

# Removal of Interferences and Easier Metabolite Detection by Ion Mobility Mass Spectrometry

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The task of identifying drug metabolites from complex biological matrices such as bile, plasma, feces, and urine with traditional techniques can be difficult. One of the typical problems when running *in vivo* samples is that without the use of radiolabeled compounds, there are no reference points to look for xenobiotics. Therefore in the vast majority of cases, the analyst relies heavily upon personal experience and customized analytical strategies to detect and identify low-level metabolites from high endogenous backgrounds. The complexity of this analysis could be significantly reduced by 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 possesses this capability is ion mobility spectrometry (IMS). IMS separates ionic species as they drift through a gas under the influence of an electric field. For any particular ion, the rate of drift depends on its mobility, which in turn is dependent on factors such as mass, charge state and the interaction cross-section of the ion with the gas. This additional dimension of separation fidelity leads to improved specificity and sample definition so that more information about the sample can be extracted. The multi-dimensional data produced by the Waters® Synapt™ High Definition MS™ (HDMS™) system is visualized and manipulated using DriftScope™ Mobility Environment software.

In this work, UPLC®/IMS-TOF-MS (Figure 1) analysis was conducted on a rat bile sample. By using the DriftScope software, the metabolites from this complex matrix were easily visualized, as the drift time was used to separate background ions from real drug-related metabolites. Further extracted ion chromatograms were also obtained by selecting specific ions with the software. As a result, the extracted ion chromatogram and MS spectrum for each metabolite were attained without interference from the endogenous compounds.

## Experimental

The *in vivo* ketotifen sample was obtained from rat bile. The drug was dosed intraperitoneally at 10 mg/kg and bile was collected from 0 to 3 hours after drug administration. The sample was diluted 1/10 using water with 0.1 % formic acid prior to the UPLC/MS analysis.

### UPLC conditions

LC System: ACQUITY UPLC  
Column: ACQUITY UPLC BEH C<sub>8</sub>  
2.1 x 100 mm, 1.7 μm  
Temperature: 45 °C

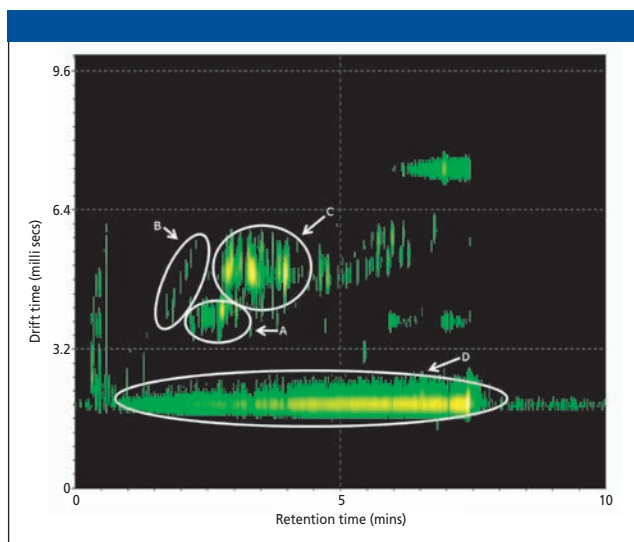


**Figure 1:** The Waters ACQUITY UPLC system with the Synapt High Definition Mass Spectrometry system.

Flow Rate: 600 μL/min  
Mobile Phase A: Water + 0.1% Formic Acid  
Mobile Phase B: Acetonitrile  
Gradient: 95% A to 20% A in 6.5 min., hold at 0% A for 0.5 min. before returning to 95% A for re-equilibration

### MS conditions

MS System: Synapt HDMS  
Ionization Mode: ESI Positive  
Capillary Voltage: 3200 V  
Cone Voltage: 35 V  
Desolvation Temp: 400 °C  
Desolvation Gas: 800 L/Hr  
Source Temp: 120 °C  
Acquisition Range: 100-1000 m/z  
Mobility Carrier Gas: Nitrogen at 32 mL/min.



**Figure 2:** DriftScope software's dt (msec.)/rt (min.) plots for rat bile dosed with ketotifen. Various regions are easily visualized from the plots: (A) metabolites and the parent drug, (B) PEG, (C) bile salts, and (D) solvent ions.

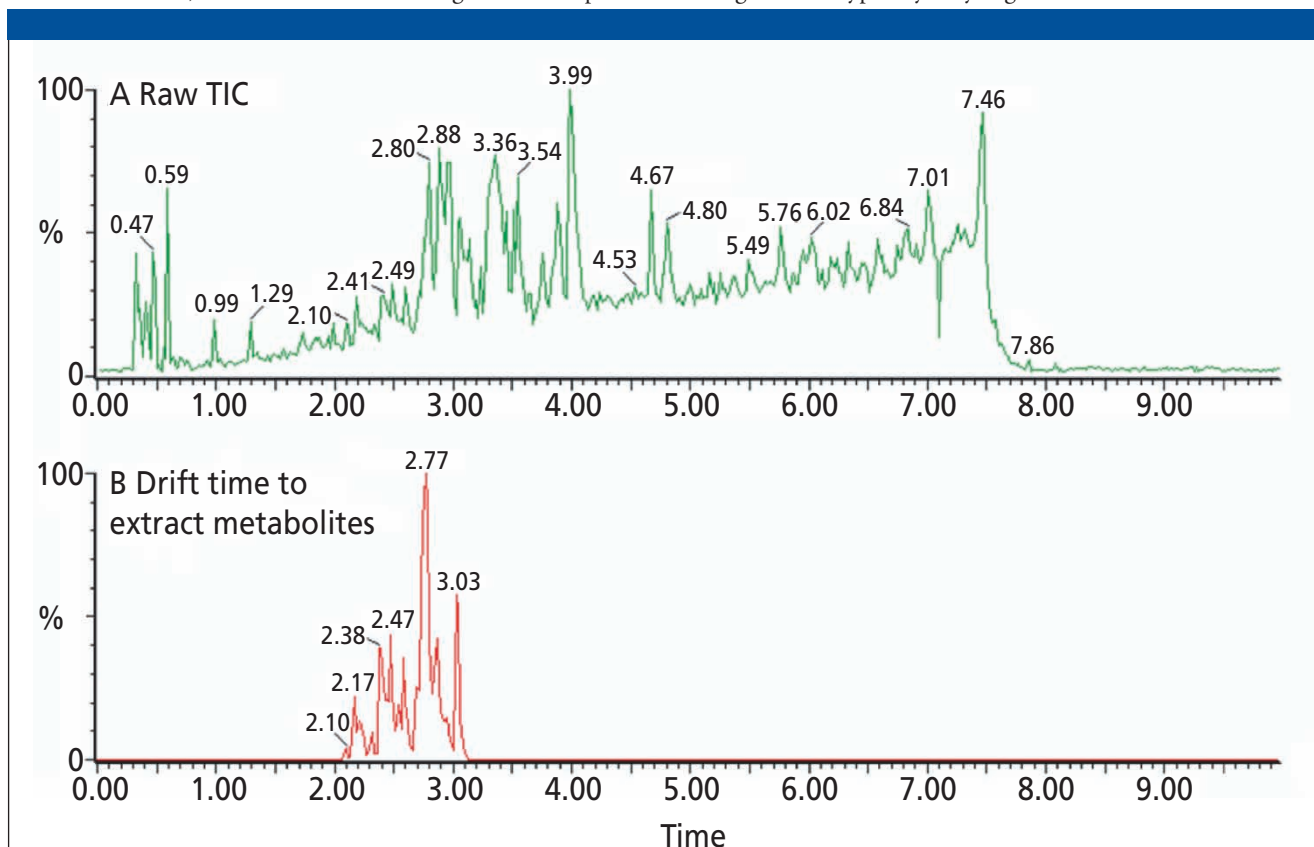
## Results

The UPLC/IMS-TOF-MS results were reviewed using the DriftScope software. It allowed sample data to be visualized as  $mz/dt$ , or  $dt/rt$ , or  $mz/rt$  ( $mz=m/z$ ,  $dt$ =drift time, and  $rt$ =retention time). The initial review using the  $dt/rt$  option

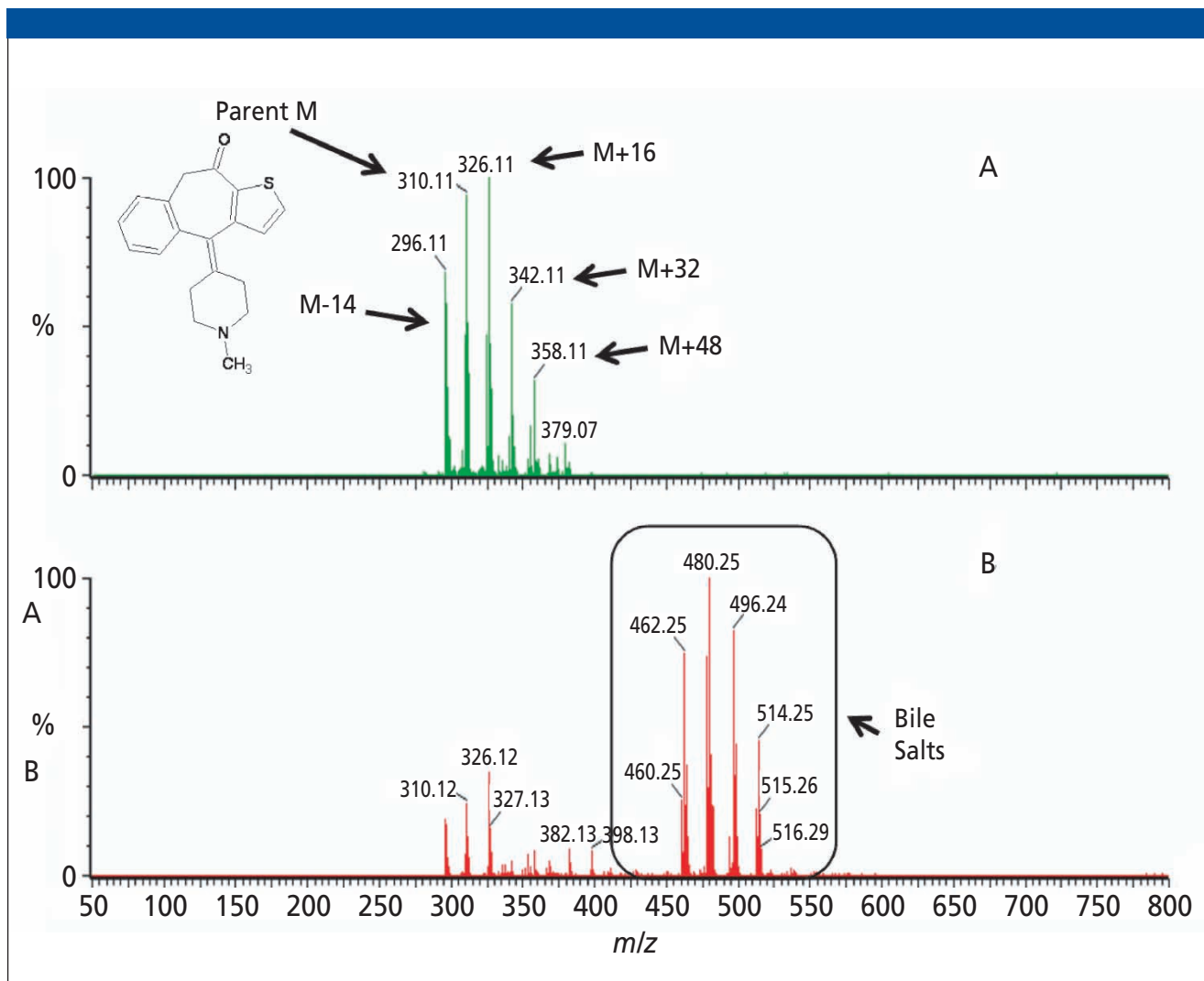
enabled the visualization of all components by using drift time, which was used to exclude false positives and consequently remove interfering ions that masked the detection of smaller metabolites. As shown in Figure 2, this was a very complex sample. But due to the fact that the drift time information was available, this data dimension was used to select the areas of interest. This can be seen in area A of the figure, where all of the metabolites and the parent drug reside. A simple lasso around this region within the software allowed efficient export of the corresponding TIC to MassLynx™ software for further data processing.

Also in Figure 2, when focusing on areas A and B it was observed that even though some of the metabolites co-eluted chromatographically with bile salts, they both exhibited different drift times. This indicated a very selective technique to remove the background ions (D), PEG (B), and bile salt interference (C). Figure 3 shows that once the area of interest had been selected and exported to MassLynx, substantial differences were seen in the cleanup of background and bile salt interferences when drift time was used to extract the metabolite information (B) vs. a normal TIC without the use of IMS (A).

Another interesting observation in Figure 2 was that as only area A was selected, we were consequently above the background noise which was constituted mainly by solvent ions (D) so the noise was zero. Therefore, it becomes even easier to detect very small components in complex samples where the background is typically very high.



**Figure 3:** TICs for ketotifen rat bile sample: (A) TIC without the use of drift time to extract the information, and (B) TIC with the use of drift time to extract the information..



**Figure 4:** Spectra combined over retention times from 2 to 3.2 min. for both TICs: (A) with the use of drift time to extract the information, and (B) without the use of drift time to extract the information.

Further, we combined the TICs from the non-IMS acquisition vs. those from the IMS-based acquisition from 2 to 3.2 minutes where both the metabolites and the parent drug eluted. As seen in the results displayed in Figure 4, it is clear how much more visible the metabolites were, showing an overall improvement in the corresponding spectra when drift time was employed. Even though some of the bile salts had the same retention times, drift time very effectively removed them from the spectra to further aid in the detection of metabolites.

Therefore, from Figure 4 (A) it was deduced that ketotifen at  $m/z$  310 was detected together with all of its major metabolites in bile corresponding to N-dealkylation  $m/z$  296 (-CH<sub>2</sub>), hydroxylation  $m/z$  326 (+O), double hydroxylation  $m/z$  342 (+2O) and triple hydroxylation  $m/z$  358 (+3O).

Other minor metabolites were also detected, indicating hydroxylation and reduction to ketone  $m/z$  324 (+O -2H), and double hydroxylation and reduction to ketone  $m/z$  340 (+2O - 2H).

## Conclusion

Ion mobility capabilities with the Synapt HDMS system offer an additional fourth dimension of information in the analysis of very complex samples by UPLC/MS. The DriftScope Mobility Environment software is an essential tool to visualize the data and serves as a very powerful aid to extract the information required, allowing only relevant drug-related information to be extracted. IMS technology could significantly accelerate the identification of metabolite peaks in "cold" *in vivo* metabolism picture earlier on during drug discovery, where radiolabeled material is usually not available.

## Acknowledgements

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