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Short communication

Determination of phenolic preservatives in gelatin and vacant capsules for medicine use by ion-suppression reversed-phase high performance liquid chromatography

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Abstract

A reliable method for the simultaneous determination of phenolic preservatives parahydroxybenzoic acid, methyl, ethyl, propyl, butyl and pentyl parahydroxybenzoates in gelatin and vacant capsules for medical use has been developed by ion-suppression reversed-phase high performance liquid chromatography. Separation was carried out on a Kromasil C_{18} column by isocratic elution using methanol–perchloric acid (pH 2.0; 10 mM) (60:40, v/v) at a flow-rate of 1.0 ml min^{-1}, and detection by UV absorbance at a wavelength of 254 nm. This method has been successfully applied to the routine analyses of these preservatives in the real samples.

Keywords: Phenolic preservative; Gelatin; Vacant capsule; Ion-suppression; Reversed-phase high performance liquid chromatography

1. Introduction

Capsules are preparations consisting of medicaments with or without excipients enclosed in shells. Capsule shells are mainly made of gelatin which is a purified protein obtained by the partial hydrolysis of animal collagen in the skin, bone, tendon and ligament. Because being easily decomposed by microorganisms, gelatin is allowed to contain suitable antimicrobial agents put in such as phenolic preservatives [1–4]. Sometimes these preservatives may also be added in the manufacture of capsule shells. Phenolic preservatives may exist as parahydroxybenzoic acid (PHBA) or its methyl, ethyl, propyl, butyl and pentyl esters, which can be used not only independently but also as a mixture. However, since the extra-use and abuse of phenolic preservatives is harmful to human health, these preservatives must be limited in gelatin and vacant capsules. They are permitted 20 μg g^{-1} in gelatin by British Pharmacopoeia. The allowable content of 500 μg g^{-1} in vacant capsule is still under discussion in China [5]. The statutory method for the examination of phenolic preservatives in gelatin is thin-layer chromatography (TLC) [1]. The most commonly employed procedure for the determination of alkyl esters of PHBA is reversed-phase high performance liquid chromatography (HPLC) [6,7], but most of previous works aimed at these esters, other than PHBA, in cosmetic and household products. No reports did not regard to the simultaneous determination of PHBA and its esters so far. In this paper, a reversed-phase ion-suppression HPLC method is presented for the determination of phenolic preservatives. PHBA and its methyl, ethyl, propyl, butyl and pentyl esters in gelatin and vacant capsules for medicine can be determined in a single step.

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2. Experimental

2.1. Apparatus

The liquid chromatograph was a Varian 5060 HPLC (Varian, Walnut Creek, CA, USA) coupled to a Rheodyne 7725i injector valve equipped with a 10-μl loop (Rheodyne, Rohnert Park, CA, USA), and a Waters 486 Tunable UV Absorbance detector (Waters, Milford, MA, USA). The chromatograms were recorded on a Model-JS3030 chromatographic work station (Johnsson Separation Science & Technology Corporation, Dalian, PRC).

2.2. Reagents and chemicals

Methanol (HPLC grade) was obtained from Hanbang Science & Technology Co. Ltd. (Huaiyin, PRC). Perchloric acid (70–72%, guaranteed reagent) was obtained from Tianjing Third Reagent Factory (Tianjin, PRC). All other reagents were of analytical-reagent grade unless stated otherwise. Water (>18 MΩ cm) used for all solutions and mobile phase was prepared from Aquapro ultrapure water systems for the laboratory-scale (Ever Young Enterprises Development Co. Ltd., Chongqing, PRC). PHBA and its esters without further purification were used as reference substances: PHBA, methyl parahydroxybenzoate (Me-PHBA), ethyl parahydroxybenzoate (Et-PHBA), propyl parahydroxybenzoate (Pr-PHBA), butyl parahydroxybenzoate (Bu-PHBA) and pentyl parahydroxybenzoate (Pe-PHBA) were purchased from Aldrich (Milwaukee, WI, USA), Tianjing Second Reagent Factory (Tianjing, PRC) or Shanghai First Reagent Factory (Shanghai, PRC).

Gelatin and vacant capsule samples were kindly supplied by various manufactures.

2.3. General procedure

2.00 g of gelatin or vacant capsule sample to be examined was soaked in 30 ml of water for swelling at room temperature, and then heated at 60 °C in a water bath until complete dissolution. The solution was transferred into a 120-ml separatory funnel, 30.00 ml of ethyl ether accurately measured was added, and the funnel was shaken gently for 2 min and allowed to stand. After the aqueous layer was discarded, 5.00 ml of the extract in ethyl ether was transferred accurately and evaporated carefully to dryness in a water bath and the residue was dissolved in 1.00 ml of methanol. The final sample solution was filtered through a Millipore membrane filter (0.5 μm) (Milipore, Bedford, MA, USA) by means of a syringe before injection.

The column used was a Kromasil C_{18}, 5 μm, 4.0 mm i.d. × 150 mm (Hanbang Science & Technology Co. Ltd.). The mobile phase was methanol–perchloric acid (pH 2.0; 10 mM) (60:40, v/v) at a flow-rate of 1.0 ml min^{-1}. The column temperature was held at 30 °C. The injection volume was 10 μL. The UV detector was set at 254 nm.

Quantitation was performed by calibration curve method based on peak area measurement. The standard solutions used for calibration purpose were prepared by dissolving the appropriate amount of each preservatives in methanol, and serially diluting to the final concentration of 0.0001–0.08 mg ml^{-1}.

3. Results and discussion

3.1. Chromatograms

An HPLC chromatogram demonstrating the separation of a mixture of the studied phenolic preservatives is shown in Fig. 1. As can be seen the retention times increase with the increase of alkyl chain length (*n*), i.e. the order is PHBA, Me-PHBA, Et-PHBA, Pr-PHBA, Bu-PHBA and Pe-PHBA with *n* of 0–5, respectively.

Perchloric acid has been frequently employed as ion-suppressant for determination of organic acids by reversed-phase HPLC due to its strong acidity and powerful suppressive action at low application concentration (mM level) [8,9]. In this present experiment, if no perchloric acid existed in the mobile phase, not only the peak of PHBA was disrupted drastically and just emerged in the dead time (t_0), but also those of alkyl parahydroxybenzates became somewhat wide and asymmetrical, because of –COOH and/or –OH groups. Perchloric acid could suppress their dissociation during chromatographic process.

3.2. Calibration curves and detection limits

A linearity has been obtained for each phenolic preservative inside the range studied with a correlation coefficient (*r*) higher than 0.9990. The regression equations ($A_n = a + bC_n$) with limits of detection (LOD) are presented in Table 1.

3.3. Analyses of samples

In the present study, 15 random samples of industrial products were analyzed for these phenolic preservative contents.

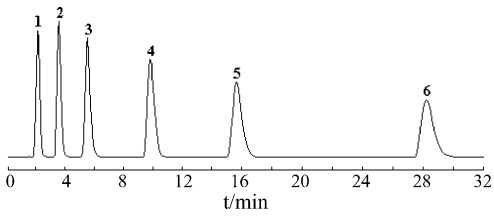

Fig. 1. Chromatogram of a standard solution containing six phenolic preservatives. They are: (1) PHBA; (2) Me-PHBA; (3) Et-PHBA; (4) Pr-PHBA; (5) Bu-PHBA; and (6) Pe-PHBA. Column: Kromasil C_{18}, 5 μm, 4.0 mm i.d. × 150 mm. Column temperature: 30 °C. Mobile phase: methanol–perchloric acid (pH 2.0; 10 mM) (60:40, v/v). Flow-rate: 1.0 ml min^{-1}. Injection volume: 10 μL. Detection wavelength: 254 nm. Sensitivity: 0.04 aufs.