Analysis of Volatile Organic Compounds in Human Breath by FAIMS/MS

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Overview

- Direct detection of VOCs in exhaled breath by MS and standalone FAIMS
- Comparing the performance of APCI-MS, ESI-MS, and FAIMS for breath analysis

Introduction

Modern breath analysis techniques can be traced to the work of Pauling in the early 1970s.[1] Over the past few decades many advancements have been made; however, a single method is yet to provide adequate sensitivity, selectivity, and speed of analysis. Field-asymmetric ion mobility spectrometry (FAIMS) coupled to mass spectrometry (MS) has the potential to achieve all three. Recently discovered was the dramatic increase in selectivity of FAIMS when the carrier gas is saturated with solvent vapor.[2] As human breath is already saturated with water, analysis by FAIMS/MS seems to prove an ideal pairing. By analyzing breath with FAIMS/MS, we may achieve data in real-time with resolution on par with capillary gas chromatography.

Instrumentation and Methods

Data were collected on two instrumental setups: a Thermo Scientific LTQ XL mass spectrometer using corona-discharge atmospheric pressure chemical ionization (APCI), and a standalone micromachined FAIMS chip (Owlstone Lonestar Portable Gas Analyzer) using ^{63}Ni ionization. The breathing apparatus used was an Intoximeters, Inc. mouthpiece with a one way check valve. For MS, the apparatus fit into a home-made ceramic face plate on the front of the IonMax ionization chamber. For FAIMS, the apparatus was held orthogonal to, and a few centimeters above, the gas inlet.

APCI-MS Settings:
- Sheath Gas Flow Rate = 20 arb.
- Auxiliary Gas Flow Rate = 0 arb.
- Discharge Current = 20 µA
- Capillary Temperature = 100 °C
- APCI Source kept at room temperature

FAIMS Settings:
- Analytical gap = 35 µm
- Length of cell = 300 µm
- FAIMS cell heated to around 55.5 - 60 °C

Standards Analysis

APCI-MS: (Fig. 1)
Blank of 95% Ethanol, with all neat standards diluted to 0.25M in 95% ethanol
Standard solutions were injected at a flow rate of 5µL/min.

FAIMS: (Fig. 2-4)
Neat standard was placed 5cm from gas inlet and the vapors were pulled through the FAIMS cell by a mechanical pump attached to the exhaust, at a flow rate of 1.50L/min.

Breath Analysis*

APCI-MS: (Fig. 5)
95% Ethanol solvent was injected at a flow rate of 5µL/min, while the breath sample was introduced to the system through the valve mentioned above. Neat standards were diluted in 95% Ethanol to 250 ppm.

FAIMS: (Fig. 6)
Breath sample was blown through valve toward gas inlet, as mentioned above. Flow rate through the cell was 1.50 L/min. Neat standards were diluted in 95% Ethanol to 250 ppm.

**All breath sampling conducted by placing 10µL of standard solution directly onto tongue about 10 seconds prior to sampling.*

Results and Discussion

Direct Standard Analysis

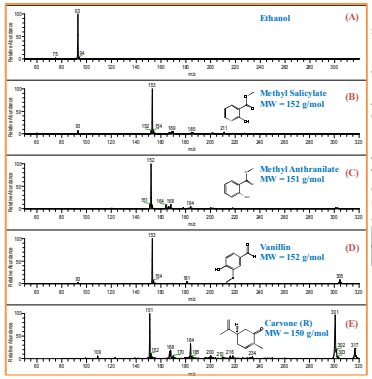

Figure 1: MS spectra of specified standard solutions.

Ethanol (A) yielded an ion at m/z 93, corresponding to $[2M+H]^+$. Methyl Salicylate (B), Methyl Anthranilate (C), Vanillin (D), and Carvone (E) all demonstarte an $[M+H]^+$.
Both Vanillin (D), and Carvone (E) yielded dimer peaks at $[2M+H]^+$. Various oxidation products were observed for all four standards (B) - (E) at $[M+O+H]^+$, $[M+O_2+H]^+$, and $[2M+O+H]^+$.
Each standard solution demonstrates most abundant peak at $[M+H]^+$.

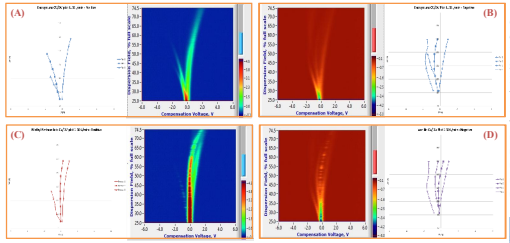

Figure 2: 3D plots of Dispersion Field (DF) vs Compensation Voltage (CV) vs Ion Current (IC) -- The unit of DF for these 3D plots are in "% full scale," where 100% is equal to 275V. The 3D plots were generated by standalone FAIMS.
2D plots of Dispersion Voltage (DV) vs CV, where the CV is taken at the IC peak maximum of the respective plot. The 2D plots were created in Microsoft Excel to facilitate visual peak separation.
(A): Positive Mode, Background (no sample)
(B): Negative Mode, Background (no sample)
(C): Positive Mode, Methyl Anthranilate
(D): Negative Mode, Vanillin
(E): Overlay of 2D plots from (A) & (C) (left); Overlay of 2D plots from (B) & (D) (right)
Demonstrates ability of standalone FAIMS to distinguish standard from background air, at a specific DV

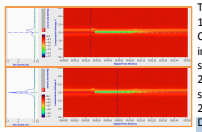

Figure 3: 3D plots (right) of CV vs Time vs IC (right) – Taken at DV 145.75V; and 2D plots (left) of IC vs CV. 3D plots are the same, but indicate the point from which the 2D signal is taken.
2D plot (top) shows air background signal
2D plot (bottom) shows Vanillin signal.
Demonstrates that standalone FAIMS can distinguish standard from background air in real time.

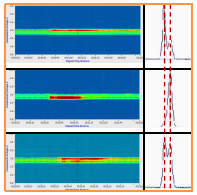

Figure 4: 3D plots (left) of CV vs Time vs IC – Taken at DV 154V; and 2D plots (right) of IC vs CV, where the CV (x-axis) of each peak is equal to the CV in the corresponding 3D plot (y-axis).
*The vertical dashed red line (right) corresponds to horizontal dashed red (left)
Plots of Methyl Anthranilate only (top), Carvone only (middle), both Methyl Anthranilate and Carvone (bottom)
Demonstrates that standalone FAIMS can distinguish between two similar compounds in the same standard solution in real time.

Breath Analysis

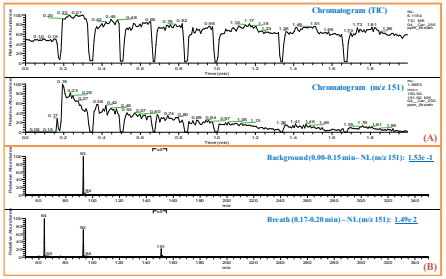

Figure 5: APCI-MS chromatograms and spectra collected by spraying Ethanol solvent and breathing into instrument at specific intervals – Each decrease in intensity in the chromatograms (A) corresponds to single breath.
Peak at m/z 151 (Carvone $[M+H]^+$ peak) is initially insignificant ((B) top), but upon collection of breath sample, m/z 151 increases dramatically ((B) bottom).
Demonstrates that APCI-MS can detect ppm quantities of standard in breath sample in real time.

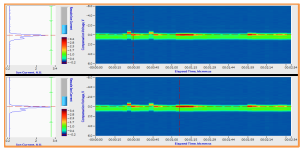

Figure 6: 3D plots (right) of CV vs Time vs IC – Taken at DV 145.75V; and 2D plots (left) of IC vs CV.
3D plots are the same, but indicate the point from which the 2D signal is taken. Produced by running no sample to collect background signal, collecting breath samples at 20 and 40 sec, then collecting neat standard at 60 sec.
2D plot (top) shows Methyl Anthranilate signal in breath.
2D plot (bottom) shows Methyl Anthranilate neat standard signal.
Demonstrates that standalone FAIMS is capable of real-time breath analysis for volatile organic flavorants.

Conclusions

- Standalone FAIMS can detect VOC in breath (Fig. 2)
  - Distinction possible between different compounds (Fig. 3)
  - Separation/distinction possible between compounds in a single mixture(Fig. 4)
- APCI-MS and standalone FAIMS provide the capability for detection of VOCs in breath (Figs. 5 & 6)
  - Both can perform this breath analysis in real time
  - Distinction possible between different samples
  - ESI-MS also performed and capable of VOC detection and breath analysis in real-time, results very similar to APCI-MS so not shown

Future Directions

- Coupling FAIMS and MS together will allow for determination of specific FAIMS peaks.

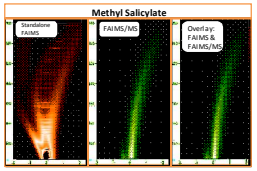

Figure 7: Plots of Dispersion Field (in Td) vs Compensation Field (in Td).

FAIMS/MS data in figure obtained on a custom Owlstone FAIMS chip interfaced to an Agilent 6400 triple quadrupole mass spectrometer, operating in APCI with single ion monitoring of m/z 153.

- Detection of nonvolatile compounds (biomarkers of disease) with standalone FAIMS and FAIMS/MS could prove of biomedical significance.
- Use of different FAIMS cells will allow performance of carrier gas saturation in order to improve selectivity of neat standards, and standards in breath.
- Use of different solvent modifiers in FAIMS has shown potential for improved selectivity.

Acknowledgements

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References

1. Pauling, L.; Robinson, A.; Teranishi, R; Cary, P. *Proc. Natl. Acad. Sci.* **1971**, 68, 2374-2376
2. Rorrer, L.; Yost, R. *Int. J. Mass Spectrom.* **2011**, 300, 173-181