COLLEGE OF SCIENCE
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Mass Spectrometric Analysis of Biologically Relevant Compounds Hyphenated by Gas And High Performance Liquid Chromatography

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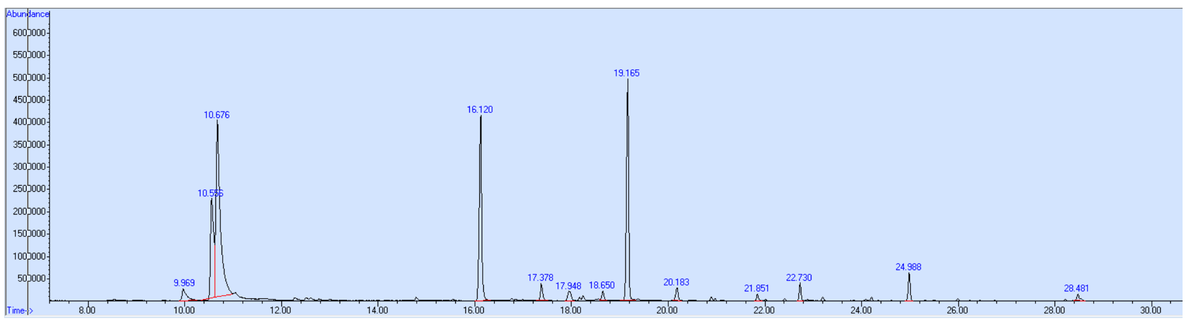
Overview

When dealing with biological samples scientist are typically faced with complex mixtures. Currently there are estimated to be 40,000 identified metabolites. Yet, a vast majority of unknown metabolites still exist. One solution to this is chromatography. Chromatography can be implemented as Gas Chromatography or as Liquid Chromatography. For detection purposes we use Mass Spectrometry (MS). The advantage of MS is that the technique can be applied to extremely small sample sizes (*μg* and below*).* This allows to detect a range of compounds from complex biological mixtures. Using GC-MS the emphasis is separation based on volatility and identification of volatile compounds through their retention times (GC) and fragment mass spectra (EI-MS). HPLC allows to interface with a high resolution mass spectrometer. Compounds get separated based on polarity. In ESI-MS a high resolution spectrum allows identification of overlapping peaks. Subsequent MS-MS spectrometry allows to identify individual components.



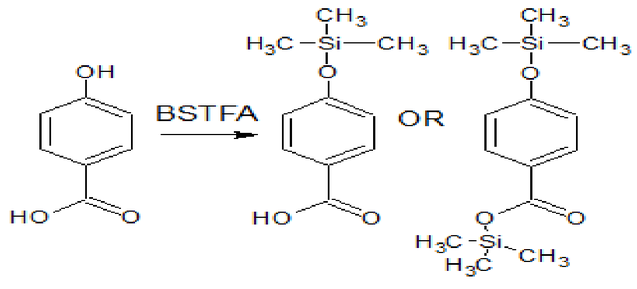
(Figure 1) This GC-MS TIC or total ion current, successfully separates the mixture into individual components. With retention times labeled in blue above each peak which help to verify correctness when identifying the compounds.

Experimental

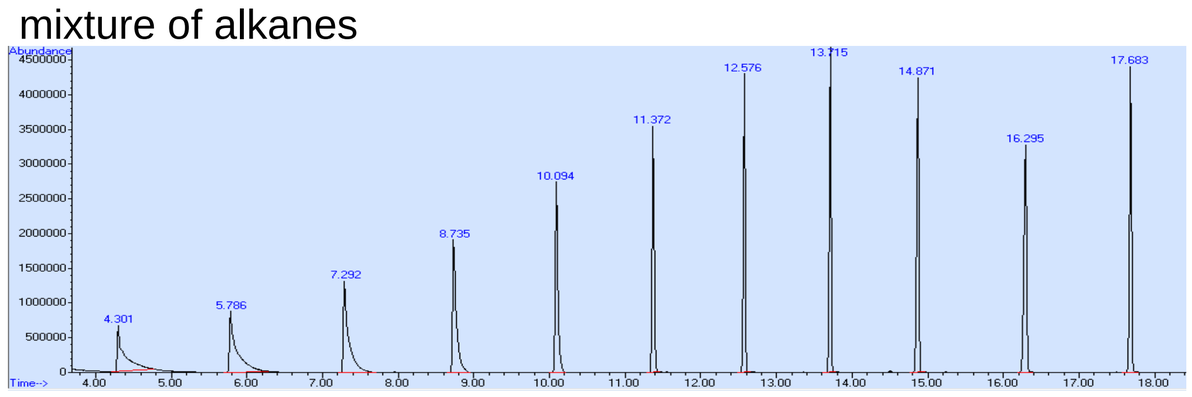
GC-MS

When conducting runs for the GC-MS the sample of metabolites was ran over a linear temperature gradient, 80° C to 325° C, and for a total time of 25 minutes. The pressure was 9.4 psi for the entire run, and the injection volume into the GC was 1 μL. Also the selected mass range was 50 to 450 mass units, where the resolution was 1 amu. When using GC some polar mixtures are not volatile enough due to their polarity. To fix this problem, a derivatization reaction is conducted where the biological mixture is mixed with BSTFA to change the polarity. This allows the mixture to change more easily from liquid to gas phase. A test mixture of known metabolites was derivatized to test the protocol and also to establish a database of expected spectra that allow identification using the AMDIS software from NIST.

(Figure 2) BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) reaction shows the how trimethylsilyl (TMS) groups replace the hydrogen on alcohols, amino acids and amines.



Retention times in GC were established relative to a standard mixture of alkanes

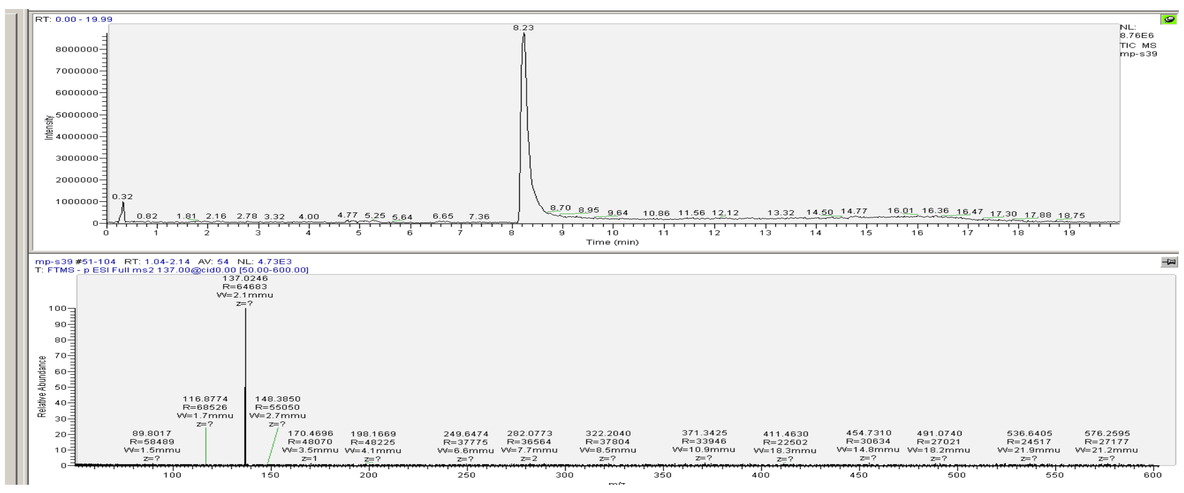


RT	RI	Identification
4.2	800	Octane
4.302	1000	Decane
5.787	1100	Undecane
7.294	1200	Dodecane
8.736	1300	Tridecane
10.095	1400	Tetradecane
11.372	1500	Pentadecane
12.577	1600	Hexadecane
13.714	1700	Heptadecane
14.87	1800	Octadecane
16.294	1900	nonadecane
17.862	2000	eicosane

(Figure 3) This shows the elution of a hydrocarbon mixture from C8 to C20. These retention times allow for a calculation of retention indicies. These values allow for a two step check to verify the Identification of a component in the biological mixture.

LC-MS

Due to the nature of the compounds pH is critical when running HPLC-MS tests. This ensures that peak splitting does not occur, instead one peak is found giving an accurate retention time. For UV-detection each sample was ran with a pH 6 phosphate buffer 20 mM and methanol as solvents. Each solution was held at 25° C and the ratio of buffer to methanol changed from 100% buffer to 15% buffer.



(Figure 4) LC-MS run of 1μg/mL Hydroxybenzoic acid

Key Findings

GC-MS

The collected mass spectra revealed the success of the derivatization by showing the [M+] plus either 1,2 or 3 TMS groups and their respective RI, allowing for the successful identification of biological compounds.

LC-MS

HPLC conditions for LCMS runs were established, retention times recorded and samples successfully detected at below μg/mL levels.

Acknowledgements

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