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DETERMINATION OF PETHIDINE IN PLASMA BY ELECTRON CAPTURE GAS CHROMATOGRAPHY AFTER REACTION WITH TRICHLOROETHYL CHLOROFORMATE

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SUMMARY

Pethidine (meperidine) is determined in plasma by electron capture gas chromatography after derivatization with trichloroethyl chloroformate. The analytical procedure involves extraction of pethidine and the internal standard from plasma and their separation from metabolites by partition chromatography. After purification of the eluate, the derivatization is accomplished with trichloroethyl chloroformate in the presence of anhydrous sodium carbonate. The reaction mixture is further purified with methanolic alkali before gas chromatographic analysis.

Optimum conditions for extraction and derivatization, as well as the sensitivity and selectivity of the method are discussed. Owing to the high sensitivity the pethidine levels are determined in 0.1 ml of plasma. The smallest amount of pethidine determined by the method was 100 ng/ml. The relative standard deviation at the 50-ng level of pethidine added to 0.1 ml of plasma was 5.8% ($n = 8$).

INTRODUCTION

Pethidine (ethyl 1-methyl-4-phenyl-4-piperidinecarboxylate) has been used for more than 30 years for the relief of acute pain, especially in obstetrics, and for post-operative analgesia. However, owing to the low sensitivity of existing analytical methods for pethidine relatively little is known about the pharmacokinetics of the drug.

Several methods for the determination of pethidine in biological material have been based on its complexation with methyl orange and determination by spectrophotometry[1–4]. However, these methods lack sufficient sensitivity and selectivity, pethidine metabolites and other amines being co-determined. A fluorimetric procedure

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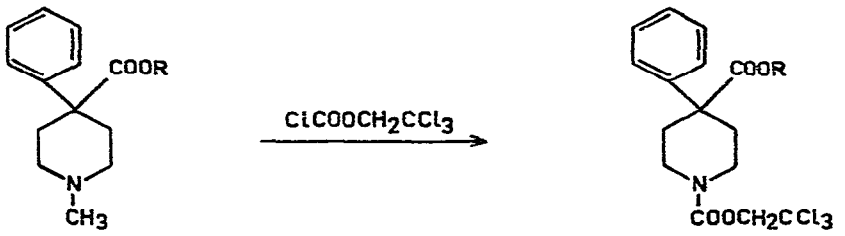
has also been used[5], but this method could not be used for concentrations of pethidine below 300 ng/ml. Recently, a sensitive radioimmunoassay for pethidine has been described[6], but the possible interference from norpethidine was not evaluated. Gas chromatography with flame ionization detection is by far the most commonly used method[7–10]. Pethidine is determined without derivatization, and the poor chromatographic properties of the drug, due to the amine function, limit the sensitivity and precision of the methods.

By the introduction of the two reagents, pentafluorobenzyl chloroformate[11] and trichloroethyl chloroformate[12], many tertiary amines can now be determined as the corresponding carbamates in the ng/ml range by electron capture gas chromatography. This paper presents a sensitive and selective procedure for the determination of pethidine in plasma samples. The method is based on the formation of the trichloroethyl carbamates of pethidine and the internal standard (Fig. 1). The high electron capture response of the derivatives permits the use of very small sample volumes.



Pethidine R=C_2H_5

Internal standard R=C_4H_9

Fig. 1. Reaction of pethidine and butyl 1-methyl-4-phenyl-4-piperidinecarboxylate with trichloroethyl chloroformate.

EXPERIMENTAL

Gas chromatography

Evaluation of reaction conditions was performed in a Varian 1400 gas chromatograph with flame ionization detector. The glass column (90 × 0.18 cm) was filled with 3% OV-17 on 80–100 mesh Gas-Chrom Q. The column temperature was 270° while injector and detector temperatures were maintained at 300°. Nitrogen was used as carrier gas at a flow-rate of 30 ml/min.

Studies in the nanogram range were performed in the same instrument with a nickel-63 or a tritium electron capture detector. The nickel-63 detector was operated at 300° and the tritium detector at 225°. The glass column (150 × 0.18 cm) contained 3% OV-17 and 0.3% Carbowax–terephthalic acid on 80–100 mesh Gas-Chrom Q and was operated at 225°. Other conditions were as stated above.

Reagents and chemicals

Trichloroethyl chloroformate was obtained from E.G.A. Chemie (Steinheim bei Heidenheim, G.F.R.) Cellulose, Munktell 410, was purified by washing with ethanol

and heptane before use. Methanolic alkali solution was prepared by dissolving 2.8 g of potassium hydroxide in a mixture of 75 g of methanol (Merck pro analysi quality) and 22 g of water and saturated methanolic alkali solution was a saturated solution of potassium hydroxide in methanol.

The internal standard (butyl 1-methyl-4-phenyl-4-piperidinecarboxylate hydrochloride) was prepared from pethidinic acid, obtained by hydrolysis of 1-methyl-4-phenyl-4-piperidinecarbonitrile[13], by refluxing it with butanol saturated with dry hydrogen chloride (m.p. 158–160°; reported m.p. 160–162°)[14]. The infrared and mass spectra were in accordance with the expected structure.

For the standard solution of the internal standard, sufficient butyl 1-methyl-4-phenyl-4-piperidinecarboxylate hydrochloride was dissolved in phosphate buffer (pH 7.4) to give a concentration of 500 ng/ml.

For the standard solution of pethidine, pethidine hydrochloride was dissolved in 0.1 *M* phosphoric acid and the solution diluted to 5 μg/ml concentration with water. Aliquots of this solution were diluted with plasma to give final concentrations of 100, 250, 400 and 600 ng/ml. In the preparation of the standard curve 0.1 ml of each were taken for analysis.

Buffer solutions were phosphate buffers of pH 2.0 ($\mu = 0.1$) and pH 7.4 ($\mu = 1$). Organic solvents were distilled before use.

METHODS

Evaluation of reaction conditions

The derivatization reaction was studied in the following manner. To 0.1–0.2 mg of pethidine in 0.2 ml of toluene, 10–50 μl of trichloroethyl chloroformate and about 10 mg of sodium carbonate were added; 0.5 mg/ml of dotriacontane was present in the organic solvent as internal standard. The mixture was heated in a metal block for the time indicated below. Before injection into the gas chromatograph excess of reagent was destroyed by treatment with 1.0 ml of methanolic alkali solution for 5 min followed by the addition of 1.0 ml of water. The peak height ratio carbamate/internal standard was calculated.

Determination of pethidine in plasma samples

A separation column was prepared by mixing 2 g of cellulose with 1.3 ml of phosphate buffer (pH 7.4, $\mu = 1$) and packing the mixture in a 30-cm glass column. The plasma sample (0.1 ml) and 0.1 ml of the internal standard solution were mixed with 0.3 g of cellulose and packed on the top of the column. Pethidine and the internal standard were eluted with *ca.* 10 ml of heptane and re-extracted into 1.0 ml of phosphate buffer (pH 2.0). The aqueous phase was made alkaline, toluene (0.25 ml) was added and the mixture was shaken in a tube for 15 min and centrifuged (10 min at 2000 rpm). The organic phase was transferred to another tube and 25 μl of trichloroethyl chloroformate and 10 mg of anhydrous sodium carbonate were added. An air condensor was attached to the tube and the mixture was heated for 1 h at 125° in a metal block.

Methanolic alkali solution (1.0 ml) was added to the reaction mixture and the tube was shaken for 10 min. Water (1.0 ml) was then added and the tube was shaken for another 10 min. After centrifugation, the aqueous phase was discarded and 0.5 ml

of saturated methanolic alkali solution was added. The tube was shaken vigorously for 15 sec and 1.0 ml of water was added. A volume of 1–2 μl of the organic phase was injected into the gas chromatograph.

A standard curve was prepared by treating 0.1 ml of the standard solutions of pethidine in plasma according to the procedure described above.

RESULTS AND DISCUSSION

Extraction conditions

The partition coefficients of pethidine and the internal standard to heptane and toluene are given in Table I. The $-\log k_d \times K_{HA}$ value corresponds to the pH at which equal concentrations of the compound are obtained in organic and aqueous phases. The pH values for quantitative extraction from water into organic phase can also be seen in Table I.

TABLE I

PARTITION OF PETHIDINE, NORPETHIDINE AND BUTHYL 1-METHYL-4-PHENYL-4-PIPERIDINECARBOXYLATE

$k_d = A_{org}/A_{aq}$ = partition coefficient of amine between organic and aqueous phases; K_{HA} = acid dissociation constant of amine; pK_{HA} (pethidine) = 8.63, pK_{HA} (norpethidine) = 9.68 (from ref. 3).

Compound	*Organic phase*	*Log $k_d \times K_{HA}$**	*>99% in organic phase at pH***	*<1% in organic phase at pH***
Pethidine	Heptane	−6.96	8.6***	5.0
Norpethidine	Heptane	−9.20	—	7.0
Butyl 1-methyl-4-phenyl-4-piperidinecarboxylate	Heptane	−5.79	7.8	3.8
Pethidine	Toluene	−5.96	8.0	4.0
Butyl 1-methyl-4-phenyl-4-piperidinecarboxylate	Toluene	−4.74	6.8	2.8

* Photometric determination according to ref. 15.
** Equal phase volumes.
*** 98% in organic phase.

Norpethidine, a major metabolite of pethidine[2], must be excluded before derivatization is carried out as it will form the same carbamate as the parent drug. A chromatographic step was elaborated which completely separated norpethidine from pethidine. As can be concluded from Table I, the partition into heptane is more than one hundred times greater for pethidine than for norpethidine at pH 7.4. At this pH value of the stationary phase both pethidine and the internal standard have a retention volume of less than 0.5 ml. In a series of experiments it was shown to be sufficient to collect 10 ml of heptane from the column. Under the conditions described above norpethidine has a retention volume of approx. 80 ml.

Adsorption losses of the amines were minimized by using cellulose as support for the stationary phase. It was also found essential to use a high content of the stationary phase in the column as well as a high ionic strength. If cellulose was substituted with silica gel a considerably lower yield was obtained (50%).

Purification and concentration of the eluate was accomplished by extracting

the amines into an acidic aqueous phase followed by making the solution alkaline and extracting into a small toluene phase in which the reaction with trichloroethyl chloroformate was performed. As can be concluded from Table I, these two extractions are quantitative.

Studies on reaction conditions

Trichloroethyl chloroformate has been found to react with pethidine three times faster than pentafluorobenzyl chloroformate[12]. The yield of the reaction was found to be 104% by comparison with an equivalent amount of synthetic trichloroethyl carbamate of pethidine.

The reaction conditions have been studied in the following respects:

Solvent. The reaction between pethidine and trichloroethyl chloroformate in heptane has previously been studied[12]. Owing to low partition of pethidine into this solvent, toluene was chosen as reaction medium. A quantitative yield of the carbamate was obtained in 30 min at 125°. In heptane under the same conditions only 20 min were required[12].

Temperature. The time dependence of the reaction at different temperatures is shown in Fig. 2, showing the highest rate at 125°.

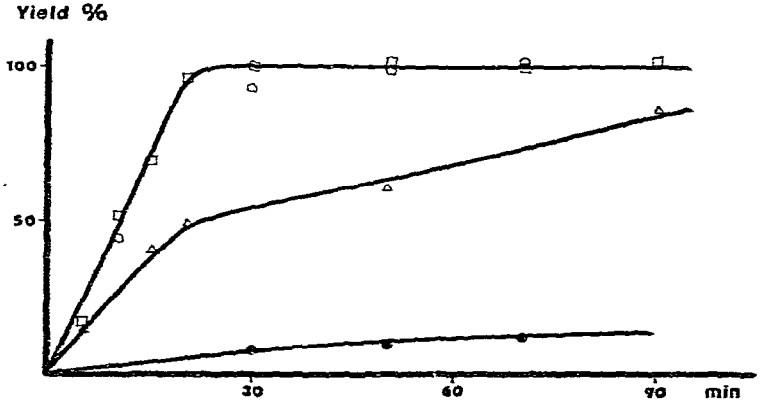


Fig. 2. Time dependence of the formation of trichloroethyl carbamate of pethidine. Reaction conditions: 0.2 mg of pethidine in 0.2 ml of toluene is treated with 25 µl of trichloroethyl chloroformate. Reaction temperature: □, 125° with Na_2CO_3; ○, 125° without Na_2CO_3; △, 110° with Na_2CO_3; and ●, 80° with Na_2CO_3.

Effects of base. In the studies on pentafluorobenzyl chloroformate, the addition of sodium carbonate greatly improved the reaction rate. A very small rate increase was observed in the reaction of trichloroethyl chloroformate with pethidine.

Concentration of trichloroethyl chloroformate. The above studies were performed with an addition of 25 µl of trichloroethyl chloroformate to 0.2 ml of solvent. If the amount of reagent was 50 µl, the reaction time was not influenced but with only 10 µl of reagent, 60 min were required for quantitative reaction.

Precision. The reaction of pethidine with trichloroethyl chloroformate was very reproducible. The standard deviation in the reaction of ten identical samples of 100 µg was 1.6%.

Selectivity of the method

Pethidine is extensively metabolised in man, the main routes being hydrolysis of the ester group to pethidinic acid, N-demethylation to norpethidine and subsequent hydrolysis to norpethidinic acid[16], hydroxylation of the phenyl ring[17] and formation of the N-oxide[18]. Of these metabolites only norpethidine has been found in plasma[9].

In the present method, acidic metabolites are excluded, if present, in the extraction step. Norpethidine forms the same carbamate as pethidine in the reaction with trichloroethyl chloroformate and must be excluded. This was achieved in a chromatographic step, taking advantage of the difference between pethidine and norpethidine in their partition into heptane. No interference from norpethidine was observed even if large amounts were added (> 200 ng) in the absence of pethidine.

The N-oxide of pethidine was also tested for possible interference. In the chromatographic system used this compound was strongly retarded and did not contribute to the levels of pethidine found in plasma.

Sensitivity of the method

The electron capture response of the trichloroethyl carbamate of pethidine in the nickel-63 detector at 320° was 3×10^{-16} mol/sec (*cf.* ref. 12). This corresponds to a minimum detectable amount of 5 pg of the derivative in an injected sample. As the plasma levels of pethidine were usually in the order 100–500 ng/ml, the high sensitivity and good gas chromatographic properties of the derivative made it possible to analyse samples as small as 0.1 ml. The small sample volume needed will be of great value when repeated blood sampling is required and will also facilitate the study of pethidine levels in infants and small animals.

Purification of the reaction mixture

The advantage of trichloroethyl chloroformate over pentafluorobenzyl chloroformate was the cleaner reaction mixture obtained[12]. The mixture was purified with two kinds of methanolic alkali before gas chromatographic analysis. The excess of reagent as well as the side product, bis(trichloroethyl) carbonate, were completely removed by this procedure. The cellulose caused several disturbing peaks, but the purity of the cellulose was greatly improved by washing with heptane. A chromatogram from an analysis of a sample (40 ng in 0.1 ml of plasma) is shown in Fig. 3.

The stability of the trichloroethyl carbamate of pethidine was good and no degradation was noticed in dilute toluene solutions (20 ng/ml). On treatment with methanolic alkali for 1 h a slight decrease was observed ($< 10\%$).

Choice of internal standard

An ideal internal standard should be extracted, derivatized and chromatographed in as similar a way to the substance under test as possible. In the present method the butyl ester analogue of pethidine fulfils these requirements in most respects. Pethidine and the internal standard both require 30 min for complete reaction in the derivatization step. The partition into organic solvents is somewhat higher for the internal standard than for pethidine (*cf.* Table I) but this difference was found not to be critical.

The trichloroethyl carbamate of the internal standard has a retention of 1.5 relative to the pethidine derivative.

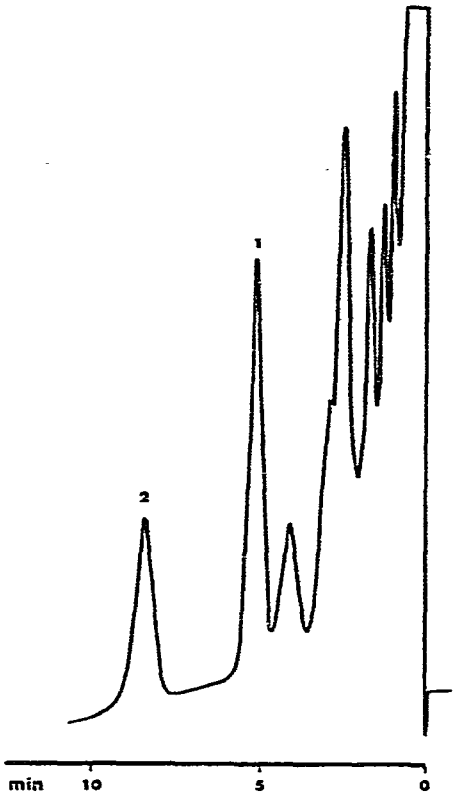


Fig. 3. Gas chromatogram from plasma sample containing 400 ng/ml of pethidine, analysed according to the general procedure. Peak 1, trichloroethyl carbamate of pethidine; peak 2, trichloroethyl carbamate of the internal standard.

Precision and yield of the method

The choice of internal standard contributed to the high precision of this method. The relative standard deviation was 5.8% ($n = 8$) when 50 ng of pethidine were added to 0.1 ml of plasma.

The total yield of pethidine as the trichloroethyl carbamate taken through the whole method was estimated to be 70% by comparision with a known amount of the synthetic derivative.

Application to plasma samples

The present method was used to establish plasma levels of pethidine after intramuscular administration of 200 mg to post-operative patients. The plasma levels found as well as the extension of the method to the determination of norpethidine levels will be published elsewhere.

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