Research Article **CODEN: AJPAD7** **ISSN: 2321-0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

UPLC ESTIMATION OF SIMULTANEOUS DETERMINATION OF EZETIMIBE AND GLIMEPRIDE IN PHARMACEUTICAL DOSAGE FORM AS PER ICH GUIDELINES

Alagar raja. M[1*], Namratha. CH[1], David banji[1], Rao. K.N.V[1], Selva Kumar. D[2]

[1]*Department of Pharmaceutical Analysis and Quality Assurance, Nalanda College of Pharmacy, Nalgonda, Telangana, India.

[2]School of Pharmacy, Subang Jaya, Taylors University, Malaysia.

ABSTRACT

A simple, accurate, precise, sensitive, rapid UPLC method has been developed and validated for determination of Glimepride and Ezetimibe in its pharmaceutical dosage form. Chromatographic separation was achieved on a inspire C18 column (2.1×50mm,18), by a mobile phase consisted of buffer (PH 2.5,maintained with ortho phosphoric acid) and Acetonitrile in 60:40(V/V) ratio with a flow rate of 0.25 ml/min. The detection wavelength was set at 242 nm. Glimepride and Ezetimibe were subjected to different stress conditions. The degradation products, when any, were well resolved from the pure drug with significantly different retention time values. The method was linear (r^2=0.999).The intra and inter day precisions were satisfactory the relative standard deviations did not exceed 2%. The accuracy of the method was proved the mean recovery of Glimepride and Ezetimibe was 100.27-101.72%. The proposed method has high throughput as the analysis involved short run-time (2mins). The method met the ICH/FDA regulatory requirements. The proposed method was successfully applied for the determination of Glimepride and Ezetimibe with acceptable accuracy and precisions the results demonstrated that the method can be applied successfully for routine use in quality control industry laboratories.

KEY WORDS

Glimepride and Ezetimibe, UPLC, Forced degradation, ICH and FDA.

Author for Correspondence:

Alagar raja. M,
Depatment of Pharmaceutical Analysis and Quality Assurance, Nalanda College of Pharmacy, Nalgonda, Telangana State, India.

Email: madurairaja@hotmail.com

INTRODUCTION

Ezetimibe, 1- (4-fluorophenyl) -3 (R) n [3 n (4 nfluorophenyl) -3 (S) n hydroxyl propyl] - 4 (S) n (4 nhydroxyphenyl) -2-azetidione. A new lipid-loweringagent, which inhibits the absorption of cholesterol from intestine. Ezetimibe localizes and appears to act at the brush borader of small intestine the absorption. Literature reveals that various methods like HPLC, UV spectroscopy and Massption of cholesterol. This leads to a decrease in

the delivery of intestinal cholesterol to the liver. Glimepiride (GLM) is sulfonylurea derivative that is chemically named (1-[[p-[2-(3-ethyl-4-mehyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl]sulfonyl]-3-(trans-4-methylcyclohexyl) urea. It stimulates the release of insulin from functioning pancreatic beta cells, and increases sensitivity of peripheral tissues to insulin.

Literature reveals that various methods like RP-HPLC, UV spectroscopy were reported for the determination of EZETIMIBE and GLM alone and in combination. Even though various methods were reported in the literature for estimation of Ezetimibe and Glimepiride alone and in combination with other drugs no method has been reported for simultaneous estimation of these drugs by using UPLC. Present study was aimed for the simultaneous estimation of Ezetimibe and Glimepiride by UPLC method. The method was validated according to the ICH guidelines.

MATERIALS AND METHODS

Reagents required

Ortho phosphoric acid (AG grade)
Acetonitrile (HPLC grade)
Phosphate Buffer (HPLC grade)
HPLC grade water (Milli Q or equivalent).

Drugs used

The gift samples of and Ezetimibe and Glimepiride were kindly provided by Dr.Reddys Laboratories and Kytross Health Care Limited and the marketed formulations containing Ezetimibe (1 mg), Glimepiride (10 mg) were procured from local pharmacy.

Preparation of Potassium Phosphate buffer

Weighed 6.8grams of Potassium dihydrogen orthophosphate into 1000ml beaker dissolved and diluted to 1000ml with HPLC water. Adjusted the pH to 2.5 With Ortho phosporic acids.

Preparation of mobile phase

Mix a mixture of above buffer 600 mL (60%) and 400 ml Acetonitrile HPLC (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 4.5 µ filter under vacuum filtration. Instrumentation and Chromatographic Conditions. Ultra performance liquid chromatography (UPLC) equipped with auto Sampler and PDA or UV detector.

Chromatographic separations achieved by using inspire C18 (2.1 x 50mm, 1.8m) column or equivalent. Mobile phase was phosphate buffer ph 2.5 600 ml (60%), 400 ml of Acetonitrile HPLC grade (40%). Other parameters were set as flow rate of 0.25 ml per min, for a runtime of 2 min, and detection at a wavelength of 242 nm.

Preparation of solutions

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Glimepride and 10 mg of Ezetimibe working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.1 ml of Glimepride and 1.0 ml Ezetimibe of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 0.3 ml and 3.0ml of Glimepride and Ezetimibe of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 10 mg of Glimepride and 10mg Ezetimibe equivalent weight of the sample into a 10ml clean dry volumetric flask add about 70mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution

Further pipette 1.0 ml of Glimepride and 1.0 ml Ezetimibe of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 0.3 ml and 3.0ml of Glimepride and Ezetimibe of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure

Inject 20µL of Standard preparation ion five times and Sample preparation ion in the chromatograph. Record the chromatograms and measure the peak responses for Ezetimibe, and Glimepiride. The System suitability parameters should be met. From the peak responses calculate the % Assay formulae in content of Ezetimibe and Glimepiride in the sample.

Evaluation of system suitability

The % RSD for the peak area of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %.

The number of theoretical plates (N) for the Ezetimibe and Glimepiride peaks should be not less than 2000.

The Tailing factor (T) for the Ezetimibe and Glimepiride peaks should be not more than 2.0.

RESULTS AND DISCUSSION

Method development

A variety of mobile phases were investigated in the development of a method for the analysis of Ezetimibe and Glimepiride in tablet dosage form. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay. The maximum absorption wavelength of the reference drug solution was found to be 242 nm. This was observed from the UV absorption spectrum and was selected as detection wave length for LC analysis. As the main objective of this chromatographic method was separation of the drugs. During the optimization of the method, different ratios of phosphate buffer (PB), water, methanol and Acetonitrile were tried as mobile phase to get optimal retention time and other peak parameters. The composition and pH of mobile phase was optimized by several preliminary experimental trials to achieve good peak symmetry and short retention time. After several trials, using inspire C18 (2.1 x 50mm, 1.8m) column and the mobile phase consisting phosphate buffer (pH 2.5) and CAN (60:40% v/v), and the flow rate of 0.25ml/min was considered optimum to achieve adequate retention time and sharp peaks of all the two drugs. System suitability parameters (Tailing factor, HETP, Resolution, Theoretical Plates, Asymmetry) for analyte peaks were evaluated.

Validation of UPLC method

System suitability

System suitability parameters, such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table.

Optimized conditions

Equipment : Ultra performance liquid chromatography equipped with Auto Sampler and PDA detector

Column : Inspire C18 (2.1 x 50mm, 1.8µm,) or equivalent

Flow rate : 0.25 mL per min

Wavelength : 242 nm

Injection volume : 5 µl

Column oven : Ambient

Run time : 2 min.

Accuracy (Recovery Studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Ezetimibe and Glimepiride were added to pre-analyzed samples and were subjected to proposed method.

Precision

The standard solution was injected for five times and measured the area for all five injections in UPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. The % RSD for the area of five standard injections results should not be more than 2.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times and measured the area for all five injections in UPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. The % RSD for the area of five standard injections results should not be more than 2.

Linearity

The linearity of the method is the ability to elicit test results that are directly proportional to the concentration of the analyte in samples. The linearity study was made from a series of mixed standard solutions of Ezetimibe and Glimepiride. Since in the available tablet dosage forms contain Ezetimibe and Glimepiride in the ratio 1:10 Individual stock solutions of Ezetimibe and Glimepiride was prepared by dissolving 10mg of

Ezetimibe in 10ml (1000mcg/ml) and 10 mg GLM in 10 ml mobile phase (1000mcg/ml). Further dilution was done to get a concentration of (3mcg/ml and 30mcg/ml).Then suitable volumes of the above stock solutions were mixed together in each case to obtain a series of mixed standard solutions with a concentration range 1-5of mcg/ml for Ezetimibe, 10-50mcg/ml for Glimepiride. Each solution was injected in replicate and chromatogram was recorded. The average peak areas were plotted against to obtain calibration curves. Slopes and intercepts were obtained by using regression equation (y= mx +c) and least square treatment of the results used to confirm linearity of the method developed.

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

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. The Quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%. Standard solution 30 µg/ml of Glimepride and Ezetimibe was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method

Table No.1: Accuracy results for Glimepride

S.No	% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1	50%	44737	5	5.021	101.91%	101.72%
2	100%	90769	10	10.18s	101.68%	
3	150%	136029	15	15.24	101.58%	

Table No.2: Accuracy results for Ezetimibe

S.No	% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
1	50%	354264	5	5.06	101.26%	100.27%
2	100%	707120	10	10.11	101.06%	
3	150%	1033510	15	14.77	98.47%	

Table No.3: Precision of Glimepride and Ezetimibe

S.No	Injection	Area of glimepride	Area of ezetimibe
1	Injection-1	85056	711580
2	Injection-2	86689	703785
3	Injection-3	87941	706453
4	Injection-4	84988	711211
5	Injection-5	86448	712516
6	Average	86224.4	709109
7	Standard Deviation	1235.6	3792.5
8	% RSD	1.432956	0.534821

Table No.4: Intraday Precision (Ruggedness)

S.No	No. of Injection	Area of Glimepride	Area of Ezetimibe
1	Injection-1	84104	716311
2	Injection-2	85918	713654
3	Injection-3	85632	707674
4	Injection-4	87690	713735
5	Injection-5	87344	708578
6	Injection-6	86391	712808
7	Std.deviation	1292.9	3326.0
8	% RSD	1.500261	0.467053

Table No.5: Linearity Results of Glimepride and Ezetimib

S.No	Linearity level	Glimepride		Ezetimibe	
		Conc. (ppm)	Area	Conc. (ppm)	Area
1	I	10 ppm	25658	1 ppm	228083
2	II	20ppm	62140	2 ppm	487422
3	III	30 ppm	93111	3 ppm	710199
4	IV	40 ppm	125624	4 ppm	916177
5	V	50 ppm	153761	5 ppm	1142222
Correlation Coefficient		0.999		0.999	

Table No.6: LOD Results of Glimepride and Ezetimibe

S.No	Drug	LOD	
1	Glimepride	Concentration (µg/ml)	0.4
		Retention time(t_R)	0.774
		Height(µv)	169
		Area(µv)	434
2	Ezetimibe	Concentration(µg/ml)	0.21
		Retention time(t_R)	0.919
		Height(µv)	167
		Area(µv)	490

Table No.7: LOQ Results of Glimepride and Ezetimibe

S.No	Drug	LOQ	
1	Glimepride	Concentration (µg/ml)	0.47
		Retention time(t_R)	0.776
		Height(µv)	569
		Area(µv)	1463
2	Ezetimibe	Concentration(µg/ml)	0.070
		Retention time(t_R)	0.920
		Height(µv)	568
		Area(µv)	1668

Table No.8: System Suitability and Robustness Results for Glimepride

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.225	2653	1.26
2	0. 25	2831.01	1.17
3	0.275	2689	1.26

Table No.9: System Suitability and Robustness Results for Ezetimibe

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.225	3086	1.38
2	0.25	3082.09	1.42
3	0.275	3002	1.41

Table No.10: Robustness Results for Glimepride

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2713	1.27
2	*Actual	2831.01	1.17
3	10% more	2587	1.31

Table No.11: Robustness Results for Ezetimibe

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3865	1.37
2	*Actual	3082.09	1.42
3	10% more	3104	1.42

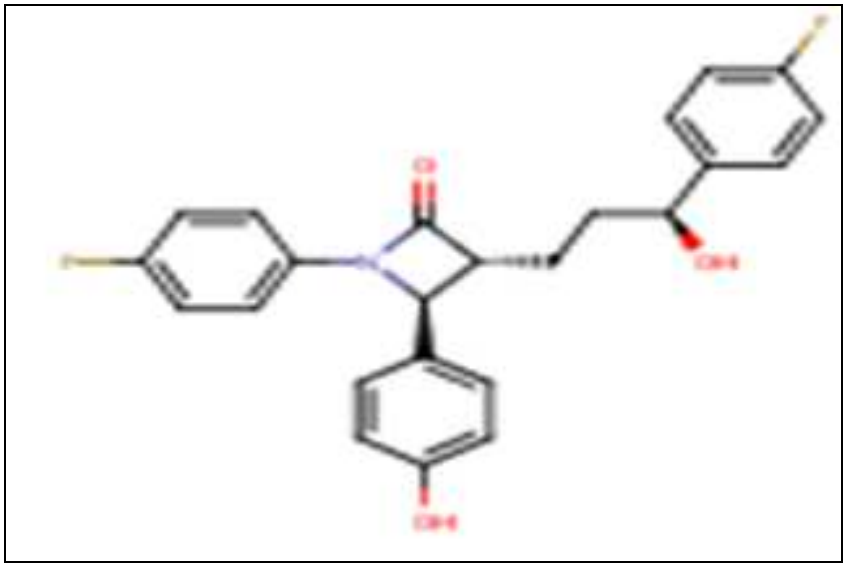


Figure No.1: Structure of Ezetimibe

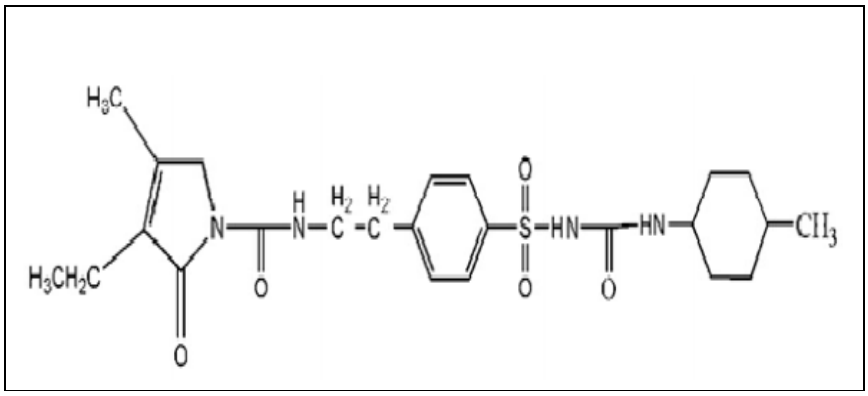


Figure No.2: Structure of Glimepride

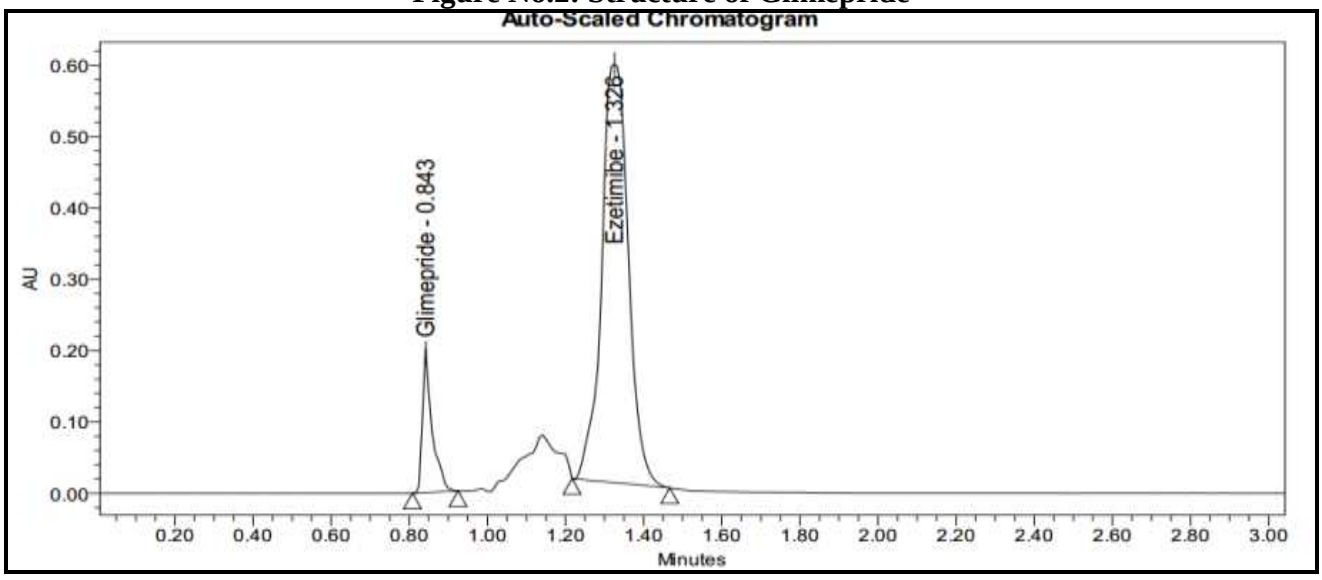


Figure No.3: Chromatogram of Glimepride and Ezetimibe

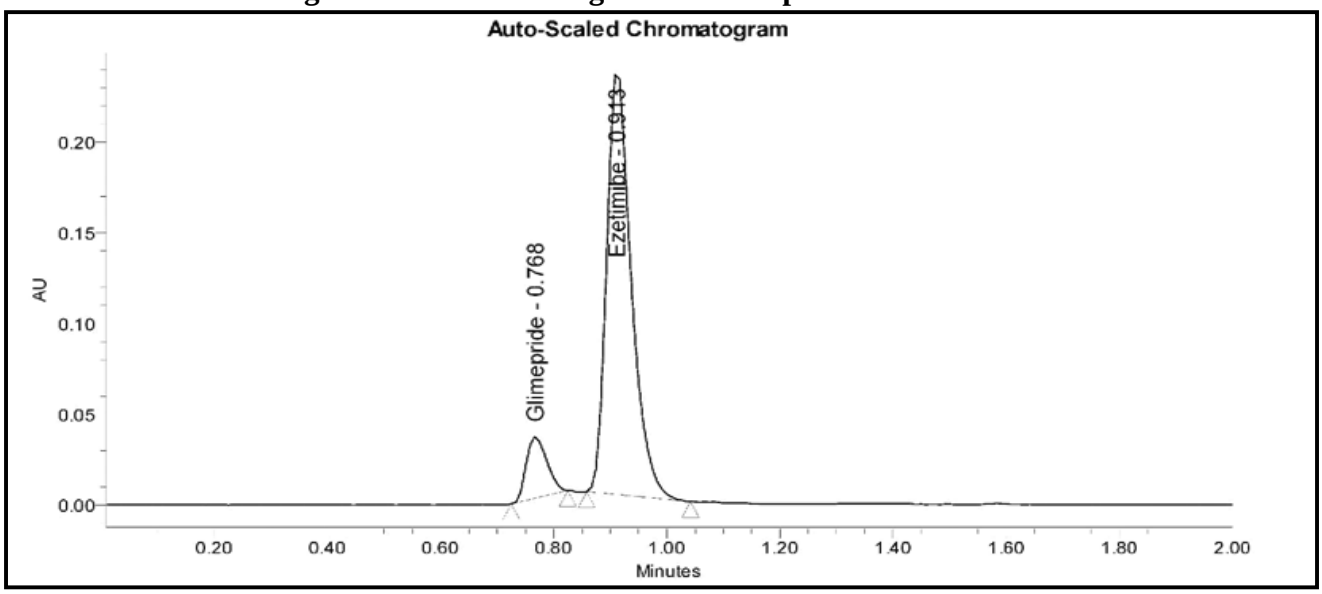


Figure No.4: Chromatogram for precision

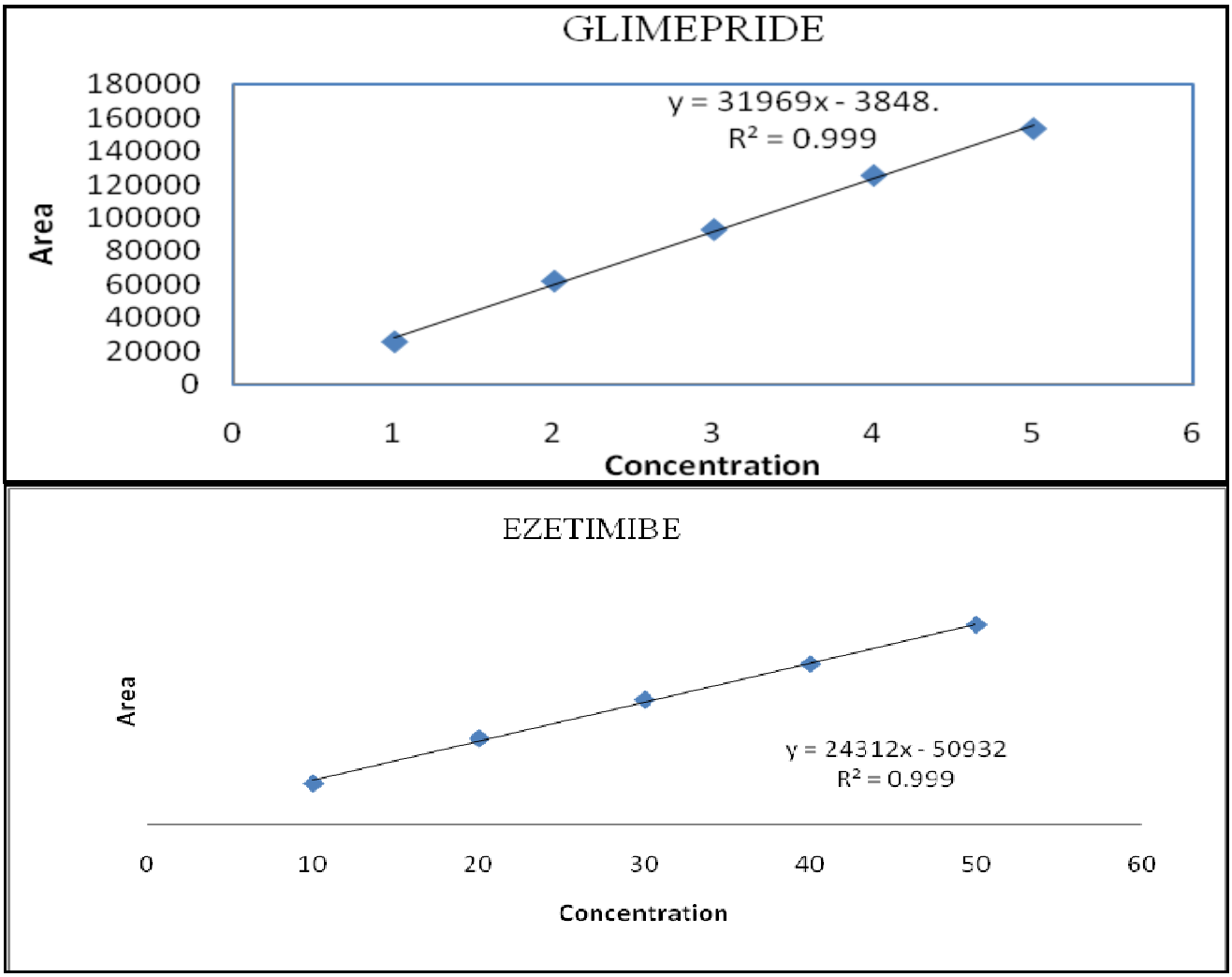
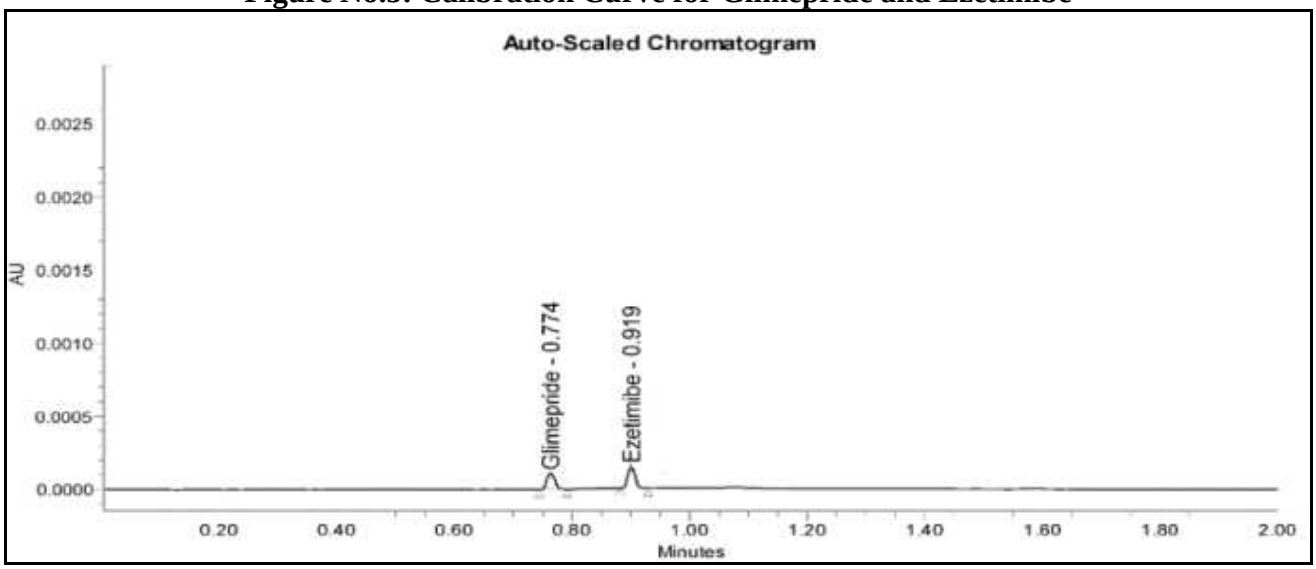


Figure No.5: Calibration Curve for Glimepride and Ezetimibe



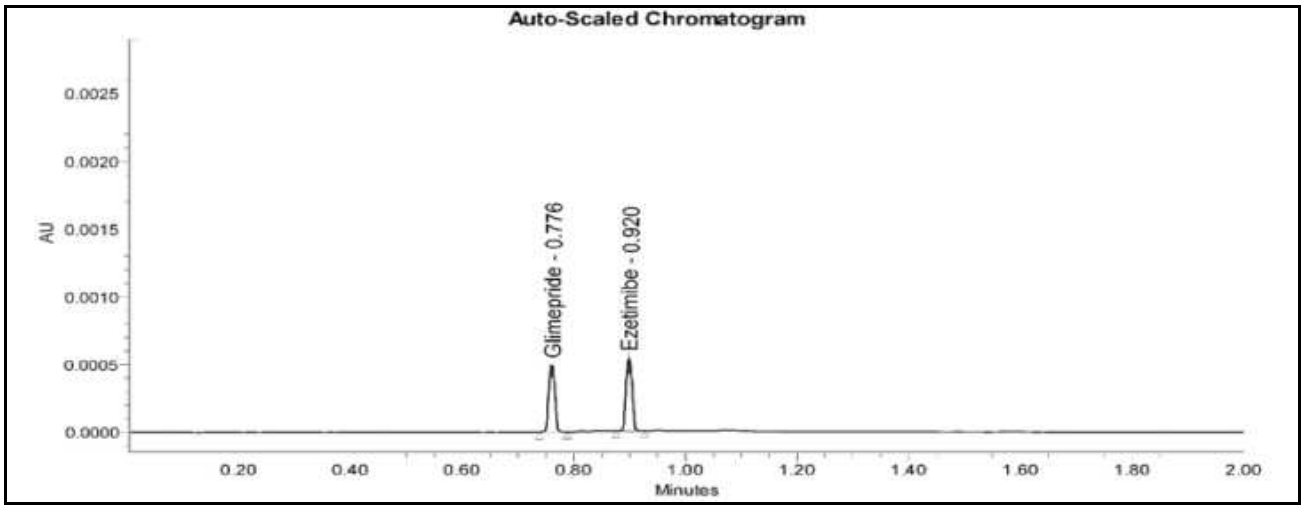


Figure No.6: Chromatogram for LOD and LOQ

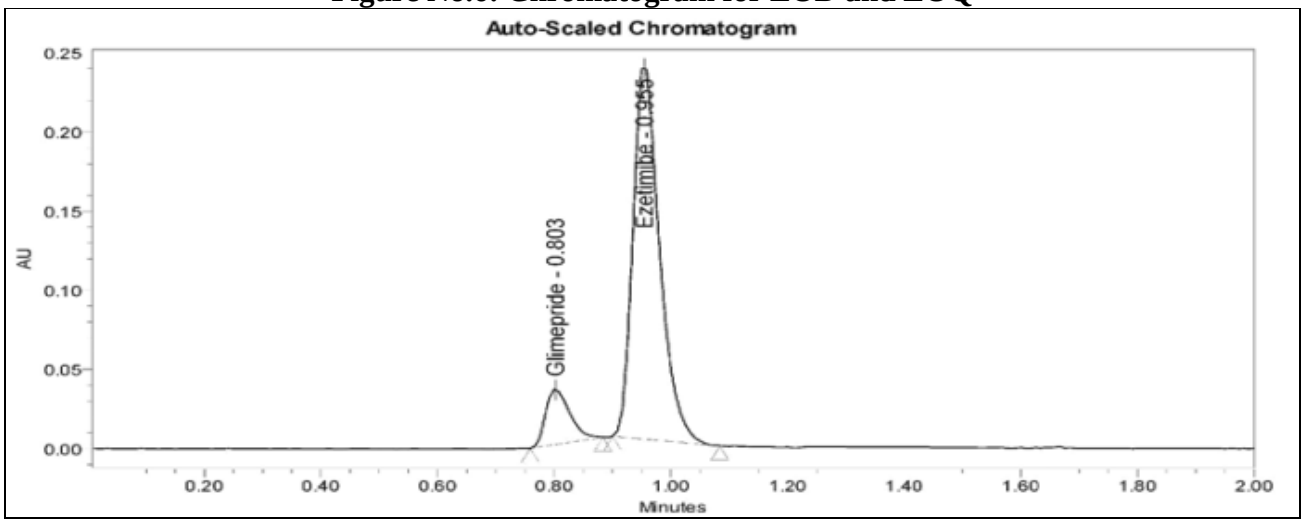


Figure No.7: Chromatogram for robustness less flow

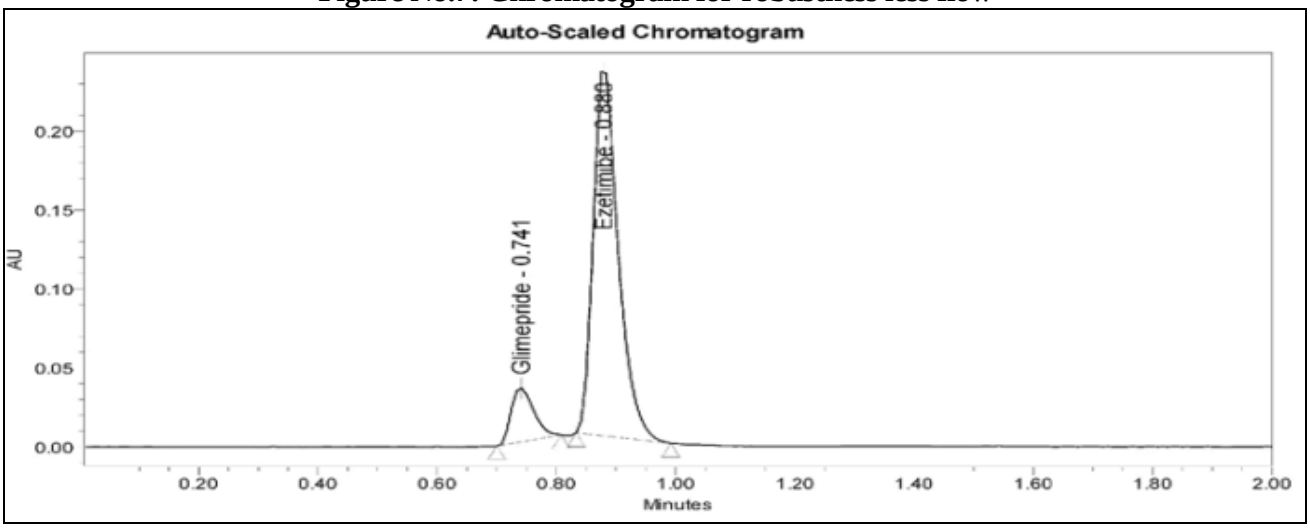


Figure No.8: Chromatogram for robustness more flow

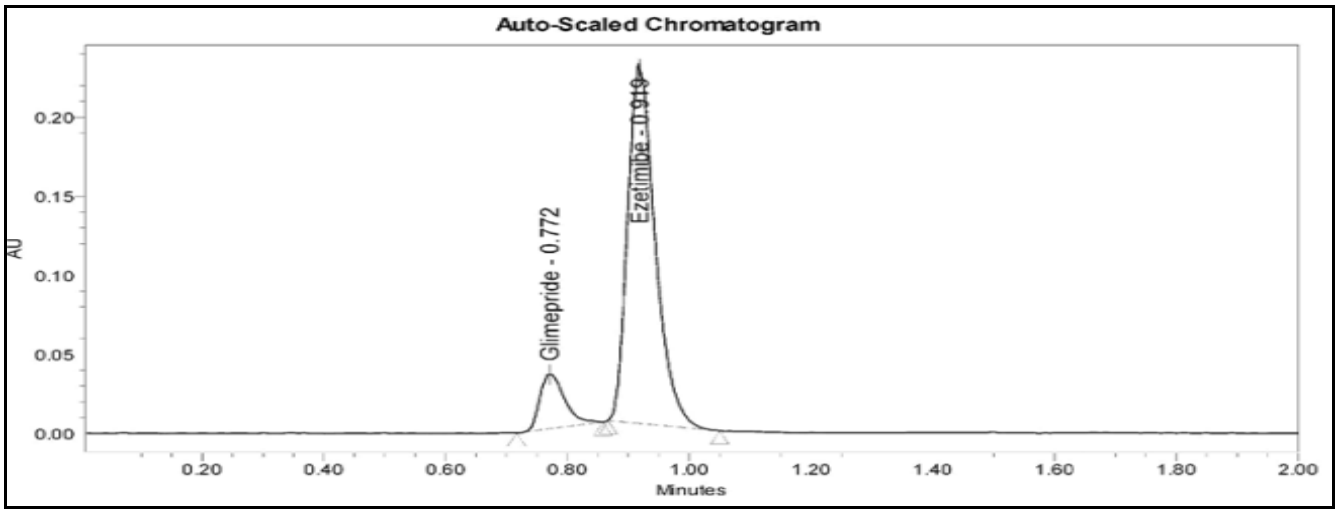


Figure No.9: Chromatogram for Less Organic

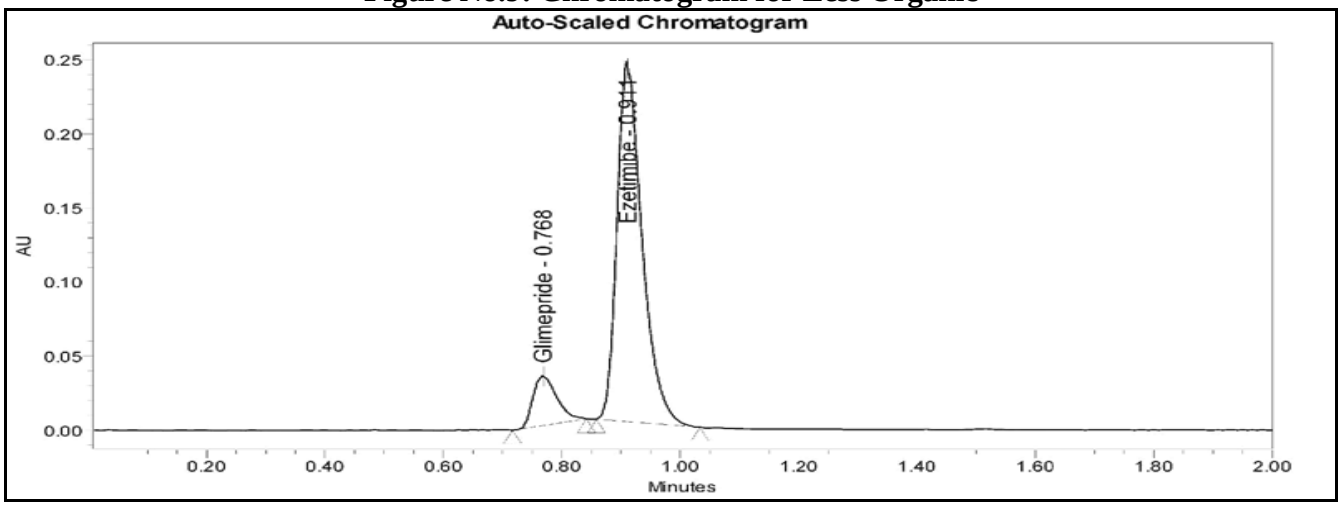


Figure No.10: Chromatogram for more Organic

CONCLUSION

A simple specific stability indicating UPLC method has been developed for the quantification of Ezetimibe and Glimepiride. This method has been validated and found to be specific, precise, accurate, linear, robust and linear for the detection and quantification of Ezetimibe and Glimepiride. This method exhibited an excellent performance in terms of sensitivity and speed as it could estimate Glimepiride concentration, which is far less when compared to the other component. The major advantage of this technique is that it is less time consuming and also eco-friendly because of its low consumption of organic solvents as compared to other analytical techniques. It helps simultaneous estimation of Ezetimibe and Glimepiride in pharmaceuticals i.e., in combination drugs. This method is suitable for routine analysis and quality control of pharmaceuticals.

ACKNOWLEDGEMENT

I take this privilege and pleasure to acknowledge the contributions of many individuals who have been inspirational and supportive throughout my work. I specially thank to my guide and others pharmaceutical Analysis Department for their moral support and encouragement during the work and to the Pharm train laboratories, Hyderabad, for providing the necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Skoog, West, Holler. Fundamental of Analytical chemistry, *Har court publishers, New Delhi*, 2(1), 2007, 644-655.
2. Verma R M. Analytical chemistry, *CBS publishers, New Delhi,* 3rd edition, 3(3), 2007, 3-3.
3. Sharma Y R. Elementary Organic Spectroscopy, *Goel publishers, New Delhi*, 23(1), 2012, 3-9.
4. Chatwal G R, Sham K, Anand. Instrumental method of chemical analysis, *Himalaya publishers, Mumbai,* 2, 2012, 2.149-2.184, 2.624-2.631.
5. Beckett A H, Stenlake J B. Practical Pharmaceutical chemistry, 4th edition, *CBS Publishers and Distributors, New Delhi,* Vol. 2(2), 1997, 296-300.
6. Jag Mohan. Whole Spectroscopy, *Narosa publishers, New Delhi,* 2(2), 2011, 119-175.
7. Sethi P D. High Performance Liquid chromatography, 1st edition, *CBS Publishers, New Delhi*, 1(1), 2010, 11-19, 161-190.
8. Kenneth A, Connors. A text book of Pharmaceutical Analysis, *Wiley India pvt.ltd New Delhi*, 3, 2009, 373-437.
9. Hobart H, Willard. Instrumental method of analysis, *CBS publishers, New Delhi*, 7(2), 2007, 513, 587-600, 614-650.
10. Vidya sagar G. Basics of Drug Analysis, *Pharma med press Hyderabad*, 2(1), 2009, 21-27.
11. Mahadik M V, Dhaneshwar S R. Quantitative determination of ezetimibe by HPTLC, *Asian Journal of Pharmaceutical Sciences*, 2(5), 2007, 182-190.

Please cite this article in press as: Alagar Raja. M *et al*. UPLC Estimation of Simultaneous Determination of Ezetimibe and Glimepride in Pharmaceutical Dosage Form as Per ICH Guidelines, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 3(3), 2015, 117 - 127.