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Liquid chromatography/tandem mass spectrometry for the determination of changrolin in rat plasma: Application to a bioavailability study

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ABSTRACT

A sensitive and specific LC–CMS/MS method was developed for the quantification of changrolin, an anti-arrhythmic drug, in rat plasma using tiapride as internal standard. Liquid–liquid extraction was employed for sample preparation and analyzed using a multiple reaction monitoring mode with electrospray positive ionization source. The calibration curve for changrolin was linear over the range 5–1600 ng/mL with coefficients of correlation >0.99. The intra- and inter-batch precision was less than 8.6%, and accuracy ranged from 94.6% to 104.5%. This validated LC–MS/MS method was successfully applied to a bioavailability study of oral and intravenous administration of changrolin with 20 mg/kg dosage in SD rats.

1. Introduction

Changrolin, 2,6-bis(pyrrolidin-1-ylmethyl)-4-(quinazolin-4-ylamino) phenol, is an anti-arrhythmic drug originated from Chinese medicinal herb of *Dichroa febrifuga* Lour. Scientists have reported its therapeutic effects against experimental arrhythmias [1] and electrophysiological effects [2–6]. Clinical trials also revealed its significant therapeutic effects on relieving ventricular premature beat [7,8] and paroxysmal supraventricular tachycardia [9].

The clinical application of anti-arrhythmic drug has decreased over the past 15 years because of problems with side effects [10]. In order to avoid adverse effects and obtain desirable clinical benefits, therapeutic drug monitoring (TDM) is essential for optimal therapy with anti-arrhythmic drugs [11,12]. Changrolin, now available in clinical application in China, has been found some side effects such as heart conduction blockade and cardiac depression [13]. Therefore, it is necessary to develop a method to monitor therapeutic blood concentration in clinical practice. In addition, studies on changrolin derivatives have already initiated [14–17]. The proposed LC–MS/MS method for determination of changrolin may be applicable for comparing study between changrolin and its derivatives.

In previous reports, the pharmacokinetics and pharmacodynamics of changrolin were studied in dogs with experimental arrhythmias by using HPLC method to determine the serum drug concentration [1]. The method presented low sensitivity of 0.1 μg/mL and large sample volume of 1 mL. However, to our knowledge, no publication describing quantitative analysis of changrolin using mass spectrometry has been published. In this paper, we developed and validated a LC–MS/MS method for analysis of changrolin in plasma with the capacity of covering a wide range from 5–1600 ng/mL required only 100 μL of sample. Subsequently, we successfully applied the method to pharmacokinetics and bioavailability study. This method, being fully validated to be simple and sensitive, may be applicable to clinical practice as well.

2. Experimental

2.1. Materials and chemicals

Changrolin (>99% purity) was provided by Department of Medicinal Chemistry, Shanghai Institute of Materia Medica. Tiapride (>99% purity, internal standard) was purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from Dikma (Richmond hill, ON, USA), and cyclohexane and trifluoroacetic acid (HPLC grade) from Tedia (Fairfield, OH, USA). Double distilled water was used throughout the study. All other reagents were of analytical grade.

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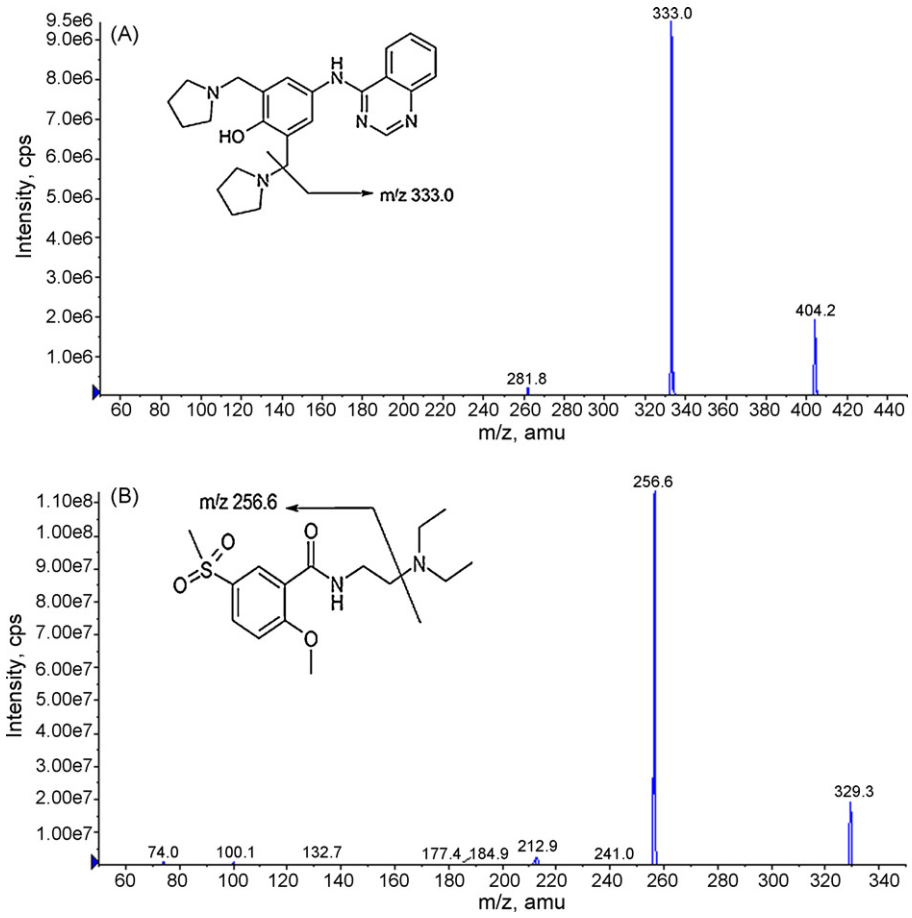

Fig. 1. Product ion mass spectra of changrolin (A) and internal standard tiapride (B).

2.2. Sample preparation

Plasma samples collected from rats were stored at −80 °C until analysis. To a 2 mL polypropylene test tube, 100 μL of the plasma sample, 10 μL of the IS solution (300 ng/mL of tiapride) and 110 μL of borax (0.05 M)-sodium hydroxide (0.5 M) buffer solution (4:1, v/v) (pH* 11.7) were added and briefly vortexed. Then 1 mL of cyclohexane–dichloromethane–ethyl acetate (25:20:7, v/v/v) was added for liquid–liquid extraction. The mixture was vortexed for 5 min, followed by centrifugation at 16,000 × *g* for 3 min. The organic layer was transferred to another tube and dried under a flow of nitrogen gas at 40 °C. The residue was reconstituted with 500 μL of mobile phase. After centrifugation at 16,000 × *g* for 3 min, 5 μL of the supernatant was injected into the LC–MS/MS system.

2.3. Instrumentation

The HPLC system, which consists of two LC-20AD pumps, a SCL-10Avp system controller, a DGU-20A_3 degasser (Shimadzu Corporation, Kyoto, Japan) and a HTC PAL autosampler (CTC Analytics AG, Switzerland), was used. Chromatographic separation was performed on Gemini C_{18} column (50 mm × 2 mm, 5 μm; Phenomenex, USA) at room temperature with a Phenomenex C18 guard column. The mobile phase was composed of 84% water and 16% methanol (both containing 0.1% trifluoroacetic acid) and run at an isocratic flow of 0.3 mL/min. By valve switching, the HPLC flow was diverted into waste before 0.6 min and after 3.0 min, and meanwhile a flow of 16% methanol was infused into MS.

The API-3000 triple quadrupole tandem mass spectrometer (Applied Biosystems, Toronto, Canada) was equipped with Turbo Ionspray source and operated in positive ionization. Analyst 1.4 software package was used for instrument control and data acquisition. The ion spray voltage was set at 1.8 kV and source temperature at 450 °C. The collision activated dissociation (CAD) was set at 12, using nitrogen as the collision gas. The quantification was performed on multiple reaction monitoring (MRM) with ion transitions of *m*/*z* 404.3 → 333.2 for changrolin, *m*/*z* 329.1 → 256.2 for tiapride, respectively.

2.4. Standard curves and quality control samples

Standard stock solutions of changrolin and tiapride prepared in methanol at 1.0 mg/mL and stored at 4 °C, were serially diluted with methanol–water (50:50, v/v) to be working solutions. Blank plasma of 990 μL was mixed with 10 μL of appropriate working solutions. The resultant concentrations were 5, 20, 100, 200, 400, 800, 1600 ng/mL for the calibration standards, and 10, 640 and 1280 ng/mL for the quality control samples. Calibration curve was fitted with a linear regression and weighted by 1/*x*.

2.5. Validation procedures

Intra-batch precision and accuracy was test on six different rat QC samples together with two sets of calibration standards. Inter-batch variability was assessed by analysis of six sets of quality control samples on three different batches. The lower limit of quantification (LLOQ) was defined as the lowest calibrator with an inter-batch coefficient of variation (CV, usually below 20%).

Recoveries were calculated from the three different quality control samples ($n = 6$ for each concentration). The mass responses of

the extracted samples were compared with the response of respective amounts of analyte or internal standard spiked in extracted blank plasma. Matrix effect was assessed by comparing the peak areas of spike-after-extraction samples to its neat standard solutions in mobile phase.

The stability of changrolin in plasma was studied by subjecting QC samples to a variety of storage and handling conditions. The short-term temperature stability was assessed by analyzing QC samples kept at 25 °C for 0, 2.5, 5 h. The autosampler stability was assessed by analyzing extracted quality control samples in autosampler at 25 °C for 0, 4, 8, 12, 16 h. Freeze–thaw stability was evaluated after three cycles from −80 °C to 25 °C. Long-term stability was evaluated by placing plasma at −80 °C for a month. The stability of stock solution in methanol was also evaluated at 4 °C for a month.

2.6. Bioavailability study

The developed LC–MS/MS procedure was used to investigate the plasma concentration–time profile after oral or intravenous administration of 20 mg/kg changrolin to six SD rats. The oral dose solutions were prepared in water and the intravenous dose solutions in saline. Blood (about 0.3 mL) was withdrawn from the vein at predose, 0.16, 0.33, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h after oral administration, and predose, 0.03, 0.13, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 24 h after intravenous administration.

The pharmacokinetic parameters were calculated from plasma levels applying a non-compartmental statistic using Drug and Statistics version 2.0 software (Anhui, China). The oral bioavailability was calculated: $F = AUC_{(0-t)oral}/AUC_{(0-t)iv} \times 100\%$.

3. Results and discussion

3.1. LC–MS/MS detection

Fig. 1 displayed the MS/MS spectra of changrolin and tiapride. The representative LC–MS/MS chromatogram of plasma samples were shown in Fig. 2. Changrolin and tiapride had short retention times about 1.3 and 1.8 min, respectively. The total run time is 3.5 min per injection. In this experiment, trifluoroacetic acid (TFA) was added in mobile phase to form ion pairs with changrolin in order to obtain higher retention and better peak shape. Considering TFA is corrosive to ion source, we used valve-switching to divert the HPLC flow into waste before 0.6 min and after 3.0 min. TFA in the mobile phase can suppress the ESI signals [18], therefore the baseline disturbance was observed in the Fig. 2 as a result of valve-switching.

3.2. Linearity and LLOQ

The linearity of the calibration curve was evaluated from three different batches. In each batch two sets of calibration standards and six sets of quality control samples were included. The calibration curve was linear over the changrolin concentration range of 5–1600 ng/mL with coefficient of correlation (r) >0.99. The regression equation of calibration curves from three different batches was: $y = (0.00820 \pm 0.000597)x - (0.0117 \pm 0.00229)$, $r = (0.9988 \pm 0.00042)$. The LLOQ was 5 ng/mL for changrolin with signal to noise ratio >10 and accuracy 100.4% and precision (CV, coefficient of variation) 4.8% ($n = 6$).

Table 1
Intra- and inter-batch assay validation for changrolin in SD rat plasma.

Nominal Concentration (ng/mL)	Intra-batch		Inter-batch	
	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
Calibration standards	($n = 2$)		($n = 6$)	
5	99.9	3.3	100.4	4.8
20	99.3	7.5	99.6	6.5
100	102.5	9.0	103.8	4.8
200	98.5	10.8	98.3	6.5
400	97.5	3.3	97.0	6.7
800	103.3	4.9	100.4	3.3
1600	98.8	5.4	100.3	5.2
Quality control samples	($n = 6$)		($n = 18$)	
10	101	7.4	103.2	6.5
640	104.5	1.3	101.3	8.6
1280	94.6	6.9	95.8	7.5

3.3. Precision and accuracy

The intra- and inter-batch precision and accuracy were evaluated by assaying the QC samples (Table 1). In this assay, the intra-batch precision (CV) was less than 7.4% for each of the three QC samples; and the accuracy ranged 94.6–104.5%. The inter-batch precision (CV) was less than 8.6% for all the QC samples, and the corresponding accuracy was 95.8–103.2%. The values were within the acceptable range, demonstrating the method was sufficiently accurate and precise.

3.4. Recovery, matrix effect and stability

The extraction recoveries of changrolin were in the range of 74–76% at concentrations of 10, 640, and 1280 ng/mL ($n = 6$). The value of IS was 73%. No significant matrix effect was observed on the analyte response during the analysis of QC samples (Table 2).

Samples placed in autosampler at 25 °C for 0, 4, 8, 12 and 16 h exhibited good stability with accuracy 92.8–98.5% and CV 4.4–8.3%. Three cycles of freeze–thaw stability was excellent, the CVs were determined to be <7.3%. After exposure to 25 °C for 5 h, the plasma samples remained stable with accuracy 91.7–98.1% and CV 2.8–7.2%. Long-term stability results also showed that there was no significant change in the concentration of analyte investigated (Table 3). The stock solution of chagnrolin in methanol was placed at 4 °C for a month and no significant degradation observed.

Table 2
Recovery and matrix effect of changrolin and IS in rat plasma ($n = 6$).

Statistical variable	Nominal changrolin concentration (ng/mL)			Nominal IS concentration (ng/mL)
	10	640	1280	30
Extraction recovery				
Mean (%)	74.3	75.2	76.0	73.0
CV (%)	6.5	8.8	5.3	9.4
Matrix effect				
Mean (%)	95.5	100.5	92.2	95.4
CV (%)	6.3	7.3	8.6	6.4

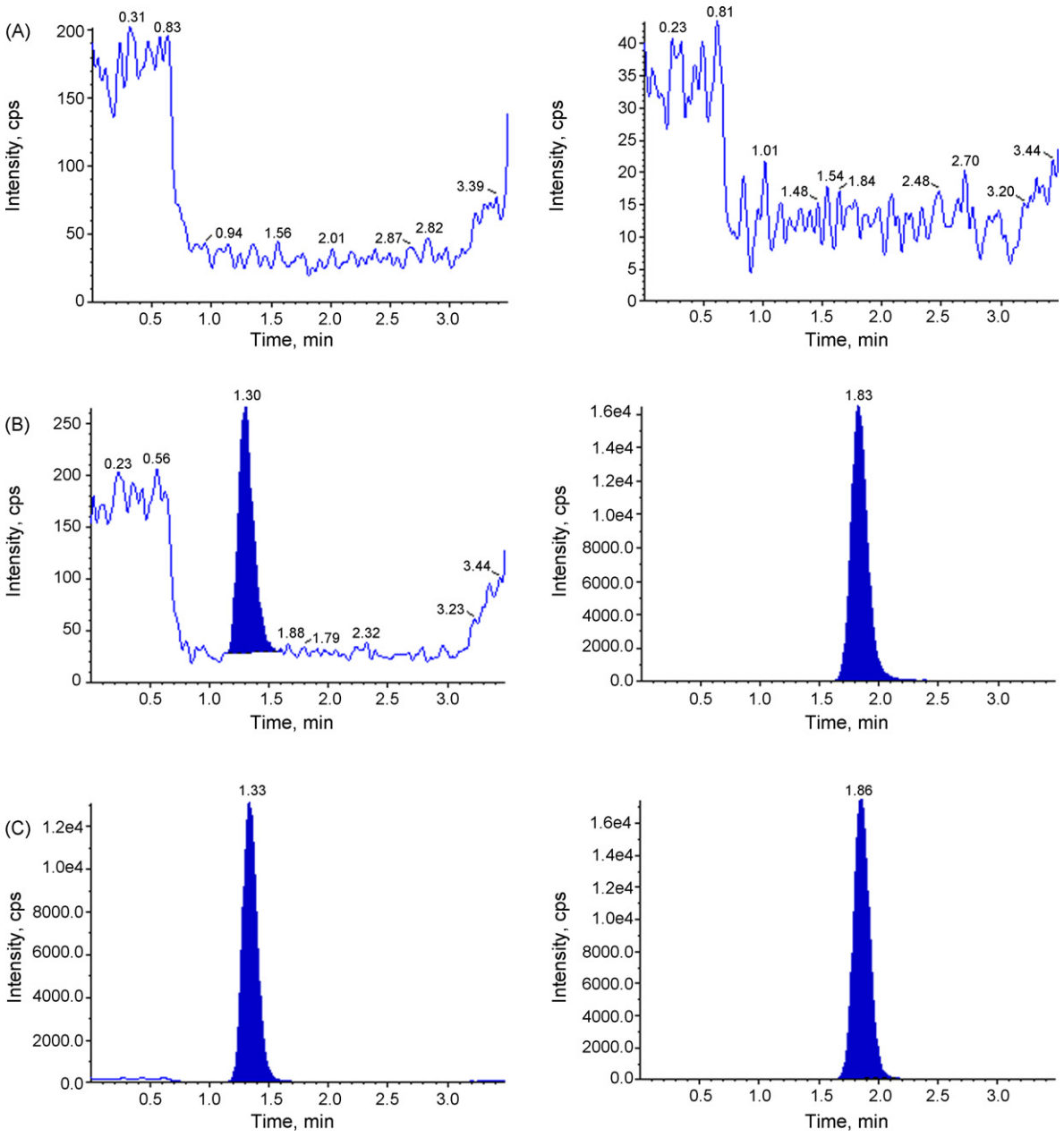

Fig. 2. Chromatograms of changrolin (left panel) and tiapride (right panel) in SD rat plasma. (A) Blank plasma sample; (B) LLOQ plasma sample spiked with changrolin (5 ng/mL) and tiapride; (C) plasma sample 2 h after intravenous administration of changrolin 20 mg/kg to a SD rat. The baseline disturbance in the figure was caused by valve-switching from 0.6 to 3.0 min.

Table 3
Stability results of changrolin under various storage conditions.

Storage conditions	Nominal concentration (ng/mL)	Accuracy (%)	CV (%)
Three freeze–thaw cycles (from −80 to 25 °C, $n = 3$)	10	94	7.3
	640	96.8	3.4
	1280	107.8	6.3
Freezing for a month (−80 °C, $n = 3$)	10	102.3	1.5
	640	108	5.6
	1280	107	2.9
Autosampler for 16 h (25 °C, $n = 5$)	10	98.5	5
	640	94.6	4.4
	1280	92.8	8.3
Short-term for 5 h (25 °C, $n = 3$)	10	98.1	7.2
	640	93	3.3
	1280	91.7	2.8

3.5. Bioavailability study

After oral and intravenous administration of 20 mg/kg changrolin to six SD rats, plasma concentrations of changrolin

Table 4
Pharmacokinetic parameters of changrolin after oral or intravenous administration to SD rats ($n = 6$, mean ± S.D.).

Parameter	Oral administration	Intravenous administration
T_{max} (h)	2.2 ± 0.4	NA
C_{max} (μg/L)	187.0 ± 99.3	NA
$AUC_{(0-tn)}$ (μg/L h)	832.7 ± 246.5	2778.5 ± 341.6
$AUC_{(0-\infty)}$ (μg/L h)	916.5 ± 223.8	2797.6 ± 360.6
$MRT_{(0-t)}$ (h)	6.6 ± 0.7	3.3 ± 0.3
$T_{1/2}$ (h)	8.1 ± 5.6	3.3 ± 0.9
F (%)	30	NA

NA: not applicable.

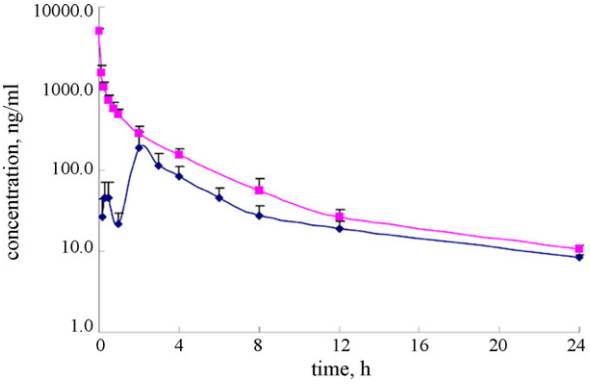

Fig. 3. Mean plasma concentration-time profile of changrolin after intravenously (■) and orally (◆) administration of changrolin 20 mg/kg to SD rats (mean ± S.D.).

were determined by the described LC–MS/MS method. Fig. 3 shows the mean plasma concentration–time curves of changrolin after oral and intravenous administration. The corresponding pharmacokinetic parameters (C_{max}, T_{max}, $T_{1/2}$, $MRT_{(0-t)}$, F, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$) were presented in Table 4. The T_{max}, C_{max}, $AUC_{(0-t)}$ of changrolin after oral administration were 2.2 h, 187.0 μg/L, and 832.7 μg/L h, respectively. Following intravenous administration, the $AUC_{(0-t)}$ was 2778.5 μg/L h. $T_{1/2}$ of changrolin for oral and intravenous administration were 8.1 and 3.3 h, respectively. The oral bioavailability (F) was 30%, showing the necessity to improve the bioavailability by means of chemical or formulation modification.

4. Conclusions

In conclusion, the use of LC–MS/MS allows an accurate, precise and reliable measurement of changrolin concentrations in rat plasma. The method exhibited significant advantages over other techniques previously described for measuring changrolin in biological fluids. The sensitivity of the assay is sufficient to follow accurately the pharmacokinetics and bioavailability study for this drug.

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