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Formation of formaldehyde adducts from various drugs by use of methanol in a toxicological screening procedure with gas chromatography–mass spectrometry

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ABSTRACT

Use of methanol as a solvent in a toxicological screening procedure with gas chromatography–mass spectrometry may be associated with artifact formation. Artifacts with a molecular ion of $[M+12]^+$ are formed from various drugs, such as amphetamine, propafenone, flecainide, β-blockers and prilocaine. The mechanism of artifact formation was studied by mass spectral techniques, labelling and nuclear magnetic resonance spectroscopy. It was shown that the artifacts were generated by the addition of formaldehyde and subsequent loss of water. Formaldehyde is probably formed by thermal dehydrogenation of methanol in the injection port of the gas chromatograph.

INTRODUCTION

Screening procedures using gas chromatography–mass spectrometry (GC–MS) have proved to be most sensitive, specific and reliable for identifying drugs and their metabolites in cases of drug overdose on an emergency basis [1–3]. Methanol is frequently used to dissolve urine or plasma extracts prior to injection into the injection port of the gas chromatograph–mass spectrometer. In general, methanol is considered to be an inert solvent that reacts only exceptionally or not at all with constituents of the extracts. Formation of methyl esters from carbonic acids or methyl ethers from phenols has been reported [1].

A molecular ion of an artifact with the mass $[M+12]^+$ has been observed after injection of extracts containing flecainide in methanol [4]. It was demonstrated

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that the $[M+12]^+$ peak was due to the addition of formaldehyde to flecainide combined with loss of water (Fig. 1) [4]. Artifacts with a molecular ion of $[M+12]^+$ have also been observed after GC–MS analysis of β-blockers dissolved in methanol [1] (M. Donike, personal communication). Replacement of the hydroxyl group and the hydrogen of the amino group by two methyl groups, accompanied by loss of two molecules of water, has been suggested as the underlying reaction mechanism [1]. Since the postulated replacement of a hydroxy group by a methyl group seems to be a rather unusual, we reinvestigated the structure and formation of this type of reaction product.

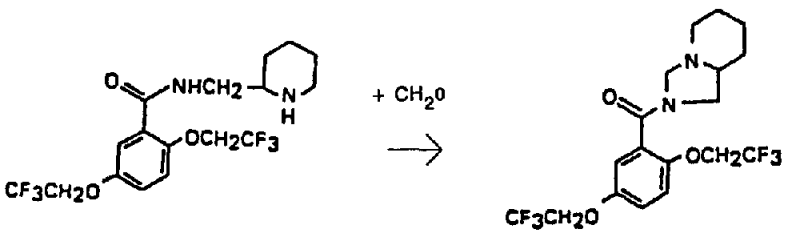

Fig. 1. Formation of an artifact from flecainide by the use of methanol as a solvent in GC–MS.

EXPERIMENTAL

Reagents and chemicals

Pure samples of β-blockers were analysed with the direct probe prior to their use. All reagents, analytical-reagent grade or better, were purchased from commercial sources and used without further purification.

In vitro experiments

Standard solutions (0.1 mg/ml) were prepared of mepindolol, carazolol, carteolol, bunitrolol, sotalol, bupranolol, atenolol, metipranolol, timolol, alprenolol, betaxolol, bisoprolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, metoprolol, acebutolol, flecainide, propafenone, prilocaine and amphetamine (Fig. 2) in methanol, ethyl acetate and diethyl ether (Nanograde, Mallinckrodt, St. Louis, MO, U.S.A.), respectively. These solutions were analysed by GC–MS in the electron-impact (EI) and chemical ionization (CI)-modes. Some of the drugs were additionally dissolved in $[^2H_4]$methanol (Merck, Darmstadt, F.R.G.), and mass spectra measured after the injection port of the GC–MS system had been rinsed three times with 1 μl of $[^2H_4]$methanol.

In addition, 2 ml of an aqueous solution of the drugs (1 mg/ml) was treated with 0.5 ml of aqueous formaldehyde (formaline) and then extracted with diethyl ether (Nanograde, Mallinckrodt). The resulting products were analysed by GC–MS. The reaction products of metoprolol, propranolol and prilocaine with form-

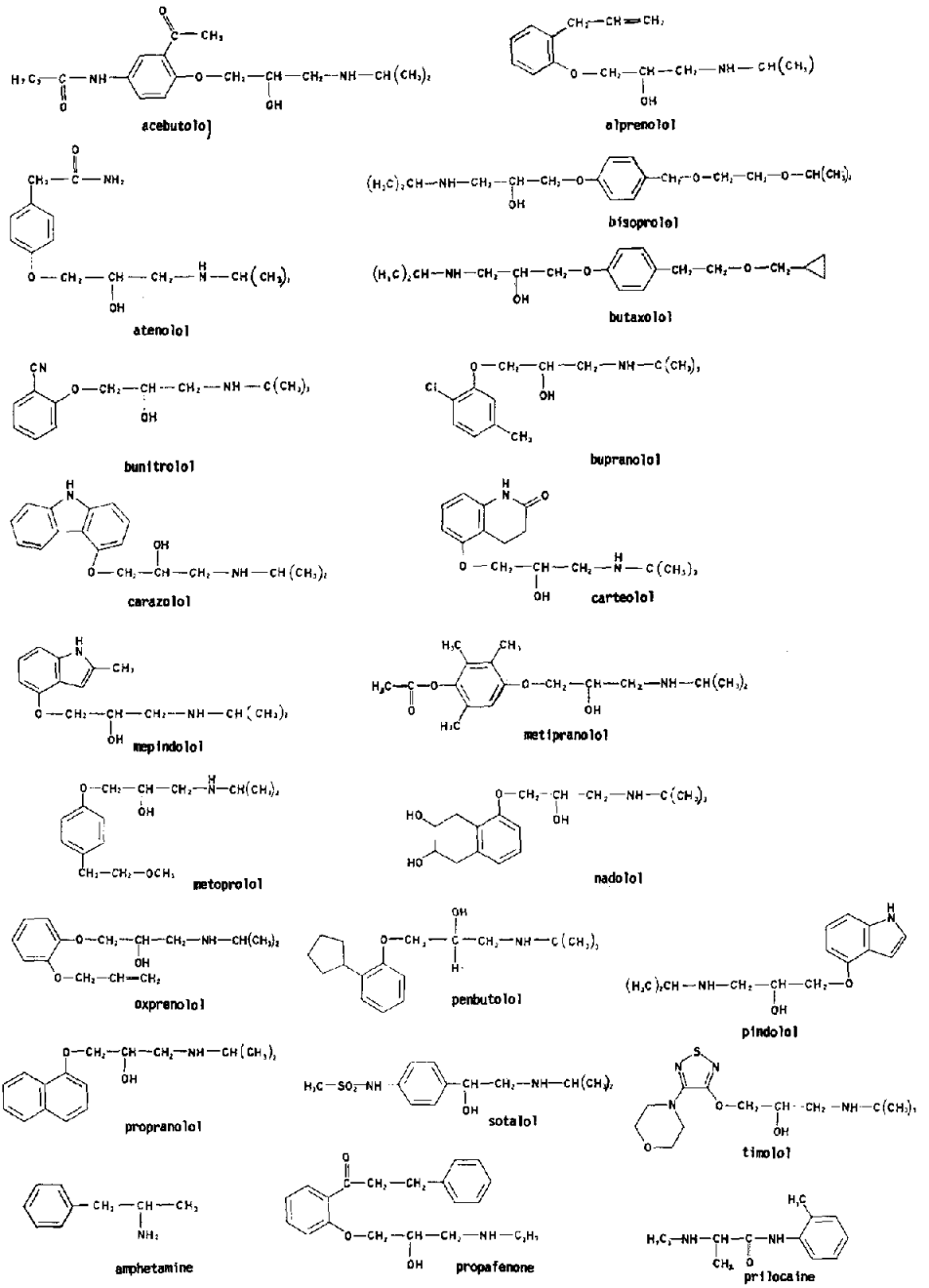

Fig. 2. Structures of drugs forming artifacts with a molecular ion of $[M+12]^+$.

aldehyde were also analysed by high-resolution MS (static high resolution; resolution $\geq$ 20 000) with a 311 mass spectrometer (Varian MAT; Bremen, F.R.G.), and by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy (WHM 270, Bruker, Darmstadt, F.R.G.).

Clinical studies

Urine was collected from patients with suspected overdoses of β-blockers, propafenone, flecainide or prilocaine. All urine samples were stored at $-20°C$ prior to analysis. A 20-ml urine sample was extracted at pH 9 with diethyl ether (Nanograde, Mallinckrodt). The organic solvent was removed with a dry stream of nitrogen. The residue was dissolved in 100 μl of methanol (Nanograde, Mallinckrodt), and a 1–3 μl aliquot was used for GC–MS. Urine was extracted similarly after hydrolysis with concentrated hydrochloric acid at 100°C for 30 min.

GC–MS analysis

EI mass spectra were run on a 4021 gas chromatograph–mass spectrometer with an Incos data system (Finnigan, San Jose, CA, U.S.A.). For GC a fused-silica capillary column (SE54, 25 m $\times$ 0.32 mm I.D., film thickness 0.3 mm; Macherey & Nagel, Düren, F.R.G.) was used with an injection port temperature of 295°C, splitless injection and a column temperature programme of 75–300°C at 15°C/min. The carrier gas was helium with a flow-rate of 1.7 ml/min. The column was directly coupled to the mass spectrometer. The ion source pressure was $4 \cdot 10^{-5}$ Pa. The ion-source temperature was 220°C. The multiplier voltage was 1200 V.

CI mass spectra (reagent gas methane) were run on an ion-trap detector (ITD 800) with software revision 4.1 (Finnigan MAT, Bremen, F.R.G.). An ARC programme with variable ionization time was used. The GC conditions were the same as described for the GC–MS 4021. The gas chromatograph was directly coupled to the ion-trap detector. The temperature of the transline was 290°C. The multiplier voltage was 1700 V.

All samples were run in the EI mode at 70 eV (GC–MS 4021) and in the CI mode (ITD 800). Structure elucidation was based on reference mass spectra, confirmation of the molecular ion by CI, fragmentation pattern and formation of the corresponding derivatives after use of deuterated methanol.

RESULTS

No $[M+12]^{+}$ artifacts of the drugs were observed when standard solutions of the drugs in diethyl ether or ethyl acetate were analysed by GC–MS. The mass spectra of the original drugs dissolved in diethyl ether or ethyl acetate were identical with mass spectra published in the literature or obtained with the direct probe. Analysis of the drugs dissolved in methanol with the direct probe at 50–100°C gave no evidence for artifact formation.

$[M+12]^+$ artifacts, with the exception of sotalol, and small amounts of the original drugs were observed after GC–MS analysis of the drugs dissolved in methanol. The EI mass spectra of the $[M+12]^+$ artifacts are summarized in Table I. Dissolving the drugs in $[^2H_4]$methanol led to the formation of artifacts with a molecular ion of $[M+14]^+$. The mass spectra of the artifacts are characterized by formation of $[OCH_2\overset{+}{N}H=R]$ and $[\overset{+}{N}H=R]$ ions from the five-membered ring (Fig. 3).

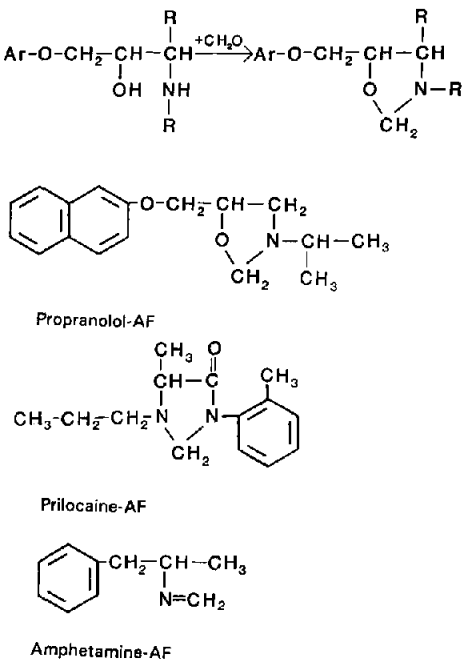

Fig. 3. Mechanism of formation of the $[M+12]^+$ artifacts from β-blockers and structures of artifacts (AF) from propranolol, prilocaine and amphetamine.

Reaction of the drugs, except acebutolol and sotalol, with formaline led to formation of the artifact with a 100% yield. Only small amounts of the artifact were observed after the *in vitro* reaction of acebutolol with formaldehyde. No artifact was found after the reaction of sotalol. Since the highly polar sotalol itself hardly passes the GC column, it might be that the artifact of sotalol could not be detected owing to its high polarity.

High-resolution mass spectra of the artifacts of propranolol and metoprolol synthesized by reaction of the drugs with formaline showed that the $[M+12]^+$ artifact was formed by "addition" of a carbon atom and not by addition of two methyl groups and loss of two molecules of water. The ^1H and ^{13}C NMR spectra

TABLE I

RETENTION TIMES AND MASS SPECTRA OF $[M+12]^+$ ADDUCTS OF VARIOUS β-BLOCKERS, PROPAFENONE, FLECAINIDE, PRILOCAINE AND AMPHETAMINE

	Retention time (s)	*m/z* (% intensity)
Acebutolol	776	M^+ 348 (2), 221 (22), 151 (11), 98 (10) *86 (100)*, 72 (61), 56 (45)
Alprenolol	478	M^+ 263 (6), 260 (7), 146 (31), 232 (2), 218 (3), 173 (11), 145 (12), 126 (83), 114 (24), 112 (26), 98 (13), 91 (21), 84 (36), *56 (100)*
Alprenolol (+ $C^2H_3O^2H$)	480	M^+ 265 (6), 262 (3), 261 (3), 148 (40), 146 (6) 234 (2), 220 (3), 175 (6), 174 (6), 173 (7), 147 (6), 145 (13), 128 (28), 127 (64), 116 (22), 115 (12), 114 (11), 113 (20), 100 (11), 98 (4), 86 (16), 85 (20), *56 (100)*
Atenolol	645	M^+ 278 (10), 263 (40), 249 (4), 190 (10), 145 (13), 128 (24), 127 (81), 114 (33), 112 (36), 86 (40), 70 (36), *56 (100)*
Atenolol (+ $C^2H_3O^2H$)	647	M^+ 280 (9), 265 (33), 251 (3), 190 (10), 145 (12), 128 (70), 127 (33), 116 (31), 112 (26), 88 (22), 86 (18), *56 (100)*
Bisoprolol	628	M^+ 337 (4), 336 (4), 322 (21), 308 (6), 234 (16), 190 (4), 128 (53), *127 (100)*, 114 (51), 112 (61), 86 (30), 56 (52)
Bisoprolol (+ $C^2H_3O^2H$)	628	M^- 339 (12), 338 (4), 337 (4), 336 (2), 324 (31), 310 (8), 236 (12), 191 (2), 150 (30), 129 (20), *128 (100)*, 116 (65), 115 ((30), 114 (44), 113 (31), 88 (30), 87 (30), 56 (56)
Bunitrolol	538	M^+ 260 (1), *245 (100)*, 215 (2), 203 (3), 158 (6), 102 (9), 96 (18), 70 (30), 57 (38)
Bupranolol	518	M^+ 283 (4), *268 (100)*, 226 (2), 181 (8), 155 (7), 141 (13), 134 (9), 96 (10), 72 (18), 70 (80), 57 (93)
Bupranolol (+ $C^2H_3O^2H$)	519	M^+ 285 (3), *270 (100)*, 181 (7), 155 (6), 141 (8), 135 (7), 96 (8), 74 (11), 70 (52), 57 (37)
Butaxolol	627	M^+ 319 (26), 318 (20), 304 (54), 290 (13), 128 (38), 127 (81), 114 (48), 112 (40), 86 (28), 56 (71), *55 (100)*
Butaxolol (+ $C^2H_3O^2H$)	629	M^+ 321 (22), 320 (8), 319 (4), 306 (54), 292 (11), 130 (18), 129 (17), 128 (82), 127 (16), 116 (56), 113 (36), 88 (24), 87 (23), 86 (16), 56 (65), *55 (100)*
Carazolol	752	M^+ 310 (30), 295 (4), 281 (3), 222 (13), 196 (4), 104 (12), *183 (100)*, 166 (9), 154 (22), 148 (31), 128 (22), 127 (67), 114 (33), 112 (48), 98 (22), 86 (37)
Carteolol	700	M^+ 304 (33), *289 (100)*, 247 (3), 240 (16), 202 (43), 176 (6), 163 (8), 141 (88), 130 (18), 126 (38), 114 (31), 96 (10), 85 (40), 70 (80), 57 (98)
Mepindolol	636	M^+ 274 (41), 259 (3), 245 (5), 200 (6), 186 (13), 160 (7), 148 (11), *147 (100)*, 146 (24), 129 (33), 128 (19), 127 (36), 114 (31), 112 (28), 98 (19), 86 (42)
Metipranolol	612	M^+ 321 (19), 306 (38), 292 (6), 278 (3), 264 (11), 191 (11), 152 (38), 128 (50), *127 (100)*, 114 (91), 112 (74), 98 (20), 86 (60), 72 (30), 56 (73)
Metoprolol	553	M^+ 279 (20), 278 (12), 264 (41), 250 (6), 159 (11), 127 (68), 114 (40), 112 (40), 86 (44), *56 (100)*

TABLE I (*continued*)

	Retention time (s)	*m/z* (% intensity)
Metoprolol (+ $C^2H_3O^2H$)	553	M^+ 281 (21), 280 (8), 279 (8), 266 (42), 252 (10), 159 (11), 128 (80), 116 (58), 113 (40), 88 (40), 87 (36), 86 (18), *56 (100)*
Nadolol	689	M^+ 321 (10), *306 (100)*, 288 (6), 264 (4), 201 (8), 141 (21), 86 (14), 70 (42), 57 (50)
Nadolol (+ $C^2H_3O^2H$)	689	M^+ 323 (10), *308 (100)*, 306 (17), 290 (6), 266 (4), 201 (9), 142 (18), 86 (10), 70 (30), 57 (41)
Oxprenolol	505	M^+ 277 (6), 276 (7), 262 (56), 248 (60), 220 (8), 148 (30), 127 (32), 114 (70), 112 (48), 86 (53), 57 (87), *56 (100)*
Oxprenolol (+ $C^2H_3O^2H$)	505	M^+ 279 (4), 278 (1), 277 (4), 264 (60), 250 (60), 249 (30), 148 (30), 128 (20), 127 (16), 116 (50), 115 (20), 114 (25), 113 (21), 112 (22), 88 (22), 87 (21), 86 (21), 72 (36), *56 (100)*
Penbutolol	579	M^+ 303 (14), *288 (100)*, 201 (6), 141 (21), 86 (16), 70 (44), 57 (43)
Penbutolol (+ $C^2H_3O^2H$)	579	M^+ 305 (14), *290 (100)*, 201 (6), 142 (19), 86 (6), 70 (32), 57 (39)
Pindolol	609	M^+ 260 (31), 245 (4), 231 (4), 172 (14), 133 (80), *127 (100)*, 114 (34), 112 (42), 86 (47), 72 (26), 56 (52)
Pindolol (+ $C^2H_3O^2H$)	609	M^+ 262 (31), 247 (4), 233 (4), 172 (12) 134 (60), *128 (100)*, 116 (42), 115 (18), 114 (14), 113 (28), 88 (41), 87 (11), 86 (18), 74 (22), 56 (54)
Propranolol	578	M^+ 271 (12), 256 (9), 183 (8), 144 (12), *127 (100)*, 114 (33), 112 (42), 86 (40), 72 (23), 56 (66)
Propranolol (+ $C^2H_3O^2H$)	581	M^+ 273 (16), 258 (14), 183 (12), 145 (11), 144 (9), *128 (100)*, 116 (42), 115 (40), 113 (30), 88 (36), 87 (18), 86 (12), 74 (20), 56 (56)
Timolol	633	M^+ 328 (20), 313 (42), 297 (10), 283 (6), 271 (12), 239 (12), 188 (6), 154 (13), 142 (20), 128 (13), 112 (13), 96 (26), 86 (94), 72 (28), 70 (30), *57 (100)*, 56 (81)
Amphetamine	110	M^+ 147 (4), 146 (3), 132 (8), 91 (13), *56 (100)*
Amphetamine (+ $C^2H_3O^2H$)	110	M^+ 149 (4), 148 (2), 134 (11), 91 (13), *58 (100)*
Propafenone	688	M^+ 353 (3), 338 (1), 324 (11), 265 (3), 262 (12), 248 (8), 226 (13), 208 (13), 175 (4), 161 (4), 144 (6) *128 (100)*, 127 (50), 121 (37), 98 (95), 91 (71), 84 (48)
Propafenone (+ $C^2H_3O^2H$)	684	M^+ 355 (3), 338 (2), 326 (11), 265 (3), 264 (8), 262 (2), 250 (5), 248 (1), 228 (1), 227 (9), 226 (3), 210 (3), 209 (8), 208 (9), 176 (2), 175 (1), 161 (3), 146 (4), 145 (2), 144 (2), *130 (100)*, 129 (13), 128 (57), 127 (10), 122 (10), 121 (26), 100 (60), 99 (23), 98 (21), 91 (68), 86 (44)
Flecainide	568	M^+ 426 (28), 301 (50), *125 (100)*, 97 (31), 84 (20), 69 (20)
Flecainide (+ $C^2H_3O^2H$)	570	M^+ 428 (20), 301 (40), *127 (100)*, 125 (30), 99 (50), 84 (20), 69 (21)
Prilocaine	449	M^+ 232 (22), 217 (13), 203 (12), 147 (6), 120 (8), 119 (28), 118 (30), 99 (24), 91 (16), 86 (29), *84 (100)*, 71 (22), 56 (71)

as well as the ^{13}C DEPT (distortionless enhancement by polarization transfer) spectra of the artifacts gave evidence for the presence of an additional methylene group (Fig. 3).

$[M+12]^+$ artifacts were also observed after the injection of urine extracts in methanol in all cases of propranolol (three patients), metoprolol (two patients), atenolol (one patient), alprenolol (one patient), propafenone (one patient), flecainide (three patients) and prilocaine (one patient) overdose.

DISCUSSION

The artifacts with a molecular weight of $[M+12]^+$ were obviously all due to the addition of formaldehyde to the drugs. Formaldehyde is probably formed by thermal dehydrogenation of methanol in the injection port of the GC.

$$CH_3OH \xrightarrow[-H_2]{295°C} CH_2O$$

Formaldehyde may react with amphetamine, flecainide, propafenone, β-blockers and prilocaine in different ways. From amphetamine, which is a primary amine, a Schiff's base is formed (Fig. 3). A similar artifact has been observed from amantadine [5]. It should be noted that many drugs with a dimethyl or a diethylamino group are metabolized to primary amines, which may react in a similar way with formaldehyde. Addition of formaldehyde to flecainide leads to formation of a six-membered ring. In general, formation of a five- or six-membered ring is a thermodynamically favoured reaction. Five-membered tetrahydroxazole rings were formed by the addition of formaldehyde to β-blockers and propafenone. Addition of formaldehyde to prilocaine yielded a methylene group linking the two nitrogens (Fig. 3). No evidence was found for the addition of two methyl groups and loss of two water molecules, which has been postulated by other authors [1]. The labelling experiments with deuterium, the high-resolution mass spectra, the fact that the artifacts are quantitatively formed by the reaction of formaldehyde with the β-blockers and the NMR spectra of the artifacts support the structure suggested in Fig. 3. No artifact formation was observed when diethyl ether or ethyl acetate was used as the solvent.

The formation of artifacts in the GC–MS screening procedure can be viewed in two ways. One aspect is that the formation of artifacts may complicate the interpretation process and might lead to misinterpretations. The formation of artifacts can easily be eliminated by using solvents other than methanol, such as diethyl ether or ethyl acetate. However, these solvents do not have the universal solubility properties of methanol. The other aspect is that formation of artifacts may yield additional information for the identification of certain drug in a blood or urine extract. Furthermore, the almost complete yield of artifact formation

from most β-blockers makes reaction of the drugs with formaldehyde a suitable derivatization technique for β-blocker assays with GC or GC–MS.

Nevertheless, it should be emphasized that careful interpretation of GC–MS results is required, especially with respect to artifact formation. Despite the support from computer-aided interpretation of mass spectra, the careful interpretation of mass spectra is still a challenge to the analytical toxicologist, especially when performing emergency analysis.

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