Comparison of Time-of-Flight and Double Focusing Mass Spectrometry for Reaching Tentative Identifications of Unanticipated Compounds Added to Drinking Water by Terrorists

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Introduction

Local monitoring of post-treatment drinking water using bench-top mass spectrometers could identify target compounds in a mass spectral library. However, a terrorist might seek to incite greater hysteria by injecting or infusing a mixture of unanticipated compounds of unknown toxicity. Authorities will want to know the identities and the toxicities of the additives as soon as possible. Unanticipated compounds of unknown polarity can be rapidly separated from other components of complex mixtures using chromatographic techniques and analyzed through mass spectrometry. Determining the molecular and fragment ion compositions in a mass spectrum constrains the number of possible isomers and can lead to compound identification based on modest literature searches.

Methods

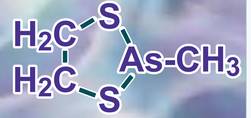
The exact mass of an ion measured with an error limit of 5 ppm that contains C, H, N, O, P, or S atoms usually corresponds to multiple possible compositions for ions higher in mass than 150 Da. Determination of the exact masses of the +1 and +2 mass peak profiles and their abundances relative to the monoisotopic ion provide four additional measurements for rejecting incorrect compositions.

In Figure 1a are displayed calculated profiles (Gaussian distributions) for the $C_{23}H_{28}O_2Br^+$ ion, the three most abundant +1 and +2 ions, and the composite +1 and +2 profiles. The theoretical exact masses and relative abundances of the +1 and +2 profiles are listed under the profiles.

In Figure 1b, these values were obtained from the top portions of the mass peak profiles, which were plotted from selected ion recording data (MPPSIRD) acquired as the two isomers evident in Figure 1c eluted into a Finnigan MAT 900S double focusing mass spectrometer (1). The chromatographic peak areas in Figure 1c provided the maxima of the partial profiles. Each of 31 m/z ratios was monitored for 20 msec during each 1-s SIR cycle. Each partial profile was plotted from 7 m/z ratios and 5 m/z ratios were monitored for each of two partial profiles for calibrant ions (not shown).

A profile generation model (PGM) automatically determines the correct ion composition by rejecting all compositions with calculated values of these three exact masses and two relative abundances that are inconsistent with the measured values (2). Use of MPPSIRD and the PGM in concert is Ion Composition Elucidation (ICE).

Table 1 lists possible compositions for a 6 ppm error limit about the measured mass of an arsenic containing compound found in a monitoring well at a landfill. The two additional exact mass measurements and two relative abundance measurements provided compelling evidence for the last composition, the only one for which all five measured and calculated values agreed. Also evident from the table, accurately measured relative abundances were more discriminatory against incorrect compositions than the two additional exact masses.



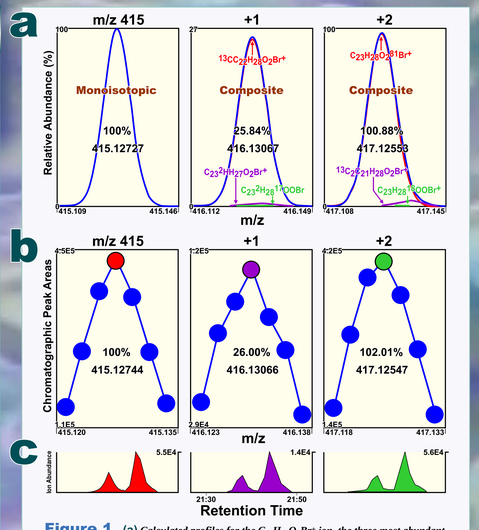


Figure 1. **(a)** *Calculated profiles for the $C_{23}H_{28}O_2Br^+$ ion, the three most abundant +1 and +2 ions, and the composite +1 and +2 profiles.* **(b)** *Partial m/z 415, +1, and +2 profiles plotted from chromatographic peak areas under ion chromatograms for 7 m/z ratios across each profile.* **(c)** *Ion chromatograms for the m/z ratios at the maxima of the partial profiles.*

Table 1. *Possible Compositions for m/z 181.92056 ± 6 ppm (1.09 mDa).*

	Mass Defects			Relative Abundances		
Composition	**182**	**+1**	**+2**	**%+1 (%+1 Range)**		**%+2 (%+2 Range)**	
$H_6O_2S_4$	.91998	.91962	.91594	2.84 (1.85-3.82)	X	17.88 (15.35-20.45)	X
$HNOF_2P_2S$	.92062	.91947	.91646	1.01 (0.64-1.40)	X	4.50 (3.84 - 5.17)	X
$HNOF_2As$	.92102	.91852	.92526	0.30 (0.17-0.45)	X	0.05 (0.00 - 0.11)	X
$H_2NO_2F_2S_3$	.92157	.92082	.91751	2.67 (1.78-3.63)	X	13.33 (11.42-15.26)	X
$CHN_2O_3P_4$	.92000	.92149	.92420	1.65 (1.35-1.99)	X	0.18 (0.02 - 0.41)	X
$C_2H_3O_4As$	.91964	.92311	.92393	2.45 (1.94-2.97)	X	0.35 (0.05 - 0.75)	X
$C_2H_3N_2PS_3$	.91960	.92035	.91542	4.59 (3.50-5.71)		13.36 (11.59-15.12)	X
$C_2NF_2P_2S$	.91948	.92175	.91535	4.19 (3.54-4.89)		4.49 (3.84 - 5.17)	X
C_2NF_2As	.91988	.92295	.92548	3.50 (2.91-4.12)	X	0.01 (0.00 - 0.03)	X
C_2HNOFS_3	.92043	.92192	.91634	5.60 (4.53-6.70)		13.42 (11.56-15.30)	X
$C_2H_4S_2As$ ✓	.92051	.92272	.91636	4.71 (3.99-5.45)		8.89 (7.70-10.09)	
Exp'l Values:	**.92056**	**.92265**	**.91625**	**4.72**		**8.54**	

An X indicates inconsistency between the measured and calculated values.

Instrumental Requirements

To measure the three exact masses and two relative abundances listed in Table 1 as ions produced from eluting compounds enter the mass spectrometer, the mass analyzer must provide rapid scanning, accurate masses, a wide linear dynamic range, and resolving power sufficient to distinguish between analyte and interfering ions.

Table 2. *oa-TOF MS vs. MPPSIRD with a double focusing mass spectrometer.*

Instrumental Characteristic	oa-TOF MS	MPPSIRD
Scan Speed To delineate partially overlapping ion chromatographic peaks and correctly correlate fragment and molecular ions, a scan speed of 1 s or less is required.	oa-TOF mass spectrometers acquire thousands of scans each second. In recent journal articles, where oa-TOF was used after chromatographic separations, individual scans were summed to provide a mass spectrum every 0.2 to 2 s (3-11). ★★	MPPSIRD utilized a SIR cycle of 1 s or less using VG 7OSE and Finnigan MAT 900S double focusing mass spectrometers. ★
oa-TOF provides faster scan speeds, but a double focusing mass spectrometer using MPPSIRD is also adequate.		
Mass Accuracy The number of possible compositions is roughly proportional to the error limit.	Most exact masses listed in the recent oa-TOF MS articles were accurate to within 5-10 ppm. ★	Most exact masses measured using MPPSIRD are accurate to within 2 ppm. ★★
The double focusing mass spectrometer provides greater mass accuracy and fewer possible compositions for higher-mass ions, but oa-TOF instruments provide exact masses accurate enough to exclude many compositions possible based on nominal masses.		
Linear Dynamic Range To make relative abundance measurements of 1% or less for +2 profiles from ions not containing Cl, Br, S, or Si practical, a linear dynamic range of at least 3 orders of magnitude is needed. If compounds in complex mixtures with very different concentrations are studied during the same data acquisition, a still wider range is important.	Linear dynamic ranges of only 50, 100, and 200 were demonstrated in the oa-TOF articles. Exact masses of +1 and +2 profiles were not considered. In addition, the oa-TOF mass spectra in many figures did not provide accurate relative abundances.	With a linear dynamic range of at least 10^4, MPPSIRD with a double focusing mass spectrometer provides more accurate relative abundances for ions from eluting analytes than other types of scanning and other types of mass spectrometers. ★★
MPPSIRD provides highly useful, accurate relative abundances; oa-TOF MS does not.		
Mass Resolving Power The greater the mass resolving power, the fewer interferences from column bleed or coeluting compounds will be observed. With lower abundances than the monoisotopic ion, interferences (overlapping profiles) with the +1 and +2 profiles are more common.	The resolving powers used in the surveyed oa-TOF MS articles ranged from 3500 to 7000 full width at half maximum (FWHM) or 1700 to 3400 with the 10% valley definition. Interferences from calibrant ions are few, since only one calibrant ion is required for internal calibration while acquiring data. ★	Mass resolving powers of 21,000 and 42,000 (FWHM) or 10,000 and 20,000 (10% valley) are used routinely with MPPSIRD. ★★
MPPSIRD using a double focusing mass spectrometer provides 3-fold greater resolving power than oa-TOF MS.		

★ **Adequate** ★★ **More Than Adequate**

Two Real-World Examples

An Arsenic Containing Compound

The mass spectrum for a trace-level compound in an extract of water from a monitoring well at a landfill displayed only m/z 182 and 167 ions above the chemical noise. The compound was hypothesized to be 2-methyl-1,3,2-dithiarsolane (structure shown to the left of Table 1), a standard was synthesized, and its identity confirmed. ICE confirmed the molecular ion composition of the trace-level compound was $C_3H_7S_2As$. All three exact masses and both relative abundances were consistent with this composition alone as illustrated in Table 1. A conservative error limit of 6 ppm was assumed for MPPSIRD with 10,000 resolving power (10% valley).

But what if a terrorist added this compound, which is not in the NIST mass spectral library on our data system, to drinking water and the presence of an arsenic atom was not suspected? Consideration of C, H, N, O, F, P, and S atoms and an exact mass correct to within 5 ppm determined by oa-TOF MS would provide four compositions: $H_6O_2S_4$, $HNOF_2P_2S$, $CHN_2O_3P_4$, and C_2HNOFS_3. The PGM would find no viable compositions based on three exact masses and two relative abundances. However, the relative abundances would lead to the correct composition deductively. In Table 1, the %+2 value of 8.54% suggests two S atoms, which contribute 1.58% to the %+1 value. The remaining %+1 of 3.14% corresponds to three C atoms. Two S atoms and three C atoms account for 100 out of 182 Da. One or more monoisotopic atoms are present. As has an atomic mass of 75 Da and 7 H atoms would account for the remaining mass. The composition $C_3H_7S_2As$ would then be confirmed experimentally.

MPPSIRD provided data that would lead deductively to the correct composition, even though an element was overlooked. A single exact mass provided by oa-TOF MS would provide no such clues.

A High-mass Disinfection Byproduct

The low resolution mass spectrum in Figure 2a with two mass peaks at m/z 415 and 417 visible above the chemical noise suggested a mono-brominated compound might be present in a chlorine-disinfected, well-water extract.

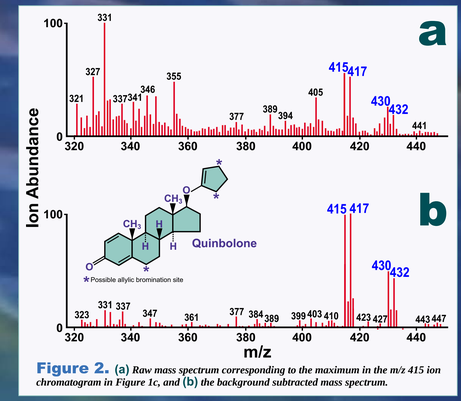


Figure 2. **(a)** *Raw mass spectrum corresponding to the maximum in the m/z 415 ion chromatogram in Figure 1c, and* **(b)** *the background subtracted mass spectrum.*

Related ions with m/z 430 and 432 became apparent in the background subtracted mass spectrum in Figure 2b for which no library matches were found. The exact mass of the apparent molecular ion was determined to be 430.15123 Da. Assuming the presence of a single Br atom, this exact mass with a presumed error limit of 5 ppm for oa-TOF MS corresponds to 41 possible compositions. For the fragment ion (m/z 415.12744 ± 5 ppm), 50 compositions would be possible. Table 3 provides the last seven possible compositions listed by the PGM for both the apparent molecular ion and the fragment ion using MPPSIRD and 20,000 resolving power (10% valley). The partial profiles for m/z 415 and its isotopic profiles are shown in Figure 1b. For the list of possible compositions based on the exact masses of these ions, the relative abundance of the +1 profile arising primarily from ^{13}C atoms rejected all but the correct composition in both cases.

ICE provided a unique composition for the molecular and fragment ions, while oa-TOF would have left 41 and 50 viable compositions, respectively.

Table 3. *Possible compositions for a fragment ion and the apparent molecular ion for two Br containing isomers.*

m/z = 415.12744 ± 3 ppm (1.25 mDa)

Composition	0	+1	+2	%+1 (%+1 Range)		%+2 (%+2 Range)	
$C_{19}H_{28}N_4SBr$	.12795	.13070	.12591	20.20 (16.65-23.85)	X	103.20 (88.77-117.94)	
$C_{17}H_{29}N_2OPBr$	.12623	.12931	.12430	19.65 (16.18-23.14)	X	96.96 (83.22-110.57)	
$C_{18}H_{24}F_3SBr$	.12819	.13148	.12616	20.90 (17.97-23.85)	X	102.42 (90.13-114.90)	
$C_{18}H_{27}N_2O_2FBr$	.12707	.13023	.12515	20.90 (17.64-24.20)	X	98.37 (86.63-110.29)	
$C_{19}H_{31}NO_2PBr$	.12758	.13092	.12567	21.64 (18.61-24.70)	X	98.43 (86.59-110.46)	
$C_{20}H_{35}O_2FBr$	.12841	.13182	.12652	22.55 (19.41-25.69)	X	98.86 (86.79-111.17)	
$C_{23}H_{28}O_2Br$ ✓	.12727	.13067	.12541	25.80 (22.22-29.39)		98.57 (86.49-110.93)	
Exp'l Values:	**.12744**	**.13066**	**.12547**	**26.00**		**102.01**	

m/z = 430.15123 ± 3 ppm (1.29 mDa)

Composition	0	+1	+2	%+1 (%+1 Range)		%+2 (%+2 Range)	
$C_{17}H_{33}NO_2FPBr$	.15220	.15554	.15027	19.53 (16.80-22.30)	X	98.65 (86.85-110.62)	
$C_{17}H_{31}N_4SBr$	.15143	.15420	.14939	21.41 (17.63-25.28)	X	103.37 (88.90-118.16)	
$C_{19}H_{34}F_3SBr$	.15167	.15496	.14965	22.05 (18.96-25.16)	X	102.59 (90.25-115.13)	X
$C_{19}H_{37}N_2O_2FBr$	.15054	.15372	.14864	22.07 (18.61-25.56)	X	98.53 (86.76-110.50)	
$C_{20}H_{34}NO_2PBr$	.15105	.15440	.14916	22.80 (19.60-26.01)	X	98.59 (86.72-110.68)	
$C_{21}H_{38}O_2FBr$	.15188	.15530	.15001	23.69 (20.40-27.00)	X	99.04 (86.94-111.40)	
$C_{24}H_{31}O_2Br$ ✓	.15074	.15415	.14890	26.95 (23.21-30.69)		98.78 (86.68-111.19)	
Exp'l Values:	**.15123**	**.15536**	**.14945**	**27.60**		**89.47**	

An X indicates inconsistency between the measured and calculated values.

Library Searches Based on One Composition

With a single composition to consider, $C_{24}H_{31}O_2Br$, for the apparent molecular ion, chemical reasoning and searches of the chemical and commercial literature can lead to compound identification. Chlorination of the well water containing bromide ions could brominate organic compounds. The structure of Quinbolone, an anabolic steroid, is shown in Figure 2b and has three possible allylic bromination sites, which can account for the two isomers observed in the ion chromatograms in Figure 1a. Substitution of a Br atom for an H atom would provide the observed composition. A feed lot was located near the well and anabolic steroids are often used to stimulate growth. Purchase of Quinbolone, its chlorination in the presence of bromide ions, and examination of the mass spectra of the products would be logical next steps in the identification process for this compound.

Conclusion

MPPSIRD with a double focusing mass spectrometer provides more accurate measurement of exact masses and relative abundances than the current generation of oa-TOF mass spectrometers. Consequently, MPPSIRD is better able to determine compositions of ions in mass spectra that can lead to compound identifications.

Speculations

Full-size double focusing mass spectrometers cost about $500,000 and have large foot prints. However, a bench-top double focusing mass spectrometer with a price more similar to those of oa-TOF instruments is commercially available. With lower mass resolution than the larger instruments, but the same linear dynamic range advantage, would this instrument also be superior to oa-TOF MS for determining ion compositions?

Multiple MPPSIRD experiments are required to determine the composition of an ion, while oa-TOF MS can acquire data for all prominent ions in one mass spectrum. oa-TOF instruments will become more useful for determining ion compositions as their specifications for mass accuracy, linear dynamic range, and resolving power improve. Ultimately, the two types of instruments may complement each other. Determination of molecular ion compositions using MPPSIRD will set limits for the elements and atoms of each element, which will in turn limit the list of possible compositions provided by an oa-TOF MS for the fragment ions. Fewer experiments would be needed to reveal the compositions of the prominent fragment ions. Knowledge of the fragment ion compositions limits the number of possible isomers.

Ice + Containment

Only the Environmental Chemistry Branch (ECB) now performs ICE. This lab is not equipped to work with unanticipated compounds that could be extremely toxic. ECB is ready and willing to transfer ICE technology to containment labs within secure facilities. If necessary, ECB will adapt the ICE code for the data systems of other models of double focusing mass spectrometers. The labs expected to identify compounds added to water supplies would then have a powerful new analytical tool for doing so.

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