Journal of Chromatography, 223 (1981) 213—218
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 781

Note

Quantitative gas—liquid chromatographic method for the determination of phenoperidine in human plasma

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(First received August 28th, 1980; revised manuscript received November 10th, 1980)

The 4-phenylpiperidine synthetic analogues of narcotic analgesics, for instance pethidine and phenoperidine (ethyl-4-phenyl-1-(3-hydroxy-3-phenylpropyl)-piperidine-4-carboxylate) have been the subject of some major research programmes in our laboratories. Recent studies on the use of narcotics in hospital practice showed that pethidine, morphine, diamorphine and phenoperidine were the four most widely used potent analgesics [1]. In particular, phenoperidine which is 50 times more potent than pethidine, was used exclusively in surgical theatres and intensive care units.

The metabolic fate and disposition of pethidine, the oldest synthetic narcotic, in both animal and human subjects have been extensively studied [2, 3]. However, very little is known about the biotransformation and pharmacokinetics of its closely related analogue, phenoperidine. The purpose of this paper is to present a report on the choice of some phenoperidine analogues and the development of one for use as an internal standard to determine plasma levels of phenoperidine after intravenous administration to healthy volunteers.

EXPERIMENTAL

Apparatus

A Sigma 3 gas chromatograph, all glass system, fitted with a phosphorus—

0378-4347/81/0000—0000/$02.50

nitrogen detector and linked to a Hitachi chart recorder Model 56 (Perkin-Elmer, Beaconsfield, Great Britain) was used. This instrument was operated with coiled glass column (1 m × 4 mm O.D.) silanised with hexamethyldisilazane (HMDS; Chromatography Services, Merseyside, Great Britain) before use and packed with various stationary phases. The operating temperature for the injector and detector was 350°C, and also a suitable one for the column. Gas flow-rates were nitrogen (carrier gas), 30 ml/min; hydrogen, 5 ml/min; and air, 100 ml/min. The temperature of the rubidium bead in the phosphorus—nitrogen detector was adjusted to optimum condition before use. Other apparatus used: 10-ml and 15-ml capacity centrifuge tubes with well fitting screw caps (Sovirel, Levallois-Perret, France); 15-ml capacity stoppered evaporation tubes with finely tapered bases. All this glassware was cleaned by soaking overnight in a 2% solution of RBS 25 (Chemical Concentrates, RBS, London, Great Britain) in water, then rinsed thoroughly with methanol and hot tap water followed by distilled water. These tubes were subsequently silanised by rinsing with HMDS in re-distilled chloroform (3%, v/v), and dried at 250°C overnight. This treatment of glassware was found to be necessary to eliminate possible loss of drug due to adsorption to the glass wall [4].

Materials

The following materials were used: chloroform, dichloromethane, diethyl ether, methanol and *n*-hexane, all of AnalaR grade and freshly re-distilled; sodium hydroxide solutions (5 *N* and 0.1 *N*, ether-washed); phenoperidine hydrochloride and R3757 hydrochloride, a phenoperidine analogue (Janssen Pharmaceutica, Beerse, Belgium); practolol hydrochloride and propranolol hydrochloride (I.C.I., Macclesfield, Great Britain); oxprenolol hydrochloride (Ciba Geigy, Macclesfield, Great Britain) a *n*-butyl reversed ester analogue of phenoperidine (synthesised in own laboratory).

Gas—liquid chromatography

Methanolic solutions of phenoperidine and possible internal standards were injected onto the following chromatographic systems at various oven temperatures.

System 1. Supelcoport (80—100 mesh) coated with 3% OV-1, in a coiled glass column (1 m × 4 mm O.D.).

System 2. Supelcoport (80—100 mesh) coated with 10% SP-2100, in a coiled glass column (1 m × 4 mm O.D.).

The columns were conditioned at 20°C below that of the maximum recommended temperature of the relevant stationary phase for 24 h. Each column was then silanized twice in situ with 20-μl aliquots of HMDS before use.

Compounds which were investigated as possible internal standards were: practolol, propranolol, oxprenolol, metoprolol, a reversed ester analogue of phenoperidine [1-(3-hydroxy-3-phenylpropyl)-4-phenyl-4-piperidinol-*n*-butyl ester] and R3757, a phenoperidine analogue with longer side chain [ethyl-4-phenyl-1-(5 hydroxy-5-phenylpentyl)-piperidine-4-carboxylate].

Retention times, resolution and symmetry factors of the analytical peaks of these compounds for various gas—liquid chromatography (GLC) systems were determined and the most suitable compound for use as internal standard was then established.

General procedure for the determination of phenoperidine in plasma

Blood samples were obtained by venous puncture after intravenous administration and collected in heparinised polythene tubes. The red blood cells were separated from the plasma by centrifugation (3000 *g* for 10 min). A plasma sample (2.5 ml) diluted with distilled water (2.5 ml) in a 15-ml glass centrifuge tube was made alkaline (pH 10—12) with 20 μl 5 *N* sodium hydroxide solution and the internal standard R3757 (60 μl of a methanolic solution equivalent to 50 ng/ml) was added. The alkaline solution was extracted with 12 ml of diethyl ether—dichloromethane (4:1) using an automatic shaker at a speed of 40 rpm for 10 min. The organic layer was then separated from the aqueous by centrifugation (2500 *g* for 15 min) and was transferred carefully into a 15-ml evaporation tube. The extract was then evaporated to dryness at 50—55°C on a water bath. An aliquot of re-distilled *n*-hexane (20 μl) was added to dissolve the dried extract. The stoppered tubes were then stored at −20°C before GLC analysis. An aliquot (1—2 μl) of the final concentrate was injected into the GLC system. The concentration of phenoperidine present in the plasma sample was determined from the ratio of the peak height of phenoperidine to that of the internal standard. Calibration graphs were prepared as follows: methanolic standard solutions of phenoperidine and internal marker were added to the drug-free plasma to cover a concentration range of 5—80 ng/ml).

Recovery, selectivity, reproducibility and storage

Recovery. Eight replicate samples of phenoperidine in plasma (20 ng/ml) were extracted and evaporated to dryness as described in General procedure. The internal standard, R3757 (50 ng/ml in methanol), was then added to each tube and evaporated to dryness and 1.5 μl aliquots injected onto GLC System 2 as described earlier. These results were then related to the 100% value obtained from standard methanolic solutions containing phenoperidine 20 ng/ml and R3757 50 ng/ml.

Selectivity. Samples of plasma from patients on a variety of drugs were analysed to find out if they produced peaks after chromatography which interfered with those of phenoperidine and R3757.

Reproducibility. Seven replicate samples of phenoperidine in plasma (20 ng/ml) were assayed by the General procedure and the peak height ratios of the drug to the marker were calculated.

Storage. Samples of plasma were analysed immediately and after storage at −20°C for seven days.

RESULTS AND DISCUSSION

Choice of GLC system

Two GLC systems were investigated and their performance is summarised in Tables I and II and Fig. 1. Both System 1 (3% OV-1) and System 2 (10% SP-2100) were satisfactory for the analysis of phenoperidine. The reversed ester analogue of phenoperidine was not resolved by any of the GLC systems investigated, and considered unsuitable for use as an internal standard. Compounds such as propranolol, practolol and metoprolol were considered un-

TABLE I

PERFORMANCE OF GLC SYSTEM 2

Glass column (1 m × 4 mm O.D.) packed with 10% SP-2100 on Supelcoport (80—100 mesh); column temperature, 275°C.

Drug	Retention time (min)	Symmetry factor (limit 0.95—1.05)	Resolution between marker (> 1.0)
Phenoperidine	6.0	1.03	—
Propranolol	1.2	1.00	2.8
Practolol	2.1	0.98	2.7
Metoprolol	0.8	1.01	3.1

TABLE II

PERFORMANCE OF GLC SYSTEM 1

Glass column (1 m × 4 mm O.D.) packed with 3% OV-1 on Supelcoport (80—100 mesh); column temperature, 235°C.

Drug	Retention time (min)	Symmetry factor (limit 0.95—1.05)	Resolution between marker (> 1.0)
Phenoperidine	3.0	1.00	3.8
R3757 (internal standard)	5.3	0.98	—

suitable as internal standards because their retention times in the systems investigated were much shorter than that of phenoperidine (Table I).

Recovery, selectivity, reproducibility and storage

The GLC System 1 (3% OV-1 on Supelcoport, 80—100 mesh) was chosen for routine analysis because phenoperidine had a shorter retention time at a lower operating column temperature (Tables I and II).

The relative recovery of phenoperidine from human plasma was 88.8 ± 2.7%. During the course of these studies, it was established that substances in samples obtained from patients on a variety of drugs, particularly morphine, diamorphine, pentazocine, pethidine and methadone did not interfere in the analysis of phenoperidine.

Repeated assays of the same plasma samples containing phenoperidine indicated that the reproducibility of the peak height ratio of phenoperidine to internal standard was 100 ± 1.42%. Calibration graphs for phenoperidine were linear over the range of 2—80 ng/ml (Fig. 2). The graphs were found to be reproducible when repeated ten times during the studies.

Samples of plasma whether fresh or stored at —20°C for seven days did not give peaks that would interfere with the measurement of peaks corresponding to phenoperidine and the internal standard in the chromatogram (Fig. 1). There was no appreciable loss of the drugs from the samples after storing at —20°C for seven days.

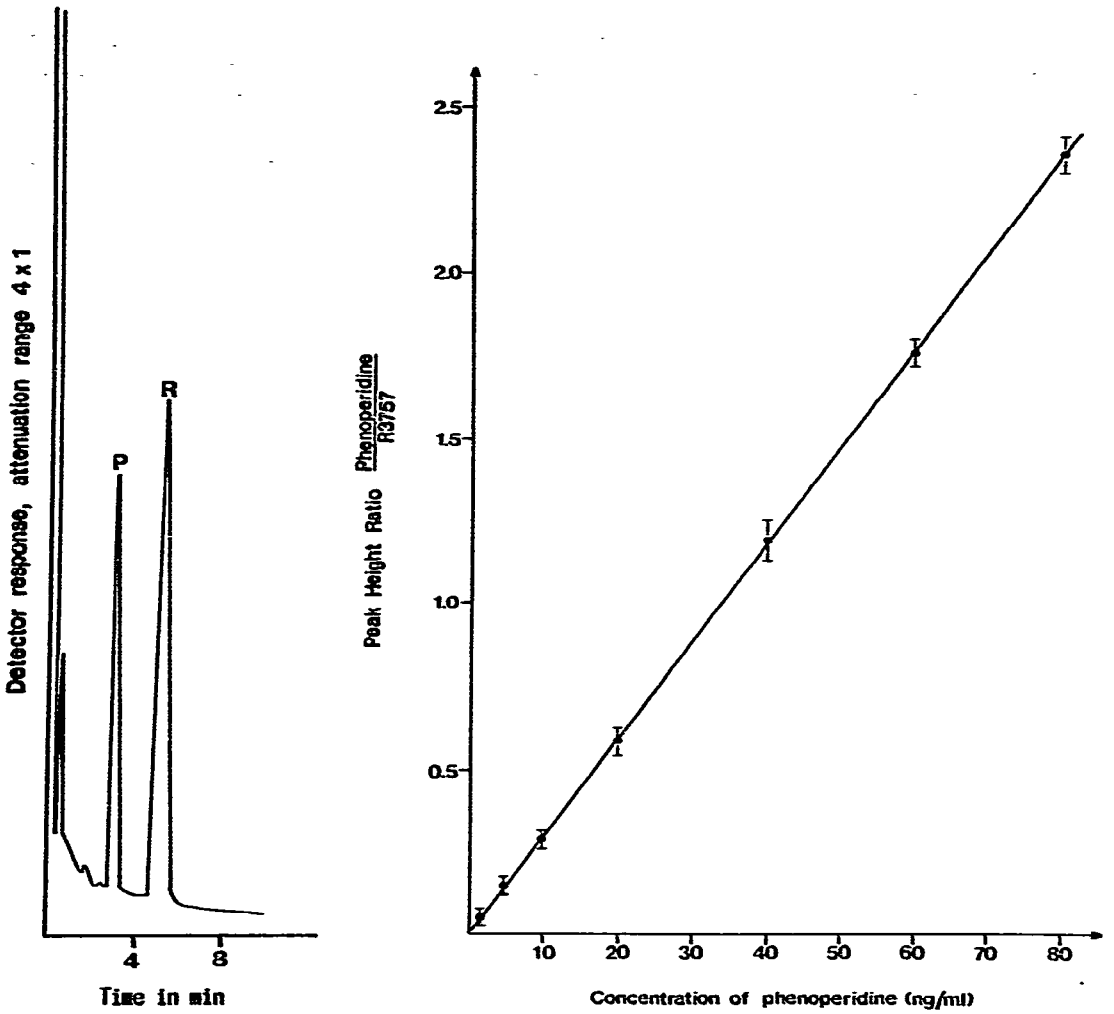

Fig. 1. Chromatogram of phenoperidine (P) 25 ng per ml and internal standard, R3757 (R) 50 ng/ml after extraction from plasma, using GLC System 1 (3% OV-1) with column temperature at 235°C.

Fig. 2. Typical calibration graph of phenoperidine in plasma.

APPLICATION

This procedure has been used in preliminary studies on the plasma concentration of phenoperidine in volunteer subjects. These studies were carried out during the administration of repeated doses of ammonium chloride since previous investigations have shown that the disposition of pethidine is influenced by urinary pH [5]. After overnight fasting, subjects were given a bolus dose of phenoperidine (15 μg/kg, intravenous) and blood samples (approximately 8 ml) were removed at frequent intervals from an intravenous cannula. The concentration of phenoperidine in plasma was measured by the General procedure.

An example of the plasma profile of a male volunteer subject (age 26 years,

weight 65 kg) obtained in this preliminary study is shown in Fig. 3. There is a rapid decline in the plasma concentration of phenoperidine followed by a secondary rise between 30 and 40 min. The concentration of the drug then declined more slowly and phenoperidine was still detected after 120 min. A separate study has shown that the secondary rise was largely abolished by concurrent treatment with antacid (Andursil, 10 ml at 10-min intervals for at least 1 h during the course of the study). It is probable that the secondary peak may be due to the elimination of the drug into the acidic gastric fluid, followed by its subsequent re-absorption in the small intestine.

A more detailed report of this work is in preparation.

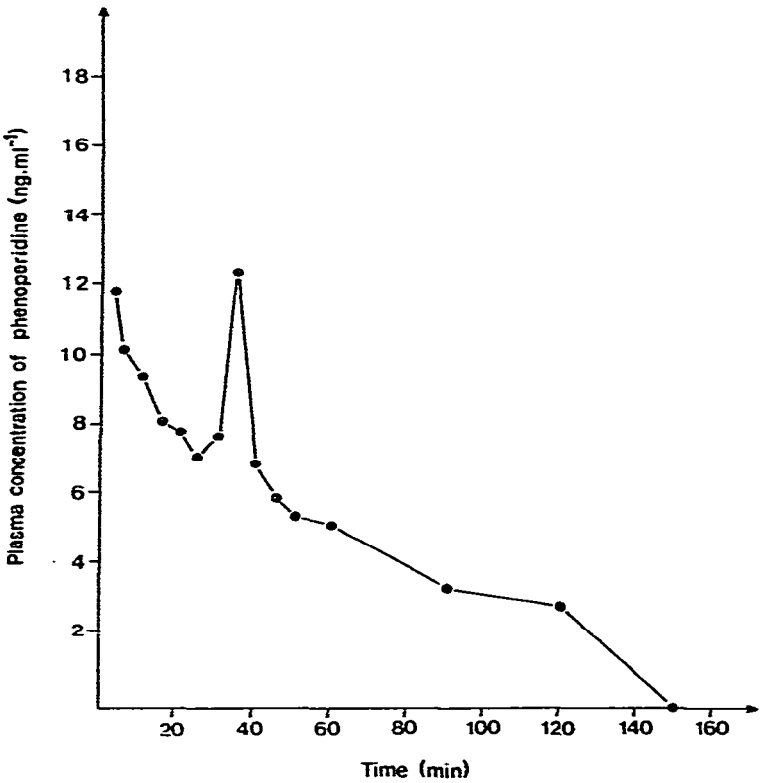

Fig. 3. Profile of plasma concentration of phenoperidine with time after an intravenous dose of 15 μg/kg to a male subject.

ACKNOWLEDGEMENT

The financial assistance of the Mersey Regional Health Authority and Janssen Pharmaceutical Ltd. is gratefully acknowledged.

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