On the Origin of Some Controversial Ions (m/z 59, 60, 77, and 119) in the Thermospray Reagent Plasma from Ammonium Acetate

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The origin of ions at m/z 60, 77, and 119 in the thermospray (TSP) reagent plasma is reconsidered. It is demonstrated that these major ions in the TSP spectrum of ammonium acetate are not due to dehydration processes in the gas or liquid phase, as is generally accepted, but to the preexistence of acetamide as an impurity in the commercial salts. Acetamide, characterized by TSP/tandem mass spectrometry, gas chromatography-electron impact ionization mass spectrometry, ^{1}H-NMR, and ^{13}C-NMR, is responsible for the $[M + 60]^+$ and $[M + 77]^+$ adducts observed in some spectra. The buffer ion at m/z 59 is also due to impurities in the ammonium acetate salts. Washing the solid salt with chloroform eliminates most of these impurities. Examples using the pesticides linuron, monuron, and carbaryl show that the ions observed at m/z $M_r + 60$ and $M_r + 59$ disappear when a buffer obtained from acetic acid and ammonia is used instead of the commercial salts. *(J Am Soc Mass Spectrom 1994, 5, 186–193)*

The thermospray (TSP) technique is one of the more widely used methods for on-line coupling of liquid chromatography with mass spectrometry. Ionization in TSP is controlled by the gas-phase proton affinities of the analyte molecules and the reagent plasma ions. Several buffers for TSP have been tested and the corresponding reagent ions generated in the TSP source have been described. Ammonium acetate (AMAC) buffers are by far the most accepted for TSP work. The plasma ions generated by this buffer alone or in mixtures with methanol or acetonitrile have been described extensively by different authors [1–4]. Major signals in the spectra from aqueous AMAC solutions have been reported at m/z 18, 35, 36, 59, 60, 77, 78, 95, 96, and 119. Sometimes, structural assignment of these ions or the corresponding adduct ions generated with the analyte has created some controversy. For example, the ion at m/z 77 has been assigned in the literature as a $[CH_3COONH_4]^{+\cdot}$ ion [5], and the same assignment has been suggested for the adducts $[M + 77]^{+\cdot}$ [4]. However, the formation of odd electron structures is not expected when working in the so-called pure TSP ionization mode where radical processes seem to be unimportant. A more likely composition for the signal at m/z 77 could be that of the $[CH_3COOH + NH_4 + NH_3 - H_2O]^+$ ion, as suggested by the work of Maeder [1] where this equation is used to explain the $[M + 77]^+$ ions observed in some spectra. This structure agrees with the data presented previously by Vestal [3] in the study of some metastable transitions in the TSP mass spectrometry of AMAC.

During our TSP work we observed important differences in the pattern of the TSP buffer spectra depending on the experimental procedure used to obtain the buffer solutions. In this work we show that these differences are due to the widespread presence of acetamide impurities in commercial AMAC salts. Acetamide, with a higher gas-phase proton affinity than ammonia (863 KJ/mol for acetamide versus 854 KJ/mol for ammonia) [6], generates the more abundant signals in the spectra. These ions, and not only the NH_3 derived adducts, act as reagent ions in the TSP source and intervene in the chemical ionization processes responsible for the ultimate analyte ionization.

Experimental

Materials

AMAC salts were obtained from Fluka (Madrid, Spain) (p.a. grade, > 99%, H_2O < 1%; and BioChemika MicroSelect grade, > 99%, H_2O < 1%); Merck (Darmstadt, Germany) (p.a. grade, > 98%, H_2O < 2%); and Panreac (Barcelona, Spain) (p.a. grade, no specified purity). Ammonium formate (p.a. grade, > 98%) was from Fluka. The salts H_3AsO_4, $CrCl_3$, $Mn(NO_3)_2$, $Co(NO_3)_2$, $SnCl_4$, $CuCl_2$, $Al(NO_3)_3$, $Hg(NO_3)_2$, and

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1044-0305/94/$7.00

Received July 19, 1993
Revised November 8, 1993
Accepted November 8, 1993

$ZnSO_4$ were obtained from J.T. Baker (Phillipsburg, NJ). $FeCl_3 \cdot 6H_2O$, $AgNO_3$, and $CdCl_2 \cdot 2\ 1/2\ H_2O$ were obtained from Carlo Erba (Milano, Italy).

Acetamide (p.a. grade, > 99.5%) was obtained from Panreac. Acetic acid (p.a. grade, solution 96%) and ammonium hydroxide solutions (p.a. grade, solution 25%) were obtained from Merck. Deuterated acetic acid (d_4, 99.5%) was obtained from Cambridge Isotope Laboratories (Woburn, MA). Water was obtained by purifying deionized water through a Milli Q (Waters-Millipore, Milford, MA) system provided with a 0.45 μm pore filter.

1-Naphthyl N-methylcarbamate (carbaryl, 99.9%) and 1,1-dimethyl-3-(p-chlorophenyl)urea (monuron, 99.9%) were from Dr. Ehrenstorfer (Augsburg, Germany). N′-(3,4-dichlorophenyl)-N-methoxy-N-methylurea (linuron, 99%) was from Riedel-De Haen (Seelze-Hannover, Germany).

Preparation of Buffers

Buffers obtained from commercial salts were prepared by dissolving the salt in water to give a 0.1 M buffer and filtering the solution through a 0.5 μm Millipore filter.

Buffers obtained from acetic acid and ammonia solutions were prepared by addition of 0.1 mol of ammonium hydroxide to 0.5 L water and then adding 0.1 mol of acetic acid and adjusting to 1 L final volume.

In both cases the pH was adjusted, if needed, to around 5.5–5.8 with acetic acid. The 0.05 M solutions of ammonium acetate in water or in water/methanol 1/1 were obtained from these 0.1 M buffers by diluting with the same volume of H_2O or methanol, respectively.

In some cases, 0.05 or 0.1 M AMAC solutions in different mixtures of water/methanol were also prepared as described above starting from more concentrated (0.2 M) initial aqueous solutions.

TSP Mass Spectrometry

Three instruments have been used in this work: the Finnigan (San Jose, CA) TSQ-70 and TSQ-700 triple quads and the Hewlett-Packard (Palo Alto, CA) 5970 quadrupole instrument; each with the originally provided TSP interface and source. The particular analytical conditions are indicated in captions in tables and figures.

Mass spectra of the pesticides were obtained after liquid chromatography using a Hypersil ODS column (3 μm, 6 × 0.46 cm) (Tracer Analitica, Barcelona, Spain) and an eluent consisting of 0.05 M AMAC buffer in water/methanol (1/3) at a flow rate of 1 mL/min. Mass spectra were obtained from 2 μg of each pesticide injected on column.

Tandem Mass Spectrometry

Collision-activated (CAD) tandem mass spectrometry was performed by using the TSQ-70 or the TSQ-700 instrument. In both cases argon at 0.5 mtorr was used as the collision gas. Collision energy was set at 30 or 50 eV.

Nuclear Magnetic Resonance

^{13}C- and ^1H-NMR were recorded on a Varian (Varian Associates, Walnut Creek, CA) Unity 300 apparatus. The sample was dissolved in CD_3OD and the spectra were recorded at 37 °C.

Gas Chromatography / Mass Spectrometry

Gas chromatography/mass spectrometry analysis was carried out with a Finnigan INCOS system coupled to a Varian 3400 gas chromatograph. Ion source, injector, and transfer line temperatures were set at 180, 150, and 250 °C, respectively. A temperature gradient from 40 to 200 °C in 16 minutes with 2 minutes initial delay time was used.

Results and Discussion

In Figure 1 the TSP spectra of the reagent gas obtained with two different AMAC buffers are presented. When AMAC salt (AMAC-S) was used, the generally reported spectrum for AMAC [3, 4] was obtained (Figure 1a), but when AMAC was prepared from the ammonia and acetic acid reagents (AMAC-R), some signals such as those at m/z 77 and 119 practically disappeared from the spectrum (Figure 1b). This effect can also be observed in Table 1 where tabulated spectra of these buffers in water or in water/methanol mixtures are presented. TSP spectra can be very dependent on instrumental and analytical conditions, as illustrated in Table 1 where very different relative patterns can be observed depending only on which one of the scanning quadrupoles (Q1 or Q3) in the TSQ-700 is used for analysis. The spectra obtained with the third quadrupole Q3 [Q3 scanning and Q1 and Q2 in the radiofrequency(rf)-only mode] clearly show more abundant low mass adduct ions than the spectra obtained with the first quadrupole (Q1 scanning, Q2 and Q3 in the rf-only mode). This effect is probably due to different transmission efficiencies or to the effect of collisions during ion transmission and can be observed for both AMAC-R and AMAC-S buffers. Instrumental differences are thus a determinant factor in some of the observed changes in the reagent plasma patterns. However, preliminary experiments carried out with three different quadrupole mass spectrometers and in two independent laboratories indicated that the relative abundances of the ions at m/z 77 and 78 could not be explained as the result of different analytical conditions or slight variations in the buffer preparation. This was supported when trying to justify the assignments of some of these plasma ions by comparing the spectrum of AMAC-S in water/methanol eluents with the one obtained with deuterated acetic acid and ammonia, also in water/methanol, using the

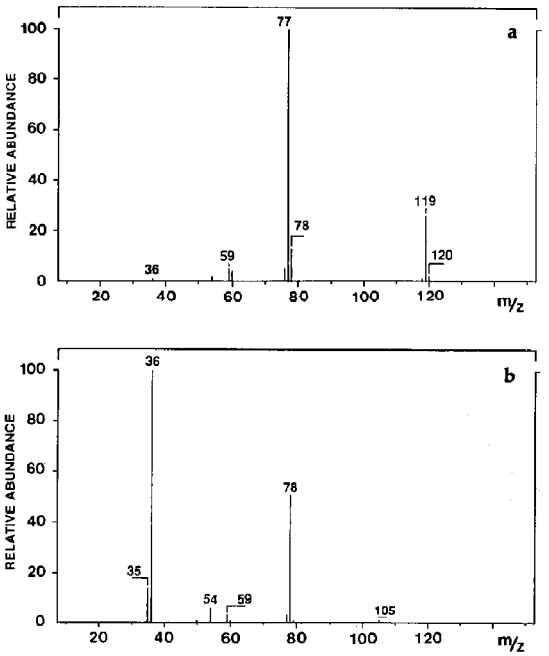

Figure 1. TSP spectra of different 0.1 M aqueous AMAC buffers. (a) Merck salt; (b) buffer generated with ammonia and acetic acid. Finnigan TSQ-700 instrument scanning with the third quadrupole. Spectra are the average of about 20–30 scans. Scan range, 10–200 u; scan rate, 0.5 scan/s. Source 200 °C, interface 100 °C. Flow rate 1 mL/min. Buffer pH adjusted to 5.5 with acetic acid.

Hewlett-Packard instrument (Figure 2). In the first case major ions were observed at m/z 77 and 119. In the second case major ions were at m/z 50 ($[CH_3OH + NH_4]^+$) and 82 ($[2CH_3OH + NH_4]^+$) with no signals at m/z 77 or 119 or at m/z 80 (the expected $[CD_3COOH + NH_4 + NH_3\text{-}H_2O]^+$).

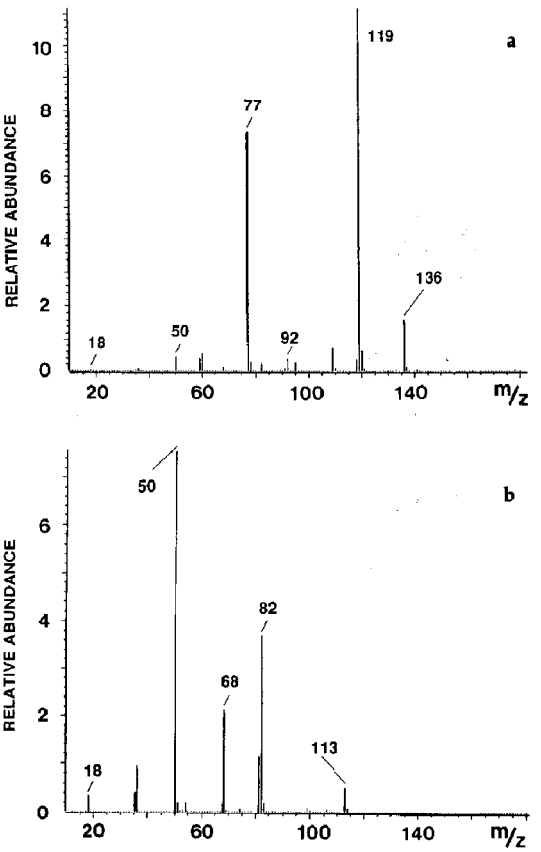

Figure 2. TSP spectra of 0.05 M AMAC solutions in methanol/water 1/1. (a) Salt from Fluka; (b) buffer generated with deuterated acetic acid and ammonia. Hewlett-Packard instrument. Conditions as in Figure 1.

Table 1. Reagent ion spectra obtained from two different 0.05 M AMAC buffers in water or in 1/1 water/methanol mixtures in a TSQ-700 mass spectrometer

	H_2O				H_2O/MeOH		Tentative
m/z	SQ1[a]	SQ3	RQ1	RQ3	SQ1	RQ1	Assignment[b]
35	—[c]	3	—	14	—	20	$[NH_4 + NH_3]^+$
36	—	15	5	100	11	100	$[NH_4 + H_2O]^+$
50	—	—	—	—	56	3	$[NH_4 + CH_3OH]^+$
54	1	1	15	6	—	26	$[NH_4 + 2H_2O]^+$
59	5	7	2	6	8	—	?
60	4	40	—	—	5	—	?
77	100	100	6	3	100	—	?
78	12	10	100	51	6	35	$[NH_4 + AcOH]^+$
82					3	—	$[NH_4 + 2CH_3OH]^+$
92	—	—	—	—	9	—	?
119	25	33	—	—	32	—	?

[a] S and R indicate the AMAC-S (Merck) or AMAC-R buffers, respectively. Q1 and Q3 indicate which of the quadrupoles is scanned for ion analysis.
[b] Based on previously published data [1–3]. Ions marked with ? are controversial and discussed in this work.
[c] No detected ions or signals with relative intensities below the selected cutoff (1%).

Table 2. Daughter ion spectra of the major reagent ions formed from aqueous 0.05 M AMAC-S (Merck salt) and AMAC-R buffers on a TSQ 70 instrument[a]

AMAC	Parent ion[b]	Daughter spectrum[c]
S	77(100)	77(100), 76(-), 60(32), 59(-), 43(2), 18(61)
R	77(< 1)	77(100), 76(2), 60(24), 59(1), 43(1), 18(62)
S	78(30)	78(100), 61(2), 60(2), 18(97)
R	78(100)	78(100), 61(-), 60(-), 18(88)
S	95(2)	95(100), 94(3), 78(4), 77(69), 60(21), 36(51), 35(26), 18(40)
R	95(1)	95(72), 94(3), 78(13), 77(-), 60(-), 36(1), 35(100), 18(33)
S	119(22)	119(19), 96(1), 60(100), 43(3)
R	119(-)	No signal at 119

Relative abundances (cutoff 1%) are indicated in parentheses.
[a] Source 200 °C, vaporizer 90 °C, collision energy 50 eV, collision pressure 0.5 mtorr.
[b] Relative abundance of the selected parent ion in the TSP reagent plasma.
[c] Relative abundances indicated as (-) correspond to no detected or low abundance ions that are included for better comparison.

The same results were obtained with different brands of salts and liquid reagents. The source of the salt (commercial brand), the mass spectrometer, and the analytical conditions used to obtain the spectra affected the relative abundance of the buffer ions. However, the presence of the 77 and 119 ions was always characteristic of a buffer obtained from the commercial solid salt.

These observations drove us to check different possible explanations. More plausible justifications should lie in the presence of impurities in the reagents used for the TSP buffers.

The accepted structure of the ion at m/z 77 corresponds to that of an ammonium adduct with the product of dehydration of AMAC [1–3]. From the data available from the manufacturers, it was calculated that the concentration of several ionic impurities in the buffers could be one to three orders of magnitude higher when the solutions were made up with commercial salts than when made up with acetic acid and ammonia of similar quality grade. The presence of metallic impurities in these salts could be responsible for a catalytic effect that could explain, at least in part, the formation of the dehydration-derived ions. However, when AMAC-R solutions were supplemented with 50 ppm of 11 different metals (see Experimental) to mimic the AMAC-S solution (as far as it was ascertained from the manufacturers' quality reports) and analyzed by TSP, no variation in the spectral pattern was observed.

The two different buffer spectra also could be explained by the presence in the AMAC salts used for TSP of some impurities directly generating the ions at m/z 77 and 119. CAD spectra of these and other ions in the reagent plasma are presented in Table 2. The tentative structure of the ion at m/z 77 that could be derived from these data agrees with the results reported by Vestal [3]. Thus, the ion at m/z 77 could be assigned to the $[NH_4 + NH_3(CH_2CO)]^+$ adduct [3]. It is generally accepted that this adduct results from the loss of water from the adduct $[NH_4 + NH_3(CH_3COOH)]^+$ at m/z 95 [3]. The latter has a low relative abundance (< 2%) in the spectra of both AMAC-S and AMAC-R buffers on the TSQ instruments. For AMAC-R buffers, the CAD of the ion at m/z 95 generated signals at m/z 18 ($[NH_4]^+$), 35 ($[NH_4 + NH_3]^+$), and 78 ($[NH_4 + CH_3COOH]^+$) which allow the tentative characterization of the ion as the adduct $[NH_4 + NH_3 + CH_3COOH]^+$. CAD of this ion did not produce any daughter signal at m/z 77, suggesting that loss of water from this adduct is not an important process. On the other hand, CAD of the ion at m/z 95 in the spectrum of the AMAC-S buffer showed a loss of water to give the ion at m/z 77, loss of NH_3 and water to give the ion at m/z 60, and other signals due to $[NH_4]^+$ adducts with water and ammonia. The easy loss of water in this case suggested a different structure for this adduct that could be tentatively explained as $[H_2O + NH_4 + NH_3(CH_2CO)]^+$.

The tentative structures of the ions at m/z 77 and 95 suggested the possible presence of acetamide (CH_3CONH_2) in the salt. In fact, the spectrum of the AMAC salt could be mimicked by adding as little as 0.1% acetamide to an AMAC-R buffer (not shown). The spectrum of acetamide was dominated by the ions at m/z 77 ($[M + NH_4]^+$) and 119 ($[2M + H]^+$) and the CAD spectra of these ions were identical with those discussed before (not shown).

Standard procedures for the preparation of acetamide involve strong heating of AMAC at high pressure or passing ammonia gas through aliphatic acids at a suitable temperature [7]. Interestingly, the methods to obtain AMAC-S are based on bubbling ammonia gas over glacial acetic acid [8]. Although there is no indication of the presence of impurities of this kind in any of the quality control reports from the suppliers, it seemed probable that, in the exothermic reaction indicated above, the generation of some acetamide could take place.

To test for the presence of acetamide, AMAC-S (Fluka) was mixed with chloroform (1/3 w/w) and, after 5 minutes of sonication or 30 minutes of shaking,

the chloroform was removed and evaporated under He. When the residue of the evaporation was dissolved in water and injected in the TSP using an AMAC-R buffer as carrier, intense signals at m/z 77 and 119 were observed (not shown). When 5 g of AMAC-S were washed with chloroform, 10 mg of a solid residue were obtained after chloroform evaporation. This material was submitted to ^{13}C- and ^{1}H-NMR. Data in Figure 3 indicated that the residue consisted of acetamide and free acetic acid. The identification was confirmed by comparison with authentic standards (not shown). Acetic acid was always present in the residue in very variable relative amounts (about 40% in Figure 3a) depending on the extent of the chloroform evaporation process. The electron impact spectrum obtained by injection of a chloroform solution of the extract into a gas chromatograph-mass spectrometer also confirmed the identity of the compound by comparison with library data (not shown). After extraction, solutions of the washed AMAC salt show a slightly more basic pH (pH 6.9, 0.05 M) than the unextracted salt (pH 6). The TSP spectrum of the washed salt still showed signals at m/z 77 and 119 (not shown). After a second washing, the ion at m/z 119 was eliminated from the spectrum and the ion at m/z 78 was higher than the ion at m/z 77 (Figure 4).

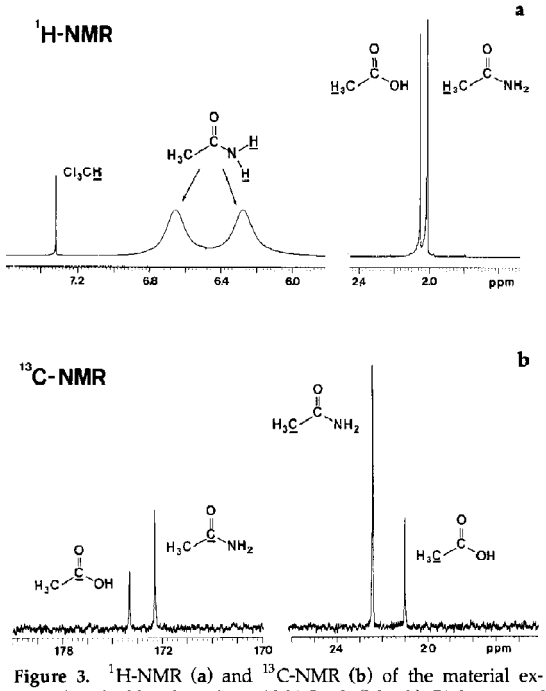

Figure 3. ^{1}H-NMR (a) and ^{13}C-NMR (b) of the material extracted with chloroform from AMAC salt (Merck). Right trace of the ^{1}H-NMR has been scaled by a factor of 15 for graphic purposes. Integration of the signals assigned to acetamide give the expected 1:1:3 ratio. The ratio of acetamide to acetic acid was calculated to be 6:4 from the signals at 2 ppm. The acidic proton of acetic acid was observed as a very wide signal around 11 ppm (not shown).

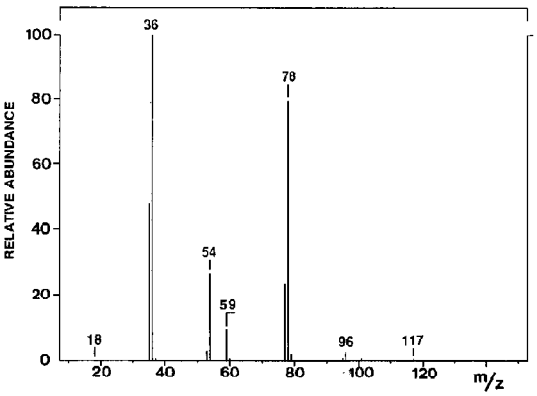

Figure 4. TSP of 0.05 M aqueous AMAC-S (Merck) after two extractions of the solid salt with chloroform. The pH was not adjusted in this case. TSQ-700 instrument.

A rough estimation of the amount of acetamide in the salts was carried out by extracting 30 g of AMAC with chloroform (50 mL × 2) and weighting the dry residue obtained after filtering (0.5 μm pore) and evaporation of the solvent. Parallel experiments were performed by adding known amounts of acetamide to AMAC-R solutions and comparing the resulting spectra with those obtained with the salts. Values obtained by the two methods were comparable and indicated amounts ranging from about 0.1 to 0.3% (Fluka BioChemika salt) to 1 to 3% (Merck salt).

The presence of acetamide as a synthetic impurity compelled us to check the appearance of the reagent plasma from ammonium formate, another commonly used buffer for TSP. Major ions in the spectra of this buffer alone or in mixtures with methanol (Figure 5) could be explained as adducts of protonated ammonia with ammonia (m/z 35), water (m/z 36, 54), and, when present, methanol (m/z 50). In addition, a signal at m/z 63 observed in both spectra could arise from the corresponding adduct of protonated ammonia with formamide, the homologue of acetamide. The daughter ion CAD spectrum of the ion at m/z 63 showed only daughter signals at m/z 18 ($[NH_4]^+$, RA 55%) and 46 (the expected $[HCONH_2 + H]^+$, RA 5%), and thus agree with the above tentative assignment. No further efforts were taken to isolate this compound from the ammonium formate salt.

It could be expected that for high proton affinity compounds the presence of acetamide, with a proton affinity slightly higher than ammonia, would not affect their TSP response relative to the pure buffer. A different situation could be encountered with compounds with a proton affinity near or below that of acetamide. In these cases, acetamide could compete with the analyte for ionization and also contribute to the spectrum of the analyte by adduct formation. In this respect, previous work with the Hewlett-Packard TSP instrument has shown that pesticides such as monuron (M_r 198), linuron (M_r 248), and carbaryl (M_r 201) show spectra with abundant adduct signals [4, 9]. Some of

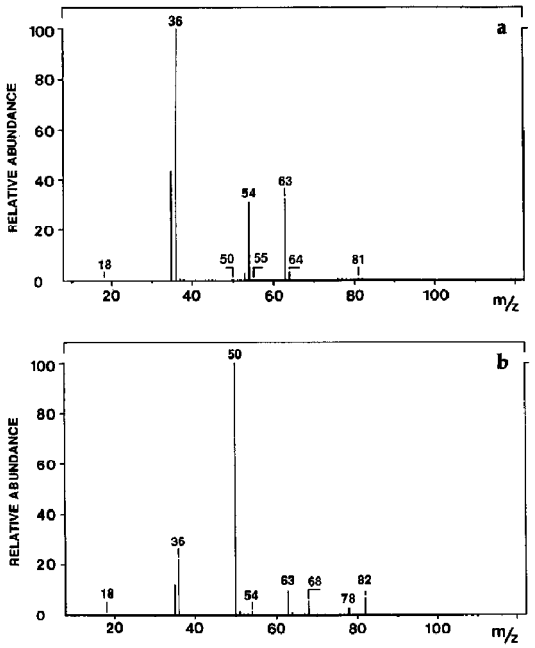

Figure 5. TSP spectra of (a) a 0.1 M solution of ammonium formate in water; (b) a 0.06 M solution of ammonium formate in water/methanol 6/4. TSQ-700 instrument. Conditions as in Figure 1.

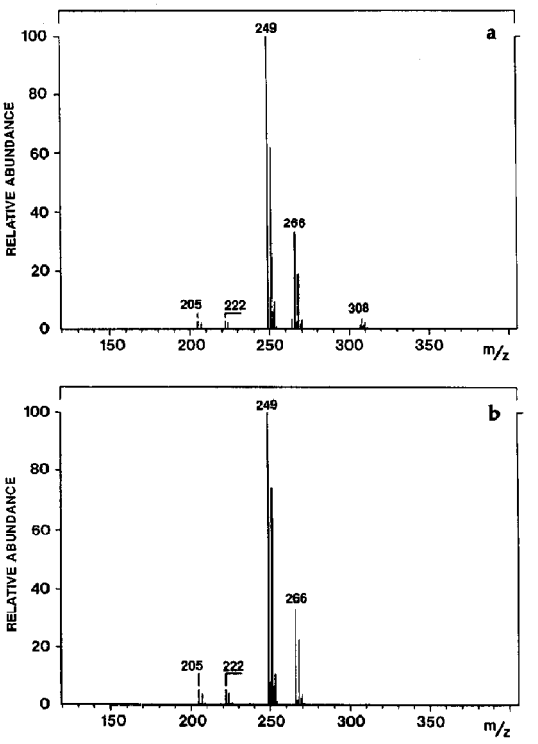

Figure 6. TSP high-performance liquid chromatography mass spectra of linuron using two different AMAC buffers in 1/3 water/methanol mixtures. (a) 0.05 M AMAC-S from Fluka; (b) 0.05 M AMAC-R buffer. TSQ-700 instrument. Conditions as in Figure 1. Chromatographic conditions as indicated in Experimental.

these signals (such as the ones at m/z $M_r + 60$ and $M_r + 77$) could be clearly related to adduct formation with acetamide. When linuron was assayed by TSP high-performance liquid chromatography/mass spectrometry using a TSQ-700 instrument and conditions similar to those in refs 4 and 9, a signal at m/z $M_r + 60$ (m/z 308, Figure 6a) could be clearly observed although formation of adducts was not as dominant as in the Hewlett-Packard instrument. The formation of these intense adduct signals by dehydration of the acetic acid while no dehydration signals from the analyte itself can be observed is hard to explain. When the same sample was analyzed by using AMAC-R, the same spectrum was obtained except for the absence of the adduct signals around m/z 308 (Figure 6b). These results indicated that the signal at m/z $M_r + 60$ could be better explained as the protonated adduct with acetamide, $[M + CH_3CONH_2 + H]^+$.

Although the $[M + 60]^+$ ions were not observed in the spectra of the pesticides monuron and carbaryl (Figure 7), acetamide adducts could be responsible for the ion at m/z 230 in Figure 7a. Monuron showed losses of 28 mass units (probably through loss of CO or due to demethylation processes) from the $[M + H]^+$ and $[M + NH_4]^+$ ions (ions at m/z 171 and 188, respectively). In this way the signal at m/z 230, that disappeared when AMAC-R was used, could be explained by the ion $[M\text{-}28 + 60]^+$.

During these assays it was observed that monuron and carbaryl showed a signal at $M_r + 59$ (m/z 257 and 260, respectively, in Figure 7a, c). An $[M + 59]^+$ signal (m/z 307) was also present in the spectrum of linuron (Figure 6a). The $[M + 59]^+$ adduct has been reported as a characteristic ion of several carbamate pesticides including carbaryl [10]. In this work it was suggested that this ion originates from an adduct of the carbamate with the ion radical $[CH_3NH_2CO]^{+\cdot}$. This odd electron ion structure itself would originate from the fragmentation of another carbamate molecule. The $[M + 59]^+$ signals clearly disappeared from the spectra when an AMAC-R buffer was used, suggesting that these ions could also be artifacts from AMAC impurities. The ion at m/z 59 was observed in the AMAC-S as well as in the AMAC-R buffers (Table 1 and Figures 1 and 2). In the spectra of the latter, however, this ion is not always detected. Further, ions at m/z 57, 59, 75, and 77 were also observed with straight water (Millipore quality water with no added salts or buffers) (not shown). These ions probably correspond to potassium (57 and 75) and sodium (59 and 77) adducts with water. The presence of these cations in the spectrum of water could result from various sources of contamination such as the glassware or from contaminated ion source or probe surfaces. The possibility of formation of adducts of carbamates with

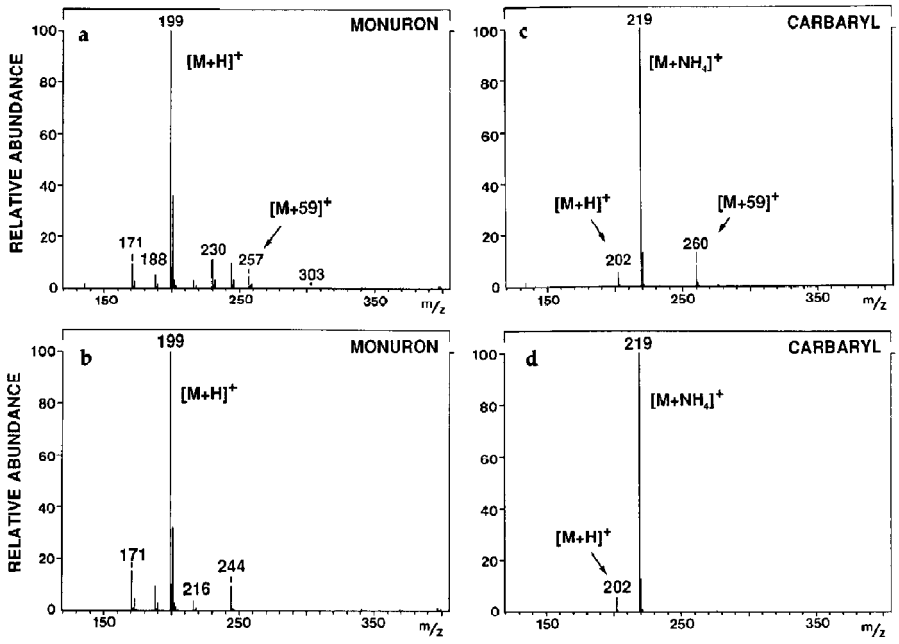

Figure 7. TSP high-performance liquid chromatography mass spectra of monuron and carbaryl using two different 0.05 M AMAC buffers in 1/3 water/methanol mixtures. (a) monuron with the AMAC-S buffer (Fluka); (b) monuron with the AMAC-R buffer; (c) carbaryl with the AMAC-S; and (d) monuron with the AMAC-R buffer. TSQ-700 instrument. Conditions as in Figure 1. Chromatographic conditions as indicated in Experimental.

these ions was not taken into account in the work of Saar and Salomon [10], although the ions at $[M + Na]^+$ and $[M + K]^+$ were reported as present in the spectrum of the carbamate anginin. The CAD of the ion at m/z 59 in the spectrum of allegedly pure water (Figure 8a) showed ions at m/z 41 ($[Na + H_2O]^+$) and at m/z 23 ($[Na]^+$) that justify its assignment as the $[Na + 2H_2O]^+$ adduct. CAD of the $[M + 59]^+$ ion of monuron and carbaryl (m/z 257 and 260) showed an easy fragmentation to give exclusively a signal at m/z 59 without signals at m/z 23 or m/z 41, suggesting that Na^+ adducts are not contributing to this signal. CAD of the ion at m/z 59 from the AMAC-R buffer suggests that it has the same structure as that in the case of water (Figure 8b). In addition, a small signal at m/z 18, which was not present in the spectrum of the $[Na + 2H_2O]^+$ adduct observed with straight water, indicated that this signal could probably have a small contribution from another unidentified ionic species. The parent ion spectrum of the signal at m/z 18 confirmed this assumption and precluded the possibility of contribution of other signals around the ion at m/z 59 to its daughter ion spectrum (not shown). The ion at m/z 59 observed in the spectra of the AMAC-S buffers showed a different CAD spectrum (Figure 8c). Besides signals at m/z 23, 41, and 18, intense signals were observed at m/z 42 (59-17), 44 (59-15), and 15. Composition of this signal corresponds thus to a mixture of sodium adducts, as in the water and AMAC-R buffer spectra, and an unknown compound that generates at least the 15, 42, and 44 fragments by CAD. These results indicate that the ions at m/z $M_r + 59$ in the spectra of these pesticides are artifactual signals due to an unknown impurity in the AMAC salts. Formation of this signal by loss of water from the ion at m/z 77 was considered unimportant because the ion intensity at m/z 59 actually diminished when acetamide was added to the buffer and because the ion is not observed (< 1%) in the CAD mass spectrum of the ion at m/z 77. It could be suggested from the CAD spectrum and from considerations of the AMAC synthetic process that this impurity has an acetamidine ($CH_3C(NH)NH_2$) structure. However, the identification could not be confirmed as the compound eluded our extraction attempts to separate it from AMAC salt and acetamide. Characterization of this impurity is still in progress and will be reported elsewhere.

Conclusions

From its inception 10 years ago, a large amount of work has been done in TSP high-performance liquid chromatography mass spectrometry both from the theoretical and the practical points of view. In most cases, buffers consisting of AMAC or ammonium formate solutions have been used.

We demonstrate in this work the presence of relatively high proportions (~ 1 to 3% for the salt from

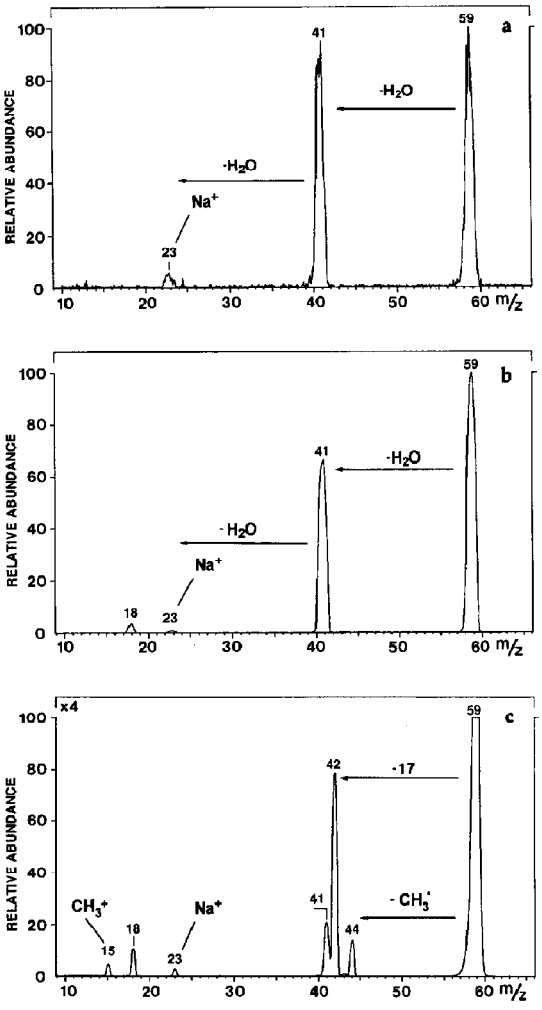

Figure 8. Daughter ion spectra of the signal at m/z 59 in the spectra of (a) straight water (see text); (b) aqueous 0.05 M AMAC-R; and (c) aqueous 0.05 M AMAC-S. TSQ-700 instrument. Collision pressure, 0.5 mtorr; collision energy, 30 eV.

Merck) of acetamide impurities in commercial AMAC salts. These impurities are responsible for some major reagent ions observed in the TSP spectra, such as the signals at m/z 60 ($[M + H]^+$), 77 ($[M + NH_4]^+$), and 119 ($[2M + H]^+$). The loss of water from the AMAC adducts during TSP analysis, the process generally accepted until now as the source of these ions, only accounts for a small fraction of these signals. In addition, an unidentified compound with a molecular weight of 58 is responsible for the $[M + 59]^+$ signals observed in the spectra of the pesticides tested in this work. To obtain comparable interlaboratory results in TSP, it should be necessary to use buffers from pure liquid reagents or chloroform-washed commercial salts. Impurities in AMAC could cause analytical artifacts in other mass spectrometry techniques such as electrospray and particle beam, where this salt is often used.

Acknowledgments

We gratefully acknowledge Teodora Tornos for providing the metallic ion solutions, Dra. Gael Durand for the deuterated acetic acid buffer spectrum, and M. Rosa Alonso and Roser Chaler for their help obtaining the electron ionization spectra. Also, Montse Carrascal and Albert Llonch for their skillful technical help and Dr. J. O. Bulbena for the graphic artwork. This work has been supported, in part, by the Project #CIPA-CT92-3005 of the Commission of the European Communities and the Spanish FIS Project 92/0281.

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