Improved performance of elemental composition determination through true internal calibration on a compact MS system

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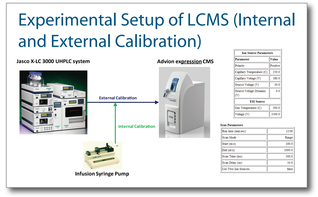
Introduction

A recently introduced and more economical Quadrupole compact mass spectrometer affording unit mass resolution was used to achieve elemental composition determination of unknown compounds through instrument line shape calibration technology. Briefly, this comprehensive mass spectral technology calibrates not only *m/z* values, but more importantly, the mass spectral peak shape. Consequently, symmetrical and mathematically well-defined mass spectral peak shape is obtained to allow accurate mass measurement on unit resolution mass spectra even when the monoisotopic peak (A) is overlapped with the corresponding A+1 peak. In addition to the high mass accuracy thus achieved, the high spectral accuracy available from a quadrupole system allows for exact isotope modeling to achieve elemental composition determination, much like one would achieve with a high resolution MS system such as TOF or qTOF. In this work, both external and internal calibration standards were investigated with their respective mass accuracy as well as the effectiveness of elemental composition determination.

Experimental Setup of LCMS (Internal and External Calibration)

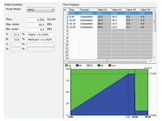


Methods

1. Infusion of calibrant (Agilent ESI Tune Mix G2421) – 5 minute Acquisition
   Followed by LCMS analysis of a custom standard mix (External Calibration) - Separate Analysis
2. Infusion of Agilent ESI Tune Mix G2421 using a syringe pump (25µL/min) into the LCMS flow (post-column – using a zero dead volume Tee with micro-flow check valve).
   LCMS analysis of custom standard mix (Internal Calibration) – Simultaneous Analysis

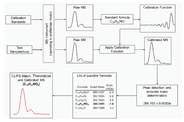
Custom Standard Mix Compound List
Caffeine
Terfenadine
Acetaminophen
Sulfadimethoxine
Verapamil
Reserpine
Indomethacin

Agilent Zorbax XDB-C18
2.1x50mm 5µm
10µL Injection

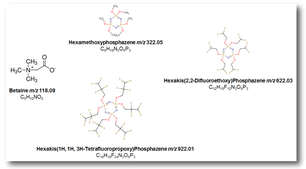


MassWorks Calibration+CLIPS Formula ID

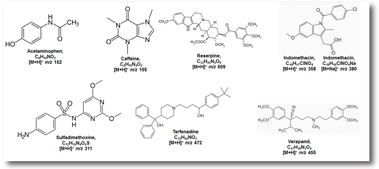
All data files of samples and calibration standards were acquired in profile mode and directly read by MassWorks (Cerno Bioscience, Norwalk, CT) for accurate mass measurements and elemental composition determination. The instrument line shape calibration must be first generated either with external or internal calibration. The calibration is then applied to sample data files to achieve high mass and spectral accuracy for elemental composition determination.



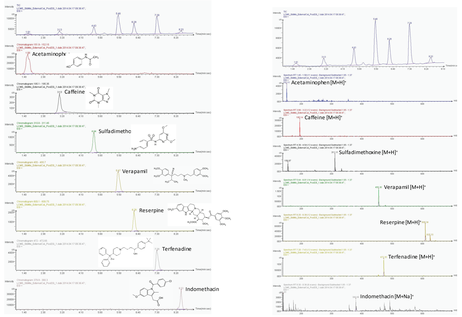
Agilent ESI Tune Mixture Compounds



LCMS Analysis Mixture



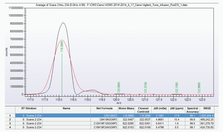
LCMS Analysis of Custom Standard Mix



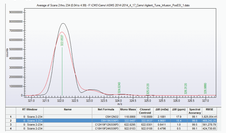
Results

LCMS – External Calibration

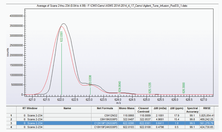
Calibration Standard - Betaine *m/z* 118.09 $C_5H_{12}NO_2$



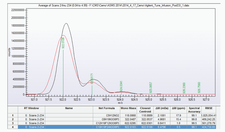
Calibration Standard - Hexamethoxyphosphazene *m/z* 322.05 $C_6H_{19}N_9O_6P_3$



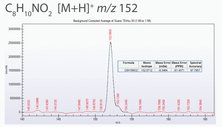
Calibration Standard - Hexakis(2,2-Difluoroethoxy)Phosphazene *m/z* 622.03 $C_{12}H_{19}F_{12}N_9O_6P_3$



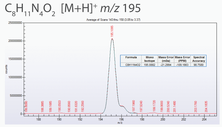
Calibration Standard - (1H, 1H, 3H-Tetrafluoropropoxy)Phosphazene *m/z* 922.01 $C_{18}H_{19}F_{24}N_9O_6P_3$



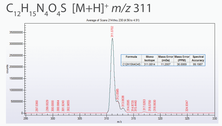
Acetaminophen - $C_8H_{10}NO_2$ $[M+H]^+$ *m/z* 152



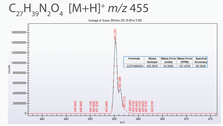
Caffeine - $C_8H_{11}N_4O_2$ $[M+H]^+$ *m/z* 195



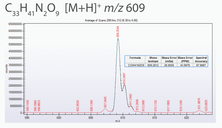
Sulfadimethoxine - $C_{12}H_{15}N_4O_4S$ $[M+H]^+$ *m/z* 311



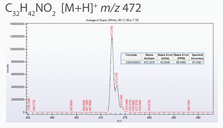
Verapamil - $C_{27}H_{39}N_2O_4$ $[M+H]^+$ *m/z* 455



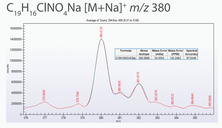
Reserpine - $C_{33}H_{41}N_2O_9$ $[M+H]^+$ *m/z* 609



Terfenadine - $C_{32}H_{42}NO_2$ $[M+H]^+$ *m/z* 472

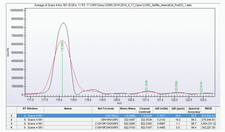


Indomethacin - $C_{19}H_{16}ClNO_4Na$ $[M+Na]^+$ *m/z* 380



LCMS – Internal Calibration

Calibration Standard - Betaine *m/z* 118.09 $C_5H_{12}NO_2$



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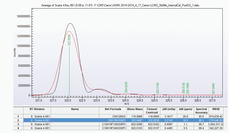

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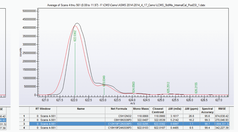

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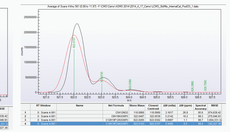
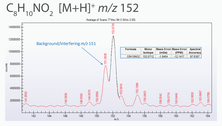
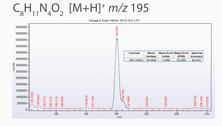


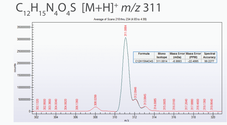
Acetaminophen - $C_8H_{10}NO_2$ $[M+H]^+$ *m/z* 152



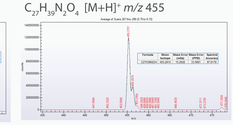
Caffeine - $C_8H_{11}N_4O_2$ $[M+H]^+$ *m/z* 195



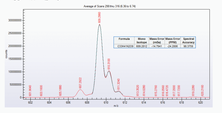
Sulfadimethoxine - $C_{12}H_{15}N_4O_4S$ $[M+H]^+$ *m/z* 311



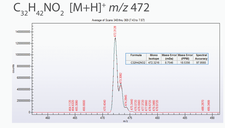
Verapamil - $C_{27}H_{39}N_2O_4$ $[M+H]^+$ *m/z* 455



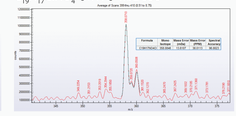
Reserpine - $C_{33}H_{41}N_2O_9$ $[M+H]^+$ *m/z* 609



Terfenadine - $C_{32}H_{42}NO_2$ $[M+H]^+$ *m/z* 472



Indomethacin - $C_{19}H_{17}ClNO_4$ $[M+H]^+$ *m/z* 358



Summary

Compound	External Calibration (mDa)	Internal Calibration (mDa)
Acetaminophen	-9.35	-1.85
Caffeine	-21.30	-7.10
Sulfadimethoxine	11.20	-7.00
Verapamil	55.28	15.28
Reserpine	26.81	-14.79
Terfenadine	42.05	8.75
Indomethacin*	54.06	13.61

Conclusion

With the novel instrument line shape calibration technology embedded in the MassWorks software, the expression compact mass spectrometer can achieve sufficient high mass and spectral accuracy to enable elemental composition determination of unknown compounds or for compound confirmation. While the utilization of internal calibration requires additional experimental setup, it does provide better mass accuracy to help minimize false positives when compared to external calibrations.