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Note

Analysis of procyclidine in human plasma and urine by gas—liquid chromatography

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Procyclidine [1-cyclohexyl-1-phenyl-3-(pyrrolidin-1-yl)-propan-1-ol hydrochloride; Kemadrin, Fig. 1a] is a synthetic anticholinergic compound therapeutically useful in the treatment of idiopathic and neuroleptic induced Parkinson's disease as shown in controlled trials [1,2]. The only existing method [3] for the quantitative determination of procyclidine in either plasma or urine is based on a gas—liquid chromatographic (GLC) procedure which includes an inconvenient initial isolation step and lacks adequate sensitivity for detailed pharmacokinetic studies. The GLC method described here has adequate sensitivity for bioavailability and pharmacokinetic studies. It involves a solvent extraction under alkaline conditions with imipramine (Fig. 1b) as internal standard, followed by quantitation on a gas—liquid chromatograph fitted with a nitrogen—phosphorus detector.

EXPERIMENTAL

Reagents

Procyclidine·HCl was obtained from the Wellcome Foundation (Dartford, Great Britain) and imipramine·HCl from Sigma (Poole, Great Britain). Cyclohexane and toluene, both of Analar grade (Fisons, Loughborough, Great Britain), were glass distilled before use. Freshly deionised water was used for rinsing glassware and in all aqueous solutions.

Glassware

Stoppered test tubes (20 ml, Sovirel type 611-03; V.A. Howe, London, Great Britain) were used for the extraction and autosampler microtubes (tube 834078-902; Du Pont (U.K.), Hitchin, Great Britain) were used in the drying

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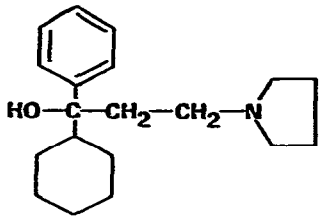
(a)



(b)

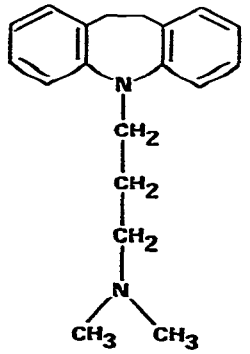


Fig. 1. Molecular structures of procyclidine (a) and imipramine (b).

stage. All glassware was washed with 2 *M* hydrochloric acid and rinsed with water before use. The microtubes were dried in a vacuum oven before use (210°C, <80 mm Hg vacuum for 1 h minimum).

Method

Standard solutions of procyclidine were prepared by dilution of a 1 mg/ml aqueous stock solution with either plasma or urine, depending on the nature of the samples to be assayed. The standards in plasma and urine covered the ranges 0—1 μg/ml and 0—2 μg/ml respectively.

A 1-ml aliquot of standard or sample was placed into an extraction tube and to it were added 40 μl of a 10 μg/ml aqueous solution of imipramine (as internal standard), 1 ml of 1 *M* sodium hydroxide and 4 ml cyclohexane. The tube was then tightly stoppered and mixed for 20 min along its long axis at 25 oscillations per min. The liquid phases were then separated by centrifugation at 1200 *g* for 10 min. A 3-ml aliquot of the cyclohexane layer (top) was then transferred to a microtube and the cyclohexane evaporated at room temperature under a stream of nitrogen.

A further 3 ml of cyclohexane were added to the aqueous layer and the extraction procedure repeated. Cyclohexane (3 ml) from the second extraction was then added to the residue in the corresponding microtube and dried as before. The resultant, combined residue was taken up in 50 μl glass-redistilled toluene about 10 min before injection into the gas chromatograph. All samples were analysed in duplicate.

Gas—liquid chromatographic conditions

The gas chromatograph used was a Model F30 Perkin-Elmer (Beaconsfield, Great Britain) equipped with a nitrogen—phosphorus detector (Perkin-Elmer).

A 1.8 m × 4 mm I.D. glass column was hand packed with 5% OV-17 on Chromosorb W HP (100—120 mesh) and conditioned at 310°C with a 50 ml/min helium carrier gas flow for 24 h before use.

The detector was used in the nitrogen mode under the following conditions: gas flow-rates, hydrogen (8.5 ml/min); air (92 ml/min); carrier gas (helium, 50 ml/min). Manifold, oven and injection port temperature were 321°C, 246°C and 310°C respectively.

Under these conditions procyclidine was well resolved from imipramine, these two components having retention times of 5.85 min and 7.10 min respectively. No interfering peaks were seen in either human plasma or urine.

RESULTS

Calculation of results

A Hewlett-Packard Model 3352 data system was used to calculate the peak areas of procyclidine and the internal standard and their ratios. A known mass of the internal standard (0.4 μg of imipramine) was added to a range of procyclidine standard solutions. A calibration curve was constructed by plotting the concentrations of procyclidine on the abcissa against the ratio of the peak area of procyclidine to that of imipramine on the ordinate. Since the same known mass of internal standard was added to the unknown samples (urine or

TABLE I

METHOD PRECISION AND REPRODUCIBILITY WHEN KNOWN AMOUNTS OF PROCYCLIDINE WERE ADDED TO HUMAN PLASMA OR URINE AND REPEATEDLY ANALYSED

$n = 6$.

Standard concentration of procyclidine (ng/ml)	Ratio of peak area of procyclidine to peak area of internal standard		
	Mean	±S.D.	S.D. of mean (%)
Plasma			
20	0.021	0.0025	11.9
50	0.059	0.0034	5.8
100	0.129	0.0062	4.8
200	0.260	0.0140	5.4
500	0.651	0.0282	4.3
1000	1.298	0.0739	5.7
Urine			
50	0.0613	0.0048	7.83
100	0.1362	0.0034	2.52
200	0.2743	0.02295	8.37
500	0.6940	0.01691	2.44
1000	1.4473	0.1063	7.34
2000	2.971	0.2178	7.33

plasma) the amount of procyclidine in the samples could be calculated from the calibration curve.

Validation

The method was validated by analysing samples of plasma to which known quantities of procyclidine had been added. Six determinations were made for each sample and the precision obtained for concentrations of procyclidine between 50 and 2000 ng/ml is shown in Table I; percentage mean S.D. ranged from 2.5 to 8.4%. At maximum sensitivity (20 ng/ml) the percentage mean S.D. was 11.9. The results show that the recovery of procyclidine added was

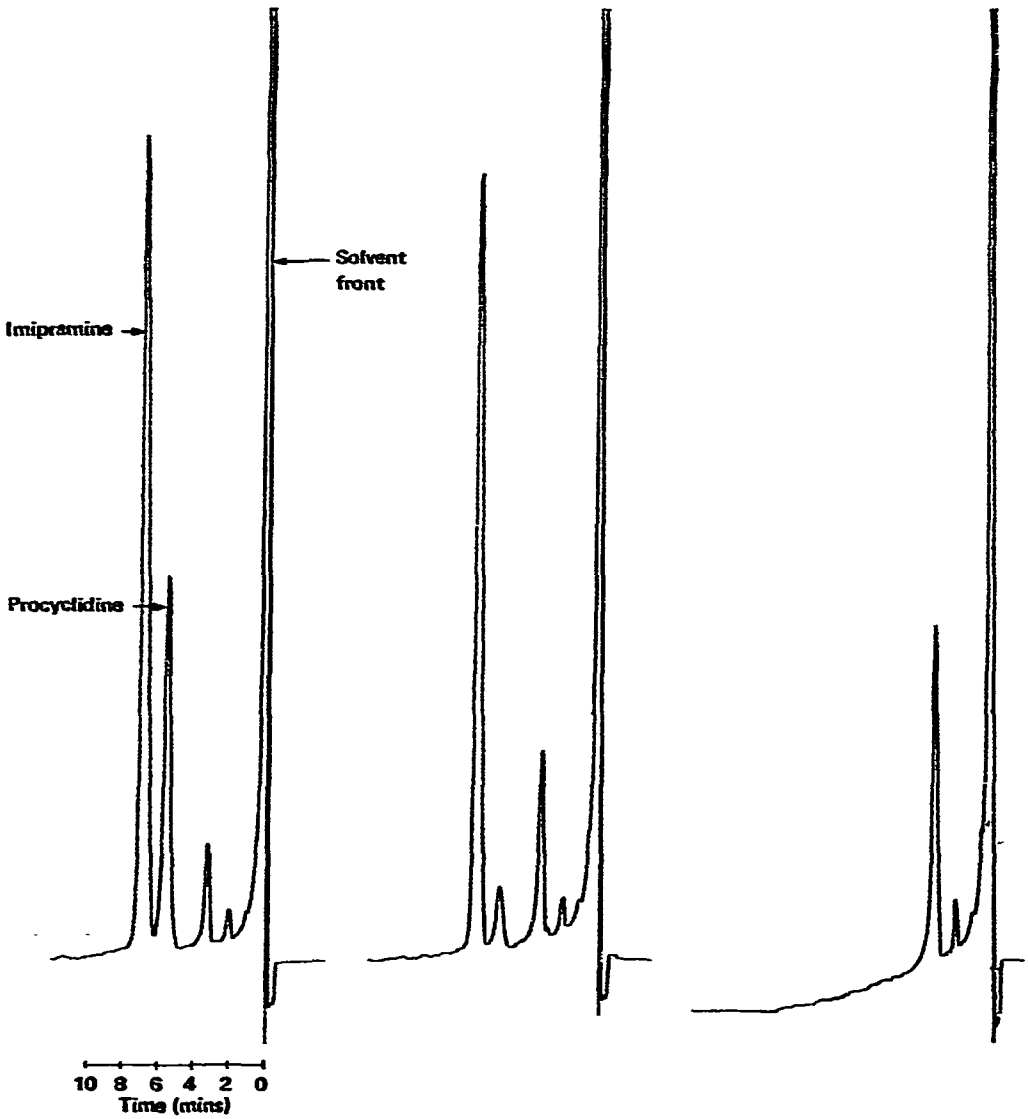


Fig. 2. Chromatograms produced from urine containing (left) 500 and (middle) 100 ng/ml procyclidine with internal standard and (right) a blank urine extract.

linear relative to the drug standards. The calibration curve was described by a straight line using a linear regression programme, Texas TI-51 (III) calculator. For plasma $y = 0.00126x - 0.0018$; for urine $y = 0.00149x - 0.0266$ with correlation coefficients of 0.9979 and 0.9960 respectively, where y = ratio of peak areas of procyclidine and imipramine and x = plasma concentration of procyclidine (ng/ml).

The detector response was linear from 1.6 to 160 ng procyclidine injected onto the column. The lower limit of sensitivity was 1—2 ng injected on column under the standard conditions. No interfering peaks were seen (Figs. 2 and 3) even at high sensitivity. Under the operating conditions described the method is capable of detecting as little as 20 ng/ml of procyclidine in plasma or urine.

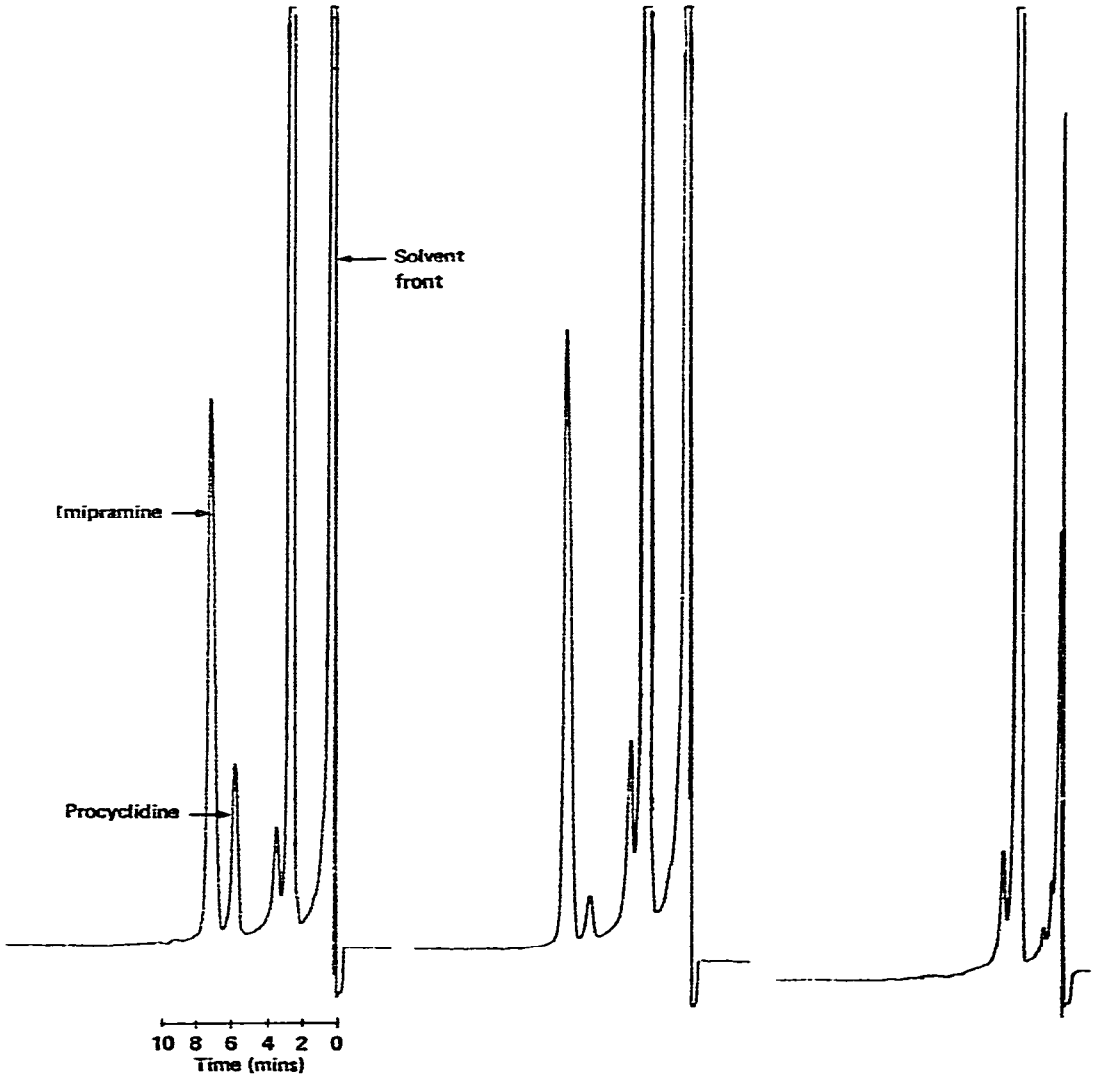


Fig. 3. Chromatograms produced from plasma containing (left) 200 and (middle) 50 ng/ml procyclidine with internal standard and (right) a blank plasma extract.

An example of the plasma concentration curve from a healthy male volunteer after receiving 10 mg of procyclidine orally is shown in Fig. 4.

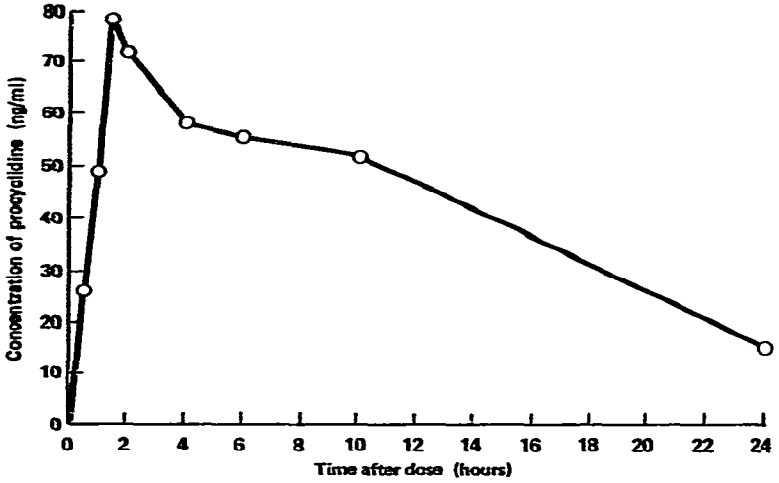


Fig. 4. Plasma profile from a healthy adult male volunteer after 10 mg of procyclidine was given orally.

DISCUSSION

The GLC method described had good precision, was specific for unchanged procyclidine and was also extremely sensitive, 1.6 ng of procyclidine injected onto the column was readily detected. Several precautions were necessary to ensure reproducible results. Some procyclidine is adsorbed onto glass; this causes carry-over from the glass syringe used to inject the samples. This problem can be overcome by washing the syringe in chloroform between injections, as previously reported for the analysis of trimethoprim [4]. Also the extraction tubes and the microtubes used during the extraction procedure should always be soaked in HCl between assays in order to prevent any carry-over of procyclidine. The use of an internal standard reduces error from transfer losses.

The method described is used routinely for the analysis of procyclidine in human plasma and urine and is sufficiently sensitive for bioavailability and pharmacokinetic studies.

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