



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**

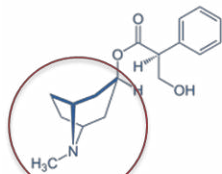
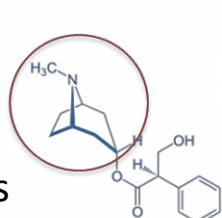
**BOLOGNA**

20-22 Febbraio 2023

[www.sitox.org](http://www.sitox.org)

# Tropane alkaloids (TAs)

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

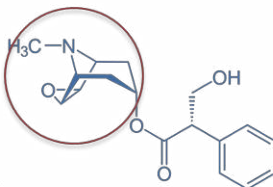


**Atropine**

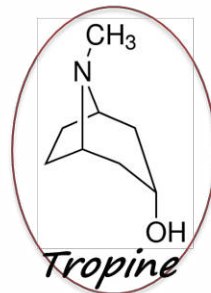
racemic mixture of (-)-hyoscyamine and (+)-hyoscyamine



*Hyoscyamus Niger*



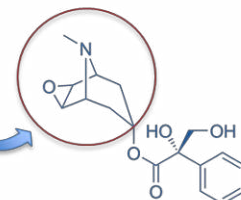
**Scopolamine**



**Tropine**



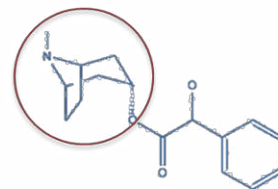
*Datura Stramonium*



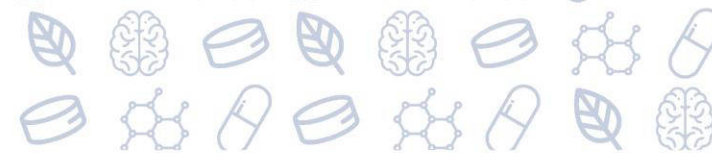
**Anisodamine**



*Atropa Belladonna*



**Homatropine**



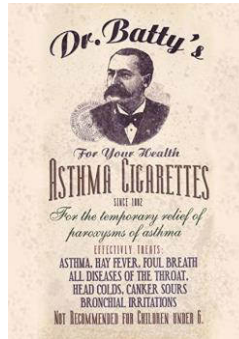
# Use of tropane alkaloids

## In Past....

**Folk medicine:** “magic potions”

**India:** In low doses “asthma cigarettes”

**Chinese medicine:** atropine to treat arthritis



## Today....

### Treatment

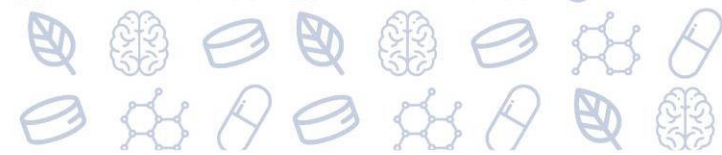
Nausea, motion sickness, intestinal cramping (antispasmodic properties), heart and respiratory problems.



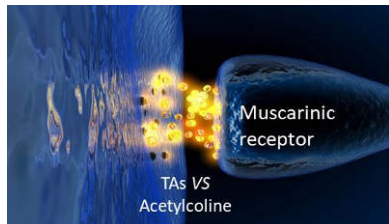
Oftalmic atropine, dilated-pupil fundus examination (DFE)

Butylscopolamine, treating crampy abdominal pain





## Poisoning cases...

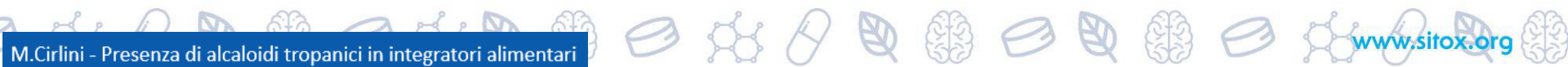


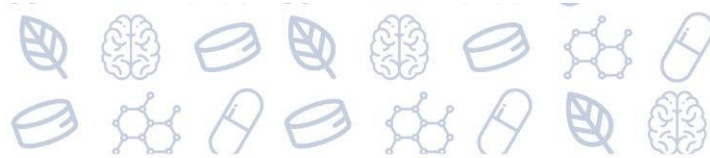
Atropine and Scopolamine are **STRONG ANTIMUSCARINIC AGENTS = ANTAGONISTS OF HUMAN CENTRAL NERVOUS SYSTEM RECEPTORS**

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);





# 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



Herbal teas and infusions



Food supplements



Frozen Vegetables



Cereal-based food; Gluten free products



Honey



Baby food



**SCIENTIFIC OPINION**

**Scientific Opinion on Tropane alkaloids in food and feed<sup>1</sup>**

EFSA Panel on Contaminants in the Food Chain (CONTAM)<sup>2,3</sup>

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 cereals for infants and young children  
(Text with EEA relevance)

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

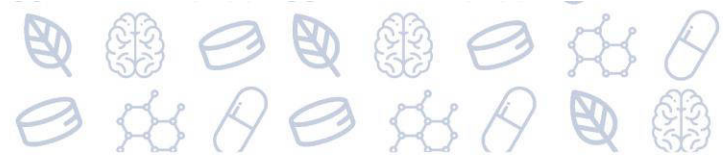
Alimento	Concentrazione massima ammessa (µg/kg)
Cereali/alimenti a base di cereali (infanti)	1 µg/kg di ATP e SCP
Cereali/alimenti a base di cereali e pseudo-cereali	5 - 15 µg/kg di ATP + SCP
Infusioni a base di erbe (secco)	25 µg/kg di ATP + SCP
Infusioni a base di semi di anice (secco)	50 µg/kg di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



## Aim of the study



Herbal teas sold in European markets: the 70% contaminated by atropine and scopolamine (429 µg/Kg) (Mulder et al. 2016)



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

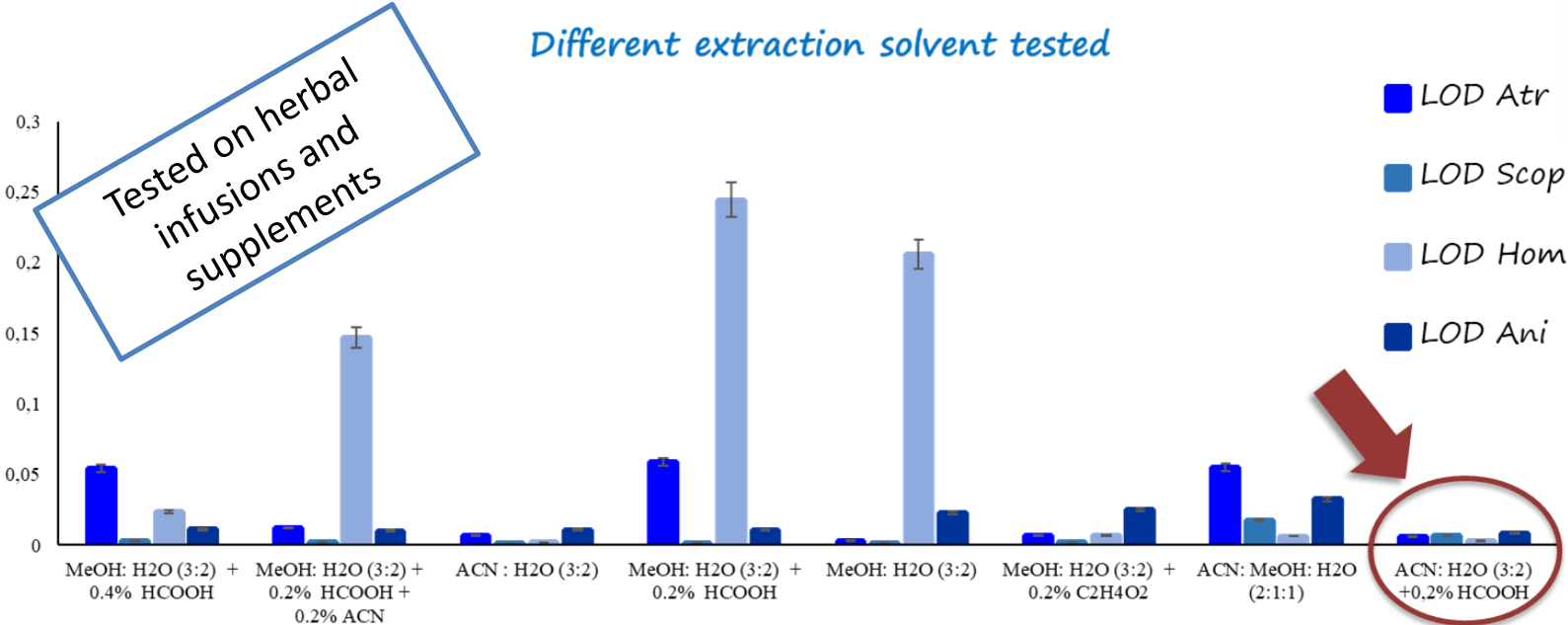


Pepper mint

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<sup>+</sup>-MS/MS (Triple Quadrupole, SRM acquisition mode)

Mobile phase: H<sub>2</sub>O (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



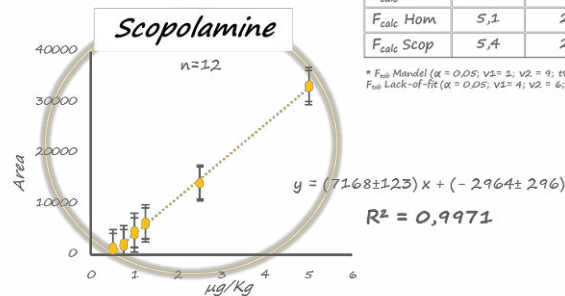
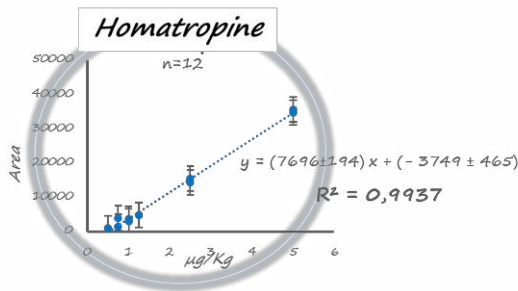
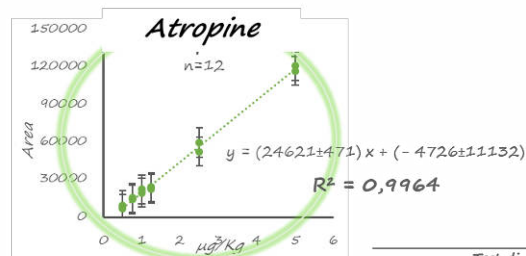
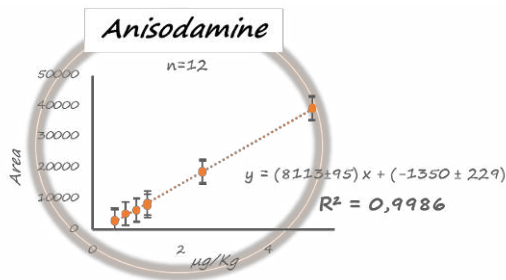
RP-C18 Kinetex  
(40°C)



	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

## Method development: linearity, matrix effect, LOD, LOQ, recovery rate, accuracy

Linearity: 0,5 – 5 µg/kg



	Test di Mandel	Test Lack-of-Fit
F tabulato	7,2	5,3
F <sub>calc</sub> Ani	2,5	2,8
F <sub>calc</sub> Atr	1,0	0,7
F <sub>calc</sub> Hom	5,1	2,4
F <sub>calc</sub> Scop	5,4	2,7

\* F<sub>tab</sub> Mandel (α = 0,05; v1 = 2; v2 = 9; two tails)  
F<sub>tab</sub> Lack-of-fit (α = 0,05; v1 = 4; v2 = 6; one tail)

**Racc. (EU) 2015/976:**  
LOQ for food supplements and herbal infusions =  
 $5 \mu\text{g/Kg} < 10 \mu\text{g/Kg}$

**RR% comprised between 80 and 120 %**

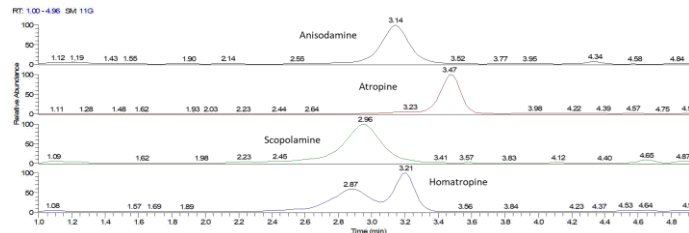
*RR% low conc.  
(10  $\mu\text{g/Kg}$ )*

*RR% high conc.  
(20  $\mu\text{g/Kg}$ )*

Ani	$106\% \pm 22\%$	$89\% \pm 12\%$
Atr	$113\% \pm 19\%$	$110\% \pm 15\%$
Hom	$94\% \pm 21\%$	$91\% \pm 10\%$
Scop	$107\% \pm 24\%$	$81\% \pm 14\%$



	LOD	LOQ
Ani	$0,48 \pm 0,03 \mu\text{g/Kg}$	$0,60 \pm 0,10 \mu\text{g/Kg}$
Atr	$0,75 \pm 0,10 \mu\text{g/Kg}$	$2,27 \pm 0,33 \mu\text{g/Kg}$
Hom	$0,40 \pm 0,04 \mu\text{g/Kg}$	$0,47 \pm 0,13 \mu\text{g/Kg}$
Scop	$0,49 \pm 0,01 \mu\text{g/Kg}$	$0,53 \pm 0,04 \mu\text{g/Kg}$



**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

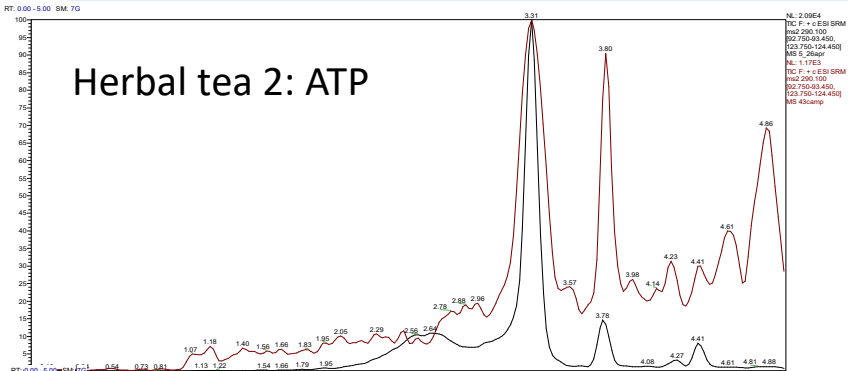
Maximum levels for  
herbal teas and  
infusions:

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

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



## Determination of TAs in samples: contamination source



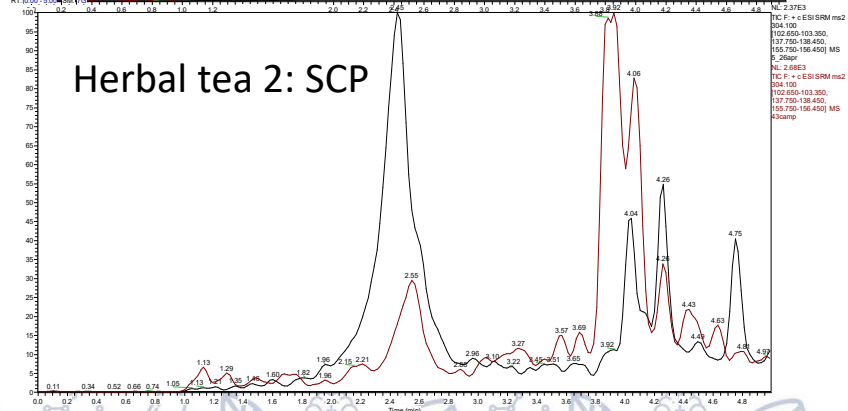
**Herbal inf 2: ATP 37%, SCP 63%**



**Datura stramonium**

**ATP 69%, SCP 31%**

*Difficult to get the guilty*



**Atropa Belladonna**

**ATP 45%, SCP 55%**

## Determination of TAs in samples: real risk?

$\mu\text{g/Kg}$	ATP	SCP	ANI
Maca	< LOD	< LOD	<b>13 ± 3</b>
Dandelion, chamomile, fennel, curcuma	<b>77 ± 9</b>	< LOD	< LOD
Lemon balm, fennel, dog rose, mallow	<b>28 ± 6</b>	<b>48 ± 25</b>	< LOD
Chamomile (pure)	<b>19 ± 2</b>	< LOD	< LOD
Betula pendula, nettle, Giava tea, chamomile	<b>22 ± 2</b>	< LOD	< LOD



MAN: 333 ml/day  
WOMAN: 360 ml/day



Intake TAs :  
0.0004-0.0024  $\mu\text{g/Kg b.w.}$

Acute reference dose  
(EFSA) :  
0,016  $\mu\text{g/Kg b.w.}$

Leclercq et al. 2009



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

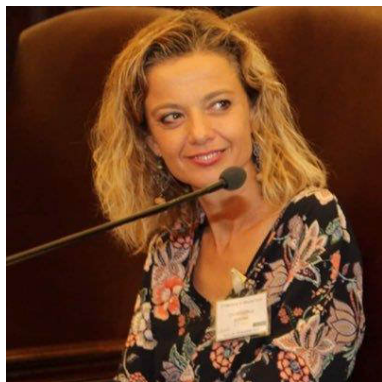
Constant monitoring of these substances is needed



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



Martina Cirlini<sup>a,\*</sup>, Valentina Cappucci<sup>a</sup>, Gianni Galaverna<sup>a</sup>, Chiara Dall'Asta<sup>a,b</sup>, Renato Bruni<sup>a,b</sup>



Chiara Dall'Asta

Thanks!!



Lorenzo Del Vecchio



Gianni Galaverna



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