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Quantitative determination of compounds in tobacco essential oils by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry

Shukui Zhu [a], Xin Lu [b], Li Dong [a], Jun Xing [a], Xueli Su [a], Hongwei Kong [b], Guowang Xu [b], Caiying Wu [a,*]

[a] *College of Chemistry and Molecular Science, Wuhan University, Wuhan 430072, China*
[b] *National Chromatographic R&A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116011, China*

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Abstract

A quantitative analysis of the individual compounds in tobacco essential oils is performed by comprehensive two-dimensional gas chromatography (GC × GC) combined with flame ionization detector (FID). A time-of-flight mass spectrometer (TOF/MS) was coupled to GC × GC for the identification of the resolved peaks. The response of a flame ionization detector to different compound classes was calibrated using multiple internal standards. In total, 172 compounds were identified with good match and 61 compounds with high probability value were reliably quantified. For comparative purposes, the essential oil sample was also quantified by one-dimensional gas chromatography–mass spectrometry (GC/MS) with multiple internal standards method. The results showed that there was close agreement between the two analysis methods when the peak purity and match quality in one-dimensional GC/MS are high enough.

Keywords: Comprehensive two-dimensional gas chromatography; Time-of-flight mass spectrometry; Gas chromatography–mass spectrometry; Multiple internal standards calibration; Essential oils; Tobacco

1. Introduction

Many tobacco essential oils have a popular and easily recognizable fragrance, which has been attributed to the volatile and semi-volatile components. In order to understand the relationship between constituents and the aroma of essential oils, analysis of these constituents is necessary. The result obtained from this may be used to answer the research or industrial analysis questions, such as for comparative purposes, where one essential oil is contrasted with others for quality control or investigation of adulteration, to discover new components, or to characterize the chemical classes of compounds present. By their nature, essential oils almost range from volatile through to semi-volatile compounds. This range is particularly suited to gas chromatographic analysis. However, Adams [1] reveals an enormous number of compounds that are present in essential oils and like materials. The similarity of retention indices of many related components will result in overlapping peaks. The presence of unsaturated bonds, various branched and cyclic compounds, and oxygenated analogues (e.g., alcohols and ketones) will further complicate the issue. One-dimensional gas chromatography cannot provide sufficient separation for a complete qualitative, let alone, quantitative analysis, so it is desired to develop some new separation technologies to achieve improved analysis.

Comprehensive two-dimensional gas chromatography (GC × GC), which may be considered the most powerful separation tool in GC [2], is a technique highly suited for

* Corresponding author. Tel.: +86 27 8768 4244; fax: +86 27 8764 7617.
E-mail address: cywu@whu.edu.cn (C. Wu).

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the separation of complex mixture such as petroleum, environmental, tobacco smoke and essential oil samples. With this new technique, the peaks eluting from the first GC column enter a cold-jet modulator, which traps each subsequent small portions of eluate, focuses these portions and introduces them into a second column for further separation. GC × GC differs from conventional multidimensional separations in that the whole sample is subjected to both dimensions of the separation processes in a single run. The theoretical peak capacity that can be achieved is the product of the peak capacities of the two individual GC separations [3].

For an essential oil, different content ratio among the components will strongly influence the quality of flavor; meanwhile, even some minor components should not be overlooked as they also contribute to the overall qualities of an essential oil. Therefore, the analysis of essential oil should not only provide sufficient separation, but also accurate quantitation of all individual components. With respect to the quantitative determination of all individual components in complex mixture, the traditional method is normalization of peak area [4–6] and single internal standard calibration without considering calibration factors (i.e. supposing the relative response factors equate to one for all compounds) [7]. Normalization of peak area requires that the whole components in the sample could elute from the capillary column and be detected, but it was not true for some non-volatile compounds in the essential oil. Internal standard calibration requires that the functional groups of internal standard molecular should be similar with those of compound determined, in addition, the response factor of flame ionization detector (FID) to different component class is distinctively different, so the single internal standard calibration seems unsuitable for the quantitation of compounds in such a complex sample. For answering this question, the experiments to quantify 10 compounds with known content in tobacco essential oil were done using normalization of peak volume, single internal standard calibration and multiple internal standards calibration, respectively. The results showed that the data from multiple internal standards calibration were closer to the true value than the other two. That is to say, in order to reliably quantify the compounds in essential oil, multiple internal standards calibration should be used.

In this paper, the components in tobacco essential oil were identified by GC × GC time-of-flight mass spectrometer (TOF/MS), and all the individual components in essential oil were quantified by GC × GC-FID using multiple internal standards calibration for the first time, which provided a method to quantify all individual components in complex mixtures. In addition, this paper also compared GC × GC and gas chromatography–mass spectrometry (GC/MS) for the identification and quantitation of the components in tobacco essential oil. The results showed GC × GC had great advantage, especially in its reliable and reproducible quantitative analysis.

2. Experimental

2.1. Instrumentation

The Comprehensive two-dimensional gas chromatograph system consisted of an HP6890GC equipped with a flame ionization detector (Agilent Technologies, Wilmington, DE, USA) and a cold-jet modulator KT-2001 Retrofit prototype (Zoex Corp., Lincoln, NE, USA). The cold-jet modulator consisted of two cold- and two hot-jets, with the nozzles providing the cold-jets mounted orthogonally to the hot-jets. Nitrogen gas was cooled by a heat exchanger through copper tubing immersed in liquid nitrogen outside the GC system and delivered through vacuum-insulated tubing to the cold-jets, which provided two continuous jets of cold nitrogen gas. The GC oven contained two capillary columns connected serially via the cold-jet modulator. The modulator focuses and reinjects the analyte as a sharp chemical pulse into the second dimension GC column. A time-of-flight mass spectrometer (Pegasus, Leco Corporation, St. Joseph, MI, USA) was used to acquire mass spectral data from the GC × GC using 70 eV electron impact ionization, which has a maximum spectral acquisition rate of 500 spectra/s.

Either GC × GC-FID data (100 Hz) or GC × GC-TOF/MS data (50 Hz) may be exported in ASCII file format (*.csv files). The *.csv files were converted into a two-dimensional matrix by a homemade conversion program based on the modulation frequency and sampling rate. The matrix files were read into Transform (part of Noesys Software Package, Research Systems International, Crowthorne, UK) to generate a contour plot. The peaks in contour plot can be integrated and quantified by Zoex software (Zoex Corp., Lincoln, NE, USA).

The one-dimensional GC/MS system used consisted of an HP6890 gas chromatograph and an HP5973N mass spectrometer (Agilent Technologies, Wilmington, DE, USA).

2.2. Columns and conditions

In the analysis of GC × GC-FID and GC × GC-TOF/MS, the columns were connected by means of a press-fit connector, and the two columns were installed in the same oven. The column sets used are listed in Table 1.

The inlet pressure used in this experiment was 600 kPa. Helium was used as the carrier gas, which had a purity of 99.9995%. Injections were performed in the split mode, at a split ratio of 1:30. The injection volume of the essential oil sample was 0.5 μl. The total modulation time was 5 s. The oven temperature program was: 40 °C (1 min hold), at 3 °C/min to 220 °C (30 min hold). The mass spectrometer was operated at an acquisition rate of 50 spectra/s, with an ion-source temperature of 220 °C and a transfer-line temperature of 250 °C. The pressure inside the flight tube was about 10^{-7} Torr. The scanned mass range was from 35 to 500 u.

Table 1
GC × GC column sets

	First column	Second column
Set 1		
Length (m)	50	2.5
Diameter (mm)	0.2	0.1
Stationary phase	DB-petro (100% dimethylpolysiloxane)	DB-17ht (50% phenylmethylpolysiloxane)
Film thickness (μm)	0.5	0.1
Corporation	J&W Scientific, Folsom, CA	J&W Scientific, Folsom, CA
Set 2		
Length (m)	60	3.0
Diameter (mm)	0.25	0.1
Stationary phase	DB-wax (polyethylene glycol)	DB-1701 (14% cyanopropylphenylmethylpolysiloxane)
Film thickness (μm)	0.25	0.4
Corporation	J&W Scientific, Folsom, CA	J&W Scientific, Folsom, CA

In the analysis of GC/MS, a (50 m × 0.20 mm × 0.33 μm) capillary column (HP-5MS, J&W Scientific, Folsom, CA, USA) was used. The column oven was programmed from 40 °C (1 min hold) to 250 °C at 10 °C/min and the final temperature was held for 20 min. The carrier gas was helium with a flow rate of 0.8 ml/min. The split mode (1:30) was used. The injection volume of essential oil sample was 1.0 μl. The mass spectrometer was operated in the electron impact mode (70 eV). The ion source temperature was held at 230 °C. The transfer-line was maintained at 280 °C. The scanned mass range was from 30 to 500 u.

2.3. Analytes and samples

Six calibration solutions containing ethyl acetate, 3-(1-methyl-2-pyrrolidinyl)-pyridine, 1-propanol and 2-ethyl-3-hydroxy-4H-pyran-4-one were prepared in ethanol at a concentration (w/w%) of 0.0005, 0.001, 0.01, 0.1, 1.0 and 2.0%. These calibration compounds were selected because they were present in the essential oil sample and resolved from other components in the GC × GC two-dimensional retention time plane. Four internal standards, acetic acid pentyl ester, 5-ethyl-2-methyl-pyridine, 1-octanol and acetophenone were added in the calibration solutions at a concentration of 0.05% each. These internal standards were selected because they were not present in essential oil and resolved from all essential oil components. A standard solution containing 10 compounds was prepared in ethanol at the following concentration (w/w%): acetic acid (0.218%); ethyl acetate (0.193%); 2-pentanone (0.168%); pentanoic acid, ethyl ester (0.228%); 1-heptanol (0.224%); α-lonone (0.232%); 1-decanol (0.227%); 2,6-dimethyl pyridine (0.186%); vanillin (0.205%); decanoate (0.159%).

The acetic acid, ethyl acetate, 1-propanol and ethanol were of analytical grade quality. The other standard compounds were all in GC purities. Essential oil samples were provided by Wuhan Tobacco Company. Samples were prepared by diluting 0.2500 g of essential oil in 1ml of ethanol. All solutions were refrigerated at 4 °C during storage.

3. Result and discussion

3.1. Selection of GC × GC-TOF/MS column system

Usually, the first column is non-polar, and the second is medium-polar (such as column set 1 in Table 1). Considering that the majority of components in essential oils are polar compounds, we also used a polar column as the first column and a different polar column as second column (column set 2 in Table 1) to improve the separation and symmetry of peaks on the first dimension. The GC × GC-TOF/MS contour plots of essential oil under different column systems are depicted in Fig. 1.

It can be seen from Fig. 1A that an apparent group-type separation of some major components in the sample was obtained when using column set 1. The components identified as esters were found in the region marked (a). Likewise, some components identified as alcohols, ketones and pyridines were located in the region marked (b), (c) and (d), respectively. The group-type separation facilitated the identification of unknown components. When using column set 2, the group-type separation was unapparent. However, the major components were found to spread throughout a wider region of the 2D plane, and the peak shape was better and more individual components were resolved. In this study, the individual components are more interesting to us, so column set 2 was selected to quantify the components in essential oil. Certainly, the group-type separation was also useful, so we also used column set 1 to support the results of column set 2 during the characterization of the essential oil.

3.2. Identification of peaks by GC × GC-TOF/MS

The GC × GC-TOF/MS software was used to find all the peaks in the raw GC × GC chromatogram. A library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0, and the results were combined in a single peak table. The mass spectral match factors include similarity, reverse, and probability. The similarity and reverse factors indicate how well a mass spectrum matches the library spectrum. Be-

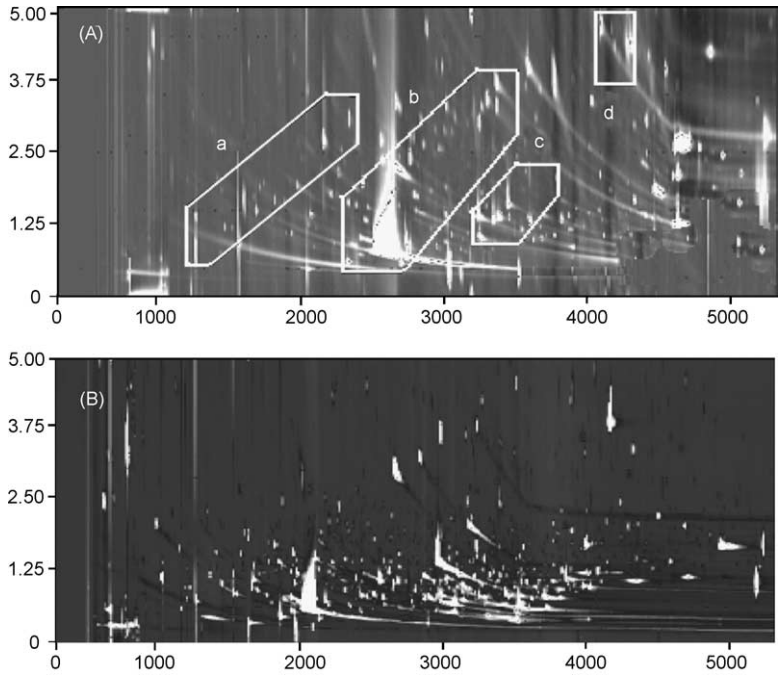

Fig. 1. The GC × GC-TOF/MS contour plots of tobacco essential oil under different column systems. (A) Column set 1, (B) column set 2. *X*-axis: first dimension retention time(s). *Y*-axis: second dimension retention time(s). Zones (a–d) are esters, alcohols, ketones and pyridines, respectively.

cause isomers have similar mass spectra, the information of probability and apex plots was added to determine whether the peaks with the same name belong to one compound or several compounds. According to the literature [8–11], a similarity and reverse number above 800 and 900, respectively, indicates that an acquired mass spectrum usually shows a good match with the library spectrum. A probability value above 9000 means that the mass spectrum is highly unique, and provisional identification based on mass spectra is possible. In total, 172 compounds with good match and 65 compounds (including 4 internal standards) with high probability value were found in the peak table. Table 2 is a detailed list of the 65 compounds. It should be pointed out that the results in Table 2 were obtained from column set 2 and most of the identified compounds were further confirmed by results from column set 1, and the handbook (in Chinese) [12] on flavor chemistry were also referred to for the identification of some components.

3.3. Quantitation of compounds in essential oil by GC × GC-FID

3.3.1. Validation of the quantitative method

A multiple internal standards method was used for quantitative determination of compounds in tobacco essential oils. The relative response factors, linearity, limit of detection (LOD) and the precision of the results were studied with the calibration solution as described in the previous experimental section.

As has been discussed in the previous section, many chemical classes of compounds are present in essential oils. It is difficult to determine the FID response factors for all the individual compounds, owing to the lack of their pure compounds. Fortunately, the response of FID to large numbers of compounds within a component class is very similar and highly constant, so the response factors of a compound in every component class can be used to quantify the individual component in the same component class [13].

In this paper, the response factors for ethyl acetate, 3-(1-methyl-2-pyrrolidinyl)-pyridine, 1-propanol and 2-ethyl-3-hydroxy-4H-pyran-4-one were selected to represent the response factors for ester, pyridine, alcohol and ketone classes, respectively. Acetic acid pentyl ester, 5-ethyl-2-methyl-pyridine, 1-octanol and acetophenone were selected as internal standards. Three GC × GC-FID chromatograms were obtained for each calibration solution. The three-dimensional volume of individual peaks was determined by integration. A baseline region next to each peak was averaged and subtracted from the data before integration. The relative response factor was described as the following equation:

$$f'_{\mathrm{i}} = \frac{V_{\mathrm{s}} W_{\mathrm{i}}}{V_{\mathrm{i}} W_{\mathrm{s}}}$$

where f'_{i} is the relative response factor of a target component; V_{i} and V_{s} are the integrated peak volume of a target component and internal standard, respectively; W_{i} and W_{s} are the mass of a target component and internal standard, respectively.

According the equation above, a plot of average peak volume ratio ($V_{\mathrm{i}}/V_{\mathrm{s}}$) versus mass ratio ($W_{\mathrm{i}}/W_{\mathrm{s}}$) was made for each peak. The slope of linear regression equation was the relative response factors of compounds. The results were pre-

Table 2
Identification by GC × GC-TOF/MS and comparison of quantitation results between GC × GC-FID and 1DGC

No.	^1t_R (s)	^2t_R (s)	Compound name	Similarity	Reverse	Probability	CAS	Content[a] (w/w%)		Relative deviation (%)
								1DGC	GC × GC-FID	
1	497.18	2.50	Ethyl acetate[b]	959	974	9654	141-78-6	1.73	1.72	0.29
2	612.58	3.22	Propanoic acid, ethyl ester[b]	951	951	9240	105-37-3	1.66	1.62	2.57
3	700.66	0.64	Camphene[b]	933	937	9000	79-92-5	0.017	0.012	34
4	723.28	0.85	Butanoic acid, ethyl ester	949	949	9821	105-54-4		0.19	
5	746.48	1.16	1-Propanol[b]	941	944	9666	71-23-8	0.030	0.033	−9.52
6	790.52	1.08	1R-alpha-Pinene[b]	926	926	9548	7785-70-8	0.021	0.014	40
7	913.74	1.26	1-Butanol, 3-methyl-, acetate[b]	845	900	9413	123-92-2	0.97	0.97	0.21
8	913.86	1.29	1-Butanol, 2-methyl-, acetate[b]	826	924	9015	624-41-9	0.011	0.011	0
9	1043.50	2.26	Acetic acid, pentyl ester[c]	909	911	9026	628-63-7	1.11	0.43	
10	1161.30	1.56	1-Butanol, 3-methyl-[b]	932	932	9554	123-51-3	0.032	0.029	9.84
11	1375.66	1.28	Acetic acid ethenyl ester	850	908	9251	108-05-4		0.068	
12	1435.52	1.15	2-Propanone, 1-hydroxy-[b]	927	927	9865	116-09-6	0.037	0.037	0
13	1540.70	1.36	Propanoic acid, 2-hydroxy-, ethyl ester	928	928	9654	97-64-3		0.031	
14	1761.58	1.26	Pyridine, 5-ethyl-2-methyl-[c]	921	921	9542	104-90-5		0.72	
15	1795.88	0.88	Ethane, 1,1-diethoxy-[b]	858	900	9215	105-57-7	0.023	0.006	117
16	1839.50	1.62	Acetic acid[b]	967	967	9514	64-19-7	0.17	0.17	0.59
17	1840.04	1.06	Propanoic acid, 2-oxo-, methyl ester	908	915	9214	600-22-6		0.088	
18	1865.12	1.24	Furfural[b]	913	913	9545	1998-1-1	0.004	0.004	0
19	1908.34	1.58	2-Heptenal, 2-propyl-	916	916	9352	34880-43-8		0.10	
20	2059.24	0.76	Propanoic acid	929	929	9254	1979-9-4		0.032	
21	2070.90	1.02	1,6-Octadien-3-ol,3,7-dimethyl-	911	911	9555	78-70-6		0.005	
22	2079.46	0.64	2,3-Butanediol	943	953	9556	19132-06-0		0.006	
23	2105.56	0.92	1-Octanol[c]	911	911	9542	111-87-5	1.11	0.14	
24	2139.26	1.06	Propylene glycol[b]	892	900	9212	57-55-6	15.33	14.53	5.35
25	2178.02	1.28	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-,acetate[b]	927	927	9110	5655-61-8	0.032	0.020	46
26	2189.70	3.46	2-Cyclopentene-1,4-dione[b]	875	910	9028	930-60-9	0.008	0.006	28
27	2309.56	2.50	Propanoic acid, methyl ester	874	900	9024	554-12-1		0.056	
28	2324.70	2.06	Butyrolactone	892	927	9240	96-48-0		0.032	
29	2365.00	1.32	Acetophenone[c]	950	950	9521	98-86-2	1.27	0.54	
30	2369.00	4.65	2-Furanmethanol[b]	910	913	9620	98-00-0	0.015	0.014	6.90
31	2485.20	0.98	Borneol	900	900	9210	10385-78-1		0.003	
32	2553.64	1.22	Pentanoic acid	911	911	9521	109-52-4		0.047	
33	2623.92	1.14	2(5H)-Furanone	860	900	9510	497-23-4		0.006	
34	2643.66	0.86	2-Cyclopenten-1-one, 2-hydroxy-	905	950	9356	10493-98-8		0.093	
35	2783.64	0.94	2-Propanol, 1,1′-oxybis-[b]	894	900	9515	110-98-5	0.050	0.046	8.33
36	2793.36	1.21	Hexanoic acid	922	946	9432	142-62-1		0.080	
37	2853.68	1.18	2,5-Dimethyl-4-hydroxy-3 (2H)-furanone	838	900	9542	3658-77-3		0.001	
38	2868.48	2.66	Benzyl Alcohol[b]	928	931	9564	100-51-6	0.24	0.20	20
39	2903.46	3.42	1-Propanol,2-(2-hydroxypropoxy)-	913	913	9518	106-62-7		0.006	
40	2910.04	3.68	Pyridine,3-(1-methyl-2-pyrrolidinyl)-[b]	945	945	9235	1954-11-5	0.46	0.46	1.31
41	3009.34	4.02	Triethylene glycol	840	900	9056	112-27-6		0.004	
42	3050.02	1.22	1-Dodecanol	916	916	9345	112-53-8		0.033	
43	3097.88	3.54	Ethanol, 2,2′-oxybis-	961	961	9532	111-46-6		0.008	
44	3118.18	1.28	Maltol	906	944	9423	118-71-8		0.059	
45	3200.16	1.44	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-[b]	943	943	9321	40716-66-3	0.033	0.034	-2.98
46	3208.28	0.95	4H-Pyran-4-one, 2-ethyl-3-hydroxy-[b]	854	900	9233	4940-11-8	1.07	1.07	0.09
47	3217.74	0.98	2,5-Dimethyl-4-hydroxy-3 (2H)-furanone	895	905	9312	3658-77-3		0.024	
48	3317.28	3.86	Propanal, 2,3-dihydroxy-	857	900	9351	367-47-5		0.006	
49	3317.46	3.75	2-Propanone, 1,3-dihydroxy-	872	900	9215	96-26-4		0.37	
50	3408.84	1.38	2-Propenoic acid, 3-phenyl-, ethyl ester	929	952	9853	103-36-6		0.074	
51	3438.58	1.50	2-Propen-1-ol, 3-phenyl-, acetate	944	951	9756	103-54-8		0.13	
52	3439.06	1.13	Acenaphthene	961	962	9335	83-32-9		0.010	
53	3474.86	1.32	Pentaethylene glycol	838	900	9215	4792-15-8		0.016	
54	3478.08	1.68	Eugenol[b]	905	943	9153	97-54-1	0.13	0.12	5.62
55	3492.32	4.02	1,3-Propanediol	890	910	9352	504-63-2		0.005	
56	3682.04	3.84	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-[b]	896	906	9324	28564-83-2	0.008	0.005	46
57	4021.54	1.16	Benzoic acid[b]	874	914	9432	65-85-0	0.019	0.019	0

Table 2 (*Continued*)

No.	^1t_R (s)	^2t_R (s)	Compound name	Similarity	Reverse	Probability	CAS	Content[a] (w/w%)		Relative deviation (%)
								1DGC	GC × GC-FID	
58	4087.96	1.18	2H-1-Benzopyran-2-one[b]	889	900	9002	91-64-5	0.072	0.071	1.40
59	4171.72	0.82	2-Furancarboxaldehyde, (5-(hydroxymethyl)-[b]	869	905	9543	67-47-0	0.036	0.010	113
60	4316.34	3.74	Benzeneacetic acid	912	912	9351	103-82-2		0.038	
61	4357.14	2.52	Vanillin[b]	908	908	9021	121-33-5	0.060	0.059	1.68
62	4371.22	1.84	1,2-Ethanediol, 1-(2-furanyl)-	861	900	9315	19377-75-4		0.013	
63	4461.40	2.28	2(3H)-Furanone, dihydro-4-hydroxy-	864	900	9135	5469-16-9		0.066	
64	4579.42	1.26	Benzyl benzoate[b]	941	941	9532	120-51-4	1.33	1.32	0.30
65	4700.22	1.18	Glycerin[b]	897	900	9201	56-81-5	0.23	0.22	3.56

[a] Content is the gravimetric percent of compounds in the ethanol solution, the relative response factor of ethyl acetate, 1-propanol, 3-(1-methyl-2-pyrrolidinyl)-pyridine and 2-ethyl-3-hydroxy-4H-pyran-4-one was used to quantify the ester, alcohol, pyridine and ketone group, respectively.
[b] Peaks were also identified by one-dimensional GC/MS.
[c] Internal standards.

sented in Table 3. From Table 3, it can be seen that the linearity is good in the following concentration range: 0.001–2.00% for ethyl acetate, 0.0005–0.30% for 1-propanol, 0.001–1.00% for 3-(1-methyl-2-pyrrolidinyl)-pyridine and 0.001-1.00% for 2-ethyl-3-hydroxy-4H-pyran-4-one, the correlation coefficients (R^2) were between 0.9950–0.9997. The LOD was in the 0.1 ppm level. The relative standard deviation (RSD) was below 8.1%.

In order to compare the three quantitation methods (normalization of peak volume, single internal standard calibration and multiple internal standards calibration) and explain the superiority of multiple internal standards calibration, a standard solution containing 10 compounds were analyzed and quantified using three methods. When using single internal standard calibration, 1-octanol was selected as internal standard. The quantitation results were listed in Table 4. It can be seen from Table 4 that the results from multiple internal standards calibration were closer to the true value than that from the other two.

3.3.2. Quantitation of the individual compounds in essential oil sample

It had been proved in the previous section that multiple internal standards calibration was more suitable for the quantitation of the individual compounds in essential oil. In this study, each compound in the GC × GC chromatogram in Fig. 1 was quantified using the relative response factor of the representative compound within the class. For example, the relative response factor of ethyl acetate was used to quantify the ester group. Likewise, the relative response factor of 1-propanol was used to quantify the alcohol group, the relative response factor of 3-(1-methyl-2-pyrrolidinyl)-pyridine was used to quantify the pyridine group, and the relative response factor of 2-ethyl-3-hydroxy-4H-pyran-4-one was used to quantify the ketone group. As for some minor components (such as aldehyde and hydrocarbon classes), on the one hand, only a few of them existed in essential oils, on the other hand, their calibration factors were close to ketone and alcohol classes, respectively [14], so we used the relative response factor of ketone and alcohol classes to quantify the aldehyde and hydrocarbon classes, respectively. The individual peaks were integrated and quantified in terms of w/w%. The quantitation results were listed in Table 2.

For comparative purposes, the essential oil sample was also quantified by one-dimensional GC/MS with multiple internal standards method. The total ion current (TIC) chromatogram of tobacco essential oil is shown in Fig. 2. The numbered peaks refer to the identities of some of the components listed in Table 2 (some minor components not labeled). In total, 29 components with match quality >80% were identified and quantified by one-dimensional GC/MS (see Table 2). All this 29 components can be identified and quantified credibly by GC × GC-TOF/MS. The quantitation results were also listed in Table 2. From Table 2, it can be seen that there was close agreement (relative deviation <10%) for 21 of 29 components between the two analysis methods.

According to Table 2, it can also be seen that the content value from one-dimensional GC/MS is usually higher than that from GC × GC-FID for most components. It is probably because more overlapping peaks exist in one-dimensional

Table 3
Linear ranges, relative response factors, correlation coefficients (R^2), limit of detection (LOD), and relative standard deviation (RSD)

Compound	Internal standard	Linear range (w/w%)	Relative response factors	R^2	LOD[a] (w/w%)	RSD%
Ethyl acetate	Acetic acid pentyl ester	0.001–2.00	1.08	0.9992	8.8×10^{-5}	3.5
1-Propanol	1-Octanol	0.0005–0.30	0.97	0.9997	2.5×10^{-5}	7.1
3-(1-Methyl-2-pyrrolidinyl)-pyridine	5-Ethyl-2-methyl-pyridine	0.001–1.00	1.02	0.9950	1.0×10^{-5}	6.3
2-Ethyl-3-hydroxy-4H-pyran-4-one	Acetophenone	0.001–1.00	1.13	0.9980	1.1×10^{-5}	8.1

[a] LOD: S/N = 3.

Table 4
Quantitation results of 10 compounds in the standard solution using three methods

Compound	Content (w/w%)	Method 1[a]		Method 2[b]		Method 3[c]	
		Content (w/w%)	Relative deviation (%)	Content (w/w%)	Relative deviation (%)	Content (w/w%)	Relative deviation (%)
Acetic acid	0.218	0.205	−5.9	0.198	−9.2	0.221	1.4
Ethyl acetate	0.193	0.167	−13.6	0.161	−16.6	0.190	−1.6
2-Pentanone	0.168	0.171	1.8	0.165	−1.8	0.165	−1.8
Pentanoic acid, ethyl ester	0.228	0.195	−14.6	0.188	−17.5	0.229	0.4
1-Heptanol	0.204	0.216	5.7	0.208	2.0	0.200	−2.0
α-Lonone	0.232	0.234	1.0	0.226	−2.6	0.230	−0.9
1-Decanol	0.227	0.238	5.0	0.230	1.3	0.230	1.3
2,6-Dimethyl pyridine	0.186	0.216	15.9	0.208	11.8	0.190	2.2
Vanillin	0.205	0.223	8.7	0.215	4.9	0.212	3.4
Decanoate	0.159	0.150	−5.5	0.145	−8.8	0.162	1.9

[a] Normalization of peak volume (100% − method).
[b] Single internal standard calibration.
[c] Multiple internal standards calibration.

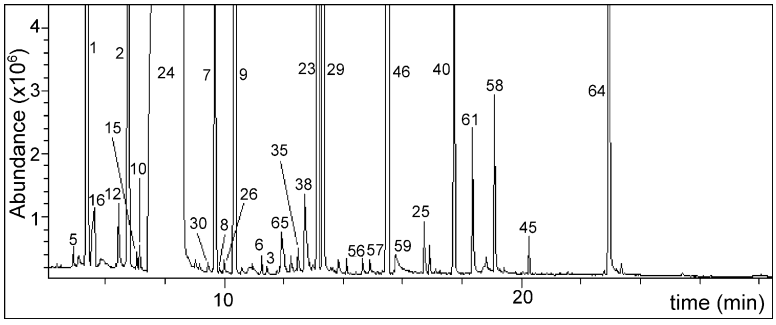

Fig. 2. The one-dimensional GC/MS chromatogram (TIC) of tobacco essential oil. The numbers refer to those in Table 2 (some minor components not labeled).

GC/MS, which can interfere with the peak integration. But in GC × GC, higher resolving power and less co-elution leads to a more accurate and precise peak integration.

Considering the different results obtained from the two analysis methods, we found the fact that the higher the peak purity is in one-dimensional GC/MS, the closer the result from the two analysis methods is. That is to say, what resulted in the significant difference (relative deviation >10%) for the eight components is that the peaks are impure, as can be seen from the mass spectra of the compounds. According to our experiences, the additional fragments in the mass spectrum of compound are probably fragments of other compounds. In order to confirm the opinions described above, the AMDIS analysis program of GC/MS was used for the deconvolution of the individual peaks. The result showed that the amount of component in peak numbered 6 and 59 was 2 and 3, respectively. It can also be seen from Fig. 2 that peak numbered 6 and 59 overlapped with their neighboring peaks and tailed in a certain extent.

Another conclusion could be obtained from the discussion above that the peak purity in one-dimensional GC/MS was not always high when the match quality was high between mass spectrum and library spectrum. But in GC × GC, the mass spectral match factors include similarity, reverse, and probability. When the three factors are all high enough, the mass spectrum will be highly unique and the peak will be highly pure [8–10]. This also leads to a more accurate and reliable quantitative determination of individual components in GC × GC-FID.

4. Conclusions

In this study, a powerful separation of tobacco essential oil has been performed by GC × GC-TOF/MS and the multiple internal standards calibration was used to quantify all the individual components in essential oil for the first time. Compared to one-dimensional GC/MS, GC × GC showed higher resolving power and peak capacity. Moreover, there was close agreement between the two analysis methods when the peak purity and match quality in one-dimensional GC/MS are high enough, even though the system used in the two methods is completely different. By comparing the quantitative results obtained from the two methods, it could be concluded that the GC/MS caused an overestimation of the concentration of some compounds, owing to the existence of more overlapping peaks.

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References

[1] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, Allured Publ., Carol Stream, IL, 1995.
[2] P. Marriott, R. Shellie, Trend Anal. Chem. 21 (2002) 573.
[3] J.B. Phillips, J. Beens, J. Chromatogr. A 856 (1999) 331.
[4] R. Shellie, L. Mondello, P. Marriott, G. Dugo, J. Chromatogr. A 970 (2002) 225.
[5] J. Rohloff, J. Agric. Food Chem. 50 (2002) 1543.
[6] A.R. Bilia, G. Flamini, V. Taglioli, I. Morelli, F.F. Vincieri, Food Chem. 76 (2002) 307.
[7] J. Cai, B. Liu, P. Ling, Q. Su, J. Chromatogr. A 947 (2002) 267.
[8] X. Lu, J. Cai, H. Kong, M. Wu, R. Hua, M. Zhao, J. Liu, G. Xu, Anal. Chem. 75 (2003) 4441.
[9] M. van Deursen, J. Beens, J. Reijenga, P. Lipman, C. Cramers, J. High Resol. Chromatogr. 23 (2000) 253.
[10] J. Dalluge, L.L.P. van Stee, X. Xu, J. Williams, J. Beens, R.J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 974 (2002) 169.
[11] J. Wu, X. Lu, W. Tang, H. Kong, S. Zhou, G. Xu, J. Chromatogr. A 1034 (2004) 199.
[12] Handbook of Tobacco Industry, Chinese Light Industry Press, Beijing, 1999, p. 351.
[13] P.J. Schoenmakers, J.L.M.M. Oomen, J. Blomberg, W. Genuit, G. van Velzen, J. Chromatogr. A 892 (2000) 29.
[14] Handbook of Analytical Chemistry, vol. 5, Chemical Industry Press, Beijing, 1999, p. 698.