Spectral Accuracy Approach to Counting Sulfurs in Unknown Compounds by Unit Mass Resolution Single Quadrupole Systems

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Overview

➢Spectral accuracy calculation was performed to determine number of sulfur atoms in 18 small molecules acquired by either GC/MS or LC/MS from three different unit mass resolution quadrupole systems. The molecular weights of the molecules ranged from 110 to 697 amu.

➢Number of sulfur atoms from zero to three in various sulfur containing compounds have been determined by spectral accuracy through comprehensive mass spectral calibration not only on mass correction but also, more importantly, on mass spectral peak shape correction.

➢Selected overlays of calibrated spectra against theoretical spectra of both correct and incorrect compounds are presented to demonstrate obvious spectral mismatch when incorrect number of sulfur atoms are included in proposed elemental composition determination.

Introduction

Positively determining and accurately counting sulfur atoms has been performed to study a variety of molecules including P16 tumor suppressor protein, glycosphingolipids, sea lamprey pheromones, and dye powder by high resolution MS, which require resolving power of 60K or higher to physically separate A+2 doublet of 34S and 13C13C. According to natural abundance of 34S about 4.4% relative to a single monoisotope 32S, the number of sulfur atoms in unknowns can be easily determined by measuring relative peak area of baseline resolved 34S peak. While sulfur-counting by the high resolution MS is quite effective, it is too expensive for routine analysis. Here we propose an alternative approach to counting sulfur atoms through spectral accuracy (1) on unit mass resolution MS.

Methods

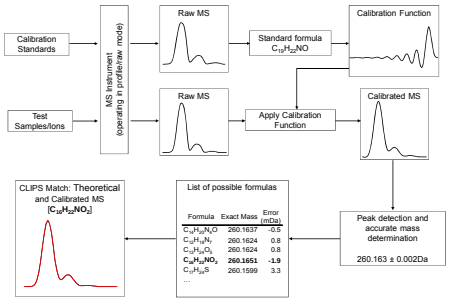
➢**Data Acquisition:** All data were acquired either from a Waters SQD LC/MS system or Agilent GC/MS system. They were all in positive ionization and continuum mode.

➢GC/MS data were acquired in a Raw Scan (profile, 10 measures per mass spectral peak) Acq. Mode with the Threshold set to 0, a scan range of 50 to 500 m/z units, and a sampling rate of 2^3, which results in 1.66 spectra per second. Mass spectral instrument line-shape calibration and sulfur counting were performed with commercially available software, a post-acquisition data processing package soft. For all the compounds under study, search parameters for sulfur counting were selected with few constraints to mimic real unknown identification situations. Additional search parameters included double-bond equivalent values from -1 to 50, mass tolerance of 20 mDa, electron state as “odd” for the molecular ions and “both” for fragments.

➢GC/MS calibration was performed with PFTBA and LC/MS calibration employed home made mixture of small molecules (2)

Results and Discussion

Fig.1 MassWorks Calibration+CLIPS Formula ID



All profile data were calibrated by instrument line shape calibration as illustrated in Fig.1. This unique calibration will not only correct for mass errors but also transform asymmetric peak shape from raw spectra to mathematically well-defined symmetric peak shape to achieve high spectral accuracy. The determination of number of sulfurs in unknown samples will automatically result from spectral accuracy calculation on calibrated spectra of the unknowns against possible theoretically calculated spectra. The advantage of this method is that the spectral accuracy measurements take the characteristics of an entire profile spectrum into consideration to determine sulfur number even though 34S and 13C13C are un-resolved. For example, through elemental composition determination including possible elements of C/H/N/O/S, two sulfur atoms were found in the molecular ion of a highly toxic rodenticide, tetramethylenedisulfotetramine (C4H8N4O4S2) which ranked on top with spectral accuracy at 99.2%. On the other hand, under the same search conditions, even the best match for any molecules

Table 1. Spectral Accuracy for Molecules with Different Sulfur Atoms

Ranking	Formula	Mono Isotope	Mass Error (mDa)	Spectral Accuracy	Comments
1	C4H8N4O4S2	239.9987	-13.0	99.2	The best with 2 S
2	C3H8N6O3S2	240.0099	-1.8	99.1	
3	C2H8N8O2S2	240.0212	9.5	98.9	
4	C6H12N2O4S2	240.0238	12.1	98.8	
5	C7H12O5S2	240.0126	0.9	98.6	
6	H4N10O2S2	239.9960	-15.7	98.1	
7	C5H4N8S2	240.0000	-11.7	97.7	
8	C4H12N6S3	240.0286	16.9	97.6	The best with 3 S
9	C5H12N4OS3	240.0173	5.6	97.5	
	:				
14	C2H8N8S3	240.0034	-8.3	97.1	
15	CH4N8O5S	240.0025	-9.2	96.7	The best with 1 S
16	C6H8O8S	239.9940	-17.7	96.7	
	:				
34	C7H4N4O4S	239.9953	-16.4	95.0	
35	C2H8O13	239.9965	-15.2	95.0	The best with 0 S

containing 3 or 1 sulfur atom(s), C4H12N6S3 and CH4N8O5S, achieved spectral accuracy of only 97.6% and 96.7% respectively. With greater than 1.5% spectral accuracy difference compared with that of the true elemental composition, C4H8N4O4S2, both C4H12N6S3 and CH4N8O5S can be confidently ruled out as possible candidates containing correct number of sulfur atoms. As the match almost perfectly shown in overlays (Fig.2) of calibrated spectra (red) of unknown and theoretical spectra (green), the elemental composition of the unknown compound is correctly determined including number of sulfur atoms. In the insert of Fig.2, the overlay represents the best match of theoretical calculated spectra of any single sulfur containing molecules against calibrated spectra under the search conditions, however, clearly indicates the mismatch at A+2 peak: any single sulfur containing molecule will result in incorrect identification.

Similarly, spectral accuracy also will reveal if more sulfur atom(s) are determined in the elemental composition of unknowns than truly existed in molecules. In Fig. 3, unknown compounds (C19H23N6O4) do not contain any sulfur and will show significant

Fig.2 Spectral Overlays of C4H8N4O4S2

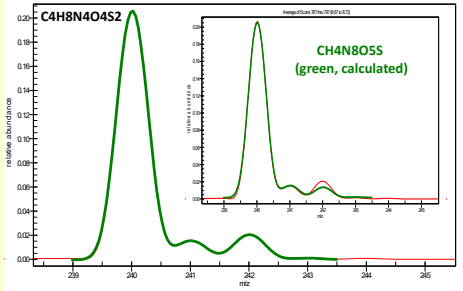
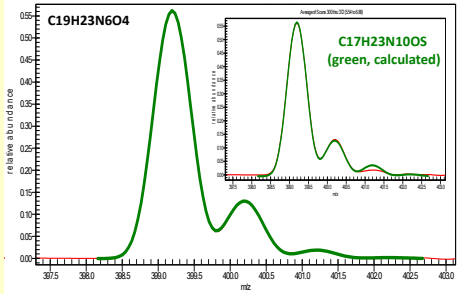


Fig. 3 Spectral Overlays of C19H23N6O4



difference when its calibrated spectra overlaid with any calculated spectra containing even on sulfur atom as illustrated in the insert of Fig.3.

Additionally, various GC/MS and LC/MS (some of them were reported on accurate mass measurements previously (2)) data acquired from different instrument were evaluated on the performance of sulfur counting through spectral accuracy calculation as summarized in Table 2. All the compounds under investigation were identified with not only correct number of sulfur atoms but complete elemental composition with most of them achieving high spectral accuracy of greater than 98% and top rankings.

Table 2. Summary of Sulfur Counting on Spectra from Various MS Systems

Formula	AM [M+H]+	Ranking	Instrument	Spectral Accuracy (%)
C18H18N4O3S	371.1178	2	Waters TSQ	98.7
C19H19FN4O4S	*419.1189*	2	Waters TSQ	98.5
C21H19Cl2N9O3S	548.0787	5	Waters TSQ	99.1
C23H19F3N6O5S	*549.1168*	9	Waters TSQ	99.5
C25H21ClN8O3S	*549.1224*	4	Waters TSQ	99.0
C24H29ClN6O5S	*549.1687*	4	Waters TSQ	98.9
C24H22ClN9O3S	552.1333	3	Waters TSQ	98.7
C23H29ClN6O6S	553.1636	1	Waters TSQ	99.3
C23H27Cl2N7O5S	*584.1250*	6	Waters TSQ	98.3
C24H23Cl2N11OS	*584.1263*	1	Waters TSQ	99.1
C26H26F3N6O7PS	655.1352	1	Waters TSQ	99.1
C33H35F3N8O4S	*697.2532*	2	Waters TSQ	99.1
C25H23N2OS	399.1531	2	Waters ZQ	99.3
C21H27N4S2	399.1677	2	Waters ZQ	99.5
C2H6O3S	110.0038	1	Agilent GC/MS	95.7
C8H20O5S2P2	322.0227	1	Agilent GC/MS	98.0
C5H11NO5S2	229.0079	2	Agilent GC/MS	96.5
C8H19O4S2P	274.0462	1	Agilent GC/MS	97.1

Conclusions

➢Through comprehensive mass spectral calibration, high spectral accuracy can be obtained and utilized to successfully determine number of sulfur atoms in unknown compounds with unit mass resolution mass spectral data.

➢This method is effective to determine number of sulfur atoms in compounds with mass range up to 700 amu according to current stidies. Further work will include higher molecular weight molecules.

➢The same spectral accuracy not only resulted in correct number of sulfur in compounds but also determine their complete elemental composition.

References

1. Y. Wang, M. Gu. *Anal. Chem*. **2010**, *82*, 7055.

2. V. Čápka , S. Tentarelli, *Proc. 58th ASMS Conference on Mass Spectrometry and Allied Topics,* Salt Lake City, UT. May 23-27, **2010**.