Journal of Chromatography, 231 (1982) 361—376
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1317

LIQUID CHROMATOGRAPHIC ASSAY OF PHENOTHIAZINE, THIOXANTHENE AND BUTYROPHENONE NEUROLEPTICS AND ANTIHISTAMINES IN BLOOD AND PLASMA WITH CONVENTIONAL AND RADIAL COMPRESSION COLUMNS AND UV AND ELECTROCHEMICAL DETECTION

STEPHEN H. CURRY*, EDGAR A. BROWN, OLIVER Y.-P. HU and JOHN H. PERRIN

Department of Pharmaceutics, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610 (U.S.A.)

(First received December 23rd, 1981; revised manuscript received April 14th, 1982)

SUMMARY

An assay strategy for determining a wide range of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines both alone and in combination in blood and plasma is described. The general method employs liquid chromatography with both conventional and radial compression nitrile bonded columns. Detection is by ultraviolet absorption spectrophotometry or by amperometry depending on the concentrations to be measured. Ultraviolet absorption is suitable down to 10 ng/ml. Below this level amperometry is preferable. The various compounds are used as internal standards for each other. The lower limit of detection is approximately 0.1 ng ml^{-1} with a 10-ml sample. The within-run coefficient of variation is a maximum of 7.3%.

INTRODUCTION

The phenothiazines, thioxanthenes, dibenzazepines and butyrophenones which are used as neuroleptics, antidepressants and antihistamines are weak bases with highly lipophilic non-ionized species. The standard approach [1] to their assay in human plasma involves alkalinization of the sample, extraction of the drug into an organic solvent, reduction of the extract to small volume, and assessment of the drug content by gas chromatography (GC). In the case of the neuroleptics, GC using electron-capture, flame ionization, or thermionic sensitive detection has been successful with chlorpromazine, fluphenazine, perphenazine, thioridazine, butaperazine and haloperidol, while dibenzazepine antidepressants have been successfully assayed by both GC and liquid chromatography (LC) [2—8]. LC was recently successfully applied to biological samples of promethazine, one of the phenothiazine antihistamines

0378-4347/82/0000—0000/$02.75

[9] using electrochemical detection [10, 11]. Between 20 and 30 other drugs within this classification are available world-wide, either as marketed drugs or as drugs under investigation in humans [12]. There is a need for specific methods of assay for all of these compounds [13, 14]. The development of an LC approach with applicability to all of them at concentrations found or anticipated in clinical samples seems desirable. The research described in this paper is a step towards this. A preliminary report of this work has already been presented [15].

EXPERIMENTAL

Apparatus

A Varian 5000 liquid chromatograph (Varian, Palo Alto, CA, U.S.A.) with a Valco injection valve, or, in a few experiments, a Waters Model 440 liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) were used, with detection at a fixed wavelength of 254 nm. A Varian Micropak CN (10 μm) column was routinely used. However, a Waters radial compression separation system with a nitrile-bonded column was also used. Electrochemical detection was achieved using a glassy carbon electrochemical (amperometric) detector (Bioanalytical Systems, Lafayette, IN, U.S.A.) employing an LC-4A controller, and a silver—silver chloride reference electrode. The electrochemical detector potential was +0.9 V. Detector response was recorded using (i) a 1-mV or 10-mV strip chart recorder (Varian), or (ii) a CDS-111 integrator (Varian).

Mobile phase

The mobile phase consisted of 90% acetonitrile or methanol and 10% aqueous ammonium acetate. The ammonium acetate concentration was varied from 0.005 *M* to 0.2 *M*. A flow-rate of 2.0 or 2.5 ml/min was used.

Chemicals and reagents

Reference samples of marketed drugs were obtained from the manufacturers and used as supplied. Small quantities of model metabolites, principally of chlorpromazine, were obtained from the U.S. National Institute of Mental Health and used as supplied. Pesticide-grade mixed hexanes and LC-grade acetonitrile and methanol were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). All other chemicals were analytical grade from Fisher Scientific. The diethyl ether was used from freshly opened small volume containers to ensure that it was peroxide free.

Stock solutions of marketed drugs and their metabolites

These were prepared by dissolving weighed quantities (generally approximately 10 mg) in sufficient methanol to give 1 mg/ml solutions. Dilutions were prepared in methanol to 10 μg/ml. Plasma standards were prepared by diluting appropriate volumes of these solutions with plasma such that the methanol content was never more than 1%. Stock solutions and plasma standards were stable for at least one month stored in the dark at 4°C (methanol solutions) or frozen (plasma solutions).

Extraction procedure using hexane

Plasma samples (1—5 ml) were made alkaline with 1 ml of 1 *N* sodium hydroxide solution, and extracted with mixed hexanes for 30 min. After centrifugation, 9-ml aliquots of the hexane layers were removed and evaporated to dryness at 30°C under a stream of nitrogen. The residues were redissolved in the mobile phase (100 μl) and chromatographed (50 μl samples).

Extraction procedure using diethyl ether

Plasma samples (1—5 ml) were added to tubes containing phosphate buffer (pH 7.4, 0.1 *M*) and extracted with diethyl ether (10 ml) for 30 min. After centrifugation, 9-ml aliquots of the ether layer were treated as described above for hexane extracts.

Extraction procedures for subnanogram levels and for whole blood

Up to 10 ml of plasma or whole blood were added to tubes containing 1 ml of 1 *N* sodium hydroxide solution and extracted twice with 10-ml quantities of mixed hexanes for 30 min, or once with 15 ml of mixed hexanes for 1 h. Measured aliquots of the hexane extracts were combined and evaporated to dryness. The residue was redissolved in 1 ml of 0.1 *N* hydrochloric acid and the compound of interest was extracted into 5 ml of chloroform by shaking gently for 10 min or by vortexing for 1 min, followed by centrifugation. A 4.5-ml aliquot of the chloroform layer was evaporated to dryness and redissolved in 10 μl as described above.

Calibration graphs

These were prepared from spiked plasma containing the appropriate drug at concentrations ranging from 0 to a level above that expected in clinical samples. Quantitation was by measurement of (i) peak height, (ii) the peak height ratio of drug of interest/internal standard, or (iii) integrator response, against drug concentration.

Internal standards

Where appropriate, internal standards were included in the sample of mobile phase used at the re-solution stage, or were added to the plasma to be extracted alongside the drug of interest.

RESULTS

Retention volumes

Table I shows the retention times recorded for 21 marketed drugs, as a function of ammonium acetate concentration in the aqueous solution used in preparation of the mobile phase. Each compound had shorter retention times at higher concentrations of ammonium acetate but no regular pattern was observed. For example the retention time of mesoridazine varied from 81.6 to 8.3 min while that of fluphenazine varied from 8.7 to 2.4 min. There was no case of compounds with identical retention volumes at all ammonium acetate concentrations. The retention volumes of the various compounds make possible the choice of an ammonium acetate concentration and mobile

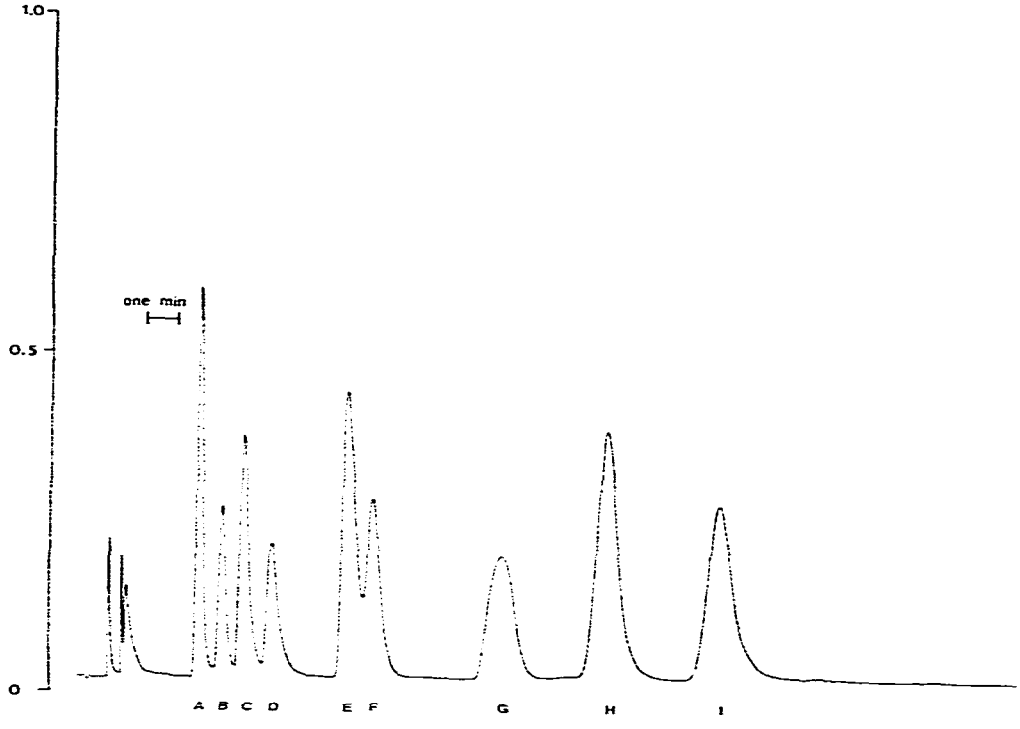

Fig. 1. LC separation of eight phenothiazine drugs plus haloperidol. Peaks and quantities: A = fluphenazine, 50 ng; B = carphenazine, 100 ng; C = trifluperazine, 50 ng; D = butaperazine, 100 ng; E = haloperidol, 250 ng; F = promethezine, 50 ng; G = chlorpromazine, 50 ng; H = promazine, 50 ng; and I = thioridazine, 100 ng. Detection by UV (254 nm); *y* axis in absorbance units.

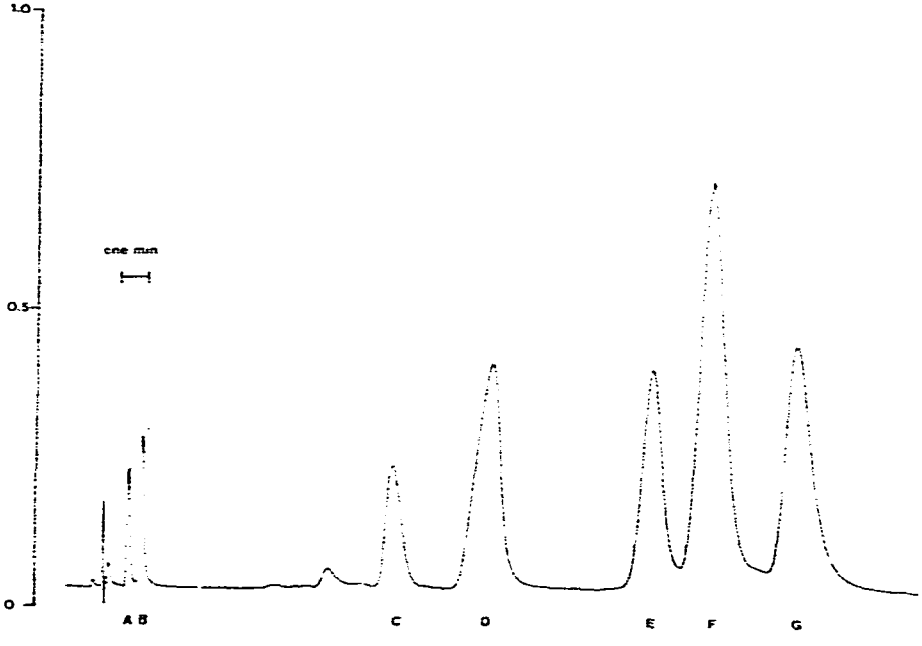

TABLE I

RETENTION TIMES (min) OF 21 MARKETED DRUGS AS A FUNCTION OF AMMONIUM ACETATE CONCENTRATION IN THE AQUEOUS PORTION OF THE MOBILE PHASE

The flow-rate was 2.5 ml/min.

Compound	Concentration of ammonium acetate (*M*)					
	0.005	0.01	0.02	0.05	0.1	0.2
	pH					
	6.24	6.72	6.89	6.91	6.94	6.99
Acetophenazine	18.4	10.1	7.4	5.8	5.1	3.5
Amitriptyline	27.2	13.2	8.8	6.0	5.0	3.3
Benztropine	46.3	20.4	13.2	8.8	7.5	4.8
Butaperazine	28.1	15.6	12.0	8.9	7.4	4.3
Carphenazine	15.5	8.6	6.4	4.8	4.3	3.1
Chlorpromazine	21.7	11.5	8.0	5.4	4.5	3.0
Eluphenazine	8.7	7.0	5.4	3.6	2.9	2.4
Haloperidol	14.0	7.4	5.1	3.4	2.7	2.0
Imipramine	31.6	15.3	10.1	6.8	5.7	3.6
Mesoridazine	81.6	43.3	23.9	16.5	14.0	8.3
Nortriptyline	40.7	21.4	10.7	7.0	6.1	3.9
Ophenadrine	24.6	14.3	8.2	5.4	4.5	3.0
Piperacetazine	34.5	20.3	11.1	7.4	6.2	4.0
Promazine	27.5	16.8	10.0	6.9	5.7	3.7
Promethazine	13.4	8.7	5.7	4.2	3.6	2.5
Thioridazine	29.5	16.7	9.2	6.1	5.3	3.2
Thiothixene	22.1	15.1	10.0	7.5	6.4	3.9
Trifluoperazine	20.1	13.7	9.3	6.9	5.8	3.6
Triflupromazine	17.9	14.0	6.6	4.5	3.7	2.5
Trihexylphendyl	21.1	12.3	7.3	4.8	3.9	2.6
Trimeprazine	17.0	10.2	6.6	4.4	3.7	2.5

phase flow-rate suitable for successful assay of any one of the compounds in the presence of another. Supportive evidence for the identification of a drug in this group is also obtainable. Fig. 1 shows a chromatogram when a mixture of nine of the compounds was examined. The use of methanol in place of acetonitrile gave essentially similar chromatographic results.

Fig. 2 shows the chromatographic separation of eight model metabolites of chlorpromazine. These compounds are produced by ring sulfoxidation, ring hydroxylation, amine oxidation, and N-demethylation of chlorpromazine. Only one pair of compounds was not resolved but they are two relatively minor polar metabolites. Four other model metabolites had retention volumes when 0.005 *M* ammonium acetate solution was used as follows: fluphenazine

Fig. 2. LC separation of eight model metabolites of chlorpromazine. Peaks: A = chlorpromazine N-oxide 5-oxide; B = chlorpromazine N-oxide; C = 7-hydroxychlorpromazine; D = chlorpromazine 5-oxide; E = dedimethylchlorpromazine; F = dedimethylchlorpromazine 5-oxide and demonomethylchlorpromazine unresolved; G = demonomethylchlorpromazine 5-oxide. Detection by UV (254 nm); *y* axis in absorbance units.

5-oxide, 5.2 ml; trifluperazine 5-oxide, 9.5 ml; demonomethylpromazine, 12.0 ml; demonomethylpromazine 5-oxide, 17.3 ml. This evidence shows adequate resolution of the marketed drugs and their metabolites.

Radial compression columns

Fig. 3 shows a sample chromatogram using the radial compression column. Separation was again satisfactory, and chromatogram quality was superior to that obtained with the conventional columns in that the peaks were more symmetrical and had a higher height/width ratio. A listing of retention times using this system is available in a preliminary communication [15].

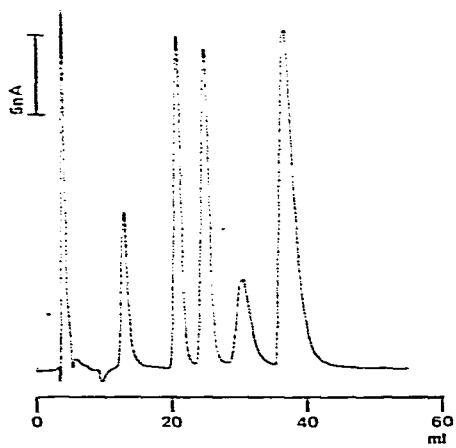

Fig. 3. LC separation of five compounds on a radial compression column. Detection was electrochemical (50 nA full scale deflection with 10-mV recorder). The mobile phase flow-rate was 5 ml/min. The compounds and their retention volumes were: carphenazine (13.0 ml), trifluprομazine (20.5 ml), chlorpromazine (25.0 ml), butaperazine (30.5 ml), benztropine (37.0 ml). The first four compounds were at 10 μg/ml; benztropine was at 1 mg/ml. Injection volume 10 μl.

Blank plasma and recovery experiments

Figs. 4 and 5 show chromatograms for blank extracts. Figs. 6 and 7 show chromatograms for two recovery experiments with spiked plasma. The results for blank samples demonstrate a lack of interfering peaks in the area of the chromatogram in which experimental peaks are expected. The spiked standards demonstrate recovery and clarity of the chromatographic result.

Quantitative data

Table II shows the essential features of a series of calibration graphs for eight phenothiazine drugs extracted from plasma. Table III shows that only with carphenazine was there a significant decrease in recovery with increased plasma volume. The apparent increase in recovery of trifluoperazine is on the borderline of statistical significance. Similar or analogous equations and other observations were obtained with the different extraction modes, the electrochemical detection system, and whole blood up to 10 ml in volume.

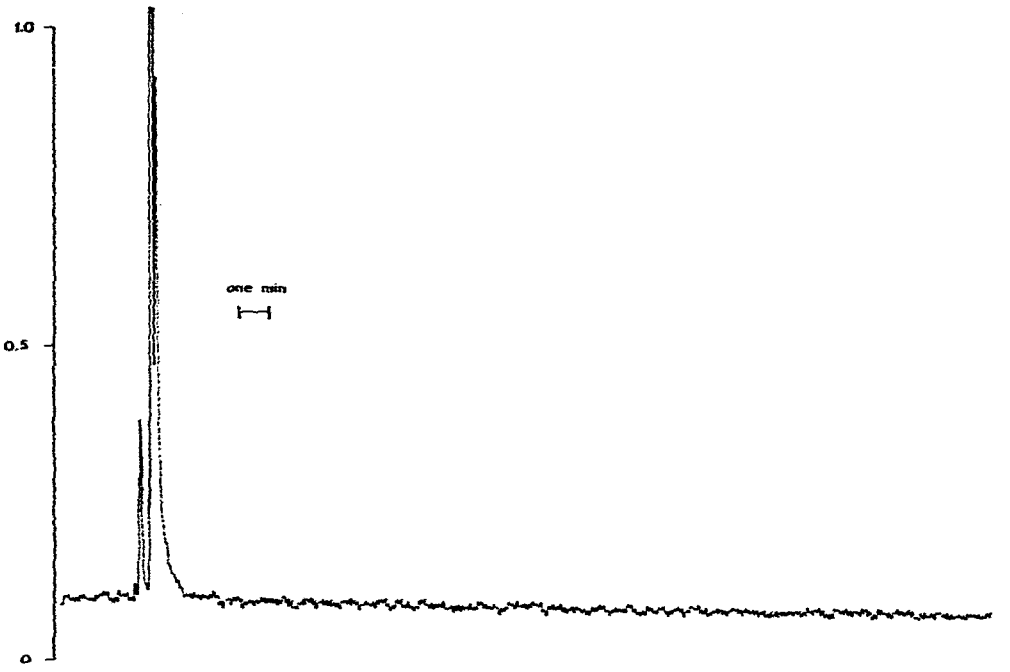

Fig. 4. LC trace of a concentrated hexane extract from blank plasma (5 ml). Detection by UV (254 nm); y axis in absorbance units.

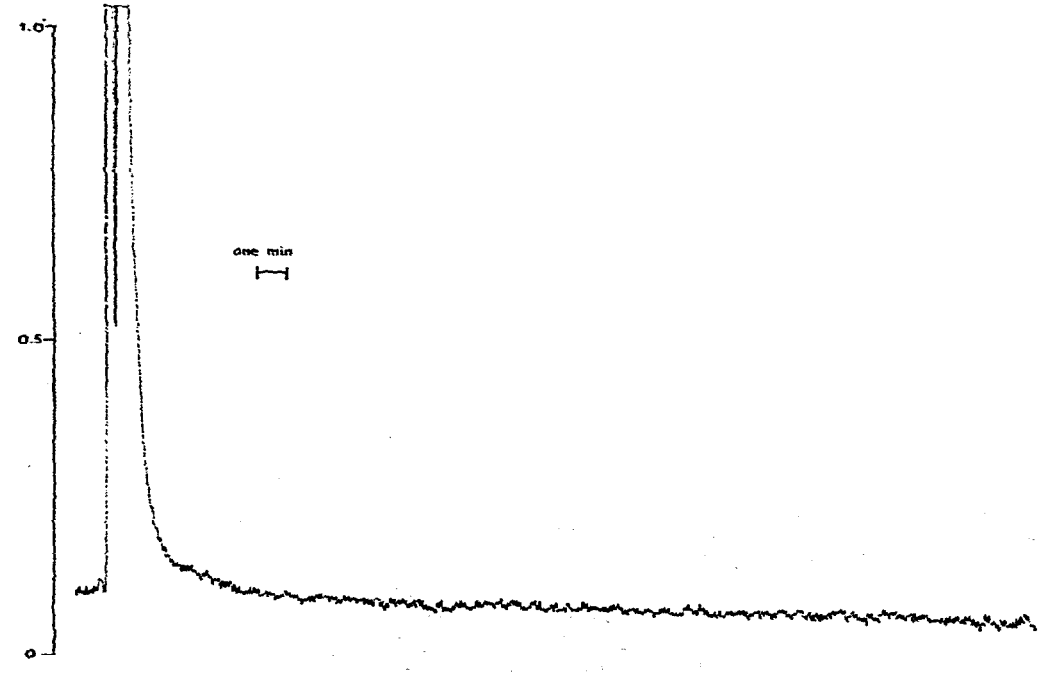

Fig. 5. LC trace of a concentrated diethyl ether extract from blank plasma (5 ml). Detection by UV (254 nm); y axis in absorbance units.

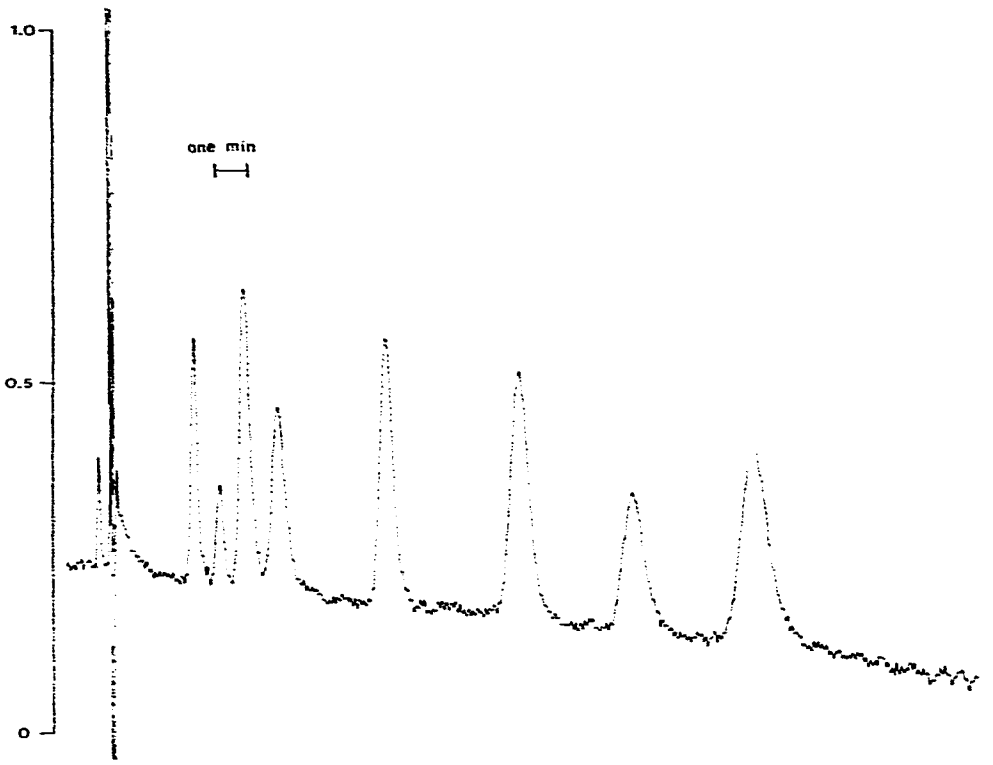

Fig. 6. LC trace of a concentrated hexane extract from plasma spiked with the eight phenothiazine drugs shown in Fig. 1 (haloperidol excluded). Sample was 5 ml containing the drugs at 10 ng/ml. Detection by UV (254 nm); y axis in absorbance units.

TABLE II

CALIBRATION EQUATIONS FOR ASSAY METHODS (HEXANE EXTRACTION FROM 1-ml SAMPLES) FOR EIGHT PHENOTHIAZINE DRUGS IN PLASMA

Model $y = a + bx$ where y is absorbance of the LC response based on peak height, x is the amount (ng) of the drug in the plasma, a is the intercept on the y axis and b is the slope of the straight line graph. The last column indicates the square of the correlation coefficient for each line. Data collected over the range x = 10—500 ng.

Compound	a	x	r^2
Fluphenazine	—0.000735	0.0000365	0.968
Chlorpromazine	—0.000005	0.0000116	0.986
Thioridazine	—0.000722	0.0000084	0.987
Trifluoperazine	—0.001071	0.0000246	0.976
Carphenazine	—0.000574	0.0000053	0.952
Promazine	—0.000151	0.0000159	0.961
Promethazine	—0.000114	0.0000123	0.976
Butaperazine	—0.000988	0.0000078	0.962

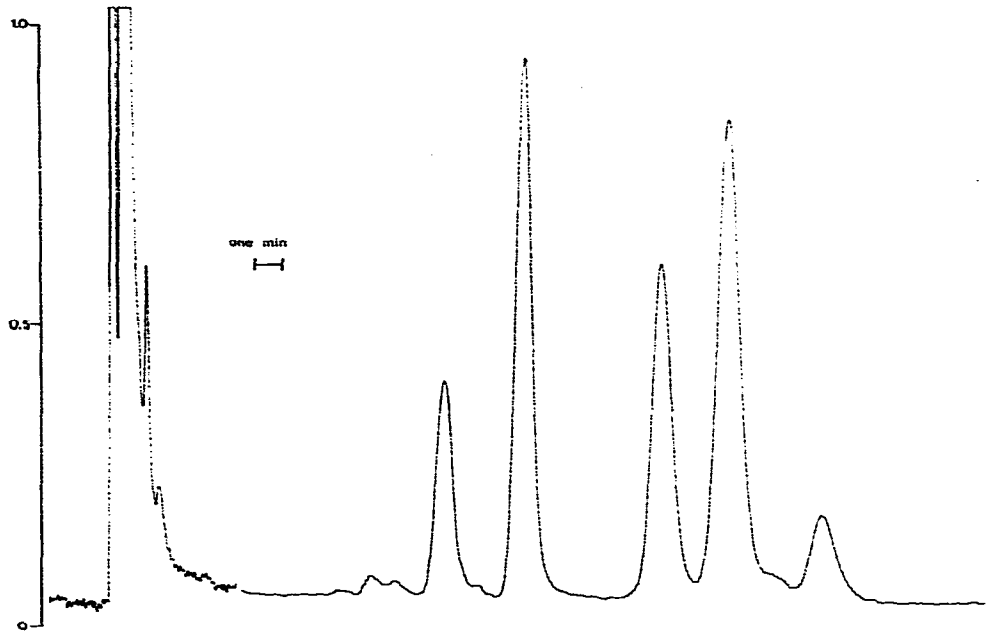

Fig. 7. LC trace of a concentrated diethyl ether extract from plasma containing five of the model metabolites of chlorpromazine shown in Fig. 2. Sample was 5 ml containing the compounds at approximately 10 ng/ml. Detection by UV (254 nm); *y* axis in absorbance units.

TABLE III

INFLUENCE OF VOLUME OF PLASMA ON EXTRACTION AND INSTRUMENT RESPONSE

Measurements are of peak height at 0.02 a.u.f.s. Plasma at various volumes containing 100 ng of the eight compounds was extracted with hexane as described under Experimental. Carphenazine showed reduced recovery with increased plasma volume (Spearman rank correlation coefficient, $r_s = 1$). The other relationships were clearly non-significant, except for trifluoperazine ($r_s = 0.7, p < 0.1$).

Compound	Volume of plasma (ml)				
	1	2	3	4	5
Fluphenazine	90	79	94	93	86
Carphenazine	54	32	29	22	21
Trifluoperazine	120	120	136	145	146
Butaperazine	72	69	76	80	75
Promethazine	109	130	139	147	141
Chlorpromazine	92	111	107	115	111
Promazine	61	66	66	71	71
Thioridazine	82	90	92	97	96

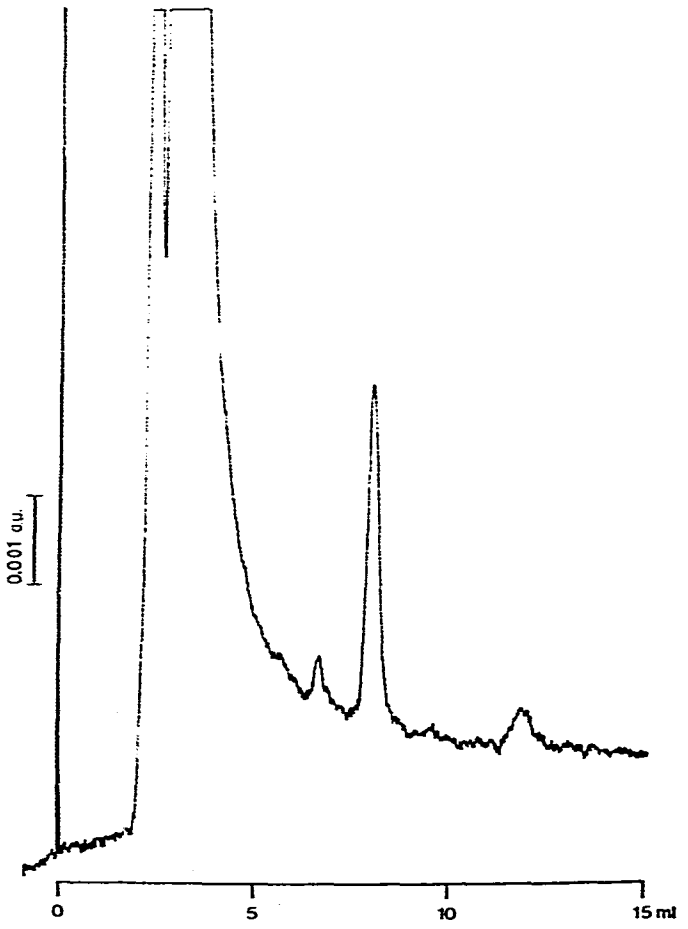

Fig. 8. LC trace of a concentrated hexane extract from a patient receiving chlorpromazine. The patient was receiving 100 mg three times a day and the sample was obtained approximately 2 h after a dose. Sample volume, 2 ml; detection by UV (254 nm); y axis in absorbance units. The trace shows chlorpromazine (8 ml) and small amounts of chlorpromazine sulfoxide (12 ml) and demonomethylchlorpromazine (6 ml to low recovery). The chlorpromazine concentration was 56.0 ng/ml.

At best, the coefficient of variation on repeated assay of plasma or blood containing known quantities of the various drugs was 1.3% (within-day). At worst this value was 7.3% (n = 5 in each case). Between-day coefficient of variation values varied over a wide range (5.0—60.1%) so that daily standardization with both pure drug solutions and spiked plasma or blood was essential.

Detection limits

From Table II, a simple calibration shows that a 1 ng/ml solution of fluphenazine in plasma, when 1 ml was sampled, gave a peak with a height equivalent to 0.0000365 absorbance units. Similarly, a 10 ng/ml solution, when 5 ml were sampled, gave a peak with a height equivalent to 0.0018 absorbance units. This is approximately 40% of full scale deflection when the instrument

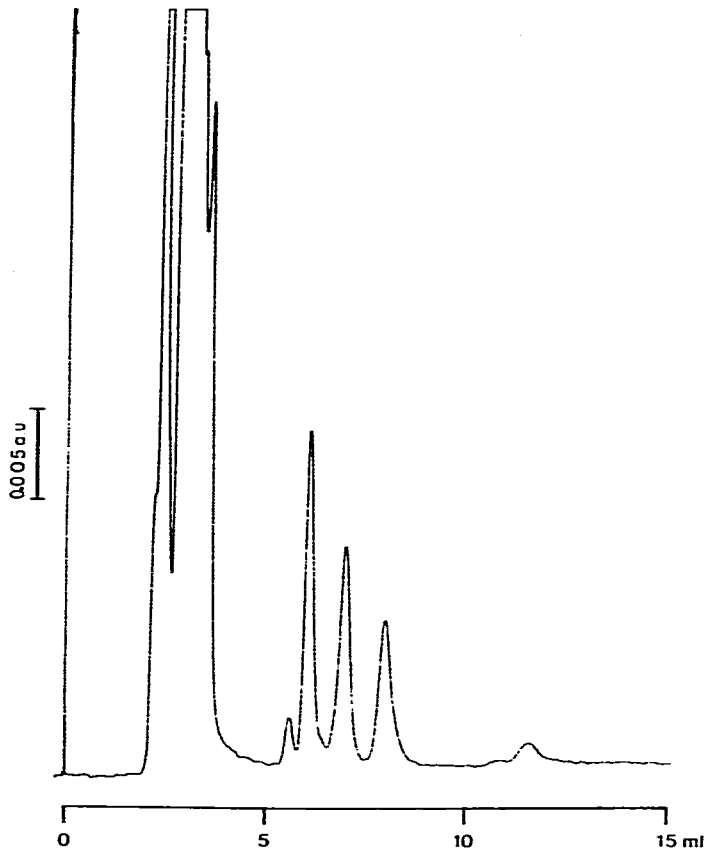

Fig. 9. LC trace of a concentrated diethyl ether extract from the patient of Fig. 8. Sample volume and other details as in Fig. 8. The trace shows chlorpromazine (8 ml), 7-hydroxychlorpromazine (7 ml), demonomethylchlorpromazine (6 ml) and chlorpromazine N-oxide (5.5 ml). The chlorpromazine concentration was 58.3 ng/ml. The metabolites are shown for identification only.

is set at 0.005 a.u.f.s. However, it is necessary to consider the noise level of the instrument response, and also to note that fluphenazine had the highest value of x in Table II. While we consider that in certain circumstances, 1 ng/ml can be assayed by UV detection, generally speaking 10 ng/ml should be considered as the lower limit for this detector. The exact lower limit will vary from laboratory to laboratory. A similar argument leads to the conclusion that electrochemical detection adds approximately two orders of magnitude. The exact detection limit may also be affected by the strength of ammonium acetate used in any particular application.

Clinical samples

Figs. 8—13 show a selection of chromatograms from patients treated with six of the drugs examined in this system. Details are given in the legends. Figs. 11 and 13 were redrawn from LC traces. All others in this paper are direct photographs of the traces.

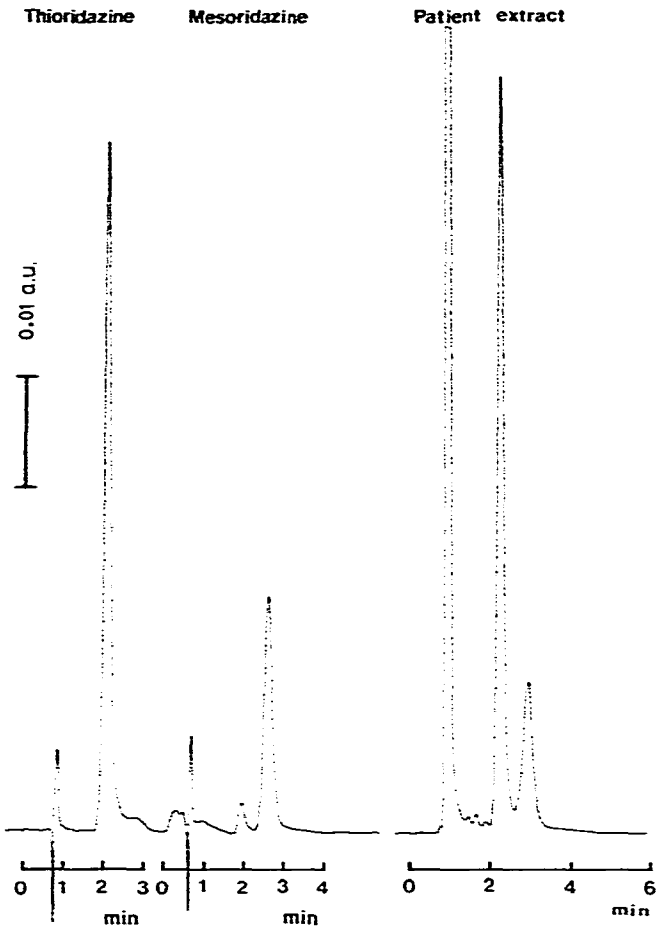

Fig. 10. LC trace of an investigation of a patient receiving thioridazine. The trace shows the drug and its metabolite as standards and in a hexane extract from plasma. The patient was receiving 200 mg thioridazine daily and the sample was collected 20 h post dosage. Detection by UV (254 nm); *y* axis in absorbance units. The thioridazine concentration was 26.1 ng/ml.

DISCUSSION

The compounds considered in this paper are unusual in that such a large number of closely related chemicals is in use in medicine. The drugs are sometimes used alone, and sometimes used in combinations of two or three, and occasionally four. Drug interactions are common and patient monitoring using drug measurements in biological fluids is often sought. Basic principles involved in the assay of these drugs are well established.

The research described in this paper could provide a system for measurement of all of these compounds individually and for most of the likely combinations of drugs. It could also provide a system for obtaining evidence supportive of a drug identification in cases when the drug ingested is unknown. It is based on published principles, and employs, to some extent, established

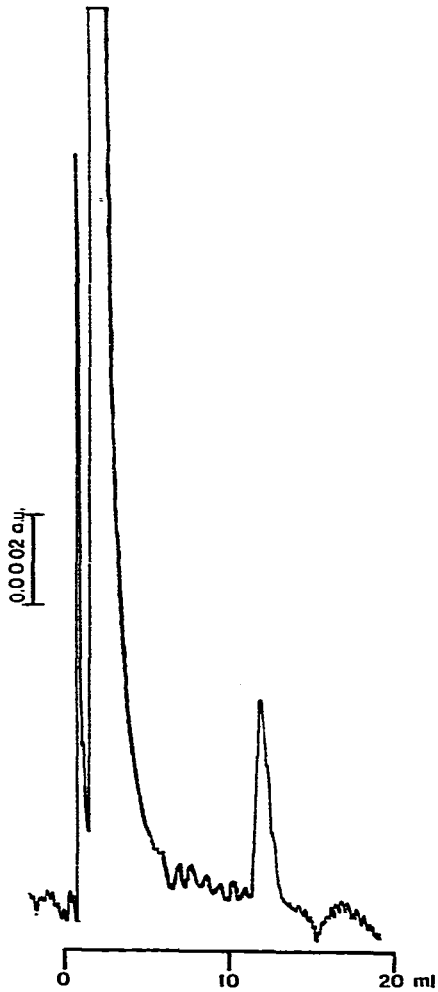

Fig. 11. LC trace of a concentrated hexane extract from a patient receiving trifluoperazine. The last dose was 60 min before the blood sample. Detection by UV (254 nm); *y* axis in absorbance units. The trifluoperazine concentration was 14.4 ng/ml.

LC conditions. We have validated the extractions for a selection of the possible compounds to which they can be applied. It is recommended that the system be used with the following guidelines: (1) for drugs known to occur at concentrations of 10 ng/ml and above, such as chlorpromazine and thioridazine, use UV detection, reserving amperometric detection for low-dose drugs such as trimeprazine; (2) choose an ammonium acetate concentration giving convenient retention times in any particular case, but note that amperometric detection requires relatively high ionic strengths; (3) when an internal standard is needed, choose any compound, from the list, known to be absent from the plasma and to have a retention volume different from that of the compound(s) to be analysed, but note that internal standards must be used with great care [16]; (4) use hexane extracts unless metabolite assays are sought; for the metabolites diethyl ether extracts are essential as many of the metabolites do not extract into hexanes; (5) reserve the hexane—chloroform approach for difficult problems, low concentrations or whole-blood

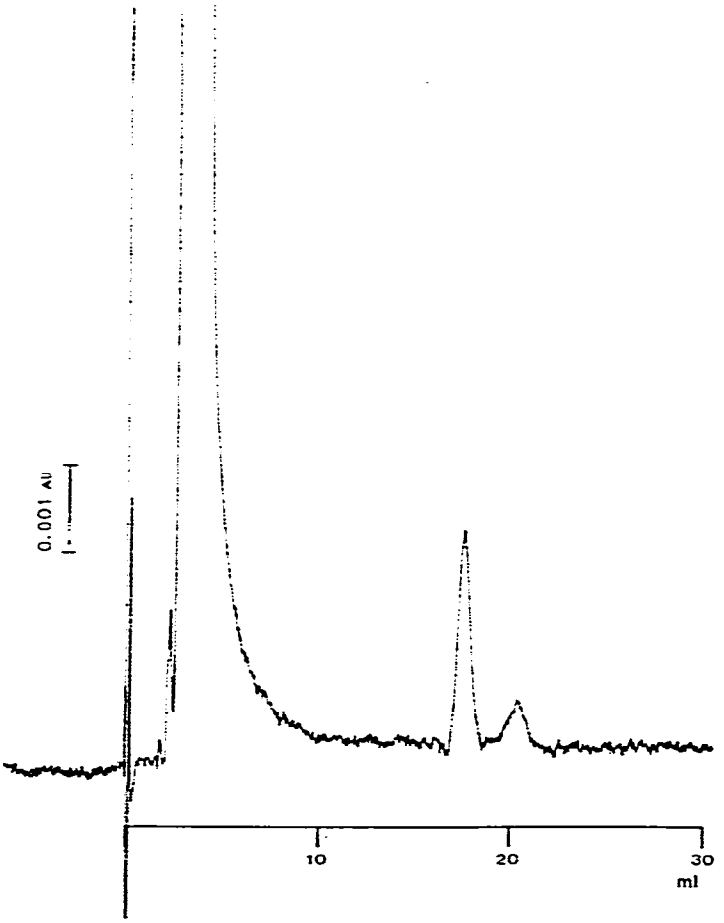

Fig. 12. LC trace of a concentrated hexane extract from a patient overdosed with amitriptyline. Dosage and timing unknown. The trace shows both amitriptyline and nortriptyline. Detection by UV (254 nm); *y* axis in absorbance units. The amitriptyline concentration was 37 ng/ml; the nortriptyline concentration was below 10 ng/ml.

samples; (6) use radial compression columns for speed and high-quality LC traces, but note that the flow-rates and therefore the solvent costs are high with this system. Quite obviously, an assay for a particular compound will have to be validated in the laboratory of application.

The expanded rationale behind some of these recommendations is as follows. UV detection has been found to be more stable than amperometry, which requires time to "warm up", gives drifting baselines, wider solvent bands, and a "noisier" trace, and shows a decay of sensitivity during the working day leading to heavy reliance on the use of the internal standard. However, amperometry is notably more sensitive. As regards extraction, very small quantities were found (< 1 ng/ml) to require prolonged extraction times and the use of large volumes of biological material. This led to "dirty" extracts. The chloroform extraction, which presumably employs an ion-pair principle was found to overcome these problems.

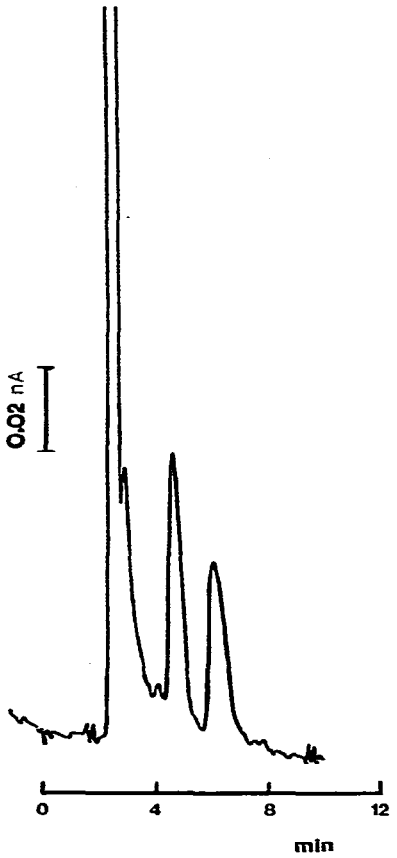

Fig. 13. LC trace of a concentrated hexane—chloroform extract from an experimental subject receiving trimeprazine. The trace shows both trimeprazine (4.5 min) and imipramine (used as an internal standard; 6.5 min). The dose of trimeprazine was 5 mg and the sample was collected 5 h post dosage. Detection by amperometry. The trimeprazine concentration was 0.8 ng/ml.

The use of peak heights, peak areas, as computed by the integrator, and ratios involving internal standards for quantitation purposes were investigated. The peak height ratio method seemed to be more successful for general application in the current study. These matters have been investigated in earlier contributions to this literature [1—9].

The LC approach has a number of advantages over other methods, as well as speed and convenience. For example with chlorpromazine, a serious problem with the GC approach involves the N-oxide. This compound reduces in GC conditions, to both parent drug and demethylated analogues; presumably this is also true for other N-oxides. Unless considerable care in choosing the extraction conditions is exercised, the GC assay of an unchanged drug can be adversely affected by its metabolites. This problem also makes the metabolites themselves difficult to assay, and is compounded by the availability of only a limited supply of model metabolites. In the LC system, all of the compounds are stable, and assayable from the same extraction.

The LC approach is superior to biological assays because of better specificity. For example, while radioimmunoassay methods published for drugs in this group are, in general, of reasonably high specificity, there remains the serious problem of cross-reactivity between unchanged drugs, their demethylated metabolites, and their 7-hydroxylated analogues [17, 18]. Radioreceptor assays are designed to detect the "total neuroleptic" content of biological samples, mixed drugs and their metabolites, and so are by definition non-specific [19, 20]. This makes them unsuitable for studies in pharmacokinetics where data are only of value if they are for a specific known compound.

ACKNOWLEDGEMENTS

We are grateful to the various drug manufacturers for samples of their products: to Dr. A.A. Manian of the U.S. National Institute of Mental Health for samples of model metabolites; and to various members of the staff at the J. Hillis Miller Health Center for assistance with patient and experimental subject samples. All investigations involving human subjects were approved by the relevant human experimentation committees.

REFERENCES

1 S.H. Curry, Anal. Chem., 40 (1968) 1251—1255.
2 A.G. Butterfield and R.W. Sears, J. Pharm. Sci., 66 (1977) 1117—1119.
3 W.F. Heyes and J.R. Salmon, J. Chromatogr., 156 (1978) 309—316.
4 W.F. Heyes, J.R. Salmon and W. Marlow, J. Chromatogr., 194 (1980) 416—420.
5 B.B. Wheals, J. Chromatogr., 177 (1979) 263—270.
6 S.H. Curry and G.P. Mould, J. Pharm. Pharmacol., 21 (1969) 674—677.
7 J.I. Javaid, J. Chromatogr. Sci., 17 (1979) 666—670.
8 C. Eggert-Hansen, Brit. J. Clin. Pharmacol., 3 (1976) 915—923.
9 J.E. Wallace, E.L. Shimek, J. Starchansky and S.C. Harris, Anal. Chem., 53 (1981) 960—962.
10 P. Kissinger, Anal. Chem., 46 (1974) 15R—20R.
11 D. Koch and P.T. Kissinger, Anal. Chem., 52 (1980) 27—29.
12 H.M. van Praag, Psychotropic Drugs: A Guide for the Practitioner, Macmillan, London, 1978.
13 T.B. Cooper, Clin. Pharmacokin., 3 (1978) 14—38.
14 S.H. Curry, in G.D. Burrows and T. Norman (Editors), Psychotropic Drugs: Plasma Concentrations and Clinical Response. Marcel Dekker, New York, 1980.
15 S.H. Curry, E.A. Brown and J.H. Perrin, IRCS Med. Sci., 9 (1981) 166—169.
16 S.H. Curry and R. Whelpton, in E. Reid (Editor), Blood Drugs and Other Analytical Challenges, Ellis Horwood, Chichester, 1978.
17 D.H. Wiles, Psychol. Med., 6 (1976) 407—415.
18 A. Jorgensen, Life Sci., 23 (1978) 1533—1542.
19 I. Creese and S.H. Snyder, Nature (London), 270 (1977) 180—182.
20 A.S. Papadopoulos, Brit. J. Psychiat., 136 (1980) 591—596.