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Analytical Methods

Optimization of sample preparation by central composite design for multi-class determination of veterinary drugs in bovine muscle, kidney and liver by ultra-high-performance liquid chromatographic-tandem mass spectrometry

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ABSTRACT

In this study a simple and fast multi-class method for the determination of veterinary drugs in bovine liver, kidney and muscle was developed. The method employed acetonitrile for extraction followed by clean-up with EMR-Lipid® sorbent and trichloracetic acid. Tests indicated that the use of TCA was most effective when added in the final step of the clean-up procedure instead of during extraction. Different sorbents were tested and optimized using central composite design and the analytes determined by ultra-high-performance liquid chromatographic-tandem mass spectrometry (UHPLC-MS/MS). The method was validated according the European Commission Decision 2002/657 presenting satisfactory results for 69 veterinary drugs in bovine liver and 68 compounds in bovine muscle and kidney. The method was applied in real samples and in proficiency tests and proved to be adequate for routine analysis. Residues of abamectin, doramectin, eprinomectin and ivermectin were found in samples of bovine muscle and only ivermectin in bovine liver.

1. Introduction

Veterinary drugs are frequently used in animals to prevent and combat infections, induce growth and for maintaining the cattle and herd. These drugs can be easily accumulated in animal tissues and the residues can cause several risks to human health as toxic effects, allergic reactions and hypersensitivity (Aguilera-Luiz, Romero-González, Plaza-Bolaños, Martínez Vidal, & Garrido Frenich, 2013). Other consequences are the development of resistant bacteria that might interfere in the efficiency of antibiotics, difficult diseases treatment and can cause negative effects in animal welfare. All these concerns present serious consequences for productivity and economy (Dahiya, Dubey, Singh, & Singh, 2013). In order to increase food safety, maximum residue levels (MRLs) are established for veterinary drugs in different kinds of food (Moreno-Bondi, Marazuela, Herranz, & Rodriguez, 2009).

Sample preparation methods to determine veterinary drugs in foods from animal origin such as bovine muscle, liver and kidney are highly complex since these matrices present high concentrations of myoglobin, fat and proteins (Aerts, Hogenboom, & Brinkman, 1995; Stolker & Brinkman, 2005). The limited sensitivity, chemical stability and the different physicochemical properties of veterinary drugs can also affect results (Kaufmann, 2009). Most of the traditional sample preparation methods includes solvent extraction (Jank et al., 2015) or QuEChERS method (Bandeira et al., 2017) followed or not by a cleanup step. Matrices of animal origin are very complex considering the large amounts of lipids, proteins, phospholipids, pigments and other interferents. Thus, several types of sorbents can be used for cleaning extracts of these matrices, for example: Supel™ QuE Z-Sep + and octadecylsilane (C_{18}) (Geis-Asteggiante et al., 2012), solid phase extraction (SPE) using Oasis® HLB cartridge (Tang, Lu, Lin, Shin, & Hwang, 2012), diatomaceous earth (De Oliveira et al., 2017) and Florisil® (Orso et al., 2016).

Recently, the sorbent Enhanced Matrix Removal-Lipid (EMR-Lipid®) was introduced as a material that can selectively remove different lipid classes and interferences avoiding the retaining of unwanted analytes. The structure of EMR-Lipid was not disclosed, however it is mentioned that its mechanism involves size exclusion and hydrophobic interactions. Long-chain hydrocarbons that presents association with lipids can fit inside EMR-Lipid structure where they are trapped. Besides, the lipid-EMR-Lipid® complex can precipitated out of solution or remain in the aqueous phase during the final salting-out step. One of the main advantages of the EMR-Lipid® is that this sorbent can remove lipids selectively from extracts of fatty foods such as animal tissues, without the loss of common veterinary drugs (DeAtley, Zhao, & Lucas, 2015).

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The use of ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) has been increasing in the field of veterinary drugs analysis since promotes narrower peaks with high intensity, improving significantly the sensitivity and selectivity of the methods and enables faster analysis (Jakimska, Kot-Wasik, & Namieśnik, 2014).

Due to advances in the science fields, including automation in data generation and acquisition, a large amount of numerical data and information is generated. In order to facilitate the best conditions for sample preparation and for the best understanding of the data generated during this step, statistical tools are indispensable. The optimization by experimental designs is very important to reduce cost and time required for experimental development and to decrease the number of experiments to be performed in the laboratory (Kemmerich et al., 2015).

The aim of this study was to develop and validate a simple, selective, sensitive and reliable method based on solid–liquid extraction and d-SPE for determination of multi-class veterinary drugs residues in bovine muscle, kidney and liver. For sample preparation optimization, a central composite design was used followed by analysis with ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The use of statistical tools allowed to obtain the best conditions for sample preparation.

2. Material and methods

2.1. Chemicals and materials

Acetonitrile and methanol LC-MS grade were purchased from J.T. Baker (Phillipsburg, USA). Formic acid and ammonium acetate (≥98% purity) were obtained from Sigma-Aldrich (St. Louis, USA). Trichloracetic acid (TCA, 99.5%) and Millex-GN nylon filters (0.20 μm) were from Merck (Darmstadt, Germany). Ultrapure water was provided by a DirectQ-UV system (resistivity of 18.2 MΩ·cm) from Millipore (Molsheim, France).

Bondesil octadecylsilane (C_{18}), with particle size of 40 μm and Bond Elut Enhanced Matrix Removal-Lipid (EMR-Lipid®) were purchased from Agilent Technologies (Santa Clara, USA). Florisil® with particle size of 60–100 mesh, activated at 675 °C before use, was obtained from J.T. Baker (Phillipsburg, USA); Supel™ QuE Z-Sep+ and diatomaceous earth were acquired from Sigma Aldrich (St. Louis, USA). SPE cartridges of Strata™ C_{18} 500 mg/3 mL was purchased from Phenomenex (Torrance, USA) and Oasis® HLB 60 mg/3 mL from Waters (Taunton, USA).

Analytical standards with purity between 95 and 99% were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Witega (Berlin, Germany) and Sigma Aldrich (St. Louis, USA). Individual stock solutions were prepared at the concentration of 1000 mg L^{-1} for each veterinary drug under study, considering the purity of the solid standards. The compounds were dissolved in water, acetonitrile, methanol or tetrahydrofuran in accordance with the respective solubility. From these individual stock solutions, a standard mixture was prepared in acetonitrile for each matrix at a concentration proportional to the MRL established for each analyte. The stock solutions diluted in organic solvent were stored in amber flasks at −18 °C. Diluted solutions and the ones that were prepared in water were stored at a refrigerator (0–10 °C). The stock solutions diluted in organic solvent were stored in amber flasks at −18 °C and diluted solutions and the ones that were prepared in water were stored at a refrigerator (0–10 °C). The stock and diluted solutions validity were 6 and 1 month, respectively.

In order to verify instrument stability, the internal standard triphenylphosphate, purchased from Sigma Aldrich (St. Louis, USA), was used. The deuterated standards albendazole-d_3, clembuterol-d_9 and sulfadimethoxine-d_6 were purchased from Witega (Berlin, Germany) and were added before the extraction step to evaluate sample preparation.

2.2. Instrumentation

Chromatographic analyses were carried out using an UHPLC-MS/MS system from Waters (USA) equipped with Acquity UPLC™ liquid chromatograph, Xevo TQ™ MS/MS with triple quadrupole detector, autosampler, binary pump, column temperature controller and the data acquisition software MassLynx V4.1. Separation was performed with Waters Acquity UPLC™ analytical column HSS-T3 (100 × 2.1 mm i.d., 1.8 μm particle size) maintained at 60 °C. Nitrogen with purity ≥99% from a Peak Scientific (Inchinnan, Scotland) generator model NM30L-MS was used in the electrospray ionization (ESI) source.

Chromatographic separation was carried out with gradient elution using the mobile phase: (A) water:acetonitrile (98:2, v/v), containing 10 mmol L^{-1} of aqueous ammonium formate, and (B) methanol:acetonitrile (75:25, v/v), with 0.1% (v/v) formic acid. The target compounds were separated in two different gradients: method 1 (class of avermectins, milbemicins, benzimidazoles, imidazolothiazoles, nitroimidazoles, salicylanilides, coccidiostats, ionophores, diaminopyrimidine derivatives, β-agonists, sedatives, anabolics, nonsteroidal anti-inflammatory, steroid) and method 2 (class of amphenicols, diterpenes, quinolones, lincosamides, macrolides, sulfonamides, tetracyclines). For method 1, the gradient program was started with 20% B and the proportion was increased linearly to 80% B in 1.5 min, then to 100% B in 3.5 min, at which it was held for 2.0 min before it was returned to the initial condition. For method 2, the gradient program started at 1% B (held 0.25 min) increased to reach 30% B in 1.5 min (held 3.0 min), then to 100% B in 4.5 min (held 1.0 min) and decreased to reach 1% B in 5.51 min (held 0.49 min). The diverter valve was programmed to send the LC flow to waste between 0 to 0.5 min and 4.5 to 6 min for method 1 and from 0 to 1 min and 4.5 to 6 min for method 2. In both methods, the total chromatographic run time, flow-rate and injection volume were 6 min, 0.400 mL min^{-1} and 10 μL, respectively.

Instrumental parameters for mass spectrometric analyses were: nitrogen (N_2), employed as desolvation and cone gas, at flow-rate of 500 and 80 L h^{-1}, respectively; capillary voltage, 2.0 kV; source temperature, 150 °C; desolvation temperature, 500 °C; and argon gas (Ar), used in the collision cell for ion fragmentation, at 0.15 mL min^{-1}. The quadrupole mass spectrometer was operated using the ESI source in positive and negative mode. Selected reaction monitoring (SRM) mode was selected by scanning two precursor/products ion transitions for each target analyte according to Tables S1 and S2 shown in Supplementary data.

2.3. Sample preparation

Blank samples of bovine muscle, kidney and liver used for method establishment were acquired in a slaughterhouse from Santa Maria (Brazil), that commercialize these products from animals without conventional treatments.

An aliquot of 2 g of sample was weighed in 50 mL polypropylene centrifuge tubes, then 6 mL of acetonitrile was added to each tube and minced samples were immediately homogenized in ultra-turrax during 20 s at 24000 rpm and centrifuged for 8 min at 2420*g*. After that, 500 μL of extract was transferred to an Eppendorf tube containing 500 μL of TCA 5% (w/v). The tube was vigorously vortexed for 30 s and centrifuged at 13,300*g*, for 10 min at 0 °C. After this, the extract was filtered in a 0.2 μm nylon membrane syringe filter and transferred to a vial for UHPLC-MS/MS analysis. The use of 12 mL of hexane:acetonitrile (1:1, v/v) and 12 mL of aqueous solution of TCA 5% (w/v):acetonitrile (1:1, v/v) in the extraction step were also evaluated.

For the clean-up step, the sorbents Bondesil C_{18} (50 mg) and Supel™ QuE Z-Sep+ (50 mg) were evaluated by d-SPE, as well Oasis HLB and Strata-C_{18} in cartridges. The extract in acetonitrile and TCA 5% (w/v) were transferred to an Eppendorf tube containing C_{18} or Supel™ QuE Z-Sep+, tube was vigorously vortexed for 30 s and centrifuged at 13,300*g* for 10 min at 0 °C. For cartridges, 3 mL of extract in acetonitrile were