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Short communication

Analysis of sofalcone in human plasma by high performance liquid chromatography

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Abstract

A simple, rapid, sensitive and reliable high performance liquid chromatography (HPLC) method for the determination of the anti-ulcer drug sofalcone in human plasma was developed. Plasma was extracted with ethyl acetate under acidic conditions and sofalcone was determined by HPLC using a C18 column and (methanol–0.1% formic acid aqueous 80:20) mobile phase. The linear calibration curves of sofalcone in human plasma were obtained over the concentration range of 0.01–5.0 μg/ml. The lower limit of quantitation (LLOQ) was 10 ng/ml in human plasma. The precision measured for plasma was within 15%. Extraction recovery was over 85% in blood. The method was successfully applied to the identification and quantification of sofalcone in pharmacokinetic studies.

Keywords: Sofalcone; High performance liquid chromatography; Plasma

1. Introduction

Sofalcone, 2′-carboxymethoxy-4,4′-bis(3-methyl-2butenyloxy)chalcone (Fig. 1), is an anti-ulcer agent with mucosa protective effect and directly inhibits growth of *Heliobacter pylori*. It is therefore useful for the treatment of gastric ulcer. Sofalcone is a type of flavonoid and a synthetic derivative of sophoradine which is isolated from the root of the Chinese medicinal plant *Sophora subprostrata*.

An analytical method for the measurement of sofalcone in human plasma by high-throughput liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been reported [1,2]. However, this procedure was still complicated. The purpose of our study was to develop a simple, rapid, sensitive method for the determination of sofalcone in human plasma with high performance liquid chromatography (HPLC).

2. Experimental

2.1. Chemicals and reagents

Sofalcone standard was provided by Qirui Co., Ltd. (Wuhan, China). Fenofibrate, the internal standard (IS), was purchased from Enhua Pharma Co., Ltd. (Xuzhou, China). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Acetonitrile (HPLC grade) was purchased from Sigma (St Louis, MO, USA). Formic acid and ammonium acetate were both of HPLC grade and purchased from Tedia (Fairfield, USA). Water was triply distilled. Blank plasma and urine were supplied by Xijing Hospital, Fourth Military Medical University, China.

2.2. Preparation of calibration curve and QC samples

Stock solutions for sofalocne and IS were prepared by dissolving an accurately weighted standard of the compound (sofalcone and IS) in methanol to give the final concentration of 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0, 25.0 and 50.0 μg/ml. Solutions were stored at −20 °C until required for evaluation.

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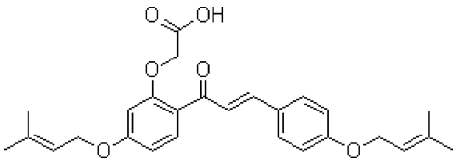

Fig. 1. Structure of sofalcone 2′-carboxymethoxy-4,4′-bis(3-methyl-2butenyloxy)chalcone.

Calibration curve samples were prepared by spiking 1 ml portions of blank plasma with 9 ml of each working standards (0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0, 25.0 and 50.0 μg/ml) to give final sofalcone concentrations of 0.010, 0.020, 0.050, 0.10, 0.20, 0.50, 1.0, 2.0, 2.5 and 5 μg/ml. Quality control (QC) samples were similarly prepared at concentrations of 0.02, 0.50 and 2.50 μg/ml (or 0.50 μg/ml for validation of stability) in plasma. All the biological samples were stored at −20 °C until analysis.

2.3. Sample preparation

Plasma specimens (0.5 ml) were transferred into plastic centrifuging tubes, and spiked with 50 μl of 5 μg/ml internal standard solution. Then 200 μl of 0.1 mol/L HCl was added into the solution and mixed on a vortex mixer for 3 min. After mixing, 5 ml of ethyl acetate was added and the samples were again mixed by vortexing for 3 min. After centrifugation at 3500 × *g* for 5 min, 4 ml of the supernatant liquid was transferred to a centrifuging tube and completely evaporated at 40 °C under a stream of nitrogen. The residue was reconstituted with 150 μl mobile phase (methanol–0.1% formic acid, 80:20). After centrifugation at 16,000 × *g* for 5 min, a 20 μl supernatant was injected into the HPLC column.

2.4. HPLC conditions

An Agilent HPLC system (Series 1100, Agilent Technologies, Palo Alto, CA, USA) was used and was comprised of a HP 1100 G1311A quaternary pump, G1379A vacuum degasser, G1313A autosampler and G1316A column oven. The chromatography was performed on a C_{18} column (150 mm × 4.6 mm i.d., 5 μm; Waters, Milford, MA, USA). The column temperature was set at 35 °C. The flow rate was 1 ml/min and the injection volume was 20 μl. The mobile phase was consisted of methanol (A) and 0.1% formic acid (B), using a linear gradient elution of 80:20 (0 min) to 97:3 (10 min) to 80:20 (13.5 min) buffer A to B. The absorption wavelength was set at 280 nm.

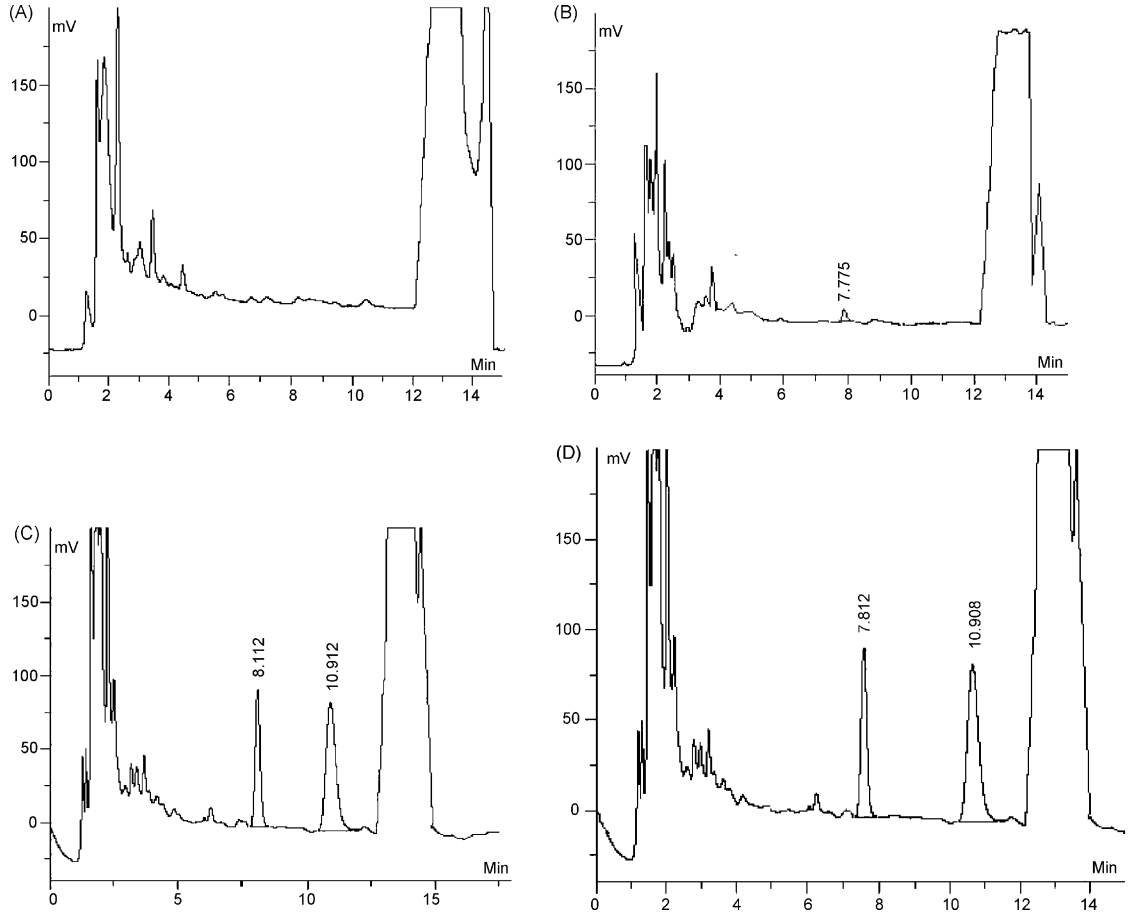

Fig. 2. Representative HPLC chromatograms of sofalcone and internal standard (fenofibrate) in human plasma samples: (A) a blank plasma sample; (B) an LLOQ sample of sofalcone (10 ng/ml); (C) a blank plasma sample spiked with sofalcone (t_R = 10.912 min, C = 0.5 μg/ml) and internal standard (t_R = 8.112 min, C = 0.5 μg/ml); and (D) a plasma sample from a volunteer 1.5 h after oral administration of sofalcone (internal standard t_R = 7.812 min, C = 0.5 μg/ml, sofalcone t_R = 10.908 min, C = 0.95 μg/ml).

2.5. Validation procedures and calibration curves

QC plasma samples were used to construct the calibration curve. Five duplicate samples were prepared for each concentration. Linear equations (weighted 1/quadr-concentration, i.e. $1/x^2$) between the peak area ratio and the concentrations were constructed. The lower limit of quantitation (LLOQ) of sofalcone in plasma was set at the concentration of the lowest non-zero calibration samples. To assess the intra-day precision and accuracy of the method, five replicated analyses were performed on plasma standards containing three different concentrations (0.02, 0.50 and 2.50 μg/ml) of sofalcone. Five replicate analyses of the same three samples were also performed to determine the initial interday precision and accuracy. The accuracy was expressed as (mean observed concentration/spiked concentration) × %, and the precision was expressed as relative standard deviation (R.S.D., %). The extraction recovery of sofalcone in plasma was determined by calculating the peak area ratio of extracted low, medium and high QC samples against unextracted standard solutions at the same concentrations. Long-term stability was estimated by assessing a QC sample of 0.50 μg/ml stored at −20 °C for 35 days. Freeze–thaw stability was studied after one, two and three cycles.

2.6. Pharmacokinetic assay

For the pharmacokinetic assay in humans, three different doses of solfacone (50, 100 or 200 mg, respectively) were administered orally to 30 volunteers who were advised about the nature and purpose of the study. Volunteers (15 males and 15 females) between 18 and 45 years old (30.25 ± 3.16) with a body weight 57.78 ± 4.84 kg participated in this study. Selection criteria excluded subjects with acute or chronic infection, hepatic, renal or gastrointestinal disorder, mental disease, metabolic disease, history of drug allergy, abuse of alcohol or other drugs, or those that were likely to violate the protocol. All volunteers avoided using other drugs from 2 weeks prior to the study until after its completion, and all were non-smokers.

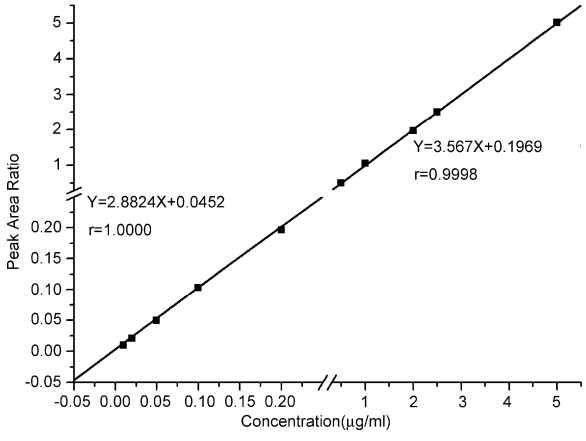

Fig. 3. The standard curve of sofalcone in plasma.

Table 1
Results of accuracy, the within-assay and between-assay precision evaluation experiments of sofalcone (0.02, 0.50 and 2.50 μg/ml) in plasma samples

Plasma samples	Sofalcone (μg/ml)		
	0.02	0.50	2.50
Intra-day ($n=5$)			
Mean	0.0204	0.501	2.502
R.S.D. (%)	16.478	9.542	12.251
S.D. (μg/ml)	0.001	0.003	0.071
Trueness (%)	2.24	3.73	−1.13
Inter-day ($n=5$)			
Mean	0.0205	0.5176	2.532
R.S.D. (%)	2.750	9.201	5.649
S.D. (μg/ml)	0.001	0.042	0.083
Trueness (%)	0.17	−0.90	3.36

To avoid absorption anomalies resulting from inhibition of intestinal CYP3A4 [3], subjects abstained from consumption of orange and grapefruit products for 2 weeks before and during the trial.

Venous blood samples (3 ml) were collected in disposable heparin tubes pre-dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after oral administration. After centrifugation at 11,000 × g for 10 min, the plasma was transferred to plastic tubes and stored frozen at −20 °C until analysis.

3. Results and discussion

3.1. Specificity

Representative HPLC chromatograms of extracted blank samples, extracted QC samples and unknown samples from a subject after oral administration of 100 mg sofalcone are shown in Fig. 2. The retention times were 7.770 min for sofalcone and 10.278 min for the internal standard (fenofibrate). It was demonstrated that there was no endogenous substances in human plasma interfering with the samples or the IS.

3.2. Linearity

The calibration curves ($y = mx + b$) were generated by a weighted linear least-squares regression of the peak area ratios of the specimens to their internal standards (y) versus the concentrations (x) of the calibration standards. Sofalcone in plasma were validated over the concentration ranges of 0.01–0.50 μg/ml and 0.50–5.0 μg/ml, respectively. The correlation coefficient of determination (r^2) was >0.999 in both cases. Typical equations

Table 2
The recovery of sofalcone (0.02, 0.50 and 2.50 μg/ml) and IS in plasma samples ($n=5$)

	Sofalcone (μg/ml)			IS (μg/ml)
Recovery (%)	0.02	0.50	2.50	0.50
Mean	86.01	93.78	89.78	91.76
S.D. (%)	2.88	3.23	6.52	2.86

Table 3
The stability of sofalcone (0.50 μg/ml) in plasma samples ($n = 5$)

			Three freeze–thaw		
	−20 °C for 35 days	20 °C for 24 h	1week	2 weeks	3 weeks
Mean	0.4748	0.4836	0.462	0.474	0.472
S.D. (μg/ml)	0.0220	0.0162	0.020	0.029	0.029
R.S.D. (%)	4.944	4.041	4.441	4.972	3.859

Table 4
Mean pharmacokinetic parameters of sofalcone following single oral doses of sofalcone at 50, 100 and 200 mg in healthy volunteers ($n = 30$)

Dose (mg)	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	MRT (h)	$AUC_{0–12}$ (h μg/ml)	$AUC_{0-\infty}$ (h μg/ml)
50	0.39 ± 0.18	0.8 ± 0.1	3.29 ± 1.93	4.27 ± 0.90	1.26 ± 0.54	1.35 ± 0.57
100	1.27 ± 0.51	0.9 ± 0.3	2.77 ± 0.32	3.76 ± 0.81	3.99 ± 1.02	4.13 ± 1.08
200	2.11 ± 0.68	0.7 ± 0.2	3.38 ± 0.97	4.12 ± 1.30	5.48 ± 1.92	5.95 ± 2.18

Each value represents the mean ± S.D.

of calibration curves were as follows (Fig. 3):

C : 0.01–0.50 μg/ml $Y = 2.8824X + 0.0452$, $r = 1.0000$;

C : 0.50–5.0 μg/ml $Y = 3.5675X - 0.1969$, $r = 0.9998$

3.3. Lower limit of quantitation (LLOQ)

The lower limit of quantitation (LLOQ) of sofalcone was set at the concentration of the lowest non-zero calibration sample, 0.01 μg/ml for plasma. Five replicate LLOQ samples were assayed. Intra-batch precision (R.S.D., %) of samples was 11.4%, and the accuracy was 8.9% in plasma. The LLOQ was sensitive enough for the pharmacokinetic study of sofalcone.

3.4. Precision and accuracy

0.02, 0.50 and 2.50 μg/ml QC samples were assayed for five samples in three independent batches in plasma. Inter-and intra-day accuracy and precision data are shown in Table 1.

3.5. Extraction recovery

The extraction recovery in plasma was determined for five samples at three concentrations of sofalcone. The results are summarized in Table 2. The data indicated that the recovery of sofalcone was over 85% in blood in the concentration range evaluated.

3.6. Stability

The stability of sofalcone in plasma was determined by assessing replicate 0.50 μg/ml QC samples ($n = 5$ for each concentration). Sofalcone was found to be stable in plasma sample for at least 35 days at −20 °C freezing condition and following three cycles of freeze–thaw (Table 3).

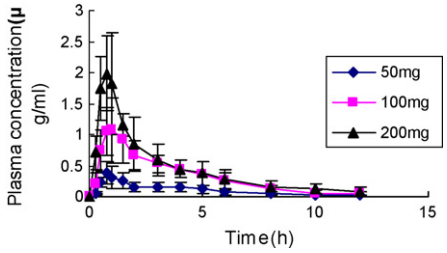

Fig. 4. Mean plasma concentration–time profiles of sofalcone following single oral doses of 50, 100 and 200 mg in healthy volunteers ($n = 30$). Data are means ± S.D. of three experiments.

3.7. Application in pharmacokinetic study of sofalcone

The above method for the quantitation of sofalcone in human plasma was applied in a pharmacokinetic study. The mean plasma concentration–time profiles of sofalcone in 30 different healthy volunteers, administered one of three different doses of solfacone (50, 100 or 200 mg, respectively), are shown in Fig. 4. Pharmacokinetic parameters including maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), area under plasma concentration–time curve from time 0 to the time for the last quantifiable concentration ($AUC_{0\text{-}tlqc}$), area under the plasma concentration–time curve from time 0 to infinity ($AUC_{0-\infty}$), terminal half-life ($t_{1/2}$), and mean residence time (MRT) were calculated using standard noncompartmental methods. Table 4 shows the pharmacokinetic parameters of sofalcone.

4. Conclusion

The method described in this paper can be used to determine the concentration of sofalcone in human plasma. Validation experiments have shown that the assay has good precision and accuracy over a wide concentration range (0.02–2.5 μg/ml), with no observable interferences caused by endogenous compounds. Simple performance, sensitivity and good validation criteria results make the method suitable for pharmacokinetic studies.

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