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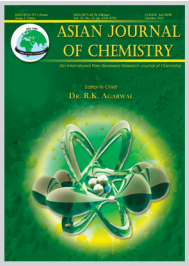

Determination of Benzoic Acid and Sorbic Acid in Tobacco Sauce by Ultra Performance Liquid Chromatography

ZHANGYU CHEN[1,2], WEI LIU[1], ZHUWEN CUI[1], GUANGYU YANG[1], YONGKUAN CHEN[1,*] and TIANFEI LI[1,2,*]

[1]Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P.R. China
[2]China Tobacco Yunnan Industry Company (Ltd.), Kunming 650000, P.R. China

*Corresponding author: E-mail: cyk1966@163.com, ygy1110@163.com

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A rapid and reliable method is presented for the determination of the preservatives (benzoic acid and sorbic acid) in tobacco sauce. The procedure utilizes ultra performance liquid chromatography followed by UV diode array detection for identification and quantitation of the two preservatives. The benzoic acid and sorbic acid were extracted from tobacco sauce samples with methanol/ammonium acetate buffer solution (25:75) by high speed homogenization and all samples were filtered to remove particulate matter prior to analysis. The ultra performance liquid chromatography determination of the preservatives was performed using a waters ACQUITY UPLC™ BEH C_{18} (2.1 × 50 mm, 1.7 mm) column and UV detection at 226 nm for benzoic acid and 258 nm for sorbic acid. The contents of preservative in the sample were be quantized by external standard method. The relative standard derivation of overall intraday variations were less than 1.8 % and the relative standard derivation of interday variations were less than 2.2 %. The standard recoveries (three different concentrations of markers: 0.5, 1.0 and 2.0 mg) were ranged from 97.8-102.4 %.

Key Words: Benzoic acid, Sorbic acid, Ultra performance liquid chromatography, Tobacco sauce.

INTRODUCTION

Tobacco sauce is an important additive for cigarette industry. The composition of the tobacco sauces includes moisture-retaining and taste-improving substances for cigarette. To prevent spoilage, the tobacco sauce also contains preservatives[1,2]. Typically, sodium benzoate and/or potassium sorbate are the preservatives that are used in sauce to inhibit mold growth, prevent spoilage and preserve freshness[3,4].

Under the provisions set forth by the US food and drug administration (FDA) in the code of federal regulations, food additives can be used if they are generally recognized as safe (GRAS) and declared on the label[5,6]. For instance, sodium benzoate may be used as a preservative, however, its usage should not result in levels exceeding 0.1 % in the sauce[5], while potassium sorbate may be used at levels of 0.1-0.2 %.[5,6] Therefore, the accurate determination of benzoic acid and sorbic acid is important.

Previous studies have reported the determination of benzoic acid and sorbic acid using high-performance liquid chromatography (HPLC) in orange juice, jelly or jam and various whole fruits[7,8]. A major drawback to this method was the use of tetrahydrofuran in the mobile phase, which presents a problem with the plastics used in filtration, along with added expense for hazardous waste disposal. The method reported for fruits, jellies and jams involves lengthy clean up steps prior to HPLC analysis, or long HPLC separation time[7-12].

In this study, a simple ultra performance liquid chromatography (UPLC) method that provides accurate results for benzoic acid and sorbic acid in tobacco sauce is presented. The UPLC technology fulfilled the promise of increased speed, resolution and sensitivity predicted for liquid chromatography[13,14]. The benzoic acid and sorbic acid can achieve a baseline separation within 2.5 min. The high speed homogenization was used as sample preparation method. Only 2 min was needed for preparing a set of samples. Compared to the previous literatures[7-12], this is one of the most simple and rapid methods for the determination of benzoic acid and sorbic acid.

EXPERIMENTAL

The HPLC analysis was performed on a waters ACQUITY UPLC system equipped with photodiode array detector and autosampler (Waters Corporation, USA) and a ACQUITY UPLC™ BEH C_{18} (2.1 × 50 mm, 1.7 mm) column was used. IKA T-25 Digital High-Speed Homogenizer (IKA Laboratory Equipment, Germany). HPLC grade methanol was provided by Fisher Scientific Inc. The ultrapure water used was obtained from a Milli-Q50 SP Water system (Millipore Inc, USA).

Ammonium acetate buffer solution was prepared by dissolving 3.855 g of ammonium acetate in 900 mL of water

TABLE-1
REGRESSION EQUATION, COEFFICIENT AND DETECTION LIMIT

Components	Regression equation C (μg mL^{-1})	Linearity range (μg mL^{-1})	Coefficient	Detection limits (μg mL^{-1})	RSD% (n=9)
Benzoic acid	A = 13.6 + 456.1 C	0.4 - 100	r = 0.9999	0.082	0.72
Sorbic acid	A= 32.12 1123 C	0.2 - 100	r = 0.9999	0.035	0.63

and adjusted to a pH of 4.5 with concentrated acetic acid; the buffer solution was then transferred to 1 L volumetric flask and brought to the volume. The mobile phase was prepared by exactly mixed 750 mL of the acetate buffer solution with 250 mL of HPLC-grade methanol. This solution also used for sample extraction.

Benzoic acid and sorbic acid stoking standard solution (1 mg/mL), this solution was prepared by accurately weighed 100 mg of benzoic acid (≥ 98 %) and sorbic acid (≥ 98 %) and dissolved in 100 mL of methanol. The five concentrations of work solution (0.5-100 μg/mL) was prepared by diluting the stock solution with methanol/ammonium acetate buffer solution (25:75).

Preparation of sample: A 1 g of sample was extracted with 50 mL of methanol/ammonium acetate buffer solution (25:75) by high speed homogenization for 2 min at the speed of 20000 rpm. This solution was filtered through a 0.45 μm syringe filter and afford to HPLC analysis.

RESULTS AND DISCUSSION

Optimal of chromatographic separation: The optimum separation and detection of benzoic acid and sorbic acid was achieved by regulating the pH, the percentage of methanol and the ammonium acetate concentration in the mobile phase. A significant change in the retention times of the analytes, along with changes in analytes resolution occurred when the pH, the percentage of methanol and the ammonium acetate concentration in mobile phase was changed. Among various mobile phases examined, buffer solution of pH 4.5 with ammonium acetate concentration of 3.855 g/L and the methanol/buffer solution (25:75) were found to be the best separation. Therefore, methanol/buffer solution (25:75) was selected as mobile phase in this experience.

To shorten the chromatographic separation time, a waters ACQUITY UPLC system with BEH C_{18} (2.1 × 50 mm, 1.7 μm) column was used in this experiment. With this UPLC system, the benzoic acid and sorbic acid were separated completely within 2.5 min (Fig. 1).

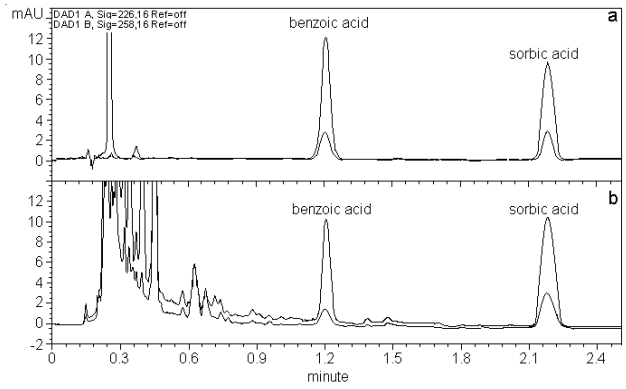

Fig. 1. Chromatogram of standard (a) and the real sample (b)

High sensitivity of the method was obtained by detecting analytes benzoic acid and sorbic acid at their wavelength maxima of 226 nm and 258 nm, respectively. UV spectra for the analytes are shown in Fig. 2. Obtaining a spectral scan at the top of the analyte peak was also used to confirm the presence of the analytes. It was considered that a preservative was present in a sample when a retention time and simultaneous spectral lmax match were obtained.

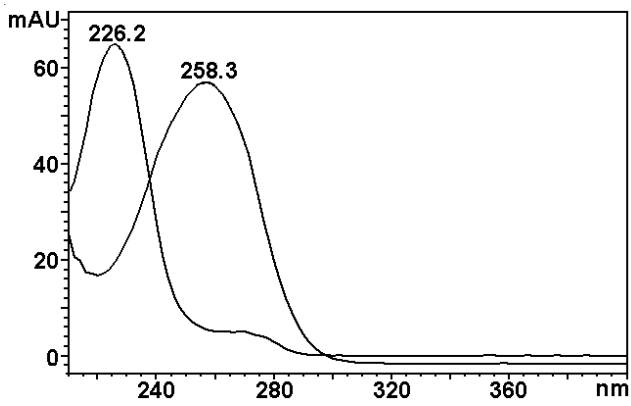

Fig. 2. UV spectra of benzoic acid (226.2 nm) and sorbic acid (258.3 nm)

Optimal of sample preparation: The tests on the composition of the extraction solvent, the time of extraction, the number of repetitions and the extraction method (ultrasonic extraction, high speed homogenization and oscillation extraction) were evaluated to develop a quantitative method by which extraction till total exhaustion is guaranteed. The results showed that among the various tests examined, high speed homogenization with methanol/ammonium acetate buffer solution (25:75) as sample extraction solvent can achieve the optimum efficiency. For 0.5 - 5 g of samples, 50 mL of methanol/ammonium acetate buffer solution used, the benzoic acid and sorbic acid can be extracted from the samples completely within 2.0 min by high speed homogenization at a speed of 20000 rpm. Therefore, high speed homogenization at a speed of 20000 rpm was selected for the samples preparation in this method.

Calibration graphs: Under the optimum conditions, the regression equations of benzoic acid and sorbic acid were established based on the standard samples injected and their peak area. The limits of detection are calculated by the ratio of signal to noise (S/N = 3). The results were shown in Table-1. The reproducibility of this method was also examined for 10 μg/mL of benzoic acid and sorbic acid. The relative standard deviations (n = 9) were shown in Table-1.

Method recovery and precision: The recovery test was carried out by adding benzoic acid and sorbic acid to the samples (three different concentrations of markers: 0.5, 1 and 2 mg). The sample was prepared as above preparation of

sample procedure and injected for UPLC analysis to calculate the amount of the benzoic acid and sorbic acid founded. The results shown that the recoveries (n = 7) were ranged from 97.8-102.4 %. This method involves in high recovery.

The measurements of intra and interday variability (determination of the same samples for seven times) were utilized to determine the precision of the developed method. The results shown that the relative standard derivation of overall intraday variations were less than 1.8 % and the relative standard derivation of interday variations were less than 2.2 %. This method is high precision.

Analysis of benzoic acid and sorbic acid in samples: A total of 256 samples were tested in this study. 43 samples were found to be containing benzoic acid (20-215.8 μg/g). 17 samples were found to be containing sorbic acid (20-187.4 μg/g). All of the samples tested were in compliance with their labels (1.0 mg/ L)[5].

Conclusion

In this manuscript, a waters ACQUITY UPLC system equipped BEH C_{18} (2.1 × 50 mm, 1.7 μm) column was used. The benzoic acid and sorbic acid can achieve baseline separation with 2.5 min on this system. Compared to the routine chromatographic method, 70 % of separation time was saved. The high speed homogenization was used for sample preparation. The benzoic acid and sorbic acid were extracted from the samples with solvent and can directly afford to HPLC analysis. Only 2.0 min is needed for preparing a set of samples. In a word, this method is rapid, high sensitive and provides good reproducibility and accurateness for the quantification of benzoic acid and sorbic acid in tobacco sauce.

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