Journal of Chromatography, 273 (1983) 453—457
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1554

Note

Determination of metoclopramide in human plasma by high-performance liquid chromatography

G.B. BISHOP-FREUDLING and H. VERGIN*

Biochemical Department, Heumann-Pharma, P.O. Box 2260, D-8500 Nürnberg (G.F.R.)

(First received June 18th, 1982; revised manuscript received October 14th, 1982)

Metoclopramide (4-amino-5-chloro-2-methoxy-N-2-diethyl-aminoethylbenzamide) is recommended for use in gastrointestinal diagnostics and in treating various types of vomiting and a variety of functional gastrointestinal disorders. The drug increases the motility of the stomach and gastric-emptying rates. It may provide symptomatic relief in dyspepsia and possibly in vertigo and reflux oesophagitis [1, 2]. Behavioural studies in animals suggest that metoclopramide is a central dopaminergic antagonist exerting its anti-emetic effects by blocking the chemoreceptor trigger zone [3]. When given orally metoclopramide is readily absorbed but shows a wide range in bioavailability which might be due to interindividual first-pass metabolism [4].

Quantitative thin-layer chromatographic methods for the determination of metoclopramide in human plasma are described by Schuppan et al. [5] for the 40—320 ng/ml range and by Berner et al. [6] up to a detection limit of about 4 ng/ml serum using an improved extraction procedure. An electron-capture gas chromatographic assay of the drug in plasma was developed by Ross-Lee et al. [7]. This analytical technique provides sufficient specificity and sensitivity up to a detection limit of 5 ng/ml plasma.

A high-performance liquid chromatographic (HPLC) assay for the analysis of the drug in serum has been published by Block and Pingoud [8]. The procedure, however, requires large sample volumes of 10 ml of whole blood, which are not possible to obtain from patients for routine use in monitoring plasma metoclopramide levels in a clinical laboratory.

This paper describes a newly developed HPLC method which requires only small volumes of plasma (2 ml), and assesses its accuracy, sensitivity and reproducibility in the determination of plasma metoclopramide concentrations.

0378-4347/83/0000—0000/$03.00

EXPERIMENTAL

Materials

All chemicals and solvents were of at least p.a. quality, and water was glass-distilled; all were prefiltered using a GV 100/1 glass filtration apparatus (Ref. No. 392700) and filter-discs, RC 58, 0.2 μm (Ref. No. 371628) both from Schleicher and Schüll (Dassel, G.F.R.). Chloroform, sodium hydroxide, methanol and acetic acid were obtained from E. Merck (Darmstadt, G.F.R.) and acetonitrile HPLC grade S from Rathburn Chemicals (Walkerburn, Great Britain). For preparing standard curves, metoclopramide hydrochloride monohydrate BP 80, charge No. 07581 was used (Heumann-Pharma, Analytical Department).

Extraction procedure

A 2-ml aliquot of plasma was added to 20-ml Sovirel culture test tubes. Sodium hydroxide (1 *N*) was then added up to 20 ml final volume. The resulting solution was carefully mixed and then applied to an Extrelut® column (Merck) of 20 ml bed-volume. Metoclopramide was eluted by rinsing the column with 70 ml of chloroform. The organic eluate was evaporated to dryness. The residue was dissolved in 4 ml of methanol by ultrasonification (Bransonic 221, Branson Europa, Soest, The Netherlands). The solution was transferred to 15-ml Pyrex/Sovirel test tubes and evaporated to dryness again. After redissolving the residue in 0.1 ml of 1% acetic acid, the solution was placed in small reaction vessels of 1.5 ml volume, centrifuged at 6400 *g* (Eppendorf 5412, Eppendorf, Hamburg, G.F.R.) and 10-μl aliquots of the clear supernatant were injected into the chromatograph.

Instrumentation and chromatography

The chromatographic system consisted of a Hewlett-Packard liquid chromatograph 1084 A. A Schoeffel variable-wavelength UV detector SF 770 (Kratos, Karlsruhe, G.F.R.) monitored the effluent at 273 nm. The mobile phase of 32% acetic acid (1%) and of 68% acetonitrile—methanol (3.7:1, v/v) was pumped through a Nucleosil C_{18}, 250 × 4.6 mm, 5 μm particle size, column at a flow-rate of 1.5 ml/min (Macherey and Nagel, Düren, G.F.R.). As a guard column a hyperchrome precolumn cartridge (Bischoff, Analysentechnik, Leonberg, G.F.R.) packed with the same material as the analytical column was used. Chromatography was performed at 50°C.

Calculations

A series of calibration experiments was carried out for the quantitative determination of metoclopramide. Defined amounts (10, 20, 40, 80, 100, 200 and 400 ng/ml) of metoclopramide were added to drug-free human plasma and processed as above. Standard curves were generated daily by plotting peak areas against the known metoclopramide concentrations. The stability of samples was tested from spiked human plasma and from samples obtained after oral metoclopramide administration to healthy subjects. The samples were stored deep-frozen for three months.

RESULTS

Typical chromatograms for blank plasma and for a plasma sample obtained from a volunteer 0.5 h after taking a single oral dose of 20 mg of metoclopramide hydrochloride are presented in Fig. 1. The total elution time per assay is 8 min. As can be seen from Fig. 1A, no plasma constituent peak extracted from the blank interferes with that of metoclopramide; there is merely an increased noise level present, which is well below our detection limit. The plasma standard curves were linear over the range of 10—400 ng/ml. The limit of detection of the method was 8 ng/ml when 2 ml of plasma were used. The between-day precision of the method, expressed as coefficient of variation (C.V.) ranged from 3.8 to 5.8% over the concentration range of 10—400 ng/ml, while the within-day precision ranged from 2.4 to 4.1% over the same concentrations chosen (Table I). The chromatographic procedure is reproducible with retention time of 5.60—5.64 for metoclopramide. This, and the fact that the compound is not destroyed in the columns, make it possible to determine metoclopramide without the use of an internal standard.

Total recovery of metoclopramide was 89.9 ± 1.9% ($\bar{x}$ ± S.D., n = 28) for plasma. Frozen plasma samples remained stable for at least three months.

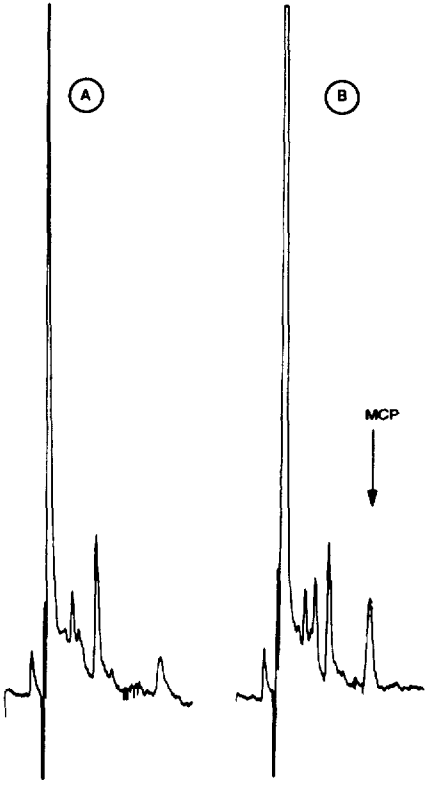

Fig. 1. Chromatograms of extracts from (A) drug-free control plasma and (B) plasma sample obtained from a healthy volunteer 0.5 h after taking a single oral dose of 20 mg of metoclopramide hydrochloride; this corresponds to 24 ng/ml.

TABLE I

VARIATION IN THE ASSAY OF PLASMA METOCLOPRAMIDE

Drug concentration (ng/ml)	Between-day		Within-day	
	n	C.V. (%)	*n*	C.V. (%)
10	8	5.2	5	3.1
20	10	4.7	5	2.9
40	8	5.8	5	3.6
80	6	3.9	4	2.7
100	6	4.4	4	2.4
200	5	4.1	4	4.1
400	5	3.8	4	3.1

DISCUSSION

The wide range in bioavailability makes metoclopramide a difficult drug to use orally. A clinically applicable and rapid procedure for the determination of metoclopramide in small plasma specimens would allow detailed pharmacokinetic studies to be carried out. The assay described in this paper has proved sufficiently sensitive and reliable for research and clinical use. As an example, the results of a bioequivalence study in volunteers who had received single doses of 20 mg of metoclopramide hydrochloride of two different liquid formulations in a cross-over design are shown in Fig. 2. Plasma levels of the unchanged drug and the main pharmacokinetic parameters, half-life of elimination and volume of distribution were in correlation with the literature values [9].

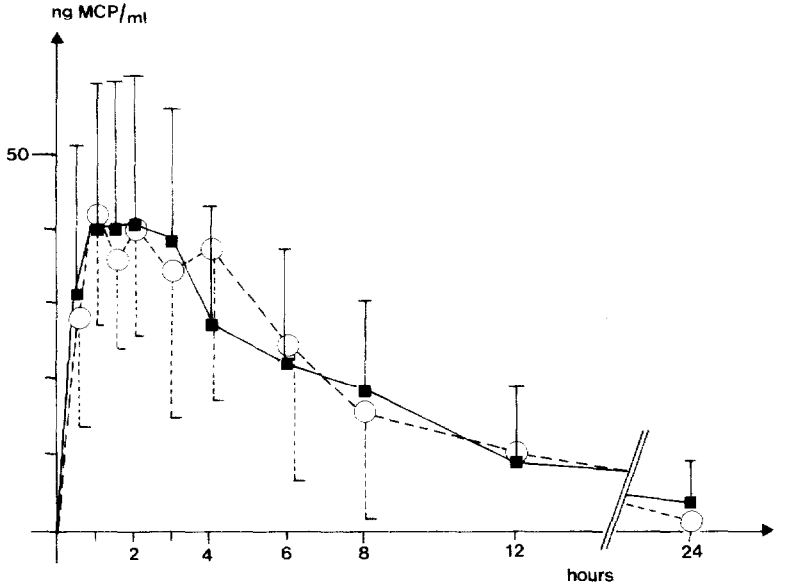

Fig. 2. Metoclopramide (MCP) concentrations in plasma from ten healthy volunteers after oral administration of two different liquid formulations of 20 mg of MCP in a cross-over design. (■), Test formulation; (○) standard formulation.

With respect to sensitivity, the gas chromatographic method of Ross-Lee et al. [7] for determining metoclopramide in serum is slightly more sensitive with a detection limit of 5 ng/ml compared to a detection limit of 8 ng/ml in the HPLC assay presented here. However, the total sample clean-up requires at least 7 h due to a multitude of diverse extraction, reextraction and centrifugation steps. The HPLC method described here involves a single extraction procedure followed by a twofold dissolution and centrifugation step. Therefore the GC assay does not appear to be a suitable method for routine drug monitoring in the clinical laboratory.

Due to the selectivity of the C_{18} 5-μm material used in this investigation, a separation of the drug from the plasma constituents was possible without an additional acidic extraction step as described in the HPLC assay of Block and Pingoud [8].

The present HPLC method demonstrates sensitivity and reproducibility over a wider concentration range than the previous assay mentioned [8]. Our procedure can be recommended for routine drug monitoring and would allow pharmacokinetic studies especially in, for example, patients with renal and/or hepatic failure and infants in whom sampling of large blood volumes would be deleterious.

REFERENCES

1 M. Eisner, Digest. Dis., 16 (1974) 409.
2 A.G. Johnson, Postgrad. Med. J., 49 (Suppl. 4) (1973) 29.
3 O.P.W. Robinson, Postgrad. Med. J., 49 (Suppl. 4) (1973) 77.
4 D.N. Bateman and D.S. Davies, Lancet, i (1979) 166.
5 D. Schuppan, I. Schmidt and M. Heller, Arzneim.-Forsch., 29 (1979) 151.
6 G. Berner, B. Emschermann, E. Gladtke, W. Haase, F. Leuschner, U. Vögtle-Junkert and H.H. Wagener, Arzneim.-Forsch., 32 (1982) 169.
7 L.M. Ross-Lee, M.J. Eadie, F. Bochner, W.D. Hooper and J.H. Tyrer, J. Chromatogr., 183 (1980) 175.
8 W. Block and A. Pingoud, Z. Anal. Chem., 301 (1980) 109.
9 W. Block, A. Pingoud, M. Khan and P. Kjellerup, Arzneim.-Forsch., 31 (1981) 1041.