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COMPREHENSIVE REVIEW

Reports of tropane alkaloid poisonings and analytical techniques for their determination in food crops and products from 2013 to 2023

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Abstract

Food safety is crucial to attaining food security and sustainability. Unsafe foods for human and animal consumption lead to product recalls and rejection, negatively impacting the global economy and trade. Similarly, climate change can adversely affect the availability of safe and nutritious food at the table. The changing climatic conditions and global food trade and transport can make the movement of toxic plants possible, resulting in food crops being increasingly invaded by some species of plants that produce toxic secondary metabolites, such as tropane alkaloids (TAs). *Datura stramonium* from the *Solanaceae* plant family is an invasive and virulent plant that produces high amounts of two TAs, atropine and scopolamine. Various food poisoning events following accidental or deliberate ingestion of foods contaminated by atropine and scopolamine from seeds of *D. stramonium* have been recorded in different locations globally. Due to these incidents, regulatory agencies require the development of plant toxin detection methods that can be used in the food chain as early as possible. This systematic review thus focuses on the TA determination techniques in food and feeds published between 2013 and 2023. A particular focus was given to the sample preparation methods, the improvements of each technique claimed, and data to support the performance of each method, especially the ability to measure at or below the maximum level. The review concludes with other technological advancements, including rapid spectroscopy, electrophoresis, and colorimetric methods, as well as the possibility of coupling with smartphones for use in on-farm detection and the challenges in applying them.

KEYWORDS

chromatography, immunoassays, scopolamine, spectroscopy, tropane alkaloids

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1 | INTRODUCTION

Tropane alkaloids (TA) are plant secondary metabolites that occur in several plant families such as *Brassicaceae*, *Proteaceae*, *Euphorbiaceae*, *Rhizophoraceae*, *Convolvulaceae*, and *Cruciferae*, but high concentrations of TAs are primarily found in *Erythoxylaceae* and *Solanaceae* (Abia et al., 2021; Kohnen-Johannsen & Kayser, 2019). There are at least 200 TAs known in nature characterized by their bicyclic tropane ring systems, such as cocaine, calystegines, atropine (hyoscyamine), and scopolamine, with the latter two produced significantly by the *Solanaceae* plants (Figure 1—Chemical structure of atropine and scopolamine) (Adamse et al., 2014; Klein et al., 2022; Kohnen-Johannsen & Kayser, 2019; Vuković et al., 2022). Atropine is the racemic mixture of (–)-hyoscyamine and (+)-hyoscyamine, with (–)-hyoscyamine and (–)-scopolamine the ones produced in TA synthesis in plants instead of (+)-hyoscyamine and (+)-scopolamine (EU, 2021). For consistency, in this review, (–)-hyoscyamine will be referred to as atropine, and (–)-scopolamine will be referred to as scopolamine.

The most common sources of TAs in the *Solanaceae* family are *Datura* (originating in Asia and America; Figure 2—image of *Datura* species found in a forest in Nigeria), *Duboisia* (found in Australia and New Caledonia), *Hyoscyamus*, and *Atropa* (originating in Europe, Asia, and North America and introduced in the United States, Canada, and Australia) (Griffin & Lin, 2000; Kohnen-Johannsen & Kayser, 2019). These plants proliferate anywhere, with the exception of the polar and subpolar climate zones (Griffin & Lin, 2000; Kohnen-Johannsen & Kayser, 2019). Among the *Solanaceae* plants, *Datura stramonium*

is the most invasive and virulent species and is frequently found growing in cereal fields (Abia et al., 2021).

TAs are synthesized in the roots of *Datura* plants and then transported into the xylem, with the precursor for the TA synthesis as putrescine through the arginine pathway, and the process was described by some published articles cited herein (Kohnen-Johannsen & Kayser, 2019; Shi et al., 2022; Zeng et al., 2021). The N-methyltransferase (PMT) enzyme methylates putrescine to N-methylputrescine, which forms 4-methylaminobutanal through oxidative deamination catalyzed by N-methylputrescine oxidase. The 4-methylaminobutanal spontaneously cyclizes to form N-methyl- Δ^1 -pyrrolinium cation, which, in the presence of tropinone synthase enzyme, tropinone is generated as a result of condensation of acetoacetate. Tropinone is reduced to tropine by tropinone reductase I (TRI). Tropinone reductase then catalyzes the reduction of tropinone to tropine and pseudotropine. Tropine is esterified with phenyllactic acid to form littorine, which is then converted to hyoscyamine through hydroxylation and rearrangement steps catalyzed by enzymes such as hyoscyamine 6 β -hydroxylase (H6H). Hyoscyamine is hydroxylated by H6H to form 6 β -hydroxyhyoscyamine, which spontaneously rearranges to form scopolamine. This critical step often targets metabolic engineering to enhance scopolamine production (Kohnen-Johannsen & Kayser, 2019; Shi et al., 2022; Zeng et al., 2021).

Methyl jasmonate, a plant hormone integral to physiological regulation and stress response mechanisms, can either regulate or downregulate the synthesis of scopolamine and atropine (Rasi et al., 2024), that is, at methyl jasmonate concentration of 150 μ M, the production of TA increased. In comparison, methyl jasmonate had an adverse effect at a concentration of 300 μ M, affecting the critical enzymes in atropine and scopolamine synthesis or translocation of precursors for scopolamine synthesis (Rasi et al., 2024). H6H is inhibited by ethylenediaminetetraacetic acid (EDTA) and entirely by other divalent cations, including Ca^{2+} , Cd^{2+} , Co^{2+} , Cu^{2+} , Hg^{2+} , Mn^{2+} , Ni^{2+} , Zn^{2+} , as well as by Fe^{3+} by binding to the enzyme resulting in the change in the conformation impacting the catalytic function (Kohnen-Johannsen & Kayser, 2019). In addition, salicylic acid and acetylsalicylic acid as elicitors can potentially inhibit the production of TAs by affecting the expression of genes involved in TA biosynthesis, such as PMT and H6H (Kang et al., 2004). Last, by genetic engineering, that is, by ribonucleic acid (RNA) interference such as silencing the genes encoding the key enzymes methyltransferase (PMT or H6H) can significantly reduce the production of TAs (Zhang et al., 2023).

All parts of the *Solanaceae* plants contain significant amounts of TA (Adamse et al., 2014; Vuković et al., 2022). Since TA plant sources grow alongside other crops,

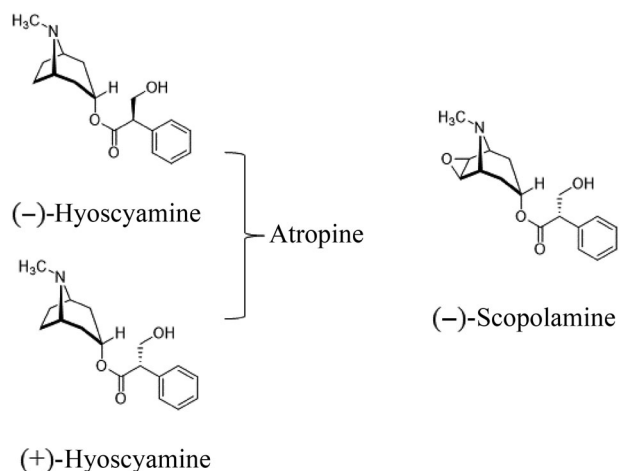


FIGURE 1 Chemical structure of atropine (the racemic mixture of (–)-hyoscyamine and (+)-hyoscyamine) and (–)-scopolamine.

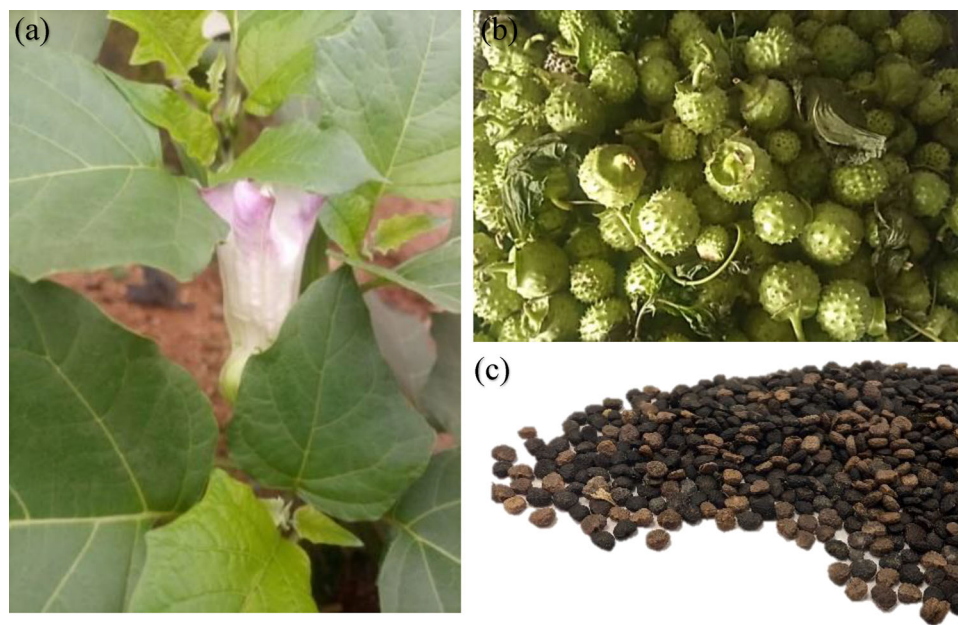


FIGURE 2 *Datura stramonium* (left) and *Datura metel* (middle and right) from a forest in Nigeria.

contamination of food, feed, and related crops can occur by accidental co-harvesting or processing. In contrast, accidental ingestion, primarily by children (e.g., berries-like fruits of *Solanaceae* plants) or abuse by adolescents or recreational use (due to hallucinogenic effects), can also result in TA poisoning (Abia et al., 2021; Adamse et al., 2014; Chan, 2017; Vuković et al., 2022).

Atropine and scopolamine are dose-dependent anticholinergics or nonselective competitive antagonists at muscarinic acetylcholine receptors in the central and nervous systems (Shim et al., 2022). They can compete with acetylcholine by binding at the muscarinic receptors or nicotinic acetylcholine receptors, to a lesser extent, in the central and autonomic nervous system (Adamse et al., 2014; EU, 2021; Kohnen-Johannsen & Kayser, 2019; Shim et al., 2022). The antimuscarinic effects of TAs on the peripheral system include dilated pupils, blurred vision, hyperthermia, dry mouth, dry flushed skin, tachycardia, reduced bowel motility, and urinary retention, while the central effects include hallucinations, delirium, drowsiness, amnesia, ataxia, seizures, and coma (Chan, 2017). The classic manifestation of TA poisoning can be identified from the mnemonic “red as a beet (flushing), dry as a bone (dry mouth), blind as a bat (mydriasis), mad as a hatter (mental confusion), hot as a hare (fever), as full as a flask (urinary retention) (Shim et al., 2022).

It was reported that the positive (+)-enantiomers have weaker pharmacological and toxicological effects than the (–)-enantiomers, such that (+) hyoscyamine had 10–40-fold weaker potency in comparison with (–)-hyoscyamine (EFSA, 2013). The lowest therapeutic doses are 1.4 µg/kg

body weight (b.w.) for (–)-hyoscyamine, 6 µg/kg b.w. for atropine (corresponding to about 3 µg/kg b.w. (–)-hyoscyamine) and 2.5 µg/kg b.w. for (–)-scopolamine (EFSA, 2013). These doses can be associated with adverse side effects, such as cardiac slowing or dryness of the mouth, and do not apply to individuals with contraindications who are likely to be more sensitive to some effects (EFSA, 2013). The lowest dose associated with the biological activity of a mixture of (–)-hyoscyamine and (–)-scopolamine in food is 0.48 µg/kg b.w., which resulted in a transient lowering of the heart rate in healthy volunteers, which could be adverse in susceptible individuals, such as those with bradycardia (EFSA, 2013). This study’s no-observed-adverse-effect level (NOAEL) is 0.016 µg/kg b.w., expressed as the sum of (–)-hyoscyamine and (–)-scopolamine, was considered to be the most appropriate basis for deriving a group acute reference dose (Codex, 2024).

As a result, Commission Regulation (EC) No 1881/2006 was amended to EC 2021/1408 and EC 2023/915 regarding the maximum level (ML) of atropine and scopolamine in certain foodstuffs (Table 1) (EU, 2023). Additionally, products intended for animal feed can contain weed seeds and unground and uncrushed fruits containing alkaloids, glucosides or other toxic substances separately or in combination to a maximum content of 3000 mg/kg, and *Datura sp.* to a maximum of 1000 mg/kg per Directive 2002/32/EC amended to Commission Regulation (EU) 2019/1869. Moreover, international guidance on the levels of atropine and scopolamine was recommended by the Joint Food and Agriculture Organization/ World Health

TABLE 1 Maximum levels of atropine and scopolamine in foods based on the Commission Regulation (EU), 2023/915 (EU, 2023).

Product	Sum of atropine and scopolamine (µg/kg) ^a	Remarks
Baby food and processed cereal-based food for infants and young children containing millet, sorghum, buckwheat, corn, or their derived products	1 ^b	Derived products relate to products containing at least 80% these cereal products. The sampling for the control of compliance with the maximum level shall be performed in accordance with the provisions provided for in point J of Annex I to Regulation (EC) No 401/2006. The maximum level applies to the product as placed on the market.
Unprocessed millet grains and sorghum grains	5	The maximum level applies to unprocessed cereal grains placed on the market before first-stage processing.
Unprocessed corn	15	Except unprocessed corn grains for which it is evident, for example, through labelling, destination, that it is intended for use in a wet milling process only (starch production) and except unprocessed corn grains for popping. The maximum level applies to unprocessed corn grains placed on the market before first-stage processing.
Unprocessed buckwheat	10	The maximum level applies to unprocessed buckwheat grains placed on the market before first-stage processing.
Corn for popping Millet, sorghum, and corn placed on the market for the final consumer Milling products of millet, sorghum, and corn	5	
Buckwheat placed on the market for the final consumer Milling products of buckwheat	10	
Herbal infusions (dried product) and ingredients used for herbal infusions	25	“Herbal infusions (dried product)” refers to: — herbal infusions (dried product) from flowers, leaves, stalks, roots, and any other parts of the plant (in sachets or in bulk) used for the preparation of herbal infusion (liquid product); and — instant herbal infusions. In the case of powdered
Herbal infusions (dried product) and ingredients used for herbal infusions (dried products) of exclusively anise seeds	50	“Herbal infusions (dried product)” refers to: — herbal infusions (dried product) from flowers, leaves, stalks, roots, and any other parts of the plant (in sachets or in bulk) used for the preparation of herbal infusion (liquid product); and — instant herbal infusions. In the case of powdered extracts, a concentration factor of 4 has to be applied.
Herbal infusions (liquid)	0.20	

^aFor the sum of atropine and scopolamine, maximum levels refer to lower bound concentrations, which are calculated assuming all the values below the limit of quantification are zero

^b1 µg/kg atropine and 1 µg/kg scopolamine.

Organization (FAO/WHO) Expert Meeting in 2020 on Super Cereal, Super Cereal Plus and Lipid-Based Nutrient Supplements based on an intake of 100 g/day (FAO/WHO, 2020). Super Cereal is used in nutrition and humanitarian programmes that assists approximately 4.9 million people globally with high nutrient needs every year in developing countries (FAO/WHO, 2020). Super Cereal is fortified flour of corn or wheat and soy—it is prepared from heat-treated corn and de-hulled soya beans, sugar, dried skim milk, and refined soya bean oil (UNICEF, 2018). The recom-

mended levels of atropine and scopolamine were 30 µg/kg (in Super Cereal) for adults or 10 µg/kg (in Super Cereal Plus and Lipid-based Nutrient Supplements) for children (FAO/WHO, 2020). These are operational limits applicable to other cereals and grains provided similar intake. In addition, in situations like food insecurity, the guidance levels for atropine and scopolamine would be 90 µg/kg (in Super Cereal) for adults and 30 µg/kg (Super Cereal Plus and Lipid-based Nutrient Supplements) for children (FAO/WHO, 2020).

TA poisoning symptoms show within five to 60 min and can last up to 24 to 48 h (Chan, 2017; Disel et al., 2015). The antidote against scopolamine and atropine intoxications is physostigmine (Kohnen-Johannsen & Kayser, 2019). This pyrrole-indole alkaloid naturally occurs in the Calabar bean (*Physostigma venenosum*, *Fabaceae*) and acts as a parasympathomimetic drug by reversible inhibition of the cholinesterase (Kohnen-Johannsen & Kayser, 2019) (Robenshtok et al., 2002). Other treatments for TA poisoning include gastric emptying, the use of activated charcoal (0.5–1 g/kg in children or 25–100 g in adults), and benzodiazepines for managing agitation (Shim et al., 2022).

A Joint FAO/WHO Expert Meeting was convened virtually on March 30 – April 3 2020 to provide risk assessments for TAs, atropine and scopolamine, and according to this meeting there are no international regulations regarding TAs (FAO/WHO, 2020). However, regulations that define the limits for noxious seeds, including *Datura* spp. in grains, are available (Codex, 2024). In addition to Codex Standards for the latter, the China Grain Standard (GB 2715-2016), 7 CFR Part 810 Subparts B, D-M in the United States, and the Republic of South Africa Foodstuffs, Cosmetics and Disinfectants Act are also available (Codex, 2024). An electronic working group prepared a discussion paper on TA in foods during the Codex Committee on Contaminants in Foods session in April 2024, which indicates that the development of global ML for TAs is limited due to a lack of occurrence data, validated official methods (including sampling method) and international laboratory proficiency testing for analytical methods, and that most of the TA data come from Europe (Codex, 2024).

A dataset retrieved from a food risk intelligence platform FOODAKAI (2023) (Table 2) shows a wide variation in the levels of TA detected from different food commodities. Hulled millet seeds distributed in Denmark, Germany, and Sweden contained atropine and scopolamine ranging from 23 to 29 µg/kg; soybean meal contained 56 µg/kg; while herbs and spices contained 106 ± 21.2 µg/kg (both commodities were distributed in Germany) (Table 2; (FOODAKAI (2023)). Our review paper identified several peer-reviewed studies between 2013 and 2023, and a summary of TAs detected in various commodities is presented in Table 3. There are substantial differences in TA levels; the buckwheat from Hungary processed into flour contained 26,000 µg/kg atropine and 12,000 µg/kg scopolamine (Perharič et al., 2013), while buckwheat or buckwheat flour from Spain did not contain atropine or scopolamine (Table 3) (Chen et al., 2017). The concentrations of TA present in their plant sources are influenced by geographical location, environmental conditions and growing conditions (FAO/WHO, 2020). The presence of TA-producing invasive weed plants that grow with crops differs based on the agricultural practices and the weed

management system (Abia et al., 2021; Stratton et al., 2017). The effectiveness of quality control or inspection of raw materials and monitoring of processing can impact the final concentration of TAs in food (Adamse et al., 2014; Codex, 2024). Since there are no international regulations on TAs, it is expected to have varying levels found in food in different countries due to their testing method and compliance with the country's requirements (FAO/WHO, 2020). Currently, no standard reference method is used to quantify TAs in food (Codex, 2024), which can also contribute to differences in the reported TA levels.

In 2023, Food Safety News reported that a cornbread (broa de milho) consumption made more than 200 people in Portugal ill; the bread was found to be contaminated by high levels of atropine and scopolamine from the seeds of *Datura* sp. (Figure 2) (Whitworth, 2023). The victims were reported to experience symptoms, including a dry mouth, vision issues, dizziness, mental confusion, and decreased muscle strength. Symptoms were observed between 30 min to 2 h after eating, and in most cases, they were classified as mild, but more than 40 people required hospital care (Whitworth, 2023). In October 2022, food poisoning incidents involving 13 individuals from different regions in Italy (Lombardy, Lazio, Campania) were reported following consumption of spinach and spinach-based foods; the symptoms were dry mouth, difficulty in speaking and swallowing, tachycardia, drowsiness, dizziness, hallucinations, and delirium (Caprai et al., 2024). In 2019, approximately 300 people suffered from severe food-related illnesses, and five died in the Karamoja region, Republic of Uganda, following the consumption of Super Cereals. The victims experienced headaches, abdominal pain, dry mouth, sticky saliva, body itching, mental confusion, dizziness, paleness, and dilated pupils (Abia et al., 2021; Haughey et al., 2021). WHO, WFP, the Ministry of Health in Uganda, and the US Centre of Disease Control led the investigation of the Super Cereal-related food poisoning outbreak by contacting some of the world's expert laboratories, including the author's research group at the Institute for Global Food Security, Queen's University Belfast in the United Kingdom. Results of the investigations indicated that the source of poisoning could be the TA-contaminated soya bean, specifically atropine and scopolamine from the seeds of *D. stramonium* (thorn-apple or Jimson weed; Figure 2) (Haughey et al., 2021). In the same year, 2019, another food poisoning incident involved the consumption of TA-contaminated unprocessed sorghum, which was distributed as food aid to the Republic of South Sudan (FAO/WHO, 2020). A family in Turkey was reported to be poisoned after eating a traditional dish, dolma, into which *Datura* flowers were accidentally used as an ingredient (Disel et al., 2015). TA poisoning events have been reviewed by Adamse et al.

TABLE 2 Some food safety incidents related to tropane alkaloids in the last 10 years (2013–2023) retrieved from the FOODAKAI (2023) platform.

Date (dd-mm-yyyy)	Hazards	Products	Origins	Type	Announced by	Notification from	Distribution countries	Remarks
6-1-2023	Scopolamine, atropine	Hulled millet seeds	Poland	Alert	RASFF	Belgium	Denmark, Germany, Sweden	Atropine = 29 µg/kg scopolamine = 23 µg/kg
29-12-2022	Tropane alkaloids, foodborne outbreak	Pickled vegetables	China	News item	ProMED			
23-12-2022	Tropane alkaloids	Porridge—baby food	Denmark	Alert	Ministry of Environment and Food of Denmark			
28-7-2022	Tropane alkaloids	Corn snacks	Belgium	Alert	Food Safety Authority of Ireland, Centre for Food Safety Hong Kong			Recall of batches of corn organic corn snacks
27-7-2022	Atropine, scopolamine	Tortilla chips	Denmark	Alert	Ministry of Environment and Food of Denmark			
27-7-2022	Atropine, scopolamine	Tortilla chips	Germany	Alert	Centre for Food Safety Hong Kong	Hongkong		
27-7-2022	Tropane alkaloids	Tortilla chips	Belgium	Alert	Food Safety Authority of Ireland		Ireland	
27-7-2022	Tropane alkaloids	Tortilla chips	Netherlands	Alert	RASFF	Belgium	Austria, Germany, Netherlands	Tropane alkaloids in corn tortilla chips, via the Netherlands
26-7-2022	Tropane alkaloids	Tortilla chips	Luxembourg	Alert	Food Safety Authority of Luxembourg	Luxembourg		
1-7-2022	Tropane alkaloids	Fried corn chips	Germany	Alert	RASFF	Belgium	Austria, Belgium, Croatia, Czech Republic, France, Hungary, Luxembourg, Netherlands, Romania, Switzerland	
1-7-2022	Tropane alkaloids	Fried corn chips	Luxembourg	Alert	Food Safety Authority of Luxembourg	Luxembourg		
6-5-2022	Atropine	Herbs and spices	Austria	Alert	RASFF	Belgium	Germany	Sum of atropine and scopolamine = 106 ± 21.2 µg/kg
7-4-2022	Atropine	Soybean flour, meal, or powder	Austria	Alert	RASFF	Belgium	Germany	Atropine = 56 µg/kg soy meal
31-3-2022	Tropane alkaloids	Food products	Uganda	News item	ProMED			

(Continues)

TABLE 2 (Continued)

Date (dd-mm-yyyy)	Hazards	Products	Origins	Type	Announced by	Notification from	Distribution countries	Remarks
9-11-2021	Tropane alkaloids	Herbal infusion	Netherlands	<i>Alert</i>	RASFF	Belgium	Belgium	
9-11-2021	Atropine, scopolamine	Corn flour	France	<i>Alert notification</i>	RASFF	Belgium	Belgium	
8-11-2021	Aflatoxin, tropane alkaloids	Flaxseed	Germany	<i>Alert</i>	RASFF	Belgium	Germany	
12-8-2021	Atropine, scopolamine	Buckwheat flour	Germany	<i>Alert</i>	RASFF	Belgium	China, Hong Kong, Netherlands, Norway, Poland	
20-7-2021	Tropane alkaloids	Parsley	Hungary	<i>Alert</i>	RASFF	Belgium	Germany	
7-4-2021	Atropine, scopolamine	Organic millet	Netherlands	Food	RASFF	Germany	Germany	
18-11-2021	Atropine, scopolamine	Popcorn maize	Spain	Food	RASFF	Germany	Germany	
11-12-2020	Scopolamine, atropine	Brown millet	Germany	Food	RASFF	Germany	Germany	
1-12-2020	Tropane alkaloids	Popcorn	Belgium	<i>Alert</i>	Federal Agency for the Safety of the Food Chain of Belgium			
27-11-2020	Scopolamine	Maize for pop corn	Belgium, Netherlands	Food	RASFF	Belgium, Europe	France, Luxembourg, Netherlands	
4-11-2020	Atropine, scopolamine	Peppermint	Turkey	Food	RASFF	Germany	Germany	
5-9-2020	Tropane alkaloids, pyrrrolizidine alkaloids (pas)	Tea	Germany	<i>Alert</i>	German Federal Office of Consumer Protection and Food Safety			
25-5-2020	Atropine, scopolamine	Blackberries	Bulgaria	Food	RASFF	Germany	Australia, Australia/Oceania, Germany	
29-1-2020	Tropane alkaloids	Soy flakes	Germany	<i>Alert</i>	German Federal Office of Consumer Protection and Food Safety			
24-1-2020	Atropine, scopolamine	Soy flakes	Austria	Food	RASFF	Germany	Germany, Greece, Hungary, Netherlands, Poland	

(Continues)

TABLE 2 (Continued)

Date (dd-mm-yyyy)	Hazards	Products	Origins	Type	Announced by	Notification from	Distribution countries	Remarks
6-8-2019	Atropine, scopolamine	Porridge	Spain	Food	RASFF	Netherlands	Netherlands	
1-4-2019	Atropine, scopolamine	Maize grits	Serbia	Food	RASFF	Croatia	Croatia	
29-1-2019	Atropine, scopolamine	Tea	Serbia	Food	RASFF	Croatia	Croatia, Germany	
21-12-2018	Tropane alkaloids	Buckwheat flour	Belgium	Alert	Federal Agency for the Safety of the Food Chain of Belgium			Recall of buckwheat flour (500 g) due to the presence of <i>Datura</i>
20-12-2018	Atropine, scopolamine	Organic buckwheat flour	France	Food	RASFF	France	Belgium, Luxembourg, Morocco, Africa, Portugal, Switzerland	
26-9-2018	Atropine, scopolamine	Muesli, organic muesli	Austria	Food	RASFF	Germany	Hungary, Austria	
21-9-2018	Tropane alkaloids	Muesli	Germany	Alert	German Federal Office of Consumer Protection and Food Safety			
20-8-2018	Atropine, scopolamine	Herbal tea	Poland	Alert	Czech Agriculture and Food Inspection Authority			
22-6-2018	Tropane alkaloids	Pop corn	Germany	Alert	German Federal Office of Consumer Protection and Food Safety			
5-6-2018	Scopolamine	Maize for pop corn	Argentina	Food	RASFF	Germany	Austria, Czech Republic, Germany	
24-5-2018	Atropine, scopolamine	Popcorn	France	Food	RASFF	Germany	Germany	
21-3-2018	Atropine, scopolamine	Cumin seeds	Hungary	Food	RASFF	Hungary	Serbia, Hungary	
20-9-2016	Atropine, scopolamine	Millet flour	Hungary	Food	RASFF	Austria	Austria, Poland	
22-7-2016	Atropine, scopolamine	Corn	Germany	Food	RASFF	Austria	Austria, Slovakia	

(Continues)

TABLE 2 (Continued)

Date (dd-mm-yyyy)	Hazards	Products	Origins	Type	Announced by	Notification from	Distribution countries	Remarks
13-4-2016	Atropine	Baby porridge without milk	Spain	Food	RASFF	Czech Republic	Andorra, Bosnia and Herzegovina, Croatia, Czech Republic, Netherlands, Romania, Serbia, Slovakia, United Kingdom	
1-2-2016	Atropine, scopolamine	Gluten-free baking mix and related products based on sorghum	Czech Republic	Food	RASFF	Czech Republic	Czech Republic, Slovakia	
26-11-2015	Atropine, scopolamine	Sorghum flour	Czech Republic	Food	RASFF	Czech Republic	Czech Republic, Germany, Poland, Slovakia	
18-9-2015	Atropine, scopolamine	Microwave popcorn	Spain	Food	RASFF	Germany	Austria, Germany	
4-6-2015	Atropine, scopolamine	Organic polenta cornmeal	Germany	Food	RASFF	Germany	Austria, Germany	
30-3-2015	Atropine, scopolamine	Gluten-free organic millet	Austria	Food	RASFF	Austria	Slovakia, Austria	
20-3-2015	Atropine, scopolamine	Organic millet dumplings	Hungary	Food	RASFF	Austria	Austria	
20-2-2015	Atropine, scopolamine	Brown millet	Austria	Food	RASFF	Austria	Belgium, France, Germany, Hungary, Italy, Netherlands, Portugal, Slovakia, Sweden, Switzerland, Austria	
18-12-2014	Atropine	Millet/cereal porridge with rice	Germany	Food	RASFF	Germany	Austria, Czech Republic, Slovakia, Slovenia, Switzerland, Germany	
17-12-2014	Atropines, tropane alkaloids, scopolamine, alkaloids, unauthorized ingredient (fraud)	Infant food	Switzerland	Alert	Singapore Food Agency	Singapore		(Continues)

TABLE 2 (Continued)

Date (dd-mm-yyyy)	Hazards	Products	Origins	Type	Announced by	Notification from	Distribution countries	Remarks
12-12-2014	Atropine, scopolamine	Baby foods	United Kingdom	Alert	UK Food Standards Agency	United Kingdom		
11-12-2014	Atropine, thorn-apple	Organic baby food apple pear millet	Germany	Food	RASFF	Germany	Andorra, Australia/Oceania, Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hong Kong, Hungary, Iceland, Ireland, Italy, Japan, Latvia, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Singapore, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, United Kingdom, Republic of North Macedonia	
4-12-2014	Atropine, scopolamine	Brown millet flour	Austria	Food	RASFF	Germany	Germany, Hungary, Austria	
21-11-2014	Atropine	Porridge - baby food	Austria	Food	RASFF	Germany	Croatia, Czech Republic, Germany, Hungary, Luxembourg, Romania, Slovenia, Austria	
13-6-2013	Atropine, scopolamine	Buckwheat flour	Austria, Slovakia	Food	RASFF	Slovenia	Slovenia	
22-5-2013	Atropine, scopolamine	Buckwheat flour	Austria, Czech Republic	Food	RASFF	Slovenia	Slovenia	

TABLE 3 Extraction (LLE, SLE, SALLE, SPE, QuEChERS³¹) of atropine, scopolamine and other contaminants in food and feeds, instrumental requirements for liquid chromatographic analysis, analytical performance and levels detected.

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/confirmatory References
Urine, herbal decoction	22 toxic plant alkaloids	Urine or herbal decoction: sodium carbonate and bicarbonate in a mixture of heptane, dichloromethane, and dichloroethane (contents of a Toxi-Tube A (Varian) herbal samples (e.g., unused herbs, herbal powder, herbal pill): 70% ACN, then the extract was added with water before transferring to Toxi-Tube A	LC-MS/MS C8; (150 mm × 4.6 mm, 5 µm particle size) A: 1 mM AF and 0.1% FA in H ₂ O B: 1 mM AF and 0.1% FA in ACN	Both = 5	NA (identification)	Validated, screening Ng et al. (2013)
Cow's milk	AT and SC	Methanol/FA (99/1, v/v); 1:2 (sample: solvent ratio)	LC-MS/MS Luna 5 µm phenyl-hexyl-column ID 2 mm × 150 mm A: H ₂ O/FA (999/1, v/v) B: MeOH	AT = 0.075 SC = 0.075	1.60 ± 0.07	Validated, confirmatory Lamp et al. (2021)
Cereal-based products for infants and young children; breakfast cereals, biscuits, and cookies for children	Tropane and ergot alkaloids	MeOH:H ₂ O:FA (60:40:0.4, v/v/v); 1:10 (sample:solvent ratio) → ultrafiltration over a 30 kDa ultrafilter	LC-MS/MS C18 (150 × 3.1 mm, 5 µm particle size) A: 6.5 mM NH ₄ OH in H ₂ O B: 6.5 mM NH ₄ OH in 90% aq. ACN	Based on LOD: AT = 0.2 SC = 0.5 LOQ: AT = 0.67 SC = 1.67	Mean: AT = 0.09–2.41 SC = 0.28–0.63 Maximum AT = 1.2–65.6 SC = 3.7–15.2	Validated, confirmatory Mulder et al. (2015)
Raw, pasteurized cow's milk	51 pyrrolizidine alkaloids, AT, and SC	2% aqueous FA and n-hexane; 1:15 (sample: solvent ratio) → SPE polymeric cation exchange (PCX) cartridges	LC-MS/MS C18 (120 × 2.1 mm, 2.6 µm particle size) A: aqueous ammonium carbonate (pH 9.0) B: ACN	AT = 0.021 SC = 0.027	None detected	Validated, confirmatory Klein et al. (2022)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/ confirmatory	References
Honey	AT and SC	Concentrated sodium acetate solution, then ACN	LC-MS/MS HILIC core-shell column (100 × 2.1 mm, 1.7 µm) and HILIC security guard ULTRA guard cartridge (2.1 mm) A: 5 mM AF in 0.1% (v/v) FA in H ₂ O B: 5 mM AF in 0.1% (v/v) FA in 95:5 (v/v) ACN: H ₂ O	Both = 0.01	AT and SC = <LOD-0.012	Validated, confirmatory	Thompson et al. (2020)
Buckwheat (flour and noodle), wheat, soy, amaranth grain, chia seeds, and peeled millet	AT and SC	H ₂ O then ACN solution with 1% (v/v) acetic acid + 4 g of anhydrous sodium sulfate and 1 g of ammonium acetate; 1:2:2 (sample: solvent:H ₂ O) → dSPE (GBC and PSA) → filtered through 0.22 µm nylon filter syringe	UHPLC-MS/MS C18 (100 × 2.1 mm, 1.8 µm particle size) A: ACN B: H ₂ O + FA (0.1%, v/v)	AT = 0.4 SC = 2.0	<LOD	Validated, confirmatory	Chen et al. (2017)
Honey	Pyrrolizidine and TAs	1.5 g sample + 10 mL 0.1 mol/L H ₂ SO ₄ with Zn dust (acid treatment) → supernatant + 10 mL ACN and Q-sep QuEChERS extraction salts → Q-sep dSPE (PSA 0.15 g and magnesium sulfate 0.9 g)	LC-HRMS C8 (150 × 3 mm, 2.7 µm particle size) A: 0.1% FA in H ₂ O B: MeOH/ACN (1:1, v/v)	Both = 0.5	AT = 1.4–3.8 SC = not observed	Validated, confirmatory	Martinello et al. (2017)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/confirmatory	References
Bee pollen, teas, herbal tea infusion	Pyrrolizidine and TAs	1 g bee pollen + 10 mL 0.1 mol/L H ₂ SO ₄ with Zn dust (acid treatment) → super-natant + 10 mL ACN and QuEChERS extraction salts (MgSO ₄ and PSA) → filtration in a PTFE 0.2 µm Mini-UniPrep® syringeless filter 2 g tea infusion + 150 mL boiling water + H ₂ SO ₄ + Zn dust → SPE (C18 column) → filter	UHPLC-MS/MS RP-MS A: 0.1% FA in H ₂ O B: MeOH/ACN (1:1, v/v)	AT = 0.2 SC = 0.5	AT = 0.011–6.7 SC = 0.007–4.3	Validated, confirmatory	Martinello et al. (2022)
Maize semolina, wheat flour, cereal flakes, oat flour, quinoa flour, soybeans, infant cereals	AT, SC, and mycotoxins	5 g sample + 10 mL H ₂ O + 10 mL ACN: acetic acid (995:5) + QuEChERS salt mixture	LC-MS/MS C18 (2.1 × 100 mm, 1.7 µm particle size) A: 1.5 mL/L FA and 10mM AF in H ₂ O B: 0.5 mL/L FA MeOH	Both = 0.5	Rice: AT = 9.6 SC = 2.6	Validated, confirmatory	Baslé et al. (2020)
Pea, soy, wheat flour, quinoa	156 plant toxins, mycotoxins, and phytoestrogens	1 g sample + 10 mL H ₂ O + 10 mL ACN → QuEChERS salts	LC-HRMS C18 (150 × 2.1 mm, 1.7 µm) A: H ₂ O B: MeOH with 10 mM ammonium bicarbonate	Both = 1	Scopoline in quinoa: 38	Validated, confirmatory	Bessaire et al. (2021)
Unprocessed maize crops		1 g sample + 10 mL H ₂ O + ACN with 1% acetic acid + QuEChERS extraction packets EN method					

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/confirmatory	References
Tea, herbal tea infusion	Dehydro-pyrrolizidine and tropane alkaloids ((-)-SC and their N-oxides, and AT)	Acetic acid:MeOH (1:2, v/v); 1:10 (sample: solvent ratio)	LC-MS/MS C18 (50 × 2.1 mm, 1.9 µm particle size) A: 99.5% H ₂ O and 0.5% FA B: 94.5% MeOH and 5% H ₂ O and 0.5% FA both added with 0.1 mM oxalic acid + 2.0 mM ammonium acetate	Both = 1	AT = 83–171 (±53) SC = 2–81 (±69)	Validated, confirmatory	Shimshoni et al. (2015)
Maize products: maize products, maize grits, polenta, semolina	AT and SC	10 g sample + 10 mL H ₂ O followed by 40 mL ACN with 1% FA + QuEChERS extraction packets EN method → dSPE (150 mg MgSO ₄ + 25 mg PSA + 25 mg C18)	LC-MS/MS C18 (50 × 2.1 mm, 1.8 µm particle size) A: H ₂ O B: MeOH both with 0.1% FA	Both = 1	AT = 1.2–58.8 SC = 1.07–10.20	Validated, confirmatory	Vuković et al. (2022)
Leafy vegetables, and tropane-alkaloid producing plants: <i>Brugmansia versicolor</i> , <i>Solantra maxima</i> , and <i>Convolvulus arvensis</i>	AT and SC	(u-QuEChERS) 0.1 g sample + 0.5 mL H ₂ O + 1 mL ACN and 0.65 g of the partitioning salts mixture formed by MgSO ₄ , NaCl, trisodium citrate dihydrate, and sodium hydrogen citrate sesquihydrate (4:1:1:0.5) → Eppendorf with 150 mg MgSO ₄ + 25 mg PSA and 0.45 µm nylon syringe filter	Eppendorf with 150 mg MgSO ₄ + 25 mg PSA and 0.45 µm nylon syringe filter C18 (150 mm × 2.0 mm, 3.5 µm particle size) A: H ₂ O B: ACN both with 0.1% FA	AT = 2.3 SC = 2.2	AT = 2.7–3.4 SC < LOQ	Validated, confirmatory	González-Gómez et al. (2022)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/ confirmatory	References
Frozen spinach and Spinach-based infant food products (no cereals)	AT and SC	10 g sample + 10 mL ACN + MgSO ₄ , 1 g NaCl, 1 g trisodium citrate dihydrate, and 0.5 g sodium hydrogen citrate sesquihydrate → dSPE (sorbents: 750 mg anhydrous MgSO ₄ and 250 mg PSA) → filtration through a 0.45 µm PTFE	UHPLC-MS/MS C18 (2.1 mm × 50 mm, 1.8 µm particle size) A: H ₂ O B: MeOH both containing 0.1% FA and 5 mM AF	Frozen spinach: AT = 0.018 SC = 0.015 Spinach-based infant food product: AT = 0.014 SC = 0.018	AT = 0.02–4.52 SC = 0.04–8.19	Validated, confirmatory	Castilla-Fernández et al. (2021)
Poultry, swine, cattle, horse and lamb feed samples	AT, SC, 77 banned veterinary drugs, mycotoxins, ergot alkaloids, plant toxins, posttarget screening for 425 substances including pesticides and environmental contaminants	H ₂ O followed by ACN with 1% acetic acid, then QuEChERS extraction salt packet → QuEChERS extract pouches-EN method (salt packet containing 4 g MgSO ₄ , 1 g NaCl, 1 g sodium citrate, 0.5 g disodium citrate sesquihydrate)	UHPLC-HRMS Hypersil Gold column (100 × 2.1 mm, 1.9 µm) A: H ₂ O with 0.1% acetic acid B: MeOH:ACN (90:10, v/v) containing 0.1% acetic acid	Both = 5	AT = 7.2	Validated, confirmatory	León et al. (2016)
11 commercial samples of tea and herbs for infusions (dried product), including rooibos, chamomile; red tea, black tea, three green teas, and white tea; linden, horsetail, infusion containing a mixture of herbs	30 tropane and pyrrolizidine alkaloids and their N-oxides	1 g sample + 30 mL ACN: H ₂ O (75:25, v/v) with 0.5% FA + QuEChERS extraction packet (6 g MgSO ₄ , 1.5 g sodium acetate) → d-SPE tube with 1.2 g MgSO ₄ , 0.4 g PSA, 0.4 g C18, 0.4 g GBC → filtration through a 0.22 µm nylon filter	UHPLC-HRMS C18 (100 × 2.1 mm, 1.9 µm particle size) A: 0.1% FA in H ₂ O B: 0.1% FA in MeOH	Both = 5	None detected at the cut-off level	Validated, confirmatory/screening	León et al. (2022)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/ confirmatory	References
Milk, porcine muscle, egg	SC, L-hyoscyamine (AT), and sparteine residues	2 g or 2 mL sample + 10 mL EDTA buffer, then ACN with 0.5% trifluoroacetic acid → QuEChERS reagent (4 g of MgSO ₄ , 1 g NaCl, 1 g sodium citrate tribasic dihydrate and 0.5 g sodium citrate dibasic sesquihydrate) → QuEChERS dSPE kit (consisting of 150 mg of C18 sorbent and 900 mg of MgSO ₄) → 0.22 µm nylon filter	LC-MS/MS C18 A: 0.1% FA, 10 mM AF in H ₂ O B: MeOH	AT = 2 SC = 5	None detected	Validated, confirmatory	Zheng et al. (2019)
Goji berries	AT, SC, and anisodamine	1 g sample + 10 mL H ₂ O, MeOH with 1% formic acid + 4 g MgSO ₄ + 1 g NaCl → centrifuge tube with 900 mg of MgSO ₄ and 60 mg of sorbent, graphene/hexagonal boron nitride hybrids sorbent (G/h-BN) → filtration through a 0.22 µm nylon filter	UHPLC-MS/MS HSS T3 column (50 × 2.1 mm, 1.8 µm) B: ACN A: H ₂ O water (0.1% FA) with 5 mM ammonium acetate	AT = 3.2 SC = 4.5	AT = 1.7–23.4	Validated, confirmatory	Zhao & Shi (2020)
Maize flour (breadsticks) with poppy seeds and contaminated by Datura species	Three tropane and five opium alkaloids	MeOH with 0.1% acetic acid; 2.5:10 (sample:solvent ratio)	HPLC-diode array detector C18 column (3.0 mm i.d., 150 mm, x2.7 µm particle size) C18 guard column (3.0 mm i.d., 2.7 µm particle size) A: H ₂ O with 0.1% trifluoroacetic acid B: ACN	AT = 8 mg/kg (ppm) or 1 mg/L SC = 1.6 mg/kg (ppm) or 0.2 mg/L	AT = 0.94 (±0.13)–5.20 (±0.21) µg/g (ppm) SC = 0.08 (±0.01) µg/g (ppm)	Validated, confirmatory	Vera-Baquero et al. (2022)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/ confirmatory	References
Buckwheat, millet, soy, linseed	AT, SC, anisodamine, tropine, lit-torine, homatropine, apoatropine, apocopolamine, scopoline, tropinone, physoperuvine, pseudotropine, and cuscohygrine	H ₂ O: MeOH (2:1, v/v) with 0.5% acetic acid; 1:10 (sample:solvent ratio)→ SCX-SPE	LC-MS/MS C18 (100 × 2.1 mm, 1.8 µm particle size) followed by HILIC (100 × 2.1 mm, 3.5 µm particle size) A: ACN B: 0.1% FA in H ₂ O	With SPE: AT = 1 SC = 2 Without SPE: AT = 2 SC = 5	AT = 13±6 SC = 23±6 Intentionally contaminated millet flour (by Brugmansia and Stramonium)	Validated, confirmatory	Marín-Sáez et al. (2017)
Baby food: buckwheat, millet or a combination of cereals, including sorghum or wheat, were the main ingredients of samples.	AT, SC, anisodamine, homatropine, tropine, tropinone, and tropine	H ₂ O:MeOH (2:1, v/v) + 0.5% acetic acid; 1:10 (sample:solvent ratio) → 0.22 µm nylon syringe filter then online SPE Strata XC Polymeric SCX- SPE coupled to mass spectrometry (MS) analyzer	MS (Orbitrap or QqQ (triple quadrupole))	Orbitrap: AT = 1 SC = 5 QqQ: AT = 0.5 SC = 2.5	AT = 11.5 SC = 2.8	Validated, screening/confirmatory	Marín-Sáez et al. (2019b)
Green tea, boiled buckwheat pasta, water from boiling pasta	TAS	Tea and pasta: MeOH:H ₂ O 2:1 (v/v) + 0.5% acetic acid → tea and pasta: filtration through a 0.22 µm nylon syringe filter H ₂ O from boiling or tea infusion: acidified with 1% acetic acid → boiling or tea infusion: SPE tea solid residue: MeOH/H ₂ O/ A (75:25:0.4, v/v/v)	LC-MS/MS C18 (100 × 2.1 mm, 1.8 µm particle size) A: 0.1% FA in H ₂ O B: MeOH	AT = 2.5 SC = 5	AT = 1788.18 (±8)–4629.14 (±4) SC = 319.75 (±4)–4689.49 (±6) Intentional contamination by <i>Solanaceae</i> seeds	Validated, confirmatory	Marín-Sáez et al. (2019)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/confirmatory	References
Bread: buckwheat and millet flour contaminated with <i>Datura stramonium</i> and <i>Brugmansia arborea</i>	Aminobenzotropine, benzatropine, anisodamine, SC, AT, littorine, aposcopolamine, homatropine, apotatropine, cuscohygrine, calystegine C, calystegine B, calystegine A, scopoline, tropine, tropinone, tropanebenztropine	MeOH:H ₂ O (2:1, v/v) with 0.5% acetic acid; 3:30 (sample:solvent ratio)	LC-MS/MS C18 (100 × 2.1 mm, 1.8 µm particle size) A: 0.1% FA in H ₂ O B: MeOH	AT = 1 SC = 2	AT = 642.4 (±5)–4629.1 (±4) SC = 51.2 (±13)–4689.5 (±6)	Validated, confirmatory	Marín-Sáez et al. (2019a)
Bread made from wheat, multigrain, rye and wheat-rye	20 ergot alkaloids and six TAS	MeOH:H ₂ O: FA (60:40:0.4, v/v/v); 1:10 (sample: solvent ratio) → ultrafiltration over a 30 kD ultrafilter	UPLC-MS/MS C18 (150 × 2.1 mm, 1.7 µm particle size) A: 10 mM Ammonium carbonate (pH 9) buffer B: ACN	AT = 1 SC = 0.8	AT and SC at <LOQ SC = 0.22	Validated, confirmatory	Veršilovskis et al. (2020)
Black tea, peppermint leaves, fennel seeds, cereal products (biscuits for infants, cereals for porridge)	TAS	MeOH/H ₂ O/FA (39:60:1, v/v/v); 1:10 (sample:solvent ratio) → filtration	LC-MS/MS Pentafluoro phenyl, F5 column (10 cm × 2.1 mm, 2.7 µm particle size) A: H ₂ O B: aqueous MeOH, both with 2 M AF and 0.2% FA	AT ~ 0.4 SC = 1.2	N/A	Validated, confirmatory	Gonçalves et al. (2020)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/ confirmatory	References
Oats and wheat (whole grains)	370 pesticides, 2 TAs, 30 pyrrolizidine alkaloids, two plant growth regulators, and nine regulated mycotoxin	ACN:H ₂ O (80:20, v/v) → 0.45 µm PTFE filter	2D-LC-MS/MS HILIC, and RP of a Phenomenex Synergi Fusion-RP HILIC A: H ₂ O B: ACN and H ₂ O (90:10, v/v) both 5 mM AF and 0.1% AA RP column A: H ₂ O; B: MeOH both containing 5 mM AF and 0.1% AA	Oat: AT = 0.20 SC = 0.40 Wheat: AT = 0.16 SC = 0.27	N/A	Validated, confirmatory	Urban et al. (2019)
Wheat, maize, soybean	350 pesticides, 16 mycotoxins, and other contaminants including AT and SC	ACN/H ₂ O (80:20) → 0.20 µm PTFE filter	2D-LC-MS/MS HILIC, packed loop interface C8, and C18 HILIC A: H ₂ O B: 90% aqueous ACN with AF (5 mMol/L) and FA (0.1%) C18 column A: H ₂ O B: MeOH with 5 mMol/L AF and 0.1% FA	Both = 0.005	N/A	Validated, confirmatory	Kresse et al. (2019)
Buckwheat flour, pasta and bakery products	AT and SC	MeOH:H ₂ O (3:2, v/v) acidified with 0.2% FA + 0.2% ACN (pH 3.2); 1:5 (sample: solvent ratio)	UHPLC-MS/MS C18 Kinetex column (2.6 µm, 100Å; 100 × 2.10 mm) A: H ₂ O B: MeOH Both acidified with 0.2% FA	AT = 0.30 SC = 0.10	AT = 13.9–83.9 SC = 5.7–10.4	Validated, confirmatory	Cirlini et al. (2018)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/confirmatory	References
Herbal teas and infusions (both mono- or multi-ingredients), herbal extracts (hydro-alcoholic extracts) and supplements (tablets)	Anisodamine, AT, SC, homatropine	ACN/bi-distilled H ₂ O (3/2, v/v) acidified with FA (0.2%, v/v); 1:10 (sample: solvent ratio)	UHPLC-MS/MS C18 (2.6 µm, 100Å; 100 × 2.10 mm) A: H ₂ O B: MeOH Both acidified with 0.2% FA	Instrumental LOQ = 0.5	AT = 25 (±6)–69 (±9) SC = 50 (±25)	Validated, confirmatory	Cirlini et al. (2019)
Wheat, barley, rice, oats, spelt, rye	40 (modified) mycotoxins, two plant growth regulators, two TAs, and 334 pesticides	ACN:H ₂ O:FA (79:20:1, v/v/v) → Chromafil RC-20/15 filters	2D-LC-MS/MS HILIC (5 µm, 120 Å, 100 × 2.1 mm) FluoroPhenyl (2.7 µm, 50 × 2.1 mm) Biphenyl (2.7 µm, 50 × 2.1 mm) HILIC A: H ₂ O with 0.1% FA, 10 mM AF B: ACN/water (90:10, v/v) with 0.1% FA, 10 mM AF RP A: H ₂ O with 0.001% FA and 5 mM AF B: MeOH, 0.001% FA, 5 mM AF	Both = 5	None detected	Validated, confirmatory	Rausch et al. (2021)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/confirmatory	References
Herbal medicine (dried sample), TCM tablet for stomach cramp	Anisodine, SC, and AT (hyoscyamine)	MeOH:H ₂ O (95:5, v/v); 1:20 (sample:solvent ratio); 1 tablet+2 mL solvent (MeOH:H ₂ O (50:50, v/v)) → 0.22 µm membrane filter	2D-LC-DAD polar group modified column (2.1 × 250 mm, 2.2 µm) and then an SCX column (4.6 × 150 mm, 5 µm) polar group column A: ACN B: 100 mM sodium perchlorate+ 50 mM monosodium phosphate (NaH ₂ PO ₄) (pH 2.8) C: H ₂ O SCX column ACN: 100 mM NaH ₂ PO ₄ (pH 2.8) (50:50, v/v)	AT = 0.268 ppm SC = 0.195 ppm	AT = 0.012–0.126 mg/tablet SC = 0.0004–0.0039 mg/tablet AT = 18.65–368.43 mg/kg SC = 3.01–558.59 mg/kg	Validated, confirmatory	Long et al. (2018)
Buckwheat and flour; quinoa and amaranth; flour (teff, refined maize, maize, blue maize, sorghum, green and red lentil, chickpea, pea); peeled millet	AT and SC	Acidified H ₂ O (1.1% HCl); 1:8 (sample:solvent ratio) → filtered and purified through SPE (SCX-SPE), with SBA-15 mesostructured silica functionalized with sulfonic acid SCX-SPE with SBA-15-SO ₃ -	LC-MS/MS C18 (150 mm × 2.0 mm, 3.5 µm particle size) A: 0.1% FA in ACN B: 0.1% FA in H ₂ O	AT = 1.5 SC = 2.4	AT = 7 (±1)–78 (±12) SC = 28 (±6)	Validated; confirmatory	González-Gómez et al. (2020)
Ginger, cloves, fennel, cumin, aniseed, coriander, mix of spices and herbs (curry)	AT and SC	Acidified H ₂ O (HCl, pH 1) → SCX-SPE mesostructured silica-based material; dSPE	LC-MS/MS C18 A: 0.1% FA in ACN B: 0.1% FA in H ₂ O	AT = 1.9–3.3 SC = 5.0–9.4	AT = 3 (±1)–19 (±6) SC = 11 (±4)–26 (±6)	Validated, confirmatory	González-Gómez et al. (2023)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/ confirmatory	References
Aromatic herb samples (dried leaves), thyme, basil, coriander	AT and SC	Acidified H ₂ O (HCl 1.1%, pH 1.0) → (HMS-SO3- packed in SPE cartridge); dSPE	LC-MS/MS C18 A: 0.1% FA in ACN B: 0.1% FA in H ₂ O	AT = 2.6–6.8 SC = 4.0–7.2	AT = <5–42 SC = <5–34	Validated; confirmatory	González-Gómez et al. (2022)
Tea and herbal infusions	AT and SC	Boiling H ₂ O (infusion) → µSPEd® and PS/DVB sorbent	LC-MS/MS C18 (150 mm × 2.0 mm, 3.5 µm particle size) A: 0.1% FA in ACN B: 0.1% FA in H ₂ O	AT = 0.09–0.12 SC = 0.06–0.15	AT = 0.08 (±0.03)–3.88 (±0.31) SC = 0.48 (±0.08)–1.13 (±0.10)	Validated, confirmatory	González-Gómez et al. (2022)
Tea and herbal teas Intentionally contaminated by <i>Datura stramonium</i> and <i>Brugmansia arborea</i>	13 TAs	MeOH:H ₂ O:FA (75:25:0.4, v/v/v); 1:10 (sample:solvent ratio) → Strata-X-Cartridge and a 0.45 µm nylon filter	LC-HRMS HILIC column (100 × 2.1 mm, 1.8 µm particle size) coupled to a C18 column (100 × 2.1 mm, 1.8 µm particle size) A: H ₂ O with 0.1% FA B: ACN	AT = 5 SC = 10	AT = 9–360 SC = 397–1725	Validated; confirmatory	Romera-Torres et al. (2018)
Animal feed (containing wheat, wheat bran, maize, barley, soy, oats, legumes, alfalfa)	Anisodamine, apoeatropine, apocopolamine, AT, homatropine, littorine, physoperuvine, pseudotropine, tropine, SC, tropane, tropinone	MeOH/H ₂ O/FA (75/25/0.4, v/v/v; pressurized liquid extraction) → cartridges (Strata-X-C); additional clean-up with 150 mg chitosan; 0.45 nm nylon filter	LC-HRMS HILIC column (100 × 2.1 mm, 1.8 µm particle size) coupled to a C18 column (100 × 2.1 mm, 1.8 µm particle size) A: H ₂ O with 0.1% FA B: ACN	AT = 5 SC = 25	AT = 5–148 SC = <LOQ–222	Validated, confirmatory	Romera-Torres et al. (2018b)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/ confirmatory	References
Honey	Anisodamine, apatropine, AT, homatropine, littorine, physoperuvine, pseudotropine, SC, scopoline, tropinone	MeOH/H ₂ O/FA (75/25/0.4, v/v/v) → supernatant mixed with 300 mg MgSO ₄ and 50 mg GBC, then filtration 0.45 µm nylon filter	LC-HRMS HILIC column (100 × 2.1 mm, 1.8 µm particle size) A: 5 mM AF and 0.1% FA in H ₂ O B: MeOH	AT = 20 SC = 20	SC = 27	Validated; confirmatory	Romera-Torres et al. (2020)
Sorghum, oregano, mixed herbal tea	21 TAs and 33 pyrrolizidine and N-oxides	MeOH:H ₂ O:FA (60:39.6:0.4, v/v/v); 1:10 (sample: solvent ratio) → dSPE, then filtration 0.22 µm	LC-MS/MS C18 (150 × 2.1 mm; 1.6 µm particle size) A: H ₂ O B: MeOH Both contained 2 mM AF and 0.2% FA	Sorghum Both = 0.5 Oregano: AT = 2 SC = 1 Herbal tea: Both = 2	N/A	Validated, confirmatory	Dzuman et al. (2020)
Millet contaminated by Datura seeds	AT and SC	MeOH:H ₂ O:98% FA (600:400:4, v/v/v); 1:10 (sample: solvent ratio) → filtration	LC-MS/MS Phenomenex RP (150 × 2.0 mm) filled with Luna phenyl hexyl material (5 µm particle size) A: H ₂ O B: MeOH Both contained 0.1% FA	AT = 0.01 SC = 0.025	AT = 0.65 (±0.02)–144.77 (±14.38) SC = 2.03 (±0.16)–53.20 (±5.45)	Validated; confirmatory	Begemann et al. (2021)
Seeds of <i>Datura stramonium</i>		MeOH/H ₂ O/98% FA 600/400/4, v/v/v; 0.1:10 (sample: solvent ratio) → matrix solid-phase dispersion material					
Feed samples, and materials such as soybean meal, rapeseed meal, and maize	AT and SC	MeOH:H ₂ O:FA (60:40:0.4, v/v/v); 1:8 (sample: solvent ratio) → PCX cartridges and filtration by PVDF filter	LC-MS/MS Kinetex C18 (2.6 mm, 100 mm × 4.6 mm column) A: 0.3% FA in H ₂ O B: MeOH	AT = 0.93 SC = 0.92	AT = 1.4–104.8 SC = 1–58	Validated, confirmatory	Kowalczyk & Kwiatek (2022)

(Continues)

TABLE 3 (Continued)

Sample	Analytes ^b	Extraction ^c	Analytical technique ^d	LOQ (ppb)	Levels detected (µg/kg)	Validated/screening/ confirmatory	References
Buckwheat grain, buckwheat food products, whole grain, groats (kasha), flour, pasta, bread, samples of a semi-prepared meal, buckwheat foods	AT and SC	Filtration	UHPLC–HRMS C18 (no information) A: 20 mM ammonium acetate B: ACN	Both = 30	AT = <30–26,000 SC = <30–12,000	Validated; confirmatory;	Perharič et al. (2013)
Granules (4), tablets (4), and solution (1) of homeopathic products and an ointment mainly produced from <i>Atropa belladonna</i> ; 1 tablet produced mainly from <i>Atropinum sulfuricum</i>	Anisodine, SC, benzoylcegonine, AT, littorine, aposcopamine, homatropine, apoatropine, cuscohygrine, calystegine, scopoline, tropine/physoperuvine, pseudoatropine, tropinone, tropane	MeOH:H ₂ O 2:1 (v/v), containing 1% acetic acid; 1.5 g solid sample or 1.5 mL liquid sample + 250 mL solvent → Strata X-C cartridges were used for the v/v) SPE → filtration 0.5 g ointment + 10 mL toluene Supernatant → 0.2 µm nylon filter <i>Atropa belladonna</i> seeds- MeOH:H ₂ O 2:1 (v/v) containing 0.5% acetic acid; 25 mg seed + 25 mL solvent	UHPLC–HRMS C18 (100 × 2.1 mm, 1.8 µm particle size) A: H ₂ O with FA (0.1%, v/v) B: ACN	AT = 1 SC = 2.5	AT = 0.03–4007 SC = 1.70–1139	Validated; confirmatory/screening	Marin-Sáe et al. (2018)
Dried plant material from different teas or from parts of Solanaceae plants	AT and SC	125 mg herbal material + 10 mL boiling H ₂ O for infusion → diluted to 5 mg sample/mL → filtration	MALDI–TOF–MS/MS	LOD: AT = 5 ng/mL SC = 0.5 ng/mL	AT = (dried plant material) 0.16–0.74 µg/mg AT = (berries) 1.1–2.4 µg/mg AT (leaves of deadly nightshade) AT (leaves of angel's trumpet) 0.05–0.15 µg/mg SC = 0.003–0.01 µg/mg (leaves of deadly nightshade) SC = 0.22–0.54 µg/mg (leaves of angel's trumpet)	Validated, confirmatory	John et al. (2018)

^aLLE, liquid–liquid extraction; SLE, solid–liquid extraction, SALLE: salt-assisted LLE, SPE, solid-phase extraction; QuEChERS, quick, easy, cheap, effective, rugged, and safe

^bSC, scopamine; AT, atropine.

^cACN, acetonitrile; MeOH, methanol; H₂O, water; H₂SO₄, sulfuric acid; Zn, zinc; EDTA, ethylenediaminetetraacetic acid; SCX, strong cation-exchange; HCl, hydrochloric acid; NaCl, sodium chloride

^ddSPE, dispersive SPE; PSA, primary secondary amine; MgSO₄, magnesium sulfate; GBC, graphitized black carbon; LOD, limit of detection; LOQ, limit of quantification; FA, formic acid; HILIC, hydrophilic interaction LC column; AF, ammonium formate; NH₄OH, ammonium hydroxide
A, mobile phase A; B, mobile phase B.

(2014), including exposure by mislabeling or mistaken identity of toxic plants *Datura* seeds mixed with frozen vegetable-bean-seed mix from Belgium with raw materials coming from Spain led to the poisoning of several people in Finland in 2013, while the presence of *D. stramonium* in canned green beans poisoned people in France in 2010. Furthermore, *Datura* leaves mistakenly as Jew's mallow poisoned some people in Japan, and mistakenly using *Datura* leaves in stew poisoned a family in the United States (Adamse et al., 2014). In 2003, 73 consumers in Slovenia experienced similar symptoms: hot red skin, tachycardia, urinary retention, ataxia, speech disturbance, and visual hallucinations following ingestion of buckwheat flour products (Perharič et al., 2013). In 1984, a poisoning outbreak happened in Ethiopia following the consumption of corn flour contaminated by *D. stramonium* (Aga & Geyid, 1992). There were 688 victims who showed symptoms such as dryness of mouth, throat, blurred vision, dry and flushed skin, restlessness, confusion, talkativeness, and delirium; 33 required hospitalization, and nine died (Aga & Geyid, 1992). Poisoning also occurred by misuse or abuse of *Datura* among adolescents in the United States, Canada, and Mexico (Kerchner & Farkas, 2020). The resemblance of *Datura* seeds to capsicum and the consumption of honey contaminated by *Datura* and Ayurvedic medicine, which involved the internal use of this plant, also poisoned some people in India (Kerchner & Farkas, 2020).

The importance of having good practices (e.g., Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP)) and food safety and quality management systems (e.g., Hazard Analysis Critical Control Point, ISO 22000) strictly implemented can help mitigate though not prevent the contamination of foods by TAs or their plant source, and occurrences of food poisoning incidents. This aspect of risk management associated with TAs was recommended by international organizations in global discussions (FAO/WHO, 2020) and was subsequently reviewed extensively by de Nijs et al. (2023), which are summarized in the following discussions: the essential components of GAP include identifying and monitoring the presence of the different invasive plants or weeds that produce toxic TAs at cultivation and harvest, implementing approved weed management, and separating contaminated crops at harvest. Education and training programs for adult learners, farmers, or producers must be integrated to realize these. For the GMP, manufacturers should choose suppliers that observe GAP. Food processors should establish standard operating procedures to prevent and detect the presence of TAs and TA-producing plants. Furthermore, strict inspection and quality control of raw materials, cleaning of equipment and facilities and continuous monitoring of manufacturing processes must be

observed. Thus, employee training is crucial to GMP. Integrating food safety and quality management systems and GMP, with continuous improvement, ensure safety from TA contaminating the food (Abia et al., 2021; Codex, 2024; de Nijs et al., 2023; FAO/WHO, 2020). Last, educating the public about the risks of consuming toxic plants is also essential; some have already been implemented, for example, distribution of information including pictures/videos such as Arvalis on YouTube, a leaflet “Managing toxic weeds in vegetable crops and beyond” on the website of PROFEL, the leaflet on “*Datura sp.* as a potential risk for agricultural production in Belgium,” and the “Guidance document on physical *D. stramonium* seed contamination” of FAO/WHO (Codex, 2024).

It is also evident from the various recorded episodes of TA poisonings that developing reliable, robust, rapid, and affordable analytical methodologies for food safety and quality control is critical to ensure that contamination of TA in food and feed does not exceed the ML set by the regulatory agencies (Table 1). The European Commission recommended developing accurate methods for detecting and quantifying atropine and scopolamine in herbal teas and their infusions and other foods likely to be contaminated by TA (Cirlini et al., 2018; EU, 2015). In the past 10 years (2013–2023), different methodologies have been developed and applied to determine TA in various matrices and have been compared in the current review. In addition, this systematic review examines immunoassays and spectroscopy as potential rapid techniques for screening and monitoring contamination by TA in food and nonfood samples. Although other reviews on TA have been published (Adamse et al., 2014; Cinelli & Jones, 2021; de Nijs et al., 2023; Dräger, 2002; Kohnen-Johannsen & Kayser, 2019; Mulder et al., 2016; Romera-Torres et al., 2018a), our current review was conducted systematically, covering studies from 2013 to 2023 in food and feed and focusing on the recent analytical techniques applied in their determination, emphasizing atropine and scopolamine.

2 | REVIEW METHODOLOGY

This review aims to understand the characteristics of TA, particularly atropine and scopolamine, as a basis for their determination in complex food and feed matrices, describing the different methods (sample preparation and instrumental analysis) used for their detection and quantification (and other contaminants, if available, in the matrix), and highlight the applicability of the methods based on the existing regulations on rapid assessment of their levels in food and feeds.

The information for the systematic review was retrieved following the Campbell Methods Guide (Kugley et al.,

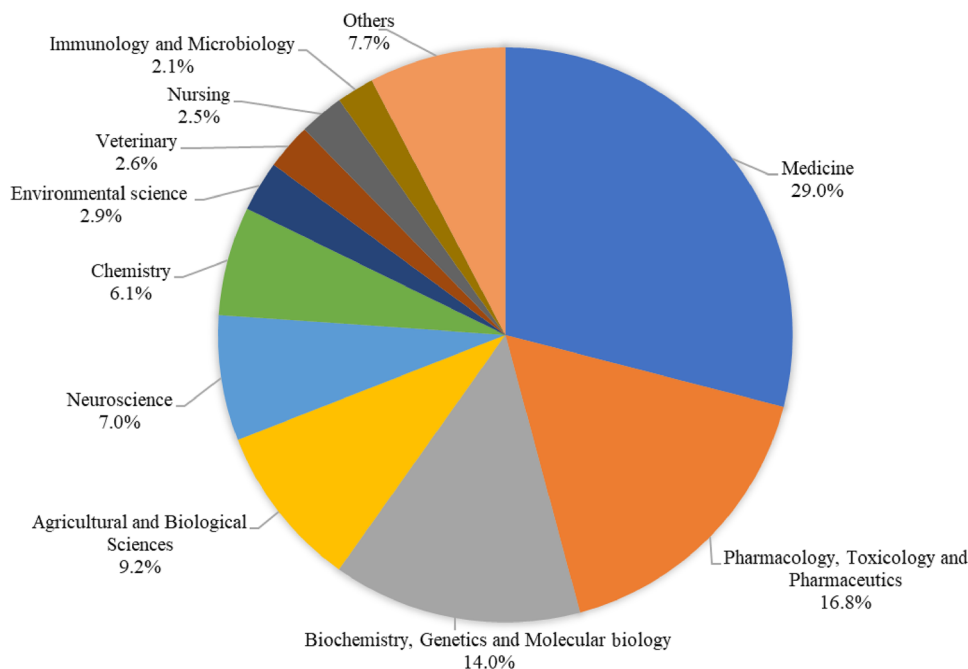


FIGURE 3 Studies on tropane alkaloids between 2013 and 2023 divided into different subject areas retrieved from Scopus.

2016). The PRISMA Extension for Reviews checklist (Tricco et al., 2018) was used to determine the different methods available for detecting and quantifying TA in food and feed, the strengths and weaknesses of the methods, and the potential for use as the standard method for quality control and monitoring purposes from farm to fork. An initial literature search was conducted by screening each article's title and abstract, followed by a full-text assessment. Search results were evaluated by two researchers and validated by another researcher to check for variations in the results obtained. All citations were exported to EndNote, and duplicates were removed before full-text screening. The screening results have been presented in a modified PRISMA chart.

A systematic review of peer-reviewed and gray literature was conducted to identify studies on the detection of TA in food and feed. Electronic searches on Scopus, Web of Science, and PubMed databases were performed using key search terms (also used to search for gray literature), which were piloted in Scopus: (tropane alkaloid*) OR (scopolamine*) OR (hyoscyne*) OR (atropine*) OR (hyoscyamine*) AND (food*). There have been many studies on these TAs in the last 10 years (2013–2023). Using these keywords, Scopus, for instance, generated a report that breaks down 706 articles into different subject areas (Figure 3—search results in Scopus by subject areas), where medicine has the highest percentage. Food and feed-related studies on determining TA fall under the chemistry, agricultural, and biological science subject areas.

In addition, the Google Scholar and Google search engines were also examined to identify further analytical methods used on TA, reports on poisoning incidents, product recalls related to TA, and relevant international and government agencies associated with food safety regulations regarding TA. Specific gray literature sources included Food Safety News, European Commission, WFP, European Food Safety Authority (EFSA), WHO/FAO, United Nations Children's Fund (UNICEF), and United States Food and Drug Administration (US FDA).

Studies were identified by searching literature published in English only between the years 2013 and 2023 of the databases. No restrictions were placed on geographical regions, and no particular study design was specified. The search strategy was piloted to test the suitability of the electronic databases and the selected keywords. Any publications relevant to TA other than atropine (or hyoscyamine) and scopolamine (or hyoscyne) in food or feed were excluded but noted. Conference proceedings and in vitro and animal/human studies were also excluded. Analytical methodologies for detecting and quantifying atropine and scopolamine in plant sources, biological samples for toxicology/forensics, and drug or pharmaceutical studies were also excluded. Still, they were recorded and mentioned in the methodology (Figure 4—other relevant publications).

The Critical Appraisal Skills Programme Tools Checklists (CASP, 2023) ensured that only relevant, high-quality studies were included in the review and low-quality

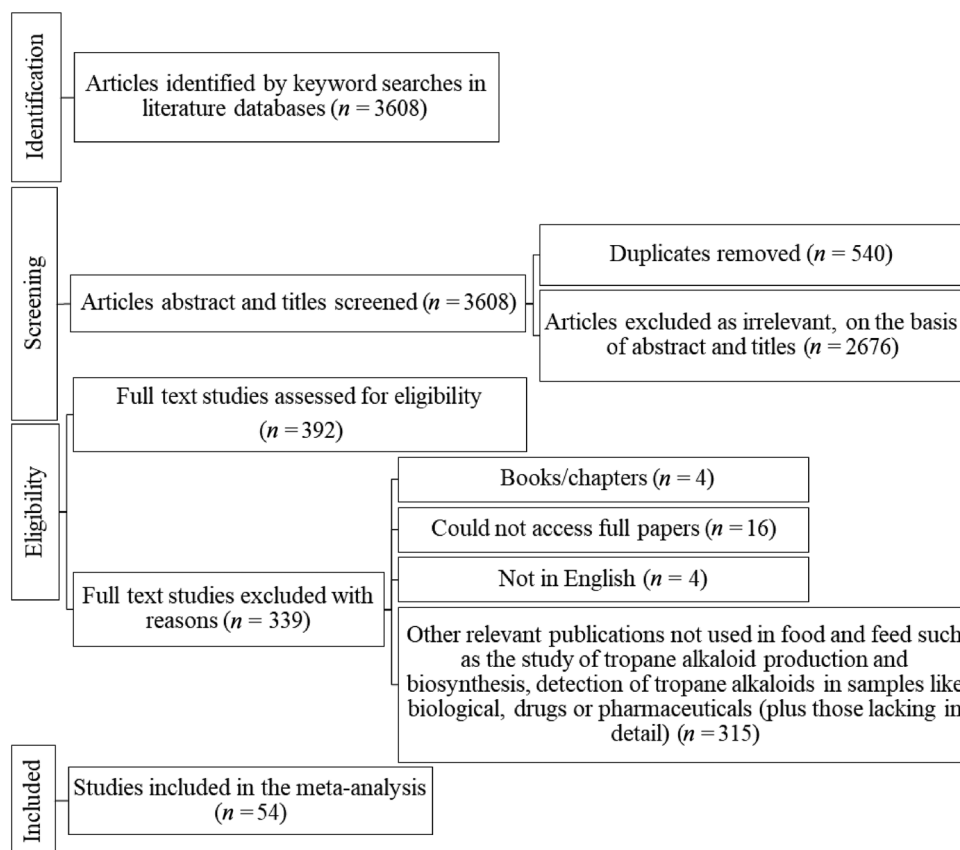


FIGURE 4 Literature search methodology.

studies were excluded, by adequately answering the following questions (Meneely et al., 2022).

1. Is the publication time within the last 10 years?
2. Are the title and abstract in English?
3. What were the aims of the study?
4. What were the main findings of the paper?
5. What are the strengths and limitations of the research and methodology?
6. Did the author acknowledge any limitations?
7. Were the findings consistent with other literature?

The studies were classified based on their relevance to the research goals using keywords, and additional queries were made. The following were examined to check the analytical methods used for TA detection in the last 10 years. Studies that used human food or animal feed as samples (the latter was included because the main ingredients were usually cereals or pseudo-cereals also consumed by humans) and TA plant sources. The extraction or sample preparation procedures for each method and the instrumentation for identification and/or quantification of atropine and scopolamine were also examined. Studies that have multiresidue (multiple analytes) determination were also considered. Method development and valida-

tion were also evaluated, including parameters such as extraction recoveries, the limit of detection (LOD) and quantification (LOQ). The scope of the search was worldwide. The significance of studying TA and establishing methods for their detection was also elaborated.

A narrative synthesis was carried out to facilitate the inclusion of all the extracted data (qualitative and quantitative). The data was obtained from scientific publications and gray literature to provide an overview of the global significance of atropine and scopolamine, or TA in general, in food safety and security. The study addressed the characteristics of these TA, the importance of developing a rapid method for their detection and quantification, and the validation of analytical methods for various purposes.

Three thousand six hundred and nine references were retrieved, of which one was retracted; thus, 3608 were subjected to screening and critical appraisal considering qualitative and quantitative studies. A summary of the critical appraisal approach has been detailed above, and the procedure summary is shown in Figure 4. Overall, 54 papers passed the critical appraisal process and were included in the review. The sources of information retrieved were international and government agency websites, providing legislative documents, reports, guidelines, and standards.

3 | ANALYTICAL METHODS FOR TA DETERMINATION

In the last 10 years, most TA analyses have been based on liquid chromatography (LC) and mass spectrometry (MS), specifically LC–tandem MS (LC–MS/MS); hence, this review will emphasize this platform (Section 3.2). Nevertheless, methods based on gas chromatography (GC), capillary electrophoresis (CE), thin-layer chromatography, or high-performance LC (HPLC) with MS or diode array detector (DAD) have been employed in studying TA, and related reviews have been published elsewhere (Dräger, 2002; González-Gómez et al., 2022; Romera-Torres et al., 2018a).

Here, the literature was also reviewed for the availability of nonchromatographic techniques, such as immunoassays and spectroscopy, which have the potential to provide sufficient information on contamination levels more rapidly, with comparable sensitivity, and at a much lower cost. Other technological advancements that involve the use of biosensors and smartphones were also reviewed. Despite the power, accuracy, and sensitivity of modern LC–MS/MS instruments, sample pretreatment can still be time-consuming and costly, sometimes leading to high operational costs. Thus, it is still challenging to use LC–MS/MS-based methods for routine, in-field, and real-time applications, especially for large sample sets. Many crops prone to contamination by TA are also susceptible to contamination by other natural and synthetic toxins, including mycotoxins and pesticides. Therefore, strategies capable of simultaneously determining their levels are highly advantageous. Furthermore, an appropriate concentration of the target compounds/analytes should be detected and quantified reliably to the lowest level possible (LOQ) to meet the ML set by regulatory agencies, outlined in Table 1. With the increasing incidences of TA-related food poisoning, a rapid, high-throughput, eco-friendly, and inexpensive approach that is flexible enough to detect them as early as the harvest and at all stages in the food chain would be ideal.

3.1 | Sample preparation for liquid chromatography

TAs typically contaminate plant-based food since the primary source of these toxins are plants/weeds that grow among crops (Chain, 2013; EFSA, 2008; Klein et al., 2022). With regards to animal-derived products, recent studies show minimal transfer of TA from animal feed to milk, meat, and eggs (Lamp et al., 2021; Zheng et al., 2019), and no MLs have yet been established for these commodities. However, for the first time, Zheng et al. (2019) reported

the LOD and LOQ of atropine and scopolamine in animal-derived products.

Physical separation of TA-containing plants (e.g., seeds or leaves of *D. stramonium*) from harvested crops (e.g., cereals or tea) may only sometimes prevent contamination since all plant organs are toxic. Begemann et al. (2021) reported that abrasion of the *Datura* plant could result in invisible TA contamination from harvest to processing. Complete removal of the plant material at harvest before further processing can eradicate TA; otherwise, a single seed can contaminate a kg of dehulled millet with 0.79 (± 0.09)–1.96 (± 1.76) $\mu\text{g/kg}$ within only 15 min of exposure (Begemann et al., 2021). Invisible TA contamination (abrasions) on dry food or feed materials and the possible heterogeneity of samples may make it difficult to get a representative sample. Thus, mixing samples thoroughly, grinding, or comminution can help alleviate the possible bias due to sampling in the analytical results. Whatever sample preparation technique is used following extraction of TAs from a sample will always lead to co-extraction of matrix components, which can result in matrix effects, either through suppression or enhancement (SSE). The amount of this SSE is very much dependent on the choice of extraction solvent and the complexity of the sample/commodity, with matrix effects giving rise to some of the most challenging issues in analytical chemistry, which include ion suppression or enhancement, poor chromatography, false positives due to the presence of isobaric compounds and can lead to poor analyte recovery due to chemisorption amongst others (Lehotay et al., 2015). Therefore, in order to maximize the levels of target analytes extracted as well as simultaneously trying to mitigate matrix effects, the choice of extraction time and solvent are vital considerations (Zhao & Shi, 2020). Furthermore, depending on the MLs required, sample clean-up may be necessary to eliminate SSE as much as possible to meet the MLs, with various sample clean-up techniques such as solid-phase extraction (SPE) and QuEChERS (quick, easy, cheap, effective, rugged and safe) discussed below.

TAs are generally small polar compounds; their ternary amine group protonates at a low pH (González-Gómez et al., 2022; Marín-Sáez et al., 2019a); thus, most extraction solvents employed in TA studies consisted of a mixture of water and organic solvents (e.g., methanol or acetonitrile), which are acidified (e.g., acetic, formic, or trifluoroacetic acid) (Table 3 shows the different extraction methods and LC–MS analysis requirements for TA). Adding water to organic solvent significantly improves the solvent polarity and extraction efficiency; the pH dependency of the partition behavior of TA between organic and water makes the presence of acid valuable in some extraction methods (Zhao & Shi, 2020).

Most sample preparations cited in this review were developed and validated according to the European SANTE/12682/2019, SANTE/11945/2015 or SANTE/11813/2017 documents or validated in-house according to Commission Decision 2002/657 requirements. Some research groups also followed the 2013 AOAC International Guidelines for Dietary Supplements and Botanicals, IUPAC harmonized guidelines for single-laboratory validation, US Department of Health and Human Services, FDA Guidance for industry bioanalytical validation 2001, or the procedures described by the Korea Ministry of Food and Drug Safety based on Codex standards. Many of the methods developed in the past number of years were not only dedicated to the determination of atropine and scopolamine but also of other analytes such as ergot, opium, and pyrrolizidine alkaloids, pesticides, growth regulators, antibiotics, and other plant toxins in solid or liquid food and feed matrices, making method development more challenging due to varying physicochemical properties of each class of analytes incorporated (Bessaire et al., 2021; Klein et al., 2022; León et al., 2016). High percentage recovery and low relative standard deviation (RSD) are metrics associated with an efficient extraction method, with recoveries between 70 and 120% deemed acceptable based on SANTE guidelines, with associated repeatability (intra- or within-laboratory precision) $RSD \leq 20\%$ for all analytes within the scope of a method (EURL, 2021). Outside this recovery rate, it can be accepted if they are consistent with RSD of $\leq 20\%$ and the basis for this is well established (e.g., due to analyte distribution in a partitioning step). However, the mean recovery must not be lower than 30% or above 140% (EURL, 2021). Depending on the nature of the samples and the analytes of concern, the typical extraction methods used are solid-liquid extraction (SLE) or liquid-liquid extraction (LLE) with (or without) SPE used as the clean-up step in order to reduce matrix effects and improve method performance (Table 3). A favorable extraction procedure should use the minimum amount of nonhazardous (organic) solvents, be inexpensive, rapid and easy to execute, be good for the environment, and result in high target analyte recoveries and reduced matrix effects (Chanoti et al., 2014). These characteristics align with meeting green analytical chemistry for food safety studies, which aim for fast, simple, and cheap analytical approaches (López-Lorente et al., 2022).

Classic LLE employs water-miscible organic extraction solvents, which can be hazardous to health and the environment (Khatibi et al., 2021). In high-fat and protein samples that are expected to contain low levels of TA, such as animal products, removing co-eluting compounds improves the accuracy and sensitivity of the detection method (Zheng et al., 2019). TA extraction in liquid sam-

ples such as milk requires degreasing steps with n-hexane (acidified), freeze-out, ultracentrifugation, SPE clean-up, or concentration steps (Klein et al., 2022). Although a long (overnight) extraction procedure, freezing high-fat milk samples in acidified methanol, followed by cooled centrifugation, resulted in clear extracts ready for injection into LC (Lamp et al., 2021). Others collected the organic phase from the LLE, which contains TA, with this dried and reconstituted in order to concentrate the analytes before filtering for LC-MS analysis (Ng et al., 2013). The drying and reconstitution steps can be time-consuming and, if not carefully performed, can result in the loss of analytes.

Salting-out LLE (SALLE) is a cost-effective, less time-consuming, eco-friendly, and simpler version of LLE that requires no clean-up, evaporation, or reconstitution steps but with enrichment capability (Rizzo et al., 2022). SALLE generates less heat and results in higher recoveries than modified QuEChERS when used to extract TAs in honey (QuEChERS will be discussed later in the section) (Thompson et al., 2020). In SALLE, a concentrated salt solution with zinc powder is used (zinc reduction is required when working with analytes of high hydrophilicity) (Rizzo et al., 2022), followed by the addition of acetonitrile (Thompson et al., 2020). The sample and SALLE system being immiscible to each other results in partitioning extracted analytes into the acetonitrile phase, with sugars and organic acids remaining in the aqueous layer (Thompson et al., 2020).

In SLE, TA in a solid matrix (e.g., cereals, spices, herbs, or tea) is extracted with a suitable solvent, similar to that used in LLE (Table 3—extraction column). Extracts containing multiple analytes may also need evaporation and reconstitution to increase analyte concentration to aid detection, followed by filtration before LC-MS analysis (Shimshoni et al., 2015). If only atropine and scopolamine are to be analyzed, a lower method LOQ can be attained due to a targeted clean-up approach being permitted, as opposed to having multiple target analytes whereby sample clean-up techniques can lead to the removal of some of the target analytes due to their varying physicochemical properties. On the other hand, SPE enriches and concentrates TA in the aqueous extracts by use of cartridges such as a strong cation exchange (SCX), C18, polymeric cation exchange (PCX), Strata-X, or OASIS mixed-mode strong cation exchange (MCX). The choice of SPE cartridges is critical since some may not retain the analytes of choice, resulting in poor recoveries. For example, PCX-SPE is based on ionic interactions and will not retain hydrophobic molecules. In the extraction of 12 TA in animal feeds, a pressurized liquid extraction (PLE) with SPE Strata-X-C cartridge was used, and an additional clean-up step with chitosan as sorbent was employed (Romera-Torres et al.,

2018b). Despite the high recoveries and low LOQ that can be obtained when using SPE with LC–MS/MS, SPE-based extraction methods can be expensive, laborious, and time-consuming.

A rapid SLE-SPE method for ultra HPLC (UHPLC)–MS/MS was developed for the detection of atropine and scopolamine in spinach and infant products with very low LOQ (0.014–0.018 $\mu\text{g/kg}$) and excellent recoveries (94.1–102.6% and $\text{RSD} < 5\%$) (Castilla-Fernández et al., 2021). SLE plus a clean-up step with magnesium sulfate and graphitized black carbon (GBC) also resulted in a clear and colorless extract for LC–MS analysis (Romera-Torres et al., 2020). Clean-up and concentration of extracts using Strata X-C or Oasis MCX cartridge was also found helpful following the use of SLE (Marín-Sáez et al., 2017; Mulder et al., 2016; Romera-Torres et al., 2018). Compared with offline SPE, online SPE is quicker since the sample extract is directly injected into the MS analyzer, with the authors suggesting it to be more advantageous due to its automation, high throughput, and simplicity and that the method could be used as a rapid screening test for detecting low concentrations of samples for TA with a LOQ (0.5–10 $\mu\text{g/kg}$) (Marín-Sáez et al., 2019b). SLE without SPE can also be used to obtain cleaner extracts containing multiple analytes, with the extracts filtered before injection to LC–MS (Marín-Sáez et al., 2019a; Marin-Saez et al., 2019), and is capable of obtaining low LOQs (0.8 and 1.0 $\mu\text{g/kg}$) (Mulder et al., 2015; Veršilovskis et al., 2020), with both these methods using standard addition to correct for both the recovery of extraction and matrix effects. However, considering the ML of 1 $\mu\text{g/kg}$ for baby food and processed cereal-based foods for infants and young children (Table 1), a method with LOQ of 1 $\mu\text{g/kg}$ is not fit for purpose. Similarly, a direct analysis for a multianalyte method was developed for various commodities, including organic buckwheat flour products, herbal teas, and their infusions, with satisfactory LOQs for cereals ($<1 \mu\text{g/kg}$) (Gonçalves et al., 2020), dried tea (25–50 $\mu\text{g/kg}$), herbal infusions (0.20 $\mu\text{g/kg}$), and cereals (5–15 $\mu\text{g/kg}$) (Cirlini et al., 2019; Cirlini et al., 2018), which showed that the method was fit for purpose.

QuEChERS has been employed in the extraction and clean-up of atropine and scopolamine, together with other alkaloids such as pyrrolizidine and their N-oxides, as well as mycotoxins among others (Baslé et al., 2020; Bessaire et al., 2021; León et al., 2022; Vuković et al., 2018; Zheng et al., 2019). In QuEChERS, extraction/partition of the sample is carried out by adding the extraction solvent and QuEChERS salts, removing residual water, and treating the extract with dispersive SPE (dSPE) to remove matrix components. An aliquot of the upper (organic) layer is transferred to a centrifuge tube containing a PSA (primary and secondary amine) sorbent and magnesium sulfate,

which is shaken before centrifugation (Anastassiades et al., 2003; Santana-Mayor et al., 2019). In the dSPE technique, the direct addition of a sample extract to the sorbent material improves the interaction area between the sorbent and analytes (González-Gómez, et al., 2022). When QuEChERS was used for animal products (muscle, egg, and milk), acetonitrile with trifluoroacetic acid was used to deproteinize the animal tissues, and EDTA buffer to improve the method accuracy (Zheng et al., 2019). For matrices rich in starch, pigment, and fat, which can all interfere with TA detection, clean-up steps using dSPE following QuEChERS were found to be optimum (Zhao & Shi, 2020), with different sorbents (C18, G, h-BN, and G/h-BN hybrids) tested. G/h-BN hybrids supplied the best clean-up efficiency and had a better affinity for fat and other lipophilic components (Zhao & Shi, 2020). A C18 sorbent in the CEN QuEChERS method used a dSPE clean-up, which effectively adsorbed fatty acids in animal-derived products (Zheng et al., 2019). A PS/DVB-RP sorbent could retain phenolic compounds (González-Gómez et al., 2022), while PSA sorbents exclude many polar matrix components, such as organic acids, certain polar pigments, and sugars in the extracts to some extent (Anastassiades et al., 2003; Santana-Mayor et al., 2019). When compared, clean extracts and best recoveries were obtained using dSPE sorbents such as PSA and GBC combined (Chen et al., 2017). Others have used Q-sep dSPE with PSA and magnesium sulfate, with the extracts evaporated and reconstituted (Martinello et al., 2017). An HMS-SO_3^- sorbent was developed as an alternative to classical commercial sorbents or other mesostructured silicas such as SBA-15 (González-Gómez et al., 2022). SPE using SBA-15- SO_3^- sorbent instead of Oasis MCX was used to eliminate the coextracted hydrophilic substances (González-Gómez et al., 2020, 2023).

A variant of SPE, a $\mu\text{SPEed}^\circledR$, uses a small volume syringe (500 μL) with PS/DVB sorbent; no wash steps were involved, and extracts from tea and herbal infusions were injected directly into HPLC (González-Gómez et al., 2022). The $\mu\text{SPEed}^\circledR$ extraction protocol is considered a green sample preparation technique and is an improvement on other traditional SPE methods that use larger amounts of sample, organic solvents, and analysis time (Mulder et al., 2016). It can be easily scaled to automatic and high-throughput systems using the ePrep $^\circledR$ Sample Preparation Workstation (EPREP, Australia) sorbent (González-Gómez et al., 2022). For a $\mu\text{-QuEChERS}$ extraction, a small quantity of sample (0.1 g) and solvent was needed; the procedure was relatively quick, although it included clean-up steps and filtration before injection into the system (González-Gómez et al., 2022). One caveat is that for the analysis of food/feed, larger sample sizes of at least 1 g are required to provide an accurate result.

The dilute and shoot technique, which aims to reduce the matrix effects for multianalyte determination in various food matrices, was reviewed extensively by Greer et al. (2021). In this technique, the sample or the extract is diluted, centrifuged, and filtered before injection into the MS systems with no further clean-up techniques necessary such as SPE, immunoaffinity columns, or QuEChERS (Greer et al., 2021). The dilute and shoot technique has been applied in the sample preparation for the determination of 295 bacterial and fungal metabolites in apple puree, hazelnut, corn and green pepper (Malachová et al., 2014), and for over 500 mycotoxins and metabolites in wheat, corn, figs, dried grapes, walnuts, pistachios, and almonds (Sulyok et al., 2020). The dilute and shoot technique was also applied to extract atropine and scopolamine in corn, wheat, soybeans, millet, and sorghum, with validation parameters that were within the acceptable limits (Gravador et al. submitted). The dilute and shoot technique is simple, resulting in minimal to no analyte losses and rapid and high throughput; however, diluting the matrix (and the analytes) could result in higher detection limits (Greer et al., 2021).

In conclusion, various extraction and clean-up methods are available depending on the matrix to be analyzed, the analytes of concern, the detection level required, and instrumentation to be used. Many of the extraction methods presented so far helped attain a LOQ lower than the ML set by the authority, indicating the method was fit for purpose. Ideally, less sample pretreatment would be advantageous if large quantities of samples are analyzed in a short period, and it would also be beneficial if other analytes that may potentially be present could be included in the analysis to future-proof the method. Thus, a dilute and shoot method following SLE or LLE would provide simple and rapid sample treatment.

3.2 | Liquid chromatography–mass spectrometry

LC–MS is often considered the gold standard in food analysis because of its accuracy, sensitivity, and specificity. In the past 10 years, only one study used LC coupled to a DAD (HPLC–DAD) in the study of TA (Vera-Baquero et al., 2022); the rest employed LC–MS in the detection and quantification of TA alone (Chen et al., 2017; Cirlini et al., 2018; González-Gómez et al., 2020; Perharič et al., 2013; Vuković et al., 2022) or with other analytes such as pesticides (Kresse et al., 2019; Urban et al., 2019), mycotoxins (Bessaire et al., 2021; Rausch et al., 2021), ergot alkaloids (Mulder et al., 2015; Veršilovskis et al., 2020), pyrrolizidine alkaloids (Dzuman et al., 2020; Klein et al., 2022; León et al., 2022; Martinello et al., 2017; Martinello

et al., 2022; Shimshoni et al., 2015), opium alkaloids and others (Bessaire et al., 2021; Vera-Baquero et al., 2022) in food and feeds. HPLC methods, including UHPLC–MS/MS (Castilla-Fernández et al., 2021; Chen et al., 2017; Cirlini et al., 2019; Irlini et al., 2018; Martinello et al., 2017; Veršilovskis et al., 2020), LC–high resolution MS (LC–HRMS) (Haughey et al., 2021; Romera-Torres et al., 2018, 2020), and a multidimensional LC such as 2D-LC–MS/MS (Kresse et al., 2019; Urban et al., 2019) have evolved to conduct faster, more efficient, high-throughput, better performance, and environmentally friendly analyses, providing LOQs below the ML. A nano-LC with an ultraviolet (UV) detector has also been developed and validated to determine scopolamine and atropine in different beverages for forensic purposes (Jornet-Martínez et al., 2021). The samples were extracted using a C18 SPE cartridge, and the separation carried out isocratically using water and acetonitrile as mobile phases on a 100 mm × 150 μm i.d.—column packed with an ODS stationary phase (1.7 μm particle size). The method could potentially be used for real-time on-site analysis, with results ready within 5 min (Jornet-Martínez et al., 2021). Although techniques such as LC–HRMS are useful in the data that can be generated, as well as having the ability to perform retrospective analysis, overall, the use of LC–MS/MS is the preferred method of choice for regulatory purposes due to its sensitivity, selectivity, and specificity. Furthermore, the use of 2D-LC, albeit in its relative infancy and potential to analyze various contaminants in one run due to the ability to use two different LC columns, it is also technically demanding and not feasible for high throughput analysis at present.

3.2.1 | Chromatographic separation

Chromatographic conditions are optimized (choice of the column, mobile phase, flow rate, injection volume, and gradient, for instance) to get the best retention time, peak shape (or symmetry), resolution (good separation), and reduced analysis time. Aqueous solutions of organic solvent (acetonitrile or methanol) with acid (acetic acid, formic acid) and/or basic or acidic additives (ammonium formate, acetate, or carbonate) are typical of the mobile phases used in the analysis of TA (Klein et al., 2022; Marín-Sáez et al., 2017; Martinello et al., 2022). The buffer system provides a more stable solution environment for the analytes (Zhao & Shi, 2020). In acidic conditions, both atropine and scopolamine are in their protonated form and are more soluble (Figure 1—the structure of atropine and scopolamine).

TAs are small-sized polar compounds, and their separation is more frequently carried out using reversed-phase (RP) chromatography (hydrophobic stationary phase),

using columns such as a C18 (Table 3) (Chen et al., 2017; Cirlini et al., 2018; Perharič et al., 2013; Vuković et al., 2022). The column length varies from 50 to 150 mm, internal diameter of 2.1–4.6 mm, and 1.8–5 μm particle size (Castilla-Fernández et al., 2021; Kowalczyk & Kwiatek, 2022; Marín-Sáez et al., 2019a, 2019b; Mulder et al., 2015). The composition of the C18 column impacts the separation of the analytes; for instance, the Waters BEH C18 column was reported to be better in terms of analytes resolution than an Agilent Bonus RP for LC–MS/MS in analyzing atropine and scopolamine in cereal and their products (Baslé et al., 2020). Also, the HSS T3 column (Waters) showed better separation of anisodamine, atropine, and scopolamine within 6 min than the BEH C18, BEH Shield RP18, and HSS C18 columns (Zhao & Shi, 2020).

One of the main challenges with the LC separation of multiple analytes is the co-elution of isomeric compounds. Several columns were tested against C18, such as pentabromobenzyl-, phenyl-hexyl-, and di-phenyl- based to attain appropriate peak, selectivity, sensitivity, and separation of positional pyrrolizidine and TA isomers in a 25 min gradient (Bessaire et al., 2021). Proper column selection and addition of 10 mmol/L ammonium carbonate to the acetonitrile mobile phase helped improve the separation of isomers, with only two minor pyrrolizidine alkaloid pairs co-eluting among the study of over 150 analytes (Bessaire et al., 2021). C8 columns have also shown well-resolved peaks and better separation efficiency for at least 13 toxic plant and pyrrolizidine alkaloids (Martinello et al., 2017; Ng et al., 2013) in comparison with an F5 or C18 column; however, diastereomers (matrine and sophoridine) could not be separated using a C8 column (Ng et al., 2013). An F5 column was a choice for the analysis of atropine and scopolamine in acidic conditions due to the limited lifetime of the C18 column at high pH and pressure build-up due to the precipitation of co-extractives from the acidic extract (Gonçalves et al., 2020). An F5 column showed excellent interaction between the analytes (k' values: scopolamine—4.9; atropine—5.7) (Gonçalves et al., 2020).

A HILIC (hydrophilic interaction LC column) (Romera-Torres et al., 2018b), HILIC-A column (Romera-Torres et al., 2020), or a combination of RP-LC and HILIC (Dzuman et al., 2020) used for atropine and scopolamine separation or multiple analytes (other TAs, pyrrolizidine, and their N-oxides) reduced co-elution issues (Dzuman et al., 2020; Romera-Torres et al., 2018b). The use of a HILIC column enables the direct injection of organic extract, so no solvent exchange is necessary (Thompson et al., 2020). If an RP column is used, organic extracts are typically diluted with water or concentrated to dryness and reconstituted in a solvent that is mainly aqueous, which can be time-consuming and may potentially result

in the partial loss of some analytes (Dzuman et al., 2020; Thompson et al., 2020), an unnecessary step when using a 2D-LC–MS/MS system. HILIC–LC–MS/MS also permits the detection of low analyte concentrations (sub- $\mu\text{g}/\text{kg}$) (Dzuman et al., 2020). The LC–MS/MS methodology using HILIC attained suitable LOQs for agricultural products (5–25 $\mu\text{g}/\text{kg}$) (Romera-Torres et al., 2020; Romera-Torres et al., 2018b). In a 2D-LC–MS/MS system, two mobile phases are also employed; thus, their immiscibility, precipitation of buffer salts, and stationary phase incompatibilities may negatively impact chromatographic performance (Tranchida et al., 2004). Furthermore, if the elution strength of the effluent of the first dimension is too high for the second dimension, this may negatively affect peak capacity and chromatographic efficiency. Nevertheless, it can be compensated by using a packed loop interface or by increasing the flow rate of the second dimension. However, the latter could lead to higher dilution factors and lower sensitivity (Wang et al., 2008). Only four research studies have been published in the last 10 years that used 2D-LC–MS/MS systems, including the simultaneous analysis of pesticides, mycotoxins, growth regulators, pyrrolizidine alkaloids, and TAs in oats, whole wheat grains and corn (Table 3) (Kresse et al., 2019; Urban et al., 2019). Overall recoveries were found to be within an acceptable range (70–119% and $\text{RSD} \leq 13\%$) for atropine and scopolamine (Kresse et al., 2019; Urban et al., 2019); however, these can likely be further improved by the use of a mixed matrix-matched approach (Kresse et al., 2019; Urban et al., 2019). A 2D-LC–MS/MS approach developed for oats and wheat had an LOQ of 0.16–0.20 $\mu\text{g}/\text{kg}$ and 0.27–0.40 $\mu\text{g}/\text{kg}$ for atropine and scopolamine, respectively (Urban et al., 2019), and for other cereals, a LOQ of 5 $\mu\text{g}/\text{kg}$ for both analytes was reported (Rausch et al., 2021).

Using a multidimensional LC approach can minimize the classical clean-up steps for the extract and has enhanced resolving power expressed as the peak capacity (Kittlaus et al., 2013; Rausch et al., 2021; Urban et al., 2019), reducing co-elution of peaks due to the two dimensions orthogonal to each other, transferring all fractions (comprehensive) or only one fraction (heart-cut) between columns (Kresse et al., 2019; Rausch et al., 2021). Furthermore, a HILIC column in a 2D-LC system has a high polarity range of the investigated analytes, huge peak capacity, and reduced sample preparation and analysis time. It can be used as a tool for high-throughput routine screening but may require confirmatory methods from time to time (Rausch et al., 2021). The disadvantages of a 2D-LC system are the high cost, particular care of the instrumentation required, limitations in method sensitivity, complexity of the analytical set-up, analysis time of the RP dimension, and solvent compatibility affecting the method validation data (Rausch et al., 2021). Using a

single column rather than a combination of two columns (a HILIC and an RP) could be more convenient for routine analysis; this would also require two different mobile phases (Dzuman et al., 2020). Although the use of 2D-LC shows great promise for the confirmatory analysis of multiple compounds of varying polarities, the complexity of this technique would not be advised for the analysis of the TAs alone.

3.2.2 | Detectors, ionizers, and mass analyzer

MS detectors have better sensitivity, selectivity, and specificity when identifying and quantifying target analytes, even with similar retention characteristics, compared with DAD. The MS/MS or tandem MS has improved sensitivity and reduced matrix interferences, enabling target compounds to be detected at low concentrations in very complex matrices (Ardrey, 2003). In LC-MS, the analytes must be ionizable, whereas in DAD or photodiode array detectors, the analyte must contain a chromophore (Ardrey, 2003). The ionization sources for LC-MS are electrospray ionization (ESI) (Klein et al., 2022; Mulder et al., 2015; Veršilovskis et al., 2020; Zheng et al., 2019) or heated electrospray ionization (HESI) (Marin-Saez et al., 2019; Marin-Saez et al., 2017, 2019a, 2019b), either in positive or negative mode (Table 3). ESI is the most widely used and has broader applications, including studying polar and thermally labile analytes of low and high molecular weight (Ardrey, 2003; Ho et al., 2003).

LC-HRMS has increased selectivity and combined advantage of full-scan acquisition, accurate mass measurement (<5 ppm), and high resolving power, allowing the separation of compounds with very close exact masses (up to several decimal places) (Bessaire et al., 2021). One notable limitation of MS/MS detection is that the method is set up for specific analytes only that you have standards for; hence, neither retrospective data evaluation nor screening of unknowns is feasible, which is an advantage of HRMS analyzers (Martinello et al., 2017). However, the sensitivity of LC-HRMS instruments may be an issue if the analytes to be detected, such as the TAs, are at low levels (sub ppb), and, with the regulatory levels of both TAs discussed being 1 µg/kg, it is unlikely that these could be detected at such low levels in complex food/feed using LC-HRMS. LC-HRMS was used to study TAs concurrently with other analytes (pyrrolizidine alkaloids and N-oxides, plant toxins) in different matrices (Table 3) (León et al., 2022; Martinello et al., 2017; Romera-Torres et al., 2018, 2020; Romera-Torres et al., 2018b). Another study using LC coupled to a Q-Exactive Orbitrap HRMS detector (Thermo Fisher Scientific) with HESI in positive ionization mode and product monitoring acquisition mode, was

used for both screening and quantification of 30 tropane and pyrrolizidine alkaloids and their N-oxides in dried teas and herbs due to the unequivocal compound identification and the isolation of the target compounds from possible matrix interferences employing their exact masses (León et al., 2022).

The use of triple quadrupole (QqQ) mass spectrometers allows for the detection of the target compounds at low concentration levels in complex matrices (Baslé et al., 2020; Chen et al., 2017; González-Gómez et al., 2020, 2023; Klein et al., 2022; Mulder et al., 2015; Shimshoni et al., 2015; Urban et al., 2019; Veršilovskis et al., 2020; Vuković et al., 2022; Zheng et al., 2019). QqQ operates in selected reaction monitoring or multiple reaction monitoring (MRM) (Cirlini et al., 2019; González-Gómez et al., 2022; Urban et al., 2019); dynamic MRM (Vuković et al., 2022) or scheduled MRM (Begemann et al., 2021) modes, which typically monitor one precursor ion to at least two product ions, giving these systems their selectivity and enhanced sensitivity. The parent/precursor ion of the compound of interest is fragmented into daughter/product ions, where one is used for quantification and the other for confirmation (qualifier) (Begemann et al., 2021; Castilla-Fernández et al., 2021; Chen et al., 2017; González-Gómez et al., 2020). Only ions from the parent to fragment pair with the exact transition will be detected. The sensitivity of the scan as the instrument is fully optimized to transmit just that parent to fragment pair.

On the other hand, for a full-scan mode for qualitative analysis, ion-trap is better than QqQ due to higher sensitivity and scan speed in full-scan mode. Q-trap can be used for screening, confirmation and identification applications by combining the MRM sensitivity of a QqQ with a multifunctional ion trap for quantitative and qualitative analyses. Quadrupole ion traps are versatile instruments that are routinely used for exploratory studies, structural characterization, and qualitative identification with high sensitivity and ability to conduct tandem MS but are not well-suited for quantitative analysis due to the possibility of space-charge ion-ion repulsion effects resulting from the limited number of charged species that can fit within the trap, which reduces the analyzer's dynamic range (Thomas et al., 2020). A mass analyzer can have a high or low resolution based on its ability to differentiate ions with a small m/z ratio difference. An orbitrap is increasingly used for this purpose where the high resolving power of these instruments is beneficial, whereas ion traps are less commonly used in the analysis of TAs but are often combined with high-resolution analyzers in powerful hybrid configurations such as ion-trap orbitrap or combination with LC-HRMS instrument.

In relation to quantification using LC-MS/MS and the absence of internal standards, the use of matrix-matched

calibration curves can be used, with the matrix spiked before extraction with various amounts of a working standard of both analytes to compensate for both the extraction of recovery (RE) and SSE. However, if the extraction efficiency is close to 100%, spiked extracts can instead be used with this compensating for the matrix effects only. Additionally, as there may be numerous varieties of each commodity study, it is known that the biggest contribution to matrix effects and method performance is due to lot-to-lot variation. Therefore, the use of a solvent calibration curve can be utilized if the method has been validated using a statistically relevant number of lots of each commodity to calculate the apparent recovery (RA). This RA includes both the extraction efficiency (RE) and matrix effects (SSE). And if the former is high, the RA is an indication of the SSE and can therefore, be applied to the final result.

The European Commission has recommended the monitoring of the presence of TAs by use of UHPLC–MS/MS (EU, 2015), which, when used in TAs studies in spinach (Castilla-Fernández et al., 2021), herbal teas, infusions, and herbal supplements (Cirlini et al., 2019), and buckwheat and baked products (Cirlini et al., 2018), the method LOQs fell within the performance characteristic set by the European Commission (Table 1). The authors described the UHPLC–MS/MS method to be sensitive, easy to perform, reliable, and serviceable for screening traces of TAs (Castilla-Fernández et al., 2021; Cirlini et al., 2019; Cirlini et al., 2018). Furthermore, the advantages of using UHPLC are higher throughput, increased sensitivity, and a reduction in the amount of solvent required.

3.3 | Immunoassays

Immunoassays have been used as a sensitive detection and screening method for biochemical analytes in clinical diagnosis, environmental monitoring, and food safety and quality. Some reviews on immunoassays for food analysis and safety evaluation are available elsewhere (Dzantiev et al., 2014; Pan et al., 2021; Xiao et al., 2021; Xing et al., 2022). The earliest application of immunoassays in the study of TA was in the 1970s (Ren et al., 2022). The application of traditional immunoassays in routine analysis has been limited due to the low sensitivity and accuracy and low sensitivity or cross-reactivity of most available monoclonal antibodies (mAbs) (Darwish, 2006; Matabaro et al., 2017; Xiao et al., 2021). From 2013 to 2023, three studies on the application of immunoassays on TA analyses in food and feed have been retrieved and discussed in this section. These papers employed an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) (Wang et al., 2021) and lateral flow immunoassay (LFIA) (Mul-

der et al., 2014; Xu et al., 2022). Immunoassays can be competitive or noncompetitive based on the size of the molecules to be detected (Matabaro et al., 2017). ELISA is the gold standard immunoassay, and along with LFIA and chemiluminescence immunoassay are the most commonly used techniques for food analyses (Dzantiev et al., 2014; Matabaro et al., 2017; Mulder et al., 2014; Ren et al., 2022; Xiao et al., 2021; Xing et al., 2022).

The fundamental principle of the immunoassay technique is the specific interactions between antibodies (or immunoglobulins) and antigens (or target analyte) to form antigen–antibody immune complexes (Matabaro et al., 2017; Rodriguez et al., 2020). Generally, the availability of antibodies with high sensitivity and specificity to the target analytes is vital to achieving reliable results. The antibodies used in immunochemical assays are either mAbs, polyclonal antibodies (pAbs) or recombinant antibodies (rAbs). While pAbs can recognize and bind to many different epitopes and thus may have poorer specificity (Rodriguez et al., 2020), the mAbs can be specific to a class of compounds, such as TA (Mulder et al., 2014; Wang et al., 2021; Xu et al., 2022). Before use, mAbs are characterized to ensure sensitivity (through the half inhibitory concentration (IC_{50})) and specificity (cross-reactivity for analyte vs. a competitor) (Table 4- examples of cross-reactivity and IC_{50} from different types of immunoassays employed for TA studies (Wang et al., 2021; Xiao et al., 2021; Xu et al., 2022).

ELISA uses biological enzymes as markers, combining the specificity of the antigen–antibody reaction with the highly efficient catalytic amplification of enzymes (Ren et al., 2022). ELISA can either be a qualitative screening test (applicable to large samples to determine the presence or absence of the target compound) or a semi-quantitative test analysis (to indicate analyte concentration level). Consequently, confirmatory tests like LC–MS/MS are essential for accurately determining analyte concentration when using ELISA. For TA analysis using ELISA, the sample preparation is straightforward: for solid samples, mechanical shaking of the sample and solvent mix (this step is skipped for liquid samples), followed by centrifugation, concentration (or dilution), and clean-up (e.g., filtration). As with other analytical tests, organic solvent solutions, lipids, vitamins, proteins, and other matrix components in the samples can influence the formation of the antigen–antibody complex (Wang et al., 2021). Consequently, depending on the sample complexity, the clean-up step is crucial to removing matrix compounds that can interfere with the antigen–antibody complex formation; for example, filtration several times may be necessary to reduce matrix effects (Wang et al., 2021).

While the sample preparation for ELISA can be straightforward, the ELISA procedure itself is time-consuming and

TABLE 4 Comparison of immunoassays used for tropane alkaloids detection in food and nonfood samples.

Method ^a	Analytes	Samples	LOD (µg/kg)	LOQ (µg/kg)	Cross-reactivity (%)	IC ₅₀ (ng/mL)	References
ic-ELISA	Atropine	Pig urine	0.18	0.45	100	0.05	Wang et al. (2021)
		Pork	0.75	2.10			
		Wheat flour	0.45	1.55			
		Buckwheat flour	0.85	2.25			
		Maize flour	0.82	2.32			
	Scopolamine	Pig urine	0.55	1.05	20.8	0.24	
		Pork	2.99	5.82			
		Wheat flour	0.73	2.43			
		Buckwheat flour	1.49	3.25			
		Maize flour	0.93	2.28			
LFIA-AuNPs or fluorescent NPs	Hyoscyamine	Pork	10		100	1.17	Xu et al. (2022)
		Pig urine Honey	(±result based on the spiking method)				
LFI (dipstick)	Scopolamine	Animal feed	800		100		Mulder et al. (2014)
	L-Hyoscyamine				~100		
ELISA	Scopolamine	Root	0.2		–		Kikuchi et al. (1991) ^b
ELISA	Scopolamine	Human serum	0.5		Scopolamine = 100 L-Hyoscyamine = 0.21		Hagemann et al. (1992) ^b
		Plant	0.1				
RIA	Atropine L-Hyoscyamine	Serum	2.5		Atropine = 100 L-Hyoscyamine = 100		Virtanen et al. (1980) ^b
ELISA	Atropine	Plant	0.1		Atropine = 100 Scopolamine = 4 L-Hyoscyamine = 4 Tropic acid = 1.6		Fliniaux et al. (1987) ^b

^aRIA, radioimmunoassay; Au, gold; NP, nanoparticles^bObtained from Wang et al. (2021)

tedious, requiring a skilled person, a plate reader and various reagents for use in the different steps of the analysis, making it a challenging assay for use outside the laboratory for field application (Wang et al., 2021; Xiao et al., 2021; Xu et al., 2022). The steps in ELISA include the addition of the coating antigen to the microtiter plate and incubating for at least 2 h before washing. The plate must be blocked for 1 h at room temperature or overnight in the fridge with a suitable blocking reagent and washed before use (Wang et al., 2021; Xu et al., 2019). Antigens in the sample solution are incubated with the antigen-specific antibodies in the microtiter plate, incubated once again, and washed to remove unbound antigen-antibody complexes and free antigens (Aydin, 2015; Darwish, 2006; Xu et al., 2022). A secondary antibody is linked to an enzyme (e.g., beta-galactosidase, glucose oxidase, peroxidase, and alkaline phosphatase), and the chromogenic substrate is added to the microtiter plate, followed by incubation. In ELISA, these enzymes or labels (such as horseradish peroxidase [HRP] and alkaline phosphatase [ALP]) conjugated to antigens or antibodies catalyze the substrate to degradation products with signal conversion and amplification (Pan et al., 2021; Xu et al., 2019; Xu et al., 2022). HRP is the most prevalent enzyme marker for the ELISA-based methods because it is cheaper, readily available, and reacts with a wide range of substrates, resulting in color changes. In contrast, ALP is very sensitive and more stable during the assay process. With these, ALP gives a more linear response and higher sensitivity with commonly available substrates than HRP. Still, ALP is more expensive and mainly applicable to high molecular weight conjugates (Matabaro et al., 2017). The choice of enzyme depends on its ability to preserve its catalytic activity when it is conjugated to probes such as antibodies (Matabaro et al., 2017). The enzyme-substrate reaction is stopped by adding hydrochloric acid, sulfuric acid, or sodium hydroxide (Aydin, 2015). Color changes (measured using a spectrophotometer) indicate the formation of an immune complex whose intensity is inversely proportional to the amount of toxin (or antigen) present in the sample (Aydin, 2015; Darwish, 2006; Z. Wang et al., 2021; Xu et al., 2019; Xu et al., 2022).

The principle of ic-ELISA is the covalent conjugation of enzyme and antibody or anti-antibody, where the enzyme-labeled antibody can specifically bind to antigen or antibody coated on a solid carrier (Xu et al., 2019). The coating antigen dissolved in a buffer is added to each of the 96 wells, incubated for 2 h at 37°C, washed and blocked, and incubated again for 30 min at 37°C, followed by the addition of HRP-labeled goat anti-mouse IgG, and incubation for 30 min at 37°C. The substrate is added following several washes and again incubated in the dark at 37°C for 15 min (Kong et al., 2016). The substrate is

a colorless solution and changes color depending on the presence of the immune response, and the color depth suggests the amount of antigen or antibody present (Xu et al., 2019). The enzyme reaction is terminated by the addition of sulfuric acid (2M). In an ic-ELISA application, the enzyme-substrate reaction finishes within 30–60 min, which is rapid considering that at least 96 samples (in a 96-well plate) can be evaluated simultaneously (Wang et al., 2021).

Small molecules such as pesticides, aflatoxins, drugs, and TA are usually not immunogenic and, hence, they do not elicit an immune response unless coupled with some macromolecules such as proteins (Matabaro et al., 2017; Rajesh et al., 2013; Wang et al., 2021). For TAs studies, a hapten is produced by reacting the 3C hydroxyl group of tropine ring typical of TAs with succinic anhydride (Wang et al., 2021) or through the carbodiimide crosslinker EDC (or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydro) cross-linked (N, N'-carbonyldiimidazolen) (Xu et al., 2022). The hapten, which mimics the physicochemical properties of the target molecules (Matabaro et al., 2017), is conjugated with proteins ovalbumin (to form the coating antigen) or bovine serum albumin (to form an immunogen); the latter is used to immunize mice to induce the production of mAbs (Wang et al., 2021). Modifying hapten by coupling with a carrier macromolecule is vital to achieving a stable carrier-hapten complex (Rajesh et al., 2013). The mAbs prepared for the study by Wang et al. (2021) were capable of recognizing seven TAs: atropine, scopolamine, homatropine, apatropine, anisodamine, anisodine, and L-hyoscyamine, of which atropine and scopolamine are the only currently regulated ones. Furthermore, their ic-ELISA approach had a LOQ of <5 µg/kg (except for pork), which is within the limit set for agricultural products, and the results had an excellent correlation with the LC-MS/MS method developed by Wang et al. (2021). This comparison is a good improvement in the process since they require no laborious sample pretreatment as in LC-MS/MS.

Similar to ELISA, the sample preparation for LFIA is quite simple for TA analysis: the target analyte was extracted from the ground solid sample with acidified extraction solvent and mixed; no clean-up step may be required (Mulder et al., 2014). On the other hand, the pretreatment for some matrices such as pork and honey has been reported to be laborious and involved the addition of extraction solvent, mixing, sonication, centrifugation, filtration, drying, resuspension, and centrifugation before analysis of the supernatant (Xu et al., 2022). The LFIA (or immunochromatography, immunochromatographic strip test, or dipstick test) combines chromatographic and immunochemical principles; it does not require reagent or instrumentation for qualitative tests (but a reader is



FIGURE 5 The basic structure of the LFIA test strip (modified image based on Xu et al., 2022).

required for quantitative tests). LFIA is suitable for rapid point-of-care (POC) diagnosis and meets the affordable, sensitive, specific, user-friendly, quick/robust, equipment-free, and deliverable (ASSURED) criteria for a test—the rapid detection can be obtained within 5–30 min using low sample concentrations such as the test strips for pregnancy, drug screening, and COVID-19 test kits (Dzantiev et al., 2014; Koczula & Gallotta, 2016; Matabaro et al., 2017; Rodriguez et al., 2020; Tripathi et al., 2018). However, the results obtained are qualitative or, at best, semiquantitative and can have variable reproducibility and sensitivity, limiting their application for trace detection and accurate analysis of complex samples. Regarding food safety detection, recent reviews on the LFIA can be used in food testing, including pathogens, aflatoxins, drug residues, food additives, and illegal additives (Dzantiev et al., 2014; Matabaro et al., 2017; Tripathi et al., 2018; Xing et al., 2022) (Rodriguez et al., 2020).

A typical LFIA test strip consists of an adsorbent sample application pad, conjugate release pad (on to which dispensed labeled antibodies, e.g., gold (Au) or enzyme-antibody labels), nitrocellulose membrane and adsorbent pad (Figure 5) (Guo et al., 2021; Matabaro et al., 2017; Xu et al., 2022). The sample extracts are applied on the sample pad and flow through the conjugate pad, along with the labeled antibodies, by capillary action (Guo et al., 2021; Tripathi et al., 2018). The antibody binds specifically the analyte, and the complex flows to the nitrocellulose membrane, and the reaction with the immobilized antigen generates a visible result on the test line for the recognition of the sample analyte (analysis result) and on the control line that indicates the proper liquid flow through the strip (test is valid) (Guo et al., 2021; Matabaro et al., 2017). The liquid will pass through the strip's test areas and finally reach the adsorption pad at the end.

LFIA-based methods have been used to detect atropine and scopolamine in animal feed (Mulder et al., 2014) and atropine in pork, pig urine and honey samples (Xu et al., 2022). Authorities have not yet set the MLs for these matrices, but generally, MLs are within 0.2–50 µg/kg depending on the commodity (Table 1). An example of a dipstick was developed in the EU project, *CONFIDENCE*, for atropine and scopolamine studies in animal feed (comprised of rapeseed oil, soya beans, palm oil by-products, beet and citrus pulp, wheat products, and corn) (Mul-

der et al., 2014). For optical test and control line intensity readings, ReadSensor®, a hand-held instrument for optical absorbance measurement, was used. The antibodies cross-reacted with both scopolamine and L-hyoscyamine (atropine) (Table 4). Despite the ease of application, the dipstick is not fit for the detection of TA contamination in food since the target level was 800 µg/kg for the sum of scopolamine and hyoscyamine, which is too high for the MLs set by the European Commission (Table 1).

In a study of TA in food and feed, fluorescent microspheres or gold nanoparticles (AuNPs) have been used for the labelling of antibodies (fluorescent-labeled mAbs and colloidal Au-labeled mAbs) (Xu et al., 2022). AuNPs are the most commonly used for their ease of conjugation, high affinity, and easy functionalization; they produce an intense red color (Mulder et al., 2014). Others include silver NPs (AgNPs), selenium NPs (SeNPs), and quantum dots, in addition to fluorescent, magnetic, and carbon NPs and Raman probes for sensitive detection (Guo et al., 2021; Koczula & Gallotta, 2016; Sajid et al., 2015; Tripathi et al., 2018). Moreover, sensitive optical and electronic components, enzymes, radioisotopes, and fluorophores were developed to enhance the signal during immunoassay detection (Sharma et al., 2014; Matabaro et al., 2017). NPs have unique physicochemical and biological properties, including their small size, large surface-to-volume ratio, biocompatibility, high stability, easy preparation, and UV–visible (VIS) solid absorption spectra; examples are semiconductor quantum dots, hybrid nanostructures, and NPs made from gold, carbon and silica and magnetic elements (Matabaro et al., 2017).

Compared with chromatographic techniques, immunoassays are more suitable for the preliminary screening of samples in large quantities. Several articles about the preparation of scopolamine mAb, the establishment of test strips, the preparation of atropine mAb and the establishment of radioimmunoassay methods have been published. However, the antibodies prepared in the cited studies demonstrated low sensitivity and poor cross-reactivity. Moreover, the procedures established needed to be improved to meet the regulatory requirements. No method for screening multiple TAs has been developed yet. An essential requirement of a screening test is that it can detect the compounds with a low rate of false negatives at the lowest appropriate level, that is,

at 1 $\mu\text{g/kg}$ ML for atropine and scopolamine (each) in food (Table 1), to include a safety margin somewhat below the ML. Nevertheless, although some immunoassays can be applied for on-site monitoring while preserving their specificity and high sensitivity, future research should focus on the optimization of the matrix components, NP technology, specific antibody production, automation, and miniaturization of equipment (Matabaro et al., 2017).

3.4 | Spectroscopy

Monitoring food quality and safety, including the detection of harmful compounds or contaminants in food before distribution to intended markets and consumers, is critical to reducing product recalls and the occurrence of food poisoning or intoxication. Due to the in-line and off-line capabilities of optical sensing techniques, they have been of interest to numerous food industries. Optical sensing-based techniques that include spectroscopy (e.g., infrared [IR] and Raman spectroscopy) and hyperspectral imaging (HSI) are emerging rapid analytical methods that can be used for objective quantification of inherent (e.g., chemical components of foods) and external attributes (e.g., trace impurities) of food, and their classification based on quality, with minimal sample preparation (Khan et al., 2020; Su & Sun, 2018). Due to food's inherent chemical and physical structure, they will either reflect, scatter, absorb or emit electromagnetic radiation in characteristic patterns at specific wavelengths—this is the underlying principle of all spectroscopic techniques, including HSI (Elmasry et al., 2012).

3.4.1 | IR spectroscopy

The IR region of the electromagnetic radiation is classified based on spectral range: (1) near-infrared (NIR) spectra with $12,821\text{--}4000\text{ cm}^{-1}$ (780–2500 nm), (2) mid-infrared spectra with $4000\text{--}400\text{ cm}^{-1}$ (2500–25,000 nm), and (3) the far-infrared spectra with $400\text{--}33\text{ cm}^{-1}$ (25,000–300,000 nm), which have been divided according to their relations to the VIS spectra within the region of $26,316\text{--}12,821\text{ cm}^{-1}$ (380–780 nm) (Su & Sun, 2018). When an IR light source is absorbed by a specific characteristic bond in the analyte molecule, it results in vibrations of atoms in the molecule, creating the IR spectra that can be used to identify the molecules in the sample or functional groups (fingerprinting) (Candoğan et al., 2021; Qi et al., 2022). The absorbance intensity is directly proportional to the concentration of a pure compound or a particular feature or functional group when analyzing mixtures (Valand et al., 2020). The near-infrared spectroscopy (NIRS) is applica-

ble for microconstituent analysis in food (usually those present at a concentration of $\sim 0.5\%$ or higher) because, at much lower concentrations, NIRS likely detects a different analyte present at macro-levels—the concentration of which is correlated with the targeted analyte (referred as the secondary, or surrogate, correlation) (Johnson et al., 2023). IR spectroscopy has been applied to studying samples in solid (e.g., powders), liquid, and solution forms; the latter should have a solvent that does not absorb in the IR region as the sample of interest. Also, when working with complex mixtures, purification is required to prevent having complex spectra that are not easy to identify.

Fourier-transform infrared spectroscopy (FTIR) has long been used to detect contaminants/adulterants in premium foods such as virgin oil, honey, beef, spices, herbs such as saffron and oregano, wine and wine products, nectars and jams (Valand et al., 2020). The interferometer of an FTIR instrument generates an interferogram, which is converted into a typical spectrum by Fourier transform. The FTIR spectroscopy has three sampling techniques: transmission, transflection (reflection), and attenuated total reflectance (ATR) (Candoğan et al., 2021). FTIR–ATR can be used to identify functional groups and chemical compounds such as primary and secondary metabolites and helps determine the IR characteristic fingerprint peaks of the compound of interest in the sample (Mardare et al., 2022). In ATR, liquid or solid samples are directly spotted or placed onto the ATR crystal, pressure is applied to the solid samples with the help of a manifold, and a beam of radiation enters the crystal and undergoes total internal reflection (Candoğan et al., 2021). During this process, the analyte absorbs IR at its contact surface with the crystal, resulting in an IR spectrum (Candoğan et al., 2021).

FTIR–ATR technique was used to study scopolamine and atropine in the different parts of *D. innoxia* (Mardare et al., 2022; Naumann et al., 2014). The objective was to identify, isolate and characterize bioactive compounds, including those that are a potential source of medicinal value. Similar to the chromatographic technique, sample preparation of *Datura* biomass required SLE or Soxhlet extraction, and for the quantification of TAs, an external calibration curve was used (Mardare et al., 2022). Others have used FTIR–ATR to directly measure fresh or dried plant tissues with spectral data representing the sample's composition, which can be further identified based on vibrational patterns by cluster analysis (Naumann et al., 2014). Due to spectral differences, FTIR–ATR was able to discriminate the plant species of the *Solanaceae* plant family, and the FTIR–ATR spectra were used to develop calibration models to predict the scopolamine and atropine contents in the samples in the plants (using the partial least square (PLS) prediction models) (Naumann et al., 2014). The results were comparable to HPLC, which was used

as a reference method, and the FTIR-ATR showed good prediction models for rapidly quantifying scopolamine and atropine without artefacts due to fluorescence observed in Raman spectra (Naumann et al., 2014). On the one hand, benchtop FTIR-ATR and NIR were used to distinguish Super Cereal samples contaminated with TAs from noncontaminated samples (Haughey et al., 2021). The non-targeted analysis could group the different suppliers or origins of Super Cereals. A benchtop NIR was also used for proximate analyses (Haughey et al., 2021). The NIR light that irradiated samples either gets reflected or transmitted, and the radiation is measured.

3.4.2 | Raman spectroscopy and surface-enhanced Raman spectroscopy

Whereas IR-based techniques are based on light absorption phenomenon, in Raman spectroscopy, the electrons in the sample molecules interact with incident light or electromagnetic radiation such as UV, VIS, and IR, resulting in light scattering (Martinez & He, 2020; Su & Sun, 2018; Wu et al., 2021). Like the other spectroscopic techniques, Raman spectroscopy is also non-destructive. It enables the rapid and accurate identification (or fingerprinting) and semi-quantification of chemical and biological substrates since it does not interact with the sample's water content (Lin et al., 2023). Raman instruments are typically user-friendly and straightforward, involve simple sample preparation, and are applicable for analyzing samples ranging from solid, liquid or gas (Martinez & He, 2020; Wu et al., 2021). It is generally accepted that the Raman scattering signal accounts for only about 10^{-6} times that of the incident light; thus, for detecting trace levels of analytes in complex matrices such as food and feeds, Raman spectroscopy is not suitable. Consequently, surface-enhanced Raman spectroscopy (SERS) was developed to increase the scattering of inelastic light (Bunaciu et al., 2020; Israelsen et al., 2015). The enhancement of light scattering signal by SERS is mainly attributed to the electromagnetic mechanism and partially to the chemical mechanism (due to charge transfer between substrate and sample) (Alonso-González et al., 2012; Bao et al., 2017; Bunaciu et al., 2020; Campion & Kambhampati, 1998; Israelsen et al., 2015; Larkin, 2017; Smith & Dent, 2019; Wang et al., 2018; Wu et al., 2021). The electromagnetic enhancement (Figure 6) arises from the increased local electric field at the metal surface, which occurs due to the generation of local surface plasmon resonance (LSPR) when the metal is illuminated with light (Lin et al., 2023). Surface plasmons refer to the collective oscillations of unbound electrons within a region confined to the interface between a metal and a dielectric material

(Lin et al., 2023). Specifically, electromagnetic enhancement accounts for a factor of 10^6 – 10^8 , while a chemical mechanism is by a factor of 10^2 (Yu Liu et al., 2017)(Wu et al., 2021). SERS can offer the capability to study analytes in ultra-trace levels due to its high sensitivity, specificity, and rapidity. Thus, it helps detect a molecule up to a single level (Lin et al., 2023). Compared with chromatographic and MS techniques, SERS is advantageous due to the portability of the instrument and the cheaper and non-destructive nature of analysis (Lin et al., 2023; Rodriguez et al., 2020; Wu et al., 2021). SERS have been employed for direct and indirect detection of single and multiple chemical and microbial contaminants, such as antibiotics (Jing et al., 2021), pesticides (Logan et al., 2022), food adulterants (Gukowsky et al., 2018), mycotoxins (Song et al., 2022), fungi, bacteria, and viruses (Xia et al., 2022).

The choice of SERS-active substrates metal colloids [or NP] in suspension and solid structures is critical for the enhancement of inelastic light scattering; the substrate is tailored to specific target substances to achieve optimal sensing performance (Guo et al., 2021; Lin et al., 2023). Metal nanomaterials, noble metal NP made from Au and Ag, and transition metal NP such as platinum (Pt), palladium (Pd), iron (Fe), cobalt (Co), and nickel (Ni) have been reported as the most widely used SERS-active substrates (Neng et al., 2020). Specifically, the AuNP and AgNP, which come in various shapes and sizes such as nanorods, nanostars, nanosheets, and nanoflowers, are highly active (Neng et al., 2020) and are also the most extensively used colloidal metal nanostructures. Other SERS structures can be prepared using copper (Cu), sodium (Na), lithium (Li) or other transition metals in the form of colloids (NP), electrodes, or metal island film (Qin et al., 2021).

In studying TAs (scopolamine hydrobromide in aqueous solution), AgNP-based substrates were used, achieving an LOD of 0.5 $\mu\text{g/mL}$ (Bao et al., 2019, 2017). In another study exploiting the use of AgNP substrate, the LOD was 5.1 and 1 $\mu\text{g/L}$ in solutions of scopolamine hydrobromide, methscopolamine bromide and scopolamine butyl bromide, respectively (Lu et al., 2019). AgNP is typically prepared using silver nitrate in water with sodium citrate solution in boiling water, producing NPs of uniform and spherical shape (Lee & Meisel, 1982). Although AgNP is easy to prepare and readily available, it aggregates and oxidizes easily compared with AuNP, which is more stable, have uniform size distributions, high enhancement factor and availability of plasmonic resonances in the visible and near-infrared (NIR) regions (Guo et al., 2021). In another study, a bimetallic substrate in the form of Ag@Au nanowires (NWs) was formed for the quantification of TA (L-hyoscyamine, anisodamine, scopolamine hydrobromide, scopolamine methyl bromide, and scopolamine butyrate bromide) (Qin et al., 2021). A bimetallic

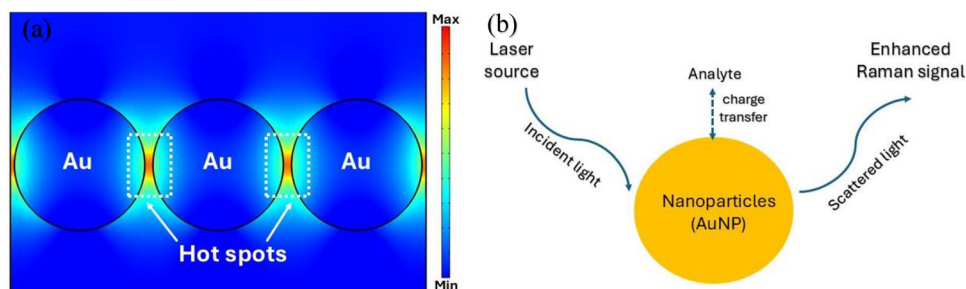


FIGURE 6 Two mechanisms contributed to the surface-enhanced Raman spectroscopy (SERS). (a) electromagnetic enhancement of SERS-active silver nanoparticles. SERS “hot-spot” is generated in the gap between two close nanoparticles. (b) chemical enhancement resulting from electron transfer between analytes and the surface of nanoparticles (K. Wang et al., 2018).

substrate was used to exploit Au’s stability, biocompatibility, straightforward surface chemistry, Ag’s LSPR, and optical efficiency (Israelsen et al., 2015). The AgNWs had excellent surface plasmon resonance characteristics, transparency, and stretchability, and they had a wide range of application prospects; however, the chemical instability hindered their practical applications (Qin et al., 2021). The SERS signal of TAs increases with an increase in concentration, and the LOD was 10^{-9} M for both L-hyoscyamine and scopolamine hydrobromide (Qin et al., 2021). Competitive adsorption on the substrates was an observed challenge when using SERS in studying TAs from more complex samples (Bao et al., 2019) reducing the amount of TAs in the hotspot region and weakening the signal, which was improved by diluting the samples (Lu et al., 2019). However, quantitative analysis of TAs in food samples with high accuracy is not available yet with a fast detection mode, although dilution can be applied (Lu et al., 2019).

3.4.3 | Hyperspectral imaging

HSI is a hybrid of spectroscopic and imaging techniques, which provides one-dimensional spectral information (wavelengths) and two-dimensional spatial information (x rows and y columns) about a sample; coupled with chemometric tools, information about spectral, textural, and morphological features can be extracted from HSI data (Khan et al., 2020; Temiz & Ulaş, 2021; D. Wu & Sun, 2013a). Specifically, HSI can provide information on the chemical composition and physical properties of the sample, and chemical maps to visualize the distribution of quality parameters in foods (Feng & Sun, 2012; Yuwei Liu et al., 2017). It helps study organic materials since NIR HSI employs a 780–2500 nm spectral range, resulting in spectra from the major X–H bonds (that is, C–H, O–H, and N–H) (Sendin et al., 2018). Differentiation of individual samples based on spectral characteristics and colors is pos-

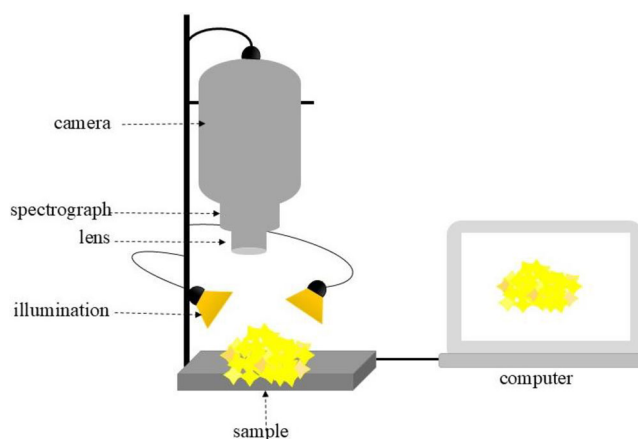


FIGURE 7 A schematic diagram of a NIR HSI with powdered corn sample, a hypercube (wavelength plus two spatial dimensions x and y), and a pixel spectrum derived from the sample.

sible with HSI by using a broad range of wavelengths of light (Schneider & Feussner, 2017). “HSI data consist of a stack of hundreds of two-dimensional spatial images of the sample, wherein each layer comprises its narrow band of wavelengths (forming a complete spectrum for each pixel), and the collection of spectral layers arranged in a three-way data matrix is called a hypercube, spectral cube, data cube or spectral volume” (Sendin et al., 2018). The HSI system is simple; it consists of an objective lens, spectrograph, camera, illumination, and computer system for data processing (Figure 7).

HSI has been employed by food industries in the classification and grading, defect and disease detection, distribution visualization of chemical attributes, and evaluations of the overall quality of meat, fish, fruits, vegetables, and other food products, with the possibility of automation for routine inspection tasks (Wu & Sun, 2013b). NIR–HSI was used to evaluate and differentiate cereal kernels for grading and milling quality assessment due to its great potential for studying heterogeneous materials (Sendin et al., 2018). The application of HSI in the food industry is essential for

its ability to carry out our online food safety and quality assessment in a non-contact, non-destructive, and continuous manner of analysis (Ma et al., 2019). There is no particular requirement for sample preparation; however, since the NIR region is sensitive to water, it is necessary to control the surface moisture of the samples (Ma et al., 2019). Using spectral characteristics and colors, HSI has also been used to discriminate between adulterants or contaminants with similar color and particle size as the food (Hashemi-Nasab & Parastar, 2022; Khamsopha et al., 2021; Laborde et al., 2021). For food where adulteration or fraud is hard to detect or discriminate visually or is expensive and time-consuming to analyze by chromatographic and immuno-techniques, HSI is a good alternative.

With the capability of spectroscopic techniques, including HSI, they can rapidly detect TA contamination without extensive sample preparation. Exposure of food and feed to plant parts say *D. stramonium*, with or without abrasion, can leave traces of TA. Contamination due to the presence of *D. stramonium* plant organs, for instance, co-harvesting the plant leaves with tea or spinach and co-harvesting the plant seeds with cereals or pseudo-cereal crops, if not completely removed, will be present in the final product reaching the consumer table. These contaminating plant parts will have different color and spectral features as the background products that can help identify their presence and concentration. Handheld or benchtop IR spectrometers, SERS, and HSI are promising and inexpensive alternatives to chromatography in the fast and in-field assessment of the contamination of toxic plant toxins in food. Despite this, these rapid analytical techniques generate massive and complex data, which requires powerful system hardware and chemometric tools for data mining and improving mathematical models for real-time applications and to take full advantage of the generated spectra (Cortés et al., 2019; Yuwei Liu et al., 2017; Su & Sun, 2018).

3.5 | Technological advancements

A CE method was developed and validated to determine atropine and scopolamine in *Solanaceae* seeds and pharmaceutical samples (Gołab et al., 2021). The method was considered to meet the green analytical chemistry requirements being low-cost, fast, selective, and sensitive and based on using a portable CE instrument integrated with a contactless conductivity detector (CE-C⁴D). The conductivity differences between the background electrolyte (0.5 M acetic acid with 0.25%, w/v) and the migrating zones of separated analytes are measured instead of detecting all ionized species with low absorbances (Gołab et al., 2021). Despite the portability of the method, it attained an LOQ of 1.5 ppm (or 1.5 µg/mL), and having

no autosampler it involves laborious manual operation; thus, further developments are necessary to meet the regulatory requirements (Gołab et al., 2021). Another portable, rapid, and low-cost, green method based on a microchip electrophoresis device integrated with C⁴-D with a running buffer composed of 40 mmol/L of butyric acid and 25 mmol/L of sodium hydroxide was also used to detect individually or simultaneously scopolamine and butylscopolamine in pharmaceuticals, beverages, and forensic samples (Santos et al., 2024). An LOD of 1.1 µmol/L (~333 ppb) was achieved, and the analysis results were obtained in less than 2 min (Santos et al., 2024).

Additionally, a bicolorimetric device was developed to detect scopolamine in beverages for forensic purposes (Jornet-Martínez et al., 2021). The method comprised a double polydimethylsiloxane sensor containing 1,2-naphthoquinone-4-sulfonate and KMnO₄. The MnO₄⁻ reacts with the scopolamine at basic conditions, resulting in a color change (magenta to green) due to the reduction of MnO₄⁻ to MnO₄⁻², where the intensity of color change depends on the concentration of the analyte (Jornet-Martínez et al., 2021). The method had an LOD of 108 µg/mL (1 ppm). The authors suggested that for on-site purposes, the color image analysis could be potentially used with a smartphone instead of a portable colorimeter (Jornet-Martínez et al., 2021). A further step in the colorimetric method was recently developed to determine atropine in baby food (Domínguez et al., 2024). The method involves enzymatic reaction (for specificity), colorimetric device (due to availability), and solid cellulose support where the RGB coordinates are collected by a smartphone lighting device and uploaded with ApK App-Colorimetry developed by the research group (Domínguez et al., 2024). Using the G coordinate, the device showed a linear response to atropine concentration from 1.2×10^{-5} to 3.0×10^{-4} M (Domínguez et al., 2024). A miniature rapid testing device electronic finger (e-finger), was developed for rapid screening of scopolamine along with other “date rape drugs” (flunitrazepam, ketamine) (Poulladofonou et al., 2022). The screening method is based on an in-house fabricated wearable 3D-printed e-finger immersed in a solution (or beverage) containing the target analytes (Poulladofonou et al., 2022). The e-finger is connected to a miniature off-the-shelf potentiostat directly connected to a smartphone and controlled by an Android app. The method was validated with different illicit alcoholic beverages, and a linear range between 2 and 26 mg/L and LOD of 0.56–0.98 mg/L were achieved (Poulladofonou et al., 2022).

An LFIA technique that was developed to detect co-contamination of 20 mycotoxins from five different classes in cereals (Liu et al., 2020), could have potential applications for TA determination. In this study, Liu et al. (2020)

developed a smartphone dual detection mode device integrated with AuNPs and time-resolved fluorescence micro-spheres LFIA, employing visible light and fluorescence modes. This device consisted of a black photosensitive resin cartridge, two optical modules with a hollow structure with white LED and UV LED fixed at the top, a sandwich-like structure of a strip holder and a strip cover inserted at the bottom of the cartridge to capture the image through a photography hole. An Android app was developed for the simultaneous detection and quantification of multiple mycotoxins (Liu et al., 2020).

In general, several challenges must be dealt with before new methods can be implemented. Investment in new equipment, such as LC–MS, ongoing maintenance, and human resources, are significant investments and hurdles for many laboratories (Savage, 2023; Ritter et al., 2016). Human resources involve training staff to ensure the proper implementation of the method and use of the equipment. A new method requires thorough validation and verification (Nieuwenhuizen, 2023) according to one of the several accepted guidelines such as Council Directive 2001/83/EC and 2001/82/EC, ICH Guideline Q2 (R1), SANTE/11813/2017, and US FDA, which are very time-consuming, laborious and costly. The laboratory must be able to properly document the protocols and validation data and show the method's performance characteristics to prove fit for the purpose. Although time-consuming and expensive, interlaboratory validation studies are essential to show reproducibility across different laboratories of a new method. One study by Gonçalves et al. (2020) conducted interlaboratory proficiency testing on TAs in cereals, tea and herbal infusions. In this study (Gonçalves et al., 2020), the EC's Joint Research Centre developed and validated an LC–MS/MS method, conducted two proficiency tests and derived interlaboratory performance characteristics. The standard operating procedure and the in-house prepared proficiency test materials were supplied to proficiency testing participants.

Therefore, while various technologies can determine TAs in foods or feeds, some have LOQs higher than the MLs set by regulatory agencies, potentially limiting their suitability for regulatory purposes. Additionally, any sample that indicates a concentration higher than the recommended level must be sent for confirmatory analysis, such as LC–MS, which is the “go-to” technique for regulatory analysis due to being a confirmatory technique. Furthermore, in comparison to LC–MS, those technologies that are in their infancy for this type of work, such as the use of biosensors and smartphones, are not feasible for multi-analyte detection.

4 | TA AND ONE HEALTH

The health of humans, animals, and the environment is interrelated, and this One Health approach is crucial to achieving sustainable food production, food safety, and security for the growing global population; otherwise, there will be a threat to public health (Garcia et al., 2020; Nwaji et al., 2022). Humans and animals are exposed to various natural toxins (e.g., phytotoxins) in the environment, which can also be a source of therapeutic agents (Nwaji et al., 2022) but could have adverse effects on health and even cause death if intake exceeds tolerable limits. TAs are secondary plant metabolites produced as their defense against environmental threats, and these plants grow among crops for human and animal consumption. Agricultural management or changing climate conditions may cause weeds such as the genus *Datura* to spread and become invasive; they can grow larger and produce more seeds even under water-limiting conditions (de Nijs et al., 2023; Jank & Rath, 2021). The major challenge related to natural toxin management in the food system is the need for standard analysis methods and databases (Nwaji et al., 2022), which have been addressed in the current review. The challenge can also be minimized by developing a rapid, affordable, user-friendly approach to tackle unsafe TAs contaminating human and animal food.

5 | CONCLUSION AND FUTURE TRENDS

Driven by climate change, invasive plant sources of TAs will soon be found in many regions growing amongst plant crops. With the knowledge that TA contamination can be visible and invisible, cleaning harvested crops and cooking can still leave deadly traces of atropine and scopolamine in food. Food safety and quality management systems (GAP, GMP, ISO 22000, HACCP), as well as training and education for public awareness of the impact of TA-producing weeds and the TAs themselves, are highly recommended. Furthermore, the development of fast, simple, rapid, and inexpensive methods that can be used for in-field applications will have a massive impact on the health and safety of society. Although laborious sample preparation for the LC–MS/MS technique has been improved to dilute and shoot, its broad application is still limited to research institutions, private laboratories and agencies with the resources to fund the expensive instrumentation and operational costs. Thus, inexpensive and straightforward spectroscopic and HSI techniques and

well-developed data mining and modelling procedures will be critical future tools for detecting TAs in food. One caveat of this at present is that as the TAs are regulated and the levels set are in the low ppb range, any analytical technique must be fit-for-purpose and, therefore have the capability of detecting and quantifying TAs at these low levels for regulatory purpose. Therefore, although some of the aforementioned techniques may be useful in a screening capacity to accurately distinguish between compliant and noncompliant, samples still need to be accurately analyzed and quantified due to the regulations in place to safeguard public health. Further work is needed to integrate screening or detection methods into smartphones that can detect atropine and scopolamine to the levels at or below the MLs recommended by the EU. The portability of the equipment and the need for less skilled operators are the main advantages of these techniques, making it the best alternative. Thus, almost real-time determination of the presence of TAs in food and feed can be achieved.

AUTHOR CONTRIBUTIONS

Rufielyn S. Gravador: Conceptualization; funding acquisition; methodology; writing—review and editing; writing—original draft; project administration; data curation. **Simon Haughey:** Conceptualization; writing—review and editing; validation; data curation. **Julie Meneely:** Conceptualization; writing—review and editing; validation; data curation. **Brett Greer:** Writing—review and editing. **Anne Nugent:** Writing—review and editing; project administration. **Christy S. Daniel:** Writing—review and editing. **Christopher Elliott:** Supervision; writing—review and editing; conceptualization; project administration; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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