Stability Indicating Assay Method for Amlodipine Tablets

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A novel stability indicating HPLC has been developed for estimation of amlodipine in tablet formulation. The method was validated using specificity, stability in analytical solution, precision, accuracy and system suitability as parameters. The mobile phase consists of 0.05 M ortho-phosphoric acid buffer, methanol and acetonitrile in the ratio of 50:35:15 and the results show that the method is reproducible and accurate. Degradation of amlodipine was performed in various conditions and the resulting solution was analyzed on HPLC using ODS column (150×4.6 mm) with a detection maxima of 361 nm. The method gave a good separation between drug and degradation peaks. Recovery studies gave results between 99.7 to 100.7 % for 5 mg tablets.

Stability indicating methods have become an important aspect of any analytical method validation and a part of USFDA requirements[1]. Amlodipine is a dihydropyridine calcium channel blocker. It is a peripheral arteriolar vasodilator[2]. A number of estimation methods have been published for the commercial formulations containing amlodipine alone and in combination with other drugs. Literature survey turned up a number of estimations procedures of various combination formulation of amlodipine by both spectroscopy and chromatography. Spectroscopic determinations include simultaneous estimation of amlodipine with losartan potassium[3], benazepril[4], lisinopril[5,6], aspartame[7] and enalapril maleate[8]. Chromatographic determinations of amlodipine combinations include benazepril by RPLC[9,10], losartan potassium[11], ramipril by RPLC[12] and atenolol by HPLC[13]. A spectroscopic method for amlodipine besylate raw material and tablets has been reported which describes formation of a colored compound which is analyzed on UV spectrophotometer[14]. An HPLC method has been reported to test Amlodipine in residues on equipment surfaces[15]. A spectroscopic method for determination of amlodipine besylate in drug formulation using substituted benzoquinone and ascorbic acid has been reported[16]. Eight other spectroscopic and chromatographic methods have been reported for analysis of amlodipine in pharmaceutical preparations[17-24]. Estimation of amlodipine in formulations by differential pulse voltametry with a glassy carbon electrode has been reported by Altiokka *et al.*[25]. A flow injection analysis of Amlodipine in colorless solution using UV detection has also been reported[26].

A derivative spectrophotometric method for the simultaneous estimation of amlodipine and its pyridine photo degradation product has also been reported. This is a stability indicating method recommended for quality procedures[27]. Stability of amlodipine besylate in two liquid dosage forms has been also studied and reported. The liquid dosage forms have been designed for the study as none were available at the time of the study[28]. It was decided to develop an estimation method for amlodipine in presence of its degradation products would add value to the well researched field of the drug. To develop such a method it would be necessary to degrade the drug in various conditions and that would enable the procedure to be used for the estimation of amlodipine in long term and accelerated studies where there is a likelihood of formation of amlodipine degradation products.

Although stress conditions under which a drug is force degraded are fairly consistent; the time of exposure and extent of stress widely vary from drug to drug. For development of stability indicating assays the general guideline is to expose drug to a stress condition to produce sufficient degradation. In some cases the degradation can be total.

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The method should be able to estimate the remaining drug without the interference of the known or unknown degraded products[29].

All solvents were of HPLC grade filtered through 0.45 μ membrane filter. Amlodipine working standard and tablets (Amtax, 5 mg/tablet) were supplied by Intas Pharmaceuticals, Ahmedabad. HPLC was performed on a Waters Alliance system with quaternary gradient and 996 PDA detector and Waters LCMI plus system with quaternary gradient and UV detector.

Orthophosphoric acid solution (0.05 M) was prepared and its pH adjusted to 3.0 using triethylamine. The mobile phase used buffer, methanol and acetonitrile in the ratio of 50:35:15. Amlodipine besylate (69.4 mg) was accurately weighed and dissolved in 50 ml of mobile phase. An aliquot (5 ml) of this solution diluted to 50 ml with the mobile phase. Finely powdered tablet sample was accurately weighed and powder equivalent to 20 mg of amlodipine was dissolved in 200 ml of mobile phase. The solution was filtered through Whatman filter paper No. 42. Prior to injection, this solution was filtered through HPLC syringe filter. The following HPLC parameters were used: ODS (150x4.6 mm) 10microm column, 40° temperature, buffer:methanol:acetonitrile (50:35:15) as mobile phase, 1.5 ml/min as flow rate and 361 nm as wavelength. The column was saturated with mobile phase for 45 min to 1 h prior to the analysis. An aliquot of 20 μl of standard and sample preparations in duplicate were injected into the liquid chromatograph and the chromatograms were recorded. Assay of amlodipine was calculated. The method was validated as per specificity, linearity, system suitability, precision and accuracy parameters.

For specificity, placebo solution and sample solution were analyzed and no peak was observed at the retention time of amlodipine in the placebo chromatogram. Peak purity data indicated that the peak is homogenous and has no co-eluting peaks. Stability of solution was performed by storing amlodipine working standard solution at room temperature for 12 h and analyzing the sample on HPLC every two hours for that period of storage. The stability table depicts the results of analysis and proves the solution to be stable for at least 12 h. Hence an extended period of analysis due to unavoidable circumstances would not interfere with results of the assay.

Linearity was determined by preparing seven different concentrations (25, 50, 75, 100, 125,150 and 175 μg/ml) of amlodipine working standard and analyzing them on HPLC. Correlation coefficient was calculated. System suitability was performed by injecting six replicate injections of amlodipine working standard solution. Relative standard deviation was calculated for the area obtained. For accuracy; the placebo tablets were powdered and three samples were weighed. The samples were spiked with different quantities of amlodipine. The samples were analyzed and recovery of the drug in percentage was calculated. Precision was performed by analyzing six different samples of amlodipine tablets on HPLC and determining their assay. The relative standard deviation was calculated for the assay obtained. Amlodipine tablets were degraded under acidic, basic, oxidative, humidity with heat, heat and sunlight conditions. The degraded sample was prepared as per the sample preparation and analyzed on HPLC using the above mentioned parameters.

Amlodipine showed good degradation in strongly acidic, basic and oxidizing conditions. It showed good stability under heat, humid and sunlight conditions. Degradation peaks were well separated. The recovery with the analytical method was obtained between 99.7 to 100.7%. The RSD of precision was 0.59. The RSD of area obtained over a period of 12 h for stability of analytical solution was 0.5 and that of system suitability 0.68. Results of recovery and precision are presented in Tables 1 and 2. Results of degradation study are presented in Table 3.

The validation data shows the method to be specific, reproducible and accurate. Peak purity studies indicate that there is no other co-eluting peak with the main peak and the method is stability indicating. The method also gives a good separation between the multiple peaks. Though no attempt was made to identify the degradation products the method can be successfully used to determine if degradation has occurred during storage of the drug. Simultaneous estimation of Amlodipine with the known oxidation product is already available and this method has covered other degradation agents. It is simple, quick and an effective stability indicating assay.

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TABLE 1: ACCURACY - AMLODIPINE TABLETS

Sample No.	Amlodipine besylate (mg.)	Theoretical amount (Amt.) (mg.)	Area added	Amount of drug recovered (mg)	Amount	Recovery %
Working Standard	-	-	1278546	-	-	-
1	70.74	50.98	743475	10.74	10.82	100.7
2	140.1	101.0	1465530	21.39	21.33	99.72
3	209.0	150.7	2207984	32.13	32.13	100.0

Aml. stands for amlodipine base. Theoretical amount of base was added to approximately 3000 mg of placebo powder to achieve 50%, 100%, 150% concentration of the drug.

TABLE 2: PRECISION-AMLODIPINE TABLETS

Sample No.	Sample Weight (mg.)	AREA		Mean	Assay (mg/tab)
Working standard	69.38	1411962	1412296	1412129	-
1	669.9	1 472 932	1 471 831	1 472 382	5.06
2	662.7	1 460 884	1 456 210	1 458 547	5.07
3	652.4	1 437 284	1 434 696	1 435 990	5.07
4	675.6	1 469 752	1 467 910	1 468 831	5.01
5	670.1	1 456 777	1 451 864	1 454 321	5
6	647.7	1 431 276	1 428 577	1 429 927	5.08
				Mean	5.05
				SD	0.03
				RSD	0.59

SD is the standard deviation; RSD is relative standard deviation; mg/tab denotes milligrams per tablet and mg denotes milligrams.

TABLE 3: AMLODIPINE FORCED DEGRADATION STUDY TABLE

Agent	Exposure Time	Condition	Physical observation	Assay %	Degradation by area normalization
None	Nil	Normal	Pink Solution		
None	Nil	Normal	Pink Solution	101.6	Nil
1N HCl*	30 min	Heat	Pink Solut ion	65.39	1.3
1N NaOH*	30 min	Heat	Pink Solution	74.14	6.3
3% H_2O_2*	30 min	Heat	Pink Solution	65.59	Nil
105°	3 d	Controlled oven	Pink Solution	100.9	Nil
75% RH	3 d	Controlled oven	Pink Solution	102.0	Nil
Sunlight	3 d	Natural	Clear Solution	101.8	Nil

*With Heat

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Antibacterial Activity of *Punica granatum* in Different Solvents

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In this study, the antibacterial activity of leaf of *Punica granatum* was investigated. Different solvents used were, water, ethanol, methanol, acetone, propanol, 1,4-dioxan, N,N-dimethylformamide (DMF) and benzyl alcohol. The selection of solvents was on the basis of their polarity. The antibacterial activity of six clinical strains (*S. paratyphi, S. aureus, S. epidermidis, E. aerogenes P. aeruginosa and B. subtilis*) was determined by Growth inhibition using Agar ditch diffusion assay. The aqueous extract was able to inhibit only *B. subtilis* and *S. aureus* and was ineffective against all the other four bacterial strains. On the other hand organic solvents proved much better in inhibiting the studied bacterial strains except benzyl alcohol extract which was ineffective against

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