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Analysis of the Essential Oil from Radix Bupleuri Using Capillary Gas Chromatography

Xiuqin LI, Ying JIA, Aihua SONG, Xiaohui CHEN, and Kaishun BI*

School of Pharmacy, Shenyang Pharmaceutical University 103 Wenhua Road, Shenyang 110016, Liaoning Provice, P. R. China

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A simple and rapid capillary gas chromatographic (CGC) method with flame ionization detection has been newly developed for analysis of the essential oil from Radix Bupleuri. Twenty components were identified with gas chromatography-mass spectrometry. *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were quantified simultaneously using the internal standard method. Decane was used as an internal standard. Separation and quantification were achieved on a DB-5 capillary column (30 m×0.25 mm i. d., 0.25-μm film thickness). The oven temperature was programmed as follows: 60℃ to 70℃ at 1℃/min rate, 70℃ for 10 min, 3℃/min to 120℃, 20℃/min to 250℃, and held at 250℃ for 5 min. The oven pressure was programmed as follows: 46.1 kPa for 25 min, 20.0 kPa/min to 77.6 kPa, and then held for 22 min. Split injection was conducted with a split ratio of 10∶1; flow-rate, 1.00 ml/min; carrier gas, nitrogen; injector temperature, 280℃; and detector temperature, 280℃. The system proved effective in resolving *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal peaks from their interfering components. The method displayed excellent linearity in the range of 26.8—1072 μg/ml (*E*-2-heptenal), 6.5—1292 μg/ml (furan, 2-pentyl), and 7.8—1564 μg/ml (*E*-2-nonenal). The average recovery rates of *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were 100.3%, 102.8%, and 97%, respectively. CGC is a quick and accurate method for analysis of the essential oil from Radix Bupleuri.

Key words——Radix Bupleuri; essential oil; GC-MS; GC-FID

INTRODUCTION

Radix Bupleuri belongs to the plant family *Bupleurum* sp., which mainly grows in northern subtropical areas. It is a well-known traditional Chinese medicine that has been used for more than 1000 years. Forty species, 17 varieties, and seven forms grow widely across China and among them about 17 species, six varieties, and one form are considered medicinal herbs.[1] According to the Chinese pharmacopoeia,[2] Radix Bupleuri is derived from the dried root of *Bupleurum Chinense DC*. and *Bupleurum scorzonerfolium Wild*. It is reported that Radix Bupleuri has anticancer,[3] antiinflammatory,[4] corticosterone-secreting,[5] and plasma cholesterol-lowering[6] activities. The essential oil is one of the important active components in Radix Bupleuri. In recent years, numerous studies have been carried out on the essential oil from Radix Bupleuri, and the results have shown that the oils have significant antifungal,[7] anticonvulsive,[8] and antiinflammatory[9—11] effects. Recently, because of the increasing demand for this crude drug as well as a shortage of the supply of quality goods, oil from many types of Radix Bupleuri, such as the roots of related *Bupleurum* sp., has been used as medicine in different areas of China. As for the chemical evaluation of this crude drug, up to now there have been no specific quantification methods to control the quality of essential oils of this medicinal herb in the Chinese pharmacopoeia. Therefore it is important and necessary to develop a method for the analysis of the essential oil from Radix Bupleuri. In this study, gas chromatography-mass spectrometry (GC-MS) was applied to identify the compositions of the essential oil. The results showed that *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were some of the main compounds of the oil. C6 and C9 aldehydes are volatile compounds that have a fresh green and cucumber-like flavor.[12] In *Phaseolus vulgaris* (L.) leaves, C6 aldehydes are rapidly formed during the hypersensitive resistance response against phytopathogenic *Pseudomonas syringae*.[13] In Arabidopsis, it has been shown that the aldehydes can induce a subset of defense-related genes.[14] From these results, it is suggested that the aldehydes have a significant role in the resistance response of higher plants against abiotic and biotic attacks. Therefore we developed a method for *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal to control the quality of the essential oil from Radix Bupleuri.

e-mail: bikaishun@yahoo.com

EXPERIMENTAL

Materials Hexane and decane were of analytical grade and purchased from Yu-Wang Chemical Company (Shandong, China). *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were supplied by J&K Chemica.

Apparatus A Shimadzu mass spectrometer (Qp5050A) equipped with Nist library software and Class-5000 data system, coupled with a Shimadzu gas chromatograph (Shimadzu17A) and Shimadzu 2010 gas chromatograph equipped with FID, was used for analysis. Data were processed on a Labsolution 2.1 workstation. GC analysis was performed on a 30 m × 0.25 mm id × 0.25 μm film DB-5 capillary column.

Operating Conditions For the GC-MS separation procedure, the instrumental parameters were: split injection was conducted with a split ratio of 10 : 1; flow-rate, 1.00 ml/min; carrier gas, helium; injector temperature, 280℃; flame ionization detection temperature, 230℃; and injected volume, 0.5 μl. The oven temperature program was 50℃ for 5 min, then raised to 170℃ at 5℃/min, and to 250℃ at 8℃/min and held for 5 min. The MS detection conditions were: interface temperature, 230℃; ionization mode, EI^+; electron energy, 70 ev; full-scan acquisition mode; and mass range, 33—500 amu. For the GC separation procedure, the oven temperature was programmed at: 60℃ to 70℃ at 1℃/min rate, 70℃ for 10 min, 3℃/min to 120℃, 20℃/min to 250℃, and held at 250℃ for 5 min; the oven pressure was programmed at: 46.1 kPa for 25 min, 20.0 kPa/min to 77.6 kPa, and then held for 22 min. Split injection was conducted with a split ratio of 10 : 1; flow-rate, 1.00 ml/min; carrier gas, nitrogen; injector temperature, 280℃; and detector temperature, 280℃.

SAMPLE PRETREATMENT

Extraction of Essential Oil Steam distillation, a typical extraction method for essential oils, was chosen, using hexane as the extraction solvent. The pulverized sample (50 g) was accurately weighed and transfered to a 500-ml round-bottomed flask soaked in 1500 ml of distilled water with 10% sodium chloride (pH 1) for 10 h. Water was added from the top of the essential oil determination apparatus until the water spilled onto the round-bottomed flask and 1.0 ml of hexane was added to the water layer. Steam distillation extraction was stopped after 8 h. Essential oil was separated from the water layer and leached into the hexane layer. Then, the hexane layer was transferred to a 5-ml measuring flask and decane standard solution was added to the solution as the internal standard.

Standard Solutions Stock standard solutions of *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were prepared by dissolving each compound in hexane separately at a concentration of 2.68, 3.23, and 3.91 mg/ml, respectively. These solutions were stored at −4℃. Decane was used as the internal standard and was dissolved in hexane at a concentration of 2.1 mg/ml.

Stock solutions of 0.2, 0.4, and 0.4 ml each of *E*-2-heptenal, furan, 2-pentyl and *E*-2-nonenal and 0.4 ml of internal standard solution were diluted to 5 ml with hexane to make standard solutions of 0.107, 0.258, and 0.310 mg/ml, respectively.

RESULTS AND DISCUSSION

Extraction Procedure Optimization In this study, soaking time (A), pH value (B), concentration of sodium chloride of water (C) and extraction time (D) affected the yield of extraction. To obtain the optimal conditions, the above factors were investigated. These four factors were arranged in an orthogonal design $L_9(3^4)$. Each factor had three levels: factor A (A_1, 20 h; A_2, 15 h; and A_3 10 h); factor B (B_1, 1; B_2, 2; and B_3, 4); factor C (C_1, 30%; C_2, 20%; and C_3, 10%); and factor D (D_1, 4 h; D_2, 6 h; D_3, 8 h). Eighteen experiments were performed. The yield of essential oil was selected as the assessment index to evaluate the extraction conditions. It was reported that UV absorbance value reflects the content of essential oil of Radix Bupleuri.[15] Therefore the UV absorbance value was used to measure the yield of essential oil from Radix Bupleuri. The results are shown in Table 1.

According to the results of the intuitive analysis, it was concluded that pH value and extraction time played more important roles than the other two factors in the extraction procedure (B > D > C > A). To shorten the experimental time, A_3 (10 h) was selected. Collectively, the optimal conditions were as follows: soaking in distilled water with a 10% concentration of sodium chloride (pH 1) for 10 h, followed by extraction for 8 h. ($A_3B_1C_3D_3$).

Chromatographic Conditions To assay the compounds of the essential oil from Radix Bupleuri

with good resolution and a reasonable elution time, different types of capillary columns, with temperature programs and pressure programs, were investigated. We analyzed the performance of DB-17, DB-5, and DB-1 columns using the same temperature and pressure programs. It was demonstrated that the furan, 2-pentyl peak and internal standard peak could be well separated on the DB-5 column. Hence further experiments were performed with the DB-5 column with different temperature and pressure programs. The peaks of *E*-2-heptenal and *E*-2-nonenal could not be well separated from their interferents with the same temperature program of GC-MS. Therefore several temperature programs between 1 and 5℃/min were tried and the pressure programs were investigated.

Table 1. Arrangment and Results of Orthogonal Test

Test no.	A	B	C	D	Absorbance value	
1	1	1	1	1	0.379	0.397
2	1	2	2	2	0.372	0.410
3	1	3	3	3	0.552	0.555
4	2	1	2	3	0.669	0.529
5	2	2	3	1	0.651	0.553
6	2	3	1	2	0.312	0.303
7	3	1	3	2	0.373	0.297
8	3	2	1	3	0.281	0.292
9	3	3	2	1	0.292	0.282
I_J	2.665	3.178	1.949	2.035		
II_J	2.578	2.067	2.676	2.437		
III_J	2.256	2.254	2.874	3.027		
R	0.409	1.111	0.925	0.992		

The optimal conditions for achieving a good chromatographic resolution were: oven temperature: 60℃ to 70℃ at 1℃/min rate, 70℃ for 10 min, 3℃/min to 120℃, 20℃/min to 250℃, and then held at 250℃ for 5 min. The oven pressure was: 46.1 kPa for 25 min, 20.0 kPa/min to 77.6 kPa, and then held for 22 min.

Identification of 14 Compounds The essential oil from Radix Bupleuri was injected manually into

Table 2. Compounds Identified in the Test Samples

Peak no.	Retention (min)	Compound
1	4.4	Hexanal
2	5.4	Furfural
3	7.8	Heptanal
4	9.9	2-Heptenal, (*E*)-
5	11.2	Furan, 2-pentyl
6	11.9	Hexanoic acid
7	13.6	2-Octenal, (*E*)-
8	14.1	*cis*-Linalool oxide
9	14.7	2-*p*-Tolylpropene
10	17.0	2-Nonenal, (*E*)-
11	17.9	Octanoic acid
12	20.2	Trans-2-decenal
13	21.9	2, 4-Decadienal, (*E, E*)
14	24.2	Bicylo [4, 1, 0] heptane, 7-bictclo [4, 1, 0] hept-7-ylidene-
15	24.5	Biphenylene, 1, 2, 3, 6, 7, 8, 8a, 8b-octahydro-4, 5-dimethyl-
16	24.6	4-Tridecen-6-yne, (*Z*)-
17	24.9	Thymohydroquinone dimethyl ether
18	25.2	Isoledene
19	27.6	Delta-cadinene, (+)-
20	35.9	*n*-Hexadecanoic acid

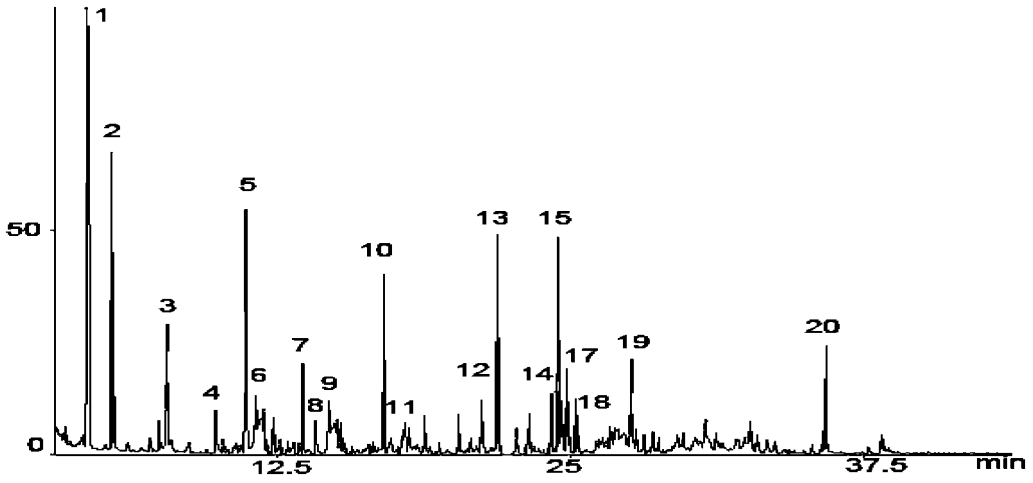

Fig. 1. Total Ion Current (TIC) Chromatogram of the Essential Oil from Radix Bupleuri by GC-MS
The main peaks were assigned as in Table 2.

the GC-MS system and analyzed in the full-scan acquisition mode. The unknown compounds were identified using online NIST library spectra, published mass spectra, and retention time.[16—19] The 14 characterized components with their retention times are listed in Table 2 and the chromatogram is shown in Fig. 1. *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were identified by comparison of their retention times and mass spectra with those obtained from the injected standards. Figure 2 shows their mass spectra.

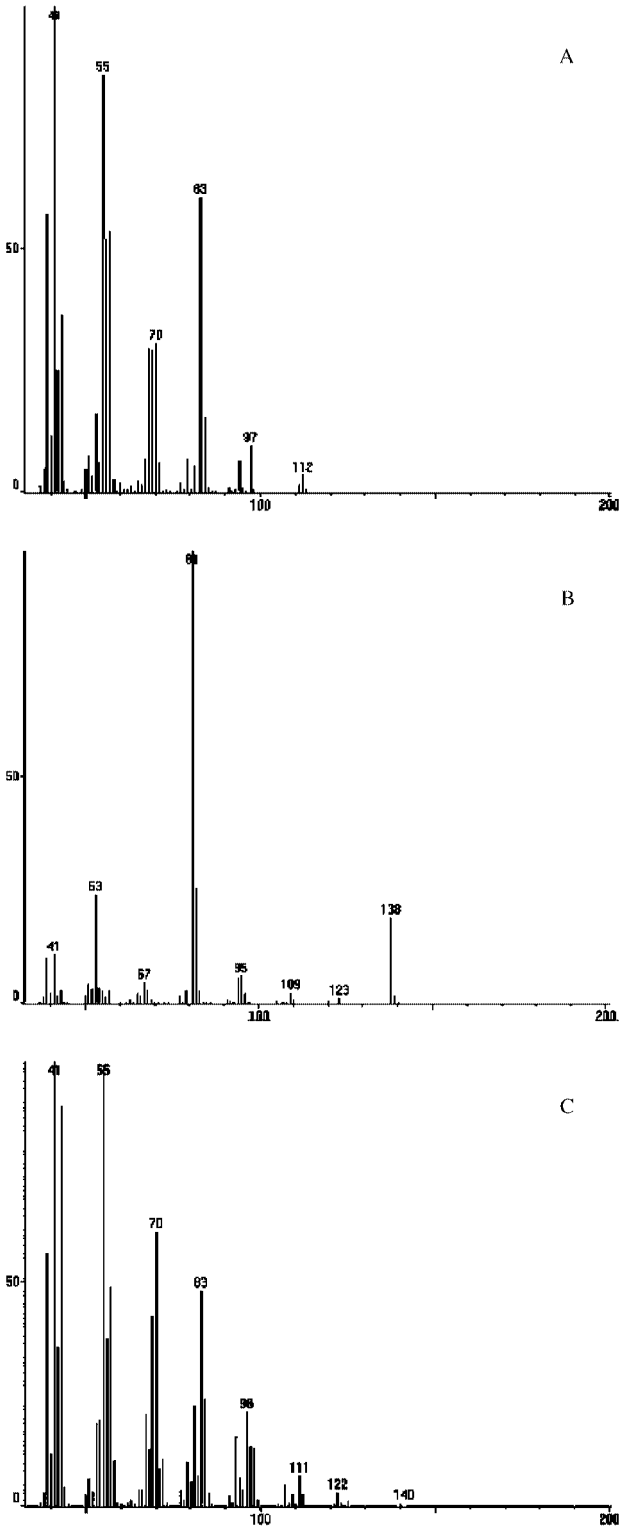

Fig. 2. A : Mass Spectrum of *E*-2-Heptenal, B : Mass Spectrum of Fural, 2-Pentyl, C : Mass Spectrum of *E*-2-Nonenal

Quantitation of *E*-2-Heptenal, Furan, 2-Pentyl, and *E*-2-Nonenal Aliquots of 0.5 μl of both the standard solution and sample solution were injected into the GC. The chromatograms are shown in Fig. 3. Calibration standards for *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were prepared from stock solutions. Calibration curves were calculated by plotting the peak area ratios of analyte to internal standard against analyte concentration. The concentration and the corresponding peak area ratio were found to be linear over the concentration range of 26.8—1072 μg/ml for *E*-2-heptenal, 6.5—1292 μg/ml for furan, 2-pentyl, and 7.8—1564 μg/ml for *E*-2-nonenal, respectively. The regression equations were:

Y=4.8465X−0.102 R=0.9996 (*E*-2-heptenal)

Y=5.0811X−0.1044 R=0.9994 (furan, 2-pentyl)

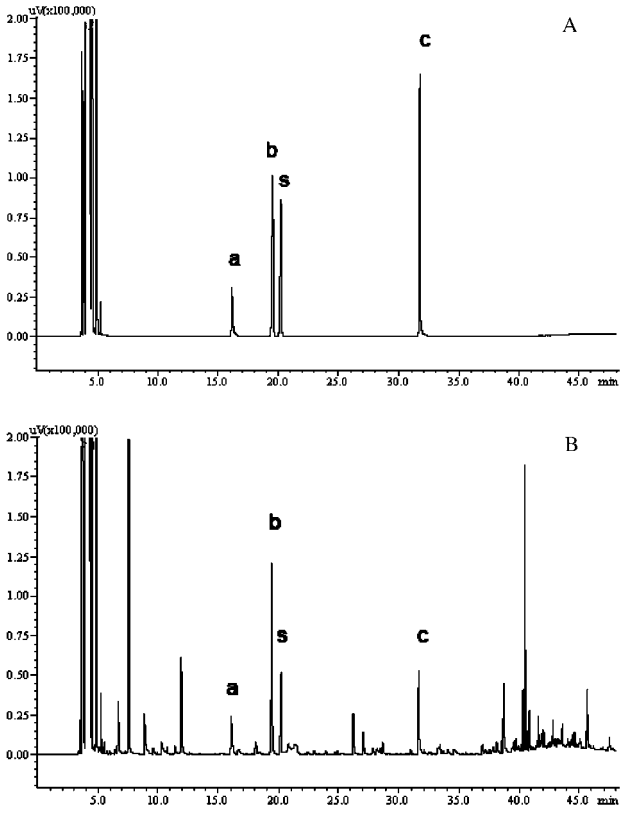

Fig. 3. A : Chromatogram of a Standard Mixture Containing *E*-2-Heptenal (a), Furan, 2-Pentyl (b), *E*-2-Nonenal (c) and Decane (s), B : Chromatogram of the Essential Oil from Radix Bupleuri

$Y=4.3198X-0.0444$ $R=0.9998$ (*E*-2-nonenal)
where Y is the peak area ratio and X is the concentration (mg/ml)

The lowest acceptable level of the calibration curve was regarded as the limit of quantitation (LOQ). In this study, the LOQ of *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were 26.8, 6.5, and 7.8 μg/ml, respectively. The intraday variability of the content was determined using the sample solution. The results showed excellent precision of the assays performed within a single day. The intraday precision of the sample was tested, and the relative standard deviations (RSD) were found to be 2.7% for *E*-2-heptenal, 0.7% for furan, 2-pentyl, and 3.1% for *E*-2-nonenal. The repeatability of the total procedure was tested using a sample of the essential oil, and the RSD values ($n=6$) were 1.6%, 0.7%, and 2.6% for *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal, respectively. The average recovery of *E*-2-heptenal, furan, 2-pentyl, and *E*-2-nonenal were 100.3% (RSD=4.2%), 102.8% (RSD=5.2%), and 97% (RSD=3.8%), respectively. These results confirmed that the present method exhibits good recovery and repeatability.

CONCLUSION

For the first time, a GC method that was applied to analyze the essential oil from Radix Bupleuri exhibited excellent resolution, recovery, and reproducibility. It can be utilized as a quality control method for this medicinal herb and preparations containing the essential oil from Radix Bupleuri.

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