Overview of “priority analytes”

Minimum list of anabolic steroids for ANP (parent compounds)

- A1 Stilbenes (3)
  - Diethylstilbestrol
  - Dienestrol
  - Hexestrol
- A2 Thyreostats (4)
  - Tiouracil
  - Methyltiouracil
  - Porpylthiouracil
  - Tapazole
- A3 Steroids (13)
  - 17β-Testosterone
  - 17β-Nortestosterone
  - 17β-Boldenone
  - Methyltestosterone
  - Stanozolol
  - 17β-Trenbolone
  - 17β-Oestradiol
  - Ethynloestradiol
  - Dexamethasone
  - Medroxyprogesterone acetate
  - Mestenolone acetate
- A4 RALs (1)
  - Zeranol
- A6 Chlorpromazine (1)
  - Sedatives

Additional compounds: Research studies performed within CRL-NRL network (parent compounds)

- A3 Steroids (5)
  - Methylandrostenedione
  - RIVM CRL document 310309-010 (August 2005)
  - Norethandrolone
  - RIVM CRL document 310309-003 (June 2004)
  - Normethandrolone
  - Clostebol
  - Norclostebol
  - RIVM CRL document 310309-008 (August 2005)

Evaluation ANPs 2006
Priority analytes in ANP

Comparing ANPs and participation in PTs

Results reported for 2005

Results reported for Boldenone (17)

Results reported for Nandrolone (17beta-Nortestosterone)
Results reported for Methyltestosterone (8 MS – 2 cases!)

Results reported for Corticosteroids

Results reported for RALs

Conclusions

• Only a limited number of non-compliant results were reported.
• Testing for methyltestosterone should be intensified
• On the basis of the results reported it remains difficult to draw conclusions when natural hormones are involved.
• Boldenone and nandrolone findings sometimes can be explained by other factors

Validation status based on CD 2002/657

• MRPL: Minimum Required Performance Limit: Minimum content of an analyte in a sample, which at least has to be detected and confirmed
• Quantitative: Detection Capability (CC β) – lowest value at which truly contaminated samples can be detected (statistical certainty 1- β)
• Quantitative: Decision Limit (CC α) – above which it can be concluded that a sample is non-compliant
Note: Guide for Analytical Validation of Screening Methods Gaudin & Sanders (CRL for Residues, Fougeres)
**Stilbenes**

<table>
<thead>
<tr>
<th></th>
<th>urine</th>
<th>muscle</th>
<th>liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>1 μg/l</td>
<td>MRPL 0.5 μg/kg</td>
<td>MRPL 2 μg/kg</td>
</tr>
<tr>
<td>SOP</td>
<td>479: GC-MS</td>
<td>method with CC &lt; 0.5 μg/l</td>
<td>SOP 113: GC-MS method currently under revision</td>
</tr>
</tbody>
</table>

**Steroids: endogenous (data for 17β-oestradiol)**

<table>
<thead>
<tr>
<th></th>
<th>serum</th>
<th>muscle</th>
<th>liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>0.04 μg/l</td>
<td>MRPL 0.1 - 0.5 μg/kg</td>
<td>MRPL 2 μg/kg</td>
</tr>
<tr>
<td>SOP</td>
<td>462: GC-MS</td>
<td>method with CC &lt; 0.1 μg/l</td>
<td>SOP 113: GC-MS method currently under revision</td>
</tr>
</tbody>
</table>

**Steroids: exogenous**

<table>
<thead>
<tr>
<th></th>
<th>urine</th>
<th>muscle</th>
<th>liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>1 - 2 μg/l</td>
<td>MRPL 0.5 μg/kg</td>
<td>MRPL 2 μg/kg</td>
</tr>
<tr>
<td>SOP</td>
<td>479: GC-MS</td>
<td>method with CC &lt; 0.5 μg/l</td>
<td>SOP 113: GC-MS method currently under revision</td>
</tr>
</tbody>
</table>

**Steroids: corticosteroids**

<table>
<thead>
<tr>
<th></th>
<th>urine</th>
<th>muscle</th>
<th>liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>2 μg/l</td>
<td>MRPL 0.5 μg/kg</td>
<td>MRPL 2 μg/kg</td>
</tr>
<tr>
<td>SOP</td>
<td>441: LC-MSMS method with CC &lt; 0.5 μg/l</td>
<td>SOP 113: GC-MS method currently under revision</td>
<td></td>
</tr>
</tbody>
</table>

**Steroids: 17β-Boldenone-(conjugated)**

<table>
<thead>
<tr>
<th></th>
<th>urine</th>
<th>muscle</th>
<th>liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>1 μg/l</td>
<td>MRPL 0.5 μg/kg</td>
<td>MRPL 2 μg/kg</td>
</tr>
<tr>
<td>SOP</td>
<td>479: GC-MS</td>
<td>method with CC &lt; 0.5 μg/l</td>
<td>SOP 113: GC-MS method currently under revision</td>
</tr>
</tbody>
</table>

**Thyreostatic compounds**

<table>
<thead>
<tr>
<th></th>
<th>urine</th>
<th>Thyroid gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>100 μg/l</td>
<td>MRPL 100 μg/kg</td>
</tr>
<tr>
<td>SOP</td>
<td>440: LC-MSMS method with CC &lt; 50 μg/l</td>
<td>Currently no validated SOP available</td>
</tr>
</tbody>
</table>

**SOPs**

- SOP 479: GC-MS method with CC < 0.5 μg/l
- SOP 490: GC-MS method with CC < 0.5 μg/kg
- SOP 440: LC-MSMS method with CC < 50 μg/l
- SOP 441: LC-MSMS method with CC < 1 μg/kg
- SOP 113: GC-MS method currently under revision
### Steroids: Stanozolol and 16βOH-Stanozolol

<table>
<thead>
<tr>
<th></th>
<th>Urine</th>
<th>Muscle</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>2 μg/l</td>
<td>0.5 μg/kg</td>
<td>2 μg/kg</td>
</tr>
</tbody>
</table>

SOP 479: GC-MS method with CC < 0.5 μg/l


### Steroids: 17α- and 17β-Trenbolone

<table>
<thead>
<tr>
<th></th>
<th>Urine</th>
<th>Muscle</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>2 μg/l</td>
<td>0.5 μg/kg</td>
<td>2 μg/kg</td>
</tr>
</tbody>
</table>

SOP 443: LC-MSMS method with CC < 0.5 μg/l

### Steroids: Gestagens

<table>
<thead>
<tr>
<th></th>
<th>Kidney Fat</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>1.5 μg/l</td>
<td>0.5 μg/kg</td>
</tr>
</tbody>
</table>

SOP 399: Analysis of samples of kidney fat with SPE LC-MS

CRL 2001 workshop

### Steroids: Zeranol and Taleranol

<table>
<thead>
<tr>
<th></th>
<th>Urine</th>
<th>Muscle</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>2 μg/l</td>
<td>0.5 μg/kg</td>
<td>2 μg/kg</td>
</tr>
</tbody>
</table>

SOP 458: GC-MS method with CC < 0.5 μg/l

### Sedatives: Chlorpromazine

<table>
<thead>
<tr>
<th></th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRPL</td>
<td>10 μg/l</td>
</tr>
</tbody>
</table>

SOP 399: Analysis of samples of kidney for sedatives with SPE LC-MS

CRL 2006 workshop

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- Can samples containing analytes at levels as low as the current MRPLs be confirmed based on CD 2002/657?
  - i.e., can these methods meet the 4 identification point criterion?
  - Yes, however…
Reliability of identification

Are we still sure?

1986: Identification criteria
2002: Identification points - 4

Extending the use of identification criteria

Willow warbler  Greenish warbler?

3 IPs  +1

Confirmation based on CD 2002/657

Criteria for Low resolution MS
- 4 ions have to be detected
- 3 ratios have to fit
- When necessary:
  - Additional detection techniques
  - GC: different derivatives
  - Different columns

Criteria for Low resolution MSMS
- 2 daughter ions have to be detected
- 1 ratio has to fit
- When necessary:
  - Additional detection techniques
  - GC: different derivatives
  - Different columns

Confirmatory analyses using lrMS: 4–ion criteria of residues in muscle tissue

Methylboldenon  methyltestosterone  Ethynyl Estradiol  β-Boldenon  β-Nortestosteron

Confirmatory analyses using lrMS

Concentratie (µg/kg)  % bevestigde ion-ratio's

Concentratie (µg/kg)  % bevestigde ion-ratio's
Confirmatory analyses using LC-MSMS (single shot analysis)

- All recently validated MSMS methods are LC-MSMS methods which allow > 95% of the samples to be confirmed during the first analyses. No additional measurements are necessary at the level of the MRL.

Proposed priorities for further method development

- Direct measurement of steroid–conjugates
- Analyses of samples of hair for esters of steroids (combined effort with RIKILT and LABERCA)
- Further development of LC-TOF-MS applications
  - From Trenbolone in urine to multi-analyte in urine
- Rewriting the procedure for gestagens in kidney fat using either GC-MS and LC-MSMS
- Modifying procedure 113 (single analyte confirmation of steroids in tissue samples). From GC-MS to GC-MSMS

2007 Proficiency test: Medroxyprogesterone in pig kidney-fat

- Currently 17 Member States have included testing for Medroxyprogesterone-acetate in their Residue Control Plan.
- Analytical methods were the topic of a CRL workshop in 2001.
- Analytical methods are available:
  - Based on SFE with In line trapping of analytes of a SPE cartridge and LC-MS (Stolker et al. The Analyst, 2002, 127,748-754)
  - Based on SPE and GC-MS. ARO SOP 399 (currently out of circulation but will be reintroduced in 2007)
  - Based on SPE and LC-MS. ARO SOP 435

2007 Proficiency test: Thyreostatic compounds in pig urine

- Currently 21 Member States have included testing for thyreostatics Residue Control Plan (Tapazole 16)
- Analytical methods for thyreostatics were the topic of a CRL workshop in 1997.
- Analytical methods are available:
  - SOP 440: Analysis of thyreostatics in bovine urine by LC-MS. Validated GC-MS method with CCl < 100 μg/kg
- Note:
  - Evidence that urinary excretion of thiouracil in adult bovine submitted to a cruciferous diet can give erroneous indications of the possible illegal use of thyreostatics in meat production
  - Pinel et al. Food additives and Contaminates, 2006 23(10) 974-980

Thank you!