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SCREENING PROCEDURE FOR DETECTION OF ANTIDEPRESSANTS AND THEIR METABOLITES IN URINE USING A COMPUTERIZED GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC TECHNIQUE*

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SUMMARY

A method for the identification of antidepressants 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 mass fragmentography with the masses 58, 84, 86, 100, 191, 193, 194, 205. The identity of positive signals in the reconstructed mass fragmentogram is established by a comparison of the entire mass spectra with those of standards. The mass fragmentogram, the underlying mass spectra and the gas chromatographic retention indices (OV-101) are documented.

INTRODUCTION

Within the scope of a screening procedure for detection of psychotropic and addictive drugs and their metabolites in urine [1, 2], screening for benzodiazepines [3], butyrophenone and bisfluorophenyl neurolectics [4, 5], anti-inflammatory analgesics [6] and opioids and other potent analgesics [7] has been described; screening for phenothiazine and analogous neuroleptics and antiparkinsonian drugs is in preparation [8]. Screening for tri- and tetracyclic antidepressants is described below. Such a screening is necessary in analytical toxicology to diagnose a probable intoxication. Furthermore, antidepressants are encountered frequently in analysis, when monitoring patients who may have taken addictive drugs and simultaneously taken antidepressants therapeutically. Only a gas chromatographic—mass spectrometric technique (GC—MS) detecting a few of these drugs as parent compounds in gastric

*These results were reported in part at the Jahrestagung der Deutschen Gesellschaft für klinische Chemie, Stuttgart, F.R.G., September 23th and 24th, 1982.

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contents or plasma after intoxications has been documented [9]. The following procedure allows rapid identification and differentiation of 21 antidepressants and their metabolites in urine after therapeutic dosage. If necessary, plasma levels of the identified drugs can be determined using a procedure (e.g. gas—liquid chromatography, high-performance liquid chromatography, radioimmunoassay) described and cited in the review article of Scoggins et al. [10].

EXPERIMENTAL

Apparatus, sample preparation (acid hydrolysis, extraction, acetylation) and the GC—MS technique used for this study have been previously described [3, 5, 6, 11].

RESULTS AND DISCUSSION

All investigations were carried out using the urine of man with the exception of dimetacrine, melitracene, noxiptyline, protriptyline and trazodone, which were detected (in the absence of human samples) in the urine of rats. Some of the antidepressants are excreted in urine completely metabolized and conjugated. Therefore, the conjugates were decomposed 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 our investigations are shown in Table I. The mass fragmentogram with the eight proposed masses allows the detection of 21 antidepressants and their metabolites. Some of these compounds are acetylated. The retention indices were determined using a gas chromatograph combined with a flame ionisation detector and a nitrogen-sensitive flame ionisation detector with a temperature programme [3]. In our experience retention indices give preliminary indications and may be useful to gas chromatographers without a GC—MS facility and so they are given here. Furthermore, they allow one to distinguish between the isomeric hydroxy metabolites which give the same mass spectra (mass spectra Nos. 9, 24 and 26 in Fig. 1). All of the mass spectra are shown in Fig. 1 for the precise identification of the compounds. Formulae are proposed for probable structures of metabolites.

Only those metabolites that were usually found are listed. Because of individual differences in metabolism, time elapsed after administration and different routes of administration, not all metabolites given in Table I were detected in each sample. Further metabolites can be found. The mass spectra of those will be included in a forthcoming handbook [12].

Dimetacrine and its metabolites are completely decomposed by the acid hydrolysis. Thus, they must be identified in a direct extract [11] of urine.

Amitriptyline, amitriptyline-N-oxide and nortriptyline lead to common metabolites. If amitriptyline-N-oxide was taken, amitriptyline and noramitriptyline (mass spectra Nos. 1 and 4) cannot be detected. If nortriptyline was taken, amitriptyline (mass spectra Nos. 1, 2 and 3) cannot be detected.

Desipramine, imipramine and lofepramine also lead to common metabolites. If desipramine or lofepramine were taken, imipramine and hydroxyimipramine (mass spectra Nos. 27 and 29) cannot be detected. Desipramine and lofe-

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MONITORING PROGRAMME FOR ANTIDEPRESSANTS AND THEIR METABOLITES

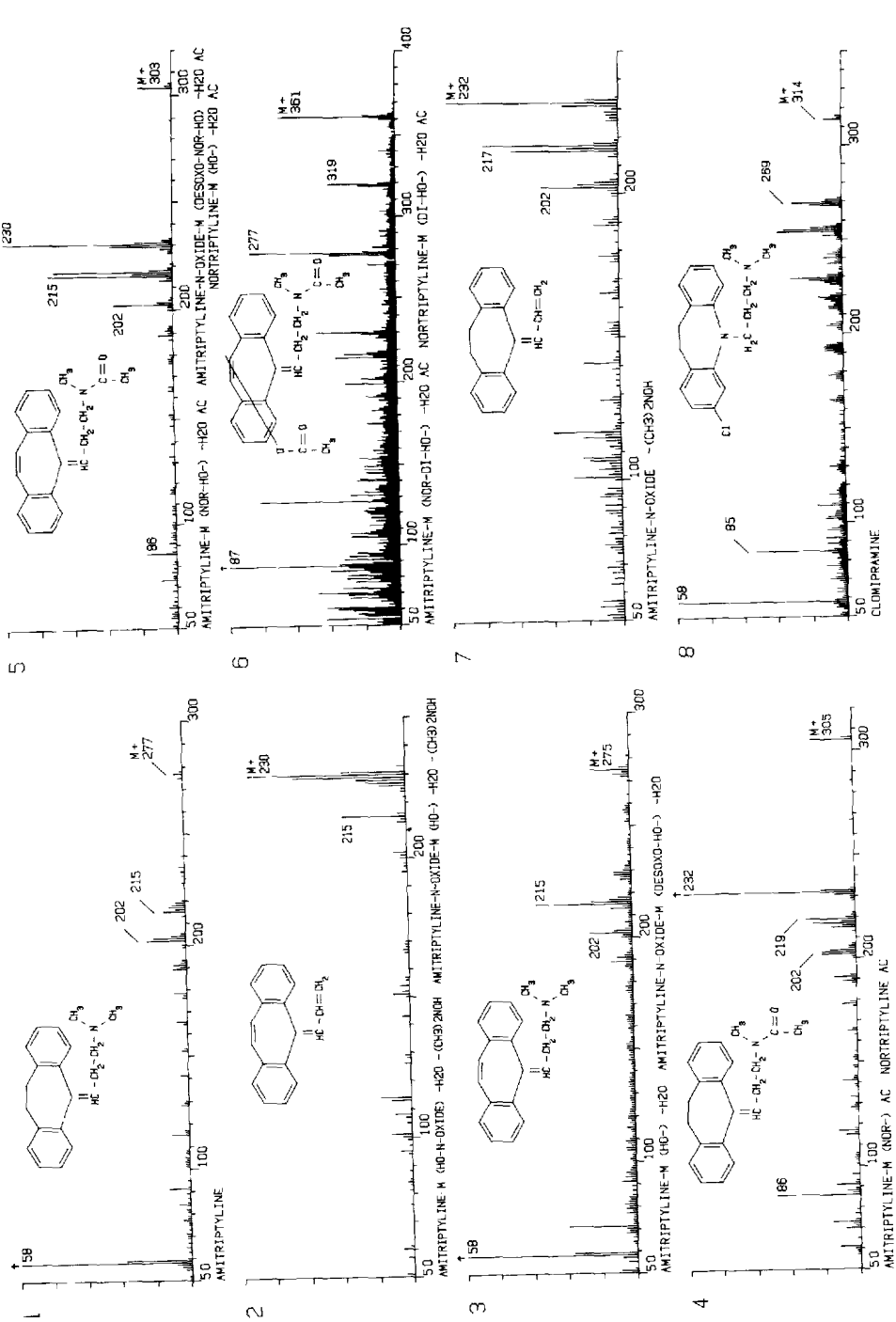
Mass spectrum No.	M^+	Drug/metabolite (M)	*m/e* (relative intensities)								Retention index (OV-101)
			58	84	86	100	191	193	194	205	
01	277	Amitriptyline	+++								2204
02	230	M (HO-N-oxide)									2000 FID
03	275	M (HO-)	+++								2236
04	305	M (Nor-)			+						2660
05	303	M (Nor-HO-)			+						2670
06	361	M (Nor-di-HO-)			+						2800
07	232	Amitriptyline-N-oxide									1976 FID
02	230	M (HO-)									2000 FID
03	275	M (Desoxo-HO-)	+++								2236
05	303	M (Desoxo-nor-HO-)			+						2670
08	314	Clomipramine	+++								2457
09	372	M (HO-) 1st isomer	+++								2806
09	372	M (HO-) 2nd isomer	+++								2906
10	342	M (Nor-)			+	+				+	2994
11	400	M (Nor-HO-)			+	+					3205
12	308	Desipramine	+		+	+	+	++	+		2669
13	195	M (ring)					+	+	++		1931
14	366	M (HO-)			+	+		+			3066
15	295*	Dibenzepin	+++								2465
16	323	M (Nor-)				+	+	+	+		2800
17	295	M (Ter-nor-)						+	+		2824
18	309	M (Bis-nor-)	+			++			+	+	2869
19	294	Dimetacrine	++		+++				+		2313
20	209	M (ring)					+	+	+++		1906
21	249	M (N-oxide)					+	++	++		2020

(Continued on p. 312)

lass pectrum o.	M^+	Drug/metabolite (M)	*m/e* (relative intensities)								Retention index (OV-101)
			58	84	86	100	191	193	194	205	
2	279	Doxepin	+++							+	2242
3	234	M (N-oxide)					+			+	1970 FID
4	337	M (HO-) 1st isomer	+++								2540
4	337	M (HO-) 2nd isomer	+++								2583
5	307	M (Nor-)			++					+	2700
3	365	M (Nor-HO-) 1st isomer			+++						2987
3	365	M (Nor-HO-) 2nd isomer			+++						3036
7	280	Imipramine	+++	+			+	++		+	2214
3	195	M (ring)					+	+	++		1931
3	193	M (Dehydro ring)					+	+++	+		1981
0	338	M (HO-)	+++	+							2610
2	308	M (Nor-)	+		+	+	+	++	+		2669
3	366	M (Nor-HO-)			+	+		+			3066
0	000	Lofepramine									0000
3	195	M (ring)					+	+	++		1931
2	308	M (Desalkyl-)	+		+	+	+	++	+		2669
3	366	M (Desalkyl-HO-)			+	+		+			3066
	319	Maprotiline	+		+	++	+			+	2800
	306	M (HO-propyl-)					++				2426 FID
	305	M (Nor-)					++				2765
	364	M (Di-HO-)									2820 FID
	377	M (HO-ethanediyl-)	+		+	++	++				2996
	377	M (HO-anthryl-)				++	+				3096
	363	M (Nor-HO-)									3150
	422	M (Tri-HO-)									3200 FID
	291	Melitracene	+++								2285
	319	M (Nor-)	+		+		+	+			2760
	379	M (Nor-HO-dihydro-)	+		+		+	+			3028
	264	Mianserin	+					+++			2208
	322	M (HO-)	+								2581

43	292	M (Nor-)			+++		++			2595
44	350	M (Nor-HO-)			+++					3005
45	280	Nomifensine						+		2470
46	338	M (HO-) 1st isomer								2850
47	338	M (HO-) 2nd isomer						++		2880
48	368	M (HO-methoxy-)								2970
04	305	Nortripytline		+						2660
05	303	M (HO-)		+						2670
06	361	M (Di-HO-)		+++						2800
49	294*	Noxiptyline	+++							2269
50	320	M (Nor-HO-)	+	+	++				++	2750
51	378	M (Nor-di-HO-)	+	+	+++	+	++	+	++	3020
52	405	Opipramol	+			+	+++	+		3171
28	193	M (ring)				+	+++	+		1980
53	305	Proptriptyline				+++				2688
54	291	M (Nor-)		+	+	+++	+	+	+	2780
55	363	M (HO-)								2895
56	175	Tranylcypromine		++						1636
57	371	Trazodone							+++	3345
58	429	M (HO-)							+++	3380
59	294	Trimipramine	+++				+	+		2224
13	195	M (ring)				+	+	++		1931
60	352	M (HO-)	+++	+						2652
61	380	M (Nor-HO-)			+					3153
62	438	M (Nor-di-HO-)			+					3556
63	279	Viloxazine		++	+++					2219
64	337	M (HO-)			+	+++				2610

*Not detectable.


1
AMITRIPTYLINE
2
AMITRIPTYLINE M (HO-N-OXIDE) -H2O -(CH3)2NOH AMITRIPTYLINE-N-OXIDE-M (HO-) -H2O -(CH3)2NOH
3
AMITRIPTYLINE-M (HO-) -H2O AMITRIPTYLINE-N-OXIDE-M (DESOXO-HO-) -H2O
4
AMITRIPTYLINE-M (NOR-) AC NORTRIPTYLINE AC
5
AMITRIPTYLINE-M (NOR-HO-) -H2O AC AMITRIPTYLINE-N-OXIDE-M (DESOXO-NOR-HO) -H2O AC
NORTRIPTYLINE-M (HO-) -H2O AC
6
AMITRIPTYLINE-M (NOR-DI-HO-) -H2O AC NORTRIPTYLINE-M (DI-HO-) -H2O AC
7
AMITRIPTYLINE-N-OXIDE -(CH3)2NOH
8
CLOMIPRAMINE

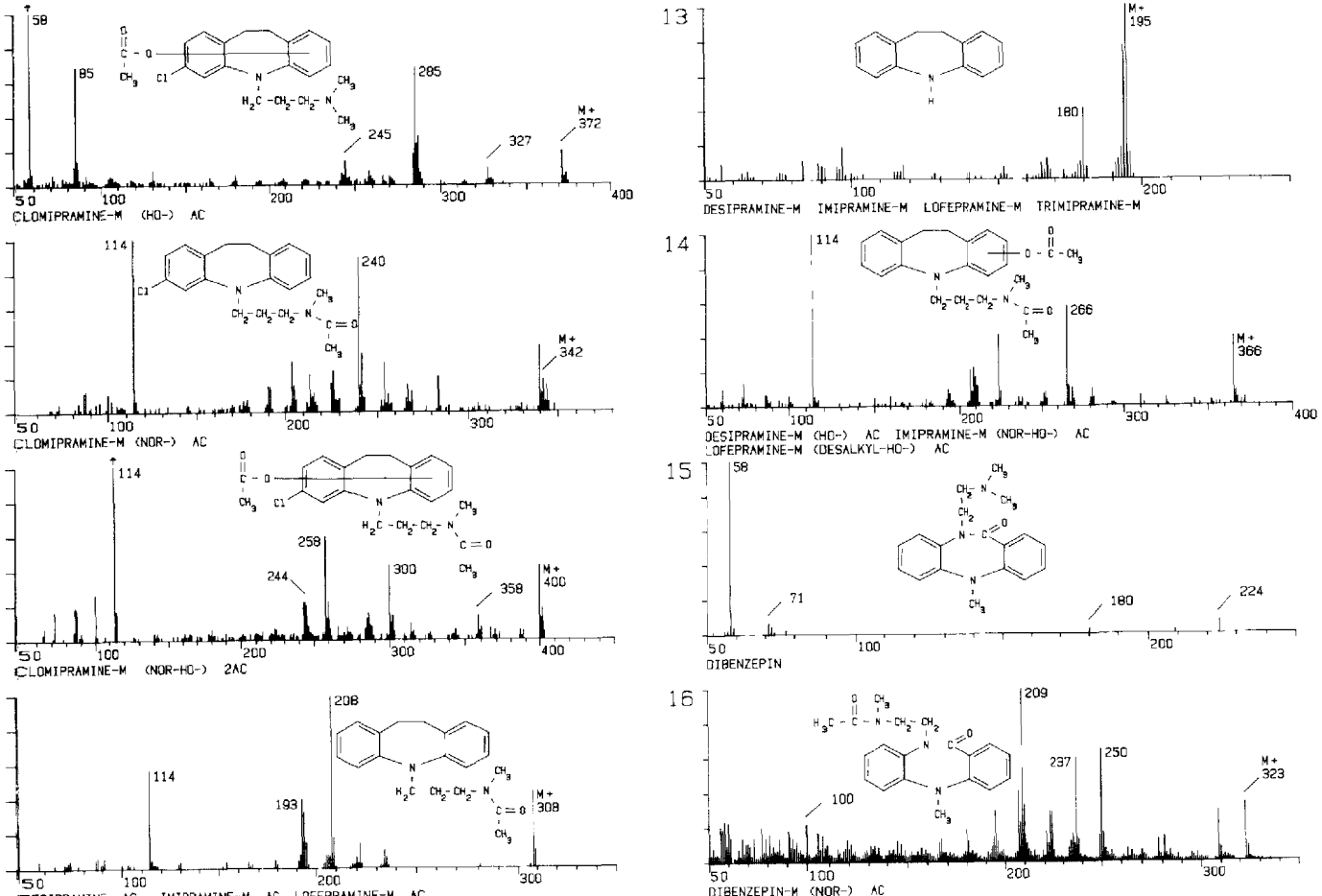
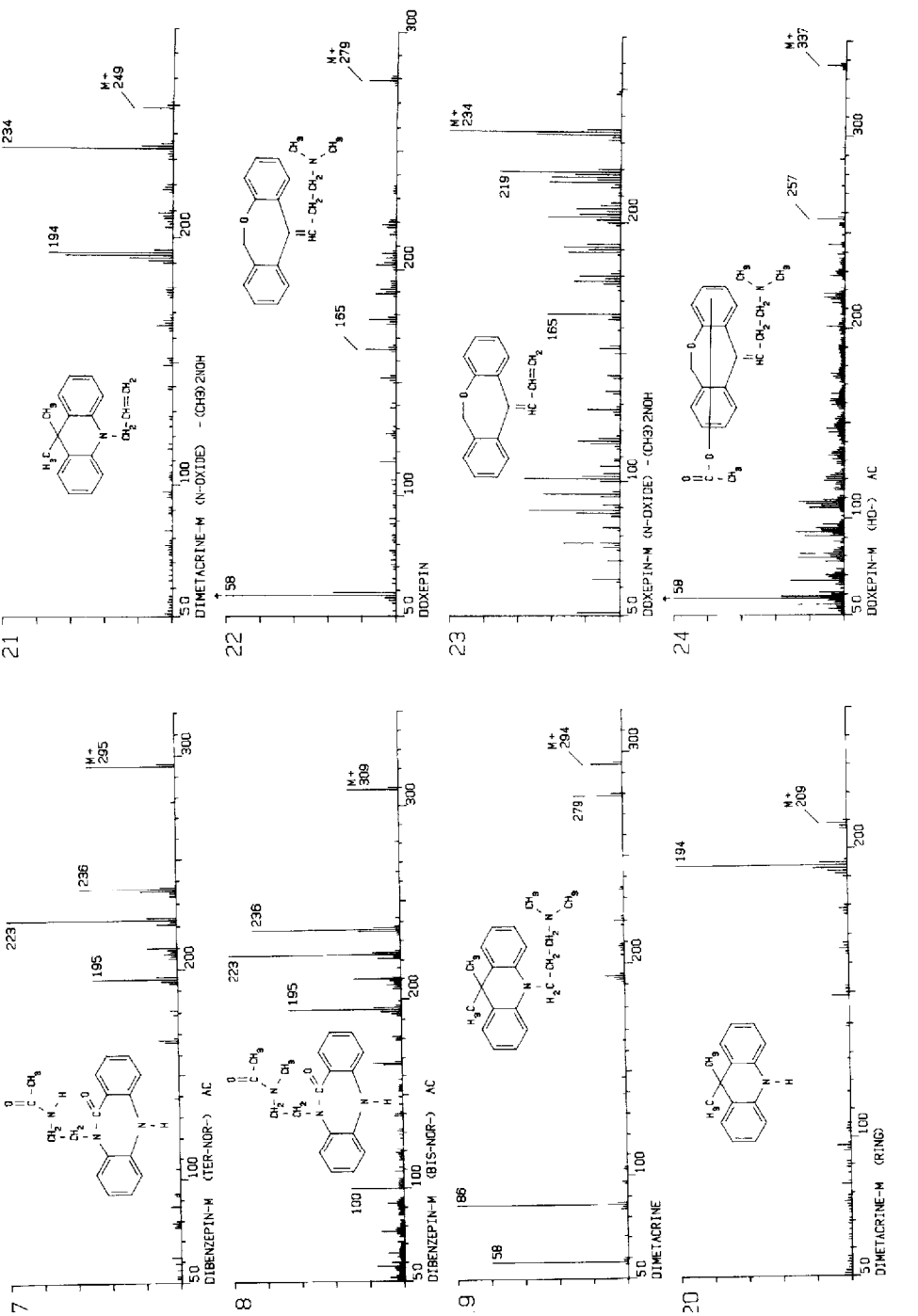


Fig. 1.

(Continued on p. 316)


7
DIBENZEPIN-M (TER-NOR-) AC
8
DIBENZEPIN-M (BIS-NOR-) AC
9
DIMETACRINE
20
DIMETACRINE-M (RING)
21
DIMETACRINE-M (N-OXIDE) - (CH3)2NOH
22
DOXEPIN
23
DOXEPIN-M (N-OXIDE) - (CH3)2NOH
24
DOXEPIN-M (HO-) AC

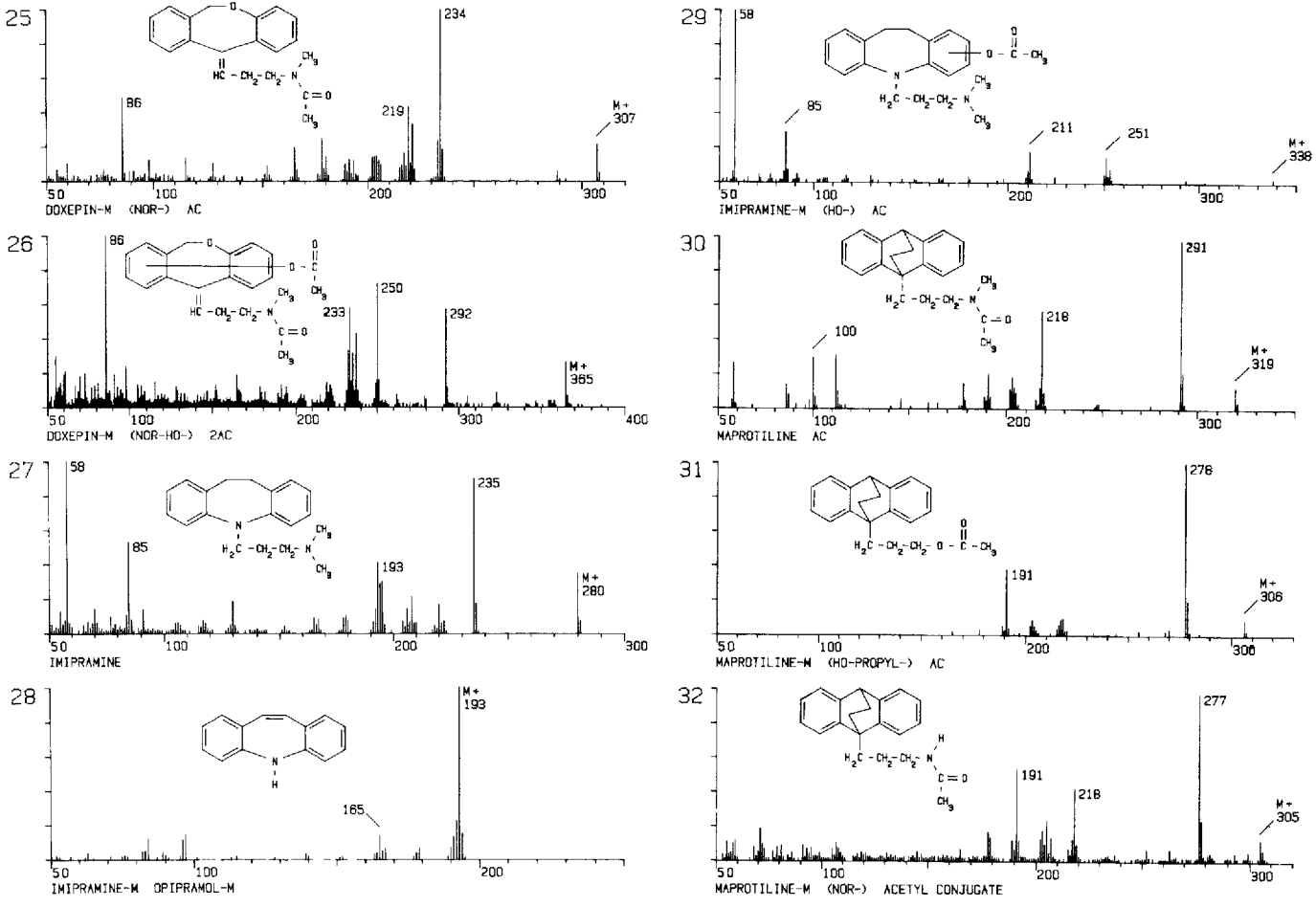
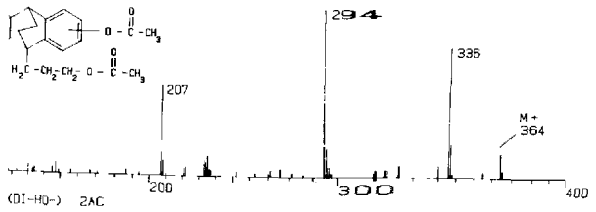
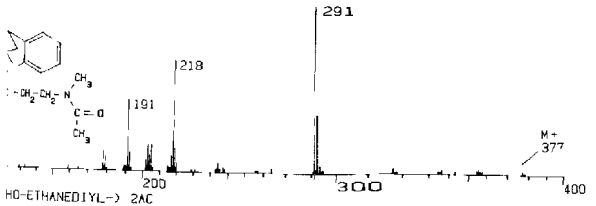
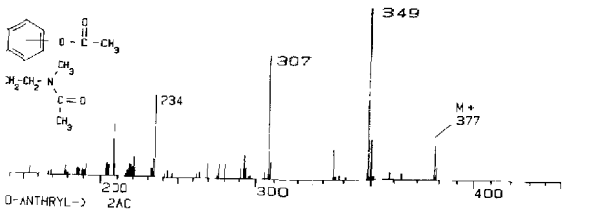


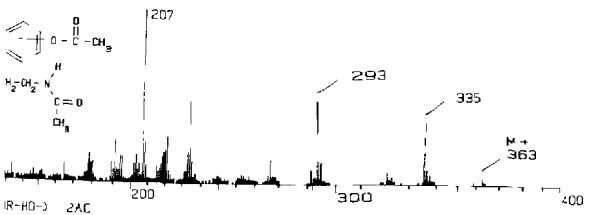
Fig. 1.

(Continued on p. 318)

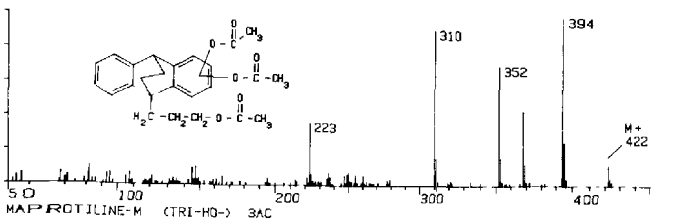

294
335
207
M+
364
(DI-HO-) 2AC


291
218
191
M+
377
HO-ETHANEDIYL-) 2AC

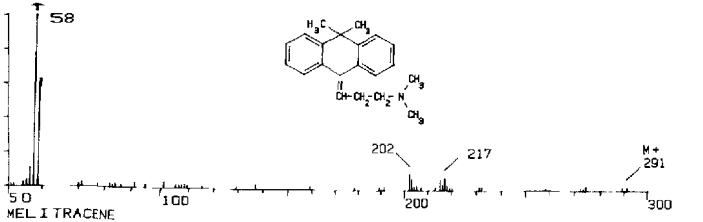

349
307
234
M+
377
O-ANTHRYL-) 2AC


207
293
335
M+
363
IR-HO-) 2AC

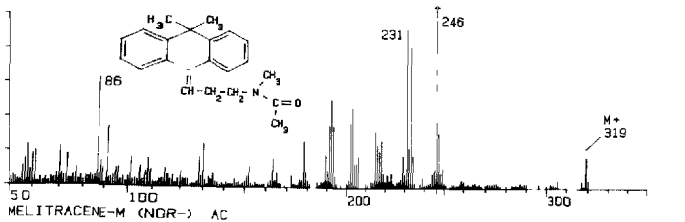
37


310
394
352
223
M+
422
MAPROTILINE-M (TRI-HO-) 3AC

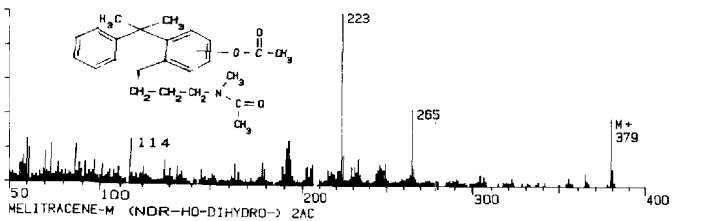
38


58
202
217
M+
291
MELITRACENE

39


231
246
86
M+
319
MELITRACENE-M (NOR-) AC

40


223
265
114
M+
379
MELITRACENE-M (NOR-HO-DIHYDRO-) 2AC

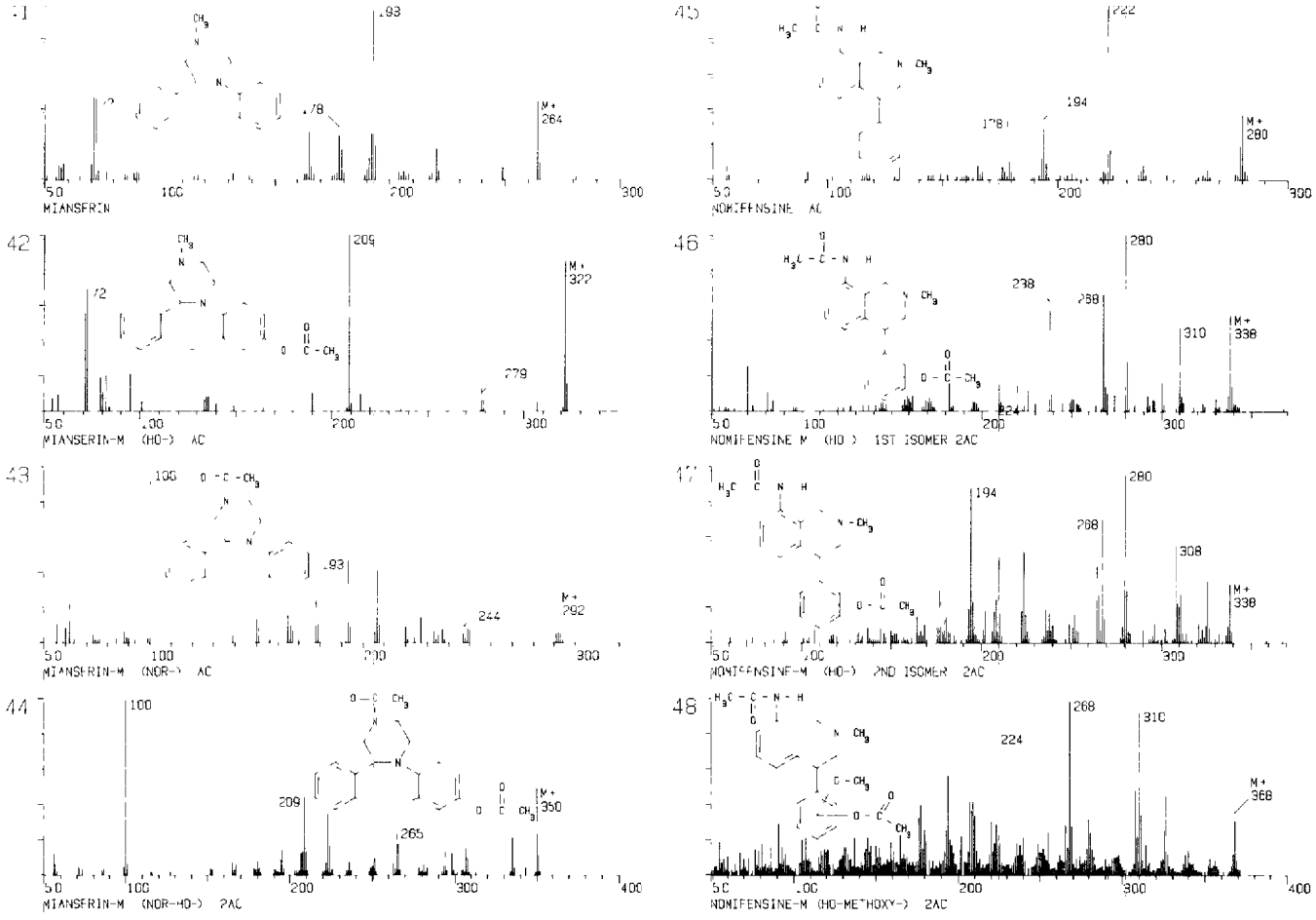
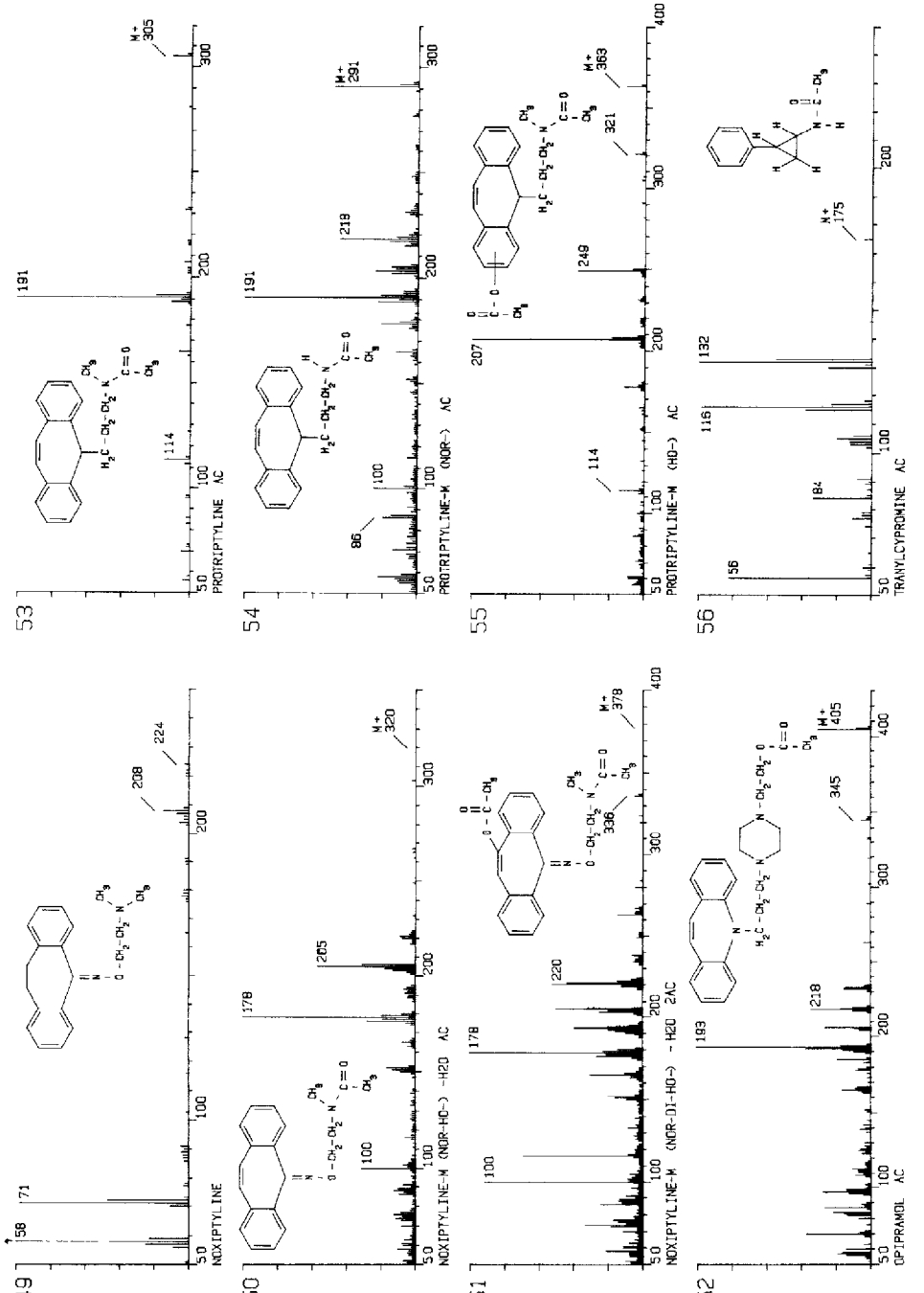


Fig. 1.

(Continued on p. 320)


49
NOXIPTYLINE
50
NOXIPTYLINE-M (NOR-HO-) -H2O AC
51
NOXIPTYLINE-M (NOR-DI-HO-) -H2O 2AC
52
OPIPRAMOL AC
53
PROTRIPTYLINE AC
54
PROTRIPTYLINE-M (NOR-) AC
55
PROTRIPTYLINE-M (HO-) AC
56
TRANYLCYPROMINE AC

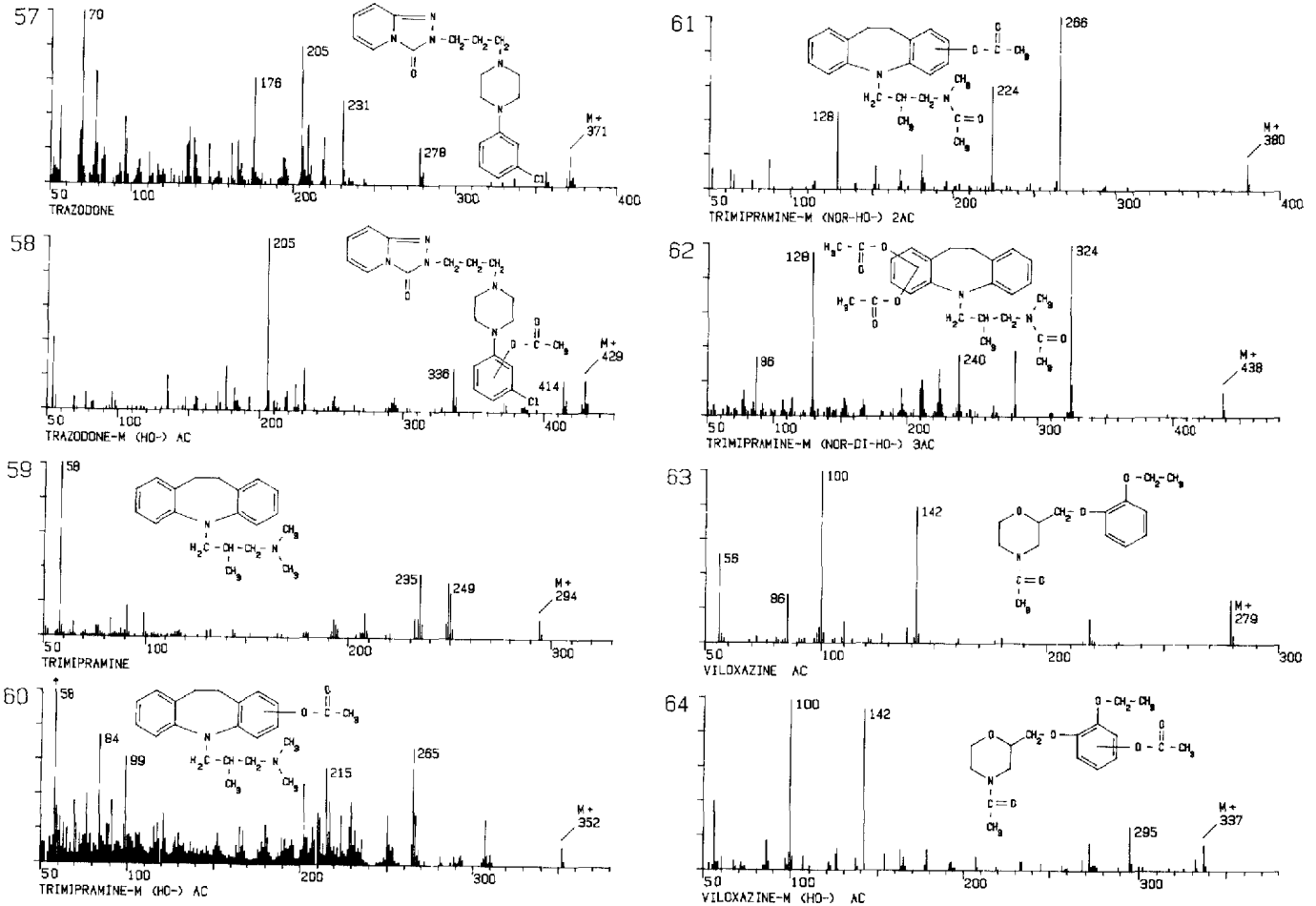


Fig. 1. Mass spectra of antidepressants and their metabolites identified in urine after hydrolysis, extraction and acetylation.

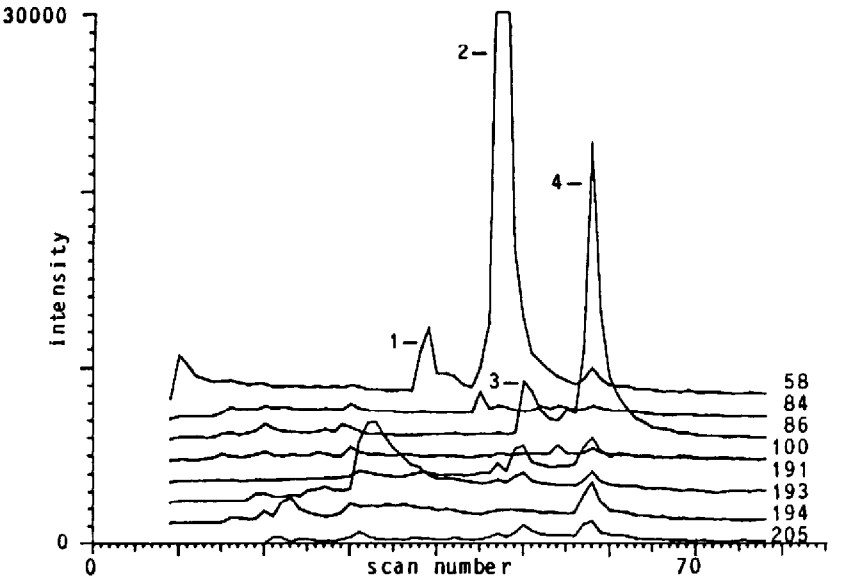


Fig. 2. Mass fragmentogram of doxepin and its metabolites. For identification of peaks, see text.

pramine cannot be differentiated by this screening procedure because lofepramine is not volatile under the GC conditions used. If necessary, the ingestion of lofepramine can be detected by identifying *p*-chlorobenzoic acid after a sample preparation described in the literature [13].

Two kinds of artifacts result from the analytical procedure used. Metabolites with alcoholic hydroxy groups eliminate water, and N-oxides undergo the Cope eliminaton reaction [14]. The mass spectra of these artifacts are included in Fig. 1 (mass spectra Nos. 2, 3, 5, 6, 50 and 51, and Nos. 2, 7, 21 and 23, respectively).

Because all compounds possibly indicated by the mass fragmentogram can be precisely differentiated by comparison of the underlying mass spectra with those of standards (Fig. 1), interference by other drugs is impossible. Furthermore, if the resolution of the peaks is imperfect, a temperature programme with a lower rate or an isothermic procedure can be used. Where there is still doubt a capillary column can help to obtain sufficient resolution.

To illustrate the method, a mass fragmentogram from a psychiatric patient is shown in Fig. 2. Peak 1 indicates doxepin (mass spectrum No. 22), peak 2 the two isomers of hydroxydoxepin (mass spectrum No. 24), peak 3 nordoxepin (mass spectrum No. 25) and peak 4 the two isomers of norhydroxydoxepin (mass spectrum No. 26). This example shows that the presented screening procedure allows a rapid and exact identification and differentiation of antidepressants and their metabolites in urine.

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