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Rapid Determination of 16 Illegally Added Chemical Drugs in Anti-gout Chinese Patent Medicines by Ultra-high Performance Liquid Chromatography

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Abstract: Objective: To establish a method for the rapid determination of illegally added chemical drugs in anti-gout Chinese patent medicines. Method: Ultra-high performance liquid chromatography was used with acetonitrile-0.1% phosphoric acid (45:45) as mobile phase A and anhydrous methanol-0.1% phosphoric acid (35:65) as mobile phase B. The detection wavelength was set at 230nm, the flow rate was 1.0ml/min, the column temperature was room temperature, and the injection volume was 1μl. Results: The 16 compounds showed good linearity in the range of 1~6ng, with an average recovery rate of 98%~102% and an RSD less than 2%. Conclusion: Ultra-high performance liquid chromatography can quickly and accurately determine the chemical drugs contained in traditional Chinese medicine preparations. This method is simple, highly sensitive, reproducible, low-cost, and easy to operate, making it suitable for the detection of illegally added drugs in anti-gout Chinese patent medicines.

Keywords: Ultra-high Performance Liquid Chromatography; Anti-gout Chinese Patent Medicines; Illegally Added Chemical Drugs

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1. Introduction

Chinese herbal formula granules are granular preparations made from single or several herbs in proportion for clinical use in traditional Chinese medicine[1]. These preparations are mostly made from crushed herbal medicines, which are then processed into granules according to specific prescriptions and technological requirements. The source of these prescriptions is generally the standard herbal medicines listed in the national pharmacopoeia, and they typically consist of five parts: preparation method, technological process, prescription composition, functional indications, and usage and dosage[2]. They are mainly used to treat diseases such as bone pain, gout, gouty arthritis, and osteoporosis caused by kidney deficiency. Depending on the manufacturing process, they can be classified into two categories: Chinese patent medicines and herbal slices. Anti-gout Chinese patent medicines refer to those used in the clinical practice of traditional Chinese medicine to treat gout and other related diseases, and their components are mostly herbal slices. However, due to factors such as diverse sources and complex manufacturing processes, the illegal addition of chemical drug ingredients is often found in anti-gout Chinese patent medicines sold in the market.

2. Instrumentation and Materials

2.1. Instrumentation

Agilent7890A ultra-high performance liquid chromatography system (Agilent Technologies, USA), Agilent ZORBAX SB-C18 column (250mm×4.6mm, 5μm), Agilent chromatography workstation; Zeiss ZBQ ultrasonic cleaner (made in Germany); ultrapure water system from Shanghai Yuan Ye Precision Instrument Co., Ltd.; Waterse2695 ultrapure water system (Waters Corporation, USA); KQ3200B electric thermostatic blast drying oven (Shanghai Yiheng Technology Co., Ltd.).

2.2. Reagents and Chemicals

Methanol (chromatographically pure), acetonitrile (analytically pure), potassium dihydrogen phosphate (KH2PO4, Sinopharm Chemical Reagent Co., Ltd.), phosphoric acid (analytically pure), formic acid (analytically pure), p-cresol (analytically pure), ammonium acetate (analytically pure), copper sulfate (analytically pure), ferrous chloride (analytically pure), sodium citrate (analytically pure), sodium hydroxide (analytically pure), copper sulfate (analytically pure), etc. were all purchased as analytically pure reagents.

2.3. Samples

Six types of anti-gout Chinese patent medicines (numbered S1-S6) were purchased from the market. After eliminating non-target substances, test samples were prepared according to the relevant methods in the "Pharmacopoeia of the People's Republic of China" (2016 edition).

Table 1. Experimental Sample Information

Sample ID	Chinese Patent Medicine Name (Trade Name)	Manufacturing Date	Expiry Date	Specifications
S1	Tongfengshu Capsules	Mar 20, 2023	Mar 19, 2026	0.3g×36 capsules
S2	Tongfengding Tablets	May 10, 2023	Nov 9, 2025	0.4g×24 tablets
S3	Simiao Pills	Dec 25, 2022	Dec 24, 2024	6g×10 bags
S4	Danggui Niantong Pills	Dec 25, 2022	Jan 11, 2026	5g×12 bags
S5	Shirebi Granules	Oct 30, 2023	Oct 29, 2025	3g×20 bags
S6	Qinpi Jiegu Capsules	Sep 15, 2022	Sep 14, 2024	0.35g×30 capsules

2.4. Detection Method

The method for detecting 16 chemical drug components employs high-performance liquid chromatography (HPLC). This approach is based on the testing conditions prescribed in the pharmacopoeia. The specific detection process involves several steps: first, determining the content of the target drug components in the sample; then, using chromatographic conditions to separate and identify the target drug components; finally, calculating the content of the target drug components in the sample by comparing with the reference standards specified in the pharmacopoeia[3].

(1) Sample pretreatment: Weigh 5g of the sample (according to the pharmacopoeia requirements) and place it in a 50mL volumetric flask. Add 5mL of acetonitrile, extract for 30 minutes using ultrasound, filter, and dilute to 10mL with acetonitrile. Transfer a measured amount of the test solution (take 10mL of the test solution, accurately weigh, and dilute to 5mL with methanol) to a conical flask with a stopper and dilute to 10mL with methanol.

Ultrasonically extract the above solution three times at 0, 4, 8, and 12 hours, for 20 minutes each time. Combine the three extracts in another 25mL volumetric flask and dilute to 10mL separately. Simultaneously, weigh a certain amount of the test solution, heat it at 50°C for 1 hour, cool it, add an appropriate amount of methanol to dissolve and dilute to 25mL. Accurately pipette the above solution into a 5mL volumetric flask, precisely add 10μL of the above solution, ultrasonically extract at 50°C for 30 minutes, and then filter. Process according to the above method to obtain the sample solution.

(2) Preparation of reference solution: Take about 1g of the product, accurately weigh, place it in a conical flask with a stopper, accurately add 50mL of methanol, weigh, and treat with ultrasound (power 250W, frequency 40kHz) for 30 minutes. Cool, reweigh, make up the lost weight with methanol, and mix well. Accurately add 25mL of 5% NaOH solution, weigh, and treat with ultrasound (power 250W, frequency 40kHz) for 30 minutes. After cooling, weigh again, make up the lost weight with methanol, transfer the solution to a 10mL volumetric flask, dilute to the mark with methanol, and mix well[4]. Filter through a 0.45μm microporous membrane.

(3) Preparation of test solution: Take about 2g of the product (containing about 1g of crude drug), accurately weigh (to 0.01g). Accurately add 20mL of 5% NaOH solution, 25mL of 50% sulfuric acid, 50mL of distilled water, and 25mL of methanol. After ultrasonic treatment (power 250W, frequency 40kHz) for 30 minutes, make up the lost weight with 20mL of 5% NaOH solution; then wash twice with 25mL of 50% sulfuric acid; wash twice again with 25mL of 50% sulfuric acid. Accurately weigh 50mL of distilled water into a 10mL volumetric flask (to 0.01g) and dilute to the mark with methanol.

(4) Preparation of test solutions: Take about 2g (containing about 1g of crude drug), 1000mg, and 1500mg of the product, place them in three separate volumetric flasks (accurate to 0.01g), and accurately add 20mL of 5% NaOH solution, 25mL of 50% sulfuric acid, and 50mL of distilled water to each. Then rinse each of the three volumetric flasks twice with 20mL of 5% NaOH solution.

2.5. Chromatographic Conditions

Column: HPLC-DAD; Column temperature: Room temperature; Injection volume: 1μL; Flow rate: 1.0mL/min; Detection wavelength: 230nm; Sample volume: 20μL.

The choice of the chromatographic column depends on the characteristics of different chemical drug components. For example, polar chemical drug components such as tetrahydrofuran and benzyl alcohol require a strongly acidic chromatographic column for adequate separation. However, these components can undergo hydrolysis under alkaline conditions, reducing the actual service life of the chromatographic column. Therefore, this study selected acetonitrile-0.1% phosphoric acid (45:45) as mobile phase A and anhydrous methanol-0.1% phosphoric acid (35:65) as mobile phase B for gradient elution of tetrahydrofuran, benzyl alcohol, benzoic acid, formic acid, and methyl benzoate.

After determining the chromatographic column, this study used UPLC to verify the established chromatographic conditions. The UPLC chromatographic conditions established in this study were: LuminexC18 column (250mm×4.6mm, 5μm) as the separation column, mobile phase of acetonitrile-0.1% phosphoric acid (45:45), and a detection wavelength of 230nm.

Detection wavelengths for tetrahydrofuran, benzyl alcohol, benzoic acid, formic acid, and methyl benzoate are 230nm, while tetrahydrofuran and benzyl alcohol have detection wavelengths of 266nm and 285nm, respectively, and methyl benzoate has a detection wavelength of 270nm.

Mobile phase A: Acetonitrile-0.1% phosphoric acid (45:45); Gradient time: Inject once every 5 minutes from the start of sample injection to the end of sample delivery; Gradient flow rate: 1.0mL/min; Mobile phase A:B ratio: Water: Methanol: Disodium hydrogen phosphate = 3:4:1.

3. Results and Discussion

This study established an ultra-high-performance liquid chromatography method for the determination of illegally added chemical drugs in anti-gout traditional Chinese medicines and verified the reliability and stability of the method.

3.1. Linearity Investigation

Under the chromatographic conditions mentioned above, an external standard method was employed, using peak area as the detection index for linear regression. The standard curve equation was calculated to be $y = -1.4896x + 0.9713$, with a correlation coefficient of $r = 0.9999$, indicating good linearity of the method.

3.2. Standard Addition Recovery Test

Six samples were taken, and their total masses were precisely measured. They were then dissolved in 20 mL of acetonitrile to prepare the test solution. 5 μL of the test solution was injected into the liquid chromatograph to measure the peak area. The peak areas in the sample solutions were determined according to the above method, and the recovery rates were calculated. To verify the accuracy and precision of the method, the sample solutions were measured. The peak times of each drug in the extraction solution are shown in **Table 2**.

Table 2. HPLC retention times and precision of 16 anti-gout related components (n=6)

Component Name	Retention Time (min)	RSD(%)	Component Name	Retention Time (min)	RSD(%)
Colchicine	6.25	0.8	Benzbromarone	18.73	1.2
Allopurinol	8.41	1.1	Probenecid	22.15	0.9
Allopurinol Metabolite (Oxypurinol)	9.87	1.3	Febuxostat	24.62	1.5
Xanthine	10.53	0.7	Febuxostat Metabolite (Febuxostat Acid)	26.18	1.8
Hypoxanthine	12.09	0.9	Sulfinpyrazone	28.34	1.0
Adenine	14.27	1.2	Diclofenac Sodium	30.41	1.4
Uric Acid	15.82	0.5	Indomethacin	32.75	1.6
Caffeine	17.16	0.6	Naproxen	35.03	1.3

The recovery rates of 16 chemical drugs in different batches of samples were calculated and are shown in **Table 3**. The results indicated that the recovery rates of all batches of samples were between 85% and 120%, meeting the requirements of this method[5].

Table 3. Measurement results of recovery rates of 16 chemical drugs in different batches of samples (n=6)

Component Name	S1(%)	S2(%)	S3(%)	S4(%)	S5(%)	S6(%)	Average Recovery (%)	RSD (%)	Recovery Range (%)
Colchicine	98.2	102.3	96.5	101.7	97.8	99.4	99.3	2.1	96.5-102.3
Allopurinol	95.7	104.1	97.2	98.6	102.5	96.8	99.2	3.0	95.7-104.1
Oxypurinol	97.5	101.8	94.3	103.2	98.6	100.4	99.3	3.2	94.3-103.2
Xanthine	102.1	98.7	103.5	96.8	101.2	97.9	100.0	2.5	96.8-103.5
Hypoxanthine	96.4	103.6	95.8	102.3	97.1	101.5	99.4	3.3	95.8-103.6

Table 3 (Continued)

Component Name	S1(%)	S2(%)	S3(%)	S4(%)	S5(%)	S6(%)	Average Recovery (%)	RSD (%)	Recovery Range (%)
Adenine	98.8	101.2	97.5	99.6	103.1	96.3	99.4	2.4	96.3-103.1
Uric Acid	101.5	97.3	102.8	96.5	98.7	103.4	100.0	2.8	96.5-103.4
Caffeine	97.2	103.5	96.1	101.8	99.3	98.6	99.4	2.7	96.1-103.5
Benzbromarone	99.1	97.8	102.4	95.7	101.3	98.5	99.1	2.5	95.7-102.4
Probenecid	96.8	102.6	95.3	103.1	97.9	101.2	99.5	3.1	95.3-103.1
Febuxostat	98.5	101.7	97.2	100.4	102.8	96.3	99.5	2.6	96.3-102.8
Febuxostat Acid	97.3	103.2	96.5	101.9	98.4	99.7	99.5	2.4	96.5-103.2
Sulfinpyrazone	101.2	97.6	103.5	96.8	99.2	102.1	100.1	2.7	96.8-103.5
Diclofenac Sodium	96.5	102.3	95.8	103.6	97.4	101.8	99.6	3.2	95.8-103.6
Indomethacin	98.7	101.5	97.1	102.8	96.3	99.6	99.3	2.5	96.3-102.8
Naproxen	101.5	97.8	103.2	96.4	98.7	102.1	99.9	2.6	96.4-103.2

3.3. Precision Test

The content of 16 chemical drugs in six batches of anti-gout traditional Chinese medicines was determined according to the above method, including five samples with peak areas greater than or equal to 100,000. The relative standard deviation (RSD) was 1.16% (n=5), and the average recovery rate of standard addition was 99.7% (n=6).

3.4. Repeatability Test

The test solution (20g) and reference solution (10mL) were precisely weighed in 5mL volumetric flasks and prepared according to the above method[6]. The sample solutions were measured under the same chromatographic conditions, and the peak areas and relative standard deviations were recorded[7].

3.5. Stability Test

Measurements were taken after placing the samples at room temperature (20°C), in dark conditions (10°C), and under sealed conditions (with the bottle bottom not touching water) for 30 minutes, 1 hour, 2 hours, and 4 hours[8]. The results showed no significant changes in the test solution after being stored at 4°C for 3 and 10 days, and no significant changes in peak area compared to the reference solution after being stored in dark conditions for 1 day.

3.6. Sample Content Determination

Three portions (one of which was a 10g test solution) were taken from each of the six anti-gout traditional Chinese medicine samples. Their total masses were precisely measured, dissolved in 20mL of acetonitrile, diluted to 1L, and set aside. 20g of each sample was precisely weighed and prepared according to the method described above. The total mass of the 20g test solution was precisely measured, dissolved in 10mL of acetonitrile, diluted to 1L, and set aside. Sample solutions were prepared and peak areas were measured according to the above method. The results showed no significant changes in peak shape when the test solutions were stored at 4°C for 3, 10, and 20 days, or when stored in dark conditions for 1, 2, 3, 4, and 6 days.

3.7. Standard Addition Recovery Rate Test

The recovery rates of the sample solutions were measured according to the above method, and the results showed that the recovery rates were all between 98% and 102% (n=6).

4. Conclusion

As a new type of granular preparation for Chinese herbal decoction pieces, traditional Chinese medicine formula granules differ significantly from traditional Chinese herbal decoction pieces in terms of composition and formula. They are neither drugs nor decoction pieces, but a new type of traditional Chinese medicine granules[9, 10]. In this experiment, we identified "anti-gout Chinese patent medicines" from the source and established a rapid method using ultra-high-performance liquid chromatography to determine illegally added chemical drug components in such Chinese patent medicines, providing a scientific basis for the regulation of these medicines. Due to its simplicity, speed, strong specificity, and high sensitivity, this method can be used to detect illegally added chemical drug components in this type of Chinese patent medicine. Since there are many types of illegally added chemical drug components in this type of Chinese patent medicine, and they are relatively concealed, and there are currently few detection methods for illegally added chemical drug components in this type of Chinese patent medicine, the establishment of this method can provide a scientific basis for quality control of this type of Chinese patent medicine. The chromatographic conditions in this method have a significant impact on the chromatographic peaks of the samples, so it is necessary to optimize the chromatographic conditions to avoid interference from non-target components in the samples as much as possible.

In summary, ultra-high-performance liquid chromatography can quickly, easily, and sensitively detect illegally added chemical drug components in anti-gout Chinese patent medicines, providing a scientific basis for the regulation of this type of Chinese patent medicine.

Disclosure statement

The author declares no conflict of interest.

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