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A simple multi-residue method for the determination of pesticides in fruits and vegetables using a methanolic extraction and ultra-high-performance liquid chromatography-tandem mass spectrometry: Optimization and extension of scope

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ABSTRACT

In 2004, a new multi-residue pesticides method had been published using methanol as extraction solvent. Our goal for this study was to optimize the analytical scheme while extending the compound scope from 19 to 200 pesticides. The main changes from the original method take place at the sample extraction and processing with a special attention to make the overall method fit for routine analysis with minimal cost. Hence, after a quick Ultra-Turrax homogenization with a methanolic solution, the sample is simply diluted before the separation and detection by ultra-high-performance liquid chromatography and MS/MS detection for quantitative and confirmatory purposes. The performance of the method including limits of quantification (LOQs), linearity, matrix effect, precision was evaluated during validation in accordance with the European Union SANCO/12571/2013 regulatory guidelines. Two representative matrices, lettuce and orange, were selected and fortified at two concentration levels for these experiments. At the LOQ and ten times the LOQ, recoveries of the analytes were mostly within 70–120%, with coefficients of variation lower than 25% in intra-day repeatability conditions. In addition to being simple and fast, these results demonstrate the suitability of the optimized method for the analysis of large scope pesticides in routine laboratories.

1. Introduction

Pesticide residue in food has to comply with the most stringent standards among food safety regulation. This is done in Europe by the European Commission which sets tolerated levels of these residues (MRL: maximum residue level) to ensure that they do not pose an unacceptable health risk for consumers. Since 2008, the MRLs have been harmonized for all the EU countries by the Regulation (EC) No 396/2005 [1]. The list of the MRLs for all the various commodities is easily accessible on the EU Pesticides database [2]. To guarantee that the food on the market is safe for consumption, official national and communitary monitoring programmes have been established on a yearly basis, with sampling targeting different commodities of the diet for evaluation. Laboratories that want to participate in the monitoring programme must demonstrate a certain level of performance (accreditation and participation in different proficiency or interlaboratory tests) with respect to EU guidelines for quality control procedures SANCO/12571/2013 [3]. A review of a decade of communitary monitoring programme results shows a considerable increase in the number of pesticides being sought in a broader range of commodities and a tendency towards reduced reporting limits due to the default MRLs (0.01 mg/kg) for non-approved pesticides. In 1997, only 13 molecules were mandatory for the monitoring programme, 17 years later the number is 188. This overall trend is a challenge for any laboratory involved in pesticide residue analysis in food.

In contrast to the 1970s, where targeted compounds were essentially amenable to gas chromatography (GC), the introduction of more polar pesticides on the market has triggered a large interest in liquid chromatography (LC) technique. Nowadays, to be able to guarantee sufficient scope for monitoring programs, routine laboratories use both techniques (GC and LC) with two analytical approaches; multi-residue method to determine hundreds of analytes in a single analysis and single residue method designed specifically to analyze compounds not compatible with the

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multi-residue method. Ideally, multi-residue method has to be as generic as possible, because performing many single residue methods on the same sample is time consuming and expensive, therefore not viable for the laboratory.

As any other analytical methods, multi-residue methods follow the basic analytical steps; sample preparation (homogenization, extraction and clean-up) and determination (separation and detection). Sample preparation is the cornerstone of any effective multi-residue method. There is a marked trend in recent years to have simple, easy to perform and fast procedures. A good example of such method is the quick, easy, cheap, effective, rugged and safe, known under its acronym QuEChERS [4]. This technique entails an acetonitrile extraction by hand shake or vortex, then partitioning is performed with the addition of anhydrous magnesium sulphate and sodium chloride followed by a clean-up to remove co-extractive compounds such as sugars and fatty acids using a dispersive solid phase extraction (d-SPE). Since its inception in 2003 [5], an adjustment was done by buffering the extract to enhance the recoveries of pH-dependant analytes. Lehotay et al. used acetate as buffer and the method became the AOAC Official Method 2007.01 [6]. Anastassiades et al. choose citrate as the buffer and this version is now the Standard Method EN 15662 [7]. Other more conventional methods have also modified their sample preparation procedure towards more straightforward protocols with fewer reduced number of steps. The popular Ethyl-acetate method has benefit from different variant since its introduction in 1987 [8]. The original version is a simple extraction with ethyl acetate, followed by gel-permeation chromatography (GPC) as clean-up. The addition of salt ($NaHCO_3$) was also necessary to yield good recovery of basic pesticides in acidic crops [9]. The method clean-up step was simplified by two approaches, the first one eliminated the GPC, and the second approach replaced the GPC with a dispersive SPE with PSA and graphitized carbon black [10]. These approaches were more environmentally friendly than GPC and helped to speed up the procedure. The QuEChERS and ethyl acetate methods are very popular in routine labs and part of that success is related to the possibility of GC and LC analysis with one extraction. In our laboratory, we already have been using a GC-based multi-residue method with acetone extraction, but it was urgent to widen our scope by implementing a LC method. With the acetonitrile shortening crisis, the QuEChERS method was less attractive in terms of cost and because we needed to weight all the different reagents for each sample the time factor was also a disadvantage. The Ethyl acetate method presented a weakness, as it required a solvent change to methanol before injection. As we were looking exclusively for a LC-based method, methanol as the extraction solvent would be a good fit to our objective. Two methanol-based multi-residue methods have been previously reported in the literature: one presented by Klein et al. [11] and the second by Granby et al. [12]. Unfortunately, the first one required a solid phase extraction clean-up with a Chem Elut column, and the elution of the analytes was done with dichloromethane. The second one, is easy to perform and straightforward: the analytes are extracted with a buffered methanol in an ultra-sonic bath for half an hour, then centrifuged and filtered before being injected. The method was validated for different matrices at different spiking levels, but only for 19 analytes. In spite of being a very attractive method, the recovery tests were conducted on spiked samples, therefore extraction efficiency on incurred pesticides was not evaluated. The present work addresses this problem and presents the adjustments made to the Granby protocol for UHPLC-MS/MS determination targeting a limit of quantification of 0.01 mg/kg for most of pesticides sought. Before implementation to routine analysis, the modified method was validated for 200 analytes in two matrices.

2. Experimental

2.1. Materials and reagents

Water was purified using a Millipore Milli-Q system (Millipore Corp., Bedford, MA, USA). Methanol and acetonitrile were Pesti-S grade from Biosolve (Nalkenwaard, The Nederlands). Ammonium acetate was of analytical grade and obtained from Fluka (Sigma, Boenem, Belgium). Pesticide analytical standards (purity >95%) were purchased from Dr. Ehrenstorfer (Ausburg, Germany). Whatman Mini-UniPrep Syringeless 0.20 μm filter vials were obtained from VWR (Leuven, Belgium). Green lettuce (*Lactua sativa)* as well oranges (*Citrus simensis*) are from organic production.

2.1.1. Standard solutions

Individual stock solutions were prepared at 1 mg/mL by weighting accurately 20 mg (0.1 mg) into a 20 mL volumetric flask. Dissolution of the compound and adjusting to volume were done with acetonitrile with 0.1% of acetic acid, methanol, methanol/water, acetonitrile, depending upon the solubility of the pesticide. Different standard mix solutions of 20–30 pesticides were prepared from the stock standard solutions at 10 μg/mL in methanol. A spike solution at 2 μg/mL was prepared with all the different mix solutions in methanol.

Internal standard stock solution of oxfendazole was prepared at 0.1 mg/mL in acetonitrile with 0.1% of acetic acid. A working internal standard solution of oxfendazole was prepared by diluting the stock solution to 10 μg/mL with methanol. All the solutions were stored at -20 °C.

2.1.2. Extraction solution & Mobile phases

The extraction solution is a 20 mM ammonium acetate in methanol/water (95:5; v/v) solution.

The composition of the mobile phases is 5 mM ammonium acetate in water/methanol (90:10 v/v) and 5 mM ammonium acetate in methanol/water (90:10; v/v) for respectively the mobile phases A and B.

2.2. Extraction procedure

A representative portion of each sample is comminuted at room temperature to a mash with a blender. In a 100 mL Erlenmeyer flask, 10 g (0.1 g) of homogenised sample is weighted. Then the extraction is carried out with 40 mL ammonium acetate 20 mM in methanol/water (95:5 v/v) blended with a high speed disperser (Ultra-Turrax®) for one minute. The extract is filtered through a glass Büchner funnel equipped with a paper filter 42.5 mm. The dispersing element of the Ultra-Turrax® is rinsed twice with 7 mL of the same extraction solution, and then passed through the same filtered for maximum recovery. The extract is transferred to a 100 mL glass cylinder with stopper, spiked with 200 μl of internal standard solution (at 10 μg/mL), volume adjustment to 60 mL with the extraction solution and shake for homogenisation. An aliquot of 3 mL is transferred to a glass tube and diluted with 2 mL of water. After 10 s of vortex, an aliquot is transferred into a mini-UniPrep Syringeless 0.20 μm filter vial ready for injection.

2.3. LC-MS/MS analysis

LC analysis was performed with an UPLC™ (Waters, Milford, MA) equipped with a mass spectrometer Quattro Premier™ (Waters). An ACQUITY™ BEH C18 column (1.7 μm; 2.1 × 100 mm) was used at 45 °C. The volume injected was 5 μL. The separation was performed at a flow rate of 0.45 mL/min, with a gradient elution starting at 0.1% of phase B, rising linearly to 99.9% phase B over