

## Perfume Analysis by means of Fast Gas Chromatography hyphenated to Time of Flight Mass Spectrometry

### INTRODUCTION

Gas chromatography (GC) and mass spectrometry (MS) are by far the most important analytical techniques in the perfumery industry. Perfumery industries and home and personal care companies rely primarily on GC and GC/MS for unravelling fragrance formulations, as well as for quality control and trace analysis.

It is worth noting that fragrance materials are composed of a wide range of compounds blended to create sophisticated perfumes. Nowadays several thousand ingredients are known and fragrances are built of 30 to 50, and sometimes up to 200 ingredients [1].

Surely, as in all other industrial sectors, perfumery industries also benefit from the use of faster analyses; applied in the continuous research for new odour-active molecules as well as for quality control purposes. The GC approaches to speed up analysis include the utilization of shorter capillary columns with decreased column internal diameter and thinner stationary phase film thickness, higher carrier gas velocities, and faster GC oven temperature programming rates.

However, even if automated instrumentation and optimized Fast GC methods are used, co-elution of some perfume ingredients is observed for almost any fragrance material. The generation of very narrow peaks (typical peak widths of 0.5 seconds or less) results in crowded chromatograms, thus requiring mass spectrometric detection with high acquisition rates. The Time of Flight Mass Spectrometer (TOF-MS) is the only technology able to provide high speed acquisition rate to collect sufficient information (15-20 points/peak) for reliable reconstruction of these very narrow peaks over the entire mass range [2, 3]. Thereby enabling accurate recognition and quantification of unknown and co-eluting peaks, even in trace-level concentrations.

The present application note investigates the suitability of an innovative Fast GC/TOF-MS system, supported by an original software, to reliably analyze perfumery materials. Two distinct methods are compared.



FIGURE 1. The MASTER TOF GC/MS equipped with MASTER AS Liquid Autosampler.

## EXPERIMENTAL

### Sample

A commercial perfumery mix was diluted in ethanol 1:100 *v/v*.

### System configuration and control

Fast GC/TOF-MS conditions are summarized in *Table 1*.

The MASTER LAB software provided the complete control of the MASTER AS Liquid Autosampler, the MASTER GC Fast Gas Chromatograph and the MASTER TOF-MS, from autotuning procedures to GC and sample sequence management. The system was also equipped with the *NIST MS Search Program*, version 2.0, 2002, the *NIST 08 Mass Spectral Library* (Gaithersburg, MD, U.S.A.) and the Adams MS Library (Allured Publishing (Carol Stream, IL, U.S.A.)).

## RESULTS

The perfumery mix was analyzed in less than 14 min on a 10 m narrow-bore column; the chromatogram is shown in *Figure 2*. On the other hand, the use of a 3 m narrow-bore column reduced the analysis time to less than 3 min; see chromatogram in *Figure 3*. The detected compounds and their relative retention times on both columns are presented in *Table 2*. Three identification masses for each compound are also listed.

Peak assignment was carried out by using a dedicated post acquisition processing procedure. At first the retention time window of each component was identified, thereafter three distinct masses were selected for each compound and their relative ratio in the mass spectra was evaluated. Finally, quantification was made using the mass values marked in bold in *Table 2*.

**TABLE 1. FAST GC/TOF-MS EXPERIMENTAL CONDITIONS**

	METHOD 1	METHOD 2
<b>MASTER GC</b>		
<b>Column</b>	DN-5 10 m x 0.1 mm, 0.1 $\mu$ m	DN-5 3 m x 0.1 mm, 0.1 $\mu$ m
<b>Oven Temp. Program</b>	50 °C, 14 °C/min, 250 °C	100 °C, 50 °C/min, 250 °C
<b>Injector</b>	Split/Splitless (250 °C)	Split/Splitless (250 °C)
<b>Split Ratio</b>	1:400	1:800
<b>Carrier Gas Flow (He)</b>	1 mL/min (constant flow)	0.5 mL/min (constant flow)
<b>Linear Velocity</b>	40 cm/s (constant mode)	40 cm/s (constant mode)
<b>Injected Volume</b>	0.5 $\mu$ L	0.5 $\mu$ L
<b>MASTER TOF-MS</b>		
<b>Transfer Line Temp.</b>	220 °C	220 °C
<b>Ion Source Temp.</b>	220 °C	220 °C
<b>Sampling Rate</b>	50 Hz (50 spectra/s)	100 Hz (100 spectra/s)
<b>Mass Range</b>	40 - 400 amu	40 - 400 amu
<b>Solvent Delay</b>	60 s	60 s
<b>Acquisition Time</b>	14 min	3 min

**TABLE 2. COMPOSITION OF THE PERFUMERY MIX.**

Compounds	Retention Time (min)		Masses
	10 m Column	3 m Column	
1 Unidentified compound 1	4.22	1.15	-
2 <i>cis</i> - $\beta$ -Terpineol	4.28	1.17	71, 93, 136
3 <i>trans</i> - $\beta$ -Terpineol	4.40	1.20	71, 93, 136
4 Unidentified compound 2	4.42	-	-
5 Unidentified compound 3	4.45	-	-
6 Acetate styrallyl	4.57	1.23	122, 104, 164
7 $\alpha$ -Terpineol	4.57	1.24	59, 93, 121
8 $\gamma$ -Terpineol	5.01	1.25	121, 93, 154
9 Unidentified compound 4	5.18	-	-
10 Nerol	-	1.27	69, 84, 93
11 Citronellol	-	1.28	69, 95, 156
12 Linalyl acetate	5.34	1.31	93, 80, 136
13 <i>trans</i> -Jasmone	6.58	1.55	79, 91, 164
14 Vanillin	6.58	1.55	151, 81, 109
15 Diethyl phthalate	8.41	2.23	149, 177, 222
16 Hedione	9.12	2.31	83, 156, 226
17 Iso E Super® (isomer 1)	9.17	2.35	191, 135, 219
18 Iso E Super® (isomer 2)	9.20	2.36	135, 191, 219
19 Hexyl salicylate	9.21	2.35	120, 138, 222
20 Hedione (isomer)	9.23	2.34	83, 156, 226
21 Iso E Super® (isomer 3)	9.31	2.39	135, 191, 219

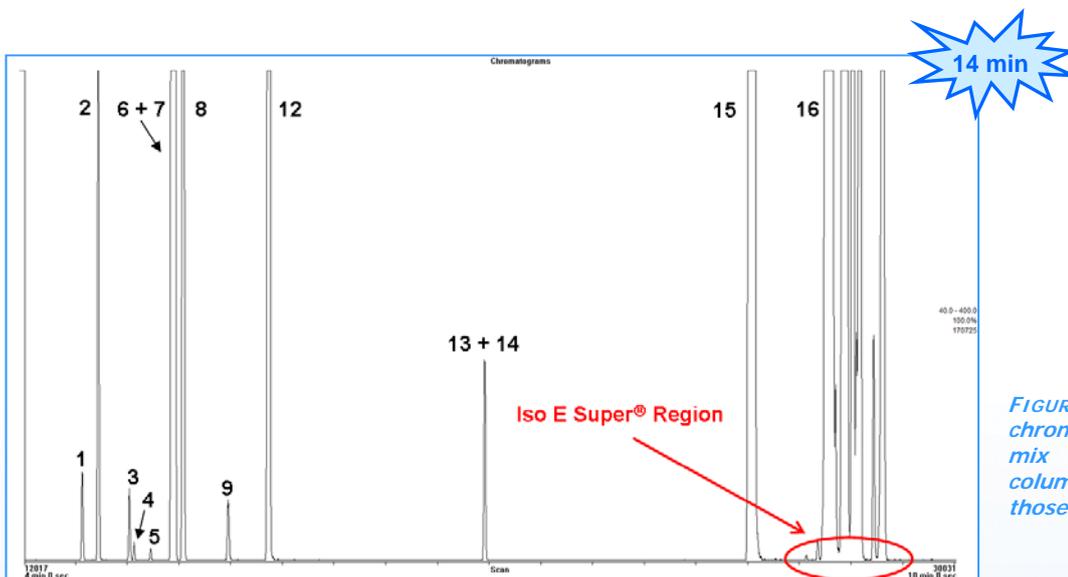


FIGURE 2. Fast GC/TOF-MS chromatogram of the perfumery mix on a 10 m narrow-bore column. The numbers refer to those in Table 2.

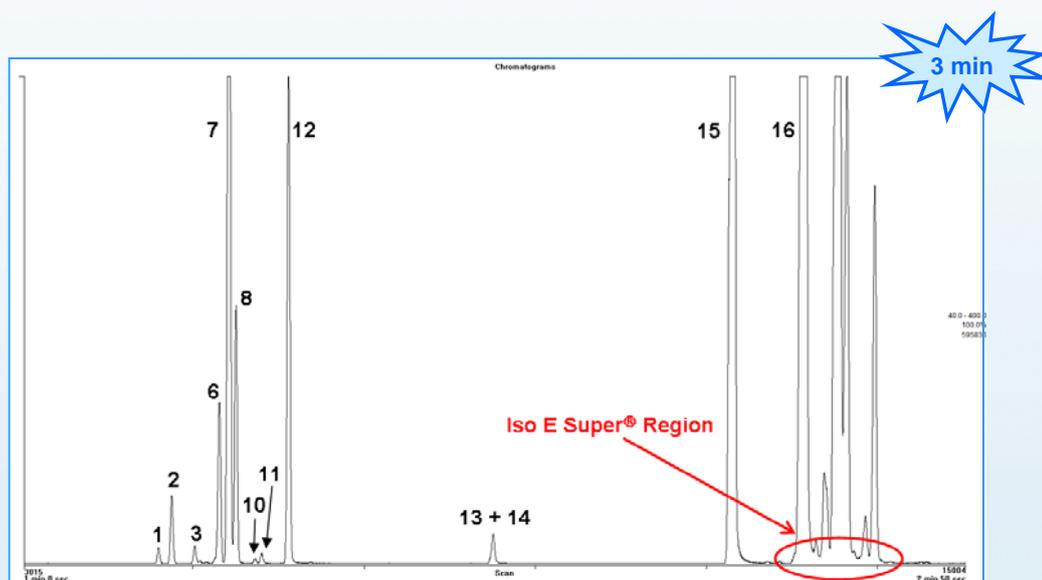


FIGURE 3. Fast GC/TOF-MS chromatogram of the perfumery mix on a 3 m narrow-bore column. The numbers refer to those in Table 2.

However, it was known that the perfumery mix under investigation contained the renowned perfumery raw material Iso E Super<sup>®</sup> (CAS No. 54464-57-2). The latter elicits woody, floral, and ambergris notes and is commonly commercialized as a mixture of its three isomers (alpha, beta, and gamma).

The expansions of the Iso E Super<sup>®</sup> region on both the columns, 10 m and 3 m, are shown in Figures 4 A and B, respectively. As can be observed, the complexity of the sample along with reduced analysis times resulted in several co-elutions.

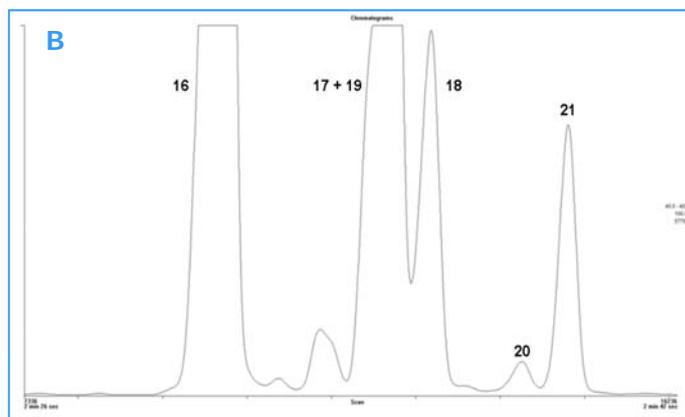
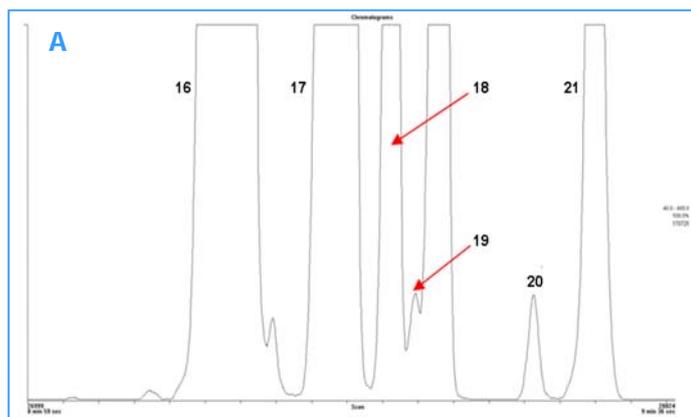


FIGURE 4. Zoomed Iso E Super<sup>®</sup> region on 10 m (A) and 3 m (B) narrow-bore columns [16: hedione, 17: Iso E Super (isomer 1), 18: Iso E Super (isomer 2), 19: salicylate hexyle, 20: hedione (isomer), 21: Iso E Super (isomer 3)].

The MASTER Lab algorithms use automatic spectral deconvolution procedures to detect and mathematically separate totally and partially overlapped peaks, refer to Figures 5 A and B.

Unresolved mass spectra were deconvoluted, pure mass spectra were generated and then submitted as unknowns to mass spectral library search.

As previously cited, the identification process was obviously supported by mass spectral library search functions. Figure 6 presents the comparison between the acquired mass spectrum of hexyl salicylate - peak 19 (top) and the spectrum stored in the NIST<sup>®</sup> MS Library (bottom).

A matching of 927 and a reverse matching of 972 were obtained. It is remarkable that spectra acquired with the MASTER TOF-MS are fully comparable with those contained in the MS Library which were obtained using quadrupole MS.

Moreover, data processing was carried out in a very short period of time requiring no particular computer specifications.

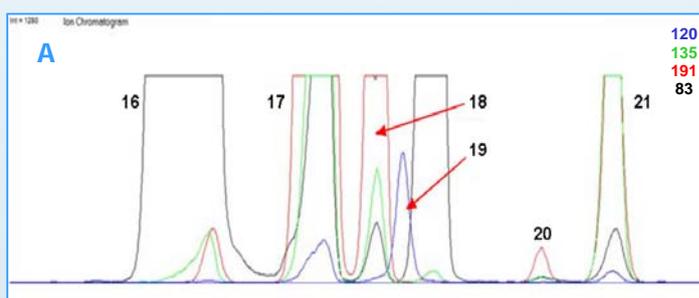
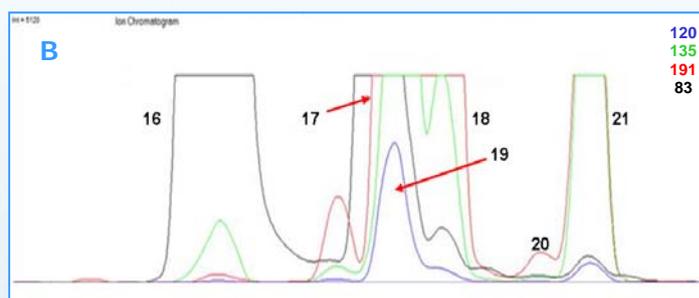


FIGURE 5. Zoom of the automatically deconvoluted Iso E Super<sup>®</sup> region on the 10 m (A) and 3 m (B) narrow-bore columns. For the numbers refer to Figure 4.

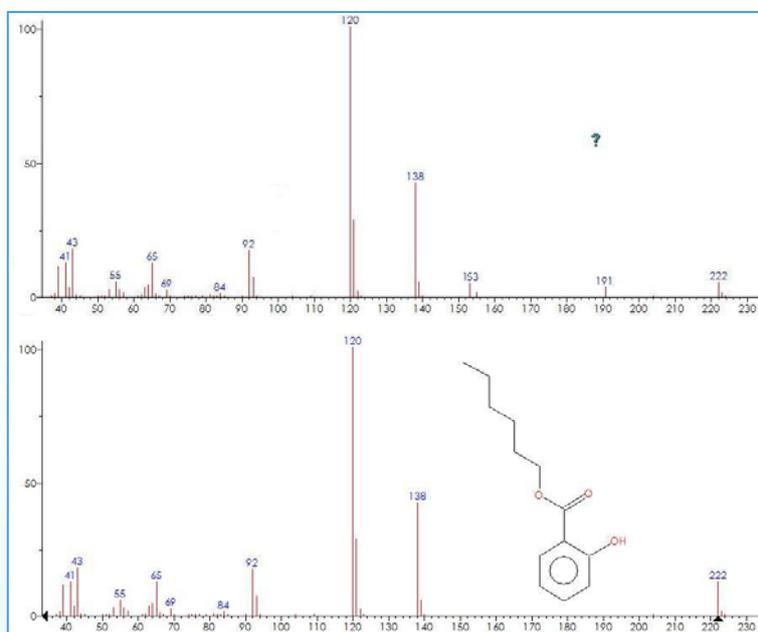


FIGURE 6. NIST® similarity search of hexyl salicylate (peak 19 - selected masses 120, 138 and 222). Acquired mass spectrum (top) and spectrum stored in the NIST® MS Library (bottom).

## CONCLUSION

It is well known that the use of modern analytical methods boosted the acquisition of higher perfumery raw material knowledge and GC/MS has been widely employed in perfumery industries.

The application of Fast GC using 0.10 mm I.D. narrow-bore columns has greatly shortened the analysis times, while the use of TOF-MS detection enabled to obtain distinguishable signals providing reliable identification even when compounds were totally co-eluting.

In addition, the MASTER Lab provides a powerful data processing solution and an original deconvolution algorithm capable to handle the large amount of information in a smart and effective way resulting in reliable identification. Furthermore, the loss in resolution is greatly compensated by automatic deconvolution procedures.

## REFERENCES

- [1] Curtis, T., Williams, D.G., *Introduction to Perfumery*, Micelle Press, New York, 2001.
- [2] Gross, J. H., *Mass Spectrometry. A Textbook*, Springer Verlag, Berlin, 2004.
- [3] Adams, R. P., *Identification of essential oil components by gas chromatography/mass spectrometry*. Allured Publishing Corp., Carol Stream, IL, U.S.A., 1995.