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# UNAMBIGUOUS CHARACTERISATION OF SYNTHETIC PATHWAYS BY EXACT MASS GC-MS ANALYSIS USING A GCT MASS SPECTROMETER


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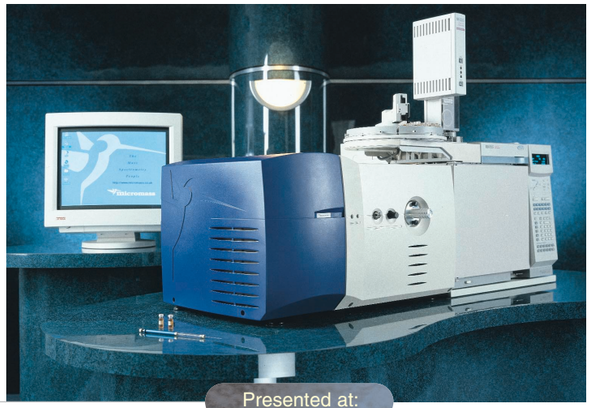

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# Overview

## PURPOSE

- Characterisation of synthetic pathways by exact mass and elemental composition determination with EI/CI/FI.
- Differentiation of compound sources/manufacture by exact mass GC-MS profiling.

## METHOD

- Orthogonal Time of Flight mass spectrometer.
- Exact mass using single point internal reference correction.

## RESULTS

- Unambiguous identification of the synthetic pathway for the effect compound Speedcure™.
- Characterisation of FAME extracts from differing batches.

## Introduction

- The identification of minor components in synthetic products has important legal and financial implications in many areas of commercial and forensic chemistry.
- These include the profiling of manufactured products in order to protect unique synthetic pathways and the determination of trace impurities in illicit drugs of abuse in order to correlate seizures with the site of production.
- In this paper two particular applications of a GCT in industrial and forensic analysis are used to exemplify the benefits of full spectra exact mass measurement.

## Instrumentation

- The orthogonal acceleration time-of-flight mass spectrometer allows fast acquisition of full spectra, with high sensitivity and elevated resolution (7000FWHM) **Figure 2**. This elevated resolution reduces mass interference's. Furthermore the precise, and stable relationship between ion arrival time and the square root of its mass allows good measurement accuracy with only a single internal reference mass.

**Figure 1** shows a schematic of the GCT system.

- Ions produced in the grounded ion source are accelerated to 40eV and focused into a parallel ion beam. As the ions 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 a host PC. The instrument is fitted with a heated GC transfer line, heated reference inlet for the introduction of volatile calibration compounds and supports direct insertion probe operation.

GCT instrument schematic

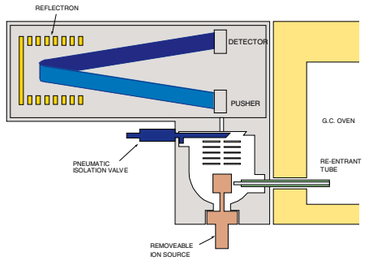



Figure 1

GCT: Doublet from a mixture of PFTBA and $C_4Cl_6$

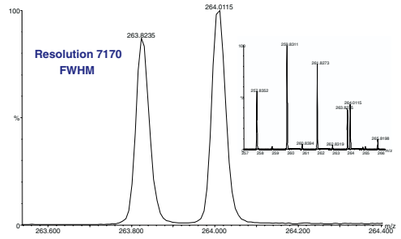



Figure 2

## Chromatographic conditions

- Analysis of Speedcure™:
  GC-EI/CI:Rtx-5-MS 30M x 0.25mm ID x 0.25µM Injection: Splitless.
  Oven: 50/0.8 -250@10

  GC-FI: HP1 30M x 0.32mm ID x 0.25µM Injection: Splitless.
  Oven: 50/0.8 -250@10
- Profiling of fatty acid methy esters.
  Rtx-5-MS 30M x 0.25mm ID x 0.25µM Injection: Splitless.
  Oven: 50/0.8 -275@25

## Characterisation of Synthetic Pathway

Speedcure™ is one of a range of polymer curing agents. Chemically it is ethyl 4-N,N-dimethylaminobenzoate. Its industrial synthesis via a reductive formylation route is subject to patent protection. An alternative common route for the synthesis of this compound is direct alkylation.

The reaction pathway for the reductive formylation route is shown in **Figure 3**.

Unique co-products, formed in minute but sufficient yield, allow the actual pathway to be identified.

Reductive Formylation Synthesis

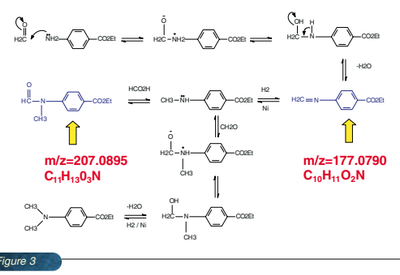



Figure 3

Speedcure™ was analysed by exact mass GC-MS in EI+, CI+ and FI (Field ionisation) modes. Characteristic reaction intermediates were then identified.

**Figure 4** shows the EI + GC-MS TIC produced.

**Figures 5 and 6** show the EI+ spectra for the components at RT 9.15 and 11.9 mins with exact mass measurement, empirical formula and proposed structure.

**Table 1** shows the statistical results from 5 repeat exact mass measurements for the two components highlighted.

**Figures 7 and 8** show the complimentary exact mass molecular ion information produced by exact mass FI and Methane CI+.

GCT: EI + GC-MS TIC produced

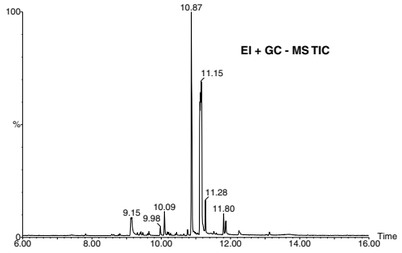



Figure 4

## Discussion

Two characteristic trace components were identified and their elemental composition determined by exact mass measurement. Complimentary exact mass CI and FI data confirm the molecular ion assignment.

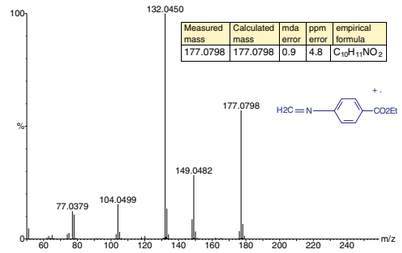
The two components identified at m/z 177.0794 and 207.0901 are unique to the reductive formylation route described in **Figure 3**, unambiguously identifying the synthetic route as reductive formylation.

The pathway for the direct alkylation route is shown in **Figure 9** for comparison. No unique by-products associated with this reaction were detected.
In the field of forensic analysis of drugs of abuse materials are often seized as tablets

## Exact mass GC MS profiling

which incorporate residues of processing reagents such as magnesium stearate. It is routine procedure to isolate the free fatty acids from a small portion of a tablet and convert to the corresponding methyl esters. As the stearate is obtained from natural sources in batch processes, the MS profile of this FAME extract can be used to correlate seizures of tablets to site(s) of production.

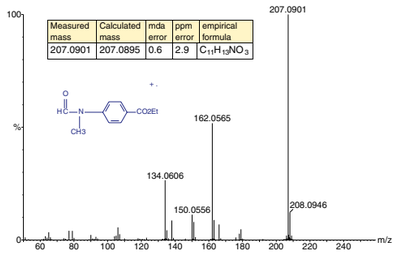
GCT: EI + GC-MS Exact mass spectrum RT 9.15 min




| Measured mass | Calculated mass | mda error | ppm error | empirical formula |
|---|---|---|---|---|
| 177.0798 | 177.0798 | 0.9 | 4.8 | $C_{10}H_{11}NO_2$ |

Figure 5

GCT: EI + GC-MS Exact mass spectrum RT 11.9 min




| Measured mass | Calculated mass | mda error | ppm error | empirical formula |
|---|---|---|---|---|
| 207.0901 | 207.0895 | 0.6 | 2.9 | $C_{11}H_{13}NO_3$ |

Figure 6

| Measurement number | Measured mass | Calculated mass | error mda | error ppm |
|---|---|---|---|---|
| 1 | 177.0798 | 177.0790 | 0.9 | 4.8 |
| 2 | 177.0794 | 177.0790 | 0.4 | 2.2 |
| 3 | 177.0799 | 177.0790 | 0.9 | 5.3 |
| 4 | 177.0801 | 177.0790 | 1.1 | 6.2 |
| 5 | 177.0802 | 177.0790 | 1.3 | 7.1 |

**RMS error ppm= 5.4 ppm**

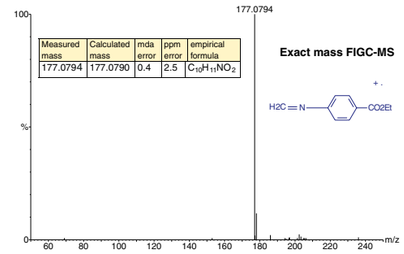
| Measurement number | Measured mass | Calculated mass | error mda | error ppm |
|---|---|---|---|---|
| 1 | 207.0901 | 207.0895 | 0.6 | 2.9 |
| 2 | 207.0922 | 207.0895 | 1.0 | 5.1 |
| 3 | 207.0901 | 207.0895 | 0.6 | 2.9 |
| 4 | 207.0895 | 207.0895 | 1 | 4.6 |
| 5 | 207.0904 | 207.0895 | 0.8 | 4.0 |

**RMS error ppm= 4.0 ppm**

Table 1

**EI+GC-MS Exact mass measurement results from five repeat injections of Speedcure™**

GCT exact mass FI GC MS peak at RT=9.15 min

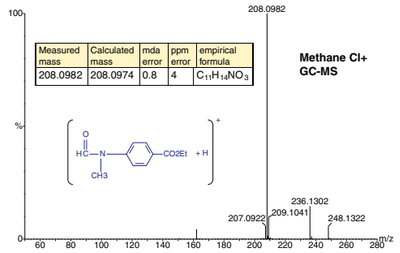



| Measured mass | Calculated mass | mda error | ppm error | empirical formula |
|---|---|---|---|---|
| 177.0794 | 177.0790 | 0.4 | 2.5 | $C_{10}H_{11}NO_2$ |

Figure 7

Two batches of Magnesium Stearate (**X** and **Y**) from differing sources were analysed using the GCT. The sample was first treated with 1M HCl, partitioned into hexane, and after drying esterified using $BF_3$ in the presence of Methanol.

GCT: Exact mass Methane CI+GC MS peak at RT=11.9 min




| Measured mass | Calculated mass | mda error | ppm error | empirical formula |
|---|---|---|---|---|
| 208.0982 | 208.0974 | 0.8 | 4 | $C_{11}H_{14}NO_3$ |

Figure 8

Direct Alkylation synthesis

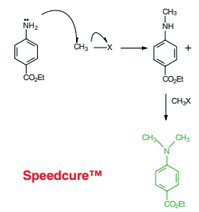



Figure 9

## Results

**Figures 10a and b** show a comparison of the TIC's from both batches.

**Figure 11** shows the library search and elemental composition data for one of the fatty acid methy esters identified.

**Figure 12** shows the the library search and elemental composition data for one of the dioates characteristic of batch **X**).

**Figure 13** Shows accurate mass chromatograms (10mda window) of a characteristic ion determined for the dioates in **X** and **Y**.

**Figure 14** shows the nominal mass (0.5da window) chromatograms of a characteristic ion determined for the dioates in **X** and **Y**.

EI+GC-MS TIC for Magnesium Stearate batch X and Y.

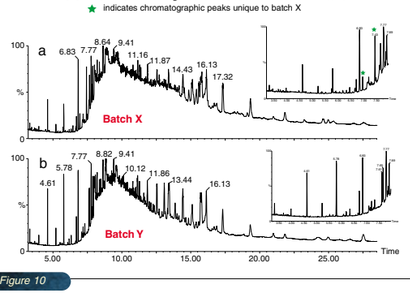
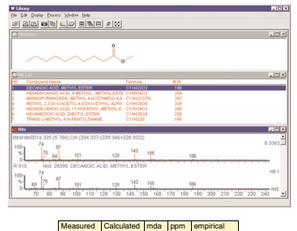



Figure 10

Library search and exact mass for peak at RT=5.78 min



| Measured mass | Calculated mass | mda error | ppm error | empirical formula |
|---|---|---|---|---|
| 186.1630 | 186.1620 | 1.0 | 5.4 | $C_{11}H_{20}O_2$ |

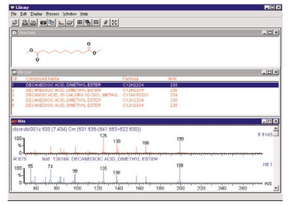
Figure 11

## Discussion

As expected many of the fatty acid methy esters found were common to both batch **X** and **Y**. However only sample **X** was found to contain a series of dioates. Library search data was compared with the exact mass measurements obtained to confirm the assignment. The ion at m/z 124.0888 corresponding to the fragment $C_8H_{12}O$, was found to be unique to these dioates.

Exact mass chromatogram, 10mda window (**Figure 13**) offer a very high degree of selectivity compared to a nominal mass chromatogram, 500mda window (**Figure 14**). In this example the selectivity is equivalent to a magnetic sector mass spectrometer, in SIR mode, at 12,400 resolution 10% valley definition.
The combination of the selectivity and specificity of exact mass and full library search using the GCT provides a clear and unambiguous differentiation of these two batches of sample.

Library search and exact mass for peak at RT=7.43 min



| Measured mass | Calculated mass | mda error | ppm error | empirical formula $[M+H_2O]^+$ |
|---|---|---|---|---|
| 199.1341 | 199.1334 | 0.7 | 3.5 | $C_{11}H_{19}O_3$ |

Figure 12

Exact mass chromatograms for m/z 124.0888 10mda window ($C_8H_{12}O$), batch X and Y

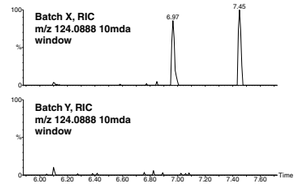



Figure 13

Nominal mass chromatograms for m/z 124.0888 0.5da window ($C_8H_{12}O$), batch X and Y

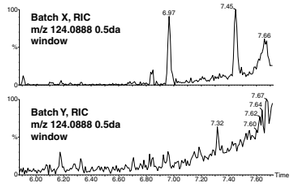



Figure 14

# Conclusion

- Exact mass measurement in conjunction with full scan library search provides unambiguous identification of trace components.
- Complimentary exact mass information from the soft ionisation techniques FI and CI allow identification of molecular ions which may be weak or absent in EI spectra.
- Exact mass chromatograms afford a very high degree of selectivity, improved signal to noise ratios, and lower detection limits
- Exact mass measurement using GCT-oaTof MS allows unambiguous and cost effective characterisation of synthetic pathways.

MMP/83/LAG/V1.00