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Accelerated solvent extraction for GC-based tobacco fingerprinting and its comparison with simultaneous distillation and extraction

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ABSTRACT

An accelerated solvent extraction (ASE) procedure has been developed as a pretreatment method for chemical fingerprinting of volatile and semi-volatile components in cut tobacco. The ASE extraction conditions including temperature, operation pressure and extraction cycles were optimized to maximize extraction yield. The method was validated with repeatability, recovery and linearity. Compared with simultaneous distillation extraction (SDE), ASE provides higher extraction yields, less extraction time, lower solvent consumption and less labor time, and is more suitable for tobacco sample preparation. A typical ASE extract was analyzed by gas chromatography/time-of-flight mass spectrometry (GC–TOFMS). A total of 305 components with signal-to-noise ratio higher than 100 were tentatively identified by NIST05 and Wiley database. Finally, 36 cigarette samples from six cigarette brands were analyzed using the developed chemical fingerprinting method. Partial least squares-discriminant analysis shows good discrimination of different cigarette brands. The results indicate that ASE method can serve as high-throughput sample preparation technique for cigarette chemical fingerprint analysis.

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1. Introduction

Volatile and semi-volatile components in cut tobacco are main contributors of cigarette flavor. Comprehensive comparison of tobacco components or tobacco chemical fingerprinting is critical for the discrimination of cigarette brand characteristics and discovery of important flavor-related components. Gas chromatography (GC)-based instrumental analysis methods, such as gas chromatography-flame ionization detection (GC-FID) [1,2], gas chromatography–mass spectrometry (GC–MS) [3,4] and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOFMS) [5,6], have been used to analyze the volatile and semi-volatile components in cigarette and its smoke. Cigarette brand characteristics investigation using tobacco chemical fingerprinting involves large number of samples. A high-throughput and reliable sample preparation method is indispensable. Many sample preparation techniques of the volatile and semi-volatile components in tobacco or cigarette have been developed including solvent extraction and distillation [4,7–9]. Simultaneous distillation and extraction (SDE) [3,10,11] and steam distillation (SD) [9,12,13] were the most widely used methods. SDE has been proved to be a better extraction method for volatile tobacco components than SD [9]. Sample preparation using accelerated solvent extraction (ASE) was firstly introduced in 1995 [14,15]. It has several advantages over traditional solvent extraction methods including less extraction time (approximately 30 min per sample) and solvent consumption, higher extraction yields, better reproducibility [16] and less extraction discrimination [17]. Additionally, automation of the ASE instrument makes it easy for batch extraction. ASE has been reported to extract tobacco components such as terpenoids and sterols [18]. In the present study, an ASE method was developed for high-throughput sample preparation of volatile and semi-volatile components in cigarettes. The developed method was compared with SDE method. The extracts were used for GC-based chemical fingerprinting, and discrimination of different cigarette brands were carried using principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA).

2. Experimental

2.1. Chemicals and reagents

Thirty-six flue-cured cigarettes from 6 Chinese well-known brands were purchased from local malls licensed by state tobacco

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monopoly administration and stored at 4 °C. Cut tobacco samples were collected and ground to pass through a 40-mesh sieve. The sealed tobacco powder was equilibrated in a sealed package at room temperature for 2 h before sample preparation. The chemical standards with purity higher than 98% were purchased from Alfa Aesar (Tianjin, China). They were used for method validation and positive identification of tobacco compounds, which included 2,3-butanediol, furfural, trans-farnesol, methyl dodecanoate, oleic acid, benzyl benzoate, vanillin, myosmine, tetradecanoic acid, 5-hydroxymethyl-2-furancarboxaldehyde, (*S*)-5-hydroxymethyl-2[5*H*]-furanone, 7-hydroxy-6-methoxy-2*H*-1-benzopyran-2-one, 2-octanone, nonanoic acid, octanoic acid, hexanoic acid, 2(5*H*)-furanone, 6-methyl-5-hepten-2-one, trans-geranylacetone, 2-furanmethanol, pyridine, cyclopentanone, decanoic acid, 3-methyl-1-butanol, acetylpropionyl, cyclohexanone, 2-octanone, β-linalool, 4-oxoisophorone, p-mentha-6,8-dien-2-one, geranylacetone (mixture of cis- and trans-), trans-β-ionone and trans-phytol. 2-Methyl-naphthalene was selected as internal standard and purchased from Sigma–Aldrich (Beijing, China).

Dichloromethane (HPLC grade, Dikma, Beijing, China) was used as extraction solvent of ASE and SDE. Anhydrous sodium sulfate and sodium chloride (analytical grade, Kermel, Tianjin, China) were used for SDE experiment.

2.2. Accelerated solvent extraction

A Dionex ASE200 accelerated solvent extractor (CA, USA) equipped with 11-mL stainless extraction cells and 60-mL glass collection bottles was used for the accelerated solvent extractions (ASEs). 4.00 g of tobacco powder was weighted and filled into a stainless steel extraction cell. 200 μL of internal standard solution (0.15 mg mL^{-1}) was spiked to the tobacco powder before extraction. Extraction conditions were as follows: dichloromethane as extraction solvent, static extraction time of 5 min, two extraction cycles (the sample was extracted 2 times with the same volume of solvent), extraction temperature at 100 °C and extraction pressure at 1000 psi. About 25 mL of extraction solution was collected and then condensed to 1 mL with a rotary evaporator at atmospheric pressure. The condensed solution was filtered and stored at a 1.5-mL screw capped vial for GC and GC–TOFMS analysis.

2.3. Simultaneous distillation and extraction (SDE)

The SDE experiment was performed with a Likens–Nickerson apparatus [19]. Before the experiment, 200 mL of redistilled water and 50 mL of dichloromethane were distilled for 1 h with the following procedure to wash up the SDE system. 4.00 g of tobacco powder was placed in a 1000 mL flask mixed with 200 mL of saturated sodium chloride solution containing 0.03 mg of 2-methyl-naphthalene as internal standard, and flask was mounted on the sample port of SDE. A 100 mL flask with 50 mL dichloromethane was linked to solvent port of the SDE. Dichloromethane solvent and sample mixture were boiled for 2 h. At last, about 50 mL extract solution was obtained and dehydrated with 3.00 g of anhydrous sodium sulfate overnight. This solution was then condensed and filtered by the method given in Section 2.2.

2.4. GC-FID and GC–TOFMS analysis

GC analysis was carried out on an Agilent 6890 GC system (Agilent Technologies, USA) equipped with a flame ionization detector (FID). A DB-5 MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies, Palo Alto, CA, USA) was used as separation column. Helium was used as the carrier gas at flow rate of 1.2 mL min^{-1}. The GC oven was programmed at 50 °C (1 min), raised to 220 °C (7 min) at 8 °C min^{-1}, then heated to 280 °C at 15 °C min^{-1}, and hold at the final temperature for 20 min. GC inlet liner with glass wool was renewed every 10 samples to prevent the contamination of the column. 1 μL sample was injected using split mode with a split ratio of 15:1. Temperature of FID detector and inlet was kept at 280 °C.

A time-of-flight mass spectrometry (TOFMS) Pegasus III (Leco Corp., St. Josephs, MI) was used to acquire mass spectra data from the GC using 70 eV electron impact ionization and −1450 V multichannel plate voltage. The temperature of transfer line was kept at 250 °C. The data acquisition rate was 5 Hz for the mass range of 35–450 amu. Data were recorded and analyzed using the ChromaTOF software Ver. 3.32 provided by Leco Corporation.

2.5. Data analysis

GC-FID peak table with comma separated values (CSV) format was aligned by home-made software. After the peak alignment, a data matrix was generated for further multivariate statistical analysis.

Tentative identification of compounds was made by matching the mass spectra of unknowns with those in the NIST05 (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 comparing the retention time of tentatively identified compounds with the authentic standards under the same analytical conditions.

Cluster heat map was made with the software MultiExperiment View Ver. 4.1 (MEV, www.tm4.org). PCA and PLS-DA were performed with SIMCA-P (Umetrics, Umea, Sweden). Peak areas of all components were divided by the peak area of the internal standard and then scaled to zero mean and unit variance (UV) [20] before PCA and PLS-DA analysis. Other tables and figures were made with Microsoft excel 2007.

3. Results and discussion

3.1. ASE method development

In order to improve ASE extraction efficiency, extraction temperature, pressure and static cycle times were investigated. Tobacco powder was extracted at 50, 80, 100, 110, 120, 130, 140 and 150 °C, respectively. The total peak area increased with temperature as shown in Fig. 1. Detailed comparisons of tobacco components with different extraction temperatures are displayed using a cluster heat map in Fig. 2. Components with similar variation of extraction yields were clustered on the heat map. Extraction yields of almost all components were improved at higher tem-

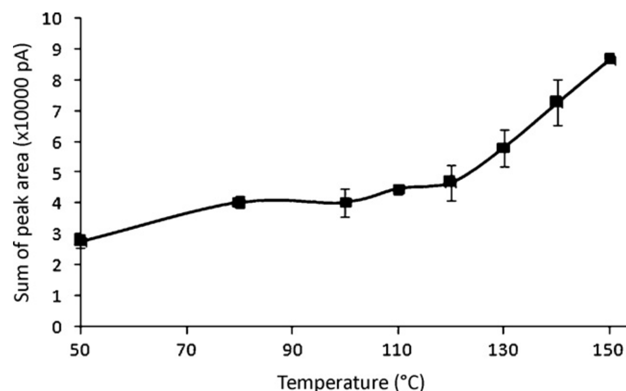

Fig. 1. Extraction yields of the sum of all components at different extraction temperatures.

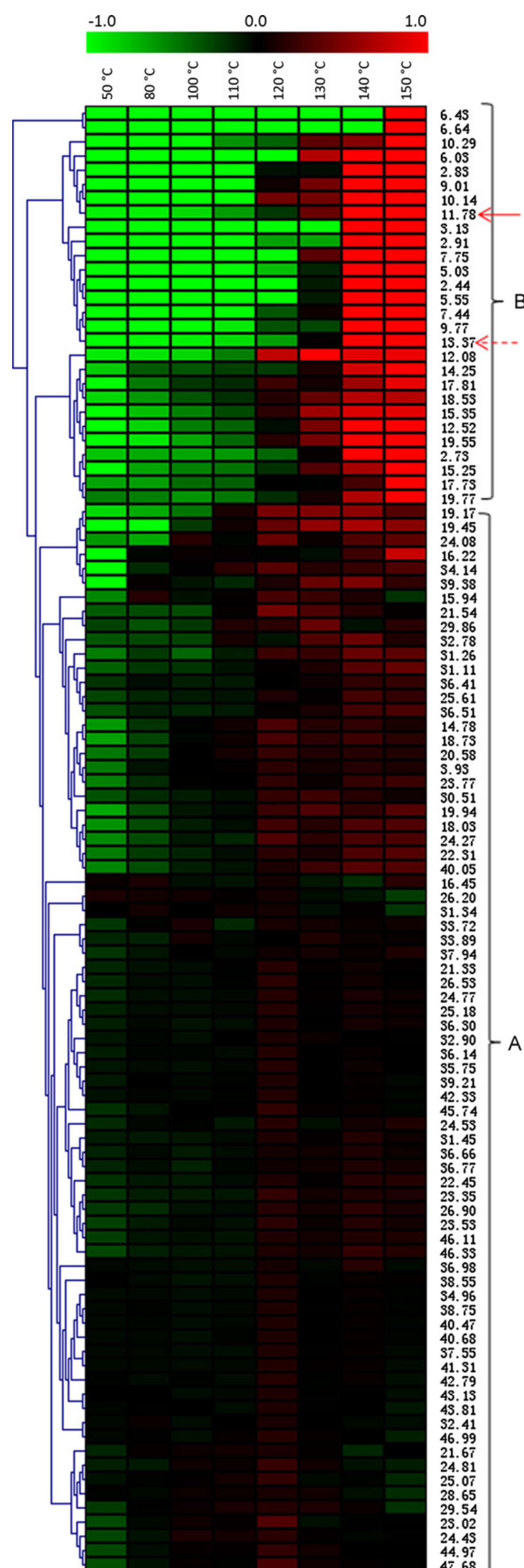

Fig. 2. Cluster heat map of tobacco components at different extraction temperatures. Columns represent different extraction temperatures, rows represent relative peak area (to internal standard) of individual tobacco component which was marked with its retention time (min) at the right side of the plot. Samples at each temperature point were analyzed in duplicate. Peak area of every component was subtracted by the mean of this component in different temperatures, then divided by the mean to make the peak area changes of most components ranged from −1 to 1. The values smaller than −1 and greater than 1 were denoted with the color of −1 and 1 in the plot, respectively. Position of DDMP and HMF on the heat map is indicated by red arrow and red dash line arrow, respectively.

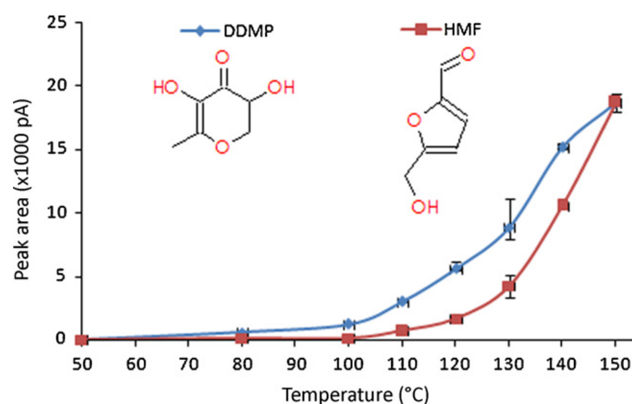

Fig. 3. Extraction yields of two Maillard reaction intermediates at different temperatures. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (DDMP) and 5-(hydroxymethyl)-2-furancarboxaldehyde (HMF) were identified by GC–TOFMS. In this experiment, the extraction pressure and cycles were set as 1500 psi and 3, respectively.

peratures. In theory, a higher temperature should be selected. However, chemical reactions were observed. As shown in Fig. 2, tobacco components are roughly clustered in two groups. Extraction yields of components in group A had little change with temperature from 50 to 150 °C. While extraction yields of components in group B increased dramatically in this temperature range, especially from 100 to 150 °C. For example, extraction yields of two Maillard reaction intermediates, 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (DDMP, marked with red arrow in Fig. 2) [21–23] and 5-(hydroxymethyl)-2-furancarboxaldehyde (HMF, marked with red dash line arrow in Fig. 2) [21,24–27], have increased about 15- and 211-fold from 100 to 150 °C (Fig. 3). It implied chemical reactions, especially Maillard reaction [28], may have occurred at a higher temperature. Other Maillard reaction products in group B were tentatively identified as acetic acid [29], 3-methyl-butanal [30], 2,3-pentanedione [30], 2-furanmethanol [31], benzeneacetaldehyde [30], furfural [31], 3,5-dihydroxy-2-methyl-4*H*-pyran-4-one [32] and 5-(hydroxymethyl)-2(5*H*)-furanone,(*S*)- [31]. Therefore, appropriate extraction temperature is the balance between higher extraction yields and less possible chemical reactions. In the meantime, extracted non-volatile components which result in contamination of GC inlet and column should be as less as possible. Finally, an extraction temperature at 100 °C was chosen.

Effects of different extraction pressures and cycles on extraction yield were also investigated. Extraction yields (the total peak area and individual peak area of scopoletin, megastigmatrienone 2, ascabin and myosmine) at different extraction pressures and cycles were displayed in Fig. 4. Using pressures 1000, 1500 and 2000 psi, extraction yields have a little change (Fig. 4a). Similarly, number of extraction cycle has no distinctive effect on extraction yield (Fig. 4b) while extraction time increased proportionally. Finally, the optimum extraction conditions were extraction pressure of 1000 psi, two cycles and 5 min per cycle.

3.2. Linearity and repeatability

To evaluate the linearity of the GC-based fingerprinting method, regression equations and correlation coefficients of tobacco components were calculated. Standard stock solution with 10 standards was spiked to a tobacco sample in the range of 0.0125–1 mg mL^{-1} and extracted with the method defined in Section 2.2. The lin-

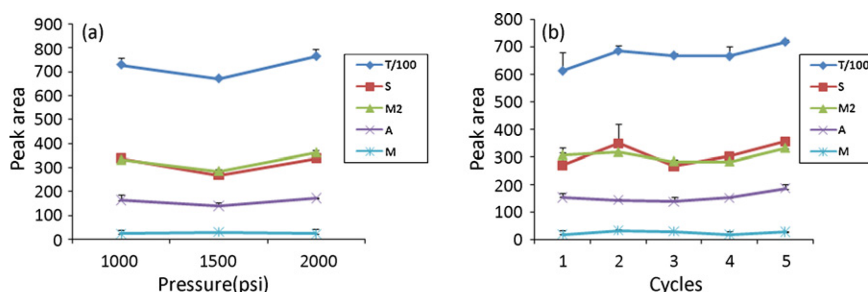

Fig. 4. Peak areas of four typical tobacco components scopoletin (S), megastigmatrienone 2 (M2), ascabin (A), myosmine (M) and total peak area (T/100, the total peak area of all extracted components divided by 100) at different extraction pressures (a) and cycles (b). The extraction temperature was set as 100 °C, and 3 cycles and 1500 psi were used in (a) and (b), respectively.

ear equation of calibration curve of six concentration points was calculated. Intra- and inter-day repeatabilities were evaluated by analyzing six duplicates of tobacco components in the same day and six consecutive days. The intra- and inter-day repeatabilities were described as the value of the relative standard deviation (RSD, %) of the mean relative peak area. Both linearity and repeatability results are shown in Table 1. The good linearity (r^2 ranged from 0.970 to 0.996) and repeatabilities (intra-day RSD ranged from 3.27 to 6.70%, inter-day RSD ranged from 5.13 to 7.81%) indicate that this method can be used to collect tobacco fingerprinting from different cigarette brands or tobacco leaves.

3.3. Identification of extracted components

GC–TOFMS was used to separate and identify tobacco components. A total of 305 components including alkaloids, ketones, phenols, esters and carboxyl acids with signal-to-noise ratio higher than 100 [33] were tentatively identified from a typical cut tobacco extract by NIST05 and Wiley database after the deconvolution with the ChromaTOF software Ver. 3.32. Fifty-four of them with higher mass spectra match factors which have similarity and reverse more than 850 and probability more than 5000 were listed in Table 2. Twenty components were further verified by authentic standards.

3.4. Comparison between ASE and SDE

SDE is one of the most widely employed methods for extraction of volatile and semi-volatile components. An overall comparison of ASE and SDE methods for tobacco chemical fingerprinting was performed. The same amount of tobacco samples (4.00 g) was extracted with SDE and ASE, respectively. Extraction solutions were concentrated and analyzed under the same instrument conditions. Typical GC-FID chromatograms of tobacco chemical fingerprinting were displayed in Fig. 5. It can be observed that more peaks and higher peak intensity can be obtained by using the ASE method than the SDE method. At the same analytical conditions, a total of 176 peaks were detected from the ASE extract, and only 66 peaks were found from the SDE extract. The difference of extraction yields between the two extraction methods may derive from their different extraction mechanisms. Extraction efficiencies of the ASE method depend on solubility of components in solvent. The extraction yields of compounds using the SDE method are based on their volatility and the distribution coefficient between water and an organic solvent [34]. The SDE method is suitable for the analytes with appropriate volatility and distribution coefficient. 1, 2-Propanediol and some semi-volatile components could not be extracted by the SDE method due to their good water solubility or low volatility (Fig. 5).

Recovery evaluation of the two methods was performed using standard-spiked samples. Recovery was determined at three levels (standard addition concentration is approximately 50, 100 and 300% of those in a flue-cured tobacco sample). The results were displayed in Table 3. Recoveries of all the components using ASE method are better than those using the SDE method. The additional lose of components using SDE method may be attributed to dissolution to saturated sodium chloride solution and escapes from the system during the SDE process. Recoveries of compounds such as 3-methyl-1-butanol (bp: 131–132 °C), 2,3-pentanedione (bp: 110–112 °C), cyclohexanone (bp: 155 °C), 2-octanone (bp: 173 °C) and β-linalool (bp: 199 °C) are lower for both ASE and SDE method.

Repeatabilities of the two methods were also investigated. Comparision of relative standard deviation (RSD) distribution using the ASE and SDE was displayed in Table 4. The repeatabilities of two methods were similar, the RSD of over 80% peaks (144 peaks of ASE and 53 peaks of SDE) were less than 20%, which accounted for about 95% of total peak area (95.5% for ASE and 94.8% for SDE). The repeatabilities of both methods meet requirment of fingerprinting analysis.

Throughput of sample preparation method was also very important and should be taken into account. Extraction time of the ASE for a tobacco sample was 30 min, which was about one-sixth of time of the SDE. Manual operation, such as solvent and sample replacement, was involved in the SDE method. The ASE instrument could automatically extract 24 samples without intervention. In addition,

Table 1
Linearity and repeatability of 10 typical tobacco components.

Compound	Retention time (min)	r^2	Intra-day repeatability (RSD, %)	Inter-day repeatability (RSD, %)
3-Methyl-1-butanol	3.15	0.983	6.70	7.81
2,3-Pentanedione	3.57	0.996	3.64	6.56
Cyclohexanone	6.12	0.996	3.93	5.13
2-Octanone	7.26	0.990	3.64	6.39
β-Linalool	8.19	0.970	3.73	6.84
Isophorone epoxide	10.82	0.983	3.27	5.92
p-Mentha-6,8-dien-2-one	12.80	0.976	3.49	5.68
cis-Geranylacetone	15.69	0.983	3.58	7.57
Trans-geranylacetone	16.01	0.980	3.41	6.52
Trans-β-ionone	16.48	0.981	3.33	6.14

Table 2
Identified analytes with good match and high probability values in a typical cut tobacco sample.

tr (s)	Name	CAS No.	Similarity	Reverse	Probability	Verified
132.1	Acetic acid	64-19-7	943	981	8691	
137.4	Butanal, 3-methyl-	590-86-3	915	915	8828	
152.4	2,3-Pentanedione	600-14-6	990	990	9735	
189.7	Propylene glycol	57-55-6	982	982	7543	√
223.5	Propanoic acid, 2-oxo-, methyl ester	600-22-6	965	965	8861	
228.9	4-Methylpent-2-enal	5362-56-1	862	871	5108	
233.7	2,3-Butanediol	513-85-9	909	909	7677	√
266.3	2-Furancarboxaldehyde	98-01-1	932	958	7952	√
292.7	2-Furanmethanol	98-00-0	962	962	8002	
324.6	Cyclohexanone	108-94-1	955	955	7656	√
360.3	2(5*H*)-Furanone	497-23-4	939	939	9453	√
384.9	Butanoic acid, 3-methyl-	503-74-2	947	947	8497	
391.5	2-Methylbutanoic acid	116-53-0	857	884	6859	
450.7	Ethanol, 2,2′-oxybis-	111-46-6	984	984	9781	
488.9	2-Butenoic acid, 2-methyl-, (*E*)-	80-59-1	884	912	5645	
500.7	Benzeneacetaldehyde	122-78-1	871	946	8496	
521.1	Glycerin	56-81-5	985	985	9837	√
540.9	Hexanoic acid	142-62-1	886	886	5521	√
587.1	2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	3658-77-3	937	937	9529	
609.4	2,6,6-Trimethylcyclohex-2-ene-1,4-dione	1125-21-9	972	972	7799	√
628.7	2-Phenylpropenal	4432-63-7	887	952	8035	
665.3	2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	28564-83-2	911	930	8486	
689.5	3-Pyridinol, 2-methyl-	1121-25-1	895	916	5409	
705.9	4-Hydroxydihydro-2(3*H*)-furanone	5469-16-9	948	948	8902	
714.1	(*S*)-5-Hydroxymethyl-2[5*H*]-furanone	78508-96-0	956	956	8311	√
717.5	4*H*-Pyran-4-one, 3,5-dihydroxy-2-methyl-	1073-96-7	926	926	9841	
719.3	5-Hydroxymethyldihydrofuran-2-one	N#: 42812	934	934	7037	
720.3	(*S*)-(+)-2′,3′-Dideoxyribonolactone	32780-06-6	941	941	7490	
720.6	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	99-49-0	967	967	5771	√
744.5	Dihydro-5-(1-hydroxyethyl)-2(3*H*)-furanone	w#: 19090	857	869	6026	
752.5	Octanoic acid	124-07-2	884	896	7867	√
760.7	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	67-47-0	898	898	9340	√
783.1	2,3-Dihydro-benzofuran	w#: 13838	898	911	6308	
827.3	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (*S*)-	54-11-5	932	935	9724	
873.9	1-(2,4,6-Trimethylphenyl)buta-1,3-diene	w#: 56052	899	932	6862	
901.9	Benzaldehyde, 4-hydroxy-3-methoxy-	121-33-5	848	848	7375	√
918.7	5,9-Undecadien-2-one, 6,10-dimethyl-, (*E*)-	3796-70-1	959	959	9661	√
926.5	Pyridine, 3-(3,4-dihydro-2*H*-pyrrol-5-yl)-	532-12-7	874	925	9450	√
950.7	Benzeneethanol, 4-hydroxy-	501-94-0	907	907	6883	
956.0	Trans-β-Ionone	79-77-6	963	963	5607	√
1028.1	2,3′-Dipyridyl	581-50-0	924	928	8452	
1093.1	Megastigmatrienone 2	w#: 73639	875	876	5307	
1114.5	(9*R*)-9-Hydroxy-4,7*E*-megastigmadien-3-one	52210-15-8	905	905	7599	
1177.1	2-Pyrrolidinone, 1-methyl-5-(3-pyridinyl)-, (*S*)-	486-56-6	919	919	9384	
1187.5	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	2478-38-8	860	864	9326	
1203.7	4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	N#: 87929	868	886	9148	
1208.1	Tetradecanoic acid	544-63-8	943	943	7735	√
1218.1	Benzoic acid, phenylmethyl ester	120-51-4	937	937	8659	√
1253.5	Neophytadiene	504-96-1	923	923	6870	
1259.5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	102608-53-7	898	898	5355	√
1312.7	Farnesyl acetone	1117-52-8	891	891	6144	
1392.5	2*H*-1-Benzopyran-2-one, 7-hydroxy-6-methoxy-	92-61-5	914	918	9751	√
1571.7	Octadecanoic acid	57-11-4	889	895	7651	
2154.7	Geranylgeraniol	24034-73-9	879	903	6235	

tr, retention time. CAS No., chemical abstracts service registry number, if CAS No. is not available, N# (identity number in NIST) or w# (identity number in Wiley) will be given. Similarity, reverse and probability are mass spectral match factors. "√" in "Verified" means the identification was verified by standards.

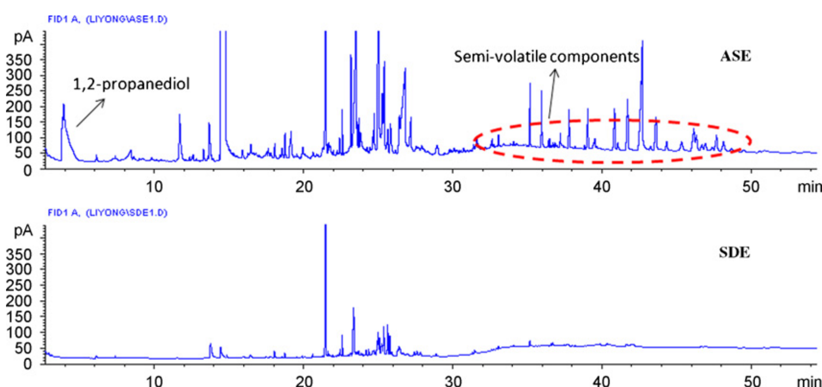

Fig. 5. GC-FID chromatogram comparison of a typical cut tobacco sample extracted by ASE and SDE.

Table 3
Absolute recovery comparison of ASE and SDE.

Name	Recovery (low, %)		Recovery (middle, %)		Recovery (high, %)	
	SDE	ASE	SDE	ASE	SDE	ASE
3-Methyl-1-Butanol	7.13 ± 4.01	15.00 ± 10.61	15.17 ± 0.34	32.35 ± 4.02	24.50 ± 1.39	51.29 ± 5.54
2,3-Pentanedione	19.38 ± 2.02	17.54 ± 5.48	24.33 ± 1.42	33.96 ± 0.39	29.81 ± 2.94	54.46 ± 4.55
Cyclohexanone	0	2.10 ± 3.28	10.41 ± 0.35	54.71 ± 3.46	28.01 ± 6.12	75.23 ± 1.00
2-Octanone	0	43.90 ± 2.28	0	63.70 ± 9.93	8.96 ± 0.82	71.22 ± 4.12
β-Linalool	0	31.42 ± 12.08	18.24 ± 13.37	66.45 ± 12.96	37.24 ± 0.71	68.93 ± 5.03
Isophorone epoxide	45.88 ± 3.81	92.02 ± 10.60	48.88 ± 0.31	96.99 ± 3.53	71.18 ± 0.80	99.01 ± 1.94
p-Mentha-6,8-dien-2-one	44.53 ± 0.09	74.84 ± 13.37	51.19 ± 1.28	76.97 ± 4.54	64.00 ± 0.68	94.92 ± 4.54
cis-Geranylacetone	63.49 ± 6.88	68.42 ± 26.32	64.40 ± 4.92	98.83 ± 28.67	75.52 ± 0.40	101.82 ± 0.71
trans-Geranylacetone	36.41 ± 6.13	62.52 ± 15.65	44.77 ± 0.50	81.34 ± 2.81	64.69 ± 0.38	92.33 ± 5.12
trans-Phytol	30.16 ± 9.63	67.35 ± 2.02	59.08 ± 8.09	77.08 ± 1.38	73.78 ± 0.96	101.40 ± 16.48
2-Methyl-naphthalene	49.24 ± 1.31	101.17 ± 7.25	54.07 ± 1.12	104.02 ± 3.36	54.95 ± 0.38	103.07 ± 1.59

Low, middle and high mean that the standard addition concentrations were approximately 0.5, 1 and 3 times of those in a flue-cured tobacco sample, respectively.

Table 4
Comparision of relative standard deviation distribution using the ASE and SDE.

Extraction method	RSD distribution ($n = 5$)						
	<5%	<10%	<15%	<20%	<25%	<30%	Total
ASE							
Count of peaks	46	101	128	144	159	166	178
Peak area percentage (%)	76.5	88.9	93.9	95.5	97.8	98.7	100
SDE							
Count of peaks	25	38	46	53	62	63	66
Peak area percentage (%)	79.4	89.1	93.3	94.8	97.6	98.6	100

ASE method in this study was water-free extraction, and no dehydration step was used. So the ASE method was more suitable for tobacco fingerprinting analysis of large number of samples.

3.5. Cigarette brand discrimination based on fingerprinting from ASE-GC analysis

Chemical fingerprintings of 36 cut tobacco samples from six different cigarette brands were analyzed using the developed ASE-GC method. The relative peak areas to internal standard were unit-variance scaled [20] and evaluated by principal components analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). PCA can basically separate the six brands of cigarette, and the first two components explained 56% of the variable information. When PLS-DA was used, the separation among different brands was improved (Fig. 6). To estimate the predictive ability of this model, 7-fold cross-validation was used [35]. The parameters for the classification from the software were $R^2Y = 0.86$, $Q^2 = 0.73$, which showed good fitness and prediction, respectively. Permutation test (Y scrambling) was performed to assess the significance of the predictive ability and to exclude overfitting due to chance correlation. According to Eriksson et al. [35], the value of R^2Y-intercept should not exceed 0.4 and the value of Q^2-intercept should not exceed 0.05 for a valid model. It shows the model (R^2Y-intercept = 0.29, Q^2-intercept = −0.63) is reliable.

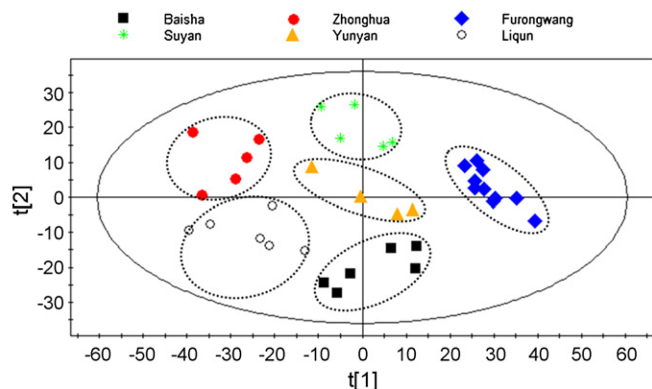

Fig. 6. PLS-DA analysis of six Chinese brands of cigarette samples. $R^2Y = 0.864$, Q^2 (cum) = 0.73, R^2Y-intercept = 0.292, and Q^2-intercept = −0.631.

4. Conclusions

Extraction of volatile and semi-volatile components from tobacco is a matrix and method-dependent process. In this study, the ASE method was optimized and validated for volatile and semi-volatile cigarette components extraction. The best extraction performance was achieved at 100 °C, 1000 psi and two extraction cycles with dichloromethane as solvent. Compared with SDE method, ASE method had high extraction yields, less time and lower solvent and labor consumption. Additionally, ASE method had better recovery for volatiles and water-soluble compounds than SDE method. Precisions of the two extraction methods were similar, and there were over 80% peaks with RSD less than 20%.

When the optimized ASE method was used for cigarette brand discrimination, samples from six tobacco brands were clearly clustered in six non-cross-regions of the PLS-DA loading plot. The model was evaluated having good prediction ability and no overfitting. The results indicated that ASE is a suitable sample preparation method for GC-based fingerprinting.

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