Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

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

	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.

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.

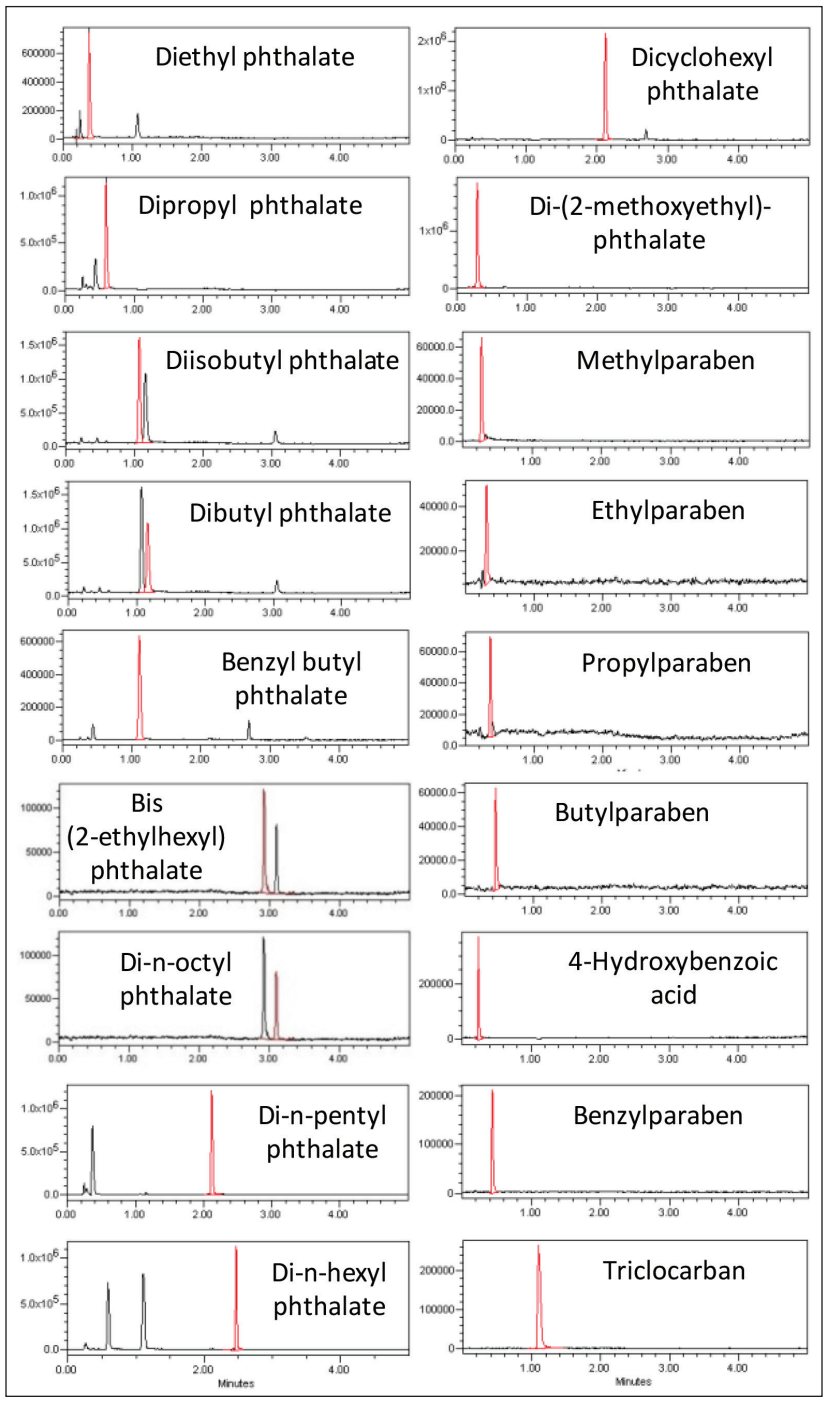

Figure 1. SIR chromatograms for phthalates, parabens, and triclocarban in a mixed 1.0 µg/mL calibration standard.

The SIR mass detection conditions detailed in 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.

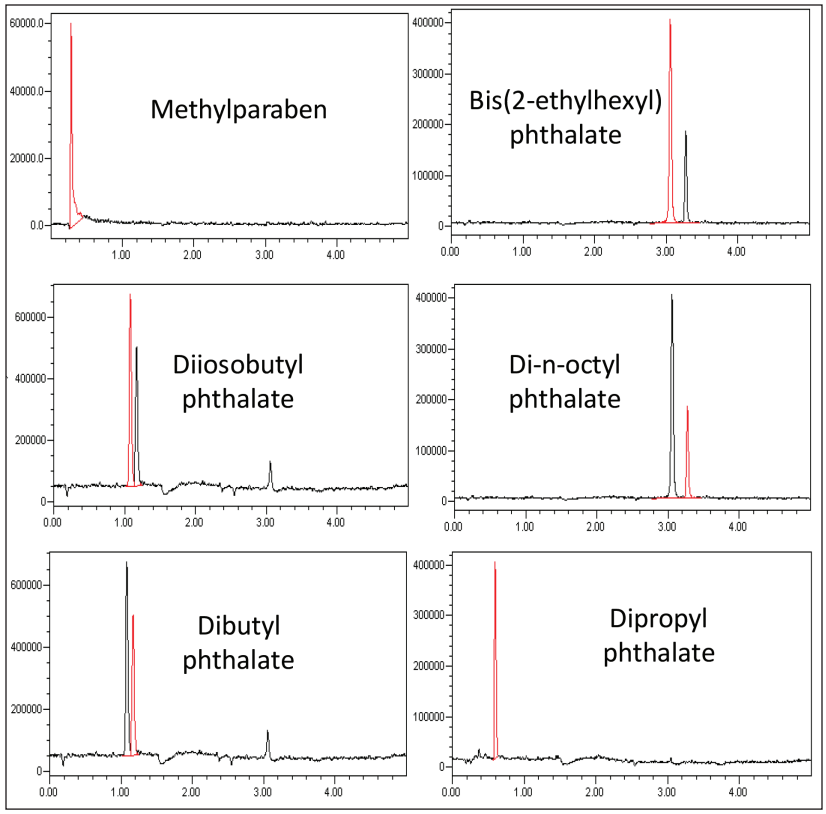

Figure 2. SIR chromatograms for selected phthalates and parabens in hair conditioner.

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.

References

1. Darbe P D. Environmental oestrogens, cosmetics and breast cancer. *Best Practice & Research Clinical Endocrinology & Metabolism*. 20(1): 121–143, 2006.
2. Golden R, Gandy J, Vollmer G. A review of the Endocrine activity of parabens and implications for potential risks to human health. *Critical reviews in toxicology.* 35(5): 435–458, 2005.
3. The European Parliament and the Council of the European Union. Regulations (EC) No 1223/2009 of The European Parliament and of the Council of 30 November 2009 on Cosmetic Products. Official Journal of the European Union. L 342: 59–209, 30th November 2009. [cited 2015 August 25]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF
4. Shen H Y, Jiant H L, Mao H L, et al. Simultaneous determination of seven phthalates and four parabens in cosmetic products using HPLC-DAD and GC-MS methods. *J Sep Sci.* 30: 48–54, 2007.
5. Farajzadeh M A, Djozan Dj, et al. Use of a capillary tube for collecting an extraction solvent lighter than water after dispersive liquid-liquid microextraction and its application in the determination of parabens in different samples by gas chromatography-flame ionization detection. *Talanta*. 81: 1360, 2010.
6. Labat L, Kummer E, Dallet P, Dubost JP. Comparison of high-performance liquid chromatography and capillary zone electrophoresis for the determination of parabens in a cosmetic product. *J Pharm Biomed Anal.* 23:763, 2000.
7. González-Mariño I, Quintana J B, Rodríguez, Cela R. Evaluation of the occurrence and biodegradation of parabens and halogenated by-products in wastewater by accurate-mass liquid chromatography-quadrupole-time-of-flight-mass spectrometry (LC-QTOF-MS). *Water Research.* 20: 6770–6780, 2011.
8. Wang Y, Li P, Liu Y, et al. Determination of Triclocarban, Triclosan and Methyl-Triclosan in Environmental Water by Silicon Dioxide/Polystyrene Composite Microspheres Solid-Phase Extraction Combined with HPLC-ESI-MS. *Journal of Geoscience and Environment Protection.* 1(2): 13–17. 2013.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com