THE USE OF A TARGETED AND A NON-TARGETED APPROACH TO DETECT GROWTH PROMOTING AGENTS

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Overview

• Introduction
• Experimental design
• Results validation, targeted approach
• Results validation, non-targeted approach
• Comparison targeted and non-targeted approach
• Conclusions
Nowadays analytical methods are:

- Limited in the number of compounds included
- Targeted to groups of compounds
- Difficult to add new compounds
- Caused by poor full-scan sensitivity and specificity of tripleQ

LC-ToF-MS equipment potentially can overcome these limitations
In the past LC-ToF-MS was considered purely a qualitative tool.

Recent advances have improved these limitations.

LC-ToF-MS is sensitive, as well as specific (exact mass) in full scan measurements.

LC-ToF-MS is outperformed by quadrupole instruments in sensitivity.

However, the full-scan capabilities, high resolution, accurate mass and improved dynamic range of LC-ToF-MS can be used to detect multiple compounds in a single extract.
Experimental design (1)

- To evaluate data obtained from LC-ToF-MS experiments two strategies can be followed

  - Targeted approach

  - Non-targeted (data evaluation) approach
Targeted approach
Non-targeted (data evaluation) approach
Other factors beside mass spectrometric detection

1. Sample clean-up should not be too specific
2. LC separation should be capable to handle large number of different compounds
3. Ionization of all compounds of interest
4. Software for data processing

Practical program will go more into details
What have we done?

Matrix

- Meat
- Hair

Analyte

- Steroid hormones
- Pro Hormones
- Gestagens
- ß-Agonist
- Steroid Esters

Detection

- LC-TOF
What have we done? (2)

- Individual analytical methods used (from tripleQ methods)
  - Clean-up method for steroid hormones, pro-hormones and gestagens in meat
  - Clean-up method for β-agonists in meat
  - Clean-up method for β-agonists in hair
  - Clean-up for steroid esters in hair
- One LC-ToF-MS Analyses
• Analysis 20 samples after spiking with the compounds of interest and 20 blank samples

• Complication no criteria are set for confirmation analysis using this type of instruments

• Validation criteria as for qualitative methods
Results validation, targeted approach

- Specific extracted ion chromatograms were constructed by extracting the mono-isotopic mass, plus/minus 10 ppm
- S/N of the signal at the expected retention time based on injection of the reference compounds exceeded six
“Detection limits” (ng/ml-g)
Number of “non-compliant” (%) results
• Sensitivity is in principle adequate

• No combination compound / matrix gives a perfect score

• Previous developed clean-up procedures are not fully transferable from triple-quad instruments, optimization is necessary

• But, these results provide proof of principle that LC-ToF-MS can be used in the veterinary residue field after some optimization
Results validation, non-targeted approach

• Discriminate between samples obtained from treated and untreated animals

• **Same data sets** of the targeted multi analyte approach were processed

• The whole process, from spiking, to data handling, is demonstrated using one compound, melengestrol-acetate
• Twenty meat samples fortified with 2 µg/kg with melengestrol-acetate and twenty blank samples of meat were analysed by using an extraction procedure followed by LC-ToF-MS

• First, the full scan data (200-500 amu) of each sample were stripped from (chemical) noise
Results validation, non-targeted approach

Original data

Stripped data
Results validation, non-targeted approach

• After peak picking all detected peaks were identified by using the Agilent Profiling software
Results validation, non-targeted approach

• To check the identity online databases can be used
Number of non-compliant (%) results
Number of non-compliant (%) results targeted and non-targeted
Results validation, non-targeted approach

- Lower non-compliant score for non-targeted
- Peak picking algorithm has to be further improved
- Measurements were performed at residues levels
- “Lack of” a human operator
- Demonstrated that it is possible to use this statistic approach
- New generations LC-ToF-MS equipment are more sensitive
Conclusion (1)

• Realistic, detection limits can be achieved using LC-ToF-MS

• In the presence of matrix the detection limits are higher

• The results obtained with a targeted approach, identify more compounds than a non-targeted approach

• The non-targeted (generic) approach, however, is still in its infancy, certainly at residue levels
• The big advantages of non-targeted software that it is possible to detect "unknowns"

• From the – statistical significant – differences, markers can be selected which can be used for monitoring purposes

• It can be concluded that LC-ToF-MS is close(r) to become a generic detector
Thank you for your attention

Questions?