*Journal of Chromatography*, 108 (1975) 370–374


CHROM. 8178

## Note

# Rapid and accurate determination of the level of carbamazepine in serum by ultraviolet reflectance photometry on thin-layer chromatograms

URSULA BREYER
*Institut für Toxikologie, Universität Tübingen, D-7400 Tübingen (G.F.R.)*
(First received October 29th, 1974; revised manuscript received January 10th, 1975)

A number of methods are available for the measurement of carbamazepine in blood, plasma or serum, including colorimetry[1], gas–liquid chromatography (see, for instance, refs. 2–4), high-speed liquid chromatography[5] and thin-layer chromatography (TLC) followed by conversion into a fluorescent compound by heating with perchloric acid[6,7]. The method described here makes use of the specificity and simplicity of TLC, but avoids the reaction with perchloric acid, as the ultraviolet (UV) absorption of unchanged carbamazepine is measured on the chromatograms.

## EXPERIMENTAL

### *Chemicals*

Carbamazepine was a gift from Dr. Karl Thomae GmbH, Biberach/Riss, G.F.R. All solvents were of ordinary grade and were distilled before use.

### *Serum samples*

Blood was drawn from patients ingesting 400–800 mg of carbamazepine (Tegretal) per day in divided doses before administration of the morning dose. Serum was obtained by centrifugation and stored at —20° for up to 2 months. A few samples were re-analyzed after longer periods of time.

### *Extraction*

Serum (1 ml) was mixed with 0.1 ml of 1 *M* potassium dihydrogen orthophosphate solution in a 15-ml centrifuge tube and shaken for 5 sec with 2 ml of ethyl acetate on a Vortex mixer, avoiding the formation of a fine emulsion. After centrifugation for 2 min at 170 *g*, as much as possible of the organic layer was transferred with a Pasteur pipette into a second tube that contained 0.5 ml of 0.02 *N* sodium hydroxide solution. After shaking and centrifugation, an aliquot of the ethyl acetate layer was weighed into a small vial (volume 3 ml) with a conical tip and a ground-glass neck. The entire procedure has to be carried out raqidly in order to avoid uncontrollable losses of ethyl acetate by evaporation. The weight of ethyl acetate transferred was divided by the weight of the original amount (1.8 g) in order to find the amount of serum represented in the final extract (usually 0.7–0.85 ml). The ethyl acetate was removed with a stream of nitrogen at 30° and the extracted

material was concentrated in the tip of the vial by rinsing the sides with 0.2 ml of ethyl acetate and evaporating again. The final residue was dissolved in 40 $\mu$l of 1,2-dichloroethane.

*Chromatography*

Glass plates (20 × 20 cm) pre-coated with non-fluorescent silica gel (Merck, Darmstadt, G.F.R.), were washed with ethyl acetate–acetone (10:10) to the upper edge, dried and stored in a closed tank. They were heated to 103° for 10 min before use. Samples and standards were applied as spots 2-cm apart. On each plate, three or four standards containing 50–600 ng of carbamazepine were spotted from a solution containing 50 $\mu$g/ml in dichloroethane. At ordinary therapeutic serum levels, 3–5 $\mu$l of the extracts had to be applied. Spotting was performed with disposable micropipettes (5 or 10 $\mu$l) equipped with ring marks (Brand, Wertheim, G.F.R.). The plates were developed in ethyl acetate–acetone (10:10) to a distance of 12 cm from the origin within about 30 min and then dried for 20 min.

The standard solution was stored in the dark at 4° and its content was checked at least once a week by diluting 0.2 ml with 3 ml of methanol and measuring the UV absorption. An extinction difference ($E_{285} - E_{320}$) of 0.444 corresponds to 10 $\mu$g/ml of carbamazepine in the diluted sample (light path 1 cm).

*Reflectance photometry*

The instrument used was a Zeiss Chromatogramm-Spektralphotometer (Carl Zeiss, Oberkochen, G.F.R.) coupled to a potentiometric recorder (Servogor S, Metrawatt, Nürnberg, G.F.R.). Light remission was recorded at 285 nm while the plate was moved in the direction of the solvent flow under a 14 × 1 mm slit. The scanning speed and chart paper speed were set at 120 mm/min. Peak areas were measured by using the triangle rule and the amounts of drug contained in the sample spots were determined graphically from the calibration graph constructed by using the standards on the same plate.

In order to measure the spectrum of a spot *in situ*, the peak heights were recorded at constant amplification from 320 to 230 nm at 5-nm intervals.

*Recovery experiments*

These experiments were carried out by dissolving 30 $\mu$g of carbamazepine in 10 $\mu$l of ethanol and adding blank serum to a volume of 3 ml. Aliquots of 50–500 $\mu$l were made up to 1 ml with blank serum and worked up as described for patients serum. All values were corrected for recovery.

## RESULTS AND DISCUSSION

A single peak at $R_F$ 0.72 corresponding to the carbamazepine standard was obtained upon photometry of thin-layer chromatograms of serum extracts (Fig. 1). Reaction with perchloric acid[6,8] led to the appearance of additional fluorescent spots due to carbamazepine metabolites, but none of these showed up in the UV traces. No UV-absorbing spots were caused by additional medication of the patients with phenytoin or barbiturates. The spectrum of the carbamazepine spot was identical with that of the standard even for a patient receiving in addition levomepromazine,

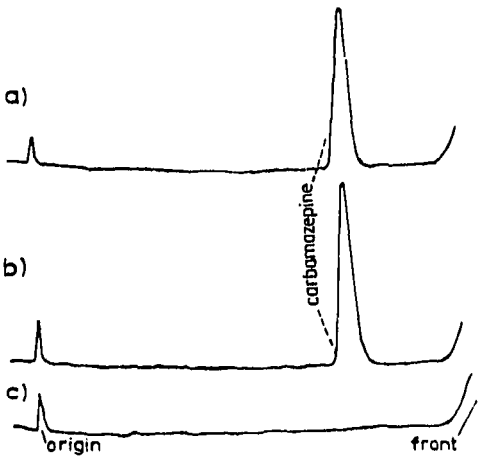


Fig. 1. Records obtained by reflectance photometry at 285 nm from thin-layer chromatograms of (a) carbamazepine standard (400 ng), (b) serum extract corresponding to 0.075 ml of serum from a patient receiving 600 mg of carbamazepine daily, and (c) blank serum extract.

promethazine, clozapine, phenytoin, acetylsalicylic acid, metamizol and coffein (Fig. 2).

The slope of the calibration graph was largely reproducible from one plate to another (Fig. 3), but it is advisable to run some standards on each plate. The optimal range for evaluation was found to be 250–500 ng per spot.

Recoveries from nine serum samples spiked with 0.5–5 $\mu$g/ml of carbamazepine were 105 $\pm$ 4% (mean $\pm$ S.D.). The mean recovery is greater than 100% probably owing to some loss of ethyl acetate by evaporation and by distribution into the

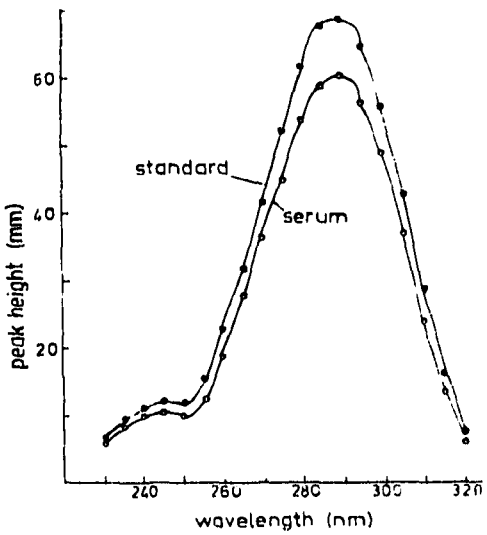


Fig. 2. "UV spectrum" of carbamazepine spots on thin-layer chromatograms of a standard (●) and of a serum extract (○) from a patient receiving in addition a variety of other drugs (see text).

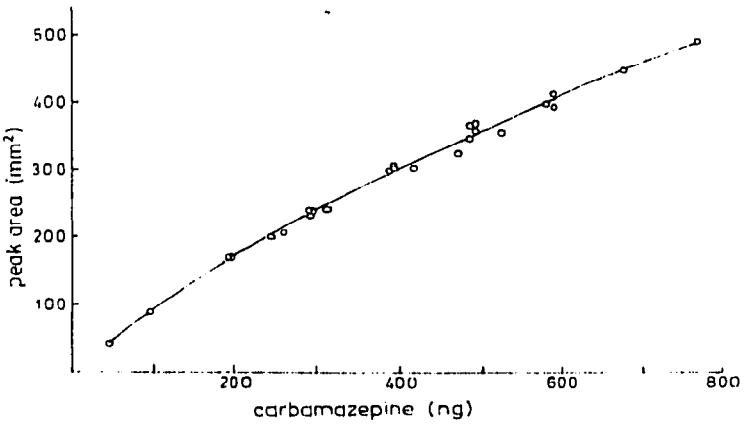


Fig. 3. Calibration graph constructed from values carbamazepine standard on different plates.

aqueous phase. The limit of detection was not reached with the concentration of 0.5 μg/ml; by increasing the volume of the serum sample, it would be possible to measure one-tenth to one-twentieth this concentration. As therapeutic serum levels are about 4–10 μg/ml (refs. 1 and 9), they can easily be assayed by this method.

Duplicate analyses performed on eleven serum samples resulted in an average deviation of 4.7% (range 0–10%); duplicate results obtained by chromatographing separate aliquots of the same extract gave an average deviation of 4.6% (0–11%, $n = 10$). No changes were observed in the values found upon replicate measurements on serum samples stored at −20° for up to 5 months. On pooled serum, ten replicate analyses were carried out. The value obtained was 4.53 ± 0.13 μg/ml (mean ± S.D.), which corresponds to a standard deviation of 2.9%.

Equilibrium concentrations were measured in sera of nine patients in whom an epilepsy or a trigeminal neuralgia was treated with daily doses of 400–800 mg of carbamazepine, partly in combination with other drugs. The values ranged from 2.6 to 7.2 μg/ml with no distinct dependence on the dose administered.

The time required for one analysis is about 2 h, of which only 40 min are actual working time. A series of ten determinations can be completed within 4 h.

The method requires neither the use of aggressive chemicals, as do the existing TLC methods[6,7], nor derivatization, which is necessary when decomposition during gas chromatography is to be avoided[3,4]. Also, the blank value is less than 20 ng/ml and is thus much lower than in recently published gas chromatographic methods[3,4]. The reproducibility compares well with those reported for other procedures[1–7]. Values found in patients' sera agreed with those obtained by other investigators[1,9].

ACKNOWLEDGEMENTS

The author thanks Dr. F. Petruch, Nervenklinik der Universität Tübingen, for supplying the serum samples. The skilful technical assistance of Miss K. Villumsen is gratefully acknowledged. The work was supported by the Deutsche Forschungsgemeinschaft.

## REFERENCES

1 S. I. Johannessen and R. E. Strandjord, *Epilepsia*, 14 (1973) 373.
2 N.-E. Larsen, J. Naestoft and E. Hvidberg, *Clin. Chim. Acta*, 40 (1972) 171.
3 J.-C. Roger, G. Rodgers, Jr. and A. Soo, *Clin. Chem.*, 19 (1973) 590.
4 R. J. Perchalski and B. J. Wilder, *Clin. Chem.*, 20 (1974) 492.
5 G. Gauchel, F. D. Gauchel and L. Birkofer, *Z. Klin. Chem.*, 11 (1973) 459.
6 J. Christiansen, *Scand. J. Clin. Lab. Invest.*, Suppl., 118 (1971) 67.
7 D. B. Faber and W. A. Man in 't Veld, *J. Chromatogr.*, 93 (1974) 238.
8 F. Scheiffarth, F. Weist and L. Zicha, *Z. Klin. Chem.*, 4 (1966) 68.
9 J. Christiansen and M. Dam, *Acta Neurol. Scand.*, 49 (1973) 543.