Are Your Herbal Teas Safe?

Tropane Alkaloid Analysis Using SPE Combined With LC-MS/MS

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Introduction

Tropane alkaloids are toxic compounds and are most abundant in the Solanaceae family of plants (deadly nightshade, henbane, mandrake and Jimson weed).[1] Atropine and scopolamine are the most common tropane alkaloids found in food samples.[2] These alkaloids can contaminate herbal teas through plant debris[3] or soil migration.[4] They cause symptoms of reduced salivation, skin dryness, pupil dilation and with higher doses drowsiness, visual disturbances, palpitations, disorientation and hallucinations.[5] The European Union (EU) has set a regulatory limit of 0.2 ng/mL[6] for the sum of atropine and scopolamine in herbal infusions.

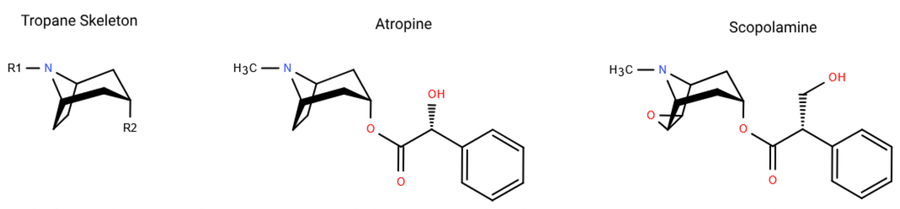

Figure 1 - Chemical structures of tropane alkaloids, including the tropane bicyclic ring structure, atropine and scopolamine

Current methods for analysis of tropane alkaloids in food use large bed weight solid phase extraction (SPE) products (>150 mg),[1] which are time consuming and less sustainable due to large solvent usage. This study validates the extraction of atropine and scopolamine with a 10 mg polymeric SCX 96 well SPE plate followed by HPLC-MS/MS analysis.

Following validation of the method, a range of herbal teas (chamomile, peppermint and green tea) sold on the UK market were analysed to assess the presence of atropine and scopolamine within the final herbal tea infusions.

Materials and Methods

Tea infusion, according to ISO 3103:1980[7]:

2.00 g of tea was added to a stainless-steel tea strainer and placed in a beaker. 150 mL of boiling ultrapure water was poured through the tea strainer into the beaker and steeped for five minutes. The final tea solution was allowed to cool to room temperature and then filtered through a 0.22 µm PES filter.

SPE Method:

A 10 mg 96 well plate (Microlute CP 10 mg SCX) with a positive pressure manifold (UltraPPM Lite) was used for processing samples.

- **Conditioning:** 1 mL of methanol
- **Equilibration:** 1 mL of 0.1% formic acid in ultrapure water
- **Loading:** 1 mL of acidified tea infusion sample (0.1% formic acid)
- **Wash 1:** 1 mL of 0.1% formic acid in ultrapure water
- **Wash 2:** 1 mL of 0.1% formic acid in methanol
- **Drying step:** Dried at 20 PSI for 2 minutes with the positive pressure manifold
- **Elution:** 2 x 0.5 mL of 0.5% ammonia in methanol
- **Reconstitution:** Eluate evaporated using a nitrogen blowdown evaporator (Ultravap Mistral) at 30°C and reconstituted with 0.2 mL of 0.1% formic acid in ultrapure water.

HPLC-MS/MS Method:

HPLC: ACQUITY Premier UPLC
Mass Spectrometer: Xevo TQ-S Micro
Column: Waters ACQUITY UPLC-BEH C18 (2.1 x 50, 1.7 µm)
Solvent A H_2O + 0.1% formic acid
Solvent B MeOH + 0.1% formic acid

Gradient

Time (min)	A (%)	B (%)
0.00	90.0	10.0
0.65	90.0	10.0
4.15	78.0	22.0
4.17	0.0	100.0
5.15	0.0	100.0
5.17	90.0	10.0
6.75	90.0	10.0

Flow Rate 400 µL/min
Column Temperature 30 °C
Capillary Voltage 1000 V
Desolvation Temperature 600 °C
Desolvation Gas Flow 1000 L/hr
Source Temperature 150 °C

MRM Transitions:

Analyte	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (V)
Atropine	289.83	124.06 (Quan ion)	30	22
		92.95		30
		90.99		42
Scopolamine	302.82	138.05 (Quan ion)	28	18
		156.03		15
		102.94		40

Table 1 - The HPLC and Mass Spectrometer conditions used for analysis and the atropine and scopolamine MRM transitions.

Validation guidelines:

Testing followed pesticide validation guidelines for food - SANTE/11312/2021[8] and Limit of Quantification was set by Regulation 2023/2783[9]:

- **Recovery:** 70-120% at three concentrations (0.2, 1.0 and 5.0 ng/mL)
- **Repeatability:** ≤20% at each level
- **Linearity:** R^2 ≥0.99 with residuals ≤±20%
- **Limit of Quantification (LOQ):** <0.05 ng/mL

Results and Discussion

Method validation – Linearity and Matrix Effects

- Good linearity was achieved for all herbal infusions (R^2 = 0.991-0.996) across 0.5-50 ng/mL range
- Low limits of quantification: 0.010 ng/mL for chamomile and peppermint, 0.025 ng/mL for green tea
- Negative matrix effects were observed for all samples (-12% to -38%)

Tea Infusion	Linearity (ng/mL)	Atropine			Scopolamine		
		R^2	LOQ (ng/mL)	ME (%)	R^2	LOQ (ng/mL)	ME (%)
Chamomile	0.5 - 50	0.992	0.010	-17	0.995	0.010	-38
Peppermint	0.5 - 50	0.996	0.010	-14	0.992	0.010	-24
Green tea	0.5 - 50	0.991	0.025	-12	0.993	0.025	-22

Table 2 – Linearity, LOQ and matrix effects for chamomile, peppermint and green tea infusions, following SPE and HPLC-MS/MS analysis

Method validation – Recovery and Repeatability

- Matrix matched calibration used due to matrix effects >20%
- High recovery rates achieved: 78-99% for both atropine and scopolamine across all matrices
- Excellent intra-day repeatability: 0.7-3.6%RSD
- Good inter-day repeatability: 2.1-15.8%RSD

Tea Infusion Sample	Atropine			Scopolamine		
	Recovery (% ± SD)	Intra-day Repeatability (%RSD)	Inter-day Repeatability (%RSD)	Recovery (% ± SD)	Intra-day Repeatability (%RSD)	Inter-day Repeatability (%RSD)
Chamomile - 0.2 ng/mL	93 ± 1.7	1.8	6.9	84 ± 3.1	3.6	4.8
Chamomile - 1 ng/mL	99 ± 1.6	1.6	9.8	94 ± 2.4	2.6	5.5
Chamomile - 5 ng/mL	98 ± 2.0	2.1	2.4	99 ± 2.1	2.2	2.5
Peppermint - 0.2 ng/mL	87 ± 0.88	1.0	2.4	85 ± 1.8	2.1	4.1
Peppermint - 1 ng/mL	95 ± 0.78	0.8	4.5	96 ± 1.6	1.7	5.7
Peppermint - 5 ng/mL	92 ± 1.6	1.7	2.2	95 ± 1.2	1.3	2.6
Green Tea - 0.2 ng/mL	78 ± 2.7	3.4	15.8	78 ± 2.5	3.2	10.8
Green Tea - 1 ng/mL	96 ± 1.2	1.3	5.1	93 ± 2.4	2.6	5.1
Green Tea - 5 ng/mL	96 ± 0.66	0.7	3.9	96 ± 1.4	1.5	2.1

Table 3 - Recovery and repeatability data for chamomile, peppermint and green tea infusions using the combined SPE and HPLC-MS/MS method for the analysis of atropine and scopolamine. Intra-day repeatability (n=8, in one day) and inter-day repeatability (n=16, on two days) SD = standard deviation

Application of the Method on Herbal Tea Infusions

- 12 commercial herbal tea samples were analysed (chamomile, peppermint, green tea)
- Most samples contained low or undetectable levels of atropine and scopolamine
- One chamomile sample (Chamomile-5) significantly exceeded EU regulatory limits:
  - Atropine: 0.59 ng/mL (~3 x EU limit of 0.2 ng/mL)
  - Scopolamine: 0.35 ng/mL (exceeds EU limit)
  - Sum of atropine and scopolamine: 0.94 ng/mL (~5 x EU limit)

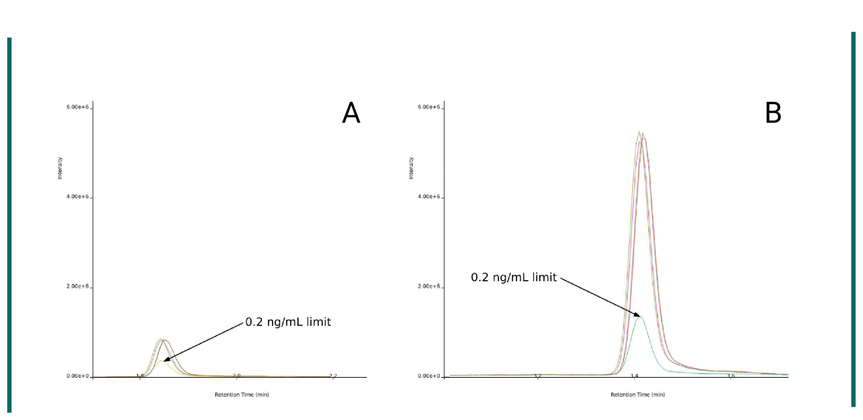

Figure 2 – Overlaid chromatograms showing the 0.2 ng/mL EU limit reference standard versus chamomile-5's chromatograms (n=4) at a mean concentration of 0.35 ng/mL for scopolamine (A) and 0.59 ng/mL for atropine (B).

Tea Infusion Sample	Concentration (ng/mL)		
	Atropine	Scopolamine	Sum of Atropine and Scopolamine
Chamomile-1	<LOQ	ND	<LOQ
Chamomile-2	<LOQ	<LOQ	<LOQ
Chamomile-3	0.013 ± 0.0035	<LOQ	0.013 ± 0.0035
Chamomile-4	<LOQ	ND	<LOQ
Chamomile-5	0.59 ± 0.0070	0.35 ± 0.012	0.94 ± 0.013
Chamomile-6	<LOQ	ND	<LOQ
Peppermint-1	<LOQ	<LOQ	<LOQ
Peppermint-2	<LOQ	<LOQ	<LOQ
Peppermint-3	<LOQ	0.011 ± 0.0014	0.011 ± 0.0014
Green tea-1	<LOQ	ND	<LOQ
Green tea-2	ND	ND	ND
Green tea-3	<LOQ	ND	<LOQ

Table 4 – Concentration values (± standard deviation) for atropine, scopolamine and sum of both analytes in herbal infusions prepared using the SPE and HPLC-MS/MS method. ND = Not detected, due to signal to noise being less than 3:1

Conclusions

- Validation was successful for the analysis of tropane alkaloids in herbal tea infusions using a 96-well SCX SPE method using a low bed weight (10 mg)
- The method meets SANTE/11312/2021 validation requirements with good linearity, low LOQs, and high recovery and good repeatability
- It provides a sustainable alternative to higher bed weight SPE methods
- Commercially available herbal teas generally contain low levels of tropane alkaloids
- However, one of the tested samples still contained a sample exceeding the EU limit showing there is still a risk to consumers

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