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DETERMINATION OF BROMOPRIDE IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Bromopride was measured in plasma and urine using reversed-phase high-performance liquid chromatography employing ultraviolet absorption detection. The limit of detection in plasma was 2 ng/ml, sufficient for pharmacokinetic studies of the drug. Plasma concentrations of bromopride reached mean peak levels (55 ng/ml) at 1 h after single oral doses of 20 mg and declined with a half-life of 4.9 h. Less than a mean of 10% of an oral dose was excreted unchanged in the urine. The assay could also be used to measure metoclopramide in these bio-fluids.

INTRODUCTION

Bromopride [4-amino-5-bromo-N-(2-diethylaminoethyl)-2-methoxybenzamide], Viaben® (Fig. 1) is an anti-emetic drug which has a selective effect on the basal motility of the gastro-intestinal tract and is used in the treatment of certain gastro-intestinal tract diseases [1, 2]. It is the bromo-analogue of metoclopramide.

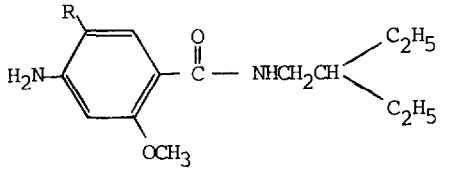

Fig. 1. Chemical structure of bromopride (R = Br) and metoclopramide (R = Cl).

Until recently, few analytical methods were available of sufficient specificity and sensitivity to measure these compounds in biological fluids. Metoclopramide has been measured by gas—liquid chromatography (GLC)

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[3, 4] as a heptafluorobutyryl derivative with a limit of detection of 5 ng/ml, and by high-performance liquid chromatography (HPLC) in an adsorption mode [5] and in a reversed-phase mode [6—8] with limits of detection of 8—10 ng/ml.

Bromopride has also been measured by GLC [9] as a heptafluorobutyryl derivative and by HPLC [9, 10] using gradient elution with limits of detection of 5—10 ng/ml. Under isocratic reversed-phase HPLC conditions, bromopride and metoclopramide elute as broad tailing peaks (Fig. 2).

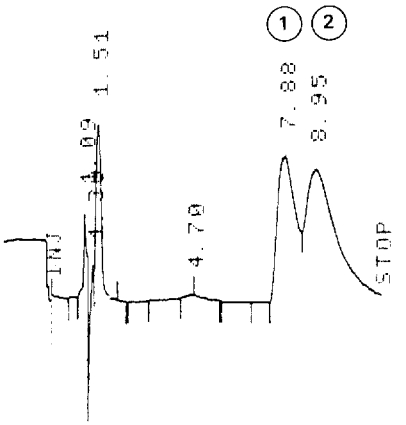

Fig. 2. Chromatogram of metoclopramide (1) and bromopride (2) under reversed-phase conditions in the absence of alkylammonium ions. Chromatographic conditions: column: μBondapak C_{18} (30 cm × 0.39 cm I.D.); flow-rate: 2 ml/min; solvent system: 40% (v/v) acetonitrile in phosphate buffer (0.1%, pH 7.5); detector: ultraviolet absorption (275 nm).

This paper describes a newly developed HPLC procedure for the measurement of bromopride under isocratic reversed-phase conditions using alkylammonium ions in the mobile phase to modify retention. Sokolowski and Wahlund [11] have previously shown that alkylammonium ions added to the mobile phase compete with sample ammonium ions for adsorption sites on the stationary phase, thus eliminating peak tailing and decreasing the retention of hydrophobic ammonium compounds under reversed-phase conditions. Metoclopramide is used as an internal standard and bromopride concentrations as low as 2 ng/ml can be detected. The method could also be used for the measurement of metoclopramide in plasma and urine.

EXPERIMENTAL

Materials and reagents

All reagents were of analytical grade and all inorganic reagents were prepared in freshly glass-distilled water. Acetonitrile was HPLC—far UV grade (Fisons Scientific Apparatus, Loughborough, U.K.). N,N-Dimethyl-3-chloropropylamine hydrochloride was obtained from Koch-Light Labs. (Colnbook, U.K.). Bromopride base and metoclopramide hydrochloride, used as internal standard, were supplied by Delagrange International (Paris, France), as were potential metabolites of bromopride, bromopride N-oxide, monodesethylbromopride and 4-amino-5-bromo-2-methoxybenzoic acid.

Standard solutions of bromopride base and metoclopramide hydrochloride were prepared at a concentration of 1 mg/ml in acetonitrile. Stock solutions were further diluted with acetonitrile to give concentrations of bromopride of 10 μg/ml and 1 μg/ml and metoclopramide of 10 μg/ml. All standard solutions were stored at 4°C in the dark under which conditions they were stable for several weeks.

Sample preparation procedures

Plasma samples (2 ml) or urine samples (200 μl) were transferred into conical centrifuge tubes and mixed with internal standard solution containing 60 ng metoclopramide hydrochloride for plasma samples or 120 ng for urine samples. The samples were extracted twice with diethyl ether (5 ml, freshly redistilled) in the presence of 0.5 *M* sodium borate buffer (1 ml, pH 10.5) using a rotary mixer for 10 min. The ether layers were separated after centrifugation, combined and extracted with 0.1 *M* hydrochloric acid. The samples were again mixed for 10 min, centrifuged and the diethyl ether layer was removed and discarded. The acid layers were then made alkaline by the addition of 0.5 *M* borate buffer (1 ml) and re-extracted with diethyl ether (5 ml). After mixing and centrifugation, the diethyl ether layers were carefully transferred to conical tubes and evaporated to dryness at 37°C under a stream of nitrogen. The residues were washed to the bottom of the tubes with a small volume of diethyl ether which was also evaporated to dryness.

The residues were dissolved in mobile phase (100 μl) and injected into the liquid chromatograph.

Calibration procedures

Samples of control (drug-free) plasma (2 ml) were mixed with bromopride at concentrations equivalent to 2, 5, 10, 20, 40, 50, 80 and 110 ng/ml and with internal standard at a fixed concentration of 30 ng/ml.

Samples of control (pre-dose) urine (200 μl) were mixed with bromopride at concentrations equivalent to 20, 50, 100, 200, 400 and 500 ng/ml and with internal standard at a fixed concentration of 600 ng/ml.

When concentrations of bromopride in urine exceeded 500 ng/ml, samples were diluted with control urine before measurement.

All calibration standards were submitted to the sample preparation procedures previously described.

Instrumentation

The liquid chromatograph consisted of a M6000A pump and WISP™ autosampler (Waters Assoc., Cheshire, U.K.) coupled to an LC-3 variable-wavelength detector (Pye Unicam, Cambridge, U.K.) operated at 275 nm and 0.01 a.u.f.s. Chromatograms were recorded on a 3380A computing integrator (Hewlett-Packard, Hitchin, U.K.) using an attenuation of 8 for plasma samples and 16 for urine samples. Peak height measurements were performed manually.

Chromatography

Chromatography was performed in a reversed-phase mode with alkylammonium ions in the mobile phase to act as a modifier of the retention of bromopride and internal standard (metoclopramide).

The mobile phase consisted of 40% (v/v) acetonitrile in phosphate buffer (0.1%, w/v) containing N,N-dimethyl-3-chloropropylamine hydrochloride (0.12%, w/v); the final pH was adjusted to 7.9 using 4 *M* sodium hydroxide solution.

The column used for the analysis was constructed of stainless steel (25 cm × 0.46 cm I.D.) and packed with Zorbax® C_8 (mean particle diameter 6 μm) (Dupont, Stevenage, U.K.). A pre-column (7 cm × 0.2 cm I.D.) constructed of stainless steel and dry-packed with pellicular Co:Pell® ODS (particle diameter 24—37 μm) (Whatman, Maidstone, U.K.) was installed in series in front of the analytical column to protect it from contamination. A mobile phase flow-rate

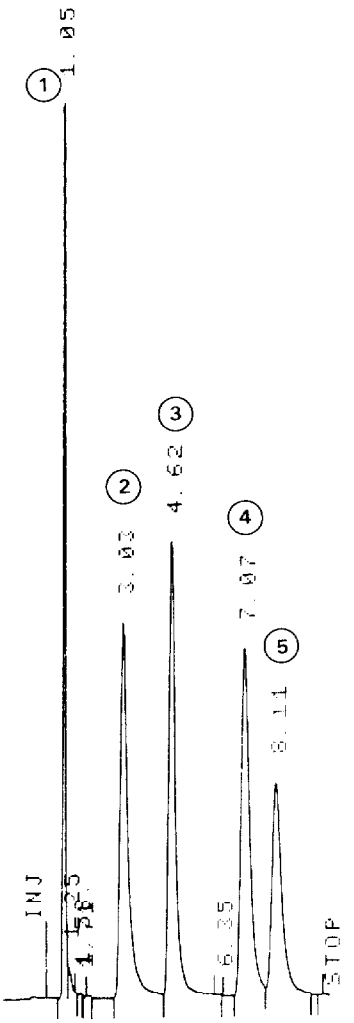

Fig. 3. Chromatogram of bromopride and its metabolites. Peaks: 1 = 4-amino-5-bromo-2-methoxybenzoic acid; 2 = bromopride N-oxide; 3 = N-desethylbromopride; 4 = metoclopramide (internal standard); 5 = bromopride. Chromatographic conditions: column: Zorbax C_8 (25 cm × 0.46 cm I.D.); flow-rate: 2.2 ml/min; solvent system: 40% (v/v) acetonitrile in phosphate buffer (0.1%, w/v) containing N,N-dimethyl-3-chloropropylamine hydrochloride (0.12%, w/v) pH 7.9; detector: ultraviolet absorption (275 nm), 0.01 a.u.f.s.

was adjusted between 2.2 and 2.7 ml/min to give retention times for bromopride and metoclopramide of approximately 8 and 7 min, respectively. Potential metabolites of bromopride, 4-amino-5-bromo-2-methoxybenzoic acid, N-desethylbromopride and bromopride N-oxide, were well resolved and did not interfere in the chromatography (Fig. 3).

Studies in human subjects

Plasma and urine samples were obtained from six human volunteer subjects dosed orally with 20 mg of bromopride as a solution formulation and analysed by the foregoing procedures. These volunteer studies were conducted under conditions similar to those described by Brodie et al. [12].

TABLE I

BETWEEN-ASSAY PRECISION MEASUREMENTS OF BROMOPRIDE IN PLASMA

Concentration of bromopride (ng/ml)	Peak height ratio ($\frac{\text{bromopride}}{\text{internal standard}}$)							Mean (± S.D.)	C.V. (%)
2	0.06	0.10	0.09	0.05	0.06	0.07	0.06	0.07 (0.02)	29
5	0.13	0.19	0.15	0.14	0.13	0.15	0.17	0.15 (0.02)	13
10	0.29	0.28	0.27	0.22	0.26	0.28	0.26	0.27 (0.02)	7
20	0.59	0.47	0.56	0.51	0.58	0.57	0.49	0.54 (0.05)	9
40	1.12	1.18	1.13	1.11	1.11	1.14	1.20	1.14 (0.04)	4
50	1.28	1.33	1.39	1.42	1.30	1.40	1.49	1.37 (0.07)	5
80	2.26	2.30	2.35	2.38	2.38	2.41	2.23	2.33 (0.07)	3
110	3.26	3.23	3.19	3.22	3.33	3.18	3.29	3.24 (0.05)	2

TABLE II

BETWEEN-ASSAY PRECISION MEASUREMENTS OF BROMOPRIDE IN URINE

Concentration of bromopride (ng/ml)	Peak height ratio ($\frac{\text{bromopride}}{\text{internal standard}}$)						Mean (± S.D.)	C.V. (%)
20	0.03	0.03	0.04	0.04	0.05	0.04	0.04 (0.008)	20
50	0.08	0.09	0.07	0.09	0.09	0.07	0.08 (0.010)	13
100	0.15	0.14	0.14	0.15	0.17	0.16	0.15 (0.012)	8
200	0.28	0.30	0.28	0.32	0.27	0.27	0.29 (0.020)	7
400	0.57	0.56	0.53	0.55	0.61	—	0.56 (0.030)	5
500	0.71	0.70	0.68	0.68	0.77	0.77	0.72 (0.042)	6

RESULTS AND DISCUSSION

Precision and accuracy of measurement

Extraction and measurement were repeated on seven occasions at each concentration over the selected calibration range in plasma and on six occasions at each concentration in urine. The between-assay precision of the method, as indicated by the coefficient of variation (C.V.) of peak height ratio measurements of drug to internal standard ranged from ± 29% at 2 ng/ml to ± 2% at 110 ng/ml for plasma samples (Table I) and from ± 20% at 20 ng/ml to ± 6% at 500 ng/ml for urine samples (Table II).

The calibration line for bromopride in plasma constructed from seven replicate measurements at eight concentrations was linear ($Y = 0.0293 - 0.020X$, $r = 0.999$) as was that for bromopride in urine, constructed from six replicate measurements at six concentrations ($Y = 0.0014X - 0.009$, $r = 0.996$). The accuracy of the method as indicated by the standard errors of the fitted least-squares regression lines was ± 2.5 ng/ml for the plasma line and ± 23 ng/ml for the urine line.

Recovery

The recoveries (extraction efficiencies) of bromopride and internal standard from plasma were 82 ± 4% S.D. and 77 ± 6% S.D., respectively, and from urine were 77 ± 2% S.D. and 82 ± 5% S.D., respectively. The bromopride concentration ranges studied were 10—50 ng/ml in plasma and 50—500 ng/ml in urine.

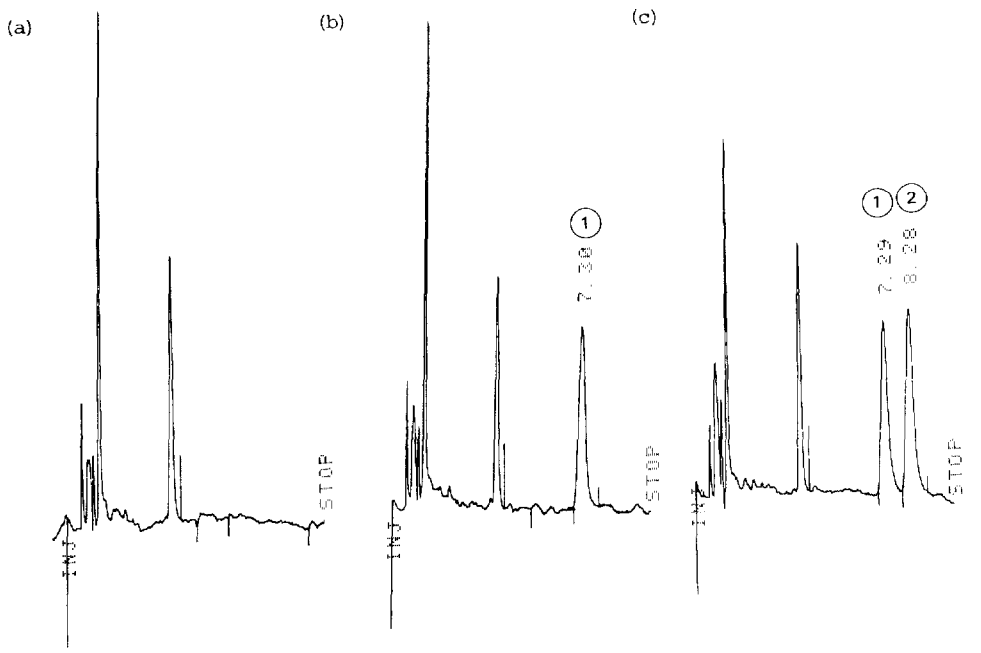

Fig. 4. Chromatograms of (a) pre-dose plasma; (b) pre-dose plasma containing added internal standard; and (c) 2-h post-dose plasma containing bromopride at a concentration of 36 ng/ml. Peaks: 1 = internal standard; 2 = bromopride. Conditions as described for Fig. 3.

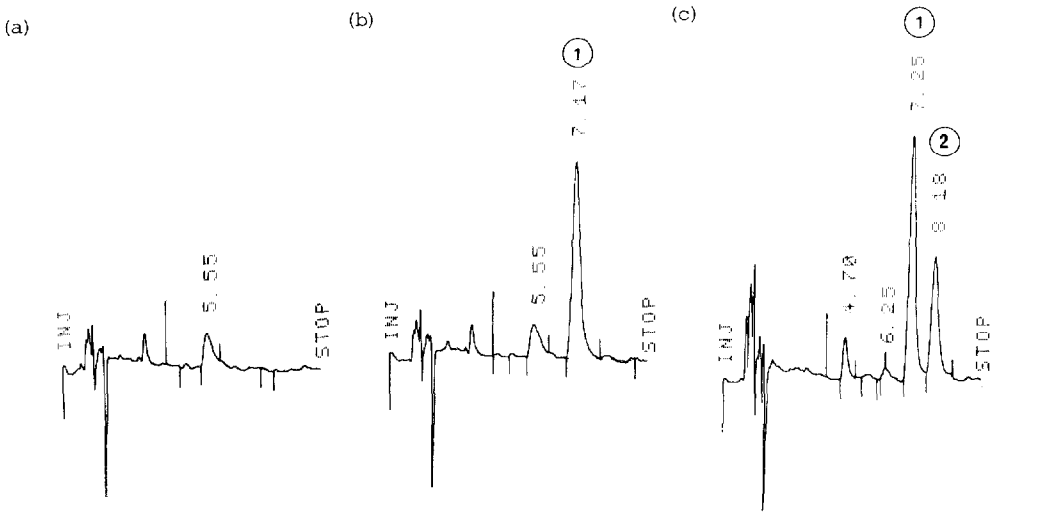

Fig. 5. Chromatograms of (a) pre-dose urine; (b) pre-dose urine with internal standard; and (c) urine collected during 0.5—1 h after dosing containing bromopride at a concentration of 336 ng/ml. Peaks: 1 = internal standard; 2 = bromopride. Conditions as described for Fig. 3.

Selectivity and limit of detection

No interfering peaks with the same retention times as bromopride or metoclopramide were present in pre-dose plasma (Fig. 4) or urine (Fig. 5) samples. Potential metabolites of bromopride were well resolved; however, 4-amino-5-bromo-2-methoxybenzoic acid and bromopride N-oxide were not extracted under the conditions used. N-Desethylbromopride was not detected in plasma samples contrary to data published elsewhere [9].

TABLE III

MEAN PLASMA BROMOPRIDE CONCENTRATIONS IN SIX HUMAN SUBJECTS AFTER SINGLE ORAL DOSES OF 20 mg IN SOLUTION

Time after dosing (h)	Concentration of bromopride (ng/ml ± S.D.)
0.5	32 ± 21
0.75	51 ± 28
1	55 ± 27
1.5	53 ± 30
2	54 ± 23
2.5	49 ± 22
3	47 ± 20
3.5	41 ± 20
4	38 ± 19
5	33 ± 17
6	29 ± 14
8	23 ± 14
10	16 ± 11
12	13 ± 9
14	9 ± 6
24	4 ± 4

The lowest calibration standard of 2 ng/ml was taken as the limit of detection in plasma and this represented a signal-to-noise ratio of 2:1.

This present bromopride assay procedure is more sensitive and simpler than previously published procedures, in that no derivatisation stage is required in the assay, and chromatography can be performed under isocratic conditions, which allows a greater throughput of samples to be analysed in a given time.

Concentrations of bromopride in human plasma and urine

After single oral doses of 20 mg of bromopride administered as a solution to six healthy human subjects, a peak of mean plasma concentrations of 55 ng/ml was reached at 1 h after dosing (Table III). After reaching peak levels, plasma concentrations declined apparently monoexponentially with a mean half-life of 4.9 ± 0.7 h S.D. A total of 1.87 ± 0.86 mg S.D. (9.4% dose) of bromopride was excreted unchanged in urine during 48 h after dosing.

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