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# Detection of Mycotoxin Patulin in Apple Juice

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## ABSTRACT

**Patulin is a mycotoxin produced mainly by *Penicillium expansum* that commonly contaminates apples. Studies with fungi or animals have demonstrated that patulin has a broad spectrum of toxicity, including mutagenicity, teratogenicity, and carcinogenicity. In human beings, it has caused nausea, vomiting and gastrointestinal disturbance. In the United States, the Office of Environmental Health Hazard Assessment has announced that patulin is a potential carcinogen. It is regulated for apple juice in several European countries at a maximum permitted level of 50 μg/L, as suggested by the World Health Organization. In the present study, patulin was extracted with ethyl acetate and was then cleaned up by extraction with sodium carbonate solution. Patulin was determined after reverse phase HPLC on a $C_{18}$ column with UV detection at 276 nm. The linear regression coefficient of the standard curve (Y= 0.013333X + 0.014789) for patulin at concentrations ranging from 1 to 2000 μg/L was 0.999995. The recovery rates for patulin at 20, 50, 100 and 200 μg/L ranged from 93.1% to 96.6% with a coefficient of variation less than 3.0%. The instrument detection limit was 0.05 ng and quantification limit was 15 μg/L. A survey with 105 retailed pure apple juice and apple juice containing mixed juice samples showed that 93 samples (88.5%) were negative for patulin and only 12 pure apple juice samples (11.4%) contained patulin at concentrations ranging from 15.4 to 39.9 μg/L. All of the detected samples contained patulin were below the level suggested by the World Health Organization.**

***Key words:*** **patulin, apple juice, high performance liquid chromatography(HPLC).**

## INTRODUCTION

Patulin [4-hydroxy-4H-furo(3, 2-c)pyran-2(6H)-one], a mycotoxin (structure shown in Figure 1), is heat resistant, stable in dilute acid, and labile in alkali. It is produced by approximately 60 species of molds belonging to over 30 genera. Many fungi found in spoiled food such as *Aspergillus* spp. and *Penicillium* spp., including *A. clavatus, A. giganteus, A. terreus, P. urticae, P. expansum* and *Byssochlamys nivea*, produce pa-

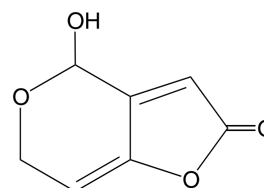


**Figure 1.** Chemical structure of patulin.

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tulin frequently when they invade apples, pears, peaches and berries. Because it is particularly produced by the apple-rotting fungus *P. expansum*, it is a toxicological concern in apple products such as apple juice[1-5]. According to studies[5] conducted over the past twenty years, only apple products, especially apple juice, were found to be easily contaminated by patulin naturally, therefore, the patulin level has been regarded as a quality indicator for fruit used in the processing of apple juice[1-5]. Patulin is quite stable in acidic media at temperature of 125˚C with pH levels ranging between 3.5 and 5.5. We are unable to get the toxin-free products by means of industrial processing for apple products through vacuum distillation concentration and high temperature short time (HTST) pasteurization treatment at 90˚C 10 second, which produce 18.4% and 18.8% respectively of patulin in the apple juice. Patulin content decrease with little extent once apple juice being packed and stored. Patulin also disappeared in three weeks with addition of ascorbic acid to patulin-contaminated apple juice, but this could be attributed to some unidentified toxins resulting from this reaction. However, fermented apple juice such as apple cider was less contaminated[5] due to degradation of patulin by yeast (*Saccharomyces* spp.) into primary non-volatile, water soluble substances.

Patulin has a broad spectrum of toxicity to bacteria, fungi, plants, protozoa and animals, and also has mutagenicity to *Saccharomyces cerevisiae* and tetratogenicity to chicken embryos. A localized tumor was found in rats at the site of injection. For human beings, patulin has caused nausea, vomiting, and gastrointestinal disturbances[1-5]. In the United States, the Office of Environmental Health Hazard Assessment has listed patulin as a potential carcinogen[6].

The British Ministry of Agriculture Fisheries and Food (MAFF, England) has monitored patulin levels in apple juice since 1980, and only trace amounts have been found. When contamination over 50 μg/L was first confirmed in 1992, the Food Advisory Committee (FAC) and Department of Health's Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment both recommended that patulin in apple juice should be reduced to minimum level and suggested a maximum permitted level at 50 μg/L[7], which was reconfirmed in 1995. In 1992, 26% fresh apple juice was reported to exceed the permitted level, with significant drop to 9%, 4%, 6% and 2% during the period of 1993 to 1996. The reduction of patulin in apple juice with at least 60% is resulted from the self regulatory program-Voluntary Code of Practice by apple processors under supervision of government authority[8-14]. The results were reported to the European Union as a reference for setting standards. Other countries like Switzerland, Sweden, Belgium and Norway that are concerned about this issue, have set a maximum permitted concentration (MPC) at 50 μg/L[4] as recommended by the World Health Organization (WHO)[2].

Following the above regulations, the FDA has launched a program in the US to monitor domestic and imported apple juice. However, apple juice of a certain brand manufactured in Washington State was found in 1997 to exceed the maximum recommended concentration for patulin established by the WHO[15]. Being frequently consumed by infants and children, contaminated apple juice might jeopardize human health. Therefore, the Codex Committee on Food Additives and Contaminants decided to offer recommendations to the draft establishing MPC[16].

In 1987, 60 imported samples obtained in Taiwan were reported free of patulin[17], possibly due to the poor recovery (78%) and not enough quantification limit (50 μg/L). Recently, the modified methods with detection limits of 2[4], 5[3,18], 10[19-21] and 20[22,23] μg/L were developed. Ethyl acetate was commonly used as solvent to extract[1-4,18-19,21-25]. Also the diphasic dialysis extraction method was used[1]. The florisil cartridge[20], silica gel cartridge[4], or sodium carbonate solution partition method[3,18,22-24] were adopted for clear up purpose. For identification and quantitation, thin layer chromatography (TLC) with various solvent systems, followed by derivatization with 0.5% 3-methyl-2-benzothiazolinone-hydrazone (MBTH)

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were coupled with fluorescence detection by LC scanner or ultraviolet examination[1,2,17]. Though gas chromatography (GC) was also adopted[19,25], reverse phase liquid chromatography/UV is considered more convenient and sensitive[3-4,17-18,20,22-24]. The method developed by Brause *et al*.[22], was used as a reference to the present study in terms of excellency in recovery, accuracy and precision, which was guaranteed by evaluation of collaborative study. Owing to the worldwide concern for the issue, this study aimed at improving the analysis method. Meanwhile, a survey of patulin in retail apple juice or apple juice containing mixed juice was conducted to create a data bank of food sanitation and provide information for health administration and trade negotiation.

## MATERIALS AND METHODS

### I. *Sources of Samples*

From supermarkets, local stores and salesrooms in schools and organizations in the Taipei area, 105 samples of retail pure apple juice and apple juice containing mixed juice were purchased. The samples included 100% pure juice, baby fruit juice, light juice and fruit juice drink packed with easy open cans, glass or plastic bottles, retort porch, tetra brik or tetra rex. All of the samples contained apple juice: 100% pure juice, 10~15% in light juice, and 30% in fruit juice drink.

### II. *Reagents*

#### (I) *Patulin Standard Purchased from Sigma (USA)*

#### (II) *Solvents and Chemicals*

LC grade acetonitrile and ethyl acetate purchased from Lab-Scan (Ireland). Analytical grade anhydrous sodium sulfate purchased form Merck (Germany), 5-Hydro-xymethyl-2-furaldehyde (5-HMF) purchased from Sigma (USA) and acetic acid purchased from Nacicalai Tesque (Japan). Reagent grade sodium carbonate purchased from Kanto Chemical (Japan).

#### (III) *Preparation of Patulin Standard Stock Solution*

Five mg of pure crystal patulin was accurately weighted into a 25 mL volumetric flask and dissolved in ethyl acetate to volume as a stock solution. The flask was tightly wrapped with aluminum foil and kept at 2-5°C. This stock solution remained stable for a month.

#### (IV) *Calibration of Patulin Standard Stock Concentration*

Two hundred and fifty μL of patulin standard stock solution was transferred into a 5 mL volumetric flask, evaporated to dryness under a stream of nitrogen, and the residue was then homogenized and diluted to volume with absolute alcohol. The UV spectrum over 250 to 350 nm was measured, while absorbance of absolute alcohol through 1 cm cell pathlength was taken as a reference. Based on the maximum absorption (A) at, ca 276 nm, concentration was calculated as follows:

$$\mu g\ Patulin/mL = (A \times MW \times 1000 \times CF)/\varepsilon$$

MW: molecular weight of patulin, which equals to 154

CF: correction factor (reasonable between 0.95 to 1.05)

ε: 14,600

#### (V) *Preparation of Patulin Standard Working Solutions*

One hundred μL of patulin standard stock solution was accurately transferred into a 10 mL volumetric flask and evaporated to dryness under stream of nitrogen at room temperature. It was then immediately diluted to volume with acetic acid solution (pH 4) to achieve a 2000 μg/L standard working solution, then diluted to a series levels of 100, 200, 500 and 1000 μg/L with addition of acetic acid solution. The flasks were tightly wrapped with aluminum foil and kept at 2~5°C. The working solutions should be prepared every week.

#### (VI) *Preparation of Mobile Phase*

Acetonitrile was mix with deionized water (10:90, v/v) and filtered through a 0.45 μm nylon filtration membrane (Xpertex®, USA). Filtrate

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was degassed in ultrasonic bath for 30 minutes.

(VII) *Preparation of 1.5 % Sodium Carbonate*

One and a half g sodium carbonate was dissolved in 100 mL deionized water.

(VIII) *Preparation of Acetic Acid Solution*

Deionized water was adjusted to pH 4 with acetic acid.

(IX) *Preparation of 5-HMF Solution*

Five mg 5-HMF was dissolved in 25 mL ethyl acetate.

III. *Apparatus*

(I) Spectrophotometer: UV-160A UV-Visible Recording Spectrophotometer obtained from Shimadzu (Japan).

(II) Extraction tube: Falcon, 50 and 15 mL polypropylene conical tube with cap obtained from Becton Dickinson (USA).

(III) Vortex mixer: Vortex Genie-2, obtained from Scientific Industries (USA).

(IV) Rotavapor: Buchi Rotavapor R-124, obtained from Buchi (Sweden).

(V) Waterbath: Buchi Waterbath B-480, obtained from Buchi (Sweden).

(VI) Circulating aspirator: Circulating Aspirator WJ-20, obtained from Sibata (Japan).

(VII) Analytical evaporator: The Meyer N-EVAP Analytical Evaporator, obtained from Organomation Associates (USA).

(VIII) High performance liquid chromatograph: Hitachi L-7100 equipped with a L-7100 liquid pump system, a L-7200 autosampler, and a L-4250 UV-VIS detector, all obtained from Hitachi (Japan).

(IX) Integrator: SISC chromatography data management system.

IV. *Methods*

(I) *Preparation of Test Solution*

A 5 mL apple juice sample and 10 mL of ethyl acetate were added into a 50 mL extraction tube and strongly vortex-mixed for 1 min. After separation of layers was completed, the upper layer was pipeted to another tube, and the original tube was re-extracted with 10 mL ethyl acetate. The combined organic solvent was again extracted with 2 mL of sodium carbonate (1.5%) using the above procedures, followed by separation of the upper layer and the lower layer. After extracting the lower layer using 5 mL of ethyl acetate, the lower sodium carbonate layer was discarded. The ethyl acetate extracts were combined and dehydrated with 1 g anhydrous sodium sulfate. The sodium sulfate solids was filtered out and rinsed with 5 mL of ethyl acetate. The ethyl acetate extracts were then collected in a 100 mL evaporation bottle, placed in 40˚C water bath and evaporated to 1~2 mL using a vacuum rotary evaporator. Subsequently, the concentrate was transferred to a 15 mL extraction tube and the bottle was rinsed twice with 2 mL of ethyl acetate. After collecting the ethyl acetate extract in the tube, the extract was evaporated to dryness in a water bath at 40˚C under a gentle stream of nitrogen. The residue was immediately dissolved in 500 μL of acetic acid solution (pH 4.0) to obtain the test solution. The test solutions were kept in deep-freezer (-20˚C) until making the chromatographic measurements.

(II) *High Performance Liquid Chromatography (HPLC) Analysis*

1. HPLC conditions

(1) Column: Inertsil ODS-2, 5 μm, 4.6 × 150 mm (GL Sciences Inc.).
(2) Mobile phase: 10 % acetonitrile solution.
(3) Flow rate: 0.5 mL/min.
(4) Detection wavelength: 276 nm.

2. Evaluation of HPLC performance

(1) Preparation of 5-HMF-patulin solution: 100 μL patulin standard stock solution and 100 μL 5-HMF were transferred into a 10 mL volumetric flask, evaporated to dryness under stream of nitrogen at room temperature, then dissolved and dilut-

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ed to volume with acetic acid solution.

(2) As 50 μL of 5-HMF-patulin solution was injected into the column, 5-HMF and patulin should elute as two separate peaks with baseline separation of at least 2 mins distance. If 5-HMF and patulin are not completely separated, different column or mobile phase should be selected to make sure the analysis is performed.

3. Standard curve graph

A series standard working solution between 100 and 500 μg/L were diluted with acetic acid to achieve 1, 2, 5, 10, 20, and 50 μg/L. The diluted standard working solutions and all original standard working solutions were obtained to prepare a series of solution between 1 μg/L and 2000 μg/L. Fifty μL of the standards were injected separately into the HPLC device. A standard curve graph was obtained by calculating linear regression of the integrated peak heights versus concentrations.

4. Determination of concentration

Fifty μL of the test solution and 500 μg/L of the standard solution were injected separately into the HPLC device and patulin was identified by comparing the retention time of the peak in the sample with that of the standard. Patulin concentration in apple juice is calculated as follows:

patulin concentration (μg/L) = $C_T$ / 10

$C_T$: patulin concentration derived from calibration graph (μg/L), "1/10"stands for the volume ratio of sample to test solution.

V. *Recovery Test*

A recovery test was carried out in triplicate for each concentration and performed by spiking 20, 50, 100 and 200 μg/L patulin in apple juice. A 5 mL solution of apple juice and a 5 mL solution of deionized water were prepared as blanks. These were extracted and analyzed using the procedure mentioned above. The blank solution should be injected prior to the test solution. The recovery rates can be calculated by comparing the quantitative results with the known concentrations.

VI. *Detection Limit*

Fifty μL of each known standard solution was separately injected. Determine the lowest concentration over the range that peak height versus concentration remains good linearity under linear regression. Calculate the weight (ng) of patulin under a certain concentration with the injection volume to acquire the detection limit under these conditions.

VII. *Quantification Limit*

Apple juice samples were spiked with appropriate levels of patulin standard followed by the above analysis procedures and determined by HPLC. The quantification limit was calculated on the basis of a signal-to-noise ratio of 10 (S/N=10).

## RESULTS AND DISCUSSION

I. *Study of HPLC Conditions and Evaluation of HPLC Performance*

Figure 2 shows the absorbance of patulin in absolute alcohol detected at wavelengths between 200 and 400 nm. The UV detector was set at 276 nm for maximum absorption. For the mobile phase, pure water used in the method developed by Möller and Josefsson (1980)[3], and tetrahydrofuran (THF) solution (0.8~1%) used by Prieta *et al.* (1992)[1], Rovira (1993)[4], Forbito and Babsky (1985)[24], both separate patulin and 5-HMF, while AOAC method[22,23] suggested acetonitrile or THF solution. Whereas, acetonitrile (1%) was suggested to use by Gökmen and Acar (1996)[18] due to the inability of pure water to resolve pat-

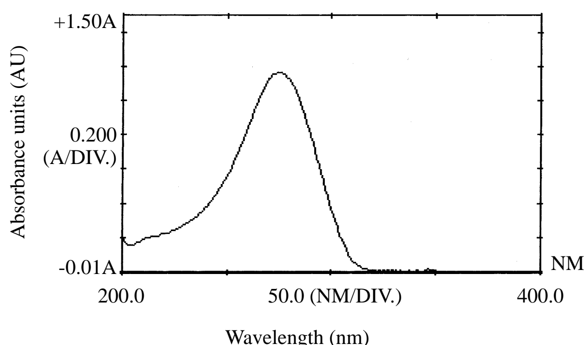


**Figure 2.** UV- absorption spectrum of patulin in absolute alcohol.

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ulin from 5-HMF. Acetonitrile is better than THF in the application of reversed phase HPLC. Consequently, this study evaluated the effectiveness of acetonitrile as a mobile phase. A $C_{18}$ column eluted with acetonitrile (5~10%) at the rate of 1.0 mL/min was determined by UV detector at 276 nm. The results showed that 5~10% acetonitrile solutions are capable of completely separating peaks of 5-HMF and patulin. The retention times decreased from 12.6 and 16.1 min to 6.3 and 8.5 min, respectively, and, as the acetonitrile concentration increased, the interval of the two peaks decreased from 3.5 to 2.2 min, respectively. Patulin resolution is commonly interfered by co-extractive 5-HMF that is formed in thermal treatment of apple juice [18,22]. Resolution of patulin from 5-HMF is the critical control point in HPLC method. In this study, the acetonitrile solution (5~10%) was effective, whereas higher concentration (3~10%) was considered superior in time of analysis, column cleaning and delay of peaks[22]. Another condition of 10% acetonitrile eluted at 0.5 mL/min flow rate was again tested; the retention times for two peaks was about 7.3 and 9.4 min, with an interval greater than 2 min. In this study, naturally coexisted phenol compounds, except patulin, were found not to interfere by comparing the chromatograms of blank solution to standard solution (Figure 3), under the condition of 10% acetonitrile as a mobile phase eluted at 0.5 mL/min.

II. *Preparation and Determination of Test Solution*

To prevent emulsion in unfiltered, directly-produced apple juice, pretreatment of pectinase at 58°C for 1 h prior to the extraction is needed[23, 27]. Since samples were mostly homogeneous in this study, 5 mL of each sample was adequate and thus less solvent was needed. In this study, disposable polypropylene extraction tube was used to replace glass tube that was used by the AOAC supplement[23] due to possible residue of toxin. For best recovery, a sample-to-solvent volume ratio of 1:2, and double extraction with ethyl acetate were suggested by Gökmen and Acar

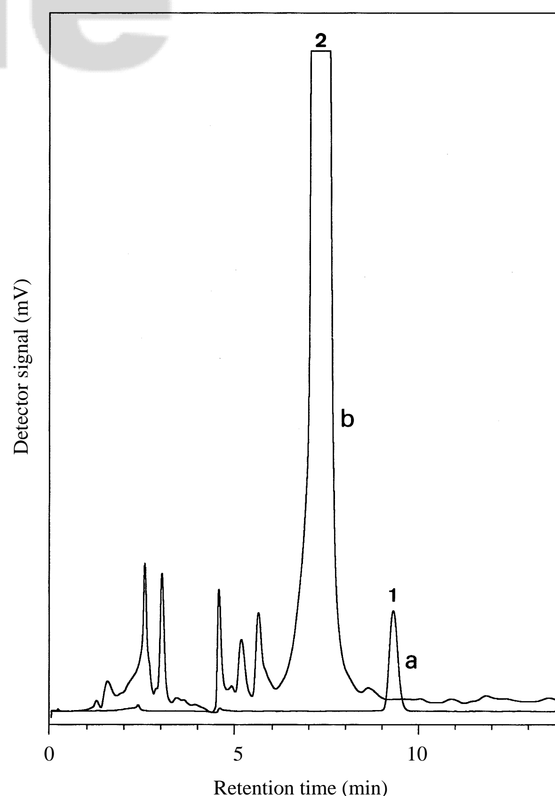


**Figure 3.** Chromatograms of patulin standard (at the 500 µg/L level, a) and an apple juice devoid of patulin (below the quantification limit, b). Peaks: 1=patulin; 2=5-hydroxymethyl-2-furaldehyde.
Chromatographic conditions: column, Inersil(15 cm×4.6 mm i.d., 5µm); mobile phase, 10% acetonitrile; flow rate, 0.5 mL/min; detection wavelength, 276 nm; temperature, ambient; injection volume, 50 µL.

(1996)[18]. To clean up co-extractives, it was easier, faster and more economic way of extraction with 1.5% sodium carbonate solution [3,18,20, 22-24] than adsorption chromatography through columns or prepacked clean-up cartridges[2,4,20]. Here, the ethyl acetate extract was cleaned up by extracting with 1.5% sodium carbonate solution. It was found to be very effective for removing most of the co-extractives from apple juice. Therefore, the use of adsorption chromatography through columns or prepacked clean-up cartridges does not seem to be necessary. Nitrogen gas is critical to recovery, Rovira *et al.* (1993) proved that, recovery decreased to 63% while prolonged evaporation took place under nitrogen of high flow

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rates, while recovery reached 99% as low rate gas was immediately turned off when reaching dryness[4]. Similar results on the importance of controlling the flow rate and time were shown in this study. Furthermore, dehydration of ethyl acetate during preparation of the test solution is also considered critical[23]. Although identical extraction and clean-up were adopted by Möller and Josefsson (1980)[3] and its modified AOAC method[23], the former method has a poor recovery (70%~75%) due to lack of consideration of dehydration. Rovira *et al.* showed that recovery would decrease to 77% if anhydrous sodium carbonate was absent, yet, the longer the dryness, the lower the recovery[4]. The method of Gökmen and Acar[18] was similar to that of Möller and Josefsson, but recovery was enhanced to 94% when 2.5 g anhydrous sodium carbonate was added to the dehydrate. For excellent recovery in this study, 1 g of anhydrous sodium carbonate was used to immediately dehydrate ethyl acetate, because patulin is likely to be destroyed in water containing ethyl acetate[23].

III. *Calibration Graph and Recovery Test*

The standard curve (Y=0.013333X+0.014789) with a linear regression coefficient ($R^2$) of 0.999995 (Figure 4), was obtained by the procedures described above. Linearity was observed over concentration ranging between 1 and 2000 μg/L. Table 1 gives the average recoveries of triplicate tests of 20, 50, 100 and 200 μg/L patulin spiked into apple juice. The average values ranged from 93.1% to 96.6%, all with coefficient of variation less than 3.0%. Results for ascorbic acid containing juice are likely to be different, due to potential interaction between ascorbic acid and patulin[23], therefore, vitamin C containing juice should be avoided in recovery test.

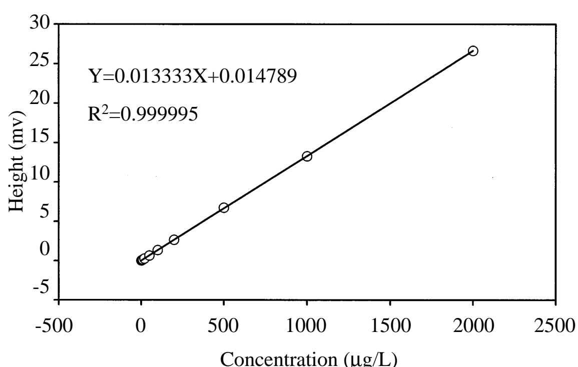


**Figure 4.** Standard curve of patulin.

IV. *Detection Limit and Quantification Limit*

Concentration of patulin was determined by HPLC following the procedures described above. Table 4 shows that the lowest concentration where the linear-regressed peak height and concentration remains good linear relation ($R^2$=0.999995) is 1 μg/L. The detection limit was estimated 0.05 ng in current analysis using injection volume of 50 μL.

As shown in Figure 5, the quantification limit of patulin in spiked apple juice was 15 μg/L, lower than 20 μg/L, as suggested by AOAC supplement[23], probably due to the consideration of generality and interchangeability demanded for AOAC methods. In England, MAFF has employed a limit of 3 μg/L[8-14], which was replaced by a stricter limit of 15 μg/L[28] after 1998, since by then the list of disqualified manufacturers was open to the public. Given the quantification limit 15 μg/L in this test method, we may follow the MPC level recommended by WHO. This method could be adopted by food industries and government authority as well to monitor patulin content in apple juice.

V. *Confirmation of Patulin*

In 1987, thermospray liquid chromatography coupled to mass spectrometry was attempted by Rajakylä *et al.* for the confirmation of patulin, but the mass chromatogram constructed was found

**Table 1.** Recovery rates of patulin from spiked apple juice

| Spike level (μg/L) | Recovery rate[a] (%) |
|---|---|
| 20 | 96.6 (2.6)[b] |
| 50 | 93.1 (1.8) |
| 100 | 94.5 (1.9) |
| 200 | 96.4 (1.1) |

[a] Average of triplicate.
[b] The value in parenthesis represents coefficient of variation (%).

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incapable of confirming due to low m/z (only 155 for its protonated molecular ion) and an inferior detection limit confined to the background matrix[29]. While in gas chromatography/MS, derivatization of patulin is necessary in order to avoid the decomposition in the processes of gasification and chromatography, in addition to meeting the MPC standard[25]. In 1991, Tarter and Scott[19] used heptafluorobutyrate (HFB), while in 1995, Sheu and Shyu[25], used anhydrous acetic acid to derivatize patulin, both followed by GC. Although the above analysis showed good quantitation results, only the derivatized patulin standard was discussed, and not the confirmation of patulin in apple juice samples. Because of different UV spectra and derived optical parameters, the diode array detector (DAD) is capable of distinguishing patulin from co-extractives. The UV spectrum of patulin contains only one unique band derived from the overlapped carbonyl group and the diene chromophores, while that of low-molecular-mass phenolics presents at least two bands within the same wavelength range. The definition of the convexity interval of the band is the distance (nm) between the inflection points at before and after the maximum absorption. The value of convexity interval of patulin is near that of a carbonylic band in a phenolic compound when this band overlaps one of the bands of the aromatic ring. In addition to this unique feature, other derived parameters such as absorbance ratios at different wavelengths and the positions of the maxima in the second-order derivative spectrum are also useful for confirmation of patulin[21].

Figure 5 shows that the nearest peak around the peak of patulin is 5-HMF, a substance that does not exist naturally but artificially in the food industry. The structure of 5-HMF is similar to patulin in furan rings but can be distinguished by characteristics such as containing two bands of the UV spectrum and unique optic parameters[21], shown in Table 2. From 1989 to 1990, Burda conducted a survey to monitor patulin levels in apple juice, pear juice and mixed fruit juice in New South Wales[30], while in 1997, Sydenham *et al.* investigated the effect of storage and pretreatment of apples on patulin levels[27]. Both adopted the diode array detector to confirm presence of patulin.

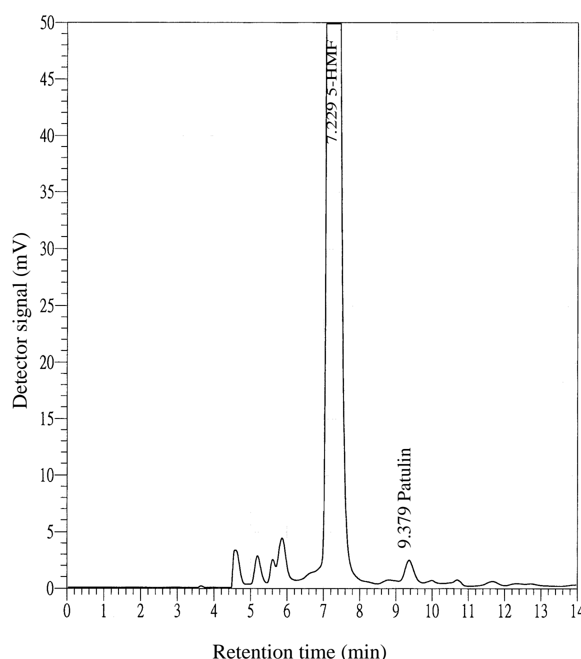


**Figure 5.** The chromatogram for quantification limit of patulin in spiked apple juice (at the level of 15 μg/L). Chromatographic conditions same as in Figure 3.

**Table 2.** Values of the chromatographic parameters obtained with photodiode-array detection

| Parameter | Patulin | | 5-hydroxymethyl-2-furaldehyde | |
|---|---|---|---|---|
| Retention time (min) | | | | |
| Absorbance maxima (nm) | 275.4 | | 231.5 | 282.2 |
| Convexity intervals (nm) | 36 | | — | 30.6 |
| 260/320 nm absorbance ratio | 13.6 | | 15.7 | |
| 270/300 nm absorbance ratio | 3.0 | | 1.46 | |
| Absorbance maxima (nm)in the second-derivative spectrum | 239.3 | 309.5 | 249.7 | 309.5 |

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VI. *Determination of Patulin in Retail Apple Juice and Apple Juice Containing Mixed Juice*

Table 3 shows the results of the analysis of 105 retail apple juice purchased in the Taipei area. All baby fruit juice (9 samples), fruit juice drink (11 samples), apple juice containing mixed juice (14 samples), and 83.1 % pure apple juice samples were reported free of patulin. Only 16.9% pure apple juice samples, which amounts to 11.4% of all samples, were contaminated, with concentration ranging from 15.4 to 39.9 μg/L. This shows that the incidence rate and content of patulin in apple juice sold in the Taipei area were of no safety concern according to the level of 50 μg/L suggested by the WHO and levels set by many European countries[2,4]. This survey data would be an excellent reference for executive management and a good marker for setting the MPC standard of patulin in apple juice by the government food safety administration.

In this study, no significant differences were found of patulin concentrations between samples containing ascorbic acid and those without. Thus, comparison maybe meaningless due to the low incidence rate and content. A survey using dialysis membrane extraction was carried out by Prieta *et al.* in 1992[31] to monitor patulin in 100 retail apple juice in Madrid, Spain, and 82% were found contaminated with the concentration mostly less than 10 μg/L, but none in baby fruit juice[31]. In 1992, Burda reported that 50% of 16 samples from New South Wales, Australia were contaminated, with its maximum reaching 646 μg/L, which revealed that the incidence rate and content were both high in this area[29]. In 1998, Gökmen and Acar[32] found 215 apple juice samples sold in Turkey from Jan. 1994 to Sep. 1996 were all contaminated, with the concentration ranging from 7 to 376 μg/L, in which 46% samples exceeded 50 μg/L. It was also mentioned in the above report that 11% of samples obtained in Ankara from 1993 to 1994 exceeded the standard. They also compared the outcome to that of other countries: 21% of samples were of trace amounts in Germany in 1982; 70% for England in 1980, with concentration ranging from 1 to 38 μg/L; 15% for New Zealand in 1981, with concentration ranging from 106 to 216 μg/L[32]. These studies showed that apple juice sold in Turkey was frequently contaminated. In a study conducted by MAFF, England, the disqualification rate was 1.7%[28] in 1998. According to the reports of MAFF, juice recombined from concentrated form were patulin undetected since 1994, and patulin detected over MPC level ones were all directly produced[10-14,28]. Juice produced from concentrate, the major retailed products processed immediately after harvest of raw apples, were diluted and packed while demanded, however, directly- produced juice was freshly made from apples, with its consumption gradually increasing in recent years. It is possible that the patulin concentration is higher in directly-produced juice because of the time lag between

**Table 3.** A survey of the patulin content in apple juice and apple juice containing mixed juice purchased from markets

| Sample type | Total No. of samples | Number of samples in each category of patulin contamination (μg/L, not corrected for recovery) | | | |
|---|---|---|---|---|---|
| | | < 15 | 15 ~ 24 | 24 ~ 49 | ≥ 50 |
| Baby juice[a] | 9 | 9(100)[b] | 0(0) | 0(0) | 0(0) |
| Juice drink[a] | 11 | 11(100) | 0(0) | 0(0) | 0(0) |
| Pure apple juice | 71 | 59(83.1) | 8(11.3) | 4(5.6) | 0(0) |
| Mixed juice[a] | 14 | 14(100) | 0(0) | 0(0) | 0(0) |
| Total | 105 | 93(88.5) | 8(7.6) | 4(3.8) | 0(0) |

[a] Apple juice is contained in all tested samples.
[b] The value in the parenthesis is the percentage(%) of samples in each category of patulin contamination.
Limit of quantification: 15 μg/L.

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harvest and processing in accordance with the demand all year round. In England, contaminated, directly-produced apple juice is mainly from small factories or farms, but it has been improved since MAFF has provided the manufacturers with guidelines since 1996[10-14,28]. Though updated reports of other countries are unavailable the condition is likely to be improved, yet patulin in directly produced apple juice is a problem worth heeded.

Theoretically, patulin is regarded as a quality indicator; therefore, spoiled apples can not be used to make apple juice. Actually, it is hard to entirely avoid spoiled apples during transportation, storage and processing, which is why processors should improve the above conditions as well as adopt an effective way to remove patulin. According to studies, patulin in apple juice could be excluded physically by removing the brown decayed areas on apples, which are likely to be contaminated. This approach enables the patulin to be reduced by 90%, only it's time-consuming. Filtration through charcoal essentially removes all patulin from apple juice[5]. On the other hand, certain substances exist in food may restrain patulin, for example, high content of sulfhydryl group ingredients such as grain, meat and cheese are not easily contaminated. On the contrary, apple juice is easily contaminated due to the small amount of these substances. Therefore, addition of 25 to 50 ppm sulfur dioxide ($SO_2$) was found to be useful in reducing patulin[5]. It is worth remembering that England has made significant improvements by devoting much energy to research and its application in the public sector with the cooperation of the industries.

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# 蘋果汁中黴菌毒素－棒麴毒素之偵測

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## 摘　要

棒麴毒素是一種黴菌毒素，主要由*Penicillium expansum*產生，特別容易污染蘋果。其致毒範圍廣大，對動物或真菌具致突變性、致畸性、產生局部腫瘤等，對人體會引起噁心、嘔吐或腸胃異常等病症。美國環境健康危害評估中心已將棒麴毒素列入引發癌症的化學物質之一。歐洲多國訂定蘋果汁中棒麴毒素之最大允許量為50 μg/L，世界衛生組織也如是建議。本研究使用乙酸乙酯萃取棒麴毒素，再以碳酸鈉溶液萃取法淨化，最後利用$C_{18}$層析管柱之逆相高效液相層析，於紫外光276 nm檢測。棒麴毒素於1～2000 μg/L濃度範圍內之標準曲線（Y = 0.013333X + 0.014789）線性迴歸係數為0.999995。蘋果汁添加20、50、100及200 μg/L棒麴毒素之平均回收率為93.1%～96.6%，變異係數均小於3.0%，儀器偵測極限達0.05 ng，檢體檢出限量為15 μg/L。針對105件市售蘋果汁及含蘋果汁之綜合果汁樣品進行檢驗，其中93件（88.5%）未檢出棒麴毒素，另有12件純蘋果汁（11.4%）雖有檢出，但毒素含量僅15.4～39.9 μg/L，均少於世界衛生組織建議的50 μg/L。

關鍵詞：棒麴毒素，蘋果汁，高效液相層析。