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Chemometrically assisted optimization and validation of reversed phase liquid chromatography method for the analysis of carbamates pesticides

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ABSTRACT

A new procedure for the simultaneous separation of six carbamates pesticides by reversed phase high performance liquid chromatography (RP-HPLC) was optimized, developed and validated. An experimental design was planned for the optimization of the chromatographic conditions. It is based on changing gradually the experimental conditions all along the chromatographic procedure as a function of the physical properties of the eluted compounds.

A two level full factorial design 2^k was applied to detect interactions among variables to be optimized: percentage of acetonitrile, flow rate and temperature of column. These significant variables were optimized using Doehlert matrix. The optimum resolution was carried out at a flow rate of 1 mL/min, column temperature of 28.5 °C and acetonitrile/water (67:33% v/v) as mobile phase. The separation method was validated according to the International Conference on Harmonization (ICH) guidelines to confirm specificity, linearity, precision, detection and quantification limits. Hence it can be employed for the routines analysis in quality control laboratories.

1. Introduction

Carbamates are one of the major classes of the pesticides that are widely used in agriculture due to their broad biological activity [1,2], low bioaccumulation potentials [3] and relatively low mammalian toxicities. However, as inhibitors of acetylcholinesterase [4,5], carbamates pesticides could affect nerve impulse transmission, inducing neurologic toxicity, chronic neurodevelopment impairment, possibly dysfunction of the immune, reproductive and endocrine systems or cancer and many others. So carbamates pesticides are included on the priority list released by the United States Environmental Protection Agency (EPA) [6]. Carbamates and their metabolites find their way into the human body through the food chain [7,8] and the water cycle [9–11]. The increasing public concern in recent years about possible health risk due to pesticide residues in the diet has deeply modified the strategy for the crop protection, with emphasis on food quality and safety, and the widespread concern for the health of society led to the strict regulation of maximum residue limits of pesticide residues in food commodities. Several analytical methods, such as gas chromatography (GC) [3,12,13], enzyme-linked immune sorbent assays (ELISAs) [14,15], micellar electrokinetic chromatography (MEKC) [16,17], biosensor [18] and high performance liquid chromatography (HPLC) [19–26], have been reported for the separation and quantification of carbamates residues in different matrices. However, the thermal instability of carbamates, limits the use of GC, which exhibits derivatization prior to analysis. For this reason, HPLC is an obvious choice for the simultaneous determination of carbamates pesticides. As regards detection, UV detection is the most widely applied in LC analysis. Chromatographic analysis of trace levels of pesticides usually depend on the step of compound separation and step of compound quantification. It is not a simple task to find optimal analytical conditions due to the large number of variables involved in the separation process. The traditional approach for optimization of experiments is time-consuming, involves a large number of runs and does not allow establishing the multiple interacting parameters. For this reason it is usually more effective and time saving to resort to experimental design procedures [27–30]. In the recent years multivariate techniques have been used for optimization of chromatographic separation methods [31–34]. These techniques allow more than one variable to be optimized simultaneously and have several advantages, such as speed of analysis, practicality, economy and reduction of number of experiments that need to be carried out [35]. In addition, these methods are able to generate mathematical models that permit to estimate the relevance as well as statistical significance of the factors effects on the processes and also evaluate the interactions effects among the factors. Factorial design is one of the available statistical processes for multivariate optimization and is widely applied in chemistry due to its usefulness in the identification of the significant variables and the best

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conditions of an experiment. However, in order to determine the real functionality established among the analytical response and the significant factors, second order designs are used.

Most of the optimized separation methods cited in the literature for the analysis of carbamates insecticides by RP-HPLC involve a variation of a large number of variables in the separation process. To our knowledge, no paper carried out changes of experimental conditions all along the chromatographic run, for the separation of carbamates pesticides. For this reason it is more effective and time saving to resort to experimental design procedure. Therefore, the objective of this work is to develop, optimize and validate a liquid chromatographic method for the separation of six carbamates pesticides on stationary phase with an embedded $C_{18,}$ by means of experimental design. A two level full factorial design was used to evaluate the experimental variables including percentage of acetonitrile, flow rate and temperature of column. The experiments for the optimization were performed according to Doehlert matrix. Responses of three factors are presented in the entire experimental studied field.

2. Experimental section

2.1. Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. HPLC acetonitrile, methanol and water were obtained from LABSCAN (Dublin, Ireland), bendiocarb (99%), carzol (99%), pirimicarb (99%), methiocarb (99%), barban (99%) and terbucarb (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The chemical structures of all studied compounds are shown in Fig. 1. Stock solutions of 1000 μg mL^{-1} of each compound were prepared in methanol. These solutions were stored in a refrigerator at 4 °C and were used to prepare the working solutions at different concentrations. The standard solutions were warmed to room temperature of 20 °C prior to injection. All solutions were degassed using a Branson ultrasonic bath sonicator. Prior to the injection of the pesticide solution, the column was equilibrated by pumping the mobile phase flowing through the system for at least 30 min.

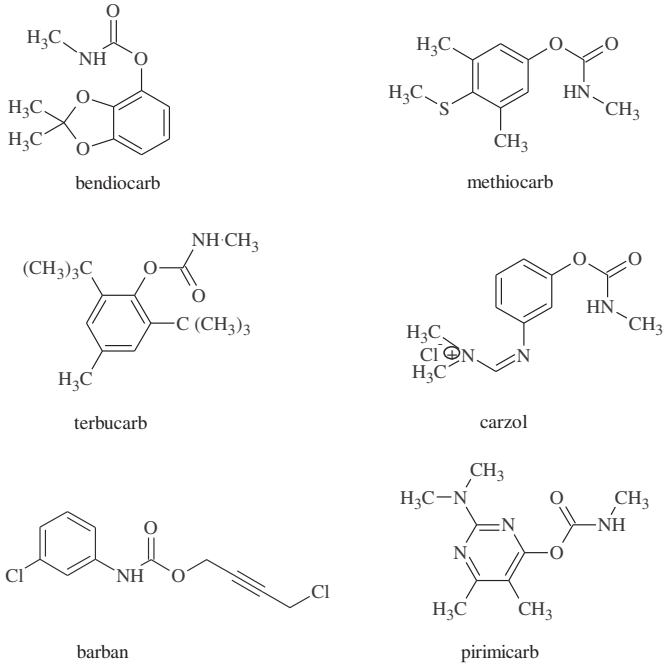

Fig. 1. Structures and common names of selected carbamates.

2.2. Instrumentation and chromatographic conditions

The analysis of each carbamate was carried out on a Varian G 1600 A HPLC system, equipped with a column oven (25 to 90 °C) and a binary gradient pump (4 to 400 bar). Injection was performed using a manual injector with a loop injector with an injection capacity of 20 μL. The detector consisted of a diode array DAD UV–Visible detector (196 to 800 nm). Separation was carried out on an Inertsil ODS-3 LC 18 column with 250 × 4.6 mm I.D. and 5 μm particle size. The work was carried out in an air conditioned room, maintained at a temperature of 22 ± 2 °C. The mobile phase for HPLC analysis was prepared from methanol and water (v/v) or acetonitrile and water (v/v). The water used for preparation of the mobile phase was sonicated using a Branson ultrasonic bath and then filtered through a Cronus Nylon 0.45 μm membrane filter. The mobile phase was also degassed using a Branson ultrasonic bath sonicator.

3. Results and discussion

3.1. Effect of the experimental parameters on the resolution by means of the experimental design methodology

The operational parameters affecting resolution, selectivity, sensitivity, and efficiency in HPLC are the column stationary phase (type and percentage of loaded carbon), the column oven temperature, the type of organic modifier in mobile phase, the amount of modifier in mobile phase, the speed flow of the mobile phase and the diameter of the stationary phase particles. As mentioned in the introduction, the column stationary phase and the diameter of the stationary phase particles are fixed. C_{18} stationary phase was usually recommended for carbamates analysis [10]. The analytical column is 25 cm in length and the size of the stationary phase particles is 5 μm, which corresponds to usual combination according to the literature [36]. The organic modifier is acetonitrile because other solvents (methanol for example) have higher viscosity causing too high pressures inside the column [37]. Three main factors were chosen in our study: amount of modifier in mobile phase (U_1), speed flow of the mobile phase (U_2) and the column oven temperature (U_3). Among all optimization of carbamates pesticides separation resolution is the selected criterion.

The experimental setup for studying factors' effects and evaluates the interactions' effects can be either sequential (e.g. simplex algorithm) or simultaneous (e.g. factorial design). A factorial model was selected for the optimization of RP-HPLC parameters, rather than simplex methods or other search algorithms, because the number of experiments is known in advance, an empirical (polynomial) model can be derived, the statistical significance of the parameters (variables) can be tested, and the optimum may be calculated by differentiation of the model functions constructed [38]. In factorial designs the variables (k) can be adjusted at fixed levels [38]. Since interactions between variables cannot be ruled out, full factorial designs with several levels for the following variables must be applied.

A two level full factorial design 2^k was carried out to determine the influence of these three selected factors and their interaction. In these types of designs, variables (k) are set at two levels (minimum) and (maximum) normalized as (−1) and (+1). The experimental response (Y) associated to a 2^k factorial design (for three variables) is represented by a linear polynomial model with interaction

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

where is the Y the experimental response the X_i the coded variable (−1 or +1) the b_i the estimation of the principal effect of the factor i for the response Y and b_{ij} the estimation of interaction effect between factor i and j for the response Y.

The coefficients of the equation model were calculated in the experimental field listed in Table 1. The choice of the limits of the investigated