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

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SUMMARY

A method for the identification of phenothiazine and analogous neuroleptics 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 *m/e* 58, 72, 86, 98, 100, 113, 114, 141 and 132, 148, 154, 191, 198, 199, 243, 267. The identity of positive signals in the reconstructed mass fragmentograms is established by comparison of the stored entire mass spectra with those of standards. The mass fragmentograms, the underlying mass spectra and the gas chromatographic retention indices (OV-101) are documented.

INTRODUCTION

Screening for phenothiazine and analogous neuroleptics is necessary in analytical toxicology to diagnose a probable intoxication. Furthermore, neuroleptics are encountered frequently in analysis when monitoring patients who may have taken addictive drugs and simultaneously take neuroleptics therapeutically. Detection of some of these drugs using ultraviolet spectrophotometry [2], paper chromatography [3], thin-layer chromatography [3—6], gas chromatography [6—8] and mass spectrometry [9] has been described. However, none of these methods allows the rapid and exact identification of all neuroleptics and their metabolites. This is important in clinical

*These results were reported in part at the Jahrestagung der Deutschen Gesellschaft für klinische Chemie in Stuttgart, F.R.G., September 23—24, 1982 [1].

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or forensic estimation of an intoxication because the various neuroleptics have different pharmacological potencies. Furthermore, all chromatographic spots or peaks must be identified because any of these may represent a potential poison. These demands are met by the computerized gas chromatographic—mass spectrometric (GC—MS) technique described below. This method has the further advantage that several other groups of drugs can be detected simultaneously by simply searching for typical fragment masses in the stored spectra.

If necessary, plasma levels of the identified phenothiazines can be determined using a GC or a GC—MS procedure described in the literature [10—13].

EXPERIMENTAL

Apparatus

A Varian Aerograph gas chromatograph series 1400 combined with a Varian mass spectrometer type 311 A, a Varian data system 111 MS and a Tektronix storage display unit type 611 was used. The GC conditions were as follows. Column: nickel-capillary 60 cm × 1 mm I.D., packed with Chromosorb G HP 100—120 mesh coated with 5% OV-101. Column temperature: programmed from 100 to 310°C at 20°C/min, final time 3 min. Injector port temperature: 270°C. Carrier gas: helium, flow-rate 7 ml/min.

The MS conditions were as follows: ionization energy, 90 eV; ion-source temperature, 200°C. The technique of open coupling was used. About 2 ml/min of gas were dosed by an SGE micro-needle-valve and an SGE shut-off valve (Scientific Glass Engineering, Ringwood, Australia) and introduced into the ion source by a nickel capillary (0.15 mm I.D., heated at 270°C).

For the exact measurement of retention indices a Varian gas chromatograph series 3700 was used. 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 nickel tube, 60 cm × 2 mm I.D., packed as for GC—MS. The column and injector temperatures were as for GC—MS; the temperature of the detectors was 270°C. Carrier gas was nitrogen at a flow-rate of 30 ml/min.

Urine samples

The investigations were carried out using urine from psychiatric in-patients, who were treated with therapeutic dosages of neuroleptics. If no human samples were available urine was used from rats that were given 20—40 mg/kg body weight of an aqueous suspension of the drug by gastric tube (see column "S" in Table I).

Hydrolysis and extraction procedure

Ten millilitres of urine were refluxed with 3 ml of hydrochloric acid (37%) for 15 min, then made basic with about 3 g of potassium hydroxide pellets and mixed with 10 ml of 30% aqueous ammonium sulphate to obtain a pH between 8 and 9. The samples were extracted twice with 10 ml each of a mixture of two parts of dichloromethane, two parts of isopropanol and six parts of ethyl acetate. After phase separation by centrifugation the combined organic extracts

were evaporated to dryness under vacuum. The residue was redissolved in 0.1 ml of methanol [14].

Acetylation

A 40-μl volume 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 in two parts of pyridine. After evaporation of the acetylation mixture the residue was redissolved in 40 μl of ethyl acetate [14]. A 1—4-μl volume of this sample was injected into the gas chromatograph.

Gas chromatographic—mass spectrometric analysis

Mass spectra were recorded at a speed of 6 sec/decade and stored on computer tape during the temperature-programmed GC analysis. Scanning at this relatively slow rate ensures at least two spectra for each GC peak and avoids excessive data accumulation. The identity of positive signals in the reconstructed mass fragmentograms was established by a comparison of the entire mass spectra with those of standards (Fig. 1).

RESULTS AND DISCUSSION

Some of the neuroleptics are excreted in urine completely metabolized and conjugated. 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 our investigations are shown in Table I. The two mass fragmentograms with eight masses each allow the detection of 29 neuroleptics and their metabolites. Some of these compounds are acetylated (see formulae in Fig. 1). Flupenthixol, homofenazine, oxypertine, sulforidazine, tetrabenazine and thiopropazate are not detected in urine because they are almost completely excreted as their metabolites or they are not volatile under the applied GC conditions which are approved for toxicological analysis.

The retention indices were determined using a gas chromatograph combined with flame-ionization detection and nitrogen-sensitive flame-ionization detection with a temperature programme. In our experience, retention indices are not necessary when employing a GC—MS technique, but since they give preliminary indications and may be useful to gas chromatographers without the latter facility, they are given here. The molecular ions and the mass spectra numbers of Fig. 1 are included.

The entire 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 which were usually found are given. Because of inter-individual differences in metabolism or the variable time elapsed after administration, not all given metabolites were detected in each sample. Further metabolites can be found. The mass spectra and retention indices of these will be included in a forthcoming handbook [15].

Although some neuroleptics lead to common metabolites (mass spectra Nos. 1, 5, 7, 8, 18, 29, 45, 70, 80, and 84 in Fig. 1), they can be differentiated because the parent compounds or additional unique metabolites are also

TABLE I

MONITORING PROGRAM FOR PHENOTHIAZINE AND ANALOGOUS NEUROLEPTICS AND THEIR METABOLITES

MS No.	M^+	Name*	Species**	*m/e* (relative intensities)***					
				58	72	86	98	100	113
25	326	Acepromazine	R	+++	+	+			
49	370	M (dihydro-)	R	+++	+	+			
60	384	M (HO-)	R	+++	+	+			
40	354	M (nor-)	R	+	+	+		+	
23	326	Acepromethazine	M	+	+++				
48	370	M (dihydro-)	M	+	+++				
39	354	M (nor-)	R	+++	+			+	
59	384	M (HO-)	M	+	+++				
13	298	Alimenazine	M	+++		+		+	
01	199	M (ring)	M						
42	356	M (HO-)	M	+++		+		+	
24	326	M (nor-)	M	+	+	++			
18	312	M (bis-nor-)	M		+				
58	384	M (nor-HO-)	M			+			
74	409	Butaperazine	R	+	+		+		++
81	437	M (nor-)	R	+		+			
22	318	Chlorpromazine	M	+++	+	++			
05	233	M (ring)	M						
29	332	M (bis-nor-)	M		+		+++		
37	346	M (nor-)	M	+	+	+		+	
21	315	Chlorprothixene	M	+++					
08	270	M (N-oxide)	M						
55	375	M (HO-dihydro-)	M	+++					
36	345	M (nor-dihydro-)	M	+					
35	343	M (nor-)	M			+			
71	403	M (nor-HO-dihydro-)	M		+	+			
83	442	Clopenthixol	R				++		
08	270	M (N-oxide)	R						
69	400	M (desalkyl-dihydro-)	R				+		
67	398	M (desalkyl-)	R			+			
26	326	Clozapine	M						
41	396	M (nor-)	M			+			
65	354	M (nor-) monoacetyl-	M						
86	469	Dixyrazine	R	+			+		+
01	199	M (ring)	R						
18	312	M (amino-)	M		+				
77	425	M (O-desalkyl-)	R				+		
57	381	M (N-desalkyl-)	R						
		Flupenthixol							
87	478	M (dihydro-)	R				+		
79	434	M (desalkyl-dihydro-)	R				+		

114	141	132	148	154	191	198	199	243	267	Retention index
						+				2757
										2765
										3041
+++						+	+			3143
						+				2626
										2690
++										2940
+										3026
						+				2313
						+	+++			2080
										2600
						++	+			2709
++						+	+			2767
										2930
	+					+				3188
	+++					++				3800
										2500
						++	+			2099
						+				2990
+++						+				3068
										2510
										2409 FID
										2800
+							+			2930
										2945
+										3194
										3462
										2409 FID
	+++									3450
	+++									3490
					+			+++		2893
					+			+	+	3492
					+			++		3650
						+	+++			3531
						+	+++			2080
++						+	+			2767
						+	+			3350
	+++					+	+			3355
									+	3004
	++									3054

(Continued on p. 130)

TABLE I (continued)

MS No.	M^+	Name*	Species**	*m/e* (relative intensities)***					
				58	72	86	98	100	113
88	479	Fluphenazine	R	+			+		+
07	267	M (ring)	R						
45	366	M (amino-)	R	+	+			+++	
80	435	M (desalkyl-)	R	+		+			
		Homofenazine							
07	267	M (ring)	R						
45	366	M (amino-)	R	+	+			+++	
85	449	M (desalkyl-)	R	+			+		+
27	328	Levomepromazine	M	+++				+	
62	386	M (HO-)	M	+++				+	
43	356	M (nor-)	M	+		+		+	
76	414	M (nor-HO-)	M	+		+			
		Oxypertine							
03	204	M (phenylpiperazine)	R						
06	262	M (HO-phenylpiperazine)	R						
16	310	Pecazine	R	+++			+		
01	199	M (ring)	R						
47	368	M (HO-)	R	+++			+		
30	338	M (nor-)	R				+		
64	396	M (nor-HO-)	R				+		
31	339	Perazine	M	+	+				+
01	199	M (ring)	M						
66	397	M (HO-)	M	++					+
46	367	M (nor-)	M	+		+		+	
73	407	Periciazine	R				+		
04	224	M (ring)	R						
84	445	Perphenazine	R			++	++		+
05	233	M (ring)	R						
29	332	M (amino-)	R		+			+++	
70	401	M (desalkyl-)	R	+					
54	373	Prochlorperazine	R	+	+				++
05	233	M (ring)	R						
29	332	M (amino-)	R		+			+++	
70	401	M (nor-)	R	+					
11	284	Promazine	M	+++	+	+			
01	199	M (ring)	M						
33	342	M (HO-)	M	+++	+	+			
19	312	M (nor-)	M					+	
52	370	M (nor-HO-)	M		+	+		+	
10	284	Promethazine	M		+++				
01	199	M (ring)	M						
17	312	M (nor-)	M	+++	+			+	
32	342	M (HO-)	M	+	+++				
50	370	M (nor-HO-)	M	+	+			+	

114	141	132	148	154	191	198	199	243	267	Retention index
									+	3169
									+++	2190
									+	2765
	+++								++	3150
									+++	2190
									+	2765
	+								+++	3240
										2542
										2747
										2970
										3220
		+++								1872
			+++							2355
						+	+			2546
						+	+++			2080
										2750
						+	+			2985
										3414
	+					+	+			2790
						+	+++			2080
	+									3190
+	+						+++			3212
+										3391
					+					2552
							+			3468
						++	+			2099
						+				2990
	+++									3500
	+					+				2983
						++	+			2099
						+				2990
	+++									3500
						+	+			2313
						+	+++			2080
										2709
+++						+	+			2804
+++										3196
						+	+			2272
						+	+++			2080
++										2640
										2690
+++										3017

(Continued on p. 132)

TABLE I (continued)

MS No.	M^+	Name*	Species**	*m/e* (relative intensities)***					
				58	72	86	98	100	113
12	285	Prothipendyl	M	+++	+	++			
02	200	M (ring)	M						
34	343	M (HO-)	M	+++	+	+			
14	299	M (bis-nor-)	M		+			+	
20	313	M (nor-)	M	+	+	+		+	
53	371	M (nor-HO-)	M					+	
		Sulforidazine							
9	277	M (ring)	M						
78	430	M (nor-)	R						
		Tetrabenazine							
28	331	M (bis-desmethyl-)	M						
44	361	M (desmethyl-HO-)	M						
63	389	M (bis-desmethyl-HO-)	M						
84	445	Thiopropazate	R			++	++		+
05	233	M (ring)	R						
29	332	M (amino-)	R		+			+++	
70	401	M (desalkyl-)	R	+					
		Thioproperazine							
15	306	M (ring)	R						
51	370	Thioridazine	M	+			+++		
68	398	M (nor-)	M						
61	384	M (oxo-)	M	+					+
72	407	Trifluoperazine	R	+					++
07	267	M (ring)	R						
45	366	M (amino-)	R	+	+			+++	
80	435	M (nor-)	R	+		+			
38	352	Triflupromazine	R	+++	+	+			
07	267	M (ring)	R						
75	410	M (HO-)	R	+++		+			
56	380	M (nor-)	R		+	+		+	
45	366	M (bis-nor-)	R	+	+			+++	
82	438	M (nor-HO-)	R	+	+	+		+	

*M ( ) = metabolite, HO- = hydroxy
**M = man, R = rat.
***+++ = > 95% relative intensity, ++ = 50—95%, + = < 50%.

excreted. Only perphenazine and its acetyl ester thiopropazate cannot be differentiated.

The Cope elimination reaction of the N-oxides of chlorprothixene and clopenthixol is the only kind of artifact which results from the analytical procedure used (mass spectrum No. 8).

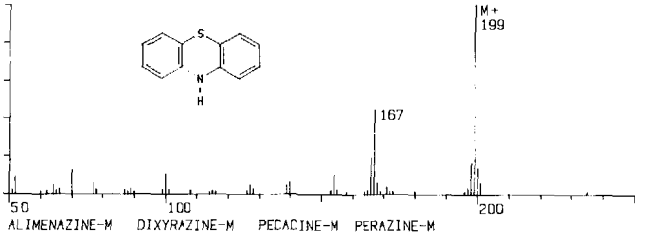
Because all compounds possibly indicated by the mass fragmentograms can be positively identified by comparison of the underlying mass spectra with those of standards (Fig. 1), interferences by other drugs are impossible.

114	141	132	148	154	191	198	199	243	267	Retention index
							+			2348
							+			2049
							+			2781
							+			2830
+++							+			2880
+++										3070
						+				3180
				+++		+				3800
					+++					2510
					+					2584
					++					2666
							+			3468
						++	+			2099
						+				2990
	+++									3500
						++	+			3200
										3125
				+++						3490
						+	+			3500
	+								+	2683
									+++	2190
									+	2765
	+++								++	3150
									+	2239
									+++	2190
										2720
+++									+	2740
									+	2765
+++									+	3120

To illustrate the method two mass fragmentograms from the urine of a psychiatric patient are shown in Fig. 2. Peak 1 indicates thioridazine, peak 2 its N-desmethyl metabolite and peak 3 its oxo metabolite (mass spectra Nos. 51, 68, 61).

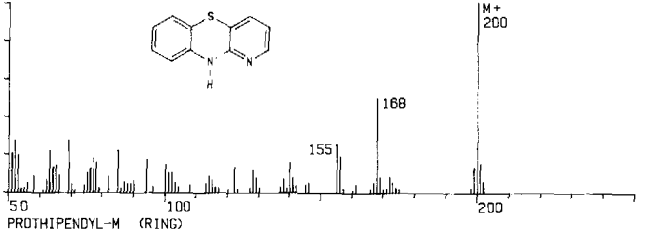
The presented screening procedure allows a rapid and exact identification and differentiation of phenothiazine and analogous neuroleptics and their metabolites in urine. It has the advantage that other groups of drugs can be detected simultaneously by simply searching for typical fragment masses in the

1



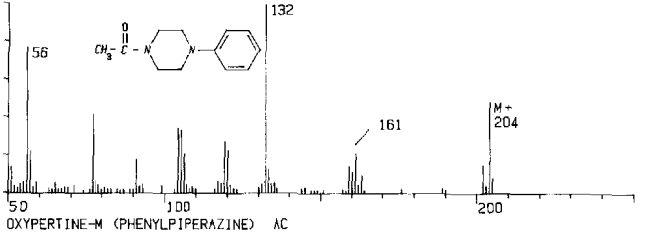
ALIMENAZINE-M DIXYRAZINE-M PECACINE-M PERAZINE-M
PROMAZINE-M PROMETHAZINE-M

2



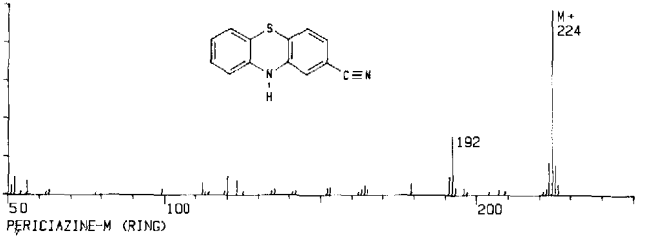
PROTHIPENDYL-M (RING)

3



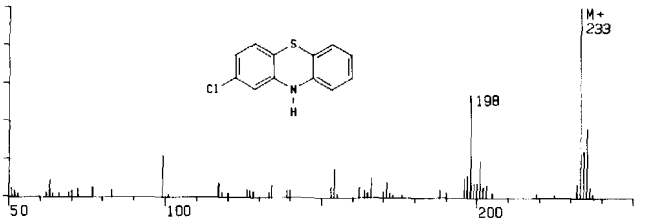
OXYPERTINE-M (PHENYLPIPERAZINE) AC

4



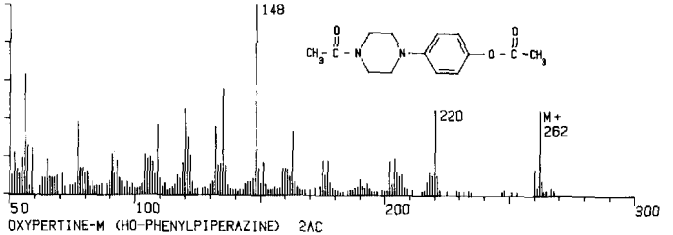
PERICIAZINE-M (RING)

5



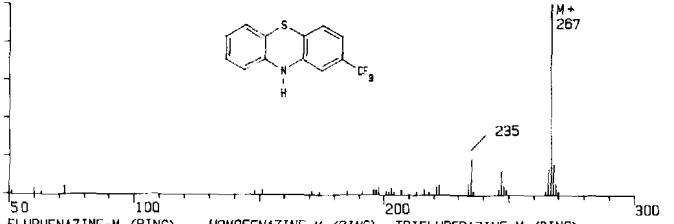
CHLORPROMAZINE-M PERPHENAZINE-M PROCHLORPERAZINE-M THIOPROPAZATE-M

6



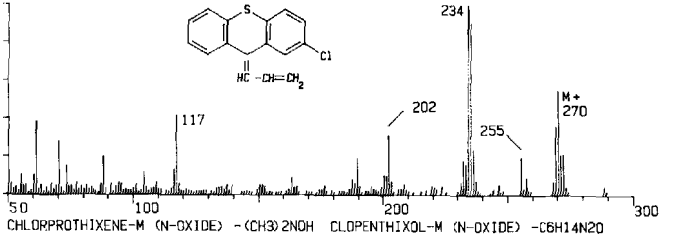
OXYPERTINE-M (HO-PHENYLPIPERAZINE) 2AC

7

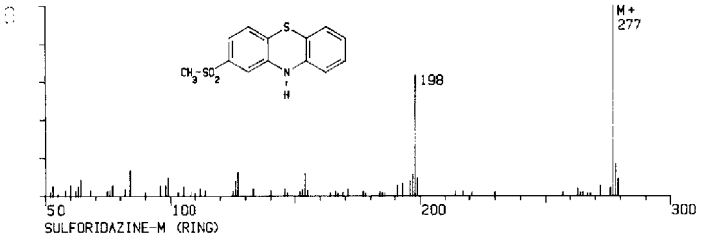


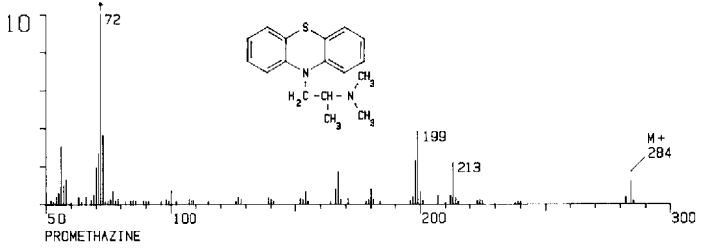
FLUPHENAZINE-M (RING) HOMOFENAZINE-M (RING) TRIFLUPERAZINE-M (RING)
TRIFLUPROMAZINE-M (RING)

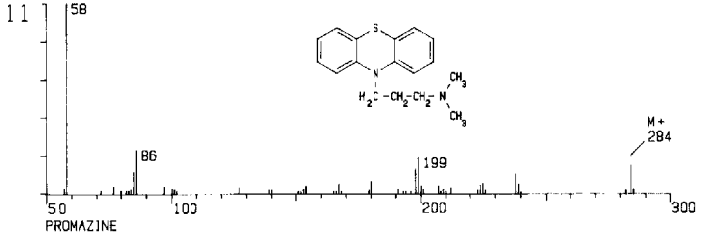
8

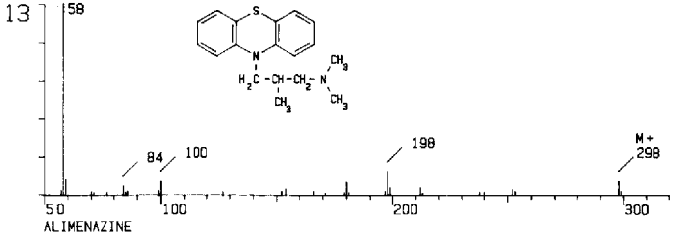


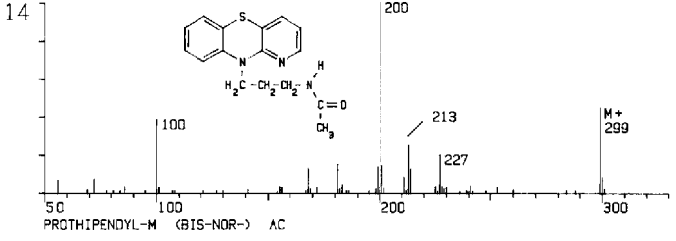
CHLORPROTHIXENE-M (N-OXIDE) -(CH3)2NOH CLOPENTHIXOL-M (N-OXIDE) -C6H14N2O

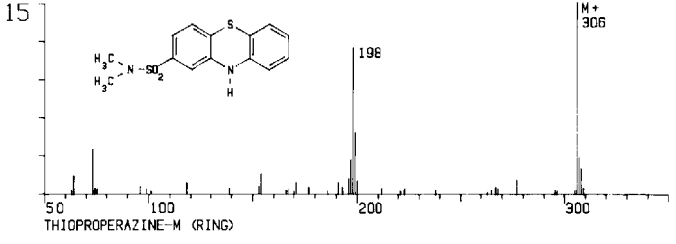












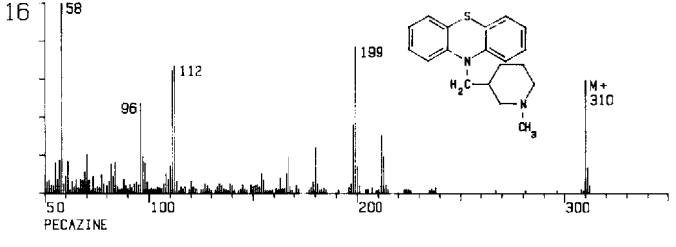
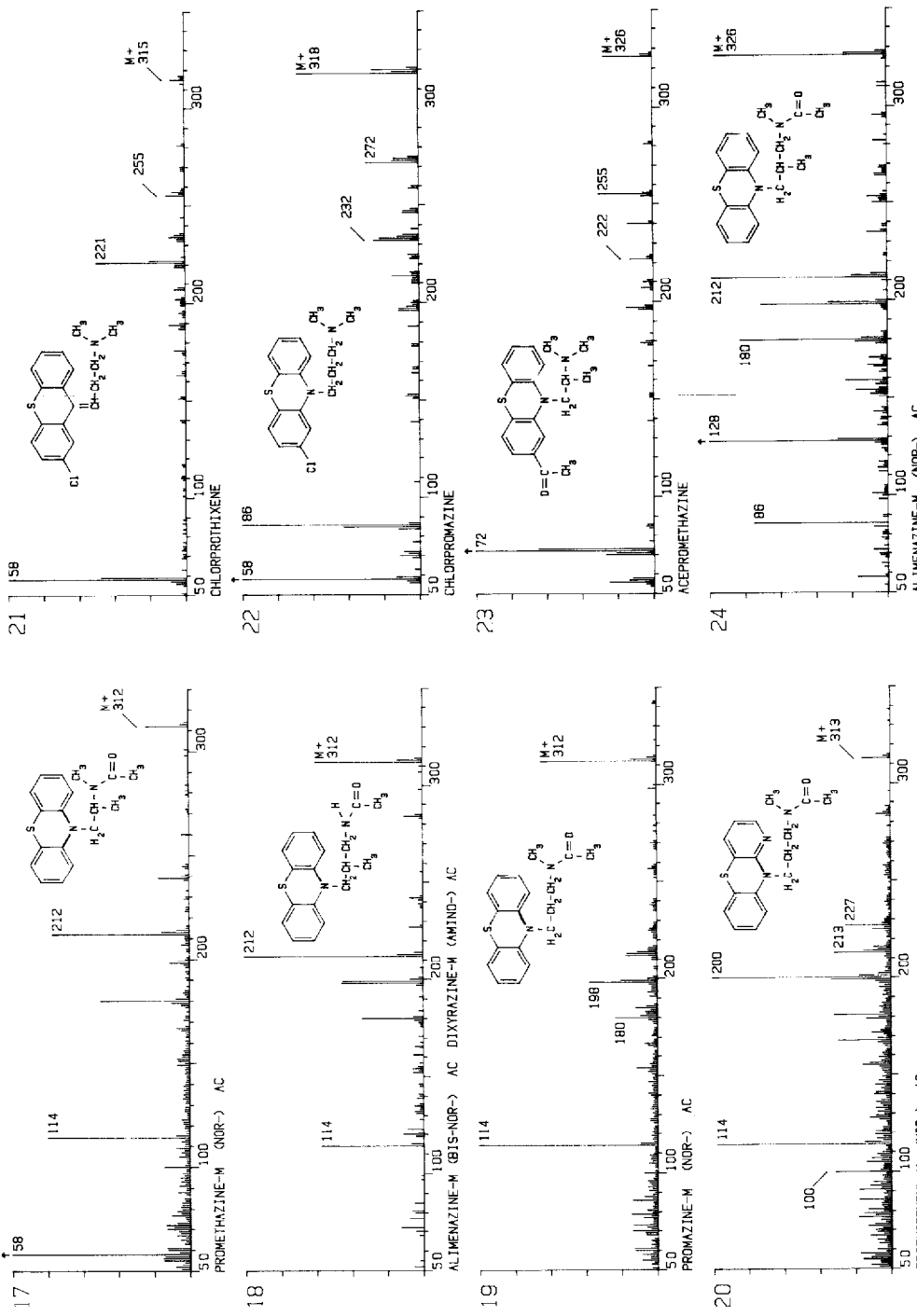


Fig. 1.

(Continued on p. 136)


17
PROMETHAZINE-M (NOR-) AC
18
ALIMENAZINE-M (BIS-NOR-) AC
DIXYRAZINE-M (AMINO-) AC
19
PROMAZINE-M (NOR-) AC
20
PROTHIPENDYL-M (NOR-) AC
21
CHLORPROTHIXENE
22
CHLORPROMAZINE
23
ACEPROMETHAZINE
24
ALIMENAZINE-M (NOR-) AC

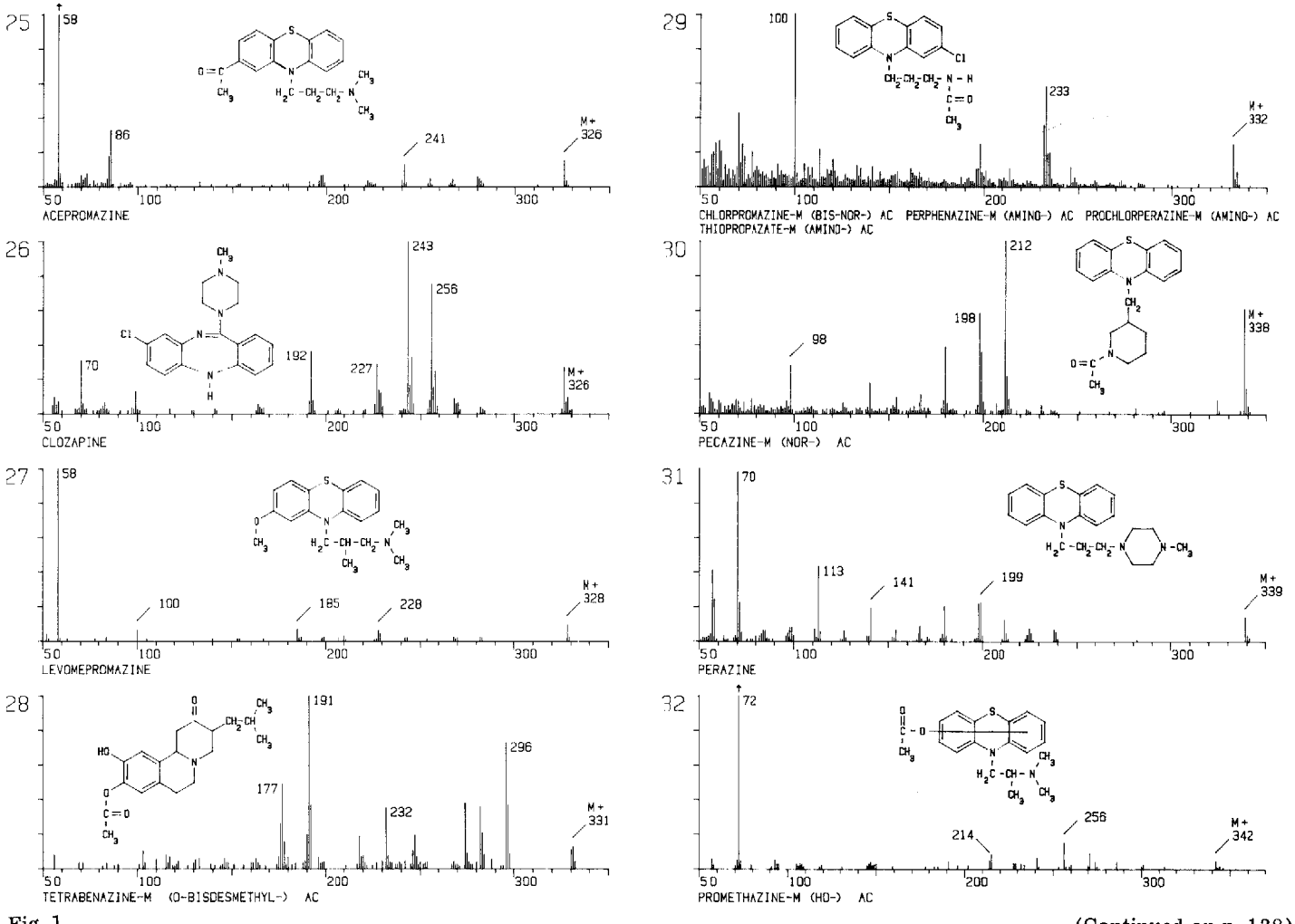
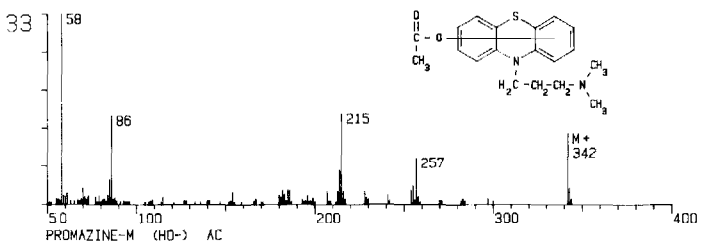
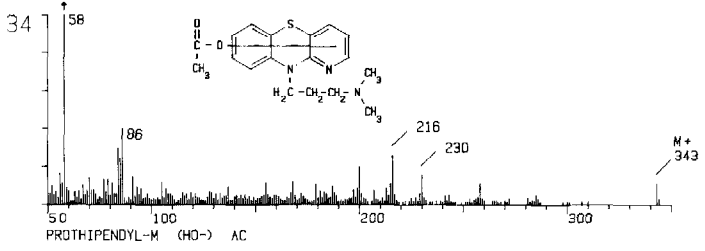
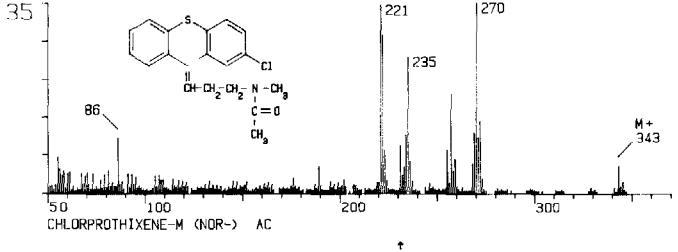


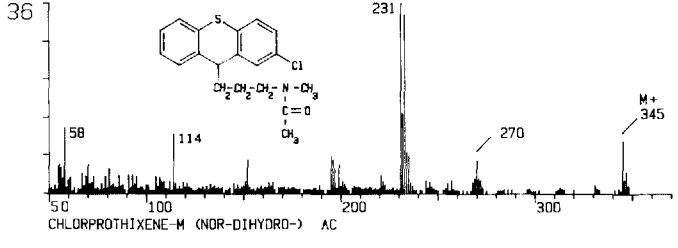
Fig. 1.

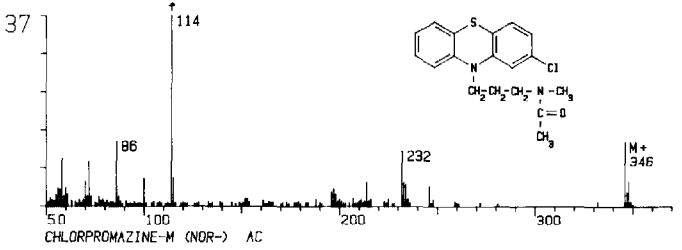
(Continued on p. 138)

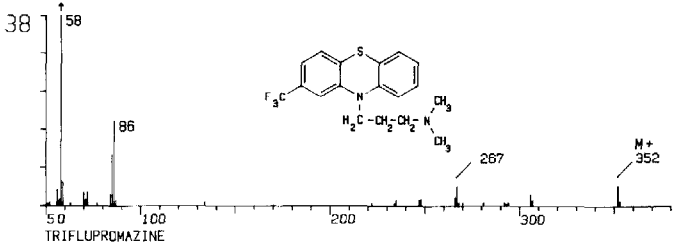

33
58
86
215
257
M+
342
50
100
200
300
400
PROMAZINE-M (HO-) AC

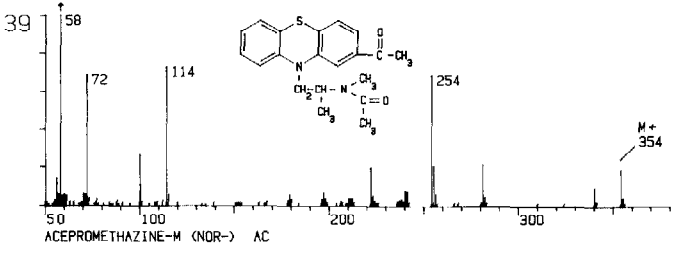

34
58
86
216
230
M+
343
50
100
200
300
PROTHIPENDYL-M (HO-) AC

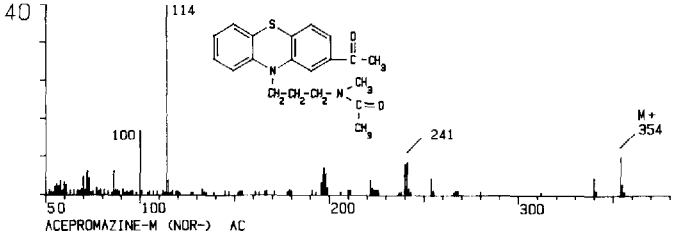

35
221
270
235
86
M+
343
50
100
200
300
CHLORPROTHIXENE-M (NOR-) AC


36
231
58
114
270
M+
345
50
100
200
300
CHLORPROTHIXENE-M (NOR-DIHYDRO-) AC


37
114
86
232
M+
346
50
100
200
300
CHLORPROMAZINE-M (NOR-) AC


38
58
86
267
M+
352
50
100
200
300
TRIFLUPROMAZINE


39
58
72
114
254
M+
354
50
100
200
300
ACEPROMETHAZINE-M (NOR-) AC


40
114
100
241
M+
354
50
100
200
300
ACEPROMAZINE-M (NOR-) AC

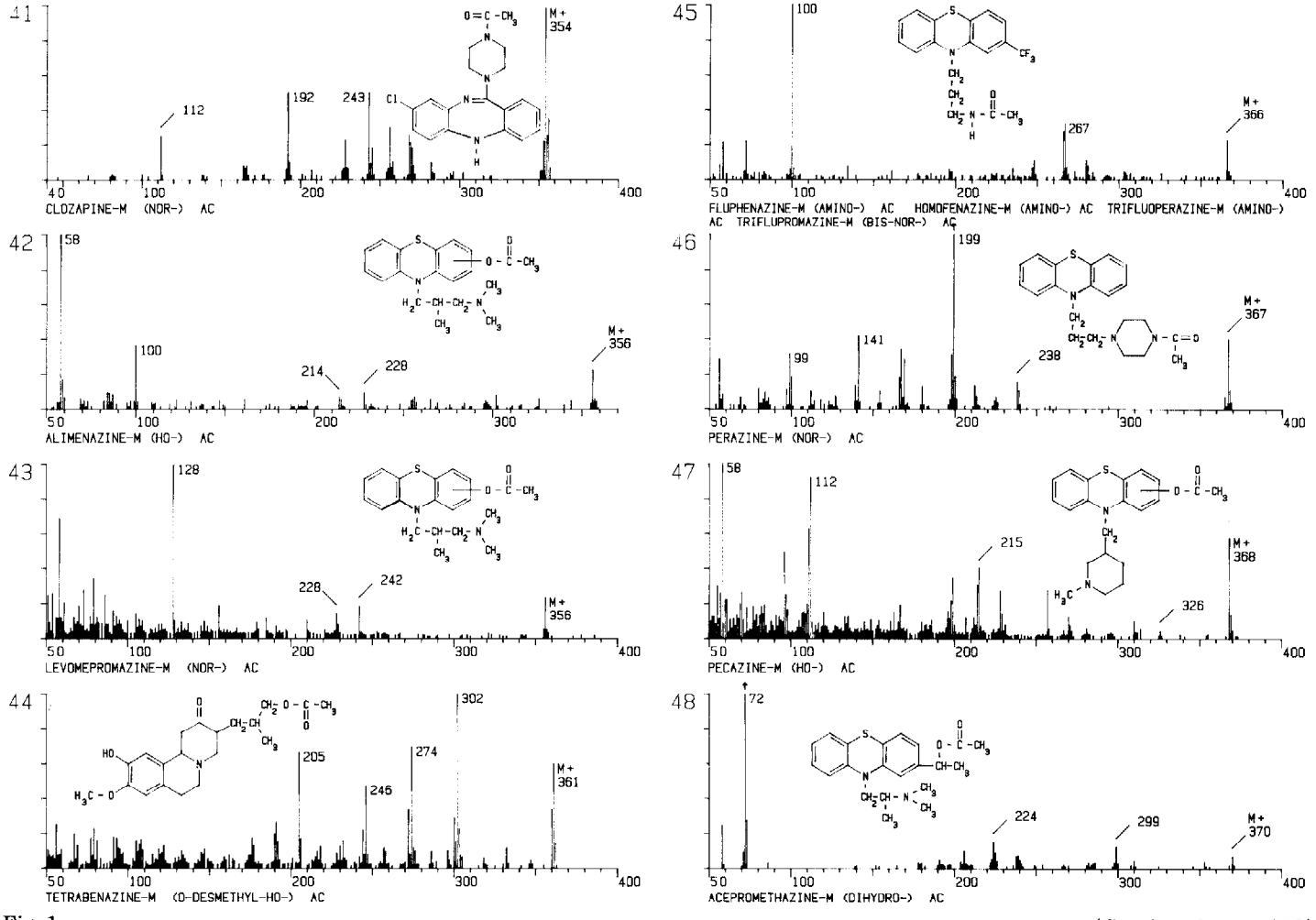
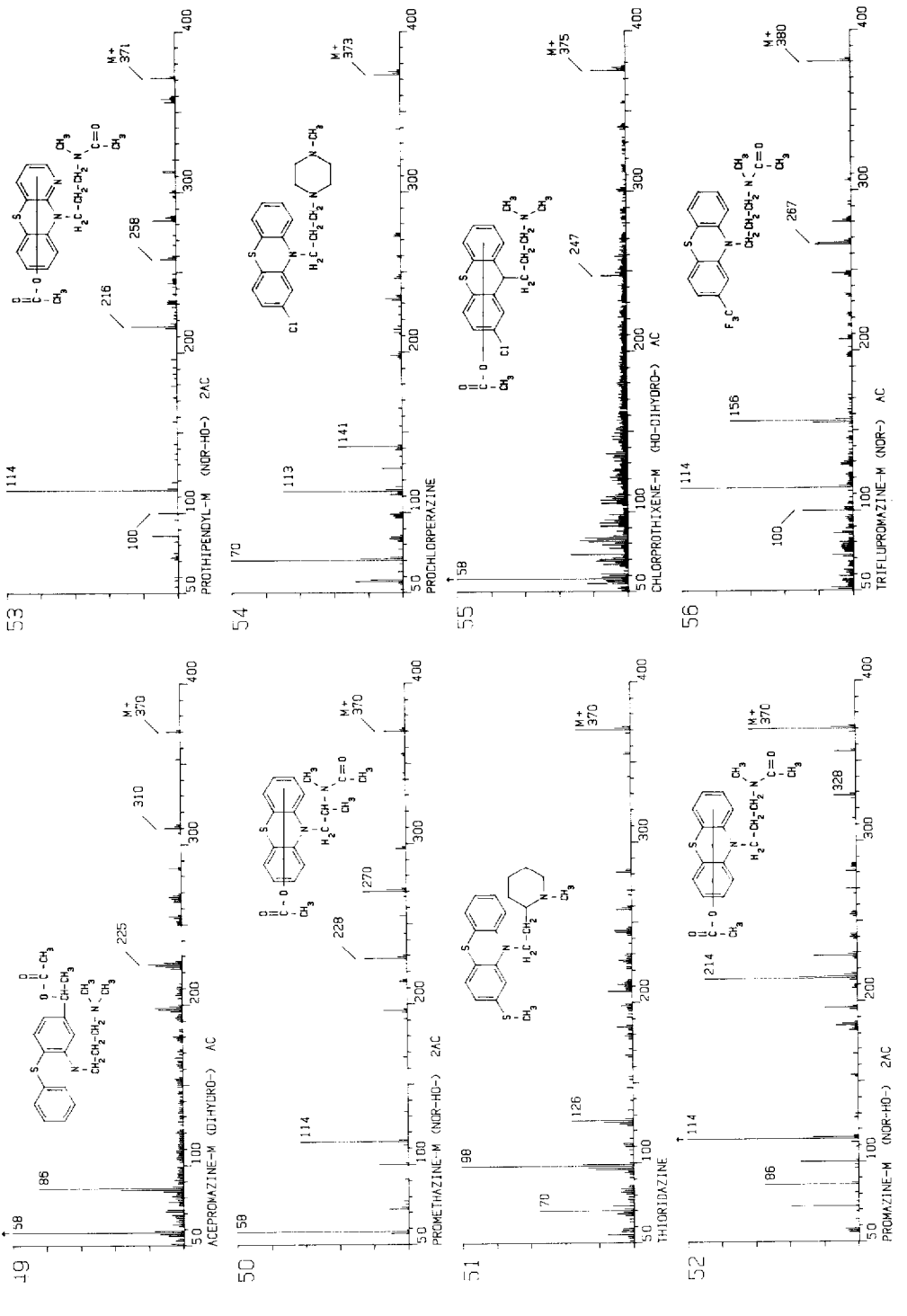
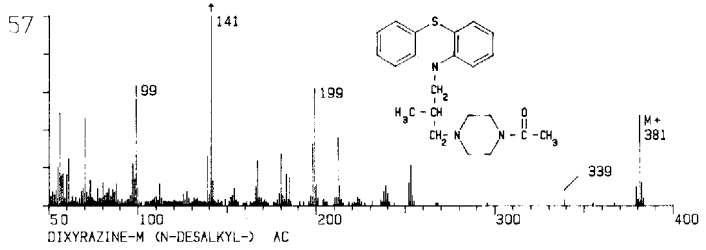
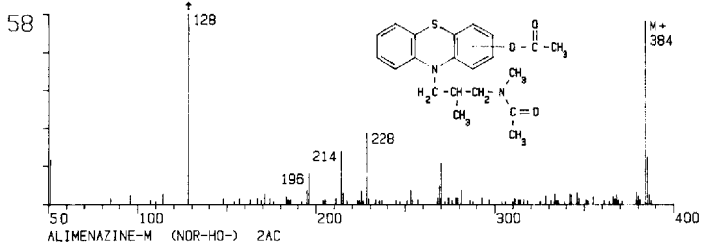


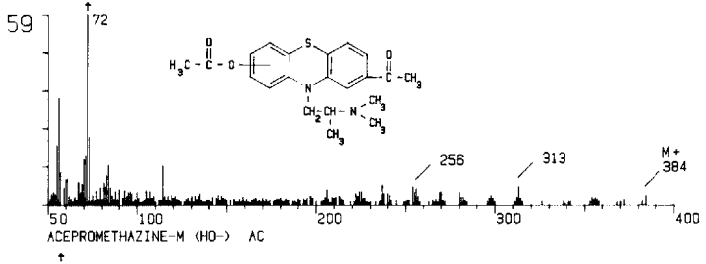
Fig. 1.

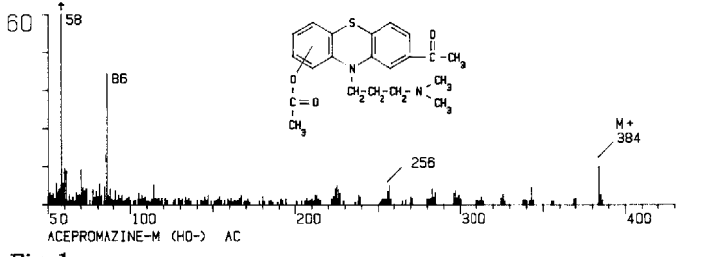
(Continued on p. 140)

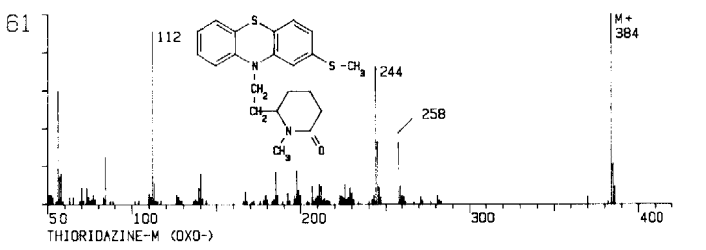

49
ACEPROMAZINE-M (DIHYDRO-) AC
50
PROMETHAZINE-M (NOR-HO-) 2AC
51
THIORIDAZINE
52
PROMAZINE-M (NOR-HO-) 2AC
53
PROTHIPENDYL-M (NOR-HO-) 2AC
54
PROCHLORPERAZINE
55
CHLORPROTHIXENE-M (HO-DIHYDRO-) AC
56
TRIFLUPROMAZINE-M (NOR-) AC

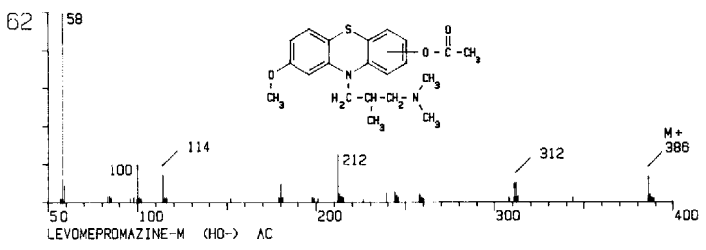


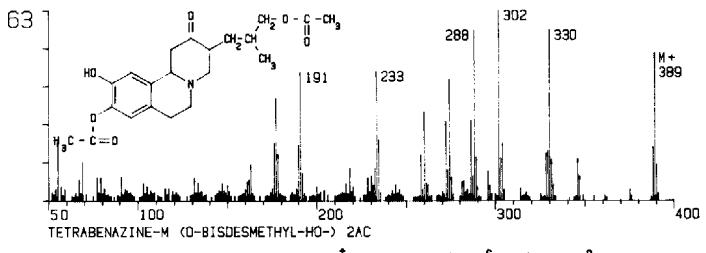












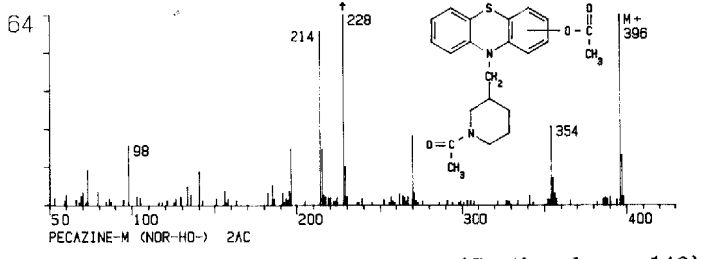
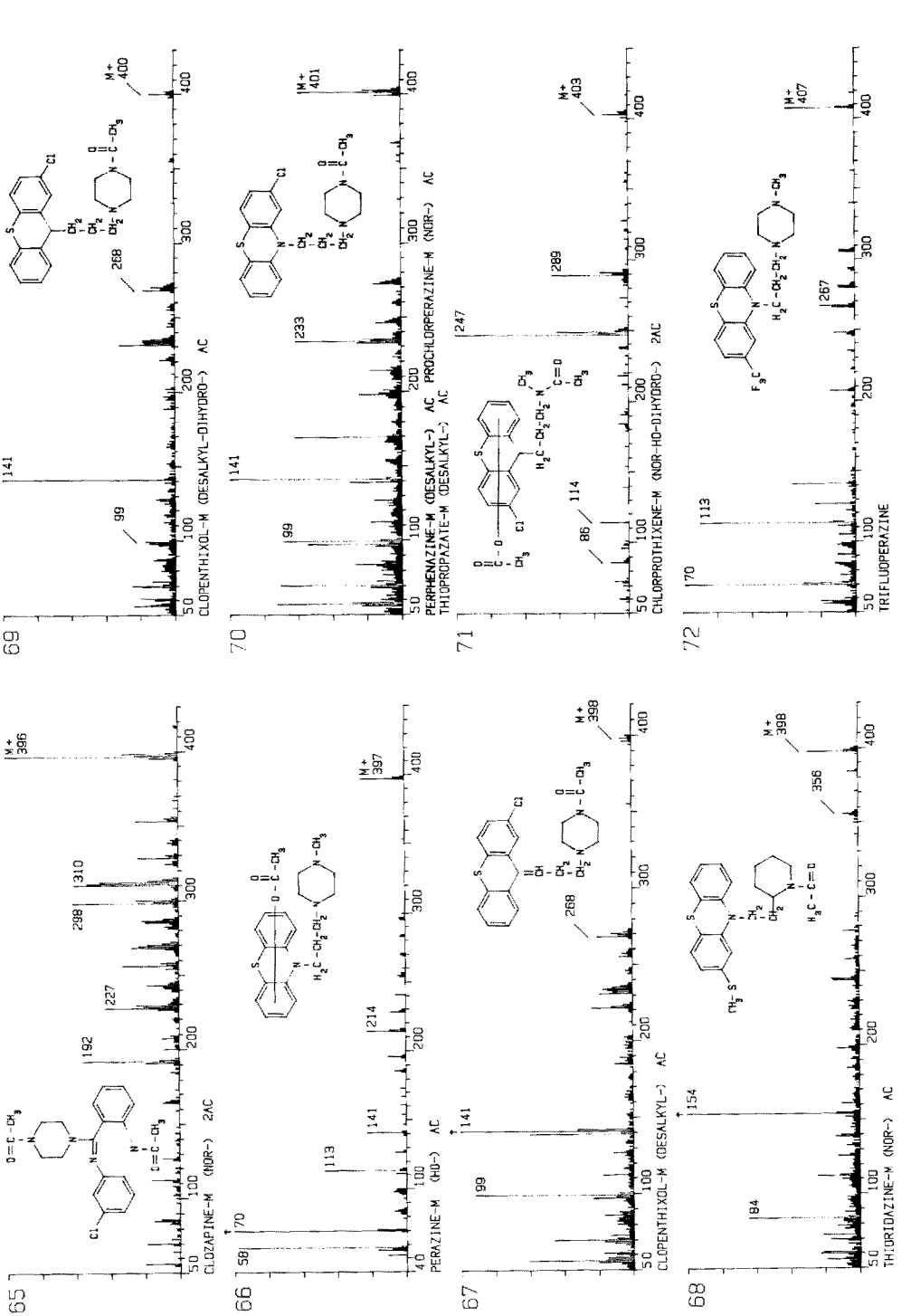


Fig. 1.

(Continued on p. 142)


65
CLOZAPINE-M (NOR-) 2AC
66
PERAZINE-M (HO-) AC
67
CLOPENTHIXOL-M (DESALKYL-) AC
68
THIORIDAZINE-M (NOR-) AC
69
CLOPENTHIXOL-M (DESALKYL-DIHYDRO-) AC
70
PERPHENAZINE-M (DESALKYL-) AC PROCHLORPERAZINE-M (NOR-) AC
THIOPROPAZATE-M (DESALKYL-) AC
71
CHLORPROTHIXENE-M (NOR-HO-DIHYDRO-) 2AC
72
TRIFLUOPERAZINE

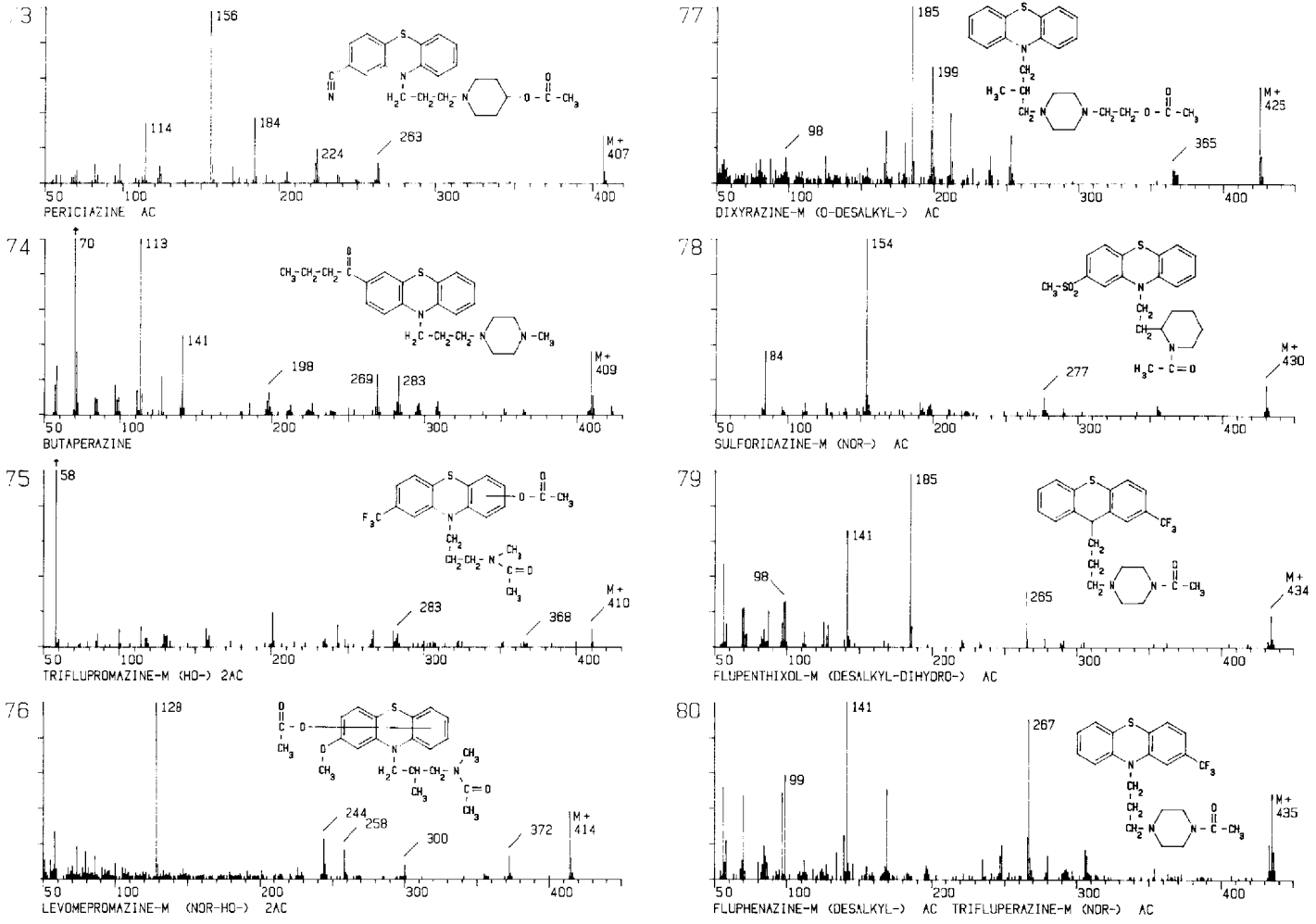


Fig. 1.

(Continued on p. 144)

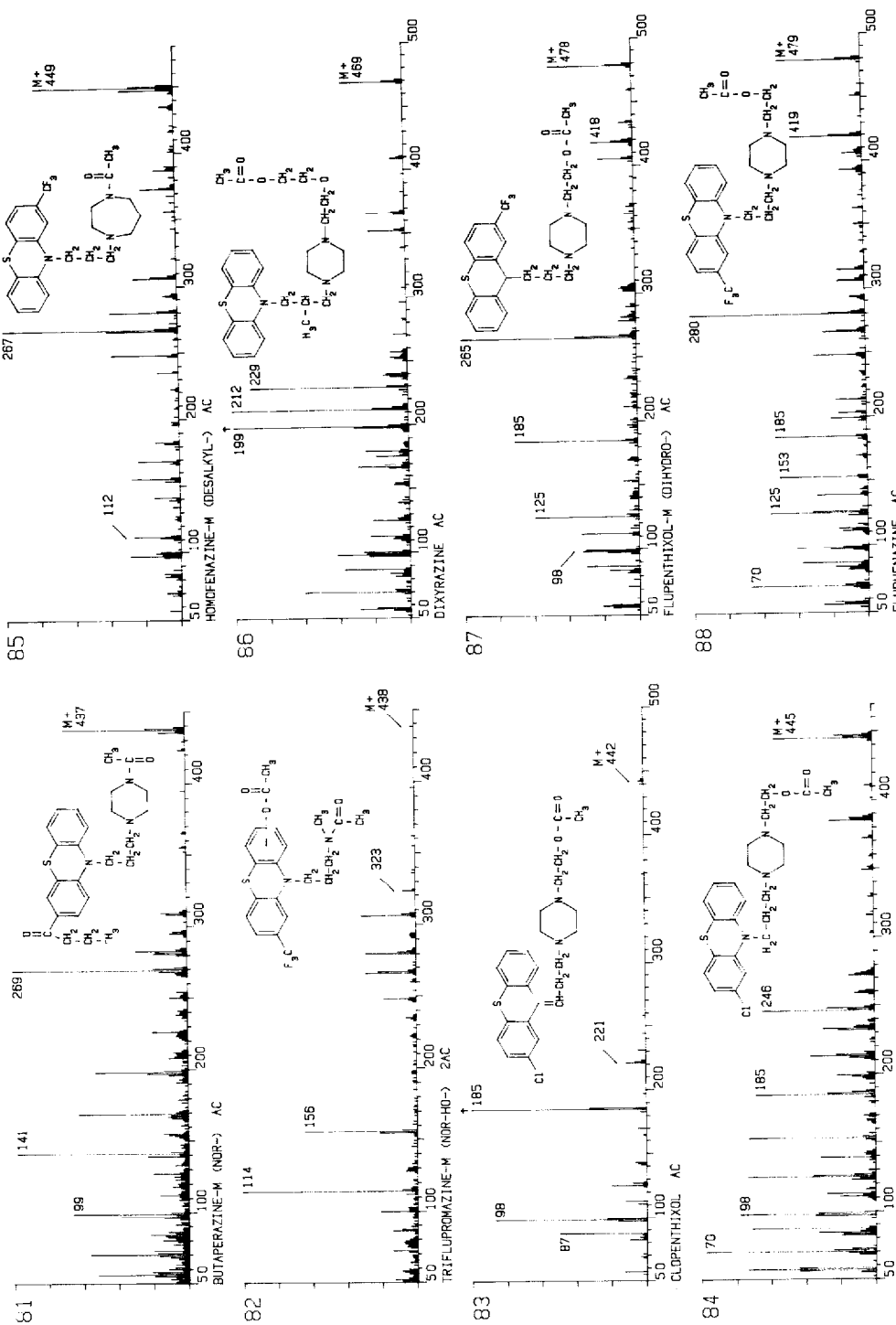


Fig. 1. Mass spectra of phenothiazine and analogous neuroleptics and their metabolites identified in urine after hydrolysis, extraction and acetylation.

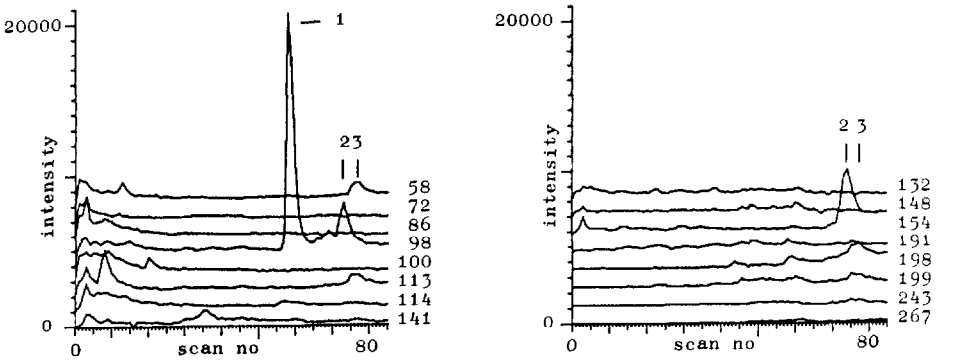


Fig. 2. Mass fragmentograms indicating thioridazine (1), nor-thioridazine (2) and oxo-thioridazine (3).

stored spectra. Such mass fragmentograms typical for benzodiazepines [16], butyrophenone and bisfluorophenyl neuroleptics [17, 18], anti-inflammatory analgesics [19], opioids and other potent analgesics [20] and antidepressants [21] have been published previously. Screening for antiparkinsonian drugs is in preparation [22]. Similar data of 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 h.

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