

High Mass Accuracy Measurements and Elemental Composition Determination of Molecular Ions of Pesticides with a Single Quadrupole GC/MS System

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Overview

➤ A comprehensive evaluation on high mass accuracy and high spectral accuracy has been performed on an Agilent 5977 GC/MS system.

➤ Elemental composition determination of molecular ions of pesticides through high mass accuracy and high spectral accuracy was demonstrated.

➤ Unique approaches to unknown ID by high mass accuracy NIST library search and elemental composition specific NIST library search were described.

Introduction

Unit mass resolution mass spectrometers have never been employed to routinely perform accurate mass measurements and elemental composition determination until recent development of innovative instrument line shape calibration. Two underlying issues that cause poor mass accuracy on the unit mass resolution MS systems are distorted and asymmetric mass spectral peak shape and overlapped monoisotope and ¹³C peaks. With advance of the instrument line shape calibration technology, these two issues can be effectively resolved. Here we will report systematic evaluation of accurate mass measurements with octafluoronaphthalene (OFN) and elemental composition determination for a mixture of pesticides acquired on an Agilent GC/MS 5977 system through internal mass spectral calibration.

Methods

➤ **Sample Preparation:** All the OFN samples were dissolved with 2,2,4-trimethylpentane at concentration level of 100 pg/μl. The pesticide mixtures (Agilent pesticide analyzer checkout solution) were prepared with acetone.

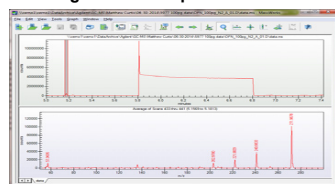
➤ **Data Acquisition:** Both OFN and pesticide data were acquired in an Agilent 5977 GC/MS system in a raw (profile) scanning mode with the threshold set to 0, and a sampling rate of 2². All data acquisition included collection on perfluorotributylamine (PFTBA) for the purpose of creating calibration. Ten injections of OFN at the volume of 1 μl were performed for each sample of two different datasets on the Agilent 5977 GC/MS system. Each dataset had its own 100 pg/μl OFN vial to minimize concentration by evaporation through the full sequence. Two time events were also included to open and close calibration gas valve to acquire MS spectra of PFTBA.

➤ **MassWorks processing:** MassWorks calibrates both the mass position and the mass spectral peak shape function, a key for achieving high mass accuracy. When applied to raw mass spectral data, the raw mass spectrum can be transformed into its calibrated version with mass spectral peaks located at accurate mass positions. Furthermore, the mass spectral peak shape would also be transformed in the same process to a mathematically definable function, a key for achieving high spectral accuracy and CLIPS formula ID.

➤ **High Performance NIST Library Search:** Launched through MassWorks, three modes of NIST library search were performed: regular search, high mass accuracy search and high spectral accuracy with elemental composition specific search.

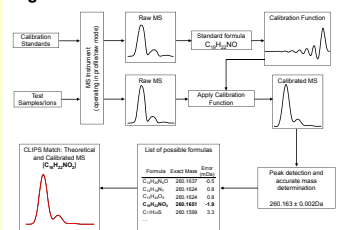
I. Performance of Accurate Mass Measurements

Fig 1. TIC and Spectra of OFN



During past a few years, numerous applications of accurate mass measurements on Agilent GC/MS systems were reported in literatures or presented in conferences. Although overall performance of determining elemental composition of unknown compounds through both high mass accuracy and spectral accuracy was excellent, achieved mass accuracy appeared to vary from low mD to 18 mD, depending on performance of instruments and signal to noise ratio of the samples analyzed. No systematic investigation has been done to evaluate high mass accuracy performance on an Agilent GC/MS with internal calibration standards by comprehensive instrumental line shape calibration. In this work, OFN was eluted at retention time (Rt) about 5.2 minute while the PFTBA appeared from Rt 5.8 to 6.8 minutes (Fig. 1). Once the calibration created by PFTBA was applied to OFN, the OFN spectra have mathematically well-defined and symmetrical peak shapes, which allows to successfully deconvolute the overlapped peaks first then perform accurate centroiding to achieve high mass accuracy. Based on the average of 10 measurements for the two datasets, the molecular ions of OFN at m/z 271 with 100 pg on column achieved high mass accuracy of 0.22 and -0.15 mD. In the same time,

Fig 2. MassWorks Calibration + Formula ID



Results and Discussion

Table 1. High Mass Accuracy and Spectral Accuracy of OFN

Sample #	Dataset 1			Dataset 2		
	Accurate Mass	Mass Errors (mD)	Spectral Accuracy	Accurate Mass	Mass Errors (mD)	Spectral Accuracy
1	271.9879	1.2	99.9	271.9866	-0.1	99.7
2	271.9857	-1	99.8	271.9865	-0.2	99.9
3	271.9871	0.4	99.7	271.9860	-0.7	99.7
4	271.9881	1.4	99.7	271.9869	0.2	99.7
5	271.9860	-0.7	99.7	271.9867	0	99.8
6	271.9860	-0.7	99.7	271.9857	-1	99.8
7	271.9867	0	99.6	271.9883	1.6	99.6
8	271.9887	2	99.7	271.9856	-1.1	99.6
9	271.9863	-0.4	99.9	271.9868	0.1	99.8
10	271.9867	0	99.7	271.9864	-0.3	99.9
Average		0.22	99.7		-0.15	99.8
Stdev		0.96	0.1		0.7	0.1

the OFN from the two datasets also achieved excellent spectral accuracy of 99.8% and 99.7%.

II. Elemental Composition Determination for m/z 376

Similar to OFN, the spectra of the pesticides were internally calibrated by PFTBA to obtain both high mass accuracy and spectral accuracy, allowing elemental composition determination of molecular ions and fragments of the pesticides. For example, molecular ions of Etofenprox (Fig. 3) with m/z 376 was determined to have correct elemental composition of C₂₅H₂₈O₃ ranked as number one out of 47 possible candidates according to its high spectral accuracy of 98.9% (Table 2) to allow almost perfect match between calibrated and calculated spectra as illustrated by spectral overlay in Fig. 4. On the other hand, elemental composition determination based on

Fig 3. TIC and Spectra of Etofenprox

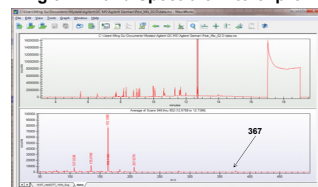


Table 2. Elemental Composition Determination on m/z 376 ranked by Spectral Accuracy

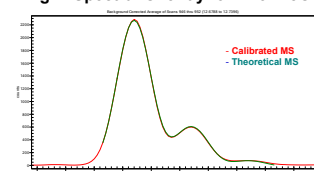
Row	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C ₂₅ H ₂₈ O ₃	376.2017	-0.792	-2.120	98.98%	10	12
2	C ₂₃ H ₂₄ N ₂ O ₂	376.1894	0.1225	324.293	98.75%	12	13
3	C ₂₃ H ₂₄ N ₂ O	376.2006	-2.1108	-5.611	98.58%	14	13
4	C ₂₀ H ₂₄ N ₂	376.2118	-13.3442	-35.4713	98.40%	16	13
5	C ₂₄ H ₂₈ N ₂ S	376.1968	1.729	4.5959	97.79%	22	12
6	C ₂₅ H ₂₈ O ₂	376.1855	12.9624	34.4562	97.720%	22	12
7	C ₁₉ H ₂₀ N ₂ O	376.1867	11.9079	31.3874	97.80%	23	14
8	C ₂₁ H ₂₈ O ₆	376.188	10.4599	27.8042	97.24%	27	8
9	C ₂₇ H ₂₄ N ₂	376.1934	5.0998	13.5561	97.1901	28	17
10	C ₂₀ H ₂₈ N ₂ O ₂	376.1993	-0.7734	-2.0559	97.0623	29	8

Table 3. Elemental Composition Determination on m/z 376 ranked by Mass Accuracy

Row	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C ₂₁ H ₁₆ N ₂	376.1984	0.0719	0.1912	89.7356	101	7
2	C ₁₂ H ₃₂ N ₂ A ₀ O ₇ S	376.1886	-0.1215	-0.323	90.1447	97	-1
3	C ₂₀ H ₂₈ N ₂ O ₂	376.1993	-0.7734	-2.0559	97.0623	29	8
4	C ₉ H ₂₄ N ₄ O ₅	376.1973	1.2264	3.26	90.874	89	5
5	C ₅ H ₂₄ N ₄ O ₆	376.1998	-1.276	-3.3918	89.1835	106	1
6	CH ₂ O ₂ N ₂ O ₄	376.1971	1.4093	3.7463	87.8878	119	2
7	C ₁₃ H ₂₈ N ₂ O ₃ S	376.2	-1.4589	-3.8781	92.1289	77	4
8	C ₂₁ H ₃₂ N ₂ S ₂	376.2001	-1.6418	-4.3643	93.7811	61	7
9	C ₉ H ₃₂ N ₂ O ₃	376.1968	1.6954	4.5067	85.6251	141	-1
10	C ₂₄ H ₂₈ N ₂ S	376.1968	1.729	4.5959	97.7936	22	12

only accurate mass could result in many choices. For example, under the same search conditions, there are at least 10 different formulas that are within mass tolerance of 5 ppm (Table 3).

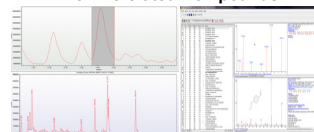
Fig 4. Spectral Overlay for C₂₅H₂₈O₃



III High Performance NIST Library Search

Taking advantages of both fragmentation patterns of EI spectra and elemental composition constraints, newly developed accurate mass and elemental composition specific NIST library significantly enhances NIST library search performance, especially for those co-eluted compounds. This is demonstrated by an example of co-eluted compounds of bis (1-methylethyl) disulfide & 2-ethyl-6-methyl pyrazine (Fig. 5)

Fig. 5 Conventional NIST Search Results on Co-eluted Compounds



Through conventional NIST library search, a wrong compound (4-ethoxy-Benzaldehyde) was found to be the most likely candidate as shown in Fig. 5. On the other hand, the elemental composition specific NIST search resulted in correct identification on one of the co-eluted compounds. This was accomplished with two steps. First, elemental composition determination

Fig 6. Elemental Composition Determination on m/z 150 ranked by Mass Accuracy

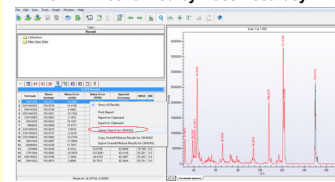
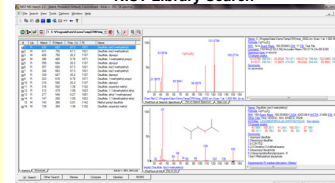


Fig 7. Correct ID for bis (1-methylethyl) disulfide by Elemental Composition Specific NIST Library Search



for m/z 150 to show C₆H₁₄S₂ is the best match with spectral accuracy of 98.6%. Secondly, additional search with elemental composition constraints of C₆H₁₄S₂, correct compound was identified as the best choice among 16 different isomers.

Conclusions

➤ With instrument line shape calibration, it is possible to achieve high mass accuracy better than 1mD and spectral accuracy better than 99% on a unit mass resolution Agilent 5977 GC/MS system to allow elemental composition determination just as good as high resolution mass spectrometers.

➤ Elemental composition specific NIST library search significantly enhances performance for identification of unknowns especially for those hard-to-determined co-eluted mixtures.

References

- Yongdong Wang and Ming Gu, The Concept of Spectral Accuracy for MS. Anal. Chem. 2010, 82, 7055-7062
- Mark Belmont, Alexander N. Semyonov, Massimo Santoro, Sergio Guazzotti and Ming Gu Identification of Co-eluted Components by High Mass Accuracy and Spectral Accuracy with Quadrupole GC-MS Systems, ASMS Poster 2014