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Determination of Astragaloside IV in rat plasma by liquid chromatography electrospray ionization mass spectrometry

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Abstract

Astragaloside IV (AGS-IV) is an active constituent of Radix Astragali used in many Traditional Chinese Medicines. This paper describes a sensitive and specific assay for the quantitation of AGS-IV in rat plasma. After solid phase extraction (SPE), samples were analyzed by liquid chromatography electrospray ionization mass spectrometry using a reversed-phase C18 column. The assay was linear in the range 1–500 ng/ml with a limit of detection of 0.5 ng/ml. The recovery was 92.5% and within-day and between-day precision were 3.7–6.0 and 2.8–9.8%, respectively. The assay was applied to a pharmacokinetic study in rat after a single oral dose. The drug was rapidly absorbed and subsequently eliminated according to a biphasic concentration–time curve.

Keyword: Astragaloside IV

1. Introduction

Radix Astragali is prepared from the roots of certain species of plants of the genus *Astragalus* (*Leguminosae*). It possesses well-documented hepatoprotective, antioxidant, antiviral, antihypertensive and immunostimulant activity [1–3] and has been widely used in Traditional Chinese Medicine (TCM) [4]. Astragaloside IV (AGS-IV) (Fig. 1), a cycloartane-type triterpene glycoside, is a characteristic and active constituent whose presence forms part of the quality assurance of Radix Astragali and products containing it [5]. Little is known about the metabolism and pharmacokinetics of AGS-IV despite the extensive consumption of Radix Astragali in TCM.

In order to investigate the potential of AGS-IV as a pure drug, a sensitive and specific assay for its quantitation in biological fluids is required. The fact that the structure of AGS-IV does not contain a chromophore makes the application of direct high-performance liquid chromatography (HPLC) with UV detection rather limited. A precolumn derivatization method [6] and the use of evaporative light-scattering detection [7] give improved sensitivity but the limit of detection of about 2 μg/ml in both cases remains inadequate for pharmacokinetic studies. This paper describes a sensitive and selective assay for AGS-IV based on liquid chromatography mass spectrometry (LC–MS) and its application to a pharmacokinetic study in rat.

2. Experimental

2.1. Reagents

AGS-IV (>99.0%) certified by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) was kindly donated by Jiangsu Pharmaceutical Research Institute (Nanjing, China). Digoxin (>99.5%) for use as internal standard (I.S.) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Distilled, deionized water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA).

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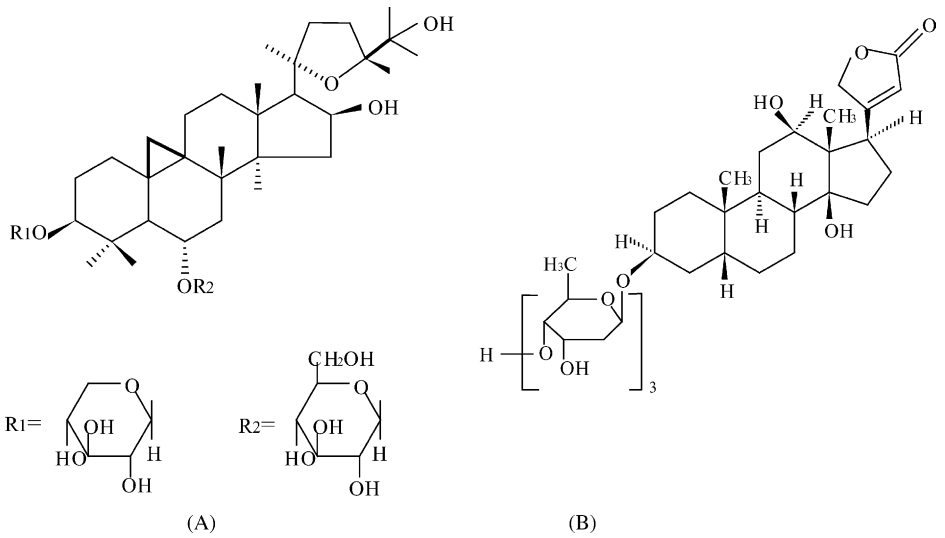

Fig. 1. Structure of (A) AGS-IV and (B) the internal standard, digoxin.

2.2. Animals

Sprague–Dawley rats (190–210 g) were obtained from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China) and housed six to a cage with unlimited access to food and water except for 12 h before and during the experiment. The animals were maintained on a 12 h light–dark cycle (light on from 8:00 to 20:00 h) at ambient temperature (22–24 °C) and ca. 60% relative humidity. Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of China Pharmaceutical University (Nanjing, China) and the protocol was approved by the Animal Ethics Committee of this institution.

2.3. Apparatus

The HPLC system was a Hewlett-Packard HP1100 LC system equipped with a binary pump and automatic sampler. Separation was carried out on a Diamonsil (Dikma, USA) C18 analytical column (Diamonsil^{TM} 150 mm × 2.1 mm, 5 μm particle size) maintained at 25 °C. The mobile phase was acetonitrile–0.01% acetic acid (42:58, v/v) and the flow rate was 0.2 ml/min. A single quadrupole mass spectrometer (Hewlett-Packard HP1100) equipped with an electrospray was used with the following parameters: fragmentor 90 V; spray voltage 4 kV; nebulizer pressure 40 psi; gas temperature 350 °C; auxiliary gas flow 10.5 ml/min. Positive ionization with selected ion monitoring (SIM) (multiplier gain set on one) was used for all analyses. Quasi-molecular ions $(M + Na)^+$ were detected at *m/z* 807.5 for AGS-IV and *m/z* 803.5 for digoxin (I.S.).

Table 1
Recovery of AGS-IV in rat plasma ($n = 5$)

Concentration (ng/ml)	1	2	3	4	5	Mean	S.D.
5	87.5	96.8	95.3	97.2	97.6	94.9	4.2
50	88.7	97.5	89.7	95.2	96.8	93.6	4.1
500	83.8	92.1	95.2	85.8	96.3	90.6	5.6

2.4. Sample preparation

Oasis HLB cartridge solid phase extraction columns (1 cc/30 mg volume, Waters, USA) were conditioned with 2 × 1 ml methanol, followed by 1 ml distilled water. Samples of plasma (500 μl) were mixed with 10 μl of I.S. solution (20 μg/ml in water) and water (500 μl) and loaded onto SPE columns. Columns were then washed with water–methanol (95:5, v/v) (1 ml) followed by 0.02% acetic acid (1 ml) before AGS-IV and the I.S. were eluted with 2 ml of methanol. Methanol extracts were evaporated to dryness in a water bath at 45 °C under a nitrogen stream after which residues

Table 2
Within-day and between-day precision for assay of AGS-IV in rat plasma ($n = 5$)

	Concentration (ng/ml)		
	5	50	500
Within-day	4.1	50.6	452.5
	4.5	54.3	485.4
	4.7	55.9	499.6
	4.7	55.1	487.2
	4.2	53.3	491.3
Mean ± S.D.	4.5± 0.3	53.8 ± 2.1	483.2 ± 18.0
CV (%)	6.04	3.85	3.73
Between-day	4.4	52.1	480.8
	4.7	53.8	483.2
	4.4	53.3	508.7
	5.2	55.8	502.2
	4.0	50.4	478.3
Mean ± S.D.	4.5 ± 0.4	53.1 ± 2.0	490.6 ± 13.8
CV (%)	9.80	3.75	2.82

were reconstituted in 200 μl aliquots of HPLC mobile phase and centrifuged at 18,000 × *g*, 4 °C for 10 min. Supernatants (10 μl) were injected into the HPLC system.

2.5. Assay validation

AGS-IV plasma standards (1.0, 5.0, 10.0, 20.0, 50.0, 100, 200 and 500 ng/ml) were prepared by dilution of an aqueous stock solution with rat plasma and then treated in the same way as samples. The calibration curve for AGS-IV was based on the peak area ratio of AGS-IV to the I.S. Recovery of the assay was based on quintuplicate analysis of plasma samples spiked with AGS-IV at concentrations of 5.0, 50.0 and 500 ng/ml and comparison with results obtained by injecting solutions containing the equivalent amount of AGS-IV in mobile phase. Precision was determined at the same three concentrations by quintuplicate analysis on one day (within-day) and again on another four

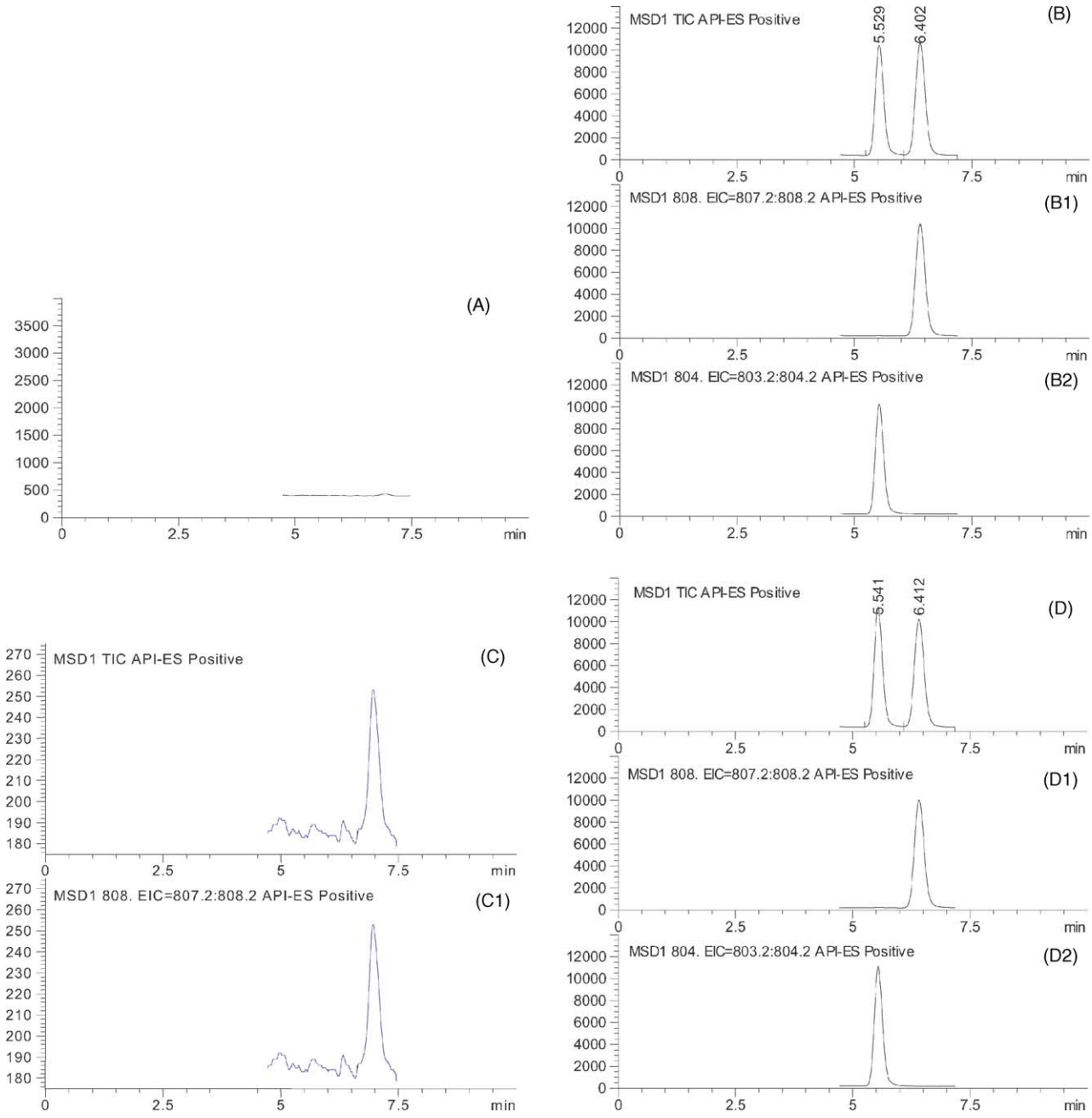

Fig. 2. Chromatograms of AGS-IV in rat plasma: (A) blank rat plasma; (B) blank plasma spiked with AGS-IV (100 ng/ml) and internal standard, (B1) AGS-IV (extract ion *m*/*z* 807.5), (B2) internal standard (extract ion *m*/*z* 803.5); (C) chromatogram of AGS-IV in blank plasma spiked with AGS-IV (1 ng/ml), (C1) AGS-IV; (D) plasma sample from a rat 3 h after oral administration of AGS-IV (the calculated concentration of AGS-IV is 91 ng/ml), (D1) AGS-IV, (D2) internal standard.

Table 3
Pharmacokinetic parameters computed using the PKAnalyst® program for rats given a single oral dose of AGS-IV (20.0 mg/kg)

Parameters	k_a (h^{-1})	t_{max} (h)	C_{max} (ng/ml)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	CL ($l\,kg^{-1}\,h^{-1}$)	V_d ($l\,kg^{-1}$)	$AUC_{0\rightarrow\infty}$ ($ng\,h\,ml^{-1}$)
	2.92	0.43	374	0.30	4.65	0.43	0.56	1062

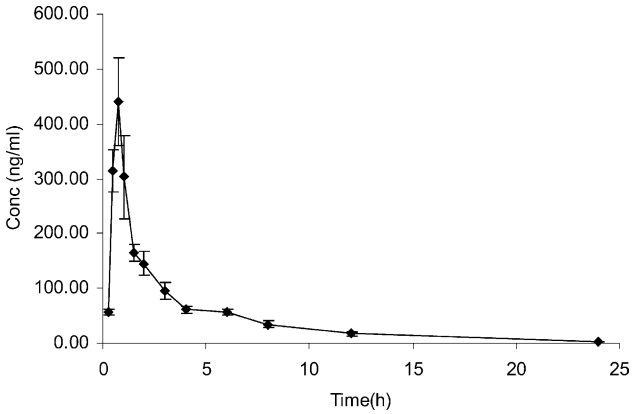

Fig. 3. Mean plasma concentration–time curve in rats after a single oral dose of AGS-IV (20.0 mg/kg). Each point represents the mean ± standard error of the mean for six rats.

different days. The limit of detection was determined at a signal-to-noise ratio of 3.

2.6. Pharmacokinetic study

Blood samples were collected from rats as part of a tissue distribution study. Groups of six rats were sacrificed by decapitation at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after oral administration of AGS-IV (20.0 mg/kg). The maximum plasma concentration (C_{max}) and the corresponding time (t_{max}), the absorption rate constant (k_a), elimination half-life ($t_{1/2}$), clearance (CL), volume of distribution (V_d) and the area under the plasma concentration–time curve ($AUC_{0\rightarrow\infty}$) were determined by PKAnalyst® for Windows (MicroMath).

3. Results and discussion

As shown in Fig. 2, the retention times of AGS-IV and the I.S. were 6.4 and 5.5 min, respectively and the peaks were free of interference from endogenous substances in plasma. Ion suppression was investigated and was not detected in the assay. The standard curve was linear in the range 1–500 ng/ml (correlation coefficient, $r^2 = 0.999$) and the limit of detection for AGS-IV was 0.5 ng/ml in rat plasma. Recovery of the assay averaged 92.5% (90.6–94.9%, Table 1) and within-day and between-day precision (CV) were 3.73–6.04 and 2.82–9.80%, respectively (Table 2).

The plasma concentration–time profile for AGS-IV in rats following a single oral dose (20.0 mg/kg) is shown in Fig. 3. Pharmacokinetic parameters based on this data are given in Table 3. AGS-IV was rapidly absorbed after which elimination was adequately described by a two compartment model. Full details of the tissue distribution study will be published elsewhere.

In conclusion, the present investigation demonstrates that AGS-IV can be accurately and precisely analyzed in rat plasma following a single oral dose. Selected ion monitoring at *m/z* 807.5 provides a limit of detection of 0.5 ng/ml in plasma with no interference from endogenous substances. The method is precise and has a favorable recovery. The method can be applied to pharmacokinetic studies in rat and may be adequate to measure pharmacokinetic parameters in clinical trials of AGS-IV.

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