** CAS: 84-69-5 $C_{16}H_{22}O_4$	**Di-n-pentyl phthalate** CAS: 131-18-0 $C_{18}H_{26}O_4$	**Di-n-hexyl phthalate** CAS: 84-75-3 $C_{20}H_{30}O_4$
Dicyclohexyl phthalate CAS: 84-61-7 $C_{20}H_{26}O_4$	**Di-(2-methoxyethyl)-phthalate** CAS: 117-82-8 $C_{14}H_{18}O_6$	**Di-n-octyl phthalate** CAS: 117-84-0 $C_{24}H_{38}O_4$	

Table 3. Phthalates, associated CAS numbers, empirical formulas, and structures.

Parabens and Triclocarban			
Methylparaben CAS: 99-76-3 $C_8H_8O_3$	**Ethylparaben** CAS: 120-47-8 $C_9H_{10}O_3$	**Propylparaben** CAS: 94-13-3 $C_{10}H_{12}O_3$	**Butylparaben** CAS: 94-26-8 $C_{11}H_{14}O_3$
4-Hydroxybenzoic acid CAS: 99-96-7 $C_7H_6O_3$	**Benzylparaben** CAS: 94-18-8 $C_{14}H_{12}O_3$	**Triclocarban** CAS: 101-20-2 $C_{13}H_9Cl_3N_2O$	

Table 4. Parabens and triclocarban, associated CAS numbers, empirical formulas, and structures.

RESULTS AND DISCUSSION

A fast, selective, and sensitive LC-MS method for the detection of a selection of phthalates, parabens, and triclocarban in cosmetic and personal care products has been developed.

The ACQUITY QDa Detector's SIR parameters were optimized, considering both negative and positive electrospray ionization modes, in order to ensure full coverage of all the compounds being analyzed (as detailed in Table 2.)

Method development was carried out using reversed phase UPLC,® where different gradient conditions, columns, and mobile phases were considered. The objective was to separate the isomeric phthalate compounds considered: di-n-octyl phthalate (DiNP), and diisobutyl phthalate (DiBP); bis(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP) – while maintaining sample throughput. This was achieved by optimizing the mobile phases and the gradient eluting conditions used. The final LC conditions used are detailed in the methods section.

The method was established over the calibration ranges of 0.01 µg/mL to 10 µg/mL for phthalates and triclocarban, and 0.05 µg/mL to 25 µg/mL for parabens, equivalent to 0.25 to 250 mg/Kg, and 1.25 to 625 mg/Kg in the extracted samples respectively. Good linearity was achieved for all the compounds considered (R^2 >0.99). SIR chromatograms for phthalates, parabens, and triclocarban in a mixed 1.0 µg/mL calibration standard are shown in Figure 1.

The developed five-minute UPLC method, is more than seven times faster than existing HPLC and GC methods, with an excess of 90% less solvent usage than existing HPLC methods.

The SIR mass detection conditions detailed in

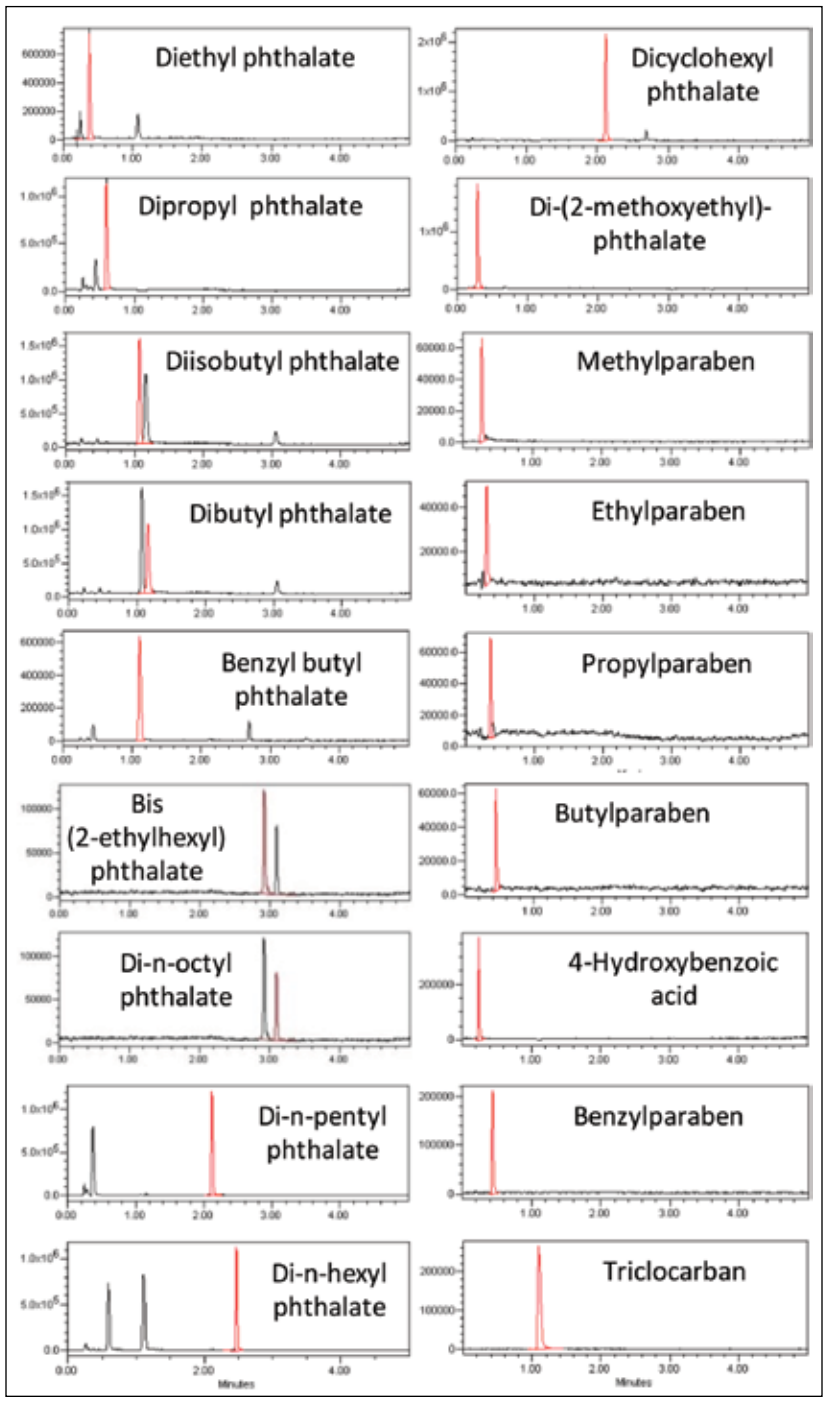

Figure 1. SIR chromatograms for phthalates, parabens, and triclocarban in a mixed 1.0 µg/mL calibration standard.

Table 2 were used after appropriate sample preparation to screen for phthalates, parabens, and triclocarban in cosmetic and personal care samples.

Cosmetic and personal care sample analysis

Samples were fortified at various levels with selected phthalates and parabens, then prepared for analysis as detailed in the experimental section. Example SIR chromatograms achieved are shown in Figure 2.

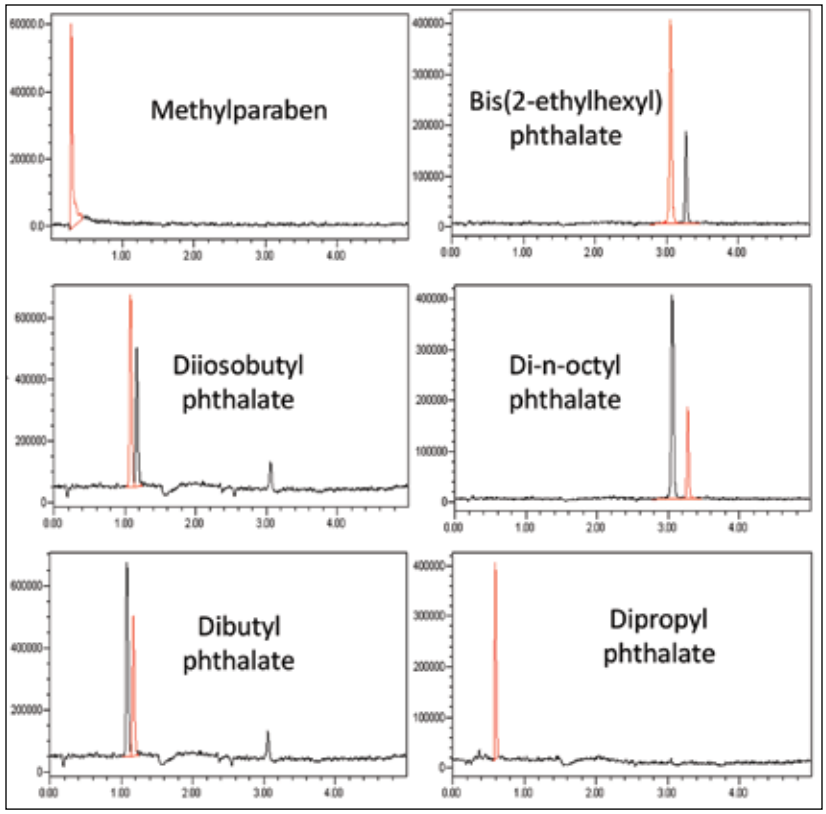

Figure 2. SIR chromatograms for selected phthalates and parabens in hair conditioner.

CONCLUSIONS

- A fast, robust, and sensitive method was developed for the combined analysis of phthalates, parabens, and triclocarban in cosmetic and personal care samples.
- The ACQUITY QDa Detector provides cost effective reliable mass confirmation, 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 Chromatography Data Software provides confidence in data management, data processing, and reporting.
- The developed 5-minute UPLC method is more than 7 times faster than existing HPLC and GC methods, with an excess of 90% less solvent usage than existing HPLC methods.
- The ACQUITY H-Class System, a quarternary system based on UPLC Technology, offers the best in chromatographic resolution, and sensitivity.

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One-Minute Method for the Screening of Phthalates in Toys at Regulatory Limits Using UPLC-MS and Empower Software

Dimple Shah, Jennifer Burgess
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- One-minute screening method for phthalates in toys at legislative limits
- Benefits of MS, such as selectivity and sensitivity, can be brought to existing Empower users, without the need for additional training
- Users can define specific limits and quickly identify samples that exceed permitted levels with Empower® Software's system suitability functionality.

WATERS SOLUTIONS

ACQUITY® SQD
Empower 3 Software

KEY WORDS

Phthalates, DEHP, BBP, DINP, DIDP, DnOP, phthalic acid, plastics

INTRODUCTION

Phthalates, esters of phthalic acid, are widely used to modify the physical properties of plastics. They are added to products to increase flexibility, transparency, softness, durability, and longevity. Owing to their unique properties, phthalates are widely used in toys, childcare items, food packages, raincoats, shower curtains, paints, lubricants, detergents, and personal care products.

Since there is no chemical bond between phthalates and the plastics, phthalates can migrate into the environment. Upon use by children or adults, they have the potential to cause serious side effects such as hormone malfunctioning, reproductive defects, and cancer.[1]

According to the Consumer Product Safety Improvement Act (CPSIA, August 2008),2 some phthalates are restricted in particular products. Since February 2009, children's toys and childcare articles cannot contain concentrations of more than 0.1% of Bis (2-ethylhexyl) phthalate (DEHP), Di-n-butyl phthalate (DBP), and Benzyl butyl phthalate (BBP). Temporary restrictions were also placed on children's toys and childcare articles that contain more than 0.1% Diisononyl phthalate (DINP), Di-isodecyl phthalate (DIDP), or Di-n-octyl phthalate (DnOP). The European Union and Japanese toy safety standards also enforce the same legislative limits for all six phthalates listed above.[3,4]

This application note describes a screening method for 14 phthalates (six of which are legislated) in one minute, using the Waters® ACQUITY UPLC® with SQ Detector and Empower 3 Software. A typical quantitative phthalate analysis using GC-MS takes approximately 30 minutes per sample.[5] Using this one-minute screening method dramatically increases the sample throughput. Those samples that show positive results can then be submitted for confirmatory analysis.

SAMPLE PREPARATION

The sample was prepared as described previously.[6] Briefly, a child's teething toy was finely chopped. Two grams of the sample was sonicated with 200 mL of methanol for 10 min. The supernatant was collected and filtered through a 0.2 µm nylon filter. The filtrate was diluted 10-fold in methanol and placed into a Waters certified vial for analysis.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC
Runtime:	1.0 min
Column:	ACQUITY UPLC BEH C_{18} 1.7 µm, 2.1 x 50 mm
Mobile phase A:	Methanol + 0.1% formic acid
Isocratic flow rate:	0.6 mL/min
Injection volume:	2 µL
Weak needle wash:	98:2 Water: Methanol + 0.1% formic acid
Strong needle wash:	Methanol + 0.1% formic acid

MS conditions

MS system:	ACQUITY SQ Detector
Ionisation mode:	ESI+
Capillary voltage:	3.5 kV
Source temp.:	150 °C
Desolvation temp.:	450 °C
Desolvation gas:	800 L/H
Acquisition:	Selected Ion Recording (SIR)

IntelliStart™ Technology was used to tune all the phthalates in this application note. IntelliStart, a standard feature of Waters MS systems automates system calibration, sample tuning, and daily checks, so that non-expert users can acquire data with confidence that the system is operating optimally. The resulting tuning parameters are shown in Table 1.

Phthalate	Parent ion	Dwell time(s)	Cone voltage (V)	Peak name
Dimethyl phthalate	195	0.02	15	a
Diethyl phthalate	223	0.02	20	b
Dipropyl phthalate	251	0.02	20	c
Di-n-butyl phtahlate	279	0.02	25	d
Diisobutyl phthalate	279	0.02	20	e
Bis (methylglycol) phthalate	283	0.02	20	f
Dipentyl phthalate	307	0.02	20	g
Benzyl butyl phthalate	313	0.02	20	h
Dihexyl phthalae	335	0.02	20	i
Butyl phthylyl phthalate	337	0.02	20	j
Bis (2-Ethylhexyl) phthalate	391	0.02	25	k
Di-n-Octyl phthalate	391	0.02	20	l

Table 1. MS tuning parameters for phthalates obtained using IntelliStart.

RESULTS AND DISCUSSION

Using the ACQUITY SQD, 14 phthalates were analyzed within 1 min. The chromatograms of each of the phthalates analyzed at 1000 ng/mL are shown in Figure 1.

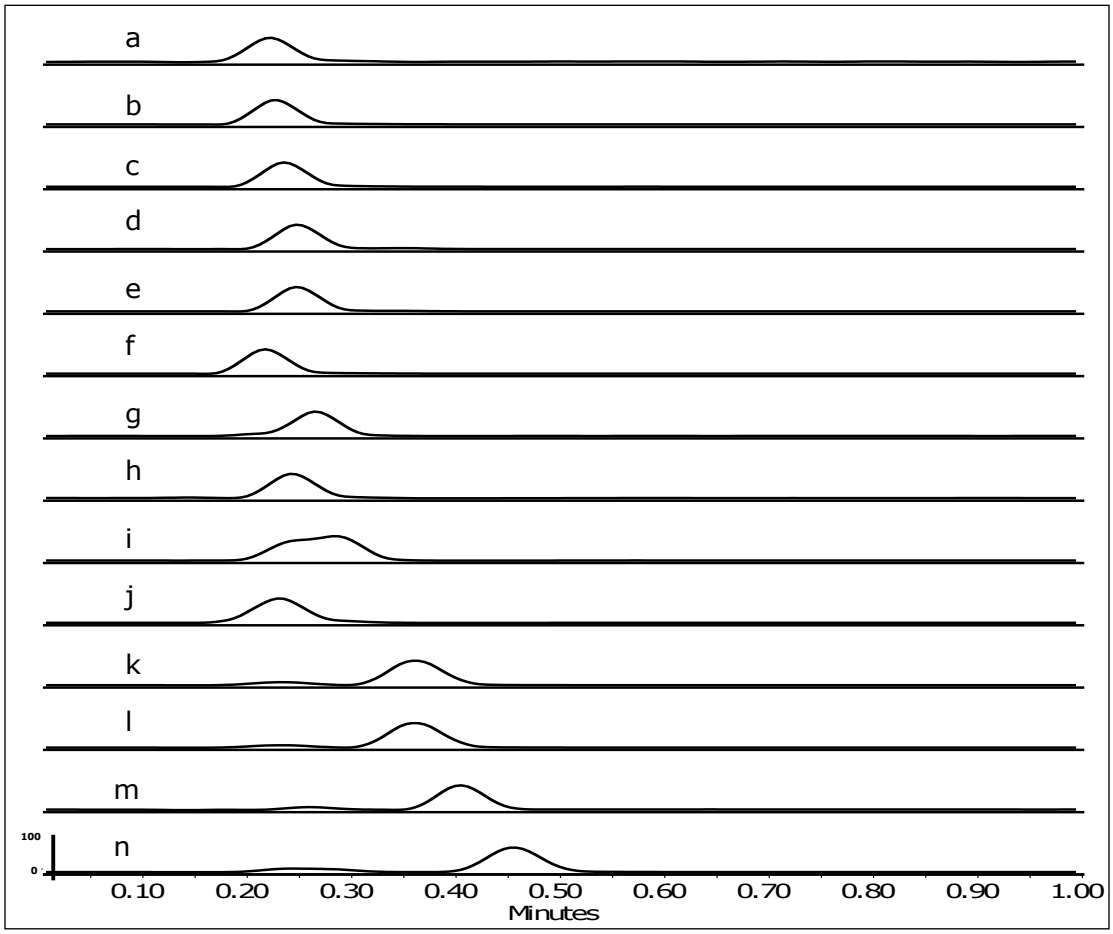

Figure 1. Chromatograms showing separation of 14 phthalates.

The results for each of the phthalates showed good injection-to-injection reproducibility. Table 2 shows the % RSD on the area count from six injections of a toy extract that was spiked at 1000 ng/mL with each of the phthalates.

Compound name	% RSD
Dimethyl phthalate	10.6
Diethyl phthalate	13.4
Dipropyl phthalate	4.1
Di-n-butyl phtahlate	3.7
Diisobutyl phthalate	8.9
Bis methylglycol phthalate	6.1
Dipentyl phthalate	8.5
Benzyl butyl phthalate	7.1
Dihexyl phthalae	4.7
Butyl phthaylyl phthalate	10.0
bis 2 Ethylhexyl phthalate	7.5
Di-n- Octyl phthalate	8.4
Di-isononyl phthalate	6.4
Di-isodecyl phthalate	3.9

Table 2. Relative standard deviation from 6 injections of a spiked toy extract at 1000 ng/mL.

According to the CPSIA legislation,[1] the concentration of phthalates should not exceed more than 0.1% of the total mass. Due to the sample preparation and dilution in the sample extraction, the legislative limit is equivalent to 1000 ng/mL. Figure 2 shows an example 5-point calibration curve of one of the phthalate standards (diisononyl phthalate) around the concentration that corresponds to the legislated level. The calibration curve showed an r^2 value of 0.9951.

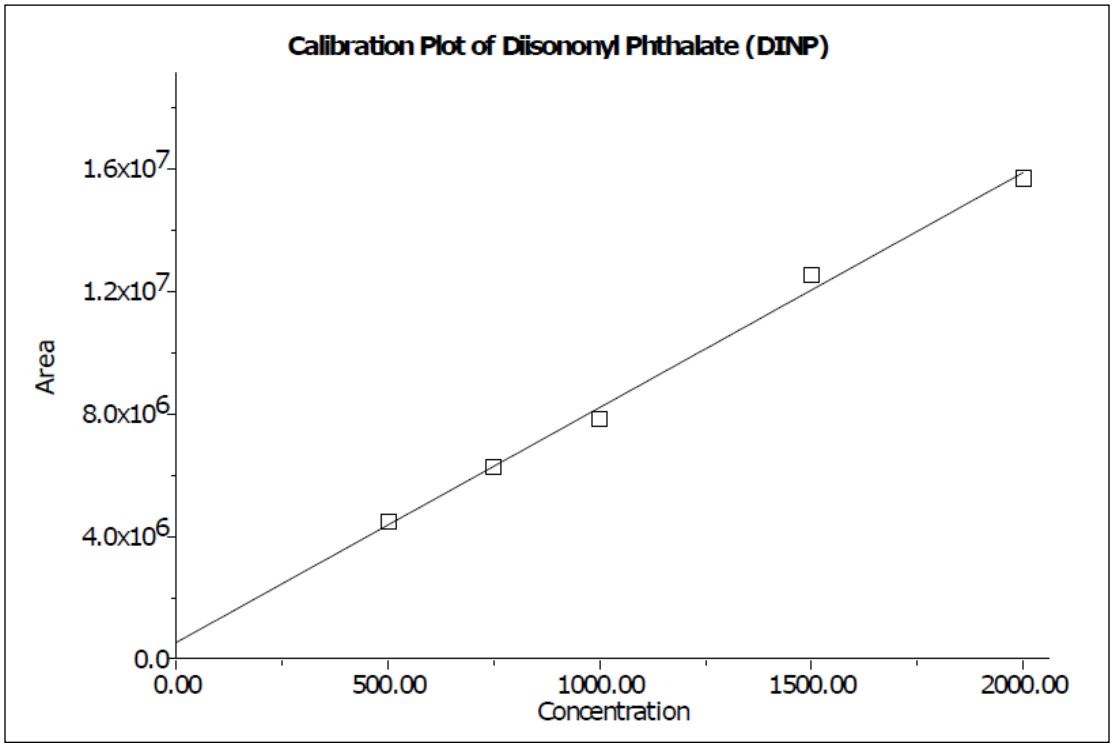

Figure 2. Five-point calibration curve for Diisononyl phthalate from 500 ng/mL to 2000 ng/mL.

The data were acquired using Empower 3 Software and processed using the system suitability function. Empower system suitability software monitors the chromatographic system automatically and provides a summary based on parameters and limits set by the user. This feature was used to set minimum and maximum values in summary charts and to flag out-of-range values.

The target limit for the phthalates is 1000 ng/mL (taking into account the sample preparation), so any sample that exceeded this limit was flagged and reported in a different color and font. A typical system suitability report is shown in Table 3. The table shows the results for diisononyl phthalate from the extracted toy sample as well as two spiked extracts from the toy sample at 500 and 1000 ng/mL. As shown in Table 3, the 1000 ng/mL spiked toy extract had a calculated concentration of 1042 ng/mL, which was flagged as being over the maximum concentration of 1000 ng/mL. The first sample (the toy extract that was not spiked) does not show a reported amount, as the detected peak for this compound was below the minimum reporting level (which was set at the lowest concentration standard, 500 ng/mL).

Component summary table
Name Diisonoyl phthalate

Sample name	Name	RT	Concentration	Units
1 TOY extracted blank	13	0.400		ng/mL
2 TOY spiked 500 ng/mL	13	0.405	542.5	ng/mL
3 TOY spiked 1000 ng/mL	13	0.405	1042.1	ng/mL

Table 3. Report table generated for the unspiked and spiked toy extracts using the system suitability function of Empower 3 Software.

CONCLUSIONS

The results presented in this application note show how the ACQUITY SQD can be used to rapidly screen for the presence of phthalates in toy samples at the regulated limits. The ACQUITY SQD can easily be added to existing laboratories using Empower Software, circumventing the requirement of dedicated MS software. The benefits of MS, such as selectivity and sensitivity, can be brought to existing Empower users without the need for additional training. IntelliStart Technology ensures ease-of-use and consistent performance. Empower's system suitability functionality enables users to define specific limits and quickly identify samples that exceed the permitted levels.

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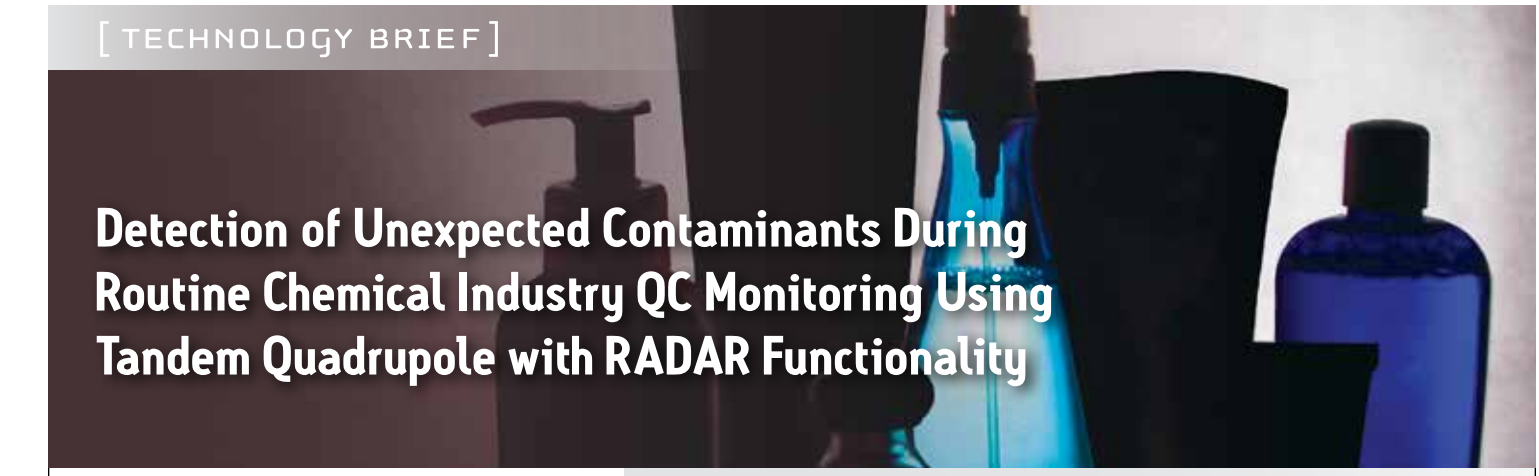

Detection of Unexpected Contaminants During Routine Chemical Industry QC Monitoring Using Tandem Quadrupole with RADAR Functionality

GOAL

To carry out QC monitoring for the manufacture of Diethylhexyl Phthalate (DEHP) using tandem quadrupole MS with RADAR™ functionality to scan for unexpected process contamination.

BACKGROUND

Bulk chemical manufacturing processes, such as DEHP, need to have quick and robust QC checks in place to ensure the purity of both the starting materials and the final product. Producing materials that are out of specification is not only expensive in terms of raw materials, but the potential cost of a plant shutdown and start up can run into millions of dollars. Early detection of contamination while still at a low level may allow corrective action to be taken rather than requiring a full shutdown.

Waters® Xevo® TQD with the ACQUITY UPLC® System allows the monitoring of known compounds using Multiple Reaction Monitoring (MRM), which delivers high sensitivity and selectivity to eliminate the risk of misidentification. Waters RADAR functionality simultaneously acquires full scan data while maintaining the quality of MRM data and allowing post analysis interrogation to identify unexpected contamination if required. This aids in complete understanding of the manufacturing process and sample purity.

The Xevo TQD with RADAR functionality provides MS full scan acquisition without compromising MRM data quality.

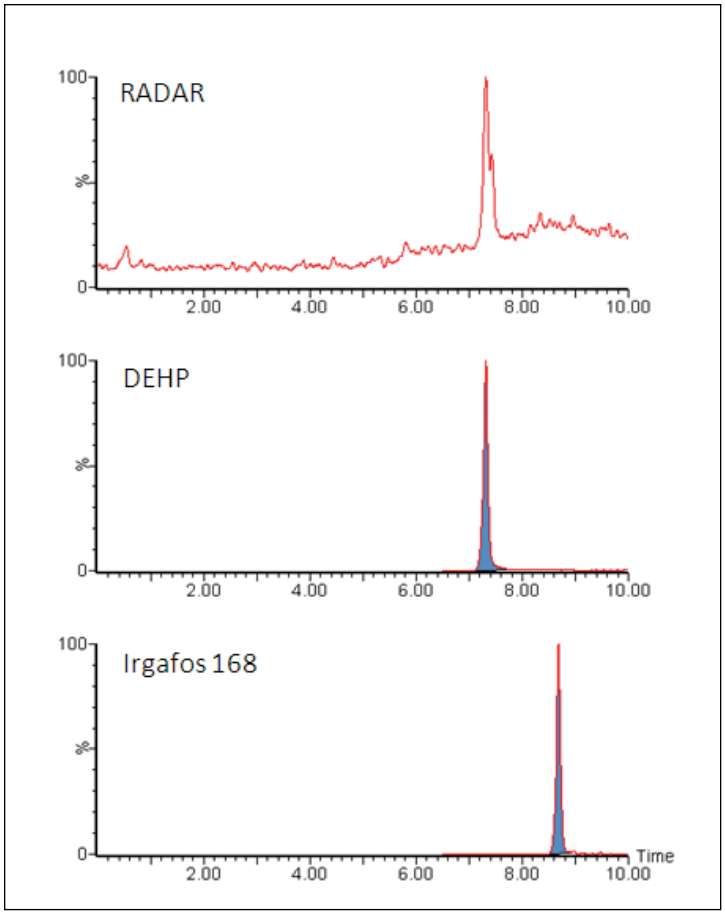

Figure 1. Xevo TQD RADAR full scan Total Ion Chromatogram (TIC) with MRM chromatograms for DEHP (plasticizer) and Irgafos 168 (stabilizer).

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THE SOLUTION

Waters Xevo TQD MS utilizing Atmospheric Pressure Photo Ionization (APPI), coupled to an ACQUITY UPLC System were used to monitor the plasticizer DEHP and the stabilizer Irgafos 168. Compounds of interest can be identified with complete confidence by operating the tandem quadrupole instrument in MRM mode. Many plasticizers, antistatic agents, UV absorbers, stabilizers, and optical brighteners commonly used in the polymer industry can be monitored in this way.

During the monitoring of DEHP and Irgafos 168, RADAR scan functionality was used. RADAR completes a full mass spectral scan every duty cycle during the analytical run without affecting the quality of the MRM data, as shown in Figure 1.

RADAR full scan data were interrogated post analysis and a low level of contamination was found. Figure 2 shows RADAR and the extracted ion chromatogram of mass *m/z* 587, with the mass spectra for the peak as an insert. Investigation into the mass *m/z* 587 shows it is likely to be the precursor ion of Irganox 245, a stabilizer commonly used in the polymer industry.

The added information that RADAR provides allows plant managers to make knowledgeable decisions if the product falls out of specification. Contamination that is considered to be "benign" and have no implication on the use of the product could allow an out of specification batch to be blended with other batches. There are clear financial and environmental advantages where this is possible.

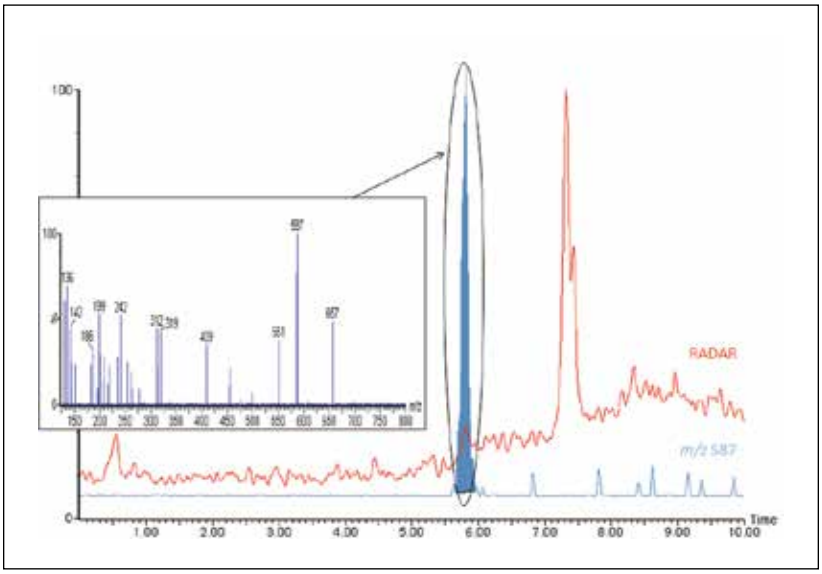

Figure 2. RADAR scan showing DEHP, Irgafos 168, and Irganox 245 (contamination), extracted ion chromatogram of m/z 587 with an insert of the full mass spectrum of the peak. (Chromatograms are not to the same scale).

SUMMARY

Waters Xevo TQD provides high-quality quantitative MRM data while simultaneously acquiring full mass spectral scans without compromising data quality. Quantification by MRM allows high sensitivity and selectivity, which eliminates the risk of misidentification.

The extra information provided by RADAR functionality offers a better understanding of how the manufacturing process is running. The identification of unknown contamination allows better business and process decisions to be made. If contamination is detected, action can be taken to address the problem and possibly save the batch, which offers significant cost savings to the business.

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PRIMARY AROMATIC AMINES/AZO DYES

Improving Sensitivity and Selectivity for Primary Aromatic Amines Analysis with ACQUITY QDa Detector

Jane Cooper

GOAL

To demonstrate the use of Waters ACQUITY® QDa Detector for the analysis of Primary Aromatic Amines with improved sensitivity and selectivity, compared with existing analogue detection methodologies.

BACKGROUND

The inks and dyes industry is highly legislated and manufacturers who use inks and dyes must monitor and quantify various regulated parameters, including the presence or absence of Primary Aromatic Amines (PAAs).

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.

Many PAAs are suspected carcinogens and therefore have a range of potential health risks, which have lead 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 PAA; whereas EU regulations (Commission Directive 2002/72/EC and Directive 19/2007/EC) state legislative limits.

Analytical laboratories require accurate and robust techniques to ensure confidence and versatility in meeting legislative requirements. Waters® ACQUITY UPLC® H-Class System with the ACQUITY QDa Detector is the solution for this industry.

Waters ACQUITY UPLC H-Class System with the ACQUITY QDa Detector provides enhanced confidence in the identification and quantification of Primary Aromatic Amines for the inks and dyes industry.

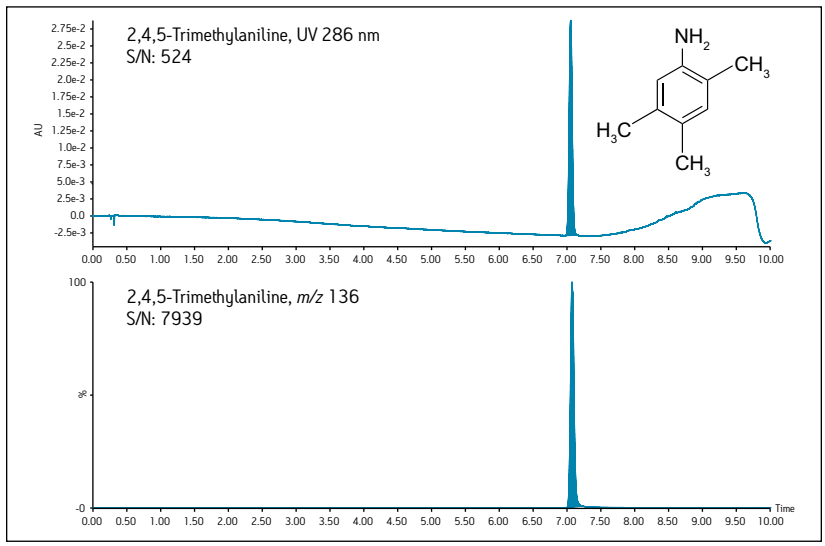

Figure 1. UV and extracted ion chromatograms for 2,4,5-Trimethylaniline.

THE SOLUTION

The ACQUITY UPLC H-Class System with the ACQUITY QDa Detector was used to monitor PAAs. The ACQUITY QDa Detector is the mass detector designed as a synergistic element of a chromatographic separations system. The ACQUITY QDa Detector complements optical data with enhanced qualitative mass spectral data to confirm the identity of components. The ACQUITY QDa Detector extends the sample coverage of optical detection to quantify compounds with no UV response or compounds at levels not amenable or accessible by optical detection.

Developing analytical method development and validation with enhanced sensitivity, selectivity, and method robustness to meet legislative requirements can be costly and time consuming. The ACQUITY UPLC H-Class with ACQUITY QDa Detector realizes multiple business benefits. Financial savings are achieved through much faster run time, reduced solvent consumption, and increased sample throughput. A method with a run time of 10 minutes was developed for PAAs, which is up to seven times faster than many existing methods. In addition, no time-consuming derivatization stages are used, affording further savings in the sample preparation stages.

Improvements in sensitivity can be demonstrated by considering the signal-to-noise (S/N), when comparing the UV and the mass spectral data for the PAA 2,4,5-Trimethylaniline. This PAA has a precursor ion at *m/z* 136 and a maximum UV absorbance at 286 nm. The increase in S/N when using mass spectral data is illustrated in Figure 1.

In order to observe improvement in selectivity, the ability to measure analytes of interest accurately and specifically in the presence of a complex matrix needs to be considered. This is demonstrated by considering the PAA 2,4,5-Trimethylaniline, which when spiked in ink cannot be distinguished due to other UV absorbing compounds present, as shown in Figure 2. However, mass detection is sufficiently sensitive and selective to enable confident detection and quantification of 2,4,5-Trimethylaniline in an ink matrix.

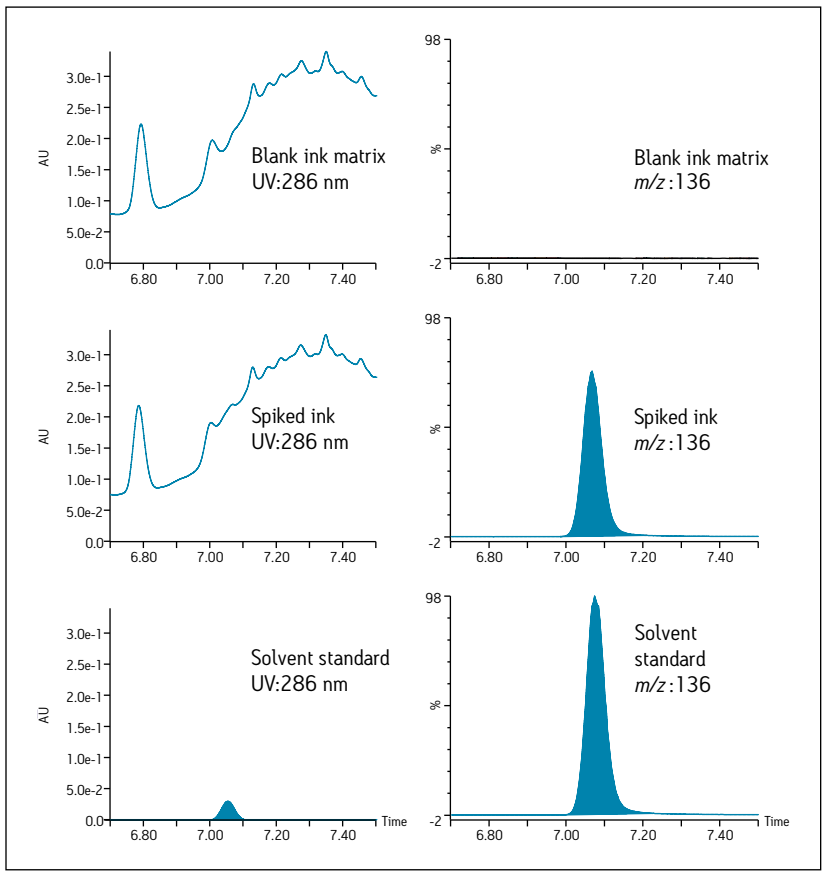

Figure 2. UV and extracted ion chromatograms for 2,4,5-Trimethylaniline spiked in ink (0.5 mg/mL), solvent standard (5.0 µg/mL equivalent to 0.5 mg/mL in spiked ink) and blank ink matrix. UV and MS data were acquired simultaneously.

SUMMARY

Waters ACQUITY H-Class System with the ACQUITY QDa Detector provides increased confidence in the identification and quantification of PAAs in ink, compared to techniques typically used for this analysis.

Due to reduced run time, UPLC® analysis for PAAs affords increased sample throughput and a reduction in solvent usage, offering businesses valuable time and cost savings.

The ACQUITY QDa Detector, provides enhanced selectivity and sensitivity of mass detection, delivering increased confidence when reporting quantitative results.

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

Jane Cooper, Eleanor Riches, and Kate Williams
Waters Corporation, Manchester, UK

APPLICATION 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.

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System
SQ Detector 2
MassLynx® Software

KEY WORDS

Primary Aromatic Amines, PAAs, azo dyes, ink

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.

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.

MS conditions

MS system:	SQ Detector 2
Ionization mode:	ESI+
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)

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.

	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.

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.

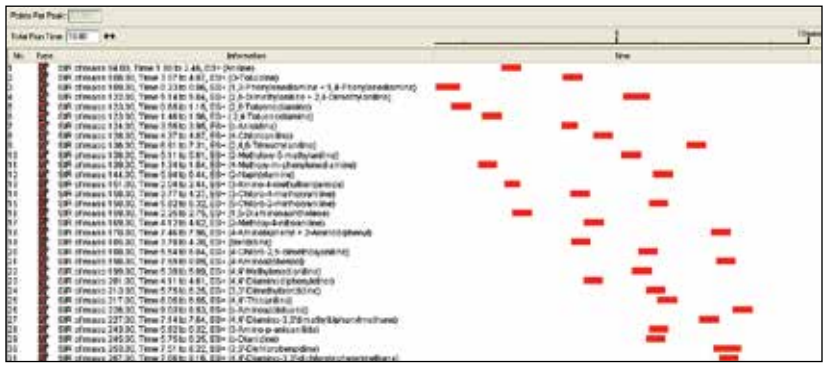

Figure 1. PAAs SIR method. 34 compounds covered over 31 channels (including 3 isomer pairs).

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.

Instrument control, data acquisition, and result processing

MassLynx Software 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.

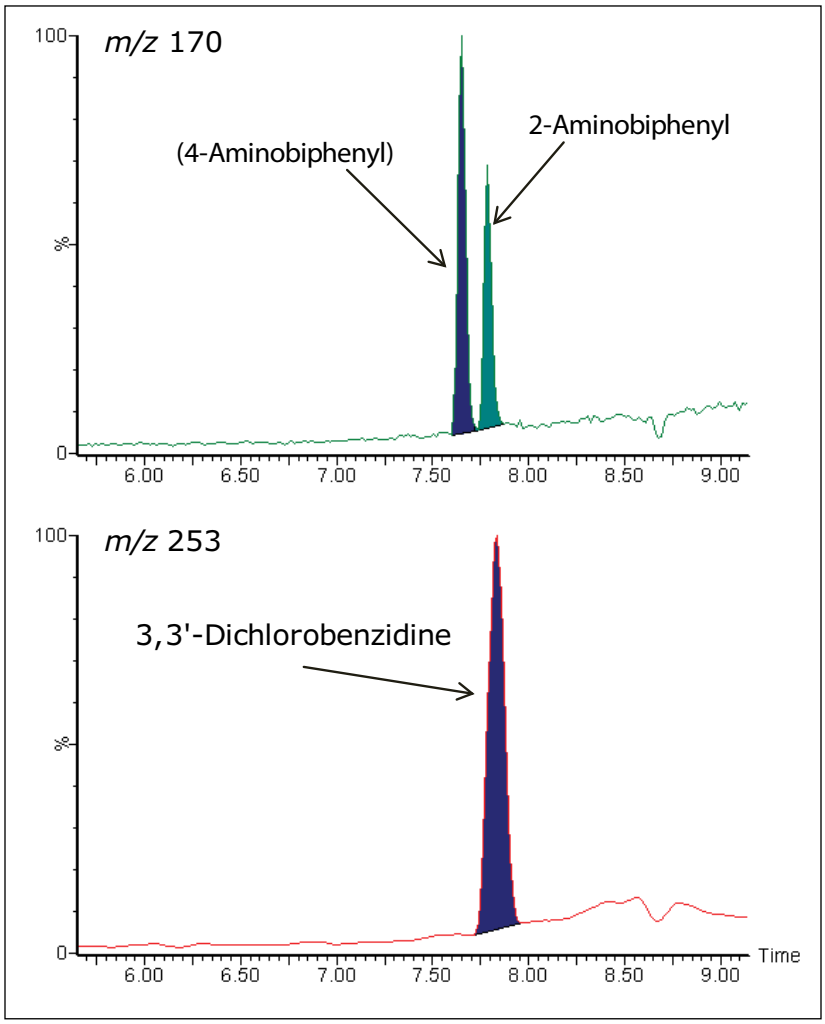

Figure 2. Extracted ion chromatograms for 2-Biphenylamine and 3,3'-Dichlorobenzidine in fortified ink (containing 4.6 µg/mL PAAs).

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.

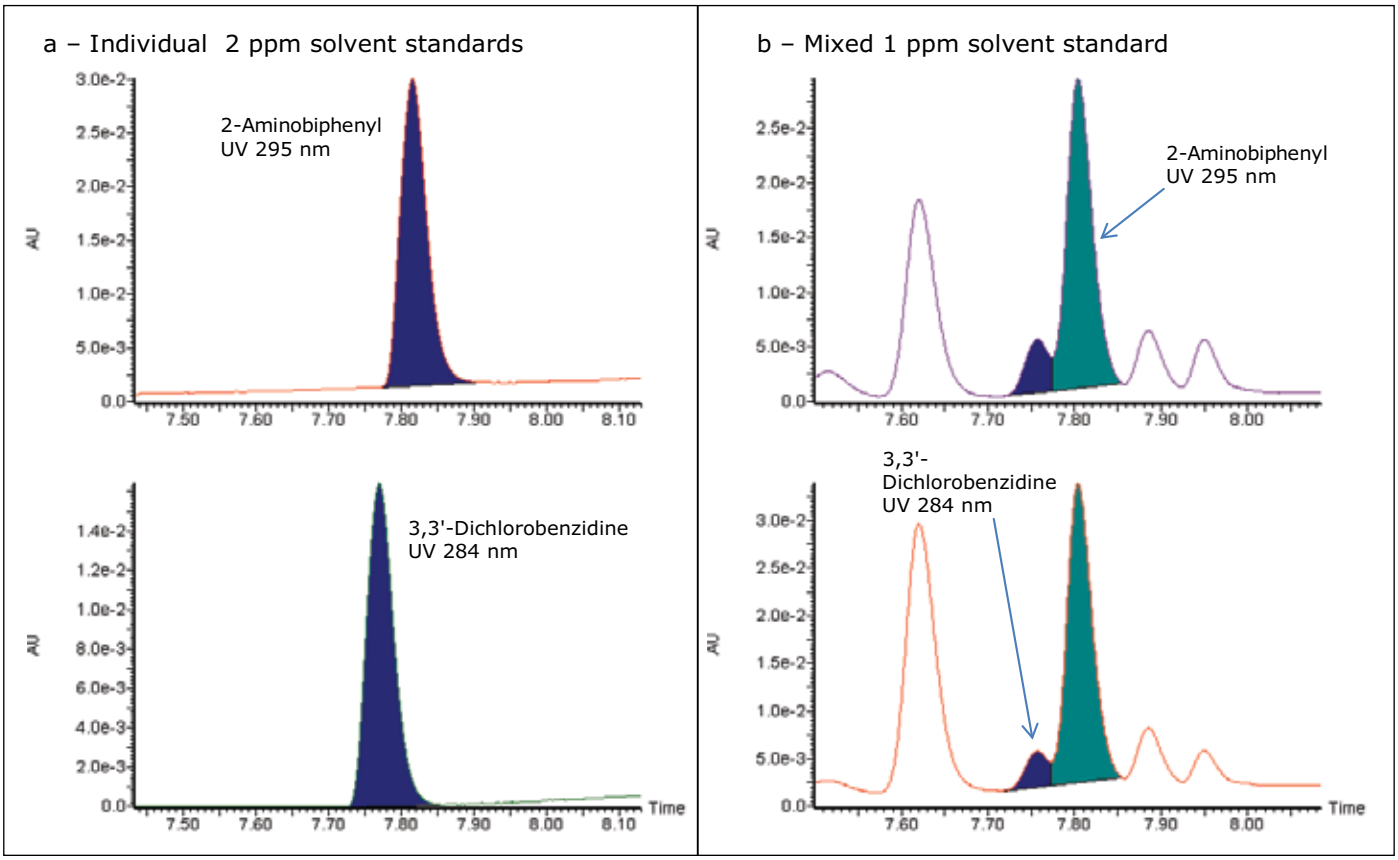

Figure 3. a) UV chromatograms for 2-Aminobiphenyl and 3,3'-Dichlorobenzidine in individual solvent standards; b) UV chromatograms for 2-Aminobiphenyl and 3,3'-Dichlorobenzidine in a mixed solvent standard.

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.

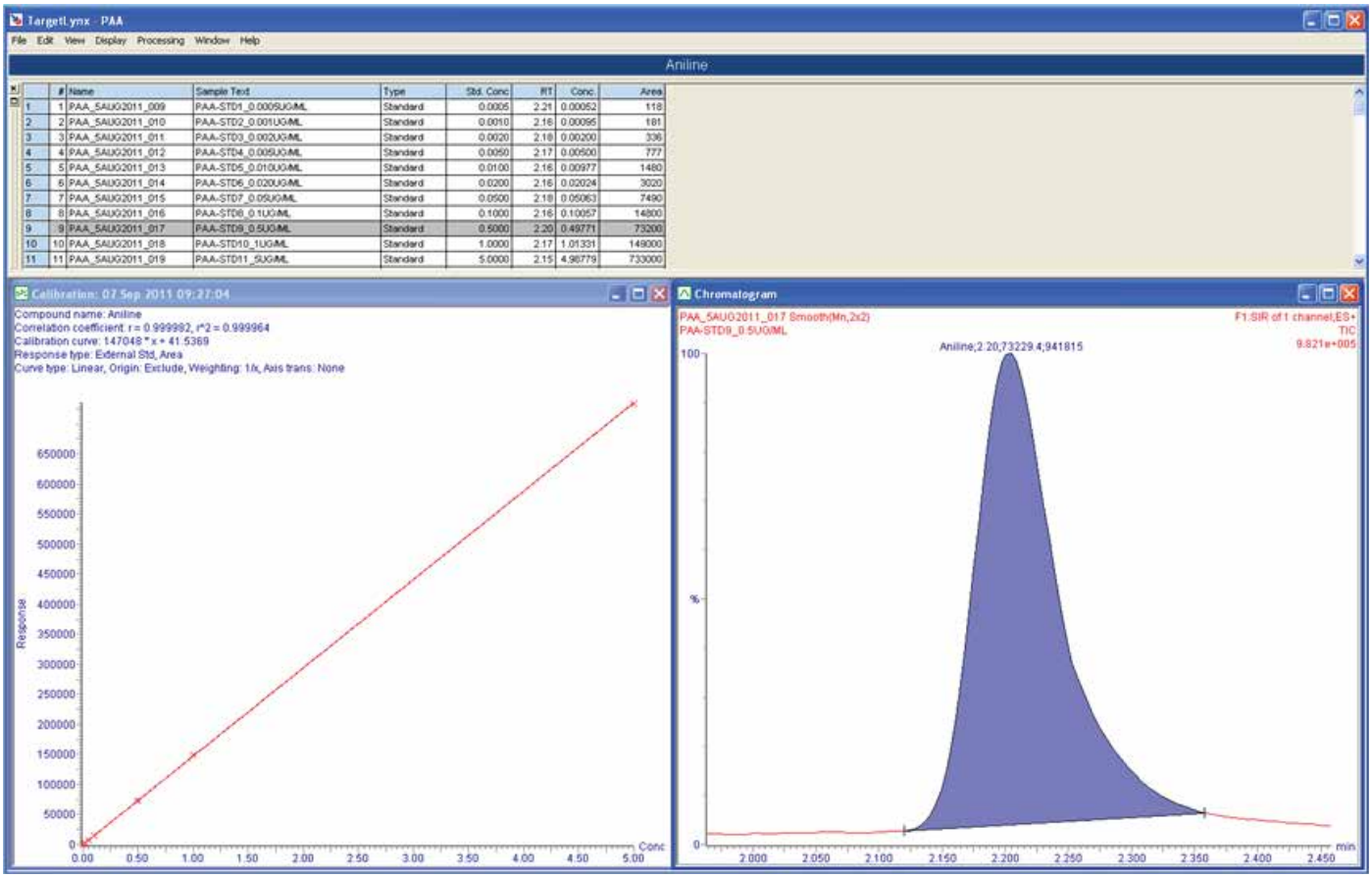

Figure 4. TargetLynx Quantify results browser showing the calibration quantitation results, calibration curve, and example SIR chromatogram for aniline.

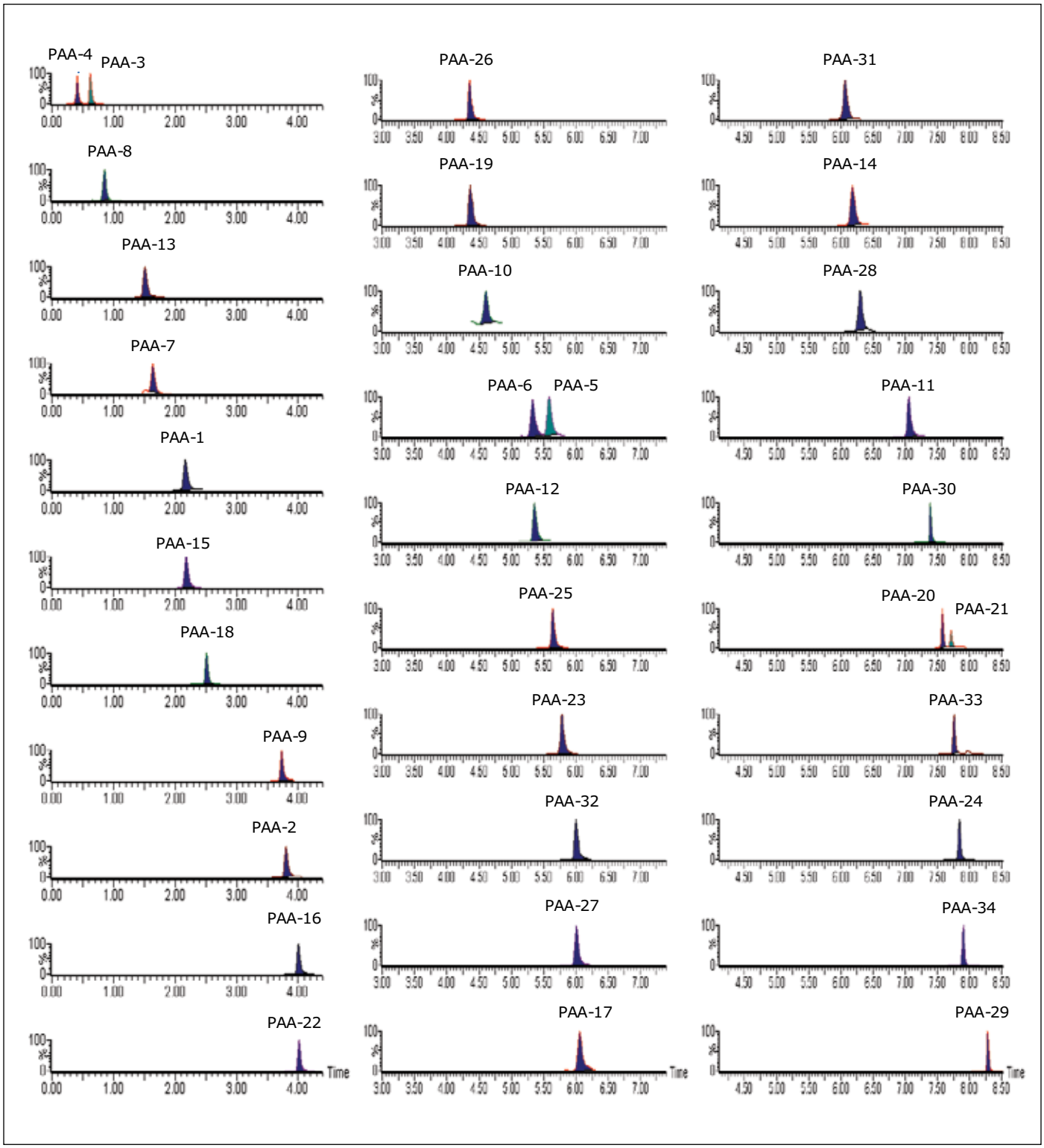

Figure 5. SIR chromatograms for 34 PAAs in a mixed 1 µg/mL calibration standard.

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 a 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.

CONCLUSIONS

- 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.

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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

Jane Cooper
Waters Corporation, Wilmslow, UK

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.

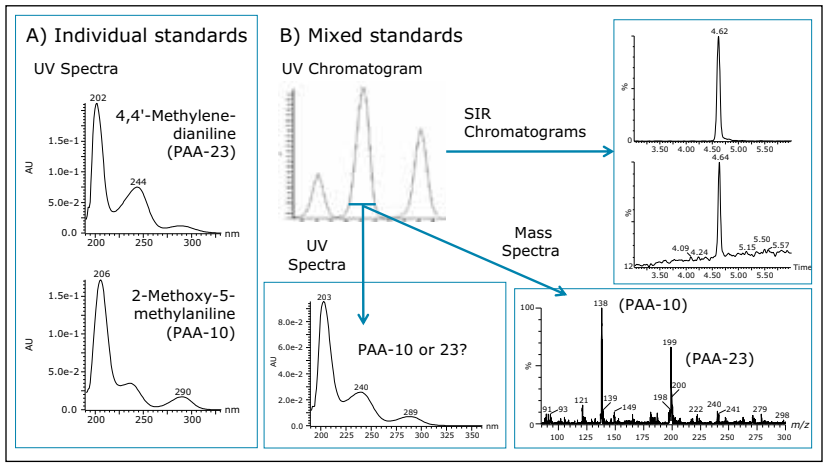

Figure 1. An illustration of the advantages of mass detection for the identification of co-eluting peaks during method development, considering two PAAs (4,4'-Methylene-dianiline and 2-Methoxy-5-methylaniline); a) UV spectra from individual standards, b) UV and mass spectra, and SIR chromatograms from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

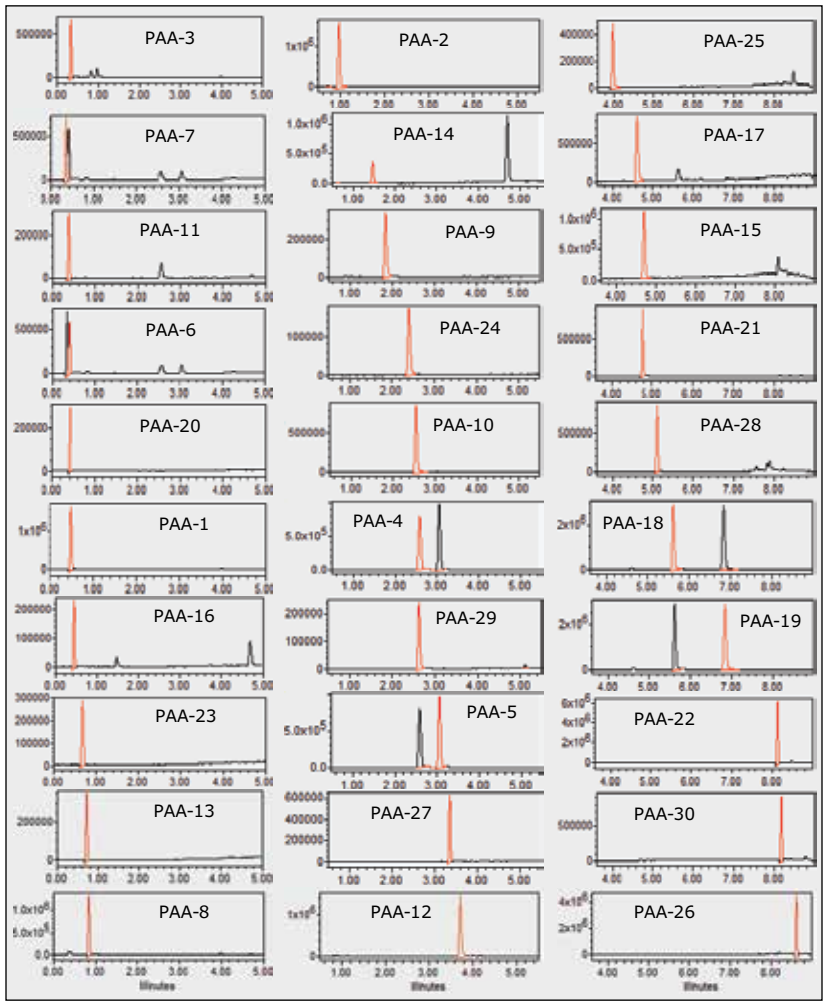

Figure 2. SIR chromatograms for 30 PAAs in a mixed 0.5 µg/mL calibration standard.

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.*

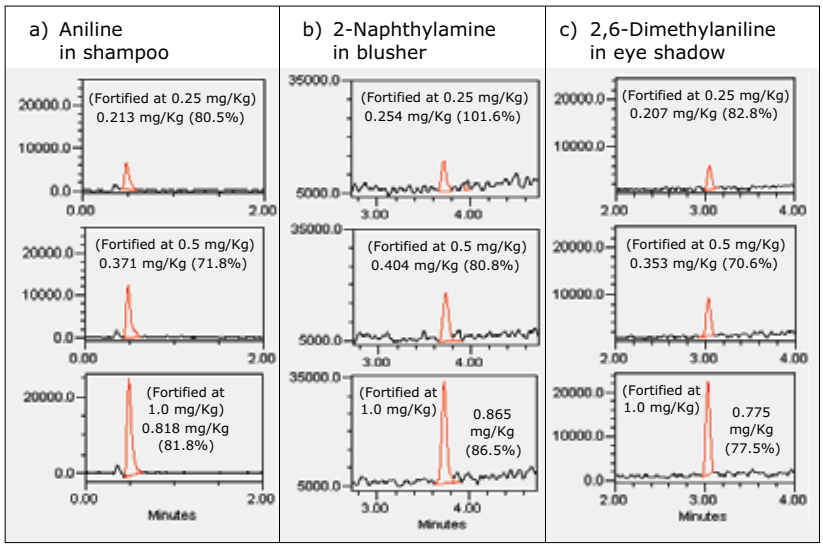

Figure 3. SIR chromatograms for selected PAAs in matrix: a) shampoo b) blush, and c) eyeshadow.

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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