Multi-Class Veterinary Drugs Analyses of QuEChERS Extracts Using an Automated Online µSPE Cleanup Coupled to LC-MS/MS

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ABSTRACT

Purpose: To demonstrate a fully automated online sample cleanup and injection solution coupled to LC-MS/MS for rapid and robust screening and quantitation of veterinary drug residues in animal tissues.

Methods: Methods were developed using an automated online cleanup and injection of QuEChERS (quick, easy, cheap, effective, rugged, and safe) extracts from various animal tissue types for LC-amenable veterinary drugs, based on the use of micro solid phase extraction (µSPE) cartridges with a PAL3-RTC autosampler from CTC Analytics (CTC Analytics AG, Zwingen, Switzerland). A total of 103 veterinary drug residues representing a variety of compound classes were spiked into bovine muscle, liver or kidney tissues to evaluate the automated µSPE-LC-MS/MS method.

Results: The automated µSPE cleanup afforded absolute spike recoveries within 30–140% with corresponding %RSDs below 20% for over 95% of the target veterinary drugs in the tissue extracts and demonstrated excellent precision and accuracy. Other benefits include reduced labor costs, automated calibration standard preparation, solvent sandwich injections, and significant reduction of matrix co-extractives due to cleanup of the extracts, resulting in robust operation for routine laboratories performing veterinary drug screening.

INTRODUCTION

A sample preparation approach often applied to veterinary drug screening in animal tissues is a modification of a QuEChERS extraction[1]. This process involves a liquid-solid extraction of the sample with acetonitrile and salts. After the extraction, sample cleanup is often preferred. One common cleanup approach is dispersive solid phase extraction (dSPE), which involves adding a fixed amount of a powdered reagent (such as C18 or PSA) to the extract, vortexing for several minutes, then centrifugation and transfer into an autosampler vial. A second approach is solid phase extraction (SPE), in which the extract is passed through a sorbent material contained in a cartridge using a vacuum manifold.

This work describes a fully automated approach to online cleanup of QuEChERS extracts of animal tissues for LC-amenable veterinary drugs, based on the use of µSPE cartridges. These µSPE cartridges are compatible with the Thermo Scientific™ TriPlus™ RSH µSPE autosampler, which was coupled to a Thermo Scientific™ Vanquish™ Flex binary UHPLC system interfaced with a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer.

MATERIALS AND METHODS

Sample Preparation

All tissues were homogenized using a laboratory blender. Five grams of tissue was added to a 50 mL Falcon tube. Next, 0.5 mL of 0.2 M ammonium oxalate/EDTA solution was added to the tube followed by acetonitrile to bring the total volume to 15 mL. The tubes were shaken at 2500 rpm on a Fisherbrand™ Digital MultiTube Vortexer for 10 minutes. Matrix extracted standards (MES) were prepared by spiking a mix of 103 veterinary drug residues prior to QuEChERS extraction into bovine muscle, kidney, and liver at different concentration levels. Matrix matched standards (MMS) were prepared by spiking the same mixture of veterinary drugs into extracts after the cleanup step. The concentration levels investigated were from 1 to 100 ng/g.

Extract cleanup approaches included manual dispersive solid phase extraction (dSPE), as well as two fully automated online µSPE cartridge cleanups utilizing the robotic TriPlus RSH µSPE autosampler. For the manual dSPE extractions, 500 mg CEC18 was added to the supernatant and shaken on a vortexer for 15 minutes, and then centrifuged at 3000 rpm for 10 minutes. These were then placed into the autosampler for injection. Automated µSPE cleanup was performed using the robotic TriPlus RSH µSPE autosampler system, based on a PAL3-RTC autosampler from CTC Analytics (CTC Analytics AG, Zwingen, Switzerland). The configuration of the system modules required to perform the automated µSPE cleanup and cartridge is shown in Figure 1.
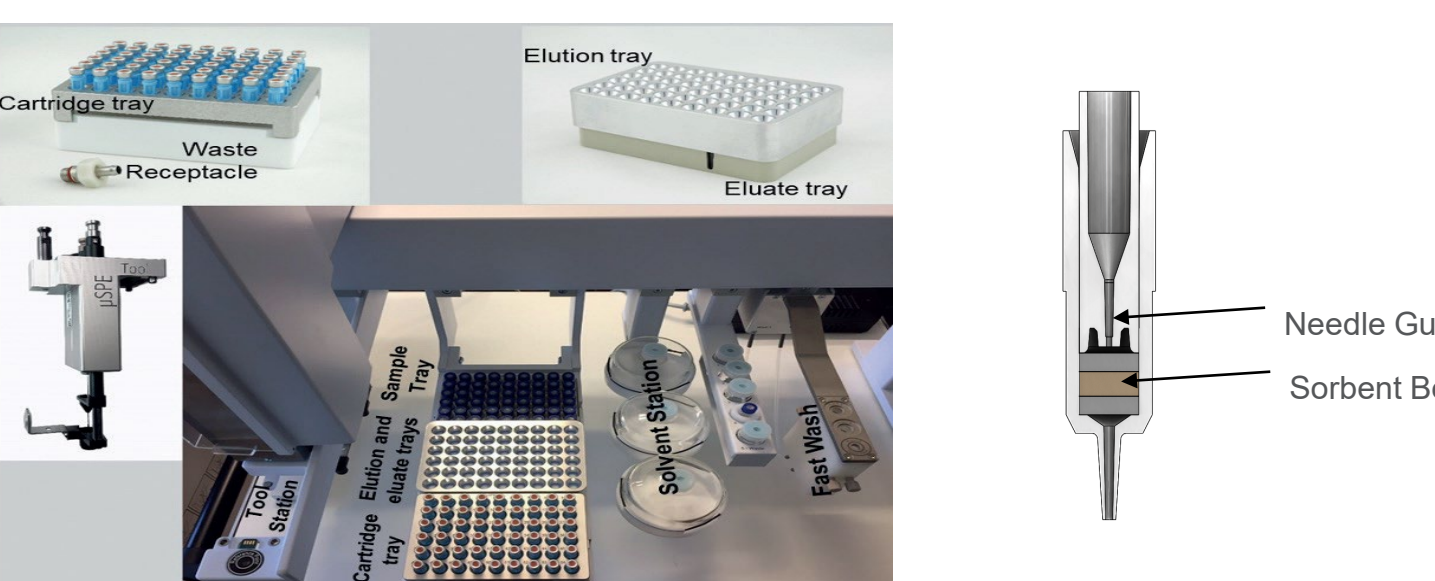

Figure 1: TriPlus RSH with µSPE capabilities system: TriPlus RSH modules required to perform the µSPE clean-up procedure (left) and µSPE cartridge design septum less seal (right)
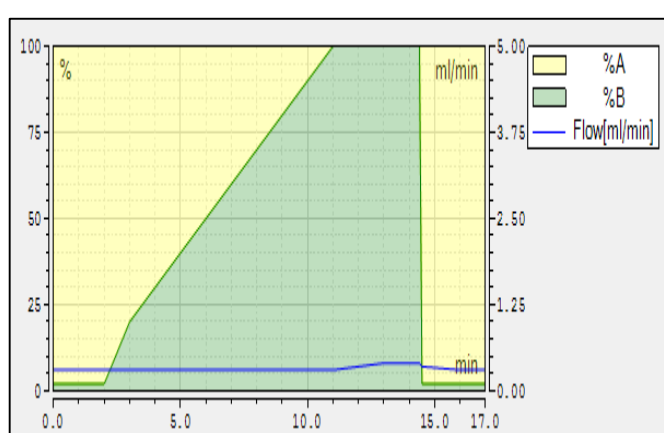
MATERIALS AND METHODS- cont.

Two miniaturized SPE cartridges, each with a different sorbent, were developed and optimized based upon experiments performed at Iowa State University or at the PAL System North America Regional Office laboratory located in Lake Elmo, MN. The result were two µSPE cartridges for evaluation: a) 15 mg CEC18 and b) 10 mg Thermo Scientific™ HyperSep™ Retain-PEP material (HRP). Procedurally, uncleaned QuEChERS extracts were transferred into 2 mL autosampler vials and placed into a 54-position tray (sample tray). The corresponding number of collection vials were placed into a second 54-position tray (eluate tray). Uncleaned extracts were loaded onto the cartridges according to the steps shown in Tables 1 for the specific µSPE cartridges, and the extracted samples were injected directly into the LC-MS/MS system.

	Action
1	Aspirate the 300uL into the syringe
2	Move µSPE cartridge to elution tray
3	Load 300 µL QuEChERS extract onto µSPE cartridge
4	Perform µSPE -push extract through cartridge
5	Move- dispose of cartridge to waste bucket
6	Change to LC/MS injection tool
7	Inject sandwich injection to LC-MS/MS
8	Change to prep syringe for next sample
9	Proceed with prep-ahead for next extract sample upon ready Signal

	Action
1	Prep syringe with elution solvent
2	Condition µSPE with 300 µL methanol
3	Condition µSPE with 300 µL water
4	Condition µSPE with 100 µL QuEChERS extract
5	Move µSPE cartridge to elution tray
6	Load 300 µL QuEChERS extract onto µSPE cartridge
7	Perform µSPE-push extract through cartridge
8	Move-dispose of cartridge to waste bucket
9	Change to LC/MS injection tool
10	Inject-sandwich injection to LC-MS/MS
11	Change to prep syringe for next sample
12	Proceed with prep-ahead for next extract sample upon ready Signal

Table 1: (Left) Steps for automated online µSPE cleanup method with LC injection using the CEC18 cartridge. (Right) Steps for automated online µSPE clean-up method with LC injection using the HyperSep Retain-PEP (HRP) cartridge.

Liquid Chromatography and Mass Spectrometry Conditions

The assays in this study were carried out using a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system and a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer. Thermo Scientific™ TraceFinder™ software was used for instrument control, analysis, data review, and reporting. The LC/MS conditions are shown in the tables 2 and 3 below..

Inj. Volume	2 µL (Sandwich Injection)
Col Temp.	40 C
Analytical Column	Thermo Scientific™ Accucore™ VDX, 100 x 2.1 mm, 2.6 µm
Run Time	17 min
Mobile Phase A	Water with 0.05% Formic Acid
Mobile Phase B	50% Acetonitrile 50% Methanol 5% Water with 0.05% Formic Acid
Neg. Voltage	2500 V
Pos. Voltage	3500 V
Sheath Gas	50 units
Auxiliary Gas	13 units
Sweep Gas	1 unit
Ion Trans Tube	310 C
Vaporizer	350 C

No	Time	Flow [ml/min]	%B	Curve
1	0.000		Equilibration	
2	0.000	0.300	2.0	5
3	0.000	0.300	2.0	5
4	New Row			
5	0.000		Run	
6	0.000	0.300	2.0	5
7	0.000	0.300	2.0	5
8	2.000	0.300	2.0	5
9	3.000	0.300	20.0	5
10	11.000	0.300	100.0	5
11	13.000	0.400	100.0	5
12	14.400	0.400	100.0	5
13	14.500	0.350	2.0	5
14	16.000	0.300	2.0	5
15	17.000	0.300	2.0	5
16	New Row			
17	17.000		Stop Run	

Tables 2 and 3: LC mobile phase, column, and gradient conditions, along with general settings for the mass spectrometer API source.

RESULTS

Sandwich Solvent Injection Technique

The selected 103 veterinary drugs represent a wide variety of compound classes and are listed in the AOAC Standard Method Performance Requirements document (SMPR 2018.010)[2]. A sandwich solvent injection technique was used to greatly improve the chromatography for early eluting veterinary drugs analyzed in this method (Figure 2).
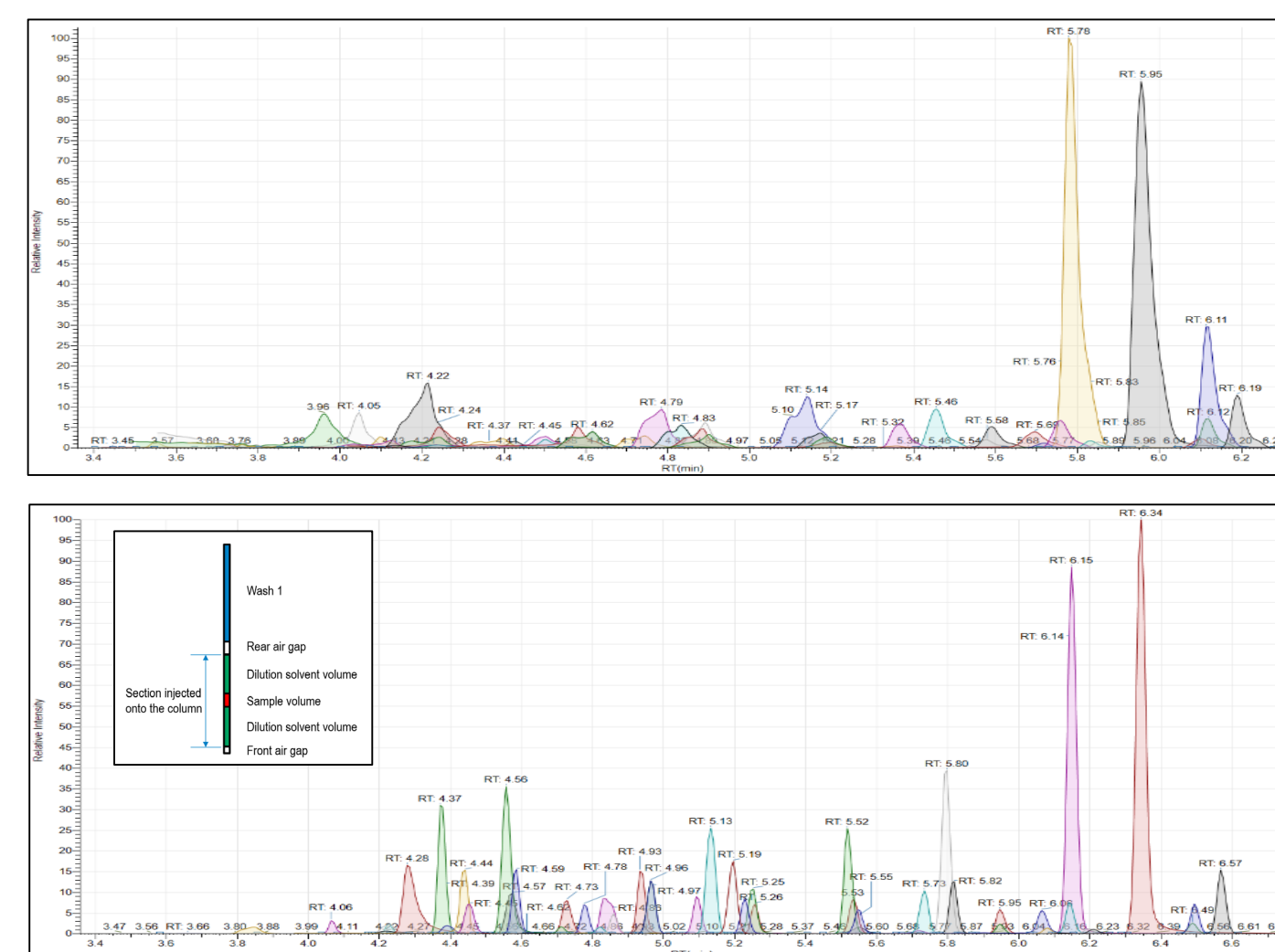

Figure 2: Dramatic peak shape improvement with sandwich technique available with the TriPlus RSH system. (Top) 2 uL injection of bovine extract in pure acetonitrile. (Bottom) Same sample with the solvent sandwich technique. (Inset) Sandwich injection schematic showing dilution solvent volumes (mobile phase A) and sample volume in syringe needle.

For screening and quantitation of the veterinary drugs, calibration levels using matrix extracted standards (MES) and/or matrix matched standards (MMS) were prepared in all three tissue types. Calibration standards ranging from 1 to 100 ng/g were analyzed and excellent linearity was achieved, with coefficient of determination R2>0.99 for most compounds. For screening workflow, the Veterinary Diagnostic Lab at Iowa State University created calibration curves using an **automated calibration script** on the TriPlus RSH µSPE system to avoid manual CAL std preparation (Figure 3).
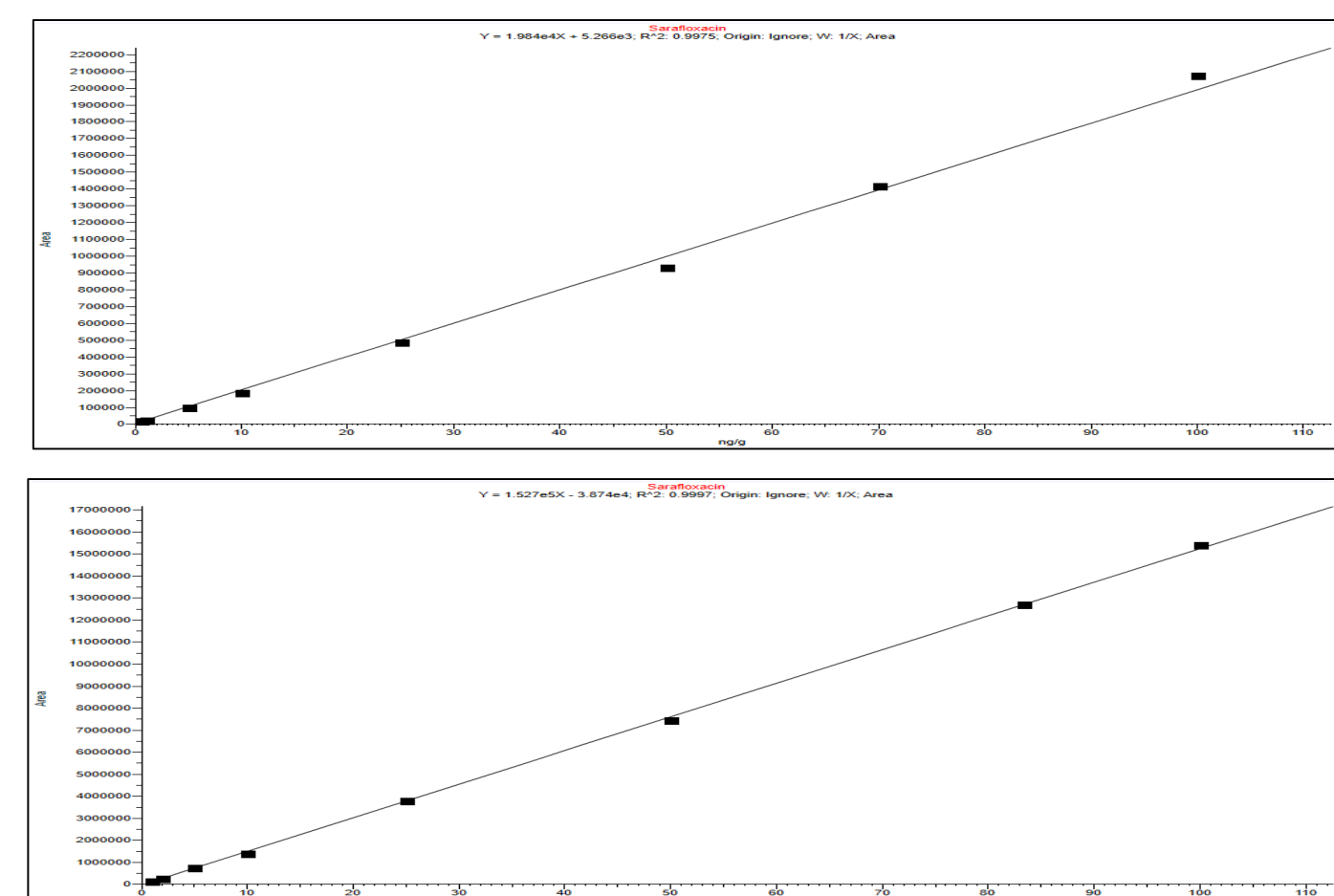

Figure 3: Calibration curves in bovine muscle with µSPE CEC18. The curve on the top of the figure was prepared manually by the lab technician. The curve on the bottom of the figure was prepared using the automated calibration script in the TriPlus RSH autosampler.

RESULTS- cont.

Recovery Experiment

The absolute recoveries of 103 veterinary drugs using the cleanups described above (manual dSPE, CEC18-µSPE and HRP-µSPE) were evaluated in bovine muscle, liver, and kidney tissues. For each experiment, five biological replicates were prepared as matrix-extracted spikes (MES) containing all the target residues at 50 ng/g. The MES were compared to standards spiked into the cleaned samples (MMS) for each of the different cleanups at the same concentrations. Recovery was calculated as the ratio of the average peak area response of the MES to the average peak area response of the MMS. Absolute recoveries within 30–140% with corresponding %RSDs less than or equal to 20% are required for satisfactory method validation according to the EU SANTE 12682/2019 document for pesticides. Recoveries within this range were achieved for over 95% of the target veterinary drugs in the three tissue extract types for the manual CEC18 dSPE procedure and automated online cleanup using either the miniaturized CEC18 µSPE or HRP cartridges.
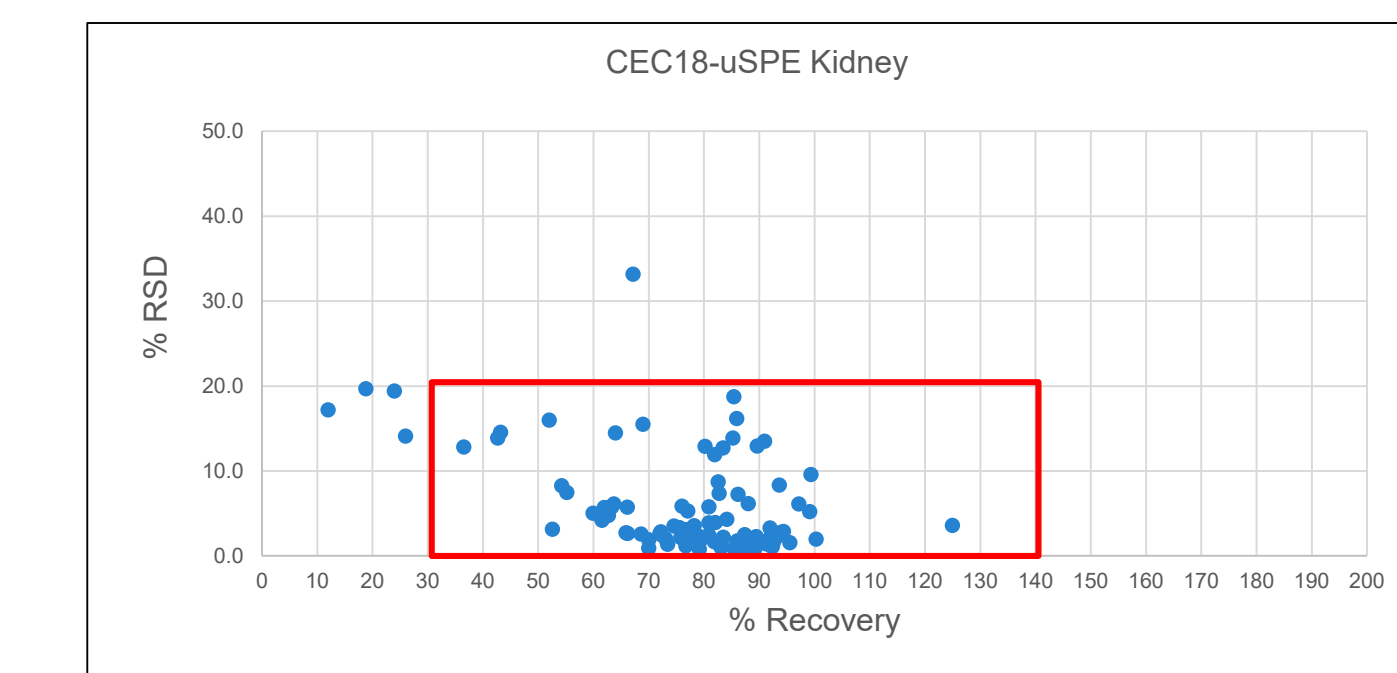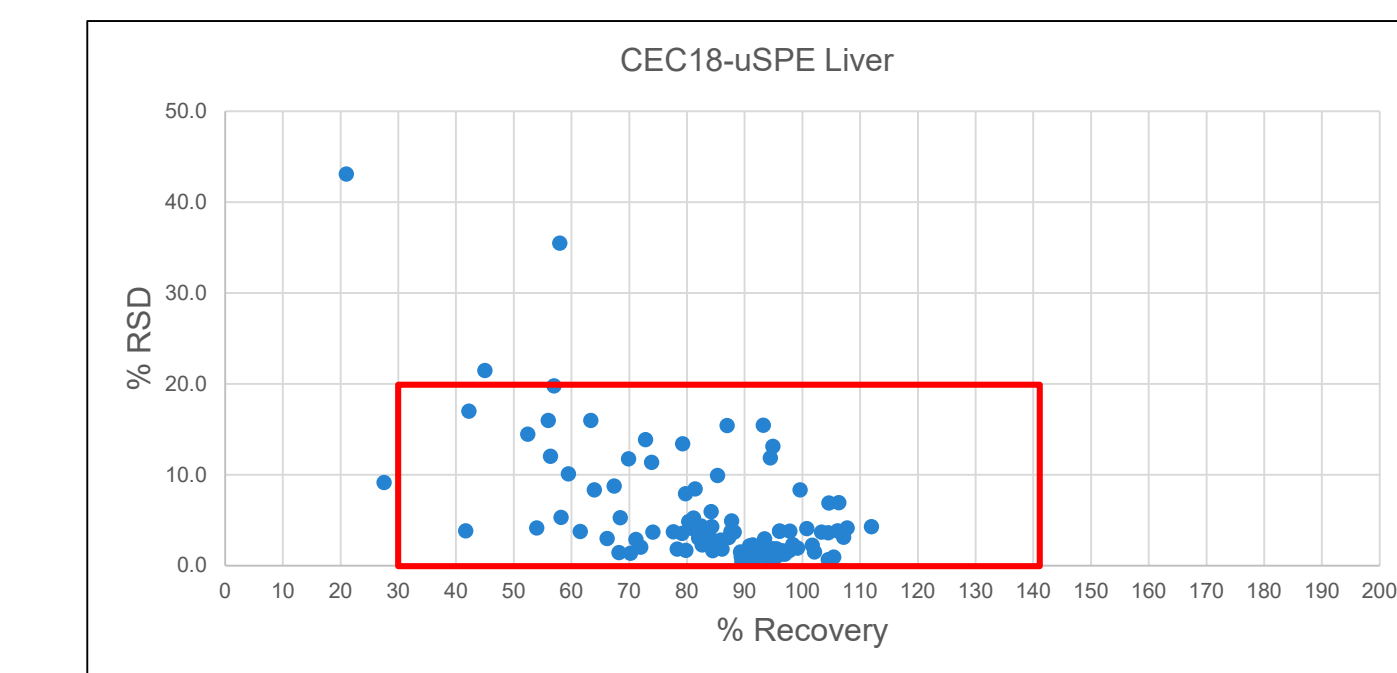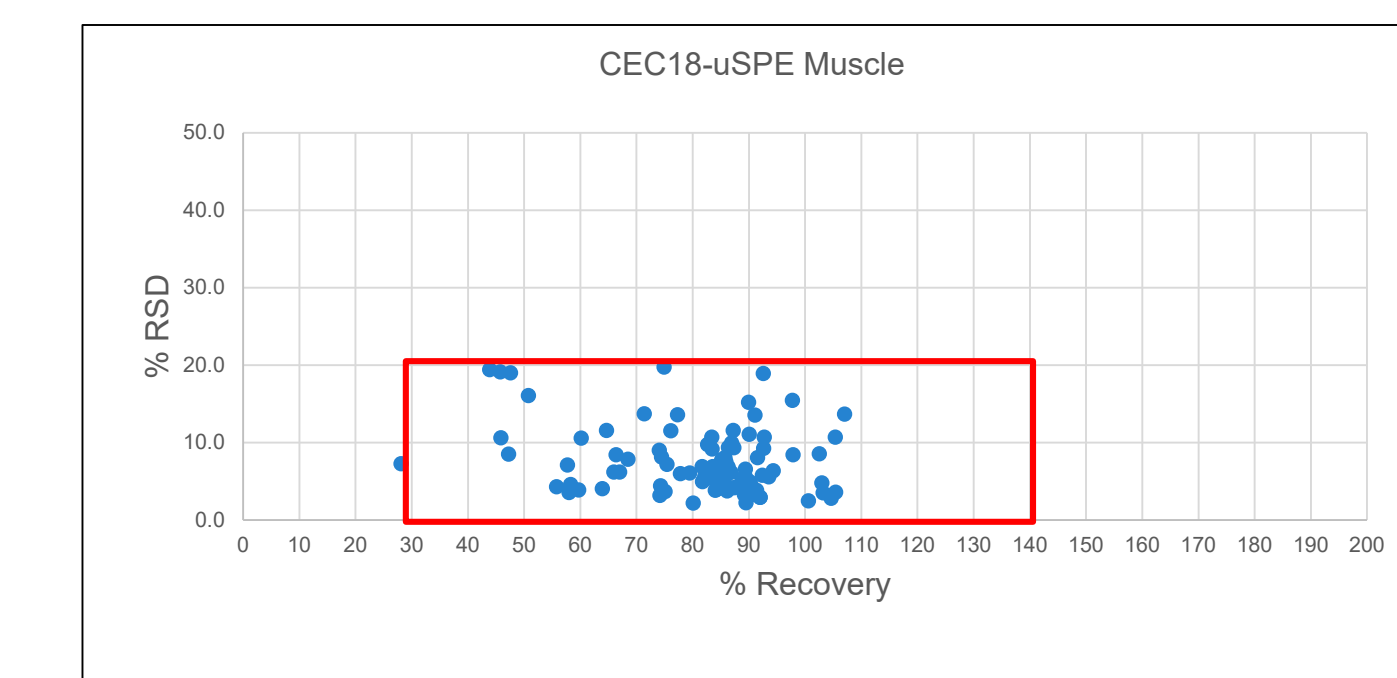

Figure 4: Percent absolute recoveries vs. %RSD of 103 veterinary drugs in bovine muscle, liver, and kidney tissue extracts at 50 ng/g (N=5) using µSPE cleanup with CEC18 cartridges. The red boxes represent recoveries within 30–140% with %RSD <20%.

RESULTS- cont.

Precision and Robustness Experiment

A precision study was also carried out using the CEC18 µSPE cartridge cleanup in all three tissue types. MES calibration curves were prepared, along with five biological replicates of each matrix at 5 ng/g. Excellent precision of less than 20% RSD were obtained for over 90% of the veterinary drugs. Method robustness is shown in an example for levamisole in bovine muscle extract, cleaned up with µSPE CEC18 cartridges. The response was well within the expected ±20% range for at least 100 consecutive injections without maintenance. At injection 50, the system was set to stand-by for 24 hours to simulate the start-up of a new batch, then resumed to complete the 100 injections. See Figure 5.
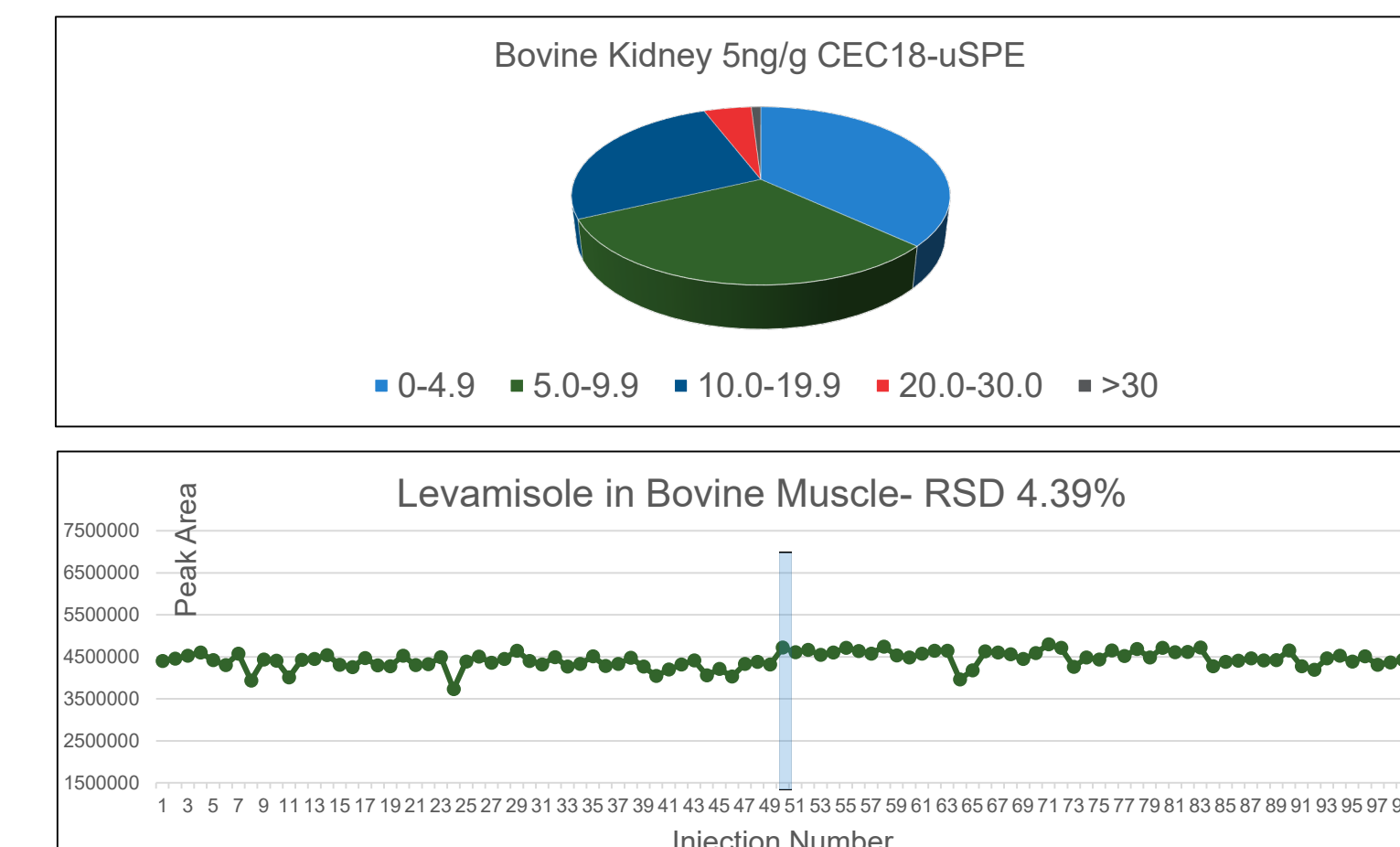

Figure 5: Top: Recoveries and %RSD for bovine kidney tissue extracts at 5 ng/g cleaned up with CEC18-µSPE miniaturized cartridges. Bottom: Levamisole in bovine muscle extract, cleaned up using the µSPE CEC18 cartridges. The blue bar represents the point where the system was set into standby for 24 hours, then resuming the analysis of the batch.

CONCLUSIONS

- On-line automation with the µSPE miniaturized cartridges saves time by reducing solvent consumption and labor, saving hours during batch sample preparation over manual methods.
- The sandwich injection technique will reduce sample handling and enable generation of excellent chromatography in pure solvents, avoiding sample dilution or solvent exchange of extracts.
- The automated µSPE cleanup afforded spike recoveries within 30–140% with corresponding %RSDs below 20% for over 95% of the target veterinary drugs in the tissue extracts.

REFERENCES

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