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Multi-residue determination of 210 drugs in pork by ultra-high-performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

This paper presents a multi-residue analytical method for 210 drugs in pork using ultra-high-performance liquid chromatography-Q-Trap tandem mass spectrometry (UPLC–MS/MS) within 20 min via positive ESI in scheduled multi-reaction monitoring (MRM) mode. The 210 drugs, belonging to 21 different chemical classes, included macrolides, sulfonamides, tetracyclines, β-lactams, β-agonists, aminoglycosides, antiviral drugs, glycosides, phenothiazine, protein anabolic hormones, non-steroidal anti-inflammatory drugs (NSAIDs), quinolones, antifungal drugs, corticosteroids, imidazoles, piperidines, piperazidines, insecticides, amides, alkaloids and others. A rapid and simple preparation method was applied to process the animal tissues, including solvent extraction with an acetonitrile/water mixture (80/20, v/v), defatting and clean-up processes. The recoveries ranged from 52% to 130% with relative standard deviations (RSDs) < 20% for spiked concentrations of 10, 50 and 250 μg/kg. More than 90% of the analytes achieved low limits of quantification (LOQs) < 10 μg/kg. The decision limit (CCα), detection capability (CCβ) values were in the range of 2–502 μg/kg and 4–505 μg/kg, respectively. This method is significant for food safety monitoring and controlling veterinary drug use.

1. Introduction

Veterinary drugs are applied in animal husbandry to promote animal growth, prevent infection and treat diseases [1,2]. However, the improper and illegal use of prohibited or permitted drugs may result in the presence of drug residues in the animal tissues [3,4], leading to serious potential hazards to consumer health [5], such as the onset of allergies in hypersensitive individuals [6], the development of antibiotic-resistant bacteria, harmonic effects interfering with the balance of human hormones, and even the generation of mutagenesis, carcinogenesis and teratogenesis.

Currently, the high-throughput screening confirmation technology used to monitor banned or unregulated multi-class drug residues in animal-derived foods still faces many challenges [7]. The aim of monitoring and screening is to determine the maximum number of target or non-target analytes. Compared to the screening quantity requirements, the recovery optimization and other analytical standards are secondary factors. Analytes belonging to different chemical groups possess different physicochemical properties and thus require different pre-treatment and analytical methods [8]. Multiple drugs present in animals derived in low levels from a variety of ways, such as therapeutic treatments, feed additives, water, and the environment, increase the difficulty of monitoring these drug residues [9]. Moreover, the complexity of the biological matrix (i.e., fats, pigments, carbohydrates, proteins, and other substances) introduces additional difficulties in the sample pre-treatment process [10,11]. Furthermore, the extraction and purification processes of traditional methods are cumbersome, costly, and time consuming and have difficulty handling large quantities of sample measurements [12]. Therefore, the establishment of a fast, effective, economical, high-throughput and wide-coverage method of screening drug residues in animal food is imperative.

Most drugs can be detected, quantified and confirmed using a popular tandem mass spectrometry (LC–MS/MS)-based procedure [13]. Marilena E. Dasenaki [14]. developed a simple multi-residue method to analyze 115 veterinary drugs and pharmaceuticals

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belonging to more than 20 different classes in butter, milk powder, eggs and fish tissue. That method employed a simple solvent extraction with 0.1% formic acid in an aqueous solution of ethylenediaminetetraacetic acid (EDTA) 0.1% (w/v)–acetonitrile–methanol (1/1/1, v/v) and further ultrasonic-assisted extraction. Then, the extraction solutions were frozen at −20 °C for 12 h. The supernatant was subjected to a series of purification and evaporation processes. Marilyn J. Schneider [15] developed a multi-class, multi-residue methods for analysis of 120 veterinary drugs from 11 different routine classes in bovine kidney. After sample extraction, hexane partitioning, and concentration procedures, samples were injected every 30 min because of the large injection volume (20 μL) with LODs ≤ 10 ng/g for 109 of the analytes. Jia Zhan [16] also published an excellent method for the determination of 220 undesirable chemical residues in infant formula by LC–MS/MS. The sample preparation for that method included 2 times extractions, 3 times centrifugations, 2 times evaporations, 1 time low temperature cleanup, and water precipitation which is a necessary and innovative cleanup. And the final data acquisition needed 3 injections for different classes of analytes. The LOQs of method ranged from 0.01 to 5 μg/kg which is ensure to verify the positive samples with legal tolerance. However, the shortcomings of these methods are that they are too time consuming for large batches of samples, or the number of detected drugs is relatively low without any drugs taken from the environment. Thus, it is difficult to meet the requirements of rapidity and large range for high-throughput screening [18].

In recent years, the Q-Trap multi-reaction monitoring (MRM) mode sensitivity has increased 10-fold, and the sensitivity of the full-scan mode has increased 100-fold with this unique MRM scanning mode. Most importantly, the advanced MRM^{TM} scheduled acquisition method can intelligently utilize the retention time of a chromatographic peak and automatically optimize the residence time of the MRM to achieve the best quantitative data. Dresen et al. [19] developed a multi-target screening method of 700 drugs and metabolites in biological fluids using a 3200 Q-Trap LC–MS/MS system. The identification of the compounds in the samples was depended on the MS/MS spectra library developed. Although the establishment of library was a very tedious matter, but there was no doubt that this study provided us some instructive ideas. Therefore, the aim of this study was to develop a new approach for the multi-residue screening of 210 veterinary drugs and pharmaceuticals in pork using ultra-high-performance liquid chromatography (UPLC) coupled to a Q-trap analyzer. The developed method simplified the current laboratory methodologies and considerably improved the analytical control strategy.

2. Experimental

2.1. Reagents and materials

All the standards were purchased from Dr Ehrenstorfer (Augsburg, German). Detailed drug information is listed in Table 1. Stock solutions of each compounds at concentration of approximately 1000 mg/L were prepared in different solvents and stored at −20 °C in brown glasses for a maximum valid time of 6 months. The dissolved solvents were chosen based on their dissolubility and purity, including methanol, acetonitrile, ethyl acetate, methanol-water (99:1(v/v)), methanol-water (70:30(v/v)), methanol-DMSO (99:1(v/v)), or methanol-acetonitrile-water (98:1:1(v/v/v)). The above compounds were divided into different groups according to their classification and stability, and then the different working standard solutions, at 10 mg/L of each compound were prepared in methanol. All solution were stored at −20 °C.

LC–MS-grade acetonitrile and methanol were purchased from Fisher Scientific (Waltham, MA, USA); formic acid 99% was purchased from Sigma-Aldrich (St. Louis, MO, USA). Na_2EDTA was purchased from SCRC (Beijing, China), and N-propylethylenediamine (PSA) was purchased from Agilent Technologies (USA). Graphitized carbon, high-purity multi-wall carbon nanotubes, N-amino modified multi-walled carbon nanotubes, and graphitized multi-walled carbon nanotubes were purchased from Nanjing XF NANO Materials Tech Co. Ltd. (Nanjing, China). Ultrapure water was produced by a Milli-Q system (Bedford, MA, USA), and citric acid monohydrate, disodium hydrogen phosphate dodecahydrate, and sodium acetate anhydrous were acquired from SCRC (Beijing, China).

A Sorvall Stratos Centrifuge (Thermo, USA), oscillator (Taitec, SR-2DS, Japan), IKA MS3 basic vortex mixer (Germany), and S220 SevenCompact^{TM} pH meter (Shanghai, China) were used for sample preparation.

2.2. Sample preparation

Pork samples were obtained from a local market. Two gram (±0.05 g) of tissue homogenate was weighed into a 50-mL polypropylene centrifuge tube. Then, 10 mL of an acetonitrile/water mixture (80/20, v/v) and 200 μL of a 0.1-M EDTA solution were accurately added. The mixture was vortexed for 30 s, shaken vigorously for 15 min and centrifuged for 15 min at 14643 × *g* (13000 rpm) at 4 °C. The supernatant was transferred to another 50-mL polypropylene centrifuge tube, preserved at −20 °C for 30 min, and then centrifuged for 10 min at 14643 × *g* (13000 rpm) at 4 °C. The supernatant (8 mL) was transferred and mixed with 4 mL of hexane that had been previously saturated with acetonitrile. After centrifugation for 5 min at 8664 × *g* (10000 rpm) at 4 °C, the *n*-hexane was discarded. The lower acetonitrile layer (1 mL) was transferred to a 1.5-mL polypropylene centrifuge tube containing 80 mg of PSA, and the mixture was vortexed for 30 s and centrifuged for 15 min at 14643 × *g* (13000 rpm) at 4 °C. The supernatant solution was filtered through a 0.2-μm filter for UPLC–MS/MS analysis.

2.3. LC–MS/MS instrumentation and conditions

Chromatographic separation was achieved with a Waters Acquity UPLC system consisting of a vacuum degasser, an autosampler, and a binary pump, and the column oven temperature was maintained at 40 °C using an Agilent Eclipse XDB-C18 analytical column (150 mm × 2.1 mm, 3.5 μm). The elution solvents were 0.1% formic acid (A) and methanol (B) with the following gradient: initial, 5% B; 0–2 min, 25% B; 2–3 min, 30% B; 3–9 min, 40% B; 9–11 min, 50% B; 11–12 min, 60% B; 16–18 min, 98% B; 18–18.1 min, 5% B with a final hold for 2 min at a flow rate of 0.3 mL/min. The injection volume was 10 μL. The weak and strong wash solvents were water/methanol (1/1, v/v).

MS was performed on an AB SCIEX Qtrap 6500 MS/MS. The resulting optimized values were as follows: source temperature 450 °C; ion spray voltage 5500 V; curtain gas 20 psi; atomizing gas (GS 1) 50 psi; and dry gas (GS 2) 50 psi. The data were acquired in a scheduled MRM mode, and the corresponding declustering potential (DP) and collision energy (CE) are presented in Table 1.

2.4. Method validation

An analyte was considered positively identified when the following criteria were met: (1) the ratio of the retention time of the analyte to that of the same analyte in the standard solution was within ± 2.5% tolerance; (2) a signal at each of the two MRM ion pairs for the analyte was present, corresponding to four identification points, as defined by the EU Commission Decision 2002/657/EC