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A Micromethod for the Determination of Carbamazepine in Blood by High Speed Liquid Chromatography

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A micromethod for the determination of carbamazepine in small blood samples by high speed liquid chromatography is reported. Carbamazepine is extracted into chloroform and determined by adsorption chromatography on Perisorb A. The minimal quantitatively detectable concentration of carbamazepine is 0.5 mg/l, requiring 100 µl of plasma, the relative standard deviation at therapeutic plasma levels being 3.8%.

Eine Mikromethode zur Bestimmung von Carbamazepin in kleinen Blutproben durch Flüssigkeitschromatographie mit hohen Eingangsdrucken wird beschrieben. Carbamazepin wird mit Chloroform extrahiert und mittels Adsorptionschromatographie auf Perisorb A analysiert. Geht man von 100 µl Plasma aus, so betragen die minimal nachweisbare Carbamazepin-Konzentration 0,5 mg/l und die relative Standardabweichung 3,8% im therapeutischen Bereich.

Carbamazepine (5-H-dibenz [b, f] azepine-5-carboxamide), an iminostilbene derivative, is widely used for the treatment of certain convulsive disorders and trigeminal neuralgia. The drug has a marked anticonvulsive and psychotropic activity in adults and children (1—4). Investigations relating dose, serum concentration, and therapeutic effects are useful in the evaluation of the drug. Analytical methodology reported for the determination of carbamazepine serum concentrations includes the use of spectrophotometric (5, 6), thin layer (7, 8), and gas chromatographic methods (9—13). This paper describes a simple, rapid and sensitive high speed liquid chromatographic estimation of carbamazepine, which requires only 100 µl of plasma.

Methods and Materials

Reagents

Carbamazepine was kindly donated by Ciba-Geigy AG (Basel), the other chemicals were obtained from Merck AG (Darmstadt). Carbamazepine standards were prepared by dilution of an ethanolic stock solution with serum.

Apparatus

A Hewlett-Packard Hupe + Busch series UFC 1000 high pressure liquid chromatograph with an UV absorbance detector (254 nm) was used. The chromatograph was equipped with a 2 × 2000 mm stainless steel column, handpacked by the tap-fill method (14) with Perisorb A (Merck).
The mobile phase was *i*-octane/ethanol/water (volumes 85.7 l + 14.0 l + 0.3 l). The flow rate was 0.40 ml/min under a pressure of 14 at. The columns were operated at ambient temperature.

Preparation of samples

50 µl of chloroform were added to 100 µl of plasma in a 300 µl plastic centrifuge microtube. The mixture was shaken vigorously for 1 min and centrifuged for 1 min at 15000 *g*. Without further separation steps 5 µl of the chloroform phase were injected into the chromatograph with a high pressure microsyringe (Scientific Glass Engineering PTY) using the continuous flow technique. To avoid any elution of UV absorbing tube materials into the chloroform layer, the whole procedure should be finished within 30 min. Two synthetic sera containing 5 and 10 mg/l carbamazepine were prepared in the same way and injected at the beginning and the end of each series. The peak areas were evaluated with a planometer (Ott Kompensationsplanometer Nr. 19).

Results and Discussion

Selection of column and solvent

Following a scheme publicated by Snyder (15) different stationary and mobile phases were examined for adequate separation conditions. The chromatographic system selected for carbamazepine analysis, was obtained by varying the relation of the solvent components *i*-octane, ethanol, and water on columns packed with irregular shaped silicagel beads of various sizes (Merckosorb SI 60; 10, 20 and 30 µm) and Perisorb A, a controlled surface porosity chromatographic support. Figure 1 illustrates a chromatogramm of a patient's plasma.

Precision

The coefficient of variation was 3.8% in 20 replicate determinations of the same plasma containing 10 mg/l carbamazepine and in 30 different duplicate plasma analyses ranging from 0.8 to 5.5 mg/l carbamazepine. The use of an internal standard (benzidine) gave no higher precision and was therefore of no advantage. Plotting the concentration vs the peak area of carbamazepine standards produced a straight line over the range of 0.5 to 50 mg/l. The calibration curve passed through the origin. The minimal quantitatively detectable amount of carbamazepine was 2.5 ng corresponding to 5 µl of a solution containing 0.5 mg/l.

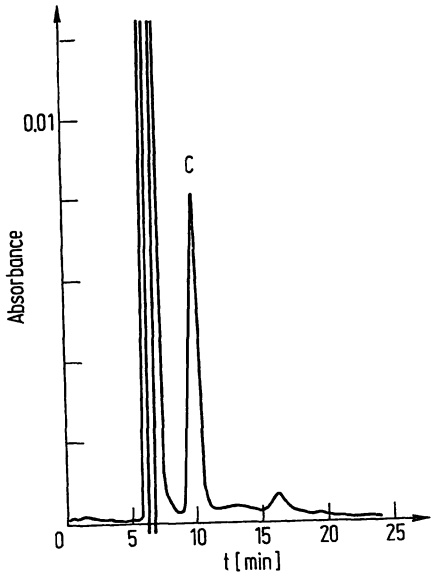

Fig. 1

Carbamazepine (C) determination in the plasma of an adult patient receiving 4 × 200 mg of carbamazepine per day. The sample was drawn 3.5 hours after the morning dose. Abscissa: time t (min). Ordinate: Absorbance A. Column: 2 × 2000 mm, stainless steel. Stationary phase: *i*-octane/ethanol/water (volumes 85.7 l + 14.0 l + 0.3 l). Flow rate: 0.4 ml/min. Inlet pressure: 14 at. UV detector: 0.02 absorbance, full scale. Sample: 36.5 ng carbamazepine, corresponding to a plasma level of 7.3 mg/l

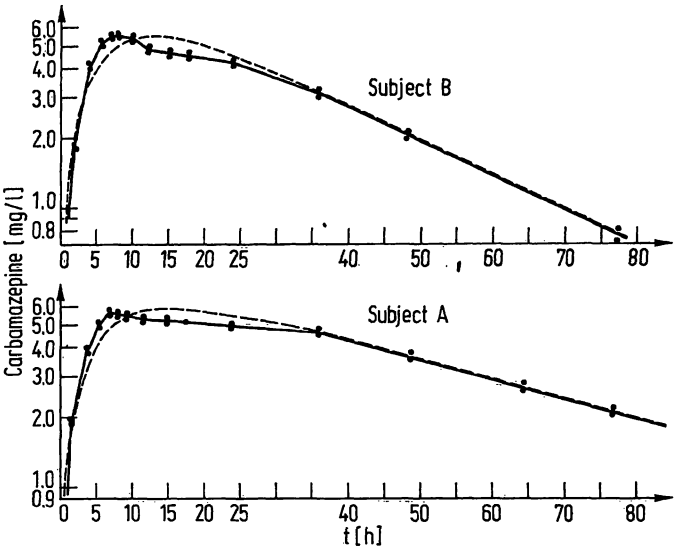

Fig. 2

Serum concentration profiles and tentatively calculated BATEMAN-functions (dotted lines) in two healthy persons receiving a single dose of carbamazepine. Abscissa: time t (h). Ordinate: serum concentration c (mg/l). — Subject A: 10.7 mg/kg carbamazepine, BATEMAN-function: $c = 9.6\ (e^{-0.018t} - e^{-0.102t})$, $t_1/_2$: 38 h. — Subject B: 10.9 mg/kg carbamazepine, BATEMAN-function: $c = 11.6\ (e^{-0.037t} - e^{-0.146t})$, $t_1/_2$: 18.5 h

Accuracy

Serum not containing carbamazepine gave no peak at the corresponding retention volume. Carbamazepine standards gave identical results whether extracted from serum or directly dissolved in ethanol. Sufficient separation of carbamazepine and its metabolites in the serum of patients under continuous therapy was proved by identification of the solute passing the detector during the carbamazepine peak. Identification was performed by UV-spectroscopy and thin layer chromatography according to a modified method of CHRISTIANSEN (8).

For a preliminary evaluation of pharmacokinetic constants of carbamazepine, the serum concentrations were determined in two healthy persons (two authors of this report) receiving a single oral dose of the drug. The experimental results fitted rather well with the data calculated by BATEMAN-functions (16) (Fig. 2). Peak serum levels occurred at six to seven hours after oral administration. Biological halflives of 18.5 and 38 h were observed. According to CEREGHINO et al. (17) who recently reviewed papers reporting pharmacokinetic studies of carbamazepine, biological halflives following cessation of therapy range from 14 to 72 h.

A major disadvantage of the method presented here may be the fact that only carbamazepine can be assayed in the same chromatographic run. In liquid-solid chromatography there is a pronounced compound type selectivity. Drugs with different functional groups generally show greatly differing retention volumes. Often they cannot be determined under identical chromatographic conditions without special programming or unacceptable loss of sensitivity. The determination of different serum components by liquid-solid chromatography generally requires the injection of discrete aliquots of the same serum sample under different chromatographic conditions. Most conveniently only solvent systems are changed, while packing materials are kept unaltered. In our laboratory diphenylhydantoin (18) and carbamazepine serum concentrations are successively determined on the same Perisorb A column.

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