

ThermoFisher SCIENTIFIC The VetDrugs Explore

The VetDrugs Explorer Collection: A Comprehensive Solution for Multi-class Veterinary Medicines Analysis by LC-MS/MS

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SciSpec co., Itd.

- Brief overview of background and challenges for the analysis of Veterinary Drugs
- Description of the VetDrugs Explorer Product
- Key benefits / features: novel column technology and H-SRM
- Method validation results for more than 170 analytes
 - Screening Target Concentration (STC) Concept and examples
 - Method Detection Limits (MDL) and Recovery Data
 - Precision and confirmation relative to global lowest Maximum Residue Limit (MRLs)
- Conclusions



Background to Veterinary Drugs

What are veterinary drugs?

- Pharmacologically active compounds which are used to treat and prevent diseases of animals
- Restore, correct or modify physiological functions by exerting a pharmacological, immunological or metabolic action
- Residues of veterinary drugs in food
 - Residues or their transformation products can remain in foods after treatment of animals
 - Frequency of residues is very low
- Approvals and usage are highly regulated and monitored
- Concerns over antibiotic resistance from over-use in farming world-wide





- Historically based on class specific methods
- Move toward use of generic extraction approaches
 - e.g. QuEChERS based approach screening as many compounds as possible in a single analysis
 - Broad scope, but less clean-up and lower recoveries for some compounds
- MS based screening approaches accepted in veterinary medicines
 - Validation of screening method based on detectability (CCß, POD)
 - Use of internal standards and matrix-extracted calibrations

• In Reality:

• Many labs use a combination of MS screening and class specific methods





Challenges of Multi-Residue Methods

- Generic enough to apply to several different matrices e.g. meat, fish, dairy.....
- Stability of Matrix Extracted Spikes (MES) and spiking standards
- Chromatography Column must handle wide polarity range; be rugged
- Sample preparation must minimize loss of analytes, be simple and cost effective
- Need sufficient sensitivity for all compounds
- Need for polarity switching
- Avoid reporting residue result not actually in sample (False +ve)
- Avoid missing residue result in a sample from not being detected (False -ve)
- Results need to be in compliance with regulations & accreditation requirements



Can we solve these challenges in a single workflow?



Balancing the Complexity of Multi-Class Veterinary Drug Method Development



Prep and stability of multi-component mixtures



Generic sample prep with good recoveries + RSDs



Wide range of chemical classes+ MRLs



Inert LC system and column for wide pKa range and good peak shape



VetDrugs Explorer: Collaborative Effort with Iowa State University and FERA

Collaborators for VetDrugs Explorer Collection Development and Validation



David J. Borts, PhD, VDPAM Iowa State Veterinary Diagnostics Laboratory Section Leader, Analytical Chemistry



George Stubbings Higher Scientific Officer Fera Science Limited





VetDrugs Explorer Collection- A Product to Address the Entire Lab Workflow



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VetDrugs Explorer Technical Highlights

Feature	Detail	Comment	
# of Compounds	170+ Analytes with acquisition and master processing method	Compound data base contains extra analytes (250 total) for labs wanting to add more compounds	
Matrices	Muscle meat (cattle), milk, and salmon (fillet)	Demonstrates broad applicability of the multi-class method approach	
QC and Analytical Standards	QC Check-20 cmpds Standards- 170 compounds	Both Included in the kit	
Sample Preparation	Generic QuEChERs extraction w/simple clean-up	Detailed procedure using included Thermo reagents	
Column	Accucore™ VDX 100mm x 2.1 x 2.6 um	Special Thermo column for optimal chromatographic performance	i Cen
UHPLC-MS system	TSQ Altis with Vanquish Flex Binary pump	Includes detailed User guide for proper installation and system check-out	
Software	TraceFinder 4.1 SP5	Master method and acquisition methods, CDB all ready-to-go	TraceFinderTM Optimized for Quantitation



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- Comprehensive User Guide
 - Installation and operating conditions for the LC and MS
 - •QC standard Check protocol with example chromatogram
 - Detailed description of sample prep for all 3 matrices
- Guide for preparation of spiking cocktails (Excel sheet)
- Overview of analyte standards included in the kit
 - Excel with Compound information, exact mass, formula, polarities, adducts, and RTs
 - Information on other analytes not included in the kit (reference material)





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Method Details and Examples of Method Performance

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Abbreviation for Veterinary Drug Explorer

- MDL Method Detection Limit
- MES Matrix Extracted Spike
- MRL Maximum Residue Limit
- STC Screening Target Concentration
 - Almost ½ of MRL
- VDX Veterinary Drug Explorer



Chemical Classes within the VDX Method





Sample Preparation and LC Conditions

QuEChERS based approach

- EDTA/NH₄ oxalate solution and acetonitrile
- Sample homogenised until fully dispersed
- Sodium sulphate added before centrifugation
- Dispersive SPE (CEC-C₁₈) clean-up
- Add 1 mL H2O to 3mL extract, filter, inject

LC conditions

- Thermo Scientific[™] Accucore[™] VDX Column
 2.1 x 100 mm, 2.6 μm
- MP A: 0.05% formic acid
- \circ MP B: 0.05% formic acid, 5% H2O in 1:1 MeOH:MeCN
- 2 μL injection

Acquire Data on TSQ Altis

- Use pos/neg switching
- Comprehensive CDB with all optimized SRMs



The development and validation of a multiclass liquid chromatography tandem mass spectrometry (LC–MS/MS) procedure for the determination of veterinary drug residues in animal tissue using a QuEChERS (QUick, Easy, CHeap, Effective, Rugged and Safe) approach

George Stubbings*, Timothy Bigwood Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK



Column-Accucore VDX- Robust and Selective for VetDrugs



Features

- Solid core particle- high resolution separations
- Column chemistry selectivity similar to C18 columns
- Optimized for MS detection
- Low column bleed
- Optimized for low tailing
- Robust against matrix extracts
- Particle size: 2.6 µm







Technology in TSQ Altis: High Sensitivity with Robustness



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Improved Sensitivity and Specificity with H-SRM (0.2 Da FWHM)



Advantage of H-SRM for Fluazuron in salmon fillet extract: Noise is significantly reduced allowing Improved signal-to-noise (selectivity) against the matrix with high ion transmission



Calibration curve for Flunixin in salmon fillet matrix using H-SRM.

Compound	MDL (0.7 SRM)	MDL (0.2 HSRM)			
Derquantel	0.42	0.04			
Fenbendazole Sulfone	0.33	0.05			
Flunixin	0.38	0.04			
Hydroxyipronidazole	0.62	0.08			
Robenidine	0.33	0.05			
Teflubenzuron	4.47	0.47			

Advantage of H-SRM for improved method detection limits (MDLs) for select compounds in a salmon fillet extract



TIC and SRMs



TIC of all SRMs @ 1xSTC in TraceFinder software

The distribution of 546 SRMs are easily visualized in the instrument software tune page

Quantitative Results- 0.2 to 5 x STC-Bovine Matrix Extracted Spike (MES)





Compound Class- Average % Recovery (Absolute-Uncorrected)

Average Absolute % Recovery by Compound Class





Compound Class - Average Calculated MDL (ng/g)

Average Method Detection Limit (MDL) by Class





Example Data : Salmon Matrix- Precision at STC Levels Across 4 LC/MS/MS Systems

	Lowest Global								
Compound	MRL	STC	System 1	System 2	System 3	System 4	Average	SDEV	%RSD
Amoxicillin	50	25	24.4	24.8	25.0	27.3	25.4	1.31	5.2
Chlortetracycline	200	100	87.3	97.1	86.4	104.0	93.7	8.39	9.0
Danofloxacin	100	50	50.9	49.8	47.3	52.3	50.1	2.09	4.2
Dicloxacillin	300	150	144.5	148.2	147.7	152.2	148.2	3.15	2.1
Difloxacin	300	150	150.0	149.2	145.6	159.3	151.0	5.80	3.8
Doxycycline	10	10	9.2	9.1	9.2	10.1	9.4	0.47	5.0
Enrofloxacin	100	50	50.2	49.4	55.0	52.3	51.7	2.51	4.9
Flumequine	500	200	200.8	201.9	201.5	199.3	200.9	1.17	0.6
Oxolinic Acid	50	25	28.2	29.3	27.5	25.8	27.7	1.48	5.3
Oxytetracycline	200	100	102.2	96.4	94.6	101.1	98.6	3.65	3.7
Penicillin G	50	25	23.6	24.0	21.6	25.4	23.6	1.57	6.7
Sarafloxacin	10	3	3.0	3.0	2.9	3.1	3.0	0.06	2.0
Sulfadoxine	SUM 100	10	9.1	8.7	9.4	10.1	9.3	0.59	6.3
Tetracycline	200	100	98.8	98.3	91.7	100.4	97.3	3.81	3.9
Thiamphenicol	50	25	23.6	24.0	22.2	26.5	24.1	1.80	7.5
Tilmicosin	50	25	27.5	28.0	25.4	23.2	26.0	2.21	8.5
Trimethoprim	50	25	21.8	18.7	24.0	22.0	21.6	2.19	10.1
Tylosin	100	50	46.8	47.5	45.9	47.7	47.0	0.80	1.7



Modular Multi-class method – can be customized as needed



- The Vetdrugs Explorer offers labs the opportunity to consolidate / aggregate single-class methods
- The method detection limits were compliant with the lowest global MRLs in all cases
- The Vetdrugs Explorer method has proven to be robust, providing stable response and retention times with good detection limits over 500 injections
- All aspects of the complete workflow are productized in a single kit for easy implementation
- The generic sample prep method demonstrates good recovery of multi-class compounds in the matrices studied
- The unique H-SRM capability of the TSQ Altis can result in increased specificity and lower method detection limits
- The VetDrugs Explorer method is consistent with the recently approved AOAC SMPR



