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QUANTITATIVE ANALYSIS OF BERBERINE IN URINE SAMPLES BY CHEMICAL IONIZATION MASS FRAGMENTOGRAPHY*

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SUMMARY

A highly specific and sensitive method has been developed for the quantitative determination of berberine in human urine. In order to carry out the microdetermination of berberine by chemical ionization mass fragmentography, berberine was reduced with sodium borohydride in methanol to tetrahydroberberine and subjected to gas chromatography–mass spectrometry. Berberine concentrations as low as 1 ng/ml urine can be measured by this method, with [$^2H_3$]berberine chloride as an internal standard.

INTRODUCTION

The Berberis (Berberidaceae) and many other plants containing berberine have been used as antidiarrhoetic agents and haemostatic agents in Chinese medicine. Since it was reported that a homologue of berberine exhibits a minor tranquillizing action in animals[1], attention has been focused on the pharmacokinetics of berberine after administration to humans as its quaternary ammonium salt.

Conventionally, berberine has been determined by UV spectrometry and fluorimetry. However, these methods have the disadvantage that they lack specificity and sensitivity for the determination of berberine in biological fluids. Therefore, there are few reports on the pharmacokinetics and metabolism of berberine in humans.

Recently, Sakurai *et al.*[2] reported the pharmacokinetics of berberine in rats using a tritiated derivative labelled by the Wurzbach method. However, this method did not differentiate the intact drug from its metabolites.

Gas chromatography–mass spectrometry (GC–MS), especially mass frag-

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mentography, in the chemical ionization (CI) mode can be used to determine very small amounts of drugs or endogenous substances in biological fluids with high reliability. However, it is difficult to apply this technique to berberine, because it is non-volatile. Thus quaternary ammonium salts must be converted into tertiary amines by means of pyrolysis[3–5] or derivatization[6,7] in order to analyse them by GC. This paper deals with the conversion of berberine into tetrahydroberberine, which is suitable for GC determination, and the microdetermination of berberine in human urine by mass fragmentography in the CI mode using [$^2H_3$]berberine as an internal standard*.

EXPERIMENTAL

Gas chromatography–mass spectrometry

A Shimadzu LKB-9000B GC–MS system equipped with multiple ion detector (LKB-9060) and data processing system (Shimadzu GC–MS PAC 300) were used. The column was 1 m × 2 mm I.D. glass, packed with 1% Dexsil-300GC on Gas-Chrom Q. The temperature of the column oven was maintained at 230°. The flow-rate of the helium carrier gas was 30 ml/min. The temperature of the injection port was 290° and of the separator 230°. The accelerating voltage was 3.5 kV. The ionization energies and trap currents were 70 eV and 60 μA for electron-impact mass spectrometry (EIMS), and 500 eV and 500 μA (this value was measured without reagent gas) for chemical ionization mass spectrometry (CIMS), respectively. For CIMS, isobutane and ammonia were used as reagent gases, at a pressure in ionization source of 1 Torr.

Samples and reagents

All reagents and solvents used in this research were of analytical grade and were used without further purification.

The berberine chloride used as a standard was obtained by successive recrystallizations of a technical grade, and its purity was determined[8].

[$^2H_3$]Berberine (5,6-dihydro-9-trideuteriomethoxy-10-methoxybenzo-[*g*]-1,3-benzodioxo-[5,6-*a*]-quinolizium chloride) was synthesized by demethylation of berberine with hydrogen bromide–acetic acid solution followed by trideuteriomethylation with trideuteriomethyl iodide[9].

Procedure

Single oral doses of 100 mg of berberine chloride were administered to healthy adult male volunteers. The urine was collected before and during the time intervals 0–12 and 12–24 h after administration. To 200 ml of the urine, 100 μg of internal standard were added, and the mixture was lyophilized. The residue was extracted with 100 ml of 100% ethanol. The extract was centrifuged for 15 min at *ca.* 1500 *g*. Then, 10 ml of the supernatant was evaporated to dryness under reduced pressure and dissolved in 1 ml of water. The solution was chromatographed over XAD-2 (1.2 × 10.0 cm; Rohm & Haas, Philadelphia, Pa., U.S.A.) with water. After washing with 20 ml

* After this paper had been prepared, another paper was published on the GC analysis of berberine and palmatine as the products of their reduction by sodium borohydride[17].

of water, the eluate with 50 ml of 1 *M* hydrochloric acid–methanol (1:1) was collected and evaporated to dryness under reduced pressure. Next, 5 ml of methanol and 10 mg of sodium borohydride were added to the residue, and the solution was allowed to stand for *ca.* 15 min. After evaporation of the methanol, 10 ml of 0.1 *M* hydrochloric acid and saturated potassium hydroxide solution were added, and the alkaline solution was extracted twice with 30 ml of chloroform. The extracts were collected, dried over anhydrous sodium sulphate and concentrated to *ca.* 0.1–0.2 ml, prior to mass fragmentographic analysis.

RESULTS AND DISCUSSION

Derivatization

The reduction product of berberine with sodium borohydride in methanol is very suitable for GC analysis if a non-polar liquid stationary phase such as OV-101 and Dexsil 300GC is used. Berberine is almost impossible to determine directly by GC because it is a quaternary ammonium salt.

Fig. 1a shows the mass spectrum of the reduction product of berberine. It is in complete agreement with that of tetrahydroberberine (THB) previously reported

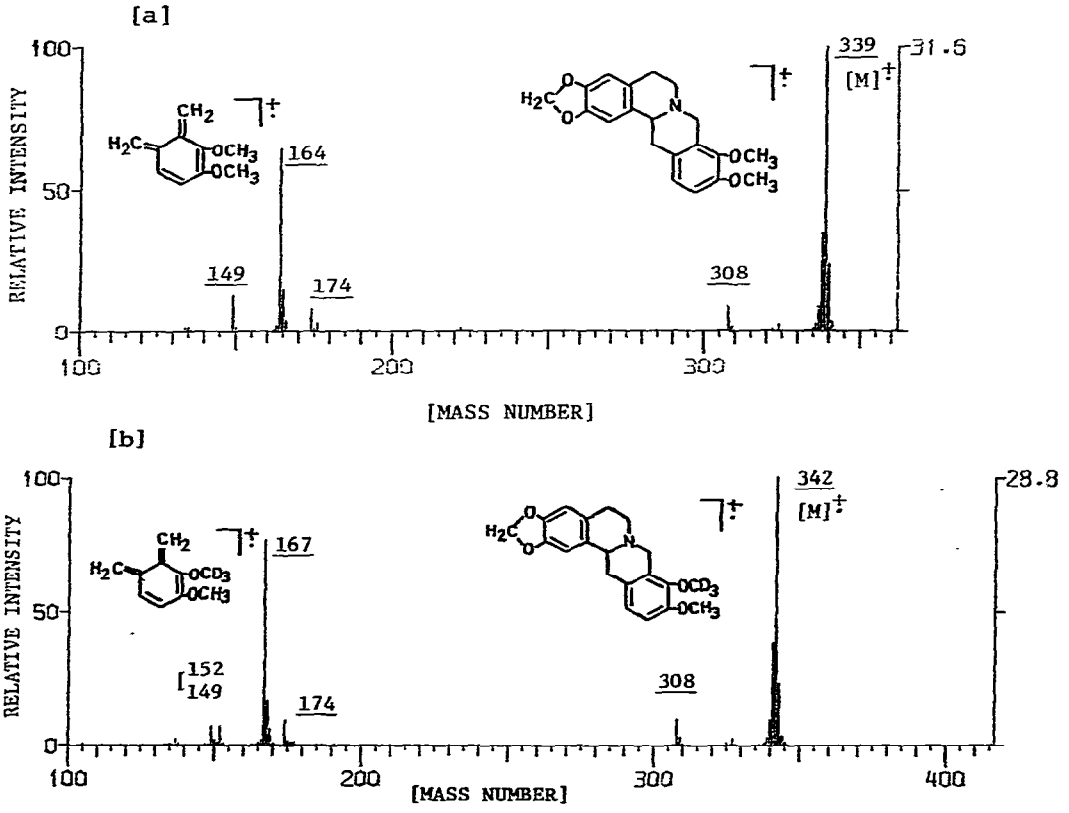

Fig. 1. Mass spectra of the reduction products of berberine (a) and its trideuterio derivative (b) with sodium borohydride in methanol. They were identified by GC–MS as tetrahydroberberine and its trideuterio derivative.

by Ohashi *et al.*[10], which suggests that berberine is reduced by sodium borohydride to THB.

When a colourless THB–chloroform solution prepared freshly was allowed to stand for 5 days it turned yellow. This yellow solution gave several spots on a thin-layer chromatogram. In contrast, when the solvent was distilled off immediately after extraction of THB from the reaction mixture with chloroform, the residue remained colourless after 10 days storage in the dark.

The storage stability of the residue was investigated by mass fragmentography. A chloroform solution containing 100 ng each of THB and cholestane as internal standard was prepared, and the residue remaining after evaporation of the solvent was kept for a definite period at room temperature. After mass fragmentography in EI mode using the ions of *m/e* 339 (of THB) and 372 (of cholestane), the residual amounts of THB were calculated from the calibration curve. There was no significant change in the amount of THB in the solution, and it decreased by less than 10% after storage in the dark at room temperature for 10 days.

Fig. 1b shows the EI mass spectrum of the reduction product of [$^2H_3$]berberine with sodium borohydride in methanol. As expected, the molecular ion of this product was shifted by three mass units from that of berberine, namely from *m/e* 339 to 342.

In order to determine the maximum permissible amounts of the trideuterio derivative as an internal standard to be added to biological fluids, the deuterium content in its reduction product was determined by monitoring the ions at *m/e* 339 and 342. From the intensity of the ion at *m/e* 339, corresponding to $[M - 3]^+$, we calculated the amount of the non-deuterated compound in the internal standard. The ratio $^2H_0$:$^2H_3$ was found to be *ca.* 0.04, indicating that it is permissible to add the internal standard to the extent of ten times the amount of berberine contained in biological fluids.

Calibration curve

Mass fragmentography in the CI mode has the advantage that it enhances the sensitivity of the compound of interest owing to the production of its quasimolecular ion[11]. Methane, isobutane and ammonia were used as reagent gases for GC–CIMS of THB. All three gases gave simple mass spectra, and each $[M + H]^+$ ion was observed as the base peak in the mass spectra of THB and [$^2H_3$]THB. Fig. 2 shows the CI mass spectra of THB and [$^2H_3$]THB with ammonia used as reagent gas.

Microdetermination of berberine in human urine was carried out by monitoring $[M + H]^+$ of THB using ammonia as reagent gas. The detection limit was 100 pg, with a signal-to-noise ratio of greater than 10:1. Fig. 3 illustrates the calibration curve for berberine obtained by plotting peak height ratio of berberine and internal standard against known amounts of berberine added to urine. A good linear relationship was obtained between the ratio of the peak heights and amount of berberine in the range 1–10 ng.

Accuracy

The accuracy of this method for the determination of urinary levels of berberine was studied. Berberine (10 μg) and internal standard (100 μg) were added to 200 ml of urine, and berberine was extracted, purified and analysed as described in Experimental.

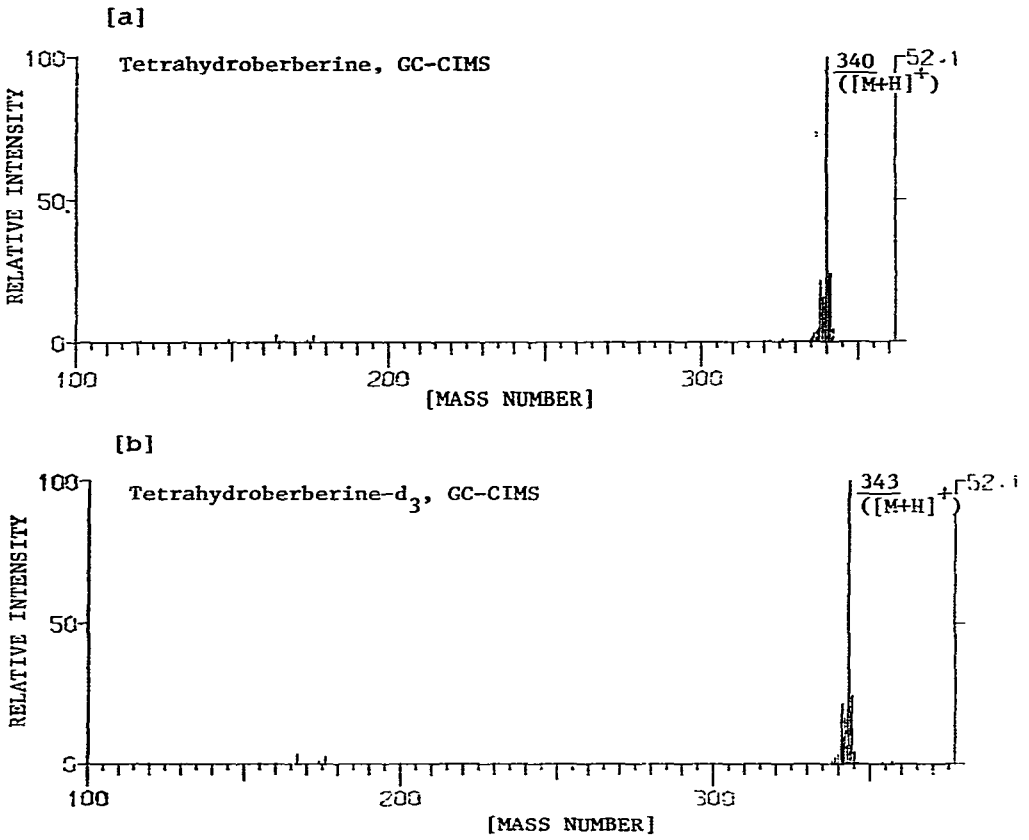

Fig. 2. Mass spectra of THB (a) and its trideuterio derivative (b) obtained by GC–CIMS with ammonia as reagent gas.

The analytical data and recoveries are shown in Table I. The average of recoveries from five drug-supplemented urines was 95.7%, and there was no significant difference between the sample preparations. These results indicate that the method is sufficiently accurate for the microdetermination of berberine in human urine.

It has been speculated[12,13] that most of the analytical errors in the determination of compounds of interest in biological fluids by mass fragmentographic analysis can be attributed to the mass fragmentographic operation itself. Use of a deuterated compound as internal standard compensates for losses of the compound during the procedures of extraction and purification[14]. The analytical data in Table I were submitted to the statistical analysis of one-way layout[15,16] in order to divide the analytical errors between the two sources of sample preparation and mass fragmentography. As shown in Table II, almost all of the total error in this method can be attributed to the mass fragmentographic process, because the error between sample preparations was negligible. The coefficient of variation in mass fragmentography was *ca.* 1.9%.

Quantitative analysis of berberine in human urine

Each of five healthy adult male volunteers received a single oral dose of 100

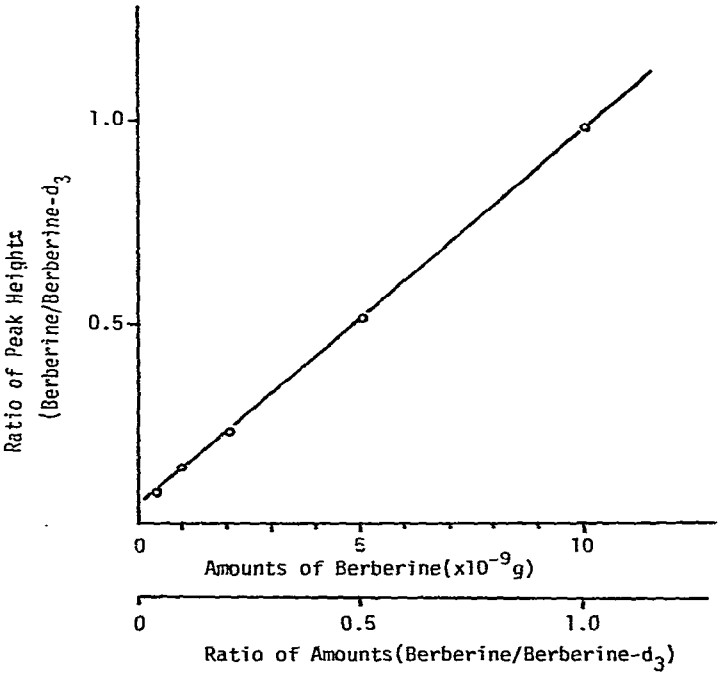

Fig. 3. Calibration curve of berberine chloride.

TABLE I

RECOVERY OF BERBERINE FROM DRUG-SUPPLEMENTED HUMAN URINE

Sample	*Added (μg/100 ml)*	*Found (μg/100 ml)*					*Recovery (%) ± S.D.*
A	10.0	9.33	9.49	9.75	9.82	9.48	95.7 ± 2.0
B	10.0	9.77	9.83	9.96	9.28	9.46	96.0 ± 2.8
C	10.0	9.53	9.49	9.47	9.34	9.62	94.9 ± 1.0
D	10.0	9.69	9.51	9.61	9.56	9.73	96.2 ± 0.9
E	10.0	9.77	9.51	9.54	9.34	9.62	95.6 ± 1.6
Mean							95.7 ± 0.5

TABLE II

ANALYSIS OF VARIANCE

Source	*ss*	*f*	*ms*	F_0
Sample preparation	4.996	4	1.249	0.378
Error	66.144	20	3.307	
Total	71.144	24		

mg of berberine chloride in the form of two 50-mg commercial sugar-coated tablets. The urine was collected and treated as described in Experimental. Berberine in the extract was determined by the peak height ratio of the $[M + H]^+$ ions of THB and $[^2H_3]$THB on the mass fragmentogram. Fig. 4 shows a typical mass fragmentogram. Table III shows the amount of berberine in human urine.

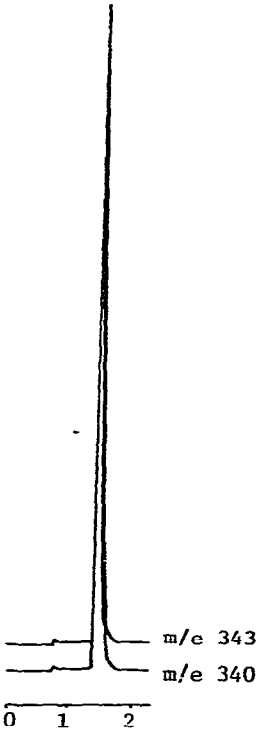

Fig. 4. Typical mass fragmentogram of the reduction products of berberine and its trideuterio derivative, in extracts from human urine.

TABLE III

URINARY LEVEL CONCENTRATION OF BERBERINE CHLORIDE IN FIVE HEALTHY ADULT MALES AFTER ORAL ADMINISTRATION OF A SINGLE 100-mg DOSE

Volunteer	*Excretion (μg)*	
	Time after administration (h)	
	0–12	*12–24*
A	48.0	36.2
B	28.6	16.9
C	30.3	9.6
D	5.2	16.3
E	11.8	12.0

CONCLUSION

This method for the microdetermination of berberine in human urine can provide accurate analytical results with high reliability through the use of CI mass fragmentography. Furthermore, it may be useful for the pharmacodynamic study of berberine chloride.

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