Vitamins and Food Contaminants
by UPLC/MS/MS

AOAC Europe / ASFILAB meeting

Renaud LE BOUQUIN - Mickael HYBOIS
LAREAL presentation

- LAREAL is a food research and analysis laboratory in food-processing:
  - Feed
  - Petfood
  - Human food
  - Health foods and nutraceuticals

- 30 compounds analysed by LC/MS/MS:
  - Food contaminants
  - Vitamins
  - Drugs

- LAREAL started with LC/MS/MS in 2004
LAREAL develops HPLC/MS/MS with Customer agreement:

- To reduce run time:
  - Simplification of sample preparation
  - Simplification of HPLC analysis (without derivatization ...)

- To improve detection limits:
  - Sensibility of mass spectrometer

- To secure the result:
  - Specificity of mass spectrometer
  - Different confirmation levels (multiple MS/MS transitions)

- To develop multi-analytes methods
Quantification by MS/MS

- Advantages of MS/MS detectors:
  - Sensibility
  - Selectivity
  - Can be coupled to high pressure LC (improve time, sensibility and selectivity)

- Disadvantage of MS/MS -> ionization pertubation
  - Competition between differents analysed molecules
  - Interference between molecule and matrix

- To have a good accuracy, we use Internal Standards:
  - The best choice is a stable isotope of the analyte (if available)
Melamine

2007 : First Melamine crisis in petfood

Development of a HPLC/UV method to detect contamination of Melamine in raw materials / petfood

Disadvantages :
- Long HPLC run time : 30 min
- Low sensibility (LOD : 500 µg/kg)
- Interferences in complex samples

Advantage :
- Easy to run
Development of a UPLC/MS/MS method to detect traces of Melamine in foods and raw materials

- Advantages:
  - Short run time: 10 min
  - No interferences
  - High sensitivity (LOD: 10 µg/kg)
  - Specificity of mass spectrometry (2 transitions MS/MS)
  - Quantification by internal standard (*stable isotope of Melamine*)
Melamine

HPLC/UV chromatogram

UPLC/MS/MS chromatogram

Soya contaminated at 1.8 mg/kg
<table>
<thead>
<tr>
<th>Material</th>
<th>HPLC/UV (mg/kg)</th>
<th>UPLC/MS/MS (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petfood</td>
<td>33.8</td>
<td>33.1</td>
</tr>
<tr>
<td>Blood meal</td>
<td>9.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>11.1</td>
<td>11.1</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>3.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Premix</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Premix</td>
<td>9.5</td>
<td>9.3</td>
</tr>
<tr>
<td>Premix</td>
<td>21</td>
<td>20</td>
</tr>
</tbody>
</table>
B vitamins

Microbiology: reference methods

- Time consuming
- Quantification of a microbiologic activity (*indirect quantification with possible interferences*)

Development of HPLC/MS/MS methods

- Vitamin B3, vitamin B5 and vitamin B9
- Specific method
- Good accuracy with an internal standard quantification (stable isotope vitamins)
B vitamins

Vitamin B3

- 2 main forms quantified: Niacin & Nicotinic Acid
- Sample preparation: acid hydrolysis + SPE
- Quantification limit: 500 µg/kg
B vitamins

Vitamin B5

- Quantification of Pantothenic Acid
- Sample extraction: enzymatic hydrolysis + SPE
- Quantification limit: 100 µg/kg
B vitamins

Vitamin B9

• 2 main forms quantified: Folic acid & 5-methyltetrahydrofolic acid
• Sample preparation: enzymatic extraction + SPE
• Quantification limit: 20 µg/kg
Vitamin D₃ (Cholecalciferol)

Reference method in Foodstuffs: NF EN 12821 (HPLC/UV)

- Long and complex sample preparation

Development of a HPLC/MS/MS method

- Simplification of sample preparation
- Quantification by internal standard (stable isotope of vitamin D3)
- Good accuracy and best reproducibility
Vitamin D₃ (Cholecalciferol)

HPLC/UV chromatogram

UPLC/MS/MS chromatogram

Petfood sample at 100 IU/100g (2.5 µg/100 g)
Comparison HPLC / UPLC

Comparison Trichothecenes (Mycotoxins) analysis by HPLC/MS/MS and UPLC/MS/MS

- Analysis time: UPLC is 4 times faster than HPLC

HPLC
run time: 32 min

1. Nivalenol
2. Deoxynivalenol
3. Fusarenone X
4. 15-acetyldeoxynivalenol
5. 3-acetyldeoxynivalenol
6. Diacetoxyisocaprenol
7. HT2 toxin
8. T2 toxin

UPLC
run time: 8 min
Comparison HPLC / UPLC

Comparison Trichothecenes (Mycotoxins) analysis by HPLC/MS/MS and UPLC/MS/MS

- Chromatographic separation: no change

- Quantification: no significant difference
  
  Intra-lab reproducibility of Deoxynivalenol, in Quality Control sample:
  
  HPLC: RSD = 8.8%
  UPLC: RSD = 9.0%

- Productivity: better with UPLC
  
  HPLC: 1.8 sample / hour
  UPLC: 7.5 samples / hour

- Sensibility: better with UPLC
## LC/MS/MS LAREAL applications

<table>
<thead>
<tr>
<th>Food contaminants</th>
<th>Melamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyanuric acid</td>
</tr>
<tr>
<td></td>
<td>Acrylamide</td>
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<tr>
<td></td>
<td>Bisphenol A</td>
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<td>Trichothecces profile</td>
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<td>Zearalenone</td>
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<td></td>
<td>Ochratoxin A</td>
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<td>Wortmannin</td>
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<tr>
<td>Vitamins</td>
<td>Vitamin B3</td>
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<td></td>
<td>Vitamin B5</td>
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<td></td>
<td>Vitamin B9</td>
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<tr>
<td></td>
<td>Vitamin D3</td>
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<tr>
<td>Others</td>
<td>Monensin</td>
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<td></td>
<td>Narasin</td>
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<tr>
<td></td>
<td>Salinomycin</td>
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<td></td>
<td>Maduramicin</td>
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<td></td>
<td>Lasalocid</td>
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<td></td>
<td>Sulfadiazine</td>
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<tr>
<td></td>
<td>Sulfadimethoxine</td>
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<tr>
<td></td>
<td>Ginkgolides (Ginkgo Biloba)</td>
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</tbody>
</table>
Thank you for your attention

Renaud LE BOUQUIN,  Technical Manager, rlebouquin@lareal.evls.net
Mickael HYBOIS,  R&D Engineer, mhybois@lareal.evls.net

www.lareal.com