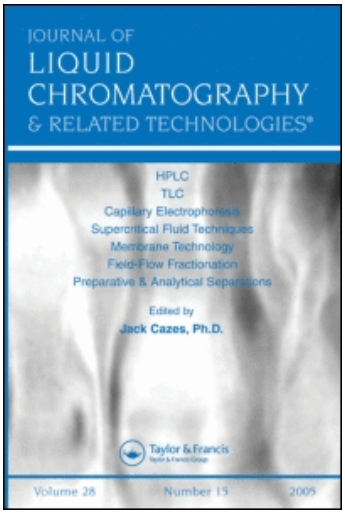

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Patulin in Apple Products using HPLC with Photodiode Array Detector and Ultra Performance Liquid Chromatography with Electrospray-Tandem Mass Spectrometry

D. Rosinská[a]; M. Vieriková[a]; J. Lehotay[b]
[a] State Veterinary and Food Institute, Janoskova, Dolny Kubin, Slovak Republic [b] Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Bratislava, Slovak Republic

To cite this Article Rosinská, D. , Vieriková, M. and Lehotay, J.(2009) 'Determination of Patulin in Apple Products using HPLC with Photodiode Array Detector and Ultra Performance Liquid Chromatography with Electrospray-Tandem Mass Spectrometry', Journal of Liquid Chromatography & Related Technologies, 32: 4, 500 — 511
To link to this Article: DOI: 10.1080/10826070802671358
URL: http://dx.doi.org/10.1080/10826070802671358

Journal of Liquid Chromatography & Related Technologies®, 32: 500–511, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802671358

Determination of Patulin in Apple Products using HPLC with Photodiode Array Detector and Ultra Performance Liquid Chromatography with Electrospray-Tandem Mass Spectrometry

D. Rosinská,[1] M. Vieriková,[1] and J. Lehotay[2]

[1]State Veterinary and Food Institute, Janoskova, Dolny Kubin, Slovak Republic
[2]Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Bratislava, Slovak Republic

Abstract: The mycotoxin, patulin (4-hydroxy-4H-furo[3,2c] pyran-2[6H]-one), is produced by a number of fungi common to fruit- and vegetable-based products, most notably, apples. A rapid, simple, and economical method was described for determination of patulin in apple products. The sample was extracted with ethyl acetate and then the extract was cleaned up by extraction with a sodium carbonate solution. Patulin was determined by reverse-phase liquid chromatography using a C_{18} column and a photodiode array detector and ultra performance liquid chromatography with electrospray-tandem mass spectrometry. Patulin was separated by an HPLC/DAD method on 125×4 mm I.D Purospher Star RP-18 endcapped column (Merck) with 5 μm C_{18} stacionary phase and it was detected at 275 nm. The mobile phase used was acetonitrile-water containing 1% acetonitrile (5/95 v/v) and the flow-rate was 0.5 mL/min. Ultra performance liquid chromatography (UPLC) was performed on an Acquity UPLC BEH C_{18} (100 mm × 2.1 mm) column. The mobile phase was acetonitrile -0,01 mol/L amonium acetate (95/5 v/v) for UPLC/MS/MS analysis and the flow-rate was 0.3 mL/min. The limits of detection for patulin were found to be 1 μg/kg for the HPLC/DAD method and 10 μg/kg for the UPLC/MS/MS method.

Keywords: Apple products, Mycotoxin, Patulin, UPLC/MS/MS

Correspondence: J. Lehotay, Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Radlinskeho 9, 812 37, Bratislava 1, Slovak Republic. E-mail: jozef.lehotay@stuba.sk

INTRODUCTION

Patulin is an unsaturated heterocyclic lactone, produced by certain fungal species of Penicillium, Aspergillus, and Byssochlamys growing on fruit.[1,2] Patulin was first isolated as an antibiotic.[3] Mycotoxin is mainly found in apple and apple products and has become one of most important quality criteria for apple products.

Patulin is formed in apples decayed by certain genus of molds, which easily transfers into apple products during processing, owing to its solubility in water.[4]

In acidic conditions, it is relatively stable to heat processes up to about 100°C. When SO_2 is used as a food preservative in fruit juice or other foods, patulin is broken down. It is not usually found in alcoholic beverages or vinegar, but has been found in 'sweet' cider in which unfermented apple juice is added to the cider. Studies show that it interacts with yeast *Saccharomyces cerevisiae* during fermentation and then patulin is destroyed.[1,5]

Patulin can be isolated as colorless white crystals from ether extracts; there is no optical activity. It melts at about 110°C and sublimes in high vacuum at 70–100°C. It is soluble in water, methanol, ethanol, acetone, and ethyl or amyl acetate and is less soluble in diethyl ether and benzene.[1]

In view of the recognized adverse effects caused by this mycotoxin and the need for regulatory control, monitoring its level in apple juice and other food products made from apples is important to evaluate the risk due to human consumption of these products.[6] For these reasons, several analytical methods have been developed for detecting and determining patulin in apple products.[7]

Various analytical methods have been reported for the determination of patulin and (hydroxymethyl)furan-2-carbaldehyde (HMF), separately in fruit juice. Patulin and HMF exhibit similar chromatographic properties, owing to their similar chemical structures (Figure 1). Therefore, HMF appears as the most encountered interference during HPLC/DAD DAD analysis of patulin.[4]

Methods are based on thin layer chromatography (TLC),[8,9] gas chromatography (GC),[10–12] and high-performance liquid chromatography (HPLC).[13–15] The LC method was described by Brause at al.[16–18]

Tandem mass spectrometry (MS/MS) has proven to be a useful and a time saving analytical tool, with many applications of direct detection of target molecules in food samples. When this method is coupled with chromatographic techniques, it combines the separation capabilities of chromatography and the power of MS/MS as an identification and confirmation method.[19] This paper reviews the use of tandem

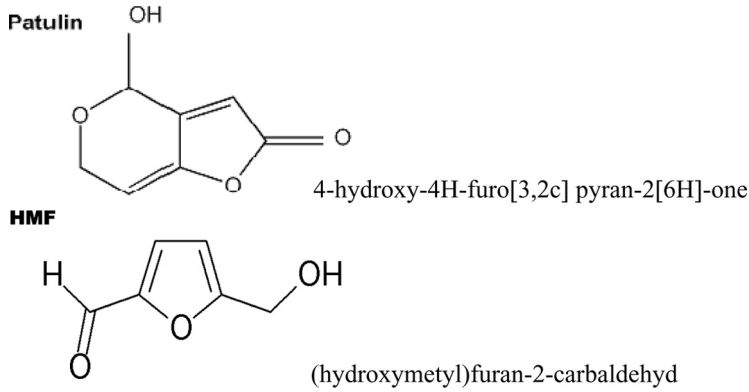

Figure 1. Chemical structure of patulin and HMF.

mass spectrometry in the determination of various food contaminants such as patulin. Sewram and others developed an HPLC/MS/MS method with selective separation monitoring to achieve a detection limit of 4 μg/kg without derivatization.[20] The MS detection was performed after atmospheric pressure chemical ionization in negative ion mode. This procedure correlated well with the standard HPLC/UV method.[20,21]

The European Commission Regulation[22] has laid down the performance criteria for sampling and method of analysis for official control of mycotoxins in foodstuff, which includes precision, repeatability (r), reproducibility (R), RSD (relative standard deviation), recovery, and uncertainty. A maximum permitted concentration has been set at 50 μg/kg in apple juice, 25 μg/kg in solid apple products and 10 μg/kg for apple baby food by the European Commission Regulation (EC).[23]

EXPERIMENTAL

Chemicals and Materials

The certified standard of patulin (concentration 100 mg/L in acetonitrile) for study was obtained from Intertec.

Acetonitrile, ethyl acetate, acetic acid (glacial) 100% anhydrous, sodium carbonate-decahydrate, and also (hydroxymethyl)furan-2-carbaldehyde were obtained from Merck (Germany). Ammonium acetate was obtained from Mikrochem (Slovak Republic).

Water was purified in a Milli-Q system with a specific resistance 18 MΩ and a total carbon value <5 ppb.

Analytical Procedure

A 5 g sample of apple product was extracted twice with 10 mL ethyl acetate by shaking vigorously for 20 minutes using a shaker (Unimax 1010, Heidolph Instrument). The organic phases were combined and extracted with 2 mL of 1.5% sodium carbonate solution by shaking, using a vortex mixer. The phases were allowed to separate and the aqueous phase was immediately extracted with 5 mL of ethyl acetate by shaking for 1 min. The organic phase was separated and mixed with first organic extract at a total volume of 25 mL. The mixed organic phases were filtered through a glass wool. A 2 mL excess of ethyl acetate was added to wash the glass wool. Then, the extract was evaporated just to dryness in a water bath at 40°C. The residue was immediately dissolved in 0.5 mL of water acidified with acetic acid on pH 4.0 and 20 μL of this solution was injected into the column.[4]

Chromatographic Conditions

High-Performance Liquid Chromatography with Photodiode Array Detector

A model Agilent 1100 series liquid chromatograph with photodiode array detector was used. Patulin was separated on an I.D. Purospher Star RP-18 endcapped column (Merck) 125×4 mm with 5 μm C_{18} spherical porous particles. Gradient elution was used (Table 1).

The flow-rate was 0.5 mL/min. Column temperature was 25°C. Injected volume was 20 μL. Patulin was detected at 275 nm.

The chromatograms were recorded with a ChemStation for LC 3D (Agilent technologies 1999–2002).

Table 1. The gradient of mobile phase for HPLC/DAD method

Time (min)	Acetonitrile (%)	Water containing 1% acetonitrile (%)
0	5	95
8	5	95
10	90	10
13	90	10
15	5	95
18	5	95

Table 2. The gradient of mobile phase for UPLC/MS/MS method

Time (min)	0.01 M amonium acetate (%)	Acetonitrile (%)
0.0	5	95
2.0	5	95
2.1	90	10
3	90	10

Ultra-Performance Liquid Chromatography with Electrospray-Tandem Mass Spectrometry

UPLC analysis was carried out using an Acquity Ultra performance LC (Waters Ltd., Watford, Herts, UK) with mass spectrometric detector Quattro Premiere XE (Micromass UK, Altrincham UK). Ultra performance liquid chromatography (UPLC) was performed with an Acquity UPLC BEH C_{18} (100 mm × 2.1 mm) with gradient elution (Table 2); the flow rate was 0.3 mL/min. Column temperature was 25°C. Injected volume was 20 μL. The chromatographic data were processed with MassLynxs 4.1.

MS-MS Parameters

A Quattro Premier XE tandem quadrupole instrument was used in UPLC-MS/MS analysis. The instrument was operated in negative ESI mode. ESI parameters, as well as selection and tuning of MS/MS transitions and analyte parameters (collision energy and cone voltage), were performed by direct infusion of patulin standard solution (concentration 1 mg/L) into the mobile phase flow. Nitrogen was used as a desolvation gas, at a flow rate of 600 L/h and also as a cone gas at a flow rate of 60 L/h. MS/MS parameters: capillary voltage 3.0 kV; cone voltage 14.0 kV; desolvation temperature 400°C; extractor 4.0 V; precursor m/z 152.8–quantification product m/z 108.7; confirmation product m/z 80.8.

RESULTS AND DISCUSSION

Figure 2 illustrates the separation of HMF and patulin standards on a Merck Purospher Star RP-18 column using gradient elution with HPLC/DAD. In Figure 3, the chromatogram of apple juice extract (concentration of patulin 72 μg/kg) is shown. Correlation coefficient based on the concentration (μg/kg) versus peak area (mAU) was 0.9992 for patulin for levels from 1 μg/kg to 1,000 μg/kg. Retention time of patulin was

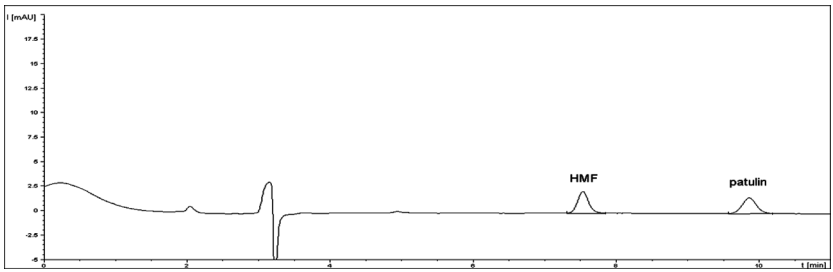

Figure 2. Chromatogram of standard solution of patulin and HMF (concentration both of them, 100 mg/L) on Merck Purospher Star RP-18 column using HPLC/DAD method, flow-rate 0.5 mL/min, detection at 275 nm, see Experimental.

approximately 9.6 min. Limit of detection for patulin was determined to be 0.5 μg/kg and limit of quantification to be 1μg/kg for the HPLC/DAD method. Samples of apple juice containing known amounts of patulin were spiked with different amounts of patulin to determine recovery of the extraction procedure (Table 3).

Apple baby puree samples containing known spiked amounts of patulin were used for determination of recovery of the extraction procedure (Table 4).

The method was useful over a wide concentration range of patulin. As low as <1 μg/kg and high as >1,000 μg/kg of patulin in apple products could be detected easily without any noticeable disadvantage.

The next step was to develop a quick and sensitive UPLC/MS/MS procedure. Figure 4 illustrates the separation of a standard solution of patulin on an Acquity UPLC BEH C_{18} column using the MS-MS method. The chromatogram of the extract of apple puree with a spiked level of 50 μg/kg of patulin is shown in Figure 3.

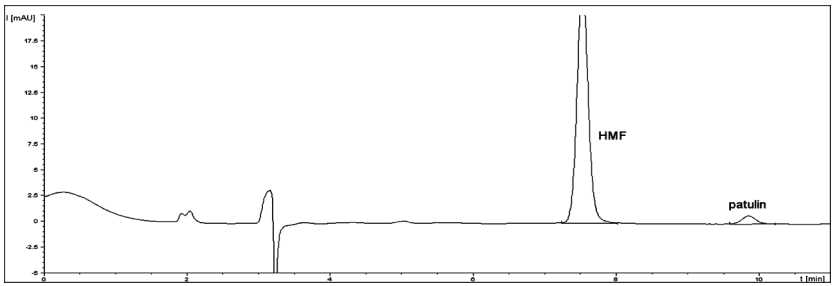

Figure 3. Chromatogram of extract of apple juice (concentration of patulin 72 μg/kg) on Merck Purospher Star RP-18 column using HPLC/DAD method, flow-rate 0.5 mL/min, detection at 275 nm, see Experimental.

Table 3. Recovery, RSDs and repeatability of juice sample analysis

Level (μg/kg)	Recovery (%)	RSDs (%)	Repeatability (%)
25	99.0	0.4	1.5
50	99.4	0.9	1.9
100	100.2	1.6	1.8

Table 4. Recovery, RSDs and repeatability of spiked puree sample analysis

Level (μg/kg)	Recovery (%)	RSDs (%)	Repeatability (%)
5	79.5	0.1	6.7
10	85.1	0.3	1.3
20	88.0	0.4	2.3

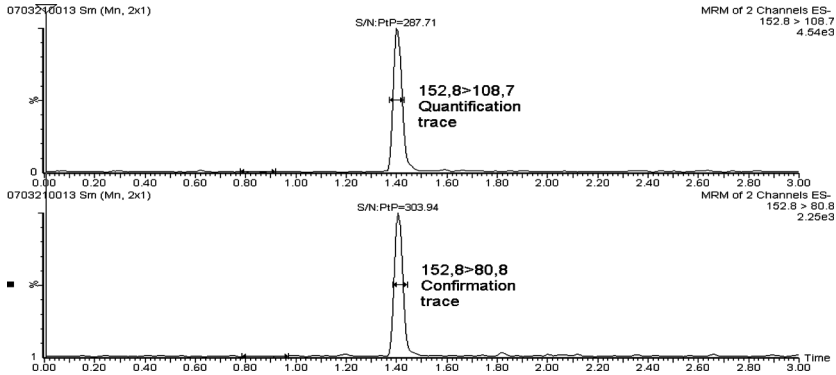

Figure 4. Chromatograms of standard solution of patulin (concentration of patulin 100 μg/L) on Acquity UPLC BEH C_{18} column using UPLC/MS/MS method, flow-rate 0.3ml/min, negative ESI mode, see Experimental.

The limit of detection for patulin was determined to be 1 μg/kg and the limit of quantification was found to be 10 μg/kg for the UPLC/MS/MS method. Samples of apple containing spiked amounts of patulin were used to determine the recovery of the extraction procedure (Tables 5 and 6).

The mass spectrometer was tuned to negative ESI mode. Full daughter scans were performed over the range m/z 20 to m/z 200

Table 5. Recovery and RSDs for juice sample

Level (μg/kg)	Recovery (%)	RSDs (%)
10	95.3	4.9
25	97.0	2.8
50	97.4	1.1

Table 6. Recovery and RSDs for apple puree sample

Level (μg/kg)	Recovery (%)	RSDs (%)
25	96.8	4.9
50	98.5	2.8
100	99.1	1.3

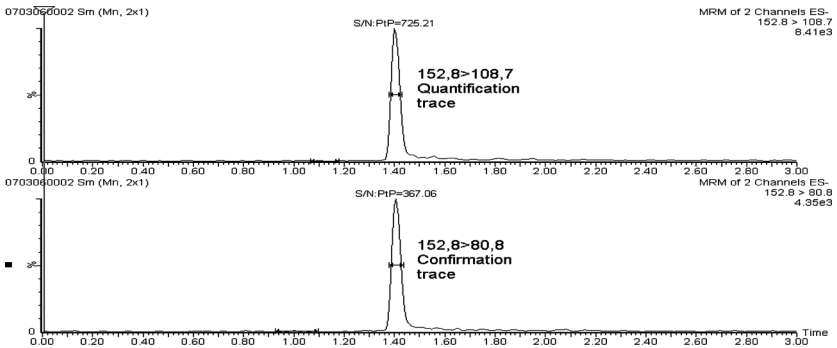

Figure 5. Chromatograms of extract of apple puree spiked with patulin at a level of 50 μg/kg, UPLC/MS/MS method was used, gradient elution, flow-rate 0.3 mL/min, negative ESI mode, see Experimental.

(Figure 6). The parent ion was determined to be m/z 152.8 and products were determined at m/z 80.8 and 108.7. The collision energy that was required for the dissociation of patulin in the negative ESI mode was 8.0 for daughter ion m/z 108.7 (quantification trace) and 11.0 for the daughter ion m/z 80.8 (confirmation trace).

The applicability of the HPLC/DAD and the UPLC/MS/MS methods was tested by analyzing large numbers of apple product samples obtained from Slovakia and another European producer (Table 7).

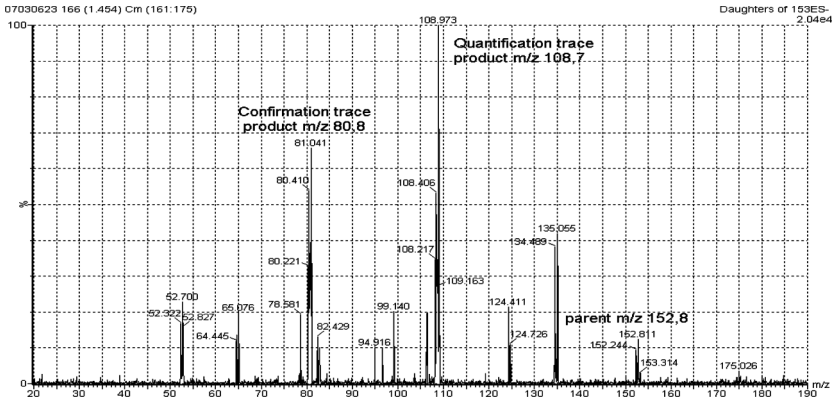

Figure 6. Daughter scan of standard patulin solution (concentration of patulin 1 mg/L), flow-rate 0.3 mL/min, negative ESI mode.

The final step was validation of the method. The limit of detection and the limit of quantification were calculated from the calibration curve using software Effi Validation 3.0. Effi Validation 3.0 is certified software

Table 7. Results of analysis of patulin per year 2007

Product	Number of analysis samples	Number of samples under LOQ	Number of samples over LOQ	Number of samples over maximum permitted concentration
Slovakia juice	28	19	9	0
Juice of European producer	45	25	20	0
Slovakia apple baby puree	9	4	2	3
Apple baby puree of European producer	1	1	0	0
Another Slovakia apple products	1	1	0	0
Another apple products of European producer	4	3	1	0
Slovakia vine	4	4	0	0
Vine of European producer	8	8	0	0
Distillate	1	1	0	0

Table 8. Validation parameters for HPLC/DAD and UPLC/MS/MS method

Parameters	HPLC/DAD method	UPLC/MS/MS method
Limit of detection	0.5 μg/kg	1 μg/kg
Limit of quantification	1 μg/kg	10 μg/kg
Scale	1–1000 μg/kg	10–1000 μg/kg
Retention time	9.6 min	1.4 min
Analysis time	18 min	3 min
Correlation coefficient	0.9994	0.9989

for validation of analytical methods according ISO 17025 for accredited laboratories and GMP quality control regulations.[24]

CONCLUSION

In this study, a simple, precise, and sensitive HPLC/DAD and UPLC/MS/MS methods for determination of patulin are described. The methods are suitable for various matrices. The extraction procedure for the samples is simple and it is the same for both methods.

The HPLC method is more sensitive than the UPLC method. The limit of quantification for the HPLC method is 1 μg/kg and, for the UPLC method, it is 10 μg/kg. The elution time of patulin for the HPLC method is 9.6 min. and, for the UPLC method, it is only 1.4 min. UPLC is a very rapid method and MS/MS detection allows identification of patulin by daughter ions m/z 108.7 (quantification trace) and m/z 80.8 (confirmation trace).

Combining the information given by both DAD and MS/MS is powerful and very useful for identification of patulin.

REFERENCES

1. European Mycotoxin Awareness Network – Basic Fact sheet 6 Patulin, 2007.
2. Mycopathologia 158, 2004, Monica Sabater-Vilar, Roel F. M. Maas, Henrik De Bosschere, Richard Ducatelle, Johanna Fink-Gremmels, Patulin produced by an Aspergillus clavatus isolated from feed containing malting residue associated with a lethal neurotoxicosis in cattle, 9 419–426.
3. *Comprehensive Reviews in Food Science and Food Safety*, Vol. 1; Matthew M. Moake, Olga I. Padilla-Zakour, Randy W. Worobo, Comprehensive reviews of patulin control methods in foods, 2005; p. 8.
4. Gokmen, V.; Acar, J. Simultaneous determination of 5-hydroxymethylfurfural and patulin in apple juice by reversed-phase liquid chromatography. J. Chromatogr. A. **1999**, *847*, 69–74.

5. Moss, M.O.; Long, M.T. Fate of patulin in the presence of the Saccharomyces cerevisiae. Food Addit. Contam. **2002**, *19* (4), 387–399.
6. Bartolomé, C. Determination of patulin in apple juice by high-performance liquid chromatography with diode-array detection. J. Chromnatogr. A. **1994**, *664* (1), 39–43.
7. Gökmen, V.; Acar, J. Simultaneous determination of 5-hydroxymethylfurfural and patulin in apple juice by reversed-phase liquid chromatography. J. Chromatogr. A. **1999**, *847* (1–2), 69–74.
8. International Organization for standardization Geneva, Switzerland, Apple juice, Apple juice concentrates and drinks containing apple juice–Determination of patulin content–Part 2: Method using TLC; International Standard ISO 8128-2:1993(E) (1993).
9. Kubachi, S.J. *Proc. Sixth Intl. Symp. Mycotoxins*, Pretoria Elsevier, Science Publishers, 1986; 295.
10. Fugimoto, Y.; Suzuki, T.; Hoshino, Y. J. Chromatogr. **1989**, *105*, 99.
11. Mortimer, D.N.; Parker, I.; Shepherd, M.J.; Gilbert, J. Food Addit. Contam. **1985**, *2*, 165.
12. Sheu, F.; Shyu, Y.T. J. Chinese Agric. Chem. Soc. **1996**, *34*, 601.
13. International Organization for Standardization, Geneva, Switzerland, Apple juice, Apple juice concentrates and drinks containing apple juice–Determination of patulin content–Part 1: Method by high-performance liquid chromatography; International Standard ISO/DIS 8128-1 (E) (1993).
14. Herry, M.P.; Lemetayer, N. J. AOAC Intl. **1996**, *79*, 1107.
15. Kubacki, S.J.; Goszcz, H. Pure Appl. Chem. **1988**, *60*, 871.
16. MacDonald, S.; Long, M.; Gilbert, J. Liquid Chromatographic Method for Determination of patulin in Clear and Cloudy Apple Juice and Apple Puree: Collaborative Study. J. AOAC Intl. **2000**, *83*, 1387–1394.
17. Brause, A.R.; Trucksess, M.V.; Thomas, F.S.; Page, S.W. J. AOAC Intl. **1996**, *79*, 451–455.
18. *Official Methods of Analysis*, 16th Ed., AOAC International: Gaithersburg, MD, 1995; Method 995.10.
19. Ito, R.; Yamazaki, H.; Inoue, K.; Yoshimura, Y.; Kawaguchi, M.; Nakazawa, H. Development of liquid chromatography-electrospray mass spectrometry for the determination of patulin in apple juice: investigation of its contamination levels in Japan. J. Agric. Food Chem. **2004**, *52* (25), 7464–7468.
20. Sewram, J., Chromatogr, A.; Nair, J.J.; Nieuwoudt, T.W.; Leggott, N.L.; Shephard, S. Determination of patulin in apple juice by high-performance liquid chromatography-atmospheric pressure chemical ionization mas spectrometry. J. Chromatogr. A. **2000**, *897*, 365–374.
21. Baranda, A.; Fundazuri, O.; Martinez de Marańón, I. Fast and reliable patulin quatification by LC-UV-MS/MS.
22. European Commission Regulation (EC) No 401/2006 of 23 February 2006.
23. European Commission Regulation (EC) No 1881/2006 of 19 December 2006.

24. Method Validation and Uncertainty software EffiValidation 3.0, designed to support accredited ISO 17025 testing and calibration laboratories and quality control GMP laboratories, EffiChem, Lesni 593, CZ-679 71 Lysice, Czech Republic, EU.

Received September 1, 2008
Accepted October 10, 2008
Manuscript 6394