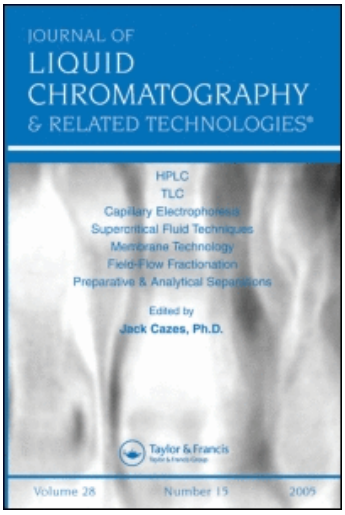

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Simple and Rapid Screening Procedure for 27 Neuroleptics using HPLC/DAD

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SIMPLE AND RAPID SCREENING PROCEDURE FOR 27 NEUROLEPTICS USING HPLC/DAD

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ABSTRACT

A HPLC/DAD method for simultaneous screening of 27 neuroleptics is presented. After single-step liquid-liquid extraction at pH 9.5 using chloroform/2-propanol/*n*-heptane (60/14/26, v/v), the substances are eluted on a Novapak C18 4µm column, with methanol/tetrahydrofuran/pH 2.6 phosphate buffer (65/5/30, v/v) as the mobile phase. Full UV spectra from 200 to 400 nm are recorded on-line during the entire analysis and may be automatically compared to spectra stored in a library. The method is simple, rapid, reproducible under local conditions, and highly specific.

INTRODUCTION

The development of reliable methods for systematic toxicological analysis (STA) has become a necessary work load for every

forensic laboratory. Such screening methods should be simple, rapid, reproducible, and able to cover a broad spectrum of toxicologically relevant substances, in one analytical run, with a sufficient specificity.

For years, gas chromatography (GC) and thin-layer chromatography (TLC) have been the most frequently used techniques for STA. TLC has gained popularity owing to its simplicity, universality, speed and low cost [1]; nevertheless, this method suffers from relatively low separating power and reproducibility [2]. Although GC shows great superiority on these two parameters, it still appears unable to provide unequivocal identification of a compound among hundreds; this necessarily leads - until GC/MS becomes a technique with sufficient flexibility for routine STA - to the use of a combination of two or more systems side-by-side [1,3], and consequently to a loss in speed and simplicity.

Due to its high separation power, universality (including thermolabile and non-volatile compounds), and ease of sample preparation, high-performance liquid chromatography (HPLC) appears as a potentially very valuable method for STA, and has been recommended for this purpose since the 1970's [4,5]. However, it first did nor receive great attention, since the lack of reproducible column material led to poor intra- and inter-laboratory reproducibility of retention times, thus to low specificity when using single-wavelength UV detection [1,6,7].

By allowing the identification of compounds not only from their retention behaviour, but also from their UV spectral properties, the introduction of UV multichannel detectors, and more recently diode-array detectors (DAD), dramatically improved the selectivity of HPLC, and afforded it a number of the advantages previously enjoyed by GC/MS solely - for a cost several times lower [8,9,10]. HPLC/DAD has proven itself for screening of benzodiazepines [11,12], laxatives [13], diuretics [14,15], and many other compounds [10,16,17].

For several months we are involved in the development of a reliable HPLC/DAD technique for STA of 200 to 300 toxicologically relevant compounds. As a preliminary result, we present in this paper a simple and rapid method for screening of the 27 neuroleptics commercially available in France.

MATERIALS AND METHODS

Materials

Methanol, tetrahydrofuran, chloroform, 2-propanol and *n*-heptane were HPLC grade (Merck, F.R.G.). All other chemicals and reagents were analytical grade and purchased from Merck (F.R.G.) and Prolabo (France).

All neuroleptics were kindly donated by the respective manufacturers, namely : Janssen, Boulogne-Billancourt, France (droperidol, haloperidol, moperone, penfluridol, pimozide, pipamperone, trifluperidol), Specia, Paris, France (chrorpromazine, levomepromazine (= methotrimeprazine), pipotiazine, prochlorperazine, promethazine, propericiazine (= pericyazine), thioproperazine), Delagrange, Chilly-Mazarin, France (amisulpride, metoclopramide, sulpiride, sultopride, tiapride), Theraplix, Paris, France (alimemazine (= trimeprazine), cyamemazine, trifluoperazine), Clin-Midy, Paris, France (acepromazine, aceprometazine, benperidol), Sandoz, Rueil-Malmaison, France (thioridazine), and Delalande, Courbevoie, France (fluanisone).

Stock solutions of pure drugs (100.0 μg/ml) were prepared in methanol and stored at 4 °C in the dark, where they were found to be

stable for several months. Work solutions at concentrations of 10.0 and 1.0 μg/ml were prepared daily by appropriate methanolic dilutions.

Chromatography

The HPLC system consisted of a quaternary low-pressure pump (model 600E, Waters, Milford, MA, U.S.A.), with a 200-μl loop volume autoinjector (model 715 Ultra Wisp, Waters). The detector was a UV/VIS diode-array spectrophotometer (model 991, Waters), with a wavelength range from 190 to 800 nm. The chromatographic system was linked to a computer (PowerMate SX Plus, NEC, Boxborough, MA, U.S.A.), with a software (Waters PDA) allowing the creation of a personal library of substances, and automatic comparison of current analytical data (retention time and UV spectra) with the data stored in the library.

The column was a NovaPak C18 (Waters) 4 μm (300 x 3.9 mm, i.d.), set at the constant temperature of 30 °C during all experiments.

The mobile phase consisted of methanol, tetrahydrofuran, and 10^{-2} M KH_2PO_4, pH 2.6 buffer (65/5/30, v/v). The elution was achieved isocratically, with a flow rate of 0.8 ml/min and average operating pressure of 2850 lb/in^2 (196.5 bar); it was degassed and filtered through 0.45 μm Durapore filters (GVWP 047) with a pyrex filter holder (Millipore, Milford, MA, U.S.A.). The equilibration time of the system was 30 min before analysis.

Procedure

To 2.0 ml sample (blood or urine) were added 5.0 ml of chloroform/2-propanol/*n*-heptane (60/14/26, v/v) and 1.5 ml of a saturated

NH_4Cl, pH 9.5 buffer solution. The mixture was shaken on an horizontal agitator for 10 min, then centrifuged at 2800 *g* for 10 min. The lower organic phase was removed and evaporated to dryness at 45 °C in a rotary evaporator (Speed Vac Concentrator A290, Savant Instruments, Farmingdale, NY, U.S.A.). The residue was then dissolved in 100 µl of the mobile phase, from which 50 µl were injected into the chromatographic system.

The eluent was monitored at 220 nm, while full spectra from 200 to 400 nm were recorded on-line during the entire analysis time, with a spectral resolution and sampling interval (delay between acquisition of two successive spectra) set at 1.3 nm and 1.0 s, respectively.

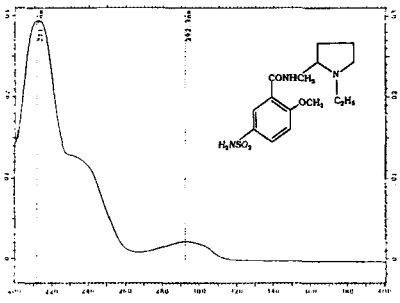
RESULTS AND DISCUSSION

The retention behaviours and UV spectra of 27 substances belonging to three classes of neuroleptics (13 phenothiazines, 9 butyrophenones, and 5 substituted benzamides) are summarized in Table I. The analysis is performed in 21 min. The drugs are well distributed across the entire length of the chromatogram. However, confusion due to peak overlapping might occur in 5 cases, namely between compounds 3-4, 6-7, 16-17, 18-19 and 25-26 (numbers referring to Table I). Excepted for one case (benperidol and droperidol), the respective UV spectra exhibit differences that are sufficient to ensure easy identification of the close-eluted compounds.

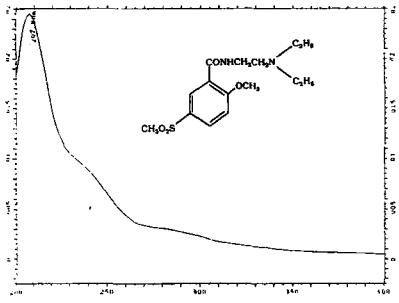
Chloroform/2-propanol/*n*-heptane (60/14/26) was chosen as the extraction solvant since it is little prone to emulsion and allows good to excellent recoveries, not only for neuroleptics, but also for a great variety of other drugs [18]. Absolute recoveries were determined by extracting pure biological samples spiked with the drugs at a concentra-

- TABLE I -
RETENTION AND SPECTRAL DATA FOR 27 NEUROLEPTICS

For each compound are given : chemical structure and UV spectrum, retention time (RT), capacity factor (k'), and the main UV absorption peaks (UV).
Drugs are listed according to their elution order.



1. SULPIRIDE
RT = 3.26 min k' = 0.09
UV = 292.3 nm



2. TIAPRIDE
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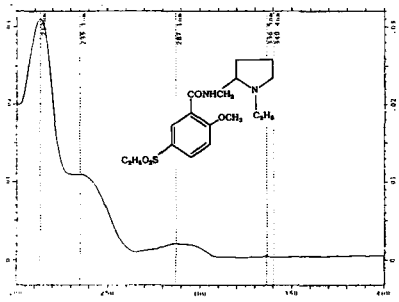
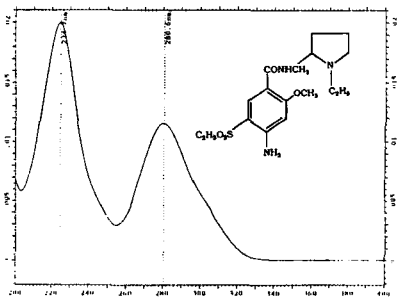
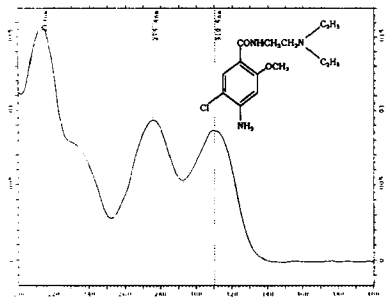
UV = no marked peak



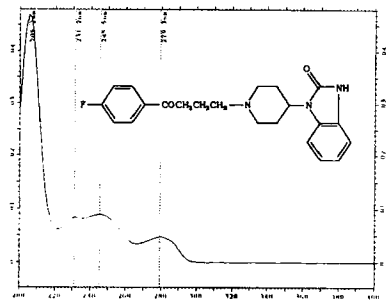
3. SULTOPRIDE
RT = 3.47 min k' = 0.16
UV = 235.1 nm; 287.1 nm



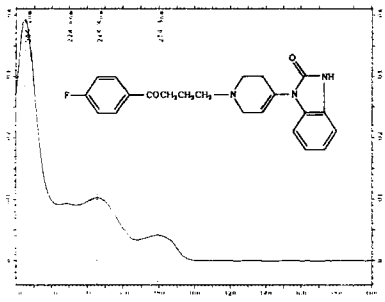
4. AMISULPRIDE
RT = 3.48 min k' = 0.17
UV = 224.7 nm; 280.6 nm



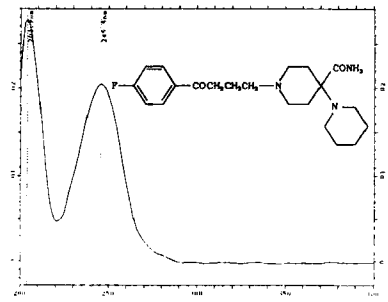
5. METOCLOPRAMIDE
RT = 3.92 min k′ = 0.32
UV = 275.4 nm; 310.5 nm



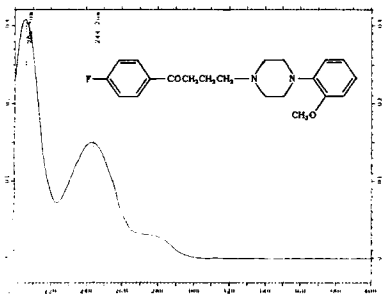
6. BENPERIDOL
RT = 4.62 min k′ = 0.55
UV = 231.2 nm; 245.5 nm; 279.3 nm



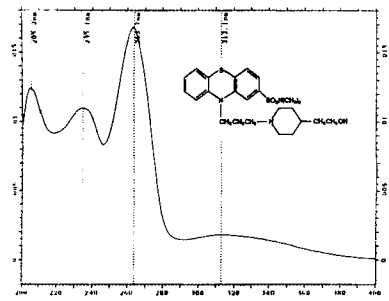
7. DROPERIDOL
RT = 4.65 min k′ = 0.56
UV = 228.6 nm; 245.5 nm; 279.3 nm



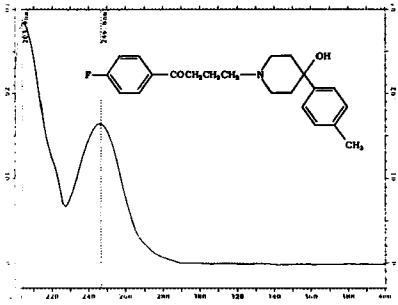
8. PIPAMPERONE
RT = 4.98 min k′ = 0.67
UV = 245.5 nm



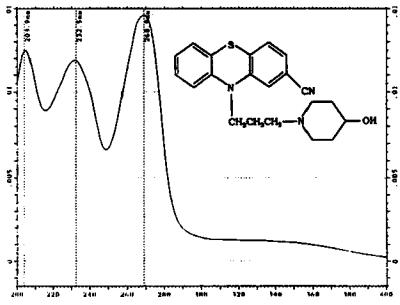
9. FLUANISONE
RT = 5.24 min k′ = 0.76
UV = 244.2 nm



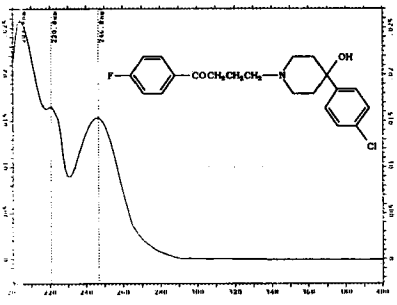
10. PIPOTIAZINE
RT = 5.50 min k′ = 0.85
UV = 235.1 nm; 263.7 nm; 313.1 nm



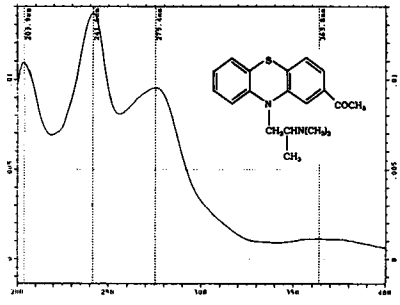
11. MOPERONE
RT = 5.62 min k′ = 0.89
UV = 246.8 nm



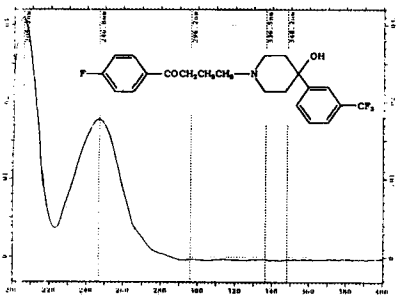
12. PROPERICIAZINE
RT = 5.94 min k′ = 0.99
UV = 232.5 nm; 268.9 nm



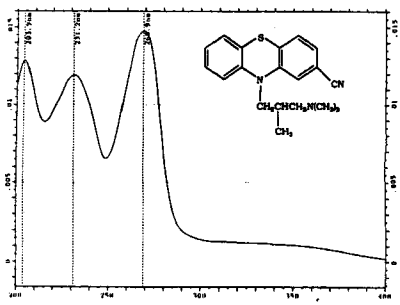
13. HALOPERIDOL
RT = 6.08 min k′ = 1.04
UV = 220.8 nm; 246.8 nm



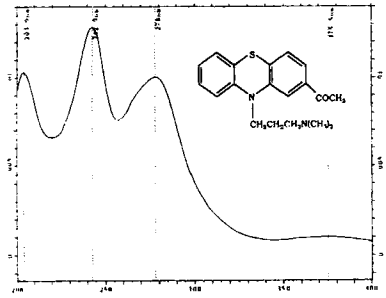
14. ACEPROMETAZINE
RT = 6.20 min k′ = 1.08
UV = 241.6 nm; 275.4 nm; 363.8 nm



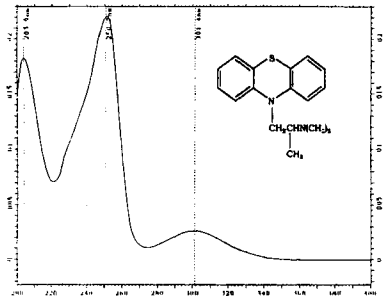
15. TRIFLUPERIDOL
RT = 6.61 min k′ = 1.22
UV = 246.8 nm



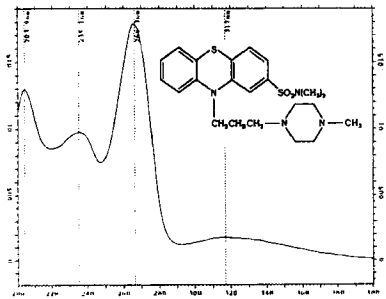
16. CYAMEMAZINE
RT = 6.71 min k′ = 1.25
UV = 231.2 nm; 268.9 nm



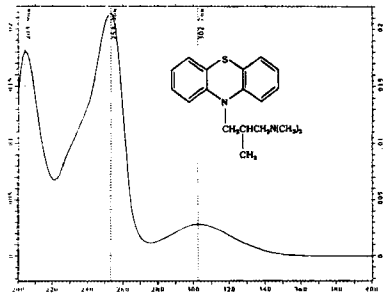
17. ACEPROMAZINE
RT = 6.77 min k′ = 1.27
UV = 242.9 nm; 278.0 nm; 375.5 nm



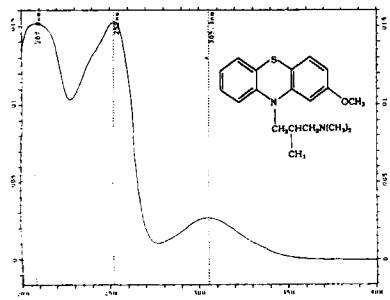
18. PROMETHAZINE
RT = 7.46 min k′ = 1.50
UV = 250.7 nm; 301.4 nm



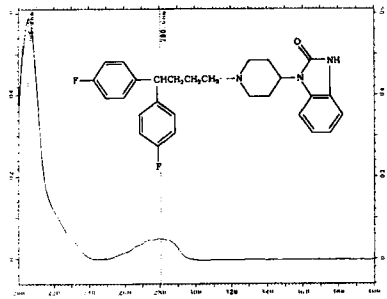
19. THIOPROPERAZINE
RT = 7.47 min k′ = 1.51
UV = 235.1 nm; 266.3 nm; 317.0 nm



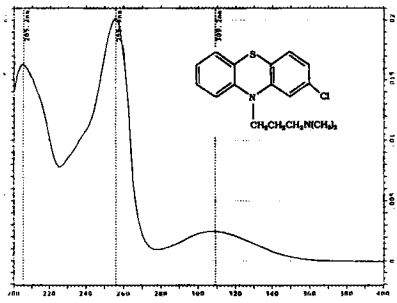
20. ALIMEMAZINE
RT = 8.35 min k′ = 1.80
UV = 255.3 nm; 302.7 nm



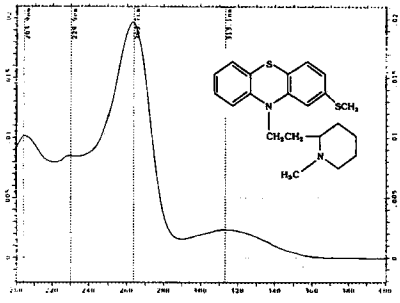
21. LEVOMEPROMAZINE
RT = 8.59 min k′ = 1.88
UV = 252.0 nm; 305.3 nm



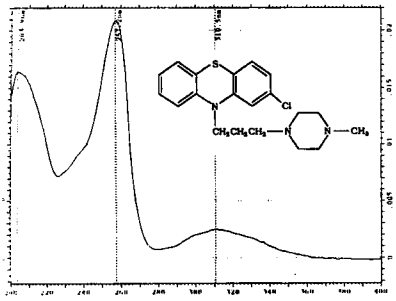
22. PIMOZIDE
RT = 8.77 min k′ = 1.94
UV = 280.6 nm



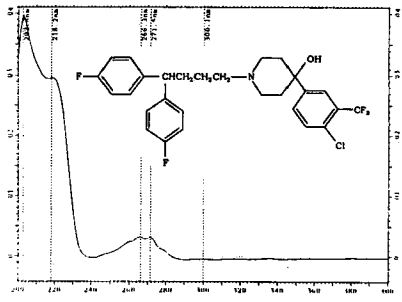
23. CHLORPROMAZINE
RT = 11.50 min k′ = 2.86
UV = 255.9 nm; 309.2 nm



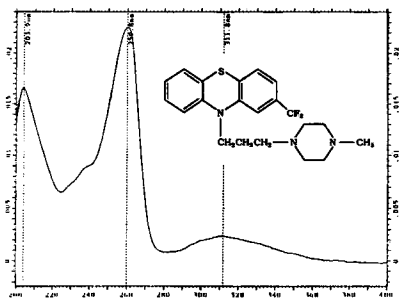
24. THIORIDAZINE
RT = 13.43 min k′ = 3.51
UV = 229.9 nm; 263.7 nm; 313.1 nm



25. PROCHLORPERAZINE
RT = 18.25 min k′ = 5.12
UV = 257.2 nm; 310.5 nm



26. PENFLURIDOL
RT = 18.25 min k′ = 5.12
UV = 266.3 nm; 271.5 nm



27. TRIFLUOPERAZINE
RT = 19.78 min k′ = 5.64
UV = 259.8 nm; 311.8 nm

tion of 0.1 μg/ml, and by comparing the obtained peaks with those of unextracted standards; each analysis was performed in triplicate for each drug, in blood and in urine (Table II). The results were found to be excellent for benzamides, acceptable to excellent for butyrophenones and phenothiazines; only 8 drugs (6 phenothiazines and 2 butyrophenones) displayed extraction rates below 60 %.

Photodiode-array detectors are generally claimed to be of less sensitivity than classical spectrophotometers [19]. The detection limits (corresponding to a peak equivalent to 3 times the background noise) were determined by analyzing pure blood spiked with decreasing amounts of the drugs tested. The results were in the range 0.009 μg/ml (haloperidol) to 0.087 μg/ml (aceprometazine). In all cases, these limits were sufficient to allow identification of the drugs at steady-state therapeutic concentrations and, *a fortiori*, in poisoning cases.

A severe criticism aimed at drug screening by HPLC is its poor reproducibility.The intra-laboratory day-to-day variation of the retention times (RT) was investigated for the 27 compounds tested by performing weekly analyses of methanolic standards over a period of 6 weeks. The coefficients of variation of the RT ranged from 1.56 % (sultopride) to 4.10 % (penfluridol). However, the comparison of our data with those obtained on a C18-type column from another manufacturer (Table III) shows not only an absence of proportionality between both retention behaviours, but even changes in the very elution order.

When considering, in addition, that UV spectral data of a drug may present significant differences from one spectrophotmeter to another, we totally agree with the recommandation for each individual laboratory to prepare its own database of retention times and UV spectra under local conditions [10].

- TABLE II -

EXTRACTION RECOVERIES FOR 27 NEUROLEPTICS

(%; all values are average of 3 measures)

	BLOOD	URINE
1. Sulpiride	91.3	90.1
2. Tiapride	84.5	92.6
3. Sultopride	95.2	87.5
4. Amisulpride	89.8	90.0
5. Metoclopramide	85.4	89.1
6. Benperidol	77.1	70.8
7. Droperidol	57.3	61.7
8. Pipamperone	42.0	37.2
9. Fluanisone	87.1	94.4
10. Pipotiazine	52.9	55.0
11. Moperone	94.5	95.5
12. Propericiazine	48.9	53.0
13. Haloperidol	96.6	92.4
14. Aceprometazine	86.6	94.2
15. Trifluperidol	65.4	60.8
16. Cyamemazine	55.3	61.9
17. Acepromazine	75.4	79.0
18. Promethazine	38.4	43.9
19. Thioproperazine	80.8	87.9
20. Alimemazine	70.7	67.1
21. Levomepromazine	52.1	43.5
22. Pimozide	75.7	83.6
23. Chlorpromazine	74.6	82.8
24. Thioridazine	63.3	70.8
25. Prochlorperazine	50.6	55.2
26. Penfluridol	76.9	82.0
27. Trifluoperazine	65.4	73.9

- TABLE III -

RETENTION BEHAVIOUR OF NEUROLEPTICS ON 2 REVERSED-PHASE H.P.L.C. C18 COLUMNS FROM DIFFERENT MANUFACTURERS

	Our study (k')[1]	Daldrup [20] (RRT)[2]
Sulpiride	0.09	0.20
Tiapride	0.13	0.22
Sultopride	0.16	-
Amisulpride	0.17	-
Metoclopramide	0.32	0.27
Benperidol	0.55	0.41
Droperidol	0.56	0.40
Pipamperone	0.67	0.19
Fluanisone	0.76	0.63
Pipotiazine	0.85	-
Moperone	0.89	0.60
Propericiazine	0.99	0.55
Haloperidol	1.04	0.67
Aceprometazine	1.08	-
Trifluperidol	1.22	0.50
Cyamemazine	1.25	-
Acepromazine	1.27	-
Promethazine	1.50	0.63
Thioproperazine	1.51	-
Alimemazine	1.80	0.84
Levomepromazine	1.88	0.94
Pimozide	1.94	-
Chlorpromazine	2.86	1.14
Thioridazine	3.51	1.88
Prochlorperazine	5.12	-
Penfluridol	5.12	-
Trifluoperazine	5.64	0.78

[1] k' = capacity factor.

[2] RRT = relative retention time, with 5-(p-methylphenyl)-5-phenylhydantoin (= MPPH) as the reference compound. Chromatography : isocratic conditions; column : Nucleosil C18 10 µm (250 x 4 mm, i.d.); temperature : 26 °C; eluent : acetonitrile/pH 2.3 phosphate buffer (312/688, w/w); flow : 1 ml/min.

CONCLUSION

The present method is the first described for convenient screening of neuroleptics in blood or urine by means of HPLC. Due to single-step liquid-liquid extraction and on-line detection and acquisition of spectral data using a photodiode-array detector, it is particularly simple, rapid, and specific. It proved to be useful in some clinical and forensic cases.

HPLC/DAD is gaining more and more in importance for drug screening purposes. This paper presented a preliminary application of such a technique. Further results will be subsequently published.

REFERENCES

1. De Zeeuw, R.A., Modern chromatographic procedures in systematic toxicological analysis, J. Chromatogr., 488, 199, 1989.

2. Bogusz, M., Klys, M., Wijsbeek, J., Franke, J.P., and de Zeeuw, R.A., Impact of biological matrix and isolation methods on detectability and interlaboratory variations of TLC Rf-values in systematic toxicological analysis, J. Anal. Toxicol., 8, 149, 1984.

3. Turcant, A., Premel-Cabic, A., Cailleux, A., and Allain, P., Screening for neutral and basic drugs in blood by dual fused-silica column chromatography with nitrogen-phosphorus detection, Clin. Chem., 34/7, 1492, 1988.

4. Twitchett, P.J. and Moffat, A.C., High-pressure liquid chromatography of drugs. An evaluation of an octadecylsilane stationary phase, J. Chromatogr., 111, 149, 1975.

5. Kabra, P.M., Stafford, B.E., and Marton, L.J., Rapid method for screening toxic drugs in serum with liquid chromatography, J. Anal. Toxicol., 5, 177, 1981.

6. Baker, J.K., Cates, L.A., and Corbett, M.D., Laboratory to laboratory reproducibility of high performance liquid chromatographic retention indices, J. Liquid Chromatogr., 5, 829, 1982.

7. Smith, R.M., Hurdley, T.G., Gill, R., and Moffat, A.C., The application of retention indices using the alkylarylketone scale to the separation of barbiturates by HPLC. The effect of the stationary phase, Chromatographia, 19, 407, 1984.

8. Minder, E.I., Schaubhut, R., Minder, C.E., and Vonderschmitt, D.J., Identification of drugs in human serum by high-performance liquid chromatography with photodiode array detection and a search algorithm for ultraviolet spectra, J. Chromatogr., 419, 135, 1987.

9. Jinno, K., Kuwajima, M., Hayashida, M., Watanabe, T., and Hondo, T., Automated identification of toxic substances in poisoned human fluids by a retention prediction system in reversed-phase liquid chromatography, J. Chromatogr., 436, 11, 1988.

10. Logan, B.K., Stafford, D.T., Tebbett, I.R., and Moore, C.M., Rapid screening for 100 basic drugs and metabolites using cation exchange solid-phase extraction and high-performance liquid chromatography with diode-array detection, J. Anal. Toxicol., 14, 154, 1990.

11. Minder, E.I., Schaubhut, R., and Simmler, F., Toxicological screening for benzodiazepines in urine : Emit-st versus high-performance liquid chromatography with photodiode array detection, Toxicol. Letters, 45, 93, 1989.

12. Mura, P., Piriou, A., Fraillon, P., Papet, Y., and Reiss, D., Screening procedure for benzodiazepines in biological fluids by high-performance liquid chromatography using a rapid-scanning multichannel detector, J. Chromatogr., 416, 303, 1987.

13. Fullinfaw, R.O., Bury, R.W., and Moulds, R.F.W., Screening procedure for stimulant laxatives in urine using high-performance liquid chromatography with diode array detection, J. Chromatogr., 433, 131, 1988.

14. Park, S.J., Pyo, H.S., Kim, Y.J., Kim, M.S., and Park, J., Systematic analysis of diuretic doping agents by HPLC screening and GC/MS confirmation, J. Anal. Toxicol., 14, 84, 1990.

15. Cooper, S.F., Massé, R., and Dugal, R., Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, J. Chromatogr., 489, 65, 1989.

16. Bogusz, M. and Wu, M., Standardized HPLC/DAD system, based on retention indices and spectral library, applicable for systematic toxicological screening, J. Anal. Toxicol., 15, 188, 1991.

17. Overzet, F., Rurak, A., van der Voet, H., Drenth, B.F.H., Ghijsen, R.T., and de Zeeuw, R.A., On-line diode array UV-visible spectrometry in screening for drugs and drug metabolites by high-performance liquid chromatography, J. Chromatogr., 267, 329, 1983.

18. Kintz, P., Tracqui, A., Lugnier, A.J., Mangin, P., and Chaumont, A.A., Simultaneous screening and quantification of several nonopiate narcotic analgesics and phencyclidine in human plasma using capillary gas chromatography, Meth. Find. Exp. Clin. Pharmacol., 12, 193, 1990.

19. Pickering, M.V., Diode array : new dimensions in detection for high performance liquid chromatography, LC-GC Intl., 4, 20, 1991.

20. Daldrup, T., Michalke, P., and Szathmary, S., HPLC in forensic chemistry, Practice of High Performance Liquid Chromatography, Engelhardt, H., ed., Springer, Berlin, 1986, p. 241.

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