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TITLE

ELECTROSPRAY MASS SPECTRA OF THERAPEUTIC OXIMES: HI-6, HS-6, OBIDOXIME, 2-PAM, TMB-4 ABD HLo-7

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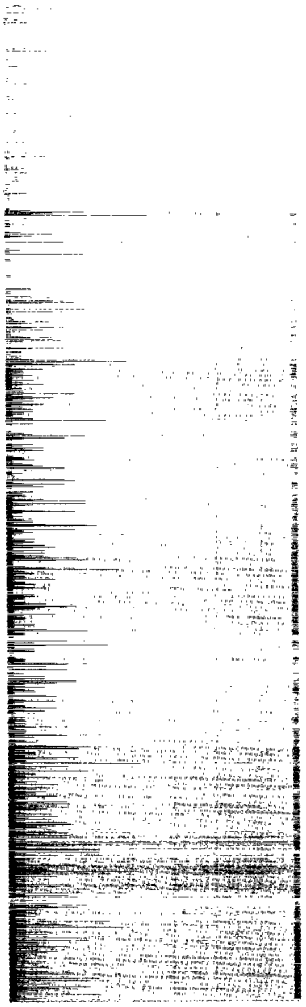
Electrospray Mass Spectra of Therapeutic Oximes: HI-6, HS-6, Obidoxime, 2-Pam, TMB-4 and HLö-7



BY

P. A. D'Agostino, L. R. Provost and J. R. Hancock
(Agent Detection Group)

and

C. A. Boulet (Agent Identification Group)

December 1995

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Suffield Memorandum No. 1475

Electrospray Mass Spectra of Therapeutic Oximes: HI-6, HS-6, Obidoxime, 2-PAM, TMB-4 and HLö-7

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P. A. D'Agostino, L. R. Provost and J. R. Hancock (Agent Identification Group)

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ABSTRACT

Pyridinium and bis-pyridinium oxime salts are currently in use or under development for treatment of patients exposed to organophosphorus nerve agents. The limited volatility and thermal lability of these compounds limits the number of mass spectrometric approaches that may be used to characterize these important compounds. Electrospray mass spectrometry (ESI-MS), a relatively new ionization approach, was investigated as a possible technique for the identification of these oximes and their degradation products. Six therapeutic oxime salts, the bis-pyridinium oximes, HI-6, HS-6, Obidoxime, TMB-4 and HLö-7, and the pyridinium oxime, 2-PAM, were analysed by ESI-MS, making use of the collisionally activated dissociation (CAD) opportunities afforded by the ESI interface. The CAD/MS (MS^2) spectra of each oxime were acquired under two different sampling cone voltage settings and the quadrupole mass analyser associated with a hybrid tandem mass spectrometer was utilized for MS/MS and CAD/MS/MS (MS^3) applications including the reliable differentiation of similar bis-pyridinium oximes and the identification of a HI-6 decomposition product.

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Executive Summary

Title: P.A. D'Agostino, L.R. Provost, J.R. Hancock and C.A. Boulet, "Electrospray Mass Spectra of Therapeutic Oximes: HI-6, HS-6, Obidoxime, 2-PAM, TMB-4 and HLö-7", Suffield Memorandum No. 1475, 1995, UNCLASSIFIED.

Introduction: The Canadian Forces (CF) may be called on to perform peacekeeping or peacemaking operations in regions of the world where there is a significant threat of chemical/biological warfare agent use. To treat nerve agent intoxication, the CF carry autoinjectors containing the bis-pyridinium oxime salt, HI-6. H-series oximes, including HI-6, HS-6, and more recently HLö-7, have been the focus of research directed towards the development of a new generation of more effective nerve agent antidotes. As part of DRES's ongoing studies on HI-6, there is a requirement for the development of analytical methods for identification of this drug, its metabolites and decomposition products.

Results: Six therapeutic oximes, the bis-pyridinium oximes, HI-6, HS-6, Obidoxime, TMB-4 and HLö-7, and the pyridinium oxime, 2-PAM, were analysed by electrospray mass spectrometry (ESI-MS), making use of the collisionally activated dissociation (CAD) opportunities afforded by the ESI interface. The CAD/MS (MS^2) spectra of each oxime were acquired under two different sampling cone voltage settings and the quadrupole mass analyser associated with a hybrid tandem mass spectrometer was utilized for MS/MS and CAD/MS/MS (MS^3) applications including the reliable differentiation of similar bis-pyridinium oximes and the identification of an HI-6 decomposition product.

Significance of Results: The CF made use of autoinjectors containing HI-6 during the Gulf War and are currently using this compound as a therapeutic drug for nerve agent intoxication. Prior mass spectrometric methods have aided in the detection and identification of these oximes, their metabolic or decomposition products but none offer the advantages of ESI-MS for the analysis of these thermally and hydrolytically labile drugs.

Future Goals: The ESI-MS data presented would allow the identification of HI-6 and five other therapeutically active oxime salts either in use or under investigation for treatment of nerve agent intoxication. Continued use of these drugs or development of more effective antidotes requires the development of the best possible analytical methods for the detection and identification of these drugs and their metabolic or decomposition products. ESI-MS is the most promising mass spectrometric technique for oxime salt analyses to date. Future analyses will make use of this technique for the analysis of this important class of compounds.

INTRODUCTION

Pyridinium and bis-pyridinium oxime salts have been used extensively for the treatment of nerve agent and organophosphate poisoning, as this class of compounds reactivates acetylcholinesterase, an enzyme critical for nerve impulse regulation. The pyridinium oxime, 2-PAM, and the bis-pyridinium oxime, Obidoxime, are therapeutic agents for the treatment of organophosphate poisoning. Newer oximes, such as those in the H-series, have been studied extensively as potential replacements for 2-PAM and Obidoxime because of their superior efficacy against soman poisoning (1). H-series oximes, HI-6 (2), HS-6 (3), and more recently, HLö-7 (4-6), have been the focus of research directed towards the development of new generation of more effective nerve agent antidotes. As part of DRES's ongoing studies on HI-6, there is a requirement for the development of analytical methods for identification of this drug, its metabolites and decomposition products.

Mass spectrometry plays a key role in oxime analyses as this analytical technique enables characterization and identification of these compounds. The traditional means of MS ionization, electron impact and chemical ionization, decompose bis-pyridinium compounds, leading to mass fragments indicative of each pyridinium ring but not representative of the intact compound (7). Only through the use of secondary ion (SI) (8,9), field desorption (10), fast atom bombardment (FAB) (9,11), laser desorption (12) or thermospray (7) mass spectrometry was more complete information accessed for these compounds. Both secondary ion and fast atom bombardment mass spectrometry, two techniques that are usually used in a static mode, tend to produce mass spectra that contain significant chemical background associated with the liquid matrix (9) which could hinder detection at low levels. Cations for pyridinium oximes (2-PAM and 4-PAM) were reported during SIMS analysis (8). Dications of the bis-pyridinium oximes were not generally detected during SIMS or FAB-MS analyses, but deprotonated dications and anion reduced dications were observed (9,11).

Thermospray-MS, a technique that has been interfaced to liquid chromatography (LC) for on-line component separation, has been investigated by DRES (13) and others (7,14) for characterization these compounds. The intact cation and characteristic ions due to loss of H_2O and HCN were observed for the pyridinium oxime, 2-PAM (13). HI-6 and Obidoxime did not exhibit a dication during thermospray-MS analysis during our study (13), but conditions were later optimized by Wils and Hulst to produce dication base ions (7). Reproducibilty difficulties were encountered during thermospray-MS, particularly at higher mass (13), and thermal decomposition of oximes (e.g., HI-6) in the thermospray interface was suspected (7,13,14).

More recently, atmopheric pressure ionization techniques, including electrospray-MS (ESI-MS) (15,16), have superseded thermospray-MS for direct or on-line LC-MS characterization of polar and often thermally labile compounds. This technique interfaces easily to LC and the gentle nature of ionization process suggested investigation of this technique for the detection and identification of these thermally labile compounds. ESI-MS was explored for several oximes in a preliminary note that compared thermospray-MS results with those obtained by ESI-MS (16), but no comprehensive study has been done on the analysis of these important therapeutic compounds by ESI-MS.

Six therapeutic oximes (Figure 1), the bis-pyridinium oximes, HI-6, HS-6, Obidoxime, TMB-4 and HLö-7, and the pyridinium oxime, 2-PAM, were analysed by ESI-MS, making use of the collisionally activated dissociation (CAD) opportunities afforded by the ESI interface. The CAD/MS, or MS^2, spectra of each oxime were acquired under two different sampling cone voltage settings and the quadrupole mass analyser associated with a hybrid tandem mass spectrometer was utilized for MS/MS and CAD/MS/MS, or MS^3, applications including the reliable differentiation of similar bis-pyridinium oximes and the identification of a HI-6 decomposition product.

EXPERIMENTAL

Samples

HI-6, HS-6, Obidoxime and TMB-4 were synthesized at DRES as the dichloride salts. HLö-7 was synthesized at DRES as the diiodide salt and 2-PAM chloride was purchased from Aldrich (Milwaukee, WI, USA). The six oximes were prepared as individual standards in distilled water at an initial concentration of 1 mg/mL and diluted to 0.1 mg/mL for ESI-MS analysis. All standards were stored at −20°C and thawed just prior to use.

Instrumental

All electrospray mass spectra were acquired using a VG (Fisons) Autospec-Q mass spectrometer (Manchester, UK) equipped with a VG (Fisons) Mark II electrospray interface. The electrospray needle was operated at 7.6 kV and ions were accelerated into the mass spectrometer at 4 kV. Sampling cone voltages up to 150 volts were initially investigated, with all subsequent data being acquired with a sampling cone voltage of 50 volts or 75 volts. Nitrogen (Very Dry, Liquid Carbonic Inc., Scarborough, Ont., Canada) bath gas was introduced into the interface (80°C) at a flow rate of 500 L/hr. Nitrogen nebulizer gas was introduced at a flow rate of 14 L/hr. The electrospray interface was pumped with both a rotary and a turbomolecular pump, which enabled maintenance of a $3x10^{-6}$ and $5x10^{-8}$ Torr within the source and analyser regions of the instrument, respectively.

Electrospray data were acquired in the continuum mode by scanning the magnet from 400 to 95 Da exponentially at a scan rate of 25 sec/decade. Five to ten scans were typically averaged to enhance the signal-to-noise ratio and the data were smoothed using VG (Fisons) OPUS software. A resolution of 2500 to 3000 (10% valley definition) was employed during magnetic sector scanning

to facilitate accurate mass measurement of the ions formed during ESI-MS analyses. The resultant CAD/MS (MS^2) data collected under these conditions for the oximes contained both the dication (or cation in the case of 2-PAM) and ions formed as a result of collisionally activated dissociation in the ESI interface. External calibrations were performed with a solution of polyethylene glycol 200 (Aldrich) in distilled water.

CAD/MS/MS (MS^3) data were acquired with a quadrupole CAD cell energy of 75 volts and a CAD cell argon pressure of approximately 1×10^{-4} Torr. This condition resulted in 60% to 70% attenuation of the precusor ion and represented a good compromise between sensitivity and spectral content for these analyses. CAD/MS/MS spectra were obtained under these quadrupole CAD cell conditions for several key product ions formed in the ESI interface by selecting the precursor with the magnetic sector and scanning the quadrupole for product ions. The quadrupole was operated at unit resolution and scanned from 400 to 100 u at 25 sec/scan and the magnetic sector resolution was set at 1000 (10% valley definition).

Oximes were introduced into the ESI interface with an Applied Biosystems Model 140B dual syringe pump (Foster City, CA). Water was distilled-in-glass and filtered through a Millipore (Bedford, MA, USA) 0.45 μm filter prior to use. Acetonitrile (UV grade) was obtained from Burdick and Jackson (Muskegon, MI, USA) and HPLC grade trifluoroacetic acid (TFA) was purchased from Pierce (Rockford, IL, USA). The following solvent compositions were prepared for ESI-MS sample introduction: Solvent A (0.05% TFA in water) and Solvent B (0.05% TFA in acetonitrile/water (80:20)). Loop injections (20 μL) of the 0.1 mg/mL oxime standards and calibration standard were made under isocratic conditions with 50% B at a flow rate of 10 μL/min.

RESULTS AND DISCUSSION

CAD/MS (MS^2) Analysis of Oximes

The electrospray mass spectra of six oximes were investigated under CAD conditions that favour the formation of product ions in the ESI interface. By increasing the sampling cone voltage in the ESI interface it was possible to produce CAD/MS or MS^2 mass spectrometric data containing both the dication and structurally significant product ions. With a lower sampling cone voltage, 50 volts, the five bis-pyridinium oximes exhibited an intense dication and several less intense singly charged product ions (Table I). Increasing the sampling cone voltage led to a significant increase in the relative intensity of product ions and a marked reduction in the presence of the dication, but at the expense of the total-ion-current. A good compromise between spectral content and sensitivity was achieved with a sampling cone voltage of 75 volts, a setting which produced similar relative intensities for both the dication and product ions. The pyridinium oxime, 2-PAM, produced just the cation with a sampling cone voltage of 50 volts, but on increasing the sampling cone voltage a product ion was observed.

Figures 2 and 3 illustrate typical CAD/MS data acquired with a sampling cone voltage of 75 volts and a magnetic sector resolution of 2500 (10% valley definition) for the five bis-pyridinium oximes, HI-6, HS-6, Obidoxime, TMB-4 and HLö-7, and the pyridinium oxime, 2-PAM. The intact dication (or cation) for each oxime was observed with between 80% and 100% relative intensity, along with a significant $(Dication-H^+)^+$ and other structurally significant product ions. In each case the dication isotopic cluster for the bis-pyridinium oximes were well resolved with mass spacings of 0.5 Da being observed. Table II lists the masses observed at 2500 resolution during CAD/MS analysis of the oximes. Errors between calculated masses and those observed for the dications (or cation) and the bis-pyridinium $(Dication-H^+)^+$ ions were generally within 0.003 Da., providing strong evidence for the assigned structures.

The CAD/MS data for the three bis-pyridinium oximes of identical mass, HI-6, HS-6 and

Obidoxime (Figure 2), exhibited essentially the same ions but with different relative intensities. Cleavage of the oxydimethylene bridge of the dication at m/z 144 with proton transfer could give rise to the cyclic and bicyclic product ions at m/z 123 and m/z 165, respectively (7,13). Alternatively, these could arise from the m/z 287 ion, $(Dication-H^+)^+$, in a similar manner with charge retention on either the pyridinium ring with or without the oxydimethylene bridge. The product ion at m/z 257 results from neutral loss of formaldehyde (CH_2O) from the m/z 287 ion, while the ions at m/z 229 and 214 suggest losses of CO and HNCO, respectively, from the m/z 257 ion. Lower mass ions at m/z 135, 136 and 137 were likely due to $C_7H_7N_2O$, $C_8H_{10}NO$ and $C_7H_9N_2O$, respectively. The use of moderate magnetic sector resolution aided in the assignment of elemental compositions and was particularly useful in assigning the mass losses, such as the loss of 28 Da to CO as opposed to C_2H_4. Product ions at m/z 135 and 137, because of their odd mass, likely contain two nitrogen atoms, one contained in either the oxime or amide substituent and the other within the pyridinium ring. A six member ring with methyl substitution at the pyridinium ring would explain the m/z 137 ion while incorporation of CH into the pyridinium ring, giving rise to a seven member ring, would account for the ion at m/z 135. The ion at m/z 136 likely contains a single nitrogen since it is of even mass, as opposed to a suggested structure containing two nitrogen atoms that would result from β-cleavage of the oxydimethylene bridge (9). A structure containing the oxydimethylene bridge unit with an expanded pyridinium ring (without amide or oxime substitution) seems probable since TMB-4, which contains a trimethylene bridge, formed a similar ion 2 Da lower at m/z 134.

The CAD/MS spectrum of 2-PAM (Figure 3a) contains the intact cation at m/z 137 and a single product ion at m/z 119 due to loss of H_2O. TMB-4 exhibited CAD/MS data similar to Obidoxime, the differences being associated with the type of bridge (Figure 3b). Both compounds contain oximes at the para position on the pyridinium rings, the difference in structures being that Obidoxime contains an oxydimethylene bridge, while TMB-4 contains a trimethylene bridge. This difference is reflected in the CAD/MS spectrum for Obidoxime which contains a significant ion at m/z 257 due to loss of CH_2O from the $(Dication-H^+)^+$ ion, while the TMB-4 data does not contain

an ion due to loss of CH_2O (or C_2H_4) from the $(Dication-H^+)^+$ ion. This evidence would suggest that the loss of CH_2O from the $(Dication-H^+)^+$ ions of HI-6, HS-6, Obidoxime and HLö-7 is associated with the oxydimethylene bridge. In addition to these ions, both TMB-4 and Obidoxime also exhibited weak $(Dication+Cl^-)^+$ isotopic ion clusters at m/z 321 and 323, respectively.

HLö-7 differs from the other bis-pyridinium oximes in that one of the rings contains two oxime substituents. This results in a dication of fractional mass, m/z 165.563. As was the case for the other oximes with an oxydimethylene bridge, product ions at m/z 330, due to the $(Dication-H^+)^+$ ion and at m/z 300, due to loss of CH_2O from the $(Dication-H^+)^+$ ion, were observed. The product ions at m/z 123 and m/z 208 could have resulted from cleavage of the dication with proton transfer, or from fragmentation of the $(Dication-H^+)^+$ ion in a manner similar to already discussed for the other oxydimethylene bridged bis-pyridinium oximes. The ions at m/z 305 and m/z 261 did not appear to be consistent with the structure of HLö-7 and neither ion was observed in the CAD/MS/MS data acquired for the m/z 300 ion. A minor impurity(s) was suspected.

CAD/MS/MS (MS^3) Analysis of Oximes

CAD/MS/MS or MS^3 experiments, were performed by making use of the quadrupole CAD cell and mass analyser associated with the Autospec-Q. The bis-pyridinium oximes of the same mass, HI-6, HS-6 and Obidoxime, exhibited slightly different relative ion intensities during CAD/MS analysis under identical CAD conditions. However, over the longer term, differentiation might be difficult, particularly if different instrumentation were used or if slightly different CAD conditions were employed. CAD/MS/MS analysis of the $(Dication-H^+)^+$ and $(Dication-H^+-CH_2O)^+$ for each of the three compounds was investigated in an effort to develop a reliable means of compound differentiation. Product ions at m/z 257, 165, 136, 135, 123, 118 and 105, with similar relative intensities, were observed for both HI-6 and HS-6 following transmission of m/z 287 ions into the quadrupole CAD cell (Figure 4a). However, Obidoxime exhibited a significantly different

CAD/MS/MS spectrum containing only two major product ions at m/z 135 and 105 (Figure 4b). Under these CAD/MS/MS conditions, reliable differentiation between Obidoxime and HI-6 or HS-6 would be possible. The structure of the observed product ions likely follows those postulated during CAD/MS, with the ions at m/z 118 (for HI-6 and HS-6 only) being due to a loss of H_2O from m/z 136 and the ion at m/z 105 being due to loss of CH_2O from m/z 135. Mass spectrometric differentiation was also possible for m/z 257, $(\text{Dication}-H^+-CH_2O)^+$, but these product ion spectra were not preferred over those of higher mass, since interferences in mass spectrometry decrease with increasing mass.

HI-6 Decomposition

HI-6 decomposes under alkaline conditions (11) and metabolizes (17), with the pyridinium-2-aldoxime being converted to the 2-pyridone moiety (11). During thermospray-MS analysis of HI-6 a significant ion at m/z 260 was observed (13) and it was suggested by Wils and Hulst (7) that HI-6 was decomposing in the heated interface, however loss of HCN from the m/z 287 ion could not be completely discounted.

Several lots of HI-6 at DRES were analysed by ESI-MS and during these analyses the m/z 260 was not significant in purified, fresh HI-6 samples. However, older standards exhibited significant m/z 260 ion content, and a lower mass ion at m/z 138 (Figure 5a). At a magnetic sector resolution of 3000 (10% valley definition) a mass of 260.1010 Da was recorded which compares favourably with the calculated mass of 260.1035 expected for the 2-pyridone. During CAD/MS/MS of the m/z 287 ion in this HI-6 sample, no product ions were observed at either m/z 260 or 138, which strongly suggested the presence of an additional sample component, likely the 2-pyridone. This was confirmed by looking for m/z 260 product ions following transmission of this ion into the quadrupole CAD cell. Two product ions at m/z 138 and 108, neither of which was associated with HI-6, were observed (Figure 5b). The product ion at m/z 138 results from the neutral loss of the

amide substituted pyridine ring (loss of 122 Da). This ion at m/z 138, containing the oxydimethylene bridging unit, may then lose CH_2O to form an ion at m/z 108. The acquisition of this data provides strong evidence for the presence of the 2-pyridone impurity in this HI-6 sample and supports the use of ESI-MS for analysis of this thermally labile compound, which likely decomposes under thermospray-MS conditions.

CONCLUSIONS

Electrospray mass spectrometry, a relatively new ionization approach, was investigated as a possible technique for the detection and identification of oxime salts and their degradation products. Six therapeutic oxime salts, the bis-pyridinium oximes, HI-6, HS-6, Obidoxime, TMB-4 and HLö-7, and the pyridinium oxime, 2-PAM, were characterized by ESI-MS, making use of the collisionally activated dissociation (CAD) opportunities afforded by the ESI interface. The CAD/MS, or MS^2, spectra of each oxime were acquired under two different sampling cone voltage settings and the quadrupole mass analyser associated with a hybrid tandem mass spectrometer was utilized during MS/MS and CAD/MS/MS, or MS^3, analyses. Obidoxime could be reliably differentiated by CID/MS/MS from either HI-6 or HS-6, all three of which have the same elemental formula. In addition a HI-6 decomposition product was identified by MS/MS.

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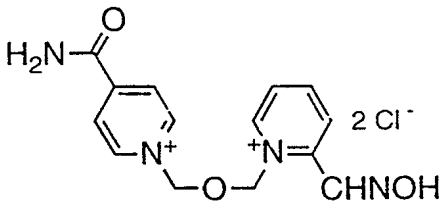
Table I: Collisionally activated dissociation mass spectrometric (CAD/MS) data acquired with an ESI sampling cone voltage of 50 volts.

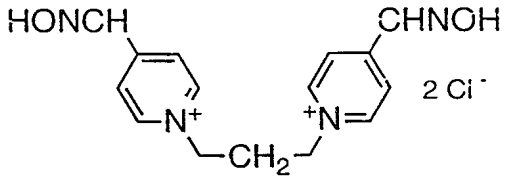
Principal CAD/MS Ions, m/z (% Relative Intensity)						
Ion Identity	HI-6	HS-6	Obidoxime	TMB-4	HLö-7	2-PAM
$(Dicat)^{2+}$	144 (100)	144 (100)	144 (100)	143 (100)	165.5 (100)	
$(Dicat-H^+)^+$	287 (13)	287 (9)	287 (7)	285 (10)	330 (13)	
$(Dicat-H^+-CH_2O)^+$	257 (29)	257 (20)	257 (2)		300 (13)	
$(Cat)^+$						137 (100)

Table II: Mass accuracy during ESI-MS analysis with a magnetic sector resolution of 2500 (10% valley definition).

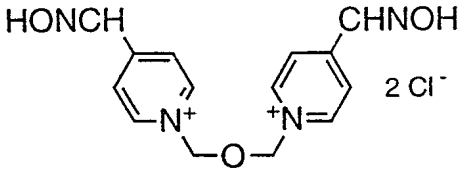
Oxime	Calculated Mass	Observed Mass (ESI Sampling Cone, 75 volts)	Observed Mass (ESI Sampling Cone, 50 volts)	Mass Difference
	$(Dication-H^+)^+$	**$(Dication-H^+)^+$**	**$(Dication-H^+)^+$**	
HI-6	287.1144	287.1178	287.1184	0.0034/0.0040
HS-6	287.1144	287.1121	287.1127	0.0023/0.0017
Obidoxime	287.1144	287.1178	287.1127	0.0034/0.0017
TMB-4	285.1352	285.1384	285.1388	0.0032/0.0036
HLö-7	330.1202	330.1213	330.1170	0.0011/0.0032
				Average ± SD 0.003 ± 0.001 (n=10)
	$(Dication)^{2+}$	**$(Dication)^{2+}$**	**$(Dication)^{2+}$**	
HI-6	144.0611	144.0656	144.0655	0.0045/0.0044
HS-6	144.0611	144.0656	144.0627	0.0045/0.0016
Obidoxime	144.0611	144.0656	144.0627	0.0045/0.0016
TMB-4	143.0715	143.0747	143.0775	0.0032/0.0060
HLö-7	165.5640	165.5635	165.5668	0.0005/0.0028
				Average ± SD 0.003 ± 0.002 (n=10)
	$(Cat)^+$	**$(Cat)^+$**	**$(Cat)^+$**	
2-PAM	137.0715	137.0749	137.0627	0.0034/0.0088



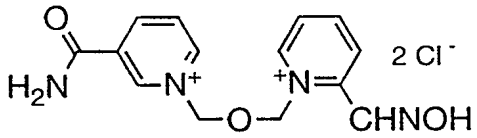
HI-6



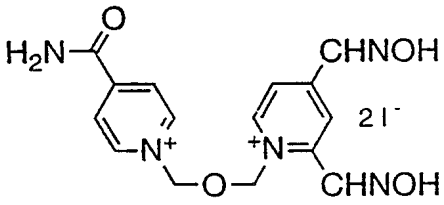
TMB-4



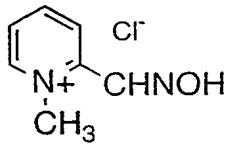
Obidoxime



HS-6



HLö-7



2-PAM

Figure 1: Pyridinium and bis-pyridinium oxime salts.

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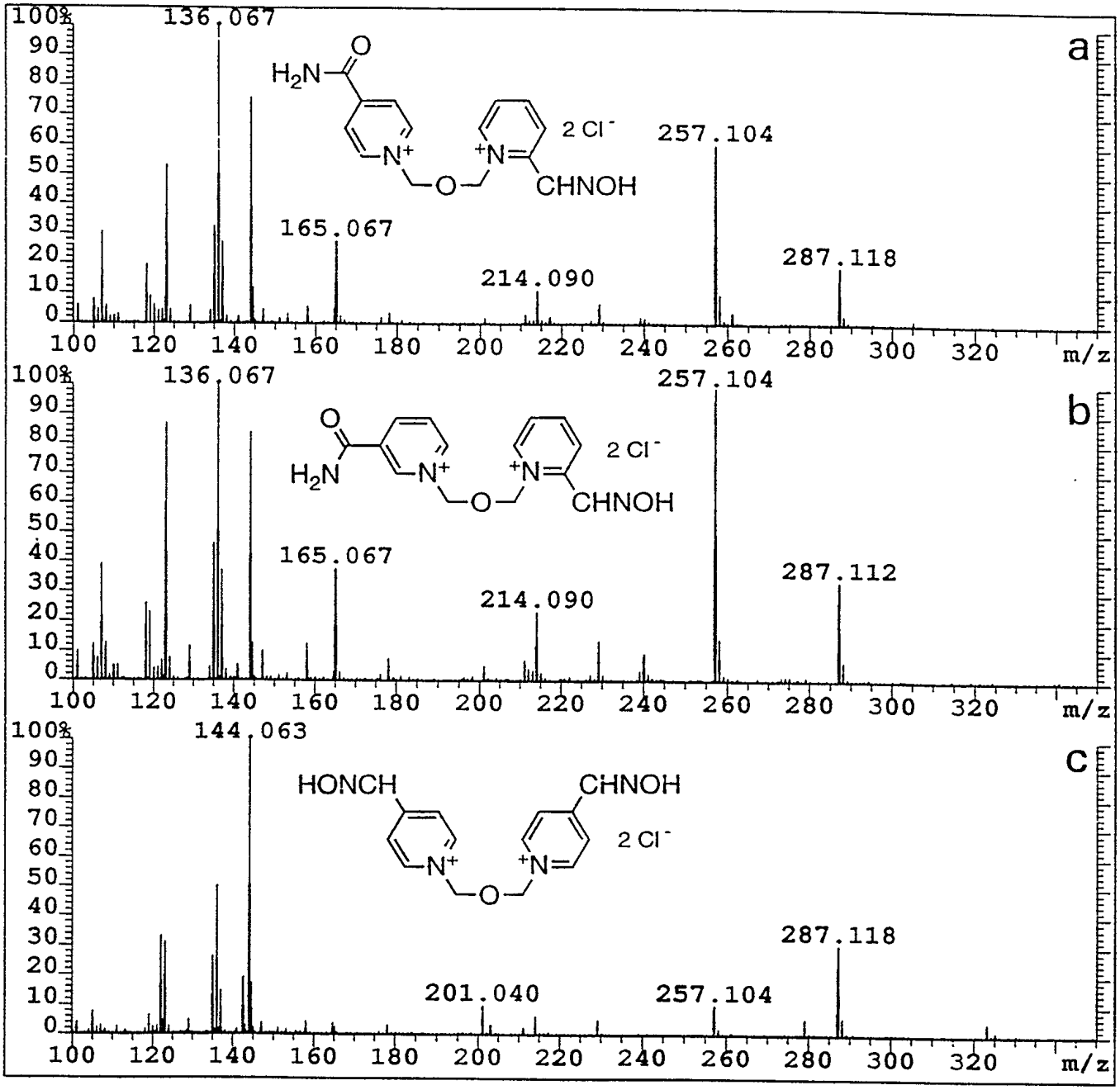

Figure 2: Collisionally activated dissociation mass spectrometric (CAD/MS) data acquired for a) HI-6, b) HS-6 and c) Obidoxime following electrospray introduction (ESI sampling cone voltage, 75 volts; Magnetic sector resolution, 2500).

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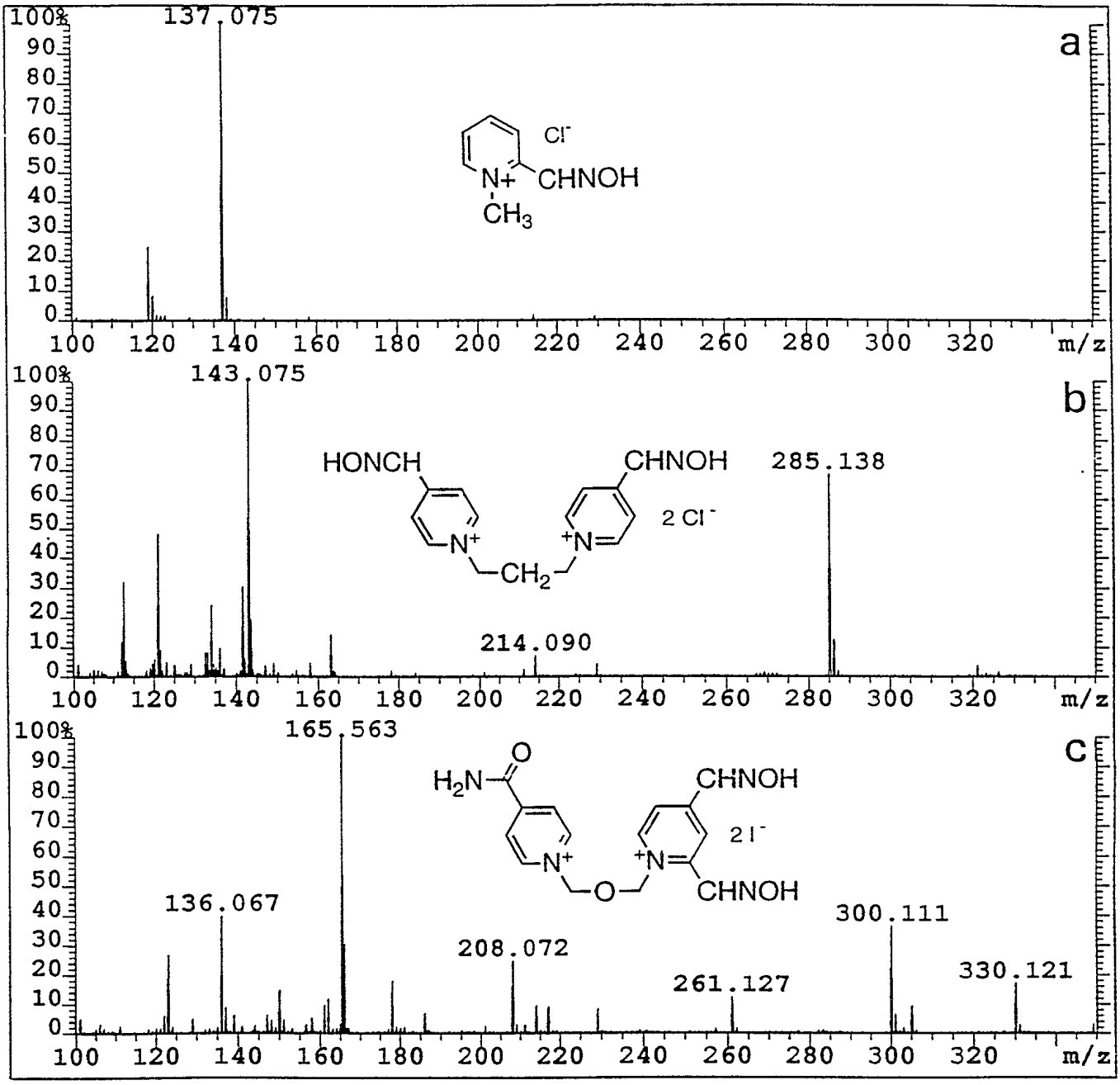

Figure 3: Collisionally activated dissociation mass spectrometric (CAD/MS) data acquired for a) 2-PAM, b) TMB-4 and c) HLö-7 following electrospray introduction (ESI sampling cone voltage, 75 volts; Magnetic sector resolution, 2500).

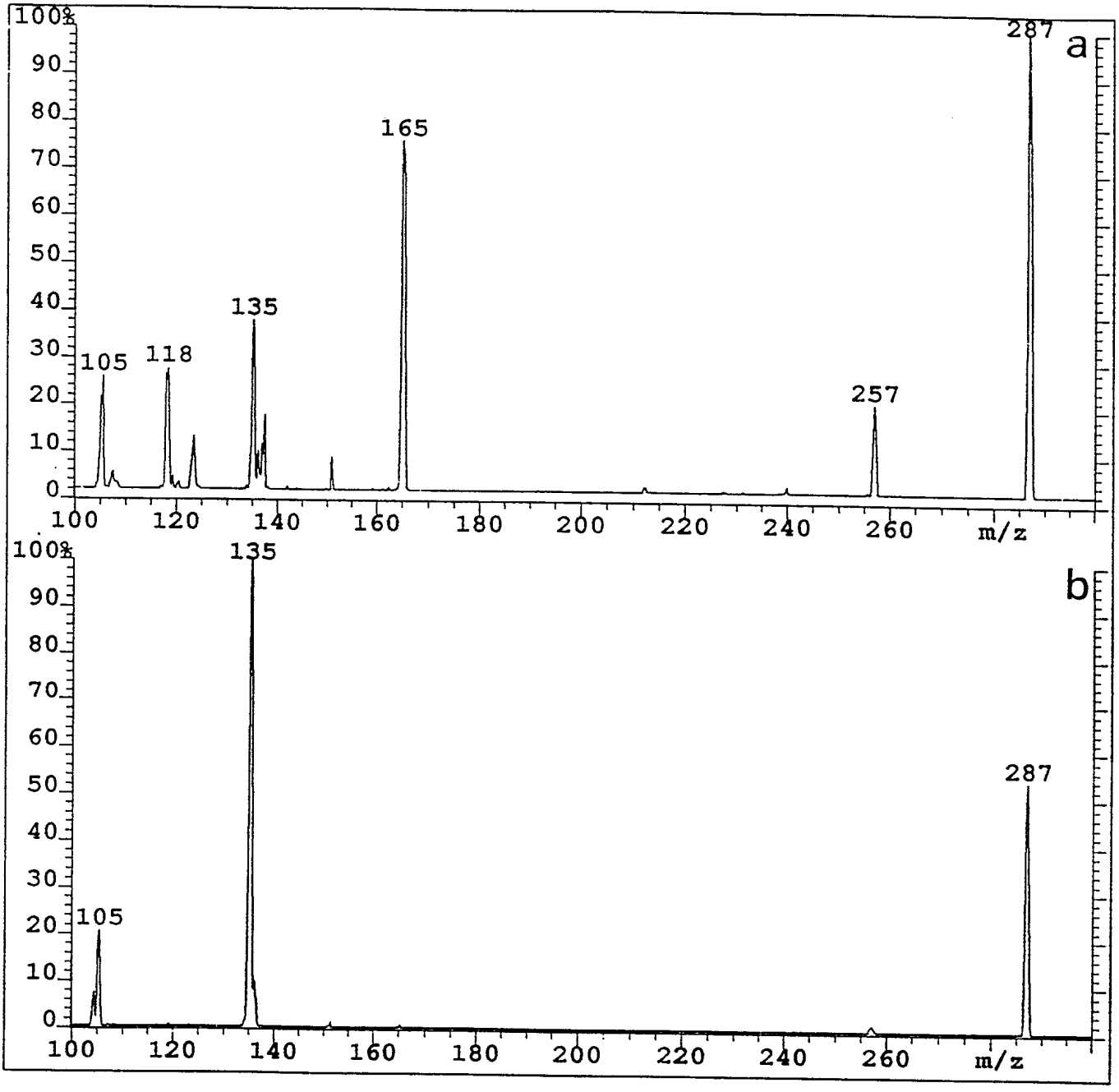

Figure 4: Collisionally activated dissociation tandem mass spectrometric (CAD/MS/MS) data for m/z 287 following electrospray introduction of a) HI-6 and b) Obidoxime (ESI sampling cone voltage: 50 volts; Magnetic sector resolution, 1000; Quadrupole resolution, unit; Quadrupole CAD cell: 1.1×10^{-4} Torr Ar and 75 volts).

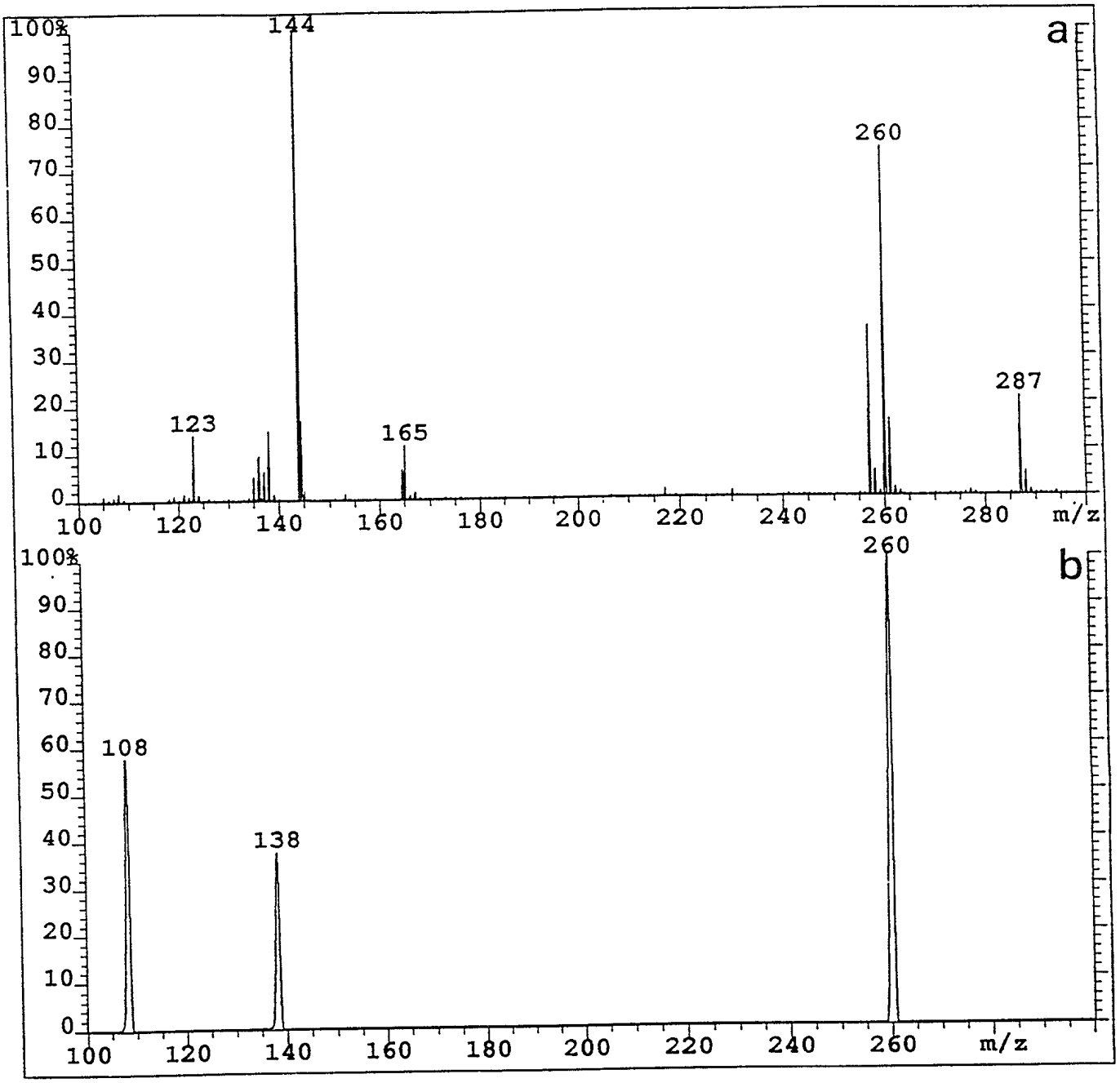

Figure 5: a) Collisionally activated dissociation mass spectrometric (CAD/MS) data acquired for a partially decomposed sample of HI-6 following electrospray introduction (ESI sampling cone voltage: 50 volts; Magnetic sector resolution, 3000). b) Collisionally activated dissociation tandem mass spectrometric (CAD/MS/MS) data for m/z 260, the cation formed following conversion of the pyridinium-2-aldoxime of HI-6 to 2-pyridone (ESI sampling cone voltage: 50 volts; Magnetic sector resolution, 1000; Quadrupole resolution, unit; Quadrupole CAD cell: 1.1×10^{-4} Torr Ar and 75 volts).

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Pyridinium and bis-pyridinium oxime salts are currently in use or under development for treatment of patients exposed to organophosphorus nerve agents. The limited volatility and thermal lability of these compounds limits the number of mass spectrometric approaches that may be used to characterize these important compounds. Electrospray mass spectrometry, a relatively new ionization approach, was investigated as a possible technique for the identification of these oximes and their degradation products. Six therapeutic oxime salts, the bis-pyridinium oximes, HI-6, HS-6, Obidoxime, TMB-4 and HLö-7, and the pyridinium oxime, 2-PAM, were analyzed by ESI-MS, making use of the collisionally activated dissociation (CAD) opportunities afforded by the ESI interface. The CAD/MS (MS^2) spectra of each oxime were acquired under two different sampling cone voltage settings and the usefulness of the second (quadrupole) mass analyzer associated with a hybrid tandem mass spectrometer was demonstrated for MS/MS and CAD/MS/MS (MS^3) applications including the reliable differentiation of similar bis-pyridinium oximes and the identification of a suspected HI-6 decomposition product.

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