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Chemometric Optimization, Development, and Validation of RP-HPLC Method for The Simultaneous Estimation of Hydrochlorothiazide, Amlodipine Besylate, and Telmisartan in Bulk and Pharmaceutical Tablet Dosage Form Using Experimental Design

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ABSTRACT

In the present study, an isocratic RP-HPLC method was developed for the simultaneous determination of Hydrochlorothiazide, Amlodipine besylate, and Telmisartan in bulk and tablet dosage form using statistical experimental design. Three independent factors Acetonitrile concentration, Buffer pH, flow rate were used to design. Central composite design(CCD) was used to study the response surface technique and to determine in-depth the effects of these independent factors. These three responses were simultaneously optimized by using Derringer's desirability function. The optimized assay conditions were ACN: Triethylamine (47.15:52.85)%V/V (pH-3.0) as mobile phase and flow rate of 1.2ml/min. The optimized procedure was validated according to ICH guidelines to confirm specificity, linearity, accuracy, and precision

INTRODUCTION

Chromatography is a non-destructive procedure for resolving a multi-component mixture of trace, minor, or major constituents into its fractions. Different variations may be applied to solids, liquids, and gases. While chromatography may be applied quantitatively, it is primarily a separation tool. High-Performance Liquid Chromatography (HPLC) is an analytical separation technique and is considered as the gold standard used in nearly all analytical laboratories in the pharmaceutical industry throughout the whole lifecycle of a drug product. Since HPLC utilizes a wide selection of chromatographic factors, viz., the type and concentration of organic modifier, pH, buffer molarity, temperature, flow rate, etc., and simultaneous optimization of resolution and analysis time, optimization of the experimental conditions is a complicated process.

Therefore, method development has been performed traditionally by varying one factor at the time (OFAT), or by a more systematic approach, e.g. Design-of-Experiments and software programs, as an efficient and fast tool for method development. In the present study using DOE approach, the optimization is done for the developed method which involves the identification of the method with higher accuracy and specificity for routine QC analysis, then evaluation of factors affecting separation like resolution, retention time, and tailing factor and also the development of method using statistical model possessing relation between factor and response. The chromatographic factors were selected based on knowledge from the literature review and optimized using central composite design i.e to identify the optimum flow rate, mobile phase concentration, pH. For three independent variables, a partial factorial design with five replicates of center points and five axial points is combined. From the results, the qualities of the fitted second-order polynomial models were calculated using the coefficient of determination. Then by applying derringers desirability function the flexible optimized chromatographic conditions were selected for the determination of drugs in a variety of samples.

Hydrochlorothiazide, a diuretic mainly used in the treatment of high blood pressure increases the urine output, thereby causing the elimination of extra salts and water in the body. It is also used to get rid of the excess fluid condition in the body caused by edema in several cardiovascular diseases. Amlodipine besylate, a calcium channel blocker chemically a dihydropyridine is also one of the drugs used directly or in combination as antihypertensives. It acts as a peripheral arterial vasodilator. Telmisartan is an orally active nonpeptide

angiotensin II antagonist of benzimidazole category. The combination of these three drugs Hydrochlorothiazide, Amlodipine besylate, and Telmisartan is available as Dynatel trio which is mainly used in the treatment of hypertension.

The literature review reveals that only a few methods are available for the simultaneous estimation of these three drugs by spectroscopy using chemometrics. No method has been reported for the simultaneous estimation of the Hydrochlorothiazide, Amlodipine besylate and Telmisartan using chemometrics. Hence an attempt was made to develop, optimize and validate an accurate and sensitive HPLC method for the simultaneous determination of the above drugs in tablet dosage form using experimental design.

MATERIALS AND METHODS

Chemicals and reagents

Pure standards of Hydrochlorothiazide (HCT), Amlodipine Besylate (AMB), and Telmisartan (TEL) were gifted from Aristo Pharmaceutical, Chennai. The tablet formulation Dynatel Trio containing Hydrochlorthiazide HCT (12.5 mg), Amlodipine Besylate AMB (5 mg), and Telmisartan TEL (40 mg) was purchased from a local pharmacy. Methanol (AR grade), Methanol (HPLC grade), Acetonitrile (HPLC grade), Water (HPLC grade), Orthophosphoric acid (AR grade), and triethylamine (AR grade) were purchased from Qualigens India Pvt. Limited, Mumbai and Loba chemical India Limited, Mumbai.

Instrumentation

Chromatographic measurements were made on Shimadzu HPLC having detector with deuterium lamp source in the range 190 to 600nm with double reciprocating plunger pump with constant flow and pressure delivery. The mobile phase was degassed by using Ultrasonicator (3.5L100) PCI Analytics private ltd., Mumbai. The UV spectrum was recorded using a UV-Visible spectrophotometer (Model Shimadzu, Japan (Model UV 1800) - software UV probe 2.32 version.

Software

Experimental design, data analysis, and desirability function calculations were performed by using Design -Expert trial version 12.0 (State- Ease Inc., Minneapolis). The rest of the

calculations for the analysis were performed by the use of Microsoft excel 2007 software (Microsoft USA).

Mobile phase selection

The main requirement of the mobile phase is that it has to dissolve the analytes up to the concentration suitable for detection. The mobile phase absorbance should usually be less than 0.5 at the wavelength used for detection. When the absorbance of the mobile phase exceeds a value of about 1.0 the detector may become unusable. Hence the mobile phase suitable for samples is selected by performing trials with different ratios of the mobile phase.

Preparation of Mobile phase

The mobile phase was prepared by mixing 50.0 ml of acetonitrile with 50.0 ml of 1% triethylamine (pH adjusted to 3.5 with orthophosphoric acid). This mobile phase was filtered through a 0.42 µ membrane filter and then it was ultra-sonicated for 30 minutes.

Preparation of standard stock solution

About 25 mg of the reference standard of HCT, 10mg of AMB, and 80 mg of TEL were accurately weighed separately and transferred into 100 ml volumetric flasks. The drugs were dissolved in 50 ml of mobile phase with shaking and then the volume was made up to the mark with the mobile phase. Finally, the concentration of the solution was to get 250 µg/ml for HCT, 100 µg/ml for AMB, and 800µg/ml for TEL.

Preparation of Sample solution

Marketed tablet formulation Dynatel Trio contains (Hydrochlorthiazide12.5 mg), Amlodipine Besylate 5 mg, and Telmisartan 40 mg). Twenty tablets were weighed accurately; the average mass per tablet was determined and finely powdered. The powder equivalent to 80 mg TEL was accurately weighed and transferred into a 100 ml volumetric flask containing 50 ml of mobile phase and then ultrasonicated for 20 min. Finally, the volume was made up to the mark with mobile phase (250 µg / ml for HCT, 100 µg/ml for AMB, and 800 µg/ml for TEL). The solution was filtered through Whatman filter paper No. 42. Insouble excipients were separated out. The filtrate was collected after rejecting the first portion of the filtrate. 6.0ml `of the clear solution was further diluted and made up to 50 ml with mobile phase to obtain 30 µg / ml for HCT, 12 µg / ml for AMB, and 96 µg / ml for TEL). 20 µl of each solution

was injected and the chromatogram was recorded. The analysis was repeated six times. The content of the drug was calculated from the peak area recorded.

Selection of wavelength

For many samples, good analytical results will be obtained only by careful selection of the wavelength used for detection. The sensitivity of HPLC depends upon the proper selection of the wavelength of detection. To determine the proper wavelength of hydrochlorothiazide (HCT), Amlodipine Besylate (AMB), and Telmisartan (TEL) in the mobile phase, spectra were scanned on UV-Visible spectrometer in the range of 200-400 against diluent as blank. The Isobestic point of wavelength 237nm was selected for the analysis.

Method Validation

The RP-HPLC method was validated in terms of parameters like accuracy, linearity, precision, range, detection limit, quantification limit, ruggedness, robustness and system suitability, etc. For all the parameters percentage relative standard deviation values were calculated.

RESULTS AND DISCUSSION

To understand the sensitivity of the chromatographic factors on the separation of analytes and for simultaneous optimization of resolution and analysis time, Chemometric protocols of Response surface design and Derringer's desirability function were successfully employed. The central composite design can be applied to optimize the separation and to assist the development of a better understanding of the interaction of several chromatographic factors on separation quality. The selection of factors for optimization was based on preliminary experiments and prior knowledge from the literature. Therefore, the key factors selected for the optimization process were Acetonitrile concentration (*A*), Buffer pH (*B*), and Flow rate (*C*). Table 1 shows the levels of each factor studied for finding out the optimum values and responses.

The ranges of each factor used were MeCN concentration (40–60%), Phosphate buffer pH (3.0–4.0), and Flow rate (0.8–1.2 mL/min). As response variables, the capacity factor of Hydrochlorothiazide (*k*1), the resolution between two pairs amlodipine and telmisartan ($Rs_{2,3}$), and the retention times of telmisartan (tR_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.

Table 1 Central composite arrangement and responses

Run	Type	A(%v/v)	B(pH)	C(ml/min)	Capacity factor (k1)	Resolution (Rs2,3)	Retention Time (tR3)
4	Center	50.00	3.5	1.00	0.91	3.82	9.30
8	Centre	50.00	3.5	1.00	0.93	3.83	9.32
11	Centre	50.00	3.5	1.00	0.94	3.80	9.30
17	Centre	50.00	3.5	1.00	0.92	3.82	9.33
19	Centre	50.00	3.5	1.00	0.91	3.84	9.34
20	Centre	50.00	3.5	1.00	0.92	3.80	9.30
2	Axial	33.18	3.5	1.00	0.91	3.54	6.68
5	Axial	63.41	3.5	1.00	0.83	6.21	11.56.
9	Axial	50.00	2.69	1.00	1.10	2.26	4.58
10	Axial	50.00	3.84	1.00	0.91	5.00	7.50
13	Axial	50.00	3.5	0.66	1.14	5.57	14.7
16	Axial	50.00	3.5	1.33	1.00	1.84	4.36
1	Factorial	60.00	3.0	0.8	0.95	4.75	6.0
3	Factorial	40.00	3.0	0.8	1.00	5.26	6.11
6	Factorial	60.00	4.0	0.8	0.94	5.87	11.96
7	Factorial	60.00	4.0	1.2	0.92	5.71	8.2
12	Factorial	40.00	4.0	0.8	1.21	9.15	21.0
14	Factorial	60.00	3.0	1.2	0.87	3.57	3.96
15	Factorial	40.00	3.0	1.2	1.0	4.15	4.10
18	Factorial	40.00	4.0	1.2	1.25	9.05	14.28

Central composite design with quadratic equation was represented as

$Y = \beta_0 + \beta 1X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta 11X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$where Y is the response to be modeled, β is the regression coefficients and X_1, $X_2$and X_3

represent factors A, B and C respectively. Statistical parameters obtained from ANOVA for the reduced models were given in table 2. The insignificant terms ($p>0.05$) were eliminated from the model through a 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.

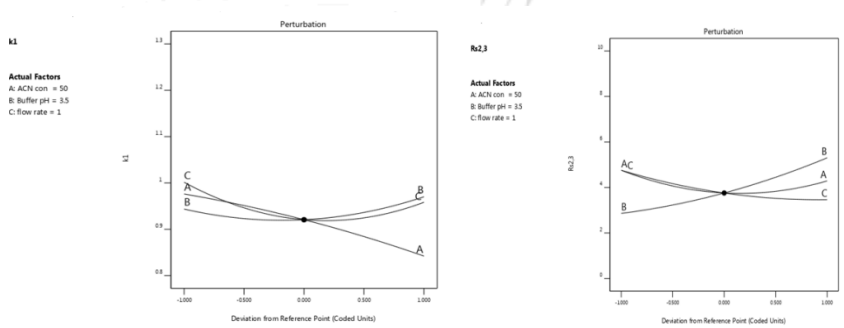
Table 2 Reduced Response Surface Models and Statistical Parameters obtained from ANOVA

Responses	Regression model	Adjusted R^2	Model p-value	% C.V	Adequate Precision
K_1	+0.920-0.067*A+0.013*B-0.021*C-0.052*AB-0.017*AC+0.012*BC-0.011*A^2+0.036*B^2+0.059*C^2	0.9947	<0.0001	8.73	6.1340
$Rs_{2,3}$	+3.76-0.235*A+1.22*B-0.646*C-0.691*AB-0.016*AC+0.253*BC+0.764*A^2+0.324*B^2+0.351*C^2	0.9241	<0.0001	5.65	14.571
tR_3	+9.27-0.524*A+2.94*B-2.34*C-0.086*AB+0.036*AC-0.038*BC+0.202*A^2-0.886*B^2+0.347*C^2	0.9388	<0.0001	7.33	7.4834

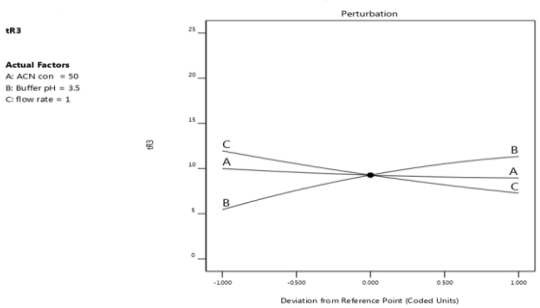
The adjusted R^2values were well within the acceptable limits of $R^2 \geq 0.90$ which revealed that the experimental data showed a good fit with second-order polynomial equations. For all the reduced models, the *p*-value of < 0.05 was obtained, implying these models were significant. The adequate precision value is a measure of the signal (response) to noise (deviation) ratio. A ratio greater than 4 is desirable. The ratio was found to be in the range of 5.65 – 8.73 which indicated an adequate signal and therefore the model was 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%.

In table 2 the interaction terms with the largest term coefficient among the fitted model were BC (+ 0.25) of $Rs_{2,3}$ model. The positive interaction between B and C was statistically significant (< 0.0001) for $Rs_{2,3}$. The existence of such interactions emphasizes the necessity to carry out active multifactor experiments for the optimization of chromatographic separation. To gain a better understanding of the results the predicted models were presented in the form of perturbation plot figure 1 and 3D response surface plot figure 2. Variables giving quadratic and interaction terms with the largest absolute coefficients in the fitted models were chosen for the axes of the response surface plots. Consequently, factors A and C were selected for the response plots of k_1, $Rs_{2,3}$, and tR_3 with factor B held constant usually at a central value of buffer pH 3.5. All these three-dimensional plots were beneficial to gain an overall understanding of the influence of phosphate buffer pH and flow rate on analysis time ($Rs_{2,3}$). Perturbation plots provide silhouette views of the response surface plots, where it shows how the response changes as each factor move from a chosen reference point, with all other factors, held constant at the reference value.

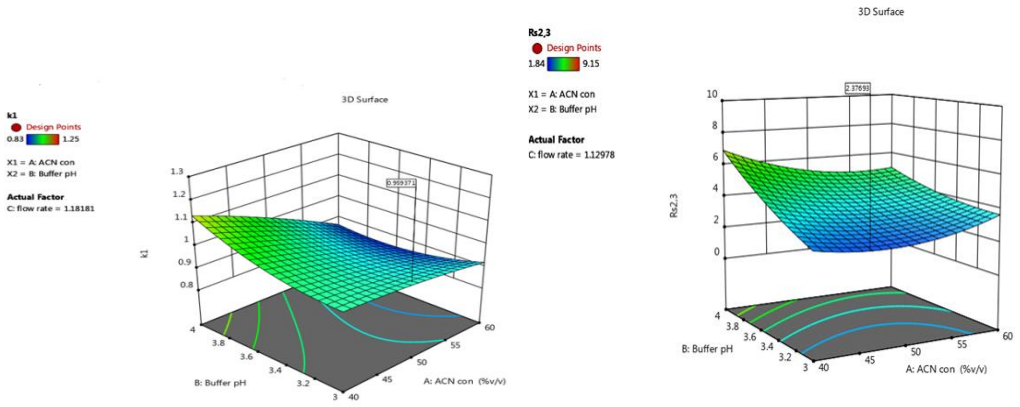


(a) Capacity factor (b) Resolution

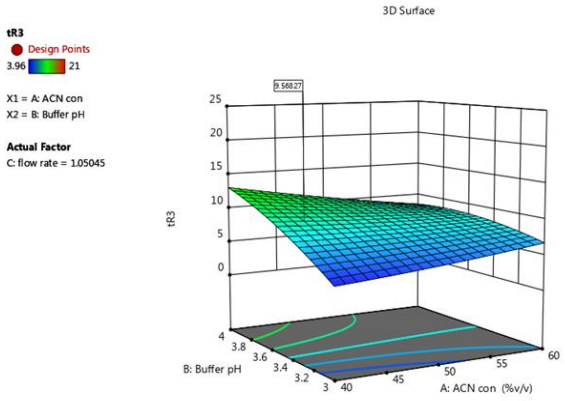


(C) Retention time

Fig 1 Perturbation plots for Responses



(a) Capacity factor **(b) Resolution**



(c)Retention time

Fig 2 Response surface plots for Responses

The steepest slope or curvature indicates the sensitiveness of the response to a specific factor. Figure 2b showed that 1% triethylamine buffer pH (factor B) had the most important effect on resolution between Amlodipine and Telmisartan $Rs_{2,3}$ followed by factor C and then factor-A. The rest of the factors (MeCN concentration and flow rate) had a significant effect on tR_3 and k_1. When k_1 and tR_3 values were increased, the level of MeCN concentration (factor A) increased, and when k_1 and tR_3 values decreased, the level of flow rate (factor C) increased. Analysis of the perturbation plot and response surface plot of optimization models revealed that factors B and C had a significant effect on the separation of analytes, whereas the factor A, MeCN concentration was of little significance. The criteria for the optimization of each response were shown in table 3.

Derringer's desirability function was employed for the global optimization of three responses and to select different optimal conditions for the analysis of formulation in the present study. The identified criteria for the optimization were resolution between the peaks, capacity factor, and elution time.

Derringer's desirability function, D, is defined as the geometric mean, weighted or otherwise of the individual desirability functions. The expression that defines Derringer's desirability function is:

$$D = [d_1^{p2} \times d_2^{p2} \times d_3^{p2} \times \ldots\ldots\ldots \times d_n^{pn}]^{1/n}$$

where pi is the weight of the response, n is 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. The criteria for the optimization of each response were shown in table 3.

Table 3 Criteria for the Optimization of the Individual Responses

Response	Lower limit	Upper limit	Criteria/Goal
k_1	0.83	1.25	Is in range
$Rs_{2,3}$	1.84	9.15	Minimize
tR_3	3.96	21	Minimize

From the above table it could be seen under the column criteria that the response of tR_3 was minimized to shorten the analysis time and the response of $Rs_{2,3}$ was minimized to allow the baseline separation of Amlodipine and Telmisartan. To separate the first eluting peak of Hydrochlorothiazide from the solvent front, k_1 was is in range. The importance could range from 1 to 5 which emphasized a target value. Following the conditions and restrictions above, the optimization procedure was carried out. The response surface obtained for the global desirability function was presented in figure 3.

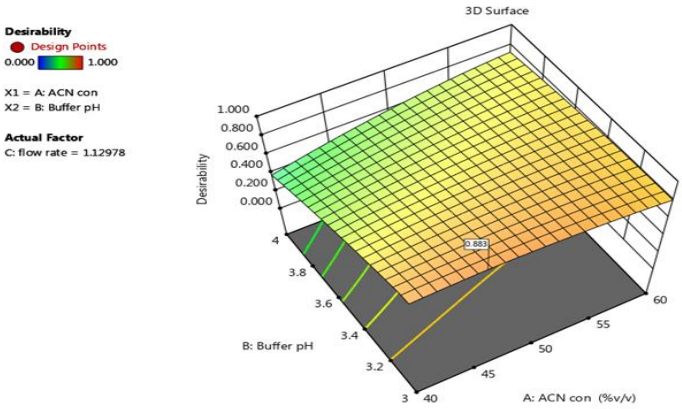
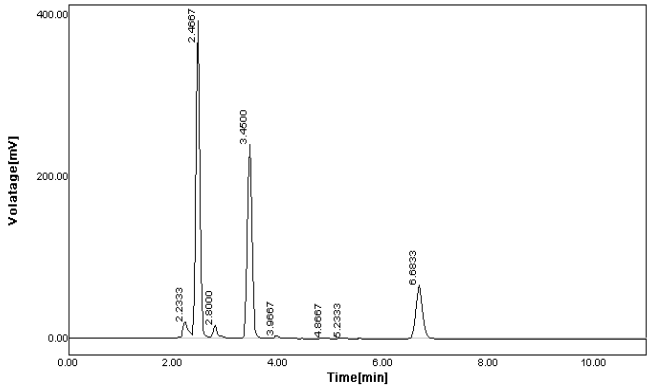


Fig 3 Graphical representation of overall desirability function

From figure 3 it could be concluded that there was a set of coordinates producing a high desirability value (D = 0.971) were MeCN concentration of 47.15%, buffer pH of 3.0, and flow rate of 1.2 ml/min. The optimized assay conditions were MeCN: 1% triethylamine buffer (47.15: 52.85%v/v) (pH 3.0) as mobile phase at a flow rate of 1.2 ml/min. and UV detection at 234 nm. The predicted response values corresponding to the later value of D were k_1 = 0.97, $Rs_{2,3}$= 2.25 and tR_3 = 6.42 min. The prediction efficiency of the model was confirmed by experimenting with the optimal condition and the corresponding chromatogram as shown in figure 4. The observed difference between the predicted and experimental responses was found to be in good agreement, within a difference of 4.0% was shown in table 4.



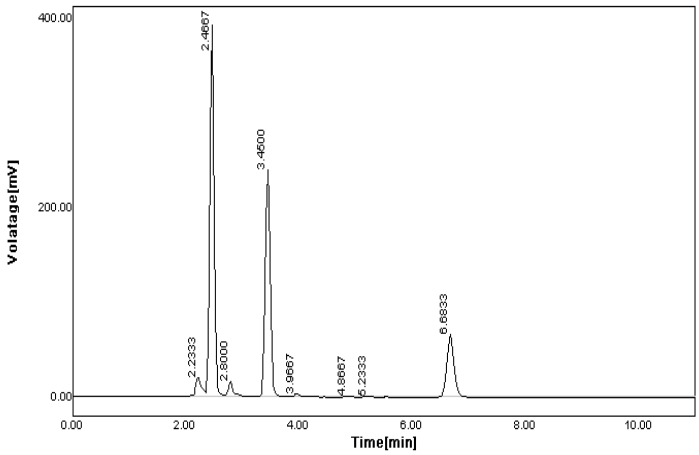


Fig 4 Chromatograms for comparison of Experimental and Predictive Value of different Functions

Table 4 Comparison of Experimental and Predictive values of different functions under Optimal Conditions.

Optimum conditions	Acetonitrile(%v/v)	Buffer (pH)	Flow rate (ml/min)	K_1	$Rs_{2,3}$	tR_3
Predictive	47.15	3.00	1.2	0.97	2.25	6.42
Experimental	69.7	2.52	1.2	0.92	2.14	6.68
Average error				5.15	4.48	4.05
Desirability value= 0.971						

Table 5 System suitability parameters

Parameters	Compound		
	Hydrochlorothiazide	Amlodipine	Telmisartan
Capacity factor (K')	0.91	1.08	1.05
Retention time (Rt) in min	2.45	3.45	6.68
Theoretical plates (N)	4785.3	6701.3	11218.3
Resolution (Rs)	-	2.15	5.41

The optimized assay method was specific about the placebo used in this study because there was no excipients peak co-eluted with the analytes. No interferences were observed as shown in figure 10. The method was also selective because there were no interferences observed from any of the excipients in the tablet formulation tested.

Report for validation parameter

Parameter	Hydrochlorthiazide	Amlodipine besylate	Telmisartan
Linearity range (µg/ml)	10 - 50	4 - 20	32 - 160
Slope	24209	53714	14121
Intercept	14506	17553	14121
Regression coefficient	0.996	0.992	0.991
Accuracy %recovery	100.85	100.52	99.96
Precision % RSD	0.3711	0.2379	1.1224
Assay %	99.64	99.83	99.90
LOD	0.0230	0.0270	0.0542
LOQ	0.0607	0.0820	0.1642

CONCLUSION

A simple, rapid, and accurate RP-HPLC method was developed for the simultaneous estimation of Hydrochlorothiazide, Amlodipine Besylate, and Telmisartan in bulk and combined pharmaceutical tablet dosage form using experimental design. Response surface methodology and central composite design were used to find out the optimized assay conditions of Acetonitrile: 1% triethylamine buffer (pH 3.0) 47.15:52.85%v/v as mobile phase at a flow rate of 1.2 ml/min and UV detection at 237 nm. With the optimized conditions, the drugs were linear with the concentration range of 10 to 50 µg/ml for Hydrochlorothiazide, 4 to 20 µg/ml for Amlodipine, and 32 to 160 µg/ml for Telmisartan. The correlation coefficients of the Hydrochlorothiazide, Amlodipine and Telmisartan were found to be 0.9996, 0.9992, and 0.9991 respectively. The percentage purity was found to be 99.64 ± 0.4213 for Hydrochlorothiazide, 99.83 ± 1.0252 for Amlodipine, and 99.90 ± 1.5938 for Telmisartan. The precision was confirmed by repeating the analysis six times. The accuracy was confirmed by recovery studies. The % recovery was found to be 100.85 for Hydrochlorothiazide, 100.52 for Amlodipine, and 99.96 for Telmisartan respectively.

Therefore, this proposed HPLC method can be used routinely for the quality control analysis of simultaneous estimation of hydrochlorothiazide, Amlodipine besylate, and Telmisartan in the bulk and pharmaceutical tablet dosage form.

REFERENCES

1. Deepali Gangrade, Priyanka KulkarniSimultaneous estimation of Hydrochlorothiazide, Amlodipine Besylate, and Telmisartan in combined tablet dosage form by using RP- HPLC method. IJPSR.1: 2017; 268 – 276.
2. Jabir Aboobacker O, Venkatachalam T,SenthilkumarN,Vijayamiruthraj R, Kalaiselvi P. Method Development and Validation of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan in tablet dosage form by RP-HPLC method. Res J Pharma, BiolChem Sci. 3 (3): 2012; 509 – 517.
3. Karmarkar S, Garber R, Genchanok Y, George S, Yang X, Hammond R. Quality by Design (QbD) Based development of a stability indicating HPLC method for drug and impurities, J ChromatoSci, 49: 2011.
4. Lundstedt T, Seifert E, Abramo L, Thelin B, Nystrom Å, Pettersen J, Bergman R.Experimental Design and optimization. Chemom. Intell. Lab. Syst. 42: 1998; 3−40.
5. Manish A. Raskar, Ashok B.Chitale, PritamD.Giri. Validated Simultaneous Derivative Spectrophotometric estimation of Telmisartan, Hydrochlorothiazide, and Amlodipine Besylate in combination tablet dosage form.Inter J PharmaPharma Res. 4(1): 2015
6. MadhukarA, Kannapan N, Mahendrakumar CB. Analytical Method development and validation for the determination of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan Hydrochloride in multicomponent tablet dosage form and biorelevant media (Fassif) by RP-HPLC techniques. Int J Pharm PharmSci, 7(1): 2015; 218-225.
7. Nethercote P, Borman, P Bennett, T, Martin G, McGregor P. QbD for Better Method Validation and Transfer, Pharm. Manufact. 37:2010
8. Raissi S, EslamiFarsani R. Statistical process optimization through the multi-response surface methodology. World Academy of science. Eng Tech 51; 2009: 267-271.
9. SasidharRLC, Vidyadhara S, Deepti B, Tejaswi K, SuhasiniJ. Development and Validation of RP - HPLC Method for the simultaneous determination of Hydrochlorothiazide, Amlodipine Besylate, and Telmisartan in bulk and pharmaceutical formulation.Oriental Jchem30(4): 2014; 1815-1822.
10. Said A. Hassan, Hany W. Darwish, Maissa Y. SalemandBadr A. El-Zeany. Sequential spectrophotometric method for the simultaneous determination of Amlodipine, Valsartan, and Hydrochlorothiazide in co-formulated tablet Int J Spectroscopy 8: 2013
11. Santajinalwade, Vangalarangareddy, Dantudurgarao, Inabathinekoteswararao. Rapid Simultaneous Determination of Telmisartan, Amlodipine Besylate, and Hydrochlorothiazide in a combined poly pill dosage form by stability-indicating ultra-performance liquid chromatography. Scientia Pharmaceutica, 79(1): 2011; 69–84
12. Sivakumar T, Manavalan R, Muralidharan P, Valliappan K. Multi-criteria decision-making approach and experimental design as chemometric tools to optimize HPLC separation of Domperidone and Pantoprazole. J pharma Biomed Ana 43; 2007: 1842–1848
13. International Conference on Harmonization guidance for Industry. (1994) Q2A Text on validation of analytical methods. Switzerland, IFPMIA, 1,4.