Drug Metabolite Identification: Increasing the Separating Power of LC-MS With Differential Mobility Spectrometry and Chemical Modifiers

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OVERVIEW:

- LC-MS has long been employed to profile metabolic pathways for drug molecules. By combining LC elution, accurate MS, and MS/MS fragmentation, a degree of structural information can be gathered. However, some challenges remain, especially when structurally similar metabolites co-elute or nearly co-elute, i.e. isomers of single oxidation metabolites. As a result, convoluted fragmentation spectra can be produced, leading to lower fidelity identifications of isomeric metabolites.
- To try to remedy these challenges, we have combined differential mobility spectrometry (DMS) with the LC-MS/MS experiments to provide more chemistry-based separations of the drug metabolites and/or potential isomers. The observed separation voltage (SV) and compensation voltage (CoV) behaviours of drug metabolites can serve to separate isobaric species and provide improved MS/MS fidelity toward structural ID. [1, 2]
- While separation was generally achieved using the LC, these DMS results demonstrate an overall enhanced peak capacity that can be used to (a) *expand the method's ability to survey additional metabolites as an unbiased approach*, and (b) to *increase confidence in coverage by providing an orthogonal detection parameter* via specific CoV's that enhance the understanding of metabolite profiles for potentially co-eluting metabolites.
- These results were possible due to the acquisition speed of the TripleTOF® 6600, which employed rapid loop scanning of CoV's while simultaneously acquiring molecular or product ion spectra. This approach to method development need not rely on optimization of drug or metabolite, but simply covers a range of CoV's.
- For demonstration of the applied technique, buspirone and verapamil were incubated in human liver microsomes and their metabolite profiles were evaluated. [3, 4]

METHODS & PROCEDURES:

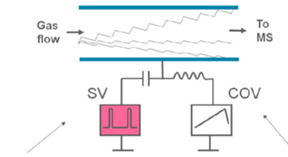
In vitro Incubation and Extraction of Human Liver Microsomes:

- Human liver microsomes (1 mg/mL protein) were incubated at 10 µM substrate concentrations at 37° for 1 hr in 100 mM phosphate buffer (1 mL), with and without the cofactor NADPH.
- At 1 hr, samples were denatured with 4 mL of MeCN, vortexed, centrifuged and transferred for evaporation under 37° N_2.
- Sample residues were reconstituted in 250µL mobile phase, vortexed and injected to the LC/MS system with DMS.

HPLC & Mass Spectrometer:

- Agilent 1290 Infinity integrated system containing binary solvent manager, auto-sampler, column heater, and diode array detector
- Reverse Phase = Halo C18 column, d_p =2.7 µm, 2.1 x 100mm
- HPLC flow rate of 0.5 ml/min & linear gradients
- Mobile phases A&B of 2 mM NH4OAc (pH 6.8) and MeCN/MeOH (1/1)
- SCIEX TripleTOF® 6600 system, ISV = 5000 V + ion
- Curtain gas = 25, Source temp = 500° C
- Gas 1 & Gas 2 = 50 & 50
- SelexION® Technology: SV = 3500 to 3800 V; ramp CoV -30 to 10 V in steps of 1.5 to 3V with 35-50 ms accumulation times for each step
- DP = 80, CE = 35 eV with CES = 25 eV for product ion scanning expts

Separation Principles of Differential Mobility Spectrometry



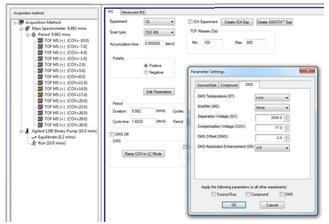
Separation waveform (SV):

Radially displaces ions towards one or the other electrode, depending upon high and low field mobility characteristics

Compensation voltage (COV):

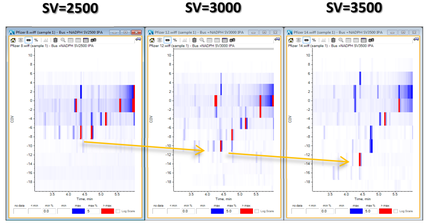
Restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer

DMS SCANNING PARAMETERS:



Rapid scanning of CoV across a wide range affords detection of similar molecular motifs, negating the need to optimize conditions for specific drug or metabolites.

IMPACT of SV on RESULTING CoV's:



Impact of Separation Voltage on CoV's of Buspirone Metabolites. As SV increases, the resolution in CoV increases (expansion of y axis) for single oxidation metabolites, providing analyte specificity.

RESULTS: MS/MS FIDELITY of ISOMERIC BUSPIRONE METABOLITES USING LOOPED SCAN DMS PARAMETERS

Figure 1. Consider 6 prominent single oxidation buspirone metabolites and 5 different resultant CoV's, affording a mobility separation parameter.

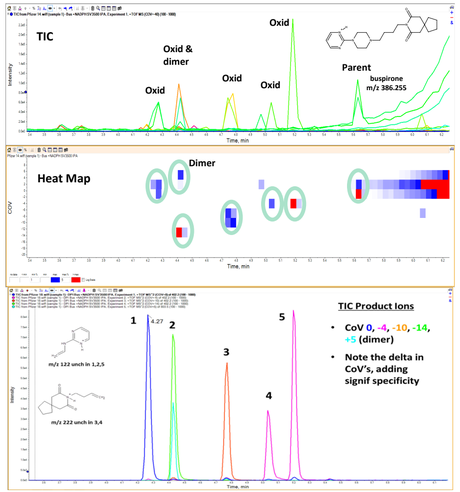


Figure 2. MS/MS spectra of metabolite #2 at two different CoV's (see heat map), yielding independent spectral qualities.

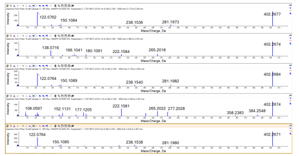


Figure 3. Product ions of m/z 402, each acquired at specific CoV's (listed to right of each panel) for buspirone M+16 metabolites #1-5.

RESULTS: SCANNING DMS PROVIDES ENHANCED PEAK COVERAGE and CONFIDENCE as an ORTHOGONAL DETECTION PARAMETER

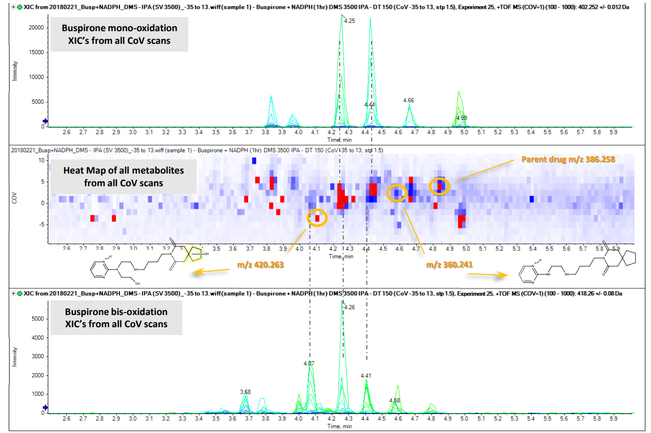


Figure 4. Buspirone metabolite analysis was performed on a different LC/TripleTOF analytical system from that used in Figure 1. Loop scans of CoV were performed at 1.5 V steps with SV=3500V and IPA modifier. The majority of peak intensities are shown to be mono- and bis-oxidations (panels A & C, respectively). But the enhanced peak detection capacity of the method is evident in the Heat Map (panel B), where intense metabolites are shown to elute but do not correspond to the chromatographic traces of A and C; these representative metabolites were oxidative and hydrolysis products of the piperidine moiety, m/z 360 and m/z 420.

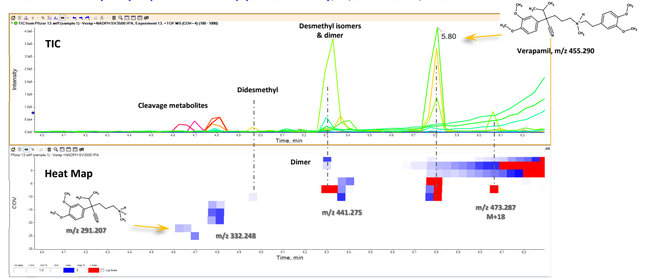


Figure 5. Verapamil metabolite profile demonstrates chromatographic and DMS separation of numerous drug metabolites, achieved in an unbiased manner. Panel A shows the TIC of all metabolites at all CoV's looped at steps of 2 V with SV=3500 and IPA as modifier. Panel B exhibits the various CoV's for each metabolite.

CONCLUSIONS:

- Coupling DMS to the TripleTOF® 6600 makes this workflow extremely practical. Primary benefits include the following:
  a) application of generic settings accommodate a wide range of chemical diversity and experimental matrices,
  b) ease of method development that uses existing discovery chromatography and MS methods, yielding the application of ion mobility separations to a drug metabolite ID study with resultant discriminating parameters.
- Scanning a range of CoV's in looped fashion captures optimal transmission characteristics for analytes of interest *without the need to optimize* individual parameters for metabolite specificity. These qualities may be combined with high resolution TOF scans, IDA or dedicated product ion scans, or other specific MS methods.
- A Heat Map of CoV vs. retention time (Figs. 1, 4B and 5B) can be very helpful in locating and assigning an orthogonal separation parameter to particular analytes, *imparting another specific variable* to include with m/z, MS/MS, retention time and /or UV λ_{max} values.
- Applying DMS with product ion scanning provides specificity of MS/MS spectra for closely eluting or co-eluting metabolites. The resulting spectra are not confounded if different CoV's are exhibited (Figs. 2 & 3).
- Comparing a CoV Heat Map to chromatographic data allows a researcher to observe the elution of isobaric analytes and/or other metabolites for which they may be unaware, offering an unbiased approach to evaluate a metabolic profile (Figs 4 & 5).
- Use of DMS suffers some sensitivity loss if compared to operation with out DMS; source tuning can improve signal but it is not practical for analysis of unknowns.
- Combined with the application of biotransformation knowledge, DMS offers an increased separation parameter for metabolite ID studies and a more complete picture of the acquired data.

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