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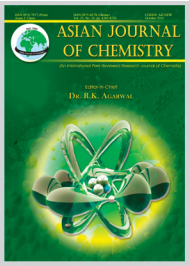

Determination of Four Parabens in Tobacco Sauce by Ultra Performance Liquid Chromatography

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A new method utilizes ultra performance liquid chromatography followed by UV diode array detection is presented for the determination of four parabens (methylparaben, ethylparaben, propylparaben, butylparaben) in tobacco sauce. The four parabens were extracted from tobacco sauce samples with 50 % methanol solution (containing 0.1 % acetic acid) by high speed homogenization and the samples were filtered to remove particulate matter prior to analysis. The UPLC determination was performed using a waters ACQUITY UPLC™ BEH C^{18} (2.1 × 50 mm, 1.7 μm) column and UV detection at 257 nm. The contents of four parabens in the sample were quantized by external standard method. The standard recoveries (three different concentrations of markers: 0.2, 0.4 and 0.8 mg) were ranged from 96.8-103.5 %. The relative standard derivation of overall intraday variations were less than 1.64 % and the relative standard derivation of interday variations were less than 2.23 %. Results showed this method is rapid and reliable and UPLC method provided a powerful tool for the analysis of parabens in tobacco sauce samples.

Key Words: Parabens, Ultra performance liquid chromatography, High speed homogenization, Tobacco sauce.

INTRODUCTION

Tobacco sauce is an important cigarette additive. The composition of the tobacco sauces includes moisture-retaining and taste-improving substances. To prevent spoilage, the tobacco sauce samples also contains preservatives[1,2]. Para-hydroxybenzoates (parabens) are the most commonly used preservatives to avoid microbial contamination, mainly because of the broad antimicrobial, good stability, nonvolatility and effectivity in a wide pH range[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, parabens may be used as a preservative, however, its usage should not result in levels exceeding 0.1 % in the sauce[5]. Therefore, the accurate determination of routine parabens (methylparaben, ethylparaben, propylparaben, butylparaben) is very important for food safety guarantee system.

In previous studies, various literatures have reported for the determination of parabens using high-performance liquid chromatography (HPLC) in foods, cosmetics and drugs. However, the method reported involves lengthy clean up steps prior to HPLC analysis, or a long HPLC separation time[7-12].

In this study, a simple ultra performance liquid chromatography (UPLC) method that provides accurate results for parabens (methylparaben, ethylparaben, propylparaben, butylparaben) in tobacco sauce is presented. The UPLC technology fulfilled the promise of increased speed, resolution and sensitivity predicted for liquid chromatography[13]. The four parabens can achieve a baseline resolution within 3.5 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 parabens.

EXPERIMENTAL

The HPLC determination 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 μm) column was used for parabens separation.

An IKA T-25 Digital High-Speed Homogenizer (IKA Laboratory Equipment, Germany) was used for sample preparation. HPLC grade methanol (provided by Fisher Scientific Inc) was used as mobile phase and sample extraction. The ultrapure

water used was obtained from a Milli-Q50 SP water system (Millipore Inc, USA). 50 % Methanol (containing 0.1 % acetic acid) was used for sample extraction.

The mobile phases used were methanol and 0.1 % acetic acid solution, with the gradient change from 0 min (40 % methanol) to 3 min (60 % methanol) at a flow of 0.5 mL/min.

Parabens stoke standard solution (1 mg/mL) was prepared by accurately weighed 100 mg of methylparaben (≥ 98 %), ethylparaben (≥ 98 %), propylparaben (≥ 98 %), butylparaben (≥ 98 %) and dissolved in 100 mL of methanol. The five concentrations of work solution (0.5-100 μg/mL) were prepared by diluting the stoking solution with 50 % methanol.

Preparation of sample: A 0.5 g of sample was extracted with 25 mL of 50 % methanol (containing 0.1 % acetic acid) 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 afforded to HPLC analysis.

RESULTS AND DISCUSSION

Optimal of chromatographic separation: 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.

The optimum separation of the four parabens was achieved by regulating the pH, the percentage of methanol and water 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 and the percentage of methanol in mobile phase was changed. When methanol and 0.1 % acetic acid solution used as mobile phase, the isocratic elution was effective for the separation of four parabens, but the butylparaben was eluted too long. Therefore, the gradient elution was used to shorten the elution time of butylparaben. Among various mobile phases examined, the gradient change of 0 min (methanol and acetic acid solution 40:60) to 3 min (methanol and acetic acid solution 60:40) were found to be the best separation and the chromatographic separation time was conveniently shortened. Therefore, this gradient change was selected as mobile phase in this experience. With the selected conditions, four parabens can achieve a baseline resolution within 3.5 min (Fig. 1).

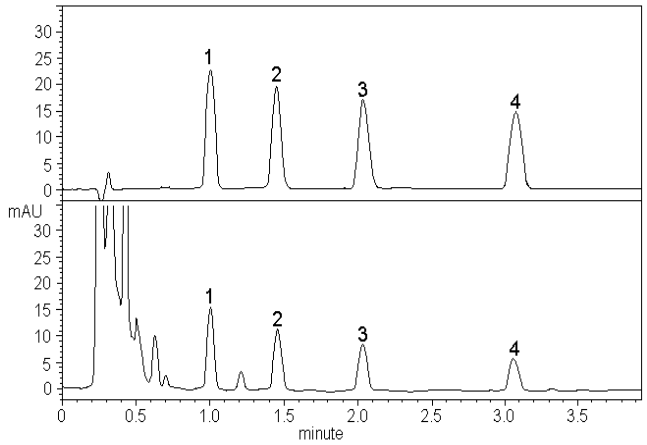

Fig. 1. Chromatogram of standards (a) and the real sample (b); (1) methylparaben; (2) ethylparaben; (3) propylparaben; (4) butylparaben

The UV spectrums of the four parabens are shown in Fig. 2. The greatest sensitivity of the method was obtained by detecting analytes at their wavelength maxima of 257 nm. Obtaining a spectral scan of the analyte peak was also used to confirm the presence of the analytes. It was considered that a paraben was present in a sample when a retention time and simultaneous UV absorption spectrum match were obtained.

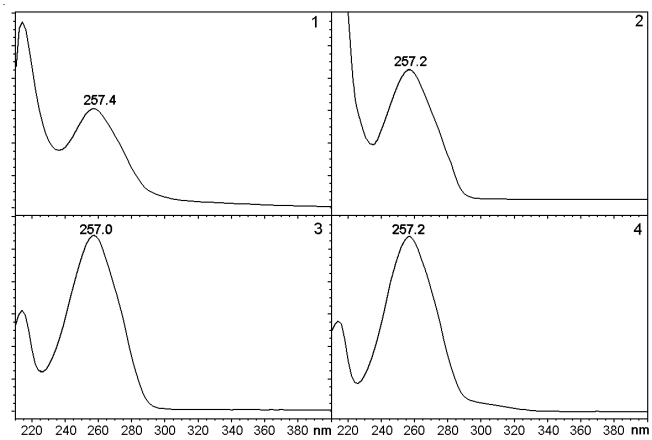

Fig. 2. UV spectra of parabens; (1) methylparaben; (2) ethylparaben; (3) propylparaben; (4) butylparaben

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 50 % methanol (containing 0.1 % acetic acid) as sample extraction solvent can achieve the optimum efficiency. For 0.25-2.5 g of samples, 25 mL of 50 % methanol (containing 0.1 % acetic acid) used, the parabens can be extracted from the samples completely with in 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 four parabens 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 parabens. The relative standard deviations (n = 9) were shown in Table-1.

Method recovery and precision: The recovery test was carried out by adding parabens to the samples (three different concentrations of markers: 0.2, 0.4 and 0.8 mg). The sample was prepared as above preparation of sample procedure and injected for UPLC analysis to calculate the amount of the parabens founded. The results shown that the recoveries (n=7) were ranged from 96.8-103.5 %. This method is 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 1.64 % and the relative

TABLE-1
REGRESSION EQUATION, COEFFICIENT AND DETECTION LIMIT

Components	Regression equation C ($\mu g\ mL^{-1}$)	Linearity range ($\mu g\ mL^{-1}$)	Coefficient	Detection limits ($\mu g\ mL^{-1}$)	RSD % (n = 9)
Methylparaben	A = -13.4 + 1263 C	0.2 - 100	r = 0.9999	0.055	0.64
Ethylparaben	A = 18.7 + 1108 C	0.2 - 100	r = 0.9999	0.070	0.73
Propylparaben	A = 10.6 + 987.2 C	0. 15 - 100	r = 0.9999	0.084	0.70
Butylparaben	A = 9.87 + 876.3 C	0. 15 – 100	r = 0.9999	0.095	0.68

standard derivation of interday variations were less than 2.23 %. This method is high precision.

Analysis of parabens in samples: A total of 256 samples were tested in present study. Twelve samples were found to be containing methylparaben (20-164 μg/g). 18 samples were found to be containing ethylparaben (20-156.3 μg/g). Five samples were found to be containing propylparaben (20-106.3 μg/g). No samples were found to be containing butylparaben. All of the samples tested were in compliance with their labels (1 g/ L) of US food and drug administration[5].

Conclusion

In this manuscript, a waters ACQUITY UPLC system equipped BEH C_{18} (2.1 × 50 mm, 1.7 μm) column was used for the determination of the parabens in tobacco sauce. Four parabens can achieve baseline resolution with 3.5 min on this system under the optimum conditions. Compared to the routine chromatographic method[7-11], more than 70 % of separation time was saved. The high speed homogenization was used for sample preparation. The parabens 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. This method is rapid and reliable, and UPLC method provided a powerful tool for the analysis of parabens in tobacco sauce samples.

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