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QUANTIFICATION OF THE MYCOTOXIN PATULIN AND HYDROXYMETHYLFURFURAL IN SOME JUICE IN THE EGYPTIAN MARKETS

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ABSTRACT

A rapid and simple method is described to determine patulin (PAT) and 5- hydroxy methyl furfural (5-HMF) in some fruit juices in the Egyptian market. The target compounds were extracted with ethyl acetate using vortex followed by high-performance liquid chromatographic separation. It was found that the 5-HMF concentration in the tested samples was ranged between 0.53 and 477 mg/L, and no patulin was detected.

Key Words: HPLC, juice, 5-HMF, patulin

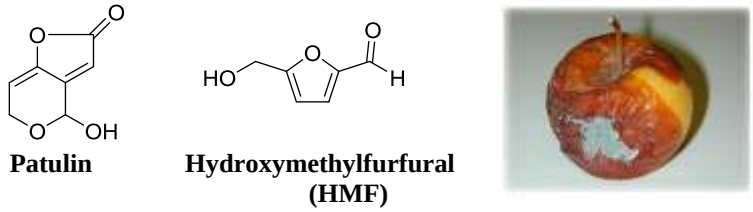
INTRODUCTION

The word mycotoxin mostly is related to chemicals produced by mould, which is toxic to vertebrates, mammals, and other animal groups in low concentrations **(Bennett and Klich 2009)** capable of producing acute or chronic toxic effects (e.g, carcinogenic, mutagenic, and teratogenic) on animals. The Greek word "mykes" meaning "fungi," and the Latin word "toxicum" meaning "poison," are the origin of the word mycotoxin **(Agriopoulou *et al.*, 2020)**. Twenty-five per cent of the world's food sources may be contaminated with mycotoxins **(Park *et al.*, 1999)**, and according to climate change, it is expected to increase contamination of human and animal foods with these products **(Krogh, 1978)**. The most important toxigenic fungal species are in the categories of *Aspergillus*, *Penicillium*, and *Fusarium* **(Agriopoulou *et al.*, 2020)**. They are also resistant to most cooking, processing, fermentation techniques, and even pasteurization process.

Patulin (4-hydroxy-4H-fural[3,2-c] pyran-2(6H)-one) **(Figure 1)** is a mycotoxin produced by various fungi, in particular by *Aspergillus*, *Byssochlamys*, and *Penicillium*. It was first isolated from *Penicillium griseofulvum* in 1943 by Harold Raistrick **(Muir 1943)**. Soon after its identification, Patulin was studied at the British Medical Research Center under the name of "tercinin" as an antimicrobial agent against some gram-positive and gram-negative bacteria, however, it was not long until the researchers at the center has identified its toxic effects in 1944

(Chalmers and Clarke 2004). Patulin has been identified in various food products, including fruits and vegetables. However, it is mostly found in apples, in which its level is applied as a quality indicator of apples used in juice or compost production **(Zhong *et al.*, 2018)**. Patulin is mainly a metabolite of *Penicillium expansum* grown on the spoilage apple (**Figure 1**). Different spoilage microorganisms have different nutrient requirements, so specific fungal types contaminate select food products and fruits. *P. expansum* has a preference to pome and stone fruits, such as peaches, cherries, and fruits in which they cause "blue rot" **(Moss 2008 and Tannous *et al.*, 2018)**.

On the other hand, 5-hydroxymethylfurfural, HMF **(Figure 1)** is considered a thermal decomposition product of fructose **(Rupp and Turnipseed 2000**). It is a dark yellow liquid or powder with the odour of chamomile flowers **(Baykoucheva 2010)**. HMF is formed during the thermal treatment of carbohydrates, particularly under acidic and high-temperature conditions. HMF is an indicator of quality in several food products such as juice, molasses, and honey. It has adverse effects on human health like cytotoxic, mutagenic, genotoxic, and carcinogenic consequences (**Türköz *et al.*, 2018)**. The present study was aimed to investigated the presence of patulin and HMF in different types of juice in the Egyptian market using HPLC to compare their allowed percentage according to the global levels.



Patulin **Hydroxymethylfurfural (HMF)**

A **B**

Figure 1.(**A**) Chemical structures of the patulin and 5-hydroxymethylfurfural, and (**B**) an apple contaminated by *Penicillium expansum*.

MATERIALS AND METHODS

1- Collection of samples

Eight juice samples were collected from different supermarkets in Cairo, Egypt. Among the collected samples, three were 100% fruit juice, three were approximately 50% fruit juice, and the other two were concentrated. These samples were in Tetra Pack containers or bottles of 1000 ml. The collected samples were stored at room temperature and

analyzed before expiration date. Different brands were selected to have a market representative sampling of products sold on the local market.

2- Reagents

In this study, we used ethanol 99.7% (v/v), glacial acetic acid ≥99.5% (v/v), acetonitrile ≥99.5% (v/v), formic acid (extra pure grade), ethyl acetate, 5-hydroxymethyl furfural (HMF), patulin, sodium carbonate anhydrous, and sodium sulphate anhydrous. These reagents were purchased from Sigma Aldrich and Al - Gomhouria Company, Egypt.

3- Preparation of standard solutions

Five mg of patulin were dissolved in 5 mL ethyl acetate to prepare a standard patulin solution. The solution was transferred to a 25 mL volumetric flask and completed with ethyl acetate. On the other hand, the stock solution of HMF was prepared by dissolving 5.0 mg of HMF in a small amount of deionized water, using a 25 mL volumetric flask, and then adjusting its volume with acidified deionized water (pH 4.0) to 25 mL. The stock solutions of patulin and 5-HMF were stored at –20^{o}C for prpration of calibration curves.

4- HPLC:

The HPLC instrument was Sykam system, equipped with a diode array detector (DAD), Clarity Software, The S 1125 Isocratic HPLC pump with a vacuum degasser, an injector, S 3345 PDA detector system and column (ReproSil 100 C18, 5µm, 250×4.6mm). The mobile phase consisted of an isocratic mixture of water/acetonitrile/formic acid (95:5:1, v/v/v) for 15 min at a 1.0 ml/min flow rate. A 100 µl of standards and samples were injected into the HPLC column. The spectra were recorded at 276 and 285 nm wavelengths. Identification of 5-HMF and patulin in samples were carried out by comparing their retention times and UV spectra with the authentic samples.

5- Extraction of patulin and 5 - HMF from fruit juice

Extraction of patulin and HMF were performed according to the **AOAC (2012).** About 5 ml of juice were extracted with 10 ml ethyl acetate and shaken vigorously for 1 min using a vortex mixer. The mixture was separated for 10 minutes, and the upper organic layer was transferred to a glass tube. This extraction method was repeated three times and combined in the glassy tube. Two mL of 1.5% Na_2CO_3 solution was added to the combined ethyl acetate layer and mixed vigorously on a vortex mixer. The upper ethyl acetate layer was transferred to a clean vial, and 1g of Na_2SO_4 was added. The tube was

shaken vigorously for 30 seconds and decanted into a 25 mL round flask. The solvent was evaporated on a rotary evaporator at 40°C. The residue was dissolved in 0.5 mL pH 4 water, filtered through a membrane filter (0.45 µm), and analyzed by HPLC.

RESULTS AND DISCUSSION

The standard mixture of 5-HMF and patulin was used to establish optimum separation conditions. It was found that water/acetonitrile/formic acid (95:5:1, v/v/v) as the mobile phase at a flow rate of 1.0 have shown a good separation of 5-HMF (R_t=5-8 min) and patulin (R_t=9-11min) (**Figures 2-4**) in a noticeably shorter time than the previously reported results **(Moss, 2008)**. A total of 8 samples of juice were analyzed for detection of 5-HMF and patulin (**Table 1 and Figures 5-12**). It was found that 5-HMF is present in all examined samples with concentrations ranging from 1.35-23.30 mgL^{-1} for 100 % juice, 0.53-11.97 mgL^{-1} for 50 % juice, and 9.24-477.41 mgL^{-1} for fruit concentrates. The International Federation of Fruit Juice Processors (IFFJP) has suggested that the maximum level for HMF in fruit juice is 5-10 mgL^{-1} and 25 mgL^{-1} for fruit concentrates **(Ioi *et al.*, 2017)**. Results of the current study also showed that the patulin level in the analyzed juice samples doesn't exceed the legal limits established by the European Union 50 mg Kg-1 for fruit juices. The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives established a provisional maximum tolerable daily PAT intake (PMTDI) at 0.4 µg/kg of body weight (BW) **(Oroian *et al.*, 2014)**.

Table 1. Result of analyzed juice for the presence of patulin and 5-HMF

NO	Sample	Average ± SD	
		PAT (µg L^{-1})	5-HMF (mg L^{-1})
1- G1	Guava juice (100%)	<LOD	4.20±0.10
2-P1	Pineapple juice (100%)	<LOD	23.30±0.90
3-O1	Orange juice (100%)	<LOD	1.35±0.07
4-Pe1	Peach juice (50%)	<LOD	2.69±0.06
5-O2	Orange juice (50%)	<LOD	0.53±0.20
6-A1	Apple juice (50%)	<LOD	11.97±1.98
7-T1	Tamarind (CONC)	<LOD	477.41±20.04
9-C1	Carob (CONC)	<LOD	9.24±0.82

LOD means Limit of detection

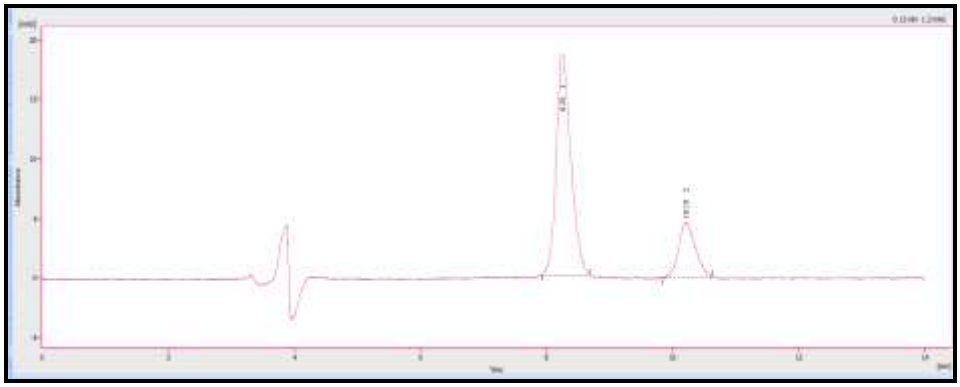


Figure 2. HPLC chromatogram of a standard mixture of 5-HMF and patulin.

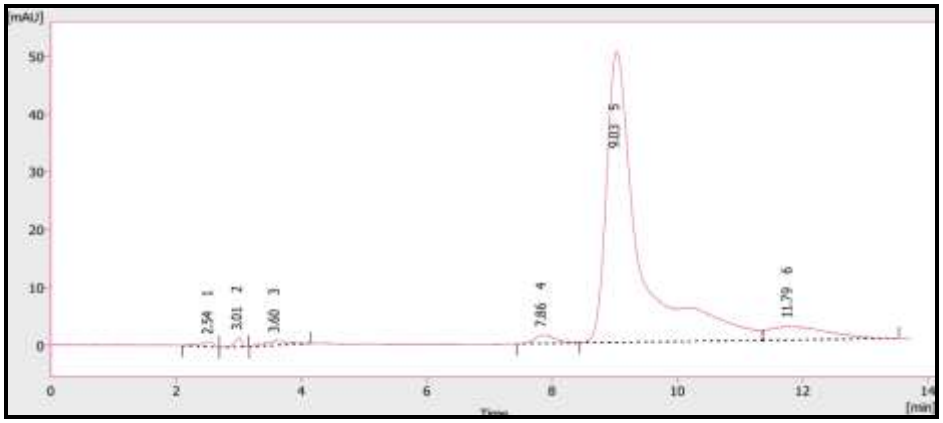


Figure 3. HPLC chromatogram of patulin.

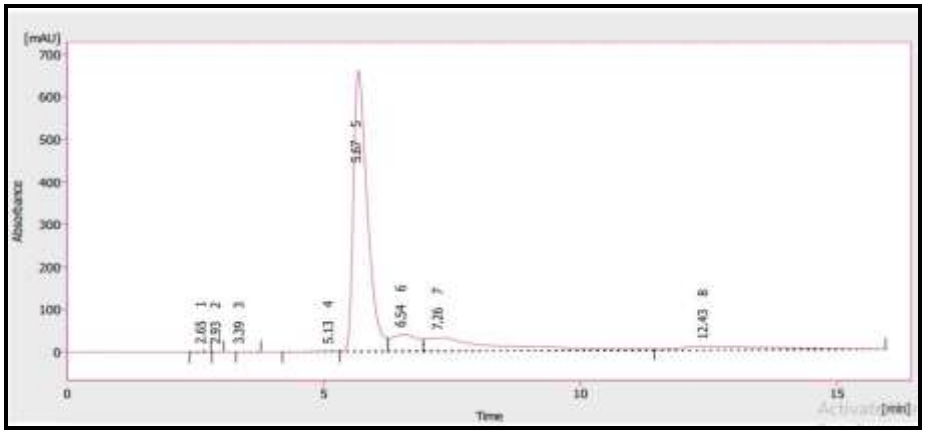


Figure 4. HPLC chromatogram of 5-HMF.

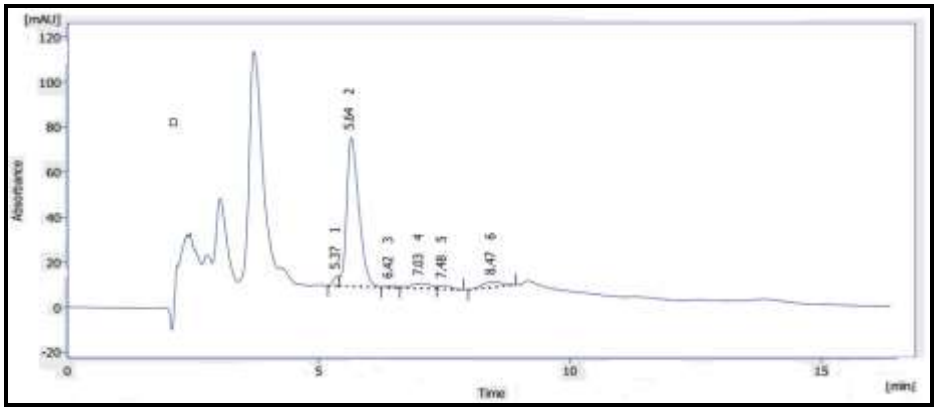


Figure 5. HPLC chromatogram of (G1) guava juice.

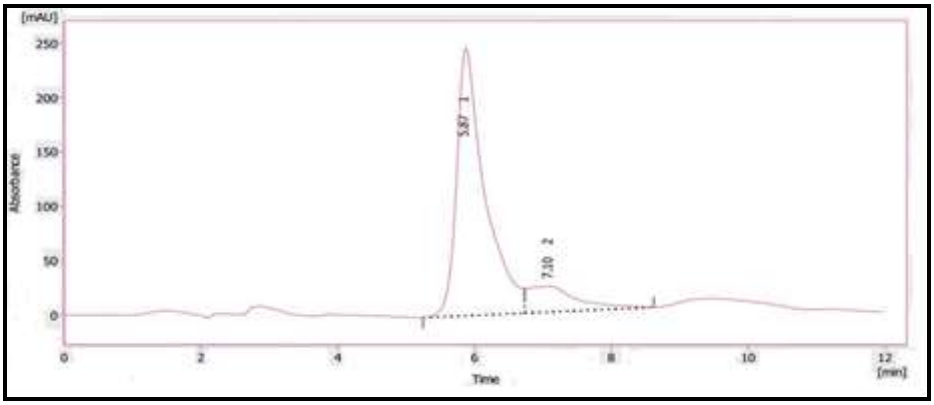


Figure 6 HPLC chromatogram of 5-HMF for (P1) pineapple juice (100%)

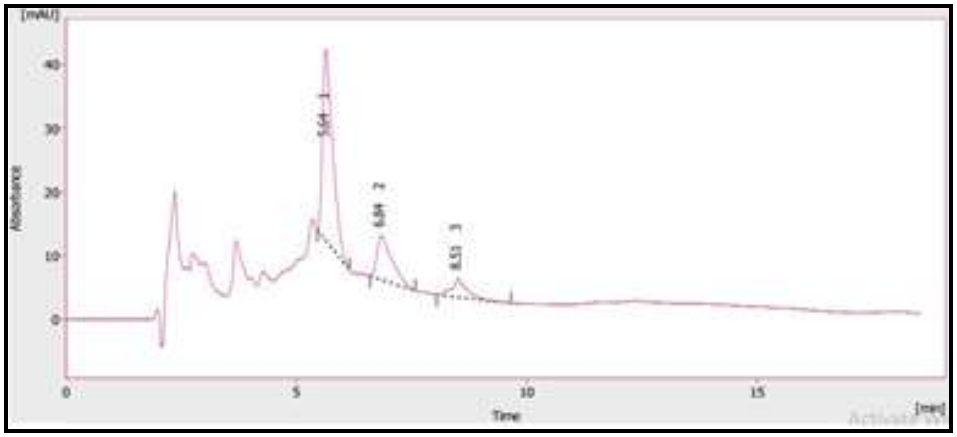


Figure 7. HPLC chromatogram of (O1) Orange juice (100%).

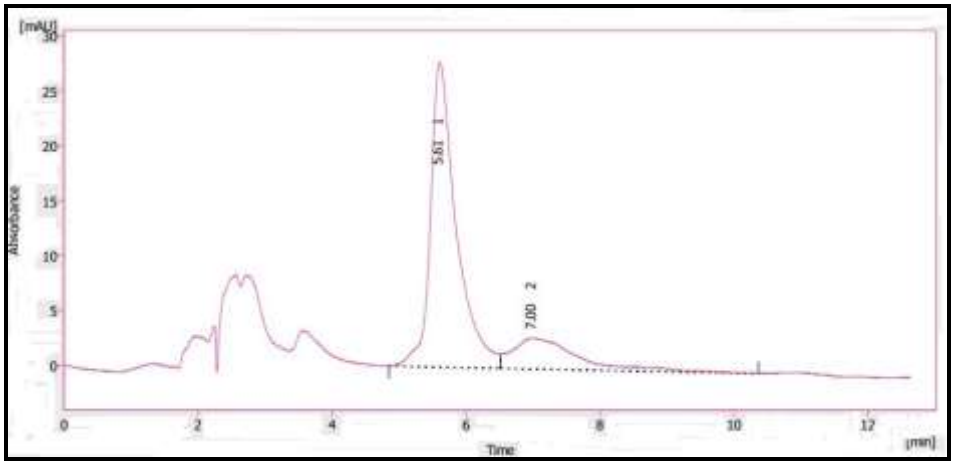


Figure 8. HPLC chromatogram of (Pe1) Peach juice (50%).

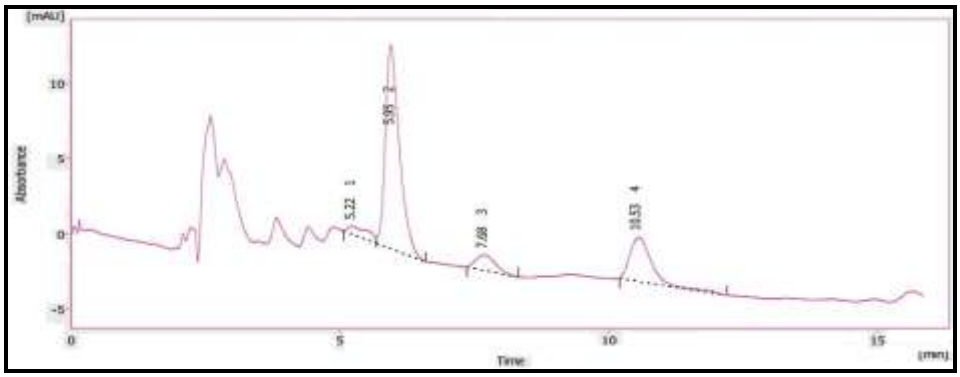


Figure 9. HPLC chromatogram of (O2) Orange juice (50%).

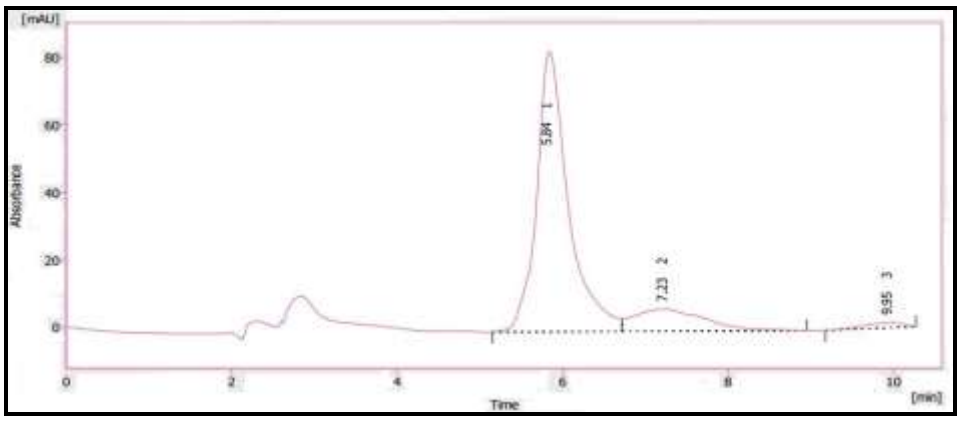


Figure 10 HPLC chromatogram of (A1) Apple juice (50%).

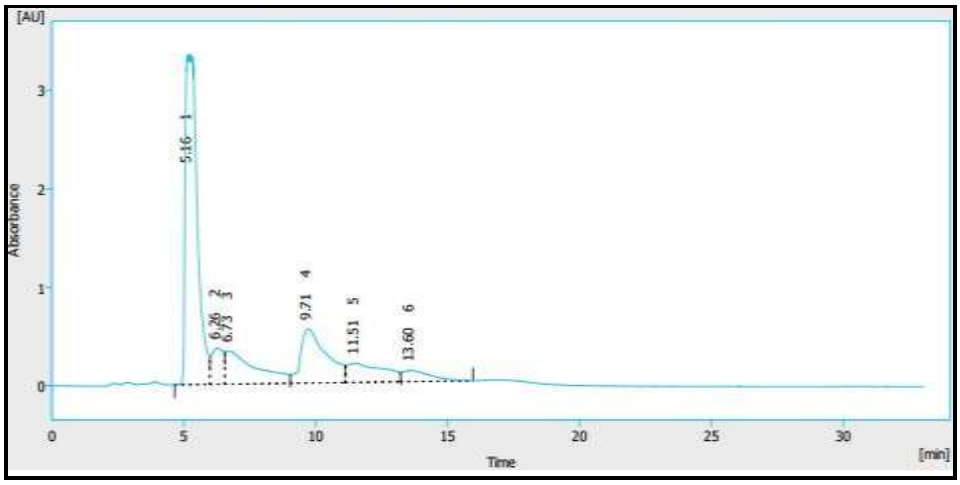


Figure 11. HPLC chromatogram of (T1) Tamarind.

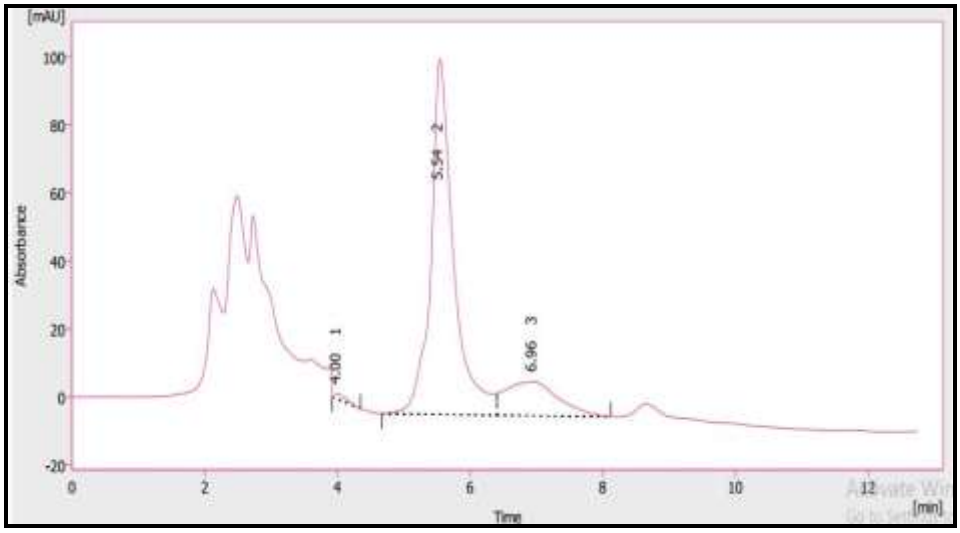


Figure 12. HPLC chromatogram of (C1) Carob.

CONCLUSION

In this study, eight samples of fruit juices found in the Egyptian market were analyzed by HPLC to detect the level of patulin and HMF. It was found that the 5-HMF concentration in the tested samples is ranged between 0.53 and 477 mg/L, and no patulin was detected. This method is useful for routine quality control of fruit juice and provides the understanding of PAT and 5-HMF levels in various fruit juices.

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التقدير الكمي للسم الفطري الباتيولين، وهيدروكسي ميثيل فورفورال في بعض العصائر في الأسواق المصرية

أيه رمضان محمود ، علي محمد صالح حبيشي ، ايهاب عبد الرؤوف العسوي ، محمد صالح عبد الفتاح

قسم الكيمياء– كلية العلوم – جامعة حلوان

الطريقه المبينه هي طريقه بسيطه و سريعة لتقدير كميه السم الفطري الباتيولين، و هيدروكسي ميثيل فورفورال في بعض العصائر في الأسواق المصرية . تم استخلاص المركبات المستهدفة باستخدام أسيتات الإيثيل متبوعة بفصل كروماتوغرافي السائل عالي الأداء (HPLC) . أظهرت النتائج ان تركيز هيدروكسي ميثيل فورفورال في العينات المختبر يتراوح بين 0.53 و477 مغ/لتر ، ولم يتم اكتشاف أي باتولين في العينات المختبرة .