THE APPLICATION OF ASAP TO THE ANALYSIS OF COMPLEX MIXTURES

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Hilary Major, Martin Green
Waters Corporation, Floats Road, Wythenshawe, Manchester, M23 9LZ, UK

INTRODUCTION

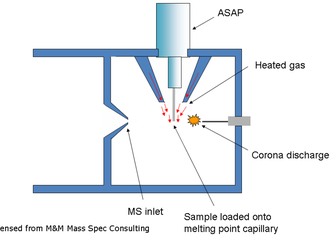
ASAP (Atmospheric Solids Analysis Probe) developed by McEwen et al[1] has been shown to be a useful tool for the rapid direct analysis of volatile and semi-volatile solid and liquid samples using atmospheric pressure ionization. ASAP analysis is a useful alternative to direct analysis in real time (DART) and desorption electrospray ionization (DESI) for the analysis of small molecules.

The ASAP technique is capable of ionizing low polarity compounds not amenable to ESI, APCI and APPI at high sensitivity and can also be used for the analysis of complex samples without the need for any sample preparation.

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 for the analysis of complex mixtures.

METHODS

All analyses were performed using either a Waters LCT Premier XE or a Synapt HDMS system. A prototype ASAP device was used which replaced the instruments electrospray probe (Figure 1). The source was operated in ESCi mode to facilitate the use of the electrospray desolvation heater in conjunction with a corona discharge. This configuration also allowed the LockSpray interface to be used for exact mass measurements (Figure 2).



Licensed from M&M Mass Spec Consulting

Figure 1. Schematic diagram ASAP

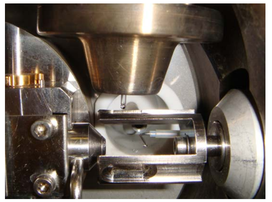


Figure 2. Photograph of ASAP on the LCT Premier XE with LockSpray fitted

Samples were introduced on a sealed glass melting point tube and vaporized in a stream of heated nitrogen. The temperature of the nitrogen was ramped to control the vaporization of components in the complex mixtures. The sample in the gas phase was ionized by proximity to a corona discharge needle. Ions then passed from the atmospheric pressure region into the mass spectrometer.

The pharmaceutical tablets were cut with a scalpel to expose a clean surface with no tablet coating. The sample capillary was then wiped across this surface and analysed directly. All other samples were loaded directly onto the glass capillary without any sample preparation.

RESULTS AND DISCUSSION

TABLET FORMULATIONS

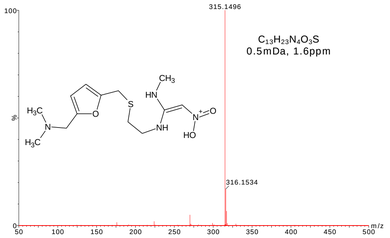


Figure 3. Direct analysis of Zantac™ (ranitidine) 75mg tablet used in the treatment of indigestion

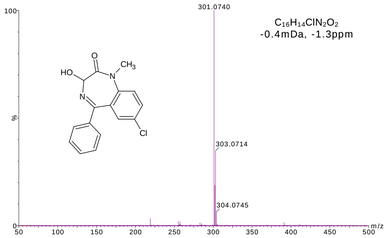


Figure 4. Direct analysis of a 10mg tablet of the anti-anxiety drug Temazepam

Without any sample preparation or chromatographic separation the active ingredients can be easily detected even when present at only a few mg in the formulated tablet. This shows the potential of using ASAP in the fast identification of unknown tablets in forensic applications and in combating the problem of counterfeit drugs which contain no active ingredient.

URINE METABOLITES

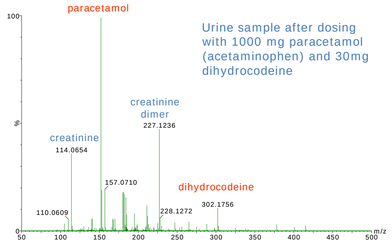


Figure 5. ASAP analysis of neat urine (1µL) after dosing with paracetamol (1000mg) and dihydrocodeine (30mg)

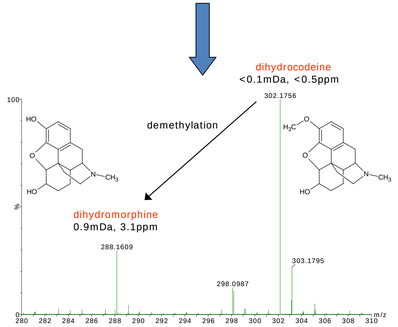


Figure 6. Expanded region of Figure 5 (m/z 280-310) showing the spectrum of dihydrocodeine and the dihydromorphine metabolite formed by demethylation

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
323 formula(e) evaluated with 2 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-200 O: 0-200

Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT (Norm)	C	H	N	O
152.0711	40.98	152.0712	-0.1	-0.7	4.5	C8 H10 N O2	372.1	0.0	8	10	1	2
195.0888	100.00	195.0882	0.6	3.1	5.5	C8 H11 N4 O2	414.2	0.0	8	11	4	2

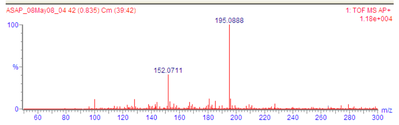


Figure 7. Elemental composition report for paracetamol (m/z 152) and caffeine (m/z 195) from an average of 4 scans from the neat urine

Figure 5 shows data from 1µL of neat urine, spotted onto the glass capillary, from a patient taking both paracetamol (1000mg) and dihydrocodeine (30mg). Paracetamol shows as the base peak of the spectrum with the naturally occurring metabolite creatinine also being significant. The dihydrocodeine can also be readily detected and magnification of the m/z 280-310 region of the spectrum (Figure 6) shows the presence of the metabolite dihydromorphine formed by demethylation.

Figure 7 shows the spectrum and elemental composition report from an average of 4 scans taken earlier in the evaporation profile showing the presence of caffeine and paracetamol.

This clearly shows that the ASAP technique is very sensitive and does not suffer from the same suppression effects associated with electrospray and APCI and that valuable information on the drugs taken and metabolites formed can be obtained from 1µL of a neat urine sample.

POLYMERS

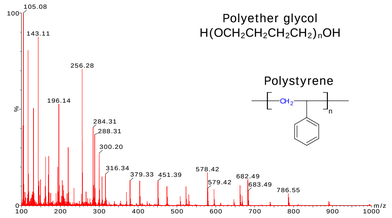


Figure 8. ASAP analysis of polystyrene 1000 and polyether glycol 1000 mix

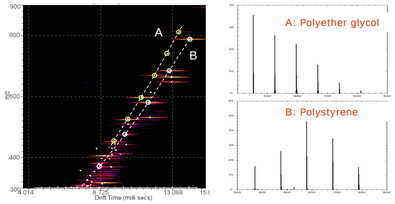


Figure 9. Drift time vs m/z plot for ASAP IMS of polystyrene 1000 and polyether glycol 1000 mix and extracted spectra

The polymer mixture was analysed by ASAP on a Synapt HDMS system (Figure 8) and the 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 9). Ion mobility separated spectra of the polyether glycol and polystyrene were readily extracted using this development software. This approach has potential for wider application in the rapid characterisation of polymeric mixtures.

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.

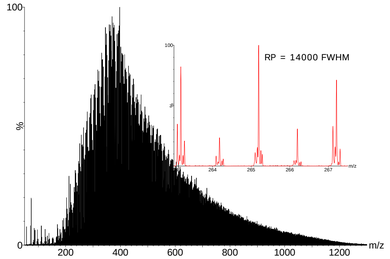


Figure 10. ASAP MS spectrum of crude oil. Expanded region, showing multiple peaks at each m/z value, is inset.

Figure 11 shows the m/z vs drift time plot obtained for the data shown in figure 10 along with the position of detected peaks.

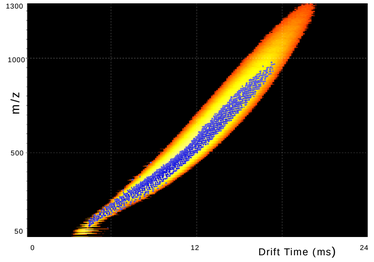


Figure 11. Drift time vs m/z plot for ASAP IMS MS of crude oil.

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

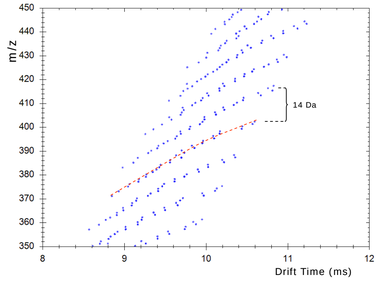


Figure 12. Expanded region drift time vs m/z plot ASAP IMS MS of crude oil

The major peaks shown in Figure 12 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.

CONCLUSION

- **ASAP provides a rapid method for the direct analysis of complex mixtures, such as urine and plasma, without any sample preparation**
- **Non-polar compounds, such as those present in crude oil, which are not amenable to analysis by ESI or APCI are readily detected with good sensitivity**
- **ASAP IMS-MS shows potential in the rapid fingerprinting of complex polymeric samples**
- **The added dimension of IMS-MS in the analysis of a complex crude oil sample reveals clear patterns indicating structurally related components**

References

1. C. McEwen et al, Anal. Chem. 2005, 77,7826-7831

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

72000275 9EN