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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1842–1848
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Multi-criteria decision making approach and experimental design as chemometric tools to optimize HPLC separation of domperidone and pantoprazole

T. Sivakumar [a], R. Manavalan [a], C. Muralidharan [b], K. Valliappan [a,*]

[a] *Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalainagar, TN 608 002, India*
[b] *Department of Manufacturing Engineering, Faculty of Engineering and Technology, Annamalai University, Annamalainagar, TN 608 002, India*

Received 6 October 2006; received in revised form 24 November 2006; accepted 9 December 2006
Available online 16 December 2006

Abstract

This paper deals with multiple response simultaneous optimization using the Derringer's desirability function for the development of a reversed-phase HPLC method for the simultaneous determination of domperidone and pantoprazole in commercial pharmaceutical preparations. Twenty experiments, taking the retention factor of the first peak, the two resolutions, and three retention times as the responses with three important factors, mobile phase composition, buffer molarity and flow rate, were used to design mathematical models. The experimental responses were fitted into a second order polynomial and the six responses simultaneously optimized to predict the optimum conditions for the effective separation of the studied compounds. The optimum assay conditions were: methanol–acetonitrile–dipotassium hydrogen phosphate (pH 7.0; 15.3 mM) (20:33:47, v/v/v) as the mobile phase and at a flow rate of 1.19 ml/min. While using this optimum condition, baseline separation with a minimum resolution of 2.0 and a run time of less than 6 min were achieved. The method showed good agreement between the experimental data and predictive value throughout the studied parameter space. The optimized assay condition was validated according to ICH guidelines to confirm specificity, linearity, accuracy and precision.

Keywords: Multiple response optimization; Derringer's desirability function; Reversed-phase HPLC; Central composite design; Domperidone; Pantoprazole

1. Introduction

Reversed-phase high performance liquid chromatography (RP-HPLC) is a well-known technique exceptionally for the simultaneous determination of pharmaceutical dosage forms. Since HPLC utilizes a wide selection of chromatographic factors, viz., the type and concentration of organic modifier, pH, buffer molarity, temperature, flow rate, etc., optimization of the experimental conditions is a complicated process. Therefore, a systematic approach such as experimental design to optimize chromatographic separations is more essential [1,2]. The best experimental design approach for the purpose of modeling and optimization are the response surface design [3].

However, the HPLC method intended to be applied for the pharmaceutical or industrial environment, the analysis time is usually optimized without losing resolution [4]. When one needs to optimize more than one response at a time the use of multi-criteria decision making (MCDM), a chemometric technique is the best choice. Chemometrics can be used to accomplish a variety of goals in chromatography laboratory: (i) speeding methods development, (ii) make better use of chromatographic data and (iii) explain the chromatographic process [5]. The different approaches of MCDM [6] include the path of steepest ascent, constrained optimization procedure, Pareto-optimality, utility function, Derringer's desirability function. The path of steepest ascent can be employed only when all the response models are linear. Constrained optimization procedure can be used when all response models are non-linear, or when there is a mix of linear and non-linear responses. However, this method optimizes only one response by targeting all other responses to appropriate constraints. When there is a mix of linear and

* Corresponding author. Tel.: +91 4144 239738; fax: +91 4144 238145.
E-mail address: kvalliappan@gmail.com (K. Valliappan).

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doi:10.1016/j.jpba.2006.12.007

non-linear responses, or when all response models are of linear or non-linear, Pareto-optimality, utility function or Derringer's desirability function can be used. Pareto-optimality method can basically identify the Pareto optimal region by graphical means, but requires some additional criterion or the advice of an expert to select one particular Pareto optimum point [7]. The Pareto-optimal method and the Derringer's approach have their own advantages and that the decision on which method to use depends on the problem and the availability of chromatographic expertise.

There are many ways in which the individual desirabilities can be combined. If the combined criterion is a simple arithmetic average, it is called as utility function and if it is a geometric mean it is referred as Derringer's desirability function. The idea of combining desirabilities as geometric mean was first presented by Harrington [8] but it was put into a more general form by Derringer [9]. The advantage of the Derringer's desirability function is that if one of the criteria has an unacceptable value, then the overall product will also be unacceptable, while for the utility functions, this is not the case. Further, Derringer's method offers the user flexibility in the definition of desirability functions. Derringer's desirability function was introduced in chromatography by Deming [4], implementing resolution and analysis time as objective functions to improve separation quality. Safa and Hadjmohammadi [10] employed Derringer's desirability function for the simultaneous optimization of resolution and analysis time in micellar liquid chromatographic separation of a group of nine phenyl thiohydantoin amino acids. Recently, Hayashi and Matsuda [11] proposed a chemometric tool based on the Function of Mutual Information (FUMI) theory to improve prediction of the uncertainty in HPLC. Kotani et al. [12] employed FUMI theory for the prediction of measurement R.S.D. and detection limits in HPLC-electrochemical detection of catechins without repetitive measurement of chromatograms, saving considerable amounts of chemicals and experimental time. Among the various above options, the Derringer's desirability function was applied to explore the user flexibility of this technique in selecting optimum chromatographic conditions for the determination of drugs in a variety of sample matrices.

Domperidone (DP) (Fig. 1) is a potent dopamine antagonist used for the treatment of nausea and vomiting, and pantoprazole (PP) (Fig. 1) is a selective and long-acting proton pump inhibitor used for the treatment of acid-related gastrointestinal disorders. Nowadays, the mixtures of these active components are present in pharmaceutical formulations as capsules and tablet forms. DP maleate is official in British Pharmacopeia [13] in which a HPLC-UV method is available for its separate determination in tablets. PP sodium is not official in any of the pharmacopoeias. On the other hand, several methods have been cited in the literature for the estimation of DP [14–17] and PP [18–20] individually. Owing to the presence of interferences or time-consuming analysis, the determination of these analytes in samples containing mixtures is not possible if analytical methods cited in the monograph and literature are followed. Therefore, the routine quality assurance of these products represents a difficult analytical task to be accomplished.

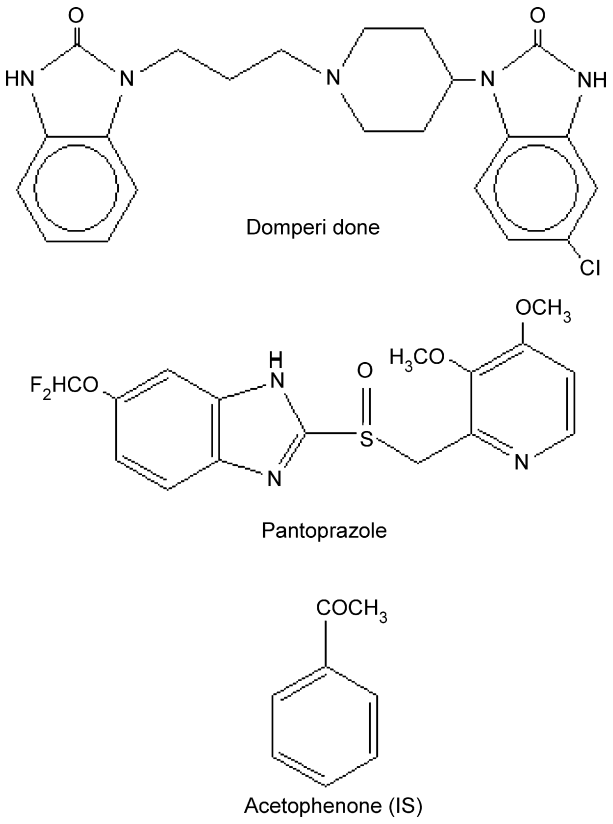

Fig. 1. The chemical structures of analytes and internal standard (IS).

Nevertheless, to the best of our knowledge, there seems to be no reports concerning methods for the simultaneous determination of DP and PP in the commercial pharmaceutical preparations.

In the present work, a HPLC method was developed, optimized and validated for the determination of DP and PP present in commercial preparations (tablets and capsules). In order to understand the sensitivity of the chromatographic factors on the separation of analytes and to simultaneous optimization of resolution and analysis time, chemometric protocols of response surface design and Derringer's desirability function were successfully employed.

2. Experimental

2.1. Apparatus

Chromatographic measurements were made on a Shimadzu (Tokyo, Japan) model which consisted of a LC10AD and LC10 ADvp solvent delivery module, SPD 10A UV–Visible detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20 μl loop, and UV detector (SPD-10A). The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1.11SP1) installed on it. The mobile phase was degassed using Branson sonicator (Branson Ultrasonics Corporation, USA). Absorbance spectra were recorded using an UV–Visible spectrophotometer (Model

UV-1601PC, Japan) employing quartz cell of 1.00 cm of path length.

2.2. Softwares

The homoscedasticity for the calibration curves was tested by Cochran's test using Matlab® version 5.1.0.421 (The Math Works Inc.). Experimental design, data analysis and desirability function calculations were performed by using Design-Expert® trial version 7.0.0. (Stat-Ease Inc., Minneapolis).

2.3. Chemicals and reagents

Working standards of domperidone (99.79%) and pantoprazole (99.76%) were donated by M/S The Madras Pharmaceuticals, Chennai, India. Acetophenone (IS) (≥99%) was purchased from Fluka, Buchs, Switzerland. Acetonitrile (MeCN) and methanol (MeOH) were of HPLC grade and dipotassium hydrogen phosphate and phosphoric acid were of analytical-reagent grade supplied by M/S SD Fine Chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, Bangalore, India. The pharmaceuticals (containing DP—10 mg and PP—20 mg), Pantop-D® capsules and Dompan® tablets were purchased from Aristo Pharmaceuticals and Medley Pharmaceuticals, Mumbai, India, respectively.

2.4. Standard solutions

Stock standard solutions of DP and PP (1 mg/ml) were prepared in mobile phase. The prepared stock solution was stored at 4 °C protected from light. Working standard solutions were freshly obtained by diluting the stock standard solutions with mobile phase during the analysis day. Calibration curves reporting peak area ratios of PP or DP to that of the IS versus drug concentrations were established in the range of 1.0–10 μg/ml for PP and 0.5–5 μg/ml for DP, in presence of acetophenone (12.5 μg/ml) as internal standard. Standard solution prepared for the optimization procedure constituted PP, DP and IS at 5.0, 5.0 and 12.5 μg/ml, respectively.

2.5. Sample preparation

Twenty tablets were weighed and finely powdered. In the case of capsule dosage, the contents of the capsule were mixed thoroughly. An amount of capsule/tablet powder equivalent to 10 mg of DP and 20 mg of PP were accurately weighed and transferred in a 50 ml volumetric flask; suitable quantity of IS was added followed by 25 ml of mobile phase. This mixture was subjected to sonication for 10 min for complete extraction of drugs and the solution was made up to the mark with mobile phase to obtain a concentration of PP, DP and IS as 5.0, 2.5 and 12.5 μg/ml, respectively. The solution was centrifuged at 4000 rpm for 10 min; the clear supernatant was collected and filtered through a 0.2 μm membrane filter (Gelman Science, India) and 20 μl of this solution was injected for HPLC analysis.

2.6. Chromatographic procedure

Chromatographic separations were carried out on a Phenomenex® C18 analytical column (150 mm × 4.6 mm i.d., 5 μm) connected with a Phenomenex® C18 guard cadridge (4 mm × 3 mm i.d., 5 μm). The mobile phase consisted of MeOH–MeCN–dipotassium hydrogen phosphate buffer (pH 7.0), adjusted with 10% phosphoric acid. In order to increase the sensitivity for the less concentrated compound (i.e., PP) and to decrease the background from mobile phase a wavelength of 285 nm was selected for detection. An injection volume of the sample was 20 μl. The HPLC system was used in an air-conditioned laboratory atmosphere (20 ± 2 °C).

2.7. Validation

Validation studies were conducted using the optimized assay conditions based on the principles of validation described in the ICH guidelines "Text on Validation of Analytical Procedures" [21] and "Q2B, Validation of Analytical Procedures: Methodology" [22]. Key analytical parameters, including, specificity, accuracy, precision, linearity, detection limit and quantitation limit were evaluated. For specificity study, placebo containing starch, lactose monohydrate, aerosil, hydroxy propyl methylcellulose, titanium dioxide and magnesium stearate was used. The homoscedasticity for the calibration curves was tested using Cochran's test [23] at the level of 95% significance. Calibration curves were constructed in a low region of 0.05–1.0% of the target analyte concentration for the limit of detection and quantification [24]. Also, robustness of the proposed method was assessed with respect to small alterations in the MeCN concentration (33 ± 0.5%), the pH value (7.0 ± 0.2) and the buffer concentration (20 ± 2.0 mM).

3. Results and discussion

3.1. Optimization design and analysis

The central composite design can be applied to optimize the separation and to assist the development of better understanding of the interaction of several chromatographic factors on separation quality [25]. In this work, the important chromatographic factors were selected and optimized by a central composite design experiment. The selection of factors for optimization was based on preliminary experiments and prior knowledge from literature, as well as certain instrumental limitations. For instance, the mobile phase pH was fixed at 7.0 as this could influence the stability of PP [26]. From preliminary experiments, ternary mobile phase consisted of MeOH, MeCN and phosphate buffer was employed in which concentration of MeOH in the mobile phase was fixed at 20%, and only MeCN content was varied [27]. The mobile phase flow rate could also moderately influence selectivity in HPLC analysis. Therefore, the key factors selected for optimization process were MeCN concentration (*A*), buffer molarity (*B*) and flow rate (*C*). Table 1 shows the levels of each factors studied for finding out the optimum values and responses. As can be seen in this table, the ranges

Table 1
Central composite rotatable design arrangement and responses[a]

Design points	Factor levels			Responses					
	A (%, v/v)	*B* (mM)	*C* (ml/min)	k_1	tR_1	tR_2	tR_3	$Rs_{1,2}$	$Rs_{2,3}$
1	30.22	12.03	0.88	2.08	5.24	5.72	12.34	2.11	18.84
				2.02	5.14	5.65	12.29	2.28	18.91
2	33.78	12.03	0.88	1.40	4.09	4.90	8.12	4.11	12.02
				1.43	4.13	4.94	8.16	4.08	12.00
3	30.22	17.97	0.88	2.12	5.30	5.69	11.30	1.73	16.91
				2.11	5.29	5.67	11.30	1.70	16.92
4	33.78	17.97	0.88	1.49	4.24	4.92	7.94	3.39	11.41
				1.51	4.27	4.98	8.00	3.49	11.29
5	30.22	12.03	1.12	2.10	4.16	4.54	9.88	2.01	17.92
				2.12	4.19	4.56	9.92	1.97	17.99
6	33.78	12.03	1.12	1.44	3.27	3.91	6.48	3.76	11.32
				1.43	3.26	3.89	6.46	3.73	11.25
7	30.22	17.97	1.12	2.12	4.18	4.48	8.95	1.55	15.99
				2.10	4.15	4.44	8.92	1.53	16.05
8	33.78	17.97	1.12	1.48	3.32	3.87	6.23	3.19	10.53
				1.48	3.32	3.87	6.27	3.21	10.59
9	29.00	15.00	1.00	2.35	5.02	5.02	11.25	0	16.85
				2.38	5.06	5.06	11.42	0	17.07
10	35.00	15.00	1.00	1.26	3.38	3.99	5.94	3.49	8.88
				1.25	3.38	3.99	5.92	3.5	8.77
11	32.00	10.00	1.00	1.65	3.98	4.42	7.94	2.33	13.53
				1.67	4.00	4.44	8.00	2.34	13.61
12	32.00	20.00	1.00	1.81	4.22	4.57	7.70	1.85	12.07
				1.81	4.22	4.57	7.68	1.82	12.00
13	32.00	15.00	0.80	1.74	5.14	5.66	9.87	2.26	13.56
				1.76	5.18	5.70	9.84	2.27	13.50
14	32.00	15.00	1.20	1.75	3.43	3.76	6.58	1.95	12.25
				1.71	3.39	3.72	6.51	1.94	12.22
15	32.00	15.00	1.00	1.72	4.09	4.51	8.09	2.22	13.35
16	32.00	15.00	1.00	1.74	4.11	4.53	8.11	2.19	13.32
17	32.00	15.00	1.00	1.73	4.10	4.52	8.09	2.21	13.38
18	32.00	15.00	1.00	1.72	4.09	4.51	8.07	2.19	13.37
19	32.00	15.00	1.00	1.72	4.08	4.50	8.06	2.20	13.41
20	32.00	15.00	1.00	1.71	4.06	4.48	8.03	2.22	13.43

[a] Randomized.

of each factors used were: MeCN concentration (29–35%), buffer molarity (10–20 mM) and flow rate (0.8–1.2 ml/min). As response variables, the retention factor of PP (k_1), the retention times of PP (tR_1), IS (tR_2) and DP (tR_3), and the resolution between two pairs, PP-IS ($Rs_{1,2}$) and IS-DP ($Rs_{2,3}$) were chosen. All experiments were performed in randomized order to minimize the effects of uncontrolled variables that may introduce a bias on the measurements. Replicates ($n=6$) of the central points were performed to estimate the experimental error. For an experimental design with three factors, the model including linear, quadratic, and cross terms can be expressed as

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

where Y is the response to be modeled, β is the regression coefficient and X_1, X_2 and X_3 represents factors A, B and C, respectively. To obtain a simple and yet a realistic model, the insignificant terms ($P>0.05$) are eliminated from the model through 'backward elimination' process. The statistical parameters obtained from the ANOVA for the reduced models are given in Table 2. Since R^2 always decreases when a regressor variable is eliminated from a regression model, in statistical modeling the adjusted R^2 which takes the number of regressor variables into account, is usually selected [28]. In the present study, the adjusted R^2 were well within the acceptable limits of $R^2 \geq 0.80$ [29] which revealed that the experimental data shows a good fit with the second-order polynomial equations. For all the reduced models, P value of <0.05 are obtained, implying these models are significant. The adequate precision value is a measure of the "signal (response) to noise (deviation) ratio". A ratio greater than 4 is desirable [30]. In this study, the ratio was found to be in the range of 26.24–125.14, which indicates an adequate signal and therefore the model is significant for the separation process. The coefficient of variation (C.V.) is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if it is less than 10% [30]. The C.V. for all the models was found to less than 10%, except for $Rs_{1,2}$ (17.17%). Hence, the diagnostic plots, (a) normal probability plot of residuals [31] and (b) plot of residuals versus predicted values [32] were analyzed for response $Rs_{1,2}$. Since, the assumptions of normality and constant variance of the resid-

Table 2
Reduced response models[a] and statistical parameters obtained from ANOVA (after backward elimination)

Response	Regression model	Adjusted R^2	Model P-value	%C.V.	Adequate precision
k_1	$1.74 - 0.32A + 0.033B - 0.013BC + 0.03A^2$	0.994	0.000	1.29	124.74
tR_1	$4.10 - 0.49A + 0.05B - 0.50C + 0.046AC - 0.026BC + 0.044A^2 + 0.07C^2$	0.997	0.000	0.82	125.14
tR_2	$4.57 - 0.33A - 0.57C + 0.092C^2$	0.972	0.000	2.17	58.12
tR_3	$8.11 - 1.66A - 0.21B - 1.01C + 0.20AB + 0.33A^2 + 0.18C^2$	0.957	0.000	4.48	34.09
$Rs_{1,2}$	$2.38 + 0.95A - 0.22B$	0.822	0.000	17.17	26.24
$Rs_{2,3}$	$13.66 - 2.80A - 0.58B - 0.40C$	0.907	0.000	6.13	32.78

[a] Only significant coefficients with $P < 0.05$ are included. Factors are in coded levels.

uals were found to be satisfied, the fitted model for the $Rs_{1,2}$ was accepted.

As can be seen in Table 2, the interaction term with the largest absolute coefficients among the fitted models is AB (+0.20) of tR_3 model. The positive interaction between A and B is statistically significant ($P = 0.04$) for tR_3. The study reveals that changing the fraction of MeCN from low to high results in a rapid decline in the retention time of DP both at the low and high level of buffer molarity. Further at low level of factor A, an increase in the buffer molarity results in a marginal decrease in the retention time. This may be due to reduced silanol effects as a result of higher buffer molarity used. Therefore, when the MeCN concentration is set at its lowest level, the buffer concentration has to be at its highest level to shorten the run time. Especially this interaction is synergistic, as it led to a decrease in run time. The existence of such interactions emphasizes the necessity to carry out active multifactor experiments for optimization of the chromatographic separation.

In order to gain a better understanding of the results, the predicted models are presented in Fig. 2 as the perturbation plot [33]. For an optimization design, this graph shows how the response changes as each factor moves from a chosen reference point, with all other factors held constant at the reference value. A steep slope or curvature in a factor indicates that the response is sensitive to that factor. Hence, the plot shows that factor A mostly affected the analysis time (tR_3), followed by factor C and then B.

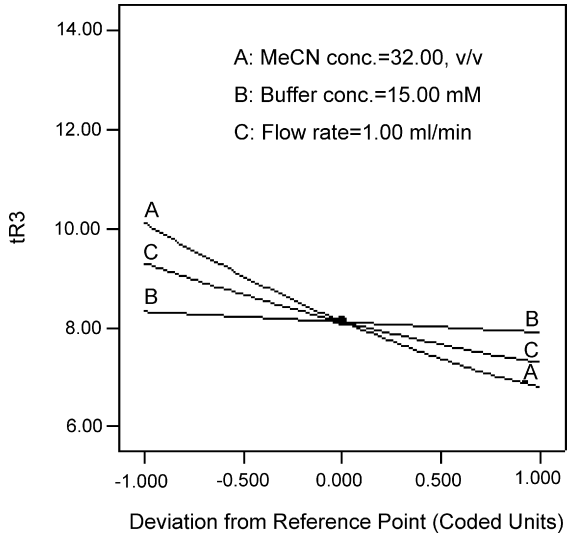

Fig. 2. Perturbation plot showing the effect of each of the independent variables on tR_3 while keeping other variables at their respective mid-point levels.

3.2. Multi-criteria decision making

In the present study, to optimize six responses with different targets, Derringer's desirability function, was used [9]. The Derringer's desirability function, D, is defined as the geometric mean, weighted, or otherwise, of the individual desirability functions. The expression that defines the Derringer's desirability function is:

$$D = [d_1^{p1} \times d_2^{p2} \times d_3^{p3} \times \cdots \times d_n^{pn}]^{1/n} \quad (2)$$

where pi is the weight of the response, n the number of responses and d_i is the individual desirability function of each response obtained from the transformation of the individual response of each experiment. The scale of the individual desirability function ranges between $d_i = 0$, for a completely undesired response, to $d_i = 1$ for a fully desired response. Weights can range from 0.1 to 10. Weights lower than 1 give less emphasis to the goal, whereas weights greater than 1 give more emphasis to the goal (in both cases, d_i varies in a non-linear way while approaching to the desired value). But with a weight of 1, d_i varies in a linear way. In the present report we chose weights equal to 1 for all the six responses. A value of D different to zero implies that all responses are in a desirable range simultaneously and consequently, for a value of D close to 1, the combination of the different criteria is globally optimal, so as the response values are near target values.

The criteria for the optimization of each individual response are shown in Table 3. Criteria I have been proposed for selecting an optimum experimental condition for analyzing routine quality control samples. As can be seen under criteria I, two responses tR_3 and $Rs_{2,3}$ were minimized, in order to shorten the analysis time. On the other hand, $Rs_{1,2}$ was targeted at 2.00 to allow baseline separation of PP and IS. In order to separate the first eluting peak (PP) from the solvent front, k_1 was targeted at 1.25. Importance can range from 1 (the least important) to 5 (the most important), which gives emphasis to a target value. For instance, high importance value of 4 was assigned to tR_3 response as short analysis time is usually preferred for routine analysis. Following the conditions and restrictions above, the optimization procedure was carried out. The response surface obtained for the global desirability function is presented in Fig. 3. The coordinates producing the maximum desirability value ($D = 0.845$) were

Table 3
Criteria for the optimization of the individual responses

Response	Lower limit	Upper limit	Criteria I		Criteria II	
			Goal	Importance	Goal	Importance
k_1	1.25	2.38	Target = 1.25	2	Target = 2.00	4
tR_1	3.26	5.3	Range	1	Range	1
tR_2	3.72	5.72	Range	1	Range	1
tR_3	5.92	12.34	Minimize	4	Minimize	1
$Rs_{1,2}$	0	4.11	Target = 2.00	2	Target = 2.50	4
$Rs_{2,3}$	8.77	18.91	Minimize	1	Minimize	1

MeCN concentration of 33%, buffer molarity of 15.3 mM and flow rate of 1.19 ml/min. The predicted response values corresponding to the latter value of D were: $k_1 = 1.59$, $tR_1 = 3.27$ min, $tR_2 = 3.72$ min, $tR_3 = 5.97$ min, $Rs_{1,2} = 2.54$ and $Rs_{2,3} = 10.40$. The prediction efficiency of the model was confirmed by performing the experiment under the optimal condition and the corresponding chromatogram is shown in Fig. 4.

To substantiate the flexibility of the optimization strategy and to search for an optimum experimental condition for analyzing plasma samples, criteria II was established by varying the response goals and their importance values (Table 3). For instance, large value of k_1 has to be selected for the separation of PP from the initial disturbances of plasma components. Therefore, k_1 was targeted at 2.00 and high importance value of 4 was assigned. Following the response goals above, the optimization procedure was carried out for which optimal condition II with the maximum desirability value of $D = 0.785$ was obtained. The agreement between experimental and predicted responses for both the predicted optimums I and II are shown in Table 4. The average errors for retention factor, retention time and resolution were 4.41, 4.69 and 5.97%, respectively which were found to be in good agreement [34], with a difference of 1–6%.

3.3. Assay method validation

The optimized assay method is specific in relation to the placebo used in this study because there was no excipients peak co-eluted with the analytes and IS (Fig. 4). An excellent linearity was established at five levels in the range of 1–10 μg/ml for PP and 0.5–5.0 μg/ml for DP, with R^2 of more than 0.999. The slope and intercept of the calibration curve were 0.322 and −0.005 for PP, and 0.239 and 0.013 for DP, respectively. The homoscedasticity of the calibration curves were tested and in that no statistical difference ($P > 0.05$) was found between variances. The LOD and LOQ were estimated as 1.89 and 5.73 ng/ml for PP, and 3.86 and 11.70 ng/ml for DP, respectively. Accuracy ($n = 9$), assessed by spike recovery, were found to be 99.66 and 99.70% for PP and DP, respectively, which were within acceptable ranges of 100 ± 2% [35]. The intra and inter-assay precision ($n = 6$) was confirmed since, the %C.V. were well within the target criterion of ≤2 and ≤3, respectively [35]. Robustness study reveals that small changes did not alter the retention times, retention factor and resolutions more than 4% and therefore it would be concluded that the method conditions are robust.

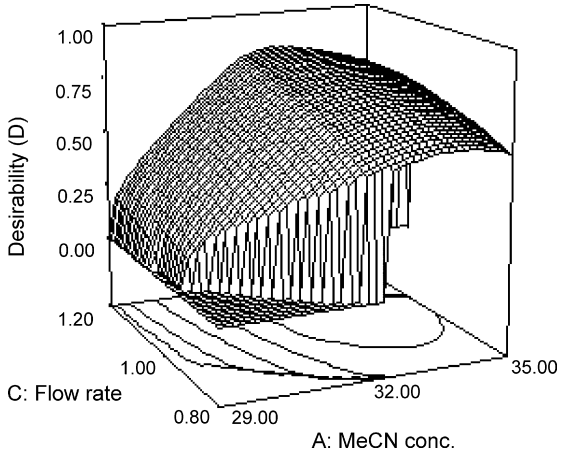

Fig. 3. Graphical representation of the overall desirability function D. MeCN concentration (A) is plotted against flow rate (C) with factor B held constant at 20.00 mM.

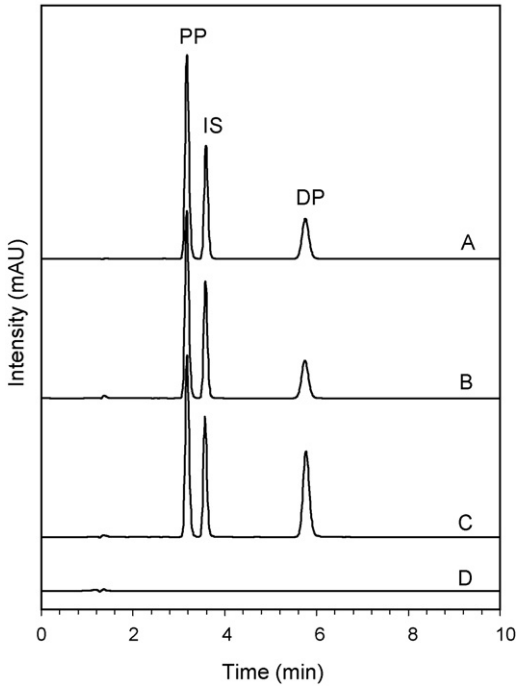

Fig. 4. Chromatograms corresponding to (A) a real sample of Pantop-D® capsules containing PP (4.96 μg/ml), IS (12.13 μg/ml) and DP (2.48 μg/ml); (B) a real sample of Dompan® tablets containing PP (4.98 μg/ml), IS (12.13 μg/ml) and DP (2.51 μg/ml); (C) a synthetic mixture of PP (4.94 μg/ml), IS (12.13 μg/ml) and DP (4.92 μg/ml) and (D) a placebo solution under optimum assay conditions.

Table 4
The comparison of experimental and predictive values of different objective functions under optimal conditions

Optimum conditions	MeCN (%)	Buffer (mM)	Flow (ml/min)	k_1	tR_1	tR_2	tR_3	$Rs_{1,2}$	$Rs_{2,3}$
I	Desirability value (D) = 0.845								
	33.00	20.00	1.19						
		Experimental		1.52	3.18	3.57	5.76	2.38	10.31
		Predictive		1.59	3.27	3.72	5.97	2.54	10.40
II	Desirability value (D) = 0.785								
	31.54	10.00	1.20						
		Experimental		1.73	3.41	3.78	7.09	2.22	13.86
		Predictive		1.81	3.55	3.97	7.80	2.50	14.67
	Average error			4.41%	4.69%			5.97%	

3.4. Application of the method

The proposed RP-HPLC method was applied to the quantitative analysis of real samples (Pantop-D® capsules and Dompan® tablets) containing PP and DP. Representative chromatograms are presented in Fig. 4. The results achieved when analyzing Pantop-D® capsules were, 20.05 (0.69) mg of PP and 10.1 (1.14) mg of DP; and Dompan® tablets were, 19.97 (1.67) mg of PP and 10.07 (1.34) mg of DP, with the values within parenthesis being the %C.V. of the six replicates. Good agreement was found between the assay results and the label claim of the product. The %C.V. for both capsules and tablets were <2, indicating the precision of the analytical methodology.

4. Conclusion

The analytes PP and DP has been simultaneously analyzed in pharmaceutical formulations (tablets and capsules) by using HPLC. Time of analysis, resolution and quality of the peaks were simultaneously optimized by applying useful tools of chemometrics: response surface design and Derringer's desirability function. The validation study supported the selection of the assay conditions by confirming that the assay was specific, accurate, linear, precise, and robust. Therefore, this HPLC-UV method can be used as a routine quality control analysis in a pharmaceutical environment.

The results of the study demonstrate the benefit of applying this approach in selecting optimum conditions for the determination of drugs in pharmaceutical formulation and plasma samples. This method reduces overall assay development time and provides essential information regarding the sensitivity of various chromatographic variables on separation attributes.

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