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Development of QuEChERS-based extraction and liquid chromatography–tandem mass spectrometry method for quantifying flumethasone residues in beef muscle

Ki Hun Park [a], Jeong-Heui Choi [b], A.M. Abd El-Aty [c,*], Soon-Kil Cho [d], Jong-Hyouk Park [a], Ki Sung Kwon [e], Hee Ra Park [e], Hyung Soo Kim [e], Ho-Chul Shin [f], Mi Ra Kim [g], Jae-Han Shim [a,**]

[a] Natural Products Chemistry Laboratory, Biotechnology Research Institute, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju 500-757, Republic of Korea
[b] Institute of Environmental Research (INFU), Faculty of Chemistry, TU Dortmund University, Otto-Hahn Str. 6, Campus North, D-44227 Dortmund, Germany
[c] Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt
[d] National Agricultural Products Quality Management Service, Products Safety Inspection Laboratory, Gwangsan-Gu, Gwangju, Republic of Korea
[e] Korea Food & Drug Administration, Food Chemical Residues Division, Osong Health Technology Administration Complex, 187 Osongsaengmyeong2(i)-ro, Osong-eup, Cheongwon-gun, Chungcheongbuk-do, Republic of Korea
[f] Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Kwangjin-gu, Seoul 143-701, Republic of Korea
[g] Regional FDA Hazardous Substances Analysis Division, Daejeon, Republic of Korea

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ABSTRACT

A rapid, specific, and sensitive method based on liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS) in the positive ion mode using multiple reaction monitoring (MRM) was developed and validated to quantify flumethasone residues in beef muscle. Methods were compared between the original as well as the EN quick, easy, cheap, effective, rugged, and safe (QuEChERS)-based extraction. Good linearity was achieved at concentration levels of 5–30 μg/kg. Estimated recovery rates at spiking levels of 5 and 10 μg/kg ranged from 72.1 to 84.6%, with relative standard deviations (RSDs)<7%. The results of the inter-day study, which was performed by fortifying beef muscle samples (n = 18) on 3 separate days, showed an accuracy of 93.4–94.4%. The precision (expressed as relative standard deviation values) for the inter-day variation at two levels of fortification (10 and 20 μg/kg) was 1.9–5.2%. The limit of detection (LOD) and limit of quantitation (LOQ) were 1.7 and 5 μg/kg, at signal-to-noise ratios (S/Ns) of 3 and 10, respectively. The method was successfully applied to analyze real samples obtained from large markets throughout the Korean Peninsula. The method proved to be sensitive and reliable and, thus, rendered an appropriate means for residue analysis studies.

1. Introduction

Corticosteroids are commonly used in veterinary practice for therapeutic purposes to treat inflammatory reactions, disorders of the musculoskeletal, respiratory, and gastrointestinal disorders, bovine ketosis, and many other diseases of farm animals (O'Keeffe, Martin, & Regan, 2003). The most administered corticosteroids include prednisolone, methylprednisolone, flumethasone, and dexamethasone (Tolgyesi, Sharmab, & Feketec, 2011). Corticosteroids may accumulate in non-polar fatty tissues or in fat of the body (Tolgyesi et al., 2011). Besides their therapeutic use, synthetic corticosteroids, including dexamethasone, betamethasone, prednisone, prednisolone, methylprednisolone, flumethasone, triamcinolone, and triamcinolone acetonide can be illegally administered to livestock to improve feed intake and body weight gain (Baiocchi et al., 2003). They are often administered together with other drugs (anabolic steroids and β-agonists) that act on water retention as well as lipid, protein, and carbohydrate metabolism (Brambilla et al., 2001; Sangiorgi, Curatolo, Assini, & Bozzoni, 2003).

The use of corticosteroids in veterinary medicine is strictly regulated by the European Union (EU), constituted by residual levels in animal tissues and/or products (Council Directive 96/22/EC, 1996; Van Peteghem & Daeselaire, 2004). It should be noted that the use of corticosteroids for fattening purposes, has been banned in EU member states (Council Directive 96/22/EC). Withdrawal periods and maximal residue limits must be established to prevent the presence of potentially harmful residues in animal derived products (Antignac, Le Bizec, Monteau, & Andre, 2002).

To date, gas chromatography–tandem mass spectrometry (GC–MS/MS) has been proposed to analyze corticosteroids in various biological matrices. Although the method is sensitive, it seems to be somewhat impractical, as corticosteroids are slightly volatile and can be denatured with heat (Delahaut et al., 1997) or require time-consuming derivatization or oxidation steps (Bevalot, Gaillard, Lehermitte, & Pepin, 2000; Courtheyn et al., 1994; Rodchenkov, Vedenin, Uralets, & Semenov,

* Corresponding author. Tel.: +20 2 27548926; fax: +20 2 35725240.
** Corresponding author. Tel.: +82 62 530 2135; fax: +82 62 530 0219.
E-mail addresses: abdelaty44@hotmail.com (A.M. Abd El-Aty), jhshim@chonnam.ac.kr (J.-H. Shim).

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1991). The best alternative to GC–MS/MS is liquid chromatography (LC)–MS (Marquet & Lachatre, 1999; Stanley, Wilhelmi, & Rodgers, 1994), in particular, reversed-phase (RP)-LC–MS (Bevalot et al., 2000; Fiori, Pierdominici, Longo, & Brambilla, 1998). Additionally, LC–MS/MS is the best candidate to increase specificity (Antignac, Le Bizec, Monteau, Poulain, & Andrè, 2000; Rizea Savu, Silvestro, Haag, & Sorgel, 1996; Tolgyesi et al., 2011). The sample preparations described in the above mentioned techniques use liquid–liquid extraction and single or multiple SPE cartridges. All procedures using more than one clean-up step are and/or time-consuming. To the best of our knowledge, no LC–MS/MS study using a quick, easy, cheap, effective, rugged and safe (QuEChERS)-based extraction method for analyzing flumethasone in beef muscle has been reported. Therefore, the aim of the present study was to develop a simple and reliable QuEChERS method to quantitatively analyze flumethasone in beef muscle by LC–MS/MS.

2. Materials and methods

2.1. Chemicals and reagents

Flumethasone (purity, 96.1%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical-grade acetonitrile, water, anhydrous magnesium sulfate ($MgSO_4$), and sodium chloride (NaCl) were obtained from Merck KGaA (Darmstadt, Germany). C_{18} (40 μm), and a QuEChERS Extraction kit (4 g $MgSO_4$, 1 g NaCl, 1 g citrate, 0.5 g disodiumcitrate sesquihydrate) were supplied by Agilent Technologies (Santa, Clara, CA, USA). Analytical-grade ammonium formate was supplied by Yakuri Pure Chemicals (Osaka, Japan). All solvents and reagents used were of high performance liquid chromatography or analytical grades.

2.2. Standard solutions

A 100 μg/mL stock standard solution of flumethasone was prepared in MeCN. Working standard solutions were prepared by diluting stock solutions with blank sample extracts, which were confirmed in advance to be free of the tested analyte. Matrix-matched compound calibration standard solutions were prepared by mixing the matrix-matched working standard solutions and additional blank sample extracts to reach appropriate compound calibration concentrations. All standard solutions were stored at −20 °C in dark amber bottles.

2.3. Sample preparation

Samples were prepared through a minor modification of the QuEChERS method reported previously by Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003. Ten grams of homogenized beef sample was placed in a 50 mL Teflon centrifuge tube to which 20 mL acetonitrile was added along with 4 g $MgSO_4$, 1 g NaCl (Anastassiades et al., 2003) or 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, and 0.5 g disodiumcitrate sesquihydrate (EN QuEChERS, European Standard EN 15662, 2008), and then the tube was vigorously shaken for 1 min followed by centrifugation for 5 min at 966×*g*. An approximate 6 mL portion of the upper layer was transferred to a 15 mL centrifuge tube containing 0.9 g $MgSO_4$ and 0.15 g C_{18}, and the tube was vigorously shaken for 1 min followed by centrifugation for 5 min at 966×*g*. The final extract was analyzed by LC–ESI–MS/MS (Scheme 1).

2.4. LC–electrospray ionization MS/MS

A MS/MS detector was equipped with an Agilent 1200 Series Rapid Resolution LC System (CA, USA), which consisted of a binary pump, autosampler, vacuum degasser, thermostated column compartment, and a diode array detector. The analytes were separated on a Gemini C_{18} (50×2.0 mm i.d., 3 μm, Phenomenex, Torrance, CA, USA) column kept in an oven at 40 °C. The binary solvent system consisted of 10 mM ammonium formate in water (A) and acetonitrile (B), with a linear gradient. The linear mobile phase gradient started at 10% B (0–10 min), increased to 90% B (10–13 min), maintained at 90% B (13–14 min), ramped back to 10% B (14–18 min), and maintained at 10% B (18–23 min). The flow rate was 0.25 mL/min, and the injection volume was 5 μL. MS/MS detection using an Agilent 6410 Triple Quadrupole LC/MS (QQQ) was conducted in the positive electrospray ionization mode using multiple reaction monitoring (MRM) with two mass transitions. In the two mass transitions, one product ion with the most intensity and the other lower intensity were used as quantifier and qualifier ions, respectively (Fig. 1). Pesticide standard solutions were directly infused into the QQQ for the optimal MS instrument parameters. Nitrogen was employed as nebulizer and a drying gas at 11 psi, 11 L/min, and 300 °C. Capillary and cell accelerator voltages were set to 4000 and 4 V, respectively. All dwell times for MRM transitions of the analytes were set to 200 ms, and other conditions are presented in Table 1. Both MS1 and MS2 quadrupoles were maintained at unit resolution. Mass Hunter Workstation Software (B.01.03) controlled the LC–ESI–MS/MS system and processed the data.

2.5. Validation

Parameters considered were instrumental linearity, specificity, precision, recovery, and limits of detection and quantification. Recovery was estimated by fortifying blank samples in six replicates with flumethasone standard solution at two different concentrations (5 and 10 μg/kg). The fortified samples were allowed to equilibrate for 1 h so that the spiked solution could penetrate the matrix, which was followed by extraction as described above. Recovery was expressed in terms of the percentage of measured concentration to fortified concentration ratio and precision is reported as a relative standard deviation (RSD).

3. Results and discussion

3.1. Optimization of sample preparation

We used the QuEChERS method to extract the target compound from beef muscle, which has been preferentially used for pesticide residue analysis (Kim et al., 2012). QuEChERS methods can be sorted into three versions; original, acetate-buffering, and citrate-buffering QuEChERS methods. The acetate- and citrate-buffering QuEChERS methods were nominated as AOAC Official Method 2007.01 (Lehotay, Kateřina, & Lightfield, 2005) and European Standard EN 15662 (2008), respectively. In the present study, the original QuEChERS provided an average recovery (means ± SD) ranging from 65.48 ± 9.13 to 80.56 ± 5.82, whereas the EN QuEChERS method provided a recovery rate ranging from 72.14 ± 0.89 to 84.62 ± 5.13. Based on these findings, we used the EN QuEChERS method throughout this study.

3.2. Method validation

3.2.1. Specificity

Specificity was tested by analyzing blank beef samples of different origins to verify the absence of potential interfering compounds at flumethasone retention times. No interfering peaks from endogenous compounds were observed at the flumethasone retention time (Fig. 2A).

3.2.2. Linearity

The linearity of the developed method was evaluated using the squared correlation coefficients (r^2) of six-point matrix-matched calibration curves obtained by analyzing blank beef extracts with analytes of 5–30 μg/kg, in quadruplicate. Favorable linearity was achieved within the concentration range, with a correlation coefficient (r^2) of 0.995. Calibration with matrix-matched standard solutions resulted in minimizing