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# Determination of patulin in commercial apple juice by micellar electrokinetic chromatography

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**Abstract**

A novel and validated micellar electrokinetic capillary chromatography (MEKC) method using ultraviolet detection (UV) has been applied to the quantitative analysis of patulin (PAT) in commercial apple juice.

Patulin was extracted from samples with an ethylacetate solution. The micellar electrokinetic capillary chromatography (MECK) parameters studied for method optimization were buffer composition, voltage, temperature, and a separation between PAT and 5-hydroxymethylfurfural (HMF) (main interference in apple juice PAT analysis) peaks until reaching baseline.

The method passes a series of validation tests including selectivity, linearity, limit of detection and quantification (0.7 and 2.5 μg $L^{-1}$, respectively), precision (within and between-day variability) and recovery (80.2% RSD = 4%), accuracy, and robustness. This method was successfully applied to the measurement of 20 apple juice samples obtained from different supermarkets. One hundred percent of the samples were contaminated with a level greater than the limit of detection, with mean and median values of 41.3 and 35.7 μg $L^{-1}$, respectively.


*Keywords:* Mycotoxins; Patulin; Micellar electrokinetic chromatography; Apple juice; Validation

## 1. Introduction

Mycotoxins are toxic secondary metabolites of fungi. Patulin (PAT) is an unsaturated heterocyclic lactone, produced by certain fungal species of Penicillium, Aspergillus and Byssochlamys growing on fruit (Ritieni, 2003). Patulin has been mainly found in apple and apple products and occasionally in pears, grapes, apricots, strawberries, blueberries and peaches (Majerus and Kapp, 2002). As PAT is very soluble in water and very stable in aqueous acid mediums, it reaches apple derivative products, such as juices (Gökmen and Acar, 2000; Armentia et al., 2000). Apple juice contaminated with PAT continues to be a problem for human health, not only due to the effects of PAT but also due to the toxicity produced when PAT is combined with other mycotoxins. Patulin is toxic for animals; it induces intestinal injuries, including epithelial cell degeneration, inflammation, ulceration, and haemorrhages; it has also been shown to be mutagenic, carcinogenic and teratogenic (Mahfoud et al., 2002).

In 2003, the European Union established maximum permitted levels of patulin in different apple products which ranged between 10 and 50 μg $kg^{-1}$, depending on the foodstuff and on the group of consumers (Commission Regulation (EC) No 1425/2003).

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*Abbreviations*: CE, capillary electrophoresis; DAD, diode array detection; GC–MS, gas chromatography/mass spectrometry; HMF, 5-hydroxymethylfurfural; HPLC-UV, high-performance liquid chromatography with ultraviolet detector; LC, liquid chromatography; MEKC, micellar electrokinetic capillary chromatography; PAT, patulin; PMTDI, provisional maximum tolerable daily intake; SDS, sodium dodecylsulphate; TLC, thin layer chromatography; UV, ultraviolet detection.

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(JECFA, 1995) established a provisional maximum tolerable daily PAT intake (PMTDI) at 0.4 μg kg of body weight.

Several PAT quantification and determination methods in apple juice have been reported in reference literature. They include thin layer chromatography (TLC) (Association of official analytical Chemists (AOAC), 1984), liquid chromatography (LC) (MacDonald et al., 2000), and gas chromatography/mass spectrometry (GC–MS) (Sheu and Shyu, 1999). The main disadvantage of TLC methods is that the detection limit is normally quite high. In general, HPLC-UV is the most frequently used technique; its range of quantification limits is between 25 μg $L^{-1}$ (Boonzaaijer et al., 2005) and 2.21 μg $L^{-1}$ (Tangni et al., 2003). No commercial ELISA-kits are available.

Of particular importance in the analysis of PAT in apple juices by UV is the separation of patulin from 5-hydroxymethylfurfural (HMF). The use of photodiode array detection to spectrally distinguish patulin from HMF has considerable application in providing confirmation regarding the purity of the chromatography peak (Bartolomé et al., 1994).

In comparison with LC methods, capillary electrophoresis methods have some advantages such as being able to use a smaller volume of organic solvent and producing less waste volume. With regard to the use of capillary electrophoresis for mycotoxin quantification in food, in the reference literature, we have found it is carried out for the determination of aflatoxins in corn and feed samples (Cole et al., 1992; Maragos and Greer, 1997; Peña et al., 2002), ochratoxin A in wine, coffee, corn and sorghum (González-Peñas et al., 2006; Corneli and Maragos, 1998) and fumonisin B1 in corn (Holcomb and Thompson, 2005; Hines et al., 1995; Maragos, 1995). Terabe et al. (1984) introduced the new technique called micellar electrokinetic chromatography (MEKC). In 1993, Holland and Sepaniak proposed a micellar electrokinetic method for the qualitative analysis of standard solutions of 10 different mycotoxins not including PAT. Therefore, the application of micellar electrokinetic chromatography for carrying out quantitative analysis of mycotoxins is not widespread.

Only one method for PAT determination by micellar electrokinetic chromatography in apple derivatives foodstuff has been published (Tsao and Zhou, 2000) although the samples analyzed were not commercial apple juices but rather the product obtained from the first hydraulic press, filtration and centrifugation of apples without suffering the process of transformation into apple juice or the storing process before being consumed by the population; therefore, the chemical composition of these ''apple cider'' samples could be different from apple juice samples, as indicated by Spanos et al. (1990). Nevertheless, this technique has never been used for the quantitative analysis of patulin in commercial apple derivative foodstuffs such as apple juice, which is the product that people consume; therefore, there is a need to determine the levels of toxin in this type of foodstuff.

Method validation is an important requirement in the practice of chemical analysis for obtaining reliable data. It is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with that which the application requires. It is also needed for transferring analytical methods and carrying out the legal requirements.

In this paper, a MEKC method with DAD detection for the quantitative analysis of PAT in commercial apple juice is described and evaluated according to the following validation criteria: selectivity, linearity, limit of detection and limit of quantification, recovery and precision (within-day and between-day variability), accuracy and robustness (buffer stability, PAT stability in water at pH 4.0, and precision of the injector system). This method has been applied to the measurement of 20 commercial apple juice samples.

## 2. Materials and methods

### 2.1. Samples

Twenty apple juice samples, obtained randomly from different supermarkets within Navarra (Spain), were analyzed. Different brand names were selected in order to have a market-representative sampling. The samples were stored in their original packets at 4–5 °C until analysis was carried out.

A stock standard solution of PAT 0.2 mg $mL^{-1}$ was prepared by dissolving 10 mg PAT in 50 mL ethylacetate. Next, 1.3 mL volumes were aliquoted in 1.5 mL eppendorfs which were stored at −20°C. It has been reported that PAT solutions in ethylacetate stored at −20°C are stable for several months (MacDonald and Felguieras, 1997).

A working solution of PAT (10 μg $mL^{-1}$ approximately) was prepared after evaporating 1 mL of the stock solution and redissolving the residue in 20 mL of ethanol. The accurate concentration of PAT was determined by ultraviolet light at 276 nm (MW = 154.12; $\varepsilon = 14{,}600$ L $mol^{-1}$ $cm^{-1}$) (MacDonald and Felguieras, 1997) and by applying the Lambert–Beer equation. This solution was stored between 0 and 4 °C and at room temperature for 30 min before each use.

A stock standard solution of HMF of 0.2 mg $mL^{-1}$ was prepared by dissolving 5 mg HMF in 25 mL of ethylacetate, after which four volumes of 8 mL were aliquoted in tubes and stored at −20 °C. A working solution of HMF was prepared by evaporating 7 mL of the stock solution under a stream of nitrogen at 40 °C and then by dissolving the residue in 20 mL of ethanol, obtaining an HMF solution of 70 μg $mL^{-1}$. This solution was stored between 0 and 4 °C and at room temperature for 30 min before each use. Calibration samples were prepared by evaporating the adequate volume of PAT and HMF working solutions under vacuum at 40 °C, followed by dissolution in 200 μL of water at pH 4.0 acidified with 0.1% aqueous acetate acid solution.

### 2.2. Extraction of PAT from apple juice samples

Twenty milliliter of apple juice were mixed with 20 mL of ethylacetate, and the solution was shaken for 30 min in a vertical shaker model ABT4 (SBS®). The sample was then set aside for 30 min so as to allow the two layers to separate. Ten milliliter of the organic phase was evaporated in an evaporator vortex (Labconco) under vacuum at 40 °C. The residue was then dissolved in 400 μL of water acidified at pH 4.0 with a 0.1% solution of acetic acid; afterwards, it was filtered through a 0.45 μm filter (Syringe Driven Filter, Millipore, Corporation Bedford, USA).