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Chemometrical evaluation of ropinirole and its impurity's chromatographic behavior

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ABSTRACT

The aim of this study was the chemometrical evaluation of ropinirole and its impurity's (4-[2-(dipropylamino)ethyl]-1H-indol-2,3-dione) chromatographic behavior in systematic and the most efficient way. For that purpose, as very descriptive, response surface designs are most preferable. Face-centered central composite design (CCD) with 2^3 full factorial design, ±1 star design and four replication in central point was applied for a response surface study, in order to examine in depth the effects of the most important factors. Factors—independent variables (acetonitrile content, pH of the mobile phase and concentration of sodium heptane sulfonate in water phase) were extracted from the preliminary study and as dependent variables five responses (retention factor of ropinirole, retentin factor of its impurity, resolution, symmetry of ropinirole peak and symmetry of impurity peak) were selected. For the improvement of method development and optimization step, Derringer's desirability function was applied to simultaneously optimize the five chosen responses. The procedure allowed deduction of optimal conditions and the predicted optimum was acetonitrile–5 mM of sodium heptane sulfonate (21.6:78.4, v/v), pH of the mobile phase adjusted at 2.0 with *ortho* phosphoric acid. By calculating global desirability's determination coefficients (R_D^2), as well as by the visual inspection of 3D graphs for global desirability, robustness of the proposed method was also estimated.

1. Introduction

In the past few years the most important problem in liquid chromatography became the proper evaluation of analyte's chromatographic behavior and the optimization of chromatographic conditions, as well as the estimation of the robustness of selected optimal conditions. As it could be expected the defined goals should be reached in the most efficient and reliable way. The statistical experimental designs are most widely used for this purpose in order to avoid too expensive and time-consuming trials [1]. The data are collected following an experimental design and models fitted to the data usually by multiple polynomial regressions. Also, regulatory guidelines for the drug quality control strictly require robustness testing of the proposed methods and for that reason many authors used experimental design in robustness/ruggedness testing [2,3].

The aim of this paper was the evaluation of chromatographic behavior of ropinirole (4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one) and its impurity *C* (4-[2-(dipropylamino)ethyl]-1H-indol-2,3-dione) using the appropriate experimental design. The analysis of ropinirole and structurally related impurities originating from the synthesis procedure, as well as the impurity *C* as the possible degradation product, has already been published in the paper describing capillary zone electrophoresis (CZE) for dissociation constants determination, separation and quantification [4]. For the separation and quantification of ropinirole and some impurities capillary liquid chromatography was also proposed [5]. Three impurities in ropinirole hydrochloride drug substance were isolated and analysed by RP-HPLC, as well as characterized on the basis of appropriate IR, NMR and MS spectra [6]. For the analysis of ropinirole in tablets spectrofluorimetric [7] and spectrophotometric [7,8] methods were used. For the analysis of ropinirole in biological samples [9] and tablets [10] HPLC method was developed.

Impurity *C* is the oxidative product of ropinirole and structurally most similar with it. Hence, their chromatographic separation may be problematic, especially burdened by the difference in the concentration between ropinirole and impurity *C*. For the evaluation of their chromatographic behavior face-centered central composite design (CCD) was selected because of its efficiency and flexibility. The novelty was the improvement of method development and optimization step accomplished by the application of Derringer's desirability function to simultaneously optimize the five chosen responses. The procedure allowed not only the deduction of optimal

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Table 1
Plan of experiment for face-centered CCD and experimentaly obtained responses.

Factor levels			Responses				
x_1	x_2	x_3	k_C	k_R	R	Sym_C	Sym_R
15 (−1)[a]	2.0 (−1)	1.5 (−1)	12.35	14.43	3.01	1.03	2.28
25 (+1)	2.0 (−1)	1.5 (−1)	1.51	1.69	0.48	1.14	0.91
15 (−1)	3.0 (+1)	1.5 (−1)	20.39	23.11	2.90	1.09	4.04
25 (+1)	3.0 (+1)	1.5 (−1)	2.23	2.27	0.07	1.38	1.66
15 (−1)	2.0 (−1)	6.0 (+1)	20.85	24.66	3.47	1.05	2.29
25 (+1)	2.0 (−1)	6.0 (+1)	2.04	2.12	0.00	1.06	1.30
15 (−1)	3.0 (+1)	6.0 (+1)	27.34	31.68	2.89	1.07	2.85
25 (+1)	3.0 (+1)	6.0 (+1)	2.62	2.64	0.00	1.09	1.58
15 (−1)	2.5 (0)	3.0 (0)	20.28	24.32	4.34	0.98	1.24
25 (+1)	2.5 (0)	3.0 (0)	3.39	3.64	0.97	1.00	1.62
20 (0)	2.0 (−1)	3.0 (0)	7.72	9.13	3.57	0.99	1.17
20 (0)	3.0 (+1)	3.0 (0)	11.94	13.90	3.45	1.01	1.32
20 (0)	2.5 (0)	1.5 (−1)	6.34	7.43	1.92	0.99	1.25
20 (0)	2.5 (0)	6.0 (+1)	12.14	14.22	1.31	1.00	1.22
20 (0)	2.5 (0)	3.0 (0)	5.10	5.67	1.15	1.10	2.10
20 (0)	2.5 (0)	3.0 (0)	5.05	5.12	1.45	0.80	1.80
20 (0)	2.5 (0)	3.0 (0)	4.23	4.95	1.36	0.95	2.00
20 (0)	2.5 (0)	3.0 (0)	5.35	5.92	1.4	1.20	2.20

x_1: content of acetonitrile (%); x_2: pH of the mobile phase; x_3: content of sodium heptane sulfonate (mM); k_R: retention factor for ropinirole; k_C: retention factor for impurity *C*; R: resolution; Sym_R: symmetry of ropinirole peak; Sym_C: symmetry of impurity *C* peak.

[a] In the parentheses coded values for factor levels are given.

conditions, but also the estimation of method robustness by calculating global desirability's determination coefficients (R_D^2), which is used as the measure of global desirability function statistical quality, and by constructing the appropriate 3D graphs for global desirability.

2. Experimental

2.1. Chromatographic conditions

The chromatographic system *Waters* Breeze consisted of Waters 1525 Binary HPLC Pump, *Waters* 2487 UV/VIS detector and Rheodyne injector valve with 20 μL sample loop. Breeze Software, Windows XP was used for data collection. All reagents used were of an analytical grade. Working standards of ropinirole and impurity *C* were obtained from the *GlaxoSmithKline*, London, UK. Separations were performed on the X-Bridge™ 3.0 mm × 100 mm, 3.5 μm particle size column with UV detection at 250 nm. Mobile phases were prepared according to plan of experiments given in Table 1. The resulting mobile phases were filtered through a 0.45 μm membranes filter *Alltech* (Loceren, Belgium). Flow rate was 1 mL min^{-1} and the column temperature 30 °C.

2.2. Software

Experimental design, data analysis and desirability function calculations were performed by using Design-Expert® 7.0.0 (Stat–Ease Inc., Minneapolis).

2.3. Standard solutions

Stock solutions of ropinirole and impurity *C* were prepared in the mixture of acetonitrile–water (25:75, v/v) to obtain the concentration of 1 mg mL^{-1} and 10 μg mL^{-1}, respectively. The prepared stock solutions were stored at 4 °C. Solutions for the chemometrical evaluation were prepared in the appropriate mobile phase to obtain the concentration of 100 μg mL^{-1} of ropinirole and 1 μg mL^{-1} of impurity *C*.

3. Results and discussion

The majority of very important aspects of method presumed for drug quality control may be evaluated by response surface designs, i.e. methodologies. Response surface methodology (RSM) is a collection of mathematical and statistical techniques based on fit of the polynomial equation to the experimental data, which must describe the behavior of the data set with the objective of making the statistical provisions [1]. It can be especially valuable in case when a set of responses of interest is influenced by several variables. The objective is to simultaneously weight the levels of these variables to attain the best system performance defined by the researcher.

The first step is the selection of the appropriate experimental design that will define which experiments should be carried out in the experimental region of interest. The simplest model which can be used in RSM is based on linear function when the responses obtained are well fitted to the following equation:

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \varepsilon \quad (1)$$

where k is the number of variables, b_0 is the constant term, b_i represents the coefficient of the linear parameters, x_i represents the variables, and ε is the residual associated to the experiments. In such case the response should not present any curvature.

To evaluate curvature, a second-order model must be used. Two-level factorial designs are used in the estimation of first-order effects, but the addition of central points in two-level factorial designs is used to evaluate curvature. Polynomial model (Eq. (2)) obtained in such a way contains additional term which describes the interaction between the different experimental variables (second-order effects):

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{1 \le i \le j}^{k} b_{ij} x_i x_j + \varepsilon \quad (2)$$

where b_{ij} represents the coefficients of the interaction parameters.

In order to determine the critical point (maximum, minimum or saddle), it is necessary for the polynomial function to contain quadratic terms according to the equation presented below:

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} b_{ij} x_i x_j + \varepsilon \quad (3)$$

where b_{ii} represents the coefficients of the quadratic terms.

To estimate the parameters in Eq. (3) the experimental design must assure that all studied variables are examined in at least three factor levels. Adequate designs are second-order symmetrical designs such as three-level factorial design, Box–Bahnken design, CCD and Doehlert design [11]. In this study face-centered CCD with 2^3 full factorial design, ±1 star design and four replication in central point was used because of its efficiency and flexibility. Plan of experiments is presented in Table 1.

The independent variables were defined during the preliminary study. Some chromatographic parameters were excluded from the beginning, such as flow rate and column temperature, as their influence can usually be easily predicted by the common sense and chromatographic theory knowledge. Acetonitrile content should normally be always evaluated, but the selection of other two factors was questionable. The pH of the mobile phase proved to have important influence on the resolution of analytes. Retention factors were not so affected by this variable, but the difference in their concentration stipulated the investigation of resolution as well as the retention factors. The investigation of analyte's peak symmetry was