Journal of Chromatography, 154 (1978) 272–274

CHROM. 10,887

Note

Simultaneous determination by gas–liquid chromatography of carbamazepine and carbamazepine-10,11-epoxide in plasma

ROBIN E. CHAMBERS

Department of Chemical Pathology, The Royal Infirmary, Bristol BS2 8HW (Great Britain)

(Received January 12th, 1978)

Carbamazepine-10,11-epoxide is a metabolite of carbamazepine that has been identified in the urine of the rat and in the urine and plasma of man[1,2] and which, in the rat, has been shown to have antiepileptic properties comparable to those of the parent drug[2]. It has therefore been suggested[3] that, in human plasma, the epoxide should be measured in addition to carbamazepine in order to correlate better the drug level with therapeutic effect. The simple gas–liquid chromatographic (GLC) procedure described recently by Chambers and Cooke[4] for monitoring levels of carbamazepine may readily be adapted for the simultaneous determination of carbamazepine and carbamazepine-10,11-epoxide in plasma. In this paper, the modified procedure is described.

EXPERIMENTAL

Materials and equipment

Carbamazepine, carbamazepine-10,11-epoxide and imipramine were supplied by Geigy Pharmaceuticals (Macclesfield, Great Britain). Diethyl ether and acetone were redistilled before use. GLC analyses were carried out with a Pye Unicam Series 104 chromatograph equipped with an alkali flame-ionization (organic-nitrogen-specific) detector.

Method

Carbamazepine and carbamazepine-10,11-epoxide together with added internal standard (imipramine, 0.02 μmole) were extracted from plasma (1 ml) by shaking with diethyl ether (9 ml) for 5 min. After the addition of ammonium sulphate (2 g, washed with diethyl ether) and further shaking for 30 sec, the organic phase was decanted into a conical centrifuge tube and evaporated to dryness under nitrogen at 40°. The residue was dissolved in acetone (50 μl) and an aliquot (3 μl) injected into the chromatograph. Chromatograms were run at 250° with a glass column (1.5 m × 4 mm I.D.) packed with 5% Apiezon L–0.5% potassium hydroxide on Diatomite CLQ (J J's Chromatography, King's Lynn, Great Britain) and a carrier gas (argon) flow-rate of 45 ml/min. The injection port and detector temperature was 260°. The concentrations of carbamazepine and carbamazepine-10,11-epoxide were determined by calculating the peak-height ratio of both compounds with respect to the internal standard

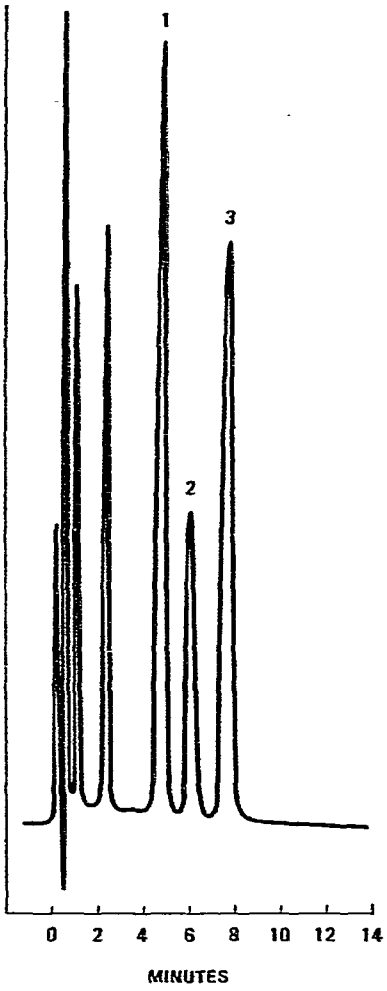

Fig. 1. GLC analysis of an extract of human plasma containing 42 μmole/l of carbamazepine (peak 1), 19 μmole/l of carbamazepine-10,11-epoxide (peak 2) and 20 μmole/l of imipramine (internal standard, peak 3).

and relating these values to a calibration graph derived from plasma standards analysed at the same time.

Reproducibility

Within-batch precision was determined from replicate ($n = 20$) analyses carried out simultaneously on a plasma pool to which carbamazepine (42.4 μmole/l) and carbamazepine-10,11-epoxide (39.6 μmole/l) had been added. Between-batch precision was determined from serial analyses ($n = 20$) of the same pool. The coefficient of variation (standard deviation divided by the mean) was then calculated in each instance.

RESULTS AND DISCUSSION

In the GLC system described by Chambers and Cooke[4], a 5% Apiezon L–1% potassium hydroxide column packing was recommended for the determination of carbamazepine. Unfortunately, when chromatographed on this column, carbamazepine-10,11-epoxide decomposes to multiple products and reproducible results cannot be obtained. If, however, the potassium hydroxide content of the column packing is reduced, both carbamazepine and the epoxide metabolite can be measured satisfactorily in a single procedure. Several concentrations of potassium hydroxide (0.7, 0.5 and 0.3) were investigated and, of these, a 0.5% loading was found to be the most suitable. Thus, as is shown in Fig. 1, both carbamazepine (peak 1, retention time 4.6 min) and carbamazepine-10,11-epoxide (peak 2, retention time 6.0 min) emerge as single, sharp and symmetrical peaks which are well separated from each other and from that of the internal standard, imipramine (peak 3, retention time 7.6 min).

Imipramine was chosen as the internal standard because amitriptyline, the internal standard previously recommended[4], is not separated adequately from the epoxide. In this system, imipramine has a longer retention time than amitriptyline and therefore does not overlap with the epoxide peak. The recovery of imipramine and carbamazepine from plasma was 80% whereas that of the epoxide was 70%.

The accuracy and reproducibility of the modified procedure are similar to those of the original system[4]. Thus, the precision figures as determined from the plasma pool (weighed-in values: carbamazepine, 42.4 μmole/l, carbamazepine-10,11-epoxide, 39.6 μmole/l) were as follows: within-batch, carbamazepine 39.6 $\pm$ 1.4 μmole/l (coefficient of variation 3.5%) and epoxide 42.5 $\pm$ 1.6 μmole/l (coefficient of variation 3.8%); and between-batch, carbamazepine 40.8 $\pm$ 2.2 μmole/l (coefficient of variation 5.4%) and epoxide 42.0 $\pm$ 2.8 μmole/l (coefficient of variation 6.7%).

Although the epoxide has been found in all plasma samples so far analysed from patients receiving carbamazepine, no clear correlation seems to exist between the level of the metabolite and that of the parent drug. This finding confirms a previous report[2] that the ratio between carbamazepine and carbamazepine-10,11-epoxide is not constant and that the levels of the epoxide in plasma do not appear to be related to those of carbamazepine itself.

REFERENCES

1 A. Frigerio, R. Fanelli, P. Biandrate, G. Passerini, P. L. Morselli and S. Garattini, *J. Pharm. Sci.*, 61 (1972) 1144.
2 P. L. Morselli and A. Frigerio, *Drug. Metab. Rev.*, 4 (1975) 97.
3 M. Eichelbaum and L. Bertilsson, *J. Chromatogr.*, 103 (1975) 135.
4 R. E. Chambers and M. Cooke, *J. Chromatogr.*, 144 (1977) 257.