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Pharmacokinetics, bioavailability and metabolism of rhaponticin in rat plasma by UHPLC-Q-TOF/MS and UHPLC-DAD-MSⁿ

Background: Rhaponticin (*Rheum L*.) demonstrates a variety of pharmacological activities, including antitumor, antithrombotic and antioxidant effect. However, there is no information describing the pharmacokinetics, bioavailability and metabolism of rhaponticin after intravenous administration. **Results:** UHPLC–Q-TOF/MS and UHPLC–multistage tandem MS methods were developed for the pharmacokinetics, bioavailability and metabolism of rhaponticin, rhapontigenin, a potent inhibitor of cytochrome P450, was confirmed by UHPLC–multistage tandem MS. The plasma profile of rhaponticin and rhapontigenin was determined by UHPLC–Q-TOF/MS. The results showed that rhaponticin was rapidly distributed and eliminated from rat plasma. The absolute oral bioavailability of rhaponticin was calculated to be 0.03%. The plasma concentrations of rhapontigenin rapidly increased and gradually eliminated after intravenous administration. **Conclusion:** The present pharmacokinetics, bioavailability and metabolism studies of rhaponticin will provide helpful information for development of suitable dosage forms and clinical references on rational administration.

(3,3',5-trihydroxy-4'-Rhaponticin methoxystilbene-3-O-β-D-glucoside), a major compound in the stilbene glucoside family, exists widely in medicinal plant of Rheum L., such as Rheum officinale Baill., Rheum undulatum Linn., Rheum hotaoense CY Cheng et CT Kao and Rheum palmatum Linn. [1,101]. Rhaponticin is recommended by health professionals in Asian countries for the treatment and prevention of allergies [2,3] and employed in Korea, Japan and China as an oral hemostatic agent in treating Oketsu [4]. Previous studies reported that rhaponticin have a potent antitumor, antithrombotic, antioxidant, vasorelaxant and α -glucoamylase inhibitory effect and alleviate liver steatosis and improve blood glucose and lipid profiles in transgenic diabetic mice [5-12]. These pharmacological studies demonstrate the various pharmacological effects of rhaponticin with an urgent need to examine the pharmacokinetic behavior of rhaponticin in vivo in more detail.

Analytical techniques such as LC, micellar electrokinetic capillary chromatography, gas-LC and thin-layer chromatography have been applied to the quantification of rhaponticin in traditional Chinese medicinal plant and Chinese traditional patent medicine [13–22]. Our previous publication have reported methods for the determination of rhaponticin in biological samples using LC coupled with ultraviolet detection (LC–UV) [23]. Presently, the preliminary results show low detection of rhaponticin in rat plasma within 30 min after oral (p.o.) administration (100 mg/kg). The cumulative amount of rhaponticin excreted in the faeces and urine within 24 h was also very low. The LLOQ of rhaponticin in rat plasma was 55 ng/ml, so the published LC–UV method may not be sensitive enough for the determination of rhaponticin in rat plasma [23].

Recently, the search for a method with reduced analysis time, enhanced retention time reproducibility, high chromatographic resolution, improved sensitivity and increased operation speed has resulted in the development of a new technology termed UPLC or rapid-resolution LC (RRLC) [24-29]. Quadrupole TOF-MS (Q-TOF/MS) allows the generation of mass information with a higher accuracy and precision. In 2005, Wrona et al. introduced MSE (where E represents collision energy) technique for the first time [30]. Two alternative scan functions are utilized for data acquisition in MS^E technique. Namely, the first function, typically set at 5 eV, collects low energy or unfragmented data while the second function collects high energy or fragmented data typically set by using a collision energy ramp from 20-30 eV.

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Key Terms

Rhaponticin: A stilbene glucoside compound. Used as antitumor and antioxidant agents.

Rapid-resolution LC: Fast separation technique based on the difference in analyte distribution between a mobile and a stationary phase.

UHPLC: LC using columns with <2 µm particles, resulting in better and/or faster separations.

Pharmacokinetics: Study of the time course of a drug within the body and the processes of absorption, distribution, metabolism and excretion.

Metabolite: Intermediates and products of metabolism. The term is usually restricted to small molecules.

Rhapontigenin: Main metabolite of rhaponticin, a potent inhibitor of cytochrome P450. This scan function allows for the collection of fragment ion data based on all ions in the first scan. In other words, MS^E can provide parallel alternating scans for acquisition at either low collision energy to obtain precursor ion information, or ramping of high collision energy to obtain full-scan accurate mass fragment, precursor ion and neutral loss information [31,32]. The structural confirmation of compounds, using accurately measured mass values, can be used to produce candidate empirical formula, which significantly reduces the number of possible structures of putative compounds.

To the best of our knowledge, the use of UPLC coupled with quadrupole TOF tandem MS (UHPLC-Q-TOF/MS) and UHPLC coupled with diode-array detection and multistage tandem MS (UHPLC-DAD-MSⁿ) has not been demonstrated in the study of rhaponticin pharmacokinetics. The objective for this project was to develop, first, a UHPLC-DAD-MSⁿ method to confirm rhaponticin and its metabolite, rhapontigenin and, second, a UHPLC-Q-TOF/MS method to quantify rhaponticin and its metabolite in the low-volume biological sample. This method was fully validated and applied to the pharmacokinetics and structural confirmation of metabolites in rat plasma following intravenous (iv.) administration of rhaponticin (10 mg/kg). The applicability of this method was determined for preclinical pharmacokinetic studies in the Sprague–Dawley rat.

Experimental sectionChemicals & materials

The rhaponticin standard (FIGURE 1A) was isolated from *R. hotaoense* using a method previously reported [33]. Analysis showed that its purity was above 99% (HPLC). Resveratrol (batch No.: 111535–200502) was used as an internal standard (IS) – a gift from Rui-Chao Lin (National Institute for Food and Drug Control, State Food and Drug Administration, Beijing, China). Methanol and acetonitrile were of LC grade. Double-distilled water was used throughout the work.

Preparation of standards & quality control samples

Stock solutions of rhaponticin and IS were prepared in methanol, and stored in darkness at 4°C. Working solutions were obtained by serial dilution of stock solution with methanol. Calibration standards containing 2, 5, 10, 50, 200, 400, 800, 1000 ng/ml of rhaponticin, and quality control (QC) samples containing 5 (low), 200 (medium) and 800 ng/ml (high) of rhaponticin were prepared with blank plasma to heparinized tubes.

Plasma sample preparation

A 50 μ l aliquot of plasma standard or sample was transferred to a 1.5 ml centrifuge tube, spiked with 25 μ l of IS working solution and vortexmixed (MS1 minishaker, IKA Instruments, China) for 1 min. An additional 125 μ l aliquot of methanol was added to the sample and vortex-mixed for 2 min. After vortex mixing, the mixture was centrifuged at 10,000 × g for 10 min (Eppendorf centrifuge 5424; Eppendorf Instruments, Germany). For analysis, 2.5 μ l of the supernatant were injected into the UHPLC for analysis.

UHPLC-Q-TOF/MS analysis

The UHPLC analysis was performed with a Waters AcquityTM UPLC system (USA) equipped with a Waters XevoTM QTof MS, a quadrupole and orthogonal acceleration TOF-MS/MS, which was equipped with a LockSprayTM interface and an ESI interface. Chromatographic separation was carried out at 40°C on an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm). The mobile phase consisted of 0.1% formic acid water (A) and ACN (B). The optimized UHPLC elution conditions were: 0–0.5 min, 1%B; 0.5–11 min, 1–20% B; 11–13 min, 20–90% B; 13–16 min, 90–1% B and 16–18 min, 1% B.





The autosampler was maintained at 4°C. The sample volume injected was 2.5 μl and the flow rate was 0.5 ml/min.

Detections were performed by a Waters Xevo[™] QTOF-MS equipped with an ESI source. High-purity nitrogen was used as the nebulizer and auxiliary gas. Argon was used as the collision gas. The TOF/MS analysis was performed in negative ion mode, and mass range was set at m/z 100–1000. The ESI capillary voltage was set at -2.5 kV in negative ion mode. The source and desolvation temperature were set at 120 and 450°C, respectively. The desolvation and cone gas flows were 700 and 30 l/h, respectively. The sample cone voltage, extraction cone voltage and lockspray capillary voltage was set at 40 V, 4.0 V and 2.0 kV, respectively. The collision energy was set at 30-45 eV. The data acquisition rate was set to 0.1 s, with a 0.02 s interscan delay. Mass accuracy was maintained using a lock spray with leucine enkephalin for negative ion mode ($[M-H]^- = 554.2615$) at a concentration of 300 ng/ml and a flow rate of 10 µl/min as reference. Data were collected in continuum mode, the lockspray frequency was set at 10 s, and data were averaged over 10 scans. All the acquisition and analysis of data were controlled by Waters Masslynx v4.1 software and Applied Waters Metabolynx XS software, respectively.

Structural confirmation of rhaponticin metabolite rhapontigenin by UHPLC-DAD-MSⁿ

Qualitative analysis of rhaponticin metabolite (rhapontigenin, FIGURE IB) from rat plasma was performed on an Agilent G6320 series LC/ MSD Trap MS system. The chromatographic separation was performed on an Agilent 1200 Series Rapid Resolution LC system (Agilent Technologies, USA), equipped with a binary pump, a microvacuum degasser, a high-performance autosampler, a column compartment, a diode array detector and a MS detector. The samples were separated on an Agilent Zorbax SB-C18 (50 mm \times 2.1 mm, 1.8 μ m) with an isocratic mobile phase consisting of 0.1% formic acid solution-methanol (20:80, v/v) at a flow of 0.4 ml/min. DAD spectra were acquired over a scan range of 190-400 nm. The sample volume injected was 10 µl.

MS experiments were performed with ESI source in positive ion mode. The vaporizer temperature was maintained at 300°C. The temperature of the drying gas was set at 350°C. The flow rate of the drying gas and the pressure of the nebulizing gas were set at 6 l/min and 60 psi, respectively. The capillary voltage was kept at 3.5×103 V. Full-scan spectra were acquired over a scan range of m/z 100–600. Optimization of the ESI/MSⁿ parameters and the qualitative analysis of rhaponticin and rhapontigenin were performed using the selected ion monitoring mode of the base ion peak at (m/z) 421 for rhaponticin and (m/z) 259 for rhapontigenin. Agilent ChemStation was used to control and process the data the Agilent 6320 Series Ion Trap LC–MS.

Method validation

The method was validated for selectivity, linearity, precision, accuracy, extraction recovery and stability according to the US FDA guideline for validation of bioanalytical methods [34].

The selectivity for compound separation was evaluated by comparing the chromatograms of five different batches of blank plasma obtained from five rats with those of corresponding standard plasma samples spiked with rhaponticin, IS and plasma sample obtained after iv. administration.

Calibration curve was prepared by assaying standard plasma samples at a concentration range (2–1000 ng/ml). The linearity of calibration curve was determined by plotting the peak area ratio (y) of rhaponticin to IS versus the nominal concentration (x) of rhaponticin. The calibration curve was constructed by weighted (1/x) least square linear regression. The LOD was considered as the final concentration producing a S/N ratio of three. The LLOQ was considered as the lowest concentration on the calibration curve where precision was within 20% and accuracy was within 100 \pm 20% [35,36].

Intra- and inter-day precision and accuracy were determined by five replicates of each LLOQ, low-, medium- and high-concentration quality control samples. within a day or during three consecutive days. Accuracy was calculated as the percentage of the concentration of drug measured from calibration curve to the theoretical concentration value of drug added to the blank plasma. Precision was expressed as the relative standard deviation(%), of measured concentrations for each QC samples. The values within $\pm 15\%$ for accuracy and precision were considered acceptable.

Recovery of rhaponticin was evaluated at three concentration levels of QC samples on the same day. Recovery was evaluated by comparing the analyte peak areas obtained from the QC samples after extraction with those obtained from the corresponding unextracted reference standards prepared at the same concentrations, respectively.

The matrix effect was determined by the ratio of the amounts of rhaponticin dissolved with blank matrix extract against those dissolved with methanol. The procedure was repeated three times.

To ensure the reliability of the results in relation to handling and storing of biological samples and stock standard solutions, stability studies were carried out at three different concentration levels. The stability of rhaponticin and IS stock solutions was evaluated after storage at room temperature and at 4°C for 30 days. The stability of rhaponticin and IS working solutions was investigated at room temperature for 6 h. QC plasma samples of three concentration levels were subjected to the conditions below. Short-term stability was assessed by analyzing QC plasma samples kept at room temperature for 6 h. Long-term stability was determined by assaying QC plasma samples after storage at -20°C for 15 days. Freeze-thaw stability was investigated after three freeze (-20°C)-thaw (room temperature) cycles.

• Evaluation of pharmacokinetics of rhaponticin & its metabolite in rats

The UHPLC-Q-TOF/MS method was applied for the pharmacokinetic studies after iv. and p.o. dose of rhaponticin. Male Sprague-Dawley rats $(200 \pm 10 \text{ g})$ were purchased from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). Rhaponticin dissolved in 2% Tween®80 was given orally (100 mg/kg) and via the caudal vein (10 mg/kg) exposed for rhaponticin injection. The blood samples were collected from retro-orbital puncture with ether and transferred into heparinized tubes at control and 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300 and 360 min after iv. or p.o. administration. Samples were immediately centrifuged at 5000 × g for 10 min and the plasma was stored at -20°C until analysis. Pharmacokinetics analysis by one-compartmental method was determined using DAS 2.0 software.

The oral bioavailability (F) is defined as the fraction of unchanged drug reaching the systemic circulation following administration through the oral route. The area under the drug concentration-time curve (AUC) is used as a measure of the total amount of unaltered drug that reaches the systemic circulation. The



Figure 2. Mass spectrum of rhaponticin and rhapontigenin. (A) *m/z* 421.0 for rhaponticin and (B) 259.0 for rhapontigenin, showing the suggested structures of the parent ion (red squares) and the daughter fragment ion (blue diamonds).





Figure 3. Typical MS spectra. (A) Rhaponticin and (B) the internal standard in rat plasma.

absolute oral bioavailability of rhaponticin after the p.o. administration compared to the iv. administration was calculated as follows:

$$F = \frac{(AUC_{p.o.}/dose_{p.o.})}{(AUC_{iv.}/dose_{iv.})}$$

All statistical analyses were performed using one way ANOVA test and p-values <0.05 were considered significant.

Results & discussion

Structural confirmation of rhaponticin metabolite rhapontigenin by UHPLC-DAD-MSⁿ

The mass spectra of rhaponticin and metabolite rhapontigenin are shown in **FIGURE 2A & B**. Peaks at m/z 421.0 (**FIGURE 2A**) and m/z 259.0 (**FIGURE 2B**) corresponded to protonated rhaponticin and its metabolite, rhapontigenin in the ESI+ source. The analytes formed were predominantly protonated molecular ion $[M+H]^+$. The most abundant ion originating from precursor ions at m/z 421.0 is a characteristic daughter ion (MS2) fragment ion $[M+H-C_6H_{10}O_5]^+$ at m/z259.1. The MS2 at m/z 259.1 was isolated and further fragmented (MS3), resulting in ions at m/z 241.0, 226.9, 199.0, 149.0, 135.0 and 107.1. Loss of one H₂O (18 Da) and one CH₂OH (32 Da) from fragment ion $[M+H-C_{\ell}H_{10}O_{\ell}]^{+}$ yields the product ions $[M+H-C_{k}H_{10}O_{s}-H_{10}O]^{+}$ $(m/z \ 241.0)$ and $[M+H-C_6H_{10}O_5-CH_3OH]^+$ (m/z 226.9), respectively. Loss of CH₂OH+CO from the precursor ion m/z 259.1 produces ion $[M+H-C_6H_{10}O_5-CH_3OH-CO]^+$ (*m*/*z* 199.0). Other fragments are a result of: loss of $C_{\alpha}H_{\alpha}O_{\gamma}$ (110 Da) from m/z 259.1 yields [M+H-C, H, O, - $C_{L}H_{C_{1}}^{+}$ (*m/z* 149.0); double bond break from m/z 259.1 yields [M+H-C₆H₁₀O₅-C₇H₈O₂]⁺ $(m/z \ 135.0)$; loss of C₉H₁₂O₂ (152 Da) from m/z 259.1 yields $[M+H-C_{6}H_{10}O_{5}-C_{9}H_{12}O_{2}]^{+}$ (m/z 107.1). The product ions and corresponding neutral fragment loss mentioned above are characteristic of the rhaponticin structure, and are needed to identify further metabolites. The most abundant ion of metabolite (rhapontigenin) originating from precursor ion at m/z 259.0 (FIGURE 2B) generated a series of fragment ions at m/z 241.0, 227.0, 199.0, 181.0, 149.0, 135.0 and 107.1, which were consistent with rhaponticin fragment ions (MS3). The UV spectra of rhapontigenin and of rhaponticin are similar with an absorption wavelength maximum (λ max)

at 220 and 324 nm. A recent report showed rhapontigenin to be enzymatically synthesized from the glycosylated parent compound rhaponticin [37]. Therefore, rhapontigenin was identified as an aglycone of rhaponticin and the structure is shown in **FIGURE IB**. Rhapontigenin is suggested to be the active moiety in anticancer, antiallergic, anticoagulant and anti-inflammatory

compounds [3,37–40]. The literature reports that rhapontigenin can inhibit human cytochrome P450 1A1 enzyme and CYP 1B1 of cancers, such as prostate and breast cancers [41,42].

UHPLC-MS condition optimization

UHPLC-Q-TOF/MS operation parameters were carefully optimized for the determination



Figure 4. Representative UPLC–Q-TOF/MS base peak intensity chromatogram. (A) Blank plasma spiked with rhaponticin LLOQ concentration; **(B)** blank plasma spiked with rhaponticin (400 ng/ml) and **(C)** IS; **(D)** plasma sample at 60 min following intravenous administration of rhaponticin (527 ng/ml) at a single dose of 10 mg/kg (1, rhaponticin; 2, IS; M1, rhapontigenin). IS: Internal standard.

of rhaponticin. A standard solution of rhaponticin was infused along with the mobile phase into the mass spectrometer with ESI as the ionization source. The most abundant fragmentation ions of rhaponticin at m/z 257.08 $([M-H-C_{\ell}H_{10}O_{\ell}]^{-})$ was selected for quantification under extracted ion chromatogram mode in a mass window of m/z 257.04–257.13. The MS spectra of rhaponticin and IS are shown as FIGURE 3. UHPLC-MS analysis of the blank plasma, blank plasma spiked with rhaponticin and IS, and a plasma sample after iv. administration showed no endogenous peak interference with the quantification of rhaponticin and the IS (FIGURE 4). In order to improve ionization efficiency and peak shape, 0.1% formic acid was added to the aqueous phase.

Method validation

No interferences from endogenous substances were observed at the retention time regions of the rhaponticin and IS. A linear calibration curve was obtained over the concentration range 2-1000 ng/ml for rhaponticin in rat plasma. The calibration curve showed good linearity (r > 0.9994) over the concentration range tested. The LLOQ for rhaponticin in rat plasma was 2 ng/ml. The results of precision and accuracy were presented in TABLE I. All the values of precision and accuracy were within the specified ranges and therefore acceptable. The results showed that extraction recoveries of rhaponticin from rat plasma were 90.0 ± 4.9 , 91.9 ± 4.1 , and $94.3 \pm 2.3\%$ at concentrations levels of 5, 200 and 800 ng/ml, respectively. The mean recovery was 92.1 ± 3.6%. Recovery of the IS was $82.5 \pm 4.3\%$. To evaluate the effect of the sample matrix on MS response, we compared the instrument response for quality control by injecting samples into the mobile phase with responses for the same amount of rhaponticin with samples that were not extracted and extracted samples. All the ratios were between 88.2 and 109.5%. No significant matrix effect for rhaponticin was observed. TABLE 2 summarizes the results of short-term, long-term and freeze-thaw stability of rhaponticin in rat plasma. All the results met the criterion for stability measurements. The data suggested no significant loss of analyte during sample storage and processing, which are consistent with our previous method [23].

Pharmacokinetic studies

The present method allowed successful quantification of rhaponticin in plasma following iv.

Table 1. Precision and accuracy for the determination of rhaponticin in rat plasma

Nominal concentration (ng/ml)	Calculated concentration (ng/ml)	Accuracy (%)	Precision, RSD (%)						
Intra-day ⁺									
2	2.1	105.0	11.2						
5	5.1	102.0	8.7						
200	189.2	94.6	6.1						
800	783.8	98.0	3.2						
Inter-day [≠]									
2	2.1	104.2	10.6						
5	4.7	94.0	10.8						
200	200.6	100.3	8.6						
800	784.6	98.1	3.9						
[†] n = 5. [‡] n = 5 series per day, 3 days.									

and p.o. dose. The concentration of rhapontigenin in rat plasma was indirectly quantified by referring to the rhaponticin calibration curve recorded with the described UHPLC-MS method. With the similar chemical structures compared with rhaponticin, the UV absorption of the metabolite rhapontigenin was similar to that of rhaponticin. Therefore, the concentration of rhapontigenin in rat plasma was also determined using the regression equation of rhaponticin from our previous method [23]. The results of two determination methods showed almost agreement. The plasma concentrationtime profiles of rhaponticin and rhapontigenin are shown in FIGURE 5A & B. TABLE 3 summarizes the pharmacokinetic parameters of rhaponticin and rhapontigenin after iv. (10 mg/ kg) and p.o. (100 mg/kg) dose. After iv. dose of

Table 2. Stability of rhaponticin in rat plasma at three QC levels (n = 5).

QC concentration (ng/ml)	Calculated concentration (ng/ml)	RSD (%)
Short-term stability		
5	5.1	10.1
200	204.4	6.2
800	806.8	3.8
Long-term stability		
5	5.1	8.5
200	193.6	9.2
800	797.4	4.6
Freeze-thaw stability		
5	4.8	9.4
200	205.4	6.1
800	794.2	3.2

Table 3. Pharmacokinetic data of rhaponticin after intravenous or oral administration (10 and 100 mg/kg, respectively) and pharmacokinetic data of metabolite rhapontigenin after an intravenous administration (10 mg/kg) of rhaponticin in rats.

	T _{max} (min)	C _{max} (µg/ml)	AUC _(0-t) (µg min/ml)	AUC _(0-∞) (μg min/ml)	V _d (l/kg)	C _L (ml/min/kg)		
Intravenous route [†]	N/A	8.91 ± 0.12	215.8 ± 25.7	216.9 ± 25.0	6.43 ± 3.42	0.047 ± 0.005		
Oral route ⁺	10.32 ± 0.12	1.71 ± 0.13	0.70 ± 0.08	0.88 ± 0.16	62.02 ± 5.58	18.76 ± 6.36		
Rhapontigenin	52.5 ± 12.5	0.18 ± 0.01	23.42 ± 2.67	23.94 ± 2.86	0.011 ± 0.001	0.42 ± 0.06		
^t Data are expressed as mean ± SD C : Total clearance: V : Volume of distribution.								

rhaponticin, the metabolite rhapontigenin was detected at 5 min, indicating rapid metabolism of rhaponticin. The plasma levels of rhaponticin after the iv. dose decreased rapidly in rat ($T_{max} = 10$ min) whereas plasma concentrations of rhapontigenin gradually increased to reach $C_{max} = 0.18$ µg/ml (range 0.14–0.20 µg/ml) at approximately 52 min after iv. administration and slowly began to decline thereafter (FIGURE 5B).

Concentration levels of rhaponticin in rat plasma after an p.o. dose were lower than quantified in rat plasma after iv. administration (FIGURE 5A). The absolute oral bioavailability of rhaponticin was calculated to be 0.03%. Highly lipophilic stilbenes, such as rhaponticin, possess a partition coefficient that is highly distributed in tissues [43]. High hepatic clearance combined with biotransformation attributed by digestive enzymes produced by enteric microbial flora in the rat digestive tract may convert rhaponticin to other active metabolites [3,43]. The change in behavior of the parent compound, rhaponticin, and the metabolite rhapontigenin due to the moieties demonstrates the change in pharmacokinetics [44]. We are currently conducting further experiments to clarify the metabolism of rhaponticin.

Conclusion

A reliable and sensitive UHPLC–Q-TOF/ MS and UHPLC–DAD–MSⁿ method was established for the quantitative determination of rhaponticin and its metabolite rhapontigenin in rats to investigate the pharmacokinetics, bioavailability and metabolism.



Figure 5. Mean plasma concentration–time curves of rhaponticin. After **(A)** an intravenous (10 mg/kg) or oral (100 mg/kg) administration and **(B)** metabolite rhapontigenin after an intravenous administration (10 mg/kg) of rhaponticin.

UHPLC-DAD-MSⁿ was successfully used to confirm rhaponticin and its metabolite, rhapontigenin, with accuracy in rat plasma. UHPLC-O-TOF/MS method was validated for quantitative analysis of rhaponticin in low volume of plasma samples. Following a single iv. or p.o. dose of rhaponticin, rapid distribution and elimination from rat plasma was detected. The plasma concentrations of rhapontigenin rapidly increased and gradually eliminated after iv. administration. The assay showed good sensitivity and reproducibility for the determination of low volume biological samples. The selectivity of this method did not have interference from other analytes. The present in vivo pharmacokinetics, bioavailability and metabolism studies of rhaponticin will provide helpful information for the development of suitable dosage forms and clinical references on rational administration.

Future perspective

We believe that UHPLC–Q-TOF/MS and UHPLC–DAD–MSⁿ method will be an important tool in understanding the pharmacokinetics, bioavailability and metabolism of rhaponticin in plasma after iv. administration. The achieved pharmacokinetics, bioavailability and metabolism results may be useful for further study of the tissue distribution, excretion (bile, faeces and urine) and bioactive mechanism of rhaponticin.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Aim of the study

- Rhaponticin demonstrates a variety of pharmacological activities, including antitumor, antithrombotic and antioxidant effect.
- These multiple pharmacological activities of rhaponticin make it worth carrying out further comprehensive studies on pharmacokinetics, bioavailability and metabolism of rhaponticin.

Methods

- A sensitive and reliable UHPLC–Q-TOF/MS method has been developed for the pharmacokinetics, bioavailability and metabolism studies of rhaponticin in rat plasma.
- UHPLC–DAD–MSⁿ is becoming a useful technique for drug metabolites detection and structural confirmation, and the method has been developed for the structural confirmation of rhaponticin and its metabolite rhapontigenin in rat plasma.
- The methods were validated as per US FDA guidelines.

Results

- The developed UHPLC-Q-TOF/MS method is free from matrix effects.
- Stability of rhaponticin is good in plasma and various other solutions.
- The metabolite of rhaponticin, rhapontigenin, a potent inhibitor of cytochrome P450, was identified and confirmed by UHPLC–DAD–MSⁿ.
- Following a single intravenous or oral dose of rhaponticin, the plasma profile of rhaponticin was determined by UHPLC–Q-TOF/MS. The results showed that rhaponticin was rapidly distributed and eliminated from rat plasma.

Conclusion

 The reported method provides a novel and useful analytical tool for pharmacokinetics, bioavailability and metabolism of rhaponticin in rat plasma.

References

(2007).

- Papers of special note have been highlighted as: • of interest
- of interest
- of considerable interest
 Zhang R, Kang KA, Piao MJ *et al.* Rhapontigenin from *Rheum undulatum* protects against oxidative-stress-induced cell damage through antioxidant activity. *J. Toxicol. Environ. Health* 70(13), 1155–1166
- 2 Matsuda H, Tomohiro N, Harima K *et al.* Study on anti-Oketsu activity on rhubarb II. Antiallergic effects of stilbene components from *Rhei undulati Rhizoma* (dried rhizome of *Rheum undulatum* cultivated in Korea). *Biol. Pharm. Bull.* 24(3), 264–267 (2001).
- 3 Kim DH, Park EK, Bae EA, Han MJ. Metabolism of rhaponticin and chrysophanol 8-O-glucopyranoside from the rhizome of *Rheum undulatum* by humam intestinal bacteria and their anti-allergic actions. *Biol. Pharm. Bull.* 23(7), 830–833 (2000).
- 4 Matsuda H, Kageura T, Morikawa T, Toguchida I, Harima S, Yoshikawa M. Effects of stilbene constituents from rhubarb on nitric oxide production in lipopolysaccharideactivated macrophages. *Bioorg. Med. Chem. Lett.* 10(4), 323–327 (2000).
- 5 Ryu SY, Choi SU, Lee CO, Lee SH, Ahn JW, Zee OP. Antitumor activity of some phenolic components in plants. *Arch. Pharm. Res.* 17(1), 42–44 (1994).
- 6 Misiti F, Sampaolese B, Mezzogori D et al. Protective effect of rhubarb derivatives on amyloid beta (1–42) peptide-induced apoptosis in IMR-32 cells: a case of nutrigenomic. Brain Res. Bull. 71(1–3), 29–36 (2006).
- 7 Hibasami H, Takagi K, Ishii T, Tsujikawa M, Imai N, Honad I. Induction of apoptosis by rhapontcin having stilbene moiety, a component of rhubarb (*Rheum officinale Baillon*) in human stomach cancer KATO III cells. *Oncol. Rep.* 18(2), 347–351 (2007).
- 8 Park EK, Choo MK, Yoon HK, Kim DH. Antithrombotic and antiallergic activities of rhaponticin from *Rhei Rhizoma* are activated by human intestinal bacteria. *Arch. Pharm. Res.* 25(4), 528–533 (2002).
- 9 Ngoc TM, Minh PTH, Hung TM et al. Lipoxygenase inhibitory constituents from rhubarb. Arch. Pharm. Res. 31(4), 598–605 (2008).
- 10 Yoo MY, Oh KS, Lee JW *et al.* Vasorelaxant effect of stilbenes from rhizome extract of rhubarb (*Rheum undulatum*) on the contractility of rat aorta. *Phytother. Res.* 21(2), 186–189 (2007).

- 11 Choi SB, Ko BS, Park SK, Jang JS, Park S. Insulin sensitizing and a-glucoamylase inhibitory action of sennosides, rheins and rhaponticin in *Rhei Rhizoma. Life Sci.* 78(9), 934–942 (2006).
- 12 Chen JL, Ma MM, Lu YW, Wang LS, Wu C. Duan HF. Rhaponticin from rhubarb rhizomes alleviates liver steatosis and improves blood glucose and lipid profiles in KK/Ay diabetic mice. *Planta Med.* 75(5), 472–477 (2009).
- 13 Wang LX, Zhang BC, Li LP, Zhou Z. Quantitative determination of rhapontin in root of *Rheum hotaoense* by TLC-scanning. *China J. Chin. Mater. Med.* 19(1), 37–62 (1994).
- 14 Xu LN, Han X, Qi Y et al. Multiple compounds determination and fingerprint analysis of Lidanpaishi tablet and keli by high-performance liquid chromatography. *Anal. Chim. Acta* 633(1), 136–148 (2009).
- 15 Gocan S. Analysis of stilbenes in wine by HPLC: recent approaches. J. Liq. Chromatogr. Related Technol. 32(11), 1598–1643 (2009).
- 16 Yi T, Leung KSY, Lu GH, Zhang H, Chan K. Identification and determination of the major constituents in traditional Chinese medicinal plant *Polygonum multiflorum* Thunb by HPLC coupled with PAD and ESI/MS. *Phytochem. Anal.* 18(3), 181–187 (2007).
- 17 Qu J, Xiao SY, Wang YM, Luo GA. Screening and identification of glycosides in biological samples using energy-gradient neutral loss scan and liquid chromatography tandem mass spectrometry. *Anal. Chem.* 76(8), 2239–2247 (2004).
- 18 Shang XY, Yuan ZB. Determination of six components in rhubarb by cyclodextrinmodified micellar electrokinetic chromatography using a mixed micellar system of sodium cholate and sodium taurocholate. *Anal. Chim. Acta* 456(2), 183–188 (2002).
- 19 Shang XY, Yuan ZB. Separation of effective constituents from rhubarb by micellar electrokinetic capillary chromatography. *Anal. Lett.* 35(1), 195–202 (2002).
- 20 Shang XY, Yuan ZB. Determination of active components in rhubarb and study of their hydrophobicity by micellar electrokinetic chromatography. *Bioorg Med. Chem. Lett.* 13(4), 617–622 (2003).
- 21 Shang XY, Yuan ZB. Separation and determination of active components in rhubarb by cyclodextrin-modified micellar electrokinetic chromatography using a mixed micellar system. *Anal. Lett.* 36(1), 203–214 (2003).
- 22 Hemingway RW, Hillis WE, Bruerton K. A gas-liquid chromatographic examination of stilbene derivatives. *J. Chromatogr. A* 50(1), 391–399 (1970).

- 23 Zhao YY, Wang LL, Miao H *et al.* LC method for the determination of rhaponticin in rat plasma, faeces and urine for application to pharmacokinetic studies. *Chromatographia* 73(5–6), 599–603 (2011).
- First paper to report a LC method for quantification of rhaponticin in rat plasma, faeces and urine.
- 24 Hsieh Y, Gaviz G, Hwa J. Ultra-performance hydrophilic interaction LC–MS-MS for the determination of metformin in mouse plasma. *Bioanalysis* 1(6), 1073–1079 (2009).
- 25 Marcelín-Jiménez G, Moreno Alionka CPA, Mendoza-Morales L, Rivera-Espinosa L, Martínez MM. Development of an ultraperformance liquid chromatography-tandem mass spectrometry micromethod for quantification of lamotrigine in human plasma and its use in a bioequivalence trial. *Bioanalysis* 1(1), 47–55 (2009).
- 26 Szapacs ME, Urbanski JJ, Kehler JR et al. Absolute quantification of a therapeutic domain antibody using ultra-performance liquid chromatography-mass spectrometry and immunoassay. *Bioanalysis* 2(1), 1597–1608 (2010).
- 27 Zhao YY, Qin XY, Cheng XL et al. Rapid resolution liquid chromatography– mass spectrometry and high-performance liquid chromatography-fluorescence detection for metabolism and pharmacokinetic studies of ergosta-4,6,8(14),22-tetraen-3-one. Anal. Chim. Acta 675(2), 199–206 (2010).
- 28 Zhao YY, Cheng XL, Liu R et al. Pharmacokinetics of ergosterol in rats using rapid resolution liquid chromatography– atmospheric pressure chemical ionization multi-stage tandem mass spectrometry and rapid resolution liquid chromatography/ tandem mass spectrometry. J. Chromatogr. B 879(21), 1945–1953 (2011).
- 29 Li F, Hsieh Y, Kang L *et al.* MALDI-tandem mass spectrometry imaging of astemizole and its primary metabolite in rat brain sections. *Bioanalysis* 1(2), 299–307 (2009).
- 30 Wrona M, Mauriala T, Bateman KP, Mortishire-Smith RJ, O'Connor D. 'All-in-One' analysis for metabolite identification using liquid chromatography/hybrid quadrupole TOF mass spectrometry with collision energy switching. *Rapid Commun. Mass Spectrom.* 19 (18), 2597–2602 (2005).
- 31 Zhao YY, Cheng XL, Wei F *et al.* Serum metabonomics study of adenine-induced chronic renal failure rat by ultra performance liquid chromatography coupled with quadrupole TOF mass spectrometry. *Biomarkers* doi:10.3109/1354750X.637180 (2012) (Epub ahead of print).

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- 32 Zhao YY, Liu J, Cheng XL, Bai X, Lin RC. Urinary metabonomics study on biochemical changes in an experimental model of chronic renal failure by adenine based on UPLC Q-TOF/MS. *Clin. Chim. Acta* 413(5–6), 642–649 (2012).
- 33 Zhao YY, Qin XY, Chen SP *et al.* Crystal and molecular structure of rhaponticin from *Rheum hotaoense. J. Chem. Crystallogr.* 41(3), 409–414 (2011).
- 34 U.S. Department of Health and Human Services, Food and Drug administration, Centre for Drug Evaluation and Research (CDER), Centre for Veterinary Centre (CVM). Guidance for industry: bioanalytical method validation (2001).
- 35 Zhao YY, Cheng XL, Zhang YM *et al.* A fast and sensitive HPLC-MS/MS analysis and preliminary pharmacokinetic characterization of ergone in rats. *J. Chromatogr. B* 878(1), 29–33 (2010).
- 36 Zhao YY, Qin XY, Zhang YM *et al.* Quantitative HPLC method and pharmacokinetic studies of ergosta-4,6,8(14),22-tetraen-3-one, a natural product with diuretic activity from *Polyporus umbellatus. Biomed. Chromatogr.* 24(10), 1120–1124 (2010).

- 37 Roupe KA, Helms GL, Halls SC, Yáñez JA, Davies NM. Preparative enzymatic synthesis and HPLC analysis of rhapontigenin: applications to metabolism, pharmacokinetics and anti-cancer studies. *J. Pharm. Pharmaceut. Sci.* 8(3), 374–386 (2005).
- A simple HPLC method was developed for the determination of rhapontigenin. The assay was successfully applied to both the *in vitro* and *in vivo* metabolic kinetic study of rhapontigenin.
- 38 Kageura T, Matsuda H, Morikawa T *et al.* Inhibitors from rhubarb on lipopolysaccharide-induced nitric oxide production in macrophages: structural requirements of stilbenes for the activity. *Bioorg. Med. Chem.* 9(7), 1887–1893 (2001).
- 39 Cheong H, Ryu SY, Kim KM. Anti-allergic action of resveratrol and related hydroxystilbenes. *Planta Med.* 65(3), 266–268 (1999).
- 40 Aburjai TA. Anti-platelet stilbenes from the aerial parts of *Rheum palaestinum*. *Phytochemistry* 55(5), 407–410 (2000).
- 41 Chun YJ, Ryu SY, Jeong TC, Kim MY. Mechanism-based inhibition of human Cytochrome P450 1A1 by rhapontigenin. *Drug Met. Disp.* 29(4), 389–393 (2001).

- Rhapontigenin is a potent mechanism-based inactivator of human P450 1A1 and may be considered as a good candidate for a cancer chemopreventive agent in humans.
- 42 Guengerich FP, Chun Y, Kim D, Gilliam EMJ, Shimada T. Cytochrome P450 1B1: a target for inhibition in anticarcinogenesis strategies. *Mut. Res.* 523–524(1–2),173–182 (2003).
- 43 Roupe KA, Yáñez JA, Teng XW, Davies NM. Pharmacokinetics of selected stilbenes: rhapontigenin, piceatannol and pinosylvin in rats. J. Pharm. Pharmacol. 58(11), 1443–1450 (2006).
- Rhapontigenin was highly distributed into tissues and were highly extracted by the liver. The estimates of oral bioavailability characterize rhapontigenin as poorly bioavailable compounds.
- 44 Sale S, Verschoyle RD, Boocock D et al. Pharmacokinetics in mice and growthinhibitory properties of the putative cancer chemopreventive agent resveratrol and the synthetic analogue trans 3,4,5,4'-tetramethoxystilbene. Brit. J. Cancer 90(3), 736–744 (2004).

Patent

101 Lee GM, Lee HJ. KR 2002088243 (2002).