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Note

A thin-layer chromatographic method for determining carbamazepine in blood

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In order to determine the correlation between the blood level of carbamazepine [5-carbamyl-5H-dibenzo(*b*,*f*)azepine] and the clinical response in treated patients, it is necessary to have a sensitive, specific method of analysis. Kupferberg[1] described a method that involved an extensive extraction procedure followed by formation of the trimethylsilyl derivative and analysis by gas–liquid chromatography (GLC). For routine purposes, this method is time consuming and its intricacy leads to the risk of mistakes occurring. Spectroscopic methods have also been reported but they have the disadvantage that the metabolites are determined simultaneously. Indirect methods such as scraping off the spot obtained by thin-layer chromatography (TLC), or column chromatographic methods followed by GLC analysis are often used to give reliable determinations in plasma. Direct measurement of fluorescing spots on a TLC plate, reported by Laufer and Schmid[2] for medazepam and by Faber[3] for cyclobenzaprine, seems to have better prospects. In this paper, a method is described that consists of a single extraction and TLC followed by densitometry.

EXPERIMENTAL

Apparatus

The densitometer used was a Vitatron TLD-100. The operating conditions and settings were as follows: light source, mercury lamp; mode, In II(+); level, f; coarse zero, 7; damping, 2; span, 9.5; excitation filter, 365 nm; emission filter, 543 nm; diaphragm, 0.5; swing, 5 mm; scanning speed, 1 cm/min; paper speed, 0.5 cm/min; integrator, 8.

The centrifuge was a Christ Model UJ1, with a maximum speed of 3200 rpm. The other apparatus used consisted of silica gel TLC plates 60; dimensions 20 × 20 cm (Merck, Darmstadt, G.F.R.), a Hamilton dosing syringe (100 μl), a Desaga chromatographic tank and a Vortex-Genie Vibromixer mixer.

Reagents

Toluene, p.a., min. 99.5%; isobutanol, p.a., min. 99%; isopropanol, p.a., min. 99.7%; and chloroform, p.a., 99–99.5% (all from Merck). Perchloric acid,

70–72%; and ethanol, 99.5% (both from J. T. Baker Chemicals N.V.). Water, demineralized.

Carbamazepine (Tegretol), GP 40923 (carbamazepine-10,11-epoxide) and CGP 5924 (10,11-dihydroxycarbamazepine) (were obtained from Ciba-Geigy, Basle, Switzerland).

Immersion liquid. Add carefully 500 ml of perchloric acid to 590 ml of demineralized water plus 590 ml of ethanol.

Carbamazepine stock solution. Dissolve 50 mg of carbamazepine in 100 ml of ethanol. For recovery experiments, the stock solution is diluted with water to the required concentration.

Carbamazepine standard solution. Dilute 5 ml of stock solution to 50 ml with ethanol, take 4 ml of the diluted solution and make the volume up to 50 ml with ethanol.

Extraction

A 2-ml volume of plasma is shaken for 2 min with 3 ml of toluene, containing 10% of isobutanol, in a 10-ml glass-stoppered centrifuge tube. After centrifugation at 3200 rpm for 10 min, 30 μl (x) of the supernatant are applied directly three times to a thin-layer plate, together with 8 ng (s_1) and 16 ng (s_2) of a 0.004 mg/ml solution of carbamazepine in ethanol in the sequence s_1, x, s_2, s_1, x, s_2, s_1, x, s_2.

Chromatography

The eluent used is chloroform–isopropanol (9:1). The development takes place in a saturated tank and the elution time is about 25 min. The distance run is 10 cm. After drying under a stream of air, the TLC plate is immersed in a 20% solution of perchloric acid in ethanol–water (1:1) and subsequently heated at 120° in a drying oven for 7 min. This procedure converts carbamazepine into a fluorescent compound, which can be measured directly and quantitatively with the Vitatron TLD-100 densitometer. The concentration of the sample is obtained by interpolation of the two reference standards.

RESULTS AND DISCUSSION

Recoveries

The aqueous carbamazepine solution is added to plasma by means of the 100-μl syringe. Five recovery experiments were carried out at a concentration in plasma of 0.5 μg/ml in order to obtain an indication of the recovery achieved with the extraction method (Table I). Although an experiment with plasma that did not contain carbamazepine did not show any visible impurities at this particular R_F value, the average recovery was just over 100%.

The lowest demonstrable concentration of carbamazepine in plasma is less than 0.5 μg/ml, but one must take into account the standard deviation. The recovery of carbamazepine-10,11-epoxide ranged from 80 to 100% and that of 10,11-dihydroxycarbamazepine was less than 10%.

It appeared that there was a linear relationship between the amount of carbamazepine (10–50 ng) and the integrated peak areas (Figs. 1 and 2).

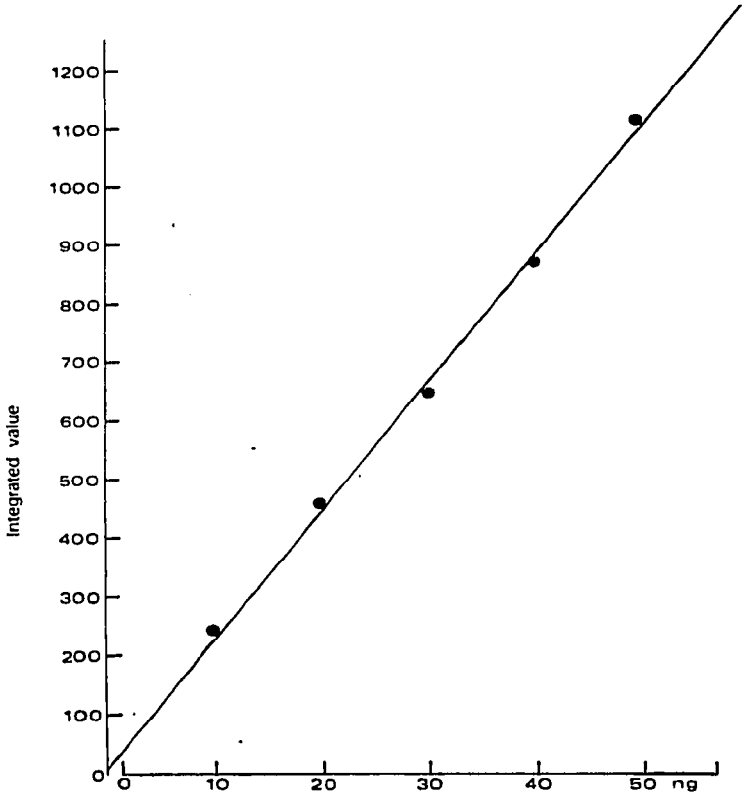

Fig. 1. Calibration curve for carbamazepine (10–50 ng).

Specificity of the method

According to Hecke-Seibicke[4], the principal metabolite is 10,11-dihydroxy-carbamazepine. Frigerio *et al.*[5] also identified carbamazepine-10,11-epoxide as a metabolite of carbamazepine. These two metabolites have different R_F values than carbamazepine. Weist and Zicha[6] described TLC studies on carbamazepine in urine

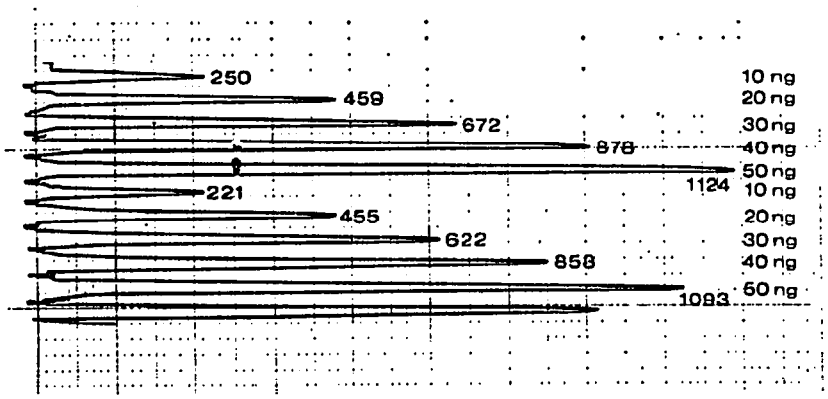

Fig. 2. Densitogram of different amounts of pure carbamazepine isolated from plasma.

TABLE I

RECOVERY OF CARBAMAZEPINE FROM PLASMA IN DIFFERENT EXPERIMENTS AT THE 0.5 μg/ml LEVEL

Experiment No.	*Recovery (%)*
1	98
2	104
3	111
4	105
5	97
Average	103

and liquor for new applications, while our investigation was particularly concerned with metabolic studies in blood for therapy control. A number of medicines are used in conjunction with carbamazepine. In some experiments their R_F value and their fluorescence were investigated and it appeared that all these medicines fluoresce. However, only three of them had almost the same R_F value as carbamazepine and could influence its determination (Table II); but their fluorescence was so small that the amounts in the extract correlated with their toxic levels were not visible on the TLC plate. Moreover, it is not certain they are extracted completely.

The reproducibility is reasonable and the method is sufficiently sensitive and specific for therapy control purposes. The time needed for an analysis is about 2 h.

TABLE II

R_F VALUES AND FLUORESCENCE OF CARBAMAZEPINE AND ITS MAJOR METABOLITES AND OF SOME OTHER COMPOUNDS

Compound	R_F	*Fluorescence/colour*	*Toxic level*	*Visibility of toxic amounts*
Carbamazepine	0.43	Blue-green		
Carbamazepine-10,11-epoxide	0.35	Blue-green		
10,11-Dihydroxycarbamazepine	0.10	Blue-green		
Chlordiazepoxide	0.34	Pink		
Diazepam	0.57	Yellow		
Nitrazepam	0.41	Brown-green	0.5 μg/ml–10 ng/30 μl	Invisible
Oxazepam	0.32	Blue		
Primidon	0.15	White		
Diphantoine	0.45	Pink	30 μg/ml–0.6 μg/30 μl	Invisible
Phenobarbital	0.45	White	40 μg/ml–0.8 μg/30 μl	Hardly visible

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