Isocratic HPLC determination of preservatives in beverages

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A high-performance liquid chromatographic (HPLC) technique was proposed for the simultaneous determination of five frequently used preservatives (benzoate, sorbate, methyl, ethyl and propyl esters of p-hydroxybenzoic acid). The optimal conditions for the separation were established by varying mobile phase parameters such as acetonitrile concentration, pH and ionic strength. The optimized separations were performed on a reversed-phase C_{18} column within 28 min by an isocratic elution with 5 mmol/l aqueous acetate buffer (pH 5.0) containing 40% (v/v) acetonitrile, and UV detection at 254 nm. The detection limits for all analytes were in the range 0.25–2.8 mg/l. The proposed method was applied for determination of common preservatives in beverages. The recovery tests established for three samples were within the range 100 ± 5%.

Key words: high-performance liquid chromatography, preservatives, beverages

INTRODUCTION

Preservatives having antimicrobial properties are permitted food additives in various food products to preserve them from decay [1–4]. For instance, benzoic acid or sodium benzoate inhibits bacterial development. Sorbic acid or potassium sorbate is an antifungal preservative against molds and yeasts [1, 2]. Esters of p-hydroxybenzoic acid, such as methyl, ethyl, and propyl p-hydroxybenzoate, also possess antifungal properties. The antimicrobical activities of these esters increase with increasing alkyl chain length, but esters with longer alkyl chains are of limited application owing to their lower solubility in water [3, 4]. Although these preservatives are frequently used in various food products, they are harmful at higher than permitted safety levels. For instance, sodium benzoate may be used as a preservative in beverages, however, its usage should not result in levels exceeding 0.1% in the beverage, while potassium sorbate and esters of p-hydroxybenzoic acid may be used at the levels of 0.1–0.2% [5].

The most common analytical technique used for the determination of preservatives in food is spectrophotometry [6]. Generally, both benzoate and sorbate are distilled with water or extracted with diethyl ether and then measured directly at 225 and 255 nm, respectively [7, 8]. Spectrophotometric determination of p-hydroxybenzoates is based on their extraction with $CHCl_3$, followed by the reduction with hydroxylamine and complexation with iron(III). The coloured complex is monitored at 525 nm [9]. Spectrophotometric methods show a good sensitivity (detection limits are in the range 10^{-6} mol/l), however, they involve many labor-intensive and as time-consuming procedures and can result in a loss of the analytes during sample preparation. Moreover, in many instances more than one preservative are added to a product. Spectrophotometric methods, however, are not suitable for the simultaneous determination of more than one analyte present in the sample.

Therefore, developing an appropriate analytical method to separate and determine common preservatives in a single analysis is essential. High-performance liquid chromatography (HPLC) with UV detection has been found appropriate for the above purposes, although most isocratic HPLC techniques may be employed to analyse only two or three kinds of preservatives [10–12]. Ion-pair HPLC, however, can simultaneously analyze all five compounds, but the separation takes about 50 min [13].

The aim of the present study is to develop a simple isocratic HPLC technique for the separation and determination of all five preservatives in one chromatographic run.

EXPERIMENTAL

The HPLC instrumentation consisted of a HPP 5001 series high-pressure pump with an injection valve

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equipped with a 25-µl sample loop (Laboratorni Pristroje, Prague). The detector was an UV/VIS LCD 2563 photometer (Laboratorni Pristroje, Prague) set to absorb at 254 nm. The results and data were collected and plotted on an SP 4290 plotter/integrator (Spectrophysics CA, USA).

Separations were performed on a 5-µm Separon SGX C_{18} (150 × 3 mm i. d.) column (TESSEK Ltd., Prague). The mobile phase flow rate was 0.2 ml/min.

All mobile phase and standard solutions were prepared using doubly distilled water. HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical-reagent grade and obtained from Aldrich (Milwaukee, Wis., USA).

Standard stock solutions (0.01 mol/l) of the preservatives were prepared from sodium benzoate (SB), potassium sorbate (PS), methyl (MeHB), ethyl (EtHB), and propyl (PrHB) p-hydroxybenzoates in water. All working solutions were prepared by suitable dilution. The mobile phase was prepared by neutralization of 5 mmol/l CH_3COOH solution in acetonitrile–water mixture with 0.1 mol/l NaOH to pH 5.0.

All mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed by ultrasonication.

RESULTS AND DISCUSSION

Since all the analytes studied have UV chromophores, UV detection was chosen for this investigation. Differences between three esters of p-hydroxybenzoic acid exist only in their alkyl groups. Therefore they exhibited a quite similar absorbance spectrum in the UV region: two absorbance maxima for each spectrum were around 195 and 295 nm. The other two analytes possessed obviously different UV spectra. Sorbic acid showed maximum absorbance at 255 nm, while benzoic acid at 195 and 225 nm. For simultaneous detection of the analytes, 254 nm was selected as a compromise. At this wavelength all five preservatives exhibited the absorbance sufficient for sensitive detection.

Initially, several mobile phases consisting of acetic acid, H_2O and CH_3CN (pH 3.5) were tested for their ability to separate the analytes. The total concentration of acetic acid was kept constant (5 mmol/l), while the ratio of the two solvents (H_2O : CH_3CN) varied from 80:20 to 40:60 (v/v). By increasing the acetonitrile concentration, the retention times of all analytes decreased (Fig. 1). When the acetonitrile concentration was too low, the separation time was unnecessarily prolonged and the peaks of strongly retained compounds, such as PrHB and EtHB, were seriously tailing. When the acetonitrile concentration was too high,

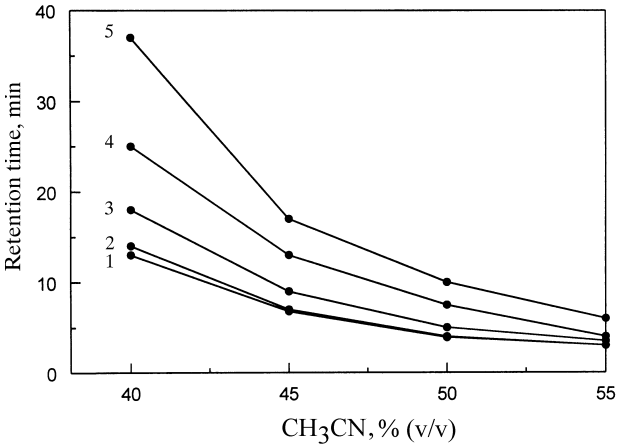

Fig. 1. Effect of acetonitrile concentration in the mobile phase on the retention times of benzoate (1), sorbate (2), methyl-p-hydroxybenzoate (3), ethyl-p-hydroxybenzoate (4) and propyl-p-hydroxybenzoate (5). Mobile phase: 5 mmol/l acetic acid, pH 2.5

the baseline separation could not be achieved for MeHB, PS and SB. Unfortunately, none of the mobile phase systems tested resolved PS and SB. Finally, acetonitrile concentration was chosen at 40% for all further investigations.

Because the pK^a values of benzoic and sorbic acids are 4.19 and 4.76, respectively, their separation can be improved by varying the pH of the mobile phase. In this experiment, the pH of the mobile phase was adjusted by neutralization of CH_3COOH with 0.1 mol/l NaOH. Figure. 2 illustrates the effect of the mobile phase pH on the retention times of the analytes studied. By increasing the pH value due to deprotonation the negative charge of both benzoate and sorbate increased, resulting in

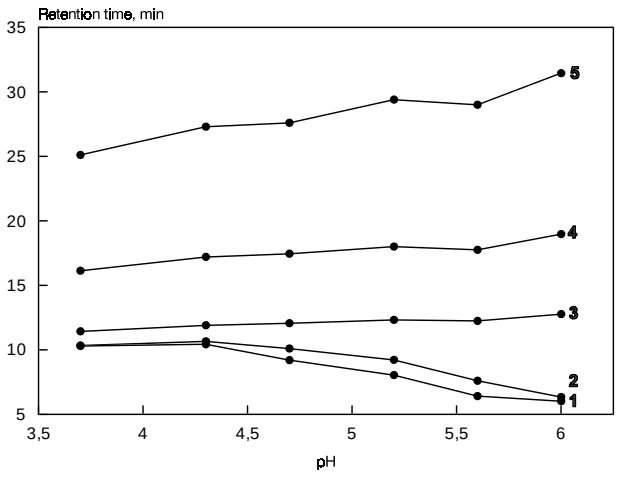

Fig. 2. Effect of mobile phase pH on the retention times of benzoate (1), sorbate (2), methyl-p-hydroxybenzoate (3), ethyl-p-hydroxybenzoate (4) and propyl-p-hydroxybenzoate (5). Mobile phase: 5 mmol/l acetic acid, 40% (v/v) CH_3CN

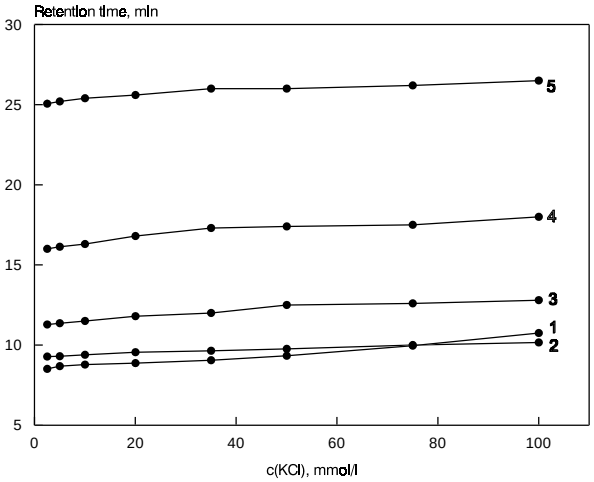

Fig. 3. Effect of KCl concentration in the mobile phase on the retention times of benzoate (1), sorbate (2), methyl-p-hydroxybenzoate (3), ethyl-p-hydroxybenzoate (4) and propyl-p-hydroxybenzoate (5). Mobile phase: 5 mmol/l acetic acid, 40% (v/v) CH_3CN, pH 5.0

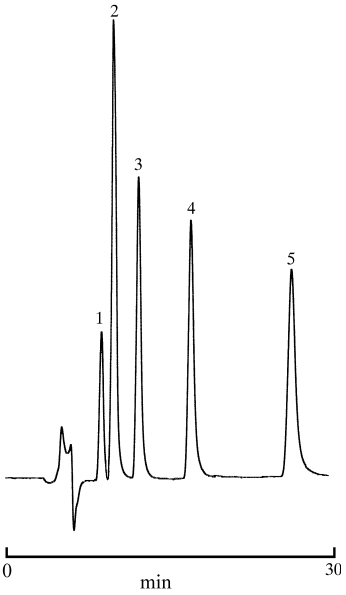

Fig. 4. Chromatogram of a standard solution under optimum conditions. Mobile phase: 5 mmol/l acetic acid, 40% (v/v) CH_3CN, pH 5.0. Peaks: *1* – benzoate, *2* – sorbate, *3* – methyl–p-hydroxybenzoate, *4* – ethyl-p-hydroxybenzoate, *5* – propyl-p-hydroxybenzoate

a reduced retention time. Moreover, the net charge and, consequently, the polarity of slightly stronger benzoic acid begin to increase at lower pH than that of sorbic acid, causing a significant improvement in the resolution of these analytes. In the pH range studied, MeHB, EtHB and PrHB existed in the molecular forms (pK^a~8.4). On this account, the varying of pH had but a little effect on their retention. The best separation was obtained at pH 5.0.

Another possible approach to improving the resolution between SB and PS was to increase the ionic strength of the mobile phase. Figure 3 displays the results for these experiments, showing that the best resolution of benzoate and sorbate was achieved between 0 and 20 mmol/l KCl concentrations.

The HPLC system was also optimized for peak shape by varying the mobile phase flow rate from 0.1 to 0.6 ml/min. An optimum flow rate of 0.2 ml/min achieved a complete resolution with a minimal bandwidth and reasonable elution time. A representative chromatogram for a standard mixture is shown in Fig. 4. One can see that an excellent separation of all five preservatives was obtained in less than 30 min.

Table 1. **Calibration data and detection limits for the preservatives studied (n = 3)**

Analyte	Linearity range, mol/l	Equation of regression lines	Detection limit, mg/l
SB	$5 \cdot 10^{-5}$–$2 \cdot 10^{-3}$	$y = 0.093 + 1.75 \times 10^4 c$	2.8
PS	$1 \cdot 10^{-5}$–$2 \cdot 10^{-4}$	$y = 2.55 + 5.71 \times 10^5 c$	0.25
MeHB	$1 \cdot 10^{-5}$–$2 \cdot 10^{-4}$	$y = 2.57 + 4.00 \times 10^5 c$	0.46
EtHB	$1 \cdot 10^{-5}$–$2 \cdot 10^{-4}$	$y = 1.46 + 3.4 \times 10^5 c$	0.50
PrHB	$1 \cdot 10^{-5}$–$2 \cdot 10^{-4}$	$y = 2.33 + 4.41 \times 10^5 c$	0.54

The linearity of the calibration curves was measured by triplicate injections of standards at seven different concentration levels. As a criterion of linearity, deviation within 5% of the mean response factor was used. The detection limits were determined, based on 3 times the baseline noise. Table 1 summarizes the results of the calibration curves and detection limits for all five analytes. A significantly higher detection limit for benzoate is caused by its poorer absorptivity at 254 nm. The detection limits achieved by the proposed method were suitable for beverage samples, and further optimization was not performed.

To evaluate the proposed method for real samples, it was applied for determination of preservatives in beverages. Figure 5 shows an electropherogram obtained for a 1:10 diluted beverage "Natūralūs gėrimai" sample. One can see that the matrix components do not interfere with the determination of preservatives. A recovery study was carried out with three different samples. The results are given in Table 2 and show that the recoveries in all the cases were within the range 100 ± 5%. The analysis does not require any preliminary treatment of the samples, except filtration and appropriate dilution.

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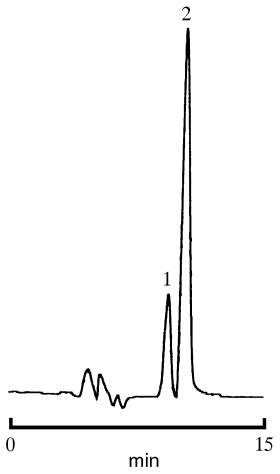

Fig. 5. Chromatogram of 1:10 diluted beverage "Natūralūs gėrimai" sample. Peaks: *1* – benzoate, *2* – sorbate. Other conditions as in Fig. 4

Table 2. **Determination of preservatives in beverages (n = 3)**

Sample	Analyte	Found, g/l	Added, g/l	Found total, g/l	Recovery, %
Sprite	SB	0.085	0.05	0.142	105
			0.10	0.185	100
Fanta	SB	0.108	0.05	0.155	98
			0.10	0.216	104
Natūralūs gėrimai	SB	0.062	0.05	0.108	96
			0.10	0.159	98
	PS	0.071	0.05	0.116	96
			0.10	0.174	102

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KONSERVANTŲ NUSTATYMAS GĖRIMUOSE IZOKRATINĖS EFEKTYVIOSIOS SKYSČIŲ CHROMATOGRAFIJOS METODU

S a n t r a u k a

Optimizuotos penkių konservantų (benzoato, sorbato, metil, etil ir propil p-hidroksibenzenkarboksirūgšties esterių) atskyrimo ir nustatymo izokratinės efektyviosios skysčių chromatografijos metodu sąlygos: kolonėlė – 150 × 3 mm Separon SGX C_{18} (5 μm); judrioji fazė – 5 mmol/l acetatinis buferis, 40% (v/v) CH_3CN, pH 5,0; UV detektavimas esant 254 nm bangos ilgiui. Išmatuotos pagrindinės analizinės charakteristikos: kalibracinės kreivės yra tiesinės koncentracijų intervale $1 \cdot 10^{-5}$–$2 \cdot 10^{-4}$ mol/l sorbatui ir metil, etil ir propil p-hidroksibenzenkarboksirūgšties esteriams bei $5 \cdot 10^{-5}$–$2 \cdot 10^{-3}$ mol/l benzoatui; aptikimo ribos yra lygios 2,8 mg/l benzoatui, 0,25 mg/l sorbatui, 0,46 mg/l, 0,50 mg/l ir 0,54 mg/l atitinkamai metil, etil ir propil p-hidroksibenzenkarboksirūgšties esteriams. Metodas pritaikytas nustatyti konservantams nealkoholiniuose gėrimuose.