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Article

In vitro skin retention and drug permeation study of Tongluo-Qutong rubber plaster by UPLC/UV/MS/MS

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Tongluo-Qutong rubber plaster (TQRP), a typical Chinese patent medicine that contains 13 different herbal remedies, is widely used in clinical practice for the treatment of cervical spondylosis and osteoarthritis. However, due to a lack of *in vitro* transdermal studies, the active ingredients of TQRP have not been fully elucidated. This presents a huge obstacle for quality evaluation, pharmacokinetic studies and clinical safety assessment of TQRP. In this work, a UPLC/UV/MS/MS method was established and validated to evaluate five analytes in TQRP. The validation demonstrated linearity ($r > 0.99$), specificity (no co-eluting peaks at the retention times of the analytes), and precision (RSD < 15%) within acceptable parameters. A skin permeation study was performed to determine the concentrations of drugs delivered to the dermis. The 24-hour cumulative permeation of ferulic acid, aleo-emodin, emodin and piperine were 303.68, 709.31, 671.06 and 25561.01 ng/cm^2, respectively. According to the fitting data of the TQRP active components, skin permeation was mainly due to a combination of passive diffusion and drug release after matrix erosion.

Keywords: Tongluo-Qutong rubber plaster, Skin permeation, UPLC/UV/MS/MS, Piperine.

Abbreviations:

TCM Traditional Chinese Medicine

TQRP Tongluo-Qutong rubber plaster

LLOQ Lower limit of quantification

INTRODUCTION

In recent years, traditional Chinese medicine (TCM) has played an important role in the treatment of joint pain (Lao *et al.*, 2015). It has been reported that TCM showed clear effects in acute gouty arthritis and protection of cartilage matrix (Shi *et al.*, 2016). In the treatment of rheumatoid arthritis, unlike western medicines that contain non-steroidal anti-inflammatory drugs (NSAIDS) and biological agents (such as anti-tumor necrosis factor-a) (Hennekens *et al.*, 2014), the widely used natural plant products in TCM can exert synergistic therapeutic effects through multiple components, targets and actions, thus reducing the incidence of serious adverse side effects and toxicity (Wu *et al.*, 2015).

Tongluo-Qutong rubber plaster (TQRP), a typical Chinese patent medicine, is recorded in the Pharmacopoeia of The People's Republic of China 2015 edition (Committee, 2015) and was authorized for clinical use in China in 2000 (National Drug Approval No. Z20000065). In the theory of traditional Chinese medicine, it has the functions of promoting blood circulation, clearing cold and dehumidifying, reducing swelling and relieving pain. TQRP contains 13 different TCM (Angelicae Sinensis Radix, Chuanxiong Rhizoma, Carthami Flos, Kaempferiae

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Rhizoma, Zanthoxyli Pericarpium, Piperis Fructus, Caryophylli Flos, Cinnamomi Cortex, Piperis Longi Fructus, Zingiberis Rhizoma, Rhei Radix et Rhizoma, Borneolum Syntheticum and Camphor) that have been widely used in clinical practice for the treatment of cervical spondylosis and osteoarthritis. Until now, studies on the finished TQRP preparation have been limited to determination of the content of a single indicator component, and simple pharmacodynamic evaluation. In the Chinese Pharmacopoeia, only the lowest levels of borneol and camphor detected by gas chromatography are specified, which is insufficient as a quality control standard. Active TQRP components with anti-inflammatory and analgesic effects have previously been studied, such as ferulic acid from Angelicae Sinensis Radix, aleo-emodin and emodin from Rhei Radix et Rhizoma, piperine from Piperis Fructus and cinnamic acid from Cinnamomi Cortex (Bae *et al.*, 2010; Kong *et al.*, 2014; McCarty, Assanga, 2018; Silveira *et al.*, 2018; Vazquez *et al.*, 1996). Although these components are believed to be pharmacologically active agents in TQRP, their skin permeation or transdermal absorption have not been determined. Furthermore, because of the lack of *in vitro* or *in vivo* permeation data, the active ingredients of TQRP have not been fully elucidated, which presents a huge obstacle for quality evaluation, pharmacokinetic studies and clinical safety assessment of TQRP.

Therefore, based on these considerations, the present work established a UPLC/UV/MS/MS method to investigate and quantify the in vitro transdermal permeation of TQRP components. Skin retention of materials in the plaster was also examined. To the best of our knowledge, this is the first time that the transdermal activity of TQRP active ingredients has been studied.

MATERIAL AND METHODS

Material

Tongluo-Qutong rubber plaster (TQRP, batch number:161206) was provided by Henan Lingrui Pharmaceutical Co., Ltd (Henan, China). Ferulic acid (99.0% purity, batch No. 110773-201614), aleo-emodin (98.5% purity, batch No. 111595-201306), emodin (98.7% purity, batch No. 110756-201512), cinnamic acid (98.8% purity, batch No. 110786-201604) and piperine (98.9% purity, batch No. 110775-201706) were all purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and methanol of HPLC grade were obtained from Fisher Scientific Co. (Fair Lawn, NJ, USA). Formic acid was supplied by Tokyo Chemical Industry Co., Ltd. (Shanghai, China). All reagents were of analytical grade. Purified Milli-Q water (Millipore, Billerica, MA, USA) was used for all experiments.

Balb/c nude mice (weighing 25 ± 5 g; Vital River Experimental Animal Tech. Co. Ltd; Tianjin, China; License: SCXK 2016-0006) were housed at room temperature with free access to drinking water. The protocol and any amendments or procedures involving the care or use of animals in this study were in accordance with the regulations for animal experimentation issued by the State Committee of Science and Technology of China and approved by the Tianjin University of Traditional Chinese Medicine Institutional Animal Care and Use Committee (Document number TCM-LAEC 2018020).

UPLC/UV/MS/MS instrumentation

All samples were analyzed using a UPLC/UV/MS/MS system consisting of an Agilent series 1290 UPLC system and an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The chromatographic separation of analytes was performed on an Acquity UPLC®HSS T3 C_{18} column (100 × 2.1 mm, 1.8 μm, Waters, CA, USA) at a column temperature of 40°C. Chromatographic separation was achieved by gradient elution using a mobile phase comprised of 0.1% formic acid in water (A) and acetonitrile (B). The UPLC gradient schedule was set as follows: 20%→70% B at 0.0–15.0 min; 70%→90% B at 15.0–16.0 min; 90%→90% B at 16.0–19.0 min; 90%→20% B at 19.0–20.0 min; 20%→20% B at 20.0–23.0 min. Efficient separation and symmetrical peaks were obtained at a flow rate of 0.3 mL/min. The sample

injection volume was set at 5 μL. The concentration of piperine exceeded the upper limit of the linear range by mass spectrometry in a preliminary experiment, so UV detection at 265 nM was employed to determine this analyte. The detection of other analytes was in multiple reaction monitoring mode (MRM) using electrospray negative ionization (ESI). The ESI configuration was set as follows: gas temperature 350°C; gas flow rate 11 L/min; nebulizer 20 psi; capillary 4000 V (Agilent Technologies). Data acquisition and processing were performed using the Agilent Mass Hunter Workstation.

Preparation of standard solutions

The stock solutions of ferulic acid, aleo-emodin, emodin, cinnamic acid and piperine were prepared in methanol and diluted with saline/ethanol (70:30, v/v). All solutions were kept at 4°C and brought to room temperature before use.

Method validation

Validation of the method was conducted according to the China Food and Drug Administration (CFDA) guidelines, including specificity, linearity, accuracy, precision, and stability during sample storage and processing.

Interfering peaks at the elution times of ferulic acid, aleo-emodin, emodin, cinnamic acid and piperine were evaluated for specificity. To evaluate linearity, calibration curves were constructed using standard solutions of 15–3000 ng/mL for ferulic acid, 40–8000 ng/mL for aleo-emodin, 5–1000 ng/mL for emodin, 250–50000 ng/mL for cinnamic acid and 2500–500000 ng/mL for piperine. Linear regression was subsequently carried out using 1/x as a weighting factor (except for piperine). The lower limit of quantification (LLOQ) was used to determine the sensitivity of the method with a signal-to-noise ratio of 10:1. Relative error (RE) and relative standard deviation (RSD) on the same day and on three consecutive days were analyzed to assess intra- and inter-day accuracy and precision, respectively.

Reflux sample extraction

Three different solvents (50%, 70%, and 90% ethanol) were evaluated for extraction of TQRP index components using the reflux heating method. Two pieces of TQRP (70 cm^2 for each plaster) were cut into small pieces and placed in a 250 mL round-bottomed flask and extracted with solvent under a reflux condenser for 2 h. After cooling and filtration, the solution was passed through a 0.45 μm filter. The TQRP extracts were analyzed and quantified using the validated UPLC/UV/MS/MS method. The extracts were stored at 4°C until analysis.

Preparation of skin samples

The nude mice were sacrificed by cervical dislocation, and non-injured skin was peeled off. Subcutaneous fat and blood vessels were carefully removed with a blade, and the skin washed repeatedly with normal saline. The clean skin was wrapped in aluminum foil and stored at -20°C. Samples were defrosted before conducting experiments at room temperature (used within 3 days).

Skin permeation study

The skin permeation study was performed in Franz diffusion cells with a diffusion area of 3.14 cm^2 and a receptor compartment of 7 mL. Skin samples were thawed at room temperature prior to mounting in the diffusion cells with the dermal side facing the receptor chamber. TQRP was placed directly on the mouse skin in the donor compartment. The concentrations of ferulic acid, aleo-emodin, emodin, cinnamic acid and piperine in TQRP were obtained from reflux samples in our preliminary study. The receptor solution was physiological saline containing 30% (v/v) ethanol and kept at 37 ± 1 °C under magnetic stirring during the entire experiment. An aliquot (2 mL) of receptor solution was collected at different time points (1, 2, 3, 4, 6, 8, 10, 12, and 24 h) and replaced with an equal volume of fresh receptor solution. After centrifugation (12,000 rpm for 10 min), the receptor samples were assayed by UPLC/UV/MS/MS to determine the cumulative amounts of ferulic acid, aleo-emodin, emodin, cinnamic acid and piperine that had permeated through the skin.

Skin retention study

At the end of the permeation experiment (24 h), the skin was removed from the equipment. The formulation was removed from the surface of the skin, which was then washed repeatedly with pure water. Cleaned epidermis membrane was cut into small pieces and added to normal saline containing 30% (v/v) ethanol. The suspension was sonicated for 30 min, cooled, and then filtered. The skin sample extracts were assayed by UPLC/UV/MS/MS to determine the concentrations of ferulic acid, aleo-emodin, emodin, cinnamic acid and piperine retained in the skin. All values were the average of five parallel experiments.

Data calculation

Individual permeation profiles were obtained for all the conditions under study by fitting the empirical diffusion equation derived from Fick's Second Law of Diffusion (see Equation 1) to the accumulated amounts of ferulic acid, aleo-emodin, emodin and piperine (ng/cm^2) against time (hours).

$$Q_n = \frac{C_n V + \sum_{i=1}^{n-1} C_i V_i}{A}$$

Where Q_n is the cumulative amount of drug permeated per area unit, C_n is the concentration of drug in the donor vehicle at the nth sampling, C_i is the concentration of drug in the donor vehicle at the ith sampling, V is the volume of receptor cell, V_i is the replaced volume and A is the area of drug permeation.

In order to study the four analytes transdermal kinetic from the TQRP, four mathematical models were used to analyze the experimental data. The applied mathematical models based on each diffusion, erosion and degradation mechanism are summarized below. Zero-order kinetic model for drug delivery is represented by the following equation

$$Q = k_0 t$$

Where, Q is the cumulative amount of drug permeated per area unit at time t, k_0 is the zero-order kinetic dissolution constant and t is the release time.

First-order kinetic model, presented as follows, can also be used to describe the drug release

$$\ln Q = k_1 t$$

Where, Q is the cumulative amount of drug permeated per area unit at time t, k_1 is the first-order kinetic constant and t is the release time.

Higuchi kinetic model is based on the Higuchi equation and describes the Fickian diffusion of the drug (Higuchi, 1963).

$$Q = k_h t^{1/2}$$

Where, Q is the cumulative amount of drug permeated per area unit at time t, k_h is a Higuchi kinetic constant and t is the release time.

Ritger-Peppas kinetic model is represented by the following equation

$$Q = k_p t^n$$

Where, Q is the cumulative amount of drug permeated per area unit at time t, k_p is a Ritger-Peppas kinetic constant, t is the release time and n is the diffusion exponent related to the drug release mechanism.

RESULTS AND DISCUSSION

LC-MS/MS instrumentation and chromatographic conditions

In this study, a UPLC/UV/MS/MS method was developed and validated for the determination of five major active ingredients (shown in Figure 1) of TQRP, using UV to determine the concentration of piperine and tandem mass spectrometry to analyze the other four components. UV detection was used to determine

piperine rather than tandem mass spectrometry because the concentration of piperine was significantly higher than other analytes. In addition, the MRM positive ion mode for piperine ionization severely affected the response of the other analytes in negative ion mode detection. Therefore, high-performance liquid chromatography with UV detection was appropriately applied to analyze piperine in TQRP, and the analytical conditions were set as previously reported (Alomrani *et al.*, 2018).

The selection of mobile phase was based on chromatographic peak shape and retention time. Accordingly, acetonitrile was found to be superior to methanol with lower column pressure and lower background noise. The addition of 0.1% formic acid was used to obtain a higher response (Wang *et al.*, 2017). The wavelength was set at 265 nm for piperine determination. Precursor-to-product ion transitions at *m/z* 193.1→114.1, 268.8→239.1, 269.04→224.9 and 147.04→102.9 were used for quantification of ferulic acid, aleo-emodin, emodin and cinnamic acid, respectively, in MRM mode.

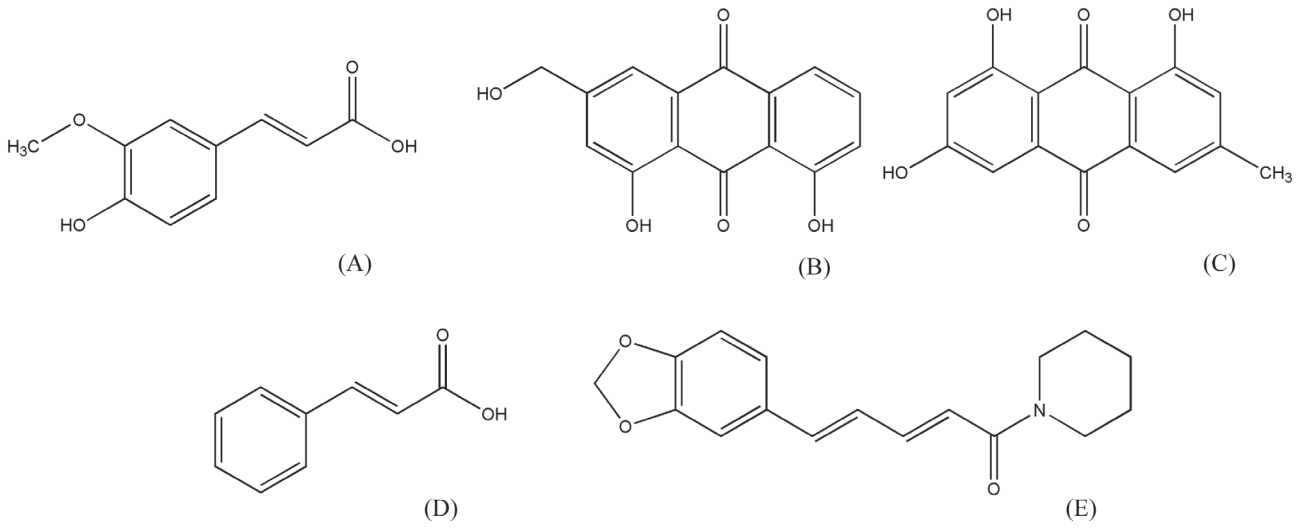

FIGURE 1 - Chemical structures of five analytes (A) FA, (B) AE, (C) EMO, (D) CA, and (E)PIP

Method validation

The typical chromatograms of blank receptor solution, standard solution and receptor solution from the tested skin are shown in Figures 2 and 3. No endogenous interference was observed at the retention time of the analytes in the blank receptor solution, which proved the specificity of the method. Regression equations, linear ranges and correlation coefficients are listed in Table I. All calibration curves showed good linearity, with correlation coefficients > 0.99. The LLOQ values of ferulic acid, aleo-emodin, emodin, cinnamic acid, and piperine were 15, 40, 5, 250, and 2,500 ng/mL, respectively. Precision and accuracy data for the five analytes are summarized in Table II. Intra- and inter-day precision were < 5%, and accuracy was within 8.24%. The results indicated that the UPLC/UV/MS/MS method was accurate, sensitive and reproducible.

The stabilities of ferulic acid, aleo-emodin, emodin, cinnamic acid, and piperine in the standard solution under the described conditions are listed in Table III. The results demonstrated that all analytes were stable in stock solution during storage at room temperature for 4 h and at 4°C for 30 days. The stability data satisfy the requirements for a routine skin permeation/retention study.

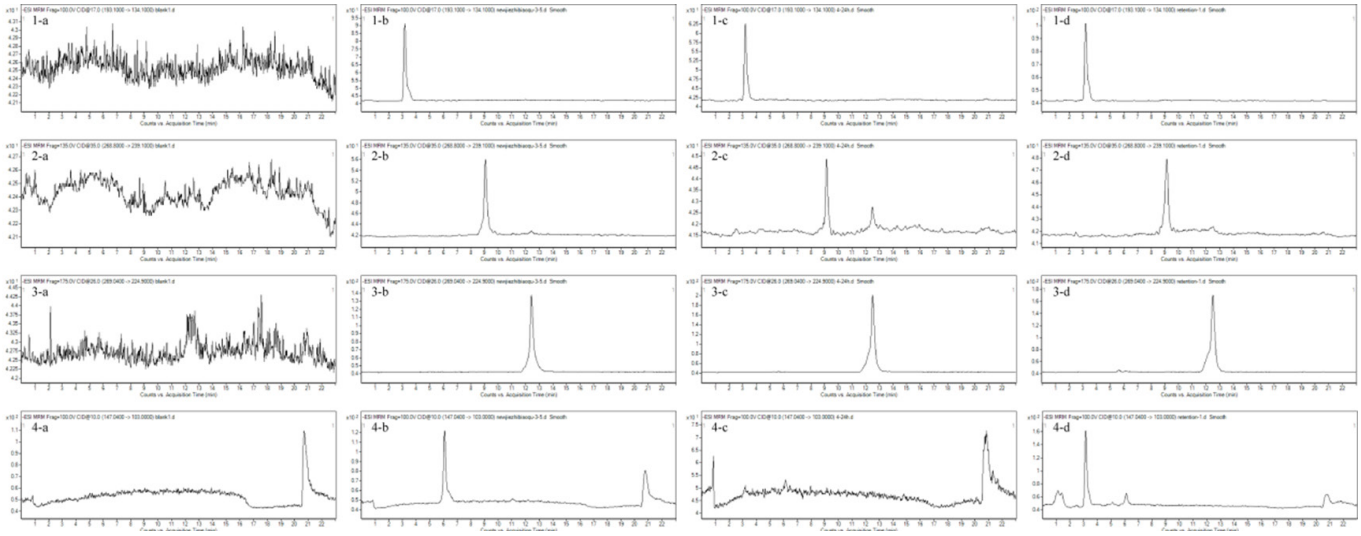

FIGURE 2 - Chromatograms of skin permeation samples by MS/MS detector (a-blank receptor solution; bstandard solution; c-receptor sample; d-skin sample; 1-FA; 2-AE; 3-EMO; 4-CA)

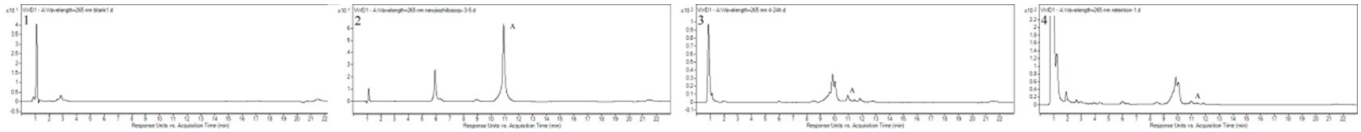

FIGURE 3 - Chromatograms of PIP skin permeation samples by UV detector (1-blank receptor solution; 2-standard solution; 3-receptor sample; 4-skin sample; Peak A-PIP)

TABLE I - Linearity for assay of FA, AE, EMO, CA and PIP in standard solution

Analyte	Linear range (ng/mL)	Regression equation	Correlation coefficient	weight
FA	15~3000	Y=4.07*X-7.98	0.9995	1/X
AE	40~8000	Y=0.90*X-38.81	0.9967	1/X
EMO	5~1000	Y=34.72*X+17.34	0.9996	1/X
CA	250~50000	Y=0.58*X-97.76	0.9979	1/X
PIP	2500~500000	Y=33.87*X-16.57	1	None

TABLE II - Precision and accuracy (Mean±SD, n=6)

Analyte	Concentration level (ng /mL)		Precision		Accuracy (RE, %)
	added	measured	Intra-day (RSD, %)	Inter-day (RSD, %)	
FA	30	32.70±1.63	1.15	4.98	8.24
	300	300.05±8.78	4.74	2.93	0.02
	2400	2252.31±18.81	0.76	0.84	-6.56
	80	78.35±2.02	2.57	2.58	-2.11

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Analyte	Concentration level (ng /mL)		Precision		Accuracy (RE, %)
	added	measured	Intra-day (RSD, %)	Inter-day (RSD, %)	
AE	800	741.09±18.01	1.47	2.43	-7.95
	6400	6170.84±9.63	0.69	0.16	-3.71
	10	9.63±0.40	2.31	3.93	-3.86
EMO	100	103.83±1.62	2.04	1.55	3.69
	800	837.32±12.12	0.57	1.45	4.46
	500	505.80±5.03	1.57	0.99	1.15
CA	5000	4760.20±100.08	3.70	2.10	-5.04
	40000	37449.99±318.84	0.50	0.85	-6.81
	5000	5161.24±101.24	2.08	1.96	3.12
PIP	50000	50216.51±670.78	3.08	1.34	0.43
	400000	393449.74±586.85	0.14	0.15	-1.66

TABLE III - Stability of five analytes in stock solution (Mean±SD, n=6)

Analyte	Added (ng/mL)	Short-term stability (at room temperature for 4h)		Long-term stability (at 4°C for 30 days)	
		Measured (ng/mL)	RE (%)	Measured (ng/mL)	RE (%)
	30	30.91±0.84	2.94	31.61±0.61	5.09
FA	300	296.70±6.81	-1.11	305.08±1.81	1.67
	2400	2208.63±16.47	-8.66	2252.16±43.26	-6.56
	80	78.90±2.20	-1.39	78.00±3.92	-2.56
AE	800	788.29±27.43	-1.49	767.10±9.93	-4.29
	6400	6150.20±74.68	-4.06	6241.93±42.52	-2.53
	10	9.50±0.42	-5.26	9.73±0.34	-2.81
EMO	100	103.92±1.42	3.77	101.95±2.46	1.91
	800	806.82±52.35	0.85	823.52±2.47	2.86
	500	507.47±15.27	1.47	504.58±11.44	0.91
CA	5000	4812.50±148.47	-3.9	4732.61±60.43	-5.65
	40000	37791.14±185.34	-5.84	37622.51±298.05	-6.32
	5000	5439.30±94.52	8.08	5511.65±104.34	9.28
PIP	50000	50729.92±247.48	1.44	51152.97±293.43	2.25
	400000	396046.32±8371.91	-1.00	405224.23±1617.90	1.29

Skin permeation study

As shown in Table IV, the five active ingredients of TQRP were effectively extracted by all three solvents. The concentrations of ferulic acid and piperine were highest in 50% ethanol, the concentration of cinnamic acid was highest in 70% ethanol and the concentrations of aleo-emodin and emodin were highest in 90% ethanol, due to the different hydrophilicities of each index component. The total concentrations of the five analytes in the three extraction solvents (50%, 70%, and 90% ethanol) were 48888.46, 49143.40, and 47382.30 ng/cm^2, respectively. Since 70% ethanol is the recommended solvent for extraction of TQRP in the Pharmacopoeia (Committee, 2015), this solvent was chosen as the extraction solvent to measure the drug content of TQRP and calculate cumulative penetration in the subsequent skin permeation test.

TABLE IV - The contents of five analytes in the sample solution of a different concentration of the solvent (ng/cm², Mean±SD, n=3)

Analyte	solvents		
	50% methanol	**70% methanol**	**90% methanol**
FA	1070.70±49.52	949.20±9.75	658.44±3.75
AE	1505.27±9.77	2004.43±5.13	2049.23±11.30
EMO	1344.62±110.04	1710.46±171.82	1822.40±102.32
CA	2261.68±10.80	2272.15±41.68	2038.69±99.68
PIP	42706.20±1442.14	42207.16±2107.02	40813.54±3605.42

The *in vitro* drug permeation test was conducted using the analytical method to determine whether all five components could permeate skin. The hairless mouse species was used because of similarities to human skin in the absence of hair (Nam *et al.*, 2011). A preliminary experiment was conducted before the main experiment to optimize the receptor solution. Saline/ethanol (70:30, v/v) was used as the receptor solution. The cumulative permeation of ferulic acid, aleo-emodin, emodin, cinnamic acid and piperine through the skin over time was profiled. It can be seen from Figure 4 that the amounts of the permeated agents increased over time during the 24-hour experiment. In the nude mouse skin permeation study, the 24-hour cumulative permeation amounts of ferulic acid, aleo-emodin, emodin and piperine per unit area were 303.68, 709.31, 671.06, and 25561.01 ng/cm^2, respectively. The concentration of cinnamic acid in the samples was below the limit of quantitation, so the cumulative permeation of cinnamic acid could not be accurately measured.

Additionally, the mechanisms for transdermal permeation of the four analytes from TQRP were studied by fitting the experimental data to zero-order, first-order, Ritger-Peppas and Higuchi mathematical models. The results are presented in Table V. The correlation coefficients in the Ritger-Peppas equation for ferulic acid, aleo-emodin, emodin and piperine were all greater than 0.98, indicating a good linear relationship. This suggested that the drugs were mainly released by matrix erosion, since the permeability coefficient in the Ritger-Peppas equation was greater than 0.89 (Peppas, 2014; Siepmann, Peppas, 2001). On other hand, the correlation coefficients of the zero-order kinetic fitting equation for emodin and piperine were better than those of the Ritger-Peppas equation, indicating a passive diffusion mechanism for skin permeation of emodin and piperine. According to the fitting data, skin permeation of active components from TQRP was a combination of passive diffusion and drug release after matrix erosion.

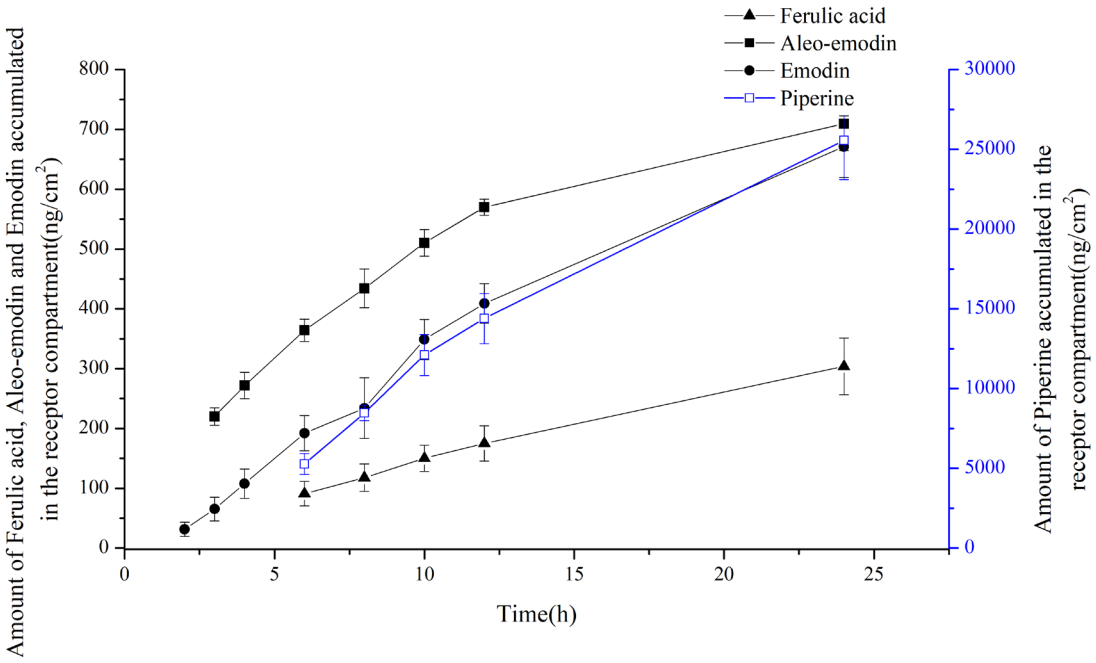

FIGURE 4 - Amounts of four analytes accumulated in the receptor compartment at different time points (mean ± SD, n=5).

TABLE V - Fitting results of drug release model of four analytes

kinetics model	FA		AE		EMO		PIP	
	Fitting equation	r	Fitting equation	r	Fitting equation	r	Fitting equation	r
Zero-order	Q=11.64t+27.81	0.997	Q=22.71t+222.57	0.941	Q=29.54t+2.56	0.986	Q=1085t+140.40	0.989
First-order	lnQ=0.03t+1.86	0.967	lnQ=0.02t+2.40	0.877	lnQ=0.05t+1.79	0.853	lnQ=0.03t+3.66	0.922
Higuchi	Q=-7038.80t1/2+254.86	0.849	Q=-3699.40t1/2+564.45	0.857	Q=-1719.40t1/2+361.75	0.685	Q=-688737t1/2+21712	0.884
Ritger-Peppas	lnQ=0.87lnt+1.29	0.998	lnQ=0.58lnt+2.09	0.985	lnQ=1.25lnt+1.23	0.984	lnQ=1.11lnt+2.92	0.980

Skin retention study

The concentrations of ferulic acid, aleo-emodin, emodin, cinnamic acid and piperine from TQRP retained in the skin were also determined. The ratios of total released drug retained and passing through the skin are presented in Table VI. The release ratios of ferulic acid, aleo-emodin, emodin, cinnamic acid and piperine were 41.22%, 38.66%, 41.68%, 8.34%, and 70.10%, respectively. The release ratio of cinnamic acid was lower than those of the other analytes, indicating lower permeation (not detected in our experiments) and higher retention on the skin (Spagnol *et al.*, 2017). This result suggests that cinnamic acid may be a promising topical agent to provide a local effect due to its antioxidant and anti-inflammatory activity. Ferulic acid, aleo-emodin, emodin and piperine, the release ratios of which were 38.66%–70.10%, were revealed as the most effective components of TQRP, providing a foundation for future in vivo studies.

The natural volatile oil components contained in the plaster, such as eugenol and cinnamaldehyde, may act as transdermal enhancers. Chen suggested that the volatile oil component of the plaster promotes transdermal absorption

of the indicator component (Chen *et al*., 2014). Moreover, piperine has been explored as a bioenhancer for transdermal delivery and shown to augment drug permeation 2–8-fold (Jantarat *et al*., 2018). It can be considered that permeability of the other analytes may be improved by the synergistic effect of volatile oils and piperine.

TABLE 6 - Skin concentrations per unit area and release ratio of five analytes

Analyte	Skin retention (ng/cm²)	Permeation in 24 h (ng/cm²)	Content in plaster (ng/cm²)	Release ratio (%)
FA	87.6	303.68	949.20	41.22
AE	65.64	709.31	2004.43	38.66
EMO	41.93	671.06	1710.46	41.68
CA	189.53	n.d.	2272.15	8.34
PIP	4028.10	25561.01	42207.16	70.10

CONCLUSIONS

Five pharmacologically active ingredients of TQRP were determined using a UPLC/UV/MS/MS analytical method. In the skin permeation study, the 24-hour cumulative permeation amounts of ferulic acid, aleo-emodin, emodin and piperine per unit area were 303.68, 709.31, 671.06, and 25561.01 ng/cm^2, respectively. According to the fitting data, skin permeation of the active components from TQRP resulted mainly from a combination of passive diffusion and drug release after matrix erosion. A total of 38.66%–70.10% of the four active agents in TQRP reached the skin tissue where they could exert their pharmacological effects. To the best of our knowledge, this is the first transdermal study of traditional Chinese medicine rubber plaster.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding this manuscript.

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