ELSEVIER

Journal of Chromatography B, 665 (1995) 117–123

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Determination of berberine in plasma, urine and bile by high-performance liquid chromatography

Chi-Ming Chen*, Horng-Chih Chang

Department of Medicinal Chemistry, School of Pharmacy, Taipei Medical College, 250 Wu-Hsing Street, Taipei, 110, Taiwan

First received 17 August 1994; revised manuscript received 13 October 1994; accepted 1 November 1994

Abstract

A high-performance liquid chromatographic method for the determination of berberine in plasma, urine and bile samples is described. Plasma samples were pretreated by protein precipitation with acetonitrile and urine and bile samples were pretreated by organic solvent extraction using 5% 2-propanol in methylene chloride. Berberine was determined in all samples using an octyl reversed-phase column with a mobile phase of 60–63% acetonitrile in 0.1% phosphoric acid (pH 6.0) and with UV detection at 267 nm. The detection limits for berberine in plasma, urine and bile were 18.1 ng/ml, 2.3 ng/ml and 90.4 ng/ml, respectively. The recoveries of berberine by simple deproteinization of plasma and by solvent extraction of urine were 78.3 and 82.9%, respectively. The intra-day and inter-day accuracy and precision for plasma reported as coefficients of variation and relative errors were both less then 6%. The applicability of the assay to pharmacokinetic and bioavailability studies was demonstrated by the determination of berberine in plasma, urine and bile after intravenous and intramuscular administration to rabbits at a dose of 2 mg/kg.

1. Introduction

Berberine (Fig. 1), a quarternary protoberberine-type alkaloid, is widely distributed in nature [1]. Berberine and its preparations have been used intensively by orientals as intestinal antiseptics [2], by oral and parenteral administration. The commercially available berberine sulfate injection [3] in Japan and Taiwan is used for intestinal infection, when the patient is incapable of accepting this drug orally. Other systemic effects, such as hypotensive [4], bilirubin excretion enhancing [5], inotropic [6], sedative [4] and anti-inflammatory [7] effects, have also been reported. The absorption and distribution of berberine have been studied in rats [8] and rabbits [9] by application of non-specific UV spectrophotometric and fluorimetric assays. Berberine absorption by the oral route in rats [10] and human subjects [11] using tritium-labelled berberine or by gas chromatography–chemical

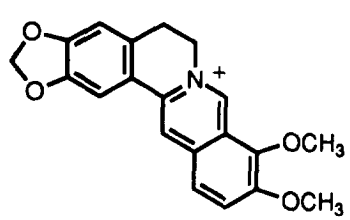

Fig. 1. Structure of berberine salt.

* Corresponding author.

0378-4347/95/$09.50
SSDI 0378-4347(94)00517-6

ionization mass spectrometry have been described. These methods, however, are not practical for the routine determination of berberine in biological samples. Further, no detailed pharmacokinetic information about berberine has been reported in the literature. In this paper, we report on the development of a specific and sensitive method to determine berberine in biological samples of plasma, urine and bile by high-performance liquid chromatography.

2. Experimental

2.1. Chemicals and reagents

Berberine chloride was purchased from Aldrich (Milwaukee, WI, USA). All organic solvents and reagents were of either LC or analytical-reagent grade. Water was produced using a Milli-Q reagent water system (Millipore). Stopin Injection (berberine sulfate; 0.25%, 2 ml) was supplied by Kyorin Pharmaceutical (Taipei, Taiwan).

2.2. Instrumentation and chromatographic system

The HPLC system consisted of a Model 110A pump (Beckman, Fullerton, CA, USA), a Model 638-41 UV monitor (Hitachi, Tokyo, Japan), a Rheodyne Model 7125 injector and a Model D5000 recorder (Omniscribe, Austin, TX, USA). The stationary phase utilized an octyl column (5 μm, 250 × 4.6 mm I.D.) (Altex, Berkeley, CA, USA). The mobile phase was prepared by mixing 60–63% acetonitrile in 0.1% phosphoric acid solution and adjusting the pH to 6.0 using concentrated ammonia solution, which gave sharp peaks resolved from the other peaks. The chromatogram was monitored by UV detection at the maximum wavelength of 267 nm, with a sensitivity setting of 0.0025 AUFS.

2.3. Preparation of biological samples

Plasma samples

Plasma samples (0.2 ml) from rabbits were precipitated with 0.5 ml of acetonitrile containing levomepromazine maleate (0.5 μg) as an internal standard. After vortex mixing and centrifuging at 2000 *g*, the supernatant was evaporated to dryness under nitrogen. The residue was reconstituted with 0.2 ml of the mobile phase (63% acetonitrile in 0.1% H_3PO_4; pH 6.0) and an aliquot (20 μl) was injected into the HPLC system.

Urine samples

Rabbit urine samples (10 ml) were mixed with 2 μg of levomepromazine maleate as an internal standard. The solution was extracted twice with 5% 2-propanol in methylene chloride (10 ml). The organic layer was separated and evaporated to dryness under nitrogen. The residue was dissolved in 0.2 ml of the mobile phase (60% acetonitrile in 0.1% H_3PO_4; pH 6.0) and an aliquot (5 μl) was injected into the HPLC system.

Bile samples

Bile samples (0.5 ml) were mixed with levomepromazine maleate (3 μg) as an internal standard and dipotassium hydrogenphosphate buffer solution (pH 9.0) (2 ml). The solution was extracted with 5% 2-propanol in methylene chloride (2 ml). The organic layer was collected and evaporated to dryness under nitrogen. The residue was mixed with 0.2 ml of the mobile phase (60% acetonitrile in 0.1% H_3PO_4; pH 6.0) and a 20-μl aliquot was injected into the HPLC system.

2.4. Preparation of calibration graphs for berberine in biological samples

Plasma samples

Blank plasma samples (0.2 ml) were spiked with berberine chloride corresponding to berberine concentrations of 18.1, 45.2, 90.3, 181.0, 451.7 and 903.5 ng/ml. The spiked plasma samples were then treated as described in Section 2.3.

Urine samples

Blank urine samples (10 ml) were spiked with berberine chloride equivalent to concentrations of 2.3, 4.5, 9.0, 22.0 and 90.4 ng/ml. These

samples were then treated as described in Section 2.3.

Bile samples

Blank bile samples from rabbits (0.5 ml) were spiked with berberine chloride corresponding to concentrations of 90.4, 180.8, 481.6, 903.4 and 1810.0 ng/ml. These bile samples were then treated according as described in Section 2.3.

2.5. Administration of berberine by different routes to rabbits

Intravenous bolus administration

Berberine sulfate (2 mg/kg) was injected into the ear vein of healthy male albino rabbits weighing 2–3 kg. Blood samples were withdrawn at 1/24, 1/12, 1/8, 1/6, 1/4, 1/3, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h. The samples were spun at 2000 *g* to separate the plasma, which was then frozen until analysis. The urine samples were also collected 48 h after spiking.

Intramuscular administration

Berberine sulfate was injected intramuscularly into the upper legs of six rabbits at a dose of 2 mg/kg. Blood samples were collected at 1/24, 1/12, 1/4, 1/2, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h after injection. The whole blood was spun at 2000 *g* to separate the plasma, which was then frozen until analysis.

2.6. Biliary excretion of berberine after i.v. bolus administration to rabbits

Rabbits were anaesthetized with 30 mg/kg of pentobarbital and the common bile duct was cannulated with a polyethylene tube cannula. Blank samples of bile were then collected for 30 min. Berberine sulfate (2 mg/kg) was injected into the ear vein. Pentobarbital was given continuously to keep the animal unconscious. Bile samples were collected every 0.5 h for 5.5 h after injection. The bile samples were diluted with water to 5 ml and kept until analysis.

3. Results and Discussion

3.1. Stability of berberine in plasma

Spiked plasma samples containing 44.1, 164.6 and 516.3 ng/ml of berberine were stored at −10°C and analysed after various time intervals. No significant degradation of berberine was found during storage for a 2-week period.

3.2. Sample treatment

To eliminate interfering peaks, plasma samples were pretreated by simple precipitation of plasma proteins using acetonitrile. However, to eliminate the interfering peaks in urine and bile samples, these samples were purified by solvent extraction with 5% 2-propanol in methylene chloride before injection into the HPLC system. The chromatograms of berberine in plasma, urine and bile samples are shown in Figs. 2–4.

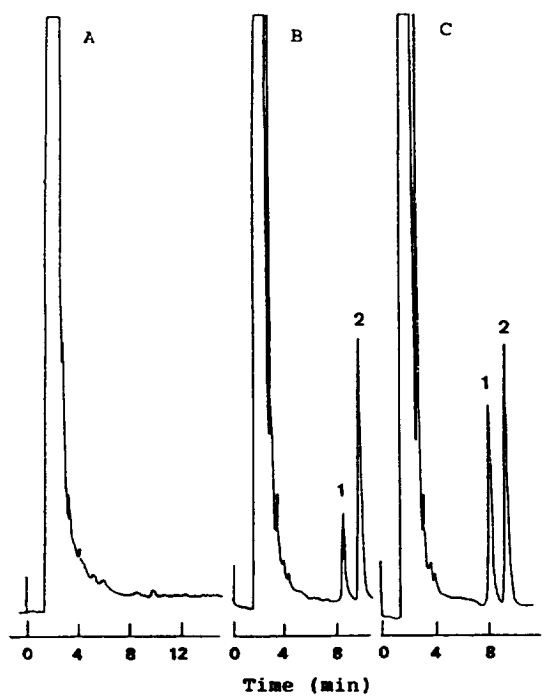

Fig. 2. Chromatograms of (A) drug-free plasma, (B) plasma spiked with 90.3 ng/ml of berberine and (C) berberine plasma sample (231.8 ng/ml) after an i.v. bolus administration of 2 mg/kg of berberine sulfate. Peaks: 1 = berberine; 2 = internal standard.

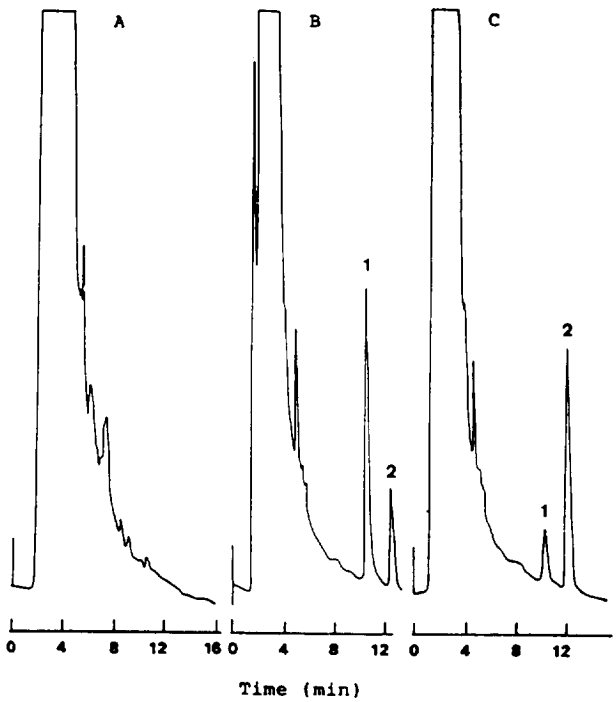

Fig. 3. Chromatograms of (A) drug-free urine, (B) urine spiked with 271.2 ng/ml of berberine and (C) berberine urine sample (22.6 ng/ml) after an i.v. bolus administration of 2 mg/kg of berberine sulfate. Peaks: 1 = berberine; 2 = internal standard.

3.3. Calibration graphs and assay precision and extraction recovery of berberine in biological samples

The calibration graph for plasma was linear over the range 18.1–903.5 ng/ml, using 0.2-ml samples, and could be expressed by the equation $y = 0.6519x - 0.0079$ ($r^2 = 0.9996$). The calibration graph for urine samples was linear over the range 2.3–90.4 ng/ml, using 10-ml urine samples, could be expressed by the equation $y = 0.0464x + 0.0001$ ($r^2 = 0.9999$). The calibration graph for bile samples was linear over the range 90.4–1810 ng/ml, using 0.5-ml bile samples, and could be expressed by the equation $y = 0.16514x - 0.0212$ ($r^2 = 0.9992$). The detection limits of berberine in plasma, urine and bile were 18.1 ng/ml, 2.3 ng/ml and 90.4 ng/ml, respectively.

The coefficient of variation (C.V.) and the relative error (R.E.) of the mean measured concentration served as measures of the accuracy and precision for validation of the assay procedure. The inter-day and intra-day assay precision and accuracy for plasma are summarized in Table 1. The C.V. and R.E. values were both less than 6%. The reproducibility of the assay meth-

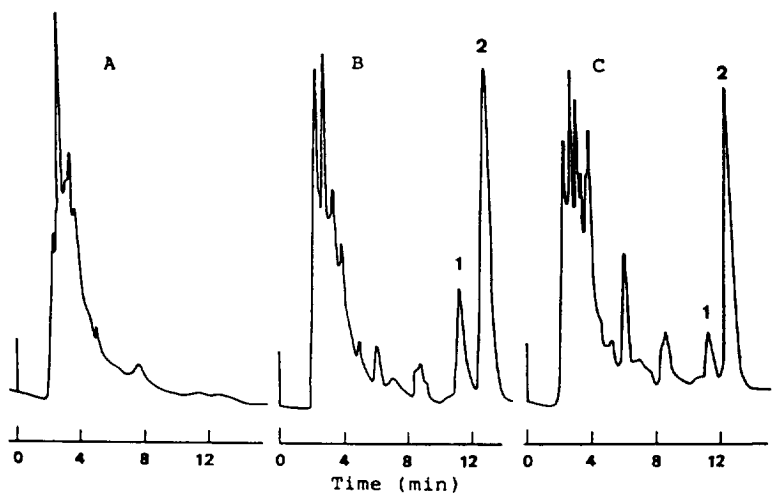

Fig. 4. Chromatograms of (A) drug-free bile, (B) bile spiked with 451.6 ng/ml of berberine and (C) berberine bile sample (223.2 ng/ml) after an i.v. bolus administration of 2 mg/kg of berberine sulfate. Peaks: 1 = berberine; 2 = internal standard.

Table 1
Precision and accuracy tests for berberine in rabbit plasma ($n = 5$)[a]

Spiked concentration (ng/ml)	Measured concentration (mean ± S.D.) (ng/ml)	C.V. (%)	R.E.[b] (%)
Inter-day			
26.9	28.3 ± 1.2	4.2	−5.20
179.1	178.9 ± 3.9	2.2	+0.11
716.5	714.6 ± 14.0	2.0	+2.65
Intra-day			
26.9	26.0 ± 1.7	6.5	+3.35
179.1	183.3 ± 13.4	7.6	−2.35
716.5	721.3 ± 13.3	1.8	−0.67

[a] Five analyses of the same samples at three different concentrations of berberine in rabbit plasma were performed.
[b] Relative error of the mean (%) = $\frac{\text{true concentration} - \text{mean measured concentration}}{\text{true concentration}} \cdot 100$.

od for plasma, urine and bile was assessed by calculating the variation of the regression slope from the calibration graph, which was prepared daily for each type of sample for five consecutive days. The C.V. for the regression slopes were 2.1, 3.8 and 6.7% for plasma, urine and bile samples, respectively. The average recovery of berberine by simple protein precipitation of plasma and by solvent extraction of urine were 78.3% and 82.9%, respectively.

3.4. Pharmacokinetic studies on rabbits

The assay method for berberine was then applied to pharmacokinetic studies on rabbits, using intravenous and intramuscular administration at a dose of 2 mg/kg. The mean plasma concentration of berberine versus time is plotted in Fig. 5. The plasma concentration as a function of time can be described by the equation

$$C = A\,e^{-\alpha t} + B\,e^{-\beta t}$$

where C is the plasma concentration of berberine at time t, A and B are constants related to the first-order distribution between central and peripheral compartments and α and β are exponents representing the distribution and elimination phases. The rate constants k_{12} and k_{21} for transfer between the central and peripheral compartments and the elimination rate constant k_{10} were calculated. The data could be expressed by a bi-exponential decline with a terminal elimination half-life [$t_{1/2(\beta)}$] of 5.28 h, a total plasma clearance (CL) of 5.64 l/h and an apparent volume distribution at the steady state (V_{ss}) of 38.30 l. The pharmacokinetic parameters of berberine, based on a two-compartmental open model, were calculated using the PCNONLIN

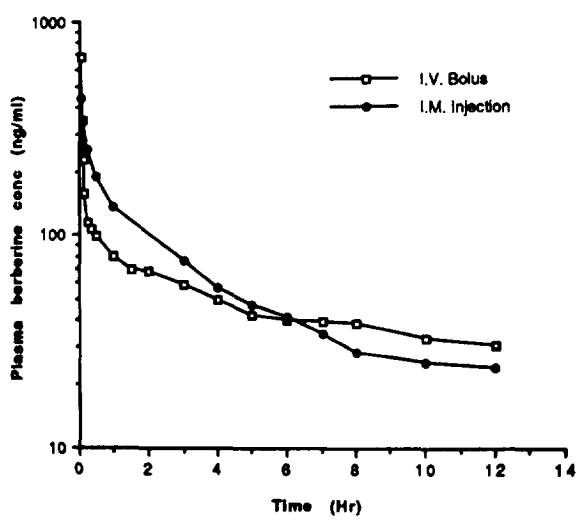

Fig. 5. Mean plasma berberine concentration versus time profile after (□) i.v. and (●) i.m. administration of a dose of 2 mg/kg to rabbits.

Table 2
Pharmacokinetic parameters of berberine following an intravenous dose of 2 mg/kg of berberine sulfate to six rabbits

Pharamacokinetic parameter	Value (mean ± S.D.)	Pharmacokinetic parameter	Value (mean ± S.D.)
A(μg/ml)	1.57 ± 1.62	CL(l/h)	5.46 ± 1.62
B(μg/ml)	0.10 ± 0.03	$k_{10}(h^{-1})$	1.75 ± 1.17
$\alpha(h^{-1})$	22.33 ± 11.04	$k_{12}(h^{-1})$	18.72 ± 10.41
$\beta(h^{-1})$	0.14 ± 0.02	$k_{21}(h^{-1})$	1.99 ± 0.79
$V_{ss}(1)$	38.30 ± 12.24	$t_{1/2(\alpha)}$(min)	2.32 ± 1.18
AUC(μg h/ml)	0.84 ± 0.27	$t_{1/2(\beta)}$(h)	5.28 ± 1.00

program (SCI Software, Lexington, KY, USA) and are given in Table 2.

Berberine was also given by the intramuscular route. The absolute bioavailability by intramuscular administration was then evaluated in rabbits by a cross-over method and by comparison with an i.v. bolus dose of 2 mg/kg. The absolute bioavailability of i.m. administration estimated from the AUC (area under the plasma level–time curve) was 99.77%.

The amount of berberine excreted unchanged into urine was measured from a 48-h urine collection after i.v. bolus administration at a dose of 2 mg/kg. The cumulative urinary excretion of berberine was found to be only 4.93% of the dose given. Biliary excretion of berberine into the intestine may be one of the other major elimination pathways after parenteral administration. Most of the berberine excreted from the bile was found during the first 3 h and the results are shown in Fig. 6. The cumulative biliary excretion of berberine within 5.5 h was calculated to be only 0.5% of the dose given by an i.v. bolus administration of 2 mg/kg. Only about 5.5% of the dose was eliminated unchanged from the urine and the bile after i.v. bolus administration of berberine to rabbits. The fate of most of the berberine administered remains unknown. Metabolism seems the most likely mechanism but requires further investigation.

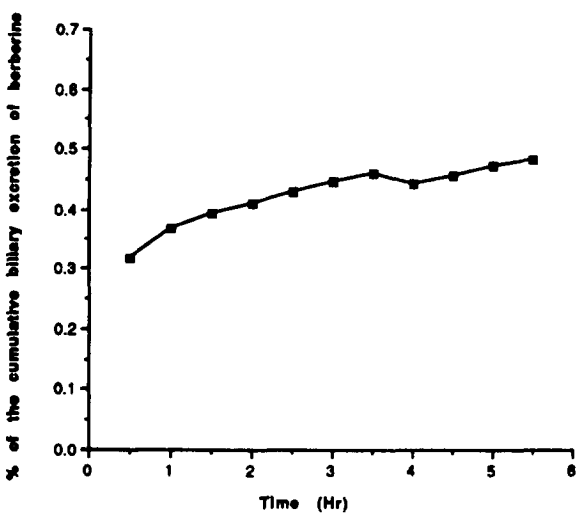

Fig. 6. Mean percentage of the cumulative biliary excretion of berberine versus time profile following an i.v. bolus administration of a dose of 2 mg/kg to cannulated rabbits.

In conclusion, the HPLC assay used in this study has high specificity, sensitivity, accuracy and reproducibility, and is suitable for the determination of berberine in plasma, urine and bile samples.

Acknowledgements

Berberine sulfate injection (Stopin) was kindly supplied by Kyorin Pharmaceutical (Taiwan). This work was supported by a grant from the National Science Council of the Republic of China (NSC-79-0412-B-038-14).

References

[1] G.D. Pandey and K.P. Tiwari, *Heterocycles*, 14 (1980) 59.

[2] K. Takagi, *Pharmacology of Medicinal Herbs in East Asia*, Nanzando, Tokyo, 1982, p. 95.

[3] M. Ozawa, K. Tanno, M. Ikeda and K. Sugahara (Editors), *Practice in Drug Therapy, Part 2*, Asahi Medical, Tokyo, 3rd ed., 1986, p. 362.
[4] M. Sabir and N.K. Bhide, *Indian J. Physiol. Pharmacol.*, 15 (1971) 111.
[5] M.T. Chan, *Comp. Med. East West*, 292 (1977) 161.
[6] J.A. Marin-Neto, B.C. Maciel, A.L. Secches and L. Gallo, Jr., *Clin. Cardiol.*, 11 (1988) 253.
[7] H. Otsuka, H. Fujimura, T. Sawada and M. Goto, *Yakugaku Zasshi*, 101 (1981) 883.
[8] F.T. Schein and H. Calvin, *Arch. Intern. Pharacodyn.*, 124 (1960) 317.
[9] M.B. Bhide, S.R. Chavan and N.K. Dutta, *Indian J. Med. Res.*, 57 (1969) 2128.
[10] S. Sakurai, M. Tezua and O. Tamemasa, *Oyo Yauri*, 11 (1976) 351.
[11] H. Miyazaki, E. Shirai, M. Ishibashi and K. Niizima, *J. Chromatogr.*, 152 (1978) 79.