



UNIVERSITÀ DI PARMA



21° Congresso Nazionale

PRESENZA DI ALCALOIDI TROPANICI IN
INTEGRATORI ALIMENTARI

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Dip. Scienze degli Alimenti e del Farmaco

Società Italiana di Tossicologia

**Pericolo, rischio
e rapporto
rischio-beneficio**

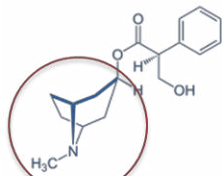
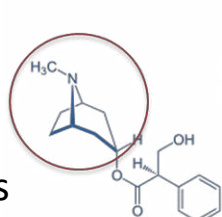
BOLOGNA

20-22 Febbraio 2023

www.sitox.org

Tropane alkaloids (TAs)

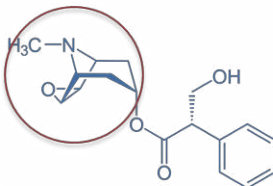
- Natural toxic compounds
- Infesting plants
- More than 200 different molecules found in seeds, flowers, leaves, stems and fruits



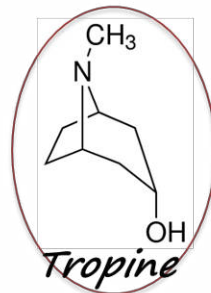
Atropine
racemic mixture
of (-)-
hyoscyamine and
(+)-hyoscyamine



*Hyoscyamus
Niger*



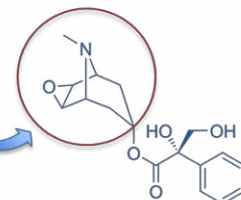
Scopolamine



Tropine



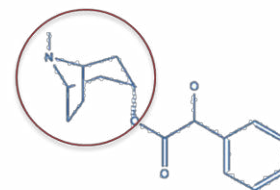
*Datura
Stramonium*



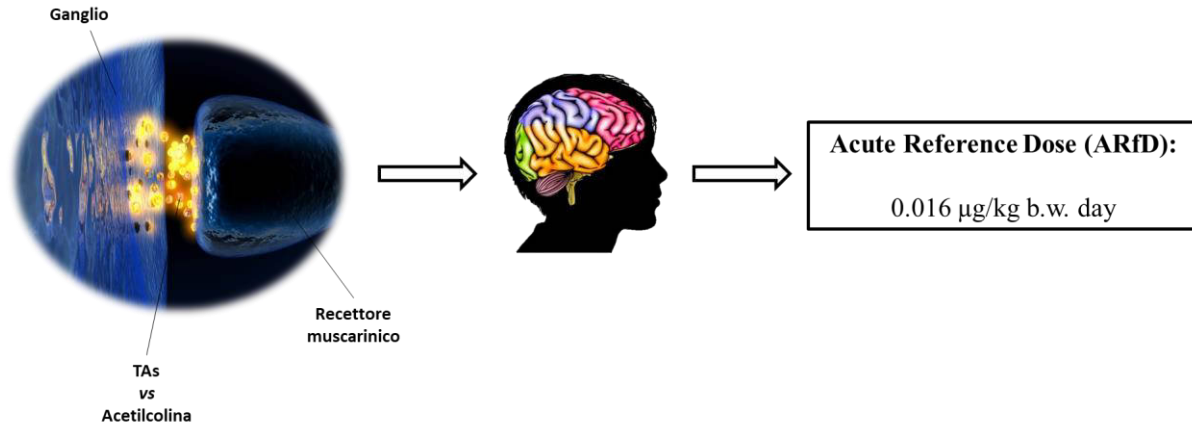
Anisodamine



*Atropa
Belladonna*



Homatropine



Poisoning cases...

Several poisoning cases from 1978 to 2013 due to presence of TAs in different food preparations or ingredients (beans, vegetables, herbal preparations, honey, buckwheat flour, teas, berries, etc.) (Adamse et al. 2014)

RASFF Notifications: Tropane alkaloids in peppermint from Turkey (Germany; 2020); frozen vegetable-bean-seed mix from Belgium, with raw material from Spain (Finland; 2013)

Uganda 2019: humanitarian food aid (soy and corn based food) contaminated with TAs caused 300 hospitalizations and 5 deaths (Abia et al. 2019);

SCIENTIFIC OPINION

Scientific Opinion on Tropane alkaloids in food and feed¹EFSA Panel on Contaminants in the Food Chain (CONTAM)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

Serious risk!
10 mg = fatal dose

Acute Reference Dose di 0.016 µg/Kg b.w. (Sum of (-) hyoscyamine (ATP) and (-) scopolamine (SCP))

Suggested LOQ of 5 µg/kg for food supplements, herbal infusions, vegetables, etc.

RECOMMENDATIONS

COMMISSION RECOMMENDATION (EU) 2015/976
of 19 June 2015
on the monitoring of the presence of tropane alkaloids in food
(Text with EEA relevance)

REGOLAMENTO (UE) 2016/239 DELLA COMMISSIONE

del 19 febbraio 2016

recante modifica del regolamento (CE) n. 1881/2006 per quanto riguarda i tenori massimi di alcaloidi tropanici in determinati alimenti a base di cereali destinati ai lattanti e ai bambini

REGULATIONS

COMMISSION REGULATION (EU) 2016/239

of 27 February 2016

amending Regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain foodstuffs

(Text with EEA relevance)

**Cereal and pseudo-cereal based food
Herbal infusions**

Cereals, pseudo-cereals, cereal based food



Food supplements



Gluten free products



Herbal infusions



Vegetables, legumes



RECOMMENDATIONS

COMMISSION RECOMMENDATION (EU) 2015/976
of 19 June 2015
on the monitoring of the presence of tropane alkaloids in food
(Text with EEA relevance)



Honey

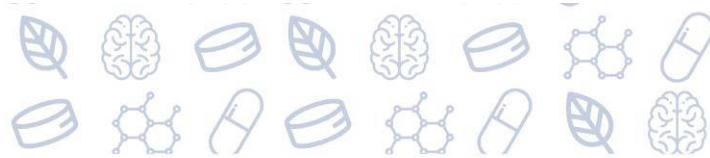
Alimento	Concentrazione massima ammessa (µg/kg)
Cereali/alimenti a base di cereali (infanti)	1 µg/kg di ATP e SCP
Cereali/alimenti a base di cereali e pseudo-cereali	5 - 15 µg/kg di ATP + SCP
Infusioni a base di erbe (secco)	25 µg/kg di ATP + SCP
Infusioni a base di semi di anice (secco)	50 µg/kg di ATP + SCP
Infusioni (liquido)	0,2 µg/kg di ATP + SCP

REGULATIONS

COMMISSION REGULATION (EU) 2021/1408
of 27 August 2021

amending Regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain foodstuffs

(Text with EEA relevance)



Tropane alkaloids (TAs): contamination



Datura Stramonium

mg/Kg	Atropine	Scopolamine	Sum
Roots	n.d. - 121	n.d. - 14	n.d. - 135
Stem	1 - 915	n.d. - 129	1 - 1044
Leaves	134 - 831	16 - 73	172 - 378
Flowers	270 - 299	66 - 106	336 - 405
Seeds	170 - 387	12 - 89	182 - 476

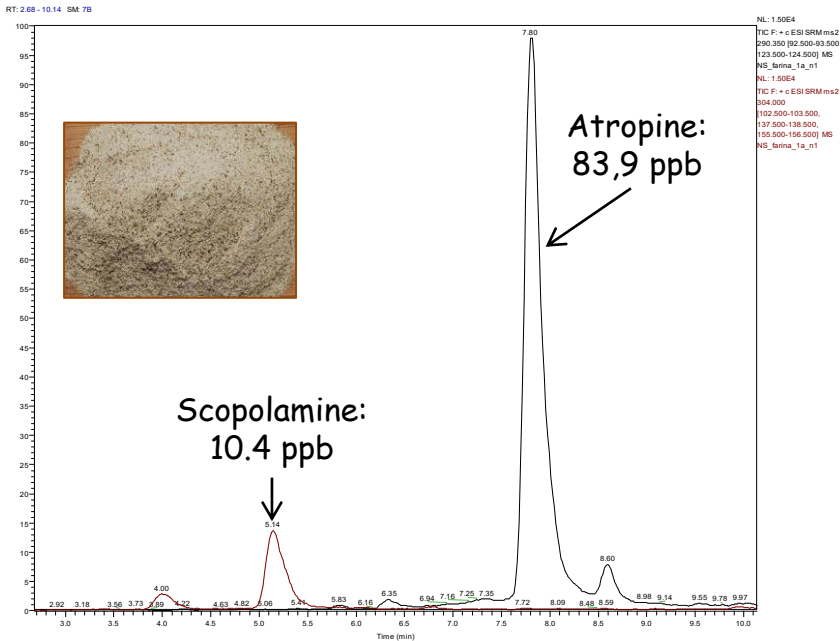


Datura seeds



Buckwheat seeds





Remarks:

Among the 26 tested samples, 3 resulted positive to the contemporary presence of atropine and scopolamine. ATP (13,9-83,9 $\mu\text{g}/\text{kg}$); SCP (5,7-10,4 $\mu\text{g}/\text{kg}$)

Max level: 5 - 15 $\mu\text{g}/\text{kg}$ di ATP + SCP

Buckwheat from organic agriculture



Analytical Methods

Are tropane alkaloids present in organic foods? Detection of scopolamine and atropine in organic buckwheat (*Fagopyron esculentum* L.) products by UHPLC–MS/MS



Martina Cirlini^a, Teresa M. Demuth^{a,b}, Alberto Biancardi^c, Michael Rychlik^b, Chiara Dall'Asta^{a,*}, Renato Bruni^a



Development of an analytical method based on the use of UHPLC-MS/MS technique for the determination of the main representative TAs in food supplements, herbal infusions

Atropine (ATP), Scopolamine (SCP), Anisodamine (ANI) and Homatropine (HOM)

Application on 60 samples



- 15 supplements (tablets and herbal extracts)
- 18 mono-component herbal infusions
- 27 pluri-component herbal infusions

Method development:



Several different
matrices



Acai



Grindelia



Noni



Food supplements



Chamomile



Green tea



Fennel

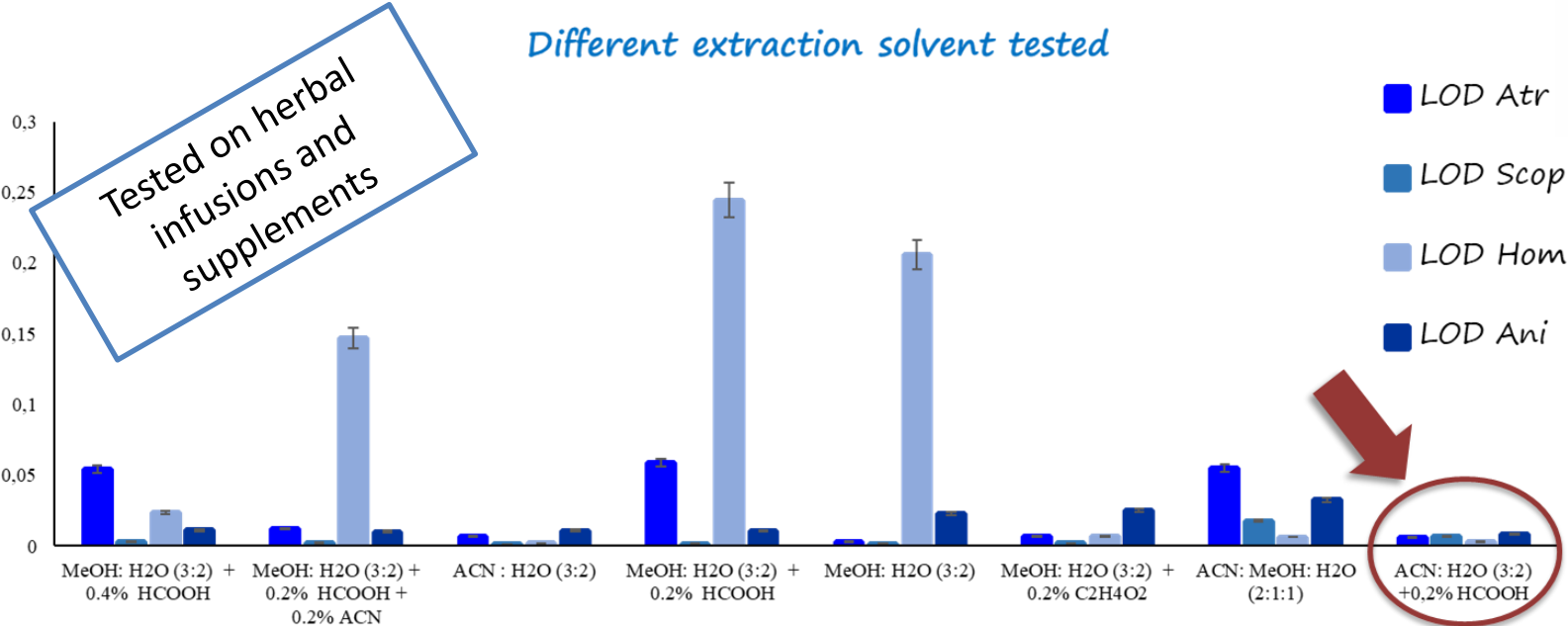


Pepper mint

Herbal infusions

Method development: extraction solvent

Different extraction solvent tested



Cirlini et al. 2018, Food Chemistry



Extraction: shaker 200 strokes/min, room temp. 90 min

Centrifuge

Dilution

Analysis

UHPLC-ESI⁺-MS/MS (Triple Quadrupole, SRM acquisition mode)

Mobile phase: H₂O (A) and ACN (B) both acidified with 0,2 HCOOH

Gradient:

Time (min)	% B
0	3
3	80
6	80
6,5	3
15	3



RP-C18 Kinetex (40°C)



	Transitions (m/z)	CE (eV)
ATP	290 → 124	23
	290 → 93	31
SCP	304 → 156	16
	304 → 138	20
ANI	306 → 140	25
	306 → 91	25
HOM	276 → 142	29
	276 → 124	23

Racc. (EU) 2015/976:
LOQ for food supplements and herbal
infusions =
5 µg/Kg < 10 µg/Kg

Linearity ok: 0,5 – 5 µg/kg

RR% comprised between 80 and 120 %

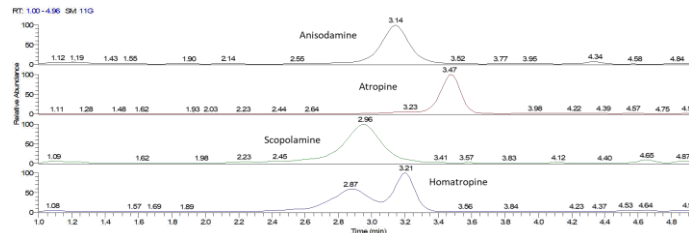
RR% low conc.
(10 µg/Kg)

RR% high conc.
(20 µg/Kg)

	RR% low conc. (10 µg/Kg)	RR% high conc. (20 µg/Kg)
Ani	106% ± 22%	89% ± 12%
Atr	113% ± 19%	110% ± 15%
Hom	94% ± 21%	91% ± 10%
Scop	107% ± 24%	81% ± 14%



	LOD	LOQ
Ani	0,48 ± 0,03 µg/Kg	0,60 ± 0,10 µg/Kg
Atr	0,75 ± 0,10 µg/Kg	2,27 ± 0,33 µg/Kg
Hom	0,40 ± 0,04 µg/Kg	0,47 ± 0,13 µg/Kg
Scop	0,49 ± 0,01 µg/Kg	0,53 ± 0,04 µg/Kg



0,8 < slope ratio < 1,2
NO matrix effect

	Slopes ratio
Ani	0,9
Atr	0,8
Hom	0,8
Scop	0,8

Determination of TAs in samples

15 food supplements
18 mono-component herbal infusions
27 pluri-component herbal infusions

8% of samples resulted positive to TA presence

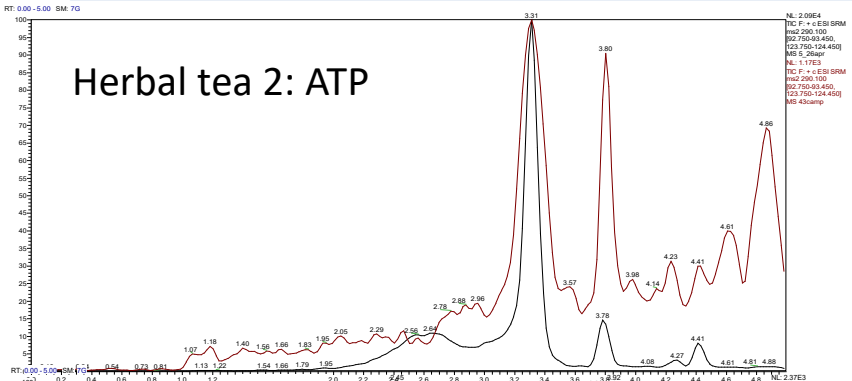
Maximum levels for
herbal teas and
infusions:

- 25 µg/Kg
- 50 µg/Kg (fennel)
- 0,2 µg/Kg beverage

µg/Kg	ATP	SCP	ANI	HOM
Supplem.1	< LOD	< LOD	13 ± 3	< LOD
Herbal inf 1	77 ± 9	< LOD	< LOD	< LOD
Herbal inf 2	28 ± 6	48 ± 25	< LOD	< LOD
Herbal inf 3	19 ± 2	< LOD	< LOD	< LOD
Herbal inf 4	22 ± 2	< LOD	< LOD	< LOD



Determination of TAs in samples: contamination source

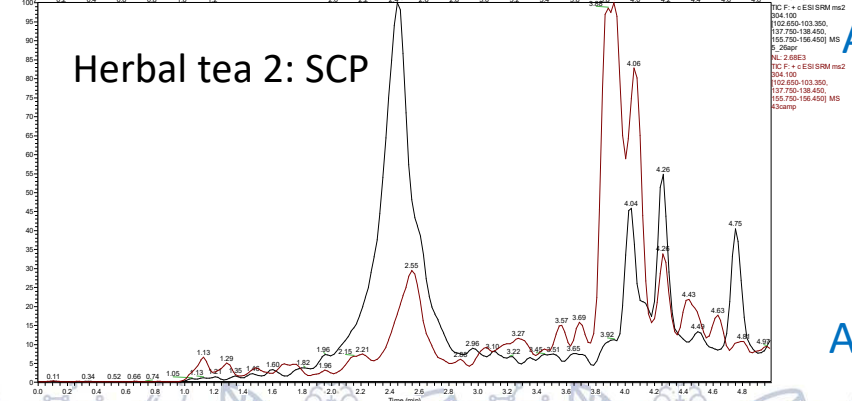


Herbal inf 2: ATP 37%, SCP 63%



Datura stramonium

Difficult to get the guilty



ATP 69%, SCP 31%



Atropa Belladonna

ATP 45%, SCP 55%

DNA barcoding is applied to exclude the presence of toxic species (taxonomic identification)

Toxins **2017**, 9, 284

LOD..?

1. Development and optimization of an analytical method for the determination of TAs in herbal supplements, herbal teas and infusions: the obtained parameters fitted with those recommended;
2. Results on tested samples allowed to obtain more detailed information about the occurrence of TAs in supplements, herbal teas, infusions;
3. The contamination found did not pose a real risk, but exceeded limits

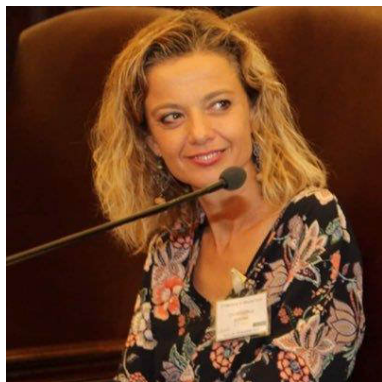
Constant monitoring of these substances is needed.
Sensitive methods have to be applied.



A sensitive UHPLC-ESI-MS/MS method for the determination of tropane alkaloids in herbal teas and extracts

Martina Cirlini^{a,*}, Valentina Cappucci^a, Gianni Galaverna^a, Chiara Dall'Asta^{a,b}, Renato Bruni^{a,b}





Chiara Dall'Asta

Thanks!!



Lorenzo Del Vecchio



Gianni Galaverna



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