

Evaluation of Patulin Contamination of Apple Juice, Using High Performance Liquid Chromatography

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Abstract: Patulin is a mycotoxin produced by molds *Penicillium patulum*, *Penicillium expansum*, *Penicillium urticae*, *Penicillium claviforme*, *Aspergillus clavatus* and *Byssoschlamys* spp., with toxic effects on human and animal body. Patulin is stable, especially in apples processed products: apples juice, apples concentrate juice, apples nectar, fruits nectar (which also has as ingredient the apples juice), apples piuree, apples in syrup, etc. In the paper are presented results of performed researches for patulin determination from apple juice, through high performance liquid chromatography. It was achieved an inhouse study for validation of method for patulin determination from apples juice, by high performance liquid chromatography. In concentration range 6.25 – 400 µg/L, the average recovery is 90.50%. Limit of detection (LOD) is 2.91 µg/L, and limit of quantification (LOQ) is 9.71 µg/L. The contamination degree of apple juice from commerce was evaluated using high performance liquid chromatography. Patulin concentrations of apple juice samples analyzed were under 50 µg/L, the maximum allowed limit by the legislation in force.

Keywords: patulin, apples juice, high performance liquid chromatography

1. Introduction

Patulin is a mycotoxin produced by molds *Penicillium patulum*, *Penicillium expansum*, *Penicillium urticae*, *Penicillium claviforme*, *Aspergillus clavatus* and *Byssoschlamys* spp., with toxic effects on human and animal body. Patulin was identified, especially, in spontaneous damaged fruits and vegetables by micotoxigen fungus: apples, apricots, bananas, pineapples, grapes, black currants, raspberry, wild strawberries, cucumbers, tomatoes, green peppers, carrots. Patulin is stable, especially in processed products from apples: apples juice, apples concentrate juice, apples nectar, fruits nectar (which has as ingredient the apples juice also), apples piuree, apples in syrup, etc. [1].

From chemical point of view, patulin is furo-piranone, with molecular mass 154, stable in acid medium, but unstable in alkaline medium.

It is presented as uncolored crystals form, rhombics or prismatic, with melting point 110.5°C, with absorption maximum at 276 nm, in alcoholic solution.

In the Institute of Food Bioresources it was developed a method for patulin determination from apples juice by high performance liquid chromatography.

2. Experimental

For determination of patulin content were analyzed samples of clear apples juice, from commerce.

Determination of patulin from apples juice was performed by high performance liquid chromatography. Steps of method for patulin determination: preparation of test sample, patulin extraction, extract purification, extract

evaporation to dryness, residue re-dissolution, HPLC detection of patulin.

Patulin extraction was achieved in acetonitrile, and purification of the obtained extract, using cleanup (C.U.) Patulin columns (MycoSep®228).

The obtained solution is evaporated to dryness under nitrogen and redissolved. Patulin was separated on chromatographic column C18, 150 x 4 mm, 5 µm (high performance liquid chromatograph Thermo Finnigan), eluted in mobile phase (acetonitrile/water) and detected on 276 nm, using an UV-VIS „DIODE ARRAY” detector [2].

In figure 1 are presented the clean-up (C.U.) Patulin columns used for purification, and in figure 2 is presented the purified patulin extract.

HPLC analysis conditions:

- Chromatographic column C18, 150 x 4 mm, 5 µm;
- Mobile phase: acetonitrile:water = 5:95 (v/v);
- Mobile phase volume: 1 mL/minute;
- UV-VIS „DIODE ARRAY” detector, $\lambda = 276$ nm;
- Injection volume: 25 µL;
- Temperature: 25°C.



Figure 1. C.U. Patulin columns

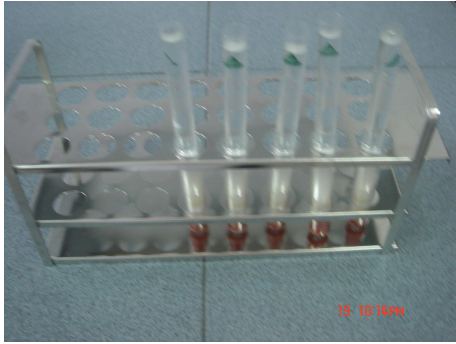


Figure 2. Purified patulin extract

3. Results and Discussion

For patulin determination from apples juice by high performance liquid chromatography, it was realized a calibration curve with 7 patulin standard levels (with each three repetitions), in concentrations range 0.00625 – 0.400 µg/mL. Calibration curve is presented in figure 3.

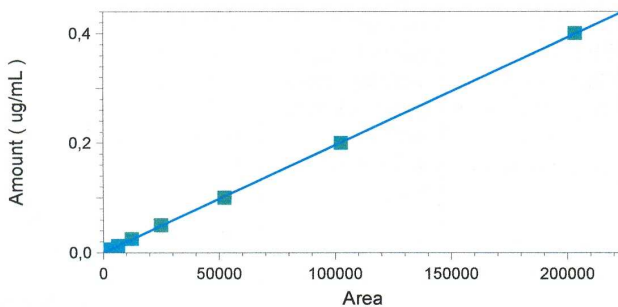


Figure 3. Calibration curve

All tests for validation of method were achieved with apples juice samples (without patulin), which, were "artificially" contaminated using standard solution with 1 µg/mL patulin concentration.

For *linearity* domain establishment were obtained apples juice samples, with the following patulin concentrations: 10, 15, 25, 50, 60, 80, 100, 125, 200 and 400 µg/L.

These samples were analyzed for determination of patulin concentration.

By plotting the concentration of patulin in the sample injected according to peak area, obtain a linear function (equation $y = 0,0020x$) and correlation coefficient (r^2) 0.9997. According to the results, the linearity of the method of patulin determination in apple juice by high performance chromatography is between 5 and 218 ppb

For *recovery* establishment were obtained apples juice samples, with the following patulin concentrations: 6.25, 15, 25, 50, 125, 200, 315 and 400 µg/L.

These samples were analyzed for patulin concentration determination.

In figure 4 the patulin peak is presented, in case of apple juice artificial contaminated with patulin.

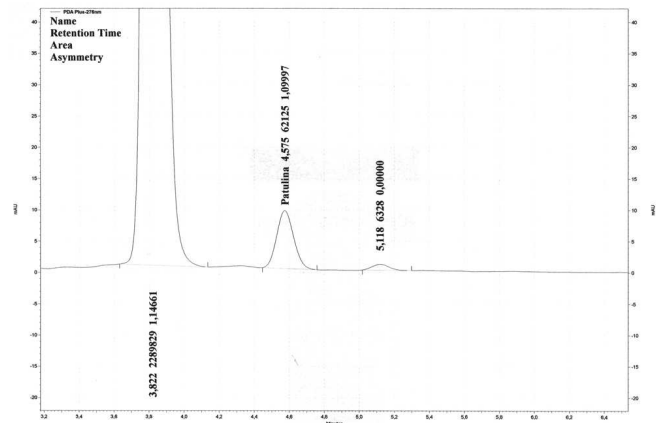


Figure 4. Chromatogram with patulin peak

Between concentrations 6.25 µg/L and 400 µg/L, mean *recovery* had the following values:

- 96.64%, for patulin concentrations < 25 µg/L;
- 90.31%, in patulin concentrations range 25 - 50 µg/L;
- 88.40%, for patulin concentrations > 50 µg/L.

In concentration range 6.25 – 400 µg/L, mean recovery is 90.50%.

For *repeatability* estimation, there were analyzed apples juice samples with patulin concentration 25, 50 and 100 µg/L and were calculated:

- relative standard deviation value RSD(r);
- expanded uncertainty;
- repeatability limit.

In case of 10 parallel samples of apples juice with 25 µg/L patulin concentration relative standard deviation RSD(r), for determined concentration, is 3.18%, and expanded uncertainty is 4.20 µg/L. Confidence interval is $24.99 \mu\text{g/L} \pm 4.20 \mu\text{g/L}$. Repeatability limit is: 2.22 µg/L.

In case of 10 parallel samples of apples juice with 50 µg/L patulin concentration relative standard deviation RSD(r), for determined concentration, is 1.84 %, and expanded uncertainty is 4.53 µg/L. Confidence interval is $49.20 \mu\text{g/L} \pm 4.53 \mu\text{g/L}$. Repeatability limit is: 2.53 µg/L.

In case of 8 parallel samples of apples juice with 100 µg/L patulin concentration relative standard deviation RSD(r), for determined concentration is 1.31 %, and expanded uncertainty is 5.36 µg/L. Confidence interval is $100.36 \mu\text{g/L} \pm 5.36 \mu\text{g/L}$. Repeatability limit is: 3.67 µg/L.

Intralaboratory *reproducibility* estimation, was performed by three analysts who have examined, in the same laboratory, with the same equipment, 8 apples juice samples (analyst A – 3 samples, analyst B – 2 samples, analyst C – 3 samples) with patulin concentration 50 µg/L.

There were calculated:

- relative standard deviation value RSD(R);
- expanded uncertainty;
- reproducibility limit.

In these case relative standard deviation RSD(R), for determined concentration, is 4.75 % and expanded uncertainty is 4.53 µg/L.

Confidence interval is $49.77 \mu\text{g/L} \pm 4.53 \mu\text{g/L}$. Reproducibility limit is: 6.62 µg/L.

Using "signal-to-noise ratio", recorded by high performance liquid chromatograph, in case of apple juice samples with patulin concentration between 6.25 and 100 µg/L, were calculated limit of detection and limit of quantification.

Limit of detection (LOD) is defined as being the concentration of which „signal-to-noise ratio” is bigger than 3 ($S/N > 3$). Limit of detection is the value which fixes inferior limit of work domain.

Limit of quantification (LOQ) is defined as being the lowest concentration of analyst, which can be determined with acceptable precision, under conditions of analysis method, at a „signal-to-noise ratio” bigger than 10 ($S/N > 10$).

In case of proposed method for patulin determination by high performance liquid chromatography, limit of detection (LOD) is 2.91 µg/L, and limit of quantification (LOQ) is 9.71 µg/L.

Proposed method for patulin determination is *selective*. Peak corresponding to patulin is separated by baseline and by other compounds peaks. Resolution (DAB) is min. 2.7.

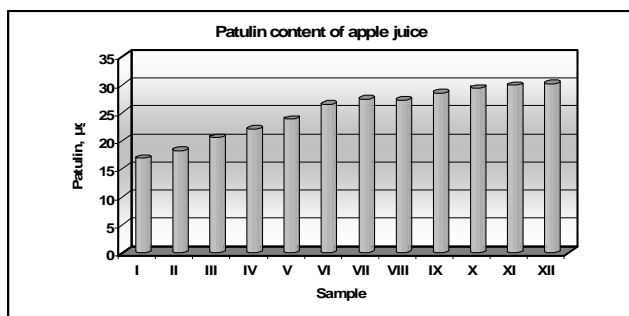


Figure 5. Patulin content of apple juice

Using this method there were analysed clear apple juice samples, from commerce, in order to determine patulin content. Over 80 % of the analyzed samples had not patulin, and within rest of them, patulin concentration was under 50 µg/L, maximum allowed limit by the legislation

in force [3]. In figure 5 it is presented patulin content of apple juice samples, from different producers, in which was detected patulin (16.85 – 30.15 µg/L).

4. Conclusions

In the Institute of Food Bioresources it was developed a method for patulin determination from apples juice by high performance liquid chromatography.

Patulin was separated on chromatographic column C18, 150 x 4 mm, 5 µm (high performance liquid chromatograph Thermo Finnigan), eluted in mobile phase and detected at 276 nm, using an UV-VIS „DIODE ARRAY” detector.

It was achieved an inhouse study for validation of method for patulin determination from apples juice, by high performance liquid chromatography.

In concentration range 6.25 – 400 µg/L, the average recovery was 90.50%. Limit of detection (LOD) and limit of quantification (LOQ) were 2.91 µg/L, and 9.71 µg/L, respectively.

The contamination degree of apple juice from commerce was evaluated using high performance liquid chromatography. Patulin concentrations of the analyzed apple juice samples, were under 50 µg/L, the maximum allowed limit by the legislation in force [3].

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