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Analysis of allergens found in cosmetics using MDGC-GCMS (Multi-Dimensional Gas Chromatograph Mass Spectrometer)

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Introduction

Cosmetics, fragrances and toiletries (Figure 1) are used safely by millions of people worldwide. Although many people have no problems, irritant and allergic reactions may occur. Irritant and allergic skin reactions are the types of contact dermatitis. Essential oils present in fragrance contain some natural and synthetic compounds, which may cause allergic reactions to the end user after application. There are 26 potential allergens listed by European Directive (EU) 2003/15/EC and International Fragrance Association (IFRA)[1] labeled on cosmetics. Shimadzu MDGC-GCMS technology facilitates the identification and quantification of these allergens to comply with the threshold limits of 100 ppm for rinse-off products.
Co-eluting peaks were resolved completely with the help of MDGC-GCMS heart-cut technique.

Figure 1. Cosmetics, fragrances and toiletries

Method of Analysis

Extraction of allergens from shampoo sample

Shampoo samples were collected from local market. Standard solutions of 23 allergens were procured from ACCU Standard and dilutions were carried out in Ethanol/Acetonitrile to yield 1000 ppm concentration. Further dilutions were made in methanol.
MDGC-GCMS technique was effectively used to minimize matrix effect. Co-eluting peaks were resolved with heart-cut technique using two columns of different polarities. In MDGC-GCMS, 1st instrument was GC-2010 Plus equipped with FID as a detector and 2nd instrument was GCMS-QP2010 Ultra with MS as a detector. Columns in both the instruments were connected with Deans switch. Allergens in shampoo samples were determined by using this technique. For sample preparation, following methodology was adopted.

1) Blank Solution : 10 mL of methanol was transferred in 20 mL centrifuge tube and vortexed for 5 minutes. The mixture was then centrifuged for 5 minutes at 3000 rpm. This solution was filtered through 0.2 µm nylon syringe filter. Initial 2 mL was discarded and remaining filtrate was collected.
2) Sample Solution : 1 g of shampoo sample was weighed in 10 mL volumetric flask and diluted up to the mark with methanol. Above mixture was transferred in 20 mL centrifuge tube. Further processing was done as mentioned in blank solution.
3) Spike Sample Solution : For recovery study, 1 g of sample was spiked with different volumes of standard stock solution. The above procedure was repeated for preparing different concentration levels of allergens in samples. These spiked samples were treated as mentioned in sample solution.

Part method validation was carried out by performing system precision, sample precision, linearity and recovery study. For validation, solutions of different concentrations were prepared using 40 ppm (actual concentration) standard stock solution mixture of allergens.

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Table 1. Method validation parameters

Parameter	Concentration
System Precision	10 ppm
Sample Precision	10 % in Methanol
Linearity	2.5, 5, 7.5, 10, 15 (ppm)
Accuracy / Recovery	5, 10, 15 (ppm)

MDGC-GCMS Analytical Conditions

The instrument configuration used is shown in Figure 2. Samples were analyzed using Multi-Dimensional GC/GCMS as per the conditions given below.

Figure 2. Multi-Dimensional GC/GCMS System by Shimadzu

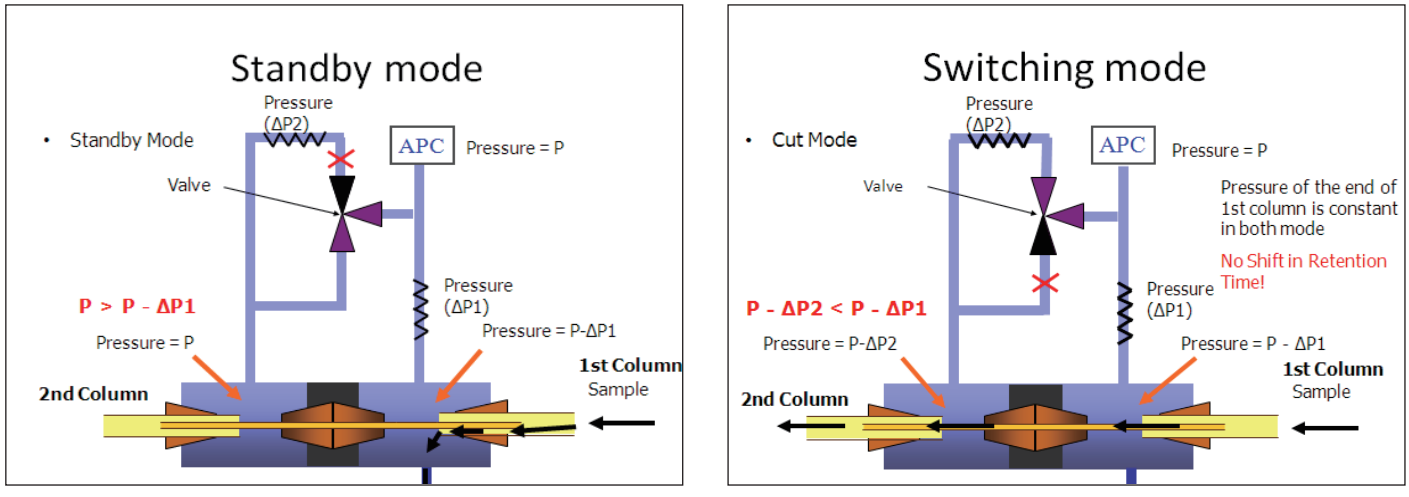

Figure 3. Schematic diagram of multi-Deans switch in MDGC-GCMS

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MDGC-GCMS analytical parameters

Chromatographic parameters (1st GC : GC-2010 Plus)

• Column	: Stabilwax (30 m L x 0.25 mm I.D.; 0.25 µm)		
• Injection Mode	: Split		
• Split Ratio	: 5.0		
• Carrier Gas	: Helium		
• Column Flow	: 2.27 mL/min		
• Detector	: FID		
• APC Pressure	: 200 kPa (For switching)		
• Column Oven Temp.	: Rate (°C /min)	Temperature (°C)	Hold time (min)
		50.0	0.00
	15.00	100.0	0.00
	5.00	240.0	43.67

Chromatographic parameters (2nd GCMS : GCMS-QP2010 Ultra)

• Column	: Rxi-1ms (30 m L x 0.25 mm I.D.; 0.25 µm)		
• Detector	: Mass spectrometer		
• Ion Source Temp.	: 200 °C		
• Interface Temp.	: 240 °C		
• Ionization Mode	: EI		
• Event Time	: 0.30 sec		
• Mode	: SIM and SCAN		
• Column Oven Temp.	: Rate (°C /min)	Temperature (°C)	Hold time (min)
		80.0	13.00
	3.00	180.0	0.00
	10.00	260.0	20.67
• Total Program Time	: 75.00 min		

Results

Sample analysis using MDGC-GCMS

MDGC-GCMS technique was used to avoid matrix interference from sample. Using multi-Deans switch and heart-cut technique (Figure 3), co-eluted components from the 1st column were transferred to the 2nd column with different polarity.

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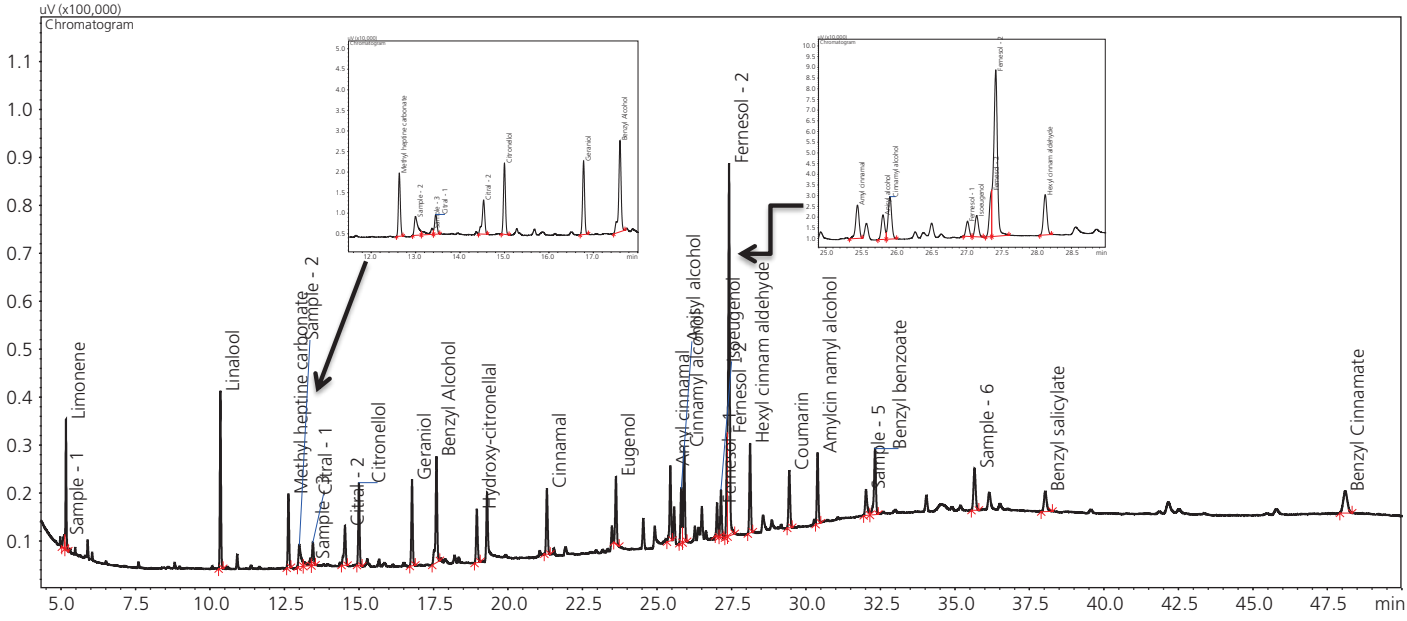

Figure 4. Chromatogram of spiked sample solution before switching

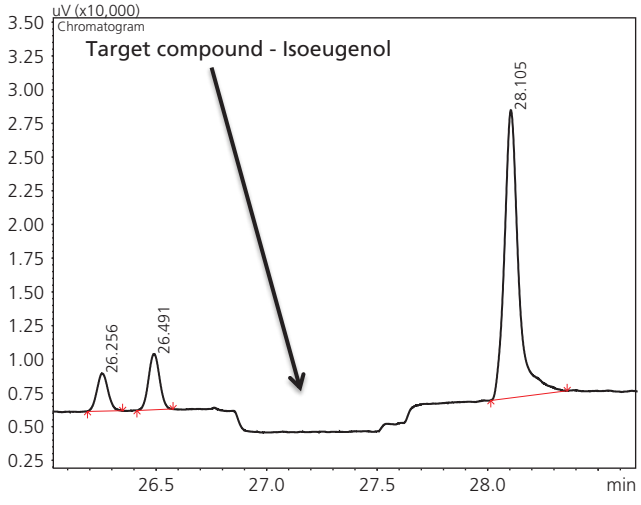

Figure 5. Chromatogram with 1st column (FID)

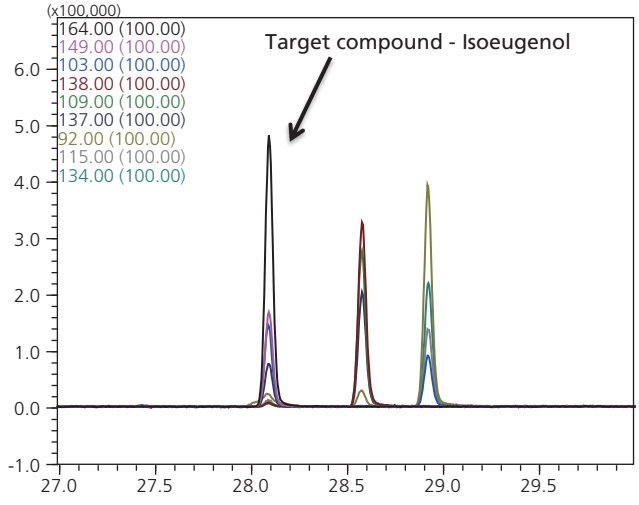

Figure 6. SIM chromatogram with 2nd column (MS)

Summary of results

Table 2. Summary of results for precision on GC and GCMS

Sr. No.	Type of sample	Sample name	Concentration	Result
1	Standard	23 Allergens mixture	10 ppm	% RSD for area (n=6) < 2.0
2	Cosmetic	Shampoo	Unknown	% RSD for area (n=6) < 2.0

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Table 3. Linearity by GC

Sr. No.	Name of allergen	Linearity (R^2)
1	Linalool	0.9945
2	Methyl heptine carbonate	0.9949
3	Citronellol	0.9965
4	Geraniol	0.9962
5	Hydroxy citronellal	0.9973
6	Cinnamal	0.9959
7	Amyl Cinnamal	0.9976
8	Coumarin	0.9971
9	Amylcin namyl alcohol	0.9983
10	Benzyl benzoate	0.9979

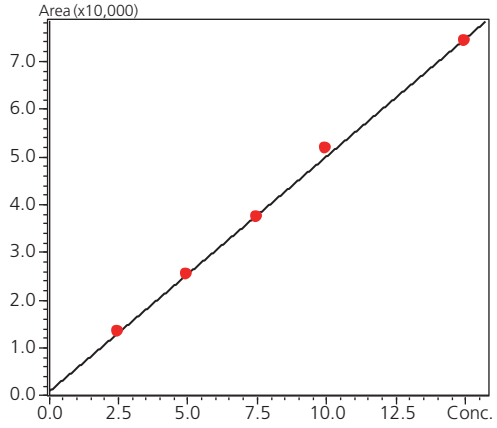

Figure 7. Linearity graph for linalool

Table 4. Linearity by GCMS

Sr. No.	Name of allergen	Linearity (R^2)
1	Limonene	0.9945
2	Benzyl alcohol	0.9871
3	Citral - 1	0.9889
4	Citral - 2	0.9902
5	Eugenol	0.9894
6	Anisyl alcohol	0.9916
7	Cinnamyl alcohol	0.9937
8	Isoeugenol	0.9902
9	Farnesol - 1	0.9919
10	Farnesol - 2	0.9929
11	Hexyl cinnam aldehyde	0.9932
12	Benzyl salicylate	0.9853
13	Benzyl cinnamate	0.9927

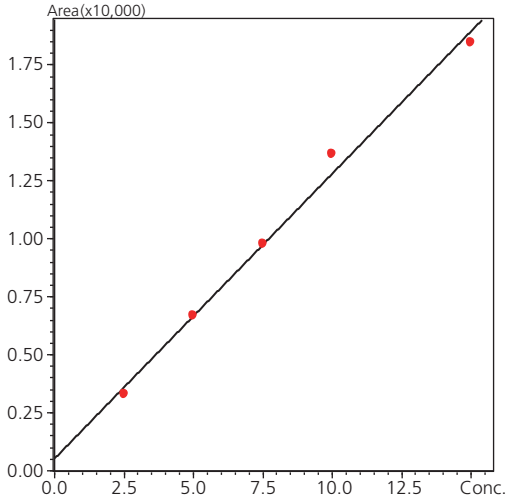

Figure 8. Linearity graph for benzyl cinnamate

Quantitation of allergens in shampoo sample

For the quantitation studies, the shampoo sample was spiked with allergens standard to achieve 5, 10 and 15 ppm concentrations. Recovery studies were performed on 13 allergens, having co-elution or matrix interference, using heart-cut technique. The quantitation of these allergens was carried out using 2nd detector (MS) in SIM mode.

In below recovery study, some allergens had recovery value out side the acceptance limit (70-130 %). Optimization can be done by means of change in sample clean up procedure and filtration study.

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Table 5. Quantitation of allergens – Recovery Study

Sr. No.	Name of allergen	% Recovery		
		Level -1 5 ppm	Level -2 10 ppm	Level -3 15 ppm
1	Limonene	127	126	129
2	Benzyl alcohol	114	114	123
3	Citral - 1	101	106	114
4	Citral - 2	97	103	112
5	Eugenol	96	105	116
6	Anisyl alcohol	94	105	116
7	Cinnamyl alcohol	98	106	115
8	Isoeugenol	103	108	118
9	Farnesol - 1	83	95	107
10	Farnesol - 2	84	95	106
11	Hexyl cinnam aldehyde	121	122	130
12	Benzyl salicylate	63	47	32
13	Benzyl cinnamate	66	61	56

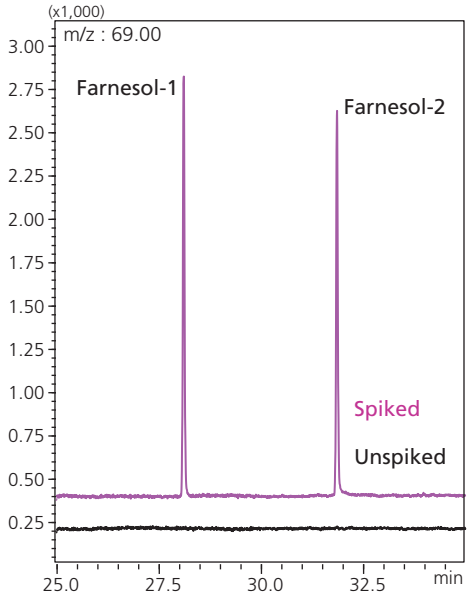

Figure 9. Overlay SIM chromatogram of unspiked and spiked sample

Conclusion

- MDGC-GCMS method was developed for quantitation of allergens present in cosmetics. Part method validation was performed as per ICH guidelines.[2] Results obtained for reproducibility, linearity and recovery studies were well within acceptable limits.
- Simultaneous SCAN/SIM and high-speed scan rate 20,000 u/sec are the characteristic features of GCMS-QP2010 Ultra, which enables quantitation of allergens at very low concentration level.
- Matrix effect from cosmetics was selectively eliminated using MDGC-GCMS with multi-Deans switching unit and heart-cut technique.
- MDGC-GCMS was found to be very useful technique for simultaneous identification and quantitation of components from complex matrix.

Reference

[1] IFRA guidelines (International Fragrance Association), GC/MS Quantification of potential fragrance allergens, Version 2, (2006), 6.
[2] ICH guidelines, Validation of Analytical Procedures: Text And Methodology Q2(R1), Version 4, (2005).

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