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Development and Validation of a Fully Automated Solid-Phase Microextraction High Throughput Method for Quantitative Analysis of Multiresidue Veterinary Drugs in Chicken Tissue

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ABSTRACT

This paper presents the development and validation of a fully automated, high-throughput multiclass, multiresidue method for quantitative analysis of 77 veterinary drugs in chicken muscle via direct immersion solid phase microextraction (DI-SPME) and ultra-high pressure liquid chromatography-electrospray ionization - tandem mass spectrometry (UHPLC-ESI-MS/MS). The selected drugs represent more than 12 different classes of drugs characterized by varying physical and chemical properties. A Hydrophilic–lipophilic balance (HLB)/polyacrylonitrile (PAN) extraction phase, prepared using HLB particles synthesized in-house, yielded the best extraction/desorption performance among four different SPME extraction phases evaluated in the current work. The developed SPME method was optimized in terms of SPME coating and geometry, desorption solvent, extraction and rinsing conditions, and extraction and desorption times. Multivariate analysis was performed to determine the optimal desorption solvent for the proposed application. The developed method was validated according to the Food and Drug Administration (FDA) guidelines, taking into account Canadian maximum residue limits (MRLs) and US maximum tolerance levels for veterinary drugs in meat. Method accuracy ranged from 80 to 120% for at least 73 compounds, with relative standard deviation of 1 to 15%. Inter-day precision ranged from 4–15% for 70 compounds. Determination coefficients values were higher than 0.991 for all compounds under study with no significant lack of fit ($p > 0.05$) at the 5% level. In terms of limits of quantitation, the method was able to meet both Canadian and US regulatory levels for all compounds under study.

1. Introduction

As per current agricultural practices, animals raised for food are often housed and transported in high densities, which makes them more prone to increased levels of stress and disease. Aiming to minimize livestock losses and increase production, veterinary drugs (VDs) are thus frequently used to prevent and treat diseases, as well as promote weight gain [1,2]. In this regard, illegal or improper dosage of VDs as well as failure to comply with stipulated withdrawal dates may lead to the presence of drug residues in the edible tissue of the treated animal. These residues, even at low concentrations, may in turn pose a risk to human health [2]. In this respect, one of the main concerns arising from the overuse of antibiotics in animals is the emergence of resistant bacteria [3].

Aiming to protect human health, most governments and associated agencies have established monitoring and regulation laws, standards, and procedures with respect to veterinary drug residues in the edible tissues of food-producing animals [4,5]. To this end, maximum residue levels (MRLs), defined as the maximum concentration of residue that can safely remain in the edible tissue of an animal that has been treated with a veterinary drug, are established to set and enforce these regulatory standards [6]. In Canada, VD residues are regulated by MRLs established by the Veterinary Drugs Directorate of Health Canada. Likewise, in the USA, regulatory tolerances of registered veterinary drugs are set by the Food and Drug Administration (FDA) Center for Veterinary Medicine [7]. In the European Union (EU), the use of veterinary drugs is also strictly regulated through EU Council Regulation 2377/90/ EC [8]. At the international level, MRLs are established by Codex Alimentarius, a joint initiative between the Food and Agricultural Organization and the World Health Organization [9]. MRLs are also

55 employed in the establishment and monitoring of trading standards so as to ensure the quality of
56 imported and exported meat [10,11].

57 Given the growing public interest and concern regarding food safety, and taking into account the
58 importance of the meat industry to the global economy, the demand for simple, automated high-
59 throughput analytical procedures for monitoring of drug residues in meat is expected to continue
60 growing. Within this same context, cost-effectiveness plays a large role in analytical method
61 selection, particularly for laboratories that provide regulatory testing for VD residues. In this
62 respect, one approach to increase the efficiency and cost-effectiveness of an assay is to increase
63 the number of analytes that can be determined in a single run or method. Consequently, multi-
64 residue, multi-class analytical methods have been gaining popularity as cost-effective methods
65 for screening, identification, and quantification of drug residues in food [12].

66 Despite the high selectivity and specificity afforded by LC-MS/MS platforms, analysis of VDs at
67 trace levels in complex matrices, such as animal tissues, still requires extensive sample
68 preparation procedures aimed at isolating target analytes from complex matrix constituents, and
69 minimizing interferences and matrix effects [13]. In this respect, multiresidue sample preparation
70 poses a large challenge to analysts due to two main factors: the large quantity and variety of
71 analytes under consideration, and the complexity of the matrix under study. A suitable sample
72 preparation method must enable the detection and quantification of a large quantity of analytes,
73 encompassing a wide range of physical/chemical properties, while offering sufficient sample
74 clean-up so as to minimize interferences and matrix effects – a challenging prospect, given that
75 the studied matrix is characterized by the presence of endogenous compounds as well as
76 macromolecules such as proteins and lipids. One of the first and most commonly employed
77 sample preparation techniques for analysis of tissues is solvent extraction (SE), which involves

liquid extraction of analytes from homogenized animal [9]. While this method offers a quick and simple workflow, it also involves the co-extraction of a high number of endogenous sample compounds, which may cause matrix effects in LC-MS/MS analysis. Other disadvantages include the possibility of emulsion formation and the use of large volumes of toxic organic solvents [14]. To minimize interferences and matrix effects, solid phase extraction (SPE) is commonly used for further sample pre-treatment [15]. Another extraction method that offers purification of sample interferences in complex matrices is dispersive SPE (dSPE), which is widely applied in the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method [16–18]. However, while SPE and dSPE techniques are widely employed for sample preparation, such approaches do not always effectively eliminate matrix effects, and may sometimes retain target analytes, thus leading to analyte loss [3,13]. Further, owing to the multiple steps often involved in such methods, employment of such sample preparation strategy may involve long analysis times, the introduction of errors, as well as large costs [19].

As a well-established and environmentally friendly sample preparation technique, SPME enables the attainment of clean sample extracts while minimizing matrix interferences from biological samples [20–22]. Matrix-compatible SPME coatings offer adequate robustness for direct immersion in complex matrices and balanced extraction coverage of compounds with a wide range of polarities [23].[24]. [25]. Matrix-compatible coatings, when combined with the open-bed configuration of SPME, facilitate extraction of multiresidue compounds from complex matrices without being burdened by the clogging issues typical of conventional SPE packed bed systems [26]. Boyaci et al. recently developed a fully automated high-throughput thin-film solid phase microextraction (TF-SPME) method where TF-SPME blades coated with C_{18} particles/PAN were utilized to extract 110 doping compounds banned by the World Anti-Doping

Agency (WADA) from urine [27]. Among the many configurations offered by SPME, SPME thin-films are consisted of a higher surface area as compared to the conventional SPME fiber format, and a comparable or lower thickness [28]. Further, as thin-film SPME offers high-throughput compatibility, use of thin-film SPME offers enhanced throughput of the extraction process due to the simultaneous extraction of 96 individual samples, with minimal use of organic solvents. Another approach to increase surface area and enhance recovery is the use of round SPME devices with larger outer diameters [29,30]. The purpose of the presented work entailed the development of a simple, green, automated, high throughput sample preparation method for determination of a wide range of veterinary drugs at regulatory levels in chicken muscle. Automated sample preparation improves precision and reproducibility due to the elimination of human error from the procedure. Thus, an automated high throughput DI-SPME LC-MS/MS method is presented in this paper for analysis of 77 veterinary drug compounds in homogenized tissue from chicken.

2. Experimental

2.1. Chemicals and Materials

The veterinary drugs under study were selected from the list of standards specified in the official method for screening and confirmation of animal drug residues developed by the United States Department of Agriculture (CLG-MRM1.08) [31] and referred to by Schneider et al. [12]. Depending on availability of standards, we aimed to include as many analytes from the list which cover a wide range of polarities representing at least 12 classes. Standards were obtained from suppliers listed in the Electronic *Supplementary Data* in Table S-1. The corresponding class of each target analyte is presented in Table S-2. LC-MS grade acetonitrile (MeCN), methanol (MeOH), isopropyl alcohol (IPA), water, and formic acid (FA) were purchased from Fisher

Scientific (Mississauga, ON, Canada). Polyacrylonitrile (PAN), Dimethyl sulfoxide (DMSO), and N, N-dimethylformamide (DMF), divinylbenzene, N-vinylpyrrolidone, and 2, 2-azobis (isobutyronitrile) were obtained from Sigma Aldrich (Oakville, ON, Canada). Nunc U96 Deep Well 2 mL and 1 mL plates made of polypropylene were purchased from VWR International (Mississauga, ON, Canada). Polypropylene inserts (600 µL) for the 1mL plates were purchased from Analytical Sales and Services (NJ, USA). For preparation of SPME coatings, C_8-benzenesulfonic acid (Mix-mode) was obtained from Supelco (Bellefonte, PA, USA), PS-DVB was purchased from Chromabond, Germany, and HLB particles were synthesized in-house as per the protocol discussed in section 2.3. High-tolerance 304 stainless steel rods (1/16" diameter) were obtained from McMaster-Carr (Aurora, OH, USA) for use as SPME pins.

Stock solutions of veterinary drugs and deuterated internal standard were prepared by weighing approximately 5 mg of each individual standard, and dissolving individual quantities in 5mL of either MeCN, MeOH, water, or 10% DMSO in MeCN, in accordance with the solubility of each compound. An internal standard (IS) solution, containing flunixin-*d3* at 10 ng mL^{-1}, was prepared by appropriate dilution of IS stock solution in MeCN. All target analytes were mixed in a composite solution and diluted to 100X, where X represents the MRLs listed in Table 1 with MeCN, with the exception of the β-lactams/cephalosporins composite solution, which was prepared and diluted in water at a concentration of 200X. All stock and composite solutions were stored at −30 °C. Spiking solutions and their dilutions were prepared daily for validation experiments.With the exception of β-lactams/cephalosporins solutions, which were stored in plastic, all other standard and composite solutions were stored in amber glass vials closed with fitted PTFE caps.

The MRL values displayed in Table 1 were based primarily on Canadian MRL values [6] in poultry, or US tolerance levels [32] in cases where MRL values corresponding to certain analytes were not available in the Canadian database. In cases where MRL values were unavailable for poultry in either database, values in other tissues, such as bovine tissue, were selected instead. In cases where Canadian MRLs were higher than US tolerance levels, such as that established for Ketoprofen, the US regulatory value was selected.

Antibiotic free chicken breast, thighs, and liver from five different sources were purchased from local grocery stores to serve as matrix. Chicken thighs and liver tissue were used only for matrix effects experiments. Each sample was homogenized separately with dry ice, using a Vitamix blender to obtain a uniform powder. All samples were then combined and ground again with dry ice to produce a pooled matrix. All homogenized samples were first stored in glass jars covered by loose lids overnight at −30 °C to allow for sublimation of dry ice to occur, then subsequently stored at −80 °C until analysis.

2.2. LC-MS/MS method

Experiments were performed with the use of a Thermo Accela 1250 pump with an on-line vacuum degasser liquid chromatography system coupled to a triple quadrupole mass spectrometer TSQ Vantage (Thermo Scientific, San Jose, USA). Further instrumental details and optimized LC and MS/MS parameters are provided in *Supplementary data*, Tables S-2, S-3, and S-4. The autosampler, thermostated at 5 ºC, was used for high throughput, 10 µL sample injections in full loop mode. A Waters (Mississauga, ON, Canada) Acquity UPLC HSS T3 (100×2.1 mm, 1.7µm) analytical column connected to a guard column (HSS T3, 2.1 x 5mm, 1.7µm) was used for separation of the targeted analytes. The column compartment was maintained at 40 °C, and the flow rate was 0.3 mL/min. MeCN/water (70:30, v/v) was used to

clean the injection system (flush and wash volumes were 1000 µL and 200µL, respectively). The mobile phases were water (solvent A) and MeCN (Solvent B), each containing 0.1% (v/v) formic acid. The gradient was run at 3% B for 1 min, ramped linearly to 100% B until 11 min, and then held at 100% B until 13 min. The column was then returned to 3% B over 2 min, where it was allowed to re-equilibrate for 3 min. MS data was processed using Xcalibur software v.2.1 (Thermo Fisher Scientific, San Jose, USA). Mobile phases were degassed for 30 min in a VWR Scientific, Aquasonic model 75HT (West Chester, PA, USA) ultrasonic bath before use.

2.3. Synthesis of Hydrophilic-Lipophilic Balance (HLB) particles

HLB particles were synthesized via precipitation polymerization by modifying the procedure described elsewhere [33,34], however it was scaled up and toluene was used as a porogen in order to prepare mesoporous particles. First, 450 mL MeCN and 150 mL toluene were added in a 2L three-necked round bottom flask equipped with a mechanical stirrer and an inlet for nitrogen gas purging, then purged with nitrogen for 30 min. Following, 42 mL of the monomer/cross linker (DVB) and 18 mL of the functional monomer (N-VP) were added to the solvent mixture. AIBN (500mg) was used as an initiator for the polymerization reaction. Particles were rinsed and dried as per previous methods.

HLB particles were characterized by UltraPlus field emission scanning electron microscopy (FE-SEM, Carl Zeiss, Germany), specific surface area analysis (Autosorb iQ-MP by Quantachrome (Boyton Beach, Florida)), for determinations of size and shape, and surface area, respectively.

2.4. Automated Concept 96-pin SPME System

The Concept 96 robotic sample preparation station (Professional Analytical System (PAS) Technology, Magdala, Germany) used in this work for SPME sample preparation is a fully

automated, software-operated, off-line bench top robotic station (Figure. 1). The device executes all steps of the SPME protocol, including preconditioning of the sorbent, SPME extraction, rinsing, and solvent desorption. In addition, the system also contains an evaporation unit that allows for optional drying and reconstitution of extracts and/or preconcentration of analytes. The SPME brush is comprised of 96 SPME pins coated with extraction phase (Figure. 1). The brush fits standard commercial 96-well-plates, which can be accommodated on the agitators used during subsequent steps of the method, and is compatible with most autosamplers available for standard LC systems.

Stainless steel rods for the SPME brush were cut to a length of 50 mm at the University of Waterloo's Science Technical Services. The SPME coating was comprised of HLB particles suspended in PAN, and immobilized on the stainless steel rod surfaces of the 96-pin SPME brush by dip coating, as per the protocol developed by Gomez-Ríos at al [35]. All prepared coatings had the same length (20mm) and thickness (60µm).

2.5. Automated SPME Procedure for High-Throughput Analysis

Spiked chicken samples were prepared by adding 100 µL of the working solution, containing all analytes under study at their respective designated concentrations, and 30 µL of the internal standard working solution to 2.0 g of homogenized chicken tissue. Samples were vortexed manually for 1 min, then placed on a benchtop agitator for 1h. Samples were then placed in a 4ºC fridge overnight so as to allow for binding equilibria between matrix and analytes to be established. Following overnight refrigeration, chicken samples were placed on the benchtop agitator for 1h prior to extraction so as to allow samples to reach room temperature. 6 mL of water were added to each individual spiked chicken sample; samples were then vortexed for 1

min in order to attain homogeneous consistency, and a viscosity suitable for pipetting of samples to wells of the 96 well-plates. Next, 1.5 mL of the diluted chicken samples were transferred to 96 well plates. All final diluted spiked samples had an organic solvent content equivalent to less than 2%.

The Concept 96 system was programmed to consecutively perform the preconditioning of fibers, SPME extraction, fiber rinsing, and solvent desorption. The SPME protocol was executed as follows: prior to extractions, SPME rods were conditioned for 30 min with 1 mL of methanol/water (50:50, v/v) in 96-well-plates with agitation (900 rpm). Next, extractions were performed from 1.5 mL of diluted chicken tissue samples spiked with the target compounds. For coating and desorption solvent selection, sample matrix was 1 mL of PBS spiked with each target analyte at 50 ng mL^{-1}. Chicken matrix was used for all subsequent steps of the study. The final optimized extraction parameters were static extraction for 60 min at 50 ºC. In the fiber rinsing step, SPME pins were rinsed with 1 mL of water for 10 s with agitation (900 rpm). Following, desorption of analytes was carried out in 400 µL of desorption solvent (in the final method) for 20 min with agitation (1200 rpm) in a new 96-well-plate containing desorption solvent. In order to evaluate carryover for each pair of sorbent and desorption solvents, second and third sequential desorption steps were carried out under the same conditions. Lastly, the 96-well-plate containing final extracts was covered with the 96-well-plate lid, and placed in the LC-MS/MS autosampler for further analysis.

The final optimized SPME conditions for preconditioning, extraction, rinsing, and desorption steps are shown in Figure. 1, while protocols for preparation of the used solutions as well as further details regarding the full analytical procedure can be found in *Supplementary data,* Figure S-1.

2.6. Optimization of the desorption solvent by experimental design

The composition of the desorption solvent was optimized for effectiveness with respect to ratios of water, methanol, and acetonitrile. Experiments to optimize the desorption solvent were designed based on a simplex-lattice mixture design (SLMD) introduced by Scheffé [36]. The design consists of a symmetrical arrangement of points, referred to as {k, m}-lattice, where k is the number of components, and m is the polynomial model degree. According to Scheffé, in order to better elucidate the shape of the response surface, the best design option comprises the use of a design where points are spread evenly over the whole simplex. The uniformly spaced distribution of points on a simplex is known as a lattice. In addition, the summation of the three portions (factors) in the SLMD must be equal to one. In cases where the optimization process involves multiple responses, it is not feasible to individually optimize each response, as such would necessitate the use of a large number of samples, equal to the dependent variable under study [37]. The Derringer & Suich approach presents an alternative to overcome this drawback, as it allows for the discovery of the best compromised conditions among all investigated responses through the desirability function [38,39]. In total, 14 experiments in triplicate were performed as listed in *Supplementary data,* Table S-5. Solvent ratios were established through an SLMD with three components, and the polynomial model degree equal to three. Statistical evaluation of data from these experiments was performed with the use of Statistica 13.0 software (TIBCO® Statistica™, CA, USA).

2.7. Validation of the method

The developed method was validated following the guidelines established by the FDA for Method Validation for Drugs and Biologics [40]. The developed method was validated in terms

of selectivity, linearity, accuracy, intra- and inter-day precision, stability, and limits of quantification (LOQs). Calibration curves as well as statistical tests were attained with Origin 2018 software (OriginLab Corporation®, MA, USA).

The first step in the validation procedure entailed an evaluation of the selectivity of the method. Method selectivity was assessed through an analysis of 10 blank chicken tissue samples.

Matrix-matched calibration with internal standard (IS) correction was selected as a calibration method for the current work. The matrix-matched calibration curve was prepared by spiking analytes in one lot of blank pooled chicken. Flunixin-*d3* was added to samples as internal standard to compensate for sample variations with respect to matrix, variations from pin to pin, as well as variations in desorption solvent loss due to evaporation during the desorption step.

The linear dynamic range of the LC-MS instrument was determined for each analyte by direct injection of the neat standards prepared in desorption solution. In order to determine the linearity of the method, calibration curve solutions were prepared in a range of 0.1X to 3X in pooled chicken, and analyzed with the proposed SPME-LC-MS method in triplicate. For determinations of accuracy and precision, target analytes were spiked at low-, mid- and high concentration levels (0.3X, 0.9X and 2.5X) in pooled chicken matrix. Internal standard (Flunixin-*d3)* was spiked in pooled chicken matrix at 30 ng mL^{-1}. These extractions were performed in six replicates for intraday precision. Interday precision was evaluated by running three different experiments over three different days.

The stability of the analytes in the desorption solvent for the duration of the analysis period was also assessed. Supposing that one run of the experiment utilizes every one of the 96 SPME pin spaces available in the Concept 96, and taking into account that a total chromatographic time of 18 min is needed per sample, an approximate period of 48 h was calculated as required to

complete analysis for each dataset. In addition to the 48 h period, periods of 72 h and one week were also considered in the stability evaluation. Stability samples were prepared in desorption solvent, stored at 5ºC, and analyzed after 48 h, 72 h, and 1 week; the attained results were then compared with those of freshly prepared standards.

3. Results and discussion

3.1. LC-MS method

The method was optimized with respect to run time, retention time stability, chromatographic separation, carryover, and sensitivity. Chromatographic separation is especially important in the presence of isobaric compounds such as sulfadoxine and sulfadimethoxine, tetracycline, and doxycycline [41]. To this end, different chromatographic columns with different chemistry from various suppliers were tested with respect to their performance for the analytes under study. Based on the results attained in this comparative study, a Waters Acquity T3 C18 HSS (100x2.1 mm, 1.7 µm) column was selected for further experiments, as it provided the best performance compared to the other evaluated columns in terms of retention of target analytes, as well as peak shapes and resolution. Quality control (QC) samples were run to verify retention time, reproducibility, and instrumental performance. QC samples constituted of extracted blank chicken matrix spiked at 0.5X. The criteria used for verification were: retention time to verify that all analytes fall in retention time windows, peak shape, as well as peak area of the internal standard to make sure it does not deviate by more than 20% from the averages obtained during the initial calibration.

3.2. Development of the SPME method

Method optimization was carried out as per the protocol suggested by Risticevic et al [42]. Evaluated parameters included coating chemistry, desorption solvent, extraction and desorption times, agitation rates for extraction and desorption, extraction temperature, and sample and desorption solvent volumes. The first, and most important step in SPME method development entails the selection of a suitable coating in terms of extraction efficiency and carryover. Coating selection is especially important when targeting a large number of analytes with a wide range of polarities such as the ones targeted in this work, which present log P values ranging from -1.50 to 8.67. For this purpose, different polymer chemistries characterized by both polar and nonpolar functional groups to facilitate extraction of compounds with a wide range of polarities were selected for evaluation. Evaluated polymer chemistries included Hydrophilic-Lipophilic Balance (HLB) particles synthesized in-house, commercially available SPE particles, a polar modified polystyrene-divinylbenzene copolymer (PS-DVB), a mix-mode (C_8-SCX), and a 50:50 (w/w) HLB:PS-DVB. At the beginning of the study, preparation of the above listed SPME coatings was first attempted by spraying the particle slurry on the SPME blade's surface, as described in earlier work by Mirnaghi et al [43]. However, due to the sharp edges of the blades and the roughness of the sprayed coating surface, attachment of matrix components, especially fatty residue was observed on the coating surface following extraction. To avoid coating fouling, the geometry of the SPME device was modified to include rounded rods, while smaller HLB particles (1-5 µm) were used to yield a smoother extraction phase surface, which prevented any further attachment of matrix constituents. A rounded SPME geometry has been previously utilized for extraction from complex biological matrices such as blood by Reyes-Garcés et al. [30] and Vuckovic et al. [44]. In addition, static extraction was carried out to totally prevent any mechanical attachment of the sample to the SPME device.

When performing SPME in complex matrices, a rinsing step is usually added after the extraction step and prior to desorption so as to avoid fouling on the sorbent surface. In the current work, water was selected as rinsing solvent. Agitation rate was set up at 900 rpm, and rinsing time was set at 10 seconds so as to avoid loss of polar analytes.

Desorption-solvent effectiveness was assessed by evaluating desorption solutions with varied composition of the MeCN, MeOH, isopropanol, and water content. The effect of adding formic acid to the desorption solvent was also evaluated. Target analyte extraction amounts by each coating/desorption solvent pair are shown in Table S-6. These results were then compared with the aim of selecting the combination of extraction phase and desorption solvent composition that offers the highest extraction efficiency for most of the analytes, and the least carryover. The attained results, as summarized in Figure 2, showed that HLB provided the highest extraction recoveries for most of the target analytes, while mix-mode yielded the poorest recoveries. HLB yielded higher extraction recoveries for hydrophobic analytes, while PS-DVB yielded higher extraction recoveries for more polar compounds. Although the PS-DVB and 50:50 [w/w] HLB:PS-DVB coatings offered higher extraction efficiencies for polar compounds, they were also shown to be characterized by higher carryover effects for a number of compounds when submitted to second and third desorption cycles. As a result, HLB was selected for further method development as the functional coating polymer.

3.3. Synthesis of Hydrophilic-Lipophilic Balance (HLB) particles

FE-SEM images of the prepared HLB particles show that the attained particles are characterized by a uniform spherical shape, and are monodisperse in nature. Surface area analysis of the HLB particles revealed that the particles were microporous and mesoporous in nature, although most of the observed pores were in the mesoporous range (2-8 nm).The specific surface area of the

HLB particles, calculated via the Brunauer–Emmett–Teller(BET) method with nitrogen gas used as adsorbate at 77.35 K, was measured at 816.78 m^2g^{-1}.The SEM images in Figure S-3 (A and B) illustrate how particles initially agglomerated when dried under vacuum. The SEM images show the obtained particles were spherical in shape and of a size between 1-5 µm. Although the HLB particles are embedded in PAN glue (Figure S-3, C and D), pores present in PAN allow analytes to access the different layers of the HLB coating. These pores enable the diffusion of analytes of interest into the coating but restrict access of macromoleculess such as proteins and lipids, resulting in satisfactory extraction of compounds of interest and minimal background interferences. At the same time, the smooth layer of PAN hinders attachment of matrix constituents to the SPME coating.

3.4. Optimization of the desorption solvent by experimental design

In order to optimize the desorption of the analytes under study, which are characterized by a wide range of polarities and physical-chemical properties, after extraction by the HLB coating, optimum desorption solvent composition was investigated via experimental design. In total, 14 experiments in triplicate were performed, and the solvents portions were established through an SLMD with three components and the polynomial model degree equal to three. The use of SLMD for optimization of desorption solvent composition enables a much more efficient optimization process as compared to sequential testing, particularly when the goal of analysis is detection of a wide range of analytes. Figure 3 demonstrates the attained results for the tested compositions with respect to the HLB coating. In this work, the optimum desorption composition was selected as 25:37.5:37.5, v/v/v water: MeCN: MeOH. The optimum desorption solvent composition was in agreement with expected results; considering the wide range of Log P values studied, a considerable amount of water would be required to desorb polar compounds from the

fiber coating, while a sufficient amount of organic solvent would be needed to desorb non-polar compounds (Figure 3A). The same experimental design was used to assess carryover values; not surprisingly, the attained results converged with the above discussed results. The best conditions to minimize carryover were water (25%), MeCN (75%), and MeOH (0%) (Figure 3B). As this test was performed by using the inverse of values obtained for carryover, the maximum point denotes the lowest carryover obtained. Although the attained carryover percentage (less than 3% for all analytes, except for Phenylbutazone and Tolfenamic acid, at 5 and 8%, respectively) in the final desorption solution is considered acceptable in terms of quantitative analysis requirements, potential false positive results should be kept in mind in cases where extractions from samples characterized by high concentrations of target compounds are followed by extractions from low concentration samples or blanks. Therefore, it is recommended that an additional desorption step is performed prior to the next SPME cycle. This additional desorption step, when combined with the conditioning step, allows for effective removal of residual analytes from the SPME coating. While addition of formic acid to the desorption solvent was found to improve overall desorption efficiency for most compounds, certain compounds, including lactams and fluoroquinolones, failed to present enough stability under such acidic conditions. Erythromycin, in particular, was observed to be very unstable under acidic conditions [45]. Thus, formic acid was not added to the final desorption solution selected in the developed method.

3.5. Time profiles

Extraction time profiles were determined by extracting spiked chicken samples at 1 MRL level at different time points, within the range of 10 – 60 minutes, under optimized conditions. According to the attained results, most polar compounds reached equilibrium within 60 minutes, while the majority of the hydrophobic compounds under study did not reach equilibrium within

this time period. As a compromise between extraction efficiency and overall analysis time, 60 minutes was selected as the final extraction time. Figure S-4, in *Supplementary data*, presents the extraction time profiles of representative compounds from each class of veterinary drugs. Thus, under the selected extraction conditions, extraction of most hydrophobic compounds would occur under the pre-equilibrium regime. However, owing to the automation of the method, which enables precise control over extraction time, carrying out pre-equilibrium extractions will not affect the precision of the method. Desorption time profiles were determined by extracting spiked PBS samples at the 1 MRL level at various times (10 – 120 minutes). Although the results showed that most compounds reached quantitative desorption within only 15 minutes, 20 minutes were selected as desorption time in order to minimize carryover of the most hydrophobic compounds.

The final optimized SPME parameters presented in Figure 1 yielded sufficient extraction efficiency, minimum carryover, and minimum use of organic solvents, while affording minimum manual handling during the sample preparation steps. A total time of 1h 21m is needed to achieve SPME extraction and desorption; supposing the 96-pin system is fully utilized within a run of the proposed workflow (i.e., 96 samples per run), the proposed method thus offers a time per sample of less than 1 min.

3.6. Matrix effect

The presence of matrix effects (ME) is considered one of the main challenges in the multiresidue determination of drugs in tissue by LC-MS due to the complexity of the matrix under study. Matrix effects were calculated by the equation: ME% = (slope of matrix-matched calibration curve − slope of reagent-only calibration curve) × 100% / slope of reagent-only calibration curve

[12]. Matrix effects were evaluated for three different types of chicken tissues; breast, liver, and thighs. Figure 4 shows the matrix effects for the analytes studied versus their retention time. Absolute matrix effects were determined by the ratio of the peak areas of analytes spiked at three levels, low (0.3X), medium (0.9X), and high (2.5X), with extracts from the pooled matrix and neat standards at the same concentrations, as described by Matuszewski et al. [46]. Absolute matrix effects values are presented in Table 1 and Figure 5. Absolute matrix effect values for the majority of compounds were within the range of 80-120%. Only florfenicol amine displayed significant signal suppression (31%, 45% and 56% at 0.3X, 0.9X and 2.5X respectively), while six other compounds displayed significant ion enhancement at the 0.3X concentration level. No significant differences were observed when the results were calculated without normalization with internal standard. This is mainly due to the use of matrix-matched calibration and minimal matrix effects offered by SPME which is capable of isolating and enriching target analytes with effective and efficient sample clean-up. However, we selected to use one internal standard in this method in order to compensate in case of variations from pin to pin, as well as variations in desorption solvent loss due to evaporation during the desorption step. The results confirm that SPME provides clean sample extracts, thus offering reliable results while minimizing analytical instrument maintenance requirements [26,27].

3.7. Validation of the method

The target analytes in this work were selected to represent more than 12 classes of veterinary drugs varying in physiochemical properties. The developed method was validated following FDA guidelines for methods validation for drugs and biologics [40]. The selectivity of the method was evaluated via an analysis of blank chicken samples (n=10). No background peaks, above a signal-to-noise ratio of 3, were present at the same elution time as the target analytes,

showing that the method is free of endogenous interferences. Figure S-5 displays an example of obtained total ion chromatograms of blank chicken and blank chicken spiked at 0.1X and 1X levels.

Method linearity was evaluated individually for each compound through the establishment of matrix-matched calibration curves, which were prepared in a range of 0.1-3X in pooled chicken, and analyzed with the proposed SPME-LC-MS/MS method in triplicate. Pearson's coefficient (R) values ranged from 0.9956 to 0.9999 for all analytes under study, indicating good correlation between both axes. Furthermore, the determination coefficient (R^2) was higher than 0.991 for all compounds, and a lack of fit (LOF) test performed at the 5% level presented no significance difference (p> 0.05), indicating that well-adjusted models were obtained for all target compounds.

Linear ranges, limits of quantitation, determination coefficients (R^2), and lack of fit test results are presented in Table 1.

The accuracy and precision of the method were calculated using six replicates per concentration (n=6) at three levels; low, mid, and high (0.3X, 0.9X and 2.5X). To evaluate the accuracy of the method, the mean relative recovery of the analyte was calculated by fortifying blank chicken samples at the three concentration levels mentioned above. The spiked samples were quantified using the matrix matched calibration curves. Accuracy of the method as presented in Table 1 and Figure 5 was within 80-120% for all analytes except for Desethylene Ciprofloxacin (73%) Sulfadimethoxine (74%) and Tetracycline (63%) at low level concentration (0.3X), Oxyclozanide (60%) at mid level concentration (0.9X), 6-phenylthiouracil (134%), Albendazole (121%), and Oxytetracycline (121%) at high concentration level (2.5X). Intra-day and inter-day precision are presented in Figure 6.

LOQs were calculated as the lowest point of the matrix matched calibration curves with RSD ≤20%. LOQs equal to 0.1X were achieved for all analytes with the exceptions of 6-Phenylthiouracil, Danofloxacin, Desethylene Ciproflaxin, Norfloxacin, and Triclabendazole with LOQ of 0.25X and Oxyclozanide 0.9X. This could be attributed to the low affinity of these analytes to the coating or due to poor ionization in electrospray ionization mass spectrometry.

Stability results were evaluated by calculating the percentage decrease in calculated concentrations of analytes after 48 h, 72 h, and one week of storage in the desorption solvent relative to the concentration results from the first day of preparation. A statistically significant decrease was only observed after 1 week of storage for Tetracyclines and Lactams. All other analytes were found to be stable within the studied conditions.

3.8. Application of final method towards analysis of chicken samples

The final developed method was used to analyze chicken samples purchased from five local grocery stores. Three different types of chicken tissues were selected: breast, liver, and thighs. Most of the samples analyzed were free from the target analytes, except for chicken samples from one supplier, where the following analytes were present at levels below established MRLs: sulfapyridine, sulfamerazine, enrofloxacin, orbifloxacin, sulfamethizole, sulfamethazine, sulfamethoxypyridazine, sulfachloropyridazine, doxycycline, sulfadoxine, sulfamethoxazole, sulfaethoxypyridazine, sulfadimethoxine. Although the amounts detected were below limits of quantification, chicken thigh tissue was observed to present consistently higher values of the detected analytes in comparison to the other tested tissues.

4. Conclusion

The developed method is the first and most comprehensive SPME approach to date for analysis of multiresidue veterinary drugs in meat in terms of the number of analytes tested as well as with respect to the range of physical and chemical properties covered. The method is fully automated, allowing for simultaneous analysis of up to 96 samples. Therefore, it offers a cost-effective alternative for analysis of veterinary drug residues in meat, additionally offering improved precision and shorter analysis times as compared to traditional sampling procedures.

Given the growing public interest and concern regarding food safety, and taking into account the importance of the meat industry to the global economy, the demand for sophisticated, automated high-throughput analytical procedures for monitoring of drug residues in meat is expected to continue growing. The method is ideal for large-scale monitoring of multiresidue drugs, and is thus proposed as a valuable tool for regulatory monitoring and enforcement of MRLs. In addition to its superior performance and wide coverage, it is environmentally friendly due to the minimum amount of organic solvents needed as compared to traditional methods. Furthermore, it can be potentially adopted for other high-throughput analyses in biological, pharmaceutical, food science, and metabolomics applications. The proposed SPME method for analysis of multiresidue veterinary drugs in meat offers many advantages in comparison to currently adopted approaches. Notably, it offers fully automated and high-throughput monitoring, thus allowing for shorter analysis times per sample as compared to traditional sampling procedures. Further, the range of compounds detectable by the method include analytes from several drug classes, and of varying physical and chemical properties. The validation results and minimal matrix effects demonstrate that the method is suitable, reliable, and offers cleaner extracts as opposed to traditional methods. It is expected that the developed method will be tested in the near future in different food matrices and for ultra-fast screening of multiclass multiresidue drugs via direct

506 interface to MS technologies Furthermore, studies to compare results of this method to other
507 established methods for incurred samples will also be considered in the future.

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Appendix A. Supplementary data: Additional information is available as noted in text.

514 **Table 1.** Validation of the developed method under optimized conditions (conditioning: 1.0 mL, 50/50 (MeOH/H_2O, v/v) 30 min; extraction: 1.5mL diluted homogenized chicken, 60 min
515 extraction time; rinsing: 1.0 mL H_2O, 10 sec with agitation; desorption: 0.4 mL 37.5/37.5/25 (MeOH/MeCN/H_2O, v/v/v), 20 min.) Accuracy (%) and intra-day precision (%RSD)
516 calculated for n=6 replicates, and inter-day precision (%RSD) calculated for n=3 days.

Compound	MRL (X) ng g^{-1}	Linearity				Accuracy (%)			Intra-day precision (%)			Inter-day precision (%)			Absolute matrix effect (%)		
		Range	LOQ	R^2	LOF	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X
2-Amino flubendazole	10	0.1 – 3X	0.1X	0.9968	0.1668	105	91	92	10	8	7	9	6	9	107	105	100
5-HydroxyThiabendazole	100	0.1 – 3X	0.1X	0.9996	0.6585	90	98	107	3	3	4	5	6	8	102	100	102
6-Phenylthiouracil	400	0.25 – 3X	0.25X	0.9991	0.7475	102	102	134	15	15	6	12	15	9	96	100	102
Acepromazine	10	0.1 – 3X	0.1X	0.9993	0.9666	97	91	93	12	6	6	8	6	10	111	113	107
Albendazole	50	0.1 – 1X	0.1X	0.9963	0.3941	91	94	121	4	6	3	11	16	17	86	93	96
Albendazole 2 aminosulfone	50	0.1 – 3X	0.1X	0.9986	0.313	98	98	110	9	2	2	9	6	10	95	98	100
Albendazole Sulfone	50	0.1 – 3X	0.1X	0.9996	0.9327	96	97	101	4	2	4	5	4	7	100	101	101
Albendazole Sulfoxide	50	0.1 – 3X	0.1X	0.9997	0.9808	99	99	106	5	3	3	8	5	7	97	97	101
Azaperone	10	0.1 – 3X	0.1X	0.9995	0.9611	90	88	96	6	1	5	6	5	8	105	92	99
Betamethasone	100	0.1 – 3X	0.1X	0.9987	0.2617	103	103	98	3	3	4	6	4	10	111	110	109
Cambendazole	10	0.1 – 3X	0.1X	0.9996	0.5943	97	98	102	3	2	3	6	6	4	111	104	101
Carbadox	30	0.1 – 3X	0.1X	0.9994	0.888	90	93	105	3	5	6	6	8	11	93	104	102
Chlorpromazine	10	0.1 – 3X	0.1X	0.9984	0.2482	94	95	114	11	7	2	10	11	8	110	107	104
Chlortetracycline	200	0.1 – 3X	0.1X	0.9989	0.7779	104	94	94	10	9	6	11	7	10	106	108	111
Clenbuterol	10	0.1 – 3X	0.1X	0.9981	0.17	95	101	101	4	8	8	6	9	8	106	103	106
Clindamycin	100	0.1 – 3X	0.1X	0.9993	0.071	102	101	106	6	4	9	7	6	10	103	103	102
Cloxacillin	10	0.1 – 3X	0.1X	0.9995	0.9664	100	96	102	6	4	4	9	7	10	119	98	100
Danofloxacin	70	0.25 – 3X	0.25X	0.9971	0.0611	85	96	115	10	7	6	13	10	7	131	116	109
Desethylene Ciproflaxin	100	0.25 – 3X	0.25X	0.9973	0.6129	73	98	114	26	8	3	31	13	10	130	130	106
Diclofenac	200	0.1 – 2X	0.1X	0.9985	0.0504	110	92	100	3	4	2	6	7	5	99	100	99
Dicloxacillin	100	0.1 – 3X	0.1X	0.9998	0.9945	102	99	109	9	6	6	7	6	8	131	100	100
Compound	**MRL**	**Linearity**				**Accuracy (%)**			**Intra-day**			**Inter-day**			**Absolute matrix**		

	(X) ng g^{-1}							precision (%)			precision (%)			effect (%)		
		Range	LOQ	R2	LOF	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X
Difloxacin	50	0.1 – 3X	0.1X	0.9994	0.8808	103	97	100	8	5	6	7	6	7	104	106	108
Dimetridazole	10	0.1 – 3X	0.1X	0.9982	0.4102	96	97	104	4	6	9	8	7	11	98	97	98
Doxycycline	100	0.1 – 3X	0.1X	0.9993	0.7341	97	96	100	6	9	4	9	8	8	116	107	106
Emamectin	10	0.1 – 3X	0.1X	0.9922	0.3439	110	105	107	4	5	6	13	12	9	101	99	102
Enrofloxacin	20	0.1 – 3X	0.1X	0.9996	0.9574	97	99	114	7	6	4	8	6	9	114	106	105
Erythromycin	125	0.1 – 3X	0.1X	0.9987	0.7575	90	94	98	11	8	7	9	6	10	109	113	109
Fenbendazole	100	0.1 – 3X	0.1X	0.9934	0.0541	100	94	99	8	8	7	11	12	12	82	95	97
Fenbendazole Sulfone	400	0.1 – 3X	0.1X	0.9991	0.8673	102	96	106	8	3	3	14	6	5	77	93	95
Florfenicol amine	100	0.1 – 3X	0.1X	0.9966	0.5284	89	101	100	6	7	9	9	9	10	31	45	56
Flubendazole	10	0.1 – 3X	0.1X	0.9993	0.4049	101	94	104	6	2	2	7	5	4	98	99	98
Flunixin	10	0.1 – 2X	0.1X	0.9993	0.1371	102	94	103	2	1	2	8	8	8	97	97	99
Haloperidol	10	0.1 – 3X	0.1X	0.9974	0.1244	106	92	106	6	8	8	9	6	9	105	105	104
Hydroxy dimetridazole	50	0.1 – 3X	0.1X	0.9991	0.6471	100	95	104	6	3	9	10	5	11	97	101	101
Hydroxy Ipronidazole	10	0.1 – 3X	0.1X	0.9995	0.6841	95	97	92	6	8	5	7	8	8	102	103	101
Ipronidazole	10	0.1 – 3X	0.1X	0.9958	0.8951	102	97	105	3	7	11	9	7	10	99	98	101
Ketoprophen	10	0.1 – 3X	0.1X	0.9996	0.7832	100	98	107	4	1	3	8	7	8	98	99	99
Levamisole	100	0.1 – 3X	0.1X	0.9985	0.1678	92	96	106	3	3	5	4	6	9	99	102	101
Lincomycin	100	0.1 – 2X	0.1X	0.9989	0.885	93	95	112	9	6	3	9	8	4	103	102	102
Mebendazole	10	0.1 – 3X	0.1X	0.9988	0.1994	102	95	107	5	3	3	7	4	5	79	82	85
Mebendazole amine	10	0.1 – 3X	0.1X	0.9996	0.7758	92	91	104	5	4	6	8	8	7	107	107	110
Melengestrol Acetate	25	0.1 – 3X	0.1X	0.999	0.4051	108	90	98	3	2	5	11	9	9	94	93	92
Morantel	150	0.1 – 3X	0.1X	0.9988	0.3643	94	94	98	4	7	6	5	6	8	103	105	111
Norfloxacin	50	0.25 – 3X	0.25X	0.9972	0.1145	89	96	114	13	6	5	25	9	8	119	107	100
Orbifloxacin	50	0.1 – 3X	0.1X	0.9997	0.9792	106	101	109	7	9	3	8	8	8	111	105	103

Compound	MRL (X)	Linearity	Accuracy (%)	Intra-day precision (%)	Inter-day precision (%)	Absolute matrix effect (%)

	ng g^{-1}	Range	LOQ	R2	LOF	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X
Oxacillin	100	0.1 – 3X	0.1X	0.9969	0.1026	98	100	103	6	4	2	7	5	8	130	101	101
Oxfendazole	800	0.1 – 3X	0.1X	0.9996	0.958	100	98	108	5	2	3	6	4	5	98	99	96
Oxyclozanide	10	0.9 – 3X	0.9X	0.9912	0.267	ND	60	86	ND	15	13	ND	51	30	98	95	96
Oxyphenylbutazone	100	0.1 – 3X	0.1X	0.9981	0.6933	102	101	109	6	7	2	9	8	5	96	97	98
Oxytetracycline	200	0.1 – 3X	0.1X	0.994	0.349	88	104	121	9	12	7	14	12	12	120	118	114
Phenylbutazone	100	0.1 – 3X	0.1X	0.9987	0.5731	92	101	114	4	10	3	8	8	5	97	99	97
Pirlimycin	300	0.1 – 3X	0.1X	0.9992	0.4285	100	97	107	5	2	6	8	5	11	100	101	98
Prednisone	100	0.1 – 3X	0.1X	0.998	0.9244	92	102	101	3	4	3	8	7	8	95	92	97
Promethazine	10	0.1 – 3X	0.1X	0.9988	0.1956	98	97	110	10	9	6	9	8	8	112	112	108
Propionylpromazine	10	0.1 – 3X	0.1X	0.9998	0.9644	94	94	115	7	9	5	6	9	8	107	106	100
Ractopamine	30	0.1 – 3X	0.1X	0.9984	0.0569	94	97	107	4	4	5	6	7	8	98	101	104
Sarafloxacin	50	0.1 – 3X	0.1X	0.9987	0.0651	90	107	100	6	6	7	20	8	13	114	111	104
Sulfachloropyridazine	100	0.1 – 3X	0.1X	0.9986	0.2934	96	99	103	6	3	2	6	5	8	101	100	101
Sulfadimethoxine	100	0.1 – 3X	0.1X	0.9927	0.583	126	96	113	24	15	12	34	16	17	126	114	106
Sulfadoxine	100	0.1 – 3X	0.1X	0.9988	0.3358	99	100	107	3	2	4	5	5	6	104	102	101
Sulfaethoxypyridazine	100	0.1 – 3X	0.1X	0.9998	0.7936	92	96	98	3	4	3	4	4	6	101	101	102
Sulfamerazine	100	0.1 – 3X	0.1X	0.9991	0.5314	98	97	108	7	3	4	7	5	9	99	103	103
Sulfamethazine	100	0.1 – 3X	0.1X	0.9986	0.2373	108	99	106	4	6	2	6	4	6	106	102	102
Sulfamethizole	100	0.1 – 3X	0.1X	0.9977	0.1043	96	102	111	7	4	2	7	7	8	99	103	102
Sulfamethoxazole	100	0.1 – 3X	0.1X	0.9987	0.7362	91	99	104	6	3	2	6	7	8	101	102	100
Sulfamethoxypyridazine	100	0.1 – 3X	0.1X	0.9988	0.6857	103	99	100	5	3	1	6	5	5	107	107	104
Sulfapyridine	100	0.1 – 3X	0.1X	0.9993	0.5457	94	99	108	4	4	3	5	6	7	101	100	101
Sulfaquinoxaline	100	0.1 – 3X	0.1X	0.9998	0.9357	96	101	109	4	2	3	5	4	4	98	100	100
Sulfathiazole	100	0.1 – 3X	0.1X	0.9992	0.0588	99	96	105	4	3	8	8	6	10	102	99	103

Compound	MRL	Linearity				Accuracy (%)			Intra-day precision (%)			Inter-day precision (%)			Absolute matrix effect (%)		
	ng g^{-1}	Range	LOQ	R2	LOF	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X	0.3X	0.9X	2.5X

Tetracycline	200	0.1 – 3X	0.1X	0.9952	0.6236	63	92	107	20	7	8	21	7	7	123	119	118
Tolfenamic Acid	200	0.1 – 2X	0.1X	0.9993	0.2278	103	90	98	3	2	2	5	7	6	99	99	100
Triclabendazole	50	0.25 – 2X	0.25X	0.9972	0.0772	106	84	97	4	4	6	8	12	10	96	96	97
Triclabendazole Sulfoxide	50	0.1 – 2X	0.1X	0.9952	0.0621	103	88	103	9	6	4	18	12	9	97	98	99
Trifluropromazine	10	0.1 – 3X	0.1X	0.9986	0.5501	101	93	110	8	7	7	10	9	8	100	103	100
Tylosin	200	0.1 – 3X	0.1X	0.9962	0.0558	105	94	105	9	5	7	11	7	8	111	107	103
Virginiamycin	100	0.1 – 3X	0.1X	0.9953	0.118	103	98	100	3	2	3	7	5	9	100	103	102
Xylazine	10	0.1 – 3X	0.1X	0.999	0.1677	91	90	93	4	7	7	4	8	9	104	103	112

517

518 **List of Figures**

519 **Figure 1**. Concept 96-SPME device and SPME brush with 96 pins. Optimized conditions
520 (time, agitation, solvent, and volume) for conditioning, extraction, rinsing, and desorption
521 steps are shown, respectively, under each agitator-station.

522 **Figure 2**. Evaluation of 4 SPME coatings (HLB, mix-mode, PS-DVB and 50:50 HLB:PS-
523 DVB) in different desorption solutions (Des 1: MeCN/H_2O 50/50, Des 2:
524 MeCN/MeOH/H_2O/FA 40/40/20/0.1 and Des 3: MeCN/IPA/H_2O/FA 40/40/20/0.1).
525 Extraction volume:1000 µL, extraction time:120 min, concentration of analytes: 50 ng mL^{-1}
526 , extraction matrix: 1X PBS. Desorption time: 120 min, desorption volume: 1000 µL.

527 **Figure 3**. Optimum contour plot for special cubic model to fit experimental data for all
528 compounds under study. (A: Desorption, B: Carryover).

529 **Figure 4**. Matrix effects of the final method for target analytes in three chicken tissues
530 (breast, liver, and thigh) plotted vs. retention time.

531 **Figure 5.** Absolute matrix effects of the final method for target analytes.

532 **Figure 6.** Accuracy of the final method for target analytes.

533 **Figure 7.** Intraday (A) and interday (B) precision of the final method for target analytes.

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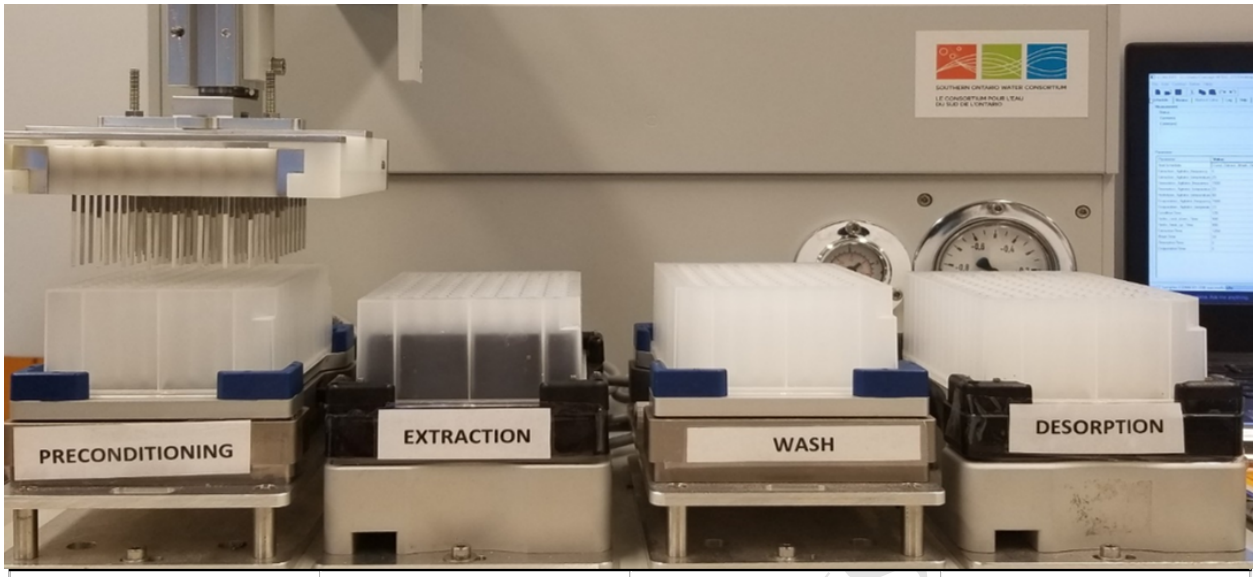

Conditioning	Extraction	Rinsing	Desorption
30 min	60 min	10 s	20 min
900 rpm	static	900 rpm	1200 rpm
MeOH/H_2O (50/50 v/v)	Chicken sample	H2O	MeCN/MeOH/H_2O (37.5/37.5/25 v/v/v)
1000 µL	1000 µL	1000 µL	400 µL