biology and life sciences forum

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Proceeding Paper

Rapid Identification of the Mycotoxin Patulin by Gas Chromatography–Mass Spectrometry †

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† Presented at the 2nd International Electronic Conference on Toxins, 14–28 July 2023. Available online: https://iect2023.sciforum.net/.

Abstract: Patulin (PAT) is one of the most common mycotoxins produced by *Penicillium* and *Aspergillus* species and is often associated with fruits and fruit by-products, mostly apple derivatives, although it has been detected in infant food and cereals. This toxin has shown a mutagenic and carcinogenic effect. Thus, the development of rapid and accurate methods for PAT detection is of utmost importance. Currently, the most widely used methods for the analysis and detection of mycotoxins are based on chromatography, including liquid chromatography (HPLC) and gas chromatography coupled to a mass detector (GC–MS), since these techniques provide high precision, selectivity, and sensitivity. In this work we show the preliminary results in the development of a GC–MS method for the detection of PAT without derivatization. Usually, the detection of mycotoxins by GC–MS needs a derivatization of all non-volatile and polar compounds. This is one drawback of gas chromatography compared to the liquid chromatographic technique for the determination of mycotoxins. In this sense, the PAT monitoring method by GC–MS proposed here is an alternative and useful technique to maintain high-quality foodstuffs and to ensure food safety.

Keywords: mycotoxins; GC–MS; patulin; food safety

Citation: Monteagudo, A.A.; Torres, A.; Chamorro, F.; Prieto, M.A.; Perez-Gregorio, R.; Simal-Gandara, J.; Otero, P. Rapid Identification of the Mycotoxin Patulin by Gas Chromatography–Mass Spectrometry. *Biol. Life Sci. Forum* **2023**, *24*, 6. https://doi.org/10.3390/IECT2023-14813

Academic Editor: Marc Maresca

Published: 20 July 2023

1. Introduction

Food safety is a growing concern in our society due to the increase in chemical and biological contaminants in the environment. Among these contaminants, mycotoxins are highly toxic secondary metabolites of low molecular weight and volatility, synthesized by certain groups of fungi [1,2]. The contamination of agricultural and other food products with this type of toxins poses serious problems for both human and animal health [1]. Inadequate collection, drying, handling, packaging, storage, and distribution are some of the factors that contribute to mycotoxin contamination the most [1,3]. Among mycotoxins, patulin (PAT) is a highly polar lactone with low molecular weight (154.12 g/mol) (Figure 1). It is produced by many species of the genera *Penicillium*, *Aspergillus* and *Byssochlamys*, *Penicillium expansum* being one of the main producers and responsible for PAT contamination of apples and their derived products [2,4]. PAT is soluble in water and in various solvents such as ethanol, methanol, acetone, ethyl acetate, etc. It is slightly soluble in sulfuric acid and

benzene, and stable under acidic conditions [1]. Compared to raw foods, the presence of PAT in processed foods is not frequent, since the clarification, filtration, and enzymatic treatment during juice processing and fermentation during winemaking significantly reduce its content [1,5]. Acute exposure to PAT can cause gastrointestinal symptoms including nausea, vomiting, ulcers, intestinal bleeding, and duodenal lesions, as well as impaired intestinal barrier function accompanied by kidney damage [2], while long-term (chronic) exposure includes neurotoxic, immunotoxic, immunosuppressive, genotoxic, teratogenic, and potentially carcinogenic effects [4]. In relation to the analytical determination, methods to confirm the presence of PAT usually include specific detection techniques, such as mass spectrometry (MS), previous separation by liquid chromatography (LC) or gas chromatography (GC). Today, with the majority of detection methods using the GC–MS methodology, PAT is detected as its trimethylsilyl derivative (TMS-patulin) [6–8]. In general, mycotoxins' detection by GC implies their derivatization. However, the derivatization process takes time and leads to a delay in analysis, especially when a high number of food samples have to be monitored. In this context, the objective of this work is to collect information regarding the available chromatography methods for PAT detection using GC and go further in the use of this technique to detect PAT without derivatization, so that it will be the starting point for the development of a method which could be improved in speed.

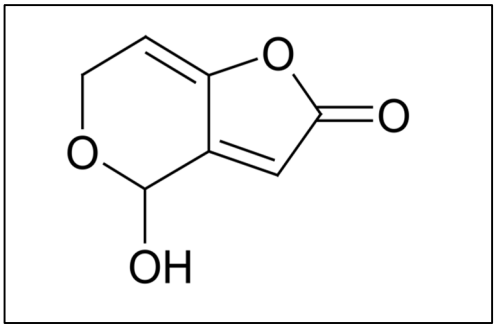

Figure 1. Patulin structure.

2. Material and Methods

2.1. Chemicals and Materials

Dichloromethane (CH_2Cl_2), acetonitrile (MeCN), and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). All chemical and reagents were of analytical grade (GC). The standard of PAT was obtained from Sigma-Aldrich (5 mg). A stock solution was prepared by dissolving 5 mg of PAT in 1 mL of pure methanol, obtaining a 5 mg/mL solution. Then, this stock solution was used in further dilutions in dichloromethane, acetonitrile, and methanol in order to obtain the appropriate working standard solutions (10 µg/mL and 100 µg/mL). All solutions were kept at −20 °C before analysis.

2.2. GC–MS Equipment and Methodology

The analysis of patulin was performed on the Thermo Scientific Trace 1300 GC system coupled with a Thermo Scientific ISQ 7000 single quadrupole mass spectrometer. The Trace 1300 GC module is integrated with the Chromeleon chromatography data system software. The separation was achieved on a HP-88 30 m × 0.25 mm × 0.2 µm capillary column from—Agilent. Helium was used as the carrier gas with a constant flow of 1 mL/min. The oven temperature program was initially set at 80 °C for 1 min, and increased to 245 °C at 60 °C/min. After 3 min of hold time at 245 °C, the temperature was increased to 260 °C at 3 °C/min and then increased to 270 °C at 10 °C/min. Finally, 270 °C was held for 10 min. Patulin was dissolved in dichloromethane, acetonitrile, and methanol; the injection volume was 1 µL and the solvent delay was 2 min. The method operated in split injection mode (1:50) with pulse injection at 35 psi for 0.5 min. The ion source temperature was 250 °C and the transfer line temperature was 250 °C.

3. Results and Discussion

Although PAT detection by GC is usually performed by derivatization, the bibliography includes some works in which mycotoxins are not derivatized. To our knowledge, there are just two studies in which GC–MS with negative ion chemical ionization allowed the detection of underivatized PAT [9,10]. PAT without derivatization was successfully analyzed and this fact encouraged us to work in this line and develop a method for rapid detection of PAT without derivatization. For the method development, we first evaluated the influence of the sample solvent on the chromatogram signal. For this, three commonly used solvents were selected and compared; they were dichloromethane, acetonitrile, and methanol [5,6,11,12]. For each solvent, two working standard solutions of 10 µg/mL and 100 µg/mL were prepared and injected. Out of the three solvents used, dichloromethane produced the higher signal of PAT (Figure 2).

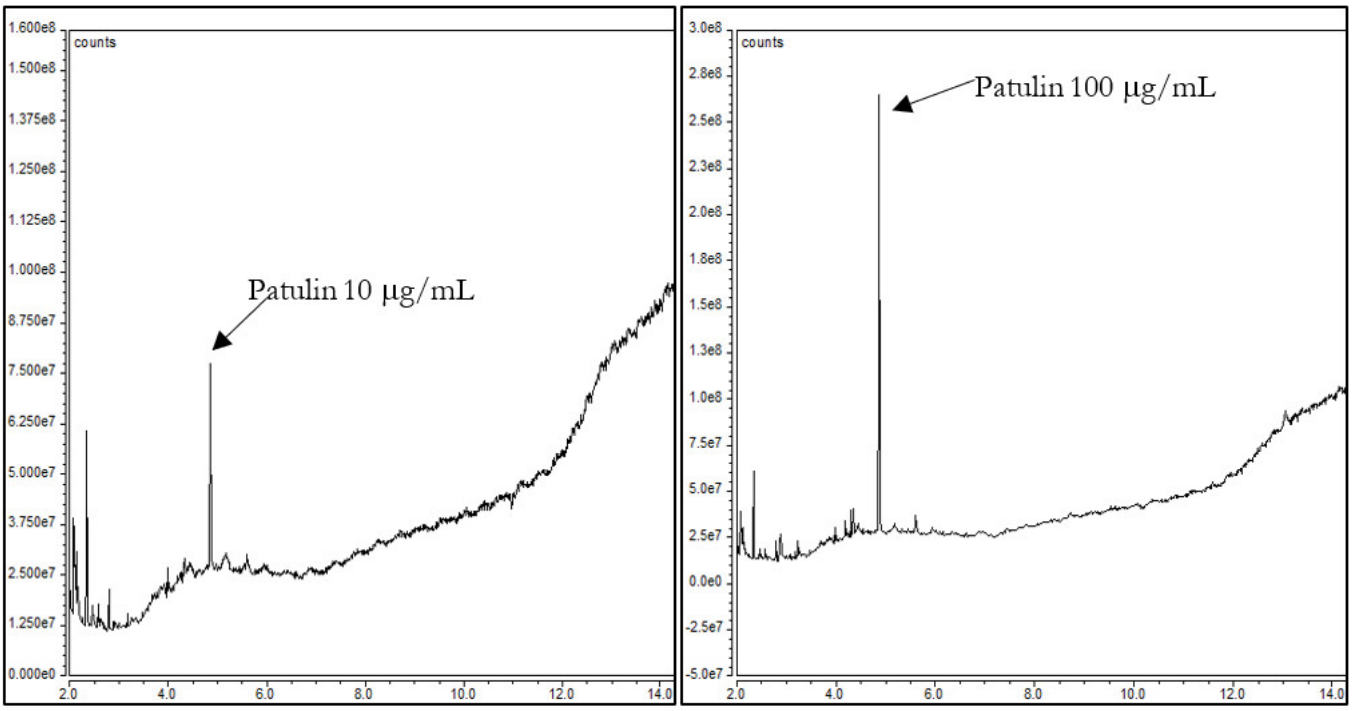

Figure 2. Standard of patulin at 10 µg/mL and 100 µg/mL in dichloromethane without derivatization.

In this work, we showed that PAT can be analyzed without derivatization using a HP-88 30 m × 0.25 mm × 0.2 µm capillary column from Agilent. Different columns have been used to detect PAT. Table 1 shows the available GC–MS methodology for PAT. The most used column for identifying PAT in apple juice is the capillary column HP-5ms [8,9,11,13] with limits of detection (LOD) between 0.4 and 3 µg/L. Another study employs three capillary columns of moderate polarity, DB-17 (0.25 µm i.d. × 30 m, 0.25 µm J&W), DB-1701 (0.25 µm i.d. × 30 m, 0.25 µm, J&W), and BPX-35 (0.22 µm i.d. × 25 m, 0.25 µm, BSE), obtaining better LODs of 0.1 µg/L [7]. Future research will test the method with different food samples which will determine the sensitivity of our methodology. Most available methods for PAT are focused on apple and apple derivatives, although in the last decade, the GC method has been developed for the detection of PAT and other mycotoxins in cereals, wheat, rice, maize, spelt, oat, soy, and tapioca [14,15].

Table 1. Available methodology for patulin detection by GC–MS.

Type of Food	Type of Column	Operating Conditions	LODs	LOQs	Reference
		With derivatization			
Fruit juice	Column 50% phenyl, 50% methyl-polysiloxane, SGE, capillary (30 m × 0.25 mm × 0.25 µm)	Initial T: 100 °C for 2 min, ramped at 10 °C min^{-1} to 200 °C and then 20 °C min^{-1} to 300 °C, held for 3 min. Carrier gas: He (constant flow: 1.42 mL/min).	5.8 µg/kg^{-1}	13.8 µg/kg	[12]
Apple juice	Three capillary columns (moderate polarity) (1) DB-17 (0.25 µm i.d. × 30 m, 0.25 µm J&W) (2) DB-1701 (0.25 µm i.d. × 30 m, 0.25 µm, J&W) (3) BPX-35 (0.22 µm i.d. × 25 m, 0.25 µm, BSE)	Oven T: 80 °C (2 min) → 150 °C (10 °C/min) → 230 °C (5 °C/min, 15 min) Carrier gas: He at constant pressure of 100 kPa.	0.1 µg/kg	1 µg/kg	[7]
Apple, Quince	Supelco SLB-5MS Column (30 m × 0.25 mm ID × 0.25 µm film thickness)	Oven T: 100 °C held for 1 min, ramped to 180 °C at 10 °C/min, finally ramped to 280 °C at 30 °C/min and held for 12.67 min (25 min total run time). Carrier gas: He (constant flow: 1 mL min).	0.4 µg/kg^{-1}	1.6 µg/kg	[5]
Apple juice	Column HP-5MS, crosslinked 5% phenylmethyl silicone (30 m × 0.25 mm id × 0.25 µm film thickness, Agilent)	Oven T: 100 °C for 2 min, ramped at 10 °C/min to 200 °C, and then 200 to 300 °C, held for 3 min.	3 µg/L	10 µg/L	[11]
Apple/Pear Juice	Column J&W DB-5MS (30 m × 0.25 mm id; 0.25 µm film thickness)	70 °C (held for 1 min) to 320 °C at 25 °C/min (held for 2 min) at a constant flow regime of 1 mL/min	n.i.	n.i.	[16]
Apple juice	Agilent HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness)	Oven T initially held at 50 °C (3 min) and programmed to 280 °C at a rate of 10 °C/min, then held for 5 min. Total run time was 31 min. Carrier gas: He (constant flow: 1 mL min).	0.4 µg/L	1.3 µg/L	[13]
Apple juice	Column HP-5MS 5% phenyl methyl siloxane cross-linked capillary GC column (15 m × 0.25 mm i.d., × 0.25 µm film thickness)	Oven T: initially at 100 °C (2 min) and programmed at 15 °C/min to 210 °C, then at 50 °C/min to a final T of 300 °C, which was held for 2 min. Total run time: 13 min. Inlet: 280 °C. Transfer line: 250 °C.	2 µg/L	5 µg/L	[8]
Apple juice	HP Ultra 2 crosslinked 5% phenyl methyl silicone (25 m × 0.2 mm), with 0.33 µm film	Oven T: 100 °C (2 min), ramped at 10 °C/min to 200 °C and then 20 °C/min to 300 °C, held for 3 min. Detector T: 300 °C. Injector T: 280 °C. Carrier gas: He (constant flow: 1 mL min).	n.i.	n.i	[6]

Table 1. *Cont.*

Type of Food	Type of Column	Operating Conditions	LODs	LOQs	Reference
		Without derivatization			
Apple Juice	Column HP-5MS cross-linked methyl silicone capillary (30 m × 0.25 mm id)	T held at 80 °C for 1 min after injection, programmed to 250 °C at 15 °C/min, and held for 5 min. Carrier gas: He	n.i	n.i	[9]
Apple Juice	Capillary column (15 m × 0.53 mm id) with a 1.5 µm film of bonded phase methyl polysiloxane	T held at 60 °C for 1 min after on-column injection, heated at 15 °C/min to 260 °C, and held at 260 °C for 5 min. Transfer lines: 260 °C. Carrier gas: He at gas flow of 25 cm/s.	n.i	n.i	[10]
Apple Juice	Capillary column (30 m × 0.25 mm id) with a 0.25 µm film of bonded phase trifluoropropylmethyl polysiloxane	T programmed from 60 °C to 260 °C 1 min after injection at a rate of 20 °C/min. Transfer lines: 260 °C, ion source: 200 °C. Injection: 260 °C. Carrier gas: He at constant flow: 40 cm/s (1.21 mL/min).	n.i	n.i	[10]

Abbreviations: T (Temperature), n.i. (not included), LOD (limit of detection), LOQ (limit of quantification).

4. Conclusions

PAT is one of the most toxic secondary metabolites produced by fungi. Their occurrence is growing significantly on vegetables and fruits like apples, pears, and grapes [1]. Toxicology data revealed that acute PAT intoxication results in ulceration, agitation, convulsions, oedema, vomiting, and DNA damage in the brain, kidney and liver. Thus, the EU Commission has established maximum levels for PAT in some foods including fruit juices, spirit drinks and cider (50 µg/kg), solid apple products like compote and puree (25 µg/kg), and products for infants and young children like apple juice and solid apple (10 µg/kg) [2], and FAO/WHO have suggested a provisional maximum tolerable daily intake (PMTDI) of 0.4 mg/kg body weight/day. Available data show that at least 30% of apple juices may be contaminated with PAT at levels higher than 50 mg/L and that consumers ingest as much as 250 mL of apple juice per day [1]. PAT has also been found in seafood such as shellfish and cereals, which is a concern for several sectors of the food industry. Thus, the monitoring of the contamination of PAT in several kinds of foods is of the utmost importance to guarantee the products' safety and quality. In this context, we have found that the detection of PAT by GC–MS without derivatization can be a solid analytical procedure for their qualitative detection.

Author Contributions: Conceptualization, P.O.; methodology, A.A.M.; formal analysis, P.O.; investigation, A.T., F.C., M.A.P. and R.P.-G.; resources, J.S.-G.; data curation, P.O.; writing—original draft preparation, A.A.M.; writing—review and editing, P.O.; visualization, P.O.; supervision, P.O.; project admin-istration, J.S.-G. and P.O.; funding acquisition, J.S.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by HORIZON-CL6-2022-FARM2FORK-01-09 grant number 101084344 (WHEATBIOME). Funder under Food, Bioeconomy Natural Resources, Agriculture and Environment.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to WHEATBIOME under grant agreement ID: 101084344 (HORIZON-CL6-2022-FARM2FORK-01) and to Bio Based Industries Joint Undertaking (JU) under

grant agreement No 888003 UP4HEALTH Project (H2020-BBI-JTI-2019). The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium. The project SYSTEMIC Knowledge hub on Nutrition and Food Security has received funding from national research funding parties in Belgium (FWO), France (INRA), Germany (BLE), Italy (MIPAAF), Latvia (IZM), Norway (RCN), Portugal (FCT), and Spain (AEI), in a joint action of JPI HDHL, JPI-OCEANS, and FACCE-JPI launched in 2019 under the ERA-NET ERA-HDHL (n° 696295).

Conflicts of Interest: The authors declare no conflict of interest.

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