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Note

A simplified method for the gas chromatographic determination of pethidine and norpethidine after derivatization with trichloroethyl chloroformate

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Several analytical procedures have been developed for the assay of plasma samples of the narcotic analgesic drug, pethidine. The majority of these are based on gas chromatography with flame ionization detection without prior derivatization [1—4]. The sensitivity of the methods is low and does not enable the determination of the major plasma metabolite, norpethidine. The simultaneous determination of pethidine and norpethidine has been achieved using gas chromatography—mass spectrometry with selected ion monitoring after acylation of the secondary amine [5, 6]. Recently, an analytical method for the assay of the two amines from plasma samples was presented which used gas chromatography with thermionic detection [7]. The poor gas chromatographic properties of pethidine may limit both precision and sensitivity of the method.

We have previously developed a procedure for the simultaneous assay of pethidine and norpethidine in plasma after conversion to the corresponding carbamate with trichloroethyl chloroformate [8, 9]. The method was extremely sensitive and made analysis possible in 0.1-ml plasma samples. Norpethidine could also be determined after a single therapeutic dose of pethidine [9]. However, since both pethidine and norpethidine form the same derivative with trichloroethyl chloroformate, the two amines must be separated before derivatization. This was accomplished by partition chromatography in a simple column separation.

The separation step was time-consuming and the success of analysis was dependent on the quality of cellulose used in the separation column. Therefore an easier method was required for the processing of a high number of samples [10].

This present paper presents a simplified procedure, where pethidine and norpethidine are separated in the extraction procedure. The secondary amine reacts readily with alkyl chloroformate at room temperature in a two-phase

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system, whereas the tertiary amine only forms carbamate in organic solvent [11, 12].

EXPERIMENTAL

Gas chromatography

A Pye GCV gas chromatograph with flame ionization and electron-capture detectors was used. The glass column (150 cm × 0.2 cm I.D.) was filled with 3% OV-17 on Gas-Chrom Q 100—120 mesh (Supelco, Bellefonte, PA, U.S.A.). The column temperature was 260°C, while injector and detector temperatures were 300°C and 290°C, respectively. Flow of nitrogen carrier gas was 30 ml/min.

Reagents and chemicals

Trichloroethyl chloroformate was purchased from EGA Chemie, Steinheim bei Hedidenheiml, G.F.R. Ethyl chloroformate was from Fluka, Buchs, Switzerland. Alcoholic alkali consisted of 2.8 g of potassium hydroxide in a mixture of 75 g of methanol and 22 g of water. Saturated alcoholic alkali was a saturated solution of potassium hydroxide in methanol. Tetrabutylammonium iodide was from Labkemi, Stockholm, Sweden. *n*-Butanol, methylene chloride and methanol (E. Merck, Darmstadt, G.F.R.) were used without purification, whereas toluene (E. Merck) was distilled before use. Sodium hydroxide 0.5 *M* and phosphate buffers (μ = 0.1) were also used.

Internal standard in the assay of pethidine was the O-butyl analogue (butyl-1-methyl-4-phenyl-4-piperidine carboxylate as hydrochloride) [8]. A solution in water containing 4 μg/ml was used. In the analysis of norpethidine, the O-propyl analogue, propyl-4-phenyl-4-piperidine carboxylate [9], was used in a concentration of 2 μg/ml in water.

Methods

Evaluation of reaction conditions. The derivatization of pethidine and norpethidine was studied as described previously [8]. To 0.25 ml of amine (2×10^{-3} *M*) in toluene, trichloroethyl chloroformate was added in a concentration of 0.4—10% together with about 10 mg of tetrabutylammonium iodide. Hexaeicosane, 0.5 mg/ml, was present as internal marker. The mixture was heated in a metal block and the reaction was quenched by washing with 0.1 *M* sulphuric acid. Analysis was performed by gas chromatography with flame ionization detection and the peak height ratio of formed carbamate to internal marker was calculated.

Determination of pethidine in plasma samples. To a 0.5-ml plasma sample, 0.1 ml of internal standard solution, 0.5 ml of 1 *M* phosphate buffer pH 8.3, and 2 ml of water were added. This mixture was gently shaken for 20 min with 5 ml of toluene containing 50 μl of ethyl chloroformate. After centrifugation at 500 *g* for 10 min, the organic phase was transferred to another tube. One ml of 0.1 *M* phosphate buffer pH 1.9 was added and the tube shaken for 10 min and centrifuged. The aqueous phase was made alkaline, 0.25 ml of toluene was added and the mixture was shaken for 15 min and thereafter centrifuged for 5 min. The organic phase was transferred to another tube and

10 μl of trichloroethyl chloroformate and about 10 mg of tetrabutylammonium iodide were added. The reaction tube was heated for 1 h at 100°C in a metal block. Alcoholic alkali solution, 1.0 ml, was added to the reaction mixture and shaken for 10 min. After that, 1 ml of water was added and the mixture shaken for another 10 min. After centrifugation, the aqueous phase was discarded followed by the addition of 0.5 ml of saturated alcoholic alkali. The tube was shaken vigorously for 15 sec and 1 ml of water was added. A 1—2 μl volume of the organic phase was taken to analysis with electron-capture gas chromatography.

Determination of norpethidine in plasma. A 0.5 ml plasma sample containing norpethidine was taken to analysis. To the sample 0.1 ml of the internal standard solution, 0.5 ml of 0.5 *M* sodium hydroxide and 2 ml of water were added and shaken with 5 ml of a mixture of *n*-butanol—methylene chloride (1:4) for 10 min. After centrifugation, the organic phase was transferred to another tube and 1 ml of 0.1 *M* sulphuric acid was added. The mixture was shaken for 10 min and after centrifugation the aqueous phase was transferred to another tube containing 0.5 ml of 1 *M* phosphate buffer pH 7.3, and thereafter 0.3 ml of toluene containing 1% trichloroethyl chloroformate was added. The mixture was shaken for 20 min and excess reagent was removed as described above. Analysis was made as for pethidine.

Standard curves for pethidine and norpethidine, respectively, were prepared in parallel by treating known concentrations of pethidine and norpethidine in blank plasma according to the procedures above.

RESULTS AND DISCUSSION

Separation of pethidine and norpethidine

Since both pethidine and its N-demethylated metabolite, norpethidine, form the same carbamate with trichloroethyl chloroformate, the two amines must be quantitatively separated before derivatization. This was easily achieved in the first extraction step from plasma, as the secondary amine readily reacted with ethyl chloroformate in the two-phase system, while the tertiary amine was left underivatized. A quantitative removal of norpethidine with this method was performed even with a very low reagent concentration, e.g. $> 2 \times 10^{-5}$ *M*. pH 8.3 was chosen in order to obtain a quantitative extraction of pethidine as base into the organic phase [8]. At pH > 12, hydrolysis of the ester function of both pethidine and norpethidine occurred giving the corresponding ethoxycarbonyl esters on reaction with ethyl chloroformate.

The ethyl carbamate of norpethidine has a high distribution to organic phase while pethidine was quantitatively extracted to acidic aqueous phase in the second extraction step.

The different reactivity between a secondary and a tertiary amine to chloroformate has been used previously for separation before derivatization [13]. This method is very time-saving compared to the previous separation by partition chromatography [8]. The number of samples processed in one day is increased to twenty samples plus the standard samples.

Trichloroethyl chloroformate reaction with pethidine

Pethidine reacts with chloroformate reagent to the formation of the corresponding carbamate and methylamine. The reaction proceeds via a reactive intermediate ion [12]. The reaction is promoted by the addition of anhydrous sodium carbonate. The effect of anhydrous sodium carbonate is most likely to be a removal of hydrolytic compounds, which will compete in the reaction with the intermediate ion.

The formation rate of carbamate with trichloroethyl chloroformate was low, probably due to the degradation of the intermediate ion by a competing and rapid hydrolysis or by other mechanisms [12]. By addition of a strong nucleophilic agent such as iodide in the form of tetrabutylammonium iodide to the reaction mixture, the reaction rate for the carbamate formation was increased at the expense of the hydrolysis reaction [11].

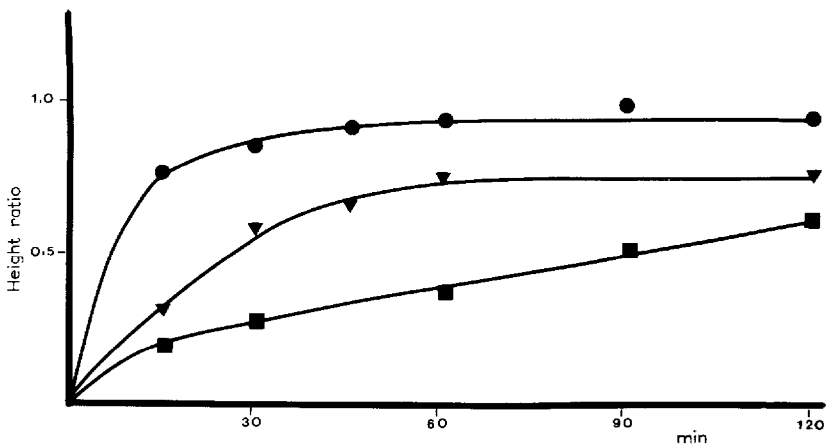

Fig. 1. Time dependence of trichloroethyl carbamate formation from pethidine. Sample concentration 2 × 10^{-3} *M*. Temperature 80°C. (•———•), 10% reagent with tetrabutylammonium iodide present; (▾———▾), 10% reagent with sodium carbonate present; (▪———▪), 1% reagent with tetrabutylammonium iodide present.

The favourable effect of iodide on the reaction rate as compared to anhydrous sodium carbonate is shown in Fig. 1, where the formation of carbamate was studied at 80°C. Although the reaction rate was increased after the change of catalyst, a quantitative formation of carbamate required a reaction time of 1 h at 100°C. The reagent concentration was lower, 0.3 *M* compared to 0.7 *M* used previously. In the concentration range 0.03—0.3 *M* of reagent, the time for quantitative reaction was almost identical. At higher concentrations of trichlorethyl chloroformate the reaction rate decreased. This is probably due to the liberation of acid in the reaction which may hamper the carbamate formation. This low reagent concentration increased both sensitivity and selectivity of the method owing to less disturbance in the chromatogram. Purification of the reaction mixture was performed with alcoholic alkali solution in order to hydrolyze bis(trichloroethyl) carbonate formed in the reaction [8].

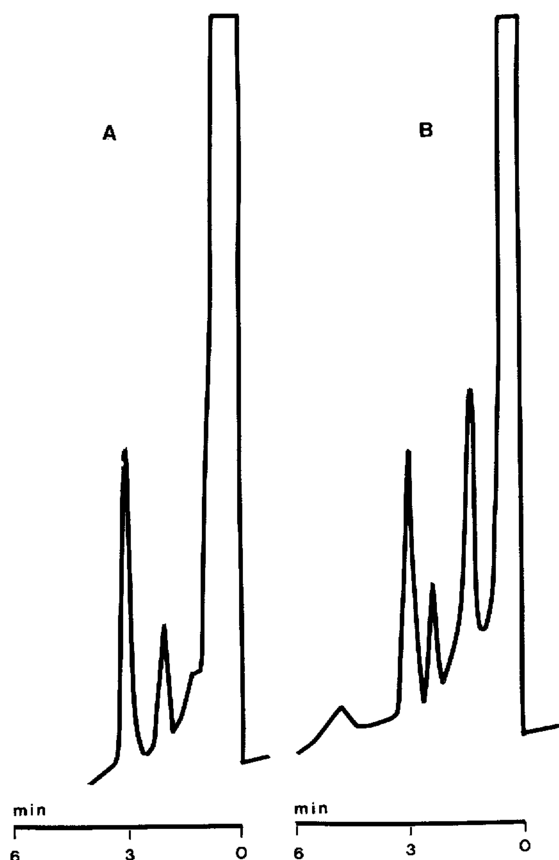

Fig. 2. Gas chromatograms of plasma samples with: (A) pethidine as trichloroethyl carbamate. Internal standard: O-butyl analogue of pethidine. (B) norpethidine as trichloroethyl carbamate. Internal standard: O-propyl analogue of norpethidine.

Application to the analysis of plasma samples of pethidine and norpethidine

The simplified method has now been used for the determination of pethidine and norpethidine in patient plasma samples for more than two years [10]. Plasma norpethidine can be determined in a separate run after selective isolation from plasma by solvent extraction.

Derivatization of norpethidine with trichloroethyl chloroformate was achieved using a two-phase procedure with an aqueous phase of pH 8.3. It was shown that pethidine did not interfere in the assay of norpethidine. Norpethidine is the only metabolite from pethidine which has been found in plasma. On continuous therapy with pethidine for several days, the plasma concentrations of norpethidine are low.

Pethidine and norpethidine could be detected below 10 ng in a 0.5-ml plasma sample and quantitative determinations were performed above 20 ng/ml with a relative standard deviation less than 10% (n = 8) for each compound. A chromatogram is shown in Fig. 2. The absolute recovery for pethidine and norpethidine through the respective methods was 92% and 86%, respectively. A comparison between this simplified method and the previous method with

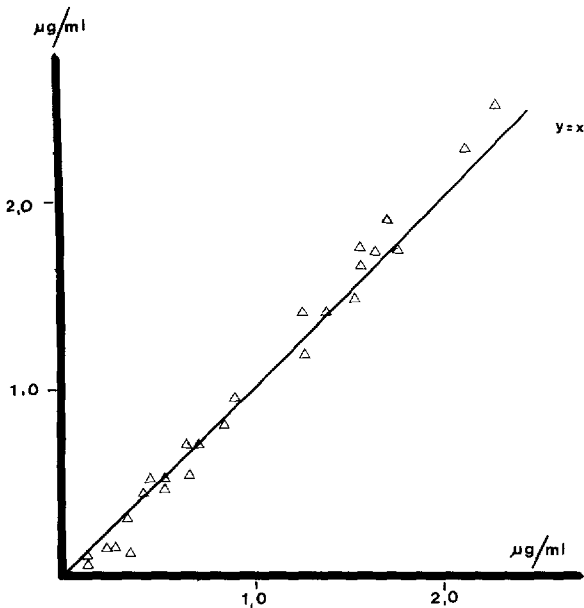

Fig. 3. Plasma concentration of pethidine obtained by the method with chromatographic separation (ordinate) and the present simplified method (abscissa). Line of best fit $Y = 1.09X - 0.07$ ($n = 27$, $r = 0.994$).

chromatographic isolation of pethidine was carried out in the analysis of a number of patient plasma samples. After plotting the results of the present method against those from the old method (Fig. 3), a line of best fit $Y = 1.09X - 0.07$ ($n = 27$, $r = 0.994$) was obtained. Thus, the agreement between the two methods was close.

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