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Quality assessment of *Flos Chrysanthemi Indici* from different growing areas in China by solid-phase microextraction-gas chromatography-mass spectrometry

Shun Shen[a], Yunfei Sha[b], Chunhui Deng[a], Xiangmin Zhang[a,*], Daxi Fu[c], Jiakuan Chen[c]

[a] *Department of Chemistry, Fudan University, Shanghai 200433, People's Republic of China*
[b] *Department of Pharmacy, Fudan University, Shanghai 200032, People's Republic of China*
[c] *Natural Pharmacy Research Center, Fudan University, Shanghai 200433, People's Republic of China*

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Abstract

Flos Chrysanthemi Indici is a common traditional Chinese medicine (TCM). In this paper, headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) was developed for quality assessment of *Flos Chrysanthemi Indici* from different growing areas in China. SPME parameters such as extraction fibers, extraction temperature, extraction time and sample mass were investigated to achieve identical results to those obtained by the steam distillation (SD). The selected SPME conditions were as follows: SPME fiber coated with 65-μm PDMS/DVB, extraction temperature of 60 °C, extraction time of 30 min and sample mass of 1.0 g. Furthermore, four active compounds (eucalyptol, camphor, borneol and bornyl acetate) presented in the TCM were applied to evaluating the quality of *Flos Chrysanthemi Indici* from 20 various areas. The quality assessment was successfully performed to compare the similarity value (*S*) between different sample vector of *Flos Chrysanthemi Indici* and the standard profile vector (SPV). The results showed that the proposed HS-SPME-GC-MS was an alternative technique for quality assessment of *Flos Chrysanthemi Indici* samples.

Keywords: Traditional Chinese medicine; *Flos Chrysanthemi Indici*; Headspace solid-phase microextraction; Active compounds; Quality assessment

1. Introduction

Historically, especially in China, traditional Chinese medicines (TCMs) have played an important role in clinical therapy because of their high pharmacological activity, low toxicity and rare complication [1]. In recent years, more and more interests have been re-attracted in this field. *Flos Chrysanthemi Indici*, anthotaxy of *Chrysanthemum indicum* L. (Asteraceae) is used as a heat-clearing and detoxication herb. It can also inhibit the agglutination of blood platelet and promote the myocardial blood circulation and white cell phagocytosis; thus it has been used to treat many diseases such as furuncle and skin nodules [2,3]. Lately, it has been found to show inhibitory activity against nitric oxide (NO) production in lipopolysaccharide-activated macrophages as well [4].

Dry *Flos Chrysanthemi Indici* contains about 0.5% of essential oil. The routine method for its essential oil analysis is based on oil isolation by steam distillation (SD) followed by GC-MS, and about 34 components have been identified [5–7]. Four active constituents, namely eucalyptol, camphor, borneol and bornyl acetate [8-23], are found to be present in the TCM. Quality difference of *Flos Chrysanthemi Indici* from five growing areas in China has been already investigated for the oil obtained by steam distillation [7]. As we know, different natural conditions, including soil and climate, lead to discrepancy in quality of the TCMs. So, quality assessment of *Flos Chrysanthemi Indici* is important in TCM industry production. In general, quality monitoring of *Flos Chrysanthemi*

* Corresponding author. Tel.: +86 21 6564 3983; fax: +86 21 6564 1740.
E-mail address: xmzhang@fudan.edu.cn (X. Zhang).

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Indici is performed by analysis of its active components by using steam distillation, which requires a relatively larger amount of sample (50–1000 g) and is a time-consuming procedure (6–8 h of distillation). For the quality evaluation of *Flos Chrysanthemi Indici*, a rapid, simple and sensitive analytical method for investigation and determination of volatile constituents is indispensable.

Solid-phase microextraction (SPME), introduced by Pawliszyn's group in 1990, is a relatively new sampling and concentration technique [24]. It has been widely adopted for the determination of chemical components of plant essential oils [25–35]. In our previous studies, this technique has also been applied to analysis of volatile constituents in TCMs. SPME has been proved to be a simple, rapid, sensitive and solvent-free method suitable for determination of volatile compounds in TCMs [36,37].

In this contribution, four main active constituents (eucalyptol, camphor, borneol and bornyl acetate) were selected for the adjustment of SPME conditions and quality assessment of *Flos Chrysanthemi Indici*. SPME parameters affecting the extraction efficiency such as extraction fibers, sample mass, extraction time and temperature were studied to give identical results to the classical steam distillation method. After adjustment, it was applied to real samples from 20 different areas in China to assess their quality.

2. Experimental

2.1. Material and reagents

Flos Chrysanthemi Indici samples were collected from 20 different areas in China (HS: Huangshan, XN: Xiuning, BZ: Bozhou, AQ: Anqing, QJ: Quanjiao, YX: Yuexi, JZ: Jinzhai, SC: Shucheng, XC: Xuancheng, NP: Nanping, PT: Putian, LC: Liancheng, SZ: Suizhou, WH: Wuhan, MC: Macheng, NZ: Nanzhang, YC: Yancheng, GX: Guangxi, ZZ: Zhenzhou, NMG: Nei Menggu), respectively. Eucalyptol, camphor, borneol and bornyl acetate standards were all provided by the National Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China. The extraction fibers: 100-μm polydimethylsiloxane (PDMS), 65-μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65-μm carbowax/divinylbenzene (CW/DVB) and 85-μm polyacrylate (PA) were purchased from Supelco, Bellefonte, PA and USA, and were conditioned prior use according to supplier's prescriptions.

2.2. Sample preparation

Fifty grams of *Flos Chrysanthemi Indici* was ground to fine powder, and then put into a 1000-ml distillation flask. Five hundred ml of distilled water was added and volatile oil distillation apparatus was set according to the Chinese pharmacopoeia (Chinese Pharmacopoeia Committee Publishing House of People's Health, 2000, Appendix 64) [38]. The mixture was distilled for 6 h. Oil was collected from the condenser, dried over anhydrous sodium sulfate, and the yield of the sample was 0.47%. The obtained essential oil was stored at −10 °C until analysis.

HS-SPME sample preparation was as follows: 1.0 g of each *Flos Chrysanthemi Indici* sample was ground to fine power, and then introduced into a 15-ml headspace glass bottle. The bottle was immediately sealed by silicone septa and stored at −10 °C until used.

Standard stock solution was prepared with 0.1 g of eucalyptol, camphor, borneol and bornyl acetate, respectively, and dissolved in a 100-ml volumetric flask with ethanol. The working analytical standard solution (5 μg/ml for each compound) was made by diluting the stock solution 200 times with distilled water. It was stored at −10 °C until used.

2.3. Adjustment of SPME conditions

Flos Chrysanthemi Indici sample (2.0 g) from Bozhou in China was used for investigation of the proper extraction conditions. At first, selection of the optimum fiber was performed by extraction of the volatile compounds of the sample using PDMS, PDMS/DVB, CW-DVB and PA fibers simultaneously in the same conditions (extraction temperature of 60 °C and time 60 min). Next, adjustment of extraction temperature (30–70 °C), extraction time (20–60 min) and sample weight (0.5–2.0 g) was carried out by extraction of the above four main active volatile compounds in the sample, comparing the relative peak areas with those of the routine steam distillation to achieve similar results. The analytes adsorbed on the fibers were then desorbed in the GC injection liner at 250 °C for 2 min.

2.4. SPME of the volatile constituents in Flos Chrysanthemi Indici samples

The PDMS/DVB fiber was applied to extraction of the volatile constituents in these *Flos Chrysanthemi Indici* samples. Extraction was carried out at the temperature of 60 °C and fiber exposure time of 30 min, and then introduced into the GC injection liner and desorbed at 250 °C for 2 min.

2.5. GC analysis

GC analyses were accomplished with an HP-5890 series II instrument equipped with HP-WAX and HP-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). The following temperature program was used: 50 °C for 2 min, ramp of 6 °C/min up to 270 °C; injector temperature, 250 °C; detector temperature, 300 °C; carrier gas, nitrogen (2 ml/min); detector dual FID; split ratio, 20:1; injection, 0.5 μl. Identification of the components was performed for both columns by comparison of their retention times with those of pure authentic samples and by means of their retention indices relative to the series of *n*-hydrocarbons.

2.6. GC-MS analysis

Volatile compound desorption and analyses were carried out on a HP 6890 GC system, coupled with a HP MD5973 quadrupole mass spectrometer. The compounds were separated on a HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). Split injection was employed for both distillation and SPME samples with a ratio of 20:1. The column oven temperature was programmed to rise from an initial temperature of 50 °C (2 min) to 200 °C at 6 °C/min, then to 270 °C at 10 °C/min. The injection temperature and ion source temperature were 250 and 230 °C, respectively. Helium was used as the carrier gas with a flow rate of 1 ml/min. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 40–350 amu. Compounds were identified using the Wiley 6.0 (Wiley, New York, NY, USA) mass spectral library.

3. Results and discussion

3.1. Comparison of different fibers

To select the most appropriate fiber, four different fibers were exposed at 60 °C for 60 min to a 2.0 g of *Flos Chrysanthemi Indici* sample from Bozhou (China) in headspace mode. The peak areas of the four main active components (eucalyptol, camphor, borneol and bornyl acetate) were obtained and presented in Fig. 1. The results proved that PDMS/DVB was the most effective coating for essential analytes of *Flos Chrysanthemi Indici*. Hence, the PDMS/DVB fiber was selected for further studies.

3.2. Selection of extraction temperature, extraction time and sample mass

In general, the quality control of *Flos Chrysanthemi Indici* is carried out by analysis of the four active compounds in the TCM by steam distillation method. It is demonstrated that SD is a reliable method for assessment of TCMs [7,39]. However, this method requires a tedious and time-consuming procedure. The TCM quality control demands development of a simple and rapid method for analysis of the four active compounds in *Flos Chrysanthemi Indici*. And SPME is proved to be a good choice for analysis of volatile constituents in TCMs [36]. Due to the different nature of the two extraction processes (SPME and SD), adjustment of the SPME parameters (extraction temperature, extraction time and sample mass) should be performed to make the results by SPME identical to those by SD.

A sample of *Flos Chrysanthemi Indici* from Bozhou (China) was analyzed by the SPME method at different extraction temperatures using 2.0 g of sample and extraction time of 60 min. In all cases, we found that the sum of the essential oil peaks in the chromatogram varied only by ±7.5%, which meant that the fiber was saturated with *Flos Chrysanthemi Indici* volatiles. Different temperatures affected the competition among volatiles with different affinities towards the fiber. Therefore, the relative peak areas of the four major active components extracted by SPME method were selected to make a comparison with those obtained by the SD method. The result is shown in Fig. 2. On the whole, the relative peak areas of the four main active components decrease as the extraction temperature increases, especially for camphor and borneol. The highest extraction efficiency for the above four active components achieves at temperatures from 30 to 40 °C. However, we are not supposed to reach the highest extraction efficiency, but to obtain a result identical to that of SD. Consequently, 60 °C seems to be a better choice for this system due to the fact that the relative peak areas of four active volatile constituents all show identical results to the classical SD method.

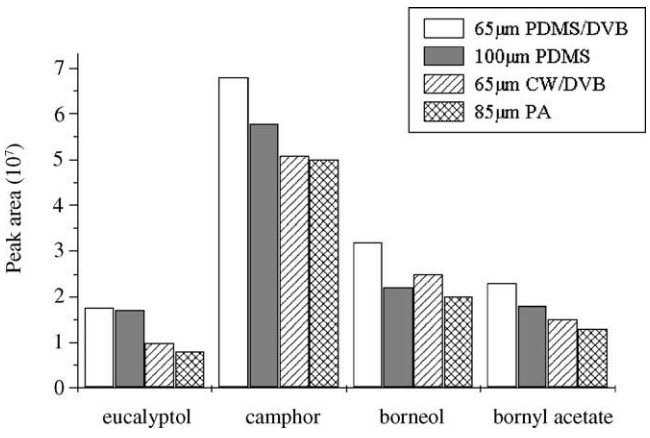

Fig. 1. Extraction profile obtained with different fibers for four active compounds in *Flos Chrysanthemi Indici*. Extraction conditions: sample mass of 2.0 g, extraction time of 60 min and temperature of 60 °C.

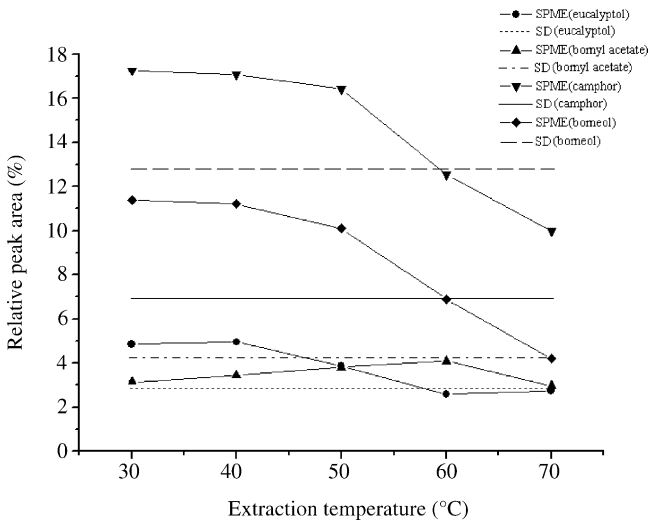

Fig. 2. Headspace SPME-GC-MS analysis of *Flos Chrysanthemi Indici* volatiles. Relative areas for four main active components (eucalyptol, camphor, borneol and bornyl acetate) at different extraction temperatures compared with relative areas for the same compounds in essential oil obtained by steam distillation. Extraction time = 60 min, sample weight = 2 g.

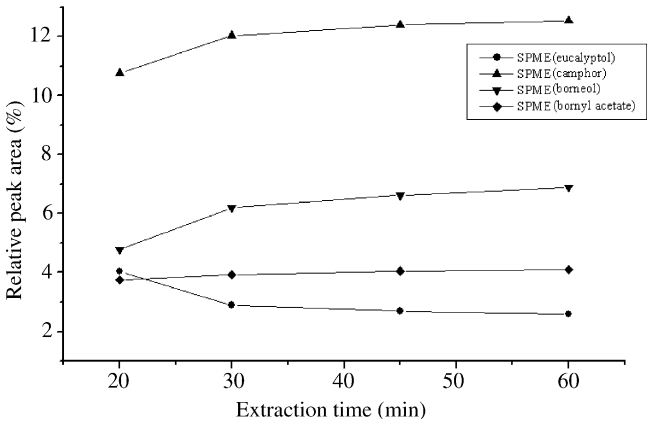

Fig. 3. Headspace SPME-GC-MS analysis of *Flos Chrysanthemi Indici* volatiles. Relative areas for four main active components (eucalyptol, camphor, borneol and bornyl acetate) at different extraction time. Extraction temperature = 60 °C, sample weight = 2 g.

Since a short time of analysis was desired in sample pretreatment, a series of extraction time was investigated at 60 °C. Fig. 3 shows that the relative peak areas of the four active constituents obtained under extraction time of 30 min are very close to those under 45 and 60 min. Besides, extraction periods of 30 min were approximately equivalent to the time required to run GC in this experiment. Therefore, 30 min was chosen as the adsorption time.

The profile of a series of sample mass studied using 30 min of extraction at 60 °C is shown in Fig. 4. The fiber concentrations of eucalyptol, camphor and borneol increase when sample mass increases, and that of bornyl acetate decreases with increasing sample mass. When sample mass increases from 1.0 to 2.0 g, the relative peak areas of four active compounds maintain constant. In the further study, 1.0 g was chosen as SPME sample mass.

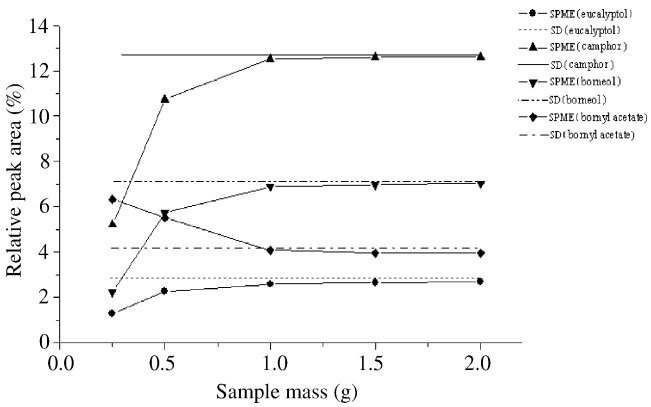

Fig. 4. Headspace SPME-GC-MS analysis of *Flos Chrysanthemi Indici* volatiles. Relative areas for four main active components (eucalyptol, camphor, borneol and bornyl acetate) for different amounts of sample compared with relative areas for the same compounds in essential oil obtained by steam distillation. Extraction time = 30 min, extraction temperature = 60 °C.

3.3. Comparison of the results by SPME and SD

As is shown in Table 1, 24 compounds in *Flos Chrysanthemi Indici* from Bozhou were identified by SD coupled with GC-MS, while 21 constituents of *Flos Chrysanthemi Indici* volatiles could be determined using the selected SPME conditions. The results obtained by the two methods were in agreement with those reported in literature [5]. Compared with SPME, SD seems more powerful to extract some high-molecular mass and low volatility compounds such as tetradecanoic acid, heneicosane, docosane and tricosane in essential oils of *Flos Chrysanthemi Indici* sample. With other things being equal, SPME appears to be a good alternative for its oil determination. As a consequence, SPME is capable of processing the subsequent quality assessment for *Flos Chrysanthemi Indici* instead of SD.

3.4. Repeatability

The repeatability of the method was studied. *Flos Chrysanthemi Indici* samples from 10 different growing areas were analyzed under the selected SPME conditions (PDMS/DVB fiber, extraction temperature of 60 °C, extraction time of 30 min and sample weight of 1 g). Three replicative analyses for each sample were carried out, and the obtained peak areas of the four active compounds were used for calculation of relative standard deviations (RSDs). The RSDs are: eucalyptol, from 6.84 to 10.04%; camphor, from 3.32 to 9.61%; borneol, from 3.55 to 8.93% and bornyl acetate, from 4.28 to 9.07%, respectively.

3.5. SPME-GC-MS determination of volatile compounds of Flos Chrysanthemi Indici

The selected extraction conditions were applied to SPME of volatile constituents of *Flos Chrysanthemi Indici* from 20 different regions in China. Twenty-one compounds were identified (Table 1). Four active constituents (eucalyptol, camphor, borneol and bornyl acetate) among them were recognized by retention time and EI mass spectrum of standard compounds with those in the TCM sample, respectively.

The identified 21 compounds, including four active compounds (eucalyptol, camphor, borneol and bornyl acetate), were detected in all 20 *Flos Chrysanthemi Indici* samples. Their peak areas of the above four constituents were chosen from the GC chromatograms and used for quality assessment of *Flos Chrysanthemi Indici*.

3.6. Quality assessment of Flos Chrysanthemi Indici from 20 different areas in China

Flos Chrysanthemi Indici from different growing areas do not always show the same intensity of its effect owing to their discriminating content of active volatile constituents. Different natural conditions, including soil and climate, lead to discrepancy in quality of the TCM. In China,

Table 1
GC-MS identification of *Flos Chrysanthemi Indici* volatiles from Bozhou in China and peak area percentages

Peak number	Retention index*	Compound	Relative percent content (%)	
			65-μm PDMS/DVB	SD
1	939	α-Pinene	1.6	1.7
2	954	Camphene	1.7	1.4
3	979	β-Pinene	0.7	0.5
4	1033	Eucalyptol	2.6	2.8
5	1061	γ-Terpinene	0.5	0.3
6	1108	Thujone	1.0	1.7
7	1144	*cis*-β-Terpineol	0.2	ND
8	1146	Camphor	12.5	13.1
9	1174	Borneol	6.9	6.9
10	1181	4-Terpineol	1.9	1.5
11	1195	α-Terpineol	1.2	0.9
12	1209	Verbenone	1.1	0.9
13	1238	*trans*-Chrysanthenyl acetate	2.6	2.8
14	1291	Bornyl acetate	4.1	4.2
15	1373	Copaene	1.3	1.6
16	1399	Isocaryophyllene	1.5	0.9
17	1418	β-Caryophyllene	7.9	8.4
18	1456	β-Farnesene	8.1	8.5
19	1480	Germacrene D	6.4	6.8
20	1495	α-Farnesene	2.2	2.4
21	1581	Caryophyllene oxide	3.5	3.9
22	1767	Tetradecanoic acid	ND	2.2
23	2100	Heneicosane	ND	1.0
24	2200	Docosane	ND	1.1
25	2300	Tricosane	ND	0.6

* Retention indices on an HP-5 column; ND, not detected.

no overall investigation has been reported about quality of the widespread *Flos Chrysanthemi Indici* and people could only tend to buy it of known variety and origin such as HS due to its recognized high quality in the folk. Therefore, it is a relevant task to establish a set of quality appraisal of *Flos Chrysanthemi Indici* by comparing their contents of active components.

For the quality assessment of *Flos Chrysanthemi Indici*, it is very significant to obtain an authentic sample (AUS). Generally, there are two ways to acquire AUS. Selection of the samples, which are well recognized to have good quality, is the first way. And the second way is to blend many samples from many different growing areas, or calculate the mean chromatographic parameters of all samples to serve as the results of AUS [40]. In our present work, the following Eq. (1) was adopted, where n is the number of samples, X_i means the peak area of the i chromatographic peak and $\bar{X}$ is the mean value vector in the system.

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n}(x_{i1}, x_{i2}, x_{i3}, \ldots, x_{in}) \quad \text{for } i=1 \text{ to } 4 \tag{1}$$

The total profile vectors, in which each datum was the mean value of the peak areas for all the four active compounds of 20 samples from different growing areas, could be acquired using Eq. (1). And their mean values were marked as the results of AUS1.

The contents of four active compounds (eucalyptol, camphor, borneol and bornyl acetate), indicated by their respective peak area and AUS1, are plotted in Fig. 5. To our knowledge, higher contents of active constituents mean better quality for a certain crude herb. Fig. 5 shows apparently

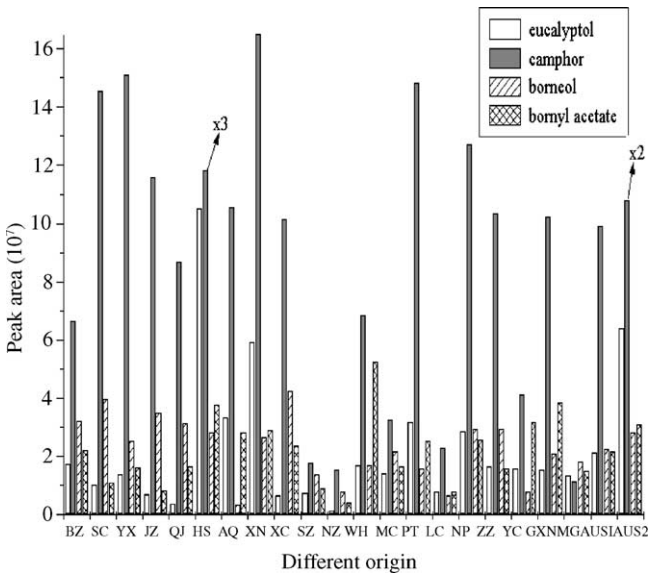

Fig. 5. Concentration comparison of four active compounds in *Flos Chrysanthemi Indici* from different growing areas in China and those of AUS.

that HS, XN and NP are the ideal growing areas for *Flos Chrysanthemi Indici* among the 20 samples as their entire target constituents present much higher peak areas than the AUS1, while *Flos Chrysanthemi Indici* from SZ, NZ, LC and NMG are considered to be relatively inferior for their extraordinarily lower contents of the above active components compared with AUS1. Nevertheless, the 13 remaining growing areas cannot be properly evaluated in this way, since not all of their content of the four active compounds show coherent results compared with AUS1 (some of the above four components are higher while the others are lower for each area). And Eq. (2) [41] gives us the calculation method to appraise their quality comprehensively, where $\vec{X}_s$ is served as the standard profile vector (SPV); $\vec{X}_t$ is the sample vector; $\rho(X_s, X_t)$ is the spatial distance between $\vec{X}_s$ and $\vec{X}_t$, and $\rho(X_s, 0)$ between $\vec{X}_s$ and $\vec{0}$. We calculate spatial distance using Euclidean distance.

$$S(X_s, X_t) = e^{-\rho(X_s, X_t)/\rho(X_s, 0)} \quad (2)$$

As is mentioned above, we calculated the SPV $\vec{X}_s$, in which each datum was the mean value of the peak areas for all the four active compounds of the best samples from HS, XN and NP using Eq. (1). And the mean values were marked as the results of AUS2 (Fig. 5), which was considered as the reliable contrast in the following analyses.

In this work, the value of *S* represents how similar a test sample is to AUS2, that is, the nearer the value of *S* is to 1, the closer the sample is to AUS2. It was also studied by Cheng et al. that '*S*' had integral measurement capability, which could be used successfully for determining the similarity of chemical fingerprint [41]. Therefore, the parameter of *S* was selected to quality evaluation of *Flos Chrysanthemi Indici* from the remaining 13 growing areas.

Next, Eq. (2) was employed to calculate the similarity between $\vec{X}_s$ and $\vec{X}_t$. According to the concept of *S* proposed above, the order in Table 2 from the top down shows the quality assessment on the left 13 areas from better to worse. By the way, parameter *S* was also applied to the forenamed inferior regions among the 20 samples, and their similarity values were 0.412, 0.404, 0.398 and 0.395 for LC, SZ, NZ and NMG, respectively, all less than the *S* values of the upper 13 areas. This accordant outcome confirmed strongly that this method could be competent for quality assessment of *Flos Chrysanthemi Indici* in our present experiment.

Table 2
Results of '*S*' value

Origin	*S*
PT	0.717
YX	0.695
SC	0.670
JZ	0.600
AQ	0.599
ZZ	0.585
GX	0.583
XC	0.570
QJ	0.535
BZ	0.505
WH	0.505
YC	0.452
MC	0.440

S: similarity value.

By and large, through comparison of *Flos Chrysanthemi Indici* from the above 20 different areas in China, HS, XN and NP were found to show the ideal quality of the TCM; *Flos Chrysanthemi Indici* from LC, NZ, SZ and NMG relatively represented the poorer quality and the others from PT, YX, SC, AQ, JZ, ZZ, GX, XC, QJ, BZ, WH, YC, MC in China were shown in Table 2 arranged from better to worse.

4. Conclusion

Compared with classical methods such as SD, HS-SPME is a relatively simple, rapid, sensitive and solvent-free method for analysis of the volatile constituents in *Flos Chrysanthemi Indici*, and is quite suited for the application of quality evaluation of the TCM using similarity value '*S*'. Moreover, we believe that this approach is potentially useful for the quality assessment of other TCMs.

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References

[1] K.C. Wen, C.Y. Huang, F.L. Lu, J. Chromatogr. 631 (1993) 241.
[2] F.K. Zhang, Measurement of Chemical Components in Common Chinese Herds, People Sanitary Press, Beijing, China, 1997, pp. 606–610.
[3] Y.C. Yan, X.E. Lou, H.T. Jiang, Chin. J. Pharm. 16 (1998) 16.
[4] M. Yoshikawa, T. Morikawa, I. Toguchida, Chem. Pharm. Bull. 48 (2000) 651.
[5] R.G. Ma, J.B. Guan, X.M. Wang, H.J. Fang, Chin. J. Chromatogr. 12 (1994) 47.
[6] Y.F. Huang, Y.M. Zhang, L. Tao, H.Q. Wu, J. Instrum. Anal. 20 (2001) 40.
[7] Y.M. Zhang, Y.F. Huang, L. Tao, J.W. Huang, J. China, Chim. Mater. Med. 27 (2002) 265.
[8] U.R. Juergens, U. Dethlefsen, G. Steinkamp, A. Gillissen, R. Repges, H. Vetter, Resp. Med. 97 (2003) 250.
[9] S. Lahlou, A.F. Figueiredo, P.J.C. Magalhaes, J.H. Leal-Cardoso, Can. J. Physiol. Pharm. 80 (2002) 1125.
[10] A. Sokmen, G. Vardar-Unlu, M. Polissiou, D. Daferera, M. Sokmen, E. Donmez, Phytother. Res. 17 (2003) 1005.
[11] T.J. Park, H.K. Seo, B.J. Kang, Biochem. Pharmacol. 61 (2001) 787.
[12] B. Tirillini, E.R. Velasquez, R. Pellegrino, Planta Med. 62 (1996) 372.
[13] D.P. Kamdem, D.A. Gage, Planta Med. 61 (1995) 574.
[14] F.J. Hammerschmidt, A.M. Clark, F.M. Soliman, Planta Med. 59 (1999) 68.

[15] E.A. Laude, A.H. Morice, T.J. Grattan, Pulm. Pharmacol. 7 (1994) 179.
[16] G. Vardar-Unlu, F. Candan, A. Sokmen, D. Daferera, M. Polissiou, M. Sokmen, E. Donmez, B. Tepe, J. Agric. Food Chem. 51 (2003) 63.
[17] T.J. Park, Y.S. Park, T.G. Lee, H. Ha, K.T. Kim, Biochem. Pharmacol. 65 (2003) 83.
[18] N. Tabanca, N. Kirimer, B. Demirci, F. Demirci, K.H.C. Baser, J. Agric. Food Chem. 49 (2001) 4300.
[19] R.C. Mühlbauer, A. Lozano, S. Palacio, A. Reinli, R. Felix, Bone 30 (2003) 372.
[20] F. Juteau, V. Masotti, J.M. Bessière, M. Dherbomez, J. Viano, Fitoterapia 73 (2002) 532.
[21] S. Savelev, E. Okelloa, N.S.L. Perryb, R.M. Wilkinsa, E.K. Perryb, Pharmacol. Biochem. Be. 75 (2003) 661.
[22] E. Tachikawa, M. Takahashi, T. Kashimoto, Biochem. Pharmacol. 60 (2000) 433.
[23] G. Buchbauer, W. Jager, L. Jirovetz, F. Meyer, H. Dietrich, Pharmazie 47 (1992) 620.
[24] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[25] J.C. Verdonk, C.H. Ric de Vos, H.A. Verhoeven, M.A. Haring, A.J. van Tunen, R.C. Schuurink, Phytochemistry 62 (2003) 997.
[26] G. Flamini, P.L. Cioni, I. Morelli, J. Chromatogr. A 998 (2003) 229.
[27] L. Jirovetz, G. Buchbauer, M.P. Shafi, M.M. Kaniampady, Eur. Food Res. Technol. 217 (2003) 120.
[28] P. Bartak, P. Bednar, L. Cap, L. Ondrakova, Z. Stransky, J. Sep. Sci. 26 (2003) 715.
[29] B.J. Savary, A. Nunez, J. Chromatogr. A 1017 (2003) 151.
[30] C.A. Zini, K.D. Zanin, E. Christensen, J. Agric. Food Chem. 51 (2003) 2679.
[31] J. Rohloff, J. Agric. Food Chem. 50 (2002) 1543.
[32] L. Jirovetz, G. Buchbauer, M.B. Ngassoum, M. Geissler, J. Chromatogr. A 976 (2002) 265.
[33] J.B. Cai, B.Z. Liu, Q.D. Su, J. Chromatogr. A 930 (2001) 117.
[34] C.A. Zini, F. Augusto, E. Christensen, Anal. Chem. 73 (2001) 4729.
[35] C. Bicchi, S. Drigo, P. Rubiolo, J. Chromatogr. A 892 (2000) 469.
[36] C.H. Deng, G.X. Song, Y.M. Hu, Chromatographia 58 (2003) 289.
[37] S. Gallori, G. Flamini, A.R. Bilia, J. Agric. Food Chem. 49 (2001) 5907.
[38] Chinese Pharmacopoeia Committee, Chinese Pharmacopoeia, Publishing House of People's Health, 2000 (Appendix 64).
[39] T.L. Liu, Q. Qiu, Z.J. Cui, Chin. Tradit. Herbal Drugs 30 (1999) 903.
[40] G.X. Sun, Y. Wang, Y.Q. Sun, K.S. Bi, Anal. Sci. 19 (2003) 1395.
[41] Y.Y. Cheng, M.J. Chen, Y.J. Wu, Acta Chim. Sinica. 60 (2002) 2017.