Research Article

HPTLC Method Development and Validation of Indapamide in Bulk Drugs and Formulation Form

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ABSTRACT

The present work describes a simple, precise and accurate HPTLC method for estimation of Indapamide as bulk and in tablet dosage form. Validation was carried out in compliance with International Conference on Harmonization guidelines. It employs aluminium backed silica gel 60 F254 TLC plates, (20 cm × 10 cm, layer thickness 0.2 mm) pre-washed with methanol and mobile phase comprising *of Ethyl acetate/Water/25% Ammonia (8: 2: 0.1, v/v/v).* The developing solvent was run up to 80 mm in *CAMAG* chamber previously saturated with 20.0 ml of solvent mixture for 20 min. Densitometric scanning was then performed with *CAMAG* TLC Scanner-IV equipped with win CATS (version 1.4.6) at λ max 241 nm. *This solvent system was found to give compact spots for Indapamide with Rf value 0.62 ± 0.03.* The limit of detection and limit of quantitation were found to be 40ng/spot & 120ng/spot for Indapamide. *Linear regression analysis showed good linearity (r2= 0 .9989) with respect to peak area in the concentration range of 150–900 ng per spot. The method was validated for precision, accuracy, specificity, and robustness.* The proposed method can also be used for routine quality control to accurately determine Indapamide in bulk and tablet dosage form.

Keywords: Densitometric scanning, HPTLC, Indapamide, Method development, Method validation.

INTRODUCTION

Indapamide ,4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)- 3-sulfamoyl-benzamide is having marked effect on hypertension, as well as decompensated cardiac failure which is given orally.[1] It is a non-thiazide sulphonamide diuretic. Its molecular formula is C16H16CIN3O3S and it has a molecular weight of 365.84 g/mol3. The drug is official in United States Pharmacopoeia (USP). [2] 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.

Various techniques have been reported for the determination of Indapamide, including colorimetry,[3,4] fluorimetry,[5,6] electrochemical methods,[7,8] Spectrophotometric method,[9] Four HPLC methods[10-13] and few HPTLC methods.[14,15] It is necessary to develop simple, precise and accurate HPTLC method for estimation of Indapamide as bulk and in tablet dosage form. So we develop this method which can be used for routine quality control to accurately determine Indapamide in bulk and tablet dosage form.

Indapamide is a thiazide diuretic drug which is used alone or in combination with other with other antihypertensive drugs.

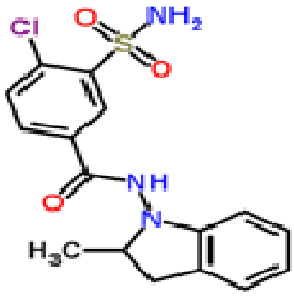

Figure 1: Structure of Indapamide

MATERIALS AND METHODS

Instruments and Apparatus

A *CAMAG* HPTLC instrument consisting of Linomat V Semi-automatic spotter equipped with a 100 µL syringe, Scanner-IV, Twin-trough developing chambers, and viewing cabinet with dual wavelength UV lamps (*CAMAG*, Muttenz, Switzerland) were used. HPTLC plates used were of aluminium backed silica gel 60F254 with 0.2 mm thickness, 20 × 20 cm (E. Merck, Darmstadt). Sartorius CP224S analytical balance (Gottingen, Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

Reagents and Materials

Indapamide pure powder with 99.98% purity was kindly gifted by Dishman pharmaceuticals and chemicals Ltd. All the reagents used during the study were procured from Merck (chemicals) Pvt. Limited, Germany and were of analytical grade. Tablets were purchased from the local pharmacy.

Chromatographic Conditions

Before analysis, HPTLC plates were cleaned by predevelopment with methanol and activated at 110°C for 5 min for solvent removal. Solutions of Indapamide were applied to plates (10 × 10 cm) by means of a Linomat V semiautomatic spotter equipped with a 100 µL syringe and operated with settings of band length, 8 mm; distance between bands, 10 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10mm. The plate was developed in a twin-trough chamber previously saturated for 20 min with the mobile phase, Ethyl acetate/Water/25% Ammonia (8: 2: 0.1, v/v/v) to 8 cm. Densitometric scanning was performed using a CAMAG TLC scanner IV in the reflectance-absorbance mode at 241 nm for all measurements and operated by the WinCATS software.

Preparation of Standard Solution

Accurately weighed 10mg of Indapamide was transferred to a 10 mL volumetric flask then from this stock solution 1.5ml diluted to 10 ml (150 ng/µl).

Method Validation

The HPTLC method was validated as per ICH guidelines.[16,17]

Linearity

Accurate quantities from working standard solutions (1, 2, 3, 4, 5, 6, 7and 8 µL) were applied to the TLC plate to give bands containing 150–1200 ng of INDAPAMIDE per spot, and the plate was developed, using the previously described optimized mobile phase, and scanned. The experiment was repeated for five times. The calibration curves were constructed by plotting peak areas versus concentrations.

Accuracy (% Recovery)

Accuracy of the method was determined by standard addition method in which the known amount of standard INDAPAMIDE solutions were added to pre analyzed tablet solution. These amounts corresponded to 80, 100, and 120% of the amounts claimed on the label. The amounts of INDAPAMIDE were estimated by applying these values to the regression equation of the calibration curve. Accuracy study was performed for five times, and % recovery of INDAPAMIDE was calculated.

Method Precision (Repeatability)

The precision of the instruments was checked by repeated spotting of same standard solution of INDAPAMIDE and repeated scanning of the same spot (n = 6) of INDAPAMIDE without changing the position of plate for the HPTLC method. Repeatability is reported in terms of relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)

The intra-day and inter-day precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days for 3 different concentrations of INDAPAMIDE (60, 100, and 140 ng/spot). The results are reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the both drugs were found by visual inspection.

Robustness of the Method

Robustness of the proposed method was estimated by changing different conditions like mobile phase composition (±0.2 ml for each component), mobile phase volume (varied ±3%), scanning wavelength ±1 nm, peak areas were measured after development of plate, and % RSD was calculated. A concentration level of 1000 ng per band was employed.

Solution stability study

The results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 hour at ambient temperature with the consideration of RSD < 2.0 % in % assay value difference against interval value.

Analysis of Indapamide in Tablets

To determine the content of Indapamide in tablet (label claim: 50mg per tablet) 10 tablets were weighed, their mean weight determined and finely powdered. Powder equivalent to 1.5mg of Indapamide was transferred into a 10mL volumetric flask containing 5mL methanol, sonicated for 30min, and diluted to the mark with methanol. This volumetric flask was kept covered with aluminum foil (150 ng/µl). Appropriate volume of solution was applied on TLC plate followed by development and scanning. 4 µL of this solution was applied to the HPTLC plate at 0.6 µg per spot and followed by development. Analysis was carried out in triplicate, peak areas were measured at 241nm, and sample concentrations calculated. The potential interference from excipients was also examined.

Validation of the Method

The calibration plot was linear over a concentration range of 150–900 ng per spot for Indapamide. A good linear relationship observed over this range (r^2= 0. 99890± 0.0069) indicated that the method is linear. Repeatability of sample application and sample measurement was expressed as % RSD and was found to be 0.71% and 1.28% for six replicate determinations. The low values of % RSD indicate that the proposed method is repeatable. The % RSD value obtained for intra-day and inter-day variation were 0.648% and 1.012%, respectively, which indicates that proposed method is precise. RSD of peak areas during robustness studies were calculated for changes in parameters and were less than 2% which indicates that this method is robust and reproducible. LOD and LOQ values were found to be 40 and 120 ng per spot, respectively, and pointed towards adequate

sensitivity of the method. Peak purity for INDAPAMIDE was assessed by comparing spectra acquired at the start (s), apex (m), and end (e) of the peak obtained from the scanning of spot, that sis, r (s,m) = 0. 9994 and r (m, e) = 0. 9996. The high value of r^2 indicates specificity of the method. Accuracy was determined on previously analysed formulations after spiking with 80, 100, and 120% of the additional drug. Mean recovery obtained is 99.8 1 % ± 0 .697. Validation parameters are summarized in Table 1.

Table 1: Validation parameters

Validation Parameter	Indapamide
Linearity Equation (r^2) Range	Y = 621.181 + 7.894 * X (0.9989) 150 –900 ng per band
Repeatability- sample application sample measurement	 NMT 0.71% NMT 1.28%
Precision (% RSD)- Intra-day Inter-day	 0.648% 1.012%
Accuracy (% mean recovery)	99.8 1 % ± 0 .697
LOD	40ng
LOQ	120ng
Specificity	Specific
Peak Purity	r (s,m) = 0. 9994 r (m,e) = 0. 9996
Stability of solution	Stable up to 48 Hrs
Robustness	Robust

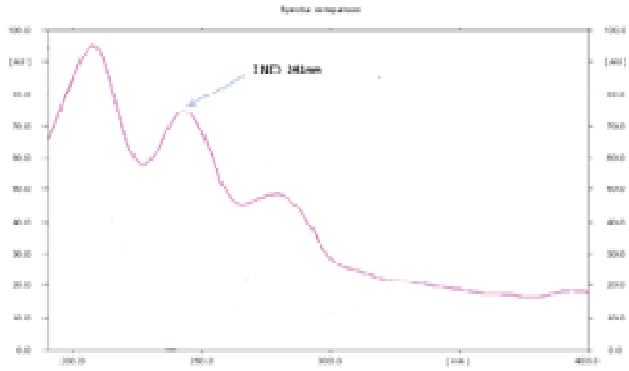

Figure 2: UV-Visible spectrum of Indapamide 241nm

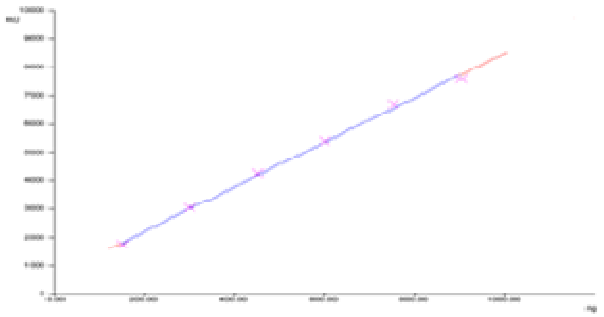

Figure 3: Linearity of Indapamide

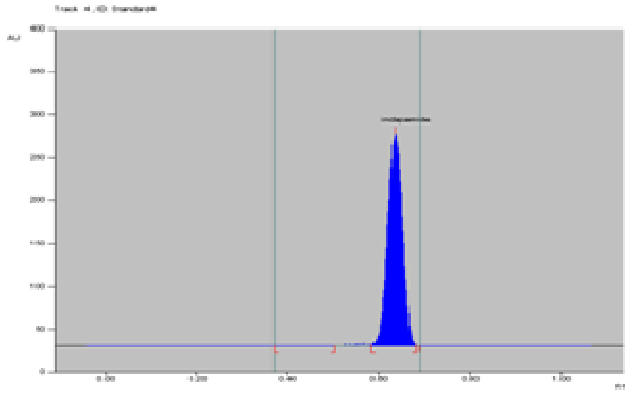

Figure 4: Representative Densitogram of Indapamide @241nm

Analysis of Formulated Tablets

A single band was observed in samples extracted from tablets, and there was no interference from the excipients which might have been present in the tablets. The amount of Indapamide obtained in tablets is in good agreement with label claim. The content of INDAPAMIDE in tablets was found to be 99.81%**.** It was therefore inferred that there is no intereference of excipients during the analysis of Indapamide normally present in tablets. Thus the method can be applied for the routine analysis of INDAPAMIDE in pharmaceutical formulations.

CONCLUSION

The developed HPTLC procedure was simple, precise, specific and accurate. Statistical analysis indicated that the method was reproducible and selective for the analysis of Indapamide in bulk drug and in tablets without interference from excipients. This methodology may also be applied to the study of degradation kinetics and for its determination in plasma and other biological fluids.

REFERENCES

1. Martindale - The Complete Drug Reference, Pharmaceutical Press, London, UK, 32nd Ed, 1999, 890.
2. United States Pharmacopeia and National Formulary (USP 30-NF 25), Rockville, MD: United States Pharmacopoeial Convention, 2, 2007, 2340.
3. Guven KC, Bergisadi N, Peremeci E, Acta Pharm. Turc, 28, 1986, 99–102.
4. Agrawal YK, Majumdar FD, Anal. Lett, 28, 1995, 1619–1627.
5. Grebow PE, Treitman JA, Yeung AK, J.Pharm. Sci, 67, 1978, 1117–1120.
6. Grebow PE, Treitman JA, Yeung AK, Johnston MM, J. Pharm. Sci, 70, 1981, 306–308.
7. Legorburu MJ, Alonso RM, Timenez RM, Electroanalysis (NY) 8, 1996, 280–284.
8. Radi A, J. Pharm. Biomed. Anal, 24, 2001, 413–419.
9. J.Pai, SK Shetty, G Chenna, B. Gopinath, M. Ahmed, Development of New Spectrophotometric methods for the determination of Indapamide in Bulk and Pharmaceutical formulations, International Journal of ChemTech Research, 2011, 755-760.

10. J. Pai, S. Shetty, B. Gopinath, G. Chenna, Development and Validation of RP-HPLC Method for Quantitative estimation of Indapamide in Bulk and Pharmaceutical dosage forms, International Journal of ChemTech Research, 2011, 1482-1487.

11. M.J. Legorburu, R.M. Alonso, R.M. Jiménez, E. Ortiz, Quantitative Determination of Indapamide in Pharmaceuticals and Urine by High-Performance Liquid Chromatography with Amperometric Detection, Journal of Chromatographic Science, 37(8), 283-287.

12. Tai-Jun Hanga, Wei Zhaoa, Jie Liua, Ming Songa, Ying Xiea, Zhengxing Zhanga, Jianping Shenb, Yingdi Zhang, A selective HPLC method for the determination of Indapamide in human whole blood: Application to a bioequivalence study in Chinese volunteers, Journal of Pharmaceutical and Biomedical Analysis, 40(1), 2006, 202-205.

13. X.Gao, J. Chen, N. Mei, W. Tao, W. Jiang, Xingu Jiang. HPLC Determination and Pharmacokinetic Study of Indapamide in Human Whole Blood, Chemistry and Materials Science Chromatographia, 61(11- 12), 2005, 581-585.

14. Dewani MG, Bothara KG, Madgulkar AR, Damle MC, Simultaneous Estimation of Perindopril Erbumine and Indapamide in Bulk Drug and Tablet Dosage Form by HPTLC, International Journal of Comprehensive Pharmacy, 2(1), 2011, 1-4.

15. Yadav SS, Rao JR, Simultaneous hptlc analysis of atenolol and Indapamide in tablets, Pharma Globale: International Journal of Comprehensive Pharmacy, 2(9), 2011, 1-4.

16. ICH, Q2B, Validation of analytical procedures: Methodology, in proceedings of the international conference on Harmonization, Geneva, November 1996.

17. ICH, Guidance on Analytical Method Validation, in: Proceedings of International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, September, 2002.

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