**Original Article****Simultaneous Determination of Four Preservatives in Foodstuffs by High Performance Liquid Chromatography**Mohammad Faraji\*<sup>1</sup>, Farzaneh Rahbarzare<sup>2</sup>

1- Dept. of Food Science and Technology, Faculty of Food Industry and Agriculture, Standard Research Institute (SRI), Karaj, Iran

2- Dept. of Food Science and Technology, Faculty of Science and Innovative Technology, Islamic Azad University of Pharmaceutical Sciences, Tehran, Iran

**Received:** February 2016**Accepted:** March 2016**ABSTRACT**

**Background and Objectives:** Food preservatives have become an essential element nowadays; they play an important role during food transportation. This will preserve the food for a long duration from the spoilage. High concentration of preservatives in food may result in gastrointestinal disturbances whereby some patients suffering from asthma, rhinitis, or urticaria. The aim of this study is introduction and optimization of a new method for simultaneous determination of four preservatives (SB, PS, MP and PP) in foodstuff by high performance liquid chromatography (HPLC).

**Materials and Methods:** Important factors in extraction, separation and determination processes were optimized using the one-variable-at-a-time method. For optimization, all tests were performed two times. Figures of merit of the proposed method were evaluated. The amount of SB, PS, MP and PP in some food samples was determined using the proposed method.

**Results:** The results showed that the obtained chromatogram of extract was free of significant interference. The preservatives' recoveries ranged from 88% to 110%. Limit of detection and limit of quantitation for the preservatives were 0.2 mg kg<sup>-1</sup> and 0.5 mg kg<sup>-1</sup>, respectively. Concentration of SB, PS, MP and PP in the 20 studied samples was in the range of N.D-639.9, N.D -214.5, N.D -579.8 and N.D -30.5 mg kg<sup>-1</sup>, respectively.

**Conclusions:** The acceptable performance and reliability of the proposed method as a simple, efficient and fast method for determination of SB, PS, MP and PP in the food samples were demonstrated.

**Keywords:** Sauce samples, Sodium benzoate, Potassium sorbate, Parabens, High performance liquid chromatography (HPLC)

**Introduction**

Food preservatives have turned into an essential element nowadays so that they play an important role during the food transportation. This will preserve the food for a long duration from the spoilage. According to directive 95/2/EC dated on 20.02.1995, "the preservatives are substances that increase the food preservation time by protecting them against the damages caused by micro-organisms" (1,2).

Food preservatives aim to preserve the appearance of food, preserve the food characteristics like odor and taste, and food is preserved for a long time.

Benzoic acid (BA) and sorbic acid (SA) are generally effective to control molds, inhibit yeasts growth, and act against a wide range of bacterial attack. Effective pH for BA and SA is 2.5-4 and more than 6, respectively (3-6). On the other hand, Parabens, alkyl esters of Para Amino Benzoic Acid (PABA), are a class of antimicrobial agents used singly or in combination to exert the intended antimicrobial effects against molds and yeasts. These substances can have multiple biological effects but it is, generally, considered that their inhibitory effect on

membrane transport and mitochondrial function processes is crucial for their actions. The parabens meet several of the criteria of an ideal preservative, in that they have a broad spectrum of antimicrobial activity, are safe to use (i.e. relatively non-irritating, non-sensitizing, and of low toxicity), are stable over the pH range, and are sufficiently soluble in water to produce the effective concentration in aqueous phase. Appreciable hydrolysis occurs at pH above 7 (7,8). Antimicrobial activity of paraben increases as the chain length of the ester group increases; however, since solubility decreases with increasing the chain length, the lower esters (methyl and propyl) are the practical choices for use in foods. MP and PP has been used as antimicrobial preservatives in foods, drugs and cosmetics for over 50 years. There have been several previous safety assessments undertaken on these substances by several agencies, including Food and Agriculture Organization/World Health Organization (FAO/WHO), Food and Drug Administration (FDA), and Federal Emergency Management Agency (FEMA) (9).

The use of food additives in different countries is limited by specific regulations. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the safety in use of an additive can be expressed in terms of its acceptable daily intake (ADI), which represents the amount of the substances that can be daily consumed, even for a lifetime, without health hazards (10). Group ADIs of 0–5 and 0–25 mg/kg of body weight have been established by JECFA for BA and benzoates salts and for SA and sorbates salts, respectively (11). According to consumer council article, the acute intoxication due to BA is unclearly identified. However, the article emphasizes that excessive daily intake of BA in a long-term may result in gastrointestinal disturbances whereby some patients suffering from asthma, rhinitis or urticaria may experience exacerbation of these symptoms after ingesting foods with BA (12).

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 determination of BA, SA and parabens is reversed-phase HPLC (13-18). Although other analytical methods such as TLC (19,20), capillary electrophoresis (21-24), gas

chromatography, and spectrophotometry (25-28) have also been reported.

The aim of this work was to develop a method to simultaneously detect the four preservatives including sodium benzoate (SB), potassium sorbate (PS), methyl paraben (MP) and propyl paraben (PP) based on ultrasonic assisted solvent extraction and reversed phase-high performance liquid chromatography (RP-HPLC) technique. A simple extraction procedure was developed in order to extract all the preservatives in a single step. Then the analytes were separated by RP-HPLC, and identified by means of a multi-channel detector (UV) using  $\lambda = 225$  nm and  $\lambda = 254$  nm detection. Preservative amount was obtained by reference to a matrix-matched standard curve due to a relevant matrix effect. Accuracy (recovery), precision, method detection limit (MDL), method quantification limit (MQL), linearity range, and ruggedness of the method were evaluated. Afterwards, the method was tested on several kinds of food samples in order to check its versatility.

## Materials and Methods

### Experimental

**Reagent and chemicals:** All chemicals and solvents used were of analytical grade and/or HPLC grade. Acetic acid glacial (99.8%), potassium hexacyanoferrate (98%), zinc acetate (98%), SB (99.5%), PS (99%), MP (99%), PP (99%) and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany).

Stock standard solutions ( $1000 \text{ mg L}^{-1}$ ) of the four mentioned food preservatives were separately prepared by dissolving 50 mg of SB, PS, MP and PP in 50 ml HPLC grade methanol. The solutions were stored in a brown glass bottle and kept at  $4^\circ\text{C}$ . Mixed working standard solutions of the above preservatives were prepared daily by appropriate dilution of the stock solutions in methanol. 100 ml of standard stock solutions were prepared by mixing the four preservatives in HPLC grade methanol.

Carrez I was prepared by dissolving 15 g of potassium hexacyano ferrate in deionized water, and for preparing 100 ml of Carrez II, 22 g of zinc acetate was mixed with 3 ml of acetic acid in water.

**Chromatographic conditions:** The chromatographic analysis was carried out in an HPLC (Knauer, Germany) equipped as follows: ultimate 3000 pump,

K1100 Automated sample injector, Knauer UVD 170U detector and thermostatted column compartment oven TCC-100. The HPLC operating mode was isocratic, the injection volume was 20 $\mu$ L, and the column temperature was adjusted at room temperature. The chromatography column was a Supelcosil LC-18: 25cm  $\times$  4.6mm, 5 $\mu$ m (Supelco, Bellefonte, PA, USA). Sample data collection was optimized to 25 min per sample with UV detection at the wavelength of maximum absorption of the compounds, 225 nm for SB, and 254 nm for PS, MP and PP. Mobile phase used was combination of methanol–acetate buffer = pH 4.4 (phase A; 70:30, v/v) and methanol–acetate buffer = pH 4.4 (phase B; 35:65v/v). Elution program are shown in Table 1. Moreover, the mobile phase flow rate was set to 1.0 mLmin<sup>-1</sup>.

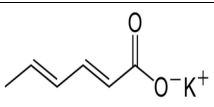
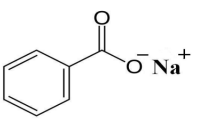
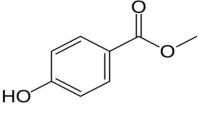
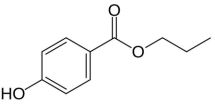
**Preparation of sample:** Solid food samples were finely ground prior to the extraction. About 1.0 g of the sample was accurately weighed in a screw-capped test tube. 5.0 mL of methanol, 2.5 mL of sodium hydroxide (0.2 mol L<sup>-1</sup>) and 5mL of deionized water and 0.5 mL of Carrez I, and 0.5 mL of Carrez II were added and placed in a sonicator (ULTRASONIK Model

28X, Ney Dental, Yucaipa, California) that was maintained at 50 °C for 10 min. The test tube was next subjected to centrifugation (Beckman, Avanti™ J-25 I, Rotor JA-21, Netherlands) for 10 min. The supernatant was filtered through a 0.45- $\mu$ m 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. The liquid extract samples were filtered through a 0.45  $\mu$ m membrane. If the concentration of the preservatives in the samples was higher than the largest one used to build the calibration curve, the samples were diluted in water, and sodium hydroxide and Carrez I and II were used to clearing the samples.

**Food samples:** A total of 20 food samples were purchased from the local supermarkets in Karaj, Iran. The samples were categorized as: soft drinks (2), canned foods (4), sauces and ketchups (5), tomato paste (4), fruit juice (2) and lemon juice (3).

**Statistical methods:** Excel software was applied to calculation of mean and relative standard deviation of results and also plot curves and so on.

**Table 1.** Name, chemical structure, E number, status and maximum level of the preservatives used

Compound	Chemical structure	E number	Status <sup>a</sup>	Maximum level <sup>b</sup> (mg/Kg)
Potassium sorbate		E202	Approved in the EU Approved in Iran	200-3000
Sodium benzoate		E211	Approved in the EU Approved in Iran	200-5000
Metylparaben		E218	Approved in the EU Banned in Iran	36-1500
Propylparaben		E216	Not reported	Not reported

<sup>a</sup>Current EU approved additives and their E Numbers, Food Standards Agency, 26 November 2010

<sup>b</sup>CODEX GENERAL STANDARD FOR FOOD ADDITIVES, CODEX STAN 192-1995

## Results

The purpose of this study was to develop a simple and rapid method using HPLC to simultaneously determine multiple preservatives (including sodium benzoate, potassium sorbate and parabens) in some food samples (soft drinks, canned vegetables, sauces and ketchups, tomato paste and lemon juice).

**Choosing the best conditions for detection and separation of the preservatives:** In order to achieve the highest sensitivity in determination of the preservatives, the effect of detection wavelength was investigated in 220, 225, 230, 235 and 254 nm wave lengths. The results showed that the optimized wave length for SB was noticed at 225 nm. However, the optimized wavelength for PS, MP and PP was found at 254 nm, and in continuation of the program, these wavelengths showed the best sensitivity of the peaks. This result is confirmed by other scientists (29).

During our preliminary experiments, several different mobile phases were tested including methanol–acetate buffer (35:65), methanol–acetate buffer (40:60), methanol–acetate buffer (50:50), and methanol–acetate buffer (30:70). The results showed that in low percent of methanol (methanol–acetate buffer (35:65)), the resolutions of SB, PS and MP were suitable; however, under this circumstance, PP was eluted so late. On the other hand, in high percent of methanol (such as methanol–acetate buffer (50:50)), the resolutions of SB, PS and MP were too bad though PP was eluted properly. So, by evaluating these results, gradient elution program was followed. Finally, the optimized gradient elution program (as shown in Table 2) was applied in order to obtain good resolution of the peaks and also reasonable run time. Under optimum conditions, the retention time for SB (first peak), PS (second peak), MP (third peak) and PP (fourth peak) is about 6, 7.5, 10.3, and 16.8 min, respectively.

**Table 3.** Analytical characteristics of the HPLC method

Preservatives	RSD%		LOQ (mg/L)	LOD (mg/L)	Linear range (mg/L)	R <sup>2</sup>
	Inter-days	Intra-day				
Sodium benzoate	6.7	4.3	0.5	0.2	1.0-100	0.9908
Potassium sorbate	7.2	4.7	0.5	0.2	1.0-100	0.9981
methyl paraben	5.6	4.5	0.5	0.2	1.0-100	0.9994
Propyl paraben	6.1	4.4	0.5	0.2	1.0-100	0.9907

RSD: relative standard deviation; LOD: limit of detection; LOQ: limit of quantization

**Table 2.** Elution program of the preservatives by HPLC

Time	% phase A	% phase B
0	0	100
8	0	100
18	100	0
20	100	0
21	0	100
25	0	100

**Choosing the best conditions for extraction of samples:** In a preliminary test, different solvents were checked. The extract solvents included: methanol–NaOH–water 40:20:40, (V/V/V), 60:0:40, (V/V/V), 20:20:60, (V/V/V) and methanol–NaOH–water–Carrez I–Carrez II 40:16:40:2:2, (V/V/V), and 38:16:38:4:4 (V/V/V). Of these, methanol–NaOH–water–Carrez I–Carrez II 40:16:40:2:2, (V/V/V), and 38:16:38:4:4 (V/V/V) solutions provided rapid extraction and better chromatographic peak resolutions.

**Method validation:** 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 decreasing the concentration of the prepared standards down to the smallest detectable peak. Other important analytical characteristics of the method are summarized in Table 3.

**Linearity:** To carry out this study, solutions with eight levels of concentration within the range of 0.5, 1, 2, 5, 20, 80, 100 and 200 mg/kg were prepared. All analyses were performed in triplicate. The linearity range and correlation coefficients are listed in Table 3.

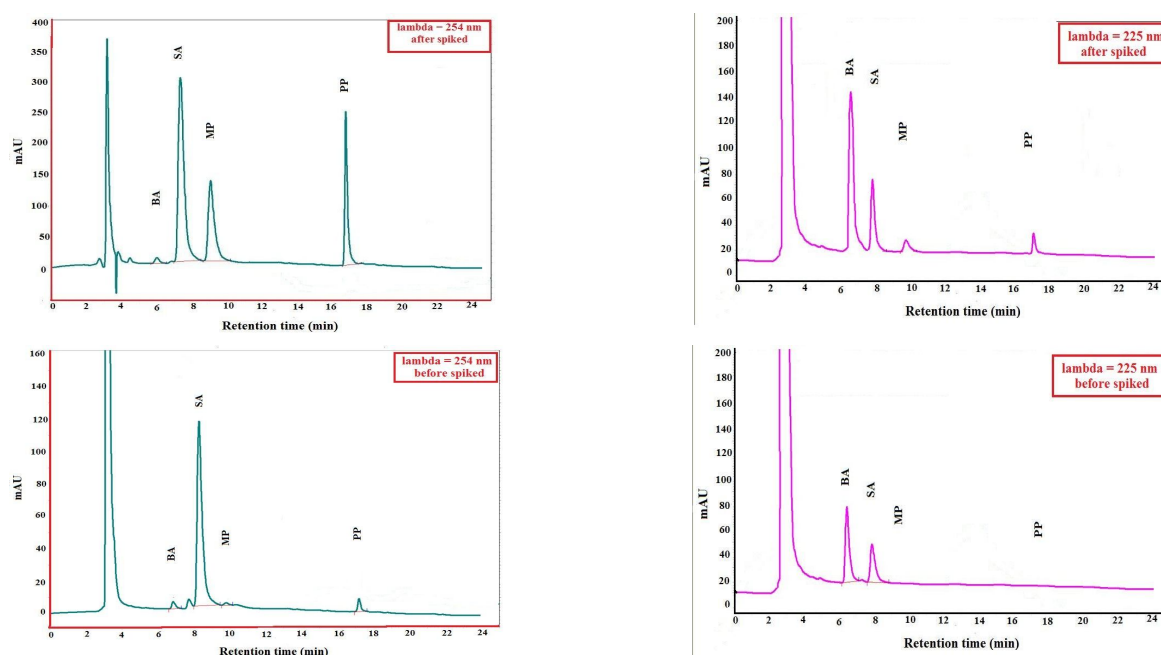
**Recovery:** The recovery of the method was studied where a known concentration of the analytes was added to the sample pre-treatment method and it was calculated by the concentration of the analytes recovered in relation to that added as a spike sample. The results obtained for the accuracy study (recovery method) from 10 samples ( $n=3$  for each concentration level) are presented in Table 4. According to Table 4, it can be concluded that the recovery study of the preservatives in the food stuffs matrix was correct. Therefore, the proposed analytical method was

sufficiently accurate for simultaneous determination of the four preservatives by the average recoveries of 88-110%; indicating the correction and accuracy of the method.

Peak identification of the preservatives in various foodstuffs was based on the comparison between the retention times of standard compounds, and was confirmed by spiking the known standard compounds to the sample. Chromatogram of one preservative-positive sample is shown in Fig. 1.

**Table 4.** Results of food preservatives' recovery study

Sample name	$C_{\text{added}}$ (mg L <sup>-1</sup> )	Recovery (%)			
		Sodium benzoate	Potassium sorbate	Methyl paraben	Propyl paraben
Sauce 1	25	112.5	111.8	102.6	91.9
Sauce 2	25	109.0	110.2	105.6	95.1
Sauce 3	25	109.0	107.6	90.9	97.6
Ketchup 1	25	108.0	101.2	105.6	92.7
Ketchup 2	25	99.7	102.7	107.8	93.4
Tomato paste 1	25	93.4	96.6	107.0	109.4
Tomato paste 2	25	100.6	100.4	86.3	69.6
Tomato paste 3	25	88.3	101.8	80.0	94.8
Tomato paste 4	25	89.9	93.2	86.00	100.4
Soft drink 1	25	90.9	93.9	91.2	96.6
Soft drink 2	25	105.1	103.3	100.4	100.6
Fruit juice 1	25	90.8	92.9	91.3	96.4
Fruit juice 2	25	100.0	98.8	101.1	99.4
Lemon juice 1	25	97.9	107.6	101.3	102.8
Lemon juice 2	25	103.6	103.1	92.0	96.5
Lemon juice 3	25	100.3	96.0	97.4	97.9
Canned food 1	25	101.1	101.6	98.8	97.2
Canned food 2	25	97.2	99.3	96.6	93.6
Canned food 3	25	100.9	100.0	96.8	102.4
Canned food 4	25	92.9	99.8	99.5	98.0



**Figure 1.** HPLC chromatograms of sauce sample 3 before and after spiking by monitoring using multi-channel detector (UV) in  $\lambda = 225$  nm and  $\lambda = 254$  nm.

**Food samples analysis:** In order to evaluate applicability of proposed method, the food samples were analyzed under optimum condition. Results for real samples are shown in Table 5. Concentration of SB, PS, MP and PP in the 20 studied samples was in the range of N.D-639.9, N.D -214.5, N.D -579.8 and

N.D -30.5 mg/kg, respectively (Table 5). Fig. 1 shows the HPLC chromatograms of sauce sample 3, before and after spiking ( $10 \text{ mg L}^{-1}$ ) by monitoring using multi-channel detector (UV) in  $\lambda = 225 \text{ nm}$  and  $\lambda = 254 \text{ nm}$ .

**Table 5.** Concentration of food preservatives in food samples

Sample	Preservatives ( $\text{mg kg}^{-1}$ )			
	Sodium benzoate	Potassium sorbate	Methylparaben	Propylparaben
Sauce 1	178.8	175.9	45.4	5.9
Sauce 2	168.9	18.9	141.8	17.2
Sauce 3	639.9	214.5	45.7	4.1
Ketchup 1	259.4	29.1	579.8	N.D
Ketchup 2	N.D	N.D	N.D	N.D
Tomato paste 1	8.7	N.D	47.2	37.3
Tomato paste 2	N.D	3.6	15.0	N.D
Tomato paste 3	N.D	N.D	N.D	N.D
Tomato paste 4	N.D	N.D	N.D	N.D
Soft drink 1	359.9	62.5	21.7	27.2
Soft drink 2	34.7	77.1	24.9	30.5
Fruit juice 1	N.D	37.2	5.6	20.5
Fruil juice 2	N.D	N.D	N.D	N.D
Lemon juice 1	N.D	N.D	N.D	N.D
Lemon juice 2	N.D	N.D	N.D	N.D
Lemon juice 3	N.D	N.D	N.D	N.D
Canned food 1	N.D	N.D	N.D	N.D
Canned food 2	N.D	N.D	N.D	N.D
Canned food 3	N.D	N.D	N.D	N.D
Canned food 4	N.D	N.D	N.D	N.D

## Discussion

It is well known that the detection wavelength is one of the most important factors affecting the sensitivity of the method. The results showed that the optimized wavelength for SB was at 225 nm. However, the optimized wavelength for PS, MP and PP was found at 254 nm. This result is confirmed by other scientists (29).

Composition of mobile phase is a key factor in resolution of chromatographic separation the same as the run time of analysis. Mobile phases containing acetate buffer (as buffering compounds) are obviously recommended as the most suitable to assure the very good chromatographic separation of preservatives (SB, PS, MP and PP) (29).

The findings showed that in low percent of methanol (methanol-acetate buffer (35:65)), the resolutions of SB, PS and MP were suitable; however, PP was eluted so late. On the other hand, in high percent of methanol (such as methanol-acetate buffer (50:50)), the resolutions of SB, PS and MP were too bad but PP was eluted properly. Therefore, a gradient

elution program (Table 2) was applied in order to have the best separation with good resolutions and also short run time.

Extraction of the preservatives from the samples should be undertaken prior to chromatographic analysis. Extraction of liquid and solid foods with methanol, sodium hydroxide and deionized water is a good alternative for the routine analysis of BA, SA, MP and PP in food samples. In spite of the complexity of some food matrixes, extraction with this method is economic, time saving, and easy to carry out. UV-vis detector is a useful tool for testing the specificity of the HPLC method herein presented for routine quantitative analyses of BA, SA, MP and PP in food.

Since most of the interference proteins present in the samples were precipitated in the presence of Carrez solutions, extraction solvent without Carrez solutions (methanol-NaOH-water 40:20:40 (V/V/V), 60:0:40 (V/V/V), and 20:20:60 (V/V/V)) did not lead to obtaining a good peak shape and effective

extraction used for complex samples such as sauce, ketchup, canned foods; this can probably be attributed to incomplete extraction and formation of additional peaks at the same retention time. Moreover, the results showed that presence of NaOH in the extraction solvent was effective based on the chemical properties of the preservatives (Table 1).

Analysis of the preservatives (SB, PS, MP and PP) showed a linear relationship with high linear regression coefficients of determination for all of them ( $R^2 > 0.99$ ). The complete description of standard linearity supported by regression data is shown in Table 1. The results indicated that the developed extraction method provided a reasonably good accuracy for the analysis of the preservatives in the food samples.

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 offers acceptable recoveries to all the food items tested. Except sauce 1, sauce 2 and carbonated drink that are free from preservatives, the sample sauce 3 includes 579.8 mg/kg of these preservatives.

Recommend maximum level for sum of SB and PS in sauce samples based on Iranian regulations is 750 mg/kg. Also recommended maximum level for SB and PS is 150 and 500 mg/kg, respectively. On the other hand, based on Iranian regulations, use of MP and PP is banned in foodstuffs. However, according to the CODEX STAN 192-1995, methylparaben and ethylparaben can be used as food preservatives in foodstuffs. So, the survey of the analyzed samples showed that the used levels of SB and PS in the food samples are in the recommended levels; however, in spite of their limitation, MP and PP are also used in some food samples.

Some analysis results of preservatives in the food samples are shown in Table 3. BA seems to be the most popular preservative in fruit juice (ranging from 178.8 to 168 mg kg<sup>-1</sup> for positive samples) while the legal maximum limit of BA in fruit juice has been reported as 150 mg kg<sup>-1</sup>. The major violation of the Act, however, was found in one sauce sample containing MP (579.8 mg kg<sup>-1</sup>) while using this

preservative is prohibited in the food standards of Iran.

### Financial disclosure

The authors declared no financial interest.

### Funding/Support

This work was supported by the Standard Research Institute, and the authors would like to thank them for financial supports.

### References

1. European Parliament and Council Directive no. 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners.
2. Institute of Standards and Industrial Research of Iran. Food preservatives ISIRI no. 950. 2nd revision, Karaj: ISIRI 1997 [in Persian].
3. Ali MS. Rapid quantitative method for simultaneous determination of sorbic acid, benzoic acid and four parabens in meat and nonmeat products by liquid chromatography. *J Assoc Off Anal Chem* 1985; 68(3): 488-492.
4. Lin JH, Choong YM. A Simple Method for the Simultaneous Determination of Various Preservatives in Liquid Foods. *J Food Drug Anal* 1999; 7: 291-304.
5. Leuenberger U, Gauch R, Baumgartner E. Determination of food preservatives and saccharin by high-performance liquid chromatography. *J Chromatogr* 1979; 173: 343-348.
6. Bui LV, Cooper C. Reverse-phase liquid chromatographic determination of benzoic and sorbic acid in foods. *J Assoc Off Anal Chem* 1987; 70: 892-896.
7. Seetaramaiah K, Smith AA, Murali R, Manavalan R. Review on Preservatives in Food Products. *Pharm Biol Archiv* 2011; 2(2): 583-599.
8. Howlett JF. The regulation of preservatives in European community. *Food Addit Contam* 1992; 9 (5): 607-614.
9. Barbas C, Ruperez J. Separation of paraben preservatives by reversed-phase HPLC application in foods, beverages, cosmetics. *Agilent Technologies*, 2005; 1-4.
10. WHO, Principles for the safety assessment of food additives and contaminants in food. *Environmental Health Criteria*, 1987; 70.
11. Cheung PCK. Food Preservatives, Food and nutritional sciences. *The Chinese University of Hong Kong*, 2003; 1-53.
12. Anaskhan HM. Headspace solid phase microextraction applied to simultaneous determination of sorbic and benzoic acids in beverages. *Sciences University Technology of Mara*, 47, 111.
13. Guarino C, Fuselli F, La Mantia A, Longo L. Development of an RP-HPLC method for the simultaneous determination of benzoic acid, sorbic acid,

- natamycin and lysozyme in hard and pasta filata cheeses. *Food Chem* 2011; 127: 1294-1299
14. Ferreira IMPLVO, Mendes Brito E, Ferreira PMA. Simultaneous determination of benzoic and sorbic acids in quince jam by HPLC. *Food Res Int* 2000; 33: 113-117.
  15. Chu TY, Chen CL, Wang HF. A rapid method for the simultaneous determination of preservatives in soy sauce. *J Food Drug Anal* 2003; 11(3): 246-250.
  16. Lee HS. Liquid chromatographic determination of benzoic acid in orange juice: interlaboratory study. *J AOAC Int* 1995; 78(1): 80-82.
  17. Lee HS, Rouseff RL, and Fisher JF. Determination of food preservatives in orange juice by reversed-phase liquid chromatography. *J Food Sci* 1998; 51(3): 568-570.
  18. Lee MH, Choong YM, Ku KL. and Wang ML. Simple and rapid method for the determination of sorbic acid and benzoic acid in foods. *J Chin Agric Chem Soc* 1995;33(2): 247-261.
  19. Coverly J, Peters L, Whittle E, Basketter DA. Susceptibility to skin stinging, non-immunologic contact urticaria and acute skin irritation; is there a relationship? *Contact Dermatitis* 1998; 38(2): 90-95.
  20. Mandrou B, Bressolle F. Thin layer chromatography reflectometric determination of benzoic and sorbic acids in fruit beverages. *J Assoc Off Anal Chem* 1980; 63: 675-678.
  21. Li R, Wei W, Yang L, Jiang Y, Xie T. Online pre concentration in capillary electrophoresis with contactless conductivity detection for sensitive determination of sorbic and benzoic acids in soy sauce. *Talanta* 2011; 83(5): 1487-1490.
  22. Costa ACO, Perfeito LS, Micke MFMGA. Determination of sorbate and benzoate in beverage samples by capillary electrophoresis, Optimization of the method with inspection of ionic mobilities. *J Chromatogr A* 2008; 1204(1): 123-127.
  23. Waldron KC, and Li J. Investigation of a pulsed-laser thermo-optical absorbance detector for the determination of food preservatives separated by capillary electrophoresis. *J Chromatogr* 1996; B 683: 47-54.
  24. Pant I, and Trenery C. The determination of sorbic acid and benzoic acid in a variety of beverages and foods by micellarelectrokinetic capillary chromatography. *Food Chem* 1995; 53: 219-226.
  25. Sen I, Shandil A, Shrivastava VS. Determination of benzoic acid residue from fruit juice by gas chromatography with mass spectrometry detection technique. *Archiv Appl Sci Res* 2011; 3(2): 245-254.
  26. Castro JC, Delgado MA, Sánchez MJ, Montelongo FG. Simultaneous 2nd order derivative spectrophotometric determination of sorbic and benzoic acids in soft drinks. *Anal Let* 1992; 25(12): 2357-2376.
  27. Ochiai N, Yamagami T, and Daishima S. Simultaneous analysis of preservatives in foods by gas chromatography mass spectrometry with automated sample preparation instrument. *Bunseki Kagaku* 1996; 45(6): 545-550.
  28. Valcarcel M, Gonzalez M, and Gallego M. Simultaneous gas chromatographic determination of food preservatives following solid-phase extraction. *J Chromatogr* 1998; A 823: 321-329.
  29. Saad B, FazlulBari MD, Saleh MI, Ahmad K, Khairuddin M, Talib M. Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatograph. *J Chromatogr A* 2005; 1073: 393-397.