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Fast Analysis of Cosmetic Allergens Using UltraPerformance Convergence Chromatography (UPC²) with MS Detection

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APPLICATION BENEFITS

ACQUITY® UPC²® with MS detection offers:

- Efficient, cost effective analysis of cosmetic allergens, compared to standard methodology.
- Greater than six-fold increase in sample throughput, and greater than 95% reduction in toxic solvent usage than existing HPLC methods.
- The ability to handle traditional GC and LC amenable compounds in a single analysis using UPC.[2]

WATERS SOLUTIONS

ACQUITY UPC² System

Xevo® TQD

ACQUITY UPC² C_{18} HSS Column

MassLynx® MS Software

KEY WORDS

Allergens, cosmetics, perfume, Convergence Chromatography, supercritical fluid chromatography, SFC, personal care products, mass spectrometry

INTRODUCTION

Fragrances are complex combinations of natural and/or man-made substances that are added to many consumer products to give them a distinctive smell, impart a pleasant odor, or mask the inherent smell of some ingredients, but ultimately to enhance the experience of the product user. Fragrances create important olfactory benefits that are ubiquitous, tangible, and valued. Fragrances can be used to communicate complex ideas such as creating mood, signaling cleanliness, freshness, softness, alleviating stress, creating well-being, or to trigger allure and attraction.

In most types of cosmetics and skin care products, including perfumes, shampoos, conditioners, moisturizers, facial cosmetics, and deodorants, there are more than 5000 different fragrances present. Many people suffer from allergies, which are caused by an abnormal reaction of the body to a previously encountered allergen that can be introduced in a number of ways such as by inhalation, ingestion, injection, or skin contact. Allergies are often manifested by itchy eyes, a runny nose, wheezing, skin rashes (including dermatitis[1]), or diarrhea.

In the EU Cosmetic Regulations (1223/2009),[2] there are 'currently' 26 fragrance ingredients, 24 volatile chemicals, and two natural extracts (oak moss and tree moss), that are considered more likely to cause reactions in susceptible people. These 26 fragrance ingredients must be indicated in the list of ingredients of the final product, if the concentration exceeds 0.001% (10 mg/kg) in leave-on products, e.g. moisturizers, or 0.01% (100 mg/kg) in rinse-off products, e.g shampoos. Listing the regulated allergens on products can help identify the cause of an allergic reaction and also aids people to make informed choices about what they buy, particularly if they have a diagnosed allergy to a specific fragrance ingredient.

Current analytical methods used for the analysis of cosmetic allergens include Gas Chromatography Mass Spectrometry[3-5] (GC-MS), Headspace-GC-MS,[6] GC-GC/MS, Liquid Chromatography-UV (LC-UV),[7] and LC-MS,[8] which all have run times of approximately 30 to 40 minutes.

The current 24 regulated volatile cosmetic allergens contain compounds from different classes and different polarities (phenols, cyclic hydrocarbons, alcohols, carbonyl compounds, esters, and lactones). Many are small molecules with similar structures that often produce non-specific fragment ions for mass spectrometric detection.

There are many challenges that need to be addressed for any method used for allergen analysis. For example, the resolution achieved between analyte, isomer, 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 the option if required to use an additional co-solvent such as acetonitrile or methanol to give similar selectivity as normal phase LC.

This application note will consider how hyphenating Waters®UltraPerformance Convergence Chromatography™ (UPC^2) with MS detection can be used to achieve specificity, selectivity, and sensitivity for the analysis of fragrance allergens in perfume, cosmetics, and personal care products in a fast 7-minute run.

EXPERIMENTAL

Sample preparation

Cosmetic and personal care sample analysis

- 0.2 g sample was added to 2.5 mL water and 2.5 mL (methanol + 20 mM ammonium hydrogen carbonate).
- Mixture vortexed for 2 min (1600 rpm).
- Mixture further extracted in an ultrasonic bath for 30 min.
- Approximately 1-mL of extract centrifuged for 5 min (10,000 rpm).
- Centrifuged extract transferred to LC vials ready for analysis.

Perfume

100 µL sample + 900 µl (methanol + 20 mM ammonium hydrogen carbonate).

UPC^2 conditions

System:	ACQUITY UPC^2
Run time:	7.0 min
Column:	ACQUITY UPC^2 C_{18} HSS, 3.0 mm x 150 mm, 1.8 µm
Column temp.:	60 °C
CCM back pressure:	1500 psi
Sample temp.:	15 °C
Mobile phase A:	CO_2
Mobile phase B:	Methanol (0.1% formic acid)
Flow rate:	1.5 mL/min
Injection volume:	3 µL
Isocratic solvent manager solvent:	Methanol
Isocratic solvent manager flow rate:	0.4 mL/min
Vials:	Waters Amber Glass 12 x 32 mm Screw Neck, 2 mL, part no. 186007200C

Mobile phase gradient is detailed in Table 1.

	Time (min)	Flow rate (mL/min)	%A	%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.

MS conditions

MS system:	Xevo TQD
Ionization mode:	APCI (+ and -)
Corona voltage:	10 µA
Source temp.:	150 °C
APCI probe temp.:	600 °C
Desolvation gas:	1000 L/hr
Cone gas:	15 L/hr
Acquisition:	Multiple Reaction Monitoring (MRM)

The MS conditions were optimized for the analysis of 24 currently regulated cosmetic allergens. 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). CAS numbers, empirical formulas, and structures are detailed in Table 2 and Table 3 respectively. The established MRM method (Table 4) utilizes fast polarity switching available on the Xevo TQD, which enables the analysis of positive and negative allergens within the same analytical analysis.

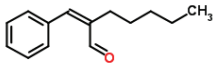
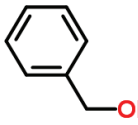
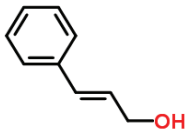
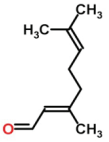
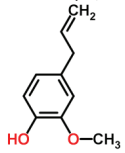
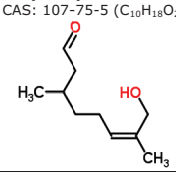
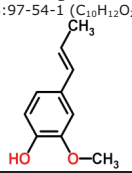
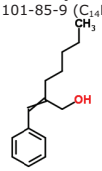
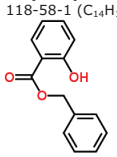
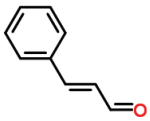
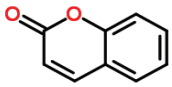
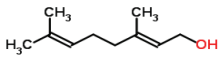
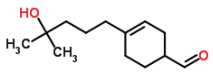
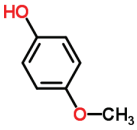
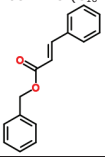
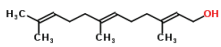
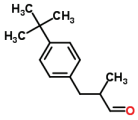
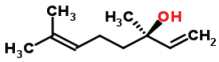
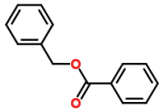
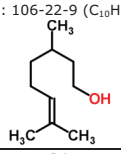
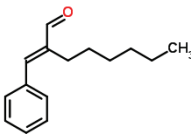
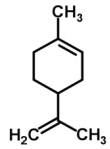
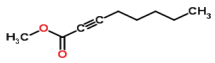
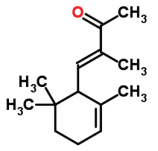
Regulated Allergens[2]			
1. **Amyl Cinnamaldehyde** CAS: 122-40-7 ($C_{14}H_{18}O$) 	**2.** **Benzyl alcohol** CAS: 100-51-6 (C_7H_8O) 	**3.** **Cinnamyl alcohol** CAS: 104-54-1 ($C_9H_{10}O$) 	**4.** **Citral** CAS: 5392-40-5 ($C_{10}H_{16}O$) 
5. **Eugenol** CAS: 97-54-0 ($C_{10}H_{12}O_2$) 	**6.** **Hydroxy-citronellal** CAS: 107-75-5 ($C_{10}H_{18}O_2$) 	**7.** **Isoeugenol** CAS:97-54-1 ($C_{10}H_{12}O_2$) 	**8.** **Amyl cinnamyl alcohol** CAS: 101-85-9 ($C_{14}H_{20}O$) 
9. **Benzyl salycilate** CAS: 118-58-1 ($C_{14}H_{12}O_3$) 	**10.** **Cinnamaldehyde** CAS: 104-55-2 (C_9H_8O) 	**11.** **Coumarin** CAS: 94-64-5 ($C_9H_6O_2$) 	**12.** **Geraniol** CAS: 106-24-1 ($C_{10}H_{18}O$) 
13. **Lyral** CAS: 31906-04-4 ($C_{13}H_{22}O_2$) 	**14.** **Anisyl alcohol** CAS: 105-13-5 ($C_7H_8O_2$) 	**15.** **Benzyl cinnamate** CAS: 103-41-3 ($C_{16}H_{14}O_2$) 	**16.** **Farnesol** CAS: 4602-84-0 ($C_{15}H_{26}O$) 
17. **Lilial** CAS: 80-54-6 ($C_{14}H_{20}O$) 	**18.** **Linalool** CAS: 78-70-6 ($C_{10}H_{18}O$) 	**19.** **Benzyl benzoate** CAS: 120-51-4 ($C_{14}H_{12}O_2$) 	**20.** **Citronellol** CAS: 106-22-9 ($C_{10}H_{20}O$) 
21. **Hexyl cinnamaldehyde** CAS: 101-86-0 ($C_{15}H_{20}O$) 	**22.** **Limonene** CAS: 5989-27-5 ($C_{10}H_{16}$) 	**23.** **Methyl heptine carbonate** CAS: 111-12-6 ($C_9H_{14}O_2$) 	**24.** **Alpha isomethyl ionone** CAS: 127-51-5 ($C_{14}H_{22}O$) 

Table 2. Cosmetic allergens considered, as regulated under current EU Cosmetic Regulations 1223/2009,[2] associated CAS numbers, empirical formulas, and structures.

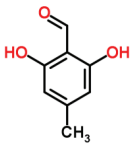
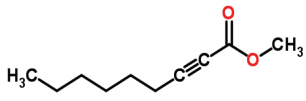
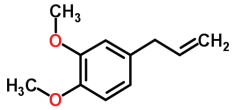
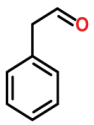
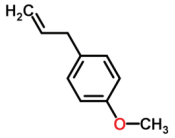
Additional compounds considered		
25. **Atranol** CAS: 526-37-4 ($C_8H_8O_3$) 	**26.** **Chloratranol** CAS: 57074-21-2 ($C_8H_7ClO_3$) 	**27.** **Methyl-2-nonynoate** CAS: 111-80-8 ($C_{10}H_{16}O_2$) 
28. **Methyl eugenol** CAS: 93-15-2 ($C_{11}H_{14}O_2$) 	**29.** **Phenylacetaldehyde** CAS: 122-78-1 (C_8H_8O) 	**30.** **4-Allyl anisole** CAS: 140-67-0 ($C_{10}H_{12}O$) 

Table 3. Additional compounds considered, associated CAS numbers, empirical formulas, and structures.

No	Chemical substance	Retention time (min) #isomers	APCI (+/-)	Cone voltage (V)	Transition	Collision energy
1	Amyl cinnamaldehyde	1.84	+	30	203.0>129.0*	18
					203.0>147.0	16
2	Benzyl alcohol	1.86	+	8	155.0>91.0*	8
					155.0>123.0	4
3	Cinnamyl alcohol	2.78	+	25	133.0>185.0*	18
4	Citral	1.58	+	15	153.0>69.0*	6
					153.0>95.0	15
5	Eugenol	1.68	+	20	165.1>124.0	20
					165.1>137.1*	12
6	Hydroxy-citronellal	3.37	+	18	171.0>111.0	15
					171.0>153.0*	10
7	Isoeugenol	1.90	+	25	165.1>105.0	20
					165.1>133.0*	20
8	Amyl cinnamyl alcohol	2.84	+	25	187.0>117.0*	20
					187.0>131.0	16
9	Benzyl salycilate	1.86	+	15	229.0>91.0*	12
					229.0>151.0	12
10	Cinnamaldehyde	1.75	+	25	133.0>55.0*	18
					133.0>115.0	14
11	Coumarine	2.52	+	40	147.0>91.0	28
					147.0>103.0*	23
12	Geraniol	1.59	+	20	137.0>81.0*	14
					137.0 >95.0	16
13	Lyral	3.24	+	20	193.0>111.0	18
					193.0>175.0*	12
14	Anisyl alcohol	2.79	+	40	121.0>77.0*	25
					121.0>78.0	25
15	Benzyl cinnamate	2.31	+	25	221.0>105.0	6
					221.0>193.0*	8
16	Farnesol	2.61/2.76/2.83#	+	25	205.1>109.0	20
					205.1>121.0*	20
17	Lilial	2.31	+	10	221.2>90.9*	30
18	Linalool	2.23	+	20	137.0>81.0*	20
					137.0>95.0	20
19	Benzyl benzoate	1.87	+	8	213.0>91.0*	8
20	Citronellol	2.19	+	18	157.1>57.0	10
					157.1>83.0*	10
21	Hexyl cinnamaldehyde	1.94	+	30	217.4>129*	20
					217.4>147	14
22	Limonene	0.67	+	20	137.0>81.0*	14
					137.0>95.0	16
23	Methyl heptine carbonate	0.72	+	30	155.0>67.0*	24
					155.0>123.0	15
24	Alpha isomethyl ionone	1.65	+	20	207.2>111.1*	20
					207.2>123.1	20
25	Atranol	4.57	-	18	151.0>78.94*	20
					151.0>123.09	20
26	Chloratranol	2.90	-	18	185.0>121.17*	20
					185.0>156.99	20
27	Methyl-2-nonynoate	1.53	+	34	153.0>42.9	22
					153.0>97.0*	16
28	Methyl eugenol	1.78	+	25	179.0>138*	16
					179.0>164	14
29	Phenylacetaldehyde	0.70	+	2	121.0>56.9	4
					121.0>89.0*	10
30	4-Allyl anisole	2.52	+	30	146.9>76.9	28
					146.9>90.9*	32

Table 4. Expected retention times, ionization mode, cone voltages, MRM transitions, and associated collision energy values for 24 regulated cosmetic allergens and six additional compounds.

Instrument control, data acquisition, and results processing

MassLynx Software was used to control the ACQUITY UPC^2 and the Xevo TQD, and also for data acquisition. Data quantitation was achieved using the TargetLynx™ Application Manager.

RESULTS AND DISCUSSION

The analysis of the 24 regulated 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.

Mixed calibration standards, 0.25 to 25 ppm, were prepared and analyzed. An example calibration curve generated for cinamyl alcohol, shown in Figure 1, with an r^2 value of 0.9999. The MRM chromatograms for each compound are shown in Figure 2.

The developed 7-minute UPC^2 method, is more than six times faster than existing HPLC and GC methods, with an excess of 95% less solvent usage than existing HPLC methods.

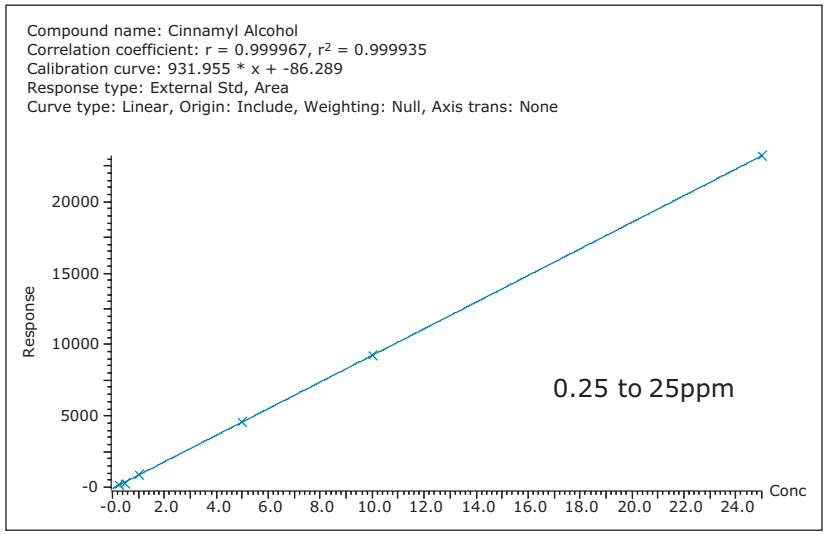


Figure 1. TargetLynx Quantify results browser showing the calibration curve for cinnamyl alcohol.

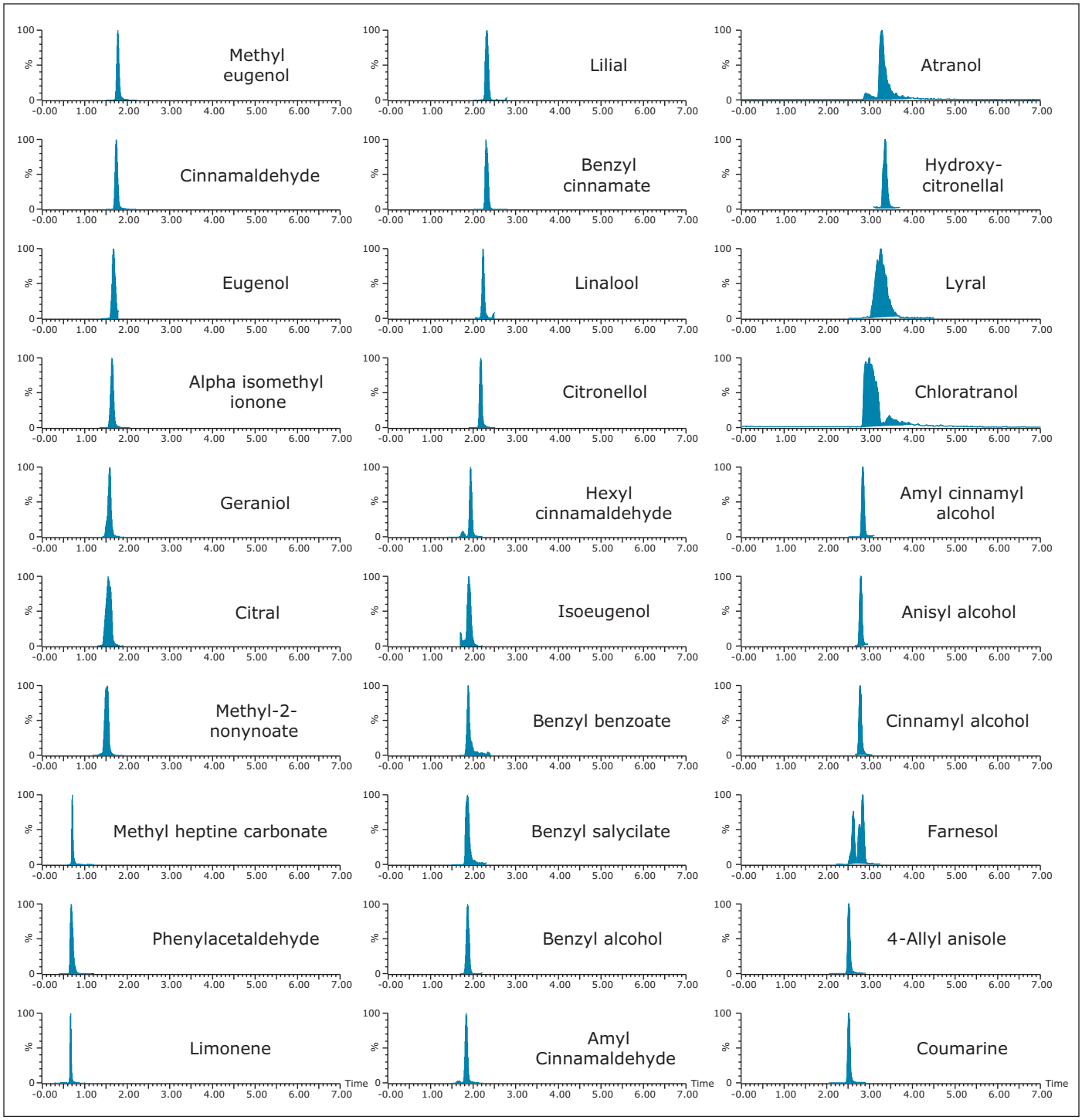


Figure 2. MRM chromatograms for 24 regulated cosmetic allergens and six additional compounds in 10 ppm calibration standards (1 ppm for chloratranol and atranol).

Shampoo and perfume analysis

The MRM mass detection method (Table 4) was used after appropriate sample preparation for the analysis of the 24 regulated and four additional compounds in shampoo and perfume samples.

Perfume samples were fortified at 10 mg/kg (0.001%) with 24 cosmetic allergens, and four additional compounds. They were then prepared for analysis as detailed in the Experimental section. Example MRM chromatograms achieved for fortified perfume are shown in Figure 3.

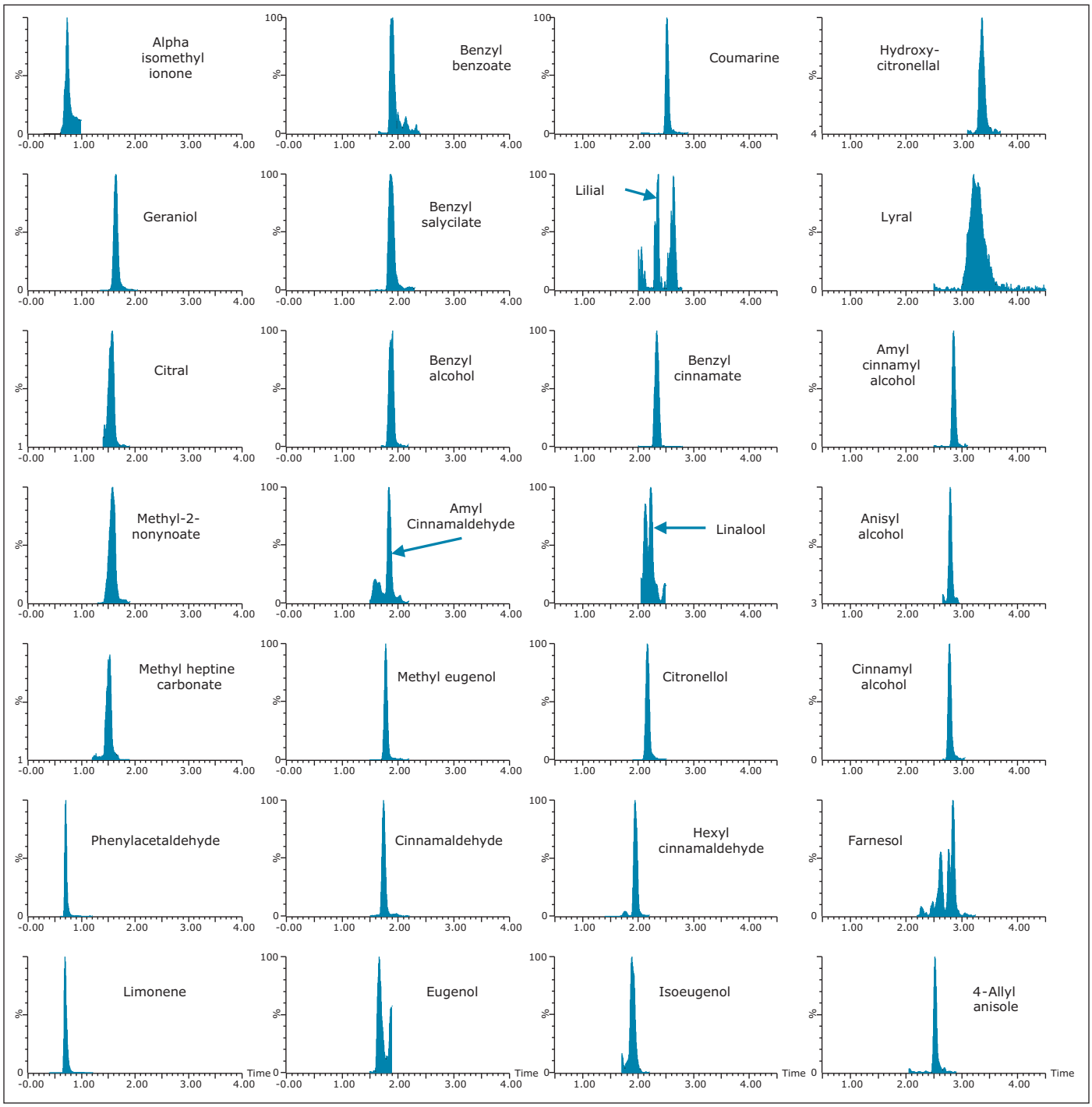


Figure 3. MRM chromatograms for 24 cosmetic allergens and four additional compounds in perfume, fortified at 10 mg/kg (0.001%).

Shampoo samples were fortified at 100 mg/kg (0.01%) with 24 cosmetic allergens and 4 additional compounds, then prepared for analysis as detailed in the Experimental section. Example MRM chromatograms achieved for fortified shampoo are shown in Figure 4.

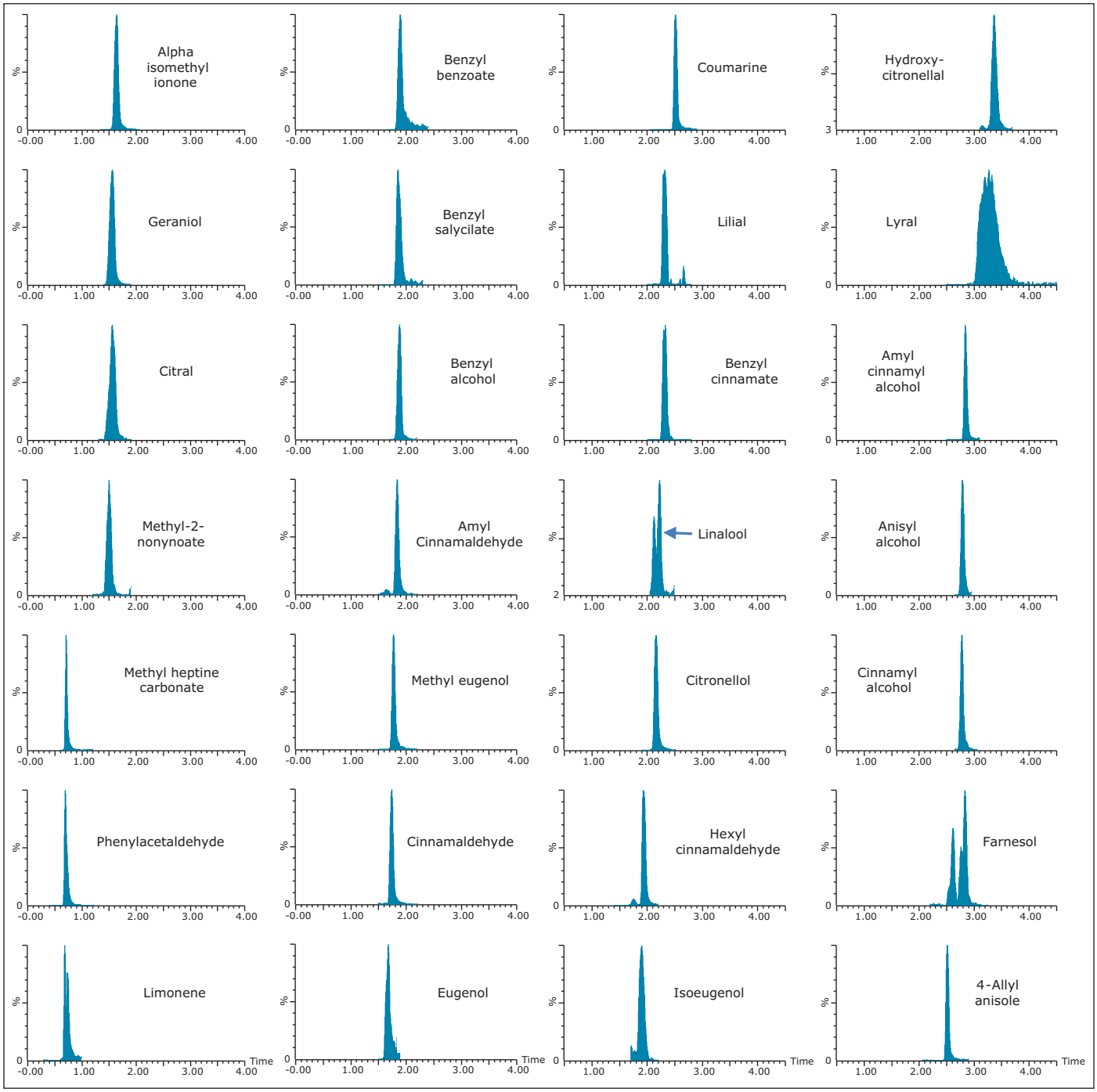


Figure 4. MRM chromatograms for 24 cosmetic allergens and 4 additional compounds in shampoo fortified at 100 mg/kg (0.01%).

Various cosmetic allergens compounds are isomeric, for example Farnesol where potentially four isomeric forms can be produced (Figure 5). 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 6) for farnesol in a shampoo sample fortified at 10 mg/Kg (one tenth of the regulated limit of 0.01%), and the nearest equivalent standard (0.5 ppm), which illustrated several isomeric farnesol peaks. For comparison, a blank shampoo sample MRM chromatogram for farnesol is also shown in Figure 6.

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).

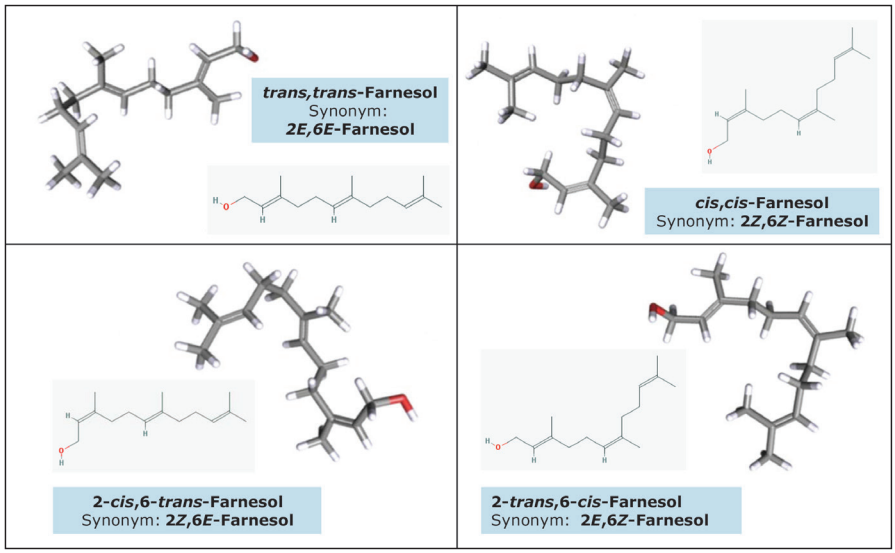


Figure 5. Four isomers of farnesol.

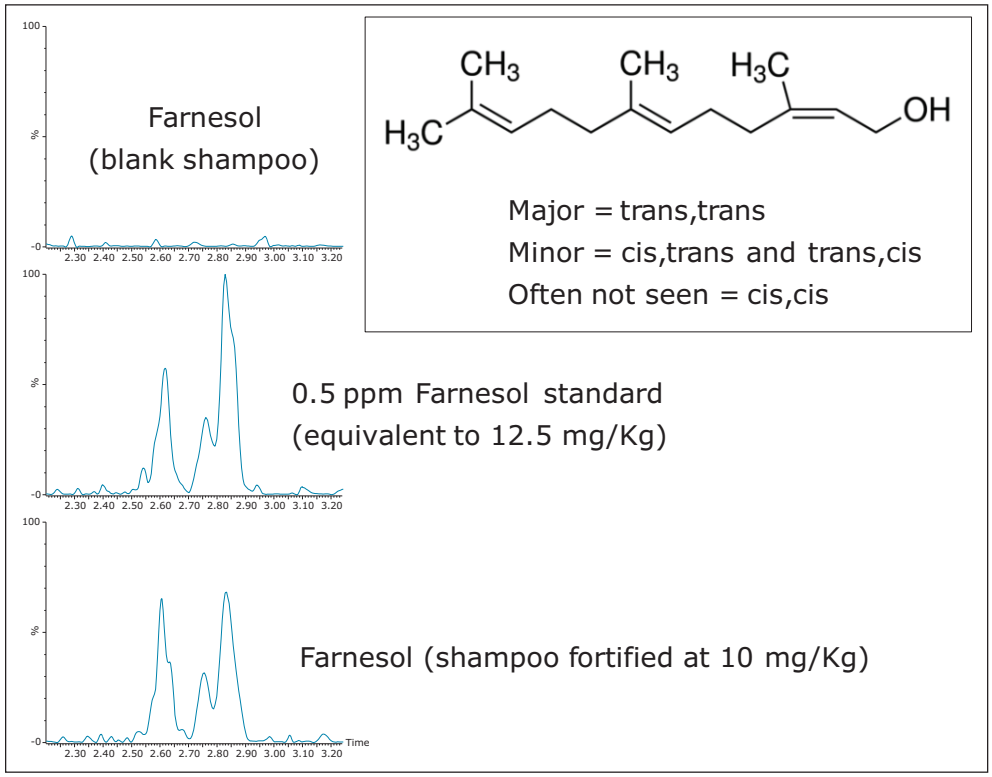


Figure 6. MRM chromatograms for shampoo fortified at 10 mg/Kg (one-tenth of the regulated limit of 0.01%), the nearest equivalent standard (10 mg/Kg), and a blank shampoo sample.

CONCLUSIONS

- 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 cosmetic allergens, 4 non-regulated cosmetic allergens, and 2 potential carcinogenic compounds 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 more than six times faster than existing HPLC and GC methods, with 95% less solvent usage than existing HPLC methods.

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