Talanta 132 (2015) 443–450

Contents lists available at ScienceDirect

Talanta

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

Analytical quality assurance in veterinary drug residue analysis methods: Matrix effects determination and monitoring for sulfonamides analysis

Rodrigo Barcellos Hoff [a,b,*], Gabriel Rübensam [b], Louise Jank [a,b], Fabiano Barreto [b,c], Maria do Carmo Ruaro Peralba [a], Tânia Mara Pizzolato [a], M. Silvia Díaz-Cruz [d], Damià Barceló [d,e]

[a] Instituto de Química, UFRGS, Porto Alegre, RS, Brazil
[b] Ministério da Agricultura, Pecuária e Abastecimento, Laboratório Nacional Agropecuário—LANAGRO/RS, Porto Alegre, RS, Brazil
[c] Faculdade de Farmácia, UFRGS, Porto Alegre, RS, Brazil
[d] Department of Environmental Chemistry, IDAEA, CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[e] Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, C/Emili Grahit, 101 Edifici H2O, E-17003 Girona, Spain

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form
15 August 2014
Accepted 17 August 2014
Available online 27 August 2014

Keywords:
Quality control
Veterinary drug residues
Matrix effects
Validation
Ion suppression
Control charts
Antibiotics
Sulfonamides

ABSTRACT

In residue analysis of veterinary drugs in foodstuff, matrix effects are one of the most critical points. This work present a discuss considering approaches used to estimate, minimize and monitoring matrix effects in bioanalytical methods. Qualitative and quantitative methods for estimation of matrix effects such as post-column infusion, slopes ratios analysis, calibration curves (mathematical and statistical analysis) and control chart monitoring are discussed using real data. Matrix effects varying in a wide range depending of the analyte and the sample preparation method: pressurized liquid extraction for liver samples show matrix effects from 15.5 to 59.2% while a ultrasound-assisted extraction provide values from 21.7 to 64.3%. The matrix influence was also evaluated: for sulfamethazine analysis, losses of signal were varying from −37 to −96% for fish and eggs, respectively. Advantages and drawbacks are also discussed considering a workflow for matrix effects assessment proposed and applied to real data from sulfonamides residues analysis.

1. Introduction

Food containing veterinary drug residues above maximum residue limit (MRL) is of major concern, since it is related directly to public health as well as international trade relationships. The demand in food regulatory control has expanded dramatically in recent decades, and residues surveillance became an important factor to be considered in international trade of commodities [1,2].

In Brazil, veterinary drug and pesticide residues analysis in animal (and also in vegetable) products are under the Ministry of Agriculture, Livestock and Supply (MAPA) management [3]. Routine analysis and methods development and validation are attributed to MAPA official laboratories network – National Agricultural Laboratories (Lanagro) – and MAPA accredited private laboratories [4]. MAPA's demand on method development and validation in residue analysis has been increased in the last decade due to the increased role of the Brazilian livestock products in national and international markets and meanly to ensure that the products traded are compliant with the safety and quality criteria required by consumers [4,5]. Wherefore, our laboratory has absorbed one important fraction of this demand in developing, validating, and submitting for accreditation methods for analysis of antimicrobial and non-antimicrobial residues in different matrices, such as milk and edible tissues of different animal species including cattle, pork, poultry, and even fish [6–10]. For these purposes, international guidelines, such as Commission Decision 2002/657/EC concerning the performance of analytical methods and the interpretation of results, and others from the US Food and Drug Administration (FDA) and the International Conference on Harmonization (ICH), are used in order to obtain methods validated according to the most stringent international criteria [11–14]. Within this issue, especial attention is paid to matrix effect (ME), which is a fundamental parameter to be determined, assessed and minimized especially when

* Corresponding author at: Ministério da Agricultura, Pecuária e Abastecimento, Laboratório Nacional Agropecuário—LANAGRO/RS, Estrada da Ponta Grossa, 3036 CEP, 91780-580 Porto Alegre, RS, Brazil. Tel.: +55 51 3248 2133; fax: +55 51 3248 2690.
E-mail address: rodrigo.hoff@agricultura.gov.br (R.B. Hoff).

http://dx.doi.org/10.1016/j.talanta.2014.08.046
0039-9140/

liquid chromatography–mass spectrometry (LC–MS) and/or tandem mass spectrometry (LC–MS/MS) methods are used [15–19]. The conceptualization of this phenomenon has been comprehensively reviewed by a number of authors [15,16,20,21]. Briefly, ME is related to the alteration of ionization efficiency in the ionization source by the presence of coeluting substances: the occurrence of endogenous substances originally present in the sample itself and that remains in the final extract, are appointed as the major source. A wide scope of molecules can lead to signal suppression or enhancement, especially when occurs in high concentration in the extract and elute in the same retention time window than the analyte [22]. A secondary cause are substances not originally present in the samples but able to migrate to extracts during sample preparation process as polymer and phthalates or material released by stationary phases, in bulk or in solid phase extraction (SPE) cartridges, for instance [20]. Normally, this alteration affects dramatically the method accuracy and precision and has been regarded as a critical validation item by most guidelines consulted. However, there is no consensus on how this phenomenon should be assessed during method validation. Beside, different experienced approaches of ME evaluation, based on procedures published in the scientific literature such as post-column infusion, calibration curves comparison, quantitative estimation based in standards, spiked samples and matrix-matched control comparison and control charts evaluation, has been experienced [23–27].

Although the knowledge on ME in mass spectrometry analysis has been improved in recent years, only few practical approaches has been reported for routine analysis [28–31]. In the present work, practical approaches to detect and estimate the occurrence of ME in qualitative and quantitative terms in LC–MS/MS methods for veterinary drugs residues analysis are presented and discussed. Tools for monitoring ME along the execution of routine methods are also reported. Without the purpose to exhaust the issue, the present study is proposed as a walkthrough based in relatively simple and easy techniques to be applied to analytical chemistry laboratories to deal with the critical theme of matrix effects in residues analysis.

2. Materials and methods

2.1. Analytical standards and reagents

Analytical standards with high purity ($\geq$99%) were obtained from Sigma-Aldrich (St Louis, MO, USA) namely sulfamerazine (SMR), sulfamethazine (SMZ), sulfamethoxazole (SMA), sulfamethoxypyridazine (SMPZ), sulfadiazine (SDZ), sulfapyridine (SPY), sulfadimethoxine (SDMX), sulfaguanidine (SGA), sulfacetamide (SCA), sulfabenzamide (SBZ), sulfisomidin (SIM), sulfamethizole (SMTZ), sulfaquinoxaline (SQX), sulfathiazole (STZ), sulfaisoxazole (SIX) and sulfadoxin (SDX). The metabolite N^4-acetyl-sulfamerazine (N^4-SMR) and the isotopically labelled compounds d^4-sulfamethoxazole (d^4-SMA), d^4-sulfamethazine (d^4-SMZ) and d^4-sulfadiazine (d^4-SDZ), used as surrogate and/or internal standards, were purchased from Toronto Research Chemicals (North York, Ontario, Canada).

Methanol (MeOH), acetonitrile (ACN), hexane and acetone of HPLC-grade were supplied by J. T. Baker (Deventer, The Netherlands). Diatomaceous earth was supplied by Agilent Technologies. Acetic acid and water (HPLC grade) were purchased from Merck (Darmstadt, Germany).

Individual stock standard solutions were prepared in MeOH: acetone (50:50) at 1 mg mL^{-1} and stored at −4 °C until use. Standard solutions of the mixtures of all compounds in appropriate concentrations were prepared by stock solutions dilutions using MeOH or acetone. Aliquots of each stock standard solution were diluted to obtain final concentrations of 10 μg mL^{-1} and 1 μg mL^{-1} and were stored at −20 °C.

2.2. Samples and sample preparation

Liver of different food production animals, chicken eggs, and fish muscle were obtained from Federal Inspection Service (SIF) or collected from treated animals in a farm. Liver and muscle samples were manual and finely chopped and homogenized in order to avoid slurring. Egg samples were manual and gently homogenized in order to avoid protein denaturation. After these processes, all samples were stored at −20 °C before extraction step.

Liver and fish samples were extracted by two different methods based on pressurized liquid extraction (PLE) and by ultrasounds-assisted extraction (US). A detailed discussion about these methods and validation results were submitted to publication. For PLE, an ASE 350 accelerated solvent extractor (Dionex, Sunnyvale, CA, USA) was used. Prior to extraction, d^4-SMA, d^4-SMZ and d^4-SDZ were added as surrogate standards in a concentration of 100 ng g^{-1}. Samples (0.5 g) were mixed into the PLE cells with diatomaceous earth as dispersing agent. Prior to extraction, the cells were submitted to a clean up method in order to remove lipids from the samples using hexane as solvent. PLE parameters were as follows: temperature 60 °C, 4 cycles of 5 min each one. Total flush volume of 80% and 300 s of purge with nitrogen flow were applied.

After that, the same PLE cells were submitted to a second extraction process using ACN with 0.2% acetic acid as extraction solvent. In this case, the extraction temperature was optimized at 90 °C. A preheating period of 8 min was selected and 3 cycles of 7 min each were carried out. A total flush volume of 80% and 60 s of purge with nitrogen flow were applied. Pressure was set at 1500 psi as it has been demonstrated that this parameter is not decisive in PLE.

The extracts were maintained in freezer by 1 h (at −18 °C) in order to promote protein precipitation. Following, samples were centrifuged at 1500 × *g* for 10 min in a 5810R centrifuge (Eppendorf). The supernatant was evaporated at 40 °C under nitrogen flow using a Turbo-Vap system (Zymark) until dryness. Extracts were redissolved in 1.0 mL of mobile phase mixture (water-ACN, 85:15) and transferred to a HPLC vial.

In ultrasound-assisted extraction, samples (0.5 g) were weighted in 15 mL polypropylene tubes and spiked as described for the PLE method. Following, 10 mL ACN were added and tubes were mixed in a mechanical vortex by 10 s. Afterwards all samples were placed into an ultrasonic bath for 1 h. and then stored in freezer (−18 °C) for 1 h. to promote protein precipitation. Then, samples were centrifuged at 1500 × *g* for 10 min. Supernatant was brought to dryness at 40 °C under a gentle nitrogen stream. The extracts were redissolved in 2.0 mL of the mobile phase mixture. An aliquot of 2 mL of hexane was added to remove the fat content. Afterwards, tubes were mixed in a vortex for 5 s followed by centrifugation (3500 rpm for 10 min). The lower layer was carefully transferred to a HPLC vial.

Sulfonamides analysis in eggs samples was performed as described elsewhere [32]. Briefly, samples were extracted with ACN and concentrated before reconstitution with mobile phase.

2.3. Instrumentation

LC analysis was performed with a Symbiosis™ Pico System (Spark Holland, Emmen, The Netherlands), equipped with a HPLC system consisting of an Alias™ autosampler, a loop injector and two binary pumps with a four-channel solvent selector for each one. Chromatographic separation was performed using a HPLC column Purospher® STAR (C18, ec, 150 × 4.6 mm, 5 μm) preceded by a guard column with the same packing material. The flow rate was set to 0.2 mL min^{-1}, being eluent (A) HPLC grade water acidified with 10 mM of formic acid, and eluent (B) ACN with