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Establishment of fingerprints and determination of various ingredients of yanlishuang pills by GC-MS

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Abstract

Yanlishuang Pills is a kind of traditional Chinese medicine used to treat pharyngitis widely. In this study, we used gas chromatography tandem mass spectrometry (GC-MS) to establish a method for the fingerprint and quantitative analysis of the four major components of Yanlishuang Pills, which can provide a more reliable method for its quality control. We used the software "Chromatographic Fingerprint Similarity Evaluation System for Traditional Chinese Medicine", version A, 2004, to obtain fingerprint using the averaging method with a time width of 0.1. The peak with the largest peak area was used as the reference peak to determine the shared peaks and generate the common pattern. Then the main components of the Yanlishuang Pills were identified and their contents were determined in GC-MS SIM mode using internal standard method.The fingerprint established by GC-MS were reproducible, and a total of 18 common peaks were identified in the fingerprint of 13 batches of samples, and the similarity of the fingerprint of each batch of samples was above 0.99. The concentrations of camphor, menthone, borneol and menthol of the four main ingredients of the Yanlishuang Pills were linearly well within the range of 25.13-150.78 μg/mL (r = 0.9995), 28.77-172.62 μg/mL (r = 0.9991), 299.70-1798.20 μg/mL (r = 0.9997), 121.98-731.88 μg/mL (r = 0.9997), and the average recoveries were 102.02% (RSD of 1.3%), 96.10% (RSD of 1.0%), 102.71% (RSD of 1.3%), 102.58% (RSD of 1.1%), respectively, with good precision, reproducibility, and stability within 16 h. The camphor content of the 13 batches of samples was 5.6025-8.3662 mg/g, menthone content was 4.7871-5.8936 mg/g, borneol content was 88.0034-133.0969 mg/g and menthol was 40.2017-61.9466 mg/g. The fingerprints of the Yanlishuang Pills established by GC-MS were characterized by a common pattern, and the simultaneous determination of camphor, menthone, borneol and menthol in the Yanlishuang Pills was rapid, simple and accurate. In conclusion, the determination of the content of multiple ingredients combined with fingerprinting can provide a more comprehensive control of the quality of Yanlishuang Pills.

Keywords: Yanlishuang Pills; *Blumea balsamifera*; fingerprint analysis; quantitative determination.

Practical Application: The investigation provides the important information for the quality control of Yanlishuang Pills and *Blumea balsamifera*.

1 Introduction

Blumea balsamifera was used as tea in China and Southeast Asian countries such as Malaysia, Philippines, Vietnam, and Thailand for the treatment of many diseases (Tan et al., 2020). The two main ingredients (L-Borneol and Blumeae balsamiferae oleum) in *Blumea balsamifera* are the main components of Yanlishuang Pills. In addition, Yanlishuang Pills also contain oleum menthae dementholatum, menthol, glycyrrhizic acid ammonium salt and the excipient PEG-6000. It has a special aroma, sweet taste and a little bitter. Yanlishuang Pills is used for the treatment of acute pharyngitis, acute attacks of chronic pharyngitis, sore throat, redness and swelling of the pharyngeal mucosa, dry throat, and bad breath because of its ability to reduce fever, relieve swelling and pain, refresh pharyngeal. L-Borneol are crystals made by extracting and processing the fresh leaves of *Blumea balsamifera* of the family Asteraceae, the main component of which is borneol, and also contains small amounts of camphor and DL-Isoborneol (Zhang et al., 2017), which have the effect of waking the mind, clearing heat and relieving pain. Pharmacological studies have shown that ice chips have anti-cancer (Li et al., 2022), neuroprotective (Ma et al., 2021) and improving cerebral effects (Li et al., 2021; Zhang et al., 2021). The oil obtained by pressing and separating the crude extract in the process of refining L-Borneol is Blumeae balsamiferae oleum (Hu et al., 2021), the main components of which are β-pinene, β-caryophyllene, camphor, α-caryophyllene, and borneol (Qin et al., 2020), which is used for the treatment of sore throat, mouth sores, skin sunburn (Li et al., 2017), eczema, colitis and mosquito bites because of its clearing heat and anti-inflammatory properties (Cai et al., 2021; Wang et al., 2021; Yi et al., 2016), antibacterial and antipruritic effects (Gao et al., 2021), and local anesthesia. Oleum menthae dementholatum is a volatile oil obtained by water steam distillation, freezing and

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processing of fresh stems and leaves of Mentha haplocalyx Briq. of the family Labiatae (Zeng et al., 2021), the main components are menthol, menthone, menthol acetate, limonene, which has anti-inflammatory, antibacterial, antioxidant (Shao et al., 2022), choleretic used to produce a cooling sensation on the skin or mucous membranes to reduce discomfort and pain (Chen et al., 2016).

The quality research of traditional Chinese medicine has been the focus and hot spot of the modernization research of traditional Chinese medicine. Some chemical components of traditional Chinese medicine will change during the storage process and the control of these components that are prone to qualitative and quantitative changes has become the main focus of the quality control of traditional Chinese medicine (Guo et al., 2023; Chen et al., 2023). The chemical composition of the Yanlishuang Pills is complex and easily volatile, and only the joint monitoring of multiple components can achieve quality control of the active ingredients. However, the existing quality control standards only have the content index of borneol and menthol by gas chromatography (requiring that each gram of pills contains not less than 30.0 mg of menthol and not less than 75.0 mg of borneol), which cannot fully control the quality of this compound preparation.

Traditional Chinese medicine fingerprinting is a multi-indicator quality control model for traditional Chinese medicines (Liu et al., 2023). The analysis of shared peaks can reflect the type and quantity of chemical components contained in the drug more comprehensively and thus the quality of the drug, and it is widely used in the quality evaluation of Chinese pharmaceutical preparations (Telloli et al., 2023; Wei et al., 2021). In recent years, GC-MS has been widely used to analyze volatile components (Pascoal et al., 2022; Garruti et al., 2021). Therefore, in this study, GC-MS was used to establish fingerprint of the Yanlishuang Pills and to determine the content of the main active ingredients camphor, menthone, borneol, and menthol, with the aim of more comprehensively ensuring the stable quality, batch-to-batch consistency, and safe and effective clinical use of this preparation by combining multi-indicator component content determination with fingerprint analysis.

2 Materials and methods

2.1 Chemicals and reagents

Yanlishuang Pills were manufactured by Guizhou Huangguoshu Lishuang Pharmaceutical Co (Batch numbers S1-S13:20220414001, 20220415001, 20220418001, 20220421001, 20220422001,20220425001, 20220427049, 20220428110, 20220505017, 20220506081, 20220509111, 20220510088, 20220511022). Methanol, anhydrous sodium sulfate (AR grade) were purchased from Chengdu Jinshan Chemical Reagent Co(Chengdu, China). Acetone (AR grade) was purchased from Chongqing Chuandong Chemical Reagent Co (Chongqing, China). Naphthalene (internal standard) (batch number:84679-1G, mass fraction of 99.9%) was purchased from Sigma-Aldrich Reagent Co(Shanghai, China). Camphor (batch number: 111749-201702, mass fraction of 99.9%), menthone (batch number: 111705-202106, mass fraction of 99.1%), borneol (batch number: 111688-201602, mass fraction of 99.6%), menthol (batch number: 110728-201707, mass fraction of 99.8%) were all purchased from the National Institute for Food and Drug Control (Beijing, China) for content determination.

2.2 Fingerprint

Instrument and equipment

Gas chromatography tandem mass spectrometry (model: GCMS-TQ8040NX, Shimadzu, Japan)

Gas chromatography conditions

We used a SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm, 0.25 μm) with a high-purity helium carrier gas (≥99.999%). The column flow rate was 1.0 mL/min, the injection port temperature was 250 °C, the injection volume was 1.0 μL. The split injection was used, and there was a split ratio of 30:1. The Program heating up conditions were an initial column temperature of 70 °C and maintained for 4 min, ramp-up to 90 °C at 1.5 °C/min and maintained at 90 °C for 5 min, ramp-up to 120 °C at 3 °C/min, ramp-up to 250 °C at 20 °C/min and maintained at 250 °C for 2 min until the analysis was completed.

Mass spectrometry conditions

The bombardment energy of the electron bombardment ion source was 70 eV, the ion source temperature was 200 °C, the interface temperature was 250 °C, the solvent delay time was 2 min, the mode was Q3 Scan, the mass scan range was 30-700 amu, detector voltage was 0.1 kV.

Preparation of sample

The Yanlishuang Pills were accurately weighed 0.15 g and then 10 mL of methanol was added, then weighed precisely again and extracted by sonication (power was 200 W, frequency was 40 kHz) for 10 min with an ultrasonic machine, let cool and then methanol was added to make up the weight loss, and anhydrous sodium sulfate was added to help dehydration, and finally filtered by 0.22 μm microporous filter membrane.

Validation of analytical methods

Precision

The samples (batch number was 20220425001) were prepared according to the method in "Preparation of sample", and the solutions were measured six times continuously according to the conditions in "Gas chromatography conditions" and "Mass spectrometry conditions". The peak number 9 with moderate retention time, good separation and maximum peak area were used as the reference peaks. The RSD of the relative retention time and the relative peak area of the main characteristic peaks were calculated for examining the consistency of the relative retention time and the relative peak area of the peaks.

Stability

The samples (batch number was 20220425001) were weighed and prepared according to the method in "Preparation of sample",

and then placed at room temperature for 0, 2, 4, 6, 8, 16 and 24 h, respectively, and measured according to the conditions in "Gas chromatography conditions" and "Mass spectrometry conditions". The RSD of the relative retention time and relative peak area of the main characteristic peaks were calculated using peak number 9 as the reference peak.

Repeatability

Six solutions (batch number was 20220425001) were prepared according to the method in "Preparation of sample", and measured according to the conditions in "Gas chromatography conditions" and "Mass spectrometry conditions", and the relative retention time and relative peak area of the main characteristic peaks were calculated using peak number 9 as the reference peak.

Establishment of the fingerprint

Thirteen batches of Yanlishuang Pills were prepared according to the method in "Preparation of sample" and measured according to the conditions in "Gas chromatography conditions" and "Mass spectrometry conditions". The fingerprints of the volatile components in the Yanlishuang Pills of different batches were detected by GC-MS. The peak number 9 was used as the reference peak to identify the shared characteristic peaks with stable retention time and peak area. The fingerprint data of the 13 batches of Yanlishuang Pills were imported into the software "Chromatographic Fingerprint Similarity Evaluation System for Traditional Chinese Medicine", version A, 2004, and a control fingerprint (R) was generated using the mean method with a time window width of 0.1. The fingerprint of the 13 batches of samples (S1-S13) were compared with the control fingerprint R to calculate the similarity, and the fingerprint of the 13 batches of samples and the control fingerprint R were fitted to obtain GC-MS superimposed fingerprint.

2.3 Determination of the content of camphor, menthone, borneol and menthol

GC-MS conditions

The conditions in "Gas chromatography conditions" and "Mass spectrometry conditions" were further optimized for better separation and shorter analysis time of the peaks for quantitative analysis in the fingerprint. The column flow rate was 0.78 mL/min. A split injection was used and the split ratio was 30:1. The Program heating up conditions were an initial column temperature of 75°C and maintained for 2 min, ramp-up to 110°C at 2.0°C/min, the analysis was completed in 19.50 min. The solvent delay time was 5 min, the mode was Q3 Scan, the mass scan range was 45-200 amu, detector voltage was 0 kV. Other conditions are the same as "Gas chromatography conditions" and "Mass spectrometry conditions".

Preparation of internal standard solution and sample

Weigh 80 mg of the internal standard naphthalene accurately, put it in a 100 mL brown volumetric flask, add methanol solution to dissolve and dilute to the scale, shake well. In this way, an internal standard solution of 800 ug/mL was prepared. The method for preparing the sample is the same as in "Preparation of sample" but with some differences. After weighing 0.15 g of the Yanlishuang Pills accurately, 8.75 mL of methanol and 1.25 mL of the internal standard solution were added and then extracted by sonication. The rest of the operations are the same.

Preparation of standard stock solution

Precisely weigh 25 mg of camphor standard, add it into 5 mL brown volumetric flask and dissolve it with methanol and dilute it to the scale, shake well, then 5 mg/mL of camphor standard stock solution was prepared. The same method was used to prepare 5 mg/mL of menthone standard stock solution, 20 mg/mL of borneol standard stock solution and 8 mg/mL of menthol standard stock solution.

Preparation of mixed standard solutions

Add 1, 1, 3 and 3 mL of each of camphor, menthone, borneol and menthol standard stock solution in "Preparation of standard stock solution" to a 10 mL brown volumetric flask, add methanol to dissolve and dilute to the scale, shake well, and prepare a mixed standard solution containing camphor at the concentration of 0.5 mg/mL, menthone at the concentration of 0.5 mg/mL, borneol at the concentration of 6.0 mg/mL and menthol at the concentration of 2.4 mg/mL.

Identification and designation of chromatographic peaks.

Prepare the test solution according to the method in "Preparation of internal standard solution and sample", add the internal standard solution in "Preparation of internal standard solution and sample" to the mixed standard solution in "Preparation of mixed standard solutions" and dilute it. The sample was injected and determined according to the conditions in "GC-MS conditions". The peaks were identified and by the combination of NIST standard mass spectrometry database search and standard comparison.

Preparation of series mixed standard solutions

Add 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 mL of the mixed standard solution in "Preparation of mixed standard solutions" to a 5 mL brown volumetric flask, respectively, and add 0.625 mL of internal standard solution in "Preparation of internal standard solution and sample", then add methanol and dilute to the scale, shake well.

Establishment of content determination procedure in SIM mode

Any one of the series of mixed standard solutions was determined in Scan mode according to the conditions in "GC-MS conditions", and the data obtained were used to create a program for content determination in SIM mode. The selected ion pairs were: z 95→110/67; menthol, m/z 81→71/95.

Validation of analytical methods

Linearity

The peak areas were determined by injecting 1 μL of the series mixed standard solutions according to the method in "Establishment of content determination procedure in SIM mode". Using the ratio of the peak area of each standard to the peak

area of the internal standard as the vertical coordinate (Y) and the ratio of the mass concentration as the horizontal coordinate (X), the standard curves of camphor, menthone, borneol and menthol were plotted, and the regression equations and linear ranges were obtained.

Precision

The test solution was prepared by extracting the Yanlishuang Pills (batch number was 20220425001) according to the method in "Preparation of internal standard solution and sample", and the peak areas of components were measured six times continuously according to the method in "Establishment of content determination procedure in SIM mode". Calculate the relative standard deviation (RSD) value of the ratio of the peak area of each component to the peak area of the internal standard.

Stability

The test solution was prepared by extracting the Yanlishuang Pills (batch number was 20220425001) according to the method in "Preparation of internal standard solution and sample". After 2, 4, 6, 8 and 16 h at room temperature, the peak areas were determined according to the method in "Establishment of content determination procedure in SIM mode". Calculate the RSD value of the ratio of the peak area of each component to the peak area of the internal standard.

Repeatability

A total of 6 test solutions of the Yanlishuang Pills was prepared (batch number was 20220425001) according to the method in "Preparation of internal standard solution and sample", and the peak areas of components were measured according to the method in "Establishment of content determination procedure in SIM mode". Calculate the RSD of each component content separately.

Recovery rate

Accurately weigh 0.075 g of the Yanlishuang Pills in which the content of each component has been determined and prepare the test solution according to the method in "Preparation of internal standard solution and sample" (batch number was 20220414001, the content of camphor was 6.551 mg/g, the content of menthone was 5.353 mg/g, the content of borneol was 89.244 mg/g,the content of menthol was 40.646 mg/g), added with the same content of camphor, menthone, borneol and menthol standard respectively, determined the peak area according to the method in "Establishment of content determination procedure in SIM mode", and calculated the recovery of each component.

Determination of the content of each component in the Yanlishuang Pills.

The test solutions were prepared according to the method in "Preparation of internal standard solution and sample" for 13 batches of Yanlishuang Pills. Three copies of each sample were prepared and determined according to the method in "Establishment of content determination procedure in SIM mode", then the contents of camphor, menthone, borneol and menthol in the samples were calculated separately.

3 Results

3.1 Validation of analytical methods of fingerprint

The results are shown in Table 1. In the precision test, the RSD of the relative retention time of each characteristic peak (RRT) was less than 0.1% (n = 6) and the RSD of the relative peak area (RPA) was less than 8% (n = 6). In the stability test, the RSD of RRT of each characteristic peak was less than 0.1% (n = 7) and the RSD of RPA was less than 8% (n = 7). In the repeatability test, the RSD of RRT of each characteristic peak was less than 0.1% (n = 6) and the RSD of RPA was less than 7% (n = 6). The similarity of the fingerprint was calculated by using the fingerprint of the first injection as a reference, and the similarity was 1.000. The results showed that the precision of the instrument was good, the test solution was basically stable within 24 h at room temperature, the method was reproducible and could meet the requirements of fingerprint analysis. The GC-MS total ion chromatogram of the Yanlishuang Pills is shown in Figure 1.

3.2 Establishment of the fingerprint

The control fingerprint (R) generated is shown in Figure 2. The similarities between the fingerprint of the 13 batches of samples (S1-S13) and R were calculated. The fingerprint of the 13 batches of samples and R were fitted to obtain the GC-MS superimposed fingerprint as shown in Figure 3. It was found that the similarity between the fingerprint of each batch of samples and R was 1.000, indicating that the volatile components of the

Table 1. Validation of analytical methods of fingerprint.

Peak Number	Precision (n = 6, RSD/%)		Stability (n = 7, RSD/%)		Repeatability (n = 6, RSD/%)	
	RPA	RRT	RPA	RRT	RPA	RRT
6	1.8	0.02	3.0	0.01	3.1	0.01
7	2.5	0.02	4.7	0.01	3.5	0.02
8	2.3	0.03	3.7	0.03	2.9	0.03
10	1.2	0.01	1.8	0.01	3.2	0.03
13	3.5	0.04	5.2	0.06	4.6	0.06
15	2.9	0.04	4.0	0.07	6.7	0.07
17	7.4	0.05	7.8	0.08	3.1	0.07

Yanlishuang Pills of different batches were basically same and the generated control fingerprint were well representative.

3.3 Identification and designation of chromatographic peaks

The total ion chromatograms of Yanlishuang Pills, mixed standard solutions and blank solvent are shown in Figure 4.

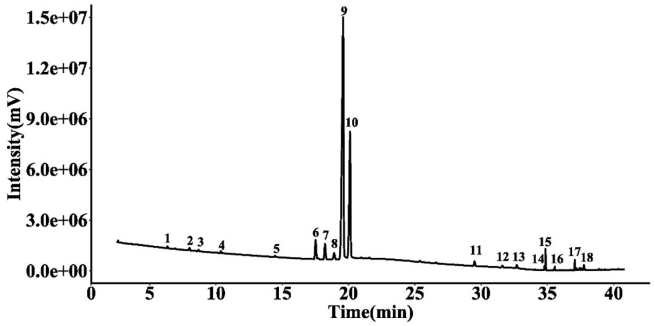

Figure 1. The GC-MS total ion chromatogram of the Yanlishuang Pills.

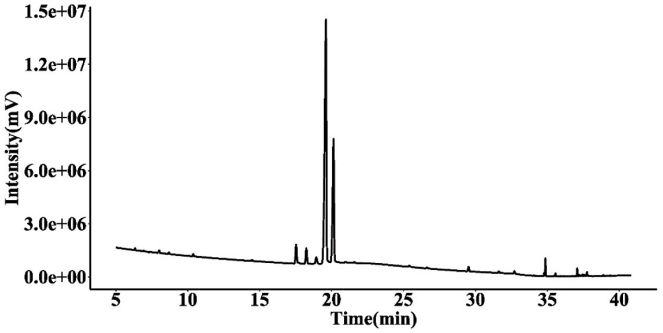

Figure 2. The GC-MS control fingerprint of the Yanlishuang Pills.

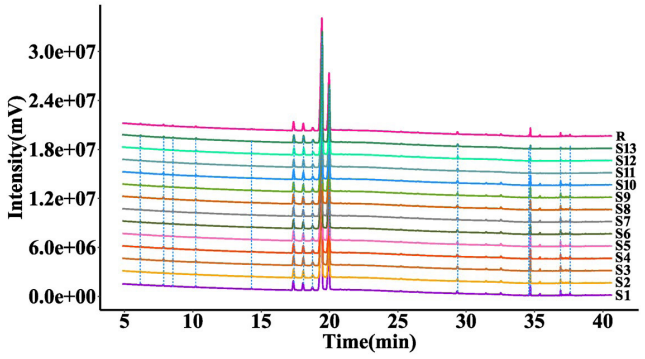

Figure 3. Overlay of GC-MS fingerprints of 13 batches of Yanlishuang Pills (S1-S13) and control fingerprints (R).

3.4 Validation of analytical methods of determination of the content

Linearity

The regression equations of the standard curves of camphor, menthone, borneol and menthol and their linear ranges are shown in Table 2. Camphor showed good linearity in the range of concentration of 25.13-150.78 μg/mL, menthone showed good linearity in the range of concentration of 28.77-172.62 μg/mL, borneol showed good linearity in the range of concentration of 299.70-1798.20 μg/mL, and menthone showed good linearity in the concentration range of 121.98-731.88 μg/mL, with linear correlation coefficients all greater than 0.9990.

Precision and stability and repeatability

The results are shown in Table 3. The RSDs of the ratios of the peak areas of camphor, menthone, borneol and menthol to the peak areas of the internal standards in the precision test were 2.3%, 2.0%, 2.7% and 2.4%, respectively, indicating good precision of the instrument. The RSDs of the ratios of the peak areas of camphor, menthone, borneol and menthol in the stability test were 2.1%, 2.4%, 2.8% and 2.6%, respectively, indicating that the stability of the components in the test solution was good at room temperature for 16 h. The average content of camphor in the repeatability test was 6.551 mg/g with RSD value of 1.4% (n = 6), the average content of menthone was 5.353 mg/g with RSD value of 2.2% (n = 6), the average content of borneol was 89.244 mg/g with RSD value of 2.9% (n = 6), the average content of menthol was 40.646 mg/g with RSD value of 2.4% (n = 6), indicating good method reproducibility.

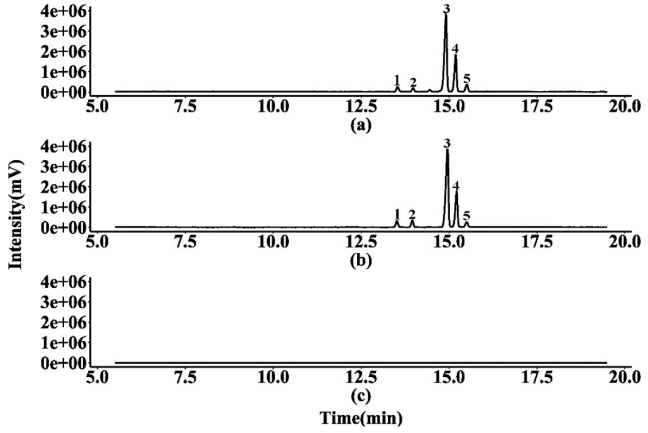

Figure 4. Total ion chromatogram of Yanlishuang Pills (a), mixed standard solutions (b), blank solvent (c) in Scan mode (Note: 1. camphor; 2. menthone; 3. borneol; 4. menthol; 5. naphthalene).

Table 2. Regression equation and linear ranges of camphor, menthone, borneol and menthol.

Component	Regression equations	r	Linear ranges (μg/mL)
Camphor	Y = 0.2389X + 0.008	0.9995	25.13-150.78
Menthone	Y = 0.1862X + 0.0095	0.9991	28.77-172.62
Borneol	Y = 0.7715X - 0.6088	0.9997	299.70-1798.20
Menthol	Y = 0.2409X - 0.0482	0.9997	121.98-731.88

Recovery rate

The results are shown in Table 4. The mean recovery rate of camphor in the Yanlishuang Pills were 102.02% with an RSD of 1.3%, the mean recovery rate of menthone were 96.10% with an RSD of 1.0%, the mean recovery rate of borneol were 102.71% with an RSD of 1.3%, and the mean recovery rate of menthol were 102.58% with an RSD of 1.1%. It indicates that the method recovery was good.

3.5 Determination of the content of each component in the Yanlishuang Pills

The results of the determination of the contents of camphor, menthone, borneol and menthol in 13 batches of Yanlishuang Pills are shown in Table 5.The results indicate that there is some variation in the contents of camphor, menthone, borneol and menthol in this Pills, with the camphor content fluctuating between 5.6025-8.3662 mg/g, menthone content fluctuating between 4.7871-5.8936 mg/g, borneol content fluctuating between 88.0034-133.0969 mg/g,and menthol content fluctuating between 40.2017-61.9466 mg/g. The contents of menthol and borneol in the 13 batches of samples were all in compliance with the provisions of the 2020 edition of the Pharmacopoeia that the content of menthol should not be less than 30.0 mg and the content of borneol should not be less than 75.0 mg per g of Yanlishuang Pills.

4 Discussion

4.1 Preparation of the test solution and selection of internal standard.

In this experiment, methanol, anhydrous ethanol, acetonitrile, ethyl acetate, acetone, n-hexane and dichloromethane were investigated as extraction solvents for the test solution. The results showed that the total ion chromatograms of several extracts had the same number of peaks and no significant difference in peak separation. The methanol extract was transparent and stable, with higher extraction efficiency than other solvents, and the total

Table 3. Precision and stability and repeatability of camphor, menthone, borneol and menthol.

Component	Precision (n = 6, RSD/%)	Stability (n = 6, RSD/%)	Repeatability	
			Average content (mg/g)	n = 6, RSD (%)
Camphor	2.3	2.1	6.551	1.4
Menthone	2.0	2.4	5.353	2.2
Borneol	2.7	2.8	89.244	2.9
Menthol	2.4	2.6	40.646	2.4

Table 4. Recovery rate of camphor, menthone, borneol and menthol.

Component	Sample weight (g)	Component content (mg)	Addition amount (mg)	Measured amount (mg)	Recovery rate (%)	Mean recovery rate(%)	RSD (%) (n = 6)
Camphor	0.0721	0.4724	0.4740	0.9496	100.68	102.02	1.3
	0.0707	0.4631	0.4624	0.9321	101.42		
	0.0717	0.4698	0.4649	0.9523	103.80		
	0.0725	0.4749	0.4729	0.9616	102.92		
	0.0720	0.4717	0.4750	0.9541	101.58		
	0.0728	0.4770	0.4785	0.9572	100.36		
Menthone	0.0721	0.3860	0.3510	0.7327	98.79	96.10	1.0
	0.0707	0.3784	0.3544	0.7212	96.73		
	0.0717	0.3838	0.3671	0.7331	95.14		
	0.0725	0.3880	0.3815	0.7552	96.25		
	0.0720	0.3854	0.3660	0.7334	95.11		
	0.0728	0.3897	0.3711	0.7508	97.28		
Borneol	0.0721	6.4354	6.6916	13.4351	104.60	102.71	1.3
	0.0707	6.3087	6.5540	13.0065	102.20		
	0.0717	6.3997	6.6175	13.3375	104.84		
	0.0725	6.4693	6.5857	13.1480	101.41		
	0.0720	6.4256	6.6599	13.2134	101.92		
	0.0728	6.4979	6.6069	13.3154	103.19		
Menthol	0.0721	2.9310	3.1339	6.1260	101.95	102.58	1.1
	0.0707	2.8732	2.9192	5.8993	103.66		
	0.0717	2.9147	3.0967	6.1306	103.85		
	0.0725	2.9464	3.1087	6.0896	101.11		
	0.0720	2.9265	3.0566	6.0574	102.43		
	0.0728	2.9594	3.1425	6.1602	101.86		

Table 5. Determination of the content of camphor, menthone, borneol and menthol.

Batches	Camphor (mg/g)	Menthone (mg/g)	Borneol (mg/g)	Menthol (mg/g)
20220414001	6.5265	5.3201	88.0034	40.2017
20220415001	6.7980	5.4783	109.9322	49.6444
20220418001	6.0287	4.8268	95.8047	40.8219
20220421001	7.4117	5.5873	120.4817	54.2017
20220422001	6.9928	5.1047	108.6183	48.3590
20220425001	6.7373	5.0023	101.4465	45.5393
20220427049	6.6385	4.7871	97.2396	43.1160
20220428110	8.4024	5.8936	133.0969	60.6985
20220505017	8.0242	5.5661	130.8904	61.1590
20220506081	8.3662	5.4843	132.4455	61.9466
20220509111	5.7714	5.3027	120.5725	52.6810
20220510088	6.4519	5.2764	129.3254	59.5073
20220511022	5.6025	5.1046	122.2064	54.3413

ion chromatograms had better peak shapes, so methanol was chosen as the extraction solvent. When naphthalene was selected as the internal standard, the peaks of the internal standard were completely separated from the peaks of all the components in the sample, and the retention times of the peaks of the target components were close to each other.

4.2 Selection of GC-MS conditions

In this experiment, three column flow rates of 0.78, 1.0 and 1.5 mL/min were investigated, and it was found that the retention time of the peaks advanced with the increase of the flow rate, and the separation of the peaks did not change significantly, the signal was best at the flow rate of 0.78 mL/min, so 0.78 mL/min was selected as the column flow rate. Then three split ratios of 10:1, 30:1, and 50:1 were examined, and it was found that the detector saturated at a split ratio of 10:1, and 30:1 had a better signal than 50:1, so a split ratio of 30:1 was chosen. Since the samples are traditional Chinese medicine with complex composition, the program heating up was chosen for the investigation, while making the samples peak in a short time as possible. Firstly, 70, 75 and 80°C were examined as the initial temperatures of the program heating up, and it was found that as the initial temperature increased, the separation between borneol and menthol was decreased, while the separation between menthol and internal standard naphthalene was increased. Nextly, 1.0, 1.5, and 2.0°C/min were examined as the warming rates for the program heating up. It was found that, as the same results as the initial temperature examination, the separation between borneol and menthol decreased and the separation between menthol and internal standard naphthalene increased with the increase of the warming rates. Considered in an integrated manner, 75°C was chosen as the initial temperature of the program heating up, and 1.5°C/min was chosen as the warming rates, and the separation between borneol and menthol and between menthol and internal standard naphthalene met the analytical requirements.

5 Conclusion

In summary, this study established a fingerprint of the Yanlishuang Pills by GC-MS, labeled a total of 18 shared peaks, identified four characteristic peaks reflecting their substance bases and determined the contents, combined fuzzy identification with precise quantification, and provided a systematic and rapid method to evaluate the quality of the Pills. This method is simple, reproducible and specific, and has the advantages of time saving and high sensitivity compared with the current gas chromatography analysis (You et al., 2016), providing a scientific basis for the elucidation of the substance basis of the drug effect and quality control based on volatile components.

Conflict of interest

The authors declare no conflict of interest.

Data availability

The data generated and analyzed in this study are available from the corresponding author on request.

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