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Nota de aplicación

Optimized Extraction and Cleanup Protocols for LC-MS/MS Multi-Residue Determination of Veterinary Drugs in Edible Muscle Tissues

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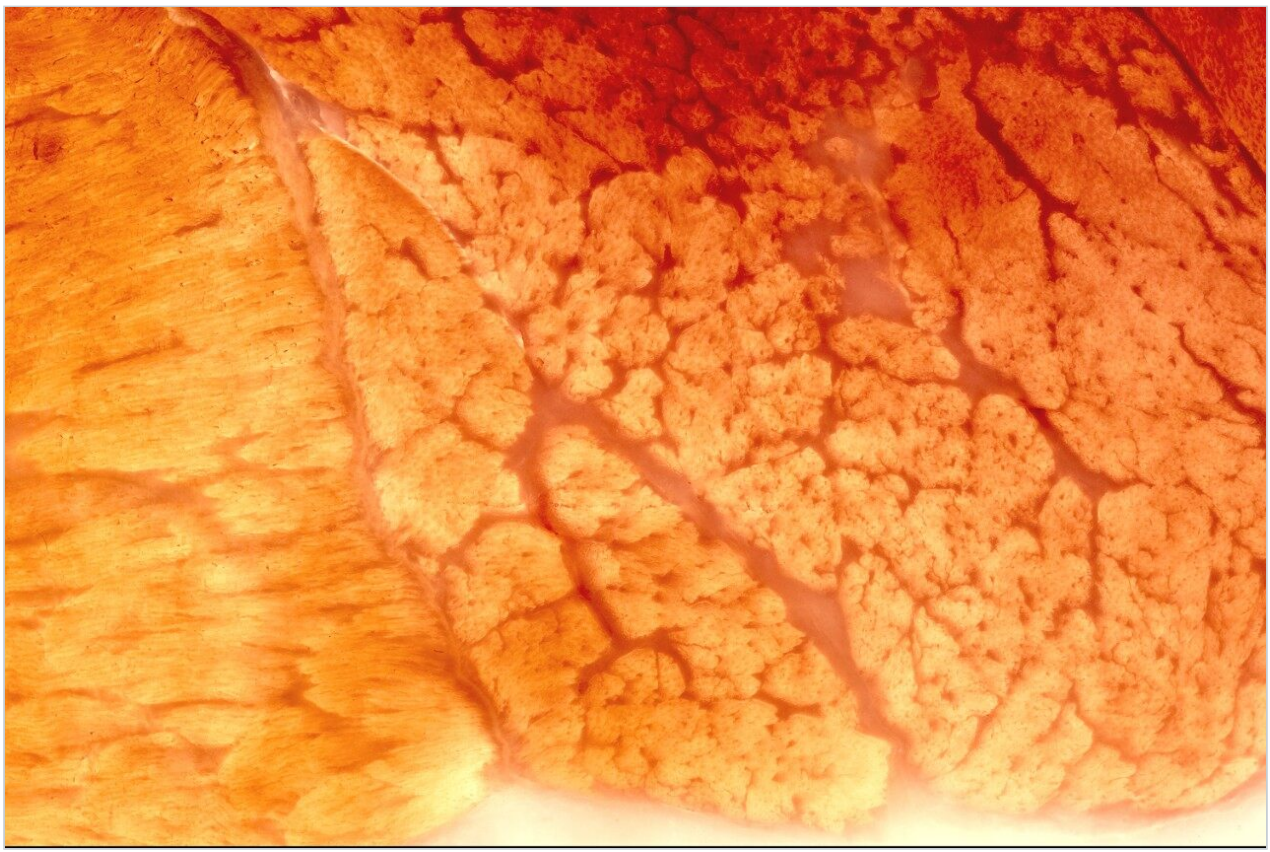

Abstract

In order to ensure public health and safety, a reliable screening analysis is necessary to determine veterinary drug residue levels in meat and other edible tissue samples. The compounds of interest range from highly polar water-soluble compounds to very non-polar, fat-soluble compounds. There exist very effective extraction and cleanup procedures for individual compounds or compound classes, but these methods are not well suited for a multi-class, multi-residue screening analysis.

- Solvent extraction (with excess acetonitrile or methanol) can be effective for many veterinary drug residues in milk, but highly water soluble drugs such as salbutamol are not well recovered using this approach.
- Aqueous buffer extraction can also be effective for many compounds, but fat-soluble compounds, such as dexamethasone, are not well recovered using this approach.
- Traditional solid-phase extraction (SPE) enrichment and cleanup (retention/ wash/elution) has limited utility for multi-residue analysis. Because the range of acidity/polarity/solubility among the compounds is so broad, dispersive, or pass-through SPE is preferred for multi-residue methods.

Benefits

- Efficient, timesaving multi-class/multi-residue methodology
- Straightforward sample preparation for diverse range of analytes
- Fast, sensitive UPLC-MS analysis

Introduction

Optimized sample preparation and analysis protocols were developed for tandem LC/MS determination of a wide variety of veterinary drug residues in tissue samples. Three types of muscle tissue samples (pork, chicken, and salmon) were chosen to demonstrate the suitability of the methodology. Samples are treated with an acidified acetonitrile/water solvent to precipitate proteins and to extract the veterinary drugs of interest. Then, a simple SPE cleanup is performed using a Sep-Pak C_{18} cartridge or 96-well plate. After evaporation and reconstitution, the sample is analyzed using tandem LC-MS. Representative compounds

were chosen from major classes of veterinary drugs including tetracyclines, fluoroquinolones, sulfonamides, macrolides, beta-lactams, NSAIDS, steroids, and beta-andrenergids.

Experimental

LC Conditions

System:	ACQUITY UPLC system
Column:	ACQUITY UPLC CSH C_{18}, 1.7µm, 100 mm x 2.1 mm (i.d.)
Mobile Phase:	A: 0.1% formic in water B: 0.1% formic acid in acetonitrile
Injection Volume:	7 µL
Injection Mode:	Partial loop injection
Column Temp.:	30 °C
Weak Wash:	10:90 acetonitrile:water (600 µL)
Strong Wash:	50:30:40 water:acetonitrile:IPA

	(200 µL)
Seal Wash:	10:90 acetonitrile:water

Gradient

Time (Minutes)	Flow (mL/min)	Solvent A (%)	Solvent 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 Conditions

Detector:	Waters Xevo TQ
Ionization:	Positive Electrospray (except negative for chloramphenicol)
Source Temp.:	150°C
Desolvation Temp.:	500°C

Desolvation Gas Flow:	1,000 L/hr
Cone Gas Flow:	30 L/hr
Collision Gas Flow:	0.15 mL/min
Data Management:	MassLynx v4.1

Sample Preparation

1. Initial 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.

2. SPE Cleanup

Take 1 mL of the supernatant (from step 1) for SPE cleanup using a Sep-Pak C_{18} cartridge or plate (see SPE details in Figure 1).

This step removes fats and non-polar interferences.

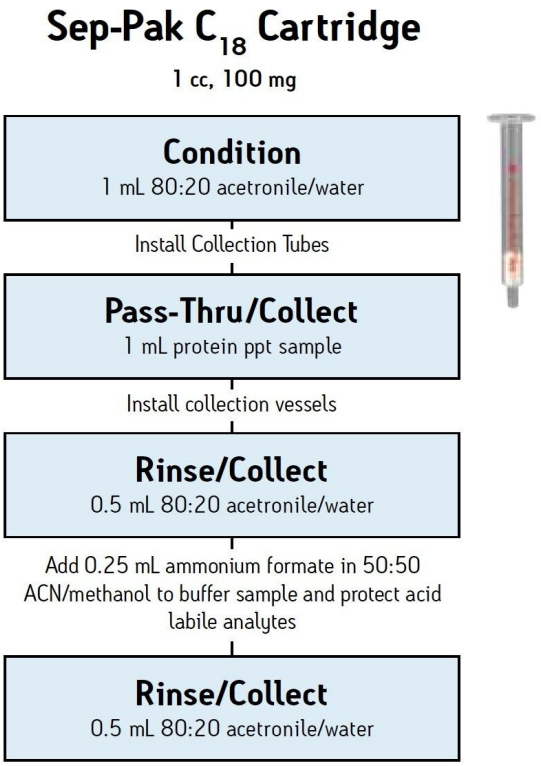

Figure 1. SPE Cleanup Protocol

Table 1 summarizes the MRM transitions and instrument parameters used for this study. Also presented in Table 1 are typical matrix matched calibration data for each compound (calculated using the primary transition in pork matrix).

Compound	MRM	Cone	CID	Calibration Range (ppb)	Corr R^2	LOQ ppb
Carbadox	263 > 231 263 > 145	25 25	15 25	6-200	0.9926	12.5
Ciprofloxacin	332 > 288 332 > 314	28 28	18 22	6-200	0.9911	6
Chloramphenicol	321 > 152 321 > 257	20 20	15 12	6-200	0.9912	25
Chlortetracycline	479 > 444 479 > 462	25 16	25 16	6-200	0.9923	6
Dexamethasone	393 > 355 393 > 373	18 18	13 10	6-200	0.9935	6
Enrofloxacin	360 > 316 360 > 342	30 30	25 25	12-400	0.9911	12
Erythromycin	735 > 158 735 > 576	30 30	25 15	0.6-20	0.9912	1.25
Lincomycin	407 > 126 407 > 359	30 30	25 20	3-100	0.9935	6.25
Oxacillin	402 > 160 402 > 243	30 30	15 10	6-200	0.9902	6
Oxytetracydine	461 > 426 461 > 381	22 22	20 20	6-200	0.9902	6
Penicillin-G	335 > 160 335 > 176	15 15	15 15	3-100	0.9913	3
Phenylbutazone	309 > 160 309 > 146	15 15	25 27	6-200	0.9915	6
Ractopamine	302 > 107 302 > 284	22 22	25 15	18-600	0.9905	18
Salbutamol	240 > 148 240 > 222	20 20	25 10	6-200	0.9915	25
Sulfamerazine	265 > 92 265 > 156	25 25	25 20	6-200	0.9917	6
Sulfamethazine	279 > 92 279 > 186	32 32	30 15	6-200	0.9901	6
Sulfanilamide	173 > 156 173 > 93	25 25	10 15	6-200	0.9928	50
Tetracydine	445 > 154 445 > 410	25 25	25 20	6-200	0.9909	6
Tylosin	917 > 174 917 > 772	25 25	25 20	1.2-40	0.9909	2.5

Table 1. MRM Transitions and Calibration Data Obtained in Chicken Matrix (Other Matrixes Similar).

Results and Discussion

Figure 2 shows a typical LC-MS chromatogram obtained from analysis of a matrix matched standard of erythromycin at 10 ng/g. Performance of the other compounds was similar. Table 2 shows the recovery data obtained from replicate analysis of spiked tissue samples. Table 3 shows the observed matrix effects for the multi-residue tissue analysis.

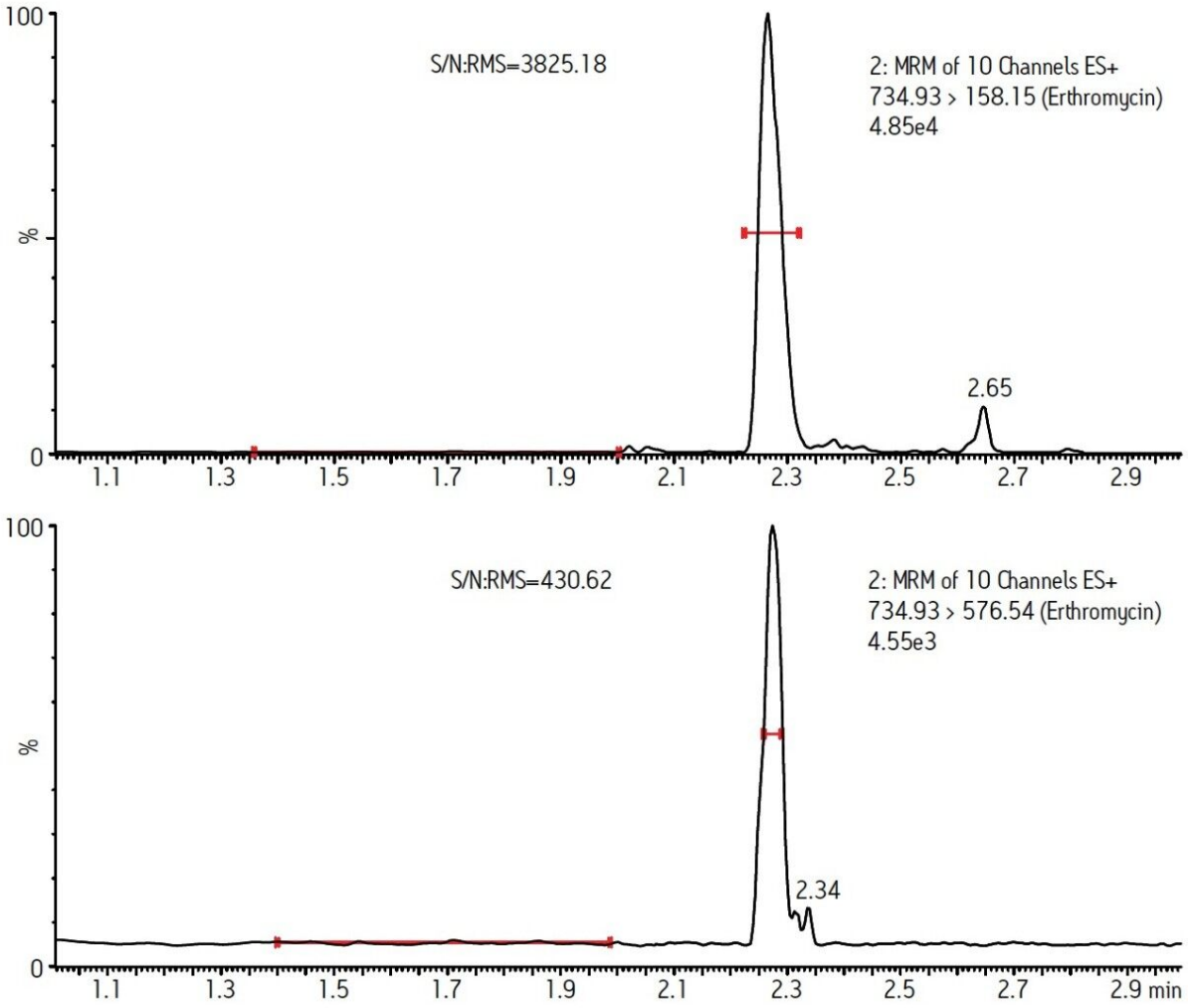

Figure 2. Typical LC-MS/MS Chromatogram Obtained from Pork Spiked with Erythromycin at 10 ng/g (Primary MRM Transition on Top).

The procedure utilized in this study was developed from methods presented by Lehotay et.al.[1] and Martos et.

al.[2]. The method used in reference 1 uses no acid in the tissue extraction solvent; under these conditions we observed recovery of tetracyclines below 5% and the RSD for recovery of fluoroquinolones was greater than 50%. The method used in reference 2 prescribes the acidification of the extract to 1% formic acid prior to centrifugation; under these conditions penicillin recovery was under 10% compared with 48% using our approach. Our extraction procedure is a compromise of the methods presented in reference 1 and 2. A similar acetonitrile/water based extraction is used but is acidified only to 0.2% with formic acid; more balanced recovery and minimized degradation of labile compounds was achieved.

Another approach was considered based on the method of Kauffman et. al.[3] by which two separate extractions were performed. The first extraction, for the water soluble compounds, was accomplished using aqueous succinic buffer. The second, performed on the re-suspended pellet, was with acetonitrile. This approach requires that each fraction be worked up independently before ultimately combining fractions for a single injection. Performance was only marginally better than the chosen procedure but at a much greater cost of time and materials. The extraction, cleanup, and analysis protocols chosen for this study provide a good balance of preparative time, cost and method performance.

Compound	Spike Level (ng/g)	Pork % Rec (%RSD) n=5	Chicken % Rec (%RSD) n=6	Salmon % Rec (%RSD) n=6
Carbadox	100.00	9 (36)	17 (14)	21 (13)
Chloramphenicol	100.00	57 (20)	51 (20)	89 (2)
Chlorotetracyline	100.00	42 (11)	49 (6)	54 (7)
Ciprofloxacin	100.00	130 (21)	61 (8)	88 (2)
Dexamethasone	100.00	70 (7)	61 (8)	91 (4)
Enrofloxacin	200.00	106 (4)	62 (9)	90 (2)
Erythromycin	10.00	36 (9)	33 (4)	43 (8)
Lincomycin	50.00	64 (17)	59 (10)	83 (8)
Oxacillin	100.00	51 (4)	48 (6)	55 (4)
Oxytetracycline	100.00	51 (8)	50 (10)	60 (5)
Penicillin	50.00	46 (7)	45 (8)	54 (9)
Phenybutazone	100.00	16 (16)	44 (10)	38 (8)
Ractopamine	300.00	74 (7)	62 (11)	88 (3)
Salbutamol	100.00	71 (14)	66 (12)	78 (7)
Sulfamerazine	100.00	63 (5)	59 (7)	82 (3)
Sulfamethazine	100.00	67 (5)	60 (8)	84 (3)
Sulfanilamide	100.00	74 (21)	65 (21)	74 (11)
Tetracycline	100.00	58 (10)	53 (8)	69 (2)
Tylosin	20.00	47 (11)	36 (12)	63 (14)

Table 2. Recovery data obtained from three types of spiked tissue samples

Compound	Pork % Suppression	Chicken % Suppression	Salmon % Suppression
Carbadox	63	33	49
Chloramphenicol	7	-17	-19
Chlorotetracyline	6	-0.15	32
Ciprofloxacin	86	62	58
Dexamethasone	37	6	32
Enrofloxacin	70	46	48
Erythromycin	4	-36	16
Lincomycin	93	88	92
Oxacillin	25	-2	32
Oxytetracycline	9	-90	18
Penicillin	11	-41	13
Phenybutazone	53	7	55
Ractopamine	81	38	50
Salbutamol	97	95	95
Sulfamerazine	57	29	44
Sulfamethazine	54	30	45
Sulfanilamide	72	78	78
Tetracycline	1	-60	25
Tylosin	8	14	51

Table 3. Matrix effects (% ion-suppression) observed for three types of spiked tissue samples (negative value indicates ion enhancement).

Conclusion

- A simple extraction/protein precipitation procedure was developed and demonstrated for analysis of meat, chicken and salmon muscle tissue.
- The procedure was suitable for screening for a wide range of veterinary drug residues.
- Recoveries averaged 60% and were similar for all tissues.
- A pass-through SPE cleanup protocol using Sep-Pak C_{18} was utilized for effective removal of residual fats.
- The sample preparation methodology produced an extract that was free of particulates and required no subsequent filtration prior to LC/MS analysis.

References

1. S. Lehotay, “High-Throughput Screening Analysis by UHPLC-MS/MS of >60 Veterinary Drugs in Animal Tissues”, 125th AOAC Annual Meeting, Presentation 2303, 21 September, 2011.

2. P. A. Martos, F. Jayasundara, J. Dolbeer, W. Jin, L. Spilsbury, M. Mitchell, C. Varilla, B. Shurmer, “Multiclass, Multiresidue Drug Analysis, Including Aminoglycosides, in Animal Tissue Using Liquid Chromatography Coupled to Tandem Mass Spectrometry”, *J. Agric. Food Chem*. 58, (2010), 5932–5944.

3. A. Kaufmann, P. Butcher, K. Maden, M. Widmer, “Quantitative Multiresidue Method for About 100 Veterinary Drugs in Different Meat Matrices by Sub 2-Micron Particulate High-Performance Liquid Chromatography Coupled to Time of Flight Mass Spectrometry”, *J. Chromatogr. A.* 1194 (2008), 66–79.

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720004144, November 2011