

## SPECTROPHOTOMETRIC SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE AND HYDROCHLOROTHIAZIDE IN COMBINED TABLET DOSAGE FORM BY SIMULTANEOUS EQUATION, ABSORPTION RATIO AND FIRST ORDER DERIVATIVE SPECTROSCOPY METHODS

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### ABSTRACT

Three sensitive, precise, accurate and simple UV spectrophotometric methods have been developed for simultaneous estimation of Amlodipine (AMLO) and Hydrochlorothiazide (HCT) in tablet dosage forms. Method A involved simultaneous equation method. The two wavelengths 238.5 nm ( $\lambda_{\max}$  of AMLO) and 271 nm ( $\lambda_{\max}$  of HCT) were selected for the formation of Simultaneous equations. Whereas method B involved formation of Q-absorbance equation at isobestic point (257.5 nm). Method C is First order Derivative Spectroscopy method in which derivative amplitudes were measured at selected wavelengths (238.5 nm for HCT and 271 nm for AMLO). Linearity was observed in the concentration range of 1-10, 1-20, 1-20 mcg/ml for AMLO and 2.5-25, 2.5-50, 2.5-50 mcg/ml for HCT by method A, B and C respectively. The proposed methods have been applied successfully to the analysis of cited drugs in pharmaceutical formulations. Recovery study was performed to confirm the accuracy of the methods. The methods were validated as per ICH guidelines.

**Keywords:** Amlodipine, Hydrochlorothiazide, simultaneous estimation, validation

### INTRODUCTION

Amlodipine (as besylate, mesylate or maleate), chemically (Fig 1.) is 3-Ethyl-5-methyl ( $\pm$ ) -2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl) -1, 4- dihydro-6-methyl-3, 5-pyridinedicarboxylate benzenesulfonate<sup>1</sup>. Amlodipine is a dihydropyridine derivative with calcium antagonist activity<sup>2</sup>. It is used in the management of hypertension, chronic stable angina pectoris and Prinzmetal variant angina<sup>3</sup>. Amlodipine acts by inhibiting the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle and also acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure<sup>4</sup>.

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiazine-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<sup>5</sup>.

Literature survey reveals few analytical methods for the determination of Amlodipine alone and in combination with other drugs in pharmaceutical preparations and biological fluids, viz. spectrophotometry<sup>6-9</sup>, HPLC<sup>10</sup> and HPTLC<sup>11-13</sup>.

Also there are some analytical methods reported for determination of HCT alone and in combination<sup>14-18</sup>.

No method has been reported for the estimation of Amlodipine (AMLO) and Hydrochlorothiazide (HCT) in combined dosage form. Present work emphasizes on the quantitative estimation of Amlodipine and Hydrochlorothiazide in their combined dosage form by UV Spectroscopic methods.

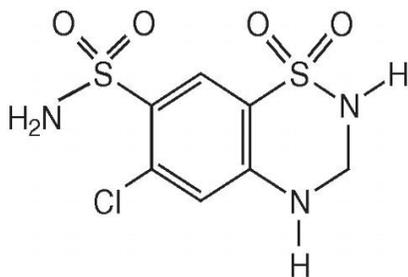


Fig 1 Amlodipine Besylate

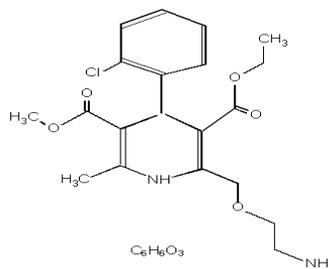


Fig 2 Hydrochlorothiazide

### MATERIALS AND METHODS

#### Instrumentation

A Double beam UV-Visible spectrophotometer (Jasco V 530) with 10 mm matched quartz cells was used. All weighing were done on single pan balance (Shimadzu).

#### Reagents and chemicals

AMLO and HCT reference standards were kindly provided by Emcure Pharmaceuticals Pvt. Ltd, Pune. Analytical grade methanol was purchased from Merck Specialities Private Ltd., Mumbai. All the

reagents were of analytical grade. Glass double distilled water was used throughout the experiment.

Tablets were purchased from local market each containing 5 mg of AMLO and 12.5 mg of HCT. AMLO and HCT are available in the ratio of 1:2.5 respectively in the formulation and were used in same ratio for preparation of calibration curves.

#### Determination of absorptivity values

Standard stock solutions of AMLO (100  $\mu\text{g/ml}$ ) and HCT (100  $\mu\text{g/ml}$ ) were prepared in methanol. For the selection of analytical wavelength solutions of AMLO (10  $\mu\text{g/ml}$ ), HCT (10  $\mu\text{g/ml}$ ) were

prepared separately by appropriate dilution of standard stock solution with distilled water and scanned in the spectrum mode from 200 to 400 nm. From the overlain spectra of these drugs

[Figure 3], wavelengths 238.5 nm ( $\lambda_{\max}$  of AMLO), 271 nm ( $\lambda_{\max}$  of HCT) AND 257.5 nm (isobestic point) were selected for analysis.

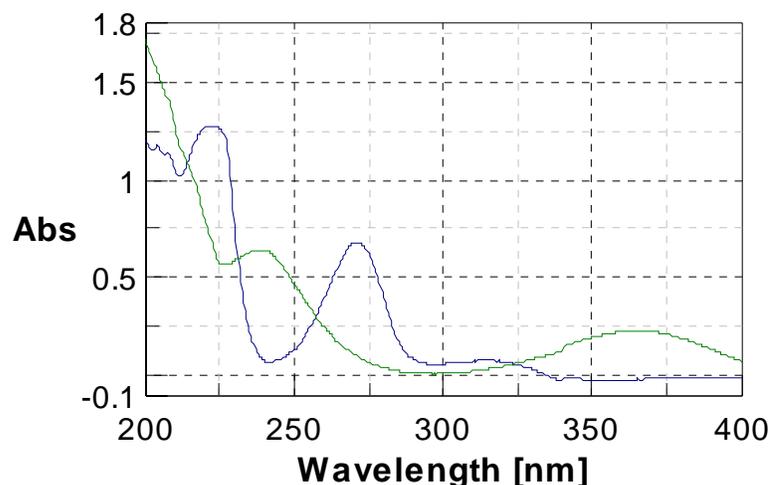


Fig. 3: It shows overlay spectrum of AMLO and HCT

The calibration curves (Figure 4,5) for AMLO and HCT were prepared in the concentration range of 1-25  $\mu\text{g/ml}$ , 1 -50 $\mu\text{g/ml}$  respectively at the selected wavelengths. Absorptivity values were

determined for AMLO and HCT. These were found to be 42.66/19.81/6.719 and 6.474/20.50/46.16 at 238.5/257.5/271 nm, respectively for AMLO and HCT.

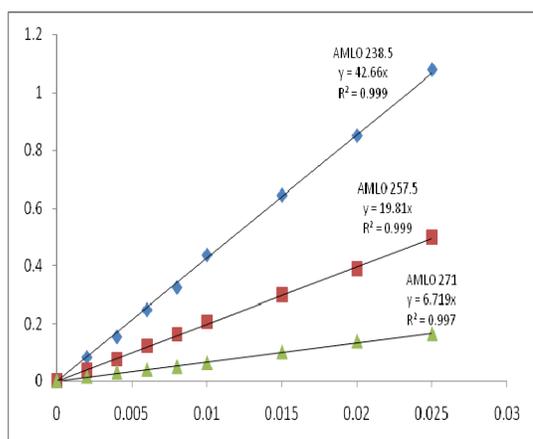


Fig. 4: Calibration curve for AMLO

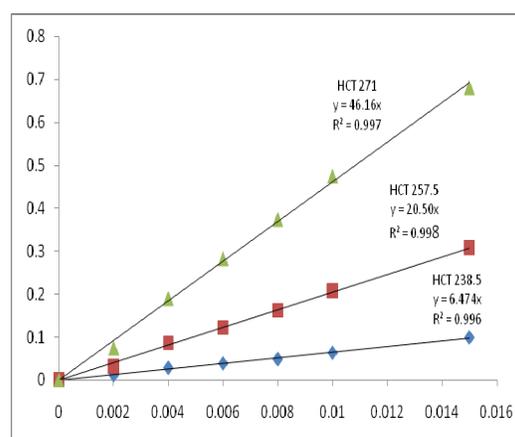


Fig. 5: Calibration curve for HCT

#### Determination of linearity

Standard stock solution of pure drugs containing 50 mg of AMLO and 125 mg of HCT/100 ml was prepared in methanol. The working standard solutions were obtained by dilution of the stock solution in the distilled water. Series of solutions with conc. 1-20 $\mu\text{g mL}^{-1}$  and 2.5 - 50  $\mu\text{g mL}^{-1}$  of AMLO and HCT respectively were used to determine linearity by three methods. Solutions were scanned and Beers Lambert's law limit was determined.

#### Formulation analysis

For estimating AMLO and HCT in tablet formulation, twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 50 mg of AMLO and 125 mg of HCT was transferred to 100 ml volumetric flask. 75 ml of methanol was added and sonicated for 15 min and

volume was made up to the mark with methanol. The solution was then filtered through Whatmann filter paper No. 41. Appropriate aliquots were taken for further analysis.

#### Method A: simultaneous equation method

Sample stock was appropriately diluted with distilled water to obtain final concentration of 4  $\mu\text{g/ml}$  for AMLO and 10  $\mu\text{g/ml}$  for HCT. Absorbance of diluted sample solution was measured at selected wavelengths. The concentration of drugs was determined by using the Equations 1 and 2.

Using absorptivity values following Eqns. were developed for determining concentration of AMLO and HCT in tablet sample solution.

$$A_1 = 42.66 C_{\text{AMLO}} + 6.474 C_{\text{HCT}} \quad (1)$$



Table 3: It shows result of recovery studies by the proposed methods

Amount added ( $\mu\text{g/ml}$ )	Amount recovered (Method A)	% Recovery (Method A) +S.D.	Amount recovered (Method B)	% Recovery (Method B) +S.D.	Amount recovered (Method C)	% Recovery (Method C) +S.D.
<b>AMLO</b>						
2.0	1.95	97.5+0.64	1.95	97.5 + 0.42	1.98	99.0 + 0.47
4.0	3.92	98.2+0.87	4.91	98.2 + 0.55	3.97	99.25 + 0.56
6.0	5.91	98.5+0.37	7.88	98.5 + 0.49	5.96	99.33 + 0.42
<b>HCT</b>						
5.0	4.95	99.00+0.53	4.92	98.4 + 0.43	4.96	99.2 + 0.36
10.0	9.76	97.60+0.41	9.86	98.6 + 0.47	9.91	99.1 + 0.42
15.0	14.72	98.13+0.31	14.81	98.73 + 0.62	14.90	99.33 + 0.51

n=6

Table 4: It shows Intra and Interday Precision

Conc. ( $\mu\text{g/ml}$ )	Intraday precision		Interday precision		Intraday precision		Interday precision		Intraday precision		Interday precision	
	Method A		Method A		Method B		Method B		Method C		Method C	
	S.D.	%R.S.D.										
<b>AMLO</b>	0.40	0.41	0.75	0.78	1.14	1.15	1.17	1.18	0.39	0.42	0.52	0.61
<b>HCT</b>	0.94	0.96	0.45	0.48	1.72	1.75	1.14	1.15	0.78	0.84	0.42	0.45

n=6

## CONCLUSION

Simple UV spectrophotometric methods were developed for the simultaneous determination of Amlodipine and Hydrochlorothiazide in bulk and tablet formulation without any interference from the excipients. To the best of our knowledge, the present study is the first report for the purpose. The present methods succeeded in adopting a simple sample preparation that achieved satisfactory extraction recovery and facilitated its application in coformulated formulation.

The results of our study indicate that the proposed UV spectroscopic methods are simple, rapid, precise and accurate. Statistical analysis proves that, these methods are repeatable and selective for the analysis of AMLO and HCT. It can therefore be concluded that use of these methods can save much time and money and they can be with accuracy.

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