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A SIMPLE GRADIENT RP-HPLC METHOD FOR THE QUANTIFICATION OF HYDROCHLOROTHIAZIDE, AMLODIPINE BESYLATE AND NEBIVOLOL HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Reverse phase HPLC method has been developed for the simultaneous quantification of Hydrochlorothiazide, Amlodipine besylate and Nebivolol in pharmaceutical dosage forms. chromatographic test was carried out by using a Develosil C-8 UG-5, 150x3mm column with the gradient mobile phase composed of Sol-A: ammonium acetate buffer (1.4g of ammonium acetate in to 1000ml of HPLC water) and sol-B: acetonitrile with simple gradient program (0-4min, sol-A:86-86; 4-8min- sol-A:86-63; 8-13min- sol-A:63-63; 13-15min- sol-A:63-86 and 15-20min- sol-A:86-86) with 1.0ml per min flow rate. Column oven temperature maintained at 35°C and UV absorbance measured at 225nm. The retention time of Hydrochlorothiazide is 2.28min, Amlodipine besylate is 11.24 min and Nebivolol is 12.56min, respectively. Area percent RSD (relative standard deviation) for five replicate standard injections is below 1.5percent. Method validation was performed with specificity, precision, linearity, accuracy, ruggedness and robustness. The response was linear over the concentration range of 10 to 60 microgram per mL for each ingredient, with correlation coefficients value is greater than 0.999. Recovery results were satisfactory. The developed method is simple, reproducible and accurate.

Key words: RP-HPLC method, Hydrochlorothiazide, Amlodipine besylate and Nebivolol HCl.

INTRODUCTION

Amlodipine is a calcium channel blocker which inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle and used for anti-hypertension and angine pectoris (chest pain). Amlodipine is a chiral calcium antagonist, in therapeutic use as a racemate [1,2]. The recommended dose for adults is 5-10 mg once daily and pediatric patients is 2.5 mg to 5 mg once daily[3, 4].

Hydrochlorothiazide (HCTZ) [5, 6] is a thiazide class diuretic drug. This reduces the volume of the blood, decreasing blood return to the heart. Hydrochlorothiazide is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones. The recommended dose of hydrochlorothiazide for treating high blood pressure is hydrochlorothiazide 25 mg to 50 mg per day.

Nebivolol [7-11] is a lipophilic Beta-blocker. It works by exhibiting a high selectivity for Padrenergic receptors and also by reducing the peripheral vascular resistance by modulating. Nebivolol is used to treat high blood pressure (Essential Hypertension). It is available with the combination of chlorthalidone, hydrochlorthiazide, theophylline or digoxin. The starting nebivolol dosage to treat high blood pressure is 5 mg once daily and a maximum of nebivolol 40 mg once daily. For kidney impairment or moderate liver impairment, the recommended starting dose of nebivolol is 2.5 mg once daily.

The chemical structures of all the active ingredients were represented in figure-1. Hydrochlorothiazide, Amlodipine besylate and Nebivolol are available in individual and combination dosage forms and have individual and single combination products methods [13 to 14]. In the present study developed a single RP-HPLC method for the simultaneous determination of three active ingredients and validated the method as per ICH and FDA guidelines with specificity, linearity, accuracy and reproducibility.

MATERIALS AND METHOD

Instruments

A waters HPLC system consisting of alliance 2695, agilent 1200 series HPLC instrument with UV-Visible detector, two systems were operated by empower software. A Develosil C8, UG-5 150mm x 3.0mm column, Mettler Toledo analytical balance were used for this study

Materials

Pure (not less than 98.5%) standards of all active ingredients, HPLC grade acetonitrile and water; AR grade of ammonium acetate were used.

Mobile phase

Sol-A: weighed accurately 700mg of ammonium acetate, transferred in to 500ml of HPLC water and mixed. Filtered the final solution through a 0.4µ membrane filter; Sol-B: HPLC grade acetonitrile.

Diluent

Mixed the HPLC water and acetonitrile in the ratio of 1:1 (v/v) and degassed.

Standard solution

Prepared the standard solution to get each active ingredient equal to 40microgram per mL with diluent.

Test solution: Prepared the all dosage forms to get each active ingredient equal to 40microgram per mL with diluent and analyzed.

Chromatographic conditions

Chromatograph: Waters/ Agilent HPLC system with Empower software.
Mobile phase: Solution-A and solution-B with gradient elution.
Gradient program: (0-4min, sol-A: 86-86; 4-8min- sol-A: 86-63; 8-13min- sol-A: 63-63; 13-15min- sol-A: 63-86 and 15-20min- sol-A: 86-86)
Column: Develosil C8, UG-5 150mm x 3mm.
Flow rate: 1.0 mL per min
Detection: 225nm
Injection volume: 20 µl
Retention time: Hydrochlorothiazide - 2.28 min, Amlodipine besylate- 11.24 min and Nebivolol-12.56min.
Run time: 20 min.

Calculation: All active ingredients were quantified with the following calculation.

Sample area x standard concentration x Potency of standard

Standard area x sample concentration

RESULTS AND DISCUSSIONS

Method development:

Several systematic trials were performed to optimize the chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of Hydrochlorothiazide, Amlodipine besylate and Nebivolol in pharmaceutical dosage forms. The present method contains mobile phase acetate buffer and acetonitrile with gradient elution. Under the above conditions the retention time obtained for Hydrochlorothiazide - 2.28 min, Amlodipine besylate- 11.24 min and Nebivolol-12.56min. Diluent and standard solution crhromatograms were represented in figure-2 and 3. All the active ingredients were well separated and the well peak shape, resolution (not less than 4.0) and tailing factor (not less than 1.5) were also within the limit.

System suitability:

System suitability parameters were established by injecting the freshly prepared standard solution (each active 40microgram per mL/five replicate injections) in to the chromatographic system. The percent relative standard deviation for peak area and retention time results found to be satisfactory. System suitability chromatograms were represented in figure-4 and tabulated the results in table-1 and 2.

Method validation:

Validated the finalized method as per ICH and FDA [15- 16] guidelines with parameters like specificity, precision, linearity and range, accuracy, ruggedness and robustness etc.

Specificity

Different forced degradation studies were performed with acid, alkali, peroxide, UV and photo degradation conditions. All samples were passed the purity test. The purity angles for drug components in all stress conditions were found to be less than the threshold angle and no interference was observed with diluent, placebo and other degraded products.

Precision:

Precision was evaluated by carrying out six different sample preparations for all ingredients in individual and combination dosage forms. Percentage relative standard deviation (% RSD) was found to be less than 1.5% for within a day and day to day variations, which proves that the developed method is precise and accurate. Precision results were tabulated in Table-3.

Linearity:

Linearity is determined by calculating the regression line using a mathematical treatment of the linearity results vs analyte concentration (10microgram per mL to 60microgram per mL for each ingredient) of the standard solution. Linearity graph was plotted against peak area and concentration of solution. The correlation coefficient value found to be within the limit 0.999. The linearity chromatograms shown in figure-5 and linearity results tabulated in table-4 and linearity plots were represented in graph-1.

Accuracy:

Accuracy represents the deviation between the mean value found and the true value. It is determined by performing known concentration of analyte. Accuracy was carried out with a known concentration of the pure standard was added to the placebo sample at the levels between 25% and 150% of the test concentration. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The mean recoveries were in range of 97.0-103.0 % which shows that there is no interference from excipients. Table-5 represents the recovery results.

Ruggedness and Robustness:

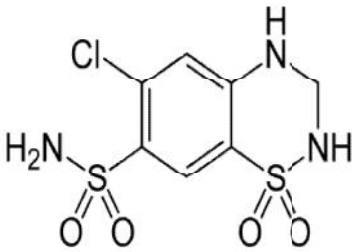
Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions (different analysts, laboratories, instruments, reagents, assay temperatures, small variations in mobile phase, different days etc). The ruggedness of the method was determined by carrying out the experiment on different instruments like waters HPLC and Agilent HPLC by different analysts using different columns of similar types. The percent RSD of six different preparations assay values with two different instruments, analysts and columns were satisfactory.

Robustness of the method was validated with slight variations in the chromatographic conditions, such as flow rate and column temperature and found that there were no major changes in the chromatograms, which demonstrated that the developed RP-HPLC method is rugged and robust. Robustness results were tabulated in table-6.

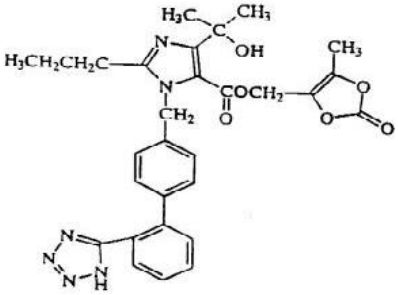
CONCLUSION

The validation results reveals that the proposed method has applicable for the quantification of Hydrochlorothiazide, Amlodipine besylate and Nebivolol in pharmaceutical drug products. The developed method is precise, linear and accurate and applicable for regular analysis.

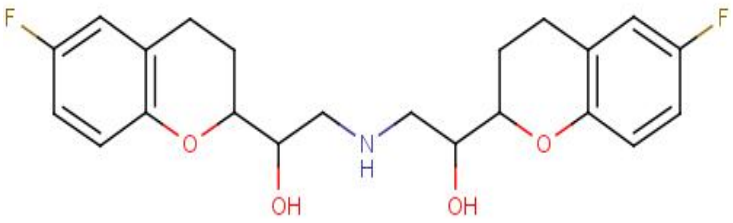
Figures and Tables



Hydrochlorothiazide



Amlodipine besylate



Nebivolol

Figure-1: Chemical structures of active ingredients

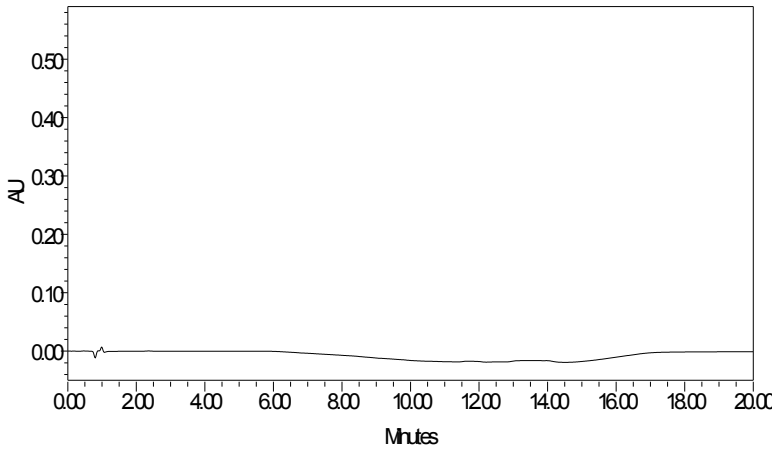


Figure-2: Diluent chromatogram

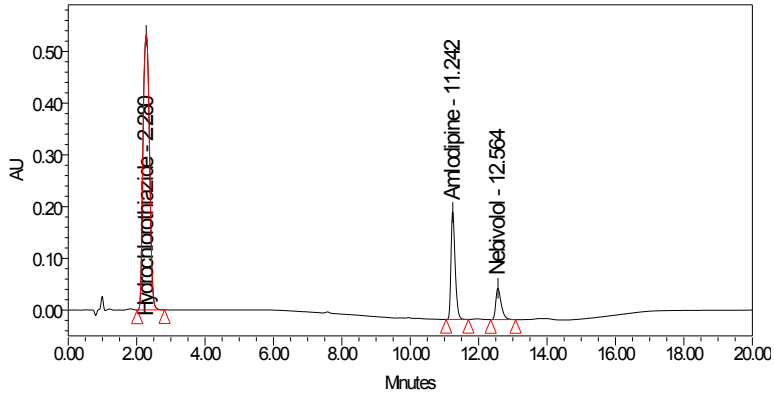


Figure-3: Standard chromatogram

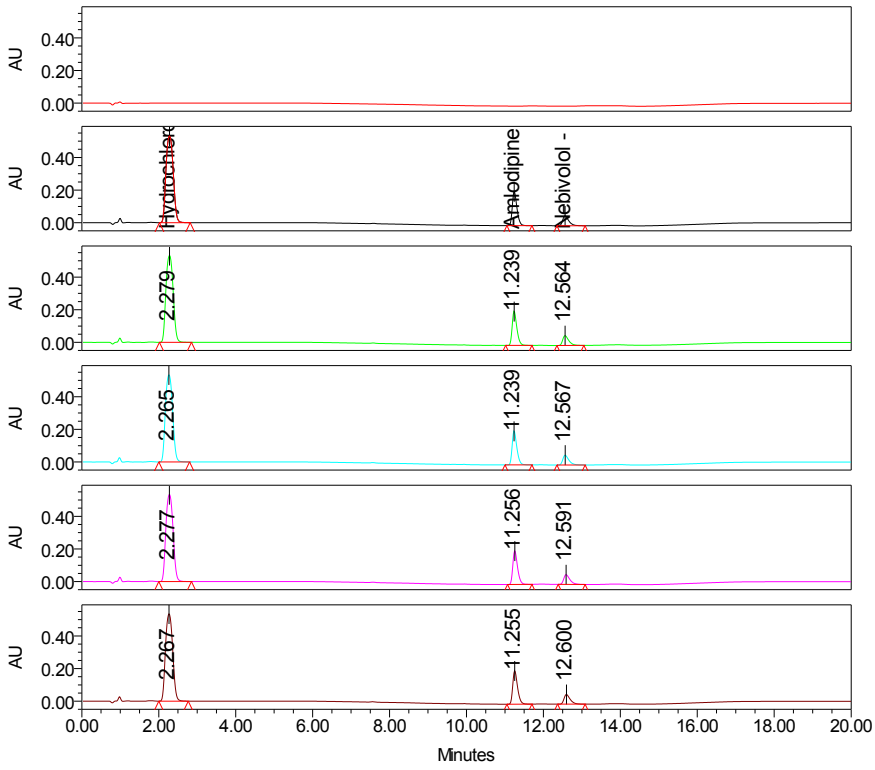


Figure-4: System suitability chromatograms

Table-1: System suitability (Area %RSD)

Active Ingredient Name	Standard solution Area						
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Average	%RSD
Hydrochlorothiazide	6876693	6989055	6978664	6984022	6983303	6962347	0.62
Amlodipine besylate	1770850	1782282	1780187	1773850	1779058	1777245	0.24
Nebivolol	666878	665147	667242	666255	665906	666286	0.11

Table-2: System suitability (~Retention time %RSD)

Active Ingredient Name	Standard solution Retention time (min)						
	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Average	%RSD
Hydrochlorothiazide	2.28	2.27	2.26	2.27	2.26	2.27	0.33
Amlodipine besylate	11.24	11.23	11.23	11.25	11.25	11.24	0.08
Nebivolol	12.56	12.56	12.56	12.59	12.60	12.57	0.14

Table-3: Precision Results.

Active Ingredient Name	Sample preparations						Average (%)	%RSD
	Prep-1	Prep-2	Prep-3	Prep-4	Prep-5	Prep-6		
Hydrochlorothiazide	99.80	99.50	100.56	101.10	100.89	100.37	99.80	0.62
Amlodipine besylate	100.20	101.20	99.30	99.90	101.21	100.36	100.20	0.74
Nebivolol	98.60	100.56	98.79	100.10	99.84	99.58	98.60	0.76

Figure-5: Linearity chromatograms

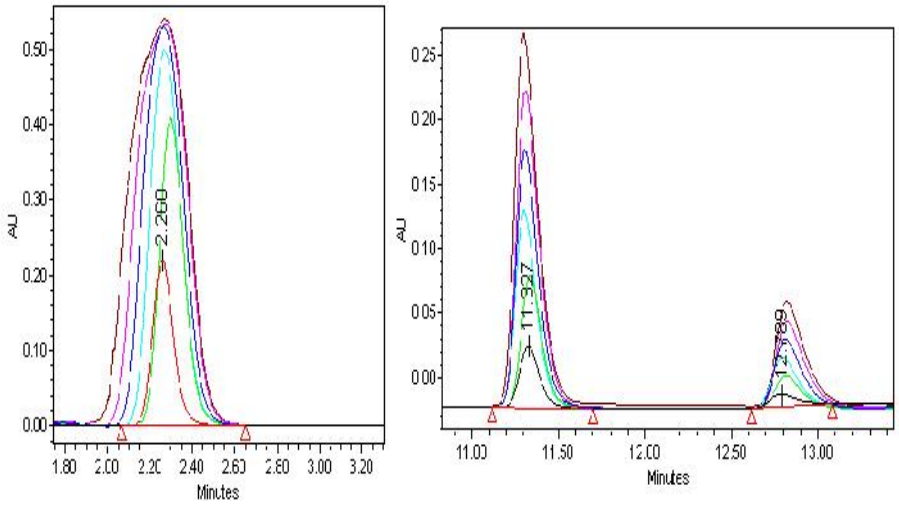
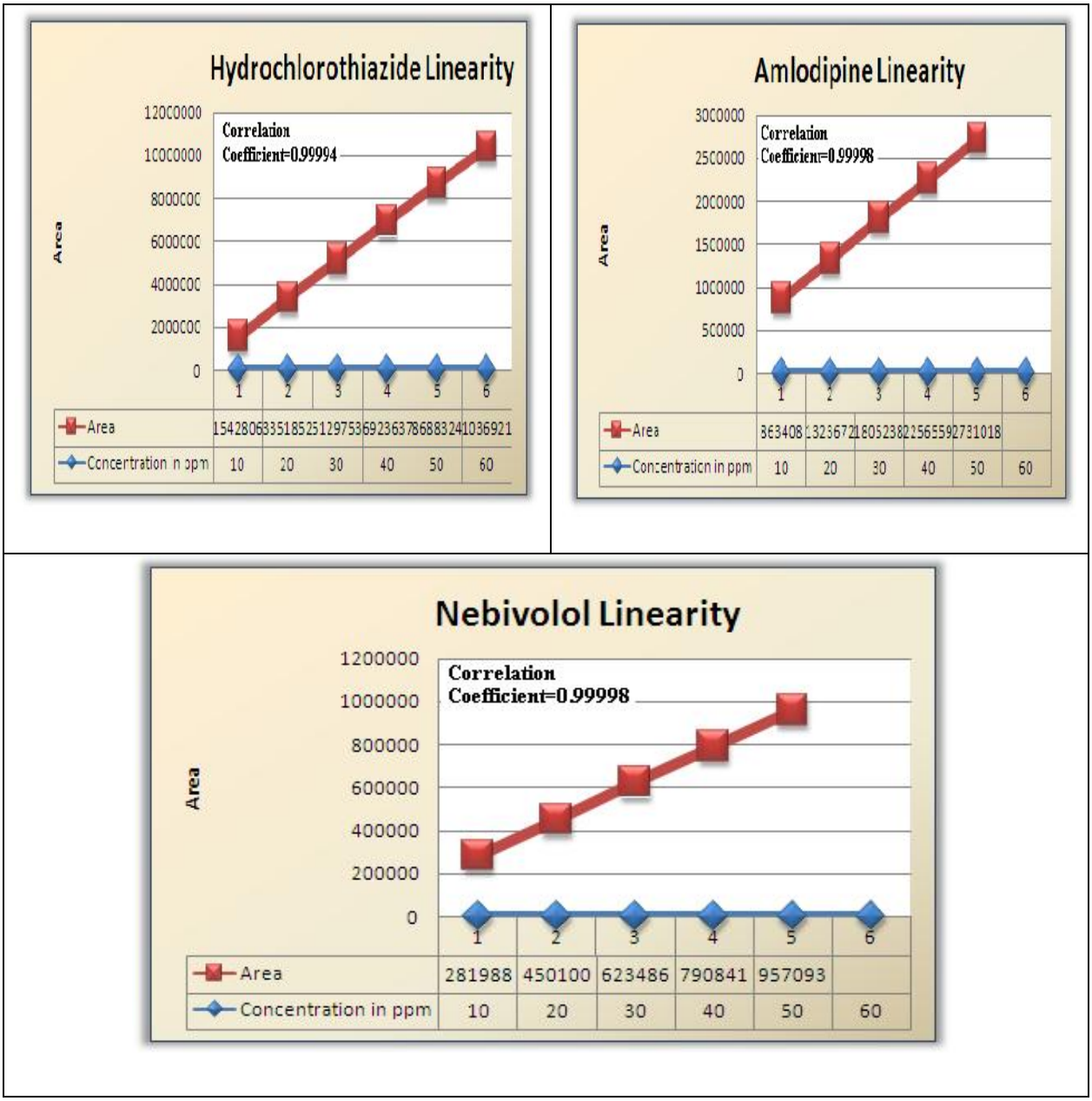


Table-4: Linearity Results.

Active Ingredient Name	Linearity solutions area						
	10ppm	20ppm	30ppm	40ppm	50ppm	60ppm	Correlation Coefficient
Hydrochlorothiazide	1542806	3351852	5129753	6923637	8688324	10369216	0.99994
Amlodipine besylate	394432	863408	1323672	1805238	2256559	2731018	0.99998
Nebivolol	114756	281988	450100	623486	790841	957093	0.99998



Graph-1: All active ingredients linearity graph.

Table-5: Accuracy (recovery) Results.

Active Ingredient Name	Spike level						Average % Recovery
	25%	50%	75%	100%	125%	150%	
Hydrochlorothiazide	100.20	99.80	100.56	101.10	100.80	100.49	100.49
Amlodipine besylate	101.30	101.60	101.21	99.79	100.50	99.91	100.7
Nebivolol	99.90	100.50	99.6	100.10	99.84	99.99	99.99

Table-6: Robustness Results.

Parameter	System suitability	
	Tailing factor	Percent (%) RSD
Standard solution	1.2-1.0	1.6-1.0
Column Oven Temperature		
+5°C	1.1-1.3	1.1-0.9
-5°C	1.2-1.1	1.3-1.5
Flow Rate		
+0.1mL per min	1.2-1.5	1.3-1.2
-0.1mL per min	0.9-1.4	0.9-1.2

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