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Application Note

Ion Mobility for Metabolite Characterization: Improving Spectral Clarity of Data Independent Acquisitions

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

Abstract

This application brief demonstrates that spectral alignment applying drift time alongside retention time can significantly improve spectral clarity aiding structural characterization for the interpretation of metabolites/degradants of dextromethorphan incubations.

Benefits

Utilizing ion mobility separation with MS^E acquisitions ($HDMS^E$) can significantly improve metabolite identification confidence in data independent acquisitions (DIA) within drug discovery or development analyses.

Introduction

Sensitive and accurate assignment of drug metabolites and/or drug degradant products is crucial when performing DMPK studies. Improving confidence in identifications allows for the quick and accurate assessment of efficacy and safety of a compound prior to candidate advancement. Identification of metabolites can be challenging due to the complex nature of the matrices with which they are contained. These may include compounds that co-elute with or are of a similar mass to analytes of interest. This can result in interference, affecting peak area estimation or casting doubt over correct identifications.[1]

HDMS^E acquisition using ion mobility separation coupled with MS^E, separates co-eluting constituents based on their gas-phase drift time (measured in ms).[2] Processing the data with the Waters UNIFI Scientific Information System (or other CCS processing software) to incorporate precursor separation using drift time can greatly improve spectral clarity and provides an additional unique compound identifier comparable against standards or theoretical values. As a result, compounds can be identified based upon LC retention time, accurate mass, collisional cross section (CCS) value, and more specific and selective precursor and fragment ion spectra.

In terms of structural elucidation, showing only fragments unique to each precursor ion provides added confidence in compound identification, assignment and quantification. This allows analysts to gain the spectral clarity seen with data dependent acquisition (DDA), without the need to generate exclusion or inclusion lists, which have the potential to remove ions of significance.

Results and Discussion

Ion mobility separation (IMS) allows separation of co-eluting analytes based on their structural conformation altering the speed at which they pass through a gas filled cell. This additional separation means that analytes sharing an LC retention time, or even isomers of the same compound, can be distinguished from one another. By applying this technique prior to collision-induced dissociation (CID) fragmentation ($HDMS^E$), it is possible to produce individual fragment ion spectra for each analyte with unique drift times through the cell, thus providing a clean fragmentation pattern without the interference of fragment ions from co-eluting matrix compounds.

When this analysis is performed to discover potential metabolites or degradation products of a candidate drug, these compounds could be at a very low abundance. Therefore, co-eluting interference from the matrix can mask the presence of the analyte of interest or add a level of complexity to confident assignment. Figure 1a shows spectra of the dextromethorphan metabolite 2x + O without the use of IMS. The high energy (fragment) spectra (bottom) shows significant interference from co-eluting matrix compounds, predominantly from ion signals that have a higher intensity than the true product ions of the analyte of interest. Figure 1b shows spectra of the same metabolite with IMS functionality active. The number of interfering fragments within the high energy spectra (top) has been greatly reduced using drift time as a unique identifier for the analyte of interest. This cleaner high energy spectra increases speed of data interpretation and provides improved confidence in identification and structural assignment of the analyte.

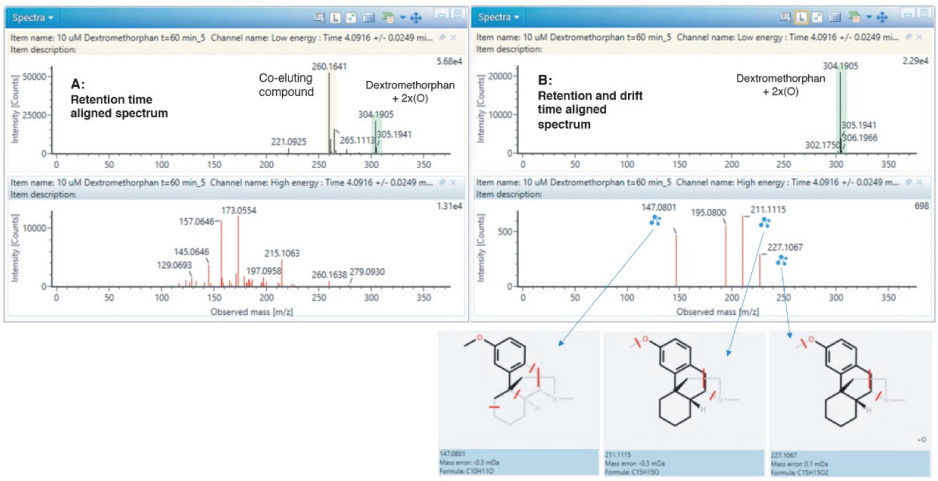

Figure 1. Spectra of dextromethorphan 2x + O metabolite, produced with and without using drift time as a filter. Top trace is the MS (low energy) spectra and the bottom trace is the HDMS^E (high energy) spectra. In a) only retention time has been used to align the spectra, b) both retention time and drift time values have been used to align the spectra. There is a marked improvement in spectra quality, and therefore ease of fragment ion assignment, when drift time of the precursor ion is used as a filter.

Conclusion

This data demonstrates the advantage of ion mobility separation combined with DIA for DMPK studies. Use of this orthogonal separation technique is capable of significantly improving the confidence and speed with which drug metabolism and degradation products can be identified. The dextromethorphan metabolite 2x + O product ion signals are relatively minor when compared to interfering matrix ions, these are not separated using traditional LC-MS analysis and therefore can complicate assignment. When this same data is analyzed incorporating the ion mobility dimension, matrix product ions are removed, and therefore, metabolite product ions are easily identified and confidently assigned.

References

1. Schadt S. *et al.* (2017) A Decade in the MIST: Learnings from Investigations of Drug Metabolites in Drug Development under the "Metabolites in Safety Testing" Regulatory Guidance. *Drug Metab Dispos.* 46, 865-878.

2. Waters Corporation (2011) An Overview of the Principles of MS^E, the Engine That Drives MS Performance. 720004036en. https://www.waters.com/webassets/cms/library/docs/720004036en.pdf

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