Time and Money Savings by the Implementation of Automated µSPE for Cleanup of QuEChERS Extracts of Veterinary Drugs

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PAL SYSTEM Ingenious sample handling

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Overview

Purpose: Demonstrate the use of automated µSPE cleanup Vs. manual cleanup of animal tissue extracts to save time and money in a veterinary drug research laboratory.

Methods: Automated µSPE and manual dispersive SPE are compared via LC-MS/MS analysis.

Results: Short synopsis of the results.

Introduction

In this poster presentation, we compare two workflows for the cleanup of multi-class veterinary drugs in animal tissue matrices, based on the Thermo Scientific™ VetDrugs Explorer Collection (P/N VDX-TSQ02-10001, Thermo Fisher Scientific, Waltham MA, USA). A manual sample cleanup using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction is performed. The final cleanup steps and dilution with mobile phase prior to injection into an LC-MS/MS are amenable to automation. By automating these steps, we save time and labor, solvent volumes, and introduce a more consistent and reproducible cleanup and dilution, thus improving the analytical results. The amount of time, labor, and solvents saved are quantitated for a typical lab and presented in this poster.

Methods

Sample Preparation

Samples were prepared following the protocol included with the VetDrugs Explorer Collection. Tissue samples were prepared by weighing 5g of bovine muscle and placing it in a 50mL centrifuge tube. 2mL of acetonitrile was added as well as 0.5mL of 0.2M ammonium oxalate and 0.1M disodium EDTA solution. An additional 13mL of acetonitrile is added and the sample is shaken to homogenize the mixture. Next, 5g of anhydrous sodium sulfate is added and shaken. After a 30-minute wait, samples are centrifuged for 10 minutes (4500 rpm), then the supernatant is decanted. At this point, samples for automated µSPE are placed on a robot for automated cleanup, while the samples for manual cleanup are mixed with a dispersive SPE material, filtered, and diluted.

Manual SPE Cleanup

The decanted supernatant is placed in a centrifuge tube and 500mg of CEC18 (Carbon End Capped C18) dispersive SPE (dSPE) is added to the supernatant. The tube is shaken a minimum of 4 times at intervals over at least 15 minutes. The tube is then centrifuged, (4500 rpm at 20°C) for 5 minutes. 3mL of the extract is added to 1mL of Mobile Phase A (see below for composition) and mixed. Finally, the final extract is filtered with a 0.45 µm PTFE syringe filter into and amber 2mL autosampler vial for analysis.

Automated µSPE Cleanup

The automated µSPE cleanup and sample injection is carried out using the Thermo Scientific™ TriPlus™ RSH autosampler. The decanted supernatant is placed in a 2mL amber autosampler vial, and placed in the cooled Peltier autosampler rack at 8°C. The autosampler aspirates 300µL of supernatant using the µSPE tool, selects a µSPE cartridge packed with 15 mg of CEC18 from the cartridge tray, and elutes the extract through the cartridge at a flow rate of 2 µL/sec. This loading amount and flow rate was based on the work of Lehotay et al. (2016) [1]. The extract is collected at the elution tray in a 2nd 2 mL autosampler vial with a 350 µL glass insert. After the extract is collected, the µSPE cartridge is returned to the cartridge tray. The autosampler then transfers 100 µL of the extract to a 3rd vial with insert in the cooled stack, then adds 33 µL of Mobile Phase A from the solvent module to the 3rd vial and mixes the solution. The autosampler exchanges the µSPE tool for the LCMS injection tool. The autosampler aspirates 6 µL of the final diluted extract and injects into a 2 µL sample loop on the injection valve. The whole process takes 8.5 minutes to complete. A photograph of the autosampler with its modules is shown in Figure 1, while a detail of the extraction is shown in Figure 2.

LC Separation

HPLC Thermo Scientific Ultimate™ 3000 HLPC pump, column oven, and degasser unit.

Column Thermo Accucore™ VDX column (100 mm x 2.1 mm x 2.6 µm, P/N VDX-102130).

Mobile Phase A Water with 0.05 % Formic Acid

Mobile Phase B 47.5% Methanol, 47.5% Acetonitrile, 5% Water, 0.05% Formic Acid

HPLC Gradient

Time (min)	Flow (mL/min)	%B
0	0.3	2
2	0.3	2
3	0.3	20
11	0.3	100
13	0.4	100
14.4	0.4	100
14.5	0.35	2
16	0.3	2
17	0.3	2

Mass Spectrometry

Thermo Scientific TSQ Fortis™ triple quadrupole mass spectrometer operating in SRM mode to monitor ~130 Veterinary Drug compounds. A total ion chromatogram is shown in Figure 3.

Data Analysis

Samples were acquired and processed with Thermo Scientific™ TraceFinder 4.1 Software.

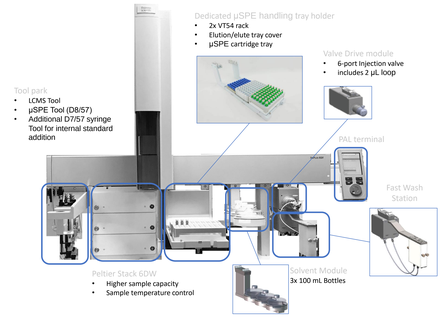

Figure 1. TriPlus RSH autosampler. From left to right, Tool Park Station, Peltier Stack, µSPE tray holder, Solvent Module, Valve Drive Module, and Wash Station.

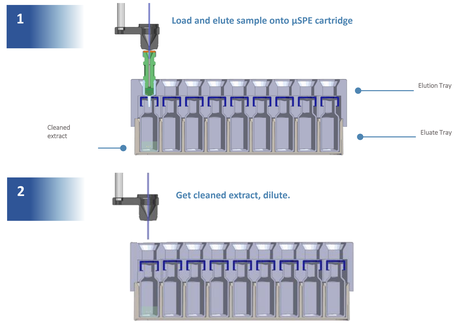

Figure 2. Side profile of automated sample loading onto µSPE cartridge, elution, and transfer of cleaned extract to dilution vial (not pictured) prior to dilution and injection.

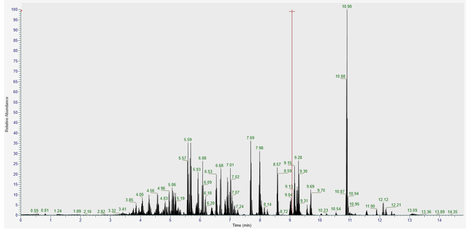

Figure 3. Total Ion Chromatogram for the µSPE cleaned sample at a concentration of 100 ng/g.

Results

Timing of 15 Sample Extracts

A batch size of 15 samples was timed to compare manual to automated SPE cleanup. The time required for each step is listed in Table 1.

Step	Step Description	Time (min)
1	Prep and Label Tubes	40
2	Weigh Samples	45
3	Spike Samples	25
4	Add Ammonium Oxalate/EDTA	6
5	Add Acetonitrile	10
6	Multi Tube Vortex Homogenization	15
7	Add $NaSO_4$	30
8	$NaSO_4$ Wait, then Centrifuge	50
9*	Decant to Falcon Tube, add dSPE	20
10*	Multi Tube Vortex with dSPE	15
11*	Centrifuge	15
12*	Dilute 3:1 Extract:Mobile Phase A	15
13*	Transfer to Autosampler Vial	15

Table 1. Extraction steps and timing for each step. *Steps 9-13 are automated in the automated µSPE experiment.

Time Savings From Automated µSPE

The TriPlus RSH Autosampler takes care of extraction steps 9-13 listed in Table 1. This results in a time savings of 80 minutes for every 15 samples prepared. These 80 minutes are converted into a single 8.5 minute extraction, a nearly 10x time savings. The very first sample of a given sequence starts with the first extraction, taking 8.5 minutes prior to LC-MS/MS injection, however, every subsequent sample in the sequence is prepared during the LC runtime of the previous sample through “look ahead” or overlapping sample preparation. By utilizing look ahead sample prep, the next sample is ready for injection as the previous sample’s LC-MS/MS run is completed. This saves 8.5 minutes per sample in our automated process. As sample batch sizes are increased, this same time savings is realized for additional samples. Additionally, a more consistent extraction is achieved due to the constant 2 µL/min flow rate through the µSPE cartridge. Finally, each extract is analyzed in near “real-time,” immediately after cleanup. This helps with compounds that are susceptible to degradation.

Money Savings From Automated µSPE

The monetary savings gained from µSPE are largely due to labor costs, and smaller solvent volumes that can be employed. Labor costs of 80 minutes per 15 sample batch. While the initial 8 steps of the sample preparation are identical in this study, further studies will investigate smaller initial sample sizes to scale down the homogenization, which uses substantial amounts of costly acetonitrile. If the sample could be scaled down by a factor of 10, the subsequent solvent usage would correspondingly be reduced by the same factor.

Results, continued

Recovery

Extraction recovery was calculated for 13 compounds, shown in Table 2. Extraction recovery was determined by comparing the quantitated amount for triplicate injections at a concentration of 5 ng/g cleaned up using µSPE compared to a direct injection of the same extract. The intended veterinary use for each compound is also listed. For the 5 ng/g concentration, recoveries ranged from 49.2% for Clopidol, to 144.5% for Ketoprofen. Recoveries are skewed high because analyzing raw extracts that were not subjected to SPE cleanup of any kind resulted in ion suppression compared to the samples that were cleaned up via µSPE.

Veterinary Drug	Usage	Recovery %
Acepromazine	Sedative	88
Albendazole	Anthelmintic	120
Azaperone	Sedative	92
Brilliant Green	Dye	123
Chlorpromazine	Anti-nausea/Muscle relaxant	114
Clopidol	Coccidiostat	84
Derquantel	Anthelmintic	69
Difloxacin	Antibiotic	75
Ipronidazole	Antiprotozoal	95
Ketoprofen	NSAID	128
Thiabendazole	Anthelmintic	175
Victoria blue bo	Dye	175
Xylazine	Adrenegic agonist	117

Table 2. Extraction recoveries for a range of veterinary drug compounds calculated at the 5 ng/g concentration level.

Conclusion

Automated µSPE is a viable alternative for cleanup of QuEChERS extracts of veterinary drugs.

- For a batch of 15 samples, 80 minutes of time is saved, a 26% time savings.
- Each sample is analyzed immediately after cleanup, reducing degradation time by sitting in an autosampler.
- Automated µSPE delivers consistent flow rates for each sample.
- Recovery will be compared to manually extracted dSPE samples in future work, rather than to spiked bovine matrix samples.

References

1. Lehotay, S. J., Han, L., Sapozhnikova, Y. (2016). Automated Mini-Column Solid-Phase Extraction Cleanup for High-Throughput Analysis of Chemical Contaminants in Foods by Low-Pressure Gas Chromatography—Tandem Mass Spectrometry. *Chromatographia,* DOI 10.1007/s10337-016-3116-y