Development and Validation of Diazoxide in Bulk and Pharmaceutical Dosage Form by RP-HPLC Method

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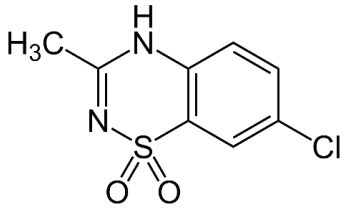
***Abstract*—This assay method was development and validation of Diazoxide in bulk and pharmaceutical dosage form by RP-HPLC method. The drug separate by using RP-HPLC on a RP-Cosmosil C_{18} (250 mm ×4.6 mm I.D.) with particle size 5 µm was selected. The mobile phase was MEOH: water 0.1% PH 3 Adjusted with OPA, (85:15 % v/v), at flow rate 0.7 ml/min. The UV detector was operated the overlain spectra 268 nm was selected for the estimation of the drug. Linearity, accuracy, precision, ruggedness and system suitability testwas found to be acceptable concentration ranges 5-25µg/ml with a $R^2$0.9996value respectively, in the drug.**

***Key Words*—Diazoxide, Validation, RP-HPLC, Simultaneous estimation.**

I. INTRODUCTION

Diazoxide (7-Chloro-3-methyl-4H-1,2,4-benzothiadiazine 1,1-dioxide) is a benzothiadiazine derivate with antihypertensive and hyperglycemic activities. Diazoxide increases membrane permeability to potassium ions in vascular smooth muscle, thereby stabilizing the membrane Action potential and preventing vascular smooth muscle contraction. This results in peripheral vasodilatation and decreases in peripheral vascular resistance. This agent also inhibits insulin release by interacting with ATP-sensitive potassium channels of pancreatic islet beta-cells.



Diazoxide inhibits insulin release from the pancreas, by opening potassium channels in thebeta cell membrane. Diazoxide is chemically related to thiazide diuretics but doesnot inhibit carbonic anhydrase and doesnot have chloriuretic or natriuretic activity. It also exhibits hypotensive activity by reducing arteriolar smooth muscle and vascular resistance [1,2,3].

II. MATERIAL AND METHOD

Chemical and reagent

Diazoxide was kindly supplied as a gift sample by Jolly Healthcare Jaipur. This drug was used as working standard. All the chemicals used were of HPLC grade (Merck Chem. Ltd., Mumbai) used without further purification. Double distilled water was used for mobile phase preparation.

Apparatus

The chromatographic system Agilent Technologies 1100 series (Gradient System)with a 20µL fixed loop and UV730D absorbance detector. The separation was performed on a RP- Cosmosil C_{18} column (250 mm ×4.6 mm I.D.) with particle size 5 µm was selected. Chromatographic data were recorded and processed using Chemstation.

Chromatography Conditions

Chromatographic separations of active substances were obtained by using RP- Cosmosil C_{18} (250 mm ×4.6 mm I.D.) with particle size 5 µm. Mobile Phase MEOH: water 0.1% PH 3 Adjusted with OPA pH 3, (85:15 % v/v), at flow rate of 0.7mL/min. The detection at 268 nm. The total time of analysis was less than 10 min.

Standard solution

The stock standard solution of Diazoxide (20 µg/mL) was prepared by dissolving10 mg of Diazoxide in 10 ml methanol (prepared solution is 1mg/ml) and take 0.2ml in 10ml with mobile phase MEOH: water 0.1% PH 3 Adjusted with OPA, (85:15 % v/v).

Sample solution

Accurately weighed quantity of 10 mg (Diazoxide) was transferred to 10 ml volumetric flask containing 10 mL methanol and volume was adjusted up to mark. It was further diluted to get concentration 20 µg/ml of Diazoxide. Constant volume 20 µl was injected into column and peak area was recorded.

Selection of Detection wavelength

From the overlain spectra 268 nm was selected for the estimation of the drug simultaneously (Figure-1)

Validation of Proposed Method[4-10]

The developed method was validated as per ICH guidelines. Analytical method was carried out as per ICH method validation guidelines Q2 (R1).

Calibration curve (linearity)

From the stock standard solution, aliquots portions (10mg) were transferred into a series of 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentration in the range of 5-25µg/ml Diazoxide. A constant volume of 20 µl of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *versus* the drug concentration.

Accuracy (% recovery)

It was done by recovery study using standard addition method at 80%, 100% and 120 % level; known amount of standard Diazoxide were added to pre-analyzed sample (20 µg/mL of Diazoxide) and subjected them to the proposed HPLC method.

Method precision (repeatability)

Precision of the method was verified by repeatability and intermediate precision studies.

Intra-day precision was studied by analyzing 5, 15, 25 µg/mL of Diazoxide for three times on the same day.Inter-day precision was checked analyzing the same concentration for three different days over a period of week.Repeatability was measured by analyzing 20 µg/mL of Diazoxide for five times.

Robustness

Robustness of the method was studied by making deliberate changes in few parameters *viz*; change in mobile phase composition, pH, and flow rate. The effects on the results were studied by injecting 20 µg/mL for Diazoxide; one factor was changed at one time to estimate the effect.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of the drug were calculated using the equations according to International Conference on Harmonization (ICH) guidelines.

Specificity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analytes in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analytes in presence of components that may be expected to be present in the sample matrix.

System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

Validation of the Proposed Method

Linearity

The concentration in the range of 5-25µg/ml Diazoxide separately. The linearity of calibration curves was found to be acceptable over the concentration ranges of for Diazoxide with a R^2 0.9996 values respectively.The results are shown in (Table No.1)

Accuracy

The recoveries obtained were 99.20% for Diazoxide, respectively (Table 2). The high values indicate that the method was accurate. The recovery studies showed that the results were within acceptable limits, above 99.5% and below 100.5%.

Method precision

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit 0.03 i.e. % RSD below 2.0 indicating that the method is reproducible. The results are shown in (Table No.2)

LOD and LOQ

LOD values for Diazoxidewasfound to be 0.02389 µg/ml respectively. LOQ values for Diazoxide was found to be 0.072 µg/ml respectively.

Robustness

Robustness of the method was studied by making deliberate changes in few parameters*viz*; change in mobile phase composition, pH and flow rate. Standard deviation was found to be Bellow 1 and % RSD is less than 2 for all results.

System Suitability Test

A sample solution of 20 µg/ml of Diazoxide (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated.

III. RESULTS AND DISCUSSION

The absorption spectra of Diazoxide significantly overlap. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Diazoxide was obtained with a mobile phase consisting of MEOH: water 0.1% PH 3 Adjusted with OPA 3 pH, (85:15 % v/v) 268 nm at flow rate 0.7ml/min. Quantification of the drug was performed at 268 nm. Resolution of the components with clear baseline separation was obtained.

IV. CONCLUSIONS

The proposed RP-HPLC method presented in this paper has compensations of simplicity, precision and convenience for separation and quantitation of Diazoxide and can be used for the assay of their particular dosage form. Moreover, the proposed method is a stability indicating assay method that can determine Diazoxide.

V. ACKNOWLEDGEMENTS

The authors are thankful toJolly Healthcare Jaipur, for supplying API as generous gift sample.

Table 1. Regression analysis of the calibration curves for Diazoxide in the proposed HPLC Method.

Parameter	Diazoxide
Linearity Range (µg/mL)	5-25
Detection Wavelength (nm)	268
Slope ± SD	87.0004
Intercept ± SD	143.518
Correlation coefficient	0.9996

SD- Standard deviation

Table 2. Summary of the validation parameters for the proposed HPLC method

Parameter	Diazoxide
LOD	0.02389 µg/ml
LOQ	0.072 µg/ml
Accuracy	99.20
Repeatability (%RSD, n = 5)	0.0348
Precision (%RSD)	0.0326
Inter-day, n = 3	100.40 (0.30)
Intra-day, n = 3	100.53 (0.04)

LOD = Limit of detection.

LOQ = Limit of quantification

RSD = Relative standard deviation.

Table 3. Assay results for the combined dosage form using the proposed HPLC method

Formulation	Diazoxide
Edumin25 mg	101.93 ±0.02

SD =Standard deviation, 5 determinations

Table 4. System suitability test

System suitability Parameters	Diazoxide
Retention time (t_R)	4.28 min
Theoretical plate (N)	4308
Area	1607.44
Resolution	0

Figure 1: An λ Spectra of Diazoxide

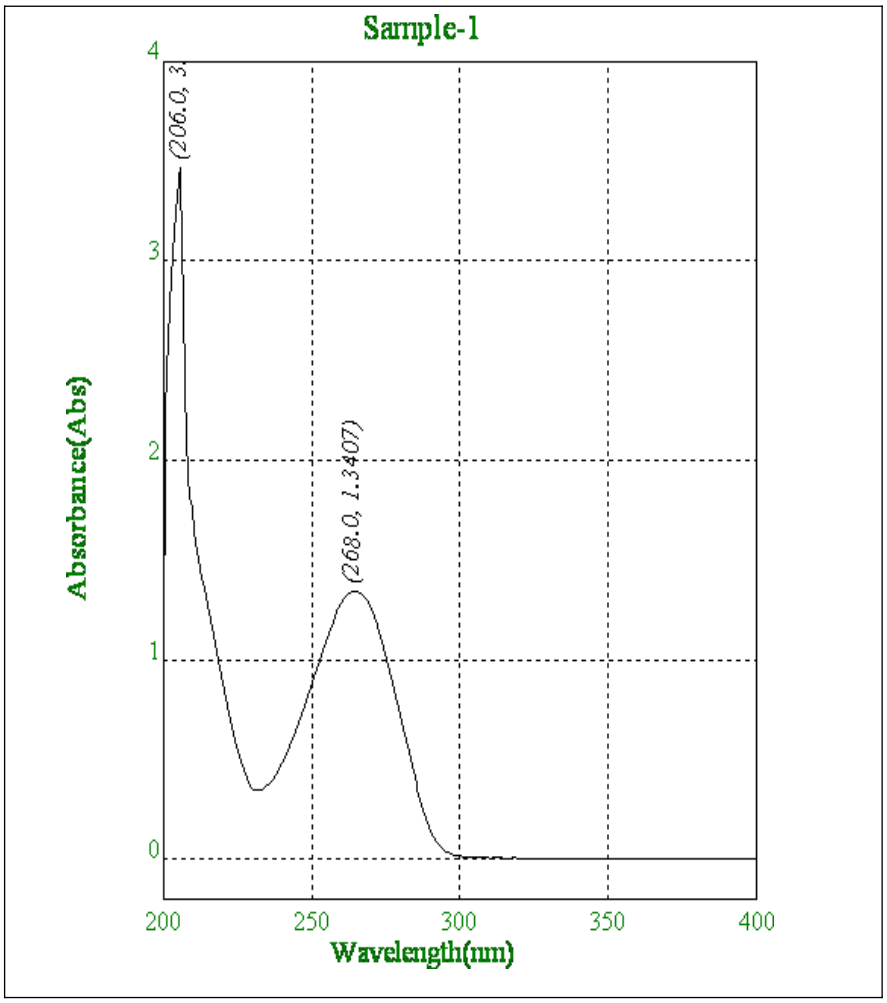


Figure 2: Chromatogram of Diazoxide.

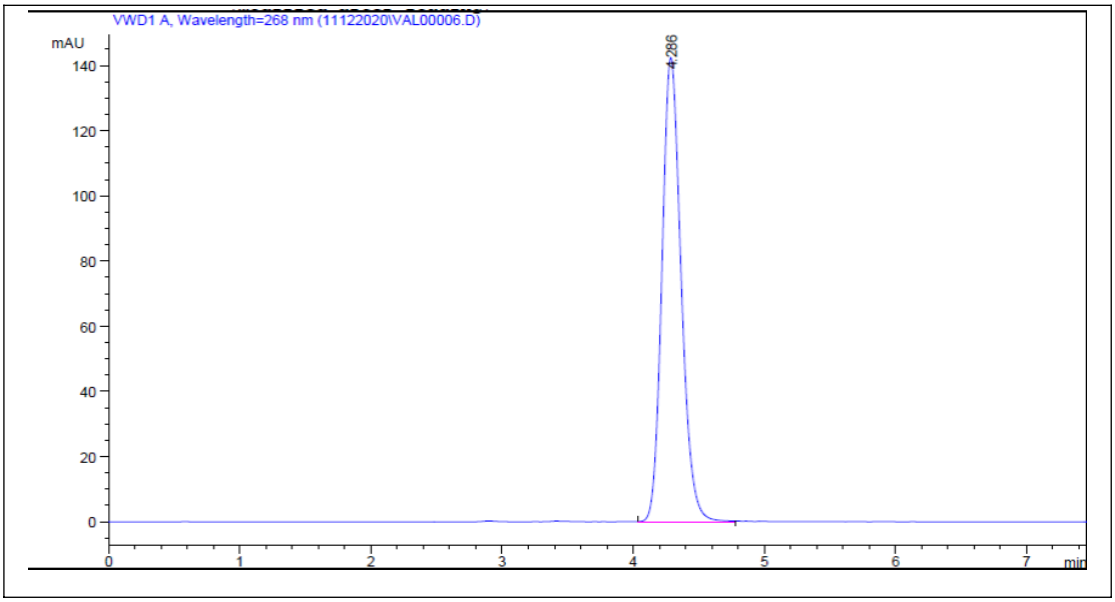
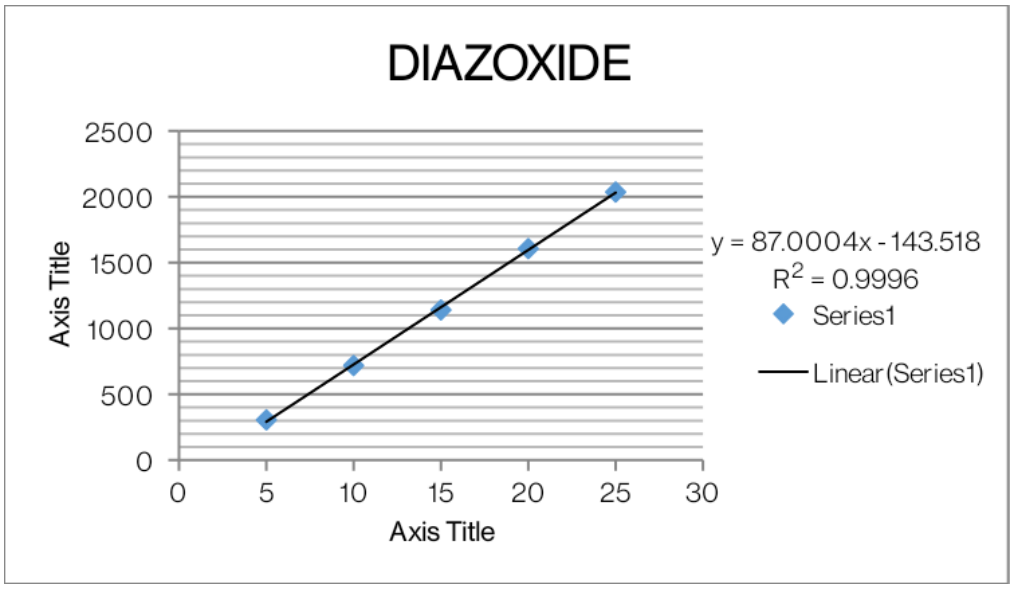


Figure 3. Calibration curve for Diazoxide



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