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(Article begins on next page)

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

3  
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8

9 **Abstract**

10 A closer monitoring of tropane alkaloids (TA) in foods is now recommended by the European Commission, following a series of alerts related to the  
11 contamination of buckwheat with weeds of the genus *Datura*. A novel, accurate UHPLC-MS/MS method was developed and validated for the rapid  
12 detection of scopolamine and atropine in buckwheat foods. A suitable extraction protocol was set up to maximize recoveries and detection limits in  
13 different raw, processed and baked foods. The method offers good sensitivity, as LODs for scopolamine and atropine in buckwheat flour were obtained  
14 at 0.03 and 0.09 µg/kg respectively, with LOQs at 0.04 and 0.10 µg/kg. Precision (%RSD range) and accuracy (% recovery range) were 2.7-3.4% and  
15 83-103%, respectively. The established method is suitable for routine determination of trace levels of TA and was applied to 26 different buckwheat-  
16 derived organic foods, detecting TA in 3 samples (13.9-83.9 µg/kg for atropine and 5.7-10.4 µg/kg for scopolamine). Only in one case the level of  
17 contamination was relevant in terms of food safety.

18

19 **Keywords:** food safety, buckwheat, tropane alkaloids, mass spectrometry, organic food.

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## 28 **1. Introduction**

29 Alkaloids are well-known for their remarkable toxicity and for a consolidated history of poisoning, due to incidental or deliberate food contamination  
30 (Koleva et al., 2012). In this regard, the once neglected presence of tropane alkaloids (TA) in foods has been recently reconsidered, leading to a specific  
31 recommendation by the European Commission (Adamse et al., 2014; Beuerle et al. 2013; EFSA 2015). Clear goals in this respect are the estimation of  
32 the actual level of human exposure by the western diet, the definition of foods most involved and the ascertainment of maximum levels for most  
33 common TA, namely atropine and scopolamine (Figure 1) (EFSA 2015). Such recommendations arise from the evidence that plants containing TA are  
34 intrinsic constituents of some food chains, in which distinct patterns were found for cereal based products, infant formulas and herbal teas. (Mulder et  
35 al., 2016). Cereal-based products, in particular, were mostly characterized by the presence of atropine and scopolamine, while ready-to-eat meals for  
36 children contained also tropine and pseudotropine. A wider range of TA including also convolvine was found in teas and infusion. These results  
37 highlight the need to include specific TA as targets in screenings, according to each food product group. Moreover, this evidence is the consequence not  
38 only of the presence of TA in specific developmental stages of certain crops (e.g. calystegines in *Solanum tuberosum* L.), but also of co-harvesting  
39 crops and specific weeds, drawing the attention for cereals and buckwheat-derived foods mostly on atropine and scopolamine (Griffin & Lin, 2000;  
40 Aehle & Drager, 2010). Plant families such as Cruciferae and Convolvulaceae may contain TA, but their agronomical relevance is lower and the  
41 knowledge regarding their toxicity is limited. According to EFSA opinion, TA can actually enter the food chains as a result of contamination especially  
42 due to seeds and fruits of some species of the Solanaceae family. In fact, in the recent past and on different continents, occurrence of TA in food was  
43 mainly associated to the cross-contamination of Solanaceae family, among them mainly the genera *Hyoscyamus*, *Atropa* and *Datura* (Kitano et al.,

44 2003; Chang et al., 1999; Van Raamsdonk et al., 2009; Fretz et al., 2007). Albeit whole *Datura* plants are known to contain TA (atropine and  
45 scopolamine, in particular), seeds are usually deemed to be the most frequent cause of contamination, due to their size and to the simultaneous  
46 maturation with crops like grains, cereals, legumes and pseudocereals sharing the same cultural cycle.

47 A simple seed contamination may be detected by visual inspection or reduced through sorting and cleaning by physical methods, but yet a minimal  
48 presence is relevant in flours and other processed materials, in which the presence of a single undetected *Datura* seed per million of crop seeds/fruits  
49 may pose relevant health risks. (Miraldi et al., 2001; Friedman & Levin, 1989; Lawrence et al., 1994). A variety of analytical methods have been set up  
50 in recent years, encompassing different approaches and matrices, thus allowing different degrees of sensitivity and workload (Aehle & Drager, 2010;  
51 Christen et al., 2013; Caligiani et al., 2011; Muder et al., 2015; Mulder et al., 2014; Shimshoni et al., 2015; Rancic & Spasic, 2009; Jakabová et al.,  
52 2012; Chow et al., 2012; Mol et al., 2011). Within this context, the recourse to liquid chromatography emerges as the leading approach for TA analysis  
53 and represents the method of choice by many laboratories, with numerous advantages following its combination with a MS detector (Christen et al.  
54 2013). As a consequence of the high human sensitivity to these anticholinergic alkaloids, extremely low amounts of atropine derivatives may trigger  
55 severe poisoning symptoms and, therefore, analytical methods should be extremely sensitive and reliable. For such reason, the EU is fostering the  
56 availability of methods with LOQ for atropine and scopolamine “*preferably below 5 µg/kg and not higher than 10 µg/kg for agricultural commodities,*  
57 *ingredients, food supplements and herbal teas and lower than 2 µg/kg for finished foods and 1 µg/kg for cereal-based foods for infants and young*  
58 *children*” (European Commission, 2015). The literature at present only offers methods matching the upper limits of the requested sensitivity, with few  
59 exceptions (Mulder et al., 2015, Chen et al., 2017).

60 Following few episodes of mass intoxication from TA in buckwheat products in 2005 (in Slovenia) and in 2012 (in France), different surveys have been  
61 performed in European countries, namely Germany, Hungary, Slovenia, France and the Netherlands. The results highlighted different levels of  
62 contamination in a wide range of plant foods as in the case of flours, infant formulas, feed, silage products, botanicals or honey, both in terms of  
63 positive samples and of total TA (Perharič, 2005; Perharič et al., 2013; EFSA, 2008; Mulder et al., 2015; Mol et al., 2011; AFSSA, 2008). In particular,  
64 due to the concurrent fruit maturation and the similar seed size with *Datura* species, crops aimed at human consumption like linseed, soybean, millet,

65 pulses, sorghum and particularly buckwheat (*Fagopyrum esculentum* Moench) have been more often related to TA contamination and are, therefore,  
66 listed as targets for more careful scrutiny (EFSA, 2008; European Community, 2015). Due to the absence of gluten and to an encouraging nutritional  
67 profile, buckwheat is experiencing a renewed commercial interest and is more frequently sold as a flour. Moreover, it is increasingly used as a healthy  
68 ingredient in different processed foods including bread, pasta, snacks, cakes and specialty food for infant nutrition (Giménez-Bastida, & Zieliński,  
69 2015). In most temperate climates, annual species known for atropine and scopolamine biosynthesis and accumulation like *D. stramonium*, *D. innoxia*  
70 and *D. ferox*, can easily thrive as weeds in buckwheat fields. Despite an adequate management, post-harvest handling and control, some seeds may go  
71 undetected to subsequent stages of the food chain making a certain degree of contamination by these TAs unavoidable (EFSA, 2008). Both *Datura* and  
72 *Fagopyrum* species in fact produce, and maturate almost simultaneously, a dehiscent fruit harboring seeds with similar size and weight. This may be  
73 particularly relevant for organic agriculture, in which a less strict weed management may allow an increased in-field presence of potentially dangerous  
74 plants alongside with crops (Bond & Grundy, 2001).

75 Therefore, in order to provide a new tool meeting EFSA requirements for the screening of tropane alkaloids in flour-based foods, we set up an accurate  
76 but simple UHPLC-MS/MS method, optimized for different food matrices. Being atropine and scopolamine the most prevalent TA in cereals and  
77 buckwheat flour, these compounds were chosen as targets. The fully validated protocol was then applied to a range of buckwheat flour samples and to  
78 different buckwheat containing foods, with the aim to provide a survey of the eventual presence of atropine and scopolamine in the organic food chain  
79 in Italy.

80

## 81 **2. Materials and Methods**

### 82 **2.1 Chemicals and solvents.**

83 Acetonitrile, Ethyl acetate, Methanol, formic acid,  $\geq 99\%$  HPLC grade, were purchased by Sigma Aldrich (Milan, Italy). Ultra-pure water,  $\geq 99\%$   
84 HPLC grade, was produced in house by ultrafiltration using an Alpha-Q system (Millipore, Marlborough, MA, USA). Furthermore atropine, ( $\geq 95\%$   
85 (TLC), and scopolamine hydrobromide, ( $\geq 90\%$  (TLC), obtained from Sigma Aldrich (Milan, Italy) were used for preparing standard solutions. For the

86 preparation of the extraction solvent with a final pH of 3.2, methanol and pure water with 0.2% acetonitrile and 0.2% formic acid were combined in a  
87 volume ratio 3:2 and prepared weekly.

88

## 89 **2.2 Plant and food material**

90 To obtain an actual TA profile from a supposed in field contaminant, a sample of dried *Datura stramonium* seeds was obtained by the Botanical Garden  
91 of the University of Parma from plants grown in 2014 and stored at -20°C until analysis. Twenty-five commercial samples, namely 12 flours, 6 pasta  
92 and 8 bakery products, belonging to different brands and containing different amounts of buckwheat were purchased during 2015 from different  
93 groceries and supermarkets in Northern Italy. The sample size for flour, pasta, and bakery products was about 1 kg, 0.5 kg, and 0.25 – 0.5 Kg,  
94 respectively. For each sample, information on the formulation and on the percentage of buckwheat were recorded, when available, together with the  
95 actual weight of package (Table 1). For sample preparation, a subsample (20 g) was obtained from the whole content of the package, after grinding (0.2  
96 mm mesh) and mixing. *Datura* seeds were finely ground and carefully mixed, before storage. All ground materials were subsequently kept at -20° C  
97 and in the dark until analysis.

98

## 99 **2.3 Preparation of standard solution for atropine and scopolamine**

100 A standard stock solution at the concentration of 5.0 mg/L was prepared, by dissolving 0.50 mg of atropin-/scopolamin powder in 100 mL methanol, in  
101 a volumetric flask. standard working solutions at 5.0 µg/L was then prepared by proper dilution of the stock solution. Both standard solutions were  
102 stored at 4° C in the dark and used for 3 months. A six-point calibration curve was obtained for both atropine and scopolamine (Figure X), by injecting  
103 in triplicate solution at the following concentrations: 0.05, 0.10, 0.25, 0.50, 0.75, 1.00 µg/L prepared in a blank extract, obtained from TA-free  
104 buckwheat flour and pasta samples, corresponding to a contamination range of 2.5 – 50 µg/kg.

105 **2.4 Sample preparation**

106 For sample extraction, 5.00 g of each ground sample was mixed with 25.0 mL of extraction-solvent (dilution factor: 1:5, v/v), then placed in a shaker  
107 for 90 minutes at room temperature. After that, the samples were centrifuged (15 min, 4000 rpm). A volume of 5.00 mL of the clear solution was  
108 collected and diluted 1:10 with the extraction solvent before analysis (total dilution factor: 1:50 v/v).

109 **2.4 Instrumental analysis**

110 The chromatographic analysis was performed by UHPLC-MS/MS. The set up consisted from an UHPLC Ultimate 3000 separative module (Dionex,  
111 Sunnyvale, CA, USA), coupled with a TSQ Vantage triple quadrupole (Thermo Fisher, Waltham, MA, USA). The separation of tropane alkaloids was  
112 achieved by using a RP-C18 Kinetex column (2.6 $\mu$ , 100A; 100x2.10 mm) from Phenomenex (Torrance, CA, USA) operated at a column temperature  
113 of 40° C.

114 The mobile phase consisted of bi-distilled water (Eluent A) and methanol (eluent B). Both eluents were acidified with 0.2% formic acid. The flow rate  
115 was set at 0.35 mL/min, column oven temperature was set at 40°C, sample temperature was 20°C and the injection volume was 4 $\mu$ L. The gradient was  
116 set as follows: the first step was a linear gradient from 3% B to 20% B in 6 min, followed by a second step to 80% B in 6 min. The eluent B was  
117 decreased to the initial conditions in 4 min, and the reconditioning step at 3% eluent B was kept for 4 min. The total run time was 20 min. Compounds  
118 were monitored by ESI ionization in positive mode (spray voltage = 3200 V), with the capillary temperature at 270 °C. The vaporizer temperature was  
119 kept at 200 °C, the sheath gas flow was set at 50 units and the auxiliary gas flow at 5 units. Detection was obtained in multiple reaction monitoring  
120 (MRM) modality. The following transitions were monitored: 290  $\rightarrow$ 93 (CE = 33 eV) and 290  $\rightarrow$  124 (CE = 25 eV) for atropine; 304  $\rightarrow$ 103 (CE = 35  
121 eV), 304  $\rightarrow$ 138 (CE = 19 eV) and 304  $\rightarrow$ 156 (CE = 16 eV) for scopolamine..

122

123 **2.5 Method performance parameters**

124 Method performance was evaluated by assessing several parameters, i.e. linearity, specificity, limit of detection, limit of quantification, accuracy as  
125 recovery, matrix effect, and precision (RSD%). Specificity was evaluated by analysing six blank samples and verifying the absence of interferences in

126 the atropine/scopolamine retention time range ( $t_R \pm 2.5\%$ ). Linearity was verified in the 0.05–10  $\mu\text{g/L}$  range. In particular, different solutions were  
127 obtained by diluting the standard stock solution in three different not contaminated sample extracts (buckwheat flour and pasta) at 8 concentration  
128 levels. Quantification was performed on the basis of calibration curves constructed by linear regression analysis of the area versus the concentration of  
129 the injected analytes. Moreover, for sample concentrations outside the calibration and linearity range, a proper dilution with extraction solvent must be  
130 done in order to interpolate the results. Matrix effect was defined in the 0.05 – 10  $\mu\text{g/L}$  range using three different not contaminated sample extracts  
131 (buckwheat flour, pasta and cake) spiked with increasing concentration of atropine and scopolamine, by the quotient of the slope of the matrix-matched  
132 regression curve and the slope of standard regression curve. A value  $> 100\%$  indicates signal enhancement, whereas a value  $< 100\%$  shows signal  
133 suppression (Sulyok et al., 2007). Limit of detection (LOD) and the limit of quantification (LOQ) were derived from the signal-to-noise ratio (3/1 and  
134 10/1, respectively). Repeatability, expressed as relative standard deviation (RSD%), was evaluated at three different spiking levels evenly distributed  
135 along the linearity range (0.05, 0.5 and 10  $\mu\text{g/L}$ ). Each concentration level was injected 6 times over 3 days ( $n = 18$ ). The RSD% values corresponding  
136 to intra- e inter-day precision (day 2 and day 3, respectively) below 15%, were considered as acceptable (Shah et al. 2000). Accuracy, in terms of  
137 trueness, was assessed by the analysis of spiked samples at six different concentration levels. Calculated and nominal concentrations were then  
138 statistically compared. Recovery was evaluated by analyzing blank samples spiked at 6 concentration levels in the range 0.05 – 10  $\mu\text{g/L}$ , and by  
139 statistically comparing the calculated and the nominal values.

140

## 141 **2.6 Statistical analysis.**

142 Calibration data were statistically evaluated by an ANOVA test ( $\alpha = 0.05$ ), performed with the Statistical Analysis Tool of Microsoft Excel 2003,  
143 Microsoft Office Professional Edition 2003 (Microsoft Co., Santa Rosa, CA, USA).

144

## 145 **3. Results and Discussion**

146 The presence of tropane alkaloids deriving from incidental contamination of seed crops by plants of the *Datura* genus has been reported in different

147 countries. Authorities are actively promoting a more careful screening of those food chains most likely to be exposed to TA contamination, but a  
148 widespread monitoring is precluded by the limited availability of reliable methods combining sensitivity and speed. As a consequence, maximum  
149 levels of exposure to TA in foods are not yet available, so as a more precise knowledge of their actual occurrence in western diet. Thus, we focused our  
150 effort on a flexible protocol and on optimizing results in terms of sensitivity and recovery on different food matrices. Tropane alkaloids are small-sized,  
151 polar analytes that are easily resolved by high performance liquid chromatography-mass spectrometry, whose quantification may take particular  
152 advantage from their sensitive detection in positive ion electrospray ionization (ESI+). Encouraged by the recent success obtained by Adamse and Van  
153 Egmond in 2010, and more recently by Mulder et al. in 2015, we focused our attention on a similar strategy (Adamse and Van Egmond, 2011, Mulder  
154 et al., 2015).

### 155 **3.1 Method development**

156 Different approaches were undertaken to maximize extraction yields while minimizing processing steps and reducing the time needed for sample  
157 preparation. Different combinations of solvents were tested using a spiking concentration of 10  $\mu$ /Kg (Table 3) and the best combination in terms of  
158 recovery was obtained using CH<sub>3</sub>OH: H<sub>2</sub>O (3:2 v/v) acidified with 0.2% HCOOH + ACN 0.2% solution, which was therefore chosen as the elective  
159 extraction solvent. As the literature frequently reported on the degradation of tropane alkaloid reference compounds (Kirchoff et al., 2004), we tested  
160 their stability under laboratory conditions. Both analytes showed excellent stability at room temperature for 48 hours, revealing an revealing an RSD%  
161 of 2.4 % for scopolamin and 10.5 % for atropine at the lowest concentration of the calibration curve (0.05  $\mu$ g/L for both TA) . Frozen TAs stock  
162 solutions were stable over 3 months of observation; in contrast, refrigerated TAs stock solutions (+ 4°C) showed a sharp degradation after 90 days.

163 **3.1.1.Method validation.** Optimized UHPLC-MS/MS method was subjected to validation in terms of precision, accuracy, linearity, detection and  
164 quantitation limits, following recommendations of the International Conference on Harmonisation (ICH, 2005). Full validation data for the method are  
165 reported in Table 2. Limits of detection (LOD) and of quantification (LOQ) were determined in blank extract, and were found to be 0.03  $\mu$ g/Kg and 0.1  
166  $\mu$ g/Kg for scopolamine, and 0.09  $\mu$ g/Kg and 0.30  $\mu$ g/Kg for atropine, respectively. Linearity, determined in blank matrix over the range 0.05  $\mu$ g/L to  
167 10  $\mu$ g/L (8 concentration levels, each injected in triplicate), was fully satisfactory, with a  $r^2 > 0.99$  for both scopolamine and atropine in all the

168 considered matrices. Matrix effect was calculated by matrix-matched experiments over the calibration range, using blank samples spiked at the  
169 required levels. Signal suppression or enhancement (SSE%) was calculated according to Sulyok et al. (2007), by comparing the slope of the regression  
170 curve obtained for standard solution and the slope of the regression curve obtained in the matrix. The SSE% values obtained for scopolamine and  
171 atropine provided different results according to each matrix, pointing out the necessity of a matrix-matched calibration for each category of commercial  
172 products (flour, bakery, pasta).

173 Recovery for TAs calculated by matrix-matched calibration experiments was in the range 78 – 103% for all considered categories. Recovery rates  
174 decreased at low spiking concentration, leading to a lower accuracy for concentrations close to the LOQ. This effect could be a consequence of the  
175 already discussed influence of matrix interferences. Considerably the recovery was constant during the whole validation period. Intra- and interday  
176 repeatability were calculated in terms of RSD % at three different concentration levels (0.05, 0.5 and 10 µg/L for both TAs); the results are showed in  
177 Table 2.

178

179 **3.1.2.Advances provided by the method.** Figures 2 and 3 depict respectively a typical chromatogram of a buckwheat flour spiked with atropine and  
180 scopolamine (Fig. 2) and with Datura seeds (Fig. 3). The sensitivity of the presented method is largely compliant with limits suggested by EFSA and  
181 better than those recently provided by means of methods based on Orbitrap-LC, GC-MS or LC-MS/MS and developed for food matrices (Caligiani et  
182 al., 2011; Mulder et al., 2015; Mol et al., 2011; Chen et al., 2017). Given their small molecular size and polarity, for the routine analysis of TA in food  
183 both GC and HPLC methods are available, with sensitivities ranging between 0.3 and 5 µg/Kg for LOD, and between 1 and 6 µg/Kg for LOQ. The  
184 most sensitive, multi-analyte HPLC-MS/MS method published was focused on grain-based products for infants and children, with LOD in the range of  
185 0.3 µg/Kg and recoveries exceeding 88% for both atropine and scopolamine, but being the target of this method the detection of both ergot and tropane  
186 alkaloids in a largest array of matrices, no precise tailoring on buckwheat was performed and the source of the contaminated commercial samples was  
187 not determined (Mulder et al., 2015). As described in Table 2, our protocol provides a net improvement to the reported methods in the literature in  
188 terms of sensitivity, with LOD for scopolamine and atropine in the range of 0.03 µg/Kg and 0.09 µg/Kg respectively, while LOQ were obtained at 0.10

189 and 0.30 µg/Kg.

190

### 191 **3.2 Screening of different commercial organic samples**

192 A further goal of our work was to provide a first estimate of the presence on TA in organic buckwheat products from the Italian market. A previous  
193 survey conducted in our laboratory did not detect TA in conventional buckwheat foods purchased in Italy in 2011, but the inter-year variability  
194 observed in other countries and the improved sensitivity of the present method prompted us to a more careful inspection (Mulder et al., 2015; Caligiani  
195 et al., 2011). Organic foods were chosen as, on a theoretical basis, organic agriculture may be more prone to TA contamination due to a different weed  
196 management in crop fields that, if not properly conducted, may increase the risk of co-harvesting *Datura* and *Fagopyrum* seeds. The results of our  
197 survey involved 26 samples, 3 of which were positive for TA (Table 3). The most contaminated sample was a buckwheat flour, in which 83.9 µg/Kg of  
198 atropine and 10.4 µg/Kg of scopolamine were detected. Minor amounts of atropine were found in a pasta sample (21.3 µg/Kg), and in one bakery  
199 snack (13.9 µg/Kg). Scopolamine was found in buckwheat flour and pasta, together with high levels of atropine (10.4 µg/Kg and 5.7 µg/Kg,  
200 respectively).

201 The atropine:scopolamine ratio of 20:80 in positive samples and the comparison of the UHPLC-MS/MS profile of pure jimsonweed seeds (Figure X)  
202 are coherent with a potential *Datura stramonium* contamination (Steenkamp et al., 2004).

203 Despite the limited numbers, our data are in agreement, both in terms of overall incidence and of quantitative amounts, with those emerging from  
204 recent surveys conducted elsewhere in Europe, in which TA were present in approximately 20% of the samples with the highest occurrence level at 100  
205 µg/Kg for atropine and 47 µg/Kg for scopolamine (EFSA, 2008 ; Mulder et al., 2015). The contamination level recorded in sample BF2 was close to  
206 the limit set by French authorities in 2008, defining an intervention threshold at 100 µg/kg buckwheat flour for the sum of atropine and scopolamine  
207 (Afssa, 2008). On the contrary, values recorded for Italian samples are remarkably lower than those detected in Slovenia in 2013 by Peharic *et al.* and  
208 in 2007 and 2008 by AFSSA in France. In particular, on these occasions TA contamination concerned 40% of the samples and total TA exceeded 7400

209  $\mu\text{g}/\text{kg}$  in one french sample and 38000  $\mu\text{g}/\text{kg}$  in one sample from Hungary (Peharic et al., 2013; AFSSA 2008). Nevertheless, a 94.3  $\mu\text{g}/\text{kg}$  TA content  
210 cannot be considered negligible from a food safety standpoint and reinforces the need for a punctual monitoring of the buckwheat market and the  
211 enforcement of precise management of *Datura* weeds in buckwheat fields, both organically or conventionally grown. In fact, assuming mean thousand  
212 seed weight values for buckwheat and *Datura* seeds (<http://data.kew.org/sid/weight.html>) and a standardised alkaloid content in the latter, the addition  
213 of one *Datura* seed per million buckwheat seeds would result into 7.4 mg/kg of atropine and 1.8 mg/kg of scopolamine (Friedman & Levin, 1989), a  
214 value that translates to approximately 10 seeds per 200 kg in our most contaminated flour sample. As this ratio may be easily achievable by co-  
215 harvesting a single *Datura stramonium* plant in a buckwheat field, a careful scrutiny at field level must be encouraged to prevent the contamination at  
216 its source. For instance, a full-grown *Datura stramonium* plant may produce approximately between 1000 and 2000 seeds, whose presence in a  
217 buckwheat crop may lead to a critical contamination of approximately 20-40 tons of flour. Furthermore, TA are fairly stable during drying and heat  
218 treatment and may be present at 72–100% after baking bread from wheat flour contaminated with *Datura stramonium* (Friedman and Levin, 1989). As  
219 a consequence, a daily portion of bread (160 g) prepared with the most contaminated flour detected in this survey would result in an intake of  
220 approximately 17  $\mu\text{g}$  of TA. This amount is sufficient to exceed the NOAEL for atropine and the Acute Reference Dose (ArfD) recently established by  
221 CONTAM panel for atropine and scopolamine, according to the same elegant rationale suggested elsewhere (Mulder et al., 2015). A similar approach,  
222 however, cannot be applied to the contaminated pasta sample, for which the likely dilution and degradation of TA in boiling water would reduce  
223 considerably the actual intake.

224

#### 225 **4. Conclusions**

226 There are actually two major sources of human exposure to TA: accidental contamination of foodstuffs and the deliberate use of *Datura* plants in herbal  
227 preparations. The former falls in turn into two categories, one involving continuous low-level contamination due to reiterated ingestion and one  
228 involving occasional, acute intoxication due to the ingestion of massively contaminated foods. Both require the availability of sensitive and easy-to-  
229 perform analytical methods. The validated UHPLC-MS/MS ESI+ method presented herein proved to be both reliable and serviceable for screening

230 traces of tropane alkaloids in buckwheat not only as a raw material, but also as an ingredient in processed and baked foods. It can be thus applied as a  
231 screening tool for a large number of matrices along the buckwheat supply chain, to monitor the potential presence of these harmful contaminants. It  
232 must be noted that the low percentage of contaminated samples in the limited screening hereby provided is undoubtedly a favourable event, but the  
233 severity of potential intoxications and in particular the high level reported for one organic buckwheat flour sample suggest the adoption of precaution  
234 and underlines the focus put by EFSA on this topic. In particular, organic buckwheat samples in Italy may present a degree of atropine and scopolamine  
235 contamination comparable to the one recently described for various cereal-based products in Europe (Mulder et al., 2016). The availability of a method  
236 capable of spotting trace levels of tropane alkaloids also in baked products is relevant as these substances are known to survive bread-baking  
237 conditions. Given the limited number of producers, we cannot exclude that the positive samples detected in our survey may have been originated by a  
238 single in-field contamination, leading to suggest the pertinence of a closer control at the very beginning of the food chain.

239

240 **5.Conflict of interests.** Having read and understood the journal policy on declaration of interests, the authors declare no competing interests.

241

## 242 **6. References**

243

244 Adamse, P., van Egmond, H. P., Noordam, M. Y., Mulder, P. P. J., & de Nijs, M. (2014). Tropane alkaloids in food: poisoning incidents. *Quality*  
245 *Assurance and Safety of Crops & Foods*, 6, 15-24.

246 AFSSA (2008). Avis de l'Agence française de sécurité sanitaire des aliments relative à la présence d'alcaloïdes (atropine et scopolamine) en tant que  
247 substances indésirables dans la farine de sarrasin destinée à la consommation humaine et à la pertinence du seuil de gestion provisoire proposé par la  
248 DGCCRF. Saisine n° 2008-SA-0221. 11 pp.

249 Beuerle, T., Benford, D., Brimer, L., Cottrill, B., Doerge, D., Dusemund, B. & Mulder, P. P. J. (2013). Scientific Opinion on Tropane alkaloids in food  
250 and feed. *The EFSA Journal*, 11, 1-113.

- 251 Bond, W., & Grundy, A. C. (2001). Non-chemical weed management in organic farming systems. *Weed research*, *41*, 383-405.
- 252 Caligiani, A., Palla, G., Bonzanini, F., Bianchi, A., & Bruni, R. (2011). A validated GC–MS method for the detection of tropane alkaloids in buckwheat  
253 (*Fagopyron esculentum* L.) fruits, flours and commercial foods. *Food Chemistry*, *127*, 204-209.
- 254 Chang, S. S., Wu, M. L., Deng, J. F., Lee, C. C., Chin, T. F., & Liao, S. J. (1999). Poisoning by *Datura* leaves used as edible wild vegetables. *Veterinary  
255 and human toxicology*, *41*, 242-245.
- 256 Chen, H., Marín-Sáez, J., Romero-González, R., & Frenich, A. G. (2017). Simultaneous determination of atropine and scopolamine in buckwheat and  
257 related products using modified QuEChERS and liquid chromatography tandem mass spectrometry. *Food Chemistry*, *218*, 173-180.
- 258 Christen, P., Bieri, S., & Berkov, S. (2013). Methods of Analysis: Tropane Alkaloids from Plant Origin. In *Natural Products* (pp. 1009-1048). Springer  
259 Berlin Heidelberg.
- 260 EFSA (2008). Tropane alkaloids (from *Datura* sp.) as undesirable substances in animal feed- Scientific Opinion of the Panel on Contaminants in the  
261 Food Chain. *EFSA Journal*, *691*, 1-55.
- 262 European Commission (2015) Commission recommendation 2015/976 of 19 June 2015 on the monitoring of the presence of tropane alkaloids in food.  
263 *Official Journal of the European Union*, *157*, 97-98.
- 264 Fretz, R., Schmid, D., Girsch, L., Pichler, A. M., Riediger, K., Safer, M., Allerberger, F. (2007). Food poisoning due to Jimson weed mimicking  
265 *Bacillus cereus* food intoxication in Austria, 2006 *International Journal of Infectious Diseases*, *11*, 557-558.
- 266 Friedman, M.; Levin, C. E. (1989). Composition of jimson weed (*Datura stramonium*) seeds. *Journal of Agricultural and Food Chemistry*, *37*, 998-  
267 1005.
- 268 Giménez-Bastida, J. A., & Zieliński, H. (2015). Buckwheat as a Functional Food and Its Effects on Health. *Journal of Agricultural and Food  
269 Chemistry*, *63*, 7896-7913.

- 270 Griffin, W. J., & Lin, G. D. (2000). Chemotaxonomy and geographical distribution of tropane alkaloids. *Phytochemistry*, *53*, 623-637.
- 271 Jakabová, S., Vincze, L., Farkas, Á., Kilár, F., Boros, B., & Felinger, A. (2012). Determination of tropane alkaloids atropine and scopolamine by liquid  
272 chromatography–mass spectrometry in plant organs of *Datura* species. *Journal of Chromatography A*, *1232*, 295-301.
- 273 Kirchhoff, C., Bitar, Y., Ebel, S., & Holzgrabe, U. (2004). Analysis of atropine, its degradation products and related substances of natural origin by  
274 means of reversed-phase high-performance liquid chromatography. *Journal of Chromatography A*, *1046*, 115-120.
- 275 Kitano, R., Chatani, Y., Ogawa, T., & Takemae, M. (2003). Determination of tropane alkaloids in the boiled rice mixed with vegetables. *Kyoto-fu*  
276 *Hoken Kankyo Kenkyusho Nenpo* *47*, 52-55.
- 277 Koleva, I. I., van Beek, T. A., Soffers, A. E., Dusemund, B., & Rietjens, I. M. (2012). Alkaloids in the human food chain–natural occurrence and  
278 possible adverse effects. *Molecular Nutrition & Food Research*, *56*, 30-52.
- 279 Lawrence, O. R., Chandler, J. M.; Buchanan, G. A. (1994). Influence of geographic region on jimsonweed (*Datura stramonium*) interference on  
280 soybeans (*Glycine max*) and Cotton (*Gossypium hirsutum*). *Weed science*, *39*, 585-589.
- 281 Miraldi, E.; Masti, A.; Ferri, S., Barni Comparini, I. (2001). Distribution of hyoscyamine and scopolamine in *Datura stramonium*. *Fitoterapia*, *72*, 644-  
282 648.
- 283 Mol, H. G. J., Van Dam, R. C., Zomer, P., & Mulder, P. P. (2011). Screening of plant toxins in food, feed and botanicals using full-scan high-resolution  
284 (Orbitrap) mass spectrometry. *Food Additives & Contaminants: Part A*, *28*, 1405-1423.
- 285 Mulder, P. P., Pereboom-de Fauw, D. P., Hoogenboom, R. L., de Stoppelaar, J., & de Nijs, M. (2015). Tropane and ergot alkaloids in grain-based  
286 products for infants and young children in the Netherlands in 2011–2014. *Food Additives & Contaminants: Part B*, *8*, 284-290.
- 287 Mulder, P. P., von Holst, C., Nivarlet, N., & van Egmond, H. P. (2014). Intra-and inter-laboratory validation of a dipstick immunoassay for the  
288 detection of tropane alkaloids hyoscyamine and scopolamine in animal feed. *Food Additives & Contaminants: Part A*, *31*, 1165-1176.

289 Mulder, P. P., Nijs, M., Castellari, M., Hortos, M., MacDonald, S., Crews, C., Hajslova, J., Stransk, M. (2016). Occurrence of tropane alkaloids in  
290 food. *EFSA Supporting Publications*, 13, 1-200.

291 Perharič L, (2005). Mass tropane alkaloid poisoning due to buckwheat flour contamination. *Clinical Toxicology*, 43, 413.

292 Perharič, L., Koželj, G., Družina, B., & Stanovnik, L. (2013). Risk assessment of buckwheat flour contaminated by thorn-apple (*Datura stramonium*  
293 L.) alkaloids: a case study from Slovenia. *Food Additives & Contaminants: Part A*, 30, 321-330.

294 Rancic, D., & Spasic, A. (2009). Identification of atropine and scopolamine by HPLC in buckwheat flour contamination with *Datura stramonium*  
295 seeds. *Toxicology Letters*, 189, S234.

296 Shah, V.P., Midha, K.K., Findlay, J.W.A., Hill, H.M., Hules, J.D., McGilveray, I.J., Mckay, G., Miller, K.J., Patnaik, R.N., Powell, M.L., Tonelli, A.,  
297 Viswanathan, C.T. & Yacobi A. (2000) Bioanalytical method validation - A revisit with a decade of progress, *Pharmaceutical Research*, 17 (12), 1551-  
298 1557.

299 Shea, Y. F., Chow, T. Y. A., Chiu, P. K. C., Chan, C. K., Mak, T. W., & Chu, L. W. (2012). Delirium due to herbal tea contaminated with tropane  
300 alkaloid. *Journal of Clinical Gerontology and Geriatrics*, 3, 110-112.

301 Shimshoni, J. A., Duebecke, A., Mulder, P. J., Cuneah, O., & Barel, S. (2015). Pyrrolizidine and tropane alkaloids in teas and the herbal teas  
302 peppermint, rooibos and chamomile in the Israeli market. *Food Additives & Contaminants: Part A*, 1-10.

303 Steenkamp, P. A., Harding, N. M., Van Heerden, F. R., & Van Wyk, B. E. (2004). Fatal *Datura* poisoning: identification of atropine and scopolamine by  
304 high performance liquid chromatography/photodiode array/mass spectrometry. *Forensic Science International*, 145, 31-39.

305 Van Raamsdonk, L. W. D., Vancutsem, J., & Jorgensen J. S. (2009). A survey on the presence of undesirable botanical substances in feed in the  
306 European Union. *Base*, 13, 33-38.

307 Aehle, E., & Drager B. (2010). Tropane alkaloids analysis by chromatographic and electrophoretic techniques: an update. *Journal of Chromatography*

308 *B*, 878, 1391–1406.

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329 **Table 1: Commercial, organic food samples and their ingredients**

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	<b>Sample</b>	<b>Description</b>
<b>Buckwheat flour</b>	BF1	100% buckwheat
	BF2	100% buckwheat
	BF3	100% buckwheat
	BF4	100% buckwheat
	BF5	100% buckwheat
	BF6	100% buckwheat
	BF7	100% buckwheat
	BF8	100% buckwheat
	BF9	100% buckwheat
	BF10	10% buckwheat, 90% maize
	BF11	100% buckwheat
	BF12	100% buckwheat
<b>Buckwheat pasta</b>	BP1	30% buckwheat flour, 70% durum wheat
	BP2	25% buckwheat flour, 75% durum wheat
	BP3	100% buckwheat
	BP4	100% buckwheat
	BP5	100% buckwheat
	BP6	100% buckwheat
<b>Buckwheat bakery</b>	BB1	Crackers: 100% buckwheat
	BB2	Crackers: 100% buckwheat
	BB3	Crackers: 40% buckwheat, 40% rice
	BB4	Plumcakes: 20% buckwheat
	BB5	Crackers: 100% buckwheat
	BB6	Crackers: 100% buckwheat
	BB7	Crackers: 100% buckwheat
	BB8	Crackers: 100% buckwheat

331 **Table 2: Quality parameters of the method, calculated from blank samples spiked with alkaloid standards**

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	<b>Scopolamine</b>	<b>Atropine</b>
<b>Linearity range (µg/Kg)</b>	0.05 - 10	0.05 - 10
<b>RR% Flour</b>	78	83
<b>SSE% Flour</b>	119	126
<b>RR% Pasta</b>	99	103
<b>SSE% Pasta</b>	133	141
<b>RR% Bakery products</b>	102	88
<b>SSE% Bakery products</b>	75	77
<b>Slope</b>	135928 ± 2982	154054 ± 2810
<b>Intercept</b>	5810 ± 262	18119 ± 1220
<b>LOD (µg/Kg)</b>	0.03	0.09
<b>LOQ (µg/Kg)</b>	0.10	0.30
<b>Intraday Repeatability at 0.05 µg/Kg (RSD%)</b>	3.4	10.7
<b>Intraday Repeatability at 0.5 0.05 µg/Kg (RSD%)</b>	5.2	1.8
<b>Intraday Repeatability at 10 0.05 µg/Kg (RSD%)</b>	2.3	2.8
<b>Interday Repeatability at 0.05 µg/Kg (RSD%)</b>	2.4	10.5
<b>Interday Repeatability at 0.5 µg/Kg (RSD%)</b>	9.5	6.9
<b>Interday Repeatability at 10 µg/Kg (RSD%)</b>	1.8	7.1

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342 **Table 3: Optimization of the extraction method: recovery rates of blank samples spiked with alkaloid standards**

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Extraction Solvent	Scopolamin		Atropine	
	Recovery rate (%) Flour	Recovery rate (%) Pasta	Recovery rate (%) Flour	Recovery rate (%) Pasta
CH <sub>3</sub> OH: H <sub>2</sub> O (3:2)	45	82	61	86
CH <sub>3</sub> OH: H <sub>2</sub> O (3:2) + 0.2 % HCOOH	75	92	110	54
CH <sub>3</sub> CN: H <sub>2</sub> O (3:2)	69	81	87	92
CH <sub>3</sub> CN: H <sub>2</sub> O (3:2) + 0.2 % HCOOH	88	89	111	79
CH <sub>3</sub> OH: H <sub>2</sub> O (3:2) + 0.2 % HCOOH + 0.2% CH <sub>3</sub> CN	98	102	105	104

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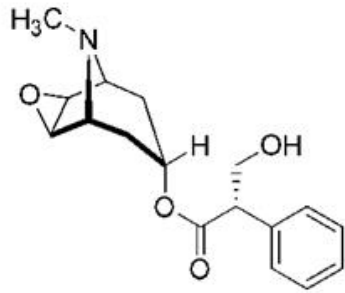
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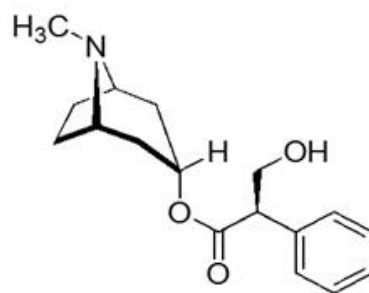
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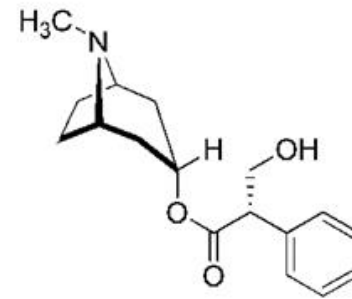
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**(-)- scopolamine**



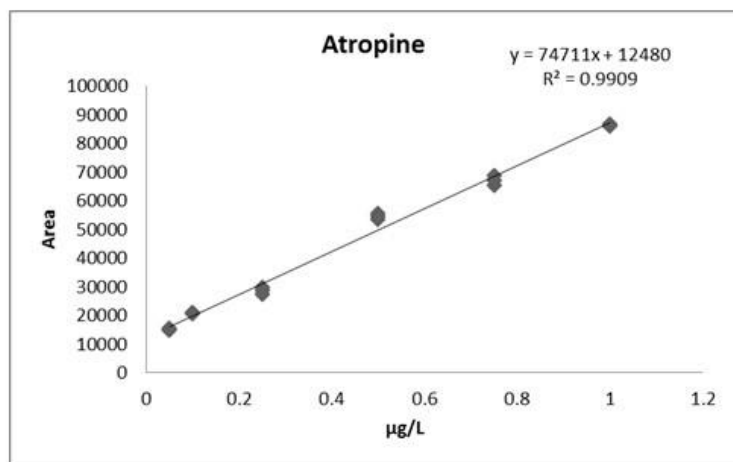
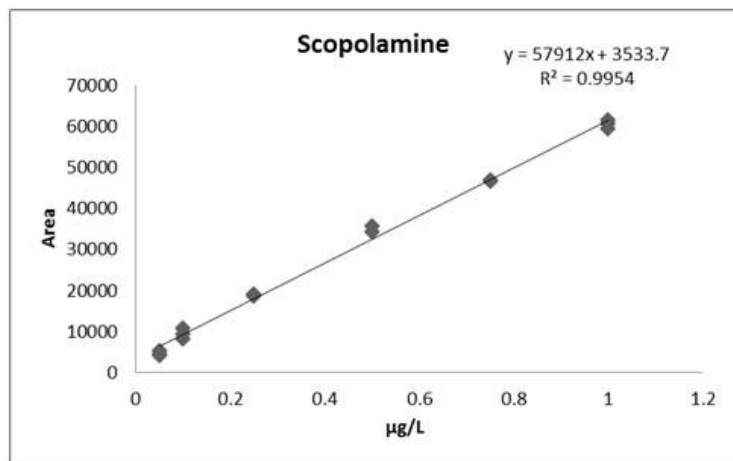
**(-) – Hyoscyamine**



**(+) – Hyoscyamine**



**atropine**



Calibration parameters	scopolamine	atropine
b	3533.7	12480
$S_b$	572.7	1004.5
a	57912	74711
$S_a$	1014.9	1790.9
$S_{y/x}$	1487.4	2626.7
df	15	16
$r^2$	0.9954	0.99089
F	3255.7	1740.3
SSreg	7202704342	1.2E+10
SSresid	33184841.6	1.1E+08

