Using a New High Capacity Lipid Depletion Material in Comparison to a C18 Adsorbent During Dispersive SPE Cleanup for Analysis of Veterinary Drugs in Animal Samples

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Introduction

In recent years the concept of using QuEChERS for sample cleanup has been successfully applied to foods other than fruits and vegetables such as breads, milk, and oils. In addition, the range of analyzed compounds has been broadened from pesticides to other types of contaminants (e.g., veterinary drugs). LC-MS methods have become main-stream for analysis.

The SPE phases that were used as QuEChERS sorbents to date included primary-secondary amine (PSA) for the removal of acids, polar pigments and sugars; graphitized carbon black (GCB) for the removal of color pigments such as chlorophyll; and C18 for the removal of lipid and non-polar components.

The C18 sorbent was, until recently, the only one that was available for the removal of fats and non-polar compounds from samples.

In this work we evaluated the use of a new lipid-removal sorbent (Z-Sep$^+$) vs. a DSC-18 for analysis of a number of veterinary drug residues in milk and kidney samples.

Experimental

Evaluation of SPE Sorbents for Fat Removal

A standard mix of oleins in acetonitrile (100-200 µg/mL each) was used as a test sample to evaluate the performance of different phases for the removal of fats.

1 mL of the prepared solution was mixed with 25 mg of the different SPE sorbents, then centrifuged and the resulting sample was analyzed by LC-ELSD for the removal of oleins.

HPLC Conditions:

column:	Ascentis® Express C18, 5 cm x 2.1 mm, 2.7 µm particles
flow rate:	0.5 mL/min.
temp.:	30 °C
det.:	ELSD (evap 30, neb 50, gas 1.50)
injection:	3 µL
gradient:	0 min. 100% methanol, 0-3 min. 0-100% isopropanol, 3-6 min. 100% isopropanol, 6-10 min. 100% methanol

Experimental (contd.)

Analyses of Veterinary Drugs

Table 1. Classes for Veterinary Drug Compounds used in the Current Study

Compound	Class
Abamectin B1a	Avermectins (anthelmintics)
Amoxicillin	Antibiotics (beta-lactams)
Chloramphenicol	Antibiotics (phenicols)
Ciprofloxacin	Antibiotics (fluoquinolones)
Furazolidone	Nitrofuranes
Levamisole	Anthelmintic
Lincomycin	Antibiotics (macrolides)
Salbutamol	Beta-blockers
Sulfanilamide	Sulfonamides

MS Conditions

Compound	Q1	Q3	ESI
Abamectin B1a	890.9	567.7	+
Amoxicillin	366.22	208.06	+
Chloramphenicol	321.1	152.1	-
Ciprofloxacine	332.2	288.2	+
Furazolidone	226.2	122.1	+
Levamisol	205.2	178.2	+
Lincomycin	407.2	126.2	+
Salbutamol	240.3	148.1	+
Sulfanilamide	173.1	93.1	+

A separate injection was done for analysis of Chloramphenicol.

LC Conditions

Both C18 and RPA columns were tested for this separation. The RPA column was used because it provided better retention for more polar analytes, such as salbutamol and sulfanilamide.

instrument:	AB QTRAP 3200, Agilent 1100-1200 Stack
column:	Ascentis Express RPA, 5 cm x 2.1 mm, 2.7 µm particles
mobile phase A:	5 mM, 10 mM ammonium acetate buffer, pH 7
mobile phase B:	5 mM acetonitrile
flow rate:	0.5 mL/min.
temp.:	35 °C
injection:	5 µL

gradient:

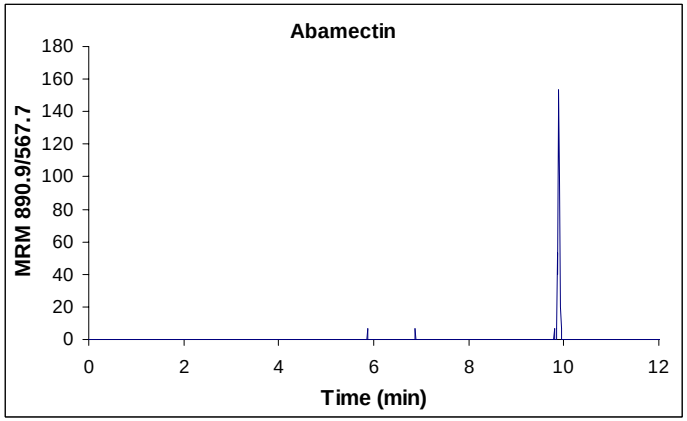
Min.	**%B**
0	2
1	2
5	60
8	100
10-14	2

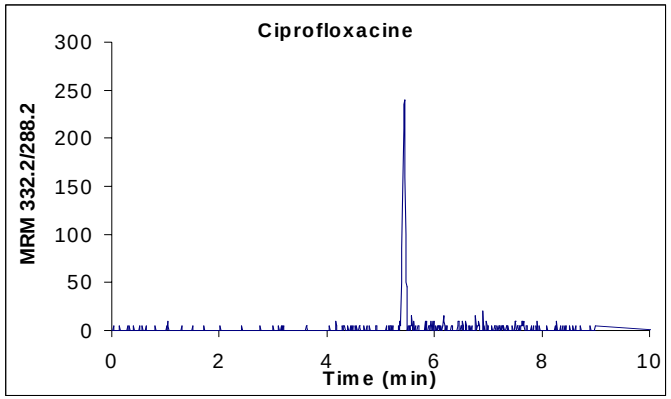
For chloramphenicol injection:

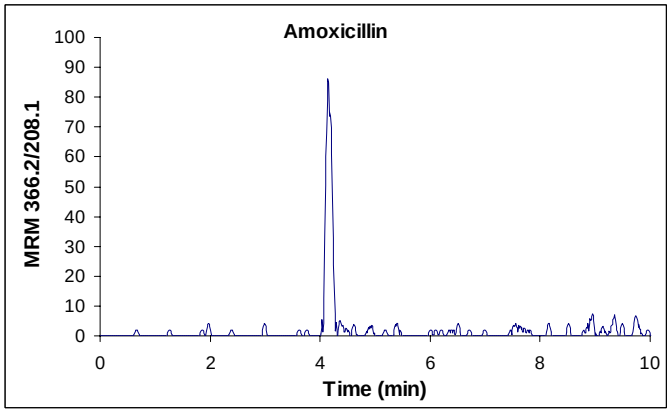
Same column and mobile phase as above was used with a different gradient:

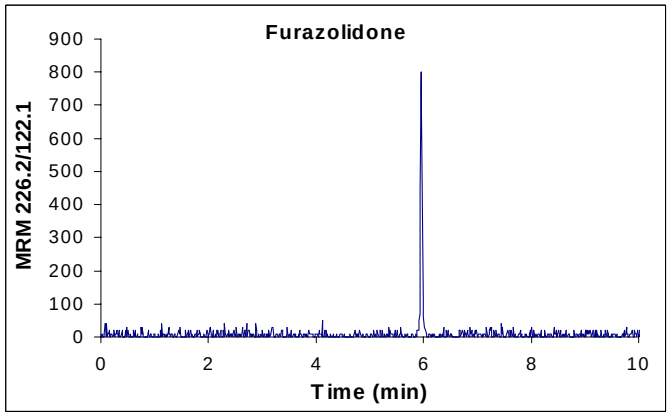
Min.	**%B**
0	20
1	20
5	50
5-9	20

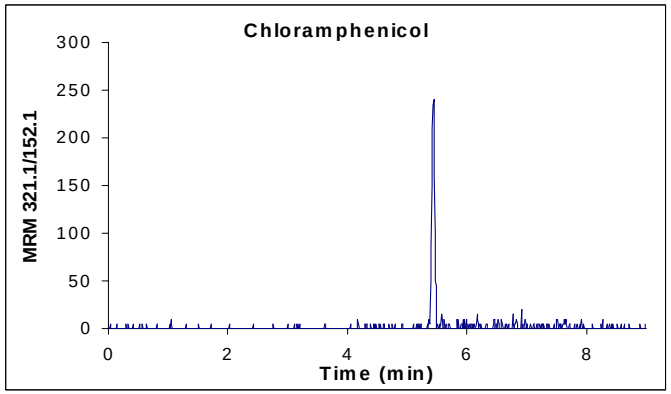
Figure 1. Extracted MRMs of Compounds











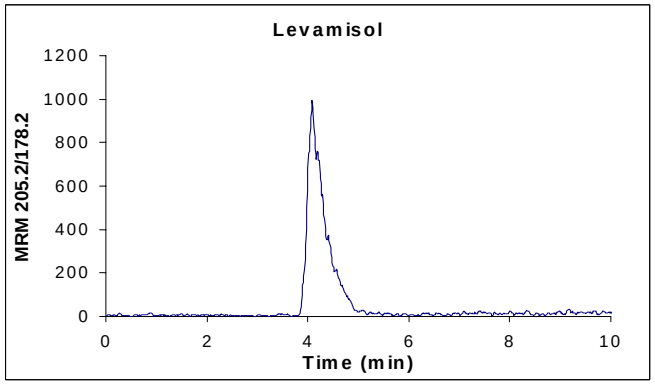
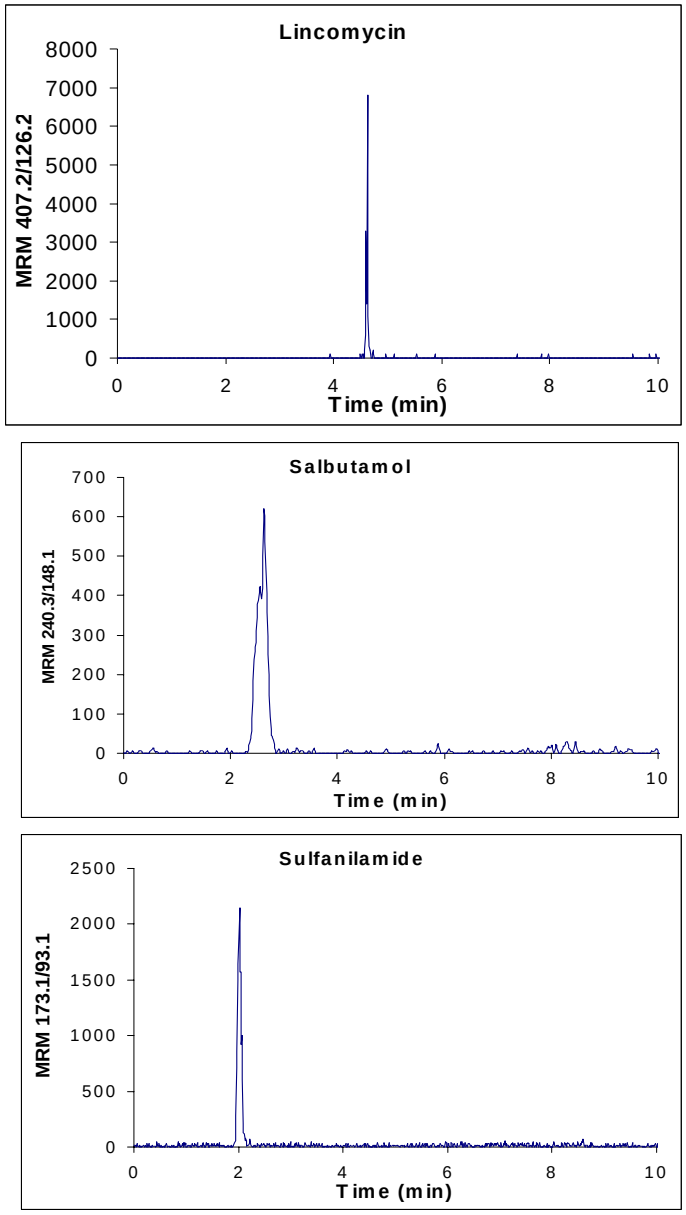
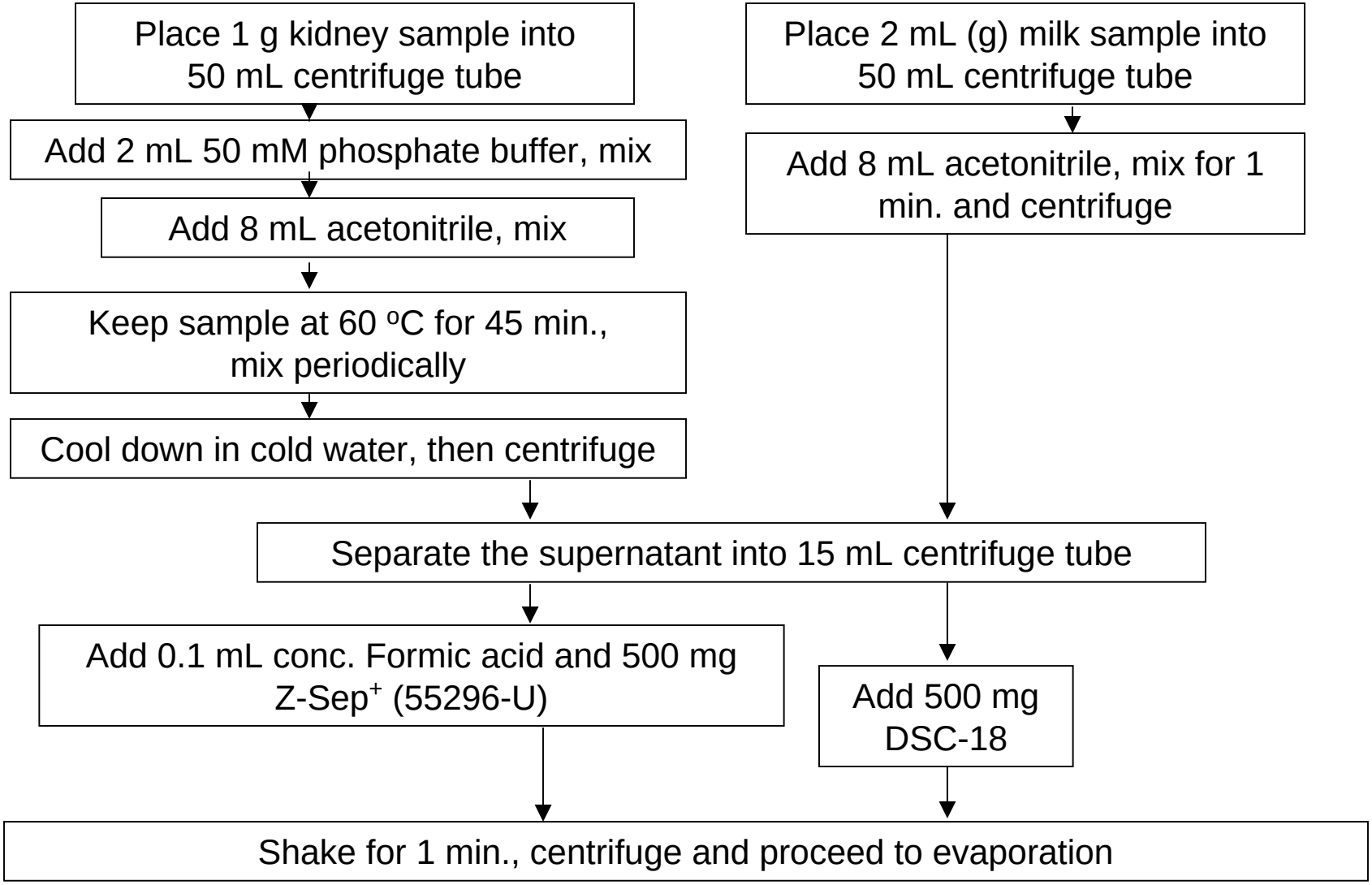


Figure 1. Extracted MRMs of Compounds (contd.)



Standard injection at the following concentrations: ciprofloxacin, levamisol, lincomycin, salbutamol at 3 ng/mL, chloramphenicol at 0.75 ng/mL, furazolidone at 7.5 ng/mL, amoxicillin at 9 ng/mL, sulfanilamide at 12 ng/mL, and abamectin at 15 ng/mL.

Extraction and Cleanup Method



Extraction and Cleanup Method (contd.)

Evaporate at 50 ^{o}C to 0.75 mL.

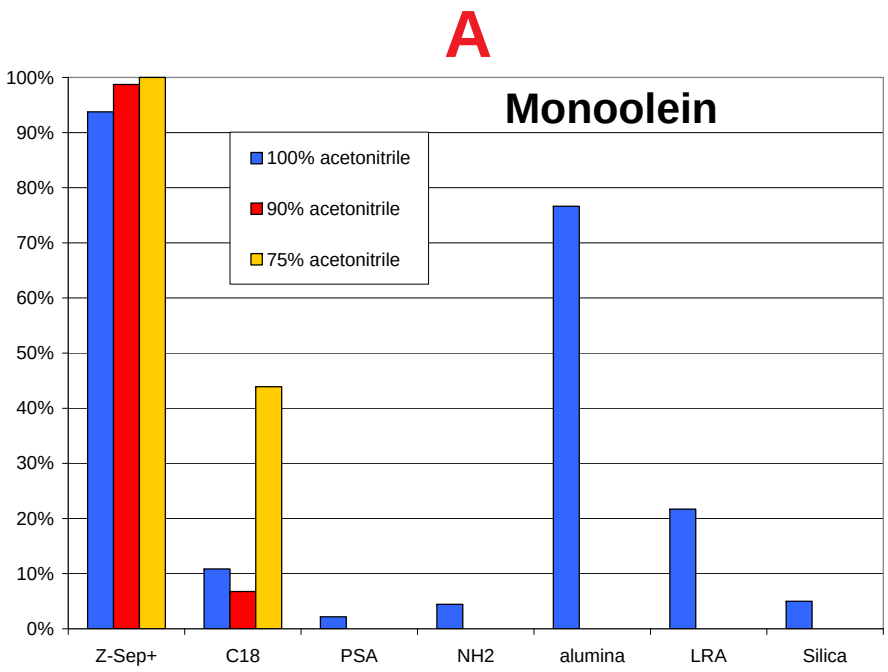
Add 0.15 mL acetonitrile and water to total volume of 1.0 mL.

Filter the sample prior to LC-MS analysis using 0.45 µm filter.

Results

A new sorbent (Z-Sep^{+}) displayed a better capacity for removal of mono-, di- and tri-oleins from standard solutions in acetonitrile and acetonitrile:water. The retention is not dependent on the %water, like that for C18 sorbent (Figures, 2A, 2B).

Figure 2. Removal of (A) Monoolein (B) Diolein and (C) Triolein from Solution by Different Sorbents



B

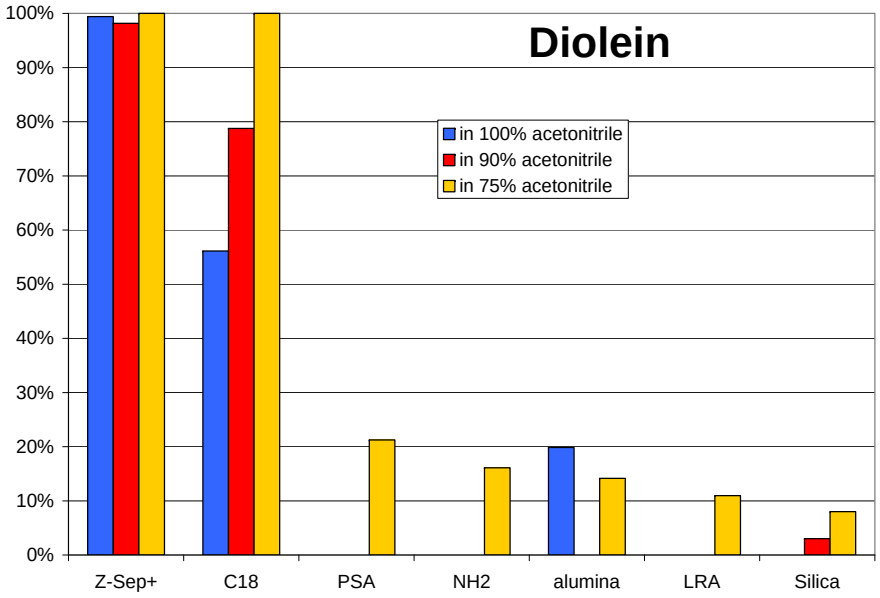


Figure 2. Removal of (A) Monoolein (B) Diolein and (C) Triolein from Solution by Different Sorbents (contd.)

C

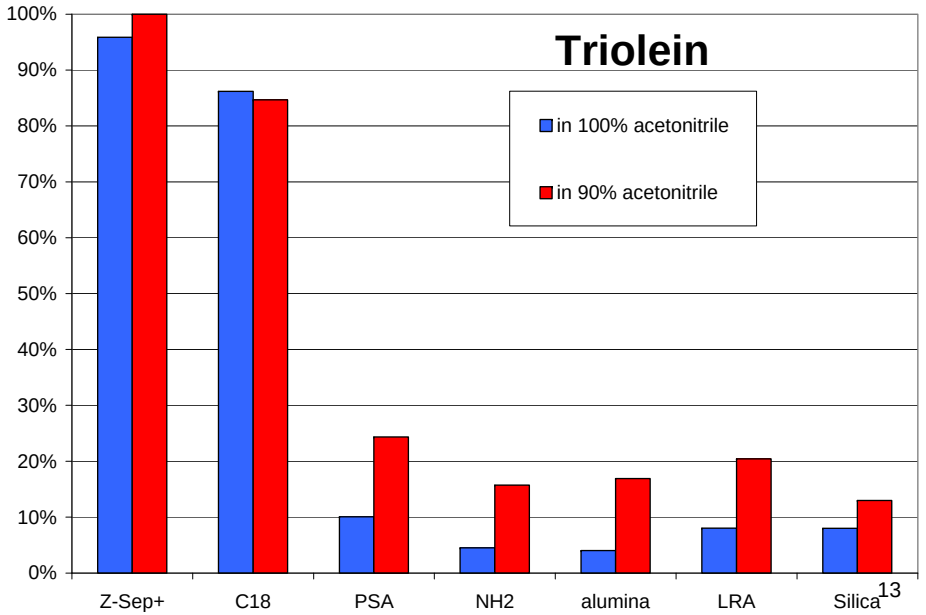


Table 2. Recoveries of Drugs Spiked into the Blank Extract. The difference from 100% is due to the presence of Ionization Effects

	milk		kidney	
Compounds	**Z-Sep$^+$ Blank**	**C18 Blank**	**Z-Sep$^+$ Blank**	**C18 Blank**
Abamectin	85%	10%	10%	9%
Amoxicillin	102%	116%	120%	41%
Chloramphenicol	106%	94%	108%	98%
Ciprofloxacine	102%	119%	112%	64%
Furazolidone	113%	97%	80%	74%
Levamisol	101%	93%	106%	77%
Lincomycin	62%	86%	150%	167%
Salbutamol	39%	58%	93%	96%
Sulfanilamide	77%	89%	77%	61%

Table 3. Recoveries of Veterinary Drugs Spiked into the Matrix Samples at the indicated Levels (n=3). The Calibration Curve Standards were made in Solvent

	Spike level (µg/kg)		Recoveries milk (%RSD)		Recoveries kidney (%RSD)	
Compounds	**milk**	**kidney**	**Z-Sep⁺**	**C18**	**Z-Sep⁺**	**C18**
Abamectin	25	50	61%(10)	3%(100)	44%(8)	4%(86)
Amoxicillin	15	30	31%(7)	40%(16)	63%(3)	19%(98)
Chloramphenicol	1.25	2.5	106%(4)	100%(6)	95%(3)	110%(6)
Ciprofloxacine	5	10	22%(26)	73%(5)	57%(22)	54%(22)
Furazolidone	12.5	25	98%(13)	90%(3)	73%(15)	70%(21)
Levamisol	5	10	98%(7)	95%(9)	82%(4)	64%(6)
Lincomycin	5	10	63%(11)	75%(9)	128%(11)	132%(12)
Salbutamol	5	10	37%(17)	80%(13)	79%(9)	88%(9)
Sulfanilamide	20	40	62%(6)	77%(8)	58%(5)	47%(9)

Discussion

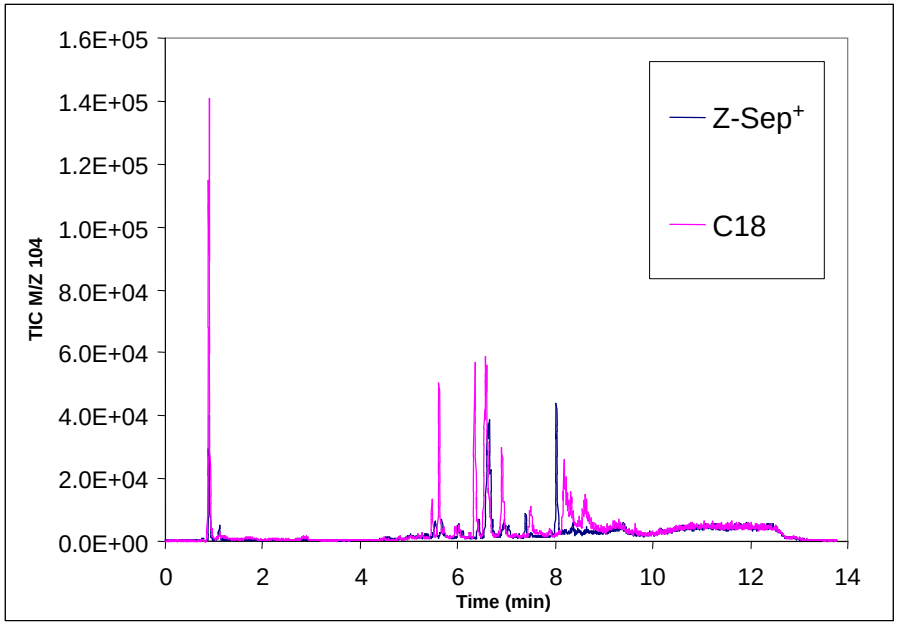
Addition of 1% formic acid to the extract was necessary to get better recoveries for some compounds during cleanup using the new lipid removal material.

The removal of phospholipids (phosphatidylcholine – PC) from milk samples was better when using Z-Sep$^+$ in comparison to using C18. The PC from milk samples is shown in Figure 3.

The recoveries of drug compounds from milk did not follow the trend for PC removal. The cleanup using C18 gave better recoveries from milk samples except for abamectin. Abamectin could not be recovered using C18 cleanup (due to ion suppression) but was better recovered using the new lipid removal material. Abamectin is the late-eluting compound and, possibly, the phospholipids contribute strongly to the ionization supression.

An additional benefit for using the new lipid-removal phase is the more efficient color removal which is shown in Figure 4 for kidney samples. Also, the recoveries of drugs from kidney samples were better when using Z-Sep$^+$ during cleanup (for amoxicillin, sulfonilamide and levamisol).

Figure 3. Comparison of Phosphatidylcholine in Milk Samples Cleaned Using Z-Sep$^+$ or C18 dSPE. 2 Ions-M/Z 104 and 184 - were monitored



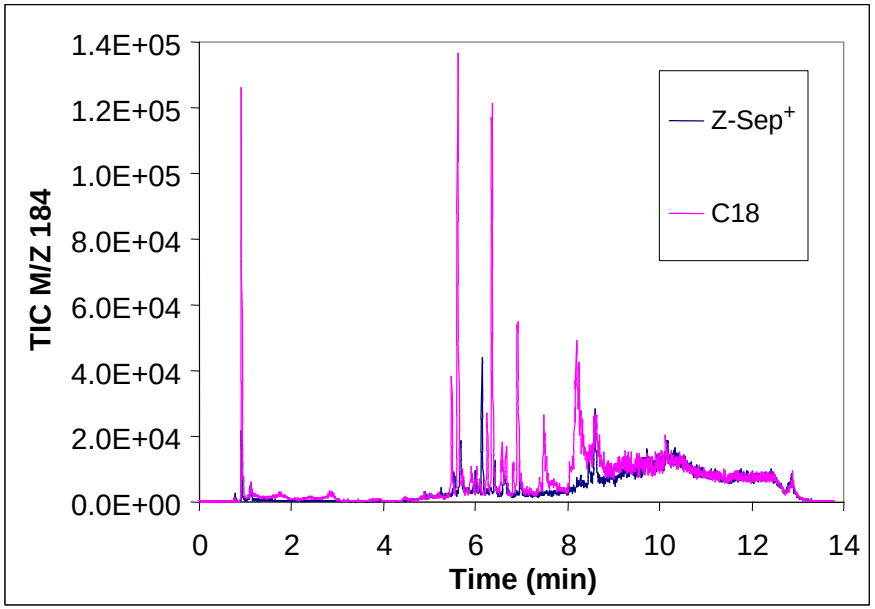
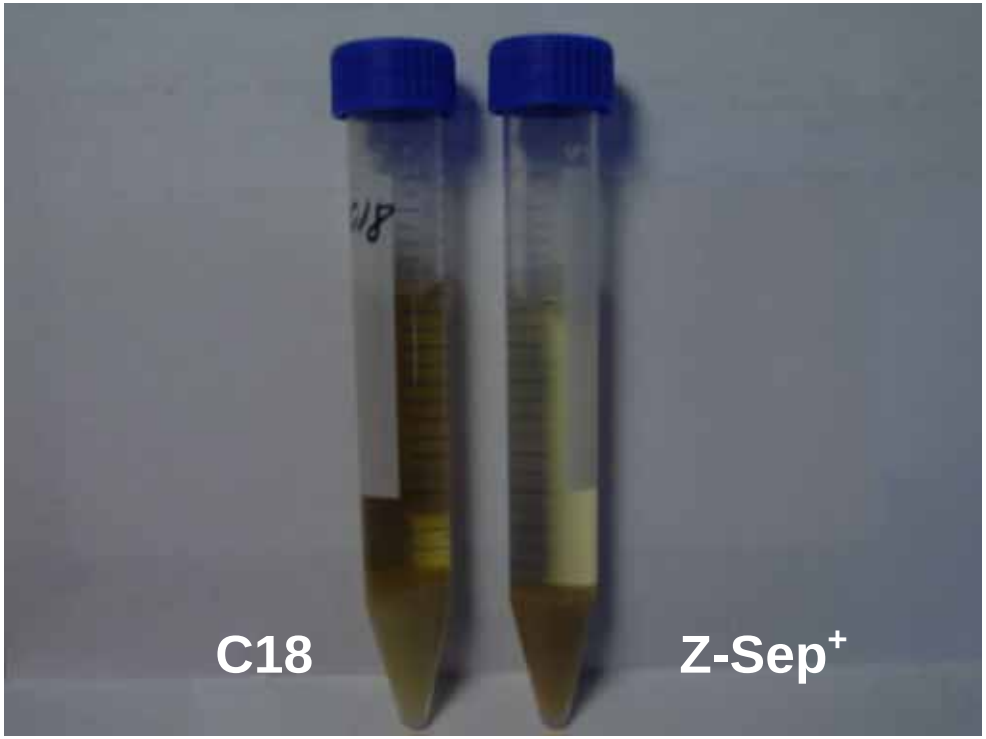


Figure 4. Extracted Kidney Samples cleaned using C18 or Z-Sep+



Conclusions

When evaluated, the new material Z-Sep$^+$ as a QuEChERS cleanup reagent compared to a C18:

1. Is better for removal of especially fatty compounds that contain single fatty acid chains.
2. Both positive and negative comparisons to the C18 sorbent were made when analyzing kidney and milk samples for monitored veterinary drugs:
   - The new sorbent is better for removing color compounds.
   - The new sorbent requires addition of formic acid during the cleanup step to avoid retaining more acidic and chelating compounds (e.g.ciprofloxacine).
   - The final recoveries from **milk** were better for this method when using C18 sorbent with exception of abamectin.
   - The final recoveries from **kidney** were better for this method when using the new sorbent with exception of salbutamol.

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