Microchemical Journal 134 (2017) 131–139

Contents lists available at ScienceDirect

Microchemical Journal

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

A chemometric cleanup using multivariate curve resolution in liquid chromatography: Quantification of pesticide residues in vegetables

Emanuella Santos Sousa, Licarion Pinto, Mario Cesar Ugulino de Araujo *

Laboratório de Automação e Instrumentação em Química Analítica e Quimiometria (LAQA) Universidade Federal da Paraíba, CCEN, Departamento de Química, Caixa Postal 5093, CEP 58051-970 João Pessoa, PB, Brazil

ARTICLE INFO

Article history:
Received 13 March 2017
Received in revised form 25 May 2017
Accepted 27 May 2017
Available online 29 May 2017

Keywords:
Pesticide residues
Vegetables
Liquid chromatography
Multivariate curve resolution
Chemometric cleanup

ABSTRACT

Both extraction and chemical cleanup are steps performed when the official AOAC 2007.01 QuEChERS method is used. The chemical cleanup step is required because many co-extractives may still be present after the whole samples extraction; however, it may reduce recovery of certain analytes due to non-selectivity of this step. In addition, unexpected constituents may still interfere with the analysis even when chemical cleanup is carried out, impairing univariate quantification. A chemometric cleanup using MCR-ALS is proposed as an alternative to the QuEChERS chemical cleanup. The performance of the proposed approach was demonstrated through quantification of seven pesticide residues (Carbendazim, Thiabendazole, Fuberidazole, Carbofuran, Carbaryl, 1-naphthol, and Flutriafol) in four non-spiked vegetable samples using HPLC-DAD. With the proposed strategy, it was possible to perform a reliable quantification despite the presence of co-eluted constituents (analytes and interferents), peak shift, band shape changes and avoiding the chemical cleanup with low analyte losses and LOD.

1. Introduction

Pesticides helps to increase the production of high quality fruits and vegetables by controlling the spread of pests during growth [1–2]. The benefits that pesticides bring to the world food supply are undoubted, but incorrect application brings harmful problems to human health. International regulatory and monitory agencies like, the European Commission (EC) [3], the Food and Drug Administration (FDA) [4], and the United States Environmental Protection Agency (EPA) [5] therefore regulate pesticide maximum residue limits (MRL) of many food products. In Brazil, a program of pesticide residue analysis under ANVISA management [6] does this control. Table 1S shows the MRL as established by the EC, the EPA and ANVISA for the studied analytes and samples.

Due to the ability to enhance detector selectivity by separation of constituents present in the sample, chromatography is the most often used technique for quantification of multiple pesticide residues in many samples [7,8,9]. However, when it comes to complex samples analysis, develop a method that assure full selectivity for the analytes in the detector is difficult, especially if a simple and non-selective diode array (DAD) or fluorescence (FLU) detectors are used. In chromatography, co-elution impairs univariate analysis if the detector cannot assure full selectivity, however, such detectors can still generate a huge amount of data that can be used to both qualify and quantify complex mixtures, even without full selectivity [9,10,11]. In a High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) hundreds spectra per second can be registered during a sample elution. The data can be arranged in a three-way array, that can be handled with proper algorithms and make it possible to quantify the analytes, even if they are co-eluted with an unexpected constituent [10].

Certain algorithms can be used to model second order data. However, to achieve good results, the analyst should use an algorithm that fits well with the obtained data. In liquid chromatographic data, a common drawback is the time misalignment frequently present between elutions, especially for complex samples [10–13]. To overcome this drawback, the analyst can use an algorithm that aligns the chromatograms [14,15], or use an algorithm that allows trilinearity deviation in this mode [10,11]. Second order chromatograms alignment is a challenge task, so it is preferable to use algorithms that are capable of suitably dealing with as chromatographic data that presents trilinearity deviation in the time mode as MCR-ALS, which is the only one with this characteristic and have figure of merit implemented [10,11].

Traditionally for liquid chromatography data, when second or higher order treatment is used to overcome the presence of unexpected constituents and background drawbacks, an isocratic elution is employed to avoid baseline profiles [11,13,16]. When it is necessary to quantify several analytes that have different retentions in the chromatographic column, a gradient elution is preferred. In cases of analytes having different retentions, the analyst can achieve narrower peaks and smaller chromatographic runs with a gradient elution that yields faster analyses and less solvent consumption as well, which is in accordance with green

* Corresponding author.
E-mail addresses: laqa@quimica.ufpb.br, mariougulino@gmail.com (M.C.U. de Araujo).

http://dx.doi.org/10.1016/j.microc.2017.05.017
0026-265X/

analytical chemistry principals [17]. However, as compared to isocratic runs, the MCR-ALS retrieved profile can be impaired by changes in gradient elution baseline profiles, due to the mixture of the solvents in the pump.

Most of the pesticides are retained in the fruit and vegetable peels, therefore, the QuEChERS method is a better approach to analyze food samples, due to its ability to extract the pesticide presence from the whole food (juice and peel), and not only from the liquid extracted from the sample. The official AOAC 2007.01 QuEChERS method [7,18,19] is employed for multiple pesticide residue quantification in solid, semisolid and liquid samples, and it is performed in two steps: extraction and cleanup [7]. In the extraction step, acetonitrile is used as an extractor solvent, magnesium sulfate for partition of the organic from the aqueous phase, and, sodium acetate to increase recovery (of some pesticides), and to adjust the extract's pH [7]. Since acetonitrile is a non-selective solvent, many co-extractives could be present in the sample extract, which may coelute and interfering with the analyte, impairing a univariate quantification. Therefore, sample cleanup is required as a second step. In this second step, Primary Secondary Amine (PSA), Octadecyl (C18), and Graphitized Carbon Black (GCB) are commonly used. These reagents are more expensive than those used in the extraction step, and increase the cost of analysis. This chemical cleanup step also increases analyte loss and reduces recovery for some analytes and samples due to the non-selectivity of the procedure [20], which is used to remove a great variety of interfering constituents. Avoiding this step therefore may result in better recoveries and better preservation of the sample integrity.

In the present study, HPLC-DAD with a chemometric approach is proposed as an alternative to LC-MS (liquid chromatography-mass spectrometry) for the quantification of pesticides residue in vegetables. To overcome interferences a chemometric cleanup using MCR-ALS modeling is proposed to substitute the official AOAC 2007.01 QuEChERS (post extraction) chemical cleanup step by chromatography analysis. In addition, a gradient elution is used in order to obtain faster analyses, less organic solvent waste, and narrower peaks for a lower limit of detection (LOD), especially of the constituents with higher retention times. Changes in gradient elution baseline impair the MCR-ALS convergence and retrieved profile, a blank solution was therefore eluted and its signal was subtracted from the sample data.

To demonstrate the performance of the proposed strategy, quantification of seven analytes, Carbendazim (CBZ), Thiabendazole (TBZ), Fuberidazole (FBZ), Carbofuran (CBF), Carbaryl (CBY), 1-naphthol (NPH) and Flutriafol (FLT) in un-spiked tomato, carrot, beet and lettuce was performed. To achieve a limit of detection (LOD) consistent to the MRL established by national and international official regulating agencies, the final extract was concentrated five times.

2. Theory

2.1. Multivariate curve resolution - alternating least squares

For more details about the MCR-ALS algorithm and the figure of merit applied to this model, see Tauler [21–22] and Olivieri [22–23]. Briefly, MCR-ALS is a bilinear decomposition method, which assumes additivity of the recorded signal for each individual constituent; similar to the generalized Lambert-Beer's law, it can be mathematically represented as in Eq. (1).

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{\mathrm{T}} + \mathbf{E} \quad (1)$$

For a single sample, **D** is the matrix of experimental data of size $j \times k$. For LC-DAD data j corresponds to the elution times (matrix rows), and k to the absorption wavelengths (matrix columns); **C** is the matrix containing the optimized concentration profiles of size $j \times n$; and $\mathbf{S}^{\mathbf{T}}$ is the matrix containing the optimized spectral profiles of size $n \times k$, where n is the number of factors for the MCR model, (informed by the analyst). The matrix **E** contains the data not modeled by MCR-ALS, of size $j \times k$. The **D** matrix should always be column-wise augmented with different samples, at the mode that breaks the tri-linearity of the data.

MCR-ALS is an algorithm that retrieves profiles for each individual constituent present at the data matrices. Therefore, it can be used for many purposes. When the objective is to predict the concentration of a chemical species, the **Daug** matrix of size $j \times k$, is composed of several i calibration standards with known compositions, and a sample with an unknown concentration. The scores (defined as the area under the concentration profile), or time in case of LC data, can be used to estimate the analyte concentration in the unknown sample likewise in a univariate chromatography. A linear fit is built between i scores of the calibration standards and their nominal (known) concentrations. This procedure is known as pseudo-univariate calibration [10,23], and the unknown sample concentration value is obtained by interpolation.

3. Materials and methods

3.1. Reagents

The standards of Carbaryl (CBY), Carbofuran (CBF), Carbendazim (CBZ), Flutriafol (FLT), Fuberidazole (FBZ), Thiabendazole (TBZ), and 1-Naphthol (NPH) were all of analytical grade and purchased from Sigma Aldrich. Acetonitrile of HPLC grade was purchased from J. T. Baker, and Milli-Q water (Millipore) was used in all experiments. Nitrogen 99.9% was purchased from the Linde Group. The AOAC 2007.01 commercial Kit for QuEChERS extraction method was purchased from Phenomenex.

3.2. Chromatographic runs

Chromatographic runs were performed on an Ultimate 3000 Dionex chromatograph, consisting of a quaternary pump, a manual injector fitted with a 20 μL fixed loop, and a UV–visible diode array detector. A Dionex Acclaim® 120 C18 column of 100 mm × 2.1 mm, 5 μm particle sizes, and 120 Å pore size was employed. A gradient elution was performed with purified Milli-Q water (solvent A) and acetonitrile (solvent B) as follows: 0–1 min: 10% B, from 1 to 6 min: linear gradient from 10 to 35% B and hold until 10 min, finally ramped to 10% B at 10–14 min. The temperature was fixed at 35 °C, and a flow rate of 0.5 mL min^{-1} was used. The data were collected using the software Chromeleon, Version 6.80 with a spectra acquisition frequency of 5 Hz.

3.3. Software

The data were handled using the MATLAB® 2010a computer environment. The calculations involving MCR-ALS and figure of merits were made using mvc2 [24], a MATLAB graphical user interface available at www.iquir-conicet.gov.ar/descargas/mvc2.rar. The rotational ambiguity calculations were made using the MCR-ALS graphical user interface 2.0 [21] available at http://www.mcrals.info/.

3.4. Calibration and test samples sets

A 320, 232, 250, 128, 126, 300, and 536 μg mL^{-1} stock standard solution (stock 1) was prepared for CBZ, TBZ, FBZ, CBF, CBY, FLT and NPH, respectively, by weighing an appropriated amount of each pesticide in a 100 mL volumetric flask, and then brought to the mark with methanol. A 50 μg mL^{-1} stock standard solution (stock 2) was prepared by taking an appropriated aliquot and diluting with acetonitrile. The standard solutions were stored at −20 °C until use. The calibration and test samples sets were prepared in acetonitrile/water (10:90 *v*/v) solution by diluting an appropriated aliquot of stock 2. Before use, the samples sets were always stored at 4 °C.

For the calibration set, eight mixtures were prepared for the seven pesticides according to each linear range. The concentrations varied