Review Article

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# Critical review of the analytical methods for determining the mycotoxin patulin in food matrices

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**Abstract:** This manuscript is a critical review of the analytical methods reported in the existing literature for the determination of mycotoxin patulin at trace/ultra-trace levels in food matrices. The article starts focusing on what mycotoxins are, their "analytical history" (more than 21,000 articles published in Scopus database): each mycotoxin is specific for a given fungus and shows toxic effects, some even being carcinogenic. Most International regulations on mycotoxins are also reported, which pertain official controls in the food chain as well as the sampling methods and the maximum tolerable limits of mycotoxins. Then the manuscript is focused on patulin, a mycotoxin that is mainly produced by the fungal species *Penicillium expansum*. The main characteristics and properties of patulin are discussed, including its biosynthesis, especially on stored fruits infected by *P. expansum* and derived products, its toxicology, and some strategies aiming at preventing and/or reducing its presence. The description of the analytical procedure for patulin starts from sampling: the extraction and analytical methods reported are based on the official protocol of the Association of Official Analytical Chemists, which relies on the high-performance liquid chromatography-ultraviolet/diode array detector (HPLC-UV/DAD). Furthermore, an in-depth discussion of the most suitable analytical methods is reported. The first analytical step regards the analyte(s) extraction from the sample, followed by a clean-up phase, and by a final quantitative determination. This last section is divided into reference or confirmation methods, rapid screening and new methods and expected results, i.e., qualitative, quantitative, or semi-quantitative. Reference methods include TLC, GC, HPLC, and MS, whereas rapid methods include enzyme immunoassay tests, dipsticks, and lateral flow tests. Novel analytical methods include fluorescence, near infrared spectroscopy, capillary electrophoresis, and biosensors. Finally, the official method is compared with others present in the literature allowing a multi-target analysis, and its use in combination with other techniques of molecularly imprinted polymers is discussed.

**Keywords:** patulin, mycotoxin, analytical methods, chromatography, molecularly imprinted polymers

## 1 Introduction

In the last few decades, consumers' awareness on food quality and safety has been steadily increasing, especially on the possible hazards for their health deriving from toxic contaminants, rather than the chemical composition of food.

Foods are matrices that can be easily contaminated by microorganisms, pests, and chemicals that can be harmful to human health. Their determination is crucial in order to guarantee food safety, especially in the case of chemical contaminants. In this regard, the development of suitable analytical procedures for their determination, especially when chemical contaminants are present at (ultra)trace levels, is a key issue. Food contamination has acquired significant importance since a definition of food contaminant was given [1,2]. Among the numerous contaminants, mycotoxins can be dangerous and elusive [3–6]. Mycotoxins are secondary metabolites that result from attacks on crops both in the field and during storage by mycotoxigenic fungal phytopathogens [7–9].

Mycotoxins can cause poisoning (mycotoxicosis) and represent severe health hazards to animals and humans

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[10,11]. The Food and Agriculture Organization (FAO) has estimated that about 25% of the global crops are contaminated with molds and therefore potentially affected by mycotoxins, and relevant economic losses are estimated to be billions of dollars [12]. Many mycotoxins are stable and remain in contaminated products for long time even after the death or inactivation of the respective mycotoxigenic fungus. A survey on scientific databases such as Scopus or Web of Science reveals that the interest on mycotoxins has been increasing over the years. To date, there are more than 21,000 publications on this topic and this trend is expected to further increase.

At present, multiple controls are available in the food sector. The Rapid Alert System for Food and Feed (RASFF) was created by the European Union (EU) with the aim of keeping under control the risks that can impact food safety. Every year, the RASFF publishes a report on the alerts and on the type of contaminant. In the latest available report ref. [13], mycotoxins are listed among the main ten categories of hazards on food products from three countries. Indeed, 542 serious risk warnings were reported. All these food warnings can be notified only if there are reliable methods to determine the presence and quantity of mycotoxins.

Among the different mycotoxins, patulin is an important mycotoxin. It is mainly produced by *P. expansum* that attacks different fruits, especially apples and pears during storage. A major concern about this mycotoxin is that it can be found in baby food, from homogenized to fruit juices. In addition, it is listed in Group 3 by the International Agency for Research on Cancer, i.e., those substances that could induce carcinogenicity but, as there is insufficient evidence in this regard, they are not yet among the verified carcinogens.

The aim of this review is to discuss the different analytical methods that allow qualitative and quantitative detection of patulin at low concentrations in several food matrices. Novel or confirmation methods giving qualitative–quantitative results with high recovery will be commented along with other methods allowing a user-friendly and quick determination, such as rapid screening tests. Currently, highly specific, easy to use, and low-cost methods that can also be used by non-expert personnel are being tentatively developed. These innovative methods are validated with the confirmation methods for giving qualitative and/or quantitative results. Their use will be assessed in relation to their regulatory and scientific acceptability, also taking into account their difference in field or laboratory application.

# 2 Patulin: general information

## 2.1 Physical and chemical properties

Patulin (IUPAC name 4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one, CAS # 149-29-1) (Figure 1), a heterocyclic lactone (molecular weight, MW, 154.12; low volatility, i.e., melting point at 110°C), is a heat-resistant toxin [14] and is stable in aqueous media between 105 and 125°C in the pH range from 3.5 to 5.5 [15], whereas it undergoes spontaneous degradation at more alkaline pH [16]. About 50% of patulin degrades within 1 h at 100°C in an aqueous medium at pH 6, whereas sulfites promote its destruction after exposure at pH 7 and 25°C for 3 h by adding 50 ppm of sulfur dioxide [17].

In fruits, the main matrix in which the toxin is found, the sulfhydryl groups (SH–), especially those of glutathione, react with patulin leading to its inactivation. However, this also leads to a significant decrease in glutathione concentration, with consequent alteration of the cellular redox homeostasis, thus making the fruit more susceptible to possible oxidations [18,19]. Patulin is produced by several species of fungi belonging mainly to the genera *Aspergillus*, *Penicillium*, and *Byssochlamys* [20–23]. Among these, the major producer is *P. expansum*, which causes the blue mold disease of many stored fruits, especially pome fruits [24–27]. Patulin production depends on several factors, such as temperature, pH, and other physiological parameters [28]. *P. expansum* is a psychrotolerant fungus, its growth optimum is ~25°C, but it is able to grow even at temperatures close to 0°C. For this reason, prevention is problematic as the fungus grows and produces patulin also at temperatures used for cold storage of fruits [29].

## 2.2 Contamination source

Humans are exposed to patulin mainly through contaminated food products, a result of fungal infections of stored fruits [30]. *P. expansum* is a fungus that penetrates and develops starting from conidia that germinate in

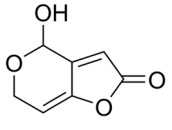


**Figure 1:** Chemical structure of patulin.

superficial fruit wounds caused by manipulations during harvest, transport, and/or storage. Rotting and soft notches develop from small roundish notches and grow rapidly. At the fungus penetration site, in the center of the rotting area, a whitish mold develops, and will later take on the characteristic green–blue color of conidia. These conidia can therefore be responsible for further infections as they are transported by wind, water, and insects to other fruits. More rarely, the penetration of the fungus can occur through the pedicel. In this case, the decay develops internally, and no symptoms are visible on the fruit surface. As a consequence, these fruits are not discarded and can contribute to patulin contamination [31,32].

## 2.3 Patulin biosynthesis

The patulin biosynthetic pathway consists of about ten steps, as suggested both by numerous biochemical studies and by identification of different mutants blocked at various steps of the patulin biosynthetic pathway [33,34].

The first step is the formation of 6-methyl salicylic acid (6-MSA) by condensation of an acetyl-CoA and three malonyl-CoA units. This reaction is catalyzed by a single multifunctional enzyme which has several enzymatic activities: acetyl and malonyl transferase, ketoacyl synthase, ketoreductase, and dehydratase [35]. Bu'Lock and Ryan [36] and Tanenbaum and Bassett [37] have shown that radiolabeled 6-MSA is converted into patulin. Studies with $^{14}C$ and $^{3}H$ radioisotopes and $^{13}C$ and $^{2}H$ stable isotopes have shown that 6-MSA is extensively modified to form patulin. Studies using radioisotopes have shown that *m*-cresol and gentisylaldehyde are structurally similar to 6-MSA [38–40]. In fact, the 6-MSA is modified into *m*-cresol by 6-MSA decarboxylase, then the methyl group of *m*-cresol is oxidized to form an aldehyde group. This step is followed by a hydroxylation reaction that leads to the formation of gentisylaldehyde. Subsequently, gentisylaldehyde is converted into a two-ring structure, i.e., patulin, which requires the opening of a ring by a mechanism mediated by a monooxygenase or a dioxygenase (Figure 2) [34].

## 2.4 Toxicological aspects

Like other mycotoxins, patulin causes the lowering of the immune defenses [41]. Acute exposure to patulin can cause gastrointestinal symptoms including nausea, vomiting, ulcers, intestinal bleeding, and duodenal injury. As

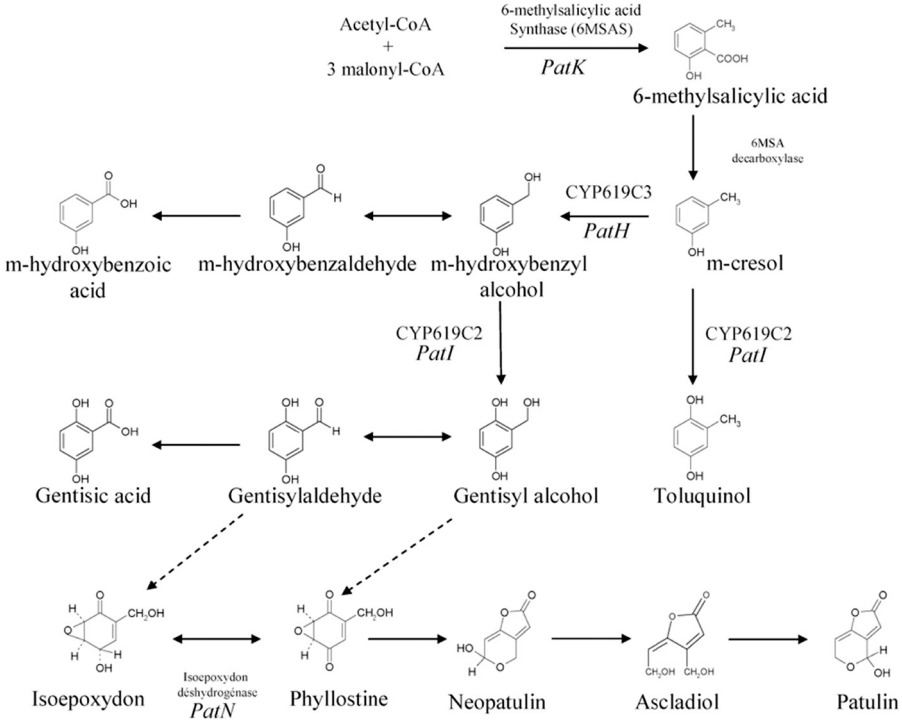


**Figure 2:** Patulin biosynthesis; from ref. [34].

mentioned, patulin is included by International Agency for Research on Cancer (I.A.R.C.) in Group 3 as potential carcinogen because animal and epidemiological studies are not enough to support its carcinogenicity, although data from *in vitro* and cell culture studies revealed its genotoxicity [42]. In addition, the World Health Organization (WHO) considers patulin a genotoxic compound [44]. The affinity of patulin with the sulfhydryl groups explains its inhibitory effect toward many enzymes (ATPase, lysosomal enzymes, RNA polymerase, etc.) [34]. At present, the toxin is known to be linked to adverse neurological, gastrointestinal, and immunological effects [43]; assessments of its toxicity have shown damages to organs such as liver, kidneys, and others [41]. Furthermore, the adverse effects of mycotoxins in general, and patulin in particular, on sensitive structures of the intestine have been widely studied, and its harmful effects on the function of the intestinal barrier have been demonstrated [45]. The intestinal barrier has two other partners in the digestive system, mucus, and microbiota. Chronic exposure to mycotoxins including patulin could significantly change the normal composition of the intestinal microflora. The effect of patulin on the gut microbiota is clearly demonstrated, but further investigations are needed [46]. All these effects have to be taken into careful consideration because patulin is resistant to industrial transformation processes (juices, purees, etc.) and it can be detected in homogenized fruits and in fruit juices for children. For this reason, the EU has established maximum tolerable levels in juices and other derived products [47]. According to the WHO [44], the maximum acceptable level in apple juice is set at 50 $\mu g\,L^{-1}$. This level is in agreement with the United States Food and Drug Administration and the EU legislative measures. Furthermore, EU also limited the highest tolerable patulin level in apple fruit to 50 $\mu g\,kg^{-1}$ and in apple-based baby food up to 10 $\mu g\,L^{-1}$ [44,48]. According to the European Commission (EC) recommendations and based on the patulin levels established by the No Observed Adverse Effect Level (NOAEL) (i.e., 43 $\mu g\,kg^{-1}$ body weight), the provisional maximum tolerable daily intake (PMTDI) is set at 0.4 $\mu g\ kg^{-1}$ body weight. This level has been adopted by most of the health risk assessment analyses conducted on patulin [49].

## 2.5 Regulations and limits

The regulation EC 466/2001 [50] establishes the maximum limits of various contaminants, including mycotoxins, whereas the regulation CE 1881/2006 [49] defines the maximum limits. The second regulation considers the information and developments of the Codex Alimentarius, the presence of some contaminants in food products above which limits the foodstuffs must not be commercialized and standards desired by FAO and WHO. In particular, these standards define the limits of mycotoxins, but these limits are less restrictive than those contained in the EU regulations. The use of non-compliant food is banned, as well as it is specified that the same non-compliant products must not be mixed with other compliant products and that the products intended for decontamination cannot be mixed with food intended for direct consumption. The limits must be respected also taking into account the sampling method defined by regulation CE 401/2006 [51]. Table 1 shows the maximum limit for patulin, as reported in annex 2 of regulation CE 1881/2006 [49].

The EU limits are intended to define whether a food may or may not be marketed. Finally, according to the regulation CE 882/2004 [52] the competent authorities of each Member State must check if these limits are respected.

## 2.6 Preventive measures for controlling patulin

*Penicillium expansum*, the main producer of patulin, is able to grow in multiple conditions. Basically, damaged

**Table 1:** Maximum patulin levels in some foods according to ref. [49]

| Food commodity | Maximum tolerable levels ($\mu g\,kg^{-1}$) |
|---|---|
| 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 labeled and sold as such | 10 |
| Baby foods other than processed cereal-based foods for infants and young children | 10 |

or aging fruits certainly favor its attack and development. For this reason, prevention is the most recommended method to limit patulin contamination [31]. Therefore, adequate harvesting and adequate storage methods are needed to protect fruit quality [53]. Studies have shown that washing fruits with high pressure water and removing rotten fruits before storage help reducing fungal contamination and, therefore, toxin production [54]. For some fruits, UV-C light has shown efficacy in limiting the growth of *P. expansum* in postharvest conditions without damaging the fruit itself [55]. To limit fungal contamination, fungicides can also be used [31]. Recently, the exogenous treatment of potassium phosphide has shown promising results in controlling the growth of patulin-producing fungi [56]. Combination of sodium hypochlorite (NaClO), hydrogen peroxide ($H_2O_2$), and copper sulfate ($CuSO_4$) resulted in complete inhibition of *P. expansum* growth, spore germination, and patulin synthesis [57]. Chlorine dioxide ($ClO_2$) has shown antifungal activity against *P. expansum* in apple samples and in potato dextrose broth medium [58]. On the other hand, propolis, natural extract produced by bees, has been applied as a natural antifungal agent to limit patulin production [59]. Finally, the chemical adsorption of patulin has been recently studied: a new organic metal-based adsorbent (UiO-66($NH_2$)@Au-Cys) showed very high efficacy in removing patulin from apple juice, ten times higher than that of microbe-based bio-adsorbents [60]. Ultraviolet (UV) irradiation has been shown to be effective to inhibit fungal development and also to directly reduce patulin levels in contaminated samples. The results were promising in the case of filtered juice. However, irradiation does not work for fresh juice containing many suspended particles that absorb UV light before it reaches patulin [61]. In addition, several chemicals, such as ammonia, potassium permanganate, vitamin B, sulfur dioxide, ozone, pyridoxine hydrochloride, and calcium D-pantothenate, have shown promising results in decontamination; however, only a few of these substances can be used in contaminated food products because they are toxic to humans [28,62]. For the patulin reduction in apple juice, other methods have been considered, such as the addition of citric acid, sodium bicarbonate, vinegar, a combination of sodium bicarbonate and citric acid, baking powder, and UV irradiation. UV irradiation and sodium bicarbonate have shown an effect in reducing patulin [63]. UV rays and sodium bicarbonate negatively affect the quality of juice; however, these effects are reversible with the addition of citric acid. Therefore, a combination of sodium bicarbonate and citric acid could be considered as an additive to apple juice to reduce patulin levels [63]. Finally, microbial agents were shown to detoxify patulin, such as the baker yeast *Saccharomyces cerevisiae*, which is the most commonly used organism for fermentations (e.g., cider production) and food preparations [64]. Other biocontrol yeasts belonging to the Pucciniomycotina within the fungal phylum Basidiomycota, such as *Rhodotorula kratochvilovae*, *R. paludigenum*, *R. mucilaginosa*, and *Sporobolomyces* sp., displayed the ability to protect stored apples from *P. expansum* infections and to decrease patulin contamination by direct enzymatic metabolization. The patulin degradation process consists of two pathways that act on separate ends of the patulin molecule and have different regulation in these yeast species. One pathway leads to the opening of the lactone ring with the formation of desoxypatulinic acid, while another pathway operates on the hemiacetal group with the formation of ascladiol, which is also the last product of the patulin biosynthetic pathway (Figure 2). Several enzymes have been predicted to be involved in the patulin degradation pathways, such as a glucose–methanol–choline oxidoreductase, a protein subunit of aromatic ring-opening dioxygenase, the vacuolar proteins Env9 and Ycf1, and several short and medium chain dehydrogenases [18,65,66]. A recent study reported that an *S*-adenosylmethionine-dependent methyltransferase plays a role in the process of patulin degradation by the biocontrol agent *Pichia caribbica* [67]. The broad interest of patulin biodegradation relies on the reduced or null toxicity of the patulin degradation products, which in the case of desoxypatulinic acid is due to the low reactivity with –SH bearing glutathione [19,68,69]. Heat-inactivated cells and spores of the genus *Alicyclobacillus* have been tested for the ability to adsorb patulin from contaminated juice samples [70]. Among seven bacterial strains tested, *Alicyclobacillus acidocaldarius* showed the best results achieving adsorption up to 12.6 $\mu g\, g^{-1}$ of patulin from apple juice samples. A recent study also showed that the probiotic bacterium *Lactobacillus plantarum* ATCC 8014 efficiently reduces patulin content in contaminated apple juice and improves the safety of fruit juices for human consumption [71].

# 3 Sampling and analytical methods for patulin determination

The regulation CE no. 401/2006 [51] establishes the criteria for sampling and analytical methods for determining the mycotoxin levels in foodstuffs. From an analytical viewpoint, "where no specific methods for the determination of

mycotoxin levels in foodstuffs are required by EU legislation, laboratories may select any method provided the selected method meets the [...] criteria" [51]. It means that there is no official method for any mycotoxin analysis [72–74], patulin in this case, and each laboratory can apply its own methodology but respecting some specific analytical parameters. In particular, these parameters are reported in the annex 2 of the regulation for the analysis of patulin in foodstuffs. The samples must be carefully prepared and homogenized since the mycotoxin distribution is not homogeneous. The analytical methods refer to some definitions with the aim of standardizing the investigation of the different laboratories [51]:

- $r$ = repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions, namely, same sample, same operator, same apparatus, same laboratory, and short interval of time, may be expected to lie within a specific probability (typically 95%) and hence $r = 2.8 \times s_r$;
- $R$ = reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions, namely, on identical material obtained by operators in different laboratories, using the standardized test method may be expected to lie within a certain probability (typically 95%); $R = 2.8 \times s_R$;
- $s_r$ (or $s_R$) = standard deviation, calculated from results generated under repeatability (or reproducibility) conditions;
- $RSD_r$ (or $RSD_R$) = relative standard deviation, calculated from results generated under repeatability (or reproducibility) conditions.

The criteria for patulin determination are reported in Table 2.

**Table 2:** Analytical method criteria for patulin determination

| Level (μg kg$^{-1}$) | $RSD_r$ (%) | $RSD_R$ (%) | Recovery (%) |
|---|---|---|---|
| <20 | ≤30 | ≤40 | 50–120 |
| 20–50 | ≤20 | ≤30 | 70–105 |
| >50 | ≤15 | ≤25 | 75–105 |

## 3.1 Sampling

In the analytical determination of patulin, sampling is one of the most important issues due to its possible irregular distribution, even if this mycotoxin is mainly found in liquid products such as fruit juices (where distribution is more homogeneous than in fruits) [75]. For the sampling procedure in EU, reference is Reg. 401/2006 [51], which incorporates Directive 2003/78/EC: it specifies the sampling and analysis methods for the official control of the levels of patulin in food products. In the case of liquid products, the batch must be possibly mixed, in an accurate way, with manual or mechanical means, immediately before sampling. In the event that such mixing is possible, it can be assumed that patulin is evenly distributed within the batch. Therefore, it is sufficient to take three samples to form the overall sample and the volume should be 1 L (Table 3).

In addition, a sampling method is also required for the official control of the maximum tolerable levels established for patulin in apple fruit, apple juice, and apple-based products intended for infants and children. In the specific case, the aggregate sample must weigh 1 kg and, depending on the lot, definite numbers of elementary samples must be withdrawn (Table 4) [51].

## 3.2 Sample extraction and clean-up

It is important to underline that in the vegetal matrices, in particular fruit, externally to primary and secondary cell wall there is a structure called middle lamella, which consists mainly of pectin and pectic acids that hold neighboring cells together. The prevalent monomers of these polymers are polygalacturonic acid and other polysaccharides that can be more or less methylated. Pectin, in particular, is widely used in the food industry as a gelling agent, stabilizer, and thickener for jams, jellies, confectionery, and fruit juice production [76]. Citrus fruits (grapefruits, lemons, oranges) and apples contain much more pectin than cherries or grapes [77]. The enzymatic hydrolysis of pectin improves both the patulin recovery and the fruit juice and puree clarity [78,79]. As suggested in the official protocol of the Association of Official Analytical Chemists (AOAC 2000/02), solubilization of pectin has to be performed before the chromatographic analysis of turbid juices and solid apple-based products. Enzymatic hydrolysis of pectin is performed by using pectinase [80] both in liquid and solid samples [81]. A depectinization followed by a solid-phase extraction (SPE) allows the recovery of about 100% of patulin from apple jam and pear jelly [81]. It is important to carry out the depectinization step because it prevents a significant loss of patulin, which would take place due to the bonds occurring between the mycotoxin and the proteins present in the solid residues of the cloudy apple juice [82]. In this way, the recoveries obtained (73–75%) still

**Table 3:** Minimum number of incremental samples to be taken from the lot (for liquid samples)

| Form of commercialization | Volume of lot (L) | Minimum number of incremental samples to be taken | Minimum volume (L) or weight (kg) of aggregate sample |
|---|---|---|---|
| Bulk (fruit juices, alcoholic drinks, cider, wine) | — | 3 | 1 |
| Bottles/packages (fruit juice, alcoholic drinks, cider) | ≤50 | 3 | 1 |
| Bottles/packages (fruit juice, alcoholic drinks, cider) | 50–500 | 5 | 1 |
| Bottles/packages (fruit juice, alcoholic drinks, cider) | >500 | 10 | 1 |
| Bottles/packages wines | ≤50 | 1 | 1 |
| Bottles/packages wines | 50–500 | 2 | 1 |
| Bottles/packages wines | >500 | 3 | 1 |

**Table 4:** Minimum number of incremental samples to be taken from the lot (for solid samples)

| Weight of lot (kg) | Minimum number of incremental samples to be taken | Aggregate sample weight (kg) |
|---|---|---|
| <50 | 3 | 1 |
| 50–500 | 5 | 1 |
| >500 | 10 | 1 |

comply with the EU recommendations [79]. After carrying out this first clarification treatment, patulin extraction is carried out; this step affects the concentration and purity of the target compound. One of the commonly used extraction methods is the liquid–liquid extraction (LLE). According to the 2000/02 official protocol of the AOAC, LLE takes place with ethyl acetate three times. The final extract is then cleaned with sodium carbonate. As already pointed out, patulin is not stable in an alkaline environment, and therefore the latter step has to be performed as quickly as possible [78]. Recently, this method has been applied, with or without some modifications, for the extraction of mycotoxins from apple juices and other fruit juices [24,81,83–88] as well as from solid samples (apples, pears, grapes, pineapples, and baby food) [89–91]. To avoid the problem of pH increasing during the extract cleaning procedure, other salts such as sodium sulfate and hydrogen sodium carbonate, have been tested and incorporated in the extraction procedure [84,85], as well as the SPE cleaning steps as an alternative system [92]. The LLE disadvantage is the high consumption of organic solvents and a relatively long time of sample preparation before the analysis. A good alternative for traditional LLE during the pretreatment of juices can be the ultrasound technique [93]. In this method, the mixture of two nonmiscible solvents (water contained in the fruit and an organic solvent) is sonicated. The technique allows rapid extraction of numerous samples simultaneously and reduces the amount of material and organic solvent [93]. The technique was successfully applied for the extraction of patulin from apple juice using ethyl acetate [93] and from the apple fruit with a solution of ammonium acetate–acetic acid in methanol–water (95:5) [94].

Another promising and environmentally friendly method is the dispersive liquid–liquid microextraction (DLLME). This technique is based on the injection of the extraction mixture and dispersion solvent in the aqueous sample containing the target compounds [95,96]. Consequently, the generated fine droplets show a greater surface area between the extraction solvent and the aqueous sample. This effect improves extraction efficiency. After centrifugation of the turbid solution, the sedimented phase is collected for the analyte determination. The advantages of this method are its simplicity, the reduction to microliters of the quantities of organic solvents to be used, and the short time needed for sample preparation. Furthermore, this methodology offers high extraction efficiency and good reproducibility. The method was used for patulin quantification in apple juice and concentrates: acetonitrile as a dispersive solvent (1 mL) and chloroform (0.5 mL) was used as an extraction solvent [97]. SPE is another example of eco-friendly approach in preparing sample for chromatographic analysis [80]. SPE is based on dissolving or suspending the sample in a solvent (such as acetonitrile) and eluting the mixture through a solid phase. The analyte separation will take place on the base of the affinity of different compounds with the adsorbing solid phase. This approach allows isolation, concentration, and purification of the target

molecule. SPE has been widely applied by researchers studying patulin contamination in several fruit-based products (juices, purees, jams) and has been used separately [79,83,98,99] or in combination with LLE [84,85,90,92,100–102].

## 3.3 Importance of pH

As mentioned above, patulin is more stable in a slightly acidic environment [103], an optimal pH that guarantees a satisfactory recovery of this mycotoxin. There are different critical points throughout the analytical steps in which the pH value should be double checked. The initial pH adjustment could be ensured in the extraction phase because it has been found that extraction using acetonitrile acidified with acetic acid (1% v/v) provides higher patulin recoveries from strawberry samples as compared to the use of acetonitrile alone [104]. An improvement using mixture of $NaH_2PO_4$ and ethyl acetate–hexane (94:6) has been found in the mycotoxin recovery in honey compote and jelly [103]. A slight acidification of the sample protects the patulin molecule by degradation [105]. On the other hand, samples with lower pH show a tendency to produce extracts with a greater quantity of co-extracted compounds [106]. After the extract clean-up, it is still necessary to check the sample pH to avoid the analyte loss: indeed, some steps could increase the pH and compromise the stability of alkaline-pH-sensitive compounds such as the patulin.

## 3.4 Patulin quantification

### 3.4.1 Official analysis, HPLC-UV(DAD), and improved methods

The official method for the patulin determination in different matrices such as fruits and their derivatives are based on high-performance liquid chromatography (HPLC) analysis coupled with UV or diode array detector (DAD). In particular, according to the protocol by the AOAC [107,108], after cleaning the extract, this must be evaporated until dryness. One milliliter (or 500 µL in the case of puree samples) of water at pH 4 is then added to the dry sample. The solution is eluted through an HPLC column for analysis. The column must be octadecylsilane (ODS); the end capped must have a length of 250 mm and an internal diameter of 4.6 mm, and the stationary phase must have a particle size of 5 µm with a carbon percentage of 12–18.5%. The ODS pre-column must have a length of 10 mm and an internal diameter of 4.6 mm with a stationary phase with a particle size of 5 µm. An acetonitrile and perchloric acid (0.095%) solution (7:93, v:v) is used as the mobile phase. Perchloric acid is added to acidify the solution, thus conferring higher stability to patulin [84,85,109]. The flow used is 0.75 mL $min^{-1}$ and the injected volume is 50 µL. The detector can be a UV detector, set at 275 nm, or a DAD. The detection at this wavelength is characterized by low selectivity due to the interference of the phenolic compounds and, in particular, of 5-hydroxymethylfurfural (5-HMF) that is also present in the analyzed sample. This aldehyde is formed during the heat treatment of food as an intermediate product of the degradation catalyzed by acidic compounds and by the decomposition of 3-deoxyosone in the Maillard reaction [110]. Patulin and 5-HMF show a strong UV absorption, similar retention time in several chromatographic conditions, and consequently the tendency to produce overlapping peaks. Improvements in the separation of patulin and 5-HMF peaks proved to be a challenge in the analysis undertaken in many HPLC-UV studies. Using these analytical conditions, the authors achieved a mean recovery of 84.8%, a limit of detection (LOD), and a limit of quantification (LOQ) of 3 and 7 µg $L^{-1}$ for patulin and 5-HMF, respectively, and a precision <7.7% in the linear range of 2.6–650 µg $L^{-1}$. The authors would like to remember that LOD and LOQ are defined as the quantities of analyte that produces a signal equal to three and ten times the standard deviation of the gross blank signal, respectively, [111] as well as the recovery is the yield of a preconcentration or extraction stage of an analytical process for an analyte divided by amount of analyte in the original sample [112].

Katerere et al. investigated the performances of four analytical methods for the determination of patulin in apple juice. They compared three different SPE methods, i.e., by using: (i) the Oasis hydrophilic–lipophilic balance (HLB) copolymer cartridge; (ii) the Arranz method based on C18 SPE [113]; and (iii) the Romer Labs method based on passage onto MycoSep 228 AflaPAt tube and on one LLE (using ethyl acetate as the extraction solvent [114]) [115]. The authors found good recoveries (>70%, except at low spiked concentration, i.e., 10 ng $g^{-1}$) in all the methods and no-interfering peaks, even if the SPE showed better recoveries at lowest concentration levels. Furthermore, the SPE methods required less solvents than LLE and were faster, whereas the LLE one showed a higher precision.

In other papers, optimal conditions for patulin determination in other fruits were reported. Gaspar and

Lucena [109] reported a HPLC-DAD method for the simultaneous determination of patulin and three different furfurals in several matrices such as honey, white and demerara table sugars, white and red balsamic vinegars, caramel, nutritional supplement, sugar substitute, apple juices (clear and cloudy), and also mold infected apple. They used a $C_{18}$ column with a mobile phase composition (A: water–acetonitrile–perchloric acid; B: acetonitrile–perchloric acid) which gave stability to the investigated compounds, especially patulin: recoveries range between 91 and 94% with RSDs <6%, a correlation coefficient ($R^2$) of 0.9993 in the range of 0.05–10 mg $L^{-1}$ and LOD and LOQ of 0.09 and 0.26 μg $L^{-1}$, respectively. In 2014, Abu-Bakar et al. applied DLLME followed by HPLC-DAD to fruit juice samples (apple, mango, and grape) [116]. The method is simple, fast (extraction time 45 s), and does not need evaporation steps, avoiding artifact formation. Furthermore, the gradient elution is based on a mobile phase of acetonitrile and water, i.e., with no acidic mobile phase. Along with patulin, the authors determined also four furfurals (2-F, 3-F, 5-MF, and 5-HMF): they achieved good LODs in the different matrices (0.28, 0.31, and 0.28 μg $L^{-1}$ in apple, mango, and grape juice, respectively), good recoveries (90–103%, 96–107%, and 98–99%, respectively) with RSD 7.6% and $R^2$ of 0.9998 in the range 0.5–100 μg $L^{-1}$.

In the same year, Lee et al. proposed a routine quality control method based on extraction with ethyl acetate and analysis by HPLC-UV and confirmation by HPLC-tandem mass spectrometry with triple quadrupole analyzer (HPLC-MS/MS) operating under multireaction monitoring mode [117]. The authors applied the protocol to 56 commercial fruit juices available from Malaysian market; by means of this approach (LOD 0.25 ng $mL^{-1}$, LOQ 0.76 ng $mL^{-1}$, recoveries from 92.8% to 108%), the authors managed to quantify patulin at very low levels in some samples (13.1–33.1 μg $L^{-1}$). Karakose et al. developed a faster, easier, reliable, and inexpensive SPE-LC-DAD method for analyzing patulin in fruit-based baby foods (homogenized purees and infant beverages) present in the Turkish markets [90]. Following method optimization, the analytical parameters managed to reach recovery between 92.85% and 100.45% with an RSD < 3.0%, LOD and LOQ $9.66 \times 10^{-6}$ and $2.93 \times 10^{-5}$ μg $mL^{-1}$, respectively.

### 3.4.2 LC-MS(/MS) methods

LC-MS method is frequently used in food analysis due to its versatility, specificity, and selectivity. Most studies focusing on the monitoring of patulin in fruits use LC coupled with triple-quadrupole tandem electrospray mass spectrometry (LC-QQQ) operating in negative ion mode. The ion with the charged mass ratio ($m/z$) of 153 (corresponding to a patulin molecule after the loss of the proton, $[M-H]^-$) is frequently chosen as a characteristic of the precursor ion for patulin [118]. For the patulin analysis, a mobile phase consisting of water and methanol or water and acetonitrile with the addition of acetic acid [25,100] or ammonium acetate is used [94,102,119,120]. Unfortunately, due to the high polarity of patulin the LC-MS analysis suffers from low sensitivity [104]. The matrix effect, observed as suppression or increase of the signal from the target molecule, must also be considered when performing LC-MS analysis of complex matrices, e.g., fruit samples. The application of an isotopically labeled patulin standard and an instrument calibration minimize these effects and are generally used in the development of analytical methods for the quantification of patulin. Analytical protocols for LC-MS are very often developed for multi-target analysis. This approach compromises the sensitivity of the method and may not be sufficient for the quantification of patulin within the legislative limits established by the EU for baby food. For instance, LC-QQQ has been applied for the simultaneous determination of 33 pesticides or degradation products together with patulin in apples [94]. The LOQ that was obtained for patulin was 40 μg $kg^{-1}$, with recoveries ranging between 86.0 and 101.0%. In another paper, the LC-MS/MS approach was used for the determination of 295 fungal and bacterial metabolites together with the patulin quantification [119]. The method was validated on four different food matrices: apple puree, hazelnuts, corn, and green pepper. The LOD and LOQ obtained in the apple matrix were 35.9 and 119.7 μg $kg^{-1}$, respectively, with 77–82% recoveries and RSD < 5.3%.

To overcome the sensitivity disadvantage, several single-target LC-MS methods have been proposed that involve sample pretreatment for patulin quantification. The application of in-tube solid-phase microextraction (in-tube SPME) coupled with LC-MS and the isotope patulin labeled internal standard ($^{13}C_3$-patulin) allowed us to obtain excellent LOD and LOQ (23.5 and 100 ng $L^{-1}$, respectively) [121]. The method was validated for the determination of patulin in various fruit juices (apple, grapes, orange, blueberry, lemon, pear, mango, and coconut) and dried fruit (apple, apricot, kiwi, plum, pineapple, papaya, mango, and fig). The estimated recoveries for the apple juice matrix were greater than 92%. Beltrán et al. coupled the most advanced LC method, i.e., ultra-

high-performance liquid chromatography (UHPLC) with the triple quadrupole analyzer (UHPLC-MS/MS with QqQ analyzer) for the only determination of patulin in four different apple matrices (juice, fruit, puree, and compote) [122]. The LOQs obtained for all the matrices were in the range 2–15 μg $kg^{-1}$ with average recoveries between 71% and 108% and RSDs <14%. LC-MS/MS has been used successfully for the patulin monitoring in several varieties of fresh fruit (for a total of 135 samples), including apples, pears, apricots, peaches, bananas, grapes, plums, strawberries, raspberries, blueberries, blackberries, black cherries, and their products (juices, pulps), showing LODs and LOQs between 0.5–1 and 1–2.5 μg $kg^{-1}$, respectively, and recoveries between 70% and 110% [120]. In some papers, authors have used LL extraction with ethyl acetate followed by a clean-up using SPE (Oasis HLB cartridge) and quantification by means of a LC-MS/MS method for the patulin determination in apple products (i.e., juices, concentrates, ground fresh fruit, and jams) [102]. The authors developed a method based on isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC-MS/MS) achieving satisfactory LODs and LOQs in the range 0.2–0.7 and 0.8–2.4 μg $kg^{-1}$, respectively, and recoveries above 95%. More recently, Wang et al. applied a single-step SPE for determining patulin along with other seven mycotoxins (*Alternaria* toxins, namely, alternariol, alternariol monomethyl ether, altenuene, tentoxin, tenuazonic acid, ochratoxin A, citrinin, and patulin) in different fruit matrices, including apple, sweet cherry, tomato, and orange fruits [123]. The authors compared six various adsorbents (octadecylsilyl C18, primary secondary amine, mixed-mode cationic exchange [MCX], HLB, silica, and amino-propyl $NH_2$): the combination of MCX and $NH_2$ gave the best performances and allowed the determination of all the eight mycotoxins. The authors determined LOQs (1–5 ng $mL^{-1}$), recoveries (74.2–102.4%), precision (as RSD < 4.7%), and correlation coefficient ($R^2 > 0.9921$) in the linear range of 5–200 ng $mL^{-1}$. LC-MS/MS has also been applied for single-target monitoring of the content of patulin in apples and apple-based products [25]. More recently, Li et al. developed a rapid and easy method coupling single-drop liquid–liquid–liquid microextraction with isotope dilution ultra-high performance liquid chromatography-mass spectrometry (SD-LLLME with ID-LC-MS/MS) [124]. The authors managed to perform the whole analytical procedure in 20 min, with low solvent consumption, high accuracy, and low manipulation, achieving LOD of 0.5 ng $mL^{-1}$, LOQ of 2 ng $mL^{-1}$, RSD < 3.6%, and recoveries between 83.6 and 96.3% with a $R^2$ of 0.9997 in the range 2–2,000 ng $mL^{-1}$.

Finally, Cunha et al. proposed an LLE (ethyl acetate and *n*-hexane, 95:5) followed by a gas chromatography-mass spectrometry method (GC-MS) using a stable isotope labeled internal standard ($^{13}C_{5-7}$ patulin) for evaluating patulin content in apple and tomato products [125,126]. Recoveries ranging between 64–80% in apple juice and 75–78% in tomato pulp were obtained as well as LOD and LOQ of 0.4 and 1.6 μg $kg^{-1}$, respectively, RSD < 12% and $R^2$ of 0.9894 and 0.9931, respectively. Table 5 resumes all the main analytical parameters of the methods cited.

### 3.4.3 Molecularly imprinted polymer (MIP) methods

Techniques based on MIPs allow the formation of specific recognition sites in synthetic polymers through the use of templates or imprinting molecules [127]. They represent an alternative to biological binding molecules (such as antibodies) that are based on the use of functional and cross-linking monomers (allowing creation of bonds between polymer chains) which are copolymerized in the presence of the target analyte acting as a molecular model [128,129]. These polymers can be used to obtain both quantitative and qualitative results if associated with analytical (extraction and analysis steps) techniques: they are useful to separate and analyze complex samples such as biological fluids and environmental samples. At the beginning, functional monomers form a complex with the template molecule (target); after the polymerization process the template is removed from the polymer leaving specific recognition sites that are complementary in shape, size, and chemical functionality to the template molecule (highly crosslinked polymer structure; Figure 3) [130].

By using liquid extraction, the target molecule is removed, and the binding sites that are stereochemically complementary to the shape of the analyte are then revealed [131]. In this way, a “molecular memory” is introduced into the polymeric structure that is able to selectively bind the analyte. The binding between the molecule and functional monomers can be based on covalent or non-covalent bonding [132]. Stability, ease of preparation, and low material cost make this technique particularly attractive [127]. MIPs, due to their high affinity with the target molecule, could perform the same functions as antibodies in immunological tests and could also have a greater tolerance at extreme pH and ionic strength. The use of MIPs can be associated with extraction and analysis techniques.

In the Scopus database, 25 papers are present with the keywords “patulin” and “molecularly imprinted

**Table 5:** Comparison of recovery (%), LODs (a: μg $L^{-1}$), and LOQs (b: μg $kg^{-1}$) and repeatability (expressed as relative standard deviation, RSD,%) of analytical methods for determining patulin in different matrices

| Method | Matrix | Recovery | LOD/LOQ | RSD | Ref. |
|---|---|---|---|---|---|
| LLE-HPLC-UV (or DAD) | Different fruits and their derivatives | 84.8 | 3/7[a] | <7.7 | [115] |
| SPE-Oasis-HPLC-DAD | Apple juice | 75.3–90.6 | –/– | <23.8 | [115] |
| SPE-C18-HPLC-DAD | Apple juice | 75.4–101.6 | –/– | <19.6 | [115] |
| SPE-MycoSep228-HPLC-DAD | Apple juice | 89–176 | –/– | <12.7 | [115] |
| LLE-HPLC-DAD | Apple juice | 72–192 | –/– | <15.2 | [115] |
| SPE-C18-HPLC-DAD | Honey, white/demerara table sugars, vinegar, caramel, apple juice | 91–94 | 0.09/0.26[a] | <6 | [109] |
| DLLME-HPLC-DAD | Fruit (apple, mango, grape) juices | 90–107 | 0.28–0.31/–[a] | <7.6 | [116] |
| LLE-HPLC-UV (or MS/MS) | Fruit juices | 92.8–108 | 0.25/0.76[a] | — | [117] |
| SPE-HPLC-DAD | Fruit-based baby foods (homogenized puree and infant drink) | 92.8–100.4 | 0.00966/0.0293[a] | <3.0 | [90] |
| LLE-HPLC-MS/MS (QqQ) | Apple fruits | 86.0–101.0 | –/40[b] | <25.7 | [116] |
| LLE-HPLC-MS/MS | Apple puree, hazelnuts, maize, and green pepper | 77–82 | 35.9/119.7[b] | <5.3 | [119] |
| SPME-HPLC-MS/MS ($^{13}C_3$-patulin) | Fruit juices and dried fruit | >92 | 0.0235/0.01[a] | <5.0 | [121] |
| LLE-UHPLC-MS/MS (QqQ) | Apple juice, fruit puree, and compote | 71–108 | –/2–15[b] | <14 | [122] |
| QuEChERS-SPE-UHPLC-MS/MS | Fresh fruit | 92–109 | 05–1/1–2.5[b] | <10 | [120] |
| LLE-SPE-Oasis-ID-HPLC-MS/MS ($^{13}C_7$-patulin) | Apple-based products | >95 | 0.2–0.7/ 0.8–2.4[b] | <38 | [102] |
| SPE-UHPLC-MS/MS | Fresh fruits | 74.2–102.4 | –/1–5[a] | <4.7 | [123] |
| LLE-HPLC-MS/MS | Fresh apples and apple-based products | — | 1/5[b] | — | [25] |
| SD-LLLPME-ID-HPLC-MS/MS | Apple juice | 83.6–96.3 | 0.5/2[a] | <3.2 | [124] |
| LLE-GC-MS ($^3C_{5-7}$ patulin) | Apple and tomato products | 64–80 | 0.4/1.6[b] | <12 | [125,126] |

"–": the information is absent.

polymer". Among them, to our opinion few papers deserve to be cited as important improvements in the patulin detection (Table 6). The first paper dealing with the use of an MIP for determining patulin in apple juice is by De Smet and coworkers in 2011 [133]. These authors optimized a molecularly imprinted solid-phase extraction (MISPE) procedure, which resulted more selective than the SPE-C18 clean up step, so that the chromatograms

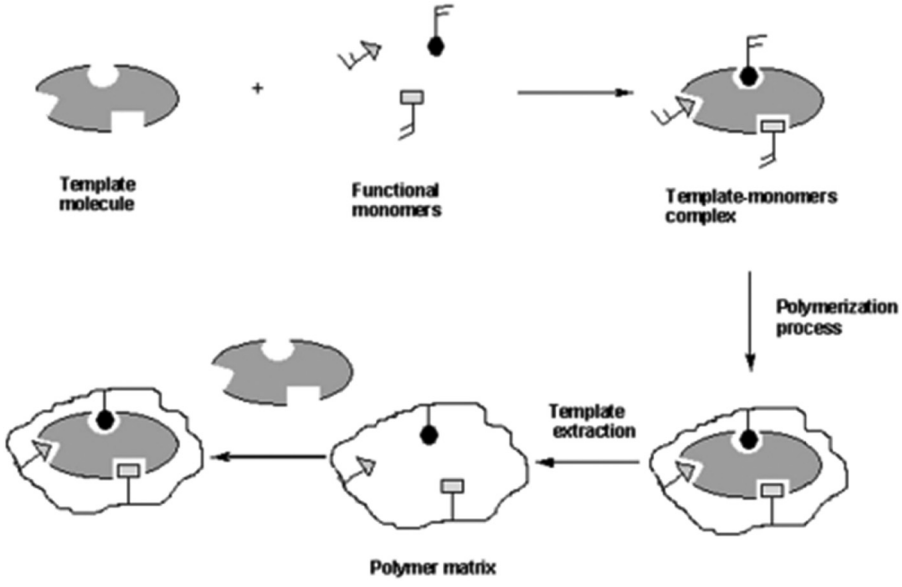


**Figure 3:** Molecular imprinting process; from ref. [130].

displayed fewer interferences, even if poor recoveries were obtained (40–66%) and LOD and LOQ were 10.0 and 33.3 μg $kg^{-1}$, respectively. In the same year, Khorrami and Taherkhani synthesized an MIP using oxindole as a dummy template [134]. The authors used this non-covalent molecular imprinting approach to extract patulin from apple juices: this method yielded recoveries > 80%, LOD and LOQ of 5 and 16 μg $g^{-1}$, respectively, intra- and inter-day RSD <7 and <19%, respectively, an overall RSD in the range 11–19%. Five years later, Anene et al. developed a new MIP, i.e., $SiO_2$maleicpolymer@MIP, in a two-step process [135]. These authors determined the optimal conditions (i.e., mass of adsorbent, washing solvent, eluting solvent, and its volume) for the patulin extraction (MIP@SPE followed by HPLC/UV): best results were obtained by using 50 mg of adsorbent, sodium bicarbonate with (1% w/w) acetic acid as a washing solvent, and 5 mL of acetonitrile as an eluting solvent. Under these conditions, recoveries ranged between 82 and 98%, with RSD < 3.8%, LOD and LOQ 8.6 and 28.6 μg $L^{-1}$, respectively, $R^2$ 0.999 in the range 0.1–10 mg $L^{-1}$, and an analysis time of 10 min.

Particularly interesting is the paper by Fang et al.: they developed a simple quartz crystal microbalance (QCM) sensor based on a sol–gel MIP film and compared their results with those obtained by the HPLC-MS validated method [136]. The proposed approach (MIP-QCM), applied to apple juice, concentrated pear juice and haw flakes resulted in high adsorption capacity, good affinity, good reproducibility, and long-term stability along with recoveries ranging between 86% and 91.2%, 76.9% and 89.1%, and 79.1% and 86.9% and RSDs 3.2–8.3%, 1.2–7.7%, and 2.6–8.4%, respectively, and LOD of $3.1 \times 10^{-3}$ μg $mL^{-1}$. Lucci et al. coupled an SPE-MIP method with UHLC-UV for detecting patulin in apple-based food products [137]. The method validated ($R^2$ 0.965 in the linear range 2–100 μg $kg^{-1}$, recoveries >77%, RSD < 11%, LOD and LOQ 25 and 82 pg injected, respectively) and successfully compared with dispersive SPE (Quick, Easy, Cheap, Effective, Rugged and Safe, QuEChERS method) and octadecyl sorbent, fulfilled the performance criteria required by the Commission Regulation No. 401/2006 [51]. Another group developed a phosphorescent nanosensor (a Mn-doped ZnS quantum dots [QDs]) for patulin detection in apple juice samples [138]. The MIP-QD method, showing $R^2$ of 0.9945 in the range 0.43–6.50 μmol $L^{-1}$, LOD of 0.32 μmol $L^{-1}$, recoveries in the range 102.9–127.2% and RSD < 4.95%, is in good agreement with the HPLC-UV analysis. Bagheri et al. used Ag nanoparticles encapsulated in the pores of flake-like metal organic frameworks (MOFs), i.e., Zn, for obtaining a fluorescent Ag nanoparticle/flake-like Zn-based MOF nanocomposite (AgNPs@ZnMOF) for selective patulin detection in environmental water and apple juice samples [139]. The fluorescence method allowed us to reach recoveries in the range 102.9–104.1% with RSD < 2.0% and LOD of 0.06 μmol $L^{-1}$.

Huang et al. developed an electrochemical sensor for detecting patulin at ultra-trace levels: specifically, they arranged a molecularly imprinted film fabricated on a thionine platinum nanoparticle (PtNP)-nitrogen-doped graphene (thionine-PtNP-NGE) modified electrode [140]. The proposed electrochemical sensor shows a wide linear range ($R^2$ 0.995 in 0.002–2 ng $mL^{-1}$ range), low detection limit (0.001 ng $mL^{-1}$), high selectivity, good recoveries (99.8–113.0% for apple juice and 95.4–104.8% for grape juice samples), and excellent repeatability (RSD < 5%).

A new MIP with specific adsorption for patulin has been successfully polymerized using 2-oxindole (2-oxin) and 6-hydroxynicotinic acid (6-HNA) as molecular

**Table 6:** Comparison of recovery (%), LODs and LOQs (a: μg $L^{-1}$; b: μg $kg^{-1}$; c: pg injected; d: μmol $L^{-1}$) and repeatability (expressed as relative standard deviation, RSD%) among analytical methods based on MIPs for determining patulin in different matrices

| Method | Matrix | Recovery | LOD/LOQ | RSD | Ref. |
|---|---|---|---|---|---|
| MISPE-C18-HPLC-UV | Apple juice | 40–66 | 10.0/33.3[b] | — | [133] |
| MIP-SPE-HPLC-UV | Apple juice | >80 | 500/16000[b] | 11–19 | [134] |
| MIP@SPE-HPLC-UV | Apple fruit, apple juice | 82–98 | 8.6/28.6[a] | <3.8 | [135] |
| MIP-QCM | Apple juice, hew flakes | 76.9–91.2 | 0.0031/–[a] | 1.2–8.4 | [136] |
| MIP-SPE-UHPLC-UV | Apple juice, jam, and puree | 77–108 | 25/82[c] | <12 | [137] |
| Mn-ZnS-QD-HPLC-UV | Apple juice | 102.9–127.2 | 0.32/–[d] | <4.9 | [138] |
| AgNPs@ZnMOF-Fluorescence | Environmental water and apple juice | 102.9–104.1 | 0.06/–[d] | <2.0 | [139] |
| Thionine-PtNP-NGE-DPV | Apple and grape juice | 99.8–113.0 | 0.001/–[a] | <5.0 | [140] |
| MIP-SPE-HPLC-MS/MS | Multiple fruit matrices | 81.3–106.3 | 0.05–0.2/0.2–0.5[b] | <4.5 | [141] |
| MMIP-HPLC-DAD | Apple, grape, orange, and pear juice | 86.4–95.5 | 3/10[b] | <3.2 | [142] |

"–": the information is absent.

models, methylacrylic acid as a functional monomer, trimethylolpropane trimethacrylate as a crosslinker, 2,2-azobis-(2-methylpropionitrile) as an initiator, and methanol as a porogen solvent. This MIP has been associated with an SPE column with the function of selective sorbent molecule. The results show that the MI-SPE method has a high selectivity for patulin with average recoveries between 81.3% and 106.3% and RSD < 4.5%. When this MI-SPE method was coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS), LOD ranged between 0.05 and 0.2 ng $g^{-1}$ and LOQ between 0.2 and 0.5 ng $g^{-1}$ [141]. In general, these molecules polymerized on a target molecule can be used on multiple matrices and associated with a considerable variety of analytical methods.

Finally, a recent study by Fu et al. showed a highly efficient and selective method using magnetic molecularly imprinted polymers (MMIPs) followed by HPLC-DAD [142]. MMIPs, prepared by surface imprinting method using $Fe_3O_4$ nanoparticles as a supporter, 2-oxindole as a virtual template, (3-aminopropyl)triethoxysilane as the functional monomer, and tetraethyl orthosilicate as the crosslinking agent, were characterized by vibrating sample magnetometer, Fourier transform infrared spectroscopy, X-ray diffraction, and thermogravimetric analysis. Under the optimal conditions, the authors achieved good LOD and LOQ (3 and 10 μg $kg^{-1}$, respectively), acceptable recoveries (ranging between 86.4% and 95.5%), high stability (1.1–3.2%), and accuracy (0.6–1.9%), thus demonstrating the applicability of such protocol to real samples.

# 4 Conclusion

This review aims to cover traditional and/or advanced methodologies based on chromatographic analysis reported in the literature for detecting the mycotoxin patulin in food matrices. This determination in foods is a really important issue: the importance of continuous monitoring of such compound is well known by scientists and consumers worldwide. The suggestion is to continuously develop new methods, more accurate and sensitive, based on GC-MS/MS or LC-MS/MS analysis but also routine methods based on inexpensive or use-friendly detectors (FID, FPD, or NPD).

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