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Research Article

Method development and validation of Amlodipine Besylate and Hydrochlorthiazide by RP-HPLC Method

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Abstract

A simple, sensitive, rapid and selective isocratic reversed phase High Performance Liquid Chromatographic method has been developed for simultaneous estimation of Amlodipine Besylate & Hydrochlorthiazide from pharmaceutical dosage form using a mobile phase consisting mixture of triethylamine Buffer: acetonitrile:Methanol (25:37.5:37.5), (pH adjusted to 3.0 using ortho phosphoric acid) at the flow rate of 1.0 mL/min. A Kromasil C-8 (Intersile250 x 4.6 mm,5 µm.) column was used as stationary phase. The retention time of Amlo and Hydro was 3.97 min. and 2.79 min. respectively. Linearity was observed in the concentration range of 10-80 µg/ml,The recovery studies ascertained the accuracy of the proposed method and the results were validated as per ICH guidelines. The eluent were detected at 230 nm.The Results were found to satisfactory and reproducible. The proposed method is precise, accurate, selective and rapid for the simultaneous determination of Amlodipine Besylate & Hydrochlorthiazide.The method can be used for routine analysis of these drugs in bulk and in formulation.

Keywords: Amlodipine Besylate, Hydrochlorthiazide, HPLC Method, Mobile phase.

Introduction

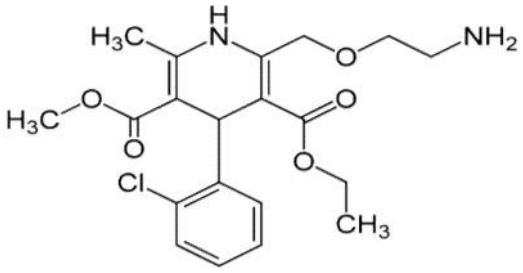
Cardiovascular diseases (CVDs) are the disorders of heart and blood vessels and primarily include coronary heart disease, hypertension, cerebrovascular disease, peripheral artery disease, rheumatic heart disease, congenital heart disease and heart failure. CVDs are the major cause of death in developed countries and also are rapidly emerging as a main cause of death in the developing world. An estimated 17.5 million people died from CVDs till 2005, representing almost 30% of all the global deaths. It is projected that almost 20 million people will die from CVDs by 2015. The major risk factors involved in CVDs are high low density lipoprotein (LDL) cholesterol, raised blood pressure, increased serum homocysteine level and platelet aggregation, which are primarily caused by unhealthy diet, physical inactivity and tobacco use. A formulation is developed using drugs Amlodipinebesylate and Hydrochlorothiazide for CVDs.[1]

S(-) amlodipine is a potent calcium channel blocker used for the treatment of hypertension, congestive heart failure and angina pectoris. S(-) amlodipine avoids the adverse effect of amlodipine in racemic mixtures.Hydrochlorothiazide is a first line diuretic drug of the thiazide class used for the treatment of hypertension, congestive heart failure and symptomatic edema.[2]

Amlodipine (as besylate, mesylate or maleate), chemicallyis 3-Ethyl-5-methyl (±) -2-[(2-aminoethoxy) methyl] -4- (2-chlorophenyl) -1, 4-dihydro-6-methyl-3, 5-pyridine dicarboxylate, monobenzenesulphate.. Amlodipine is a dihydropyridine derivative with calcium antagonist activity. Amlodipine acts by inhibiting the transmembrane influx of calcium ions into vascular smooth muscles.

Amlodipine is a dihydropyridine derivative with calcium antagonist activity. Amlodipine acts by inhibiting the transmembrane influx of calcium ions into vascular It inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes. The decrease in intracellular calcium inhibits the contractile processes of the myocardial smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased systemic blood pressure, and decreased after load.[3]

Structure of Amlodipine[4]



Hydrochlorothiazide (H) is a thiazide diuretic (water pill) that decreases the amount of fluid in the body by increasing the amount of salt and water lost in the urine. Hydrochlorothiazide is used to lower blood pressure and to decrease edema (swelling), it is chemically described as 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7 sulfonamide 1,1-dioxide-33 Hydrochlorothiazide is a Loop Diuretics used as an antihypertensive by reducing symptomatic oedema. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance.-36 Hydrochlorothiazide is a Loop Diuretics used as an anti hypertensive by reducing symptomatic oedema. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, isbelieved to lower peripheral vascular resistance.[5]Literature survey reveals that there was no method established for the RP-HPLC method development for this combination . The present work describes the development of validated RP-HPLC method, which can quantify these components simultaneously from a combined dosage form. The present RP-HPLC method was validated following the ICH guidelines

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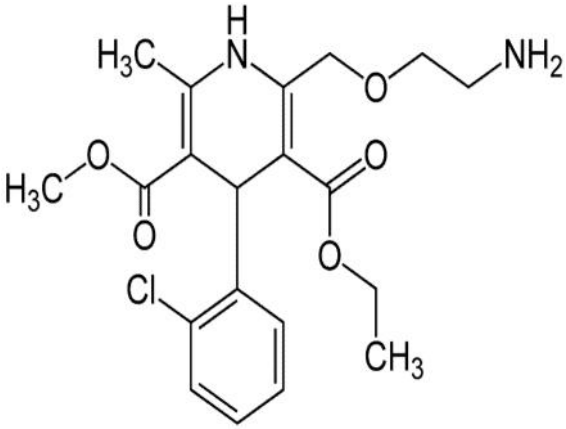
Structure of Hydrochorthiazide[6]



Materials and Methods

Materials

Amoldifine Besylate and Hydrochlor thiazide pure standards were received as gift samples from micro labs Pharmaceuticals GOA (India). All other reagents used were AR grade.

Instruments:

HPLC, Centrifuge,

Standard stock solution of Amlodipine Besylate:

Accurately weighed quantity of about 50mg of Amlodipine was taken in 100 ml volumetric flask dissolved in sufficient quantity of double distilled water, then sonicated for 15 min and diluted to 100ml with the same solvent. 1 ml of above solution transferred in 100 ml volumetric flask and the volume was made with diluents.

Standard stock solution of Hydrochlorthiazide:

An accurately weighed quantity of about 125mg of hydrochlorthiazide was taken in 100 ml volumetric flask dissolved in sufficient quantity of D.Water, then sonicated for 15 min and diluted to 100ml with the same solvent . 1 ml of above solution transferred in 10 ml volumetric flask and the volume was made with diluents.

Apparatus and Chromatographic conditions

Chromatographic separation was performed by RP-HPLC-Shimadzu module prominence with LC-UV-2000 detector. Column-Kromasil C-18 (250×4.6mm×5µ) was used for the separation. Mobile phase of buffer,methanol and acetonitrile were mixed in the ratio 25:37.5:37.5, filtered through 0.45µ membrane filter, degassed and used for the separation. The flow rate was 1ml/min and inject volume was 20µL with detection at 230nm and analysis was performed at ambient temperature.

Assay method

With the optimized chromatographic condition, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of Amlodipinebesylate and Hydrocholrthiazide was found to be 2.79 and 3.97 respectively. This procedure was repeated for the sample solution obtained from the formulation. The concentration of the drug was calculated using the following formulae.

$$\% \text{ Assay} = \frac{AT \times \text{Std dilution} \times \text{Potency} \times \text{Average Wt of the tablet}}{\text{Std Area} \times \text{Sample Dilution} \times 100} \times 100$$

Method validation [7,8]

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in table 1.From data obtained, added recoveries of standard drugs were found to be accurate.

Table no. 1 Accuracy (Recovery Studies)

Drug	Accuracy Levels	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	% RSD
AMD	15% of label claim	345154.86	57.5	53.46	99.97	1.64	1.63
	25% of label claim	400654.00	62.5	62.06	99.29		
	50% of label claim	495945.77	75	76.82	100.56		
HCT	15% of label claim	3120620.06	143.75	144.19	100.30	0.181	0.180
	25% of label claim	3381243.60	156.25	156.23	99.98		
	50% of label claim	4070009.43	187.5	188.06	100.29		

System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and presented in table 2. The values obtained demonstrated the suitability of the systefor the analysis of the drug combinations. System suitability parameter may fall within ±3% standard deviation range during routine performance of the method.

Table No. 2: Analytical Parameters

System suitability	Amlodipine besylate	Hydrochlorothiazide
%RSD	0.39	0.22
Tailing factor	1.67	1.42
No. of theoretical plates	6846.41	7450.90
Resolution	4.37	4.37
LOD(µg/mL)	0.141	0.167
LOQ(µg/mL)	0.429	0.507
Retention Time	3.97	2.79

Specificity

There is no interference in the standard peak of Amlodipine besylate and Hydrochlorothiazide. It shows that developed analytical methods was specific in the tablet dosage form of the drug and were presented in table 3.

Table no.3 Specificity studies of Amlodipine besylate and Hydrochlorothiazide in dosage form

Drug	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
AMD	400654.00	62.5	62.06	99.29	1.07	1.08
	322259.31	50	46.91	99.83		
	236065.71	37.5	36.56	97.49		
HCT	3381243.60	156.25	156.23	99.98	1.54	1.55
	2700920.17	125	124.79	99.83		
	1964414.97	93.75	90.76	96.81		

Precision

The Relative Standard Deviation should not be more than 2% for test result. The RSDs for intra-day and inter-day precision were not more than 2.0% for both AMD and HCT. The low RSD values indicate the repeatability and reproducibility of the method. Therefore, as per the ICH guidelines, this HPLC method for the determination AMD and HCT was precise.

Results for Intra-day precision:

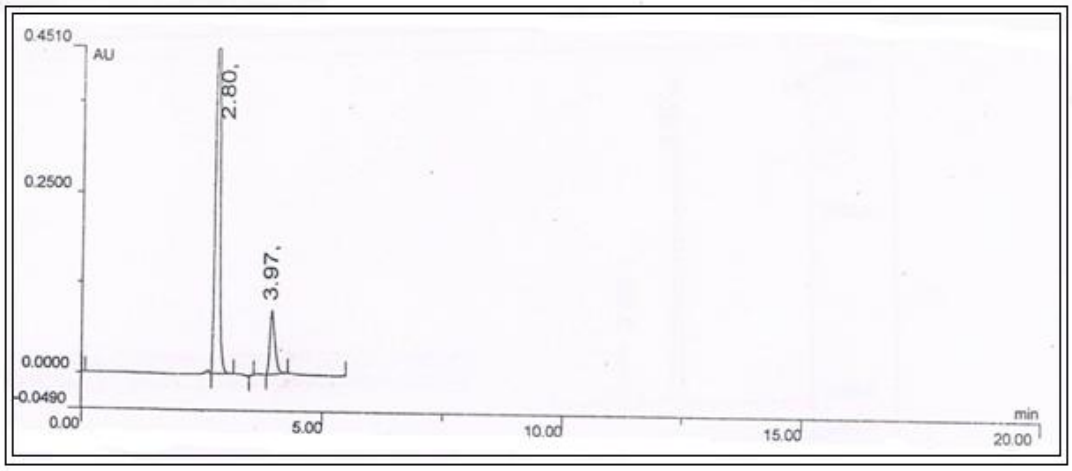


Fig.1: Chromatogram for Intra-day Precision study; Trial-1

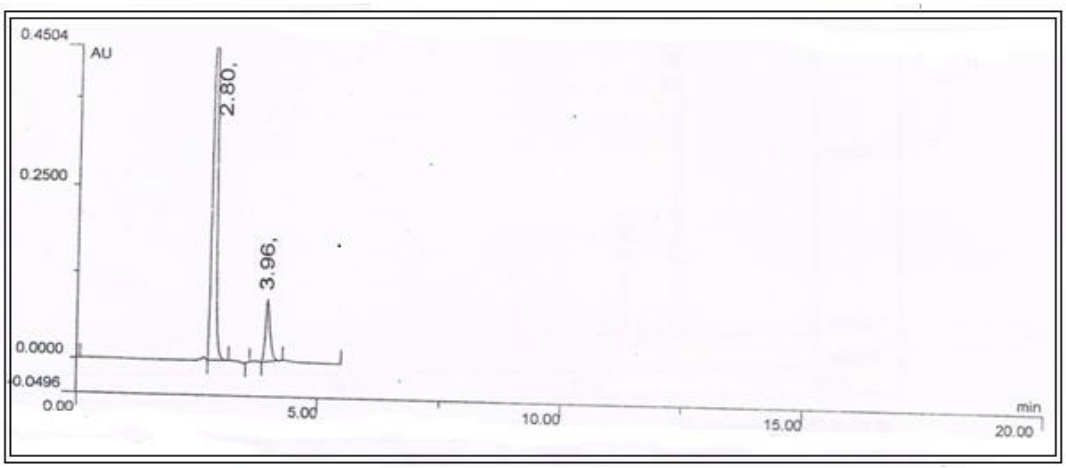


Fig.2: Chromatogram for Intra-day Precision study; Trial-2

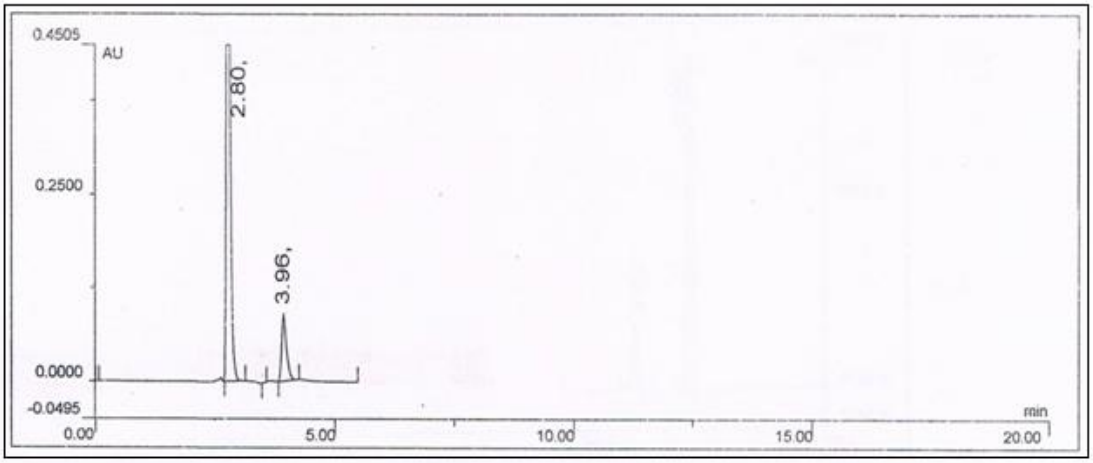


Fig.3: Chromatogram for Intra-day Precision study; Trial-3

Table No.4: Intra-Day variability for AMD and HCT

Drug	Trial No	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
AMD	1	322259.31	50	49.91	99.83	0.09814	0.09843
	2	321697.09		49.83	99.66		
	3	321713.03		49.83	99.66		
HCT	1	2700920.17	125	124.79	99.83	0.07505	0.07518
	2	2698897.26		124.70	99.76		
	3	2703009.03		124.89	99.91		

Results for Inter-day Precision:

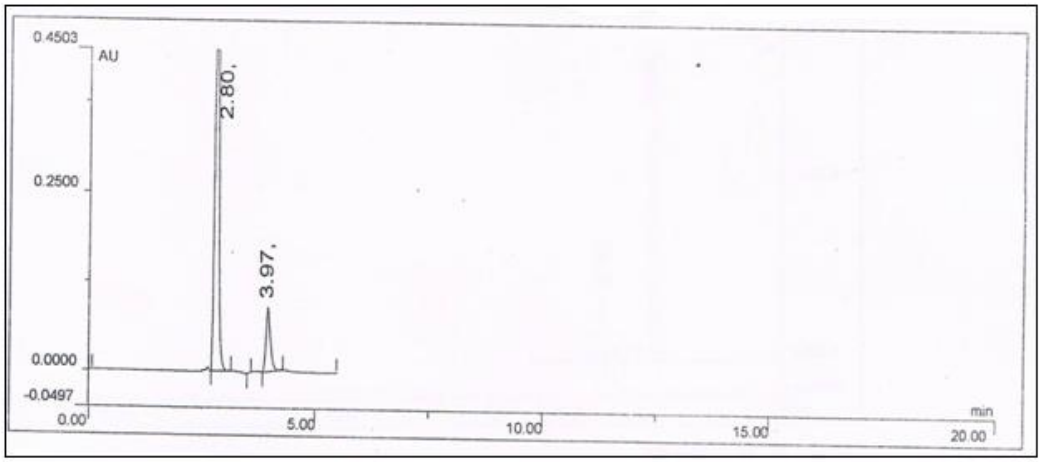


Fig.4: Chromatogram for Inter-day Precision study; Day-1

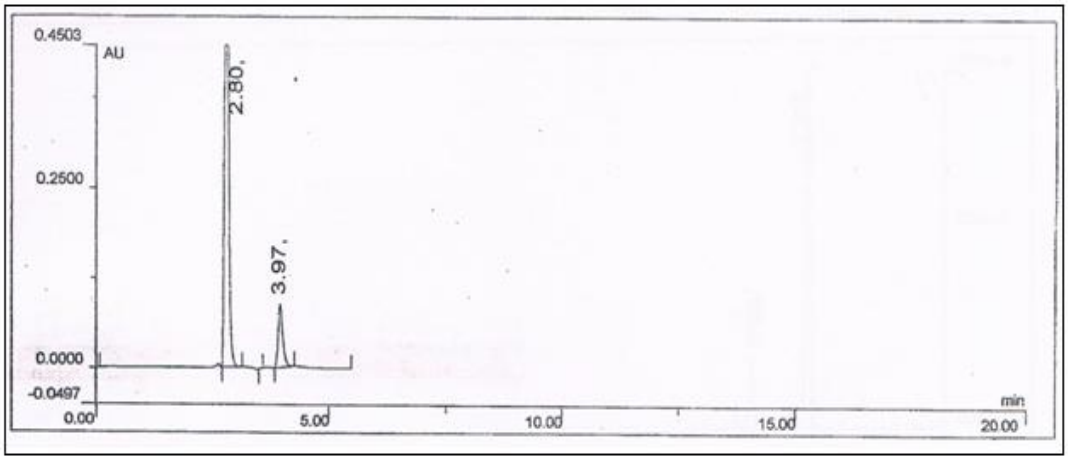


Fig.5: Chromatogram for Inter-day Precision study; Day-2

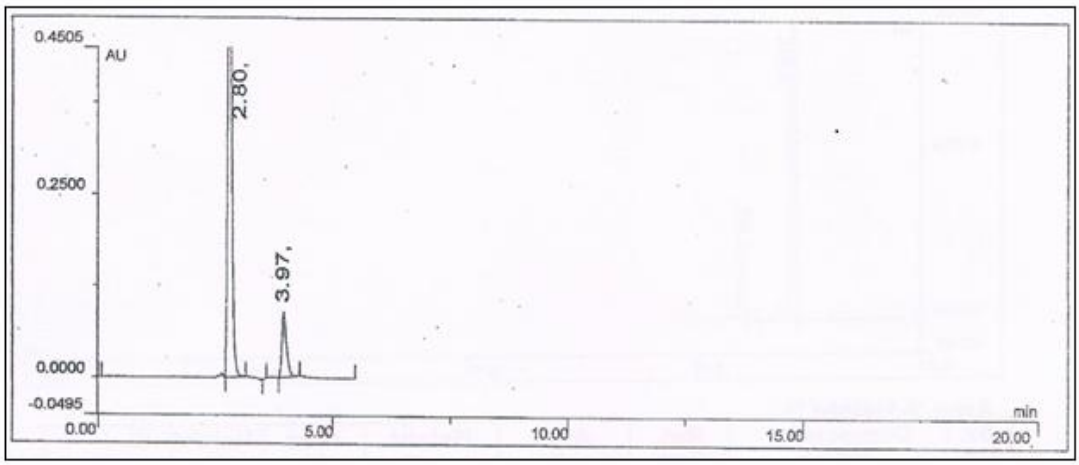


Fig.6: Chromatogram for Inter-day Precision study; Day-3

Table No-5-Inter Day Precision Study

Drug	Trial No	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
AMD	1	321552.69	50	49.80	99.60	0.16165	0.16
	2	321554.06		49.80	99.60		
	3	322413.77		49.94	99.88		
HCT	1	2698165.03	125	124.67	99.73	0.04041	0.041
	2	2699370.97		124.72	99.78		
	3	2700199.66		124.76	99.81		

Linearity

- The plot should be linear passing through the origin.
- Correlation Coefficient should not be less than 0.999.

The calibration curves exhibited linear relationship of peak area to concentration in the range 10-80 µg/mL for AMD and 10-80 µg/mL for HCT. The regression coefficients (r^2) for AMD and HCT were 0.998 and 0.996, respectively, maintaining good correlation close to unity. The graph of concentration Vs Average area was plotted which is showing straight line passing through all points. So as per ICH guidelines, the proposed HPLC method for the determination of AMD and HCT was found to be linear.

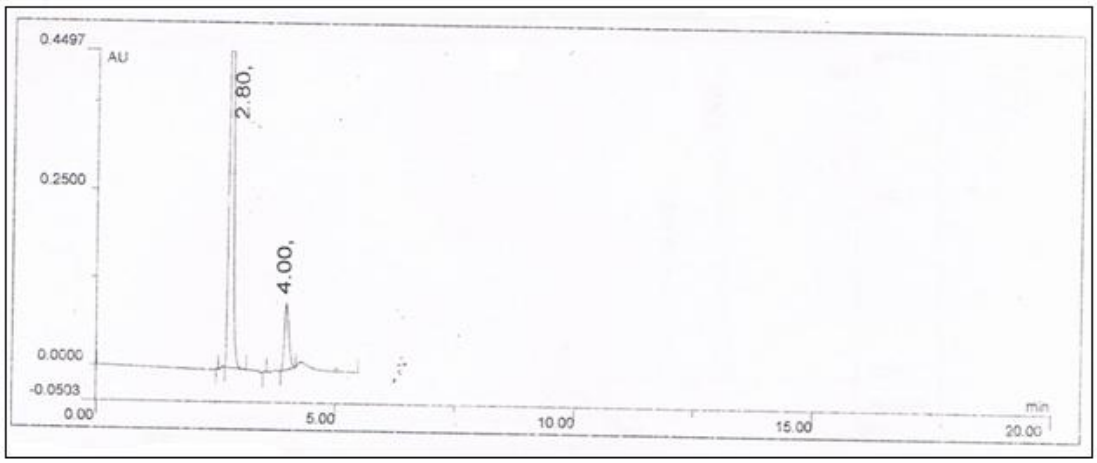


Fig-7: Chromatogram for Sample Containing 10µg/ml of AMD and HCT

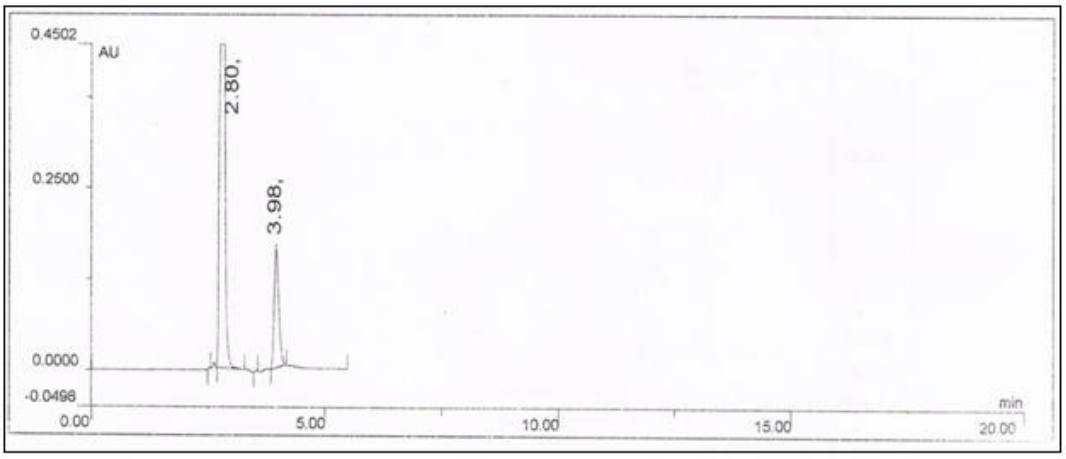


Fig.8: Chromatogram for Sample Containing 20µg/ml of AMD and HCT

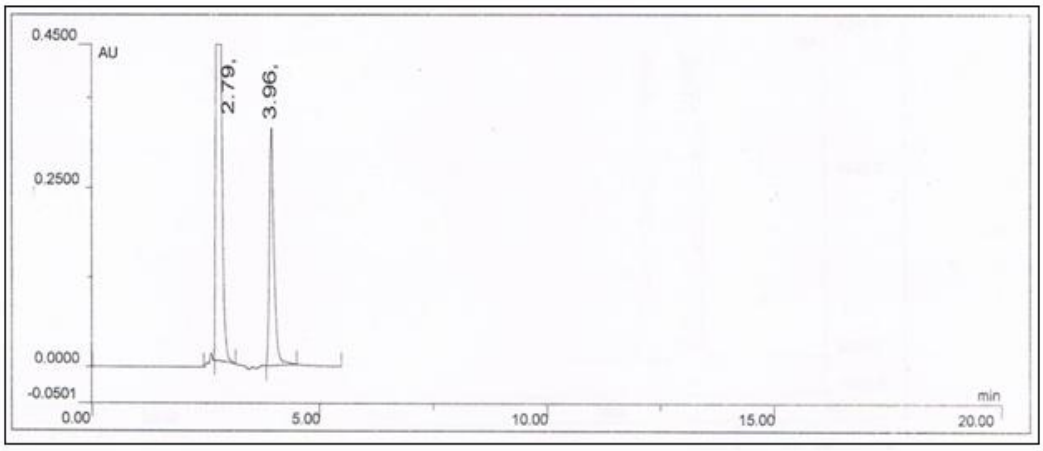


Fig.9: Chromatogram for Sample Containing 40µg/ml of AMD and HCT

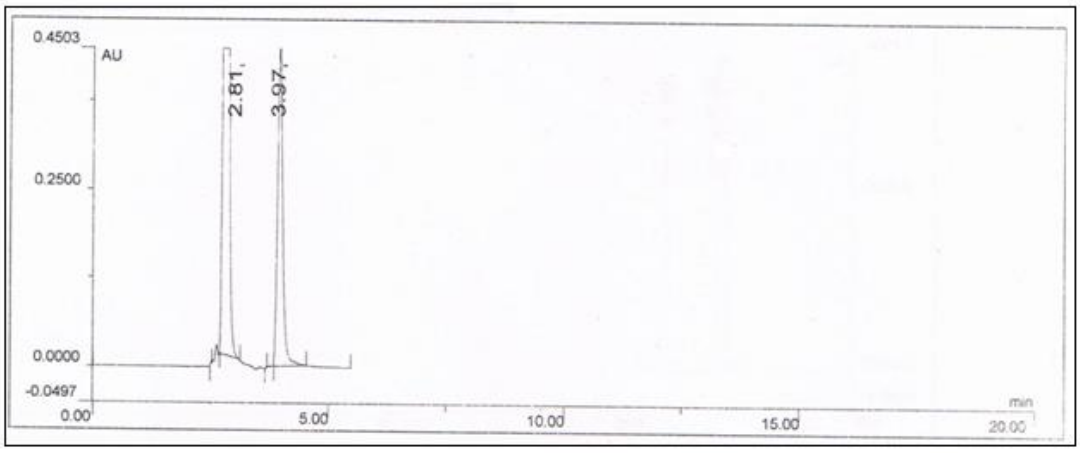


Fig.10: Chromatogram for Sample Containing 60µg/ml of AMD and HCT

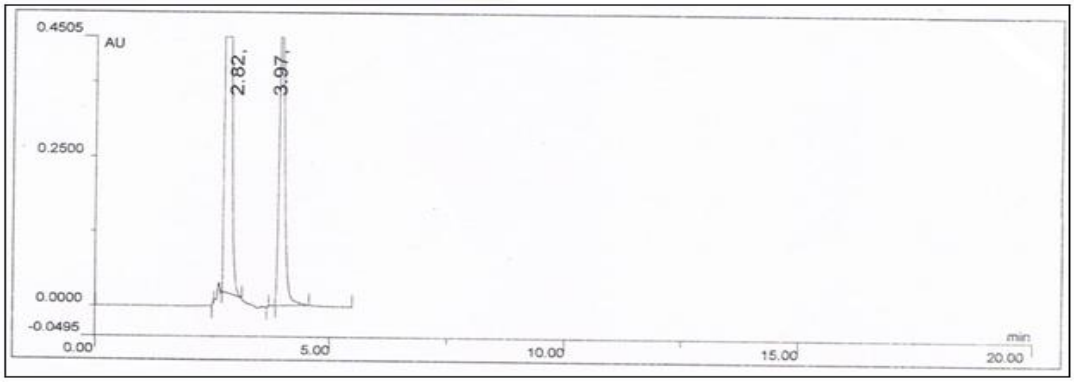


Fig.11: Chromatogram for Sample Containing 80µg/ml of AMD and HCT

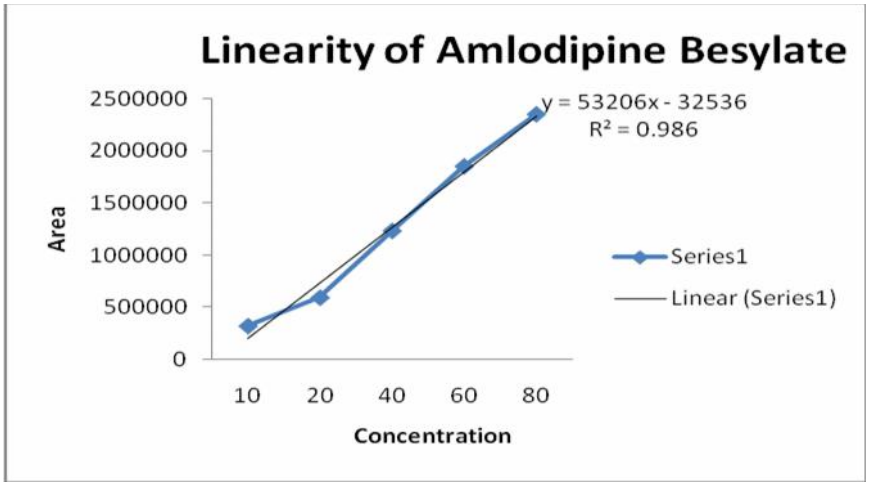


Fig.12: Linearity graph for AMD

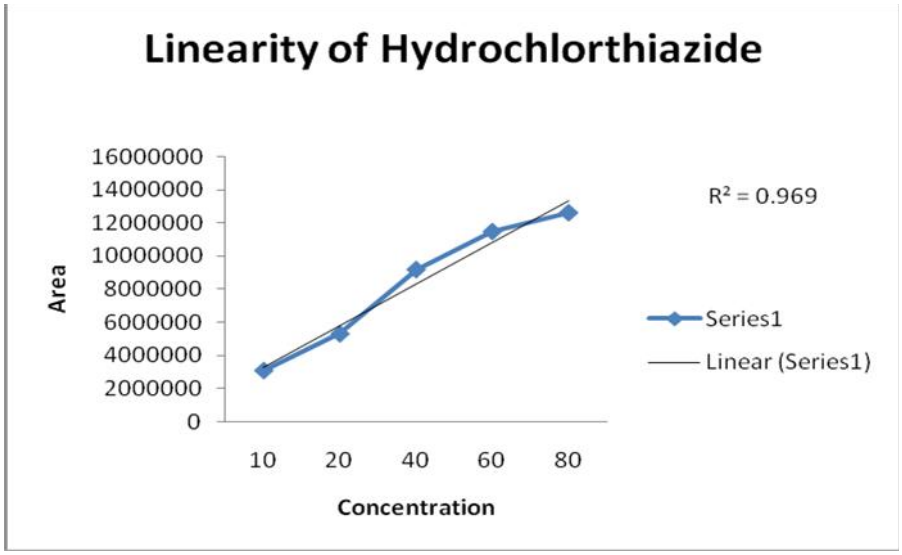


Fig.13: Linearity graph for HCT

Ruggedness:

% RSD between the test result obtained should not be more than 2% for assay method.

To evaluate the ruggedness of the proposed RP-HPLC method, the analysis was performed by different analysts and employing different brands of chemicals and solvents. Overall RSD for results obtained fromdifferent analysts are within limits.Therefore, the HPLC method for the determination of AMD and HCT was found to be Rugged.

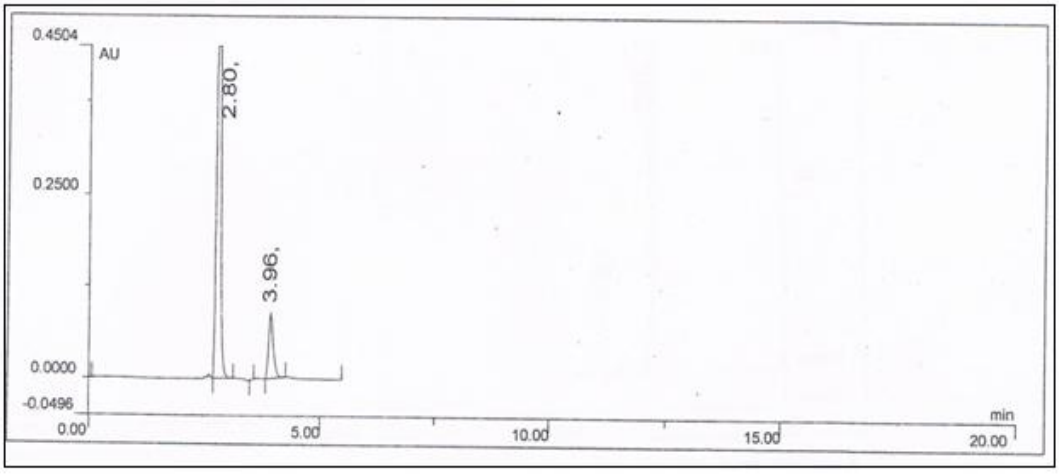


Fig.14: Chromatogram for Ruggedness of Sample no1

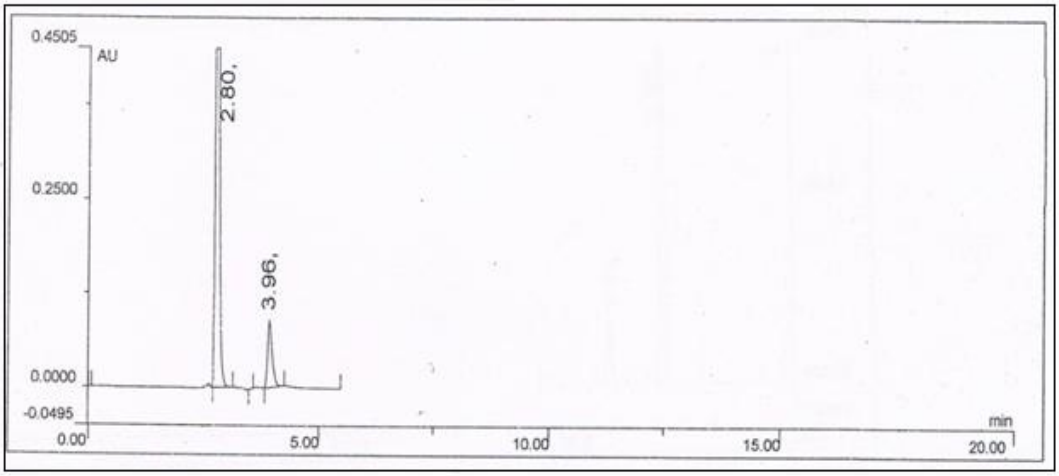


Fig.15: Chromatogram for Ruggedness of Sample no2

Table No.6-Data for Ruggedness

Drug	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	% RSD
AMD	321697.09	50	49.82	99.64	0.0841	0.0842
	321713.03	50	49.88	99.76		
HCT	2698897.26	125	124.75	99.80	0.070	0.070
	2703009.03	125	124.65	99.70		

Robustness

% RSD should be NMT 2.0%.

Results of the robustness study showed that the elution order and resolution for both components were not significantly affected. RSD of both components were examined and found to be well within the limit of 2.0%. The plate count and asymmetry factor was well within the acceptable USP limits, ensuring that the proposed method was robust and was capable of providing data of acceptable quality.

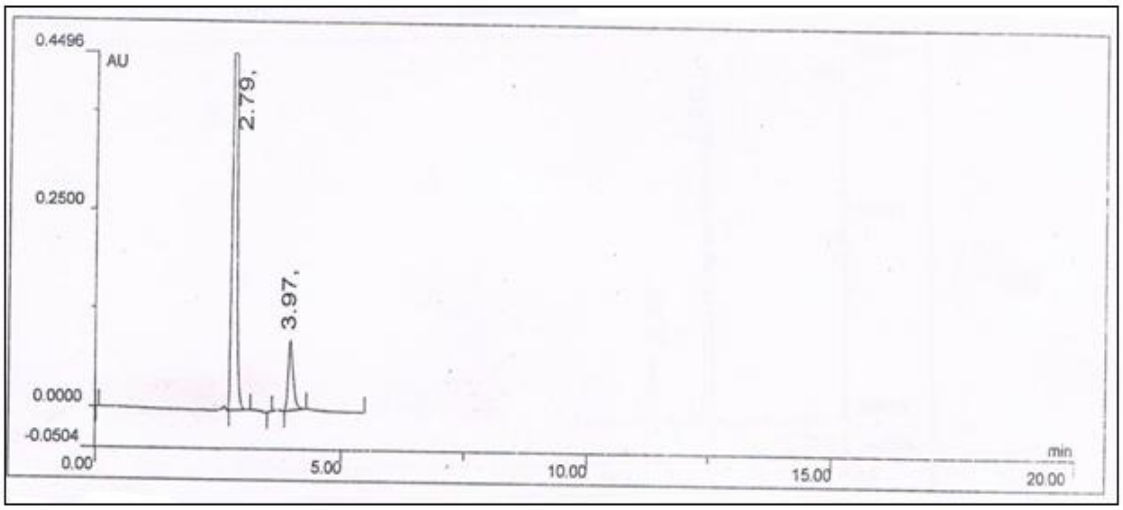


Fig.16: Chromatogram for Robustness at a low p^H value

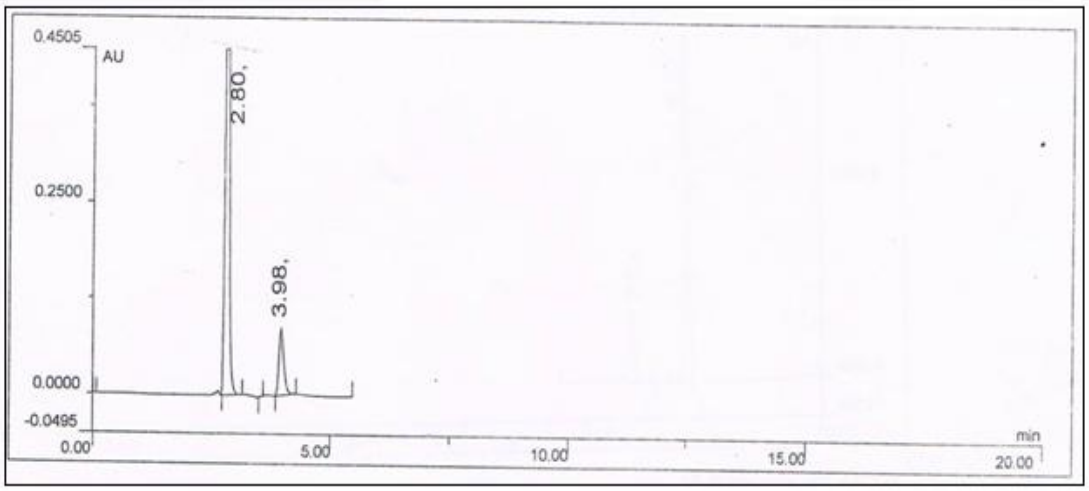


Fig.17: Chromatogram for Robustness at a High p^H value

Table No.7-Data for Robustness

Drug	Robustness Test	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	% RSD
AMD	At low p^H	321441.20	50	49.79	99.58	0.2616	0.2622
	At high p^H	322628.86	50	49.97	99.95		
HCT	At low p^H	2708759.77	125	125.16	100.12	0.141	0.140
	At high p^H	2703307.03	125	124.90	99.22		

Results and Discussion

HPLC method was developed. It was validated for the estimation of Amlodipine besylate and Hydrochlorothiazide in tablet dosage form .The Chromatographic separation was performed by RP-HPLC-Shimadzu module prominence with LC-UV-2000 detector. Column-Kromasil C-18 (250×4.6mm×5µ) was used for the separation. Mobile phase of buffer ,methanol and acetonitrile were mixed in the ratio 25:37.5:37.5, filtered through 0.45µ membrane filter, degassed and used for the separation. The flow rate was 1ml/min and inject volume was 20µL with detection at 230nm and analysis was performed at ambient temperature. The developed method was validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity, Specificity, Ruggedness, Robustness, LOQ and LOD.

Conclusions

The high performance liquid chromatographic method for the determination of amlodipine and hydrochlorothiazide from their fixed dosage form was found to be accurate and precise.

The elution of the analytes by thus developed and validated isocratic method is shorter than the gradient method reported so far. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of amlodipine, hydrochlorothiazide and losartan from their fixed dosage form and analysis of raw materials.

References

1. SantajiNalwade,Rapid Simultaneous Determination of Telmisartan, Amlodipine Besylate and Hydrochlorothiazide in a Combined Poly Pill Dosage Form by Stability-Indicating Ultra Performance Liquid Chromatography 2011 page no-69-84
2. Bagyalakshmi ,Development of UV spectroscopic method for the estimation of s(-) Amlodipine and Hydrochlorothiazide in dosage forms by simultaneous equation method Scholars Research Library Der PharmaChemica, 2011, 3(3): 246-252
3. Janhavi R Rao Simultaneous Quantitation of Olmesartanmedoxomil, Amlodipine besylate and Hydrochlorothiazide in Pharmaceutical dosage form by using HPLC International
4. Journal of PharmTech Research CODEN (USA): IJPRIF ISSN : 0974-4304 Vol.3, No.3,pp 1435-1440, July-Sept 2011
5. Wilson Organic medicinal and Pharmaceutical Chemistry Page No.642
6. Jabir Aboobacker O,Method Development and Validation of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan in Tablet Dosage Form by RP-HPLC Method Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012 vol-3 page no 509-517
7. Ashutosh Kar Medicinal Chemistry, Fifth Edition New Age International Publishers Page No.447
8. United States Pharmacopeia 2007 .
9. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology, ICH - Q2 (R1), Geneva 2005.