Chromatography Technical Note No AS136

The Automated Derivatisation and Extraction of Fatty Acids using the Gerstel MPS Autosampler and Agilent 7890 / 7200 GC/Q-TOF

Sean O'Connor and Nathan Hawkins, Anatune Ltd. Girton, Cambridgeshire (UK).

Introduction

Precise extraction and measurement is essential for any method used in metabolomics as significant metabolic effects can be observed as small chemical changes which can go unnoticed if the data collected is variable. The measurement of fatty acids is a well-established metabolomic profiling technique. The simplest method for looking at these profiles is to derivatise the sample of interest, trans-esterifying lipids to form the methyl esters of the fatty acids, with a subsequent liquid-liquid extraction (LLE) clean-up step.

Utilising the Dual Head Gerstel MultiPurpose Sampler (MPS) sample preparation system with a heated agitator which was integrated with an Agilent 7200 quadrupole time of flight mass spectrometer (Q-TOF) and a 7890B Gas Chromatography system, (Figure 1) it was possible to automate this derivatisation and extraction. The advantages of this was that all of the samples were prepared in exactly the same way every time and using the prep-ahead feature the extracts were injected almost instantly after preparation.

Figure 1: Agilent GC/Q-TOF system with a Gerstel MPS System

Large volume injection (LVI) is a technique which enables the injection of larger aliquots of sample onto a GC without exceeding the liner capacity and avoiding sample loss. This technique uses the Gerstel cooled injection system (CIS) to maintain a low temperature allowing solvent venting. The sample is injected slowly, at a rate which allows the solvent to be vented marginally faster than it is introduced to the GC inlet maintaining a temperature which focuses the analytes of interest in the liner. Once the injection is completed the temperature of the CIS is rapidly increased and the analytes are transferred to the column.

Instrumentation

Agilent GC 7890B and Agilent 7200 Q-TOF
Gerstel Dual Head MPS 2 XL-*xt*
Gerstel CIS4,
Gerstel TDU
Gerstel Heated Agitator
Agilent MassHunter software (version B07.00)
Maestro software integrated (version 1.4.18.25/3.5)

Method

An aliquot of approximately 5-6 mg of the freeze dried oregano was added to a 2 mL vial. These vials were place on the vial tray of the MPS system and the following steps were automated. Utilising the larger syringe (1 mL) of the right hand head of the MPS system, an aliquot of 500 µL of methanolic hydrochloric acid was added to the vial and the vial was placed in the heated agitator for 15 minutes, agitated at 500 rpm and 70 °C. This allowed the methylation reaction to complete. Once cooled, 500 µL of hexane and 500 µL water were added to the vial. A 10 µL aliquot from the top layer, which contained the FAME's, was taken using the smaller syringe (10 µL) on the second head and was injected on to the GC/Q-TOF using LVI.

Results

The extractions were automated successfully in 2 mL vial (Figure 2).

Figure 2: Extraction mixture with the FAME's in the top hexane layer

Injecting the upper layer into the GC/Q-TOF gives a total ion chromatogram (TIC) (Figure 3) which not only contains the fatty acid methyl esters but also the hexane soluble components from the original oregano. As such, this method may prove useful as the basis of a non-polar metabolic fingerprinting method. The base ion for the fatty acid methyl esters is formed by a McLafferty re-arrangement of the ester group. By extracting the exact mass of this ion (m/z 74.0366) with a 20 ppm extraction window (Figure 4), it is possible to rapidly screen for the fatty acids present in this complex sample extract.

Anatune Ltd, Unit 4, Wellbrook Court, Girton Road, Cambridge, CB3 0NA, UK
Tel: +44 (0) 1223279210 Fax: +44 (0) 1223279253 Email: info@anatune.co.uk Internet: www.anatune.co.uk

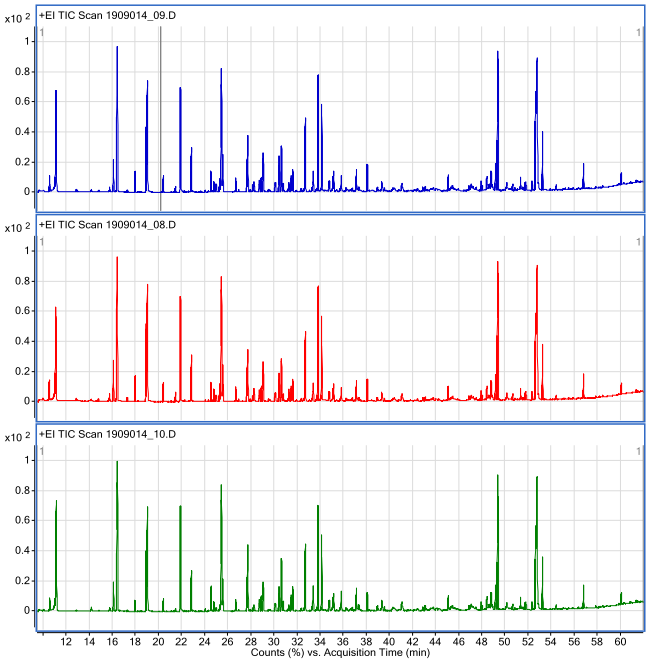

Figure 3: Total ion chromatogram of the hexane layer from the three extracts.

.

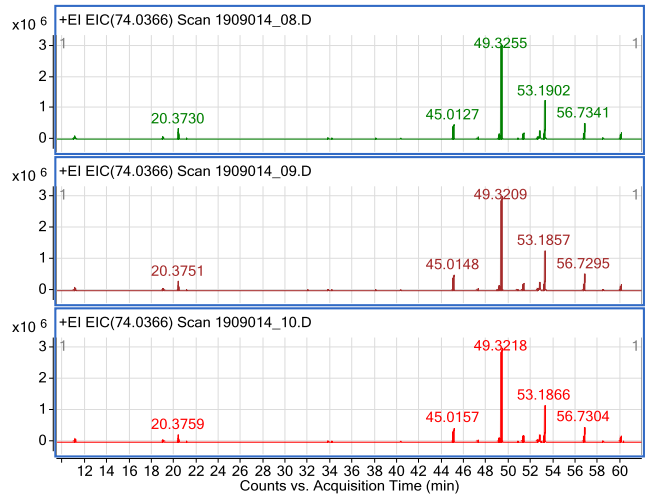

Figure 4: Extracted Ion Chromatogram of m/z = 74.0377 (McClafferty re-arrangement of the ester grouping)

Integration of the base ion for each of the methyl esters shows that over the three extractions gave a mean %cv was 8.2% for the fatty acids present at 1 % or more of the most abundant FAME, methyl hexadecanoate (retention time - ~49.32). Table 1 shows the %CV for each of the straight chain fatty acid methyl esters present

Table 1: %CV of C14 to C22 Straight chain fatty acid methyl esters

FAME	%CV
Methyl tertradecanoate	6.9
Methyl hexadecanoate	4.7
Methyl octadecanoate	6.2
Methyl eicosanoate	5.8
Methyl docosanoate	3.9

Identification of the individual fatty acid methyl esters was confirmed by comparison of the peak spectra against the NIST library.

Discussion

Overall the use of automated sample preparation with a Gerstel Dual head MPS system and analysis by GC/Q-TOF provides a number of benefits including reduction of solvent use, reduced labour and time taken per extraction. Both the derivatisation and extraction are reproducible ensuring that data obtained can be used for fingerprinting or comparative metabolomics studies. Using the Q-TOF not only allows specific target ion extraction for the methyl esters but also enables confirmation of analyte identities from the full scan, high resolution, accurate mass spectra. This feature can also be used for the structural elucidation of unknown or novel metabolites. Q-TOF data also has the added advantage of being able to be searched retrospectively for different non-target analytes, as all of the ions which are formed are collected.

The Gerstel system and Agilent GC/Q-TOF could be used for a number of derivatisation and extraction steps especially when combined with an *m*Vorx vortexer and centrifuge. Although the data is not shown here both the Folch extraction and Fiehn derivatization protocol have been automated in a similar manner to the work carried out in this application note.

Anatune Ltd, Unit 4, Wellbrook Court, Girton Road, Cambridge, CB3 0NA, UK
Tel: +44 (0) 1223279210 Fax: +44 (0) 1223279253 Email: info@anatune.co.uk Internet: www.anatune.co.uk