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Differential mobility spectrometry: a valuable technology for analyzing challenging biological samples

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Abstract

"Implementing differential mobility spectrometry in an LC–MS bioanalytical assay can simplify sample preparation methods and enhance selectivity at the same time."

Keywords

chemical noise reduction; differential mobility spectrometry; fluxomics; isomer separation

Recently, differential mobility spectrometry (DMS) has proven to be a valuable addition to mobility spectrometry (MS) analyses. It provides separations that are orthogonal to both the MS and the LC that accompany most bioanalytical workflows. In addition, using DMS can solve problems upstream of LC–MS analyses, allowing for streamlining of sample preparation by virtue of DMS's unique gas-phase separations. Generally, sample preparation, derivatization and cleanup can cost the most time and money in bioanalytical workflows. For example, many small, polar analytes are not easily separated from isobaric chemical noise (same molecular weight) during sample preparation or LC. For larger analytes, like peptides and proteins, endogenous species (other peptides, proteins) are sampled during `cleanup' and LC elution. This editorial focuses on the use of planar DMS for improving such bioanalytical workflows; microscale DMS and cylindrical DMS (or FAIMS) are not included for brevity. Here, we detail the benefits of using DMS, which provides rapid

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separations for analytes that can be difficult or impossible to separate by LC and where isobaric or isomeric chemical noise overwhelms analytical signal.

A brief description of DMS

DMS separates ions in the gas phase based upon subtle differences in their chemical structures [1–4]. This separation occurs at atmospheric pressure, between the ESI source and the MS sampling orifice, [5] allowing for three consecutive and orthogonal separations in an LC–DMS/MS workflow. After the ESI source, ions are swept by carrier gas (N_2) through the DMS cell – two planar electrodes between which a high-voltage radio frequency asymmetric waveform is applied (separation voltage – SV) [1–5]. The SV causes ions to oscillate toward one electrode or the other depending upon the difference in the ion's mobility during the high- and low-field portions of the waveform. To insure that an ion is detected by the MS, a direct current voltage (compensation voltage – CoV) deflects ions away from collisions with the electrodes and toward the MS.

Another critical factor is the gas-phase environment of the DMS cell, which can be changed by adding volatile chemical modifiers (≥1.5% v/v), such as isopropanol, to the carrier gas [6,7]. Clustering interactions between the modifier molecules and analyte ions [6–8] can cause large shifts in their CoV values; oftentimes, the analyte's CoV is shifted from the CoVs of isomeric or isobaric impurities. Also, the addition of chemical modifiers spreads the analytical signals across a wider range of CoV values (compared with pure N_2) while maintaining the peak widths of the analytes, thereby increasing the overall peak capacity of the DMS experiments [9]. The choice of chemical modifier and the degree of separation depends upon the ion's structure, the gas-phase properties of the modifier and the binding energies between the ion and modifier [8]. In fact, even the site of protonation of some molecules can be determined using DMS [10,11].

When does it make sense to use DMS?

Generally, DMS finds its greatest utility in the most challenging analytical workflows where: high levels of debilitating isobaric chemical noise reduce analyte detection limits, isomeric interferences are present, or exhaustive sample preparation, derivatization and/or chromatography are required to combat the preceding issues. DMS's capability to solve these challenges has been recently demonstrated by several groups.

> Using differential mobility spectrometry in more advanced bioanalytical workflows (e.g., fluxomics) will enable biologists to answer more complex questions about cellular processes.

Using DMS can sometimes present opportunities to circumvent the use of LC while still providing reduced isobaric and isomeric chemical noise levels. For the analysis of isomeric glucuronides of propranolol from mouse liver, Parson and coworkers [12] employed a DMS-multiple-reaction-monitoring workflow that separated these isomers using DMS with acetonitrile as a chemical modifier. While a similar LC-based separation required approximately 5 min of run time, the DMS required approximately 19 s – a time saving of approximately 15×. In addition to similar time savings, Porta and coworkers [13] described a

more convenient experimental setup with their integration of a surface sampling technique (LESA – liquid extraction surface analysis) with DMS instead of LC. Since LESA can use high levels of organic solvent for its extraction step, injection of such a solution directly on to a LC column could yield variable retention times; the DMS does not suffer the same effect. With DMS, they mapped the distributions of 30 drugs of abuse (including isomeric cocaine metabolites) from human kidney and muscle tissue cross sections. Again, LESA with DMS required 1.5 min total analysis times (3–6× time savings over LC–MS). Dharmasiri and colleagues [14] also avoided using LC to separate cancer antigen peptides from a test matrix of 94 similar peptides; without DMS, the antigen could not be detected by direct infusion. Similarly, Coy and coworkers [15] employed direct infusion with DMS to survey for biomarkers for radiation exposure in mouse urine. They noted the presence of *N*-hexanoylglycine (*m/z* 172) and suberic acid (*m/z* 173) – two biomarkers whose isotopic envelopes convolved without DMS; these species separated cleanly with DMS. While use of MS/MS or MRM transitions may have made DMS unnecessary, they demonstrated that DMS with single-stage MS could have provided similar results.

Implementing DMS in an LC–MS bioanalytical assay can simplify sample preparation methods and enhance selectivity at the same time. Ray and coworkers [16] developed an LC–MRM panel for detecting five steroids, including two pairs of isomers: corticosterone and 11-deoxycortisol, 11-deoxycorticosterone and 17-hydroxyprogesterone, and progesterone. With DMS, the MRMs that were hampered by poor selectivity due to high levels of chemical noise now yielded unequivocal responses with desired limits of quantification. They also redesigned and simplified their sample preparation method, streamlining the assay for maximum efficiency and economy [16]. However, some steroid analyses require derivatization, [17] such the isomeric allopregnanolone and pregnanolone – important neurosteroids present at low levels in blood. While the derivatization increased ionization efficiency, it did not solve the problem that both isomers presented similar fragment ions at each other's LC retention times. However, when Jin and colleagues [17] integrated DMS into their LC–MRM method, they observed that derivatized allopregnanolone and pregnanolone had unique CoV values, thereby yielding clean MRM channels only at the correct retention times for each steroid.

Future outlook for DMS in bioanalytical workflows

As more bioanalytical workflows develop and the marketplace for these assays expands, DMS can provide streamlining for these workflows with unique and rapid separations where none are available. Beyond the small molecule and peptide-based assays we described here, there are also examples of DMS's benefits to lipidomics workflows, whether using a shotgun-style approach [18], an LC-based method [19] or more in-depth examinations of isomeric species [20].

Using DMS in more advanced bioanalytical work-flows will enable biologists to answer more complex questions about cellular processes. One such advancement is fluxomics [21,22] in which scientists monitor the rates of flux, the sources and the sinks of individual interacting molecules within a cellular pathway (e.g., metabolism). By adding stable isotope labeled analogs (isotopologs) of a known pathway interactor (e.g., citrate in Krebs cycle),

one can observe how quickly different sinks of the isotopologue incorporate stable isotopic labels themselves. DMS enhances fluxomics studies by its ability to separate small polar molecules (whose LC retention is often suboptimal) from high levels of chemical noise [21,22]. The overlap in this small molecule *m/z* space is considerable, and incorporating additional isotopes only exacerbates this problem. However, using DMS, isotopologues of a given molecule will have very similar CoV shifts – thereby separating the signals of these species from potential chemical interference [18].

A similar cellular pathway study by Israelsen and coworkers [23] demonstrated the use of DMS in understanding the role of pyruvate kinase M2 (PKM2) in the metabolism of glucose by breast cancer tumors in mouse models. By monitoring the levels of ^{13}C-labeled lactate formed from *in vivo* metabolism of ^{13}C-labeled glucose infused into the mice, they determined that PKM2-null tumors and PKM2-expressing tumors both exhibited similar levels of ^{13}C-isotope incorporation in lactate – confirming that tumors produce lactate at rates independent of PKM2 [23]. Here, specific measurements of pyruvate and lactate in samples from plasma, tumors and normal tissues were all hampered by the difficulty fragmenting these ions. Given that pyruvate is two mass units below lactate, the incorporation of ^{13}C label into pyruvate and other known and unknown ionic species in this *m/z* window potentially leads to a restrictively high degree of spectral overlap unique to each sample matrix. Again, DMS served to focus the lactate isotopologues within a narrow band of CoV values, preventing isobaric chemical noise from skewing the data and the underlying conclusions on these cells' glucose-to-lactate metabolism.

In subsequent studies involving more complex cellular pathways, DMS was employed to filter the isotopic signatures of labeled molecules, thereby revealing the specific roles of those molecules. Stark and coworkers [24] examined the role of mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M) on the basal turnover of gluconeogenic (glucose-forming) substrates by monitoring the flux of isotopologues of glucose, glycerol, lactate, glutamine, alanine; and urea. By monitoring the DMS-filtered MRMs of these species, they determined that, when PEPCK-M is silenced *in vivo*, glucose production remains stable despite a reduction in the turnover of lactate - the primary gluconeogenic precursor. Instead, glycerol turnover (not amino acids) took up the slack to provide gluconeogenic support to maintain euglycemia. In total, a more complete picture of the true role of PEPCK-M was obtained by monitoring the flux of many potentially contributing species than would have been if just glucose production were measured alone.

Conclusion

In the future, we will see many more examples of the utility of DMS in challenging assays (e.g., isomer identification, metabolic pathway analyses) and in cost- and time-reduction strategies for conventional workflows (e.g., offsetting exhaustive sample preparation, etc.). As the community of DMS users grows and the technology evolves, many exciting and unforeseen opportunities will undoubtedly be discovered.

Biography

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