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Short communication

Optimization of chemometric approaches for the extraction of isorhamnetin-3-*O*-rutinoside from *Calendula officinalis* L.

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ABSTRACT

The application of Design of Experiments (DoE) to the determination of optimum conditions for an extraction process relies on the correct selection of mathematical models. The linear model is the one typically used; however, in some cases it does not always have superior performance, ignoring the real nature of the data and its appropriate descriptive model. In order to evaluate the extraction efficiency of isorhamnetin-3-*O*-rutinoside from flowers of *Calendula officinalis* L. a multivariate factorial analysis was used. Simulations were conducted using linear, quadratic, full cubic and special cubic models. A Simplex-Centroid design was chosen as it delivered greater precision with only minor errors versus other models tested. Analyses were performed by capillary zone electrophoresis using sodium tetraborate buffer (40 mmol L^{-1}, pH 9.4) containing 10% methanol. The detection was linear over a range of 8.0–50.0 mg L^{-1} ($r^2 = 0.996$), and the limits of detection (LOD) and quantification (LOQ) for isorhamnetin-3-*O*-rutinoside were 3.44 mg L^{-1} and 11.47 mg L^{-1}, respectively. The full cubic model showed the best extraction results, with an error of 3.40% compared to analysis of variance, and a determination coefficient of 0.974. The difference between the responses at the reference point, calculated by the model, and the experimental response, varies around 2.72% for full cubic model. Comparison of the four models showed the full cubic model was the most appropriate one, allowing greater efficiency in the extraction of isorhamnetin-3-*O*-rutinoside. Selection of the model made it possible to obtain a 60% increase in sensitivity compared to the linear model.

1. Introduction

Calendula officinalis L. (Asteraceae) or marigold, is a versatile plant used in pharmaceutical and cosmetic products because it has many pharmacological properties [1–3]. The extract is obtained from dried flowers and is rich in flavonoids, especially isorhamnetin-3-*O*-rutinoside [4–8] that is found in high concentration and is responsible for antioxidant and wound healing activities [3].

Extraction of the active ingredient is a critical point as it directly influences the therapeutic effects of the crude extract. The nature and proportion of each solvent has direct influence on the extraction efficiency [9,10]. Arend et al. [9] used a quadratic model to evaluate the interactions between the ethanol concentration, time of drug:solvent contact, temperature and the presence of a preservative to determine the optimum conditions for the extraction yield on chlorogenic acid and caffeic acid in *Cecropia glaziovii* Sneth extracts. The statistical model showed a variation between the predicted and measured properties of less than 3%. Gong et al. [10] used Response Surface Methodology (RSM) to optimize the extraction parameters for total flavonoids and total phenolics in extracts of marigold in which the antioxidant activity was the strongest. They used ethanol concentration, temperature and extraction time as independent process variables, also applying a quadratic model, and concluded that ethanol concentration and temperature were the dominant factors in increasing antioxidant activity of the marigold extracts. However, Muley et al. [3] detected antioxidant activity in propylene glycol extracts of the petals and flower heads of marigold. In addition to ethanol concentration, we also evaluated the influence of propylene glycol and water as independent process variables. The results were evaluated using four different models of experimental design to determine the optimal solvent proportions

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producing the greatest yield of flavonoids in the marigold flower extract.

Analyses of flavonoids and phenolic compounds in marigold have been performed by chromatographic techniques, particularly high performance liquid chromatography [4,6,11,12], using reverse phase usually. Capillary electrophoresis (CE) has also been used for the analysis of plant extracts, since it allows direct injection of the sample without the need for time-consuming cleaning procedures [4,13–16]. The analysis of flavonoid has been conducted by CZE or MEKC using borate buffer. Borate can form complexes with vicinal hydroxyl from carbohydrates and has also an ability in the complexation of polyphenols aglycones forming charged ions with five or six membered-ring complexes, increasing the selectivity of separation [16,17].

Several multivariate chemometric-based techniques have been developed to aid in the optimization of a given system's performance [18,19]. There are several strategies used for the implementation of chemometric experimental design. In this case, we applied the technique of mixture design,

$$\sum_{i=1}^{q} x_i = 1$$

where q is the number of mixture components.

One simple way in which the proportions of the mixture may be displayed is through triangular graphs (ternary) [18,19]. The effects of components of the ternary mixture can be described by typical regression models:

$$\textit{Linear}: \hat{y} = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$$

$$\textit{Quadratic}: \hat{y} = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 \, \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

$$\textit{Special Cubic}: \hat{y} = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3$$

$$\textit{Full Cubic}: \hat{y} = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \delta_{1\,2} x_1 x_2 (x_1 - x_2) + \delta_{1\,3} x_1 x_3 (x_1 - x_3) + \delta_{2\,3} x_2 x_3 (x_2 - x_3) + \beta_{123} x_1 x_1 x_2 x_3$$

where $\hat{y}$ is the true response function, βij are called regression coefficients, xi are called "coded variable", δij's are also parameter of the model. This empirical model is called a 'response surface model' or Triangular Surfaces for Mixture Designs.

Methods commonly employed for experimental design are Simplex-Lattice and Simplex-Centroid [20]. Simplex is a strategy where m + 1 equally spaced ratios are tested for each factor or component in the model: x_i = 0, 1/m, 2/m,..; where i = 1,2,. . ..,q and all combinations of levels of factors are tested. Simplex-Centroid design points correspond to all permutations of the pure blends (e.g., 0 1 0, 1 0 0, 0 0 1), the permutations of binary blends (0.5 0 0.5, 0 0.5 0.5, 0.5 0.5 0), the permutations of mixtures involving three component and central points. The addition of points near the central point generally improves the resolution of the model and enables the plotting of points on a scatter chart with triangular coordinates, which allows verification of whether the experimental design covers the region defined by the triangle uniformly [21].

The linear model, due to its simplicity, is the one typically used to describe the variability of response in the design space as a function of the investigated factors. However, in some cases, the linear model does not always have superior performance and often seeks adjustment strategies for linearization, ignoring the nature or the real characteristics of the data and its appropriate descriptive model. In this study, we have shown the importance of evaluating different models for Design of Experiments (DoE) and how they can influence the quality of response for flavonoid extraction from marigold. The Simplex-Centroid approach was applied using water, ethanol and propylene glycol as independent process variables. The linear, quadratic, full cubic and special cubic models were evaluated in the optimization of extraction mixtures for isohramnetin-3-*O*-rutinoside in extracts of marigold flowers.

2. Experimental

2.1. Materials

2.1.1. Chemicals

Ethanol, methanol and propylene glycol were obtained from Synth (Rio de Janeiro, Brazil). Rutin, chlorogenic acid, quercetin, caffeic acid, kaempferol, *p*-hydroxycinnamic acid, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-glucoside, and isorhamnetin aglycone were obtained from Merck (Darmstadt, Germany). Sodium tetraborate was purchased from Mallinckrodt (Paris, France). Ultrapure water was generated using a Milli-Q system from Millipore (Bedford, USA). All reagents were of analytical grade.

2.2. Equipment

CE analyses were performed using an HP^{3D} CE capillary electrophoresis system (Agilent; Palo Alto, USA), equipped with a diode array detector monitored at 350 nm with the temperature controlled at 25 °C and an HP ChemStation. Samples were injected by pressure at 50 mbar for 3 s and the electrophoresis system was operated at constant voltage (+20 kV). The fused-silica capillary with the following dimensions: total length 63 cm; effective length 55 cm; 75 μm i.d; 365 μm o.d. was obtained from Polymicro Technologies (Phoenix, USA). CE running buffer consisted of sodium tetraborate 40 mmol L^{-1}, pH 9.4; 10% MeOH.

2.3. Methods

2.3.1. Extraction

Dried marigold flowers were obtained from Farma Service (São Paulo, Brazil). 2.2 g of dried marigold flowers were extracted with 48 mL of solvent consisting of different proportions of ethanol, water and propylene glycol. Extracts were prepared using an IKA T25 Ultra-Turrax Homogenizer (Labortechnik; Wasserburg, Germany) at a speed of 11,000 rpm. The mixture was shaken for 1 min in 15-min intervals for 1 h. All samples were subsequently filtered through a 0.22 μm membrane filter (Millipore Corporation; Bedford, USA). Extractions were performed in duplicate and each extracts analyzed in triplicate. After extraction, all extracts were diluted 1:5 with water.

2.3.2. Experimental design

The three solvents examined in the experimental design for extraction optimization were Ethanol (E), Propylene glycol (P) and Water (W). The dependent variable (I) was the concentration of isorhamnetin 3-*O*-rutinoside. Three replicates for each solvent system were performed (Table 1).

2.3.3. Analytical procedures

Before CE analyses, the capillary was flushed with 1 mol L^{-1} NaOH solution (15 min), followed by deionized water (15 min) and electrolyte (30 min). Between each run, the capillary was reconditioned by flushing with the electrolyte solution (high pressure, 3 min). Stock solution of isorhamnetin-3-*O*-rutinoside (1000 mg L^{-1}) in ethanol was prepared and stored at −18 °C.