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Optimization of Headspace Solid-Phase Microextraction Coupled with Gas Chromatography−Mass Spectrometry for Detecting Methoxyphenolic Compounds in Pu-erh Tea

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ABSTRACT: A method based on headspace solid-phase microextraction coupled with gas chromatography−mass spectrometry was developed for the analysis of volatile methoxyphenolic compounds in pu-erh tea. Six fibers with different polarities were initially evaluated. The 75 μm carboxen/polydimethylsiloxane fiber exhibited the highest extraction efficiency and was selected for further optimization. A Plackett−Burman design was used to screen for the brewing proportion of tea and water, amount of pu-erh tea, ionic strength, extraction time, extraction temperature, desorption time, rate of agitation, and equilibrium time. A Box−Behnken design was then applied to optimize the significant factors. Under optimal conditions, the proposed method affords a wide range of linearity, high linear regression coefficients (0.996−0.999), less than 9.0% repeatability of relative standard deviation, and limits of detection ranging from 2.31 to 21.80 ng/g. The proposed method has satisfactory accuracy, with recoveries of 79.08−113.9%. This method was successfully applied for the analysis of pu-erh tea samples.

KEYWORDS: *Methoxyphenolic compounds, Plackett−Burman design, Box−Behnken design, pu-erh tea, solid-phase microextraction, gas chromatography−mass spectrometry*

INTRODUCTION

Pu-erh tea is a popular, special post-fermented tea that is mainly produced in the Yunnan province of China. This tea is traditionally made from leaves from mature wild "broad-leaf tea" trees, *Camellia sinensis* (L.) O. Kuntze var. *assamica* Kitamura, in southwestern China.[1] The unique method of tea fermentation involves microorganisms that enter the fermentation process as a natural flora. Complex changes from the coordinated microbial metabolic action and natural oxidation occur during the post-fermentation and piling process to produce the special quality and flavor characteristics of pu-erh tea.[2] Pu-erh tea has attracted much attention, particularly in China and other Asian countries, because of its health benefits and special aroma.[3] Nonvolatile substances in pu-erh tea contribute to these health benefits and biological activities. These compounds in tea include polyphenols, polysaccharides, tea pigments, etc. Several studies have demonstrated that pu-erh tea has various biological activities, including its hypolipidemic,[4,5] antioxidant,[3,6,7] oxidative-damage-preventing, nitric-oxide-scavenging,[8] hypocholesterolemia,[9] antiobesity,[10] antimutagenic, and antimicrobial[11] effects. In contrast, the special aroma of pu-erh tea is due to its volatile compounds. The volatiles of pu-erh tea were similar to other teas, generally including alcohols, hydrocarbons, aldehydes, ketones, and esters. In addition, pu-erh tea also contains some special methoxyphenolic compounds. These methoxyphenolic compounds have been reported to be the characteristic aroma components of pu-erh tea.[2,12,13]

Fermented pu-erh tea samples contain amounts of methoxyphenolic compounds, such as 1,2-dimethoxybenzene, 1,2,3-trimethoxybenzene, 1,2,4-trimethoxybenzene, etc. Only a few of these components appear to originate from the raw materials of the tea. These compounds are considered to be produced by the microbial degradation and methylation of tea catechins during the post-fermentation process. In addition to microbial metabolism, thermal degradation may have contributed to the formation of these compounds as a result of the heat produced within the piling tea leaves.[2] The volatile methoxyphenolic compounds are major contributors to the special "stale" aroma, which is unique to the organoleptic odor qualities of pu-erh tea. Moreover, its special "stale" aroma is also one of the most important indicators for its market price. However, only a few studies have been carried out on the contents of its volatile methoxyphenolic compounds, and the exact content of those compounds remain unknown.

The determination of volatile compounds includes an extraction step, which is followed by the analytical quantification of the individual compounds using gas chromatography (GC) or gas chromatography−mass spectrometry (GC−MS). The accurate and complete extraction of volatiles is critical. Over the past few decades, the studies of volatile constituents of various teas are very popular as well as the kind of extraction methods that were applied by different researchers. The most notable among these methods is direct solvent extraction,[14,15] simultaneous distillation−extraction (SDE),[2,13,16−20] steam distillation−liquid/liquid extraction,[17] Soxhlet extraction,[17] vacuum hydrodistillation,[18] thermal desorption,[18,21] and solid-

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phase microextraction (SPME).[12,22,23] For pu-erh tea analysis, Xu et al.[2] studied the influence of fungal fermentation on the development of volatile compounds during the pu-erh tea manufacturing process. Gong et al.[13] investigated the change in the volatile components in pu-erh tea during processing using SDE and GC−MS analysis. Liang et al.[16] studied the volatile constituents of pu-erh tea and their correlation to its sensory quality using SDE and GC analysis. Gu et al.[17] studied the SDE of some volatile flavor components from pu-erh tea samples, which were compared to the results of steam distillation−liquid/liquid extraction and Soxhlet extraction. Wang et al.[20] compared the volatile compounds of different types of tea using SDE and GC−MS analysis. These methods based on distillation (SDE, steam distillation−liquid/liquid extraction, and Soxhlet extraction) are hazardous because of the required amounts of toxic and expensive solvents. Furthermore, these methods are labor-intensive, time-consuming, and require extract preconcentration. In comparison to the well-established techniques, SPME is a sensitive, solvent-free, and economical method of sample preparation before GC analysis. SPME combines sampling, extraction, and concentration in a single step, which substantially shortens the analysis time. In the last few years, this method has been widely used for determining the volatile flavor profiles of food, such as fruit,[24] wines,[25,26] coffee, [27] and sausages.[28]

This work aimed to develop an analytical method for the direct determination of volatile methoxyphenolic compounds in pu-erh tea using the headspace solid-phase microextraction (HS-SPME) technique coupled with GC−MS. This study assessed the possible of this method to quantify pu-erh tea samples.

MATERIALS AND METHODS

Chemicals. Ethanol (≥99.5%), ethyl decanoate (internal standard, ≥99%), 1,2,3-trimethoxybenzene (98%), 1,2,3-trimethoxy-5-methylbenzene (97%), 1,2,4-trimethoxybenzene (97%), 1-methoxy-4-(1-propenyl)-benzene (≥99%), 1,2-dimethoxybenzene (99%), and 3,4-dimethoxytoluene (≥97%) were purchased from Sigma-Aldrich (St. Louis, MO). Potassium chloride (99.5%, analytical quality) was purchased from Aladdin (Shanghai, China). Ultrapure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA).

Samples. The pu-erh tea samples 7572, 7562, and JZBL (Jinzhenbailian) were obtained from Dayi Ltd., Inc. (Menghai, Yunnan, China), whereas CN, GP, and TF were obtained from Yunnan Tasly Biology Tea Technology Ltd., Inc. (Simao, Yunnan, China). All of these pu-erh tea samples were picked and processed in 2011.

Sample and Standard Preparation. All of the pu-erh tea samples were ground to pass through 30−60 mesh and sealed for future use. The normal pu-erh tea sample (JZBL) was selected as a control for the optimization process. "Volatile-free" pu-erh tea was prepared to generate a matrix identical to the commercial pu-erh tea that was free of volatile methoxyphenolic compounds. First, JZBL was removed of volatile methoxyphenolic compounds and other volatile compounds using an EYELA N-1100 rotary evaporator (Tokyo Rikakikai Co., Ltd., Japan) at 40 °C, and then the "volatile-free" pu-erh tea was used as a blank matrix to obtain the calibration curves.

The stock solution was prepared by dissolving the solid/liquid standard in ethanol, which contained 9.008 g/L 1,2-dimethoxybenzene, 4.240 g/L 3,4-dimethoxytoluene, 1.980 g/L 1-methoxy-4-(1-propenyl)-benzene, 10.000 g/L 1,2,3-trimethoxybenzene, 8.672 g/L 1,2,4-trimethoxybenzene, and 3.000 g/L 1,2,3-trimethoxy-5-methylbenzene. The stock solution of the internal standard was serially diluted in ethanol to yield a working standard of 172.40 mg/L. All of these solutions were stored at 4 °C before used. A series of working standard solutions were prepared from the stock solutions by dilution with ethanol.

Selection of SPME Fibers. The SPME fiber coating is an important factor that controls the extraction efficiency.[29−31] Six types of fibers were investigated to obtain a suitable fiber for the determination of volatile methoxyphenolic compounds in pu-erh tea. The fiber performance was evaluated using 6 g of pu-erh tea in a 100 mL headspace vial. After adding 18 mL of water, the vial was tightly sealed with a polytetrafluoroethylene (PTFA) septum. The sample vial was equilibrated for 15 min in a 60 °C water bath at 400 rpm, followed by fiber exposure to the headspace over the sample for 60 min. Finally, each fiber was exposed in the GC injector for 5 min. The total peak areas were used to assess the effect of fiber coatings on the extraction efficiency of the volatile compounds. The evaluated fibers were purchased from Supelco (Bellefonte, PA) and were coated with different stationary phases: polydimethylsiloxane/divinylbenzene (PDMS−DVB, 65 μm), polyacrylate (PA, 85 μm), carboxen/polydimethylsiloxane (CAR−PDMS, 75 μm), divinylbenzene/carboxen/polydimethylsiloxane (DVB−CAR−PDMS, 50/30 μm), polydimethoxysiloxane (PDMS, 100 μm), and carbowax/divinylbenzene (CW−DVB, 70 μm). All fibers were conditioned before the first extraction in accordance with the specifications of the manufacturer by inserting them into the GC injector port.

Optimization Strategy. The parameters that influence the extraction procedure were considered and optimized to obtain the appropriate HS-SPME-based method for determining the volatile methoxyphenolic compounds in pu-erh tea. An experiment based on the Plackett−Burman (PB) design was performed to screen for the significant factors during extraction of volatile methoxyphenolic compounds. These factors included the brewing proportion of tea (in g) and water (in mL), amount of pu-erh tea, ionic strength, extraction time, extraction temperature, desorption time, rate of agitation, and equilibrium time. Two levels were considered for each factor high and low, which were denoted by (+1) and (−1). A total of 12 experimental runs were performed. The list of the different experimental designs with their respective name, symbol code, and coded level of the variables is presented in Table 1. After the factors that significantly affected the extraction process are determined, the

Table 1. Variables, Coded Levels, and PB Design Matrix

		coded levels	
symbol	independent variables	low (−1)	high (+1)
X_1	brewing proportion of tea and water (g and mL)	1:3	1:5
X_2	amount of pu-erh tea (g)	4	6
X_3	ionic strength (g/mL)	0	0.3
X_4	extraction time (min)	50	70
X_5	extraction temperature (°C)	50	70
X_6	desorption time (min)	4	8
X_7	rate of agitation (rpm)	300	600
X_8	equilibrium time (min)	0	20

runs	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
1	1	−1	1	−1	−1	−1	1	1
2	1	1	−1	1	−1	−1	−1	1
3	−1	1	1	−1	1	−1	−1	−1
4	1	−1	1	1	−1	1	−1	−1
5	1	1	−1	1	1	−1	1	−1
6	1	1	1	−1	1	1	−1	1
7	−1	1	1	1	−1	1	1	−1
8	−1	−1	1	1	1	−1	1	1
9	−1	−1	−1	1	1	1	−1	1
10	1	−1	−1	−1	1	1	1	−1
11	−1	1	−1	−1	−1	1	1	1
12	−1	−1	−1	−1	−1	−1	−1	−1

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Box−Behnken design (BBD) was used to optimize the extraction process and to evaluate the effects and interaction of the variables. The coded and uncoded independent variables used in the response surface methodology (RSM) design are summarized in Table 2. A total of 15

Table 2. Factors, Coded Levels, and BBD Matrix

factors		coded levels		
		low (−1)	central (0)	high (+1)
(X_1) brewing proportion of tea and water (g and mL)		1:2	1:3	1:4
(X_3) ionic strength (g/mL)		0.25	0.3	0.35
(X_5) extraction temperature (°C)		60	70	80
runs	X_1	X_3	X_5	
1	−1	−1	0	
2	1	−1	0	
3	−1	1	0	
4	1	1	0	
5	−1	0	−1	
6	1	0	−1	
7	−1	0	1	
8	1	0	1	
9	0	−1	−1	
10	0	1	−1	
11	0	−1	1	
12	0	1	1	
13	0	0	0	
14	0	0	0	
15	0	0	0	

experiments were randomly performed, which included 12 factorial points and 3 center points. Experiments at the center point were conducted for the evaluation of the experimental error.[32] The sum of the peak areas of the methoxyphenolic compounds were used to determine the response to optimization of both PB and BBD.

GC−MS Analysis. After extraction by the HS-SPME-based procedure, the analytes from pu-erh tea samples were identified using an Agilent 7890A GC that was directly interfaced with an Agilent 5975C mass selective detector (MSD) (Agilent, Santa Clara, CA). An Agilent HP-5MS capillary column (30 m × 0.25 mm inner diameter, 0.25 μm film thickness) was used for the GC separation. Helium (percentage purity > 99.999%) was used as the carrier gas at a flow rate of 1 mL min^{-1}. Desorption of analytes from the SPME fiber occurred at 250 °C in a splitless mode. The employed temperature program had the following settings: An initial temperature of 50 °C was held for 3 min and then increased to 125 °C at 2 °C min^{-1}; this temperature was held for 5 min and then increased to 180 °C at 6 °C min^{-1}; and this temperature was held for 3 min and then increased to 250 °C at 15 °C min^{-1}. The total GC runtime was 62.333 min. The mass spectrometer was operated in an electron-impact mode of 70 eV. The mass scan range was 30−500 atomic mass units (amu). The temperatures of the interface, ion source, and quadrupole were 280, 230, and 150 °C, respectively. The volatile methoxyphenolic compounds were determined by comparing the MS fragmentation pattern to those of the standards and mass spectrum of the unknown peaks to those stored in the National Institute of Standards and Technology (NIST) 08 GC−MS library, with the retention time of the standards obtained under the same conditions.

Calibration of Standard Curves. Five standard solutions of different analytes at increasing concentrations were prepared by diluting the stock solutions in ethanol. Under the optimized conditions, the calibration curves were performed using "volatile-free" pu-erh tea sample, to which was added 20 μL of standard solution and 20 μL of the internal standard solution.

Quantification of Samples and Calculation of Recovery. Known amounts of the standard (20 μL) and internal standard (20 μL) solutions were added to the pu-erh tea sample to produce the spiked samples. The recovery rate of each analyte in the pu-erh tea sample was estimated as [(the concentration of the detected amount in the spiked sample − the concentration of the detected amount in the sample)/the concentration of the added amount] × 100%.

Determination of Pu-erh Tea Samples. On the basis of the optimized conditions, the methoxyphenolic compound contents of the six kinds of pu-erh tea samples (JZBL, 7572, 7562, CN, GP, and TF) were determined via the addition of 20 μL of internal standard solution to a 100 mL vial capped with a polytetrafluoroethylene (PTEF)-lined cap.

All studies were conducted in triplicate, and the average values were calculated.

Statistical Analysis. The PB design matrix was generated, and the results were evaluated using Minitab.16 software (Minitab, Inc., State College, PA). Design Expert 8.0.6 software (Stat Ease, Inc., Minneapolis, MN) was used to generate the BBD matrix and quadratic models that fit the experimental data as well as to draw the response surface plots. Analysis of the relative standard deviation (RSD) was used to determine the accuracy of HS-SPME by the SPSS statistics software (version 17.0, SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Screening of the Fiber Coating. In the HS-SPME procedure, the type of sorbent used as the fiber coating is the key parameter that governs the selectivity and recovery of SPME.[33−35] Thus, the selection of a suitable fiber coating is an important step in SPME optimization.[24,34] In this study, six different fiber coatings (100 μm PDMS, 75 μm CAR−PDMS, 85 μm PA, 70 μm CW−DVB, 65 μm PDMS−DVB, and 50/30 μm DVB−CAR−PDMS) were evaluated. The extraction efficiency of different fibers was expressed as the GC peak area in Figure 1a. The higher extraction efficiency of 1,2-dimethoxybenzene, 3,4-dimethoxytoluene, 1,2,3-trimethoxybenzene, and 1-methoxy-4-(1-propenyl)-benzene was achieved with CAR−PDMS. However, DVB−CAR−PDMS and PA had higher extraction capacities for 1,2,4-trimethoxybenzene and 1,2,3-trimethoxy-5-methylbenzene, respectively. In comparison to the six adsorption fibers, the CAR−PDMS fiber demonstrated the highest extraction efficiency for volatile methoxyphenolic compounds, which was followed by DVB−CAR−PDMS (Figure 1b). This observation can be attributed to the higher proportion of micropores in the CAR−PDMS fiber, which makes it more suitable for the analytes. The DVB−CAR−PDMS fiber had higher extraction efficiency than the PDMS−DVB fiber, which can be attributed to the predominant meso- and macropores in the PDMS−DVB fiber, with lower efficiency for the analytes. In contrast, the two-layered DVB−CAR−PDMS fiber has a larger number of micropores, thereby making it more effective for analytes than the PDMS−DVB fiber. On the basis of the total peak areas of the volatile methoxyphenolic compounds, the CAR−PDMS fiber was selected for further optimization.

PB Design for Screening Variables. The reduced factorial PB design is usually employed for screening when a large number of variables are involved. The aim of this design is to detect the variables with the greatest influence on the selected response by calculating the main factors.[36] Eight variables were analyzed with regard to their effect on the total peak area of the volatile methoxyphenolic compounds. Analysis of variation (ANOVA) was used to evaluate the data, and effects were deemed statistically significant at the 5% level. The results were visualized using the Pareto chart. The bar beyond the line corresponds to the effects that are statistically significant at the 95% confidence level.

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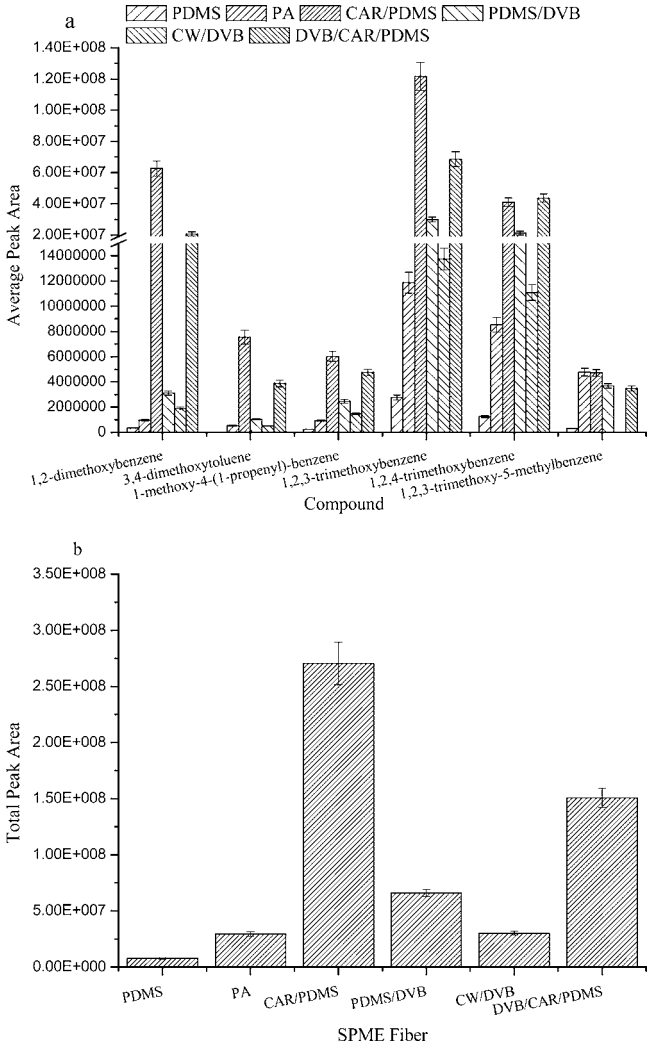

Figure 1. Comparison of SPME efficiencies for six fibers on the extraction of (a) different and (b) total analytes of pu-erh tea.

According to Figure 2, the most significant factor is the temperature, which displays a positive effect. This result is based on the enhanced diffusion transference caused by the increasing temperature. Ionic strength is the next most important positive significant variable. The addition of salt in the analytical matrix could increase the ionic strength of the matrix and affect the solubility of the organic analytes via the salting-out effect.[37−39] The proportion of tea and water (tea/water in Figure 2) is another significant factor that negatively affects the extraction process. This phenomenon may be due to the excessive water that reduces the headspace above the liquid surface, which was not conducive to the balance of adsorption. The amount of pu-erh tea and desorption time are shown to have non-significant negative effects on the extraction efficiency. The extraction time, rate of agitation, and equilibrium time are shown to have non-significant positive effects on the extraction efficiency.

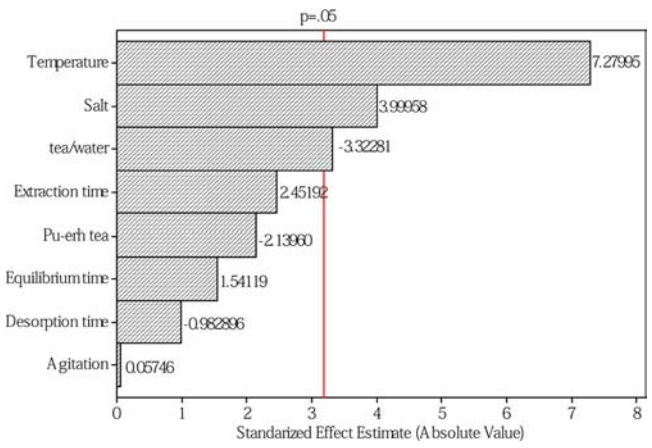

Figure 2. Standardized main effect Pareto chart for the PB design of the screening experiment. The vertical line in the chart defines the 95% confidence level.

On the basis of these results, the variables that were considered in the optimization step were the extraction temperature, ionic strength, and brewing proportion of tea and water. The amount of pu-erh tea, extraction time, desorption time, rate of agitation, and equilibrium time did not have significant effects and were all set at low levels.

Optimization Design. To obtain the best response, the BBD design was used to optimize the values of the significant variables (the extraction temperature, ionic strength, and brewing proportion of tea and water). The examined levels of the different variables are provided in Table 2. The levels of the significant variables were set according to the path of steepest ascent (data not shown).

The model coefficients were calculated by backward multiple regression and validated by ANOVA. By applying multiple regression analysis to the experimental data, the following second-order polynomial equation was found to explain the extraction efficiency of the volatile methoxyphenolic compounds:

$$Y = 525196286 - 18967738X_1 + 17843447X_3 + 39415105X_5 - 58711011X_1^2 - 30898021X_3^2 - 140142991X_5^2 - 15074808X_1X_3 - 3085847X_1X_5 - 7749136X_3X_5 \quad (1)$$

where Y is the total peak area of the volatile methoxyphenolic compounds and X_1, X_3, and X_5 are the brewing proportion of tea and water, ionic strength, and extraction temperature, respectively.

The ANOVA of the optimization study showed that X_1, X_3, X_5, X_1^2, X_3^2, and X_5^2 were significant ($p < 0.05$). X_1X_3, X_1X_5, and X_3X_5 were not significant ($p > 0.05$), which implied there was no interaction between variables. Meanwhile, the lack of fit was not significant ($p > 0.05$), with high values for R^2 (0.9877) and adjusted R^2 (0.9656). The closer the R^2 value is to 1.00, the stronger the model and the more accurate the predicted response.[40] The adjusted R^2 value is the R^2 value with adjustment for the number of terms in the respective model. The large adjusted R^2 value indicated the correspondence between the experimental data and the fitted model.[36] The values of R^2 (0.9877) and adjusted R^2 (0.9656) indicated that the response equation provided a suitable model for the BBD and the analysis of the response trends using the model was reasonable.

Three-dimensional response surface plots were constructed to determine the optimal levels of each factor for the maximum peak area of the total volatile methoxyphenolic compounds. The response (peak area of the total volatile methoxyphenolic compounds) was plotted on the z axis against any of the two

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independent factors while maintaining other factors at their optimal levels. The three-dimensional response surface plots were shown in panels a–c of Figure 3. The peak area of the total volatile methoxyphenolic compounds has a maximum point in the studied region, as seen from the plots. The experimental data were fitted in eq 1, with the following optimum levels: a brewing proportion of tea and water of 1:2.8, an extraction temperature of 71 °C, and an ionic strength of 0.316 g/mL.

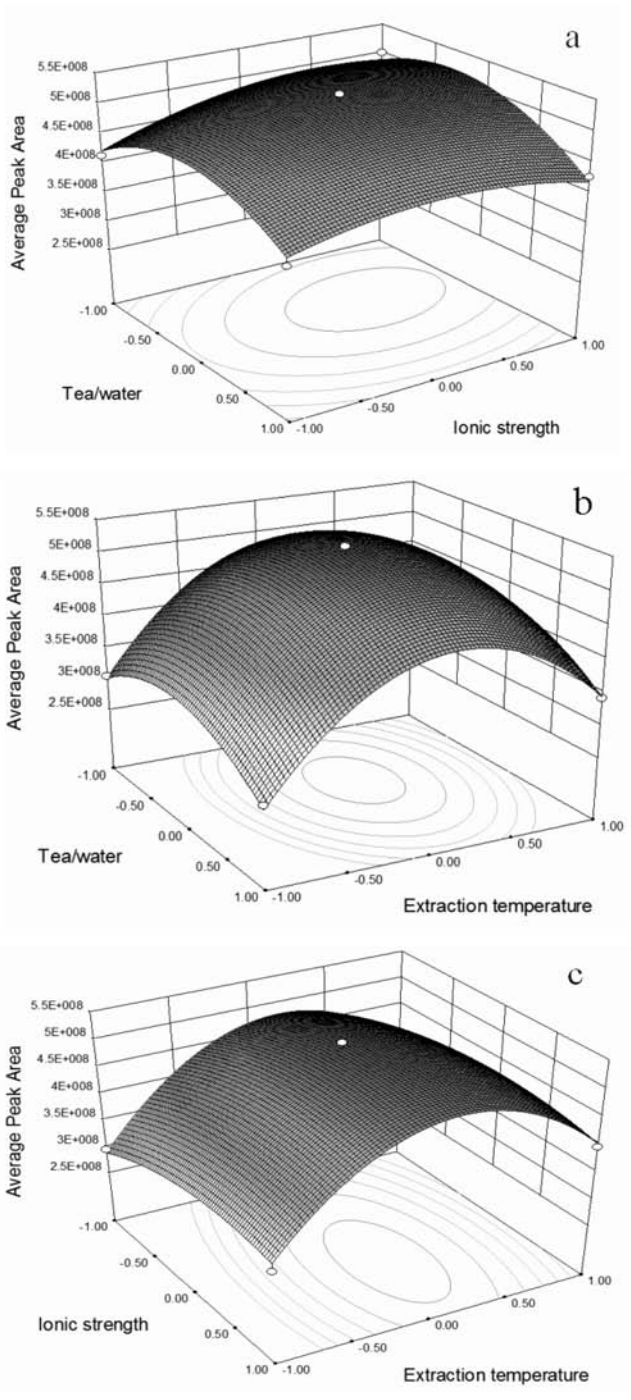

Figure 3. Response surface plots on the sum of the peak area of methoxyphenolic compounds in pu-erh tea as affected by the brewing proportion of tea and water, ionic strength, and extraction temperature: (a) brewing proportion of tea and water and ionic strength at a constant extraction temperature (°C), (b) brewing proportion of tea and water and extraction temperature at a constant ionic strength (g/mL), and (c) ionic strength and extraction temperature at a constant brewing proportion of tea and water (g and mL).

Method Validation. A linear regression analysis of the relative peak areas in the internal standard versus the analyte concentrations in "volatile-free" pu-erh tea was performed to decrease the influence of the matrix effect. The matrix structure of the "volatile-free" pu-erh tea sample is similar to that of the real pu-erh tea samples. The linear ranges of the concentration, intercept, slope, and correlation coefficient (R^2) for the calibration curves of six methoxyphenolic compounds are listed in Table 3. The linearity is satisfactory in almost all cases, with R^2 values ranging from 0.996 (1,2-dimethoxybenzene) to 0.999 (3,4-dimethoxytoluene).

The repeatability of the method was determined on the basis of the RSD using the "volatile-free" pu-erh tea sample with 4.504 μg/g for 1,2-dimethoxybenzene, 10.6 μg/g for 3,4-dimethoxytoluene, 4.95 μg/g for 1-methoxy-4-(1-propenyl)-benzene, 25.0 μg/g for 1,2,3-trimethoxybenzene, 21.68 μg/g for 1,2,4-trimethoxybenzene, and 7.5 μg/g for 1,2,3-trimethoxy-5-methylbenzene. The RSD values were below 9% for all analytes, ranging from 4.52% for 1,2,3-trimethoxy-5-methylbenzene to 8.22% for 1,2-dimethoxybenzene, which were considered satisfactory for this type of analysis.

The limits of detection (LODs) were obtained from the "volatile-free" pu-erh tea sample. The LODs of six methoxyphenolic compounds were estimated on the basis of the lowest detectable peak that had a signal 3 times that of the background noise (signal/noise = 3). The high selectivity and sensitivity of the CAR–PDMS coating allowed for the achieved low detection limits of the six volatile methoxyphenolic compounds, which ranged from 2.31 ng/g for 1-methoxy-4-(1-propenyl)-benzene to 21.80 ng/g for 1,2-dimethoxybenzene (Table 3). In this study, the values obtained were low enough to permit the determination of these compounds in real pu-erh tea samples.

A recovery study for each analyte was performed using a real pu-erh tea sample to evaluate the accuracy of the optimized method. For this purpose, known quantities of the six analytes at two concentration levels (Table 4) were added to the pu-erh tea sample. The recoveries and repeatability of the method are listed in Table 4. The low-level recoveries ranged from 79.08 to 110.0%, whereas the high-level recoveries ranged from 80.02 to 113.9%. The RSD values were below 10%. The recoveries and RSD of 1,2,3-trimethoxybenzene and 1,2,4-trimethoxybenzene were slightly better than the values obtained when working with SDE combined with GC and GC–MS.[17]

Analysis of Tea Samples. The established HS-SPME–GC–MS method was used to determine the volatile methoxyphenolic compounds in pu-erh tea samples. The mean values for these compounds (μg/g) were obtained for the six methoxyphenolic compounds in the pu-erh tea samples (Table 5). 1,2,3-Trimethoxybenzene, 1,2,4-trimethoxybenzene, and 1,2-dimethoxybenzene were the observed as the major components in all pu-erh tea samples. In comparison to these three compounds, the levels of 3,4-dimethoxytoluene, 1-methoxy-4-(1-propenyl)-benzene, and 1,2,3-trimethoxy-5-methylbenzene were relatively low. However, 1-methoxy-4-(1-propenyl)-benzene was not detected in the samples that were supplied by the Yunnan Tasly Biology Tea Technology Ltd., Inc. The 1,2,3-trimethoxybenzene and 1,2,4-trimethoxybenzene contents agreed with the result found in the literature.[17] A typical chromatogram from the HS-SPME–GC–MS analysis of a pu-erh tea sample (JZBL) is shown in Figure 4.

Table 3. Ranges of Concentration (n = 5), Intercept (a), Slope (b), Regression Coefficient (R^2), Limit of Detection (LOD), and RSD (%) of Repeatability

compound	linear range (μg/g)	a	b	R^2	LOD (ng/g)	RSD (%)
1,2-dimethoxybenzene	0.2252−45.04	0.081	0.701	0.996	21.80	8.22
3,4-dimethoxytoluene	0.106−10.6	0.060	1.590	0.999	3.45	6.85
1-methoxy-4-(1-propenyl)-benzene	0.0495−4.95	0.091	13.50	0.997	2.31	6.20
1,2,3-trimethoxybenzene	0.25−50	−0.014	0.941	0.996	4.51	5.71
1,2,4-trimethoxybenzene	0.2168−43.36	0.062	1.093	0.997	5.09	8.18
1,2,3-trimethoxy-5-methylbenzene	0.075−15	0.078	1.320	0.999	3.87	4.52

Table 4. Recoveries and RSD (%) of the Volatile Methoxyphenolic Compounds

	high level			low level		
compound	added content (μg/g)	recovery (%)	RSD (%)	added content (μg/g)	recovery (%)	RSD (%)
1,2-dimethoxybenzene	4.504	102.4	9.06	0.4504	104.8	7.55
3,4-dimethoxytoluene	10.6	96.3	7.10	1.06	93.0	6.18
1-methoxy-4-(1-propenyl)-benzene	4.95	107.5	6.67	0.495	109.7	8.98
1,2,3-trimethoxybenzene	25.0	113.9	6.02	2.5	110.0	5.77
1,2,4-trimethoxybenzene	21.68	81.06	8.52	2.168	79.08	9.15
1,2,3-trimethoxy-5-methylbenzene	7.5	80.2	6.53	0.75	82.8	6.04

Table 5. Content of Volatile Methoxyphenolic Compounds in Pu-erh Tea Samples (μg/g)

	pu-erh tea samples					
compound	7572	7562	JZBL	CN	GP	TF
1,2-dimethoxybenzene	9.01	9.23	11.28	15.79	7.50	5.97
3,4-dimethoxytoluene	0.52	0.32	1.51	0.97	0.74	1.78
1-methoxy-4-(1-propenyl)-benzene	0.31	0.21	0.34	ND[a]	ND	ND
1,2,3-trimethoxybenzene	29.30	26.33	32.11	27.04	14.50	20.51
1,2,4-trimethoxybenzene	10.52	12.22	20.53	10.47	4.97	5.30
1,2,3-trimethoxy-5-methylbenzene	1.32	0.73	0.74	2.26	0.88	4.88

[a]ND = not detected.

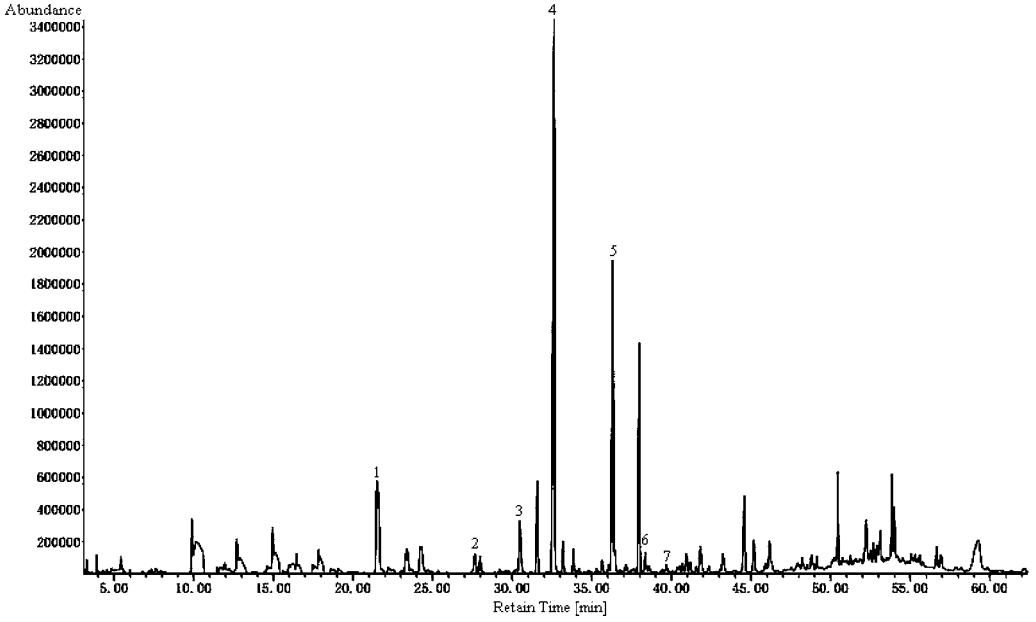

Figure 4. Total ion chromatogram of HS-SPME−GC−MS analysis of a pu-erh tea sample (JZBL) using the CAR−PDMS fiber. Peaks: (1) 1,2-dimethoxybenzene, (2) 3,4-dimethoxytoluene, (3) 1-methoxy-4-(1-propenyl)-benzene, (4) 1,2,3-trimethoxybenzene, (5) 1,2,4-trimethoxybenzene, (6) ethyl decanoate (IS), and (7) 1,2,3-trimethoxy-5-methylbenzene.

In conclusion, a method for the determination of volatile methoxyphenolic compounds in pu-erh teas using HS-SPME combined with GC−MS was optimized. The CAR−PDMS fiber performed best during the optimization process. The following optimized parameters influenced the extraction: a brewing proportion of tea and water of 1:2.8, a pu-erh tea sample of 4 g, an ionic strength of 0.316 g/mL, an extraction time of 50 min, an extraction temperature of 71 °C, a

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desorption time of 4 min, and an agitation of 300 rpm. Under optimal conditions, the method showed satisfactory linearity, repeatability, detection limits, and recoveries. The precision of the determination of the pu-erh tea samples was likewise satisfactory for the six methoxyphenolic compounds. All RSD values were below 10%. Thus, the developed method was successfully applied to quantify the volatile methoxyphenolic compounds in the samples of commercial pu-erh tea.

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Notes

The authors declare no competing financial interest.

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