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Research Article

HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF PROTON-PUMP INHIBITORS WITH DOMPERIDONE IN HUMAN PLASMA EMPLOYING RESPONSE SURFACE DESIGN

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ABSTRACT

Multiple response simultaneous optimizations employing the Derringer's desirability function for the development of reversed-phase HPLC methods for the simultaneous determination of pantoprazole, rabeprazole and lansoprazole with domperidone in human plasma samples is described. The ranges of the independent variables used for the optimization were MeCN: 30-35%, buffer conc.: 10-20 mM and flow rate: 0.9-1.1 ml/min. The influence of these independent variables on the output responses: capacity factor of the first peak (k_1), resolutions ($Rs_{2,3}$), Retention time (tR_5) and the Chromatography optimization function (COF) were evaluated. Using this strategy, mathematical model were defined and response surface were derived for the separation. The coefficients of determination R^2 were more than 0.92 for all the models. Optimum conditions chosen for assay were MeCN, MeOH, 18.65 mM K_2HPO_4 (pH 7.0 ± 0.5) solution (31.41:20: 48.59 v/v/v) and flow rate 1.10 ml/min. The eluate was monitored using an UV detector set at 280 nm. Peak area ratio of the analyte and internal standard was used for the quantification of plasma samples. Total chromatographic analysis time per sample was approximately 9 min. The validation of the proposed analytical method was conducted in accordance to the recommendations of the guidelines ''Bioanalytical method validation'' [FDA-CDER, 2001]. The method was found to be simple, sensitive and hence it could be applied in bioavailability studies.

Keywords: Central composite design, Derringer's desirability function, Domperidone, HPLC, Lansoprazole, Pantoprazole, Rabeprazole

INTRODUCTION

Domperidone (DP) (Fig. 1) is a potent dopamine antagonist used for the treatment of nausea and vomiting. Pantoprazole (PP), Rabeprazole (RP) and Lansoprazole (LP) (proton-pump inhibitors) (Fig. 1) belong to a class of antisecretory compounds, the substituted benzimidazoles that suppress gastric acid secretion by specific inhibition of the H^+/K^+ ATP^{ase} enzyme system at the secretory surface of the gastric parietal cell[1]. They are used for the treatment of acid-peptic diseases such as duodenal, gastric and oesophegeal ulceration[2]. Nowadays, the mixtures of these active components are present in pharmaceutical formulations as capsules and tablet forms. Further, in India, domperidone is predominantly prescribed in combination with different proton-pump inhibitors for the treatment of acid related disorders, and dyspepsia. Therefore the simultaneous determination of these analytes becomes motivating and significant. Domperidone is official in British Pharmacopoeia[3] in which a HPLC-UV method is available for its separate determination in tablets and also in European Pharmacopoeia 6.0.[4] Pantoprazole sodium is official in BP[3] and USP.[5] Lansoprazole is official in BP,[3] USP[6] and EP[7]and the Rabeprazole is official in IP.[8] Although there is a crescent number of works describing the determination of PP,[9-11] RP,[12-13] LP[14-16]and DP[17-20] there seems to be no reports concerning methods for the simultaneous determination of all the four analytes (PP, RP, LP and DP) using HPLC in the human plasma samples.

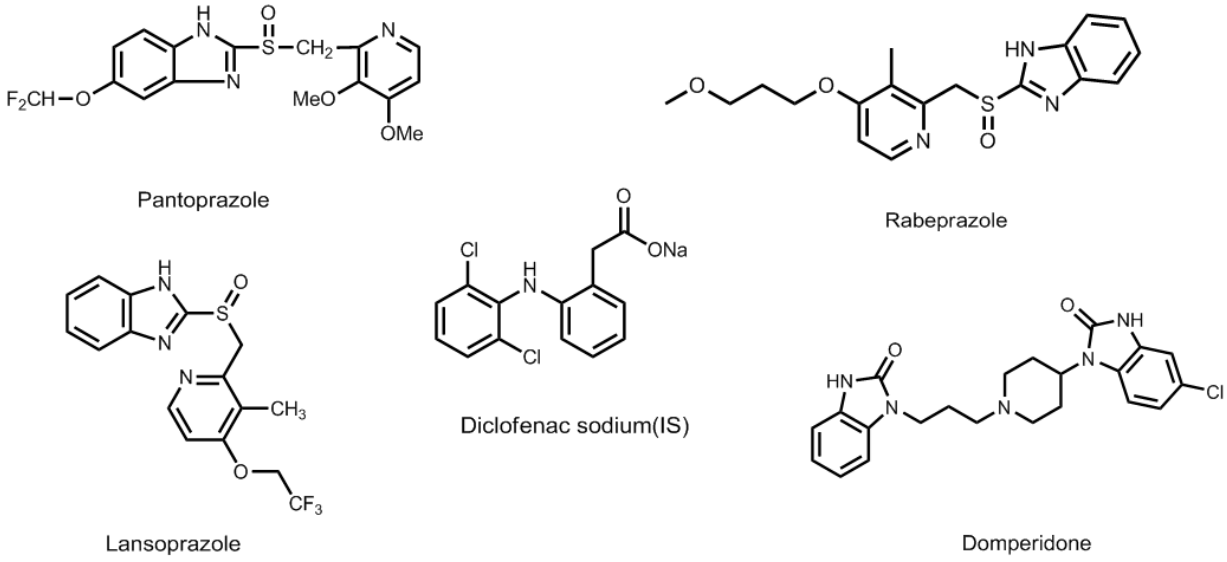

Fig. 1: The chemical structures of analytes and internal standard (IS)

HPLC method development[21-23] and optimizing is a complex procedure that requires simultaneous determination of several factors, viz., the type and composition of the organic phase, column temperature, flow rate, pH, type of the stationary phase, etc. For decades HPLC separations were based on a trial and error methodology, but employing a time-consuming trial-and-error approach resulting only in an apparent optimum and information concerning the sensitivity of the factors on the analytes separation and interaction between factors is not available. To achieve this objective, any one of the chemometric methods which includes the overlapping resolution maps [24] and response surface methodology[25-29] can be applied. In general, the 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.[30] This kind of knowledge provides important clues in the attainment of optimum experimental conditions in the development of chromatography methods.[31] The best experimental design approach for the purpose of modeling and optimization are the response surface design.[25] However, the HPLC method intended to be applied for the pharmaceutical or industrial environment, the analysis time is usually optimized simultaneously without losing resolution.[32] 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. The different approaches of MCDM[33] 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 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.[34] he 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[35] but it was put into a more general form by Derringer.[36] 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,[32] implementing resolution and analysis time as objective functions to improve separation quality. Safa and Hadjmohammadi[37] 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[38] proposed a chemometric tool based on the Function of Mutual Information (FUMI) theory to improve prediction of the uncertainty in HPLC. Kotani et al.[39] 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. We have recently employed the same MCDM approach (Derringer's desirability function) for the development and optimization of a HPLC method for the simultaneous estimation of pantoprazole and domperidone, [29] amlodipine and atorvastatin[28] in quality control and plasma samples.

In the present work, a HPLC method was developed, optimized and validated for the Simultaneous determination of pantoprazole, rabeprazole and lansoprazole with domperidone using chemometric procedure. The optimum chromatographic conditions were estimated by a central composite design using both a graphical and a mathematical (Derringer's desirability function) global optimization approach. Finally, the validation of the proposed analytical method was conducted in accordance to the recommendations of the guidelines ''Bioanalytical method validation'' [FDA-CDER, 2001].

MATERIAL AND METHODS

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 20µl 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.

Softwares

Experimental design, data analysis and desirability function calculations were performed by using Design-Expert® trial version 7.0.0. (Stat-Ease Inc., Minneapolis). The rest of the calculations for the analysis were performed by use of Micro soft Excel 2007 software (Microsoft, USA).

Chemicals and reagents

Working standards of domperidone, pantoprazole, rabeprazole lansoprazole and diclofenac sodium (IS) were donated by M/S. Pharma analytical Lab., Puducherry, India. Acetonitrile (MeCN) and methanol (MeOH) were of HPLC grade and dipotassium hydrogen phosphate, phosphoric acid, potassium hydroxide and ethyl acetate 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 drug free human plasma was purchased from blood bank of Rotary Central TTK VHS (Chennai, India).

Stock and working standard solutions

Stock standard solutions of PP, RP, LP, and DP (1mg/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, RP, LP and DP to that of the IS versus drug concentrations were established at six levels; 10, 25, 50, 250, 500 and 1000 ng/ml for PP, RP and LP and 15, 25, 50, 250, 500 and 1000 ng/ml for DP in presence of diclofenac Sodium (250 ng/ml) as internal standard. Standard solution prepared for the optimization procedure

constituted PP, RP, LP, DP and IS at 250, 250, 250, 250 and 750 ng/ml respectively.

Extraction procedure for plasma sample

The 1ml blank plasma in a glass-stoppered 15 ml centrifuge tube were spiked with the working solutions of PP, RP, LP, DP and IS to achieve a concentration of 250 ng ml each. The samples were then alkalinized by addition of 100 µl of 3M KOH, vortex-mixed for 30 seconds and a certain volume of extraction solvent was added. The mixture was gently shaked for 5 min and centrifuging on a laboratory centrifuge (Remi®, R&C, Remi Equipment, Mumbai, India) at 3500 rpm (1878 × g) for 5 min. The supernatant organic layer was transferred to eppendorf tubes and the contents were evaporated to dryness under vacuum at 60°C using an Eppendorf concentrator. The residue was reconstituted in 100 µl of mobile phase and vortex mixed for 30 seconds.[40] Aliquots of 20 µl were injected into the chromatographic system. The same procedure was carried out for blank plasma samples to check the cleanness of the extracts. To assess the efficiency of the extraction procedure, the spiked plasma sample was extracted according to the above procedure, but the addition of IS after extraction. The percentage recovery was estimated by comparing the peak areas of each analyte spiked sample with that from the blank plasma sample to which the drug was added previous the evaporation step.

$$\% \text{ Recovery} = \frac{\text{E (spike)/IS}}{\text{E(non spike)/IS}} \text{ X } 100$$

Where, E (spike) is the area of the each analyte in spiked plasma sample; E (non spike) is the area of each analyte obtained by addition of the drug previous to the evaporation step.

Chromatographic procedure

Chromatographic separations were carried out on a Phenomenex® C18 analytical column (150mm×4.6mm i.d., 5µm) connected with a Phenomenex® C18 guard cadridge (4mm×3mm i.d., 5µm). The mobile phase consisted of MeOH-MeCN-dipotassium hydrogen phosphate buffer (pH 7.0), adjusted with 10% phosphoric acid. Wavelength of 280 nm was selected for detection. An injection volume of the sample was 20µl. The HPLC system was used in an air conditioned laboratory atmosphere (20 ± 2°C).

Validation

The plasma assay method was validated in accordance to the recommendations of the guidelines ‘‘Bioanalytical method validation’’ [FDA-CDER, 2001].

RESULTS AND DISCUSSION

Optimization design and analysis

The central composite design (CCD) is employed, which is a design type under Response surface methodology (RSM). CCD is chosen due to its flexibility and can be applied to optimize an HPLC separation by gaining better understanding of factor’s main and interaction effects.[41, 42]. The selection of key factors examined for optimization was based on preliminary experiments and prior knowledge from literature The factors selected for optimization process were MeCN concentration (*A*), buffer molarity (*B*) and flow rate (*C*). The capacity factor for the first eluted peak (k_1), the resolution of the critical separated peak, RP and IS, ($Rs_{2,3}$), the retention time of the last peak, DP,(tR_5), and the chromatographic optimization function (COF) were selected as responses. COF is calculated according to Eq. (1) [43]:

$$\text{COF} = \sum_{i=1}^{k} Ai \ln\left(\frac{Rs_i}{Rs_{id}}\right) + B(t_M - t_L) \quad (1)$$

Where *Ai* and *B* are weighted parameters, Rs_i is the resolution of the *i*th pair, Rs_{id} is the desired resolution for the specific pair, t_M represents the desired maximum analysis time (here assumed 10 min), and t_L is the actual time of the last eluted peak. All experiments were conducted 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. (Table 1), summarizes the conducted experiments and responses. The quadratic mathematical model for three independent factors is given in Eq. (2):

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

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. Statistical parameters obtained from ANOVA for the reduced models are given in (Table 2). The insignificant terms (P > 0.05) were eliminated from the model through backward elimination process to obtain a simple and realistic model. 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.[44]

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	$Rs_{2,3}$	tR_5	COF
1	30.00	10.00	0.90	1.67	4.31	11.65	-2.15
2	35.00	10.00	0.90	0.93	1.86	6.84	17.39
3	30.00	20.00	0.90	1.61	4.49	10.70	1.29
4	35.00	20.00	0.90	1.05	2.26	6.85	15.88
5	30.00	10.00	1.10	1.57	4.1	9.57	5.96
6	35.00	10.00	1.10	0.92	1.77	5.61	20.42
7	30.00	20.00	1.10	1.62	4.22	8.78	8.83
8	35.00	20.00	1.10	1.01	2.07	5.49	20.62
9	28.30	15.00	1.00	2.03	5.38	12.77	-6.51
10	36.70	15.00	1.00	0.81	1.27	5.29	21.02
11	32.50	6.59	1.00	1.19	2.34	7.85	12.35
12	32.50	23.41	1.00	1.27	3.14	7.28	14.07
13	32.50	15.00	0.83	1.6	2.86	9.25	6.781
14	32.50	15.00	1.17	1.29	2.74	6.63	16.81
15	32.50	15.00	1.00	1.26	2.85	7.75	12.54

16	32.50	15.00	1.00	1.27	2.78	7.73	12.64
17	32.50	15.00	1.00	1.27	2.78	7.73	12.64
18	32.50	15.00	1.00	1.27	2.78	7.73	12.64
19	32.50	15.00	1.00	1.27	2.78	7.73	12.64
20	32.50	15.00	1.00	1.27	2.78	7.73	12.64

[a] Randomized.

Table 2: Reduced response models[a] and statistical parameters obtained from ANOVA for CCD

Responses	Regression modle	Adjusted R^2	Model *P* value.	%C.V	Adequate precision
K_1	+1.31-0.34A+0.025B-0.048C	0.9120	<0.0001	6.80	28.41
$Rs_{2,3}$	+2.98-1.18A+0.17B-0.065C	0.9443	<0.0001	8.22	36.24
tR_5	+7.74- 2.09A-0.20B-0.81C+0.20AB +0.18AC+ 0.45A^2	0.9929	<0.0001	2.05	72.11
COF	+12.39+7.23A-7.705E-003B+2.37C-1.95AB-1.99AC-2.00A^2	0.9549	<0.0001	13.54	28.73

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

Table 3: Criteria for the optimization of the individual responses

Responses	Lower limit	Upper limit	Criteria	
			Goal	Importance
K_1	0.814	2.027	Target = 1.5	5
$R_{2,3}$	1.267	5.38	Target = 2	3
tR_5	5.287	12.768	Minimize	3
COF	-6.5095	21.0197	Target = 13	4

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

Optimum conditions	MeCN (%)	Buffer (Mm)	Flow (ml/min)	K_1	$Rs_{2,3}$	tR_5	COF
	Desirability Value (D) = 0.778						
	31.41	18.65	1.10				
	Experimental value			1.45	3.21	7.86	12.46
	Predicted value			1.41	3.40	7.673	12.99
	Average error			5.83	-3.31	4.38	-2.65

In the present study, the adjusted R^2 were well within the acceptable limits of $R^2 \geq 0.80$ [45] 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 is 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 [46] In this study, the ratio was found to be in the range of 28.42–72.12, 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%.[46] The C.V. for all the models was found to less than 10%, except for COF (13.54%). Hence, the diagnostic plots, (a) normal probability plot of residuals[47] and (b) plot of residuals versus predicted values [48] were analyzed for response COF. Since, the assumptions of normality and constant variance of the residuals were found to be satisfied, the fitted model for the COF 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_5 model. The positive interaction between *A* and *B* is statistically significant (< 0.0001) for tR_5. The non-parallel lines obtained for the AB interaction plot (Fig. 2) support this observation. 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.

In (Fig. 3) perturbation plots are presented for predicted models in order to gain a better understanding of the investigated procedure. This type of plots show the effect of an independent factor on a specific response, with all other factors held constant at a reference point.[24] A steepest slope or curvature indicates sensitiveness of the response to a specific factor. and shows that MeCN (factor *A*) had the most important effect on Retention time tR_5 followed by factor *C* and then *B*. Response surfaces plots for k_1, $Rs_{2,3}$, tR_5 and COF are illustrated in (Fig.4). (% acetonitrile concentration is plotted against the flow rate with buffer concentration held at constant at the center value). Analysis of the perturbation plots and response plots of optimization models revealed that factor *A* and *C* had the significant effect on separation of the analytes, whereas the factor *B*, i.e. the buffer molarity, is of little significance.

Global Optimization

In the present study, the identified criteria for the optimization were: resolution between the critical peaks, capacity factor, elution time and COF. Derringer's desirability function was used to optimize four responses with different targets [33] 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:

$$D = [d_1^{p_1} \times d_2^{p_2} \times d_3^{p_3} \times \ldots \times d_n^{p_n}]^{\frac{1}{n}} \quad (3)$$

Where *pi* is the weight of the response, *n* the number of responses and *di* is the individual desirability function of each response. Desirability

function (*D*) can take values from 0 to 1. Weights can range from 0.1 to 10. Weights lower than 1 give less importance to the goal, whereas weights greater than 1 give more importance to the goal. In the present study, *pi* values were set at 1 for all the four responses. A value of *D* close to 1, indicates that the combination of the different criteria is matched in a global optimum[24].The criteria for the optimization of each individual response are shown in (Table 3). Optimum condition for analyzing the plasma samples, Criteria were established by varying the response goals and their importance values. For instance, larger value of k_1 has to be selected for the separation of PP from the initial disturbance of plasma components. There, k_1 was targeted at 1.5 and high importance value of 5 was assigned. Following the conditions and restrictions above, the optimization was carried out. The function is maximized at an overall desirability of about D = 0.778, is presented in (Fig. 5) which provides an optimum condition for the analysis of plasma samples. The predicted response values corresponding to the latter value of D were: k_1 = 1.31, $Rs_{2,3}$ = 3.40, tR_5 = 7.67 min, and COF = 12.99. The prediction efficiency of the model was confirmed by performing the experiment under the optimal condition and the corresponding chromatogram is shown in (Fig.6).The observed difference between the predicted and experimental responses are found to be in good agreement, within a difference of 5.83% is shown in (Table 4). This approach offers flexibility to the chromatographer to slide k_1 values depending upon of the analyte under consideration.

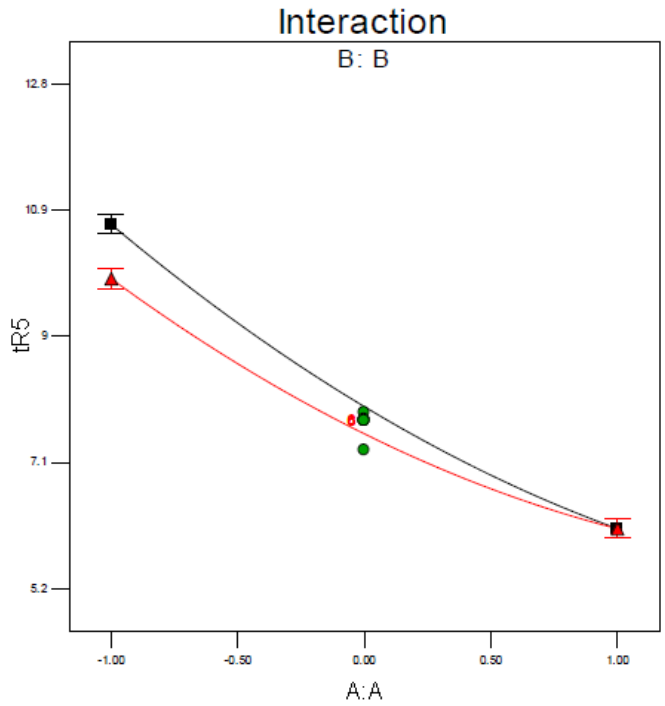

Fig. 2: AB interaction plot for tR_5 response. The line with square ends is the effect of B at low level and the line with diamond ends is the effect of B at high level combined with varying of factor A. The vertical bars represent the least significant difference intervals

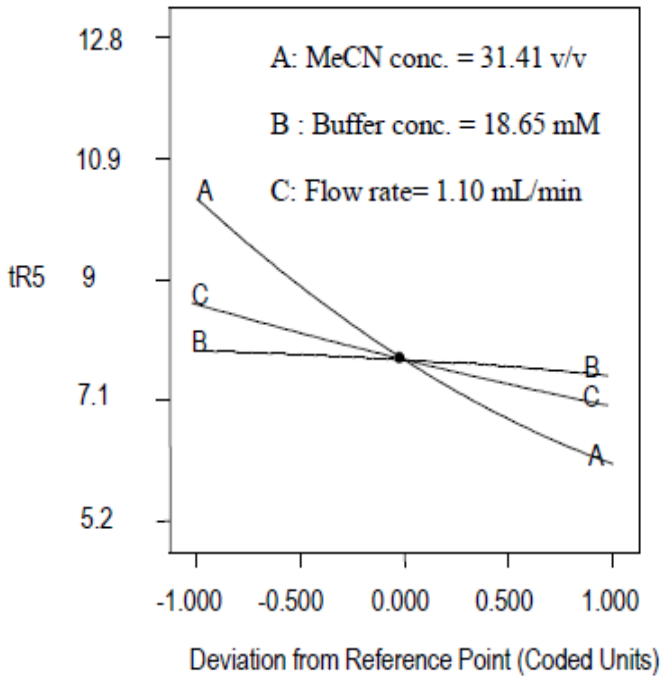

Fig. 3: Perturbation plot for tR_5 response. It shows the effect of each of the independent factor on tR_5, while keeping other factors at their respective mid-point levels

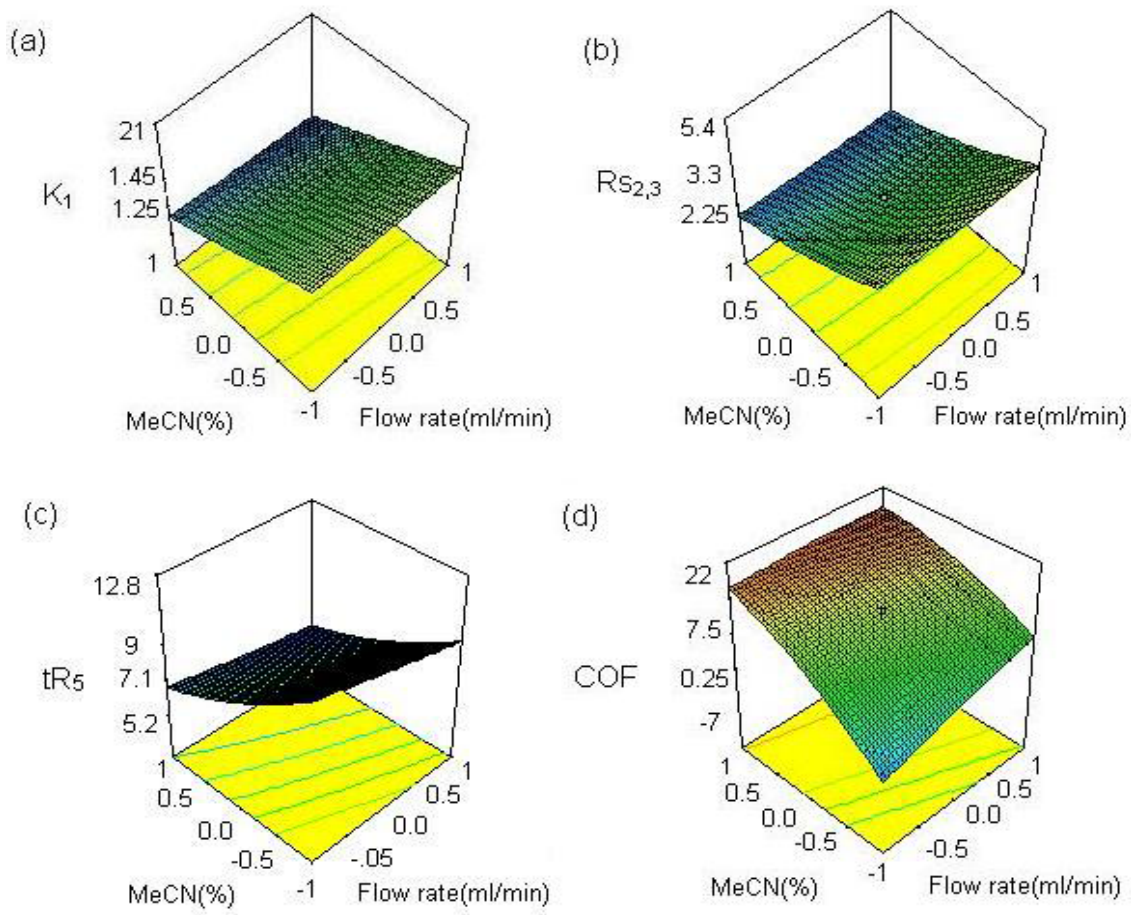

Fig. 4: Response surfaces related to percentage acetonitrile concentration (*A*) and Flow rate (*C*): (a) capacity factor of the first peak (k_1), (b) resolution of the critical pair ($Rs_{2,3}$), (*C*) retention time of the last peak (tR_5) and (d) chromatographic optimization function (COF). Buffer molarity (*B*) was kept constant at the center value

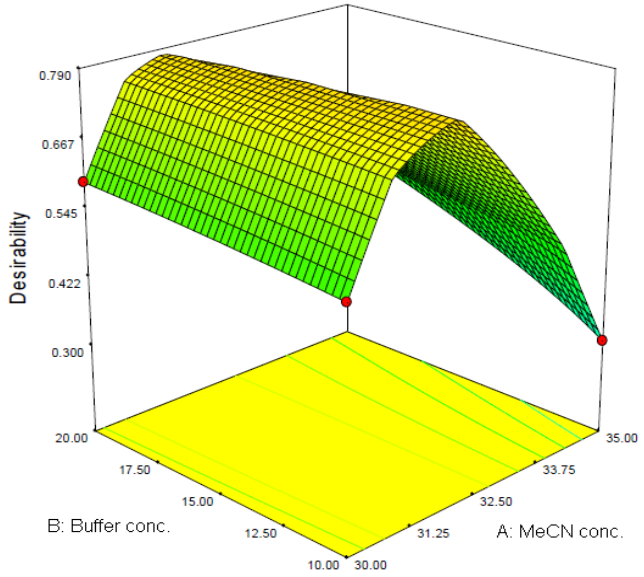

Fig. 5: Graphical representation of the maximum global desirability function. The best compromise is obtained at the top of the graph, *D* = 0.778

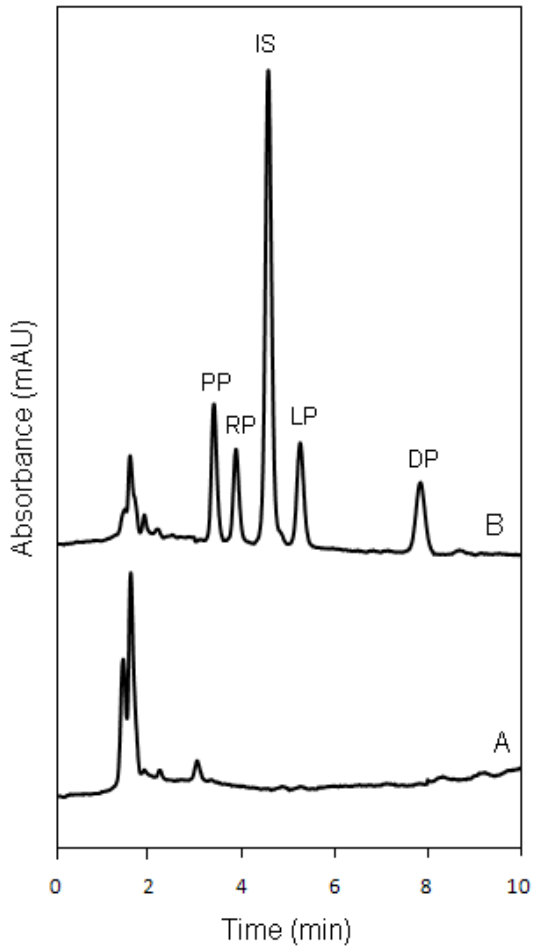

Fig. 6: Chromatograms of pantoprazole (PP), Rabeprazole (RP), Lansoprazole (LP), Domperidone (DP) and Diclofenac sodium (IS) obtained under optimal separation and extraction conditions. (A) extract of human blank plasma (B) spiked plasma sample with 250 ng/ml each of PP, RP, LP, DP and 750 ng/ml of IS

Validation of plasma assay method

Linearity was established at six levels in the range of 10, 25, 50, 250, 500, 1000 ng mL^{-1} for PP, RP, and LP and 15, 25, 50, 250, 500, 1000 ng ml for DP. Typically, the mean (n = 6) regression equations were: y = 0.003 x – 0.015 for PP with R^2 more than 0.997, y = 0.001 x – 0.005 for RP with R^2 more than 0.998, y = 0.002 x + 0.008 for LP with R^2 more than 0.999, and y = 0.002 x + 0.048 for DP, with R^2 more than 0.999 for the analytes. The LOQ values for PP, RP, LP, and DP were 5.45 ng ml, 7.7ng ml, 8.45 ng ml and 15.2 ng ml, respectively. In the optimized chromatographic and extraction conditions, specificity was indicated by the absence of any endogenous interference from plasma matrix at retention times of PP, RP, LP, DP and IS peaks (Fig. 6). Accuracy and precision was determined by replicate analysis (n = 6) of 3 concentration levels of each analyte (25, 250 and 1000 ng ml). The accuracy and precision were well within the acceptance criterion of ±15%. Stability of PP, RP, LP and DP in the spiked plasma samples was examined by replicate analysis (n = 6) at three concentration levels: 25, 250 and 1000 ng ml. The stability of analytes and the IS stock solution in MeCN (250 ng ml each) was also checked over a 12 h period, at 3 h sampling interval. The percentage responses for the aged solutions were calculated using freshly prepared solutions. The results shows that sample and standard solutions of analytes and IS were stable for 12 h, as during this time the result does not decrease below the minimum percentage (95%).

CONCLUSION

In this study, an isocratic RP-HPLC-UV method for the simultaneous determination of PP, RP, LP and DP in human plasma samples was developed and optimized. Time of analysis and resolution were simultaneously optimized by applying chemometrics tools: CCD and Derringer's desirability function. The results of the study demonstrate the benefit of applying this approach in selecting optimum conditions for the determination of drugs in plasma samples. Total chromatographic analysis time per sample was approximately 9 min. The validation study supported the selection of the assay conditions by confirming that the assay was specific, accurate, linear, precise, and robust. The method was found to be simple, sensitive and can be applied successfully in routine analysis for the estimation of PP, RP, LP and DP in biological samples.

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