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RESEARCH ARTICLE

Comparison of plasma pharmacokinetics of Tanreqing solution between intratracheal aerosolization and intravenous injection in rats

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Abstract

A rapid ultra high performance liquid chromatography tandem mass spectrometry method was developed for the simultaneous analysis of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid in rat plasma, and applied to comparison of pharmacokinetics of Tanreqing solution between intratracheal aerosolization and intravenous injection. Results of the analytical method validation assay showed high sensitivity, accuracy and suitable recovery. Results of pharmacokinetics showed similar decline phases for baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid in two different delivery routes. The half-lives of intratracheal aerosolization and intravenous injection were 0.90 and 1.22 h for baicalin, 0.47 and 0.17 h for oroxylin A-7-O-β-D-glucoronide and 0.22 and 0.13 h for chlorogenic acid, and this implies that compounds were retained in the lung for a relatively short time. This study was the first to provide important pharmacokinetics information for traditional Chinese medicine delivery to the lung.

KEYWORDS

intratracheal aerosolization, intravenous injection, plasma pharmacokinetics, Tanreqing solution, UHPLC–MS/MS

1 | INTRODUCTION

Ever since the successful development of inhaled insulin, drug delivery via lung administration has become an attractive route to treat diseases (Patton & Byron, 2007). Drug delivery to the lung is advantageous to avoid the extensive first-pass metabolism, increasing the bioavailability and reducing the time of pharmacological onset of activity (Brillault, Tewes, Couet, & Olivier, 2017; Dugas et al., 2013; Gaspar et al., 2015; Patton & Byron, 2007). As a noninvasive and easy drug administration route, drug delivery via lung administration also has the potential to minimize toxicity without compromising efficacy and improve patient compliance (Fu & de Lannoy, 2002). Nowdays, it is preferably used in the treatment of pulmonary disease such as respiratory tract infections, asthma and chronic obstructive pulmonary disorder in the clinic (Li et al., 2016; Nahar et al., 2013).

Traditional Chinese medicines have been used to treat respiratory diseases for thousands of years (Wong, Lam, & Fong, 2012; Zhang et al., 2014). Although the enormous gas-exchange surface of the lung represents a versatile and highly promising market, exploitation of this route for drug delivery of traditional Chinese medicine has been rare until recently. Traditional Chinese medicine Tanreqing solution (TRQ) is a famous traditional Chinese medicine prescription. Consisting of five kinds of traditional Chinese medicines: Radix Scutellariae, *Forsythia suspense*, Flos Lonicerae, bear gall powder and Cornu Gorais. In clinical practice TRQ is mainly utilized to cure upper respiratory tract infections (Dong, Zhong, Yang, Xiong, & Mao, 2013; Wang et al., 2011, 2016). In this work, TRQ was selected as the research object for drug delivery via lung administration. Baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid are the main active compounds in TRQ (Liu et al., 2014; Zhang, Sun, Gao, Chen, & Chai, 2016). These compounds are associated with a variety of biological activities, including anti-inflammatory, antipyretic and anti-tumor (Farah, Monteiro, Donangelo, & Lafay, 2008; Liao et al., 2005; Wei et al., 2017; Zhou et al., 2013). A rapid ultra-high-performance liquid chromatography

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Abbreviations: MRM, multiple reaction monitoring; MRT, mean residence time; TRQ, Tanreqing solution.

tandem mass spectrometry (UHPLC–MS/MS) method was developed for the simultaneous analysis of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid in rats plasma, and used for pharmacokinetics studies of both drug delivery to the lung and intravenous administration of TRQ. By evaluating plasma pharmacokinetics data from intravenous and inhalational administration of TRQ, our aim was to increase the mechanistic understanding of the lung absorption processes and provide support for the clinical potential of traditional Chinese medicine in the inhalation route.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Both baicalin and chlorogenic acid were purchased from the National Institutes for Food and Drug Control (Beijing, China). Oroxylin A-7-O-β-D-glucoronide and apiin were obtained from Jiangsu Yongjian Pharmaceutical Technology Co. Ltd (Jiangsu, China). The purities of all standard drugs were >98% and met the requirements of ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) analysis. The chemical structures of all compounds are shown in Figure 1. HPLC-grade acetonitrile, formic acid and methanol were purchased from Fisher Scientific (FairLawn, NJ, USA). Ultra-pure water was supplied by a Cascada™ IX water purification system obtained from Pall Corporation (Port Washington, NY, USA). Tanreqing solution was supplied by Shanghai Kaibao Pharmaceutical Co. Ltd (Shanghai, China). A 10% chloral hydra solution was purchased from Beijing Dingguochangsheng Biotecnology Co. Ltd (Beijing, China). All other chemicals were analytical grade and implemented without further purification.

2.2 | Animals

All male Sprague–Dawley rats (210–220g) were supplied by Vital River Laboratories (Beijing, China), and were kept in a temperature- and humidity-controlled environment with free access to food and water in a 12 h light–dark cycles. All experimental procedures were approved by the Committee on Animal Care and Usage of the China Academy of Chinese Medical Sciences. All of the operations in experiments followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3 | Size of the particles of TRQ

TRQ was delivered to the lung as particles. The size of the particles is the major factor that affects the deposition. A miniature liquid aerosolizer (Beijing Huironghe Technology Co. Ltd, Beijing, China) was used in the study. The size of the particles was measured on a Spraytec laser diffraction system from Malvern Instruments Ltd (Worcestershire, UK). All measurements were made at room temperature (25°C) at a relative humidity close to 60% (Dayal, Shaik, & Singh, 2004; Nahar et al., 2013). The focal length of the lens was 1.0 m, which has a particle size range of 0.5–200 mm. Measurements were conducted at 1 cm from the laser beam six times. Data was reported as Dv50, defined as 50% of the cumulative volume undersize.

2.4 | Pharmacokinetics study

Twelve rats were evenly divided into two groups, and were anesthetized using an intraperitoneal injection of 3 mL/kg body weight 10% chloral hydrate. TRQ was analyzed by Shanghai Kaibao Pharmaceutical Co. Ltd. The concentrations of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid in TRQ were 853.75, 44.24 and 6.28 μg/mL. Group 1 was given an intravenous bolus injection of TRQ in a tail vein at a dose of 1 mL/kg of body weight. Group 2 was set on the operating table at 20° angle, and then was treated intratracheally with aerosolized TRQ using a miniature liquid aerosolizer at a dose of 1 mL/kg of the body weight. TRQ was atomized via the trachea of rats into the lung as previously described (Sakagami, 2006). A blood sample (0.3 mL) was collected from the ocular fundus veins of rats before administration and after 10, 20, 30, 45 min, 1, 1.5, 2, 3, 6, 12 and 24 h. To prevent blood from clotting, all collected blood samples were placed in a plastic centrifuge tube prefilled with EDTA anticoagulant. Then blood samples were centrifuged at 4000 rpm for 15 min at 4°C, and stored in a low-temperature refrigerator (−20°C) until analysis.
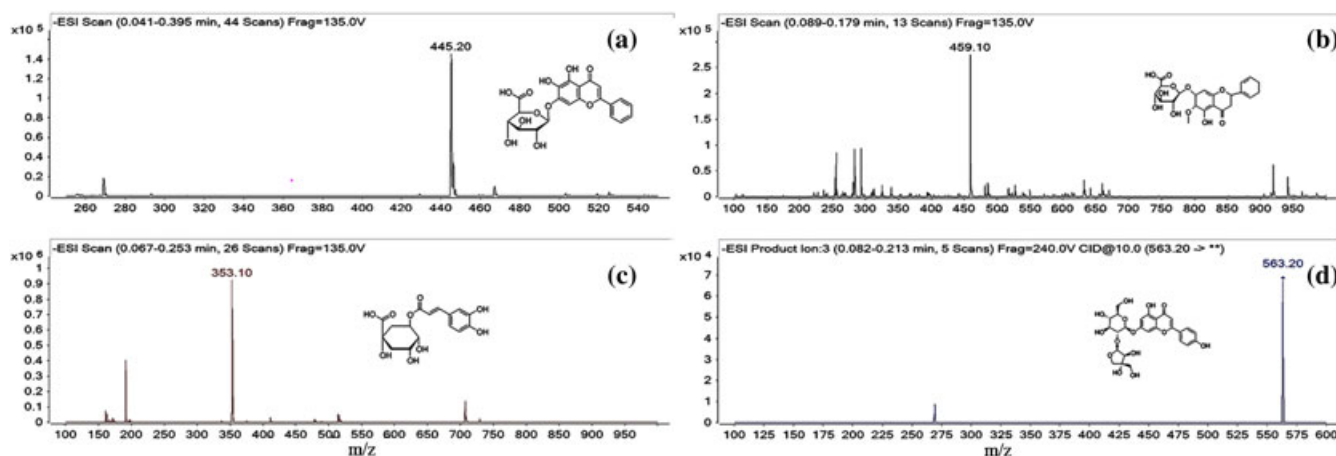

FIGURE 1 MS spectra and chemical structures of baicalin (a), oroxylin A-7-O-β-D-glucoronide (b), chlorogenic acid (c) and apiin (d)

2.5 | Determination of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid

2.5.1 | Instrumentation and chromatography

An Agilent 1290 liquid chromatograph equipped with an Agilent 6460 triple quadrupole tandem mass spectrometer (California, USA) was used to identify and quantify analytes. The separation of the samples was performed with the help of an Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm). The temperature of the autosampler was set at 20°C, and the column was conditioned at 30°C. Gradient elution was employed with acetonitrile as solvent A and 0.1% formic acid aqueous solution as solvent B. The gradient program was as follows: 0–3 min, 10% A; 3–5 min, 10–40% A; 5–7 min, 40% A; 7–9 min, 40–20% A; 9–10 min, 90–100% A; 10–11 min, 100% A; 11–11.10 min, 100–10% A; 11.10–15 min, 10% A. The total run time of each sample was 15 min. The flow rate was set at 0.2 mL/min. The sample (10 μL) was injected directly into the UHPLC–MS/MS system. Negative ion ionization was applied for the detection of baicalin, oroxylin A-7-O-β-D-glucoronide, chlorogenic acid and apiin. Apiin was selected as the internal standard (IS) owing to its structural similarity to the analytes and high recovery.

High-purity nitrogen served as both the nebulizing and the dry gas. The pressure of the nebulizer was 45 psi, and the capillary voltage was 4000 V. Gas temperature was held at 325°C, and gas flow was set at 6 min/L. All data were acquired in centroid mode by software. Additional parameters were also optimized for maximum sensitivity as shown in Table 1.

2.5.2 | Preparation of calibration standards and quality control samples

To obtain primary stock solutions of baicalin, oroxylin A-7-O-β-D-glucoronide, chlorogenic acid and IS, all standard compounds were dissolved in methanol at a concentration of 1 mg/mL and stored at −20°C. The retention period was <30 days. Working standard solutions were prepared ranging from 1 ng/mL to 10 μg/mL by diluting with methanol–water (1:4, v/v) respectively; the concentration of the IS solution was 5 μg/mL. Calibration standards were prepared using blank rat plasma spiked with baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid working solutions. Quality control (QC) samples were obtained in the same way, representing three different levels of concentrations of baicalin, oroxylin A-7-

O-β-D-glucoronide and chlorogenic acid in plasma at 5, 100 and 800 ng/mL respectively.

2.5.3 | Sample separation

A volume of 50 μL rat plasma was collected in a plastic centrifuge tube (1.5 mL) to which had been added 5 μL IS already. After vortexing for 0.5 min, 150 μL of acidified methanol–acetonitrile (3:1, v/v, 0.1% formic acid) was inserted for protein precipitate. Then, after vortexing for 1 min and centrifugating at 14,000 rpm for 10 min, the supernatant was transferred to a new centrifuge tube, and was evaporated to dryness using a ZymarkTurboVap LV drying system (Westborough, MA, USA). The residue was dissolved with 50 μL methanol and ultrapure water (1: 4, v/v). After vortexing for 1 min and centrifugating for 10 min at 14,000 rpm, the solution was transferred into a sample vial for UHPLC–MS/MS analysis.

2.5.4 | Method validation

The analytical method was validated according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use document in including selectivity, linearity, precision, accuracy, recovery, matrix effects and stability.

Selectivity and specificity

Selectivity and specificity tests can help eliminate the probable interference in plasma samples. This method was established by comparing the blank plasma from six separate sources with that containing standard compounds.

Linearity and LLOQ (lower limit of quantification)

Linearity reflects the concentration and response value of the drugs in samples, and was generally evaluated by the regression equation obtained by regression analysis. The linearity was obtained by the observed peak area ratios of analytes to IS vs the spiked concentrations of analytes based on a calibration curve. The correlation coefficient must be >0.99. The quantitative ranges of the standard curves covered the ranges of concentrations of all of the samples. The LLOQ was <5% of the peak concentration.

Precision and accuracy

Precision and accuracy were studied at three QC levels. Each level was tested six times. The work was carried out on the same day (intra-day) and in three batches on three consecutive validation days (inter-day). Precisions are expressed as relative standard deviation (RSD) and accuracies are expressed in terms of relative error (RE). The precisions did not exceed 15%,and the accuracies were required to be within ±15%.

Extraction recovery and matrix effect

The extraction recovery of analyte was determined by comparison between the responses from blank plasma samples spiked with analytes before extraction and those from blank plasma samples spiked after extraction. Matrix effects were analysed by comparison between the peak areas of standards in the blank plasma and the standards in

TABLE 1 Optimized multiple reaction monitoring (MRM) parameters for the detection of analytes

Analyte	Precursor ion (m/z)	Product ion (m/z)	Fragmentor	CE	Dwell
Baicalin	445.2	269.1[a]	100	20	35
Oroxylin A-7- O-β-D-glucoronide	459.1	283.1[a]	100	10	16
		268	100	40	16
Chlorogenic acid	353.1	191[a]	100	10	35
		85.2	100	40	35
Apiin (IS)	563.2	269.1[a]	240	40	16

[a]Quantitative ion.

the solvent, as shown by the equation: matrix effect (%) = (area of the standard in matrix/area of the standard in the solvent × 100. Three different levels need to be analyzed with six replicates for each level.

Stability

Plasma sample stability verification was performed under various conditions including: at 20°C for 24 h; after three freeze–thaw cycles; after storage at −20°C for a month; and post-preparation stored at 4°C for 24 h. The chemical compounds were considered stable when the obtained concentrations were in the range of 85–115%.

2.6 | Data analysis method of pharmacokinetics

Software kinetica 4.4 (Thermo Scientific, USA) was used to calculate the pharmacokinetic parameters of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid. Noncompartmental analysis was selected to determine standard pharmacokinetics parameters of all compounds. The measured values and analytic results were presented as mean ± SD.

3 | RESULTS AND DISCUSSION

3.1 | Size of the particles of TRQ

Figure 2(a) shows a typical time–history profile during administration. Figure 2(b) shows the particle size data of TRQ as determined. The value of Dv50 is 19.19 μm and the RSD is 1.53%. It is clear from the results that the miniature liquid aerosolizer was able to transform TRQ solution into stable aerosol particles.

3.2 | Determination of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid

3.2.1 | Method development

The mobile phase was optimized through comparisons of different solvents, solvent ratio and gradient elution condition to achieve satisfactory resolution of adjacent peaks within a short analysis time. Acetonitrile and acidic aqueous solution made an obvious improvement in the resolution of diverse constituents and minimized peak tail-
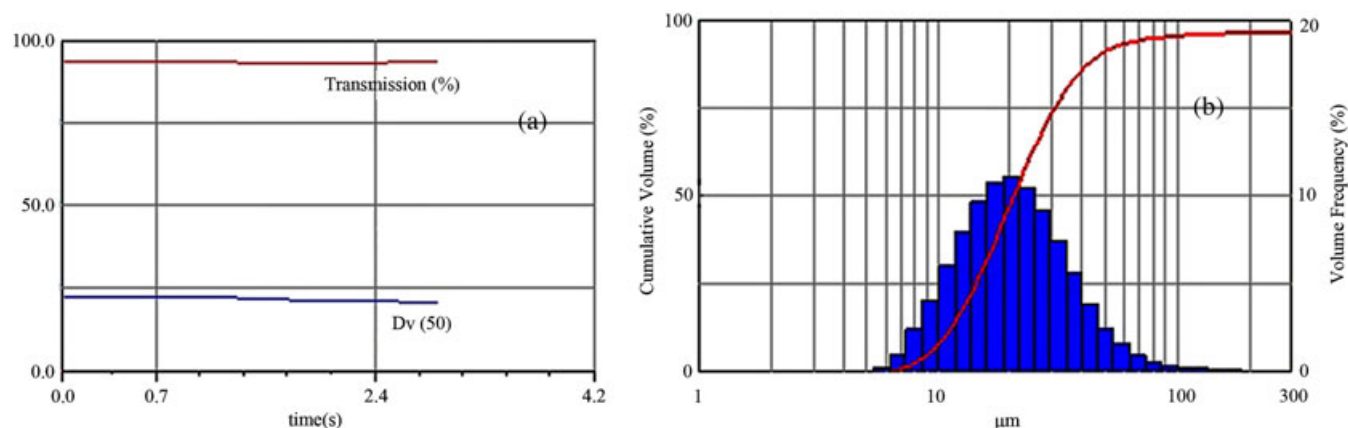

FIGURE 2 Time–history profile of Tanreqing solution (TRQ) as determined (a) and particle size data (b)
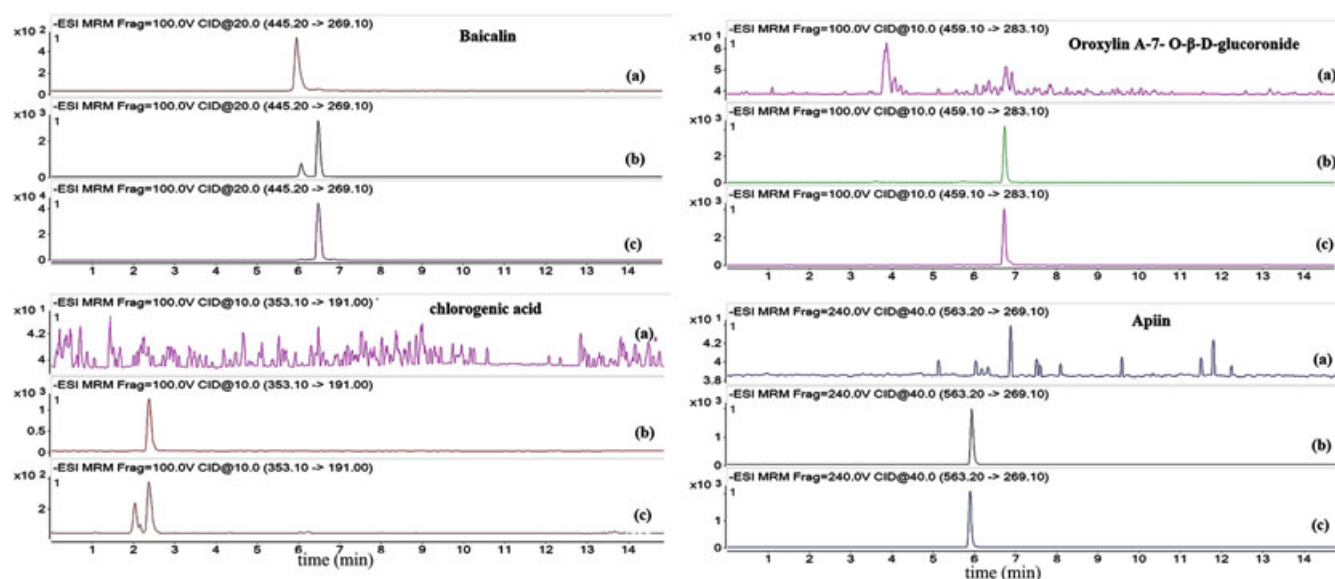

FIGURE 3 Representative multiple reaction monitoring chromatograms of baicalin, oroxylin A-7- O-β-D-glucoronide,chlorogenic acid, and apiin. (a) Blank plasma; (b) blank plasma spiked analytes and IS (100 ng/mL); (c) plasma sample after the lung administration for 30 min

ing, compared with methanol and ammonium acetate aqueous solution. The Acquity UPLC HSST3 columns proved to be the most suitable among three brands of reversed-phase columns tested: Acquity UPLC HSS T3 (2.1 × 100 mm, 1.8 μm); Acquity UPLC BEH C18 (2.1 × 50 mm, 1.7 μm); and Phenomenex Hydro-RP C_{18} (150 × 2.0 mm, 4 μm). It is important to use an IS to get high levels of accuracy during MS quantitation. Hyperoside, chloramphenicol, quercetin, rutin and apiin were tested and compared. The results show that the response and sensitivity of apiin were better than others owing to its structural similarity to the analytes and high recovery.

3.2.2 | Method validation

Six individual blank plasma samples from different sources were analyzed in order to evaluate the specificity of the method. All samples were found to have no interferences from endogenous substances at the retention times of the analytes. The result is illustrated in Figure 3.

Plasma calibration curves were constructed using the peak area ratios of the analytes to IS. Typical equations of the calibration curves are displayed in Table 2. The LLOQ for baicalin, oroxylin A-7-O-β-D-

glucoronide, chlorogenic acid was 1 ng/mL. The precision and accuracy at each concentration level were acceptable (see Table 3). Results of recovery and matrix effects of baicalin, oroxylin A-7-O-β-D-glucoronide, chlorogenic acid and IS are shown in Table 4. The extraction recoveries were between 78.69 and 91.35%. The matrix effects ranged from 86.30 to 102.12% at different concentration levels.

Table 5 shows the results of stability. Stability results showed that concentrations of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid did not obviously change in plasma stored at −20°C for 30 days, at 20°C for 24 h, after three freeze–thaw cycles and in post-preparation samples saved at 4°C for 24 h.

3.2.3 | Pharmacokinetics data analysis

The validated UPLC–MS/MS method described above was applied to pharmacokinetics study of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid in rats plasma. Plasma samples were obtained from intratracheal aerosolization and intravenous administration of TRQ. Single dose 24 h pharmacokinetics studies were performed in rats. A noncompartmental approach was used to estimate pharmacokinetics parameters.

TABLE 2 The calibration curves and lower limit of quantification (LLOQ) for baicalin, oroxylin A-7-O-β-D-glucoronide,chlorogenic acid. (data are mean ± SD, n = 3)

Analyte	Typical equations	R^2	Concentration range	LLOQ (n = 3) Found concentration (ng/mL)	Accuracy (%)	Precision (RSD,%)
Baicalin	y = 0.9645× + 0.0384	0.9996	1–1000 ng	0.98 ± 0.095	90.33 ± 9.97	10.34
OroxylinA-7-O-β-D-glucoronide	y = 0.7634× + 0.0644	0.9981	1–1000 ng	1.04 ± 0.099	109.57 ± 9.86	10.75
Chlorogenic acid	Y=0.9503×X + 0.0072	0.9998	1~1000ng	1.17±0.126	96.89±8.68	6.82

TABLE 3 Accuracy and precision for the analysis of baicalin, oroxylin A-7-O-β-D-glucoronide,chlorogenic acid in plasma sample

Analyte	Added concentration (ng/mL)	Intra-day (n = 6) Found concentration (ng/mL) (mean ± SD)	Re (%)	Precision (RSD, %)	Inter-day (n = 18 Found concentration (ng/mL) (mean ± SD)	Re (%)	Precision (RSD,%)
Baicalin	5	4.61 ± 0.41	−7.80	8.89	4.52 ± 0.38	−9.60	8.48
	100	105.73 ± 4.78	5.73	4.26	96.78 ± 8.21	−3.22	8.21
	800	764.79 ± 20.54	−4.40	2.69	727.09 ± 43.70	−0.91	6.01
OroxylinA-7-O-β-D-glucoronide	5	4.35 ± 0.56	−5.00	12.78	4.47 ± 0.23	−0.18	9.45
	100	98.27 ± 2.47	−2.86	2.51	103.34 ± 5.34	−0.64	5.16
	800	687.45 ± 10.29	−14.06	1.52	680.29 ± 21.41	−14.96	3.24
Chlorogenic acid	5	4.55 ± 7.31	−9.00	6.76	4.82 ± 0.28	−3.60	8.92
	100	90.88 ± 8.78	−9.12	5.86	89.42 ± 3.28	−10.58	8.22
	800	695.81±51.28	-13.02	4.22	689.35±36.92	-13.83	3.23

TABLE 4 Recovery and matrix effects of baicalin, oroxylin A-7-O-β-D-glucoronide,chlorogenic acid and apiin (data are mean ± SD, n = 6)

Analyte	Concentration(ng/mL)	Recovery (%)	Matrix effects (%)
Baicalin	5	85.02 ± 5.94	99.11 ± 4.34
	100	89.33 ± 5.54	101.32 ± 8.71
	800	91.35 ± 6.96	97.46 ± 5.92
OroxylinA-7-O-β-D-glucoronide	5	83.45 ± 4.64	86.30 ± 9.62
	100	88.20± 4.72	89.92 ± 9.84
	800	89.61 ± 8.92	88.43 ± 8.62
Chlorogenic acid	5	78.69 ± 5.33	97.37 ± 8.96
	100	80.90 ± 4.72	102.12 ± 7.86
	800	85.00 ± 8.87	98.13 ± 8.58
Apiin	500	86.82 ± 6.98	93.64 ± 2.63

TABLE 5 Stability of baicalin, oroxylin A-7-O-β-D-glucoronide, chlorogenic acid (data are mean ± SD, n = 6)

Compound	Concentration (ng/mL)	Long-term (−20°C) Found concentration (ng/mL)	Re (%)	Short-term (20°C) Found concentration (ng/mL)	Re (%)	Freeze–thaw Found concentration (ng/mL)	Re (%)	Post-preparation (20°C) Found concentration (ng/mL)	Re (%)
Baicalin	5	4.53 ± 5.36	−9.40	4.61 ± 0.41	−7.80	4.92 ± 7.28	−1.60	4.88 ± 5.26	−2.40
	100	87.22 ± 4.66	−12.78	99.73 ± 4.78	0.27	89.84 ± 6.94	−10.36	97.70 ± 3.87	−2.30
	800	687.89 ± 26.65	−14.01	764.79 ± 20.54	−4.40	712.16 ± 7.32	−10.98	803.98 ± 76.99	0.49
OroxylinA-7-O-β-D-glucoronide	5	4.84 ± 6.26	−3.20	4.39 ± 0.56	−12.2	4.87 ± 6.25	−2.60	4.83 ± 8.29	−3.40
	100	88.72 ± 4.21	−11.28	98.27 ± 2.47	−2.86	97.87 ± 6.53	−2.13	97.56 ± 9.77	−2.44
	800	697.24 ± 35.78	−12.84	687.45 ± 10.29	−14.06	792.24 ± 33.72	−0.97	784.38 ± 22.71	−1.95
Chlorogenic acid	5	4.84 ± 5.23	−0.32	4.56 ± 7.31	−8.80	5.02 ± 6.22	0.40	4.87 ± 8.17	−2.60
	100	91.56 ± 4.75	−8.44	90.88 ± 8.78	−9.12	98.98 ± 4.29	99.92	101.12 ± 8.39	1.12
	800	718.06 ± 28.26	−10.24	695.81 ± 51.28	−13.02	720.06 ± 56.84	−1.02	765.12 ± 21.06	−4.36

TABLE 6 Pharmacokinetic parameters of baicalin, oroxylin A-7-O-β-D-glucoronide, chlorogenic acid after the lung administration of Tanreqing solution (TRQ) (data are mean ± SD, n = 6)

	Parameters	Unit	Baicalin	Oroxylin A-7-O-β-D-glucoronide	Chlorogenic acid
Intratracheal aerosolization	C_{max}	Ng/mL	1792.27 ± 171.22	104.97 ± 12.69	54.19 ± 17.18
	AUC_{0-24}	Ng h/mL	927.35 ± 107.96	54.01 ± 3.38	10.87 ± 2.19
	$AUMC_{0-24}$	Ng h²/mL	84.12 ± 30.63	7.24 ± 4.93	1.37 ± 0.83
	$T_{1/2}$	h	0.90 ± 0.24	0.47 ± 0.14	0.22 ± 0.08
	MRT	h	0.91 ± 0.10	0.79 ± 0.10	0.37 ± 0.09
Intravenous injection	C_{max}	Ng/mL	1457.12 ± 145.37	58.80 ± 8.73	14.00 ± 3.50
	AUC_{0-24}	Ng h/mL	893.49 ± 69.01	26.42 ± 5.77	7.49 ± 3.06
	$AUMC_{0-24}$	Ng h²/mL	74.28 ± 43.20	0.98 ± 0.58	0.33 ± 0.32
	$T_{1/2}$	h	1.22 ± 0.57	0.17 ± 0.01	0.13 ± 0.04
	MRT	h	0.64 ± 0.12	0.24 ± 0.01	0.19 ± 0.06

C_{max}, Highest compound concentration observed after administration; T_{max}, time at which C_{max} is observed; AUC, area under the compound concentration vs time curve; AUMC, area under the first moment curve; $T_{1/2}$, half-life; MRT, mean residence time.
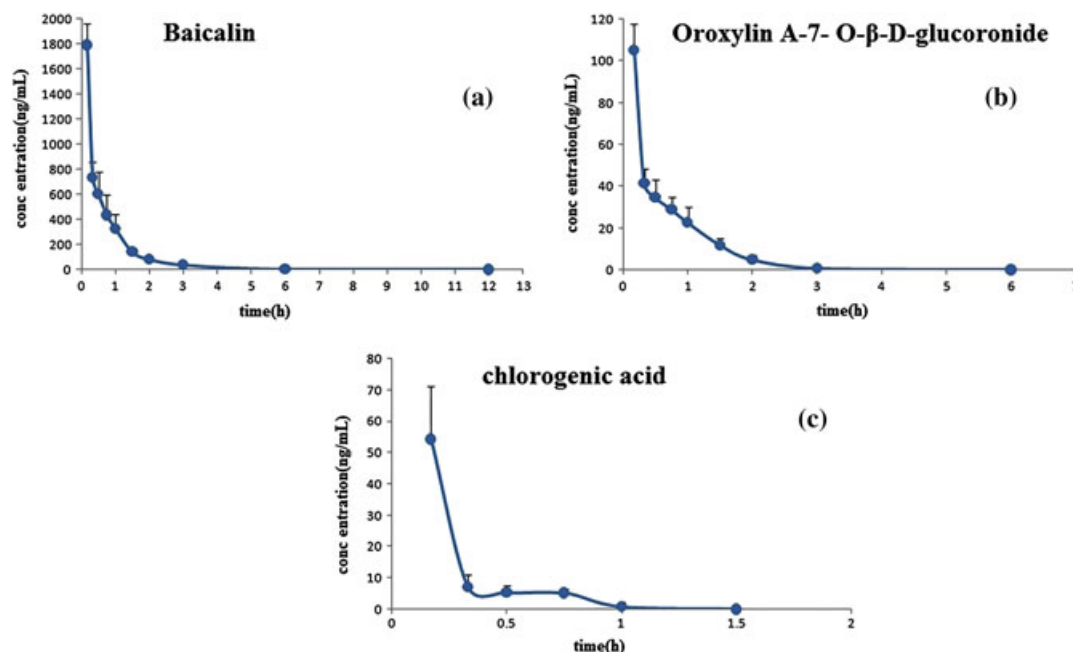
FIGURE 4 Mean (± SD) plasma concentration–time profiles after administration of baicalin (a), oroxylin A-7- O-β-D-glucoronide (b) and chlorogenic acid (c)

The main pharmacokinetic parameters obtained from the study are presented in Table 6. Pharmacokinetics parameters determined included C_{max}, which is the highest compound concentration observed after administration, and T_{max}, which is the time at which C_{max} is observed (Benet & Zia-Amirhosseini, 1995). With the current data, the values of T_{max} of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid are similar; C_{max} appears about 10 min after administration. The half-life ($T_{1/2}$) is the time it takes for the plasma concentration to decrease by as much as 50%, and represents the rate of compound distribution and elimination. The $T_{1/2}$ (h) of intratracheal aerosolization and intravenous injection are 0.90 and 1.22 for baicalin, 0.47 and 0.17 for oroxylin A-7-O-β-D-glucoronide, and 0.22 and 0.13 for chlorogenic acid, respectively. The mean residence time (MRT) is the arithmetic mean of the duration that a compound resides in the body for before being eliminated. The values of MRT_{0-24} (h) of intratracheal aerosolization were estimated to be 0.91, 0.79 and 0.37 for baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid, respectively, and were more than the values for intravenous injection (0.64, 0.24, 0.19, respectively). The MRT_{0-24} (h) of chlorogenic acid was the lowest among the three compounds. Baicalin and oroxylin A-7-O-β-D-glucoronide are flavone glycosides, and chlorogenic acid is a phenolic acid. Phenolic acid usually has a shorter biological half-life owing to its chemical structures of two adjacent phenolic groups. Therefore distribution and elimination of chlorogenic acid are more rapid than those of baicalin and oroxylin A-7-O-β-D-glucoronide.

The area under the compound concentration vs time curve (AUC) estimated for compound plasma concentrations is the primary measure of overall compound exposure following intravenous injection or extravascular administration, which reflects the total amount of the drug in the body for a period of time. AUC_{0-24} (ng h/mL) values of intravenous injection were 893.49, 26.42 and 7.49 for the three compounds, and AUC_{0-24} (ng h/mL) values of intratracheal aerosolization were 927.35, 54.01 and 10.87. The total amount of the test analytes

in the body after intratracheal aerosolization was higher compared with intravenous injection during 24 h.

Four fundamental processes which influence *in vivo* pharmacokinetics of the drug are absorption, distribution, metabolism and excretion. After intravenous administration, the drug is not subject to first-pass elimination and is introduced directly into the venous circulation. The arterial circulation will then distribute the drug to the various tissues and organs, some of which (e.g. the kidney), in addition to the liver, may eliminate the compound by metabolism and/or excretion (Fan & de Lannoy, 2014). With regard to the lung administration, drug absorption was achieved into the venous circulation quickly owing to excellent blood perfusion of this organ, large surface area of the alveoli and the thin barrier for absorption. Without first-pass elimination, the drug is introduced directly into the venous circulation, and then delivered to the site of action (Borghardt et al., 2016). Therefore lung delivery is useful for the treatment of local disorders, e.g. asthma and upper respiratory tract infections. The geometric mean plasma concentration–time profiles (Figure 4) showed a multi-exponential decline for baicalin and oroxylin A-7-O-β-D-glucoronide with two distinct routes. Lung administration has an analogous steeply declining phase immediately to intravenous injection owing to the rapid distribution of baicalin, oroxylin A-7-O-β-D-glucoronide from plasma to tissues and organs, whereas the terminal straight line, which is shallower, is primarily due to elimination of the compounds from the body.

4 | CONCLUSION

We have in this study developed a highly sensitive, rapid and specific UHPLC–MS/MS method and validated it for quantification of baicalin, oroxylin A-7-O-β-D-glucoronide and chlorogenic acid. The method showed suitability for pharmacokinetics studies in rats plasma.

The lung administration has gained increasing interest in recent years, but traditional Chinese medicine delivery to the lung is still in the primary stage of development. This study provided additional insights into traditional Chinese medicine delivery to the lung, and a valuable reference for clinical applications.

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