Determination of ephedrine, pseudoephedrine, sinapine thiocyanate, tetrahydropalmatine and amygdalin by HPLC-MS/MS after oral administration of extracts of Majie cataplasm in rabbits plasma: Application to a pharmacokinetic study

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Research

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Abstract

Background

This study aimed to develop HPLC-MS/MS method for quantification of ephedrine, pseudoephedrine, sinapine thiocyanate, tetrahydropalmatine and amygdalin of extracts of Majie cataplasm after oral administration in rabbits plasma and describe the pharmacokinetics of this five components.

Methods

The qualitative detection of the five compounds was accomplished by two methods. One method for the simultaneous determination of ephedrine, pseudoephedrine, sinapine thiocyanate and tetrahydropalmatine was developed and validated for the first time, while the other method was for amygdalin. Chromatographic separations were achieved on a C18 column using gradient elution with the mobile phase consisting of acetonitrile and water (containing 0.1% formic acid and 5mM ammonium formate) for ephedrine, pseudoephedrine, sinapine thiocyanate and tetrahydropalmatine, while acetonitrile and water for amygdalin, at a flow rate of 0.4 mL/min. The initial gradient was extended in order to isolate ephedrine and pseudoephedrine better. The detection was performed in multiple-reaction monitoring (MRM) mode using electrospray ionization (ESI).

Results

All calibration curves showed good linearity (r^2 0.996) within the test ranges. The lower limit of quantification was 0.01 ng/mL for all five analytes. Reproducibility for five analytes ranged from 0.79 to 2.3% (Ephedrine), 0.51 to 3.4% (pseudoephedrine), 2.8 to 5.0% (Sinapine thiocyanate), 2.6 to 6.3% (Tetrahydropalmatine) and 1.1—2.5% (amygdalin) respectively. The extraction recoveries were within the acceptable range.

Conclusion

The two methods were successfully applied to pharmacokinetic study of rabbits after oral administration. The two methods has several advantages including good chromatographic resolution, specific and reproducibility and superior minimum detection limit. The results provided a basis for further study on the bioactivity of Majie cataplasm.

Background

Majie cataplasm consisting of Mahuang (Herba Ephedrae), Kuxingren (Semen Armeniacae Amarum), Baijiezi (Semen Sinapis) and Yanhusuo (Rhizoma Corydalis), is a classical transdermal administration dosage form employed in treating asthma (Zhang, 2009; Kong et al., 2016). Such topical cataplasm has been widely and clinically applied in China for great effect. Millions of people used it to prevent asthma during the period of hottest days each year (Liu et al., 2014; Liu et al., 2016; Chen, 2005). Pharmacokinetics play a key role in the evaluation of drug action and are regarded as available means in elucidating the mechanism of traditional Chinese medicine (TCM) (Yan et al., 2015). In order to explore the possible mechanism and the time-

concentration relation of absorption of Majie cataplasm, there is a need to do the pharmacokinetic analysis. In the present study, HPLC-MS/MS methods were validated for quantification of five components.

From the perspective of TCM theory, some traditional Chinese herbs, such as Semen Sinapis and Rhizoma Corydalis cannot pass into the bloodstream through skin but have the effects of local stimulation and promoting blood circulation when use externally (Li et al., 2014; Wang et al., 2011). So in the present study, the rabbits were given extracting solution of Majie cataplasm by gavage to make sure that the active ingredients can pass into the bloodstream. Then the blood samples were taken from the rabbits as the research objects, and the determination method were established so that to measure the condition of the active ingredients by transdermal administration.

As we all know, complexities are the basic characters of Chinese herbal medicine. The pharmacodynamics of Majie cataplasm are generated from the synergistic effects of different herbal and components. Consequently, it is not enough to select monomeric component as analyte to conduct the pharmacokinetic study of Majie cataplasm. Thus, it is better to select several effective ingredients as the targets to investigate the pharmacokinetics of the whole prescription (Wang XQ, 2014). Ephedrine (E), pseudoephedrine (PE), Sinapine thiocyanate (ST), Tetrahydropalmatine (THP) and amygdalin (AG) (Fig. 1) are major pharmacologically active compounds and serve as the quality control standard of Ephedra Herb, Semen Sinapis and Semen Armeniacae Amarum listed in the Pharmacopeia of the People's Republic of China, respectively (China Pharmacopoeia Committee, 2010; Tang et al., 2016; Tanaka et al., 2014; Guo et al., 2013; Lee et al., 2014). In addition, as the major active components of Herba Ephedrae, Semen Armeniacae Amarum and Semen Sinapis, E, PE, ST, THP and AG have been exhibited to have antiasthmatic effects (Song et al., 2014; Song et al., 2015; Guo et al., 2013; Lin et al., 2014). As a promising antiasthmatic agent, a better understanding of its pharmacokinetics is necessary for correlation of its therapeutic effect. Therefore, in the present study, we choose E, PE, ST, THP and AG as research subjects to established HPLC-MS/MS method.

In the previous studies, several detection methods have been developed, including LC-MS/MS method for E, PE and AG in rats (Jiang et al., 2015)**,** LC-MS/MS method for ST and THP in plasma (Wang et al., 2015), LC-ESI-MS/MS method for E and THP in human blood (Wu et al., 2013). However, to our best knowledge, these methods had limitation such as high limit of detection, which was not adequate to meet current needs. And there was no attempt to establish HPLC-MS/MS method for the determination of the five representative compounds in one study. Therefore, this study explored two HPLC-MS/MS methods for the determination of the E, PE, ST, THP and AG in rabbits plasma, and has been successfully applied to characterize oral administrations of them in pharmacokinetic study.

Experimental

2.1 Chemicals and reagents

Herba ephedrae, Semen Armeniacae Amarum, Semen Sinapis and Rhizoma Corydalis which were purchased from Guoyitang (Beijing, China). Reference-standard E, PE, ST, THP and AG (certified to contain 99.8%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing,China). Acetonitrile and methanol (HPLC grade) were purchased from EMD Millipore Corporation

(Millipore, Milford, MA). Formic acid was obtained from Dikma technologies (Shanghai, China). Purified water used throughout the study and ammonium formate were obtained from Sigma-Aldrich (St. Louis, USA). All other reagents were of analytical grade. All solutions and sample aliquots were filtered through a 0.22um nylon filter membrane manufactured by the Jinteng Corporation (Tianjin, China).

2.2 Preparation of herbal aqueous extracts

Following the extractive method of Majie cataplasm, MaHuang (2.5 g), baijiezi (2.5 g), yanhusuo (2.5 g) and kuxingren (2.5 g) were boiled twice with 80ml of 80% ethanol of 1.5 hours for each time. An aqueous solution by combine all the above mentioned extracts was obtained by ltration and concentrated under reduced pressure at 60℃(Wang et al., 2013). According to the method described above, sufficient extracts were prepared in this experiment.

2.3 Preparation of stock and working dilution and quality control samples

Master stock solutions were prepared by dissolving E, PE, ST, THP and AG in methanol at concentrations of 2 mg/ml, each stock solution was stored in tube after a brief vortex. A series of working solutions of these analytes were obtained by diluting these stocks with methanol at appropriate concentrations of 1000ng/ml, 500ng/ml, 100 ng/ml, 50 ng/ml, 10 ng/ml, 5ng/ml, 1 ng/ml, 0.5 ng/ml, 0.1 ng/ml. Calibration standard samples were prepared by spiking 90ul blank rabbits plasma with standard mixture working solutions (10ul), at final plasma concentrations of 0.01 ng/ml, 0.05 ng/ml, 0.1 ng/ml, 0.5 ng/ml, 1 ng/ml, 5 ng/ml, 10 ng/ml, 50 ng/ml, 100 ng/ml.

Low, medium and high level of Quality control (QC) working solutions at the desired concentrations (1, 5, 20ng/ml and 0.1, 5, 50 ng/ml) were prepared. All samples were vortexed and stored at -80℃ until analysis.

2.4 Sample preparation

Frozen plasma samples were thawed at 25℃. To a 100 uL of rabbits plasma in 3mL tube, 350uL of methanol was added. The samples were vortexed for 30s after centrifugation at 13000 rpm for 10 min, 60ul of the upper organic layer was then transferred into another set of tube, and 140 uL of water was added and vortexed, then filtrated it with a 0.2um micro filter, the supernatant (20ul) was injected into the HPLC–MS/MS system for analysis(Huang et al., 2009).

2.5 Instrumentation and conditions

Plasma samples were analyzed by HPLC–MS/MS method. The HPLC-MS/MS system was composed of Agilent 1290 Infinity liquid chromatography instrument (Agilent, Waldbronn, Germany) and an Agilent 6490 QQQ triple-quadrupole mass spectrometer equipped with an AJS electrospray ionization source (Agilent Technologies, Inc., CA, USA). Two analytical methods were developed for analysis of these five compounds. An Agilent MassHunter Workstation Software (Agilent Technologies, USA) was used for all data acquisition and processing.

2.6 Method

2.6.1 Method 1

Chromatographic separation was achieved on a ZORBAX RRHD Eclipse Plus C18 column (3.0×100 mm, 1.8 μm) using gradient elution with the mobile phase consisting of acetonitrile and water (containing 0.1% formic acid and 5mM ammonium formate) for E, PE, ST and THP. The temperature of the analytical column was set at 40℃. The elution conditions applied for E, PE, ST, THP were: 0.0 min, 3%B; 10.0 min, 6%B; 12.0 min, 40%B; 13.0 min, 98%B; 14.0 min, 98%B; 14.1 min, 3%B. The flow rate was 0.4 mL/min and the injection volume was 5μL.

The capillary voltage and the nozzle voltage were set at 4000V and 0V for E, PE, ST and THP, respectively. The drying gas flow and temperature were set at 15.0 L/min and 200℃. The nebulizer gas pressure was set at 35.0 psi. The sheath gas flow and temperature were set at 11.0 L/min and 350℃.

2.6.2 Method 2

Chromatographic separation was achieved on a ZORBAX RRHD Eclipse Plus C18 column (3.0×100 mm, 1.8 μm) using gradient elution with the mobile phase consisting of acetonitrile and water for AG, the temperature of the analytical column was set at 40℃. The gradient elution conditions for amygdalin was: 0.0 min, 10%B; 3.0 min, 40%B; 5.0 min, 98%B; 6.0 min, 98%B; 6.1 min, 10%B. The flow rate was 0.4 mL/min and the injection volume was 5μL.

The capillary voltage and the nozzle voltage were set at -3500V and -1500V for amygdalin. The drying gas flow and temperature were set at 15.0 L/min and 200℃. The nebulizer gas pressure was set at 35.0 psi. The sheath gas flow was set at 11.0 L/min.

The tandem mass spectrometer was operated under the multiple reaction monitoring (MRM) mode using electrospray source in positive ion mode for E, PE, ST and THP, and negative ion mode for AG. The MRM parameters for the different analytes are shown in Table 1.

2.7 Method validation

2.7.1 Specificity

Specificity was assessed by analyzing blank plasma, blank plasma spiked with E, EP, THP, ST and AG and real plasma samples from rabbits after oral administration of extracting solution of Majie cataplasm.

2.7.2 Calibration curves

The calibration curves were prepared by assaying standard plasma samples at concentrations as described in the section of Preparation of stock and working dilution. Each calibration curve was constructed based on the peak-area ratios of analyte (y) vs the concentration of analyte(x) using a 1/x weighting.

2.7.3 Reproducibility

The reproducibility was determined by analysis of three replicate QC samples at low, medium and high concentration(0.1, 5, 50 ng/ml) in three independent analysis batches. Reproducibility was expressed as

R.S.D.%

2.7.4 Extraction recovery

The extraction recovery at the three concentration levels(1, 5, 20ng/ml) was determined by comparing the peak areas ratios of QC samples that had been spiked prior to extraction, to QC working solutions had been added post-extraction.

2.7.5 Pharmacokinetic study

Six rabbits were housed at Beijing Jinmuyang animal breeding center (license number: SCXK (Beijing) 2010-0001). Environmental controls for the animal room were set at 22 ± 3℃ with 50 ± 20% relative humidity. The animal studies were approved by China national legislation. The rabbits were given herbal aqueous extracts (2.725g /Kg) via intragastric administration.

Blood samples were collected at 10, 30, 60, 90, 120, 150, 180, 360, 480, 540, 720, 1440 and 2880min post administration. Blank rabbits plasma was taken from other blank rabbits. The samples were immediately transferred to tubes and centrifuged at 4000 rpm at 4℃ for 10 min, the plasma supernatant was transferred to clean tubes and stored at 20℃ until analysis.

2.7.6 Pharmacokinetic data analysis

The plasma concentrations versus time profiles were analyzed. Pharmacokinetic parameters of E, PE, ST, THP and AG were calculated using the extravascular non-compartmental analysis tool of Kinetica software (Version 5.0). The pharmacokinetic parameters include the maximum plasma concentration (Cmax), the time to reach maximum concentration (Tmax),the area under the curve from 0 to 2880min (AUC0–2880) andmean retention time from 0 to last infinity (MRT0-∞).

Results

3.1 Method validation

3.1.1 Specificity

The specifiity of the assay was evaluated using blank plasma samples. Blank rat plasma was spiked with the working solutions of E, EP, THP, ST and AG. Under these conditions, the retention times of E, EP, THP, ST and AG were at 8.9, 9.5, 13.3,12.5 and 2.5 min, respectively (Fig.2). There was no interference observed from endogenous substances in the plasma at the retention time of the analytes.

3.1.2 Calibration curves

The calibration curves of E, EP, THP, ST and AG were obtained by weighted (1/x) linear regression analysis, and showed good linearity. The calibration curves, correlation coefficients and linear ranges of these five analytes in plasma were listed in Table 2. The calibration curves of these five analytes showed good linearity ($r > 0.99$) over the concentration ranges.

3.1.3 Extraction recovery

Across the concentration range studied, the relative recovery at the three concentrations of 1.0, 5.0 and 20 ng/mL in rabbits plasma showed in Table 3.

3.1.4 Reproducibility

Reproducibility was determined by analysis of the low, medium and high QCs

(1, 50, and 500 ng/mL, n = 6) on three different assays. The results were showned in Table 4.

3.2 Pharmacokinetic study

The validated method was successfully applied to the pharmacokinetic study of Majie cataplasm in rabbits following oral administration (n = 6 for each administration). The mean plasma concentrations vs time profiles of E, EP, THP, ST and AG following i.g. administration of herbal aqueous extracts were shown in Fig 3.

And its pharmacokinetic parameters using non compartmental analysiswere summarized in Table, the results of PK parameters include Cmax, Tmax, (AUC0–2880)and (MRT0-∞) were shown in Table 5.

Conclusions

The two HPLC-MS/MS methods for the determination of E, PE, ST, THP and AG in rabbits plasma was developed and validated. To the best of our knowledge, this is the first report of oral administration of extracts of Majie cataplasm using HPLC-MS/MS method. Compared with the analytical method reported in the literature, the methods offered superior minimum detection limit and reproducibility, and better separation of ephedrine and pseudoephedrine. We report a fully validated (LC/MS/MS) method for the simultaneous determination of E, PE, ST and THP in rabbits plasma. The method meets the requirement of high sample through-put in bioanalysis and has been successfully applied to a pharmacokinetic study of Majie cataplasm in rabbits.

Abbreviations

HPLC: High Performance Liquid Chromatography

MS: Mass Spectrometry

LC: Liquid Chromatography

MRM: multiple-reaction monitoring

ESI: electrospray ionization

TCM: traditional Chinese medicine

E: Ephedrine

PE: pseudoephedrine

ST: Sinapine thiocyanate

THP: Tetrahydropalmatine

AG: amygdalin

QC: Quality control

AUC: area under the curve

MRT: mean residence time

PK: pharmacokinetic

Declarations

Ethics approval and consent to participate

Experiments were approved by the Ethics Committee on Animal Experiments of the Beijing University of Chinese Medicine.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

name	Contributor Role & Degree of Contribution
Changxiang Li	Formal analysis (Lead) Writing-original draft (Lead)
Hanfen Shi	Formal analysis (Lead) Writing-original draft (Supporting)
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Wenting Ji	Formal analysis (Supporting) Writing-original draft (Supporting)
Qianyi Zhang	Formal analysis (Supporting) Writing-original draft (Supporting)
Xilei Du	Formal analysis (Supporting) Writing-original draft (Supporting)
Tianyi Feng	Formal analysis (Supporting) Writing-original draft (Supporting)
Xueqian Wang	Writing-original draft (Supporting) Writing-review & editing (Lead)

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Changxiang Li and Hanfen Shi designed and performed the experiments, analyzed the data, prepared figures and drafted the manuscript. Fafeng Cheng, Wenxiang Zhu, Yuanjun Liu, Wenting Ji, Qianyi Zhang, Xilei Du and Tianyi Feng performed the experiments. Professor Xueqian Wang designed the study, developed and revised the manuscript, and is the corresponding author. All authors read and approved the final manuscript.

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Tables

Table 1. Mass spectrometry Parameters for E, PE, ST, THP and AG

Compound Name	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Ephedrine	166.1	Unit	148.1	Unit	50	380	12	3	Positive
Ephedrine	166.1	Unit	117.1	Unit	50	380	20	3	Positive
Pseudoephedrine	166.1	Unit	148.1	Unit	50	380	12	3	Positive
Pseudoephedrine	166.1	Unit	117.1	Unit	50	380	20	3	Positive
Sinapine thiocyanate	310.1	Unit	251.1	Unit	50	380	15	3	Positive
Sinapine thiocyanate	310.1	Unit	207.1	Unit	50	380	25	3	Positive
Tetrahydropalmatine	356.2	Unit	192.1	Unit	50	380	30	3	Positive
Tetrahydropalmatine	356.2	Unit	165.1	Unit	50	380	20	3	Positive
Amygdalin	502.2	Unit	179	Unit	200	380	20	3	Negative
Amygdalin	502.2	Unit	323	Unit	200	380	20	3	Negative

Table 2. The regression equation, the correlation coefficients (r2) and the linear range of the 5 analytes in Majie cataplasm

Compounds	Regression equation	Linear range(ng/mL)	Correlation coefficient
E	y = 1613599.57 * x - 7784.34	0.01-100	0.9989
PE	y = 1312891.28 * x -6499.92	0.01-100	0.9987
THP	y = 1151049.52 * x + 1739.22	0.01-100	0.9983
ST	y = 624780.89 * x - 170173.38	0.01-100	0.9994
AG	y = 24980.19 * x + 109.16	0.01-100	0.9964

Table 3 Parameters of extraction recovery for E, EP, THP, ST and AG

Component	Extraction recovery (%RSD, n=6)		
	1 ng/ml	5 ng/mL	20 ng/mL
ephedrine	99.9	109	97.9
pseudoephedrine	102	105	96.0
sinapinethiocyanate	92.1	104	88.3
tetrahydropalmatine	102	105	97.4
amygdalina	84.7	101	93.5

Table 4 Parameters of reproducibility for E, EP, THP, ST and AG

Component	Reproducibility(%RSD, n=6)		
	100 pg/mL	5 ng/mL	50 ng/mL
ephedrine	2.3	0.79	2.2
pseudoephedrine	3.4	0.51	0.83
sinapinethiocyanate	5.0	2.8	3.9
tetrahydropalmatine	6.3	2.6	3.6
amygdalina	2.5	2.2	1.1

Table 5 Pharmacokinetic parameters of E, EP, THP, ST and AG in rabbits after oral administration(mean ± SD, n = 6)

Parameters	P	EP	THP	ST	AG
Cmax(ng/ml)	1.86+0.84	0.49+0.095	0.27+0.23	0.29+0.15	0.98+1.27
Tmax(min)	285+566.91	45+36.75	80+24.50	150+0	55+12.25
AUClast(min*ng/ml)	1237.90+199.53	421.36+47.97	19.27+13.04	201.73+22.14	58.67+59.13
MRT(min)	1717.37+188.90	3462.29+566.24	771.44+657.36	12294.28+7313.09	87.90+34.05

Figures

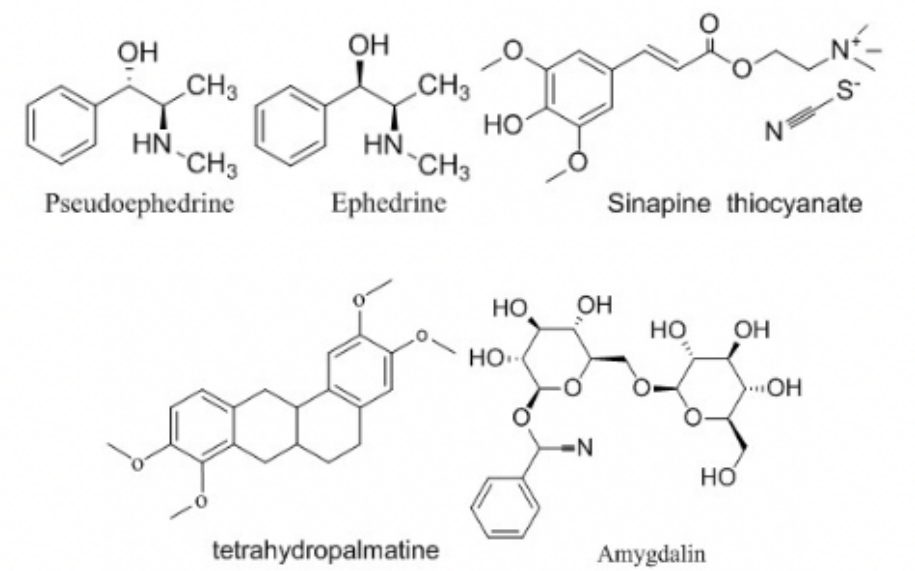

Figure 1

The chemical structure of ephedrine, pseudoephedrine, sinapine thiocyanate, tetrahydropalmatine and amygdalin.

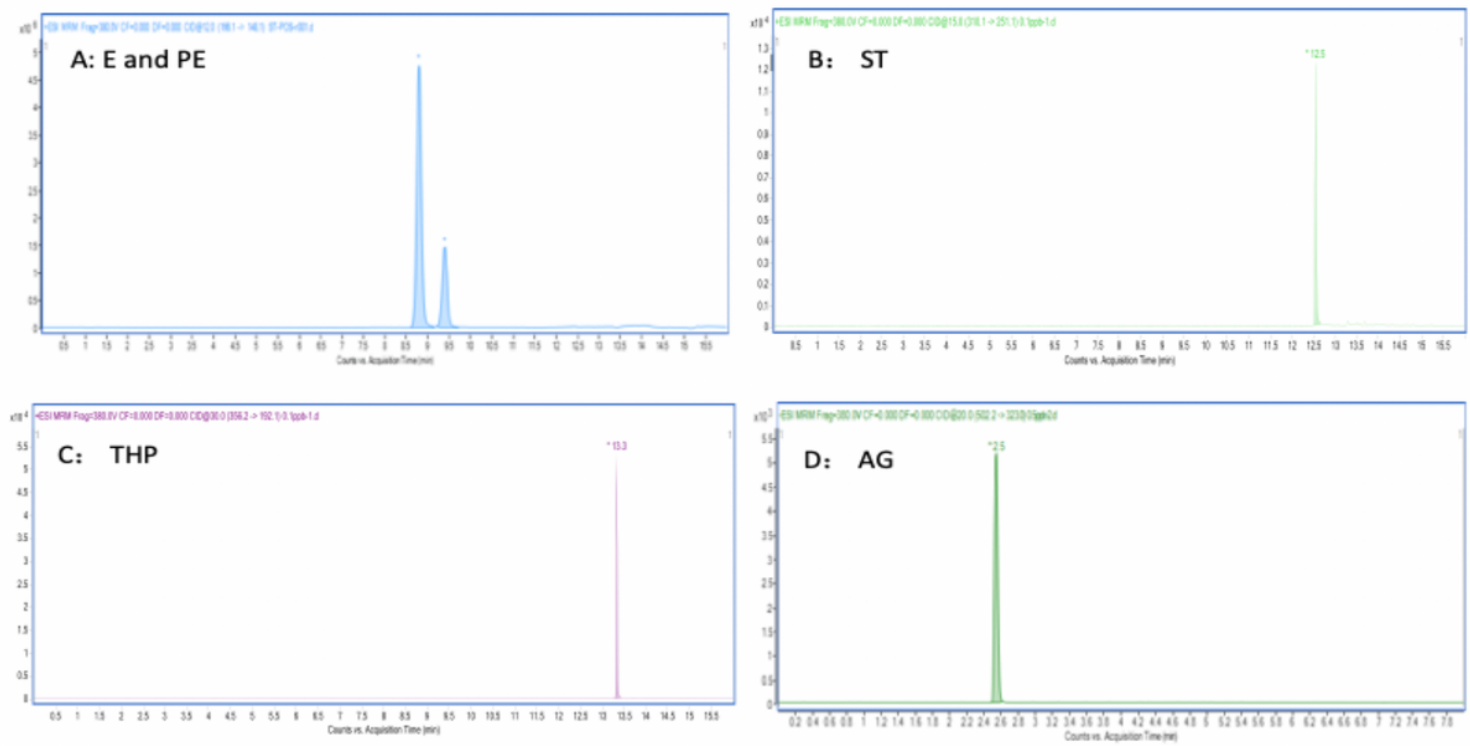

Figure 2

Chromatograms of plasma for ephedrine and pseudoephedrine (A), sinapinethiocyanate (B), tetrahydropalmatine (C), amygdalina(D)

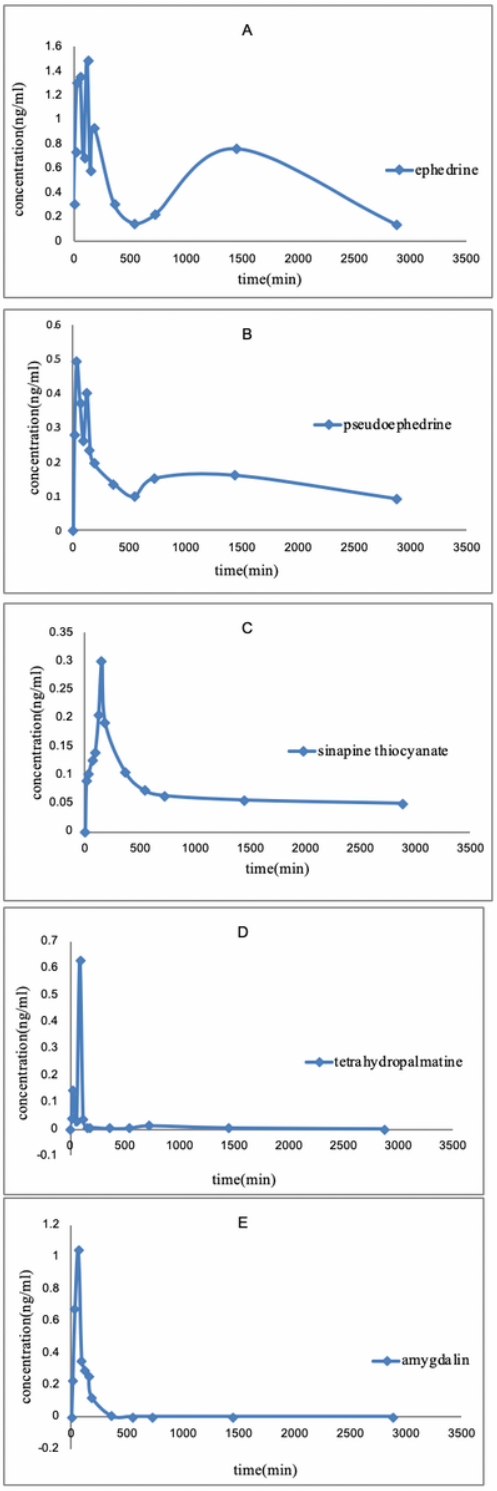

Figure 3

The mean plasma concentrations vs time profiles of E(A), EP(B), THP(C), ST(D) and AG(E) following oral administration of extracts of Majie cataplasm in rabbits plasma