HPTLC-bioluminescence screening for residues of antibiotics in food of animal origin

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Prof. Dr. Wolfgang Schwack
Research background

Reports for 2011 on the veterinary antibiotics monitoring program in EU

<table>
<thead>
<tr>
<th>Number of analyses</th>
<th>Non-compliant rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild game</td>
<td>0.00%</td>
</tr>
<tr>
<td>Farmed game</td>
<td>0.00%</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.12%</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.12%</td>
</tr>
<tr>
<td>Milk</td>
<td>0.09%</td>
</tr>
<tr>
<td>Aquaculture</td>
<td>0.35%</td>
</tr>
<tr>
<td>Poultry</td>
<td>0.07%</td>
</tr>
<tr>
<td>Horse</td>
<td>0.00%</td>
</tr>
<tr>
<td>Sheep/goat</td>
<td>0.40%</td>
</tr>
<tr>
<td>Pigs</td>
<td>0.15%</td>
</tr>
<tr>
<td>Bioven</td>
<td>0.30%</td>
</tr>
</tbody>
</table>

Therefore, the dramatic contrast between enormous sampling numbers and constantly low non-compliant rates (<0.5%) implies that screening-oriented methods are highly required.
Our strategy: screening-oriented

HPTLC + Biolumincent bioautography

450nm
Challenges faced in screening

1. Demanding sensitivity
2. Poor separation
3. Bio-active matrix

Methods should be highly sensitive: < 0.5 mg/kg

Tolerance limits (mg/kg)

United States

EU & China

Max. residue limits (mg/kg)

0.6 0.3 0.1 0.1
Challenges faced in screening

1. Demanding sensitivity
2. Poor separation
3. Bio-active matrix
Challenges faced in screening

1. Demanding sensitivity
2. Poor separation
3. Bio-active matrix

After QuEChERS extraction:

Milk  Kidney
Detection optimization

OD 600 nm

Pre-incubation time (h)

0,06
0,11
0,19
0,33
0,47
1,00
1,33
1,38
1,42

Active cells were used

Logarithmic phase

Harvest point
Detection optimization

Screening on bio-compatibility of HPTLC Layers
Detection optimization

Time-dependent cytotoxicity of antibiotics below the acute lethal level.
Detection optimization

Layer moisture is a vital factor for living bacteria!

Incubation chamber  Detection chamber
Window separation

- Bioactive matrix
- Nonpolar analyte
- Polar/tailing analyte

Window 2

Window 1

Sample 1  Standards  Blank  Sample 2
Window separation

Kidney matrix

Tetracyclines  Quinolones

Samples  Blank  Samples

Amphenicol
**Real sample application**

**Milk**

- Window 1
- Window 2

**Kidney**

- Window 1
- Window 2
## Detection spectrum

<table>
<thead>
<tr>
<th>Group</th>
<th>Milk</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biolum</td>
<td>Premi®*</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>😊</td>
<td>😞</td>
</tr>
<tr>
<td>Quinolones</td>
<td>😊</td>
<td>😞</td>
</tr>
<tr>
<td>Amphenicols</td>
<td>😊</td>
<td>😞</td>
</tr>
<tr>
<td>Macrolides</td>
<td>😊</td>
<td>😊</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>😊</td>
<td>😊</td>
</tr>
<tr>
<td>Penicillines</td>
<td>😞</td>
<td>😊</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>😞</td>
<td>😊</td>
</tr>
</tbody>
</table>

* H Cantwell, Food Additives and Contaminants 23 (2006) 120.
Identification by TLC-MS

TLC-MS interface

Electrospray Mass spectrometry
### Conclusion

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Biolum</th>
<th>Premi®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>Amino F$_{254}$S plates</td>
<td>Agar tubes</td>
</tr>
<tr>
<td>Operation</td>
<td>Automatic</td>
<td>Manual</td>
</tr>
<tr>
<td>Quantitation</td>
<td>Semi-quantitative</td>
<td>Semi-quantitative</td>
</tr>
<tr>
<td>chromatography</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>MS compatibility</td>
<td>Yes</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Figure:**

- Bioluminometer with chromatogram overlay.
- Premi® equipment with sample preparation instructions.

**UNIVERSITÄT HOHENHEIM**
**INSTITUT FÜR LEBENSMITTELCHEMIE**
Thank you for your attention!

International Symposium for Thin-Layer Chromatography