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Analytical Methods

An environment-friendly procedure for the high performance liquid chromatography determination of benzoic acid and sorbic acid in soy sauce

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ABSTRACT

A rapid, accurate and environment-friendly procedure has been developed for the HPLC-based determination of benzoic acid and sorbic acid contents in soy sauce. A C18 column served as the stationary phase, methanol–ammonium acetate buffer (0.02 M) (30:70, v/v) was used as the mobile phase, the flow rate was 1 mL/min, the UV detector was set at 225 nm and cinnamic acid was selected as an internal standard. Under such optimized conditions, benzoic acid, sorbic acid and the internal standard were separated within 8.1 min. This newly developed procedure also showed excellent recurrence, with a relative standard deviation of less than 3% and recoveries were 96.1–104.3%.

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1. Introduction

Soy sauce is a condiment that originated in ancient China and is extensively used in modern China and Southeast Asia. Benzoic acid and sorbic acid are prevalently used as preservatives in soy sauce. However, adverse effects have been associated with benzoic acid at low doses. In sensitive individuals, conditions such as asthma, urticaria, metabolic acidosis and convulsions have been observed (Tfouni & Toledo, 2002; WHO, 2000). Some clastogenic activity has also been observed with in vitro assays, therefore, the use of benzoic acid must be controlled. Sorbic acid and its leopoldite are safe and effective preservatives recommended by the Food and Agriculture Organization (FAO) and World Health Organization (WHO). However, strict standards have been set by the China Health Ministry to monitor its usage, which have been implemented since 2004. The maximum allowed combined content of benzoic acid and sorbic acid in soy sauce is 1.0 g/kg (GB/T 5009.29, 2003). Therefore, it is necessary to ensure that low levels of these preservatives are used in soy sauce to meet regulatory standards.

Many methods for the determination of benzoic and sorbic acids in soy sauce are available in the literature. HPLC is the most popular method for their simultaneous determination (Kritsunankul & Jakmunee, 2011; Nour, Trandafir, & Ionica, 2009; Tfouni & Toledo, 2002; Wu, He, & Guo, 2012). In addition, gas chromatography (Jurado-Sanchez, Ballesteros, & Gallego, 2011; Sun, Wang, Huang, Pan, & Wang, 2013), capillary electrophoresis (Hsu, Hu, & Chiu, 2014; Wei, Li, Yang, Jiang, & Xie, 2011) and supercritical fluid chromatography (Berger & Berger, 2013) have also been utilized. However, the extensive use of organic solvents in sample preparation or in the preparation of standard solutions of the preservatives is potentially harmful to environment and the operators. In this study, we developed an HPLC-based method using small amounts of organic solvents in the liquid–liquid extraction (diethyl ether, 5 mL) and using no organic solvents in the preparation of the standard solutions. Meanwhile, benzoic and sorbic acids were determined by the internal standard method which eliminated target losses during liquid–liquid extraction, thus making the procedure more accurate.

2. Experimental

2.1. Materials and reagents

Different brands of soy sauce samples were purchased from local supermarkets. Standards of benzoic acid (BA, 99.5%), sorbic acid (SA, 99%) and cinnamic acid (CA, 99%) were purchased from

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Fluka (Shanghai, China). Sodium hydroxide (99%), ammonium acetate (98%) and hydrochloric acid (12 mol/L) were obtained from Hewei Chemical Co. (Shanghai, China). Methanol (HPLC grade) was purchased from Zhengxin Chemical Co. (Shanghai, China).

2.2. Preparation and storage of standards

Standard stock solutions containing 1000 mg/L each of BA or SA was prepared in water (Milli-Q). The mixture was shaken well until a homogenous and clear solution formed. If necessary, a small amount of methanol was added to help dissolution. The same method was applied to prepare a 500 mg/L internal standard CA stock solution. All stock solutions were covered with aluminum foil, stored in a freezer (4 °C) and away from light (ready for a maximum usage of one month). Before each experiment, BA and SA standard solutions were prepared in the same volumetric flask, and then diluted to five different concentrations in the range 20–100 mg/L at constant intervals, where 50 mg/L internal standard (CA) was added to every diluted standard solution.

2.3. Sample preparation

2.3.1. Extraction procedure

Approximately 1 g of sample was accurately weighed in a 10 mL capped test tube. A 0.5 mL aliquot of the internal standard CA stock solution, 0.5 mL hydrochloric acid–water (1:1, v/v) and 5 mL diethyl ether were then added successively. The mixture was then sonicated for 1 min before being allowed to separate into layers. The upper ether layer was quantitatively transferred by an injector to another 10 mL capped test tube.

2.3.2. Back extraction procedure

A 1 mL aliquot of aqueous sodium hydroxide (0.1 M) was added to the diethyl ether extracts. The resulting mixture was sonicated for 1 min, and then allowed to separate into layers. The upper ether layer was discarded, and the lower aqueous layer was placed in a 40 °C water bath to evaporate any residual diethyl ether. Finally, the residue was transferred to a 5 mL volumetric flask and diluted with water to scale.

2.4. Chromatographic conditions

Analytical separation was carried out on an Agilent 1100 HPLC unit using a Supelco 516 C18 column (25 cm × 4.6 mm, 5 μm) at room temperature. The detector used was an UV–vis spectrophotometer set at 225 nm and the volume of sample injected was 20 μL. The mobile phase used was methanol–ammonium acetate buffer (0.02 M) (30:70, v/v); the flow rate was 1 mL/min.

3. Results and discussion

3.1. Calibration curve and detection limit

The calibration curve was constructed by plotting the peak area ratio of BA or SA and the internal standard CA against the concentration of BA or SA. Excellent linearity was obtained within the concentration range of 20–100 mg/L (the wavelength used to perform the calibration was 225 nm), giving both samples a correlation coefficient of 0.9996.

The detection limit of BA, SA and CA was found to be 0.2, 0.1 and 0.5 mg/L respectively in the final solution when the signal-to-noise ratio was greater than 3:1.

3.2. Optimization of analysis conditions

In order to obtain maximum sensitivity, it is optimal to detect each compound at its maximum absorbance wavelength. The UV–vis spectra of the compounds are shown in Fig. 1. It can be anticipated that a small peak would be obtained for BA if the detection wavelength was fixed at 275 nm. On the other hand, small peaks for BA and CA would be obtained if the detection wavelength was set at 235 nm. The maximum peak for BA could be obtained if the detector was set at 225 nm where peak intensities for BA, SA and CA were the same. Therefore, 225 nm was selected as a suitable detection wavelength. In order to verify this prediction, a standard mixture of BA, SA and CA was injected into the HPLC column at maximum absorption wavelengths of 235 and 225 nm separately. As shown in Fig. 2(a), it is evident that the peaks of BA and CA were too small when the detector was set at 235 nm. However, in Fig. 2(b), all peaks were of satisfactory intensity when the detector was set at 225 nm.

To protect the environment and the health of the operators, the use of organic solvents (especially highly toxic solvents such as methanol), should be reduced as much as possible. It is generally acknowledged that to minimize matrix effects, the solvent for preparation of samples and standard solutions should be identical. In this work, the extraction-stripping two-step procedure in the sample preparation step eventually drove the sample into water matrix. This allows water to be used for preparing the BA and SA standard solutions, thus decreasing the quantity of organic solvent.

To select a suitable internal standard, an extensive literature survey was done. However, no suitable internal standard has been reported so far, for the determination of BA and SA content by HPLC. Phthalate, hendecanoic acid and decylic acid are common internal standards for the determination of BA and SA content in soy sauce by gas chromatography (in China). However, these internal standards were not suitable for the technique in this study. Phthalate could get hydrolyzed by aqueous sodium hydroxide in the back extraction process. Furthermore, hendecanoic acid and decylic acid have poor UV absorption in the 190–400 nm range and are unsuitable for HPLC with an UV detector. Cinnamic acid was selected for this study due to its similar structure with benzoic acid. The initial results were promising, so cinnamic acid was chosen as the internal standard in this new developed method.

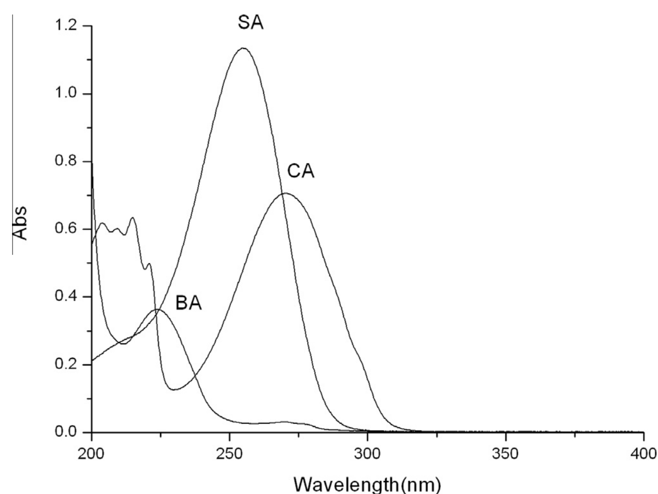

Fig. 1. UV spectra of BA, SA and CA (10 mg/L each); the solvent was methanol–ammonium acetate buffer (0.02 M) (30:70, v/v).

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