

Accurate Mass Measurement Using Single Quadrupole GC/MS for Structure Elucidation of Unknowns

Joseph Mick; Todd Gillespie
Eli Lilly & Company, Indianapolis, IN

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Overview

> Accurate mass measurement attained on single quadrupole GC/MS instruments providing elemental composition for compound identification and/or confirmation.

> A second dimension of identification using calibrated isotopic peak shape to rank the calculated elemental formulas, improving the ability to identify the correct elemental composition.

> A novice spectroscopist now has the ability to obtain accurate mass identification of compounds on a routine basis using only a nominal mass instrument.

Introduction

Single quadrupole GC/MS instruments are commonly used across the industry today. Even with the plethora of spectral databases on the market, a large number of compounds and impurities observed in research and development do not generate a computer library match.

User-friendly software is available which will convert LC/MS & GC/MS nominal mass data to accurate mass data, providing identification of unknown compounds. Benefits of this post acquisition software include time-reducing data analysis and a cost effective alternative to purchasing high resolution mass spectrometers.

Presented is the GC/MS evaluation of this software.

Instrumentation & Method

GC/MS: Agilent 6890N GC / 5973Network MSD ChemStation D.02.00.275

Originally, 11 different functional compounds were analyzed at different concentrations. Later, a pharmaceutical raw material was analyzed to determine the identification of observed impurities.

Data Collection Parameters

Data acquisition: Raw scan
Data Threshold: 0
Acquisition rate: ≤ 0.1
Mass Range: 30-400

Steps For Data Processing

- 1) Data acquired for system calibrant.
- 2) Data acquired for sample.
- 3) Simple import of calibration data file into MassWorks™ software.
- 4) Select mass spectra range and create calibration ion list.
- 5) Software creates a calibration file.
- 6) Import sample data file and select recently created calibration file.
- 7) Data file is automatically converted and accurate mass data can be viewed.

Results & Discussion

To generate plausible elemental formulas from the calculated accurate mass data, the following parameters were used:

Charge	1	
Mass Tolerance (mDa)	9	
Electron State	Both	
Profile Mass Start (Da)	-0.5	
Profile Mass End	3.5	
Element	Minimum	Maximum
Carbon	0	34
Hydrogen	0	50
Nitrogen	0	10
Oxygen	0	10
Chlorine*	0	3
Fluorine**	0	3

*Fluorine was added and chlorine was removed from the table for the analysis of the pharmaceutical raw material

To demonstrate the ruggedness of the search algorithms, the elemental table was given extremely wide limits.

When performing a formula search, the isotopic profile can be adjusted to counter contributions from interfering ions near the ion of interest. This will affect the ranking of the chemical formula.

For some of the pharmaceutical impurities no molecular ion was observed. For these impurities, identification was achieved on the accurate mass of the fragment ions.

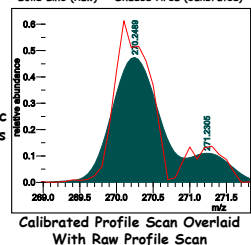
Overview Of 8 Test Compounds Processed By The Software

GC/MS	Ranking of Correct Formula	Formula	Theoretical	Measure	Mass Error (mDa)	Mass Error (PPM)
Hexadecanoic acid methyl ester	1st of 68	C ₁₇ H ₃₄ O ₂	270.2559	270.2489	-7.0	-25.8
4-chloro-1,1-biphenyl	1st of 41	C ₁₂ H ₉ Cl	188.0393	188.0339	-5.4	-28.6
biphenyl	1st of 25	C ₁₂ H ₁₀	154.0783	154.0816	3.3	21.7
methylene chloride	1st of 2	CH ₂ Cl ₂	83.9534	83.9458	-7.6	-90.0
THF	1st of 7	C ₄ H ₈ O	72.0575	72.0507	-6.8	-94.6
Heptane	1st of 6	C ₇ H ₁₆	100.1252	100.1232	-2.0	-20.0
xylene	1st of 12	C ₈ H ₁₀	106.0783	106.0794	1.1	10.8
anisole	1st of 10	C ₇ H ₈ O	108.0575	108.0582	0.7	6.3

It is known and observed that the lower the S/N, the lower the mass accuracy and the more difficult it is to identify the correct elemental formula. As shown below, examples of low S/N illustrate a lower ranking in the identification of the correct formula. Several standards were reanalyzed at a higher concentration for comparison.

GC/MS	Ranking of Correct Formula	Formula	Theoretical	Measure	Mass Error (mDa)	Mass Error (PPM)	Peak to Peak S/N
t-butyl methyl ether	2nd of 5	C ₄ H ₈ O	73.0653	73.0682	2.9	39.1	53
t-butyl methyl ether	1st of 4	C ₄ H ₈ O	73.0653	73.0652	-0.1	-1.9	1923
2-butanone	3rd of 7	C ₄ H ₈ O	72.0575	72.0508	-6.7	-93.2	40
2-butanone	1st of 4	C ₄ H ₈ O	72.0575	72.0567	-0.8	-11.3	431
dodecane	11th of 137	C ₁₂ H ₂₆	170.2035	170.1543	-49.2	-288.9	23

Molecular ion of Hexadecanoic acid methyl ester
Solid Line (Raw) Shaded Area (Calibrated)



An additional software feature is the ability to rank the possible elemental formulas by calibrating the line shape of the theoretical isotope profile to a known mathematical algorithm

Observe the correct formula is the top "hit" even though the mass error is larger than other possible formulas.

Spectral Accuracy	Formula	Theoretical	Mass Error (mDa)	Mass Error (ppm)
98.4169	C ₁₇ H ₃₄ O ₂	270.2559	-7.0	-25.8
98.2368	C ₁₆ H ₃₂ NO ₂	270.2433	5.6	20.7
98.1412	C ₁₆ H ₃₂ N ₂ O	270.2545	-5.6	-20.9
97.9524	C ₁₄ H ₃₀ N ₄ O	270.2420	6.9	25.7
97.8514	C ₁₃ H ₃₀ N ₆	270.2532	-4.3	-15.9

Top five hits for Hexadecanoic acid methyl ester (measured mass m/z 270.2489)

Overview Of Impurities Observed In The Pharmaceutical Raw Material (4-Fluorophenylethyl Alcohol) Processed By The Software

Impurities	Ranking of Correct Formula	Formula	Theoretical	Measure	Mass Error (mDa)	Mass Error (PPM)
fluorobenzyl alcohol	2nd of 24	C ₇ H ₇ OF	126.0481	126.0428	-5.3	-42.0
fluorobiphenyl	2nd of 45	C ₁₂ H ₉ F	172.0688	172.0774	8.6	49.8
difluorobiphenyl	2nd of 57	C ₁₂ H ₇ F ₂	190.0594	190.0624	3	15.7

Fragment of impurity m/z 184*	1st of 22	C ₉ H ₇ F	122.0532	122.056	2.8	23.1
Fragment of impurity m/z 184*	1st of 16	C ₉ H ₇ F	109.0454	109.0521	6.7	61.9
Fragment of impurity m/z 184*	1st of 16	C ₈ H ₇	103.0548	103.0551	0.3	3.2
impurity m/z 156**	1st of 38	C ₈ H ₉ O ₂ F	156.0587	156.0678	9.1	58.6
impurity m/z 166***	1st of 40	C ₁₀ H ₁₁ OF	166.0794	166.0843	4.9	29.5
Fragment of impurity m/z 166***	1st of 42	C ₁₀ H ₁₀ OF	165.0716	165.0814	9.8	59.6

* Fragment ions were observed for this impurity rather than the molecular ion m/z 184. Was able to synthesize material to confirm structure and formula

** Believed to be the correct formula based on known chemistry of the original material

*** Believed to be the correct formula based on known chemistry of the original material. The isotopic profile range was changed to 0-3.5 due to isotopic interference of the "loss of a hydrogen" fragment. If the profile range was held to -0.5-3.5 the rank of the formula would have been 8th out of 40.

Conclusions

The software provides elemental composition determination on nominal mass GC/MS systems.

The software ranked the correct formula in the top three hits for every compound except one, due to low S/N. The lower limit for peak to peak S/N was observed to be approximately 100.

The accuracy for the data was less than 10 mDa error. This error could potentially be lowered if an internal calibration was performed, but due to the additional dimension of the accurate line shape of the isotope profile this is not necessary except when low S/N is observed.

Though not shown here, a parallel analysis was achieved on single quadrupole LC/MS systems with similar results. The ability to provide the elemental identification of unknown compounds on a routine basis by any scientist is now within ones reach.

Acknowledgement

Cerno BIOSCIENCE is the maker of MassWorks™