Journal of the Korean Society of
Tobacco Science Vol. 35, No. 1, 7~12(2013)
Printed in Republic of Korea

Optimization of Headspace Sampling Using Solid Phase Microextraction For Volatile Organic Acids in Different Tobacco Types

Jang-Mi Lee, Jeong-Min Lee, Seong-Ae Son, Young-Ju Kwon, Gi-Chul Jang, Young-Ho Kim

KT&G Research Institute, Taejeon 305-805, Korea
(Received Nov 5, 2013; Revised Nov 14, 2013; Accepted Nov 20, 2013)

ABSTRACT : A Solid-phase micro extraction(SPME) was evaluated as a tool for headspace sampling of tobacco samples. Several experimental parameters (sampling temperature, pH, and type of SPME fibers) were optimized to improve sampling efficiency in two aspects ; maximum adsorption and selective adsorption of volatile organic acids onto SPME fibers. Among four types of SPME fibers such as PDMS(Polydimethylsiloxane), PA(Polyacrylate), Car/PDMS (Carboxen/Polydimethylsiloxane) and PDMS/DVB(Polydimethylsiloxane/Divinylbenzene) which were investigated to determine the selectivity and adsorption efficiency. A variety of tobacco samples such as flue cured, burley and oriental were used in this study. The effect of these parameters was often dominated by the physical and chemical nature (volatility, polarity) of target compounds. This method allowed us to make important improvements in selectivity and sensitivity. The Car/PDMS fiber was shown to be the most efficient at extracting the 10 selected volatile organic acids. The parameters were optimized: 80℃ adsorption temperature, 30 min of adsorption time, 240℃ desorption temperature, 1 min of adsorption time.

Key words **: Solid-phase microextraction(SPME), volatile organic acids, Selectivity, tobacco, adsorption efficiency**

Volatile organic acids mainly affect the cigarette tastes and flavors. Volatile organic acids are organic compounds with acidic properties. The most common acids are the carboxylic acids, such as acetic acid and valeric acids. Traditionally, for analyzing volatile organic acids was prepared by base-catalyzed or acid-catalyzed esterification. Boron fluoride is a commonly used acid catalyst for methylation and methanolysis, but it is harmful, and boron and fluorine are also both restricted by local drainage laws. In addition, the methanolic boron fluoride reagent has a limited shelf life. A sulfuric acid is also an effective acid catalyst for volatile organic acid methyl esters synthesis, but it is a very corrosive liquid and must be handled with care. When the acid

*연락저자 : 305-805, 대전광역시 유성구 가정로 30, KT&G 연구원
*Corresponding author : *KT&G Research Institute, 30 Gajeong-ro, Yuseong-gu, Daejeon 305-805, Korea*
(phone : 82-42-866-5527; fax: 82-42-866-5544; e-mail:20060057@ktng.com)

catalysts, boron fluoride and sulfuric acid are used at high concentrations or at high temperatures, artifacts derived from organic acids can be produced.

So, Solid-phase micro-extraction which allows the extraction, concentration, and sample injection steps to be combined into one single step, is a relatively new sample preparation technique. Solid-phase micro-extraction (SPME) analytical system is a fast, simple and solvent-free method for qualifying and quantifying volatile organic acids from tobacco samples.

Gas chromatography-mass spectrometry (GC-MS) has been widely used for volatile organic acids analysis, because of its high specificity and sensitivity.

Therefore we developed an SPME-based method for selected volatile organic acids, choosing GC-MS as the instrumental technique with a single quadrupole MS using the selected ion monitoring (SIM) mode.

MATERIALS AND METHODS

Reagents and Materials

Methanol(HPLC grade) was obtained from JT Baker(USA). Selected 10 volatile organic acids and the internal standard (ISTD, anethol) were obtained from Sigma Aldrich. Stock standard solutions of individual compounds with concentration 100 μg/mL were prepared by weighing of the powder or liquid and dissolution in 100 mL of methanol, which were then stored in a refrigerator at 4℃. A multi-compounds working standard solution (approximately 1 μg/mL concentration of each compound) was prepared by making appropriate dilutions of the stock solutions with methanol, and the dilutions were stored in a refrigerator at 4℃. Flue-cured tobacco (B1O, Korea), burley tobacco (B1T, Korea), oriental tobacco were used in this study.

Sample Preparation

Ground tobacco (1.0 g) was exactly weighed into a 20 mL headspace amber vial, and 50 μL of ISTD solution were added. The vial was then placed to SPME device.

SPME Procedure

The SPME device was automated and on-line coupled to a gas chromatograph with mass spectrometer. Four types of SPME fibers, 65 μm polyacrylate (PA), 100 μm poly-dimethylsiloxane (PDMS), 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB) and 75 μm carboxen-polydimethylsiloxane(Car-PDMS) were tested for an initial fiber selection (Supelco, USA). All extractions were conducted in 20 mL amber glass vials. The sample was agitated and the fiber was inserted into the vial in the headspace over the sample. After exposure, the fiber was immediately desorbed in the GC injection port for analysis.

The parameters that affect the SPME process were optimized for the type of fiber, the extraction temperature, the extraction time and the thermal desorption temperature and time of fiber.

GC-MS analysis

The GC-MS analysis was carried out on an Agilent 6890 gas chromatograph with a 5973 mass spectrometer(USA). This instrument was equipped with a CTC CombiPAL autosampler (CTC Analytics, USA), which automated the SPME analysis. An analytical capillary column was used VF-5ms (30m × 0.25mm i.d., 0.25 μm film thickness, Varian, USA) was used. Helium was used as the carrier gas at flow rate of 1.0 mL/min. The injector temperature was set at 270℃ and 1 μL was injected in the splitless mode

for 1 min. The GC oven temperature program was used as follows : initial temperature 40℃ (hold for 3 min), ramp to 230℃ at 3℃/min and finally ramp to 240℃ held for 10 min. The ion source was set at 230℃.

Quantitative analysis

The calibration curves were determined by analyzing the solution spiked with different levels of the volatile organic acids after that they were subjected to the overall treatment; extraction, absorption and desorption from the fiber mentioned above. The calibration curves were linear over the range of 1.0 to 10.0 μg/mL. These linearity tests were repeated on three different days to obtain mean values. The limit of detection (LOD) for the procedure was calculated on a signal-to-noise basis of 3:1. Recovery tests were carried out at three spiking levels and 3 replicates. An analyte solution was spiked to the tobacco to achieve concentrations of 0.1, 0.5 and 1.0 μg/g for each volatile organic acids.

RESULTS AND DISSUSSION

Optimization of MS conditions

The MS worked in selected-ion monitoring (SIM) mode and from the mass spectra of individual compounds, two characteristic fragments were identified and were used for quantification purposes. To optimized the MS conditions, fragmentation of volatile organic acids was performed by using single quadrupole MS in the full scanning mode. From the mass spectra of each analyte, qualification and quantification ions were identified (Table 1).

Table 1. Mass ions for qualification and quantification by GC-MS

	VOAs* Ingredient	Retention (min)	MS Fragmentation ions(m/z)
1	Acetic acid	9.819	60, 43
2	Propionic acid	14.967	74, 45
3	Isobutyric acid	18.905	73, 88
4	Butyric acid	20.659	60, 73
5	Isovaleric acid	24.342	60, 87
6	2-Methyl butyric acid	24.99	74, 87
7	Valeric acid	26.906	60, 73
8	3-Methyl butyric acid	30.935	60, 87
9	Hexanoic acid	32.905	60, 73
10	Benzoic acid	44.121	105, 122
ISTD	Anethol	49.573	148, 133

* VOAs : Volatile Organic Acids

Optimization of SPME conditions

1. Comparison of fiber types : Four types of SPME fibers, 65 μm polyacrylate (PA) of a polar coating phase, the most common non-polar phase coating currently used 100 μm poly-dimethylsiloxane (PDMS), a mixed-phase fiber consisting of porous polymer particles of 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB) and 75 μm carboxen- polydimethylsiloxane(Car-PDMS) were tested for an initial fiber selection .The adsorption efficiency of the four different SPME fibers was determined for the 10 volatile organic acids (Fig. 1).

As a result, the Car-PDMS fiber seems to be

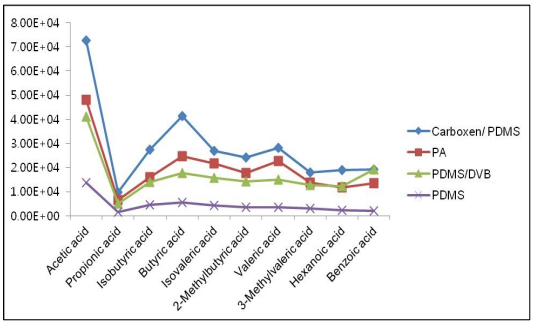

Fig. 1. Responses of the selected volatile organic acids by using four different SPME fibers.

more effective than the PDMS-DVB or PDMS fiber for extraction of the selected volatile organic acids. The 75 μm Car-PDMS fiber was used for the remainder of the study.

2. Extraction temperature : In order to study the effect of temperature on the extraction process, the samples were heated to temperatures ranging from 60℃ to 110℃ for 10 min, with continuous agitation. Figure 2 shows the effect of extraction temperature on extraction efficiency. The extraction temperature of 80℃ was selected for optimum extraction efficiency.

Since the SPME technique involves the adsorption of analytes from a liquid sample into the polymeric phase according to their partition coefficients, it is important to determine the temperature and time required to reach this equilibrium for each analyte.

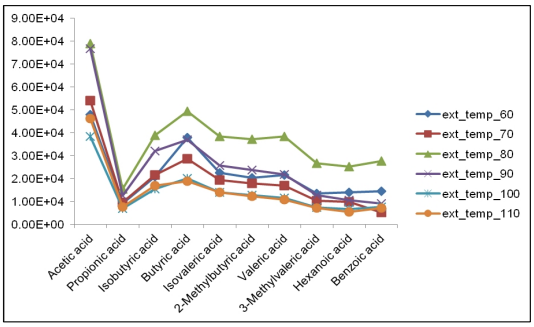

Fig. 2. Extraction temperature profile of of 10 volatile organic acids

3. Extraction time : The extraction time ranged from 20 min to 60 min. Figure 3 shows the adsorption time profiles obtained for the selected volatile organic acids. Six compounds reached equilibrium after 30 min (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, 2-methyl butyric acid), while the others already reached equilibrium after 20 min. The relative peak response of some volatile organic acids decreased after a long extraction.

The time profile of adsorption was studied by monitoring the area of each peak as a function of exposure time.

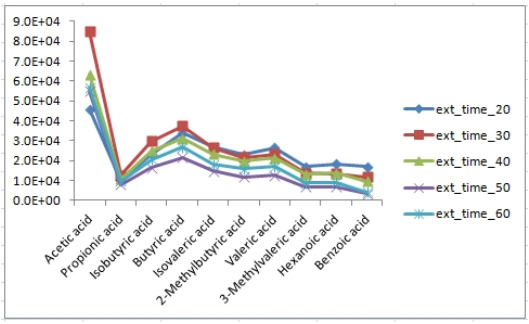

Fig. 3. Extraction time profile of of 10 volatile organic acids.

4. Desorption temperature : The analyte can be desorbed effectively under a higher temperature in a shorter time, but the stability and the life-time of the fiber will be affected. Also the analyte may be decomposed if the desorption temperature is too high. Figure 4 shows the profile of the analyte desorption in response to increasing temperature (230℃ to 270℃). A desorption temperature of 240℃ showed no carryover effects.

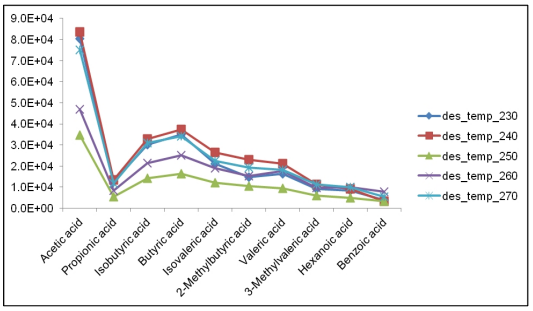

Fig. 4. Desorption temperature profile of of 10 volatile organic acids

5. Desorption time : We tested a range of desorption time from 1 min to 10 min to determine the optimal condition. Figure 5 shows the desorption time profile of the analytes. One minute of desorption time was sufficient to allow the complete desorption of analytes from the fiber.

Another important parameter is the desorption time of the analytes from the SPME fiber in the injection port of the GC.

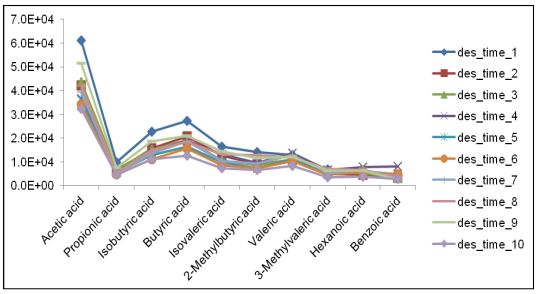

Fig. 5. Desorption time profile of of 10 volatile organic acids

Comparison of the selected volatile organic acids in different tobacco

We tested the comparison of the selected volatile organic acids in different tobacco types. Korea flue cured, B1O, korea burley, B1T and basma, oriental tobacco were used this study. Volatile organic acids mainly affect the cigarette tastes and flavors.

Comparing the analysis of the total volatile organic acids content, the quantity order was oriental, flue-cured, and burley. Especially 3-methylvalericacid and isovalericacid are the characteristic components of oriental tobacco. (Fig. 6)

Acetic acid is the main component in the volatile organic acids, and is mostly contained in the flue-cured tobacco. (Fig. 7)

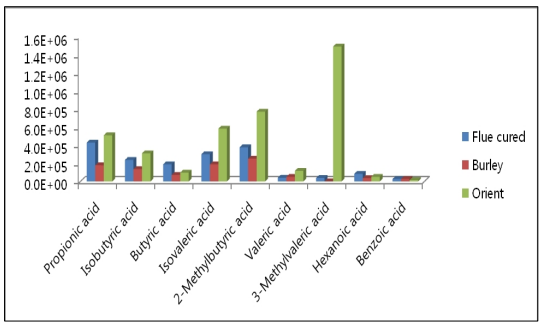

Fig. 6. Comparison of of 10 volatile organic acids in different tobacco

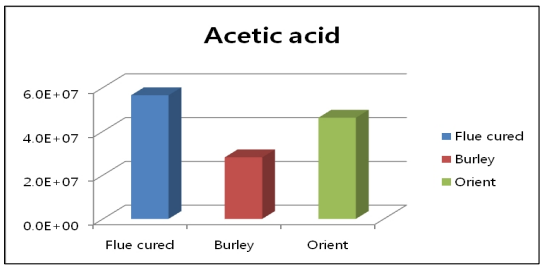

Fig. 7. Comparison of of acetic acids in different tobacco

Linearity, Limit of Detection, Precision and Recoveries

The linearity of the method was studied over the range of 1.0 μg/mL to 10.0 μg/mL. Each concentration was analyzed three times. We have good linearities for most of the selected volatile organic acids over this range (Table 2).

The precision of the method, repeatability, was calculated on a single-day basis. The relative standard deviations, calculated by using the mean of the peak areas of five consecutive SPME extractions of the same standard solutions, varied from 2.7% (acetic acid) to 9.1% (benzoic acid) in GC-MS (Table 2).

The limits of detection were calculated by comparing the signal-to-noise ratio(S/N) of the

Table 2. Linearity, LOD, precision and recovery rate by using the proposed method

Active ingredient	Linearity (R^2)	LOD (μg/mL)	Recovery (%)	Precision (RSD%)
Acetic acid	0.9994	0.005	82.3	2.7
Propionic acid	0.9994	0.002	95.1	5.4
Isobutyric acid	0.9989	0.010	91.7	6.9
Butyric acid	0.9981	0.007	88.2	3.5
Isovaleric acid	0.9982	0.005	91.0	7.0
2-Methyl butyric acid	0.9966	0.010	80.5	5.8
Valeric acid	0.9991	0.010	90.9	7.6
3-Methyl butyric acid	0.9989	0.010	93.2	9.0
Hexanoic acid	0.9990	0.010	89.1	7.1
Benzoic acid	0.9994	0.010	81.4	9.1

lowest concentration to the limit S/N=3.

The recoveries obtained from the analysis of tobacco samples are from 80.5% to 95.1% for each volatile organic acid at each concentration (Table 2).

CONCLUSIONS

This study shows that a combination of SPME with a gas chromatograph with mass spectrometer can be used to accurately determine 10 selected volatile organic acids in tobacco. This method allowed us to make important improvements in selectivity and sensitivity.

The Carboxen/PDMS fiber was shown to be the most efficient extraction. The parameters were optimized: 80℃ adsorption temperature, 30 min of adsorption time, 240℃ desorption temperature, 1 min of adsorption time.

SPME with the mass spectrometric analytical system is a fast, simple and solvent-free method for qualifying and quantifying volatile organic acids from tobacco samples.

Comparing the analysis of the total volatile organic acids content, the quantity order was oriental, flue-cured, and burley. Especially 3-methylvalericacid and isovaleric acid are the characteristic components of oriental tobacco.

REFERENCES

D.D. Robert, P. Pollien, C. Milo(2000) Solid-Phase Microextraction Method Development for Headspace Analysis of Volatile Flavor Compounds. *J. Agric. Food Chem.*, 48. 2430-2437

Jibao Cai, et al.(2001) Comparison of simultaneous distillation extraction and solid-spase microextraction for the determination of volitile flavor components. *Journal of Chromatography A*, 930. 1-7.

Mookherjee, B. D. and Wilson, R. A. (1988) Tobacco constituents - Their importance in flavor and fragrance chemistry *Rec. Adv. Tob. Sci.* Vol. 14, Chemical and sensory aspects of tobacco flavor, 42nd TCRC, 49-78.

Roberts, D. L. (1988) Natural Tobacco Flavor. *Rec. Adv. Tob. Sci.* Vol14, Chemical and sensory aspects of tobacco flavor, 42nd TSRC, 114-158.

S.S.Yang, et al.(2002) Optimizition of headspace sampling using solid-phase microextraction for volitile components in tobacco. *Journalof Chromatography A*, 942. 33-39.

Stephen B. S. and David L. A.(1999) Solid-phase microextraction of alkenylbenzenes and other flavor-realted compounds from tobacco for analysis by selected ion monitoring gas chromatography-mass spectrometry. *Journal of Chromatography A*, 858. 79-89.

Weeks, W. W. (1985) Chemistry of tobacco constituents influencing flavor and *aroma. Rec. Adv. tob. Sci.* 11:175-200.