

Proceeding

Rapid identification of the Mycotoxin Patulin by Gas Chromatography-Mass Spectrometry[†]

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[†] Presented at the 2nd International Electronic Conference on Toxins, online, 14–28 July 2023.

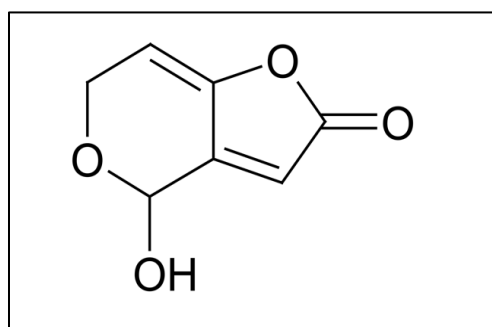
Abstract: Patulin (PAT) is one of the most common mycotoxins produced by *Penicillium* and *Aspergillus* species and are often associated with fruits and fruit by-products, mostly apple derivatives, although it has been detected in infant food and cereals. This toxin has shown a mutagenic and carcinogenic 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 about 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 all non-volatile and polar compounds. This is one drawback of gas chromatography against liquid chromatographic technique for the determination of mycotoxins. In this sense, the PAT monitoring method by GC-MS here proposed is an alternative and useful technique to maintain high quality foodstuffs and to ensure food safety.

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

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 most contribute to mycotoxin contamination [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 *Byssoschlamys*, being *Penicillium expansum* one of the main producer 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, the majority detection methods using 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 derivation process takes time and leads in an analysis delay, 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 a method development 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 (5mg). 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 to further dilutions in dichloromethane, acetonitrile and methanol in order to obtain the appropriate working standard solutions (10 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$). All solutions were kept at -20°C before analysis.

2.2. GC-MS equipment and methodology

The analysis of patuline was performed on 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 \times 0.25 mm \times 0.2 μm capillary column 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 a 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 hold for 10 min. Patuline was dissolved in dichloromethane, acetonitrile, and methanol and the injection volume was $1\mu\text{L}$ and the solvent delay was 2 min. 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

Despite, PAT detection by GC is usually performed by derivatization, the bibliography shows some works in which mycotoxins are not derivatized. From our knowledge, there are just 2 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, first we evaluated the influence of sample solvent on chromatogram signal. For this, three commonly used solvents were selected and compared, which were dichloromethane, acetonitrile, and methanol [5,6,11,12]. For each solvent, two working standard solutions of 10 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ were prepared and injected. From three solvents used, dichloromethane produced the higher signal of PAT (Figure 2).

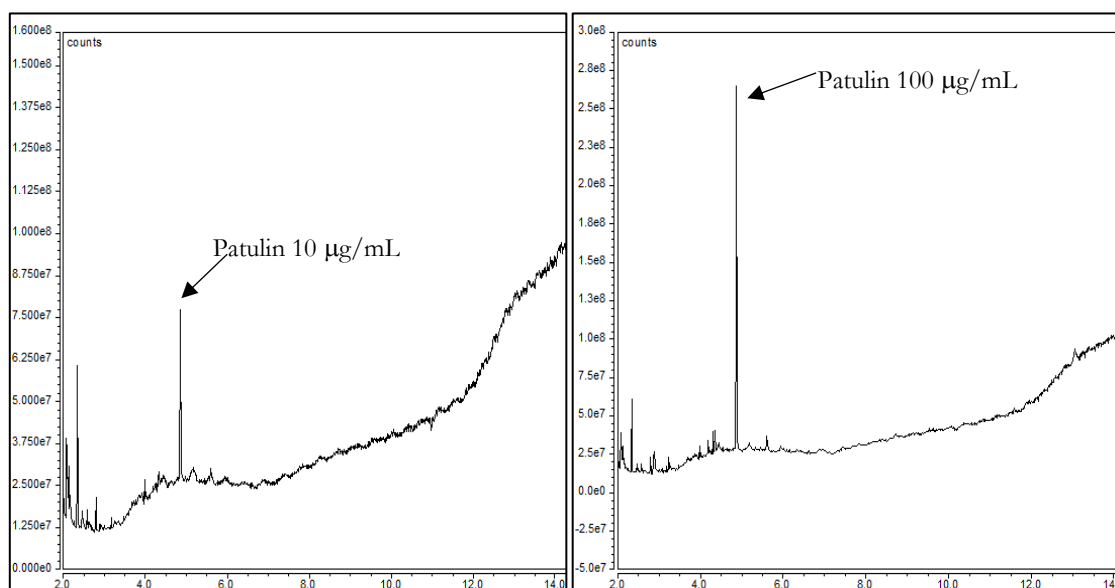


Figure 2. Standard of Patulin at 10 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ in dichloromethane without derivatization.

In this work we showed that PAT can be analysed without derivatization using a HP-88 30 m \times 0.25 mm \times 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 columns for identify PAT in apple juice is the capillary column HP-5ms [8,9,11,13] with limits of detection (LOD) between 0.4 and 3 $\mu\text{g}/\text{L}$. Other study employs 3 capillary columns of moderate polarity, DB-17 (0.25 μm i.d. \times 30 m, 0.25 μm J&W), DB-1701 (0,25 μm i.d. \times 30 m, 025 μm , J&W) and BPX-35 (0.22 μm i.d. \times 25 m, 0.25 μm , BSE), obtaining better LODs of 0.1 $\mu\text{g}/\text{L}$ [7]. Future research direction is to 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 development for the detection of PAT and other mycotoxins has increased 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
<i>With derivatization</i>					
Fruit juice	Column 50% phenyl, 50% methyl-polysiloxane, SGE, capillary (30 m x 0.25 mm x 0.25 µm)	Initial T: 100°C for 2 min, ramp at 10°C min ⁻¹ to 200°C and then 20°C min ⁻¹ to 300°C, hold for 3 min. Carrier gas: He (constant flow: 1.42 mL/min).	5.8 µg/Kg ¹	13.8 µg/Kg	[12]
Apple juice	3 capillary columns (moderate polarity) (1) DB-17 (0.25 µm i.d. x 30 m, 0.25 µm J&W) (2) DB-1701 (0,25 µm i.d. x 30 m, 025 µm, J&W) (3) BPX-35 (0.22 µm i.d. x 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 x 0.25 mm ID x 0.25 µm film thickness)	Oven T: 100 °C held for 1.0 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 x 0.25 mm id x 0.25 µm film thickness, Agilent)	Oven T: 100°C for 2 min, ramp at 10°C/min to 200°C, and then 200 to 300°C, hold for 3 min.	3 µg/L	10 µg/L	[11]
Apple / Pear / Juice	Column J&W DB-5MS (30 m x 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 x 0.25 mm x 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 and 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 x 0.25 mm i.d., x 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 x 0.2 mm), with 0.33 µm film	Oven T: 100°C (2 min), ramp at 10°C/min to 200°C and then 20°C/min to 300°C, hold 3 min. Detector T: 300°C. Injector T: 280°C. Carrier gas: He (constant flow: 1 mL min).	n.i.	n.i.	[6]
<i>Without derivatization</i>					
Apple Juice	Column HP-5MS cross-linked methyl silicone capillary 30 m x 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 was 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 apple juice per day [1]. PAT has also been found in seafood such as shellfish and cereals, being a concern for several sectors of the food industry. Thus, the monitoring of the contamination of PAT in several kinds of foods is of utmost importance to guarantee the products' safety and quality. In this context, we have found that the detection of PAT by GC-MS without derivation can be a solid analytical procedure for their qualitative detection.

Acknowledgments: 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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