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Der Pharma Chemica, 2012, 4 (3):996-1002
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Validated RP-HPLC Method for the Determination of Indapamide in Bulk and Tablet Dosage Form

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ABSTRACT

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of Indapamide in bulk and tablet dosage form with greater precision and accuracy. Separation was achieved on C18 column (250X4.6mm i.d.,5µm) in isocratic mode using Acetonitrile:Methanol:Water in the ratio of 40:50:10 (v/v/v) as mobile phase, pumped in to the column at flow rate of 1.0 mL $min^{-1}$and the detection of eluent from the column was carried out using variable wavelength UV detector at 242 nm. The total run time was 10 min and the column was maintained at ambient temperature. The retention time of Indapamide was 3.233 min. The standard curves were linear over the concentration range of 10-60 µg mL^{-1} with R^2 9995 and the LOD and LOQ values for Indapamide were 0.52 µg $mL^{-1}$and 0.78 µg mL^{-1}, respectively. The percentage recovery was found to be 98.16 – 100.12%, the % RSD of intraday and inter day precision was found to be 0.4404 and 0.5588, respectively. The percentage amount of a marketed tablet formulation of Indapamide was found to be 99.12 %. The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. Hence the proposed method can be applied for the routine quality control analysis of Indapamide in bulk and tablet dosage forms.

Key words: Indapamide (IND), RP-HPLC, Method Development, Validation, ICH guideline

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INTRODUCTION

Indapamide is a non-thiazide sulphonamide diuretic drug, generally used in the treatment of hypertension, as well as decompensated cardiac failure. Chemically Indapamide is 3-(amino sulfonyl)-2methyl-4-imdol-1-yl) benzamide. (Fig.1) The drug is official in United States Pharmacopoeia (USP). [1] .It is a white to off- white crystalline powder that is soluble in methanol, ethanol, acetic acid and ethyl acetate, very slightly soluble in ether, chloroform and benzene and practically insoluble in water. Indapamide belongs to a class of medications called Diuretics. Literature survey reveals that one Spectrophotometric method [2], Four HPLC methods [3-6] have been developed for the estimation of Indapamide in human serum and tablet formulation. The objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC method for the estimation of Indapamide in bulk and tablet. The method was further validated as per ICH guidelines [8] for the parameters like precision, accuracy, sensitivity, and linearity. The results of analysis were validated statistically and by recovery studies. These methods of estimation of Indapamide were found to be simple, precise, accurate and economic.

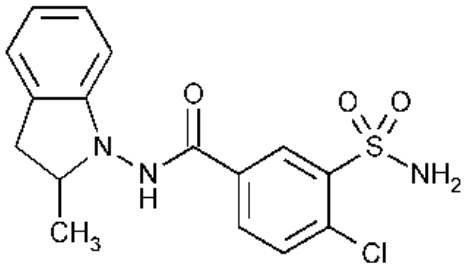

Fig 1: Structure of Indapamide

MATERIALS AND METHODS

Samples

Indapamide, 3-(amino sulfonyl)-2methyl-4-imdol-1-yl) benzamide, was kindly provided by Cadilla Pharmaceuticals Ltd. Gujarat, India. The pharmaceutical formulation used was Natrilix, was procured from local market.

Reagents

Methanol and acetonitrile were of HPLC grade, from Thomas Baker (India). Water used was bidistilled.

Apparatus

A Jasco HPLC system (Japan) composed of a PU-2080 plus pump equipped with a 7725i Rheodyne (CA, USA) injector, an UV-2075 plus UV–vis detector and a LC-Net II/ADC with inbuilt Borwin software.

Chromatographic conditions

The separation was performed on a 25 cm×4.6mm i.d. HiQ Sil-C18 HS column (Kya Tech, Japan). The flow rate was 1.0 mL min^{-1}. The injection volume was 20µl. The detection wavelength was set at 242 nm. The mobile phase consisted of Acetonitrile: Methanol: Water in the ratio of 40:50:10 (v/v/v). The run time was set at 10 min and column temperature was maintained at ambient. Prior to injection of analyte, the column was equilibrated for 30 min with mobile phase. The mobile phase was premixed, filtered through 0.45 µm membrane filter and degassed by sonication

3.0 Method Validation

3.1 Linearity

A stock solution of IND (1000 µg mL^{-1}) was prepared by dissolving 100 mg drug in 100 ml mobile phase then solutions of different concentrations (10–60 µg mL^{-1}) for construction of calibration plots were prepared from this stock solution. The mobile phase was filtered through a 0.45 µm membrane filter and delivered at 1.0 mL min^{-1} for column equilibration; the baseline was monitored continuously during this process. The detection wavelength was 242 nm. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.

3.2 Accuracy

Accuracy was determined by the standard addition method. Previously analyzed samples of IND (10 µg mL^{-1}) were spiked with 80, 100, and 120% extra IND standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%), RSD (%), and standard error (SE) were calculated for each concentration.

3.3 Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability of sample injection was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. For both intra-day and inter-day variation, solutions of IND at single concentrations was determined.

3.4 Reproducibility

The reproducibility of the method was checked by determining precision on a different column, analysis being performed by another analyst. For both intra-day and inter-day variation, solutions of IND at single concentrations (10μg mL^{-1}) were determined six times.

3.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by the standard deviation ($S_{y/x}$) method. LOD and LOQ were determined from the slope, S, of the calibration plot, $S_{y/x}$, by use of the formulae

LOD = 3.3 × $S_{y/x}$/S and

LOQ = 10 × $S_{y/x}$/S.

3.6 Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of IND. Robustness was determined by changing the mobile phase flow rate to 0.9 and 1.1 mL $min^{-1}$and the concentration of methanol in the mobile phase to 48 and 52%.

3.7 Stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 48 hrs. under laboratory bench conditions (33 ± 1°C) and under refrigeration (8 ± 0.5°C).

3.8 Procedure for pharmaceutical formulation

For tablets, 20 units were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 2.5 mg of Indapamide was transferred into a 100 ml volumetric flask and sonicated for 15 min with 100 ml of mobile phase. The resulting suspension was filtered through 0.22μm membrane filter. A suitable aliquot of this filtrate was diluted with mobile phase in order to obtain a final concentration of 10 to 60 μg mL^{-1}. A 10 μl of the obtained solution was chromatographed.

RESULTS AND DISCUSSION

4.1 Method Development

The HPLC procedure was optimized with a view to developing a method. From several solvents and solvent mixtures investigated Acetonitrile: Methanol: Water in the ratio of 40:50:10 (v/v/v) was found to furnish sharp, well-defined peak with very good symmetry and low t_R (3.233 min) (Fig. 1). Various other mobile phases tried earlier either did not give well defined peak in a short time, therefore were not considered. The final selection on mobile phase composition and flow rate was made on the basis of peak shape (peak area, peak asymmetry & tailing factor), baseline drift, time required for analysis, and cost of solvent, and Acetonitrile:Methanol:Water in the ratio of 40:50:10 (v/v/v) was selected as the optimum mobile phase. Under these conditions the retention time was 3.233 ± 0.01 min.

Table 1. Optimized Chromatographic Conditions

Parameters	Conditions
Stationary phase (column)	HiQ Sil-C18 HS
Mobile Phase	Acetonitrile:Methanol:Water in the ratio of 40:50:10 (v/v/v)
Flow rate (ml min^{-1})	1.0
Runtime (min)	10
Column Temperature(ºC)	Ambient
Volume of Injection (μl)	20
Detection wavelength (nm)	242nm
Drug Retention Time(min.)	3.233

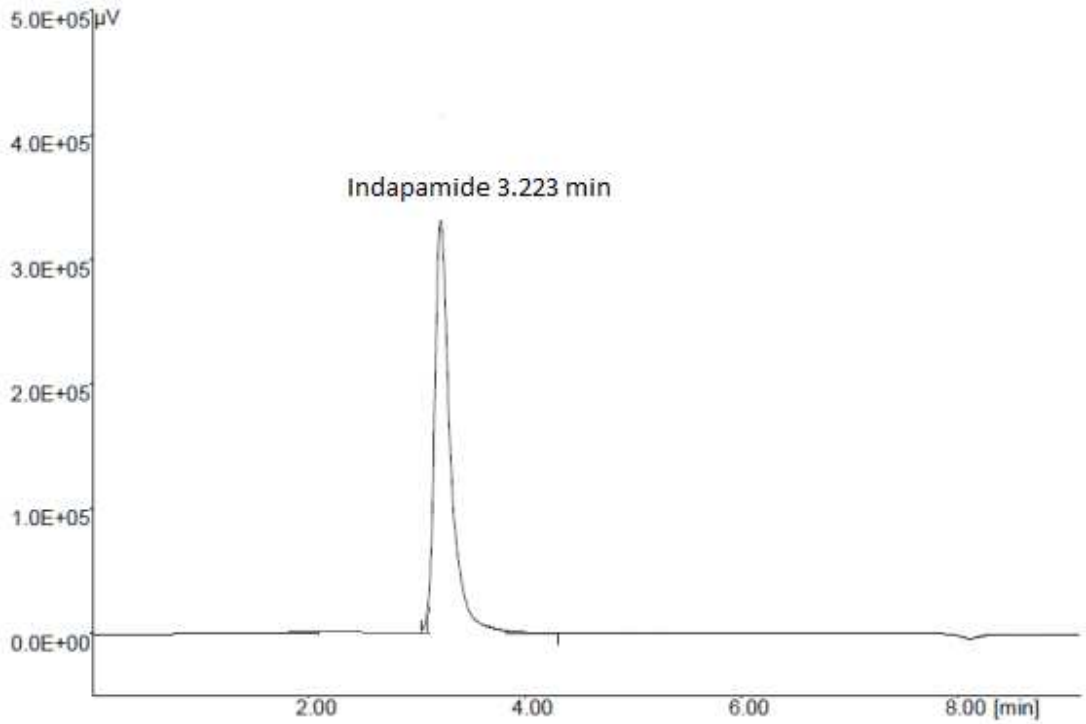

Figure 2. Typical chromatogram of IND in Acetonitrile: Methanol: Water (40:50:10) t_R 3.225 min

5.0 Validation of the Method

5.1 Linearity

The calibration plot of peak area against concentration was linear in the range investigated (10–60 µg mL^{-1}). The low values of RSD and standard error show the method is precise. Statistical calculations were performed at the 5% level of significance. The linear regression data for the calibration plot are indicative of a good linear relationship between peak area and concentration over a wide range. The linear regression equation was y = 0.00598x+ 0.00236and the regression coefficient was 0.9995. The correlation coefficient was indicative of high significance. The low values of the standard deviation, the standard error of slope, and the intercept of the ordinate showed the calibration plot did not deviate from linearity. There were no significant differences between the slopes of standard curves constructed on different days.

Table 2. Statistical data of calibration curves of IND

Parameters	IND
Linearity (µg mL^{-1})	10 - 60
Regression equation	23273x + 20134
Correlation coefficient (R^2)	0.9995

Table 3. System Suitability Parameters

Parameters	Obtained Values
Theoretical plates (N)	3024
Tailing Factor	1.23
LOD (µg mL^{-1})	0.52
LOQ (µg mL^{-1})	0.78

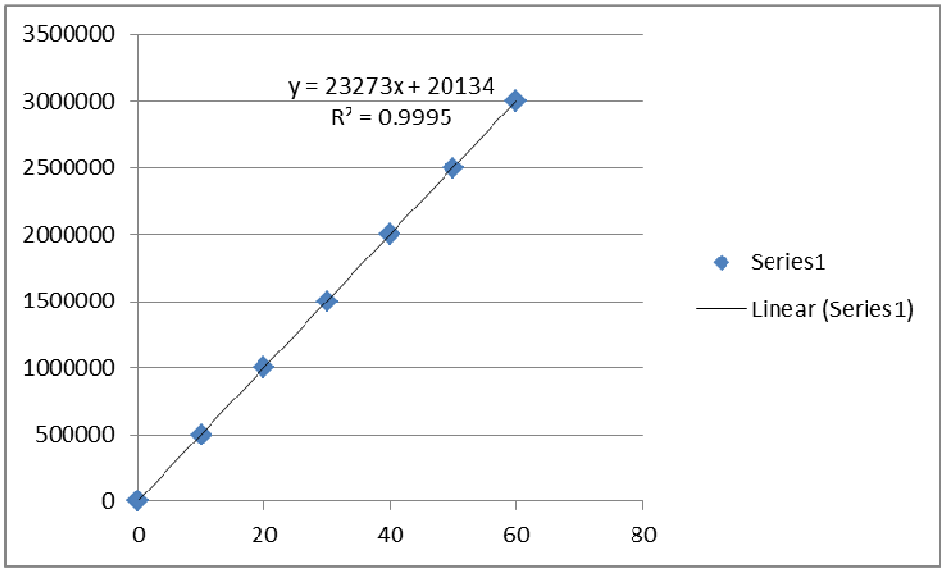

Fig 3: Calibration Curve of Indapamide

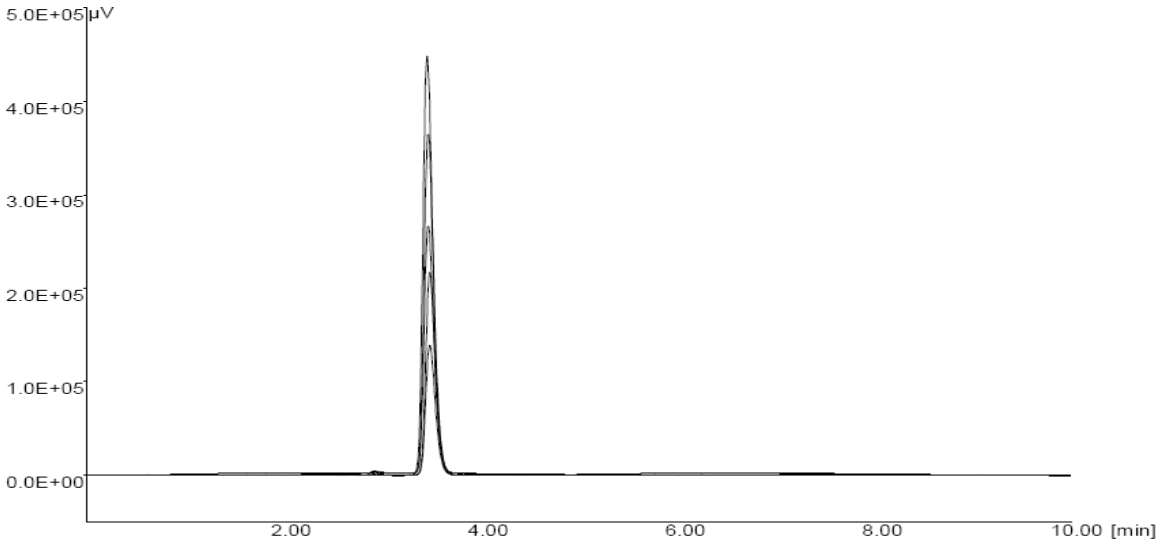

Figure 4. An overlain chromatogram of IND

5.2 Accuracy

The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was 98.16 – 100.12%. The values of recovery (%), RSD (%), indicate the method is accurate.

Table 4. Result of Recovery Studies of Indapamide

Level of Recovery	Amount Present in formulation (µg mL^{-1})	Amount of pure drug Added (µg mL^{-1})	% Recovery*	R.S.D.	S.E.
80	10	8	98.16	1.50	0.029
100	10	10	99.48	0.96	0.035
120	10	12	100.12	1.70	0.039

** Indicates mean of three determinations, , R.S.D. =Relative Standard Deviation, S.E. =Standard Error*

5.3 Precision

Intraday and inter-day precision were carried out for the various concentrations of the sample at different time intervals in the same day and at same time on different days. The concentration of the sample solution was

determined as per the procedure given for the tablet formulation by determining peak area at selected analytical wavelength 242 nm. The variation of the results within the same day was analysed and statistically validated.

Table 5. Results Analysis of Precision Studies

Concentration ($\mu g\ mL^{-1}$)	Repeatability (intra day precision) *		Intermediate precision (inter day) *	
	% RSD	SE	% RSD	SE
10	0.4404	0.60	0.5588	0.81

** Indicates mean of six determinations, R.S.D. =relative Standard Deviation, S.E. =Standard Error*

5.4 Reproducibility

Reproducibility was checked by measuring the precision of the method on another column with analysis performed by another person. Both intra-day and inter-day precision were determined. There were no significant differences between RSD (%) values for intra-day and inter-day precision, which indicates the method, is reproducible.

5.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the method, determined by the standard deviation method, as described above, were 0.52 and 0.78 $\mu g\ mL^{-1}$, respectively, which indicated the method can be used for detection and quantification of IND over a very wide range of concentrations.

5.6 Robustness

There was no significant change in the retention time of IND when the composition and flow rate of the mobile phase were changed. The low values of the RSD indicated the robustness of the method.

Table 6. Robustness of the method

System suitability parameters	Normal condition	Change in condition	Change in % RSD
Flow Rate	1.0 mL min^{-1}	0.9 mL min^{-1} 1.1 mL min^{-1}	0.034 0.026
Mobile phase ratio (Acetonitrile: Methanol: water)	40:50:10	38:54:08 52:42:06	0.050 0.039

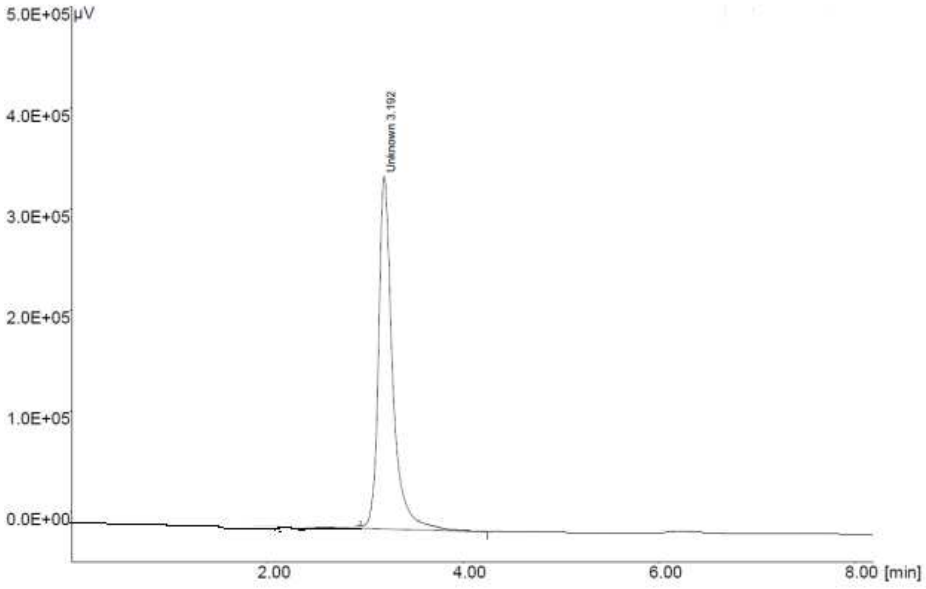

Figure 3. A chromatogram of IND formulation

5.7 Analysis of IND from tablet formulation

The proposed method was applied to the determination of Indapamide in tablets formulation (2.5 mg). The mean average (three replicates) was found to be 2.48 mg corresponding to a mean recovery of 99.12% with an R.S.D. of 0.030%. This result was in good agreement with the label value. It should be pointed out that the chromatogram of the solution of excipients is absolutely free of any peak indicating thus that no interference from the excipients is encountered.

Table7. Analysis of commercial formulation

Commercial formulation	Label claim (mg)	% Label claim estimated*	S.D.	%RSD
Tablet	2.5	99.12	0.050	0.030

*SD= Standard deviation, RSD = Relative standard deviation, *Average of six determinations*

CONCLUSION

A simple and rapid HPLC method has been developed for the determination of Indapamide. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The method is reliable and convenient for routine control and stability assays of Indapamide in both raw material and tablets.

Acknowledgement

The authors are thankful to Cadilla Pharmaceuticals Ltd., Gujarat, India for supplying gift sample of Indapamide and to the management, Sinhgad Technical Education Society's , Smt. Kashibai Navale College of Pharmacy, Kondhwa (Bk.), Pune-48, for providing excellent research facilities.

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