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Article

A Rapid Determination of Patulin Using Capillary Zone Electrophoresis and its Application to Analysis of Apple Juices

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This study describes a capillary zone electrophoretic method for the determination of patulin. An optimum run buffer was found to be 25 mM of sodium tetraborate and 10% acetonitrile (v/v) at pH 10. Optimum conditions were determined to be: an applied voltage of 25 kV (normal polarity), temperature of 25°C and injection time of 10 s at 50 mbar; the signals of patulin and phenobarbital as internal standard were detected at 276 nm. The method was highly reproducible, with relative standard deviations of 0.02–0.85 for intra-day and 0.04–0.42 for inter-day for standard patulin. Acceptable linearity [$y = 0.0020C$ (μg/L) − 0.0680 ($r = 0.9999$)] was obtained over a concentration range of 0.25 to 4.99 μg/mL of patulin. The limits of detection and quantification were calculated to be 5.9×10^{-3} and 1.79×10^{-2} μg/mL, respectively. Recovery was 68.0%. The proposed method was applied to 21 apple juice samples purchased from different Turkish markets, and two were found to contain higher than the limits of the European Union Directive for patulin.

Introduction

Mycotoxins, potentially hazardous to humans and domestic animals, are toxic metabolites produced by fungi, mostly by saprophytic molds growing on a variety of foodstuffs, including animal feed, and by many plant pathogens. Patulin (PAT) is one of the most important mycotoxins. Its chemical structure [4-hydroxy-4-H-furo (3,2-c)pyran-2(6H)-one] is that of an unsaturated heterocyclic lactone (Figure 1). It is produced by certain species of microorganisms; namely, *Aspergillus*, *Penicillium* and *Byssochlamys*. The most important producer is *Penicillium expansum*, which grows on apples and other fruits (1–3).

The PAT molecule contains a hemiacetal lactone structure and is instable in neutral and basic media (2, 4).

PAT is considered to be mutagenic, although it may not be carcinogenic (2). Recent papers concerning PAT toxicology report that this mycotoxin may increase the risk of the development of allergies (5) and can induce DNA damage (6). Animal experiments have also found that PAT had adverse effects on the developing fetus and the gastrointestinal tract. Additionally, PAT might behave as immunotoxic and neurotoxic agents (7). The occurrence of PAT as a natural contaminant of apple juice is a worldwide problem; international recommendations and regulations have been created to determine the maximum levels permitted in consumer products. The maximum level of PAT permitted in the EU has been defined as 10 μg/kg in apple products and juice for infants and young children and 50 μg/kg in apple juice for adults. In the USA, a level of 50 μg/PAT kg in apple juice has been under discussion (8, 9).

PAT has been detected in many foods, including fruit, vegetable, juices, jam, cooked corn and meat (10, 11). Apple juices are the most important source of PAT in the human diet (12). The juice production requires the use of ripened fruit, which is normally stored at low temperature before processing. Certain species of *Penicillium* are able to grow and produce PAT even at temperatures below 5°C (13). The contamination of apples with PAT is normally associated with spoiled tissue areas, and although removing rotten tissue from the fruit can reduce PAT levels, penetration nevertheless occurs up to approximately 1 cm into the surrounding healthy tissue (14). PAT and certain furfural derivatives are the parameters related to the freshness and quality of certain fruits, such as apples and apple juices. They are used to evaluate both the quality of the processing method and the organoleptic characteristics of the final products (15–17).

An important interference was reported between PAT and hydroxymetyl furfural (HMF) when a high-performance liquid chromatography (HPLC) method was employed; this can be attributed to their close polarities (16, 18–20). The measurement of HMF is accepted to evaluate both the quality of the processing method and the organoleptic characteristics of the final products like PAT in certain foods such as biscuits, bread, marmalade, breakfast cereals and honey (21). The International Federation of Fruit Juice Processors (IFFJP) recommends a maximum concentration of 5–10 mg/L of HMF in fruit juices and 25 mg/L in fruit concentrates (22).

Previously reported determination methods for PAT include gas chromatography–mass spectrometry (GC–MS) (18, 20, 21), HPLC (4, 23, 24) and liquid chromatography–mass spectrometry (LC–MS) (25) in fruits and fruit juices. The details of the determination of PAT in fruit and fruit juices have also been summarized in certain reviews (2, 17).

The goal of this study is to develop a capillary zone electrophoresis (CZE) method for the analysis of PAT without any interference from HMF. CZE is the easiest mode of CE and has been recognized as a suitable technique that is increasingly used for the analysis of many different compounds in various food samples that have complex matrices (26–29). CZE offers many advantages over commonly used HPLC methods, including reduction in the use of organic solvents, low sample

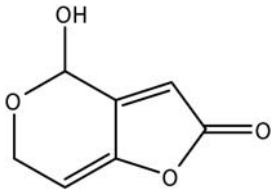

Figure 1. Chemical structure of patulin.

volume and increased separation efficiency and resolution (30). Thus far, the determination of PAT has not been reported using CZE.

HPLC with ultraviolet (UV) detection most often used for the PAT determination. Because PAT and furfural derivatives (especially HMF) have similar chromatographic properties such as polarity (16, 18, 19), HMF interferes with the determination of the PAT when HPLC analysis is utilized. Thus, it is important to determine PAT without any interfering effect from HMF (16, 31). So far, PAT has been separated from HMF by reversed-phase HPLC using a time-consuming gradient elution and complex mobile phases (19, 24).

A few studies using CE with capillary electrokinetic chromatography have been described for the determination of PAT in fruit juices (23, 26, 31, 32); however, no PAT analysis has been achieved by CZE. CE is very satisfactory method for the complicated matrix and separation mechanism, which is different from HPLC. Therefore, it was decided that PAT could be analyzed by CZE.

Experimental

Chemicals and samples

PAT, phenobarbital sodium (internal standard: IS), ascorbic acid (AA), HMF and methanol were obtained from Sigma-Aldrich (St. Louis, MO). Sodium tetraborate and sodium phosphate were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical reagent grade and used without further purification.

Different brands of commercial apple juices and fresh apples were purchased from local markets. Homemade apple juice obtained from apples, typically left at 80% humidity and 30°C in the laboratory, was prepared as follows: some apples (free of rot) were transferred to a container, where they were partially covered by soil and then left at room temperature for a week. Apple juice was obtained by pressing the ground apples.

Apparatus and CE conditions

A CE system (Agilent Technologies, Waldbronn, Germany) was utilized, equipped with a diode array UV detector (model G1600 A) and a fused silica capillary of 75 μm id and 50 cm effective length (Agilent, Portland, OR).

A ThermoOrion 720A pH/Ion meter (Beverly, MA) cable to an Orion 71-03 glass electrode was employed to measure the pH of the solution. A Sonorex ultrasonic bath (Bandelin, Berlin, Germany) degassed all of the solutions after centrifugation before analysis. An RE 100 rotary evaporator with an RE 100B heating bath from Bibby Scientific(Staffordshire, UK) was used for the evaporation procedures of the extracts. Ultrapure water (18.2 μS/cm) was supplied from Millipore (Molsheim, France).

Preparation of solutions

Stock PAT solutions were prepared from the original packaging (5 mg). PAT was dissolved in 10 mL chloroform and that solution was divided into 1 and 0.5 mL portions. The solvents were evaporated in a vacuum desiccator and stored in a deep freeze at −22°C in darkness. The contents of each tube were dissolved in methanol before use. Further dilutions, e.g., calibration solutions (0.25, 0.50, 1.00, 2.50 and 4.99 μg/mL) were daily prepared freshly in methanol and run buffer solutions. PAT was stable in the presence of AA for at least six hours during the experiments.

The run buffer solution was prepared by using an appropriate volume of sodium tetraborate or sodium phosphate stock solutions and acetonitrile (ACN).The pH of the solutions was adjusted to the desired value with 0.1 M HCl or 0.1 M NaOH.

CE procedures

The system was thermostated at 25°C and conditioned before each run by flushing at high pressure (930 mbar) with distilled water for 2 min, 0.1 M NaOH for 1 min, and distilled water for 3 min, and finally with a run buffer for 5 min. Run buffer and sample solutions were filtered through 0.45 μm membrane filters (La-Pha-Pack, Rockwood, TN) before analysis. Solutions were sonicated for 5 min before injection into the CE column.

All standards or samples were injected through the capillary for 10 s using a hydrodynamic injection mode applying low pressure (50 mbar). Signals were detected at 276 nm and the total run time of the analysis was 10 min.

Sample preparation

PAT was extracted from the samples by using liquid–liquid extraction (LLE) according to the method described in a previous publication, with minor modifications (16). Although no reports have been made concerning the photosensitivity of PAT, a sample preparation was carried out in a dimly lit working environment. Five milliliters of homogenized apple juice (commercial or homemade) was extracted twice with 10 mL of ethyl acetate by shaking vigorously for 1 min in a vortex-mixer. The organic phases were combined and left on the table (Phase 1). Then, 2 mL of 1.5% (m/v) sodium carbonate solution was added to the remaining apple juice and the solution was shaken for 1 min. The aqueous phase was immediately extracted with 5 mL of ethyl acetate by shaking for 1 min (Phase 2). The two organic phases (Phase 1 and 2) were combined. The total volume of 25 mL of organic phases was dried over 2.5 g of anhydrous sodium sulfate. The dried extract was filtered through a filter paper (Macherey-Nagel; Ø: 90 mm, type: 640 w, thickness: 0.2 mm, A781.1). Two milliliters of excess of ethyl acetate was added to wash the filter cake layer and the obtained filtrate was combined with the filtered extract. The extract was evaporated to dryness at 40°C under a gentle stream of nitrogen using a rotavapor. The residue was immediately dissolved in 0.5 mL of methanol. One-half milliliter of AA (7.5 mg/mL) and 50 μL of IS (2.3×10^{-3} M) were then added to this solution, which was filtered through the 0.45 μm membrane filter. The solution was either stored in a deep freezer at −22°C or injected through the CE.

Validation studies

The method was validated in terms of precision of peak area response, limit of linearity, specificity and accuracy, limit of detection (LOD), limit of quantification (LOQ), recovery, robustness and ruggedness in accordance with International Conference on Harmonization (ICH) Guidelines (33).

Results and Discussion

Considering the PAT molecule, a buffer system at a high pH of approximately 10 was chosen to reveal the negative charge by the deprotonation of the hydroxy group of the molecule. The selection of this pH and the deprotonation of the hydroxyl proton were supported by a calculating the theoretical pKa (11.65) by using the Marvin Beans 5.7.0 computational chemistry program (34).

The PAT molecule showed some interesting behavior during the preliminary experiments: when the standard PAT was injected through the capillary, an M-shaped peak appeared. Depending on time, the area of the first peak decreased, and the second peak area decreased (Figure 2A). It was thought that this variation could be sourced from the degradation of the PAT molecule. Some chemicals were attempted to prevent the degradation of the PAT molecule and it AA was found to be the best compound. The addition of AA has two effects on the stability of PAT. The acidity in the medium (35, 36) and the antioxidant effect of AA (37) provide the stability for PAT. This aspect is confirmed by a report in which the two primary degradation products of PAT were identified as 3-keto-5-hydroxypentanal and glyoxylic acid (37).

The effect of the addition of AA into the PAT solution was examined in the range of 1–10 mg/mL. A half portion of AA solution was added to the PAT solution and injected through the capillary. A morphologically symmetric and maximum peak was obtained when a final concentration of 3.75 mg/mL AA was used. The reduction was completed in approximately 5 min, monitoring the electropherogram in the stated conditions (Figure 2B).

HMF, which may exist in fruit or fruit juice, is an important compound exhibiting certain problems because of its neutral behavior. When using CZE, PAT was not affected by the peak of HMF, because HMF appeared with the electroosmotic flow (EOF) peak in the optimum electrophoretic conditions (Figure 2C).

Certain parameters such as mobile phase composition, species of buffer, pH, AA amount and sample injection were examined to determine the optimum conditions for the rest of the study.

Investigations of optimum conditions

Effect of buffer type and buffer components

Certain buffer species were utilized, such as sodium tetraborate and sodium phosphate, and the most satisfying buffer agent was sodium tetraborate.

The variation of sodium tetraborate concentrations at pH 10.0 were investigated in the range of 15–35 mM and a gradual increase was exhibited in both the migration time and the peak area of PAT. Moreover, the best peak morphology was observed in 25 mM borate. It was decided that 25 mM borate was the best concentration for the run buffer system.

Although it has been stated that PAT is unstable in neutral and basic media (2, 4), no decomposition was observed in the run buffer at pH 10 with the addition of AA to the standard or sample solutions.

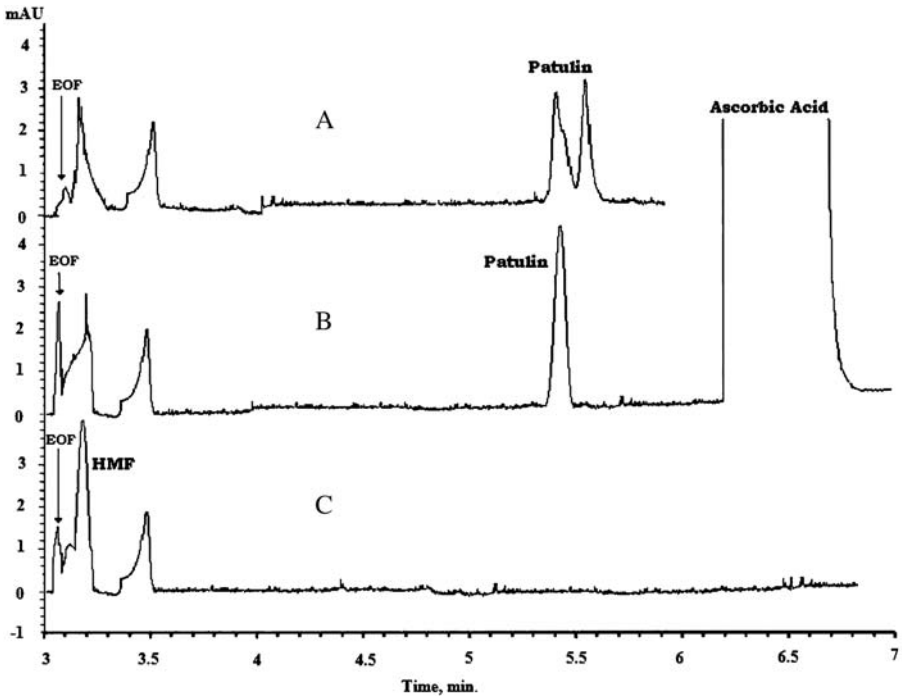

Figure 2. Typical electropherograms: before AA addition for standard PAT (4.99 μg/mL) (A); after AA addition (3.75 μg/mL) for standard PAT (4.99 μg/mL) (B); for HMF (1.26 μg/mL) by direct injection employing 25 mM sodium tetraborate buffer containing 10% (v/v) ACN at pH 10.5, applying 25 kV, hydrodynamic injection time of 10 at 50 mbar, detection at 276 nm (C).

Effect of pH

Buffer pHs in the range of 7.5–10.5 were tested to investigate the effect of buffer pH in the mobile phase using phosphate and sodium tetraborate buffers at a final concentration of 25 mM. Small peaks were observed for PAT under a pH of 8.5 and morphologically good peaks were monitored at approximately pH 10.

Effect of organic modifier

Keeping the concentration of sodium tetra borate constant at 25 mM and pH at 10.0, the percentage of ACN was varied in the range of 5–25%, v/v. The most convenient organic modifier was 10%, v/v, ACN, regarding the peak morphology and peak area of PAT.

Effect of injecting mode and injection time

Electrokinetic and hydrodynamic injection modes with different injection times were applied for the determination of PAT to enhance the method sensitivity. When using hydrodynamic injections, much higher peak areas and better precision were obtained than when using electrokinetic injection. The electrokinetic injection mode caused small peak areas due to low mobility and hydrodynamic injections. It has been reported that electrokinetic injection is relatively unaffected by sample composition and sample matrix (38). Therefore, hydrodynamic injection was utilized throughout the study.

Different hydrodynamic injection times were applied to the instrument in the range of 5–15 s and the best peak morphologies and highest peak areas appeared at 50 mbar × 10 s.

Effect of wavelength

The effect of wavelength on the peak area and peak morphology was investigated in the UV wavelength zone of 200–360 nm and 276 nm was selected as an optimum wavelength at which PAT absorbed the maximum amount of light and obtained a symmetrical peak.

Investigation of internal standard

To increase the repeatability and sensitivity of the method, certain chemicals (methyl, ethyl, propyl and butyl paraben and phenobarbital) were applied to the capillary to determine the most suitable IS under optimal conditions. The most suitable IS was determined to be phenobarbital.

PAT and IS appeared at 5.81 min [relative standard deviation (RSD) 0.31%] and 6.32 min (RSD% 0.24), respectively (Figure 3).

The optimum run buffer and CE conditions were found to be 25 mM sodium tetraborate, 10%, (v/v) ACN at pH 10.0 and +25 kV applied voltage, and a hydrodynamic injection time of 10 s at 50 mbar and detection at 276 nm.

A reasonable analysis time was exhibited without any interference in the use of standard and sample analysis. In the given conditions, the mean electrophoretic mobilities (m^2/sV) of PAT and IS were calculated to be 5.13×10^{-6} (RSD: 2.42%) and 4.28×10^{-6} (RSD: 1.83%), respectively (after six experiments). Additionally, CZE parameters were calculated to be $\alpha = 1.20$ (RSD: 0.69%), $N_{PAT} = 2.43 \times 10^5$, $N_{IS} = 6.05 \times 10^5$, where α is selectivity of PAT, and N_{PAT} and N_{IS} are the theoretical plate numbers of PAT and IS, respectively.

Method validation

Precision of the method

Three groups of PAT (n = 3), each with five dilutions (l = 5) (0.25, 0.50, 1.00, 2.50 and 4.99 μg/mL), and fixed concentrations

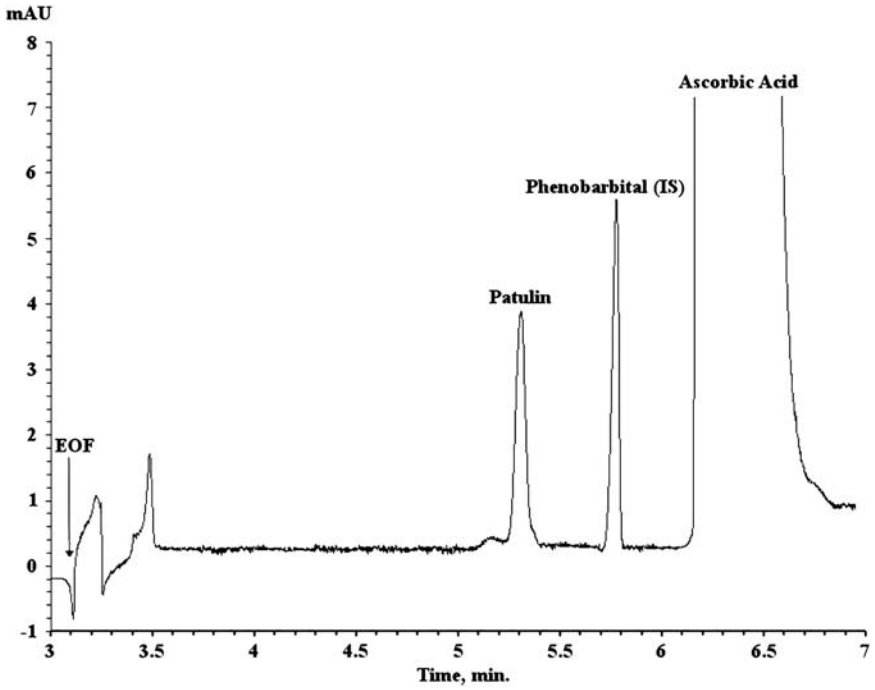

Figure 3. Electropherograms of standard patulin (4.99 μg/mL), ascorbic acid (3.75 mg/mL) and phenobarbital (IS) (26.7 μg/mL) with 25 mM borate buffer [pH 10.0, 10 ACN (% v/v)], 10 s hydrodynamic injection at 50 mbar, 25 kV, 276 nm.

of IS (26.7 μg/mL) were prepared from the stock solution. These were consecutively injected six times for three days. Repeatability, regarding the intra-day and inter-day results, was evaluated using the peak-normalization values (PN = peak area/peak migration time), and the rate of the peak normalizations (rPN = PN_{PAT}/PN_{IS}) was evaluated. The results of the repeatability tests, executed as intra-day and inter-day precision, show that this method is highly precise (Table I).

The repeatability results, as represented with RSD values, are in the range of 0.02–0.85 for intra-day and 0.04–0.42 for inter-day, indicating that the method is highly reproducible for standard PAT.

Precision tests of the study indicate that the PAT molecule is also stable in the given conditions. This is confirmed by intra-day and inter-day precisions, as described previously.

Although the PAT molecule is instable in neutral or basic media (2, 4) due to the opening of the lactone ring (39), it is thought that the lactone ring can be preserved by the addition of AA in the present study.

Linearity of the method

The linearity of the method was examined by preparing three sets of five dilutions in the range of 0.250 to 4.99 μg/mL of PAT and a fixed amount of 26.7 μg/mL IS solution. These were consecutively injected through the capillary for three days. The results were evaluated by the ratio of the peak normalization values for PAT and IS, and these values were used for the linear regression analysis and other statistical calculations. The relevant statistical results are demonstrated in Table I.

As shown in Table I, the relationship between detector response and concentration is completely linear [rPN = 0.0020C (μg/L) − 0.0680] in the mentioned range with a good correlation coefficient (r = 0.9999).

The results of precision and accuracy also show that the PAT molecule is stable in the run buffer and in the injection solution containing AA.

Table I
Results of Repeatability and Calibration Data at Five Different Concentrations Regarding the Rate of Inter-Day Peak Normalization Values for under Optimum CE Conditions*

0.25 μg/mL	Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	Inter-day
$\bar{X}$	0.470	0.470	0.470	0.470
SD	0.004	0.002	0.001	0.002
RSD	0.851	0.425	0.213	0.425
0.50 μg/mL	Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	Inter-day
$\bar{X}$	0.955	0.955	0.953	0.954
SD	0.002	0.004	0.002	0.003
RSD	0.209	0.419	0.210	0.314
1.00 μg/mL	Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	Inter-day
$\bar{X}$	1.717	1.726	1.726	1.723
SD	0.002	0.003	0.002	0.005
RSD	0.116	0.174	0.116	0.290
2.50 μg/mL	Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	Inter-day
$\bar{X}$	4.656	4.664	4.658	4.659
SD	0.002	0.002	0.003	0.004
RSD	0.043	0.043	0.064	0.086
4.99 μg/mL	Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	Inter-day
$\bar{X}$	10.124	10.133	10.126	10.128
SD	0.003	0.002	0.002	0.004
RSD	0.030	0.020	0.020	0.039

*Note: n is the number of experiments, $\bar{X}$ is the mean ratio of peak-normalization, SD is the standard deviation of the mean response and RSD is the relative standard deviation as percent.

LOD and LOQ of the method

For standard PAT, the LOD and LOQ were calculated as follows: standard deviation of the repeatability = standard deviation of the regression equation / slope of the linearity, multiplied by 3.3 and 10, respectively (33). These were calculated to be 5.9×10^{-3} and 1.79×10^{-2} μg/mL as inter-day and intra-day, respectively. These are very reasonable and comparable those of the other HPLC and CE studies (1, 16, 26). The results show reasonably low concentrations of LOD and LOQ for the determination of PAT.

Recovery of the method

The recovery of PAT was investigated by using the standard addition method. PAT standards at three different concentrations were spiked into commercial apple juice, which does not contain PAT, and extracted by the LLE technique, as described previously. The results were obtained by using the calibration equation. The recovery and RSD values were then calculated and given in Table II.

The recovery was found to be 68.0% as an average of 18 experiments employing three different PAT concentrations. This recovery value complies with the EU Directive (50–120%) (40). The RSD values are distributed between 0.32 and 1.10. The RSD values in Table II are highest at low concentrations of PAT, which can be attributed to the dilution process.

Robustness

Robustness measures the ability of the method to remain unaffected by small but deliberate variations in the optimization parameters such as pH, mobile phase composition and temperature, and instrument settings such as voltage, wavelength, column temperature and injection time. Robustness provides an indication of the method's reliability during usage. Parameters concerning robustness are given in Table III. RSD values for robustness parameters are smaller than 1, which demonstrates that the developed method is highly reliable.

All of the small changes are in the acceptable range regarding RSD values and the method is highly robust.

Table II
Recovery and Accuracy of Patulin in Commercial Apple Juice

spiked PAT (μg/mL)	Found PAT (μg/mL) ($\bar{X} \pm$ SD, each n = 6)	Recovery (%)	RSD (%)
2.49	1.81 ± 0.02	72.49	1.10
3.74	2.60 ± 0.01	69.41	0.38
4.99	3.10 ± 0.01	62.09	0.32

Table III
Robustness Parameters for the Determination of PAT in Apple Juices (3.24×10^{-5}M PAT)

Parameter	SD	RSD (%)
pH (9.9, 10, 10.1)	0.009	0.799
Concentration of sodium tetra borate buffer (24.9, 25, 25.1 mM)	0.007	0.606
ACN (9.9, 10, 10.1%, v/v)	0.006	0.487
Applied potential (24.8, 25, 25.2 kV)	0.009	0.824
Wavelength (275, 276, 277 nm)	0.004	0.353
The column temperature (24, 25, 26°C)	0.010	0.857
Injecting time (9.9, 10, 10.1 s)	0.008	0.713

Ruggedness

Ruggedness is a dated term, now commonly accepted as intermediate precision. Intermediate precision refers to variations within a laboratory, such as experiments on different days by different analysts. During the validation procedure, a second analyst repeated the repeatability analysis on a different day under different conditions. A statistical comparison is made between the two analysts' results (Table IV).

A non-statistically significant difference was observed between the two analysts' results, according to the *t*-test and *F*-test.

Application of the CZE method to real samples

PAT was determined in apple juice samples as described in the following. First, the sample was concentrated 10 times, as described previously, in which 5 mL of homogenized apple juice (commercial or homemade) was extracted and the final volume was completed 0.5 mL. Therefore, the amount of PAT was calculated by dividing by 10. Second, the determined PAT was converted to a definite amount (corrected) considering the recovery value. Some of the PAT amounts in the samples seem to be lower than the smallest concentration value in the validated range. The problem has been solved by concentrating the samples 10 times.

This method was applied to 21 apple juice samples, which were purchased from different Turkish markets and subjected to previously described sample preparation procedures. PAT was measured in six of the apple juices in the range of 0.092 to 0.228 μg/mL. The PAT amounts and their RSD values are shown in Table V. Two juices contained PAT amounts that were higher than the maximum level of PAT permitted in the EU (8, 9). PAT might be determined at a lower concentration in real apple fruit juices, compared to the amount of PAT corresponding to the LOD value, because the extracted materials may be concentrated.

Additionally, some analysis was conducted on the apple juice made in the laboratory. The typical electropherogram of this apple juice is shown in Figure 4.

As shown in the electropherogram, both peaks (the PAT and the IS) clearly appeared. The definite PAT amount, which was calculated regarding the recovery value, was 2.62 μg/mL with 0.22 RSD (n = 6). The result shows that the method is reasonably applicable for the determination of PAT. Because the peak of HMF appeared with the EOF peak, the probable interference of HMF is not observed, which is different from earlier studies by micellar electrokinetic chromatography (17, 31).

Table IV
Ruggedness Results for the Developed Method for PAT in Apple Juices

	Analyst 1	Analyst 2
Mean ± SD for (PN_{PAT}/PN_{IS})	1.993 ± 0.003	1.990 ± 0.005
RSD (%)	0.126	0.238
t-test ($p < 0.05$)	0.0034 (experimental difference)	0.0049 (computed from *t*)
F-test ($p < 0.05$)	0.183	Table $F_{0.05} = 4.28$

Table V
Definite PAT Levels in Apple Juice Samples Purchased in Turkish Markets ($n = 3$ each)

Sample number	PAT level, μg/mL (RSD%)
4	0.092 (4.93)
9	0.093 (2.23)
12	0.228 (0.67)
13	0.181 (1.10)
18	0.092 (2.75)
21	0.094 (2.21)

Conclusion

In this study, a simple, precise and sensitive CZE method has been developed, which requires fewer chemicals and less time for the determination of PAT. The method was validated using a series of validation tests, including repeatability, accuracy,

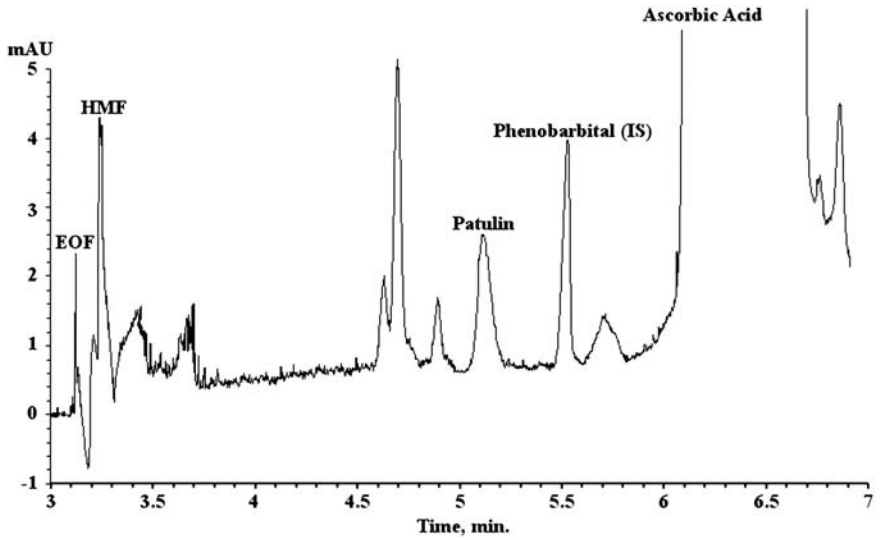

Figure 4. Typical electropherogram of homemade apple juice (prepared in the laboratory) obtained under optimum conditions.

linearity, LOD and LOQ, robustness and ruggedness, and applied for the determination of PAT in apple juice without any interference from HMF. It is concluded that the method might be useful for the routine analyses of fruit juices and apple products.

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