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Determination of vinpocetine and its primary metabolite, apovincaminic acid, in rat plasma by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A precise and sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for simultaneous determination of vinpocetine (VP) and its primary metabolite, apovincaminic acid (AVA), in rat plasma was developed and validated. The analytes and the internal standard-dimenhydrinate were extracted from 50 μL aliquots of rat plasma via solid–liquid extraction. Chromatographic separation was achieved in a run time of 3.5 min on a C_{18} column under isocratic conditions. Detection of analytes and IS was done by tandem mass spectrometry, operating in positive ion and multiple reaction monitoring (MRM) acquisition mode. The protonated precursor to product ion transitions monitored for VP, AVA and IS were m/z 351.4 → 280.2, 323.2 → 280.2 and 256.2 → 167.3 respectively. The method was fully validated for its sensitivity, selectivity, accuracy and precision, matrix effect, stability study and dilution integrity. A linear dynamic range of 0.5–500 ng/mL for both VP and AVA was evaluated with mean correlation coefficient (r) of 0.9970 and 0.9984 respectively. The precision of the assay (RSD%) was less than 8.55% at all concentrations levels for both VP and AVA. This method was successfully applied to a pharmacokinetic study of VP in rats after intravenous (1 mg/kg) and oral (1 mg/kg) administration.

1. Introduction

Vinpocetine (VP), ethyl (3a,16a)-eburnamenine-14-carboxylate, is a semi-synthetic derivative of the Vinca minor (common periwinkle) plant alkaloid, vincamine. The drug improves the cerebral utilization of oxygen and protects the brain cells against ischaemic anoxia [1]. It dilates the cerebral blood vessels and increases the cerebral blood flow [2–4]. It has been used in cerebrovascular and cognitive disorders [5–8]. In human vinpocetine is rapidly absorbed and undergoes extensive metabolism, during which approximately 75% of the substance is hydrolysed into its main active metabolite (3a,16a)-eburnamenine-14-carboxylic acid (apovincaminic acid, AVA), leading to an absolute bioavailability of (6.2 ± 1.9)% in man [9]. Since VP and AVA have significant association of those diseases which have be talked above, the analysis of VP and AVA in plasma is of great clinical importance.

In order to perform pharmacokinetic studies, there is a need for analytical methods that quantify VP and AVA in plasma. The pharmacokinetic investigations of VP have been conducted by direct mass fragmentography [10,11], HPLC [12–15], sensitive enzyme immunoassay [16], gas chromatography (GC) with nitrogen–phosphorus detection [17–21] and GC–MS methods [22–24,25–27]. Also, several methods have been developed for the determination of AVA. GC methods based on prior derivatization had been used for the determination of AVA in biological samples [28,29]. While determination of AVA by HPLC with ultraviolet detection [30–33] and LC–MS [34] was more common. However, all these methods suffered from various drawbacks namely sensitivity, long chromatographic run times, large sample volume for processing or a cumbersome extraction procedure, which prevented their use for routine biological sample analysis. To our knowledge, simultaneous analysis of VP and its active metabolite AVA in biological fluids by HPLC have not been reported. It is well known that the use of mass spectrometry interfaced with HPLC helps to improve the selectivity and sensitivity compared to traditional HPLC–UV and GC methods. Thus, the aim of the present study was to develop and validate more sensitive, specific and rapid method for the simultaneous estimation of VP and its active metabolite AVA in rat plasma by LC–MS/MS. Also, the method should be simple, rugged and suitable for routine measurement of biological samples for pharmacokinetic study.

2. Experimental

2.1. Reagents and chemicals

VP and AVA were obtained from Gedeon Richter Ltd. (Budapest, Hungary). Dimenhydrinate, used as internal standard (IS), was obtained from National Institute for the Control of Pharmaceu-

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tical and Biological Products (Beijing, China). The purity of AVA, VP and IS were all >99.5% (as determined by HPLC). HPLC grade methanol was purchased from Tedia Company (Fairfield, OH, USA). Ammonium acetate and formic acid of AR grade were purchased from Shanghai Chemical Reagent Company (Shanghai, P.R. China). Other chemicals were of analytical reagent grade and purchased from commercial sources. Double distilled water was purified by Millipore Simplicity™ (Millipore, Bedford, MA, USA).

2.2. Instrument

An Agilent 1100 system consisting of a G1312A quaternary pump, a G1379A vacuum degasser, a G1316A thermostated column oven (Agilent, Waldbronn, Germany) and a HTS PAL autosampler (CTC Analytics, Switzerland) was used. Mass spectrometric detection was performed on an API 3000 triple quadrupole instrument (Applied Biosystems, Toronto, Canada) in multiple reaction monitoring (MRM) mode. A TurboIonSpray ionization (ESI) interface in positive ionization mode was used. Data processing was performed with Analyst 1.4.1 software package (Applied Biosystems).

2.3. Chromatographic conditions

The chromatographic separation was achieved on a Gemini C18 column (100 mm × 2.0 mm i.d., 3.0 μm, Phenomenex, Torrance, CA, USA) with a SecurityGuard C18 guard column (4 mm × 3.0 μmm i.d., Phenomenex, Torrance, CA, USA). A mixture of methanol–2 mM ammonium acetate–formic acid (40:60:0.1, v/v/v) was used as mobile phase at a flow rate of 0.3 mL/min. The temperature of column and autosampler were maintained at 40 and 4 °C respectively. The chromatographic run time of each sample was 3.5 min.

2.4. Mass spectrometric conditions

The mass spectrometer was operated using ESI source in the positive ion detection. Quantitation was done using multiple reaction monitoring (MRM) mode to monitor protonated precursor → product ion transition of m/z 351.4 → 280.2 for VP, 323.2 → 280.2 for AVA and 256.2 → 167.3 for IS (Fig. 1). All the parameters of LC and MS were controlled by Analyst software version 1.4.1.

Turbo spray voltage (IS) was set at 5500 V. Source temperature was maintained at 500 °C. Entrance potential (EP) was set at 10 V. Nitrogen was used as nebulizing gas (8 L/min) and curtain gas (8 L/min). For collision activated dissociation (CAD), nitrogen was employed as the collision gas at a pressure of 4 L/min.

The compound dependent parameters like declustering potential (DP), focusing potential (FP), collision energy (CE) and cell exit potential (CXP) were optimized at 75, 250, 43 and 20 V for VP, 65, 200, 39 and 18 V for AVA and 31, 150, 15 and 11 V for IS respectively. Quadrupole 1 and quadrupole 3 were maintained at unit resolution. Dwell time set was 200 ms for all the analytes.

2.5. Preparation of standard stocks and plasma samples

The standard stock solutions of 1 mg/mL for VP, 1 mg/mL for AVA and 1 mg/mL for IS were prepared by dissolving requisite amount of VP, AVA and IS in methanol respectively. The combined working solution of VP (100 μg/mL) and AVA (100 μg/mL) was prepared from the stock solutions by diluting 1.0 and 1.0 mL respectively in 10 mL volumetric flask using methanol:water (50:50, v/v). This combined working solution was used for spiking plasma calibration and quality control samples using methanol:water (50:50, v/v). IS working solution of 100 ng/mL was prepared using the stock of 1 mg/mL in deionized water. All the standard stock, intermediate stock and working stock solutions were prepared and stored at 4 °C until use. Drug free plasma, i.e. control (blank) plasma was withdrawn from the deep freezer and allowed to get completely thawed before use. The calibration standards (CS) and quality control (QC) samples (LQC, low quality control; MQC, middle quality control; HQC, high quality control) were prepared by spiking 100 μL of blank plasma with 5 μL of combined working solution (5% of total volume of plasma). Calibration standards were made at 0.5, 1, 5, 10, 50, 100, 500 ng/mL for VP and AVA. Quality controls were similarly prepared at 0.5 ng/mL (LOQ), 10 ng/mL (MQC), 500 ng/mL (HQC) for VP and AVA. The spiked plasma samples at all the levels were stored at −20 °C for validation and subject sample analysis.

2.6. Sample preparation

A 12-tube solid-phase extraction vacuum manifold (Agilent, USA) was used for sample preparation. Satisfactory values for recovery of both VP and AVA were obtained with a single extraction with OASIS® HLB solid-phase extraction cartridge (1 cc, 30 mg, Waters Corporation) for isolation of the analytes and the internal standard from plasma samples. Before sample preparation, the cartridge was preconditioned with 1.0 mL of methanol followed by 1.0 mL of water. For sample preparation, 50 μL aliquot of plasma was added into a 0.5 mL Eppendorf microvial, then 10 μL of phosphoric acid (20%, v/v) and 50 μL of IS solution were added. The resulting solution was vortexed and centrifuged for exactly 5.0 min at 10,000 × g at 4 °C. The spiked sample (approximate volume 0.11 mL) was introduced into the cartridge under vacuum. The cartridge was washed with 0.5 mL of water. VP, AVA and IS were then subsequently desorbed with 1.0 mL of methanol. The eluted solution was evaporated to dryness in a water bath at 40 °C under nitrogen steam and the residue reconstituted in 50 μL of mobile phase. The resulting solutions were carefully vortexed and centrifuged at 10,000 × g for 10 min at 4 °C then a volume of 10 μL was subsequently injected into the chromatographic system for LC–MS/MS analysis.

2.7. Methodology for validation

A thorough and complete method validation of VP and AVA in rat plasma was validated for selectivity, sensitivity, linearity, precision and accuracy, recovery, matrix effect, ion suppression/enhancement, cross-specificity, stability and dilution integrity.

Test for selectivity was carried out in 6 different lots of blank plasma (with heparin sodium as anticoagulant), processed by the same extraction protocol and analysed to determine the extent to which endogenous plasma components may contribute to the interference at the retention time of analytes and the internal standard.

The linearity of the method was determined by analysis of standard plots associated with a seven-point standard calibration curve. Five linearity curves containing seven non-zero concentrations were analysed. Best-fit calibration curves of peak area ratio versus concentration were drawn. The concentration of the analytes were calculated from calibration curve ($y = mx + c$, where y is the peak area ratio) using linear regression analysis with reciprocate of the drug concentration as a weighing factor ($1/x^2$) for VP and AVA. The regression equation for the calibration curve was also used to back-calculate the measured concentration at each QC level. The peak area ratio values of calibration standards were proportional to the concentration of the analytes in plasma over the range tested. Intra-batch and inter-batch (on five consecutive days) accuracy and precision were evaluated at three different concentrations levels (LQC, MQC and HQC) in five replicates for both the analytes. Mean values were obtained for calculated analytes concentration over these batches. The accuracy and precision were calculated and

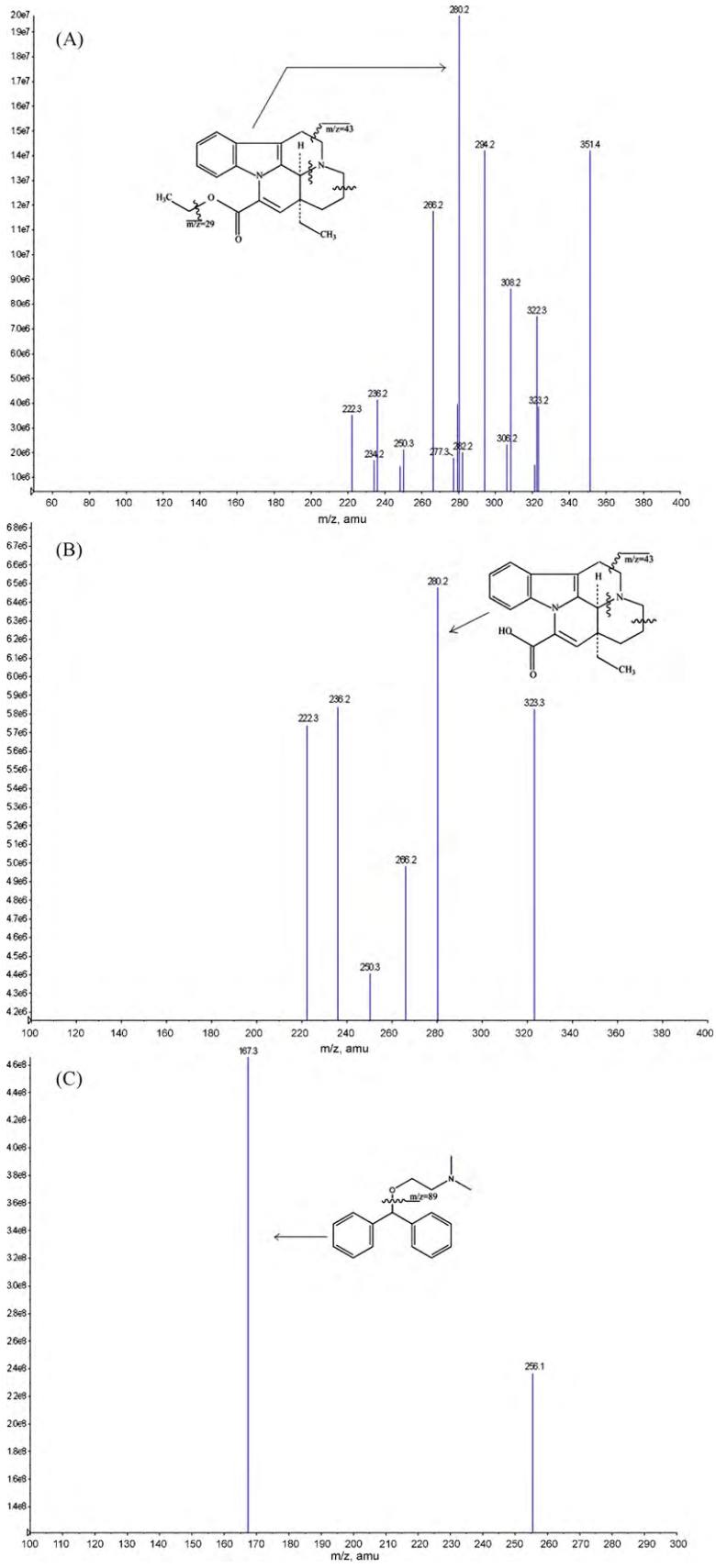

Fig. 1. Product ion spectra of (A) VP (351.4 → 280.2), (B) AVA (323.2 → 280.2) and (C) IS (256.2 → 167.3).

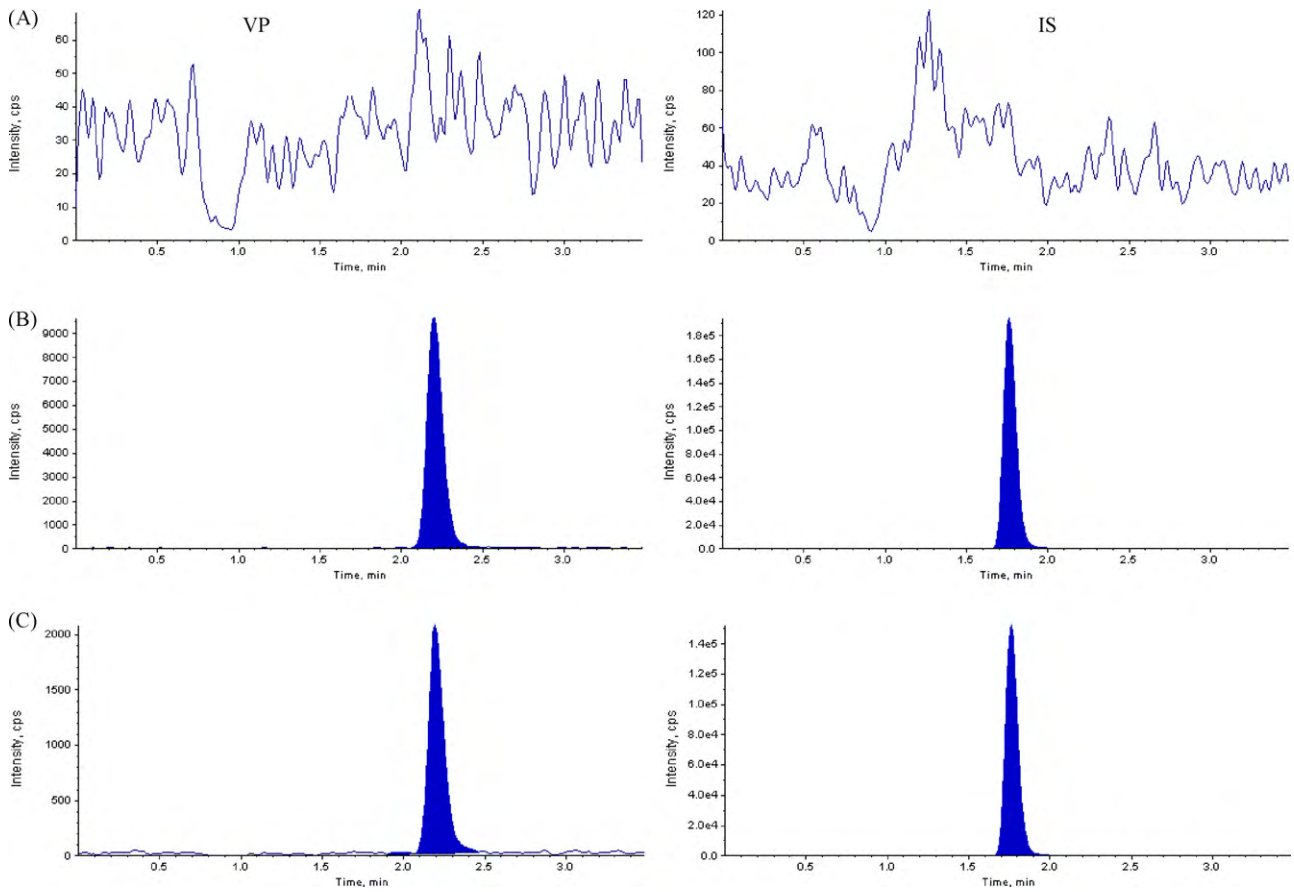

Fig. 2. Chromatograms for VP (351.4 → 280.2) and IS (256.2 → 167.3) in (A) double blank plasma, (B) a blank plasma sample spiked with VP at 10 ng/mL and IS, (C) a real subject sample. The retention time of VP and IS were 2.2 and 1.8 min respectively.

expressed in terms of %bias and coefficient of relative standard deviation (RSD%) respectively.

Recovery of the analytes from the extraction procedure was performed at LQC, MQC and HQC levels. It was evaluated by comparing peak area of extracted samples (spiked before extraction) to the peak area of unextracted samples (quality control working solutions spiked in extracted plasma).

The matrix effect was evaluated at three concentrations (0.5, 10 and 500 ng/mL in plasma). Two groups of samples were prepared: group 1 was prepared to evaluate the MS/MS response for a pure standard of VP and AVA dissolved in the mobile phase (A); group 2 was prepared in plasma originating from six different donors and submitted to the sample purification process and spiked with VP and AVA after processing (B). The value (B/A × 100) was considered as an absolute matrix effect. The inter-subject variability of matrix effect at every concentration level should be less than 15%.

Stability experiments were performed to evaluate the analyte stability in stocks solutions and in plasma samples under different conditions, simulating the same conditions, which occurred during study sample analysis. Stock solution stability was performed at room temperature and at 4 °C by comparing area response of stability sample of analytes and internal standard with the area response of sample prepared from fresh stock solutions. The results should be within the acceptable limit of ±10% change for stock solution stability experiment. Bench top stability of extracted samples (BTS), room temperature stability (stability in biological matrix, SBM), refrigerated stability of extracted sample (RSS) at 4 °C, freeze–thaw stability (FTS) and long term stability (LTS) at −20 °C were performed at LQC, MQC and HQC levels using five replicates at each level. To meet the acceptance criteria the %bias should be within ±15%.

The dilution integrity experiment was performed with an aim to validate the dilution test to be carried out on higher analyte concentrations (above ULOQ), which may be encountered during real samples analysis. Dilution integrity experiment was carried out at 10 times the HQC concentration i.e. 5000 ng/mL for VP and AVA and also at HQC level for both the analytes. Six replicate samples each of 1/10 of 10 × HQC (5000/500 ng/mL) concentration were prepared and their concentrations were calculated, by applying the dilution factor of 10 against the freshly prepared calibration curve for VP and AVA.

2.8. Application of LC–MS/MS analysis

Sprague–Dawley rats (female and male, 14 weeks old, 250–270 g) were purchased from the Experimental Animal Center of School of Pharmacy, Fudan University (the experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of School of Pharmacy, Fudan University). Before the day of administration, the rats were fasted 10 h but were allowed water ad libitum. The solution of VP was administered to rats (1 mg/kg) by oral gavage or by intravenous administration via the tail vein. Blood samples were drawn from the carotid vein at 0.083, 0.25, 0.5, 0.75, 1, 2, 3, 4, 8, 12 h after oral administration or after intravenous administration. As soon as possible, the heparinized blood was centrifuged for 10 min at 2000 × *g*, and plasma was obtained and stored frozen at −20 °C until analysis. The plasma concentra-

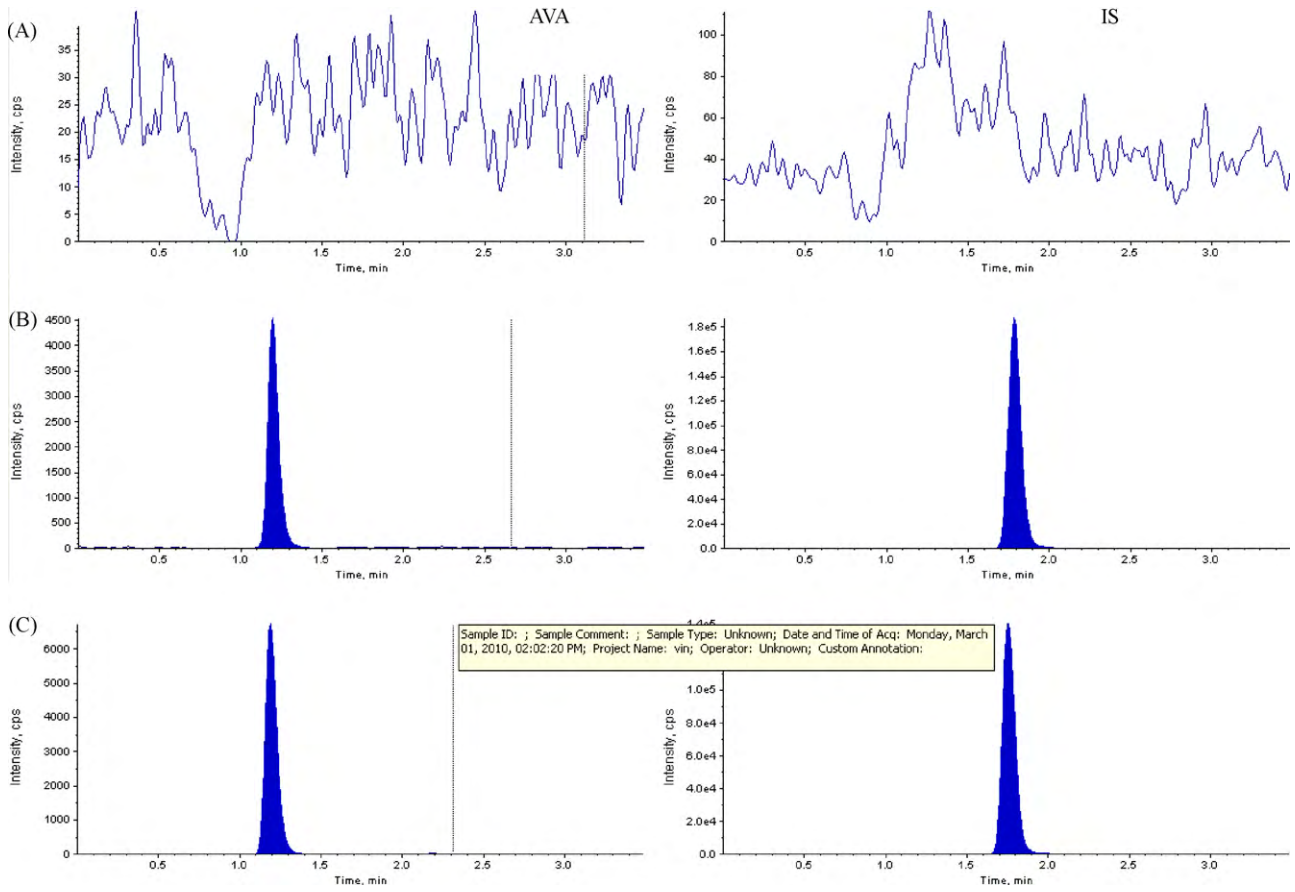

Fig. 3. Chromatograms for VP (323.2 → 280.2) and IS (256.2 → 167.3) in (A) double blank plasma, (B) a blank plasma sample spiked with AVA at 10 ng/mL and IS, (C) a real subject sample. The retention time of AVA and IS were 1.2 and 1.8 min respectively.

tions of VP and AVA at different times were expressed as mean ± SD and the mean concentration–time curve was plotted. The pharmacokinetic parameters for VP and AVA were evaluated by analyzing the data of plasma concentration–time profiles, which was calculated by the pharmacokinetic software, DAS 2.0 (issued by the State Food and Drug Administration of China for pharmacokinetic study).

3. Results and discussion

3.1. Method development

As the literature reveals, there is no report yet on the simultaneous determination of VP and AVA in biological fluids by LC–MS/MS. Thus, in the present study method development was initiated to realize a rugged, sensitive, and specific LC–MS/MS method with a short overall analysis time for the simultaneous quantification of VP and AVA in rat plasma. To accomplish this aim it was imperative to have a simple, inexpensive and an efficient extraction procedure, with a short chromatographic run time. Also, the sensitivity should be adequate enough to monitor at least three to five half-lives of VP and AVA concentration with good accuracy and precision for rat plasma samples.

The tuning of MS parameters was carried out in positive as well as negative ionization modes for VP, AVA and IS using 500 ng/mL tuning solution. The response observed was much higher in positive ionization mode for all three compounds compared to the negative mode. Moreover, use of ammonium acetate and formic acid in the mobile phase further enhanced the response for both the analytes and the IS with low background noise, resulting in higher sensitivity. The analytes and IS gave predominant singly charged protonated precursor $[M+H]^+$ ions at m/z of 351.4, 323.2 and 256.2 for VP, AVA and IS respectively in Q1 MS full scan spectra. The most abundant ions found in the product ion mass spectra were m/z 280.2, 280.2 and 167.3 at 43, 39 and 15 V collision energy for VP, AVA and IS respectively. It was observed that ion spray voltage had a significant effect on the response of both the analytes and the IS. At high voltage the response was drastically enhanced and hence an optimum potential of 5500 V was kept which gave consistent and stable signal. The ion source chamber temperature had little effect on the signal and thus was maintained at 500 °C. A dwell time of 200 ms was adequate and no cross talk was observed between the MRMs of analytes and IS.

To develop an accurate, valid and optimal chromatographic condition, the different HPLC parameters including mobile phase (methanol–0.1% formic acid aqueous solution, methanol–water, methanol–2 mM ammonium acetate aqueous solution containing 0.1% formic acid, acetonitrile–0.1% formic acid aqueous solution), category of column (Agilent Zorbax SB-C18 column, 100 mm × 3.0 mm, 3 μm, Agilent Zorbax Eclipse XDB-C18 column, 150 mm × 2.1 mm, 5 μm, or Waters symmetry C18 column, 50 mm × 2.1 mm, 5 μm, Gemini C18 column, 100 mm × 2.0 mm, 3.0 μm), column temperature (30, 40, 45 or 50 °C) and flow rate of mobile phase (0.2, 0.3 or 0.4 mL/min) were all examined and compared. Finally, plasma samples were separated by HPLC on a Gemini C18 column (100 mm × 2.0 mm, 3.0 μm) using a solvent system consisting of methanol and 2 mM ammonium acetate aqueous solution (containing 0.1% formic acid) at 40 °C and the flow rate was set at 0.3 mL/min. The system provides higher resolution, greater baseline stability and higher ionization efficiency.

Table 1
Intra- and inter-day precision and accuracy of VP spiked in rat plasma ($n = 5$).

Added concentration (ng/mL)	Detected concentration (ng/mL)	Mean ± SD (ng/mL)	Mean accuracy (%)	RSD (%)
Intra-day				
0.5	0.52	0.52 ± 0.04	100.4	8.49
	0.52			
	0.54			
	0.5			
	0.43			
10	11.2	10.5 ± 0.54	105.0	5.13
	10.8			
	10.2			
	10.5			
	9.8			
500	506	489 ± 27	97.8	5.55
	492			
	450			
	476			
	520			
Inter-day				
0.5	0.46	0.50 ± 0.03	99.2	5.81
	0.47			
	0.52			
	0.51			
	0.52			
10	10.2	10.1 ± 0.60	100.8	5.93
	10.7			
	9.2			
	10.5			
	9.8			
500	455	484 ± 18	96.7	3.82
	504			
	478			
	492			
	489			

VP was insoluble in water whereas AVA was a hydrophilic acid and soluble in water. AVA could not be extracted with organic solvents from plasma, so it was difficult to extract both of the analytes by liquid–liquid extraction. In the present study, we develop a solid–liquid extraction method with high recovery. OASIS® HLB cartridges (in which the sorbent was poly(divinylbenzene-N-vinylpyrrolidone)) were used for solid-phase extraction for the purpose of obtain satisfactory values for recovery of both VP and AVA.

3.2. Selectivity and sensitivity (LLOQ)

The aim of performing selectivity check with 6 different types of plasma samples was to ensure the authenticity of the results for study sample analysis. Figs. 2 and 3 demonstrate the selectivity results with the chromatograms of double blank plasma (without IS), and the peak response of VP and AVA at 10 ng/mL concentration. Also, the real rat plasma chromatograms are presented for VP and AVA at 4.0 h respectively after oral administration of 1 mg/kg VP in these figures. The solid–liquid extraction method employed gave very good selectivity for the analytes and IS in the blank plasma. The chromatograms show excellent peak shape for both the analytes and the IS. No endogenous interferences were found at the retention times of VP (2.2 min), AVA (1.2 min) and IS (1.8 min) in the blank plasma. The retention time was short for both the analytes, which made it suitable for routine analysis. Under the optimized conditions, the limit of detection ($S/N \geq 4$) and the limit of quantification ($S/N \geq 10$) of both VP and AVA observed to be 0.25 and 0.5 ng/mL respectively.

3.3. Linearity, accuracy and precision

The calibration curves for VP and AVA were linear from 0.5 to 500 ng/mL with correlation coefficient $r \geq 0.9970$ and $r \geq 0.9984$ respectively across five regression curves. The equations for mean ($n = 5$) of five calibration curves for the analyte were: VIN, $y = 0.000891x + 0.00084$; AVA, $y = 0.000272x + 0.00018$. The intra-assay precision and accuracy were evaluated in five replicates were presented in Tables 1 and 2. These tables indicated that intra-assay RSDs were between 3.82 and 8.49% for VP and lower than 8.55% for AVA. The mean accuracy was ranged from 96.7 to 105.0% for VP and from 95.6 to 105.6% for AVA.

3.4. Recovery and matrix effect

The overall mean recoveries for VP at LQC, MQC and HQC levels were 93.9, 87.1 and 88.2% and that for AVA were 85.2, 80.2 and 82.0% respectively with RSD% between them of 6.8% for VP and 7.6% for AVA. Thus, the consistency in recoveries of VP and AVA supported the extraction procedure for its application to routine sample analysis.

The absolute matrix effects for VP and AVA at concentrations of 0.5, 50 and 500 ng/mL were all within 85–115% respectively. The absolute matrix effects for IS (100 ng/mL in plasma) was 98%. These results showed that ion suppression or enhancement from plasma matrix was negligible in the present condition.

3.5. Stability and dilution integrity

The stability experiments were performed thoroughly to evaluate their stability in stock solutions and in plasma samples under

Table 2
Intra- and inter-day precision and accuracy of AVA spiked in rat plasma ($n = 5$).

Added concentration (ng/mL)	Detected concentration (ng/mL)	Mean ± SD (ng/mL)	Mean accuracy (%)	RSD (%)
Intra-day				
0.5	0.49	0.48 ± 0.04	95.6	8.55
	0.54			
	0.47			
	0.46			
	0.43			
10	10.3	10.0 ± 0.2	100.4	2.4
	10.3			
	9.8			
	9.9			
	9.9			
500	488	501 ± 7.8	100.2	1.55
	503			
	501			
	503			
	509			
Inter-day				
0.5	0.54	0.53 ± 0.04	105.6	7.01
	0.58			
	0.53			
	0.51			
	0.48			
10	10.7	10.3 ± 0.3	102.6	3.28
	9.9			
	10.5			
	10.2			
	10.0			
500	505	499 ± 6.3	99.7	1.27
	503			
	490			
	494			
	501			

different conditions. The stability of spiked QC samples was compared with freshly prepared quality control samples. Stock solution of VP, AVA and IS were stable at room temperature for 12 h and at 4 °C for 30 days for VP, AVA and IS with mean %change well within 0.7–1.4%. The intermediate solution of VP, AVA and IS in methanol–water (50:50, v/v) was stable for 30 days. Both the analytes were found stable in controlled plasma at room temperature up to 24 h and for at least three freeze and thaw cycles. The analytes in extracted plasma samples were stable for 24 h under refrigerated condition of 4 °C. Bench top stability of extracted samples was also up to 24 h. The VP and AVA spiked plasma samples stored at −20 °C for long term stability were found stable for minimum period of 30 days. The values for the percent change for the above stability experiments are compiled in Table 3.

The precision for 1/10 dilution samples was within 85–115% of their nominal values. The RSD for 1/10 dilution samples was <1.8 for both the analytes.

3.6. Application

This new developed method was applied to determine the plasma concentration of VP and AVA in rats following i.v.

Table 3
Stability results for VP and AVA ($n = 3$).

Stability	Storage condition	Level	VP			AVA		
			ng/mL	RSD (%)	%Bias	ng/mL	RSD (%)	%Bias
Stability in biological matrix (SBM)	Room temperature (24 h)	LQC	0.48	3.52	−4.0	0.47	3.02	−6.0
		MQC	10.6	1.2	6.0	10.4	2.31	4.0
		HQC	523	1.31	4.6	537	3.11	7.4
Refrigerator stability of extracted samples (RSS)	Autosampler (4 °C, 24 h)	LQC	0.51	2.42	2.0	0.51	3.55	2.0
		MQC	10.2	1.01	2.0	10.6	2.34	6.0
		HQC	508	1.4	1.6	511	1.04	2.2
Bench top stability of extracted samples (BTS)	Room temperature (24 h)	LQC	0.48	3.75	−4.0	0.52	4.76	4.0
		MQC	9.8	1.07	−2.0	9.4	1.3	−6.0
		HQC	470	2.37	−6.0	508	2.08	1.6
Freeze and thaw stability (FTS)	After 3rd cycle at −20 °C and room temperature	LQC	0.46	7.9	−8.0	0.57	5.1	14
		MQC	9.6	6.41	−4.0	10.4	2.27	4.0
		HQC	508	2.55	1.6	474	4.02	−5.2
Long term stability (LTS)	30 Days at −20 °C	LQC	0.54	7.24	8.0	0.51	1.67	2.0
		MQC	10.8	2.81	8.0	9.5	3.82	−5.0
		HQC	518	1.7	3.6	523	2.04	4.6

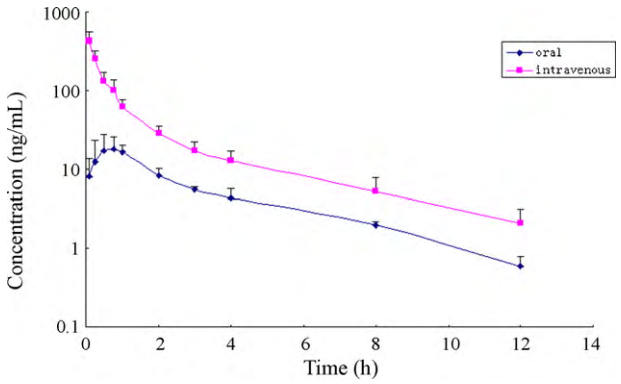

Fig. 4. Mean plasma concentration–time profiles of VP in rats following intravenous (1 mg/kg) and oral (1 mg/kg) administration. All data are expressed as mean ± SD (n = 5).

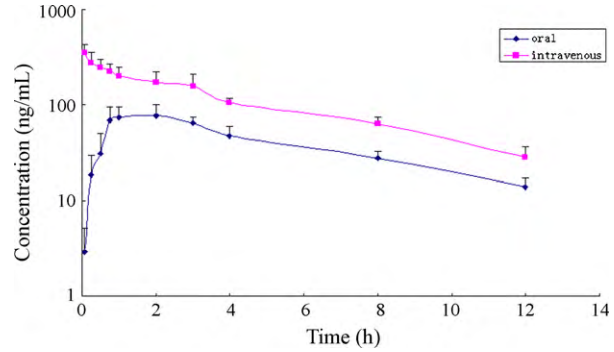

Fig. 5. Mean plasma concentration–time profiles of AVA in rats following intravenous (1 mg/kg) and oral (1 mg/kg) administration. All data are expressed as mean ± SD (n = 5).

Table 4
Pharmacokinetic parameters of VP in rats following intravenous (i.v., 1 mg/kg) and oral (1 mg/kg) administration.

Parameters	The route of dosing	
	i.v.	Oral
C_{max} (ng/mL)	–	23.8 ± 6.3
T_{max} (h)	–	0.75 ± 0.25
$t_{1/2}$ (h)	3.0 ± 0.6	2.9 ± 0.6
AUC_{0-t} (ng h/mL)	315.4 ± 79.4	57.4 ± 10.3
F (%)		19.5 ± 7.8

All data were expressed as mean ± SD (n = 5).

Table 5
Pharmacokinetic parameters of AVA in rats following intravenous (i.v., 1 mg/kg) and oral (1 mg/kg) administration.

Parameters	The route of dosing	
	i.v.	Oral
C_{max} (ng/mL)	–	86.6 ± 22.6
T_{max} (h)	–	1.25 ± 0.7
$t_{1/2}$ (h)	2.8 ± 0.6	3.2 ± 0.6
AUC_{0-t} (ng h/mL)	1253.6 ± 259.6	471.6 ± 89.0

All data were expressed as mean ± SD (n = 5).

(1 mg/kg) and oral (1 mg/kg) administrations. The mean plasma concentration–time profiles of VP and AVA after oral administration and intravenous administration were illustrated in Figs. 4 and 5 and its estimated pharmacokinetic parameters were presented in Tables 4 and 5. It was found that VP was rapidly absorbed into the circulation system and reached its peak concentration at around 0.75 h after oral administration, then rapidly metabolized to the main metabolite AVA. The oral bioavailability of VP was 19%.

4. Conclusion

The objective of this work was to develop a simple, cost effective, rugged and rapid method for simultaneous estimation of VP and its active metabolite AVA in rat plasma, especially to meet the requirement for biological sample analysis. The simple solid–liquid extraction employed in the present work gave consistent and reproducible recoveries for both the analytes. The limit of quantification was low enough to monitor at least four half-lives of VP and AVA concentration with good intra- and inter-assay reproducibility for the quality controls. From the results of all the validation parameters, the method also could be useful for VP pharmacokinetics study and for therapeutic drug monitoring in human with desired precision and accuracy.

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