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ORIGINAL ARTICLE

Pharmacokinetic analysis and tissue distribution of Vam3 in the rat by a validated LC-MS/MS method

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KEY WORDS

Vam3;
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LC-MS/MS

Abstract Vam3 is a potential pharmacologically active ingredient isolated from *Vitis amurensis* Rupr. A rapid, simple and sensitive method to determine Vam3 levels in rat plasma and tissue was developed based on LC-MS/MS. Vam3 and an internal standard (IS) were chromatographed on a C18 short column with acetonitrile–0.1% formic acid in water by gradient elution. MS detection was performed by electrospray ionization in negative ion multiple reaction–monitoring modes. This method monitored the transitions *m/z* 451.0→345.0 and *m/z* 301.0→164.0 for Vam3 and IS, respectively. The calibration curve was linear over a concentration range of 1.64–1000 ng/mL. The inter-day and intra-day variabilities in precision was less than 12.8%, while the inter-day and intra-day accuracies ranged from –10.60% to 9.08% in plasma and tissue homogenates. This method was applied to investigate the pharmacokinetics and tissue distribution of Vam3 in rats. The results indicated that Vam3 had poor absorption into systemic circulation and extensive tissue distribution after oral administration, and the absolute bioavailability was low (0.79%). Vam3 had a relatively long terminal elimination half-life in lung, and the highest concentration was found in small intestinal tissue. The developed method and the pharmacokinetic data can provide a basis for further studies on the bioactivity of Vam3.

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1. Introduction

More than 400 new naturally occurring stilbenes have been isolated and identified from January 1995 to now[1]. Stilbenes are of particular interest to chemists because of their range of biological activities[2]. Resveratrol, the most representative compound, has been associated with several biological activities such as anti-oxidant, anti-inflammatory, analgesic, cardio-protective, neuro-protective, chemo-preventive, anti-ageing and antimicrobial activities[3–5]. In recent years some stilbene oligomers, especially resveratrol oligomers, have attracted attention because of their wider range of biological activities compared with the monomer[6–8]. However, studies on resveratrol oligomers are limited to some preliminary pharmacological research at present.

Vam3 (Amurensin H), a resveratrol dimer, was isolated from ethanol extracts of *Vitis amurensis* Rupr (Fig. 1). *V. amurensis* Rupr, belonging to a family of Vitaceae, grows in the northeast and central parts of China. The roots and stems have been used in traditional Chinese medicines for many years. Some mature synthetic routes for Vam3 have been achieved, too[9]. Previous *in vivo* and *in vitro* studies have found that Vam3 had anti-asthmatic[10–12] and anti-chronic obstructive pulmonary disease (COPD) effects[13,14]. COPD is characterized by progressive, partially reversible airflow obstruction associated with abnormal airway inflammation and oxidant/antioxidant imbalance[15], and is predicted to become the third leading cause of mortality by 2030[16]. Vam3 can inhibit autophagy in cigarette smoke condensate (CS)–treated human bronchial epithelial cells and in CS-exposed mouse lung by preventing mitochondrial dysfunction and restoring the levels of SIRT1 and FOXO3a, which are implicated in pulmonary emphysema in COPD. Some studies have shown that resveratrol may be superior to other drugs for COPD therapy[17,18], yet research has shown that Vam3 was more effective than resveratrol in inhibiting autophagy induced by CS[13].

So far, no method has been published for the quantitation of Vam3 in biological samples. The objective of this study was to develop and validate a highly sensitive LC-MS/MS method for the determination of Vam3 and to investigate its pharmacokinetic properties in rat plasma and tissues.

2. Experimental

2.1. Chemicals and materials

Vam3 (purity > 96.0%) was provided by the Institute of Materia Medica of the Chinese Academy of Medical Sciences (Beijing, China). Hesperetin, the internal standard (IS), was purchased from Sigma (St. Louis, USA). LC–MS grade formic acid was purchased from ROE Scientific Inc. (Newark, USA). HPLC grade acetonitrile and methanol were purchased from ANPEL (Shanghai, China). Water was purified by a Milli-Q system from Millipore (Billerica, USA).

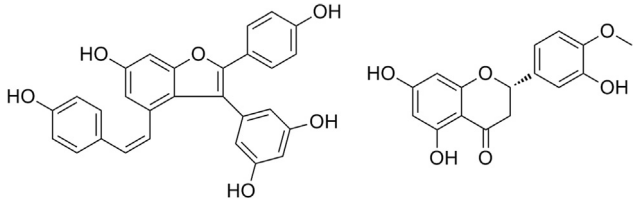

Figure 1 Chemical structure of Vam3 and IS.

2.2. Preparation of calibration and quality control samples

Stock solutions were prepared by dissolving Vam3 and IS in methanol to obtain 0.2 mg/mL for each. A series of working standard solutions were prepared by diluting the Vam3 stock solution with methanol to concentrations of 16.4–10,000 ng/mL. The IS stock solution was diluted with methanol to a final concentration of 200 ng/mL. All solutions were stored at –20 °C before use.

Calibration curves and quality control (QC) samples were prepared by adding Vam3 working standard solutions into 100 μL of blank rat plasma or tissue homogenate. Calibration curves were prepared at concentrations of 1.64, 4.10, 10.24, 25.6, 64, 160, 400, 1000 ng/mL for plasma, heart, liver, lung, kidney and small intestine. QC samples of Vam3 were prepared at four different levels in rat plasma or tissue homogenate at concentrations of 1.64, 4.10, 400 and 800 ng/mL (namely LLOQ, LQC, MQC and HQC). Standard calibration samples and QC samples were stored at −20 °C until analysis.

2.3. Sample preparation and extraction

An aliquot of 100 μL of each plasma or tissue homogenates sample was mixed with 20 μL of working IS solution (200 ng/mL). The mixture was vortexed for 30 s and then ethyl acetic (800 μL) was added for extraction. After vortexing for 3 min and centrifugation at 10,000 × *g* for 15 min, 750 μL of the supernate was transferred to a new tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was then reconstituted in 200 μL methanol. After centrifugation at 10,000 × *g* for 15 min, 10 μL of the supernate was injected for LC-MS/MS analysis.

2.4. LC-MS/MS method

The LC-MS/MS system consisted of a Shimadzu LC-20AD UPLC system and an Applied Biosystems Sciex Qtrap5500 (MDS-Sciex, Concord, Canada). A shim-pack XR-ODS column (2.3 mm × 75 mm, 3 μm, Shimadzu, Tokyo, Japan) was used for separation. The mobile phase was composed of A (0.1% formic acid–water, *v/v*) and B (acetonitrile). The column temperature was maintained at 40 °C and the injection volume was 10 μL. The optimal gradient elution condition for plasma, heart, liver, lung and kidney was as follows: 0–0.03 min, 50% B; 0.03–0.3 min, 50%–75% B; 0.3–1.8 min, 75% B; 1.8–2.1 min 75%–50% B; 2.1–6 min, 50% B, and the flow rate was 0.2 mL/min.

The mass spectrometer was operated by electrospray ionization in negative mode for analyte and IS. The typical MS/MS spectra for Vam3 and IS are shown in Fig. 2. Quantification was performed using multiple reaction monitoring (MRM) mode of the transitions *m/z* 451.0 → 345.0 for Vam3 with a declustering potential (DP) of −180 V and *m/z* 301.0 → 164.0 for hesperetin (IS) with a DP of −100 V, respectively. The dwell time was set at 200 ms per transition. The optimized collision energy (CE) and collision exit potential were −32 eV and −10 eV, respectively. The MS parameters were as follows: ion spray voltage: –4500 V; temperature: 550 °C; nebulizer and turbo gases (nitrogen) were set at 50 and 50 psi, respectively. The curtain gas using nitrogen was

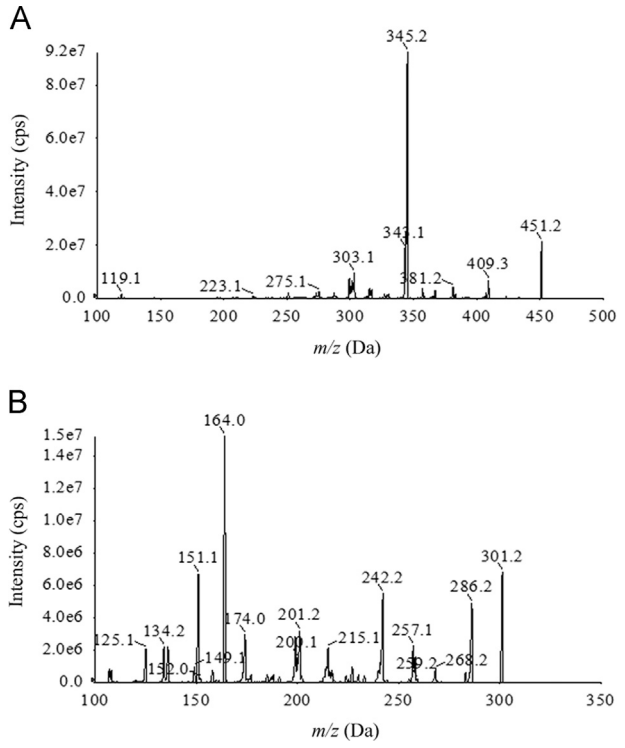

Figure 2 The typical MS/MS spectrum for Vam3 (A) and IS (B).

set at 35 psi. Data acquisition and integration were performed using the Analyst 1.5.1 software (Applied Biosystems, Foster City, USA).

2.5. *Method validation*

The LC-MS/MS method was validated in accordance with the Guidance for Industry, Bioanalytical Method Validation of FDA.

2.5.1. *Selectivity*

The selectivity was investigated by comparing blank plasma samples and tissue homogenates from six individual rats with the corresponding spiked plasma samples and tissue homogenates at the LLOQ level of the analyte (Vam3 and IS) and actual samples.

2.5.2. *Linearity and LLOQ*

The calibration curve was acquired by plotting the peak area ratio of Vam3 to IS (Y) against the corresponding nominal Vam3 concentration (X) by weighted least-squares linear regression ($1/X^2$ as a weighting factor). The LLOQ was defined as the lowest concentration of the calibration curve and produced a signal-to-noise (S/N) ratio of 10. The acceptance criteria of accuracy and precision were $\pm 15\%$ deviation from the nominal value except LLOQ, which was set at $\pm 20\%$.

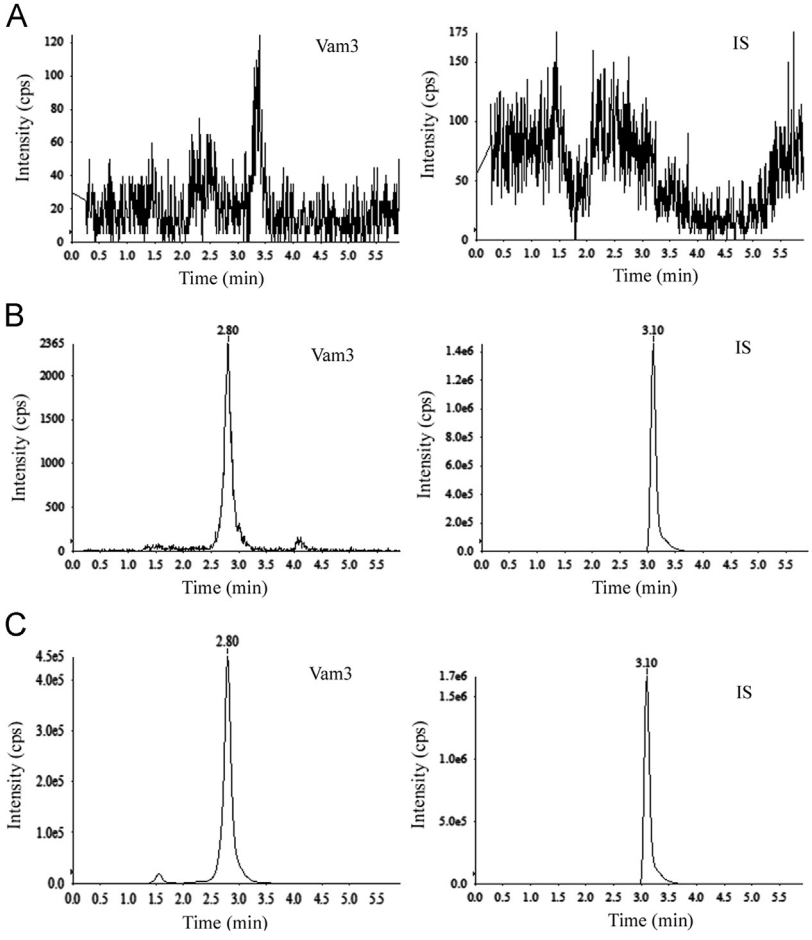

Figure 3 Representative MRM chromatograms of (A) blank rat plasma, (B) rat plasma spiked with Vam3 at LLOQ, and (C) Vam3 in plasma profiles 1 h after oral administration of 70 mg/kg.

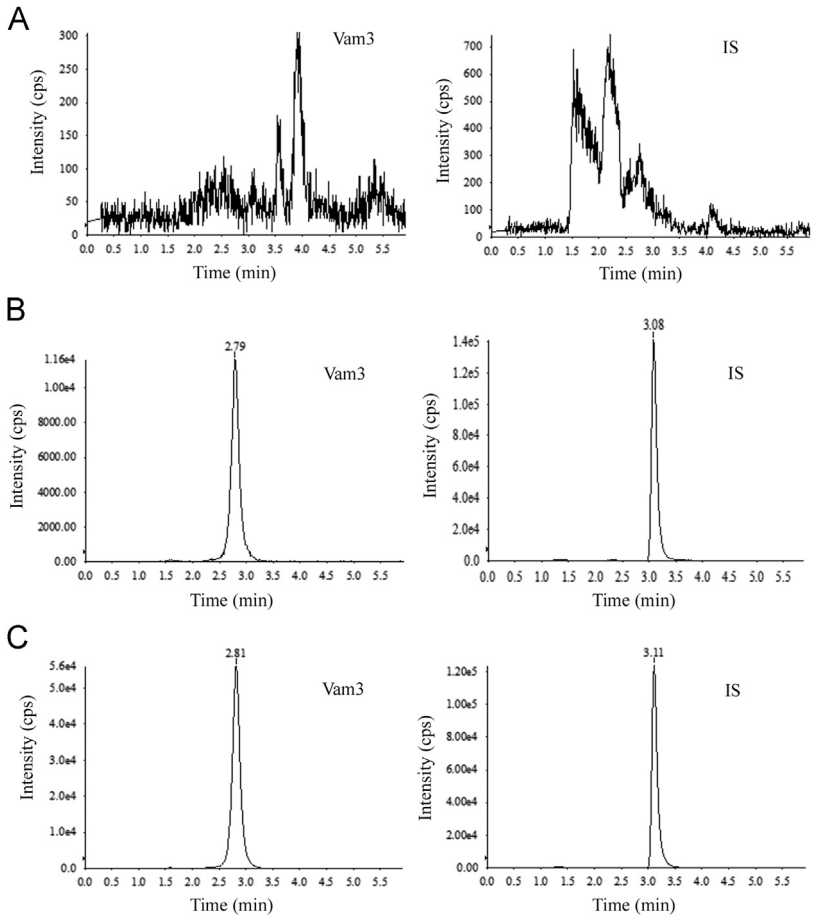

Figure 4 Representative MRM chromatograms of (A) blank lung tissue, (B) lung tissue spiked with Vam3 at LLOQ, and (C) Vam3 in lung tissue profiles 1 h after oral administration of 70 mg/kg. The retention time for Vam3 and IS were 2.80 min and 3.10 min, respectively.

2.5.3. Accuracy and precision

The accuracy and precision were determined by analyzing the QC samples of plasma, heart, liver, small intestine, kidney and lung at four concentrations ($n=5$ each) on three consecutive days. The accuracy and precision were expressed in terms of relative error (R.E.%) and relative standard deviation (R.S.D.%), respectively. The intra- and inter-day precision should not exceed 15% and accuracy should be within $\pm 15\%$ for the QC samples (LQC, MQC and HQC), except for LLOQ, whose accuracy and precision should be within $\pm 20\%$. The intra-day assay precision and accuracy were estimated in the same day.

2.5.4. Recovery and matrix effect

The extraction recovery was determined by comparing peak area of Vam3 from spiked in blank matrix (plasma or tissue homogenate) with the peak area of analyte spiked in post-extracted matrix at four QC concentrations ($n=5$). The matrix effect was evaluated at four QC concentrations ($n=5$) by comparing the peak areas of post-extraction plasma or tissue homogenate spiked with analyte *versus* the standard solution of the analyte at corresponding concentrations. The extraction recovery and matrix effect of IS (200 ng/mL) also was investigated in a similar way. The precision of extraction recovery or matrix effect should be no more than 15% (LQC, MQC and HQC), while for LLOQ it should not exceed 20%.

2.5.5. Stability

The stability of Vam3 in plasma and tissues homogenate was investigated by analysis of four levels of QC samples stored at $-20\ ^{\circ}C$ for one month (long-term stability), at room temperature for 12 h (short-term stability) and after three freeze-thaw cycles. Post-preparative stability was evaluated by analyzing the ready-to-inject samples placed in an autosampler at room temperature for 24 h. The recovery stability was evaluated by $\pm 15\%$ bias of the actual value, except that the LLOQ level should not exceed 20%.

2.5.6. Dilution integrity

The levels of some plasma, liver and small intestine samples exceeded the highest concentration of the calibration curve. To investigate the effect of diluting over-range samples into the calibration range, the accuracy and precision of dilution samples at 20,000 ng/mL (plasma samples), 10,000 ng/mL (small intestine homogenate) and 5000 ng/mL (liver homogenate) (each $n=5$) were assessed by performing a 20-fold dilution. The accuracy and precision was required to be within the range of $\pm 15\%$.

2.6. Pharmacokinetic study and data analysis

Wistar rats (body weight 200 ± 20 g, 8 weeks) were purchased from Beijing Military Medical Sciences Experimental Animal Co., Ltd. (Beijing, China). All animals were kept in an environmentally controlled room. The room was maintained at a temperature of

Table 1 Intra-day and inter-day accuracy and precision of Vam3 ($n=5$). [a]

Matrix	Nominal concentration (ng/mL)	Intra-day			Inter-day		
		Measured concentration (ng/mL)	Precision R.S.D. (%)	Accuracy R.E. (%)	Measured concentration (ng/mL)	Precision R.S.D. (%)	Accuracy R.E. (%)
Plasma	1.64	1.56	9.3	–4.52	1.57	9.3	–4.19
	4.10	3.91	8.7	–4.44	3.79	8.7	–7.57
	400	398.80	7.5	–0.30	390.27	7.5	–2.43
	800	761.80	5.0	–4.78	751.20	5.0	–6.10
Liver	1.64	1.56	9.3	–4.52	1.57	9.3	–4.19
	4.10	4.12	9.1	0.54	4.13	8.4	0.94
	400	392.80	5.0	–1.80	406.00	4.5	1.50
	800	872.20	3.3	9.03	830.93	8.9	3.87
Heart	1.64	1.61	11.1	–1.83	1.59	9.6	–3.13
	4	3.96	8.3	–3.37	3.92	5.7	–4.39
	400	377.40	2.5	–5.65	395.07	7.6	–1.23
	800	796.40	12.8	–0.45	764.73	8.4	–4.41
Kidney	1.64	1.63	8.2	–0.49	1.58	7.3	–3.58
	4.10	4.47	6.2	9.08	4.37	5.1	6.64
	400	410.00	6.7	2.50	418.00	4.5	4.50
	800	795.40	3.4	–0.58	785.67	5.4	–1.79
Lung	1.64	1.63	8.5	–0.24	1.66	7.7	1.51
	4.10	4.42	11.3	7.86	4.19	9.2	2.36
	400	357.60	4.8	–10.60	388.13	7.3	–2.97
	800	787.80	4.0	–1.53	795.07	4.6	–0.62
Small intestine	1.64	1.67	9.4	1.71	1.66	7.6	1.06
	4.10	4.22	12.4	3.13	4.06	8.2	–0.96
	400	378.20	4.1	–5.45	397.07	7.3	–0.73
	800	754.80	7.7	–5.65	784.07	8.6	–1.99

[a]R.E. (%)=(Measured concentration–Nominal concentration)/Nominal concentration × 100.

24±2 °C with a relative humidity of 55%±10% and an approximately 12 h light/dark cycle.

Twelve Wistar rats (6 female, 6 male) were used for the pharmacokinetic and bioavailability study. All rats had free access to water and food. Diet was prohibited for 12 h before the experiment while water was taken freely. All rats were randomly assigned to two groups ($n=6$) for intravenous or oral administration, respectively. Blood samples (0.3 mL) were collected from the ocular vein into 1.5 mL Eppendorf tubes at 0.25, 0.5, 1, 1.5, 2, 2.25, 2.5, 3, 4, 6, 8 and 12 h after oral (singe dosage, 70 mg/kg) administration of Vam3. Similarly, 0.3 mL blood samples were collected from the ocular vein into 1.5 mL Eppendorf tubes at 0.033, 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8 and 12 h after intravenous administration (singe dosage 2 mg/kg) of Vam3. The samples were immediately centrifuged at 3000 × *g* for 15 min. The plasma obtained (100 μL) was stored at −20 °C until analysis.

The pharmacokinetic parameters of Vam3 were calculated by DAS Software (version 3.2.4, China State Drug Administration) using non-compartmental methods. Based on the calculated AUC values, absolute bioavailability (F) was calculated as $F\% = (AUC_{p.o.} \times dose_{i.v.})/(AUC_{i.v.} \times dose_{p.o.}) \times 100$.

2.7. Tissue distribution study

Thirty Wistar rats (15 female, 15 male; weight 200±20 g) were randomly assigned to five groups (6 rats/group) to carry out a tissue distribution study. Tissue samples, including the heart, liver, lung, kidney and small intestine, were collected at 1, 2.5, 4, 6 and 12 h after oral administration (70 mg/kg). Tissue samples were rinsed with 0.9% physiological saline solution to remove the blood and blotted dry with filter paper. An accurately weighed amount of the soft tissue samples (0.5 g) was individually homogenized with normal saline (0.5 mL) and stored at −20 °C until analysis.

3. Results and discussion

3.1. Method development

The ESI conditions for Vam3 and IS were optimized in both positive and negative mode, but more stable and intense ions were observed in negative mode. Protonated precursor $[M - H]^-$ ions at *m/z* 451.0 and 301.0 were obtained for Vam3 and IS respectively in Q1 full scan mode. The most abundant ions in the product ion mass spectra were 345.0 and 164.0 for Vam3 and IS, respectively. The MRM transitions of *m/z* 451.0→345.0 and *m/z* 301.0→164.0 were found for Vam3 and IS, respectively. MS/MS operating conditions were optimized to obtain the greatest intensity of the most abundant product ion by infusing the standard solution (500 ng/mL) of analyte or IS into the ESI source *via* a syringe pump.

Different mobile phases (methanol–water and acetonitrile–water in various proportions) were tested during method development. It was found that acetonitrile selected as the organic phase could enhance the sensitivity in the negative ions mode and improve the peak shape. Both Vam3 and IS have higher response signals and decreased tailing of the chromatographic peak when 0.1% formic acid was added in. Reduction in the proportion of organic phase enhanced the sensitivity of analyte, but the retention time also was extended. Finally, considering symmetrical peak shapes, good resolution, and a short chromatographic run time, the gradient

Table 2 Recovery and matrix effect of Vam3 and IS in rat plasma and tissue homogenates ($n=5$).

Compound	Matrix	Nominal concentration (ng/mL)	Extraction recovery[a] (%)	R.S.D. (%)	Matrix effect[b] (%)	R.S.D. (%)
Vam3	Plasma	1.64	66.1	7.6	81.4	4.7
		4	66.0	11.8	80.2	11.2
		400	64.5	9.4	81.6	7.7
		800	66.1	10.3	79.1	11.8
	Liver	1.64	62.2	19.6	107.1	2.5
		4	61.6	13.7	93.1	10.4
		400	59.3	6.1	93.0	3.2
		800	57.4	6.5	91.7	4.3
	Heart	1.64	60.7	12.7	96.7	10.5
		4	59.2	14.7	93.1	10.4
		400	59.3	6.1	91.1	3.2
		800	57.9	6.6	89.2	4.3
	Kidney	1.64	71.3	4.6	94.4	13.2
		4	71.3	13.3	93.4	4.3
		400	66.8	8.6	102.3	3.6
		800	65.1	7.7	92.8	2.5
	Lung	1.64	77.8	19.0	105.5	12.6
		4	78.2	13.1	106.1	12.0
		400	78.0	8.0	107.6	4.1
		800	73.5	3.4	90.5	9.5
	Small intestine	1.64	52.5	15.3	81.4	13.6
		4	61.8	10.4	74.0	11.6
		400	52.3	9.0	72.1	13.2
		800	56.4	8.5	89.5	12.0
IS	Plasma	20	68.4	7.2	78.5	5.3
	Liver	20	64.9	10.3	91.8	11.7
	Heart	20	66.4	9.1	88.7	3.6
	Kidney	20	76.3	5.6	85.3	4.5
	Lung	20	65.0	10.9	95.5	7.2
	Small intestine	20	58.9	10.5	86.6	6.5

[a]Extraction recovery (%)=Peak area of analyte spiked in blank matrix/Peak area of analyte spiked in post-extracted matrix × 100.
[b]Matrix effect (%)=Peak area of analyte spiked in post-extracted matrix/peak area of analyte spiked in solvent × 100.

mobile phase with acetonitrile–water containing 0.1% formic acid (50:50, *v/v*) was selected, and the flow rate was set at 0.2 mL/min. The gradient mode proved to be stable according to method validation results.

3.2. *Sample preparation*

Sample preparation by an efficient extraction step is important in determining Vam3 content in biological matrices. A protein precipitation method was first considered for the sample preparation for this work. Methanol and acetonitrile were used for protein precipitation solution to investigate the matrix effect and extraction recovery, respectively. But a serious ion suppression or matrix effect for analyte was observed in the LC-MS/MS analyses, which could not meet the requirements of the pharmacokinetic study. Therefore, liquid-liquid extraction (LLE) was chosen to prepare sample using ethyl acetate as extraction solvent. The results indicated that it could provide satisfactory recovery and minimal matrix effects for analyte and IS.

3.3. *Selectivity and specificity*

The assay condition provided adequate specificity for Vam3 and IS analysis while no interfering peaks were observed. Typical chromatograms of blank rat plasma, blank rat plasma spiked with the analyte (at LLOQ level) and IS, and plasma sample at 1 h after oral administration of Vam3 are shown in Fig. 3. The representative chromatograms acquired from blank lung tissue, blank lung tissue spiked with the analyte (at LLOQ level) and IS, and lung tissue sample at 1 h after oral administration of Vam3 are shown in Fig. 4. The retention time of Vam3 and IS were 2.80 min and 3.10 min, respectively, in both plasma and tissue samples.

3.4. *Calibration curve and LLOQ*

For all matrices, calibration curves were determined from the peak area ratio of Vam3 to IS (Y) against concentration (X) using $1/X^2$ weighting factor. The calibration curves were linear over the following concentration ranges: 1.64–1000 ng/mL for plasma and tissue samples. Typical regression equations for Vam3 in plasma and each tissue are as follows: plasma, $Y=0.0117X+0.00521$; liver, $Y=0.00375X+0.00130$; heart, $Y=0.00347X+0.00393$; kidney, $Y=0.00740X+0.0125$; lung, $Y=0.00721X+0.0196$; small intestine, $Y=0.0122X+0.0150$. The correlation coefficients (r) were greater than 0.99 for both analytes. The LLOQ of Vam3 was 1.64 ng/mL (S/N > 10) for all biological samples. Accuracy and precision at LLOQ concentration did not exceed ±20% R.S.D. and R.E.

3.5. *Intra- and inter-day precision and accuracy*

Precision and accuracy were evaluated at four levels of QC samples on three consecutive days. The results for intra- and inter-day precision

Table 3 Stability data of Vam3 in tissue and plasma. [a]

Matrix	QC (ng/mL)	Short-term (12 h) at room temperture		Processed sample (12 h at auto-sampler)		Three freeze-thaw cycles		Long-term stability	
		R.S.D. (%)	R.E. (%)	R.S.D. (%)	R.E. (%)	R.S.D. (%)	R.E. (%)	R.S.D. (%)	R.E. (%)
Plasma	4.10	2.3	0.59	3.8	–10.40	1.1	–7.23	13.7	–0.96
	400	5.3	–7.83	7.4	2.25	0.2	–5.14	2.3	11.17
	800	1.2	–5.71	1.8	–0.04	4.6	–0.92	3.2	9.00
Liver	4.10	4.0	–6.33	0.8	0.02	4.9	–9.97	2.8	7.42
	400	5.3	0.08	1.7	–8.92	6.8	–5.36	4.5	2.00
	800	6.1	–5.33	5.6	–6.42	1.6	–7.14	5.3	–1.50
Heart	4.10	7.8	–1.04	2.4	–4.54	1.3	–11.11	5.4	–7.55
	400	3.3	11.33	1.4	–12.42	2.3	–2.89	9.2	2.92
	800	1.2	–3.63	4.2	–2.33	6.1	–5.13	5.8	–1.88
Kidney	4.10	9.4	0.10	4.2	–0.07	2.2	–0.09	5.2	6.53
	400	9.3	9.42	3.1	–8.25	6.5	3.81	3.6	6.33
	800	6.7	–0.50	2.5	–8.88	3.3	4.97	7.4	7.33
Lung	4.10	5.2	0.10	3.6	5.71	4.7	4.82	6.2	–4.05
	400	4.1	–0.08	6.3	–4.75	6.7	–6.33	2.7	2.00
	800	4.8	–0.08	5.5	–4.83	2.0	–11.79	11.7	–2.88
Small intestine	4.10	2.4	0.10	1.7	–10.64	10.1	–2.75	4.5	–1.04
	400	4.7	–3.50	4.0	8.08	5.3	6.00	7.0	7.25
	800	4.2	5.00	8.0	–4.54	11.1	–1.50	0.9	–7.46

[a]R.E. (%) = (Measured concentration–Nominal concentration)/Nominal concentration × 100.

and accuracy are presented in Table 1. The intra-day accuracies ranged from –4.78% to –0.30% for plasma and from –10.60% to 9.08% for tissue homogenates (heart, liver, lung, kidney and small intestine), throughout the four QC concentrations examined. The intra-day precision in R.S.D. varied by less than 12.8% for all rat bio-samples. The data of the inter-day accuracy were within −7.57% to −2.43% for plasma, −4.41% to –1.23% for heart, −4.19% to 3.87% for liver, −2.97% to 2.36% for lung, −3.58% to 6.64% for kidney and –1.99% to 1.06% for small intestine, respectively, while the inter-day precision varied by less than 9.6%. These results revealed that the precision and accuracy of this assay were within the acceptable range for the analysis of the rat bio-samples.

3.6. *Extraction recovery and matrix effect*

The extraction recoveries and matrix effects determined for Vam3 and IS are shown in Table 2. The extraction recoveries of Vam3 in plasma at four QC concentrations ($n=5$) were more than 64%. The extraction recoveries of Vam3 in tissue samples were also more than 52% at four QC concentrations ($n=5$). The extraction recovery rates of IS also exceeded 58% in the above samples. The absolute matrix effect of Vam3 ranged from 79.1% to 81.6% in plasma and from 72.1% to 107.6% in tissue samples, respectively. The absolute matrix effect of IS at 200 ng/mL level ranged from 78.5% to 95.5% in the above biological matrixes. Values < 100% indicated that there was ionization suppression for Vam3 and the IS under the present conditions, but their values were similar and kept consistent at the four QC concentrations. The relative matrix effects expressed as R.S.D. (%) were acceptable with < 13.6% for Vam3 and < 11.7% for IS. The above results confirm that the presence of matrix effects had practically no influence on the determination of Vam3 in different rat matrices, and that the present LC-MS/MS method is reliable.

3.7. *Stability and dilution*

The stability tests were carried out under various conditions that the samples might experience. In rat plasma and tissue homogenate, Vam3 was found to be stable for three freeze-thaw cycles,

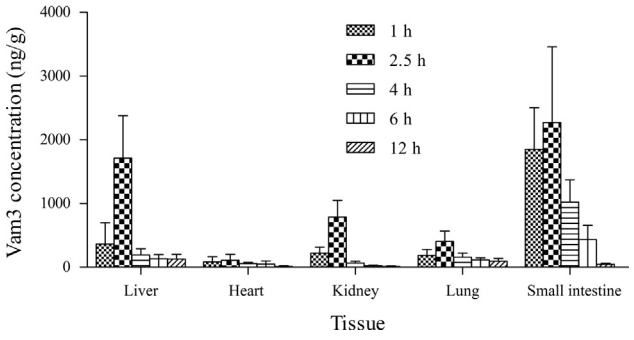

Figure 6 Vam3 concentrations (ng/g) in rat tissues 1, 2.5, 4, 6 and 12 h after oral administration of 70 mg/kg Vam3 ($n=6$). Data are means ± SD.

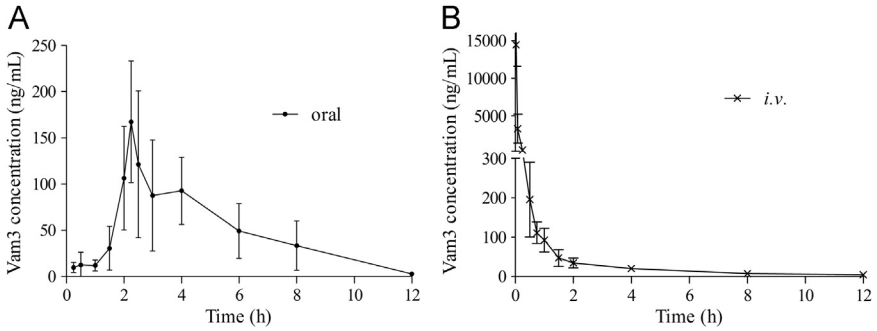

Figure 5 The mean plasma concentration-time profile of Vam3 in rats after oral administration of 70 mg/kg (A) and intravenous administration of 2 mg/kg (B) Vam3 ($n=6$). Data are means ± SD.

Table 4 Non-compartmental pharmacokinetics parameters of Vam3 after a single oral and intravenous administration in rat plasma.

Parameter	Unit	Mean ± SD	
		Intravenous	Oral
		(2 mg/kg, $n=6$)	(70 mg/kg, $n=6$)
$AUC_{(0-t)}$	μg/L·h	2046.7 ± 315.0	565.1 ± 148.2
$AUC_{(0-\infty)}$	μg/L·h	2059.4 ± 311.2	570.0 ± 150.3
$MRT_{(0-t)}$	h	0.4 ± 0.2	4.4 ± 0.8
$MRT_{(0-\infty)}$	h	0.52 ± 0.23	4.4 ± 0.8
$T_{1/2}$	h	2.9 ± 0.4	1.4 ± 0.2
T_{max}	h	–	2.3 ± 0.1
V	L/kg	4.2 ± 1.2	269.6 ± 100.8
CL	L/h/kg	1.0 ± 0.2	130.9 ± 37.6
C_{max}	μg/L	14460.0 ± 2854.3	197.3 ± 41.4
F		–	0.79%

Table 5 Vam3 concentrations (ng/g) in rat tissues 12 h after two different administration methods ($n=6$). Data are means ± SD.

Time	Concentrations of Vam3 (ng/g)				
	Lung	Heart	Kidney	Liver	Intestine
12 h (oral 70 mg/kg)	93.8 ± 44.6	13.3 ± 9.6**	11.1 ± 6.2**	127.6 ± 74.8	44.5 ± 18.6*
12 h (*i.v.* 2 mg/kg)	83.2 ± 49.8	4.5 ± 2.9**	1.69 ± 0.71**	3.0 ± 3.7**	6.1 ± 3.1**

The significance of the differences between groups was determined by one-way ANOVA.
*$P<0.05$ compared with lung.
**$P<0.01$.

at room temperature for 12 h, in the auto-sampler for 24 h and in a long term freezer set at −20 °C for 30 days. As shown in Table 3, that predicted concentrations for analyte at LQC, MQC and HQC samples deviated within the assay variability limits (±15%) of the nominal concentration. In addition, dilution integrity experiments were carried out with five replicates by a 20-fold dilution with blank rat plasma, liver and small intestine. The results indicated that the accuracy was within ±13.2% and the variation in precision was less than 7.59%.

3.8. *Pharmacokinetic and tissue distribution studies*

The assay method was applied in pharmacokinetic studies after oral administration of 70 mg/kg or intravenous injection of 2 mg/kg Vam3 to rats ($n=6$). The mean plasma concentration–time curves are illustrated in Fig. 5, and the major pharmacokinetic parameters of Vam3 from non-compartment model analysis are presented in Table 4.

A relatively slow absorption, extensive distribution, as well as quick elimination and clearance of Vam3 were indicated by the pharmacokinetic parameters. After oral administration Vam3 at 70 mg/kg, the time to peak concentration (T_{max}) was observed at about 2.3 ± 0.1 h, and peak plasma concentrations (C_{max}) were 197.3 ± 41.4 μg/L. The mean area under the plasma concentration–time curve from time zero to the last measurable plasma concentration point (AUC_{0-t}) and the mean area under the plasma concentration–time curve from time zero to time infinity ($AUC_{0-\infty}$) were 565.1 ± 148.2 and 570.0 ± 150.3 μg/L·h, respectively. The apparent volume of distribution (V) value was 269.6 ± 100.8 L/kg for the oral group, suggesting that this compound could extensively distribute into organs and tissues. Clearance, MRT and $T_{1/2}$ values were estimated at 130.9 ± 37.6 L/h/kg, 4.4 ± 0.8 h and 1.4 ± 0.2 h, respectively. That indicated that Vam3 could be quickly eliminated from the circulatory system. Low oral absolute bioavailability (0.79%) of Vam3 was calculated after intravenous and oral administration. It could be seen from the concentration-time curve that there were double peaks (approximately at 4 h) of Vam3 after oral administration in rat plasma. Because Vam3 has low bioavailability, data points showed significant variability, and enterohepatic circulation might play a role. The homolog resveratrol has been reported to undergo enterohepatic circulation which can result in a concentration-time curve with two peaks[19]. However, the double-peak feature of Vam3 needs further study.

The distribution of Vam3 in the various tissues, namely heart, liver, lung, kidney and small intestine is listed in Fig. 6. After oral administration of Vam3 at 70 mg/kg, results indicated that Vam3 was widely distributed in the tissues, and the concentration of Vam3 in certain tissues was much higher than that in the plasma. The maximum concentration was observed at 2.5 h in all tissues after oral administration. The highest tissue concentration of Vam3 was observed in the small intestine (2273.3 ± 1187.3 ng Vam3/tissue (g)), followed by the liver (1144.4 ± 667.5 ng/g), the kidney (787.0 ± 259.8 ng/g), lung (404.0 ± 163.2 ng/g) and heart (109.9 ± 90.2 ng/g). The data indicated that small intestine might be the main absorption sites of Vam3. The dense blood vessel network with high blood flow may have relevance for the high distribution of Vam3 in the liver and kidney. Twelve hours after oral administration there was little in other tissue samples except for lung and liver. Meanwhile, we also studied the distribution of Vam3 in these tissues 12 h after intravenous administration. The result (Table 5) showed that very little Vam3 could be detected in other tissue samples beyond 12 h, but the concentration of Vam3 in the lung was 83.2 ± 49.8 ng/g. The level in lung remains significantly higher than in other tissues. Through cop analysis for tissue by DAS Software using non-compartmental methods, we found Vam3 had a relatively long terminal elimination half-life in lung. It indicated that Vam3 may have certain affinity for lung tissue and provided the material basis for its pharmacological actions in the treatment of COPD. However, the reasons for this distribution profile still need further investigation.

4. Conclusions

A rapid and sensitive method by LC-MS/MS for the quantification of Vam3 was developed and validated in rat plasma and tissue. The advantages of this method included short analysis time, high sensitivity and simple sample preparation procedure. The pharmacokinetic data showed that Vam3 displayed negligible oral bioavailability, but it had a relatively long terminal elimination half-life in lung tissue following oral administration. The results help us understand the pharmacological action of Vam3 for COPD *in vivo*, even if its blood concentration is low. The reported pharmacokinetic parameters of Vam3 in the present work will provide helpful information for further development. How to improve the oral bioavailability of Vam3 will be the focus in further bio-pharmaceutics research.

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