Journal of Chromatography, 174 (1979) 341–349

CHROM. 11,812

DETERMINATION OF BROMOCRIPTINE IN PLASMA: COMPARISON OF GAS CHROMATOGRAPHY, MASS FRAGMENTOGRAPHY AND LIQUID CHROMATOGRAPHY

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(Received February 20th, 1979)

SUMMARY

Gas chromatographic, mass fragmentographic and liquid chromatographic techniques for the determinations of bromocriptine (2-bromo-α-ergocriptine; Parlodel) in human plasma are described. These methods were found to be suitable for determining concentrations of bromocriptine down to 0.5, 1.0 and 10.0 μg/l, respectively. Accuracy, specificity and analytical capacity were satisfactory for all three methods.

Gas chromatography was compared with liquid chromatography, and the two methods were demonstrated to give identical results in patients treated with bromocriptine for Parkinson's disease. Gas chromatography was also compared with mass fragmentography, and the results from these two assays were also in agreement.

INTRODUCTION

Bromocriptine (BCT) (2-bromo-α-ergocriptine; Parlodel) has been demonstrated to have therapeutic potency in the treatment of hyperprolactinaemia[1–3], and in high doses has been shown to have significant anti-Parkinson effects[4–6]. Treatment with BCT might be improved by monitoring plasma concentrations, but the first prerequisite is reliable analytical procedures for its determination.

More than one method might be suitable, and it is of importance to compare different assays. The aim of this paper is to describe a comparison of three different

methods for the determination of BCT in plasma from patients undergoing ordinary treatment with this drug. The gas chromatographic (GC) and mass fragmentographic (MF) methods were developed at Glostrup Hospital, and the liquid chromatographic (LC) method was developed at Lillhagen's Hospital. The GC method has recently been used in an investigation of the pharmacokinetic properties of BCT in patients with Parkinson's disease[7].

MATERIALS AND METHODS

Chemicals

Concentrated borate buffer (pH 12) of analytical reagent grade was obtained from E. Merck (Darmstadt, G.F.R.), hexamethyldisilazane from Pierce (Rockford, Ill., U.S.A.) and 1-heptanesulphonic acid (PIC-B7) from Waters Assoc. (Gothenburg, Sweden). Other commercial chemicals and solvents used were of analytical-reagent grade.

Stock solutions (1 g/l) of BCT and of the internal standards ergotamine and dihydroergocristine were prepared in ethanol. When kept in a refrigerator at 4° they were stable for at least 1 year.

Gas chromatography

Extraction procedure. To a 500-μl plasma sample were added 100 μl of borate buffer (pH 12) and 10 ng of dihydroergocristine (DHEC) as internal standard. Toluene (6 ml) was added and the sample mixed for 5 min at 20 rpm on a rotary mixer. The specimen was then contrifuged for 3 min at 1000 *g*, after which 5 ml of the organic phase were transferred into another centrifuge tube containing 1 ml of 1 *M* sulphuric acid. The contents were mixed for 5 min, centrifuged, and the organic phase was discarded. The original plasma phase was re-extracted by mixing with a further 6 ml toluene for 5 min. After centrifugation, 5 ml of this toluene phase was transferred to the sulphuric acid phase, mixed and centrifuged, then the toluene phase was discarded. A volume of 400 μl of 6 *M* sodium hydroxide solution was added. Toluene (3 ml) was added to the aqueous phase and mixed for 5 min. After centrifugation for 1 min, the organic phase was transferred into a tapered tube moistened with hexamethyldisilazane (HMDS) in ethanol (0.5, w/w). The toluene phase was evaporated to dryness under a stream of nitrogen. The aqueous phase was re-extracted with 3 ml of toluene, centrifuged, and the toluene transferred into the tapered tube and evaporated to dryness. The final residue was dissolved in 50 μl of a mixture of HMDS in ethanol (0,5, w/w). A few microlites of this solution were injected into the chromatograph.

Apparatus and conditions. A Pye Series 104, Model 74, gas chromatograph equipped with an electron-capture detector was used. A glass column (0.9 m × 4 mm I.D.) was used. The stationary phase was 1% (w/w) OV-17 on Celite JJ CQ (100–120 mesh B.S.). The amount of column filling was 7 g. The column was conditioned at 350° for 48 h. The column temperature was 245°, injection block temperature 250°. detector temperature 350°, carrier gas (argon–methane, 90:10) flow-rate 50 ml/min and quench gas flow-rate 10 ml/min. The detector was run in a pulsed mode (150 μsec). The attenuation of the recorder was $5 \cdot 10^2$. The retention time of BCT was 2 min.

Calculations. The concentrations were read from a calibration graph constructed on the basis of a series of standard samples containing known amounts of BCT. The ratio of the peak height of BCT to that of DHEC was plotted against concentration.

Mass fragmentography

Extraction procedure. The procedure was similar to that used in the GC assay except that ergotamine (EGT) was used as the internal standard.

Apparatus and conditions. An LKB 9000 mass spectrometer was used. A glass column (0.5 m × 2 mm I.D.) was used. The stationary phase was 3% (w/w) OV-101 on Celite JJ CQ (100–120 mesh B.S.). The amount of column filling was 1.5 g. The column was conditioned at 350° for 48 h. The column temperature was 180°, injection block temperature 200°, molecule seperator temperature 260°, ion source temperature 290°, carrier gas (helium) flow-rate 20 ml/min, electron energy 27 eV and trap current 60 μA. The mass fragments used were 308 and 314 for BCT and EGT, respectively. The retention time of BCT was 2 min.

Calculations. The concentrations were read from a calibration graph constructed on the basis of a series of standard samples containing known amounts of BCT. The ratio of the peak height of BCT to that of EGT was plotted against concentration.

Liquid chromatography

Extraction procedure. A 2-ml volume of serum or plasma was used for each analysis. BCT (10, 20 and 30 ng/ml) was added to standard samples of pooled blank serum, 400 μl of 2.5 *M* potassium carbonate solution and 1.5 ml of diethyl ether were added and the sample was mixed for 30 sec on a rotary mixer, followed by centrifugation for 5 min at 1000 *g*. The ether phase was withdrawn and the aqueous phase re-extracted with 1 ml of diethyl ether. A 100-μl volume of 0.05 *M* sulphuric acid was added to the combined organic phase (2 ml), followed by mixing and centrifugation. A 75-μl volume of the aqueous phase was collected and injected for liquid chromatography.

Apparatus and conditions. A Waters Assoc. liquid chromatograph equipped with a Model 440 UV detector (254 nm) was used, with a U6K injector a 6000A pump and a Tarkan 600 recorder. The column (30 cm × 3.9 mm I.D.) was packed with μBondapak C_{18}. The mobile phase was methanol–water (65:35) that was 0.01 *M* in 1-heptanesulphonic acid). The column flow-rate was 1.5 ml/min, sensitivity 0.005 a.u.f.s., full recorder deflection 2 mV and recorder speed 0.5 cm/min. The retention time of BCT was 7 min.

Calculations BCT peaks were identified and peak heights plotted against concentration of BCT on a standard graph.

Plasma samples

Venous blood samples were drawn from patients undergoing continuous treatment with BCT for Parkinson's disease. The plasma was immediately separated by centrifugation and stored at −20° until required for analysis. Samples were transported between laboratories in containers packed with dry-ice, which kept the samples deep frozen at all times.

RESULTS

Chromatograms

Fig. 1 illustrates chromatograms of plasma samples. Fig. 1a and b demonstrate the GC of plasma extracts containing 0 and 20 μg of BCT per litre of plasma, respectively. Under the conditions described BCT and DHEC appeared 2 and 5 min after the injection, respectively. Fig. 1c and d demonstrate the LC of plasma extracts containing 0 and 10 μg of BCT per litre of plasma, respectively, and Fig. 1e and f demonstrate the MF of plasma extacts containing 0 and 4 μg of BCT per litre of plasma, respectively.

Fig. 2a is a calibration graph constructed on the basis of plasma samples analysed by GC containing various concentrations of BCT in the range from 0 to 30 μg/l. A linear graph was obtained. Fig. 2b and c are similar graphs for plasma samples analysed by LC and MF, respectively.

Sensitivity

For all three analytical procedures the sensitivity was defined as the lowest concentration giving a peak at least 10 times higher than the noise on the baseline. In this way the lowest concentrations giving reliable safe quantitation were 0.5 (GC), 1.0 (MF) and 10.0 μg/l (LC).

Accuracy and reproducibility

For clinical use both accuracy and reproducibility were found to lie within acceptable limits for all three methods (Table I).

Specificity

Tests were not carried out by adding different drugs to the samples. However, plasma samples used for the comparison of the methods were drawn not only from patients receiving BCT as the sole medication, but also from patients on concomitant medication with L-dopa and diazepam. None of these drugs interfered with the determination owing to complete separation from BCT and the applied internal standards in the chromatographic systems. Furthermore, no interfering peaks were detected in blank plasma samples. Finally, the specificity was confirmed by MF (see below).

Capacity

As illustrated in Fig. 1, the chromatogram from a sample can be run with an interval of 8 min in all three assays. This gives a (theoretical) maximal capacity of about seven injections into the chromatographic system per hour.

Inter-method correlations

Ten plasma samples drawn from patients undergoing commonly applied dose regimens with BCT (for Parkinson's disease) were analysed both by GC and LC. The correlation between the results of the two methods is demonstrated in Fig. 3. The methods gave almost identical results for the concentration range between *ca.* 12 and 26 μg/l. As one sample deviated significantly, the correlation coefficient between the two methods is 0.862. If the result for this sample was dis-

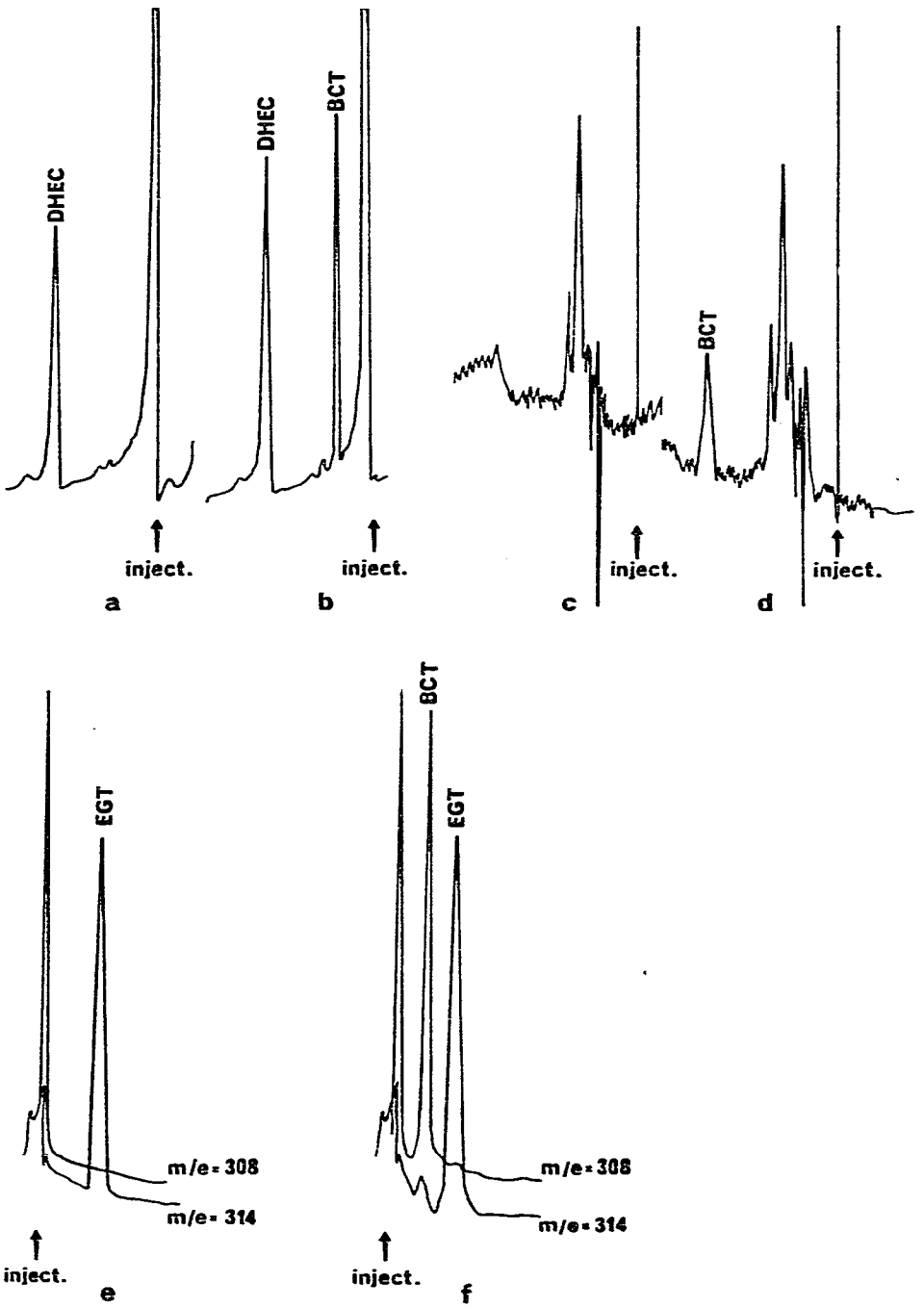

Fig. 1. Gas chromatograms, liquid chromatograms and mass fragmentograms from plasma samples containing different concentrations of bromocriptine (BCT). Concentrations in plasma: GC, (a) 0 and (b) 20 μg/l; LC, (c) 0 and (d) 10 μg/l; MF, (e) 0 and (f) 4 μg/l.

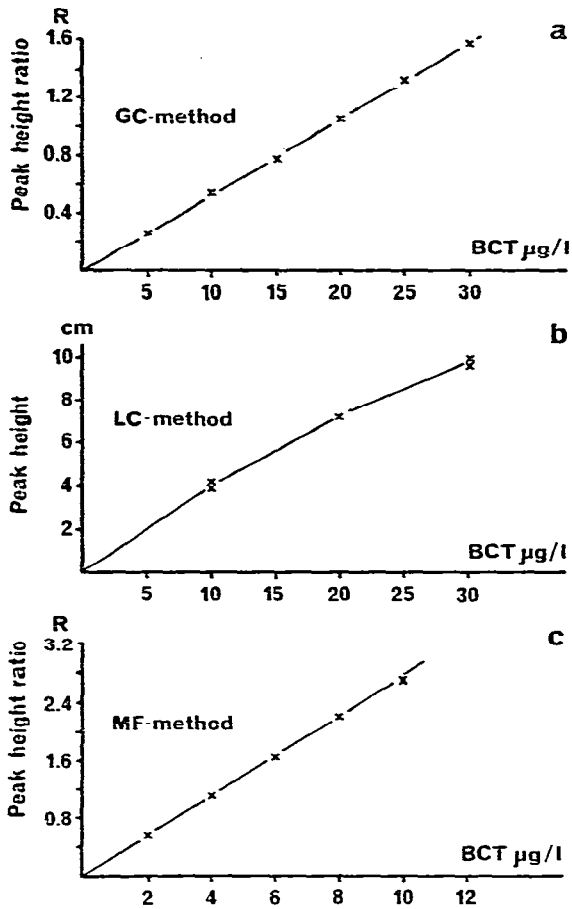

Fig. 2. Calibration graphs. Ordinate: (a) GC, the ratio (*R*) of the peak height of BCT to that of DHEC; (b) LC, the peak height of BCT in centimetres; (c) MF, the ratio (*R*) of the peak height of BCT to that of EGT.

TABLE I

ACCURACY OF BROMOCRIPTINE DETERMINATIONS

Method	*Concentration added (µg/l)*	*Number of samples*	*Calculated mean concentration (µg/l)*	*Coefficient of variation (%)*
GC	1.00	10	1.02	6.8
	5.00	10	4.95	1.8
	10.00	10	9.91	1.0
	20.00	10	20.15	0.6
	30.00	10	30.11	0.6
MF	2.00	10	2.01	2.0
	4.00	10	3.98	2.3
	6.00	10	6.02	0.5
	8.00	10	8.00	0.3
	10.00	10	10.01	0.6
LC	10.0	10	10.0	5.4
	20.0	10	19.9	6.0
	30.0	6	29.9	3.0

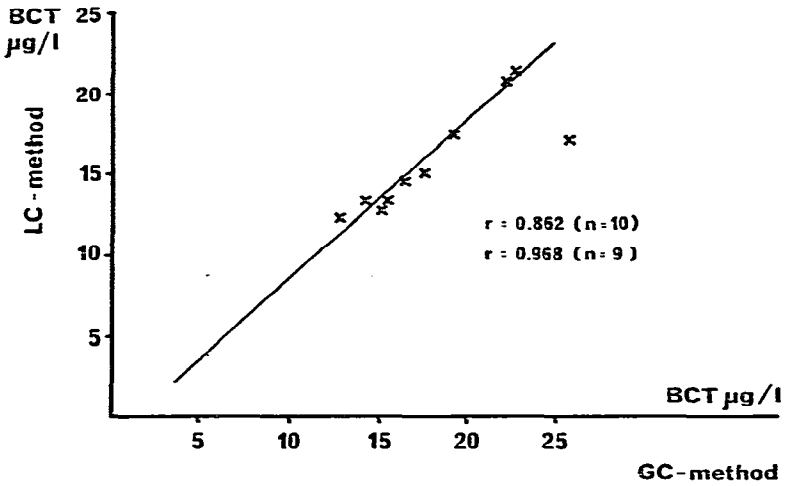

Fig. 3. Correlation between the GC and LC procedures. Ten plasma samples measured by each assay.

regarded ($n = 9$), a correlation coefficient of 0.968 was obtained. In order to verify further the specificity of the GC assay, another ten plasma samples from BCT-treated patients were analysed both by GC and MF. The selected fragments in the MF method were $m/e = 308$ and 314 for BCT and EGT, respectively. As demonstrated in Fig. 4, they produced identical results ($r = 0.996$) for the concentration range 1.5–8.3 μg/l.

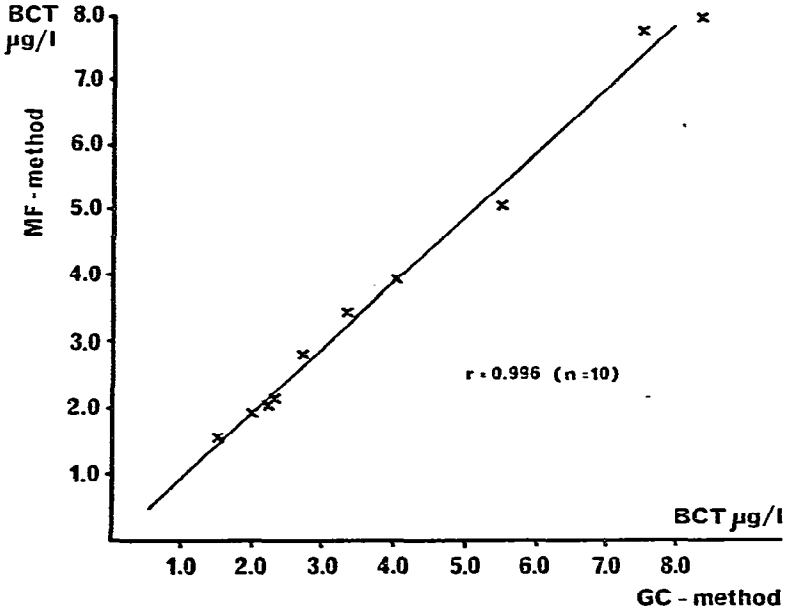

Fig. 4. Correlation between the GC and MF procedures. Ten plasma samples measured by each assay.

The GC method was also compared with a radioimmunoassay (RIA) method developed by Price *et al.*, and ten plasma samples were analysed by both methods. The comparison revealed some deviations, particularly for concentrations exceeding 4–5 μg/l. This might be due to the fact that in the RIA method different plasma was used for the calibration and for the measurements (Fig. 5).

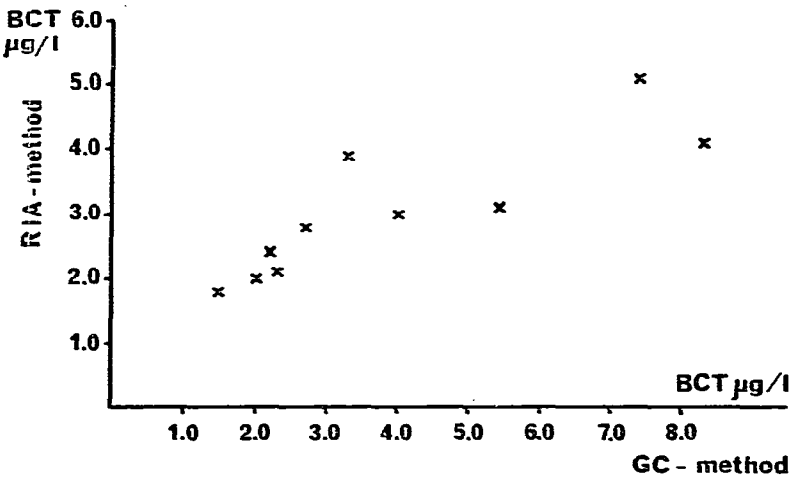

Fig. 5. Relationship between the GC procedure and a radioimmunoassay (RIA) method. Ten plasma samples measured by each assay.

DISCUSSION

The development of the three different assays for BCT in plasma revealed some interesting data. The retention time of BCT in the GC method, using a column temperature of 245°, is only 2 min (Fig. 1). This is surprisingly short considering the molecular structure of BCT, which is a tripeptide alkaloid with a molecular weight of 654.5. α-Ergocriptine demonstrated, under identical conditions, an identical retention time in spite of the lack of a bromine atom. This strongly indicates cleavage of the BCT molecule when exposed to the column temperature. Therefore, the peak representing BCT on the chromatogram is probably only the tripeptide moeity. Such a rearrangement would explain the apparently short retention time of BCT. Injection of EGT revealed a much longer retention time compared with that of BCT, which supports the above theory of the cleavage of BCT. For the MF method the fragments $m/e = 308$ and 314 for BCT and EGT, respectively, were selected. These fragments correspond exactly to the tripeptide moiety for the two drugs. Further, the mass spectrum of BCT did not indicate the presence of a bromine atom, with equal intensity for M and M + 2.

None of the three methods can be claimed to be superior to the others when concentrations above 10 μg/l have to be measured, which are relevant to plasma levels (therapeutic levels) seen during BCT treatment in Parkinson's disease[7]. Each method has particular advantages, and the best choice depends on the specific use and on the facilities and experience of the laboratory. As the results of these three assays are comparable, the outcome of different investigations can be compared.

Figs. 3 and 4 show that in our hands these analytical procedures give almost identical results when the assays are applied to the same plasma samples. The RIA method is incomparable to the others as the method does not seem to be fully developed and further comparisons have to be done. As investigations carried out with different methods that do not give comparable results are bound to cause confusion, inter-laboratory comparisons of methods for drugs determinations are desirable, especially when assays are being developed.

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