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A HPLC method for simultaneous determination of 5-aminoimidazole-4-carboxamide riboside and its active metabolite 5-aminoimidazole-4-carboxamide ribotide in tumor-bearing nude mice plasma and its application to pharmacokinetics study

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ABSTRACT

A HPLC method with on-line solid phase extraction (SPE) and column switching was developed for simultaneous determination of 5-aminoimidazole-4-carboxamide riboside (AICA riboside) and its active metabolite 5-aminoimidazole-4-carboxamide ribotide (AICA ribotide) in nude mice plasma. Plasma sample was deproteinized by adding a half volume of 10% trichloroacetic acid (TCA), and the resulting supernatant was extracted with diethyl ether to remove TCA. 50 μl aqueous fraction was injected onto a WAX-1 SPE column, and AICA ribotide was trapped on the SPE column, while AICA riboside was eluted from the SPE column. The chromatographic separation of AICA riboside was achieved on CG16 column, and separation of AICA ribotide was performed on HILIC-10 and WAX-1 column. The columns temperature was maintained at 40 °C, and the optimal detection wavelength was 268 nm for both AICA riboside and AICA ribotide. The total analytical run time was 40 min. The proposed method was linear over the range of 0.1–500 μg/ml for AICA riboside and 0.03–50 μg/ml for AICA ribotide. The lower limit of quantification (LLOQ) was 100 and 30 ng/ml for AICA riboside and AICA ribotide, respectively. The sensitivity, accuracy and precision of this method were within acceptable limits during validation period. The method was successfully applied to investigate the pharmacokinetics characteristics of AICA riboside and its active metabolite AICA ribotide in nude mice bearing MCF-7 cell xenografts.

1. Introduction

5-Aminoimidazole-4-carboxamide riboside (AICA riboside, Fig. 1A), a cell permeable nucleoside, was the first compound reported to activate AMP-activated protein kinase (AMPK) [1]. AICA riboside is taken up into cells by adenoside transporters and is then converted by adenoside kinase to the monophosphorylated derivative AICA ribotide which mimics the effect of AMP in activation of AMPK [2,3]. AICA riboside has been shown inhibitory effects on proliferation of a variety of human cancer cell lines in vitro [4–9] and in vivo [10,11]. The first patient was enrolled in a phase I/II trial of AICA riboside treatment for B-cell chronic lymphocytic leukemia in 2008 [12]. To evaluate the pharmacokinetics of AICA riboside and its active metabolite AICA ribotide, it is very important to establish a sensitive and reliable analytical method for determination of the plasma concentration of AICA riboside and AICA ribotide.

High-performance liquid chromatography (HPLC) with ultraviolet detection has been developed for determination of either AICA riboside or AICA ribotide in biological fluids separately [13–15]. A capillary electrophoretic method was reported to detect 5-aminoimidazole-4-carboxamide and AICA riboside in untreated urine [16]. A LC–MS method has been used for the quantification of urinary AICA riboside [17]. To the best of our knowledge, no method for simultaneous determination of AICA riboside and its active metabolite AICA ribotide in biological fluids has been reported. This is because AICA ribotide is an anionic compound while AICA riboside is a cationic compound, and they are difficult to be separated simultaneously by one column.

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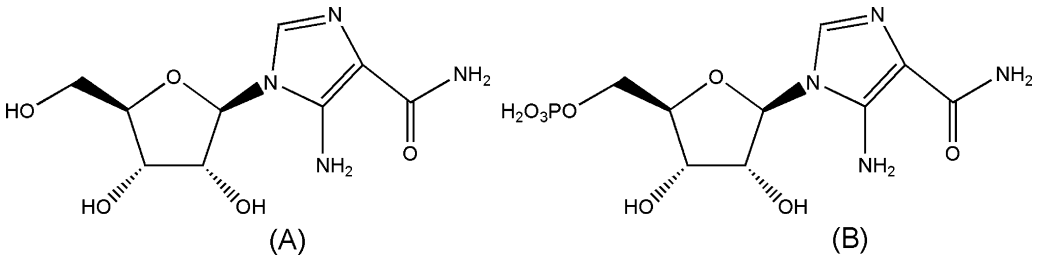

Fig. 1. Chemical structure of 5-aminoimidazole-4-carboxamide riboside (A) and 5-aminoimidazole-4-carboxamide ribotide (B).

In the present study, we developed a HPLC analytical method with on-line solid phase extraction (SPE) and column switching for simultaneous determination of AICA riboside and its active metabolite AICA ribotide in nude mice plasma. The results showed that this method was sensitive, accurate and reliable, and has been successfully applied to the study of the pharmacokinetics of AICA riboside and its active metabolite AICA ribotide in nude mice bearing MCF-7 cell xenografts.

2. Experiments

2.1. Chemical and reagents

AICA riboside (98%) and AICA ribotide (95%) were purchased from Toronto Research Chemicals (Toronto, Canada). Acetonitrile was HPLC grade and was obtained from Merck Serono Co., Ltd. (Darmstadt, Germany). HPLC quality water was prepared using a Nanopure water purification system (Thermal Fisher Scientific, USA). All other chemicals used in this study were analytical grade.

2.2. Instruments

The HPLC system was composed of a Dionex (USA) Ultimate 3000 system equipped with DGP-3600A dual-gradient pumps (left pump and right pump, Fig. 2), an SRD-3600 solvent rack with integrated vacuum degasser, a WPS-3000TSL autosampler equipped with a 100 μl loop, a TCC-3200 thermostatted column compartment with a two-position, ten-port (2P-10P) valve, a DAD detector and a Chromeleon chromatography data system.

2.3. Chromatographic conditions

Acclaim Mixed-Mode WAX-1 (4.3 mm × 10 mm, 5 μm, Dionex, USA) was tested as the solid phase extraction (SPE) column for analyte loading. An IonPac CG16 Guard Column (5 mm × 50 mm, 5.5 μm, Dionex, USA) was used as the analytical column for AICA riboside. Acclaim Mixed-Mode WAX-1 column (4.6 mm × 150 mm, 5 μm, Dionex, USA) and Acclaim HILIC-10 column (4.6 mm × 150 mm, 3 μm, Dionex, USA) were used as the analytical column for AICA ribotide. The column temperature was maintained at 40 °C.

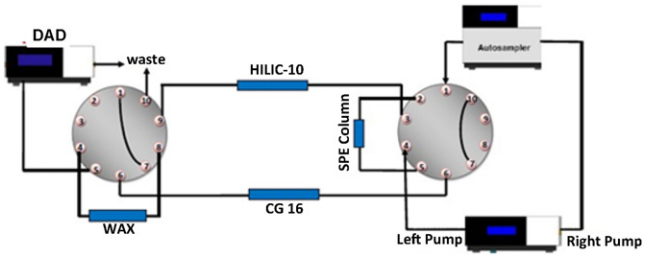

Fig. 2. Schematic of devices for the determination of AICA riboside and its active metabolite AICA ribotide by HPLC with on-line SPE and column switching.

Table 1
Gradient program of left and right pump.

Pump	Time (min)	CH_3CN (%)	KH_2PO_4 (pH 2.5; 100 mM) (%)	H_2O (%)	Flow rate (ml/min)
Left pump	0	5	13	82	0.8
	13.7	5	13	82	0.8
	14	5	55	40	1
	30	5	55	40	1
	35	20	13	67	1
	38	5	13	82	1
	40	5	13	82	1
Right pump	0	2	1	97	1
	40	2	1	97	1

The mobile phases A and B was pure water and acetonitrile respectively, and the mobile phase C was KH_2PO_4 (pH 2.5; 100 mM). The mobile phases A–C were for both left and right pump. The optimal detection wavelength was 268 nm for both AICA riboside and AICA ribotide.

The schematic of HPLC device was illustrated in Fig. 2, and gradient program of left and right pump and program of valve switching was presented in Tables 1 and 2 respectively. The steps are as follows:

Step 1. With the left and right valve in the 1-2 position, 50 μl sample was injected into the system. AICA ribotide was trapped on the SPE column, while AICA riboside was eluted from the SPE column by the right pump with CH_3CN–KH_2PO_4 (pH 2.5; 100 mM)–H_2O (2:1:97, v/v/v) at the flow rate of 1 ml/min and subsequently separated by CG16 Column.
Step 2. At the time of 6 min, the right valve was switched to the 10-1 position to connect the SPE column and HILIC-10 column. AICA ribotide was eluted from the SPE column onto the HILIC-10 column by the left pump with CH_3CN–KH_2PO_4 (pH 2.5; 100 mM)–H_2O (5:13:82, v/v/v) at the flow rate of 0.8 ml/min.
Step 3. At the time of 11.7 min, the left valve was switched to 10-1 position to connect HILIC-10 column and WAX-1 column. AICA ribotide was eluted from the HILIC-10 column onto the WAX-1 column by the left pump with CH_3CN–KH_2PO_4 (pH 2.5;

Table 2
Program of valve switching.

Time (min)	Left valve location	Right valve location
0	1-2	1-2
6	1-2	10-1
11.7	10-1	10-1
13.7	1-2	10-1
19	10-1	10-1
30.5	1-2	1-2

Note: When valve is in the 1-2 position, valve 1 connects to valve 2, valve 3 connects to valve 4, valve 5 connects to valve 6, valve 7 connects to valve 8 and valve 9 connects to valve 10, respectively. With valve in the 10-1 position, valve 1 connects to valve 10, valve 2 connects to valve 3, valve 4 connects to valve 5, valve 6 connects to valve 7 and valve 8 connects to valve 9, respectively.

100 mM)–H_2O (5:13:82, v/v/v) at the flow rate of 0.8 ml/min. At the time of 13.7 min, AICA ribotide was completely eluted from HILIC-10 column and the left valve was switched to 1-2 position to flush endogenous substance to waste.

Step 4. At the time of 19 min, the left valve was switched to 10-1 position to separate AICA ribotide on WAX-1 column with CH_3CN–KH_2PO_4 (pH 2.5; 100 mM)–H_2O (5:55:40, v/v/v) at the flow rate of 1 ml/min.

Step 5. After analysis was completed, the left and right valves were switched to 1-2.

2.4. Stock solutions and calibration standards

Stock solution of AICA riboside was prepared in 0.1% H_3PO_4 (v/v) at 10 mg/ml. The solution was further diluted with 0.1% H_3PO_4 to concentrations of 2, 10, 20, 200, 1000, and 5000 μg/ml. AICA ribotide stock solution was prepared in 0.1% H_3PO_4 at 1 mg/ml. The solution was further diluted with 0.1% H_3PO_4 to concentrations of 0.6, 2, 10, 20, 100, and 500 μg/ml. Blank blood was collected from the ocular artery of BALB/c nude mice after eyeball removal and placed into heparinized tube, and then centrifuged at 1110 × *g* for 10 min at 4 °C to obtain blank plasma. Plasma calibration standards containing 0.1, 0.5, 1, 10, 50, 250, 500 μg/ml AICA riboside and 0.03, 0.1, 0.5, 1, 5, 25, 50 μg/ml AICA ribotide were prepared by adding 15 μl AICA riboside and 15 μl AICA ribotide working solutions into 270 μl blank plasma. Quality control (QC) samples were prepared by spiking blank plasma with independently prepared standard stock solution to achieve final concentrations of 0.2, 10 and 400 μg/ml AICA riboside and 0.06, 1 and 40 μg/ml AICA ribotide.

2.5. Sample preparation

100 μl 10% (w/v) trichloroacetic acid (TCA) was added to 200 μl plasma, the mixture was vortex-mixed for 30 s before centrifugation at 11,750 × *g* for 30 min. 200 μl supernatant was placed into an eppendorf tube and 800 μl diethyl ether was added. The mixture was vortex-mixed, centrifugated at 11,750 × *g* for 1 min, and the upper organic layer was discarded. 800 μl diethyl ether was added to the remaining lower layer, and this extraction procedure was repeated a second time [18,19].

2.6. Method validation

Method validation was performed according to the guidelines suggested by the U.S. Food and Drug Administration. QC samples were processed in five replicates at each concentration level for five different analytical runs to validate this method in precision, accuracy, recovery and stability.

2.6.1. Selectivity and specificity

Interference by endogenous compounds in plasma was assessed by comparing chromatograms of blank plasma, blank plasma spiked with AICA riboside or AICA ribotide, and plasma samples obtained from pharmacokinetics studies.

2.6.2. Linearity

Calibration standards were prepared and analyzed in triplicate to establish the calibration curves range from 0.1 to 500 μg/ml for AICA riboside and from 0.03 to 50 μg/ml for AICA ribotide. The peak areas of AICA riboside and AICA ribotide were plotted against the spiked AICA riboside or AICA ribotide theoretical concentrations in blank plasma. Least-squares linear regression with $1/C^2$ as weighting factor was used for curve fitting.

2.6.3. Sensitivity

The lower limit of detection (LLOD) was defined as the concentration with a signal of three times the background noise. Similarly, the lower limit of quantification (LLOQ) which was the lowest level in the calibration curve corresponds to a signal/noise ratio of 10.

2.6.4. Precision and accuracy

Intra-day precision and accuracy were evaluated by analysis of the three QC samples in five replicates on a single day. Inter-day precision and accuracy were measured by assaying the three QC samples on five consecutive days. Precision and accuracy was expressed as relative standard error (RSD) and relative error (RE), respectively.

2.6.5. Recovery

The extraction recovery of AICA riboside and AICA ribotide was assessed by comparing the peak areas of extracted spiked plasma samples with the peak areas of pure compounds with the same concentrations in solvent.

2.6.6. Stability

Short-term, long-term and freeze–thaw cycles stability was assessed by analyzing the three QC samples for five replicates. The QC samples were analyzed after storage at 4 °C for 2 h and at −80 °C for 2 weeks. Freeze–thaw stability was evaluated after three cycles (−80 °C/room temperature). Deterioration of each analyte was defined as a greater than 15% difference in the analyzed sample versus the control at the nominal sample concentration.

2.7. Pharmacokinetics study

Female BALB/c nude mice (5-week-old, 18–22 g) were purchased from the Experimental Animal Center, Peking University Health Science Center. Mice were housed under constant temperature, humidity and lighting (12 h light per day) and were allowed free access to food and water. 2×10^6 MCF-7 cells (purchased from cell bank of Cancer Institute & Hospital, Chinese Academy of Medical Science) were suspended in 200 μl PBS, and inoculated subcutaneously in mice's right flank. When tumor volume reached about 1000 mm^3, the mice were randomly divided into three groups (n = 8 per group) and were injected intravenously AICA riboside (dissolved in PBS) at the dose of 200 mg/kg via the tail vein. Blood samples were obtained from ocular artery after eyeball removal and placed into heparinized tube at 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 3 h and 4 h after injection, and then centrifuged at 1110 × *g* for 10 min at 4 °C. The upper plasma was collected and stored at −80 °C before analysis. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals.

Pharmacokinetics parameters of AICA riboside and its active metabolite AICA ribotide in nude mice plasma after intravenous administration were estimated by non-compartment analysis. C_{max} was the observed maximum concentration, and T_{max} was the time taken to reach the maximum concentration. Mean residence time (MRT) was calculated as the ratio of the area under the first moment versus time curve ($AUMC_{0-\infty}$) to the area under the plasma concentration versus time curve ($AUC_{0-\infty}$). Half-life ($t_{1/2}$) was calculated as 0.693 × MRT, and systemic clearance was obtained from the equation $CL = D/AUC_{0-\infty}$ where D was the dose of AICA riboside. The apparent volume of distribution was calculated from the equation $V = D \times AUMC_{0-\infty}/AUC_{0-\infty}^2$.

3. Results and discussion

3.1. Development of the HPLC method

The aim of this study was to develop a HPLC method for simultaneous determination of AICA riboside and its active metabolite AICA ribotide in nude mice plasma. Previous reported methods for separation of AICA ribotide which is an anionic compound was achieved on anion exchange column [13–15], while AICA riboside, a cationic compound, can not be separated simultaneously by anion exchange column. Thomas et al. have developed LC–MS method for determination of AICA riboside in human urine, and the LLOQ was 100 ng/ml [17]. However, to date, no LC–MS method for determination of AICA ribotide in biological fluids has been reported. At first we tried LC–MS/MS method to analyze AICA riboside and AICA ribotide in nude mice plasma, but both positive and negative ionization efficiency were very low especially for AICA ribotide, so LC–MS/MS was not suitable for simultaneous determination of AICA riboside and AICA ribotide in biological fluids. So we used SPE for selective retention of either AICA riboside or AICA ribotide and applied column switching for separation of them by different columns.

We used Acclaim Mixed-Mode WAX-1 column (4.3 mm × 10 mm, 5 μm) which has anion-exchange property as SPE column for selective retention of AICA ribotide, while AICA riboside, a cationic compound, was eluted from SPE column. After elution from SPE column, AICA riboside was separated on IonPac CG16 Guard Column (5 mm × 50 mm, 5.5 μm) which has cation exchange property. In addition, Acclaim HILIC-10 column (4.6 mm × 150 mm, 3 μm) which was particularly promising for the separation of polar compounds [20] was selected for separating AICA ribotide. At the time of 6 min, the right valve was switched to 10-1 position to connect the SPE column and HILIC-10 column. AICA ribotide was eluted from the SPE column onto the HILIC-10 column by the left pump with CH_3CN–KH_2PO_4 (pH 2.5; 100 mM)–H_2O (5:13:82, v/v/v) at the flow rate of 0.8 ml/min. As shown in Fig. 3, after separation by HILIC-10 column the retention time of AICA ribotide was 12.6 min. But assessment peak purity by relative absorption spectra [21] revealed that the peak at 12.6 min was not pure (as depicted in Fig. 4A), so the switching window was set between 11.7 and 13.7 min to elute AICA ribotide from HILIC-10 column onto the WAX-1 column. After separation by WAX-1 column, AICA ribotide was completely separated from endogenous substances. As illustrated in Fig. 4 B, after separation by HILIC-10 and WAX-1 column, the relative absorption spectra at the peak start point, the peak maximum ascending slope, the peak maximum descending slope and the peak end point were super imposed on each other, and the peak purity was more than 99.5%. Although the total analytical run time of 40 min was a little long, this method can simultaneously determine AICA riboside and AICA ribotide in plasma and the analytical run time was much shorter than the most cited method which had an analytical run time of 35 min for AICA riboside and of 40 min for AICA ribotide separately [15]. The plasma pretreatment of two times extraction with diethyl ether to remove TCA was relatively complex, but the previous reported pretreatment method also included removing TCA with tri-N-octylamine [15].

3.2. Method validation

3.2.1. Selectivity and specificity

Representative chromatograms of blank plasma, blank plasma spiked with 10 μg/ml AICA riboside or 0.5 μg/ml AICA ribotide, and plasma collected at 15 min after intravenous administration of AICA riboside at a dose of 200 mg/kg were shown in Fig. 5. The retention times of AICA riboside and AICA ribotide were 9.1 and 25.4 min, respectively. The identity of AICA riboside and AICA ribotide in the endogenous samples were demonstrated by comparison of retention times with known external standards. No significant interferences by endogenous substances were observed at the retention times of AICA riboside and AICA ribotide.

Table 3
Intra-day accuracy and precision of AICA riboside and AICA ribotide in plasma ($n = 5$).

Analytes	Nominal concentration (μg/ml)	Mean ± SD (μg/ml)	RE (%)	RSD (%)
AICA riboside	0.2	0.20 ± 0.01	0.04	2.85
	10	10.20 ± 0.18	2.03	1.74
	400	441.45 ± 6.43	10.36	1.46
AICA ribotide	0.06	0.062 ± 0.004	0.13	6.26
	1	1.07 ± 0.01	7.22	0.91
	40	42.82 ± 0.53	7.06	1.24

3.2.2. Linearity

Calibration curves of this method covered the range from 0.1 to 500 μg/ml for AICA riboside and from 0.03 to 50 μg/ml for AICA ribotide. Using weighted least-squares regression with $1/C^2$ as weighting factor, calibration equations $A = 1.5120 \times C - 0.0308$ ($r = 0.9977$) and $A = 0.9527 \times C + 0.0053$ ($r = 0.9994$) were obtained for AICA riboside and AICA ribotide, respectively.

3.2.3. LLOD and LLOQ

In this study, the LLOD was 30 ng/ml for AICA riboside and 10 ng/ml for AICA ribotide and the LLOQ was 100 ng/ml for AICA riboside and 30 ng/ml for AICA ribotide. The LLOQ of both AICA riboside and AICA ribotide were much lower than the previous reported HPLC-UV method with LLOQ of 0.1 mg/ml for AICA riboside and of 2.5 mg/ml for AICA ribotide [14].

3.2.4. Precision and accuracy

The results of intra- and inter-day precisions and accuracies were listed in Tables 3 and 4 respectively. The intra- and inter-day accuracy was within ±15% for all QC samples of both AICA riboside and AICA ribotide. The intra- and inter-day assay precision (RSD) was also within the acceptable range of 15% for all samples. The precision and accuracy results indicate that the method had remarkable reproducibility and accuracy.

3.2.5. Recovery

The recovery of AICA riboside from plasma was 71.56 ± 2.04%, 66.41 ± 1.16% and 67.61 ± 0.99% at low, medium and high concentrations, respectively. The recovery of AICA ribotide from plasma was 65.94 ± 4.13%, 69.06 ± 0.63% and 66.82 ± 0.83% at low, medium and high concentrations, respectively.

3.2.6. Stability

Stability results of AICA riboside and AICA ribotide under various conditions were summarized in Table 5. Freezing and thawing did not have any detrimental effect on absolute concentrations of AICA riboside and AICA ribotide spiked in plasma. No obvious trend

Table 4
Inter-day accuracy and precision of AICA riboside and AICA ribotide in plasma ($n = 5$).

Analytes	Nominal concentration (μg/ml)	Mean ± SD (μg/ml)	RE (%)	RSD (%)
AICA riboside	0.2	0.19 ± 0.01	−2.25	6.61
	10	10.07 ± 0.78	0.77	7.72
	400	427.37 ± 25.95	6.84	6.07
AICA ribotide	0.06	0.055 ± 0.004	−7.93	7.37
	1	1.05 ± 0.03	0.43	3.25
	40	42.77 ± 2.53	6.93	5.92

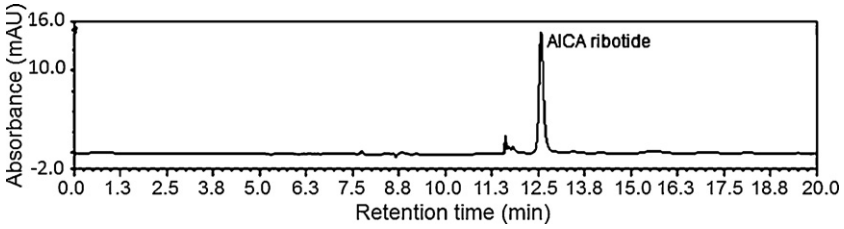

Fig. 3. The HPLC chromatograms of AICA ribotide after separation by HILIC-10 column.

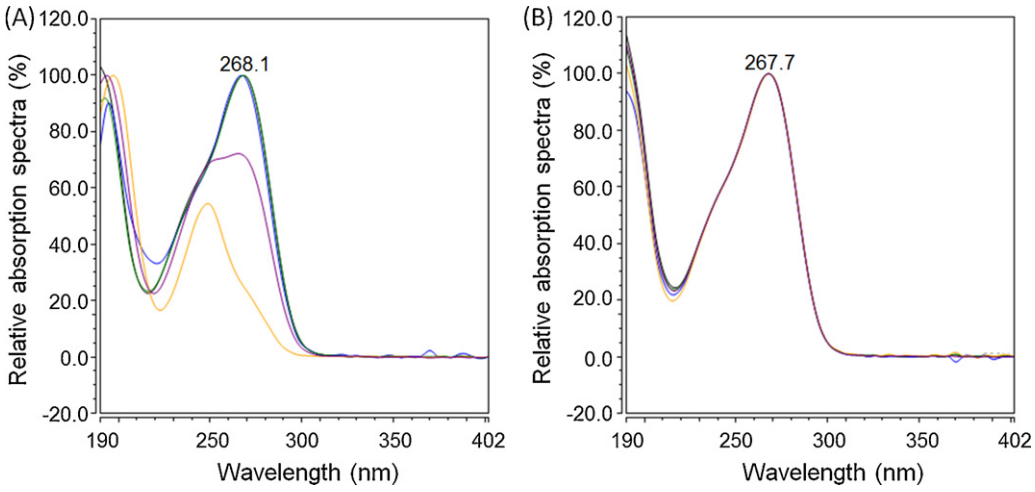

Fig. 4. Relative absorption spectra of AICA ribotide after separation by HILIC-10 column alone (A) or by combination of HILIC-10 and WAX-1 column (B) using DAD detector. The blue, green, purple and yellow curve was the relative absorption spectra at the peak start point, the peak maximum ascending slope, the peak maximum descending slope and the peak end point of AICA ribotide, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of concentration change was observed during storage at −80 °C for 2 weeks. AICA riboside was stable at 4 °C for 2 h, while AICA ribotide spiked in plasma at low, medium and high concentrations degraded approximately 15%. In this study we found that AICA ribotide hydrolyzed to AICA riboside and H_3PO_4, but in acidic condition AICA ribotide was stable. So we immediately added 10% TCA into plasma sample after thawing to keep AICA ribotide stable and precipitate proteins.

3.3. Application to pharmacokinetics study

The proposed method was applied to analyze plasma samples obtained from nude mice after intravenous administration of AICA riboside (200 mg/kg). By interpolating peak areas on the calibration curves, mean plasma concentration–time curves were constructed for AICA riboside and AICA ribotide, as shown in Fig. 6. The pharmacokinetics parameters of AICA riboside and its active

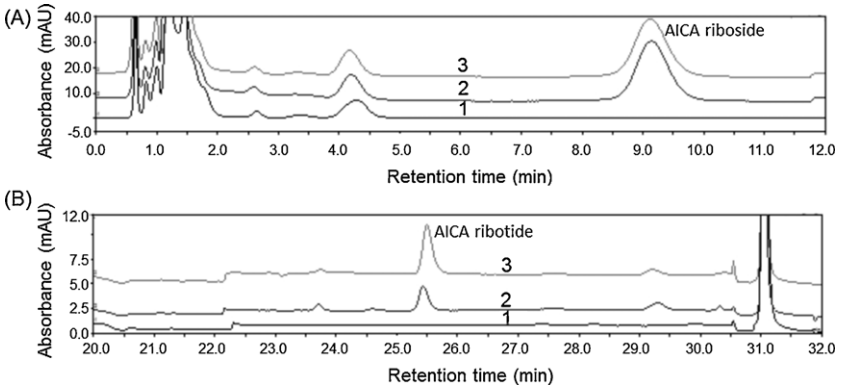

Fig. 5. The HPLC chromatograms of AICA riboside (A) and its active metabolite AICA ribotide (B) in nude mice plasma: blank nude mice plasma (A-1 and B-1), blank nude mice plasma spiked with 10 μg/ml AICA riboside (A-2) or 0.5 μg/ml AICA ribotide (B-2), and AICA riboside (A-3) and AICA ribotide (B-3) in a plasma sample from a nude mice at 10 min after intravenous administration of AICA riboside at the dose of 200 mg/kg.

Table 5
Stability results of AICA riboside and AICA ribotide in spiked plasma samples (n = 5).

Stability	Analytes	Nominal concentration (μg/ml)	Mean ± SD (μg/ml)	RE (%)	RSD (%)
Freeze–thaw stability (three cycles)	AICA riboside	0.2	0.20 ± 0.01	−1.92	5.74
		10	10.64 ± 0.21	6.41	1.99
		400	449.22 ± 4.77	12.3	1.06
	AICA ribotide	0.06	0.054 ± 0.001	−9.64	1.38
		1	1.07 ± 0.01	6.58	0.61
		40	42.16 ± 0.42	5.4	0.99
Short-term stability (2 h at 4 °C)	AICA riboside	0.2	0.19 ± 0.02	−5.2	9.08
		10	10.31 ± 0.10	3.07	0.94
		400	422.66 ± 20.85	5.67	4.93
	AICA ribotide	0.06	0.051 ± 0.007	−14.5	14.07
		1	0.85 ± 0.01	−15.38	1.47
		40	34.20 ± 1.49	−14.5	4.34
Long-term stability (14 days at −80 °C)	AICA riboside	0.2	0.20 ± 0.01	1.76	3.79
		10	10.46 ± 0.28	4.6	2.69
		400	431.20 ± 4.11	7.8	0.95
	AICA ribotide	0.06	0.054 ± 0.004	−9.78	8.4
		1	0.97 ± 0.05	−2.53	4.94
		40	39.66 ± 1.23	−0.84	3.11

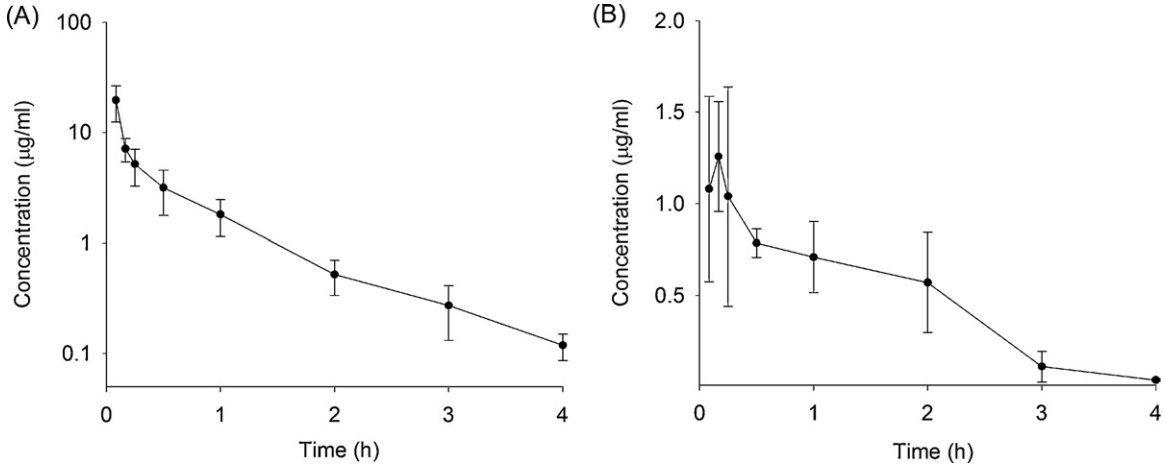

Fig. 6. Concentration–time profiles of AICA riboside (A) and its active metabolite AICA ribotide (B) in bearing MCF-7 xenografts nude mice plasma after intravenous administration of AICA riboside at the dose of 200 mg/kg ($n = 3$).

Table 6
Pharmacokinetics parameters of AICA riboside and AICA ribotide in nude mice bearing MCF-7 xenografts after intravenous administration of AICA riboside (200 mg/kg) ($n = 3$).

Parameter	AICA riboside (mean ± SD)	AICA ribotide (mean ± SD)
AUC_{0-t} (μg h/ml)	7.905 ± 1.985	1.891 ± 0.417
$AUC_{0-\infty}$ (μg h/ml)	8.013 ± 1.957	1.992 ± 0.459
$MRT_{0-\infty}$ (h)	0.636 ± 0.035	1.206 ± 0.039
$t_{1/2}$ (h)	0.441 ± 0.024	0.836 ± 0.027
C_{max} (μg/ml)	19.537 ± 7.095	1.530 ± 0.185
CL (l/h/kg)	24.959 ± 6.096	–
V (l/kg)	16.707 ± 5.006	–

metabolite AICA ribotide were presented in Table 6, respectively. After intravenous administration, the plasma AICA riboside concentration declined rapidly in a biphasic fashion with a short MRT of 0.636 ± 0.035 h which was similar to the previous study in healthy volunteers [22]. T_{max} of AICA ribotide was 0.167 ± 0.083 h, suggesting AICA riboside was rapidly metabolized to AICA ribotide.

4. Conclusion

In this study, a selective and sensitive HPLC method involving SPE and column switching was developed for simultaneous determination of AICA riboside and its active metabolite AICA ribotide in plasma. This method was validated for its selectivity, accuracy, precision and sensitivity as well as stability, and was successfully applied to pharmacokinetics study of AICA riboside and AICA ribotide in nude mice bearing MCF-7 cell xenografts.

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