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Preparation of quality control materials for the determination of sulfonamides in animal feed

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Abstract

A feasibility study of the preparation of quality control materials for the analysis of medicated feeds has been carried out. Two analytical methodologies for the analysis of sulfonamides in feeds were developed, validated and applied to homogeneity and stability studies. Pig feeds spiked with sulfadiazine and sulfadimidine were prepared. The drugs were spiked at 500 µg g\(^{-1}\), representing what can be expected in a commercial medicated feed, and at 2 and 5 µg g\(^{-1}\) which roughly correspond to drug-free feeds cross-contaminated during the fabrication process. The homogeneity of both the bulk and the bottled materials was verified. A stability study of the materials containing 2 and 5 µg g\(^{-1}\) of sulfonamides has been carried out over an 18-month period at room temperature, at 4°C and at -20°C. The determination of sulfadiazine and sulfadimidine in samples coming from these homogeneity and stability studies of the quality control materials was carried out by HPLC with either UV or fluorimetric detection, depending on the concentration of the analytes in the samples.

Keywords: Sulfonamides, animal feed, reference material, quality control material, liquid chromatography
Introduction

Food safety includes all steps in the food production chain. In the case of food from animal origin, it covers from the feed the cattle are given through to the final products reaching the consumers. In the European Union, the use of antimicrobial agents in animal feeds is regulated by law. At present, the use of antibiotics as growth promoters is forbidden (EC No 1831/2003), as they can be directly related with a detected increase in cases of resistance to antimicrobial drugs employed in medicine (Turnidge 2004; Phillips et al. 2004; Emborg and Hammerun 2006). This is why animal feeds can only contain antibiotics for therapeutic or prophylactic reasons. Medicated feeds, which contain active principles at therapeutic levels, are prepared under veterinary prescription from authorised medical pre-mixtures that comply to the requirements of European Directive 90/167 (EC No 90/167 1990).

Medicated feeds are prepared in the same production lines as regular feeds, and this leads to a high risk of carry-over, as antibiotic concentrations in medicated feed are quite high (100-1000 µg g\(^{-1}\)) and full elimination of their residues before switching back to a normal production is very difficult. This kind of contamination may affect cattle producers, as the consumption of feed that has become contaminated with antibiotic residues by animals about to be slaughtered may lead to food products containing concentrations exceeding the corresponding Maximum Residue Limits (MRLs). Moreover, the presence of antimicrobial residues in food of animal origin can also come from fraudulent uses of medicated feeds, and in order to detect these illegal practices, sanitary authorities carry out compulsory inspections in farms, in which samples of feeds are taken.

Ensuring safety in this first stage of the food chain requires reliable and rugged analytical methodology, suitable for an efficient control of the presence of antibiotics in animal feed. In order to validate analytical methods, or to control the quality of the analytical results, the availability of reference materials (RM) is a key point. These
materials can also be used in intercomparison exercises for the external assessment of laboratory quality. Due to the variety and complexity of the matrices found in feeds, RMs must be as similar as possible to the samples analysed, both in matrix composition and analyte concentration. Additionally, analytes should have been incorporated in the same way as at industrial level.

Because of the variety in the characteristics and in the applications of RM, there is some confusion in the terminology used to designate them (Quevauviller 1999; Walker et al. 1999; Zschunke 2000; Barwick 2001; Emons et al. 2004). In the present paper, the terms proposed by Emons et al. (Emons et al. 2004) are used. According to this terminology, quality control materials (QCMs) intended for the statistical control of the quality of the analytical results were prepared. These materials can also be used for method development and validation and as samples for interlaboratory comparisons. Although certified reference materials (CRMs) are at the top of the metrological hierarchy, their scarcity in most areas of food analysis means that QCM must frequently be used. Moreover, QCMs can be prepared in such a way that they match the samples analysed by a laboratory, or a group of laboratories, better than the commercially available CRMs. Additionally, they are a lower cost alternative when frequent use is required.

The objective of the research work described in this paper has been to study the feasibility of the preparation of two QCMs, consisting of an animal feed used for pig rearing spiked with two sulfonamides: sulfadiazine (SDZ) and sulfadimidine (SDD). The drugs have been spiked at several concentration levels, representing what can be found in a medicated feed (500 µg g\(^{-1}\)) and in a drug-free feed that has been contaminated during the fabrication process (2 and 5 µg g\(^{-1}\)).

Sulfonamides are among the most widely used veterinary drugs in the European Union. In Spain, only two of them, sulfadiazine (SDZ) and sulfadimidine (SDD), can be added to medicated pre-mixtures, and they are mainly used for pig rearing. This is why they have been selected for the preparation of the QCMs.
In recent years, several analytical methods for the determination of antimicrobials in animal feed have been proposed. In the case of sulfonamides, the methods found in the literature for their analysis in feeds are scarce and quite old (Stringham et al. 1982; Torel et al. 1985; Smallidge et al. 1988). They are based on HPLC with spectrometric detection at 254 nm or at 450 nm, after derivatization with dimethylaminobenzaldehyde, the target compounds being SDD, sulfathiazole and sulfamethoxypyridazine. An ELISA screening assay for SDD in feeds has also been proposed (Dixon-Holland and Katz 1991). More recently, a collaborative study, launched by AOAC, on the determination of SDD in swine and cattle feed was carried out (Albert and Smallidge 2000). In the present work, the analytical methodology required for the determination of the concentrations of SDZ and SDD for the assessment of the homogeneity and stability of the prepared QCMs has been developed and validated.

Materials and methods

**Chemicals and solutions**

Sulfadiazine and sulfadimidine, Vetranal grade, supplied by Riedel-de Haën (Buchs, Switzerland), Fluorescamin, ≥99.0%, supplied by Fluka (Buchs, Switzerland), acetonitrile and methanol, LC gradient grade (Merck, Darmstadt, Germany), and doubly de-ionised water (Milli-Q, Millipore, Molsheim, France) of 18.2 mΩ cm⁻¹ resistivity were used. All other reagents were of analytical reagent grade.

Stock solutions (50 mg l⁻¹) of each sulfonamide in methanol were prepared monthly from the solid compounds. Standard solutions were freshly prepared by dilution of the stock solutions with the mobile phase (LC-UV method) or acetonitrile (LC-fluorescence method). A 0.2% (w/v) fluorescamine solution in acetonitrile was prepared daily.

Mobile phases of several compositions were prepared by mixing acetonitrile with the appropriate volumes of 0.01 mol l⁻¹ acetic acid/sodium acetate or 0.01 mol l⁻¹
formic acid/sodium formiate buffer solutions, previously filtered through a 0.22 µm nylon filter (Lida, Kenosha, WI, USA).

**Animal feed**

Pig-rearing feeds were kindly supplied by the Associació Catalana de Fabricants de Pinsos (ASFAC). They were not granulated and were used as received. On arrival, they were stored at 4º C in PVC flasks. Information about their composition is shown in Figure 1a. Particle size distribution was determined by sample sieving, and the obtained curve is shown in Figure 1b. Feed moisture, determined as the weight loss at 103ºC for 4 hours, was about 11%.

**Apparatus**

Chromatographic analysis was carried out in a Shimadzu system (Kyoto, Japan) consisting of a LC-10AD VP quaternary pump, a SIL-10AD VP automatic injector, a SPD-M10A VP diode array detector with deuterium and tungsten lamps and a RF-10A XL fluorescence detector with a 150 w Xenon lamp. The analytical column was a Supelcosil (Bellefonte, PA, USA) LC-PAH RP-18 column (150 mm × 4.6 mm, 5 µm particle-size), equipped with a Supelcoguard LC-18 2 cm guard column.

For microwave assisted extraction, a Milestone ETHOS E system (Sorisole, Italy) was used. It consists of a microwave unit designed for extraction with organic solvents, a twelve-position segmented rotor (MPR-600/12S), twelve teflon vessels (100 ml) with magnetic stirring, an optical-fibre temperature probe and an ETHOS E 320 touch-screen terminal.

Mechanical shaking was carried out with a Breda 34526 (Breda Scientific, Breda, Netherlands) rotary mixer. A Heraeus Christ Labofuge 400 centrifuge (Osterode am Harz, Germany), with a 600 - 6000 rpm speed range, was used. The pH was measured with a Crison GLP21 pH-meter (Alella, Spain) equipped with a Crison 52-02 Ag/AgCl combined glass electrode.
For the preparation of the QCM, two cylindrical PVC containers of five and ten litres, respectively, were used. Ceramic balls (3 cm diameter) were mixed with the feed to ease the homogenization process. The containers were placed on a specially designed roller table (Metalitzats Cat, Barcelona, Spain) that makes controlled rotation speed possible.

**Procedures**

*Determination by HPLC with ultraviolet detection*

Feed (1 g) and acetonitrile (10 ml) were placed in the reactors of the microwave system and kept at 110º C for 20 min with magnetic stirring. After allowing the system to cool, the resulting mixture was transferred to a centrifuge tube and centrifuged at 3500 r.p.m. for 7 min. Aliquots of the clear extracts thus obtained were diluted ten-fold with acetonitrile/0.01 mol l⁻¹ acetic acid/acetate buffer (13:87) mobile phase. The resulting solutions were filtered through a 0.45 µm nylon membrane and injected into the chromatographic system.

The gradient elution program used was as follows: keep a 13:87 mixture of acetonitrile and 0.01 mol l⁻¹ acetic acid-sodium acetate buffer solution (pH 4.7) for 4 min, switch to a 10:90 ratio and maintain it up to minute 10, return to the initial composition and equilibrate for 5 min.

The mobile phase flow rate was 1 ml min⁻¹, the injection volume was 20 µl and the diode array detector was set at 268 nm.

*Determination by liquid chromatography with fluorimetric detection.*

Sulfonamides were extracted from the feed as described in section “Determination by liquid chromatography with ultraviolet detection”. Feed extract (1 ml) or 1 ml of a standard solution in acetonitrile were mixed in an amber-coloured vial with 2 ml of formic acid-sodium formiate aqueous buffer solution (pH 3.4) and 1 ml of 0.2% fluorescamine solution in acetonitrile. The mixture was left to stand at least 2 hours at
room temperature, filtered through a 0.45 \( \mu \)m nylon membrane and 50 \( \mu l \) were injected into the chromatographic system. An isocratic elution with a 29:71 mixture of acetonitrile and 0.01 \( \text{mol l}^{-1} \) formic acid-sodium formiate buffer at pH 3.4 was used. The mobile phase flow rate was 1.2 \( \text{ml min}^{-1} \). The fluorescence detector was set at \( \lambda_{\text{exc}}: 405 \text{ nm and } \lambda_{\text{em}}: 485 \text{ nm} \). The fluorescence of the derivatised sulfonamides remained constant for about 8 h.

**Preparation of the quality control material**

The schema followed is shown in Figure 2. For the preparation of the 500 \( \mu \text{g g}^{-1} \) material, 1.5 g of sulfadiazine (SDZ) and of sulfadimidine (SDD) were mixed with 3 Kg of animal feed. The mixture was placed in a PVC flask containing ceramic balls and made to roll on a rolling table for at least 90 hours. Homogeneity was checked by periodically taking samples from different parts of the material and determining the concentration of the sulfonamides by liquid chromatography with UV detection. After the homogeneity of the material was confirmed, 100 g were taken and diluted with 900 g of animal feed to obtain the 50 \( \mu \text{g g}^{-1} \) material. Homogenization and homogeneity checking of this material were made as previously described.

In the subsequent step, 300 g of the 50 \( \mu \text{g g}^{-1} \) material were mixed with 2.7 Kg of feed to obtain the 5 \( \mu \text{g g}^{-1} \) QCM, while 120 g were mixed with 2.88 Kg of feed to obtain the 2 \( \mu \text{g g}^{-1} \) QCM. Homogenization was as previously explained. After checking their homogeneity, both QCMs were placed in shuttered, screw-stopped, amber-coloured glass-flasks, each containing 10 g of material. After testing the homogeneity of the bottled materials, 10 flasks of each QCM were stored at three different temperatures (room temperature, 4\(^\circ\)C and –20\(^\circ\)C) and their stability was tested for 18 months. Samples of the homogeneity and stability studies of the 2 and 5 \( \mu \text{g g}^{-1} \) materials were analysed by a liquid chromatography with fluorimetric detection method.

**Results and discussion**
Analytical methodology

The objective of this part of the work was to optimize and validate an as simple as possible methodology for the determination of SDZ and SDD in feed samples coming from the homogeneity and stability studies included in the preparation of QCMs. The concentration levels of sulfonamides present in medicated or contaminated feeds are high enough to make a dilution of the extracts before injection in the chromatographic system possible. This avoids a clean-up step, which would have lengthened the analysis and increased their cost.

Liquid chromatography-UV detection

Mixtures of acetonitrile and aqueous acetic acid-sodium acetate buffers have been frequently used as mobile phases for the analysis of sulfonamides by LC-UV. In the present research work, the composition of the mobile phase has been optimised for the analytes of interest. Acetic acid-sodium acetate buffer solutions at pH 3.5, 4 and 4.7 were tested, using a 20:80 acetonitrile/buffer solution mixture. No influence of pH on sulfonamide retention time was detected. A pH of 4.7 was used for further work, to take advantage of the higher buffer capacity. Acetonitrile percentage was varied between 30% and 10%. A 22:78 acetonitrile/acetic acid-sodium acetate buffer mobile phase was initially selected, as this led to good analyte separation and short analysis times.

However, when blank feed extracts were injected in these experimental conditions, two peaks having virtually the same retention times as the sulfonamides were observed. Different mobile phase compositions were tested again and the problem was solved with a gradient that decreased acetonitrile percentage in the mobile phase from 13% to 10% between minutes 4 and 5. No significant matrix interferences were observed in these conditions. In Figure 3 (a and b) the chromatograms of a standard solution and of extracts of a blank and a spiked feed are shown.

Six standard solutions of SDZ and SDD, with concentrations ranging from 50 to 1000 µg l⁻¹ were injected in these conditions. Plots were linear for both SDZ (r=0.9998)
and SDD (r=0.9995). The relative standard deviations of the slopes of calibration lines obtained in four different days were 4% for SDZ and 6% for SDD. From the injection of six standards of 250 µg l⁻¹, in conditions of repeatability, the precision of retention time (< 1%) and peak areas (2% - 3%) was assessed. Limits of detection (LOD) and limits of quantification (LOQ) were calculated on the basis of 3 and 10 times, respectively, the standard deviation of the base line of the chromatographed blank feed extract, using low concentration standard solutions. LOD and LOQ values are shown in Table 1. Therefore, liquid chromatography with UV detection at 268 nm offers the sensitivity and precision required for the analysis of samples of feeds spiked with SDZ and SDD at 50 and 500 µg g⁻¹.

**Liquid chromatography-fluorimetric detection**

A method described in a previous paper (Raich-Montiu et al. 2007) was suitably modified for the determination of sulfonamides in feeds. The percentage acetonitrile was varied between 36% and 29%. Results indicated that a 29:71 acetonitrile/formic acid-sodium formiate aqueous buffer solution led to adequate analyte separation and short analysis times. The pH of the aqueous buffer solution was then varied between 3 and 4, while keeping a 29% acetonitrile content in the mobile phase. An increase in pH considerably decreased analyte retention times. A pH of 3.4, a value giving short retention times and suitable separation, was selected for all further work. When blank feed extracts were injected using the above mentioned conditions, no matrix interferences were observed at the retention times of the analytes (Figure 3, c and d). No modifications were required for the derivatization process with fluorescamine.

Six standard solutions of SDZ and SDD, with concentrations ranging from 5 to 200 µg l⁻¹ were injected in these conditions. Plots were linear for both SDZ (r=0.9997) and SDD (r=0.9997). The slopes of calibration lines obtained in four different working sessions showed a good reproducibility for both analytes: 2% for SDZ and 7% for SDD. The injection of six standards of 100 µg l⁻¹, in conditions of repeatability, led to
estimates of the precision of retention time (< 1%) and peak areas (2%). The LOD and LOQ were calculated as described in section “Liquid chromatography-UV detection”. Their values are shown in Table 1. Therefore, liquid chromatography with fluorimetric detection has the sensitivity and precision required for the analysis of samples of feeds spiked with SDZ and SDD at 2 and 5 µg g\textsuperscript{-1}.

**Extraction**

First attempts at sulfonamide extraction from animal feed were carried out with mechanical shaking. Three different extracting agents were tested: acetonitrile, 0.1 mol l\textsuperscript{-1} HCl aqueous solution and 10\textsuperscript{-3} mol l\textsuperscript{-1} NaOH aqueous solution. Acetonitrile was found to be the most suitable, as both HCl and NaOH gave extracts containing many co-extracted matrix components, which prevented their direct injection and would have required a clean-up step. Extraction time was varied between 1.5 and 3 hours, but recoveries after 3 h shaking were only 5% higher than those obtained with just 1.5 h. For a feed spiked at 50 µg g\textsuperscript{-1}, recoveries with a 1.5 h extraction time were 62% for SDZ and 36% for SDD.

Microwave-assisted extraction was then tested and found to be more efficient than mechanical shaking, as higher recoveries were obtained. Optimum temperature and irradiation time were determined with a feed spiked at 50 µg g\textsuperscript{-1}. Triplicate extractions of 1 g of feed with 10 ml acetonitrile were carried out at 80ºC, 90ºC, 100ºC and 110ºC, with 15 minutes irradiation time. The effect of 20 and 30 minutes irradiation times was then checked at 90ºC, 100ºC and 110ºC. As shown by results given in Figure 4, no significant differences in recoveries were observed. Finally, 110º C and 20 minutes irradiation time were selected as working conditions for all further extractions. Recoveries in these conditions were 104% for SDZ and 89% for SDD.

Precision of the whole method was assessed analysing materials spiked at 50 µg g\textsuperscript{-1}, 5 µg g\textsuperscript{-1} and 2 µg g\textsuperscript{-1} in five different sessions, with the same technician and the same instrument. Three determinations were carried out in each session, so that fifteen
results for each analyte were available. A one way (day) ANOVA for a 95% confidence level was applied to obtain intersession variation ($s_L$), intrasession variation ($s_r$) and intralaboratory total standard deviation ($s_R$) (ISO 1994). Results are shown in Table 2 as relative standard deviation. The HorRat values were calculated to confirm that the precision was adequate for the intended purpose (Horwitz and Albert 2006).

**Preparation of the QCMs**

**Spiking**

Several procedures for the addition of the sulfonamides to the feed matrix were tested. Initially, sulfonamides were added in methanolic solutions and methanol was subsequently evaporated in a vacuum desiccator. Later, sulfonamides dissolved in a 90:10 water/methanol mixture were added to the feed and the solvent was eliminated by lyophilization. However, these spiking procedures were found to be inadequate, because they either induced changes in the matrix of the spiked samples, which difficulted the extraction process (effect of lyophilization) or led to extracts containing co-extracted components that interfered in sulfonamide determination (effect of spiking with sulfonamides in methanolic solution).

Finally, the decision was taken to spike samples by mixing solid sulfonamide standards with the feed; besides making sulfonamide determination easier, this had the added advantage that analytes were incorporated to the feed matrix in a way similar to the industrial preparation of medicated feeds.

As described in section “Preparation of the quality control material”, and schematically shown in Figure 2, a feed containing 500 µg g⁻¹ of both SDZ and SDD was first prepared, and then it was diluted with blank feed to obtain materials containing 2 µg g⁻¹ and 5 µg g⁻¹.

**Homogeneity study**
Homogeneity of the different bulk materials was tested after each dilution step. For this purpose, 5 sub-samples (1 g each) were taken every 24 hours in different positions of the homogenization container during the homogenization process. The sub-samples were analysed, making use of the suitable methodology, and the average concentration, standard deviation and relative standard deviation for each sulfonamide were calculated. The criterion for homogeneity was that the variation coefficient for the determinations should be lower than, or similar to, the method RSD for each analyte.

Once the homogeneity of the bulk materials was verified, they were stored in bottles, each unit containing 10 g of material. To assess the homogeneity of the bottled materials containing 2 and 5 µg g\(^{-1}\), ten bottles were randomly selected and analysed in duplicate in a single working session. One way ANOVA for a 95 % confidence level was performed with the results, and the conclusion was that there were no significant differences in the concentrations found in the different units in which both materials were bottled. The results of the ANOVA are shown in Table 3 as the relative standard deviations obtained within and between units. Since the calculated F values are lower than the critical value, it can be concluded that the material is homogeneous.

**Stability study**

Having confirmed the homogeneity of the 2 and 5 µg g\(^{-1}\) materials, their stability was studied. Among the experimental designs used for stability studies of reference materials, the model in which different portions of the material are stored at several temperatures and samples are periodically taken and analyzed in reproducibility conditions has been chosen (Lamberty et al. 1998). In the present study, room temperature, 4°C and -20°C have been assessed as storage temperatures. In order to minimize the influence of the long-term analytical fluctuations on the conclusions of the stability study, results obtained on the materials stored at room temperature and 4°C have been referred to those corresponding to the material stored at -20°C. The stability at this temperature has been previously verified by means of a regression model.
(Linsinger et al. 2001): if the slopes of the regression lines representing analyte concentration vs. storage time do not differ significantly from zero, a dependence between these variables can be discarded and the materials can be considered to be stable. In order to ascertain whether the slope significantly differs from zero, an ANOVA has been applied to the regression model for each sulfonamide and each concentration. The results obtained have confirmed the null hypothesis and thus both QCMs can be considered to be stable at -20°C along the eighteen-month period studied. As an example, the regression lines corresponding to the 5 µg g\(^{-1}\) material are shown in Figure 5.

For each storage time, the ratios between the sulfonamide content in each material stored at either 4°C or room temperature \(X_{t(T)}\) and the corresponding values for the materials stored at -20°C \(X_{t(-20°C)}\) are obtained by equation (1)

\[
R_t = \frac{X_{t(T)}}{X_{t(-20°C)}}
\]

(1)

Estimated uncertainty for \(R_t\) \((U_t)\) is obtained by means of equation (2) (Kramer et al. 2001):

\[
U_t = \frac{R_t \times (CV_{t(T)}^2 + CV_{t(-20°C)}^2)^{1/2}}{100}
\]

(2)

where \(CV_t\) is the variation coefficient at each time. Materials stored at 4°C and room temperature can be considered to be stable if \(R_t\) values are randomly distributed about 1 and their \(U_t\) lie within the range around 1 determined by the method intralaboratory reproducibility (average of RSD\(_R\) values obtained for the 2 and 5 µg g\(^{-1}\) materials).

Results obtained with this criterion along the eighteen-month stability study are shown in Figure 6. Materials stored at 4°C can be considered to be stable, both at the 5 µg g\(^{-1}\) and 2 µg g\(^{-1}\) levels, over the whole period tested. In the case of materials stored at room temperature, stability is reduced in all cases to just over 6 months.

Conclusions
From the middle scale feasibility study carried out in the present work, the conclusion can be reached that spiking animal feeds with solid sulfonamides and successive dilutions with blank feed to the desired level of concentration, including a thorough homogenization on a rolling table in each step, is a suitable and quick method for the preparation of homogeneous materials. In order to guaranty the stability of the QCM for a period of at least eighteen months, a storage temperature of 4°C, or lower, is mandatory. At room temperature, the contents of SDZ and SDD slowly decrease after six months, and additionally, the risk of biological degradation cannot be discarded in these conditions.

There is a clear lack of reference materials for the analysis of veterinary drugs in feeds, which hinders method validation and quality control activities in the feed industry laboratories and also in those devoted to food quality control. This work could be considered to be a first step for a future development and commercialisation of this kind of materials, which would be useful tools for the analytical laboratories involved in feed analysis.

Acknowledgements

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References


Emborg HD, Hammerun AM. 2006. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. (Eds.) DANMAP2006. Copenhagen: Danish Veterinary and Food Administration; p. 12-14


Table 1. LOD and LOQ determined for SDZ and SDD with each method.

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<th>LC – Fluorimetry method</th>
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<td>SDZ</td>
<td>SDD</td>
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<td>LOD Solution</td>
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<tr>
<td>LOD In feed</td>
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<td>730</td>
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<tr>
<td>LOQ Solution</td>
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<td>28.1</td>
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<td>LOQ In feed</td>
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<td>3160</td>
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Table 2. Precision of the methods in repeatability (RSD$_{r}$) and intralaboratory reproducibility conditions (RSD$_{R}$).

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<td></td>
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<td>SDZ</td>
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<td>50 µg g$^{-1}$</td>
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<td>3</td>
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<tr>
<td>5 µg g$^{-1}$</td>
<td>LC - fluorimetry</td>
<td>6</td>
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<tr>
<td>2 µg g$^{-1}$</td>
<td>LC - fluorimetry</td>
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Table 3. Homogeneity results obtained from the ANOVA between the units in which both materials were bottled.

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<td>RSD_within units</td>
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*Critical F value = 3.02
Compound feed composition (a). Particle size distribution curve of the compound feed (b).
59x64mm (300 x 300 DPI)
Schema of the procedure followed for the preparation of the quality control materials.

96x82mm (305 x 305 DPI)
LC - UV chromatograms of a 500 µg l⁻¹ standard and of a blank animal feed extract (a) and an extract of an animal feed containing 50 µg g⁻¹ of SDZ and SDD (b). LC - fluorimetry chromatograms of a 125 µg l⁻¹ standard and of a blank animal feed extract (c) and an extract of an animal feed containing 5 µg g⁻¹ of SDZ and SDD (d).

172x124mm (305 x 305 DPI)
Effect of temperature and irradiation time of the microwave-assisted extraction on the recoveries of SDZ (a) and SDD (b).
Results of the regression analysis of the material of 5 µg g^{-1} stored at -20°C. SDZ (a) and SDD (b).
Variation of the Rt values with time for the materials stored at room temperature and at 4°C. SDZ (2 µg g⁻¹) (a), SDD (2 µg g⁻¹) (b), SDZ (5 µg g⁻¹) (c) and SDD (5 µg g⁻¹) (d).

181x123mm (305 x 305 DPI)