

## Simultaneous Determination of Preservatives (Sodium Benzoate and Potassium Sorbate) in Soft Drinks and Herbal Extracts Using High-Performance Liquid Chromatography (HPLC)

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### Abstract

**Background:** Chemical preservation has become an increasingly important practice in modern food technology and herbal medicinal products with the increase in production of processed and convenience products.

**Objective:** In the present study, a reversed-phased HPLC method for simultaneous determination of the preservatives sodium benzoate (SB) and potassium sorbate (PS) is described.

**Methods:** The separation of SB and PS were performed on the C<sub>18</sub>- column and acetonitrile – ammonium acetate buffer as mobile phase. The detector wavelength was set at 254 nm.

**Results:** Separation of the two components (SB and PB) was achieved in less than 6 min. Analytical characteristics of the separation such as limit of detection, limit of quantification, accuracy, precision and reproducibility were evaluated. The range of preservatives found were from not detected (nd) — 2477 mg l<sup>-1</sup>, nd — 328 mg l<sup>-1</sup> for SB, PS respectively.

**Conclusion:** In This study, has shown that the concentration of SB and PS in the soft drink samples is higher that ADI even for normal consumers, based on maximum limits specified in national standards and on model diets.

**Keywords:** Preservatives, Sodium benzoate, Potassium sorbate, Soft –drinks, Herbal extracts

## Introduction

Chemical preservation has become an increasingly important practice in modern food technology and herbal medicinal products with the increase in production of processed and convenience products. These preservatives are deliberately added to stop or delay nutritional and pharmaceutical product losses due to microbiological, enzymatic or chemical changes and thus increasing its shelf life. Benzoic acid (E210), sorbic acid (E200) and their corresponding salts are generally effective to control mold and inhibit yeast growth, and against a wide range of bacterial attack [1 – 5].

The analytical determination of these preservatives is not only important for quality assurance purposes but also for consumer interest and protection. The most common analytical method for the determination of benzoic acid (BA) and sorbic acid (SA) or sodium benzoate (E211) and potassium sorbate (E202) has been reversed-phase HPLC [2 – 6, 8 – 16], although other analytical methods such as TLC [8], capillary Electrophoresis [7, 13] and gas chromatography [14] have also been reported. Such a method is important as there seem to be an increasing trend in using combination of preservatives, in soft drinks and herbal extracts. Here we report on a simplified procedure followed by HPLC separation of a mixture of sodium benzoate (E211) and potassium sorbate (E202). The method was applied to the analysis of these preservatives in 50 soft drinks and 55 herbal extracts.

## Experimental

### Chemicals

Sodium benzoate (>99%), potassium sorbate (>99%), acetic acid glacial and

methanol were obtained from Merck (Darmstadt, Germany). Acetonitrile was obtained from Caledon (Georgetown, Canada). Ammonium acetate was obtained from Mallinckrodt chemical works (st. Louis). The water used for preparing the buffer and the standard solutions by the UHQ-II-MK3 water purification system of Millipore (High Wycombe, Buck, UK).

### Apparatus

The chromatographic analysis was carried out in a Dionex high-performance liquid chromatograph (München, Germany and Sunnyvale, CA, USA) equipped with a Dionex P680 Pump and a Dionex rodyne valve injector. The analytical column which operates at room temperature was ACE C<sub>18</sub>-A<sub>3681</sub>, 250×4.6mm ID from Dionex, and the analysis involving UV detection was performed in a Dionex UVD 170U/340U UV/Vis absorbance detector. The Chromeleon (version 6.60, 2002, Dionex) software was used to control the system.

### Chromatographic conditions

The mobile phase contains a mixture of acetonitrile–ammonium acetate buffer adjusted to (40: 60). The buffer was prepared by dissolving 3.84 g of ammonium acetate in one liter of water and adjusting the pH to 4.4 with acetic acid. Before use, the effluent was filtered through a 0.45-mm membrane filter and degassed in an ultrasonic bath. The separation was achieved with isocratic elution at a flow-rate of 0.8 ml min and 50 µl of sample were injected into the chromatographic system.

### Calibration curve of standard solutions

Mixed standards containing 1, 2, 5, 10, 20 and 40 mg l<sup>-1</sup> of SB and 0.1, 0.2, 0.5, 1, 2, and 4 mg l<sup>-1</sup> of PS was prepared. 50 µl of each standard was injected. The peak areas were

measured and those of analytes ( $y$ ) were plotted against the respective concentration ( $\text{mg l}^{-1}$ ) of SB and PS ( $x$ ). Least square linear regression analysis was used to determine the slope,  $y$ -intercept and the correlation coefficients of the standard plots [16].

### Preparation of sample

The soft drink and herbal extract samples were filtered through a  $0.45\text{-}\mu\text{m}$  membrane. If the concentration of the preservative in the beverage and herbal extract are higher than the largest one used to build the calibration curve, the drink and extract samples were diluted in water.

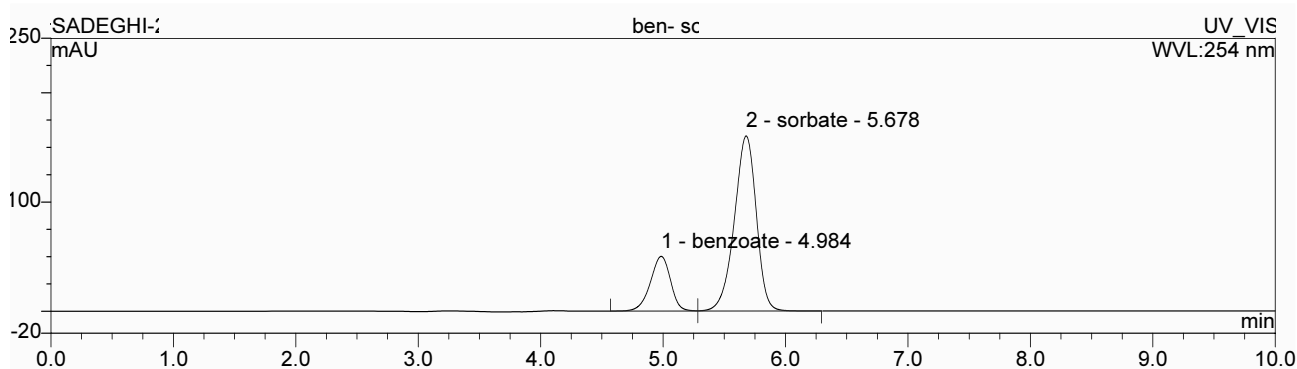
### Samples

This study was performed on 50 soft drinks and 55 herbal extracts. 50 soft drink samples were purchased from supermarkets, 55 herbal extract samples were obtained from two companies, Tehran, Iran.

## Results

Fig. 1 illustrates the chromatogram of a standard solution, which contains  $40\text{ mg l}^{-1}$  of sodium benzoate and  $4\text{ mg l}^{-1}$  of potassium sorbate; it was obtained with the experimental conditions indicated in section 2.3. The retention times for SB and PS are about 4.9 and 5.6 min respectively. The elution order is (1) sodium benzoate (retention time, 4.98 min) and (2) potassium sorbate (retention time, 5.67 min). Values found in the separation and the resolution of the column, indicate that the analytical method proposed in this work completely separates the analytes.

The limit of detection (LOD) is defined as the smallest peak detected with a signal height three times that of the baseline while the limit of quantization (LOQ) referred to the lowest level of analyte which can be determined with an acceptable degree of confidence [17]. Important analytical characteristics of the method are summarized in Table 1.



**Fig. 1 - Illustrates the chromatogram of a standard solution, which contains  $40\text{ mg l}^{-1}$  of Sodium benzoate and  $4\text{ mg l}^{-1}$  of Potassium sorbate**

**Table 1- Analytical characteristics of HPLC method**

Parameter	Preservative	
	SB	PS
Limit of detection ( $\text{mg l}^{-1}$ )	0.05	0.005
Limit of quantification ( $\text{mg l}^{-1}$ )	0.1	0.01

SB: Sodium benzoate; PS: Potassium sorbate

The reliability of the chromatographic method was tested for the determination of the same standard mixtures that was stored in a refrigerator, but analyzed about a 1-month period. The results are shown in Table 2.

An R.S.D. of less than 4 % was found, which not only indicate the high reproducibility of the method but also indicates that these preservatives are stable for at least 1 month when stored in refrigerator.

Reproducibility was determined by standard solutions of SB and PS at concentration levels of 1, 0.5 mg l<sup>-1</sup> for, SB and at concentration levels of 0.1, 0.05 and 0.01 mg l<sup>-1</sup> for PS and analyzed accordingly to the method. Relative standard deviation

(R.S.D.) data are presented in Table 3. Precision calculated as RSD was <4.5%.

Peak identification of the preservatives in various samples was based on the comparison between the retention times of standard compounds and was confirmed by spiking known standard compounds to the sample. Quantification was based on the external standard method using calibration curves fitted by linear regression analysis. Chromatogram of 2 preservative-positive sample is shown in Fig. 2, 3 and 4.

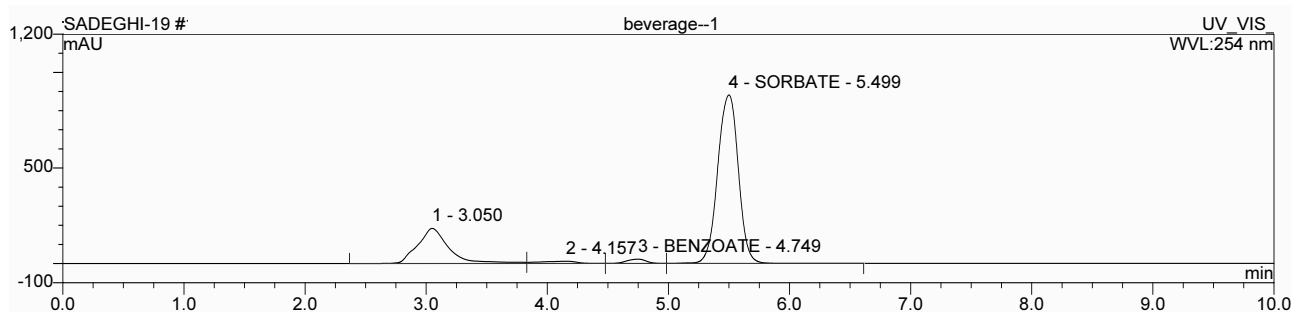
The average concentrations of sodium benzoate and potassium sorbate in soft drinks and extracts were determined and given in Table 4 and 5.

**Table 2 - Intraday reproducibility on the determination of standard mixtures of SB and PS Intra day (n=6)**

Concentration (mg l <sup>-1</sup> )	SB		PS	
	Concentration mean ± SD	RSD (%), Intra day (n=6)	Concentration mean ± SD	RSD (%), Intra day (n=6)
10	11.02 ± 0.40	3.63	10.71 ± 0.20	1.90
20	20.60 ± 0.29	1.41	20.07 ± 0.21	1.04
40	39.44 ± 0.20	0.51	39.79 ± 0.09	0.23

**Table 3 - Precision for preservative of standard solutions (n=5)**

SB			PS		
Amount prepared in water (mg l <sup>-1</sup> )	mean ± SD	RSD (%) (n=5)	Amount prepared in water (mg l <sup>-1</sup> )	mean ± SD	RSD (%) (n=5)
1	1 ± 0.03	3	0.1	0.11 ± 0.002	1.8
0.5	0.49 ± 0.02	4.1	0.05	0.053 ± 0.0006	1.1
			0.01	0.015 ± 0.0001	0.7



**Fig. 2 - Chromatogram carbonated multi fruit beverage**

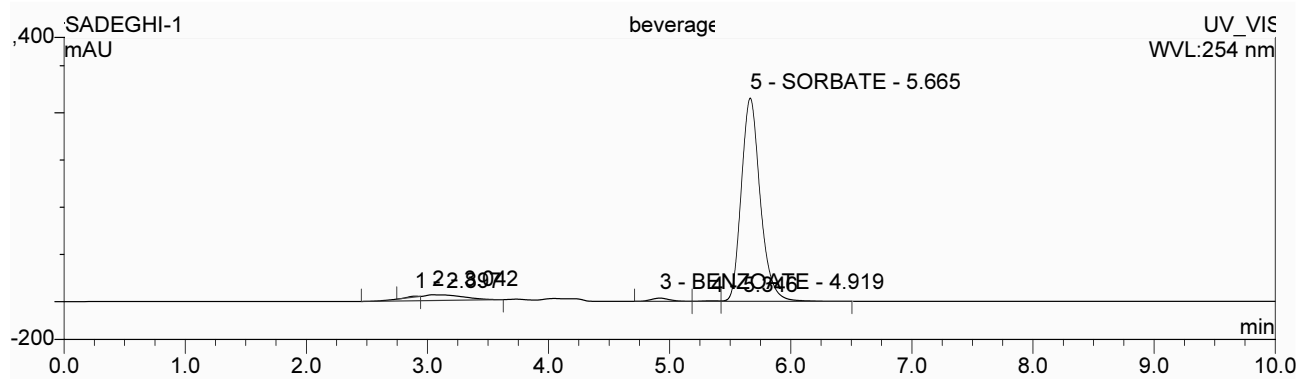


Fig. 3 - Chromatogram carbonated sour cherry beverage

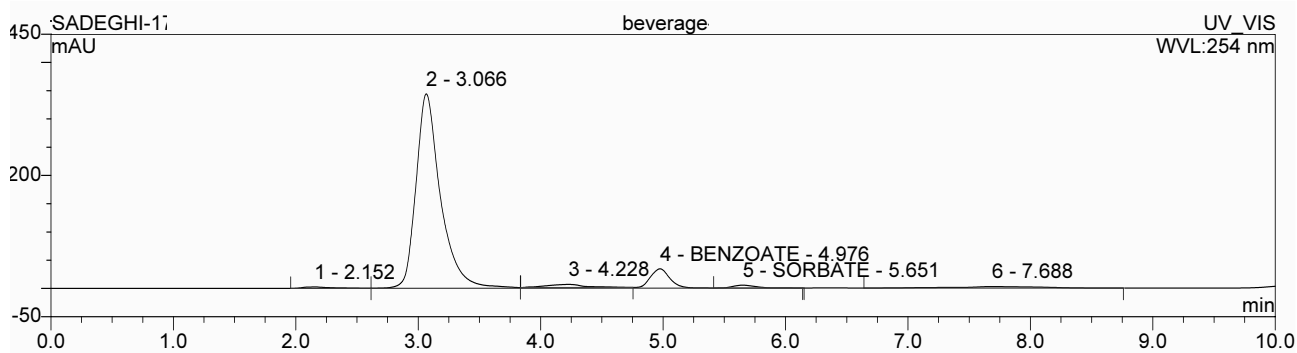


Fig. 4 - Chromatogram orange extract

Table 4 - Determination of Sodium benzoate and Potassium sorbate in soft drinks and extracts (n=25)

Soft drinks	SB		PS	
	Concentration mean ± SD (mg l <sup>-1</sup> )	RSD(%), n=25	Concentration mean ± SD (mg l <sup>-1</sup> )	RSD(%), n=25
Carbonated Lemon Beverage	163.8 ± 3.6	2.2	263.8 ± 5.7	2.2
<b>Extracts <sup>c</sup></b>				
Orange	2312.7 ± 174.9	7.5	Not detected	-

C: from company B

**Table 5 - Determination of Sodium benzoate and Potassium sorbate in soft drinks and extracts (n=5)**

Soft drinks	SB		PS	
	Concentration <sup>a</sup> mean ± SD (mg l <sup>-1</sup> )	RSD (%), n=5	Concentration <sup>a</sup> mean ± SD (mg l <sup>-1</sup> )	RSD (%), n=5
Carbonated sour cherry Beverage	131.9 ± 2.6	2	257.8 ± 5.3	2
Carbonated apple Beverage	164 ± 3.2	1.9	316.6 ± 6.8	2.1
Carbonated cola Beverage	3.9 ± 0.1	2.6	Not detected	-
Carbonated multi fruit Beverage	163.9 ± 4.4	2.7	206.2 ± 3.5	1.7
Carbonated orange Beverage	103.7 ± 0.7	0.7	1.2 ± 0.04	3.3
<b>Extracts<sup>b</sup></b>				
Orange 1	1394.3 ± 20.8	1.5	2.4 ± 0.2	8.3
Orange 2	1555.5 ± 55.3	3.6	6.2 ± 0.5	8.1
Orange 3	1212.6 ± 46.9	3.9	6.5 ± 0.3	4.6
Pine apple 1	1549.6 ± 16.6	1.1	45.2 ± 0.5	1.1
Pine apple 2	1491.6 ± 42.9	2.9	41.9 ± 1.7	4
Pine apple 3	1326.1 ± 36	2.7	33.8 ± 1.5	4.4

a: average of five concentrations

b: from company A

## Discussion

Many of the reported methods use complicated and labor-intensive pre-treatment procedures such as steam distillation multiple-steps and solid-phase extractions [7, 8, 13, 14]. Comparing to the previous methods [2, 6, 8 and 16], the presented analysis method simplifies considerably the analysis, reducing its cost and time (4-6 min) also encompasses higher level of sensitivity and lower level of LOD and LOQ.

A HPLC method has been developed and validated for the joint quantitative determination of sodium benzoate and potassium sorbate in soft drinks and extracts.

This information shows that the concentration of benzoates and sorbates in the soft drink samples is higher than ADI even for normal consumers, based on maximum limits specified in national standards and on model diets (WHO, 2000). Therefore, in order to reduce an overestimation, it is recommended that whenever possible the intake estimates of additives be refined by a more precise approach using analytical data. The use of sorbate and benzoate should be regulated and used only as a means to control yeast at concentrations not exceeding the actual need.

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