ANALYSIS OF ALLERGENS IN PERFUMES, COSMETICS AND PERSONAL CARE PRODUCTS USING ULTRA PERFORMANCE CONVERGENCE CHROMATOGRAPHY (UPC^2) WITH MS DETECTION

Authors: Jane A. Cooper[a], Michael Jones[a], and Isabelle François[b]

Affiliations: [a]Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX, UK.
[b]Waters EHQ, 5 rue Jacques Monod, 78280 Guyancourt, France.

INTRODUCTION

Volatile cosmetic allergens encompass compounds with different polarities from a wide range of different classes (phenols, cyclic hydrocarbons, alcohols, carbonyl compounds, esters and lactones) also many are small molecules with similar structures which often produce non-specific fragment ions for mass spectrometric detection.

Current analytical methods used for the analysis of cosmetic allergens include: Gas Chromatography Mass Spectrometry[1-3] (GC/MS), Headspace-GC/MS,[4] GC-GC/MS, Liquid Chromatography-UV (LC-UV),[5] and LC-MS,[6] which all have run times between approx. 30 and 40 minutes. But in various cases existing methods lack selectivity, sensitivity, resolution, and do not cover the full range of allergens required in a single run.

There are many challenges that need to be addressed for any method used for allergen analysis. For example, the resolution achieved between analytes, isomers and matrix components all need to be optimized, and the sensitivity of the method should be at least 1 ppm (greater preferred).

Convergence Chromatography (CC) is a separation technique that uses carbon dioxide as the primary mobile phase, with a co-solvent such as methanol or acetonitrile to give similar selectivity as normal phase LC.

This poster will consider the analysis of cosmetic allergens using UltraPerformance Convergence Chromatography (UPC^2) with MS detection (see Figure 1).

Figure 1. The Xevo TQD and the ACQUITY UPC^2.

METHODS

UPC^2 Conditions

- System: ACQUITY UPC^2
- Run times: 7 min
- Column: ACQUITY UPC^2 C18 HSS, 3.0 mm x 150 mm, 1.8 µm
- Injection volume: 3.0 µL
- CCM back pressure: 1500 psi
- Mobile phase A: CO_2
- Mobile phase B: Methanol (0.1% Formic Acid)
- Isocratic Solvent Manager Solvent: Methanol
- Isocratic Solvent Manager Flow Rate: 0.4 mL/min
- UPC^2 mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A (CO_2)	%B	Curve
1	Initial	1.5	99.5	0.5	-
2	4.50	1.5	85.4	14.6	6
3	4.60	1.5	80.0	20.0	6
4	5.00	1.5	80.0	20.0	6
5	5.05	1.5	99.5	0.5	6
6	7.00	1.5	99.5	0.5	6

Table 1. ACQUITY UPC^2 mobile phase gradient.

Mass Detector Conditions

- Mass Detector System: Xevo TQD
- Ionisation mode: APCI (+ and –)
- Corona voltage (APCI): 10 µA
- Source temperature: 150 °C
- APCI Probe temperature: 600 °C
- Desolvation gas: 1000 L/hr
- Cone gas: 15 L/hr
- Acquisition: Multiple Reaction Monitoring (MRM)[7]

RESULTS AND DISCUSSION

The MS conditions were optimized for the analysis of 24 currently regulated cosmetic allergens.[8] Six additional compounds were also analyzed, considering cosmetic allergens that could potentially be added during future regulation changes, and two compounds that are potential carcinogens (methyl eugenol and 4-allyl anisole). The established MRM method[7] utilizes fast polarity switching available on the Xevo TQD, which enables the analysis of positive and negative allergens within the same analytical analysis.

The analysis of the 24 regulated[8] and 6 additional compounds was achieved using the Xevo TQD in MRM mode with APCI ionization (+/-), coupled to an ACQUITY UPC^2 System. Optimum MRM and UPC^2 conditions were developed with the elution of all compounds within a 7-minute run.[7] Mixed calibration standards, 0.25 to 25 ppm, were prepared and analyzed.

Shampoo and Perfume Analysis

The MRM mass detection method was used after appropriate sample preparation for the analysis of shampoo and perfume samples.

Perfume samples were fortified at the regulated labelling limit of 0.001% for leave on products (10 mg/kg) with 24 cosmetic allergens, and four additional compounds. Example MRM chromatograms achieved for fortified perfume are shown in Figure 2.

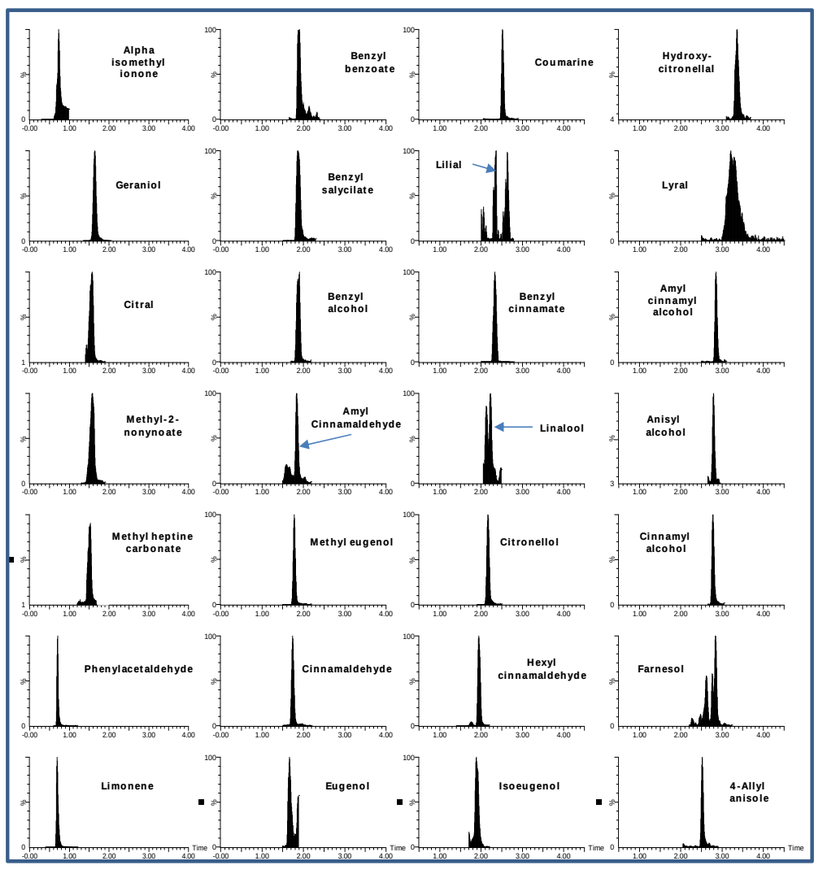

Figure 2. MRM chromatograms for 24 cosmetic allergens and four additional compounds in perfume, fortified at 10 mg/kg (0.001%).

Various cosmetic allergens compounds are isomeric, for example Farnesol where potentially four isomeric forms can be produced (Figure 3).

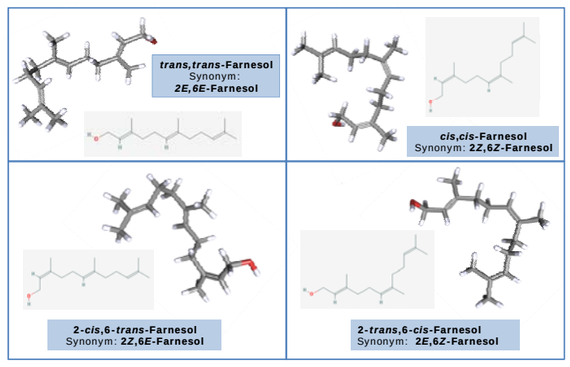

Figure 3. Four isomers of Farnesol.

For the example of farnesol, normally trans,trans-farnesol is the major isomer, with trans,cis-farnesol and cis,trans-farnesol being the minor forms, leaving cis,cis-farnesol which is rarely seen. This is demonstrated by the MRM chromatograms (Figure 4) for farnesol in a shampoo sample fortified at 10 mg/Kg (one tenth of the regulated labelling limit of 0.01% for rinse off products), and the nearest equivalent standard (0.5 ppm), which illustrated several isomeric farnesol peaks.

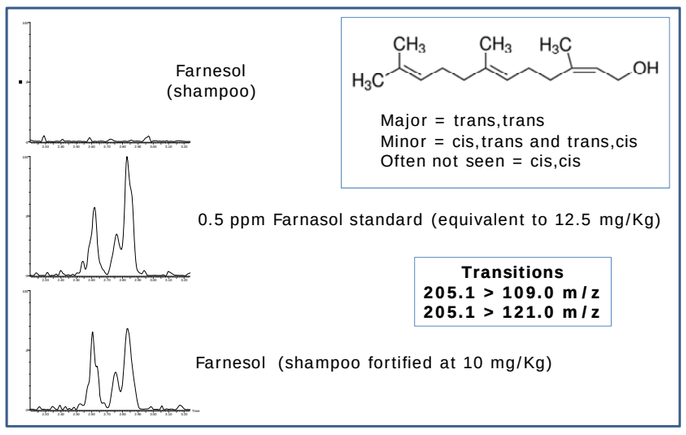

Figure 4. MRM chromatograms for shampoo fortified at 10 mg/Kg (0.01%), the nearest equivalent standard (0.5 ppm), and a blank shampoo sample.

Additional benefits of using ACQUITY UPC^2 coupled to the Xevo TQD over previous methodology include improved selectivity and sensitivity for the analysis of cosmetic allergens. The established method achieves resolution between analytes, isomers, and matrix. Additionally, the attained sensitivity is four times less than required (0.25 ppm).

CONCLUSION

- **Separation by UPC^2 is an ideal alternative to both HPLC and GC analysis.**
- **Ability to run LC and GC amenable compounds in a single analysis.**
- **Fast 7 minute analysis of the 24 regulated and 6 non-regulated volatile allergens containing:**
  - **different classes of compounds;**
  - **different polarities.**
- **UPC^2 with MS detection offers an orthogonal technique, which enables greater selectivity and specificity compared to either HPLC or GC analysis alone.**
- **The developed 7 minute UPC^2 method, is greater than 6 times faster than existing HPLC and GC methods, with 95% less solvent usage than existing HPLC methods.**

References

1. Debonneville C, Chaintreau A. Quantitation of suspected allergens in fragrances: evaluation of comprehensive GC- conventional MS. J Chromatogr A. 1027: 109-15, 2004.
2. Rastogi S C. Analysis of Fragrances in Cosmetics by Gas Chromatography-Mass Spectrometry. J. High Resolution Chromatography. 18: 653-658, 1995.
3. López-Nogueroles M, Chisvert A, Salvador A. Determination of atranol and chloratranol in perfumes using simultaneous derivatization and dispersive liquid -liquid microextraction followed by gas chromatography-mass spectrometry. Analytica Chimica Acta. 826: 28-34, 2014.
4. Desmedt B, Canfyn M, Pype M et al. HS-GC-MS method for the analysis of fragrance allergens in complex cosmetic matrices. Talanta. 131: 444-451, 2015.
5. Villa C, Gambaro R, Mariani E, Dorato S. High-performance liquid chromatographic method for the simultaneous determination of 24 fragrance allergens to study scented products. Journal of Pharmaceutical and Biomedical Analysis. 44: 755-762, 2007.
6. Rudback J, Islam N, Nilsson U et al. A sensitive method for determination of allergenic fragrance terpene hydroperoxides using liquid chromatography coupled with tandem mass spectrometry. J. Sep. Sci. 36:1370-1378, 2013.
7. Cooper J, Jones M, Dubant S. Fast Analysis of Cosmetic Allergens Using UltraPerformance Convergence Chromatography (UPC^2) with MS Detection. Application note 720005553en. Feb 2016.
8. 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: 59-209, 22nd Dec 2009. [cited 2015 January 15]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS