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Simultaneous determination of synthetic food additives in kimchi by liquid chromatography-electrospray tandem mass spectrometry

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Abstract A new analytical method was developed for the simultaneous determination of seven food additives (Ponceau 4R, Allura Red AC, Amaranth, 4-hydroxymethyl benzoic acid, ethyl-4-hydroxybenzoate, butyl-4-hydroxybenzoate, and saccharin sodium) in kimchi using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. The linearity, sensitivity, selectivity, precision, and accuracy of the method were validated. The limit of detection was 0.00004–0.24 μg/mL, and the limit of quantification was 0.00012–0.8 μg/mL. Recoveries ranged from 85.65 to 120.82%. The method was successful and may help to ensure food safety.

Keywords Food additives · Kimchi · HPLC–MS/MS · Food analysis · Food safety

Introduction

Kimchi is a traditional Korean fermented food that is rich in vitamin C, dietary fiber, β-carotene, minerals, phytochemicals, and Lactobacillus species. Most Koreans eat kimchi every day as a side-dish [1]. Consumption of kimchi is increasing worldwide [2, 3]. Various benefits of kimchi, including anticarcinogenic [4], antioxidant [5], and immune-stimulating effects [6], have been reported.

The prevention of excessive fermentation by *Lactobacillus* is a major concern of the kimchi industry [3]. Food additives such as preservatives, nutrients, colors, flavors, texturizing agents, and natural additives, are used to improve taste or storage life [8]. Food companies use synthetic food additives for economy and efficiency, and kimchi is no exception [7].

Food additives used in kimchi include synthetic dyes, preservatives such as para-hydroxybenzoic acid, and saccharin, an artificial sweetener. However, synthetic dyes can cause cancer [8, 9] if taken in excess, and may also cause allergies, asthma [10–12], DNA damage [13], hyperactivity [14], and other disorders. Therefore, restrictions on synthetic colors and food additives apply internationally [15, 16]. Simultaneous analysis of multiple food additives in foods is important in order to comply with regulations on the import and export of processed foods, including kimchi, to investigate the intake of food additives, to provide a basis for risk evaluation, and to respond to the increasing demand for safe food.

A number of methods for the determination of preservatives have been reported, including high-performance liquid chromatography (HPLC) [17, 18], gas chromatography (GC) [19], thin-layer chromatography (TLC) [20, 21], capillary electrophoresis (CE) [22, 23], liquid chromatography-mass spectrometry (LC–MS) [24, 25], and gas chromatography-mass spectrometry (GC–MS) [26].

In this study, we developed the first highly sensitive and accurate LC–MS/MS method for the simultaneous analysis of 7 food additives from kimchi: Ponceau 4R, Allura Red AC, Amaranth, 4-hydroxymethyl benzoic acid, ethyl-4-hydroxybenzoate, butyl-4-hydroxybenzoate, and saccharin sodium. We also validated analytical parameters such as sensitivity, precision, mass accuracy, selectivity and linearity. This method has been successfully applied to

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monitoring and ensuring the safety of kimchi samples from a local market.

Materials and methods

Chemicals

Ponceau 4R, Allura Red AC, Amaranth, 4-hydroxymethyl benzoic acid, ethyl-4-hydroxybenzoate, butyl-4-hydroxybenzoate, saccharin, ammonium formate, and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were obtained from Fisher (Pittsburgh, PA, USA). Membrane filters (0.22 μm) were purchased from Whatman (Maidstone, UK).

Sample extraction

Kimchi samples were purchased from local supermarkets in Korea. Approximately 5 g was weighed into a 50 mL flask; 25 mL of 60% acetonitrile were added, followed by sonication for 10 min. The extract was centrifuged at 3000 rpm (10 min) then filtered through a 0.22 μm membrane prior to LC–MS/MS.

LC–MS/MS analysis

LC–MS/MS analysis was performed on an Agilent 1260 HPLC system coupled with an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Diegem, Belgium). We used a Cadenza CD C18 column, 100 mm × 2 mm with a 3-μm particle diameter (Imtakt, Kyoto, Japan). The injection volume was 3 μL, and the oven was held at room temperature. Mobile phase A was 20 mM ammonium formate and 0.1% formic acid in water, mobile phase B was 7:3 (by volumn) acetonitrile:methanol with 0.1% formic acid. The optimized chromatographic conditions were as follows: 10% B to 0.1 min, linear gradient to 100% B between 0.1 and 10.0 min, 100% B until 13.0 min, and then return to 10% B in 1.0 min. The run time was 20.0 min, and the flow rate was 0.4 mL/min. The triple quadrupole tandem mass spectrometer operated under multiple reaction monitoring mode (MRM) for quantitative and qualitative analysis. The optimized electrospray ionization(ESI) conditions were as follows: ESI was carried out at atmospheric pressure in negative ion mode; gas temperature, 150 °C; gas flow, 9 L/min; sheath gas temperature, 350 °C; sheath gas flow, 11 L/min; capillary voltage, 3000 V. Detailed MRM settings are illustrated in Table 1.

Method validation

The analytical method was validated in terms of linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). Quantitative analysis was carried out using external standard calibration. Calibration solutions were prepared by appropriate dilution of intermediate mixed standard solutions in acetonitrile.

Accuracy was evaluated in terms of percentage recovery. For recovery studies, blank kimchi samples were spiked prior to the extraction step (*sample extraction*). A weighed sample was added to a small volume of working solution of analyte. The extraction was then carried out as previously described. For precision, three concentrations (2, 10, and 20(μg/mL) were investigated. Each experiment was conducted three times, and the data were averaged. The average recovery and relative standard deviation (RSD) were calculated. The sensitivity of the method was evaluated by estimating LOD and LOQ at signal-to-noise ratios of 3 and 10, respectively. The intra- and inter-day precisions of the methods were examined by analysis of six replicates of standard solution for each of three concentrations (2, 10 and 20 μg/mL) over 3 days. Intra-day and inter-day was assessed by analyzing standard solutions.

Results and discussion

Method validation

Individual analyte solutions were directly infused into the mass spectrometer in positive and negative ion scan modes. Under the selected ESI conditions, all compounds showed higher sensitivity in negative than in positive mode; the most abundant ion was $[M–H]^-$ for all analytes. Therefore, the deprotonated form of each compound (the $[M–H]^-$ ion) was selected as the parent ion for Q1 spectra and used as the precursor for Q3 product ion spectra. The detailed data was shown in Fig. 1. Total ion chromatogram (TIC) and MRM chromatogram of the compounds are shown in Fig. 1. Figure 1(A) is the TIC of the seven food additives standards in acetonitrile and Fig. 1(B) is the seven food additives standards in blank kimchi sample.

To evaluate the feasibility of HPLC–MS/MS for quantitative analyses of food additives in kimchi, the analytical performance of the proposed method was examined. Linear range, correlation coefficient (r^2), LOD, LOQ, and recovery are listed in Table 2. To test linearity, three concentrations of food additives within the range 2–10 μg/mL were prepared. Analysis was performed in triplicate. Excellent linearity was achieved for each food additive, with a linear regression coefficient of $r^2 \geq 0.9947$. There was no difference of primary slope and intercept of the

Table 1 Tandem mass spectrometry parameters of seven food additives

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Fragmentation voltage (V)	Collision energy (V)
Allura Red AC	5.2	451	207	190	15
		451	371	190	35
Ponceau 4R	4.2	537	302	150	10
		537	429	150	15
Amaranth	3.3	537	317	230	25
		537	457	230	25
Saccharin	3.5	182	42	130	25
		182	106	130	25
4-hydroxymethyl benzoic acid	6.7	151	92	90	15
		151	136	90	15
Ethyl-4-hydroxybenzoate	7.6	165	92	90	20
		165	137	90	20
Butyl-4-hydroxybenzoate	9.2	193	92	110	25
		193	137	110	25

The first transition was used for quantification and the second as a qualifier

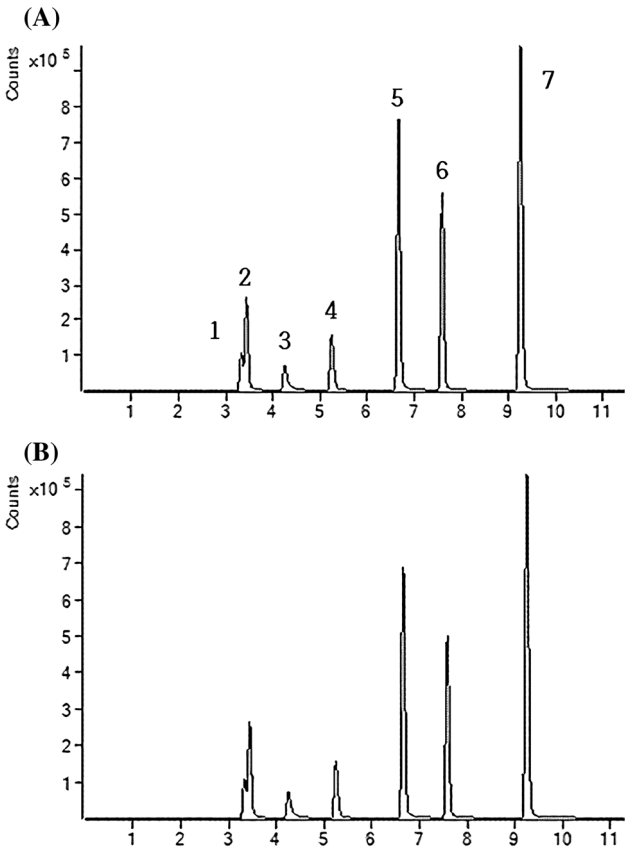

Fig. 1 HPLC-MS/MS total ion chromatogram (**A**) TIC of standards in acetonitrile, (**B**) TIC of standards in blank kimchi sample, (**C**) fragment ion (*1* Amaranth, *2* Saccharin, *3* Ponceau 4R, *4* Allura Red AC, *5* 4-hydroxymethyl benzoic acid, *6* Ethyl-4-hydroxybenzoate, *7* Butyl-4-hydroxybenzoate)

calibration equation $y = ax + b$ of the between kimch matrix matched calibration and acetonitrile calibration.

The sensitivity of the method was assessed by determining the LOD and LOQ. The LOD and LOQ were estimated with concentrations giving a signal-to-noise ratio of 3 and 10, respectively. As shown in Table 2, the LOD ranged from 0.00004 to 0.24 μg/mL, while the LOQ ranged from 0.00012 to 0.8 μg/mL.

Precision is expressed as percent relative standard deviation (% RSD). Overall intra- and inter-day RSDs were less than 5.0%. The results showed good reproducibility and precision (Table 3). RSD is less than 5% may be found more satisfactory than in SANCO guidelines, it can be said that the precision of this method is acceptable. Recovery was tested by adding mixed standard solutions at three different concentrations (2, 10 and 20 μg/mL) to kimchi samples. The results are shown in Table 3: recoveries were in the range of 85.65–120.82%. It can be concluded that the recovery values for the food additives in kimchi were acceptable. In addition, in the SANCO guideline, the recovery of the simultaneous multi component analysis is acceptable 70–120% with slight difference range [27]. Therefore, the proposed analytical method was sufficiently accurate. The validation data showed that the method provides good linearity, sensitivity, selectivity, precision, and accuracy for simultaneous analysis of seven food additives in kimchi.

Application to real samples

In Korea, synthetic food additives are not permitted in kimchi. To detect illegal food additives in kimchi and to

Fig. 1 continued

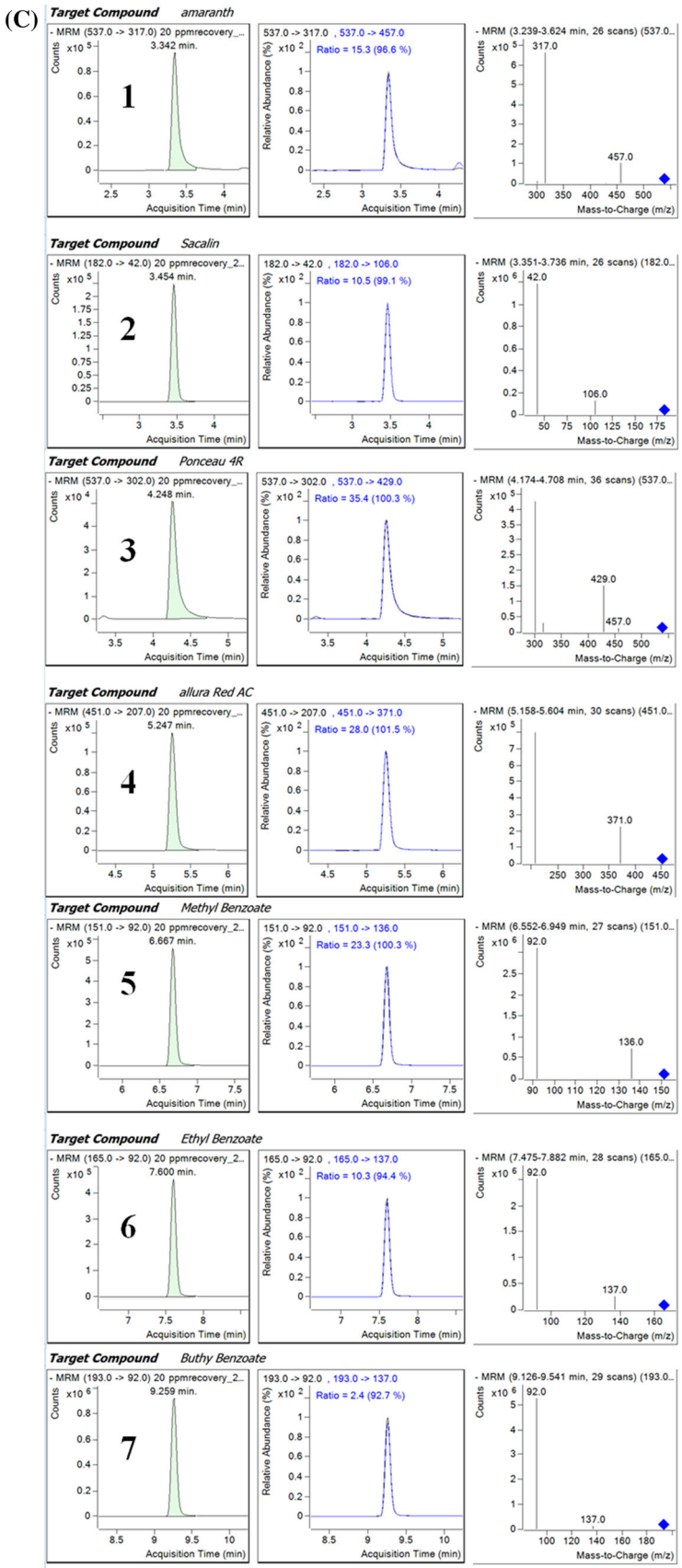

Table 2 Linear equation, correlation coefficient, LOD, and LOQ for detection of seven food additives

Compound	Linear equation	Correlation coefficient	LOD[a] (μg/mL)	LOQ[b] (μg/mL)
Allura Red AC	Y = 35,306.53X + 57,300.64	0.9961	0.00042	0.00140
Ponceau 4R	Y = 20,443.12X + 4974.43	0.9963	0.24000	0.80000
Amaranth	Y = 28,053.05X − 9582.95	0.9986	0.24000	0.80000
Saccharin	Y = 48,924.87X + 25,177.49	0.9968	0.03000	0.10000
4-hydroxymethyl benzoic acid	Y = 146,657.51X + 40,424.35	0.9974	0.00070	0.00230
Ethyl-4-hydroxybenzoate	Y = 104,848.55X + 42,716.31	0.9947	0.00004	0.00012
Butyl-4-hydroxybenzoate	Y = 214,361.07X + 202,419.14	0.9978	0.00011	0.00037

[a]Limit of detection

[b]Limit of quantification

Table 3 Recoveries and relative standard deviations (%) of seven food additives (n = 6, n = 18)

Compound	Concentration (μg/mL)	Precision (% RSD[a])				Recovery (%)[b] ± SD[c]
		Intra-day (n = 6)	Inter-day (n = 18)			
		Day	Day 1	Day 2	Day 3	
Allura Red AC	0.002	5.6	4.8	4.2	6.1	105.79 ± 1.4
	0.02	4.2	4.8	4.7	5.1	
	0.1	5.3	5.0	5.2	3.8	
Ponceau 4R	1	4.6	3.8	3.1	4.0	85.65 ± 3.5
	10	4.8	3.5	4.5	4.3	
	50	4.9	4.1	4.0	4.3	
Amaranth	1	3.6	3.8	3.1	4.0	96.78 ± 3.0
	10	4.0	3.5	4.5	4.3	
	50	4.2	4.1	4.0	4.3	
Saccharin	0.2	0.6	3.8	3.9	4.1	110.99 ± 2.4
	2	0.9	3.2	4.0	3.9	
	10	1.1	4.1	3.3	4.3	
4-hydroxymethyl benzoic acid	0.005	1.5	1.5	1.4	1.4	101.15 ± 4.2
	0.05	1.6	1.6	1.3	1.3	
	0.25	1.4	1.1	1.4	1.1	
Ethyl-4-hydroxybenzoate	0.0002	1.4	1.5	1.5	1.3	111.40 ± 1.5
	0.002	1.1	1.7	1.8	1.4	
	0.01	1.5	1.4	1.3	1.2	
Butyl-4-hydroxybenzoate	0.0005	1.4	1.9	2.0	1.2	120.82 ± 1.3
	0.005	1.4	2.0	2.5	1.8	
	0.025	1.6	2.0	2.3	1.7	

[a]Relative standard deviation (%)

[b]Average recoveries at three spike levels

[c]Standard deviation

evaluate the effectiveness of the proposed method, the developed HPLC–MS/MS method was applied to the analysis of seven food additives (Ponceau 4R, Allura Red AC, Amaranth, 4-hydroxymethyl benzoic acid, ethyl-4-hydroxybenzoate, butyl-4-hydroxybenzoate, and saccharin sodium) in kimchi purchased from local supermarkets in Korea. Samples were analyzed in triplicate. In most cases, the seven food additives were not found.

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