Journal of Chromatography A, 1216 (2009) 3746–3750

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of patulin in fruit juice and dried fruit samples by in-tube solid-phase microextraction coupled with liquid chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 15 December 2008
Received in revised form 25 February 2009
Accepted 6 March 2009
Available online 13 March 2009

Keywords:
In-tube solid-phase microextraction
Automated sample preparation
Liquid chromatography–mass spectrometry
Patulin
Mycotoxin
Apple juice

ABSTRACT

A simple and sensitive method for the determination of patulin in fruit juice and dried fruit samples was developed using a fully automated method consisting of in-tube solid-phase microextraction (SPME) coupled with liquid chromatography–mass spectrometry (LC–MS). Patulin was separated within 5 min by high-performance liquid chromatography using a Synergi MAX-RP 80A column and water/acetonitrile (80/20, v/v) as the mobile phase. Electrospray ionization conditions in the negative ion mode were optimized for MS detection of patulin. The pseudo-molecular ion $[M-H]^-$ was used to detect patulin in selected ion monitoring (SIM) mode. The optimum in-tube SPME conditions were 25 draw/eject cycles of 40 μL of sample using a Carboxen 1006 PLOT capillary column as an extraction device. The extracted patulin was readily desorbed from the capillary by passage of the mobile phase, and no carry-over was observed. Using the in-tube SPME LC–MS with SIM method, good linearity of the calibration curve ($r = 0.9996$) was obtained in the concentration range of 0.5–20 ng/mL using $^{13}C_3$-patulin as an internal standard, and the detection limit (S/N = 3) of patulin was 23.5 pg/mL. The in-tube SPME method showed >83-fold higher sensitivity than the direct injection method (10 μL injection volume). The within-day and between-day precision (relative standard deviations) were below 0.8% and 5.0% ($n = 6$), respectively. This method was applied successfully for the analysis of fruit juice and dried fruit samples without interference peaks. The recoveries of patulin spiked into apple juice were >92%, and the relative standard deviations were <4.5%. Patulin was detected at ng/mL levels in various commercial apple juice samples.

1. Introduction

Patulin, 4-hydroxy-4*H*-furo[3,2*c*]pyran-2(6*H*)-one (Fig. 1), is a toxic mycotoxin secondary metabolite produced by a variety of molds, particularly *Aspergillus*, *Penicillium*, and *Byssochlamys* [1,2]. *Penicillium expansum*, the blue mold that causes soft rot of apples, pears, cherries, berries and other fruits, is one of the most common sources of patulin contamination [3,4]. The amount of patulin in apple products can generally serve as an indicator of the quality of fruit used in their production as an appreciable concentration of the mycotoxin remains in the product after processing [5]. Patulin exhibits mutagenic and carcinogenic properties in several animal species and induces intestinal injuries, including epithelial cell degeneration, inflammation, ulceration, and haemorrhage [6]. Therefore, patulin contamination is a worldwide problem with regard to food and feed safety, and several countries have instituted patulin restrictions in apple products. In 1995, a provisional maximum tolerable daily patulin intake of 0.4 μg/kg body weight was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [7]. The World Health Organization also recommends a maximum concentration of 50 μg/L in apple juice [8]. In view of the recognized adverse effects of patulin and the need for regulatory control, monitoring of its level in apple juice and other food products is important to evaluate the risks associated with human consumption of these products. Therefore, a sensitive, selective, and simple method to determine the presence and contents of patulin in these food samples is required.

Analyses of patulin have been carried out mainly by gas chromatography–mass spectrometry (GC–MS) [9–12], high-performance liquid chromatography (HPLC) [13–21], liquid chromatography–mass spectrometry (LC–MS) [22–25], and micellar electrokinetic chromatography (MEKC) [26]. The details of the determination of patulin in food samples have been summarized in some reviews [27,28]. Although GC–MS methods are highly sensitive, they require derivatization of patulin prior to analysis. HPLC with UV detection is the most widely used method and has been validated as an AOAC International (AOAC Int.) official method [14]. However, several HPLC methods reported previously are less

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doi:10.1016/j.chroma.2009.03.017

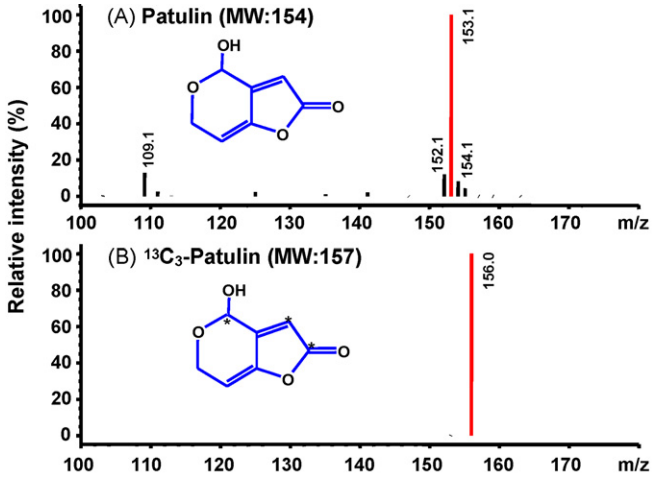

Fig. 1. ESI-mass spectra of patulin and $^{13}C_3$-patulin obtained by direct injection.

sensitive and many interfering peaks such as 5-hydroxymethylfurfural on the chromatogram often make it difficult to determine the content of patulin in apple juice precisely. Furthermore, it is also difficult to confirm patulin by UV spectroscopy, because the UV spectra of neighboring interfering peaks are very similar to that of patulin. MEKC with UV detection is also less sensitive. On the other hand, LC–MS methods are specific and sensitive, and are becoming increasingly common. However, most of the above methods require sample preparation steps, such as extraction, concentration, and isolation. Usually ethyl acetate extraction and solid-phase extraction have been used as sample preparation techniques. Several methods use stable isotope-labeled patulin as an internal standard to improve recovery and matrix interference [28]. However, most of these sample preparation techniques are both complicated and time-consuming. Complicated pretreatment methods may introduce errors, and the use of large volumes of organic solvent poses a health hazard to those performing the analyses and contributes to environmental pollution. Therefore, it is important to develop an efficient sample pretreatment method, and automation will reduce both labor and costs. A routine analysis method will also facilitate the processing of large numbers of samples.

In-tube solid-phase microextraction (SPME), using an open tubular fused-silica capillary with an inner surface coating as the SPME device, is simple and can be easily coupled on-line with HPLC and LC–MS. In-tube SPME allows convenient automation of the extraction process, which not only reduces the analysis time, but also provides better accuracy, precision, and sensitivity than manual off-line techniques. We have already developed an in-tube SPME method for the determination of various compounds in food samples by coupling with HPLC [29,30] and LC–MS [31,32]. The details of the in-tube SPME technique and its applications have been summarized in a number of reviews [33–36]. Here, we report an automated on-line in-tube SPME/LC–MS method for the determination of patulin in food samples.

2. Experimental

2.1. Materials

Patulin was purchased from Wako Pure Chemicals (Tokyo, Japan) and dissolved in methanol to make a stock solution at a concentration of 1 mg/mL. $^{13}C_3$-patulin (100 μg/mL) as an internal standard (IS) was purchased from Hayashi Pure Chemicals (Osaka, Japan) and diluted in methanol to make a stock solution at a concentration of 1 μg/mL. The solutions were stored at 4 °C and diluted to the required concentrations with pure water prior to use. LC–MS grade acetonitrile and water used as mobile phases were purchased from Kanto Chemical (Tokyo, Japan). All other chemicals were of analytical grade.

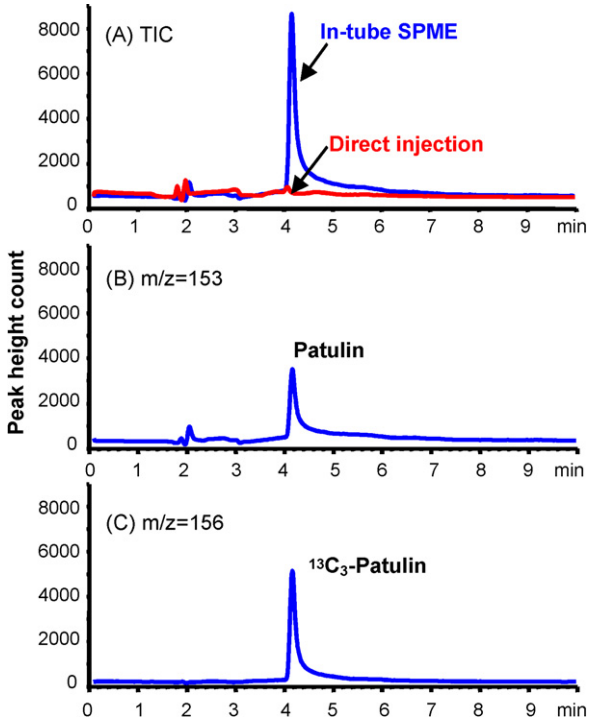

Fig. 2. Typical total ion and selected ion chromatograms obtained from standard patulin (20 ng/mL) and $^{13}C_3$-patulin (20 ng/mL) by direct injection and in-tube SPME LC–MS in negative ion mode. LC–MS conditions: see Section 2.

2.2. Instrument and analytical conditions

The LC–MS system was a Model 1100 series LC coupled with an atmospheric pressure (AP) electrospray ionization (ESI) MS (Agilent Technologies, Boeblingen, Germany). A Synergi 4u MAX-RP 80A column (150 mm × 4.6 mm, particle size of 4 μm; Shimadzu GLC Ltd., Kyoto, Japan) was used for LC separation under the following conditions: column temperature, 40 °C; mobile phase, water/acetonitrile (80/20, v/v); flow rate, 0.75 mL/min with a run time of 10 min. ESI-MS conditions were as follows: nebulizer gas N_2 (50 psi); drying gas, N_2 (12 L/min, 350 °C); fragmentor voltage, 110 V; capillary voltage, 2000 V; ionization mode, negative mode; mass scan range, 100–180 amu; selected ion monitoring (SIM), *m/z* 153 (patulin) and 156 ($^{13}C_3$-patulin); dwell times for the ions in SIM, 289 ms. LC–MS data were processed with an HP ChemStation.

2.3. In-tube solid-phase microextraction

A GC capillary column (60 cm × 0.32 mm i.d.) was used as the in-tube SPME device, and placed between the injection loop and injection needle of the autosampler. The injection loop was retained in the system to avoid fouling of the metering pump. Capillary connections were facilitated by use of a 2.5 cm sleeve of 1/16-in polyetheretherketone (PEEK) tubing at each end of the capillary. PEEK tubing with an internal diameter of 330 μm was suitable to accommodate the capillary used. Standard 1/16-in stainless steel nuts, ferrules, and connectors were used to complete the connections. CP-Sil 5CB, CP-Sil 19CB, CP-Wax 52CB, CP-Pora PLOT amine (Varian Inc., Lake Forest, CA), Supel Q PLOT and Carboxen 1006 PLOT