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An Improved Analytical Approach Based on μ-QuEChERS Combined with LC-ESI/MS for Monitoring the Occurrence and Levels of Patulin in Commercial Apple Juices

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Abstract: Patulin (PAT) is a mycotoxin produced in fruits, especially in apples, by diverse fungal species that can be transferred into industrial apple juice during processing. An accurate, effective, and selective method has been validated for the quantification of PAT in different commercial apple juices by combining a modified μ-QuEChERS procedure with high-pressure liquid chromatography (LC) equipped with a triple quadrupole mass spectrometer (QqQMS). This sample extraction procedure reduced interference from the sugar-rich matrix, and the separation was performed using the C18 Atlantis T3 column within 10 min. PAT was found by MS with electrospray negative ionization (ESI^-) in the mode of multiple reaction monitoring (MRM). The correlation coefficient (R^2 = 0.999) satisfied the prerequisite of linearity for PAT in the concentration range of 2–50 μg/kg. The limits of detection (LOD) and quantification (LOQ) of PAT were 0.32 and 1.15 μg/kg, respectively, which were compliant with the maximum levels settled in Commission Regulation (EC) No. 1881/2006. The recoveries were within the 92–103% range, at three fortified levels of 2, 20 and 50 μg/kg, with relative standard deviations lower than 7%. Based on analytical validation, it was confirmed that the μ-QuEChERS/HPLC-MS/MS method is an enhanced, reliable, and quick approach for determination of PAT in apple juice. The current approach proposes reduced sample preparation and analysis time. In addition, it is economical, environmentally friendly, and simpler to implement in comparison to traditional approaches.

Keywords: mycotoxin; patulin; apple juice; μ-QuEChERS; LC-MS/MS

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1. Introduction

Food contamination with toxigenic molds has attracted rising attention, especially over the past decade. Mycotoxins are secondary metabolites of fungi that are well-known to contaminate several food products, both at pre-harvest and/or during storage [1]. Patulin (4-hydroxy-4H-furo[3,2c]pyran-2(6H)-one, PAT) is an acetate-derived tetraketide mycotoxin generated by numerous molds, particularly *Aspergillus*, *Penicillium* and *Byssochlamys* species, in several food products (e.g., apples, apricots, grapes, peaches, pears, olives, cereals). It is the most common mycotoxin detected in apple-derived products (e.g., cider, compotes, fruit, juice) and other food intended for young children. Exposure to PAT is correlated with mutagenic and carcinogenic properties in numerous animal species and causes intestinal injuries, including epithelial cell degeneration, inflammation, ulceration, and hemorrhage [2–5]. In addition, PAT can attack cellular DNA in bacteria and humans, which can lead to the progression of tumors and cancer (e.g., esophageal, intestinal) [6]. The biosynthetic pathway of patulin (Figure 1) comprises of several stages as indicated by

numerous biochemical investigations and by the identification of numerous mutants that are stopped at various stages in the patulin biosynthetic pathway.

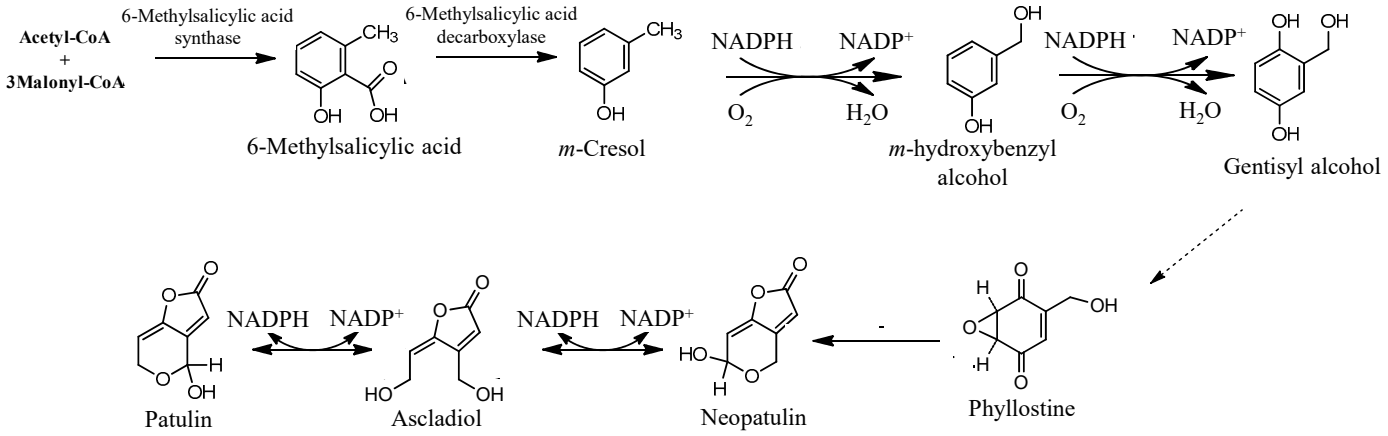

Figure 1. A possible patulin biosynthetic pathway. Adapted from [7,8].

In view of the accepted harmful consequences caused by PAT and the demand for regulatory control, monitoring its level in apple-derived products is crucial to assess the hazard due to human consumption of these products. Since 2003, European regulation 1425/3003 has set a maximum level of 50 μg/kg for fruit juices and derived products, 25 μg/kg for solid apple products and 10 μg/kg for juices and foods intended for infants and young children [9]. The Joint Food and Agriculture Organization Health Organization Expert Committee on Food Additives (JECFA) recognizes a temporary maximum tolerable daily intake (PMTDI) of 0.4 mg/kg body weight/day [10].

To ensure that the levels set by the European Union are respected, the use of an analytical approach capable of guaranteeing reliable results is of utmost importance, which is why many laboratories desire to develop robust analytical approaches that are highly sensitive and specifically enable accurate results for mycotoxins. Achieving accurate results for mycotoxins is no easy task, because there are several factors that complicate this type of analysis: for example, the non-uniform distribution of mycotoxins in the contaminated lots; the fact that the concentrations of mycotoxins are extremely low; extracts are accompanied by lipids and interfering pigments, requiring a cleaning phase; and the varied nature of the samples, requiring different extraction procedures. Typically, mycotoxins have been detected and quantified by physicochemical and biological techniques. Different chromatographic techniques (e.g., thin layer, liquid chromatography) beyond fluoro-densitometry and spectrophotometry have also been used. The biological techniques include bioassays (e.g., tissue culture, animals, microorganisms) and immunoassays such as radioimmunoassay (RIA), affinity chromatography and enzyme-linked immunosorbent assay (ELISA) [11–14]. However, several of these extraction procedures are extremely costly, time consuming, and involve complex running of sophisticated instrumentation. A few years ago, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS), which combined the extraction and isolation of agrotoxics in food matrices as well as cleaning of extracts, emerged as a suitable extraction procedure. This extraction procedure provides reliable results, reduces the number of analytical tests, solvent amounts, and laboratory equipment used, simplifies the extraction of the analytes, and requires fewer stages of extraction and cleaning of extracts, without damaging the extent of recovery [1,2]. However, as far as we know, miniaturization of QuEChERS (μ-QuEChERS) for the extraction of PAT from apple-derived products has not been reported. This extraction procedure, compared to original QuEChERS, is economical and environmentally friendly, requires smaller amounts of salt mixtures and solvents, and produces less waste. Moreover, analytical approaches such as liquid chromatography (LC) are rarely applied because of lower selectivity and sensitivity provided by the detectors used, such as ultraviolet light (UV). The potential coupling of

LC and tandem mass spectrometry (MS/MS) presents numerous benefits, among them high sensitivity and selectivity. Since the development of sources of ionization operating at atmospheric pressure (API), such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), various robust and reliable instruments have become available. HPLC-MS/MS allows increased selectivity and sensitivity by providing monitoring of mycotoxins in a single analysis. This equipment has become an indispensable tool in laboratories. Despite the high cost of acquisition and maintenance of equipment, these tools have several advantages, namely high efficiency analysis, reduced time to method development, and robustness [15,16]. However, PAT determination requires certain LC-MS/MS conditions due to its superior polarity and small molecular mass, which can result in minimal recovery and/or low sensitivity of the analysis. Moreover, significant PAT ion suppression occurs when ESI mode is applied, which can also influence the recovery, precision, and sensitivity of the method [2].

The main aim of the current research was to avoid the coelution of interfering peaks by improving an appropriate chromatographic method for PAT determination without extensive and time-consuming cleanup steps. Thus, to the best of our knowledge, this is the first report of a quick, precise, reliable, and high-throughput μ-QuEChERS-based extraction procedure combined with HPLC-MS/MS for determination of PAT in apple juices. The proposed analytical method was adequately validated in terms of linearity, accuracy, precision, sensitivity, and selectivity and effectively employed for the analysis of different commercial apple juices to demonstrate its feasibility.

2. Materials and Methods

2.1. Chemicals and Reagents

Patulin analytical standard with a purity of 98% was obtained from Sigma Aldrich (Madrid, Spain). Acetonitrile (MeCN, LC-MS grade), methanol (MeOH, LC-MS grade), and acetic acid (analytical grade) were acquired from Fisher Scientific (HPLC grade). Magnesium sulphate anhydrous ($MgSO_4$), sodium chloride (NaCl), sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$), disodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_7{\cdot}1.5\ H_2O$), and ammonium acetate ($C_2H_4O_2{\cdot}NH_3$) were obtained from Fluka (Saint Louis, MO, USA). Ultrapure water (18.2 MΩ/cm, Milli-Q Plus system, Millipore Bedford, MA, USA) was used in all experiments. Samples extracts were filtered through a 0.22 μm polytetrafluoroethylene (PTFE) filter membrane purchased from Via Athena, (Lisbon, Portugal) and injected into the HPLC-MS/MS system.

2.2. Samples

A total of 30 apple juices were acquired in different local markets in Madeira Island (32°39′00.7″ N 16°54′29.2″ W), from July to August 2022.

2.3. Standard Solutions

The PAT standard stock solution was prepared by dissolving 0.1 mg of patulin in 20 mL of MeCN (5 mg/kg), and this solution was diluted, using a mixture of water and MeCN (H_2O:MeCN, 9:1 *v*/*v*), to a concentration of 200 μg/kg. The stock and intermediate solutions were stored at −20 °C with no exposure to sunlight. The PAT standard series were prepared at concentrations of 2 to 50 μg/kg by diluting the PAT intermediate solution in H_2O:MeCN, (9:1 *v*/*v*) to build the calibration curve.

2.4. μ-QuEChERS Extraction

For μ-QuEChERS extraction, 100 mg of sample was added to a 10 mL polypropylene centrifuge tube, and 1 mL of MeCN with 1% acetic acid was added and homogenized in a vortex for 1 min (Figure 2). The salting-out process was performed by adding 0.65 g of the partitioning salt mixture composed of 0.40 g $MgSO_4$, 0.10 g NaCl, 0.10 g $C_6H_5Na_3O_7$ $2H_2O$, and 0.05 g $C_6H_6Na_2O_7$ 1.5 H_2O. The tube was shaken for 15 s, then submitted to ultrasound agitation for 3 min and centrifugation at 5000 rpm for 5 min. The supernatants

were collected, filtered through PTFE, and inserted in vials for subsequent analysis by HPLC-MS/MS.

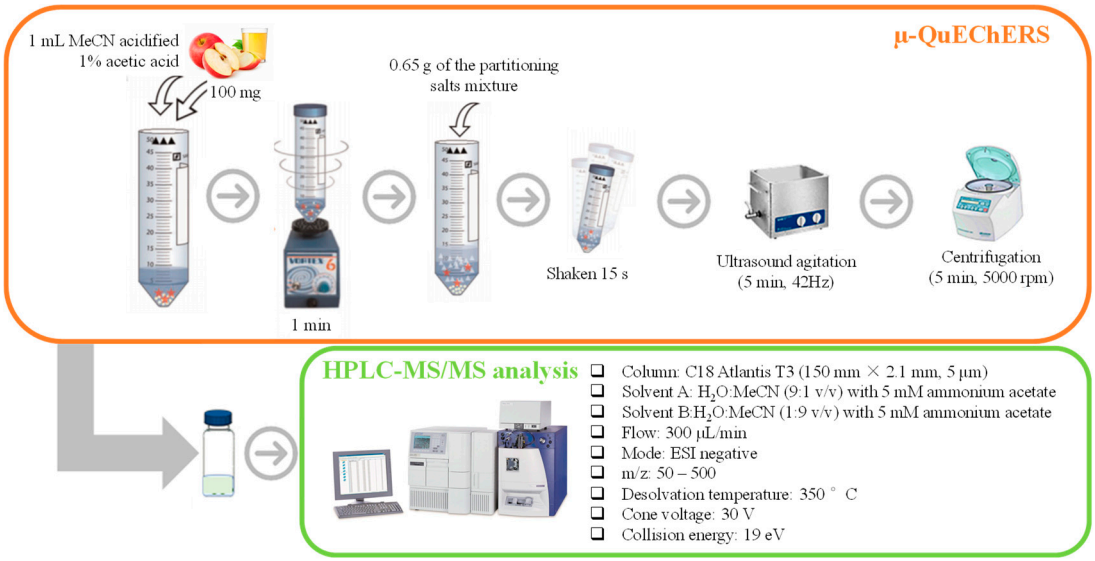

Figure 2. Schematic representation of the μ-QuEChERS/LC-MS/MS analysis.

2.5. Method Validation

The μ-QuEChERS/HPLC-MS/MS approach was fully validated in terms of linearity, matrix effect, selectivity, limit of detection (LOD), limit of quantification (LOQ), precision (expressed as relative standard deviation, % RSD), and accuracy (as recovery percentage). The linearity was evaluated by building a calibration curve for seven concentrations, of 2, 5, 10, 20, 30, 40 and 50 μg/kg. The matrix effect for PAT in apple juice was assessed using this calibration curve through the ratio between the slope of the standard in solvent (H_2O:MeCN, 9:1 v/v), and those obtained by spiking apple juice (standard addition method). Student's *t*-test was used to check if a matrix effect existed. There was a matrix effect if the calculated value of t was greater than the theoretical value of t. The LOD and the LOQ were determined utilizing the variability of peak area, injecting the analyte numerous times at the lowest calibration point.

The accuracy, expressed as recovery percentage (%), was measured through the analysis of fortified apple juice with three levels of PAT concentration: 2 μg/kg (low level), 20 μg/kg (medium level), and 50 μg/kg (high level). The recovery should be between 70–120%, and the % RSD less than or equal to 20%. The precision was calculated in terms of repeatability (intra-day) and reproducibility (inter-day), using the same concentration levels used in the accuracy analyses. For repeatability (expressed as % RSD), six replicates (n = 6) of the entire technique were carried out on the same day. The reproducibility (also expressed as RSD %) was calculated by the analysis of three replicates of a sample, which were performed in triplicate over three different days (n = 9). Based on the validation guidelines, the RSD values for these precision parameters should be lower than 20%. Selectivity was evaluated by the lack of interfering chromatographic peaks at the retention time (RT) of the PAT.

2.6. High Performance Liquid Chromatography Tandem Mass Spectrometry

Liquid chromatography was carried out on a Waters Alliance 2695 system comprising a quaternary, low-pressure mixing pump, on-line vacuum degasser, autosampler and column compartment. The PAT separation was reached on a silica-based reversed-phase C18 Atlantis T3 (150 mm × 2.1 mm, 5 μm) analytical capillary column, kept at 30 °C. A binary mobile phase with a gradient program was employed, mixing solvent A (H_2O:MeCN (9:1 v/v) with 5 mM ammonium acetate) and solvent B (H_2O:MeCN (1:9 v/v)) with 5 mM

ammonium acetate) as follows: 100% A (0 min), 100% B (15–20 min), 100% A (20–25 min). The elution was obtained at a flow rate of 300 µL/min in the gradient mode, and the injection volume was 20 µL. Prior to the following injection, the system was re-equilibrated for 5 min with the initial. Mass spectrometry analysis was carried out on a Micromass Quattro Micro triple-quadrupole equipped with a heated electrospray ionization (ESI) source, which was running in the negative ion mode. Microsoft Windows NT (v 4.1)-based MassLynx software was used in the data acquisition, data processing and instrument control. The mass spectra were attained over the mass range 50 to 500 *m/z*. The ionization source working parameters were: capillary voltage, 2.9 kV; cone gas flow rate, 80 L/h; cone voltage, 30 V; collision energy, 19 eV; desolvation gas flow rate, 650 L/h; desolvation temperature, 350 °C; and source temperature, 140 °C. Argon (99% purity) and nitrogen (>99% purity) with argon were employed as collision (product ion scan, MS/MS) and nebulizing gases, respectively. Flow injection of PAT was used to improve the multiple reaction monitoring (MRM) conditions. A dwell time of at least 20 ms was used for each MRM transition. The following MRM transitions were monitored: 152.9 > 108.9 (quantifier) and 152.9 > 80.9 (qualifier). The criteria employed to check the identification of PAT were as follows: (1) a signal for each of the two specific MRM transitions of the analyte had to be identical in the sample and in the standard; (2) the peak ratio of the confirmation transition against quantification; (3) the relative retention time of the analyte in both sample and standard solution should have a maximum difference of 0.1 min.

3. Results and Discussions

The parameters of HPLC and MS/MS were meticulously optimized to meet better analytical requirements for the detection and identification of PAT in a short chromatographic run time. For HPLC optimization, several mixtures of mobile phase solvents were considered, namely H_2O, MeCN, MeOH, ammonium acetate, and acetic acid. The data obtained demonstrated that the solvent mixture consisting of MeCN was advantageous for the ionization procedure, which led to an increase in the detected signal (peak area) with improved resolution equated to MeOH. This result agrees with those of other prior studies [16–18]. Regarding MS optimization, a standard solution of PAT at a concentration level of 2 µg/kg was injected in negative ESI mode, and the data obtained showed a better signal intensity compared to that obtained in positive ESI mode. Patulin was ionized to the deprotonated molecular ion $[M\text{-}H]^-$ at *m/z* 153, which was chosen as the precursor ion due to its superior intensity. As can be seen in Figure 3, two predominant and characteristic fragment ions were observed at *m/z* 109 and *m/z* 81, which corresponded to $[M\text{-}H\text{-}CO_2]^-$ and $[C_5H_5O]^-$, respectively. This fragmentation pathway agrees with previously conducted studies [16].

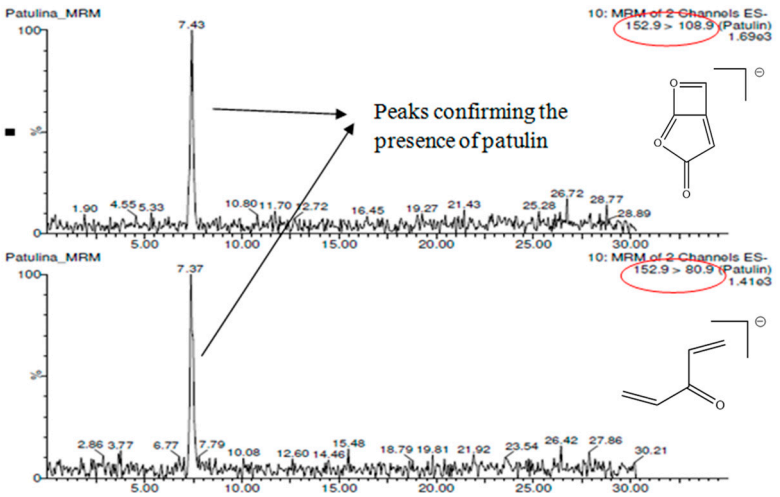

Figure 3. Typical MRM of patulin in negative ESI mode.

In sum, the identification procedure for patulin in apple juice was performed using the retention time (RT = 7.37 min) and two transitions, *m/z* 152.9 > 108.9 and *m/z* 152.9 > 80.9, with the most intense transition *m/z*, 152.9 > 108.9, used as quantifier, while the other was employed as qualifier peak for the positive assessment.

3.1. Method Validation

The µ-QuEChERS/HPLC-MS/MS method was fully validated for the following analytical parameters: linearity, matrix effect, selectivity, LOD, LOQ, accuracy, and precision. The results obtained in the method validation are shown in Table 1.

Table 1. Linearity, sensitivity, accuracy, and precision of patulin analysis using µ-QuEChERS/HPLC-MS/MS method.

Linear Range (µg/kg)	Correlation Coefficient (R^2)	LOD (µg/kg) [a]	LOQ (µg/kg) [b]	Accuracy [c]		Precision [c] (%RSD)	
				Recovery (% ± SD)		Intra-Day	Inter-Day
2–50	0.999	0.32	1.15	LL	98 ± 8	3.78	5.10
				ML	92 ± 4	4.58	6.85
				HL	103 ± 1	2.67	4.73

[a] LOD: limit of detection; [b] LOQ: limit of quantification; [c] Accuracy and precision were obtained by spiking apple juice at three concentration levels: low level (LL, 2 µg/kg), medium level (ML, 20 µg/kg) and high level (HL, 50 µg/kg).

The linearity of the calibration curve established in the range of 2 to 50 µg/kg was obtained by least-squares linear regression analysis of the data, giving an exceptional correlation coefficient (R^2) value of 0.999. Regarding the matrix effect, the calculated t value (1.42) was lower than the tabulated t value (2.57), indicating that the matrix had no influence and the MS/MS detector used in the current method was sensitive and selective. For this reason, it was chosen as in earlier investigations, not including a stable isotope-labeled internal standard of PAT [19,20].

The sensitivity of the µ-QuEChERS/HPLC-MS/MS method was determined based on LOD and LOQ following the EU Commission Decision 2002/657/EC as the minimum PAT concentration (2 µg/kg) to produce chromatogram peaks with signal-to-noise ratios of 3/1 and 10/1, respectively [21]. The LOD and LOQ of PAT were 0.32 and 1.15 µg/kg, respectively (Table 1). The LOD and LOQ obtained in the current study were lower than those reported by others [1,19,22]. Moreover, the LOD and LOQ obtained were quite similar to those reported by Gab-Allah et al. [16]. The selectivity of the µ-QuEChERS/HPLC-MS/MS method was established through the analysis of both blank and fortified apple juice based on checking the typical MRM transitions at particular elution times of PAT. The analysis of HPLC-MS/MS chromatograms obtained from blank and fortified apple juice demonstrated the lack of interference peaks in the RT of PAT. Accuracy and precision were assessed at three different concentration levels, 2 µg/kg (LL), 20 µg/kg (ML) and 50 µg/kg (HL). Accuracy was expressed as the mean recovery achieved by comparing six samples (n = 6) fortified with the corresponding values of their modeled samples. As can be observed in Table 1, suitable results were achieved, since the mean recoveries ranged from 92 to 103%, which is consistent with the range 87 to 107% obtained in previous studies using the same detection method (MS/MS) [16,19]. Regarding intra-day and inter-day precision at the three concentration levels, the RSD values ranged from 2.67 to 4.58 and from 4.73 to 6.85%, respectively. The RSD values were lower than 20%, and based on the guidelines [21], confirmed that the µ-QuEChERS/HPLC-MS/MS method is precise.

Table 2 reports analytical approaches developed to quantify PAT in apple juice and related products [1,2,16,19,20,23,24]. Gab-Allah et al. [16] developed a feasible alternative isotope dilution as an extraction procedure utilizing simple and reliable sample preparation steps and a clean-up process using molecularly imprinted polymer-solid-phase extraction (MIP-SPE). The method showed an LOD of 0.33 µg/kg, an LOQ of 1.10 µg/kg, and accuracy ranging from 98 to 102%, with RSD for inter-day and intra-day precision of lower than 3%. This method was effectively used for the accurate determination of patulin in apple

products. Taşpınar and collaborators [20] proposed a green and inexpensive air-assisted natural deep eutectic solvent-based solidified homogeneous liquid phase microextraction (AA-NADES-SH-LPME) procedure followed by spectrophotometric determination. In this study, 2 mL of sample solution was transferred in a 15 mL tube, and then 5.1 mg/L of Zn(II) solution was added to provide the complexation of patulin. Finally, the patulin was extracted using 410 µL of NADES-3. This procedure showed higher LOD (3.5 µg/kg) and LOQ (10 µg/kg) compared to those obtained in this study and the previous one [16,19], and recoveries ranging from 94 to 104%, with RSD for inter-day and intra-day precision of lower than 6%. The spectrophotometric determination used by Taşpınar and collaborators could be a possible explanation for the highest LOD and LOQ [20]. A report in the literature proved that the selection of HPLC-MS/MS was suitable for PAT determination, since it provides more sensitivity and selectivity that other analytical approaches [19]. The method was effectively used for the extraction, identification, and quantification of PAT in dried fruits (apple, fig, prune). Sadok et al. [2] and Shinde et al. [1] validated a robust and sensitive method based on QuEChERS combined with HPLC-MS/MS for rapid testing of patulin in red-pigment fruits and apple juices, respectively. For both studies, 10 g of sample was placed in a 50 mL polypropylene centrifuge tube and extracted with 10 mL ethyl acetate. However, Sadok et al. [2] added QuEChERS salt (4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, 0.5 g sodium hydrogen citrate sesquihydrate) and did not perform any clean-up process, whereas Shinde et al. [1] used 10 g of sodium sulphate and performed a dispersive solid phase extraction (dSPE) cleanup step. Both methods showed good performance in the determination of PAT in apples, with recoveries ranging from 90 to 109%, and lower LODs and LOQs, which demonstrated the feasibility of the proposed methods.

Table 2. Reported analytical approaches for patulin quantification in apple juice.

Sample Amount	Extraction Procedure	Analytical Method	LOD (µg/kg)	LOQ (µg/kg)	Rec (%)	Ref
1 g	LLE (10 mL MeCN)	LC-MS/MS	0.5	4	87–100	[19]
1 g	LLE (2 mL ethyl acetate)	LC-PDA	6	18	55–97	[24]
0.5 g	ID-MIP-SPE (10 mL H_2O:AcA (99:1 *v*/*v*)	LC-MS/MS	0.33	1.10	98–102	[16]
5 g	-	LC-MS/MS	0.5	2	94–98	[23]
2 mL	AA-NADES-SH-LPME (410 µL NADES solvent)	UV-Vis	3.5	10	94–104	[20]
10 g	QuEChERS (10 mL ethyl acetate)	UHPLC-MS/MS	-	0.65–3.01	96–109	[2]
10 g	QuEChERS-dSPE (10 mL ethyl acetate)	LC-MS/MS	-	5	90–95	[1]
0.1 g	µ-QuEChERS (1 mL MeCN)	LC-MS/MS	0.32	1.15	92–103	This work

LLE—liquid–liquid extraction; ID-MIP-SPE—isotope dilution—molecularly imprinted polymer—solid phase extraction; AcA—acetic acid; AA-NADES-SH-LPME—air-assisted natural deep eutectic solvent-based solidified homogeneous liquid phase microextraction; QuEChERS—quick, easy, cheap, effective, rugged and safe; µ-QuEChERS—micro-quick, easy, cheap, effective rugged and safe; LOD—limit of detection; LOQ—limit of quantification; LC-MS/MS—high performance liquid chromatography with tandem mass spectrometry; LC-MS/MS—liquid chromatography with tandem mass spectrometry; UHPLC-MS/MS—ultra high performance liquid chromatography with tandem mass spectrometry; LC-PDA—high performance liquid chromatography with photodiode array detection; rec.—recovery; UV-Vis—ultraviolet-visible.

In sum, the extraction procedure proposed in the current study did not present remarkable advantages in terms of LOD, LOQ and accuracy since the data obtained were quite similar to those reported in previous studies, as discussed above. Nevertheless, µ-QuEChERS/HPLC-MS/MS, compared to the extraction procedures reported in Table 2, showed several benefits, such as reduced sample amounts, washing steps, and harmful chemicals during experimental procedures. In addition, µ-QuEChERS is economical and does not require trained personnel to conduct the analysis.

Overall, the suggested analytical approach based on µ-QuEChERS following with LC-MS/MS analysis was demonstrated to be a quick, cost-effective, and high-throughput

strategy for the extraction, identification and quantification of PAT in apple juices, revealing exceptional performance in terms of linearity, sensitivity, matrix effect, accuracy, and precision.

3.2. Quantification of Patulin in Apple Juice

The validated µ-QuEChERS/LC-MS/MS method was finally used for the quantification of PAT in apple juice to show its applicability. Table 3 summarizes the results for the incidence of PAT in the examined apple juice samples.

Table 3. Concentration (µg/kg) of patulin in apple juices investigated.

Number of Samples	**30**
Incidence [a] (%)	10 (3 of 30)
Concentration range (µg/kg)	1.94–7.15
Mean ± SD (µg/kg)	4.38 ± 0.09

[a] % Incidence = (number of samples containing patulin/total number of samples) × 100.

Patulin was detected in 3 (10%) of the 30 analyzed apple juice samples, with an average concentration of 4.38 ± 0.09 µg/kg. In addition, PAT was not detected in 20 (67%) of the analyzed apple juices, whereas in 23% of the remaining apple juices, the PAT levels were detected at concentrations lower than LOD (1.15 µg/kg) (Table S1 in Supplementary Material). The results compare with other studies on apple juices collected in Korea and Brazil, where PAT was undetected or detected in levels lower than the LOD value [16,19]. For all apple juices investigated, the concentration of PAT was lower than the maximum allowable limit set by the EU for fruit juice and derived products (50 µg/kg); consequently, there was no risk to consumers through ingestion of these apple juices.

4. Conclusions

The µ-QuEChERS/LC-MS/MS method is a suitable analytical approach to determine PAT in apple juices, as it is simple, fast, requires low amounts of solvent and produces a minimal amount of waste compared to classical extraction procedures. The data obtained for linearity (R^2 = 0.999), sensitivity (LOD = 0.32 µg/kg, LOQ = 1.15 µg/kg), accuracy (92–103%), selectivity, and precision (% RSD < 7%) proved that the µ-QuEChERS/LC-MS/MS method is efficient in quantifying PAT in apple juices. Moreover, the validated analytical method was applied to 30 samples of apple juices, and none of the samples surpassed the legal European limit of 50 µg/kg; for this reason, there was no risk to consumers from ingestion of these juices.

The results obtained in this study confirmed that the analytical approach is appropriate for the purpose intended and that the risk of PAT occurrence in apple juices, as expected, is practically null. Considering the benefits of the suggested analytical approach, in addition to the low LODs and LOQs achieved, it could be employed in supervision programs and regular monitoring of PAT in apple juices, as well as derived products.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10030149/s1, Table S1. Patulin concentration (µg/kg) in apple juices investigated.

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