Determination of Elemental Compositions by High Resolution Mass Spectrometry without Mass Calibrants

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INTRODUCTION

For decades, mass calibrants have been used to determine exact masses of analyte ions using high resolution mass spectrometry (HRMS). For low-mass ions, a single elemental composition corresponds to a precisely and accurately determined exact mass. For larger-mass ions, exact masses and relative abundances of mass peak profiles that arise from ions containing less common isotopes than the molecular ion, $[M]^{+\cdot}$, provide the elemental composition of compounds.[1] Widely applicable mass calibrants, including perfluorokerosene, are available for gas-phase introduction of analytes ionized by electron impact, but no all-purpose calibrants[2] are available for recently developed liquid sample introduction techniques that use electrospray or atmospheric pressure chemical ionization. This limitation stimulated development of an alternative approach for determining elemental compositions of ions.

2. HRMS Measurements

Double focusing mass spectrometers measure ion abundances as a function of mass-to-charge (m/z) ratio with high mass resolution. Using these measurements, relative abundances of ions, the presence of multiple mass peak profiles from an analyte at a single nominal mass, and exact mass differences between ions are determinable. These data alone were used to establish elemental compositions of several compounds without using mass calibrants.

3. Relative Abundances of Mass Peak Profiles

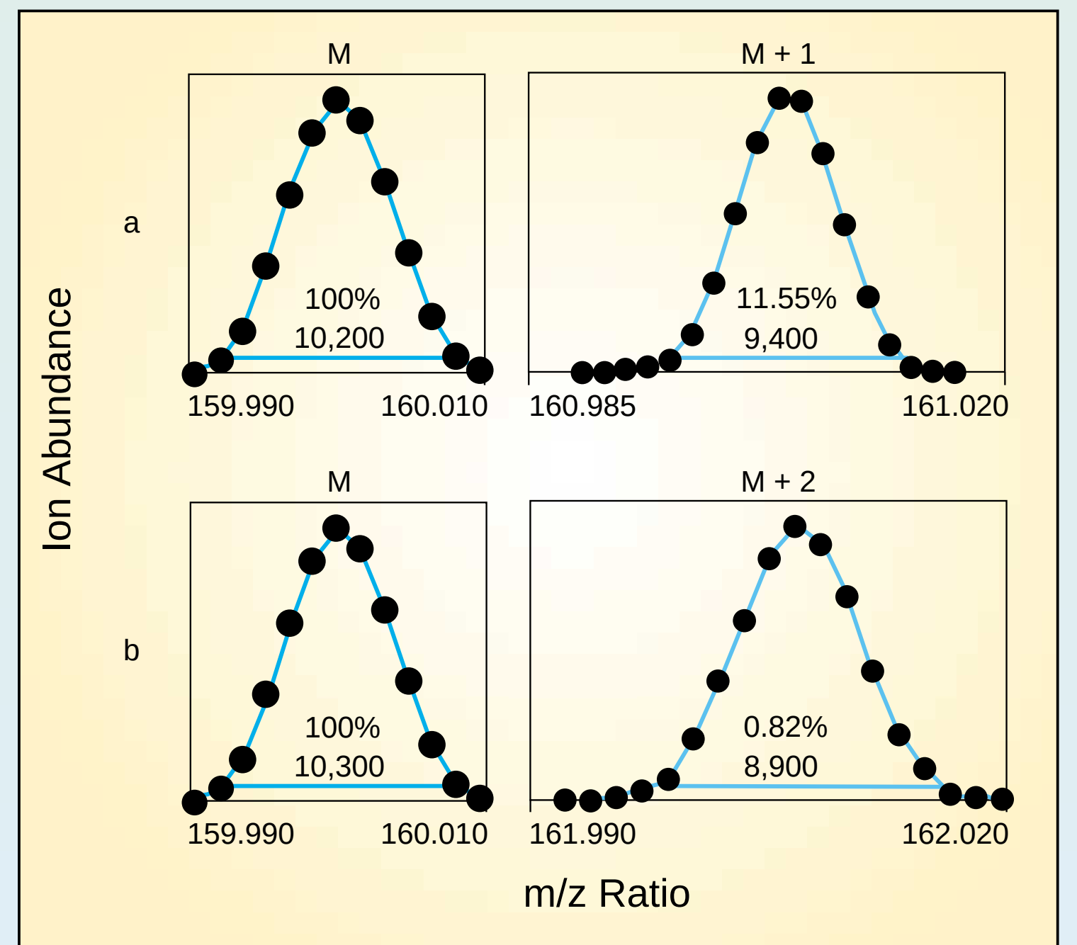
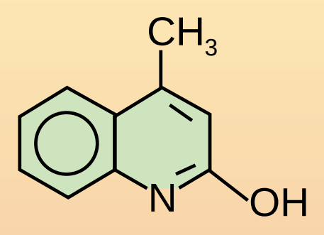


Figure 1. *M and M+1 profiles (a) and M and M+2 profiles (b) for protonated 2-hydroxy-4-methylquinoline.*

Biemann[3] and McLafferty[4] have illustrated that the relative abundances of isotopes heavier by 1 or 2 Da than the most common isotopes in an ion can be used to estimate the number of C, Cl, Br, or other atoms in the ion. Letting M and F represent the *protonated molecule, $[MH]^+$*, and fragment ions, respectively, the abundances of the M+1 and M+2 mass peak profiles relative to the M profile and of F+1 and F+2 profiles relative to F profiles limit the possible compositions for M and F's. The greater the accuracy of relative abundances determined and the smaller the error limits, the shorter will be the lists. The error cannot be less than the ranges of isotopic abundances found in nature but additional error can be limited by acquiring the data using Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD).[5] A Finnigan MAT 900S-Trap hybrid mass spectrometer was used for this work; note that Selected Ion Recording and Multiple Ion Detection (MID) are synonymous.

4. Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD)

Table 1. 2-hydroxy-4-methylquinoline



m/z 160 %M+1=11.53±0.45 %M+2=0.82±0.04

#	Composition	%M+1	%M+2	Δ%M+1	Δ%M+2
1	$C_{10}H_{10}NO^+$	11.68	0.82	-0.15	+0.00

In Figure 1, mass peak profiles were plotted from MID data acquired for 100 s at different m/z ratios across the profiles while 4 µL/min of 10 ng/µL of 2-hydroxy-4-methylquinoline in 1:1 methanol:water with 1% acetic acid was infused into an electrospray ionization (ESI) source. In Figure 1a, M and M+1 profiles are shown, and in Figure 1b are M and M+2 profiles. The data for each relative abundance was acquired using the same MID descriptor to minimize error. The relative abundance of the M+2 profile was less than 1%. The wide dynamic range afforded by double focusing mass spectrometers and the 100-fold enhancement in sensitivity provided by MPPSIRD compared to full scanning enabled accurate determination of such small relative abundances using ESI. The mass resolution of 10,000 was maintained after data acquired for 100 s was integrated because the software locked on to the maximum in the first mass peak profile before data was recorded for each 1-s MID cycle. The maximum average standard deviations (σ) obtained for 13 triplicate measurements for each of four standards were 1.3% of %M+1 and 1.8% of %M+2. Three σ of 3.9% and 5.4% were used in a modified Profile Generation Model[1] (PGM) that compiles lists of possible compositions based only on relative abundances. This precision error was added to the possible isotopic abundance error for each composition. Table 1 shows that only the correct composition, ($C_{10}H_{10}NO^+$, 160 u) was possible for the low-mass, protonated molecule from 2-hydroxy-4-methylquinoline.

5. Examination of the M+1 Profile

M+1 and M+2 mass peak profiles usually arise from multiple ions. For example, an M+1 profile can have contributions from ions containing a ^{13}C atom or a ^{15}N atom. Because the mass differences between ^{13}C and ^{12}C and between ^{14}N and ^{15}N are 1.00336 Da and 0.99704 Da, respectively, the mass difference between the two M+1 profiles is 0.00632 Da. For small ions, sufficient mass resolution is available to separate these two profiles. When a profile due to an ion containing a ^{15}N atom is observed in an M+1 or F+1 profile, at least 1 N atom is present in M or F. This is the case in Figure 2, where the calculated and observed M+1 profiles at 24,000 resolution for protonated benzidine ($C_{12}H_{13}N_2^+$) are shown. Relative abundance data alone provided the 5 possible compositions in Table 2. Only the correct composition contained one or more N atoms.

Examination of individual profiles is also used to check for interferences, which are common for small mass ions. As in Figure 1, relative abundances can be determined for F+1 and F+2 profiles using a mass resolution that separates the analyte profile from interfering profiles, but that does not resolve multiple profiles due to the analyte.

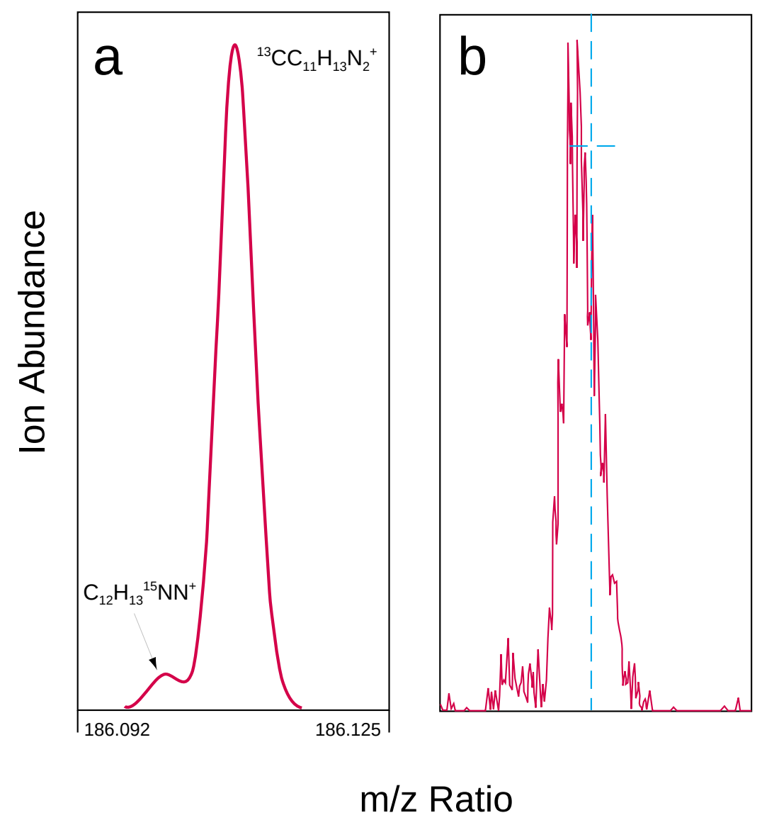
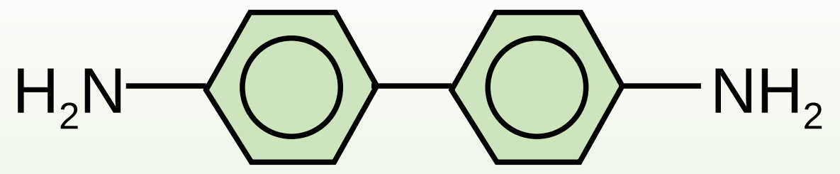


Figure 2. *Calculated (a) and observed (b) M+1 mass peak profiles for protonated benzidine at 24,000 resolution.*

6. Exact Mass Differences

Table 2. Benzidine



m/z 185 %M+1=14.13±0.55 %M+2=0.94±0.05

#	Composition	%M+1	%M+2	Δ%M+1	Δ%M+2
1	$C_{12}H_6OF^+$	13.47	1.03	+0.66	-0.09
2	$C_{12}H_{10}P^+$	13.50	0.84	+0.63	+0.10
3	$C_{12}H_{13}N_2^+$	14.28	0.94	-0.15	-0.00
4	$C_{12}H_{22}F^+$	13.68	0.86	+0.45	+0.08
5	$C_{13}H_{10}F^+$	14.61	0.99	-0.48	-0.05

In Figure 3 are shown three profiles plotted from MID data for widely separated masses. If calibrants were used, the first and third profiles would be due to calibrant ions with known exact masses and the second profile's exact mass would be determined from the masses of the other two. Here, either the mass difference between the first and second profiles or between the second and third profiles was known, and the other mass difference was determined using the known difference for calibration. Successive mass differences between pairs of ions were determined moving toward lower masses from the protonated molecule or toward higher masses starting with a fragment ion. The initial known mass difference was either the calculated mass difference between F and F+1 profiles for an F with a composition determined from relative abundances or the difference in mass between ^{13}C and ^{12}C atoms when the highest mass F and F+1 profiles were used. The error introduced by ignoring contributions from F+1 ions containing ^{15}N, ^2H, or ^{17}O to the mass of the F+1 profile was very small compared to the mass differences between possible neutral losses that produced the F ion. The sum of exact mass differences between M and F ions was determined with sufficient accuracy to determine the correct elemental compositions of the corresponding neutral losses.

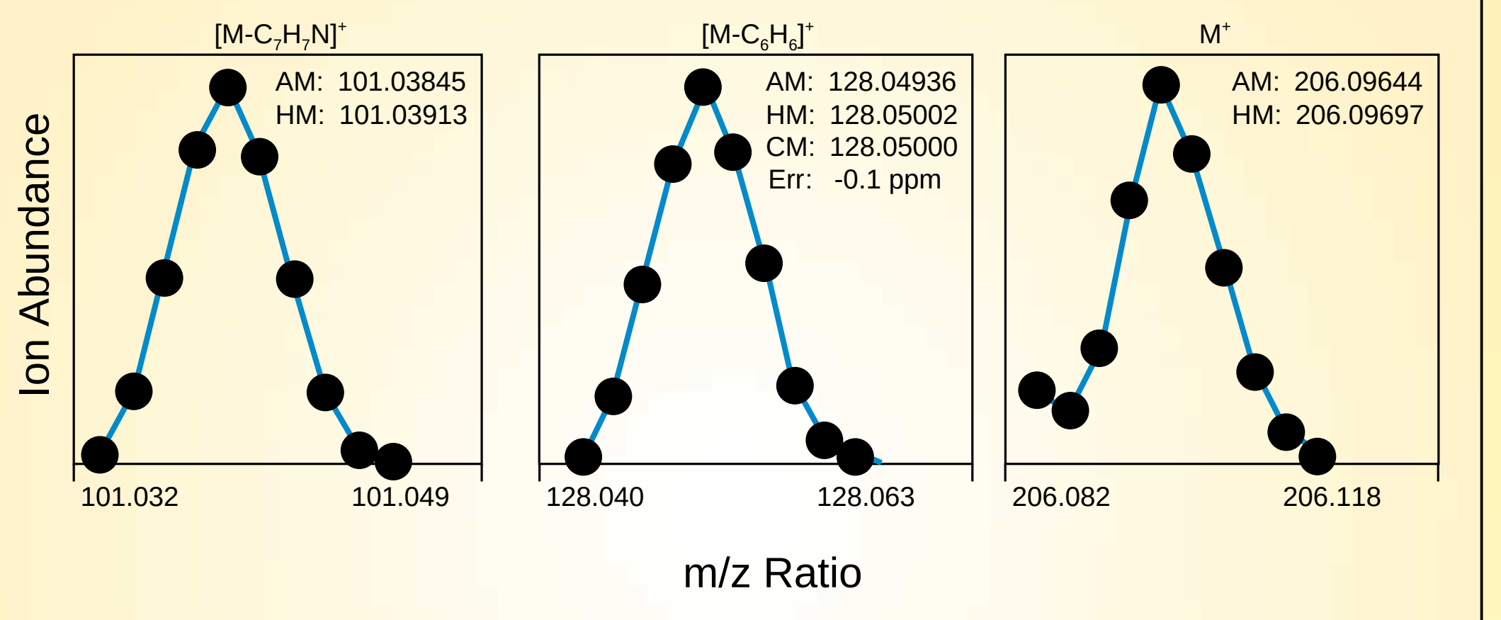


Figure 3. *Mass peak profiles for two fragment ions that are widely separated in mass and the protonated molecule from 2-phenylquinoline. The apparent masses (AM) were determined from the data; the hypothetical masses (HM) were entered as the center masses in the MID descriptor; and the corrected mass (CM) was determined using corrections for the first and third AMs.*

Figure 4 illustrates that exact mass differences for small neutral losses are determinable using the calibration mass option of the MID software, even when the correct composition of the ions is unknown. Ions with m/z 215 and m/z 230 result from successive loss of CH_3 from 3,3'-dimethoxybenzidine ($C_{14}H_{17}N_2O_2$+, 245.1290 u). A wrong composition of M that passed the relative abundance criteria was assumed ($C_{14}H_{33}ON_2^+$, 245.2596 u). In Figure 4a, its exact mass was chosen as the calibration mass in the MID descriptor; while in Figure 4b, the calibration mass option was not used. In both cases, lock on occurred and the first profile was centered within the mass range monitored. In Figure 4b, the use of incorrect exact masses caused the wrong mass range to be monitored for the second and third profiles, and they are not centered in their displays. In Figure 4a, the MID software assigned the specified calibration mass to the top of the third profile, and the error in the masses was compensated. The exact mass differences from Figure 4a are correct, even though the exact masses are not.

The largest/smallest mass ratio for Figure 3 was 2.04; 2 is the maximum normally used due to sensitivity loss for the largest mass ion at the halved accelerating potential ($V_2 = V_1 \times m_1/m_2$). Using the other two profiles to calibrate each profile in turn, the average errors in the exact masses for 15 determinations in the first, second, and third profiles were 0.3, -0.3, and 1.0 ppm, respectively. The largest errors observed were 2.8, -2.1, and 8.1 ppm. Errors of this magnitude allowed determination of the correct neutral losses between M and F ions.

In Table 3, possible compositions for M and F ions were determined from their relative abundances. A contribution from an ion containing ^{15}N was observed in the F+1 profile, which identified composition B as correct. Because compositions 1-3 all contained N atoms, the exact mass difference between the ions was determined. Based on the largest numbers of each element in compositions 1-3, only a neutral loss of C_6H_6 was possible. Addition of C_6H_6 to composition B identified composition 3 as correct for the protonated molecule from 2-phenylquinoline.

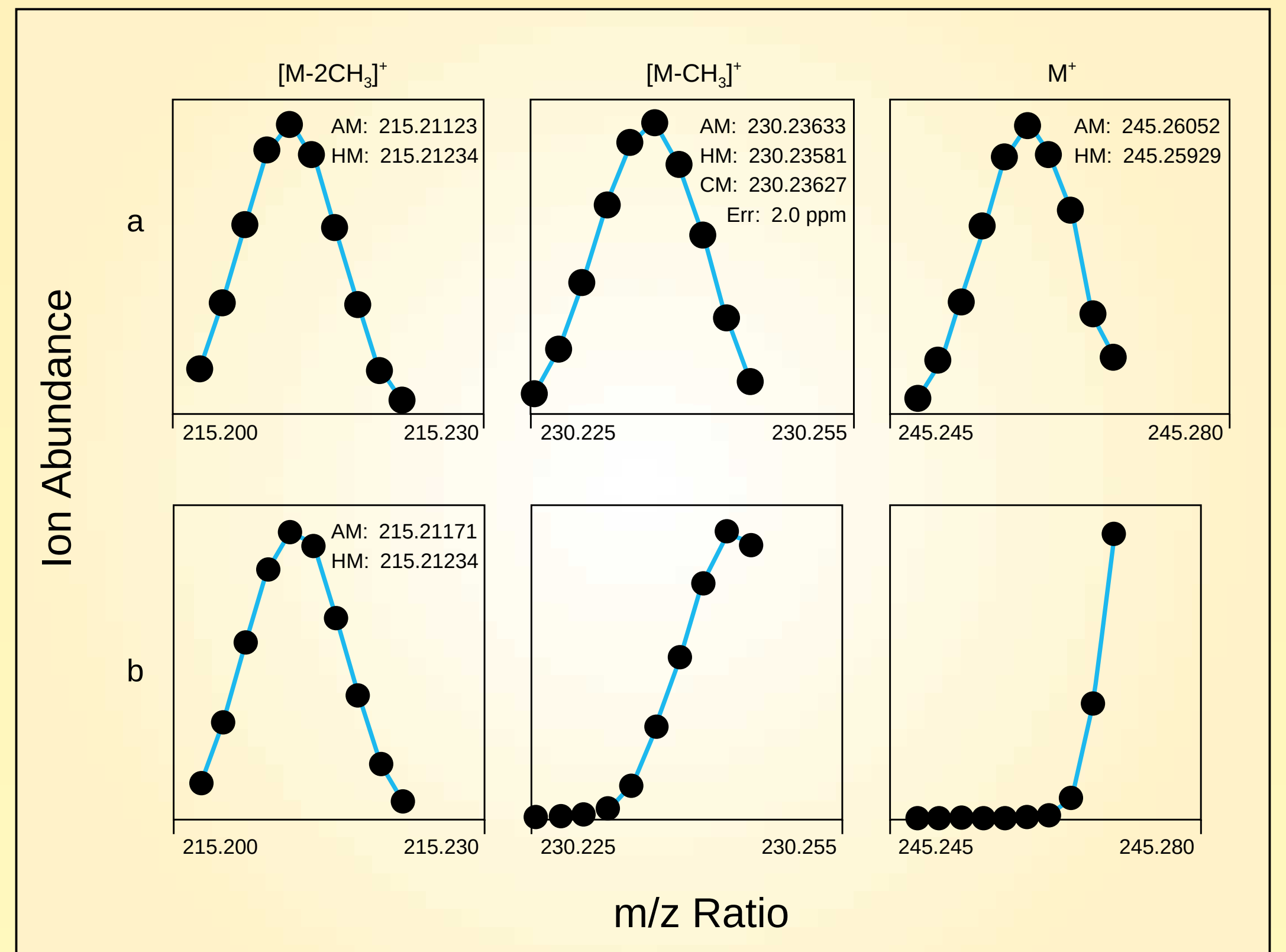
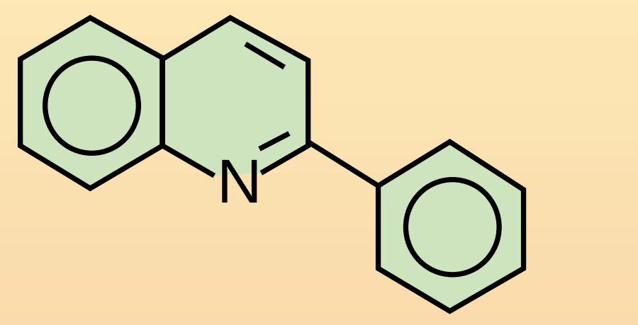


Figure 4. *Mass peak profiles for M and fragment ions formed by loss of one and two methyl groups from M. The hypothetical composition and therefore the exact mass for M was incorrect. In (a) the calibration option in the MID descriptor was used; in (b) it was not.*

Table 3. 2-Phenylquinoline



m/z 206 %M+1=16.78±0.65 %M+2=1.40±0.08

#	Composition	%M+1	%M+2	Δ%M+1	Δ%M+2
1	$C_{14}H_8NO^+$	16.10	1.41	+0.68	-0.01
2	$C_{14}H_{24}N^+$	16.30	1.24	+0.48	+0.16
3	$C_{15}H_{12}N^+$	17.23	1.39	-0.45	+0.01

m/z 128 %M+1=10.22±0.40 %M+2=0.48±0.03

#	Composition	%M+1	%M+2	Δ%M+1	Δ%M+2
A	C_9HF^+	10.03	0.45	+0.19	+0.03
B	$C_9H_6N^+$	10.47	0.49	-0.25	-0.01
C	C_9H_{20}+	10.31	0.48	-0.09	+0.00

7. Larger Protonated Molecules

Determination of relative abundances, observation of F+1 and M+1 profiles with high mass resolution, and determination of exact mass differences have been used to determine the compositions of higher mass compounds: 1-aminopyrene ($C_{16}H_{11}N$, 217 u); 3,3'-dimethoxybenzidine ($C_{14}H_{16}N_2O_2$, 244 u); cetyldimethylethylammonium ion ($C_{20}H_{44}N^+$, 298 u); and chlorpromazine ($C_{17}H_{19}N_2SCl$, 318 u). In two cases, too few fragment ions were observed to bridge the gap between the smallest F and M, while keeping the largest/smallest mass ratio below 2. In these cases, ions from the solvent or contaminants were used to establish intermediate mass differences, and the correct neutral losses were determined from the summed mass difference between M and F. The correct elemental compositions were determined for seven standards. The identity of two of the standards, 2-hydroxy-4-methylquinoline and 1-aminopyrene, were unknown to the operator.

Conclusion

When calibrants are available, elemental compositions for analyte ions are determined based on exact masses of M, M+1, and M+2 profiles and relative abundances of the M+1 and M+2 profiles. When calibrants are not available, the infusion mode of sample introduction provides a lock mass, which allows use of MPPSIRD to accurately determine relative abundances of M+1, M+2, F+1, and F+2 ions and exact mass differences between ions. Coupled with the appearance of M+1 and F+1 profiles, elemental compositions can be determined for ions, although more data must be collected and evaluated. The methodology outlined here could be useful for analyzing HPLC fractions. This work appears in more detail in Reference 5.

References

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