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Multi-residue analysis of veterinary drugs, pesticides and mycotoxins in dairy products by liquid chromatography–tandem mass spectrometry using low-temperature cleanup and solid phase extraction

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ABSTRACT

A multi-class multi-residue analysis method for determination of veterinary drugs, pesticides and mycotoxins in dairy products by liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been established. These 17 classes, a total of 40 kinds of target compounds were chosen because their administration to food-producing animals is banned or regulated in China and may be potentially abused or misused. Samples were extracted with acetonitrile–ethyl acetate–acetic acid (49.5 + 49.5 + 1, v/v/v). Most of lipids in the extract were removed by low-temperature cleanup, prior to solid phase extraction on HLB cartridges. The quantification and confirmation of the 40 analytes were performed by LC–MS/MS with electro-spray ionization (ESI) interface in multiple reaction monitoring (MRM) mode. The limits of detection (LODs) and limits of quantification (LOQs) were 0.006–0.3 μg/kg and 0.02–1.0 μg/kg, respectively. The spiked recoveries in milk, yogurt, milk powder and cheese samples were from 67.3% to 106.9%. The repeatability and the within-laboratory reproducibility were less than 12.7% and 13.9%. Applying this method, our results revealed the presences of chloramphenicol, cimeterol, and flunixin at the concentration of 0.027–0.452 μg/kg in some samples.

1. Introduction

Dairy products constitute an important source of nutrients in the daily diet of humans [1]. China's dairy consumption is about 37 million tons in 2014 [2]. However, the harmful compounds, such as veterinary drugs, pesticides and mycotoxins, are led into dairy cows in breeding farming procedures [3,4]. The possible presence of veterinary drugs, pesticides and mycotoxins in dairy products is one of the key issues for food safety which arouses great public concern [5,6]. To ensure high quality of produced milk and protect consumer health, the European Union (EU), United States (US) and China have banned the use of some veterinary drugs and pesticides in milk-producing animals [7,8] and strictly regulated for mycotoxins [9,10]. Moreover, it is important to notice that the maximum levels established by the EU for aflatoxin M_1 is 0.05 μg/kg, and it is further below the tolerance set by the Chinese administration and US Food and Drug Administration (0.50 μg/kg). Therefore, it has prompted the development of a control programme that necessitated sensitive, robust and reliable analytical method for the determination of veterinary drugs, pesticides and mycotoxins residues in dairy products.

Currently, various methods for determination of veterinary drugs, pesticides or mycotoxins residues, such as enzyme-linked immunosorbent assay (ELISA) [11], spectrophotometry [12], thin-layer chromatography [13], gas chromatography (GC) [14], high performance liquid chromatography (HPLC) with UV or fluorescence detection [15,16], gas chromatography–mass spectrometry (GC–MS) [17], and liquid chromatography–tandem mass spectrom-

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etry (LC–MS/MS) [18–27] have been reported. However, the ELISA often results in a "one drug, one test" approach, hindering multi-residue methods and preventing the detection of non-target drugs. Moreover, many chromatography methods were developed for specific analytes or groups of closely related analytes that were typically determined using instrumentation of relatively low selectivity (HPLC with UV, fluorescence or refractive index detection and GC with flame ionisation, electron capture nitrogen phosphorous or flame photometric detection). The GC–MS method usually needs derivatization procedure before analysis, which is more tedious and time-consuming. In contrast to these reports, LC–MS/MS is a powerful analytical technique, due to its high universality, sensitivity and selectivity. Some applications of this technique concerning milk or raw milk have been reported for determination of veterinary drugs [3,18], pesticides or mycotoxins [1]. Aguilera-Luiz et al. [1] developed a method for simultaneous determination of mycotoxins and pesticides (e.g., aflatoxin M_1, ochratoxin A, and carbofuran). Ortelli et al. [3] built methods for simultaneous determination of multi-veterinary drugs (e.g., clenbuterol, dimetridazole, and flunixin). Zhan et al. [18] established a method which could analyze veterinary drug residues and other contaminants (e.g., cimaterol, metronidazole, and aflatoxins). However, few work focused on simultaneouly analyzing veterinary drugs, pesticides and mycotoxins by LC–MS/MS.

Since the matrix of the dairy products samples is complicated, it is extremely important to develop effective pretreatment methods to concentrate the aim compounds and minimize the matrix interference before trace analysis. Moreover, measuring 17 classes of veterinary drugs, pesticides and mycotoxins with different chemical properties in the complex dairy matrix can be a real challenge. In recent years, the low-temperature cleanup method has been widely developed to determine veterinary drug residues and other contaminants in infant formula or muscle [28,29], veterinary drugs in animal muscles, or tomatoes [30–35], organophosphorus insecticides in olive oil [36], cocaine in postmortem human liver [37], mycotoxins in breast milk [38]. In above described studies [28–38], most of lipid components can be successfully separated from extracts with the low-temperature cleanup method. This methodology has several advantages over conventional methods with respect to effective sample clean-up and the relatively easy combination with solid phase extraction (SPE) clean-up. So far, the low-temperature cleanup method has been scarcely used simultaneously purifying multiclass veterinary drugs, pesticides and mycotoxins in milk, yogurt, milk powder, and cheese.

In this paper, we used low-temperature cleanup followed by solid phase extraction to purify dairy products, and developed a LC–MS/MS method for the determination of 40 compounds belonging to 17 different classes in milk, yogurt, milk powder and cheese samples. The established method was validated, and the results showed that the method was sensitive and reliable, and would be a promising method for multiple chemical residue analysis.

2. Experimental

2.1. Reagents and chemicals

Chloramphenicol (CAP), cimeterol (CMT), clenbuterol (CLB), ractopamine (RCT), salbutemol (SLB), phenolethanolamine A (PEA), metronidazole (MTD), dimetridazole (DMD), trenbolone (TBL), testosterone propionate (TSP), nandrolone phenylpropionate (NTPP), methyltestosterone (MTS), mengestrol acetate (MA), estradiol benzoate (EB), chlorpromazine (CPZ), diazepam (DZP), malachite Green oxalate (MG), crystal violet (CV), nitrovin (NTV), dapsone (DPS), flunixin (FLX), 4-formylaminoantipyrine (4-FAA), carbofuran (CBF), chlordimeform (CDF), standards purity ≥99%, were obtained from Dr. Ehrenstorfer (Augsburg, Germany); the standards of α-zearalanol (α-ZAL), β-zearalanol (β-ZAL), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), zearalanone (ZAN), zearalenone (ZEN), aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), aflatoxin M_1 (AFM_1), aflatoxin M_2 (AFM_2), ochratoxin A (OTA), deepoxy-deoxynivalenol (DOM-1), fumonisin B_1 (FB_1), and Fumonisin B_2 (FB_2), standards purity ≥98%, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Isotopically labeled compounds, used as internal standards, d5-chloramphenicol (d5-CAP), d7-cimetarol (d7-CMT), d9-clenbuterol (d9-CLB), d3-methyltestosterone (d3-MTS), and d6-chlorpromazine (d6-CPZ), standards purity ≥98%, were acquired from Witega (Berlin, Germany).

HPLC grade acetonitrile (MeCN) and methanol (MeOH) were supplied by J.T. Baker (USA). Ethyl acetate (EtOAc) and *n*-hexane of HPLC grade were purchased from Fisher Chemicals Co. (New Jersey, USA). Formic acid and ammonium formate of HPLC grade were obtained from ACROS ORGANICS (Brussels, Belgium). Analytical grade acetic acid and sodium chloride were obtained from Beihuajingxi Corp. (Beijing, China). Oasis HLB cartridge was purchased from Waters (Milford, MA, USA). Ultra-water was purified from a Milli-Q purification system (Millipore, Bellerica, MA, USA) at 18.2 MΩ/cm resistivity.

Individual stock standards and internal standards were prepared in MeCN at a concentration from 200 to 1000 μg/mL. Diluted mixed working standards at 2 μg/mL were prepared in MeCN from the stock solutions. All these solutions were kept at −20 °C. Working solution was renewed every month.

2.2. LC–MS/MS analysis

The LC–MS/MS analysis was performed with an Agilent (Santa Clara, CA, USA) 1290 Rid Resolution LC system equipped with a binary solvent delivery system and an autosampler. The LC system was coupled with an Agilent QQQ 6460 triple quadrupole mass spectrometer. The instrument control, data acquisition and data treatment were performed with Masshunter database software (Agilent, Santa Clara, CA, USA). Chromatographic separation was carried out using an Agilent ZORBAX SB-C_{18} column (100 × 2.1 mm id, 3.5 μm, Agilent, Santa Clara, CA, USA).

The mobile phase for ESI^+ consisted of 0.1% formic acid solution containing 5 mM ammonium acetate (mobile phase A_1) and MeCN (mobile phase B). The mobile phase for ESI^- consisted of deionized water (mobile phase A_2) and MeCN (mobile phase B). The same gradient elution program for ESI^+ and ESI^- was as follows: 0–3 min, 1–25% B; 3–3.5 min, 25–35% B; 3.5–5 min, 35–50% B; 5–6.5 min, 50–80% B; 6.5–9.5 min, 80–100% B; 9.5–10.5 min, 100% B; 10.5–11.5 min, 100–1% B; 11.5–13 min, 1% B. Flow rate was 0.4 mL/min. The column temperature was 40 °C. The injection volume was 10 μL. The column was re-equilibrated at the initial chromatographic conditions for 5 min before the next injection.

The MS/MS acquisition was carried out using ESI in both positive and negative mode. The main MS parameters were optimized and finally set as follows: nebulizer gas, N_2 (40 psi); drying gas, N_2 (10 L/min, 300 °C); sheath gas, N_2 (11 L/min, 350 °C); capillary voltage, 4.0 kV. Two MRM transitions of each analyte were chosen. The retention times and optimized MRM parameters for each analytes are presented in Table 1.

2.3. Samples

The whole milk, yogurt, milk powder, and cheese were purchased from local supermarket. All the samples were stored below 4 °C. The cheese samples were minced before storage. All kinds of samples of 5 g were taken for the analysis.