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Short communication

Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods

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Abstract

A rapid and reliable method is presented for the determination of the preservatives sodium benzoate and potassium sorbate in fruit juices, sodas, soy sauce, ketchup, peanut butter, cream cheese, and other foods. The procedure utilizes high-performance liquid chromatography (HPLC) followed by UV diode array detection for identification and quantitation of the two preservatives. Liquid samples were prepared by diluting 1 ml of the sample with 10 ml of an acetonitrile/ammonium acetate buffer solution. Samples of viscous or solid foods were prepared by blending the sample with the same buffer solution in a 1:5 ratio followed by a dilution identical to liquid samples. All samples were filtered to remove particulate matter prior to analysis. The HPLC determination of the preservatives was performed using a reversed-phase C_{18} column and UV detection at 225 nm for sodium benzoate and 255 nm potassium sorbate. The percentage of preservative in the sample was calculated by external standard using authentic sodium benzoate and potassium sorbate. Apple juice, apple sauce, soy sauce, and peanut butter, spiked at 0.10 and 0.050% for both sodium benzoate and potassium sorbate, yielded recoveries ranging from 82 to 96%. The method can detect 0.0010% (10 mg/l) of either preservative in a juice matrix.

Keywords: Food analysis; Fruit juices; Sodium benzoate; Potassium sorbate; Preservatives

1. Introduction

Processed foods, such as fruit juice, soda, soy sauce, cream cheese, and many other products are often sold with food additives to prevent spoilage. Typically, sodium benzoate and/or potassium sorbate are the preservatives that are used in juices to inhibit mold growth, prevent spoilage, and preserve freshness. 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 [1]. For instance, sodium benzoate may be used as a preservative in juices, however, its usage should not result in levels exceeding 0.1% in the beverage [1], while potassium sorbate may be used at levels of 0.1–0.2% [2].

Sodium benzoate is the sodium salt of benzoic acid and works well in acidic media to inhibit yeasts, molds, and bacterial growth. It is used in a variety of products, such as cosmetics and pharmaceuticals, but more commonly in foods like soda and fruit juice to

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preserve freshness [2–9]. Potassium sorbate is the potassium salt of sorbic acid; it is a polyunsaturated fatty acid used to inhibit mold growth in juices [2]. Previous studies have reported the determination of these preservatives using high-performance liquid chromatography (HPLC) in orange juice [3], jelly or jam and various whole fruits [4]. A major drawback to this method was the use of tetrahydrofuran in the mobile phase, which presents a problem with the plastics used in filtration, along with added expense for hazardous waste disposal [3]. The method reported for fruits, jellies and jams involves lengthy clean up steps prior to HPLC analysis [4]. Other methods have been reported for the determination of sodium benzoate and potassium sorbate using thin-layer chromatography [5] and liquid chromatography utilizing anionic resin coupled with solid-phase extraction to clean up the samples prior to analysis [6]. Both of these methods achieved good sensitivity, however, they also involve many steps, which can result in loss of the analytes during sample preparation.

The importance of food preservatives to consumers has always been a health safety issue. Consumers and scientists have raised questions about the necessity and safety of these preservatives, since in some cases allergic reactions to GRAS additives have been observed [2,7–9]. The role of preservatives has become more prominent with the increase in production of processed and convenience foods [9]. For these reasons, a rapid and accurate testing method is desired. In this study, a simple method that provides accurate results for sodium benzoate and/or potassium sorbate in foods is presented. The method uses a simple dilution for sample preparation followed by detection and determination by HPLC with UV diode array detection.

2. Experimental

2.1. Samples

Samples were obtained from food stores and restaurants and chosen to be representative of what a consumer would find in a market-basket study. Sample sizes ranged from 10 to 50 g. Each of the samples that were collected were tested for the two preservatives, sodium benzoate and potassium sorbate.

2.2. Reagents

Sodium benzoate, ACS grade, and potassium sorbate, ACS grade, were obtained from J.T. Baker, Phillipsburg, NJ, USA. Acetonitrile, HPLC grade, water, HPLC grade, ammonium acetate, ACS grade, and glacial acetic acid, ACS grade, were obtained from Fisher Scientific, Fairlawn, NJ, USA.

2.3. Filters

Nylon, 47 mm×0.45 μm, for mobile phase, and Nylon Acrodisk, 25 mm×0.45 μm for samples, were obtained from Gelman Sciences, Ann Arbor, MI, USA.

2.4. Mobile phase preparation

The mobile phase consisted of 90% ammonium acetate buffer with 10% HPLC-grade acetonitrile and was prepared in two steps.

(i) Step 1, acetate buffer. Exactly 0.30 g of ammonium acetate were dissolved in approximately 900 ml of HPLC grade water in a 1 l beaker. To this solution were added approximately 0.5 ml of glacial acetic acid and the pH adjusted to 4.2. The buffer solution was then transferred to 1 l volumetric flask, brought to volume, and filtered through a 47 mm× 0.45 μm nylon filter.

(ii) Step 2, completion. Exactly 900 ml of the acetate buffer solution were mixed with 100 ml of HPLC-grade acetonitrile. This was mixed, degassed, and used for solid sample extraction, liquid sample dilution, standard dilution, and the HPLC mobile phase. An ammonium acetate concentration of 0.30 g adjusted to a pH of 4.2 with concentrated acetic acid optimized the separation between sodium benzoate and potassium sorbate, see Discussion.

2.5. Standard preparation

Exactly 50.00 mg of either sodium benzoate or potassium sorbate were added to a 100.0 ml volumetric flask and brought to volume with HPLC-grade water. Dilutions of the stock solutions were made

into mobile phase, by addition of 0.50, 1.0, 2.0, 4.0, 10.0, and 20.0 ml of stock to a total volume of 100.0 ml mobile phase to yield 2.5, 5.0, 10.0, 20.0, 50.0 and 100.0 mg/l calibration standards, respectively. Standard solutions were not filtered prior to HPLC analysis.

2.6. Sample preparation

Beverage samples were prepared by diluting 1.0 ml of sample with 10.0 ml of mobile phase. Solid samples were prepared by blending 10 g of the sample with 50 ml of mobile phase for 2 min. The sample blend was then allowed to settle for 5 min and 1.0 ml of the supernatant liquid was diluted 1:10 with mobile phase just like the liquid samples. After dilution, all samples, solid or liquid, were filtered through a 25 mm×0.45 μm nylon Acrodisk filter to remove particulate matter from the samples and to prevent these particles from damaging the pumping or injection system, or clogging the column. For liquids, approximately 5–8 ml of sample solution were obtained, while for solid and viscous samples, about 1–2 ml of solution were obtained. These volumes, although small, were more than adequate for multiple HPLC analyses.

2.7. Apparatus

A high-performance liquid chromatograph from Hitachi, Tokyo, Japan, was used, equipped as follows: L-6300 and L-6000 dual pumps, AS-400 Auto sampler, L-3000 photodiode array detector and D-6100 data station. The HPLC operating mode was isocratic, the injection volume was 10 μl, and the column temperature 20°C (room temperature). The chromatography column was a Supelcosil LC-18, 25 cm×4.6 mm, 5 μm, Supelco, Bellefonte, PA, USA. Sample data collection was optimized to 12 min per sample with UV detection at 225 nm for sodium benzoate and 255 nm for potassium sorbate, with the detector wavelength switched between analytes during each run. The optimal flow rate (pump speed) was determined to be 0.8 ml/min. The concentration of preservative in the sample was calculated by 6 point external standard method. The method was linear over the concentration range from 2.5 mg/l to 100 mg/l, with correlation coefficients routinely ranging from 0.994 to 0.998 for either preservative. The method had a detection limit of 1.0 mg/l for both preservatives as a standard solution, 10.0 mg/l (0.0010%) for both preservatives in a juice matrix, and 50.0 mg/l (0.0050%) for both preservatives in a solid matrix, such as peanut butter.

Table 1
Products analyzed for sodium benzoate and potassium sorbate preservatives, number of each sample tested, label declaration, and range of results. A total of 65 products were tested; table lists only samples that contained a preservative

Product	Type	No. tested	No positive	Sodium benzoate declared	Sodium benzoate found (%)	Potassium sorbate declared	Potassium sorbate found (%)
Apple juice	Liquid	8	4	No	–	Yes	0.013–0.035
Grape juice	Liquid	8	4	No	–	Yes	0.023–0.045
Apple cider	Liquid	8	5	No	–	Yes	0.012–0.065
Cranberry juice	Liquid	4	4	No	0.0012–0.0038[a]	No	–
Soy sauce	Liquid	3	3	Yes	0.033–0.035	No	–
Cola soda	Liquid	5	4	Yes	0.020–0.025	No	–
Strawberry–cranberry jelly	Solid	1	1	No	0.0040[a]	No	–
Strawberry cream cheese	Solid	2	1	No	0.040[b]	No	0.041[b]
Peanut butter	Solid	3	1	No	0.032[b]	No	–
Fat free cream cheese	Solid	1	1	No	–	Yes	0.022
Chinese mustard	Viscous	1	1	Yes	0.057	No	–
Maple syrup	Viscous	1	1	No	0.020[b]	No	–

[a] Cranberry product with naturally occurring benzoic acid.
[b] Product in variance with label claim.

3. Results and discussion

The optimum separation and detection of sodium benzoate and potassium sorbate was achieved by regulating the pH and ammonium acetate concentration of the mobile phase. A significant change in the retention times of the analytes, along with changes in analyte resolution occurred when the pH of the mobile phase was changed. The HPLC system was optimized for compound separation from pH 4.0 to pH 4.5, and it was determined that an ammonium acetate concentration of 0.30 g/l and a pH of 4.2 optimized the separation between sodium benzoate and potassium sorbate.

The HPLC system was also optimized for peak shape by varying the flow rate from 0.5 to 1.0 ml/min. An optimum flow rate of 0.8 ml/min achieved 100% separation with minimal bandwidth (resolution, $R_s > 1.5$), and reasonable elution time. Representative chromatograms for a standard mixture and maple syrup sample (Table 1) are shown in Fig. 1. It can be seen, in chromatogram B, that a clean and interference-free baseline is typical for the sample matrixes tested in this study.

The greatest sensitivity of the method was obtained by detecting analytes sodium benzoate and potassium sorbate at their wavelength maximums of 225 nm and 255 nm, respectively. UV spectra for the analytes are shown in Fig. 2. Obtaining a spectral scan at the top of the analyte peak was also used to confirm the presence of the analytes. It was considered that a preservative was present in a sample when a retention time and simultaneous spectral λ_{max} match were obtained.

A total of 65 samples were tested in this study. Liquid products tested were apple, grape, grapefruit, tomato, orange, and pineapple juice, apple cider, and cranberry juice cocktail. Table 1 lists only the samples that were found to contain a preservative. The samples selected for this study were chosen to fall under three categories; products that declared the preservatives sodium benzoate and/or potassium sorbate on their labels, samples which directly stated ''no preservatives'' on the label, and samples without labels like ketchup and mayonnaise from restaurants and cafeterias having bulk dispensing devices. For a product to be in compliance of a label claim ''contains sodium benzoate and/or potassium sorbate'' the product upon testing must only contain that preservative [1]. Many of the products tested declared ''no preservatives'' on the label. These products were tested to determine if the products were in accordance with their label claims.

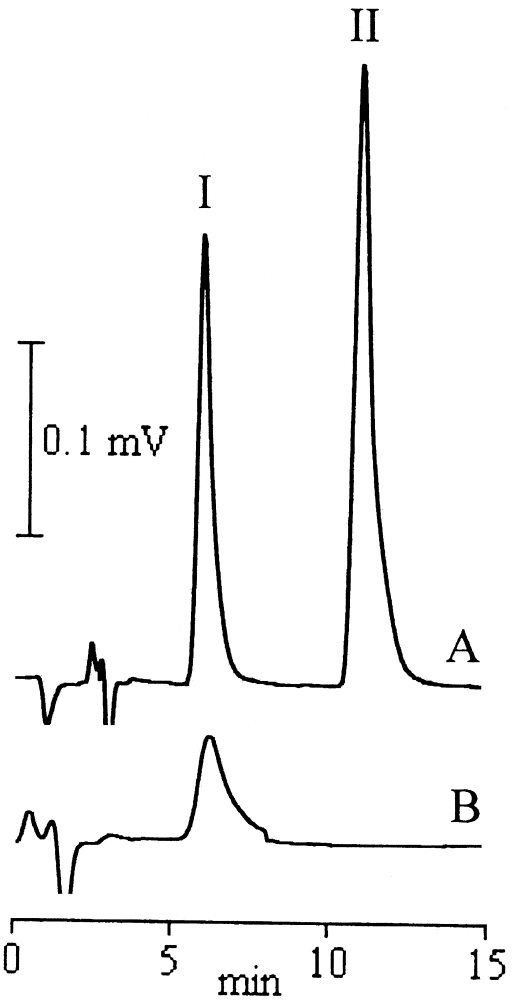

Fig. 1. HPLC chromatogram (A) of 0.050 ppm each of sodium benzoate (I), retention time 6.41 min, and potassium sorbate (II), retention time 10.17 min. Chromatogram (B) of maple syrup containing 0.020% sodium benzoate, see Table 1.

Of particular interest were juice beverages and products that contained cranberries or cranberry extract. Cranberries contain a natural amount of benzoic acid at approximately 0.0150%, when calculated as sodium benzoate [2,10]. Thus, all products

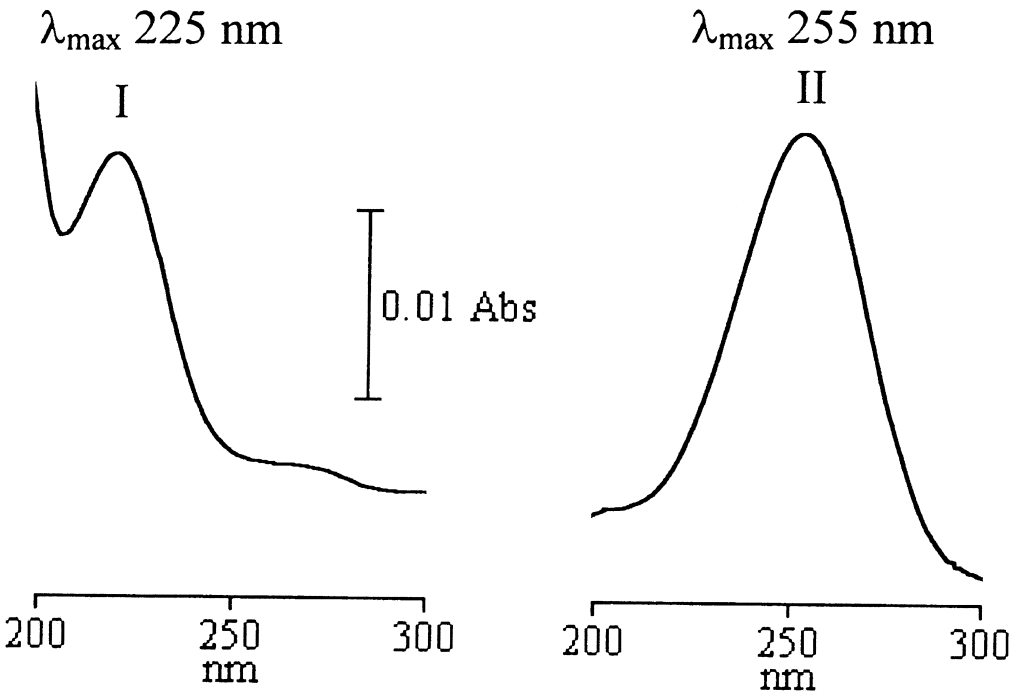

Fig. 2. Spectra of sodium benzoate (I), and potassium sorbate (II) from 200 nm to 300 nm, each at a concentration of 0.050 ppm.

that contain cranberries or cranberry extracts should have detectable amounts of benzoic acid. The levels of sodium benzoate vary depending on the amount of cranberry or extract in the product. Amounts of sodium benzoate in various cranberry products are listed in Table 1. Due to the natural occurrence of benzoic acid in cranberries, and in these juices and other cranberry products, it was concluded that these samples were not in violation of any labeling regulations.

Spiked samples were performed to validate the procedure. Samples were selected from both the ''no preservative'' category and the declared category. Tables 2 and 3 list the recoveries of spiked samples. The products tested were spiked at 0.1% (1000 mg/l) and 0.05% (500 mg/l) for sodium benzoate and potassium sorbate and yielded recoveries ranging from 83 to 96% for sodium benzoate and from 82 to 93% for potassium sorbate.

Most of the products tested, 96.4%, were in compliance with their labels, <0.1% (<1000 mg/l), while only 4.6% of the products tested deviated from

Table 2
Recovery of sodium benzoate from spiked samples with 3 replicate determinations

Sample matrix	Amount in sample before spike	Spike amount	Amount in sample after spike	Recovery (average) (%)
Apple juice	<0.0010	0.10	0.094–0.098	96
Apple juice	<0.0010	0.050	0.045–0.051	96
Apple sauce	<0.0010	0.10	0.082–0.089	86
Soy sauce	0.033–0.035	0.10	0.11–0.13	90
Peanut butter	<0.0050	0.050	0.040–0.043	83

Table 3
Recovery of potassium sorbate from spiked samples with 3 replicate determinations

Sample matrix	Amount in sample before spike	Spike amount	Amount in sample after spike	Recovery (average) (%)
Apple juice	<0.0010	0.050	0.038–0.044	82
Apple juice	0.054–0.056	0.10	0.13–0.14	87
Apple sauce	<0.0010	0.10	0.088–0.097	93
Soy sauce	<0.0010	0.10	0.089–0.096	93
Peanut butter	<0.0050	0.050	0.044–0.048	92

their label claims. In conclusion this method provides fast and convenient sample preparation that can be used to monitor the occurrence of these preservatives in samples found in the market place.

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