Journal of Chromatography B 1065–1066 (2017) 29–34

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

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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ARTICLE INFO

Keywords:
Bupivacaine
Isoflupredone
LC–MS/MS
Animal-derived food products
Determination
Residue

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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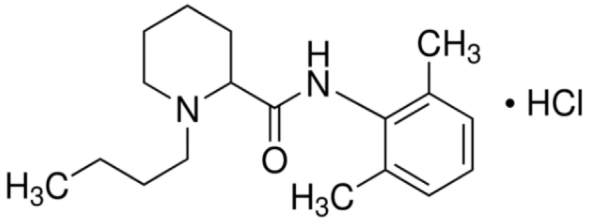
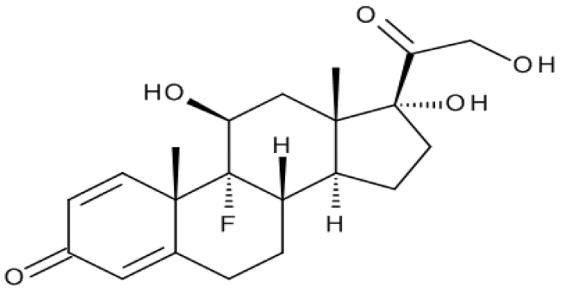
http://dx.doi.org/10.1016/j.jchromb.2017.09.026
Received 5 July 2017; Received in revised form 11 September 2017; Accepted 17 September 2017
Available online 19 September 2017



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,