Ion Mobility Orthogonal Time-of-Flight Mass Spectrometry (IMS–oaTOF-MS) for the analysis of small molecules and complex mixtures using an Atmospheric Solids Analysis Probe.

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OVERVIEW

PURPOSE- Investigate utility of IMS-MS in the characterisation of small molecules and complex mixtures.

METHODS- ASAP-IMS-MS using a Synapt HDMS Mass Spectrometer. IMS-MS data processed using 3D peak detection algorithm.

RESULTS-Separation of positional isomers demonstrated and structurally driven fragmentation pathways illustrated.

ASAP-IMS-MS characterisation of crude oil demonstrating IMS separation of homologous series.

Collision cross sections of a variety of small molecules shown.

INTRODUCTION

The ability of Ion Mobility Spectrometry (IMS) to separate ions based on their collision cross sectional area and charge state provides a powerful orthogonal separation technique when coupled with Mass Spectrometry. The Atmospheric Solids Analysis Probe (ASAP)[1] technique allows rapid, direct analysis of volatile and semi volatile, compounds of low polarity. In this paper we investigate the IMS characteristics of a variety of small molecules and complex mixtures introduced using the ASAP technique.

METHOD

All analyses were performed using a Waters Synapt HDMS system, figure 1. The ion source region was modified, figure 2, to facilitate ASAP. IMS-MS data was post-processed using a 3 dimensional peak detection algorithm 'APEX 3D' to determine m/z, drift time (DT) and intensity.

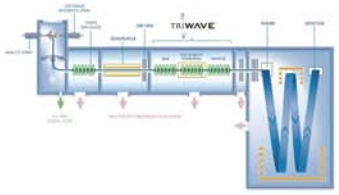

Figure 1. Schematic diagram of the Synapt HDMS system.

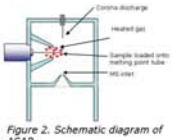

Figure 2. Schematic diagram of ASAP.

During ASAP analysis, sample was loaded onto the tip of a glass melting point tube. The tube was placed in the flow of heated gas. Sample was vaporised by controlling the temperature of the gas and ionised by proximity to the corona discharge needle. Ions then pass from the atmospheric pressure region into the mass spectrometer.

COLLISION CROSS SECTION

Figure 3 shows a map of m/z vs collision cross section (CCS) for a selection of small molecules ionised using ASAP. The ion mobility device was calibrated against literature values [2] using peptides from a typtic digest of yeast enolase in ESI mode.

This plot indicates the range of compound types which may be analyzed using the ASAP technique and the ability of the Synapt HDMS system to separate these relatively small molecules based on structure.

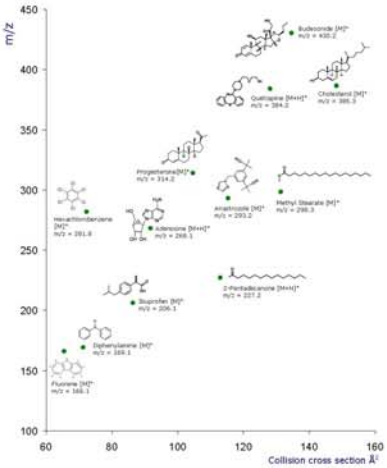

Figure 3. m/z vs collision cross section for a variety of small molecules introduced using ASAP .

SEPARATION OF ISOMERS

A mixture of 1,2 and 1,4 dinitrobenzene (Sigma Aldrich) was analysed. The $[M+H]^+$ (m/z 169) were selected using the quadrupole and subsequently separated by mobility. Post IMS CID (18eV) was performed in the transfer device and the resulting m/z vs DT plot is shown in **Figure 4**. The centroid location of each DT-m/z peak derived using APEX 3D is shown. The two distinct vertical bands of fragment ions at drift times of 4.6ms and 5.3ms correspond to fragments from the 1,2, and 1,4 substituted isomers respectively.

Figure 5 shows extracted CID spectra at these two drift times.

Figure 6 Shows mass chromatograms of the fragment ions at m/z=139.03 characteristic of 1,4 dinitrobenzene and at m/z=77.04 characteristic of 1,2 dinitrobenzene. The fragment ion at m/z=81.04 was found to be common to both isomers.

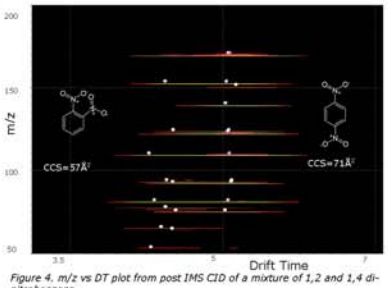

Figure 4. m/z vs DT plot from post IMS CID of a mixture of 1,2 and 1,4 dinitrobenzene.

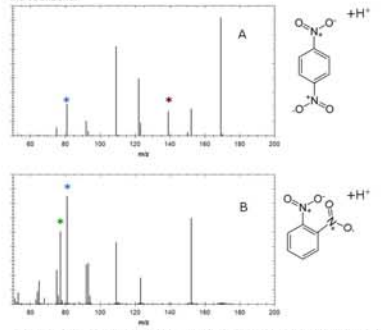

Figure 5. (A) Extracted CID spectrum of 1,4 dinitrobenzene. (B) Extracted CID spectrum 1,2 dinitrobenzene.

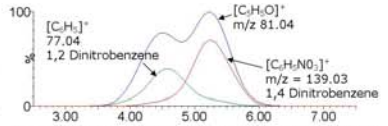

*Figure 6. Extracted mass chromatograms of species labeled with * in Figure 5.*

In this example IMS was able to partially resolve the isomers prior to fragmentation. The combination of IMS and m/z separation allows unique fragment ions to be assigned providing a method to detect and quantify the different isomeric forms in a complex mixture without chromatographic separation prior to entry into the mass spectrometer.
The combination of IMS-MS or MS-MS has potential utility for characterisation of isomers in applications including impurity profiling, and metabolite profiling.

STRUCTURALLY DIRECTED FRAGMENTATION

Animal and plant fatty acids are commonly analysed as methyl ester derivatives (FAME) and were among the first lipid structures to be characterised by Mass Spectrometry. CID pathways for these compounds have been studied previously to investigate secondary structure[3] for example branching.

Figure 7A shows the ASAP CID spectrum of the $[M+H]^+$ ion of methyl stearate at m/z 299.3.
Figure 7B shows the ASAP CID MS-MS spectrum of the M^+ radical cation of methyl stearate at m/z 298.3.

The protonated species was produced by introduction of methanol vapor into the source chamber during the ASAP process.
It is clear, from figure 7, that the fragmentation pathways are very different for these two ions.

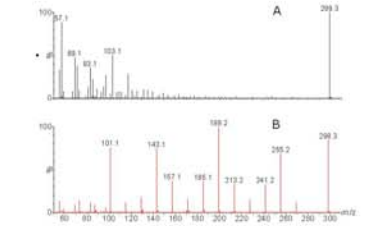

Figure 7. (A) ASAP CID spectrum of $[M+H]^+$ methyl stearate. (B) ASAP CID spectrum of M^+ methyl stearate.

Figure 8 shows ion IMS-DT profiles for the $[M+H]^+$ and M^+ of methy stearate.

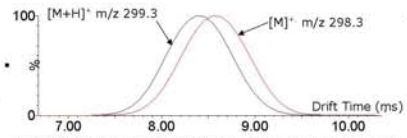

Figure 8. Extracted mass IMS chromatogram of $[M+H]^+$ ion m/z=299.3 and M^+ ion m/z=298.3 of methyl stearate.

ASAP IMS MS OF CRUDE OIL

To examine the possible application of ASAP IMS-MS in the analysis of a more complex mixture a sample of crude oil was analysed.

Figure 9 shows the mass spectrum obtained for a sample of crude oil using the ASAP technique.

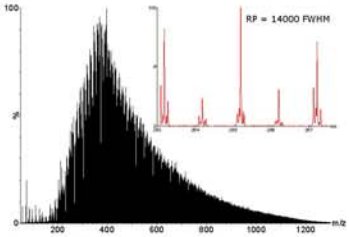

Figure 9. ASAP MS spectrum of crude oil. Expanded region, showing multiple peaks at each m/z value, is inset.

Figure 10 shows the m/z vs drift time plot obtained for the data shown in figure 9 along with the position of detected peaks.

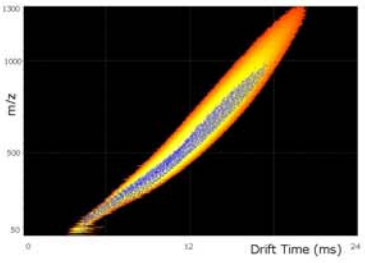

Figure 10. m/z vs DT plot ASAP IMS MS of crude oil.

Crude oil contains many different types of compounds including unsaturated and saturated, branched and straight chain hydrocarbons, aromatic and heteroaromatic compounds, nitrogen, and sulphur containing compounds.

Many of these compound types appear as homologous series with characteristic m/z and intensity profiles.

Figure 11 shows an expanded region of the peak detected data shown in Figure 10 over a m/z range from 350—450 amu and ion mobility drift time of 8 —12 ms. Very clear structure is evident in this data.

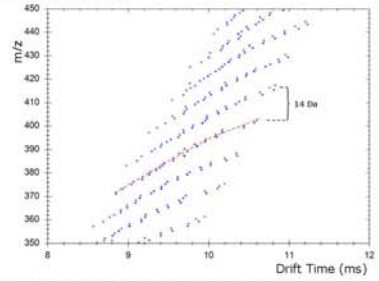

Figure 11. Expanded region m/z vs drift time plot ASAP IMS MS of crude oil

The major peaks shown in Figure 11 appear in a series of clearly defined bands running diagonally from left to right indicated by a red dotted line.

Peaks within each of these bands are separated by 14 amu, (a single CH_2 unit), from the corresponding peaks in the bands above and below.

This structure represents the IMS separation of homologous series of compounds which make up this very complex mixture.

The m/z and ion mobility information for this complex mixture may allow rapid fingerprinting of oils from different sources. This approach has potential for wider application in the rapid

CONCLUSION

- **ASAP provides a rapid method for the direct analysis of low polarity compounds and complex mixtures.**
- **Combination of IMS and MS separation has the potential to allow characterisation of mixtures of isomers without chromatographic separation.**
- **IMS-MS can afford insights into how secondary and tertiary structure of small molecules can direct fragmentation pathways.**
- **IMS -MS of a complex crude oil sample reveals clear patterns indicating structurally related components. ASAP IMS MS may have utility in rapid fingerprinting of complex polymeric samples.**

References

1. C. McEwen et al, Anal. Chem.2005, 77,7826-7831
2. D. Clemmer et al, J. Am. Soc. Mass Spectrom. 1999, 10, 1188-1211
3. M. Takayama et al. Int. J. Mass Spectrom. And Ion Proc. 148 (1995) L11-L16

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