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Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatography

Bahruddin Saad[a,*], Md. Fazlul Bari[a], Muhammad Idiris Saleh[a], Kamarudzaman Ahmad[b], Mohd. Khairuddin Mohd. Talib[b]

[a] *School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[b] *Food Quality Control Laboratory, Ministry of Health Malaysia, Bukit Kayu Hitam, Kedah, Malaysia*

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Abstract

A reversed-phased HPLC method that allows the separation and simultaneous determination of the preservatives benzoic (BA) and sorbic acids (SA), methyl- (MP) and propylparabens (PP) is described. The separations were effected by using an initial mobile phase of methanol–acetate buffer (pH 4.4) (35:65) to elute BA, SA and MP and changing the mobile phase composition to methanol–acetate buffer (pH 4.4) (50:50) thereafter. The detector wavelength was set at 254 nm. Under these conditions, separation of the four components was achieved in less than 23 min. Analytical characteristics of the separation such as limit of detection, limit of quantification, linear range and reproducibility were evaluated. The developed method was applied to the determination of 67 foodstuffs (mainly imported), comprising soft drinks, jams, sauces, canned fruits/vegetables, dried vegetables/fruits and others. The range of preservatives found were from not detected (nd)—1260, nd—1390, nd—44.8 and nd—221 mg kg^{-1} for BA, SA, MP and PP, respectively.

Keywords: Food analysis; Preservatives; Benzoic acid; Sorbic acid; Parabens

1. Introduction

Chemical preservation has become an increasingly important practice in modern food technology with the increase in production of processed and convenience foods. These preservatives are deliberately added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes and thus increasing its shelf life. Benzoic acid (BA) and sorbic acid (SA) are generally effective to control mold and inhibit yeast growth, and against a wide range of bacterial attack [1–5]. *P*-hydroxybenzoic esters (parabens) have been used as preservatives for over 70 years [6]. Methylparaben (MP) and propylparaben (PP) are the most commonly used parabens and are often used together since they have synergistic effects [7]. It had been found that the antimicrobial activities of the parabens seem to increase with increasing chain length. However, esters of longer alkyl chains are of limited applications due to their lower solubility in water [8].

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 BA and SA or the parabens has been reversed-phase HPLC [2–6,9–15], although other analytical methods such as TLC [9], capillary electrophoresis [8,14] and gas chromatography [15] have also been reported. Most of the reported methods are for the separation of benzoic and sorbic acid or amongst the parabens. However, chromatographic reports on the simultaneous determination of BA, SA and the parabens, especially in food items are scarce [5,11]. Such a method is important as there seem to be an increasing trend in using combination of preser-

* Corresponding author.
E-mail address: bahrud@usm.my (B. Saad).

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vatives, not only in the food industry but also in pharmaceutical formulations and cosmetic products [10]. Moreover, many of the reported methods use complicated and labor-intensive pre-treatment procedures such as steam distillation multiple-steps and solid-phase extractions. Here we report on a simplified methanol extraction procedure followed by HPLC separation of a mixture of benzoic acid, sorbic acid, methylparaben and propylparaben. The developed method was applied to the analysis of these preservatives in 67 different food samples.

2. Experimental

2.1. Chemicals and reagents

Chemicals and reagents used were obtained from the following sources: sodium hydroxide, acetic acid (99.8%), BA (99.5%), SA (99%), Fluka, Buchs, Switzerland; ammonium acetate (98%), Sigma (St. Louis, MO, USA); MP (99%), PP (99%), Aldrich (Steinheim, Germany); methanol (HPLC grade), Fisher (Loughborough, UK).

2.2. Preparation of sample

Solid food samples were finely ground prior to the extraction. About 1 g sample is accurately weighed in a screw-capped test tube. Twenty-five milliliters of methanol was added, and placed in a sonicator (ULTRAsonik Model 28X, Ney Dental, Yucaipa, California) that was maintained at 50 °C for 30 min. The test tube was next subjected to vortex mixing (KIKA Works, Model MS1, Malaysia) for 2 min. The contents were filtered through a 0.45-μm nylon membrane filter (Whatman, Maidstone, UK) and the clear filtrate was injected into the HPLC column. For concentrated samples, prior dilution with the mobile phase was done.

2.3. Chromatographic conditions

Analytical separation was carried out on a Jasco PV-1580 HPLC unit using a Supelco 516 C_{18} column (15 cm × 4.6 mm, 5 μm) at room temperature. The detector used was a Jasco UV-1570 UV–vis spectrophotometer set at 254 nm and the volume of sample injected was 20 μL. The aqueous phase was prepared by weighing 3.8 g ammonium acetate and dissolving in 1 L water and its pH adjusted to 4.4 using acetic acid. The mobile phase used was methanol–acetate buffer (pH 4.4) (35:65, v/v) for 9 min, after which it was changed to methanol–acetate buffer (pH 4.4) (50:50, v/v).

2.4. Food samples

A total of 67 food samples were purchased from supermarkets located in the northern states (Kedah and Perlis) of Peninsula Malaysia. The samples were categorized as: soft drinks (9), canned fruits/vegetables (19), jam/fruit jelly (11), sauces (15), dried fruits (8) and miscellaneous (5).

3. Results and discussion

The UV absorption spectrum of the preservatives are shown in Fig. 1. It can be anticipated that a small peak for BA will be obtained if the detector wavelength was fixed at 254 nm, while on the other hand, small peaks for SA, MP and PP will be obtained if the detector is set at 230 nm. Thus, in order to obtain maximum sensitivity, detecting at the respective maximum wavelengths of the preservatives can done (i.e., 230 nm for BA, 254 nm for the others). However, in this work, since the sensitivity of the BA was not an issue (legal limits on the order of 350 mg kg^{-1} or more), the detector wavelength was kept constant at 254 nm.

Under the stated experimental conditions, baseline resolution of the four components were achieved. The retention times for BA, SA, PP and MP are about 7.5, 8.5, 11.2 and 21.0 min, respectively (Fig. 2). The use of methanol–acetate buffer (35:65) was effective for the separation of BA, SA and MP, but PP was eluted too long. Attempts to shorten the elution time of PP by increasing the percentage of methanol greater than 50% was not successful as noisy baseline was obtained. Thus, the mobile phase was changed to methanol–acetate buffer (50:50) after the successful elution of BA, SA and MP using methanol–acetate buffer (35:65). The chromatographic analysis can be conveniently shortened using gradient elution, but this facility was not available to us during the course of the study.

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 quantitation (LOQ) refers to the lowest level of analyte which can be determined with an acceptable degree of confidence. LOQ value is often calculated as 10 times the signal height to the baseline. In our work, detection and quantitation limits were estimated by successively

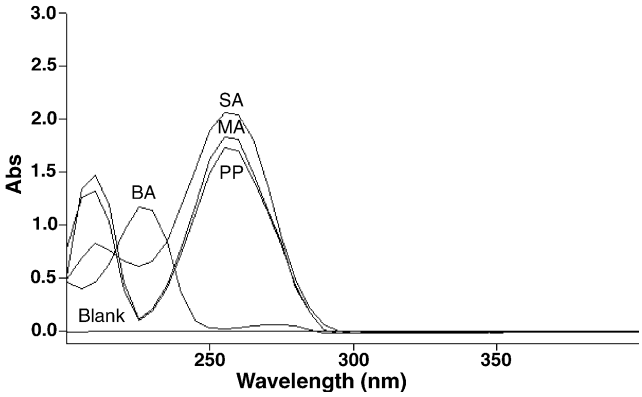

Fig. 1. UV spectrum of preservatives, mg L^{-1}: BA, 12.0; SA, 11.8; MP, 12.5; PP, 17.0.

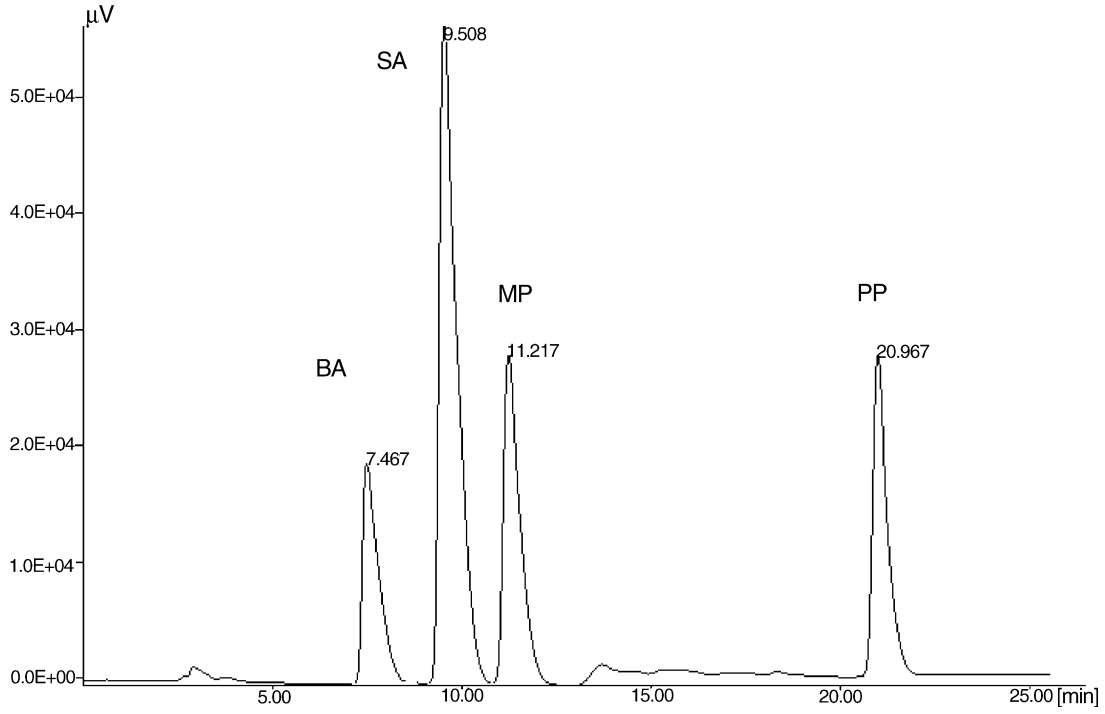

Fig. 2. Typical chromatogram of standard mixture of preservatives, mg L^{-1}: BA, 48.2; SA, MP and PP, 5.0 each.

decreasing the concentration of the prepared standards, down to the smallest detectable peak. This concentration was multiplied by 3 and 10 to obtain the detection and quantitation limits, respectively. Other important analytical characteristics of the method is summarized in Table 1.

The reliability of the chromatographic method was tested for the determination of the same standard mixtures that was stored in a refrigerator and covered by aluminium foil (to protect from light), but analyzed over a 3-month period. The results are shown in Table 2. An R.S.D. of less than 3% was found, which not only indicate the high reproducibility of the method but also indicates that these preservatives are stable for at least 3 months when stored in refrigerator and adequately protected from light.

In the development of pre-treatment procedure, due consideration was given to methods that are simple, cheap and, if possible, applicable to all types of food samples. The sample pre-treatment method that was used was tested by spiking with known quantities of preservatives to a diverse range of food samples, and analyzed using the HPLC method. Results are summarized in Table 3. It can be readily seen from this table that on the whole the pre-treatment procedures in combination with the HPLC method produce acceptable results as reflected by the average recoveries of 106, 104, 102 and 102% for BA, SA, MP and PP, respectively.

Peak identification of the preservatives in various foodstuffs was based on the comparison between the retention time 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. SA

Table 1
Analytical characteristics of HPLC method

Parameter	Preservative			
	BA	SA	MP	PP
Limit of detection (mg L^{-1})	0.5	0.1	0.3	0.1
Limit of quantification (mg L^{-1})	1.7	0.3	1.0	0.3
Linear range (mg L^{-1})	5.0–120	1.0–75	3.0–100	1.0–75

BA: benzoic acid; SA: sorbic acid; MP: methylparaben; PP: propylparaben.

Table 2
Interday reproducibility on the determination of standard mixtures of BA (96.3 mg L^{-1}) and 10.0 mg L^{-1} each of SA, MP and PP

Date	Preservative (mg L^{-1})			
	BA	SA	MP	PP
22/November/2003	97.7	10.3	10.2	10.6
23/November/2003	104	10.5	10.6	10.2
3/December/2003	97.5	10.1	10.0	9.99
7/February/2004	95.1	10.0	10.1	9.92
13/February/2004	100	10.3	10.3	10.2
20/February/2004	98.4	10.6	9.94	9.89
25/February/2004	102	9.87	10.6	9.96
28/February/2004	97.4	9.95	10.5	10.1
	98.8	10.7	9.91	10.2
Mean	99.0	10.3	10.2	10.1
Standard deviation	2.73	0.30	0.27	0.21
R.S.D. (%)	2.76	2.90	2.64	2.01

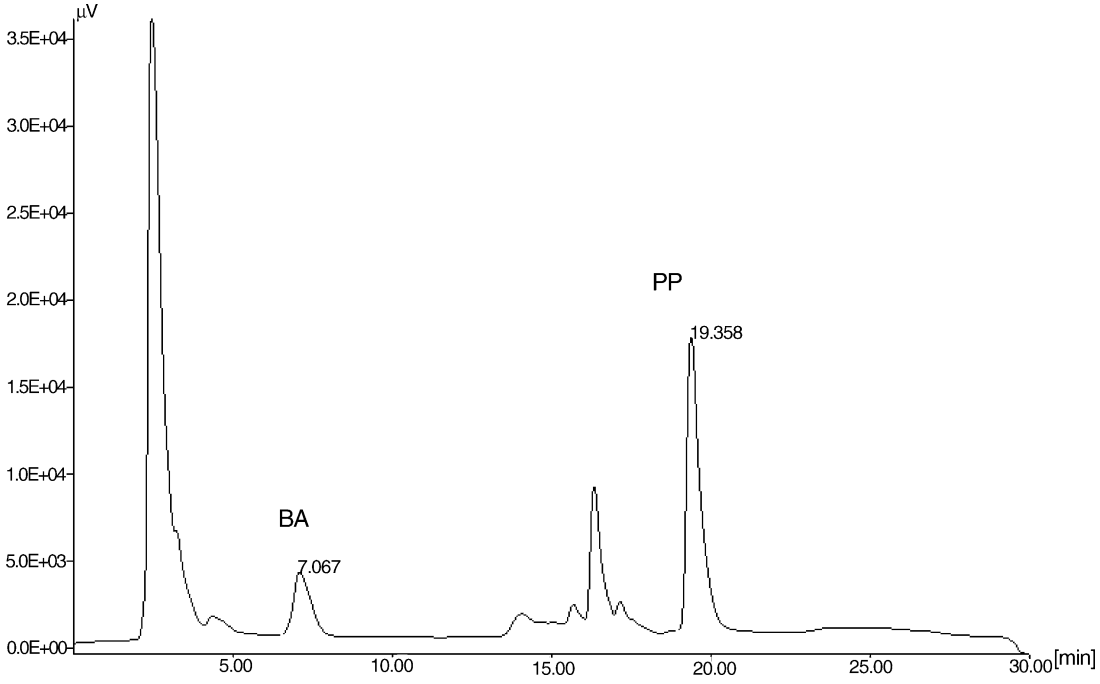

Fig. 3. Chromatogram of extract from canned vegetable product.

seem to be the most popular preservative in jams (ranging from 162 to 266 mg kg^{-1} for positive samples). None of these positive samples violate the legal limit of 450 mg kg^{-1} for BA or SA [16]. One sauce sample (1260 mg kg^{-1} BA) and two canned fruit/vegetable samples (1390 mg kg^{-1} SA, 840 mg kg^{-1} BA) was found to exceed the legal limits. BA was found to be the most common preservative in dried fruits (ranging from 390 to 730 mg kg^{-1} for positive samples). All these positive samples violate the legal requirements of a maximum of 350 mg kg^{-1} for BA or SA. It is also interesting to find that four samples contain a mixture of at least three types of preservatives. The use of parabens is not regulated under the current Malaysian Food Act. Chromatogram of one preservative-positive sample is shown in Fig. 3. The most popular chemical preservatives in soft drinks and jam are SA, while in dried fruits is BA.

Table 3
Results for recoveries of spiked standards to various samples

Sample type	Recovery (%)			
	BA	SA	MP	PP
Soft drink	112	105	109	106
Jam 1	91.0	105	102	97.0
Jam 2	97.0	98.0	103	102
Jam 3	96.0	97.0	101	104
Sauce	109	94.0	96.0	103
Canned fruit/vegetable 1	113	106	102	96.0
Canned fruit/vegetable 2	9.0	104	96.8	102
Canned fruit/vegetable 3	0.0	106	95.7	96.3
Canned fruit/vegetable 4	09	106	103	107
Dried fruit 1	14	108	97.5	96.8
Dried fruit 2	115	111	97.0	107
Dried fruit 3	106	111	103	108

Concentration of preservatives spiked, mg kg^{-1}: BA, 19.3; SA, 9.4; MP and PP, 10.0.

4. Conclusion

The sample pre-treatment procedure, in combination with the HPLC method was found to be suitable for the routine determination of these preservatives in food items. The straightforward pre-treatment method offer acceptable recoveries to all the food items tested. On the whole, except for isolated cases of sauce and canned fruit/vegetable, the levels of the preservatives tested were in compliance with the Malaysian Food Act and Regulations [16]. Major violation of the Act, however, was found in dried fruits where 62.5% (eight samples tested) contain benzoic acid that are above the legal limits of 350 mg kg^{-1}.

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