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Photochemically induced fluorescence coupled to second-order multivariate calibration as analytical tool for determining imidacloprid in honeybees

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ABSTRACT

This paper presents a method for the determination of imidacloprid in honeybees based on the measurement of excitation-emission spectra of photo-induced fluorescence (PIF-EEMs) associated to unfolded partial least squares coupled to residual bilinearization (U-PLS/RBL) algorithm. As a preliminary step, matrix solid phase dispersion (MSPD) using C18 as dispersant, combined on-line with a solid phase extraction (SPE) clean-up on graphitized carbon-amino propyl silica phase was applied to diminish the interferences presents in samples. A previous study on the photochemical induction of fluorescence of imidacloprid in presence of bee matrix was included. The second order advantage achieved with RBL permitted the determination of imidacloprid in the presence of interferences present in samples (unexpected compounds of bees), which also shows photo-induced or native fluorescence. The LOD was 20 μg kg^{-1} (2.5 ng per bee; four bees treated), which is suitable for detecting imidacloprid at the oral LD50 for the insect. The predicted U-PLS/RBL concentrations compared favorably with those measured using high-performance liquid chromatography with diode array detection. The PIF-EEMs coupled to U-PLS/RBL was applied for the determination of imidacloprid in honeybees collected in field hives. The work demonstrates the feasibility of the determination of imidacloprid in a highly complex sample matrix as bee through photochemically induced fluorescence spectroscopy coupled to multivariate calibration.

1. Introduction

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] belongs to the neonicotinoid insecticides, a new group of pesticides with properties that allow for their systemic distribution within plants after being absorbed by the leaves or roots. The major modes of application of these compounds are spraying and seed dressing, especially to control pests in crops, such as cereals, soybeans, corn and several fruits and vegetables. On the other hand, bees are the prominent and economically most important group of pollinators worldwide. The decline of pollinating species may lead to a parallel decrease of plant species or vice versa. More specifically, there is a great concern about the decline of honeybee (*Apis mellifera*) and the worldwide acute depopulation of hives called "Colony Collapse Disorder (CCD)", first named in 2007 [1]. In recent years it has been postulated that neonicotinoid pesticides could be a trigger of CCD. Some authors have done a wide overview on the effect of neonicotinoids on bees and their relation with CCD [2–4]. In the case of imidacloprid, honeybees that were feed with corn syrup containing this neonicotinoid showed symptoms consistent to CCD [5,6]; and field-realistic levels of imidacloprid reduce colony growth and queen production of bumble bee *Bombus terrestris*[7]. Thus, the use of these pesticides in agriculture has indubitable repercussions on the environment which has become a serious environmental concern.

The most relevant measures of exposure of bee to imidacloprid are the concentrations in bee-collected plant materials, such as pollen and bee products like bee bread, honey and beeswax, and in the bees themselves. The determination of pesticides in bees is difficult by the complexity of the matrix, the low levels of the analytes and the variety of interferents potentially present. Usually this entails the use of some sample pre-treatment as an essential part of the analytical process and a subsequent chromatographic determination [8,9]. Accordingly, the majority of the analyses of imidacloprid in bees involve an extraction followed by a clean-up step before a chromatographic determination using high-performance liquid chromatography with mass spectrometric (HPLC-MS) or tandem mass spectrometric (HPLC-MS/MS)

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detection [10–13]. The limits of detection reported ranged 0.2–4 μg kg^{-1}. Liquid chromatography with a diode array detector (HPLC-DAD) has also been used for the determination of imidacloprid in single bees with limits of detection of 7 ng per bee (approximately 50 μg kg^{-1}) [8] and 50 μg kg^{-1}[14]. On the other hand, the extraction and/or clean-up methods principally used to determine imidacloprid in bees are the solid-liquid extraction with acetonitrile, partition with hexane and a subsequent solid-phase extraction (SPE) with florisil [8] the QuEChERS method [8,12,13] and matrix solid phase dispersion (MSPD) [9,10].

Alternative methods based on the fluorimetry of a photoproduct of imidacloprid produced after the UV irradiation of an aqueous imidacloprid solution have been proposed for water analysis [15–18]. In aqueous media, imidacloprid does not exhibit native fluorescence; however, its irradiation with UV light results in a fluorescent signal [15]. However, the relevance of the fluorimetric methods has been limited by their lack of selectivity, especially when chemically similar compounds must be analyzed in a complex matrix. One approach to improve the analytical selectivity in this matrix would be the use of excitation-emission fluorescence measurements (three-way data set), in conjunction with different chemometric algorithms as parallel factor analysis (PARAFAC) or unfolded partial least square with residual bilinearization (U-PLS/RBL) to build a second-order calibration method. These methods permit the determination of the compounds of interest, without the use of chromatography, in a sample with overlapping spectral interferences that are not included in the calibration set (known as the second-order advantage) [19]. In a previous work we reported the use of photochemically induced fluorescence excitation-emission matrices (PIF-EEMs) coupled to PARAFAC and U-PLS/RBL for the determination of imidacloprid in water samples [20]. However, to our knowledge there are no available reports on the determination of imidacloprid in bee samples through photochemically induced fluorescence spectroscopy coupled to multivariate calibration. In this work U-PLS/RBL was applied to determine imidacloprid in bee samples using PIF-EEMs in presence of interferences from the matrix, associated to MSPD-SPE on graphitized carbon-amino propyl silica phase on-line as sample preparation step. At this point it is worth mentioning that bees is a very complex matrix, which represent an analytical challenge that we have solved presenting a new treatment of the sample and an additional study to assess whether the photo-induced fluorescence of imidacloprid is comparable in solvent and in matrix in order to validate the experimental conditions. The predicted U-PLS/RBL concentrations were compared with those obtained using high-performance liquid chromatography (HPLC) with UV–vis detection. The method was applied for the determination of imidacloprid in honeybees collected in field hives located in an area of great agricultural activity (maize and fruit trees) located in the central region of Chile.

2. Theory

2.1. UPLS/RBL

The U-PLS method is a variant of the classical partial least squares (PLS) that was proposed for second-order data where three-way data are unfolded into vectors before two-way PLS calibration. If the calibration was exact, the regression coefficients, v, could be employed to estimate the analyte concentrations in an unknown specimen using eq. (1),

$$y_u = t_u^T v \quad (1)$$

where t_u is the test sample score, which is obtained by projection of the vectorized (unfolded) data for the test sample $\mathbf{X}_u$ onto the space of the *A* latent factors, as indicated in eq. (2),

$$t_u = (\mathbf{W}^T\mathbf{P})^{-1}\mathbf{W}^T\, vec(\mathbf{X_u}) \quad (2)$$

where **P** and **W** are the matrix of loadings and weight loadings, respectively; vec(.) implies the vectorization operator and T the transposition operator.

When unexpected constituents occur in $\mathbf{X_u}$, the sample scores given by Eq. (1) are unsuitable for analyte prediction and the U-PLS method must be coupled to RBL to achieve the second-order advantage. RBL is a post-calibration procedure that is based on principal component analysis (PCA) to model the presence of unexpected constituents in a sample [21,22].

The RBL procedure consists maintaining the matrix of loadings **P** constant at the calibration values and varying t_u to minimize the norm of residual error. The standard deviation (s_{RBL}) of the residuals can be used as a measure of the goodness of fit (GOF) for the RBL procedure and to estimate the number of unexpected constituents according to Bortolato et al. [22]. In this approach the s_{RBL} is assumed to stabilize at a value compatible with the instrumental noise when the correct value of RBL components is reached [21,22].

3. Experimental

3.1. Reagents and solutions

Imidacloprid of high purity grade (99%) was obtained from Sigma-Aldrich (St. Louis. MO, USA). NaOH and Na_2HPO_4 were of analytical purity grade and obtained from Merck (Darmstadt, Germany). Hexane, dichloromethane, acetonitrile and methanol were of HPLC grade and purchased from Merck (Darmstadt, Germany). Supelclean® ENVI-Carb/aminopropyl-functionalized silica (500 mg/500 mg, 6cc) solid phase extraction (SPE) cartridges and Supelclean® ENVI-18 bulk packing for matrix solid phase dispersion (MSPD) were provided by Sigma-Aldrich (St. Louis. MO, USA).

Stock solutions of pure analyte (1000 μg mL^{-1}) and diluted solutions were prepared in acetonitrile. The stock solution was stored in amber vials at 4 °C in the dark. Under these conditions, the solution was stable for almost two months.

3.2. Apparatus and software

A Varian Cary-Eclipse luminescence spectrometer (Mulgrave, Australia) equipped with a xenon flash lamp was used to obtain excitation–emission fluorescent measurements. A Starna (Essex, England) quartz cell with a 700-μl inner volume and a 10×2-mm light path was used. The classic fluorescence spectra were recorded at λ_{exc} of 345 nm in the λ_{em} range of 365–700 nm every 2 nm at a scanning rate of 600 nm min^{-1} and 10 nm for emission slit. The EEMs were recorded in the λ_{exc} ranges of 220–400 nm every 5 nm and λ_{em} of 324–550 nm every 2 nm. The widths of the excitation and emission slits were 10 and 20 nm, respectively. The spectra were saved in ASCII format and transferred to a computer for subsequent manipulation. All chemometric computation and routines were implemented in Matlab v.7.6 (Mathworks, Natwick, MA). The routine for data pre-treatment used to eliminate Rayleigh and Raman scattering peaks from the EEMs was taken from Zepp et al., 2004 [23]. The routines used for PARAFAC [24] and U-PLS/RBL [25] are available on the internet. These algorithms were implemented using the graphical interface of the MVC2 toolbox, which is also available on the internet [26].

In order to visualize the photo-degradation of imidacloprid versus time of irradiation, the UV–vis spectra at different irradiation times were obtained. For this aim, the UV–vis absorbance spectra were recorded with an Agilet Cary 8453 spectrophotometer equipped with a photo-diode array, wavelength range from 200 to 500 nm with a 1 nm interval.

The HPLC-DAD analysis was carried out as reference method. The analyses were performed on a liquid chromatograph equipped with a Waters 600 HPLC pump, a Waters 996 diode array detector and a Waters 717 auto sampler (Milford, MA, USA). The column was an