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Development and Validation of UV Spectrophoto metric Methods for Simultaneous Analysis of Amlodipine and Indapamide in Combined Dosage forms

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ABSTRACT
Two simple UV-Vis Spectrophometric method was developed for the simultaneous determination of Amlodipine Besylate and Indapamide from their combined dosage form. The method employs formation and solving of simultaneous equation using 242 nm and 239nm as two analytical wavelengths ($\lambda_{Max}$of the drugs) of detection .An Isoabsorptive point 310nm was selected for absorbance ratio method. Both the drugs obeyed Beer-Lambert's law over the concentration range 2-12µg/mL for Amlodipine Besylateand 2-7 µg/mL for Indapamide, respectively. The developed methods were validated for Accuracy, Precision, Limit of Detection and Limit of Quantification as per ICH guidelines and results of analysis were validated statistically.

Key Words: Amlodipine, Indapamide, Simultaneous Equation, Absorbance ratio

I. INTRODUCTION

Amlodipine (AMD), 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate an antihypertensive drug belongs to the group of drugs called dihydropyridine calcium channel blockers.[1] It is commonly used in the treatment of high blood pressure and angina. It also has antioxidant properties and ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure. Amlodipine acts by inhibiting the trans membrane influx of calcium ions into vascular smooth muscle and cardiac muscle also acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.[2]

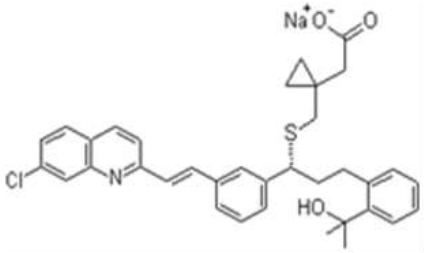

Fig1:Amlodipine,:3-ethyl5-methyl2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate

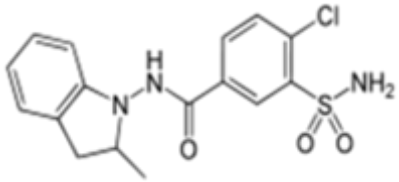

Fig 2: Indapamide ;4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoyl-Benzamide

Indapamide is a diureticacts by inhibiting the carbonic anhydrase enzyme and a significant reduction in blood pressure can be achieved. [2-3]

Literature survey reveals that Amlodipine is estimated in various combination dosage forms even with Indapamide by using methods of spectrophotometric multi component analysis techniques. But no attempts has made to estimate the combination by simultaneous equation method and absorbance ratio methods.[3]The present research work, our aim is to develop a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate routine analysis of amlodipine and Indpamide in tablet dosage forms.

II. MATERIALS AND METHODS

Drug Samples Indapamide and amlodipine were received as gift samples from Supra chemicals Mumbai and Bafna Pharmaceuticals Ltd Chennai respectively. Natrilam tablets were obtained from Serdia pharmaceuticals Mumbai.

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Solubility Test

Solubility test for the drugs Amlodipine and Indapamide were performed by using various solvents. The solvents include water, methanol, ethanol, acetonitrile, 0.1N hydrochloric acid (0.1N HCl), 0.1N sodium hydroxide (0.1N NaOH) and chloroform.[4]

Selection of Wavelength:

Indapamide and Amlodipine Besylate solutions 10µg/ml solution were prepared separately and the λ max of both drugs was scanned individually in UV-Spectrophotometer. The over lain UV spectra of both Indapamide and Amlodipine were shown in fig. 1. For estimation, the two wavelengths were selected, 242 nm for Indapamide and 239nm for amlodipine in the respective solvent and 310 for Absorbance ratio method as it is the iso-absorptive point.[5]

Preparation of standard drug solution:

A series of concentrations of 2,4,6,8,10and 12µg/ml pure drug amlodipine was prepared from a stock solution of 1000µg/ml .Similarly from a stock solution of concentration1000µg/ml of Indapamidea series concentration of 2,3,4,5 and 6 µg/ml respectively.[6]

Estimation of Amlodipine and Indapamide from formulation

Twenty Tablets each containing 5mg of Amlodipine and 1.5 mg of Indapmide were weighed and finely powdered in a mortar. From the powdered tablets, a quantity of powder equivalent to 10 mg of amlodipine was weighed accurately and treated with methanol and made up to 100 ml with same solvent to set a stock solution of (100µg/ml). The solution is then filtered through Whatmann filter to get a clear solution. From this 1ml of solution was drawn and make up to 10 ml with water.[7-8]

Method I: Simultaneous Equation Method`

The sample containing two absorbing species Amlodipine Besylate and Indapamideand (X &Y) each of which absorbs at the λ max of the other. So the absorbance of each drugs were measured at both wavelengths λ1 & λ2 respectively. The both the drugs are determined by simultaneous method (Vierodt's method). The absorptivity of amlodipine (X) at λ1 (237) and λ2 (242) is ax1 ax2 respectively. The absorptivity of Indapamide(Y) at λ1 (237) and λ2 (242) is ay1 ay2 respectively. The absorbance of the sample (formulation) at λ1 (237) and λ2 (242) is A1 and A2 respectively. The total absorbance of the mixture is equal to the sum of individual absorbance of X and Y.[9]

A1 = ax1bcx + ay1bcy

A2 = ax2bcx + ay2bcy

Cx – Concentration of Amlodipine besylate

Cy – Concentration of Indapamide

By using this formula the both drugs Amlodipine and Indapamide can be estimate.

$$Cx = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2}$$

$$Cy = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

Method II: Absorption ratio method

Absorption ratio method uses the absorbencies at two selected wave length, one of which is an isosbestic point and other being the wave length of maximum absorption of one of the two components. From the overlain spectra wave length 310nm and 237 nm were selected for absorption ratio method.The absorbance is and absorptivity were substituted in the following equation to obtain the concentration of both the drug.

$$Cy = \frac{Qm - Qz}{Qy - Qz} \times \frac{A2}{ay2} \quad Cz = \frac{A2}{ay2} - Cy$$

Where Qm = A3/A2, Qy = ay3/ay2, Qz = az3/az2

Cy, Cz –Concentration of Amlodipine and Indapamide respectively.

A2, A3 –Absorbances of sample at , 310nm and 237nm respectively.

III. RESULT AND DISCUSSION

1. Selection of wavelength:

The drugs were dissolved in methanol and further diluted with water to linearity range and 237 and 242 were found to be detection wavelength.

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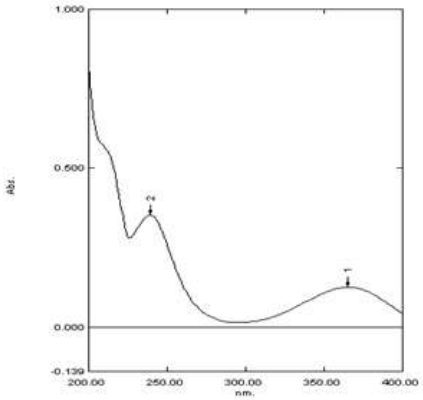

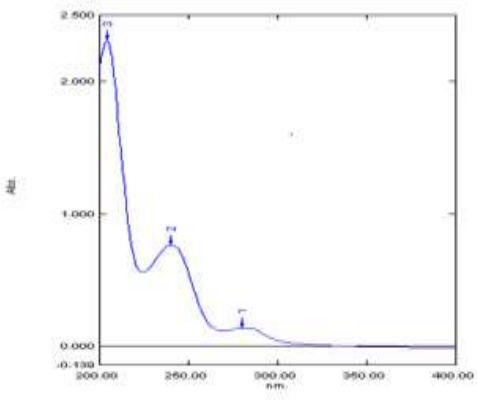

Fig 3: UV Spectrum of Amlodipine besylate Fig 4: UV Spectrum of Indapamide

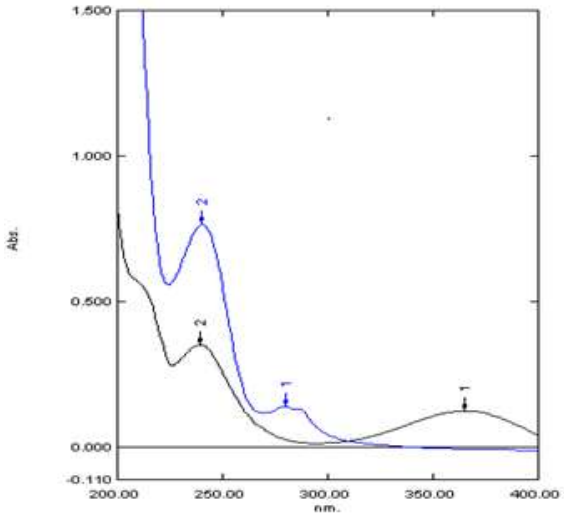

Fig 5:Over-Laid UV Spectrum of Amlodipine and Indapmide

Estimation:

Estimation of Amlodipine and Indapamide in dosage forms by UV Spectroscopic Simultaneous Equation methods (Method 1) and Absorbance ratio Method (Method II)was carried out using optimized conditions.

Table .1:- Analysis of Formulation:-

Method	Drug	Label claim mg/tab	Estimated amount mg/tab	%label claim	%SD
Method I	Amlodipine	5	5.07	100.23%	0.239
	Indapamide	1.5	1.49	98.45%	0.776
Method II	Amlodipine	5	4.992	99.84%	1.564
	Indapamide	1.5	1.498	99.313%	0.997

Validation Parameters

1 Accuracy

The accuracy of the method was studied by recovery studies at three stages 80%, 100% and120% of the assay amount.The results are depicted in Table 2

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Method	Drug	Theoretical % target level	Amount of drug recovered(mg)	Percentage recovery	% Rsd
Method II	Amlodipine	80	7.96	99.42	0.805
		100	9.8	98	
		120	12	100	
	Indapamide	80	2.5	101.42	1.138
		100	2.99	99.33	
		120	3.55	102.2	
Method II	Amlodipine	80	7.97	99.62	1.205
		100	9.92	99.2	
		120	11.89	99.08	
	Indapamide	80	2.39	99.58	0.992
		100	2.99	99.66	
		120	3.61	100.277	

Table 2 :Recovery Studies data

2. Precision

The intra-day and inter-day precision studies (intermediate precision) were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations of Amlodipine and Indapamide , and the results are reported (Table:3) in terms of relative standard deviation.[10]

Table 3: Intra-Day and Interday Precision Data

Day	Amlodipine		Indapamide		Method	Drug	Percentage Content	% RSD
	% Content	% RSD	%Content	%RSD				
DAY 1	101.4 100.4 99.3	1.0503	95.86 99.66 101.21	1.28	Method II	Amlodipine	99.92	0.876
DAY 2	100.05 99.4 97.58	1.28	98.66 100.4 99.33	0.880		Indapamide	100.23	0.288
DAY 3	100.62 100.92 100.52	0.808	99.46 99.2 98.9	0.280	Method II	Amlodipine	100.4	1.002
						Indapamide	99.33	1.214

3. Linearity

To determine linearity range for the drugs, a series of working standard solutions were prepared from the respective stock solutions of drugs

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Table 4: Summary of linearity data for Amlodipine and indapamide

Parameters	Amlodipine	Indapamide
λmax	237	242
Linearity(µg/ml)	2-12	2-7
Correlation coefficient	0.9905	0.9986
Slope	0.0263	0.075
intercept	0.0113	0.0612

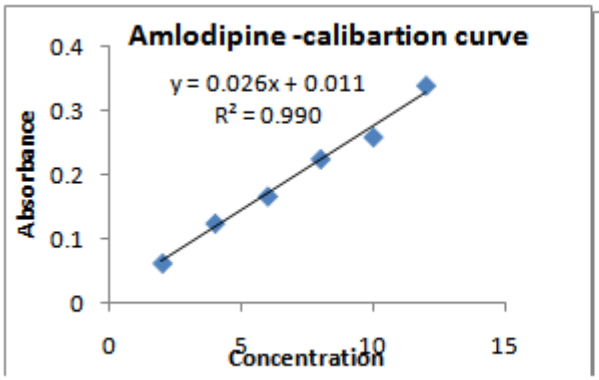

Fig 6:Calibration curve for Amlodipine

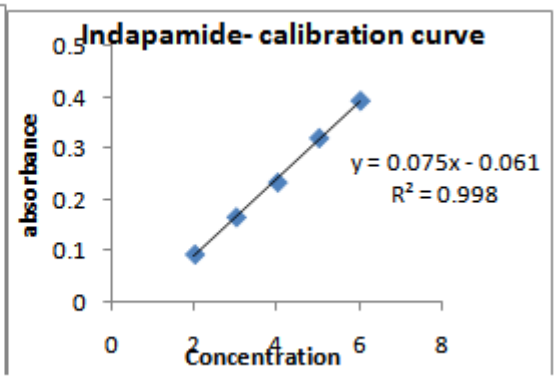

Fig 7:Calibration curve for Indapamide

4. Robustness:
The robustness of the method was determined by introducing deliberate changes in the experimental conditions during the analysis of mixed standard solution of drugs, namely use of different instrument, change of analyst and by altering composition ofdiluent. The results as depicted in Table 5

Table 5: Validation Parameter –Robustness of the method

Method	Drug	Instrument II	Instrument II	Analyst I	Analyst II
Method I	Amlodipine	101.27±1.89	99.59±1.9421	101.7+1.82	98.248+0.43
	Indapamide	99.57±0.3524	98.84±0.3712	100.57+1.432	98.8+0.4423
Method II	Amlodipine	101±0.27	99.07±0.342	100.07+1.142	99.28+0.423
	Indapamide	99.59±0.7435	99.38±0.453	100.59+1.043	100.3+0.435

IV. CONCLUSION

The Absorption ratio and simultaneous equation method was developed and validated for simultaneous determination Amlodipine besylate and Indapamide in combined pharmaceutical formulation. The method was found to be simple, precise and rapid. The assay result obtained by this method is in fair agreement.

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CONFLICT OF INTERESTS:
The authors declare no conflict of interest.

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