Analysis of Veterinary Drug Residues in China

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Shanghai Entry-Exit Inspection and Quarantine Bureau of The People’s Republic of China
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Contents

- Profile of the institution
- Analysis of veterinary drugs
- The newly research findings
The Technical Center for Animal, Plant and Food Inspection and Quarantine (AFTC) is one of the affiliated institutions of Shanghai Entry-Exit Inspection and Quarantine Bureau (SHCIQ).

**Responsibilities:** Inspect the entry-exit foodstuffs, cosmetics and their products, and quarantine the animal and plant products.
Capacities

- State Key Laboratory (Shanghai) of Food Safety.
- Authorized reference laboratory of veterinary drugs (Triphenylmethanes, β-agonists, Resorcylic acid lactones and Steroids) by General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ).
- Coordinate PT’s including Triphenylmethanes in animal feeds in 2013.

<table>
<thead>
<tr>
<th>No.</th>
<th>2013-2014 PT’s Project</th>
<th>Code</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nitrofuran in shrimps</td>
<td>FAPAS PT02229</td>
<td>Satisfied</td>
</tr>
<tr>
<td>2</td>
<td>Chloramphenicol in milk</td>
<td>FAPAS PT1870</td>
<td>Satisfied</td>
</tr>
<tr>
<td>3</td>
<td>Trifluralin in fish meat</td>
<td>FAPAS PT0588</td>
<td>Satisfied</td>
</tr>
<tr>
<td>4</td>
<td>Antibiotics in egg</td>
<td>RILILT 1227295401</td>
<td>Satisfied</td>
</tr>
<tr>
<td>5</td>
<td>β-agonist pork</td>
<td>CNCA-13-A08</td>
<td>Satisfied</td>
</tr>
</tbody>
</table>
Personnel and Equipments

- 15 chemists and 35 technicians/assistants

- GC, GC-MS, GC*GC-MS, GC-QQQ, GC-QTOF; HPLC, HPLC-QQQ, HPLC-IT-TOF, HPLC-QTOF, HPLC-Oritrap; IRMS, ICP-MS, AAS, AFS, RT-IR, GPC, ASE, IC, etc.
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Regulations

• No. 193 Announcement from Ministry of Agriculture of China: The prohibited veterinary drugs and other chemicals in food animals.

• No. 235 Announcement from Ministry of Agriculture of China: The maximum residues limits of veterinary drugs in animals origin food.
Guidance on Method Validation

- GB/T 27404-2008 Criterion on quality control of laboratories-chemical testing of food.

Reference:
- No.SANCO/10684/2009 Method validation and quality control procedures for pesticides residues analysis in food and feed.
- CRL guidelines for the validation of screening methods for residues of veterinary medicines.
- AOAC Guidelines for single laboratory validation of analytical methods for trace-level concentrations of organic chemicals.
The challenges in drug analysis

Diversity of the compounds
- More groups and classes
- Different physical/chemical properties (e.g., polarity and pKa values)
- Parent drugs and metabolites

Complex matrices
- Matrix effect
- Coextracted matrix
- Extremely low part-per-billion levels
Representative Sample

Sample Extraction

Extract Clean-up

GC/MS (PTV) – for known/unknown

SIM/Scan

Deconvolution Final Report

Library Search

LC/QQQ MRM – for known targets

Screen

Confirm

Quantify

LC/QTOF or TOF Full Spectrum – for unknowns or non-targeted

Accurate Mass Database Search

Another injection for MS/MS (QQQ or QTOF)

Molecular Formula Generation

Fragmentation

MS/MS
The advantages of QQQ and QTOF

**QTOF**
- High resolution
- Accurate mass
- High scan speed
- Unknows in one injection
- High sensitive in Full scan

**QQQ**
- MS/MS mode
- Qualify and quantify both
- Low noise and high sensitive
- Knows in one injection

Veterinary drug analysis
Methods Classification in Our Lab

1. Single(-class) Residue Methods
   GC, GC/MS, HPLC, LC-MS/MS

2. Multi-class Residue Methods
   HPLC-MS/MS

3. Non-target Screening Methods
   LC-QTOF

Single(-class) Residue Methods

- SN/T 1979-2007 Determination of praziquantel residue in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 1777.2-2007 Determination of macrolide antibiotic residues in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T2113-2008 Determination of tranquillizer residues in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 2190-2008 Determination of non-steroidal anti-inflammatory drugs residue in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 2220-2008 Determination of benzodiazepine residues in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 2222-2008 Determination of glucocorticosteroids residues in foodstuffs of animal origin for export--LC-MS/MS method
- More than 100+ other SRMs
Multi-class Residue Methods

- SN/T 2624-2010 Determination of basic veterinary drugs residues in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 2443-2010 Determination of multi-residues of acidic and neutral drugs in foodstuffs animal origin for import and export--LC-MS/MS method
- SN/T 3235-2012 Determination of multi-groups of banned drug residues in foodstuffs of animal origin for export-LC-MS/MS method
- .......
SN/T 2624-2010

- 76 basic veterinary drugs
- 6 classes (β-agonist, Benzodiazepine, Sulfonamide, Benzimidazole, Triphenylmethane, Nitroimidazole)

- Acetonitrile and Citrate buffer Extraction
- strong cation exchange SPE Cleanup
- LC-MS/MS in MRM mode
SN/T 2443-2010

- 64 acidic and neutral drugs
- 6 classes (corticosteroid, progestin, Androgens, hypoglycemic and non-steroidal anti-inflammatory drug)

- Acetonitrile extraction
- n-hexane Solvent exchange cleanup
- LC-MS/MS in MRM
SN/T 2235-2012

- 44 banned individual drugs
- 9 classes (β-agonist, Androgen, Glucocorticoid, Estrogen, Nitroimidazols, Resorcylic acid lactone, Triphenylmethane, Sedative and Cloramphenicol)

- Ammonia acetonitrile extraction
- QuEChERS cleanup
- LC-MS/MS in MRM
Non-target Screening Method

- Lab SOP: Qualitative Screening and Quantitative Determination of 100+ Veterinary Drugs in Food Using High Performance Liquid Chromatography Tandem Quadrupole Time-Of-Flight Mass Spectrometry
What's it?

What's concentration?

and/or

That's really all there is

Screening for Target /Unknown

Identification with AM(RT) or PCDL

Confirmation with MS/MS

Quantification With TOF/Q-TOF/QQQ
LRMS v.s. HRMS

Max. 8.7e5 counts.
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## Veterinaries studied (total 105) and their MRLs

<table>
<thead>
<tr>
<th>Name</th>
<th>Number</th>
<th>Maximum residue levels</th>
<th>China</th>
<th>EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta agonist</td>
<td>14</td>
<td><strong>Banned (MRPL)</strong></td>
<td>Banned (MRPL)</td>
<td></td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>13</td>
<td>60 µg/kg (Mebendazole)</td>
<td>60 µg/kg (Mebendazole)</td>
<td></td>
</tr>
<tr>
<td>Benzodioxode</td>
<td>19</td>
<td><strong>Banned (MRPL)</strong></td>
<td>Banned (MRPL)</td>
<td></td>
</tr>
<tr>
<td>Nitroimidazole</td>
<td>10</td>
<td>100 µg/kg</td>
<td>100 µg/kg (Thiabendazole)</td>
<td></td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>19</td>
<td>100 µg/kg</td>
<td>100 µg/kg</td>
<td></td>
</tr>
<tr>
<td>Triphenylmethane</td>
<td>4</td>
<td><strong>Banned (MRPL)</strong></td>
<td>Banned (MRPL)</td>
<td></td>
</tr>
<tr>
<td>Quinolone</td>
<td>14</td>
<td>10~200 µg/kg</td>
<td>10~200 µg/kg</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>5</td>
<td>100 µg/kg (chlortetracycline)</td>
<td>100 µg/kg (chlortetracycline)</td>
<td></td>
</tr>
<tr>
<td>Sugar cortical</td>
<td>7</td>
<td><strong>Banned (MRPL)</strong></td>
<td>Banned (MRPL)</td>
<td></td>
</tr>
</tbody>
</table>
Sample prepare

2.0 g Sample

10mL 0.1% formic acid/acetonitrile, 5g anhydrous NaSO4

homogeneous, shake 10min

4000 rpm for 5 min

Extracted again by 10 mL 0.1% acid/acetonitrile, followed by 10 mL ethyl acetate

Evaporating at 40°C till dryness

reconstituted with 5mL of 5% ammonia/methanol

Collect all elution (HLB functions: Retain the interferences and filtrate)

Eluted with 8mL of 5% ammonia/methanol
## Method parameters—MS condition

<table>
<thead>
<tr>
<th>Mass system</th>
<th>Q TOF MS</th>
<th>Ion source</th>
<th>ESI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebulizer gas</td>
<td>Nitrogen</td>
<td>Polarity</td>
<td>Positive/ Negative</td>
</tr>
<tr>
<td>Pressure</td>
<td>45 psi</td>
<td>Ion spray voltage</td>
<td>4500 V/4000 V</td>
</tr>
<tr>
<td>Drying gas temp</td>
<td>330 ºC</td>
<td>Drying gas flow rate</td>
<td>5L/min</td>
</tr>
<tr>
<td>Sheath Gas temp</td>
<td>400 ºC</td>
<td>Sheath gas flow</td>
<td>10mL/min</td>
</tr>
<tr>
<td>Fragmentor</td>
<td>110 V</td>
<td>Nozzle voltage</td>
<td>0 V</td>
</tr>
<tr>
<td>Mass range</td>
<td>m/z 80-1050</td>
<td>Resolution</td>
<td>4G HR mode</td>
</tr>
</tbody>
</table>
Results

TIC of 105 veterinary drugs standards (5 ng/mL) and sample (5 µg/kg)
Results

Overlaid EIC of 105 veterinary drugs standards (5 ng/mL) and sample (5 µg/kg)
Identification

An analyte was considered positively identified when criteria were confirmed:

- the accurate mass deviation of two selected ions of each analyte was less than 5ppm.
- the ratio of the chromatographic retention time of the analyte to that of the same analyte in standard solution was within 2.5% tolerance.
## Identification of compounds with the same nominal mass

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>Formula</th>
<th>Monoisotopic mass (Da)</th>
<th>Mass difference (ppm)</th>
<th>Identified by</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sulfameter</td>
<td>C11H12N4O3S</td>
<td>280.060301</td>
<td>0</td>
<td>Rt</td>
</tr>
<tr>
<td></td>
<td>Sulfamethoxypridazine</td>
<td>C11H12N4O3S</td>
<td>280.060301</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sulfamonomethoxine</td>
<td>C11H12N4O3S</td>
<td>280.060301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Temazepam</td>
<td>C16H13ClN2O2</td>
<td>300.06656</td>
<td>5.13</td>
<td>Rt and isotope match</td>
</tr>
<tr>
<td></td>
<td>Sulfaquinoxaline</td>
<td>C14H12N4O2S</td>
<td>300.06810</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Formula</td>
<td>Rt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
<td>-------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxypyridazine</td>
<td>C11H12N4O3S</td>
<td>6.35 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfametere</td>
<td>C11H12N4O3S</td>
<td>6.55 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>C11H12N4O3S</td>
<td>7.05 min</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Temazepam (C_{16}H_{13}ClN_{2}O_{2}) and Sulfaquinoxaline (C_{14}H_{12}N_{4}O_{2}S)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Std</th>
<th>Meat</th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{16}H_{13}ClN_{2}O_{2}</td>
<td>300.0661</td>
<td>300.0666</td>
<td>-1.62</td>
</tr>
<tr>
<td>C_{14}H_{12}N_{4}O_{2}S</td>
<td>300.0676</td>
<td>300.0681</td>
<td>-1.69</td>
</tr>
</tbody>
</table>
Temazepam \((\text{C}_{16}\text{H}_{13}\text{ClN}_2\text{O}_2)\) and Sulfaquinoxaline \((\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_2\text{S})\)

Isotope match
Confirmation of Temazepam

MS/MS spectrum

Information of Fragment ion
Confirmation of Sulfaquinoxaline

Cpd 4: C14 H12 N4 O2 S

ESI Product Ion (9.234-9.507 min, 13 scans) Frag=110.0V CID@15.0 (301.0745) [z=1] -> ** s...

MS/MS Formula Details: Cpd 4: C14H12N4O2S C14H12N4O2S

<table>
<thead>
<tr>
<th>m/z</th>
<th>Formula</th>
<th>Abund%</th>
<th>Diff (ppm)</th>
<th>Loss Mass</th>
<th>Loss Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>108.0439</td>
<td>C6 H6 N O</td>
<td>16.95</td>
<td>4.4</td>
<td>193.031</td>
<td>C8 H7 N3 O S</td>
</tr>
<tr>
<td>108.0439</td>
<td>C3 H10 N O S</td>
<td>16.95</td>
<td>35.59</td>
<td>193.0276</td>
<td>C11 H3 N3 O</td>
</tr>
<tr>
<td>156.0106</td>
<td>C6 H6 N O2 S</td>
<td>54.81</td>
<td>5.03</td>
<td>145.064</td>
<td>C8 H7 N3</td>
</tr>
<tr>
<td>156.0106</td>
<td>C9 H2 N O2</td>
<td>54.81</td>
<td>-16.57</td>
<td>145.0674</td>
<td>C5 H11 N3 S</td>
</tr>
<tr>
<td>241.1507</td>
<td></td>
<td></td>
<td>8.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

- Recovery and repeatability. Results with a range from 41.1–120.9% (meat), 52.4–91.9% (milk), and 57.3–118.9% (egg), and the relative standard deviation was less than 20%.

- LODs and LOQs of all drugs ranged from 0.01 µg/kg to 5.96 µg/kg and from 0.04 µg/kg to 18.45 µg/kg, respectively.
Thank you for your attention!

http://www.shciq.gov.cn/english/

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