# Analytical Methods

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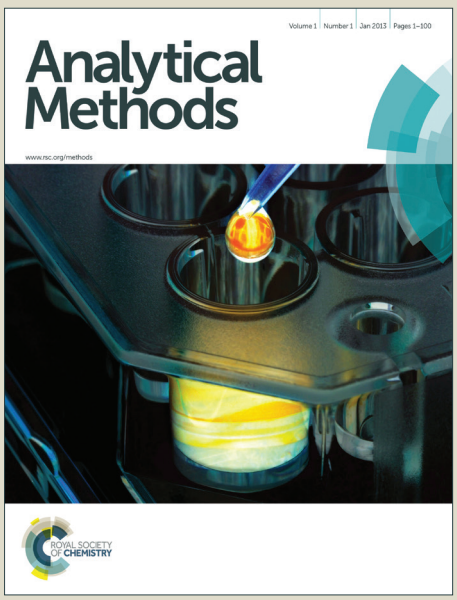





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# Simultaneous determination of six volatile components in Longhu Rendan pills using gas chromatography coupled with triple quadrupole mass spectrometry

Tian-Ming Wang[a], Li-Qin Ding[b], Yi-qun Jia[c], Hua-Jia Jin[b], Rong Shi[a], Li Zhu[b], Yue-Ming Ma[a]*

**ABSTRACT:** Longhu Rendan pills (LRPs) are one of the most widely used traditional Chinese over-the-counter medicines for the prevention and treatment of heat stroke and motion sickness. A rapid and effective GC-MS/MS method for the determination of six volatile active constituents including menthol, borneol, isoborneol, anethole, eugenol and acetyl eugenol in LRPs was developed and validated. The six compounds were separated within 8 min using a VF-WAXms capillary column, and the analytes were quantified using GC-MS/MS in multiple reaction monitoring mode. Good linearity was achieved ($r > 0.9973$). Variations in the intra- and inter-day precisions of all the analytes were below 4.32%, and the accuracy (92.44% to 103.64%) was evaluated using a recovery test. The method successfully determined six volatile compounds in three batches of LRP samples. The present study offers a highly accurate, sensitive and reliable method for the determination of six volatile active constituents in LRPs to promote the quality control investigation of LRPs.

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## 1 Introduction

Longhu Rendan pills (LRPs), which contain *Mentholum, Borneolum syntheticum, Fructus Anisi Stellati, Flos Caryophylli, Cortex Cinnamomi*, *Radix Aucklandiae*, *Fructus Piperis*, *Fructus Amomi*, *Rhizoma Zingiberis*, *Glycytthizae* and *Catechu Radix*, are one of the most widely used traditional Chinese over-the-counter medicines for the prevention and treatment of heat stroke and motion sickness. In 1911, Chujiu Huang created LRPs based on the ancient prescription "Zhuge marching powder" in Shanghai.[1] LRPs are authorised for sale by the State Food and Drug Administration (SFDA) of China (No. Z20025168), and LRPs annual sales volume has exceeded $16 million since 2011. Recent experimental studies have revealed that LRPs produce significant anti-motion sickness, anti-heat stroke and peripheral antiemetic effects in rats.[2]

In a previous study, we have developed an ultra-high performance liquid chromatography -mass spectrometry (UHPLC-MS) method for simultaneous determination of 14 major components in LRPs,[3] but LRPs also contain some volatile compounds that exhibit very important pharmacological effects. Menthol displays anti-diarrheal and antiperistaltic activities.[4] *Borneolum syntheticum*, which is a mixture of optically inactive borneol and isoborneol, shows anticoagulant,[5] neuroprotective,[6, 7] analgesic[8] and vasorelaxant[9] activities as well as inhibits acetylcholine-mediated effects.[10] In addition, borneol can easily penetrate the blood–brain barrier as well as enhance the oral bioavailability and distribution of drugs in the brain tissue.[11, 12] Anethole has antimicrobial[13] and antioxidant activities.[14] Eugenol and acetyl eugenol have antiplatelet aggregating,[15-17] antioxidant and antifungal effects.[18] Moreover, eugenol also has antipyretic,[19] neuroprotective,[20] hepatoprotective[21] and analgesic effects.[22] Hence, these volatile compounds may contribute to the effects of LRPs on the prevention and treatment of heat stroke and motion sickness. Determining the concentrations of the volatile components in LRPs could be beneficial to ensure the reliability and repeatability of treatment.

Menthol and borneol detected using gas chromatography (GC) have been chosen as "marked compounds" for the quality control of LRPs by the SFDA. GC uses a mixture of borneol and isoborneol as the reference standard, which is less accurate than separate reference standards, and requires a long time to analyse (25 min).[23] However, quantitative analysis of one or two volatile components in herbal medicine formulae may not be adequate. Therefore, more comprehensive and accurate determination of volatile components in LRPs is necessary to ensure the reliability

and repeatability of quality assessments.

In the present study, we developed a rapid, accurate, sensitive and reliable method using gas chromatography coupled with triple quadrupole mass spectrometry (GC-MS/MS) for the determination of the following multi-active volatile compounds in LRPs: (**1**) menthol, (**2**) isoborneol, (**3**) borneol, (**4**) anethole, (**5**) eugenol and (**6**) acetyl eugenol (Fig. 1). The six volatile compounds were successfully determined in three batches of LRP samples.

## 2 Experimental

### 2.1 Reagents and chemicals

Analytical reference standards of menthol, borneol, isoborneol, eugenol and naphthalene were purchased from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetyl eugenol and anethol were obtained from Nanjing Spring & Autumn Biological Engineering Co., Ltd. (Nanjing, China). The purities of all reference compounds were greater than 98%. LRPs (Chinese SFDA ratification No. Z20025168) were provided by Shanghai Zhonghua Pharmaceutical Co., Ltd. (Shanghai, China). Ethyl acetate, ethanol and n-hexane were obtained from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China). Ultra-pure water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

### 2.2 Preparation of standard solutions

The reference standards were accurately weighed and dissolved in ethyl acetate to prepare stock solutions. Naphthalene was chosen as the internal standard (IS). All standards were completely dissolved in the mixed standard working solution. Standard working solutions were obtained by diluting the stock solutions with ethyl acetate. A mixed standard working solution was prepared, and all stock standard solutions were stored at 4 ℃ in the refrigerator.

### 2.3 Chromatography and GC-MS/MS conditions

Analyses were performed using an Agilent 7890A GC interfaced to a Triple Quadrupole Mass Spectrometer Agilent 7000B (Agilent Technologies, USA) equipped with an autosampler (CTC, Switzerland). Chromatographic separation was performed on a VF-WAXms capillary column (30

m×0.25 mm ID; Agilent Technologies, USA) coated with 100% polyethylene glycol (0.25 μm film thickness). The following GC temperature program was used: 80 ℃ (0 min to 1 min), 80 ℃ to 245 ℃ (1 min to 7.6 min at 25 ℃/min) and 245 ℃ (7.6 min to 8.6 min). Solvent delay was set to 4 min, and the injection volume was set to 2 μL in splitless mode. Mass spectrometry was operated in electron impact ionisation (EI) MS/MS mode at 70 eV using multiple reaction monitoring (MRM) for all the analytes and the IS. Helium and nitrogen were used as collision cell gases at 2.25 and 1.5 mL/min, respectively, and helium was used as the carrier gas at a constant flow rate of 2.5 mL/min. The temperatures for the transfer line and the ion source were both set to 250 ℃. Using this method, the overall run time was approximately 8.6 min. The full list of the analytes with their time segments, respective retention times, monitoring ion transition, dwell times, collision energies and gains are shown in Table 1.

## 2.4 Sample preparation

The powdered LRPs (30 mg) was extracted using 6 mL ethyl acetate in an ultrasonic bath for 30 min. The extracted solution was centrifuged at 12,000 rev/min for 10 min. IS was added into the supernatant, and the mixture was stored at 4 ℃ in a refrigerator. A 2 μL aliquot of the supernatant was injected into the GC-MS/MS system for analysis. Each sample was analysed five times.

## 2.5 Method validation

### 2.5.1 Linearity, limit of detection (LOD) and limit of quantification (LOQ)

An IS method was utilised for quantification. A mixed standard working solution containing the six analytes was diluted to the appropriate concentration range and was added with IS to establish calibration curves. The linearity of the relationship between the concentration (x) and the peak area ratio of analyte/IS was analysed using weighted least square regression. The calibration curve of each compound was constructed using at least five concentrations. LOD and LOQ were determined as signal-to-noise ratios of 3 and 10, respectively.

### 2.5.2 Precision and accuracy

The intra- and inter-day variations at high, medium and low levels were chosen to determine the precision of the developed method. Intra-day variations within 1 day and inter-day variations for three consecutive days were assessed by repeatedly analysing the samples (n = 5). The recovery at all levels was used to further evaluate the accuracy of the method. Accurate amounts of six standards were added to the LRPs sample, and then, it was processed and analysed. The amount of each analyte was calculated using the corresponding calibration curve. The recovery of each analyte was calculated according to the following equation: Recovery (%) = ($Amount_{detected}$ − $Amount_{original}$)/$Amount_{spiked}$ × 100).

### 2.5.3 Repeatability and stability

To investigate the repeatability of the method, five different solutions of LRPs were analysed, and the RSD was considered as a measure of reproducibility. The same sample solution was stored at 4 °C and analysed at 0, 4, 6, 8 and 12 h to investigate the stability of the solution.

## 3 Results and discussion

### 3.1 Optimisation of the GC-MS/MS conditions

The MS/MS method development started from the analysis of the standard solution in full-scan mode to select the appropriate precursor ions for all the analytes. The abundantly generated fragment ions in the full-scan mode of menthol, borneol and isoborneol were m/z 71, m/z 95 and m/z 95, respectively. However, the molecular ions of menthol, borneol and isoborneol (m/z 156, 154 and 154, respectively) are present at a low tendency. A series of collision energies from 2 V to 45 V were also investigated in the collision cell. The product ions of menthol, borneol and isoborneol are also the predominant ions at m/z 71, m/z 95 and m/z 95, respectively. Hence, the precursors to the product ions of menthol, borneol and isoborneol are the same ions. Ions of anethole, eugenol and acetyl eugenol were at m/z 148, and m/z 164, respectively. These ions from the full-scan mass spectrum were selected as the precursor ions on the basis of highest abundance. The most intense ion of the IS naphthalene is its molecular ion at m/z 128, rather than the fragment ions. Subsequently, collision energies were tested using the selected precursor ions to obtain characteristic product ions. Different response analytes in LRPs showed similar responses

in one chromatogram by adjusting the different gain values. The optimised MS/MS parameter values are shown in Table 1. The initial temperature of the column oven was optimised to obtain good separation. Fig. 2 shows that the chromatographic peaks of menthol ($t_R$, 4.943 min), borneol (5.290 min), isoborneol (5.110 min) and naphthalene (5.579 min) are completely separated and evenly shaped under the optimal column oven heating rate of 25 ℃/min. The MRM total ion chromatograms are shown in Fig. 2, and the MRM extracted ion chromatograms of the reference compounds are shown in Fig. 3.

### 3.2 Sample extraction optimisation

The extraction solvents (ethyl acetate, ethanol and n-hexane), solvent volumes (3, 6 and 9 mL), and extraction times (10, 20, 30 and 60 min) were investigated to determine the best extraction efficiency. Optimal extraction was achieved with 30 mg of powdered sample extracted with 6 mL of ethyl acetate in an ultrasonic bath for 30 min.

### 3.3 Method validation

The regression equations, correlation coefficients and linear ranges, as well as LOD and LOQ values, of the six analytes are shown in Table 2. All calibration curves exhibit good linearity ($r$ > 0.9973) between the peak area ratio and the concentration. The precision of the methods are shown in Table 3. The precision of the intra- and inter-day variation for the detection levels of the investigated compounds is less than 4.32%. Table 4 lists the mean recoveries (92.44% to 103.64%) of the six analytes, with RSD values < 4.10%. The RSDs of the repeatability test were not over 1.2% for all analyses. When the solution was stored at 4 °C, the 6 compounds were found to be stable for 12 h (RSD < 4.30%). The results indicate that the established method was sensitive, satisfactory, accurate and reliable for the quantification of the volatile constituents of LRPs.

### 3.4 Sample analysis

The newly developed analytical method was applied to determine the six volatile compounds in three batches of LRP samples. A summary of information on the six volatile compounds is listed in Table 5. The results show that the content levels of the constituents in the three sample batches

are relatively stable. Among the six compounds, menthol (**1**) and borneol (**3**) exhibited the highest concentrations, followed by isoborneol (**2**). The amount of menthol was 21.67 ± 2.06 mg/g, and the combined level of borneol and isoborneol was 15.64 ± 0.21 mg/g, which meets the quality standard of no less than 14 mg/g and 10 mg/g in LRPs according to SFDA, respectively. However, the result also shows that the amount of anethole, eugenol and acetyl eugenol ranged from 1.38 mg/g to 1.45 mg/g, 1.23 mg/g to 1.31 mg/g and 0.10 mg/g to 0.12 mg/g, respectively. The results indicate that determining the amounts of anethole, eugenol and acetyl eugeonol in LRPs may also be necessary for more comprehensive quality assessments, because these ingredients may also exhibit pharmacological effects.[13-18] The capability of this method to analyse more components could improve the quality assessment of LRPs.

Though the well-established UHPLC-MS method may be used to determine the 14 major components of LRPs,[3] the method can not determine volatile components because of the limitation of LC-MS. Therefore, in this study, the proposed method for the simultaneous determination of six volatile compounds provides a basis for reliable quality control of volatile components, and promote a more comprehensive quality control study of LRPs.

## 4 Conclusion

A quantitative method to determine the six major volatile components in LRPs was established using GC-MS/MS. The proposed method showed high specificity and saving time. The method was used to successfully quantify the six volatile components from three batches of LRP samples, and it has demonstrated that the stability of the six target compounds. Moreover, the satisfactory results demonstrated that the proposed method is a reliable and sensitive quality control method of volatile components for LRPs.

## Acknowledgements

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**References**

1. H. Y. Zeng, *Shanghai Tycoon-Chujiu Huang*, People's Literature Publishing House, Beijing, 2004, p. 122.

2. X. H. Li, W. Pan, J. H. Jin, J. L. Du, J. Li and D. F. Song, *Pharmacol. Clin. Chin. Mat. Med*, 2009, 25, 61-62.
3. T. M. Wang, L. Q. Ding, J. H. Jin, R. Shi, J. Zhong, C. Gao, Q. Wang, Z. Li and Y. M. Ma, 2014, DOI: 10.1039/c3ay42152j, 272-278.
4. A. L. Rozza, C. A. Hiruma-Lima, R. K. Takahira, C. R. Padovani and C. H. Pellizzon, *Chemico-biological interactions*, 2013, 206, 272-278.
5. Y. H. Li, X. P. Sun, Y. Q. Zhang and N. S. Wang, *Am. J. Chinese. Med.*, 2008, 36, 719-727.
6. L. L. Tian, Z. Zhou, Q. Zhang, Y. N. Sun, C. R. Li, C. H. Cheng, Z. Y. Zhong and S. Q. Wang, *Cell. Physiol. Biochem.*, 2007, 20, 1019-1032.
7. R. Liu, L. Zhang, X. Lan, L. Li, T. T. Zhang, J. H. Sun and G. H. Du, *Neuroscience*, 2011, 176, 408-419.
8. R. E. Granger, E. L. Campbell and G. A. R. Johnston, *Biochem. Pharmacol.*, 2005, 69, 1101-1111.
9. J. C. Silva-Filho, N. N. P. M. Oliveira, D. D. R. Arcanjo, L. J. Quintans, S. C. H. Cavalcanti, M. R. V. Santos, R. D. C. M. Oliveira and A. P. Oliveira, *Basic. Clin. Pharmacol.*, 2012, 110, 171-177.
10. T. J. Park, Y. S. Park, T. G. Lee, H. Ha and K. T. Kim, *Biochem. Pharmacol.*, 2003, 65, 83-90.
11. B. Yu, M. Ruan, Y. Sun, X. B. Cui, Y. Yu, L. L. Wang and T. H. Fang, *Neural. Regen. Res.*, 2011, 6, 1876-1882.
12. Z. Cai, S. X. Hou, Y. B. Li, B. B. Zhao, Z. X. Yang, S. G. Xu and J. X. Pu, *J. Drug Targeting.*, 2008, 16, 178-184.
13. P. Pillai and K. Ramaswamy, *J. Food. Sci. Tech. Mys.*, 2012, 49, 228-233.
14. G. A. Naderi, S. Asgary, M. Ani, N. Sarraf-Zadegan and M. R. Safari, *Mol. Cell. Biochem.*, 2004, 267, 59-66.
15. S. A. Saeed, R. U. Simjee, G. Shamim and A. H. Gilani, *Phytomedicine*, 1995, 2, 23-28.
16. S. J. Chen, M. H. Wang and I. J. Chen, *Gen Pharmacol*, 1996, 27, 629-633.
17. K. C. Srivastava and N. Malhotra, *Prostaglandins, Leukotrienes Essent. Fatty Acids*, 1991, 42, 73-81.
18. K. Chaieb, T. Zmantar, R. Ksouri, H. Hajlaoui, K. Mahdouani, C. Abdelly and A. Bakhrouf, *Mycoses*, 2007, 50, 403-406.
19. J. Feng and J. M. Lipton, *Neuropharmacology*, 1987, 26, 1775-1778.
20. S. N. Prasad and Muralidhara, *Neurochem. Res.*, 2013, 38, 330-345.
21. E. Nagababu, B. Sesikeran and N. Lakshmaiah, *Free Radic Res.*, 1995, 23, 617-627.
22. S. A. Guenette, A. Ross, J. F. Marier, F. Beaudry and P. Vachon, *Eur. J. Pharmacol.*, 2007, 562, 60-67.
23. X. H. Mao, Z. Y. Wu and J. Shen, *Chin. Pharm. J.*, 2005, 1263-1266.

**Table 1** Instrument method for the GC–MS/MS analysis for the six components and IS

| Compound | Time segments | RT (min) | Precursor | Product | Dwell (ms) | CE(V) | Gain |
|---|---|---|---|---|---|---|---|
| Menthol | 4.80 | 4.943 | 71.0 | 71.0 | 100 | 2 | 5 |
| Isoborneol | 5.00 | 5.110 | 95.0 | 95.0 | 100 | 3 | 5 |
| Borneol | 5.00 | 5.290 | 95.0 | 95.0 | 100 | 3 | 5 |
| Anethole | 5.80 | 5.940 | 148.0 | 117.0 | 100 | 4 | 70 |
| Eugenol | 7.40 | 7.462 | 164.0 | 149.0 | 100 | 5 | 70 |
| Acetyl eugenol | 7.70 | 7.818 | 164.0 | 149.0 | 100 | 2 | 70 |
| Naphthalene (IS) | 5.45 | 5.579 | 128.0 | 102.0 | 100 | 25 | 5 |

**Table 2** Calibration curves, LODs and LOQs of the six components

| Compound | Calibration curve | r | Linear range (μg/mL) | LOQ (ng/mL) | LOD (ng/mL) |
|---|---|---|---|---|---|
| Menthol | *Y* =0.0507+1.1872*X | 0.9973 | 15.00 - 240.00 | 300.00 | 90.91 |
| Isoborneol | *Y* = 0.0193+4.1578*X | 0.9996 | 3.13 - 50.00 | 62.50 | 18.94 |
| Borneol | *Y* = 0.0413+5.9509*X | 0.9995 | 3.75 - 60.00 | 75.00 | 22.73 |
| Anethole | *Y* = -0.0003+0.3985*X | 0.9991 | 0.63 - 10.00 | 31.25 | 9.47 |
| Eugenol | *Y* = -0.0070+5.4679*X | 0.9993 | 0.75 - 12.00 | 15.00 | 4.55 |
| Acetyl eugenol | *Y* =-0.0029+8.9213*X | 0.9996 | 0.19 - 3.00 | 3.75 | 1.14 |

**Table 3** Intra- and inter-day variability for the assay of the six components

| Compound | Concentration (μg/ml) | Intra-day (n=5) Mean (μg/ml) | Intra-day (n=5) RSD (%) | Inter-day (n=5) Mean (μg/ml) | Inter-day (n=5) RSD (%) |
|---|---|---|---|---|---|
| Menthol | 15.00 | 13.74 ± 0.18 | 1.33 | 13.54 ± 0.28 | 2.06 |
| | 60.00 | 63.02 ± 0.98 | 1.56 | 65.39 ± 2.26 | 3.45 |
| | 240.00 | 234.52 ± 10.13 | 4.32 | 232.93 ± 7.54 | 3.24 |
| Isoborneol | 3.13 | 3.16 ± 0.02 | 0.67 | 3.13 ± 0.06 | 1.94 |
| | 12.50 | 12.39 ± 0.33 | 2.69 | 12.54 ± 0.37 | 2.94 |
| | 50.00 | 50.24 ± 1.11 | 2.21 | 50.05 ± 1.51 | 3.02 |
| Borneol | 3.75 | 3.76 ± 0.03 | 0.88 | 3.71 ± 0.08 | 2.14 |
| | 15.00 | 15.04 ± 0.36 | 2.40 | 15.30 ± 0.45 | 2.97 |
| | 60.00 | 60.05 ± 1.79 | 2.98 | 59.79 ± 1.93 | 3.23 |
| Anethole | 0.63 | 0.65 ± 0.01 | 1.10 | 0.66 ± 0.02 | 2.76 |
| | 2.50 | 2.41 ± 0.06 | 2.61 | 2.38 ± 0.09 | 3.80 |
| | 10.00 | 10.12 ± 0.29 | 2.85 | 10.15 ± 0.40 | 3.90 |
| Eugenol | 0.75 | 0.82 ± 0.01 | 0.74 | 0.82 ± 0.02 | 1.89 |
| | 3.00 | 2.98 ± 0.05 | 1.69 | 3.00 ± 0.09 | 2.91 |
| | 12.00 | 12.68 ± 0.16 | 1.26 | 12.66 ± 0.42 | 3.35 |
| Acetyl eugenol | 0.19 | 0.19 ± 0.00 | 2.16 | 0.19 ± 0.00 | 2.46 |
| | 0.75 | 0.73 ± 0.01 | 1.46 | 0.73 ± 0.02 | 2.93 |
| | 3.00 | 3.03 ± 0.05 | 1.55 | 3.03 ± 0.10 | 3.27 |

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294 **Table 4** Accuracy and reproducibility levels of the six components of LRPs

| Compound | Accuracy (n=3) | | | | Reproducibility (n=5) | | | |
|---|---|---|---|---|---|---|---|---|
| | Recovery (%) | | | RSD (%) | Mean (μg/ml) | | | RSD (%) |
| Menthol | 103.64 | ± | 3.32 | 3.20 | 113.68 | ± | 0.53 | 0.47 |
| Isoborneol | 98.98 | ± | 3.34 | 3.37 | 28.87 | ± | 0.05 | 0.16 |
| Borneol | 101.69 | ± | 3.35 | 3.29 | 48.94 | ± | 0.17 | 0.35 |
| Anethole | 97.63 | ± | 4.00 | 4.10 | 2.21 | ± | 0.03 | 1.2 |
| Eugenol | 100.26 | ± | 2.89 | 2.89 | 5.76 | ± | 0.03 | 0.55 |
| Acetyl eugenol | 92.44 | ± | 2.44 | 2.64 | 1.05 | ± | 0.00 | 0.44 |

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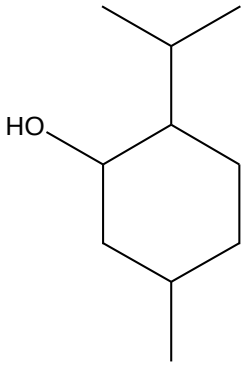
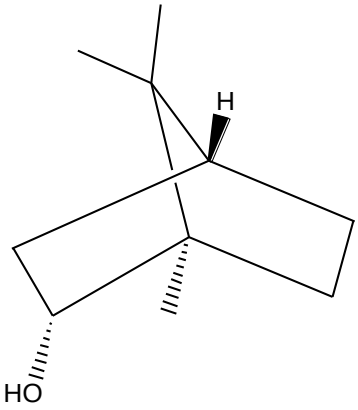
**Table 5** Contents of the six components in three batches of LRPs

| Compound | Content (mg/g) | | |
|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 |
| Menthol | 19.298 ± 1.025 | 23.004 ± 0.173 | 22.709 ± 0.226 |
| Isoborneol | 5.556 ± 0.200 | 5.949 ± 0.037 | 5.702 ± 0.076 |
| Borneol | 10.138 ± 0.729 | 9.881 ± 0.044 | 9.717 ± 0.107 |
| Anethole | 1.376 ± 0.126 | 1.425 ± 0.013 | 1.445 ± 0.020 |
| Eugenol | 1.309 ± 0.027 | 1.230 ± 0.012 | 1.293 ± 0.019 |
| Acetyl eugenol | 0.100 ± 0.009 | 0.104 ± 0.001 | 0.118 ± 0.002 |




1.Menthol

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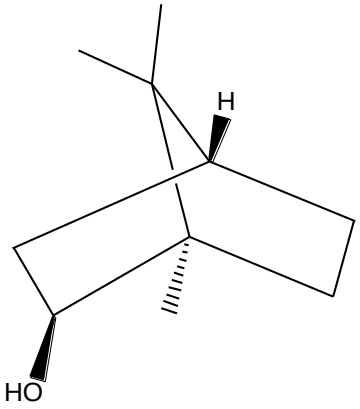
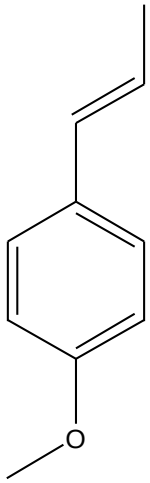
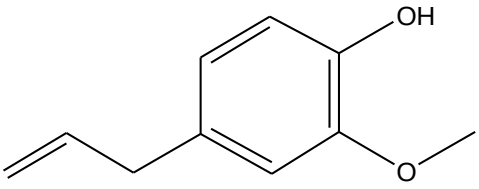


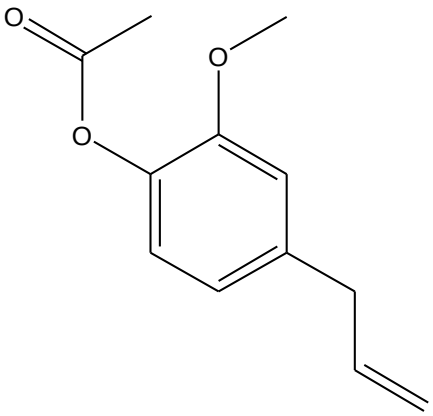

3.Borneol



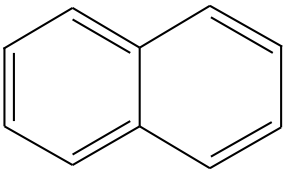

4.Anethole




5.Eugenol




6.Acetyl eugenol



7.Naphthalene

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314 **Fig. 1** Structures of the six components and naphthalene

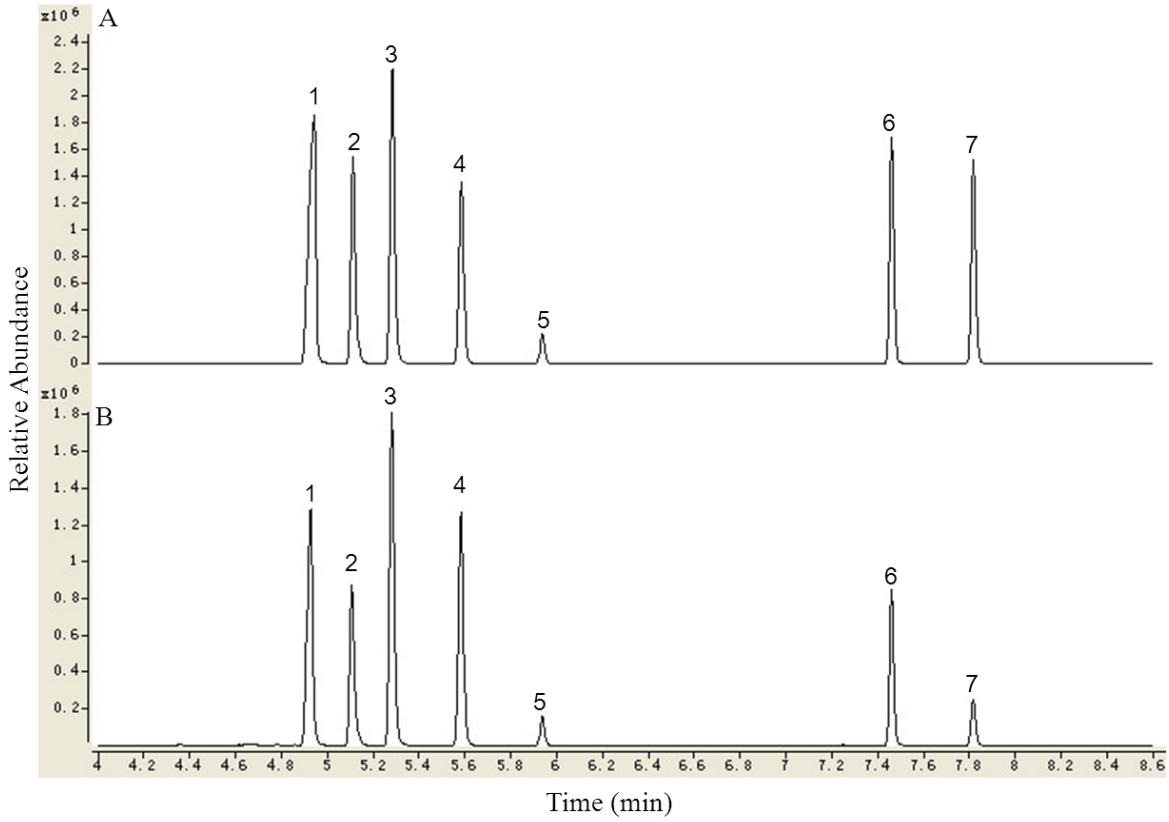


**Fig. 2** MRM total ion chromatograms of (A) reference standards and (B) LRPs sample: (1) menthol, (2) isoborneol, (3) borneol, (4) naphthalene (IS), (5) anethole, (6) eugenol and (7) acetyl eugenol

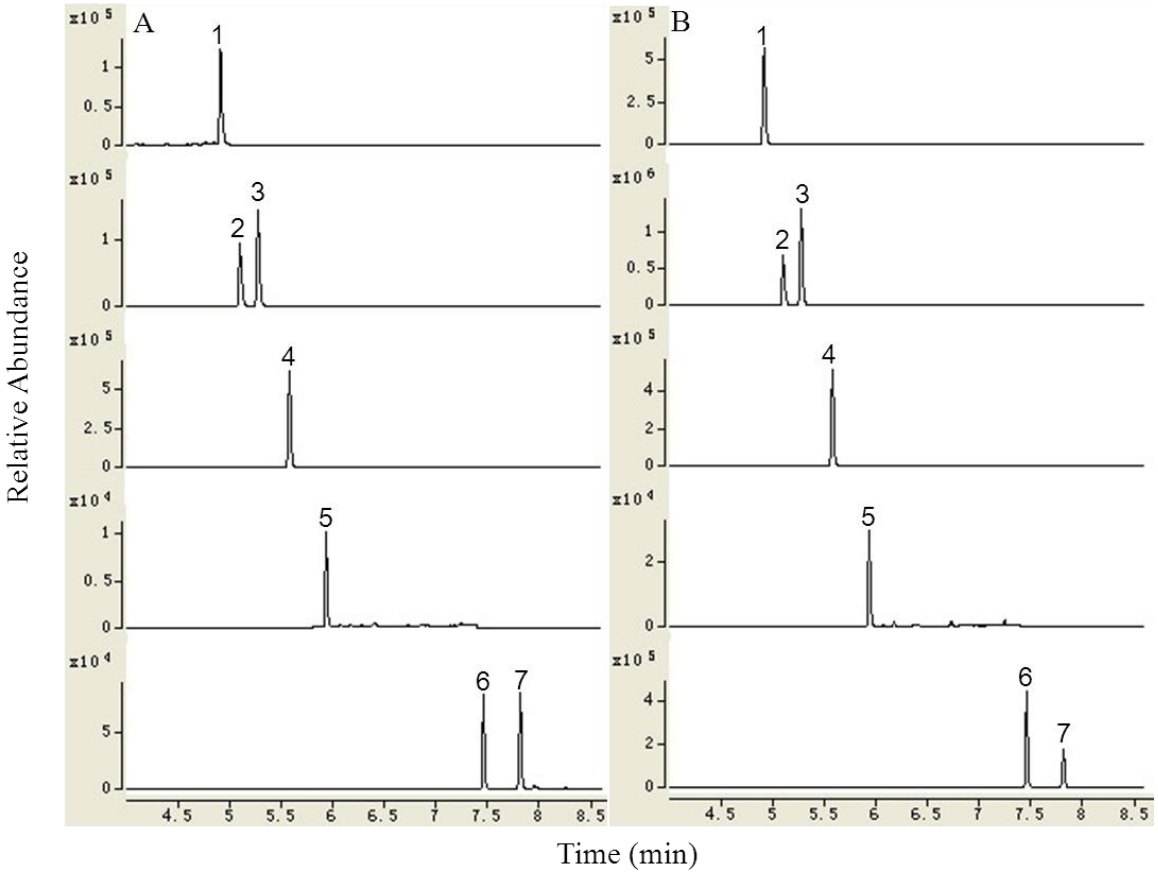


**Fig. 3** MRM extracted ion chromatograms of (A) reference standards and (B) LRPs sample: (1) menthol, (2) isoborneol, (3) borneol, (4) naphthalene (IS), (5) anethole, (6) eugenol and (7) acetyl eugenol

A rapid and effective GC-MS/MS method for the determination of six volatile active constituents in Longhu Rendan pills was developed and validated.
6x3mm (300 x 300 DPI)