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Simultaneous determination of seven toxic components in ShenFuTuoDu capsules by HPLC-MS/MS

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Abstract: An accurate and reliable HPLC-MS/MS method has been established for the simultaneous determination of seven toxic components in the Chinese medicine ShenFuTuoDu capsules. The seven toxic components were separated on a Shimadzu Shim-pack GIST C_{18} column (3.0 mm×50 mm, 3.0 μm) with methanol and water (containing 0.1% formic acid) as the mobile phase by gradient elution. The flow rate was 0.5 mL·min^{-1}. The column temperature was 25°C and the injection volume was 5μL. An ESI$^+$ scan combined with MRM was adopted and the instrument parameters were as follows: ion source voltage, 5.5 kV; ion source temperature, 600°C; curtain gas, 68.95 kPa; atomized gas, 344.75 kPa; auxiliary gas, 344.75 kPa. The linear relationships of the seven components were good (R^2>0.9937). The average recoveries were 95.2%-106.7% with RSD of 0.79%-5.27% (n=6). The seven toxic components of scopolamine, atropine, rhynchophylline, isorhynchophylline, benzoylaconine, benzoylmesaconine and benzoylhypaconine in six batches of ShenFuTuoDu capsules were 5.99-18.48μg·g^{-1}, 6.36-14.79μg·g^{-1}, 3.71-15.45μg·g^{-1}, 7.90-15.08μg·g^{-1}, 19.05-44.58μg·g^{-1}, 117.38-248.26μg·g^{-1} and 19.74-79.49μg·g^{-1}, respectively. Precision, stability and repeatability test RSDs were less than 7.17% (n=6). The method is suitable for the simultaneous determination of scopolamine, atropine, rhynchophylline, isorhynchophylline, benzoylaconine, benzoylmesaconine and benzoylhypaconine. It can be used for the quality control of ShenFuTuoDu capsules.

Keywords: ShenFuTuoDu capsules, scopolamine, atropine, rhynchophylline, benzoylaconine, HPLC-MS/MS, content determination.

INTRODUCTION

There has long been a large number of people abusing opioid drugs worldwide. In China, the number of people using heroin exceeds one-third of the total number of drug abusers (Office of China National Narcotics Control Commission, 2022). Chemical drugs used to treat heroin addiction, such as methadone and buprenorphine, often have addictive properties and multiple toxic side effects (Bishop-freeman *et al*., 2021; Darke *et al*., 2021; Errico *et al*., 2021). Traditional Chinese medicine, including ShenFuTuoDu capsules, has become a research hotspot because of its outstanding addiction-treatment properties and multitarget effect (Liu, 2019; Liu *et al*., 2020; Wang *et al*., 2022). ShenFuTuoDu capsules are composed of nine Chinese medicinal materials (Gu, 2007; Qiao *et al*., 2006), including processed Aconiti Lateralis, artificial Bovis Calculus, Datura meel Linn, Uncaria rhynchophylla, pearl, Panax ginseng, Radix Curcumae, Aloe and Licorice. It has therapeutic effects, including warming yang, benefiting qi, relieving vexation and cooling the liver (Li *et al*., 2005) and is used to treat acute withdrawal symptoms and protracted symptoms of opioid drug abuse and for rehabilitation from drug abuse (Huang *et al*., 2002; Jordan and Tu, 2008; Liu *et al*., 2009). Processed Aconiti Lateralis, Datura metel Linn and Uncaria rhynchophylla are main components in the formula of ShenFuTuoDu capsules that are toxic Chinese medicinal materials and the contents of the related toxic components, such as scopolamine, atropine, rhynchophylline, isorhynchophylline, benzoylaconine, benzoylmesaconine and benzoylhypaconine, have an important influence on the efficacy and safety of the drug.

In the Chinese Pharmacopoeia, the permissible levels of aconitine and scopolamine in ShenFuTuoDu capsules were determined by TLC. However, this detection method cannot effectively control and evaluate the quality of drugs due to its single detection index and lack of quantitative detection of the two toxic components. In this study, an accurate and reliable HPLC-MS/MS analytical method was established to quantitative analysis of seven toxic components (scopolamine, atropine, rhynchophylline, isorhynchophylline, benzoylaconine, benzoylmesaconine and benzoylhypaconine) simultaneously in ShenFuTuoDu capsule. Then the assay was carried out in different batches of ShenFuTuoDu capsules. Therefore, the developed HPLC-MS/MS analytical method is particularly suitable for the routine analysis and quality control of ShenFuTuoDu capsules and other similar traditional Chinese medicines for opioid drug addiction.

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MATERIALS AND METHODS

Chemicals and materials

HPLC-grade methanol, acetonitrile, and formic acid were purchased from Merck (Darmstadt, Germany). Ultra-pure water was prepared by using a Milli-Q purification system (Millipore, USA). Reference standards of scopolamine (Batch number 9-RFS-72-7), benzoylmesaconine (Batch number 17061904) and benzoylhypaconine (Batch number 17062204) were purchased from TRC (Toronto, Canada). Atropine (Batch number 133718) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Benzoylaconine (Batch number 111794-201304), rhynchophylline (Batch number 112028-201601), and isorhynchophylline (Batch number 111927-201403) were obtained from the National Institute for Food and Drug Control (Beijing, China). The purity of the seven compounds was ≥99.62%, ≥98%, ≥98%, ≥99.5%, ≥ 97.5%, ≥98% and ≥98%, respectively.

Six batches of ShenFuTuoDu capsules (Sample number and batch number: T1, 170101; T2, 170102; T3, 170201; T4, 170202; T5, 170401; T6, 170402) were purchased from Chengdu Zhizhi Pharmaceutical Company Limited (Chengdu, China).

Preparation of sample solution and standard solution

Accurately weigh the powder of each ShenFuTuoDu capsules (400 mg) and transfer it to a dark brown calibrated flasks, add 50% methanol aqueous solution (v/v) to a constant volume of 10mL. After that, extract for 30 min in an ultrasonic bath and centrifuge the extracted solution for 10 min at 15000 rpm. The supernatant is then used for analysis after being filtered via a 0.22μm micro-porous filter membrane.

To create stock solutions with a concentration of 20 $\mu g \cdot mL^{-1}$, seven standards are individually dissolved in methanol and subsequently dilute the mixture with 50% methanol aqueous solution (v/v). To create a series of standard working solutions for creating calibration curves, further dilute the stock solutions of each standard component in 50% methanol aqueous solution (v/v). Before use, all solutions were raised to ambient temperatures after being kept at -20°C in the dark.

Liquid chromatography

Shimadzu LC-20AD series liquid performed system (Shimadzu, Japan), which consisted of a quaternary pump, an auto-sampler, an online degasser and a column oven, was used for the chromatographic analysis. The seven compounds were separated on a Shimadzu Shim-pack GIST C18 (3.0 mm× 50 mm, 3.0μm) maintained at 25°C with the mobile phases being 0.1% (v/v) formic acid (A) and methanol (B). The flow rate was 0.5 $mL \cdot min^{-1}$. Gradient elution was employed with 0~2.0 min, 10% B; 2.0~11.0 min, 10%-30% B; 11.0~11.2 min, 30%-75%B; 11.2~12.5 min, 75%B; 12.5~13.0 min, 75%-10%B; and 13.0~16.0 min, 10%B. Each sample was injected 5μL.

Mass spectrometry

An API4000 triple quadrupole mass spectrometer (AB Sciex Company, Canada) with an electrospray ionization (ESI) source was used to perform the MS/MS analysis. Acquire mass spectra in multireaction monitoring mode operated in positive. Nitrogen served as the nebulizer and heater gas. Argon served as the collision gas. The mass spectrometry was conducted under the following optimized settings: ion source voltage, 5.5 kV; ion source temperature, 600℃; atomized gas, 344.75 kPa; curtain gas, 68.95 kPa; auxiliary gas, 344.75 kPa. The optimized MS parameters for each component are shown in Table 1.

Method validation

Specificity

Take a blank extraction solvent, standard solution and sample solution, determine them under the optimized HPLC–MS/MS conditions. Determine the specificity by analyzing the chromatograms of the seven components.

Linearity, work range and limit of detection

Calibration curves were created by plotting the chromatographic peak area(*Y*) versus the analyte concentration(*X*). The regression relationship was described by linear regression with a 1/x weighting factor. LOD and LOQ was the lowest concentrations of the analytes at signal-noise ratio (S/N) of approximately 3 and 10, respectively.

Precision, repeatability and stability

The intra-day precision was verified by measuring six replicates of mixed standard solution at three different concentrations on the same day. The inter-day precision was validated by measuring the mixed standard solutions once a day for three consecutive days. Precision was expressed as the RSD.

Accurately weigh the ShenFuTuoDu capsules (batch number 170101), prepare six sample solutions, determine the sample solutions under the optimized conditions, and then calculate the RSD of the determined results to verify the repeatability of the method. The stability was investigated at room temperature, and the samples of ShenFuTuoDu capsules were prepared in triplicate and determined at 2, 4, 8, 12 and 24h, respectively.

Recovery test

The recovery test was determined using the standard addition method. Seven standards at low, medium and high concentrations were added to the ShenFuTuoDu capsules (batch number 170101) to prepare the sample solution, which was then determined by the optimized analytical method. The mean recovery was calculated according to Eq.1.

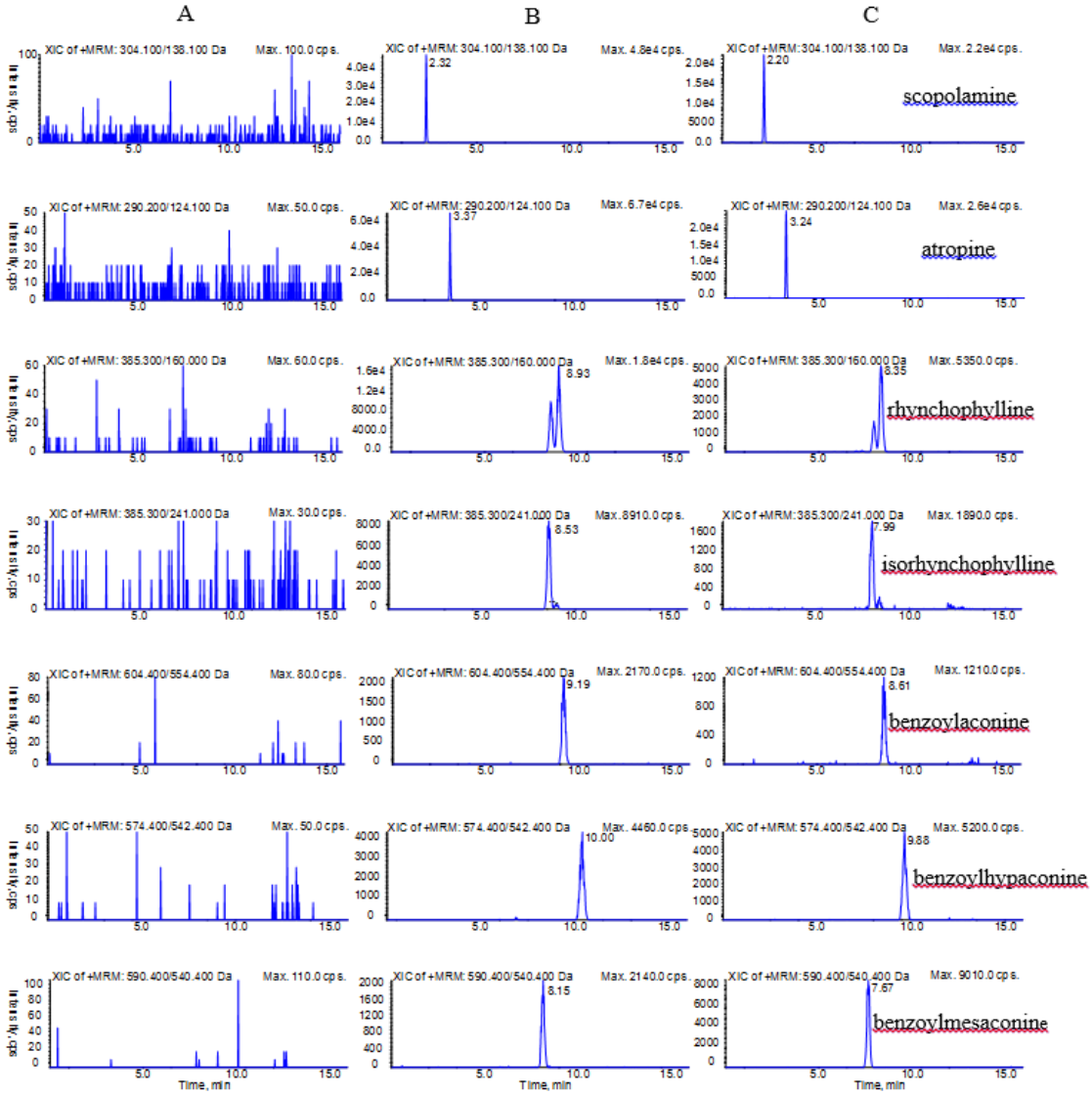

Fig. 1: MRM chromatograms of negative sample (A), reference substances (B) and sample (C)

$$\text{Recover (\%)} = \frac{\text{Amount found} - \text{Original amount}}{\text{Amount spike}} \times 100\% \quad \text{(Eq. 1)}$$

$$\text{RSD (\%)} = \frac{\text{SD}}{\text{Mean}} \times 100\%$$

STATISTICAL ANALYSIS

Microsoft Excel is used for analyzing obtained data. Percentage recovery, percentage RSD and line equantion was calculated.

RESULTS

Method validation

Specificity

As shown in the chromatograms in fig. 1, where the seven target components can be separated effectively, the blank extraction solvent does not interfere with the determination of each component to be measured, and other components in the sample do not interfere with the determination of the analyzed components. The results indicate that the HPLC-MS/MS analytical method in this research is acceptable in terms of specificity.

Calibration curves, LOD and LOQ are listed in table 2. The linear correlation coefficients were greater than 0.9937 for all calibration curves indicating that the seven components had acceptable linear correlations in the corresponding concentration ranges. The analytical method was sensitive, with the LOD and LOQ of the seven components were than 5.00 and 10.0ng·mL^{-1}, respectively.

Precision, repeatability, stability and recovery test

The seven analytes' respective intra- and inter-day precision RSD% varied from 1.66% to 4.59% and 3.16% to 6.77% (n=6), respectively. The findings show that the quantitative analytical method that was devised is precise. The RSD% of repeatability and stability were 2.20% to

Table 1: MS parameters of the seven components

Compounds	precursor ion (m/z)	product ion (m/z)	DP(V)	EP(V)	CE(V)	CXP(V)
Scopolamine	304.1	138.1	50	10	31	15
Atropine	290.2	124.1	70	10	34	15
Rhynchophylline	385.3	160.0	70	10	45	15
Isorhynchophylline	385.3	241.0	80	10	41	20
Benzoylaconine	604.4	554.4	83	11	48	17
Benzoylmesaconine	590.4	540.4	120	10	47	34
Benzoylhypaconine	574.4	542.4	105	10	46	14

Table 2: Linear equations, correlation coefficients, linear range and limit of quantity

Compounds	Regression Equation	R^2	linear range/(ng·mL^{-1})	LOD/(ng·mL^{-1})	LOQ/(ng·mL^{-1})
Scopolamine	Y=9.45×10^3X-557	0.9937	1.00~40.0	0.20	0.400
Atropine	Y=1.13×10^4X-673	0.9977	1.00~100	0.20	0.800
Rhynchophylline	Y=7.33×10^3X-2940	0.9967	3.00~150	0.0800	0.200
Isorhynchophylline	Y=3.51×10^3X+48	0.9983	1.50~300	0.0200	0.0400
Benzoylaconine	Y=522X+283	0.9990	5.00~500	5.00	10.0
Benzoylmesaconine	Y=496X-84.1	0.9983	5.00~500	0.800	4.00
Benzoylhypaconine	Y=2.03×10^3X+350	0.9995	1.50~300	2.00	4.00

Table 3: Contents in six batches of samples

Compounds	Content/(μg·g^{-1})					
	T1	T2	T3	T4	T5	T6
Scopolamine	11.23	5.99	9.52	18.48	17.95	8.70
Atropine	6.36	12.17	7.89	10.51	9.78	14.79
Rhynchophylline	6.24	15.45	10.33	8.19	8.41	3.71
Isorhynchophylline	8.91	13.06	15.08	8.65	9.55	7.90
Benzoylaconine	33.39	23.82	44.58	19.05	43.40	23.51
Benzoylmesaconine	181.02	203.90	121.16	194.13	248.26	117.38
Benzoylhypaconine	27.80	49.67	79.49	19.74	20.53	30.18

7.17% and 2.72% to 5.27% (n=6), respectively, which proved that the analytical method was sufficiently reproducible and satisfactorily stable for the quantitative analysis of the seven components in ShenFuTuoDu capsules. The RSD% for seven components ranged from 0.79% to 5.27% (n=6), while the recoveries for the components themselves ranged from 95.2% to 106.7%. The recovery test results demonstrated the analytical method established in this research is precise, accurate and sensitive and is suitable for determining all seven toxic components in ShenFuTuoDu capsules simultaneously.

Sample analysis

Finally, six batches of ShenFuTuoDu capsules were accurately weighed and determined. The determination of all samples were analyzed by optimized extraction methods and optimized HPLC-MS/MS conditions. The average contents of seven components in six batches of ShenFuTuoDu capsules are listed in table 3.

DISCUSSION

Optimization of the extraction methods

To simultaneously determine multiple toxic components in drugs, it is necessary to optimize the extraction method so that multiple toxic components can be fully extracted at the same time.

To achieve good extraction efficiency for multiple components, this study compared the extraction solvent and ultrasonic extraction time. A variety of toxic components in ShenFuTuoDu capsules (batch number 170101) were extracted with different concentrations (30%, 50%, 70% and 100%) of methanol solutions and different ultrasonic extraction times (10, 20, 30 and 40 min). The results suggest that when 50% methanol aqueous solution (v/v) was employed as the extraction solvent and the ultrasonic extraction time was 30 min, the seven toxic components in ShenFuTuoDu capsule could be extracted relatively completely.

Optimization of HPLC-MS/MS conditions

In this research, because the components contained in the drug are very complex, conventional HPLC conditions cannot completely and effectively separate all the components. In addition, the content of different components in the drug varies greatly, and the response of mass spectrometry varies greatly. It is difficult to obtain good chromatographic peaks and mass spectrometry signals of multiple components.

Different mobile phases, including acetonitrile-water,

acetonitrile-0.1% (v/v) formic acid, methanol-water, and methanol-0.1% (v/v) formic acid, were investigated to improve the chromatographic conditions and produce chromatograms with good peak shapes and high sensitivity. With methanol-0.1% (v/v) formic acid as the mobile phase, each target component could be separated well. The gradient elution approach significantly increased the separation rate and efficiency, and the seven target components were successfully separated within 16 min. While enhancing chromatographic separation, formic acid increased the abundance of $[M+H]^+$ in the positive ion mode.

The MS/MS fragmentation of each analyte was investigated by injecting a single standard solution into the mass spectrometer to develop a sensitive and accurate analytical method. Mass spectrometry was used to characterize each analyte to identify the precursor ions and product ions for MRM analysis. MRM parameters, including DP, EP, CE, and CXP, were manually optimized.

Sample analysis

The average contents of scopolamine, atropine, rhynchophylline, isorhynchophylline, benzoylaconine, benzoylmesaconine and benzoylhypaconine in six batches of ShenFuTuoDu capsules were 5.99-18.48μg·g^{-1}, 6.36-14.79μg·g^{-1}, 3.71-15.45μg·g^{-1}, 7.90-15.08μg·g^{-1}, 19.05-44.58μg·g^{-1}, 117.38-248.26μg·g^{-1} and 19.74-79.49μg·g^{-1}, respectively. The results showed that different batches of ShenFuTuoDu capsules had some differences in the contents of the seven toxic components, which may be due to the differences in the Chinese herbal medicines of the companies that produced the drugs, as well as the preparation process.

CONCLUSION

There are many studies on the treatment of opioid drug addiction with ShenFuTuoDu capsules and similar traditional Chinese medicines. However, there are very few studies on the quality control methods of ShenFuTuoDu capsules and similar traditional Chinese medicines. In this research, an accurate and reliable method for the simultaneous determination of seven toxic components (scopolamine, atropine, rhynchophylline, isorhynchophylline, benzoylaconine, benzoylmesaconine and benzoylhypaconine) in the Chinese medicine ShenFuTuoDu capsules was developed using HPLC–MS/MS. The established method is expected to improve quality control for ShenFuTuoDu capsules and other related traditional Chinese medicines used to treat opioid drug addiction.

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