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ROUTINE MONITORING OF CARBAMAZEPINE AND CARBAMAZEPINE-10,11-EPOXIDE IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING 10-METHOXYCARBAMAZEPINE AS INTERNAL STANDARD

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SUMMARY

Carbamazepine and carbamazepine-10,11-epoxide were separated by high-performance liquid chromatography (HPLC) with acetonitrile—water as mobile phase, and detection was effected by UV absorption at 215 nm with a total retention time of less than 10 min. Plasma samples were extracted with dichloromethane and 4 *M* sodium hydroxide, and 10-methoxycarbamazepine was added as internal standard.

Other commonly used anticonvulsant drugs present in plasma showed no significant interference.

The within-batch coefficient of variation for carbamazepine was 4.9% and carbamazepine-10,11-epoxide 5.9%. Between-batch coefficients of variation were 3.7% and 5.3%, respectively. Mean recovery for carbamazepine was 100.2% and for carbamazepine-10,11-epoxide 100.6%.

This HPLC method was compared with both an enzyme immunoassay procedure (EMIT) and a gas—liquid chromatographic (GLC) method. Correlation coefficient between HPLC/EMIT for carbamazepine was 0.983, HPLC/GLC carbamazepine 0.988 and HPLC/GLC carbamazepine-10,11-epoxide 0.981.

INTRODUCTION

Carbamazepine (Tegretol®, Ciba-Geigy, Basle, Switzerland) is increasingly used in the treatment of epilepsy [1] and is the drug of choice in trigeminal neuralgia [1,2]. Carbamazepine is partly converted in the body to the 10,11-epoxide metabolite which also displays anticonvulsant properties similar to those of the parent compound [3]. The plasma level of carbamazepine-10,11-epoxide is lower than that of the parent drug, the relative percentage ranging from 10—50% [4].

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There is no clear relationship between the daily dose of carbamazepine and its steady state plasma level in epileptic patients receiving long-term therapy with the drug [5,6]. Marked individual variations in the ratio of dose to plasma level [7] make it essential to measure plasma carbamazepine levels in each patient, but no firm data are yet available concerning the importance of measuring the levels of the metabolite in routine practice.

Many different methods have been used for the estimation of carbamazepine including procedures based on UV spectrophotometry [8,9], gas—liquid chromatography (GLC) [10—16], enzyme-multiplied immunoassay technique (EMIT) [17,18] and high-performance liquid chromatography (HPLC), some of which also estimate the epoxide. The subject has been reviewed recently. Nevertheless, there are disadvantages to some of the published methods. In particular the spectrophotometric methods lack specificity and may be subject to interference, and some of the GLC methods yield variable results because of decomposition of carbamazepine on column [19] or during derivatisation [20]. Some of the HPLC procedures employ as internal standards, compounds (or metabolites of drugs) such as nitrazapam [21], lorezapam or N-desmethyldiazapam [22] any of which may be present in the serum of patients in neurological hospitals or clinics, or 10,11-dihydroxycarbamazepine which is the major metabolite of carbamazepine present in urine. Others require a change in wavelength [23] during each run in order to overcome the unfavourable absorption characteristics of the epoxide. The reversed-phase HPLC procedure presented here employs 10-methoxy-carbamazepine as an internal standard, a compound which is not normally administered, nor present as a metabolite in patients' plasma or urine.

EXPERIMENTAL

Materials

Carbamazepine, carbamazepine-10,11-epoxide and 10-methoxycarbamazepine were obtained from Geigy (Horsham, Great Britain). All solvents used were HPLC grade.

Samples

Blood samples were collected from patients on carbamazepine for routine measurement, and plasma was stored at −20°C until required for analysis.

*EMIT** assay

The reagents for enzyme immunoassay of carbamazepine were obtained from Syva (Maidenhead, Great Britain) and were used according to the manufacturer's instructions. Absorption was measured at 340 nm on a Gilford System 3500 Computer Directed Analyser (Gilford Instruments, Teddington, Great Britain) and the results were directly calculated in μmol/l by a CP 5000 EMIT Clinical Processor (Syva).

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* Trade name of Syva.

GLC assay

Plasma containing carbamazepine and its metabolite was analysed by GLC using the method of Chambers [15] with 10-methoxycarbamazepine as internal standard instead of imipramine. The estimation was carried out on a Hewlett-Packard research chromatograph 5750G using an organic nitrogen specific detector (Hewlett-Packard, Winersh, Great Britain).

HPLC assay

Apparatus. The liquid chromatograph used was a Spectra Physics SP 8000 (Spectra Physics, St. Albans, Great Britain) with data processing capability, fitted with an automatic 10-μl Valco loop injector and a Schoeffel S770 variable-wavelength UV absorption detector (Kratos, Manchester, Great Britain). Chromatograms were run at ambient temperature on a column 25 cm × 4.9 mm I.D. packed with LiChrosorb RP-8 10 μm (Hichrom, Reading, Great Britain). A mobile phase (acetonitrile—water, 35:65) flow-rate of 1.8 ml/min at 60 ± 5 bar was used. The column eluate was monitored at 215 nm with sensitivity range of 0.1 a.u.f.s. and chart speed of 0.5 cm/min.

Preparation of standard solutions. The stock solutions of carbamazepine, carbamazepine-10,11-epoxide and 10-methoxycarbamazepine as internal standard were made up in methanol to a concentration of 1 mg/ml. These solutions were kept at 4°C in a sealed container and were stable for several weeks.

Working standards of carbamazepine and carbamazepine-10,11-epoxide were made by further dilution with methanol to give a range of concentrations from 2—16 μg/ml for carbamazepine and 1—8 μg/ml for carbamazepine-10,11-epoxide. The concentration of 10-methoxycarbamazepine in each tube was 4 μg.

Carbamazepine and carbamazepine-10,11-epoxide concentrations were determined by the ratio of the peak areas of each drug to the peak area of internal standard plotted against concentration of the drugs.

Procedure. Plasma or standard (250 μl) to which 10-methoxycarbamazepine (4 μg) had been added as an internal standard was made alkaline with 4 *M* aqueous sodium hydroxide (250 μl); 2 ml of dichloromethane (BDH Chemicals, Poole, Great Britain) were added and the mixture shaken at 120 cycles/min for 5 min. The mixture was then centrifuged at 3 *g* for 5 min and the top (aqueous) layer removed by aspiration. The solvent layer was then carefully transferred to a separate 15-ml conical tube and evaporated to dryness at 40°C under a gentle stream of oxygen-free nitrogen. The sides of each conical tube were washed down with 0.2 ml of acetonitrile (Fisons, Loughborough, Great Britain), which was then evaporated to dryness, the residue dissolved in 100 μl of acetonitrile and 10 μl injected in the chromatograph.

RESULTS

Table I gives the figures for precision, recovery and commercial quality control data.

Specificity

The retention times of carbamazepine and its metabolite and possible inter-

TABLE I

ANALYTICAL PARAMETERS OBTAINED BY THREE PROCEDURES

	HPLC	GLC	EMIT
Carbamazepine (pooled plasma)			
Between-batch precision	31 ± 2.0	32 ± 3.0	36 ± 3.0
Coefficient of variation (%)	3.7 (n=45)	4.2 (n=40)	6.0 (n=35)
Within-batch precision	29 ± 2.0	30 ± 3.0	35 ± 3.0
Coefficient of variation (%)	4.9 (n=20)	5.9 (n=22)	8.9 (n=35)
Carbamazepine-10,11-epoxide (pooled plasma)			
Between-batch precision	7.0 ± 1.0	7.4 ± 2.6	–
Coefficient of variation (%)	5.3 (n=40)	10.7 (n=40)	–
Within-batch precision	6.8 ± 1.0	7.2 ± 2.5	–
Coefficient of variation (%)	5.9 (n=20)	15.6 (n=22)	–
Carbamazepine (spiked plasma)			
Mean of recovery (%)	100.2 (n=20)		
Carbamazepine-10,11-epoxide (spiked plasma)			
Mean of recovery (%)	100.6 (n=20)		
Commercial quality control (Seronorm Pharmaca):			
Carbamazepine			
Recommended value	63.0 μmol/l		
Analytical value	59.07 μmol/l		
Coefficient of variation (%)	2.43 (n=15)		
Carbamazepine-10,11-epoxide			
Recommended value	6.5 μmol/l		
Analytical value	5.9 μmol/l		
Coefficient of variation (%)	3.9 (n=15)		

fering compounds are given in Table II. When these compounds are injected before extraction, it is evident that some of the drugs have very nearly the same retention times as carbamazepine and its derivatives, but after extraction of plasma spiked with (toxic) levels of the compound no peaks are apparent. Fig. 1 shows typical chromatograms of (a) a standard extract and (b) a plasma extract. It can be seen that there is adequate separation between carbamazepine-10,11-epoxide, carbamazepine and the internal standard in both cases.

Precision

Both between-batch and within-batch precision were determined from analyses made on pooled plasma. Patients' plasma samples known by previous analysis to contain carbamazepine were pooled and filtered with thorough mixing, aliquoted into bottles and stored at −20°C until required for analysis. Between-batch precision was determined on this pooled plasma on different days. In all instances the coefficient of variation for HPLC was less than 6%

TABLE II

RETENTION TIMES OF ANTIEPILEPTIC DRUGS AND OTHER POTENTIALLY INTERFERING COMPOUNDS

Compound	Retention time (min)	Retention time after extraction (min)
Carbamazepine	5.9	5.9
Carbamazepine-10,11-epoxide	3.9	3.9
10-Methoxycarbamazepine	7.4	7.4
Phenobarbitone	3.6	No peak
Primidone	3.0	No peak
Phenytoin	6.7	No peak
Ethosuximide	No peak	No peak
Valproic acid	No peak	No peak
Clonazepam	9.3	No peak
Diazepam	18.1	No peak
Desmethyldiazepam	12.4	No peak
Sulthiame	3.5	No peak
Nitrazepam	8.1	No peak
cis-Dihydroxycarbamazepine	2.7	2.6

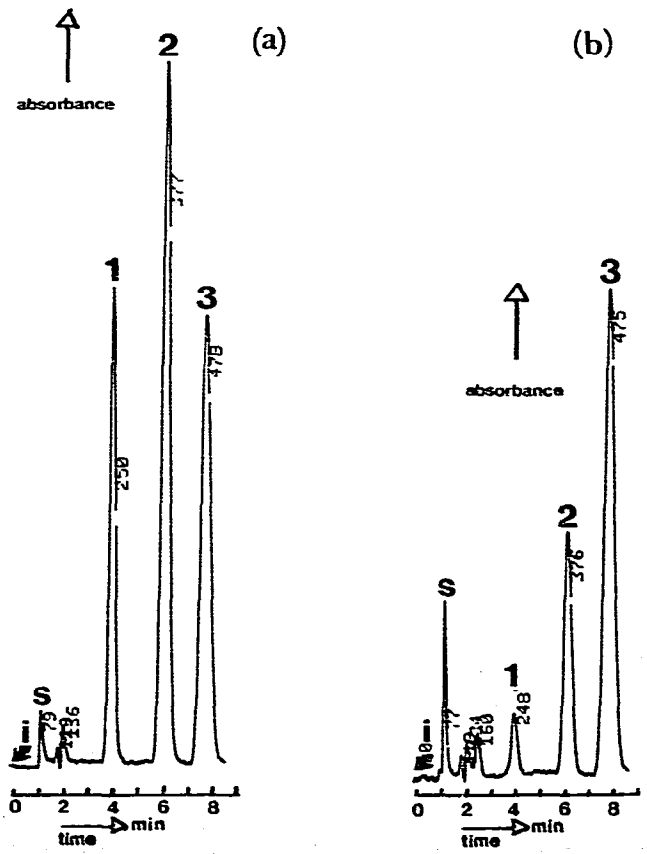

Fig. 1. HPLC chromatograms (a) showing separation of a standard solution and (b) obtained from plasma extract of a patient taking carbamazepine (600 mg/day). Peaks: (1) the metabolite, carbamazepine-10,11-epoxide, (2) carbamazepine and (3) the internal standard, 10-methoxycarbamazepine. (i) Point of injection and (s) solvent front.

which is entirely acceptable for routine measurement of carbamazepine and carbamazepine-10,11-epoxide.

Recovery

Recovery was determined by analysing drug-free plasma samples (spiked with 5 μg/ml carbamazepine and 2 μg/ml carbamazepine-10,11-epoxide) 20 times, giving measured recoveries ranging from 98.8—102.5% for carbamazepine and 97.9—103% for carbamazepine-10,11-epoxide.

Commercial quality control

The commercial quality control serum (Seronorm Pharmaca, BDH) was analysed for carbamazepine and carbamazepine-10,11-epoxide giving coefficients of variation of 2.43% and 3.0%, respectively.

Correlation

Regression analyses of the results obtained between HPLC and GLC (carbamazepine and carbamazepine-10,11-epoxide) and HPLC and EMIT (carbamazepine) are shown in Figs. 2, 3 and 4, which in addition give values for slopes of the regression lines and intercepts. There was good correlation for carbamazepine between HPLC and GLC (r=0.988) and HPLC and EMIT (r=0.983). There was also a good correlation between HPLC and GLC for carbamazepine-10,11-epoxide (r=0.981).

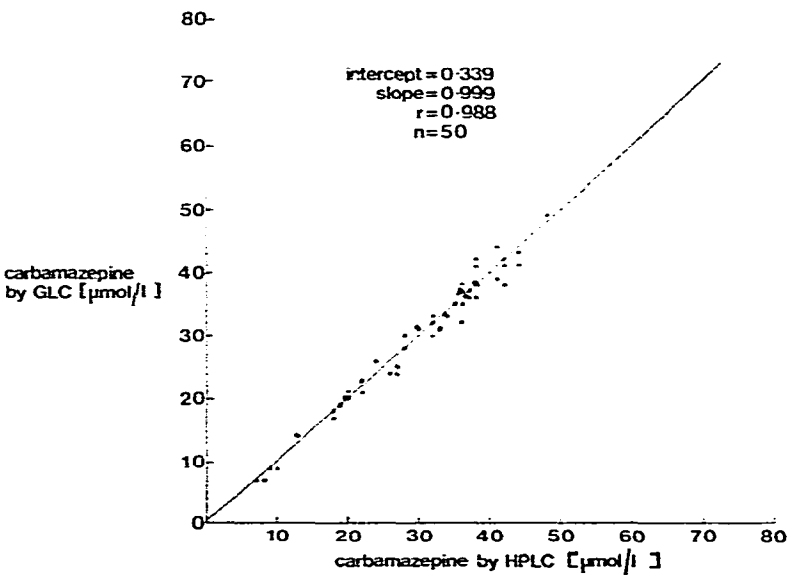

Fig. 2. Correlation of carbamazepine concentration as determined by HPLC and GLC.

Linearity

This HPLC assay is linear over the range of 0—100 μmol/l for carbamazepine and 0—50 μmol/l for carbamazepine-10,11-epoxide. This more than adequately spans the therapeutic range for carbamazepine (12—50 μmol/l).

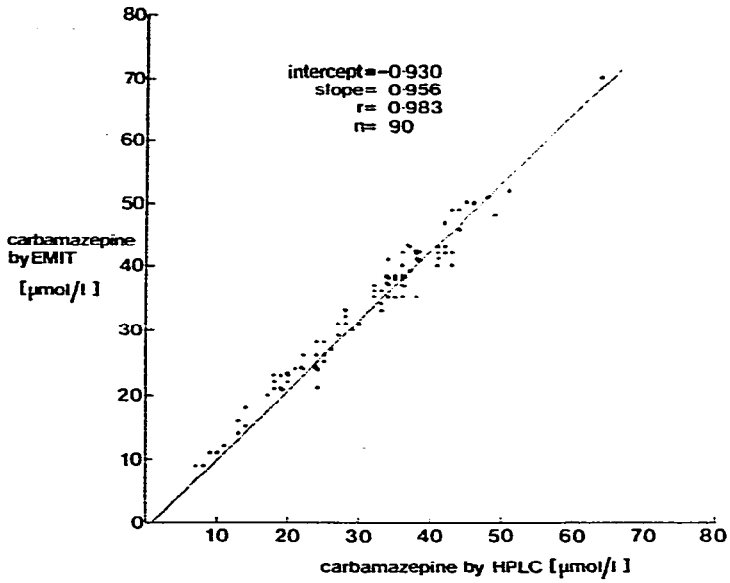

Fig. 3. Correlation of carbamazepine concentration as determined by HPLC and EMIT.

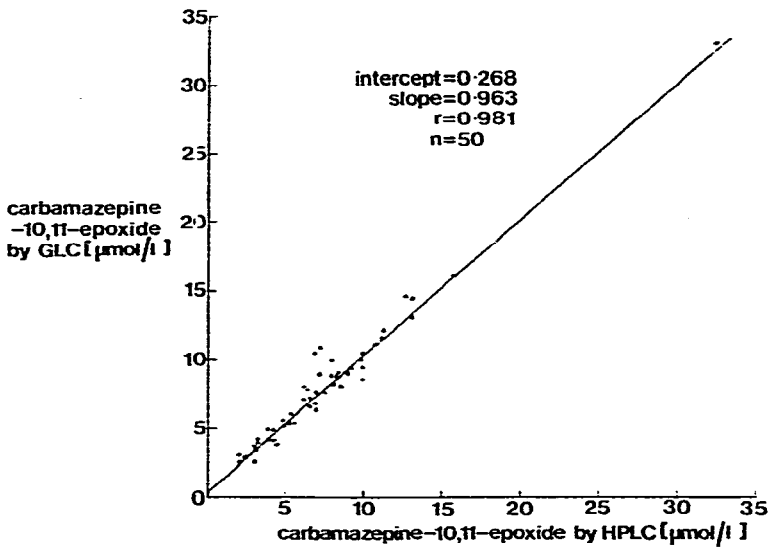

Fig. 4. Correlation of the metabolite carbamazepine-10,11-epoxide concentration as determined by HPLC and GLC.

DISCUSSION

The HPLC system described here for routine monitoring of plasma carbamazepine and its metabolite with use of 10-methoxycarbamazepine as an internal standard has some advantages over published HPLC, GLC and EMIT procedures.

The low UV absorbing properties of the epoxide have been a problem in HPLC analysis but in this HPLC procedure the use of optimum wavelength for

carbamazepine and carbamazepine-10,11-epoxide (215 nm) has contributed significantly to the sensitivity of the method. This factor eliminates the need to change the range of the detector during analysis as is the case for some HPLC procedures [1]. Furthermore, 10-methoxycarbamazepine is a suitable internal standard as it is not present in patients' plasma or urine; the use of lorezapam and N-desmethyldiazepam (a metabolite of diazapam), nitrazepam and imipramine as internal standard may pose problems in neurological hospitals where patients may be taking any one of these compounds together with the carbamazepine.

The high precision of the HPLC procedure shown in Table I as compared with GLC probably reflects better prevailing chromatographic conditions. This may be because the HPLC is carried out at ambient temperature with no possibility of on-column degradation while GLC is carried out at a relatively high column temperature (225°C). By contrast with the above the EMIT procedure is quicker than HPLC but slightly less precise (Table I) and unable to determine the epoxide at present.

No difficulties have been encountered with the HPLC technique in routine use and an interference problem from other drugs has not arisen. Very minor extraneous peaks have been observed around 2 min retention time (Fig. 1) but these peaks are probably caused by impurities from the silicone tubing used for blowing down the extract and do not interfere with the assay.

Sera from a total of 115 patients (dose ranging from 300—1800 mg per day) have been analysed for carbamazepine and its metabolite by this HPLC procedure, and of these one who had symptoms of toxicity was found to have a serum level of epoxide as high as 86.8% of the carbamazepine level (carbamazepine 38 μmol/l and epoxide 33 μmol/l). Another 20 patients with serum carbamazepine levels 50 μmol/l and above had epoxide levels of 12 μmol/l and above. The remaining 95 patients had levels of epoxide below 12 μmol/l and correspondingly serum carbamazepine levels of less than 50 μmol/l.

These results indicate that the upper limit of the therapeutic range for epoxide could be of the order of 12 μmol/l, but it is still necessary to relate the upper limit of epoxide level to clinical toxicity. Further work is necessary in order to clarify this situation and also evaluate interactions between the epoxide and other anticonvulsants co-prescribed with carbamazepine.

CONCLUSION

The HPLC, GLC and EMIT techniques evaluated in this study are all reliable, accurate, simple to perform and linear. The levels of carbamazepine and its metabolite analysed by these methods agree well over a wide range of concentrations. However, the HPLC procedure possesses the advantages of greater precision and sensitivity over the GLC technique examined here, and has the potential for determining carbamazepine and its metabolites in urine. Further it is quicker than GLC (requiring less than 1 h to perform an analysis) and in addition requires smaller plasma samples which is an advantage in paediatric practice. The EMIT technique requires even smaller plasma samples than HPLC and it is more rapid in operation but cannot be adapted at present to analyse carbamazepine-10,11-epoxide in plasma or urine.

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REFERENCES

1 M. Eichelbaum and L. Bertilsson, J. Chromatogr., 103 (1975) 135.
2 M.J. Eadie and J.H. Tyrer, Anticonvulsant Therapy, Churchill Livingstone, Edinburgh, 2nd ed., 1980, p. 132.
3 P.L. Morselli, P. Biandrate, A. Frigerio, M. Gerna and C. Tognoni, in J.W.A. Meyer, H. Meinardi, C. Gardner-Thorpe and E. van der Kleijn (Editors), Methods of Analysis of Antiepileptic Drugs, Excerpta Medica, Amsterdam, 1973, p. 169.
4 H. Kutt, in C.E. Pippenger, J.K. Penry and H. Kutt (Editors), Antiepileptic Drugs, Raven Press, New York, 1978, p. 300.
5 P.L. Morselli and A. Frigerio, Drug Metab. Rev., 4 (1975) 97.
6 M.J. Eadie and J.H. Tyrer, Anticonvulsant Therapy, Churchill Livingstone, Edinburgh, 2nd ed., 1980, p. 140.
7 M. Eichelbaum, L. Bertilsson, L. Lund, L. Palmer and F. Sjöqvist, Eur. J. Clin. Pharmacol., 9 (1976) 417.
8 J. Fuhr, Arzneim.-Forsch., 14 (1964) 74.
9 P.L. Morselli, M. Gerna and S. Garrathini, Biochem. Pharmacol., 20 (1971) 2043.
10 P.A. Toseland, J. Grove and D.J. Berry, Clin. Chim. Acta, 38 (1972) 321.
11 W.J. Kupferberg, J. Pharm. Sci., 61 (1972) 284.
12 O. Drummer, P. Morris and F. Vajda, Clin. Exp. Pharmacol. Physiol., 3 (1976) 497.
13 R. Varma and A.Y. Hoshino, Clin. Chim. Acta, 93 (1979) 165.
14 R.E. Chambers and M. Cooke, J. Chromatogr., 144 (1979) 257.
15 R.E. Chambers, J. Chromatogr., 154 (1978) 272.
16 A. Ranise, E. Benassi and G. Besio, J. Chromatogr., 222 (1981) 120.
17 D.D. Schottelius, in C.E. Pippenger, J.K. Penry and H. Kutt (Editors), Antiepileptic Drugs, Raven Press, New York, 1978, p. 95.
18 V. Goldberg, N. Ratnaraj, A. Elyas and P.T. Lascelles, Anal. Proc., 18 (1981) 313.
19 A. Frigerio, K.M. Baker and G. Belvedere, Anal. Chem., 45 (1973) 1846.
20 P. Friel and J.R. Green, Clin. Chim. Acta, 43 (1973) 69.
21 A. Astier, M. Maury and J. Barbizet, J. Chromatogr., 164 (1979) 235.
22 J.J. MacKichan, J. Chromatogr., 181 (1980) 373.
23 G.W. Mihaly, J.A. Phillips, W.J. Louis and F.J. Vajda, Clin. Chem., 23 (1977) 2283.