AOAC International
Stakeholder Panel on Strategic Food Analytical Methods:
Emerging Contaminants & Multi-Residue Analysis of Veterinary Drugs

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AOAC 130th Annual Meeting & Exposition, Dallas, TX, Sept. 18-21, 2016
Community
Chemical Contaminants and Residues in Food

► Subgroup ‘Veterinary drugs’
  Meeting on Tuesday 20 September, 11:45 am – 1:15 pm

► Subgroup ‘Metals’
  Meeting on Tuesday 20 September, 1:30 pm – 3:00 pm

► Subgroup ‘Environmental and Emerging Contaminants’
  Meeting on Tuesday 20 September, 4:30 pm – 6:00 pm

► Subgroup ‘Pesticides’
  Meeting on Tuesday 20 September, 6:15 pm – 7:45 pm

Community meeting on Monday 19 September, 5:00 pm – 7:00 pm
New Topics of Interest

► **Subgroup ‘Environmental and Emerging Contaminants’**

- Validation procedure (guidelines?) for fingerprinting-based methods
- Guidelines for untargeted analysis aimed at identifying unknowns
- Platform for suitable information in the case of a response to crisis
New Topics of Interest

► Subgroup ‘Environmental and Emerging Contaminants’

- Validation procedure (guidelines?) for fingerprinting-based methods
- Guidelines for untargeted analysis aimed at identifying unknowns
- Platform for suitable information in the case of a response to crisis

► Subgroup ‘Veterinary drugs’

- International Standard for multiresidue analysis of veterinary drugs in food
Veterinary Drugs

Definition

“Any substance applied or administered to any food-producing animal, such as meat or milk producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behaviour.”

by Codex Alimentarius

Use & Actions

- To treat an existing illness
- To prevent future diseases
- To promote growth

Main pharmacological actions:
- **Antibiotics** to control bacterial diseases
- **Sedative, pain killers and anti-inflammatory medicines**
- **Wormers** (anthelmintics) to control internal parasites
- **Coccidiostats** to control protozoal diseases in poultry
- **Carbamates and pyrethroids** to control external parasites
- **Dyes** (Malachite green) as fungicide, parasiticide, and disinfectant in aquaculture
- **Substances having anabolic effect** (Stilbenes, antithyroid agents, steroids, resorcylic acid lactones, beta-agonists)
Regulation & Health Issues

- **MRLs**: Maximum Residue Limits from mg/kg (ppm) to < µg/kg (ppb). A withdrawal period must be respected to avoid residues in animal tissues.

- **Prohibited substances**: These substances are not allowed to be administered to food-producing animals. E.g. Listed in Commission Regulation (EU) No 37/2010 under prohibited substances for which MRLs cannot be established (e.g. Chloramphenicol, Nitrofurans)

- Antibiotic used for threatening animal diseases are also applied in human medicine

- MRLs must be respected to avoid *increasing bacterial resistance to antibiotics used in therapeutics*

- **Acute**: Allergenicity/Hypersensibility/β-Agonist

- **Long term**: Teratogens/Cancer
Antimicrobial Resistance

The overlap of critical lists for human and veterinary medicine can provide further information, allowing an appropriate balance to be struck between animal health needs and public health considerations

CRITICALLY IMPORTANT ANTIMICROBIALS

- Aminoglycosides
- Carbapenems and other panams
- Cephalosporins (3rd and 4th generation)*
- Cyclic esters
- Fluoro- and other quinolones*
- Glycopeptides*
- Glycylcyclines
- Lipopeptides
- Macrolides* and ketolides
- Monobactams
- Oxazolidinones
- Penicillins (natural, aminopenicillins, and antistaphylococcal)
- Polymyxins
- Rifamycins
- Drugs used solely to treat tuberculosis or other mycobacterial diseases

* Designated by the WHO as “Highest Priority Critically Important Antimicrobials.”

OIE LIST OF ANTIMICROBIALS OF VETERINARY IMPORTANCE

“The overlap of critical lists for human and veterinary medicine can provide further information, allowing an appropriate balance to be struck between animal health needs and public health considerations”
Integrated Approach for Analytical Development

Team of experts to define an integrated approach

Early Warning/Chemical Contaminants Experts/Corporate Quality
- Assess likelihood of occurrence
- Anticipate and mitigate incidents

Market and specific needs
- Supply constraints
- Operator skills
- Specific regulation
- Restricted importation

Corporate Requirements
- Analytical Volume
- Internal vs External Approach

Internal Alerts System
- Early Warning, Positive findings data capture system

External Alerts
- Consumers, Suppliers, Contaminants network

Agricultural Services (Corporate and Zones)
- Field information
- Fraud scenarios
- Training

Regulatory
- Local regulation (e.g. EU, US, China etc…)
- Codex

Alignment on official national control plan
- Alignment with authorities control plan in global monitoring program

Analytical Development
- Early Warning
- Local Needs
- Corporate Requirements
- Official Control
- Alert System
- Agricultural
Quality Testing along the Supply Chain & Manufacturing
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- Farm
- Raw material collection center
- Arrival at factory
- Factory raw material warehouse
- Factory line
- Finished product warehouse
- External supplier
- Rapid methods for effective release
- Confirmatory methods for full compliance
- RM specification & CoA
- Quality Testing along the Supply Chain & Manufacturing
Literature Available for Veterinary Drugs by LC-MS/MS

Over 77 methods described from 2009 on ...

- ➔ developed for a single food matrix
- ➔ developed for two food matrices
- ➔ developed for ...
  - ➔ more than two food matrices

https://www.scopus.com/
Keywords: Veterinary drugs, LC-MS/MS, multi-class, validation, pub year > 2009
What About Fitness-for-Purpose?

• M. Danesaki and N. Thomaidis (Analytica Chimica Acta, 2015, pp 103-121)
  - Validated level = 100 µg/kg for all the 155 compounds i.e. far above numerous MRL
  - Incomplete and/or not compliant for some Penicillins, Cephalosporins, Tetracycline, β-Agonists, Steroids ...

• S. Chung and C.-H. Lam (Analytical Methods, 2015, pp 6764-6776)
  - 78 compounds without inclusion of Penicillins, Sulfonamides, or Tetracyclines
  - Incomplete and/or not compliant for some Amphenicols, Cephalosporins, Quinolones, β-Agonists, Steroids ...

• X.-J. Deng et al. (Journal of Liquid Chromatography and related Technology, 2011, pp 2286-2303)
  - 105 compounds without inclusion of Penicillins, Cephalosporins, Avermectins etc...
  - Incomplete scope for Tetracyclines
What About Fitness-for-Purpose?

- C. Robert et al. (Food Additives and Contaminants Part A, 2013, pp 443-457)
  - Most complete scope (154 analytes in milk, muscle, egg and honey)
  - Incomplete and/or not compliant for some Penicillins, Cephalosporins, Tetracycline, β-Agonists, Steroids

- D. Chen et al. (Journal of Chromatography B, 2016, pp 82-88)
  - Validated level claimed between 1.5 – 8 µg/kg, but validation data not shown
  - Calibration curve in solvent for matrices as different as edible muscles, hen eggs, and cow’s milk

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A Compliance-driven Approach

Multi-class
\(n = 105\)

Aminocoumarins (1), Amphenicols (3), Diaminopyrimidines (2), Lincosamides (2), Macrolides (8), Quinolones (18), Rifamycins (2), Streptogramins (1), Sulfonamides (22), Avermectins (6), Benzimidazoles (14), Diphenylsulfides (1), Halogenated phenols (1), Imidazothiazoles (1), Organophosphates (1), Salicylanilides (4), Tetrahydropyrimidines (1), NSAID (5), Coccidiostats (12), Tranquilizers (3).
A Compliance-driven Approach

**Tetracyclines**  
(n = 10)  
Chlortetracycline + 4-epi, Demeclocycline + 4-epi, Doxycycline + 6-epi, Oxytetracycline + 4-epi, Tetracycline + 4-epi.

**Aminoglycosides**  
(n = 13)  
Apramycin, Dihydrostreptomycin, Gentamycin (C1, C1a, C2), Hygromycin B, Kanamycin (A), Neomycin (B), Paromomycin, Spectinomycin, Streptomycin, Tobramycin, Amikacin.

**Beta-lactams**  
(n = 23)  
Penicillins (12), Cephalosporins (11).

**Growth pro.**  
(n = 28)  
β-Agonists (8), Anabolic steroids (6), Stilbenes (3), Resorcylic lactones (3), Corticosteroids (7).

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## A Compliance-driven Approach

**Multi-class** *(n = 105)*

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- Amphenicols (3)
- Diaminopyrimidines (2)
- Lincosamides (2)
- Macrolides (8)
- Quinolones (18)
- Rifamycins (2)
- Streptogramins (1)
- Sulfonamides (22)
- Avermectins (6)
- Benzimidazoles (14)
- Diphenylsulfides (1)
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- Salicylanilides (4)
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- Anabolic steroids (6)
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- Apramycin
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Stream for 23 β-Lactams

- Low MRL requirement e.g. 4 µg/kg in milk for Amoxicillin (Commission Regulation (EU) No 37/2010)
- Massively used as broad spectrum antibiotic. No amoxicillin = no method for β-lactams
- Polar compound(s) with multiple pKₐ but sensitive to acidic/basic conditions

→ Multiclass, Multiresidue Methods fail to cover all β-lactams at their MRL

Need a separate method ... but complete and fit-for-compliance

LC-MS/MS chromatograms of 23 β-lactams in an infant formula spiked at 1x STC level
Stream for 10 Tetracyclines

- Chlortetracycline, Oxytetracycline, Tetracycline are regulated as «the sum of parent drug and its epimer» (Commission Regulation (EU) No 37/2010)

- Chromatographic challenges:
  - Separation between parent drug and corresponding epimer
  - Chelation of compounds in the LC-MS/MS system

→ Multiclass, Multiresidue Methods fail to cover all tetracyclines and epimers

Need a separate method ... but complete and fit-for-compliance

LC-MS/MS chromatograms of 10 Tetracyclines in chicken powder spiked at 1x STC (25 µg/kg)
Relevant Food Commodities

An approach including raw materials, semi-finished and finished products

Milk-based products

Meat/Seafood-based products

The «USALLY-SHOWN» matrices

- Raw milk
- Fresh or cooked meat, fish and seafood
# Relevant Food Commodities

**An approach including raw materials, semi-finished and finished products**

<table>
<thead>
<tr>
<th>Milk-based products</th>
<th>Meat/Seafood-based products</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Raw milk" /></td>
<td><img src="image2.png" alt="Fresh or cooked meat, fish and seafood" /></td>
</tr>
</tbody>
</table>

### The «USALLY-SHOWN» matrices
- Raw milk
- Fresh or cooked meat, fish and seafood

### The «FORGOTTEN» matrices
- Milk fractions
  (e.g. Skimmed milk powder, whey protein concentrate/hydrolysate, lactose etc...)
- Formulae with milk
  (e.g. Infant, follow-on, grow-up formulae; hydrolysed formulae; adult formulae etc...)
- Infant Cereals with milk
- Meat, fish and seafood powder
  (e.g. Shrimp, duck, meat, pork, lamb, beef, chicken, veal etc...)
- Infant Cereals with meat tissues
- Babyfood in jars and pots
  (based on vegetables, meat/fish, pasta, cereals, vegetable oil etc..)

☑️ Need for «Quick Easy Cheap Rugged and Safe» like methods
Beyond Raw Milk Analysis

“Whole milk powder and skimmed milk powder will remain the most traded agricultural commodities”

Additionnal consideration: Drugs are transferred from whole milk to milk fractions during processing

Hakk et al., J. Agric Food Chem, 2016, 64, 326-335
A total of fifteen confirmed positive milk samples were identified out of the 1’912 total samples.

In 2012, there were just over 1’000 non-compliant samples from over 425’000 total samples.
Overall, non-compliance is steady or decreasing

Across the EU monitoring of the levels of these residues in food-producing animals and animal-derived foods takes place annually. The substances can be grouped into six broad categories: hormones, beta-agonists, prohibited substances, antibacterials, other veterinary drugs, and other substances/environmental contaminants. The animals and foods monitored are bovines, pigs, sheep and goats, horses, poultry, rabbit, farmed game, wild game, aquaculture, milk, eggs and honey.
Analytical Strategy

- **Aim** is to check if samples are below or potentially above the Screening Target Concentration (STC)
- **Results** are either $<\text{STC}$ (given in µg/kg) or **Suspect**
- **Response** = relative comparison between Peak Area in Unspiked Sample ($A_{us}$) vs. Peak Area in the related Spiked Sample ($A_s$)

**Validation scheme according to EU CRL 2010 / 01 / 20**

**Samples**
- **Milk-based products**
  - Milk fractions (16), infant formulae & milk powders (15),
  - milk-based infant cereals (5)
- **Meat/Seafood products**
  - Meat/seafood powders (10),
  - Meat/seafood fresh and cooked (10), meat-based baby-foods

**Design**
- 67 samples
- Fortified at 0, 1, 2 STC
- Three analyst involved
- Over 15 days

**Quality Criteria**
- Cut-off level
- False suspect rate: $< 10\%$
- False negative rate: $< 5\%$
- Retention time: $< 0.2$ min
- Identification: 2 MRM

*Full validation by the developing lab + Multi-site implementation (France, Singapore, USA)*
Take-Home Message

► Uncontrolled occurrence of veterinary drugs in food is a health concern, particularly with regard to antimicrobial resistance.

► Multiresidue analysis is needed for an effective control.

► Mass spectrometry is needed for full compliance testing.

► A single LC-MS-based method capable to demonstrate full compliance of veterinary drugs in food does not exist so far.

► Matrix scope should represent current practices in terms of trade and business.

► Method performance should fit with throughput and positive rate for a as-low-cost-as-possible analysis.