Asian Journal of Chemistry; Vol. 27, No. 5 (2015), 1903-1906

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.18454

Determination of 3-Methoxy-5-methylphenol and Veramoss in Oakmoss Essential Oil by GC-TOFMS

YANG-BIN XU[1,2], KAI WANG[1,*], RUI-ZHI ZHU[1], MAO-DE SHOU[1], WEI ZHE[1] and QIANG LIU[1]

[1]R & D Center of China Tobacco Yunnan Industrial Co., Ltd, Kunming 650231, P.R. China
[2]Faculty of Chemical Engineering, Kunming University of Science and Technology, Kunming 650500, P.R. China

*Corresponding author: Tel: +86 15087724205; E-mail: wangkai@hongta.com

Received: 16 July 2014; *Accepted*: 26 November 2014; *Published online*: 20 February 2015; AJC-16917

The volatile components of oakmoss essential oil were analyzed by using gas chromatography time-of-flight mass spectrometry (GC-TOFMS) and the peak area normalization method was used to calculate the relative content of each components. With the mass spectrometry library search, 20 compounds, which account for 95.78 % in the volatile components of oakmoss essential oil, were identified by using reference literature and retention index. Veramoss, 3-methoxy-5-methylphenol, triethylene glycol, ethyl orsellinate, methyl everninate, orcinol and linoleic acid were the main components. Compared with gas chromatography and liquid chromatography, the quick method to determine 3-methoxy-5-methylphenol and veramoss in oakmoss essential oil by GC-TOFMS was developed. The linearity of method was obtained in the range of 0.01-2 mg/mL concentrations of 3-methoxy-5-methylphenol and veramoss. The correlation coefficients were 0.9969 for 3-methoxy-5-methylphenol and 0.9960 for veramoss. The limits of detection were 0.12 mg/kg for veramoss and 0.07 mg/kg for 3-methoxy-5-methylphenol. The results showed that the relative standard deviation was less than 4 % for two compounds (n = 6), the recovery of standard addition were 91.06-105.68 % for veramoss and 96.30-107.11 % for 3-methoxy-5-methylphenol. The method can be used to determine 3-methoxy-5-methylphenol and veramoss in oakmoss essential oil or in oakmoss extract.

Keywords: Oakmoss, Gas chromatography, Time-of-flight mass spectrometry, 3-Methoxy-5-methylphenol, Veramoss.

INTRODUCTION

Oakmoss (*Evernia prunastri* (L.) Ach.), the lichen principal growing on the bark of oak trees, was especially widespread in the south-central Europe and also distributed in Yunnan province of China. The fragrance of oakmoss was very special green type and contained the aroma of fennel, bean and hay. Oakmoss essential oil was used as a good-quality perfume fixative, because its fragrance had lots of advantages such as mild, rich, smooth and persistent[1-4].

At present, the research of oakmoss were mainly centralized on the analysis of the volatile components in oakmoss extract, the deployment of oakmoss flavor, the medicinal value, *etc*.[5-11]. However, the quantitative analysis of volatile constituents and functional ingredients in oakmoss essential oil was rarely reported. In this paper, the volatile components of oakmoss essential oil were analyzed by using GC-TOFMS combined with retention index method, which improve the accuracy of the qualitative analysis of the volatile components in oakmoss extract. At the same time, the quantitative analysis method for functional ingredients in oakmoss essential oils, such as 3-methoxy-5-methylphenol, veramoss were studied. The results provided technical support for the development and quality control of oakmoss essential oil.

EXPERIMENTAL

Oakmoss essential oil was purchased from MANE Inc. Acetone, ethanol and hexane were obtained from J & K Technology Ltd, chloroform, dichloromethane, methanol and acetonitrile were purchased from Sigma-Aidrich Co., Ltd. All chemicals were chromatographic purity. Methyl nicotinate, *n*-alkane series (C7-C30), veramoss and 3-methoxy-5-methylphenol were obtained from Sigma-Aldrich Co., Ltd.

Instrument and chromatographic conditions: Waters ACQUITY UPLC system equipped with PDA eλDetector (Waters, USA) was employed for analysis. ACQUITY UPLC®BEH C_{18} column (1.7 μm, 2.1 × 50 mm) was used for the separation. The mobile phase was consisted of 1 % acetic acid aqueous solution and acetonitrile:water:acetic acid (1:30:69). The flow rate was set at 0.3 mL/min and the volatile components were detected by PDA eλDetector at 269, 274 nm, respectively. The column temperature was 30 °C and the injection volume was 5 μL. The list of mobile phase elution program is shown in Table-1.

TABLE-1
LIST OF MOBILE PHASE ELUTION PROGRAM

Time (min)	Rate of flow (mL/min)	1 % Aqueous solution of acetic acid (%)	Acetonitrile-water-acetic acid (1:30:6 9) (%)
0	0.30	95.0	5.0
3.00	0.30	95.0	5.0
3.01	0.30	60.0	40.0
5.00	0.30	15.0	85.0
7.00	0.30	15.0	85.0
7.01	0.30	95.0	5.0
10.00	0.30	95.0	5.0

GC-TOFMS Analysis was carried out with an Agilent 7890A GC equipped with time -of- flight mass spectrometry (LECO, America). The gas column was Rxi-5sil MS 60 m × 0.25 mm × 0.25 μm column. 1 μL of each sample was injected in split mode (1:10) with a column temperature program of 60 °C and increased to 120 °C at 10 °C/min and then increased to 240 °C at 2 °C/min and held at this temperature for 9 min. Injector temperature was set at 250 °C. The carrier gas was helium and the flow rate was 1 mL/min. Solvent delay time was 300 s and the transfer line was set at 250 °C. Mass spectra were acquired over 40-450 amu range at 50 spec/s with ionizing electron energy of 70 eV. The raw mass spectrograms were processed by Leco ChromaTOF software v4.33 and the mass spectral libraries were NIST08 library and Wiely9N library.

Qualitative analysis: Oakmoss essential oil was diluted by CH_3OH:CH_2Cl_2 (1:4) mixed solution (20 times) and the retention index was measured by *n*-alkane series (C7-C30) in hexane (diluting 20 times). All samples used for experiments were filtered through 0.45 μm organic phase filter. The volatile components of oakmoss essential oil were analyzed by GC-TOFMS and the peak area normalization method was used to calculate the relative content of each component. With the mass spectrometry library search, the identification of volatile components was performed by a comparison of the observed RI with those of published MS data. In addition, authentic reference compounds were used for some of the analytes to be identified. RI Values were calculated using the *n*-alkane series (C7-C30) under the same GC conditions in experiment. At present, retention index method was often used in the analysis of natural products, because it was only affected by the stationary phase and had no connect with temperature program and carrier gas flow rate. Retention index method with the mass spectrometry comparing has been increasingly applied in the analysis of complex samples.

Quantitative analysis: A stock solution was prepared by diluting 200 mg methyl nicotinate into 1000 mL volumetric flask with methanol. Methyl nicotinate was used as an internal standard. Mixed standard solution of 2 mg/mL was obtained by diluting 20 mg veramoss and 20 mg 3-methoxy-5-methylphenol into 10 mL volumetric flask with stock solution. The other concentrations of working solutions were obtained by diluting desired volumes of 2 mg/mL mixed standard solution into 10 mL volumetric flask with stock solution. Total ion chromatogram of standard sample was shown in Fig. 1. Sample solution was prepared by diluting 200 mg oakmoss essential oil into 100 mL volumetric flask with stock solution.

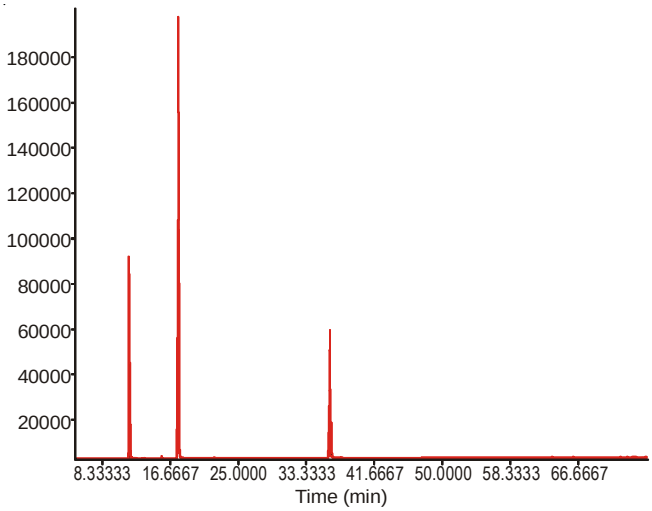

Fig. 1. Total ion chromatogram of standard sample

RESULTS AND DISCUSSION

Analysis of the volatile components in oakmoss essential oil: Through analysis of appraisal, qualitative results of volatile components in oakmoss essential oil were shown in Table-2 and total ion chromatogram of GC-TOFMS of sample were shown in Fig. 2.

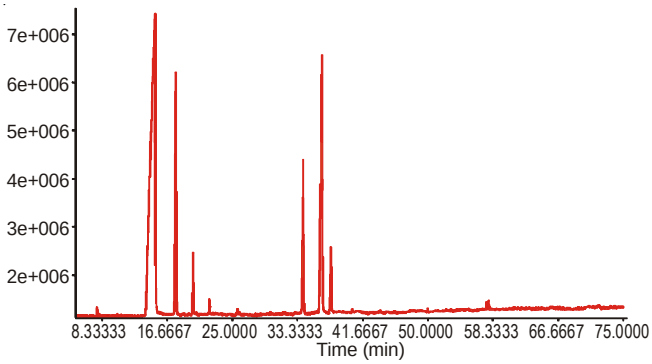

Fig. 2. Total ion chromatogram of GC-TOFMS of oakmoss essential oil

Observed RI value was automatically calculated by Pegasus 4D workstation and cited RI value was obtained from literature[12]. Among them, retention index of belting * was RI value of DB1 column or RI value of OV101 column and retention index of compound name of belting * was the observed RI value of standard substance.

According to Table-2 and Fig. 2, 20 compounds were identified by GC-TOFMS, which accounted for 95.78 % in the volatile components of oakmoss essential oil. The major components were 3-methoxy-5-methylphenol, veramoss,

TABLE-2
QUALITATIVE RESULTS OF VOLATILE COMPONENTS BY GC-TOFMS IN OAK MOSS ESSENTIAL OIL

Peak no.	Component name	Similarity	Retention time (min)	CAS	Retention index		Area (%)
					Observed	Cited	
1	Heptane	938	6.12	142-82-5	699	700	0.01
2	2-(Methoxyethoxy)ethanol	886	7.27	111-77-3	931	920*	0.01
3	Phenol	921	7.99	108-95-2	976	976	0.10
4	Acetophenone	892	9.92	98-86-2	1074	1065	0.02
5	α,α-Dimethylbenzylalcohol*	885	10.31	617-94-7	1093	1098	0.10
6	δ-Hexanolactone	914	10.73	823-22-3	1096	1064	0.01
7	Borneol	907	12.96	464-45-9	1178	1169	0.01
8	Triethylene glycol	961	15.16	112-27-6	1256	-	49.23
9	3-Methoxy-5-methylphenol*	926	17.79	3209-13-0	1326	1320	7.84
10	Orcinol	929	19.97	504-15-4	1377	-	3.91
11	2,6-Dimethoxytoluene	851	20.16	5673-7-4	1381	-	0.03
12	2,6-Dimethyl-1,4-benzenediol	861	24.69	654-42-2	1509	-	0.11
13	Methyl orsellinate	845	33.78	3187-58-4	1661	-	0.10
14	Everninate methyl	856	34.08	520-43-4	1667	-	11.31
15	Veramoss*	933	36.47	4707-47-5	1714	1708	17.34
16	Ethyl orsellinate	850	37.62	2524-37-0	1737	-	5.25
17	Ethyl myristate	900	39.52	124-06-1	1792	1795	0.01
18	Ethyl hexadecanoate	901	50.00	628-97-7	1991	1994	0.09
19	Leinoleic acid	884	57.48	60-33-3	2158	2128	0.27
20	Ethyl octadecanoate	938	60.20	111-61-5	2191	2196#	0.03

triethylene glycol, methyl everninate, ethyl orsellinate and orcinol. More content of triethylene glycol indicated that the sample oil may be the blending oil or use triethylene glycol as solvent in the preparation process.

Veramoss was a colourless to pale peach red crystalline powder. It was described as: powerful oakmoss, woody, phenolic and it offered the power and tenacity of oakmoss absolute. It was extremely versatile and was often used as an oakmoss replacement. 3-Methoxy-5-methylphenol had the aroma of natural oakmoss, sweet, phenolic and probably used in spice, roasted and smoke flavors[13].

Comparison of quantitative method: Several methods for the determination of 3-methoxy-5-methylphenol and veramoss in oakmoss essential oil had been explored. First, ultra-high performance liquid chromatography (UPLC) was used as quantitative analysis. In the experimental process, 1 % aqueous solution of acetic acid and acetonitrile:water:acetic acid (1:30:69) as mobile phase, according to the experimental conditions of ultra-performance liquid chromatography, 3-methoxy-5-methylphenol and veramoss could be well separated, but chromatographic peak existed tailing phenomenon as shown in Fig. 3.

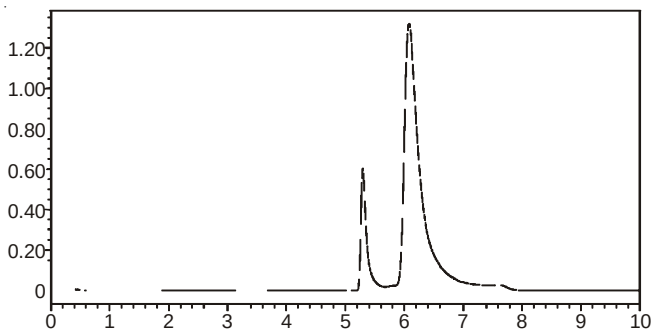

Fig. 3. UPLC Chromatography of veramoss and 3-methoxy-5-methyl-phenol

When we used this method for determination of 3-methoxy-5-methylphenol and veramoss in oakmoss essential oil, their peaks had overlap and interfered by other components as shown in Fig. 4. After adjusting the mobile phase elution process and flow rate, it was failed to effectively improve this phenomenon.

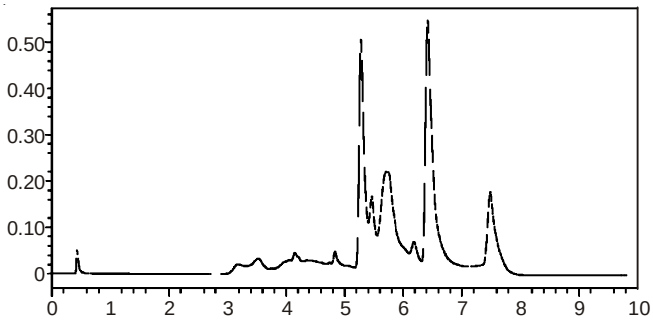

Fig. 4. Ultra high performance liquid chromatography of oakmoss essential oil

Compared to UPLC, gas chromatography had more safety and environmentally harmless (without organic solvent). A GC-TOFMS assay for the determination of 3-methoxy-5-methyl-phenol and veramoss in oakmoss essential oil was developed in this experiment.

Selection of organic solvent: Methanol, dichloromethane, acetone, ethanol and chloroform were selected as extracting solvent in the experiment. Comparison of different organic solvent on extraction effect of veramoss and 3-methoxy-5-methyl-phenol in oakmoss essential oil were shown in Table-3. Each of these solvents was conducted on three parallel experiments and the relative standard deviation was less than 3 %. Oakmoss essential oil could be completely dissolved in five kinds of organic solvents and the solution was clarified. But five kinds of organic solvents had different extraction effect on 3-methoxy-5-methylphenol and veramoss. As to 3-methoxy-

TABLE-3
COMPARISON OF DIFFERENT ORGANIC SOLVENT ON EXTRACTION EFFECT OF VERAMOSS AND 3-METHOXY-5-METHYLPHENOL

Component name	Concentration of 3-methoxy-5-methylphenol and veramoss (g/kg)				
	Methanol	Dichloromethane	Acetone	Ethanol	Chloroform
3-Methoxy-5-methylphenol	47.68	42.01	47.17	48.99	45.55
Veramoss	92.41	90.25	91.65	89.84	95.05

5-methylphenol, extraction efficiency of ethanol, methanol and acetone was higher and the difference of these three was smaller. As to veramoss, extraction efficiency of chloroform, methanol and acetone was higher and extraction efficiency of dichloromethane and ethanol was relatively lower. So, methanol was selected as extracting solvent in the next experiments.

Method validation: Linearity of method was obtained in the range of 0.01-2 mg/mL concentrations of 3-methoxy-5-methylphenol and veramoss. The equation of calibration curve of 3-methoxy-5-methylphenol was Y = 0.00365 X-0.2097 and the goodness of fit (R^2) was found to be 0.9969. The equation of calibration curve of veramoss was Y = 0.00255 X -0.1703 with R^2 = 0.9960.

In the determination of 3-methoxy-5-methylphenol and veramoss in oakmoss essential oil, the method precision was studied for five replicate experiments under optimum GC-TOFMS conditions and the relative standard deviation (RSD) obtained were 3.29 % (3-methoxy-5-methylphenol) and 3.01 % (veramoss).

3-Methoxy-5-methylphenol and veramoss of low, medium, high concentration were, respectively added to the samples of oakmoss essential oil and each of experiment was conducted with three replications. The recoveries were calculated on the basis of the results of these assays. The results showed that the recovery for 3-methoxy-5-methylphenol was in the range of 96.30-107.11 % and it was in the range of 91.06-105.68 % for veramoss.

Limit of detection (LOD) was determined as the concentration with a signal to noise ratio of 3 and the limit of detection were 0.07 mg/kg for 3-methoxy-5-methylphenol and 0.12 mg/kg for veramoss.

Sample analysis: The method of GC-TOFMS was applied to determine the concentrations of 3-methoxy-5-methyl-phenol and veramoss. Oakmoss essential oil was three times analyzed in order to assure an accurate determination. The concentrations of 3-methoxy-5-methylphenol and veramoss in oakmoss essential oil were 47.68 and 92.41 g/kg, respectively.

Conclusion

The volatile components of oakmoss essential oil were analyzed by using GC-TOFMS, with the mass spectrometry library search, 20 volatile compounds of oakmoss essential oil, were identified in combination with retention index method. A simple and rapid method for the simultaneous determination of 3-methoxy-5-methylphenol and veramoss in oakmoss essential oil using gas chromatography-time-of-flight mass spectrometry was developed. The method provided a linear range of 0.01-2 mg/mL concentrations of 3-methoxy-5-methyl-phenol and veramoss. Analytical parameters are satisfactory with linearity, recoveries, limit of quantitation and precision all suitable for determining 3-methoxy-5-methyl-phenol and veramoss in oakmoss essential oil or oakmoss extract.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Foundation of China Tobacco Yunnan Industrial Co., Ltd (No. 2013FL10).

REFERENCES

1. S. Munzi, L. Paoli, E. Fiorini and S. Loppi, *Environ. Pollut.*, **171**, 25 (2012).
2. D. Joulain and R. Tabacchi, *Flavour Fragrance J.*, **49**, 24 (2009).
3. M.L. Sanchez, Ph.D. Thesis, University of Bordeaux, France (1994).
4. C.K. Lagoo, *Indian Perfumer*., **42**, 51 (2007).
5. D.S. Mao, Q. Liu and C. Hou, *Se Pu*, **23**, 323 (2005).
6. Y. Gao, B.Z. Liu, X.L. Zhu, L. Shi, J.L. Chen, M. Gong and L.G. Zhang, *Se Pu*, **18**, 251 (2000).
7. B.F. Lutnaes, T. Bruun and H. Kjosen, *Nat. Prod. Res*., **18**, 379 (2004).
8. H. Schulz and G. Albroscheit, *J. Chromatogr. A*, **466**, 301 (1989).
9. H. Nomura, Y. Isshiki, K. Sakuda, K. Sakuma and S. Kondo, *Biol. Pharm. Bull*., **36**, 833 (2013).
10. H. Nomura, Y. Isshiki, K. Sakuda, K. Sakuma and S. Kondo, *Biol. Pharm. Bull.*, **1560**, 35 (2012).
11. C. Ehret, P. Maupetit, M. Petrzilka and G. Klecak, *J. Cosmet. Sci*., **14**, 121 (1992).
12. E.S.O. Leffingwell, 2006 [DB/CD]. Georgia: Bolens Aroma Chemical Information Service (2010).
13. Leffingwell and Associates, Flavor-base, Tabacco Edition [DB/CD] (2010).