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EXACT MASS DECONVOLUTION APPLIED TO GC/MS DATA

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OVERVIEW

Purpose

- Identification and confirmation of individual fragrance compounds in a complex mixture.

Method

- GC-MS using electron impact (EI).
- Orthogonal Time of Flight mass Spectrometer providing exact mass with single point internal reference correction.
- Deconvolution using peak apex tracking and spectrum refine function.

Results

- Demonstrated production of 'pure' spectra from unresolved peaks.
- Confirmed identity using exact mass to determine elemental composition.

INTRODUCTION

- Capillary GC-MS with its high column efficiencies and universal EI ionisation is an ideal technique to profile complicated mixtures containing thermally stable and volatile components.
- The technique is often employed in the flavour and fragrance industries where it is necessary to semi-quantitatively determine hundreds of compounds in a single injection. Extracts from metabolomic studies often put the same demands on analytical instrumentation.
- Given the complexity of the mixtures being analysed it is not surprising that many of the components remain unresolved chromatographically.
- In these circumstances it is necessary to apply software solutions in order to first identify the presence of multiple components, under seemingly a single chromatographic peak, and secondly to apply deconvolution to produce pure spectra for each of the individual components.
- This paper describes the use of deconvolution techniques applied to a fragrance sample. It also discusses the use of exact mass to confirm identities postulated by library search.

INSTRUMENTATION

Figure 1 shows a photograph of the GCT used in these studies.

Figure 2 shows a schematic of the GCT.

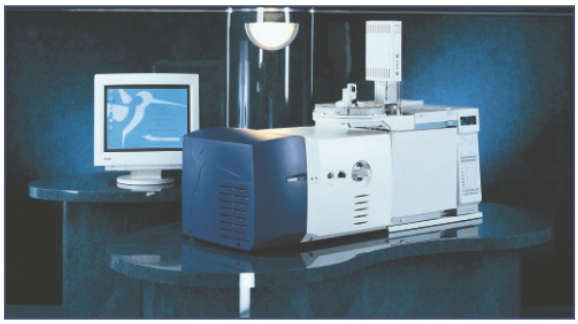

Figure 1. Photograph of GCT

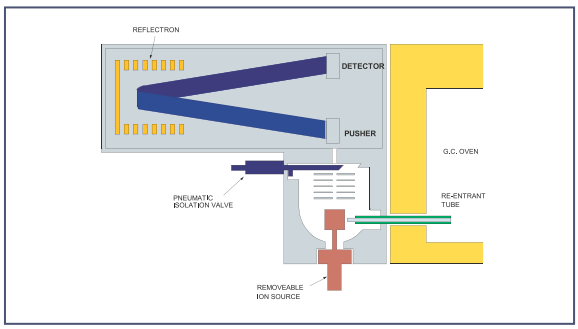

Figure 2. Schematic of GCT

MICROMASS MS TECHNOLOGIES

- Ions produced in the grounded ion source are accelerated to 40eV and focused into a parallel ion beam. As the ion traverse the pushout region, a sudden voltage pulse is applied, ejecting a portion of the beam orthogonally. A single stage reflectron reflects the ions back to a dual microchannel plate detector (MCP). Ion arrivals are recorded using a 3.6 GHz time to digital converter (TDC).

- The ion beam is sampled orthogonally at up to 30,000 times / second. Individual time of flight spectra are summed before being transferred to the host PC. The instrument is fitted with a heated GC transfer line and heated reference inlet for introduction of volatile calibration compounds.

- The GCT instrument design allows fast acquisition, with high sensitivity and elevated resolution (7000FWHM) **Figure 3**. This elevated resolution reduces mass interferences. Furthermore the precise, and stable relationship between ion arrival time and the square route of its mass allows good mass measurement accuracy with only a single internal reference mass.

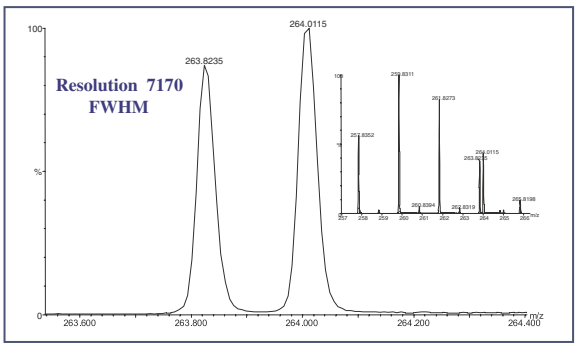

Figure 3. Resolution on GCT

SOFTWARE

- Chromatographic data was processed using GCLynx. This is a developmental applications manager designed for automated peak identification and spectral deconvolution.

EXPERIMENTAL

Sample Preparation:

A 'neat' mixture, containing in excess of 120 components was diluted 1:10000 in acetone.

Autosampler:

Agilent 7683 series 1µL injection.

Gas Chromatograph:

Agilent 6890.
Splitess Injection at 250°C
J&W DB1, 20m x 0.18mm ID.
He at 1mL/min in constant flow mode.
Program 40°C (2mins) to 250°C at 10°C/min.

EI+ Mass Spectrometry:

Micromass GCT
Acquired Mass Range 50-500 Da
Acquisition Time 5 spectrum / second
Source 180°C
Resolution 7000 (FWHM)
Lock Mass m/z 201.9609
(chloropentafluorobenzene)

RESULTS AND DISCUSSION

General

- **Figure 4** shows the TIC of the fragrance mixture. The performance of GCLynx for automated peak detection and deconvolution was assessed by examining a complex region of the chromatogram between RT 12.32 to 13.62 containing 17 expected peaks.

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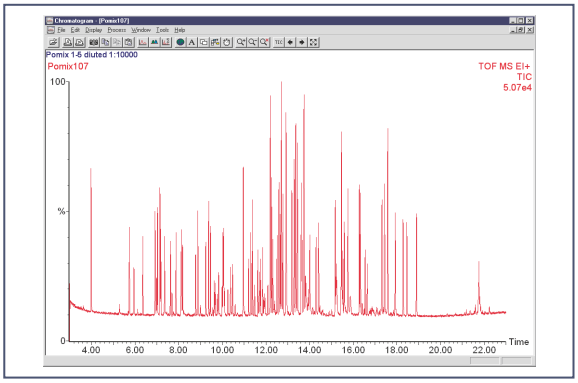

Figure 4. TIC of Fragrance Mixture

- **Figure 5** shows the GCLynx browser chromatogram trace in the region of interest. Also included is the peak list and associated library search result. All 17 compounds were identified as the number 1 library hit. **Figure 6** is a summary table of the results including forward and reverse fit factors.

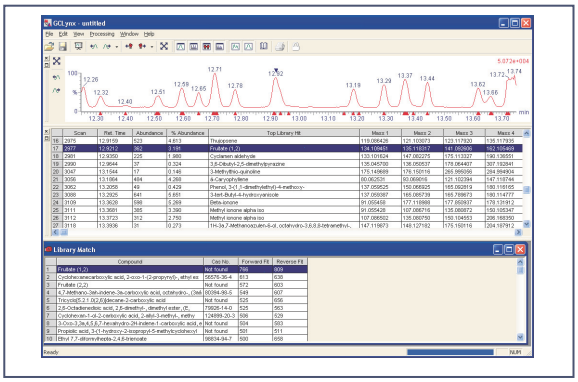

Figure 5. 'Zoomed' TIC of Fragrance Mixture

RT	Compound	Hit No	Forward Fit	Reverse Fit
12.32	coumarin	1	867	892
12.51	Jasmacyclene(1,2) and (2,2,)	1	726	778
12.59	Allyl cyclohexyl propionate	1	793	828
12.6	Floralozone (1,2)	1	790	863
12.65	alpha-ionone	1	874	885
12.7	alpha-Cedrene	1	813	828
12.72	Floralozone (2,2)	1	744	781
12.78	beta-Caryophyllene	1	878	903
12.91	Thujopsene	1	743	784
12.92	Fruitate (1,2)	1	766	809
12.94	Cyclamen aldehyde	1	722	759
13.19	Humulene	1	881	910
13.3	Butyl hydroxy anisole	1	903	919
13.34	Beta Ionone	1	814	832
13.37	Methyl Ionone alpha iso	1	725	771
13.44	Fruitate (2,2)	1	908	923
13.62	Methyl Ionone beat iso	1	848	872

Figure 6. Library Hit Summary

DECONVOLUTION

- Close examination of the results show that the chromatographic response at RT 12.92, appearing as a single component in the TIC, is in fact a triplet. The M+ mass chromatograms of each of the components are shown in **Figure 7**.

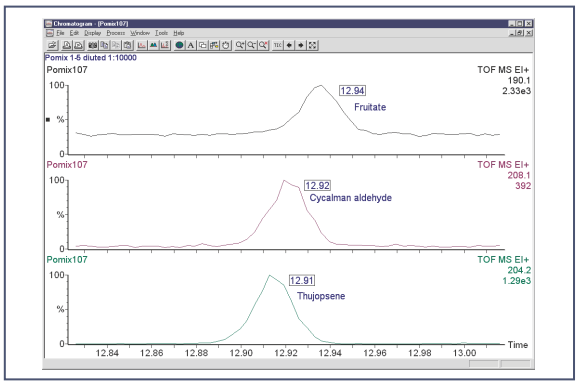

Figure 7. Mass Chromatograms of Individual Components

- These components are known to be, in retention time order, thujopsene, fruitate (1,2) and cyclaman aldehyde. Given the close proximity of elution it is difficult by 'manual' manipulation of the data to identify the presence of 3 components. It is equally difficult to produce 'pure' spectra. **Figure 8** shows the GCLynx browser output for the refined spectrum of each versus the library entry. Clearly satisfactory fits have been obtained. **Figure 9** shows exact mass chromatogram outputs for the four most intense masses for fruitate.

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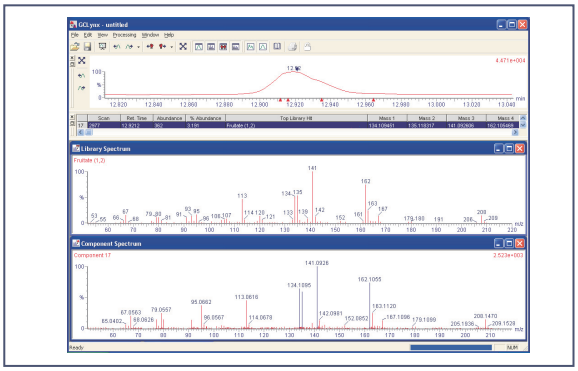

Figure 8a. GCLynx Browser Output Library Component Spectrum Versus Library Spectrum

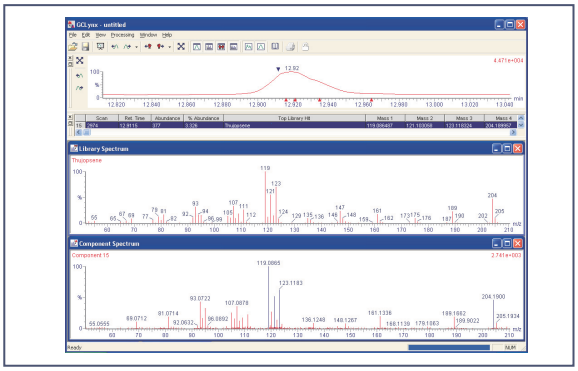

Figure 8b. GCLynx Browser Output Library Component Spectrum Versus Library Spectrum

Figure 8c. GCLynx Browser Output Library Component Spectrum Versus Library Spectrum

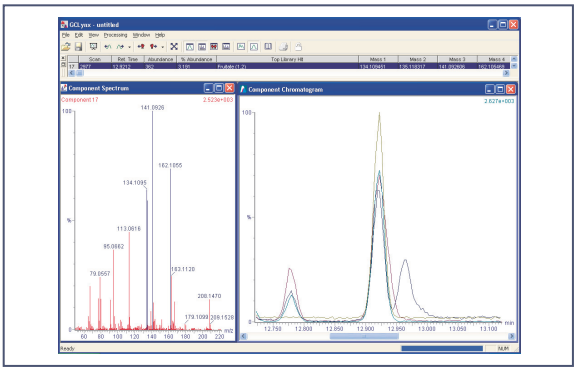

Figure 9. Mass Chromatograms for Fruitate

- In the case of metabolomic studies many extracts may not be well characterised. In these circumstances it is essential to have some form of confirmation of library hit data for instance. On many occasions this can be performed by examining the elemental composition data afforded by exact mass measurement. This obviously is also critical in the interpretation of completely unknown spectra. **Figure 10** shows a table comparing the elemental composition of the molecular ion for the proposed library structure, for all 17 compounds, with that of the calculated elemental composition from GCT exact mass measurement.

RT	Compound	Hit No	Elecomp	Calc Mass	Measured Mass	Error mDa
12.32	coumarin	1	C9H6O2	146.0368	146.0359	0.9
12.51	Jasmacyclene(1,2) and (2,2,)	1	C12H16O2	192.1150	192.1162	1.2
12.59	Allyl cyclohexyl propionate	1	C12H20O2	167.1072	167.1084(1)	1.2
12.60	Floralozone (1,2)	1	C13H18O	172.1252	172.1279(2)	2.7
12.65	alpha-ionone	1	C13H20O	192.1514	192.1524	1.0
12.70	alpha-Cedrene	1	C15H24	204.1878	204.1887	0.1
12.72	Floralozone (2,2)	1	C13H18O	190.1358	190.1346	1.2
12.78	beta-Caryophyllene	1	C15H24	204.1878	204.1888	1
12.91	Thujopsene	1	C15H24	204.1878	204.1900	1.2
12.92	Fruitate (1,2)	1	C13H20O2	208.1463	208.1470	0.7
12.94	Cyclamen aldehyde	1	C13H18O	190.1358	190.1366	0.8
13.19	Humulene	1	C15H24	204.1878	204.1887	0.9
13.30	Butyl hydroxy anisole	1	C11H16O2	180.115	180.1148	0.2
13.34	Beta Ionone	1	C13H20O	192.1514	192.1525	1.1
13.37	Methyl Ionone alpha iso	1	C14H22O	206.1671	206.1684	1.3
13.44	Fruitate (2,2)	1	C13H20O2	208.1463	208.1481	1.8
13.62	Methyl Ionone beta iso	1	C14H22O	2006.1671	206.1681	1.0
(1) Fragment ion -C2H5						
(2) Fragment ion -H2O						

Figure 10. Exact Mass Confirmation Table

CONCLUSION

- Automated peak detection and deconvolution are required to accurately profile complex chromatograms.
- The developmental GCLynx software package reliably and automatically detects chromatographic peaks.
- It is essential to perform deconvolution in complex mixture analysis in order to fully profile the extract.
- GCLynx developmental software package is able to accurately produce pure spectra from unresolved closely eluting peaks.
  Exact mass measurement aids in the confirmation of identity of components in a complex matrix.

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