Determination of Patchoulic Alcohol in Herba Pogostemonis by GC-MS-MS

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Patchoulic oil, the volatile oil of *Pogostemon cablin* (BLANCO) BENTH. and the Chinese crude drug Herba Pogostemonis, is widely used in the cosmetic and oral hygiene industries. Patchoulic alcohol is commonly used as an indicator for the quality assessment of dried *P. cablin*. However, the complexity of the herbal constituents makes it difficult for using conventional gas chromatography (GC) for analytical purpose. The present study established an accurate, sensitive and reproducible method for the quality assessment of the dried patchouli herb based on patchoulic alcohol content. A gas chromatography-tandem mass spectrometry (GC/MS/MS) method has been successfully developed and demonstrated for the determination of patchoulic alcohol content in the samples of dried *P. cablin*. The developed method was found to be convenient and facile in particular to tackle the complicated matrix problems always encountered in the herbs which contain high level of essential oils.

Key words patchoulic alcohol; Herba Pogostemonis; *Pogostemon cablin*; tandem mass spectrometry

Herba Pogostemonis is the dried aerial part of *Pogostemon cablin* (BLANCO) BENTH. (Family Labiatae), commonly known as "Guang-Huo-Xiang" or Cablin Patchouli. It is a Chinese Materia Medica traditionally used for the treatment of common cold, nausea and diarrhea.[1)]

Patchouli oil, the volatile oil of *P. cablin*, has been widely used in the cosmetic and oral hygiene industries to scent perfumes, flavor toothpaste, *etc.* Modern researches have repeatedly demonstrated various pharmacological activities of this oil including anti-emetic properties, trypanocidal activities, anti-bacterial, anti-fungal and Ca^{2+} antagonist activities.[2—5)] It was reported that *P. cablin* contains sesquiterpenes[5)] cytotoxic chalcones,[6)] antimutagenic flavones.[7)] Patchoulic alcohol (Chart 1) is a major active ingredient of Herba Pogostemonis and is the most odor-intensive component of patchoulic oil.[8)] Therefore, the patchoulic alcohol content has been widely used as an indicator for the quality assessment of Herba Pogostemonis or dried *P. cablin*.

Determination of patchoulic alcohol content, however, is not straightforward owing to the inherent chemical complexity of Herba Pogostemonis. Conventional methods always failed to give reliable results. For example, GC equipped with packed column may encounter difficulties in achieving satisfactory resolution. Capillary GC interfaced with Flame Ionization Detector (GC/FID) did not give steady outputs as a result of interfering GC peaks.[9)]

To overcome these difficulties, we have developed a GC/MS/MS method to determine the patchoulic alcohol content in the whole herbs, stems and leaves of Herba Pogostemonis. All samples were freeze-dried in advance and then extracted with liquid carbon dioxide by supercritical fluid extraction to minimize the loss or decomposition of the active ingredient in the herbs. Supercritical fluid extraction is particularly useful for the extraction of volatile oils and thermally liable compounds. It has the advantage of extraction at a relatively low temperature, negligible solvent residue and relatively high extraction efficiency.[10—12)]

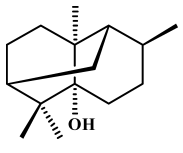

Chart 1. Structure of Patchoulic Alcohol

Quantitative analysis was conducted by measuring the abundance of product ions of patchoulic alcohol in the MS-MS spectrum. The identity of target compound was confirmed by full-scan mode and MS/MS scan mode by matching the retention time with an authentic standard. The enhanced selectivity of GC/MS/MS can eliminate the possible chromatographic interference occurred in GC/FID analysis. The minimized chromatographic interferences observed in the present study indicated suitability of the developed method for quality evaluation and quality control of Herba Pogostemonis.

Experimental

Materials. Reagents and Chemicals Chromatographic grade *n*-hexane was purchased from Merck (Darmstadt, Germany). Patchoulic alcohol, the reference standard, was obtained from the National Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China.

Equipment Supercritical Fluid extractor: ISCO SFX^{TM}2-10 (Nebraska, U.S.A.). Gas chromatography-tandem mass spectrometer: Finnigan PolarisQ equipped with AS2000 autosampler. GC Column: Alltech AT-5 capillary column, 25 m×0.25 mm ID, 0.25 μm film thickness. Freeze-drier: Model 77530, Labconco Corporation (Switzerland).

Plant Samples The fresh aerial parts of *Pogostemon cablin* (BLANCO) BENTH. were collected in September 2002 in Gaoyao, Guangdong Province, China. Five batches of samples were collected in the same cultivation area. All the samples were preserved at −20 °C before freeze-drying. The collected plants were taxonomically identified by one of the authors (Z. Zhao) and were deposited at the Bank of China (Hong Kong) Chinese Medicines Centre, School of Chinese Medicine, Hong Kong Baptist University.

Methods. GC-MS-MS Conditions One microliter of sample solution was injected in splitless mode with injector temperature maintained at 210 °C. The initial oven temperature was 60 °C and progressively heated up to 150 °C at a rate of 5 °C/min. The temperature was further ramped to 200 °C at the rate of 2 °C/min and hold for 10 min. Helium was used as the carrier gas with flow rate of 1 ml/min.

The temperature of transfer line and ion source was kept at 230 °C and 220 °C respectively. All mass spectra were acquired in electron impact mode with electron energy of 70 eV. Full scan mass spectra were acquired in the mass range within 50 to 400 (*m*/*z*). Product ion mass spectra of the base ion peak at *m*/*z* 138 of patchoulic alcohol were acquired with mass range from *m*/*z* 50—150.

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Sample Preparation The collected fresh herbs (aerial parts, stems and leaves) were preserved at −20 °C and then freeze dried. The freeze dried samples were kept sealed. The freeze-dried samples were blended and passed through a 40 mesh sieve. About 0.5 g samples were weighed and transferred into an extraction cell. Using the optimized conditions below extracts were collected and dissolved in a 50 ml volumetric flask containing 20 ml *n*-hexane. *n*-Hexane was then added and made up to volume. The solution was then filtered through a 0.2 μm membrane filter before GC injection.

The major factors affecting supercritical fluid extraction were extraction pressure, temperature, time and flow rate of the supercritical fluid. The extraction conditions were optimized empirically. The optimal conditions were determined to be 25 MPa, 40 °C for 30 min with a supercritical fluid flow rate of 2 ml/min.

Quantitation of Patchoulic Alcohol by GC-MS-MS Linearity: The calibration was performed using patchoulic alcohol as the reference standard. A series of standard solutions of patchoulic alcohol in *n*-hexane were prepared. A concentration range of 0.5 to 50 μg/ml was used to determine the linear range with 10 calibration points. A calibration curve was plotted by using the chromatographic peak area against the amount of standard on column (μg). By linear regression analysis, the calculated calibration function was $y=1.21\times10^8-48509$, with $R^2=0.9997$.

Precision: Samples of each matrix were injected in 5 replicates. The relative standard deviation (R.S.D.) of chromatographic peak areas of herb, stem and leaf samples were found to be 1.5%, 1.6% and 1.1%, respectively.

Repeatability: Five replicates of herb sample were prepared and analyzed (R.S.D.=1.2%).

Stability of Sample Solutions: Each sample solution was assayed for 5 times within 10 h. The R.S.D. of patchoulic alcohol content of herb, stem and leaf samples were 2.7%, 2.5% and 2.5% respectively. No significant difference was observed within the 10 h period.

Recovery: By spiking a known amount of reference standard into each sample, the recovery ratio was determined by using GC/MS/MS. The results shown in Table 1 are the average of five replicates ($n=5$) for herb, stem and leaf samples.

Quantification: Quantitative analysis was performed in MS/MS mode using external calibration. One microliter of all standard solutions and samples were injected in splitless mode.

Results and Discussion

Although the GC determination of patchoulic alcohol in patchoulic oil was reported previously with capillary column and Flame Ionization Detector,[9] this analytical approach always failed to give an unequivocal identification despite using the highest resolution capillary gas chromatography. For GC/MS analysis, the identity of each interested GC peak could be provided by the mass spectrometric data. However, matrix interference always led to ambiguous quantitative results. In the present GC/MS/MS analysis, the mass separation of precursor ion at *m/z* 138 from the possible interfering co-elutes was attempted and characteristic product ion mass spectra was generated by collision activated dissociation (CAD). The mass chromatograms of GC/MS and GC/MS/MS analysis of patchoulic alcohol and Patchouli herb were shown in Figs. 1 and 2, respectively. In full scan mode, the overall chromatographic pattern was relatively complicated and significant interfering peaks were observed. However, the product ion (*m/z* 138) scanning mode resulted in a highly selective detection and completely eliminated the interference peaks from matrix. Moreover, the product ion mass spectra of the samples highly resemble to that of the au-

Table 1. Recoveries for the GC/MS/MS Analysis of Patchoulic Alcohol

Sample	Average recovery % ($n=5$)	Relative standard deviation (R.S.D.) (%)
Whole Herb	98.5	2.8
Stem	98.8	2.3
Leaf	99.2	2.6

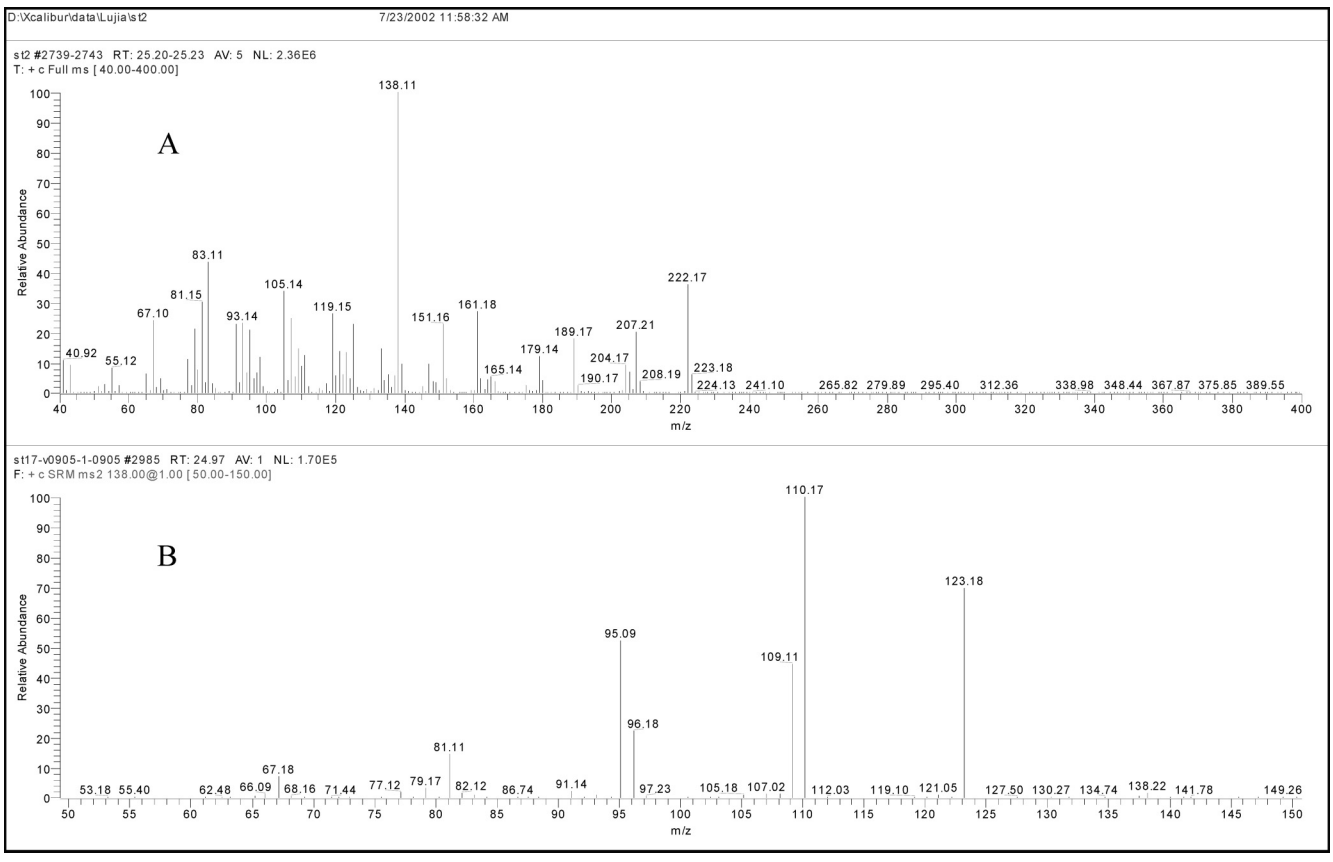

Fig. 1. EI-MS Spectrum of Patchoulic Alcohol (A) and MS/MS Spectrum Targeting *m/z* 138 (B)

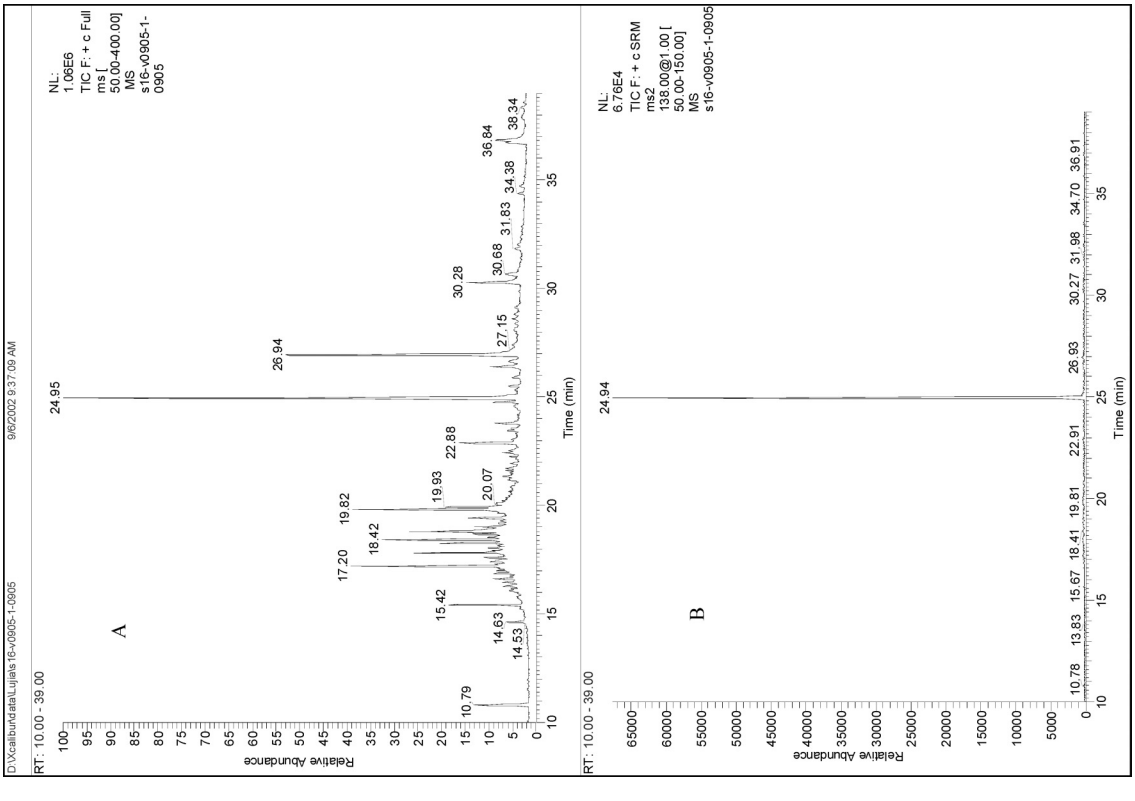

Fig. 2. Chromatogram of an Extract of Patchouli Herb: (A) Full Scan Mode with Mass Range from *m/z* 50 to 400; (B) CAD Mass Spectrum of the Base Ion Peak at *m/z* 138 of Patchoulic Alcohol Acquired with a Mass Range of *m/z* 50—150

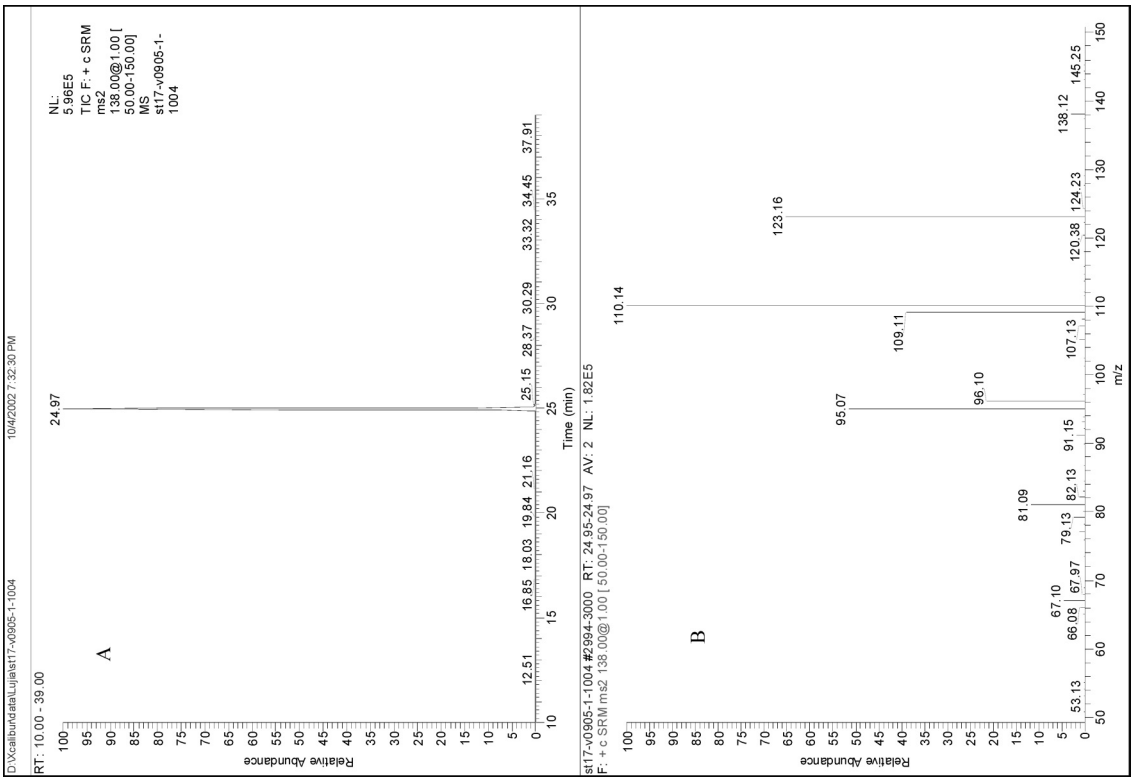

Fig. 3. GC/MS/MS Chromatogram of Patchoulic Alcohol: (A) Reconstructed Ion Chromatogram; (B) CAD Mass Spectrum of Ion Peak at *m/z* 138

thentic standard. By coupling the high resolution gas chromatography and tandem mass spectrometric techniques, unequivocal identification and accurate determination of patchoulic alcohol in the complex herbal matrix were satisfactorily achieved.

The contents of patchoulic alcohol in five batches of whole herb, stem and leaf of *Pogostemon cablin* are shown in Table 2. The result indicated that the content of patchoulic alcohol in patchouli leaves was about 30 times higher than that in stems. The mass chromatograms of patchouli herb samples, patchoulic alcohol, and patchouli stem samples were shown in Figs. 2 to 4, respectively.

The application of GC/MS/MS quantitative method described in this paper was found convenient and precise for the determination of patchoulic alcohol in Herba Pogostemonis. By using the techniques of freeze drying and CO_2 supercritical fluid extraction, any potential loss or decomposition of active ingredients in fresh herbs can be minimized. The risk of matrix interference can also be reduced greatly by using highly selective MS/MS detection. In this regard, the developed method provided a more reliable means for evaluating the patchoulic alcohol content and thus the quality of Herba Pogostemonis.

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References

1) Board of Pharmacopoeia of P. R. China (ed.), "Pharmacopoeia of the People's Republic of China," Chinese Edition 2000, Part I, Chemical Industry Press, Beijing, 2000, pp. 33—34.
2) Yang Y., Kinoshita K., Koyama K., *Phytomedicine*, **6**, 89—93 (1999).
3) Kiuchi F., Matsuo K., Ito M., Qui T. K., Honda G., *Chem. Pharm.*

Table 2. Contents of Patchoulic Alcohol (mg/g) in Different Parts of *Pogostemon cablin*

Samples	Whole Herb	Stem	Leaf
1	0.302	0.0218	0.736
2	0.386	0.0220	0.741
3	0.294	0.0196	0.725
4	0.225	0.0207	0.711
5	0.306	0.0227	0.752
Mean±S.D.	0.303±0.057	0.0214±0.0012	0.733±0.016

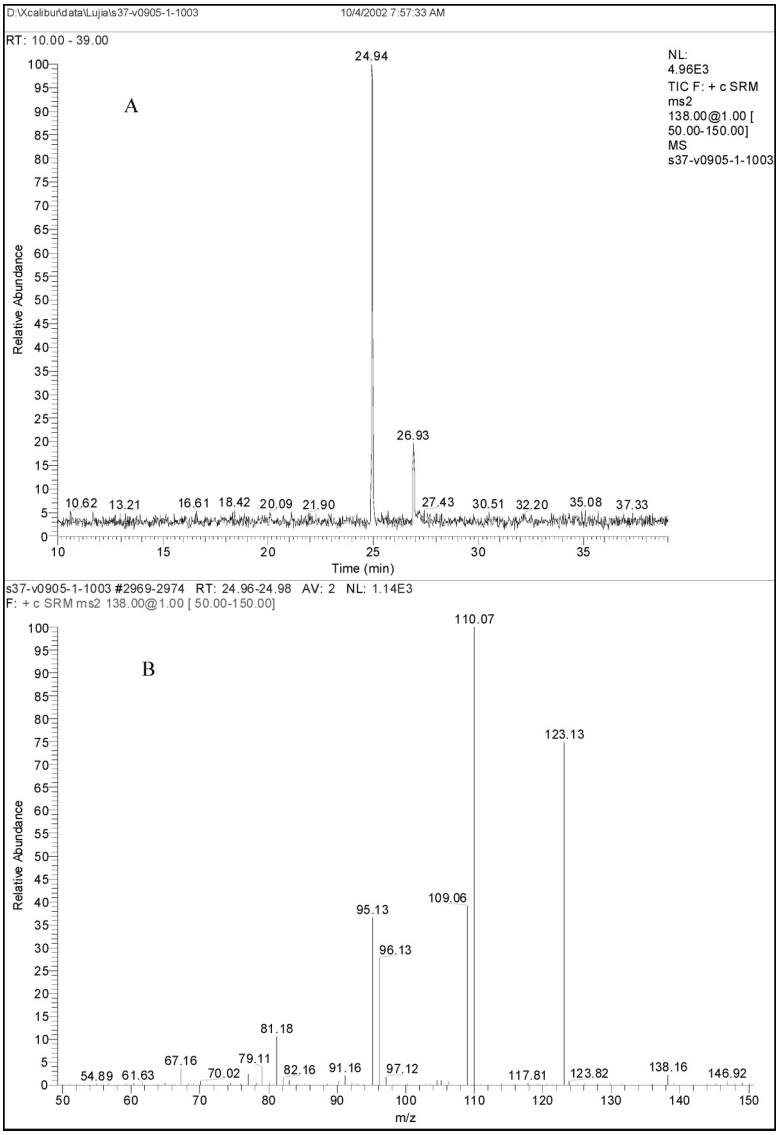

Fig. 4. GC/MS/MS Chromatogram of Patchouli Stem Samples: (A) Reconstructed Ion Chromatogram; (B) CAD Mass Spectrum of Ion Peak at *m*/*z* 138

Bull., **52**, 1495—1496 (2004).
4) Ichikawa K., Kinoshita T., Sankawa U., *Chem. Pharm. Bull.*, **37**, 345—348 (1989).
5) Yang D., Chaumont J.-P., Millet J., *Zhongguo Yaoxue Zazhi*, **35**, 9—11 (2000).
6) Park E. J., Park H. R., Lee J. S., Kim J., *Planta Medica*, **64**, 464—466 (1998).
7) Miyazawa M., Okuno Y., Nakamura S., Kosaka H., *J. Agric. Food Chem.*, **48**, 642—647 (2000).
8) Nikiforov A., Jirovetz L., Buchbauer G., *Monatsh. Chem.*, **117**, 1095—1098 (1986).
9) Analytical Methods Committee, Royal Society of Chemistry, *Analyst*, **112**, 1315—1318 (1987).
10) Sun Y., "Modern Chromatography and Its Application in Medicine," Press of People's Health, Beijing, 1998, p. 499.
11) Wei G., Fu H., Wang S.-Y., Li W., *Chin. Trad. Patent Med.*, **24**, 407—410 (2002).
12) Huang L.-S., Wu H.-Q., Zhang G.-Y., Xiao R., Huang F., Wang P., Zhang Z.-Y., *Fenxi Ceshi Xuebao*, **20**, 79—81 (2001).