Application Note

Food Testing

Multiclass Multiresidue Veterinary Drug Analysis in Beef Using Agilent Captiva EMR—Lipid Cartridge Cleanup and LC/MS/MS

Authors

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Abstract

Agilent Captiva Enhanced Matrix Removal—Lipid (EMR—Lipid) cartridges are the second generation of Agilent EMR—Lipid products, and are implemented in a solid phase extraction (SPE) format for highly selective lipids removal without impacting analyte recovery. The SPE cartridges provide a simple pass-through cleanup workflow that requires minimal method development. The tube is optimized to facilitate hands-free gravity elution for large volume sample cleanup without the hassle of control on vacuum or pressure. To facilitate lipid capture and increase hydrophobic compound recoveries, the Captiva EMR—Lipid sorbent requires the addition of 20 % water to activate the EMR sorbent for cleanup. This study demonstrates the application of Captiva EMR—Lipid in the analysis of 39 representative multiclass, veterinary drugs in beef. A two-step sample extraction was used to achieve satisfactory recoveries for both hydrophilic and hydrophobic compounds. The extracts were then combined and applied to a Captiva EMR—Lipid cartridge for cleanup. The method was assessed for matrix effect, analyte recovery, and method reproducibility. When compared to other cartridge pass-through cleanup products, the Captiva EMR—Lipid cartridge provided more efficient matrix cleanup and better recovery of hydrophobic analytes.

Introduction

Veterinary drugs are widely used in animal food to prevent animal diseases, or as a growth promoter. These drugs can accumulate in animal tissues, and improper use can result in drug residues in edible tissues, resulting in risks to human health. With increased public attention on food safety, regulation of veterinary drugs used in animal food production is imposed in most countries[1,2]. Animal foods, such as muscle, liver, and eggs, are complex matrices; thus it is critical to use an efficient preparation method for sample extraction, cleanup, and concentration (when needed) before instrumental analysis. The established sample pretreatment methods include traditional solvent extraction, solid phase extraction (SPE), or a combination of techniques. These methods are usually labor-intensive, time-consuming, and only suitable for limited classes of compounds, requiring method development.

Multiclass, multiresidue methods are increasingly becoming popular in regulatory monitoring programs due to their increased analytical scope and laboratory efficiency. Analysis of more than 100 veterinary drugs has been reported in literature in the past few years[3-5]. The sample pretreatment usually involves pre-extraction with a mixture of acetonitrile (ACN)/water, followed by C18 cleanup or a combination of other cleanup techniques. However, current cleanup techniques have limitations such as inefficient lipid removal and unwanted analyte loss. The ACN/water mixture direct extraction can compromise the protein removal efficiency and hydrophobic analyte extractabilities during the extraction step.

Agilent Enhanced Matrix Removal—Lipid (EMR—Lipid) dSPE cleanup has gained a lot of attention since it was introduced in 2015. The EMR—Lipid sorbent specifically interacts with the unbranched hydrocarbon chains of lipid compounds using a combined mechanism of size exclusion and hydrophobic interaction. This combined mechanism provides highly selective lipid removal without unwanted impact on target analytes. This technology has been used for multiclass, multiresidue pesticides analysis in complex matrices, providing superior matrix cleanup and optimum results[6,7]. The second-generation product, Agilent Captiva EMR—Lipid cartridges, reduces the water percentage needed for sorbent activation, and eliminates the need for a polishing step subsequently. This simplifies the workflow and improves the solubility of hydrophobic compounds during cleanup.

This study investigates the use of Captiva EMR—Lipid cartridge cleanup during sample preparation for the analysis of 39 representative and challenging veterinary drugs in beef. The selected representative veterinary drugs were from 17 different classes including hydrophilic and hydrophobic drugs, acidic, neutral, and basic drugs, and some of the most difficult classes, such as tetracycline and β-lactam. Table 1 shows the drug class, regulatory information, retention time, and MS/MS conditions for analysis of these veterinary drugs.

Experimental

Reagent and Chemicals

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN) was from Honeywell (Muskegon, MI, USA). Dimethyl sulfoxide (DMSO) and ethylenediaminetetraacetic acid, disodium salt, dehydrate (NaEDTA) were from Sigma-Aldrich (St Louis, MO, USA). Reagent grade formic acid (FA) was from Agilent (p/n G2453-86060). The veterinary drugs standards and internal standard were purchased from Sigma-Aldrich (St Louis, MO, USA).

Solution and Standards

Standard and internal standard (IS) stock solutions were made in DMSO at 2.0 mg/mL, except for the following:

- Danofloxacin stock solution was made in DMSO at 1.0 mg/mL.
- Ciprofloxacin stock solution was made in DMSO at 0.25 mg/mL.

All the β-lactam drugs and cefazolin stock solutions were made in water at 2.0 mg/mL. All stock solutions were prepared in amber glass vials, except for β-lactam drugs, Cefazolin, and tetracycline drugs stock solutions, which were prepared in polypropylene plastic tubes. All solutions were stored at –20 °C. The 39 compounds were separated into two groups: group 1 (G1) and group 2 (G2), based on their instrument response. Two combined standard working solutions, 25/5 µg/mL and 5/1 µg/mL (G1/G2), were prepared in 1:1 ACN/water. A 25 µg/mL working solution of Flunixin-d3 IS was prepared in 1:1 ACN/water.

A cold extraction solvent was prepared daily by adding 2 mL of formic acid and 2 mL of DMSO into 100 mL of precooled ACN. A 0.1 M Na EDTA solution was made by dissolving 1.8612 g of NaEDTA powder in 50 mL Milli-Q water. The solution was stored at room temperature. A 80:20 ACN/water was made by combining 80 mL of ACN with 20 mL of Milli-Q water.

Table 1. List of selected veterinary drugs for analysis; drug class, US tolerance, retention time, and MRM conditions.

Analyte	Drug class	US tol. (µg/g)	Retention time (min)	Polarity	Precursor ion (*m/z*)	Product ion			
						Quant ion	CE (v)	Qual ion	CE (v)
2-Thiouracil	Thyreostat	–	1.41	NEG	127	57.9	17	–	–
Amoxicillin	β-Lactam	0.01	1.94	POS	366.1	349.2	5	114	25
Metronidazole-OH	Nitroimidazole	[d]	2.21	POS	188.1	123.1	9	126.1	13
Lincomycin	Lincosamide	0.1[c]	3.80	POS	407.2	126.1	37	70.1	80
Levamisole	Anthelmintic	0.1[f]	3.90	POS	205.1	178.1	21	91.1	41
Minocycline	Tetracycline		4.14	POS	458.2	440.9	17	282.9	49
Ampicillin	β-Lactam	0.01	4.15	POS	350.1	106	33	79.1	61
Norfloxacin	Fluoroquinolone	[d]	4.36	POS	320.1	276.1	17	302.2	21
Oxytetracycline	Tetracycline	2[e]	4.42	POS	461.2	426.1	17	443.2	9
Ciprofloxacin	Fluoroquinolone	[d]	4.43	POS	332.1	231	45	314.3	21
Tetracycline	Tetracycline	2e	5.37	POS	445.2	409.9	17	153.9	33
Danofloxacin	Fluoroquinolone	0.2[b,f]	4.53	POS	358.2	340.2	21	81.9	53
Ractopamine	β-Agonist	0.03[f]	4.55	POS	302.2	107	33	77	77
Cefazolin	Cephalosporin	–	4.78	POS	455	323.1	9	156	13
Sulfamethizole	Sulfonamide	–	4.88	POS	271	156.1	13	92	29
Sulfamethoxypyridazine	Sulfonamide	–	4.91	POS	281.1	92	33	65.1	57
Demeclocycline	Tetracycline	–	4.94	POS	465.1	429.9	21	448.0	13
Difloxacin	β-Lactam	–	4.97	POS	400.2	356.3	17	382.0	25
Morantel	Anthelmintic	–	5.08	POS	221.1	123.1	37	76.9	80
Gamithromycin	Macrolide	0.15	5.22	POS	777.6	157.9	41	83.1	65
Chlortetracycline	Tetracycline	2[e]	5.24	POS	479.1	444.2	21	462.1	17
Doxycycline	Tetracycline	–	5.36	POS	445.2	428.1	17	410.2	25
Florfenicol	Phenicol	0.2[c]	5.69	NEG	356.0	336.0	5	185.1	13
Chloramphenicol	Phenicol	[d]	5.86	NEG	321	152	17	257.1	9
Tylosin	Macrolide	0.2[g]	5.94	POS	916.5	173.9	45	772.5	33
Prednisone	Corticosteriod	–	6.02	POS	359.2	147.2	33	341.2	9
Clorsulon	Flukicide	0.1[f]	6.09	NEG	377.9	341.9	9	–	–
Acetopromazine	Tranquilizer	–	6.09	POS	327.2	86	21	58	45
Chlorpromazine	Tranquilizer	–	6.69	POS	319.1	86	21	58.1	45
Penicillin V	β-Lactam	0.05[a]	6.70	POS	351.6	160.1	9	113.9	45
Oxacillin	β-Lactam	–	6.93	POS	402.1	160.0	17	242.9	9
Fenbendazole	Anthelmintic	–	6.98	POS	300.1	268.1	25	159.1	41
Cloxacillin	β-Lactam	0.01[a]	7.20	POS	436.1	159.9	9	276.8	13
Nafcillin	β-Lactam	–	7.35	POS	415.1	199.0	13	171.0	41
Ketoprofen	Tranquilizer	–	7.44	POS	255.1	208.9	13	77	57
Oxyphenbutazone	NSAID	–	7.47	NEG	323.1	295	17	133.9	25
Flunixin-d3 (NEG)	–	–	7.81	NEG	298.1	254.2	17	192	37
Flunixin-d3 (POS)			7.81	POS	300.1	282	25	264	41
Melengestrol acetate	Other	0.025[h]	9.05	POS	397.2	279.2	21	337.4	13
Niclosamide	Flukicide	–	9.07	NEG	325	170.9	25	289.1	13
Bithionol	Flukicide	–	9.07	NEG	352.9	161	21	191.8	25

[a] Tolerance in uncooked edible tissue of cattle
[b] Tolerance in cattle liver
[c] Tolerance in swine muscle
[d] Banned for extralabel use
[e] Tolerance is for the sum of residue of tetracycline including chlortetracycline, oxtetracycline, tetracycline in muscle
[f] Tolerance in cattle muscle
[g] Tolerance in uncooked cattle fat, muscle, liver, and kidney
[h] Tolerance in cattle fat

Equipment and Material

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of an:

- Agilent 1290 Infinity binary pump (G4220A)
- Agilent 1290 Infinity high performance autosampler (G4226A)
- Agilent 1290 Infinity thermostatted column compartment (G1316C)

The UHPLC system was coupled to an Agilent G6490 Triple Quadrupole LC/MS system equipped with an Agilent Jet Stream electrospray ionization source. Agilent MassHunter workstation software was used for data acquisition and analysis.

Other equipment used for sample preparation:

- 2010 Geno/Grinder (Metuchen, NJ, USA)
- Centra CL3R centrifuge (Thermo IEC, MA, USA)
- Multi Reax Test Tube Shaker (Heidolph, Schwabach, Germany)
- Eppendorf pipettes and repeater
- Agilent Captiva EMR—Lipid cartridge, 6 mL, 600 mg (p/n 5190-1004) and, 3 mL, 300 mg (p/n 5190-1003)
- Agilent Vac Elut SPS 24 Manifold with collection rack for 16 × 100 mm test tubes (p/n 12234004)

Instrument Conditions

Figure 1 shows the typical chromatograms for A) beef extract matrix blank, and B) beef extract fortified with 5/1 ng/g (G1/G2) veterinary drug standards (limit of quantitation level).

Sample Preparation

Figure 2 shows the final sample preparation procedure to prepare beef samples. The following points need to be emphasized for the optimized extraction and cleanup method for the beef samples:

- Beef purchased from a local grocery store was used for method development and the validation study. Samples were homogenized and stored at –20 °C.
- After prespiking the standard and IS into the homogenized beef sample, the samples stood at room temperature for 20 minutes. This allowed the spiked standards to infiltrate the sample matrix and equilibrate prior to sample extraction.
- The use of water for sample extraction is necessary to achieve homogeneous mixing with the beef, and ensure the recovery and stability of the polar drug compounds.

HPLC conditions

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 150 × 2.1 mm, 2.7 µm (p/n 693775-902) Agilent InfinityLab Poroshell 120 EC-C18 UHPLC guard, 5 × 2.1 mm, 2.7 µm (p/n 821725-911)
Flow rate	0.3 mL/min
Column temperature	40 °C
Autosampler temperature	4 °C
Injection volume	3 µL
Mobile phase	A) 0.1 % FA in water B) 0.1 % FA in acetonitrile
Needle wash	1:1:1:1 ACN/MeOH/IPA/H_2O w/ 0.2 % FA
Gradient	Time (min) %B Flow rate (mL/min) 0 10 0.3 0.5 10 0.3 8.0 100 0.3
Stop time	12 minutes
Post time	3 minutes

MS conditions

Parameter	Value
Positive/negative mode	
Gas temperature	120 °C
Gas flow	14 L/min
Nebulizer	40 psi
Sheath gas heater	400 °C
Sheath gas flow	12 L/min
Capillary	3,000 v
iFunnel parameters	Positive Negative High-pressure RF 90 V 90 V Low-pressure RF 70 V 60 V

A one-step extraction with a 20:80 water/ACN mixture greatly reduces the solvent extractability of hydrophobic compounds and protein removal efficiency. Therefore, a two-step extraction protocol was used: a 2 mL aqueous extraction followed by an 8 mL solvent extraction.

- To prevent loss of tetracycline compounds due to chelation, a 0.1 M EDTA buffer solution was used for aqueous extraction.
- To improve solvent extractability for difficult drug compounds, such as tetracycline, β-lactam, and fluoroquinolones, 2 % formic acid and 2 % DMSO was added into the extraction solvent, ACN.
- To improve the phase separation with solid residues, especially in the first aqueous extraction step, a cooled centrifugation (4 °C) was used.
- To ensure the complete elution of analytes from the cartridge, a secondary elution after EMR—Lipid cartridge cleanup was performed.

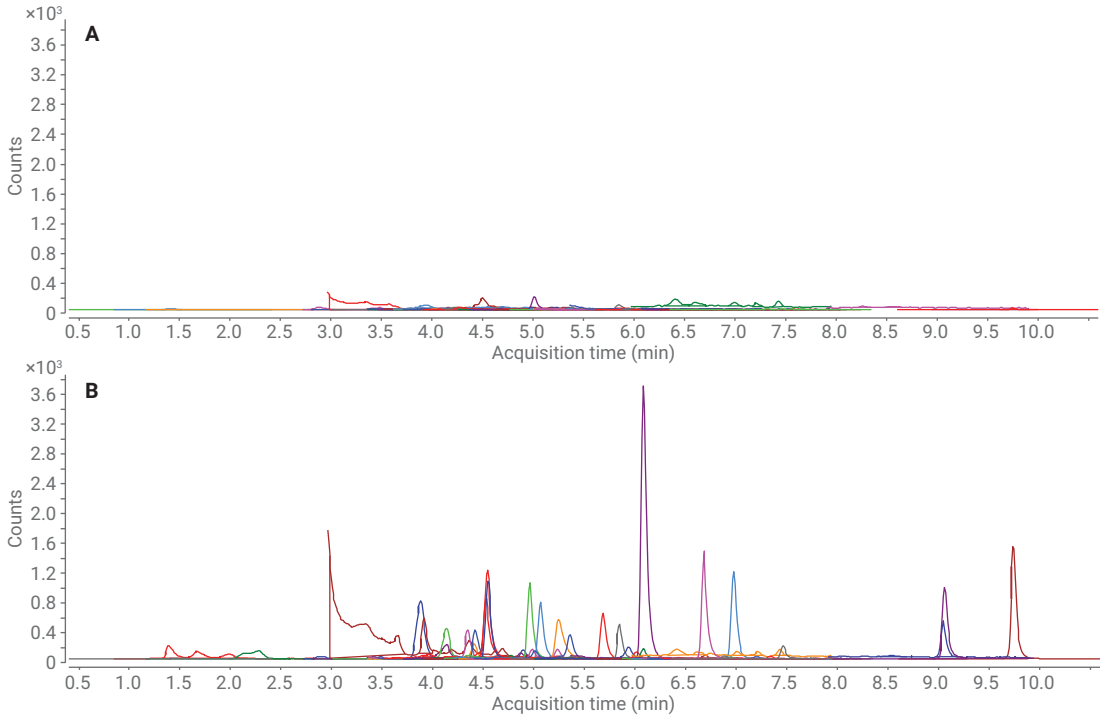

Figure 1. LC/MS/MS chromatograms for A) beef extract matrix blank, and B) beef extract fortified with 5/1 ng/g (G1/G2) vet drug standards. Group 1 (G1) analytes correspond to a 5 ng/g fortification level, while group 2 (G2) compounds correspond to a 1 ng/g fortification level. Refer to Table 1 for analyte identification with elution order, and Table 2 for compound group identification.

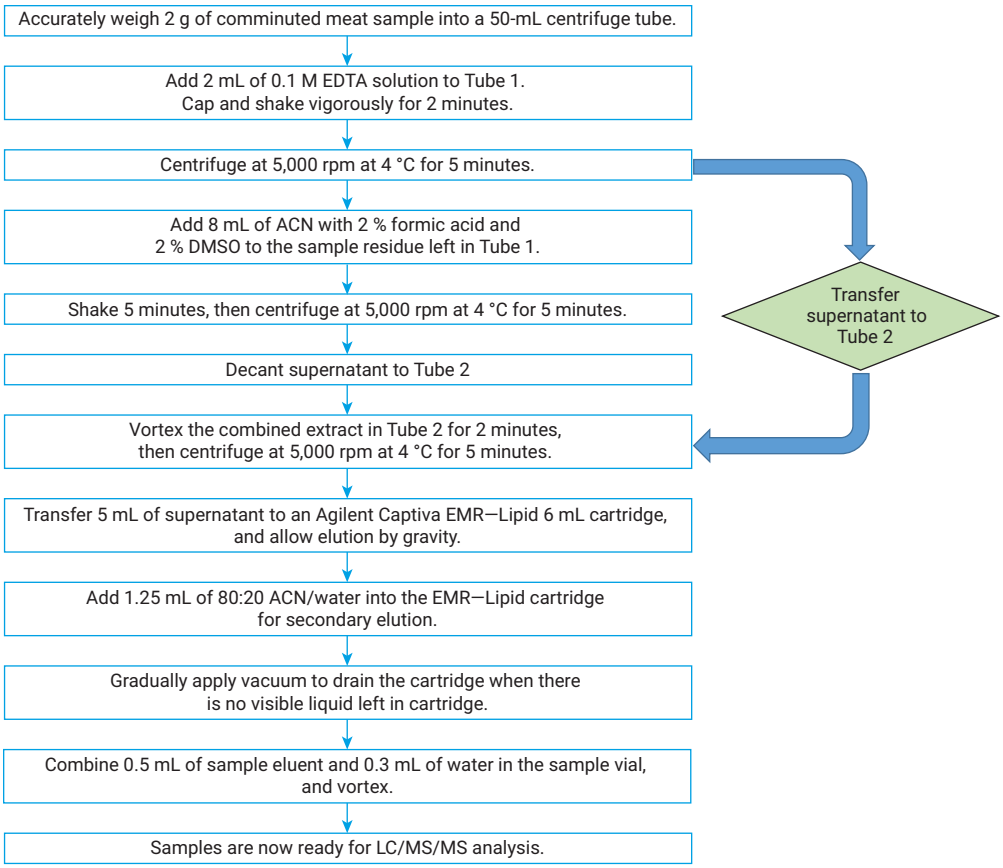

Figure 2. Beef sample extraction and following cleanup procedure using an Agilent Captiva EMR—Lipid 6 mL cartridge.

Calibration Standards and Quality Control (QC) Samples

Prespiked QC samples were fortified by spiking appropriate standard working solution into the homogenized beef samples with six replicates of low, mid, and high levels.

- For G1 analytes, the spiking levels were 10, 50, and 750 ng/g.
- For G2 analytes, the spiking levels were 2, 10, and 150 ng/g.

A standard 25/5 µg/mL (G1/G2) working solution was used to spike high-level QC samples; while a 5/1 µg/mL (G1/G2) standard solution of was used to spike low and mid QC samples. The IS solution was also spiked into all samples except matrix blank, corresponding to 200 ng/g of Flunixin-d3.

Matrix-matched calibration standards and postspiked QC samples were prepared by spiking appropriate standard and IS working solutions into the matrix blank eluent after cartridge cleanup. The spiking concentrations for calibration standards were 5, 25, 50, 250, 750, and 1,000 ng/g (G1) or 1, 5, 10, 50, 150, and 200 ng/g in beef (G2), and 200 ng/g IS; spiking concentrations for postspiked QC samples were 10, 50, and 750 ng/g (G1) or 2, 10, and 150 ng/g (G2).

Determine the Amount of Co-extractives

The amount of co-extractive residue was determined by gravimetric measurements[5] for EMR—Lipid cartridge and other manufacturer's cartridge cleanup. The co-extractive residue weight was collected based on 1 mL of ACN final extract, while the matrix co-extractives removal efficiency by cleanup was calculated by comparing the ratio of the difference of co-extractive residue weight with and without cartridge cleanup.

Matrix Effect Assessment

Chromatographic matrix effect was assessed by a post column infusion test. The matrix blank samples were injected with simultaneous post column infusion of a 10 ng/mL neat standard veterinary drug solution at 90 µL/min. All compound transitions were monitored through the chromatographic window.

Analyte Recovery Assessment by Cartridge Cleanup

Cartridge cleanup impact on analyte recovery was evaluated by prespiking standards into beef extract blank before cartridge cleanup, and postspiking standards into beef extract blank eluent after cartridge cleanup. The collected recovery results only reflect the impact of cartridge cleanup on analyte recovery, and exclude other contributions from the extraction procedure. It is a more direct comparison of cartridge cleanup impact on analyte recovery. The EMR—Lipid cartridges, 3 mL and 6 mL, were compared to corresponding other manufacturer's cartridges. For 3 mL cartridges, the sample loading volume was 2.5 mL, and the secondary elution volume was 0.625 mL.

Method Validation

To ensure calibration reproducibility, the developed method was validated by running a full quantitation batch with two separate calibration curves run before and after the QC samples.

Results and Discussion

Ease of Cartridge Cleanup

An important feature of using Captiva EMR—Lipid cartridges for complex sample matrix cleanup is ease-of-use. The EMR—Lipid sorbent targets unwanted lipid interferences instead of analytes, and implements a pass-through approach. The sample mixture is loaded onto the cartridge and is allowed to pass through the packed Captiva EMR—Lipid sorbent in the cartridge. Lipids are trapped in the sorbent, while target analytes pass through the cartridge, foregoing the need for traditional SPE steps such as conditioning, washing, and elution. Therefore, the use of Captiva EMR—Lipid cartridges is greatly simplified, saving a significant amount of time and solvent. The pass-through cleanup does not need traditional SPE method development for washing and elution steps. A possible method modification for Captiva EMR—Lipid is the use of a secondary elution step to achieve complete elution. It is recommended to use a 20:80 water/ACN mixture at approximately 20–25 % of the sample loading volume for secondary elution (for example, a 5 mL load followed by a 1–1.25 mL second elution). Lastly, the product design for gravity elution allows hands-free operation once the sample is loaded onto EMR—Lipid cartridges. Control of the elution flow rate by manipulating vacuum or positive pressure is not required. These features provide increased lab productivity when using Captiva EMR—Lipid cartridge cleanup to prepare complex food samples.

Amount of Co-extractives

Table 2 shows the sample co-extractives gravimetric test results. The co-extractive residue weight study is an important method to evaluate how efficient sample extraction and cleanup method can control the residue of matrix co-extractives, including proteins, lipids, salts, and other matrix components in the final sample being injected onto the instrument. Matrix co-extractives residue weight shows the entire co-extractives amount, whether they are detectable on an instrument or not. Co-extractive residues, whether being detectable on an instrument or not, can introduce matrix effects, impact method reliability and data quality, accumulate on the instrument flow path, such as the column and MS source, and deteriorate the detection system's long-term performance.

The less matrix co-extractive residue, the better method reliability and instrument performance. The results clearly demonstrated that Captiva EMR—Lipid cartridge cleanup provided better matrix cleanup efficiency with less co-extractive residue weight than the other manufacturer's cartridge cleanup.

Matrix Effect Assessment

Postcolumn infusion (PCI) of veterinary drug standards was used to evaluate matrix effects in beef extract with and without Captiva EMR—Lipid cartridge cleanup. All analytes were monitored through the entire acquiring window. The PCI profiles reflect the matrix impact for analytes monitored under both positive and negative mode. Figure 3 shows the PCI profiles.

Table 2. Beef matrix co-extractives residue amount and matrix removal by cartridge cleanup.

Cleanup technique	Co-extractives per 1 mL of ACN final extract (mg)	Matrix co-extractives removal efficiency by cleanup (%)
No further cleanup	7.68	–
Agilent Captiva EMR—Lipid 3 mL cartridge	4.38	43
Agilent Captiva EMR—Lipid 6 mL cartridge	4.03	48
Other manufacturer's 3 mL cartridge	5.91	23
Other manufacturer's 6 mL cartridge	6.30	18

$$\text{Matrix co-extractives removal efficiency (\%)} = \frac{(\textit{Amount of co-extractives without cleanup} - \textit{Amount of co-extractives with cleanup})}{\textit{Amount of co-extractives without cleanup}} \times 100$$

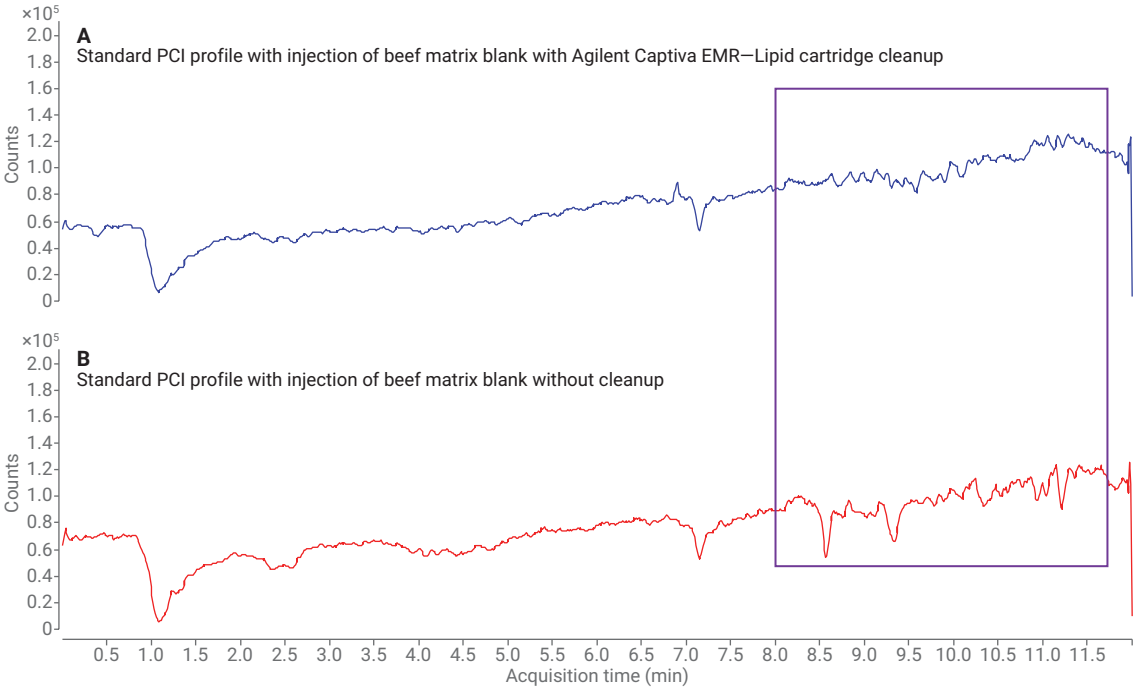

Figure 3. Matrix effect study by standard PCI with the injection of beef matrix blank with Agilent Captiva EMR—Lipid cartridge cleanup (A) and without any cleanup (B).

Figure 3B shows the PCI profile in red, matrix ion suppressions (overall low baseline) observed with the injection of beef extract without cleanup. Matrix ion suppression can dramatically impact the method sensitivity, reliability, and data quality for analytes within the coelution window. Conversely, Figure 3A shows that the PCI profile (in blue) becomes much smoother and more consistent with fewer troughs when injecting beef extract with EMR—Lipid cartridge cleanup. The highlighted RT window in Figure 3 shows the reduced matrix ion suppression effect comparison.

Cartridge Cleanup Recovery

Traditionally, the mechanism for lipid removal is based on hydrophobic interaction between lipids and sorbent. This mechanism can be efficient, especially when using strong hydrophobic interaction as the major sorbent function mechanism to trap and remove lipids. However, this interaction mechanism is not selective, and it does not differentiate unwanted lipids and wanted hydrophobic analytes from sample. Therefore, while the sorbent works on trapping lipids, it can also strongly interact with hydrophobic analytes, resulting in dramatic analyte loss during the cartridge cleanup. Furthermore, not all classes of lipids can be removed efficiently through hydrophobic interactions (for example, phospholipids).

Captiva EMR—Lipid sorbent uses a novel chemistry that combines size exclusion and hydrophobic interactions to significantly improve lipid removal selectivity. Only the lipid-like molecules containing straight unbranched hydrocarbon chains, preferably with more than six carbons, have access to EMR—Lipid sorbent pores. Once the lipids enter the EMR—Lipid sorbent, they are trapped inside with strong hydrophobic interaction. Other hydrophobic molecules that do not resemble lipids and are too bulky to enter the EMR—Lipid sorbent will stay in solution for subsequent analysis. Thus, EMR—Lipid sorbent can efficiently differentiate lipids from other hydrophobic molecules, dramatically improving selectivity, and decreasing hydrophobic compound loss during cleanup.

This mechanism has been well proven by the cartridge cleanup recovery study. In this study, the standards were prespiked into the beef blank extract before cartridge cleanup, and postspiked into the blank eluent after cartridge cleanup. The recovery data only indicate the cartridge cleanup impact on analytes. The comparison study included four types of cartridges: Captiva EMR—Lipid 3 mL (300 mg) and 6 mL (600 mg) cartridges, and other manufacturer's 3 mL (60 mg) and 6 mL (500 mg) cartridges. Figure 4 shows the study results. The analytes shown in the comparison are more hydrophobic compounds, eluting later on the C18 column. EMR—Lipid 3 mL and 6 mL cartridges provide consistent superior cartridge cleanup recoveries for compounds from mid to high hydrophobicity. However, for the other manufacturer's cartridge cleanup, which uses hydrophobic interaction mostly for lipids removal, the more hydrophobic (late eluting) analytes had lower recovery. When the comparable sorbent bed mass contained in 6 mL cartridges (500 mg) was used, the medium to high hydrophobic compounds were significantly retained. For example, as the last two most hydrophobic compounds, niclosamide and bithionol with log P >5, the other manufacturer's 6 mL cartridge cleanup recoveries were single digits, indicating substantial analyte loss on-cartridge. Their 3 mL cartridges use much less sorbent to balance hydrophobic analyte loss. In summary, the other cartridge cleanup tube with 60 mg sorbent sacrifices the cartridge matrix cleanup efficiency, and increasing to a higher bed mass lowers the recoveries for hydrophobic compounds to unacceptably low levels (<40 %). This study clearly demonstrates that EMR—Lipid sorbent provides a highly selective interaction mechanism for lipids, ensuring acceptable target analyte recoveries, especially for hydrophobic analytes.

Method Validation

The optimized extraction and cleanup method was validated by running a full quantitation batch. The methodology is described in the Experimental section. Internal standard (Flunixin-d3 for both positive and negative mode) was used for quantitation. However, as the absolute recoveries are the greatest concern when evaluating a new sample preparation method, the prespiked and postspiked QCs at three levels were included in the validation run. Table 3 lists the quantitation results in detail, and a summarized figure (Figure 5) was generated by average recovery and precision at each level. Acceptable recoveries (60–120 %) were achieved for most analytes at three levels (94 %), with the exception of two outliers, acepromazine and chlorpromazine. A confirmatory study reveals that these compounds can undergo analyte loss during the extraction step of the protocol. However, the RSD values for six replicates of these two compounds at each level were exceptional, with <10 % RSD for 91 % of analytes, and 10–20 % RSD for the remaining 9 % of analytes.

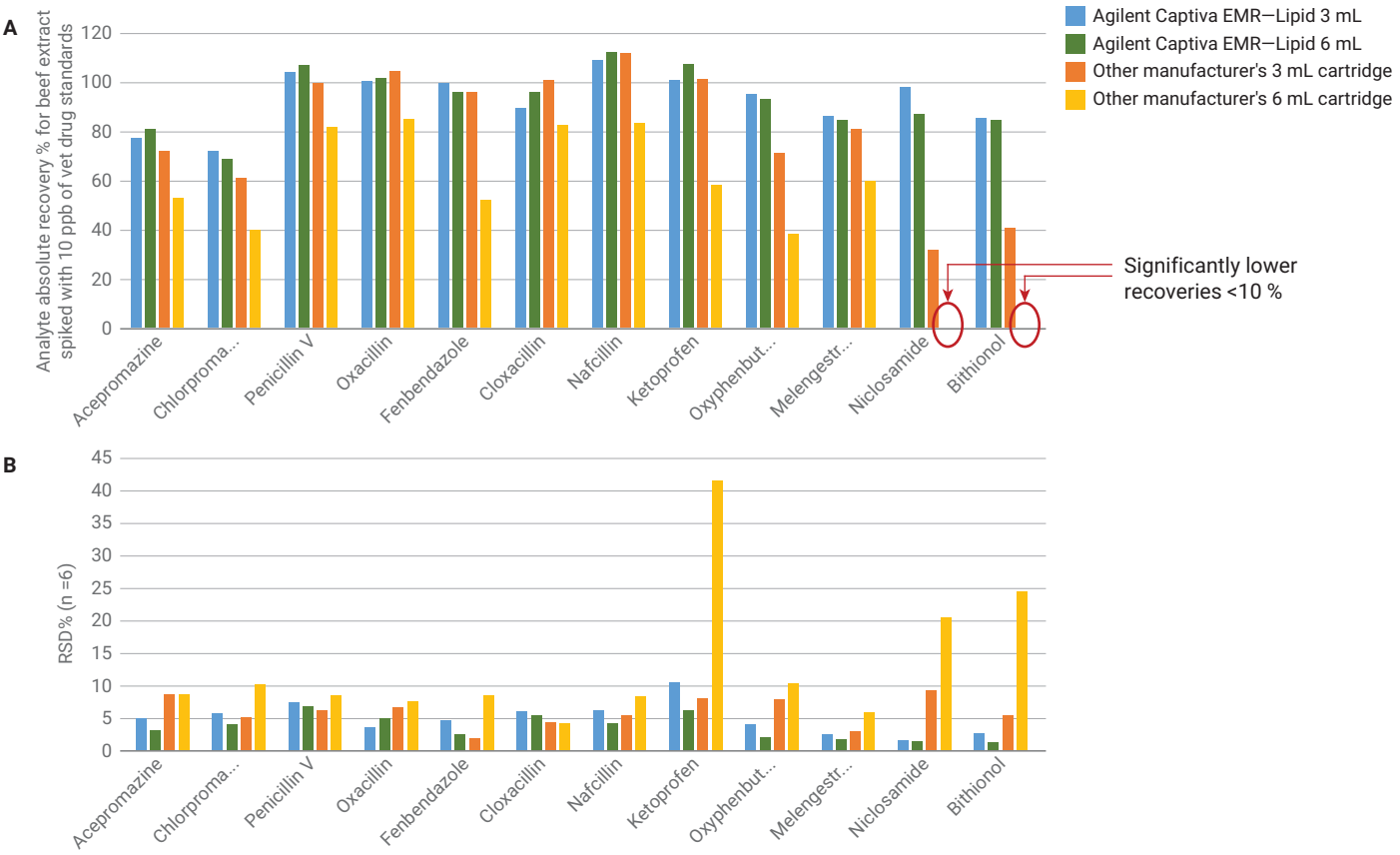

Figure 4. Cartridge cleanup comparison for hydrophobic analyte recovery (A) and reproducibility (B) from beef extract. The standard was spiked into beef extract before cartridge cleanup at 10 ng/mL. Analytes order from left to right with increasing hydrophobicity.

Table 3. Method quantitation results for veterinary drug analysis in beef.

Group no.[a]	Analyte	Calibration curve		Method absolute recovery and precision									
				2 ng/g QCs (n = 6)		10 ng/g QCs (n = 6)		50 ng/g QCs (n = 6)		150 ng/g QCs (n = 6)		750 ng/g QCs (n = 6)	
		R^2	Cal. range (ng/g)	Rec%	RSD	Rec%	RSD	Rec%	RSD	Rec%	RSD	Rec%	RSD
1	2–Thiouracil	0.9862	5–1,000	–	–	94	6.7	116	2.2	–	–	103	4.1
1	Amoxicillin	0.9964	5–1,000	–	–	88	8.6	77	3.9	–	–	69	2.7
1	Metronidazole–OH	0.9963	5–1,000	–	–	112	3.8	108	1.8	–	–	103	2.1
1	Ampicillin	0.9926	5–1,000	–	–	88	9.6	84	3.8	–	–	82	4.2
1	Minocycline	0.9943	5–1,000	–	–	72	13.3	69	11.2	–	–	62	2.9
1	Oxytetracycline	0.9941	5–1,000	–	–	84	6.2	87	11.0	–	–	72	5.1
1	Tetracycline	0.9919	5–1,000	–	–	87	9.0	86	5.2	–	–	90	4.1
1	Cefazolin	0.9933	5–1,000	–	–	109	5.8	94	2.8	–	–	87	5.4
1	Demeclocycline	0.9966	5–1,000	–	–	80	17.6	86	3.4	–	–	86	3.8
1	Difloxacin	0.9824	5–1,000	–	–	122	7.3	102	5.9	–	–	102	5.0
1	Gamithromycin	0.9901	5–1,000	–	–	100	8.9	92	5.3	–	–	89	6.1
1	Chlortetracycline	0.9976	5–1,000	–	–	80	7.8	86	8.5	–	–	81	4.1
1	Doxycycline	0.9936	5–1,000	–	–	77	11.2	70	5.4	–	–	73	4.7
1	Florfenicol	0.9920	5–1,000	–	–	116	4.4	110	3.8	–	–	99	7.1
1	Chloramphenicol	0.9928	5–1,000	–	–	113	7.6	104	1.9	–	–	103	3.4
1	Prednisone	0.9932	5–1,000	–	–	110	6.7	110	5.5	–	–	106	5.4
1	Clorsulon	0.9927	5–1,000	–	–	114	12.1	97	4.8	–	–	98	5.7
1	Penicillin V	0.9952	5–1,000	–	–	97	4.3	100	6.3	–	–	100	7.1
1	Oxacillin	0.9942	5–1,000	–	–	96	12.0	99	8.2	–	–	99	5.1
1	Cloxacillin	0.9932	5–1,000	–	–	103	8.1	101	6.0	–	–	97	5.7
1	Nafcillin	0.9926	5–1,000	–	–	107	8.9	110	6.5	–	–	95	5.6
1	Oxyphenbutazone	0.9910	5–1,000	–	–	106	8.1	98	3.0	–	–	86	2.8
1	Melengestrol acetate	0.9942	5–1,000	–	–	117	7.0	114	3.0	–	–	102	5.1
1	Bithionol	0.9807	5–1,000	–	–	63	8.2	81	5.7	–	–	92	1.4
2	Lincomycin	0.9961	1–200	94	8.5	99	3.0	–	–	88	6.4	–	–
2	Levamisole	0.9942	1–200	111	2.1	109	3.0	–	–	99	1.3	–	–
2	Norfloxacin	0.9974	1–200	111	5.5	91	4.9	–	–	100	8.0	–	–
2	Ciprofloxacin	0.9965	1–200	114	11.8	103	6.9	–	–	103	4.0	–	–
2	Danofloxacin	0.9969	1–200	101	8.3	94	5.8	–	–	99	5.6	–	–
2	Ractopamine	0.9858	1–200	120	6.5	110	5.5	–	–	109	3.2	–	–
2	Sulfamethizole	0.9950	1–200	102	11.0	105	2.5	–	–	97	5.0	–	–
2	Sulfamethoxypyridazine	0.9949	1–200	118	9.7	106	6.3	–	–	86	4.6	–	–
2	Morantel	0.9965	1–200	107	7.8	112	6.1	–	–	109	6.5	–	–
2	Tylosin	0.9946	1–200	125	5.3	105	4.8	–	–	98	7.5	–	–
2	Acetopromazine	0.9942	1–200	66	7.9	52	3.2	–	–	56	3.7	–	–
2	Chlorpromazine	0.9944	1–200	50	9.2	36	3.2	–	–	43	4.1	–	–
2	Fenbendazole	0.9910	1–200	76	5.6	99	1.6	–	–	90	4.9	–	–
2	Ketoprofen	0.9911	1–200	112	9.4	102	7.0	–	–	103	1.9	–	–
2	Niclosamide	0.9964	1–200	120	10.2	85	8.5	–	–	89	2.1	–	–

[a] Group 1 analytes have calibration range of 5–1,000 ng/g, QC spiking level of 10, 50, and 750 ng/g; while group 2 analytes have calibration range of 1–200 ng/g, QC spiking level of 2, 10, and 150 ng/g.

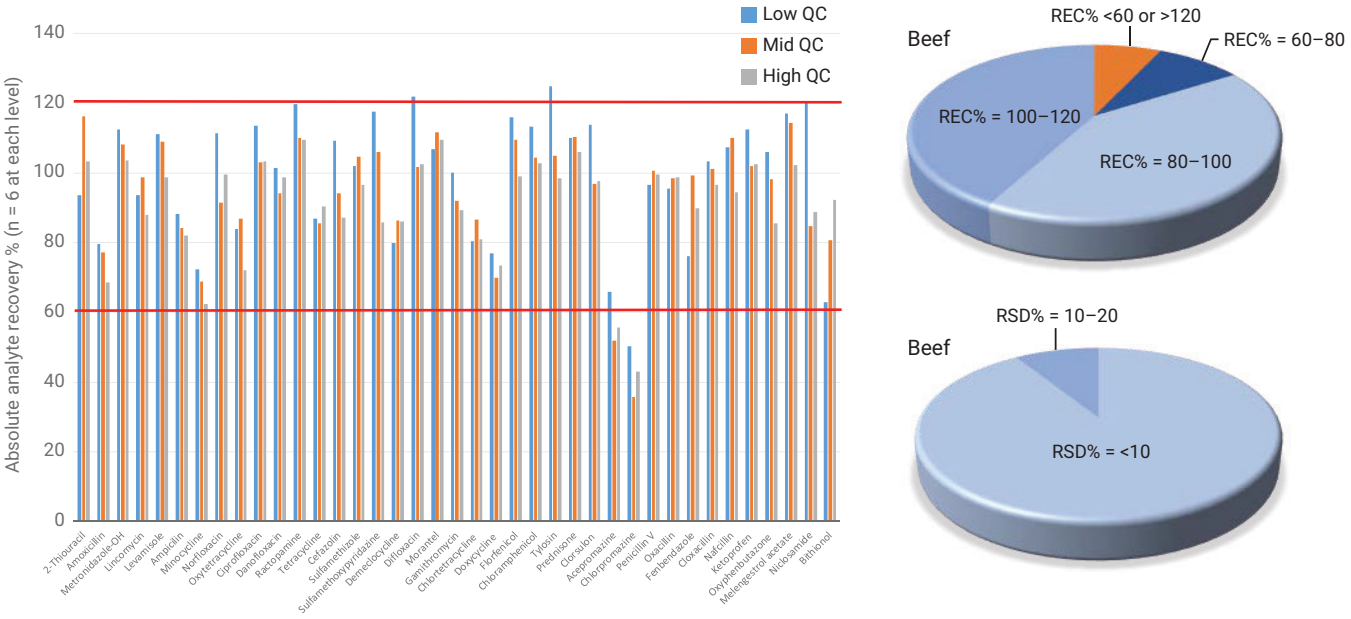

Figure 5. Absolute analyte recoveries and statistical summary of the analysis of veterinary drugs in beef method validation. Refer to Table 3 for more detailed information.

Conclusions

A rapid, reliable, and robust method using solid-liquid extraction followed by Agilent Captiva EMR—Lipid cartridge cleanup was developed and validated for the analysis of veterinary drug multiresidues in beef. A modified extraction procedure applied an aqueous extraction step, followed by an organic solvent extraction step to optimize analyte recovery. These extracts were combined and applied to the Captiva EMR—Lipid cartridge for cleanup. Matrix co-extractives and matrix effect was carefully assessed and compared with similar cartridges from another manufacturer. A study of the impact of cartridge cleanup on analyte recovery shows that the EMR—Lipid sorbent provides highly selective lipids removal, and does not cause unwanted target analytes loss. Results demonstrate that optimized solid-liquid extraction followed by the Captiva EMR—Lipid cartridge cleanup method provides superior matrix cleanup, excellent recovery, and precision results for this type of application.

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