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Electron-impact mass spectrometry of diuretic agents

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Abstract: The mass spectrometric behaviour of nineteen diuretic agents in clinical use under conditions of 70 eV electron impact is analysed from reported and novel mass spectra. The molecular compositions of fragment ions are supported by high resolution data in selected cases, and a scheme presented for the rapid identification by MS of diuretics within the group.

Keywords: *Diuretic agents; mass spectrometry; accurate mass determinations.*

Introduction

Apart from inclusions in data collections of therapeutic agents [1, 2] and certain volumes of *Analytical Profiles* [6–10], little attention has been paid to the mass spectrometry of diuretics. Since therapeutic agents of this kind are in widespread use it was felt that a survey which included the mass spectrometric behaviour of the most commonly encountered diuretics would be of value for analytical purposes in regard to the raw drug substances, pharmaceutical formulations and samples isolated from the urine and other biofluids of patients undergoing diuretic therapy. The nineteen examples included cover a wide range of structures and little correlation in their 70 eV EI MS behaviour is possible apart from that of the thiazide group. Data used for the analysis include those obtained from specially recorded spectra and literature sources [1, 2]. All the compounds except chlorthiazide (see later) and polythiazide displayed molecular ions of intensities ranging from high (e.g. dichlorphenamide, hydroflumethazide, bumetanide, triamterene: 90–100%) to low (e.g. bendrofluazide, chlorthalidone, spironolactone; 1–6%). The molecular composition of fragment ions is supported by accurate mass determinations in many cases and probable mechanisms are presented where possible.

Experimental

Low resolution MS spectra were obtained using a 7070E VG Analytical instrument operating at 70 eV in the EI mode (this instrument recognizes no metastable ions).

High resolution (accurate mass) spectra were run using perfluorokerosene (PFK) as the reference substance. Differences were found between high and low resolution spectra in several cases and are due to differences in the operating conditions; the high

resolution spectra required a narrower beam and longer scan time (loss of sensitivity) and were influenced by the inherent vapour pressure of the reference compound. Reference peaks were automatically deleted from the mass spectral data.

Samples of diuretics were obtained from various pharmaceutical companies as listed in the acknowledgements.

Results and Discussion

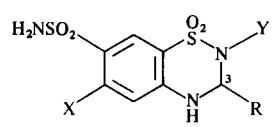
In this analysis of the MS data, the compounds are grouped according to structural similarities as far as possible, a classification most meaningful in the case of the thiazide diuretics.

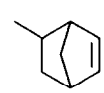
Thiazides

Of the seven dihydrothiazides (**1–7**) only hydrochlorthiazide ($M^{+\cdot}$ 297, 55%) and hydroflumethazide ($M^{+\cdot}$ 331, 90%)* gave prominent molecular ion peaks; the rest (all with R substituents) gave mass spectra with $M^{+\cdot}$ intensities in the range 5–8%. Fragmentations of the molecular ions of **1** and **2** probably proceed via the M-1 ion followed by the sequential loss of HCN (base peak, M-28) and SO, or SO_2 and HCN (Schemes 1 and 2). The latter pathway may involve a benzimidazole intermediate [3]. It is not easy to formulate the HCN/SO losses.

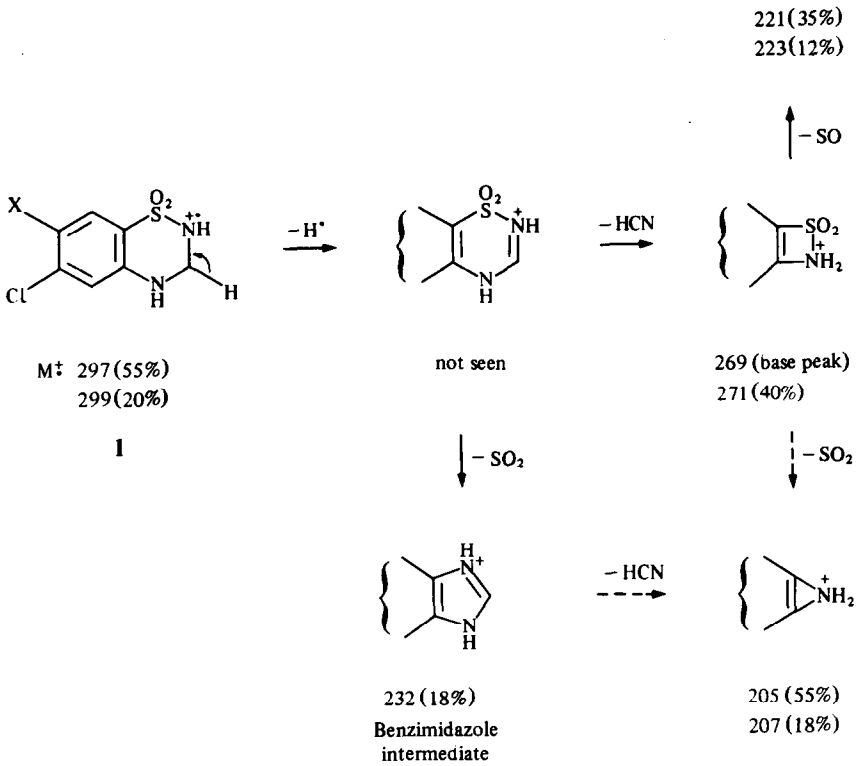
The fragmentation of bendrofluazide **3** is dominated by the 3-benzyl substituent. Loss of the benzyl radical gives the base peak while the tropylium ion itself has an abundance of about 40%. The presence of a 3-substituent also appears responsible for formation of the ion *m/z* of 118 (25%) (Scheme 3).

Cyclopenthiazide (**5**) and polythiazide (**6**) behave like bendrofluazide in losing their 3-R substituent to produce the base peaks (*m/z* 296 for **5** and *m/z* 310 for **6**) [2]. Loss of 3-R is minor for cyclothiazide (**4**) (M-93, 7%); instead the 3-(2-norbornen-5-yl) substituent

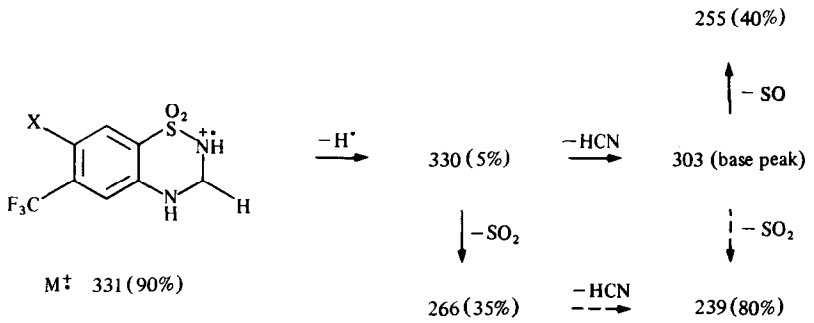


		X	Y	R
1	hydrochlorthiazide	Cl	H	H
2	hydroflumethazide	F_3C	H	H
3	bendrofluazide	F_3C	H	CH_2Ph
4	cyclothiazide	Cl	H	
5	cyclopenthiazide	Cl	H	CH_2 
6	polythiazide	Cl	Me	$CH_2SCH_2CF_3$
7	methyclothiazide	Cl	Me	CH_2Cl

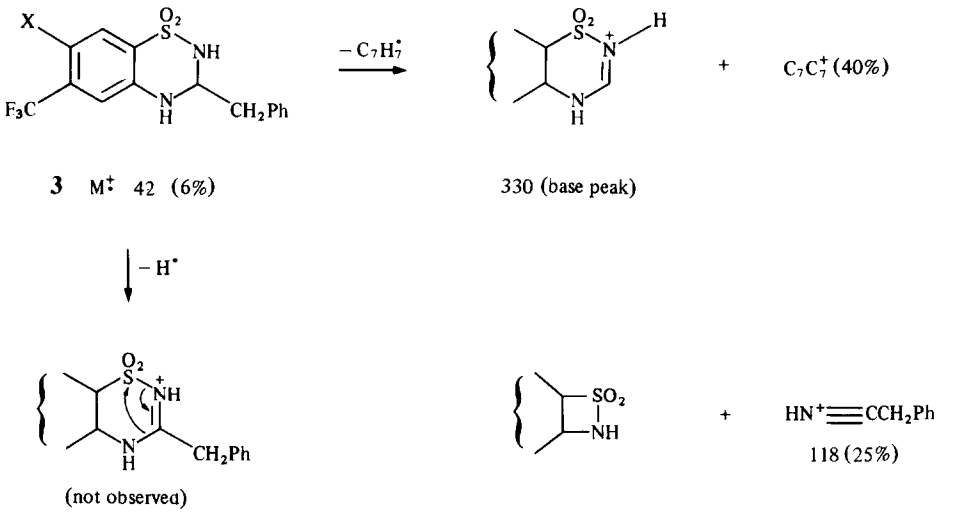
*Unless otherwise stated MS details refer to low resolution 70 eV EI spectra specially recorded for this paper. Data from other sources are referenced. For chlorine-containing compounds *m/z* ions quoted generally refer to those containing the ^{35}Cl isotope.



Scheme 1
MS behaviour of hydrochlorthiazide. General notes: Data from 70 eV EI spectra unless otherwise stated. Numerical values refer to *m/z* ions. High resolution evidence of ion formulations noted where applicable. In the formulae X = SO_2NH_2 throughout. Hydrochlorthiazide: 70 eV and high resolution spectra differed sharply; in the latter the molecular ion was the base peak, while ions *m/z* 269/271, 232 and 205 were not recorded. The ion *m/z* 220.9786 ($M-CH_2NOS$ requires 220.9785) had an intensity of 67%. The ions *m/z* 205.9918 (46%) and 189.9698 (43%) corresponded with $M-CHNO_2S$ (205.9914) and $M-CH_3N_2O_2S$ (189.9729), respectively; both these ions had low intensities in the 70 eV spectrum.



Scheme 2
MS behaviour of hydroflumethazide. High resolution evidence obtained for *m/z* values 331, 303, 266, 255 and 239.

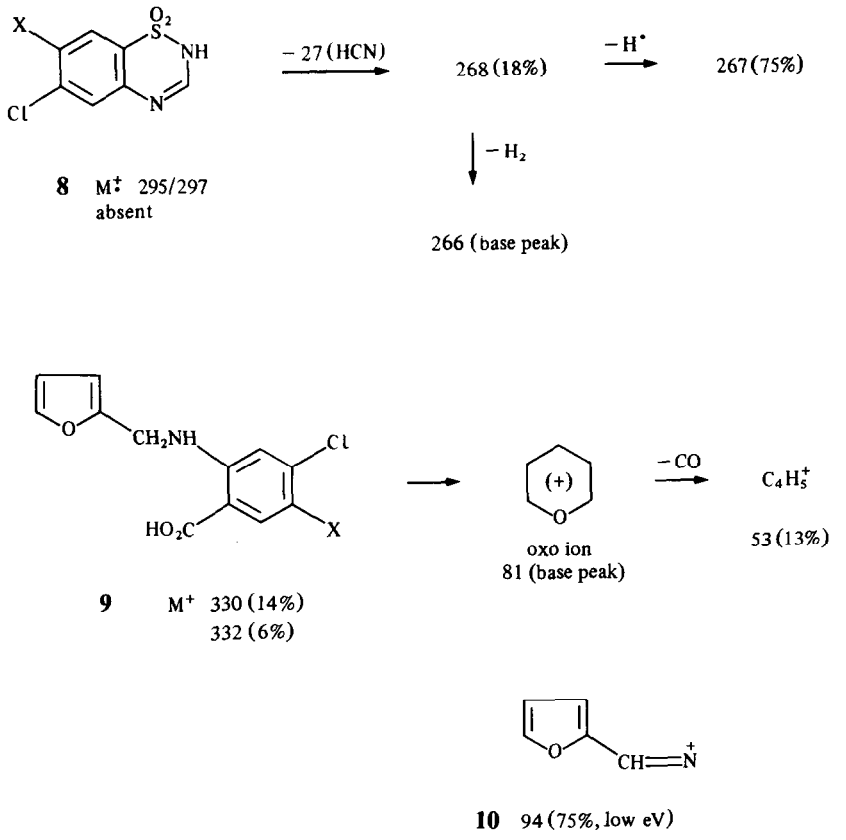


Scheme 3
MS behaviour of bendrofluazide. High resolution evidence obtained for all *m/z* values.

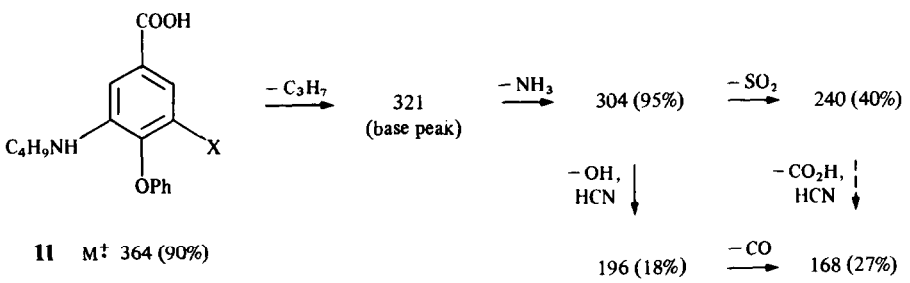
undergoes a retro Diels–Alder reaction followed by loss of a hydrogen radical to give a base peak of *m/z* 66 ($C_5H_6^+$). The pathway leading to formation of an $HN^+ \equiv CR$ fragment, as followed by bendrofluazide, is also favoured for molecular ions of cyclopenthiazide (*m/z* 110, 46%) and cyclothiazide (*m/z* 120, 78%), but not for polythiazide. Ions rationalized by loss of HCN, and HCN plus SO_2 from $(M-R)^+$ ions as observed for **1** and **2** (R=H in these cases), are only prominent in the cases of cyclopenthiazide **5** (*m/z* 269, 29%; *m/z* 205, 30%) and cyclothiazide (*m/z* 269, 13%; *m/z* 205, 12%). In mass spectra of all thiazides with 6-chloro substituents (**1**, **4–7**), ions containing ^{35}Cl were all accompanied by their heavier isotope counterparts in appropriate intensity ratios. The mass spectrum of methylchlorthiazide (**7**) likewise displays low intensity molecular ions (*m/z* 359, 4%; *m/z* 361, 2.9%) which fragment preferentially by loss of the 3-substituent (CH_2Cl) to give the base peak *m/z* 310 and corresponding ^{37}Cl isotope ion *m/z* 312 (43%) [1].

Chlorthiazide (**8**), the di-3,4-dehydroanalogue of hydrochlorthiazide failed to yield a molecular ion (contrary to a CRC listing) [1] and the only prominent lines above 100 in its mass spectrum were at *m/z* 149 (40%), 266 (base peak), 267 (75%) and 268 (18%). The ions of higher mass probably arise through loss of HCN followed by H$^\cdot$ and H_2 from the molecular ion.

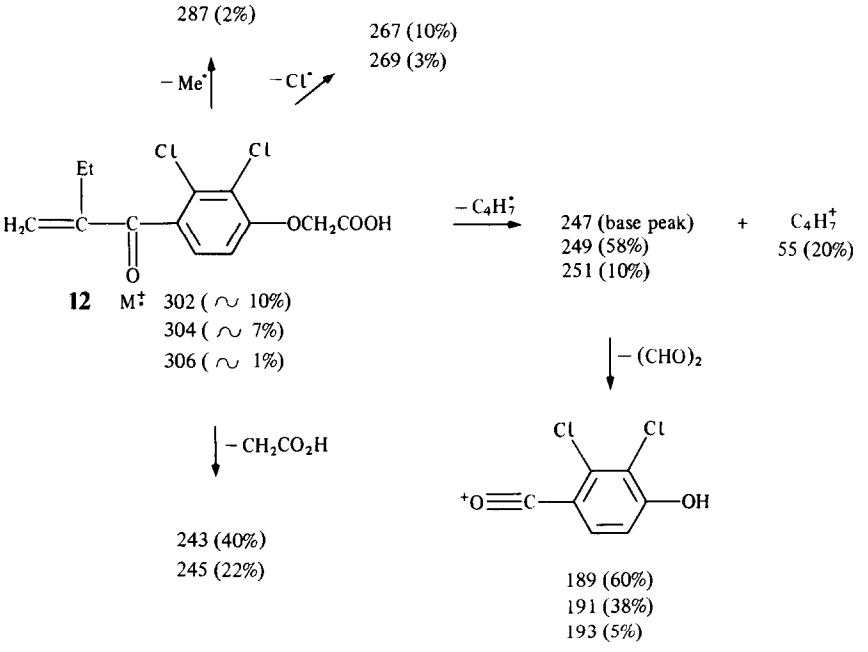
Two sulphonamide carboxylic acids are considered next. The mass spectrum of *frusemide* (**9**, Scheme 4) showed weak molecular ions (330, 13.7%; 332, 5.7%) and a base peak at *m/z* 81 due to the oxo ion which lost CO to give a *m/z* 53 fragment. The ion (**10**) was prominent in a low eV EI spectrum. *Bumetanide* (**11**, Scheme 5) provided an intense molecular ion (90%, base peak in high resolution run) which lost a propyl radical to form the base peak (*m/z* 321), followed by removal of ammonia to yield a prominent *m/z* 304 ion. Losses of other small molecules are also evident (Scheme 5). *Ethacrynic acid* (**12**, Scheme 6), another carboxylic acid but a non-sulphonamido diuretic, yields a trio of low intensity molecular ions. Loss of a butenyl radical (C_4H_7) from the ^{35}Cl molecular ion, followed by loss of $C_2H_2O_2$ (glyoxal?) produces the base peak (*m/z* 247)



Scheme 4
MS behaviour of frusemide. High resolution evidence obtained for *m/z* values 332, 330, 81 and 53.



Scheme 5
MS behaviour of bumetanide. High resolution evidence obtained for *m/z* values 364, 321, 304, 240 and 168.



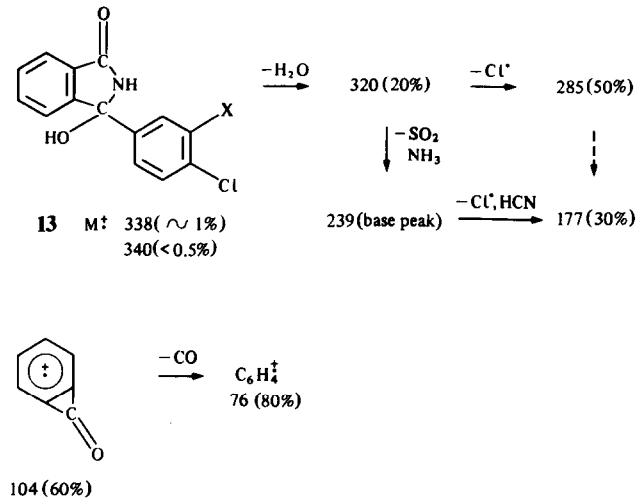
Scheme 6
MS behaviour of ethacrynic acid. High resolution evidence obtained for *m/z* values 302, 245, 247, 249, 189, 191 and 55.

and (*m/z* 189, 60%) ions, respectively; these two lines form part of the cluster pattern of three diagnostic ions of a disubstituted chlorine derivative. Loss of methyl (*m/z* 287, 2%) and chlorine radicals (*m/z* 267, 10%) from the molecular ion are also evident together with that of $C_2H_3O_2$ (*m/z* 243, 40%). The last fragment was the base peak in the low eV (<70 eV) EI spectra, and was accompanied by two isotope peaks (*m/z* 245, 247). The butenyl cation (*m/z* 55, 20%) also had a significant presence in the 70 eV spectrum.

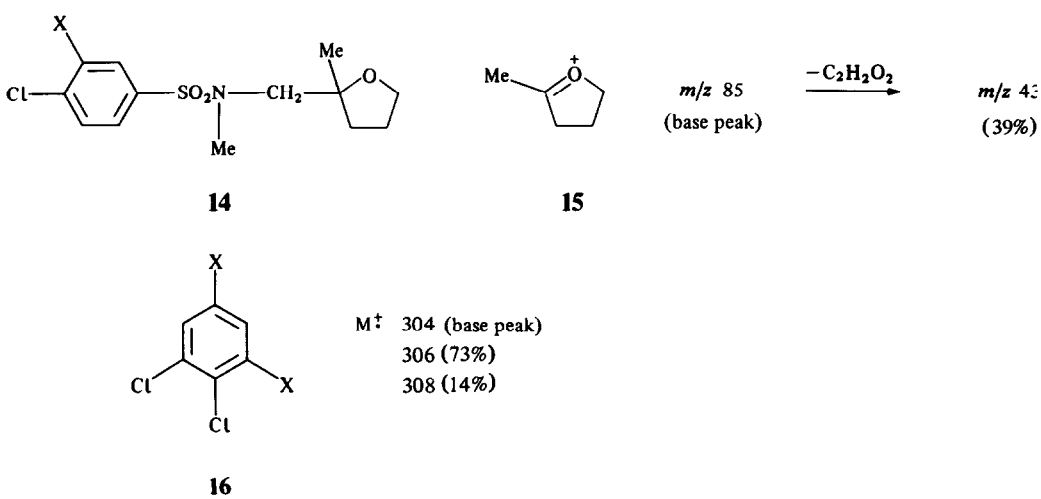
Returning to the sulphonamido derivatives, *chlorthalidone* (**13**, $M^{+\cdot}$ intensity ~1%) readily fragments by loss of water and other small molecules and radicals (Scheme 7); the base peak (*m/z* 239) corresponds to loss of H_2O, SO_2 and NH_3. The accurate mass of the prominent ion *m/z* 104 (60%) corresponds to C_7H_4O which suffers loss of CO to form the ion *m/z* 76 (80%) — the latter is the base peak in a reported spectrum [2]. The SO_2 ion (*m/z* 64), a low intensity line (5%) in the 70 eV EI spectrum, surprisingly formed the base peak in the high resolution spectrum. The mass spectrum of *mefruside* (**14**) showed only two features, namely the base peak *m/z* 85 assigned to the tetrahydrofuran ion **15**, and the ion *m/z* 43 (39%) probably derived from it by loss of C_2H_2O (ketene?) [2].

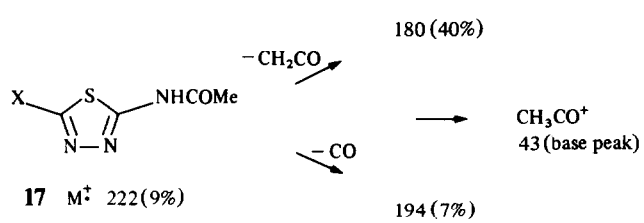
The molecular ion of the disulphonamide *dichlorphenamide* (**16**) is of high stability since the ^{35}Cl ion (*m/z* 304) is the base peak and is accompanied by its isotopic partners in intensities appropriate to a dichloro derivative [2]. Loss of 1 mole (*m/z* 224, 44%) and 2 moles (*m/z* 144, 28%) of SO_2NH_2 are evident, while the latter ion appears to release both 1 mole (*m/z* 109, 62%) and 2 moles (*m/z* 74, 63%) of chlorine.

Acetazolamide (**17**) yields a low intensity molecular ion (*m/z* 222) (not observed in a reported spectrum, ref. 2) and is characterized by a base peak at *m/z* 43 due to CH_3CO. Molecular ion loss of ketene (*m/z* 180, 40 — base peak in high resolution spectrum) and



Scheme 7
MS behaviour of chlorthalidone. High resolution evidence obtained for *m/z* values 340, 338, 320, 285, 239, 177, 104 and 76.





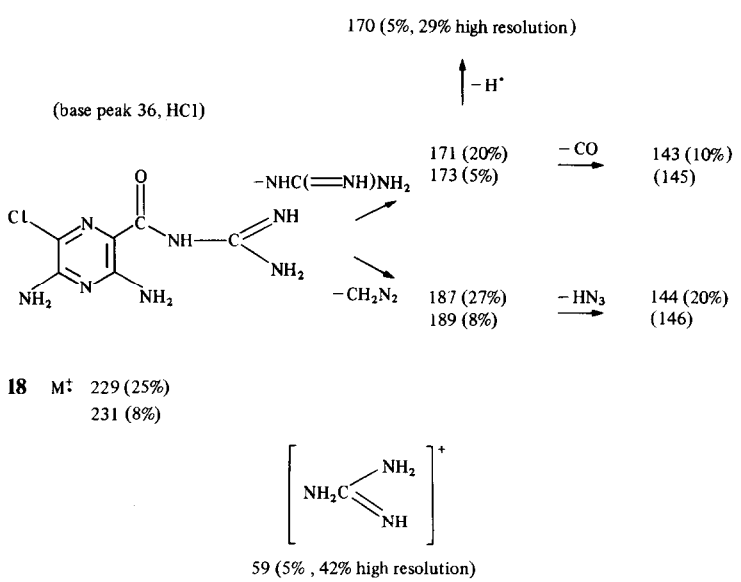
Scheme 8
MS behaviour of acetazolamide. High resolution evidence obtained for *m/z* values 222, 194 and 180.

CO (more probable than N_2 from accurate mass data) is also seen (Scheme 8). Under low eV EI, (<70 eV), the molecular ion is the base peak while fragments *m/z* 194 (70%) and 180 (80%) are both prominent.

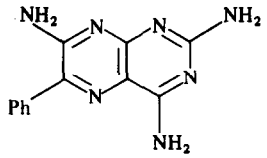
Amiloride (**18**) hydrochloride and triamterene (**19**) are two diuretics notably rich in nitrogen. The base peak of the spectrum of *amiloride hydrochloride* (*m/z* 36) is indicative merely of the acid component of the salt. No other prominent lines were found in the spectrum recorded for this work. The molecular ion (*m/z* 229, 25%, base peak in low eV spectrum) suffers loss of a variety of neutral molecules, including diazomethane to give the ion *m/z* 187 (27%, 45% in low eV spectrum) as supported by accurate mass measurements (Scheme 9).

The ion *m/z* 59 ($CH_5\dot{N}_3$, guanidine) was prominent in the high resolution spectrum (42%) but of low intensity in that recorded at 70 eV. In a collection of spectra [2], that of amiloride showed a base peak at *m/z* 43 ($HN{=}C{=}\dot{N}H_2$ or N_3H^+) and a prominent (75%) *m/z* 187 peak. The *triamterene* molecular ion (*m/z* 253) is stable with an intensity just below that of the M-1 ion (*m/z* 252, base peak). The only significant lines above *m/z* 100 are those at *m/z* 104 (18%, accurate mass 104.0488, $PhC \equiv \dot{N}H$ requires 104.0500) and 235 (30%, 252 − NH_3). An intense low mass line at *m/z* 43 (80%) may be due to hydrazoic acid (N_3H).

Spironolactone (**20**, Scheme 10), the only steroidal diuretic examined, yields a weak molecular ion (*m/z* 416, 2%) under 70 eV EI which loses its 7-thioacetyl substituent in two stages to give ions at *m/z* 374 (40%, loss of C_2H_2O) and *m/z* 341 (base peak, further loss of SH). The small molecule/radical losses shown in Scheme 10 are supported by accurate mass data. The usual ion diagnostic of acetyl (*m/e* 43) is present in 40% intensity.



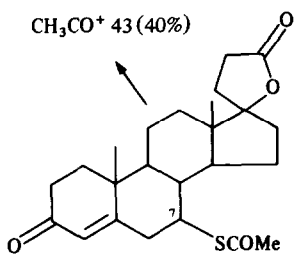
Scheme 9
MS behaviour of amiloride hydrochloride. High resolution evidence obtained for *m/z* values 231, 229, 189, 187, 171, 170, 144 and 59.



$-H^{\cdot}$ → 252 (base peak) $-NH_3$ → 235 (30%)

19 $M^{\ddagger}$ 253 (99%) *m/z* 43 (80%) HN_3?

(High resolution evidence obtained for *m/z* values 253, 252 and 235)



$-CH_2CO$ →

359 (20%)
↑ $-Me^{\cdot}$
374 (40%) $-SH^{\cdot}$ → 341 (base peak)
↓ $-H_2S$ ↓ $-C_2H_4O_2$
340 (45%) 267 (20%)

$M^{\ddagger}$ 416 (2%)

20

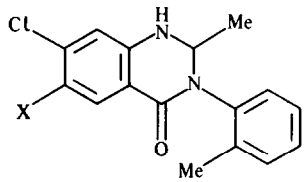
↓ $-HC{=}O$, $HC{=}S$

342 (20%)

Scheme 10
MS behaviour of spironolactone. High resolution evidence obtained for *m/z* values 416, 374, 359, 342, 341, 340 and 267.

Metolazone (**21**), the last example, is a 1-oxo analogue of the thiazide diuretics. Its mass spectrum [2] displays low intensity molecular ions (*m/z* 365, 12%; *m/z* 367, 4%) and a base peak *m/z* 350 formed by loss of the 3-methyl substituent. The ion *m/z* 259 (36%) probably results from further loss of the *o*-tolyl substituent at N-2 (350-91); the tropylium ion itself is present at an intensity of 57%.

Apart from resort to searching collections of abbreviated mass spectral data [4, 5], several schemes may be devised for the rapid identification of a diuretic from the information collated in this paper provided it is in common clinical use. One approach may be based on molecular ion intensities which provides an initial subdivision into three groups, namely those with prominent (>50%), moderate (10–50%) and feeble (<10%) $M^{\ddagger}$ intensities. Diagnostic ions could then be sought amongst each group. Thus, spectra of both bendrofluazide (**3**) and metolazone (**21**) include a prominent *m/z* 91 (tropylium) ion, while recognition of isotopic chlorine cluster ions differentiates monochloro, dichloro and non-chloro analogues (allowance should be made for distortion of calculated intensity ratios by contributions from minor isotopes of other atoms). The



M‡ 365 (12%)
367 (4%)

21

Table 1
Scheme for identification of 19 clinical diuretics by mass spectrometry based on molecular ion intensities

Compound*	Molecular ion (>50%)	Base peak	Selected diagnostic ions
bumetanide (**11**)	364	321	304
hydroflumethazide (**2**)	331	303	239
dichlorphenamide (**16**)	304	304	306, 308 (Cl isotopes) 224, 109
hydrochlorthiazide (**1**)	297	269	205
triamterene (**19**)	253	252	43
	Molecular ion (10–50%)		
metolazone (**21**)	365	350	259, 91
amiloride HCl (**18**)	229 (base m.wt)	36	187
	Molecular ion (<10%)		
polythiazide (**6**)	439	310	206
bendrofluazide (**3**)	421	330†	91
spironolactone (**20**)	416	341‡	374
cyclothiazide (**4**)	389	66§	120
mefruside (**14**)	382	85	43
cyclopenthiazide (**5**)	379	296	110
methyclothiazide (**7**)	359	310¶	312, 314 (Cl isotopes)
chlorthalidone (**13**)	338	239‖	104, 76
frusemide (**9**)	330	81	53
ethacrynic acid (**12**)	302	247**	189**
chlorthiazide (**8**)	295††	266	267
acetazolamide (**17**)	222	43	180

*Each group in descending order of molecular weight.
†~20% intensity in literature report [6] which gives *m/z* 319 as base peak.
‡As literature report [10].
§As literature report [7].
¶As literature report [8], supported by high resolution data.
‖68% intensity in literature report [9] which gives *m/z* 148 (M^+:$C_6H_3ClSO_2NH_2$) as base peak.
**Plus two Cl isotope lines.
††Base peak in CRC listing [1].

spectrum of frusemide (**9**) is characterized by a base peak *m/z* 81 due to the 2-furfuryl substituent, and that of ethacrynic acid by one at *m/z* 247 due to unique loss of C_4H_7 from the molecular ion. A procedure of this kind is presented in Table 1.

While other analytical techniques are valuable for the identification of diuretic agents, e.g. TLC [11] and other forms of chromatography, mass spectrometry has the virtue of providing specific characterization without the need for reference samples, and allows the ready differentiation of materials which have similar chromatographic retention times. The variability of mass spectra dependent on the instrument used and operating conditions (some differences for diuretics are noted in Table 1) precludes application of a rigid analytical MS scheme. However, since variations mostly relate to intensities of the more prominent ions rather than major fragmentation pathways (as footnotes to Table 1 illustrate), a data compilation as presented here should have analytical value provided consideration be given to these limiting factors.

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