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Presence of plant toxins in food and food supplements

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Introduction

Plant toxins are secondary plant metabolites that exhibit acute or chronic toxicity. Plant toxins can be inherently present in our food as constituents of edible crops, aromas, food supplements and (traditional) herbal medicines, or end up in our food through contamination or adulteration.

Objective

Investigate the occurrence of selected plant toxins in herbal products (food and food supplements) available on the Dutch market using a multi-plant toxin method based on LC-MS/MS.

Experimental

Samples: 100 herb-based products

Analytes: 64 plant toxins including:

I) Plant toxins/plant species regulated in the Netherlands [1]
   a) toxic pyrrolizidine alkaloids, yohimbe alkaloids, aristolochic acids
   b) plant toxins known to occur in regulated plants species

II) Selected plant toxins ('natural substances of possible concern') from the EFSA compendium of botanicals [2]

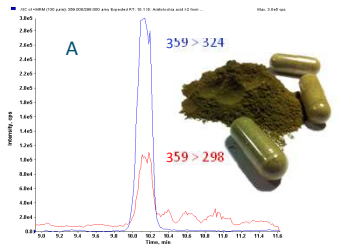
Sample preparation ('QuEChERS'):

- 2.5 g sample + 7.5 mL water + 10 mL acetonitrile/1% acetic acid
- Extraction 30 min end-over-end
- Salt-induced phase partitioning (4 g $MgSO_4$ + 1 g NaAc)
- Dilution of acetonitrile extract with water (1:1)
- Final extract 0.125 g/ml

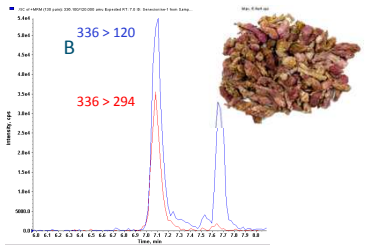
LC-MS/MS analysis:

- 5 µL injection
- HPLC: column: 100 mm x 3 mm ID, 3 µm Atlantis T3
- Gradient of MeOH/H_2O (5mM ammonium formate, 0.1% formic acid)
- MS/MS detection: API5500 Qtrap (2 transitions for each toxin)
- Quantification: single point standard addition

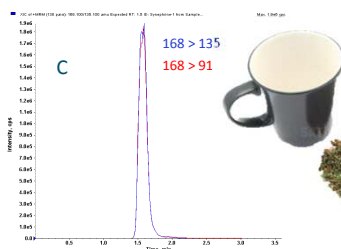
Example extracted ion chromatograms



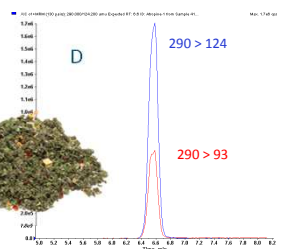
A) Aristolochic acid I in Chuan Xiong Cha Tia Wan (0.68 mg/kg),



B) Senecionine in Kuan Dong Hua (0.30 mg/kg)



C) Synephrine in hemp tea (2.1 mg/kg)



D) Atropine in hemp tea (0.24 mg/kg)

Plant toxins confirmed and quantified

Product categorie/sample				Toxins found (levels in mg/kg)
Herbal teas		1	herbal infusion ('anti-stress')	Synephrine 14
		2	herbal infusion ('breakfast mixture)	Erucifoline 0.031, Senecionine-N-oxide 0.048,
		3	herbal infusion (hemp)	Atropine 0.24, Synephrine 2.1
		4	herbal infusion (honeybush)	Atropine 0.009
		5	herbal infusion ('mint & cinnamon')	Erucifoline 0.086, Senecionine-N-oxide 0.13,
		6	herbal infusion	Senecionine 0.013, Senecionine-N-oxide 0.056,
		7	herbal infusion ('slim-maté)	Erucifoline 0.033, Retrorsine-N-ox 0.066,
		8	herbal infusion ('spring')	Synephrine 0.42
		9	herbal infusion ('sterrenmix')	Atropine 0.022, Podophyllotoxin 0.34,
		10	herbal infusion ('sterrenmix')	Podophyllotoxin 1.3
		11	herbal infusion ('sterrenmix')	Erucifoline 0.032, Seneciphylline-N-oxide 0.029
Food supplement (single herbs)		12	ashwaganda (tablets)	Atropine 0.013, Heliotrine 0.041
		13	common hop (capsules)	Kavain 0.013
		14	feverfew (capsules)	Synephrine 2.9
		15	opium oil (essential oil)	Ricinine 0.008
		16	St. John's wort (dried plant)	Atropine 0.02, Synephrine 0.17
		17	wild yam (capsules)	Strychnine 0.073
Food supplements (herbal mixtures)	'intestinal health'	18	capsules-	Heliotrine 0.011, Ricinine 0.044
		19	capsules-(re-sampled 1.5 years later)	Heliotrine 0.017, Ricinine 0.11
		20	capsules	Atropine 0.12, Heliotrine 0.014, Monocrotaline 0.036, Ricinine 0.078, Scopalamine 0.25
		21	dried plant material	Podophyllotoxin 0.42
		22	powder-	Heliotrine 0.007, Monocrotaline 0.007, Ricinine 0.17
		23	powder-(re-sampled 1.5 years later)	Monocrotaline 0.052, Ricinine 0.011
		24	tablets	Ricinine 0.032
		25	tablets	Ricinine 0.016
	'weight management'	26	capsules	Synephrine 0.07
		27	capsules	Senecionine 0.005, Senkirkine 0.013
		28	capsules	Heliotrine 0.006, Monocrotaline 0.006
		29	capsules	Erucifoline 0.038, Retrorsine-N-ox 0.016, Senecionine-N-oxide 0.052, Seneciphylline 0.013
		30	tablets	Erucifoline 0.036, Senecionine-N-oxide 0.028, Seneciphylline-N-oxide 0.038
		31	tablets	Kavain 0.008, Synephrine 0.024
		32	tablets	Synephrine 0.015
	Other	33	capsules ('prostate health')	Kavain 0.012, Senecionine-N-oxide 0.010
		34	mixture of dr. plant material ('blood purifier')	Podophyllotoxin 0.22
		35	tablets ('anti-stress')	Atropine 0.008, Heliotrine 0.007, Scopalamine 0.005
Traditional Chinese Medicins		36	Ba Zhen Nang (capsules)	Ricinine 0.12
		37	Cao Wu (dr. plant, *Aconitum Kusnezoffii*)	Aconitine 6.4, Synephrine 3.7
		38	Chuan Wu (dr. plant, *Aconitum Carmichaeli*)	Aconitine 0.13
		39	Chuan Xiong Cha Tiao Wan (capsules)	Aristolochic acid-I 0.68, Lycopsamine 0.026, Podophyllotoxin 0.33, Synephrine 0.058
		40	Kuan Dong Hua (dried plant, *Tussilago arfara*)	Senecionine 0.3, Senecionine-N-oxide 3.1, Senkirkine 57
		41	Pei Lan (dried plant, *Herba Eupatorii*)	Lycopsamine 37
		42	Qian Li Guang (dried plant, *Senecionis Scandentis*)	Erucifoline 0.53, Senecionine-N-oxide 0.27, Seneciphylline 0.047, Seneciphylline-N-ox 0.55
		43	Xiao Pang Mei Nang (capsules)	Synephrine 14

red = regulated plant toxin [1]
green = plant toxins known to occur in regulated plants species [1]
black = other natural substances of concern

Conclusions

- The targeted plant toxins were detected in 43 out of 100 samples
- Pyrrolizidine alkaloids were most frequently detected
- Based on Dutch legislation [1], 21 samples were non-compliant
- Furthermore, in 16 other samples plant toxins were found that indicated potential presence of forbidden plant species
- Detection of forbidden plant species through their plant toxins is a feasible alternative to current visual/microscopic methods
- The results call for extension of the survey
- The establishment of maximum limits, or -in absence of sufficient data for full risk assessment- threshold levels, is desirable for enforcement

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[1] Dutch Commodities Act Decree 'Herbal preparations'. 2001. Besluit van 19 januari 2001, houdende vaststelling van het Warenwetbesluit kruidenpreparaten. Staatsblad. 2001:56.
[2] EFSA Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements. EFSA Journal 2012;10(5):2663