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Optimization of headspace sampling using solid-phase microextraction for volatile components in tobacco

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Abstract

Solid-phase microextraction (SPME) was evaluated as a tool for headspace sampling of tobacco samples. Several experimental parameters (e.g. sampling temperature, pH, moisture, and the type of SPME fibers) were optimized to improve sampling efficiency in two aspects; maximum adsorption and selective adsorption of volatile components onto SPME fibers. The effect of these parameters was often dominated by the physical and chemical nature (e.g. volatility, polarity) of target compounds, thus, SPME sampling conditions can be adjusted to favor a selected group of compounds, such as organic acids in tobacco.

Keywords: Optimization; Headspace sampling; Tobacco; Solid-phase microextraction; Volatile compounds

1. Introduction

Since solid-phase microextraction (SPME) was introduced in 1989 [1], it has been increasingly accepted as a sensitive sampling technique for volatile organic analysis in environmental [2,3] and food [4,5] industries among others [6,7]. The key configuration of SPME device is a silica fiber coated with a polymer phase (e.g. polydimethylsiloxane or carbowax). Adsorption of target compounds onto the polymer coated silica fiber can be done either from the headspace of solid or liquid sample or by directly dipping the fiber into an aqueous solution using a variety of mixing techniques [8]. The loaded fiber is then inserted into the GC injection port at an elevated temperature, in which the absorbed volatile compounds are thermally desorbed into the head of a capillary column for gas chromatographic analysis with various detectors.

The sampling mechanism has made SPME a concentration tool for trace analysis. In some cases, its solvent-free nature has proved to be unique and beneficial. For example, it happened so often that early eluted peaks of interested were masked by the huge solvent peak in the practice of fast GC analysis, particularly under low split ratio or splitless mode for a greater sensitivity. This masking problem did not exist when a solvent-free injection technique such as SPME was employed [9]. Another frequently used technique for the combined technique of SPME and fast GC was the use of cryotrapping followed by ballistic thermal desorption to improve the refocusing effect and the resolution of early eluted peaks. Usually, a mini-cryotrap was installed at the initial section of the capillary column for this purpose [4,10,11]. The solvent-free condition of SPME made multiple injections an easy task in the above cryo-

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trapping procedure and was thus beneficial in the improvement of sensitivity. In addition, the solvent-free injection using SPME helped eliminate the solvent-related carryover problem in alkaloid analysis using a purge-and-trap device [12]. Recently, Pawliszyn and Martos developed an on-fiber derivatization technique to quantitate gaseous formaldehyde in ppbv levels [13]. All of these advantages plus its simple set-up, low cost and easy operation make SPME a valuable alternate to other analytical tools such as static headspace sampler, purge and trap, steam distillation, and short path thermal desorption device.

Previously, we had evaluated the use of SPME for the quantitation of alkaloids from aqueous tobacco extract [14]. Tobacco samples were extracted with 1% NH_4OH in water. SPME fiber was directly dipped into the tobacco extract for 12 min, then inserted into the injection port of GC for 60 s. Nicotine and a group of minor alkaloids (i.e. nornicotine, myosmine, anabasine and anatabine) were separated with baseline resolution in 3.5 min. It was found that directly dipping a SPME fiber into an aqueous tobacco extract at ambient conditions provided reasonable sensitivity and better precision. However, there were two disadvantages: matrix effect and fiber aging. The use of an internal standard (2,4′-dipyridyl) for the compensation of these effects was limited by the difference of chemical nature between the internal standard and the individual alkaloid.

A variety of tobacco samples, such as burley, bright and oriental, were used in the study. Bright tobacco is known as flue-cured or Virginia tobacco. It possesses a sweet aroma and slightly acidic taste. It is high in sugar content and low to average in acids and nicotine. Burley tobacco is an air-cured tobacco. It is grown in rich limestone soils, primarily in Kentucky and Tennessee areas. It is low in sugar, but high in alkaloid content. Oriental tobacco is a class of tobacco grown in Turkey, Greece, and neighboring areas. It is mostly sun-cured. It has strong characteristic flavor, low in nicotine, and high in reducing sugars, acids, and volatile flavor oil. Adjusting the pH of aqueous samples prior to SPME sampling improved sensitivity and selectivity for the analysis of organic acids and bases [15]. The influence of extraction time and temperature as well as medium on SPME sampling in analysis of aldehydes was also studied [16]. In this work, experimental parameters were optimized for SPME headspace sampling in the determination of volatile components in a variety of tobacco samples. Attention was focused on the adjustment of sampling temperature, moisture level and pH during the headspace sampling process. Temperature profile was adjusted to cover compounds with a broader range of volatility, while moisture and pH was optimized to enhance the sampling of a certain class of compounds based on the acidity or ionic nature of analytes. Two types of SPME phases, polydimethylsiloxane and carbowax, were tested under the optimized experimental conditions.

2. Experimental

2.1. Chemicals

SPME fibers coated with 100-μm polydimethylsiloxane–divinylbenzene(PDMS–DVB) and 65-μm carbowax–divinylbenzene (CW–DVB) were purchased from Supelco (Bellefonte, PA, USA). Water was obtained from a Milli-Q water purification system (Millipore, Redford, MA, USA). Sodium hydroxide and hydrochloric acid were purchased from Fisher (Pittsburgh, PA, USA). Normal paraffins, C_8–C_{20} were obtained from Alltech (Deerfield, IL, USA).

2.2. Sample preparation

Completely cured bright, burley and oriental tobacco leaves received in1996 were ground to a $\leq$1 mm size at ambient temperature using a Thomas Wiley mill (Model ED-5).

A 2.10-g amount of the ground tobacco sample was weighed, placed in a 60-ml serum bottle and clamped with a PTFE-lined cap for headspace analysis. A mixture of paraffins (C_8–C_{20}) was prepared by weighing 100 mg of each hydrocarbon in a 100-ml volumetric flask, which was filled to capacity with methylene chloride. A 2-μl volume of the paraffin mixture solution was injected into a 60-ml serum bottle and clamped with a PTFE-lined cap for headspace analysis.

2.3. Instrument and procedure

The instrument consisted of a Hewlett-Packard 6890 gas chromatography equipped with a 5973 mass selective detector (MSD). A SPME fiber was inserted into the headspace of sample vial containing 2.1 g of ground tobacco or 2 μl of paraffin mixture. The whole set-up was placed in a GC oven and heated at 100°C. After 12 min, the set-up was removed from the oven and allowed 20 min to cool down to ambient temperature. The SPME fiber was then removed from the sample vial and immediately inserted into the GC injection port at 250°C for 60 s. The used SPME fiber was conditioned at 250°C for 5 min prior to the next sampling. Conditions of GC and MSD are outlined below:

GC column	DB-5MS, 30 m×250 μm×25 μm
Flow rate (He)	0.8 ml/min
Oven temperatures	40°C (hold for 3 min) to 250°C, 6°C/min
Injection temperature	250°C
Detector temperature	280°C
Total running time	41 min
MS scan	35–550

3. Results and discussion

3.1. Effect of sampling temperature

The SPME procedure was developed as an alternate sampling technique to replace micro-steam distillation extraction technique for the analysis of volatile organic components in tobacco. Since GC results of the micro-steam distillation extraction were based on liquid injection of methylene chloride extract, a mixture of C_8–C_{20} normal paraffins was prepared using methylene chloride as a solvent and was used for the test of SPME sampling under three different conditions. GC–MSD chromatograms obtained from these tests were compared to that of a direct liquid injection (Fig. 1a). By comparing Fig. 1b and c, headspace sampling by SPME under isothermal condition could only absorb compounds with boiling points within a certain temperature range. At an elevated temperature of 100°C, SPME could effectively absorb hydrocarbons at C_{15}–C_{20} range, however, the adsorption of more volatile hydrocarbons (e.g. C_8–C_{14}) under such a high level of thermal energy was not as effective. Under ambient conditions, the sampling results were opposite with better adsorption for more volatile hydrocarbons. In order to allow for the adsorption of the

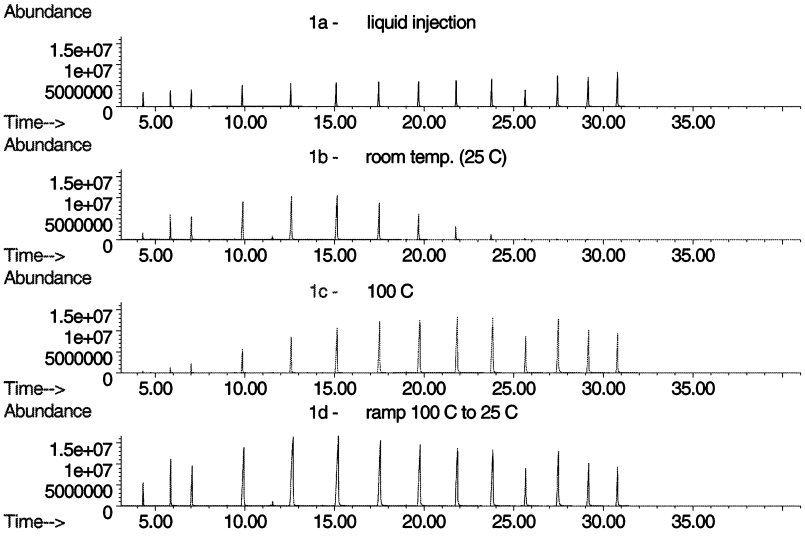

Fig. 1. (a–d) GC–MSD chromatograms of C_8–C_{20} normal paraffins at various SPME sampling temperatures. (a) Direct liquid injection; (b) headspace sampling at 25°C; (c) headspace sampling at 100°C and (d) headspace sampling at temperatures ramping from 100 to 25°C.

whole range of hydrocarbons from C_8 to C_{20}, a 'cooling temperature ramping' was tested. A set-up with a SPME fiber inserted in a glass sample vial containing 2 μl of hydrocarbon mixture was heated to and stayed at an elevated temperature (e.g. 100°C) for 12 min. The whole set-up was then cooled and stayed at end temperature (e.g. ~25°C) for 20 min. The GC results in Fig. 1d showed that the hydrocarbon distribution profile obtained from SPME sampling with temperature ramping from 100 to 25°C was qualitatively similar to that of the direct liquid injection. The length of heating and cooling time periods (i.e. 12 and 20 min) were determined by the equilibrium of thermal adsorption of hydrocarbons on the fiber. The cooling and heating temperatures measured inside an empty sample vial are shown in Fig. 2. Based on the temperature curves, it took approximately 8 and 14 min, respectively, just for the surface temperature of SPME fiber to reach equilibrium during the heating and cooling cycles.

To evaluate the SPME sampling with 'temperature ramping' for the analysis of tobacco volatiles, bright tobacco samples were analyzed under two sampling conditions: under isothermal condition at of 100°C, and by temperature ramping from 100 to 25°C. Results similar to the above hydrocarbon test were obtained. GC chromatogram of 'temperature ramping' shows a profile with a broader range of tobacco components, particularly those more volatile components at the front part of the chromatogram (Fig. 3).

3.2. Moisture and pH

When the newly harvested tobaccos were subjected to a drying or curing process, the cells of tobacco tissue collapsed. By adding a small amount of water to the dried tobacco sample, it was expected that the high moisture level could swell up the tobacco tissue and helped release volatile components from tobacco matrix, however, that was not exactly what happened as shown in Fig. 4. In fact, the nicotine peak decreased in the presence of high moisture content, which suggested that in addition to the effect of high moisture level, the nature of the target compounds could be an important factor as well. After all, the volatility of ionic compounds like nicotine is mainly dominated by pH, not just by moisture level. It was known that nicotine can exist either as protonated ions or as a free base molecule, depending on the pH. Since nicotine is highly soluble in aqueous solution, adding water to tobacco samples reduced nicotine content in the vapor phase, thus unfavorable to the SPME headspace sampling. As shown in Fig. 4, maximum SPME adsorption of nicotine was obtained by adding a basic solution to tobacco

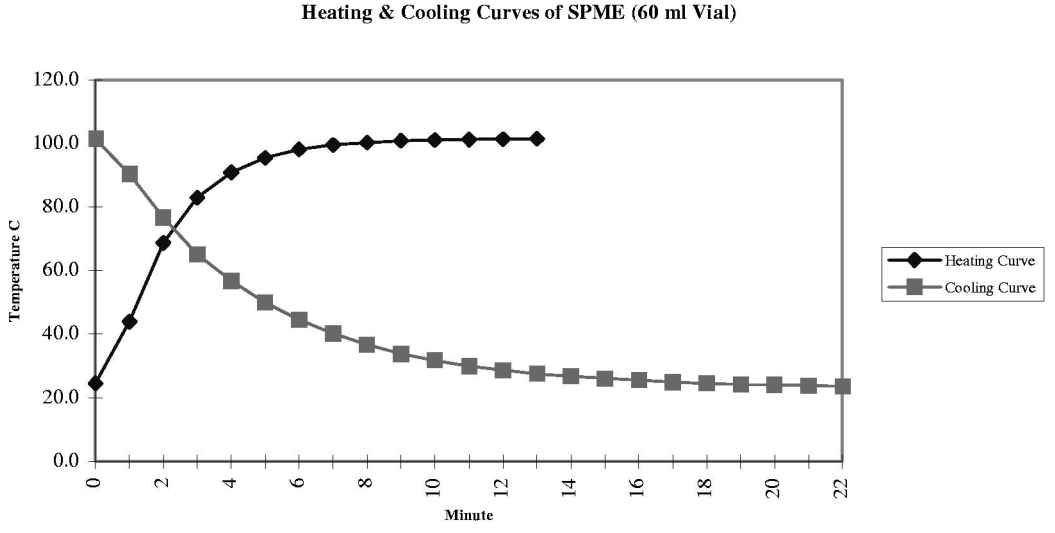

Fig. 2. Heating and cooling curves of air temperature inside the sample vial.

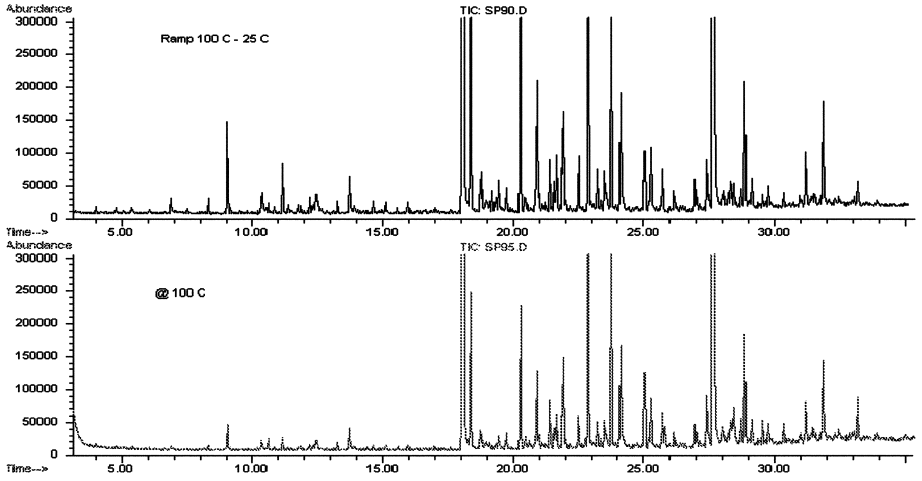

Fig. 3. GC–MSD chromatograms of tobacco samples with different SPME headspace sampling temperatures (ramp 100–25°C vs. 100°C).

samples, followed by the 'as is' condition. The addition of an acidic solution converted nicotine into protonated ions and resulted in minimal adsorption. The 2nd peak in Fig. 4 was solanone which is close to neutral with weak acidity. Adding water did help improve SPME sampling of solanone, but the addition of an acidic solution was still a better choice. For neutral compounds (e.g. megastigmatrienone, neophytadiene) moisture level which swelled up the tobacco tissue to release volatile components from tobacco cells, could be the dominating factor for headspace sampling using SPME. Increasing moisture level can help release this group of compounds from tobacco tissue. It was interesting to notice that

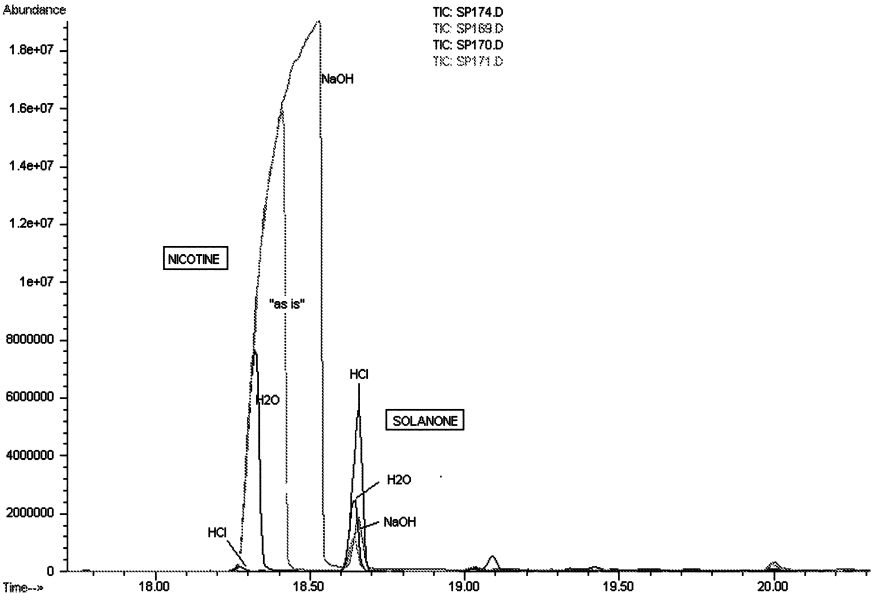

Fig. 4. pH and moisture effects on the efficiency of SPME headspace sampling.

the adsorption of neophytadiene on SPME fiber remained quite constant, either in acidic or in basic solution, due to its neutral property.

3.3. Selection of SPME Fibers

Two SPME fibers, polydimethylsiloxane–divinylbenzene (PDMS–DVB) and carbowax–divinylbenzene (CW–DVB) were evaluated for the analysis of tobacco samples. As shown in Fig. 5, the more polar CW–DVB fiber provided better adsorption for more volatile components, most of them are flavor related compounds and are eluted as early peaks on the GC chromatogram. CW–DVB fibers were also an appropriate tool for the analysis of a group of organic acids, particularly β-methylvaleric acid (BVA). BVA is unique to oriental tobacco, almost non-existent in bright or burly tobacco (Fig. 5). Therefore, BVA can be used as a chemical marker for tobacco identification in the American cigarette blends. However, it should made clear that SPME sampling can be applied to those BVA molecules in a free base form, but not those in 'bound form'. The 'bound form' of BVA is the binding complex of amines and BVA in cured tobacco. The non-polar PDMS–DVB fiber provides better selectivity and sensitivity than the CW–DVB fiber toward neutral organics, such as hydrocarbons.

Fiber to fiber variation has been recognized as a problem in quantitative analysis using SPME. A bright tobacco was sampled using five different CW–DVB fibers and significant fiber to fiber variation, >20%, on the peaks of interest was observed, which required the use of a monitor sample and an internal standard for quantitative analysis. However, replicate analyses using the same fiber generated reproducible results.

4. Conclusion

The effects of several experimental parameters on the efficiency of SPME headspace sampling for tobacco analysis were studied. Using a 'temperature ramping' approach enables the adsorption of volatile compounds with a broader range of volatility. High moisture content helps loosen tobacco tissue and the release of volatile molecules from tobacco matrix. Under the experimental conditions in the study, pH appears to be the dominant factor for the SPME headspace sampling of polar or ionic compounds.

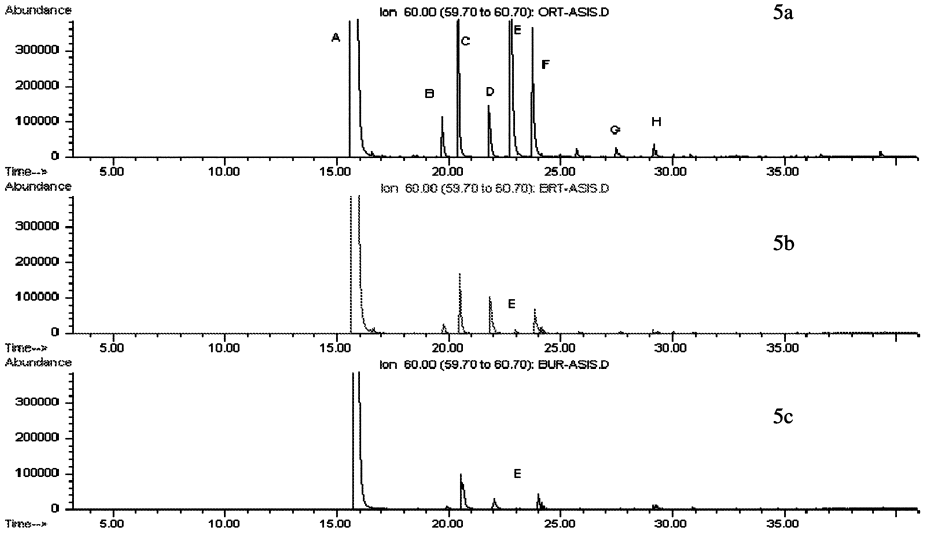

Fig. 5. Analysis of organic acids in oriental (a), bright (b) and burley (c) tobacco samples using SPME fibers for headspace sampling. SPME fiber: 65-μm carbowax–divinylbenzene. Sampling temperature: ramping from 100°C to 25°C. GC–MSD conditions: as described in the text. Peaks: A=acetic acid; B=butanoic acid; C=isovaleic acid; D=valeic acid; E=β-methylvaleic acid; F=hexanoic acid; G=heptanoic acid; H=octanoic acid.

Choosing a fiber with suitable polarity, depending on the nature of target compounds, is another important factor. A few limitations, such as the fiber to fiber variation, were also realized.

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