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IDENTIFICATION AND DIFFERENTIATION OF BETA-BLOCKERS AND THEIR METABOLITES IN URINE BY COMPUTERIZED GAS CHROMATOGRAPHY—MASS SPECTROMETRY*

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SUMMARY

A method for the identification and differentiation of beta-blockers and their metabolites in urine after acid hydrolysis is described. The acetylated extract is analysed by computerized gas chromatography—mass spectrometry. An on-line computer allows rapid detection using ion chromatography with the ions of *m/z* 72, 86, 98, 140, 151, 159, 200 and 335. The identity of positive signals in the reconstructed ion chromatogram is confirmed by a comparison of the stored entire mass spectra with the reference spectra The ion chromatogram, the reference mass spectra and the gas chromatographic retention indices (OV-101) are documented References for the quantitation of the single beta-blockers are cited.

INTRODUCTION

In the last few years, more and more beta-blockers have been made commercially available. Because of their widespread application, these drugs are frequently encountered in clinical and forensic toxicological analysis. Numerous methods for the quantitation of single beta-blockers have been published (see References column in Table I). However, in analytical toxicology, usually unknown drugs or poisons must be identified before quantitation in plasma. Because the concentrations in urine are higher than those in plasma and metabolites can in addition be detected, it is preferable to use urine for the identification. The detection of some beta-blockers in urine using thin-layer chromatography [2, 3] or gas chromatography [4] has been described. None of these

*These results were reported in part at the Jahrestagung Klinische Chemie, Mannheim, F.R.G., September 25—28, 1985 [1] Dedicated to Prof. Dr. Joachim Knabe, Saarbrücken, on the occasion of his 65th birthday.

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methods allows the rapid and selective identification and differentiation of all beta-blockers and their metabolites, which is the prerequisite of quantitation. Further, all chromatographic zones or peaks have to be identified because any of them may represent a potential poison. These demands are met by the computerized gas chromatographic—mass spectrometric—data system (GC—MS—DS) technique described here. This method has the further advantage that several other categories of drugs can be detected simultaneously by simply searching for typical fragment ions in the stored spectra.

EXPERIMENTAL

Apparatus

A Hewlett-Packard (HP) Series 5790 A gas chromatograph combined with an HP MSD Series 5970 A mass spectrometer and an HP Series 59970 A workstation was used. The GC conditions were as follows: column, HP capillary (12 m × 0.2 mm I.D.), cross-linked methylsilicone, 0.33 μm film thickness; column temperature, programmed from 100 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, 2 ml/min.

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

For the exact measurement of retention indices a Varian Series 3700 gas chromatograph was used. The column effluent passed 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 as for GC—MS and the temperature of the detectors was 270°C. The carrier gas was nitrogen at a flow-rate of 30 ml/min.

Urine samples

The investigations were carried out on urine from in-patients treated with therapeutic dosages of beta-blockers. If suitable human samples were not available, urine from rats was used. Rats were administered 20—80 mg/kg body weight of drugs in aqueous suspension by gastric tube (see Species column in Table I).

Hydrolysis and extraction procedure

A 10-ml volume of urine was refluxed with 3 ml of 37% hydrochloric acid for 15 min, then basified with about 3 g of potassium hydroxide pellets and mixed with 10 ml of 30% aqueous ammonium sulphate to give a pH between 8 and 9. These solutions were extracted twice with 10-ml portions of dichloromethane- isopropanol—ethyl acetate (1:1:3). After phase separation by centrifugation the combined organic extracts were evaporated to dryness under vacuum and the residue was dissolved in 0.1 ml of methanol.

Acetylation

A 40-μl aliquot of extract was evaporated and then acetylated for 30 min

at 60°C with 40 μl of a mixture of three parts of acetic anhydride and two parts of pyridine. After evaporation of the acetylation mixture the residue was dissolved in 40 μl of methanol. A 0.5—2 μl volume of this sample was injected into the gas chromatograph.

Gas chromatographic—mass spectrometric analysis

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 chromatogram was confirmed by comparison of the entire mass spectra with reference spectra (Fig. 1).

RESULTS AND DISCUSSION

Some of the beta-blockers were excreted in urine in a completely metabolized and conjugated form. Therefore, the conjugates were cleaved by acid hydrolysis, which can be completed more quickly than enzymatic hydrolysis. To improve their GC characteristics hydroxy and amino groups were acetylated.

The results of the investigations are shown in Table I. The ion chromatogram with the eight proposed ions allows the detection of 23 beta-blockers and their metabolites. The empirical formulae of the ion fragments are as follows: $C_4H_{10}N$ for the ion of m/z 72, $C_5H_{12}N$ for 86, $C_6H_{12}N$ for 98, $C_8H_{14}NO$ for 140, $C_8H_9NO_2$ for 151, $C_7H_{11}O_4$ for 159 and $C_{10}H_{18}NO_3$ for 200. The empirical formula of the ion of m/z 335 is different for the different compounds. Most of these compounds are acetylated (see formulae in Fig. 1). Atenolol, carazolol, mepindolol and pindolol are completely decomposed by acid hydrolysis, so they must be identified in a direct extract of urine [5]. The data for glucose, which is the only endogenous biomolecule that was indicated in the ion chromatogram, are included. Because oxprenolol has a common metabolite with guaifenesin (mass spectrum No. 30 in Fig. 1), the data for guaifenesin itself are added for the differentiation. The mass spectra numbers in Fig. 1 and the species from which the urine was assayed are given. The molecular masses are included because they are helpful in verifying the metabolite data if the molecular ion is not observable in the spectrum (mass spectra Nos. 3, 9, 14, 22, 25, 34, 36, 38, 44, 49, 53, 56 and 60).

The retention indices were determined using gas chromatography combined with flame ionization detection and nitrogen-sensitive flame ionization detection with temperature programming. In our experience, retention indices provide preliminary indications and may be useful to workers without a GC—MS facility and hence they are included in Table I. Further, they allow one to distinguish between isomeric hydroxy metabolites, which give very similar mass spectra. For the quantitation of the beta-blockers identified in urine, publications that describe suitable methods for the determination of plasma levels using fluorimetry [6, 7], thin-layer chromatography [8—10], GC [11—21], GC—MS [22—28], high-performance liquid chromatography [29—65], radioreceptor assay [66, 67] or radioimmunoassay [68—70] are cited in the last column.

The entire mass spectra are shown in Fig. 1 for the selective identification

TABLE I

MONITORING PROGRAMME FOR BETA-BLOCKERS AND THEIR METABOLITES

Mass spectrum No.	Mass	Drug/metabolite (M)	Species	*m/z* (relative intensities)*								Retention index	References
				72	86	98	140	151	159	200	335		
02	235	Acebutolol hydrolysed	Man					+++				1875	8, 29, 30
37	333	Alprenolol	Man	+		+	+			+++		2275	11, 31
23	292	M (desaminohydroxy-)	Rat						+++			1850 FID	
51	352	M (desaminohydroxy-) + H20	Rat						+++			2100 FID	
50	350	M (desaminodihydroxy-)	Rat						+++			2220 FID	
68	410	M (desaminodihydroxy-) + H20	Rat						+++			2450 FID	
63	391	M (hydroxy-)	Man	+		+	+			+++		2575	
09	266	Atenolol**	Man	+++								2380	9, 12, 29, 66,
16	278	Artifact	Man	+++	++	+						2400	13, 32, 33
36	391	Betaxolol	Rat	+		+	+			+++		2680	14, 22, 34
33	319	M (O-desalkyl-) — H20	Rat	+		+++	++		+			2570	
34	379	M (O-desalkyl-)	Rat	++		+	++		+	+++		2620	
14	290	Bunitrolol	Rat	+	+++	+						2070	35
15	276	M (desisobutyl-)	Rat	+	+++	+						2040	
38	348	M (hydroxy-)	Rat	+	+++	+						2300	
56	378	M (hydroxymethoxy-)	Rat		+++	+						2480	
25	313	Bupranolol	Man		+++	+						2370	36
53	371	M (hydroxy-)	Man	+	+++	+						2260	
26	298	Carazolol**	Rat	+								2810	6, 68
31	310	Artifact	Rat	+	+	+						2830	
41	334	Carteolol	Man		+++	+					+	2700	37, 69
64	392	M (hydroxy-)	Man		+++	+					+	2800	

60	454	Labetalol	Man								+++	3400		38—40
01	191	Artifact	Man	+	+							1780		
07	249	M (hydroxy-) isomer I artifact	Man	+	++	+						1940		
08	249	M (hydroxy-) isomer II artifact	Man	+	+							2000		
39	333	Levobunolol	Man	+	+++	+				+		2460		41
32	315	—H20	Man			+				+		2570		
11	262	Mepindolol**	Rat	+								2390		42
13	274	Artifact	Rat	+	+	+						2410		
65	393	Metipranolol + M (desacetyl-)	Rat	+		++	++	+		+++		2670		23
52	352	M (desaminohydroxy-)	Rat					+	+++			2240	FID	
40	333	M (desacetyl-) — H20	Rat	+		++	+++	+				2660		
22	351	Metoprolol	Man	+++		+	+			++		2480		15, 16, 24, 29,
21	291	—H20	Man	+++		+	+		+			2330		31—33, 43—45
34	379	M (O-desmethyl-)	Man	++		+	++		+	+++		2620		
49	409	M (hydroxyethyl-)	Man	++		+	++		+	+++		2730		
70	435	Nadolol	Man	+	+++	+						2650		10, 25, 46—49
29	308	Nifenalol	Rat	+++								2305		
06	248	—H20	Rat	+++								2265		
48	349	Oxprenolol	Man	+++		+	+			+++		2390		17, 18, 29, 50, 66
28	308	M (desaminohydroxy-)	Man						+++			1900	FID	
30	310	M (desaminohydroxydesalkyl-)	Man						+++			1920	FID	
20	289	—H20	Man	+++		+	++					2260		
46	347	M (hydroxy-) — H20 isomer I	Man	+++		+	+		+	++		2520		
47	347	M (hydroxy-) — H20 isomer II	Man	+++		+	+		+	+		2570		
66	407	M (hydroxy-) isomer I	Man	+++		+	+					3050		
67	407	M (hydroxy-) isomer II	Man	+++		+	+			++		3100		
59	375	Penbutolol	Man	++	+	+				+		2205		51, 52
62	391	M (hydroxy-)	Man		++	+						2520		
72	449	M (dihydroxy-)	Man		+++	+						2890		

(Continued on p. 152)

TABLE I (continued)

Mass spectrum No.	Mass	Drug/metabolite (M)	Species	*m/z* (relative intensities)*								Retention index	References
				72	86	98	140	151	159	200	335		
05	248	Pindolol**	Rat	+++								2240	19, 53, 54
10	260	Artifact	Rat	+	++	+						2250	
58	365	Propafenone — H20	Man	+		+	+++					2930	55—57
61	384	M (desaminohydroxy-)	Man			+			+++			2715 FID	
71	442	M (desaminodihydroxy-)	Man	+					++			2950 FID	
69	423	M (hydroxy-) — H20	Man	++		++	++					3050	
18	283	Propranolol — H20	Man	++		++	+++		+			2330	20, 26, 29, 32, 47,
27	302	M (desaminohydroxy-)	Man						+++			2195 FID	58—61, 66, 67, 70
55	360	M (desaminodihydroxy-)	Man	+		+			+++			2565 FID	
42	341	M (hydroxy-) — H20 isomer I	Man	+		++	+++		+			2750	
43	341	M (hydroxy-) — H20 isomer II	Man	+		++	+++		+			2900	
44	401	M (hydroxy-)	Man	+		++	+++		+	+		2940	
24	296	Sotalol — H20	Man	+					+			2675	29, 62, 63
54	358	Timolol	Rat	+	++	+						2290	21, 27, 38, 64—66
19	284	M (desisobutyl-) — H20	Rat	+	+	++						2205	
45	344	M (desisobutyl-)	Rat	+		+			+			2620	
04	247	Toliprolol — H20	Rat	+++		+	+			++		2230	7
12	266	M (desaminohydroxy-)	Rat						+++			1820 FID	
35	324	M (desaminodihydroxy-)	Rat						+++			2200 FID	
57	365	M (hydroxy-)	Rat	+		+	+			+++		2550	
03	390	Glucose	Man		+	+++	+			+		2010 FID	
17	282	Guaifenesin	Man						+++			1865 FID	

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

**These beta-blockers can only be detected in a direct extract of urine [5].

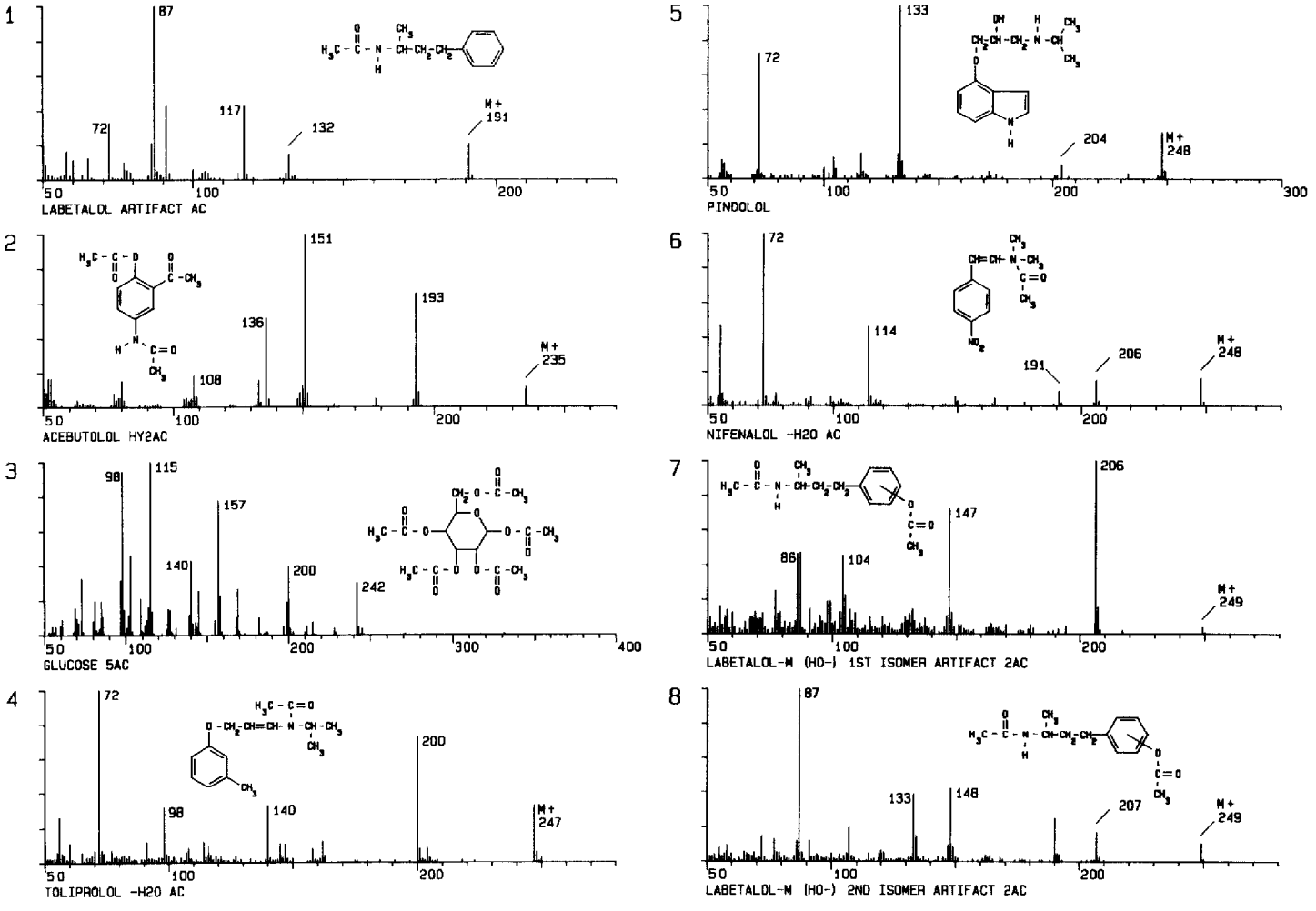
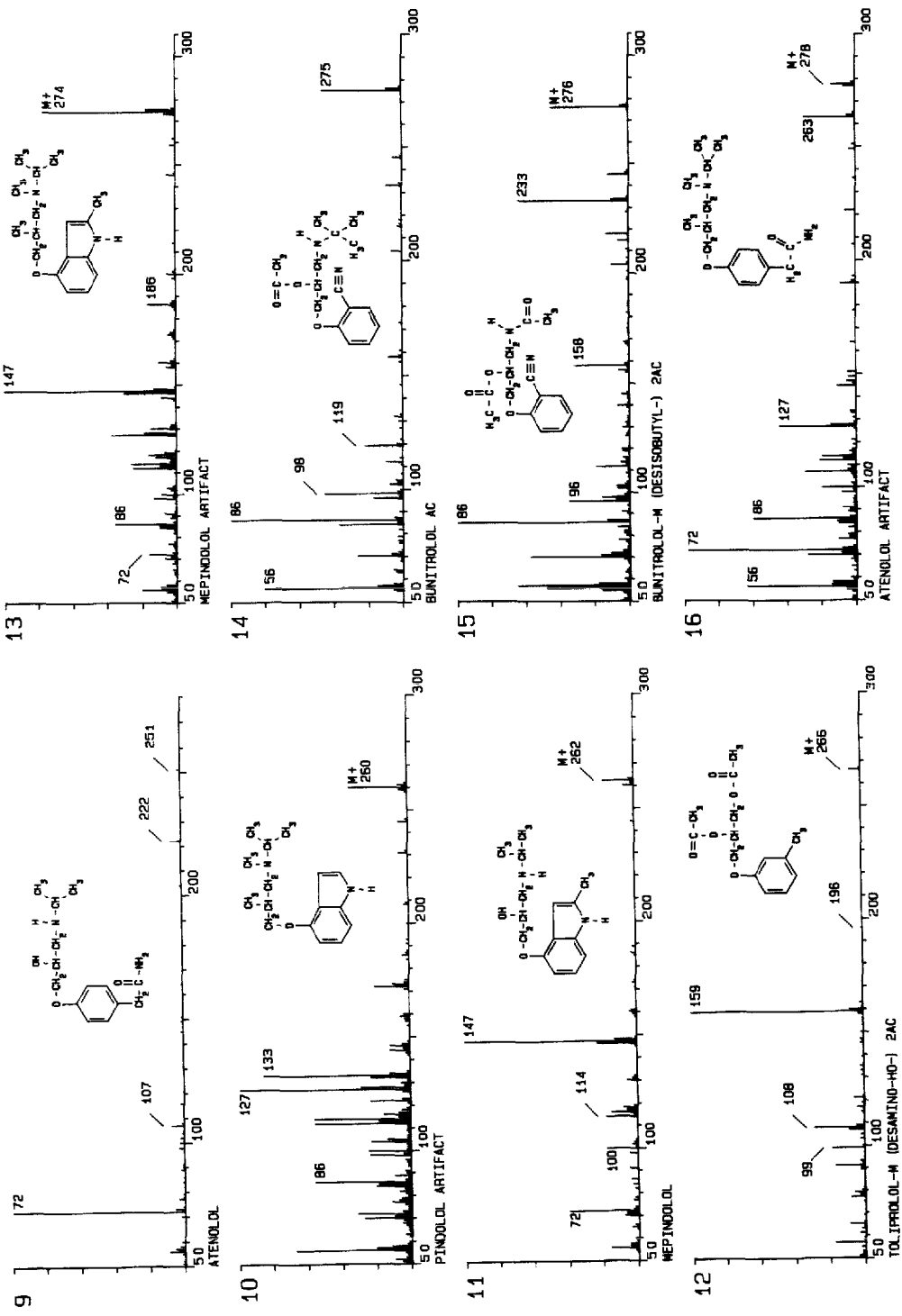


Fig 1

(Continued on p 154)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ATENOLOL
10
PINODOLOL ARTIFACT
11
MEPINDOLOL
12
TOLIPROLOL-M (DESAMINO-HO-) 2AC
13
MEPINDOLOL ARTIFACT
14
BUNITROLOL AC
15
BUNITROLOL-M (DESISOBUTYL-) 2AC
16
ATENOLOL ARTIFACT

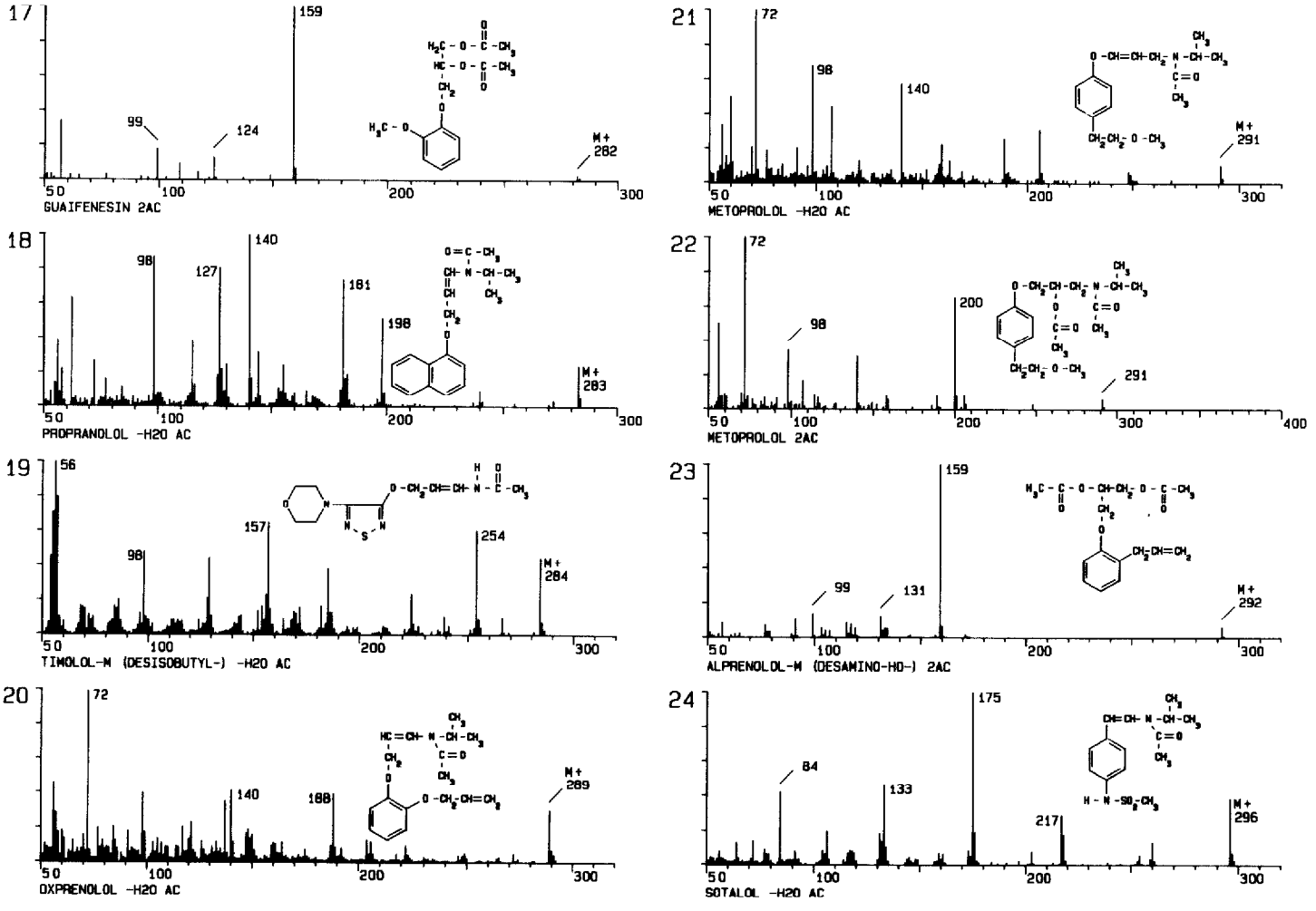
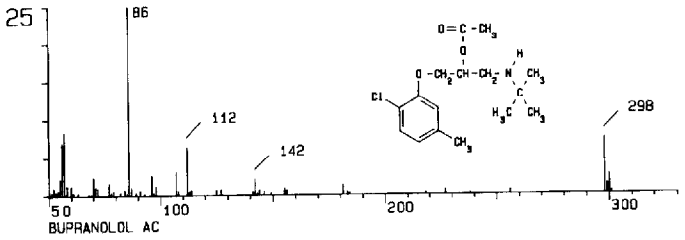


Fig. 1.

(Continued on p. 156)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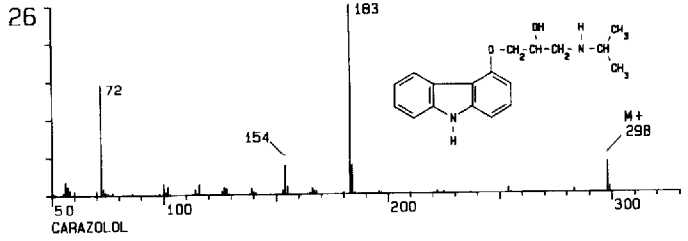
112
142
298
50
100
200
300
BUPRANOLOL AC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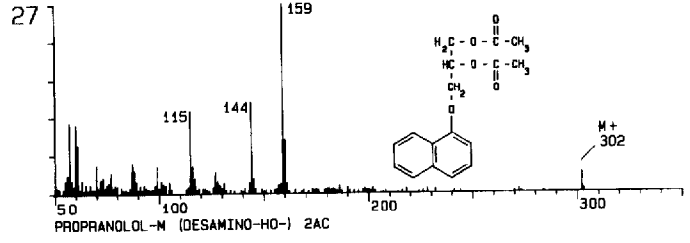
183
72
154
M+
298
50
100
200
300
CARAZOLOL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159
115
144
M+
302
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100
200
300
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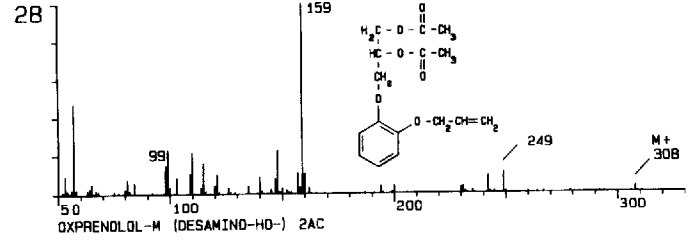
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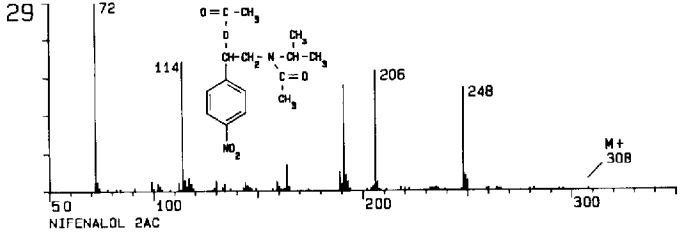
159
99
249
M+
308
50
100
200
300
OXPRENOLOL-M (DESAMINO-HO-) 2AC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206
248
M+
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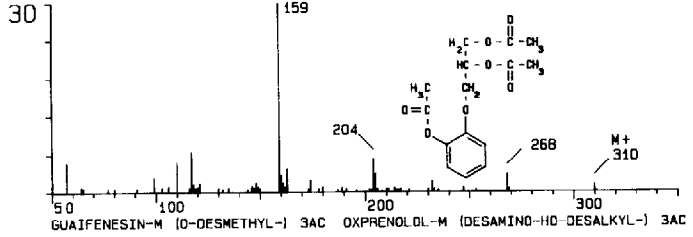
NIFENALOL 2AC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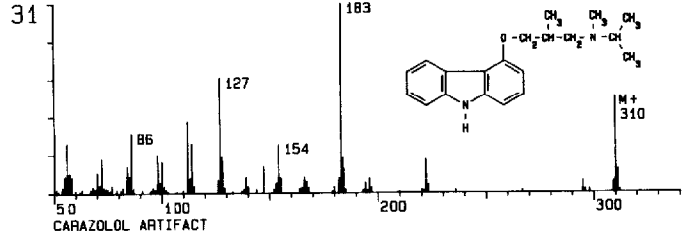
159
204
268
M+
310
50
100
200
300
GUAIFENESIN-M (O-DESMETHYL-) 3AC
OXPRENOLOL-M (DESAMINO-HO-DESALKYL-) 3AC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154
M+
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200
300
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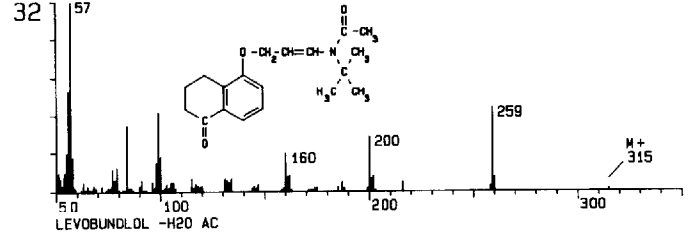

32
57
259
160
200
M+
315
50
100
200
300
LEVOBUNDLOL -H2O AC

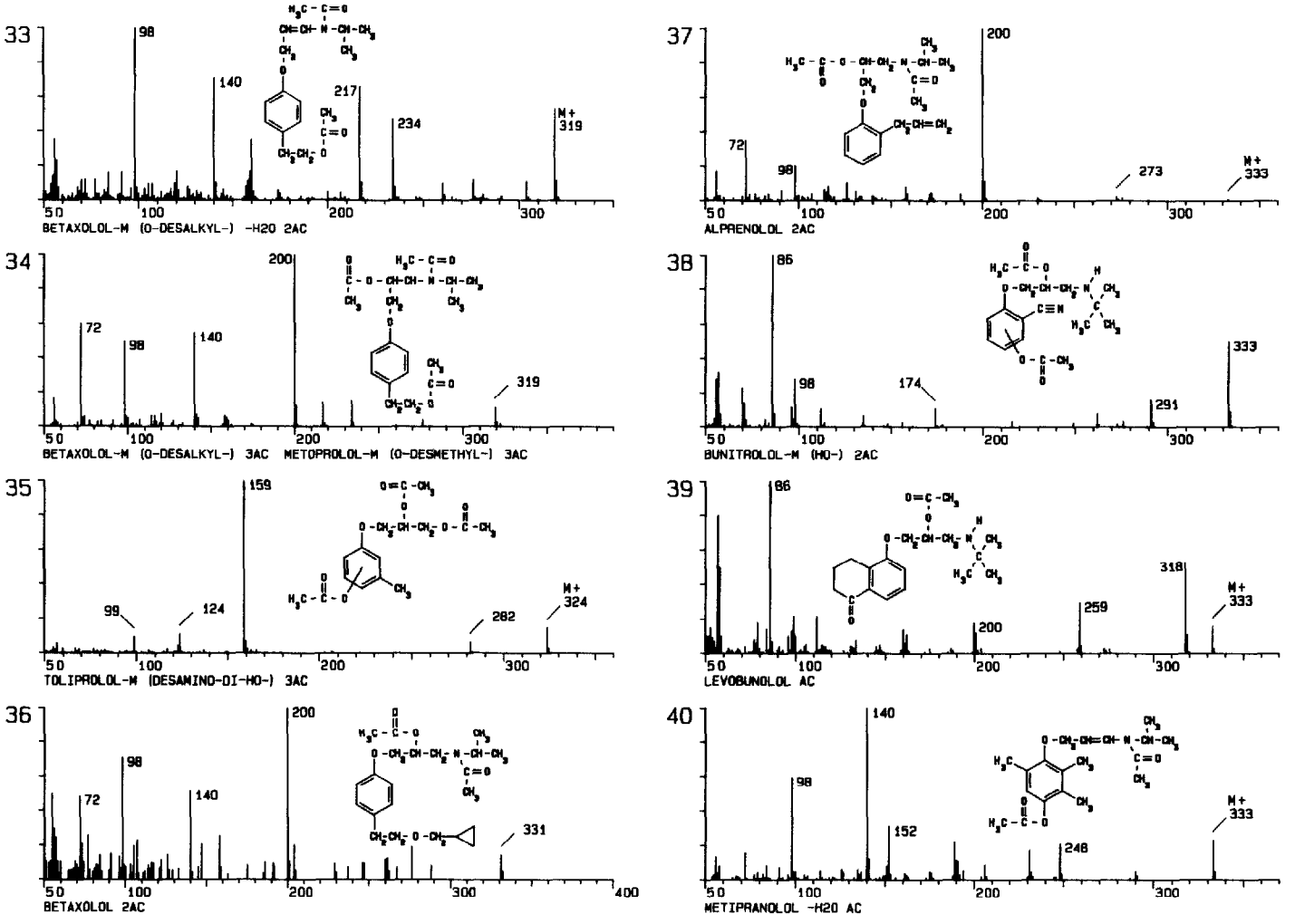
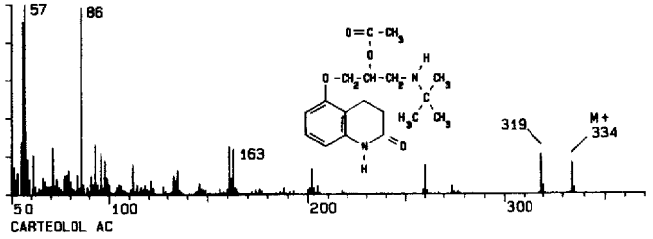


Fig. 1.

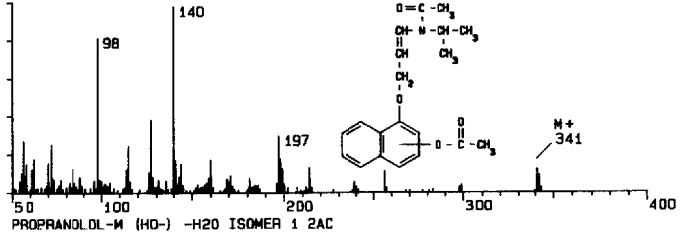
(Continued on p. 158)

41



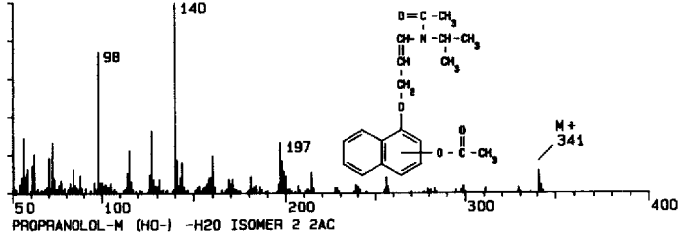
CARTEOLOL AC

42



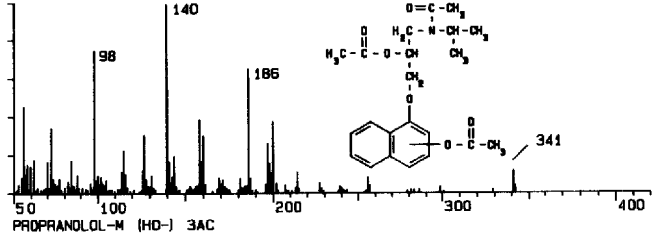
PROPRANOLOL-M (HO-) -H2O ISOMER 1 2AC

43



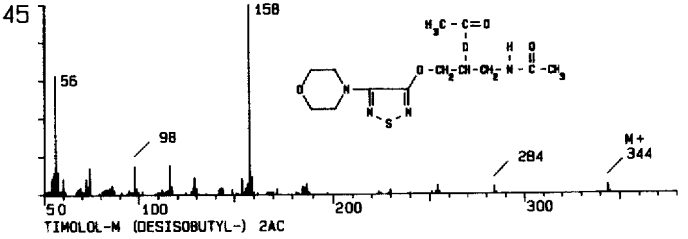
PROPRANOLOL-M (HO-) -H2O ISOMER 2 2AC

44



PROPRANOLOL-M (HO-) 3AC

45



TIMOLOL-M (DESISOBUTYL-) 2AC

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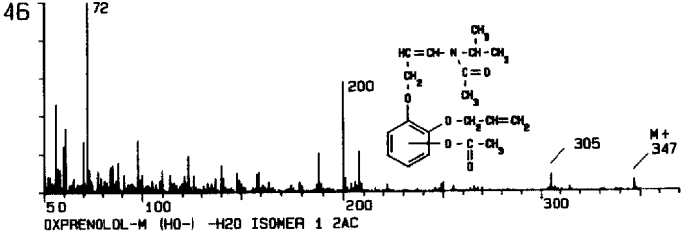
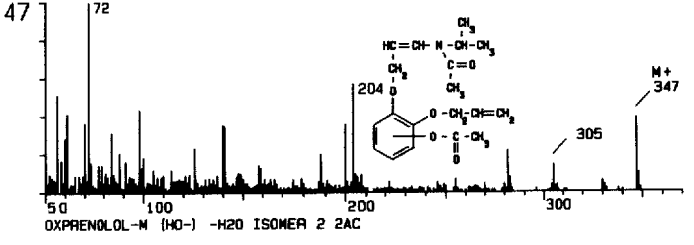


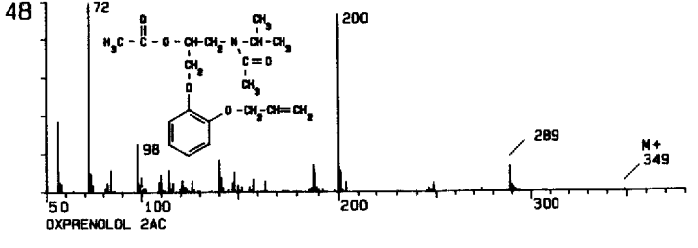
OXPRENOLOL-M (HO-) -H2O ISOMER 1 2AC

47



OXPRENOLOL-M (HO-) -H2O ISOMER 2 2AC

48



OXPRENOLOL 2AC

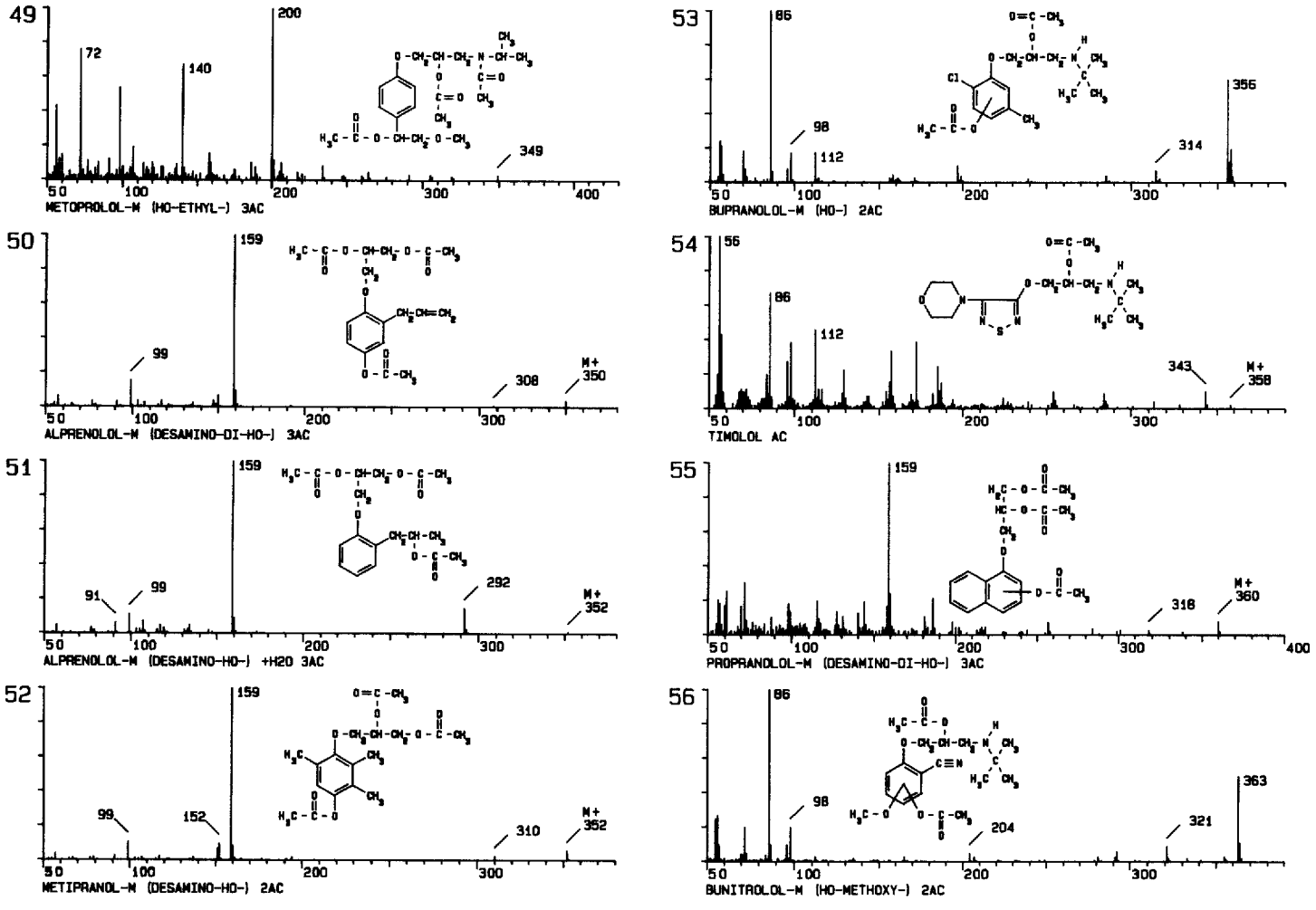
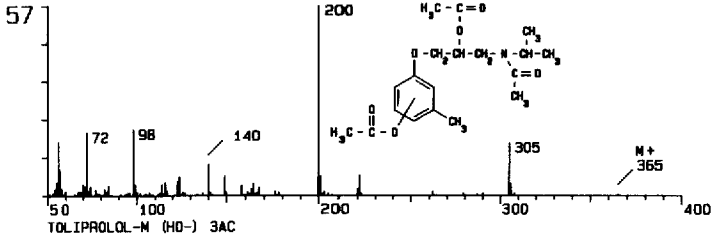


Fig. 1.

(Continued on p. 160)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200
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98
140
305
M+
365
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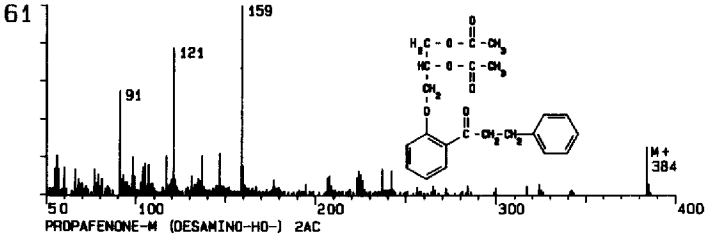
200
300
400
TOLIPROLOL-M (HO-) 3AC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159
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M+
384
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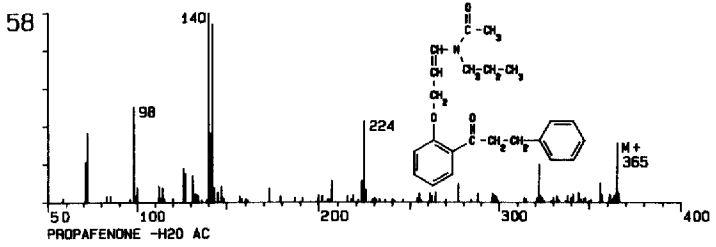
200
300
400
PROPAFENONE-M (DESAMINO-HO-) 2AC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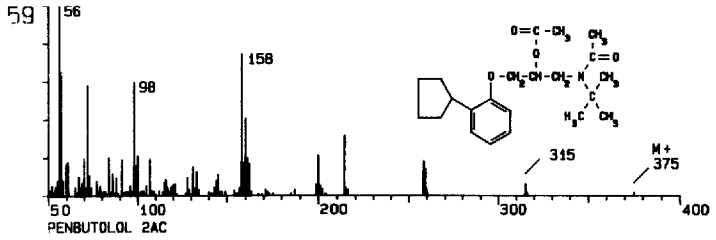
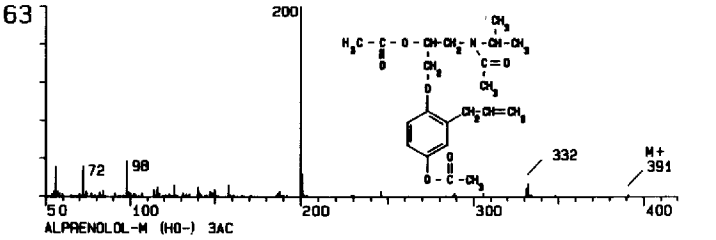
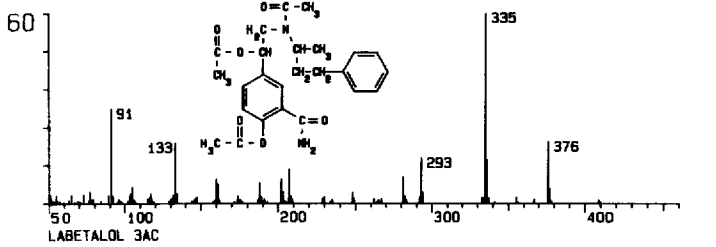
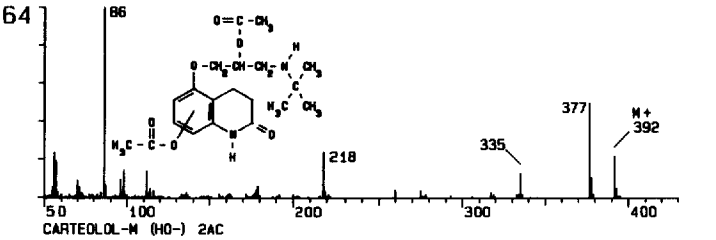
140
98
224
M+
365
50
100
200
300
400
PROPAFENONE -H2O AC


62
56
86
158
376
M+
391
50
100
200
300
400
PENBUTOLOL-M (HO-) 2AC


59
56
98
158
315
M+
375
50
100
200
300
400
PENBUTOLOL 2AC


63
200
72
98
332
M+
391
50
100
200
300
400
ALPRENOLOL-M (HO-) 3AC


60
335
91
133
293
376
50
100
200
300
400
LABETALOL 3AC


64
86
377
M+
392
335
218
50
100
200
300
400
CARTEOLOL-M (HO-) 2AC

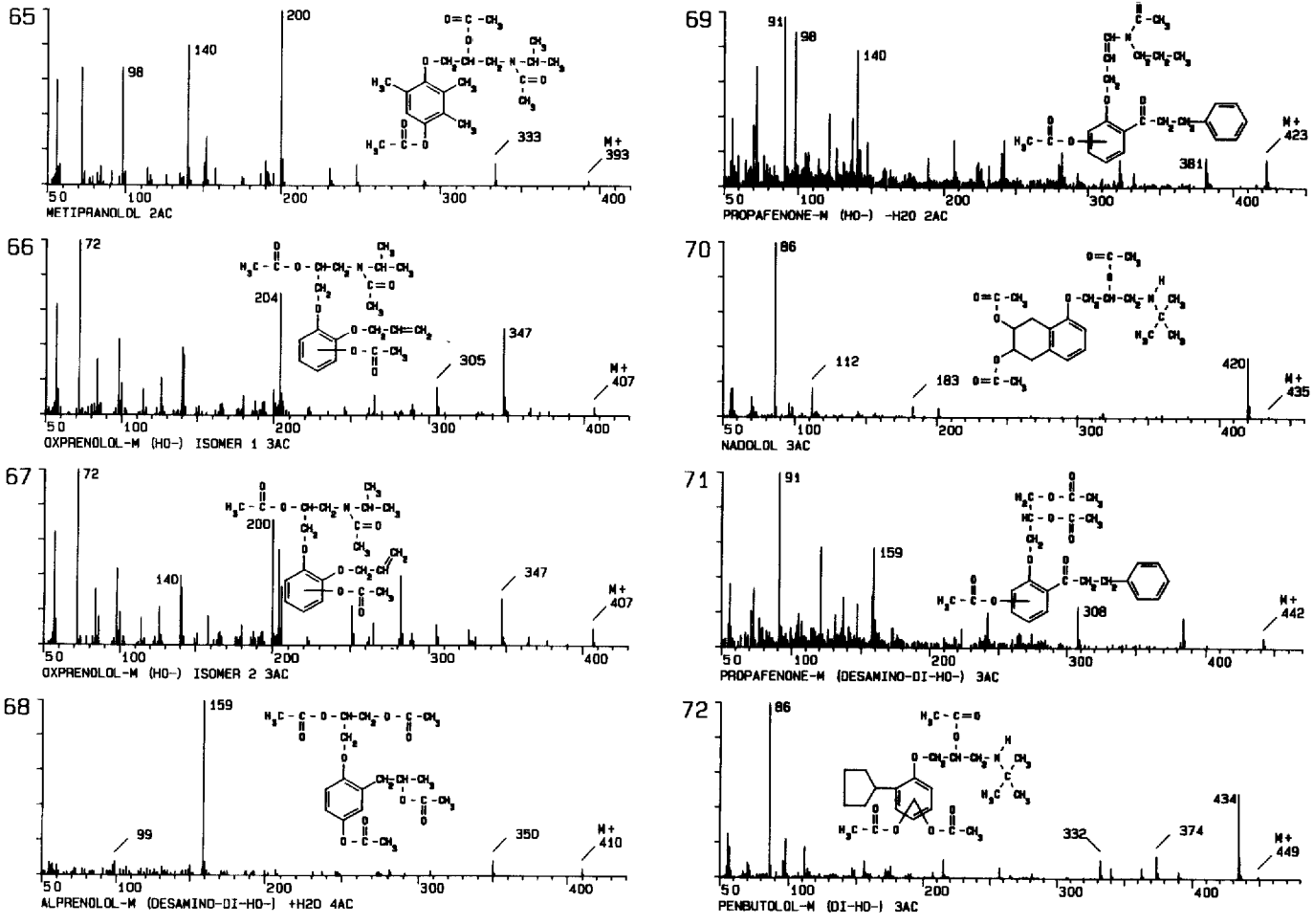


Fig. 1. Mass spectra of beta-blockers and their metabolites identified in urine after acid hydrolysis, extraction and acetylation.

of the compounds. They are arranged primarily in ascending mass of the highest ions. For the same nominal mass value the spectra are arranged in order of ascending retention index. Formulae are proposed for probable structures of metabolites. Only those metabolites which were frequently found are given. Because of inter-species (man/rat) or inter-individual differences in metabolism or variable times elapsed after administration, not all given metabolites are detected in each sample. Further metabolites can be found. The mass spectra and retention indices of these are included in a forthcoming handbook [71].

Five kinds of artifacts result from the analytical procedure used. Acebutolol is hydrolysed to an aminophenol derivative (mass spectrum No. 2). Labetalol

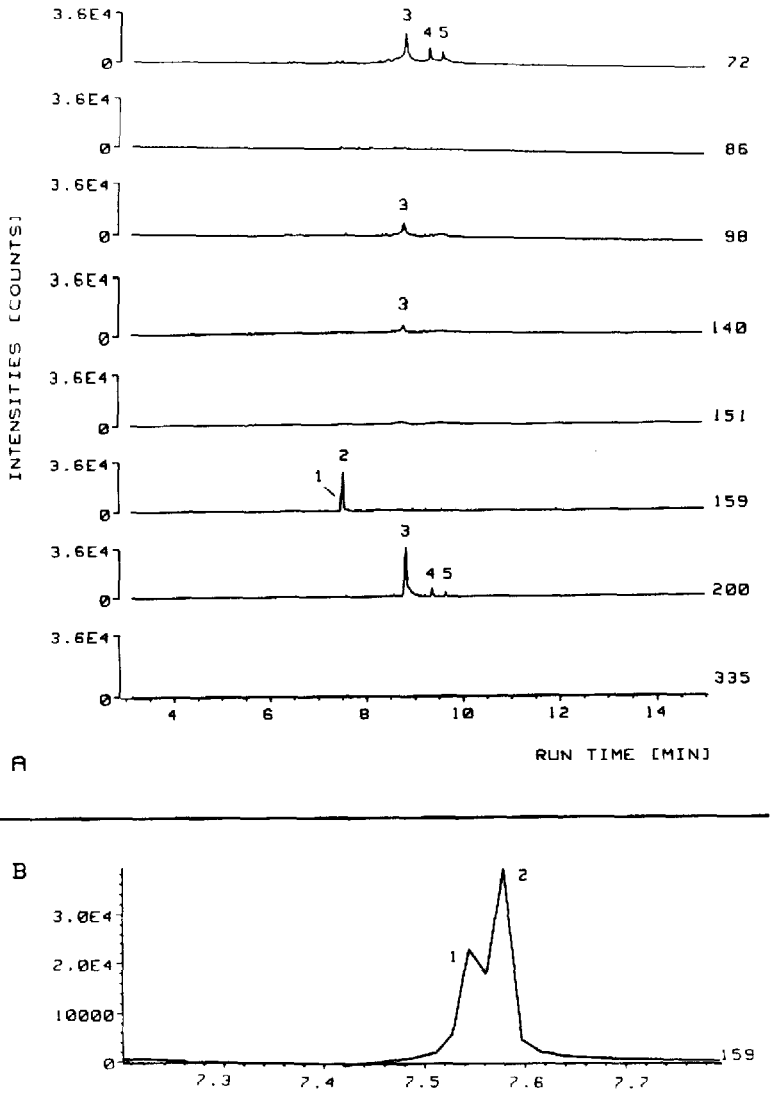


Fig. 2. (A) Ion chromatogram indicating oxprenolol (3) and its metabolites (1, 2, 4 and 5). (B) Amplified chromatogram in the region of the ion of m/z 159.

and its hydroxy metabolites are altered during GC to amines (mass spectra Nos. 1, 7 and 8). Addition of water was observed for two metabolites of alprenolol (mass spectra Nos. 51 and 68). Dehydration was observed for several of the studied compounds (mass spectra Nos. 4, 6, 18—21, 24, 32, 33, 40, 42, 43, 46, 47, 58 and 69). Beta-blockers that are analysed without derivatization are partly altered when their methanolic solutions are injected into the GC system. These artifacts were identified using high-resolution MS. The empirical formulae and the fragmentation patterns suggest that the artifacts were formed by methyl substitution (mass spectra Nos. 10, 13, 16 and 31). Further spectroscopic studies are in progress [72].

Because all compounds indicated on the ion chromatogram can be differentiated by comparison of the underlying mass spectra with those of standards (Fig. 1 and ref. 71), interferences by other drugs are improbable.

To illustrate the method, an ion chromatogram from the urine of a patient suspected of intoxication with beta-blockers is shown in Fig. 2A. Peak 3 indicates oxprenolol (mass spectrum No. 48) and peaks 1, 2, 4 and 5 indicate metabolites of oxprenolol (mass spectra Nos. 28, 30, 46 and 47). Because the chromatogram of the ion 159 is insufficiently resolved, the signal in this region can be further amplified by the data system used to ensure its identification (Fig. 2B).

The procedure presented allows the rapid and selective identification and differentiation of beta-blockers and their metabolites in urine, which is the prerequisite of quantitation in plasma. It has the advantage that other groups of drugs can be detected simultaneously simply by searching for typical fragment ions in the stored spectra. Such ion chromatograms typical of benzodiazepines [73], butyrophenones and bisfluorophenyl neuroleptics [74], anti-inflammatory analgesics [75], opioids and other potent analgesics [76], antidepressants [77], phenothiazine and analogous neuroleptics [78] and antiparkinsonian drugs [79] have been published previously. Detection of antihistamines is in preparation [72]. Similar data for other compounds of toxicological interest will be collected so that nearly all relevant drugs will be detectable in urine or other biological materials within 1—2 h.

REFERENCES

1 H Maurer, A. Weber and K. Pfleger, J. Clin. Chem. Clin. Biochem., 23 (1985) 559.
2 D.B. Jack, S. Dean and M.J. Kendall, J. Chromatogr., 187 (1980) 277.
3 D.B. Jack, S. Dean, M.J. Kendall and S. Laugher, J. Chromatogr., 196 (1980) 189.
4 L. von Meyer, G. Drasch, G. Kauert, L. Riedl and A. Riedl, Beitr. Gerichtl. Med., 37 (1979) 363.
5 H. Maurer, A. Weber and K. Pfleger, Fresenius Z. Anal. Chem., 311 (1982) 314.
6 J.W. Engelsma and J. Simons, Vet. Q., 7 (1985) 73.
7 K. Stock and E. Westermann, Biochem. Pharmacol., 14 (1965) 227.
8 J.M. Steyn, J. Chromatogr., 120 (1976) 465.
9 M. Schäfer and E. Mutschler, J. Chromatogr., 169 (1979) 477.
10 M. Schafer-Korting and E. Mutschler, J. Chromatogr., 230 (1982) 461.
11 C.F. Poole, L. Johansson and J. Vessman, J. Chromatogr., 194 (1980) 365.
12 S.H. Wan, R.F. Maronde and S.B. Matin, J. Pharm. Sci., 67 (1978) 1340.
13 M. Ervik, K. Kylberg-Hanssen and P.-O. Langerström, J. Chromatogr., 182 (1980) 341.
14 J. Ganansia, G. Gillet, P. Padovani and G. Bianchetti, J. Chromatogr., 275 (1983) 183.

15 A. Sioufi, F. Leroux and N. Sandrenan, J. Chromatogr., 272 (1983) 103.
16 O. Gyllenhaal and J. Vessman, J. Chromatogr., 273 (1983) 129.
17 P.H. Degen and W. Riess, J. Chromatogr., 121 (1976) 72.
18 A. Sioufi, D. Colussi and P. Mangoni, J. Chromatogr., 278 (1983) 185.
19 M. Guerret, J. Chromatogr., 221 (1980) 387.
20 S. Caccia, G. Guiso, M. Ballabio and P. de Ponte, J. Chromatogr., 172 (1979) 457.
21 D.J. Tocco, F.A. de Luna and A.E.W. Duncan, J. Pharm. Sci., 64 (1975) 1879.
22 Ph. Hermann, J. Fraisse, J. Allen, P.L. Morselli and J.P. Thenot, Biomed. Mass Spectrom., 11 (1984) 29.
23 R. Endele, M. Senn and U. Abshagen, J. Chromatogr., 227 (1982) 187.
24 D. Gaudry, D. Wantiez, J. Richard and J.P. Metayer, J. Chromatogr., 339 (1985) 404.
25 P.T. Funke, M.F. Malley, E. Ivashkiv and A.I. Cohen, J. Pharm. Sci., 67 (1978) 653.
26 T. Walle, J. Morrison, K. Walle and E. Conradi, J. Chromatogr., 114 (1975) 351.
27 J.R. Carlin, R.W. Walker, R.O. Davies, R.T. Ferguson and W.J.A. Vandenheuvel, J. Pharm. Sci., 69 (1980) 1111.
28 J.B. Fourtillan, M.A. Lefebvre, J. Girault and Ph. Courtois, J. Pharm. Sci., 70 (1981) 573.
29 M.A. Lefebvre, J. Girault and J.B. Fourtillan, J. Liq. Chromatogr., 4 (1981) 483.
30 J.N. Buskin, R.A. Upton, R.M. Jones and R.I. Williams, J. Chromatogr., 230 (1982) 438.
31 J. Hermansson and C. von Bahr, J. Chromatogr., 227 (1982) 113.
32 H. Winkler, W. Ried and B. Lemmer, J. Chromatogr., 228 (1982) 223.
33 P.M. Harrison, A.M. Tonkin and A.J. McLean, J. Chromatogr., 339 (1985) 429.
34 H. Caqueret and G. Bianchetti, J. Chromatogr., 311 (1984) 199.
35 A. Nagakura and H. Kohei, J. Chromatogr., 232 (1982) 137.
36 H. Winkler and B. Lemmer, J. Chromatogr., 309 (1984) 193.
37 S.-Y. Chu, J. Pharm. Sci., 67 (1978) 1623.
38 T.F. Woodman and B. Johnson, Ther. Drug Monit., 3 (1981) 371.
39 I.J. Hidalgo and K T. Muir, J. Chromatogr., 305 (1984) 222
40 K.B. Alton, F. Leitz, S. Bariletto, L. Jaworsky, D. Desriviers and J. Patrick, J. Chromatogr., 311 (1984) 319.
41 H. Hengy and E -U. Kölle, J. Chromatogr., 338 (1985) 444.
42 W. Krause, J. Chromatogr., 181 (1980) 67.
43 M.T. Rosseel, F.M. Belpaire, I. Bekaert and M.G. Bogaert, J. Pharm Sci., 71 (1982) 114.
44 M.S. Lennard and J.H. Silas, J. Chromatogr., 272 (1983) 205.
45 J.-B. Lecaillon, J. Godbillon, F. Abadie and G. Gosset, J. Chromatogr., 305 (1984) 411.
46 R.N. Gupta, R.B. Haynes, A.G. Logan, L.A. Macdonald, R. Pickersgill and C. Achber, Clin. Chem., 29 (1983) 1085.
47 V.K. Piotrovskii, V.G. Belolipetskaya, A.R. El'Man and V.I. Metelitsa, J. Chromatogr., 278 (1983) 469.
48 C.D. Kinney, J. Chromatogr , 305 (1984) 489.
49 V.K. Piotrovskii, Y.A. Zhirkov and V.I. Metelitsa, J. Chromatogr., 309 (1984) 421.
50 S E. Tsuei, J. Thomas and R.G. Moore, J. Chromatogr., 181 (1980) 135.
51 N. Bernard, G. Cuisinaud and J. Sassard, J. Chromatogr., 228 (1982) 355.
52 D.J. Miner, D.A. Binkley and L.D. Bechtol, Clin. Chem., 30 (1984) 717.
53 M. Bangah, G. Jackman and A. Bobik, J. Chromatogr., 183 (1980) 255.
54 B. Diquet, J.J. Nguyen-Huu and H. Boutron, J. Chromatogr., 311 (1984) 430.
55 E. Brode, R. Sachse and H.D. Hoffmann, Drug Res., 32 (1982) 1.
56 S.R. Harapat and R.E. Kates, J. Chromatogr., 230 (1982) 448.
57 R. Kannan, D. Tidwell and B.N. Singh, J. Chromatogr., 272 (1983) 428.
58 M. Lo and S. Riegelman, J. Chromatogr., 183 (1980) 213.
59 F. Albani, R. Riva and A. Baruzzi, J. Chromatogr., 228 (1982) 362.
60 I.W. Wainer, T.D. Doyle, K.H. Donn and J.R. Powell, J Chromatogr., 306 (1984) 405.
61 M.J. Wilson and T. Walle, J. Chromatogr., 310 (1984) 424.
62 B. Lemmer, T. Ohm and H. Winkler, J. Chromatogr., 309 (1984) 187.
63 S. Kärkkäinen, J. Chromatogr., 336 (1984) 313.

64 M.R. Gregg and D.B. Jack, J. Chromatogr., 305 (1984) 244.
65 M.S. Lennard and S. Parkin, J. Chromatogr., 338 (1985) 249.
66 S.R. Nahorski, M.I. Batta and D.B. Barnett, Eur. J. Pharmacol., 52 (1978) 393.
67 R.B. Innis, D.B. Byland and S.H. Snyder, Life Sci., 23 (1978) 2031.
68 E. Rattenberg, P. Matzke and J. Neudegger, Arch. Lebensmittelhyg., 36 (1985) 85.
69 S.-Y. Chu, S.M. Vega, A. Ali and L.T. Sennello, J. Pharm. Sci., 70 (1981) 990.
70 V. Marks, G.P. Mould, G. Stout and S. Williams, Br. J. Clin. Pharmacol., 5 (1978) 371P.
71 K. Pfleger, H. Maurer and A. Weber, Mass Spectral and GC Data of Drugs, Poisons and Their Metabolites, VCH Verlagsgesellschaft, Weinheim, Deerfield Beach, FL, Basle, 2nd ed., in preparation.
72 H. Maurer and K. Pfleger, in preparation.
73 H. Maurer and K. Pfleger, J. Chromatogr., 222 (1981) 409.
74 H. Maurer and K. Pfleger, J. Chromatogr., 272 (1983) 75.
75 H. Maurer and K. Pfleger, Fresenius Z. Anal. Chem., 314 (1983) 586.
76 H. Maurer and K. Pfleger, Fresenius Z. Anal. Chem., 317 (1984) 42.
77 H. Maurer and K. Pfleger, J. Chromatogr., 305 (1984) 309.
78 H. Maurer and K. Pfleger, J. Chromatogr., 306 (1984) 125.
79 H. Maurer and K. Pfleger, Fresenius Z. Anal. Chem., 321 (1985) 363.