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Article

Optimization of Sample Pretreatment Methods for Simultaneous Determination of Dolasetron and Hydrodolasetron in Human Plasma by HPLC–ESI-MS

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A high-performance liquid chromatographic method coupled with electrospray mass spectrometry was developed for the simultaneous determination of dolasetron and its major metabolite, hydrodolasetron, in human plasma. A new sample pretreatment method, i.e., salt induced phase separation extraction (SIPSE), was proposed and compared with four other methods, i.e., albumin precipitation, liquid–liquid extraction, hydrophobic solvent-induced phase separation extraction and subzero-temperature induced phase separation extraction. Among these methods, SIPSE showed the highest extraction efficiency and the lowest matrix interferences. The extraction recoveries obtained from the SIPSE method were all more than 96% for dolasetron, hydrodolasetron and ondansetron (internal standard). The SIPSE method is also very fast and easy because protein precipitation, analyte extraction and sample cleanup are combined into one simple process by mixing acetonitrile with plasma and partitioning with 2 mol/L sodium carbonate aqueous solution. The correlation coefficients of the calibration curves were all more than 0.997, in the range of 7.9–4750.0 ng/mL and 4.8–2855.1 ng/mL for dolasetron and hydrodolasetron, respectively. The limits of quantification were 7.9 and 4.8 ng/mL for dolasetron and hydrodolasetron, respectively. The intra-day and inter-day repeatability were all less than 10%. The method was successfully applied to the pharmacokinetic study of dolasetron.

Introduction

Dolasetron (Figure 1) is a serotonin 5-HT3 receptor antagonist used to treat nausea and vomiting (1–5). After administration, dolasetron is quickly metabolized into hydrodolasetron (6–8).

For analysis of dolasetron and its metabolites, a number of methods have been reported, such as high-performance liquid chromatography with ultraviolet detection (HPLC–UV) (9), HPLC–fluorescence detection (FL) (10, 11), gas chromatography–mass spectrometry (GC–MS) (12) and HPLC–MS(13, 14). Among these reported methods, HPLC–MS has clear, and specific advantages over other methods for the pharmacokinetic study of these compounds. In 1999, Dimmitt *et al.* (13, 14) reported the first HPLC–electrospray ionization (ESI)-MS method to simultaneously analyze dolasetron and hydrodolasetron in plasma. The method had low limits of quantification (LOQ) of 0.73 ng/mL for dolasetron and 0.92 ng/mL for hydrodolasetron. Nevertheless, the pretreatment of sample was somewhat complicated because three steps were employed, i.e., liquid–liquid extraction (LLE), evaporation and reconstitution. Furthermore, extraction recoveries of the targets in those studies were not ideal (all less than 82%).

For the plasma analysis, the sample pretreatment is very important. Different extraction techniques such as LLE or solid-phase extraction are always employed to clean-up impurities. Induced-phase separation extraction (IPSE) of water-soluble organic solvent aqueous solution is a novel technique (15–21). The extraction recovery of IPSE was much higher than others and could not be duplicated, even if multiple stages were performed when using conventional LLE. Therefore, IPSE is a significant technique for sample pretreatment for plasma.

In this paper, a new salt-induced phase separation extraction (SIPSE) method was proposed for the analysis of dolasetron and hydrodolasetron in plasma by HPLC–ESI-MS. This method was compared with four other methods, i.e., albumin precipitation (AP), LLE, hydrophobic solvent-induced phase separation extraction (HSIPSE) and subzero temperature-induced phase separation extraction (STIPSE), in terms of extraction recovery, matrix effect and convenience of operation. The results are also significant to the dolasetron and hydrodolasetron analysis

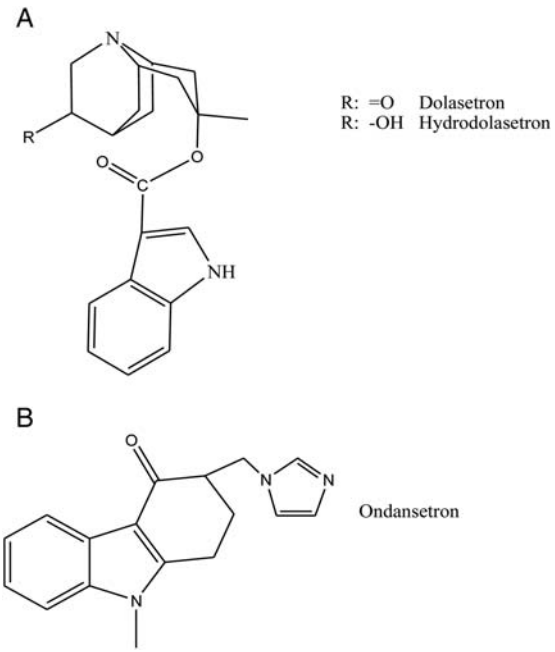

Figure 1. Structures of dolasetron, hydrodolasetron and ondansetron.

because reports about HPLC–MS analysis of the drugs in the existing literature are very limited.

Experimental

Chemicals and reagents

Standards of dolasetron mesylate and hydrodolasetron were obtained from Liaonin Haisike Pharmaceutical Co. (LiaoNin, China). Ondansetron (internal standard; IS) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade acetonitrile and methanol were purchased from Tedia Inc (Fairfield, OH). Ultra-pure water was obtained using a Millipore Milli-Q purification system (Millipore; Bedford, MA). Sodium carbonate was purchased from Tianheng Scientific Instruments Equipment Co. (Changsha, China). All other chemical reagents were of analytical grade and no further purification was required. Drug-free and drug-containing plasma were obtained from volunteers and stored below −40°C until analysis. The samples were collected in Xiangya Hospital, the protocol was approved by the Hospital Review Board for the clinical site and volunteers gave informed written consent before participating in the study.

Instrumentation

A Waters Alliance 2695 (Waters; MA) HPLC system interfaced to a 2487 UV detector (Waters) and a Micromass ZQ2000 electrospray mass spectrometer (Manchester, UK) were used. A high speed bench centrifuge (TG16, Changsha, China) was used for protein precipitation of the plasma sample. The biomedical freezer was from Sanyo Electric Biomedical Co.

HPLC–ESI-MS conditions

The chromatographic separation was carried out on an Ultimate XB-C18 analytical column (250 × 4.6 mm, Welch Materials; Shanghai, China) packed with 5-μm C18 silica. A Phenomenex C18 guard cartridge column (4 × 3 mm i.d.) was used to protect the analytical column. The mobile phase was a mixture of acetonitrile–0.8% formic acid aqueous solution–20 mmol/L ammonium formate aqueous solution (35:1:64, v/v/v). Elution was carried out under isocratic mode with flow rate set at 1.0 mL/min and column temperature maintained at 25°C.

The mass spectrometer was operated in positive ion mode over a range of m/z 100–500 amu, and selective ion monitors (SIMs) were set at m/z 325, 327 and 294 for dolasetron, hydrodolasetron and IS, respectively. The outlet of the UV detector was split, and only 0.2 mL/min of the column effluent was delivered into the ESI source. Nitrogen served as desolvation gas and cone gas with flow rates of 450 and 80 L/h, respectively. Capillary and cone voltages were 4.0 kV and 34 V, respectively. The desolvation temperature was set at 250°C and the ionization source operated at 135°C.

Standard solution and calibration curve

The standard stock solutions of dolasetron and hydrodolasetron were prepared as follows: 12.0 mg of dolasetron and 19 mg of hydrodolasetron were dissolved in 50 mL aqueous acetonitrile solution (acetonitrile–water, 1:1, v/v). In other words, corresponding concentrations of dolasetron and hydrodolasetron were 240 and 380 μg/mL, respectively. The standard stock solution of 16.0 μg/mL ondansetron (IS) was prepared in acetonitrile. All stock solutions were stored at −40°C before analysis.

A series of standard working solutions for the calibration curves of dolasetron and hydrdolasetron were prepared in the concentration ranges of 7.9–4750.0 and 4.8–2855.1 ng/mL, respectively, with drug-free plasma. Before being added into plasma, the IS working solution was prepared at a concentration of 1.6 μg/mL by diluting the standard stock solution with acetonitrile.

Quality control (QC) samples for dolasetron and hydrodolasetron were prepared at three different concentrations: 47.5 ng/mL (low), 475.0 ng/mL (medium), 2,375.0 ng/mL (high); and 28.5 ng/mL (low), 285.5 ng/mL (medium) and 1,427.5 ng/mL (high), respectively, in drug-free plasma. All QC samples were stored at −40°C until analysis.

Sample pretreatment

Different sample pretreatment methods were investigated. The detailed procedures were as follows.

AP method

Ten microliters of 1.6 μg/mL IS and 0.4 mL methanol were added into 0.2 mL of plasma. The mixture was then vortexed in a polypropylene tube on a vortex mixer for approxmiately 30 s. After this, the mixture was centrifuged at 13,400 rpm for 15 min, then 200 μL of the supernatant was transferred into separate autosampler vials. Sequentially, 10 μL of the supernatant was injected into the HPLC–ESI-MS system.

Conventional LLE

LLE was performed in the same manner as the method reported in the literature (13). First, the analytes were extracted with ethylacetate–*n*-hexane (3:1, v/v) from 0.2 mL of plasma and then the organic phase was back-extracted with 0.1 mol/L hydrochloric acid. Next, the analytes were again extracted into ethylacetate–*n*-hexane. Finally, the organic phase were evaporated with a stream of nitrogen and redissolved with mobile phase.

HSIPSE

A 0.2-mL plasma sample containing IS was mixed well with 0.2 mL of acetonitrile, and then 0.02 mL of chloroform was added. After mixing and centrifugation, clear phase separation of the mixed solution was obtained. One hundred microliters of the separated acetonitrile-rich upper phase was transferred into autosampler vials, and 10 μL of this was injected into the HPLC–ESI-MS system.

STIPSE

Plasma samples were thawed at room temperature and vortexed to ensure homogeneity of the samples. After 0.2 mL of plasma was added into a polypropylene tube, 10 μL of IS standard working solution (1.6 μg/mL) and 0.2 mL of acetonitrile were added. The mixture was then vortexed on a vortex mixer for approximately 30 s. After this, the mixture was centrifuged at

13,400 rpm for 5 min and frozen at −40°C for 8 min, and 100 μL of the separated acetonitrile-rich upper phase was transferred into autosampler vials. Ten microliters of acetonitrile-rich phase was injected into the HPLC–ESI-MS system.

SIPSE

Plasma samples were thawed at room temperature and vortexed to ensure homogeneity of the samples. After 0.2 mL of plasma was added into a polypropylene tube, 10 μL of IS standard working solution (1.6 μg/mL), 0.2 mL of sodium carbonate aqueous solution (2 mol/L) and 0.4 mL of acetonitrile were added in sequence. The mixture was then vortexed on a vortex mixer for approximately 30 s. After this, the mixture was centrifuged at 13,400 rpm for 5 min. The mixture was separated into two phases with a clear interface. One hundred microliters of the upper phase (acetonitrile-rich phase) was transferred into autosampler vials and 10 μL of the upper phase was injected into the HPLC–ESI-MS system. The final concentration of IS in the acetonitrile-rich phase was 39.0 ng/mL.

Results and Discussion

Matrix effects and extraction recoveries of different sample pretreatment methods

To evaluate the matrix effects on ESI-MS responses of two analytes and IS, the sample solutions were compared with a standard solution prepared with pure acetonitrile. Blank extract solutions were prepared from blank plasma processed by the different methods described previously. These extract solutions were used to prepare post-extraction spiked sample solutions: 10 μL of each standard solution prepared with pure acetonitrile with an appropriate concentration were mixed with 190 μL of each extract solution to result in spiked extract samples containing 0.95 μg/mL of dolasetron, 1.2 μg/mL of hydrodolasetron and 0.8 μg/mL of IS ($n = 5$ for each). Furthermore, a set of standard solutions prepared in pure acetonitrile (neat standard solution) with the same concentrations as described earlier were used as references. Then, the same volume of each solution was injected into the HPLC–MS system. Matrix effect was calculated by the following formula:

$$\text{matrix effect\%} = \frac{\text{mean post extraction peak area} - \text{mean neat solution peak area}}{\text{mean neat solution peak area}} \times 100\%$$

Positive and negative values indicate enhancement and suppression of the matrix on the ESI response, respectively. The results of matrix effects for different sample pretreatment methods are listed in Table I. The results showed that the values of matrix effect with SIPSE for dolasetron at 0.95 μg/mL, hydrodolasetron at 1.2 μg/mL and IS at 0.8 μg/mL ranged from −0.46 to 3.67%; therefore, the method is obviously superior to others.

The extraction recoveries of different methods are listed in Table I. The results show that SIPSE has the highest extraction recovery, which is higher than 96%. Compared with other methods, SIPSE is simpler and more environmental friendly than all of the others except the AP method. Compared with the AP method, SIPSE had better cleanup effect. Figure 2A shows the chromatograms detected at UV 280 nm of sample solutions prepared by the AP and SIPSE methods. The detector response results (the ordinate) show that the sample solution obtained by AP still contains a higher content of impurities,

Table I
Matrix Effect and Extraction Recovery of the Methods ($n = 5$)

		Matrix effect (%)	Extraction recovery (%)
AP	Hydrodolasetron	−5.64	89.2
	Ondansetron	−7.38	88.7
	Dolasetron	−5.23	93.5
LLE	Hydrodolasetron	−6.67	82.5
	Ondansetron	−5.95	79.8
	Dolasetron	−4.51	83.6
STIPSE	Hydrodolasetron	−0.39	68.7
	Ondansetron	−1.76	63.5
	Dolasetron	1.17	70.0
HSIPSE	Hydrodolasetron	6.03	47.6
	Ondansetron	3.05	50.2
	Dolasetron	2.67	53.1
SIPSE	Hydrodolasetron	1.45	96.7
	Ondansetron	3.67	99.2
	Dolasetron	−0.46	98.9

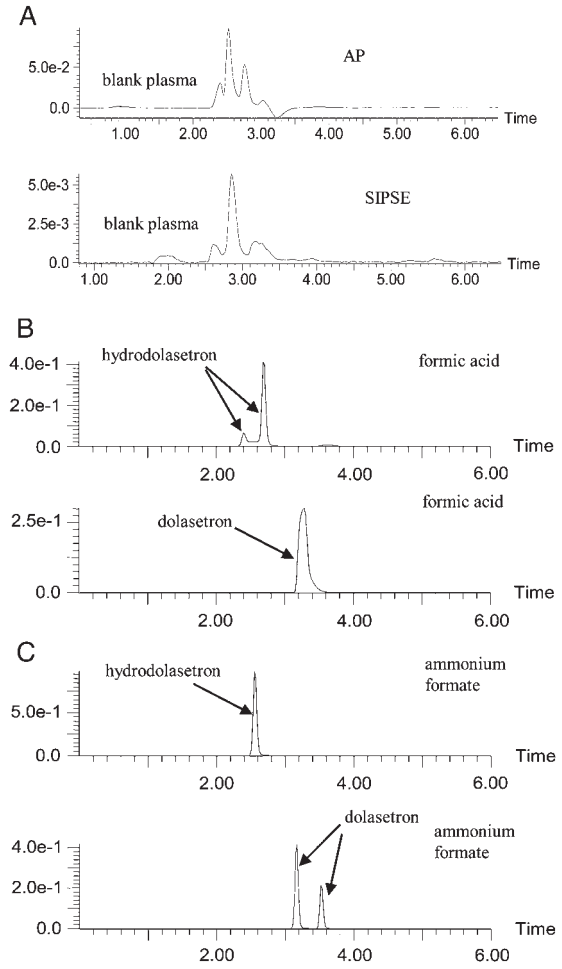

Figure 2. The UV chromatograms of blank plasma with AP and SIPSE (A); The UV chromatograms of hydrodosetron and dolasetron using formic acid and ammonium formate as a mobile phase modifier, respectively (B, C). (λ = 280 nm).

which is almost 10 times of that obtained by SIPSE. Although these endogenous impurities did not affect the determination of analytes because of differences in retention times, they could deteriorate the column.

The comprehensive comparison results show that the SIPSE method is the optimal pretreatment method for the simultaneous determination of dolasetron and hydrodolasetron in human plasma by HPLC–ESI-MS.

Optimization of SIPSE

To optimize the SIPSE method, the effects of the type of organic solvent, type of salt and concentration of salt on the extraction recovery were investigated. The organic solvents, including methanol, acetonitrile, ethanol and acetone, were assessed. Except for acetonitrile, the organic solvents could not simultaneously achieve satisfactory phase separation and high extraction recovery with water. For the acetonitrile aqueous solution, ammonium formate, ammonium acetate, sodium chloride and sodium carbonate used as inducers for the phase separation were investigated. Although sodium chloride, ammonium formate and ammonium acetate could induce the homogeneous acetonitrile aqueous solution system into phase separation, the extraction recoveries of dolasetron, hydrodolasetron and IS were low (less than 50%). On the other hand, sodium carbonate could induce phase separation at a wide concentration range (0.1–2 mol/L) and resulted in a higher extraction recovery for the analytes, with the highest recoveries (all more than 95%) obtained when using 2 mol/L sodium carbonate solution. When replacing sodium carbonate with ammonium hydroxide, the phenomenon of phase separation disappeared.

Optimization of HPLC–MS conditions

To optimize the separation of dolasetron, hydrodolasetron and ondansetron (IS), different columns, i.e., Ultimate-XB C18 (250 × 4.6 mm, Welch Materials), Johnsson Spherigel C18 (200 × 4.6 mm, Dalian Johnsson Separation Science & Technology Corporation; China) and Phenomenex C18 (250 × 4.6 mm, Phenomenex Scientific Instrument Co.), were compared under mobile phase conditions of 35:1:64 (v/v/v) of a mixture of acetonitrile–0.8% formic acid–20 mmol/L ammonium formate using isocratic mode. The results are listed in Table II, which shows that the Ultimate-XB C18 column provided the best peak shape of the analytes and IS. This may be because the Ultimate-XB C18 column has the highest carbon loading and fewer acidic silanol groups on the silica base, which result in weaker secondary interactions. Therefore, the Ultimate-XB C18 column was selected as the analytical column.

The ESI process is highly complex. Many characteristics of the solvents and additives, such as volatility and viscosity, can influence ionization process and thereby the signal response. A free selection of mobile phase composition in LC–ESI-MS is not possible because only polar solvents and volatile additives can be used in practice. The selection of the mobile phase in the development of an LC–ESI-MS method must often be balanced between ESI response and LC separation efficiency. Methanol and acetonitrile used as organic modifiers of the mobile phase were compared on the separation and ESI response. The MS response of the analytes in methanol is 1.5 times higher than that in acetonitrile on average. Although a higher ionization efficiency was obtained in methanol than in acetonitrile, the chromatographic separation was worse in methanol than in acetonitrile, i.e., complete separation of dolasetron and hydrodolasetron could not be achieved within 10 min. Thus, acetonitrile was used as the organic modifier of the mobile phase.

When the mobile phase was composed of only acetonitrile and water, the analytes were retained strongly in the column and formed a wide tailing peak of the analyte, owing to switching between the forms of ion and molecule of analyte. To improve the separation and MS response of analytes, it is necessary to add some modifier into the mobile phase. In this experiment, different modifiers, i.e., formic acid, acetic acid, ammonium formate, ammonium acetate, trifluoroacetic acid and triethylamine were investigated. The addition of formic acid and acetic acid resulted in good peak shapes of dolasetron, whereas hydrodolasetron was detected as twin peaks, as shown in Figure 2B. A reverse result was observed by addition of ammonium formate and triethylamine, i.e., good peak shape of hydrodolasetron was achieved, whereas dolasetron was detected as twin peaks (Figure 2C). The cause for this phenomenon was unclear, although a similar phenomenon was reported in a previous study (16). When formic acid and ammonium formate were simultaneously added to the mobile phase, good separation of dolasetron and hydrodolasetron was achieved without the twin peaks phenomenon. Therefore, the mixture of 0.8% formic acid aqueous solution–20 mM aqueous ammonium formate solution–acetonitrile (1:64:35, v/v/v) was chosen as the optimal mobile phase (pH = 5.08). In addition, because ondansetron has a similar structure and could be completely separated with hydrodolasetron and dolasetron, it was used as the IS in this method.

The parameters of MS, i.e., ionization mode, capillary voltage, cone voltage and source temperature, were optimized by

Table II
Parameters and Performances of Different Columns

Column	Carbon loading (%)	Particle size (μm)	Resolution*			Tailing factor*		
			Hydrodolasetron	Ondansetron	Dolasetron	Hydrodolasetron	Ondansetron	Dolasetron
Ultimate XB-C18	17	5	3.716	2.405	1.704	1.146	1.115	1.108
Phenomenex C18	10	5	3.431	2.423	1.356	1.543	1.625	1.865
Spherigel C18	12.5	5	4.345	2.645	1.432	1.367	1.531	1.365

*The results were obtained automatically by Shimadzu LCsolution workstation software (Japan) from the real chromatogram of a mixed standard solution.

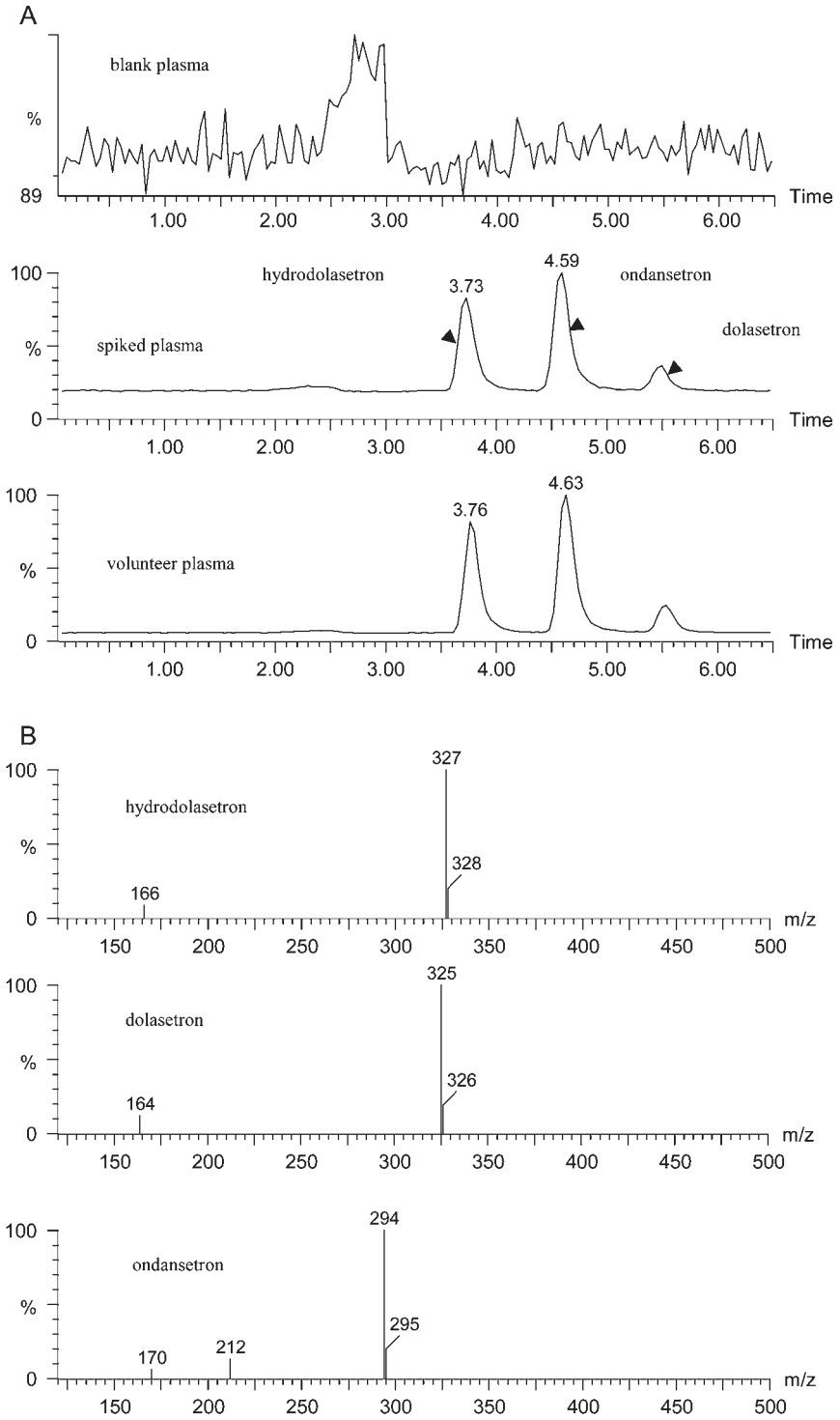

Figure 3. SIM chromatograms of samples (A) and mass spectrums of targets (B).

flow injection analysis (FIA) with dolasetron and hydrodolasetron standards at concentrations of 14.2 and 10.5 μg/mL, respectively. In the positive ionization mode, a better MS sensitivity was obtained for dolasetron, hydrodolasetron and IS; the protonated molecular ions ($[M + H]^+$) of dolasetron, hydrodolasetron and IS were detected at m/z 325, 327 and 294, respectively. To optimize cone voltage, different cone voltages increasing from 5 to 50 V, step by step at a 1-V interval, were investigated. The capillary voltage was investigated in the range from 2.0 to 5.0 kV. The results implied that the best choices of capillary and cone voltage were 4.0 kV and 34 V, respectively. The MS signal was stable and abundant when

source temperature was set at 135°C after investigating in the range of 90–150°C.

Assay validation

Linearity and limit of quantification

A calibration curve was constructed by plotting the peak-area ratios of the response of dolasetron and hydrodolasetron to IS versus the concentrations of the calibration standards. The calibration curves showed a good linearity in the concentration range of 7.9–4,750.0 and 4.8–2,855.1 ng/mL for dolasetron and hydrodolasetron, respectively. The correlation coefficients (r^2) were more than 0.997. The LOQs of the method were 7.9 and 4.8 ng/mL for dolasetron and hydrodolasetron, respectively [signal-to-noise (S/N) = 10:1; relative standard deviation (RSD), 15.16 and 3.16% for dolasetron and hydrodolasetron, respectively].

Specificity

The specificity was evaluated by comparing the chromatogram of blank human plasma with that spiked with analytes and IS at a proper concentration. Under the optimized conditions, retention times of dolasetron, hydrodolasetron, and ondansetron were 5.53, 3.73 and 4.59 min, respectively (Figure 3). No endogenous interferences were observed at the retention time of the analytes and IS in SIM mode in blank human plasma.

Accuracy and precision

The accuracy and the precision of the method were confirmed by five replicate determinations of drug-free plasma spiked with dolasetron and hydrodolasetron at three concentration levels (47.5, 475.0 and 2,375.0 ng/mL; and 28.5, 285.5 and 1,427.5 ng/mL for dolasetron and hydrodolasetron, respectively). The data are listed in Table III. The RSD values were less than 10% for precision of inter-day and intra-day precision and accuracy, which implied that the method is repeatable and accurate.

Extraction recovery

The extraction recovery was measured as follows: the upper phase was injected to the HPLC–ESI-MS for analysis after spiked plasma samples (containing 0.95 μg/mL of dolasetron, 1.2 μg/mL of hydrodolasetron and 0.8 μg/mL of IS, $n = 3$ for each) were treated with the SIPTE technique. Blank extract solutions were obtained with the SIPTE technique and used to prepare postextraction spiked samples at the same concentrations, and these samples were used as the contrast samples. Extraction recovery was calculated by comparing peak area of the spiked plasma sample with the contrast sample. The results showed that the mean extraction recovery for dolasetron, hydrodolasetron and IS was 98.5, 96.7 and 99.4%, with RSD below 10% for all samples.

Stability

The three concentration levels (28.5, 285.5 and 1,427.5 ng/mL for hydrodolasetron; 47.5, 475.0 and 2,375.0 ng/mL for dolasetron) of spiked samples were analyzed after storage at ambient temperature for 12 h, three freeze and thaw cycles and a lengthy period (29 days) frozen in the refrigerator at −40°C.

Table III
Intra-Day and Inter-Day Precision and Accuracy ($n = 5$)

		Added (ng/mL)	Measurement, mean ± SD (ng/mL)	RSD (%)	Accuracy (%)
Dolasetron	Intra-day	47.5	47.2 ± 2.0	4.24	99.4
		475.0	474.5 ± 11.8	2.49	99.9
		2375.0	2,430.2 ± 37.5	1.54	102.3
	Inter-day	47.5	46.8 ± 2.7	5.77	98.5
		475.0	489.6 ± 26.8	5.47	103.1
		2375.0	2,313.1 ± 97.4	4.21	97.4
Hydrodolasetron	Intra-day	28.5	25.9 ± 0.8	3.09	90.7
		285.5	254.3 ± 5.7	2.24	89.1
		1427.5	1,330.6 ± 63.2	4.75	93.2
	Inter-day	28.5	26.4 ± 1.1	4.17	92.5
		285.5	263.0 ± 10.8	4.11	92.1
		1427.5	1,388.8 ± 84.3	6.10	97.3

Table IV
Stability of Dolasetron and Hydrodolasetron in Plasma ($n = 5$)*

Stability test			Nominal concentration (ng/mL)		
			28.5/47.5	285.5/475.0	1,427.5/2,375.0
Short-term stability (12 h)	Dolasetron	Mean ± SD	47.8 ± 4.3	488.5 ± 22.8	2,270.7 ± 55.5
		RSD (%)	8.99	4.67	2.44
	Hydrodolasetron	Mean ± SD	26.4 ± 1.5	264.7 ± 10.0	1,356.8 ± 35.8
		RSD (%)	5.68	3.78	2.64
Freeze-thaw stability (three cycles)	Dolasetron	Mean ± SD	48.6 ± 2.4	474.4 ± 10.3	2,265.9 ± 49.2
		RSD (%)	4.93	2.17	2.17
	Hydrodolasetron	Mean ± SD	25.9 ± 1.3	257.7 ± 5.2	1,356.6 ± 36.7
		RSD (%)	5.02	2.02	2.71
Long-term stability (29 days)	Dolasetron	Mean ± SD	44.1 ± 1.7	464.2 ± 10.7	2,353.6 ± 98.3
		RSD (%)	3.85	2.31	4.18
	Hydrodolasetron	Mean ± SD	24.8 ± 1.6	262.8 ± 8.3	1,419.0 ± 94.4
		RSD (%)	6.45	3.16	6.65

*Note: when analyzing dolasetron, the three concentration levels are 47.5, 475.0 and 2375.0 ng/mL; when analyzing hydrodolasetron, the three concentration levels are 28.5, 285.1 and 1,427.5 ng/mL.

The stability data of dolasetron and hydrodolasetron are listed in Table IV. The results implied that dolasetron and hydrodolasetron in plasma were stable under the various investigated conditions.

Application

This method was applied to the determination of dolasetron and hydrodolasetron in human plasma samples for a pharmacokinetic study. Mean plasma concentration-time profiles for dolasetron and hydrodolasetron, obtained after intravenous injection at three doses, 50, 100 and 150 mg, into 12 volunteers, are shown in Figure 4. Dolasetron was quickly eliminated from human plasma after administration via intravenous injection, with an elimination half-life ($t_{1/2}$) of less than 10 min; the results were in agreement with the data previously reported by Dimmitt *et al.* (13, 14).

Conclusions

The proposed HPLC–ESI-MS method was accurate, precise and sensitive for the simultaneous determination of dolasetron and its major metabolite, hydrodolasetron, in human plasma in accordance with Food and Drug Administration criteria (22). It

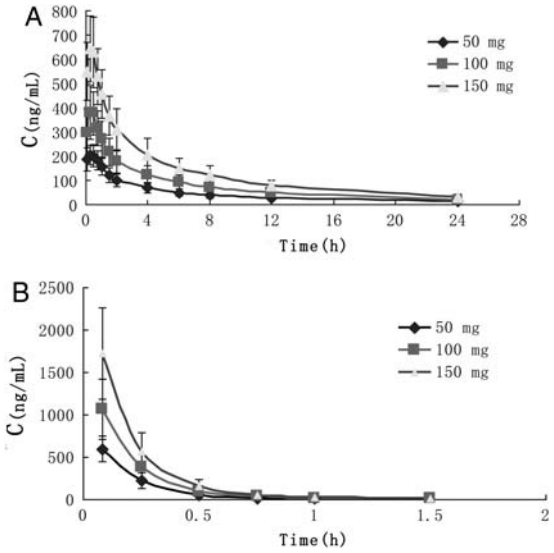

Figure 4 Mean plasma concentration-time curves for hydrodolasetron (A) and dolasetron (B) after intravenous injection.

has been successfully applied to the pharmacokinetic study of dolasetron.

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