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Comparison of veterinary drug residue results in animal tissues by ultrahigh-performance liquid chromatography coupled to triple quadrupole or quadrupole–time-of-flight tandem mass spectrometry after different sample preparation methods, including use of a commercial lipid removal product

Tarun Anumol[1] · Steven J. Lehotay[2] · Joan Stevens[1] · Jerry Zweigenbaum[1]

Abstract Veterinary drug residues in animal-derived foods must be monitored to ensure food safety, verify proper veterinary practices, enforce legal limits in domestic and imported foods, and for other purposes. A common goal in drug residue analysis in foods is to achieve acceptable monitoring results for as many analytes as possible, with higher priority given to the drugs of most concern, in an efficient and robust manner. The U.S. Department of Agriculture has implemented a multiclass, multi-residue method based on sample preparation using dispersive solid phase extraction (d-SPE) for cleanup and ultrahigh-performance liquid chromatography–tandem quadrupole mass spectrometry (UHPLC-QQQ) for analysis of >120 drugs at regulatory levels of concern in animal tissues. Recently, a new cleanup product called "enhanced matrix removal for lipids" (EMR-L) was commercially introduced that used a unique chemical mechanism to remove lipids from extracts. Furthermore, high-resolution quadrupole–time-of-flight (Q/TOF) for (U)HPLC detection often yields higher selectivity than targeted QQQ analyzers while allowing retroactive processing of samples for other contaminants. In this study, the use of both d-SPE and EMR-L sample preparation and UHPLC-QQQ and UHPLC-Q/TOF analysis methods for shared spiked samples of bovine muscle, kidney, and liver was compared. The results showed that the EMR-L method provided cleaner extracts overall and improved results for several anthelmintics and tranquilizers compared to the d-SPE method, but the EMR-L method gave lower recoveries for certain β-lactam antibiotics. QQQ vs. Q/TOF detection showed similar mixed performance advantages depending on analytes and matrix interferences, with an advantage to Q/TOF for greater possible analytical scope and non-targeted data collection. Either combination of approaches may be used to meet monitoring purposes, with an edge in efficiency to d-SPE, but greater instrument robustness and less matrix effects when analyzing EMR-L extracts.

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Electronic supplementary material The online version of this article (doi:10.1007/s00216-017-0208-y) contains supplementary material, which is available to authorized users.

✉ Steven J. Lehotay
steven.lehotay@ars.usda.gov

[1] Agilent Technologies Inc., 2850 Centerville Road, Wilmington, DE 19808, USA

[2] U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA

Introduction

Veterinary drugs are commonly used in animal production to treat disease and promote growth, which helps provide quality food products at a profit in the marketplace. However, improper administration of drugs in animal production can lead to excessive residues being present in meat or other regulatory marker tissues, which indicates illegal veterinary practices and possible human

health risks. Another public health concern involving veterinary use of antibiotics relates to antimicrobial resistance by pathogenic microorganisms [1, 2]. Thus, monitoring for veterinary drugs in foods is an important function of regulatory agencies worldwide, which can include verification of proper organic food labeling and acceptability of food commodities in international trade [3–5]. The regulations are described by the U.S. Food and Drug Administration in CFR Title 21, the EU Council Directive 96/22/EC, the Chinese Ministry of Agriculture Announcement 235, and similar laws in other countries, as well as *Codex Alimentarius* internationally [6, 7].

Analytical method development for veterinary drugs in animal tissues is highly challenging because the matrices tend to be complex with high amounts of fat and protein, and the residues of interest consist of highly diverse analytes from several different classes with widely varying chemical properties. Current methods for veterinary drugs in animal-derived foods rarely exceed 100 analytes, many of which cannot be acceptably quantified and only meet screening criteria even at >100 ng/g levels [8, 9]. In comparison, regulatory needs can be met more easily using similar analytical tools for simultaneous analysis of hundreds of pesticides and environmental contaminants in fruits, vegetables, grains, fish, and other common foods at <10 ng/g [10–13].

Hence, sample preparation needs to include satisfactory extraction of disparate compounds while also being able to provide adequate cleanup to avoid direct and indirect matrix interferences/effects. Traditionally, sample preparation techniques for veterinary drugs in food have included solid phase extraction (SPE) and liquid–liquid partitioning steps that can be time-consuming, inefficient, and may limit the scope of acceptable recoveries for some drug classes [14–16]. Currently, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach is commonly used for sample preparation of veterinary drugs among other applications to efficiently and effectively meet analytical needs [17–19]. Dispersive-SPE (d-SPE) is commonly employed in QuEChERS, which is more convenient compared to cartridge-based SPE, but despite the broader scope afforded by d-SPE to recover more analytes, it yields less effective cleanup.

A new chemical mechanism for selective removal of lipids and proteins particularly from animal-derived food extracts would be highly beneficial. In August of 2015, Agilent Technologies introduced a new product called "enhanced matrix removal for lipids" (EMR-L) that rather selectively and effectively traps the long hydrocarbon chain of lipids in solution [20–22]. EMR-L integrates well for cleanup of QuEChERS or protein precipitation extracts because lipids are extracted, and the activated EMR-L material scavenges the lipids readily in aqueous acetonitrile (MeCN) extracts. The product also works in non-aqueous solutions, but water activation helps to increase its effectiveness to remove lipids. The functional mechanism by which the EMR-L material operates includes both size exclusion and hydrophobic interactions. Unbranched hydrocarbon chains (lipids) enter the

sorbent, whereas bulky analytes containing aromatic rings and functional groups are not trapped by the material. The lipid chains that enter the sorbent are trapped by hydrophobic interaction and hydrogen bonding. Non-lipids with unbranched aliphatic groups that enter the sorbent may also be trapped, but few chemical residues in foods possess long hydrocarbon chains. For cleanup of aqueous MeCN QuEChERS or protein precipitation extracts, the EMR-L complexes with the lipids extracted into the MeCN. The EMR-L–lipid complex is removed through centrifugation from the aqueous MeCN. A subsequent salting-out step separates the MeCN extract from water. Agilent provided an EMR-L method protocol using this two-step cleanup approach for veterinary drug residues at the time of the product release [23].

For analysis, (ultra)high-performance liquid chromatography [(U)HPLC] with detection by mass spectrometry (MS) techniques offer requisite sensitivity and selectivity while saving time, labor, and costs compared to the use of multiple alternate methods that similarly would cover the same number of analytes [4, 24]. Electrospray ionization (ESI) is nearly always used after LC of veterinary drugs prior to MS-based detection, and triple quadrupole (QQQ) MS/MS is the most common analyzer currently applied in this and other chemical residue applications. Independent of sample preparation limitations, (U)HPLC-QQQ can simultaneously quantify and identify potentially hundreds of targeted drug analytes at regulatory monitoring levels of concern in foods [25–28]. However, the use of accurate mass, high-resolution (HR) MS as with Q/time-of-flight (Q/TOF) or Q/orbital ion trap instruments permits full-spectral MS/HRMS data acquisition for all ions, which allows retrospective data mining of the chromatogram to look for additional compounds of possible interest, such as metabolites. Recent studies have demonstrated the applicability of HRMS for analysis of veterinary drugs in animal food products at levels at or below those required by regulatory agencies [9, 29–32].

The aims of this study were to compare two methods of sample preparation (d-SPE and EMR-L) and analysis (UHPLC-QQQ and UHPLC-Q/TOF) for more than 120 veterinary drugs spiked at the US regulatory levels of interest in bovine kidney, liver, and muscle tissue samples. The comparison entailed assessment of co-extractives and matrix effects, analyte recoveries, precision, extent of chemical interferences, and other analytical performance parameters. This study was also intended to evaluate the feasibility of the EMR-L sample preparation and UHPLC-Q/TOF analysis approaches for regulatory monitoring of veterinary drug residues in animal-derived foods.

Materials and methods

Chemicals and reagents

Veterinary drug standards were obtained from commercial vendors, while stock solutions and mixed standards were

prepared as described previously [17]. All solutions were stored at −20 °C, and working spike solutions were prepared fresh within a week of each experiment. HPLC grade acetonitrile (MeCN) was purchased from Fisher Scientific (Pittsburgh, PA, USA) by the USDA lab (#1) and LC/MS grade Burdick & Jackson (Muskegon, MI, USA) by the Agilent lab (#2). Deionized water of 18.2 Ω-cm was prepared with an E-Pure model D4641 from Barnstead/Thermolyne (Dubuque, IA, USA) in lab #1 and by EMD Millipore (Darmstadt, Germany) Milli-Q Integral 3 system in lab #2. Methanol (MeOH) was purchased from Burdick & Jackson, and LC-MS grade formic acid (98%) and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA) in both labs. End-capped C18 sorbent and anhydrous (anh.) $MgSO_4$ were from UCT (Bristol, PA, USA). The EMR-L product from Agilent Technologies (Santa Clara, CA, USA) consisted of two 15-mL polypropylene centrifuge tubes, one of which contained 1 g EMR-L material and the other, for salt-out partitioning (called the "polishing" step), contained 1.6 g anh. $MgSO_4$ and 0.4 g NaCl (2 g of 4:1, w/w).

Bovine kidney, liver, and muscle samples were purchased from a local grocery store to serve as method validation samples. Each ≈500 g tissue sample was homogenized separately using a Robot Coupe (Ridgeland, MS, USA) RSI 2Y1 chopper with dry ice added to produce a fine powder. All comminuted samples were placed in loosely capped containers at −20 °C overnight to allow sublimation of the remaining dry ice before they were sealed.

Sample preparation

Two different sample preparation protocols were used to extract veterinary drugs from the bovine tissue in this study, which are outlined in Fig. S1 (see Electronic supplementary material, ESM). In a comparison of the two methods, 2 g (n = 6 each) of the bovine kidney, liver, and muscle samples was weighed into 50-mL polypropylene (PP) centrifuge tubes and fortified at the 1X level (Table 1 lists the concentrations of X for the 127 drug analytes tested in the study). Reagent blanks (1.54 mL water) and duplicate matrix blanks (2 g) for each bovine tissue type were also prepared at the same time for each sample preparation method. Makeup solvent volumes to match the spiking volumes were added to the blanks to ensure equivalent extraction conditions for blanks and spikes alike. At least 10 min was given after spikes and solvent additions before initiating side-by-side extractions by two teams using the different methods in the same lab at the same time.

For reagent-only (RO) and matrix-matched (MM) calibration, separate solvent solutions and matrix blank extracts, respectively, were prepared at 0X, 0.5X, 1X, and 2X levels in accordance with the appropriate equivalent sample amounts for each sample preparation method. Each sample and calibration standard was also fortified with an equivalent of 250 ng/g

flunixin-d_3, which was used as an internal standard (IS), and 250 ng/g $^{13}C_6$-sulfamethazine was included as an alternative IS, if needed. The IS solution was not added to the matrix blanks prior to extraction as in the other cases, but it was included in the 0X, 0.5X, 1X, and 2X calibration standard preparation solutions added to the final extracts for each matrix.

QuEChERS/d-SPE method

In the d-SPE method, the two 2-g matrix blanks and three spiked samples for each matrix, plus a reagent blank (16 tubes total), were shaken for 5 min at room temperature with 10 mL of 4:1 (v/v) MeCN/water using a GlasCol (Terre Haute, IN, USA) pulsating vortex platform shaker at 80% setting and maximum pulsation (up to fifty 50-mL tubes can be shaken at a time). The sample tubes were then centrifuged at 3700 rcf for 2 min at room temperature. The d-SPE cleanup was then conducted by decanting the full extract into another 50-mL PP centrifuge tube containing 500 mg end-capped C18 sorbent. The 16 tubes in a tray were shaken on the platform vortexer for 30 s and centrifuged as before for 2 min. Taking into account the 1.5 mL water in the 2-g tissue samples (yielding 11.5 mL extraction volumes), the final extracts had a sample equivalence of 0.174 g/mL in ≈7:3 MeCN/water.

Normally, a portion of this extract would be transferred directly into a PP autosampler vial, and 1 μL would be injected in (U)HPLC-QQQ for analysis, but to better match the EMR-L (U)HPLC-Q/TOF method, 115 μL of the extracts was transferred into 0.8-mL PP vials containing 385 μL water. Furthermore, all final extracts and calibration standards were prepared in duplicate autosampler vials for analysis by LC-QQQ and (U)HPLC-Q/TOF separately in each lab. For MM calibration, the duplicate blank extracts for each matrix were combined, and six extra 115-μL aliquots were transferred to additional vials for preparation of the duplicate standards. In the case of MM standards, 80 μL of the appropriate 0X, 0.5X, 1X, or 2X calibration preparation solution in 1:1 (v/v) MeCN/water was added, and to equalize volumes and MeCN/water ratios, 80 μL of 1:1 MeCN/water was also added to all other final extracts. Thus, all diluted final extracts were 0.0345 mg equivalent sample per microliter in 21:79 MeCN/water solution. For shared RO calibration standards, eight 115-μL portions of 7:3 MeCN/water were transferred to eight PP vials containing 385 μL water, and duplicate 0X, 0.5X, 1X, and 2X standards were prepared in the same way as described above for MM calibration standards.

QuEChERS/EMR-L method

In the EMR-L method, the 2-g spiked samples in triplicate for each matrix, plus two matrix blanks each and a reagent blank, were shaken for 2 min with 10 mL of MeCN containing 5% formic acid (v/v) using the same mechanical shaker and

settings as in the d-SPE method. The sample tubes were centrifuged at 3700 rcf for 5 min. Then, 5 mL of 5 mM aqueous ammonium acetate solution (measured pH of 5.1) was added to the EMR-L tube which contains 1 g of the proprietary material for cleanup and vortexed immediately for 30 s, following which 5 mL of the MeCN extract (0.87 g sample equivalent) was transferred to the buffer-activated EMR-L material in the tube. The resulting extract was vortexed immediately for 30 s and centrifuged for 5 min.

In the salting-out step, 5 mL extract (0.435 g sample equivalent in buffered ~44:56 MeCN/water) was transferred into the second 15-mL centrifuge tube containing 2 g 4:1 (w/w) anh. MgSO$_4$/NaCl, followed by vortexing and centrifuging as in the previous step. The volume of the upper phase (MeCN extract) was 2.2 mL, which yielded 0.2 g/mL equivalent sample. Lastly, 100 μL of each extract, in duplicate as in the case of the d-SPE method, was pipetted into 0.8-mL PP vials containing 400 μL water. Everything subsequently was done the same as described above for the d-SPE method, and the final diluted extracts were 0.0345 g/mL sample equivalents in 21:79 MeCN/water (note that this was not as precise in either method as implied by the calculations). The duplicate vials from both sample preparation methods for both analytical methods were capped, vortexed <1 s each, and stored at 4 °C (lab #1) and in ice (lab #2) until analysis on the next day in both labs.

In a follow-up experiment conducted only in lab #1 using UHPLC-QQQ analysis, the EMR-L method was repeated as above, except MeCN without formic acid was used. Also, the aqueous MeCN extract in the EMR-L step was split into two 4-mL portions, one of which was partitioned using 0.64 g anh. MgSO$_4$ + 0.16 g NaCl as in the original protocol and the second 4-mL portion used 0.8 g anh. MgSO$_4$ only. This was done in an attempt to improve recoveries of drug analytes that may degrade under acidic conditions and/or that did not fully partition into the MeCN phase (final extract) from the water phase during the salting-out step.

UHPLC-QQQ analysis

A Shimadzu (Columbia, MD, USA) Nexera X2 UHPLC system coupled to a Sciex (Foster City, CA, USA) 6500 QTrap mass spectrometer was used by lab #1. A Waters (Milford, MA, USA) Acquity UPLC HSS T3 5 × 2.1-mm i.d., 1.8-μm, guard column + 100 × 2.1-mm i.d., 1.8-μm, analytical column was used for UHPLC. Column temperature was 40 °C and the flow rate was 0.5 mL/min. The mobile phase consisted of (A) water and (B) MeCN, each containing 0.1% (v/v) formic acid, and the gradient was 5% B for 0.5 min ramped linearly to 100% B until 8.0 min, and then held at 100% B until 11 min. A divert valve directed column effluent to waste before 0.4 min and after 11 min. The column was returned to 5% B over 0.5 min, where it was allowed to re-equilibrate for 3.5 min. Vials were kept at

10 °C in the autosampler tray, and the UHPLC-QQQ injection volume was 4 μL (138 μg tissue sample equivalents) in both sample preparation methods.

Similarly but not entirely the same as previously [17], the QQQ instrument was operated in ESI with positive and negative switching using scheduled multiple reaction monitoring with a 90-s retention time (t_R) window, 0.5-s target scan times with 3-ms pause, and 20-ms settling times. Sciex Analyst 1.6.2 software was used for instrument control and MultiQuant 3.0 for data processing. Curtain gas was set to 25, collision gas was medium, ion spray voltage was 5000 V or −4500 V in ESI+/− switching, source temperature was 400 °C, and source gases 1 and 2 were set at 40 and 50, respectively. Other parameters for the ion transitions (typically three) monitored for each analyte are provided in Table S1 (see ESM).

UHPLC-Q/TOF analysis

Details on the analytical approach used by lab #2 are presented elsewhere [33]. Lab #2 used an Agilent 1290 Infinity UHPLC coupled to an Agilent 6550 high-resolution accurate mass Q/TOF instrument. Separation was performed using an Agilent Zorbax Eclipse Plus C18 guard column (30 × 2.1-mm i.d., 1.8 μm) + matched analytical column (150 × 2.1-mm i.d., 1.8 μm). Mobile phase solvents A and B were the same as in UHPLC-QQQ, and the gradient was 2% B for 1 min, during which the flow was sent to the waste using a diverter valve, then ramped to 100% B until 10 min, followed by a 1-min hold at 100% B before returning the column to the initial 2% B at 11.1 min. Re-equilibration time was 3.0 min, for a total analysis time of 14.1 min. The flow rate was 0.5 mL/min, while the column was maintained at 30 °C and the autosampler tray was at 4 °C. Injection volume was 3 μL (104 μg tissue equivalent).

The UHPLC-Q/TOF system equipped with an Agilent Jet Stream dual-electrospray source was operated in ESI+ mode. The Q/TOF was tuned for the 50–1700 m/z range in the 2-GHz extended dynamic range (resolution ~30,000 at m/z 922). During analysis, 5 mM purine (m/z 121.0509) and 3 mM HP-921 (m/z 922.0098) in 95:5 (v/v) water/MeCN were continually delivered as reference ions for accurate mass correction via the second nebulizer into the analyzer using an isocratic pump at 0.4 mL/min with a 1:100 split. The UHPLC-Q/TOF data were collected in the "All Ions" MS/MS mode, allowing both MS and MS/MS data to be collected in a single analytical run. In this method, collision energies (CE) of 0, 10, and 40 eV were used to give satisfactory molecular ion and fragment information for all analytes. The 0-eV CE mainly led to the detection of precursor ions, and the product ions were observed at the higher CE settings. Data collection rate was 4.5 spectra/s overall. Data processing was performed using the Agilent MassHunter (ver. B 06.01)

software, while a personal compound database library containing the exact mass, structure, MS/MS spectra at multiple collision energies, and t_R for each drug analyte built from standards was used for identification purposes. The criteria for a positive identification of a veterinary drug with the UHPLC-Q/TOF was the precursor mass within ±10 ppm mass accuracy, the presence of at least two product ions (from the database), and a t_R within ±0.2 min. In UHPLC-Q/TOF, 117 veterinary drugs were monitored in this method, while the remaining 10 were not analyzed due to unavailability of MS/MS spectra and/or t_R in the database. The Q/TOF conditions used and the compound details are presented in Tables S2 and S3 (see ESM).

Quantification and matrix effects

For UHPLC-Q/TOF, recoveries were calculated for each of the three matrices individually by a comparison of the peak areas (all normalized to the flunixin-d$_3$ IS) of fortified samples with the MM calibration standards for each matrix and sample preparation method. In UHPLC-QQQ, recoveries were determined from a single linear least square calibration line generated from all 24 MM standards in all matrices from both sample preparation methods.

Matrix effects (MEs) were calculated in both sample preparation and analytical methods for each compound and matrix as the percent difference between RO and MM calibration standards [ME = (MM slope − RO slope)/RO slope].

Determination of co-extractives and cleanup efficiencies

The co-extracted amounts of each matrix in undiluted final extracts from both sample preparation techniques were measured by gravimetric analysis. Briefly, 1.2 g sample equivalents (6 mL for EMR-L and 6.9 mL for d-SPE) of the individually combined final extracts from the reagent, kidney, liver, and muscle blanks were transferred in duplicate to oven-dried, pre-weighed glass test tubes and evaporated to dryness in a 60 °C water bath under nitrogen gas flow. The tubes were then placed for 30 min in an oven at 120 °C, and the percent co-extractives was the difference in the final and initial weights of the tubes vs. the 1.2-g sample equivalent.

Results and discussion

Comparison of co-extractives

Figure 1 is a picture and graph showing the extent of co-extractives for the three bovine tissues and the reagent (solvent) blank for both sample preparation techniques. The EMR-L method clearly gave better cleanup of matrix co-extractives compared to the d-SPE method. As also shown previously [34], the d-SPE
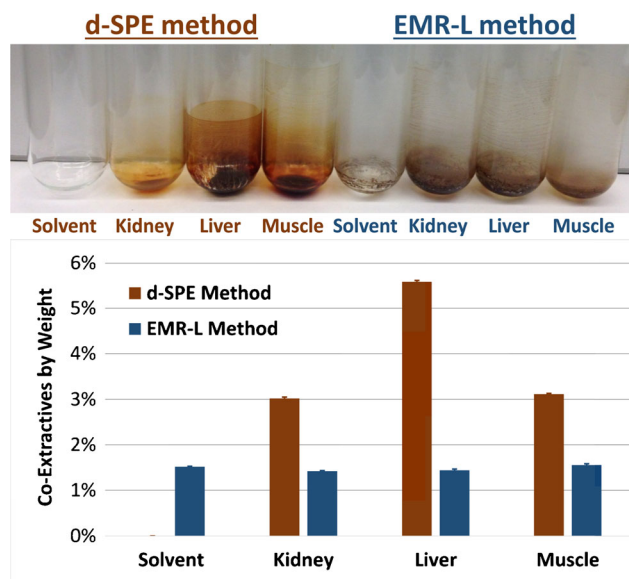
Fig. 1 Amount of co-extractives (in percent) in each bovine matrix for both sample preparation techniques

method only provided minimal cleanup of the initial extracts. In the EMR-L method, only ≈1.5% equivalent material by weight remained for all three tissue matrices, which also appeared in the reagent blank. This generally mirrors previous findings using the EMR-L approach for cleanup of QuEChERS extracts for avocado, pork, salmon, and kale [21, 35, 36]. The d-SPE extracts had 2.9–5.5% co-extractive amounts depending on the bovine matrix, which was up to threefold more than the EMR-L extracts in this study.

As it turns out, the EMR-L "co-extracted" material consisted almost entirely of NaCl from the salting-out step in the method. Even though the picture in Fig. 1 shows darker color of the dried EMR-L extracts for the matrices than the reagent blank, no measurable matrix co-extractives by weight differences occurred in the EMR-L final extracts. On average, 2.97 mg/mL salt was dissolved in the EMR-L extracts after the cleanup step, which is 4.4-fold higher than the 0.68 mg/mL NaCl dissolved in the final extracts after EMR-L cleanup in QuEChERS for pesticides [21]. The EMR-L extracts in this study were further diluted to contain 0.51 mg/mL NaCl in the injected extracts. Fortunately, the NaCl passes unretained through the chromatography columns at initial aqueous mobile phase conditions and is diverted to waste.

Matrix effects

Another way to compare the degree of cleanup in each sample preparation method is to assess MEs in the LC-MS system. ESI is notoriously susceptible to ionization suppression of analytes in the presence of charge-competing matrix components [37]. Matrix-induced enhancement effects are also known to occur in ESI, which can also introduce a large bias

in quantification. Isotopically labeled IS for each analyte would be very useful to compensate for MEs, but this is not practical for so many analytes due to aspects of availability, cost, and convenience. Thus, MM calibration is commonly used to reduce (but not eliminate) MEs in practice [38].

Two different approaches were conducted to compare MEs in the UHPLC-QQQ for each bovine matrix and sample preparation method. In the first, a concentrated solution of several drug analytes was continuously infused into the source post-column at 10 μL/min flow rate as blank final extracts were injected as usual in the UHPLC method. The MRM ion transitions of those drug analytes were monitored throughout the chromatogram to determine the extent of MEs with respect to t_R. The results of this experiment are shown in Fig. 2a for flunixin, which gives the same general chromatogram as the other analytes tested. The signal intensities of flunixin for each injected sample matrix shown in the chromatograms demonstrate the much greater ionization efficiency, but also more noise, when the mobile phase mainly consists of MeCN, due to greater desolvation in the source. The MEs (with respect to flunixin) shown in Fig. 2b demonstrate extensive suppression for all matrices and methods from 0.5 to 1 min when many polar components elute, then an enhancement spike occurs at 1.25 min particularly for the EMR-L extracts (which probably relates to small differences in injected water/MeCN ratios and pH/ionic strengths among the extracts), and then a leveling to ≈0% MEs for the remaining time. The peaks observed at
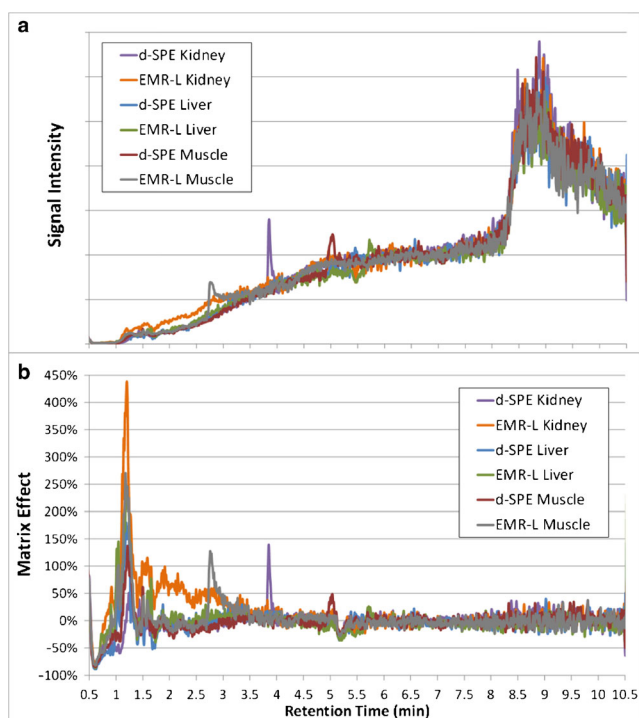
≈3 min for the EMR-L of liver and ≈4 and ≈5 min for the d-SPE of kidney and muscle, respectively, indicate chemical matrix interferences of the monitored m/z 297 → 279 transition. These did not interfere with the analysis of flunixin at t_R of 5.48 min, but notable interferences were observed in some cases, to be discussed later.

The second approach to assess and compare MEs was done in both UHPLC-QQQ and UHPLC-Q/TOF for all analytes and both sample preparation methods, as shown in Fig. 3. In this approach, the percent differences in the best-fit linear slope of the combined MM calibration curve for each analyte were calculated relative to the RO calibrations. Few major differences were observed among matrices, and the use of all MM standards for each method gave greater accuracy in the slopes (especially since normalization to the IS was not done in the ME assessment). In the UHPLC-QQQ analysis for most drug analytes, the MEs for the d-SPE extracts were consistently slightly higher than for the EMR-L extracts, which is probably just a small bias in the measurements because UHPLC-Q/TOF showed small or no differences. Also, several drugs are labeled in Fig. 3 that underwent excessive MEs. The same analytes gave poor calibrations and highly variable recoveries from the spiked samples; thus, matrix-induced ion suppressions and enhancements were probably not the main reason for the outlier results shown in Fig. 3 in either sample preparation or analytical method. In any case, the general conclusion can be made that both sample preparation methods led to similar MEs, with a few notable issues, as discussed below.

Both methods gave excessive enhancement for the late-eluting mectin anthelmintics in ESI+. In the case of UHPLC-Q/TOF, positive MEs predominated from 3.5 to 4.5 min when many drugs eluted from the column. The UHPLC conditions in the QQQ analysis distributed the analytes more evenly in a shorter time, and perhaps less bunching of analytes (and matrix components, too) in the Q/TOF method would have reduced the MEs observed. MEs result not only from matrix–analyte competitive interactions but also from other co-eluting analytes in the mixture, and the injection of up to 11,000 ng/g equivalent of tulathromycin in the $2X$ calibration standards at t_R of 4 min in the Q/TOF method and 2.9 min in QQQ very likely affected quantification, independent of matrix co-elutions. Detector saturation particularly in Q/TOF was another possible explanation, especially with the drugs run at higher concentrations giving large responses.

In the case of the UHPLC-QQQ of mectins, from 7.5 to 9 min, the high MEs were very likely due to co-elution of excessive matrix towards the end of the run. A similar trend was noticed in the UHPLC-Q/TOF method for the mectins between 9.9 and 11.5 min. This indicates that the column needed to be flushed longer with MeCN, or an extensive flushing with MeCN/MeOH to waste should have been done before conducting the experiment. In both the UHPLC-QQQ
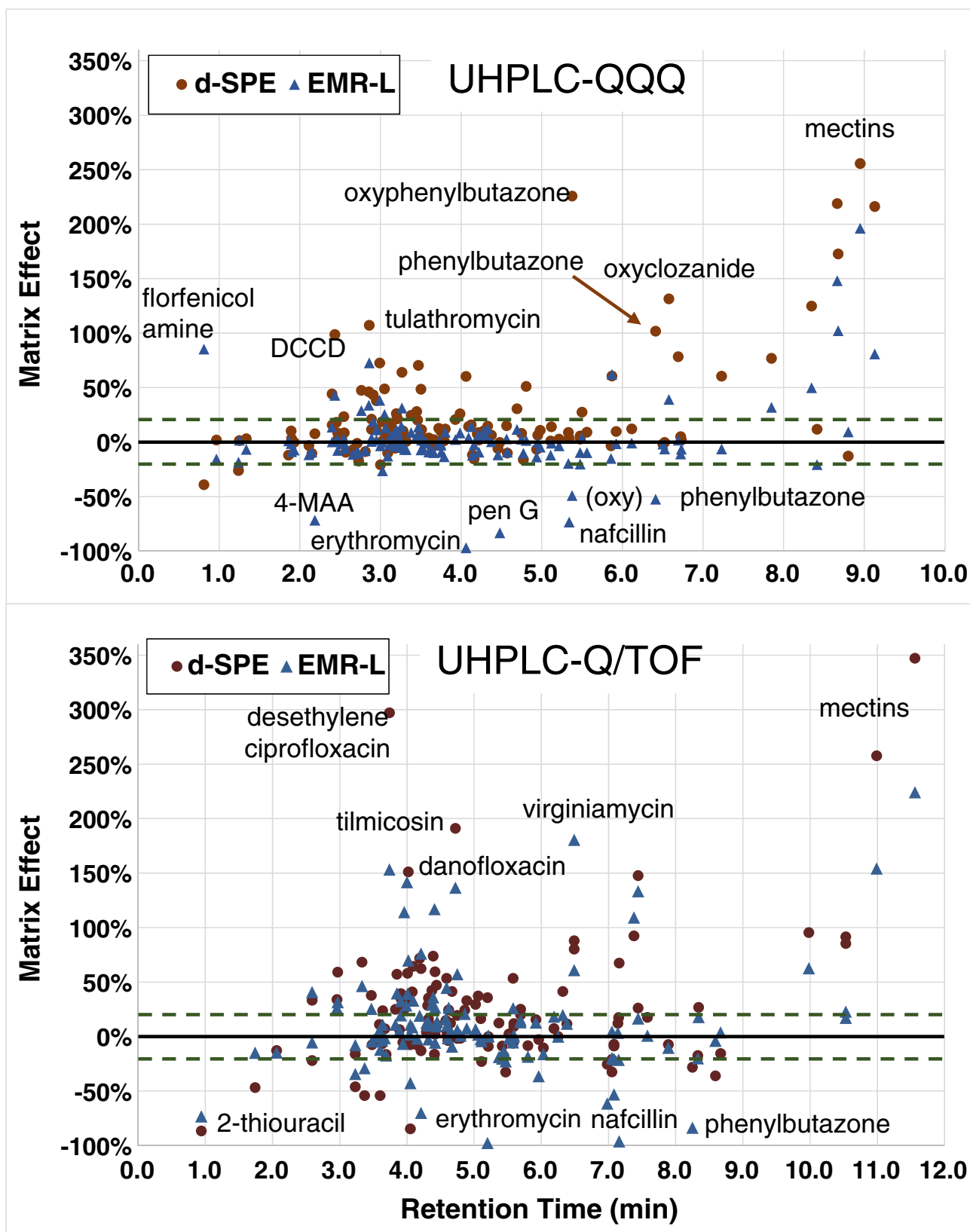

Fig. 2 **a** Signal of flunixin infused post-column in the UHPLC-QQQ of injected matrix blanks from each sample preparation method. **b** Concomitant MEs relative to the flunixin signal during the mobile phase gradient alone

Fig. 3 Averaged MEs of the three bovine tissues (kidney, liver, and muscle) of the drug analytes plotted vs. t_R in both sample preparation and analytical methods. The *dotted green lines* indicate MEs ±20%

and UHPLC-Q/TOF methods, the EMR-L extracts had lower suppression than d-SPE with respect to mectins, which could indicate better cleanup with this procedure especially for later-eluting co-extractives.

Other complications in the analysis of the mectins relate to their low concentrations and sensitivities in the analysis and their tendency to form adducts with sodium and ammonium ions. In previous studies, ammonium formate was infused post-column at the last part of the chromatogram to maximize ammonium adduct formation of mectins, but the QQQ instrument's software in this study could not control the start/stop of the infusion syringe pump during the run [17]. Thus, sodium adducts of the mectins were monitored (see Tables S1 and S3 in ESM), but their quantitative analyses were highly variable in the UHPLC-QQQ method independent of sample preparation. Other studies have recommended the use of volatile buffers in the mobile phase, such as ammonium acetate, to inhibit the formation of sodium adducts [39]. For analysis of only mectins, these options could be considered, but as we were interested in analyzing >120 drugs in a single analytical method, certain compromises had to be made.

Q/TOF advantage

A benefit of employing HRMS, such as a Q/TOF, is the ability to measure the accurate mass of detected ions. This reduces matrix interferences within one mass unit of the target analyte that are normally detected in QQQ. However, the use of accurate mass alone to identify compounds can result in false positives or misidentifications. Figure S2 (see ESM) illustrates a kidney extract at the 1X spiking level with two peaks at m/z 360.1718, which corresponds to the mass of the $[M+H]^+$ adduct of enrofloxacin. As such, it would not be possible to identify the correct peak, but with the use of fragment information from the compound library (which includes MS/MS spectra), it is clear that the peak at t_R of 4.08 min has five fragments corresponding to enrofloxacin that co-elute with the precursor, while the later peak ($t_R = 6.02$ min) does not possess any of these fragment masses. Thus, the correct peak can be identified as enrofloxacin. Furthermore, the t_R was verified with the use of an analytical standard and stored in the database for better targeted analysis.

Drug analyte recoveries

Spike recovery tests were performed at the 1X levels (see Table 1) for 127 veterinary drugs in bovine kidney, liver, and muscle, each in triplicate. The average recoveries and RSDs ($n = 9$) calculated using both analytical methods among all three matrices for each veterinary drug are presented in Table 1. The peak areas of all veterinary drugs were normalized to the flunixin-d_3 IS in each injection. Figure S3 (see ESM) displays a compilation of recoveries for between 70 and 120% and RSDs ≤20% among the different matrices and sample preparation methods using UHPLC-QQQ for analysis. Additionally, Fig. 4 compiles the results into each class of drug for each sample preparation and analytical method. As the figures show, trends between the two sample preparation and analytical methods were much the same.

The majority of analytes (103 by d-SPE and 100 by EMR-L) had acceptable average recoveries between 70 and 120% in either method. In fact, 11 drug groupings (anthelmintics, anti-inflammatories, coccidiostats, fluoroquinolones, lincosamides, macrolides, phenicols, sulfonamides, thyreostats, β-agonists, and miscellaneous) averaged within 70–120% for the two sample preparation techniques and both analytical methods. Similarly, the recoveries of many drug analytes were similar independent of kidney, liver, and muscle matrices. The overall average recoveries ranged from 4% (DCCD) to 127% (6-phenyl-thiouracil) for the EMR-L technique and from 32% (chlorpromazine) to 141% (6-phenyl-thiouracil) for d-SPE when analyzed with the UHPLC-QQQ method. Similarly, the UHPLC-Q/TOF method gave recoveries between 4% (DCCD) and 148% (6-phenyl-thiouracil) and between 37% (chlorpromazine) and 140% (6-phenyl-thiouracil) by EMR-L and d-SPE, respectively. The calculated recoveries using the two different analytical techniques were similar in most cases, with the few exceptions discussed below.

The tetracyclines had low recoveries (<65%) with both the d-SPE and EMR-L sample preparation techniques. The average recoveries with both analytical methods for the four tetracyclines tested in the bovine tissues ranged from 46% (oxytetracycline) to 83% (chlortetracycline) with the EMR-L and from 48% (chlortetracycline) to 69% (tetracycline) in d-SPE. These are typical results for tetracyclines when EDTA chelation in buffered extraction solvent is not employed [40]. The low partition coefficients (log$P$) for the tetracyclines may also result in greater partitioning into the water phase during the salting-out step in the EMR-L method. The salting-out step contributed to the overall cleanup, but it also led to lower recoveries of polar analytes. The authors suggest the use of a specific tetracycline internal standard and MM calibration for better quantification of these compounds when using the sample preparation techniques evaluated in this study.

Eleven β-lactam/cephalosporin antibiotics were evaluated using the d-SPE and EMR-L protocols (cloxacillin was not analyzed in UHPLC-Q/TOF due to lack of an individual reference standard). Recoveries of the β-lactams were higher using the d-SPE method compared to EMR-L in both types of analyses, averaging 103% and 56% in UHPLC-QQQ in d-SPE and EMR-L, respectively. Similarly, UHPLC-Q/TOF analysis gave 97% (d-SPE) and 67% (EMR-L) recoveries.

Table 1 Average %recoveries (and %RSD) for veterinary drugs spiked at the regulatory target concentrations (X) in bovine kidney, liver, and muscle ($n = 3$ each) for both sample preparation techniques (EMR-L and d-SPE) and both analytical methods (UHPLC-QQQ and UHPLC-Q/TOF)

Drug class/analyte	X (ng/g)	EMR-L method		d-SPE method	
		QQQ %Rec. (%RSD)	Q/TOF %Rec. (%RSD)	QQQ %Rec. (%RSD)	Q/TOF %Rec. (%RSD)
β-Agonists (5)					
Cimaterol	10	111 (13)	NA	95 (15)	NA
Clenbuterol	10	116 (11)	112 (19)	93 (8)	110 (8)
Ractopamine	30	102 (12)	107 (10)	106 (14)	99 (11)
Salbutamol	10	92 (9)	NA	108 (16)	NA
Zilpaterol	12	76 (9)	72 (5)	103 (16)	99 (7)
Anthelmintics (34)					
Albendazole	50	88 (22)	78 (17)	77 (15)	91 (10)
Albendazole sulfoxide	50	85 (12)	89 (13)	112 (10)	*122* (13)
Albendazole sulfone	50	100 (9)	100 (11)	95 (5)	113 (6)
Albendazole-2-aminosulfone	50	**58 (5)**	**57 (6)**	106 (5)	106 (2)
2-Amino-flubendazole	10	90 (16)	92 (7)	95 (5)	93 (15)
2-Amino-mebendazole	10	91 (12)	93 (10)	96 (15)	100 (10)
Avermectin B1	20	72 (**30**)	84 (16)	**55** (*18*)	**64** (*16*)
Bithionol	10	**67 (34)**	NA	74 (25)	NA
Cambendazole	10	108 (11)	89 (11)	101 (14)	105 (11)
Clorsulon	100	102 (14)	NA	89 (14)	NA
Closantel	50	**40 (64)**	NA	**64 (34)**	NA
Doramectin	30	86 (14)	94 (14)	**56 (33)**	**58** (14)
Emamectin benzoate	10	105 (20)	104 (14)	**50 (43)**	**64 (28)**
Eprinomectin B1a	100	93 (21)	91 (20)	78 (21)	73 (14)
Fenbendazole	400	90 (15)	94 (14)	72 (22)	97 (14)
Fenbendazole sulfone	400	96 (11)	106 (14)	*85* (*11*)	*119* (*10*)
Flubendazole	10	96 (12)	95 (11)	86 (12)	106 (8)
Haloxon	100	**44 (66)**	**44 (37)**	**56 (67)**	**61 (41)**
5-Hydroxy-thiabendazole	100	**65 (39)**	**63** (24)	83 (**31**)	94 (**28**)
Ivermectin B1a	10	91 (22)	83 (16)	**48 (29)**	**63** (14)
Levamisole	100	109 (3)	107 (12)	93 (3)	107 (4)
Mebendazole	10	98 (9)	102 (12)	87 (12)	119 (4)
Morantel	100	117 (9)	119 (14)	96 (7)	113 (4)
Moxidectin	50	73 (**32**)	81 (17)	**36 (41)**	**41** (19)
Niclosamide	10	**58 (45)**	76 (18)	75 (20)	**69 (27)**
Nitroxynil	50	89 (11)	NA	101 (7)	NA
Oxfendazole	800	86 (7)	87 (6)	94 (4)	105 (6)
Oxibendazole	10	100 (12)	100 (14)	85 (10)	105 (4)
Oxyclozanide	10	**59 (38)**	NA	81 (19)	NA
Rafoxanide	10	**33 (56)**	NA	**36 (59)**	NA
Selamectin	200	88 (27)	85 (9)	**40 (28)**	**46** (11)
Thiabendazole	100	101 (5)	90 (10)	91 (5)	109 (2)
Triclabendazole	50	95 (15)	112 (17)	**65 (27)**	*105* (*21*)
Triclabendazole sulfoxide	50	95 (12)	94 (14)	82 (22)	96 (9)
Anti-inflammatories (10)					
Betamethasone	100	102 (17)	98 (22)	102 (14)	96 (5)
Diclofenac	200	96 (16)	100 (16)	90 (3)	103 (8)
Dipyrone metabolite (4-MAA)	200	**27** (12)	NA	105 (**52**)	NA

Table 1 (continued)

Drug class/analyte	X (ng/g)	EMR-L method		d-SPE method	
		QQQ %Rec. (%RSD)	Q/TOF %Rec. (%RSD)	QQQ %Rec. (%RSD)	Q/TOF %Rec. (%RSD)
Flunixin	25	104 (3)	103 (1)	105 (3)	104 (1)
Ketoprofen	10	91 (19)	105 (18)	88 (14)	102 (14)
Meloxicam	100	91 (20)	99 (11)	100 (22)	106 (5)
Oxyphenylbutazone	100	106 (**51**)	98 (17)	101 (22)	109 (**40**)
Phenylbutazone	100	78 (**54**)	109 (16)	93 (20)	106 (24)
Prednisone	100	104 (9)	96 (14)	93 (14)	107 (5)
Tolfenamic acid	200	86 (19)	108 (20)	87 (6)	112 (9)
Coccidiostats (6)					
Dimetridazole	10	108 (5)	95 (12)	97 (19)	114 (3)
2-Hydroxy-dimetridazole	50	96 (7)	100 (13)	98 (10)	119 (9)
Ipronidazole	10	107 (7)	111 (16)	104 (11)	116 (5)
Ipronidazole-hydroxy	10	118 (11)	120 (12)	94 (10)	115 (4)
Metronidazole	10	93 (8)	90 (11)	94 (16)	111 (6)
Ronidazole	10	119 (7)	103 (0)[b]	106 (9)	90 (11)
Fluoroquinolones (8)					
Ciprofloxacin	50	**68** (16)	80 (9)	88 (12)	89 (7)
Danofloxacin	200	**65** (9)	87 (11)	82 (12)	87 (7)
Desethylene ciprofloxacin	100	**61** (15)	72 (13)	87 (12)	91 (4)
Difloxacin	50	106 (10)	94 (12)	91 (9)	83 (6)
Enrofloxacin	100	105 (9)	94 (10)	90 (9)	86 (6)
Norfloxacin	50	90 (**30**)	76 (2)	101 (11)	91 (7)
Orbifloxacin	50	115 (12)	101 (13)	107 (8)	100 (3)
Sarafloxacin	50	106 (15)	90 (9)	89 (11)	85 (4)
β-Lactams (11)					
Amoxicillin	10	76 (22)	89 (9)	104 (24)	109 (6)
Ampicillin	10	NQ	96 (15)	NQ	99 (10)
Cefazolin	100	75 (16)	82 (17)	106 (18)	92 (13)
Cephapirin[a]	100	**28** (**52**)	**21** (18)	77 (17)	**47** (**32**)
Cloxacillin	10	84 (**33**)	NA	111 (16)	NA
DCCD (marker for ceftiofur)	400	**4** (**70**)	**4** (1)	85 (**27**)	72 (6)
Desacetyl cephapirin	100	NQ	71 (14)	NQ	122 (11)
Dicloxacillin	100	80 (19)	86 (**26**)	108 (7)	108 (16)
Nafcillin	100	**41** (19)	73 (17)	112 (12)	110 (10)
Oxacillin	100	80 (18)	83 (**26**)	112 (14)	116 (13)
Penicillin G	50	**38** (**71**)	NQ	113 (10)	NQ
Lincosamides/macrolides (9)					
Clindamycin	100	101 (7)	89 (13)	105 (8)	99 (9)
Erythromycin A	100	98 (**31**)	105 (24)	110 (8)	106 (6)
Gamithromycin	100	72 (22)	102 (15)	71 (20)	93 (21)
Lincomycin	100	**44** (10)	**44** (5)	113 (18)	99 (6)
Pirlimycin	300	77 (8)	76 (12)	94 (11)	90 (9)
Tildipirosin	100	**125** (11)	**129** (**57**)	90 (**41**)	89 (22)
Tilmicosin	100	120 (10)	104 (18)	94 (12)	94 (24)
Tulathromycin A	5500	**132** (10)	**125** (22)	88 (**36**)	76 (**38**)
Tylosin	200	**55** (**32**)	**49** (18)	76 (**26**)	99 (23)

Table 1 (continued)

Drug class/analyte	X (ng/g)	EMR-L method		d-SPE method	
		QQQ %Rec. (%RSD)	Q/TOF %Rec. (%RSD)	QQQ %Rec. (%RSD)	Q/TOF %Rec. (%RSD)
Miscellaneous (6)					
Carbadox	30	87 (16)	89 (13)	102 (15)	*129 (4)*
Melengesterol acetate	25	92 (17)	95 (6)	74 (21)	90 (4)
Novobiocin	1000	85 (27)	92 (**25**)	98 (10)	111 (3)
Quinoxaline-2-carboxylic acid	30	99 (42)	NA	**125 (41)**	NA
Virginiamycin	100	95 (13)	96 (10)	91 (10)	111 (3)
Zeranol	100	101 (13)	*132 (51)*	*87 (17)*	*87 (18)*
Phenicols (4)					
Chloramphenicol	10	93 (14)	NA	*86 (11)*	NA
Florfenicol	300	106 (13)	108 (16)	88 (11)	110 (7)
Florfenicol amine	300	80 (11)	74 (22)	**51 (31)**	71 (16)
Thiamphenicol	10	105 (18)	112 (6)	118 (20)	107 (14)
Sulfonamides (16)					
Sulfabromomethazine	100	86 (11)	104 (17)	94 (11)	118 (3)
Sulfachloropyridazine	100	96 (8)	104 (12)	95 (8)	107 (1)
Sulfadiazine	100	104 (10)	99 (12)	92 (10)	109 (2)
Sulfadimethoxine	100	101 (12)	94 (11)	92 (9)	103 (3)
Sulfadoxine	100	100 (14)	103 (9)	98 (7)	110 (1)
Sulfaethoxypyridazine	100	89 (12)	95 (17)	95 (9)	111 (3)
Sulfamerazine	100	101 (9)	104 (13)	96 (6)	111 (2)
Sulfamethazine	100	95 (13)	99 (12)	92 (6)	107 (3)
Sulfamethizole	100	93 (17)	101 (20)	102 (12)	112 (4)
Sulfamethoxazole	100	103 (13)	104 (14)	95 (7)	110 (4)
Sulfamethoxypyridazine	100	103 (12)	99 (16)	96 (8)	114 (1)
Sulfanilamide	100	78 (22)	91 (13)	*85 (23)*	*120 (4)*
Sulfanitran	100	101 (13)	99 (9)	85 (19)	114 (**28**)
Sulfapyridine	100	96 (12)	96 (14)	94 (9)	114 (3)
Sulfaquinoxaline	100	95 (15)	90 (13)	90 (6)	102 (2)
Sulfathiazole	100	91 (17)	90 (13)	86 (10)	110 (5)
Tetracyclines (4)					
Chlortetracycline	1000	70 (21)	**61** (13)	**51** (12)	**48** (4)
Doxycycline	100	73 (17)	83 (21)	**66** (9)	**66** (6)
Oxytetracycline	1000	**50** (13)	**46** (9)	**59** (13)	**55** (5)
Tetracycline	1000	**59** (11)	**58** (12)	**69** (14)	**68** (4)
Thyreostats (5)					
2-Mercaptobenzimidazole	25	**50 (59)**	**54** (25)	97 (23)	90 (22)
6-Methyl-thiouracil	400	93 (7)	89 (11)	106 (8)	116 (3)
6-Phenyl-thiouracil	400	*127* (20)	*148* (25)	*141* (12)	*140* (4)
6-Propyl-2-thiouracil	50	102 (17)	108 (10)	103 (14)	118 (2)
2-Thiouracil	400	80 (6)	97 (32)	109 (11)	85 (5)
Tranquilizers (9)					
Acetopromazine	10	**61 (33)**	**61** (19)	**66** (25)	**64** (17)
Azaperone	10	90 (10)	93 (10)	74 (23)	87 (9)
Carazolol	10	109 (9)	106 (13)	84 (13)	100 (8)
Chlorpromazine	10	**54 (33)**	**54** (25)	**32** (18)	**37** (17)
Haloperidol	10	98 (8)	102 (12)	**67 (43)**	89 (**33**)

Table 1 (continued)

Drug class/analyte	X (ng/g)	EMR-L method		d-SPE method	
		QQQ %Rec. (%RSD)	Q/TOF %Rec. (%RSD)	QQQ %Rec. (%RSD)	Q/TOF %Rec. (%RSD)
Promethazine	10	79 (30)	72 (**27**)	**35** (*22*)	**58** (*30*)
Propionylpromazine	10	**56** (**32**)	**59** (21)	**41** (21)	**54** (18)
Triflupromazine	10	77 (**27**)	71 (21)	**38** (**32**)	**45** (18)
Xylazine	10	*110* (*13*)	**54** (*17*)	**69** (14)	**58** (21)

Italicized analyte names indicate >30% (absolute) differences in averaged results between sample preparation and analytical methods. Bold indicates results <70% recovery or >25% RSD. Entries in bold italics denote >120% recovery

NA not analyzed, *NQ* not quantified

[a] Liver and muscle only ($n = 6$)

[b] Muscle only ($n = 3$)
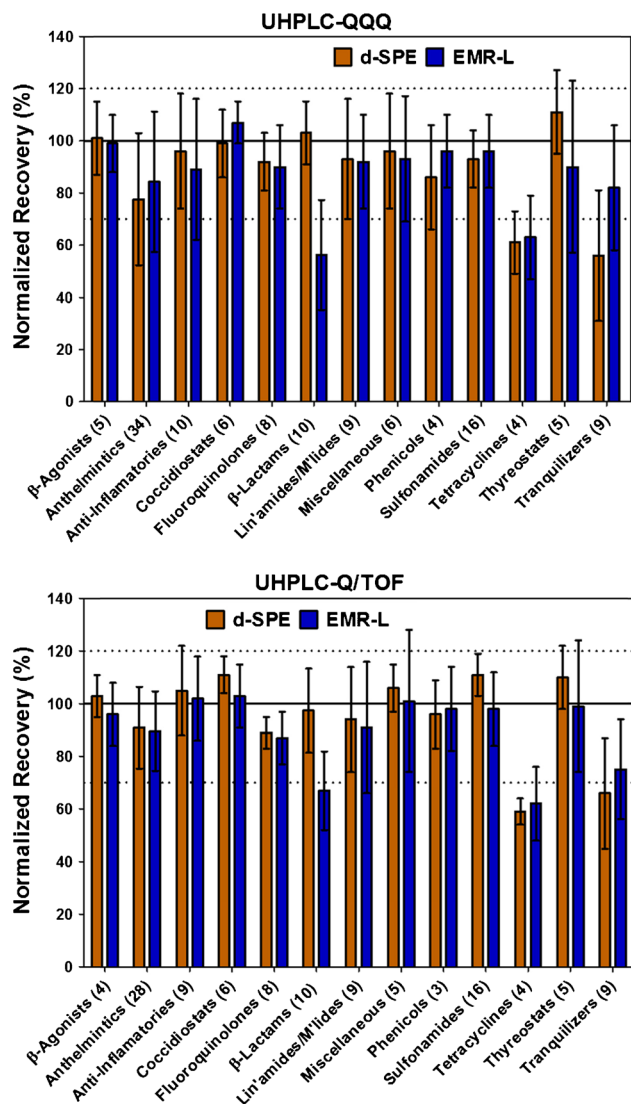
Fig. 4 Average recovery of veterinary drug classes in all three bovine matrices with both sample preparation techniques analyzed by UHPLC-QQQ and UHPLC-QTOF

The recovery of desfuroylceftiofur cysteine disulfide (DCCD), a ceftiofur metabolite, was merely 4% in the EMR-L technique in both analytical methods.

The use of MeCN without acid for extraction may have also contributed to some recovery differences vs. the results shown in Table 1, but the use of 10 mL 4:1 (*v/v*) MeCN/water for the extraction of 2 g tissue samples in the d-SPE method has been extensively validated, including incurred samples [34], and implemented by FSIS. Maštovská et al. also demonstrated that extraction with 4:1 (*v/v*) MeCN/water yielded acceptable extraction efficiencies of these antibiotics [41]. Independent to the EMR-L cleanup step, the recoveries of β-lactams (including DCCD) using the EMR-L method may be improved by avoiding acidic conditions during the extraction step. In particular, penicillin G converts to penillic acid more rapidly in acidic solutions [42], and although penicillin G was not quantified in the EMR-L Q/TOF method, its conversion product peak was monitored and identified.

The salting-out partitioning step must also be considered in the evaluation of the overall EMR-L method. Figure 5 shows how the use of $MgSO_4$ alone rather than a combination of 4:1 (*w/w*) $MgSO_4$/NaCl helps improve the recoveries of several polar drugs. Only those drugs with >10% absolute average recovery differences in the three matrices ($n = 9$) are presented, including azaperone and florfenicol amine, which gave higher recoveries when using the $MgSO_4$/NaCl mix. When developing the original QuEChERS method, Anastassiades et al. extensively studied aqueous MeCN salt-out partitioning of diverse pesticides in fruits and vegetables [43]. Although $MgSO_4$-only was shown to yield higher recoveries of the most polar analytes in the MeCN phase, it also led to higher amounts of sugars from fruits getting into the final extracts. Thus, a little NaCl was included with $MgSO_4$ in the QuEChERS method as a compromise to avoid the sugary co-extractives, yet still achieve >70% recovery of the most polar pesticide in the study (methamidophos). Sugars are not
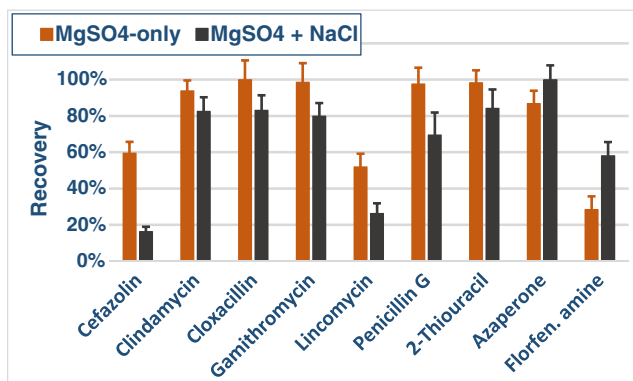
Fig. 5 Improvement in the recoveries of several drug analytes in the EMR-L method when salting out with MgSO$_4$ alone rather than 4:1 (w/ w) MgSO$_4$ + NaCl

an issue in animal tissues, nor after the initial MeCN extraction step in QuEChERS of most foods; thus, the EMR-L product should not need the 4:1 MgSO$_4$/NaCl combination in the first place. Recent commercial EMR-L kits are available using 3.5 g anh. MgSO$_4$ as an option.

Although the EMR-L method yielded lower recoveries than d-SPE for certain polar analytes, it gave improved results vs. d-SPE for many less polar drugs. For example, the nine tranquilizers had 79% overall average recovery using EMR-L in both analyses, whereas it was 61% by the d-SPE method. The late-eluting anthelmintics (avermectin, doramectin, emamectin, eprinomectin, ivermectin, moxidectin, and selamectin) also had significantly higher recoveries in the EMR-L method compared to the use of d-SPE. These less polar drugs are partially retained on the C18 sorbent during d-SPE in the aqueous MeCN extract, leading to their lower recoveries. However, they are not retained by the EMR-L material due to the different mechanism from traditional hydrophobic chemical interactions with C18. Also, these less polar analytes readily partition into the MeCN phase during the salting-out step in the EMR-L method.

While most drug analytes had similar recoveries in the kidney, muscle, and liver tissues, the recoveries of certain (sub)classes of veterinary drugs varied depending on the matrix. Figure 6 shows the recovery of six mectins tested in each of the matrices for both sample preparation techniques and two analytical methods. While the mectin recoveries were greater using EMR-L in the kidney and liver, their recoveries were almost identical in muscle for both sample preparation and analytical methods. Perhaps the enhanced matrix effects of these compounds, as shown in Figs. 2 and 3, explain this result. The recoveries for the other analytes (e.g., triflupromazine, DCCD, 6-phenyl-thiouracil, and haloxon) that had matrix-specific differences are presented in Fig. S4 (see ESM).

As shown in Fig. S5 (see ESM), ractopamine was incurred in the kidney samples used as blanks at about 3 ng/g, but not detected in the muscle and liver tissues. This amount was
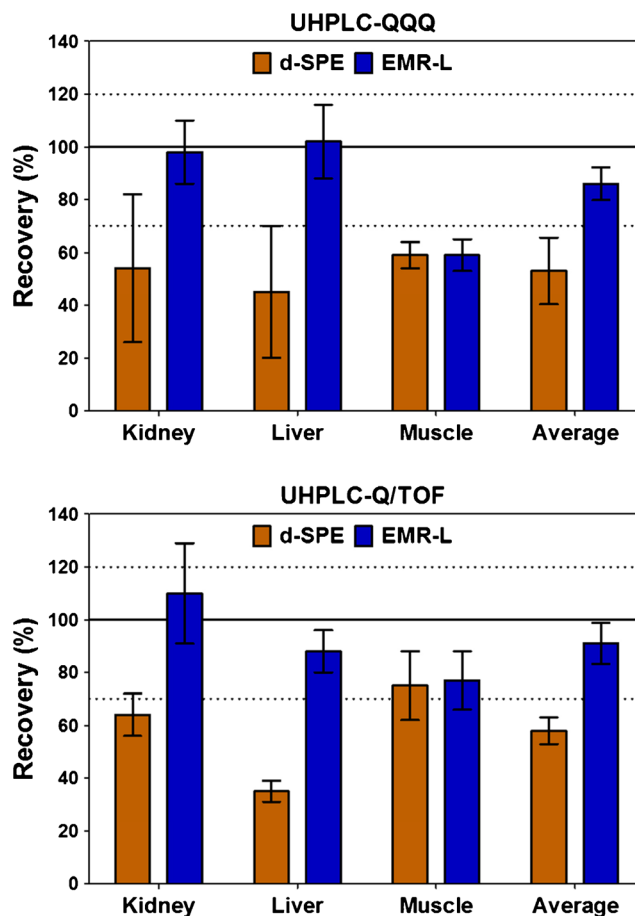
Fig. 6 Matrix-specific and average extraction recoveries of six mectins with both sample preparation techniques (d-SPE and EMR-L) and two analytical methods (UHPLC-QQQ and UHPLC-QTOF)

taken into account when calculating recoveries for this analyte in kidney. UHPLC-QQQ had the same findings for ractopamine as in UHPLC-Q/TOF, and it was also identified at low levels in a previous report [44].

In the case of ampicillin, it was identified in all reagent and bovine matrix blanks using UHPLC-QQQ, but not in Q/TOF (see ESM Fig. S5). This problem was subsequently resolved, but the recovery of ampicillin was not calculated in this experiment using the LC-QQQ. However, its recoveries at the 5- to 20-ng/g levels in bovine tissues ranged from 70 to 84%, with 18–29% RSDs, previously with the d-SPE method [34, 44].

Conclusions

The intent of this study was to compare the analytical and practical performances of two sample preparation methods and two analytical methods in the analysis of >120 veterinary drugs spiked in bovine tissues (kidney, liver, and muscle) at regulatory levels of interest. Sample preparation entailed QuEChERS-like extractions in both cases followed by clean-up using EMR-L in one case and d-SPE with C18 in the other.

The results of the side-by-side comparison showed that both sample preparation methods gave overall average recoveries between 70 and 120% in the bovine tissues for 80% of the 127 veterinary drugs. Each sample preparation method has its benefits, but on the whole, both methods performed well overall in covering a wide range of veterinary drugs and classes tested in this study. The EMR-L method provided cleaner extracts and better results for mectins and tranquilizers, but significantly lower recoveries for some β-lactams. In terms of practical aspects, the d-SPE method was easier, faster, and less expensive than the EMR-L method. However, this does not include assessment of instrument maintenance, which impacts time and cost. The authors suggest that further work be done including additional replicate testing and relevant QA/QC for long-term applicability of both the sample extraction and analysis methods before use in a regulatory environment.

The final extracts were analyzed separately by UHPLC-QQQ and UHPLC-Q/TOF instruments. The results from both instruments were very similar and gave identical trends. The optimized conditions in QQQ permitted lower limits of quantification for targeted analytes if the matrix was not the limiting source of noise, but Q/TOF successfully quantified and identified veterinary drugs at regulatory levels of concern in this study. HRMS using the Q/TOF gave greater selectivity and ability to monitor a larger number of compounds, including retroactive processing of chromatograms for previously untargeted chemicals of interest.

This study demonstrated that the new EMR-L method and analysis by UHPLC-Q/TOF independently and together meet common veterinary drug monitoring needs and serve as additional options in a variety of applications involving this type of analysis.

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