THE USE OF A PARALLEL CID-ION MOBILITY-CID FRAGMENTATION TECHNIQUE FOR STRUCTURE ELUCIDATION IN METABOLITE IDENTIFICATION STUDIES

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INTRODUCTION

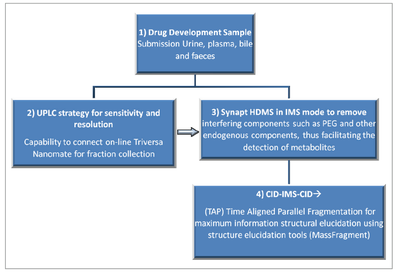
One of the typical problems when running in-vivo samples is that without the use of radiolabel compounds there are no reference points to look for xenobiotics. Therefore, in the vast majority of cases the analyst relies heavily on personal experience and analytical strategies to detect and identify low-level metabolites. In principle, the problems described above could be reduced through the use of an additional stage of separation which is orthogonal to the LC and mass spectrometric separations and occurs on a timescale that is intermediate between the two. A technique that potentially has this capability is HDMS™ or otherwise known as ion mobility spectrometry (IMS). The novel aspects of this technology are based on; an extra dimension in mass spectrometry separation with drift time and an information rich approach for metabolite detection and identification with multiple stages of fragmentation.

This technique consists on 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. Furthermore, in the majority of cases *in-vivo* sample analysis consists of many LC-MS and LC-MS/MS injections to obtain the required information about the sample in question. Ion-trap based MS technology which uses data-dependant MS^n acquisitions often requires multiple injections to obtain the desired fragment ions of interest for putative metabolites. Having said that, typically the fragmentation generated by these kinds of experiments using ion-traps is less informative than the fragmentation generated by collision induced dissociation or otherwise abbreviated as CID fragmentation which may be obtained from a triple quadrupole or a QTof hybrid instrument.

In this study we investigate the capabilities of a hybrid quadrupole/Travelling Wave IMS/ oa-TOF instrument for drug metabolite analysis. In addition to the orthogonal separation afforded by ion mobility, this current set-up has the capability for both pre-IMS and post-IMS ion fragmentation which can be selectively used to provide high sensitivity MS^3 information. Another interesting feature of this device is the fragment separation for metabolites of interest in the IMS by different drift times (Time Aligned Parallel fragmentation – TAP) which can be used very selectively for multiple stages fragmentation experiments. Furthermore, the use of structure elucidation software tools such as MassFragment™ further complemented the entire workflow reducing the bottleneck in structure elucidation. With this approach, a two injection strategy is necessary for the fraction collection resulting in more valuable time spent analyzing and optimizing the sample analysis conditions for each fraction.

METHODS

Workflow Schematic



UPLC-MS on-line Fraction Collection

UPLC settings

Column:	Waters Acquity BEH C18 column 1.7 um, 2.1x100mm
Mobile Phase A:	Water + 0.1 % formic acid
Mobile Phase B:	Acetonitrile + 0.1 % formic acid
Flow Rate:	600 µL/min
Flow Rate to MS:	289nL/min
Fraction Collected:	599.7 µL/min
Injection Volume:	10 µL

UPLC gradient settings

Time (min)	Flow Rate (µl/min)	A (%)	B (%)
0.00	600	100	0
10	600	50	50
11	600	10	90
12	600	10	90
15	600	100	0

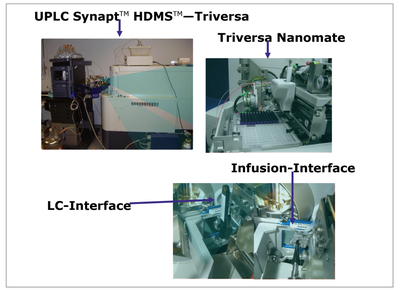


Figure 1. UPLC Synapt™ HDMS™-Triversa Nanomate set up for in-vivo sample analysis.

Advion Triversa Nanomate Settings for on-line Fractionation

- The UPLC-MS flow was split by a 2000:1 ratio in which most of the flow was collected as a fractions in a 96 well sample plate
- 70µL was collected on each well which corresponded to a 7 second fraction

Sample preparation

A time point at 4 hours rat urine sample was collected from a 5mg/kg (Verapamil) oral dose experiment. The sample was diluted 1/4 with water + 0.1 formic acid and injected directed to the LC/MS

Synapt™ HDMS™ settings

Ionization mode:	ESI +ve
Cone Voltage:	35 volts
MS scan range:	50–1000 m/z
IMS gas:	Helium
IMS scan time:	10 ms
TOF Push rate:	50 µs, 200 pushes/IMS scan
Collision Gas:	Argon

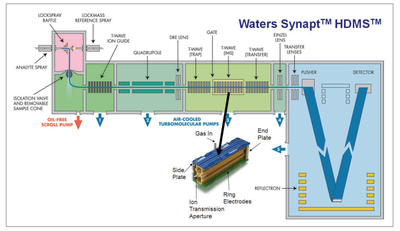


Figure 2. Schematic for Synapt™ HDMS™ system.

RESULTS

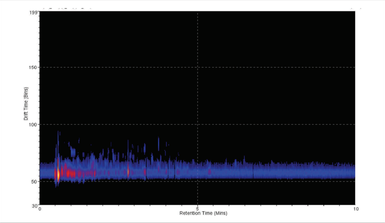


Figure 3. Drift time plot for control sample showing drift time (x-axis) vs. retention time (y-axis).

- By comparison with the control sample (Figure 3) it was possible to highlight differences between the control and analyte (Figure 4)
- Then, by lassoing the drift time regions of interest from Figure 4 it was possible to obtain a clean extracted ion TIC only corresponding to the metabolites of interest

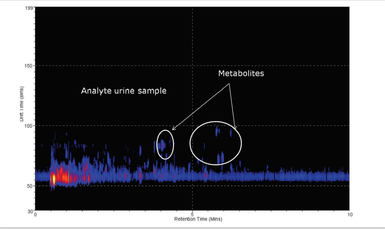


Figure 4. Drift time plot for analyte sample showing drift time (x-axis) vs. retention time (y-axis).

- It is worth noting that since the metabolites are above the chemical noise and background ions the resulting TIC was very clean with zero baseline noise making the detection of these putative metabolites easier Figure 5

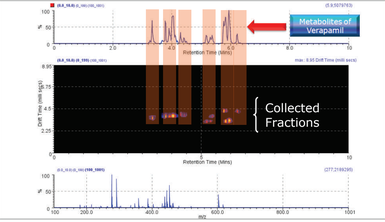


Figure 5. Excised corresponding potential metabolite drift times from comparison of control and analyte sample drift plots.

- Once the metabolites of interested were found then the fractions were collected
- For each of the collected fractions a TAP experiment was carried out which is described in more detailed below (Figure 6)
- The parent drug Verapamil was fully characterized by the TAP approach as shown below in Figure 7

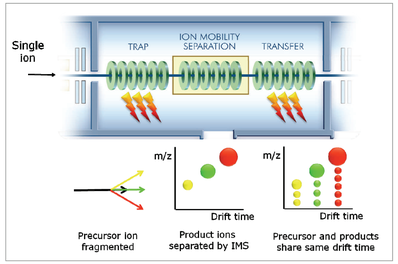


Figure 6. Time Aligned Parallel fragmentation (CID-IMS-CID) experiment description.

- After carrying out TAP fragmentation on the parent drug (Figure 7), it was possible to interrogate each one of the drift time regions independently by creating fragmentation drift time trees. Thus, maximizing the amount of information generated with no 'low mass-cut-off' and in a parallel fashion without the need to pre-select which ions required to do further MS/MS experiments

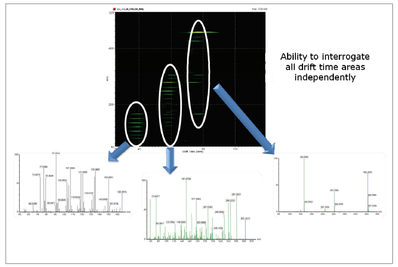


Figure 7. Time Aligned Parallel fragmentation (CID-IMS-CID) for Verapamil parent drug.

- The major fragment ions were submitted to the MassFragment™ software tool which enabled us to propose the fragment ion structures for the parent compound Figure 8

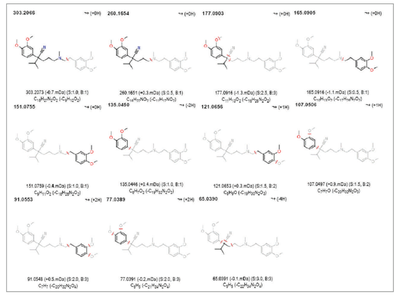


Figure 8. Assignment of major fragment ions for Verapamil using MassFragment™.

- Once the parent fragment ions were characterized it was then possible to use this information to localize the position of the of one of the glucuronidated metabolites using the TAP fragmentation (Figure 9)

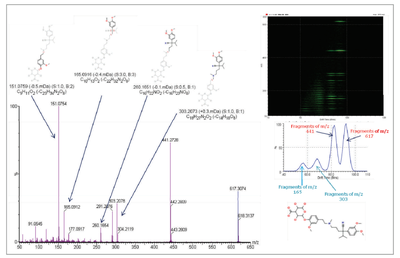


Figure 9. Glucuronidated metabolite of Verapamil submitted to TAP fragmentation and elucidation with MassFragment™.

CONCLUSION

- **IMS mode allows the user to dissect the data with greater specificity utilizing the 4th dimension (drift time). This makes it possible to remove chemical noise and other interferences such as PEG thus facilitating the search for putative metabolites**
- **The configuration of the TriWAVE allows TAP experiments in a very unique but informative approach as all ions are fragmented in a parallel fashion enabling MS^3 fragmentation**
- **The use of a chemically intelligent software algorithm (MassFragment™) for structure elucidation is very desirable as it provides all the necessary tools for rapid compound identification**

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