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Development and Validation of UV-Spectrophotometric methods for estimation of Indapamide in bulk and tablet dosage form

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ABSTRACT

Indapamide is an oral antihypertensive diuretic agent indicated for the treatment of hypertensive and edema [2]. Various methods for analysis of the same are available but are time consuming and expensive. Here we have developed two new, precise and simple UV spectrophotometric methods for estimation of Indapamide from bulk and tablet formulation in phosphate buffer 7.4. The drug obeyed the Beer's law with correlation coefficient 0.996 and 0.998 respectively for Method I and Method II. It showed absorption maxima at 240 nm and 223 nm respectively for method I and Method II; in phosphate buffer 7.4. The linearity was observed between 5 –40 µg/ml. The results of analysis were validated by recovery studies, accuracy, precision, LOD, LOQ and ruggedness. The method was found to be simple, accurate, precise, economical and robust.

Key words: Indapamide, Phosphate buffer 7.4, Zero order spectra and second order spectra, validation.

INTRODUCTION

The chemical formula of indapamide is 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-benzamide (Indapamide is an oral antihypertensive diuretic agent indicated for the treatment of hypertensive and edema [2]. Indapamide inhibits carbonic anhydrase enzyme [3]. A significant reduction in blood pressure can be achieved with daily oral dose of 2.5 mg [4]. It differs chemically from the thiazide diuretics in that it does not posses the thiazide ring system and contains only a sulfonamide groups.

Analysis is an important component in the formulation and development of any drug molecule. A suitable and validated method has to be available for the analysis of drugs in bulk, in drug delivery systems, release dissolution studies and in biological samples. If a suitable method for specific need is not available then it becomes essential to develop simple sensitive, accurate, precise reproducible method for the estimation of drug samples. Methods Several methods have been reported for the determination of indapamide in biological fluids and in pharmaceutical preparations including spectrophotometry [6], fluorimetry [7] and [8], gas chromatography–mass spectrophotometry [9], [10] and [11], high performance liquid chromatography thin layer chromatography [20], capillary electrophoresis [21] and [22], and electrochemical analysis at carbon paste electrode [23].

MATERIALS AND METHODS

Instrumentation, Reagents &Chemicals:
Instruments used were UV-Visible spectrometer**,** model *JASCO* 1505 Instrument and Shimadzu ELB 300 analytical balance, Indapamide pure drug was obtained as a gift sample from Glenmark Pharmaceutical industry Nashik. All chemicals and reagents used were of analytical grade. Formulation used for studies was developed by "SERVIER LABORATORIES SOUTH AFRICA (PVT) LTD "NATRILIX®TABLETS".

Selection of media:
Main criteria for selection of media solubility and stability i.e., drug should be soluble as well as stable for sufficient time in selected media. Indapamide was slightly soluble in distilled water and was soluble in methanol, ethanol, PEG-400/Water and ethanol-water mixture. It was freely soluble in phosphate buffer 7.4 and was considerably stable.

Preparation of standard stock solution:
Standard drug solution of Indapamide was prepared by dissolving 10mg pure Indapamide in phosphate buffer 7.4 and transferred into 100ml volumetric flask to obtain 10µg/ml of stock solution from which desired concentrations 5, 10, 15, 20, 25, 30,35,40 µg/ml of solution were prepared.

Preparation of sample solution:
Twenty tablets were weighed; average weight was determined and finely powdered. An accurately weighed quantity of tablet powder equivalent to 10mg of Indapamide was transferred to 100 ml volumetric flask and dissolved by sonication with sufficient quantity of phosphate buffer 7.4, volume was made up to mark. The solution was then filtered through whatman filter paper no.41. A 1 ml portion of the filtrate was further diluted with phosphate buffer 7.4 in a 10 ml volumetric flask up to mark (10µg/ml) on label claim basis. The absorbance of the resulting solution was measured at 240 nm (method I) and 223 nm (method II) against solvent blank. The results of estimation by proposed methods are shown in Table.2.

Determination of λmax:
A 10 µg/ml solution of Indapamide was prepared and scanned in UV range of 200-400nm and spectrum was obtained. The **λ**max was found to be at 240 nm wave length where absorbance was maximum at this wavelength for Method I, and the λmax for Method II was found to be 223 nm. Hence these are considered as absorbance maxima (λmax**)** shown in fig.1 and fig.3.

Preparation of calibration curve:
Standard stock solution was suitably diluted with phosphate buffer 7.4 to obtain concentrations ranging from 5-40 µg/ml. Absorbance of these solutions was measured at 240nm for Method I and at 223 nm for Method II using UV. The calibration curve was plotted as concentration versus absorbance over the range of 5-40 µg/ml with correlation coefficient of 0.996 and 0.998 for the proposed method I and method II (fig.4).

VALIDATION
Accuracy:
To assess the accuracy of the proposed method, recovery studies were carried out three different levels i.e. 80%, 100% and 120%. To the pre-analyzed sample solution a known amount standard drug solution was added at three different levels, absorbance was recorded. The % recovery was then calculated as % Recovery = [(A – B) / C] x 100, Where A is total amount of drug estimated; B is amount of drug found on pre analyzed basis; C is amount of pure drug added to formulation (Table No.3).

Precision:
Precision of the method is studied as intra-day and interday precision. Intra-day and Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days. In intermediate precision study, % R.S.D. values were not more than 1.0 % in all the cases (Table No.5).

Limit of detection and Limit of quantitation:
Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by using the formula based on the standard deviation of the response and the slope. Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated by using the equations LOD = 3 × s/S and LOQ = 10 × s/S, where s is standard deviation of intercept, S is the slope of the line (Table No.4).

Ruggedness:
Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions (Table No.5).

RESULTS AND DISCUSSION

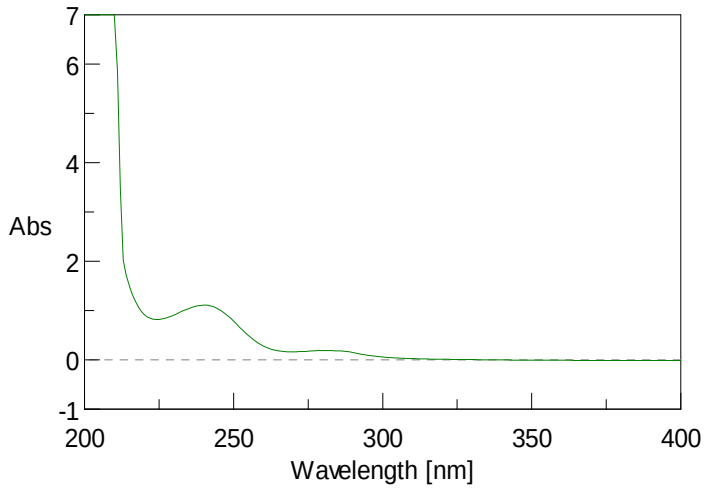

Fig.1 Absorbance maxima of Indapamide at 240 nm

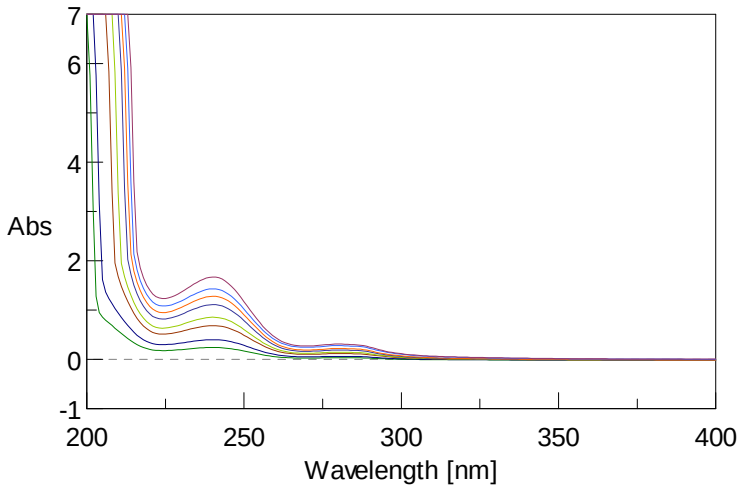

Fig.2 Overlay spectra of different concentrations of Indapamide

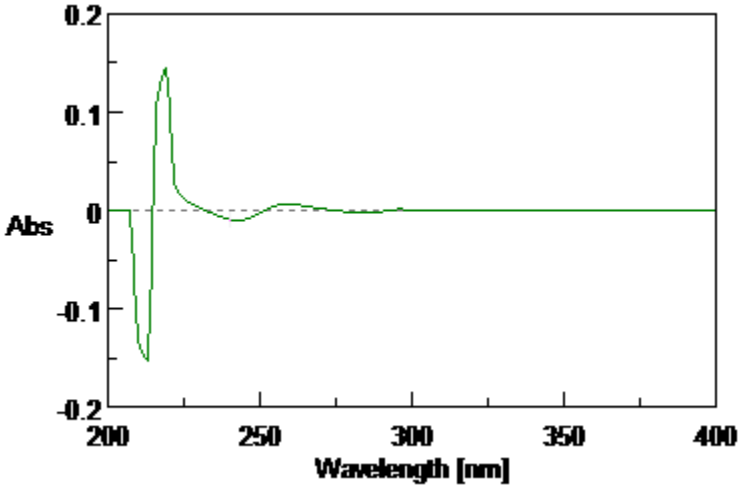

Fig.3 Second order derivative spectra of Indapamide

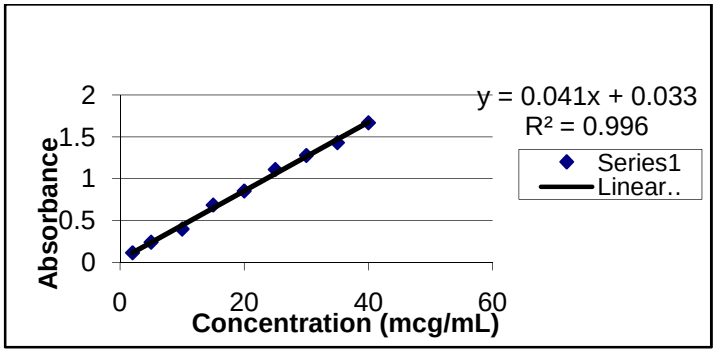

Fig.4 Calibration curve of Indapamide

Table No.1: Optical Parameters:

Sr. No.	Parameters	Observations Method I	Observations Method II (Derivative)
1	λ max	240 nm	223 nm
2	Beers range	5-40 µg/ml	5-40 µg/ml
3	Correlation coefficient	0.996	0.998
4	Intercept	0.07495	0.0892
5	Slope	24.28	22.36

Table No.2: Assay of Indapamide 2.5 mg tablets (NATRILIX 2.5):

Sr. No.	Label claim	% claim found*	
		Method I	Method II
Sample 1	2.5 mg	99.77 %	99.12 %
Sample 2	2.5 mg	99.84 %	99.35%

**mean of 5 determinations.*

Table No.3: Results of Recovery study of Indapamide 2.5 mg tablets (NATRILIX 2.5):

Labelled amount	Amount of drug Added (%)	Method I			Method II (Derivative)		
		Amount of drug Recovered(mg)	Percent Recovery (%)*	% RSD	Amount of drug Recovered(mg)	Percent Recovery (%)*	% RSD
2.5 mg	80	1.91	99.56	0.28	1.86	99.09	0.56
2.5 mg	100	2.485	99.39	0.35	2.31	99.59	0.23
2.5 mg	120	3.0156	100.54	0.29	3.0126	100.21	0.31

**mean of 4 determinations.*

Table No.4: Validation parameters:

Sr. No.	Method I		Method II (Derivative)	
	LOD	LOQ	LOD	LOQ
1	0.0646	0.189	0.0796	0.176

Table No.5: Validation parameters:

Sr. No.	Parameters	Method I	Method II (Derivative)
1	Intraday precision Amount found + %RSD (n=3)	99.32 + 0.69	98.11 + 0.99
2	Interday precision Amount + %RSD (n=3)	98.67 + 0.99	97.03 + 3.62
3	Ruggedness Amount found + %RSD (n=3)	0.141	0.169

A validated, simple, rapid sensitive and accurate UV-Spectrophotometric methods has been developed for estimation of Indapamide in bulk and pharmaceutical formulation. In phosphate buffer 7.4, Indapamide showed absorbance maxima at 240 nm and 223 nm respectively for Method I and Method II. Linearity was observed in the concentration range 5-40 µg/ml with correlation coefficient value 0.996 and 0.998 respectively for Method I and Method II. The proposed method was applied to pharmaceutical formulation and Percent amount of drug estimated was found in good agreement with the label claim. The recovery experiment was carried out at three different levels i.e., 80 %, 100 % and 120 %. The percentage recovery was found to be 99.84 % and 99.35 % respectively for

Method I and Method II; the low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day and inter-day precision. Ruggedness of the proposed method was studied with the help of two analysts. The Limits of Detection and Quantitation for Indapamide with a lower concentration were 0.0646 and 0.189 for Method I and for Method II 0.0796 and 0.176 respectively, values which are under the lowest expected concentrations in the sample.

CONCLUSION

The present study was undertaken with an objective of developing simple, sensitive and reliable analytical method like UV-Visible spectrophotometry for estimation of Indapamide in phosphate buffer 7.4 in tablet dosage form. The method has sufficiently good accuracy, precision and permitted as a cost effective as other methods. The analytical method is simple, sensitive, rapid and specific. Further it can be conveniently employed for the routine analysis and the quality control of Indapamide in tablet formulation.

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