FAIMS separation of fentanyl-related compounds using vapor modification

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Overview

An improvement on the ion selectivity capabilities of a Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) system.

A vapor modifier addition system to aid in the separation of fentanyl, alfentanil, 4-ANPP, norfentanyl, and heroin was developed.

Introduction

Prior to analysis by mass spectrometry, FAIMS provides the ability to separate analytes with respect to a voltage parameter (CV) similar to separation in time with chromatography. This analyte selection, analogous to a quadrupole mass filter, is done by exploiting the difference in high and low electric field mobilities of analytes as they flow between two electrodes. These mobilities can be significantly affected by the composition of the carrier gas in the in system. With an abundance of solvent molecules available, a clustering-declustering effect has been known to occur in low and high fields respectively[1]. This impacts the cross-sectional area and collisional dynamics of the analyte thus magnifying the differential in high and low field mobilities.

Here, we characterize the potential to implement a vapor modifier addition system for the improvement of the separation capabilities of FAIMS in the specific domain of fentanyl and other related compounds.
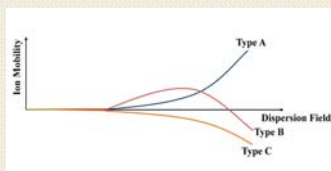

Figure 1. Diagram showing ion mobility types[2]

In FAIMS, compounds are classed into different groups based on their mobilities. These mobilities are manifested in the CV values required to facilitate transmission through the cell.
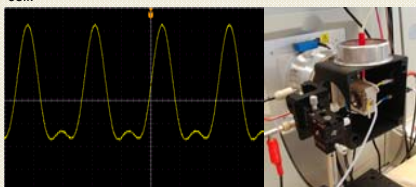

Figure 2. Bisinusoidal waveform used to supply dispersion voltage (DV) to the FAIMS cell and front end of instrument

Method

- **FAIMS/DMS system:** A planar FAIMS device (Heartland Mobility, Wichita, KS) approximately 35.5 mm long and 53 mm wide, with a gap of 1.87mm was used. The bisinusoidal asymmetric waveform applied to one of the FAIMS electrodes had an amplitude of 3.5 kV, a frequency of 870 kHz and an offset of 125 V. CV scans were performed at a rate of 12.5 V/min and applied to the electrode across from the one where the waveform is applied. CV scans were performed in triplicate and the extracted ion chromatogram for each compound was averaged with the other trials. The Dispersion plot was processed in Originlab software from spectra run in succession with 500V increments.

- **Mass Spectrometer:** A Thermo Fisher Scientific LTQ XL linear ion trap mass spectrometer was used for detection of the analytes. Injection time was set at 200 ms with one micro scan per spectrum over the 225-425 m/z range evaluated. An ESI capillary emitter was used at an effective ionization voltage of 2.5 kV and ran at 1μL min⁻¹

- **Vapor modifier addition system:** A custom modifier set up, shown in **Figure 3**, was assembled from a glass bubbler, two mass flow controllers, scientific tubing, Yor-Lok fittings, an acrylic flow meter and a humidity sensor. The nitrogen flow from the tank is split and regulated by two separate mass flow controllers. One flow controller (Alicat MC Series) supplies the nitrogen to the bubbler, where the vapor modifier is picked up, while the other (MKS GE50A) controls the flow rate of dry nitrogen that is later mixed with the humidified nitrogen flow. The desired humidity was achieved through manipulation of the flow rates through these mass flow controllers, while the final flow rate though the FAIMS cell was kept constant by venting off the excess flow.
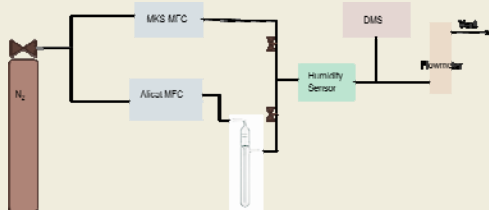

Figure 3. Schematic of the vapor modifier addition system used

- **Humidity sensor:** The relative humidity of the resulting mixture was monitored with a Honeywell HIH-4000-001 humidity sensor positioned in the gas flow. It was controlled by an Arduino Uno and was set to sample at a rate of 1 Hz, outputting units of relative humidity (RH).

- **Samples:** Norfentanyl oxalate, fentanyl, and alfentanil standards came diluted in methanol at a concentration of 1 mg/mL, heroin at the same concentration in acetonitrile, and 4-Aminophenyl-1-phenethylpiperidine (4-ANPP) at a concentration of 100 μg/mL in methanol (Cerilliant, Round Rock, Texas). Samples were created by dilution in methanol with 0.05% trifluoroacetic acid to nominal concentrations of 1 ppm.

Result

Ion Behavior

- All of the compounds analyzed exhibited multiple peaks along the CV axis. These have been attributed to the formation of multimers in the FAIMS cell that are subsequently broken into monomers prior to analysis[3].
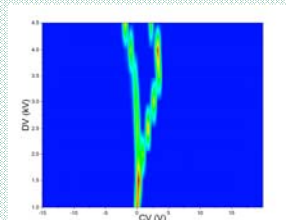

Figure 4. Shown is a dispersion plot of alfentanil obtained in humidified conditions

- These separated groups of multimers/monomers were found to exhibit a wide range of mobility behaviors even when evaluated in the same conditions. As shown in **Figure 4**, one group (left) demonstrated a decrease in mobility with respect to higher DV fields (Type C), while the other (right) initially showed the opposite behavior before a reversal (Type B) .

- The wide range of ion mobility types and magnitudes of these compounds when a modifier is used beget the potential for high resolution separation under certain conditions.

Comparison of modified vs dry

- The best separations obtained *without* the use of a modifier (**Figure 5a**) were still significantly overlapped. All were found at a negative CV, thus showing "hard sphere" type A behavior.
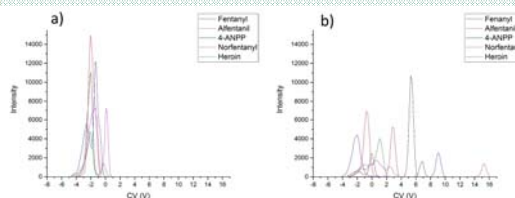

Figure 5. (a) overlaid EICs of a 5 compound mixture when evaluated at 3.5kV DV when only dry nitrogen is used as a carrier gas. (b) Humidified at 50.9% RH

- When a water modifier is added into the system, a significant improvement in the overall separation of these compounds can be observed while several of the peaks are baseline resolved.

Result

- The increased range of mobilities afforded by modifier usage is not limited to one particular DV field. Shown in **Figure 6**, significant occlusion of fentanyl and heroin occurs at every field evaluated. In comparison, a humidified system shows better separation.
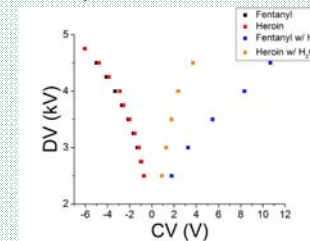

Figure 6. Dispersion tracks of heroin and fentanyl in dry and humidified conditions.

- The use of methanol as a modifier was also evaluated for this purpose and showed similar beneficial separation effects, but was not pursued further as reproducibility issues arose from the inability of this vapor modifier addition system to accurately produce and quantify the concentration of methanol in the carrier gas.

Conclusion

- Use of water as a modifier has shown and improvement in the FAIMS based separation of fentanyl and related compounds when compared to the use of dry nitrogen alone as a carrier gas.

- Implementation of this technique may be used in lieu of chromatography for rapid targeted sample analysis

Future Work

- Evaluation of more structurally similar analytes that may provide more of a challenge to traditional separation techniques.

- Use of other modifiers for further improvements on separations/ creation of a quantitative addition system for them.

Acknowledgement

References

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(3) Kolakowski, B. M.; Mester, Z. *Analyst* **2007**, *132* (9), 842–864.