Screening of Veterinary Drugs in Meat by LC/Q-TOF

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LC-MS Applications Scientist
Market Development Group

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Veterinary Drugs in Food

Definition and sources

✓ “Important tools in the prevention and control of animal diseases” – OIE (World Organization For Animal Health)
✓ Can also be used to promote and stimulate animal growth
✓ Improper administration and excessive use can lead to drug residues in food products

Veterinary Drugs  Animal contamination  Meat Processing  Contaminated Meat
What is it?

“Antimicrobial resistance is resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it.”

- World Health Organization
Veterinary Drugs in Food Contamination reports

Veterinary drug found in Asda budget corned beef

Asda is recalling all corned beef from its budget range after traces of the veterinary drug phenylbutazone were found in some batches.

Tainted meat: Banned veterinary drugs found in horse meat

Dispute Over Drug In Feed Limiting US Meat Exports

By Helena Bottemiller, On January 25, 2012
Veterinary Drugs in Food Regulation

• USA
  - Code of Federal Regulations (CFR) – Title 21 Part 556
  - “Tolerance for Residues in New Animal Drugs in Food”

• Europe (EU)
  - “monitor certain substances and residues thereof in live animals and animal products”

• China
  - Ministry of Agriculture (MOA) – Notice no. 235
  - Lists drugs that can and cannot be detected in animal meat
## Veterinary Drugs in Food Monitoring in US

![USDA Logo](image)

### FSIS Residue Violation Information System

**January 14, 2016**

**WEEKLY RESIDUE REPEAT VIOLATOR FOR USE BY FSIS INSPECTION PROGRAM PERSONNEL**

Part I: This part is intended to assist Inspection Program Personnel to identify producers with more than one residue violation in the last 12 months either in the same establishment or different establishments.

<table>
<thead>
<tr>
<th>Source Name By State</th>
<th>Plant Name / ID</th>
<th>Sample ID / Date Collected / Tags</th>
<th>Tissue</th>
<th>Residue</th>
<th>Value (ppm)</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>101043957 03/06/15 BOV VEAL EAR TAGS 13374 BACK TAGS 91 TA 7174</td>
<td>KIDNEY</td>
<td>SULFAMETHOXAZOLE</td>
<td>DETECTED</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>101041339 03/04/15 BOV VEAL EAR TAGS 13280 BACK TAGS 91 TA 7132</td>
<td>KIDNEY</td>
<td>SULFAMETHOXAZOLE</td>
<td>DETECTED</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>101023302 02/10/15 BOV VEAL BACK TAGS 91/TA 6504</td>
<td>KIDNEY</td>
<td>SULFAMETHOXAZOLE</td>
<td>DETECTED</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>101228260 11/05/15 COWS - DAIRY BACK TAGS 93zz056</td>
<td>KIDNEY</td>
<td>AMPICILLIN</td>
<td>0.06</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Veterinary Drugs in Food

Classes – List based on USDA-ARS

- Anthelmintics (27)
- Anti-inflammatories (11)
- β Agonists (5)
- β Lactams (11)
- Coccidiostats (6)
- Fluoroquinolones (8)
- Macrolides (8)
- Miscellaneous (8)
- Phenicols (4)
- Sulfonamides (16)
- Tetracyclines (4)
- Thyreostats (5)
- Tranquilizers (9)

Total: 122

Sample preparation and storage:
- Not retained on RP-HPLC without use of ion pairing agents.
Matrices Tested:

i. Muscle
ii. Kidney
iii. Liver

Sample Spikes:
0.5x, 1.0x and 2.0x TLs for each VD.

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Veterinary Drugs in Food
Overall Project Goals

1. Develop a sample preparation technique to analyze veterinary drugs in meat
   ✓ Webinar available on our website (www.agilent.com)

2. Compare EMR-Lipid extraction with currently accepted QuEChERS dSPE employed by USDA-ARS
   ✓ Both methods performed well for extraction of a large variety of VDs in kidney, liver and muscle
   ✓ Peer-review publication currently under preparation with Steve Lehotey at USDA-ARS

3. Develop a rapid, sensitive screening method for VDs with Agilent UHPLC-QTOF-MS using All Ions MS/MS workflow

4. Develop a sensitive quantitation method for VDs using Agilent UHPLC-MS/MS COMING SOON!
# Method Conditions

## UHPLC parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UHPLC Instrument</strong></td>
<td>1290 Infinity Binary LC with Intelligent System Emulation Technology (ISET)</td>
</tr>
<tr>
<td><strong>UHPLC Column</strong></td>
<td>Agilent ZORBAX Eclipse Plus C-18 (2.1x100 mm, 1.8 µm)</td>
</tr>
</tbody>
</table>
| **Mobile Phase**      | A: Water + 0.1% Formic Acid  
                        | B: Acetonitrile (MeCN) + 0.1% Formic Acid                             |
| **Flowrate**          | 0.5 mL/min                                                            |
| **Gradient**          |                                                                      |
|                       | | Time (min) | B (%)       |
|                       | 0.0         | 2           |
|                       | 1.0         | 2           |
|                       | 10          | 100         |
|                       | 11          | 100         |
|                       | 11.1        | 2           |
| **Injection Volume**  | 15 µL (80/20: H₂O/MeCN)                                              |
Agilent 6550 LC-QTOF MS

iFunnel Technology

- 5-10x increase in ions sampled
- Enhanced sensitivity
Agilent 6545 LC-QTOF MS
SWARM Tune

Advantages

- Increased Sensitivity
- Better Mass Accuracy
- Faster Tune
# Method Conditions

**QTOF-MS parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Condition</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>Agilent 6545 Accurate-Mass QToF-MS</td>
<td>Agilent 6550 Accurate-Mass QToF-MS with iFunnel technology</td>
</tr>
<tr>
<td>Ionization Mode</td>
<td>Positive electrospray ionization with jet stream</td>
<td>Positive electrospray ionization with dual jet stream</td>
</tr>
<tr>
<td>Instrument Mode</td>
<td>2 GHz extended dynamic range</td>
<td>2 GHz extended dynamic range</td>
</tr>
<tr>
<td>Instrument Tune Range</td>
<td>SWARM tune with fragile ion (m/z: 50-750)</td>
<td>Classic tune (m/z: 50-1700)</td>
</tr>
<tr>
<td>Mass Range</td>
<td>m/z: 50-1000</td>
<td>m/z: 50-1000</td>
</tr>
<tr>
<td>Drying Gas Temperature</td>
<td>200 ºC</td>
<td>130 ºC</td>
</tr>
<tr>
<td>Drying Gas Flow</td>
<td>11 L/min</td>
<td>16 L/min</td>
</tr>
<tr>
<td>Sheath Gas Temperature</td>
<td>375 ºC</td>
<td>375 ºC</td>
</tr>
<tr>
<td>Sheath Gas Flow</td>
<td>11 L/min</td>
<td>11 L/min</td>
</tr>
<tr>
<td>Nebulizer Gas</td>
<td>35 psi</td>
<td>35 psi</td>
</tr>
<tr>
<td>Fragmentor</td>
<td>135 V</td>
<td>365 V</td>
</tr>
<tr>
<td>Capillary</td>
<td>3500</td>
<td>3500</td>
</tr>
</tbody>
</table>
Method Performance

Chromatograms

Sample Chromatogram at 50 ng/g for 122 Veterinary Drugs.
Method Performance

Chromatograms

Sample: Liver (2x Tolerance Level)

Fluoroquinolones

Sulfonamides
Method Performance
Chromatograms

Sample: Liver (2x Tolerance Level)

**Anthelmintics**

**Tranquilizers**

[Chromatogram images of Anthelmintics and Tranquilizers]
Analysis of Veterinary Drugs in Meat
Traditional MS/MS

Quadrupole

Isolation of precursor ions

Collision Cell

Fragmentation of specific ion

Individual MS/MS Spectrum

Ionized species

Precursor species

Product species

Confidentiality Label
14 June 2016
Analysis of Veterinary Drugs in Meat
All Ions MS/MS

- **NO Isolation of precursor ions**
- **Collision Cell**
  - 0 eV
  - 10 eV
  - 40 eV

**Ionized species**
**Precursor species**
**Product species**

**Generic MS/MS Spectrum**
**MassHunter PCDL**
**Individual MS/MS Spectrum**

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*Agilent Technologies*

Confidentiality Label
14 June 2016

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Analysis of Veterinary Drugs in Meat
All Ions MS/MS Data Acquisition

- Three Collision energy experiments performed simultaneously at 0, 10 & 40 eV
- Spectral acquisition at 4.5 spectra/sec
All Ions MS/MS Workflow – Find By Formula

- Source of formulas to confirm:
  - Database / Library
    - D:\MassHunter\PCDL\VetDrugs\Std.cdb
  - Worklist

- Fragment ion source:
  - Use spectral library only
  - Use average fragment spectrum if spectral library not available

- Fragment ion EIC qualification settings:
  - RT difference: ±0.10 min of expected RT
  - S/N ratio: ≥90
  - Coelution score: ≥50
  - Minimum number of qualified fragments: 1
  - Minimum percent of qualified fragments: 75
Analysis of Veterinary Drugs in Meat
Personal Compound Database and Library (PCDL)
Analysis of Veterinary Drugs in Meat

All Ions MS/MS Method Conditions

**Molecular-Ion Assessment**
- Mass accuracy
- Isotope abundance
- Isotope spacing
  - Warn if <85.0  Reject if <50.0

**Find by Formula**
- Database/Library Search
- Veterinary Drugs PCDL

**Precursor Identification**
- Mass Tolerance: 10 ppm
- Retention Time Tolerance: 0.2 min
- Minimum Absolute Area Counts: 10,000

**Fragment Confirmation**
- Min. Number of fragments req.: 1
  - Minimum S/N: 9.0
  - Coelution score: >90.0
Analysis of Veterinary Drugs in Meat
All Ions MS/MS Data Analysis

Agilent Technologies
Analysis of Veterinary Drugs in Meat
All Ions MS/MS Data Analysis

H⁺ adduct

Na⁺ adduct

Average spectrum of 0,10 and 40 eV

Theoretical Isotope Abundance Isotope Spacing

Mass: 351.0356
RT: 7.146
Δ ppm: 2.3
Score: 95.07
Analysis of Veterinary Drugs in Meat
All Ions MS/MS Data Analysis

Chromatogram Results

Fragment 5
Fragment 4
Fragment 3
Fragment 2
Fragment 1
Precursor Ion
Analysis of Veterinary Drugs in Meat
All Ions MS/MS Data Analysis
Analysis of Veterinary Drugs in Meat
False Positive Identification

Compound 1
Mass: 359.1652
RT: 6.01
Δppm: 1.81
Score: 92.0

Compound 2
Mass: 359.1654
RT: 4.09
Δppm: 2.5
Score: 96.2
## Analysis of Veterinary Drugs in Meat

<table>
<thead>
<tr>
<th>Class</th>
<th>Std 0.5x</th>
<th>Std 1.0x</th>
<th>Std 2.0x</th>
<th>Liver 0.5x</th>
<th>Liver 1.0x</th>
<th>Liver 2.0x</th>
</tr>
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<tbody>
<tr>
<td>β-Lactam</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Cloxacillin</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>β-Lactam</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Amoxicillin</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Class</td>
<td>Kidney 0.5x</td>
<td>Kidney 1.0x</td>
<td>Kidney 2.0x</td>
<td>Muscle 0.5x</td>
<td>Muscle 1.0x</td>
<td>Muscle 2.0x</td>
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<td>β-Lactam</td>
<td>Cloxacillin</td>
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<td>β-Lactam</td>
<td>✓</td>
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<td>✓</td>
<td>Nafcillin</td>
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Table shows VDs not identified in spiked samples on 6550

When freshly prepared analytical standards were run, all these compounds were detected.
Analysis of Veterinary Drugs in Meat
Quantification using LC-QTOF MS

113 VDs tested with 4 or 5 point calibration curve (2, 10, 25, 50 & 100 ng/g)

>85% VDs with R2 >0.99

>93% VDs with R2 >0.90 in both matrices

Ipronidazole (0.9981)
Ipronidazole (0.9984)
Enrofloxacin (0.9997)
Enrofloxacin (0.9986)

Ground Beef
Liver
Analysis of Veterinary Drugs in Meat

ESI Negative ...

53 VDs analyzed in negative mode.
Sample: Standard at tolerance level

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<tr>
<td>Flowrate</td>
<td>0.5 mL/min</td>
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<td>Injection Volume</td>
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Analysis of Veterinary Drugs in Meat
ESI Negative …

53 VDs analyzed in negative mode. Sample: Standard at tolerance level

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Screening of Veterinary Drugs in Meat
Complete Toolbox
Analysis of Veterinary Drugs in Meat

Summary

- The current LC-QTOF MS systems can offer rapid screening of several hundred veterinary drugs in complex bovine meat matrices.

- UHPLC allows for fast separation and narrow chromatographic peaks reducing analytical method runtimes.

- All Ions MS/MS workflow allows for additional verification tools in suspect screening with fragment confirmation and retention time through personal compound database and libraries (PCDLs).

- The EMR-Lipid and QuEChERS d-SPE sample preparation methods performed well for the vast majority of analytes tested.

- Sensitive quantification of VDs in complex matrices is possible using current LC-QTOF MS.
References


- “Analysis of 122 Veterinary Drugs in Meat using All Ions MS/MS with an Agilent 1290/6545 UHPLC-QTOF System” (Application Note: 5991-6651EN)

For the full Vet Drugs eSeminar Series, including this event OnDemand and the webinar on **Optimizing Recovery and Productivity in Veterinary Drug Analysis** using EMR Lipid “

Acknowledgements

Agilent Technologies Inc.

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- Steven J. Lehotay – Lead Research Chemist
- Alan R. Lightfield – Research Chemist
For more information please visit: [www.agilent.com/chem/food](http://www.agilent.com/chem/food)

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