Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):483-494

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Validated RP-HPLC method for the simultaneous determination of amlodipine besylate, and hydrochlorothiazide using losartan potassium as working standard in bulk and pharmaceutical formulation

Babikir H. El-Rasoul[1] and Hana Tag Elsir[2] and Ahmed Elsayed[2]

[1]Quality Control Manager, Azal Pharmaceutical Company, Khartoum, Sudan
[2]Quality Control Unit Laboratories, Azal Pharmaceutical Company, Khartoum, Sudan

---

ABSTRACT

Amlodipine Besylate and hydrochlorothiazide combination is a long acting calcium channel blockers used as anti-hypertensive and for the treatment of angina. The combination is planned to be introduced very soon in the market in the Sudan. It is of vital importance that a validated and very precise analytical method should be established for the quantification of the components of this drug combination. The present study describes a reliable reverse phase high performance liquid chromatographic RP-HPLC method that has been developed and validated for the simultaneous estimation of amlodipine besylate and hydrochlorothiazide in pharmaceutical formulation. The combination were firstlyHPLC assayed and excellently resolved peaks were obtained via an RP – C_{18} column. The mobile phase (mixture of Buffer pH 3.0: Acetonitrile: Methanol) was pumped at a flow rate of 1.0 mL min^{-1} in the ratio of (500: 300: 350, v/v) and the eluents were monitored by a uv-detector set up at 240 nm. The retention time for amlodipine and hydrochlorothiazide was found to be 7.3 min and 3.1 min, respectively. Linearity was ascertained via linear calibration curves for both drugs (R^2= 0.9997 for amlopedine besylate and 0.9991 for hydrochlorothiazide) within the concentration range of 2.0–48 µg ml^{-1} for amlodipine besylate, and 10.0–120 µg ml^{-1} for hydrochlorothiazide. The method was statistically validated and RSD was found to be less than 2% indicating high degree of accuracy and precision of the proposed HPLC method. The percentage recoveries from the combined dosage form were between 99.79% to 102.84% for amlodepine and 98.16% to 100.58% for hydrochlorothiazide. The method is simple, rapid and of high degree of precision and accuracy. The method can, confidently, be applied and utilized in pharmaceutical quality control laboratories in routine analysis for determining amlodipine besylate, and hydrochlorothiazide in bulk and in pharmaceutical form.

INTRODUCTION

Amlodipine besylate AMB, 3-ethyl-5-methyl-2-[(2-aminoethoxymethyl]-4-(chloro-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate is a chiral calcium antagonist and it is a long acting calcium channel blocker used as an anti-hypertensive drug and also be used for the treatment of angina [1]. Hydrochlorothiazide, 6–chloro- 3,4–dihydro–7–sulfamoyl–2H–1,2,4–benzothia–diazine – 1, 1 –dioxide, is a thiazide diuretic [6]**.** The chemical structure of amlodipine besylate and hydrochlorothiazide. Figure 1and Figure 2, shown below**:**

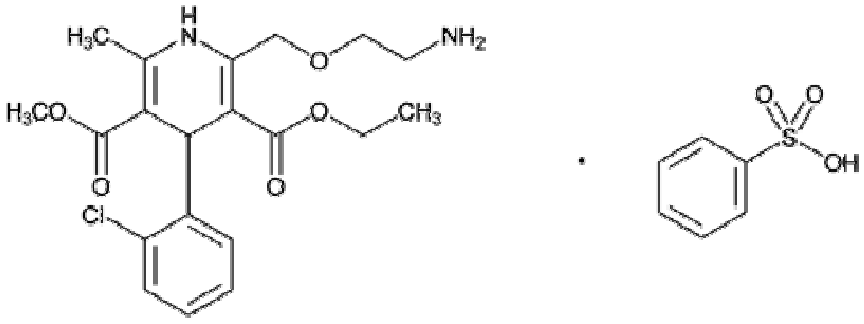


Figure 1

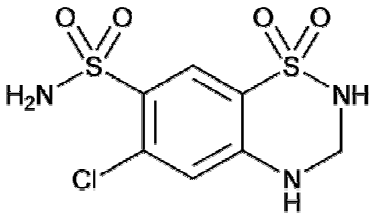


Figure 2

Various analytical methods have been attempted and reported for the assay of AMB alone and quite few in combinations with other anti-hypertensive agents in pharmaceutical formulations. These include UV spectroscopy [2-4], high performance liquid chromatography [5,6], LC-MS and LC-MS/MS [5,6]. Many analytical methods were reported for the analysis of (HCT) alone and in combination with other drugs by stability indicating methods and had been determined in plasma[5]. Amlodipine and hydrochlorothiazide are official in USP [4]. The chemical structure of amlodipine and hydrochlorothiazide. Search in literature revealed that there is no available official method for the simultaneous determination for this drug combination. Moreover, based on the fact that, currently, HPLC-analytical tool and the procedures associated with it, specifically, RP–HPLC procedure have proved to be simple, accurate and of high degree of precision. Accordingly, the present study is an attempt to develop and validate an RP-HPLC-procedure for simultaneous estimation of amlodipine and hydrochlorothiazide in bulk and in pharmaceutical preparations.

EXPERIMENTAL SECTION

Materials

All analytical runs were performed in a HPLC-Shimadzu (Japan) chromatograph equipped with an LC – 20AB solvent delivery system, a universal loop injector (SIL20A) of injection capacity of 100 µl, and an SPD – 20 AV UV–Visible detector set at 240 nm. The instrument was equipped with a GL SCIENCES C18 column of the dimensions (250mm x 4.6mm i.d., 5 µm particle size). An isocratic elution was adopted using a mixture of Buffer pH 3.0: Acetonitrile: Methanol (500: 300: 350, v/v), as a mobile phase. Flow rate of mobile phase was adjusted to 1.0 ml.min^{-1} and injection volume was 50 µl at 40°C temperature. Normal run time was chosen as 15 minutes. The equipment was controlled by a PC work station with LC solution Software. Analytically pure samples of amlodipine besylate and hydrochlorothiazide were procured from Azal Pharmaceutical Company, Khartoum north, Sudan as a gift and used as working standards. Methanol of HPLC grade from ROMIL, Acetonitrile of HPLC grade from Chemical lab (CL), Triethylamine of HPLC Grade from Sharlau, all other reagents are of analytical grade.

Methods:

Preparation of internal standard:

Weigh accurately about 25 mg of losartan potassium, as internal working standard to a 100ml volumetric flask. Add about 70 ml of Mobile Phase. Dissolve it completely and sonicate it. Make up to 100ml with Mobile Phase.

Preparation of standard solution:

Amlodipine Besylate (20 mg) and hydrochlorothiaiazide (50 mg) working standard was, accurately, weighed and introduced in to a 100ml volumetric flask. The contents were dissolved in the mobile phase (70 ml) and sonicated.

The solution was made up to 100 ml by the mobile phase. 5 ml of this solution were mixed with the internal standard (5 ml). The solution was made up to 50 ml by the mobile phase in a 50 ml volumetric flask.

Preparation of buffer solution:
Triethylamine (7 Ml) was added, with stirring, to water (800 mL). the pH of the resulting solution was adjusted to pH 3 through the drop wise addition of *ortho*-phosphoric acid. The solution was then diluted, with distilled water, to 1000 mL.

Preparation of sample solutions:
The sample drug (20 tablets, 140 mg) was, accurately, weighed and crushed to a course powder. The powder constituted 5mg of amlodepine and 12.5 mg of hydrochlorothiazide. The powder was transferred to a 100ml volumetric flask. The mobile phase (70 ml) was added and the mixture was shaken for complete solution and then sonicated for around 10 minutes with occasional shaking. The mobile was added to mark to make up to 100ml solution. Aportion of this solution (20 ml) add 5ml of internal standard solution then made up by the mobile phase to 50 ml in another volumetric flask. The final solution was filtered through 0.45 µm GHP filter.

Preparation of the Test Solutions (50%, 100% and 150% Solutions):
Amlodepine Besylate WS (2.5 mg), hydroclorothiazide WS (6.3 mg) and the placebo (122.5 mg) were thoroughly mixed and transferred into a 100 ml volumetric flask and then dissolved in the mobile phase (70 ml), sonicated to ensure complete dissolution. After cooling the volume was made up to the mark by the addition of the appropriate amount of the mobile phase. 20 ml portion of this solution add 5ml of internal standard solution then diluted to 50 ml with mobile phase to afford a 50% solution.

In a similar manner, for the preparation of a 100% and 150% different amounts (weights) of the drug combination be considered. Amlodepine Besylate WS (2.5 mg), and hydroclorothiazide WS should be 5 mg and 7.5 mg for the former drug and 12.5 mg, 16.8 mg for the latter drug, respectively. The appropriate volumes be taken and diluted to afford these two percentages

Specificity preparations:
Standard preparation:
Amlodepine besylate WS (20 mg) and hydrochlorothiazide WS (50 mg) were, accurately weighed, mixed, transferred into a 100ml volumetric flask and dissolved in the mobile phase (70 mL). The solution was sonicated for few minutes, then cooled and the volume was completed to the mark by the mobile phase. A volume (5 mls) of this solution was diluted to 50 mL by the mobile phase.

Test preparation solution:
Amlodepine Besylate WS (5 mg), hydroclorothiazide WS (12.5 mg) and placebo (122.5mg) were accurately weighed and transferred into a 100 ml volumetric flask and a 70 mL of the mobile phase was added. The contents were thoroughly mixed and sonicated for few minutes. The solution was allowed to cool and the volume was completed to the mark by the mobile phase. 10 mL of this solution was diluted to 25 mL with the mobile phase**.**

Acid hydrolysis test (0.1N hydrochloric acid):
Amlodepine Besylate WS (5mg), hydroclorothiazide WS (12.5 mg) and of placebo (122.5 mg) were accurately weighed and transferred into a 100 ml volumetric flask. An aqueous hydrochloric acid (0.1N HCl, 5 mL) was added. The solution was allowed to stand for 2 hrs and about 70 mL of the mobile phase was added. The solution was then sonicated for few minutes, allowed to cool and the volume was made up to the mark with the mobile phase. A volume of 10 mL of this solution was diluted to 25 mL. The appropriate volume of this solution was injected in the HPLC-system and the chromatogram was studied and recorded.

Base hydrolysis (0.1N sodium hydroxide):
Amlodepine Besylate WS (5 mg), hydroclorothiazide WS (12.5 mg) and placebo (122.5 mg) were weighed accurately and transferred into a 100 mL volumetric flask. An aqueous solution of sodium hydroxide (0.1 N, 5 mL) was added and the solution was allowed to stand for 2 hrs. 70 Ml of the mobile phase was added and the contents of the flask were sonicated for few minutes, allowed to cool and the volume was made up to the mark by the mobile phase. 10 ml of this solution was diluted to 25 ml by the mobile phase. The appropriate volume of this solution was injected in the HPLC-system and the chromatogram was studied and recorded.

Hydrogen peroxide oxidation test:
Amlodepine Besylate WS (5 mg), hydrochlorothiazide WS (12.5 mg) and placebo (122.5mg) were weighed accurately and transferred into a 100 ml volumetric flask. Hydrogen peroxide (5 mL, 30% solution) was added and the contents of the flask were allowed to stand for 2 hrs. 70 mL of the mobile phase was added and the contents were sonicated. It was then allowed to cool and the volume was made up to the mark by the mobile phase. 10 mls of this solution was diluted to 25 mls with the mobile phase. The appropriate volume of this solution was injected in the HPLC-system and the chromatogram was studied and recorded

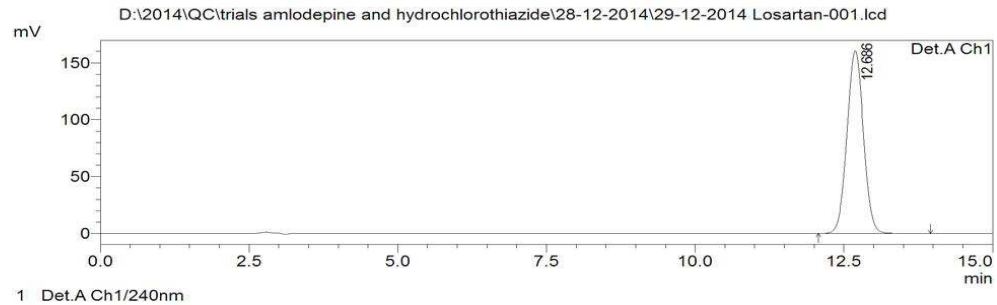
Thermal stability test: Test preparation for Heat hydrolysis (at 80ºC for 72 hours):
Amlodepine besylate WS (5.0 mg), hydroclorothiazide WS (12.5 mg) and the placebo (122.5 mg) were weighed accurately, transferred into a 100 mL volumetric flask. It was then placed into a dry oven set at 80 ºC and allowed for 72 hr. The solution was then transferred into a 100 mL volumetric flask and 70 mL of the mobile phase was added. The contents of the flask were sonicated and then allowed to cool. The volume was then made up to the mark with the mobile phase. 10 mL of this solution was diluted to 25 mL by the mobile phase. The appropriate volume of this solution was injected in the HPLC-system and the chromatogram was studied and recorded.

RESULTS AND DISCUSSION

The protocol adopted for the establishment the HPLC analytical procedure presented in this current work consisted of: choosing optimum HPLC-conditions and suitable mobile phase composition to achieve an excellent resolution of the individual working standards, amlopedine WS and hydrochlorothiazide WS drugs and thereafter the resolution of a 1:1 ratio by weight mixture of the two drugs. The second phase comprises HPLC determinations of ranges of concentration levels of each component drug working standards to establish linearity plots. The third phase involves the derivation and determination of the validation parameters associated with the results obtained in terms of linearity, accuracy, precision, coefficient of variation, reproducibility and specificity of the sample applications. The fourth phase is a preliminary attempt for the application of method in monitoring drug stability and the final phase is the statistical data study for the derivation of a number of validation parameters.

HPLC-Resolution of the Drug Combination:
The HPLC-instrument employed in this work was a Schimatzu (Japan) Model ------equipped with a UV-detector being set at λ 240 nm and an RP-C18 Column. Other HPLC-conditions were presented in the Materials and Methods Section. The first organic solvent composition of the mobile phase was: buffer pH 3.00: methanol: acetonitrile 50: 25: 25 v/v, which has given good resolution but perturbed shapes of the peaks.

HPLC-runs have been performed in which the buffer was kept constant and the composition of the organic solvents varied. An excellent resolution and best peak shapes were reached when the mobile phase composition of (Buffer pH 3.0: Methanol: Acetonitrile 50:35:30v/v) was attempted. This solvent mixture was used to resolve the individual working standards amlopedine WS and hydrochlorothiazide WS drugs at similar concentrations affording a retention time of 3.148 min for amlopedine and 7.323 min for hydrochlorodiazide. Moreover, a 1:1 ratio combination of the two drugs mixture has shown an excellent resolution as shown below.



PeakTable

Detector A Ch1 240nm

Peak#	Name	Ret. Time	Area	Height	Area %
1	Losartan Potassium	12.686	3096968	160572	100.000
Total			3096968	160572	100.000

<System Suitability Parameters>

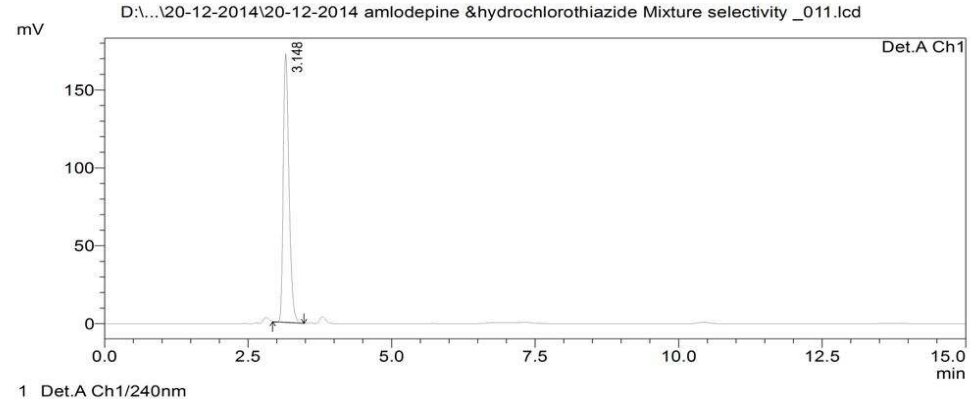
PeakTable

Detector A Ch1 240nm

Peak#	Name	Theoretical Plate#	k'	Tailing Factor	Resolution
1	Losartan Potassium	9805.521	24.371	1.041	0.000
Total					

Figure 3.a

<Chromatogram>



PeakTable

Detector A Ch1 240nm

Name	Ret. Time	Area	Height	Area %	Height %
Hydrochlorothiazi	3.148	1190998	172555	100.000	100.000
		1190998	172555	100.000	100.000

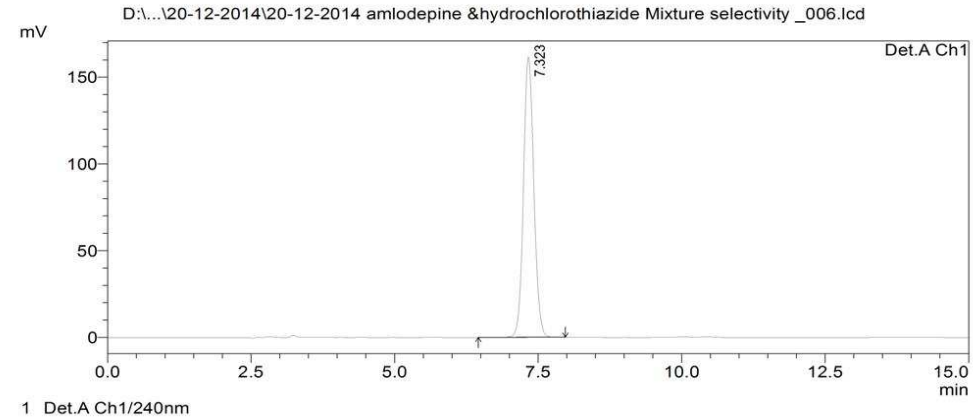
System suitability

Detector A Ch1 240nm

Peak#	Theoretical Plate#	k'	Tailing Factor	Resolution
1	4349.941	5.296	1.395	0.000
Total				

Figure 3.b

<Chromatogram>



PeakTable

Detector A Ch1 240nm

Name	Ret. Time	Area	Height	Area %	Height %
Amlodepine	7.323	2032203	161754	100.000	100.000
		2032203	161754	100.000	100.000

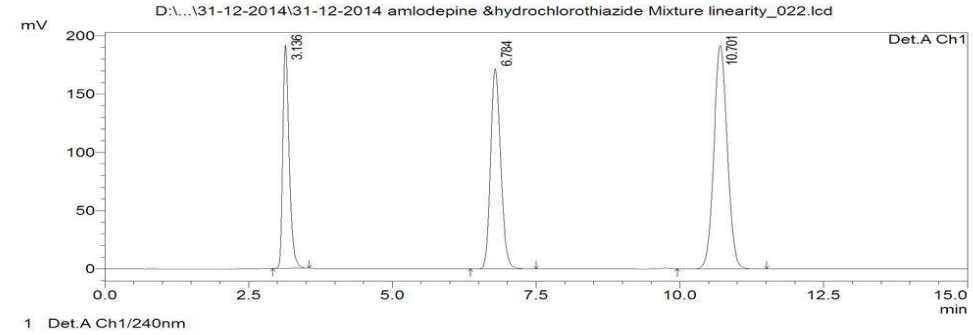
System suitability

Detector A Ch1 240nm

Peak#	Theoretical Plate#	k'	Tailing Factor	Resolution
1	7617.988	13.646	1.115	0.000
Total				

Figure 3.c

<Chromatogram>



PeakTable

Detector A Ch1 240nm

Name	Ret. Time	Area	Height	Area %	Height %
Hydrochlorothiazi	3.136	1450360	191770	21.885	34.550
Amlodepine	6.784	2070218	171536	31.238	30.904
Losartan K	10.701	3106615	191750	46.877	34.546
		6627193	555056	100.000	100.000

System suitability

Detector A Ch1 240nm

Peak#	Theoretical Plate#	k'	Tailing Factor	Resolution
1	3772.839	0.000	1.498	0.000
2	7097.720	1.163	1.219	13.863
3	9820.684	2.413	1.072	10.390
Total				

Figure 3.d

It was observed that optimizing acetonitrile composition in the mobile phase was a determining factor in improving the resolution, maintaining good peak shape and minimizing the HPLC-run time. Accordingly, the following optimum mobile phase ratio: buffer pH 3.00: methanol: acetonitrile 50: 35:30 v/v, was reached after conducting a number of HPLC-trials involving varying volumes of acetonitriles versus fixed volumes of methanol and buffer. The components of the combination drug have been resolved without any interferences, Figure 1.accordingly, the fore-mentioned composition of the mobile phase has been used throughout the work at a flow rate of 1.00 ml/min.

Determination of the Linearity Parameter:

The linearity parameter was determined by injecting a series of nine concentration levels within the range 2.0 - 48 µg/ml and 10 - 120 µg/ml, for each amlopedine WS and hydrochlorothiazide WS, respectively. The response of each of the two drugs was found to be linear within its investigation concentration range and the linear regression equation was y = 33.17648349x - 0.000105974 with a correlation coefficient 0.9997 for amlopedine and y = 8.533145578x + 0.028010921 with a correlation coefficient of 0.9991 for hydrchlorothiazide. The results obtained for both drugs have shown an excellent coefficient of variation and reproducibility, which was evident from the low relative standard deviation RSD ranging from 0.41 to 0.01 for amlopedine and 1.4 to 0.01 for hydrochlorodiazide see Table 1 and Table 2, below.

Table (1)

	Level 01	**Level 02**	**Level 03**	**Level 04**	**Level 05**	**Level 06**	**Level 07**	**Level 08**	**Level 09**
	0.004	0.008	0.012	0.016	0.02	0.024	0.03	0.04	0.048
1st	0.1348	0.2681	0.3996		0.6658	0.7838	0.9771	1.3318	
2nd	0.1350	0.2676	0.3997	0.5332	0.6664	0.7913	0.9764	1.3424	1.5900
3rd	0.1350	0.2677	0.3994	0.5330	0.6662	0.7907	0.9761	1.3416	1.5953
4th	0.1349	0.2675	0.3987	0.5331	0.6661	0.7914	0.9760	1.3410	1.5952
5th	0.1350	0.2675	0.3990	0.5331	0.6662	0.7903	0.9762	1.3421	1.5953
Average	0.1349	0.2677	0.3993	0.5331	0.6661	0.7895	0.9764	1.3398	1.5939
RSD%	0.06	0.09	0.10	0.01	0.03	0.41	0.04	0.33	0.17

Table (2)

	Level 01	**Level 02**	**Level 03**	**Level 04**	**Level 05**	**Level 06**	**Level 07**	**Level 08**	**Level 09**
Conc	0.01	0.02	0.03	0.04	0.05	0.06	0.075	0.1	0.12
1st	0.0967	0.1896	0.2865		0.4666	0.5433	0.6691	0.8867	
2nd	0.0998	0.1893	0.2868	0.3776	0.4669	0.5500	0.6725	0.8788	1.0396
3rd	0.0998	0.1894	0.2865	0.3776	0.4666	0.5512	0.6722	0.8792	1.0399
4th	0.0998	0.189986	0.2862	0.3776	0.4668	0.5508	0.6720	0.8799	1.0399
5th	0.0999	0.1912	0.2863	0.3777	0.4667	0.5506	0.6717	0.8797	1.0399
Average	0.0992	0.1899	0.2865	0.3776	0.4667	0.5492	0.6715	0.8809	1.0398
RSD%	1.40	0.41	0.08	0.01	0.03	0.60	0.20	0.38	0.01

A linearity plot of concentration versus intensity (area under the peak) was established for each of the working standards, Figure (4) and Figure (5), respectively.

Determination of Precision and Accuracy Parameters :

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing the same procedure on the different day (intraday) by another analyst under the same experimental conditions. The intermediate precision, which is less than 2.0%, is an evidence for the excellent repeatability of the results indicating that the method is of high precision. It is noteworthy, to mention that the repeatability parameter could be determined from the precision and accuracy, since all three parameters are inter-related.

Figure (4)

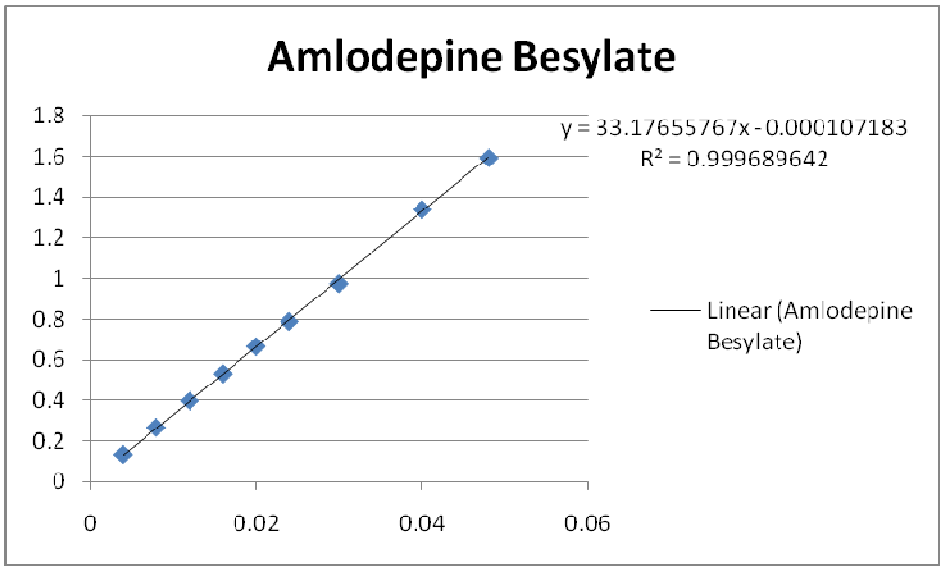


Figure (5)

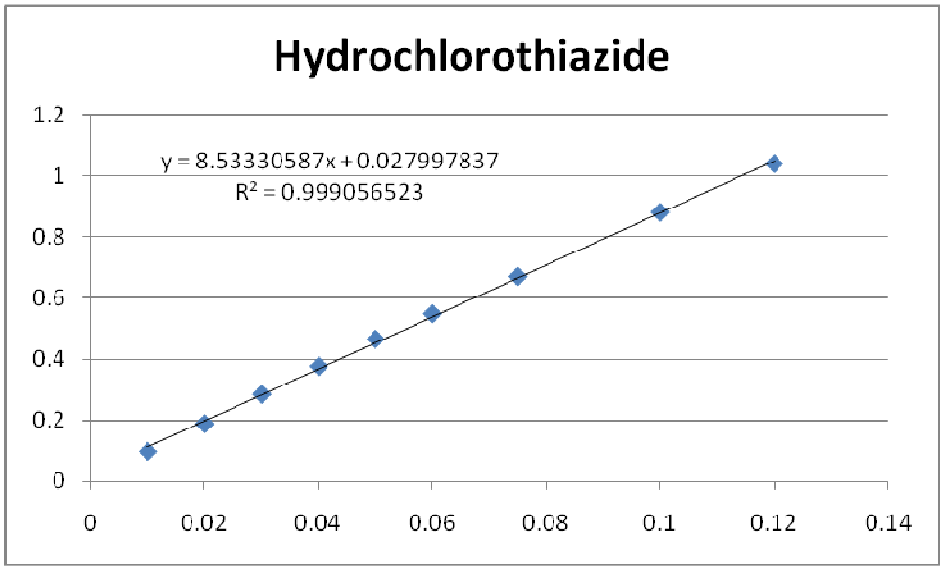


Table (3)

Precision 1									
Amlodepine					**Hydrochlorothiazide**				
P		**WC**	**average**	**Cliam**	**P**		**WC**	**average**	**Cliam**
99.16		0.17	140	5	100.32		0.01	140	12.5
M.W. Amlodepine Besylate			567.05						
M.W. Amlodepine			408.88						
	STD1	**STD2**	**Test1**	**test2**		**STD1**	**STD2**	**Test1**	**test2**
wieght	20.5	20.1	140	140	wieght	50.6	49.9	140	140
Inj#01		0.65751	0.91862		Inj#01		0.48587	0.48753	0.50184
Inj#02	0.6683	0.66795	0.92156	0.94017	Inj#02	0.48886	0.4873	0.48525	0.49154
Inj#03	0.66724				Inj#03	0.48969			
Inj#04	0.66736				Inj#04	0.48964			
Inj#05	0.66783				Inj#05	0.4903			
Average	0.66768	0.66273	0.92009	0.94017	average	0.48962	0.48659	0.48639	0.49669
RSD	0.06	0.01	0.06	0.12	RSD	0.10	0.15	0.23	1.04
Agree	100.46	**assay**	100.82	103.02	Agree	101.13	**assay**	100.84	102.98
		average	101.92				**average**	101.91	
		RSD	1.53				**RSD**	1.48	

Table (4)

Precision 2									
Amlodepine					**Hydrochlorothiazide**				
P		**WC**	**average**	**Cliam**	**P**		**WC**	**average**	**Cliam**
99.16		0.17	140	5	100.32		0.01	140	12.5
M.W. Amlodepine Besylate			567.05						
M.W. Amlodepine			408.88						
	STD1	**STD2**	**Test1**	**test2**		**STD1**	**STD2**	**Test1**	**test2**
wieght	19.5	20.1	140	140	wieght	50.1	50.2	140	140
Inj#01	0.65492	0.67627	0.96316	0.97221	Inj#01	0.46927	0.48019	0.46864	0.47096
Inj#02	0.65557	0.67802	0.99049	0.97266	Inj#02	0.47177	0.4799	0.46974	0.47296
Inj#03	0.65539				Inj#03	0.46956			
Inj#04	0.6552				Inj#04	0.46897			
Inj#05	0.65564				Inj#05	0.47164			
average	0.65534	0.67715	0.97683	0.97244	average	0.47024	0.48005	0.46919	0.47196
RSD	0.04	0.01	0.06	0.12	RSD	0.26	0.03	0.12	0.21
Agree	100.46	**Assay**	103.73	103.27	Agree	101.13	**Assay**	100.28	100.88
		Average	103.50				**Average**	100.58	
		RSD	0.32				**RSD**	0.42	

The accuracy of the method was determined by recovery of spiked pre-analyzed sample formulation of the drug in triplicate sets of concentration levels: 50%, 100%, and 150%. The robustness of procedure was investigated to evaluate the influence of small but deliberate variations in the chromatographic conditions, such as changes in the flow rate [-+0.1ml\min], a change in the wavelength [+/- 2.0 nm].

Table (5)

Accuracy of amlodepine								
	STD 1		**50% T 1**		**50% T 2**		**50% T 3**	
	Amlodepine	**Losartan K**	**Amlodepine**	**Losartan K**	**Amlodepine**	**Losartan K**	**Amlodepine**	**Losartan K**
Wt	19.8		2.3		2.5		2.2	
1st	2061540	3065129	980950	3078237	1042010	3060207	926166	3051225
2nd	2064023	3063682	982003	3077399	1041179	3059767	930910	3052224
3rd	2063564	3064864	978807	3067637	1040723	3059608	932925	3051681
4th	2063644	3067700	982370	3074346	1040808	3059280	931944	3050781
5th	2065594	3066837	983494	3075597	1040597	3057624	931864	3051096
Avg	2063673	3065642	981525	3074643	1041063	3059297	930762	3051401
RSD	0.07	0.05	0.18	0.14	0.05	0.03	0.29	0.02
%			102.06		100.09		101.95	
				AVG	101.37			
				RSD	1.09			
	STD 1		**100% T 1**		**100% T 2**		**100% T 3**	
	Amlodepine	**Losartan K**	**Amlodepine**	**Losartan K**	**Amlodepine**	**Losartan K**	**Amlodepine**	**Losartan K**
Wt	19.8		5		5.1		4.9	
1st	2061540	3065129	2073868	3092425	2159100	3096413	2014748	3004464
2nd	2064023	3063682	2106504	3090800	2130919	3101115	2001212	3003982
3rd	2063564	3064864	2106302	3091380	2140940	3097792	2000834	3001155
4th	2063644	3067700	2105124	3100426	2137518	3091735	2000845	2997495
5th	2065594	3066837	2106044	3100622	2134508	3093326	2000914	2996636
Avg	2063673	3065642	2099568	3095131	2140597	3096076	2003711	3000746
RSD	0.07	0.05	0.68	0.16	0.51	0.12	0.31	0.12
%			99.76		99.69		100.21	
				AVG	99.89			
				RSD	0.28			

Table (6)

			150% T 1		**150% T 2**		**150% T 3**	
	STD 1	**Losartan K**	**Amlodepine**	**Losartan K**	**Amlodepine**	**Losartan K**	**Amlodepine**	**Losartan K**
Wt	19.8		7.2		7		7.2	
1st	2061540	3065129	3022997	3095824	2965731	3136091	3020893	3068958
2nd	2064023	3063682	3036512	3097204	2956785	3135018	3039833	3077081
3rd	2063564	3064864	3035413	3096001	2957230	3136354	3040185	3074525
4th	2063644	3067700	3035179	3095582	2959342	3136864	3038576	3075355
5th	2065594	3066837	3035497	3095127	2960010	3135376	3037305	3075978
Avg	2063673	3065642	3033120	3095948	2959820	3135941	3035358	3074379
RSD	0.07	0.05	0.19	0.03	0.12	0.02	0.27	0.1
%			100.06		99.15		100.83	
		AVG	100.01		**Average Over All**		100.42	
		RSD	0.84		**RSD% Over All**		0.82	

Table (7)

Accuracy of Hydrochlorothiazide								
	STD 1		**50% T 1**		**50% T 2**		**50% T 3**	
	49.5		6.3		6.3		6.5	
Wt	1435598	3065129	756997	3078237	753108	3060207	778138	3051225
1st	1438372	3063682	756577	3077399	753203	3059767	772498	3052224
2nd	1439616	3064864	754269	3067637	753369	3059608	778505	3051681
3rd	1435322	3067700	757074	3074346	751357	3059280	778369	3050781
4th	1442305	3066837	749353	3075597	755227	3057624	775326	3051096
5th	1438243	3065642	754854	3074643	753253	3059297	776567	3051401
Avg	0.2	0.05	0.43	0.14	0.18	0.03	0.34	0.02
RSD			102.79		103.09		103.28	
%				**AVG**	103.05			
				RSD	0.24			
	STD 1		**100% T 1**		**100% T 2**		**100% T 3**	
	HCT	**Losartan K**	**HCT**	**Losartan K**	**HCT**	**Losartan K**	**HCT**	**Losartan K**
Wt	49.5		12.5		12.4		12.6	
1st	1435598	3065129	1473715	3092425	1444606	3096413	1465986	3004464
2nd	1438372	3063682	1473752	3090800	1440928	3101115	1464219	3003982
3rd	1439616	3064864	1473905	3091380	1445845	3097792	1464529	3001155
4th	1435322	3067700	1473666	3100426	1440086	3091735	1457460	2997495
5th	1442305	3066837	1474147	3100622	1439750	3093326	1463861	2996636
Avg	1438243	3065642	1473837	3095131	1442243	3096076	1463211	3000746
RSD	0.2	0.05	0.01	0.16	0.19	0.12	0.23	0.12
%			100.48		99.09		102.08	
				AVG	100.55			
				RSD	1.49			

Table (8)

	STD 1		**150% T 1**		**150% T 2**		**150% T 3**	
	HCT	**Losartan K**	**HCT**	**Losartan K**	**HCT**	**Losartan K**	**HCT**	**Losartan K**
Wt	49.5		18.6		18.4		18.4	
1st	1435598	3065129	2169680	3095824	2167532	3136091	2140738	3068958
2nd	1438372	3063682	2175226	3097204	2171145	3135018	2146318	3077081
3rd	1439616	3064864	2179479	3096001	2166854	3136354	2145868	3074525
4th	1435322	3067700	2179137	3095582	2167421	3136864	2141268	3075355
5th	1442305	3066837	2175064	3095127	2176486	3135376	2145699	3075978
Avg	1438243	3065642	2175717	3095948	2169888	3135941	2143978	3074379
RSD	0.2	0.05	0.18	0.03	0.19	0.02	0.13	0.1
%			99.66		99.19		99.97	
		AVG	99.61		**AVG over all**		101.07	
		RSD	0.39		**RSD over all**		1.76	

Specificity of the Method

It is noteworthy to mention that preliminary tests were performed whereby the specificity of the method was firstly determined against placebo. It was the found that there were no interferences between the drug and the excepients of the claimed placebo. Secondly the specificity of the method toward the drug was approved via the non-existence of interferences between the peaks of the drug and the degradation products resulting from exposure to forced stress conditions of acidic, alkaline, photolytic and oxidative conditions. In this context, it is important to mention that 24% and 8% of the drug was degraded during oxidative and alkaline stress conditions; while only traces of peaks were observed during exposure of the drug to photolytic and acidic conditions. In conclusion, no interferences were observed between the peaks of the drug and those of the degradation products.

CONCLUSION

A new analytical method has been developed to be routinely applied to simultaneous determination of amlodipine besylate and hydrochlorothiazide in pharmaceutical dosage form. In this study, stability of amlodipine besylate, hydrochlorothiazide in present dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear,

precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also for stability sample analysis.

Acknowledgement
The authors are thankful to Azal medical company, for supplying the gift samples, and for providing the necessary facilities to carry out the research work.

REFERENCES

[1] S. Budavari. The Merck Index, An Encyclopedia of chemical, Drugs, and Biologicals, 14thedn, Merck Research Lab, Division of Merck & Co, Inc, USA, **2006**, 83
[2] B. Wankhede, M. R. Tajane, K. R. Gupta, S. G. Wadodkar, "*Indian Journal of Pharmaceutical Sciences*, **2007**, 298-300.
[3] Erk, *Journal of Pharmaceutical and Biomedical Analysis*, **2001**, 24 (4), 603-611.
[4] S. M. Al-Ghannam, *Journal of Pharmaceutical and Bio-medical Analysis*, **2006**, 40(1), 151-156.
[5] M. del R. Brunetto, Y. Contreras, S. Clavijo, D. Torres, Y. Delgado, F. Ovalles, C. Ayala, M. Gallignani, J. M. Estela, V. C. Martin, *Journal of Pharma- ceutical and Biomedical Analysis*, **2009**, 50 (2) , 194-199.
[6] V.G. Dongre,S. B. Shah, P. P. Karmuse, M. Phadke and V. K. Jadhav, *Journal of Pharmaceutical and Bio-medical Analysis*, **2008**, 46 (3) , 583-586.