A Novel Approach for Elemental Composition Determination of Pharmaceuticals by Accurate Mass Measurements and Isotope Pattern Recognition

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Overview

- Accurate Mass measurement and elemental composition determination made available on unit mass resolution mass spectrometers
- Novel algorithms to calibrate mass spectrometers for both mass and peak shape
- Elemental composition determination facilitated by isotope pattern recognition
- Demonstrated data including infusion and loop injection of drug standards and LC/MS of microsome incubations

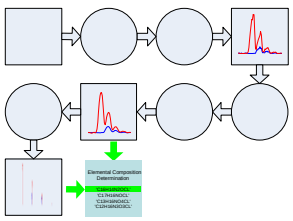
Introduction

The determination of elemental composition for compounds is usually achieved by accurate mass (AM) measurements on high resolution mass spectrometers such as qTOF, high resolution TSQ, and FTMS. These instruments deliver mass accuracy in low ppms and greatly enhance specificity of the determination of elemental compositions for small pharmaceutical molecules. However, more than often the results of elemental composition search are not unique with quite a few of possible formulas even at 1ppm mass accuracy. Obviously, AM measurements alone are not sufficient for the unique determination of the elemental composition of these molecules. We propose a novel approach to improve the determination of elemental compositions by combining the AM measurements and isotope pattern recognition.

Methods

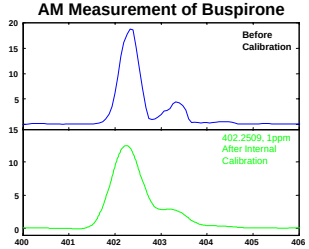
- All the Data acquired in profile mode and processed by the HAMSCA (Highly Accurate Mass Spectral Calibration Approach) implemented in the MSIntegrity™ software from Cerno Bioscience.
- Infused standards of either Poly-Alanine or sodium trifluoroacetate for external calibration combined with internal calibration for highest possible mass accuracy.
- Loop injection of a drug mixture of diazepam and sulfamethoxazole acquired on a Waters Quattro Ultima.
- LC/MS of the metabolites of rat microsome incubation of buspirone performed on Thermo Electron LCQ Classic.
- C18 column with gradient for separation of the metabolites of RM incubation of buspirone.

Calibration and Elemental Composition Determination Procedures

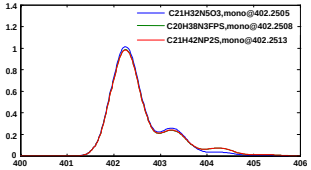


Results and Discussion

AM Measurement of Buspirone



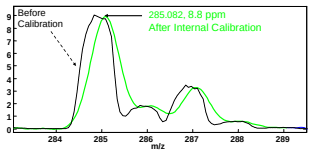
Top 3 Hits of Isotope Pattern Matching



Detailed Search Results

Formula	RMSE	PPM
C21H32N5O3	15620	1.0
C21H39NOFP2	19622	4.5
C20H35N3OF2P	21148	5.7
C21H42NP2S	23132	-1.0
C20H38N3FPS	23997	0.2
C19H34N5F2S	25033	1.5
C19H37N3O4P	25219	-3.2
C18H33N5O4F	26952	-2.0
C18H40NO2F2P2	33948	1.7
C18H43NOFP2S	34285	-4.0
C18H38NO4F2S	35279	4.7
C17H36N3O2F3P	35924	3.0
C17H39N3OF2PS	35951	-2.7
C16H35N5OF3S	37679	-1.2
C16H32N5O2F4	37931	4.2
C16H44N3P4	41375	5.7
C15H34N5O5F2	42629	-4.7

AM Measurement of Diazepam



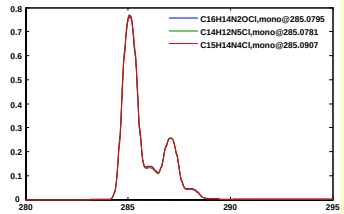
Detailed Search Results

Elemental Composition	RMSE (1.0e+007)	Mass Errors (ppm)
C16H14N2OCL	0.1400	8.8
C14H12N5CL	0.1488	13.7
C15H14N4CL	0.1527	-30.5
C7H16N5O5CL	0.261	-7.0
C12H15N4CL2	0.7239	51.2
C13H17N3CL2	0.7242	7.0
C9H19N4O2CL2	0.7282	-22.8
C8H17N5O2CL2	0.7295	21.4
C14H19N2CL2	0.7303	-36.8
C10H19N2O3CL2	0.7316	16.5
C14H17NOCL2	0.7317	46.7
C15H19OCL2	0.7341	2.5
C9H17N3O3CL2	0.7376	60.7
C11H19O4CL2	0.7393	56.1
C5H19N4O5CL2	0.7608	30.5
C19H11NO2	1.0257	10.5
C18H11N3O	1.0337	-28.8

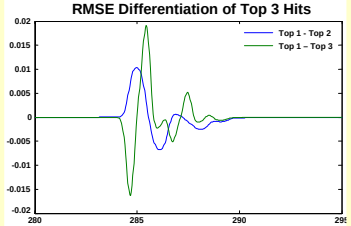
Vendor SW Search Results

Elemental Composition	Δ Mass (mmu)	Mass Errors (ppm)
C15H19OCl2	0.7	2.5
C16H14N2OCl	2.5	8.9
C17H9N4O	4.4	15.3
C10H19N2O3Cl2	4.7	16.6
C15H13N2O4	-5.5	-19.4
C16H13O5	5.7	20
C9H19N4O2Cl2	-6.5	-22.8
C11H14N4O3Cl	6.6	23
C14H18O4Cl	-7.4	-25.8
C15H14N4Cl	-8.7	-30.5
C20H13O2	-9.6	-33.5
C14H19N2Cl2	-10.5	-36.9
C17H14O2Cl	13.8	48.3

Top 3 Hits of Isotope Pattern Matching



RMSE Differentiation of Top 3 Hits



Conclusions

- AM alone is not sufficient and may lead to wrong formula even with near-perfect match in mass.
- Combining AM with profile match outperforms conventional search but requires comprehensive mass spectral calibration to calibrate peak shape in addition to mass.
- The novel algorithm makes it possible to perform the elemental composition determination on a unit mass resolution mass spectrometer.