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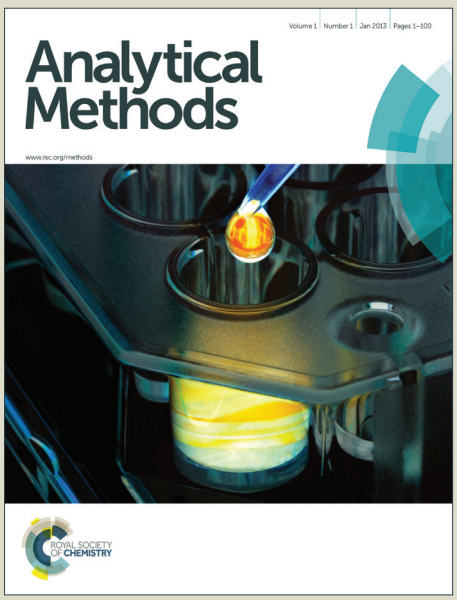

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Comparison of different sample preparation procedures for multiclass determination of selected veterinary drugs, coccidiostats and insecticides residues in eggs by liquid chromatography-tandem mass spectrometry

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Abstract

The paper presents the evaluation of extraction techniques and sample clean-up procedure for the simultaneous determination of residues of veterinary drugs (sulphonamides, fluoroquinolones, tetracyclines, macrolides, β-lactams, nitroimidazoles, benzimidazoles, amphenicoles, lincosamide, pleuromutilin,), coccidiostats and insecticides in fresh eggs samples. The study utilises the liquid chromatography-tandem mass spectrometry technique to perform proper analytical parameters for screening and confirmatory method. The results of eggs samples analysed by 8 different sample preparation schemes were compared by the mean recoveries. The recoveries of analytes showed that both SPE and modified QuEChERS have not been sufficient for the extraction of all of the analytes in the expected range of 70-120%. Additional cleaning with dispersive sorbents prolonged the time of sample preparation steps providing slightly difference in final extracts clarity. The most demanding analytes to isolate from sample were coccidiostats while sulphonamides were the group of analytes to which all of the proposed sample clean-up procedures were suitable. As a chosen method extraction with 0.1% formic acid in acetornitrile:water (8:2) and clean-up with HybridSPE can be applied as sample preparation step for screening and confirmatory method in official laboratories.

Key words: multiclass, multiresidue, veterinary drugs, coccidiostats, antibiotics, LC-MS/MS

1. Introduction

Nowadays' methods of commercial food production are oriented on high efficiency. One of the problems is that the presence of great number of animals on small area causes that the outbreaks of disease are more often, and sometimes they run out of control, thus farmers and veterinarians have to deal with many diseases including: Newcastle disease, Gumboro disease, Marek's disease, coccidiosis, E. coli infections and salmonellosis [1]. Furthermore, animals can be exposed be exposed to a range of internal and external parasites. Consequently, feed additives and veterinary medicinal products and may be required to prevent and/or treat infections.

The use of veterinary drugs causes that they residues are present in food of animal origin, like eggs, milk, honey. Also the residues of feed additives, like coccidiostats, forbidden for use in laying hens, still can be found in the eggs. Some of the drugs may possess negative influence on animals' and humans health, e.g. develop the allergies, antimicrobial resistance or act genotoxic or mutagenic [2, 3, 4, 5]. Due to that residues and the presence of many substances have to be monitored in animal tissues and products to ensure the food safety for consumers'. By the reason of potential harmful effects of many of medicinal products, toxicological limits like maximum residue limits (MRL's) were established for some of the drugs (although most of them are forbidden for use in laying hens [6, 7]. Due to

that some feed additives like coccidiostats were not subjected to the same regulations, EU maximum levels were recently established for eleven coccidiostats [8].

Due to the variety of veterinary medicinal products used in animal husbandry an appropriate methods are essential to fulfil the requirements for the survey of their residues. Depending on the application of the method for screening or confirmation of presence of substances of interest, many different techniques are used. In recent years liquid chromatography - mass spectrometry detection became a method of choice in residue analysis of food [9, 10]. Combining ultra-high pressure liquid chromatography (UHPLC) with tandem mass spectrometry (MS/MS) provides adequate sensitivity for the determination of banned substances [11, 12, 13]. Also the time-of-flight mass spectrometer (ToF-MS) was applied in the detection of veterinary drugs in eggs [14] although demonstrated the high resolution mass spectrometry often cannot replace an adequate sample preparation.

The main issue during developing of such a method is general sample preparation step suitable for dozen or even hundreds of analytes [11, 15, 16]. It is often a real challenge, because of the differences in chemical structure and properties of the analytes, as well as different performance levels. The other problem in LC-MS/MS analysis of biological sample is matrix effect caused mainly by the proteins and phospholipids [17].

Despite there are multiclass multiresidue screening methods for even over a hundreds of analytes in variety of matrices [11, 14, 18, 19, 20] which cover the antibiotics and coccidiostats, there is a few of such methods suitable for analysis of egg samples. Quantitative of such methods which fulfil the criteria of the Decision 2002/657/EC [21] for eggs were already reported [22, 23] and they cover several dozen of analytes.

A majority of extraction and clean-up techniques in multiresidue analysis of eggs were applied. Usually at first samples were extracted by liquid-liquid extraction (LLE) using some organic solvent, like: acetonitrile [24] or methanol [15] to precipitate the proteins. Further the solid-phase extraction (SPE) was applied [25] utilising the use of different types of cartridges. Sometimes *n*-hexan was used to remove the lipids by liquid-liquid [26, 27] or solid-phase extraction [28]. The other extraction techniques developed were matrix solid-phase dispersion (MSPD) [29] and pressurised liquid extraction (PLE) [30, 31]. These two techniques allow for the reduction of solvent consumption and the time of analysis. In 2010 also the QuEChERS ("Quick, Easy, Cheap, Effective, Rugged, and Safe") method, originally developed for pesticides analysis, was applied for eggs analysis and compared with LLE, SPE and MSPD technique on the basis of mean recoveries and number of veterinary drugs extracted [23]. Since then this technique was also modified and applied for veterinary drug analysis in eggs by other authors [32].

The aim of this study was to compare different types of clean-up procedures in sample preparation step for the simultaneous determination of veterinary drugs, coccidiostats and insecticides in eggs by LC-MS/MS technique. The HybridSPE (Zirconia Coated Silica) columns were used for clean-up on SPE and the usefulness of modified QuEChERS procedure was also studied. As a cleaning sorbents for dispersive SPE in this experiment octadecyl (C_{18}) sorbent and primary-secondary amine (PSA) were used. The effect of anhydrous sodium sulphate was also investigated.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (ACN), methanol (MeOH), formic acid, (99.5) (HPLC grade) were provided by J.T. Baker (Cenetr Valley, PA, USA). Anhydrous sodium sulphate and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich (Steinheim, Germany). Disodium versenate dihydrate (Na_2EDTA) was from POCH (Gliwice, Poland). Water was purified through a Mili-Q plus system from Millipore (Bedford, MA, USA). The SPE cartridges Hybrid-SPE^{TM} (30 mg/1 mL), C18 silica bonded adsorbent DISCOVERY^{TM} DSC-18 and PSA bonded silica were obtained from Supelco (Bellefonte, PA, USA) and PVDF syringe filters (0.45 µm,13 mm) were received from Restek (Bellefonte, PA, USA).

Analytical standards of ampicillin (AMPI), penicillin V (PEN V), oxacillin (OXA), cloxacillin (CLOX), nafcillin (NAF), dicloxacillin(DICLOX), ceftiofur (CFT), cephalexin (CFLE), cefquinome (CFQ), cefalonium (CFLO), cefapirin (CFP), sulfaphenazole (SPZ), sulfamerazine (SME), sulfamethazine (SMT), sulfamethoxazole (SMA), sulfamethoxypyridazine (SMP), sulfamonomethoxine (SMM), sulfadoxine (SDX), sulfaquinoxaline (SQX), sulfadimethoxine (SDMX), tylosin (TYL), erythromycin (ERY), tilmicosin (TIL), josamycin (JOS), azythromycine (AZY), roxithromycine (ROXY), danofloxacin (DAN), difloxacin (DIF), enrofloxacin (ENR), ciprofloxacin (CIP), ciprofloxacin d8 (CIP-d8), flumequine (FLU), sarafloxacin (SAR), marbofloxacin (MAR), norfloxacin (NOR), oxolinic acid (OXO), nalidixic acid (NAL), chlortetracycline (CTC), tetracycline (TC), doxycycline (DC), oxytetracycline (OTC), metacycline (MTC), demeclocycline (DMC), streptomycin (STRP), dihydrostrepromycin (DISTRP), gentamycin (GEN), paromomycin (PAR), spectinomycin (SPEC), kanamycin (KAN), neomycin (NEO), lincomycin (LIN), tiamulin (TIA), fenbendazole (FBZ), mebendazole (MBZ), flubendazole (FBZ), oxibendazole (OXBZ), tiamphenicol (TAP), florfenicol (FF) as well as dinitrocarbanilide (DNC), maduramycin (MAD), monensin (MON), narasin (NAR), nigerycin (NIG), robenidine (ROB), sali-nomycin (SAL) and lasalocid (LAS) standard solution 100 ng l^{-1} were purchased from Sigma-Aldrich (Munich, Germany). Decoquinate-d5 (DEC-d5), dinitrocarbanilide-d8 (DNC-d8) and robenidine-d8 (ROB-d8), Ipronidazole (IPZ), hydroxyipronidazole (IPZOH) fenbendazole sulfoxide (FBZ-SO), albendazole (ABZ), albendazole sufone (ABZ-SO_2), albendazole sulfoxide (ABZ-SO), hydroxymebendazole (MBZ-OH), triclabendazole sulfone (TCBZ- SO_2), triclabendazole sulfoxide (TCBZ-SO), ketotriclabendazole (TCBZ-KETO), triclabendazole d3 (TCBZ-d3) were obtained from Witega (Berlin, Germany), and decoquinate (DEC) from U.S.Pharmacopeial Convention (Rockville, USA). Clazuril (CL), diclazuril (DCL), halofuginone (HLF), methyldiclazuril (MDCL) and semduramycin (SMD) were donated from European Union Reference Laboratory (EURL) in Berlin. Fenbendazole sulfone (FBZ-SO_2) and triclabendazole (TCBZ) were obtained from National Measurement Institute (Australia), amino mebendazole (MBZ-NH) was purchased from Merck (Darmstadt, Germany), cambendazole (CBZ) was obtained from Janssen-Cillag (Neuss, Germany) whereas carnidazole (CNZ) andtinidazole (TNZ) were from Riedel-de Haën (Seelze, Germany). Phoxim, propoxur and carbaryl were purchased by Dr Ehrenstorfer (Augsburg, Germany).

2.2. Preparation of standard solutions

Coccidiostats stock standard solutions (1000 µgml^{-1}) were prepared by weighting of 10.0 mg of reference standard and dissolving in 10.0 ml of solvent. CL, DCL, DNC, DNC-d8,

MDCL, ROB and ROB-d8 were dissolvedin DMSO, HLF in acetonitrile–water (50:50, v:v), DEC and DEC-d5 in acetonitrile with formic acidaddition. The rest of stock standard solutions (MAD, MON, NAR, SAL, SMD) were prepared in acetonitrile. The stock standard solutions (1000 μg ml^{-1}) of benzimidazoles were prepared by weighting of 10.0 mg of substances and dissolving in DMSO. The stock standard solutions (1000 μg ml^{-1}) of macrolides, tetracyclines, quinolones, sulfonamides, amphenicoles, insecticides, tiamulin (TIA) and lincomycin (LIN) were prepared by weighing appropriate amount of substances and dissolved in methanol, nitroimidazoles in acetonitrile, whereas β-lactams were dissolved in ultra pure water. All of the solutions in the concentrations of 1000 μg ml^{-1} were kept in the dark below -18 °C for six months.

Working standard solutions at concentrations of fortification level (Tab. 2.) were prepared for each group of analytes by dissolving appropriate amount of stock standard solutions in acetonitrile (nitroimidazoles, benzimidazoles, coccidiostats), methanol (tetracyclines, sulphonamides, fluoroquinolones, macrolides, amphenicoles, insecticides, lincomycin, tiamulin) or water (β-lactams, IS mixture). A mixed working standard solution used for the sample fortification was prepared by the dilution of 1 ml of each working standard solutions in water up to 10 ml. A mixed solution of internal standards (IS mixture) was prepared separately. Working standard solutions were kept in the dark below -18 °C for six months, while the mixed working standard solution was kept in the dark at +2 to +8 °C for three months.

2.3. Liquid chromatography-mass spectrometry

The LC–MS/MS system consisted of an Agilent 1200 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, a degasser, an autosampler, a column heater, a switching valve (Valco Instruments Co., Inc., USA) and an triple quadrupole mass analyser QTRAP® 5500 (AB Sciex, Canada).The experiments were carried out in the positive and negative ion electrospray mode. The Turbo Ion Spray source was operated at 400 °C with the capillary voltage set at 5 500 V and -4 500 V. The Analyst 1.5.2 software controlled the LC–MS/MS system and processed the data. Nitrogen was used as a nebuliser gas, curtain gas and collision gas. The chromatographic separation was performed on a Halo® C_{18} analytical column (150 mm × 2.1 mm, 2.7 μm) with an C_{18} guard cartridge (4mm × 2mm) (Advanced Materials Technology, Inc., USA) operated at 40 °C. The mobile phase consisted of solvent A (methanol:acetonitrile 8:2, v/v) and solvent B (0.1% formic acid in water). The gradient was 5 % A at 0 to 2 min, 95 % A from 12 to 25 min and then 5 % A from 25 to 33 min. The flow rate was 250 μl min^{-1} and the injection volume was 20 μl.

The mass spectrometer working parameters (ionisation mode, capillary voltage, source temperature, sheath gas flow, nebuliser pressure, fragmentary voltage and collision energy) were optimised both with direct infusion of each standard solutions (0.1 μg ml^{-1}) from a syringe pump at the rate of 7 μl min^{-1} and with a LC-injection. The fragmentation reactions (transitions) used for monitoring were selected on the basis of their significance in the production spectra. The analytes were quantified using multiple reactions monitoring (MRM) mode. For each analyte at least two transitions were monitored, when for internal standards one transition was monitored.

2.4. Sample preparation equipment

A homogenizer Polytron PT-3100 (Kinematica, Luzern, Switzerland) operated at 7000 rpm was used to homogenise the egg yolk and albumen. Samples were weighted in 35 ml Nalgene high-speed centrifuge tubes (Thermo Fisher Scientific, Waltham, MA, USA) and the Waters (Milford, MA, USA) SPE chamber was used for sample clean-up. An ultrasound Sonorex (Bandelin electronic, Berlin, Germany) and rotator Stuart STR 4 (Bibby Scientific Limited, Stone, Staffordshire, UK) was used to support the extraction. Two centrifuges operated at 15 000 rpm, -4 °C (Beckman J2-MC, Beckman Coulter Inc., Brea, CA, USA) and at 4 500 rpm, 4 °C MPW-6K15 (MPW Med. Instruments, Warsaw, Poland) were used to remove the precipitated proteins. AVLM Eva EC1/EC2 L (VLM GmbH, Bielefeld, Germany) nitrogen evaporator operated at 45 °C was used for sample evaporation.

2.5. Sample preparation

Homogenised eggs samples (2.0 g) were fortified with 20 µl of mixed working standard solution consisted of all analytes and 20 µl of IS mixture. An amount of 8 ml of 0.1% formic acid in acetonitrile:water (8:2) was used as a extraction solution and 500 µl of 0.1 M EDTA was added. Samples were rotary shaken for 10 minutes (30 rpm) and after that ultrasonicated in water bath at ambient temperature for 15 min. This step was common for all of the preparation procedures and then the samples were proceeding as follows:

Procedure 1. The sample was centrifuged (10 min, 15 000 rpm, -4°C), passed through cartridge filled with anhydrous Na_2SO_4 (0.5 g) and further through Hybrid SPE cartridge. Additional 1 ml of 0.1% formic acid in acetonitrile was passed through the SPE cartridge and sample was collected to a glass tube for evaporation.

Procedure 2. The sample was centrifuged (10 min, 15 000 rpm, -4 °C), evaporated under the nitrogen (temperature 45 °C), reconstituted with 1 ml 0.1% formic acid in acetonitrile and passed through Hybrid SPE cartridge. Additional 1 ml of 0.1% formic acid in acetonitrile was passed through the SPE cartridge and sample was collected to a glass tube for evaporation.

Procedure 3. Anhydrous Na_2SO_4 (0.5 g) was added to the sample and sample was vortexed for 2 minutes. The sample was centrifuged (10 min, 15 000 rpm, -4°C) and passed through Hybrid SPE cartridge. Additional 1 ml of 0.1% formic acid in acetonitrile was passed through the SPE cartridge and sample was collected to a glass tube for evaporation.

Procedure 4. Anhydrous Na_2SO_4 (0.5 g) was added to the sample and samples were vortexed for 2 minutes. The sample was centrifuged (10 min, 15 000 rpm, -4°C), evaporated under the nitrogen (temperature 45 °C), reconstituted with 1 ml 0.1% formic acid in acetonitrile and passed through Hybrid SPE cartridge. Additional 1 ml of 0.1% formic acid in acetonitrile was passed through the SPE cartridge and sample was collected to a glass tube for evaporation.

Procedure 5. The sample was centrifuged (10 min, 15 000 rpm, -4°C) passed through cartridge filled with anhydrous Na_2SO_4 (0.5 g) and further through Hybrid SPE cartridge. Additional 1 ml of 0.1% formic acid in acetonitrile was passed through the SPE cartridge. Further the clean-up with 200 mg C_{18} sorbent was applied, sample was centrifuged (10 min, 4 500 rpm, 4°C) and the upper layer was transferred to a glass tube for evaporation.

Procedure 6. The sample was centrifuged (10 min, 15 000 rpm, -4°C) passed through cartridge filled with of anhydrous Na_2SO_4 (0.5 g) and further through Hybrid SPE cartridge. Additional 1 ml of 0.1% formic acid in acetonitrile was passed through the SPE cartridge. Further the clean-up with 200 mg PSA sorbent was applied, sample was centrifuged (10 min, 4 500 rpm, 4°C) and the upper layer was transferred to a glass tube for evaporation.

Procedure 7. The sample was centrifuged (10 min, 15 000 rpm, -4°C) passed through cartridge filled with anhydrous Na_2SO_4 (0.5 g) and further through Hybrid SPE cartridge. Additional 1 ml of 0.1% formic acid in acetonitrile was passed through the SPE cartridge. Further the clean-up with 200 mg C_{18} sorbent was applied, sample was centrifuged (10 min, 4 500 rpm, 4°C) and 200 mg PSA was added. After the centrifugation (10 min, 4 500 rpm, 4°C) the upper layer was transferred to a glass tube for evaporation.

Procedure 8. The sample was centrifuged (10 min, 15 000 rpm, -4°C), 1.2 g $MgSO_4$, 200 mg C_{18} sorbent and 200 mg PSA were added. Samples were vortexes, centrifuged (10 min, 4 500 rpm, 4°C) and the upper layer was transferred to a glass tube for evaporation.

After the evaporation to dryness (procedures 1-8) in nitrogen evaporator at 45°C all the samples were reconstituted with 250 µl MeOH:ACN (8:2), filtered through 0.45 µm PFDF syringe filters and analysed by LC-MS/MS. Analysis of spiked eggs sample were repeated three times for each procedure(Fig. 1.).

Figure 1

3. Results and discussion

Minding the increasing number of samples to analyse with a variety of analytes to recover we decided to follow the current trend in sample preparation and develop multiclass method covering such analytes as: antimicrobials, antiparasitic agents, coccidiostats and insecticides (both: registered and not licensed for use in laying hens). The criteria for the selection of analytes were to include analytes for which MRL's are set, to include coccidiostats and substances which potentially may contaminate the eggs. The results of non compliant samples within the European Union for eggs were also basis for selecting such analytes. Combining methods which currently work in national residue monitoring plan in one method could help to monitor more analytes simultaneously and thus increase the effectiveness in the detection of activity incompatible with the law. Additionally, this approach will help to reduce the amount of reagents used; the number of workers involved and will make the equipment more accessible for other purposes.

3.1 HPLC-MS/MS conditions

The ions selected and MS/MS parameters optimised for the method are presented in Table 1. Analyses were conducted using both positive and negative ionisation. The group of sulphonamides, fluoroquinolones, tetracyclines, macrolides, β-lactams, nitroimidazoles, insecticides, lincomycin and tiamulin were detected in positive ionisation mode. Amphenicoles were detected in negative ionisation mode, while coccidiostats and benzimidazoles were detected in both ESI^+ and ESI^-. The two Amphenicoles analytes (Thiamphenicol and Florfenicol) were assayed without internal standard (IS) as there was

problem with detection of Chloramphenicol d5 (IS). Because the recoveries were satisfactory without the use of IS, none was used for the calculations.

Table 1

3.2. Liquid-liquid extraction and drying of extract

The development of efficient extraction suitable for all of the analytes (high recoveries) is the important step in multiclass multiresidue analysis. Among others acetonitrile is one of the most often used and efficient extraction solvent in protein precipitation. Moloney et al. after testing several clean-up sorbents for the recovery of coccidiostats decided to develop method based only on acetonitrile extraction and sample concentration prior to analysis [38]. In this experiment solvent mixture consisted of 0.1% formic acid in acetonitrile:water (8:2) to extract the analytes from the samples was used. Previously a mixture of organic solvent and water with addition of formic acid for simultaneous determination of antimicrobials and mycotoxins in eggs was described by A.L.Capriotti et al. [15]. A content of 80% of organic solvent was reported not to elute non polar lipids and phospholipids from C_{18} silica bonded adsorbent [33]. Previous works also reported that the addition of EDTA as a competing agent for tetracyclines helps to achieve higher recoveries [15, 22] and due to that it was also used in this experiment.

Because the presence of proteins and phospholipids in eggs can enhance or decrease the signal during the LC-MS/MS analysis, precipitated proteins can be simply removed by centrifugation. In this experiment a centrifuge operated at 15.000 rpm at -4 °C was used for better removal of proteins and the freeze of the lipids, which retain on the wall of the centrifuge tubes. Water is usually removed from the samples by the addition of anhydrous sodium or magnesium sulphate. Recently also freezing of samples after the extraction with acetonitrile, previously developed for milk [34] was applied to remove water and matrix compounds from organic phase enriched with analytes in egg analysis [11]. In this experiment an anhydrous Na_2SO_4 was used as a drying agent.

3.2.1. Optimisation of HybridSPE clean-up

In recent years for QuEChERS phase's Z-Sep materials are used, which are reported to remove more fat and dyes from complex matrices than traditional phases. The Z-Sep material was design to replace traditional C18 and PSA phases providing better removal of matrix interferences. Combination of Z-Sep particles with C18 is recommended for analysing of hydrophobic analytes from matrices containing less than 15 % fat. Z-Sep+ which is C18 with Z-Sep dual bonded to silica are recommended for samples containing more 15 % fat.

In this experiment to remove interfering compounds – phospholipids, the clean-up procedure utilising solid phase extraction with HybridSPE columns was applied (procedure 1-7). It allows for the targeted removal of phospholipids from supernatant without any selectivity to basic, neutral and acidic compounds providing better extract clarity and minimizing or eliminating matrix effect [35]. The mechanism of action is a selective Lewis acid base interaction between the zirconia ions bonded to the silica stationary phase with phosphate moiety of phospholipids [36]. After the extraction with 0.1% formic acid in acetonitrile:water (8:2) with the addition of 500 µl of EDTA the sample was passed through

the Hybrid-SPE columns and the extract was discarded. Further the amount of 1 ml of acetonitrile with four different concentrations of formic acid (0.1%, 0.25%, 0.5% and 1%) wasadditionally passed through the Hybrid-SPE columns. The resulted extract was collected and analysed to observe if there are any analytes retained. The results showed that the concentration of 0.1% in acetonitrile eluted the biggest amount of most of the analytes and it was chosen for the procedure with SPE clean-up (Fig. 2.) for additional elution.

Figure 2

Further the influence of the time of the evaporation on the recovery range was studied (Fig. 3.). For this purpose, procedure 1 utilises the use of columns filled with 0.5 g of anhydrous sodium sulphate before loading the extracts on HybridSPE and procedure 2 utilises the evaporation of the extract before the SPE usage. The results were compared in terms of mean recoveries (Table 2.) and it demonstrated that because of the presence of water the time of evaporation is too long (about 3.5 hours) and it causes the loss of some analytes. For example fluoroquinolones, cephalosporinsand lincomycin were not extracted at all, while the penicilines were recovered at 0-3% level. Also the recoveries for insecticides and amphenicols decreased significantly (from > 80% to < 30% and from > 70% to ≤ 15%). The loss of tetracyclines was from > 90% to 0-27%. Among the benzimidazoles and coccidiostats the recoveries varied from very slight difference (triclabendazole, triclabendazolesuphone, robenidine, dinitricarbanilide) to very high loss of analytes for fenbendazole (from 101% to 2%) or decoquinate (from 76% to 0%). But there was slightly difference for sulphonamides (Tab. 2), the recoveries were even higher when the extract was not dehydrated prior to SPE clean-up. The lowest recoveries were obtained only for two sulphonamides: sulpadoxine (119% → 83%) and sulfamerazine (92% → 72%).

Figure 3

Table 2

Subsequently to verify that the analytes were lost during the evaporation, the columns filled with anhydrous sodium sulphate were replaced by the step utilising the addition of drying agent directly to the sample in centrifuge tube. Further extract were loaded on SPE for clean-up (procedure 3) or evaporated before they were cleaned using SPE (procedure 4). The results confirmed that analytes were lost during the evaporation and before loading them on HybridSPE cartridge. Despite shorter time of sample preparation the decrease in the recoveries for some analytes (florphenicol, norfloxacin, difloxacin, ceftiofur, caphalonium, cefquinome, ketotriclabendazol, halofuginone) was still observed and due to that procedure 1 was used for further experiments.

3.3. Dispersive SPE

Previously some authors reported that the use of endcapped C_{18} sorbent removes 15% of co-extracted matrix components itself and it is more effective when used in combination with other sorbents [37]. Different sorbents like aminopropyl, silica, C_8, C_{18} and PSA were also investigated in terms of its use for clean-up of egg samples for the recovery of 20

coccidiostats [38]. Authors concluded that bonded silicas resulted in the lower recovery of ionophores while silicas gave satisfactory recovery of ionophores with limited clean-up. In this work the effect of clean-up sorbents such as C_{18} (procedure 5) and primary-secondary amine (PSA, procedure 6) were tested. For this purpose clean-up agents were used after the liquid-liquid extraction and HybridSPE extraction (procedures 5, 6 and 7).

A significant loss of some fluoroquinolones (danofloxacin, norfloxacin, sarafloxacin) up to 45%, florfenicole – 23% and diclazuril- 22% was observed when C_{18} sorbent was used, causing that the recoveries for those analytes did not reach the range of recovery above 70%. But there was also a positive effect of it use on the recoveries e.g. for some sulphonamides (sulfadimethoxine 17% andsulfamethazine 26%), penicilines (cloxacilin 28%, dicloxacilin 32% and nafcilin 21%), coccidiostats (salinomycin 46% and monensin 50%), difloxacin 23% and slight increase for benzimidazoles (especially metabolites, e.g. hydroxy mebendazole 19%).

The use of PSA caused a significant loss of some tetracyclines – metacycline 49% and chlortetracycline 54% causing that they did not match the limit of expected recoveries. The recovery for oxytetracycline was also decreased but still in the expected range – 76%. Among the penicilines the effect varies for the analytes. A slight decrease in the recoveries for peniciline V 10%, ampicilin 17% and oxacilin 15% was observed and the increase in the recoveries for cloxacilin 10% and dicloxacilin 21%, what is something opposite to C_{18} sorbent. The loss of 6% of florfenicole cased that it did not match the expected limit of recoveries. Among the benzimidazoles a slight increase (up to 21% for hydroxy mebendazole and flubendazole sulfoxide) was observed for all analytes except triclabendazole and triclabendazole sulphone, but they loss was not significant (12% and 3%, respectively). In case of coccidiostats the recoveries for half of the analytes slightly decreased up to 16% for lasalocid and for other half increased up to 35% for monensin.

Also a simultaneous effect of both agents was studied (procedure 7). The results varied significantly, sometimes a positive effect resulting in slight increased recoveries was observed (like for sulphonamides), other time obtained results were centred (e.g. methacycline, chlortetracycline, nafciln). There are also examples of significant increase in the recoveries (flubendazole, robenidine, maduramycin) and significant decrease below the level of 70% (ceftiofur, cefalonium). Besides the increase or reduction of recoveries in case of some of the analytes, the visible effect of resulted extracts (clarity) after applying the additional clean-up with dispersive sorbents was very slight.

The results for all three experiments are presented in Figure 4.

Figure 4

3.4. Modified QuEChERS extraction and purification

The modified QuEChERS extraction was also designed and applied as a procedure for eggs. The extraction solvent described in Section 3.2. was used, as well as the EDTA. Samples were further purified by the addition of C_{18} and PSA sorbent and the water was removed by the magnesium sulphate (procedure 8). The resulted extracts were visibly not as clear as those obtained on HybridSPE clean-up, but the procedure was less time consuming. In comparison with the recoveries obtained in procedure 1 the recoveries were lower for some group of analytes, specially for cephalosporines, which were lost when the upper dehydrate

layer of sample was collected for the analysis (Figure 7). Among the coccidiostats the lowest recoveries were for semduramycin (28%), clazuril (50%), maduramycin and diklazuril (67%), but comparing them to those obtained when procedure 1 was applied, they were higher (except for diclazuril). Other authors previously tested different extraction solvents and dispersive agents for the application of QuEChERS procedure for veterinary drugs [39]. They found 1% (v/v) acetic acid in acetonitrile followed by dispersive SPE with NH_2 and PSA sorbent to be the most suitable solution. But the additional cation exchange cleaning was necessary to determine the nitroimidazoles at the level of 3 μg/kg. In this experiment there was no need for further clean up to determine these analytes at the same level. Moreover, the results for benzimidazoles were similar to those concluded by Whelan et al. and Kinsella et al. who demonstrated that QuEChERS reduce the extraction time with maintaining high recoveries for anthelmintic residues [12, 40].

3.5. Comparison of proposed cleaning steps

Among the proposed clean-up procedure 8 (QuEChERS) was the fastest one unlike procedure 2 (Hybrid SPE) utilising evaporation of the extracts prior to loading them onto cartridges was the most time consuming. Also the additional use of cleaning sorbents after the SPE extraction (procedures 5, 6 and 7) prolonged the time of sample preparation steps providing slightly difference in final extracts clarity. Moreover, besides the increase of the recoveries for some of the analytes they also contribute to the decrease in the recovery for others, causing that they did not match the expected range between 70-120%.

In this study the numbers of extracted compounds were investigated when the different clean-up procedures were used, as well as the mean recoveries obtained for each analyte. In Figure 5. the comparison of extraction efficiency within the preparation steps applied for all analytes is presented. It was observed, that the procedure 1. allows to extracts the highest number of analytes in the range of recoveries between 70-120 % and the lowest recoveries were obtained for procedure 2. Results given when modified QuEChERS (procedure 8) was applied were also satisfactory, but it extracted less analytes in expected range of recoveries. Sulphonamides were the group of analytes to which all of the proposed clean-up procedures were suitable.

Figure 5

The results showed, that the evaporation of extracts before applying them on SPE (while the mixture consisted of 0.1% formic acid in acetonitrile:water (8:2) to extract the analytes from the samples was used) significantly prolongs the time of evaporation and some of the analytes are lost (fluoroquinolones, some macrolides and β-lactams). The use of additional cleaning agents after the cleaning on SPE also prolongs the time of sample preparation without giving significantly improvement of samples clarity.

Obtaining satisfactory recoveries (between 70-120%) for all of the analytes used in this experiment was not possible, but as a method of choice sample procedure 1. was selected. It extracted the highest number of analytes in the expected range of recoveries.

4. Conclusions

It is difficult to fulfil all the requirements for confirmatory methods while developing multiresidue method for several dozen of analytes due to the differences between the polarities of the analytes. The other problem is a variety of MRL levels for different analytes as well as a prohibition of use for the others. Due to that a LC-MS/MS method was proposed as a technique for qualitative and quantitative analysis of several dozen of analytes from different classes after sample extraction with 0.1% formic acid in acetonitrile:water (8:2) and cleanup on Hybrid SPE columns. Developed sample preparation can be a basis for further validation of screening and confirmatory method.

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Table 1
Tandem mass spectrometry parameters used for the detection and confirmation of the selected veterinary medicinal products.

Group	Analyte	RT (min)	Parent Ion (m/z)	Daughter Ions (m/z)*	DP (eV)	CE (eV)
ESI^+						
Sulphonamides	Sulfaquinoxaline	13.6	301.3	156/92	70	23/42
IS Sulfafenazole	Sulfamethoxazole	12.4	254.3	156/92	54	21/38
	Sulfamonomethoxine	12.4	281.3	156/92	80	25/40
	Sulfamerazine	10.4	256.3	156/92	80	19/33
	Sulfamethoxypyridazine	11.8	281.3	156/92	65	23/40
	Sulfadimethoxine	13.4	311.3	156/92	80	28/45
	Sulfadoxine	12.6	311.3	156/92	80	25/46
	Sulfamethazine	11.6	279.3	108/156	60	25/36
Fluoroquinolones	Sarafloxacin	11.8	386.4	299/342	100	25/39
IS Ciprofloxacin d8	Ciprofloxacin	11.3	332.3	314/231	261	28/51
	Enrofloxacin	11.5	360.4	316/245	80	27/38
	Norfloxacin	11.1	320.3	302/231	270	30/54
	Difloxacin	11.7	400.4	356/299	80	28/40
	Danofloxacin	11.5	358.4	340/255	280	32/53
	Flumequine	14.5	262.2	244/202	60	22/44
	Marbofloxacin	10.7	363.3	72/320	100	21/28
	Nalidixic acid	14.4	233.2	215/187	60	19/35
	Oxolinic acid	13.6	262.2	244/216	235	27/39
Tetracyclines	Doxycycline	11.3	445.4	428/154	150	22/39
IS Demeclocycline	Chlortetracycline	12.5	479.8	445/463	80	31/25
	Oxytetracycline	11.3	461.4	426/443	80	27/19
	Tetracycline	11.3	445.4	410/154	80	27/34
	Metacycline	12.7	443.4	426/201	80	43/23
Macrolides	Jozamycin	14.2	828.2	174/229	100	43/76
IS Roxithromycin	Tylosin	13.8	917.1	174/83	100	50/130
	Tilmicosin	12.7	870.1	174/88	100	57/124
	Erythromycin	13.7	734.9	158/83	80	38/96
	Azithromycin	12.2	749.8	158	80	100
Penicilines	Cloxacilin	15.2	436	160/277	50	20/20
IS Sulfafenazole	Dicloxacilin	15.6	470	160/311	50	20/20
	Ampicilin	11.3	350.1	106/160	58	27/19
	Penicilin V	15.0	351.1	160/114	54	17/48
	Oxacilin	15.0	402	160/243	50	18/18
	Nafcilin	14.7	415	199/171	50	20/50
Cephalosporins	Ceftiofur	13.7	524	241/125	100	75/25
IS Sulfafenazole	Cefalonium	10.9	459	337/152	46	16/28
	Cephalexin	11.1	348.4	158/106	50	10/23
	Cefquinome	10.7	529	134/125	50	25/75
Nitroimidazoles	Carnidazol	13.2	245.27	118/75	60	21/44
IS Tinidazole	Ipronidazole	13.0	170.18	124/109	190	32/24
	Hydroxy-ipronidazole	12.2	186.18	168/121	60	19/38

Lincosamide	Lincomycin	10.0	407.5	126/359	283	33/25
IS Sulfafenazole						
Pleuromutilin	Tiamulin	13.6	494.9	192/119	100	17/35
IS Sulfafenazole						
Benzimidazoles	Flubendazole	14.7	314.28	282/123	280	31/48
IS Oxibendazole	Cambendazole	13.1	303.35	217/261	250	39/25
	Mebendazole	14.5	296.29	264/105	270	31/46
	Amino-mebendazole	12.2	238.26	105/133	130	46/81
	Hydroxy-mebendazole	12.7	298.13	266/79	280	34/49
	Fenbendazole	14.0	300.3	268/159	270	31/48
	Fenbendazole-solfone	14.0	332.35	300/159	280	35/54
	Fenbendazole-sulfoxide	13.7	316.35	159/191	260	45/30
	Albendazole	14.6	266.3	234/191	295	29/45
	Albendazole-sulfoxide	12.8	282.33	240/208	135	20/35
Insecticides	Phoxim	16.5	299.3	77/129	155	44/18
IS Sulfafenazole	Propoxur	14.3	210.25	168/111	80	20/11
	Carbaryl	14.5	202	145/127	80	13/13
Coccidiostats	Halofuquinone	13.0	416	120/100	50	29/39
IS Nigericin	Narasin	22.0	787.3	431/279	50	70/70
	Salinomycin	21.0	773.5	431/531	60	65/65
	Semduramycin	19.1	895.5	833.5/705.5	160	53/85
	Monensin	20.0	693	675/461	60	50/70
	Maduramycin	21.1	934.8	647.4/629.4	170	29/36
IS Decoquinate d5	Decoquinate	18.6	418.5	372/204	290	34/57
IS Robenidine d8 ESI-	Robenidine	14.4	334	155/138	34	35/47
Coccidiostats	Clazuril	15.7	371	300/301	-120	-24/-24
Dinitrocarbanilide d8	Diclazuril	16.2	405/407	334/336	-90	-27/-28
	Dinitrocarbanilide	15.8	301.24	137/107	-160	-24/-53
	Lasalocid	20.7	589.5	235/173	-140	-46/-67
Amphenicoles	Thiamphenicol	11.35	354	185/290	-120	-32/-19
No IS	Florfenicol	12.63	356	336/185	-80	-14/-27
Benzimidazoles	Triclabendazole	16.8	358.65	197/344	-150	-47/-37
Triclabendazole d3	Triclabendazolesulfone	16.3	389	310/149	-160	-40/-49
	Triclabendazolesulfoxide	16.4	374.66	360/181	-80	-30/-61
	Ketotriclabendazole	16.1	328.56	182/184	-160	-36/-39
IS						
	Sulfafenazole	13.2	315.4	158	80	19
	Tinidazole	10.5	248.27	121/82	60	23/48
	Demeclocycline	11.9	465.2	430	80	24
	Roxithromycin	14.3	838.4	158/680	295	42/31
	Ciprofloxacin d8	11.3	340	296	261	28
	Oxibendazole	13.2	250.26	176	240	39
	Robenidine d8	14.3	342	182	255	29
	Decoquinate d5	18.6	423	377	290	35
	Nigericin	22.0	747.4	703	10	73
	Dinitrocarbanilide d8	15.7	309.24	141	-160	-25
	Triclabendazole d3	16.8	361.65	197	-180	-45

* The first ion given is the quantitation ion

Table 2

Comparison of the mean recoveries obtained by using different clean-up procedures based on SPE and modified QuEChERS.

Class/Analyte	Fortification Level	LOQ*	Procedure applied/Mean Recoveries [%]/SD [%]**							
			1	2	3	4	5	6	7	8
SULPHONAMIDES										
Sulphaquinoxaline	10 µg/kg	1 µg/kg	92/8	131/7	105/3	94/6	97/2	95/3	92/7	111/14
Sulfamethoxazole	10 µg/kg	1 µg/kg	81/29	110/35	100/9	109/20	88/13	95/5	97/4	99/3
Sulphamonomethoxine	10 µg/kg	1 µg/kg	92/28	92/12	101/11	108/21	108/22	116/14	119/10	116/12
Sulfamerazine	10 µg/kg	1 µg/kg	92/24	72/34	96/23	107/19	116/49	104/42	104/61	120/40
Sulfamethoxypyridazine	10 µg/kg	1 µg/kg	80/27	90/17	88/11	98/15	93/28	106/10	106/9	115/8
Sulfadimethoxine	10 µg/kg	1 µg/kg	70/4	72/48	93/28	104/31	84/3	77/5	87/14	99/30
Sulfadoxine	10 µg/kg	1 µg/kg	119/3	83/55	104/10	109/3	103/25	112/22	107/10	110/1
Sulfamethazine	10 µg/kg	1 µg/kg	74//16	88/49	89/8	87/16	99/22	119/31	93/57	111/20
FLUOROQUINOLONES										
Ciprofloxacin	10 µg/kg	1 µg/kg	102/14	0	70/10	87/17	96/19	91/32	89/10	81/68
Norfloxacin	10 µg/kg	1 µg/kg	105/11	0	48/25	81/19	61/7	111/14	67/18	74/33
Danofloxacin	10 µg/kg	1 µg/kg	105/26	0	93/21	47/7	58/15	99/26	50/15	44/9
Enrofloxacin	10 µg/kg	1 µg/kg	104/21	0	106/59	99/55	119/66	127/24	109/18	124/18
Difloxacin	10 µg/kg	1 µg/kg	57/14	0	50/11	65/13	74/21	67/23	99/65	102/33
Sarafloxacin	10 µg/kg	1 µg/kg	94/57	0	117/58	61/15	54/3	72/4	54/13	77/5
Flumequine	10 µg/kg	1 µg/kg	79/18	0	105/37	110/14	89/28	71/16	90/33	99/11
Marbofloxacin	10 µg/kg	1 µg/kg	93/41	0	96/3	108/13	85/30	125/33	90/26	51/7
Nalidixic acid	10 µg/kg	1 µg/kg	77/10	0	114/54	113/33	78/35	61/19	129/29	108/12
Oxolinic acid	10 µg/kg	1 µg/kg	121/25	0	99/62	124/14	123/9	81/21	121/11	82/7
TETRACYCLINES										
Doxycycline	10 µg/kg	1 µg/kg	98/48	11/18	82/26	121/63	73/9	99/36	71/21	81/47
Methacycline	10 µg/kg	1 µg/kg	114/48	0	89/23	88/28	116/32	58/15	80/18	36/6
Tetracycline	200 µg/kg	20 µg/kg	114/16	18/20	81/4	60/44	110/21	110/8	85/12	78/23
Chlorteracycline	10 µg/kg	1 µg/kg	118/43	27/46	80/14	71/12	106/10	54/6	75/13	81/18
Oxytetracycline	10 µg/kg	1 µg/kg	99/18	0	94/17	79/27	115/22	76/16	94/15	32/0
MACROLIDES										
Tilmicosin	10 µg/kg	1 µg/kg	107/20	7/13	121/28	40/50	123/19	140/51	91/34	104/32
Erythromycin	150 µg/kg	15 µg/kg	114/11	0	104/14	66/61	98/9	82/12	99/15	95/12
Tylosin	200 µg/kg	20 µg/kg	102/2	0	110/12	94/43	107/5	120/9	80/6	99/8
Azythromycin	10 µg/kg	1 µg/kg	87/12	0	111/32	17/29	92/9	86/25	80/16	86/10
Josamycin	10 µg/kg	1 µg/kg	93/5	23/34	83/12	81/34	101/7	108/9	95/32	112/3
PENICILINES										
Penicillin V	25 µg/kg	2.5 µg/kg	106/29	0	94/28	57/7	123/24	96/27	96/30	75/19
Ampicillin	10 µg/kg	1 µg/kg	101/13	3/6	106/24	94/13	104/37	84/11	92/20	102/27
Oxacillin	10 µg/kg	1 µg/kg	101/10	1/1	112/27	107/11	88/30	86/20	104/17	104/7
Cloxacilin	10 µg/kg	1 µg/kg	79/6	0	90/17	94/1	109/16	94/2	88/9	73/19
Dicloxacilin	10 µg/kg	1 µg/kg	71/12	0	82/3	53/10	103/43	90/28	90/13	45/18
Nafcilin	10 µg/kg	1 µg/kg	83/2	0	85/11	67/12	104/29	83/11	90/8	107/13
CEPHALOSPORINS										
Ceftiofur	10 µg/kg	1 µg/kg	81/14	0	66/5	78/10	84/6	72/7	63/9	50/2
Cephalonium	10 µg/kg	1 µg/kg	102/18	0	64/20	98/6	80/40	100/16	62/9	54/8
Cefquinome	10 µg/kg	1 µg/kg	104/27	0	62/28	40/4	71/12	105/8	108/10	68/15
Cephalexin	10 µg/kg	1 µg/kg	100/34	0	99/27	79/12	86/22	99/29	86/5	105/17
NITROIMIDAZOLES										
Carnidazole	3 µg/kg	0.3 µg/kg	90/24	92/23	100/18	98/6	101/20	77/10	104/16	108/9
Ipronidazole	3 µg/kg	0.3 µg/kg	87/16	72/7	94/8	98/3	81/16	97/6	89/23	49/6
Hydroxy-ipronidazole	3 µg/kg	0.3 µg/kg	115/9	0	73/12	119/26	87/24	89/16	87/25	98/17
BENZIMIDAZOLES										
Triclabendazole	10 µg/kg	1 µg/kg	107/10	88/12	110/11	94/30	108/13	84/6	101/14	116/7
Mebendazole	10 µg/kg	1 µg/kg	95/35	9/14	89/17	113/20	105/47	114/31	92/19	85/9
Flubendazole	200 µg/kg	20 µg/kg	85/12	11/14	72/11	83/12	69/11	90/22	124/18	109/6
Cambendazole	10 µg/kg	1 µg/kg	90/24	3/6	79/17	79/27	94/20	99/24	85/11	103/25
Amino-mebendazole	10 µg/kg	1 µg/kg	79/12	1/2	86/5	90/16	85/3	90/7	83/6	97/5
Hydroxy-mebendazole	10 µg/kg	1 µg/kg	74/4	8/14	82/12	123/30	91/6	93/33	96/12	97/2
Fenbendazole	10 µg/kg	1 µg/kg	101/18	2/0	83/7	97/5	105/36	102/24	99/19	106/37
Fenbendazole-solfone	10 µg/kg	1 µg/kg	91/15	16/11	72/22	89/36	104/23	91/16	80/10	83/14
Fenbendazole-sulfoxide	10 µg/kg	1 µg/kg	85/8	3/5	82/30	82/23	100/9	108/17	88/9	97/16
Albendazole	10 µg/kg	1 µg/kg	97/12	7/13	94/10	106/13	124/16	105/9	115/23	115/36
Albendazole-sulfoxide	10 µg/kg	1 µg/kg	106/17	9/9	100/18	101/6	116/15	105/5	105/17	106/4
Triclabendazolesulfone	10 µg/kg	1 µg/kg	93/6	88/36	112/4	94/9	104/12	90/16	124/8	109/10
Triclabendazolesulfoxide	10 µg/kg	1 µg/kg	95/8	15/27	102/14	94/20	112/14	109/13	107/17	99/11

Ketotriclabendazole	10 µg/kg	1 µg/kg	39/17	5/9	39/15	40/20	53/20	48/5	49/13	64/31
COCCIDIOSTATS										
Robenidine	25 µg/kg	2.5 µg/kg	100/6	108/4	107/11	109/17	96/11	91/14	105/12	99/22
Halofuginone	6 µg/kg	0.6 µg/kg	96/25	4/5	67/13	43/31	79/21	111/35	109/8	94/20
Narasin	2 µg/kg	0.2 µg/kg	91/36	10/10	94/27	85/18	119/3	112/4	116/17	79/12
Decoquinate	20 µg/kg	2 µg/kg	76/41	0/0	77/37	44/13	67/26	64/19	54/15	80/19
Semduramycin	2 µg/kg	0.2 µg/kg	22/20	8/14	24/1	112/26	30/3	33/5	39/17	28/21
Monensin	2 µg/kg	0.2 µg/kg	52/10	23/39	57/16	85/42	103/32	80/10	97/32	89/19
Maduramycin	2 µg/kg	0.2 µg/kg	32/16	26/44	25/16	27/12	22/4	22/1	88/58	67/18
Salinomycin	3 µg/kg	0.3 µg/kg	38/10	4/6	34/11	47/32	70/29	57/18	66/9	81/15
Clazuril	10 µg/kg	1 µg/kg	46/8	26/5	42/8	35/6	39/2	44/2	32/11	50/4
Diclazuril	2 µg/kg	0.2 µg/kg	73/7	28/6	78/9	61/31	57/4	74/15	51/9	67/3
Dinitrocarbanilide	10 µg/kg	1 µg/kg	112/10	116/20	90/11	83/3	75/1	88/5	85/3	87/6
Lasalocid	150 µg/kg	15 µg/kg	100/4	2/1	80/7	86/28	85/9	84/15	96/4	100/4
INSECTICIDES										
Carbaryl	50 µg/kg	5 µg/kg	86/18	29/9	75/39	62/51	89/32	81/36	70/30	99/15
Propoxur	10 µg/kg	1 µg/kg	83/53	16/6	81/27	75/45	91/26	84/36	62/13	92/48
Phoxim	60 µg/kg	6 µg/kg	89/20	18/4	112/43	105/20	103/53	99/43	117/36	102/28
AMPHENICOLES										
Thiamphenicol	10 µg/kg	1 µg/kg	93/13	13/22	95/13	68/27	89/34	96/37	88/17	92/32
Florphenicole	10 µg/kg	1 µg/kg	71/28	15/8	64/2	70/22	55/12	67/5	77/18	68/5
LINCOSAMIDE										
Lincomycin	50 µg/kg	5 µg/kg	89/27	0	96/20	54/47	85/25	114/13	115/31	104/33
PLEUROMUTILIN										
Tiamulin	1000 µg/kg	100 µg/kg	104/23	6/8	93/5	40/27	96/51	101/50	73/53	80/25

* LOQ – Limit of Quantification

** Three replications

STEP	SAMPLE PREPARATION PROCEDURE APPLIED							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Extraction (ACN:H_2O 8:2 + EDTA)	■	■	■	■	■	■	■	■
Ekstraction with Na_2SO_4			■	■				
Ultrasonication	■	■	■	■	■	■	■	■
Centrifugation	■	■	■	■	■	■	■	■
Columns with Na_2SO_4	■				■	■	■	
Evaporation N_2, 45 °C		■		■				
Reconstitution with 1 ml 0.1% HCOOH		■		■				
Hybrid-SPE	■	■	■	■	■	■	■	
200 mg C_{18}					■			
200 mg PSA						■		
200 mg C_{18} + 200 mg PSA							■	■
1.2 g $MgSO_4$								■
Evaporation N_2, 45 °C	■	■	■	■	■	■	■	■
Reconstitution 250 µl MeOH:ACN (8:2)	■	■	■	■	■	■	■	■
PVDF filter (0.45 µm)	■	■	■	■	■	■	■	■

■ Step applied

Fig. 1. Graphic presentation of sample preparation procedures applied.

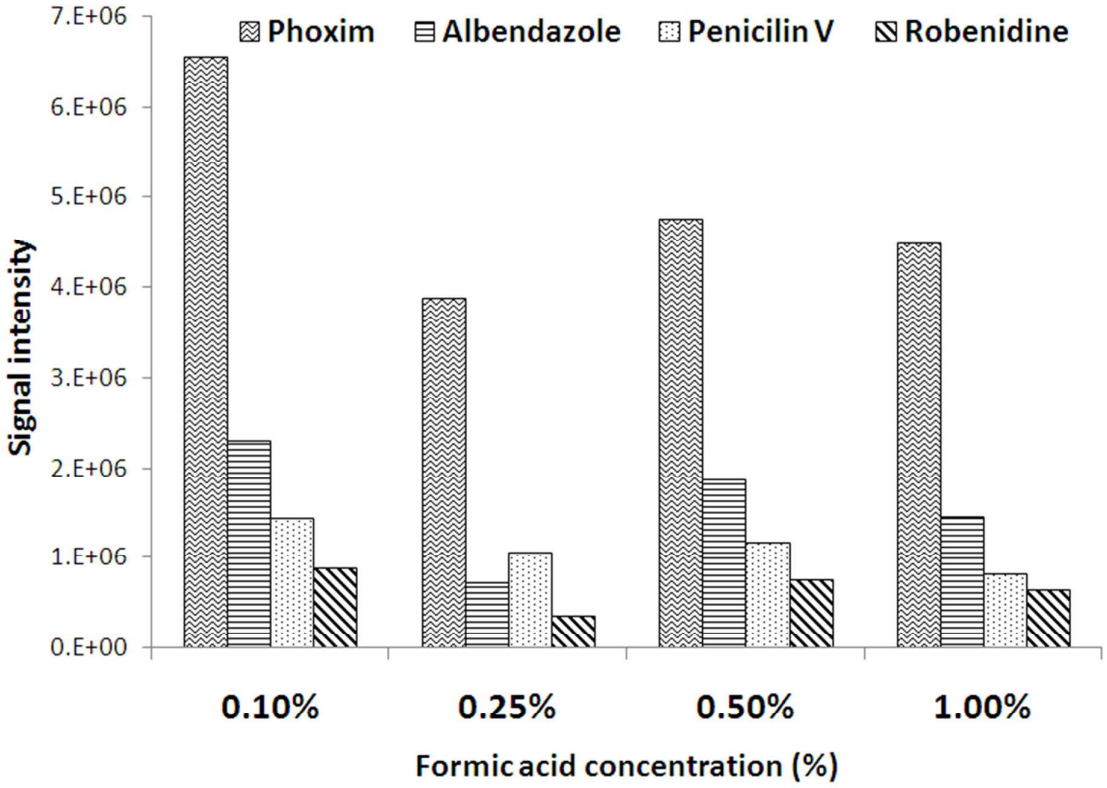


Figure 2. Evaluation of formic acid concentration in acetonitrile used to elution on SPE (selected examples)

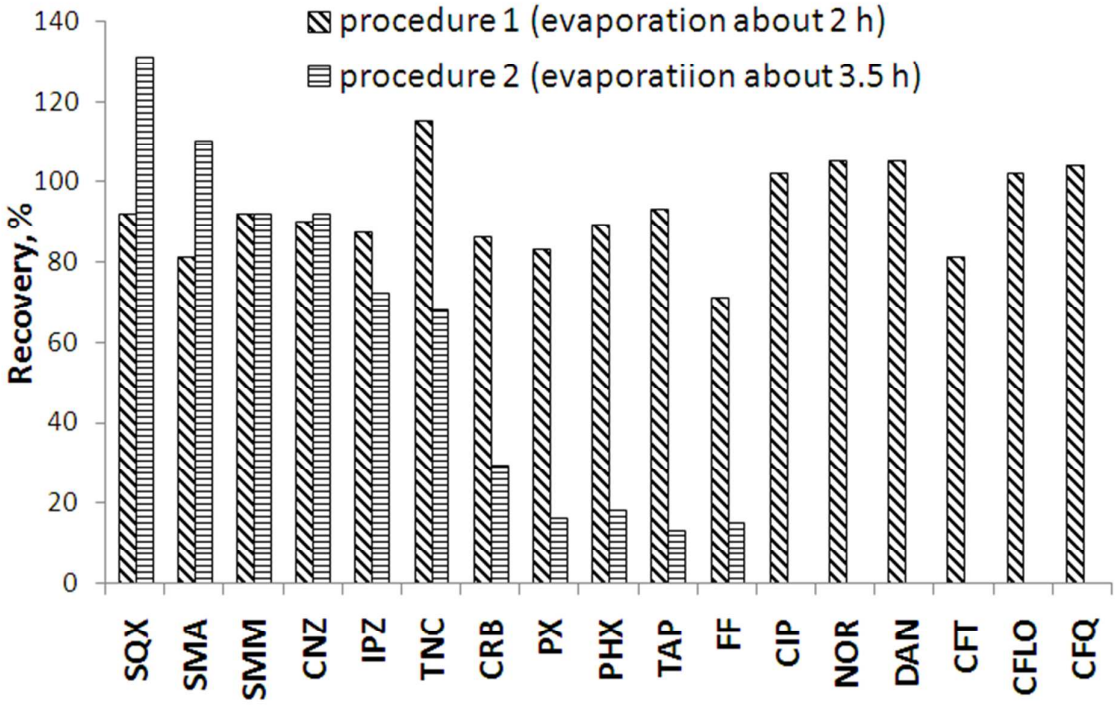


Figure 3. Effect of the time of evaporation on the analytes recoveries on selected examples

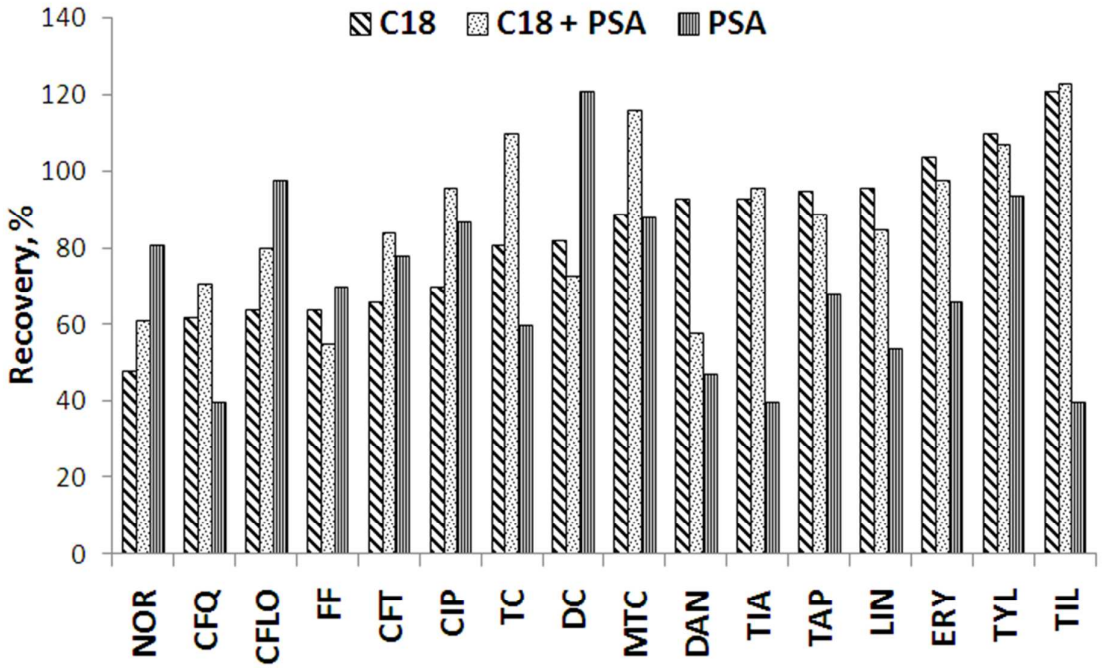


Figure 4. Comparison of the C18, PSA and C18 + PSA usage on the example of selected analytes

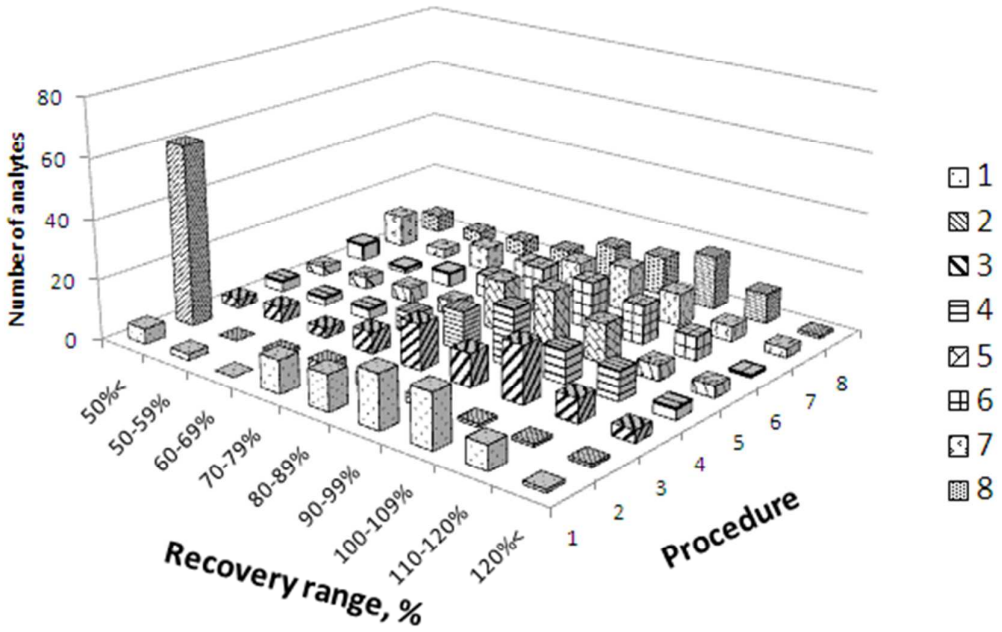


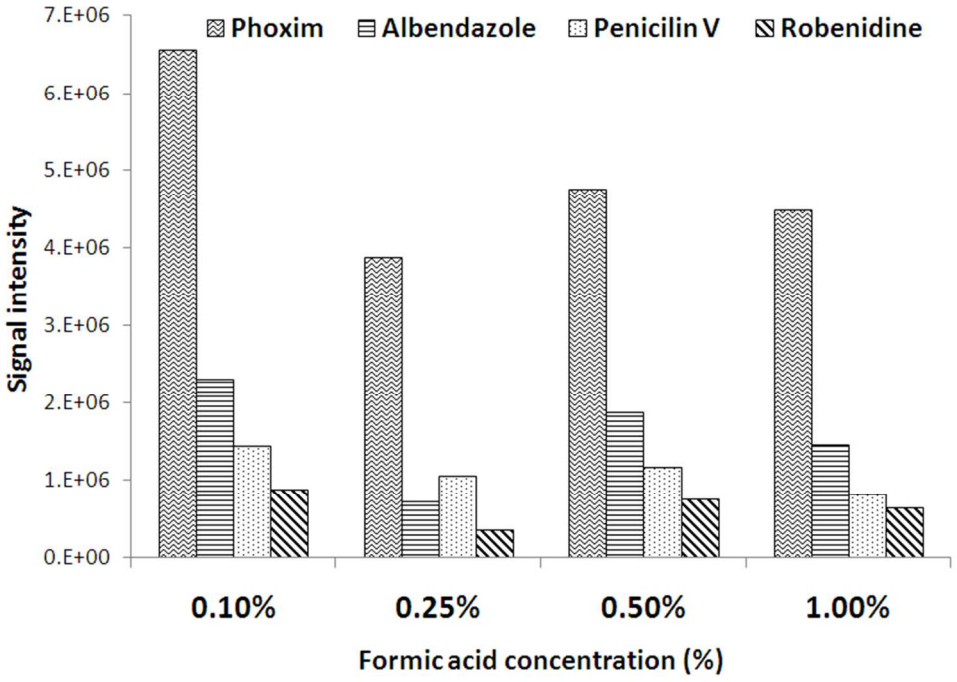
Figure 5. Comparison of extraction efficiency within the preparation steps applied for all analytes.

QuEChERS and zirconium coated silica SPE in multiresidue method for the analysis of veterinary drugs residues and other contaminates in eggs by LC-MS/MS.

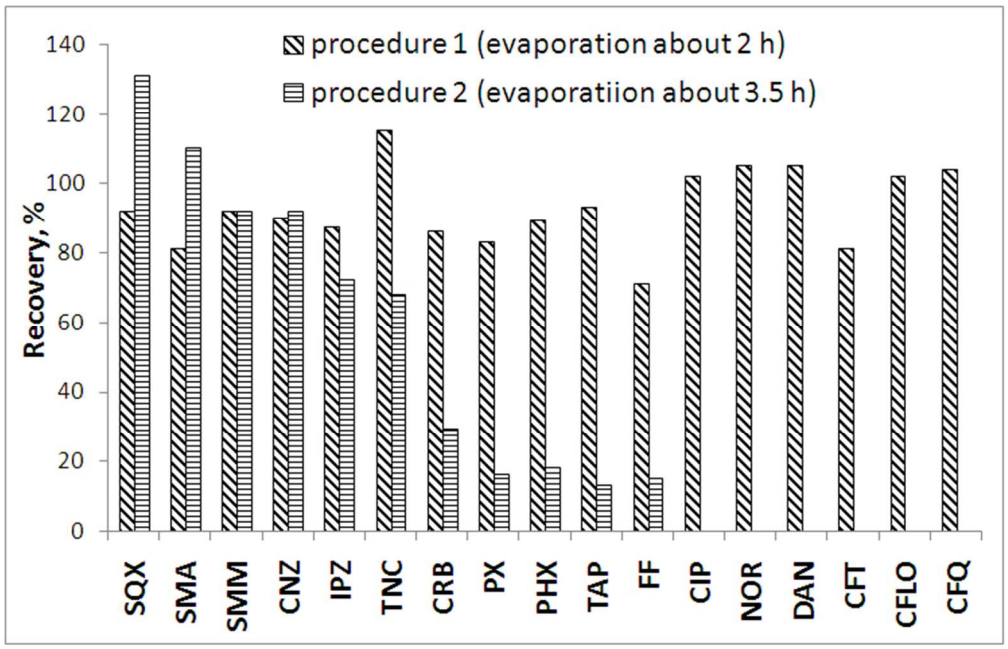
SAMPLE PREPARATION PROCEDURE APPLIED

STEP	1	2	3	4	5	6	7	8
Extraction (ACN:H_2O 8:2 + EDTA)	■	■	■	■	■	■	■	■
Ekstraction with Na_2SO_4			■	■				
Ultrasonication	■	■	■	■	■	■	■	■
Centrifugation	■	■	■	■	■	■	■	■
Columns with Na_2SO_4	■				■	■	■	
Evaporation N_2, 45 °C		■		■				
Reconstitution with 1 ml 0.1% HCOOH		■		■				
Hybrid-SPE	■	■	■	■	■	■	■	
200 mg C_{18}					■			
200 mg PSA						■		
200 mg C_{18} + 200 mg PSA							■	■
1.2 g $MgSO_4$								■
Evaporation N_2, 45 °C	■	■	■	■	■	■	■	■
Reconstitution 250 µl MeOH:ACN (8:2)	■	■	■	■	■	■	■	■
PVDF filter (0.45 µm)	■	■	■	■	■	■	■	■

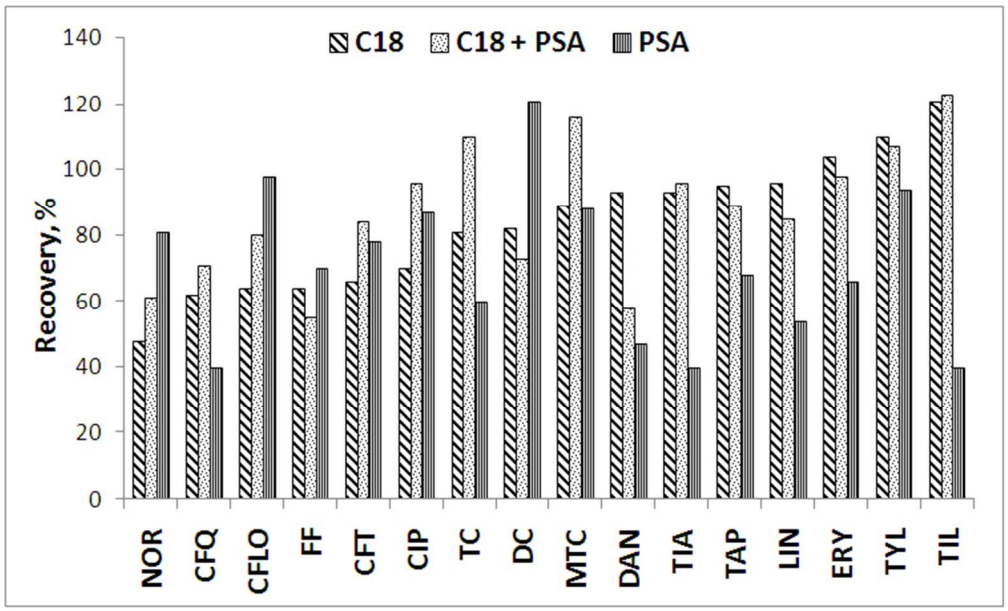
■ Step applied



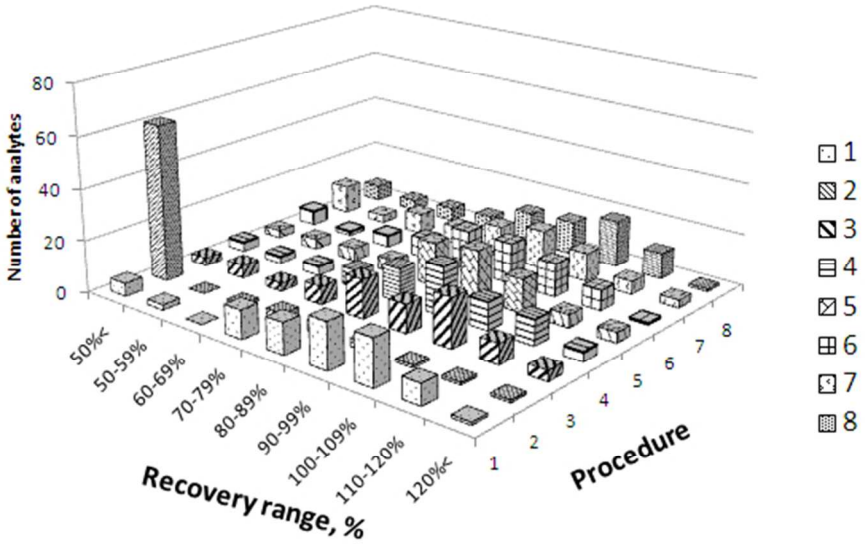
68x47mm (300 x 300 DPI)



62x40mm (300 x 300 DPI)



199x120mm (96 x 96 DPI)



53x30mm (300 x 300 DPI)