Simultaneous and rapid analysis of chemical preservatives in processed animal products by ultra-performance liquid chromatography

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Abstract An ultra-performance liquid chromatography-tunable ultraviolet method was optimized and validated for the simultaneous analysis of nine chemical preservatives in processed animal products. The limits of detection and quantification for the preservatives were within the ranges of 0.02–0.23 and 0.07–0.76 µg/mL, respectively. The relative standard deviations for intraday analyses of retention time and peak area were 0.00–0.23 and 0.03–2.93%, respectively, whereas, those for interday analyses were 0.67–2.30 and 2.12–5.37%, respectively. Of the nine preservatives spiked into six different animal products, dehydroacetic acid spiked into soft cheese exhibited the lowest recovery rate of $72.1 \pm 0.36\%$ at the lowest concentration (0.25 g/kg). Comparing data between UPLC and high-performance liquid chromatography with a 5% significance level, the t-statistic was 1.42. Moreover, sorbic acid was detected in 16 animal products (0.11–2.49 g/kg) when 278 products were analyzed for preservatives.

Keywords Ultra-performance liquid chromatography · High-performance liquid chromatography · Preservatives · Processed animal products · Simultaneous determination

Introduction

The use of preservatives to prohibit or delay the deterioration of foods has become an important treatment in modern food technology with the increase in production of processed and convenience foods [1]. Preservatives have been recognized as effective agents for increasing the shelf life of foods by controlling the growth of microorganisms and preventing contamination by a wide range of bacteria owing to their high effectiveness, inexpensiveness, and broad availability. Chemical preservatives used in commercial applications and analyzed in the laboratory include propionic acid (PA), benzoic acid (BA), sorbic acid (SA), methyl 4-hydroxybenzoate (MHB), dehydroacetic acid (DHA), ethyl 4-hydroxybenzoate (EHB), isopropyl 4-hydroxybenzoate (IPHB), propyl 4-hydroxybenzoate (PHB), isobutyl 4-hydroxybenzoate (IBHB), and butyl 4-hydroxybenzoate (BHB). If these chemicals are added to foodstuffs immoderately without regulation, they can cause harmful effects in individuals who consume the treated foodstuffs. Generally, their negative influence on human health is thought to be related to their potential capacity to induce allergic contact dermatitis, convulsion, and hives, among other conditions [2]. In particular, MHB, EHB, PHB, and BHB are thought to possess estrogenic activity in several in vitro assays and in animal models in vivo [3], and the estrogenicity of MHB has been reported to be increased by the addition of an aryl group and by lengthening or branching the alkyl group [4]. Moreover, alkyl esters of *p*-hydroxybenzoic acid (parabens) are known to be present at high levels in human breast tumors [5]. IBHB is also involved in or contributes to the occurrence of breast cancer [3]. Hence, the development of rapid and simultaneous analytical methods that can be used to quantify chemical preservatives in an effective, rapid, and

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convenient manner may be important for reducing health risks related to chemicals.

High-performance liquid chromatography (HPLC) has been used as an analytical method for preservatives [6, 7]. Additionally, gas chromatography (GC) has been recognized as a useful analytical tool [8, 9], and capillary electrophoresis [10, 11], liquid chromatography mass spectrometry [12, 13], and GC-mass spectrometry [14] have been examined as potential analytical methods for simultaneous determination of various chemicals. However, these methods are relatively time-consuming, laborious, and inefficient. The recently developed liquid chromatography-based method designated ultra-performance liquid chromatography (UPLC) can surpass existing tools with regard to speed, flow rate, resolution, and quantitative response. The use of UPLC has been reported in several studies in which chemical preservatives in foodstuffs, cosmetics, and pharmaceuticals were analyzed [15, 16].

The aim of this study was to develop an advanced simultaneous analytical method for nine different types of quasi or nonpolar preservatives (BA, SA, MHB, DHA, EHB, IPHB, PHB, IBHB, and BHB) present in processed animal products, such as cheese, ham, butter, and sausage, as stipulated by the Korean Ministry of Food and Drug Safety (MFDS) guideline (and the bylaws of other nations) for monitoring of preservatives and apply this information for field monitoring. To achieve this, the UPLC method developed in this study was validated in terms of accuracy, selectivity, detection limit, quantification limit, linearity, and recovery rate, and a total of 278 animal product samples, imported mainly from the United States of America and Europe, were analyzed by both UPLC and HPLC to monitor chemical preservatives (commonly called synthetic preservatives) and compare the analytical performance of UPLC with that of HPLC, the most commonly used analytical method for field monitoring.

Materials and methods

Food samples

A total of 278 test samples of processed animal products imported mainly from the United States of America and Europe, including 46 raw milk cheeses, 69 soft cheeses, seven rigid cheeses, 63 semi-rigid cheeses, 32 process cheeses, 16 sausages, 19 hams, eight butters, 12 seasoned meats, three bacons, two natural casings, and one beef powder, were chosen randomly using a computer to include representative samples. The samples were taken from refrigerated samples collected by the Korean Ministry of Food and Drug Safety (MFDS).

Standard preservatives and chemicals

Reference standard materials (BA, SA, MHB, DHA, EHB, IPHB, PHB, IBHB, and BHB) with purities of at least 98.0% were purchased from Sigma Chemical Co. (Steinheim, Germany). HPLC-grade acetonitrile (ACN), ethanol (EOH), and methanol were purchased from Burdick and Jackson (Muskegan, MI, USA). Analytical-grade ammonium acetate (AA) and phosphoric acid (PPA) were obtained from Merck KGaA (Darmstadt, Germany). Ultra-pure water (UW, 0.1–0.5 MΩ cm) was purified using an AQUAMAX-ULTRA (Young Lin Instrument, Anyang, Korea).

Preparation of standard stock solution and working solution

A standard stock solution was prepared at 10,000 µg/mL by dissolving 1.000 g (accurately weighed to 0.0001 g) of the reference standard materials (BA, SA, MHB, DHA, EHB, IPHB, PHB, IBHB, and BHB) in 100-mL volumetric flasks with EOH. The standard stock solutions were shaken and sonicated to obtain a homogeneous suspension. The stock solution was transferred into a brown bottle and stored at 4 °C in a refrigerator (Sanyo, Gifu, Japan). Before preparing working solutions (0.1–20.0 µg/mL), the stock solution was diluted to 100.0 µg/mL by adding EOH into a volumetric flask. Different working solutions were prepared by adding appropriate amounts of the diluted stock solution (100.0 µg/mL) into EOH.

Spiking recovery test

The recovery test was performed by spiking six different samples, including butter, mixed sausage, mixed press ham, natural casing, raw cheese, and soft cheese, in which no preservatives were detected, with nine chemical preservatives, including BA, SA, MHB, DHA, EHB, IPHB, PHB, IBHB, and BHB, at three fortification levels of 0.25, 0.50, and 1.00 g/kg. The spiked samples were pretreated and analyzed as described below. The recovery rate was calculated by comparing the amount of each preservative detected in the spiked sample with the amount of each standard material added to the sample.

Pretreatment of samples

Animal product samples were pretreated according to the MFDS's sample preparation procedure as follows. First, 1.000 g (accurately weighed to 0.0001 g) of animal product samples, such as butter, cheese, ham, and sausage, was weighed in a 50-mL conical tube. Forty milliliters of EOH was added to each sample, and samples were shaken for

15 min with an MMS-510 multi shaker (EYELA, Tokyo, Japan). The sample solution was centrifuged at 4000 rpm ($3515\times g$) for 7 min with a Combi-514R refrigerated centrifuge (Hanil Science Industrial, Inchon, Korea) and kept in a deep freezer (Sanyo, Osaka, Japan) at $-80\text{ }^{\circ}\text{C}$ for 2 h. Subsequently, about 1 mL of the upper layer was filtered through a $0.2\text{-}\mu\text{m}$ Whatman PTFE syringe filter (GE Osmonics, Trevose, PA, USA) into an LC vial.

UPLC analysis

Instrumental analysis was performed using an ACQUITY UPLC system equipped with an ACQUITY Tunable Dual Wavelength Ultraviolet/Visible (TUV) Detector (240 nm) and Empower2 Chromatography Manager software (Waters, Milford, MA, USA). An ACQUITY BEH C_{18} column ($100\text{ mm} \times 2.1\text{ mm}$, $1.7\text{ }\mu\text{m}$ particle size; Waters) was used for the separation of preservatives, and the column temperature was maintained at $40\text{ }^{\circ}\text{C}$. The optimal resolution was obtained using a dual mobile phase at a flow rate of 0.45 mL/min . Solvent A was 100% ACN and solvent B was 25 mM AA/ACN (80:20, v/v) adjusted to pH 4.5 with 10% PPA. The gradient elution mode was: 0–1.5 min, 20% A; 1.5–3.5 min, 30% A; 3.5–4.0 min, 40% A; 4.0–4.5 min, 100% A; 4.5–6.0 min, back to initial conditions and equilibration of the column. The sample temperature was $4\text{ }^{\circ}\text{C}$, and the injection volume was $2\text{ }\mu\text{L}$. The detection wavelength was set at 240 nm.

HPLC analysis

An Agilent HPLC 1200 system equipped with a multiple wavelength UV detector (MWD) was adopted for the comparative analysis of the detection ability of the UPLC system over chemical preservatives in animal products. HPLC analysis was conducted using a Shiseido Capcell-pack C_{18} column ($250\text{ mm length} \times 4.6\text{ mm i.d.}$, $5\text{ }\mu\text{m}$ particle size; Tokyo, Japan). The column temperature was maintained at $30\text{ }^{\circ}\text{C}$. Solvent A was 0.1% PPA, and solvent B was 100% ACN. The gradient elution mode was as follows: 0–10 min, 90% A; 10–26 min, 60% A; 26–28 min, 50% A; 28–30 min, 0% A; 30–35 min, back to initial conditions and equilibration of the column at a flow rate of 1.0 mL/min . The sample temperature was $4\text{ }^{\circ}\text{C}$, and the injection volume was $20\text{ }\mu\text{L}$. The detection wavelength of MWD was set at 235 nm.

Statistical evaluation

All experiments were carried out in triplicate unless stated otherwise. The results given are the average values. SPSS software (IBM Corporation, Armonk, NY, USA) was used to perform all statistical calculations and data analyses.

Results and discussion

Optimization of the mobile phase and gradient elution mode

The mobile phase conditions were modified from that described in a previous report [17], as follows: 25 mM AA-ACN (80:20, v/v) was used as solvent B in lieu of 20 mM potassium dihydrogen phosphate; the pH of the mobile phase was adjusted to 4.5 with PPA. The optimized mobile phase conditions were obtained to give the best resolution after comparing several mobile phases (data not shown). Under the optimized conditions, the gradient elution time was shortened to less than 5 min at a flow rate of 0.45 mL/min . The total run time was set at 6 min; only half the time used in the previous report [17]. Consequently, successive and rapid separation of nine chemical preservatives was accomplished by optimizing analytical conditions, as shown in Fig. 1(A). Considering the merit of the UPLC method, in terms of more rapid analysis, the optimized conditions appeared to be highly efficient for the determination of chemical preservatives.

Validation of the method

The specificity of the developed method was validated by comparing original samples (without spiking with reference standard materials) with reference spiked samples and identifying peaks for each of chemical preservatives as follows. At first, samples such as rigid processed cheese, ham, mixed processed cheese, half rigid cheese, butter, raw cheese, and soft cheese were pretreated and used for analysis. BA was detected in rigid processed cheese, mixed processed cheese, and half rigid cheese. A high level (1.43 g/kg) of SA was also identified in mixed processed cheese. No preservatives were detected in the other samples. Subsequently, a constant concentration ($500.0\text{ }\mu\text{g/mL}$) of standard materials (BA, SA, MHB, DHA, EHB, IPHB, PHB, IBHB, and BHB) was spiked into non-preservative-detected samples, including butter, raw cheese, and soft cheese. As shown in Fig. 1(B), there was no interference between peaks. Finally, the resolution between peaks was calculated. Resolution was not less than 1.25 for the individual peaks on the UPLC chromatogram (Table 1).

Sensitivity tests were carried out at eight concentration levels of chemical preservatives: 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and $20.0\text{ }\mu\text{g/g}$. Linear calibration curves were plotted between the peak area and the corresponding concentrations of respective compounds, and the resulting correlation coefficients for all nine compounds in the calibration range were over 0.99999. The linear equations, correlation

Fig. 1 Typical UPLC chromatograms of synthetic preservatives in standard solution (A) and in spiked food sample (B). The standard solution and spiked food sample (raw cheese) contained the standard materials of preservatives at final concentrations of 20.0 µg/mL and 0.01 g/kg, respectively. BA benzoic acid, SA sorbic acid, MHB methyl 4-hydroxybenzoate, DHA dehydroacetic acid, EHB ethyl 4-hydroxybenzoate, IPHB isopropyl 4-hydroxybenzoate, PHB propyl 4-hydroxybenzoate, IBHB isobutyl 4-hydroxybenzoate, BHB butyl 4-hydroxybenzoate

Table 1 Resolution, calibration factor, LOD and LOQ for nine synthetic preservatives

Compounds	Resolution ^a	Linear equations	Correlation coefficient (r)	Linear range (µg/g)	LOD (µg/g)	LOQ (µg/g)
BA	1.79	$Y = 8.32 \times 10^3 X + 2.37 \times 10^2$	0.99999	0.1–20.0	0.08	0.27
SA	1.79	$Y = 3.52 \times 10^4 X + 7.88 \times 10^3$	0.99999	0.1–20.0	0.02	0.07
MHB	2.11	$Y = 1.59 \times 10^4 X + 3.88 \times 10^2$	0.99999	0.1–20.0	0.05	0.18
DHA	2.42	$Y = 4.73 \times 10^3 X - 7.71 \times 10^1$	0.99999	0.1–20.0	0.23	0.76
EHB	3.62	$Y = 1.42 \times 10^4 X + 7.25 \times 10^1$	1.00000	0.1–20.0	0.06	0.21
IPHB	8.59	$Y = 1.33 \times 10^4 X + 2.40 \times 10^2$	1.00000	0.1–20.0	0.07	0.22
PHB	1.57	$Y = 1.37 \times 10^4 X + 2.44 \times 10^1$	1.00000	0.1–20.0	0.07	0.22
IBHB	11.50	$Y = 1.20 \times 10^4 X + 2.08 \times 10^2$	1.00000	0.1–20.0	0.08	0.26
BHB	1.25	$Y = 1.30 \times 10^4 X - 3.53 \times 10^1$	1.00000	0.1–20.0	0.08	0.26

^aResolution was calculated via Empower2 Chromatography Manager software

BA benzoic acid, SA sorbic acid, MHB methyl 4-hydroxybenzoate, DHA dehydroacetic acid, EHB ethyl 4-hydroxybenzoate, IPHB isopropyl 4-hydroxybenzoate, PHB propyl 4-hydroxybenzoate, IBHB isobutyl 4-hydroxybenzoate, BHB butyl 4-hydroxybenzoate

coefficients, and linear ranges are given in Table 1. The limit of detection (LOD) was taken at a signal to noise ratio of 3, while the limit of quantification (LOQ) was taken at a signal to noise ratio of 10. As shown in Table 1, the resulting LODs for nine chemical preservatives ranged from 0.02 to 0.23 µg/g, and the LOQs ranged from 0.07 to 0.76 µg/g.

The repeatability test for retention time (RT) and peak area was carried out by injecting the standard mixtures of nine preservatives at concentrations of 0.5, 1.0, 2.0, and 5.0 µg/mL three times a day. As shown in Table 2, the relative standard deviation (RSD) of intraday precision for RT and peak area ranged from 0.00 to 0.23% and from 0.03 to 2.93% respectively. Moreover, the reproducibility test

Table 2 The intra- and interday precision of UPLC-TUV analyzing system

Conc ($\mu\text{g}/\text{mL}$)	Intraday (% RSD), n = 3								Interday (% RSD), n = 5							
	0.5		1.0		2.0		5.0		1.0		2.0		5.0		10.0	
	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area
BA	0.23	0.70	0.06	0.12	0.17	0.41	0.11	0.20	0.78	4.05	0.74	3.57	0.67	2.94	0.71	3.46
SA	0.15	0.13	0.00	0.18	0.06	0.03	0.12	0.09	1.15	4.28	1.07	4.53	1.02	4.42	1.09	5.37
MHB	0.05	0.61	0.05	0.18	0.09	0.09	0.05	0.19	1.54	3.09	1.47	2.77	1.41	2.56	1.43	2.95
DHA	0.08	2.93	0.04	1.65	0.09	0.63	0.09	0.18	1.54	3.81	1.48	2.49	1.44	2.83	1.46	3.12
EHB	0.04	0.54	0.06	0.24	0.10	0.25	0.07	0.11	2.14	3.39	2.04	2.81	2.00	2.12	2.00	2.76
IPHB	0.03	0.38	0.05	0.26	0.07	0.41	0.05	0.19	2.26	3.47	2.21	2.91	2.17	2.35	2.18	3.08
PHB	0.03	0.22	0.05	0.52	0.09	0.22	0.07	0.28	2.30	3.17	2.22	2.36	2.16	2.20	2.16	2.57
IBHB	0.02	0.50	0.05	0.43	0.07	0.22	0.07	0.13	2.25	3.18	2.16	2.55	2.10	2.22	2.14	2.62
BHB	0.03	0.51	0.03	0.61	0.06	0.32	0.07	0.15	2.23	3.01	2.15	2.76	2.08	2.78	2.11	3.08

BA benzoic acid, SA sorbic acid, MHB methyl 4-hydroxybenzoate, DHA dehydroacetic acid, EHB ethyl 4-hydroxybenzoate, IPHB isopropyl 4-hydroxybenzoate, PHB propyl 4-hydroxybenzoate, IBHB isobutyl 4-hydroxybenzoate, BHB butyl 4-hydroxybenzoate

was performed by injecting the standard mixtures of nine preservatives at concentrations of 1.0, 2.0, 5.0, and 10.0 $\mu\text{g}/\text{mL}$ daily for 5 days. As shown in Table 2, the RSD of interday precision for RT and peak area ranged from 0.67 to 2.30% and from 2.12 to 5.37%, respectively. The RSD for peak area was found to be higher than 5% only when SA was examined at a concentration of 10.0 $\mu\text{g}/\text{mL}$. Taken together, the precision of the developed method was within the expected range and fulfilled the Association of Official Analytical Chemists (AOAC) criteria and recommendations (RSD, 5%).

The recovery test was performed to validate the accuracy of the developed method by spiking six different samples, including butter, mixed sausage, mixed press ham, natural casing, raw cheese, and soft cheese, in which no preservatives were detected, with chemical preservatives. The results of accuracy studies are given in Table 3. The recovery rates were in the range of 72.1–93.4%, with RSDs between 0.11 and 3.22%, and the lowest recovery rate was obtained from soft cheese spiked with DHA at 0.25 g/kg. Consequently, the recovery rates for all spiked samples, except natural casing and soft cheese, fulfilled AOAC criteria and recommendations, although some losses of analytes were observed in the recovery test. Therefore, the recovery study of the chemical preservatives in the matrix of most processed animal products was correct, and the developed method using UPLC was sufficiently accurate.

Levels of chemical preservatives in processed animal products in a field study

A total of 278 animal product samples, including 12 types of animal products, i.e., raw cheese, soft cheese, rigid cheese, semi-rigid cheese, processed cheese, ham, sausage, butter, seasoned meat, bacon, beef powder, and natural casing, were analyzed for nine different chemical preservatives by both UPLC and HPLC. The quantification was carried out using matrix-matched calibration curves. Of the 278 samples, as shown in Table 4, SA was detected by UPLC in 16 animal product samples (approximately 5.8% of the samples) at a range of 0.11–2.49 g/kg (nine processed cheeses, three raw cheeses, two semi-rigid cheeses, one soft cheese, and one sausage), which had been imported from the United States of America, Europe, and Australia. No other preservatives were detected in all samples. Although SA is considered generally recognized as safe for use in food [18], SA has been reported to be moderately toxic, particularly when ingested, and has irritant action on human skin [19]. In rare cases, the excessive use of SA can cause contact allergy urticaria, wheal, and flare reactions [2]. For various reasons and to avoid allergy-like symptoms, many countries have enacted regulations stipulating the maximum amount of SA permissible in specific foods. In both Korea and the United States of America, the maximum allowable level is 3.0 g/kg in cheese and related cheese products [20, 21]. The maximum level of SA is also set at 2 g/kg in processed meat products in Korea [20] and 3.0 g/kg in processed foods in Japan [22]. In the present study, two processed cheese product samples were found to contain approximately 2.4–2.5 g/kg SA, which nearly reached the limit. These results indicated

Table 3 Recoveries of the spiked preservatives from the 6 different kinds of animal products

Compounds	Spiked level (g/kg)	Recovery (%) (mean $\pm$ RSD)					
		Butter	Mixed sausage	Mixed press ham	Natural casing	Raw cheese	Soft cheese
BA	0.25	89.3 $\pm$ 1.54	84.0 $\pm$ 1.89	86.1 $\pm$ 1.37	81.0 $\pm$ 1.80	86.3 $\pm$ 0.20	79.9 $\pm$ 0.40
	0.50	85.1 $\pm$ 0.28	83.7 $\pm$ 1.03	84.4 $\pm$ 1.92	81.6 $\pm$ 0.68	86.5 $\pm$ 0.22	81.3 $\pm$ 0.23
	1.00	84.3 $\pm$ 0.57	81.8 $\pm$ 2.37	83.0 $\pm$ 1.17	81.0 $\pm$ 0.56	85.2 $\pm$ 0.72	76.7 $\pm$ 4.33
SA	0.25	90.2 $\pm$ 1.55	86.7 $\pm$ 1.84	88.1 $\pm$ 0.86	81.5 $\pm$ 0.70	86.5 $\pm$ 0.18	80.4 $\pm$ 0.41
	0.50	86.2 $\pm$ 0.36	86.2 $\pm$ 1.11	86.4 $\pm$ 2.02	82.2 $\pm$ 0.87	86.7 $\pm$ 0.16	82.1 $\pm$ 0.11
	1.00	85.7 $\pm$ 0.60	84.2 $\pm$ 2.51	85.2 $\pm$ 1.15	81.7 $\pm$ 0.26	85.5 $\pm$ 0.69	79.9 $\pm$ 0.58
MHB	0.25	90.2 $\pm$ 1.71	87.5 $\pm$ 1.74	88.8 $\pm$ 0.89	83.9 $\pm$ 1.07	85.8 $\pm$ 0.15	79.8 $\pm$ 0.55
	0.50	86.3 $\pm$ 0.39	86.7 $\pm$ 1.11	86.6 $\pm$ 1.88	86.5 $\pm$ 0.38	87.3 $\pm$ 0.22	83.2 $\pm$ 0.20
	1.00	85.8 $\pm$ 0.58	84.9 $\pm$ 2.58	85.7 $\pm$ 1.18	84.4 $\pm$ 0.19	85.9 $\pm$ 0.51	80.9 $\pm$ 0.57
DHA	0.25	87.6 $\pm$ 1.60	83.9 $\pm$ 1.89	87.5 $\pm$ 0.79	73.8 $\pm$ 1.59	82.9 $\pm$ 0.79	72.1 $\pm$ 0.36
	0.50	88.4 $\pm$ 0.29	86.8 $\pm$ 1.00	89.2 $\pm$ 1.48	76.5 $\pm$ 2.06	84.3 $\pm$ 0.62	73.6 $\pm$ 0.79
	1.00	93.4 $\pm$ 0.40	88.5 $\pm$ 2.43	91.7 $\pm$ 1.48	77.2 $\pm$ 1.11	86.0 $\pm$ 0.62	74.5 $\pm$ 0.77
EHB	0.25	89.4 $\pm$ 1.90	86.4 $\pm$ 1.72	87.3 $\pm$ 0.99	83.8 $\pm$ 0.52	86.0 $\pm$ 0.37	81.7 $\pm$ 0.45
	0.50	86.3 $\pm$ 0.50	87.1 $\pm$ 1.35	86.8 $\pm$ 2.34	85.6 $\pm$ 0.49	86.3 $\pm$ 0.23	82.9 $\pm$ 0.18
	1.00	86.3 $\pm$ 0.74	85.5 $\pm$ 2.77	86.3 $\pm$ 1.03	84.5 $\pm$ 0.28	85.8 $\pm$ 0.73	80.8 $\pm$ 0.68
IPHB	0.25	89.4 $\pm$ 1.65	86.9 $\pm$ 1.79	87.7 $\pm$ 1.10	84.5 $\pm$ 0.31	86.6 $\pm$ 0.24	80.8 $\pm$ 0.58
	0.50	85.8 $\pm$ 0.66	86.7 $\pm$ 1.63	86.6 $\pm$ 2.82	85.8 $\pm$ 0.59	86.3 $\pm$ 0.24	82.0 $\pm$ 0.16
	1.00	85.6 $\pm$ 0.83	85.0 $\pm$ 2.88	85.7 $\pm$ 0.84	84.6 $\pm$ 0.20	85.7 $\pm$ 0.83	80.5 $\pm$ 0.71
PHB	0.25	88.5 $\pm$ 1.62	86.3 $\pm$ 1.96	87.0 $\pm$ 1.00	83.6 $\pm$ 0.45	85.7 $\pm$ 0.20	79.9 $\pm$ 0.48
	0.50	86.2 $\pm$ 0.87	86.8 $\pm$ 1.81	86.9 $\pm$ 3.19	84.6 $\pm$ 0.59	85.0 $\pm$ 0.23	80.7 $\pm$ 0.16
	1.00	87.5 $\pm$ 0.78	86.6 $\pm$ 3.04	87.3 $\pm$ 0.95	85.1 $\pm$ 0.48	85.9 $\pm$ 0.80	80.8 $\pm$ 0.56
IBHB	0.25	89.3 $\pm$ 1.53	86.5 $\pm$ 1.78	87.1 $\pm$ 1.19	84.7 $\pm$ 0.45	86.5 $\pm$ 0.35	80.6 $\pm$ 0.75
	0.50	85.1 $\pm$ 0.71	86.2 $\pm$ 1.87	85.9 $\pm$ 3.08	86.0 $\pm$ 0.73	86.3 $\pm$ 0.31	81.9 $\pm$ 0.15
	1.00	85.3 $\pm$ 0.99	84.6 $\pm$ 3.04	85.2 $\pm$ 0.68	84.5 $\pm$ 0.16	85.5 $\pm$ 1.02	80.1 $\pm$ 0.89
BHB	0.25	89.3 $\pm$ 1.67	86.6 $\pm$ 1.81	87.2 $\pm$ 1.26	84.5 $\pm$ 0.48	86.2 $\pm$ 0.44	80.4 $\pm$ 0.82
	0.50	85.3 $\pm$ 0.78	86.5 $\pm$ 1.88	86.5 $\pm$ 3.12	85.7 $\pm$ 0.78	86.0 $\pm$ 0.35	81.6 $\pm$ 0.32
	1.00	87.7 $\pm$ 1.04	86.8 $\pm$ 3.22	87.3 $\pm$ 0.67	84.6 $\pm$ 0.28	85.8 $\pm$ 1.45	80.2 $\pm$ 1.20

BA benzoic acid, SA sorbic acid, MHB methyl 4-hydroxybenzoate, DHA dehydroacetic acid, EHB ethyl 4-hydroxybenzoate, IPHB isopropyl 4-hydroxybenzoate, PHB propyl 4-hydroxybenzoate, IBHB isobutyl 4-hydroxybenzoate, BHB butyl 4-hydroxybenzoate

that chemical preservatives, particularly SA, should be routinely monitored and controlled to ensure the quality and safety of food products. The method developed in the present study may facilitate rapid and reliable detection of preservatives in many types of samples, which may in turn help to minimize health risks associated with chemical preservatives.

When comparing the amounts of SA detected by UPLC with those detected by HPLC, the t-statistic was 1.42, which was lower than the critical t-value of 2.13 at a 5% significance level, indicating that the results obtained by UPLC and HPLC were statistically indistinguishable. Table 4 shows comparison data between UPLC and HPLC. The comparative analytical data clearly indicated that the performances of both UPLC and HPLC were appropriate

for the intended use, i.e., the detection and quantification of chemical preservatives in animal products. Moreover, the use of UPLC allows us to achieve much better efficiency and sensitivity than HPLC. Based on the advantages of UPLC in terms of speed, resolution, and sensitivity compared with conventional HPLC [23], the developed method may be quite beneficial for monitoring of chemical preservatives in animal products.

In conclusion, a rapid analytical method was developed and validated for the regular and simultaneous determination of chemical preservatives in processed animal products. HPLC required about 40 min of running time per sample to separate the tested preservatives, whereas the developed UPLC analytical method required less than 6 min per sample. Therefore, the developed method

Table 4 Comparative analytical data of preservatives in animal products by UPLC and HPLC

Samples no.	Type	Content (g/kg) ^a		
		Sorbic acid		Other preservatives
		UPLC	HPLC	UPLC and HPLC
1	Processed cheese	0.515	0.471	ND
2	Processed cheese	0.745	0.838	ND
3	Processed cheese	1.489	1.042	ND
4	Processed cheese	0.972	0.991	ND
5	Processed cheese	1.157	1.201	ND
6	Processed cheese	0.796	0.753	ND
7	Processed cheese	2.401	2.419	ND
8	Processed cheese	2.492	2.266	ND
9	Processed cheese	1.458	1.459	ND
10	Raw cheese	0.495	0.475	ND
11	Raw cheese	0.150	0.159	ND
12	Raw cheese	0.416	0.433	ND
13	Semi-rigid cheese	0.606	0.586	ND
14	Semi-rigid cheese	1.128	0.988	ND
15	Soft cheese	0.192	0.191	ND
16	Sausage	0.109	0.114	ND

^aSA was detected in only 16 of 278 samples, and no other preservatives were detected in all the samples. This table presents the data from food samples in which preservatives were detected

ND not detected

economized the use of organic solvents and reduced costs. The specificity, sensitivity, precision, and accuracy of the UPLC method were evaluated, and the method was seen to be effective and efficient for the determination of preservatives in animal products. Furthermore, consistent and stable results were generated by UPLC in the analysis of nine chemical preservatives in 278 animal product samples, as compared with HPLC. Moreover, two processed cheese products were found to contain considerable amounts of SA. The developed method may be beneficial for continuous and rapid monitoring of chemical preservatives in processed animal products.

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