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SCREENING PROCEDURE FOR DETECTING BUTYROPHENONE AND BISFLUOROPHENYL NEUROLEPTICS IN URINE USING A COMPUTERIZED GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC TECHNIQUE*

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SUMMARY

A method for the identification of butyrophenone and bisfluorophenyl neuroleptics and their predominant basic 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* 112, 123, 134, 148, 169, 257, 321 and 189, 191, 223, 233, 235, 245, 287, 297. The identity of positive signals in the reconstructed mass fragmentograms is established by a comparison of the entire mass spectra with those of standards. The mass fragmentograms and the underlying mass spectra are documented.

INTRODUCTION

Screening for butyrophenone and bisfluorophenyl neuroleptics is necessary in analytical toxicology to diagnose a probable intoxication. Only an assay for differentiation of some of these neuroleptics employing thin-layer chromatography and gas—liquid chromatography [2] has been described. However, this assay does not allow the rapid and exact identification of all drugs or their metabolites. This is important in clinical or forensic estimations because the various neuroleptics have very different pharmacological potencies. These demands are met by the computerized gas chromatographic—mass spectro-

*This work is part of the Thesis of H. Maurer at the Universität des Saarlandes, Saarbrücken, G.F.R. Part of these results were reported at the 23. Frühjahrstagung der Deutschen Pharmakologischen Gesellschaft (DPhG), in Mainz, G.F.R., March 16th, 1982 [1].

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metric (GC—MS—DS) technique described below. If necessary plasma levels of the identified drugs can be determined using a GC procedure [3—7] or a radioimmunoassay [8—10] described in the literature.

EXPERIMENTAL

Apparatus

The apparatus used for this study has been previously described [11].

Hydrolysis and extraction procedure

Ten millilitres of urine were refluxed with 3 ml of hydrochloric acid (37%) for 15 min, then made alkaline with about 3 g of potassium hydroxide pellets and mixed with 10 ml of 30% aqueous ammonium sulfate to obtain a pH of between 8 and 9. The extraction procedure [11] was modified so that samples were extracted twice with 10 ml each of a mixture of two parts dichloromethane, two parts isopropanol and six parts 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.

Acetylation

Forty microlitres of extract were evaporated and then acetylated for 30—60 min at 60°C with 40 μl of a mixture of three parts acetic anhydride in two parts pyridine. After evaporation of the acetylation mixture the residue was redissolved in 40 μl of ethyl acetate [12]. One to four microlitres of this were injected into the gas chromatograph.

Gas chromatographic—mass spectrometric analysis

The GC—MS—DS analysis procedure used in this study has been previously described [11].

RESULTS AND DISCUSSION

Some of the butyrophenone and bisfluorophenyl neuroleptics are excreted in urine completely metabolized and conjugated. Therefore conjugates were decomposed by acid hydrolysis which can be completed more quickly than enzymatic hydrolysis. The former extraction procedure [11] was modified to improve the extraction of polar metabolites. In addition, hydroxy and amino groups were acetylated to improve the GC characteristics. The acetylated extract was redissolved in ethyl acetate to avoid solvolysis of the acetylated compounds by methanol.

The results of our investigations are shown in Table I. The two mass fragmentograms with eight masses each allow the detection of twelve butyrophenone and bisfluorophenyl neuroleptics or their predominant basic metabolites. Some of the metabolites are acetylated.

The retention indices were determined using a gas chromatograph combined with flame ionization detection (FID) and nitrogen-sensitive FID with a temperature program [11]. In our experience retention indices are not necessary when employing the GC—MS technique but give preliminary indications

TABLE I

MONITORING PROGRAMS FOR BUTYROPHENONE AND BISFLUOROPHENYL NEUROLEPTICS

MS No.	Drug/metabolite	*m/e* (relative intensity, %)								Retention index
		112	123	134	148	169	257	259	321	
01	Benperidol M*			60				100		2750
02	Droperidol M*			100						1730
03	Fluanisone		60	10						2794
04	Fluanisone M I*			10	100					2140
05	Fluanisone M II*		100	10	40					2830
06	Melperone	100	30							1889
07	Melperone M I*	100								1837
08	Melperone M II*	100								2163
09	Moperone		95			40				2828
10	Moperone M					100				1600
11	Moperone artifact		100			10				2709
12	Penfluridol M I						100	35		1918
13	Penfluridol M II*								20	2240
02	Pimozide M I*			100						1730
01	Pimozide M II*			60				100		2750
14	Pipamperone		70							3040
15	(Androsterone)*		10	15	10		40			2581 (FID)
		189	191	223	233	235	245	287	297	
16	Bromperidol M I				100	98				1850
17	Bromperidol M II*								35	2335
18	Fluspirilene M*						15	7		2728
19	Haloperidol M I	100	35							1650
20	Haloperidol M II*	30	10			100				2150
21	Trifluperidol M I			100						1570
22	Trifluperidol M II*						5	35		2033
23	Fluorophenyloxobutanal									1490 (FID)
24	Bisfluorophenylbutyric acid									2228 (FID)

*Acetylated.

and may be useful to gas chromatographers without the latter facility and so they are given here.

The entire mass spectra are shown in Fig. 1 for the precise identification of the compounds. Formulae are proposed for probable structures of metabolites. It is possible that some of the metabolites were altered by the analytical procedures.

All investigations were carried out using the urine of man with the exception of bromperidol, fluanisone, fluspirilene, moperone and pimozide which were detected (in the absence of human samples) in the urine of rats. Benperidol, bromperidol, droperidol, fluspirilene, haloperidol, penfluridol, pimozide and trifluperidol 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.

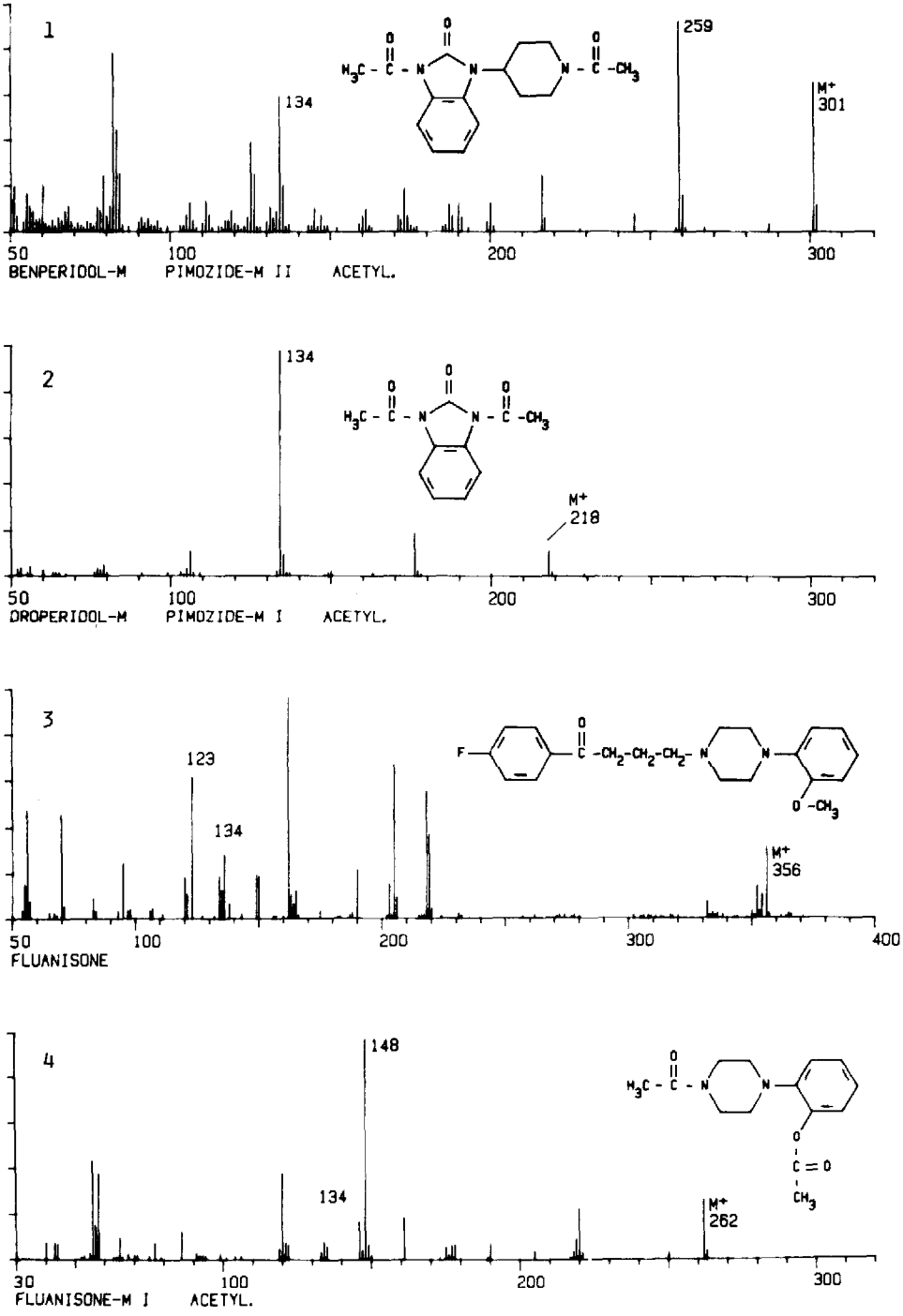
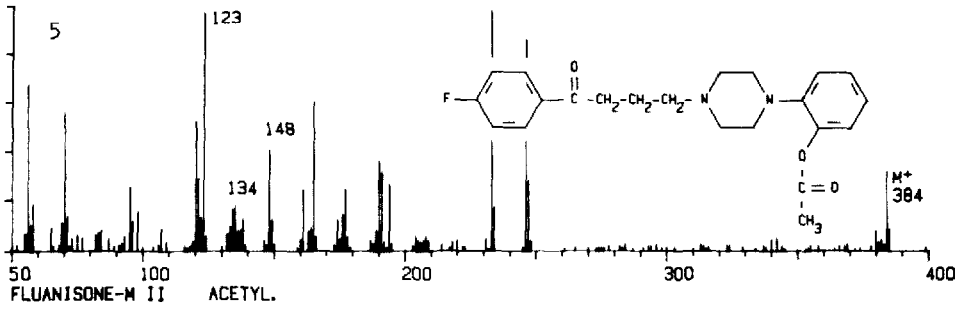
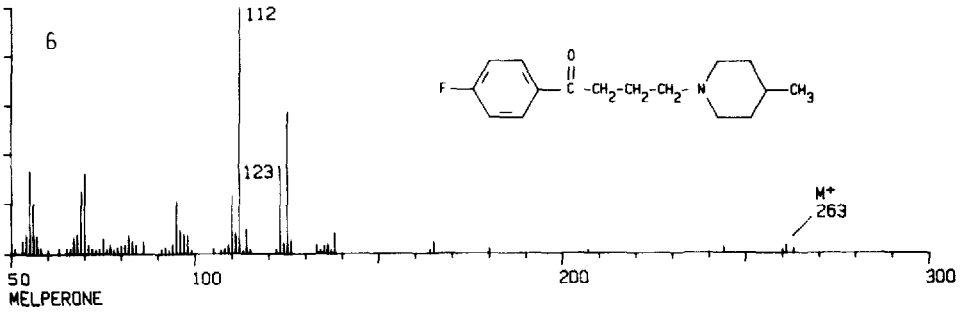
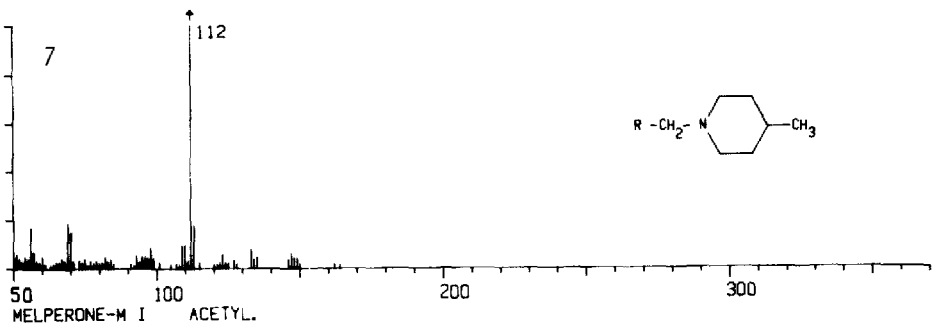

1
134
259
M+
301
50
100
200
300
BENPERIDOL-M
PIMOZIDE-M II
ACETYL.
2
134
M+
218
50
100
200
300
DROPERIDOL-M
PIMOZIDE-M I
ACETYL.
3
123
134
M+
356
50
100
200
300
400
FLUANISONE
4
148
134
M+
262
30
100
200
300
FLUANISONE-M I
ACETYL.

Fig. 1.







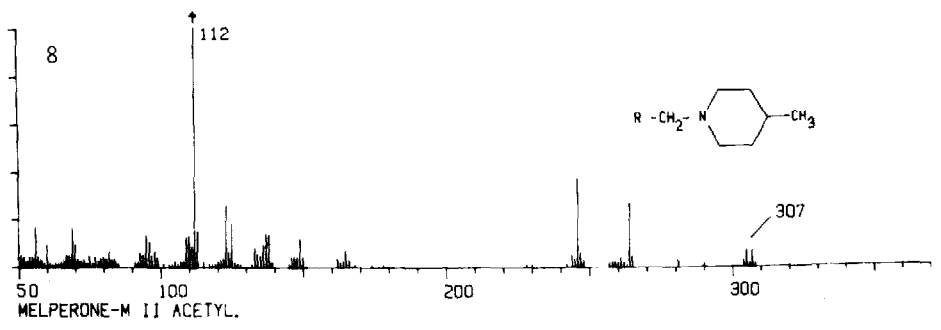


Fig. 1.

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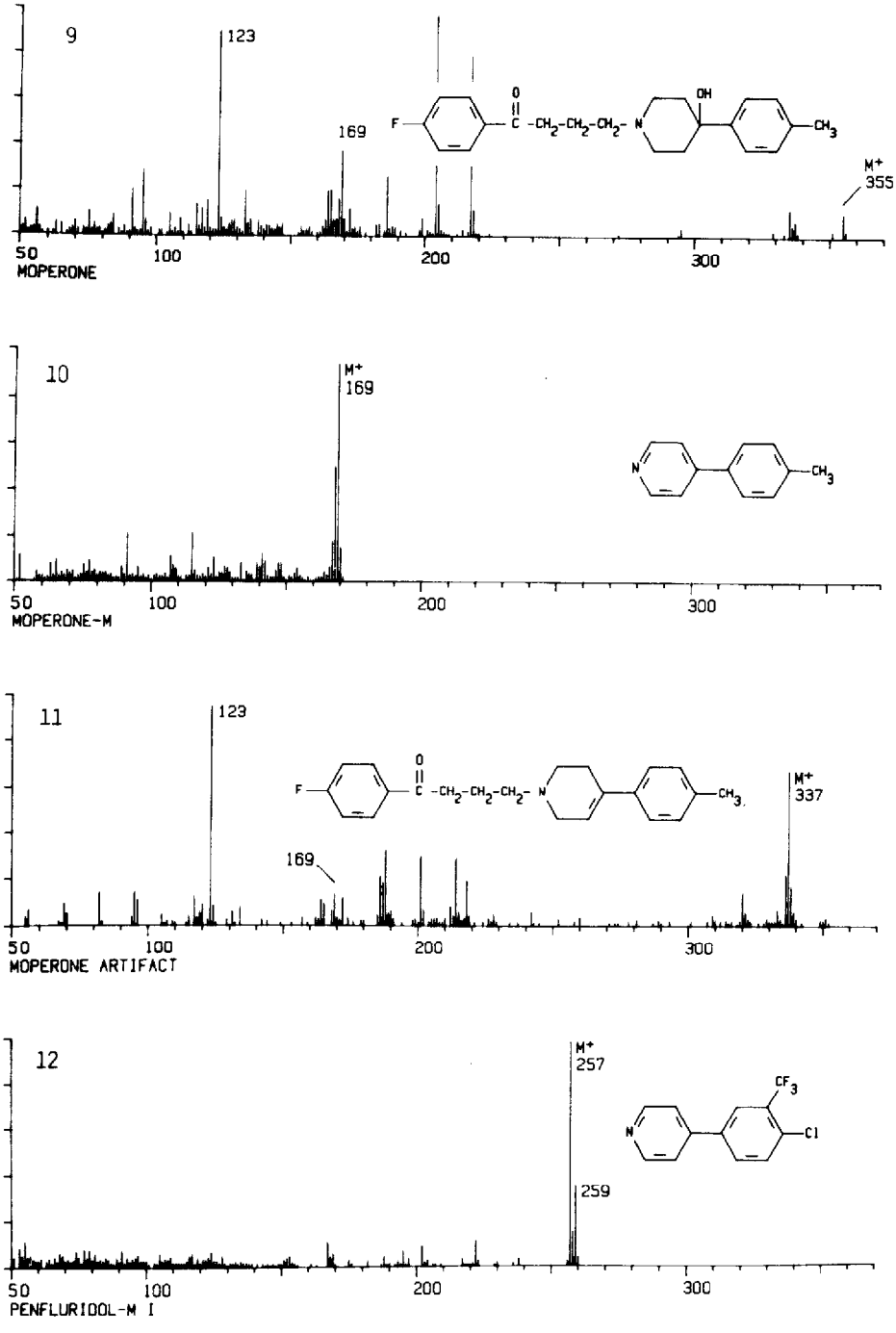
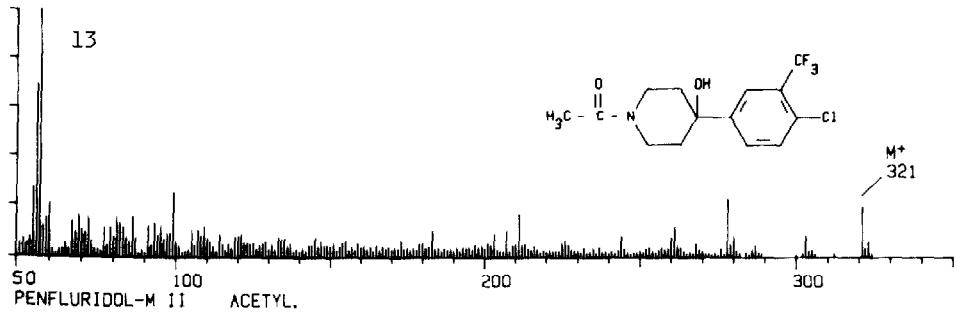
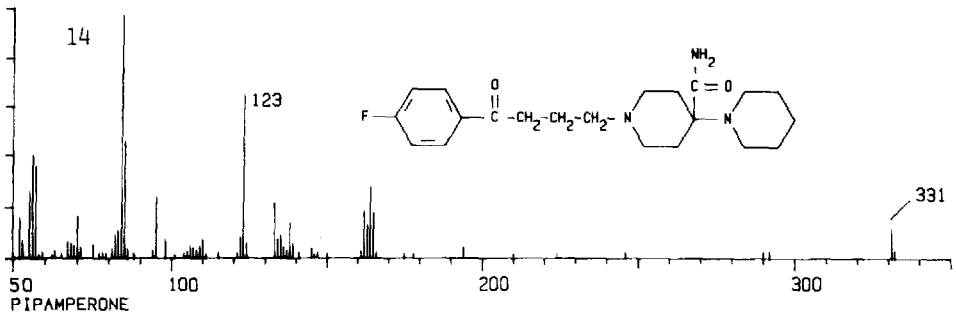

9
123
169
M+
355
50
100
200
300
MOPERONE
10
M+
169
50
100
200
300
MOPERONE-M
11
123
169
M+
337
50
100
200
300
MOPERONE ARTIFACT
12
M+
257
259
50
100
200
300
PENFLURIDOL-M I

Fig. 1.





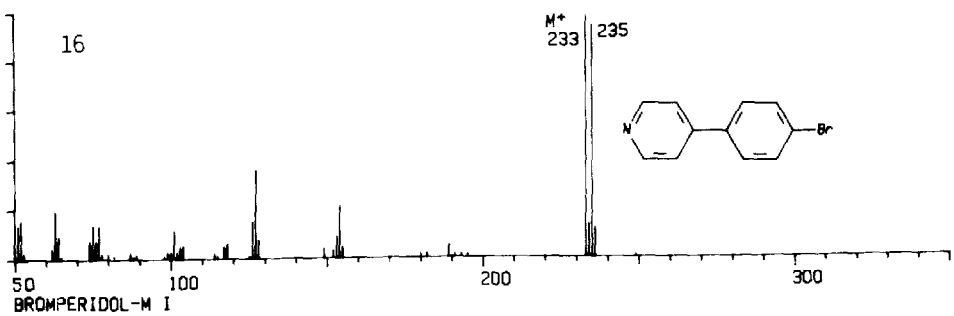
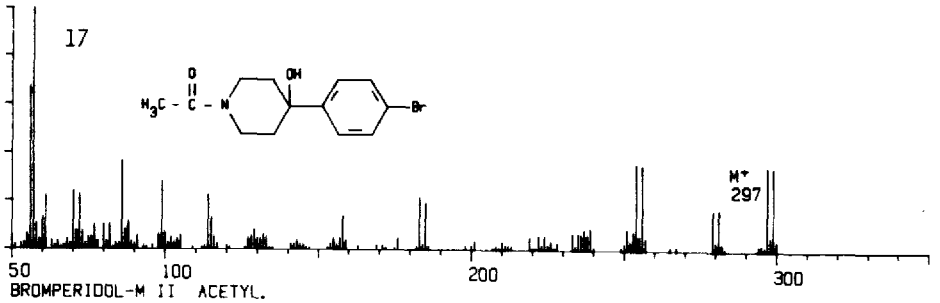
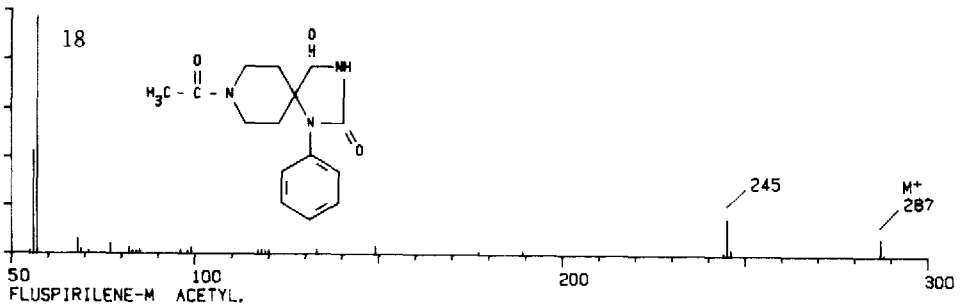
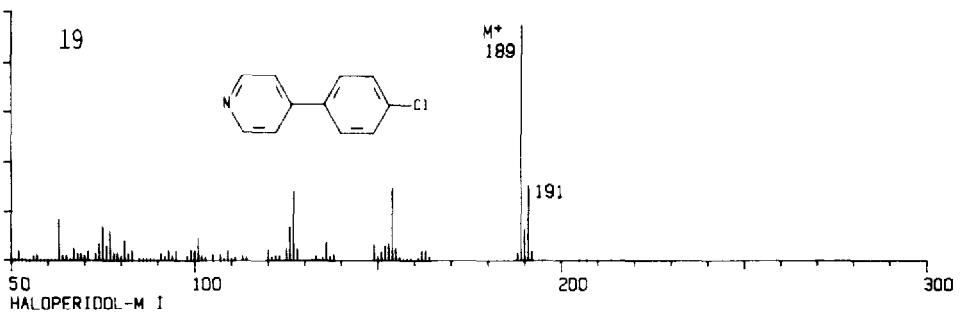


Fig. 1.

(Continued on p. 82)


17
M+
297
50
100
200
300
BROMPERIDOL-M II ACETYL.


18
245
M+
287
50
100
200
300
FLUSPIRILENE-M ACETYL.


19
M+
189
191
50
100
200
300
HALOPERIDOL-M I

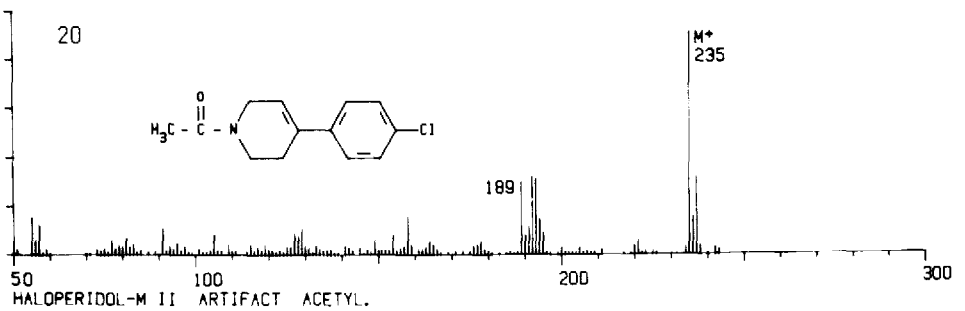

20
M+
235
189
50
100
200
300
HALOPERIDOL-M II ARTIFACT ACETYL.

Fig. 1.

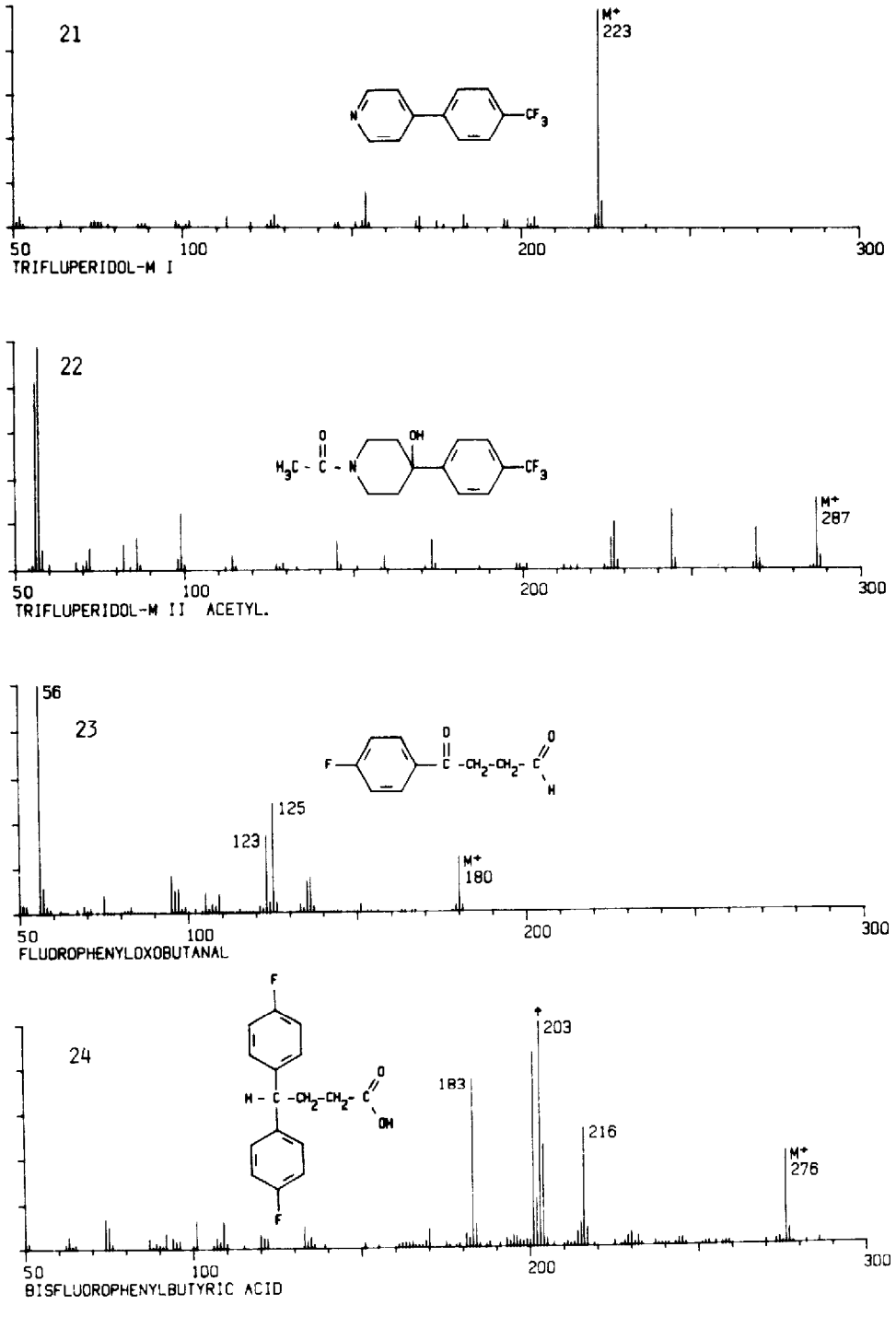


Fig. 1. Mass spectra of the compounds identified in urine after hydrolysis, extraction and acetylation.

Benperidol and droperidol each have a common metabolite with pimozide (mass spectra Nos. 1 and 2). Thus to determine if the patient has taken benperidol plus droperidol, or pimozide alone, it is necessary to analyse an acid extract. If benperidol and droperidol were taken, fluorophenyloxobutanal (mass spectrum No. 23) will be detected. If pimozide was taken, bisfluorophenylbutyric acid (mass spectrum No. 24) will be detected.

The moperone artifact (mass spectrum No. 11) is formed by the elimination of water during sample preparation because it is not present in the parent drug sample. The elimination of water was also observed for haloperidol M II (mass spectrum No. 20) but not for bromperidol M II, penfluridol M II or trifluperidol M II (mass spectra Nos. 17, 13, 22).

In our experience androsterone is the only endogenous physiological substance that appears in the mass fragmentogram. Because all compounds possibly indicated by the mass fragmentograms (e.g. the peaks at 134 and 148 in Fig. 3) can be precisely differentiated by comparison of the underlying mass spectra with those of standards (Fig. 1), interferences by other drugs are impossible.

To illustrate the method, a mass fragmentogram of a sample from a psychiatric patient administered a therapeutic dose of trifluperidol is shown in Fig. 2. The peak at *m/e* 223 indicates metabolite I (mass spectrum No. 21) and the peak at *m/e* 287 indicates the acetylated metabolite II of trifluperidol (mass spectrum No. 22).

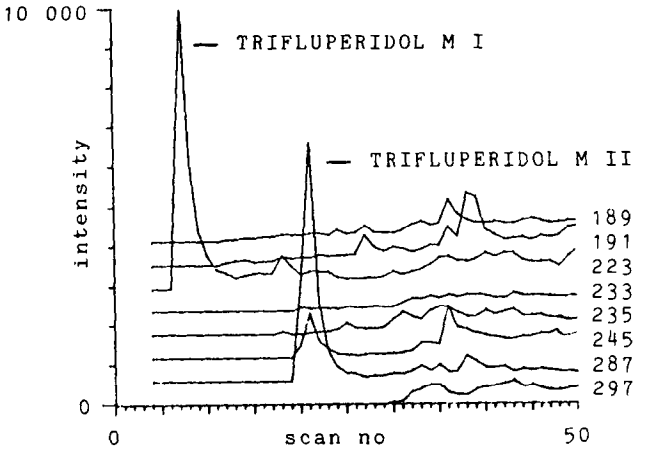


Fig. 2. Mass fragmentogram indicating metabolites of trifluperidol after a therapeutic dose.

Fig. 3 shows a mass fragmentogram of a sample from a child who had taken, accidentally, an unknown drug. The peaks at *m/e* 257, 259 indicate metabolite I (mass spectrum No. 12), the peak at *m/e* 321 the acetylated metabolite II of penfluridol (mass spectrum No. 13) and the second peak at *m/e* 257 indicates the acetylated physiological hormone androsterone (mass spectrum No. 15). The peaks at *m/e* 134 and 148 indicate unidentified compounds, which appeared in only this patient.

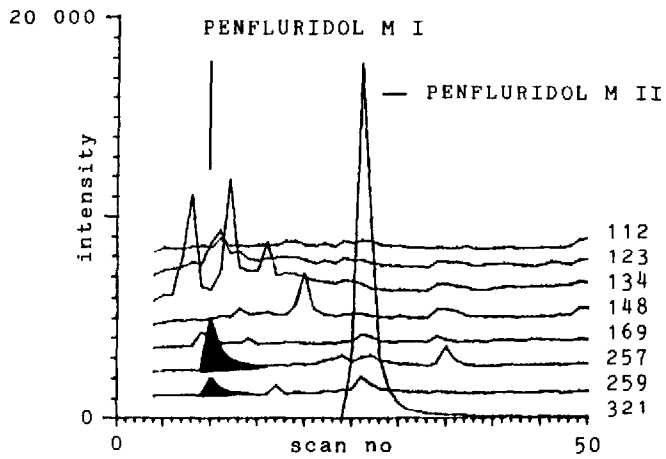


Fig. 3. Mass fragmentogram indicating metabolites of penfluridol in the urine of a child who had taken accidentally an unknown drug.

These examples show that the presented screening procedure allows a rapid and exact identification of butyrophenone and bisfluorophenyl neuroleptics and their predominant basic metabolites in urine.

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REFERENCES

1 H. Maurer and K. Pfleger, Naunyn-Schmiedebergs Arch. Pharmacol., 319 (Suppl.) (1982) R 6.
2 M.P. Quaglio, G. Sandri Cavicchi and M. Muraglia, Boll. Chim. Farm., 110 (1971) 385.
3 G. Bianchetti and P.L. Morselli, J. Chromatogr., 153 (1978) 203.
4 S.F. Cooper, J.M. Albert and R. Dugal, Int. Pharmacopsychiatry, 10 (1975) 78.
5 A. Forsman, E. Martensson, G. Nyberg and R. Öhman, Naunyn-Schmiedebergs Arch. Pharmacol., 286 (1974) 113.
6 F. Marcucci, L. Airoldi, E. Mussini and S. Garattini, J. Chromatogr., 59 (1971) 174.
7 I.A. Zingales, J. Chromatogr., 54 (1971) 15.
8 B.R. Clark, B.B. Tower and R.T. Rubin, Life Sci., 20 (1977) 319.
9 R. Hendriks, M. Michiels and J. Heykants, Preclinical Research Report of Janssen Pharmaceutica, Belgium, Serial number: R 4749/2, R 4584/2.
10 L.J.M. Michiels, J.J.P. Heykants, A.G. Knaeps and P.A.J. Janssen, Life Sci., 16 (1975) 937.
11 H. Maurer and K. Pfleger, J. Chromatogr., 222 (1981) 409.
12 H. Maurer, A. Weber and K. Pfleger, Z. Anal. Chem., 311 (1982) 414.