Determination of a Single Methodology for the Analysis and Quantitation of Multi-class Veterinary Drugs in Different Animal Matrices Used for Consumption

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Overview

Purpose: Development of a single quantitative method for multi-class veterinary drugs in animal meat products.

Methods: 3 µL injections of extracted meat (chicken, beef, and pork) spiked with veterinary drugs were injected onto a C18 reverse phase column. Compounds of interest were separated and eluted using a standardized gradient elution profile. A high performance triple quadrupole mass spectrometer with a heated electrospray source (HESI) was used to analyze the compounds of interest in positive and negative ionization, and the data were collected, analyzed, and reported using customized software.

Results: The method provided excellent result s for most compounds at or below the required Maximum Residue Limits (MRLs)[1] in the matrices studied. Several compounds could not be incorporated into the final method due to high polarity or poor chromatographic response under the proposed generic mobile phase conditions.

Introduction

Veterinary drugs are pharmacologically active compounds that are used to treat and prevent diseases of animals in livestock. Their use can result in non-desirable drug residues in the food products for consumption. Animals with high amounts of these drugs can cause harm in humans or can render the use of antibiotics in hospitals useless due to the counter effects of the drugs. Therefore, strict regulatory guidelines exist for these compounds in meat products. Required MRLs typically are in the range of sub-µg/Kg up to 20,000 µg/Kg depending on the substance and specific regulation, with some compounds set at "zero" tolerance.

The quantification of over 200 multi-class veterinary drugs from different meat products usually involves a series of different extraction methods with either SPE or LLE. Each requires substantial time in both sample preparation and analytical run time with multiple HPLC and mass spectrometer methods. A new robust method, utilizing a single chromatography run and a triple quadrupole mass spectrometer is described in this poster. It can be used to quantitate over 200 veterinary drugs by LC/MS in a variety of matrices. In addition, the instrumental method can be set up to perform targeted screening of samples.

Methods

Sample Preparation

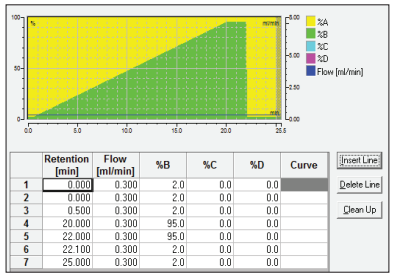
Sample preparation of blank matrices used for matrix spiked calibration curves were performed by the U.S. Department of Agriculture (USDA)[2] based upon the QueCHeRS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method. Steps below:

1. 2.0 gram sample in 10 mL of acetonitrile/water (8:2).
2. Shake for 10 minutes using platform shaker.
3. Centrifuge at 3700 G for 5 minutes.
4. Extract was filtered using 0.45 micron PVDF syringe filter.

Matrices studied: Beef muscle, chicken muscle, chicken liver, beef liver, beef kidney, pork muscle

Liquid Chromatography (See Figure 1)

Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
Column: Thermo Scientific™ Accucore™ aQ C18 Polar Endcapped (100 × 2.1 mm, 2.6 µm)
Column temperature: 30° C
Mobile phase: A = H_2O + 0.1% FA, B = MeOH + 0.1% FA
Flowrate: 300 µL/min
Inject volume: 3 µ L

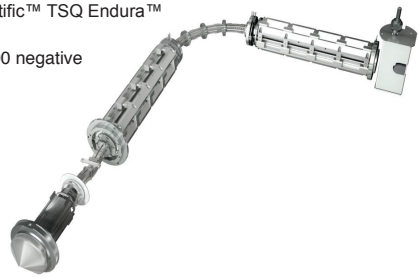


	Retention [min]	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	0.300	2.0	0.0	0.0	
2	0.000	0.300	2.0	0.0	0.0	
3	0.500	0.300	2.0	0.0	0.0	
4	20.000	0.300	95.0	0.0	0.0	
5	22.000	0.300	95.0	0.0	0.0	
6	22.100	0.300	2.0	0.0	0.0	
7	25.000	0.300	2.0	0.0	0.0	

FIGURE 1. LC Gradient Program Used for Multi-class Veterinary Drugs.

Mass Spectrometry

Mass spectrometer: Thermo Scientific™ TSQ Endura™ triple-stage quadrupole LC-MS
Spray voltage: 3500V positive, 2500 negative
Capillary temperature: 250° C
Vaporizer temperature: 300° C
Sheath gas: 40arb
Aux Gas: 5arb
Sweep gas: 1arb
Collision gas: 1.5
Q1 and Q3 (FWHM): 0.7



Data Analysis

Thermo Scientific™ TraceFinder™ software with a specific veterinary drugs compound database (283 analytes) was used for data processing. The compound database contains 3–5 SRM transitions per compound, including optimized CEs, lens voltages, and retention times. The database allows easy setup of the full method or selected compounds on the TSQ Endura triple-stage quadrupole LC-MS.

Results

Instrument Calibration

All matrices were spiked with standard mixtures prepared from individual stock solutions. Total of 11 mixes containing approximately 25 compounds per mix. Total of 255 veterinary drugs, to final volume of 500 µL with internal standards added at 20 ppb.

Calibration curve levels in each matrix: 0.05, 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 200, 300 ppb (12 point curve) and matrix blank. (See Figure 2)

Prepared in 40 % ACN:Water

Seven replicates at each concentration level were analyzed to obtain % RSD at each concentration level

r^2 for 95%+ compounds > 0.990 in all matrices

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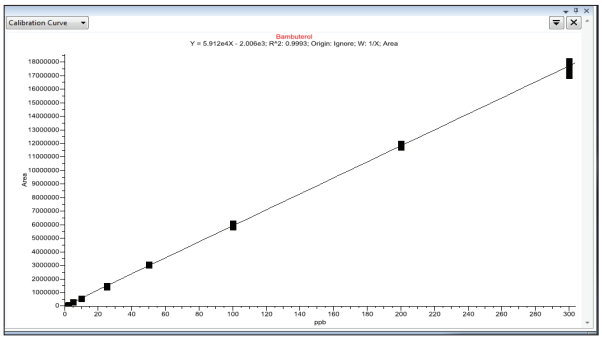
FIGURE 2. Example calibration curve for Bambuterol with seven replicates at each calibration level in the beef kidney matrix.



Analytical Challenges

Multi-Class compounds using a generic mobile phase and column resulted in some analytes being poorly retained and/or poorly ionized. As a result, some compounds were removed from the final quantitative method.

Peak shapes weak and broad: For several acidic compounds, tetracyclines, perfloxacin

Unknown stability and quality of standards: Majority of neat standards dissolved in ACN:MeOH and/or DMSO

At 40% acetonitrile, peak shapes are generally good; 3 ul is a good compromise injection volume.

The submitted matrices were not completely free of incurred veterinary drugs. All of the matrices contained nicotinamide at high concentration (>100 ppb) and Riboflavin (Vitamin B2) in beef liver, beef kidney, and chicken liver at concentrations approximately 100–500 ppb. (See Figures 3 and 4)

FIGURE 3. Nicotinamide in beef muscle compared to solvent spike. The compound was detected in all matrices studied with passing ion ratios; estimated concentration at 150 ppb.

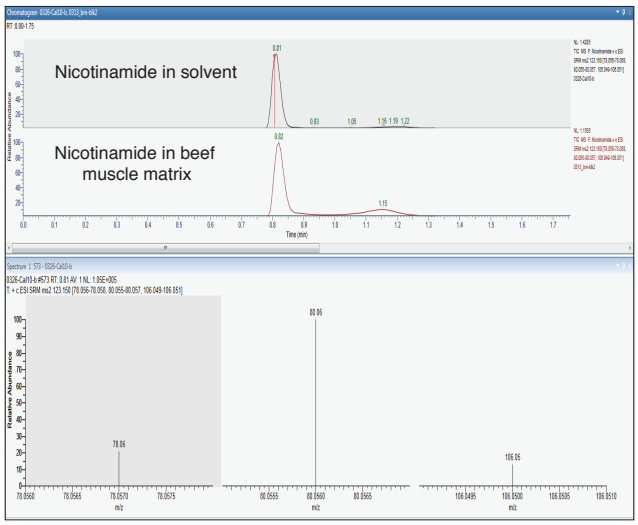
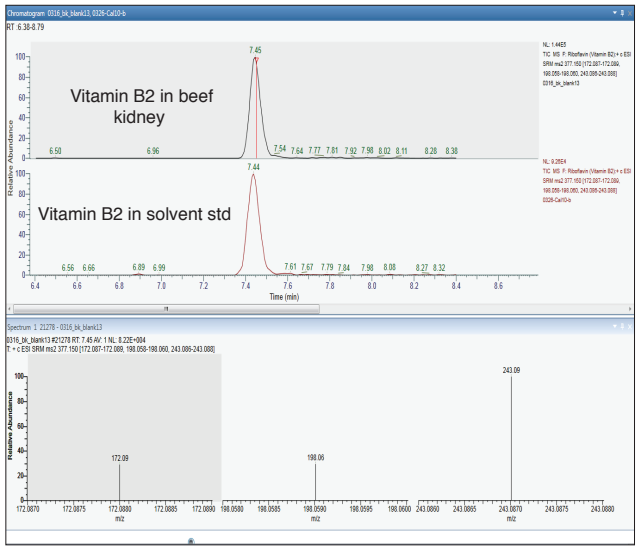


FIGURE 4. Riboflavin (Vitamin B2) in beef kidney matrix compared to solvent standard. Ion ratios passed in all samples (beef kidney, beef liver, and chicken liver).



LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by analyzing a series of 7 replicates at each calibration level for each matrix. TraceFinder software then calculates the coefficient of variation and % RSD at each level. This provides an estimate of the LOD and LOQ for each of the veterinary drugs using the following criteria:

LOD = <20% RSD for the calculated concentration

LOQ = <15% RSD for the calculated concentration

The ranges of LODs and LOQs were very similar in all the matrices studied. LODs ranged from 0.05–100 μg/Kg and LOQs ranged from 0.1–200 μg/Kg. (See Table1)

FIGURE 5. Digoxin at 10 ppb passing ion ratio qualitative confirmation. Ion ratios set to 25% (2 or 3 ions for most analytes).

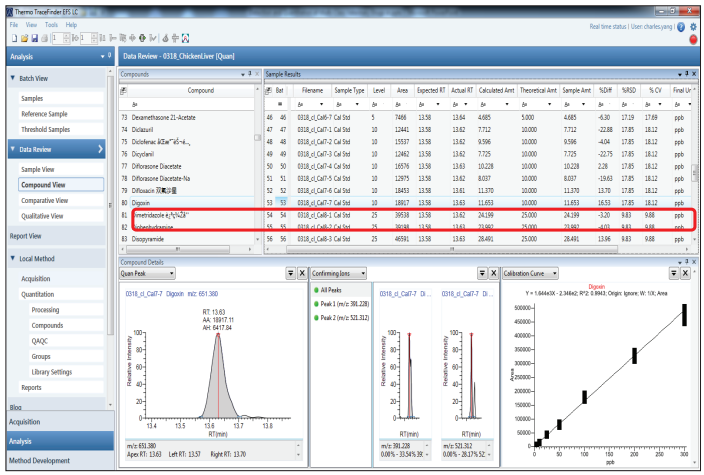


TABLE 1. LOD/LOQs (µg/Kg) for select compounds in the various matrices, with LOD in blue and LOQ in red.

Compound	Beef Muscle	Pork Muscle	Chicken Muscle	Beef Kidney	Beef Liver	Chicken Liver
5-Hydroxy-Thiabendazole	2.5	2.5	0.5	1	2.5	2.5
5-Hydroxy-Thiabendazole	5	5	1	2.5	5	5
Abamectin [M+Na]	25	25	25	10	10	10
Abamectin [M+Na]	50	50	50	25	25	25
Acriflavine	1	1	1	1	2.5	1
Acriflavine	2.5	2.5	2.5	2.5	5	2.5
Albendazole	0.05	0.05	0.5	0.05	0.5	0.5
Albendazole	0.1	0.1	1	0.1	1	1
Albendazole_Sulfone	0.5	1	0.5	0.5	0.5	1
Albendazole_Sulfone	1	2.5	1	1	1	2.5
Albendazole_Sulfoxide	1	0.5	0.5	1	0.5	0.5
Albendazole_Sulfoxide	2.5	1	1	2.5	1	1
Amcinonide	2.5	2.5	2.5	2.5	2.5	5
Amcinonide	5	5	5	5	5	10
Aminophenazone	0.5	1	2.5	2.5	2.5	1
Aminophenazone	1	2.5	5	5	5	2.5
Amoxicillin	1	2.5	1	2.5	2.5	1
Amoxicillin	2.5	5	2.5	5	5	2.5
Ampicillin	25	50	10	50	25	25
Ampicillin	50	100	25	100	50	50
Androstendione	5	5	5	2.5	2.5	5
Androstendione	10	10	10	5	5	10
Azaperol	2.5	2.5	2.5	1	1	1
Azaperol	5	5	5	2.5	2.5	2.5
Azaperone	1	1	1	2.5	2.5	0.5
Azaperone	2.5	2.5	2.5	5	5	1
Bambuterol	0.5	0.1	0.5	0.1	0.1	0.1
Bambuterol	1	0.5	1	0.5	0.5	0.5
Beclomethasone	25	10	10	25	50	50
Beclomethasone	50	25	25	50	100	100
Beclomethasone Dipropionate	2.5	2.5	1	2.5	5	2.5
Beclomethasone Dipropionate	5	5	2.5	5	10	5
Brombuterol	2.5	1	0.5	1	1	1
Brombuterol	5	2.5	1	2.5	2.5	2.5
Buspirone	0.5	0.5	1	1	1	1
Buspirone	1	1	2.5	2.5	2.5	2.5
Carbamazepine	0.5	0.5	0.1	0.5	0.5	0.5
Carbamazepine	1	1	0.5	1	1	1
Sulfadiazine	2.5	5	1	2.5	1	2.5
Sulfadiazine	5	10	2.5	5	2.5	5
Sulfadimethoxine	1	1	1	1	1	0.5
Sulfadimethoxine	2.5	2.5	2.5	2.5	2.5	1
Sulfadoxine	5	2.5	2.5	5	2.5	10
Sulfadoxine	10	5	5	10	5	25
Sulfaguanidine	1	5	1	1	5	2.5
Sulfaguanidine	2.5	10	2.5	2.5	10	5
Sulfamerazine	5	5	2.5	5	5	5
Sulfamerazine	10	10	5	10	10	10
Sulfameter	5	5	2.5	5	5	10
Sulfameter	10	10	5	10	10	25
Sulfamethazine	1	1	0.5	1	0.5	1
Sulfamethazine	2.5	2.5	1	2.5	1	2.5
Sulfamethizole	2.5	1	2.5	2.5	2.5	2.5
Sulfamethizole	5	2.5	5	5	5	5
Sulfamethoxazole	10	10	5	5	2.5	2.5
Sulfamethoxazole	25	25	10	10	5	5
Sulfamethoxypyridazine	2.5	5	2.5	5	5	5
Sulfamethoxypyridazine	5	10	5	10	10	10
Sulfamonomethoxine	5	10	5	10	5	10
Sulfamonomethoxine	10	25	10	25	10	25
Sulfanitran	25	10	5	5	10	10
Sulfanitran	50	25	10	10	25	25
Sulfaphenazole	1	2.5	1	2.5	1	1
Sulfaphenazole	2.5	5	2.5	5	2.5	2.5
Sulfapyridine	2.5	2.5	2.5	5	5	5
Sulfapyridine	5	5	5	10	10	10
Sulfaquinoxaline	1	1	2.5	1	1	0.5
Sulfaquinoxaline	2.5	2.5	5	2.5	2.5	1
Sulfasalazine	1	2.5	2.5	2.5	2.5	5
Sulfasalazine	2.5	5	5	5	5	10
Sulfathiazole	1	2.5	1	2.5	2.5	2.5
Sulfathiazole	2.5	5	2.5	5	5	5

Conclusion

- A multi-class, comprehensive quantitative LC/MS/MS method for over 200 veterinary drugs has been developed. A compound database for the method, including retention times, MRMs, and optimized instrumental parameters is built directly into the TraceFinder data processing software, making it very easy to set up and run the multi-class method.
- LODs and LOQs obtained meet or exceed established MRLs for most compounds in the meat products studied.
- Riboflavin (Vitamin B2) was detected in beef liver, beef kidney, and chicken liver extracts at approximately 100–150ppb. All matrices were found to contain high concentrations of Nicotinamide (ion ratios and RTs confirmed) at approximately 200–300 ppb.
- Analytical challenges of the multi-class method include stability of mixed stock standards, poorly retained (highly polar) analytes, poor ES ionization, and poor chromatographic performance of certain drugs. Despite these challenges, the developed method allows consolidation of several individual methods.

References

1. (EU) Regulation 1831/2003/EC; Directive 2009/8/EC
2. U.S. Department Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038

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