Journal of Chromatography B, 879 (2011) 3071–3075

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Simultaneous determination of dronedarone and its active metabolite debutyldronedarone in human plasma by liquid chromatography–tandem mass spectrometry: Application to a pharmacokinetic study

Cen Xie, Shilei Yang, Dafang Zhong, Xiaojian Dai, Xiaoyan Chen*

Center for Drug Metabolism and Pharmacokinetics, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 501 Haike Road, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 15 July 2011
Accepted 1 September 2011
Available online 7 September 2011

Keywords:
Liquid chromatography–tandem mass spectrometry
Dronedarone
Debutyldronedarone
Pharmacokinetics

ABSTRACT

Dronedarone is a derivative of amiodarone – a popular antiarrhythmic drug. It was developed to overcome the limiting iodine-associated toxicities of amiodarone. Debutyldronedarone is a major circulating active metabolite of dronedarone in humans. To investigate the pharmacokinetics of dronedarone, a rapid, simple, and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated to simultaneously determine dronedarone and debutyldronedarone in human plasma using amiodarone as internal standard (IS). Acetonitrile with IS was used to precipitate proteins from a 50-μL aliquot of plasma. Effective chromatographic separation was performed on a CAPCELL PAK C_{18} MG (100 mm × 4.6 mm, 5 μm) column with gradient elution (5 mmol/L ammonium acetate–acetonitrile, with each phase containing 0.2% acetic acid) at a flow rate of 0.7 mL/min. Complete separation was achieved within 5.5 min. Detection was carried out on an tandem mass spectrometer in multiple reaction monitoring mode using a positive atmospheric pressure chemical ionization interface. A lower limit of quantification of 0.200 ng/mL was achieved for both dronedarone and debutyldronedarone, with acceptable precision and accuracy. The linear range of the method was from 0.200 to 200 ng/mL for each analyte. Intra- and inter-day precisions were lower than 7.2% in relation to relative standard deviation, while accuracy was within ±5.1% in terms of relative error for analytes. Our findings demonstrate the successful application of the validated LC–MS/MS method to a pharmacokinetic study after a single oral administration of 400 mg dronedarone to six healthy volunteers.

1. Introduction

Amiodarone (Fig. 1) is a widely used potent antiarrhythmic agent for ventricular and supraventricular tachyarrhythmias. Due to its high iodine content (37.3% by weight), abnormalities in thyroid function are common in patients receiving long-term amiodarone therapy [1]. A derivative of amiodarone, dronedarone (marketed as Multaq® by Sanofi-Aventis, Fig. 1), was approved by the US FDA in 2009 for the treatment of nonpermanent atrial fibrillation and atrial flutter [2–4]. Dronedarone does not contain iodine atoms in its structure, thus addressing the toxicity limitation of amiodarone.

After oral administration, dronedarone undergoes extensive metabolism in humans. Debutyldronedarone (Fig. 1), formed primarily by CYP3A4, is a major circulating metabolite with similar or even higher plasma exposure than dronedarone. Debutyldronedarone has been demonstrated to be pharmacodynamically active but exhibited a potency that is 1/10 to 1/3 of that of the parent drug [5]. Guideline issued by the US FDA in 2008 recommended that any human drug metabolite formed at >10% of the parent drug systemic exposure at steady state should be subjected to a separate safety testing [6]. Therefore, the simultaneous determination of dronedarone and debutyldronedarone in human plasma is necessary to document their circulating levels.

Quantification methods for dronedarone and debutyldronedarone in human plasma are rarely available. Only one validated bioanalytical assay for dronedarone and debutyldronedarone in human plasma and myocardium was reported; this method uses high-performance liquid chromatography (HPLC) coupled with ultraviolet detection [7]. The lower limit of quantification (LLOQ) of this method is 40 ng/mL, which is not sufficient for a conventional pharmacokinetic study of dronedarone. In a study of the pharmacokinetic and pharmacodynamic interactions between metoprolol and dronedarone [8], a liquid chromatography–mass spectrometry (LC–MS) method with an LLOQ of 0.50 ng/mL was utilized to determine the plasma concentrations of dronedarone and debutyldronedarone. However, the analytical procedure and validation process were not described. In our laboratory, a liquid

* Corresponding author. Tel.: +86 21 50800738; fax: +86 21 50800738.
E-mail address: xychen@mail.shcnc.ac.cn (X. Chen).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.09.004

Table 1
Precision and accuracy data for analysis of dronedarone and debutyldronedarone in human plasma (3 days with 6 replicates per day).

Analyte	Concentration (ng/mL)		RSD (%)		RE (%)
	Added	Found	Intra-day	Inter-day	
Dronedarone	0.200	0.206	8.2	9.5	3.2
	0.500	0.525	5.3	3.5	5.1
	20.0	20.7	0.8	4.8	3.5
	160	159	1.4	5.1	−0.4
Debutyldronedarone	0.200	0.199	7.6	10.6	−0.6
	0.500	0.520	4.4	5.1	4.0
	20.0	20.5	0.8	7.2	2.6
	160	154	5.7	5.6	−3.7

chromatography–tandem mass spectrometry (LC–MS/MS) method was established to simultaneously determine dronedarone and debutyldronedarone in dog plasma [9]. After optimizing the internal standard (IS) and chromatographic conditions, we developed and validated a rapid and sensitive LC–MS/MS method for the simultaneous determination of dronedarone and its main active metabolite debutyldronedarone in human plasma using a one-step protein precipitation. The method was applied to a pharmacokinetic study of dronedarone after a single oral administration of 400 mg of dronedarone to six healthy volunteers.

2. Experimental

2.1. Chemicals and reagents

Dronedarone (purity >99%) and debutyldronedarone (purity >99%) were obtained from TLC Pharmachem., Inc. (Vaughan, Ontario, Canada). Amiodarone hydrochloride (purity >98%), methanol, and acetonitrile of HPLC grade were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ammonium acetate and acetic acid of HPLC grade were supplied by Tedia (Fairfeild, OH, USA). Deionized water (18.2 mΩ and TOC ≤ 50 ppb) was purified by a Millipore Milli-Q Gradient Water Purfication System (Molsheim, France).

2.2. Instrumentation

An Agilent 1100 liquid chromatography system consisting of a G1322A vacuum degasser, a G1311A quaternary pump, a G1316A column oven (Agilent, Waldbronn, Germany), and a 3133 HTS autosampler (Shiseido, Tokyo, Japan) was used for solvent and sample delivery. Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument (Applied Biosystems, Concord, Ontario, Canada). The Analyst software (version 1.4.1, Applied Biosystems) was used for data acquisition and system controlling.

2.3. LC–MS/MS conditions

Chromatographic separation was achieved on a CAPCELL PAK C_{18} column (4.6 mm × 100 mm, 5 μm, Shiseido, Tokyo, Japan) using a gradient elution program. The mobile phase system consisted of 5 mmol/L ammonium acetate solution with 0.2% acetic acid (A) and acetonitrile with 0.2% acetic acid (B). Run was started at 55% A followed by a linear gradient to 5% A over 1.5 min, held at 5% A for 1 min, shifted to linear to 55% A over 0.5 min, and then held constant until the end of the run for column equilibration. Total run time was 5.5 min, column temperature was maintained at 45 °C, and flow rate was set at 0.7 mL/min.

The mass spectrometer was operated in a positive ion mode using an atmospheric pressure chemical ionization (APCI) source for the analytes. The nebulizer, auxiliary, and curtain gases (nitrogen) were set at 50, 50, and 10 psi, respectively. Optimized nebulizer current and temperature were set at 3 μA and 400 °C, respectively. For collision-activated dissociation, nitrogen was used as collision gas at 4 psi. Declustering potential was set at 100 V for both analytes and IS. Optimized multiple reaction monitoring (MRM) fragmentation transitions were m/z 557 → 100 with a collision energy (CE) of 60 eV for dronedarone, m/z 501 → 114 with a CE of 48 eV for debutyldronedarone, and m/z 646 → 100 with a CE of 100 eV for IS. Dwell time for each transition was 200 ms.

2.4. Preparation of standards and quality control samples

Stock standard solutions of dronedarone and debutyldronedarone were prepared in methanol at 1.00 mg/mL for both solutions. Working solutions (0.200, 0.400, 1.00, 3.00, 10.0, 30.0, 80.0, and 200 ng/mL) of each analyte were prepared by diluting stock solutions with 50% aqueous methanol. Quality control (QC) samples were independently prepared in blank plasma at four different concentrations (LLOQ and low, medium, and high concentrations at 0.200, 0.500, 20.0, and 160 ng/mL for each analyte, respectively). A 5.00 ng/mL IS working solution was prepared by diluting 1.00 mg/mL stock solution of amiodarone with acetonitrile. All solutions were stored at 4 °C and were brought to room temperature before use. QC samples were stored at −20 °C.

2.5. Sample preparation

A 200-μL aliquot of IS solution (5.00 ng/mL amiodarone, with final plasma concentration of 20.0 ng/mL) and 50 μL of 50% aqueous methanol was added to 50 μL of plasma sample. The mixture was vortex-mixed for 1 min, followed by centrifugation at 11,000 × g for 5 min. The supernatant was then transferred to another tube and evaporated to dryness at 40 °C under a stream of air in the Turbo Vap evaporator (Zymark, Hopkinton, MA, USA). The residue was reconstituted in 150 μL of the initial mobile phase (5 mmol/L ammonium acetate solution/acetonitrile/acetic acid, 55:45:0.2, v/v/v) and an aliquot of 20 μL was injected into the LC–MS/MS system for analysis.

2.6. Method validation

The method was validated for selectivity, linearity, precision and accuracy, matrix effect, recovery, and stability according to FDA guideline [10].

Selectivity was evaluated by analyzing six different sources of human blank plasma and six spiked plasma samples at LLOQ level to test interference at the retention times of the analytes and the IS.

Linearity was assessed by plotting the peak area ratios of the analyte to the IS against the concentrations of analyte in human plasma using a linearly weighed ($1/x^2$) least squares regression method in duplicate on three consecutive validation days.

Precision and accuracy were determined by assessing six replicates of QC samples at 0.500, 20.0, and 160 ng/mL of both dronedarone and debutyldronedarone on three consecutive days. Intra- and inter-day precisions (relative standard deviation, RSD) were required to be below 15%, and accuracy (relative error, RE) within ±15%.

LLOQ was established by analyzing six blank plasma samples spiked with 0.200 ng/mL of both analytes with acceptable precision and accuracy (less than 20% for each criterion).

The matrix effect was quantitatively measured as the ratio of the peak area of the solution spiked with blank-processed plasma obtained from six individuals with the solutions containing equivalent amounts of analytes. In this study, the matrix effect of

dronedarone and debutyldronedarone was determined at two concentrations (0.500 and 160 ng/mL), while the matrix effect of the IS was determined at 20.0 ng/mL. Inter-subject variability of the matrix effect should be within ±15%.

Recoveries of dronedarone and debutyldronedarone at the concentrations of 0.500, 20.0, and 160 ng/mL ($n = 6$) were calculated by dividing the peak areas of analyte sample spiked before extraction by those of spiked after extraction. IS recovery was determined at 20.0 ng/mL similarly.

The stabilities of dronedarone and debutyldronedarone in human plasma at 0.500 and 160 ng/mL ($n = 3$) were assessed and were subjected to different conditions (time and temperature). Analytes were considered stable when accuracy bias was within ±15% of the initial concentration.

2.7. Application of method to a clinical pharmacokinetic study

The validated method was applied to determine dronedarone and debutyldronedarone concentrations in plasma following an oral administration to the participants. The pharmacokinetic study was approved by the Ethics Committee. After a single oral dose of dronedarone hydrochloride tablet containing 400 mg of dronedarone (Multaq®, Sanofi Aventis) to six healthy male Chinese volunteers, venous blood samples were collected in heparin-containing tubes at pre-dose (0 h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, and 96 h. Plasma samples were obtained by centrifugation at 2000 × *g* for 10 min and were stored at −20 °C prior to analysis. Pharmacokinetic parameters of dronedarone and debutyldronedarone were calculated by non-compartmental analysis using the WinNonlin 5.3 software (Pharsight, USA).

3. Results and discussion

3.1. Mass spectrometric condition

Dronedarone, debutyldronedarone, and amiodarone all have basic secondary or tertiary nitrogen groups in their structures and their p*K*a values were 9.4, 10.3, and 9.4, respectively (calculated by Advanced Chemistry Development (ACD/Labs) software, ADME Suite, Version 5.0). Since they are susceptible to exhibiting high mass spectrometric responses under a positive ion detection mode, the positive electrospray ionization (ESI) and APCI sources were evaluated in the current study. Signal responses for the analytes were found to be more stable with APCI than with ESI. Moreover, the utilization of the APCI interface led to elimination of the matrix effect when combined with acetonitrile protein precipitation. In the Q1 full scan mode, protonated molecules at *m*/*z* 557, *m*/*z* 501, and *m*/*z* 646 were observed for dronedarone, debutyldronedarone, and IS, respectively, and no adductive ions were detected. Fig. 1 presents the product ions spectra of $[M+H]^+$ ions from the analytes and IS, as well as their proposed fragmentation patterns. The most abundant fragment ions at *m*/*z* 100, 114, and 100 were chosen in the MRM transitions for dronedarone, debutyldronedarone, and IS, respectively.

3.2. Chromatographic condition

In our laboratory, an LC–MS/MS was established to simultaneously determine dronedarone and debutyldronedarone in dog plasma [9]. After protein precipitation, dronedarone, debutyldronedarone, and the IS (tolvaptan) were separated on a Capcell Pak C_{18} MG column using a mixture of acetonitrile–methanol–5 mmol/L ammonium acetate–acetic acid as the mobile phase. However, this method is not suitable for determining dronedarone and debutyldronedarone in human plasma. Inconsistent retention times from day-to-day and even from run-to-run were observed for IS. Therefore, the analogue amiodarone was chosen as the IS.

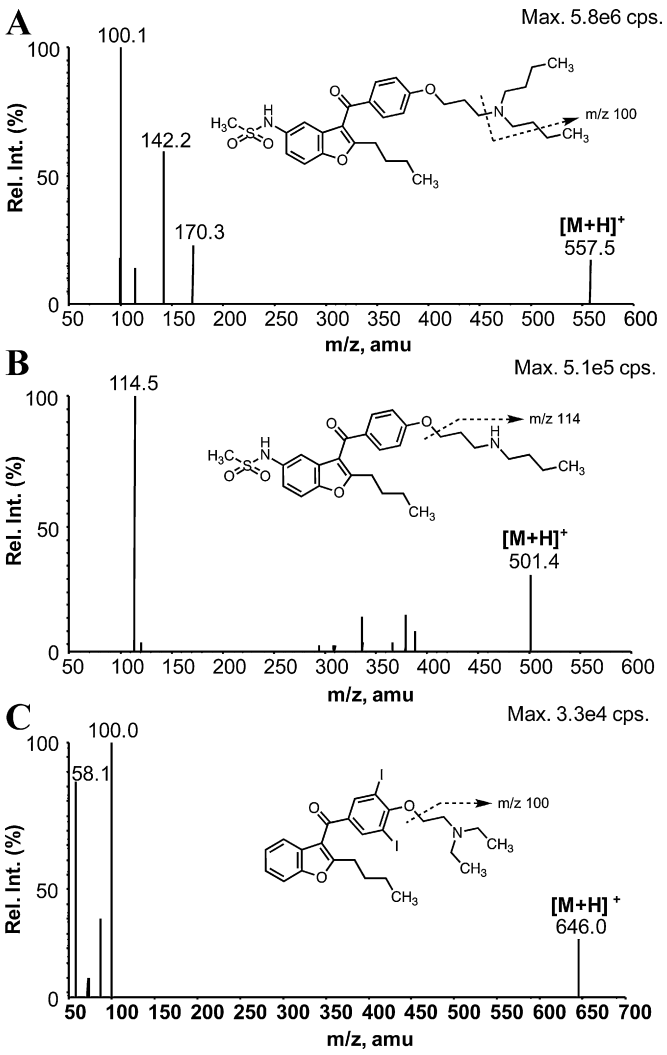

Fig. 1. Product ion spectra of $[M+H]^+$ of dronedarone (A), debutyldronedarone (B), and internal standard amiodarone (C).

In this study, higher mass spectrometric response and lower background noise were obtained using acetonitrile as the organic solvent content compared with methanol. log *P* values of dronedarone, debutyldronedarone, and amiodarone are 6.36, 4.97, and 7.41, respectively (calculated by Advanced Chemistry Development (ACD/Labs) software, ADME Suite, Version 5.0). During method development, significant differences in retention time were observed when isocratic elution of acetonitrile–water (60:40, v/v) was used for dronedarone, debutyldronedarone, and IS at 4.1, 2,7, and 8.9 min, respectively, due to their great differences in lipophilicity. Therefore, we utilized a gradient elution program. The mobile phase was composed of 5 mmol/L ammonium acetate solution (containing 0.2% acetic acid, A) and acetonitrile (containing 0.2% acetic acid, B). The ammonium acetate solution was found to be necessary to achieve good peak shape. Moreover, acetic acid did significantly improve sensitivity by promoting ionization of the analytes. We found that the analytical run time under optimized chromatographic conditions of each sample was 5.5 min and that the retention times of dronedarone, debutyldronedarone, and IS were 3.7, 3.3, and 4.3 min, respectively.

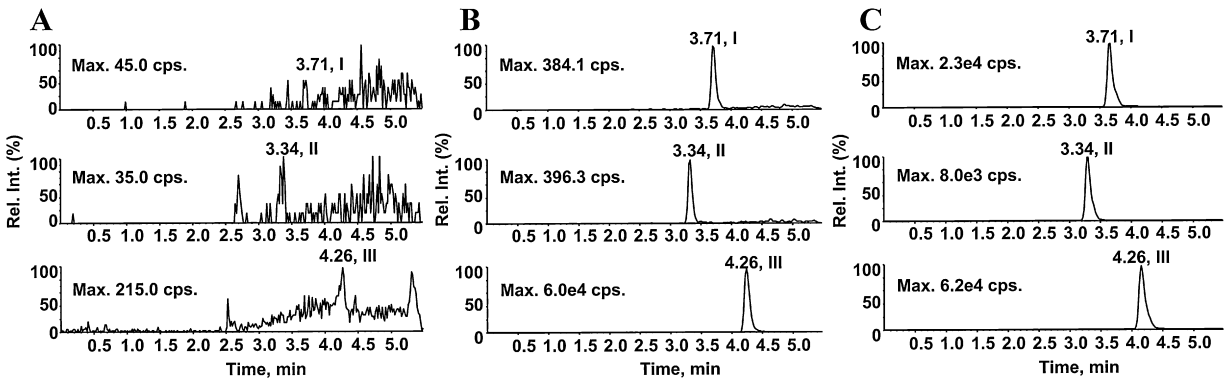

Fig. 2. Representative MRM chromatograms for dronedarone (I), debutyldronedarone (II), and amiodarone (IS, III) in human plasma: (A) blank plasma sample; (B) blank plasma sample spiked with dronedarone (0.200 ng/mL), debutyldronedarone (0.200 ng/mL), and IS (20.0 ng/mL); (C) plasma sample obtained at 2 h after oral administration of dronedarone hydrochloride tablet containing 400 mg of dronedarone (the measured concentrations for dronedarone and debutyldronedarone were at 13.2 and 4.94 ng/mL, respectively).

3.3. Sample preparation

In the HPLC–UV method described previously [7], a labor-intensive multistep extraction procedure was applied. Plasma was first precipitated with acetonitrile and the supernatant was evaporated to dryness. The residue was reconstituted with glycine buffer and was then extracted by a mixture of heptane and dichloromethane.

During method development, two sample preparation techniques were attempted: liquid–liquid extraction and protein precipitation. Low recovery and some interfering peaks for both analytes were found after liquid–liquid extraction with ethyl ether–dichloromethane (65:35, v/v). Protein precipitation by acetonitrile yielded consistent and high recovery rates without matrix interference, and could therefore provide a robust assay.

3.4. Method validation

3.4.1. Assay selectivity

Selectivity of the method was assessed by comparing the chromatograms of blank human plasma from six different sources with the corresponding spiked plasma at LLOQ concentration. Fig. 2 shows the typical chromatograms of a blank plasma sample, blank plasma sample spiked with dronedarone and debutyldronedarone at LLOQ and IS, and a plasma sample obtained 2 h after oral administration of 400 mg of dronedarone to a volunteer. No significant endogenous interference co-eluting with analytes and IS was observed in the blank human plasma.

3.4.2. Linearity of calibration curve and lower limit of quantification

Linear regression curves were obtained over the concentration range of 0.200–200 ng/mL for both analytes. The mean (±SD) regression equations from replicate calibration curves on three consecutive validation days were as follows:

$$\text{Dronedarone}: \quad y = (0.00494 \pm 0.00099)x + (0.0000718 \pm 0.0000331) \quad (r = 0.9994 \pm 0.0004);$$

$$\text{Debutyldronedarone}: \quad y = (0.00469 \pm 0.00212)x + (0.000124 \pm 0.0000649) \quad (r = 0.9991 \pm 0.0005);$$

where y is the peak area ratio of analytes to IS and x is the concentration of analytes.

LLOQs of both dronedarone and debutyldronedarone were at 0.200 ng/mL. In the current study, the intra- and inter-day precision of LLOQs for dronedarone was less than 9.5%, while accuracy was 3.2%. Intra- and inter-day precision of LLOQs for debutyldronedarone was less than 10.6%, with an accuracy of −0.6%. With the present LLOQ, the plasma concentration of dronedarone and debutyldronedarone could be determined for up to 96 h after oral administration of 400 mg of dronedarone to human volunteers, which was sensitive enough to allow for the investigation of the pharmacokinetic behavior of dronedarone.

3.4.3. Precision and accuracy

Intra- and inter-day precision and accuracy values for the QC samples are summarized in Table 1. In this assay, the intra- and inter-day precisions for dronedarone were less than 5.3%, while accuracy was within ±5.1. For debutyldronedarone, intra- and inter-day precisions were less than 7.2% and accuracy was within ±4.0%.

3.4.4. Matrix effect and recovery

In human plasma, the matrix effects of dronedarone were 104 ± 2.7% and 105 ± 0.8% at concentrations of 0.500 and 160 ng/mL, respectively. The matrix effects of debutyldronadarone were 105 ± 3.6% and 104 ± 1.4% at concentrations of 0.500 and 160 ng/ml, respectively. The matrix effect of IS was 107 ± 6.7%. Inter-subject variability of matrix effects never exceeded 5.9%, indicating that the method is not influenced by variations in the sample matrix.

Recovery levels of dronedarone were 105 ± 7.6%, 100 ± 0.8%, and 98.5 ± 1.0% at concentrations of 0.500, 20.0, and 160 ng/mL, respectively. Recoveries of debutyldronadarone were 105 ± 5.0%, 98.8 ± 0.9%, and 95.9 ± 1.3% at concentrations of 0.500, 20.0, and 160 ng/mL, respectively. Mean recovery for IS at 20.0 ng/mL was 95.2 ± 2.8%.

3.4.5. Stability

In this study, stability tests of dronedarone and debutyldronedarone were designed to simulate anticipated conditions of handling clinical samples, including being on bench top for 6 h, in the auto-sampler for 24 h, through three freeze–thaw cycles from −20 °C to ambient temperature, and a long period (20 days) of

Table 2
Stability of dronedarone and debutyldronedarone in human plasma under various storage conditions ($n = 3$).

Storage condition	Dronedarone			Debutyldronedarone		
	Concentration (ng/mL)		RE (%)	Concentration (ng/mL)		RE (%)
	Added[a]	Found[b]		Added[a]	Found[b]	
Benchtop, 6 h	0.495	0.500	1.1	0.499	0.519	4.0
	153	150	−1.7	151	158	4.6
Three freeze/thaw cycles	0.495	0.508	2.6	0.499	0.485	−2.7
	153	156	2.4	151	163	7.9
Autosampler rack, 24 h	0.500	0.500	0.1	0.500	0.468	−6.5
	160	159	−0.6	160	154	−3.8
−20 °C, 20 days	0.495	0.510	3.2	0.499	0.489	−1.9
	153	164	7.6	151	150	−0.4

[a] Concentration of each analyte measured in freshly processed plasma samples.
[b] Concentration of each analyte measured in samples under different storage conditions.

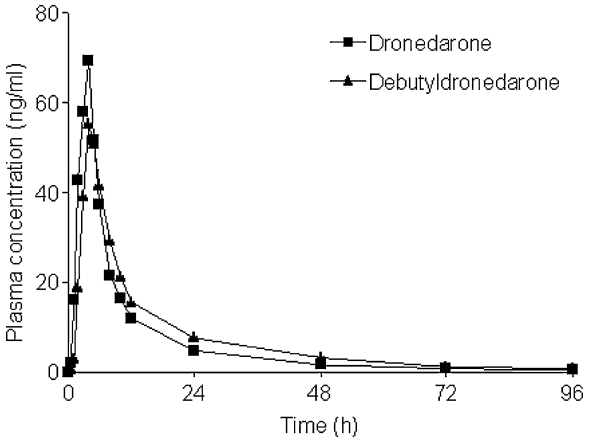

Fig. 3. Mean plasma concentration–time profiles of dronedarone and debutyldronedarone after an oral administration of 400 mg dronedarone to six healthy male Chinese volunteers.

storage at −20 °C. As shown in Table 2, more than 92.4% of initial concentrations were recovered for all post-storage samples. Findings indicate that both analytes are stable under the storage conditions described above.

3.5. Clinical application

The validated LC–MS/MS method has been successfully applied in the determination of the plasma concentrations of dronedarone and debutyldronedarone in six healthy male Chinese volunteers after a single oral administration of 400 mg of dronedarone. Mean plasma concentration–time profiles of dronedarone and debutyldronedarone is presented in Fig. 3.

4. Conclusion

We developed and validated a sensitive, accurate, and precise LC–MS/MS method for the simultaneous determination of dronedarone and its active metabolite debutyldronedarone in human plasma. The method has an LLOQ of 0.200 ng/mL for both analytes and a chromatographic run time of less than 5.5 min. Sample preparation was simple and quick with acetonitrile protein precipitation. This method provides superiority in sensitivity, analysis duration, and ease of sample preparation compared with the HPLC–UV method reported previously. The method could be applied as a routine assay in evaluating the exposure of dronedarone and debutyldronedarone in human pharmacokinetic studies.

Acknowledgment

This study was partly supported by the National Basic Research Program of China (2009CB930300).

References

[1] G.V. Naccarelli, D.L. Wolbrette, J.T. Dell'Orfano, et al., Clin. Cardiol. 23 (2000) 73.
[2] H.C. Van Beeren, W.H. Jong, E. Kaptein, et al., Endocrinology 144 (2003) 552.
[3] B.N. Singh, S.J. Connolly, H.J. Crijns, et al., N. Engl. J. Med. 357 (2007) 987.
[4] S.H. Hohnloser, H.J. Crijns, M. van Eickels, et al., N. Engl. J. Med. 360 (2009) 668.
[5] USFDA, 2011, http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/CardiovascularandRenalDrugsAdvisoryCommittee/UCM134981.pdf (accessed 19 June, 2011).
[6] USFDA, 2008, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079266.pdf (accessed 19 June, 2011).
[7] R.W. Boldermana, J.J. Hermansb, J.G. Maessena, J. Chromatogr. B 877 (2009) 1727.
[8] T. Damy, F. Pousset, H. Caplain, et al., Clin. Pharmacol. 18 (2004) 113.
[9] S. Yang, X. Dai, X. Tang, et al., Chin. J. Pharma 42 (2011) 7.
[10] USFDA, 2001, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf (accessed 19 June, 2011).