Confirming Synthesis Products Using Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD)

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1 ABSTRACT

A crucial step in developing immunoassay methods for small molecules is synthesis of haptens. Determining the exact mass of the molecular ion of synthetic products provides incomplete confirmation. To reveal structural features of the molecule, purification or chromatography is required before using NMR or FTIR. In this study, HRMS provided structural information for a thermolabile, non-ionic, phosphorothionate compound in a synthetic mixture without prior separation. A direct insertion probe introduced the product into a VG70-250SE mass spectrometer. Ions from the product and impurities were separated by mass using HRMS rather than in the time domain by using chromatography to separate the original compounds. A fragmentation scheme based on the unique compositions determined for the molecular ion and fragment ions was consistent with the structural features of the desired product.

2 INTRODUCTION

The Journal of the American Society for Mass Spectrometry[1] states that the "acceptable uncertainty" in the exact mass measurement must be assessed and all elemental compositions possible for an ion within that error range must be considered. The number of compositions possible for an ion increases rapidly for a given error limit as a function of the ion's mass and the number of elements considered.[2] When confirming identities of synthetic products, the list of elements can be limited to those in the reactants, solvents, and catalysts.

3 TOOLS FOR SELECTING THE CORRECT COMPOSITION

Recently, the Environmental Sciences Division of the Office of Research and Development, U.S. EPA, developed analytical tools used to determine the elemental compositions of ions formed from environmental contaminants in complex mixtures.[3] The tools are a new high resolution mass spectrometric technique for acquiring data, Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD),[4,5] and a Profile Generation Model (PGM)[2] that is used to plan experiments and interpret the data. The PGM considers error limits of the exact masses determined for the M, M+1, and M+2 mass peak profiles and the abundances of the M+1 and M+2 profiles relative to that of the M profile. Testing criteria based on five quantities, three exact masses and two relative abundances, rather than a single exact mass, increases the upper mass limit for which a unique composition can usually be determined from 150 Da to 600 Da for ions containing C, H, N, O, P, or S atoms.[2]

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4 APPLICATION TO IDENTIFYING A SYNTHESIS PRODUCT

In this study, MPPSIRD and the PGM were used to determine the elemental composition of a synthesis product that was prepared by the Human Exposure Research Branch (EPA) to develop a new immunoassay.[6] MPPSIRD was further employed to determine elemental compositions of fragment ions observed in the low resolution (1,000) full scan (50-700 Da) mass spectrum shown in Figure 1. From this information, structural features of the product were determined, to provide further confirmation of the product's identity.

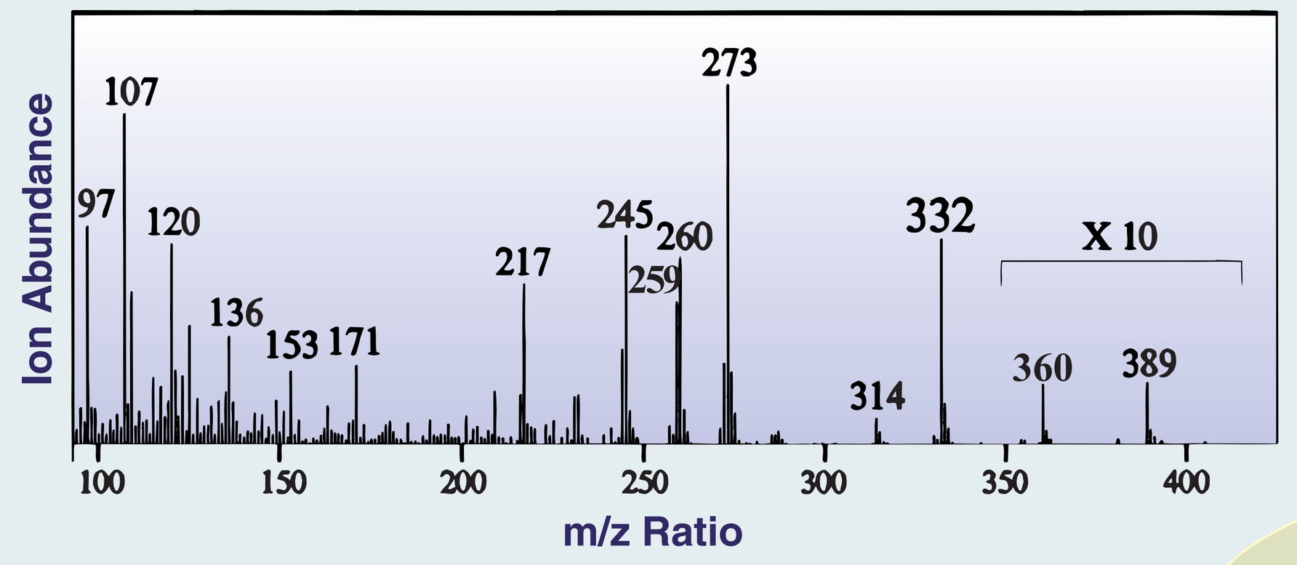

Figure 1. *Low resolution mass spectrum corresponding to the maximum in the ion chromatogram for the m/z 332 ion. The ion abundances were magnified by 10 above 350 Da.*

5 EXPERIMENTAL SAMPLE INTRODUCTION

A small amount of the synthesis product was dissolved in about 1 mL of toluene. From 0.1 - 1 µL of the solution was injected within the probe capillary and the solvent was evaporated in a roughing vacuum region before being pressed against the ion source block. The block was at 250 ºC and slowly heated the probe tip. Gentle heating made thermal decomposition of the analyte less likely. Most of the product was volatilized over a 1.5-min period.

6 MPPSIRD

In Figure 2a are displayed ion chromatograms obtained at different m/z ratios across the mass peak profile in Figure 2b that was plotted from the areas of the shaded chromatographic peaks. Figure 2b is a full mass peak profile acquired at 20,000 (±10%) resolution with 5-ppm mass increments between the points. The chromatographic peak areas used to plot the three partial profiles in Figure 2c were acquired using a single SIR descriptor, which can monitor no more than 25 m/z ratios. From the partial profiles, the exact masses of the M+1 and M+2 partial profiles and the abundances of the M+1 and M+2 partial profiles relative to the M partial profile are obtained; the exact masses as weighted averages of the top several points and the relative abundances as the appropriate ratio of the sum of the six points for each partial profile. Partial profiles were used only to identify molecular ions. The exact mass obtained from the partial profile of a single calibration ion (not shown), which is also monitored for both full and partial profiles, is used to correct the exact masses of the analyte ions.

MPPSIRD provides about 100 times more sensitivity and 3-fold faster cycle times[4] than KVE scanning at 10,000 resolution. However, these advantages were not important in these experiments. Here, MPPSIRD was used rather than KVE scanning for three other reasons. (i) With MPPSIRD, data interpretation was automated, whereas KVE scan data requires manual interaction to locate profile maxima and to enter reference masses. (ii) The error limits for exact mass determinations and relative abundances for the M+1 and M+2 profiles by MPPSIRD have been established and are incorporated in the PGM,[5] which is used to reject incorrect compositions using criteria based on these quantities automatically. (iii) The computer memory requirement for data is an order of magnitude less for MPPSIRD.

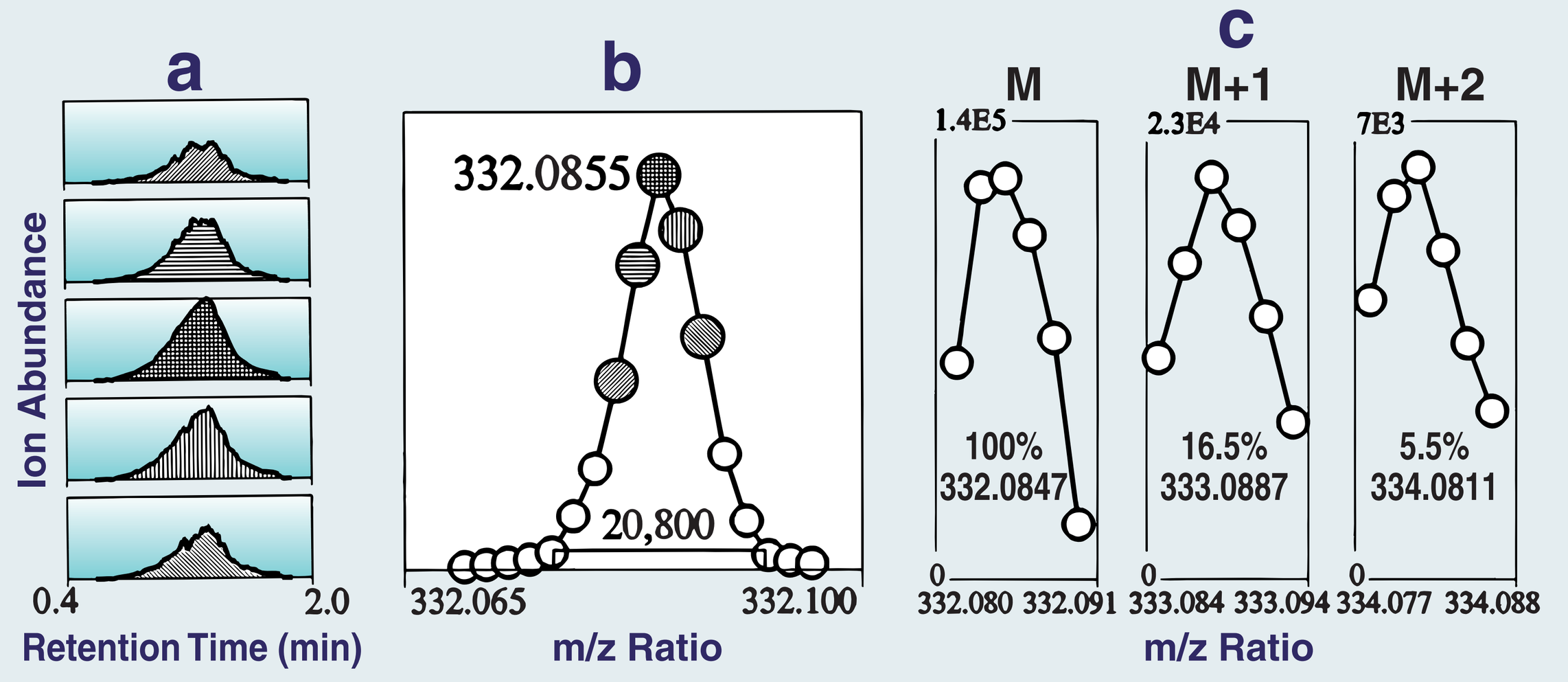

Figure 2. (a) chromatographic peak areas under ion chromatograms acquired at five m/z ratios across the top of a mass peak profile. (b) a full mass peak profile plotted from chromatographic peak areas, including those in (a). (c) partial mass peak profiles for M, M+1, and M+2 ions.

7 RESULTS AND DISCUSSION

The low resolution mass spectrum corresponding to the maximum in the total ion chromatogram for the primary compound in the synthesis product is shown in Figure 1. The presumed molecular ion of the target product at m/z 332 and numerous possible fragment ions were observed. Ions with m/z 360 and m/z 389 were also of interest, since one might be the parent ion of the m/z 332 ion.

8 ELEMENTAL COMPOSITION OF THE MOLECULAR ION

The exact mass of the m/z 332 ion was determined to be 332.0855 ±0.0010 Da (±3 ppm) from the mass peak profile in Figure 2b obtained at 20,000 resolution. The PGM determined that 53 compositions were possible within the error limits of the exact mass determination. In Table 1, 17 of the possible compositions are listed with the calculated mass defects for the M profile and the M+1 and M+2 partial profiles, as well as the calculated abundances of the M+1 and M+2 partial profiles relative to the M partial profile. The permissible ranges for %M+1 and %M+2 calculated by the PGM are shown in parentheses. The experimental values were determined from the partial profiles in Figure 2c. The error limits exceeded by the experimental results are marked with "X". Because only C, H, N, O, P, and S atoms were present during synthesis, only the expected composition, $C_{14}H_{21}O_5PS$, was consistent with the five quantities; the other 52 compositions were not.

Using full and partial profiles acquired at 10,000 and 20,000 resolution, the compositions of the m/z 360 and m/z 389 ions seen in low abundance in Figure 1 were determined using the PGM to be $C_{16}H_{25}O_5PS^{+\cdot}$ and $C_{17}H_{26}O_4PS_2^{+}$, respectively, based on the five criteria. Because the m/z 332 ion contains one more O atom than the m/z 389 ion, the m/z 332 ion was not formed from the m/z 389 ion. A linked scan for m/z 360 indicated that the m/z 332 ion was not formed from the m/z 360 ion either.

Table 1. *Elemental compositions and quantities useful for distinguishing among them.*

m/z 332.0855 ±3 ppm — Resolution: 20000

#	RDB[a]		Composition	M[b]	M+1[b]	M+2[b]	%M+1[c]	%M+1 Range[d]	%M+2[c]	%M+2 Range[d]
37	6.0	18.0	$C_9H_{18}N_8P_2S$	.0850	.0874 X	.0811	12.2	(9.8-15.2) X	4.8	(3.8- 5.7)
38	0.5	5.5	$C_{10}H_{24}O_5NP_2S$	.0850	.0882	.0816	12.3	(10.4-14.5) X	4.8	(3.9- 5.7)
39	0.5	5.5	$C_{10}H_{26}O_3NP_4$	.0863	.0896	.0918 X	11.7	(10.0-13.5) X	1.2	(0.0- 0.6) X
40	11.5	20.5	$C_{11}H_{10}O_4N_9$	.0856	.0881	.0901 X	14.0	(11.4-17.4)	1.7	(0.0- 1.2) X
41	5.5	14.5	$C_{11}H_{18}O_3N_5S_2$	.0851	.0878	.0812	14.6	(11.8-17.8)	9.3	(7.6-11.1) X
42	5.5	14.5	$C_{11}H_{20}ON_5P_2S$	.0864	.0892	.0827 X	13.9	(11.7-17.1)	4.7	(4.0- 5.9)
43	-0.5	8.5	$C_{11}H_{26}O_2NS_4$	.0846	.0872 X	.0805	15.2	(12.3-18.2)	18.1	(14.1-21.4) X
44	-0.5	8.5	$C_{11}H_{28}NP_3S_3$	.0859	.0887	.0818	14.5	(12.1-17.7)	13.5	(11.3-16.8) X
45	6.0	8.0	$C_{12}H_{16}O_9N_2$	.0856	.0888	.0905 X	14.2	(12.1-16.6)	2.7	(0.0- 1.5) X
46	5.5	11.5	$C_{12}H_{21}O_2N_3P_3$	.0847	.0879	.0904 X	14.1	(12.1-16.5) X	1.3	(0.0- 0.8) X
47	10.5	20.5	$C_{13}H_{15}N_7PS$	.0847	.0875 X	.0810	16.4	(13.4-19.5)	4.9	(4.0- 5.7)
48	5.0	11.0	$C_{13}H_{20}O_4N_2S_2$	.0864	.0893	.0826 X	16.3	(13.9-20.0)	9.3	(7.9-11.7) X
50	5.0	8.0	$C_{14}H_{23}O_3P_3$	.0860	.0894	.0920 X	16.0	(13.1-18.4)	1.7	(0.0- 0.7) X
51	10.0	17.0	$C_{15}H_{17}ON_4PS$	.0861	.0892	.0826 X	18.1	(15.4-21.9)	4.8	(4.0- 5.9)
52	4.0	11.0	$C_{15}H_{25}PS_3$	.0856	.0886	.0815	18.7	(15.8-22.1)	13.6	(11.2-16.6) X
53	14.5	19.5	$C_{19}H_{14}ON_3S$	.0858	.0888	.0825 X	22.4	(19.1-26.4) X	4.9	(4.0- 6.0)
49	5.0	8.0	$C_{14}H_{21}O_5PS$	.0847	.0880	.0813	16.6	(14.2-19.1)	4.8	(3.9- 5.9)
Experimental Values:				.0855	.0887	.0811	16.5		5.5	

[a]Rings and double bonds: minimum with valences of 3, 3, and 2 for N, P, and S, and maximum with valences of 5, 5, and 6 for N, P, and S; [b]based on partial profiles that provide maximum area; [c]based on full profiles; [d]based on partial profiles centered about the calculated mass of the hypothetical composition, ±1 mass increment at ±10% of resolution, and isotopic abundance.[5] An "x" indicates application of this criterion will reject this composition if the hypothetical composition is correct.

9 IDENTIFYING FRAGMENT IONS

The compositions of fragment ions and neutral losses produced from the molecular ion reveal structural details of a compound.[7,8] With probe introduction, composite mass spectra were observed due to impurities. A three-step process was employed to investigate prominent ions observed in Figure 1. First, the exact masses of the fragment ions at each nominal mass were determined from data acquired at 10,000, and in some cases 20,000 resolution. Second, ion chromatograms were acquired at 10,000 or greater resolution to determine which ions fragmented from the molecular ion. Linked scans acquired with low resolution (1000) were also used for this purpose. Finally, the PGM was used to determine elemental compositions of the fragment ions based on the maximum number of atoms of each element in the molecular ion. The ions labeled in Figure 1 were investigated.

10 CORRELATING FRAGMENT IONS AND THE MOLECULAR ION

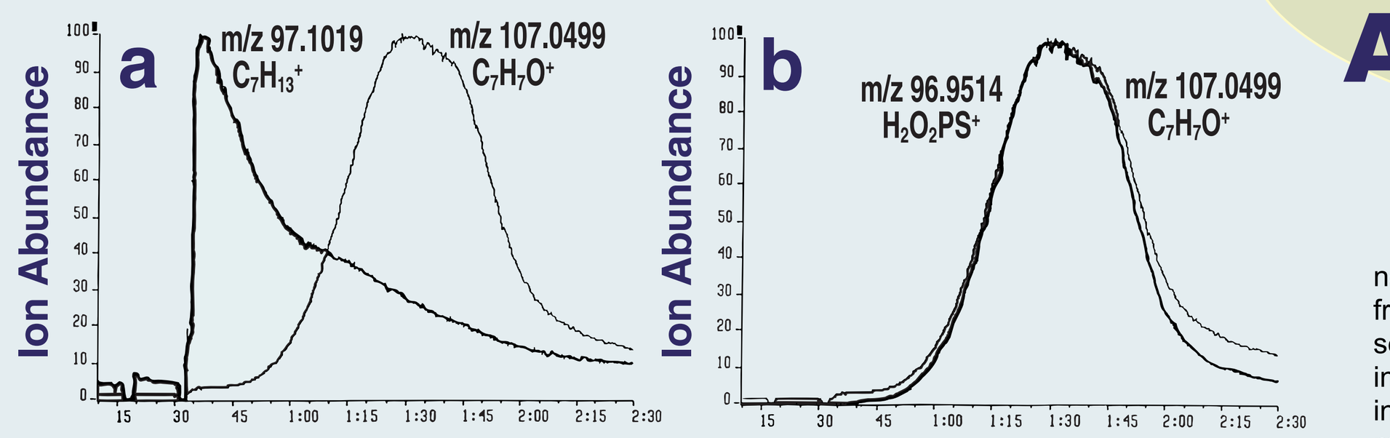

Figure 3. *Ion chromatograms acquired with 10,000 resolution for (a) m/z 107.0499 and m/z 96.514, and (b) m/z 107.0499 and m/z 97.1019.*

Ions are easily correlated with GC/MS by superimposing normalized ion chromatograms of the molecular ion and the suspected fragment ions. Volatilization from a probe, however, provides much less separation capability than a GC. To compensate for limited separation in the time domain, high mass resolution was used to provide separation in the mass domain. Hence, ion chromatograms obtained with high mass resolution were still useful for correlating ions. When two ion chromatograms did not overlap as in Figure 3a, the ions originated from different compounds. When two ion chromatograms overlapped as in Figure 3b, they usually originated from the same compound. For m/z ratios greater than 133, linked scans confirmed that several ions were daughter ions. For example, the linked scan in Figure 4 indicated that ions with m/z 314, 273, 260, 245, 217, and 171 were produced from the m/z 332 ion.

Multiple ions were found for some m/z ratios. In these cases, ion chromatograms acquired with 10,000-24,000 mass resolution alone were used to find daughter ions. For example, the ion chromatograms for two of four ions with a nominal mass of 97 Da are shown in Figures 3a and 3b. The trace for 96.9514 ($H_2O_2PS^+$, characteristic of phosphoronates) correlates well with that of the m/z 107 ion produced from the molecular ion, while the other trace for m/z 97.1019 ($C_7H_{13}^+$) does not.

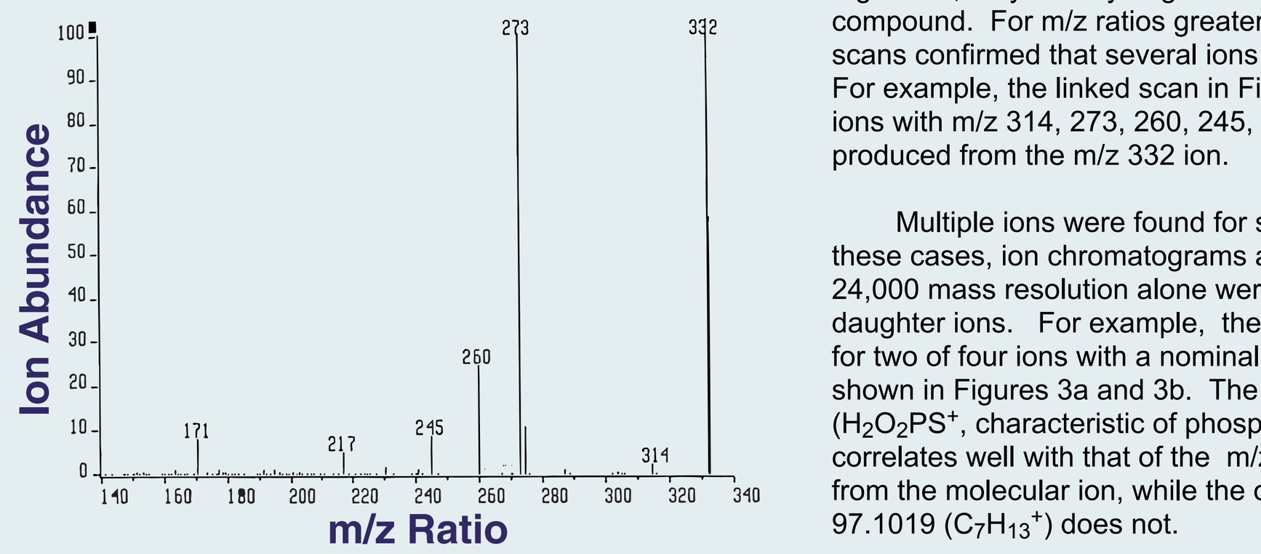

Figure 4. *A linked scan (constant B/E) for m/z 332 as the parent ion.*

11 COMPOSITIONS OF THE FRAGMENT IONS

In Table 2 are listed the exact masses of 13 ions formed by fragmentation from the m/z 332 ion, and in Table 3 are listed the exact masses of the corresponding neutral losses determined from the mass difference between M+· and the fragment ions. The compositions in the table of fragment ions are the unique elemental compositions that correspond to each exact mass based on the total number of atoms of each element in the molecular ion: 14 C, 21 H, 5 O, 1 P, and 1 S. For all neutral losses, only one composition was possible -- the one that corresponded to subtraction of the composition of each fragment ion from the composition of the molecular ion. This agreement provided a check for consistency. Obtaining exact masses eliminated numerous other plausible neutral losses based on nominal masses of the fragment ions. Simplified mass spectral interpretation and greater confidence in fragmentation schemes result when the correct composition of each ion and neutral loss is determined.

Table 2. *Exact masses for ions related to m/z 332 ion.*

Composition	RDB Range[a]		Experimental Mass	Error (mmu)
$C_{14}H_{21}O_5P^{34}S$[b]	5.0	8.0	334.0818	+0.5
$^{13}CC_{13}H_{21}O_5PS$[b]	5.0	8.0	333.0881	+0.1
$C_{14}H_{21}O_5PS$	5.0	8.0	332.0855	+0.8
$C_{14}H_{19}O_4PS$	6.0	9.0	314.0730	-1.2
$C_{12}H_{18}O_3PS$	4.5	7.5	273.0711	-0.3
$C_{11}H_{17}O_3PS$	4.0	7.0	260.0631	-0.5
$C_{11}H_{16}O_3PS$	4.5	7.5	259.0557	-0.1
$C_{10}H_{14}O_3PS$	4.5	7.5	245.0399	-0.2
$C_8H_{10}O_3PS$	4.5	7.5	217.0088	0
$C_4H_{12}O_3PS$	-0.5	2.5	171.0243	-0.2
$C_4H_{10}O_2PS$	0.5	3.5	153.0135	-0.4
$C_7H_6O_2P$	5.5	6.5	153.0104	-0.1
C_8H_8S	5.0	7.0	136.0345	-0.2
C_8H_8O	5.0		120.0573	-0.2
C_7H_7O	4.5		107.0499	+0.2
H_2O_2PS	0.5	3.5	96.9514	+0.1

[a]Rings and double bonds: minimum with valences of 3, 3, and 2 for N, P, and S, and maximum with valences of 5, 5, and 6 for N, P, and S; [b]most abundant M+1 or M+2 ion.

Table 3. *Exact masses of neutral losses determined as mass differences between the molecular and fragment ions.*

Neutral Losses	Experimental Mass	Error (mmu)
2H,O	18.0117	+1.1
2C,3H,2O	59.0136	+0.3
3C,4H,2O	72.0216	+0.5
3C,5H,2O	73.0290	0
4C,7H,2O	87.0448	+0.2
6C,11H,2O	115.0759	0
10C,9H,2O	161.0604	+0.1
10C,11H,3O	179.0712	+0.4
7C,15H,3O,S	179.0743	+0.1
6C,13H,5O,P	196.0502	+0.1
6C,13H,4O,P,S	212.0274	+0.2
7C,14H,4O,P,S	225.0348	-0.2
14C,19H,3O	235.1333	-0.1

12 POSSIBLE FRAGMENTATION SCHEME

In Figure 5 is depicted a possible fragmentation scheme that yields all of the ions shown to be associated with the molecular ion. The compositions of the fragment ions and the neutral losses, and rational explanations for formation of all the ions provide additional evidence that the target compound was synthesized. Although other fragment ions and neutral losses could be isolated, the ions with high relative abundances that were investigated were sufficient to provide structural details of the molecule. Significant structural information was deduced from HRMS alone, without resort to NMR or FTIR, which require purification of products before analysis.

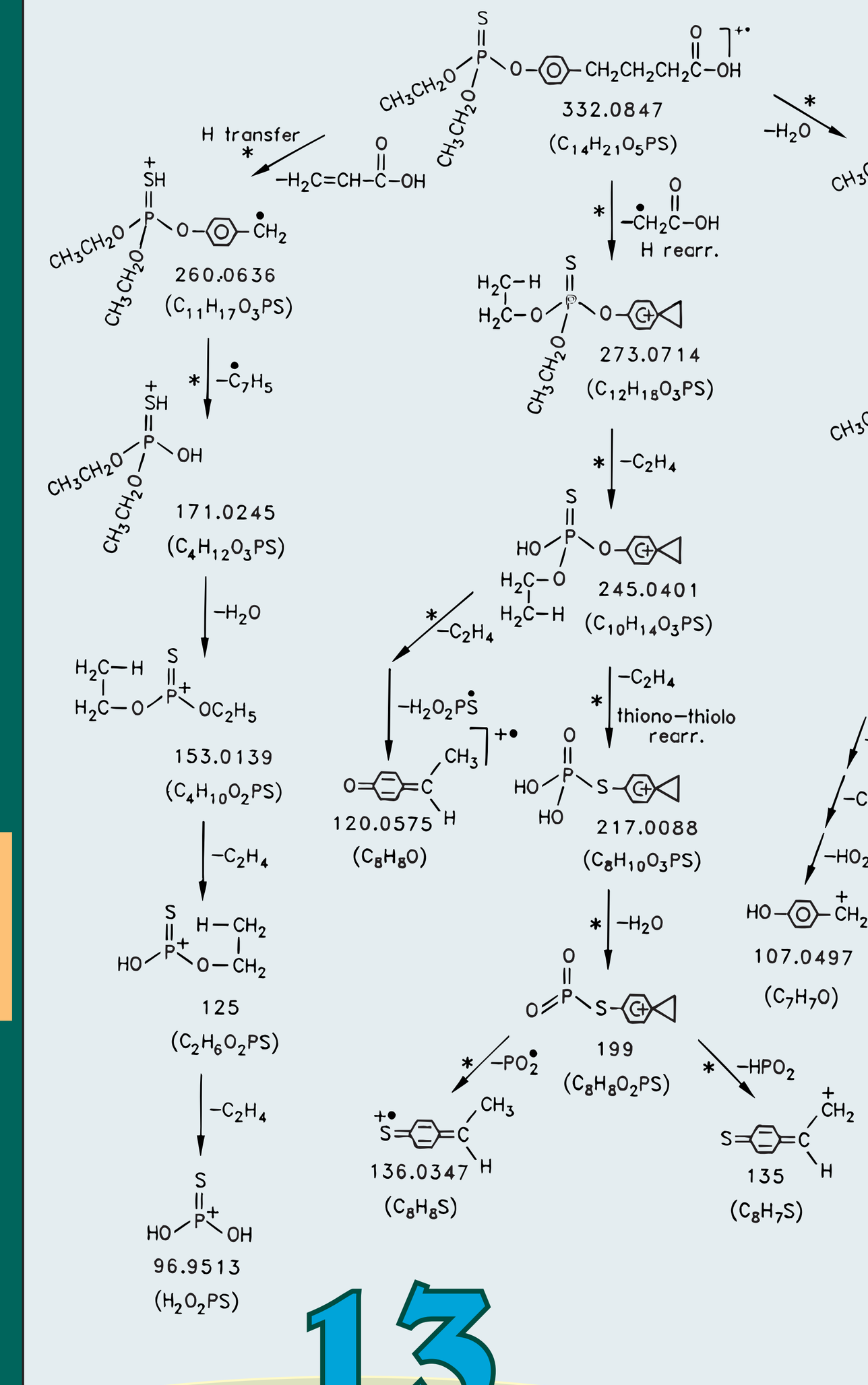

Figure 5. *Plausible fragmentation scheme for the ions investigated that were produced from the m/z 332 ion. The 7-digit masses are the theoretical masses for compositions determined using MPPSIRD and the PGM. The asterisks indicate fragmentations confirmed by linked scans.*

13 CONCLUSION

Traditionally, to provide mass spectrometric confirmation of the identity of a synthesis product, the exact mass of the molecular ion is determined and cited as consistent with the expected elemental composition within the error limits of the mass determination. In this study, the evidence that the product was made was far more complete. All other possible compositions based on the exact mass and its error limits were rejected, and structural details were deduced from the compositions of the fragment ions and neutral losses.

This study used high resolution mass spectrometry to provide compelling evidence that the desired synthesis product was made. Researchers with access to high resolution mass spectrometers should become aware that the capabilities of these machines for aiding in synthetic studies have been expanded by development of MPPSIRD, which operates with the B2.2 data system of VG-70S mass spectrometers and the PGM. These tools are available, free of charge, from the authors.

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