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Research Article

MULTICRITERIA OPTIMIZATION METHODOLOGY IN DEVELOPMENT OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF INDAPAMIDE AND PERINDOPRIL IN BULK DRUG AND ITS COMBINED DOSAGE FORM

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ABSTRACT

Multicriteria optimization methodology was applied for development of isocratic reversed-phased HPLC method for simultaneous determination of Indapamide and Perindopril. In the first stage of method development, pH value of the water phase, percentage of methanol, temperature of the column and flow rate of the mobile phase were investigated using fractional factorial design. This work is concerned with application of simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Indapamide and Perindopril in combined dosage form. Chromatographic separation was achieved isocratically at 25°C ± 0.5°C on phase Inertsil ODS C_8 [250 mm x 4.6mm] 5µm column with a mobile phase composed of Phosphate buffer with pH 2.5 and methanol in the ratio of 40:60 at flow rate of 0.5 ml/min. Detection is carried out using a UV-PDA detector at 215nm. The retention time of Perindopril at and Indapamide at 5.08 min and 6.91 min respectively. The correlation coefficients for all components are close to 1. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus the proposed method was successfully applied for simultaneous determination of Indapamide and Perindopril in routine analysis.

Keywords: Indapamide, Perindopril, R P- H PLC,Factorial design

INTRODUCTION

Indapamide (IND), 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)- 3-sulfamoyl-benzamideis a non-thiazide sulphonamide diuretic drug marketed by Servier, generally used in the treatment of hypertension, as well as decompensated cardiac failure. The US trade name for indapamide is Lozol. Indapamide is marketed as Natrilix outside of the US. There is good evidence for its effectiveness in secondary prevention after stroke. It is described as a thiazide-like diuretic. Perindopril (PER), H-Indole-2-Carboxylic Acid (trade names Coversyl, Aceon) is a long-acting ACE inhibitor. Perindopril is the free acid form of perindopril erbumine, is a pro-drug and metabolized in vivo by hydrolysis of the ester group to form perindoprilat, the biologically active metabolite. IND and PER was determined by several methods including Liquid chromatography–mass spectrometry (GC–MS), liquid chromatography with UV detection (LC–UV), HPLC and spectrophotometrically, Chemiluminescence and colourimetry[1-9] with or without combination of several drugs. Literature survey revealed that no HPLC method has been reported yet for the analysis of these two drugs in combination without preliminary separation that makes it worthwhile to pursue the present work.

EXPERIMENTAL

Instrumentation

The present work was carried out on HPLC systems consisted of Series 200 UV/Visible Detector, Series 200 LC Pump, Series 200 Auto sampler and Series 200 Peltier LC Column Oven (all Perkin Elmer, Boston, Massachusetts, USA). The data were acquired via TotalChrom Workstation (Version 6.2.0) data acquisition software (Perkin Elmer), using Nelson Series 600 LINK interfaces (Perkin Elmer). The reverse phase Inertsil ODS C_8 [250 mm x 4.6mm] 5µm column was used for all chromatographic separation. Tablets, Perigard DF containing Indapamide and Perindopril erbumine was purchased from local market.

Reagents and chemicals

IND and PER were obtained as gift sample from IPCA LABS, Pvt Ltd ,RATLAM, India. All solvents were of HPLC grade obtained from Merck Research Laboratory, Mumbai, India.

Experimental condition

The HPLC system was operated isocratically at flow rate of 0.5 ml/min. at 25°C ± 0.5°C. The mobile phase found to be most suitable for analysis was Phosphate buffer with pH 2.5 and methanol in the ratio of 40:60, detection was carried out at 215 nm

Preparation of Standard Stock Solution

Indapamide (12.5 mg) and Perindopril Erbumine (40 mg) were weighed accurately and transferred to two, separate, 10 ml volumetric flasks. They were dissolved in 10 ml of mobile phase1 ml of above Indapamide solution and 1 ml of Perindopril Erbumine solution were further diluted with mobile phase to obtain the final concentration 125 µg/ml of Indapamide and 400 µg/ml of Perindopril Erbumine respectively.

Preparation of Mixed Standard

The commercial tablet formulation Perigard DF is combined dosage dosage form containing 1.25 mg of Indapamide and 4mg of Perindopril erbumine. Based on this fact mixed standards were selected for quantitative analysis, which gave satisfactory results. During the preparation of mix standard accurately weigh quantity of Indapamide,1.25mg and Perindopril erbumine , 40 mg were dissolved in mobile phase in a 100ml volumetric flask. This solution having the concentration125µg and 400µg respectively.

Preparation of Sample Stock Solution

For preparing sample stock solution, twenty tablets were weighed (each containing 1.25 mg of Indapamide and 4mg of Perindopril Erbumine) and their average weight was calculated. The tablets were finely powdered and powder equivalent to 1.25 mg of Indapamide and 4 mg of Perindopril Erbumine was accurately weighed and transferred to 100 ml volumetric flask and diluted with mobile phase . The solution was sonicated for 15 min for the proper dissolution. The solution was filtered through syringe filter (millipore millex-HV, hydrophilic pvdf0.45micron).

Linearity

To establish the linearity a series of dilutions ranging from 1-187 µg/ml for IND and 1-800µg/ml for PER were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

Method validation

The method was validated as per the ICH guidelines in terms of linearity, accuracy and specificity, limit of detection, limit of quantitation, intra-day and inter-day precision, repeatability of measurement of peak area as well as repeatability of sample application.

Table No 1: Result of the system suitability

Parameters	Indapamide	Perindopril
Purity Threshold	0.466	1.174
Purity Angle	0.342	1.167
Resolution	1.64	0.0
Asymmetry /tailing	1.4	1.1

Table no 2: Result of Accuracy

Brand Name	Indapamide		Perindopril	
	Label Claim (mg)	% Purity (n=3)	Label Claim (mg)	% Purity (n=3)
Perigard DF	1.25	101.88	4	98.667

Experimental design and statistical analysis

The entire factorial experiment was carried out in three steps. The objective of step 1 was to perform a screening of the factors that allowed well separation of IND and PER and shortest possible total analysis run time. So, fractional factorial design was used to "screen" the following factors: pH value of the water phase, percentage of methanol, temperature of the column and flow rate of the mobile phase. The fractional factorial design was set-up in two levels, with 3 replicates. The original values of each factor were coded (1 for the high level and -1 for the low level) to remove the measurement units and to facilitate the experimental design. Table 4 shows the investigated variables and their domains. Table 5 shows matrix of the experimental design generated from combinations of all factor levels.

Based on the results of step 1, step 2 was carried out in order to evaluate the effect of the most significant factors on the resolution and run time. The full factorial design comprised four levels of pH value of the water phase, percentage of methanol. All factorial designs were outlined using SYSTAT 13 software.

In step 3, the results obtained from experimental design relative to step 2 were fitted to a response surface to generate a global optimum reaction condition. The final step in RSM is to find a suitable approximation for the true functional relationship between response Y and the set of independent variables. Usually, a low order polynomial in some region of the independent variables is employed. If the response is well modeled by linear function of the independent variables, then the approximating function is the first order model

$$Y=\beta_o+\beta_1x_1+\beta_2x_2+\ldots\ldots\beta_kx_k+E$$

If there is curvature in the system, then a polynomial of higher degree must be used, such as the second order model.

$$Y=\beta_o+\sum\beta_1x_1+\sum\beta_2x_2+\ldots\ldots\sum\beta_kx_k+E$$

All RSM problems utilize one or both of these models. RSM is a sequential procedure. The eventual objective of RSM is to determine the optimum operating conditions for the system or to determine a region of the factor space in which operating requirements are satisfied. The software (SYSTAT 13) fitted the factors to a complete quadratic response surface and analyzed the fitted surface to determine which values of each of the factors tested would give an optimum response.

RESULTS AND DISCUSSION

Chromatographic method

Initially different mobile phases containing methanol, water, acetonitrile, ammonium formate, acetate buffer, phosphate buffer of different PH in different proportions were used with different columns and finally found that the phosphate buffer having the PH 2.5 (PH adjusted with orthophosphoric acid) and methanol in proportion of 40:60was suitable for method and selected mobile phase give the good resolution with acceptable peak shape. A representative graph of this is shown in Fig.1.

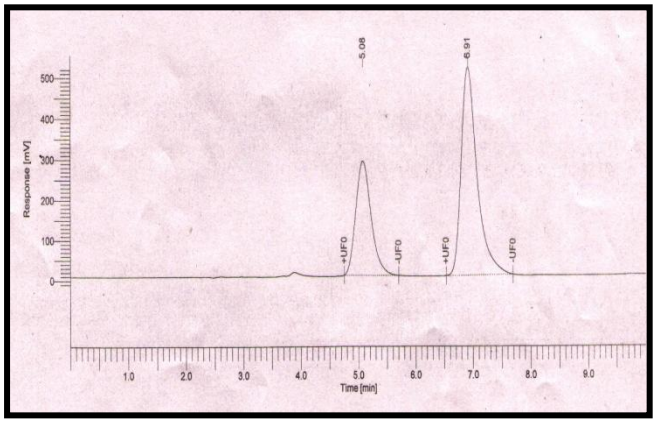

Figure 1: Sample Chromatogram with Perindopril at and Indapamide at 5.08 min and 6.91 min respectively.

System suitability

To check the system suitability 6 injections were run and percentage RSD was calculated for obtained all the responses of the injection. It also involves the parameters like retention time, resolution, symmetry factor. The result obtained is shown in Table 3 The rsd was obtained less then the 2%. All these parameters were evaluated with the background of regulatory requirements, which also suggests good chromatographic condition.

Screening design

Preliminary experiments involved the investigation of the variables that might have influence on the behavior of analyzed substances in the chromatographic system. Generally, the HPLC separation depends on the physical and chemical properties of thecompounds, composition and pH of the mobile phase, column temperature and stationary phase properties. According to this, in the screening phase all factors that could influence the separation of IND and PER as well as their domains were determined. During preliminary experiments, nature of the stationary phase was firstly investigated. The C8 packing columns showed to be the most suitable according to the nature of the compounds than C18. As the paramount of the modern pharmaceutical analysis is to provide higher column efficiency and shorter analysis time, we decided to continue our investigations on Inertsil ODS C_8 column. This enabled us to provide satisfactory peak symmetries and to work with different flow rates. For that reason, the influence of the flow rates on the chromatographic behavior of the compounds was also included in the screening design. Afterwards, the percentage of organic modifier was examined. Between different organic modifiers, methanol showed the best characteristics considering peak shape and retention parameters. As the retention time of the compounds was unreasonably prolonged when amount of methanol was changed so this factor is included in factorial design. Considering peak broadening and symmetries, the addition of phosphate buffer was necessary. Satisfactory peak symmetries were accomplished with phosphate buffer. Since lower concentrations of the phosphate buffer did not give satisfactory results and higher concentrations showed to have no influence on the retention parameters, buffer concentration was held constant and was not taken into the consideration in the further investigation. The pH values of the water phase were decided to be varied from 2.5 to 4.5. The idea was

to examine wider pH range and investigate the statistical significance of pH variable and all possible interactions between pH and all other variables, which would lead to the choice of a thorough experimental optimum. The temperature was examined in the range from 25 to 35 ^{0}C. Since the peak symmetries of both compounds were considerably worse on higher temperatures, wider range of temperature was not investigated. After preliminary experiments, 2 $^{4-1}$ fractional factorial design (FFD) was performed. FFD was employed prior to central composite design to reduce the number of variables. In that way only statistically significant variables would be studied in detail employing optimization designs. Applying FFD the number of experiments can be kept low based on the assumption that interaction effects among three or more parameters are small compared to main effects and two-variable interaction effects. From the obtained results it could be concluded that percentage of methanol and pH of the water phase showed statistically significant influence on the chromatographic behavior of both substances. The temperature of the column and flow rate showed to have no statistically significant influence on the chromatographic behavior of the investigated compounds and was further held constant. In order to reach a compromise among the responses which could better satisfy the goals, the Derringer's desirability function was used, thus converting a multi-response problem into a single response one [9]. In these situations where compromise between goals is necessary desirability function becomes an interesting and powerful tool. 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:

$$D = [dp1^1 \times dp2^2 \times dp3^3 \times \ldots \times d^{pn}n]^{1/n}$$

where *pi* is the weight of the response, *n* the number of responses and *di* is the individual desirability function of each response obtained from the transformation of the individual response of each experiment. The response surfaces corresponding to DIND and DPER are presented in Fig. 3 and 4 Considering IND, the *d* is approaching to 1 when percentage of methanol is approaching to 60 % and if at the same time pH of the water phase is approaching to 2.5. Considering PER the interaction between examined variables also exist and the influence of methanol on the retention factor of PER is much grater when working with lower pH of the water phase. The values of *d* approaching to 1 are with 60% of methanol and pH of 2.5. For final choice of optimal conditions, which would satisfy all proposed goals, the overall Derringer's desirability function is obtained and is graphically presented at Fig. 3 and 4. The satisfactory chromatographic conditions, which would fulfill both goals, could now be selected

Table no 3: Assay of indapamide and Perindopril erbumine in Tablet.

S. No	Concentration present in tablet (µg/ml)		Concentration of added drug in reanalyzed solution (mg/ml)		% Recovery		Statistical Analysis
	PDP	IND	PDP	IND	PDP	IND	**PDP**
1	240	75	0.8 mg	0.25 mg	112%	111%	**Mean** = 111.66% **S.D** = 0.57 **% R.S.D** =0.517
2	240	75	0.81mg	0.24mg	111%	112%	**IND** **Mean =**112.0% **S.D** = 1.0 **% R.S.D** = 0.892
3	240	75	0.8 mg	0.25 mg	112%	113%	
1.	240	75	1.60 mg	0.50mg	103%	94.66%	**PDP** **Mean** = 103.36 **S.D** = 0.404 **% R.S.D** =0.39
2.	240	75	1.65mg	0.55mg	103.8%	94.87%	
3.	240	75	1.61mg	0.55mg	103.3%	94.79%	**IND** **Mean =**94.75 **S.D** = 0.138 **% R.S.D** =0.146
1.	240	75	2.40mg	0.75mg	100.1%	90%	**PDP** **Mean** = 100.43 **S.D** = 0.416 **% R.S.D** =0.414
2.	240	75	2.44mg	0.75mg	100.9%	90.4%	
3.	240	75	2.40mg	0.74mg	100.3%	90.1%	**IND** **Mean =**90.16% **S.D** = 0.20 **% R.S.D** = 0.23

Table no 4: The plan of the experiments for the FFD 2^{4-1}design.

	Variables			
Experiments	**pH value of phosphate buffer**	**Methanol in the mobile phase**	**Flow rate of the mobile phase**	**Temp of the column**
1	-1	1	1	-1
2	-1	-1	1	1
3	-1	-1	-1	-1
4	-1	-1	-1	1
5	1	1	-1	1
6	1	1	1	1
7	1	1	-1	-1
8	1	-1	1	-1

Table 5: Investigated variables and their levels studied in the FFD 2^{4-1}design.

Variables	Investigated Levels		
	-1	0	+1
pH value of phosphate buffer	2.5	3.5	4.5
Methanol in the mobile phase	60	70	80
Flow rate of the mobile phase	0.2	0.5	0.8
Temp of the column	25	30	35

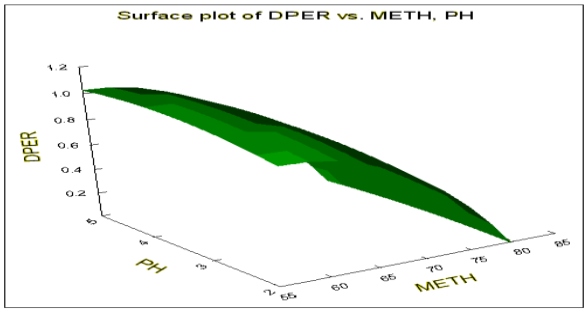

Figure 2: Response surface plot of the IND.

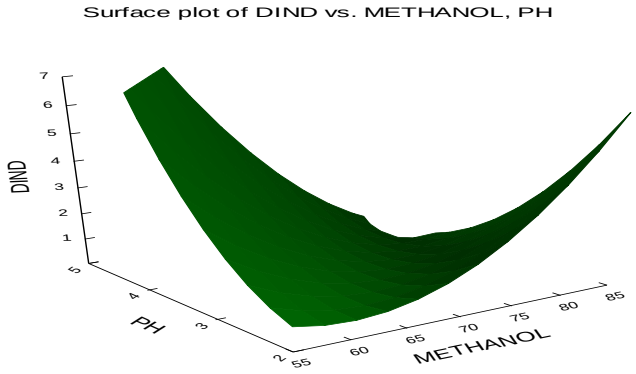

Figure 3: Response surface plot of the PER.

Linearity

The method proved to be linear with a range of 25 µg/ml to 800 µg/ml for Perindopril with r^2 = 0.9997 and 3.65µg/ml to 187.56g/ml for Indapamide with r^2 = 0.996. Values are acceptable by ICH guidelines. This linearity was represented by a linear regression equation as follows.

YIND = 84057conc. + 440334 (r2 = 0.996)

YPER = 26451 conc. + 87709 (r**2** = 0.9997)

Method Validation

The recovery experiment was carried out by spiking the already analyzed sample of the tablets with their different known concentration of standard IND and PER. The result is summarized in Table 3.The LOD / LOQ are been calculated the LOD for Perindopril-1.55µg/ml, Indapamide-0.05 µg/ml and the LOQ for Perindopril-5.12 µg/ml, Indapamide-1.6 µg/ml

Assay

The content of SIM and EZ found in the tablets by the proposed method are shown in Table 2. The low R.S.D indicates that the method is precise and accurate.

Stability of sample solution

The standard and sample solutions made in mobile phase [Phosphate Buffer (ph = 2.5 by OPA : Methanol (40:60 v/v)] was found stable for more than 24 hours.

CONCLUSIONS

With assistance of experimental design and Derringer's desirability function new chromatographic method has been developed. The proposed RP-HPLC method allows for accurate, precise and reliable measurement of IND and PER simultaneously in combined dosage form. The developed RP-HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in respective two-component tablet dosage form of IND and PER. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of IND and PER in multicomponent pharmaceutical preparation.

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