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Comparison of different extraction methods: steam distillation, simultaneous distillation and extraction and headspace co-distillation, used for the analysis of the volatile components in aged flue-cured tobacco leaves

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Abstract

Steam distillation (SD), simultaneous distillation and extraction (SDE) and headspace co-distillation (HCD) were compared here for their effectiveness in the extraction of volatile compounds from tobacco. The different grades of aged flue-cured tobacco leaves extracted by the three methods respectively were analyzed using GC–MS. Mass spectra or authentic compounds were used to identify around 408 components in various volatile fractions. On the one hand, the qualitative comparison showed that more compounds were detected in HCD extract (391 components) than in SDE extract (377 components), and the approximately quantitative analysis showed that the total amount of volatile components in SDE extract (445.48 μg/g) was much more than that in HCD extract (315.72 μg/g). But on the other hand, HCD was the most efficient for nearly all the highly volatile compounds among the three methods. As to low-volatile compounds such as lactones, long chain aldehydes, ketones, alcohols, and esters, more was detected in SDE extract than in HCD extract. The SD method (322 components, total amount 228.42 μg/g) was the lowest sensitive to all compounds except semi-volatile fatty acids among the three methods.

Keywords: Steam distillation; Simultaneous steam distillation-solvent extraction; Headspace co-distillation; Extraction methods; Tobacco; Volatile organic compounds

1. Introduction

Tobacco, whose principal tobacco type used for cigarette manufacture are flue-cured, burley and orientals, is one of the most widely consumed items. Flue-cured tobacco, which is also called Bright or Virginia, derives its name from the unique curing process. Freshly cured tobacco leaves are not suitable for use because of its pungent and irritating smoke. After the process of aging and fermentation, the leaves deliver mild, aromatic smoke. The aging process of tobacco is necessary prior to cigarette manufacturing. Thereby volatile component in aged flue-cured tobacco is a very important factor to appraise the quality and the commercial values of tobacco. Because the volatile components of tobacco are very complex and the content of many important components in tobacco are in trace level, suitable sample-preparing methods and sensitive analyzer are indispensable. So many techniques for sample preparing of volatile components in tobacco have been developed. In those studies, sample-preparing techniques were mostly based on solvent extraction and distillation. Among all the sample-preparing techniques, steam distillation (SD), simultaneous distillation and extraction (SDE) and headspace co-distillation (HCD) are the most widely used methods.

SD [1–7] provides a simple means for the recovery of volatile components of tobacco in the past but unfortunately it was overlaborate and solvent-consuming. Also the volatile components were significantly diluted by water when being

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collected. This fault was overcome by SDE method [8–11] by solvent extraction of the distillate [12]. SDE remains popular in the aroma research area and the Likens–Nickerson apparatus has been a standard for a long time [10]. HCD method [13] using a simple, easily constructed glass apparatus allows distillation of volatile from tobacco samples under the continuous stream of an inert gas. This method combines the virtues of extraction, distillation and headspace enrichment.

The different sampling techniques offer a number of individual advantages but also suffer from some specific limitations [15,16]. Here we studied qualitative and approximately quantitative comparison of volatile components of different grades of flue-cured tobacco leaves prepared by the three methods: SD, SDE, and HCD using GC–MS. The aims of our work are to compare the influence of SD, SDE, and HCD on the compounds, when they are used in the analysis of the volatile components of tobacco. The experiment shows that there are 408 volatile components detected in all; relative standard deviations (R.S.D.) were calculated for all of them to evaluate the repeatability of the three methods. Based on all of this, the effectiveness of the extraction to the volatile components of the three methods was compared in details.

2. Materials and methods

2.1. Materials

The different grades of aged flue-cured tobaccos leaves harvested in different regions including Tongxin (China), Dali (China), Qujing (China), Baokang (China) in 2001 (Xian Fan Cigarette Manufactory of China) were all collected randomly. A 1 kg aliquot of each sample was mixed thoroughly and then ground to 40–60 mesh powder.

All solvents employed were of analytical grade quality and were redistilled before use. Technology Center of Xiang Fan Cigarette Manufactory of China provided 67 kinds of authentic compounds used to confirm mass spectra and retention indices. They were marked with an asterisk (∗) in Table 1 and were purchased from Sigma–Aldrich (St. Louis, MO, USA) or Tokyo Kasei (Nihonbashi, Tokyo, Japan). All of them were refrigerated during storage.

2.2. Extraction of volatile components

2.2.1. Steam distillation/solvent extraction

A 10.0 g tobacco sample was added to 40 ml dichloromethane. Then the mixture was shaken overnight and steam distillated for 3 h to obtain 800 ml aqueous solution of volatile components using a simple apparatus [2,3]. This solution was extracted four times with 60 ml dichloromethane (60 ml × 4) and then the combined dichloromethane was concentrated to 40 ml in a rotary evaporator (R-201, Shanghai Shenbo, China) at less than 50 °C under reduced pressure.

2.2.2. Simultaneous distillation and extraction

SDE [8,9] was carried out in a micro version apparatus [17]. For each extract, 10.0 g tobacco sample, 140 g sodium sulfate and 350 ml redistilled water were placed in a 1000 ml flask; 40 ml dichloromethane in a 100 ml flask was placed in a 60 °C water-bath; and they were both distilled for 2.5 h at atmospheric pressure. At last, about 40 ml extract obtained.

2.2.3. Headspace co-distillation

A simple, easily constructed glass apparatus was used by this method [13]. A 10.0 g tobacco sample was added to 40 ml dichloromethane and the mixture was shaken overnight. Then the sample and 350 ml fresh distilled water were added into a 1000 ml flask. A stream of ultrahigh purity nitrogen (99.999%) was used to purge the sample for 10 min. And then the flask was heated in an oil bath at 130 °C with nitrogen flow at a rate of 15 ml/min. The vapor carried by nitrogen was condensed below −5 °C in cold traps. After 3 h distillation, the distillate was extracted four times with 40 ml dichloromethane (40 ml × 4). The combined dichloromethane was concentrated to 40 ml in a rotary evaporator at less than 50 °C under reduced pressure.

2.3. Preparation of the samples

The dichloromethane solutions of volatile components obtained by three methods were extracted twice with 20 ml of a 5 wt.% aqueous solution of sodium hydroxide (20 ml × 2) and reextracted twice with 10 ml dichloromethane (10 ml × 2) to obtain the acidic fraction. Then the combined dichloromethane solution was extracted twice with 20 ml of a 5 wt.% aqueous solution of hydrochloric acid (20 ml × 2) and extracted twice with 10 ml dichloromethane (10 ml × 2) to obtain the basic fraction. The residual of combined dichloromethane solution represented the neutral fraction. The acidic fraction was extracted four times with 20 ml dichloromethane (20 ml × 4) at the pH 1. In the same way, the basic fraction was extracted at the pH 13. The 1 ml internal standard (62.40 μg/ml heptadecane) was added to the neutral fraction, 200 μl to the acidic fraction, and 100 μl to the basic fraction. After dried by anhydrous sodium sulfate, the three fractions were all concentrated to 5 ml in a rotary evaporator at less than 50 °C under reduced pressure first and then concentrated to 1 ml using the a stream of ultrahigh purity nitrogen (99.999%) before GC–MS analysis. A series of five consecutive extracts were performed on different aliquots of samples in order to evaluate the repeatability of three methods.

2.4. GC–MS analysis

GC–MS was used for both qualitative and quantitative analyses. It consists of a Trace GC 2000 system and a GC–Q plus ion trap MS system (Finnigan, Thermo Electron Corporation of America) with electron impact ionization mode. Chromatographic separations were performed on a HP-5MS

Table 1
Volatile components in tobacco leaves

t_R (min)	Compound	CAS	Approx. concn. (μg/g)			R.S.D. (%, $n = 5$)			Match factor	
7			SD	SDE	HCD	SD	SDE	HCD	SI	RSI
1 Alcohols aliphatics										
4.91[n]	1,2-Propanediol*	57-55-6	0.02	0.01	0.08	6.98	8.92	4.20	926[e]	941
5.82[n]	3-Methylbutanol	123-51-3	nd	0.01	0.04	nd	6.96	23.76	813[e]	896
9.11[n]	1-Hexanol	111-27-3	nd	0.07	0.02	nd	7.14	16.07	896[e]	900
13.70[n]	6-Methyl-5-hepten-2-ol*	4630-6-2	tra	0.04	0.01	nd	7.10	25.63	917[f]	927
14.31[n]	2-Hexen-1-ol	928-95-0	nd	0.06	0.04	nd	9.17	10.42	802[g]	854
14.43[n]	2,6-Dimethyl-3,5-heptadien-2-ol	77411-76-8	tra	0.16	0.08	nd	8.50	13.66	811[e]	834
17.27[a]	2-Ethyl-1-hexanol	104-76-7	nd	0.02	tra	nd	4.93	nd	845[f]	897
25.73[n]	2-Cyclohexyl-hexan-2-ol	ID#: 76947	0.04	0.25	0.17	16.39	9.12	9.26	742[e]	753
39.20[n]	2-(2-Ethyl-1,3-dimethyl-cyclopent-2-enyl)-propan-2-ol	N#: 186824	0.15	0.23	0.20	10.70	10.18	8.26	805[g]	815
59.28[n]	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7	0.57	0.41	0.51	7.34	15.95	8.99	800[g]	833
Aromatics										
14.76[n]	Benzyl alcohol*	100-51-6	0.85	6.01	4.73	9.40	2.15	5.85	900[e]	912
17.73[n]	Phenylethyl alcohol*	60-12-8	0.58	4.28	3.45	15.52	3.35	6.96	893[f]	902
31.60[n]	1,2,3,4-Tetrahydro-2,5,8 trimethyl-1-naphthalenol terpenoids	55591-08-7	0.15	3.05	0.99	13.45	5.33	6.49	805[e]	846
16.78[n]	*trans*-Linalool oxide	23007-29-6	0.05	0.17	0.11	21.23	8.44	9.27	842[f]	849
17.33[n]	*cis*-Linalool oxide	5989-33-3	nd	0.08	0.08	nd	13.51	8.77	781[g]	829
17.82[n]	Linalyl alcohol*	78-70-6	0.01	0.10	0.04	4.58	3.24	7.45	888[e]	926
20.95[n]	Terpineol*	98-55-5	tra	tra	0.02	nd	nd	14.97	944[f]	945
23.68[n]	*p*-Menthane-1,2,3-triol	104153-60-8	0.01	0.11	0.17	17.78	11.78	8.40	791[e]	791
23.92[n]	Lemonol*	106-24-1	nd	0.04	0.03	nd	13.54	16.14	901[e]	906
23.45[a]	Isophorol	470-99-5	nd	nd	0.01	nd	nd	14.72	832[f]	872
25.19[n]	Dihydronopol	ID#: 58751	nd	0.09	0.06	nd	10.77	11.93	865[e]	882
26.20[n]	β-Cyclohomogeraniol	472-65-1	0.08	0.20	0.23	16.27	10.22	8.03	768[e]	808
26.90[n]	Artemesia alcohol	57590-19-9	0.01	0.07	0.10	24.38	17.08	8.43	804[g]	841
29.15[n]	2,3-Pinanediol	22422-34-0	0.08	0.06	0.06	7.16	14.27	9.19	800[g]	885
31.88[n]	Nerolidol	142-50-7	0.24	0.77	0.60	9.62	8.29	8.55	832[f]	885
43.61[a]	*cis*-Chrysanthemol	18383-59-0	0.05	0.15	0.07	12.84	4.99	14.98	800[e]	820
52.06[n]	Widdrol	6892-80-4	0.09	0.24	0.05	13.91	6.33	18.49	795[g]	805
52.67[n]	Hexahydrofarnesol	6750-34-1	0.57	0.76	0.61	8.97	7.00	8.58	838[e]	878
48.20[a]	Phytol	150-86-7	0.78	1.71	0.68	2.11	4.65	9.19	881[f]	887
52.86[n]	Cedrol	77-53-2	0.07	0.45	0.12	24.05	6.70	20.59	810[e]	863
53.20[n]	α-Santalol	115-71-9	0.06	0.28	tra	17.95	6.76	nd	811[g]	812
63.28[n]	Isophytol	505-32-8	nd	0.08	0.13	nd	18.70	8.56	852[e]	896
67.60[n]	Farnesol	3790-71-4	1.02	1.22	1.29	10.11	12.28	8.13	827[g]	851
86.36[n]	Geranyl linalool isomer furfurals	ID#: 201890	0.31	0.70	0.50	14.40	9.02	8.57	843[f]	844
8.28[n]	2-Furanmethanol*	98-00-0	0.24	1.81	5.50	10.22	3.04	10.33	897[e]	939
11.92[n]	5-Methyl-2-furanmethanol	3857-25-8	0.28	1.14	1.08	3.64	4.79	4.48	802[g]	861
12.78[n]	(5-Methyltetrahydro-2-furanyl) methanol	54774-28-6	0.01	0.07	0.05	24.24	15.65	17.20	838[e]	863
Ionol derivatives										
32.02[n]	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butan-2-ol	ID#: 91116	1.42	3.07	2.21	6.24	4.10	6.73	807[e]	886
37.39[n]	β-Ionol	22029-76-1	0.30	0.61	0.17	8.29	6.67	19.29	815[f]	848
41.85[n]	Isomethyl-β-ionol	70172-00-8	0.17	0.26	0.23	11.25	10.70	8.15	795[e]	795
42.52[n]	3-Hydroxy-7,8-dihydro-β-ionol	172705-13-4	0.35	1.93	0.54	3.99	4.14	6.80	851[f]	865
Others										
4.52[a]	2-Ethoxyethanol	110-80-5	0.18	0.31	0.18	9.95	5.35	8.86	852[e]	881
66.16[n]	Cembra-2,7,11-trien-4,5-diol	N#: 140923	0.81	1.00	1.06	10.36	8.11	8.11	847[e]	879
68.33[n]	β-Retinol	68-26-8	3.09	2.99	1.29	6.48	9.52	9.78	798[g]	826
69.25[n]	Contortadiol	1857-24-5	19.72	24.59	21.63	7.46	4.78	4.65	834[e]	843
70.34[n]	4,8,13-Duvatriene-1,3-diol	7220-78-2	3.57	5.21	4.33	10.05	10.02	8.36	857[f]	860
71.06[n]	4,8,13-Duvatriene-1,3-diol	7220-78-2	3.31	4.28	3.35	8.66	9.87	8.71	856[e]	874
71.50[n]	4,8,13-Duvatriene-1,3-diol	7220-78-2	2.54	7.09	2.74	11.69	6.93	12.91	861[e]	864
73.79[n]	Duvatriendiol 1	ID#: 217619	2.91	4.92	5.17	12.31	7.90	5.29	868[e]	908
75.20[n]	Duvatriendiol 2	ID#: 217619	6.02	10.74	4.83	8.50	7.38	11.66	871[f]	902
75.95[n]	Strophanthidol	560-54-3	1.52	2.00	1.58	8.82	9.85	8.67	801[g]	809
77.78[n]	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4, 5-diol	N#: 140923	0.59	1.02	0.71	10.42	8.85	12.75	802[e]	808

Table 1 (*Continued*)

t_R (min)	Compound	CAS	Approx. concn. (μg/g)			R.S.D. (%, $n = 5$)			Match factor	
7			SD	SDE	HCD	SD	SDE	HCD	SI	RSI
78.12[n]	4,8,13-Duvatriene-1,3-diol	7220-78-2	2.92	3.53	1.98	7.19	8.74	10.99	868[f]	871
78.57[n]	4,8,13-Duvatriene-1,3-diol	7220-78-2	5.71	7.56	2.40	6.98	7.65	9.46	871[e]	875
79.08[n]	4,8,13-Duvatriene-1,3-diol	7220-78-2	6.47	6.17	3.28	6.51	9.87	13.46	866[f]	877
79.25[n]	labda-8(20),14-Diene-6,13-diol	1438-66-0	1.43	2.85	0.91	8.42	6.78	6.30	823[e]	827
79.71[n]	Thunbergol	25269-17-4	1.93	2.80	1.77	8.44	8.64	9.43	812[e]	823
80.10[n]	Cedrenol	28231-03-0	1.09	1.50	0.77	7.50	8.13	11.23	801[g]	824
80.72[n]	4,8,13-Duvatriene-1,3-diol	7220-78-2	0.41	1.23	0.29	10.89	6.41	12.23	856[f]	876
80.94[n]	4,8,13-Duvatriene-1,3-diol	7220-78-2	0.21	0.97	0.21	15.55	6.45	14.68	872[f]	881
81.80[n]	Thunbergol 2 aldehydes aliphatics	25269-17-4	1.50	1.66	1.15	7.31	9.74	9.81	877[e]	877
3.27[n]	2-Butenal	4170-30-3	0.01	0.02	0.03	23.69	20.12	21.28	876[f]	905
3.37[n]	3-Methyl-butanal	590-86-3	0.02	0.02	0.03	19.50	18.59	17.94	888[e]	898
3.50[n]	2-Methylbutanal	96-17-3	tra	0.10	0.06	nd	19.43	20.25	858[f]	849
7.55[n]	4-Methylpent-2-enal	ID#: 6011	nd	nd	0.02	nd	nd	23.83	874[f]	884
8.06[n]	2-Hexenal	6728-26-3	0.02	0.03	0.05	22.91	15.52	17.97	800[e]	980
13.86[n]	2,4-Heptadienal	4313-3-5	tra	0.02	0.11	nd	18.94	12.78	811[e]	886
Aromatics										
11.72[n]	Benzaldehyde*	100-52-7	0.15	0.30	0.38	15.29	4.94	9.59	927[e]	953
14.86[n]	Benzeneacetaldehyde*	122-78-1	0.99	2.79	3.15	8.53	3.25	4.48	911[f]	923
15.88[n]	*p*-Methylbenzaldehyde	104-87-0	tra	0.22	0.02	nd	6.22	24.73	946[f]	984
22.73[n]	Aubepine*	123-11-5	nd	0.09	0.10	nd	13.58	8.76	901[g]	946
31.52[a]	2,3,4,5-Tetramethyl-benzaldehyde	29344-95-4	nd	0.23	0.71	nd	11.96	6.34	828[f]	855
33.21[a]	Vanillin*	121-33-5	0.06	0.48	0.19	23.66	5.22	3.14	905[f]	919
45.70[a]	4-Hydroxy-3,5-di-tert.-butylbenzaldehyde	1620-98-0	0.11	0.13	0.08	9.53	5.70	14.33	830[e]	862
	terpenoids									
18.96[n]	Citronellal	106-23-0	nd	0.04	0.03	nd	17.35	15.56	809[g]	813
21.06[n]	Safranal	116-26-7	0.01	0.24	tra	23.87	9.91	nd	860[f]	868
21.70[n]	1-*p*-Menthen-9-al	29548-14-9	nd	0.07	tra	nd	16.34	nd	808[e]	864
21.95[n]	β-Cyclocitral	432-25-7	0.10	0.41	0.40	3.36	4.20	7.38	807[e]	831
Furfurals										
7.18[n]	Furfural*	98-01-1	1.27	12.61	5.15	6.34	3.61	5.71	963[f]	964
11.80[n]	5-Methyl-2-frfural*	620-02-0	0.07	3.25	0.81	9.02	3.37	7.06	909[e]	920
25.26[a]	5-(Hydroxymethyl)-2-furfural	67-47-0	nd	0.26	0.35	nd	4.67	5.51	881[f]	895
Others										
20.00[n]	2-Isopropyl-5-oxohexanal	15303-46-5	0.04	0.19	0.34	13.12	10.92	8.32	803[e]	891
50.61[n]	Liguhodgsonal	64185-18-8	tra	2.08	1.80	nd	5.87	3.89	806[f]	815
55.56[n]	Isovelleral	37841-91-1	6.11	8.09	7.39	4.19	2.82	4.33	811[e]	815
70.72[n]	3 Ketones aliphatics	116-31-4	4.18	4.75	4.00	8.16	10.63	8.76	808[f]	820
3.81[n]	1-Penten-3-one	1629-58-9	0.04	0.10	0.15	22.80	8.79	9.00	871[e]	926
4.86[n]	(*E*)-3-Penten-2-one	3102-33-8	0.22	0.12	0.34	9.46	7.36	13.42	815[f]	852
6.09[a]	2,4-Pentanedione	123-54-6	0.01	0.04	0.09	21.21	15.22	13.32	841[e]	890
6.78[a]	2,3-Pentanedione	600-14-6	0.03	0.05	0.16	19.67	11.89	2.94	829[f]	885
7.32[a]	1,3-Cyclopentanedione	3859-41-4	nd	0.01	nd	nd	17.13	nd	770[g]	833
8.63[n]	2-Cyclopentene-1,4-dione	930-60-9	0.63	0.22	1.46	8.16	12.49	5.80	849[f]	922
9.87[n]	1-(2-Methyl-2-cyclopenten-1-yl)-ethanone	1767-84-6	0.04	0.18	0.10	13.11	3.07	9.68	802[e]	826
10.32[n]	2,5-Hexanedione	110-13-4	nd	0.01	0.03	nd	25.61	18.62	833[f]	836
10.47[n]	5-Hexene-2-one	109-49-9	nd	nd	0.05	nd	nd	17.02	847[e]	928
11.31[a]	1,2-Cyclopentanedione	3008-40-0	tra	0.06	0.08	nd	6.24	11.74	872[e]	887
11.33[n]	3-Hexene-2,5-dione	4436-75-3	nd	0.05	0.07	nd	19.30	18.56	865[e]	895
12.08[n]	6-Methyl-2-heptanone	928-68-7	0.22	0.13	0.13	8.24	12.06	11.30	835[f]	877
13.02[n]	2,4-Dimethyl-1-penten-3-one	3212-68-8	tra	tra	0.01	nd	nd	19.02	813[f]	874
13.22[n]	6-Methyl-5-hepten-2-one*	110-93-0	0.14	0.17	0.37	5.79	5.88	8.59	909[e]	914
14.58[n]	2,6-Dimethylcyclohexanone	2816-57-1	0.02	0.14	0.13	24.24	11.38	8.60	800[e]	844
14.50[a]	1,2-Cyclohexanedione	765-87-7	0.01	0.04	0.07	24.00	8.22	3.93	879[f]	899
15.28[n]	3-Methyl-2-cyclohexen-1-one	1193-18-6	nd	0.07	0.10	nd	16.43	8.60	914[e]	926
15.37 [n]	1-(1,2,3-Trimethyl-cyclopent-2-enyl)-ethanone	70987-81-4	0.04	0.09	0.06	16.28	11.43	13.98	820[f]	894
17.57[n]	3,4,4-Trimethyl-2-cyclo-penten-1-one	30434-65-2	nd	0.04	0.07	nd	20.94	14.55	813[e]	894
19.39[n]	(*R*,*S*)-5-Ethyl-3e-hepten-2-one	57283-79-1	0.03	0.03	0.08	19.44	26.93	10.14	800[g]	837
19.54[a]	1-Methyl-2,6-cyclohexanedione	1193-55-1	nd	nd	0.04	nd	nd	15.72	819[f]	888

Table 1 (*Continued*)

t_R (min)	Compound	CAS	Approx. concn. (μg/g)			R.S.D. (%, $n = 5$)			Match factor	
7			SD	SDE	HCD	SD	SDE	HCD	SI	RSI
21.54[a]	4,5-Dimethyl-2-cyclohexen-1-one	5715-25-3	nd	0.34	0.15	nd	5.38	7.10	846[e]	887
28.56[n]	4-Methyl-5-isopropyliden-8-oxo-nona-1,3-diene	60714-16-1	0.60	0.29	0.15	6.09	8.16	9.95	804[f]	844
33.27[n]	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)butan-2-one	20483-36-7	0.04	0.28	0.08	20.84	7.35	17.90	833[f]	860
33.41[n]	4-(3-Oxobutyl)hexahydro-3,3,4-trimethyl-l-benzoxirene-2,5-dione	N#: 196815	0.07	0.04	0.10	13.62	17.20	12.31	794[g]	796
36.04[n]	7-Neopentylidene-bicyclo[3.3.0]octan-2-one	N#: 158896	tra	1.74	0.47	nd	7.44	8.11	790[g]	812
38.18[n]	5,5-Dimethyl-2-propyl-1,3-cyclohexanedione	1919-64-8	0.41	0.75	0.56	6.62	3.19	4.50	789[g]	874
41.05[n]	Megastigmatrienone1	38818-55-2	1.17	1.84	1.77	6.43	3.46	5.43	886[e]	891
42.13[n]	Megastigmatrienone2	38818-55-2	8.14	10.33	10.29	4.02	4.38	2.74	881[e]	888
43.80[n]	Megastigmatrienone3	38818-55-2	3.32	19.13	3.76	9.90	1.71	4.92	878[f]	897
44.54[n]	Megastigmatrienone4	38818-55-2	6.52	12.69	8.05	5.70	4.34	3.76	869[f]	893
53.48[n]	2,3-Dimethyl-8-oxonon-2-enal	N#: 186826	0.45	0.59	0.95	7.11	5.75	4.96	815[e]	856
54.70[n]	4,5,6,7,8,8a-Hexahydro-8a-methyl-, (*S*)-2(1H) azulenone	55103-73-6	nd	0.06	0.16	nd	24.70	8.62	797[g]	833
61.10[n]	Longiverbenone	N#: 163074	0.02	0.37	0.38	23.40	9.66	8.68	800[g]	811
62.00[n]	1,13-Tetradecadien-3-one	58879-40-6	0.95	0.66	3.43	6.29	7.90	5.46	816[e]	875
72.62[n]	Androsta-3,5-dien-7-one	32222-21-2	25.81	33.83	28.71	1.48	4.86	4.33	863[e]	890
Ketols										
3.39[a]	Acetol	116-09-6	nd	0.03	nd	nd	22.13	nd	827[e]	938
4.12[n]	Acetoin	513-86-0	0.02	0.01	0.03	10.20	21.77	6.59	830[f]	872
16.27[a]	Corylon	80-71-7	0.06	0.27	0.33	12.26	4.98	9.18	812[f]	877
20.78[a]	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	21835-01-8	nd	0.04	0.08	nd	18.75	15.54	804[e]	891
36.84[n]	2,6-Di(*tert*-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	ID#: 140926	0.20	0.44	0.44	10.58	8.45	8.06	756[g]	800
38.49[n]	5-Hydroxy-3-methyl-1-indanone	57878-30-5	1.20	2.27	1.44	1.71	5.24	6.53	798[g]	879
40.85[n]	*trans*-5-Isopropyl-6,7-epoxy-8-hydroxy-8-methylnonan-2-one	58002-07-6	0.04	0.37	0.06	21.76	6.54	16.02	751[g]	838
42.82[n]	4,6,10,10-Tetramethyl-5-oxa-tricyclo[4.4.0.0,1,4]-dec-2-en-7-ol	97371-50-1	0.17	0.92	0.40	10.91	7.46	9.88	878[e]	884
48.38[n]	3-oxo-7,8-Dihydro-α-ionol	36151-02-7	0.26	3.38	0.39	8.89	1.56	6.08	871[e]	895
57.58[n]	3-Hydroxy solavetivone	ID#: 138383	8.49	18.66	8.83	6.43	3.51	3.49	821[f]	896
Aromatics										
15.78[n]	Acetophenone*	98-86-2	1.25	4.42	3.07	6.78	2.91	4.83	890[e]	900
1 9.30[n]	Acetyltoluene	122-00-9	0.05	0.06	0.06	11.29	7.11	7.66	881[e]	902
37. 18[a]	Acetovanillone	498-02-2	tra	0.35	0.07	nd	6.81	23.12	893[f]	906
49.17[n]	2′,5′-Dimethylcrotonophenone	15561-15-6	0.40	2.19	0.52	5.68	5.98	3.79	881[e]	891
45.18[a]	*o*-Chlorobenzophenone	5162-3-8	0.04	0.02	0.03	17.20	21.50	18.66	824[f]	913
59.80[n]	β-Methylchalcone	495-45-4	0.23	0.06	0.30	9.57	17.00	8.90	842[f]	878
Terpenoids										
17.12[n]	Isophorone epoxide*	10276-21-8	nd	0.02	0.05	nd	23.04	18.61	894[f]	906
17.93[n]	Isoacetophorone*	78-59-1	0.10	0.05	0.12	13.28	19.45	8.51	909[e]	909
18.54[n]	4-Oxoisophorone	1125-21-9	0.24	0.27	0.31	4.87	9.04	5.64	805[f]	835
19.10[n]	Dihydrooxophorone	20547-99-3	0.04	0.10	0.14	21.45	15.99	8.09	878[f]	913
20.55[n]	Pulegon	89-82-7	0.01	0.03	0.06	26.46	21.61	17.28	862[e]	893
24.30[n]	Isodiosphend	54783-36-7	0.01	0.15	0.22	21.17	12.01	8.44	893[e]	896
25.54[n]	6-Hydroxy-3-bornanone	39850-78-7	0.10	0.18	0.15	13.48	10.24	8.16	771[g]	795
25.98[n]	Diosphenol	490-03-9	0.05	0.29	0.28	19.91	11.73	8.45	789[g]	859
30.26[n]	(*E*)-Solanone	54868-48-3	9.06	14.16	13.22	4.89	2.19	5.19	892[e]	895
34.32[n]	Geranyl acetone I*	3796-70-1	0.02	0.03	0.04	12.67	17.31	15.00	879[e]	896
35.34[n]	Geranyl acetone 2*	3796-70-1	0.65	1.37	1.45	12.66	3.87	7.67	891[e]	916
36.47[n]	Norsolanadione	ID#: 90834	4.75	8.84	6.37	3.77	2.60	4.19	885[f]	896
53.70[n]	Solavetivone	54878-25-0	0.63	0.94	0.71	9.55	9.40	8.62	878[f]	898
55.10[n]	Ledene oxide(II)	N#:159367	0.72	4.74	0.65	12.17	9.58	22.60	823[f]	824
57.73[n]	Hexahydrofarnesyl acetone	502-69-2	2.24	1.45	1.93	2.78	6.31	4.59	797[g]	885
59.42[n]	Farnesyl acetone 1*	1117-52-8	nd	0.88	0.38	nd	8.06	13.21	893[e]	902
59.65[n]	Farnesyl acetone 2*	1117-52-8	0.96	0.39	0.66	5.32	8.12	6.59	886[e]	912
60.69[n]	Farnesyl acetone 3*	1117-52-8	2.09	1.71	2.28	11.23	2.64	4.67	897[e]	907

Table 1 (*Continued*)

t_R (min)	Compound	CAS	Approx. concn. (μg/g)			R.S.D. (%, $n = 5$)			Match factor	
7			SD	SDE	HCD	SD	SDE	HCD	SI	RSI
Furanones										
6.35[n]	Dihydro-2-methyl-3(2H)-furanone	3188-00-9	0.19	0.27	0.54	11.63	5.02	7.20	924[e]	934
10.01[n]	1-(2-Furanyl)-ethanone	1192-62-7	0.07	0.61	0.55	9.53	2.42	7.00	905[e]	964
11.61[n]	1-(2-Furyl)-2-propanone	ID#: 17221	nd	nd	0.03	nd	nd	9.02	851[f]	894
15.51[n]	1-(5-Methyl-2-furyl)-2-propanone	ID#: 27436	nd	0.01	0.05	nd	24.65	17.74	866[f]	963
18.72[a]	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	3658-77-3	tra	0.01	0.38	nd	26.96	4.86	848[f]	924
Pyranone										
14.06[a]	Isomaltol	3420-59-5	tra	0.01	0.03	nd	15.24	21.93	833[f]	927
20.05[a]	Maltol*	118-71-8	0.12	1.12	0.78	11.44	4.50	8.38	896[e]	912
21.86[a]	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	28564-83-2	tra	tra	0.10	nd	nd	4.88	847[f]	899
39.62[n]	2,5,5,8a-Tetramethylocta hydro-7H-chromen-7-one	5835-18-7	0.08	0.73	0.15	133.89	6.65	7.22	813[f]	848
Damascone and ionone derivatives										
31.10[n]	Damascenone	23726-93-4	1.85	4.28	3.01	4.39	1.39	6.66	938[e]	949
32.97[n]	β-Damascone*	85949-43-5	0.39	0.59	0.63	6.09	2.90	9.72	936[e]	948
33.17[n]	2,3-Dehydro-α-ionone	1203-08-3	tra	2.24	0.79	nd	3.66	5.60	835[f]	876
36.94[n]	*trans*-β-Ionon-5,6-epoxide	23267-57-4	0.19	0.40	0.40	8.08	11.45	8.03	837[f]	861
37.13[n]	*trans*-β-Ionone*	14901-07-6	0.14	0.55	0.54	10.97	6.48	8.32	901[e]	913
43.13[n]	Dihydro-β-methylionone	56763-64-5	0.05	nd	0.06	13.99	nd	11.45	786[g]	805
43.90[n]	3-Hydroxy-β-damascone	102488-09-5	1.15	0.88	0.72	2.48	6.80	8.90	845[e]	850
39.85[a]	Tetrahydroionone	60761-23-1	0.01	0.12	0.05	21.62	5.21	15.29	780[g]	830
45.06[n]	Retro-ionone	56052-61-0	tra	2.29	tra	nd	5.68	nd	848[f]	861
45.46[n]	3-Oxo-α-ionol	34318-21-3	1.99	23.34	2.48	4.25	4.44	4.65	888[e]	891
41.86[a]	3-Hydroxy-β-damascone	102488-09-5	0.02	0.01	0.04	16.68	21.93	14.17	838[e]	863
47.41[n]	α-Ionone	8013-90-9	tra	0.18	tra	nd	6.68	nd	821[f]	879
48.88[n]	6,7-Dehydro-7,8-dihydro-3-oxo-β-ionol 4 acids	110114-85-7	nd	0.46	tra	nd	7.45	nd	809[f]	811
Volatile fatty acids										
2.87[a]	Acetic acid*	64-19-7	0.01	0.01	0.08	25.92	22.72	16.44	892[e]	893
6.00[a]	Isobutyric acid*	79-31-2	tra	0.02	0.01	nd	4.56	29.55	788[g]	801
7.66[a]	Pivalic acid	75-98-9	0.02	0.02	0.04	10.83	8.25	10.67	889[e]	913
9.25[a]	Isovaleric acid*	503-74-2	0.10	0.21	0.55	12.69	6.36	18.94	877[e]	912
10.00[a]	2-Methylbutanoic acid	116-53-0	0.31	0.57	1.25	11.80	8.60	15.73	872[f]	894
11.01[a]	Pentanoic acid*	109-52-4	0.04	0.13	0.09	4.48	6.01	14.46	910[e]	965
12.69[a]	2-Methyl-2-butenoic acid	13201-46-2	0.03	0.12	0.12	4.41	10.46	14.19	838[f]	889
12.67[a]	Isosuccinic acid	516-05-2	0.01	0.01	nd	11.09	16.42	nd	784[g]	821
13.53[a]	4-Methyl-3-pentenoic acid	504-85-8	nd	0.01	nd	nd	21.13	nd	831[f]	872
13.75[a]	3-Methyl pentanoic acid*	105-43-1	0.01	0.07	0.05	20.05	5.07	15.79	819[f]	957
13.85[a]	Isobutylacetic acid	646-07-1	0.01	0.28	0.29	22.69	8.12	5.62	831[f]	969
15.40[a]	Hexznoic acid*	142-62-1	0.04	0.18	0.13	20.56	2.87	13.69	867[e]	912
16.62[a]	2,2-Dimethylvaleric acid	1185-39-3	nd	0.54	0.33	nd	6.13	7.21	863[f]	897
17.74[a]	2-Ethylbutanoic acid	88-09-5	tra	0.10	0.03	nd	5.00	12.12	860[e]	899
18.42[a]	5-Methylhexanoic acid	ID#: 21841	nd	0.03	0.03	nd	18.41	16.63	815[f]	860
18.87[a]	4-Methylhexanoic acid	1561-11-1	tra	0.24	0.47	nd	7.02	4.42	807[f]	825
19.90[a]	Heptanoic acid*	111-14-8	0.01	0.03	0.02	9.78	6.83	17.89	896[e]	923
22.10[a]	2-Ethylhexanoic acid	149-57-5	0.05	0.03	0.01	13.10	18.58	24.85	800[g]	963
24.50[a]	Octanoic acid	124-07-2	0.05	0.09	0.09	15.28	2.48	7.50	879[e]	901
25.54[a]	2-Methyloctanoic acid	3004-93-1	nd	0.08	0.09	nd	13.21	15.59	785[g]	835
28.96[a]	Nonanoic acid*	112-05-0	0.28	0.19	0.30	4.65	8.70	5.17	856[e]	911
29.19[a]	3-Nonenoic acid	4124-88-3	nd	0.05	nd	nd	4.13	nd	791[g]	807
33.79[a]	Decanoic acid*	334-48-5	0.16	0.14	0.14	8.32	2.27	5.57	863[e]	901
Semi-volatile fatty acids										
37.83[a]	Undecanoic acid	112-37-8	0.08	0.08	0.08	8.64	21.06	11.18	826[f]	851
41.08[a]	Dodecanoic acid*	143-07-7	0.28	0.50	0.50	21.57	10.75	11.31	891[e]	902
43.74[a]	Tridecanoic acid	638-53-9	0.01	tra	0.01	16.87	nd	24.40	797[g]	808
44.38[a]	4-(2,6,6-Trimethylcyclohex-1-enyl)butyric acid	54344-76-2	0.08	0.24	0.10	18.08	3.64	13.70	786[g]	821
44.48[a]	3-Methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)-2-pentenoic acid	N#:196812	0.07	0.36	0.11	14.95	4.53	9.24	784[g]	803
45.49[a]	3,7,11-Trimethyldodeca-2,6,10-trienoic acid	7548-13-2	0.07	0.16	0.07	13.28	11.99	4.76	820[f]	838

Table 1 (*Continued*)

t_R (min)	Compound	CAS	Approx. concn. (μg/g)			R.S.D. (%, $n = 5$)			Match factor	
7			SD	SDE	HCD	SD	SDE	HCD	SI	RSI
46.18[a]	Tetradecanoic acid*	544-63-8	2.12	0.91	1.61	6.64	7.91	4.59	883[e]	914
47.77[a]	12-Methyl-,(*S*)-tetradecanoic acid	5746-58-7	tra	0.21	0.51	nd	6.60	5.10	792[g]	947
47.99[a]	14-Pentadecenoic acid	17351-34-7	0.24	0.89	1.16	6.74	4.35	3.75	816[f]	884
48.38[a]	Pentadecanoic acid	1002-84-2	1.10	0.35	0.77	6.64	6.59	8.56	874[e]	914
49.99[a]	9-Hexadecenoic acid	2091-29-4	0.10	0.41	0.46	11.20	8.35	5.82	805[f]	828
50.88[a]	Palmitic acid*	1957-10-3	54.95	12.88	34.15	5.00	11.60	10.65	916[e]	965
52.00[a]	Heptadecenoic acid	26265-99-6	1.03	0.32	0.60	10.55	7.01	9.67	805[f]	847
52.51[a]	Heptadecanoic acid*	506-12-7	0.68	0.20	0.44	9.07	10.64	8.87	901[e]	923
53.70[a]	Linolenic acid*	463-40-1	25.49[c]	6.28[c]	11.42[c]	9.86	11.08	14.01	911[e]	916
53.81[a]	Linoleic acid*	60-33-3							902[f]	913
53.91[a]	Oleic acid*	112-79-8							903[e]	920
54.18[a]	Octadecanoic acid*	1957-11-4	1.70	0.33	0.68	29.31	21.57	23.42	906[e]	918
Anhydrides										
7.40[a]	Maleic anhydride	108-31-6	tra	0.01	tra	nd	17.28	nd	825[e]	908
11.64[a]	Succinic anhydride	108-30-5	nd	nd	0.01	nd	nd	25.20	826[e]	845
15.61[a]	2,3-Dimethylmaleic anhydride*	766-39-2	0.01	0.07	0.04	10.95	6.59	25.52	836[e]	928
15.94[n]	Hexanoic acid, anhydride	2051-49-2	0.36	0.90	0.72	16.36	3.03	7.18	762[g]	917
19.16[a]	Ethylmethylmaleic anhydride*	3552-33-8	0.09	0.43	0.22	9.11	3.48	5.51	869[f]	921
20.38[n]	Cantharidin	56-25-7	0.01	0.03	0.01	15.02	12.87	17.46	793[g]	971
Others										
3.88[a]	Lactic acid	50-21-5	nd	0.01	tra	nd	19.13	nd	772[g]	830
23.83[a]	Benzoic acid*	65-85-0	0.02	0.17	0.28	20.27	11.80	5.33	854[e]	897
27.42[a]	β-Toluic acid	103-82-2	nd	nd	0.21	nd	nd	4.67	845[f]	916
40.35[a]	6-Hydroxy-4-heptenoic acid	105728-84-5	0.10	0.74	0.15	6.36	5.58	3.80	886[e]	903
16.45[a]	Hydroxypivalic acid	4835-90-9	0.02	0.08	0.06	14.21	10.21	13.45	812[g]	861
30.51[a]	2,6,6-Trimethyl-cyclohex-1-enecarboxylic acid	ID#: 58372	0.04	0.29	0.11	13.81	4.87	8.56	836[f]	872
5 Esters aliphatics										
3.61[n]	Isopropyl acetate	108-21-4	0.11	0.02	0.06	5.90	19.10	12.71	869[e]	879
4.41[n]	Methyl methacrylate	80-62-6	nd	0.05	tra	nd	10.68	nd	871[e]	894
10.05[a]	Glycidyl acrylate	ID#: 19771	0.04	nd	nd	15.25	nd	nd	801[g]	874
10.71[n]	1-Ethyl-2-methylpropyl acetate	35897-16-6	nd	nd	0.01	nd	nd	20.02	808[f]	827
14.29[a]	Diethyl oxalate	95-92-1	0.01	nd	0.01	21.48	nd	16.05	866[e]	899
47.60[a]	Methyl myristate	124-10-7	tra	0.09	0.20	nd	15.88	5.05	798[g]	846
49.47[a]	4-Acetoxypentadecane	N#: 245622	0.03	nd	0.34	14.10	nd	4.82	800[g]	831
49.85[a]	Ethyl linoleate	544-35-4	2.23	2.37	0.73	19.26	10.53	9.08	819[f]	884
61.77[n]	Methyl palmitate	112-39-0	6.59	6.38	7.52	9.63	6.08	4.36	878[e]	908
67.92[n]	Methyl-14-methyl-hexadecanoate	2490-49-5	nd	0.20	0.28	nd	6.54	6.60	821[f]	834
72.95[n]	Methyl linolenate	301-00-8	4.05	4.30	3.88	4.07	3.29	4.71	821[f]	829
76.62[n]	16-Methyl-heptadecanoic acid methyl ester	112-61-8	3.32	3.68	2.92	7.76	5.39	6.06	817[f]	901
76.60[n]	11,14,17-Eicosatrienoic acid, methyl ester	55682-88-7	nd	1.15	nd	nd	6.68	nd	848[f]	918
Aromatics										
19.58[n]	Phenmethyl acetate*	140-11-4	nd	0.12	0.19	nd	10.34	7.94	925[e]	947
23.34[n]	Phenethyl acetate*	103-45-7	tra	0.08	0.13	nd	19.26	8.56	932[e]	954
38.41[a]	Ethyl vanillate	617-05-0	nd	0.12	0.03	nd	4.64	15.77	813[f]	875
41.58[a]	5-Hydroxy-2-methoxy-benzoicacid, methyl ester	87513-63-1	nd	0.03	0.09	nd	22.51	14.94	810[f]	863
46.20[b]	Dibutyl phthalate	84-74-2	0.12	0.11	0.08	9.41	10.71	13.74	886[e]	917
57.24[n]	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	84-69-5	1.02	0.93	1.11	4.60	5.31	4.38	911[e]	932
89.86[n]	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	117-81-7	1.27	1.92	2.29	4.59	3.22	7.93	864[e]	922
Terpenoids										
28.98[n]	Tetrahydrogeranyl acetate	ID#: 95679	0.22	0.38	0.39	9.39	7.47	8.00	791[g]	845
30.07[a]	Linalyl formate	115-99-1	tra	0.02	0.01	nd	6.32	25.42	763[g]	798
33.97[a]	Methyl chrysanthemate	5460-63-9	0.06	0.10	0.07	25.50	5.76	16.64	811[e]	828
37.40[a]	Ethyl chrysanthemate	97-41-6	0.19	0.22	0.16	10.92	14.34	10.67	791[g]	834
Others										
12.47[n]	Methyl 3-furoate	13129-23-2	0.02	tra	0.03	19.48	nd	19.35	792[g]	987
43.38[a]	Methyl 7-(2-furyl)heptanoate	98188-02-4	nd	0.09	nd	nd	14.13	nd	795[g]	893

Table 1 (*Continued*)

t_R (min)	Compound	CAS	Approx. concn. (μg/g)			R.S.D. (%, $n = 5$)			Match factor	
7			SD	SDE	HCD	SD	SDE	HCD	SI	RSI
6 Lactones furanone										
9.7[a]	Butyrolactone*	96-48-0	tra	0.11	0.02	nd	19.33	12.31	895[e]	905
9.78[a]	2(5H)-furanone*	497-23-4	0.02	0.18	0.14	5.59	20.50	15.31	856[e]	913
11.20[n]	5,5-Dimethyl-2(5H)-furanone	20019-64-1	0.01	0.02	0.05	20.18	15.19	13.23	818[f]	823
11.64[a]	Dihydro-3-methyl-2(3H)-furanone	1679-47-6	nd	0.02	tra	nd	14.13	nd	844[e]	878
11.85[a]	Dihydro-5-methyl-2(3H)-furanone	108-29-2	nd	0.04	0.03	nd	16.93	15.04	847[f]	931
13.02[a]	3-Methyl-2(5H)-furanone	22122-36-7	tra	0.11	0.03	nd	10.80	5.95	806[g]	883
14.16[n]	5-Ethyl-2(5H)-furanone	2407-43-4	tra	0.06	0.08	nd	16.28	12.57	893[e]	987
16.04[a]	Dihydro-3-hydroxy-4,4-dimethyl,(R)-2(3H)-furanone	52126-90-6	nd	0.02	0.05	nd	17.78	15.07	817[f]	894
16.99[n]	3-(3-Butenyl)butyrolactone	ID#: 29333	nd	0.08	0.15	nd	12.53	8.55	797[g]	865
37.90[n]	Dihydroactinidiolide	15356-74-8	1.94	3.59	2.24	5.02	3.96	3.95	844[e]	928
38.14[a]	Tetrahydroactinidiolide	16778-27-1	tra	0.17	0.04	nd	4.63	15.89	847[e]	885
Pyranone										
18.24[a]	Tetrahydro-4-methyl-2H-pyran-2-one	1121-84-2	nd	0.02	nd	nd	15.76	nd	791[g]	850
22.63[a]	5,6-Dimethyltetrahydro-2H-pyran-2-one	24405-16-1	0.01	0.12	0.08	12.62	4.85	9.09	867[e]	892
27.67[a]	Tetrahydro-6-propyl-2H-pyran-2-one	698-76-0	nd	0.06	nd	nd	10.13	nd	882[e]	902
60.55[n]	Eudesma-5,11(13)-dien-8,12-olide	N#: 140297	1.01	3.39	1.10	9.63	3.67	3.02	799[g]	845
Others										
18.40[a]	δ-Caprolactone	823-22-3	nd	0.07	nd	nd	13.23	nd	819[f]	866
7 Phenols										
11.42[n]	3,5-dimethylphenol	108-68-9	0.01	0.08	0.09	22.20	13.39	10.53	827[f]	868
14.72[a]	Phenol*	108-95-2	0.13	0.24	0.31	4.86	3.75	7.15	854[e]	881
16.58[n]	Methyl-p-hydroquinone	95-71-6	nd	1.95	0.70	nd	7.73	7.44	808[f]	816
18.13[a]	*p*-Methylphenol	106-44-5	nd	0.05	0.07	nd	16.15	3.17	868[e]	872
19.25[a]	Guaiacol	1990-5-1	0.02	0.21	0.17	12.78	5.67	6.28	810[f]	818
19.39[a]	*m*-Ethoxyphenol	621-34-1	0.04	0.27	0.08	6.07	4.78	5.75	812[f]	869
23.52[a]	*p*-Ethylphenol	123-07-9	0.01	0.25	0.19	25.27	6.71	6.15	790[g]	864
23.62[a]	*o*-Ethylphenol	90-00-6	0.03	0.02	0.01	17.20	16.50	17.41	786[g]	856
26.45[n]	1-Ethenyl-2-methoxy-phenol	7786-61-0	0.01	0.36	0.11	19.47	7.42	11.13	815[e]	887
26.27[a]	*p*-Isopropylphenol	99-89-8	0.01	0.01	0.01	4.41	21.91	9.00	823[e]	871
29.67[a]	*p*-Vinylguaiacol	7786-61-0	1.36	7.96	4.26	10.51	4.33	4.20	937[e]	947
31.13[a]	Syringol	91-10-1	tra	0.19	0.04	nd	4.53	19.06	867[e]	885
32.03[a]	Eugenol*	501-19-9	nd	0.12	0.57	nd	12.61	4.86	902[e]	915
34.68[a]	Isoeugenol*	97-54-1	tra	0.07	0.02	nd	14.79	18.59	854[f]	927
36.84[a]	4,5-Dimethoxy-2-methylphenol	72312-07-3	tra	0.17	0.12	nd	11.87	12.05	882[e]	928
39.11[a]	Butrlated hydroxytoluene	128-37-0	0.02	0.22	0.12	18.30	5.70	6.66	874[e]	878
49.31[n]	Styrolfenol	1988-89-2	nd	0.66	0.33	nd	5.20	8.37	837[f]	855
8 Hydrocarbons aliphatics										
7.65[b]	2,4-Dimethylheptane	2213-23-2	tra	0.01	0.01	nd	15.17	12.83	801[g]	820
8.92[b]	4-Methyloctane	2216-34-4	tra	0.01	tra	nd	18.08	nd	797[g]	888
19.67[a]	Dodecane	112-40-3	0.01	0.07	0.02	20.24	10.32	10.92	837[f]	882
22.18[n]	1,1,4,5-Tetramethylindan	16204-57-2	nd	0.03	0.05	nd	19.58	14.55	883[e]	903
30.84[n]	1,1,4,4,7,7-Hexamethyl-cyclononane	149331-19-1	0.26	0.64	0.63	9.41	5.12	4.02	818[g]	873
32.19[n]	1,3,5-Trimethyladamantane	707-35-7	tra	0.07	tra	nd	11.83	nd	955[e]	955
51.56[n]	2,6,10-Trimethyltetradecane	14905-56-7	0.67	0.58	0.68	4.18	4.39	5.52	810[f]	824
56.29[n]	Octadecane	593-45-3	0.42	nd	0.41	7.41	nd	6.05	839[f]	942
61.31[n]	(*E*,*E*)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10, 14-tetraene	70901-63-2	2.23	1.33	3.39	2.35	3.96	3.33	862[e]	890
67.35[n]	Eicosane	112-95-8	0.51	0.34	0.74	6.82	6.31	4.28	848[f]	846
91.7[n]	Tricosane	638-67-5	0.07	1.25	1.02	18.68	5.68	5.09	839[f]	906
93.41[n]	Tetracosane	646-31-1	1.56	5.06	2.27	4.91	3.28	5.37	845[e]	849
Aromatics										
5.70[n]	Toluene	108-88-3	0.04	0.01	0.04	17.65	2.02	19.17	942[e]	942
9.01[n]	*p*-Xylene	106-42-3	0.18	0.04	0.19	7.80	15.07	9.34	839[e]	847
8.35[b]	Ethylbenzene	100-41-4	tra	0.03	0.04	nd	19.67	14.04	798[g]	872
9.60[n]	Styrene	100-42-5	tra	0.01	0.01	nd	19.27	18.39	956[e]	967
9.79[n]	*m*-Dimethylbenzene	108-38-3	0.04	0.01	0.06	11.97	20.98	15.69	827[f]	890
10.45[a]	*o*-Xylene	95-47-6	0.01	0.03	4.54	20.75	13.70	3.21	866[e]	890

Table 1 (*Continued*)

t_R (min)	Compound	CAS	Approx. concn. (μg/g)			R.S.D. (%, $n = 5$)			Match factor	
7			SD	SDE	HCD	SD	SDE	HCD	SI	RSI
27.05[n]	1,5,8-Trimethyl-1,2,3,4-tetrahydronaphthalene	21693-51-6	0.05	0.58	0.19	5.69	11.11	15.67	886[e]	896
28.06[n]	1,6,8-Trimethyl-1,2,3,4-tetrahydronaphthalene	30316-36-0	0.02	0.14	0.05	10.98	13.27	22.66	829[f]	858
29.60[n]	1,1,5,6-Tetramethylindane	942-43-8	0.07	1.33	0.20	8.90	6.36	24.55	860[e]	890
31.50[n]	1,1,6-Trimethyl-1,2-dihydronaphthalene	30364-38-6	tra	0.03	tra	nd	12.68	nd	824[f]	860
46.07[n]	1-Ethyl-2-(1-phenylethyl)benzene	18908-70-8	0.09	0.89	0.53	10.36	7.80	9.02	781[g]	806
47.60[n]	1,1-Bis(p-tolyl)ethane	ID#:109036	0.09	0.37	0.14	16.11	7.05	11.18	864[e]	934
47.98[n]	3,4-Diethyl-1,1′-biphenyl	61141-66-0	0.47	4.01	0.41	8.60	6.02	8.25	812[f]	818
51.75[n]	Phenanthrene	85-01-8	0.34	1.11	0.56	6.28	4.63	5.36	759[g]	875
Terpenoids										
11.00[b]	Limonene	138-86-3	0.02	0.08	0.03	17.01	14.49	16.66	873[e]	827
12.83[a]	3-Thujene	2867-5-2	0.03	0.75	0.22	27.43	4.76	8.62	827[f]	856
13.29[n]	Sabinene	3387-41-5	0.11	0.10	0.36	11.51	13.94	3.94	855[f]	905
24.08[n]	Ionene	475-03-6	0.06	0.61	0.25	5.49	21.71	18.90	903[e]	941
40.10[n]	Cadinene	483-76-1	nd	0.20	nd	nd	6.41	nd	807[e]	904
56.20[n]	Elixene	3242-8-8	0.54	0.61	0.52	6.16	5.66	8.75	812[f]	854
56.91[n]	Dehydroabietane	19407-28-4	0.12	0.21	0.35	11.84	7.86	8.00	796[g]	825
56.97[n]	Farnesan	3891-98-3	0.39	0.20	0.23	6.16	9.86	11.57	811[f]	815
58.10[n]	Neophytadiene	ID#: 189154	93.40	83.41	131.94	5.49	3.47	4.19	912[e]	926
61.57[n]	Cembrene	1898-13-1	0.78	0.75	1.61	6.33	4.73	6.09	892[e]	912
62.28[n]	Valencene	4630-7-3	tra	0.07	0.34	nd	16.96	8.75	826[f]	846
62.75[n]	18-Norisopimara-4(19),7,15-triene	26549-04-2	0.28	0.40	1.06	8.87	8.23	5.87	868[e]	876
82.20[n]	Cycloisolongifolene	N#: 151997	0.16	1.19	0.20	11.98	6.50	10.61	821[f]	825
9 Miscellaneous oxygenated compounds										
4.56[n]	2,5-Dimethylfuran	625-86-5	nd	0.01	nd	nd	16.68	nd	815[f]	850
6.59[n]	*trans*-1,2-Dimethoxycyclohexane	29887-60-3	0.01	0.03	0.05	22.88	17.62	15.21	861[e]	898
11.11[n]	2-Ethylfuran	3208-16-0	nd	0.05	0.01	nd	17.20	22.34	757[g]	761
25.75[a]	Coumaran	496-16-2	0.27	1.85	1.04	10.32	5.27	3.99	893[e]	902
39.57[a]	Hymecromone	90-33-5	tra	0.14	0.04	nd	5.09	10.63	867[e]	915
42.15[a]	Asarone	2883-98-9	tra	0.04	tra	nd	14.13	nd	822[f]	873
64.43[n]	3-(4,8,12-Trimethyltridecyl) furan	N#:245551	1.83	tra	6.25	11.56	nd	4.61	821[f]	832
10 Pyrroles										
5.25[n]	Pyrrole*	109-97-7	nd	0.02	0.02	nd	17.24	19.22	869[e]	891
5.30[b]	*N*-Methylpyrrole	96-54-8	nd	nd	0.08	nd	nd	10.52	800[g]	823
9.88[b]	2-Acetyl-1-pyrroline*	85213-22-5	0.12	0.02	0.11	4.00	4.33	4.28	884[e]	855
15.70[n]	2-Acetylpyrrole	1072-83-9	0.42	4.89	2.72	14.32	2.88	7.42	872[e]	998
15.50[a]	2-Pyrrolylcarboxaldehyde	1003-29-8	0.01	0.06	0.04	9.22	8.98	8.42	806[f]	890
17.23[b]	Hygrine	496-49-1	nd	nd	tra	nd	nd	nd	781[g]	893
22.45[a]	1,5-Dihydro-1-methyl-2H-pyrrol-2-one	13950-21-5	nd	nd	0.03	nd	nd	10.87	822[f]	841
11 Pyridines										
5.00[b]	Pyridine*	110-86-1	0.13	0.23	0.48	20.45	6.06	3.95	801[g]	865
8.24[b]	4-Picoline	108-89-4	nd	nd	0.04	nd	nd	18.23	811[f]	852
9.08[b]	2,6-Lutidine*	108-48-5	0.01	0.05	0.05	7.91	19.95	4.53	803[f]	836
10.54[b]	2,4-Lutidine	108-47-4	0.02	0.06	0.07	13.70	3.27	4.42	813[f]	888
11.49[b]	4-Ethenylpyridine	100-43-6	0.04	tra	0.01	11.29	nd	13.02	826[f]	877
11.60[b]	3-Methylpyridine	108-99-6	tra	0.02	tra	nd	5.24	nd	836[f]	893
11.90[b]	Nicotinealdehyde	500-22-1	0.03	0.07	0.10	15.07	3.48	1.68	885[e]	844
12.38[b]	3-Methoxypyridine	7295-76-3	nd	nd	0.01	nd	nd	20.58	845[e]	879
12.96[b]	2,3,6-Trimethylpyridine	1462-84-6	0.01	0.01	0.02	5.37	4.21	4.54	829[f]	875
13.33[b]	2-Acetylpyridine*	1122-62-9	tra	0.01	0.04	nd	20.16	17.03	811[f]	914
13.54[b]	2-Ethyl-5-methyl-pyridine	104-90-5	tra	0.01	0.01	nd	4.69	4.32	827[f]	839
14.01[b]	2-Acetyl-3,4,5,6-tetrahydropyridine	27300-27-2	0.45	0.17	0.33	9.40	12.87	10.38	865[e]	902
15.33[b]	4-Acetylpyridine	1122-54-9	nd	tra	0.01	nd	nd	16.39	762[g]	801
18.08[n]	Nicotinyl alcohol	100-55-0	tra	0.44	0.17	nd	7.76	9.85	800[f]	863
15.85[b]	3-Acetopyridine*	350-03-8	0.05	0.15	0.11	7.99	4.10	1.66	825[f]	879
15.96[b]	*N*-Ethyl-*m*-toluidine	102-27-2	0.06	0.20	0.21	8.91	2.21	2.39	802[f]	814
18.19[b]	2-Acetyl-1,4,5,6-tetrahydropyridine	25343-57-1	0.24	0.24	0.52	17.70	5.69	5.65	796[g]	884
18.71[b]	2-Propionyl-3,4,5,6-tetrahydropyridine	80933-75-1	0.03	0.02	0.03	5.59	8.00	6.76	813[f]	890
33.77 [b]	1-Methyl-6-[2-pyridyl]-1,2,5,6-tetrahydropyridine	N#:132276	0.31	0.08	0.15	1.78	15.09	10.44	847[e]	859
34.53[b]	2,2′-Dipiperidine	531-67-9	nd	0.13	0.09	nd	7.46	12.37	786[g]	802

Table 1 (*Continued*)

t_R (min)	Compound	CAS	Approx. concn. (μg/g)			R.S.D. (%, $n = 5$)			Match factor	
7			SD	SDE	HCD	SD	SDE	HCD	SI	RSI
12 Pyrazines										
4.65[b]	Pyrazine	290-37-9	0.02	nd	0.04	6.78	nd	3.45	796[g]	967
6.95[b]	Methylpyrazine*	109-08-0	0.01	nd	0.03	18.40	nd	10.02	800[e]	887
9.77[b]	2,5-Dimethylpyrazine*	123-32-0	tra	tra	0.02[c]	21.89	18.86	16.28	802[f]	856
9.77[b]	2,6-Dimethylpyrazine*	108-50-9							832[e]	869
12.65[b]	2,3,5-Trimethylpyrazine*	14667-55-1	tra	tra	0.01	nd	nd	4.79	805[g]	865
34.62[b]	2-Methyl-6-[(1E)-1-propenyl]-pyrazine	55138-67-5	0.05	0.04	0.03	10.40	6.32	4.92	767[g]	806
13 Amides										
5.78[b]	*N*,*N*-Dimethylformamide	68-12-2	nd	tra	tra	nd	nd	nd	803[f]	872
19.44[b]	2-Methyl-6-isopropylaniline	5266-85-3	tra	tra	tra	nd	nd	nd	809[f]	885
23.05[b]	Caprolactam	105-60-2	nd	nd	tra	nd	nd	nd	779[g]	825
28.12[a]	4-Methyl-2-pyrrolidinone,	N#:197003	nd	0.19	nd	nd	4.13	nd	799[g]	819
34.84[n]	3′-Hydroxy-acetamide	621-42-1	0.22	0.54	0.44	7.91	5.86	8.31	759[g]	861
31.35[b]	7-Amino-4-methylcoumarin	26093-31-2	nd	nd	0.02	nd	nd	17.58	811[e]	860
42.51[b]	2-(Cyclohex-1-enyl)aniine	46175-80-8	nd	nd	0.03	nd	nd	15.58	819[e]	876
14 Tobacco alkaloids										
28.40[b]	Nicotine*	54-11-5	289.77	109.57	202.93	8.12	3.39	7.43	823[e]	857
30.86[b]	Myosmine	532-12-7	0.60	0.72	0.81	6.01	3.32	10.66	817[f]	895
31.72[b]	*N*-Ethyl-nornicotine	ID#: 67017	0.01	0.02	0.04	23.46	16.13	16.89	836[e]	876
32.64[b]	*N*-Methyl-anabasine	19730-04-2	0.13	0.04	0.07	10.45	9.31	7.64	768[g]	808
33.24[b]	Nicotyrine	487-19-4	0.20	0.15	0.10	7.95	6.94	12.27	842[f]	899
33.34[b]	(1′S,2′S)-Nicotine-N′-oxide	29419-55-4	0.06	0.24	0.06	0.76	4.07	2.59	881[e]	908
33.50[b]	Anabasine	494-52-0	0.18	0.02	0.01	3.22	20.04	21.58	877[e]	921
34.31[b]	Nicotine N-oxide	2820-55-5	0.23	0.01	0.02	1.21	25.54	19.88	844[e]	854
34.95[b]	2,3-Bipyridine	581-50-0	0.64	2.93	0.64	1.87	3.72	6.46	816[f]	889
36.17[b]	*N*-Propylnornicotine	91907-45-8	0.03	0.02	0.05	15.42	19.31	13.63	789[g]	895
37.81[b]	1-Acetylnicotine	ID#: 99947	0.10	0.14	0.07	5.70	8.62	11.27	809[f]	899
39.75[b]	Cotinine	486-56-6	nd	nd	0.05	nd	nd	14.58	790[g]	832
15 Nitrogenous compounds										
3.82[b]	Isoxazole	288-14-2	0.60	0.51	0.41	11.45	8.56	7.39	940[e]	970
13.18[b]	2-Methyl[1,3,4]oxadiazole	3451-51-2	0.01	nd	0.02	14.17	nd	10.05	869[e]	898
13.83[b]	3,5-Dimethylisoxazole	300-87-8	0.70	0.15	0.41	12.92	10.92	10.75	804[f]	863
17.11[b]	4,5,6,7-Tetrahydro-3-indolinone	58074-25-2	nd	nd	0.02	nd	nd	19.58	803[f]	803
21.54[n]	Benzothiazole	95-16-9	0.07	0.34	0.31	14.41	9.38	5.20	881[e]	917
24.74[n]	1H-Indole*	120-72-9	nd	0.52	0.18	nd	8.78	12.46	856[e]	878
22.50[b]	Quinoline*	91-22-5	0.02	0.03	0.04	16.68	5.69	4.74	863[f]	894
29.51[b]	1,6-Dimethyl-indazole	34879-87-3	nd	0.07	0.02	nd	14.93	15.03	803[f]	869
30.16[b]	2-Methylindoline	6872-6-6	0.05	tra	0.01	11.22	nd	17.66	793[g]	845
30.66[b]	2,5,6-Trimethylbenzimidazole	3363-56-2	0.03	nd	nd	17.25	nd	nd	796[g]	816
31.20[b]	1,3,3-Trimethyloxindole	20200-86-6	0.08	nd	tra	15.22	nd	nd	775[g]	811
32.85[b]	4-Quinolinecarboxaldehyde	4363-93-3	nd	0.07	0.02	nd	4.91	14.99	856[e]	958
34.08[b]	5-Amino-6,8-dimethoxy quinoline	N#: 213952	0.13	0.13	0.12	13.74	4.19	4.91	776[g]	816
34.40[b]	5-Amino-8-quinolinol	13207-66-4	0.37	nd	nd	1.25	nd	nd	822[f]	833
35.36[b]	2-Methylpyrido[3,2-d]pyrimidinl-4-ol	3303-26-2	tra	0.29	tra	nd	5.86	nd	746[g]	802
36.01[b]	8-Amino-2-hydroxymethyl-6-methoxyquinoline	N#: 214275	1.15	1.83	0.80	5.38	3.86	10.83	757[g]	822
38.43[b]	maltoxazine	80933-73-9	0.14	0.46	0.30	10.16	7.16	9.44	829[f]	841
39.56[b]	3,4-Dihydro-1(2H)-quinolinecarbaldehyde	2739-16-4	nd	nd	0.08	nd	nd	10.58	867[e]	885
42.58[a]	2(3H)-Benzothiazolone	934-34-9	0.02	0.59	0.26	51.32	5.30	7.58	866[e]	890
43.26[b]	1,2-Dihydro-1-demethyl-harmalol	83177-17-7	nd	nd	tra	nd	nd	nd	830[f]	882

a, b and n—detected in acidic, basic and neutral fraction respectively. (*) Positively identified compound by MS database and authentic compounds; the others identified by MS database. CAS—chemical abstracts service registry number; if CAS is not available, N# (NIST number) or ID# (Identified number in Wiley) will be given. Appro. concen.—approximate concentration, assuming all response factors of 1; nd—not detected; tra—the amount <0.01 μg/g; c—linolenic acid, linoleic acid and oleic acid could not be separated completely, so their total concentration were calculated, so did 2,5-dimethylpyrazine and 2,6-dimethylpyrazine. R.S.D.—relative standard deviation. SI—a direct matching factor for the unknown and the library spectrum; RSI—a reverse search matching factor ignoring any peaks in the unknown that are not in the library spectrum; match factor—the lowest one obtained by three methods is given; e, f and g—obtained by SDE, HCD, and SD, respectively.

column (30 m × 0.25 mm, 0.25 μm film thickness; a poly (dimethyldiphenylsiloxane) containing 5% diphenylsiloxane monomer as stationary phase; maximum temperature, 350 °C). Injector and GC–MS transfer line temperatures were 280 and 250 °C, respectively. Ultrahigh purity helium (99.999%) was used as carrier gas at a constant flow of 1.0 ml/min. The electron impact ionization mass spectrometer was operated as follows: ionization voltage, 70 eV; ion source temperature, 200 °C; scan mode, 29.0–350.0 (mass range); scan rate, 1286.1 amu/s and 3.68 scan/s; start time 2.5 min. Electron multiplier (EM) voltage was obtained from autotune.

For neutral fraction, the split injection with a ratio of 30:1 was used. The sample volume injected with an AS 2000 Autosampler was 1 μl. The oven temperature program was 40 °C (3 min)–4 °C/min–110 °C (5 min)–2 °C/min–180 °C (15 min)–5 °C/min–270 °C (5 min).

The sample volume of acidic and basic fraction was 2 μl with a split ratio of 20:1. The oven temperature program of acidic fraction was 40 °C (3 min)–3 °C/min–120 °C (2 min)–5 °C/min–210 °C–10 °C/min–250 °C (5 min); that of basic fraction was 40 °C (3 min)–5 °C/min–90 °C (5 min)–120 °C (5 min)–220 °C (5 min)–250 °C (3 min).

2.5. Compounds identification and quantification

Identification was conducted following the procedures of Chung [14]. Tentative identification of compounds was made by matching the mass spectra of unknowns with those in the NIST02 (National Institute of Standards and Technology, Gaithersburg MD, USA) mass spectral library as well as the Wiley seventh (Wiley, New York, NY, USA) mass spectral library. Positive identifications were based on comparison between the mass spectra and retention indices of unknown compounds in extracts with the authentic standards (marked with an asterisk in Table 1) under the same experimental conditions. Quantitative data for an identified compound by three methods was obtained by the internal standard method using heptadecane as internal standard, without considering calibration factor (i.e. $F = 1.00$ for all compounds). The total amount of substance groups was the result of the addition of approximate concentrations of all identified volatile components in each group.

3. Results and discussion

In this study, four grades of aged flue-cured tobacco leaves were analyzed to obtain a typical composition of volatile compounds. Similar components were detected in these samples and only different in the concentration of compounds. But the extracts of same samples prepared by different methods were greatly different not only in the number of components (components, SD 322, SDE 377, HCD 391) but also in quantity (total amount, SD 228.42, SDE 445.48, HCD 315.72 μg/g). Table 1 showed the volatile components of one grade tobacco leaves prepared by three methods and these components were listed by chemical function groups. Table 2 showed the summary of components number and concentration. There were 408 compounds identified from the combined data, including 39 hydrocarbons, 53 acids, 61 alcohols, 24 aldehydes, 92 ketones, 26 esters, 17 lactones, 17 phenols, 7 miscellaneous oxygenated compounds, 7 pyrroles, 20 pyridines, 6 prazines, 7 amines, 20 nitrogenous compounds, and 12 tobacco alkaloids. The chromatograms (Figs. 1–3) illustrated the acidic, basic and neutral volatile compounds profiles of the same tobacco samples, which were obtained by SD, SDE, and HCD. They showed some differences among the three sample-preparing methods. Due to the large number of variables (volatile compounds), many co-eluting peaks and small peaks could not be identified. If without authentic standards, those compounds were not analyzed in this study.

3.1. Qualitative and quantitative comparison of the three methods

From Table 2, we can see that SDE gained advantage over HCD and SD in general. Though there were no obvious differences between the SDE (number of components 377) and the HCD (391) in qualitative analysis, HCD (total amount of volatile components 315.72 μg/g) showed a lower level than SDE (445.48 μg/g) quantitatively. And the SD showed the lowest level among the three methods both qualitatively (322) and quantitatively (228.42 μg/g). But from Table 1, there were different details in different classes of compounds.

3.1.1. Alcohols, aldehydes, ketones, and esters

Most of the highly volatile compounds in alcohols, aldehydes, ketones, and esters were found in the largest amount in HCD extract, and most of the low volatile compounds in these classes were found in the largest amount in SDE extract. The SD extract contained the lowest amount of these classes of compounds.

Quantitatively, some highly volatile alcohols showed lower level in SDE extract than in HCD and SD, such as 1, 2-propanediol. It was less than one-sixth in HCD extract or two-thirds in SD extract. Some familiar alcohols, such as 1-hexanol, linalyl alcohol, benzyl alcohol, and phenylethyl alcohol, were detected in the largest amount in SDE extract. Moreover, the amount of 3-hydroxy-7,8-dihydro-β-ionol was more than three times of that in HCD extract and five times in SD extract. Many aliphatic alcohols and terpene alcohols, such as 3-methylbutanol, 1-hexanol, 2-ethyl-1-hexanol, cis-linalool oxide, lemonol, isophorol, dihydronopol, and isophytol, were not detected in SD extract.

Extracts by three methods all showed that furfural was the most abundant aldehyde in tobacco samples, which perhaps arose from pyrolysis or hydrolysis during the process of curing, aging and fermentation as reported [18]. And it showed

Table 2
Summary of compounds number and total amount of different groups

	Groups	Total amount (μg/g)			Number of compounds			All
		SD	SDE	HCD	SD	SDE	HCD	
1	Alcohols	74.48	123.21	84.65	52	60	61	61
2	Aldehydes	13.12	34.07	27.57	18	23	24	24
3	Ketones	88.06	191.69	118.97	74	89	91	92
4	Acids	9.33[a]	11.05[a]	13.02[a]	44	51	50	53
5	Esters	19.28	22.34	20.51	18	22	23	26
6	Lactones	2.99	8.06	4.00	9	16	13	17
7	Phenols	1.62	12.84	7.22	13	17	16	17
8	Hydrocarbons	9.64[b]	23.14[b]	21.37[b]	37	38	38	39
9	Miscellaneous oxygenated compounds	2.12	2.13	7.40	5	7	6	7
10	Pyrroles	0.56	4.99	2.99	3	4	7	7
11	Pyridines	1.40	1.88	2.44	16	18	20	20
12	Pyrazines	0.08	0.05	0.14	6	4	6	6
13	Amides	0.22	0.73	0.50	2	4	6	7
14	Tobacco alkaloids	2.18[c]	4.30[c]	1.93[c]	11	11	12	12
15	Nitrogenous compounds	3.35	5.00	3.01	14	13	18	20
	Together	228.42	445.48	315.72	322	377	391	408

Total amount—total amount of each group based on Table 1. Number of compounds—the number of compounds in each group detected by different methods. All—the total number of compounds in each class detected by three methods. Together—the total amount of all compounds detected by different methods and the total number of all compounds.

[a] Not containing palmitic acid.

[b] Not containing neophytadiene.

[c] Not containing nicotine.

highest level in SDE extract among the three methods. Some aliphatic aldehydes with high level in SDE and HCD extract, such as 2,4-heptadienal and 4-methy-2-pentenal, which could impart grassy and oily aldehydic odors to the SDE and the HCD extract, were even not found in SD extract. The principal components of aromatic aldehydes, such as benzaldehyde and benzeneacetaldehyde, showed the highest level in HCD extract and exceptionally low level in SD extract. As for an important flavor compound vanillin, the amount in SDE extract was more than two times of that in HCD, and eight times of that in SD.

Ketones were the biggest class in tobacco samples and contained 92 compounds. From 1-penten-3-one and acetoin to 3-methyl-2-cyclohexen-1-one and 3-ethyl-2-hydroxy-2-cyclopenten-1-one, most of these ketones with relatively low molecular weight showed the highest level in HCD extract. Meanwhile, about half of them showed the lowest level in SD extract. The 80 percent of ketones showed the highest level in SDE and the lowest level in SD extract. Among aliphatic ketones, many kinds of alkenyl-2-ones were detected in three extracts. The amount of aliphatic and alicyclic ketones by SD, SDE, and HCD were 51.81, 84.66, and 62.78 μg/g, respectively, and the quantitative changes in those compounds, which have woody and hay like odors must result in a great difference among the three extracts. Damascenone, 2,3-dehydro-α-ionone, tetrahydroionone, 3-oxo-α-ionol, ionone, retro-ionone and 6,7-dehydro-7,8-dihydro-3-oxo-β-ionol were exceptionally abundant in SDE extract, but dihydro-β-methylionone was even not found. A large number of β-ionone and its epoxides were found in SDE extract. Most of them increased during SDE process and showed the lowest level in SD extract.

The low molecular weight esters such as isopropyl acetate, diethyl oxalate, phenmethyl acetate and so on were detected in the largest amount in HCD extract. The medium and longer chain esters except methyl palmitate were found in the largest amount in SDE extract. Except ethyl chrysanthemate, ethyl linoleate, and methyl palmitate in the medium amount, all the others were detected in the lowest amount in SD extract.

3.1.2. Acids, lactones, phenols, and hydrocarbons

The same trend was formed by the efficient extraction of the highly volatile fatty acids by HCD (total amount 4.13 μg/g), comparing to that by SDE (3.15 μg/g) and SD (1.13 μg/g). In addition, some volatile aromatic acids such as benzoic acid and toluic acid abundant in HCD extract were hardly detected in SDE and SD extracts. It is very exceptional that more than half of the advanced fatty acids were detected in the largest amount in SD extract, and half of them were in the lowest amount in SDE extract. Meanwhile, palmitic acid, heptadecenoic acid, heptadecanoic acid, octadecanoic acid, and sum of oleic acid, linolenic acid and linoleic acid in SD extract were 4.4, 3.3, 3.6, 5.2, and 4.1 times of those in SDE respectively. Most of them were detected in the medium amount in HCD extract except medium-chain fatty acids (C_{11}–C_{13}) that were in the largest amount.

In hydrocarbons, lactones and phenols classes, the amount of compounds was detected in the largest amount in SDE extract generally. But phenol showed the highest level in

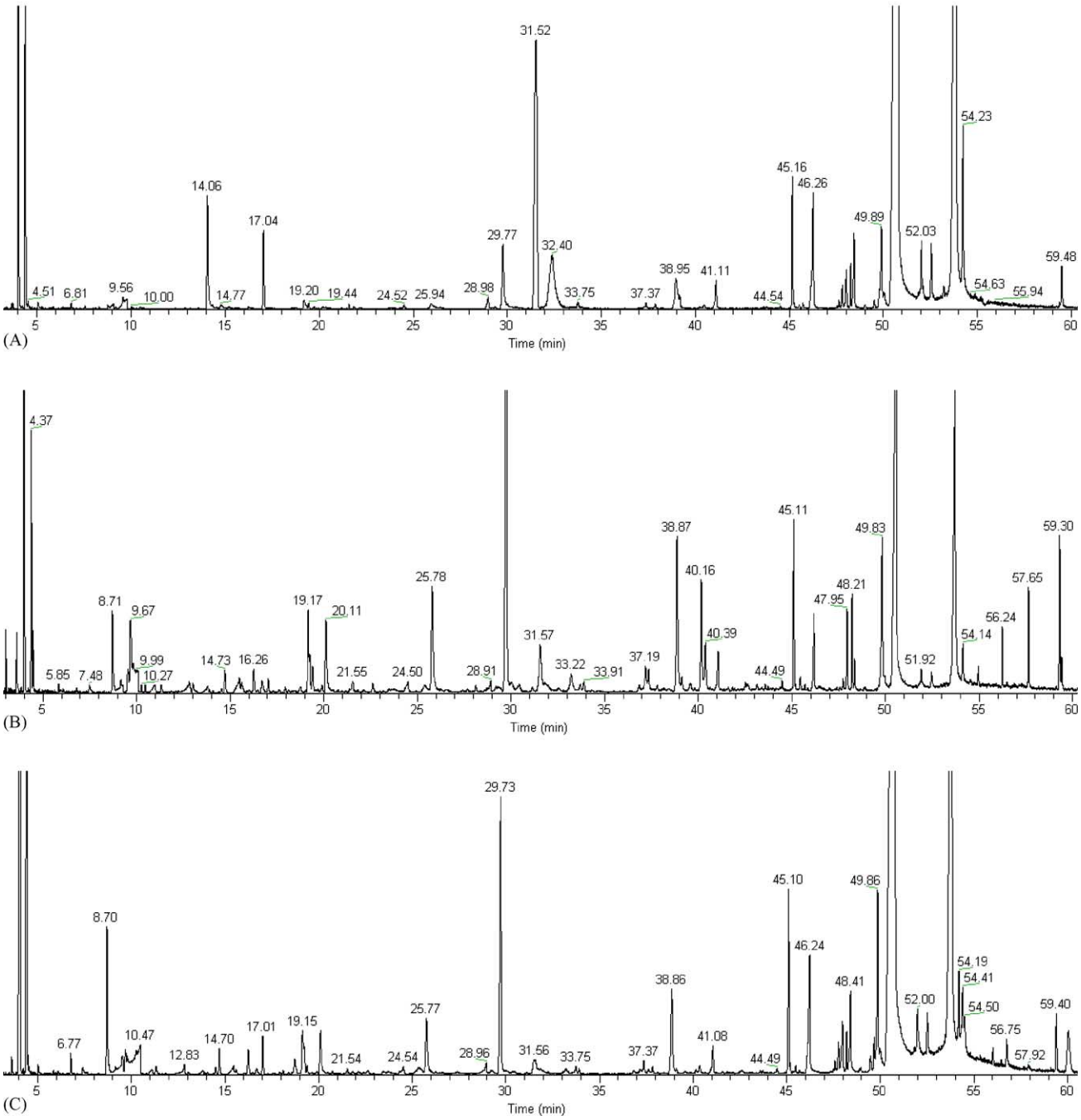

Fig. 1. GC–MS total ion chromatograms of acidic volatile components of tobacco in (A) SD, (B) SDE, and (C) HCD extracts.

HCD extract. SD did not detect several phenols such as 4,5-dimethoxy-2-methylphenol and methyl-*p*-hydroquinone, which showed higher level in SDE and HCD extracts.

Neophytadiene, which is the most abundant hydrocarbon in tobacco, showed the lowest level in SDE extract among the three. All biphenyls, several naphthalenes, and some specific miscellaneous compounds were detected by all three methods and showed the highest level in SDE extract. And as other authors had reported [19–22], they perhaps resulted from the contamination during the tobacco growth, storage, and manufacture processes.

3.1.3. Pyrroles, pyridines, pyrazines, and alkaloids

As shown in Table 2, HCD showed the highest sensitivity to basic compounds of tobacco volatile components. Almost all of the pyrroles, pyridines, pyrazines and alkaloids were detected in HCD extract. Conversely, three pyrroles, two pyridines and two pyrazines were not detected in SDE

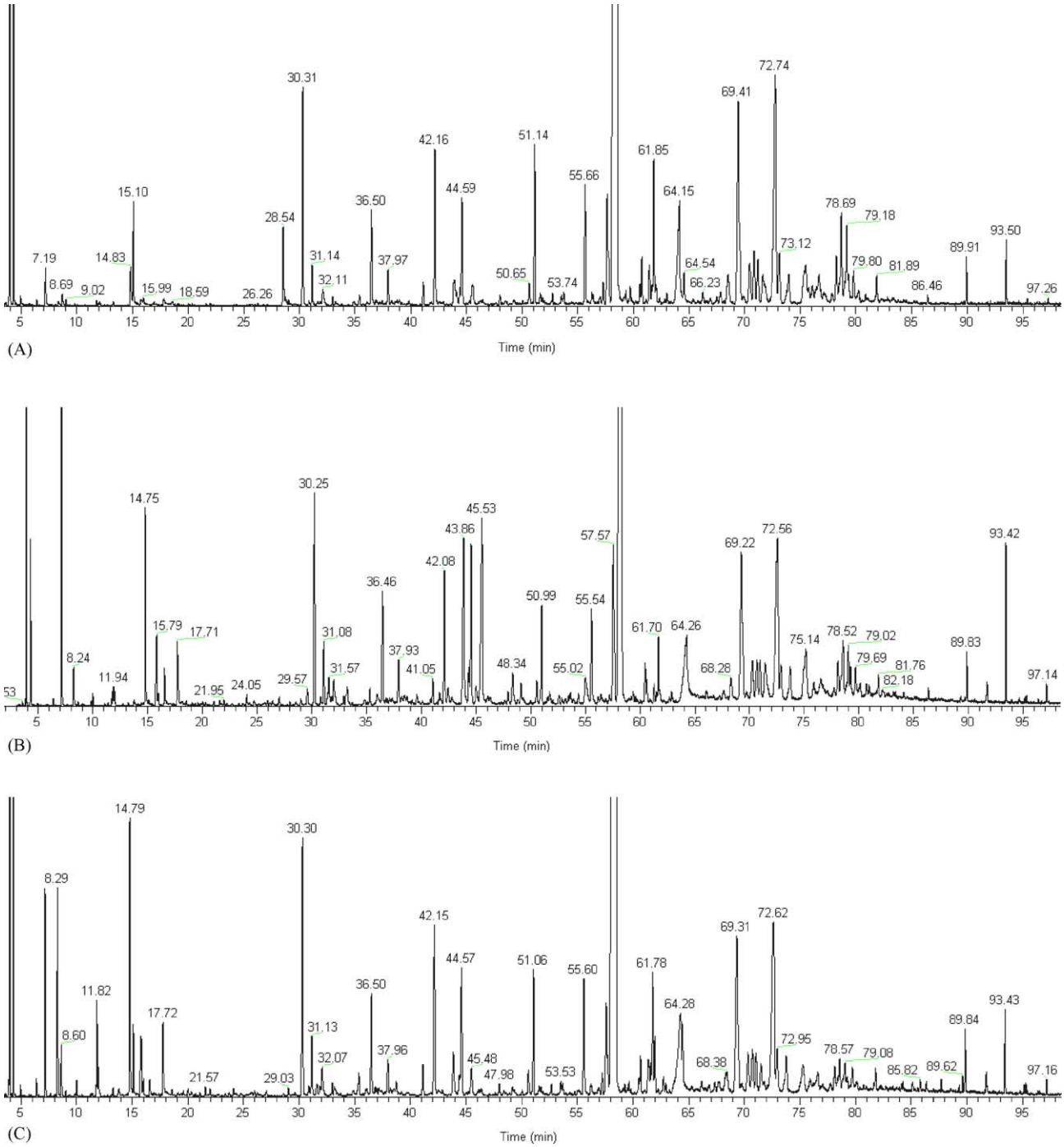

Fig. 2. GC–MS total ion chromatograms of neutral volatile components of tobacco in (A) SD, (B) SDE, and (C) HCD extracts.

extract and detected in lower level in SD extract. But the amount of pyrroles, amines, alkaloids and nitrogenous compounds were all the largest in SDE extract except pyridines and pyrazines, which were found the largest in HCD extract.

In detail, all pyrroles identified in SDE extract showed the highest level except 2-acetyl-1-pyrroline. Especially, 2-acetylpyrrole was almost two times of that by HCD and twelve times by SD. But there was a great change happened to pyridine. All pyridines were detected in the largest amount in HCD extract except 3-methylpyridine and 3-acetopyridine. The amount of pyridines detected by HCD was more than two times of that by SDE. The same as pyridines, except 2-methyl-6-[(1E)-1-propenyl]pyrazine, all the other pyrazines show the highest level in HCD extract. Especially, methylpyrazine, which showed high level

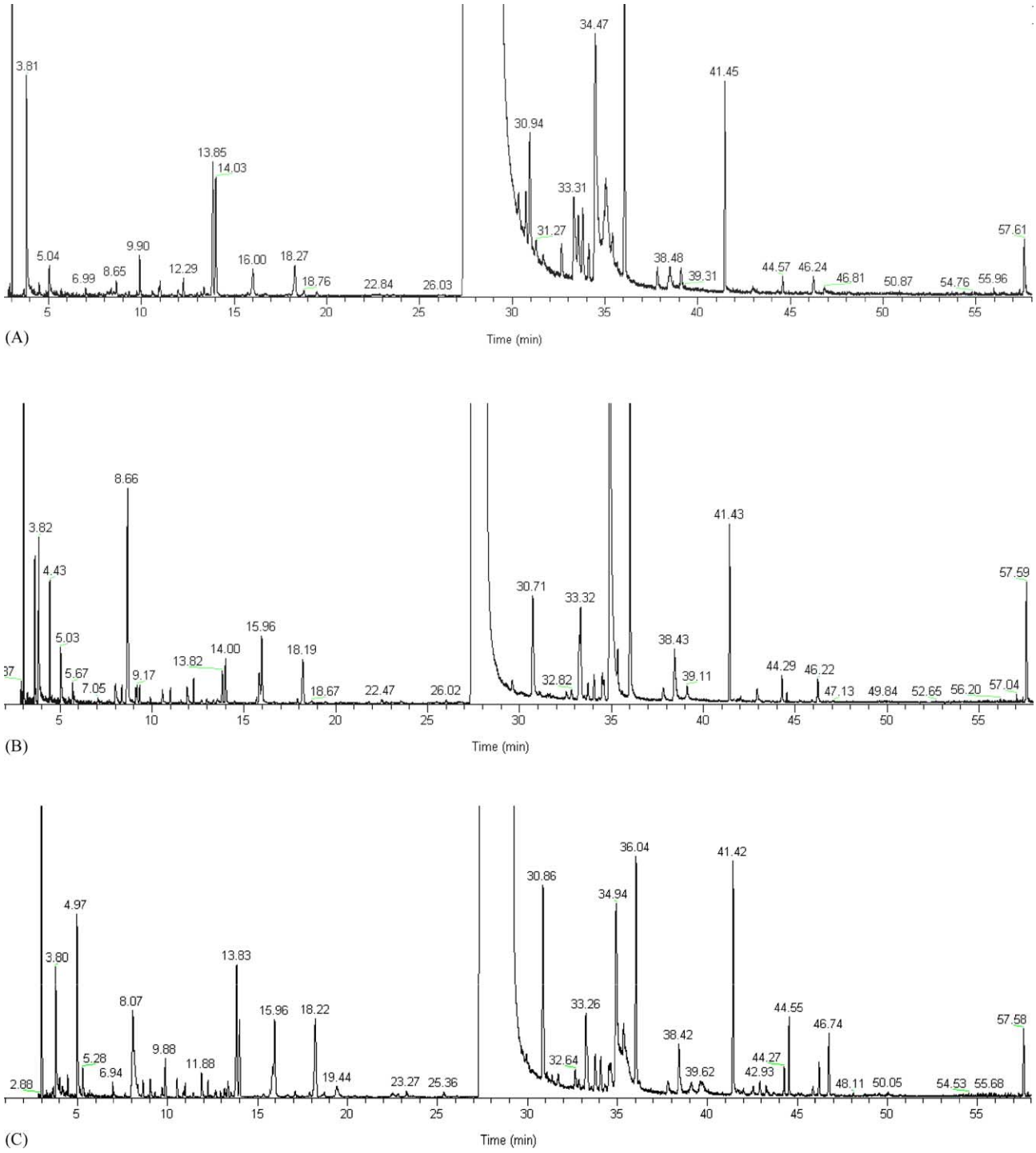

Fig. 3. GC–MS total ion chromatograms of basic volatile components of tobacco in (A) SD, (B) SDE, and (C) HCD extracts.

in HCD extract, was even not detected in SDE extract. As we know, nicotine is a main component in tobacco, and the result of it by the three methods agreed with each other. Concerning its water solubility and its stability to heating in the open air, there were great differences in content by the three methods. The amount of nicotine by SDE was only about one third of that by SD and a half of that by HCD.

3.2. Comparison of repeatability of the three methods

A series of five consecutive extracts were performed on different aliquots of tobacco in order to evaluate the repeatability of the SD, SDE, and HCD methods. As shown in Table 1, the precision of the SD, SDE, and HCD methods are satisfying. For the compounds whose concentrations

were above 0.10 μg/g, 72.27% of them had the R.S.D. below 10.00% in SD extract, 85.19% in SDE extract and 80.91% in HCD extract. And the amount of those compounds were 98.25% of total amount in SD extract, 95.45% in SDE extract and 92.26% in HCD extract (not containing palmitic acid, neophytadiene and nicotine). As far as it is concerned, SD, SDE, and HCD all can be used for the analysis of volatile components in tobacco. However, SDE was the best one. But for low-M_r (molecular mass) ketones, pyridines, and pyrazines, the precision of SDE was worse than HCD. To semi-volatile fatty acids, lactones, and phenols, the precision of SDE was worst than SD and HCD. Generally, the same thing happened to the three methods: the precision of semi-volatile fatty acids and esters was worse than any other kinds of volatile components.

3.3. Comparison of operation process of the three methods

In this study, the analysis of volatile components in tobacco was originally undertaken through the use of SD, SDE, and HCD techniques, which were referred to many references. The important parameters that might affect the methods were investigated, such as the duration of SD, the duration of SDE, the temperature of distillation and the flow rate of nitrogen in HCD. Those different parameters were experimented to determine the optimum conditions for the following analysis.

As we know, SD needs several steps in sample preparing and is time-consuming and solvent-consuming, which will lead to the sample loss and the contamination during the process.

As it is known, SDE analysis of volatile compounds is a widely used technique. It has two major advantages: only two single main operations (extraction and concentration) and giving a relatively wide spectrum of chemical compounds detected. In addition, we speculated that there was the possibility for the artifacts generated from continuous heating in an open-air condition during SDE process, which need further study in our future work. Now some authors [10,23] have also noted that low recovery of some volatile compounds might result from artifacts formation and oxidation of volatile components in SDE.

HCD use a simple, easily constructed glass apparatus, which allows distillation of volatile components from plant matrices under the continuous stream of an inert gas. With the technique, headspaces over the sample is continuously swept by an inert gas to avoid the sample heated continuously and provide an inert atmosphere to the sample which can efficiently reduce thermal degradation and interaction with air. The analytes partitioning in the headspace are subsequently trapped. Hence, HCD preparation is capable for the detection of highly volatile analytes with low molecular weight. Comparing to HCD, the other two techniques lack the sensitivity to these analytes.

4. Conclusion

In summary, both SDE and HCD contained the similar components. Though traditional SDE analysis of volatile components is a more widely used technique comparing to HCD, it shows less recovery of components having high volatility. By HCD technique, we detected the largest amount of low-M_r components in alcohols, aldehydes, ketones, acids and esters comparing to SD and SDE. In addition, pyridines and pyrazines also showed the highest level in HCD extract. Conversely, SDE provided a good recovery of those having a high M_r in alcohols, aldehydes, ketones, anhydrides, esters, lactones, phenols, tobacco alkaloids, and nitrogenous compounds. Meanwhile, we speculated that heating in the open air might lead to the oxygenation of volatile compounds during SDE process and it needs our further study to prove that. However, HCD does well in it. As to SD, it only does well in advanced fatty acids. Besides this, it cannot parallel the other two techniques either in the number of compounds or in the amount of volatile compounds detected in the tobacco.

From what we mentioned above, we suggest that SDE and HCD are both good preparation methods for comprehensive analysis of volatile compounds in tobacco and can reinforce each other.

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