

# Determination of Tropane alkaloids in feed Analytical challenges

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RIKILT – Institute of Food Safety



126th AOAC Annual Meeting & Exposition  
September 29 - October 3, 2012  
Las Vegas, Nevada

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## RIKILT – Institute of Food Safety

Wageningen UR Netherlands, about 200 employees

Chemical analyses ↔ Effect analysis (tox)

Environment & process contaminants  
Natural toxins (*phyco-, mycotoxins, plant toxins*)  
Veterinary drugs and residues  
Nutrients / Quality / Authenticity

Radioactivity  
Pesticides  
GMO's  
Allergens



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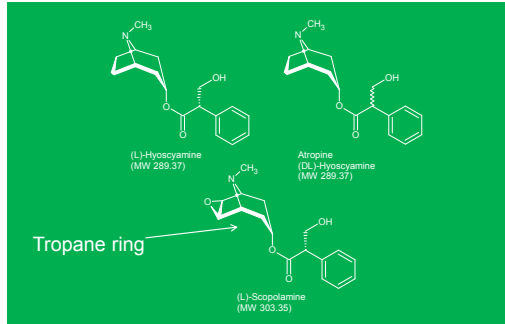
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## Tropane alkaloids

Over 200 Tropane alkaloids known



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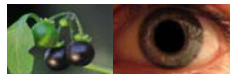
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## Effects of Tropane alkaloids

- Humans:
  - Medicine: arthritis, nausea, motion sickness, intestinal cramping and ophthalmic purposes (dilatation)
  - Drug: hallucinogenic effects
  - Poison: 10 berries of deadly night-shade (*Atropa belladonna*)



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## Effects of Tropane alkaloids

- Animals:
  - Pigs are most sensitive species, poultry least sensitive
  - Hyposalivation, tachycardia, hyperventilation, pupil dilatation, restlessness, nervousness, muscle tremor, hypothermia, convulsions, delirium and death from asphyxia
  - Off-taste milk
  - Carry over: little evidence



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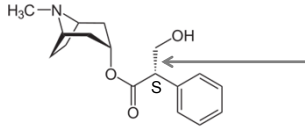
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## Tropane alkaloids

- Occurrence of enantiomers:
  - (DL)-hyoscyamine = atropine (produced by chemical synthesis)
  - L-hyoscyamine (S-enantiomer) is the active form
  - L-hyoscyamine occurs naturally while D-hyoscyamine (R-enantiomer) can be formed during isolation



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## Levels in plant materials (µg/g) DW Preliminary literature compilation

	atropine/hyoscyamine			scopolamine		total		ratio hyoscyamine/scopolamine				
	leaves	stem	seeds	leaves	stem	leaves	stem	seeds	leaves	stem	seeds	
<i>datura stramonium</i>	2038	3371	2534	412	878	314	1450	2249	1908	2.5	1.6	4.1
<i>datura stramonium tatula</i>	1969	5315	676	818	2320	684	2786	3615	1359	2.4	2.3	3.0
<i>datura innoxia</i>	185	400	1529	1129	1950	614	1314	2350	2143	0.2	0.2	2.5
<i>datura metel</i>	873	350	1179	1188	1715	1713	2061	2065	2891	0.7	0.2	0.7
<i>datura ferrox</i>	75		14	1312	900	1065	1387	900	1078	0.1	0.0	0.0
<b>all <i>datura stramonium</i></b>	<b>1135</b>	<b>2140</b>	<b>1166</b>	<b>593</b>	<b>1166</b>	<b>613</b>	<b>1728</b>	<b>3326</b>	<b>1779</b>	<b>1.9</b>	<b>1.9</b>	<b>1.9</b>
<b>all <i>datura</i> samples</b>	<b>975</b>	<b>1969</b>	<b>1199</b>	<b>1034</b>	<b>1377</b>	<b>800</b>	<b>2009</b>	<b>3346</b>	<b>1999</b>	<b>0.9</b>	<b>1.4</b>	<b>1.5</b>

- Substantial levels in all parts of the plant
  - In *Datura stramonium* (thornapple/Jimson weed) L-hyoscyamine is more prominent than scopolamine
  - In other species scopolamine is more important
  - Average of all reports: around 2000 µg/g



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## EU Legislation on Tropane alkaloids

- No EU legislation for Tropane alkaloids
- EU Regulation on *Datura* seeds in feed (Dir. 2002/32/EC):
  - Limit: 1 g plant material / kg feed 12% moisture
- Netherlands: List with banned herbal preparations from food (2001), will be updated in 2012
- Belgium: Royal Decree concerning the manufacture of and trade in foods from plants or preparations made from or containing plants, 1997
- EFSA opinion Tropane alkaloids (from *Datura* sp.) in feed, 2008
- EFSA opinion on Tropane alkaloids in food expected Q2 2013



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## Tropane alkaloids – EU RASFF notifications

- 17 Notifications since 2006
  - *Datura stramonium* seeds (10 notifications)
    - in cereals: millet (6) mostly from Austria
    - In (canned) vegetables (3)
    - In sunflower seeds (1)
 Reported levels: 1.86 – 2.76 g/kg (0.19-0.27%) for feed applications
  - Atropine and scopolamine (4 notifications)
    - In buckwheat flour (4) from Eastern Europe
 Total concentration: 26 to 157 µg/kg
  - Henbane seeds (*Hyoscyamus niger*) (3 notifications)
    - In blue poppy seeds (3) from Czech Republic
 Levels between 1.3 and 4.2 g/kg (0.13-0.42%)



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## Tropane alkaloids method *performance criteria*

- Method(s) to be developed should be fit for purpose
  - Sufficiently sensitive
  - Robust, intra/inter-laboratory validated
  - Flexible
  - As simple as possible
  - Adaptations can be made for more (or less) demanding matrices
- For Tropane alkaloids
  - Quick screening method required since incidence of occurrence is low
  - Followed by confirmation
  - Incorporate in existing methods e.g. pyrrolizidine alkaloids or ergot alkaloids
  - Requires reference standards/materials for quantification
  - Requires C<sub>13</sub> or deuterium labelled standards
  - Clean-up and sensitivity depends on matrix (food > feed > plant)
  - Detect both enantiomers (D- and L-hyoscyamine)



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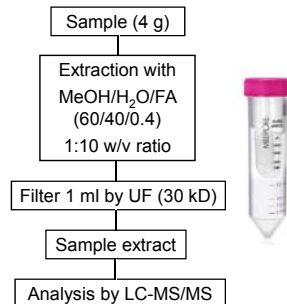
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## Methodology TA analysis animal feeds

- LC-MS/MS analysis
  - Xbridge C<sub>18</sub> 150 x 3 mm
  - Linear gradient H<sub>2</sub>O/CH<sub>3</sub>CN/NH<sub>4</sub>OH (0.05%)
  - 18 min run
  - Pos ESI
- Quantification by standard addition to each sample (100 µg/kg)
- LOD around 2 µg/kg



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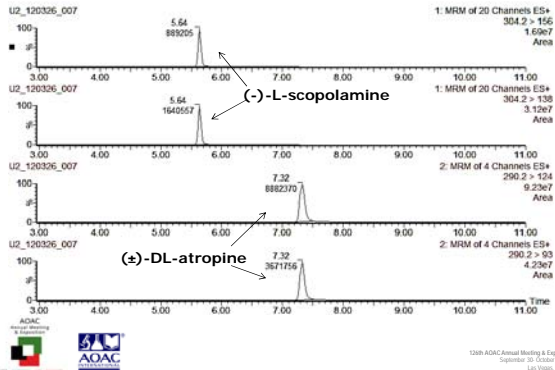
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## Separation of Tropane alkaloids

- Waters Xbridge C18 column, MRM method




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## Separation of enantiomers

- Measure exposure of humans in case of intoxication atropine
- Metabolism studies
- Racemisation during extraction/isolation from plant/seeds
- Requires the use of chiral stationary phase columns
  - Chiralpack IA (polysaccharide)
  - Cyclobond (derivatised  $\beta$ -cyclodextrin)
  - Chiral-AGP ( $\alpha$ -acid glycoprotein)
  - Chirobiotic T2 (teicoplanin)
- Columns can be run with aqueous and/or organic mobile phase, compatible with LC-MS/MS




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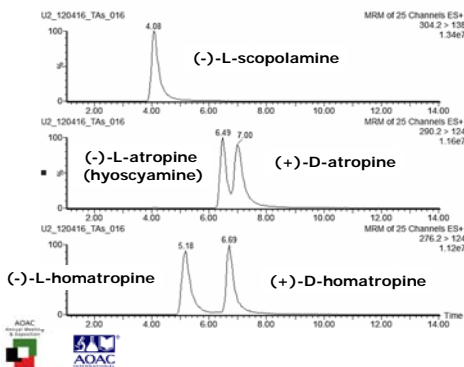
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## Separation of Tropane alkaloid enantiomers

- ASTEC Chiral-AGP column




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## Results Tropane alkaloids in animal feed

- Screening animal feed for Tropane alkaloids in 2006 – 2011
- LOD: 1-4.5 µg/kg (no correction for dry matter)
- 611 samples of feed: forage, soya, seeds, herbal products, grains and compound feeds



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## Results Tropane alkaloids in animal feed

Category	Commodity	Samples	LC	Total (µg/kg)	Atropine (µg/kg)	Scopolamine (µg/kg)
1	Cereal grains and products derived thereof	121	96%	14.9	8.5	6.4
2	Oil seeds, oil fruits, and products derived thereof	71	77%	13.1	5.9	7.2
3	Legume seeds and products derived thereof	13	100%	0.0	0.0	0.0
4	Tubers, roots, and products derived thereof	1	100%	0.0	0.0	0.0
5	Other seeds and fruits, and products derived thereof	5	80%	24.1	18.9	5.0
6	Forages and roughage and products derived thereof	302	100%	0.0	0.0	0.0
7	Other plants, algae and products derived thereof	32	88%	19.6	13.3	6.3
13	Compound feed	66	42%	8.0	5.4	2.6
1-13	All categories	611	90%	6.6	3.8	2.8

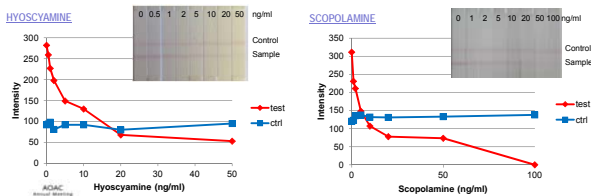
- 10% of samples contain TAs
  - Average content is low; relative few samples exceed 100 µg/kg
  - TAs appear to concentrate in specific commodities, such as cereal grains, (oil) seeds and other plants
  - Compound feeds often contain traces of Tropane alkaloids
  - Ratio atropine/hyoscyamine to scopolamine is more variable but on average 1.5:1



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## Development of a fast screening assay

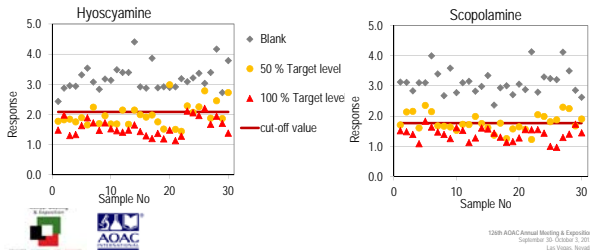
- EU FP7 Confidence project (2008-2012) <http://www.confidence.eu/>
  - Tropane sensor development
  - Dip-stick approach / competitive assay
  - (-)-L-hyoscyamine and (-)-L-scopolamine
  - Target level: 800 µg/kg
  - Both compounds show similar sensitivity



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## Validation of Tropane Sensor

- 21 different blank compound feeds
  - 3 days, 3 levels (0, 400, 800 µg/kg)
  - Ratio (intensity blank vs intensity sample)
  - Good separation between blank and target level
  - Cut-off levels established (95% confidence)




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## Conclusions - Analytical challenges

- Appropriate methods are available using LC-MS/MS
  - However, not interlaboratory validated
- No TDI or therefore, unknown LOD
- Challenges:
  - TDI will be determined by EFSA Q2 2013
  - Quick screening method
  - Development of reference material / internal standards
  - Validation of the method(-s)
  - Proficiency test




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## Mycotoxins - Plant toxins

- Several presentations on plant toxins in food and feed
- Session on method development and validation: Inexpensive detection for control of exposure (EU CONFIDENCE)




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Thank you for your attention!



More information?

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