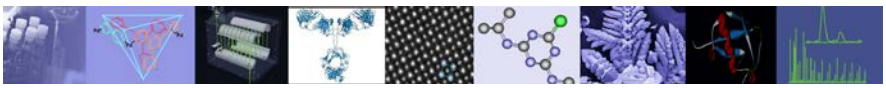
Applications Note



Accurate Isotope Data is Essential for Determining Elemental Compositions

Introduction

Elemental compositions are commonly determined from high-resolution mass spectra and accurate mass measurements. Given a measured mass (m/z) and a range of elements that can be present, software calculates the exact mass for each combination of elements and reports all elemental combinations that match the measured mass within a specified error tolerance. Improving the mass accuracy reduces the number of elemental compositions, but mass accuracy alone may not be sufficient to determine the correct elemental composition for an unknown sample.

JEOL AccuTOF™ mass spectrometers (the AccuTOF™-DART®, the AccuTOF™-GCX and the AccuTOF™-GCX Plus) are capable of accurate isotope measurements that can be used to determine elemental compositions from high-resolution mass spectra. Matching the measured abundances and exact masses for isotope peaks can be more effective than mass accuracy alone.

Experimental

A JEOL AccuTOF™-DART® mass spectrometer was used to acquire positive-ion mass spectra at a resolving power of >10,000 (FWHM). The DART was operated in positive-ion mode with helium gas and a gas heater temperature of 350°C. Chlorpromazine was analyzed by dipping a melting point tube into a dilute solution of chlorpromazine in dichloromethane and dangling the tip of the tube in the DART gas stream. Jeffamine® M-600 (Huntsman) copolymer was measured in the same data file as an external calibrant for exact mass measurements. Mass Mountaineer™ software was used for elemental composition determinations from accurate mass measurements and isotope matching.

Example

The pesticide chlorpyrifos has the elemental composition $C_9H_{11}Cl_3NO_3PS$. The major peaks in the positive-ion DART mass spectrum in Figure 1 correspond to the protonated molecule of chlorpyrifos.

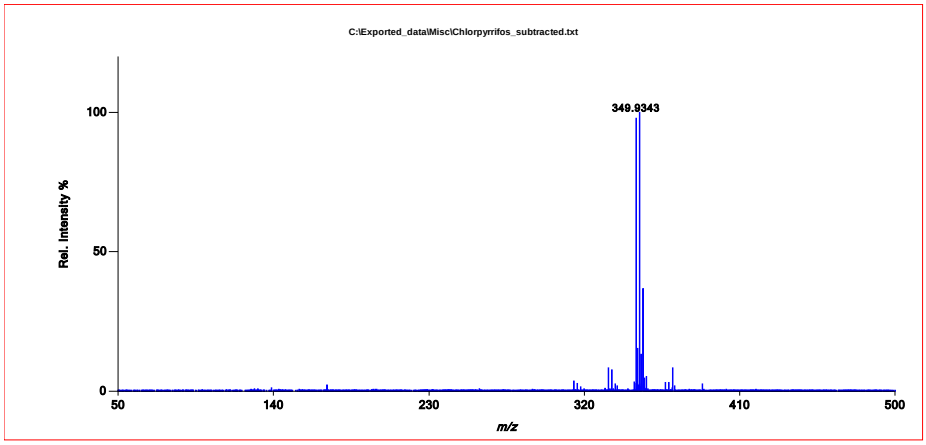


Figure 1. Positive-ion AccuTOF-DART mass spectrum of the pesticide chlorpyrifos

The measured monoisotopic mass is 349.93427, which differs from the calculated exact mass by 0.00016 u, that is, 0.16 millimass units (mmu) or 0.5 parts per million (ppm). The range of elements used for elemental compositions was:

$$C_{0\text{-}50}\ H_{0\text{-}100}\ O_{0\text{-}100}\ N_{0\text{-}100}\ Cl_{0\text{-}3}\ Br_{0\text{-}1}\ S_{0\text{-}9}\ P_{0\text{-}2}$$

with the maximum limits for the X+2 elements Cl, Br, and S determined automatically by the Mass Mountaineer program. Using the measured monoisotopic mass and an error tolerance of 2 mmu (5.7 ppm), there are 99 possible elemental compositions for even-electron ions (protonated molecules). If we let the software calculate the exact masses and theoretical isotope abundances for each of the 99 possible compositions and match these against the measured isotope masses and abundances, the correct composition is readily determined as the best match (Figure 2 and Table 1). The correct compound (chlorpyrifos) is the only entry found in the 2017 NIST Mass Spectral Database for this composition.

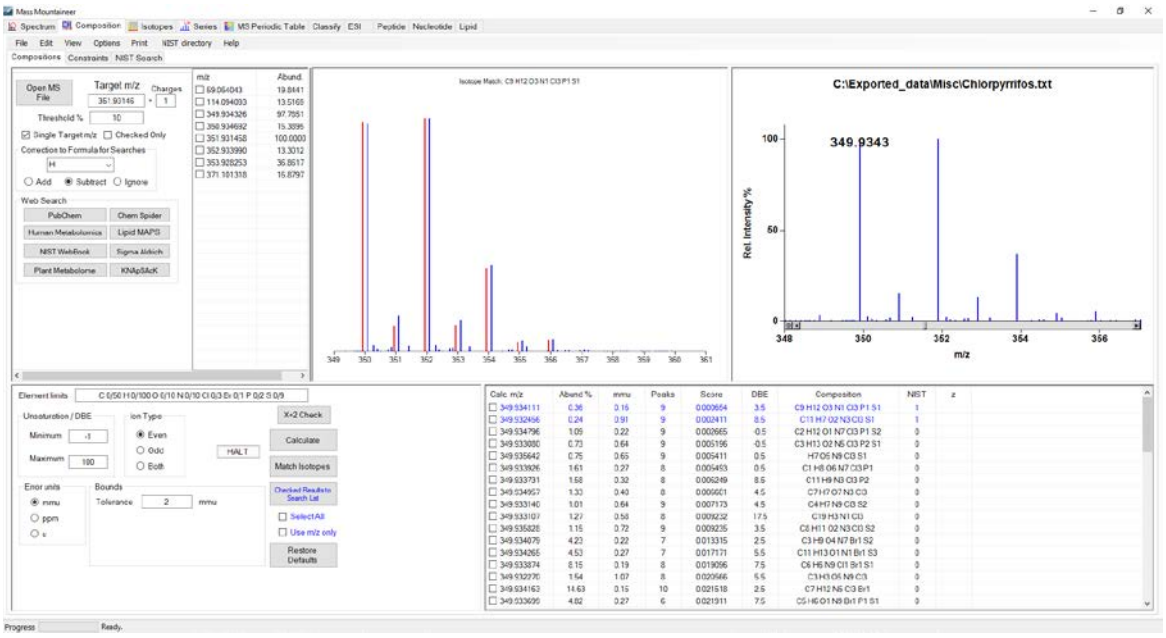


Figure 2. Mass Mountaineer elemental composition determination with isotope matching shows the correct elemental composition for chlorpyrifos.

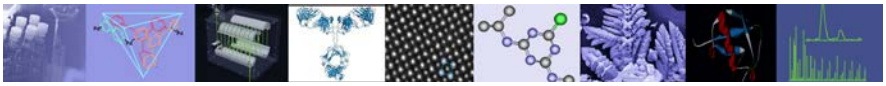


Table 1. The top 10 isotope match results for measured monoisotopic *m/z* 349.93427 using the data from the mass spectrum shown in Figure 1. The correct composition (in red) has the lowest isotope match score, indicating the best match. One entry (chlorpromazine) is found in the NIST database for this composition minus a proton.

Calc. m/z	Abund %[a]	mmu[b]	Peaks[c]	Score[d]	DBE[e]	Composition	NIST
349.93411	**0.36**	**0.16**	**9**	**0.000654**	**3.5**	**C9 H12 O3 N1 Cl3 P1 S1**	**1**
349.93246	0.24	0.91	9	0.002411	8.5	C11 H7 O2 N3 Cl3 S1	1
349.9348	1.16	0.23	8	0.003367	-0.5	C2 H12 O1 N7 Cl3 P1 S2	0
349.93373	1.58	0.32	8	0.006249	8.5	C11 H9 N3 Cl3 P2	0
349.93496	1.33	0.4	8	0.006601	4.5	C7 H7 O7 N3 Cl3	0
349.93564	0.79	0.69	8	0.006847	0.5	H7 O5 N9 Cl3 S1	0
349.93393	1.72	0.29	7	0.007164	0.5	C1 H8 O6 N7 Cl3 P1	0
349.93308	0.83	0.72	7	0.008588	-0.5	C3 H13 O2 N5 Cl3 P2 S1	0
349.93311	1.27	0.58	8	0.009232	17.5	C19 H3 N1 Cl3	0

a. RMS abundance error for 9 isotope peaks found in the measured mass spectrum
b. RMS mass error for 9 isotope peaks found in the measured mass spectrum
c. Number of isotope peaks found in the measured spectrum that match the calculated isotopes
d. Isotope match score. A lower score indicates a better match,
e. Double-bond equivalents ("unsaturation" or "rings + double bonds")

What would be the result of having better mass accuracy, but without isotope matching? Even if we were to measure the mass exactly (no error!) and limit the mass tolerance to 0.15 mmu (0.4 ppm), there are eight possible elemental compositions. Two possible compositions fall within an error tolerance of 0.1 ppm.

Calc. m/z	mmu	ppm	DBE*	Composition
349.934079	-0.03	-0.09	2.5	C3 H9 O4 N7 Br1 S2
349.934028	-0.08	-0.24	3.5	C5 H9 O7 N3 P1 S3
349.934202	0.09	0.26	3.5	C6 H9 O6 N3 Cl1 S3
349.933989	-0.12	-0.35	2.5	C6 H12 O1 N5 Cl2 Br1 P1
349.934163	0.05	0.15	2.5	C7 H12 N5 Cl3 Br1
349.933997	-0.11	-0.33	8.5	C9 H6 O2 N5 Cl2 S2
349.934111	**0**	**0**	**3.5**	**C9 H12 O3 N1 Cl3 P1 S1**
349.933983	-0.13	-0.37	13.5	C13 H1 O9 N1 Cl1

* Double-bond equivalents

Conclusion

Mass accuracy alone is often not enough to determine the elemental composition of an unknown. JEOL AccuTOF™ mass spectrometers provide accurate masses and accurate isotope data with software that provides powerful capabilities for elemental composition determinations.