An Introduction into the Role of Gas Chromatography - Mass Spectrometry (GC-MS) in Metabolomic Analysis

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While metabolomics would not intuitively be associated with volatile compounds, gas chromatography coupled to mass spectrometry (GC-MS) nonetheless has many merits which render it extensively useful in the field of metabolomic profiling. This is illustrated by a literature search for metabolomics or metabonomics, which returns a vast number of publications detailing the successful use of GC-MS to profile a wide variety of samples. These include applications ranging from the food industry and agriculture, to pharmaceutical and disease biomarker discovery applications [1-4].

As a separation method GC is renowned for its high reproducibility and powerful resolving capability. It is commonly coupled to MS analysers such as the triple quadrupole (QqQ), which can be used in both qualitative and quantitative analysis, and the time-of-flight (TOF) instrument which can determine accurate mass to 4 decimal places. Compounds eluting from a GC column are directly compatible with mass spectrometry as they are already in the gas phase and therefore can easily be ionised by an electron impact source or by chemical ionisation. Fixed electron voltages are often used to reproducibly ionise and fragment molecules, and therefore databases can be constructed which record mass spectra and also retention time to give highly accurate 2D databases for compounds which can be shared across analytical platforms. The early adoption of GC-MS as a tool for metabolomics has spurred the creation of a variety of such databases which span toxicology, forensic, and biological applications. Amongst the libraries which record retention times is FeihnLib [5], which is recognised as a leading metabolite library for GC-MS. Metabolites in this library are recorded using a retention time locking (RTL) method which includes the use of a specific column and fragmentation energy to align the compounds under slightly differing environments. This allows the GC-MS user to repeat the method on an RTL calibrated instrument and gain the same results, therefore allowing them to match unknown compounds in the library [6, 7].

Non-targeted metabolomic studies, which aim to profile the 'fingerprint' of an organism's metabolome, involve detection and often quantitation of the maximum possible number of compounds from a sample in order to compare sets of samples and gain useful biological insight [8]. In this respect detection of non-volatile compounds is limited in GC analysis unless these molecules are first derivatised to increase their volatility. Fortunately a range of selective and non-selective derivatising reagents can be employed to modify compound volatility, improve stability and also improve chromatographic behaviour such as resolution and peak tailing. Commonly derivatives are prepared by acylation, alkylation or silylation of certain functional groups [9, 10]. When applied to the analysis of bio-fluids a preliminary drying step must be performed to remove water, which would otherwise react with the derivatising reagents, effectively quenching the reaction. Derivatisation procedures for GC-MS can be performed in an aqueous environment however, with the suitable selection of reagents. Specific examples include preparation of N, S-ethoxycarbonyl methyl ester and N(S)-isopropoxycarbonyl methyl ester derivatives of sulphur amino acids which are prepared in the native aqueous environment associated with biological samples [2, 11]. Modern derivatising kits can be purchased which are highly efficient, reproducible and relatively simple to use. Furthermore, the use of reagents which produce trimethylsilyl (TMS) derivatives is advantageous when performing database searching, as vast libraries of TMS derivatives have been generated to aid identification of metabolites.

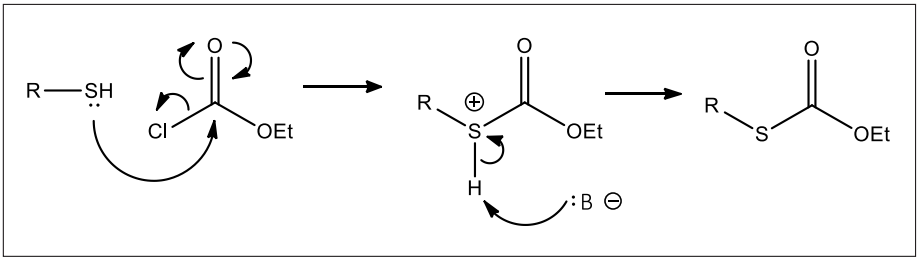

Figure 1: Derivatisation of thiols by ethylchloroformate

[2] Capitan, P., et al., Gas chromatographic–mass spectrometric analysis of stable isotopes of cysteine and glutathione in biological samples. Journal of Chromatography B: Biomedical Sciences and Applications, 1999. 732(1): p. 127-135

Following the identification of significant metabolites, it is common that quantitation of these compounds should be performed. Data collected during non-targeted studies is generally not appropriate for quantitation due to the limited or non-existing use of internal standards. Although semi-quantitative results can be extracted using a single standard if selected carefully to represent a class of compounds. This lack of full quantitation is due to the nature of the data collection and extraction in non-targeted analysis. Normally the MS analyser would be set to scan the m/z range in order to detect as many compounds as

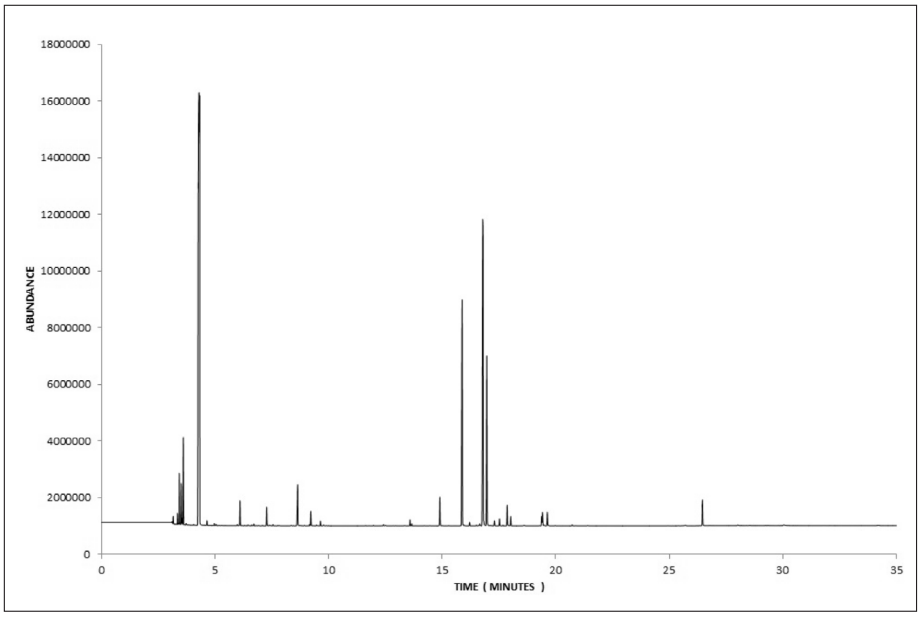

Figure 2: GCQMS chromatogram of MSTFA derivatised control serum.

GC Capillary Column : Agilent DB-5ms, 30 m, 0.25 mm, 0.25 µm, DuraGuard, 10m

possible, and from the total ion current (TIC) specific compounds can be extracted from the signal by deconvolution. The problem with this approach is that the deconvoluted signals are not a true response, but rather a mathematical product which can be affected by varying the parameters [4]. Additionally, sensitivity is reduced when MS scanning mode is used, compared to selectively setting the instrument to specific m/z values of interest, which allows more of these ions to reach the detector in the same given time. The use of QqQ detectors is well suited to this purpose as they can selectively exclude ions both before and after fragmentation to ensure the ions reaching the detector are from the expected precursor molecule. This type of tandem MS experiment is referred to as selected / multiple reaction monitoring (S/MRM), and vastly improves selectivity and sensitivity, both of which are critical in the analysis of metabolites in complicated matrixes [12].

To collect high quality quantitative data it is common practice to use an internal standard which can take into account sample preparation and injection errors. The use of internal standards in GC-MS greatly improves standard deviations, where the usual RSD is <5%, compared to external standard calibration which can be up to 20% [13]. Ionisation of molecules in the source of a mass spectrometer can be variable and also be influenced by other co-eluting compounds which can suppress the ionisation of the former. As a result, for quantitative analysis this effect should be considered when choosing an internal standard. The ideal internal standard for GC-MS mimics the compound of interest as closely as possible and for this purpose

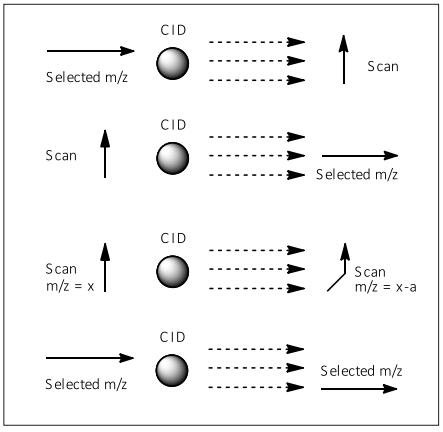

Figure 3: Different MSn modes

isotopically labelled analogues of the compound being analysed are best suited. The degree of deuteration must be sufficient to allow the deuterated molecular ions to be resolved from those of the natural isotope. Assuming the acquisition of isotopically labelled internal standards is feasible and affordable, accurate quantitation can be performed at very low levels in complex biological samples.

Despite some of the drawbacks of GC-MS such as increased sample preparation for polar molecules, the advantages of this technique in terms of reproducibility, sensitivity and compatibility with comprehensive libraries justify its continued use in metabolomic profiling. Development of robust derivatisation protocols for non-volatile molecules extends the reach of this technique to a greater variety of metabolites and provides overlap with other platforms like liquid chromatography. This allows the use of instruments working in conjunction to fully map the profile of an organism or sample by analysis of metabolites with wide-ranging physiochemical properties [8].

References

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