A Comprehensive Multi-Class Veterinary Medicines Method Using A New Best-In-Class Triple Quadrupole Mass Spectrometer

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New Thermo Scientific™ TSQ Quantis™ and TSQ Altis™ Triple Quadrupole MS Systems overview

Veterinary Medicines Methodology
- Challenges of multi-class veterinary medicines analysis and integration into a routine testing laboratory
- Thermo Scientific™ Acclaim™ Column Technology for wide range of VetDrugs
- Scope of method and results

Conclusions
Introduction to TSQ Altis and TSQ Quantis

<table>
<thead>
<tr>
<th></th>
<th>TSQ Altis High-end</th>
<th>TSQ Quantis Mid-tier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Range</td>
<td>5-2000</td>
<td>5-3000</td>
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<tr>
<td>SRM/sec</td>
<td>600</td>
<td>600</td>
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<tr>
<td>Selectivity (H-SRM)</td>
<td>0.2 Da FWHM</td>
<td>0.4 Da FWHM</td>
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<tr>
<td>Sensitivity (HESI)</td>
<td>500,000:1</td>
<td>150,000:1</td>
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</table>

Robustness, Reproducibility, Speed, Ease-of-Use, Flexibility
Technology in TSQ Quantis: Excellent Robustness, Day After Day

- Enhanced dual-mode electron multiplier detector
  - Ensures excellent linearity and dynamic range

- Segmented Quadrupoles
  - With hyperbolic surfaces for enhanced performance with both SRM and H-SRM (0.4 FWHM)

- Stacked ring ion guide (SRIG)
  - Increases ion flux

- Active collision cell with axial DC field
  - Facilitates more SRMs/sec

- Ion beam guide with neutral blocker
  - Reduces chemical background

- OptaMax™ NG
  - APCI ready

NEW!
Technology in TSQ Altis: High Sensitivity with Robustness

- **Segmented Quadrupoles**
  - with hyperbolic surface for enhanced performance with both SRM and H-SRM (0.2 FWHM)
  - NEW!

- **Electrodynamic ion funnel (EDIF)**
  - Increases ion flux

- **Active collision cell with axial DC field**
  - facilitates more SRMs/sec
  - NEW!

- **OptaMax™ NG**
  - APCI ready
  - NEW!

- **High capacity ion transfer tube (HCTT)**
  - Increases ion flux

- **Ion beam guide with neutral blocker**
  - Reduces chemical background

- **Ion beam guide with neutral blocker**
  - Reduces chemical background

- **Enhanced dual-mode electron multiplier detector**
  - Ensures excellent linearity and dynamic range
  - NEW!
Improved Sensitivity with H-SRM (0.2 Da FWHM) – GPSVFPLAPSSK

GPSVFPLAPSSK - Peptide from monoclonal antibody

- **GPS H-SRM**
  - Q1 0.2 Da FWHM
  - %CV = 1.3
  - AA: 621
  - SN: 21

- **GPS SRM**
  - Q1 0.7 Da FWHM
  - %CV = 11.3
  - AA: 2689
  - SN: 5

- **Internal Standard SRM**
  - Q1 0.7 Da FWHM
  - %CV = 3.2
  - AA: 63810

- **H-SRM**
  - LOQ = 25 ng

- **SRM**
  - LOQ = 100 ng

25 ng GPSVFPLAPSSK and IS
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 prep must minimize loss of analytes and be simple and cost effective
- Single mobile phase for all compounds
- Sufficient sensitivity for certain compounds
- Need for polarity switching
- Accurate quantification
- Identification against guideline criteria
- Can we solve these challenges in a single workflow?
Column-Acclaim Polar Advantage II (PA2)- Robust and Selective for VetDrugs

Features
- Unique selectivity- amide embedded group
- Enhanced hydrolytic stability
- 100% aqueous compatibility
- pH Range 1.5 to 10.5
- Low column bleed
- Robust against matrix extracts
- Particle size: 2.2, 3.0 or 4.5-µm
- Advanced surface technology
- Acclaim columns use innovative silane ligands - ensures unique selectivity
Column-Acclaim Polar Advantage II (PA2)- Robust and Selective for VetDrugs

Dyes- 1xSTC (1ng/g) in Salmon Fillet

Acidic Compounds- 3 x STC in Bovine Muscle
• 160+ compounds in 3 matrices: bovine muscle, salmon fillet, and milk (plus addition of labelled internal standards) included in the method from the following classes of veterinary medicines:
  • Cefalosporins, macrolides, penicillins, quinolones, sulfas, tetracyclines, anthelmintics, nitroimidazoles, NSAIDs, sedatives, avermectins and coccidiostats, dyes (applied to fish), steroids (milk)

• Experimental Design:
  • 8 x spikes @ 0.2, 0.5, 1, 3, and 5 x STC = [Screening Target Concentration] for each compound with 2 blanks and one recovery spike per batch
  • Analyze the batches on 3 separate LC/MS/MS systems
  • Use basic elements of the same sample prep applied to all 3 matrices
Compounds Studied and Chemical Classes

- Antibiotics-68
- β-agonist-11
- Coccidiostat-17
- NSAID-13
- Aquaculture (Dyes and metabolites)-12
- Antihelmintic-23
- Steroids-9
- Other-23
Sample Preparation and LC Conditions

- **QuEChERS based approach**
  - EDTA/NH₄ oxalate solution and acetonitrile added to sample
  - 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™ Vanquish™ Acclaim™ PA2, 2.1 x 150 x 2.2 um
  - MP A: 0.05% formic acid + 0.1 mM NH₄F (aq)
  - MP B: 0.05% formic acid in 1:1 MeOH:MeCN
  - 2 uL injection

- **Acquire Data on TSQ Altis**
  - *Use pos/neg switching*
  - Comprehensive CDB with all optimized SRMs

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<th>Flow [ml/min]</th>
<th>%B</th>
<th>Curve</th>
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</table>
Steps for Evaluating Method Performance

1. Prepare Matrix Extracted Spike (MES) Replicates at 0.2-5 x STC (Establish 'Calibration Line' for screening)
2. Establish Screen Target Concentration (STC) Level Levels typically ½ the MRL/MRPLs
3. Calculate %RSDs at each level to check precision
4. Calculate MDL @ or below a cut-off (Lowest xSTC factor at or below 15% RSD)
5. Calculate Absolute % Recovery Based upon A 'post spike' at 3 x STC [Recovery Std]
Extracted SRMs for Multi-Class VetDrugs

Extracted SRMs at 0.5 x STC in MES

TSQ Altis - total of 525 transitions from analysis at left
Quantitative Results - 1x to 5x STC - Bovine

- Sulfa Drugs - Sulfamerazine
- NSAIDs - Flunixin

8 replicates plotted per each point
Quantitative Results - 0.2 to 5 x STC - Bovine

Quinolones - Sarafloxacin

Nitroimidazoles - Ronidazole
Quantitative Results - 0.2 to 5 x STC-Bovine

Antibiotics - Erythromycin

Antibiotics - Oxytetracycline
Quantitative Results- 0.2 to 5 x STC-Salmon Fillet

Leucomalachite Green in salmon extract at 1 x STC, with curve representing 0.2-5 ng/g.
Quantitative Results- 0.2 to 5 x STC-Milk

Steroid hormone Megestrol acetate in milk extract at 1 x STC, with curve representing 0.04-1.0 ng/g
### Observed MDLs and % Recoveries in MES

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bovine Muscle</th>
<th>Salmon Fillet</th>
<th>Milk*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDL Average (ng/g)</strong></td>
<td>2.7</td>
<td>3.4</td>
<td>NA</td>
</tr>
<tr>
<td><strong>MDL Range (ng/g)</strong></td>
<td>0.01-76</td>
<td>0.01-126</td>
<td>NA</td>
</tr>
<tr>
<td><strong>% Recovery-Mean</strong></td>
<td>72.7</td>
<td>73.2</td>
<td>NA</td>
</tr>
<tr>
<td><strong>% Recovery Range</strong></td>
<td>39.7-97.5</td>
<td>34.4-101</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Notes:**
- *Milk results pending data reduction
- MDL based on 8 replicate injections (EPA-based Student t calculation)
- Stability of some compounds result in poor precision/higher MDLs, eg. Ampicillin, Penicillin G
- %Recovery is **absolute recovery** (no correction) based on comparison with post-spiked MES@ 3xSTC
Example comparison of matrix extracted spike vs. post-spike to show absolute recovery from the extraction process.
Compound Class- Average Calculated MDL (ng/g)

Example Albendazole in Salmon Fillet
0.2 x STC in Thermo Scientific™ TraceFinder™ software
Conclusions

• New Thermo Scientific™ TSQ Altis™ and Quantis™ triple quadrupole instruments offer advanced technology and innovative design for robust operation and high sensitivity

• A Multi-class veterinary method has been developed that shows:
  • Fit-for-purpose Acclaim PA2 column for robust analysis, great peak shape for wide range of compound classes
  • Generic QuEChERS extraction applied to bovine, salmon fillet, and milk is easy to use, low cost, with no extract concentration
  • Good results for absolute recovery, precision, and low MDLs for most analytes studied with STC screening range of 0.2 to 5x (Can easily go lower on several analytes)
  • Further optimization of the method on-going with collaborator at Iowa State
Confident Quantitation
Any compound, any matrix, any user.