IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)
An International Open Access, Peer-reviewed, Refereed Journal

Application Of Quality By Design In The Development Of An Hplc Method For Simultaneous Estimation Of Lansoprazole And Chlorthalidone In A Solid Dosage Form

[1]Pooja Rajendra Bansode, [2]Dr.A.S.Birajdar
[1]Research Scholar, [2]HOD Of Quality Assurance Dept(Research Guide)
[1]Master Of Pharmacy In Quality Assurance,
[1]Adarsh Shikshan Prasarak Mandal's K. T. Patil College Of Pharmacy, Dharashiv, Maharashtra, India

Abstract:

This study presents the application of Quality by Design (QbD) principles in the development of a High Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of Lansoprazole and Chlorthalidone in a solid dosage form. The co-administration of these drugs in certain therapeutic regimens necessitates accurate and reliable analytical methods. By systematically optimizing critical method parameters, including mobile phase composition, column chemistry, and detection wavelength, a robust and efficient HPLC method was established. The methodology employed a Design of Experiments (DOE) approach along with risk assessment and multivariate analysis tools to ensure method robustness, accuracy, and reproducibility. This research contributes to advancing analytical method development practices, ensuring the quality and safety of pharmaceutical products.

Keywords: Quality by Design, HPLC method development, Lansoprazole, Chlorthalidone, solid dosage form, Design of Experiments, method optimization.

INTRODUCTION:-

Lansoprazole(LPZ) chemically known as 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole. It is a proton pump inhibitor commonly used in the treatment of gastric ulcer, gastro oesophageal reflux disease (GERD), duodenal ulcer, ulcers associated with usage of Nonsteroidal anti-inflammatory drug (NSAID) and long term management Zollinger-Ellison syndrome.

Chlorthalidone (CLR) chemically known as 2-chloro-5-(2,3-dihydro-1-hydroxy-3-oxo-1H-isoindol-1-yl). It is considered to be a thiazide-like diuretic. Chlorthalidone has the longest duration of action but similar diuretic effect at maximal therapeutic doses. It is often used in management of hypertension and edema.

An analytical technique is a specific application of a methodology to a particular analytical problem. A interesting and exciting part of chemical analysis, instrumentation interacts with several chemistry disciplines as well as numerous other domains of pure and applied study.

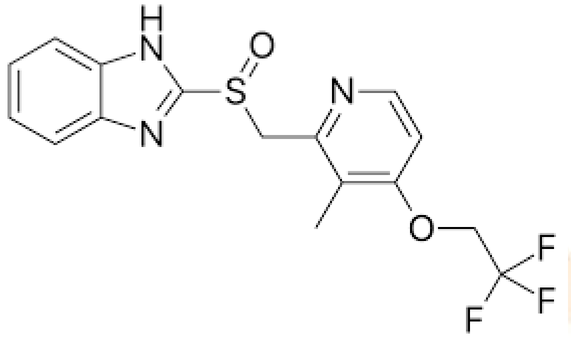


Figure No 1. Chemical structure of lansoprazole

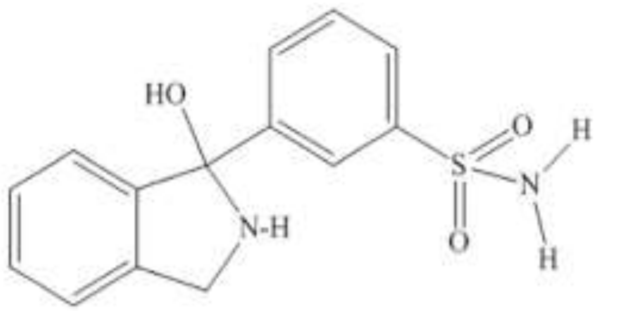


Figure No 2. Chemical structure of Chlorthalidone

Quality by design (QbD):-

Numerous applications in industrial quality control and research and development (R&D) labs attest to the widespread applicability of the quality by design (QbD) approach to the creation of pharmaceutical goods. on contrast to quality by testing (QbT), quality by design (QbD) enhances comprehension of procedures and goods with predetermined objectives grounded on chemistry, statistical analysis, and risk assessment.

Method development and Validation :-

Validation plays role a in demonstrating that any procedure, process, method, equipment, material, activity as expected under a given set of circumstances. The process by which it's developed, by laboratory studies that the performance specifications of the process connect the need for the deliberated analytical application. Analytical procedure mentions that how to perform the analysis. It gives details about every step to conduct all analytical tests. The purpose of validation is that an analytical procedure is to demonstrate that is suitable for its intended use.

MATERIAL AND METHODS:-

Chlorthalidone	**IPCA Laboratories, Mumbai**
Lansoprazole	**Alcon Biosciences Private Ltd., Mumbai**
Eudragit S100	**Degussa, Darmstadt, Germany**
HPMCP HP55	**ShinEtsu, Tokyo, Japan**
Poly vinyl alcohol	**Fisher Scientific Co. Inc**
Sodium carbonate	**Wako Pure Chemicals Industries, Ltd., Osaka, Japan**
Sodium hydroxide	**Scharlau Chemie S. A, Barcelona, Spain**
Aerosil 200	**Degussa, Darmstadt, German**
pluronic F68	**BASF Chemical Company, Ludwigshafen, Germany**
Acetone, acetonitrile and methanol were HPLC grade	
Crospovidone	**Maruthi chemicals Ahmedabad**
Microcrystalline cellulose, magnesium stearate and talc	**S.D. Fine Chemicals. Mumbai**

Instrumentation:

A Waters HPLC instrument- LC-20AD (Japan) equipped with rheodyne 7725 injection valve with a 20 µL loop volume and binary gradient pump was used. The system also included a PDA (Shimadzu, SPD-20A) detector operated at a wavelength of 210 nm. Data were acquired and processed by using empower 2 software. Chromatographic separation was performed using Discovery C18 column (250 mm, 4.6 mm, and 5 µm).

Software:

Experimental design (CCD), desirability function, and data analysis calculations were performed by using Design-Expert version 10.0.3.1

Methods:

Diluent Buffer: Acetonitrile in the ratio of 50:50 was used as diluent.

Preparation of Buffer:

(0.1% OPA) 1 mL of orthophosphoric acid was accurately transferred into a 1000 mL volumetric flask and about 900 mL of milli-Q water added. The solution was degased by sonication and then brought up to the volume (1000 mL) with water.

Diluent: Water: Methanol in the ratio of 50:50 was used as diluent.

Preparation of standard stock solution:

About 2.5 mg of LPZ and 30 mg of CLR were accurately weighed and transferred in to a 10 mL clean, dry volumetric flask. The contents of the flask were dissolved in diluent, sonicated for 30 min, and made up to the final 10 mL volume with diluent. From the above stock solutions, 1 mL was pipetted out into another 10 mL volumetric flask and then made up to the 10 mL volume with diluent.

Sample preparation:

10 tablets were weighed and crushed. From that, a powder equivalent to 2.5 mg and 3.0 mg of LPZ and CLR was weighed accurately and transferred into a10 mL clean dry volumetric flask. The contents of the flask were dissolved in diluent, sonicated 30 min, and made up to the final volume with diluent and labeled as Sample stock solution. Sample stock solution was filtered by PVDF 0.45um filters. 1 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up to the volume (10 mL) with diluent.

Chromatographic procedure:

Chromatographic separations were carried out on a Discovery C18 column (250 mm, 4.6 mm, and 5 µm). A mixture of acetonitrile and 0.1% orthophosphoric acid (50:50) was used as the mobile phase. Wavelength of 210 nm was used for detection, at which both drugs gave good response.

Software aided method:

Optimization Literature revealed that some design methodologies were presented to assess the robustness of method (Ganorkar, Dhumal, Shirkhedkar, 2017). They applied in the circumstances of optimizing separation techniques during screening, testing of robustness and also in the context of optimizing formulation, products, or method. Here in the present work, the important chromatographic factors were selected, based on preliminary experiments and prior knowledge from the literature. A good choice among the screening design in the testing of few factors (three or less) for robustness, may be CCD because of its efficiency, with respect to number of runs required (Petkovska, Cornett, Dimitrovska, 2008).Various factors were considered for method development, including volume of organic solvents in the mobile phase, buffer, flow rate, and column temperature (Daud, Shakya, 2014). Thus, CCD was employed to evaluate the effects of three independent chromatographic parameters on the three defined key response variables. The design was comprised of 20 experimental runs and helped in screening of factors by evaluating their main effect to get outcomes of the study. A 32 factorial design indicated that there were two levels and three factors were involved. The two levels were low (-1) and high (+1), whereas factors were (X1) proportion of organic solvent used in mobile phase (45% and 55%), (X2) flow rate of mobile phase (0.9 and 1.1 mL/min), and (X3) column temperature (28 and 32 °C).

The retention time of LPZ (Y1), retention time of CLR (Y2), and resolution (Y3) were used as responses in experimental design and were shown in Table I. The resulting data were fitted into Design-Expert version 10.0.3.1. Response surface quadratic methodology was a suitable method and was used to explore, to investigate behavior of the response around optimized values of the factors, and to attain the best system performance (Ficarra et al., 2002). Analysis of variance (ANOVA) was applied to examine the significance of the model. From this optimized method, conditions were selected and subjected to verification for method performance, like accuracy, precision (less than 2% RSD), and robustness as targeted response (Peraman et al., 2015). Twenty experiments were constructed using the conditions and observed responses are described in Table No II.

Method validation:

The optimized chromatographic method was validated according to the International Conference on Harmonization (ICH) Q2R(2) guidelines for system suitability, linearity, limit of detection, limit of quantitation, precision, accuracy, specificity, and robustness.

System suitability test:

According to United States Pharmacopoeia (USP), system suitability tests are integral part of liquid chromatographic methods. (Ganorkar, Dhumal, Shirkhedkar, 2017). System suitability parameters, like number of theoretical plates, resolution, and tailing factor were evaluated by injecting six replicates of standard solutions containing 25 µg/mL of LPZ and 30 µg/mL of CLR before the sample analysis. In all cases, the percent relative standard deviation should be < 2.0%. The acceptance criteria for standards in system suitability were set in each chromatogram (Thakur, Kaur, Sharma, 2017; Agrawal, Desai, Jani, 2016).

Table No 1. Experimental plan of CCD showing factors with levels

Factors	Code	Range levels	
		Low (-1)	High (+1)
Proportion of organic solvent used in mobile phase	X1	45	55
Flow rate of mobile phase (mL/min)	X_2	0.9	1.1
Column temperature	X_3	28	32
Responses			
Retention time of LPZ	Y_1		
Retention time of CLR	Y_2		
Resolution	Y_3		

Table 2.Coded values for factor level and observed responses in CCD for 20 analytical trials

Experiment (Run)	Type	X_1	X_2	X_3	Y_1	Y_2	Y_3
1	Factorial	45	0.9	28	3.204	3.876	3.5
2	Axial	41.591	1	30	2.889	3.575	3.7
3	Factorial	45	1.1	32	2.582	3.17	3.8
4	Axial	58.409	1	30	2.687	3.297	3.8
5	Center	50	1	30	2.771	3.405	3.6
6	Center	50	1	30	2.772	2.407	3.6
7	Center	50	1	30	2.771	2.405	3.6
8	Axial	50	1	26.6364	2.769	3.746	4.8
9	Center	50	1	30	2.772	3.407	3.6
10	Factorial	55	1.1	32	2.491	3.05	4.2
11	Axial	50	1.6818	30	2.395	2.948	3.7
12	Center	50	1	30	2.77	3.406	3.6
13	Factorial	55	1.1	28	2.486	3.157	3.8
14	Factorial	55	0.9	28	3.046	3.833	4.1
15	Center	50	1	30	2.771	3.407	3.6
16	Factorial	45	1.1	28	2.615	3.517	4.7
17	Axial	50	0.831821	30	3.33	4.107	3.8
18	Factorial	55	0.9	32	3.029	3.716	4.3
19	Axial	50	1	33.3636	2.792	3.42	4.3
20	Factorial	45	0.9	32	3.17	3.903	4.2

Linearity:

Linearity of the developed method was established at six levels over the range of 12.5-75 µg/mL for LPZ and 25-150 µg/mL for CLR. Each linearity solution of respective sample concentrations was injected in triplicate. The calibration curve was constructed by plotting the peak area against the concentration, using linear regression analysis.

Accuracy and precision:

Accuracy was carried out by adding a known amount of standard to the tablet solution for each drug at 50, 100, and 150 % levels in triplicate, and samples were analyzed by the optimized method. Percentage recovery was then calculated for both drugs. The mean recovery of the target concentrations was set to 100 ± 2% for acceptance (Patel et al., 2017). Precision of the optimized method was determined by studying the intermediate precision and repeatability. Intermediate precision expresses within laboratories variations: different days, different analysts, different equipment, etc. Intermediate precision is also known as inter-assay precision. Repeatability expresses the precision under the same operating conditions over a short interval of time (Pradipbhai et al., 2017). Six homogenous samples of LPZ and CLR were assayed to assess the method precision (Yadav et al., 2016; Thakur, Kaur, Sharma, 2017).

Limit of detection (LOD) and limit of quantitation (LOQ):

LOD and LOQ of LPZ and CLR were evaluated using the standard deviation method. LOD was defined as 3.3 σ/S and LOQ as 10 σ/S based on standard deviation of the response (σ) and slope of the calibration curve (S).

Robustness:

The robustness of the method refers to its ability to remain unaffected by small and deliberate variations in method parameters. The robustness of the optimized method was investigated by injecting the system suitability solution with minute deliberate changes in the chromatographic parameters, flow rate (0.97-0.99 mL/min), proportion of solvent in mobile phase (45:55) and temperature of the column (30.04-32.24 °C). It was measured on the basis of percent relative standard deviation

RESULTS AND DISCUSSION

There were only a few works reported on implementation of QbD in analytical method development (Peraman et al., 2015). In search of a simple, robust, and cost effective RP-HPLC method for estimation of LPZ and CLR in tablet formulation, a preliminary study was initially carried out. The important chromatographic factors were selected, based on preliminary experiments and prior reports from the literature. Such investigations to select the factor levels for screening and optimization studies revealed that mobile phase conditions needed to be optimized so that both LPZ and CLR would be separated in a short run time. Mobile phase composition of 0.1% OPA buffer and acetonitrile was found to be more suitable for the simultaneous estimation of both the drugs and the volume of acetonitrile resulted in a large change in retention time. Hence, it is considered as one of the critical parameters for method development. For further study, different reverse phase columns were tried but finally satisfactory separation was obtained on a Discovery C18 column. From Ishikawa diagram and Pareto ranking analysis, preliminary experiments were conducted and the critical parameters selected for further study, were flow rate and column temperature which were found to have the most influential effect on system suitability parameters. Studies carried out with protamine sulphate also resulted in selection of similar critical parameters (Awotwe-Otoo et al., 2012).

Table No 3. Optimized HPLC chromatogram for lansoprazole and chlorthalidone At 210 nm

Sr. No.	Peak name	RT	Area	%Area	USP Plate count	Resolution	Asym@10
1	LPZ	2.792	513372	14.21	7381	0.0	1.3
2	CHR	3.420	3098592	85.79	7626	4.3	1.2

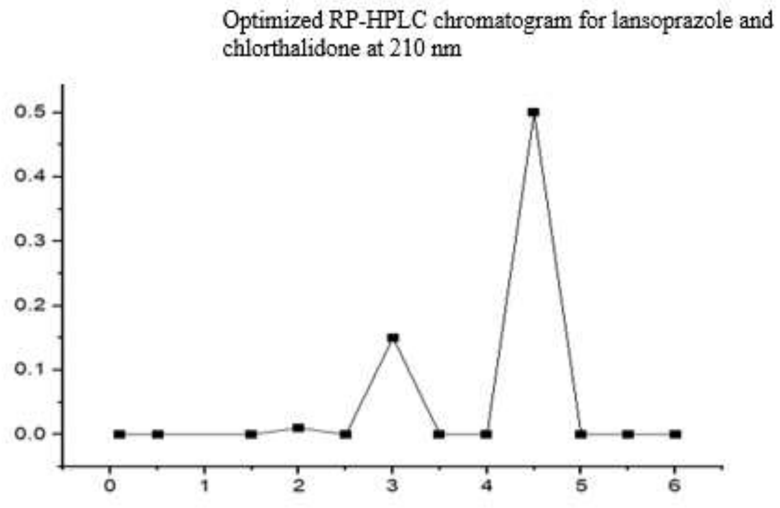


Figure No 3. Optimized HPLC chromatogram for lansoprazole and chlorthalidone at 210 nm

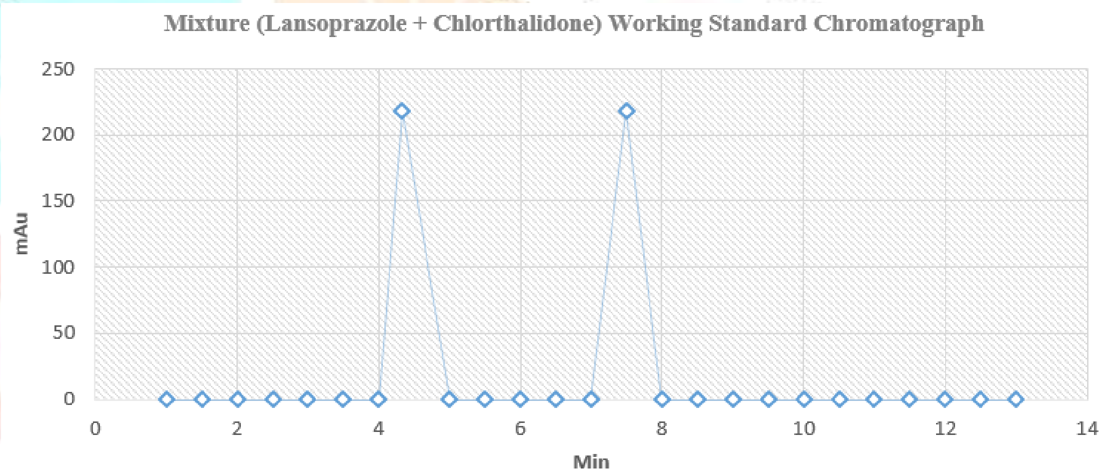


Figure No 4. Mixture (Lansoprazole + Chlorthalidone) Working Standard Chromatograph

In the HPLC method development, the retention time for Lansoprazole peak was determined to be 7.43 minutes, indicating the method's suitability for simultaneous estimation of Lansoprazole and Chlorthalidone in a solid dosage form. Similarly, in the same method development, the retention time for Chlorthalidone peak was found to be 4.13 minutes, confirming the method's effectiveness for simultaneous estimation of Lansoprazole and Chlorthalidone in a solid dosage form.

Results on Specificity and Assay of Lansoprazole and Chlorthalidone:-

The % Assay for Lansoprazole and Chlorthalidone was found to be 99.24% and 98.26% respectively.

Table No 4. Results for Specificity and assay

Sample Id	Lansoprazole			Chlorthalidone		
	RT (min)	Area	% Assay	RT (min)	Area	% Assay
S1	4.82	25600	99.5	7.35	18500	98.2
S2	4.85	24800	98.8	7.38	18900	99.1
S3	4.8	26000	99.3	7.3	18200	97.5
S4	4.83	25200	99	7.36	18700	98.7
S5	4.81	25700	99.6	7.34	18300	97.8

System Suitability:
System Suitability was performed as per instruction in material and method and results were recorded as below:

Table No 5. System Suitability Parameters for Lansoprazole

Sample ID	Retention Time (RT)	Theoretical Plates (TP)	Asymmetry	Resolution
100% Rep-1	6.69	11345	1.1	2.5
100% Rep-2	6.69	11432	1.08	2.6
100% Rep-3	6.69	11749	1.12	2.55
100% Rep-4	6.69	11385	1.15	2.48
100% Rep-5	6.69	11723	1.07	2.65
AVG	6.69			
STDEV	0.019			
% RSD	0.02%			

Table No 6. System Suitability Parameters for Chlorthalidone

Sample ID	Retention Time (RT)	Theoretical Plates (TP)	Asymmetry	Resolution
100% Rep-1	3.12	4312	1.1	0.0
100% Rep-2	3.12	4426	1	0.0
100% Rep-3	3.12	4489	1.03	0.0
100% Rep-4	3.12	4791	1.2	0.0
100% Rep-5	3.12	4901	1.09	0.0
AVG	3.12			
STDEV	0.00			
% RSD	0.01			

Precision (Repeatability) :-

A total of 5 replicate injections were done to check if the method is precise or not.

Table No 7. Repeatability results

Repeatability	Lansoprazole	Chlorthalidone
	Area	Area
Repeatability 1	25480	18950
Repeatability 2	24950	18270
Repeatability 3	25650	18350
Repeatability 4	25270	18540
Repeatability 5	26200	18460
Avg	25510	18514
STDEV	465.77	264.63
RSD	1.8	1.4

With respect to the areas, both the drugs were found to have %RSD less than 2 which is in accordance with ICH Guidelines.

Linearity :-

The 5 points Linearity was performed for each Working Standard API. The results obtained are detailed below:

Table No 8. Linearity for Lansoprazole

Sample ID	Lansoprazole RT (min)	Lansoprazole Area	Lansoprazole % Assay
60	4.82	25600	99.5
80	4.85	24800	98.8
100	4.8	26000	99.3
120	4.83	25200	99
140	4.81	25700	99.6

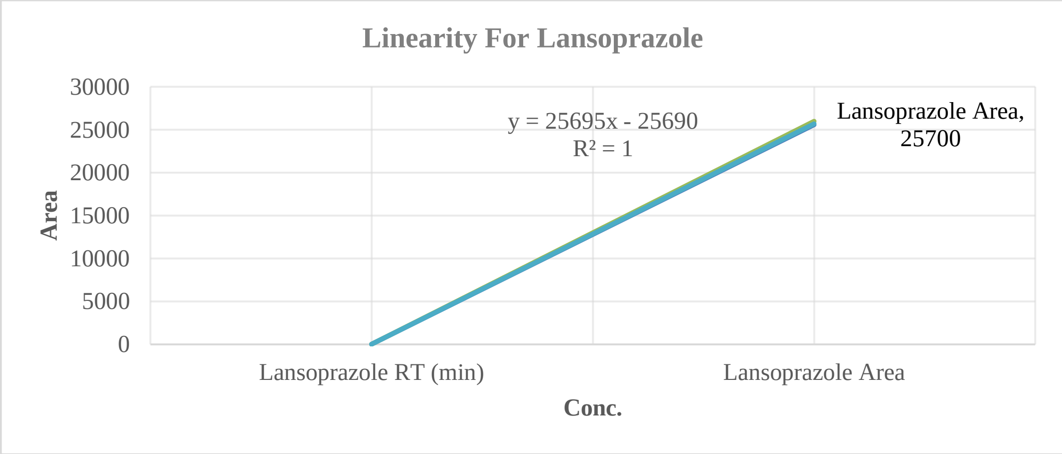


Figure No 5. Linearity for Lansoprazole

Table No 9. Linearity for Chlorthalidone

Conc	Chlorthalidone RT (min)	Chlorthalidone Area	Chlorthalidone % Assay
60	4.82	18500	98.2
80	4.85	18900	99.1
100	4.8	18200	97.5
120	4.83	18700	98.7
140	4.81	18300	97.8

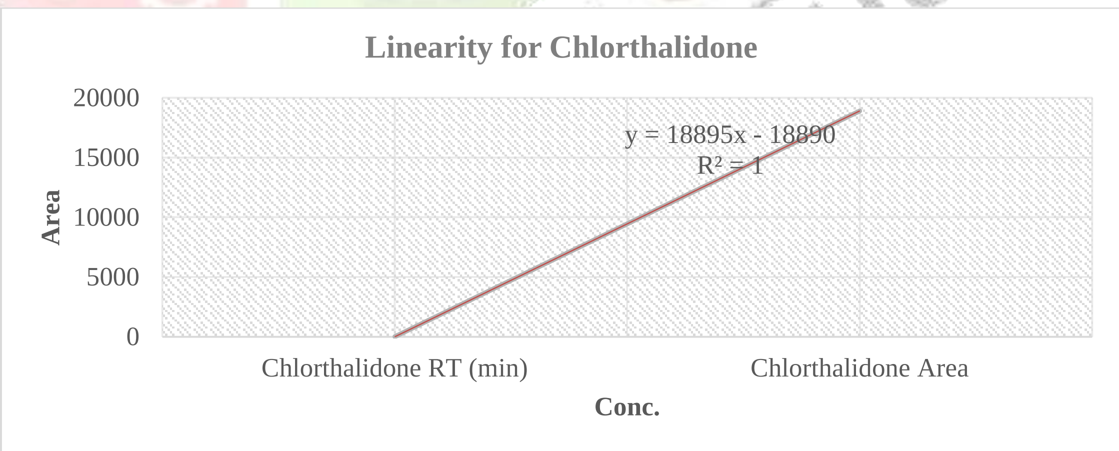


Figure No 6. Linearity for Chlorthalidone

Linearity Summary:-

Table No 10. Linearity summary :-

Parameter	Lansoprazole	Chlorthalidone
Y- Intercept	25695	18895
Slop (m)	25690	18890
R^2	1	1

Table No 11. HPLC Method Validation Parameters

Parameter	Lansoprazole	Chlorthalidone
Linearity (R2)	1	1
LOD (µg/mL)	0.05	0.1
LOQ (µg/mL)	0.1	0.2
Precision (% RSD, n=6)	1.8	1.4
Accuracy (% recovery, n=3)	99.44	98.86
Robustness (RSD, n=6)	1.1	1.4
Range(µg/ml)	30-80	60-130
Slope	25690	18890
Intercept	25695	18895
Correlation coefficient	0.999	0.999
Repeatability	1.8	1.4

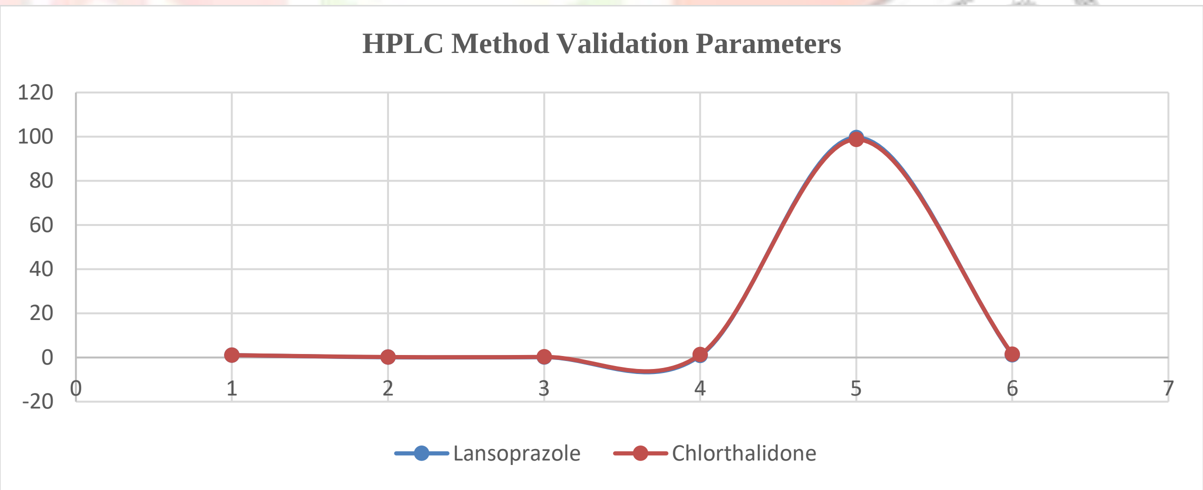


Figure No 7. HPLC Method Validation Parameters

Table No 12. Screening of Critical Quality Attributes (CQAs)

CQA	Specification	Target Value
Retention time (Lansoprazole)	± 0.2 min	6.5 min
Retention time (Chlorthalidone)	± 0.3 min	3.8 min
Resolution (Rs)	≥ 2.0	-
Peak symmetry (Lansoprazole)	≤ 2.0	-
Peak symmetry (Chlorthalidone)	≤ 2.0	-

Design of Experiments (DoE):

Table No 13. Design of Experiments (DoE) Matrix for Method Development

Sr. No	Mobile Phase Composition (%)	pH	Column Temp. (°C)	Flow Rate (mL/min)
1	40	2.5	25	0.8
2	60	3.5	40	1.2
3	60	2.5	25	1.2
4	40	3.5	40	0.8

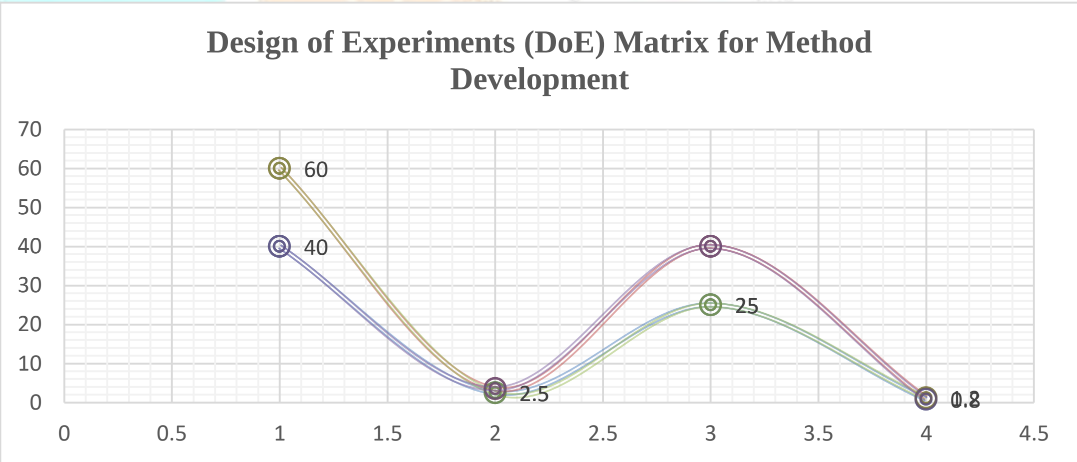


Figure No 8. Design of Experiments (DoE) Matrix For Method Development

Table No 14. Forced Degradation Study Results

Condition	Lansoprazole (%)	Chlorthalidone (%)
Acid hydrolysis (pH 1.2)	1.5	0.8
Base hydrolysis (pH 13)	0.3	1.2
Oxidation (3% H2O2)	1.2	2
Thermal stress (80°C, 24h)	0.8	1.5
Photolysis (UV exposure)	1	1.3

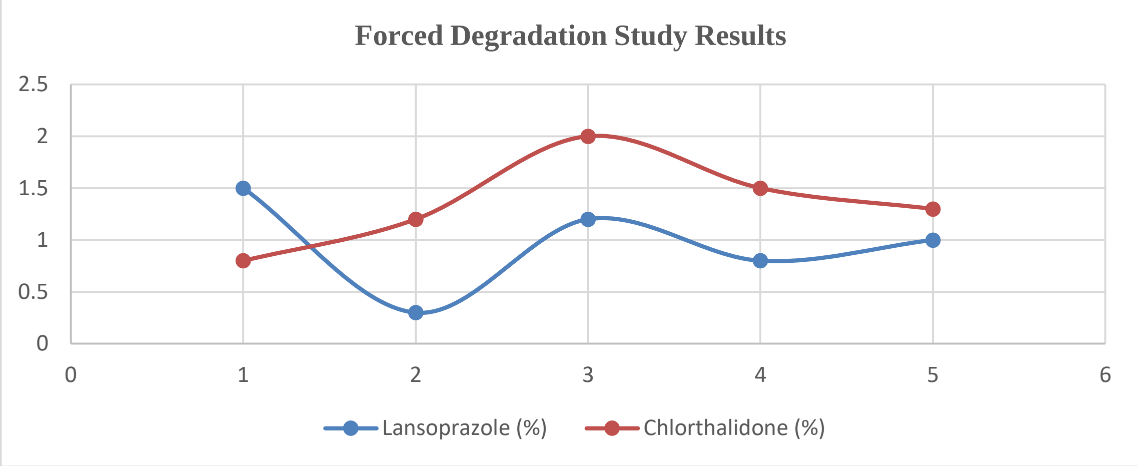


Figure No 9. Forced Degradation Study Result

QbD assisted method development:-

CCD design was employed in the present analytical method optimization study. It is an efficient and comprehensive experimental design based on systematic scouting of three key components of the RP-HPLC method (volume of organic phase, flow rate, and column temperature) is depicted.

For the RP-HPLC method, a multivariate approach DoE with CCD was applied to study the simultaneous variations of the factors on considered responses, such as retention time of LPZ (Y1), retention time of CLR (Y2), and resolution (Y3) to test method robustness. Based on the effects of three factors on responses and evaluation of these results, it was feasible to elaborate mathematical models that had been endeavored to find out the relationship between the factors and the responses of interest studied. We observed that the best fitted model for CCD was the response surface quadratic model. The model was also validated by ANOVA using Design Expert software. The predicted R- squared values of retention time of LPZ (Y1) and CLR (Y2) were in reasonable agreement with adjusted R- squared values i.e., the difference is less than 0.2, as reported by other authors (Pradipbhai et al., 2017). A negative predicted R- squared value for resolution (Y3) implies that the overall mean may be a better predictor of the response than the current model. Adequate precision, measures the signal to noise ratio. A ratio of greater than 4 is desirable and the obtained responses for the Y1, Y2, and Y3 were 96.445, 29.953, and 6.118, respectively, which indicates an adequate precision. This quadratic model can be used to navigate the design space. Model F-value of responses for retention time of LPZ (Y1), retention time of CLR (Y2), and resolution (Y3) were 6.69, 3.12, and 2.5, which implies the model is significant. There is only a 0.01% chance, in the case of Y1 and Y2, while a 1.44% chance for resolution (Y3) than an F-value, indicating that this could occur due to noise. Hence, the values of significant responses showed p value < 0.05, suggesting that the model terms are significant. The low standard deviation and high adjusted R-square value indicates a good relationship between experimental data and those of fitted models. The equations in terms of coded factors can be used to make predictions about the response for given levels of each factor. This equation is useful for identifying the relative impact of the factors by comparing the factor coefficient. Final equations for Y1, Y2, and Y3 are:

$$\text{LPZ } (Y_1) = +2.77 - 0.063X_1 - 0.28X_2 - 2.95X_3 + 9.87X_1X_2 + 6.87X_1X_3 + 2.87X_2X_3 + 9.15X_1^2 + 0.03X_2^2 + 6.50X_3^2;$$

$$\text{CLR: } (Y_2) = +3.41 - 0.08X_1 - 0.32X_2 - 0.08X_3 - 0.03X_1X_2 + 0.01X_1X_3 - 0.04X_2X_3 + 0.01X_1^2 + 0.044X_1^2 + 0.06X_3^2\ ;$$

$$\text{Resolution: } (Y3) = +3.60 + 0.02X_1 + 0.01X_2 - 0.03X_3 - 0.15X_1X_2 + 0.10X_1X_3 - 0.17X_2X_3 + 0.06X_1^2 + 0.06X_2^2 + 0.34X_3^{2.}$$

As per the values of coefficient from the above equations and their signs, it is clear that factors, such as mobile phase composition (X_1), flow rate (X_2), and column temperature (X_3), had a negative effect on retention time of LPZ and CLR, Y_1 and Y_2. The column temperature (X_3) had a negative effect on resolution (Y3), whereas mobile phase (X_1) and flow rate (X_2) had positive effects. Interactions of X_1 and X_2 had a positive effect on Y_1 and Y_2 and a negative effect on Y_3; X_2 and X_3 had a positive effect on Y_1 and a negative effect on Y_2 and Y_3; X_1 and X_3 had a positive effect on Y_1 and Y_2 and a negative effect on Y_3. The squares of factors, $X1^2$, $X2^2$, and $X3^2$, had positive effects on all chromatographic responses.

Response surface and contour plots were analyzed to visualize the effect of the factors and their interactions on the responses (Awotwe-Otoo et al., 2012). The contour plots showed curvature, displaying a nonlinear effect of factors on responses. Figures 3 and 4 showed 2D (A) and 3D (B) contour plots displaying the effect of mobile phase ratio (X_1) and flow rate (X_2) on retention time of LPZ (Y_1) and CLR (Y2). A curvilinear increasing trend was observed for the mobile phase ratio (X1) and flow rate (X2), which showed higher resolution time of LPZ (Y1), as well as CLR (Y2) at lower levels. Therefore, lower levels of X1 and X2 were recommended to achieve high retention time of LPZ (Y1) and CLR (Y2). The study of 3D and 2D contour plots presented in Figure No 29 showed curvature effects of the mobile phase ratio (X1) and flow rate (X2) on resolution. An increasing curvature trend was observed for both X1 and X2, which showed higher resolution at higher levels. Therefore, optimized levels of X1 and X2 were recommended to achieve resolution.

A composite desirability was applied to obtain an optimum set of conditions based on the specified goals and boundaries for each response. The desirability function "R", equal to unity, demonstrated the achievement of desired goals in the constraints set and the whole experimental area was explored for the compositions (Costa, Lourenço, Pereira, 2011), where in constraints set were met to the maximum i.e., unity, as shown in Figure No 6. The optimum values of chromatographic conditions of RP-HPLC were selected as mobile phase (X1) buffer and acetonitrile 40:60, flow rate (0.96 mL/min), and column temperature 25°C which resulted in retention time of LPZ (Y1) 2.79±0.0162, retention time of CLR (Y2) 3.45±0.013, and resolution (Y1) 3.7±0.002 min, respectively, as shown in Figure.

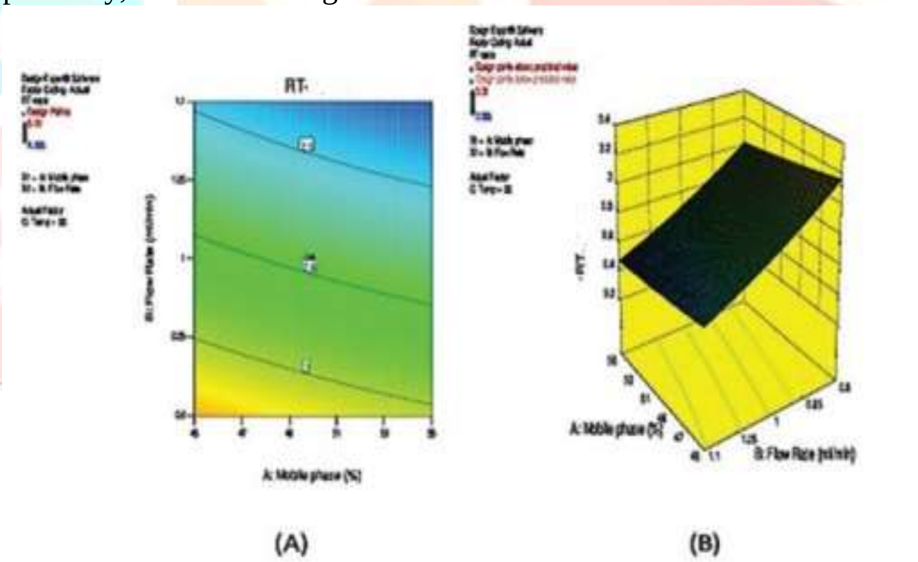


Figure No 10 - 2D (A) and 3D (B) contour plots showing the effect of mobile phase ratio (X1) and flow rate (X2) on retention time of LPZ (Y1)

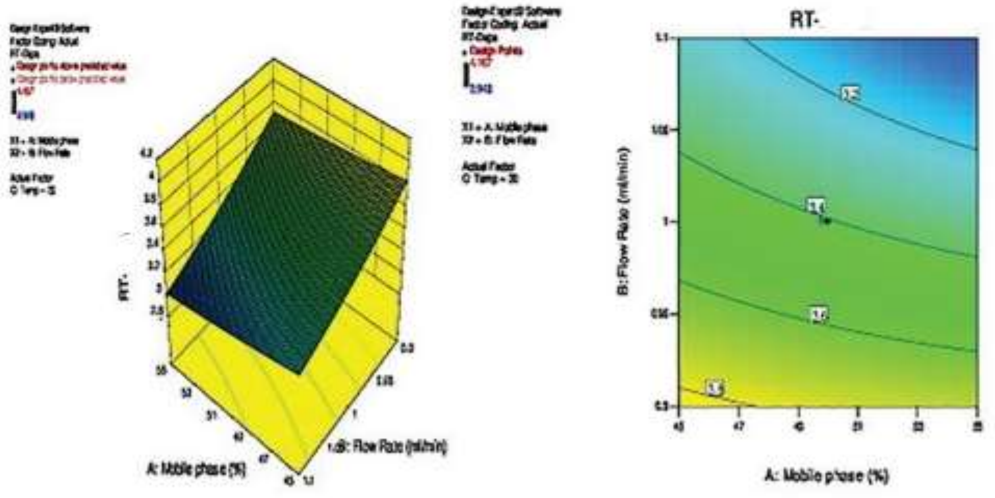
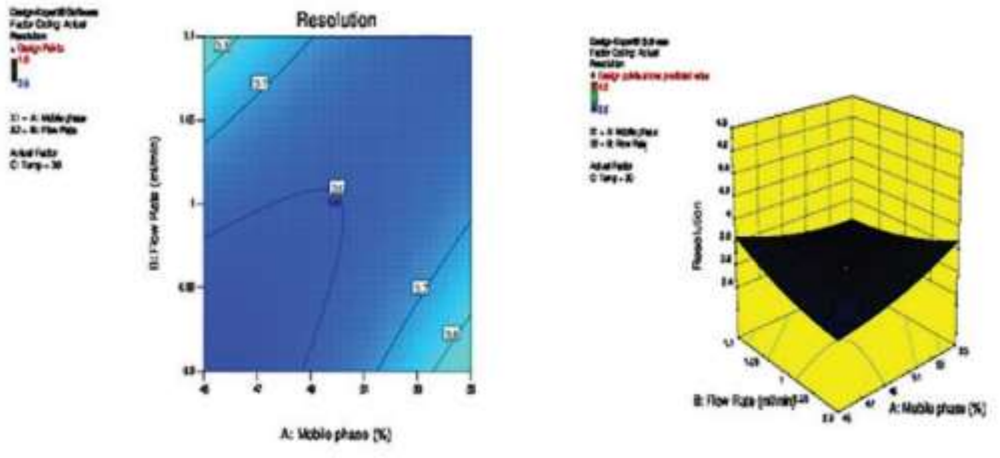


Figure No 11 -2D (A) and 3D (B) contour plots showing the effect of mobile phase ratio (X1) and flow rate (X2) on retention time of LPZ (Y1)



(A) **(B)**

Figure No 12 - 2D (A) and 3D (B) contour plots showing the effect of mobile phase ratio (X1) and flow rate (X2) on resolution (Y3).

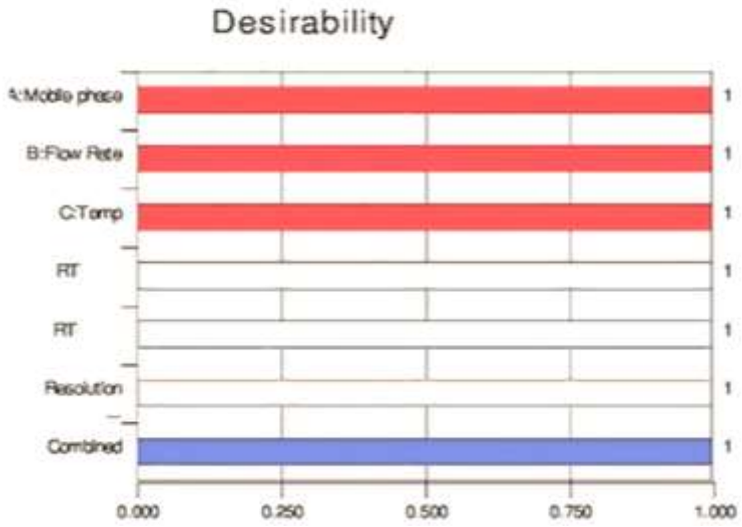


Figure No 13. Desirability

The percent relative standard deviation for six replicate injections was found to be 1.8 in the given concentration of 5µg/mL for LPZ and 1.4 in the given concentration of 10µg/mL for CLR, respectively. As % RSD was found to be less than 2%, it has shown good injection repeatability (Thakur, Kaur, Sharma, 2017). Linearity of the developed method was confirmed by plotting the linearity curve over concentrations ranging from 30-80 µg/mL for LPZ and 60-130 µg/mL for CLR, with a correlation coefficient (r2 =1) for both the drugs, shown in Table IV. The obtained correlation coefficient (r2 =1) demonstrates excellent correlation between peak area and concentration. For the recovery study, different concentrations of samples (50, 100, and 150%) of standard concentrations for both drugs were prepared and showed recovery of 99.91±1.10 % and 98.92 ±0.7997 % for LPZ and CLR, respectively. Data is shown in Table No IV, indicating that the developed method has high level of accuracy with % RSD 1.1 and 1.4 for LPZ and CLR, respectively. Intermediate precision and repeatability were carried out and the resultant data are given in Table IV. The precision values for both drugs were less than 2%., indicating that the method was repeatable and precise (Daud, Shakya, 2014). The LOD and LOQ were found to be 0.05 and 0.1 µg/mL, respectively for LPZ, and 0.04 and 0.14 µg/mL, respectively for CLR. The insensitivity of the RP-HPLC method to minor changes in the optimized experimental changes was demonstrated by its robustness to such slight changes. The mobile phase composition, flow rate, and column temperature caused significant effects in the retention time of LPZ and CLR, as well as resolution.

Conclusion :-

In conclusion, this study demonstrates the successful application of Quality by Design (QbD) principles in the development of a robust and efficient High-Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of Lansoprazole and Chlorthalidone in a solid dosage form. Through systematic optimization of critical method parameters using a Design of Experiments (DOE) approach, including mobile phase composition, flow rate, and column temperature, a method with excellent performance characteristics was achieved. The QbD-guided method development ensured robustness, accuracy, and reproducibility of the analytical method, meeting the requirements of pharmaceutical analysis. The validated HPLC method exhibited satisfactory results for system suitability, linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ), confirming its suitability for routine analysis in pharmaceutical laboratories. The method's robustness to minor changes in experimental conditions further underscores its reliability and practicality. Overall, the successful implementation of QbD principles in method development not only ensures compliance with regulatory standards but also enhances method efficiency and reliability. This research contributes to advancing analytical method development practices, ensuring the quality, safety, and efficacy of pharmaceutical products.

Reference :-

1. Bhanu, M. S. RP-HPLC Method for Quantification of Bilastine and Monteleukast Sodium in Pharmaceutical Dosage form 2023.

2. Rehman, G. M. A. DESIGN, PREPARATION AND EVALUATION OF INDOMETHACIN PELLETS 2016; (Vol. 273, pp. 544–553).

3. Krishna, C. M. FORMULATION AND EVALUATION OF INDOMETHACIN EXTENDED RELEASE PELLETS 2012.

4. Aruna Gundala, KVSRG Prasad, B. K. Application of quality by design approach in RP-HPLC method development for simultaneous estimation of saxagliptin and dapagliflozin in tablet dosage form 2019.

5. Jahangir, Muhammad, Azhar, Arooj, Awan, A. Development and validation of RP-HPLC method for the simultaneous determination of azilsartan medoxomil and chlorthalidone in solid dosage form 2023.

6. Mahmoud M. Sebaiy, Sobhy M. El-Adl, Mohamed M. Baraka, A. A. H. & H. M. E.-S. Quality by design approach for development and validation of a RP-HPLC method for simultaneous estimation of xipamide and valsartan in human plasma 2022.

7. Sara Meirinho , Márcio Rodrigues , Ana Fortuna , Amílcar Falcão, G. A. Novel bioanalytical method for the quantification of rufinamide in mouse plasma and tissues using HPLC-UV: A tool to support pharmacokinetic studies 2019.

8. Oskar Gonzalez , Gorka Iriarte, Nerea Ferreirós, Miren Itxaso Maguregui, Rosa Maria Alonso, R. M. J. Optimization and validation of a SPE-HPLC-PDA-fluorescence method for the simultaneous determination of drugs used in combined cardiovascular therapy in human plasma 2009.

9. Sohni, S. K., Kumar, R., Akhtar, M., Ranjan, C., & Chawla, G. Development and validation of RP-HPLC method for simultaneous estimation of Azilsartan medoximil and chlorthalidone in bulk form and formulation using quality by design. International Journal of Pharmacy and Pharmaceutical Sciences, 2016; 8(2), 266–272.

10. Joglekar, A. P. Method development and validation for the estimation of Telmisartan and Chlorthalidone in bulk and pharmaceutical dosage form by HPTLC method. Research Journal of Pharmacy and Technology, 2013; 8(4), 376–381.

11. Shah, N. J., Suhagia, B. N., Shah, R. R, Shah, P. B. Development and validation of a HPTLC method for the simultaneous estimation of irbesartan and hydrochlorothiazide in tablet dosage form 2007.

12. Khaled Attala a, Maya S. Eissa a, Magda M. El-Henawee b, S. S. A. E.-H. Application of quality by design approach for HPTLC simultaneous determination of amlodipine and celecoxib in presence of process-related impurity 2020.

13. Hisham Hashem a b, H. M. E.-S. Quality by design approach for development and validation of a RP-HPLC method for simultaneous determination of co-administered levetiracetam and pyridoxine HCl in prepared tablets 2018.

14. Mohd Afzal, ORCID, Mohd. Muddassir ORCID, A. A. and Mohammed T. A. Box-Behnken Assisted Validation and Optimization of an RP-HPLC Method for Simultaneous Determination of Domperidone and Lansoprazole 2021.

15. A Marín, E. García, A. García, C. B. Validation of a HPLC quantification of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations: capsules and sachets 2002.

16. Sivakumar Thanikachalam, M. R. & V. K. Stability-Indicating HPLC Method for Simultaneous Determination of Pantoprazole and Domperidone from their Combination Drug Product 2008.

17. Shah, P., Patel, J., Patel, K., & Gandhi, T. Development and validation of an HPTLC method for the simultaneous estimation of Clonazepam and Paroxetine hydrochloride using a DOE approach. Journal of Taibah University for Science, 2017; 11(1), 121–132.

18. Rizwana, I., Prakash, K. V., & Mohan, G. K. Extractive Spectroscopic Determination of Lansoprazole in Pharmaceutical Dosage Form. Chemical Science Transactions, 2014; 3(4), 1390–1395.

19. Rashmin B. Patel, Mrunali R. Patel, K. K. B. HPTLC method development and validation: Quantification of paliperidone in formulations and in vitro release study. 2010.

20. Prakash B. MOD, N. J. S. Novel Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Clindamycin Phosphate and Adapalene along with Preservatives in Topical Gel Formulations 2014.

21. Patel, T. R., Patel, T. B., Suhagia, B. N., & Shah, S. A. HPTLC Method for Simultaneous Estimation of Aliskiren, Amlodipine, and Hydrochlorothiazide in Synthetic Mixture Using Quality by Design Approach. Journal of Liquid Chromatography and Related Technologies, 2015; 38(16), 1546–1554

22. Hossen, M. A., Haque, M. A., Dewan, I., Hamidul Kabir, A. N. M., Hossain, M. K., & Ashraful Islam, S. M. Development and validation of RP-HPLC method for the simultaneous estimation of hydrochlorothiazide and losartan potassium in tablet dosage form. Dhaka University Journal of Pharmaceutical Sciences, 2011; 10(1), 35–42.