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IDENTIFICATION AND DIFFERENTIATION OF ALKYLAMINE ANTIHISTAMINES AND THEIR METABOLITES IN URINE BY COMPUTERIZED GAS CHROMATOGRAPHY–MASS SPECTROMETRY*

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SUMMARY

A gas chromatographic–mass spectrometric screening procedure is described for the identification and differentiation of the following alkylamine antihistamines and their metabolites in urine: azatadine, benzquinamide, brompheniramine, chlorphenamine, (clofedanol), cyproheptadine, dimetindene, ketotifen, mebhydroline, phenindamine, pheniramine, pyrrobutamine, terfenadine and tolpropamine. After acid hydrolysis of the conjugates, extraction and acetylation, the urine samples were analysed by computerized gas chromatography–mass spectrometry. Using ion chromatography with the selective ions *m/z* 58, 169, 203, 205, 230, 233, 262 and 337, the presence of alkylamine antihistamines and/or their metabolites was indicated. The identity of positive signals in the reconstructed ion chromatograms was confirmed by a visual or computerized comparison of the stored full mass spectra with the reference spectra. The ion chromatogams, reference mass spectra and gas chromatographic retention indices (OV-101) are documented. The procedure presented is integrated in a general screening procedure (general unknown analysis) for several groups of drugs.

INTRODUCTION

The antihistamines (histamine H_1-receptor antagonists) are one of the largest groups of drugs, usually classified into alkanolamine, alkylamine, ethylenediamine, piperazine and phenothiazine derivatives. They are widely used as antiallergics, antiemetics and hypnotics. Among the alkylamine antihistamines, cyproheptadine also has antiserotoninergic properties. It is used as an appetite stimulant. Ketotifen is a selective mast-cell stabilizer used as an antiasthmatic. Alkylamine antihistamines may cause, alone or in combination with other drugs

*Part of these results was reported at the "Symposium Klinisch-Toxikologische Analytik" of the Austrian and German Societies of Clinical Chemistry, Salzburg, September 14–16, 1987 [1].

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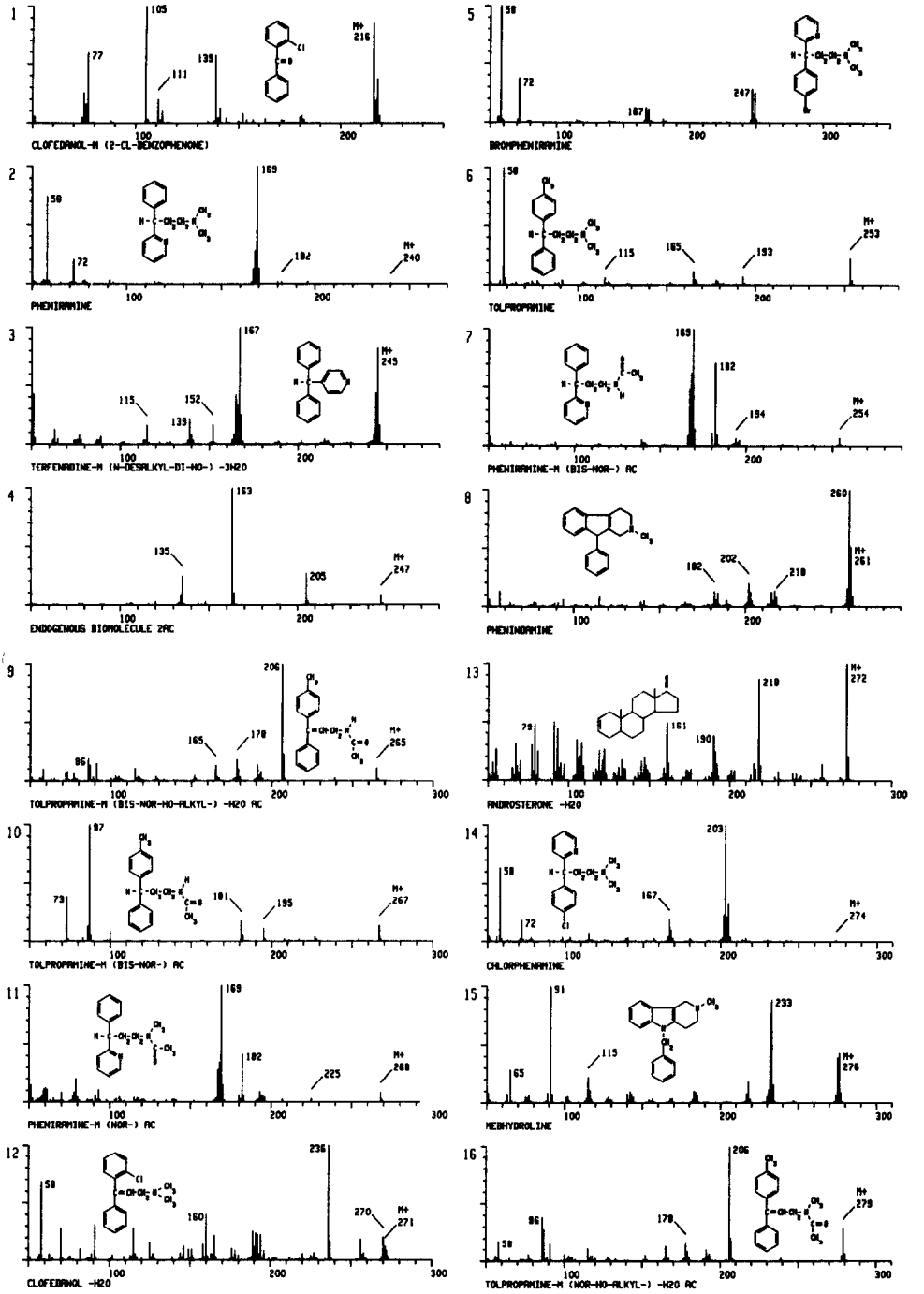
1
105
77
111
139
M+
216
CLOFEDANOL-H (2-CL-BENZOPHENONE)
5
58
72
167
247
BROMPHENIRAMINE
2
58
169
72
182
M+
240
PHENIRAMINE
6
58
115
185
193
M+
253
TOLPROPAMINE
3
167
115
152
139
M+
245
TERFENADINE-H (N-DESALKYL-DI-HO-) -3H2O
7
169
182
194
M+
254
PHENIRAMINE-H (BIS-NOR-) AC
4
163
135
205
M+
247
ENDOGENOUS BIOMOLECULE 2AC
8
260
M+
261
182
202
218
PHENINDAMINE
9
206
165
178
86
M+
265
TOLPROPAMINE-H (BIS-NOR-HO-ALKYL-) -H2O AC
13
M+
272
210
79
161
190
ANDROSTERONE -H2O
10
97
73
181
195
M+
267
TOLPROPAMINE-H (BIS-NOR-) AC
14
203
58
72
167
M+
274
CHLORPHENAMINE
11
169
182
225
M+
268
PHENIRAMINE-H (NOR-) AC
15
91
233
65
115
M+
276
MEBHYDROLINE
12
58
236
160
270
M+
271
CLOFEDANOL -H2O
16
206
86
178
58
M+
279
TOLPROPAMINE-H (NOR-HO-ALKYL-) -H2O AC

Fig. 1.

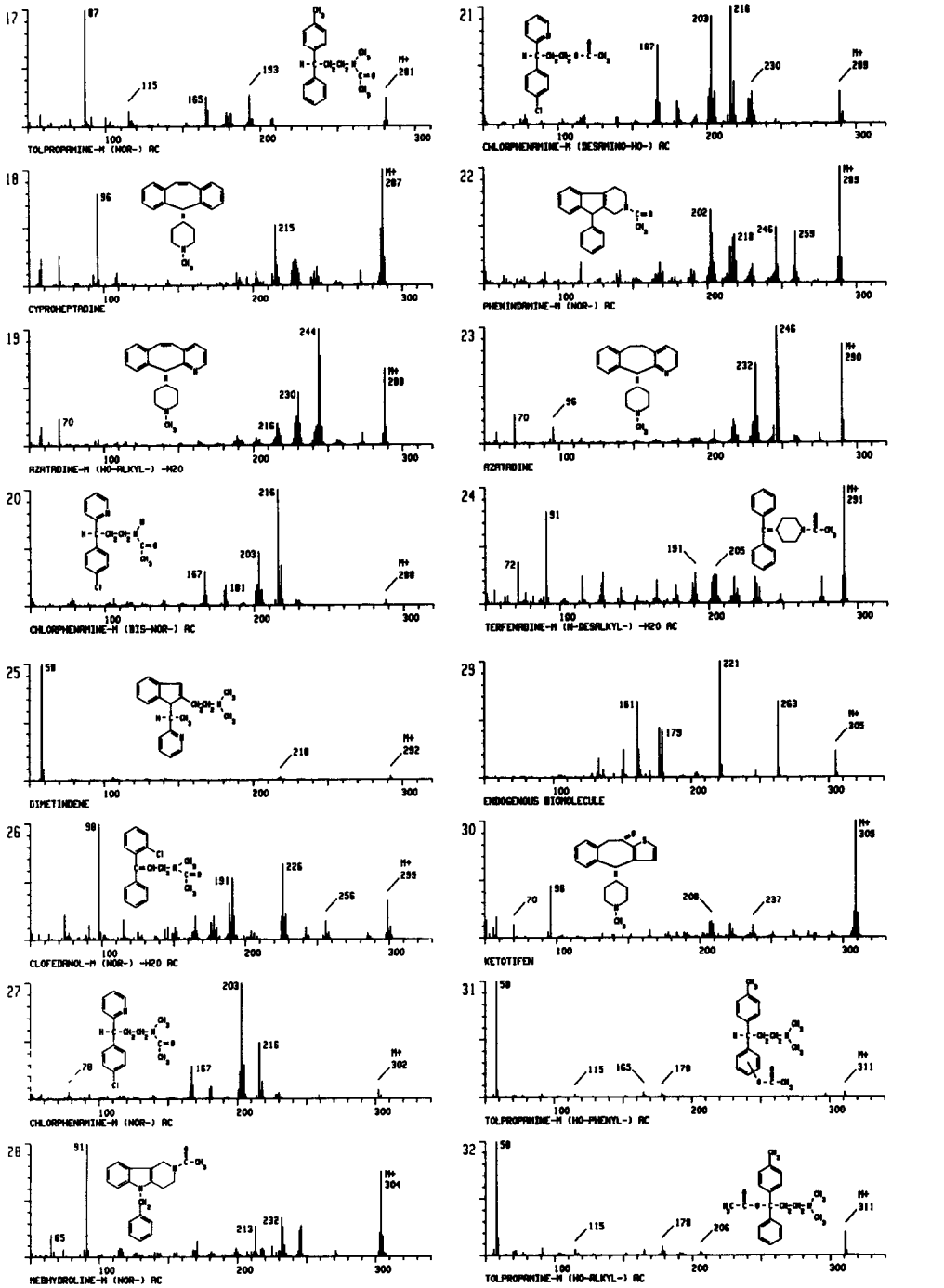

Fig. 1.

(Continued on p. 34)

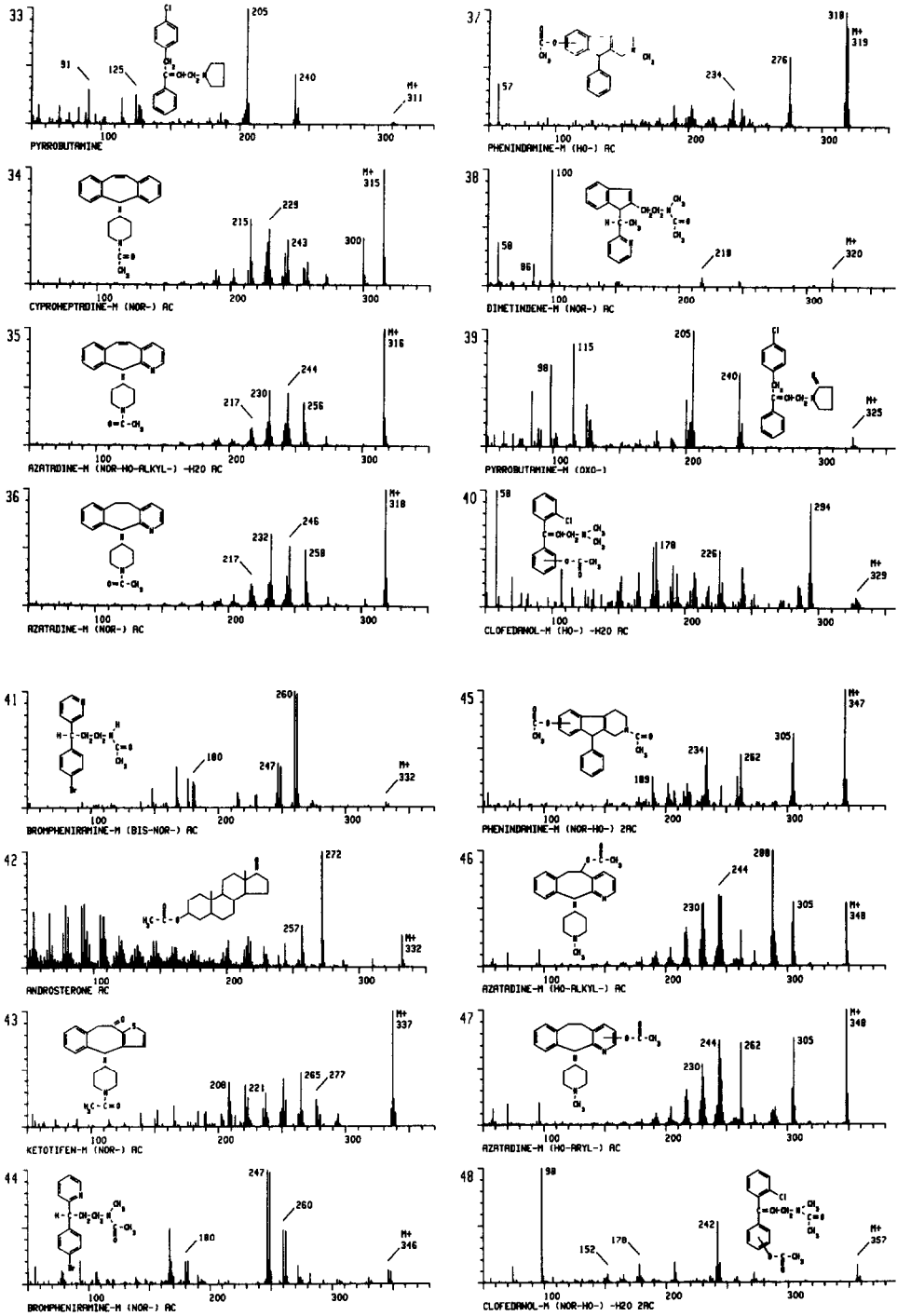
33
PYRROBUTAMINE
34
CYPROHEPTADINE-M (NOR-) AC
35
AZATADINE-M (NOR-HO-ALKYL-) -H2O AC
36
AZATADINE-M (NOR-) AC
37
PHENINDAMINE-M (HO-) AC
38
DIMETINDENE-M (NOR-) AC
39
PYRROBUTAMINE-M (OXO-)
40
CLOFEDANOL-M (HO-) -H2O AC
41
BROMPHENIRAMINE-M (BIS-NOR-) AC
42
ANDROSTERONE AC
43
KETOTIFEN-M (NOR-) AC
44
BROMPHENIRAMINE-M (NOR-) AC
45
PHENINDAMINE-M (NOR-HO-) 2AC
46
AZATADINE-M (HO-ALKYL-) AC
47
AZATADINE-M (HO-ARYL-) AC
48
CLOFEDANOL-M (NOR-HO-) -H2O 2AC

Fig. 1.

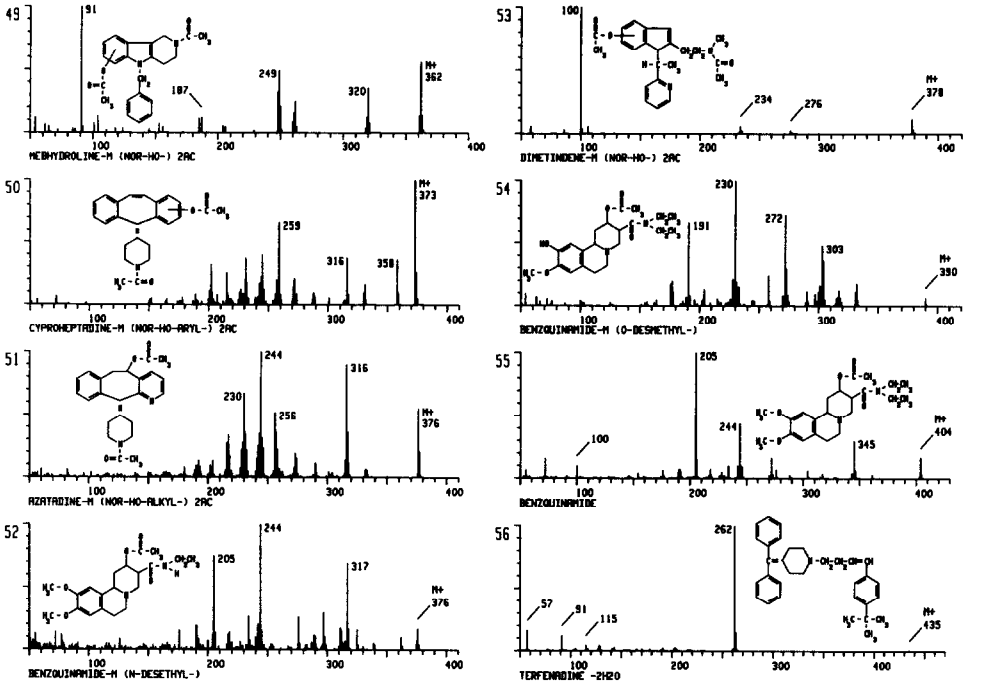

Fig. 1. Mass spectra of alkylamine antihistamines and their metabolites identified in urine after acid hydrolysis, extraction and acetylation.

and/or ethanol, an intoxication for which treatment is necessary [2, 3]. Because alkylamine antihistamines used in ointments can be absorbed transdermally [4, 5], intoxications may occur, if large areas of skin, especially of children, have been embrocated. Fitness to drive a car may be impaired even after therapeutic doses [6, 7]. For all these reasons, alkylamine antihistamines may be encountered in clinical or forensic toxicological analysis. Before quantification in plasma, the drugs, which are usually unknown, must first be identified. The detection of some of the alkylamine antihistamines using spectrophotometry [8–10], paper chromatogrpahy [11], thin-layer chromatography [12, 13], gas chromatography [9, 14–16], high-performance liquid chromatography [17] and gas chromatography–mass spectrometry (GC–MS) [16, 18–20] has been described. However, none of these procedures allows the rapid and specific identification and differentiation of all alkylamine antihistamines.

This paper describes a computerized GC–MS screening procedure for the identification and differentiation of alkylamine antihistamines and their metabolites in urine. Urine was used because the concentrations of alkylamine antihistamines and their metabolites are higher in urine than in plasma. Because some of the alkylamine antihistamines are excreted in urine in a completely metabolized and conjugated form in the later phase of excretion, the conjugates were cleaved by acid hydrolysis, which can be completed more quickly than enzymatic hydrolysis. This method has the advantage that it can be integrated in an existing

general screening procedure (general unknown analysis) for the detection of several categories of drugs [21–32].

EXPERIMENTAL

Apparatus

A Hewlett-Packard (HP) Series 5890 gas chromatograph combined with an HP MSD Series 5970 mass spectrometer and an HP Series 59970 C workstation were used. The GC conditions were as follows: splitless injection mode; column, HP capillary (12 m×0.2 mm I.D.), cross-linked methylsilicone, 0.33 μm film thickness; column temperature, programmed from 100°C to 310°C at 30°C/min, initial time 3 min, final time 5 min; injection port temperature, 270°C; carrier gas, helium; flow-rate, 1 ml/min.

The MS conditions were as follows: scan mode; ionization energy, 70 eV; ion-source temperature, 220°C; capillary direct interface heated at 260°C.

Exact measurement of retention indices was performed on a Varian Series 3700 gas chromatograph. The column effluent went to a flame ionization detector and a nitrogen-sensitive flame ionization detector after a 1:1 split by a splitter made from nickel tubing. The column was a steel tube (60 cm×2 mm I.D.) packed with Chromosorb G HP (100–120 mesh) coated with 5% OV-101. The column and injector temperatures were identical with those used for GC–MS, and the temperature of the detectors was 270°C. Nitrogen was used as carrier gas at a flow-rate of 30 ml/min.

Urine samples

The investigations were performed on urine from in-patients treated with therapeutic doses of alkylamine antihistamines. When suitable samples from humans were not available, urine from rats was used (see Species column in Table I). Rats were administered 50 mg/kg body weight of drugs in aqueous suspension by gastric intubation. Urine was collected separately from faeces [33].

Hydrolysis and extraction procedure

A 10-ml volume of urine was refluxed with 3 ml of 37% hydrochloric acid for 15 min. Following hydrolysis, ca. 3 g of potassium hydroxide pellets were added and the resulting solution was mixed with 10 ml of 30% aqueous ammonium sulphate to obtain a pH between 8 and 9. This solution was extracted with a 10-ml portion of dichloromethane–2-propanol–ethyl acetate (1:1:3). Phase separation was accomplished by centrifugation. The organic extract was transferred to a pear-shaped flask and evaporated to dryness under vacuum.

Acetylation

The extracted residue was acetylated for 30 min at 60°C with 100 μl of a mixture of three parts of acetic acid anhydride and two parts of pyridine. The acetylation mixture was then evaporated to dryness and the resultant residue was dissolved in 100 μl of methanol. A 0.5–2 μl volume of this sample was injected into the gas chromatograph.

GC–MS analysis

Full mass spectra were recorded at a speed of 1 scan/s and stored on a hard disk during the temperature-programmed GC analysis. The identity of positive signals in the reconstructed ion chromatograms was confirmed by a visual comparison of the full mass spectra with reference spectra (Fig. 1) or by a computer library search [34].

RESULTS AND DISCUSSION

The results of our studies are summarized in Table I. Using ion chromatography with the eight proposed ions, the possible presence of alkylamine antihistamines and/or their metabolites in urine could be indicated selectively. Complete or partial dehydration ($-H_2O$ in Table I and Fig. 1) for alcoholic hydroxy groups was observed. Because these artifacts were formed during acid hydrolysis, the corresponding intact molecules were found after enzymatic hydrolysis. The data of the antitussive clofedanol were included because of its structural similarity to these antihistamines. Most of the compounds are derivatized by acetylation (see Derivative column). The mass spectra numbers in Fig. 1, the molecular masses, the species from which the urine was assayed and the GC retention indices are given. These indices were determined using temperature-programmed GC combined with flame ionization detection and nitrogen-sensitive flame ionization detection. In our experience, retention indices provide preliminary indications of the possible presence of the compounds and may be useful to workers without a GC–MS facility.

Data are given for only those metabolites that were frequently found. Not all the listed metabolites were detected in each sample owing to inter-species (human/rat) or inter-individual differences in metabolism, or variable elapsed time after administration. The mass spectra and retention indices of the less abundant metabolites will be included in a forthcoming handbook and computer library [34, 35].

The data for androsterone, its dehydrated artifact and two unidentified endogenous biomolecules are included because these physiological compounds can be indicated by the ion chromatograms.

The full mass spectra for the specific identification of the selectively indicated compounds are shown in Fig. 1. They are listed primarily in order of ascending mass of the highest ion. For the same nominal mass value, the spectra are arranged in order of ascending retention indices. Formulae are proposed for probable metabolite structures.

Interferences by other drugs are improbable because the identity of the peaks observed in the ion chromatograms can be positively confirmed by a visual comparison of the underlying mass spectrum with reference spectra (Fig. 1 and ref. 35) or by a computer library search [34]. The mass spectrum of 2-chlorobenzophenone, a metabolite of clofedanol, is almost identical with that of 4-chlorobenzophenone, a common metabolite of the piperazine antihistamines buclizine, etodroxizine, hydroxyzine and meclozine [32]. However, the detection of the parent drugs and further unique metabolites allowed a differentiation.

TABLE I

MONITORING PROGRAMME FOR THE DETECTION OF ALKYLAMINE ANTIHISTAMINES AND THEIR METABOLITES IN URINE AFTER ACID HYDROLYSIS AND ACETYLATION

MS No.	Mol. mass	Drug/metabolite (M)	Derivative*	Species	*m/z* (relative intensities)**								Retention index
					58	169	203	205	230	233	262	337	
23	290	Azatadine		Rat	+		+	+	+	+			2375
19	288	M (hydroxyalkyl-) – H_2O		Rat	+		+		+				2410
46	348	M (hydroxyalkyl-)	AC	Rat	+		+	+	++	+	+		2520
47	348	M (hydroxyaryl-)	AC	Rat	+		+	+	++	+	++		2540
36	318	M (nor-)	AC	Rat			+	+	+	+			2720
35	316	M (norhydroxyalkyl-) – H_2O	AC	Rat			+	+	+	+			2750
51	376	M (norhydroxyalkyl-)	AC	Rat			+	+	++	+			2810
55	404	Benzquinamide		Rat	+			+++	+	+			2980
52	376	M (N-desethyl-)		Rat				++					2960
54	390	M (O-desmethyl-)		Rat			+		+++	+			2990
05	318	Brompheniramine		Man	+++	+							2105
41	332	M (bis-nor-)	AC	Man		+			+		+++		2170
44	346	M (nor-)	AC	Man		+					++		2195
14	274	Chlorphen(ir)amine		Man	++	+	+++	+					2020
21	289	M (desaminohydroxy-)	AC	Man			++	+	+				2130
27	302	M (nor-)	AC	Man			+++	+					2530
20	288	M (bis-nor-)	AC	Man			+	+					2535
12	271	Clofedanol – H_2O		Rat	++								2085
01	216	M (2-chlorobenzophenone)		Rat									1720 FID
40	329	M (hydroxy-) – H_2O	AC	Rat	+++		+						2370
26	299	M (nor-) – H_2O	AC	Rat									2400
48	357	M (norhydroxy-) – H_2O	AC	Rat				+					2800
18	287	Cyproheptadine		Man	+		+	+	+				2340
34	315	M (nor-)	AC	Man			+		+				2920
50	373	M (norhydroxy-)	AC	Man			+			+			3060

25	292	Dimetindene		Man	+++								2290
38	320	M (nor-)	AC	Man	+								2775
53	378	M (norhydoxy-)	AC	Man	+					+			3090
30	309	Ketotifen		Man	+					+			2600
43	337	M (nor-)	AC	Man			+					+++	3180
15	276	Mebhydroline		Rat					+	+++			2445
28	304	M (nor-)	AC	Rat					+	+			2820
49	362	M (norhydroxy-)	AC	Rat							+		3130
08	261	Phenindamine		Rat			+				+		2180
37	319	M (hydroxy-)	AC	Rat			+	+	+	+			2580
22	289	M (nor-)	AC	Rat			+		+				2640
45	347	M (norhydroxy-)	AC	Rat			+	+	+	+			3000
02	240	Pheniramine		Man	++	+++							1805
07	254	M (bis-nor-)	AC	Man		+++							2210
11	268	M (nor-)	AC	Man		+++							2250
33	311	Pyrrobutamine		Rat			+	+++					2370
39	325	M (oxo-)		Rat			+	+++					2920
56	435	Terfenadine $-2H_2O$		Man							+++		3460
03	245	M (N-desalkyldihydroxy-) $-3H_2O$		Man		+	+		+				2190
24	291	M (N-desalkyl-) $-H_2O$	AC	Man			+	+		+			2550
06	253	Tolpropamine		Rat	+++								1900
31	311	M (hydoxyphenyl-)	AC	Rat	+++								2230
32	311	M (hydroxyalkyl-)	AC	Rat	+++			+					2250
10	267	M (bis-nor-)	AC	Rat									2340
17	281	M (nor-)	AC	Rat	+								2360
09	265	M (bis-norhydroxyalkyl-) $-H_2O$	AC	Rat	+								2560
16	279	M (norhydroxyalkyl-) $-H_2O$	AC	Rat	+								2585
42	332	Androsterone	AC	Man			+		+				2580 FID
13	272	Androsterone $-H_2O$		Man			+		+				2240 FID
04	247	Endogenous biomolecule	AC	Man				+					1920
29	305	Endogenous biomolecule	AC	Man				+					1950

*AC = acetylated.

**+++ = >95% relative intensity; ++ = 50–95%; + = <50%.

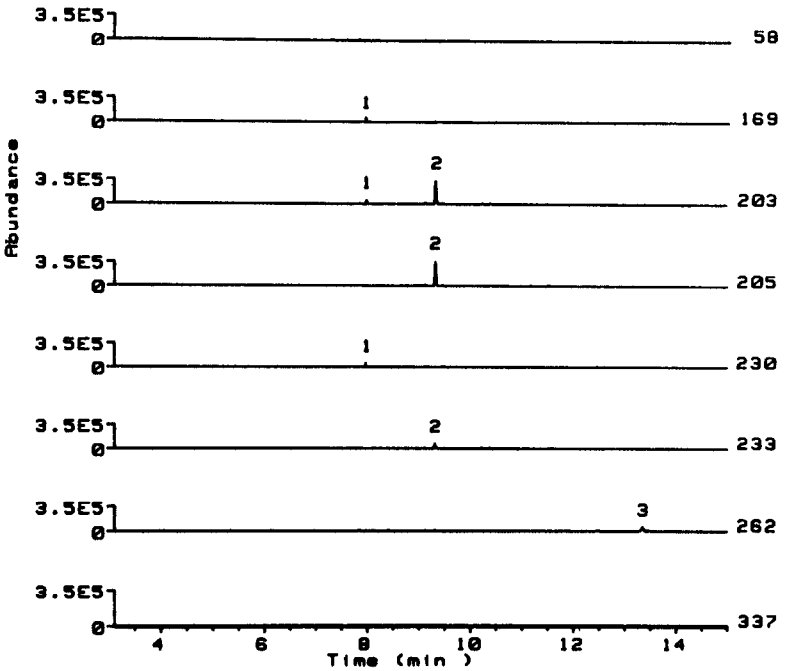

Fig. 2. Ion chromatograms indicating the dehydrated N-desalkyldihydroxy metabolite of terfenadine (1), the dehydrated and acetylated N-desalkyl metabolite (2) and dehydrated terfenadine (3) (mass spectra Nos. 3, 24 and 56 in Fig. 1).

The sensitivity of the method is sufficient to detect therapeutic concentrations of at least those drugs that were studied in human urine.

To illustrate the method, ion chromatograms from the urine of a patient treated with alkylamine antihistamines is shown in Fig. 2. Peak 1 indicates the dehydrated N-desalkyldihydroxy metabolite of terfenadine, peak 2 the dehydrated and acetylated N-desalkyl metabolite and peak 3 dehydrated terfenadine (mass spectra Nos. 3, 24 and 56 in Fig. 1).

CONCLUSIONS

The procedure presented allows the identification and differentiation of alkylamine antihistamines and their metabolites in urine to be integrated into a general screening procedure for the detection of several categories of drugs [21–32]. The identified drugs can then be quantified in plasma using methods described by Bruce et al. [36], Dieckmann et al. [37], Cailleux et al. [16] and Jane et al. [17], and reviewed by Paton and Webster [38].

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