응용 자료

An Added Dimension for Metabolite ID Studies Using Ion Mobility Combined with MS^E

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

To use $HDMS^E$ data to generate cleaner, more precise data sets and to resolve isobaric species. When LC/MS and LC-MS/MS just isn't enough, using DriftScope™ Software allows you to interrogate your data in an extra dimension, RT, *m/z*, and now drift time (ion mobility separation).

Benefits

$HDMS^E$ (Ion Mobility Mass Spectrometry) provides researchers with added orthogonal separation and peak capacity to differentiate small changes in closely eluting isobaric metabolites.

Introduction

When performing metabolite identification, it is common to observe multiple biotransformation that give the same isobaric mass. These compounds are often nearly indistinguishable and can be very difficult to resolve by chromatography alone. Extremely high levels of matrix, as is often the case with in vivo studies compound the problem. Although re-optimized chromatography, improved instrument sensitivity and careful interpretation of data can lead to resolution of these species, they are time consuming steps and often require large data sets to be reacquired with modified conditions. Additional orthogonal separation such as ion mobility introduces selectivity that can often quickly resolve these differences and further improve spectral quality, leading to higher quality data sets and interpretations. This combination of Ion Mobility Separation (IMS) and MS^E creates High Definition Mass Spectrometry,™ HDMS^E, and gives the researcher another powerful tool to understand and probe their datasets. In this technology brief we will study the applicability of HDMS^E to complex datasets.

Results and Discussion

Rat liver microsomes spiked with 10 µM buspirone were incubated for 0 and 20 min at 37 °C. Samples were quenched with one volume of cold acetonitrile + 0.1% formic acid and centrifuged. In order to evaluate the application of HDMS^E to in vivo metabolite identification studies, the above in vitro samples were diluted 10-fold with SD (Sprague Dawley) rat urine containing 0.1% PEG400 by volume. Samples were analyzed using a Waters' SYNAPT® G2 coupled with an ACQUITY UPLC® System. Data acquisition was performed with HDMS^E in positive ion, sensitivity mode. 5 µL of sample were injected onto an ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm Column and run with a 20 min gradient using a flowrate of 0.7 mL/min. The mobile phase consisted of 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). Data was processed and visualized using DriftScope Software.

Figure 1 illustrates the added dimension of separation generated by HDMS, the additional peak capacity introduced by IMS clearly elucidates an additional three metabolites versus UPLC alone.

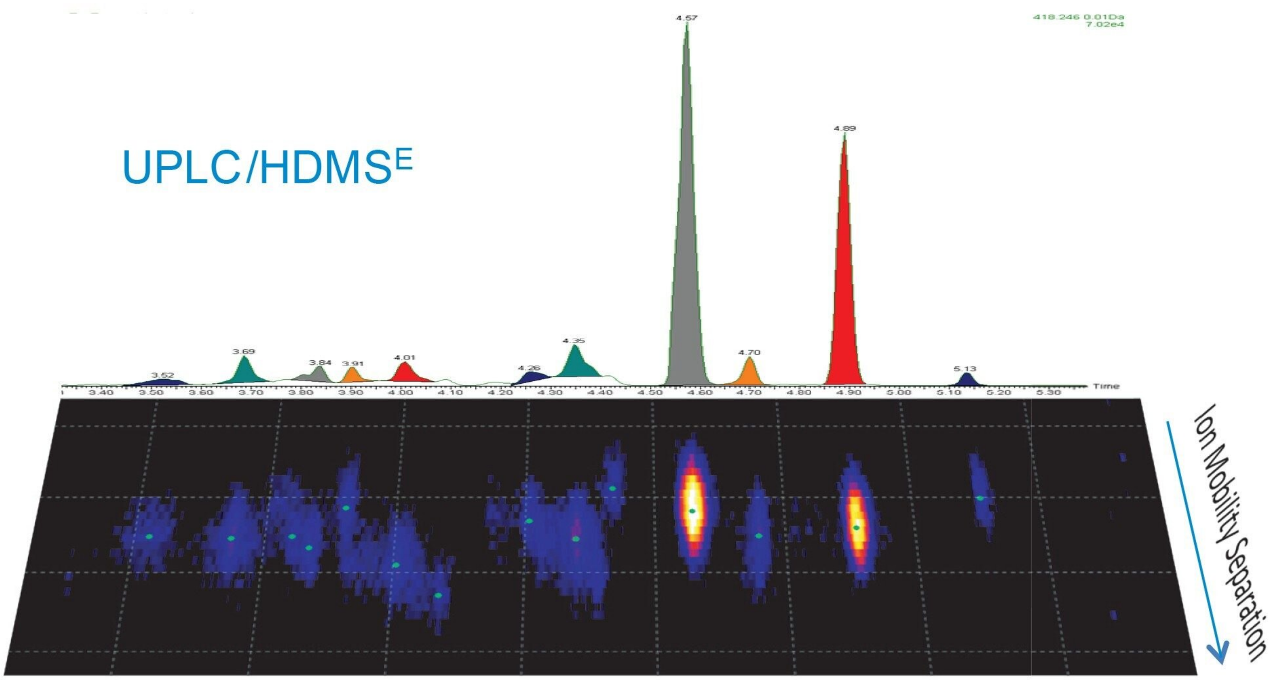

Figure 1. An additional three +32 Da metabolites of buspirone are clearly identified with the added dimension of separation generated by HDMS^E.

Figure 2 shows a comparison between MS^E data for a dealkylation metabolite generated with and without IMS separation enabled. Precursor and fragment ions that co-elute perfectly with the compound of interest can be quickly resolved using IMS techniques alone. Dedicated software using patented MS^E and IMS peak peaking algorithms (Apex 4D) leads to clear resolution of all relevant peaks. The power of peak picking in four dimensions (RT, *m/z*, ion mobility, and intensity) allows for a thorough cleanup of background noise and artifact peaks.

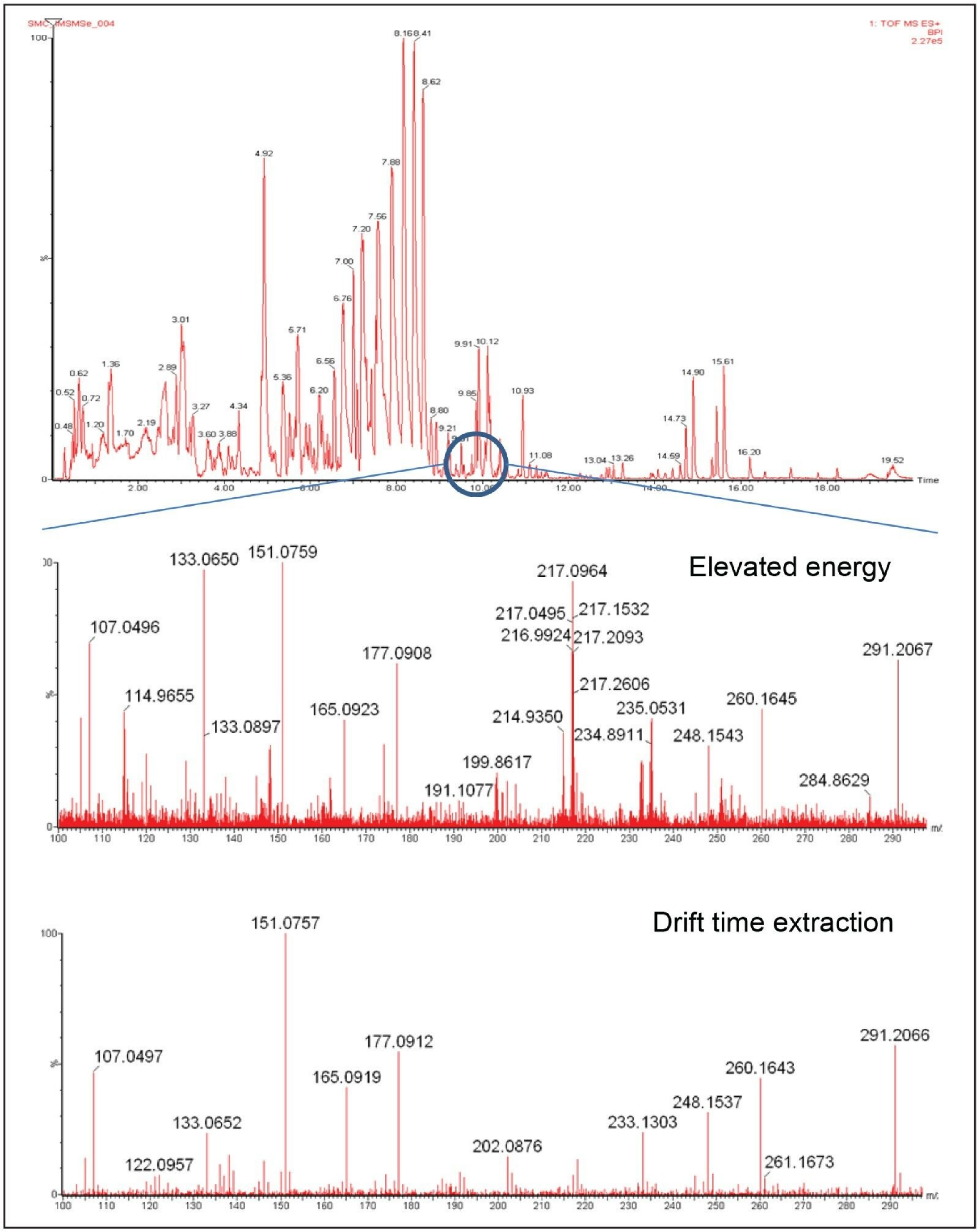

Figure 2. An illustration of the use of HDMS^E to remove background ions from a fragment ion spectrum in an in

vivo sample.

Conclusion

The advances presented in this technology brief facilitate the identification of metabolites, not only with better sensitivity and resolution, but through the unique properties of ion mobility. This allows the user to view data with less interference from matrix and other nominal mass interfering ions not separable through other methods.

Having an entirely unique mode of separation at your fingertips as an additional rich layer of information may mean the difference between an easy analysis and a costly, time-consuming revisiting of already worked out LC and MS methodology.

The benefits of UPLC coupled with SYNAPT G2 HDMS described in this technology brief are now available from Waters. As an additional weapon in your analytical toolkit, ion mobility separation can help you make insightful scientific discoveries and more keenly interpret and understand your experiments.

Featured Products

SYNAPT MS Series HDMS Upgrade <https://www.waters.com/waters/global/nav.htm?cid=10099686>

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