Talanta 149 (2016) 43–52

Contents lists available at ScienceDirect

Talanta

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

Target analysis and retrospective screening of veterinary drugs, ergot alkaloids, plant toxins and other undesirable substances in feed using liquid chromatography–high resolution mass spectrometry

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ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form
6 November 2015
Accepted 12 November 2015
Available online 14 November 2015

Keywords:
Retrospective analysis
Veterinary drugs
Ergot alkaloids
Animal feed
LC–HRMS
Orbitrap

ABSTRACT

A comprehensive strategy combining a quantitative method for 77 banned veterinary drugs, mycotoxins, ergot alkaloids and plant toxins, and a post-target screening for 425 substances including pesticides and environmental contaminants in feed were developed using a QuEChERS-based extraction and an ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC–HRMS).

The quantitative method was validated after previous statistical optimisation of the main parameters governing ionisation, and presented recoveries ranging, in general, from 80 to 120%, with a precision in terms of Relative Standard Deviation (RSD) lower than 20%. The full-scan accurate mass data were acquired with a resolving power of 50000 FWHM and a mass accuracy lower than 5 ppm. The method LOQ was lower than 12.5 $\mu g\ kg^{-1}$ for the majority of the veterinary drugs and plant toxins and 20 $\mu g\ kg^{-1}$ for ergot alkaloids.

For post-target screening a customised theoretical database including the exact mass, the polarity of acquisition and the expected adducts was built and used for post-run retrospective screening. The analytical strategy was applied to 32 feed samples collected from farms of the Valencia Region (Spain). Florfenicol, zearalenone and atropine were identified and quantified at concentrations around 10 $\mu g\ kg^{-1}$. In the post-target screening of the real samples, Sulfadiazine, Thrimetoprin and Pirimiphosmethyl were tentatively identified.

1. Introduction

Toxic substances such as veterinary drugs, mycotoxins, plant toxins and ergot alkaloids are frequently present in animal feed due to their misuse, carry-over or environmental contamination [1–3]. The direct consequence is the incorporation of these substances into the food chain, which may, therefore, present a risk for consumers [4]. In fact, carcinogenic and teratogenic effects, as well as allergies and drug resistance resulting from the presence of these molecules in the food chain have been previously reported [5,6]. Therefore, monitoring the presence of these potentially hazardous chemicals remains one of the main tasks for ensuring feed safety and human health. Several European regulations have been implemented for feed safety. In the case of veterinary drugs, all antibiotics (except coccidiostats and histomonostats) are banned for use as feed additives [7]. While many medicines are authorised for therapeutic and prophylactic purposes (provided their use is registered), others are authorised as long as their concentrations in food of animal origin remain below certain established limits. Other uses of veterinary drugs, as growth promoters to increase yield, are strongly prohibited by the European Union since 1996 altogether [8]. Nevertheless, the unintentional presence of some compounds such as coccidiostats can occur in feedstuffs as a result of the so-called cross-contamination [9]. Other undesirable substances in animal feed such as mycotoxins, plant toxins or ergot alkaloids have been included in the legislation by the directive 2002/32/EC [10]. Two recent papers describe the complicated European Legislation on these substances in feed [11,12].

Appropriate analytical methods are essential to support the enforcement of regulations. In the last 15 years the workhorse for the analysis of veterinary drugs and contaminants in animal feed and in other food safety areas has been liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS), mainly using

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http://dx.doi.org/10.1016/j.talanta.2015.11.032
0039-9140/

triple quadrupole analysers (QqQ). This is supported by the many examples of methods that have been developed for the analysis of toxic substances in feed by using this analytical platform [13–16]. Despite its high sensitivity and selectivity, the method set-up is tedious and time-consuming when wanting to determine a large number of substances. Likewise, this technique presents limitations on the number of compounds that can be analyzed in one run, and only target analytes can be detected without the possibility of retrospective data analysis [17]. Thus, nowadays, the use of liquid chromatography coupled to high-resolution mass spectrometry (HRMS) has emerged as a successful alternative for the multiclass/multianalyte analysis in food safety and environmental control that overcomes the limitations of tandem mass spectrometry analysis [18–22]. The use of HRMS is mainly driven by the advantages of using the full-scan acquisition mode with high sensitivity, combined with high resolving power >50000 FWHM and accurate mass measurement (1–5 ppm). Currently, HRMS can be performed by using time of flight (TOF) mass spectrometers, or through Orbitrap technology, permitting to combine target and post-target analysis [23–25]. This comprehensive strategy allows developing methods that cover a wide scope of compounds with different physicochemical properties (multiresidue–multiclass) with high selectivity and high or enough sensitivity. The way to achieve target analysis without information loss for the untarget analysis is to perform a generic extraction method. Dilute and shoot could be the ideal choice for the sample preparation of matrices, Some of its advantages are that being a generic method a wide range of polarities is covered, it is fast and the matrix effect decreases considerably because of the dilution of the interferences [26,27]. However, one of its common limitations is the low sensitivity for some of the investigated compounds. The QuEChERS (standing for quick, easy, cheap, effective, rugged, and safe) procedure has been used as a generic extraction method for different matrices, including feed samples because of its flexibility, which permits modifications depending on the analytes, matrices or analyst preferences [28–30]. In the same way, salting-out liquid–liquid extraction (SALLE) has also been proposed as a generic extraction approach for veterinary drugs and pesticides in urine and foods [31,32]. In the course of SALLE different salts used at diverse concentrations will alter the degree of phase separation between miscible solvents.

In comprehensive methods with a large scope of substances, optimising the spectrometric parameters, mainly those linked to the ionisation step, improves sensitivity. Optimisation of the mass spectrometric settings can be achieved using the conventional approach of change-one-separate-factor-at-a-time (COST), or using the automatic tuning procedures of each instrument. However, a better understanding of the influence of each parameter in the response, and of the interaction between factors could be achieved using a statistical design of experiments (DoE) [33].

Another key element to perform an integrated analytical strategy that combines target with post-run target analysis is the creation of a detailed database containing adequate information for the identification and confirmation of compounds, both for the target analysis (using standards) and for the retrospective analysis (without standards).

In the present study we have developed an analytical strategy for the quantitative target analysis and the post-run target analysis of banned veterinary drugs, mycotoxins, ergot alkaloids, pesticides and other undesirable substances in feed using a generic extraction method and LC–HRMS. The developed method was applied to 32 feed samples.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile and methanol were of LC–MS grade and supplied by Scharlab (Barcelona, Spain). Acetic acid (purity 98–100%) and water were of hypergrade quality and were purchased from Merck (KGaA, Darmstadt, Germany). QuEChERS Extract Pouches-EN method (salt packet containing 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, 0.5 g disodium citrate sesquihydrate) and ceramic homogenisers for QuEChERS extraction were obtained from Agilent Technologies (Madrid, Spain).

2.2. Standards and solutions

All commercial standards were of high purity and were obtained from Sigma-Aldrich (Barcelona, Spain), Witega (Berlin, Germany), USP Reference Standards (Maryland, United States), Medical Isotopes (Waltham, Massachusetts, USA), RIKILT (Community Reference Laboratory, Wageningen, The Netherlands), LGC Standards S.L.U (Barcelona, Spain) orBiopure (Tulln, Austria).

2.2.1. Ergot alkaloids

the ergot alkaloid (ergosine, ergosinine, ergocornine, ergocorninine, ergocryptine, ergocriptinine, ergocristine and ergocristinine) standards were supplied as a thin film dried-down standard and were reconstituted in 5 mL acetonitrile following manufacturer instructions. To tackle epimerisation problems between the main and the-inine forms [34], deep frozen standard solutions were prepared as reported by Diana et al. [14]. Fresh individual standard solutions were divided into several aliquots, evaporated and deep frozen at -20 °C. The exact volume of the aliquots was calculated taking into account the desired concentration of working solution to obtain the appropriate concentration in the control samples. To prevent epimerisation the working solution (containing all ergot alkaloids at 2.5 $\mu g\ ml^{-1}$) was prepared immediately before use from the individual deep frozen standards by reconstituting each one with the required volume of solvent.

2.2.2. Individual stock solutions

stock solutions containing approximately 50–1200 $\mu g\ ml^{-1}$ of the majority of analytes were prepared by weighing each compound and dissolving it in methanol or acetonitrile depending on its solubility properties. Solutions were stored at -20 °C for a maximum of 6 months. Individual stock solutions of nivalenol, deoxynivalenol, zearalenone, T-2 toxin, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, fumonisin B1 and fumonisin B2, were purchased directly in solution at concentrations ranging from 25 to100 $\mu g\ ml^{-1}$.

2.2.3. Working standard solutions

four different working standard solutions and the internal standard working solution were prepared. The analytes were distributed in the four working solutions depending on the concentration of the compound in the stock solution and the volume of addition of the working standard solution to the feed control samples to obtain the required concentration levels. For example, low concentration stock solution with high limit of quantitation may lead to a more concentrate working solution or in the other hand high concentration of the working solution with low limit of quantitation may need a diluted one. The multianalyte solutions were prepared by diluting the individual stock solutions with methanol, except for fumonisin B1, fumonisin B2 that were added directly to the control samples because of their low concentration or their high RPA. Working standard solution “1”, contained hydroxymethylclenbuterol, ractopamine, tulobuterol, clenbuterol,