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Quantification of bupivacaine hydrochloride and isoflupredone acetate residues in porcine muscle, beef, milk, egg, shrimp, flatfish, and eel using a simplified extraction method coupled with liquid chromatography–triple quadrupole tandem mass spectrometry

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ABSTRACT

In this study, a simple analytical approach has been developed and validated for the determination of bupivacaine hydrochloride and isoflupredone acetate residues in porcine muscle, beef, milk, egg, shrimp, flatfish, and eel using liquid chromatography-tandem mass spectrometry (LC–MS/MS). A 0.1% solution of acetic acid in acetonitrile combined with *n*-hexane was used for deproteinization and defatting of all tested matrices and the target drugs were well separated on a Waters Xbridge™ C18 analytical column using a mobile phase consisting of 0.1% acetic acid (A) and 0.1% solution of acetic acid in methanol (B). The linearity estimated from six-point matrix-matched calibrations was good, with coefficients of determination ≥0.9873. The limits of quantification (LOQs) for bupivacaine hydrochloride and isoflupredone acetate were 1 and 2 ng g^{-1}, respectively. Recovery percentages in the ranges of 72.51–112.39% (bupivacaine hydrochloride) and 72.58–114.56% (isoflupredone acetate) were obtained from three different fortification concentrations with relative standard deviations (RSDs) of < 15.14%. All samples for the experimental work and method application were collected from the local markets in Seoul, Republic of Korea, and none of them tested positive for the target drugs. In conclusion, a simple method using a 0.1% solution of acetic acid in acetonitrile and *n*-hexane followed by LC–MS/MS could effectively extract bupivacaine hydrochloride and isoflupredone acetate from porcine muscle, beef, milk, egg, shrimp, flatfish, and eel samples.

1. Introduction

Pharmaceuticals are a class of emerging environmental contaminants that are widely used in human and veterinary medicine. For instance, bupivacaine hydrochloride (bupivacaine) (Fig. 1), which belongs to the pipecoloxylidide group, is a well-established local anesthetic used for humans [1], while isoflupredone acetate (isoflupredone) (Fig. 1), which is a glucocorticoid, is a type of anti-inflammatory drug (AID) that is widely used in veterinary medicine to treat dairy cattle with fatty liver and clinical ketosis diseases, which result in a loss of milk production [2,3]. Bupivacaine is used in combination with ropivacaine and mepivacaine for achieving anesthesia with an appropriate duration of action in surgery as well as in obstetrics. The parent compound and its three principal metabolites (desbutylbupivacaine, 4′-hydroxybupivacaine, and 3′-hydroxybupivacaine) are normally excreted in human urine [4]. The careless and excessive use of bupivacaine and isoflupredone might lead to direct or indirect continuous contamination of food commodities. Indirectly, bupivacaine may be released to the environment through patient excrement and hospital medical waste disposal. In such cases, the trace concentrations are likely to flow into surface, ground, and drinking water, leading to contamination of the aquatic environment and potential toxicity to

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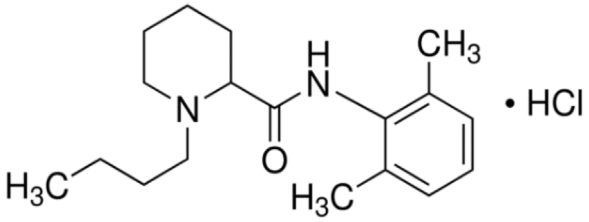
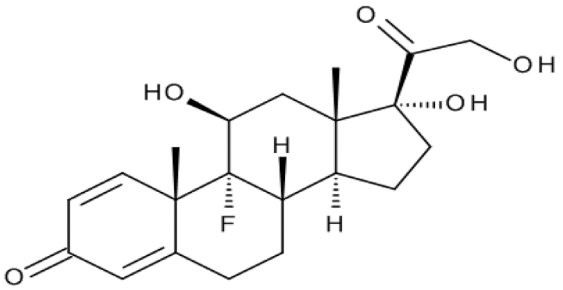


Bupivacaine hydrochloride



Isoflupredone acetate

Fig. 1. Chemical structures of bupivacaine hydrochloride and isoflupredone acetate.

human health. The contaminated water may also be used for agricultural irrigation and animal grooming, giving rise to contamination of both plants and animals such as swine, cattle and chicken, which are presumed to have residues in their muscle tissues, milk, and eggs, respectively. On the other hand, as isoflupredone is used in dairy cattle, a portion is assumed to be excreted in milk, resulting in milk contamination. The remainder is eliminated through feces and urine, and run-off to surface water, thereby indirectly contaminating both aquatic organisms and livestock animals. On the other hand, bovine blood, which possesses a considerable amount of protein, has been proposed as an additive for poultry diets, which could in turn lead to contamination of chicken products [5] such as eggs. Furthermore, certain animal bloods, such as that of swine, are intended for human consumption because of their nutritive value [6], creating another human exposure route, and thereby posing a public health hazard. For instance, bupivacaine may result in cardiotoxicity [7] and isoflupredone may have a negative impact not only on meat quality but also on human health, including hypertension, obesity, or osteoporosis [2]. Because of the variability in function, chemical structure, and physiochemical properties, the determination of the afore-mentioned drug residues in foods of animal origin is an indispensable part of food safety.

Along with ropivacaine and mepivacaine, bupivacaine is extensively metabolized before being excreted, mainly in urine. Several methods to measure bupivacaine and its major metabolites in urine [8–11] and plasma or serum [11–13] have been successfully developed. However, to date, there is no analytical method for the determination of bupivacaine in the tissues of livestock, poultry, and aquatic products. With regard to isoflupredone, gas chromatography-mass spectrometry (GC–MS) was the first technique to be successfully employed for screening and confirming glucocorticoids in complex biological matrices (milk, liver, urine, or feces) [14]. Afterwards, the development of liquid chromatography-mass spectrometry (LC–MS) techniques coupled with electrospray ionization (ESI), which provides high efficiency, specificity and sensitivity, was considered a powerful alternative to GC–MS [2] for monitoring corticosteroids [15]. A previous study has shown that bupivacaine is extensively protein bound and requires a highly sensitive detection method [16]; liquid–liquid or solid-phase extraction has been generally employed as a sample pre-treatment method to determine the metabolites of bupivacaine. Therefore, an analytical method relying on liquid–liquid extraction followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS), has been developed in this study. To the best of our knowledge, simultaneous analysis of bupivacaine hydrochloride and isoflupredone acetate in various food products has not been reported so far. Furthermore, there are no maximum residue limits (MRLs) set by regulatory authorities for bupivacaine or isoflupredone in any matrices [17–19]. Consequently, the purpose of this study is to establish an accurate and sensitive method for the quantification of bupivacaine and isoflupredone residues in porcine muscle, beef, milk, egg, shrimp, flatfish, and eel.

2. Materials and methods

2.1. Chemicals, reagents, and samples

Bupivacaine hydrochloride (CAS: 73360-54-0) and isoflupredone acetate (CAS: 338-98-7) were purchased from US Pharmacopeial Convention (Rockville, MD, USA). Acetic acid (99.5% pure) was purchased from Fluka BioChemika. Analytical grade acetonitrile (100% pure) and methanol (99.9% pure) were obtained from J.T. Baker Chemicals (Phillipsburg, NJ, USA). A 0.45 μm GH polypro (GHP) membrane and syringe filters were purchased from Pall (Michigan, USA). Ultra-high purity water used for preparation of the mobile phase was produced by an aqua MAX™ water (Young Wha, Seoul, Republic of Korea) purification system. Porcine muscle, beef, milk, egg, shrimp, flatfish, and eel were purchased from the local markets in Seoul, Republic of Korea, and all samples were stored at −4 °C prior to analysis.

2.2. Standard solutions

Stock solutions (1000 μg mL^{-1}) were prepared by weighing 10 mg of each drug (AG 285, METTLER TOLEDO, Seoul, Republic of Korea) in a 15-mL conical tube (Falcon, Corning Science Mexico S. A. de C.V., Tamaulipas, Mexico) containing 10 mL of methanol. Then, working solutions with various concentrations were prepared by further dilution in methanol. All standard solutions were stored at −20 °C and analyzed within a week after preparation.

2.3. Sample preparation

Chopped samples (5 g each) of porcine muscle, beef, shrimp, flatfish, and eel or 5 mL of homogenized samples (whole milk and egg without shell) were transferred to 50 mL conical tubes followed by fortification with 500 μL of standard solution and vortex mixing for 1 min (BenchMixer™ Multi-Tube Vortexer, Benchmark Scientific, NJ, USA). The mixtures were allowed to stand for 10 min and then 8 mL of 0.1% acetic acid in acetonitrile was added, followed by vortex mixing for 5 min and centrifugation at 2600 *g* (Union 32 R Plus, Hanil Science Industrial Co., Ltd., Incheon Republic of Korea) for 15 min at 4 °C. The upper layers (supernatant) were gently transferred to 50 mL conical tubes and the remained portions were re-extracted with an additional 7 mL of 0.1% acetic acid in acetonitrile followed by vortex mixing for 5 min and centrifugation at 2600 *g* for 15 min at 4 °C. The supernatants were collected in the same 50 mL conical tubes (approximate volume of 15 mL) and thereafter 15 mL of *n*-hexane was added and the mixtures were sufficiently vortexed and centrifuged under the same conditions stated above. Then, the lower layer (approximate volume of 15 mL) was transferred to a 15-mL centrifuge tube, followed by evaporation under nitrogen at 40 °C (TurboVap®RV, Caliper Life Sciences, MA, USA) until the residual volume was below 0.3 mL. Finally, the extract was reconstituted with the mobile phase (0.1% acetic acid (A) and 0.1% solution of acetic acid in methanol (B), 1:1, *v/v*) followed by short vortex mixing and centrifugation at 15,000*g* (MEGA 17R, Hanil Science Industrial Co., Ltd., Incheon, Republic of Korea) for 10 min at 4 °C. A syringe filter (MILLEX®-LCR, Merck Millipore Corporation, Merck KGaA, Darmstadt,

Table 1
Precursor and product ions (*m/z*), collision energy, and retention time of bupivacaine hydrochloride and isoflupredone acetate in positive ESI LC–MS/MS.

Drug	Precursor ion (*m/z*)	Product ion (*m/z*)			Collision energy (CE)	Retention time (min)
		Quantitation	Confirmation 1	Confirmation 2		
Bupivacaine hydrochloride	289.263	143.00	84.100	98.200	27.00	9.68
Isoflupredone acetate	421.246	237.300	341.300	147.100	17.00	10.51

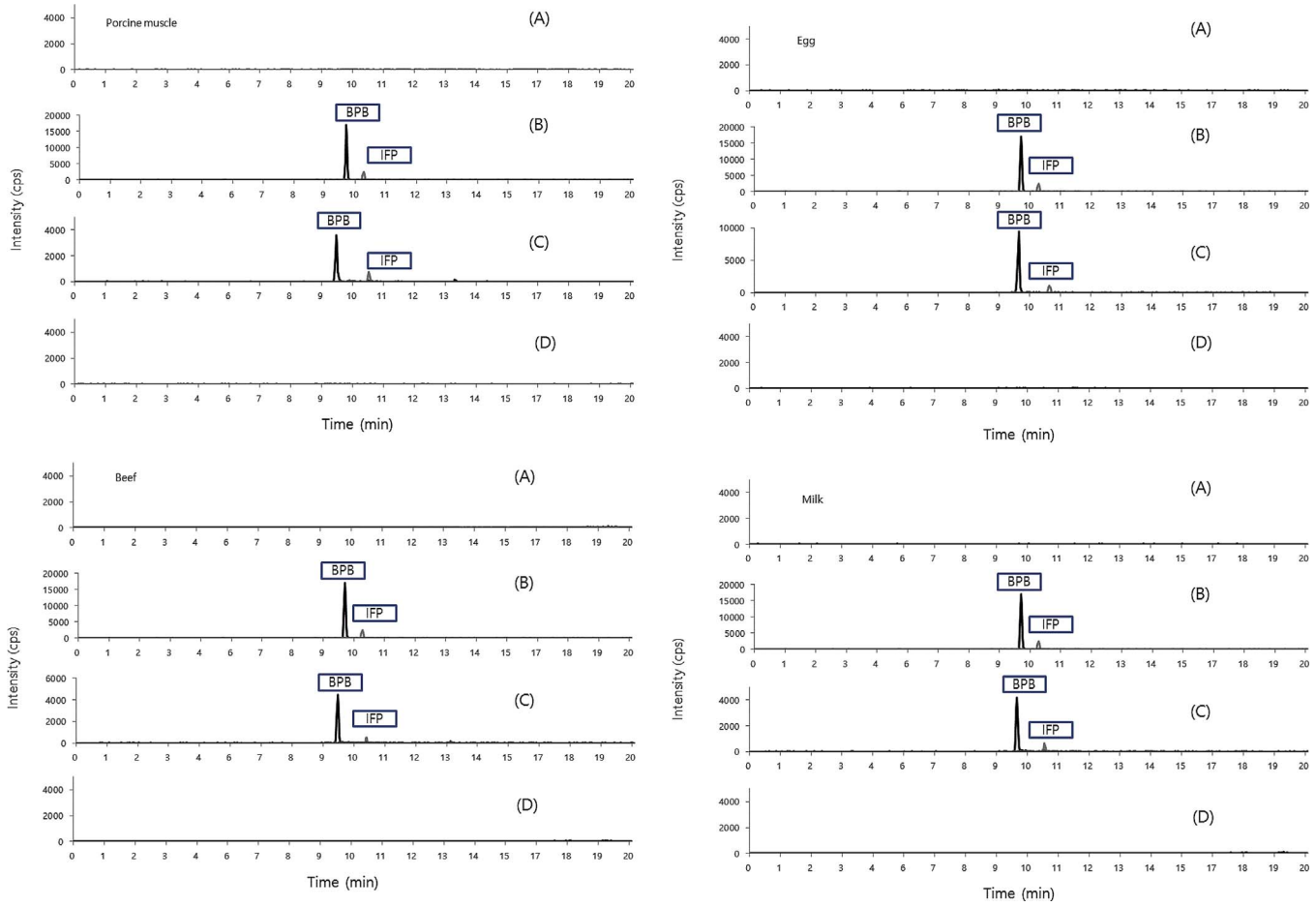


Fig. 2. Liquid chromatography-tandem mass spectrometry (LC–MS/MS) chromatograms of bupivacaine hydrochloride and isoflupredone acetate in (A) blank sample, (B) standard in solvent, (C) sample matrices spiked at 1 and 2 ng g^{-1}, respectively, and (D) market samples.

Germany) was used for filtration prior to analysis by LC–MS/MS.

2.4. LC-electrospray ionization MS/MS

The liquid chromatography system consisted of an Agilent series 1100 HPLC (Agilent Technologies, CA, USA) equipped with a G1311A quart pump, a G1313A autosampler, a G1322A degasser, a G1316A column oven and an API 3200™ LC–MS/MS system (Applied Biosystems, NY, USA). A Waters Xbridge™ C18 analytical column (2.1 × 100 mm, 3.5 μm particle size; Waters, Milford, CT, USA) was selected for the chromatographic separation. A binary solvent system composed of 0.1% acetic acid (A) and 0.1% solution of acetic acid in methanol (B), was applied in gradient pump mode. The following scheme was followed for the linear gradient: 0–3 min, 5% B; 3–5 min, increasing the solvent ratio from 5 to 95% B; 5–13 min, 95% B; 13–15 min, decreasing the solvent ratio from 95 to 5% B; and, 15–20 min, 5% B. The flow rate was 0.25 mL min^{-1} and the injection volume was 10 μL. The column temperature was maintained at 30 °C.

Triple quadrupole tandem mass spectrometric (MS/MS) analysis was performed using an electrospray ion source. Data acquisition was carried out in multiple reaction monitoring (MRM) mode and Analyst 1.4.2 software (SCIEX) was used for data management and control. The ion spray voltage was 5.5 kV and the capillary temperature was set at 350 °C. Nitrogen was used as the collision gas and ion source gas 1 (GS1) and gas 2 (GS2) were both maintained at a pressure of 50 psi. Standard solutions at a concentration of 0.1 μg mL^{-1} were used for optimization of precursor ion isolation, product ion formation, declustering potential, and collision energy via direct injection into the mass spectrometer. The positive mode was selected and the highest intensity transition was employed for quantification, while the lowest intensity transition was employed for confirmation.

2.5. Validation

According to the criteria specified by the Ministry of Food and Drug Safety (MFDS), Republic of Korea [20], the protocol was validated in terms of linearity, accuracy, precision, and limits of detection (LODs) and quantification (LOQs). The linearity was achieved by matrix-spiked

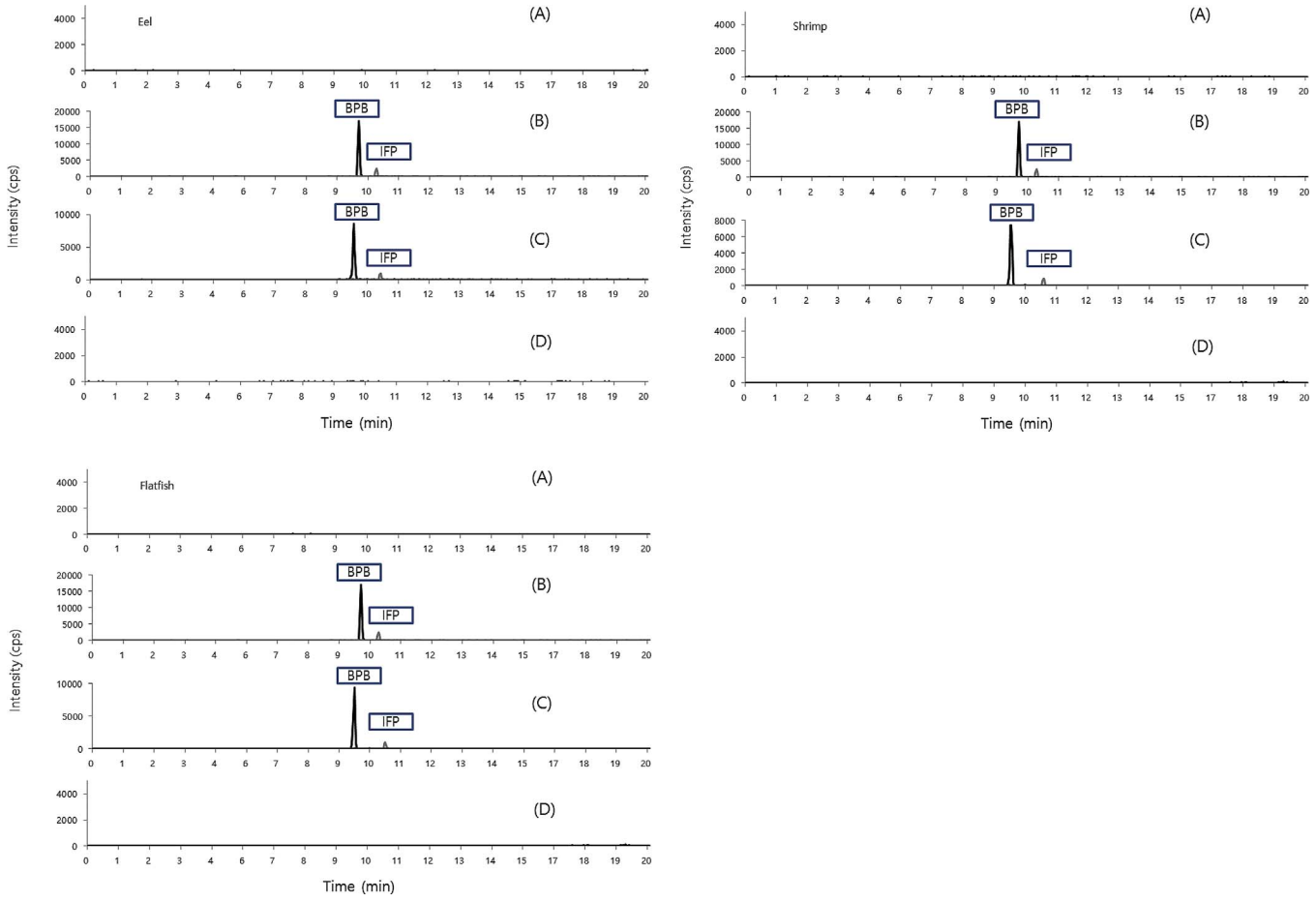


Fig. 2. (*continued*)

calibration at six concentration levels. Curves were constructed via plotting the response factor as a function of drug concentration. Recoveries (accuracy) were determined by comparing the calculated amounts of the analytes in the spiked samples with the standard solution at the same spiking level in solvent. The intra- and inter-day relative standard deviations (RSD), which represent precision, were also calculated. The calculated LOD and LOQ (n = 5) were obtained as 3 and 10 times the signal-to-noise ratio, respectively.

3. Results and discussion

3.1. Optimization of sample preparation

Small chopped samples (porcine muscle, beef, shrimp, flatfish, and eel) were employed as we reported in our previous study [21]. Acetonitrile, ethanol, and methanol in the presence of acid or base [22] are commonly used organic solvents for protein precipitation. Acetonitrile with the addition of 0.1% formic acid, 10 mM ammonium acetate or 0.1% acetic acid was tested as an extraction solvent in this study, obtaining recoveries of approximately 50, 60, and 70%, respectively. Therefore, 0.1% solution of acetic acid in acetonitrile was used as the extraction solvent. Following extraction, *n*-hexane was used for defatting to avoid disturbance from suspended endogenous interferences such as lipids, phospholipids, and fatty acids [23]. Thereafter, high-speed centrifugation was employed to remove the solidified impurities from the liquid layer and obtain a clearer solution prior to injection. In addition, a syringe filter was used for further analyte purification and to protect the instrument.

3.2. Optimization of LC-electrospray ionization MS/MS

Both positive and negative ESI modes were assayed under a full spectrum scan of *m/z* 0–300 to quantify bupivacaine hydrochloride and isoflupredone acetate standards in solvent and ESI^+ was selected as it had better signals than ESI^- throughout the experimental work. All the parameters used for quantitation and confirmation in MRM mode are summarized in Table 1. A Phenomenex Luna C18 analytical column was compared with a Waters XBridge™ C18 analytical column, and the Waters XBridge™ C18 analytical column was selected for the separation of bupivacaine hydrochloride and isoflupredone acetate because of its smoother baseline and sharper peaks compared with the Phenomenex Luna C18 analytical column. Since the effect of mobile phase composition on the ionization efficiency in analysis by LC–MS/MS methodology is significant [24], seven mobile-phase combinations were tested including: (a) 0.1% solution of formic acid in acetonitrile; (b) 0.1% solution of TFA in acetonitrile; (c) 10 mM solution of ammonium formate in methanol; (d) 5 mM solution of ammonium formate in methanol; (e) 0.1% solution of acetic acid in methanol; (f) 0.2% solution of acetic acid in methanol; and, (g) 0.1% solution of acetic acid and 0.1% solution of acetic acid in methanol. The latter mobile-phase composition of 0.1% solution of acetic acid (A) and 0.1% solution of acetic acid in methanol (B) was chosen as it provided well-resolved, high-intensity peaks.

3.3. Method validation

3.3.1. Specificity, linearity, and matrix effect

As shown in Fig. 2, no interfering peaks are observed around the retention times of bupivacaine hydrochloride and isoflupredone acetate

Table 2
Method performance of bupivacaine hydrochloride and isoflupredone acetate in spiked porcine muscle, beef, milk, egg, shrimp, flatfish, and eel samples.

Drug	Matrix	Spiking concentration (ng g^{-1})	Intra-day (n = 3)		Inter-day (n = 9)		Matrix effect % (mean ± SD)	Linear range (ng g^{-1})	R^2	LOD (ng g^{-1})	LOQ (ng g^{-1})
			Recovery (%)	RSD (%)	Recovery (%)	RSD (%)					
Bupivacaine hydrochloride	Porcine muscle	5	72.51 ± 1.00	1.38	73.17 ± 0.58	0.79	−19.45 ± 6.05	5–50	0.9993	0.3	1
		10	81.34 ± 1.53	1.88	76.92 ± 4.26	5.54	−18.55 ± 2.85				
		20	77.29 ± 4.62	5.98	77.17 ± 2.52	3.26	−12.68 ± 8.27				
	Beef	5	86.41 ± 3.75	4.34	83.24 ± 1.15	1.39	−25.09 ± 2.72	5–50	0.9989	0.3	1
		10	83.01 ± 1.89	2.27	73.21 ± 3.59	4.53	−26.20 ± 2.66				
		20	79.60 ± 2.31	2.91	74.81 ± 3.31	4.43	−15.65 ± 2.74				
	Milk	5	84.47 ± 3.82	4.52	91.47 ± 3.01	3.29	−21.91 ± 4.01	5–50	0.9975	0.3	1
		10	81.98 ± 9.38	11.44	75.73 ± 7.29	9.62	−20.42 ± 5.84				
		20	78.66 ± 2.88	3.67	80.20 ± 2.68	3.34	−12.01 ± 6.65				
	Egg	5	85.93 ± 4.62	5.37	83.71 ± 3.36	4.01	−17.68 ± 3.47	5–50	0.9959	0.3	1
		10	91.60 ± 3.84	4.2	92.93 ± 3.84	4.14	−17.73 ± 8.32				
		20	92.39 ± 3.16	3.42	89.98 ± 1.47	3.34	−13.54 ± 2.83				
	Shrimp	5	112.39 ± 1.01	0.9	110.32 ± 3.61	3.27	−19.03 ± 1.48	5–50	0.9966	0.3	1
		10	94.73 ± 2.72	2.87	92.76 ± 1.15	1.24	−11.41 ± 0.91				
		20	90.56 ± 4.48	4.95	87.90 ± 2.02	2.3	−11.15 ± 1.78				
	Flatfish	5	99.82 ± 2.90	2.9	97.24 ± 0.87	0.89	−26.97 ± 1.47	5–50	0.9888	0.3	1
		10	108.33 ± 4.37	4.03	103.12 ± 5.70	5.53	−15.24 ± 1.66				
		20	105.00 ± 1.65	1.57	98.95 ± 4.16	4.21	−11.78 ± 1.49				
	Eel	5	105.99 ± 1.59	1.5	100.86 ± 6.38	6.32	−29.15 ± 5.19	5–50	0.9986	0.3	1
		10	110.93 ± 3.68	3.32	108.96 ± 3.26	2.99	−16.83 ± 1.87				
		20	100.13 ± 3.69	3.68	97.30 ± 2.60	2.67	−10.42 ± 2.77				
Isoflupredone acetate	Porcine muscle	5	78.53 ± 9.72	12.38	72.58 ± 2.97	4.1	−28.40 ± 5.76	5–50	0.9875	0.6	2
		10	80.40 ± 1.46	1.82	74.06 ± 5.96	8.05	−24.50 ± 6.26				
		20	82.72 ± 3.72	4.49	91.90 ± 9.51	10.35	−15.23 ± 2.19				
	Beef	5	88.82 ± 13.45	15.14	96.92 ± 5.27	5.44	−24.91 ± 3.09	5–50	0.9994	0.6	2
		10	100.03 ± 7.03	7.02	85.87 ± 6.58	7.67	−14.35 ± 6.26				
		20	81.94 ± 0.82	1.00	80.85 ± 2.77	3.43	−18.66 ± 3.42				
	Milk	5	114.56 ± 6.67	5.82	111.38 ± 8.11	7.28	−29.92 ± 3.28	5–50	0.9873	0.6	2
		10	88.11 ± 8.40	9.54	87.29 ± 9.52	10.9	−28.95 ± 3.50				
		20	77.52 ± 6.35	8.19	83.14 ± 0.86	1.03	−15.84 ± 4.92				
	Egg	5	110.10 ± 13.19	11.98	111.70 ± 7.85	7.03	−29.84 ± 3.52	5–50	0.9968	0.6	2
		10	93.98 ± 0.64	0.68	88.92 ± 5.37	6.04	−27.84 ± 5.55				
		20	95.51 ± 0.50	0.52	98.51 ± 5.68	5.76	−15.38 ± 3.93				
	Shrimp	5	90.82 ± 4.87	5.96	90.22 ± 5.91	6.55	−12.29 ± 0.12	5–50	0.9986	0.6	2
		10	95.31 ± 8.71	9.14	87.34 ± 7.34	8.41	−15.86 ± 4.62				
		20	88.69 ± 0.29	0.33	87.19 ± 7.64	8.76	−11.82 ± 1.86				
	Flatfish	5	93.98 ± 13.00	13.84	89.22 ± 2.29	2.57	−27.93 ± 4.05	5–50	0.9942	0.6	2
		10	99.30 ± 1.25	1.26	97.53 ± 7.30	7.49	−10.59 ± 4.90				
		20	94.71 ± 8.12	8.57	89.66 ± 1.65	1.84	−21.29 ± 2.64				
	Eel	5	103.93 ± 10.18	9.8	100.99 ± 7.78	7.71	−29.77 ± 5.23	5–50	0.999	0.6	2
		10	101.59 ± 5.98	5.89	101.14 ± 6.76	6.69	−27.42 ± 5.92				
		20	102.84 ± 5.72	5.56	102.33 ± 5.28	5.16	−17.75 ± 3.82				

in the porcine muscle, beef, milk, egg, shrimp, flatfish, and eel blank samples, which were obtained from different markets (in triplicate); indicating method specificity.

Matrix-fortified calibration at six different concentration levels was performed for the assessment of linearity. The tested concentrations for both bupivacaine hydrochloride and isoflupredone acetate were 5, 10, 20, 30, 40, and 50 ng g^{-1} (n = 5), following the criteria of the MFDS 2015 guideline [20]. As shown in Table 2, all calibration curves possess good linearity with coefficients of determination (R^2) ≥0.9873.

The high selectivity of LC–MS/MS does not guarantee the effective elimination of interference from endogenous impurities. Additionally, electrospray ionization (ESI) is more sensitive to components (non-volatile materials) competitively co-eluted with the analytes during bioanalysis, thus producing a suppression or enhancement effect, a phenomenon commonly referred to as matrix effects (MEs). Such effects could diminish the reproducibility, linearity, and accuracy of the method and lead to erroneous quantitation. Therefore, such effects should be explored to ensure accurate quantification of the tested analytes. Herein, the MEs of the tested drugs were evaluated at 3 spiking levels (Table 2). The percentage of the ME was calculated as follows: ME (%) = B/A × 100

where A and B represent the peak area of the standard solution and standard spiked after extraction, respectively.

As shown in Table 2, the 2 drugs produced suppression effects in various matrices. Obviously, all values were < 30% and clearly low spiking levels might result in more severe suppression than high spiking levels, as reported by Wang et al. [25]. As all matrices contain different percentages of fat, the suppression effect is likely related to particular phospholipids. Phospholipids are known to produce MEs through ion suppression, i.e., they can decrease the signal intensity for measured analytes if they migrate at the same time as the analytes during chromatography [26]. Overall, matrix-matched calibrations were used throughout the experimental work for quantification of the tested drugs in various animal-based food matrices.

3.3.2. LOD and LOQ

As shown in Table 2, the LODs are 0.3 and 0.6 ng g^{-1} and the LOQs are 1 and 2 ng g^{-1} for bupivacaine hydrochloride and isoflupredone acetate, respectively. The limits obtained herein are relatively low and justify that the protocol is a sensitive and reliable method for the determination of bupivacaine hydrochloride and isoflupredone acetate in porcine muscle, beef, milk, egg, shrimp, flatfish, and eel at concentrations lower than 0.01 μg kg^{-1}, which is a globally proposed level for all chemicals having no MRL.

3.3.3. Recovery

Recovery (accuracy) and repeatability (intra-day precision) were assessed by spiking blank porcine muscle, beef, milk, egg, shrimp, flatfish, and eel samples at three concentration levels (n = 3) in a single day. The inter-day precision (reproducibility) was evaluated at the same concentrations (n = 3) over three consecutive days. As shown in Table 2, the recoveries obtained for bupivacaine hydrochloride are between 72.51 and 112.39%, with RSDs ≤ 11.44%, and the recoveries for isoflupredone acetate range from 72.58 to 114.56%, with RSDs ≤ 15.14%. All the results are satisfactory on the basis of the accepted criteria for analytical approaches prescribed by the Codex Alimentarius Commission [27]: when the spiking concentrations were between 1 and 10 ppb or 10–100 ppb, the recoveries were in the range of 60–120% and the RSDs were below 30% (70–120% and RSD% = 20% for higher spiking level). The acceptable precision and reproducibility indicate that the method developed herein is sufficiently accurate and sensitive.

3.4. Method application

The proposed method was applied to the monitoring of porcine muscle, beef, milk, egg, shrimp, flatfish, and eel collected from markets located in Seoul, Republic of Korea (three different samples of each product). The samples were extracted and analyzed following the protocol mentioned in Section 2.3. All the samples tested negative for the target analytes (Fig. 2).

4. Conclusions

A simple liquid–liquid extraction method coupled with LC–MS/MS analysis was developed for the determination and validation of bupivacaine hydrochloride and isoflupredone acetate in porcine muscle, beef, milk, egg, shrimp, flatfish, and eel. A solution of 0.1% acetic acid in acetonitrile was used for extraction and deproteinization, and *n*-hexane was used for defatting. No solid-phase extraction cartridge was deemed necessary for purification. The validation parameters, including linearity, accuracy (expressed as recovery), precision (expressed as RSD), and sensitivity (LOD and LOQ) were all satisfactory. None of the market samples tested positive for either of the drug residues. The method developed herein could be extrapolated to animal-derived products, which possess high protein and fat contents after a complementary validation.

Conflict of interest

The authors have declarde no conflict of interest

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