Journal of Chromatography, 133 (1977) 367–371

CHROM. 9797

Note

Simultaneous determination of plasma amitriptyline and nortriptyline as trichloroethyl carbamates by electron-capture gas chromatography

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(Received October 25th, 1976)

Amitriptyline is frequently used in the treatment of depressive disorders, and there are great differences in the plasma concentrations of amitriptyline and its N-demethylated metabolite, nortriptyline, in patients. A simultaneous determination of the parent drug and the metabolite in plasma is therefore desirable for adequate therapy. Several methods exist for the determination of amitriptyline in plasma, but few show sufficient sensitivity for measuring the drug after administration of a single dose[1–4]. The N-demethylated metabolite has been determined simultaneously after acylation and analysis by gas chromatography with nitrogen-specific detection[3,4]. Derivatization of tertiary amines with pentafluorobenzyl or trichloroethyl chloroformate has been found to improve the gas chromatographic properties greatly and also to increase the detectability of the amines in an electron-capture detector. The determination of low concentrations (nanograms per millilitre levels) of trimipramine as the pentafluorobenzyl carbamate has been demonstrated[5], and the simultaneous determination of pethidine[6] and its N-demethylated metabolite, norpethidine[7], in plasma as the trichloroethyl carbamates has been described.

In this paper, we describe the simultaneous determination of amitriptyline and nortriptyline by electron-capture gas chromatography after separation by column extraction and derivatization with trichloroethyl chloroformate (Fig. 1). Determination of the two amines in plasma was possible to 1 ng/ml. The method has been used in the analysis of imipramine and N-demethylimipramine and of 2-chloroimipramine and the corresponding metabolite.

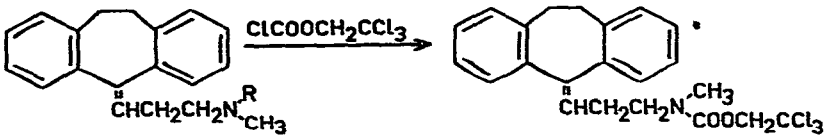

Fig. 1. Reaction of amitriptyline (R=CH_3) and nortriptyline (R=H) with trichloroethyl chloroformate.

EXPERIMENTAL

Gas chromatography

A Varian 1400 gas chromatograph was used, equipped with a ^{63}Ni electron-capture detector operated in the d.c. mode. The glass column (200 × 0.18 cm) was filled with 2% OV-17 on Chromosorb G, 100–120 mesh, and was operated at 290°. The injector and detector temperatures were 320° and 335°, respectively. The flow-rate of the carrier gas (nitrogen) was 30 ml/min.

Reagents and chemicals

Trichloroethyl chloroformate was purchased from EGA Chemie (Steinheim bei Heidenheim, G.F.R.). *n*-Heptane and toluene were distilled and a dichloromethane + *n*-butanol (4:1) mixture was equilibrated with water before use. Cellulose (Munktell 410) was washed with ethanol.

An alcoholic alkali solution was prepared by dissolving 2.8 g of potassium hydroxide in 22 g of water and adding 75 g of methanol. A saturated solution of potassium hydroxide in methanol was prepared.

A 1 *M* citrate buffer solution (pH 5.0) was used as the stationary phase in the chromatographic column.

Internal standard solution. 2-Chloroamitriptyline was used as the internal standard in the determination of amitriptyline and 2-chlorodemethylimipramine in the determination of nortriptyline. The two standards were dissolved together in citrate buffer solution (pH 5.0) to a concentration of 40 ng/ml.

Standard solution of amitriptyline and nortriptyline. Equal amounts of amitriptyline and nortriptyline were dissolved in and diluted with water to give concentrations of 5 μg/ml. Aliquots of this solution were diluted with plasma to give concentrations of 5, 20, 40 and 80 ng/ml. In the preparation of the standard graph, 0.5-ml volumes of these dilutions were taken for analysis.

Determination of amitriptyline in plasma

A small chromatographic column is prepared by mixing 0.2 g of cellulose with 0.1 ml of 1 *M* citrate buffer solution (pH 5.0) and 0.05 ml of blank plasma and packing the mixture in a glass column (30 × 1 cm). The plasma sample (0.5 ml) and 1.0 ml of the internal standard solution are carefully mixed with 2 g of cellulose and packed on top of the first filling. Amitriptyline and 2-chloroamitriptyline are eluted with 10 ml of *n*-heptane and then extracted with 1.0 ml 2 *M* of orthophosphoric acid. This solution is made alkaline and extracted with 0.25 ml of toluene for 10 min. The toluene layer is transferred into another tube and 25 μl of trichloroethyl chloroformate and 10 mg of sodium carbonate are added. An air condenser is attached to the tube, which is then heated for 1 h in a metal block at 110°. The reaction mixture is shaken with 1.0 ml of alcoholic alkali solution for 10 min, water (1.0 ml) is added and the mixture shaken for a further 5 min. After removal of the aqueous phase, 1.0 ml of saturated alcoholic alkali solution is added and the mixture shaken vigorously for 10–15 sec. Water (1.0 ml) is added and, after extraction for 10 min, the aqueous phase is discarded and 5 μl of the organic phase are injected into the gas chromatograph.

Determination of nortriptyline in plasma

Nortriptyline and its internal standard are eluted from the column with 10 ml of a mixture of dichloromethane and butanol (4:1). After addition of 10 ml of *n*-heptane to the eluate, the amines are extracted with 1 ml of 2 *M* orthophosphoric acid and, after being made alkaline, with 0.25 ml of toluene. Trichloroethyl chloroformate (20 μl) is added and the mixture allowed to stand for 30 min at room temperature. Removal of excess of reagent and the gas chromatographic analysis are performed as described for amitriptyline.

Standard graphs were constructed by treating the standard solutions in plasma according to the above procedures.

Determination of absolute yield

The absolute yield of the method at the 50 ng/ml level is determined by gas chromatography by comparison with a known amount of nortriptyline as the trichloroethyl carbamate obtained from a synthesis on the preparative scale.

RESULTS AND DISCUSSION

Extraction and separation conditions

Tertiary amines form the same carbamate as the corresponding demethylated secondary amines on treatment with trichloroethyl chloroformate. The N-demethylated metabolite of amitriptyline must be separated before the chloroformate reaction in order to avoid co-determination. In a previous study[2], amitriptyline was determined after column extraction of the plasma sample without interference from its metabolites. Separation was obtained owing to the large difference in the partition into *n*-heptane used as the mobile phase in the extraction. In the present method, a trap layer containing an aqueous phase at pH 5 with addition of blank plasma is placed below the extraction part in the column.

The yield from the column at the nanograms per millilitre level was measured after derivatization of the amine and quantitation by means of electron-capture gas chromatography. The recovery of amitriptyline in the overall method in comparison with a known amount of carbamate was 88%. It is interesting that with separation without the addition of blank plasma the yield decreased to below 20%. The absolute recovery of nortriptyline was 75%. Partition data for nortriptyline[8] indicated that elution with the solvent front occurred if methylene chloride was used as the second mobile phase.

As the principle involved seems to be of value for the rapid and simple separation of tertiary and secondary amines, some additional studies were carried out on the composition of the column. In the previous study[2], a high concentration of the buffer, used as the stationary phase, was important for a high yield. With concentrations of the buffer below 0.5 *M*, the yield of amitriptyline decreased to below 50% and that of nortriptyline to below 40%. An increase in the amount of cellulose (3 g) in the column with the same volume of stationary phase was also detrimental, particularly to the yield of amitriptyline.

Selectivity of the method

Interferences in the method due to co-determination of metabolites were stud-

ied. In the determination of amitriptyline, no interference was detected even on addition of large amounts (> 2000 ng) of metabolite to the plasma sample.

Interference from amitriptyline in the determination of nortriptyline is not possible as tertiary amines do not form carbamates at room temperature. A co-extraction of N,N-didemethyl- and 10-hydroxyamitriptyline with the nortriptyline fraction from the column may be possible. However, these two metabolites, after treatment with trichloroethyl chloroformate, did not give rise to peaks that interfered with the nortriptyline peak.

Determination of amitriptyline and nortriptyline in plasma

The present method was used to monitor plasma levels of amitriptyline and nortriptyline in patients after a single oral dose of 50 mg of amitriptyline. A chromatogram from a plasma sample containing 24 and 23 ng/ml of amitriptyline and nortriptyline, respectively, is shown in Fig. 2.

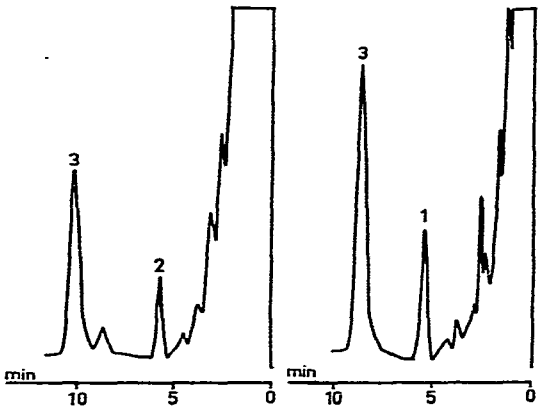

Fig. 2. Gas chromatogram from plasma sample. Peaks (trichloroethyl carbamates): 1 = amitriptyline; 2 = nortriptyline; 3 = internal standards.

Amitriptyline and nortriptyline have been determined down to 1 ng/ml by means of the present method, with relative standard deviations at the 10 ng/ml level of 5.5 and 7.5%, respectively ($n = 10$).

Application of the method to imipramine or 2-chloroimipramine

Imipramine and 2-chloroimipramine and their N-demethyl metabolites can be determined in plasma at concentrations down to a few nanograms per millilitre; this application will be reported elsewhere.

ACKNOWLEDGEMENT

Our thanks are due to Professor Göran Schill for valuable discussion on the manuscript.

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