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Simultaneous Determination of Sodium Benzoate and Potassium Sorbate Preservatives in Foodstuffs Using High Performance Liquid Chromatography

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A reversed-phase high-performance liquid chromatography (HPLC) analysis without preextraction was carried out for rapid determination of sodium benzoate and potassium sorbate which were added as preservatives to different samples commercially available in Syrian Arab Republic local markets such as tomato sauce, soft drink, beverages and energy drink. Separation and determination of sodium benzoate and potassium sorbate were performed on a 5 μm Purospher® STAR RP-18 column (25 cm × 4.6 mm), using caffeine as an internal standard (0.04 mg/mL), with UV detection at 235 nm. The column temperature was 40 °C. Mobile phase was (acetate buffer and methanol) with percentage (25:75), at a flow rate 1.2 mL min^{-1}, The injection volume was 20 μL. The two elution peaks were completely separated with a good resolution. This method has offered a determination of sodium benzoate and potassium sorbate directly with a high accuracy and authenticity for the results without extraction need.

Key Words: Preservatives, High-performance liquid chromatography, Sodium benzoate, Potassium sorbate.

INTRODUCTION

Food preservatives play a vital role in the modern food industry and generally use for maintaining food quality and its characteristics as well as promoting food safety and preventing nutritional losses from chemical alterations and preserving the products during their shelf life which prevent or inhibit the growth of bacteria, fungi and yeasts as well as preventing spoilage and decay. Food preservatives have been the subject of interest among consumers, health professionals, commercial and industrial agencies, alike because they are widely consumed in the diet by most segments of the population and can exert adverse health effects, especially for children and pregnant women. Food preservatives must be in allowable safety limits, which must not exceed the maximum allowed concentration of sodium benzoate and potassium sorbate 0.1 and 0.2 %, respectively[1].

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There are many analytical methods used in qualitative and quantitative detection of sodium benzoate and potassium sorbate in foods such as UV-vis spectrophotometry, HPLC methods, gas chromatography, capillary electrophoresis and polarography[2].

Benzoate was determined in soft drinks and fruit juices, lemon juice and soy sauce by using ion chromatography (IC)[3]. Methyl ethyl and propyl paraben have been identified in different beverages using HPLC coupled with UV detector and DAD detector[4,5]. Gas chromatography and solid-phase extraction were used to identify some preservatives such as benzoic acid and sorbic acid, as well as methyl, ethyl and propyl paraben in soft drinks, sauces and jams[6,7]. Benzoic acid and sorbic acid were identified in soft drinks using capillary electrophoresis with UV and visible detector[8]. Benzoic acid and sorbic acid and esters of *p*-hydroxy benzoate (methyl-propyl) were identified in the juices, soft drinks, cheese and meat products by using RP- HPLC with UV detector, mass spectroscopy detector (MS) and photo diode array detector[9-12]. Benzoic acid and sorbic acid were identified in Chinese meat using gas chromatography with flame ionization detector. The level of sorbic acid and benzoic acid in these samples ranged from $< 10^{-2}$ and $< 10^{-9}$ ppm, respectively[13]. Sodium benzoate was identified in different beverage samples commercially available in Riyadh local markets using spectrophotometry[14]. As well as benzoic acid levels were identified in the milk produced in China using HPLC with photo diode array detector[15].

EXPERIMENTAL

Different brands of tomato sauce, soft drinks, beverages and energy drinks were selected for this study.

Analytical separation was carried out on a (Knauer) high-performance liquid chromatography (HPLC) with detector (UV spectral photometer S-2500), (Purospher® STAR) RP-18 columns dimensions (250 mm × 4.6 mm)-5 µm (Merck), JetStream column Oven, ultrasonic bath (Daihan), analytical balance 0.1 mg (Pricesa), Germany digital pipettes (Isolab). The solvents and materials were used analytical grade: HPLC water and methanol (Isolab), sodium benzoate, potassium sorbate, ammonium acetate and caffeine (Merck), HPLC glacial acetic acid (Panreac).

Samples preparation: Five batches from each food product (tomato sauce, soft drink, beverages and energy drink) were studied. Liquid samples were prepared by transferring 5.0 mL of sample into 25 mL volumetric flask, the volume was completed to 25 mL using HPLC-grade water. Semi-solid samples (tomato sauce) were prepared by mixing 10 g of the sample with 50 mL of HPLC-grade water, then the mixture transferred into 100 mL volumetric flask, the volume was maintained to 100 mL using HPLC-grade water and placed in the ultrasonic bath for 15 min. The solution was filtered and transferred 12.5 mL of liquid filtrate (after neglecting the first 10 mL), 1 mL of internal standard solution to 25 mL volumetric flask, the volume was completed to 25 mL using HPLC-grade water. Final sample solution

filtered through a 0.45 μm and degassed by ultrasonication and then the samples were injected in the chromatograph.

Standard preparation: Standards stock solutions of both sodium benzoate and potassium sorbate were prepared at 1000 mg/L with HPLC-grade water. Combine 2, 4, 6, 8, and 10 mL of each stock standard into 100 mL volumetric flasks and dilute to final volume with HPLC-grade water. The concentration range of the standard curves was 0.02-0.10 mg/mL (Fig. 1).

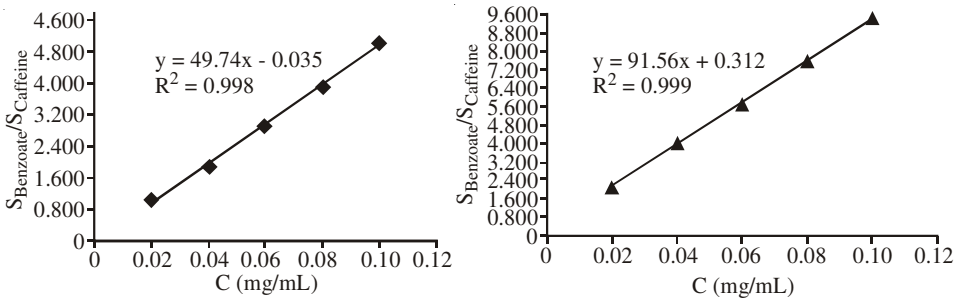

Fig. 1. Linearity graphs of Na benzoate and K sorbate

Mobile phase preparation: The mobile phase consisted of 25 % ammonium acetate buffer and 75 % HPLC-grade methanol. Mobile phase pH was adjusted to 4.4 by glacial acetic acid. All mobile phase were filtered through a 0.45 μm membrane filter and degassed by ultrasonication.

Chromatographic conditions: Isocratic mobile phase consisted of a mixture of acetic buffer (pH = 4.4) and methanol in the ratio 25:75 (v/v). Purospher® STAR RP-18, 250 mm × 4.6 mm-5 μm column, flow rate of 1.2 mL/min, variable UV detector was set at 235 nm, column temperature at 40 °C and injected sample volume was 20 μL.

RESULTS AND DISCUSSION

Determination of standard solutions for sodium benzoate and potassium sorbate: It was separated and identified the components of a mixture consisting of sodium benzoate and potassium sorbate with caffeine as internal standard (0.04 mg/mL) at five different standard concentrations and every concentration was injected five times, (Fig. 2).

Effect of mobile phase pH on the retention times t_R of sodium benzoate and potassium sorbate: The effect of mobile phase pH acidified by acetic acid in range (pH = 3.0-7.5) was studied by increasing the pH value due to deprotonation of both sodium benzoate and potassium sorbate increased and drawing the curve representing the relation between the retention times of both in terms of pH, the relation $t_R = f(pH)$ using the same mobile phase component ratios and the injected quantity. It was shown that the ionization suppression was impossible for both pre-

servative with decrease in pH and this required the work at constant pH, using a convenient buffer in the range between 3.5-4.5 to perform an accuracy mixture separation (Fig. 3).

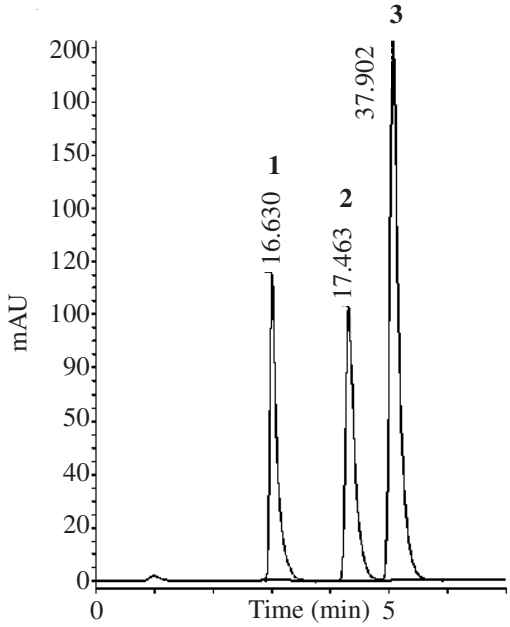

Fig. 2. Chromatogram of standard solutions under optimum conditions. Peaks: 1-caffeine, 2-Na benzoate, 3-K sorbate

Effect of changing temperature on the separation of potassium sorbate and sodium benzoate: The effects of the column temperature on the degree of separation peaks for both potassium sorbate and sodium benzoate in the range between 25 and 50 °C was studied and has been shown that the separation of potassium sorbate and sodium benzoate at 25 ºC was complete, but accompanied with long analysis time and band broadening, while at 50 °C was noted the interference of the two peaks, which necessitated selection of temperature 40 °C, where the completed separation with a suitable analysis time was *ca.* 6 min.

Peaks resolution for potassium sorbate and sodium benzoate R_s: Resolution R_s of potassium sorbate and sodium benzoate peaks was identified by changing the ratios of mobile phase components (methanol and acetate buffer) with constant mobile phase pH (pH = 4.4). It was concluded that the best ratio for the mobile phase components (25:75) and the best completed separation for all peaks begin at R_s = 1.4 (Fig. 4).

Separation of real food samples: The chromatographic separation was performed for sodium benzoate and (or) potassium sorbate in (tomato sauce, soft drink, beverages and energy drink) using the precedent chromatographic conditions. The total analysis time was less than 6 min with good resolution, peak shapes and minimal

tailing. It was determined sodium benzoate and potassium sorbate in all samples without any interference, peaks were completely separated and the resolutions were more than (1.4). The peaks of sodium benzoate and potassium sorbate in the samples were identified by comparing the retention time with that of the standards, as well as correlation coefficients and linear equations of peak areas and standard concentrations were used for the quantitative analysis, as it is shown in five representative samples Figs. (5-8).

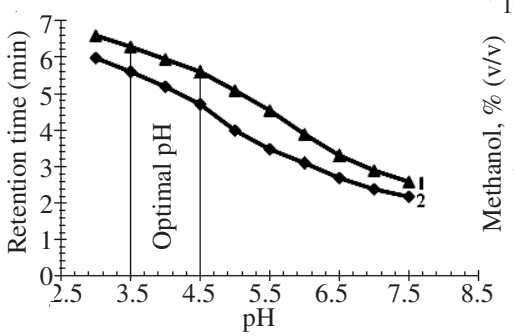

Fig. 3. Effect of mobile phase pH on the retention times of 1-K sorbate, 2-Na benzoate

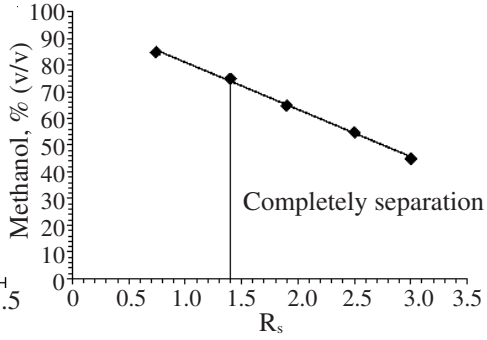

Fig. 4. Peaks resolution for K sorbate and Na benzoate in relation to methanol percentage

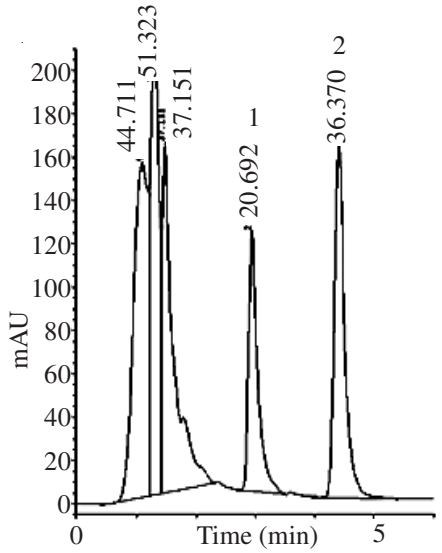

Fig. 5. Chromatogram of tomato Sauce "Saha" sample.1- caffeine, 2- Na benzoate, 3-K sorbate

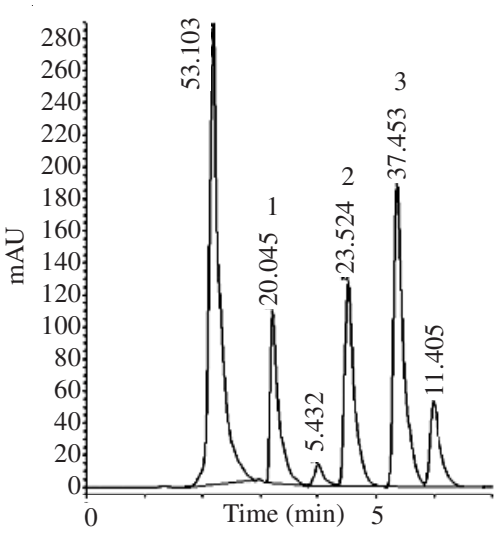

Fig. 6. Chromatogram of beverage "Original" sample, 1-caf feine, 2- sodium benzoate

Conclusion

The HPLC method with UV spectrophotometric detection on a 5 µm Purospher® STAR RP-18 column was developed for the determination sodium benzoate and

potassium sorbate in food samples, using caffeine as internal standard. The total analysis time was less than 6 min. Proposed high-performance liquid chromatography (HPLC) method is rapid, direct, accurate and precise for simultaneous determination of sodium benzoate and potassium sorbate in food samples and the matrix components do not interfere with the determination of preservatives in all samples.

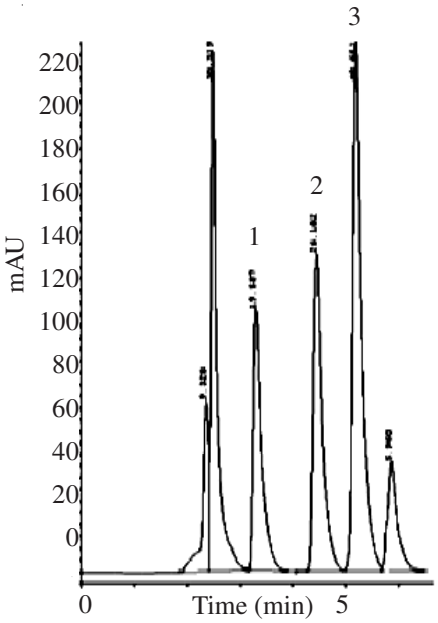

Fig. 7. Chromatogram of beverage sample, "Mirinda Orange" 1-caffeine, 2-Na benzoate, 3-K sorbate

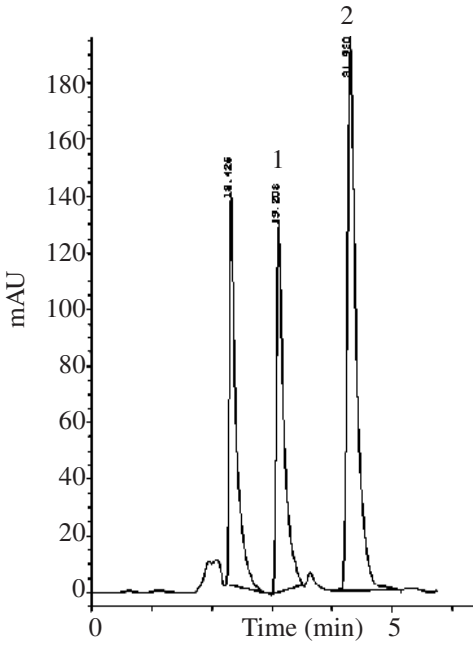

Fig. 8. Chromatogram of soft drink sample "7up"1- caffeine, 2- Na benzoate

The accuracy of the method was confirmed with an average recovery ranging between 93.8 and 98.7 %, 95.6 and 99 % of sodium benzoate and potassium sorbate, respectively.

According to the results of this study, although both preservatives are added to different foodstuffs in a wide range of concentrations, none of the samples presented levels below the range of antimicrobial activity[2].

Potassium sorbate and sodium benzoate was separated and identified directly without preextraction in different soft drink and beverages samples (Tables 1 and 2). The identified amount of preservatives in tomato sauce samples was in allowable safety limits and less than the authorized amounts, as well as a difference in amounts between different brands. While the beverages samples was in allowable safety limits and authorized amounts. In samples contain both potassium sorbate and sodium benzoate together, the added amounts of those preservative were in allowable safety limits and did not exceed the authorized limits.

TABLE-1
SUMMARIZED THE CONTENT OF PRESERVATIVES IN SEVERAL FOOD SAMPLES DISCUSSED ABOVE, THUS INDICATING THAT THIS METHOD PROVIDED GOOD QUANTITATIVE REPRODUCIBILITY

Food samples	Preservatives	[a]Concentration $\overline{X}$ (mg/mL) n = 5	RSD (%)
Orient	Sodium benzoate	0.172	1.19
Mountain dew	Sodium benzoate	0.173	1.41
Cheer up	Sodium benzoate	0.104	1.27
Ugarit	Sodium benzoate	0.139	1.22
Sprite	Sodium benzoate	0.171	1.71
Fanta orange	Sodium benzoate	0.354	1.39
Mirinda orange	Sodium benzoate	0.135	1.37
Mirinda green apple	Sodium benzoate	0.169	1.45
Canada dry apple	Sodium benzoate	0.104	1.88
7up	Sodium benzoate	0.173	1.47
Mirinda lemon	Sodium benzoate	0.132	1.02
Mandarin veneto	Sodium benzoate	0.429	1.17
Mirinda berry	Sodium benzoate	0.136	1.37
Original	Sodium benzoate	0.126	1.34
Rival	Sodium benzoate	0.131	1.53
Bison	Sodium benzoate	0.117	1.32
Pepsi diet	Sodium benzoate	0.151	1.41
Coca cola light	Sodium benzoate	0.150	1.38
Coca cola zero	Sodium benzoate	0.145	1.73
Mirinda orange	Potassium sorbate	0.133	1.66
Original	Potassium sorbate	0.107	1.15
Mandarin ice tea	Potassium sorbate	1.259	1.89

[a]: Average of five determinations.

TABLE-2
SUMMARIZED THE CONTENT OF PRESERVATIVES IN SEVERAL TOMATO SAUCE SAMPLES DISCUSSED ABOVE, THUS INDICATING THAT THIS METHOD PROVIDED GOOD QUANTITATIVE REPRODUCIBILITY

Food samples (Tomato sauce)	Preservatives	[a]Concentration $\overline{X}$ (mg/mL) n = 5	RSD (%)
Dura	Sodium benzoate	0.862	2.01
Gota flower	Sodium benzoate	0.451	1.21
Al Jabal Al Akhdar	Sodium benzoate	0.741	1.98
Rotana	Sodium benzoate	0.409	1.09
Saha	Sodium benzoate	0.712	1.91
Four Season	Sodium benzoate	0.732	1.96
Crystal	Sodium benzoate	0.843	1.62
Zamzam	Sodium benzoate	0.388	1.04
Alraffee	Sodium benzoate	0.584	1.57
Myas	Sodium benzoate	0.829	2.02

[a]: Average of five determinations.

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