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Validation and application of micro flow liquid chromatography–tandem mass spectrometry for the determination of pesticide residues in fruit jams

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ABSTRACT

In this study, a very sensitive method was validated to determine pesticides residues in fruit jams using micro flow liquid chromatography–tandem mass spectrometry (μLC–MS/MS). A slurry of the fruit jams and water was prepared to yield homogeneous samples. Because of the high sensitivity achieved with the μLC-MS/MS equipment and to minimize matrix effects, the QuEChERS extracts were diluted 30-fold before the analysis. The validation was performed analyzing spiked samples at 9 and 45 μg kg^{-1} ($n=5$). The method met validation criteria of 70–120% recovery and RSD ≤ 20% for 92% of the 107 pesticides evaluated. The reporting limit (RL) was 9 and 45 μg kg^{-1} for respectively 66% and 26% of the analytes, 5% of the compounds did not fulfill the requirements for validation and 3% were not detected at the studied concentrations. The validated method was applied to the analysis of 51 different fruit jam samples from Brazil and Spain and pesticide residues were detected in 41 samples, 26 of which contained at least one pesticide at concentration > 10 μg kg^{-1}.

1. Introduction

Historically, jams originated as an early effort to preserve fruit for consumption during the fruit off-season. It is an intermediate moisture food prepared by boiling fruit pulp with sugar, pectin, acid and other ingredients (preservatives, coloring and flavoring substances) until obtaining a reasonably thick consistency [1] and a final water content of 32–34% [2]. According to the Document SANCO 12571/2013 [3] fruit jams are classified as high sugar and low water content commodities, together with honey and dried fruits and these characters of the fruit jams can represent a challenge in pesticide determination in such matrices [4].

Pesticides are chemical substances applied to crops at various stages of cultivation and during the post-harvest storage of crops. The use of pesticides is intended to prevent the destruction of food crops by controlling agricultural pests or unwanted plants and to improve plant quality [5]. In Brazil, one of the world's major food producers, over 90% of farmers rely on pesticide use and the country has ranked first in pesticide use worldwide in recent years, with over 673 million tons applied in 2008 [6].

Although pesticides help to control agricultural pests and organisms harming human activities, they may present a risk for human health. In the European Union (EU), the evaluation of plant protection products and the monitoring of pesticide residues in food are harmonized through Regulation EC No 1107/2009 and Regulation EC No 396/2005 [7]. In Brazil, the basis for pesticide regulation was set by Federal Law No. 7802, enacted in 1989, and later by Acts 4074/2002 and 5981/2006. Two monitoring programs for pesticide residues are currently in place in Brazil that aim to evaluate compliance with national MRLs: the Program on Pesticide Residue Analysis in Food (PARA), coordinated by the National Health Surveillance Agency (ANVISA), which aims to analyze fruits and vegetables, and the National Residue and Contaminant Control Program (PNCRC), coordinated by the Ministry of Agriculture, Livestock and Food Supplies (MAPA), that intent to control animal products, fruit and vegetables products [6,8,9]. None of these programs aims to evaluate processed food products, like e.g. fruit jams, juices or tomato purees, for pesticide residues.

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Liquid chromatography (LC) is used in many analytical applications worldwide and is commonly coupled to mass spectrometry (MS) to detect, to identify and to monitor compounds [10]. The development of miniaturized LC started by the mid-1970s but the first commercially available micro (μ) LC system was announced in late 1975 [11]. μLC typically uses columns with an internal diameter (I.D.) of 0.5 to 1 mm [12], lower mobile phase flow rates (1 to 40 μL min^{-1}) and present numerous advantages compared to conventional LC [13] like the ability to work with smaller sample sizes, lower volumetric flow-rates and the improvement in detection performance with the use of concentration-sensitive detectors as a result of the reduced chromatographic dilution [12,14].

It is considered that the increase of detection sensitivity in tubing with a small inner diameter is due to reduced axial sample band diffusion [15,16]. The following rationale suggests the selection of capillary LC. During chromatographic separation, the dilution (D) of an injected sample ($D=C_{end}/C_{inj}$, where C_{end} is the concentration after chromatography and C_{inj} is the concentration injected) is given by

$$D = \frac{\in \pi r^2 (1+k)(2LH)^{\frac{1}{2}}}{V_{inj}}$$

where $\in$ is the column porosity, r is the column radius, k is the retention factor, L is the column length, H is the plate height, and V_{inj} is the injection volume. If conditions are otherwise equal, D is in direct proportion to the square of column radius. When compared to conventional LC, μLC increases the signal-to-noise ratio (S/N) drastically when electrospray ionization (ESI) coupled to MS/MS is employed [14,17]. For example, from the previous equation it can be calculated that this will result in a 235-fold increase in peak height and mass sensitivity for a reduction in the diameter of a column from 4.6 mm to 300 μm I.D. [14], when all the other parameters are kept constant.

ESI is a soft ionization technique and these techniques perform considerably better if most of the eluate solvents are removed before the ionization process takes place. μLC delivers sharper and narrower solute bands to the interface nebulizer using a minimal amount of an appropriate solvent mixture. Consequently, smaller droplets are generated carrying less solvent to evaporate. The solute, which is distributed among a larger number of lower mass particles, is rapidly vaporized into the ion source minimizing thermal decomposition [18].

Due to the increase of pesticides applied in agriculture, their potential accumulation in both the environment and foods and their toxicities to humans a stricter control of residues in food commodities should be applied. Considering the decrease of the maximum residue limits (MRL) in most countries and continuous further prohibition of older, more harmful pesticides there is a need for sensitive multi-residue methods for monitoring and enforcement of the residues that may be present in food [19] including for processed food crops like fruit jams. The goal of this work was to develop and validate a selective, robust and highly sensitive μLC–MS/MS method to determine pesticides residues in fruit jams and later apply it to the analysis of samples to verify the existence of pesticides in these commodities.

2. Material and methods

2.1. Reagents and materials

Acetonitrile, HPLC grade (99.9%), formic acid, analytical grade (≥96.0%) and magnesium sulfate (98.0%) were purchased from Sigma Aldrich (Steinheim, Germany). Water, Optima®, HPLC grade was supplied by Fisher Scientific (New Jersey, USA). Sodium chloride (99.0%) was obtained from J.T. Baker (Deventer, Netherlands). Ethyl acetate, HPLC grade, sodium citrate tribasic dehydrate (99.0%) and disodium hydrogencitratesesquihydrate (99.0%) from Fluka (Steinheim, Germany). C_{18} (40 μm) was from Varian (Middelburg, The Netherlands) and Primary-Secondary Amine (PSA) Bond-Elut from Supelco (Bellefonte, USA). Pesticides standards (purity > 98.0%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany), from Riedel-de Haën (Seelze, Germany) and from Sigma–Aldrich (Steinheim, Germany) and stored in a freezer at −30 °C.

2.2. Pesticide standard solutions

Individual pesticide standard stock solutions were prepared in acetonitrile and in ethyl acetate, at 1000 − 2000 mg L^{-1} and stored in amber screw-capped glass vials at −20 °C. A standard mixture solution of 107 pesticides was prepared in acetonitrile at the concentration of 1000 μg L^{-1}. This solution was used as spiking solution for recovery experiments and also to prepare the standard solutions in matrix (matrix-matched calibration standards) and organic solvent to obtain the calibration curves, by dilution with blank fruit jam extract or acetonitrile, respectively. The standards in blank matrix extract were used for the determination of the matrix effect and also for the recovery calculations.

2.3. Instrumentation

The chromatographic system consisted of an Eksigent ekspert™ μLC 200 (Eksigent, Redwood City, CA, USA) integrated to a hybrid quadrupole/linear ion trap mass spectrometer (QTRAP® 4500 MS/MS, AB Sciex Instruments, Foster City, CA, USA). Chromatographic separations were performed using an Halo C_{18} column 50 × 0.5 mm I.D. and 2.7 μm particle size (Eksigent, AB Sciex Instruments, Foster City, CA, USA) held at 30 °C by a column heater. The mobile phases consisted of water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The injection volume was 3 μL, the flow rate used was kept constant at 30 μL min^{-1} and the gradient program in positive mode was set as follows: 20% B (initial conditions) was kept constant for 1 min followed by a linear gradient up to 98% B in 9 min, after which the mobile phase composition was maintained at 98% A for 3 min, the re-equilibration time was 1 min and the total run time was 14 min.

The QTRAP® 4500 MS/MS system was equipped with an ESI source with μ-Flow electrode (50 mm I.D.), operating in positive and negative ionization mode, there was applied scheduled multiple reaction monitoring (sMRM) software features. The Turbo Ion Spray source settings were ion spray voltage, 5000 V; temperature, 400 °C; curtain gas flow, 20 L min^{-1}; collision gas, medium; and ion source gas (nebulizer gas and turbo gas), at a pressure of 30 psi. Nitrogen was used as the nebulizer gas, turbo gas, curtain gas and collision gas. The data were acquired and processed with the Analyst software version 1.6.2.

2.4. Selected μLC–MS/MS parameters

To optimize the mass spectrometer parameters an individual solution of each target compound was prepared in methanol at the concentration of 0.1 μg L^{-1}. Using flow injection analysis of these solutions, it was possible to optimize all the parameters including declustering potential, entrance potential, collision energy and collision cell exit potential for each single compound. The system was operated in a sMRM mode, through the acquisition of single reaction monitoring (SRM) transitions for each analyte with resolution set to Unit at the first and third quadrupoles. The time window was from 30 s for each SRM transition. According to Lozano et al. [20] the sMRM enables optimized cycle time and maximized dwell times to be used during acquisition to provide higher multiplexing with good analytical precision.