Enabling Accurate Mass Elemental Composition Determination on a New Compact Mass Spectrometer – Application to detection and identification of radiopharmaceuticals

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Overview:

Though it has been shown previously that high mass accuracy and elemental composition determination is feasible on conventional quadrupole GC/MS and LC/MS systems, it remains to be seen that such capability could be carried over to a more economical compact MS system so that more users of MS systems could have access to such capability typically reserved for high resolution MS systems such as TOF, Orbitrap, or FT ICR MS which come at significantly more cost while requiring highly trained specialists in their maintenance and operations.

Introduction:

The rapid identification of radiolabeled compounds would clearly be beneficial for applied clinical and preclinical tracers used for imaging with positron emission tomography (PET). The limited uptake of mass spectrometry in the imaging field has been due arguably to several factors including concerns regarding adequate sensitivity, instrument footprint in the highly constrained space requirements for the majority of labs and high initial capital costs plus high ongoing maintenance costs.

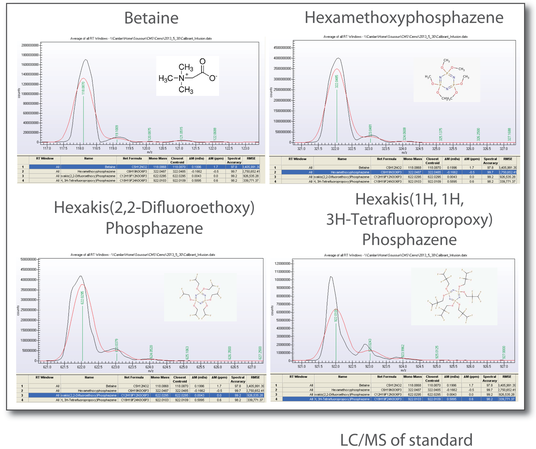
The introduction of the expression Compact Mass Spectrometer (CMS) at the 2012 Spring ACS National Meeting addressed for the first time three of the concerns, space, capital cost and maintenance costs. Adequate sensitivity is required to measure the small amounts of labeled compound involved. High spectral accuracy is required to allow the Cerno MassWorks specal matching software to precisely identify the chemical formulae of the analytes. Before testing the system on radiopharmaceuticals, the basic performance was validated using standard compounds.

Methods:

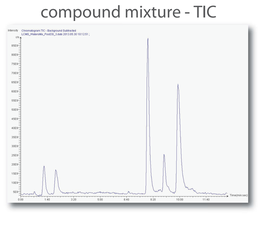
A new compact mass spectrometer is calibrated by direct infusion of a mixture of compounds spanning the m/z range of 100-1000. In this case a mixture of Betaine, Hexamethoxyphosphazene, Hexakis(2,2-Difluoroethoxy)Phosphazene, Hexakis(1H, 1H, 3H-Tetrafluoropropoxy)Phosphazene was used. This is available as the Agilent Technologies ESI tuning mix. (Agilent Technologies, P/N G2421A). By acquiring unprocessed profile mode MS data of these standards, it is possible to build a novel MS calibration which would calibrate not only the m/z, as is conventionally done, but also the MS peak shape. Such a comprehensive calibration could then be applied to a sample containing unknown ions in the calibration range of *m/z* 100-1,000 in either direct infusion or LC separation mode. The calibration of MS peak shape into known mathematical function allows for the accurate determination of m/z values even when the MS peak width is of unit mass resolution.

Results:

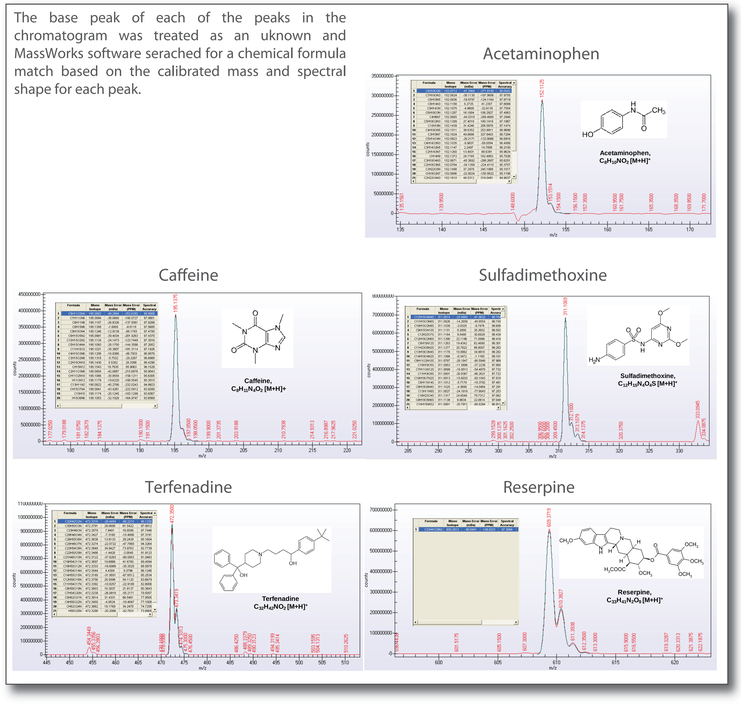
The following figues show the MassWorks (Cerno Bioscience, Norwalk, CT) mass calibration and peak shape calculation for these compounds. The average spectral accuracy is 97.8% showing a very close match between the theoretcial and actual quadrupole performance.



In order to test the accuracy of the calibration is was applied to an LC-CMS run of a standard test mix containing Acetominophen, Caffeine, Sulfadimethoxine, Reserpine and Terfenadine.



The base peak of each of the peaks in the chromatogram was treated as an uknown and MassWorks software serached for a chemical formula match based on the calibrated mass and spectral shape for each peak.



Mass confirmation of 18 PET radiopharmaceutical standards

Standards	Predicted m/z	Observed m/z	Minimum Detection Level
^{12}C-PIB	+257.1	+257.1	500 pg
^{12}C-Raclopride	+347.1	+347.2	10 pg
^{12}C-SA4503	+369.2	+369.2	50 pg
^{19}F-EF5	+303.0	+302.9	2.5 ng
^{19}F-FAZA	-282.2	-282.1	1 ng
^{19}F-FBPA	-217.1	-217.1	500 pg
^{19}F-FEAU	-237.2	-237.2	100 pg
^{19}F-FHBG	+256.1	+256.2	1 ng
^{19}F-FLT	-242.1	-243.2	1ng
^{19}F-FPPRGD2	+807.5	+807.9	2 ng
^{19}F-FSPG	-206.1	-206.2	5 ng
^{19}F-FTC-146	+337.2	+337.2	10 pg
^{19}F-FU	-129.1	-129.2	100 pg
^{19}F-P3BZA	+240.2	+240.3	50 pg
^{19}F-SPARQ	+451.1	+451.2	200 pg
^{19}F-TFSB	-347.0	-347.1	2.5 ng
6-^{19}F-fluoro-dopamine	+172.2	+172.1	1 ng
6-^{19}F-fluoro-L-DOPA	+216.1	+216.2	1 ng

- Eighteen reference compounds were detected at low mass levels (pg to ng) with ESI/MS and confirmed with >95% spectral accuracy.
- Eleven tracers (2 CA ^{18}F-labeled compounds, 6 NCA ^{18}F-labeled compounds, 3 ^{11}C-methylated compounds) were collected and tested after HPLC purification.
- Eight of these tracers 3 ^{11}C-methylated compounds (^{11}C-SA4503, ^{11}C-PIB, 11C-Raclopride), 2 CA ^{18}F labeled compounds (^{18}F-EF5, ^{18}F-TFSB) and 3 NCA ^{18}F-labeled compounds (^{18}F-FTC-146, ^{18}F-P3BZA, ^{18}F-FBPA, SR 1~3 Ci/umol), could be detected by mass identification of respective parent ions of ^{19}F and/or ^{12}C carrier compounds following HPLC elution.

Conclusion:

- The expression CMS single quadrupole LC/MS with spectrum analysis is:
  - Fast enough to measure coumpounds containing short-lived isotopes in a matter of minutes
  - Sensitive enough to measure small amounts of active raiopharamceuticals
  - Has sufficient spectral accuracy to allow accurate determination of chemical formulae
- Detection and identification of CA ^{18}F-labeled and tertiary amino compounds was straightforward.
- Ionization and chromatographic condition optimization is still required for some of the tracers evaluated.