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Contributed Paper

Validation of a HPLC Method for the Determination of Benzoic Acid and Sorbic Acid in Noodles

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ABSTRACT

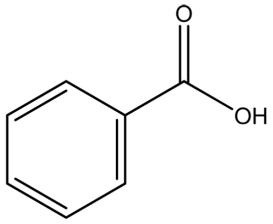
A simple, sensitive and specific HPLC method was validated for the simultaneous determination of benzoic acid and sorbic acid in noodles. Sample preparation involved the extraction with methanol and water (60:40, v/v). The separation was achieved on a Germini-C18 modified silica column (50 mm x 4.6 mm i.d., 5 μm particle diameter). The mobile phase consisted of 0.05 M ammonium acetate (pH 4.4) and methanol in the ratio of 60:40 (% v/v) at a flow rate of 1 mL/min and detection was performed at 234 nm using a diode-array detector (DAD). The method was validated with respect to specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness. The calibration curve for benzoic acid and sorbic acid was found to be linear in the concentration range of 5-200 μg/mL (r^2 0.9998) and 1-50 μg/mL (r^2 0.9998), respectively. The values of LOD and LOQ were 0.42 and 1.14 μg/mL and 0.32 and 0.99 μg/mL for benzoic acid and sorbic acid, respectively. The accuracy determined by spike recovery measurement was 85.61-102.04% for benzoic acid and 83.62-102.47% for sorbic acid. The method and intermediate precision for benzoic acid and sorbic acid were demonstrated by the relative standard deviation of 1.84% and 2.40% and 1.41% and 2.80%, respectively. The validation acceptance criteria were met in all cases. The proposed method was successively applied for monitoring of benzoic acid and sorbic acid in 5 different kinds of noodles.

Keywords: benzoic acid, sorbic acid, noodles, HPLC-DAD

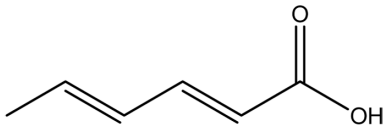
1. INTRODUCTION

Noodles (Kuy-Teaw) are generally consumed in Thailand as oriental cultures and have been exported to international markets. One of the most popular and favorite dish worldwide is Stir-fried rice noodle (Pad-Thai). Almost Thai noodles are made from rice; however, some kinds are made from wheat flour (Ramen or Ba-mee) and starches derived from mung bean (Mung bean vermicelli or Wun Sen). Rice noodles are presented in various size, shape and thickness such as Rice spaghettini (Sen lek), Rice fettuccine (Sen yai) and Rice vermicelli (Sen mee). The main ingredients are rice flour and water. Corn

starch is also added in order to increase the gelatinous and chewy texture of the noodles [1]. All types of noodles are accessible fresh, semi-dried and dried. The moisture content of fresh and semi-dried noodles leads to sensory deterioration during handling and storage, producing loss of quality and limiting shelf-life [2].



benzoic acid



sorbic acid

Figure 1. Structure of benzoic acid and sorbic acid.

Benzoic acid (C_6H_5COOH) and sorbic acid (C_5H_7COOH), organic acids, are preservatives widely employed in food, pharmaceutical and cosmetic products (Figure 1). Benzoic acid and sorbic acid exhibit inhibitory activity against a wide variety of fungi, yeasts, molds and bacteria [3]. A broader spectrum of microbicidal activity is achieved by using a combination of them, which inhibit several bacterial strains better than either of them alone [4]. Under the notification of the Ministry of Public Health of Thailand ((No.281) B.E.2547 (2004)) and announcement of the Thai Food and Drug Administration (Thai FDA), the maximum level (ML) of benzoic acid and sorbic acid for noodles permitted by law is 1,000 parts per million (ppm) or 1,000 mg/kg. According to international standards adopted Codex Alimentarius Commission (CAC), the ML of benzoic acid for noodles is 1000 mg/kg while sorbic acid is not allowed. The acceptable daily intake (ADI) values determined by the Joint FAO/WHO Expert Committee on Food Additives, for benzoic acid and sorbic acid are 0-5 and 0-25 mg/kg body mass, respectively. A small amount of benzoic acid is naturally excreted by the human body through the normal metabolic processes as hippuric acid or benzoyl glucuronide. Nevertheless, there is an evidence of health risks for consuming high amount of benzoic acid such as asthma, rhinitis, urticaria and anaphylactic shock in sensitive individuals. Cases of nausea, headache, weakness, burning and irritation of oesophagus have been reported as well [5]. Sorbic acid has a very low level of mammalian toxicity. Few cases of non-immunological contact urticaria and pseudo-allergy have been reported [6,7]. A validated quantification method for both acids is subsequently needed to facilitate consumer protection and the production of good quality noodles [8].

Various methods are available for the determination of benzoic and sorbic acid in foods products including the use of lanthanide-sensitized luminescence [9], potentiometric [10], spectrophotometric [11], capillary zone electrophoresis [12,13], high performance thin layer chromatography (HPTLC) [14], micellar electrokinetic chromatography (MECC) [15], gas chromatography (GC) [16-18] and high performance liquid chromatography (HPLC) [19-26]. The official AOAC GC method requires derivatization reaction. Some GC and HPLC methods use harmful organic solvent for extraction that can cause environmental pollution and human health risk. In addition, almost of these methods are applied for foods, fruits, beverages and drinks which are

not specific to noodle matrices. Only one previous non-validated HPLC method is operated for noodle samples [21]. However, the lengthy sample preparation by stream distillation is used. Hence, the present work was conducted in order to develop a simple and rapid HPLC-DAD method for the determination of benzoic acid and sorbic acid in noodles simultaneously. A simple extraction procedure was applied in order to extract both preservatives and clean up samples in single step. The established method was validated with respect to specificity, linearity, precision, accuracy and robustness.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Standards of benzoic acids and sorbic acid with purity greater than 99% were purchased from Sigma/Fluka (Schnelldorf, Germany). Ammonium acetate and acetic acid was from Univar Ajax Finechem (Australia). The HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Other chemicals used were of analytical grade. High purity water was prepared by using Milli-Q RO system (Millipore, Bedford, MA, USA).

2.2 Noodle Samples

Five kinds of noodles; Rice spaghettini, Rice fettuccine, Rice vermicelli, Mung bean vermicelli and Ramen, were purchased from 6 noodle shops around Silpakorn University in Amphur Muang, Nakorn prathom province, Thailand. A total of 150 samples were collected incessantly for 5 weeks and kept at 4°C in a refrigerator.

2.3 Instrumention and Chromatographic Conditions

The HPLC-DAD system consisted of an Agilent 1100 series pump, an on-line solvent degasser, an autosampler, a diode-array detector (DAD) and a Chemstation software version A.08.01 (Agilent Technologies, USA). A reversed-phase column, 50 mm × 4.6 mm packed with 5 μm, Germini-C18 modified silica (Phenomenex, USA) and a guard column, 20 mm × 3.9 mm packed with 5 μm, C18 were used. The column was operated at ambient temperature. The separation was carried out under isocratic elution with 0.05 M ammonium acetate buffer (pH 4.4 with acetic acid) and methanol in the ratio of 60:40 (% v/v). The flow rate was 1 mL/min and the wavelength was monitored at 234 nm. The injection volume was 10 μL. The mobile phase was filter through 0.45 mm Chrom Tech Nylon-66 filter and degassed in an ultrasonic bath prior to use. The samples were analyzed using a DAD detector in scan mode covering the range of 200-400 nm.

2.4 Preparation of Stock and Standard Solutions

Stock solutions of benzoic acid and sorbic acid in methanol were prepared at a concentration of 2.5 and 1.25 mg/mL, respectively. A series of standard mixture solutions was prepared by the appropriate dilution of the stock standard solutions with methanol to concentration range of 5-200 μg/mL for benzoic acid and 1-50 μg/mL for sorbic acid.

2.5 Preparation of Analytical Sample

Noodle samples were cut into small pieces and dried in a hot air oven at 50°C for 30 min. The dried samples were then homogenized to fine powder. One gram of powdered sample was accurately weighed into a 10 mL volumetric flask and added a part of extraction solvent; methanol: water (60:40, v/v). The mixture solution was shaken vigorously for 1 min by vortex mixing and made up to volume with extraction solvent.

The mixture solution was then placed in a sonicator bath for 30 min to affect complete extraction. The extracted solution was centrifuged for 5 min at 4,000 rpm. An aliquot supernatant was filtered through a 0.45 μm nylon membrane filter and subjected into HPLC column. For concentrated samples, further dilution with mobile phase was performed.

3. RESULTS AND DISCUSSION

3.1 Sample Preparation

Several types of extraction solvent; 100% methanol, 100% acetonitrile, methanol: water (60:40, (v/v)), acetonitrile: 20 mM sodium acetate (50:50, (v/v)) and 1.0 N NaOH were investigated. A blank noodle sample was spiked with a standard mixture solution containing 50 μg/mL of benzoic acid and 10 μg/mL of sorbic acid, mixed and prepared as previous above. The recovery and standard deviation were calculated and compared. The average recovery rate for benzoic acid ranged from 66-78% using 100% methanol, 100% acetonitrile, acetonitrile: 20 mM sodium acetate (50:50, (v/v)) and 1.0 N NaOH while for sorbic acid ranged from 89-105%. The mixture of methanol and water (60:40, v/v) was found to be the most suitable extracting solvent in this study with the average recovery between 96% and 103%. The result achieved indicated that there were no matrix effects on benzoic acid and sorbic acid determination.

3.2 Optimization of the HPLC Condition

The chromatographic conditions were optimized so as to obtain a good separation between benzoic acid, sorbic acid and matrix interferences in noodles. Acetate and phosphate aqueous solutions with different pH and organic solvent (methanol and acetonitrile) were investigated and system suitability test (SST) parameters were performed during the development and optimization of method. Firstly, the separation was attempted and compared on a C18 column (150 mm × 4.6 mm, 5 μm) and a C18 column (50 mm × 4.6 mm, 5 μm) with a wavelength at 224 nm (for sorbic acid), 234 nm (for benzoic acid) and 254 nm using acetonitrile and 5 mM phosphate buffer (pH 7) in the ratio range from 5:95 - 30:70. Both acids were eluted quickly and could not separate from matrix interferences. Methanol was then replaced in the same ratio range but the peaks of both acids were co-eluted. Increasing of phosphate buffer concentration (5-30 mM) led to increase in analytes' retention, decrease in tailing factor and resolution value. Consequently, ammonium acetate aqueous solution was employed instead of phosphate to achieve the best resolution. Poor resolution and peak tailing was obtained when acetonitrile was used. Separation of benzoic acid and sorbic acid was accomplished by varying the concentration of ammonium acetate (5-30 mM) and the ratio of methanol. An increase in retention and peak broadening of benzoic acid and sorbic acid was observed when higher concentration of ammonium acetate at higher pH was utilized. Hence, the aqueous component of the mobile phase was selected to be ammonium acetate 5 mM, pH 4.4. Methanol in varying ratio was studied and the resolution (R) between two acids and interferences, and the run time of the chromatogram was taken into consideration. The optimization condition was performed using an isocratic elution at the proportion of 60:40 (v/v) 5 mM ammonium acetate aqueous solution adjusted to pH 4.4: methanol at a flow rate of 1 mL/min. The C18 column with 5 cm length was preferred as the complete separation was occurred within 3 min and the mobile phase operated was also 3 times decreased.

Under this condition, benzoic acid was

well separated from sorbic acid with good peak shape (resolution 3.62). Tailing factor was 0.96 and 1.00 and the number of the theoretical plates (efficiency) was 3029 and 3599 for benzoic acid and sorbic acid, respectively. The typical retention time of benzoic acid and sorbic acid was 1.82 and 2.32 min, respectively (Figure 2). Moreover, peaks of matrix interference in noodles were observed to separate from the peak of benzoic acid and sorbic acid (resolution > 2) (Figure 2 (B) and (D)). The overlaid diode-array spectrum showed good UV absorbance at 234 nm for benzoic acid and sorbic acid, and then this wavelength has been chosen for detection.

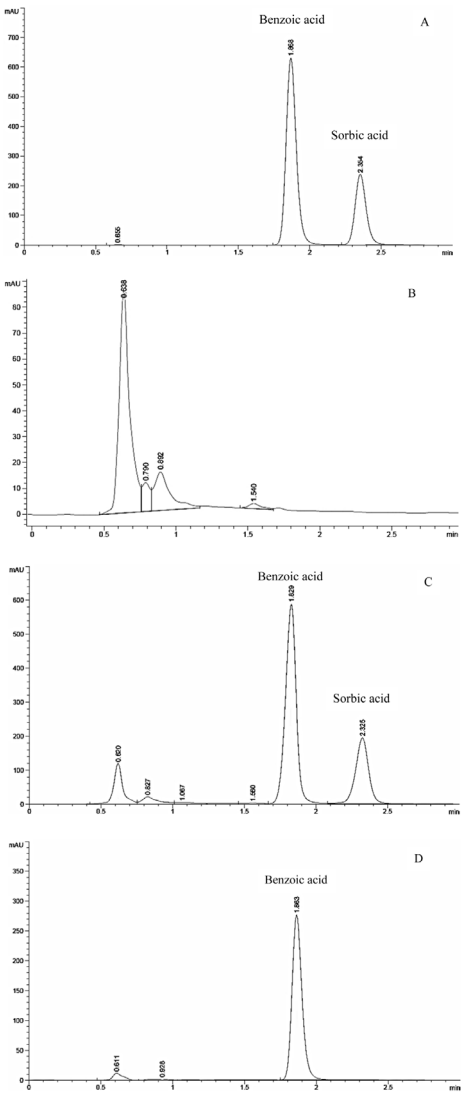


Figure 2. Typical HPLC chromatograms of (A) a standard mixture solution of benzoic acid 100 μg/mL and sorbic acid 20 μg/mL (B) a blank noodle sample (C) a blank noodle sample spiked with a standard mixture solution of benzoic acid 100 μg/mL and sorbic acid 20 μg/mL and (D) a noodle sample containing benzoic acid.

3.3 System Suitability

The system suitability test (SST) parameters were performed during the development and optimization of the method as well as through the validation procedure. SST parameters including capacity factor (k′), selectivity (a), resolution (Rs), column efficiency (number of theoretical plates, N) and tailing factor (T) listed in Table 1 were established by ten replicates. All parameters were satisfactory with good specificity for the quantitation of benzoic acid and sorbic acid in noodles. The RSD% values for the calculated SST parameters for 10 replicates were less than 2%.

Table 1. System suitability test data.

Parameter	Benzoic acid	Sorbic acid	Preferable level
Capacity factor (k')	1.70	2.68	1.0 to 10.0
Selectivity factor (α)		1.58	1.0 to 2.0
Resolution (Rs)	2.42	3.62	> 2
Theoretical plate (N)	3029	3599	$> 2{,}500$
Tailing factor (T)	0.96	1.00	1

3.4 Specificity

The specificity of the method was established through the study of resolution factor of benzoic acid, sorbic acid and peak from the nearest resolving peak. Benzoic acid and sorbic acid were completely separated from all interference matrixes with the satisfactory resolution greater than 2.

Peaks were identified with retention times compared with standards and confirmed the characteristic spectra by photodiode array detection (range 200-400 nm) in both sample and standard solutions. DAD spectral analysis was employed to verify the homogeneity of the benzoic acid and sorbic acid peaks in all solutions. The peak purity value was greater than the threshold value of 995 and thus establishing the selectivity of the assay method for noodle samples.

3.5 Linearity and Range

Linearity was established by least squares linear regression analysis of the calibration curve and evaluated by a determination coefficient (r^2) using data analysis MS excel software (2007) (ANOVA; $P < 0.05$). Standard mixture solutions of benzoic acid and sorbic acid at seven different concentrations (ranging from 5 to 200 μg/mL and 1 to 50 μg/mL, respectively) were prepared and analyzed in triplicate to prove the linearity of system. Peak area of benzoic acid and sorbic acid solution was plotted against their respective concentrations. The calibration curve of benzoic acid and sorbic acid showed good linearity over the concentration range with r^2 0.9998 and 0.9998, respectively. The statistical data of the regression equation is presented in Table 2.

3.6 Determination of Limit of Detection and Quantitation (LOD and LOQ)

Limit of detection (LOD) and limit of quantitation (LOQ) of benzoic acid and sorbic acid were calculated from the residual standard deviation of the regression line (d) of the calibration curve of each compound staged above and its slope (S) in accordance to the following equation: $LOD = 3.3(d/S)$ and $LOQ = 10(d/S)$. The results given in Table 2 suggested that this method could be used for the determination of very small concentrations of benzoic acid and sorbic acid in noodle samples.

Table 2. Linearity, statistical data of the regression analysis, LOD and LOQ.

Parameter	Benzoic acid	Sorbic acid
Calibration range (μg/mL)	5-200	1-50
Limit of detection (μg/mL)	0.42	0.32
Limit of quantitation (μg/mL)	1.14	0.99
Regression equation		
Slope	32.2190	55.2338
Standard error of mean (S.E.) of slope	0.1982	0.3275
95% confidence limit of slope	31.7096-32.7284	54.3918-56.0757
Intercept	37.4699	5.3878
Standard error of mean (S.E.) of intercept	20.5838	7.4843
95% confidence limit of intercept	-15.4424-90.3822	-13.8513-24.6268
Standard error of estimate	36.8800	14.0147
Regression coefficient (r^2)	0.999811	0.999824

3.7 Accuracy

The accuracy of the method was performed on blank noodle samples spiked with known quantities of preservatives. Five known amount of benzoic acid and sorbic acid were added in blank samples, mixed and all samples were treated and analyzed as described above. Three samples were prepared at each concentration. The percentage recovery of added preservatives was calculated by comparing the peak area of the test samples with that of the standard solutions. The results obtained from 15 samples are summarized in Table 3. The mean recoveries ranged from 98.92-102.04% for benzoic acid and 97.66-102.47% for sorbic acid excepting at the lowest spiked level of each compound that was 85.61 and 83.62%, respectively. The RSD value of each spiked concentration was < 1% and all RSD values (n=15) were < 8%.

Table 3. Recovery analysis of benzoic acid and benzoic acid.

	Actual concentration (μg/mL)	Calculated concentration (μg/mL)	Recovery (%)	Mean recovery (n=3)	RSD (%)
Benzoic acid	5.00	4.30	85.93	85.61	0.28
		4.28	85.55		
		4.27	85.38		
	10.00	9.90	98.92	98.92	0.92
		9.78	97.77		
		10.00	100.00		
	20.00	19.76	98.78	99.96	0.95
		20.22	101.10		
		20.00	100.00		
	50.00	51.05	102.09	101.12	0.78
		50.57	101.13		
		50.07	100.15		
	100.0	101.79	101.79	102.04	0.17
		102.15	102.15		
		102.17	102.17		
		Total	(n = 15)	97.53	6.25

Table 3. continue.

	Actual concentration (μg/mL)	Calculated concentration (μg/mL)	Recovery (%)	Mean recovery (n=3)	RSD (%)
Sorbic acid	1.02	0.86	84.25	83.62	0.69
		0.86	83.76		
		0.85	82.85		
	2.05	2.00	97.73	97.66	0.92
		1.98	96.53		
		2.02	98.72		
	5.12	5.03	98.31	99.46	0.89
		5.14	100.46		
		5.10	99.61		
	10.24	10.45	102.07	101.34	0.59
		10.38	101.32		
		10.30	100.62		
	20.48	20.93	102.18	102.47	0.21
		21.03	102.68		
		21.00	102.55		
		Total	(n = 15)	96.91	7.10

Method accuracy was also demonstrated by plotting the amount of benzoic acid and sorbic acid found against the amount added. Method accuracy of benzoic acid (y = 1.026x-0.615) and sorbic acid (y= 1.032x-0.172) showed good linearity with r^2 0.9990 and 0.9999, respectively. The result is found to be satisfactory for intended purpose and is adequate for routine analysis.

3.8 Precision

Precision was evaluated in term of system precision (repeatability), method precision (intra-day) and intermediate precision (inter-day).

3.8.1 System precision

System precision or repeatability was determined by performing ten consecutive injections (n=10) of a sample solution and a standard mixture solution containing 100 μg/mL of benzoic acid and 20 μg/mL of sorbic acid. The RSD% of peak area response and retention time was calculated and showed the satisfactory precision of the system (< 1 %) (Table 4).

3.8.2 Method precision

The precision of method was assessed by determining the spiked samples. Blank noodles were spiked with four known levels of each compound (10, 20, 50 and 100 μg/mL for benzoic acid and 2.05, 5.12, 10.25, and 20.48 μg/mL for sorbic acid). Three replicate of each level were prepared and analyzed within the day (intra-day). The method was found to be precise with %RSD values within 0.85-1.05% for benzoic acid and 0.43-1.55% for sorbic acid. The data is shown in Table 4.

3.8.3 Intermediate precision

The RSDs for intermediate precision was evaluated by analysis of benzoic acid and sorbic acid at the same concentration as it was performed for method precision, repeated for two different days by two analysts (inter-day). The %RSD values were 1.57-1.84% (overall = 2.40%) for benzoic acid and 1.42-1.62 (overall = 2.80%) for sorbic acid.

The precision was also investigated by analysis of selected noodle samples for 9

determinations on the same day (intra-day) and on three consecutive days (inter-day). Only benzoic acid was detected with %RSD values of 2.00 and 3.61% for intra-day and inter-day assay, respectively. All results of method and intermediate precision confirmed excellent precision of the proposed HPLC-DAD method (Table 4).

Table 4. System precision, method precision and intermediate precision data.

	Benzoic acid (mean ± SD)	%RSD		Sorbic acid (mean ± SD)	%RSD	
System Precision(n= 10) Peak Area	3317.43 ± 2.14	0.07		1113.01 ± 4.72	0.42	
Retention time	1.80 ± 0.01	0.28		2.32 ± 0.01	0.38	
Spiked sample analysis	Benzoic acid Spiked level (µg/mL)	%RSD Day 1 Analyst 1	Day 2 Analyst 2	Sorbic acid Spiked level (µg/mL)	%RSD Day 1 Analyst 1	Day 2 Analyst 2
	10	1.05	1.13	2.05	0.72	1.13
	20	0.97	1.44	5.12	0.74	1.09
	50	1.02	1.56	10.24	0.43	0.67
	100	0.85	1.05	20.48	1.55	0.25
Method precision	(n = 12)	1.84	1.57		1.41	1.62
Intermediate precision	(n = 24)		2.40			2.80
Noodle analysis	Benzoic acid Measured concentration (µg/mL)	%RSD		Sorbic acid Measured concentration (µg/mL)	%RSD	
Analyst 1 Day 1 (n= 9)	385.08±8.09	2.00		nd	-	
	400.77±8.59	2.14		nd	-	
Analyst 1 Day 2 (n= 3)	400.00±6.80	1.70		nd	-	
Analyst 2 Day 3 (n= 3)	394.83±26.05	6.60		nd	-	
Analyst 3 Day 4 (n= 3)	398.53±14.41	3.61		nd	-	
Total (n= 9)						

3.9 Robustness

The robustness is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its robustness during normal usage. The robustness was tested during the development phase by a slightly vary of volume of injection (± 5 µL), wavelength (± 2 nm), flow rate (± 0.1 mL/min), the percent of organic modifier (± 2% absolute) and pH of the mobile phase (± 0.2 pH unit). The robustness was performed on a standard mixture solution of benzoic acid and sorbic acid and the result was evaluated by calculating the SST (system suitability test) values. The SST parameters kept on unaffected over deliberate changes in the chromatographic conditions (Table 5), illustrating that the method was robust. Moreover, the noodle samples were considered under different parameters and it was observed that there was no change in the area under the peak, confirming the robustness of the method.

Table 5. Robustness data of benzoic acid (BA) and sorbic acid (SA).

Conditions	Range	T		N		Rs		Measured concentration (μg/mL) (n = 3)	
		BA	SA	BA	SA	BA	SA	BA	SA
Injection volume (μL)	5	0.96	1.00	3296	3695	6.80	3.65	381.12 ± 0.11	nd
	10[a]	0.96	1.00	3029	3599	6.77	3.62	380.22 ± 0.34	nd
	15	0.96	1.00	2706	3124	6.70	3.60	381.26 ± 0.43	nd
Wavelength (nm)	232	0.96	1.00	3029	3599	6.77	3.62	380.08 ± 0.32	nd
	234[a]	0.96	1.00	3029	3599	6.77	3.62	380.22 ± 0.34	nd
	236	0.96	1.00	3029	3599	6.77	3.62	380.12 ± 0.43	
Flow rate (mL min^{-1})	0.9	0.96	1.00	3361	3714	6.96	3.73	380.77 ± 0.38	nd
	1.0[a]	0.96	1.00	3029	3599	6.77	3.62	380.22 ± 0.34	nd
	1.1	0.97	1.01	3523	4004	6.68	3.55	379.02 ± 0.20	nd
Percent of methanol	38	0.98	1.02	3090	3636	6.85	3.84	380.31 ± 0.17	nd
	40[a]	0.96	1.00	3029	3599	6.77	3.62	380.22 ± 0.34	nd
	42	0.96	0.99	2901	3714	6.63	3.39	380.22 ± 0.17	nd
Mobile phase pH	4.2	0.98	1.01	3240	3861	7.18	3.05	383.67 ± 1.12	nd
	4.4[a]	0.96	1.00	3029	3599	6.77	3.62	380.22 ± 0.17	nd
	4.6	0.95	1.00	2898	3457	6.59	3.67	384.31 ± 1.28	nd

[a] HPLC method conditions.
T = tailing factor, N = theoretical plates and R = resolution.
nd = not detected.

3.10 Stability of Standard Solutions

The stability of benzoic acid and sorbic acid standard was assessed by analyzing the solutions at 0, 1, 2, 4, 6, 8, 12 and then every 4 h until 48 h after preparation. The %RSD value of the assay of benzoic acid and sorbic acid was 0.07% and 0.42%, respectively (< 1%). The chromatogram showed no peak corresponding to the degradation products and there was no significant change in the peak area response of benzoic acid and sorbic acid. The results reveal that standard solution is stable in methanol for at least 48 h.

3.11 Analysis of Noodle Samples

Five kinds of noodles were collected over a period of five weeks from 6 noodle shops and analyzed by the proposed validated method. No concentration level of preservative was declared on their label. The average level of benzoic acid and sorbic acid found in noodle samples is presented in Table 6. The presence and purity of benzoic acid and sorbic acid was confirmed by DAD spectra. The benzoic acid content was simultaneously detected in all Rice spaghettini and Rice fettuccine samples throughout the five weeks in a wide range of concentration. Over 90 % of both rice noodles contained benzoic acid exceeded the approved regulation limits (≤1,000 mg/kg). Higher quantity of benzoic acid added was probably to extent their shelf life of these products with a higher demand. Nevertheless, benzoic acid

found in Rice vermicelli, Mung bean vermicelli and Ramen was within the allowable regulation limits. Amounts of benzoic acid added varied amongst the kinds of noodle and with time. Wide fluctuation can be attributed due to the lack of quality control and inconsistent in benzoic acid usage. Sorbic acid was detected only in 6 samples of Ramen noodle (from totally 150 analyzed samples) in the low concentration ranging from 2.68 to 5.79 mg/kg. Moreover, the proposed method was applied for the analysis of benzoic acid and sorbic acid in 2 different noodle samples produced for exporting which were labeled as "no preservatives added". No preservative was detected.

Table 6. Quantitative analysis of benzoic acid (BA) and sorbic acid (SA) in 5 types of noodles (mg/kg of noodle sample).

Noodle Type	Nt	Week 1		Week 2		Week 3		Week 4		Week 5		Range found		Average (n=30)	
		BA	SA	BA	SA	BA	SA	BA	SA	BA	SA	BA	SA	BA	SA
Rice spaghettini	6	2082.32	nd	2541.65	nd	2534.80	nd	2958.16	nd	3023.24	nd	713.41-4607.41	nd-2.28	2414.09	0.08
Rice fettuccine	6	2669.21	nd	2137.78	nd	4525.96	nd	2763.54	nd	2480.27	nd	192.04-10499.00	-	3175.46	nd
Rice vermicelli	6	2.96	nd	3.10	nd	1.73	nd	0.87	nd	2.23	nd	nd-42.04	-	4.44	nd
Mung bean vermicelli	6	5.52	nd	7.77	nd	3.79	nd	5.51	nd	2.24	nd	nd-25.17	-	5.62	nd
Ramen	6	136.03	nd	40.20	nd	31.97	nd	47.60	nd	37.95	nd	nd-487.29	nd-5.79	58.75	0.83

Nt = number of analyzed samples per week. Each sample was determined in triplicate.

nd = not detected.

4. CONCLUSION

A simple isocratic HPLC-DAD method was successfully developed for the determination of benzoic acid and sorbic acid in noodles. The sample preparation engaged the extraction of sample with methanol: water (60:40, v/v) that was economic and easy to accomplish without using harmful organic solvent or time-consuming and expensive solid-phase extraction as previous report [19,22,24]. The complete separation of the analytes were achieved in shorter chromatographic run time (3 min) in comparison to all published literature [19-24] and found to be more specific for noodle sample. All validation parameters were within the acceptance range. This proposed method was applied for the analysis of benzoic acid and sorbic acid in the different kinds of noodles and shown satisfying results in terms of accuracy, repeatability and easiness. Additionally, reducing run time caused less in using reagents. The advantage of utilizing fewer reagents could also bring the healthier life and cleaner environment.

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