Effective Sample Preparation For Multi-Residue LC-MS Determination of Veterinary Drugs in Meat and Milk

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INTRODUCTION

To ensure food safety, there is a need for multiresidue UPLC-MS methods that can identify and quantify a wide range of veterinary drug residues from many drug classes. Solvent extraction can be effective for many of these compounds in meat and milk. However, highly water soluble drugs such as sulfanilamide and salbutamol may not be well recovered using this approach. If, instead, an aqueous buffer is used for extraction then there is poor recovery of fat soluble compounds such as phenylbutazone and dexamethasone. In this poster we will discuss effective sample preparation to maximize recovery of the widest possible range of veterinary residues in meat or milk. Optimized sample preparation and analysis protocols were developed for tandem LC-MS determination of a wide variety of veterinary drug residues in milk and meat samples. A two step extraction and precipitation procedure is used for milk; a single step extraction and precipitation procedure is used for meat. For either matrix, a simple SPE cleanup is performed using a Sep-Pak C18 cartridge. After evaporation and reconstitution, the sample is analyzed using tandem LC-MS. Representative compounds from each class of veterinary drugs used in this study are shown in Figure 1. The systems used for the LC-MS analysis are shown in Figure 2.

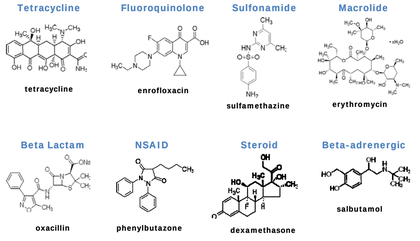

Figure 1. Representative Structures of Veterinary Drugs Classes in This Study

Figure 2. LC-MS(MS) systems used in this study; the Xevo™ TQ was used for the meat samples and the ACQUITY® TQD was used for the milk samples

SAMPLE PREPARATION

Milk

1. Initial Extraction / Precipitation

Transfer a 2 mL sample into a 15 mL centrifuge tube. Add 2 mL acetonitrile and vortex for 30 seconds. Centrifuge at 12000 rpm for 5 minutes.

- *The initial extraction step gives good recovery of most compounds of interest but also extracts significant amounts of protein and some fat.*

2. Residual Protein Precipitation

Transfer 2 mL of supernatant (from step 1, milk or meat) to a second centrifuge tube. Add 3 mL of acidified acetonitrile (0.2 % formic acid) and vortex for 30 seconds. Centrifuge the samples at 12000 rpm for 5 minutes

- *This step effectively removes most residual protein*

Tissue

Extraction / Precipitation

Place a 5 g sample of homogenized tissue into a 50 mL centrifuge tube. Add 10 mL 0.2 % formic acid in 80:20 acetonitrile/water. Vortex for 30 seconds and place on mechanical shaker for 30 minutes. Centrifuge at 12000 rpm for 5 minutes.

- *The extraction/precipitation step gives good recovery of most compounds of interest but also extracts significant amounts of fat.*

SPE Cleanup (Tissue or Milk)

Take 1 mL of the supernatant from tissue extract or milk step 2. Perform pass-thru SPE cleanup using a Sep-Pak C18 cartridge (see SPE details in Figure 3).

- *This step effectively removes fats and other highly non-polar interferences.*

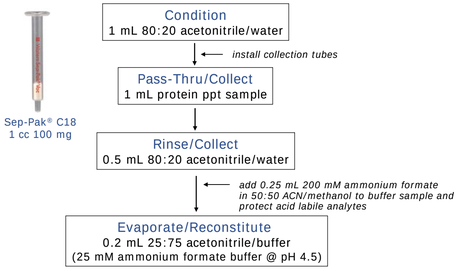

Figure 3. SPE Cleanup Protocol

UPLC-MS(MS) ANALYSIS

UPLC Conditions

- Column: ACQUITY CSH™ C18 1.7µm 100 x 2.1 mm
- Mobile phase
  - A: 0.1% formic in water
  - B: 0.1% formic acid acetonitrile
- Injection volume: 7 µL
- Injection mode: partial loop injection
- Sample diluent: 20:80 ACN:water
- Column temperature 30 °C
- Weak Needle Wash: 10:90 acetonitrile:water (600 µL)
- Strong Needle Wash: 50:30:40 water:acetonitrile:IPA (200 µL)
- Seal wash: 10:90 acetonitrile: water

Gradient Table

Time (min)	Flow (mL/min)	% A	% B	Curve
Initial	0.4	85	15	Initial
2.5	0.4	60	40	6
3.9	0.4	5	95	6
4.9	0.4	5	95	6
5.0	0.4	85	15	6
7.0	0.4	85	15	6

MS(MS) Conditions

Polarity	ES+
(Except Chloramphenicol	ES-)
Capillary (kV)	2.80
Extractor (V)	3.00
Source Temperature (°C)	150
Cone Gas Flow (L/Hr)	30
Desolvation Temperature (°C)	500
Desolvation Gas Flow (L/Hr)	1000
Collision Gas Flow (mL/Min	0.15

MassLynx V4.

Waters Xevo TQ MS:
meat analysis – conditions shown

Waters ACQUITY® TQD :
milk analysis – similar MS conditions and same transitions

Compound	Principal MRM	Cone (V)	Collision (eV)
Amoxicillin	366>113	15	20
Carbadox	263>231	25	15
Ciprofloxacin	332>288	28	18
Chloramphenicol	321>152	10	15
Chlortetracyline	479>444	25	25
Dexamethasone	393>355	20	15
Enrofloxacin	360>316	30	25
Erythromycin	734>158	30	25
Lincomycin	407>126	30	25
Oxacillin	402>160	15	15
Oxytetracycline	461>426	22	20
Penicillin-G	335>160	20	15
Phenylbutazone	309>160	20	15
Ractopamine	302>107	22	25
Salbutamol	240>148	20	25
Sulfamerazine	265>92	25	25
Sulfamethazine	279>92	32	30
Sulfanilamide	173>156	25	10
Tetracycline	445>154	25	25
Tylosin	916>174	50	30

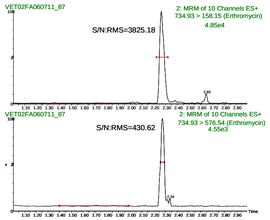

Figure 4. Typical LC-MS(MS) Chromatogram obtained from pork muscle spiked with erythromycin at 10 ng/g

Matrix matched calibration was performed for each compound.

Correlation coefficients (R^2) ranged from 0.990 to 0.998 for five point external standard calibration.

Typical LC-MS(MS) performance is shown in Figure 4.

RESULTS

Results for milk samples are shown in Table 1 and for pork muscle in Table 2.

Ion suppression (or enhancement) was calculated by comparison of analyte response for a standard prepared in sample matrix with analyte response for a standard prepared in pure solvent.

Recovery was calculated by comparison of the response shown for an analyte fortified into the sample matrix prior to all sample preparation with response shown for a blank matrix sample fortified with the analyte after all sample preparation steps.

Compound	Spike Level (ng/g)	% REC (% RSD) n=3	% Suppression
Carbadox	67.0	27 (27)	43 (enhance)
Chloramphenicol	6.7	94 (16)	10.0
Chlorotetracyline	67.0	22 (20)	7.0
Ciprofloxacin	67.0	67 (20)	32.0
Dexamethasone	67.0	87 (6)	8 (enhance)
Enrofloxacin	134.0	76 (11)	26.0
Erythromycin	6.7	59 (10)	5.0
Lincomycin	33.0	102 (9)	25.0
Oxacillin	67.0	79 (12)	9 (enhance)
Oxytetracycline	67.0	24 (16)	9 (enhance)
Penicillin	33.0	73 (8)	8(enhance)
Phenylbutazone	67.0	67 (18)	20.0
Ractopamine	200.0	65 (14)	0.0
Salbutamol	67.0	80.4 (3)	96.0
Sulfamerazine	67.0	71 (4)	16 (enhance)
Sulfamethazine	67.0	71 (6)	74 (enhance)
Sulfanilamide	67.0	110 (30)	60.0
Tetracycline	67.0	31 (18)	21 (enhance)

Table 1. Recovery data for milk samples

Compound	Spike Level (ng/g)	% REC (% RSD) n=5	% Suppression
Carbadox	100	8.9 (36)*	62.7
Chloramphenicol	10	57.5 (20)	7.1
Chlorotetracyline	100	41.9(11)	5.7
Ciprofloxacin	100	130 (21)	85.6
Dexamethasone	100	70.2 (7)	36.9
Enrofloxacin	200	106 (4)	70.3
Erythromycin	10.0	36.1 (9)	4.2
Lincomycin	50.0	64.5 (17)	93.4
Oxacillin	100	51.5 (4)	25.2
Oxytetracycline	100	51.1 (8)	9.4
Penicillin	50.0	46.8 (7)	11.3
Phenylbutazone	100	15.9 (16)	53.3
Ractopamine	300	73.7 (7)	81.1
Salbutamol	100	70.8 (14)	97.4
Sulfamerazine	100	63.4 (5)	56.7
Sulfamethazine	100	67.1 (5)	53.6
Sulfanilamide	100	74.4 (21)*	71.8
Tetracycline	100	58.3 (10)	0.4
Tylosin	20.0	46.6 (11)	8.4

Table 2. Recovery data for pork muscle samples

DISCUSSION

The purpose of this study was to evaluate simple sample preparation strategies (extraction, precipitation and cleanup) for multiresidue analysis of veterinary drugs in meat and milk. No internal standards were used in this study. The average precision for the milk and meat samples was 13 % (± 8 %), quite typical for external standard calibration.

The two step procedure chosen for milk was to initially extract and precipitate the sample with 50 % acetonitrile in water (taking into account the water already present in the sample). The remaining protein from the supernatant was then precipitated in a second step with more acetonitrile in the presence of dilute acid. A single step procedure was used for meat samples, a direct extraction/precipitation with 0.2 % formic acid in 80% acetonitrile. These procedures are straightforward and produce clean final extracts suitable for LC-MS analysis. The one-step protocol was also evaluated for milk samples. Compared with the two step protocol, there was significantly lower recovery for the most polar compounds such as sulfanilamide and virtually no recovery of chlorotetracycline. A third approach was also considered, to perform two separate extraction steps. The first step, for the water soluble compounds, was accomplished using aqueous succinic acid buffer. The second, performed on the re-suspended pellet, was with acetonitrile. This approach required a separate work up for each step before ultimately combining fractions for a single injection. Performance was somewhat better than the chosen protocols but at a much greater cost of time and materials.

CONCLUSIONS

- **Methods were demonstrated for determination of multiclass / multiresidue veterinary drugs in milk and meat**
- **A two step extraction / protein precipitation procedure was demonstrated for milk; a one step procedure for meat**
  - Recoveries in milk averaged 67 % with the lowest values for tetracyclines
  - Recoveries in meat averaged 61 % with the lowest values for carbadox and phenylbutazone
- **Many compounds show significant ion-suppression; this may not be a serious impediment to a useful screening method if matrix matched standards are employed and the response of the compound is sufficient**
- **SPE pass-thru cleanup was accomplished with Sep-Pak C18-silica cartridges**
  - Effective for removal of residual fats
  - Produced an extract for LC/MS that was free of particulates and required no subsequent filtration prior to LC/MS analysis
  - Not highly effective for reduction of matrix effects
- **Among the goals of future work will be advances in SPE cleanup to reduce matrix effects**

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