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Development and Validation of UV- Spectrophotometric Method for Simultaneous Estimation of Amlodipine Besylate and Hydrochlorothiazide in Combined Dosage Form Including Stability Study

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ABSTRACT:

Two simple, rapid, precise and accurate spectrophotometric methods have been developed for determination of Amlodipine Besylate (AMB) and Hydrochlorothiazide (HCTZ) by simultaneous equation method and stability study method in combined tablet dosage form. The simultaneous equation method is based on measurement of absorbance at 238 nm and 271 nm as two wavelengths selected for quantification of Amlodipine Besylate and Hydrochlorothiazide. The method obeyed Beer's law in the concentration range of 5-30 µg/ml for Amlodipine and 2.5-15 µg/ml for Hydrochlorothiazide. Amlodipine and Hydrochlorothiazide (Marketed Formulation) were subjected to stress degradation under different conditions recommended by ICH. The proposed methods were validated and can be applied successfully for routine quality control analysis of AMB and HCTZ in bulk and pharmaceutical formulation.

KEYWORDS: Simultaneous Equation Method, λmax, Validation, Amlodipine Besylate, Hydrochlorothiazide, Stability Study, Stress Degradation.

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INTRODUCTION:

Amlodipine Besylate, 3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzenesulfonate, is a antihypertensive and antianginal drug.[1,2] Amlodipine Besylate belongs to a class of medications called calcium channel blockers (CCBs).[3]Amlodipine Besylate having molecular formula C20H25ClN2O5 and molecular weight 408.879 g/mol. It is official in IP, BP, EP and USP[4,5](Fig. No. 1).

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-Sulphonamide 1, 1- dioxide, is a diuretic, which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co-transporter, resulting in an increase in the excretion of sodium, chloride, and water.[6,7] It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism.[8,9] Hydrochlorothiazide having molecular formula C7H8ClN3O4S2 and molecular weight 297.739 g/mol. It is official in IP, BP and USP[10] (Fig. No. 2).

The literature review reveals that few analytical techniques like RP-HPLC, HPTLC, HPLC, Spectroscopic methods have been reported for the determination of Amlodipine Besylate and its combination with other drugs in pharmaceutical dosage forms.[11-13] Estimation of

Hydrochlorothiazide in combination with other drugs by spectrophotometric methods, LC-MS, HPLC and stability study has been reported Hydrochlorothiazide in combination with other drugs by spectrophotometric methods, LC-MS, HPLC and stability study has been reported.[14,15] But there are no methods reported in any pharmacopoeia for simultaneous estimation of Amlodipine Besylate and Hydrochlorothiazidein combined dosage form including stability study using 0.1 N NaOH as a solvent.

MATERIALS AND METHODS

Instruments:

(a) Spectrophotometer -Double beam UV –Visible spectrophotometer with 1 cm matched quartz cell Model UV-1601 Shimadzu.
(b) Hot Air Oven- Dolphin.
(c) Electronic Balance-Shimadzu.

Reagents and Chemicals:

Amlodipine Besylate and Hydrochlorothiazide reference standard were kindly provided by Yarrow Chem. Products Mumbai. 0.1 N Sodium Hydroxide of analytical grade was used as a solvent throughout the analysis. All chemicals and reagents used were of analytical reagent grade.

Marketed Preparation:

The brand name of marketed combined tablet formulation is Amlong-H containing Amlodipine Besylate 5.0 mg and Hydrochlorothiazide 12.5 mg manufactured by Micro Labs Ltd. South Sikkim.

Preparation of standard stock solution:

(a) Amlodipine Besylate*:-* Standard stock solution of Amlodipine besylate was prepared by dissolving 10 mg Amlodipine besylate in 50 ml of 0.1N sodium hydroxide and sonicated for 20 min and then diluted upto 100 ml to produce a concentration of 100 µg/ml which was standard stock solution.

(b) Hydrochlorothiazide*:* -Standard stock solution of Hydrochlorothiazide was prepared by dissolving 10 mg of Hydrochlorothiazide in 100 ml of 0.1N sodium hydroxide to produce a concentration of 100 µg/ml which was standard stock solution.

Determination of λ max of Amlodipine Besylate and Hydrochlorothiazide:

The aliquot portion of stock standard solutions of AMB and HCTZ were diluted appropriately with 0.1 N sodium hydroxide to obtain concentration 5 µg/ml and 12.5 µg/ml respectively. The solutions were scanned in the range of 200-400 nm in 1 cm cell against blank. The λmax was determined on double beam UV – Visible Spectrophotometer using 0.1 N sodium hydroxide as blank. The λmax was found to be 238 nm and 271 nm respectively. The Overlain UV absorbance spectrum of AMB and HCTZ is shown in **Fig.No. 3**.

Preparation of Calibration Curve or study of Beer-Lambert's Law:

The aliquot portion of stock standard solutions of AMB and HCTZ were diluted appropriately with 0.1 N sodium hydroxide as solvent to get a series of concentration between 5- 30 µg/ml of AMB and 2.5 – 15 µg/ml of HCTZ. The absorbance of each solution was measured at 238 nm and 271 nm in 1 cm cell against 0.1 N sodium hydroxide as blank. The graphs plotted as concentration Vs absorbance at selected wavelengths for AMB and HCTZ are shown in **Fig. No. from 4 and 5**.

Determination of A (1%, 1 cm) of drugs at selected wavelengths:

Aliquot portions of AMB from stock solution were transferred to five 10 ml volumetric flasks; volume was adjusted to mark to obtain the concentration of 5 µg/ml. Similarly, aliquot portions from HCTZ stock solution were transferred to 10 ml volumetric flasks; volume was adjusted to mark to obtain concentration of 12.5 µg/ml. Absorbance of these solutions were recorded at two wavelengths 238 nm and 271 nm. A (1%, 1 cm) values of drugs were calculated using following formula-

A (1%, 1 cm) = Absorbance / Concentration (g / 100 ml)

Results of A (1 %, 1 cm) of drugs are given in **Table No. 1.**

$$Cx = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2 \quad \text{---------- (I)}$$

$$Cy = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2 \quad \text{---------- (II)}$$

Where,

A_1 and A_2 are the absorbance of the sample solution measured at 238 nm and 271 nm respectively.

Cx and Cy are concentration of AMB and HCTZ respectively.

ax_1 and ax_2 are absorptivity of AMB at 238 nm and 271 nm respectively.

ay_1 and ay_2 are absorptivity of HCTZ at 238 nm and 271 nm respectively.

Simultaneous Estimation of drugs in standard laboratory mixture:

In order to study the practicability of proposed method for simultaneous estimation of AMB and HCTZ in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture. Accurately weighed 10 mg AMB and 10 mg HCTZ were transferred to 100 ml volumetric flask individually containing 40 ml 0.1 N sodium hydroxide, shake manually for 10 minute and the volume was adjusted to the mark with the same solvent. Appropriate aliquot portion of these solutions were mixed to get the concentration 5 µg/ml AMB and 12.5 µg/ml of HCTZ. Absorbance was measured at 238 nm and 271 nm against 0.1 N sodium hydroxide as blank. Amount of each drug was estimated using (I) and (II) equation and results are given in **Table No. 2.**

Simultaneous Estimation of drugs in tablets:

Twenty tablets each containing 5 mg of AMB and 12.5 mg of HCTZ were weighed and average weight was calculated. The tablets were crushed to fine powder. The powder equivalent to 5 mg of AMB and 12.5 mg of HCTZ was transferred to 100 ml volumetric flask containing 70 ml of 0.1 N sodium hydroxide by intermittent shaking followed by sonication for 10 min and then the volume was made upto 100 ml with 0.1 N sodium hydroxide. The solution was diluted further with 0.1 N sodium hydroxide to obtain 5 µg/ml of AMB and 12.5 µg/ml of HCTZ. The solution was filtered through a Whatman filter paper (No. 41). The absorbances were recorded. The concentrations of two drugs in sample were determined using equation No. (I) and (II), results are given in **Table No. 3**. Brand Name: Amlong-H (Composition- Amlodipine Besylate: 5.0 mg, Hydrochlorothiazide: 12.5 mg), Average Weight: 250 mg.

Recovery Study:

In recovery study to the preanalysed sample solutions (5 µg/ml AMB and 12.5 µg/ml of HCTZ) a known amount of standard solutions of pure drugs (AMB and HCTZ) was added at different level. The % recovery was calculated by using formula,

$$\% \text{ Recovery} = (A / B + C) \times 100$$

Where,

A = Total amount of drug estimated,

B = Amount of drug found on preanalysed basis and

C = Amount of pure drug added. Results are shown in **Table No. 4**.

Validation of Proposed Method:

Validation of method was done as per ICH guidelines. Method was validated for various parameters such as accuracy, precision, linearity, repeatability and ruggedness.

Accuracy: Accuracy of the proposed method was ascertained on the basis of recovery studies performed by the standard addition method. The results of recovery studies are shown in **Table No. 4**.

Precision: Precision was determined by intra-day and inter-day precision. Intra-day precision was determined by analyzing the 2.5, 5, 10 µg/ml of AMB and 10, 12.5, 15 µg/ml of HCTZ drug solution for three times in the same day. Inter-day precision was determined by analyzing the same concentration at three different days. The results are given in **Table No.5**.

Linearity: The study of linearity and range was performed as per ICH guidelines. AMB and HCTZ was found to be linear at a concentration range of 5-30 µg/ml and 2.5-15 µg/ml respectively with R^2 = 0.99 at selected wavelength for both the methods.

Repeatability: Repeatability was determined by analyzing AMB (5 µg/ml) and HCTZ (12.5 µg/ml) of drug solutions for five times and results are given in **Table No. 6**.

Ruggedness: Analysis of aliquots from homogenous slot by two analyst using same operational and environment conditions was performed for determination of Ruggedness of proposed method. The results are given in **Table No. 7**.

Method for Performing Stability Study:

Stress or Forced Degradation Studies: The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The present work was performed on the stress degradation studies of Amlodipine and Hydrochlorothiazide (Tablet).

Preparation of stock solution (tablet): An accurately weighed tablet powder equivalent to 5 mg of AMB and 12.5 mg of HCTZ was transferred to 100 ml volumetric flask containing about 40 ml 0.1 N sodium hydroxide, sonicated for 10 min and volume was made upto the mark with the same solvent and further diluted to obtain 5 µg/ml of AMB and 12.5 µg/ml of HCTZ and filtered through Whatman filter paper (No. 41).

Stress degradation by hydrolysis under acidic condition: To 1 ml of stock solution of Amlodipine and Hydrochlorothiazide (tablet), 5 ml of 1N HCl was added in 10 ml of volumetric flask and kept for 3 hours. After 3 hours solution was diluted with 0.1 N sodium hydroxide up to the mark and the solution was taken in cuvette and analysed in UV spectrophotometer at 238 nm and 271 nm of AMB and HCTZ respectively.

Stress degradation by hydrolysis under basic condition: To 1 ml of stock solution of Amlodipine and Hydrochlorothiazide (tablet), 5 ml of 1 N NaOH was added in 10 ml of volumetric flask and kept for 3 hours. After 3 hours solution was diluted with 0.1 N sodium hydroxide up to the mark and the solution was taken in cuvette and analysed in UV spectrophotometer at 238 nm and 271 nm of AMB and HCTZ respectively.

Oxidative degradation: To 1 ml of stock solution of Amlodipine and Hydrochlorothiazide (tablet), 5 ml of 6% Hydrogen Peroxide (H_2O_2) was added in 10 ml of volumetric flask and kept for 3 hours. After 3 hours solution was diluted with 0.1 N sodium hydroxide up to the mark and the solution was taken in cuvette and analysed in UV spectrophotometer at 238 nm and 271 nm of AMB and HCTZ respectively.

Dry heat induced degradation: Amlodipine and Hydrochlorothiazide (tablet powder) sample was taken in a Petri plate and exposed to a temperature of 50°C for 48 hours in an oven. After 48 hours, 10 mg of the sample was diluted with 0.1 N NaOH in order to make the volume upto 100 ml. From this solution, dilutions were carried out and the solution was taken in cuvette and analysed in UV spectrophotometer at 238 nm and 271 nm of AMB and HCTZ respectively.

Determination of % Degradation:-

Record the absorbance of stressed sample then compare it with absorbance of unstressed sample to determine the % degradation.

$$\%\ \text{Degradation} = \frac{(\text{Response of unstressed sample}) - (\text{Response of stressed sample})}{\text{Response of unstressed sample}} \times 100$$

Results of the Stability studies are given in **Table No. 8**.

RESULTS AND DISCUSSION

The wavelength of Amlodipine Besylate and Hydrochlorothiazide were found to be 238 nm and 271 nm respectively in the solution of 0.1 N Sodium Hydroxide. In this method drugs obeyed Beer's law in the concentration range of 5-30 µg/ml of AMB and 2.5-15 µg/ml of HCTZ. The results showed an excellent correlation between absorbance's and concentration of the drugs. Standard calibration curves for AMB and HCTZ were linear with correlation coefficients R^2=0.99 at all the selected wavelengths. The results of analysis of the marketed formulation by Simultaneous Equation Method are shown in **Table No.3**. Method validations were done as per ICH Validation parameters like Accuracy, Precision,Linearity, Repeatability and Ruggedness. The method showed accuracy in the range of 96-100 % . Results of Validation parameters are shown in **Table No.from 4-7**. The stress degradation studies showed that Amlodipine Besylate and Hydrochlorothiazide (Marketed Formulation) i.e. tablet undergoes degradation in acidic and oxidative conditions whereas it is relatively stable when exposed to basic and dry heat conditions. Results of stress degradation studies of AMB and HCTZ are shown in **Table No.8**.

CONCLUSION

A simple, accurate, precise and rapid UV-visible spectrophotometric method was developed for simultaneous estimation of AMB and HCTZ in combined tablet dosage form. The method was validated for AMB and HCTZ in pharmaceutical dosage form. The present study was concluded to understand the degradation behavior of AMB and HCTZ under ICH recommended stress conditions. This study is also an example of the development of a stability indicating assay method for AMB and HCTZ where forced degradation was carried out under all stress conditions. These drugs showed degradation in all of the studied conditions. However the extent of degradation was different. The method developed for quantitative determination of AMB and HCTZ is rapid, precise, accurate and selective. The results

of validation tests were found to be satisfactory and therefore, these methods can be applied successfully for routine quality control analysis of AMB and HCTZ in bulk and pharmaceutical formulation. The developed method can be conveniently used for the assay determination of AMB and HCTZ in bulk drugs and pharmaceutical dosage form.

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TABLES AND FIGURES

Table No. 1: Absorptivity values of AMB and HCTZ at 238 nm and 271 nm

Sr. No.	AMB (238 nm)	AMB (271 nm)	HCTZ (238 nm)	HCTZ (271 nm)
1	149	88.3	60	158
2	149.1	87	63	157.3
3	151	93.8	57.09	158
4	157.5	91	41.3	149.5
5	160.7	91.4	49.71	151
Mean ± SD	153.46 0.475	90.3 0.832	54.22 1.03	154.76 0.884

Table No. 2: Analysis of AMB and HCTZ in Standard Laboratory Mixture

Sr. No.	Drugs	Conc. of std (µg/ml)	Amount found (µg/ml)	% Amount found	% RSD (n=5)
1	AMB	5	4.905	98.1	0.68
2	HCTZ	12.5	12.41	99.28	0.55

Table No. 3: Analysis of AMB and HCTZ in Tablet

Sr. No.	Drugs	Conc. of tablet (µg/ml)	Mean amount found (mg/tab)	% Amount found (mean)	% RSD (n=5)
1	AMB	5	4.88	97.6	0.72
2	HCTZ	12.5	12.39	99.12	0.49

Table No. 4: Results of Recovery Studies

Sr. No.	Pre-analysed samples (µg/ml)		Pure drug added (µg/ml)		Drug recovered (µg/ml)		% Recovery		% RSD (n=5)	
	AMB	HCTZ	AMB	HCTZ	AMB	HCTZ	AMB	HCTZ	AMB	HCTZ
1	5	12.5	2.5	10	2.44	9.68	97.6	96.8	0.76	1.11
2	5	12.5	5	12.5	4.90	12.37	98	98.96	0.52	0.49
3	5	12.5	10	15	9.61	14.86	96.1	99.06	1.09	1.38

Table No. 5: Results of Precision Studies (Intra-day and Inter-day)

Sr. No.	Drugs	Conc. (µg/ml)	Intra-day amount found (n=5)		Inter-day amount found (n=5)	
			Mean	**% RSD**	**Mean**	**% RSD**
1	AMB	2.5	2.36	0.88	2.20	1.20
		5	4.89	0.65	4.01	0.92
		10	9.77	0.29	8.50	1.25
2	HCTZ	10	9.58	0.94	9.27	0.41
		12.5	12.40	0.49	12.43	0.17
		15	14.93	0.22	14.70	0.31

Table No. 6: Results of Repeatability Studies

Sr. No.	Drugs	Conc. (µg/ml)	Mean conc. found (µg/ml)	% RSD (n=5)
1.	AMB	5	4.921	0.39
2.	HCTZ	12.5	12.41	0.47

Table No. 7: Results of Ruggedness studies

Sr. No.		Amount found (%)		% RSD (n=3)	
		AMB	**HCTZ**	**AMB**	**HCTZ**
1	Analyst-I	99.11	99.92	0.87	0.75
2	Analyst-II	98.13	97.3	0.95	1.16

Table No. 8: Results of Stability Studies

Sr. No.	Stress Condition	Conc. taken (µg/ml)		Conc. found (µg/ml)		% Degradation		% Assay of active substance of drugs	
		AMB	**HCTZ**	**AMB**	**HCTZ**	**AMB**	**HCTZ**	**AMB**	**HCTZ**
1	Acidic Degradation (1 N HCl, 3 hours)	5	12.5	4.49	11.29	10.11	9.66	89.89	90.34
2	Basic Degradation (1 N NaOH, 3 hours)	5	12.5	4.97	12.39	0.58	0.81	99.42	99.19
3	Oxidative Degradation (6% H_2O_2, 3 hours)	5	12.5	4.30	10.58	13.86	15.3	86.14	84.7
4	Dry Heat Induced Degradation (50°C, 48 hours)	5	12.5	4.91	12.13	1.79	2.96	98.21	97.04

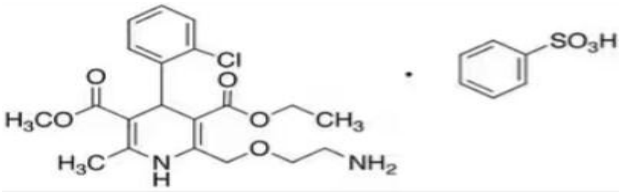

Fig. No. 1: Chemical Structure of Amlodipine Besylate

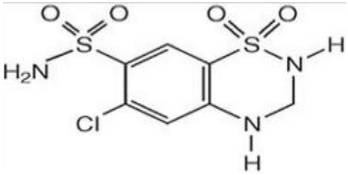

Fig. No. 2: Chemical Structure of Hydrochlorothiazide

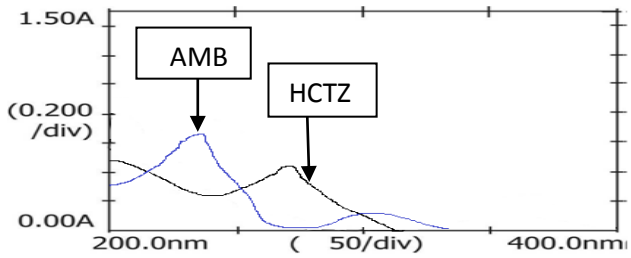

Fig. No. 3: Overlain spectra of AMB and HCTZ (5: 12.5 µg/ml)

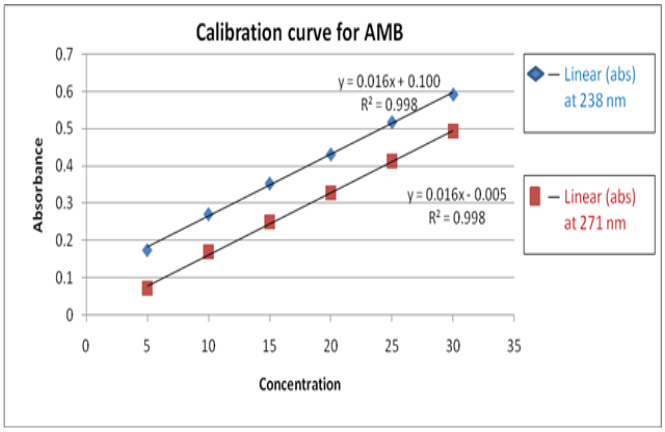

Fig. No. 4: Plot of Beer- Lambert's study for AMB at 238 nm and 271 nm

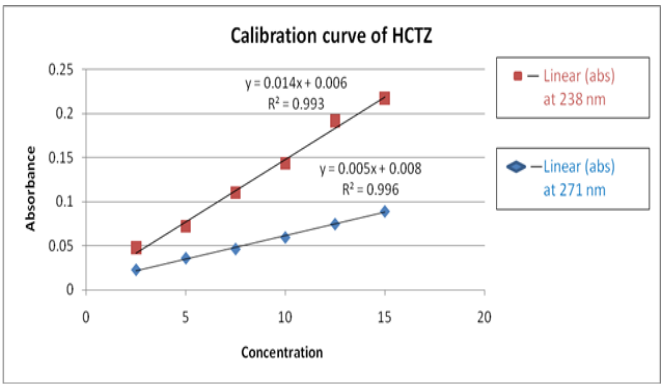

Fig. No. 5: Plot of Beer- Lambert's study for HCTZ at 238 nm and 271 nm