A NOVEL APPROACH TO METABOLITE DETECTION AND IDENTIFICATION BY THE USE OF UPLC-HYBRYD QUADRUPOLE-TRAVELLIG WAVE-IMS-OA-TOF

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INTRODUCTION

Typically one of the main problems with metabolite id samples are the biological matrices analyzed. These samples may contain a very large number of endogenous compounds which may interfere with the detection of the drug-related component or may obscure its detection. This is true for example in the case of bile where there is a high content of bile salts and sometimes these co-elute with putative metabolites. In this work we have utilized a Travelling Wave[1]-IMS-TOF (Figure1) to detect and separate endogenous metabolites from xenobiotics. IMS (Ion Mobility Spectrometry) is the separation of ionic species as they drift through a gas under the influence of an electric field. The rate of drift depends on the particular mobility of an ion species in the gas and is dependent on factors such as the mass of the ion, its particular charge state and the interaction cross-section of the ion with the gas. Consequently it is possible to separate species of nominally the same m/z ratio if they have different charges or different interaction cross-sections. Ion mobility separations are generally on the millisecond timescale and so many can be acquired over the timescale of peaks eluting from an LC separation system.

Previously, a factor that has prevented IMS from becoming a mainstream separation approach in conjunction with mass spectrometry has been the low sensitivity of conventional DC-only ion mobility spectrometers as a result of poor duty cycle and ion radial diffusion losses. However, developments in sub-ambient pressure ion mobility instrumentation have improved this situation with ion trapping prior to mobility separation improving duty cycle and the use of RF ion guides as mobility separators to minimize diffusive losses. This combination provides a system with sufficient sensitivity to be useable for sample analysis at analytically significant levels.
An advantage of this configuration is that isobaric interferences can be separated if they have different interaction cross sections.

Another useful feature of this particular device is the ability to fragment ions of interest both before and after mobility separation facilitating MS^3 type experiments through drift time correlation.

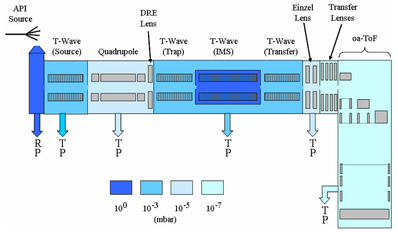

Figure 1. Schematics for Hybrid Quadrupole-Travelling Wave-IMS-oa Tof

METHODS

LC-MS Methodology

LC-conditions:	Aquity UPLC™ Acquity BEH C18 Column 100x1mm id, 1.7µm
Mobile phase A:	0.1 % formic acid
Mobile phase B:	Methanol
Flow rate:	0.11 mL/min
Gradient:	Initial 95% A, 0-2 min 85% A, 2-17 min 5% A, 17.5-20 min 95% A
Injection volume:	10 µL

Mass Spectrometer:	Experimental instrumentation incorporating IMS and TOF MS
MS scan range:	70-900 Da
Mode of Operation:	+/-ve ion mode ESI
IMS gas:	Nitrogen
IMS experiment time :	10 msec
Number of Pushes per single experiment :200	
Push rate : 50µsec	

IMS data acquisition

The IMS experiments equate to 200 oa pushes on the ToF (Figure 2) which are acquired following gated release of ions into the IMS device. Each IMS experiment is 10msec long (= 200 pushes x 50µsec) and so many can be acquired across a typical LC chromatographic peak. For instance, if a 0.1 sec acquisition time is used 10 sets of mobility data are summed.

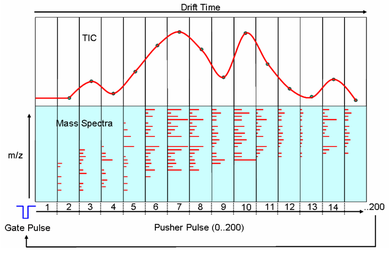

Figure 2. Diagram representing a single IMS experiment in the mass spectrometer.

RESULTS

- From Figure 3, it can be observed how Verapamil and Dextromethorphan when infused can be separated in the IMS device by different drift times.
- Ions are separated due to their different mobility and is determined by;
  - Different m/z values
  - Cross section of the molecule
  - Charge state.

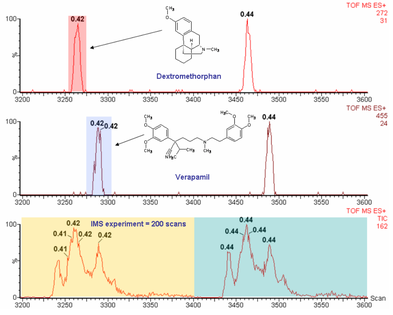

Figure 3. IMS experiment for the analysis by infusion of Verapamil and Dextromethorphan

- If the spectra is combined over the entire IMS experiment, then it will result in spectra A in Figure 4, which is typical of a standard infusion by ESI/MS
- But when the spectra is combined over the drift time regions which belongs to Verapamil or Dextromethorphan (Figure 4) then it is clear that the two compounds share different drift times and the spectra is very clean (Spectra B for Verapamil and Spectra C for Dextromethorphan)

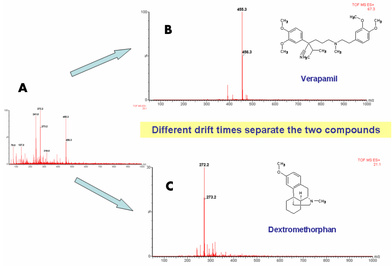

Figure 4. Spectra for Verpamil and Dextromethorphan showing different drift times after the IMS experiment

IMS with Fragmentation

- In this example Verapamil precursor ion fragmentation was carried out in the Trap T-wave after preselection in Q1
- As it can be seen in Figure 5, the fragment ions of Verapamil at m/z 303 and 165 have quite different drift times from the precursor ion at m/z 455

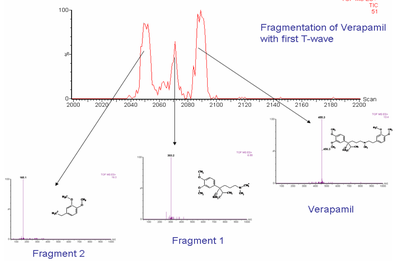

Figure 5. CID– IMS spectra for the analysis by infusion of Verapamil

- If fragmentation of the mobility separated product ions is carried out in the transfer T-wave then MS^3 type data can be obtained.
- From the first experiment the drift times are known for fragment ions m/z 303 and 165 including the parent drug (Figure 5). It is now possible to align these mobility peaks with the post-mobility fragment ion peaks, Figure 6, and infer MS^3 type fragmentation pathways.

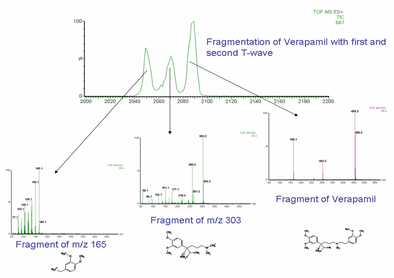

Figure 6. CID– IMS-CID spectra for the analysis by infusion of Verapamil

- In Figure 7, a typical scenario is shown where a metabolite of interest may co-elute with bile salts. Making the metabolite detection difficult as seen in Figure 8.

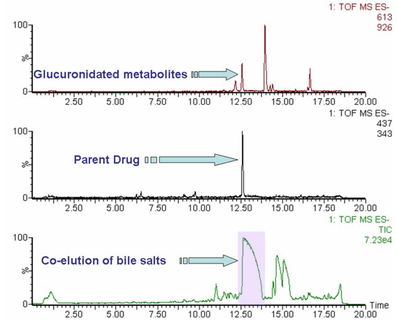

Figure 7. Typical TIC and extracted ion chromatograms for parent drug and glucuronidated metabolite representing a dog bile sample in standard mode of operation

- IMS may be a very useful technique for separating components in a complex mixtures and specially when these components are in low concentrations.
- This can be better appreciated in Figure 8 where the peak at m/z 613 corresponding to the glucuronidated metabolite under normal conditions is not the base peak ion whilst with IMS the ion of interest is the base peak ion

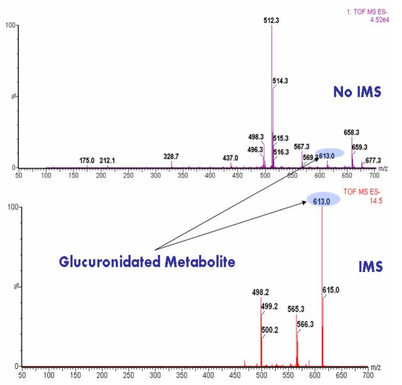

Figure 8. Spectral comparison between standard mode of operation and IMS for a dog bile sample dosed with a pharmaceutical compound of interest

CONCLUSION

- **Great potential for detecting low level components in biological samples**
- **It will add a fourth dimension to the detection of compounds by the use of different drift times**
- **Capability to fragment in the first and second T-wave device simultaneously has the potential for selective qualitative information**

References

The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

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