Automated approach to optimize DMS separations

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ABSTRACT

This presentation describes an automated workflow to determine and track the Differential Mobility Spectrometry (DMS) behavior for compounds of interest and the use of respective data to determine and apply optimum separation conditions for the DMS separation of isobaric species in routine analytical methods. In addition to the effect of separation voltage (SV) on the optimum compensation voltage (CoV), other DMS characteristics, such as the transport gas composition, transport gas flow and temperature conditions of the DMS cell influence compound behavior in the cell and all of these characteristics are essential to determine and control the compound DMS behavior. The utility of the library of DMS behavior in terms of alpha curves for series of compounds is presented through three example use cases.

INTRODUCTION

Differential Mobility Spectrometry is a powerful technique for separation of isobaric species prior to mass spectrometry (MS). In DMS, a high–field asymmetric separation voltage is applied between 2 flat plates, and this gives a net drift for ions towards one of the plates. A small DC potential, compensation voltage, corrects the trajectory for an ion to enable transmission to the mass spectrometer inlet. Separation of isobaric species requires judicious selection of an SV value and determination of the optimal CoV to transmit the selected compounds. Despite the potential for impressive separations, typical tuning approaches cannot guarantee the best possible separations for isobaric species as they focus on sensitivity, not a balance of sensitivity and specificity.

MATERIALS AND METHODS

Experiments were conducted on a QTRAP 5500 system (SCIEX, Concord, ON) with a commercial DMS interface (SelexION+ device, SCIEX, Concord, ON). The DMS MS data acquisition, extraction of the optimization and construction of the alpha curves was facilitated with a research-grade DMS Tools plug in for the PeakView software. LC-MS experiments were conducted using an Agilent 1200 system.

Samples of amobarbital, pentobarbital, morphine, hydromorphone, norhydrocodone, noroxycodone, oxymorphone, and dihydrocodeine were prepared in 50/50 water/methanol with 0.1% formic acid for infusion analysis. Optimization data for each compound were collected using series of MRM acquisitions, each at a discrete SV (from 0V to 4000V, step 250V) and CoV ramp from -50V to 20V, step 0.1V, at different gas transport conditions, adding modifiers isopropanol, acetonitrile or ethyl acetate.

For each compound of interest, the optimum CoV was determined for each SV step together with the compound response and shape of CoV profile. The resulting CoV(SV) data were converted to an alpha function[1] indicated below and stored with the DMS settings in an analytical repository.

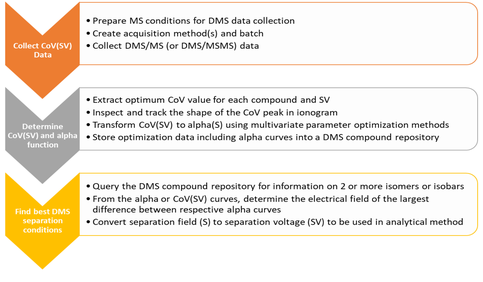
$$C = \frac{-\langle S\alpha \rangle}{1 + \langle \alpha \rangle}$$

Resulting alpha functions were queried to determine the best possible separation conditions for a mix of isobaric or isomeric compounds in the routine analytical methods.

RESULTS

Once the alpha curve is established for a given compound, the DMS behavior can be determined for the compound under the specific transport gas conditions that were used for alpha determination. In addition, known alpha function for a given compound allows to calculate the compensation voltage or field for any separation voltage or field setpoint. Established alpha functions allow to find the optimum DMS separation for a mix of compounds in an alpha dimension. This, followed by a conversion of the separation field into an SV value, enables in-silico DMS method optimization for a mix of compounds.

Figure 1: Automated DMS Optimization Workflow



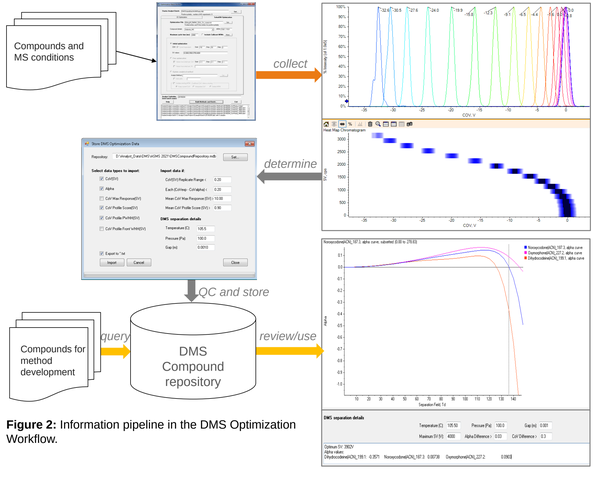
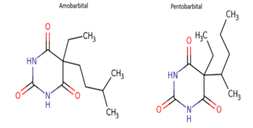


Figure 2: Information pipeline in the DMS Optimization Workflow.

Use case 1: Isomers Amobarbital and Pentobarbital



The two isomers differing in chain arrangement in position 5, exhibit similar behavior in DMS when nitrogen transport gas is applied. Adding the acetonitrile modifier causes the alpha curve differentiation leading to a difference function maximizing at separation field of 150Td. The CoV ionogram obtained at SV of 4000V shows the near-baseline DMS separation of the isomers.

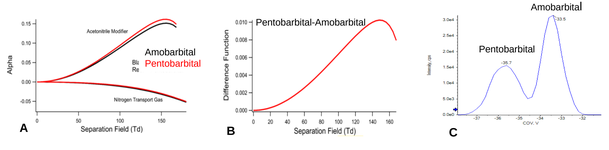
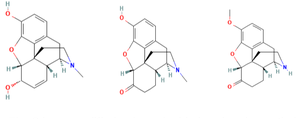


Figure 3: A – Alpha curves under different modifier conditions, B – differential alpha curve indicating optimum separation field value, C – ionogram of a mix of amobarbital and pentobarbital (SV = 4000V, modifier: ACN, 3%)

Use case 2: Resolving structural isomers ($C_{17}H_{19}NO_3$) morphine, hydromorphone and norhydrocodone by DMS to enable high throughput drug screening. Extremely similar structures result in nearly identical MS/MS spectra, making it impossible to choose unique MRM transitions for the LC/MS analysis of these 3 compounds.

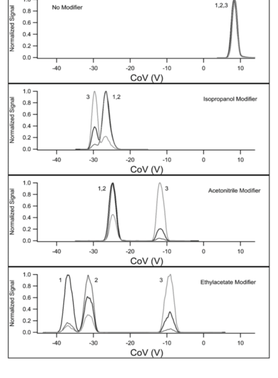
1. Morphine
2. Hydromorphone
3. Norhydrocodone



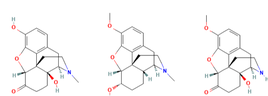
The 3 isomers differing by a double bond and a methyl group positions, could not be separated with a nitrogen transport gas.

The effect of addition of isopropanol, acetonitrile and ethyl acetate modifiers was studied. The isomers could be separated with 3% ethyl acetate addition to the transport gas.

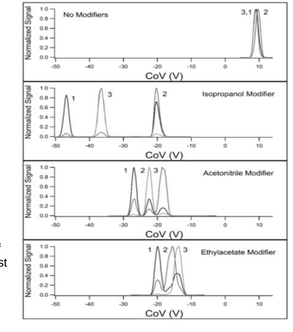
Figure 4: MRM ionograms of a mix of morphine, hydromorphone and norhydrocodone illustrating the effect of different chemical modifiers on the DMS separation.



Use Case 3: DMS optimization for separation of isobaric analogues Oxymorphone, Dihydrocodeine and Noroxycodone in support of drug screening (Figure 5).



1. Oxymorphone $C_{17}H_{19}NO_4$ (MRM 302.1/227.1)
2. Dihydrocodeine $C_{18}H_{23}NO_3$ (MRM 302.1/199.1)
3. Noroxycodone $C_{17}H_{19}NO_4$ (MRM 302.1/187.1)



The core of the structure of the 3 molecules is identical; just the position of a methyl group, double bond and substitution of methyl for hydroxy group differentiate them. Same as in the previous example, the MRM transitions use Q3 masses derived from fragments common to all 3 molecules. The addition of 1.5% isopropanol to the transport gas enables the best DMS separation.

CONCLUSIONS

- The automated approach to optimize DMS separations eliminates one of the key challenges for general applicability of DMS technology.
- Streamlined optimization method building for series of compounds saves time and prevents transcription errors.
- Optimization data extraction in parallel with the instrument performance control assures that only qualified data are used to derive compound DMS behavior.
- The results demonstrate the chemical orthogonality that can be realized in DMS separations by changing the chemical modifier.

REFERENCES

1 Schneider, B., Nazarov, E., Londry, F., Vouros, P., Covey, T. (2015). Differential mobility spectrometry/mass spectrometry history, theory, design optimization, simulations, and applications: DIFFERENTIAL MOBILITY SPECTROMETRY/MASS SPECTROMETRY. Mass spectrometry reviews. 35. DOI10.1002/mas.21453.

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