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Review

The mycotoxin patulin: An updated short review on occurrence, toxicity and analytical challenges

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ABSTRACT

Patulin (PAT) is a common mycotoxin in fruit products, especially in apples and apple-based products. The European Commission has set maximum levels for PAT in food. Nevertheless, worrying PAT levels were recently recorded in diverse foods across the world. Therefore, a worldwide follow-up of PAT-levels in foods should be considered. Because of PAT's high probability in food products, the toxicological implications for humans need to be addressed as well. Recent studies proved adverse health effects of PAT, such as hepatotoxicity, gastrointestinal alterations and inmunotoxicity. In comparison to the toxicity of other mycotoxins such as ochratoxin A, PAT's immunotoxicity can be even more outspoken destructive. In addition, PAT is a low-molecular-weight and highly polar molecule, resulting in many analytical challenges for its detection. As the analytical techniques are continuously improving, PAT determination in multi-mycotoxin analysis has advanced using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) during the last year. Finally, the presence and toxicity of PAT requires a biomarker method to assess its exposure among the population. To date, however, there is no information regarding PAT biomarkers in biological samples. This short review highlights the PAT-occurrence profile, toxicological discoveries and analytical challenges of 2014 until to date.

1. Introduction

Mycotoxins are secondary metabolites produced by fungi. These natural toxins are frequently occurring contaminants in the food chain, leading to adverse human health effects through consumption. Among mycotoxins, patulin (PAT) (4-hidroxi-4H-furo [3,2-c]piran-2(6H)-ona) is produced by at least 60 different fungal species such as *Penicillium expansum* (*P. leucopus*), *P. crustosum*, *P. patulum* (*P. urticae* & *P. griseofulvum*) and *A. clavatus* (Samson et al., 2009). *P. expansum* is the most common PAT-producing fungus (Moake et al., 2005). Different studies have clarified that acute PAT-intoxicosis leads to convulsions, agitation, ulceration, oedema, intestinal inflammation, vomiting and induces DNA damage in the brain, liver and kidneys (de Melo et al., 2012; McKinley and Carlton, 1980a, b; McKinley et al., 1982). Chronic exposure leads to neurotoxic, immunotoxic, genotoxic and teratogenic effects in rodents (Boon et al., 2009; Spadaro et al., 2007). However, based on a lack of toxicological human data, the International Agency for Research on Cancer (IARC) has classified PAT in Group 3 as not carcinogenic to humans (IARC, 2002). Maximum permitted levels were established by the European Commission (EC), and the FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) implemented a provisional maximum tolerable daily intake (PMTDI) of 0.4 mg/kg body weight (bw)/day for PAT (1881/2006/EC, 2006; JECFA, 1995) (Table 1). PAT-producing fungi have been isolated from various fruits and vegetables including apples, plums, peaches and pears (Piqué et al., 2013). In various regions of the world PAT was reported in primarily apple and apple-based commodities, and occasionally in other fruits -such as pears, oranges, grapes, and products thereof (Guo et al., 2013; Harris et al., 2009; Iha and Sabino, 2008; Lee et al., 2014; Morales et al., 2007; Moukas et al., 2008; Spadaro et al., 2007; Torović et al., 2017; Zaied et al., 2013). The reduction of PAT during food processing is challenging, and up to now it has been showed that washing and removal of decayed parts are low-cost procedures which effectively mitigate PAT in fruit products (Forouzan and Madadlou, 2014), moreover, fermentation can also cause an important reduction of PAT (> 90%) due to yeast degradation (Moss and Long, 2002; Stinson et al., 1978). Several factors make the control of PAT and the producing fungi even more complex, such as climate conditions,

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Table 1
Maximum levels for patulin in food established by the European Commission (1881/2006/EC, 2006).

Patulin	μg/kg
Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	50
Spirit drinks, cider and other fermented drinks derived from apples or containing apple juice	50
Solid apple products, including apple compote, apple puree intended for direct consumption.	25
Apple juice and solid apple products, including apple compote and apple puree, for infants and young children and labelled and sold as such.	10

geographical location, year of production, pre- and post-harvest treatments, surface damage on the fruits and storage conditions.

Sensitive, precise and robust analytical procedures are imperative for the qualitative and quantitative analysis of mycotoxins. Current methods for multi-mycotoxin extraction focus on the reduction or elimination of co-eluting matrix components; most common techniques include inmunoaffinity separation (IAC) (Vidal et al., 2016a), solid phase extraction (SPE) (Hernández et al., 2006) or liquid-liquid extraction (LLE) (Romero-González et al., 2009). However, new techniques such as 'dilute and shoot' or 'QuEChERS' gained more success for multi-mycotoxin determination (Heyndrickx et al., 2015), however, these new techniques are not able to remove the co-eluting matrix components. The selection of the extraction procedure depends on the physico-chemical properties of the mycotoxin, *in casu* PAT, solvent costs and the solubility of the non-analytes in the extraction solvent. Regarding quantification and detection methodologies, chromatographic techniques coupled to mass spectrometry were an important advance in multi-mycotoxin analysis.

The present short review covers three main topics: 1) update on the most recent PAT occurrence studies in foods (2014–2018), 2) update on the most recent toxicological studies of PAT, and 3) current analytical challenges for the determination of PAT. This review gives insight into the latest PAT scientific output, and aims to highlight the importance to include PAT in food- and bio-monitoring studies.

2. Occurrence and prevention

Recent PAT occurrence studies in fruit products highlight its common presence in these food commodities as surveys (after search in Scoups, ScienceDirect, Google Scholar and Web of Science) detailed in Table 2 showed. Despite the wide PAT occurrence, PAT levels in foods from the European Union (EU) were mostly below the limits in the last 4 years, however, different products analysed in the Czech Republic exceeded those limits (apple (122 μg/L), pear (231 μg/L) and mixed fruits (56 μg/L) juices). In most of the European countries, more than 20% of the analysed samples were positive for PAT, so this means that the population is chronically exposed to low-doses of PAT. In contrast to the *safe* PAT concentrations observed in Europe, other countries showed higher concentration levels: Japan, average of 464 μg/L in apple juice (n = 3 samples) (Li et al., 2018) or Pakistan, 270 μg of PAT/kg in apples (Iqbal et al., 2018) (Table 2).

Most studies focused on apples and apple-based products, however other fruit commodities also showed worrying levels of PAT: grapes in Pakistan (71% positive samples, average PAT concentration of 466 μg/kg and maximum concentration of 1100 μg/kg) (Iqbal et al., 2018), fufu-maize in Cameroon (30% positive samples, average PAT concentration of 105 μg/kg and maximum concentration of 890 μg/kg) (Abia et al., 2017) and dried figs in China (65% positive samples, average PAT concentration of 131 μg/kg and maximum concentration of 277 μg/kg) (Ji et al., 2017) (Table 2). Based on PAT occurrence data, risk assessments were carried out in Belgium (Baert et al., 2007), Spain (Cano-Sancho et al., 2009), Romania (Oroian et al., 2014) and Serbia (Torović et al., 2018). All studies agreed that the majority of the population did not exceed the TDI. Unfortunately, no information on PAT exposure is available from countries where PAT levels in food are higher. The authors agree that it is necessary to assess PAT exposure from these countries.

The incidence of PAT in recent researches pinpointed the importance to strengthen control strategies for PAT during food production. During food processing, mycotoxins are highly stable, however, different factors can affect the mycotoxin stability (Vidal et al., 2016b). Cleaning, washing, milling and pressing indicated a reduction of up to 55% of PAT (Acar et al., 1998). Heat treatment showed a limited effect on PAT reduction in apple juice as only 26% of PAT was reduced at 100 °C for 20 min (Kadakal and Nas, 2003). Based on this results, the reduction of PAT in apple juice in industrial conditions for pasteurization (heat treatment used in fruit and vegetable products) can not be very big and only a low PAT reduction can be expected pasteurization. By the use of several non-thermal methods a reduction of PAT was observed in fruits and vegetable products. Hence, UV-irradiation (5 min) led to a significant reduction of PAT (83%) in apple juice (Diao et al., 2018), and pulsed-high hydrostatic pressure treatment caused a reduction of 62% in apple juices (Avsaroglu et al., 2015). Both methodologies to detoxify PAT are promising techniques for the application in the fruit- and vegetable-processing industry. Also, some pre-harvest strategies pointed out their efficacy in PAT reduction. The application of non-mycotoxigenic moulds *in vitro* as biocontrol agent showed the ability to reduce PAT (> 95%): *Rhodotorula glunitis* and *Aureobasidium pullulans* (Leibinger et al., 1997), *Candida sake* and *Pantoea agglomerans* (Morales et al., 2008), *Candida membranifaciens* and *Rhodotorula mucilaginosa* (Gholamnejad et al., 2010) and *Pichia anomala* (Haïssam, 2011). The use of modern technologies, such as CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats), can be promising to improve beneficial fungi in preventing mycotoxin contamination as showed to reduce mycotoxins produced by *Alternaria alternate* (Wenderoth et al., 2017).

3. Toxicity

Based on the high incidence of PAT, concerns are raised on the toxicological effects of PAT on both humans and animals through consumption. Although information on the absorption of PAT is rare to non-existent, the observed *in vitro* bio-accessibility is large, especially in the oral (87%) and gastric phase (82%) (Torović et al., 2018). Consequently, PAT is expected to be majorly absorbed. In Table 3 a short overview is given on the toxic outcomes of PAT *in vitro* and *in vivo*. Acute PAT exposure causes nausea, vomiting and other gastrointestinal symptoms such as gastric ulcers, intestinal haemorrhages and lesions in the duodenum, as well as alterations in the intestinal barrier function accompanied with kidney damage (de Melo et al., 2012; Mahfoud et al., 2002; Speijers et al., 1988). These alterations were observed in rats after a dose of 1 mg/kg bw in rats (Table 3). PAT is able to down-regulate the expression of density-enhanced phosphatase-1 (DEP-1) and peroxisome proliferator-activated receptor gamma (PPARγ), consequently acute exposure results in the destruction of tight junctions (TJs) in the gastrointestinal tissue. DEP-1 affects the TJ function, and PPARγ may control DEP-1 expression (Katsuyama et al., 2014). Sub-acute toxicity to PAT showed a decrease in water and food intake in rats, it is believed that the decrease of water intake could be the result of unpalatability and the decrease of food intake could be secondary to the decrease of water or the stomach lesions observed after the high dose of patulin (Speijers et al., 1988), and the rat's kidney and gastro-intestinal tract were damaged by lesions (Speijers et al., 1988). PAT is an immunotoxic compound (> 10 nM) as it affects T-cell proliferation (Llewellyn et al., 1998). Compared to OTA, a major immunotoxic mycotoxin, PAT alters the lymphocytes' proliferation at lower concentrations (Stec et al., 2008). The level of toxicity also depends on the animal type; in rodents, the oral LD50 of PAT ranges from 29 to 55 mg/kg body weight (bw), while poults seem less sensitive with an oral LD50 of

Table 2
Patulin incidence of surveys (per continent & year) executed during 2014–2018 highlighting the percentage of positive samples (%), average and maximum concentrations (μg/kg or μg/L).

Product	Positives (%)	Average ± SD (μg/kg or μg/L)	Maximum (μg/kg or μg/L)	Country	Reference
Apple juice for infants	21 (44)	3.6 ± 1.9	8.3	Serbia (Europe)	Torović (2018)
Apple puree for infants	11 (17)	3.4 ± 2.0	7.7		
Apple juice for children	43 (43)	5.6 ± 6.8	30		
Cereal-based food	15 (75)	2.3	4.5	Portugal (Europe)	Assunção et al. (2016b)
Tomato concentrates	–	7.2	–	Italy (Europe)	Sarubbi et al. (2016)
Tomato sauces	–	4.1	–		
Tomato sauce of baby food	–	5.2	–		
Homogenized apple	–	0.9	–		
Homogenized pear	–	0.8	–		
Apple juice	27 (64)	46 ± 6.0	122	Czech Republic (Europe)	Vaclavikova et al. (2015)
Pear juice	20 (48)	63 ± 12	231		
Mixed juice	17 (50)	29 ± 3.9	56		
Compote	7 (20)	32 ± 9.7	77		
Apple jam	5 (33)	302 ± 9.6	554		
Pear jam	7 (44)	124 ± 41	325		
Apples	1 (33)	415	415		
Apple cider	2 (100)	30	48		
Apple juice	5 (83)	17	28		
Apple pulp	25 (78)	24	102		
Baby food apple	2 (20)	3.6	5.0		
Pear	1 (33)	42	42		
Pear juice	2 (67)	25	39		
Mix fruit salad	1 (100)	14	14		
Moulded bell peppers	5 (11)	–	–	Belgium (Europe)	Van De Perre et al. (2014)
Moulded soft red fruits	4 (8)	–	–		
Moulded tomatoes	17 (11)	–	–		
Apple juices	5 (56)	14 ± 18	46	Portugal (Europe)	Cunha et al. (2014)
Tomato pulps	7 (37)	17 ± 12	41		
Tomato juices	2 (50)	47 ± 1.0	48		
Tomato jellies	1 (25)	3.2	3.2		
Fufu-maize	15 (30)	105 ± 219	890	Cameroon (Africa)	Abia et al. (2017)
Concentrated juice	24 (80)	158 ± 47	889	Tunisia (Africa)	Zouaoui et al. (2015)
Apple juice	3 (43)	464 ± 671	1234	Japan (Asia)	Li et al. (2018)
Hawberry	19 (46)	10 ± 14	93	Polant (Europe)	Przybylska et al. (2019)
Apple juice	9 (39)	1.0	4.8	China (Asia)	Yang et al. (2017)
Hawthorn juice	5 (50)	1.4	3.7		
Apple juice	38 (53)	–	39.5	Iran (Asia)	Poostforoushfard et al. (2017)
Apple cans	17 (45)	–	34.8		
Apple concentrate	2 (50)	11.7	18.9	Spain (Europe)	Marsol-Vall et al. (2016)
Apple	24 (67)	270 ± 80	631	Pakistan (Asia)	Iqbal et al. (2018)
Grapes	22 (71)	466 ± 397	1100		
Pineapple	8 (82)	254 ± 16	460		
Pears	13 (65)	218 ± 20	620		
Tomato	12 (80)	410 ± 21	521		
Apple juice	15 (52)	26 ± 18	121		
Apple	8 (20)	4.7 ± 2.5	9.5	Thailand (Asia)	Poapolathep et al. (2017)
Apricot	4 (10)	4.5 ± 1.8	6.3		
Peach	3 (8)	4.4 ± 1.0	5.6		
Grape	7 (18)	3.0 ± 0.6	3.5		
Dried longans	19 (91)	75	194	China (Asia)	Ji et al. (2017)
Dried figs	13 (65)	131	277		
Fruit juices	3 (15)	13	17		
Jams	2 (10)	11	11		
Dried apricots	1 (2)	31	31	China (Asia)	Wei et al. (2017)
Fresh apple	5 (42)	3.7 ± 0.6	17	Qatar (Asia)	Hammami et al. (2017)
Apple juice	20 (100)	35 ± 1.7	82		
Baby apple juice	6 (100)	31 ± 6.7	61		
Baby apple compote	7 (100)	11 ± 1.2	25		
Apple concentrates	2 (33)	28 ± 37	54	Korea (Asia)	Seo et al. (2015)
Apple juice	4 (67)	11 ± 9.1	21		
Apple juice	12 (33)	53 ± 15.6	191	Iran (Asia)	Rahimi and Rezapoor Jeiran (2015)
Apple juice concentrate	5 (18)	24 ± 4.9	41		
Pear juice	2 (13)	23 ± 8.2	31		
Peach juice concentrate	3 (20)	27 ± 7.8	35		
Pomegranate juice	1 (8)	8.2	8.2		
White grape juice	3 (30)	12 ± 3.5	17		
Apple juice	1 (8)	27	27	Malaysia (Asia)	Lee et al. (2014)
Pineapple juice	1 (17)	33	33		
Lychee juice	1 (17)	13	13		
Apple juice	2 (20)	17 ± 2.1	18.0	United States (North-America)	Zhang et al. (2014)

(*continued on next page*)

Table 2 (*continued*)

Product	Positives (%)	Average ± SD (μg/kg or μg/L)	Maximum (μg/kg or μg/L)	Country	Reference
Concentrated juice	16 (25)	322 ± 2470	19622	Argentina (South-America)	Oteiza et al. (2017)
Concentrated pulp	68 (67)	327 ± 1045	7339		
Apple	1866 (40)	26 ± 330	19622		
Apricot	1 (5)	16	16		
Grape	5 (10)	283 ± 1951	13808		
Peach	9 (10)	5.0 ± 5.0	24		
Pear	122 (11)	54 ± 53	1749		

-Information not present in the article.

170 mg/kg bw (Puel et al., 2010). When PAT is administered via intravenous, intraperitoneal or subcutaneous routes, PAT is 3–6 times more toxic (Puel et al., 2010). Chronic animal studies revealed that PAT has mutagenic, neurotoxic, immunotoxic and genotoxic features with implications on the gastrointestinal system in rodents (Hopkins, 1993). There is an evident concern that similar effects could occur in humans through chronic PAT-contaminated consumption of foods and beverages. As there was inadequate evidence for the carcinogenicity of PAT in experimental animals and humans, IARC has classified PAT in group 3 (IARC, 1993). Based on a non-observed effect level (NOEL) of 43 μg/kg bw/day and a safety factor of 100, JECFA established a PMTDI of 0.4 μg/kg bw/day (JECFA, 1995). PAT also exerts cytotoxic effects such as severe impairment of plating efficiency and the formation of covalent adducts with essential cellular thiol-groups to proteins and amino acids (De Champdoré et al., 2007). The major retention sites of PAT are the erythrocytes and the blood-borne organs (spleen, kidney, lung and liver) (de Melo et al., 2012). Moreover, PAT is genotoxic, based on an observed impairment of DNA synthesis in mammalian cell assays, resulting in DNA strand breakage and inter-strand cross-links (De Ruyck et al., 2015; Schumacher et al., 2006). PAT induces oxidative damage of DNA which consequently leads to DNA-base modifications, mutagenesis and cancer initiation (De Champdoré et al., 2007). As well, PAT is able to inhibit the activity of several enzymes (Pfeiffer et al., 2005), including RNA-polymerase and aminoacyl-tRNA synthetases (de Melo et al., 2012). *In vivo* PAT administration (1.0–3.75 mg/kg bw) in mice exerted a doses-dependent DNA damage in the brain, liver and kidneys (de Melo et al., 2012). Furthermore, a glutathione (GSH) reduction followed by an increase in lipid peroxidation was observed after *in vivo* PAT administration (de Melo et al., 2012). A previous administration of N-acetyl-cysteine reduced the PAT-induced DNA damage and lipid peroxidation, establishing a strong correlation among a decreased GSH-content, increased lipid peroxidation and DNA damage. PAT also gave rise to hepatotoxic effects which could be linked to the inhibition of the enzyme hepatic-aldolase (de Melo et al., 2012). Recently, Lu et al.

Table 3
Toxicity studies on patulin: *in vitro* and *in vivo* studies targeted on different species at different doses.

Toxicity	*In vitro*/*In vivo*	Species	Dose	Reference
PAT induces DNA damage in the brain, liver and kidneys.	*In vivo*	Rat	1.0–3.75 mg/kg bw	de Melo et al. (2012)
PAT down-regulates the expression of density-enhanced phosphatase-1 (DEP-1) and peroxisome proliferator-activated receptors-γ.	*In vitro*	Human	50 μM	Katsuyama et al. (2014)
T-cell proliferation is highly sensitive to PAT.	*In vitro*	Human	50 μM	Assunção et al. (2016a)
PAT decreases the transepithelial electrical resistance.	*In vitro*	Human	95 μM	Assunção et al. (2014)
PAT causes a decrease of cell viability in a dose-dependent manner. PAT induces a significant ROS formation. PAT induces p53 expression, which is a well-known DNA damage marker. PAT increases total DNA damage. PAT affects the mitochondrial metabolism when measuring succinate dehydrogenase activity by MTT assay.	*In vitro*	Human	5–100 μM	Ayed-Boussema et al. (2013)
PAT produces a simultaneous suppression of GJIC and GSH depletion. PAT generates the presence of ROS. PAT induces mitochondrial membrane depolarization and cytoplasmic acidification.	*In vitro*	Rat	0 to 1000 μM	Barhoumi and Burghardt (1996)
PAT causes a sulfhydryl modification of membrane proteins. PAT induces the inhibition of the Na^+-K^+ ATP-ase.	*In vitro*	Rat	0.1–1 μM	Burghardt et al. (1992)
PAT increases the creatinin phosphokinase (CPK) level, induces lipoperoxydation and protein oxidation, and triggers the antioxidant enzymes such as superoxide dismutase and catalase activities. PAT induces the caspase 3-activation, and a significant decrease in CPK, ALT, AST, TG, LDH, and T-CHOL levels. PAT induces an overexpression of HSP70 genes. PAT provides an increase in PC groups. PAT exhibits the MDA formation in cardiac tissues. PAT significantly promotes SOD and CAT activities.	*In vivo*	Rat	50–250 mg/kg bw	Boussabbeh et al. (2015)
PAT treatment directly increases intracellular oxidative stress in human embryonic kidney (HEK293) and human promyelocytic leukemia (HL-60) cells. The activation of the ERK1/2 signaling pathway is correlated with PAT-mediated ROS. PAT leads to the activation of MAPKs, including extracellular signal-regulated kinase (ERK) 1/2, p38 kinase, and c-jun N-terminal kinase (JNK). The phosphorylation of ERK1/2 is a major factor contributing to PAT-induced genotoxicity.	*In vitro*	Rat and human	25–100 μM	Liu et al. (2007)
PAT-intoxication causes severe hepatotoxicity, as indicated by the significant elevation of serum ALT and AST activities	*In vivo*	Rat	25–100 mg/kg bw	Song et al. (2014)
PAT produces a phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) in human embryonic kidney (HEK293) cells, human peripheral blood mononuclear cells (PBMCs), and Madin-Darby canine kidney (MDCK) cells.	*In vitro*	Human and dog	15–100 μM	Wu et al. (2005)

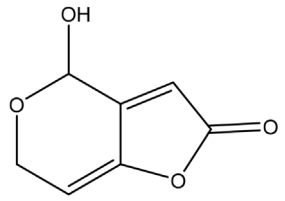
bw = body weight.

(2017) proved that methylseleninic acid is capable to protect against the hepatotoxicity produced by PAT; methylseleninic acid is a well-known essential micronutrient with certain pharmacological activities for humans and animals attributed to its biological functions in the redox regulation and defence against oxidative stress (Ip, 1998; Nido et al., 2016), (Lu et al., 2017). In conclusion, an amalgam of toxic effects are attributed to PAT, even at low concentrations. Therefore there is an urgent need to address the PAT exposure, and to reduce the toxicity of PAT by using strategies such as methylseleninic acid intake.

4. Analytical challenges

The analytical determination of PAT represents the common challenges observed in mycotoxin analysis. One of the most important issues is the sampling as mycotoxins have a mainly heterogeneous distribution in a sampling lot (Koppen et al., 2010). Several studies on the heterogeneity of mycotoxin contamination have allowed the development of statistical and mathematical models aiding in the design of sampling plans (Whitaker, 2006), luckily PAT is mainly found in liquid products like fruit juices and these samples are more homogenic than solid ones. In addition, PAT has physicochemical properties that add more difficulties in its determination: PAT is a highly-polar, low-molecular weight molecule (Fig. 1, 154 g/mol). In terms of analytical sample pre-treatment, recent studies are mostly using solid/liquid-liquid extraction in food matrices. These techniques are pinpointing a robust method for the PAT extraction in a wide range of food matrices (Table 4). Immunoaffinity columns (IAC) have been used due to their highly selective feature and they have been applied for extraction and purification of PAT. However, the high cost and the no possibility of analyse other mycotoxins cause disadvantages in their use.

Multi-mycotoxin determination is nowadays a common practice, especially for modified mycotoxins determination as mycotoxin conjugates (Malachová et al., 2018). However, PAT is not usually included in the multi-mycotoxin analysis as probably the presence of PAT in foods is limited to fruit and vegetables. For detection and identification in food, most recent studies applied HPLC-UV (Iqbal et al., 2018; Ji et al., 2017; Torović, 2018) (Table 4). Apart from its strong absorption of UV light, the detection of small molecules such as PAT by using mass spectrometry (MS) remains as a major challenge during the interpretation of MS data. However, HPLC-UV has some limitations in specificity and sensitivity compared to LC-MS/MS. Specificity is imperative to correctly identify the compound of interest: *e.g.* 5-hydroxymethylfurfulral (HMF) produces interferences and affects the PAT quantification in HPLC-UV (Da Silva et al., 2007). The lack of sensitivity and specificity present in HPLC-UV can be a serious disadvantage when low concentrations or complex matrixes are being analysed. In addition, hydrophilic compounds such as PAT are difficult to retain on column, therefore a large water content in the mobile phase is the proposed solution for polar compounds' retention. Most of the PAT analytical methods are using large percentages of water (≥90%) in the mobile phase (Table 4) (Assunção et al., 2016b; Ji et al., 2017; Lee et al., 2014; Rahimi and Rezapoor Jeiran, 2015; Torović, 2018; Van De Perre et al., 2014). The large water content of the mobile phase, however, has to be compatible with the ionization source, so previous LC-MS/MS experience is necessary. Large percentages of water in the mobile phase (≥90%) have proven to have a positive impact on the efficient negative ionization of PAT using ammonium acetate (NH_4Ac) (Li et al., 2018; Poapolathep et al., 2017; Wei et al., 2017). As commented before, LC-MS/MS provides a higher sensitivity with a factor 100, consequently a lower LOD than HPLC-UV is obtained. GC-MS/MS has also been used with success for PAT determination with a LOD of 0.4 μg/kg (Cunha et al., 2009, 2014) (Table 4). Finally, the selection of the analysis procedure for PAT determination will depend on the matrix and the sensitivity demanded.



$C_7H_6O_4$ - molecular weight = 154.12 g/mol

Fig. 1. Chemical structure of patulin. $C_7H_6O_4$ - molecular weight = 154.12 g/mol.

On the other hand, the analysis of mycotoxin biomarkers of exposure has become commonplace, and biomarker-driven research has been proposed as a successful method to assess the exposure to mycotoxins through the measurement of their metabolites in biological fluids (Atkinson et al., 2001). Among mycotoxins, only aflatoxins (Wild et al., 1990, 1992) and deoxynivalenol (Vidal et al., 2018a) biomarkers have been successfully validated in humans (Vidal et al., 2018b). Although the lack of validated mycotoxin biomarkers, multi-mycotoxin analytical methods in biological fluids has been set up (Ediage et al., 2013; Escrivá et al., 2017; Heyndrickx et al., 2015; Slobodchikova and Vuckovic, 2018; Valitutti et al., 2018). Although the robustness and solidity of solid/liquid-liquid PAT extraction in food samples, this technique is not applicable in biological samples. 'Dilute and shoot' and QuEChERS-based methodologies have been the common methods to analyse mycotoxins in biological fluids. PAT has not been included in any of these multi-mycotoxin analysis in biological samples as there is no PAT biomarker. Therefore, a PAT biomarker should be identified and validated, primarily through an *in vitro* experiment to verify PAT predominant metabolites, and secondly through *in vivo* a toxicokinetic studies to verify its metabolism and excretion profile. The PAT determination in biological fluids must be in LC-MS/MS because PAT concentrations in biological fluids are expected to be low, so LC-MS/MS will provide a better sensitivity than HPLC-UV and it will be more suitable for PAT determination in biological samples. Moreover, some more polar compounds could be formed during metabolisation, as glucuronide compounds. Hypothetic glucuronide compounds would be more polar than PAT and they could elude before than free PAT.

5. Conclusion

During the last few decades, consumers have become more aware on health and food quality, consequently research on food safety augmented. The evaluation of the PAT contamination in fruits and vegetables became an important factor to guarantee the products' quality. PAT observed in food samples points out that human PAT exposure is a non-negligible fact. Moreover, PAT has a higher inmunotoxicity level than OTA, which should be a trigger to increase PAT research. For this reason, it is imperative to improve the analytical standards to detect and quantify PAT. Although UV-based detection has become the most applied method to analyse PAT in food, a reliable MS-based method -as it exists for other mycotoxins-is necessary to improve the sensitivity and selectivity of PAT analysis. PAT exposure studies are scarce, therefore focus should be drawn towards the identification of PAT biomarkers of exposure. The latter would become a crucial step to assess PAT exposure in humans.

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Transparency document

Transparency document related to this article can be found online at

Table 4
Analytical methods for patulin (PAT) determination in foods with the limit of detection (LOD) used in studies from the last 4 years (2014–2018).

Matrix	Extraction method	Equipment	Mobile phase	Recovery (%)	LOD (μg/L or μg/kg)	Reference
Apple juice for infants Apple puree for infants Apple juice for children	SPE	HPLC-UV	Isocratic H_2O/ACN/perchloric acid (95.9/4/0.1, v/v/v)	85 72 62	5	Torović (2018)
Apple juice	Liquid-liquid microextraction	LC-MS/MS	Gradient A: 5 Mm NH_4AC in H_2O B: ACN	90.1	0.5	Li et al. (2018)
Apple, grapes, pineapple Pear, tomato & apple juice	Liquid/solid-liquid	HPLC-UV	Isocratic ACN/H_2O (90/10, v/v)	–	0.01	Iqbal et al. (2018)
Apple, apricot, peach, grape	SPE	LC-MS/MS	Gradient A: 5 Mm NH_4AC in H_2O B: MeOH	84–90	0.5	Poapolathep et al. (2017)
Fufu-maize	Solid-liquid	LC-MS/MS	–	100	5	Abia et al. (2017)
Dried longans Dried figs Fruit juices Jams	Solid-liquid Solid-liquid Liquid-liquid Solid-liquid	HPLC-UV	Isocratic ACN/H_2O (90/10, v/v)	89.9 85.4 89.9 78.8	3.2 7.5 3.5 2.6	Ji et al. (2017)
Concentrated juice & pulp Apple, apricot, grape, peach pear	Liquid-liquid Solid-liquid	HPLC-UV	Isocratic Methanol (100%)	–	–	Oteiza et al. (2017)
Dried apricots	Solid-liquid	LC-MS/MS	A: ACN B: H_2O with NH_4AC	81.8	1.5	Wei et al. (2017)
Fresh apple, apple juice Baby apple juice and compote	Solid-liquid Liquid-liquid	LC-MS/MS	A: H_2O with AA B: MeOH	–	1	Hammami et al. (2017)
Cereal-based foods	SPE	HPLC-UV	Isocratic H_2O/ACN/perchloric acid (96/4/0.1, v/v/v)	50–120	3	Assunção et al. (2016b)
Tomato concentrates, sauces Tomato sauce of baby food Homogenized apple & pear	Solid/liquid-liquid	HPLC-UV	Isocratic A: H_2O B: ACN	96.2	–	Sarubbi et al. (2016)
Apple concentrates & juice	SPE	LC-MS/MS	Gradient A: H_2O with 5 mmol/L NH_4AC and AA B: 20% MeOH	–	0.2	Seo et al. (2015)
Concentrated juice	Solid/liquid-liquid	HPLC-UV	Isocratic H_2O/ACN (90/10, v/v)	–	10	Zouaoui et al. (2015)
Apple, pear & mixed juice Compote, apple & pear jam Apple, apple cider & pulp Baby food apple, pear & mixed fruit salad	QuEChERS	LC-MS/MS	Gradient A: 5 Mm NH_4AC in H_2O B: MeOH	92	0.5	Vaclavikova et al. (2015)
Apple juice & concentrate Pear juice & concentrate Peach juice concentrate Pomegranate juice White grape juice	SPE	HPLC-UV	Isocratic H_2O/THF (95/5, v/v)	86	5	Rahimi and Rezapoor Jeiran (2015)
Apple juice	Centrifuge & shoot	LC-MS/MS	Gradient A: H_2O with 10 mM NH_4F/0.1% FA B: MeOH with 10 mM NH_4F/0.1% FA	97	4	Zhang et al. (2014)
Apple juice, pineapple juice & lychee juice	Liquid-liquid	HPLC-UV	ACN/H_2O (10/90, v/v)	99	0.25	Lee et al. (2014)
Moulded bell peppers, moulded soft red fruits & moulded tomatoes	Liquid-liquid	HPLC-UV	Isocratic ACN/H_2O 0.0175% perchloric acid (7/93, v/v)	97	13	Van De Perre et al. (2014)
Apple juices, tomato pulps, Tomato juices & tomato jellies	Liquid-liquid	GC-MS	Helium	72 77	0.4	Cunha et al. (2014)

*ACN = Acetonitrile; MeOH = methanol; AA = acetic acid; NH_4F = ammonium formate; NH_4Ac = ammonium acetate, THF = TetraHydroFuran, SPE = Solid-phase extraction, QuEChERS = Quick, Easy, Cheap, Effective, Rugged, and Safe, HPLC-UV = High Performance Liquid Chromatography with UltraViolet detector, LC-MS/MS = Liquid Chromatography coupled with double Mass Spectrometry.
-Information not present in the article.

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