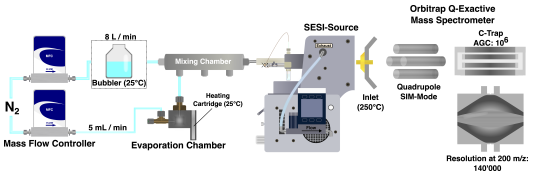
Can breath components be quantified with secondary-electrospray ionization coupled to mass spectrometry?

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Absolute quantification is desirable for any analytical technique, especially in the context of medical diagnosis. As an alternative to traditional blood analysis, on-line breath analysis using secondary-electrospray ionization (SESI) coupled to mass spectrometry (MS) could serve as a non-invasive diagnostics tool. To reach the full potential of this technique, a gas standard generation system based on the controlled evaporation of liquid analytes and their dilution in a carrier gas stream was developed to produce low-concentration (down to part-per-trillion) standards.[1] This system can operate at the same flow rates as typical human exhaled breath and under humid conditions. To test the analytical capabilities of the system, short-chain fatty acids were used to test the limits of detection and quantification, as well as the linearity. To elucidate whether this system could be used to calibrate a SESI-MS system externally, ion suppression effects also had to be characterized. Ion suppression was postulated to be potentially present in SESI[2], which was investigated using the gas generation system described above. It was shown that ion suppression in SESI is mainly a gas-phase phenomenon. For this purpose, gas standards of D_6-acetone, D_3-acetic acid and pyridine were generated and their impact on each other's signal was determined. D_3-acetic acid seemed to be most affected by rising levels of D_6-acetone and pyridine, whereas pyridine was the least affected by increasing the concentrations of the other two compounds. This indicated a mechanistic rationale for signal suppression within SESI related to gas-phase acid-base chemistry. Pyridine with the highest gas-phase basicity was not affected by increasing concentrations of D_3-acetic acid and D_6-acetone, while the other two compounds that have lower basicity are more strongly affected when the concentration of pyridine was increased. Regarding quantification of breath metabolites with SESI-MS, the use of internal standards is advised.



(1) Wüthrich, C.; Fan, Z.; Vergères, G.; Wahl, F.; Zenobi, R.; Giannoukos, S. Analysis of Volatile Short-Chain Fatty Acids in the Gas Phase Using Secondary Electrospray Ionization Coupled to Mass Spectrometry. *Analytical Methods* **2023**, *15* (5), 553–561. https://doi.org/10.1039/D2AY01778D.

(2) Bruderer, T.; Gaugg, M. T.; Cappellin, L.; Lopez-Hilfiker, F.; Hutterli, M.; Perkins, N.; Zenobi, R.; Moeller, A. Detection of Volatile Organic Compounds with Secondary Electrospray Ionization and Proton Transfer Reaction High-Resolution Mass Spectrometry: A Feature Comparison. *J Am Soc Mass Spectrom* **2020**, *31* (8), 1632–1640. https://doi.org/10.1021/jasms.0c00059.