Cerno Application Note

Extending the Limits of Mass Spectrometry

Determination of Elemental Composition with an Ion Trap at Unit Mass Resolution

While high mass accuracy is important for elemental composition determination, proper peak shape calibration can allow accurate isotope profile matching that can lead to dramatically more accurate results even on spectra of moderate mass accuracy.

Introduction

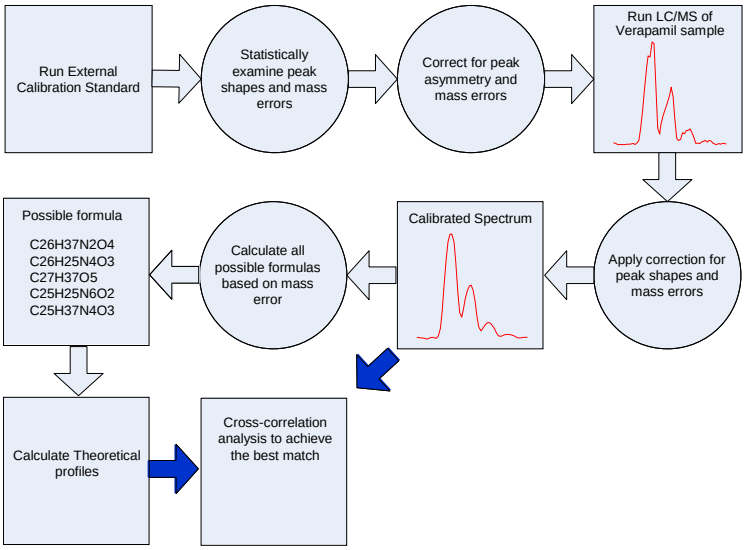
With the advent of high resolution mass spectrometers, accurate mass measurements at low parts per million (ppm) by LC/MS analysis can be achieved and allow for the possible elemental composition determination of unknown compounds. Depending critically on the mass accuracy, confident determination of elemental composition is obtained at the high cost of expensive instruments and often requires skilled instrument operators. Although high mass accuracy plays an significant role in reducing possible search hits, the mass accuracy alone often can not provide sufficient specificity to effectively determine an unknown molecule even at a very high mass accuracy of 1 ppm. For example, an elemental search for hydroxylated busprione resulted in three different molecular formula, C21H32N5O3 (1 ppm), C21H42NP2S (-1 ppm), and C20H38N3FPS (0.2 ppm). Here, a unique search strategy was developed based on a unique calibration technology, allowing for the elemental composition determination on conventional unit mass resolution instruments such as a Thermo Electron LXQ with less stringent mass accuracy requirements. This is demonstrated with LC/MS of verapamil metbolites from rat microsomal incubation.

Experimental

Sample information: Verapamil (10 µM) was incubated in 0.1 M potassium phosphate buffer (pH 7.4), magnesium chloride (4 mM), rat liver microsomes (1 mg ml-1 microsomal protein) and NADPH (1 mM). The incubation reactions lasted for one hour at 37°C. A mixture of calibration standards consisting of eight small pharmaceutical molecules with molecular ions at m/z = 158, 368, 470, 477, 508, 609, and 714 was made in water and acetonitrile.

MS conditions: A Thermo Electron LXQ was used for analysis of verapamil metabolites. The mass spectrometer was operated on positive electrospray ionization mode with the high voltage set to 4.5 kV and the capillary temperature tuned to 330 °C. Sheath gas flow and aux gas flow were adjusted to be 40 and 5 (arbitrary units), respectively. All spectra were collected in profile mode for analysis.

Data acquisition and analysis: Scheme 1 describes the data processing flow. The spectra of the calibration standards were acquired first and used for creating the calibration with MassWorks software. This calibration was then applied to LC/MS data files to produce the



Scheme 1. Data processing work flow for profile searching

spectra with a mathematically defined symmetric peak shape as well as accurate mass values. Based on the estimated mass error, a list of all possible molecular formula was determined and their corresponding theoretical profile spectra were calculated. The calibrated profile spectra were then evaluated and compared with the theoretical spectra to generate a statistical measure, RMSE (Root Mean Squared Error), based on which the possible formulas will be ranked to propose the most probable elemental compositions.

Results

Verapamil is a calcium channel blocker commonly used to treat hypertension and coronary artery disease. Its in vitro metabolism is dominated by demethylation since there are five possible sites where a methyl group can be cleaved during microsomal incubation. Two major components of verapamil and its demethylated metabolite were observed by LC/MS as shown in Figure 1. The molecular ion of verapamil and its sodium adducts were found at m/z of 455 and 477, respectively, while the demethylated metabolite at m/z of 441 was also detected with its sodium adduct at m/z of 463.

The external calibration created from the calibration mixture was then applied to the raw profile mode spectra. The MassWorks MSIntegrity algorithm not only improves the mass accuracy, but, most importantly for this work it corrects the profile mode spectra to a mathematically defined peak shape. Due to the external calibration used and the possible space charge effect intrinsic to ion trap instruments, the mass accuracy for verapamil and its related molecules can only be measured to within 50 mDa. The mass errors at this level are too large to perform effective elemental composition determination by conventional search algorithms which depend solely on mass accuracy.

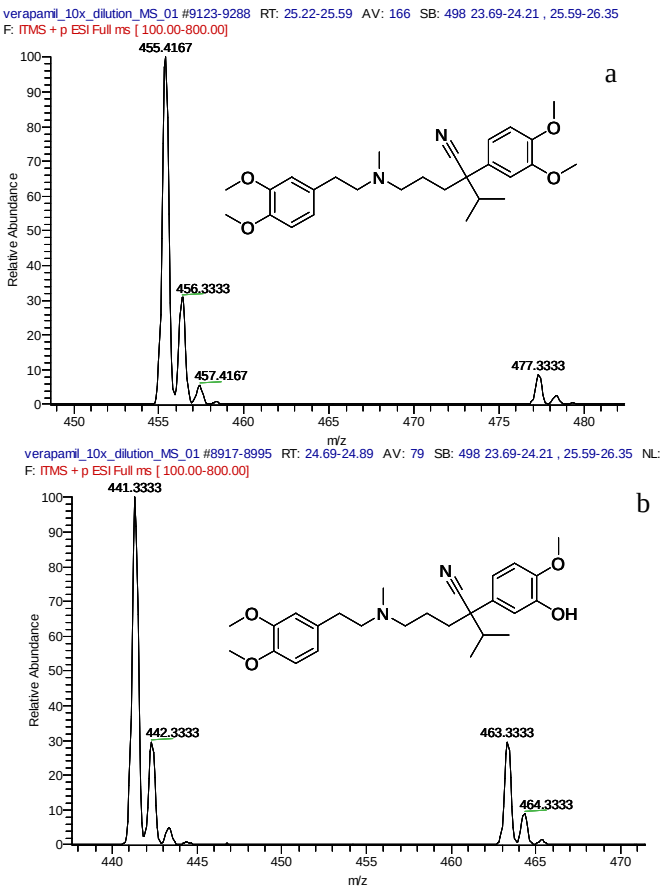


Figure 1. Full scan MS spectra of verapamil and its sodium adduct (a) and demthylated verapamil and its sodium adduct (b)

The improved mass accuracy obtained from the calibration (from a few hundred mDa to < 50 mDa) significantly reduces the number of potential candidate formulas. The candidate formulas are used to generate theoretical isotope profiles which were then matched to the experimentally measured spectra. Attempts to use isotope profile information to help identify unknown compounds have been reported previously with only limited success, primarily because these methods have to make rough assumptions on the peak shape function. A Gaussian peak shape was typically assumed, even though a finely-tuned instrument will have a mass spectral peak shape that deviates significantly from a Gaussian peak shape. This approach typically leads to poor search results, given that the isotope patterns among top hit molecules are quite similar, most of the time within 1-10% of each other (Figure 2). In the approach proposed here, the experimental spectra are calibrated such that their peak shape can be exactly described by a mathematical function. At the same time, all possible isotope profiles to be searched for are also calculated based on the identical mathematical function. As a result, this search strategy can detect small profile changes due to the changes in elemental composition. Any peak shape variation caused by instrument artifacts has been calibrated out to minimize or eliminate their interference with the search.

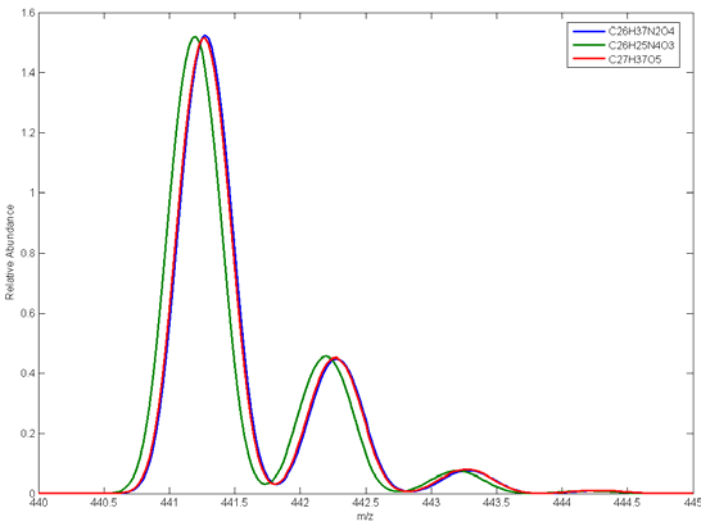


Figure 2. The profiles of top three possible elemental composition distinguished by isotope profile search

This new approach delivers superior search performance even when the mass error for unknown molecules is as much as 100 ppm, a level of mass accuracy unsuitable for elemental composition determination through conventional approaches. This is demonstrated by the search on mass spectra from LC/MS of verapamil microsomal incubation. Verapamil, verapamil sodium adduct, demethylated verapamil, demethylated verapamil sodium adduct, all calibrated to a mass accuracy of about 100 ppm, were searched to determine their elemental composition by this new approach. Three of the compounds are ranked number one and another ranked number four based on the RMSE statistics (Table 1). Searching against 544 possible formulas within the mass error window, demethylated verapamil comes out on the very top (Table 2). The ability to determine elemental composition by this isotope profile search is attributed to the MSIntegrity calibration technology used in MassWorks, as mentioned early. It is the calibration that standardizes the peak shape of both measured and theoretical spectra and makes it possible to distinguish very subtle differences as shown in Figure 2. The determination with this high level of confidence is better or similar to what can be achieved by conventional search algorithms at 5 ppm mass accuracy. For example, at less than 5 ppm mass error, the verapamil sodium adduct is ranked 3rd with possible elemental compositions of C29H37N2O4 (-0.7 ppm), C32H38O2Na (-4.1 ppm), and C27H38N2O4Na (4.3 ppm).

Conclusion

This application example illustrates that while mass accuracy is very important for elemental composition determination, it alone typically does not give the best possible results. The isotope profile contains equally important information pertinent to the elemental composition, which can only be taken advantage of when the mass spectral shape function is accurately known. When the information from iso-

tope distribution is effectively incorporated into the elemental composition search, it was found that the required mass accuracy can be significantly relaxed, making it possible to perform elemental composition determination with only moderate mass accuracy obtainable on ion trap instruments. The authors have also demonstrated the ability to obtain even higher mass accuracy on quadrupole systems which should further improve the selectivity. Such an approach has the potential to become a powerful tool in challenging areas such as proteomeincs, metabolomics, and environmental applications that require comprehensive analysis of complex mixtures.

Molecules	Exact m/z values	Measured m/z values	Mass Accuracy (mDa)	Mass Accuracy (ppm)	Formula	Search Rank
Verapamil	455.2910	455.2814	-9.6	-21.09	C27H39N2O4	#4
Verapamil sodium adduct	477.2729	477.2263	-46.6	-97.64	C27H38N2O4N	#1
Demethylated verapamil	441.2753	441.2341	-41.2	-93.37	C26H37N2O4	#1
Demethylated verapamil sodium	463.2573	463.2268	-30.5	-65.84	C26H36N2O4N	#1

Table 1. A Summary of Elemental Composition Determination for Verapamil and its Related Molecules by Profile Search

Calculated Mass	Mass Accuracy (mDa)	Mass Accuracy (ppm)	Formula	RMSE
441.2753	-41.2	-93.37	C26 H37 N2 O4	10.02
441.1927	41.4	93.84	C26 H25 N4 O3	12.45
441.2641	-30	-67.99	C27 H37 O5	13.68
441.2039	30.2	68.45	C25 H25 N6 O2	15.50
441.2866	-52.5	-118.97	C25 H37 N4 O3	15.69
441.1814	52.7	119.45	C27 H25 N2 O4	19.92
441.19	44.1	99.96	C22 H21 N10 O	20.04
441.1913	42.8	97.01	C25 H29 O7	21.14
441.2277	6.4	14.50	C26 H33 O6	21.25
441.2151	19	43.06	C24 H25 N8 O	21.50
441.1787	55.4	125.57	C23 H21 N8 O2	23.24
441.2614	-27.3	-61.87	C23 H33 N6 O3	23.29
441.2389	-4.8	-10.88	C25 H33 N2 O5	23.33
441.2502	-16.1	-36.49	C24 H33 N4 O4	23.73
441.2026	31.5	71.40	C24 H29 N2 O6	23.86
441.2726	-38.5	-87.25	C22 H33 N8 O2	23.88
441.2515	-17.4	-39.43	C25 H29 N8	25.29
441.2264	7.7	17.45	C23 H25 N10	26.19
441.2767	-42.6	-96.54	C27 H33 N6	26.29
441.2066	27.5	62.33	C29 H29 O4	27.54
441.2839	-49.8	-112.85	C21 H33 N10 O	27.97
441.2403	-6.2	-14.05	C26 H29 N6 O	28.02
441.2178	16.3	36.94	C28 H29 N2 O3	28.33
441.2291	5	11.33	C27 H29 N4 O2	28.89

Table 2. Elemental Composition Search for m/z = 441.2341. Elemental search conditions and results: mass tolerance = 60.0 mDa, double bond equivalents = 1 to 35. 544 formula were evaluated with 78 results within the mass accuracy limits and the top 25 are listed on the table.

[1]MassWorks software from Cerno Bioscience, Danbury, CT 06810.

[2]Automated interpretation of mass spectra of complex mixtures by matching of isotope peak distributions, Rapid Commun. Mass Spectrom. 2004; 18: 2465-2472.

[3]Accurate mass filtering of ion chromatograms for metabolite identification using a unit mass resolution liquid chromatography/mass spectrometry system, Rapid Commun. Mass Spectrom. 2006; 20: 764–770.