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Atropine and scopolamine occurrence in spices and fennel infusions

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ABSTRACT

Tropane alkaloids (TAs) are toxic anticholinergic compounds that appear due to accidental contamination of foods with TA-producing plants. Atropine and scopolamine are the most common TAs and their maximum levels in certain foodstuffs have been recently regulated, including food supplements. TAs occurrence in commercial products and the transfer ratio to herbal infusions prepared from contaminated spices are of interest for exposure assessment of consumers. In this study, the occurrence of atropine and scopolamine was investigated in a total of 51 commercial spices of 6 types (ginger, cloves, fennel, cumin, aniseed, coriander) and a mix of spices and herbs (curry) purchased in Portugal (19 samples) and Spain (32 samples) in 2021. Results evidenced that more than half of the samples analysed (67%) were contaminated with one or both analytes. Fennel, cloves and coriander showed the highest concentrations ranging between 5-31 μg/kg, 8–28 μg/kg and 5–45 μg/kg, respectively, for the sum of atropine and scopolamine. On the other hand, the transfer ratio of the target analytes found in four naturally contaminated fennel samples was studied. Infusions were prepared according to International Standard ISO 3103 protocol, and a transfer ratio between 64 and 88% for atropine and between 47 and 57% for scopolamine was found.

1. Introduction

Tropane alkaloids (TAs) are secondary metabolites produced by various plants families (Solanaceae, Brassicaceae, etc). About 200 different TA types, have been identified although the predominant ones are (−)-hyoscyamine and (−)-scopolamine, which in contrast to the (+)-enantiomers are formed naturally by TA-producing plants and exhibit potent anticholinergic activity. However, the (−)-hyoscyamine is not stable and undergoes racemisation over time, so both enantiomers can be found specially in older plant organs, in varying ratio, but always in favour of the (−)-form (Eich, 2008). The racemic mixture of (±)-hyoscyamine (1:1) is known as atropine (European Commission, 2013). *Datura stramonium, Atropa belladonna* or *Mandragora officinarum* are the species most often associated with poisoning after consuming of foods contaminated by TAs. The interest in these compounds is high due to their toxicity and the numerous cases of accidental poisoning through the ingestion of different foods and plants (Abia et al., 2021; Balíková, 2002; Chan, 2017; Shea et al., 2012). In this sense, in 2013 the European Food Safety Authority (EFSA) established an acute reference dose (ARfD) of 0.016 μg/kg body weight expressed as the sum of (−)-hyoscyamine and (−)-scopolamine, assuming equivalent potency (European Commission, 2013). In addition, after some considerations and opinions of the EFSA (Mulder et al., 2016), new legislation has been recently published to regulate the content of TAs in different types of foods (European Union, 2021). In this regard, as for analytical reasons is not always possible to distinguish between the enantiomers of hyoscyamine and taking into account that the synthesis of TAs in plants leads to (−)-hyoscyamine and (−)-scopolamine and not to (+)-enantiomers, it is assumed that analytical results on atropine and scopolamine in food of plant origin reflects the occurrence of (−)-forms (European Union, 2021). The maximum levels, as the sum of atropine and scopolamine, range between 5-15 μg/kg for cereals and pseudo-cereals (sorghum, corn, buckwheat, millet and their derived products) and 25–50 μg/kg for dried herbs used for infusions, showing the lowest maximum content for liquid plant infusions (0.20 μg/kg). Many other products of plant origin are susceptible to being unintentional contaminated by TAs, such as spices, aromatic herbs and other plants used for culinary or medical purposes. The presence of Solanaceae species in the crops is the most widespread source of accidental cross-contamination during harvesting, since all the parts of the TA-containing plants are toxic, including roots,

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seeds, stems, flowers and leaves. Common cleaning practices are not always sufficient to remove the weed plant parts. For example, morphological examination of different herbal medicines that caused episodes of TAs poisoning, revealed the presence of rhizomes, seeds and other impurities (leaves) originating from Solanaceae plants (Chan, 2017). In some spices, seeds of *D. stramonium* could be a cause of the contamination, due to their similar size and color (González-Gómez et al., 2022b). However, contamination of these products may also arise from deliberate adulteration or other malevolent causes. In that respect, general considerations of the spices and herbs supply chain with hypothetical points of contamination have been revised by Székács et al. (2018). In addition, Maquet et al. (2021) have recently reported a control plan coordinated with the European Union to check the authenticity of spices and herbs, showing that most of the suspicious samples contained undeclared plant material.

Spices, along with aromatic herbs, are condiments widely used in the food industry gastronomy with increasing popularity. They are added to a great variety of foods and dishes, due to interest in new tastes and the tendency to use natural ingredients rather than chemical additives. But also, some of these products (i.e., fennel, coriander, aniseed, etc.) are used to prepare herbal infusions and, at higher doses (usually between 150 and 400 mg per tablet or capsule) as dietary ingredients in many food supplements (Saldanha et al., 2016). Spices are excellent sources of bioactive compounds with antioxidant, antimicrobial, anti-inflammatory, etc. properties, among others, providing beneficial therapeutic effects (Rathore et al., 2013). They are also known to enhance digestion through the stimulation of digestive enzymes and secretion of bile, thus aiding the digestion and absorption of dietary fats (Otunola, 2022). For these reasons, the food safety of spices and herbs became an issue of high priority to protect consumers' health. In this sense, spices and aromatic herbs, have been a highly controlled food due to their vulnerability to different chemical contaminants that appear intentionally or unintentionally (Banach et al., 2016; Rozentale et al., 2018). To date, chemical compounds such as polycyclic aromatic hydrocarbons (Rozentale et al., 2018), pesticides (Goon et al., 2018), heavy metals (Aberie et al., 2021) and dyes (Moreno-González et al., 2020) have been analysed in this type of foods. Mycotoxins (El Darra et al., 2019; Potortì et al., 2020) and plant toxins, for example, alkenylbenzenes (Rivera-Pérez et al., 2021) and pyrrolizidine alkaloids (Izcara et al., 2022; Picron et al., 2018) have also received increasing attention in the last years. However, further research is needed about the presence of other plant toxins such as TAs due to their toxicological characteristics. In that respect, in a previous study by our research group (González-Gómez et al., 2022a), the presence of these compounds was verified in some aromatic herbs (coriander, basil and thyme leaves) and few studies have focused on the analysis of TAs in spices. Only a study analysed TAs in coriander and fennel seeds, finding an average of 35 μg/kg of atropine for coriander and 6.1 μg/kg of atropine for fennel (Arcella et al., 2018). On the other hand, the exposure of TAs through infusions can be greater than through their consumption as condiments. For example, Mulder et al. (2016) showed that around 50% of TAs present in dry tea were transferred to the tea infusion when a standard protocol for tea preparation was applied and recommended to study the transfer in other types of herbal infusions. Marín-Sáez et al. (2019) studied TAs degradation and migration to water during tea preparation (100 °C, 5 min). In teas contaminated with *D. stramonium* and *B. arborea,* an amount varying from 20-30% and 40–60% of the initial amount of atropine and scopolamine, was respectively found in water. For this reason, more data focusing on the transfer ratio of TAs during infusion preparation are needed.

In our research group, methods for TAs analysis in cereals, aromatic herbs, and infusions by high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS) detection have been developed and validated (González-Gómez et al., 2020; González-Gómez et al., 2022a,c). Considering these previous works, a sample preparation protocol based on solid-liquid extraction and solid-phase extraction (SLE-SPE) has been optimized for detection of atropine and scopolamine in spices. Therefore, the aim of this work was to provide useful information about the occurrence of the target TAs in 51 samples of commercially available spices that were purchased in Portugal and Spain. Additionally, herbal infusions prepared from contaminated fennel samples were analysed to estimate the transfer ratio of atropine and scopolamine, providing relevant information for risk assessment of TAs in these types of products.

2. Materials and methods

2.1. Reagents and materials

Hydrochloric acid (purity 36.5–38%) was obtained from Scharlab (Barcelona, Spain). Formic acid (purity ≥99% Optima™) LC-MS grade was purchased from Fisher Chemical (Madrid, Spain). Solvents of LC-MS grade used in this work, acetonitrile (ACN) and methanol (MeOH) were obtained from Scharlab.

Materials required for solid phase extraction such as polyethylene frits (20 μm), nylon filter membranes (0.45 μm), empty syringes (3 mL), nylon syringes filters (0.45 μm, 25 mm and 13 mm) and Scharlab ExtraVac® solid extraction vacuum manifold 12 port were obtained from Scharlab. Waters OASIS® MCX 150 mg/g 6 cc (30 μm) commercial cartridges used for comparative purposes were purchased from Scharlab. The DigiVOL® digital syringe using a 500 μL μSPEed® syringe and PS/DVB (3μm/300 Å) commercial μSPEed® cartridge used for the analysis of infusions were acquired to EPREP (Mulgrave, Victoria, Australia).

2.2. Preparation of standard solutions

Atropine sulfate (purity ≥99%; CAS 51-55-8) and scopolamine hydrobromide (purity ≥98%; CAS 6533-68-2) were purchased from Sigma-Aldrich. Stock standard solutions (1000 mg/L) were prepared by diluting 10 mg of each analyte in MeOH. The resulting solutions of atropine and scopolamine were stored at −20 °C in the dark. The working solutions containing both TAs were prepared by dilution from the concentrated solutions (1000 mg/L) dissolved in ACN/H_2O (50:50, v/v) and stored at −20 °C in the dark. Ultra-pure water (resistance 18.2 MΩ cm) was obtained from a Millipore Milli-Q-System (Billerica, MA, USA).

2.3. Samples

For this study, a total of 51 commercial samples (see Table S1 in Supplementary Material for details) were collected for the analysis of atropine and scopolamine: 9 samples of ginger (*Zingiber officinale*), 5 samples of cloves (*Syzygium aromaticum*), 9 samples of fennel (*Foeniculum vulgare*), 7 samples of cumin (*Cuminum cyminum*), 10 samples of aniseed (*Pimpinella anisum*), 6 samples of coriander (*Coriandrum sativum)* and 5 samples of curry (spices mix). Samples were purchased from local markets in Spain (Madrid) and Portugal (Madeira Island and Algarve). The format of ginger, cumin and curry samples was ground. Cloves, fennel, aniseed, and coriander samples were purchased in whole form, except one sample of each spice that was acquired in powder form (see Table S1). Whole spices (26 samples of 51) were ground and homogenized before being analysed. Finally, the samples were codified with the three initial letters of each spice and numbered according to the commercial sample. All samples were stored at room temperature in the dark until their analysis. Each sample was analysed in triplicate.

2.4. Infusion preparation

Infusions of fennel were prepared according to International Standard ISO 3103 protocol (ISO, 2019). 2 g of ground fennel were weighed into disposable tea bags. The tea bags were placed in a porcelain cup and

100 mL of boiling ultra-pure deionized water was added. The cup was covered for 5 min. After this time, the infusion was cooled at room temperature and an aliquot was filtered through a nylon filter (0.45 μm) before purification.

2.5. Analysis of tropane alkaloids

2.5.1. Sample preparation

Sample preparation for the analysis of spices was carried out according to two previously validated methods (González-Gómez et al., 2020, 2022a) with slight modifications. For this, 0.250 ± 0.001 g of the sample was treated with 8 mL of an aqueous solution of HCl (pH 1), before being purified by SPE using a mesostructured silica-based material as sorbent. Sometimes some supernatants appear cloudy depending on the sample matrix. For this reason, the fennel, cumin, aniseed, and coriander samples were frozen for 15 min at −20 °C. After this time, the frozen supernatant was centrifuged again for 10 min and the obtained supernatant was filtered with a 0.45 μm (25 mm) nylon filter. To carry out purification by the SPE, two silica-based materials with sulfonic groups (SBA-15-SO_3^- and HMS-SO_3^-) were synthesized following previous works (González-Gómez et al., 2020, 2022a) and evaluated as strong cationic exchange sorbents. After the optimization studies, 150 mg of SBA-15-SO_3^- were used as sorbent, following the protocol described by González-Gómez et al. (2020). The eluate was evaporated and reconstituted in 350 μL of ACN/H_2O (50/50, v/v) before HPLC-MS/MS analysis. For comparative purposes, the protocol established in the EFSA Supporting publication 2016: EN-1140 (Mulder et al., 2016) was tested in a fennel sample. For this, 4 g of the sample were treated with 40 mL of MeOH/water/formic acid (75/25/0.4, v/v/v), before being purified by SPE using OASIS MCX commercial cartridges (see S1 in Supplementary Material for details of the protocol).

On the other hand, TAs analysis in the fennel infusions was carried out using the validated method of González-Gómez et al. (2022c). For this, a microextraction protocol using the μ-SPEed® technique was applied with a polymeric-based cartridge (PS/DVB), followed by HPLC-MS/MS analysis. Method detection (MDL) and quantification (MQL) limits achieved were: 0.03 and 0.04 ng/mL for atropine, and 0.09 and 0.12 ng/mL for scopolamine, respectively. Recoveries (low level: 5 μg/kg) were 108 ± 3 % and 105 ± 1 % for atropine and scopolamine, respectively.

2.5.2. Identification and quantification by HPLC-MS/MS

The analysis was carried out on a Varian 1200L triple quadrupole coupled with a Varian Prostar HPLC (Varian Ibérica, Spain). The HPLC consisted of an autosampler equipped with a 100 μL loop (ProStar 410), two solvent deliver modules (ProStar 210/215) and a thermostatted compartment for the chromatographic column. The chromatographic separation was conducted with a reverse phase column C18 Kromaphase 100 column (150 mm × 2.0 mm, 3.5 μm particle size) with a C18 Kromaphase guard column (10 mm × 4.0 mm I.D., 5 μm particle size) at 30 °C acquired from Scharlab (Barcelona, Spain). The separation was performed in gradient mode with a flow rate of 0.25 mL/min. The injection volume was 10 μL. The mobile phases consisted of solvent A (ACN) and solvent B (Milli-Q water), containing both 0.1% formic acid. The following gradient was used after equilibrating the column for 2 min at 90% B: eluent A increased linearly to 70% in 10 min, returning in 1 min to the initial conditions. These conditions were maintained for 4 min. The total run-time was 15 min. For mass spectrometry acquisition, an electrospray ionization interface (ESI) was used in positive ion mode. Data acquisition in the mass spectrometry detector was carried out with a Workstation version 6.3 system. Argon was used as collision gas and nitrogen as drying gas and nebulizer gas. The conditions were as follows: N_2 drying gas (350 °C, 22 psi), nebulizer gas pressure (58 psi), capillary voltage (5000 V) and shield (600 V). Argon was set at 1.90 mTorr and detector voltage at 1480 V. For detection of atropine and scopolamine, a multiple reaction monitoring (MRM) mode was used (mass peak width Q1 2.5; mass peak width Q3 2.5; scan width in MRM 0.70 s) and the analytes were monitored at cone voltage of 70 V with the following transitions: 290 → 90.9 (CE = 34 V), 290 → 93.0 (CE = 29 V) and 290 → 124.0 (CE = 20.5 V) for atropine; 304 → 121.0 (CE = 16 V), 304 → 138.0 (CE = 12 V) and 304 → 156.0 (CE = 9.5 V) for scopolamine.

2.5.3. Method validation

The proposed methodology was validated in terms of linearity, matrix effect (ME), method detection limit (MDL) and quantification limit (MQL), selectivity, accuracy, and precision. Matrix-matched calibration curves were prepared for each spice type in order to assess linearity. For this, a blank sample (not contaminated) was selected, and the protocol for extraction and purification was applied. The extract obtained was spiked with the corresponding aliquot of the standard solution of atropine and scopolamine. Six concentration levels were evaluated, and the samples were spiked in known increasing concentrations within the evaluated linear range: 0.004–0.143 μg/mL for atropine and 0.004 (ginger)/0.007–0.143 μg/mL for scopolamine. In case of not finding a blank sample for some spice (e.g. fennel, coriander), the signal of the unspiked sample used for the matrix-matched calibration was subtracted in each point of the curve. Once the different matrix-matched calibration curves were prepared, the linear regression analysis was applied, and the linearity was expressed as a coefficient of determination denoted R^2. To verify ME, a solvent-based calibration curve was prepared in concentrations from 0.005 to 0.2 μg/mL in ACN/H_2O. The ME was calculated as follows: ((slope matrix-matched calibration/slope solvent-based – 1) x 100). Positive values obtained after the application of this formula meant an increase in the signal and negative values mean suppression of the signal. The ME is normally considered important if it exceeds ± 20% (European Union, 2019). The sensitivity of the method was calculated from the MDL and MQL as three and ten times, respectively, the signal/noise (S/N) of the response obtained in HPLC-MS/MS at the lowest concentration of the matrix-matched calibration curve. The selectivity of the method was checked at the characteristic retention time (t_R) for atropine and scopolamine with an uncontaminated sample, a contaminated sample and a spiked sample at the lowest calibrated level. Also, the ion transition ratios in unit mass resolution were verified. The accuracy of the method was evaluated by adding the standard solution of atropine and scopolamine to the spice samples to reach a final concentration of 100 μg/kg (high level) and 5 μg/kg (low level). Subsequently, the average recovery (%) and the standard deviation (SD) were determined. As to date the maximum level of TAs in these types of samples is not yet regulated, the lowest value for solid samples (as the sum of atropine and scopolamine) according to the Commission Regulation (EU) 2021/1408 (European Union, 2021) was taken as the low level (5 μg/kg). Precision was evaluated as intra- and inter-day precision at two concentration levels (5 and 100 μg/kg) and expressed as relative standard deviation (RSD %). Intra-day precision was carried out by analyzing six replicates (n = 6) in one day, spiked at three concentration levels. The inter-day precision was estimated by analyzing three different replicates on three different days spiked at three concentration levels (n = 9). In all cases, the extracts were injected into HPLC-MS/MS in triplicate.

3. Results and discussion

3.1. Optimization of the sample treatment for the analysis of spices

With the aim to select the more appropriate sample treatment protocol, two methods previously validated in our group, applied in gluten-free grains and flours (González-Gómez et al., 2020) and aromatic herbs (González-Gómez et al., 2022a), were evaluated for the spices under study. To carry out the experiments, 0.25 g of ginger, cloves, fennel, cumin, aniseed, coriander and curry samples were used. To calculate the recovery (%), two samples of each spice were spiked at the beginning of the process (pre-extraction spike) to a final concentration of 100 μg/kg

(higher level of the validation). In parallel, another non-doped sample was spiked with the same amount of the target analytes at the end of the process (post-extraction spike). Prior to SPE, samples were subject to a common SLE step (see section 2.5.1) and sample extracts were passed through SPE cartridges packed with SBA-15-SO_3^- (150 mg) or HMS-SO_3^- (75 and 150 mg) as indicated in section 2.5.1. The results showed, in general, recovery percentages between 74 and 101% for atropine and scopolamine using 150 mg of SBA-15-SO_3^- as a sorbent (Fig. 1). On the other hand, with 75 and 150 mg of HMS-SO_3^- as a sorbent, worse recoveries were found in both cases, especially for scopolamine (Fig. 1B) in most of the samples in comparison with the SBA-15-SO_3^- sorbent. For cumin and aniseed samples, recovery percentages were lower than 70% with both materials. This fact was attributed to a higher matrix complexity of these spices (Apiaceae family) since the extracts obtained after the SLE showed greater turbidity in comparison with the other samples. On the contrary, fennel (which also belongs to this family), showed good recoveries as its frozen extract showed a less cloudy appearance after centrifugation. Considering the results of these preliminary studies, 150 mg of SBA-15-SO_3^- was selected as the best sorbent. To check the effect of the purification step, a fennel sample spiked with 25 μg/kg of TAs was subject to SLE and injected into the HPLC-MS/MS without clean-up. A significant signal reduction for both analytes in the chromatograms of the non-cleaned-up extracts was observed (Fig. S1), which evidenced the need for the SPE clean-up stage.

Finally, for comparative purposes, the protocol established in the EFSA Supporting publication 2016: EN-1140 (Mulder et al., 2016) using OASIS® MCX commercial cartridges (150 mg) for SPE was tested in a fennel sample because this sample was studied by Mulder et al. (2016) (see section 2.5.1 and Supplementary Material for details). For this experiment, two samples were spiked at the beginning of the process at two concentration levels, 10 and 100 μg/kg (pre-extraction spike). The lower concentration was selected according to the study of Mulder et al. (Mulder et al., 2016). The results obtained (Fig. 2) demonstrate that at high concentration level (100 μg/kg of TAs) the OASIS® MCX commercial cartridge is not able to achieve a good recovery for scopolamine (<60%). In addition, worse recovery was found for atropine (72 ± 3%) in comparison with the SBA-15-SO_3^- sorbent (86 ± 5%).

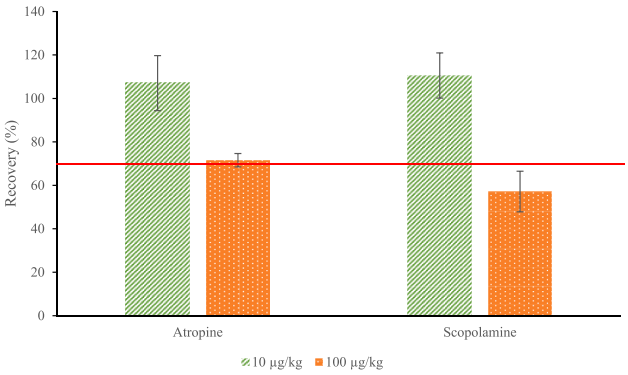

Fig. 2. Recovery percentages (% ± SD) at two concentration levels (10 μg/kg and 100 μg/kg) applying the protocol established by Mulder et al. (2016) with OASIS® MCX cartridges in fennel samples.

3.2. Method performance

As it can be seen in Table 1, good linear regression was obtained for both analytes (being $R^2 \geq 0.992$). Regarding the ME, Table 1 shows that all samples have a significant suppression of the signal for scopolamine, with exception of the coriander sample. The cloves sample showed the greatest suppression of the signal for atropine (−49%) and for scopolamine (−63%). In the case of coriander, fennel and curry samples, no significant ME was found for atropine. Therefore, to quantify the target analytes in these samples, matrix-matched calibration curves had to be used to compensate for the errors associated with these matrix effects.

For atropine, MDL was between 0.6 and 1 μg/kg and MQL between 1.9 and 3.3 μg/kg and higher limits were found for scopolamine, MDL between 1.5 and 2.8 μg/kg and MQL between 5.0 and 9.4 μg/kg (Table 1).

The selectivity of the method was also evaluated. For this, the t_R of atropine and scopolamine found in the contaminated samples showed a SD lower than ±0.1 min compared to the standard solutions and spiked extracts in the matrix-matched calibration curves. In the uncontaminated samples, no interfering peak was found at the t_R corresponding to atropine and scopolamine demonstrating the selectivity of the method. Besides, ion transition ratios in unit mass resolution MS/MS were checked in contaminated samples and compared to the spiked samples. The deviation was less than 30% (relative abundance) so the selectivity of the method is considered adequate.

Finally, accuracy and precision were evaluated. The samples of ginger, cloves, fennel, coriander and curry showed good recovery percentages at the two levels from 74 to 104% for atropine and from 76 to 101% for scopolamine, but for cumin and aniseed, the percentages were slightly lower at the two levels evaluated from 56 to 62% for atropine and from 64 to 71% for scopolamine. In general, no significant differences were found in the recovery percentages at the two evaluated levels, so the method can be successfully applied to samples with high and low contamination degree. The values obtained for precision are shown in Table S2. For intra-day precision, RSD (%) was ≤13% and for inter-day precision, RSD (%) was ≤16% for both analytes complying with the values recommended by the validation guides (RSD ≤20%) (European Union, 2019).

3.3. Sample analysis

For the determination of atropine and scopolamine, 51 samples of 6

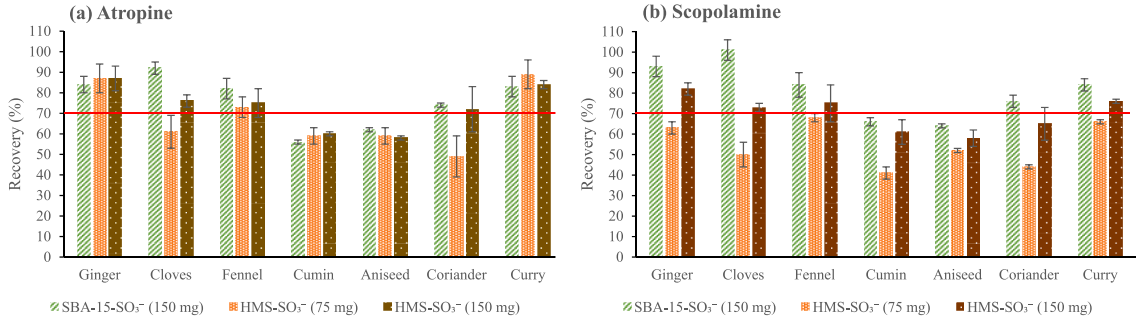

Fig. 1. Recovery percentages (% ± SD) of (a) atropine and (b) scopolamine for the studied spices spiked at 100 μg/kg using SBA-15-SO_3^- (150 mg) and HMS-SO_3^- (75 mg and 150 mg) as sorbents for solid-phase extraction.

Table 1
Results of in-house validation for the atropine and scopolamine analysis in spices.

	Atropine						Scopolamine					
Sample	Recovery High [a] Low [b] (% ± SD)	Linear Range μg/mL	Matrix matched calibration (R^2)	MDL[c] (μg/kg)	MQL[d] (μg/kg)	ME[e] (%)	Recovery High [a] Low [b] (% ± SD)	Linearity μg/mL	Matrix matched calibration (R^2)	MDL[c] (μg/kg)	MQL[d] (μg/kg)	ME[e] (%)
Gin	86 ± 2 [a] 94 ± 2 [b]	0.004–0.143	$8.6{\cdot}10^8$ x + $4.9{\cdot}10^6$ (0.999)	0.8	2.5	−34	93 ± 5 [a] 92 ± 5 [b]	0.004–0.143	$2.9{\cdot}10^8$ x + $1.4{\cdot}10^6$ (0.996)	1.5	5.0	−57
Clo	92 ± 3 [a] 88 ± 1 [b]	0.004–0.143	$6.6{\cdot}10^8$x + $1.5{\cdot}10^6$ (0.995)	0.7	2.5	−49	101 ± 5 [a] 99 ± 5 [b]	0.007–0.143	$2.4{\cdot}10^8$x +$1.1{\cdot}10^6$ (0.997)	2.2	7.2	−63
Fen	86 ± 5 [a] 88 ± 8 [b]	0.004–0.143	$1.1{\cdot}10^9$x - $2.4{\cdot}10^5$ (0.999)	0.9	2.8	−15	84 ± 6 [a] 90 ± 8 [b]	0.007–0.143	$4.4{\cdot}10^8$x −$2.8{\cdot}10^6$ (0.993)	1.8	6.1	−33
Cum	56 ± 1 [a] 62 ± 9 [b]	0.004–0.143	$1.0{\cdot}10^9$x + $4.9{\cdot}10^6$ (1.000)	0.8	2.6	−22	66 ± 2 [a] 71 ± 1 [b]	0.007–0.143	$3.9{\cdot}10^8$x + $2.1{\cdot}10^5$ (0.999)	1.9	6.3	−41
Ani	62 ± 1 [a] 60 ± 3 [b]	0.004–0.143	$9.3{\cdot}10^8$x + $1.7{\cdot}10^6$ (0.998)	0.6	1.9	−29	64 ± 4 [a] 74 ± 7 [b]	0.007–0.143	$3.4{\cdot}10^8$x + $1.2{\cdot}10^4$ (0.999)	2.2	7.2	−48
Cor	74 ± 1 [a] 96 ± 2 [b]	0.004–0.143	$1.3{\cdot}10^9$x - $2.4{\cdot}10^6$ (0.994)	1.0	3.3	2	76 ± 3 [a] 78 ± 1 [b]	0.007–0.143	$5.2{\cdot}10^8$x −$3.2{\cdot}10^6$ (0.992)	2.3	7.6	−22
Cur	83 ± 5 [a] 104 ± 4 [b]	0.004–0.143	$1.0{\cdot}10^9$x - $1.2{\cdot}10^6$ (0.998)	0.8	2.8	−20	84 ± 3 [a] 91 ± 5 [b]	0.007–0.143	$4.2{\cdot}10^8$x −$6.4{\cdot}10^5$ (0.999)	2.8	9.4	−37

[a] Recovery percentages (% ± SD) for atropine and scopolamine at high level (100 μg/kg).
[b] Recovery percentages (% ± SD) for atropine and scopolamine at low level (5 μg/kg).
[c] MDL: Method detection limit.
[d] MQL: Method quantification limit.
[e] ME: Matrix effect. To estimated ME, solvent-based calibration curves between 0.005 and 0.2 μg/mL were prepared (Atropine: $y = 1.3{\cdot}10^9x + 1.4{\cdot}10^7$; Scopolamine: $y = 6.6{\cdot}10^8x + 6.2{\cdot}10^6$). ME = ((slope matrix-matched calibration/slope solvent-based) − 1) × 100).

different spices (ginger, cloves, fennel, cumin, aniseed, coriander) and a mix of spices and herbs (curry) were analysed in triplicate. For quantification, the obtained area in the contaminated samples was interpolated in its respective matrix-matched calibration curve, and the concentration was corrected with the recovery percentages shown in Table 1.

As it can be seen in Table 2, 67% of the commercial spices under study showed contamination with one or both analytes. 34 of the 51 samples analysed were contaminated with atropine (≥MDL). In the case of scopolamine, only 9 samples presented this analyte. Of the 34 samples contaminated, 13 samples were purchased in ground format (that means 52% of the total ground samples) and 21 samples were acquired as whole spices (81% of the total whole samples). The spices with the highest levels of contamination were fennel (9 of 9 samples), coriander (6 of 6 samples) and cloves (3 of 5 samples) showing concentrations of up to 31 μg/kg, 45 μg/kg and 28 μg/kg, respectively, for the sum of atropine and scopolamine. These samples were purchased mainly in whole format. In these spices, a visual inspection of the product allowed to detect visible impurities, for example in Cor-1 (see Fig. S2). The rest of the coriander samples and some fennel samples such as Fen-3 and Fen-4 showed leaves or branches belonging to other types of plants. The spice origin was declared in only 15 of the 34 contaminated samples, and 47% of them were from Spain (1 ginger, 1 fennel, 1 cumin, 1 aniseed and 3 coriander). In cumin (7 of 7 samples) and aniseed (6 of 10 samples) samples, only atropine was found. None of the samples of curry presented TAs and in ginger only 3 samples of 9 analysed had atropine, but at a concentration lower than the MQL (2.5 μg/kg). In seven samples (1 clove, 2 fennel and 4 coriander) the sum of atropine and scopolamine was greater than 25 μg/kg (maximum level established by regulation (EU) 2021/1408 in dried products for herbal infusions) (Table 2). A sample of coriander (Cor-1) showed up to 45 μg/kg of the sum of atropine and scopolamine. For anise seeds, the level established by the legislation is 50 μg/kg, but only in three samples was it possible to quantify atropine with concentrations of 7 μg/kg (Ani-4), 12 μg/kg (Ani-5) and 4 μg/kg (Ani-10). In general, the At/Sc ratio observed in the analysed samples was higher than 1, except for three samples of fennel and five samples of coriander with a ratio between 0.7 and 0.3. All of these different ratios could be compatible with Datura, Hyoscyamus or Atropa sp. contamination, among others, as the amount of TAs is influenced not only by the plant species, but also by the part of the plant, the plant maturity and its geographical location (Adamse et al., 2014). For example, whereas D. stramonium seeds from Italy had 14 times more atropine than scopolamine, in seeds from Poland the content of atropine was between 10 and 30% lower (Abia et al., 2021). This fact shows that it is difficult to identify the possible source of contamination by TA-producing plants and evidences the importance of monitoring commercially available foods, as occurrence data are essential to assess any potential risk of human toxicity. At this point, it deserves to be mentioned that some recent works (González-Gómez et al., 2022d) have suggested that the presence of this toxins in foods of plant origin can be a consequence of the horizontal and natural transference of TAs through the soil. There are different ways in which transfer from the soil can take place, for example by living plants growing around crops or by dead plants used for composting. Also, rainwater can favour horizontal transfer because TAs are water-soluble compounds. Moreover, a similar phenomenon has been previously proposed to explain the presence of toxic pyrrolizidine alkaloids in some aromatic herbs (Izcara et al., 2022; Nowak et al., 2016). Although recently Chmit et al. (2021) in his review article concluded that horizontal transfer is not a cause for concern. More studies in this regard in TAs are necessary to clarify this issue.

There are few published data about the occurrence of atropine and scopolamine in spices. In coriander and fennel seeds, Arcella et al. (2018) found similar concentrations of atropine to that of our study (35 and 6.1 μg/kg, respectively). In fennel, Mulder et al. (2016) found atropine between 0.12 and 101.6 μg/kg and scopolamine up to 43.5 μg/kg. On the other hand, the European Rapid Alert System for Food and Feed (RASFF Portal) portal notified in 2018 an alert of whole cumin seeds from Hungary with 16178 μg/kg of atropine and 4658 μg/kg of scopolamine (RASFF Portal).

Table 2
Atropine and scopolamine content (μg/kg) in the 51 spice samples analysed.

Sample	Sample Code	Format	Atropine (μg/kg ± SD)	Scopolamine (μg/kg ± SD)	Total content (μg/kg)
Ginger	Gin-1	Ground	≤MQL	ND	≤MQL
	Gin-2	Ground	ND	ND	ND
	Gin-3	Ground	ND	ND	ND
	Gin-4	Ground	ND	ND	ND
	Gin-5	Ground	≤MQL	ND	≤MQL
	Gin-6	Ground	ND	ND	ND
	Gin-7	Ground	ND	ND	ND
	Gin-8	Ground	≤MQL	ND	≤MQL
	Gin-9	Ground	ND	ND	ND
Cloves	Clo-1	Ground	ND	ND	ND
	Clo-2	Whole	ND	ND	ND
	Clo-3	Whole	17 ± 2	11 ± 4	28
	Clo-4	Whole	8 ± 1	ND	8
	Clo-5	Whole	13 ± 1	ND	13
Fennel	Fen-1	Ground	19 ± 6	ND	19
	Fen-2	Seeds	11 ± 1	19 ± 1	30
	Fen-3	Seeds	10 ± 1	21 ± 3	31
	Fen-4	Seeds	8 ± 1	17 ± 6	25
	Fen-5	Seeds	6 ± 3	ND	6
	Fen-6	Seeds	5 ± 1	ND	5
	Fen-7	Seeds	5 ± 1	ND	5
	Fen-8	Seeds	7 ± 2	ND	7
	Fen-9	Seeds	8 ± 4	ND	8
Cumin	Cum-1	Ground	8 ± 2	ND	8
	Cum-2	Ground	≤MQL	ND	≤MQL
	Cum-3	Ground	≤MQL	ND	≤MQL
	Cum-4	Ground	3 ± 1	ND	3
	Cum-5	Ground	10 ± 2	ND	10
	Cum-6	Ground	3 ± 1	ND	3
	Cum-7	Ground	3 ± 1	ND	3
Aniseed	Ani-1	Seeds	≤MQL	ND	≤MQL
	Ani-2	Seeds	ND	ND	ND
	Ani-3	Seeds	ND	ND	ND
	Ani-4	Seeds	7 ± 1	ND	7
	Ani-5	Seeds	12 ± 3	ND	12
	Ani-6	Seeds	ND	ND	ND
	Ani-7	Seeds	ND	ND	ND
	Ani-8	Seeds	<MQL	ND	≤MQL
	Ani-9	Ground	<MQL	ND	≤MQL
	Ani-10	Seeds	4 ± 2	ND	4
Coriander	Cor-1	Seeds	19 ± 5	26 ± 6	45
	Cor-2	Seeds	8 ± 1	21 ± 2	29
	Cor-3	Seeds	9 ± 2	23 ± 5	32
	Cor-4	Seeds	5 ± 1	16 ± 1	21
	Cor-5	Ground	6 ± 2	20 ± 4	26
	Cor-6	Seeds	5 ± 1	ND	5
Curry	Cur-1	Ground	ND	ND	ND
	Cur-2	Ground	ND	ND	ND
	Cur-3	Ground	ND	ND	ND
	Cur-4	Ground	ND	ND	ND
	Cur-5	Ground	ND	ND	ND

*The results obtained correspond to the analysis of three replicates (n = 3) of each sample. ≤MQL: below or equal to the limit of quantification of the method; ND: Not detected.

3.4. Transfer ratio of atropine and scopolamine during infusion preparation

Although TAs intake from spices can be small, since spices are generally used as condiments at low amounts, a more frequent probability of intoxication can be derived from their use to prepare herbal infusions. This is due to their severe acute toxic effects and can be of concern for high-level infusion consumers and children. Therefore, TAs-content in infusions should also be determined to evaluate the transfer ratio and to assess realistic consumer exposure. In this sense, Shimshoni et al. (2015) found in a fennel infusion a high level of atropine (83 μg/kg) and scopolamine (11 μg/kg) that justifies the evaluation of TAs transfer in this type of sample.

To evaluate the transfer ratio of TAs from the dry seed to the infusion, infusions of different naturally contaminated fennel samples were analysed by the method previously validated by González-Gómez et al. (2022c). Fen-1 sample contaminated with atropine and Fen-2, Fen-3 and Fen-4 samples contaminated with both analytes were selected for this study. This spice was selected because it was the most contaminated. In addition, fennel is commercialized in tea bags and is widely used by consumers to aid digestion and to treat digestive disorders. Three infusions of each sample were prepared according to section 2.4 and then the infusions were purified as indicated in section 2.5.1. The standard addition method was used for TAs quantification in the infusions (Table S3). Table 3 shows the concentration (μg/L) of TAs found in infusion. As it can be seen, taking into account the concentration of TAs in the dry seeds (Table 2) and the amount found in the prepared infusions, a transfer between 64 and 88% of atropine was found. For scopolamine, the three contaminated samples showed a transfer between 47 and 57%. In the case of scopolamine, the lower transfer ratio can be due to an incomplete migration of the compound to the boiling water or, as indicated by other authors, to a degradation due to the extraction with hot water (Marín-Sáez et al., 2019). The results confirmed those found by Mulder et al. (2016). In this work, 20 tea infusions were prepared according to the standardized protocol ISO 3103 to calculate the extraction efficiency in boiling water. A relatively wide variation was observed for the obtained results and on average a transfer efficiency near 50% was obtained, being higher for atropine than for scopolamine (Mulder et al., 2016). In addition, besides the type of spice and the infusion preparation conditions, the transfer ratio is dependent on the type of TAs-containing plant and the source of contamination (leaves, seeds, etc.), so this can explain the different transfer ratios observed in the four fennel samples analysed.

On the other hand, as it can be seen in Table 3, the sum of atropine and scopolamine in the prepared fennel infusions was between 0.30 and 0.37 μg/L that is higher than the maximum content of 0.20 μg/kg established for liquid plant infusions (European Union, 2021). In this regard, assuming a transfer of 50–75%, in samples with 25 μg/kg (maximum content allowed in dry herbs) would result in concentrations of 0.25 and 0.375 μg/L and thus exceed the limits for infusions. As can be seen in Table 3, two fennel infusions had concentrations exceeding this maximum level (Fen-1 and Fen-3).

3.5. Assessment of atropine and scopolamine exposure from spices

Based on the results thus obtained, an evaluation of the exposure to the sum of atropine and scopolamine was carried out. For it, the Estimated Daily Intake (EDI) (ng/kg/day) was calculated with the following formula: EDI=(F•RL)/b.w., where F is the spices consumption data (g/day), RL is the mean of the sum of atropine and scopolamine in ng/g and b.w. is body weight (kg), which was fixed on average in an adult of about 60 kg. The average consumption of spices of the Spanish population between 18 and 74 years is 0.02 g/day according to the data obtained in the ENALIA 2 survey carried out by the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (Spanish Agency for Consumer Affairs, 2015a,b). The EDI results calculated for the contaminated Fen-1 – Fen 4 samples (mean concentration as the sum of atropine and scopolamine: 26,000 ng/kg) showed 0.009 ng/kg/day. This value is lower than the acute reference dose (ARfD) indicated by EFSA of 16 ng/kg b.w., expressed as the sum of (−)-hyoscyamine and (−)-scopolamine, assuming equivalent potency (European Commission, 2013). The same consideration was done for the intake of TAs due to the consumption of herbal infusions, which was around a cup per day in Portugal (77 g/day) (Lopes et al., 2017). For this purpose, we considered the weight of an herbal tea bag (2 g) and a 50% transfer ratio, so an EDI of 0.4 ng/kg/day was found (3% of the ARfD). For children, the Spanish ENALIA survey indicates a consumption of plants for infusions of 0.017 g/day (6–11 months) and 0.018 g/day (3–9 years). In this case, the EDI results calculated for the contaminated Fen-1 – Fen 4 samples showed 0.055 ng/kg/day, for a 6-month-old child (weight 8 kg) and 0.033 ng/kg/day,

Table 3
Atropine (μg/L) and scopolamine (μg/L) in infusion, in dry fennel seed and transfer ratio (%).

	Atropine				Scopolamine			
Sample	Infusion (μg/L ± SD)	Expected concentration in infusion with 100% transfer (μg/L ± SD)[a]	Found in dry seed (μg/kg ± SD)	Transfer (%)	Infusion (μg/L ± SD)	Expected concentration in infusion with 100% transfer (μg/L ± SD)[a]	Found in dry seed (μg/kg ± SD)	Transfer (%)
Fen-1	0.30 ± 0.12	0.38 ± 0.12	19 ± 6	79	N.D	N.D	ND	–
Fen-2	0.14 ± 0.05	0.22 ± 0.03	11 ± 1	64	0.18 ± 0.15	0.37 ± 0.03	19 ± 1	47
Fen-3	0.13 ± 0.05	0.19 ± 0.03	10 ± 1	70	0.24 ± 0.09	0.42 ± 0.07	21 ± 3	57
Fen-4	0.14 ± 0.01	0.15 ± 0.03	8 ± 1	88	0.16 ± 0.04	0.34 ± 0.11	17 ± 6	47

The results were obtained by analyzing 3 different infusions in triplicate.
[a]Data calculated from the value obtained in the 100 mL infusion, taking into account the concentration found from dry seed. For example: (19 μg/kg × 0.002 kg)/0.1 L.
[b]N. D.: not detected.

for a 3-year-old child (weight 14 kg). On the other hand, if the intake of spices is as food supplements for adults a higher daily range ingestion is expected (usually one capsule of 500 mg three times per day), so the EDI calculated for the contaminated Fen-1 – Fen 4 samples will be higher (0.65 ng/kg/day). With these estimations, it is possible to observe that the level of contamination found in the considered samples seemed not to pose a risk for the health of the consumers. However, if higher levels of contamination are found, an evident risk for health consumers can be produced, especially for high-level infusion or supplements consumers and if these products are combined with other TAs contaminated foods.

4. Conclusion

The occurrence of atropine and scopolamine was investigated in 51 samples of commercial spices acquired in Portugal and Spain. The results show that 67% of the samples were contaminated by one or both analytes, and 7 samples were found above the maximum level established by regulation (EU) 2021/1408 for herbal infusions (dried product) for the sum of atropine and scopolamine. In addition, the transfer of atropine and scopolamine was evaluated in 4 fennel infusions, finding a transfer of 64–88% for atropine and between 47 and 57% for scopolamine. Based on the results, strategies to reduce and analyse these contaminants in spices should be further developed and applied. It is necessary that food producers and manufacturing companies ensure, in the next years, the reduction of the amount of undesirable TA-producing plants-in crops, raw materials and finished products, following good agricultural and manufacturing practices. In addition, adequate application of food safety control measures (including the establishment of maximum limits) can aid in the reduction of TAs in foods.

CRediT authorship contribution statement

Lorena González-Gómez: Formal analysis, Investigation, Writing – original draft. **Judith Gañán:** Formal analysis, Investigation. **Sonia Morante-Zarcero:** Methodology, Validation, Resources, Supervision. **Damián Pérez-Quintanilla:** Software, Resources, Visualization, Supervision. **Isabel Sierra:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors have declared no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.109555.

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