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SIMULTANEOUS ESTIMATION AND VALIDATION FOR AMLODIPINE BESYLATE AND INDAPAMIDE IN PHARMACEUTICAL DOSAGE FORM BY ABSORPTION CORRECTION METHOD

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ABSTRACT

A Versatile, accurate, precise and economic method for simultaneous determination of Amlodipine besylate (AML) and Indapamide (IND) in fixed dose combination products was developed. Wavelengths selected for AML was 343 nm as IND shows zero absorbance. So absorbance of IND was found by subtracting absorbance of AML. Absorbance corrected for IND was measured at 263.5 nm. This method obeyed Beer's law in the concentration range of mixture of 14–38 μg/ml for AML and 4-12 μg/ml for IND. The results of analysIs have been validated for linearity, accuracy and precision, LOD and LOQ of the proposed method.

INTRODUCTION

Amlodipine besylate (AML) is chemically 2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine-benzenesulfonate.Amlodipine besylate is a Ca^{+2} channel blocker. It decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels and thus causes muscle contraction. Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle cells and results in vasodilation.

Indapamide (IND) is chemically Benzamide, 3-(aminosulfonyl)-4-chloro-N-(2,3-dihydro-2-methyl-1H-indol-1-yl)-4-Chloro-N-(2-methyl-1-indolinyl)-3-sulfamoylbenzamide and used as a diuretic. Combined dosage forms of AML and IND is available in the market. Clinical trials showed that combination therapy when used in hypertensive patient with coronary heart diseases reduced cardiovascular events.

Amlodipine is official in Indian Pharmacopeia and Indapamide is official in United states Pharmacopoeia and British Pharmacopoeia. A survey of literature revealed that no chromatographic and Spectrophotometric methods are reported for determination Amlodipine besylate and Indapamide in combined dosage form. The present work describes simple, precise, accurate and economical spectrophotometric method have been developed for simultaneous estimation of Amlodipine besylate and Indapamide in combined dosage form.
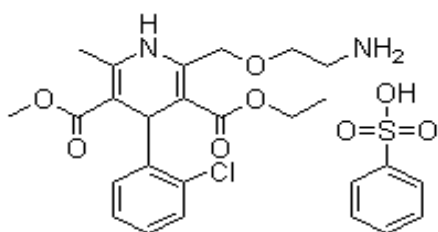

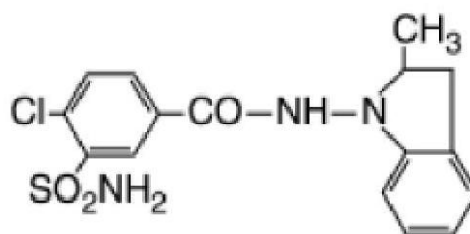Figure-1 Amlodipine besylate   Figure-2 Indapamide

MATERIAL AND METHOD

Instrument

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions.

Reagents and Chemicals

Reference Standards of AMLODIPINE BESYLATE and INDAPAMIDE were obtained as gift samples from the Torrent Pharmaceutical Ltd. The drug sample (tablets) NATRILAM manufactured by Serdia Pharmaceuticals, Mumbai, were procured from market. All other reagents were of analytical grade for Spectrophotometric method.

Procedures

Preparation of Standard Stock Solution and Calibration curve:

A mixed stock solution of AML (1000 µg/ml) and IND (1000 µg/ml) was prepared by accurately weighing AML (25 mg) and IND (25 mg), dissolving in methanol and diluted to 25ml with the same solvent in the same volumetric flask. Then AML (100µg/ml) and IND (100µg/ml) were prepared by further dilution with distilled water and again dilutions were made as such that five solutions prepared containing 14-38µg/ml AML and 4-12µg/ml IND which comes in the ratio as per the tablet (AML 5mg and IND 1.5mg).

Calibration curve were prepared for AML using absorbance of mixture at 343nm and for IND using corrected absorbance at 263.5nm.

Methodology

Absorbance spectrum of pure AML was scanned in the spectrum basic mode. Using the cursor function, the absorbance corresponding to 343 nm (wavelength $\lambda 1$, the wavelength of minimum absorbance for AML) was noted from spectrum. Then the cursor function was moved along with peak curve until the absorbance equal to that of absorbance at 343 nm was found. The wavelength obtain corresponding to this absorbance value was 263.5 nm ($\lambda 2$). The absorbance of various dilutions of AML in methanol + water was measured at 343 nm. Absorbance spectrum of pure IND was also scanned in the spectrum basic mode. IND showed some absorbance value at 263.5 nm ($\lambda 2$) while it does not show any absorbance value at 343 nm. The absorbance value at 343 nm is due to AML only in the combined mixture of both drugs. Wavelength $\lambda 1$ (343 nm) was selected for the measurement of AML and for measurement of IND corrected absorbance by subtraction of absorbance of mixture at λ_2.

Procedure for tablet formulation

Twenty tablets were accurately weighed and crushed. Average weight of the content per tablet was calculated. A quantity of tablet powder equivalent to 5mg of Amlodipine besylate and 1.5mg

of Indapamide was transferred to 50ml volumetric flask and dissolved in methanol with sonicated for 20 min, was then filtered through whatman filter. The Aliquot portion of filtrate was further diluted to get a final concentration of about 20μg/ml Amlodipine besylate and 6μg/ml of Indapamide. The absorbance of sample solution was measured at 343nm and 263.5nm in 1cm cell against the blank.
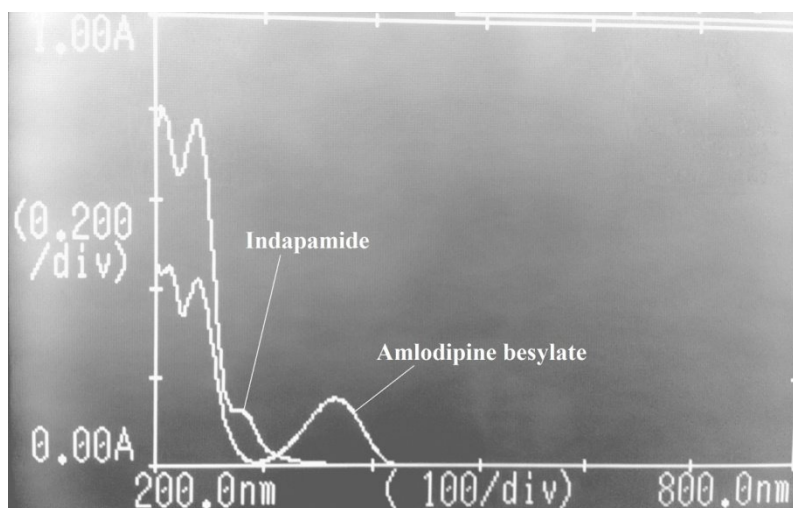

Figure-3 Overlain spectra of Amlodipine besylate (10μg/ml) and Indapamide (10μg/ml)

Table-1 Optical Characteristic:

Absorption correction method		
Parameters	**Amlodipine besylate**	**Indapamide**
Wavelength (nm)	343	263.5
Beer's law limit (μg /ml)	14-38	4-16
Regression equation (y = a + bc)	y =0.007946x-0.01171	y =0.008678x+0.008556
Slope (b)	0.007946	0.008678
Intercept (a)	-0.01171	0.008556
Correlation coefficient (r^2)	0.9992	0.9989
LOD (μg/ml)	1.001	3.03
LOQ (μg /ml)	0.7122	2.158

Table-2 Results of the recovery studies

Level of recovery (%)	Amount of pure drug added (µg/ml)		% recovery	
	AML	IND	AML	IND
80	16	4.8	100.30	100.51
100	20	6	100.59	99.95
120	24	7.2	100.08	100.27
Mean % recovery			100.323	100.243
SD*			0.255	0.280
RSD**			0.254	0.280

*SD = Standard deviation ** RSD=Relative Standard deviation

Table-3 Results of analysis of tablet formulation

Drugs	%Assay ± SD(n=6)
Amlodipine besylate(5mg)	100.9% ± 0.95
Indapamide(1.5mg)	100.5% ± 0.45

Validation of the Method according to ICH Guidelines

Validation of the method was done according to ICH guidelines for Simultaneous Equation method.

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in the samples. AML was linear with the concentration range of 14-38 µg/ml at 343 nm. IND showed the linearity in the range of 4–12µg/ml at 263.5 nm.

Precision (repeatability)

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration.

Intermediate precision (reproducibility):

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of AML and IND.

Accuracy (recovery study):

To check the accuracy of the proposed methods, recovery studies carried out at 80%, 100%, and 120% of the test concentration as per ICH Guideline. The recovery study was performed three times at each level.

Limit of detection and Limit of quantification:

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULTS AND DISCUSSION

In this method, two wavelengths were used for the analysis of the drugs. As IND is showing zero absorbance at wavelength of AML (343nm), its corrected absorbance is measured by subtracting absorbance of AML from absorbance of mixture at 263.5nm.

Linear correlation was obtained between absorbances and concentrations of AML and IND in the concentration ranges of 14-38 μg/ml and 4-12 μg/ml for both drugs respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. LOD and LOQ values for AML were found to be 1.001 and 0.7122 μg/ml and LOD and LOQ values for IND were found to be 3.03 and 2.158 μg/ml respectively. These data show that method is sensitive for the determination of AML and IND. Both drugs showed good regression values at their respective wavelengths and the results of a recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method. The proposed validated method was successfully applied to determine AML and IND in their combined dosage form. The results obtained for AML and IND were comparable with the corresponding labeled amounts (Table-3).

CONCLUSION

The proposed methods are simple, rapid and validated in terms of linearity, precision, accuracy, reproducibility, and can be used successfully for routine simultaneous estimation of Amlodipine besylate and Indapamide in pure and TABLET dosage forms.

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