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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THESIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND CLOPIDOGREL IN BULK AND TABLET DOSAGE FORMS

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Abstract

A reverse phase high performance liquid chromatography method was developed for simultaneous estimation of amlodipine and clopidogrel in table formulation. The separation and quantification was achieved by thermo hypersil BDS C18 250x4.6x5mm column in isocratic mode, with mobile phase consisting of buffer-methanol(30:70v/v). The mobile phase was pumped at a rate of 1.5 ml/min and the detection was carried out at 238nm. The retention time of amlodipine and clopidogrel was found to be 1.9 and 3.8 min, respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, LOD, LOQ and robustness. Linearity was observed in the concentration range of 20-60 µg/mL and 300-600 µg/mL. Calibration curve were linear over studies ranges with correlation co-efficient found to be 1.00. The developed method was found to ve accurate, precision and selective for simultaneous estimation of amlodipine and clopidogrel in tablets.

Key wards: RP-HPLC, Amlodipine, Clopidogrel, isocratic mode.

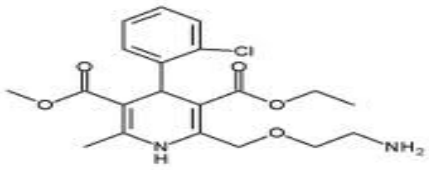
Introduction

Amlodipine besylate is the besylate salt of amlodipine, a long-acting, calcium channel blocker. Amlodipine besylate is chemically described as 3-ethyl-5-methyl(±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate. Amlodipine besylate is a white crystalline powder. It is slightly soluble in water and sparingly soluble in ethanol. Amlodipine besylate tablets are formulated as white to off-white

tablets equivalent to 2.5, 5, and 10 mg of amlodipine for oral administration. In addition to the active ingredient, amlodipine besylate, each tablet contains the following inactive ingredients: calcium phosphate dibasic anhydrous, magnesium stearate, microcrystalline cellulose, and sodium starch glycolate.

Its structural formula is:

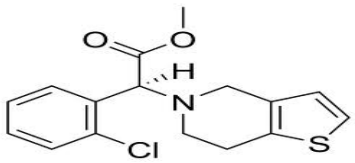
C20H25CIN2O5·C6H6O3S M.W. 567.1



Clopidogrel bisulphate (CPS) is methyl-2-chlorophenyl-(4, 5, 6, 7-tertrahydrothieno [3,2-c] pyridine-5yl)acetate bisulphate used in the treatment of cardiovascular diseases. Clopidogrel is used as platelet inhibitor.It is indicated for the reduction of atherosclerotic events in patients with atherosclerosis documented by recent stroke, recent myocardialinfraction or cardiovascular disease. It is an analogue of ticlopidine and acts by inhibiting adenosine diphosphate-mediated platelet aggregation. Clopidogrel inhibits platelet aggregation by selective preventing of the binding adenosinediphosphate (ADP) to its platelet receptor. It is a potent antiplatelet drug used in thromboembolitic disorders.

Its structural formula is:

$C_{16}H_{16}ClNO_2S$ M.W 321.05



Materials and Methods

Material

Working standards of Amlodipine and Clopidogrel were received as gift samples from Dr Reddys laboratory,Hyderabad.. Distilled Water, methanol of HPLC grade.Potassium di hydrogen phosphate,Orthophosphoric

acid,triethylamine are the other chemicals from rankem chemicals.Waters e2695 Alliance HPLC system with empower2 software connected to PDA detector2998 running under Windows XP on a Pentium PC, pH meter,electronic balance, Sonicator.

Commercial Formulation

Amlodipine and Clopidogrel Tablets were purchased at Service Medicals which are available as Numlopar-5 in composition of Amlodipine besylate-5mg and Clopidogrel-75mg. The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Chromatographic System and Conditions

Stationary phase	Thermo Hypersil BDS C18 column (250x4.6mm i.d, 5µm)
Mobile phase	Methanol : Buffer (0.01M Potassium dihydrogen phosphate + 5ml triethylamine)
PH	3.00 (adjusted with Orthophosphoric acid)
Solvent ratio	70:30
Detection wavelength	238 nm
Flow rate	1.5ml/min
Temperature	30^0C

Preparation of mobile phase

Methanol and buffer were mixed in the ratio of 70:30 and filtered through 0.45µ membrane filter and degassed in a sonicator for 10 minutes.

Preparation of buffer (0.01M)

1.3609 gm of Potassium di-hydrogen phosphate in sufficient water to produce 1000ml to this add 5ml triethylamine, pH adjusted to 3.0 with orthophosphoric acid.

Preparation of standard solution

Weight accurately about 20mg of Amlodipine and transfer into a 50ml volumetric flask.weigh 30mg of Clopidogrel and transferred in to a clean 25ml volumetric flask dissolve them in few ml of diluent(70%methanol & 30% buffer)and make up to the volume in both the flasks with diluent. Sonicate for 10 minutes and filtered through 0.45µ membrane filter and marked as standard stock solutions. Pipette out 1ml from the Amlodipine standard stock solution and 5ml from the Clopidogrrel standard stock solution into a clean 10ml standard flask and make up the volume 10 ml with mobile phase and marked as standard stock solution A.

Preparation of sample solution

Weigh and powder 20 tablets, weight accurately a quantity of powder equivalent to its average weight 390mg and transfer it into a clean 25 ml standard flask. Add few ml of diluent and dissolve it, make up the volume with diluent. The solution is sonicated for 20 minutes and filtered through 0.45µ membrane filter, and marked as sample stock solution. Pipette out 5 ml from the sample stock solution in to a clean 25 ml standard flask and make up the volume 25 ml with mobile phase. So as to give a concentration of 40µg of Amlodipine and 600µg Clopidogrel.

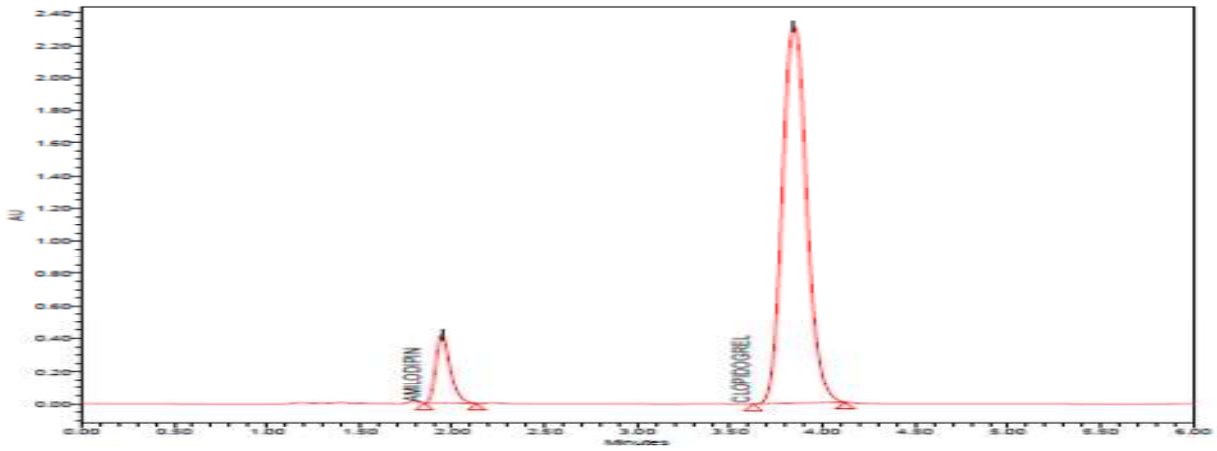
Method Development and Optimization

The wavelength for the analysis was selected by 3D view of the combined spectra using PDA detector, the maximum absorbance with good peak intensity, good peak shape and height was observed at 238nm.

For HPLC analysis, initially various mobile phases and stationary phases were tried in attempts to obtain the best separation and resolution between Amlodipine and Clopidogrel. The mobile phase consisting a combination of methanol and 0.01M potassium di hydrogen phosphate buffer of pH 3.0 in the ratio of 70:30 v/v.was found to be an appropriate mobile phase allowing adequate separation of two drugs using a C18 Thermo Hypersil BDS 5µ, 250cm x

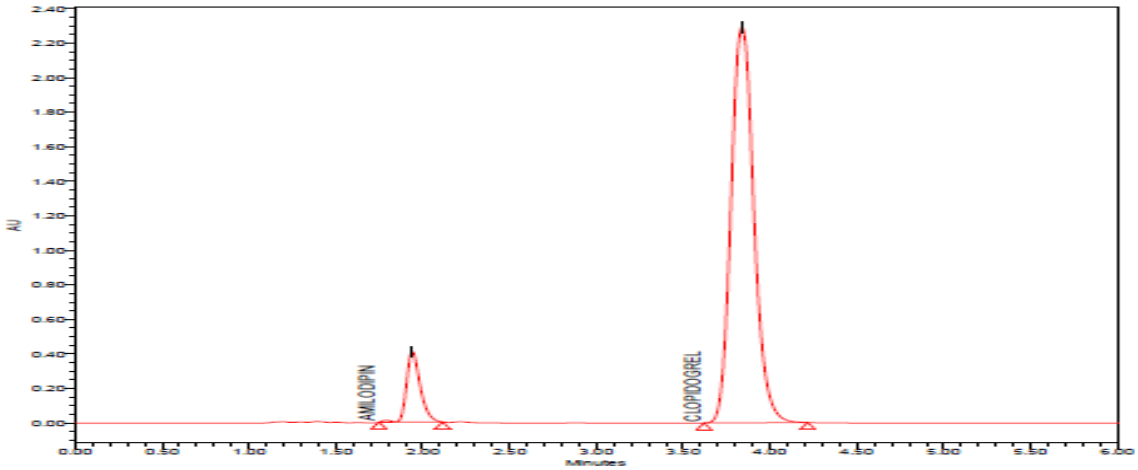
4.6mm id with flow rate of 1.5ml/min using PDA detection at 238nm. A typical chromatogram of separation of two compounds in standard and sample are shown in fig 1 and fig 2.

Figure1: Chromatogram of standard Amlodipine and Clopidogrel.



S.No	Name	Retention Time	Area	Height	USP Resolution	s/n	USP Tailing	USP Plate Count
1	Amlodipine	1.946	2249228	424751		1474.061982	1.4	3172
2	Clopidogrel	3.852	20971141	2336420	10.31	8108.346615	1.1	4737

Figure 2: Typical chromatogram of Amlodipine and Clopidogrel in marketed formulation.



S.No	Name	Retention Time	Area	Height	USP Resolution	s/n	USP Tailing	USP Plate Count
1	Amlodipine	1.941	2248848	408028	-	1454.384684	1.4	2973
2	Clopidogrel	3.838	20974978	2290735	10.31	8165.15778	1.2	4444

System suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 µg/mL of both the drugs. The resolution, correlation coefficient, number of theoretical plates, tailing factor , LOD,LOQ etc were calculated as represented in Table 1. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Method Validation

The developed method was validated for simultaneous assay determination of Amlodipine and Clopidogrel using following parameters.

Table 1: System suitability parameters for RPHPLC Method.

S. no.	**Parameters**	**Amlodipine**	**Clopidogrel**
1	Linearity range µg/ml	20-60 µg/mL	300-900 µg/mL
2	Correlation Coefficient (r2) ± S.D	1.0	1.0
3	Retention time (min) ± S.D	1.9	3.8
4	USP Theoretical plate count	3172	4737
5	Tailing factor	1.4	1.1
6	Resolution*	10.31	10.31
7	Limit of detection (µg/ml)	0.08	0.22
8	Limit of Quantification (µg/ml)	0.27	0.73
9	Precision (RSD %) intraday (n=6)	0.77	0.17

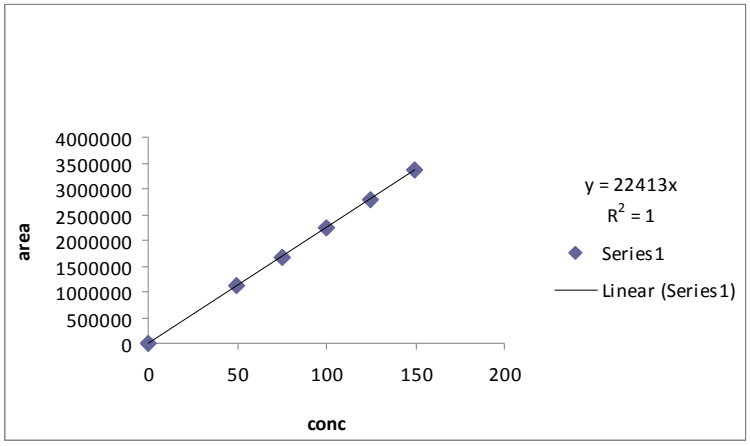
Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample Amlodipine and Clopidogrel were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

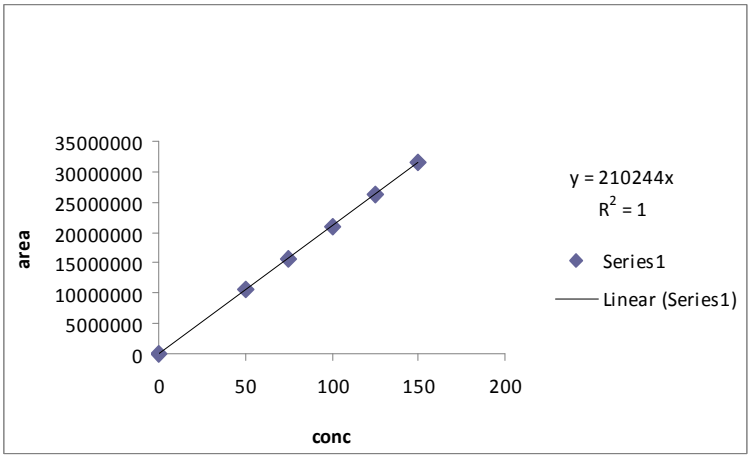
Linearity

Linearity was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of Amlodipine and Clopidogrel which were found to be linear in the range of 20-60 µg/mL and 300-900 µg/mL respectively. Coefficient of correlation was 1 and 1.these are shown in the fig 3 and fig 4 below.

Linearity ofAmlodipine



Linearity ofClopidogrel



Precision

To demonstrate agreement among results, a series of measurements are done with Amlodipine and Clopidogrel six replicate injections of the specific standard at various time intervals on the same day were injected into the chromatograph and the value of %RSD was found to be 0.77and 0.17 for Amlodipine and Clopidogrel (Table 2).

Table 2: Results of Intra-Day Precision and Inter-Day Precision For Simultaneous Determination of Amlodipine and Clopidogrel.

Drug	%RSD(intra-day)	%RSD(inter-day)
Amlodipine	0.77	1.17
Clopidogrel	0.17	0.92

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% and 150%. The percentage of recoveries were calculated, results of which are represented in Table 3.

Table 3: Accuracy (%recovery) results of Amlodipine and Clopidogrel.

AMLODIPINE				
S.NO	Spiked Amount(mg)	Recovered amount(mg)	%Recovered	%Average recovery
1.	2.5	2.47	99	98.2
2.	5	4.9	98	
3.	7.5	7.33	97.8	

CLOPIDOGREL				
S.NO	Spiked Amount(mg)	Recovered amount(mg)	%Recovered	%Average recovery
1.	37.5	37.27	99.4	98.8
2.	75	73.5	98	
3.	112.5	111.3	99	

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) of this method were determined from the known concentrations of Amlodipine,Clopidogrel.

Robustness

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following two factors were selected for change: flow rate of the mobile phase (1.5 ±0.2 mL/min) and temperature($30^0 \pm 5^0$C) .One factor at the time was changed to estimate the effect. It was observed that there were no marked changes in the chromatograms,which demonstrated that the RP-HPLC method developed is robust.It is shown in Table 4.

Table 4: Robustness

A.FLOW RATE				
DRUGS	CHANGES	RT	USP TAILING	USP PLATE COUNT
AMLODIPINE	1.3	2.2	1.5	2670
	1.7	1.7	1.5	2482
CLOPIDOGREL	1.3	4.3	1.2	4012
	1.7	3.4	1.2	3659

B.COLUMN TEMPERATURE				
DRUGS	CHANGES	RT	USP TAILING	USP PLATE COUNT
AMLODIPINE	25	2.3	1.5	2570
	35	2.6	1.5	3045
CLOPIDOGREL	25	4.8	1.3	3729
	35	5.3	1.3	4773

Results and Discussion

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both Amlodipine and Clopidogrel. The mobile phase combination of Methanol: Buffer (0.01M Potassium dihydrogen phosphate) 70:30 PH 3.00 (adjusted with Orthophosphoric acid) found to be satisfactory and gave two symmetric and well resolved peaks for Amlodipine and Clopidogrel. The retention time for Amlodipine and Clopidogrel were 1.94 and 3.83, respectively. The calibration curve for Amlodipine was obtained by plotting the peak area of Amlodipine versus the concentrations of Amlodipine over the range of 20-60µg/ml, and it was found to be linear with r2 = 1. Similarly, the calibration curve for Clopidogrel was obtained over the range of 300-900µg/ml and was found to be linear with r2 = 1. The recoveries of Amlodipine and Clopidogrel were found to be in the range of 97%-99% and 98%-100% within precision RSD of 0.77 and 0.17 for Amlodipine and Clopidogrel. The system suitability parameters such as theoretical plates and tailing factor were found to be 3172, 1.4 and 4737, 1.1 respectively for Amlodipine and Clopidogrel. The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The detection limit (LOD) was found to be 0.08µg/ml for Amlodipine and 0.22µg/ml for Clopidogrel respectively.The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The quantitation limit (LOQ) was found to be 0.27µg/ml for Amlodipine and 0.73µg/ml for Clopidogrel respectively.

Proposed study describes a new RP-HPLC method for estimation of Amlodipine and Clopidogrel combination in mixture using simple mobile phase. The method gives good resolution between both the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise. Percentage recovery shows that the method is free from interference of the excipients used in the formulation (Table **3**). Therefore, the proposed method can be used for routine analysis of Amlodipine and Clopidogrel their combined dosage form.

Conclusion

In the current study a new RP-HPLC method for estimation of Amlodipine and Clopidogrel combination in mixture using simple mobile phase was developed, optimized and validated. The developed method is simple, sensitive, accurate and precise. The developed method can be used for routine analysis of Amlodipine and Clopidogrel in a combined dosage form.

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