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

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HCL AND GLICLAZIDE BY RP-HPLC IN BULK AND TABLET DOSAGE FORMS

Vegesna Swetha*[1], N. L. Durga Bhavani[1], S. V. U. M. Prasad[2]

[1*]Department of Pharmaceutical Analysis, School of Pharmacy, Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh, India.

[2]School of Pharmaceutical Sciences and Technologies, JNTU Kakinada, Andhra Pradesh, India.

ABSTRACT

The main objective of this study was to develop a simple, precise, specific and accurate RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride (MET) and Gliclazide (GLZ) in bulk and combined dosage form. The separation method was carried out using Reverse phase C18 column; ZODIAC - 3V (250 mm x 4.6 mm x 5µm). Mixture of Phosphate buffer (1.625 gm of Potassium Di Hydrogen Ortho Phosphate and 0.3 gm of Di Potassium Hydrogen Ortho Phosphate in 550 ml water) using as mobile phase; pH 4.8 and Acetonitrile (55:45 (v/v)) at isocratic mode and eluents were monitored at 248 nm using UV- Visible spectrophotometer as the Detector with the optimized method, the Retention times of MET and GLZ were found to be 2.420 and 4.270 mnts respectively with theoretical plate count and asymmetry as per the ICH limits. The linearity range was good in this method and it was of 60-140µg/ml for Metformin and 3- 8µg/ml for Gliclazide with Regression coefficient (r2) of 0.999 and 0.999. The percentage assays were found to be 99.59% for MET and 98.13% for GLZ. Limit of detection and Limit of quantitation values were found to be 1.53µg/ml and 4.65µg/ml for Metformin Hydrochloride, 0.02µg/ml and 0.07µg/ml for Gliclazide respectively. The method was found to be Accurate (with percentage mean recoveries 101.14% for Metformin HCl and 100.22% for Gliclazide), precise, robust, stable and Specific. The proposed method was validated by using ICH guidelines and hence can be successfully applied to the simultaneous estimation of MET and GLZ in tablet formulations.

KEYWORDS

MET, GLZ, Validation and RP-HPLC.

Author for Correspondence:

Vegesna Swetha,
Department of Pharmaceutical Analysis,
School of Pharmacy, Jawaharlal Nehru Technological University, Kakinada,
Andhra Pradesh, India.
Email: sweth.analysis@gmail.com

INTRODUCTION[1,2]

Analytical chemistry is the science of making quantitative measurements, which requires broad background knowledge of chemical and physical concepts. Analytical chemistry seeks ever improved means of measuring the chemical composition of natural and artificial materials. The techniques of this science are used to identify the substances

which may be present in a material and to determine the exact amounts of the identified substances.

Analytical chemistry serves the needs of many fields.

To develop a sensitive, simple, rapid, reliable and accurate analytical method for the simultaneous estimation of Metformin Hydrochloride and Gliclazide in Bulk and Pharmaceutical dosage form and validation of developed method by using RP-HPLC technique.

1. The scope of developing and validating method is to ensure a suitable strategy for a particular analyte which is more specific, accurate and precise. Here the main focus is drawn to achieve improvement in conditions and standard operating procedures to be followed.
2. To develop a method that is rapid, sensitive and at the same time cost effective.
3. To validate the developed HPLC method for various parameters like Specificity, Linearity, Accuracy, Precision, Robustness, etc. as per the guidelines.
4. To ensure that the method can be used as quality control tool in routine analysis of Gliclazide and Metformin HCl in pure and pharmaceutical dosage forms.

MATERIAL AND INSTRUMENTS[3,4]

All the instruments and chemicals used in the work are tabulated in Table No.1 and 2.

Drugs used

METFORMIN and GLICLAZIDE bulk drugsGift Samples obtained from Chandra labs, Hyd.

Zilmet (30+500) (Gliclazide-30 mg

Metformin-500 mglabel claims). Obtained from local pharmacy

Manufactured by: FDC Limited

METHOD DEVELOPMENT[5,6,7]

Method Development Parameters

Selection of suitable solvent

According to solubility tests and literature review, Gliclazide and Metformin HCl are freely soluble in water, methanol and acetonitrile. Then it was checked with different dilutions of said solvents for solubility of Gliclazide and Metformin HCl, finally Mixed Phosphate buffer prepared with Acetonitrile and mixed with HPLC grade ACN chosen as solvent for present work.

Selection of wave length (For Detection)

In setting up the conditions for development of assay method, the choice of detection wavelength was based on the scanned absorption spectra for Gliclazide and Metformin HCl. The UV-Spectra of Gliclazide and Metformin HCl was obtained separately by scanning the sample over the wave length range 200-400 nm against blank as water. After examination of the spectra, the wave length 248 nm is selected for further analysis.

Solubility Studies

These studies are carried out at 25 0 C and tabulated in Table No.3.

Determination of Working Wavelength (λmax)[8]

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Gliclazide[9]

10 mg of Gliclazide was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Metformin HCl[10]

10mg of Metformin HCl was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS

The wavelength of maximum absorption (λmax) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the Figure No. 6.1, 6 .2, and 6.3 and the

absorption curve shows characteristic absorption maxima at 235nm for Gliclazide, 232 nm for Metformin HCl and 248nm for the combination.

Obeservation

λmax was found to be 235 nm for Gliclazide shown in the Figure No.1.

Observation

λmax was found to be 232nm for Metformin HCl shown in the Figure No.2.

Observation

The isobesticpoint was found to be 248 nm for Gliclazide and Metformin HCl in combination and was shown in Figure No.3.

Selection of chromatographic technique[11]

The choice of the method is based on the nature of the sample. The drugs are polar in nature due to presence of amino group, bromide group in molecular structure. Bromhexine HCl and Phenylpropanolamine HCl are freely soluble in high polar solvents like water, methanol and acetonitrile and practically insoluble in less polar solvents like hexane and methylene chloride. So the reverse phase chromatographic technique was selected for the present study.

In RP-HPLC technique, generally non polar columns are used as stationary phase. For the present study Kromosil 250 column is used as stationary phase.

RESULTS AND DISCUSSION

Method Development Trials

Observation

The Efficiency for both Gliclazide and Metformin HCl is within the limits (limit- >2000).

The Asyymetry factor is less than 2.0

The run time was 6 minutes.

All the system suitability parameters were satisfied.

The details are given in Table No.11.

SUMMARY AND CONCLUSION

Summary

From the reported literature, there were few methods established for the determination of Metformin HCl and Gliclazide in individual and in combination with other drugs. It was concluded that there was no method reported for the simultaneous estimation of the above selected multi component dosage form, which promote to pursue the present work. The scope and objective of the present work is to develop and validate a new simple RP-HPLC method for simultaneous estimation of Metformin HCl and Gliclazide in combined dosage form.

In simultaneous RP-HPLC method development, Waters HPLC Grade with UV detector is used. The column used is ZODIAC column, C18 (250X4.6ID), 5µm column with 5-micron particle size. Injection volume of 20 µL was injected and eluted with the mobile phase of mixed Phosphate buffer and Acetonitrile in the ratio of (55:45). The flow rate was found to be optimized at 1 ml/min. Detection was carried out at 248 nm. Quantitation was done by external standard method with the above mentioned optimized chromatographic condition. This system produced symmetric peak shape, good resolution and reasonable retention times of Metformin HCl and Gliclazide were found to be 2.420 and 4.270 minutes respectively. The Metformin HCl and Gliclazide showed linearity in the range of 60-140µg/mL and 6- 8.4 µg/mL respectively. The slope, intercept and correlation coefficient(s) were found to be 67.93, 780.5, and 0.999 respectively for Gliclazide and 254, 411.2 and 0.999 respectively for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The %RSD values for precision was found to be within the acceptable limits, which revealed that the developed method was precise. The developed method was found to be robust. The %RSD value for percentage recovery of Metformin HCl and Gliclazide was found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for simultaneous estimation of the Metformin HCl and Gliclazide.

Table No1: Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC	Shimadzu(LC 20 AT VP)
HPLC	Agilent 1200 series
Ultra Sonica tor	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	ZODIAC column,C18(250x4.6 ID) 5µm

Table No.2: Reagents used

Water	HPLC Grade
Sodium dihydrogen ortho phosphate	AR Grade
Methanol	HPLC Grade
Potassium Dihydrogen ortho Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate	AR Grade
Tetra Hydro Furan	AR Grade

Table No.3: Solubility Studies

S.No	SOLVENT	GLICLAZIDE	METFORMIN HCl
1	Water	Insoluble	Soluble
2	Phosphate	Sparingly	Soluble
3	buffer(P^H 6.8)	soluble	
4	Mixed	Soluble	Soluble
5	Phosphate	-	-
6	buffer(P^H4.8)	-	-
7	ACN	Freely Soluble	Insoluble
8	Acetone	Soluble	Insoluble
9	Methanol	Soluble	Insoluble
10	Chloroform	Soluble	Insoluble

Table No.4: Optimized chromatographic conditions

S.No	**Mobile phase**	**Mixed Phosphate Buffer: ACN(55:45)**
1	Column	Zodiac C18,250×4.6mm ID, 5µm Particle size
2	Flow rate	1.0 ml/min
3	Column temperature	Room temperature(20-25^{o}C)
4	Sample temperature	Room temperature(20-25^{o}C)
5	Wavelength	248 nm
6	Injection volume	20 µl
7	Run time	6 min
8	Retention time	About 2.420 min for Metformin HCl and 4.270 min for Gliclazide.

Table No.5: Result for trial-1 by using mobile phase

S.No	Name	Rt(min)	Peak Area	Asymmetry factor	Efficiency	Resolution
1	Metformin HCI	1.370	5892.378	2.429	346	--
2	Gliclazide	6.537	137.977	2.137	1932	11.619

Table No.6: Result for trial-2 by using mobile phase

S.No	Name	Rt(min)	Peak Area	Asymmetry factor	Efficiency	Resolution
1	Metformin HCI	2.053	1953.198	1.893	1314	--
2	Gliclazide	8.697	3903.902	1.446	4268	17.504

Table No.7: Result for trial-3 by using mobile phase

S.No	Name	Rt(min)	Peak Area	Asymmetry factor	Efficiency	Resolution
1	Metformin HCI	2.297	2546.935	1.781	1141	--
2	Gliclazide	10.373	1699.517	1.327	12317	25.013

Table No.8: Result for trial-4 by using mobile phase

S.No	Name	Rt(min)	Peak Area	Asymmetry factor	Efficiency	Resolution
1	Metformin HCI	1.963	4240.837	2.219	800	--
2	Gliclazide	6.167	988.458	1.247	1753	9.699

Table No.9: Result for trial-5 by using mobile phase

S.No	Name	Rt(min)	Peak Area	Asymmetry factor	Efficiency	Resolution
1	Metformin HCI	2.553	6617.766	2.486	966	--
2	Gliclazide	3.933	612.058	1.282	3809	4.730

Table No.10: Result for trial-6 by using mobile phase

S.No	Name	Rt(min)	Peak Area	Asymmetry factor	Efficiency	Resolution
1	Metformin HCI	2.420	5640.876	1.414	2253	--
2	Gliclazide	4.270	977.839	1.256	4115	7.869

Table No.11: All parameters result

S. No	Parameter	Result
1	System suitability	1) Tailing factor of Metformin HCl is 1.43 and Gliclazide is 1.24. 2) Theoretical plates of Metformin HCl is 2708 and Gliclazide is 6213. 3) Retention time of Metformin HCl is 2.420 and Gliclazide is 4.270.
2	Specificity Blank interference excipients interference	No peaks are observed in the blank chromatogram at the retention time of the peak. No peaks are observed in the excipients chromatograms at the retention time of the peak.
3	Precision	The % RSD for assay of six replicate preparations is 0.27 for Metformin HCl is 0.82 and Gliclazide.
4	Linearity	The Square of correlation coefficient value for Metformin HCl is 0.999 and Gliclazide is 0.999.
5	Accuracy	1) Individual % recovery is found to be in the range of 99.54 to 101.26% 2) % RSD is found to be in the range of 0.69 and 0.93(mean% recovery and %RSD are within limits)
6	Robustness i) Flow rate variation ii) Mobile phase variation iii) Temperature variation	System suitability parameters are found to be within the acceptable limits for all the robustness parameters
7	LOD and LOQ	For Metformin HCl 1.53 µg/ml and 4.65 µg/ml For Gliclazide 0.02 µg/ml and 0.07 µg/ml.

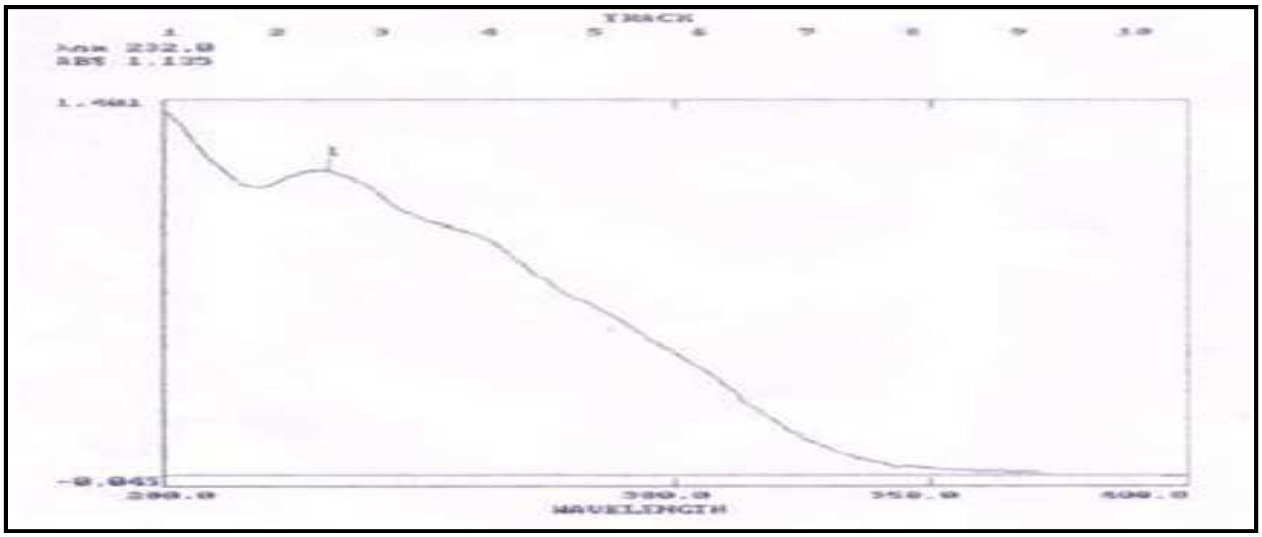

Figure No.1: UV spectrum of Gliclazide

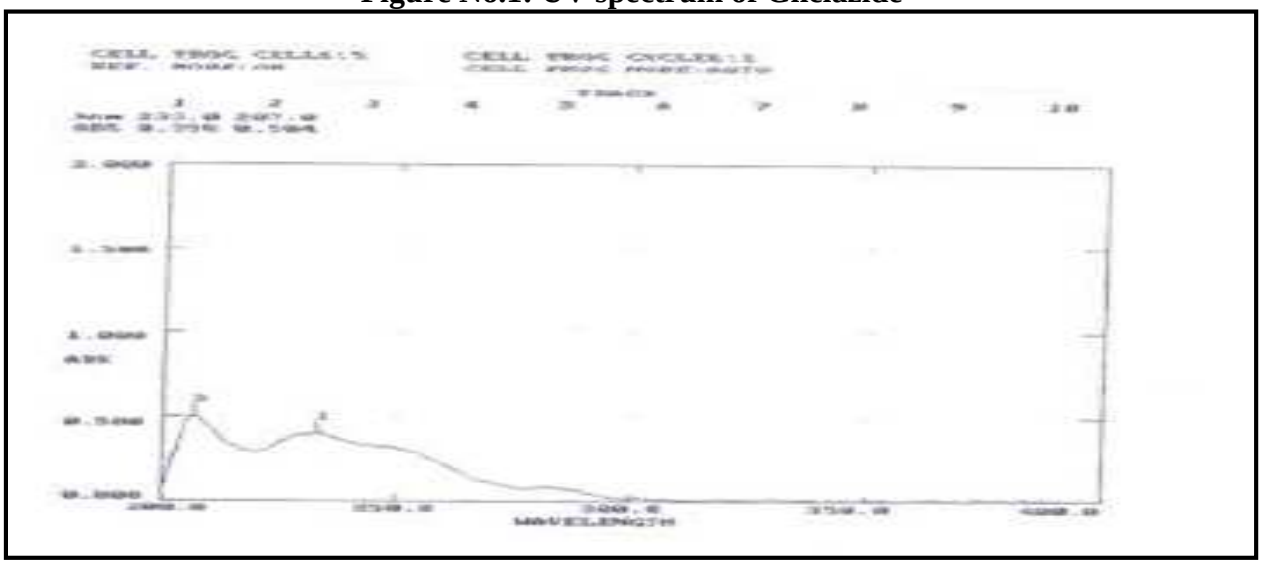

Figure No.2: UV spectrum of Metformin HCl

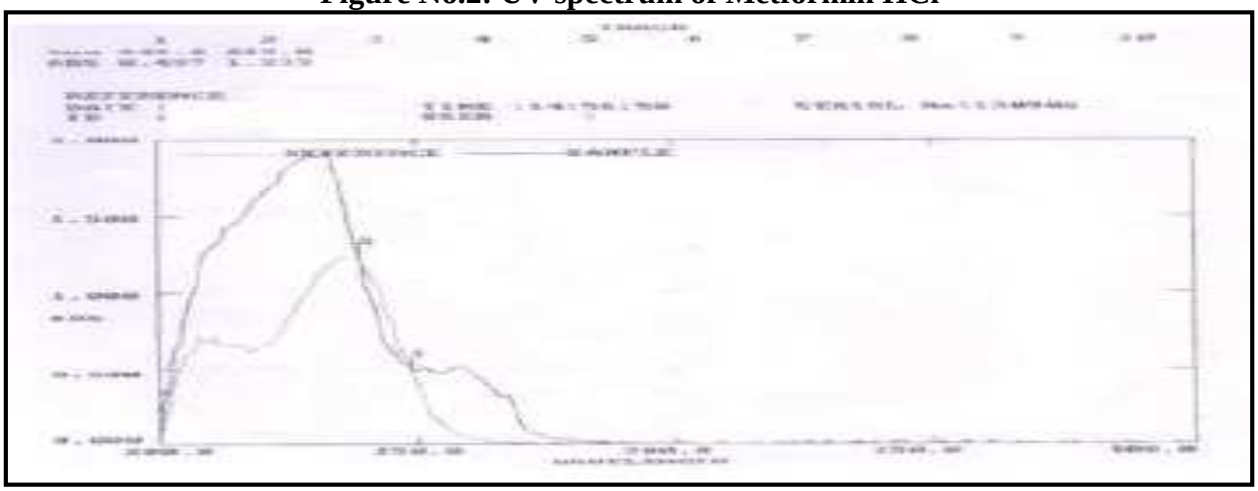

Figure No.3: UV-OVERLAP spectrum of Gliclazide and Metformin HCl

Method development trial-1

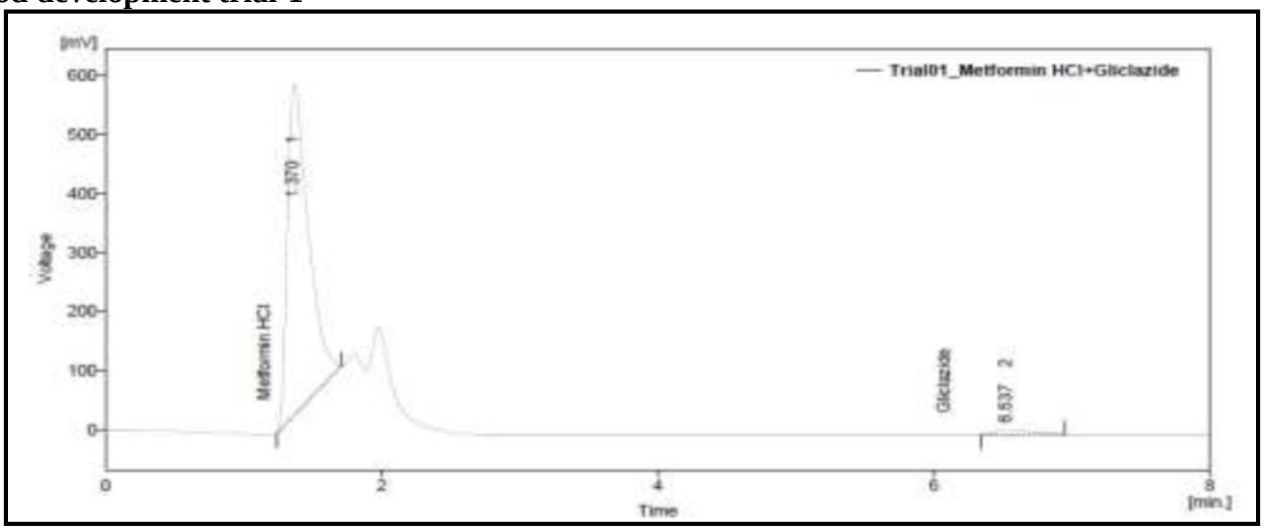

Figure No.4: Chromatogram of trial-1 by using mobile phase

Method development trial -2

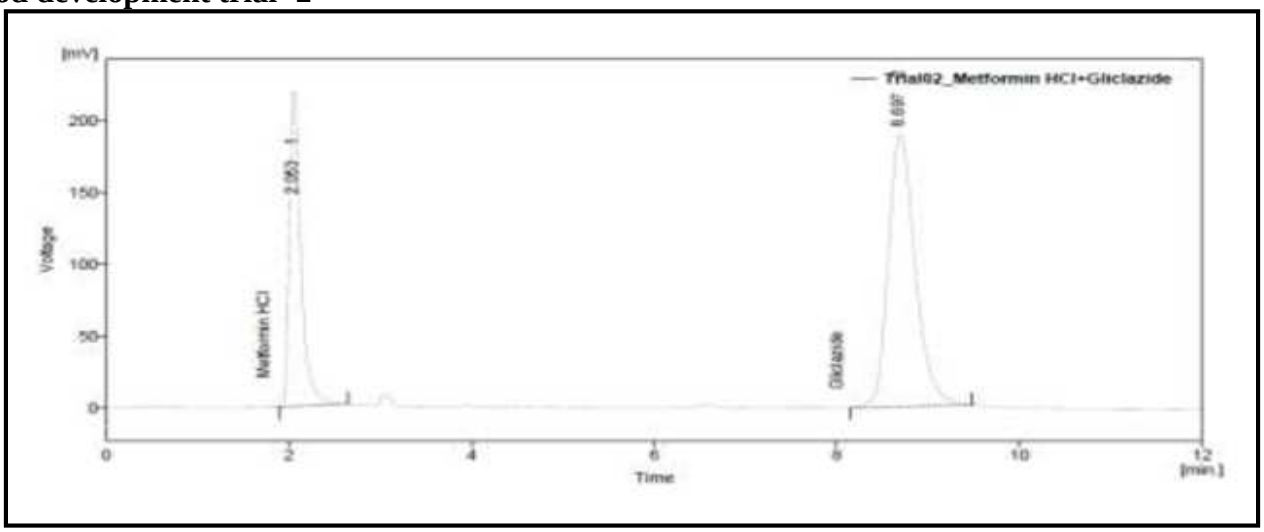

Figure No.5: Chromatogram of trial-2 by using mobile phase

Method development trial -3

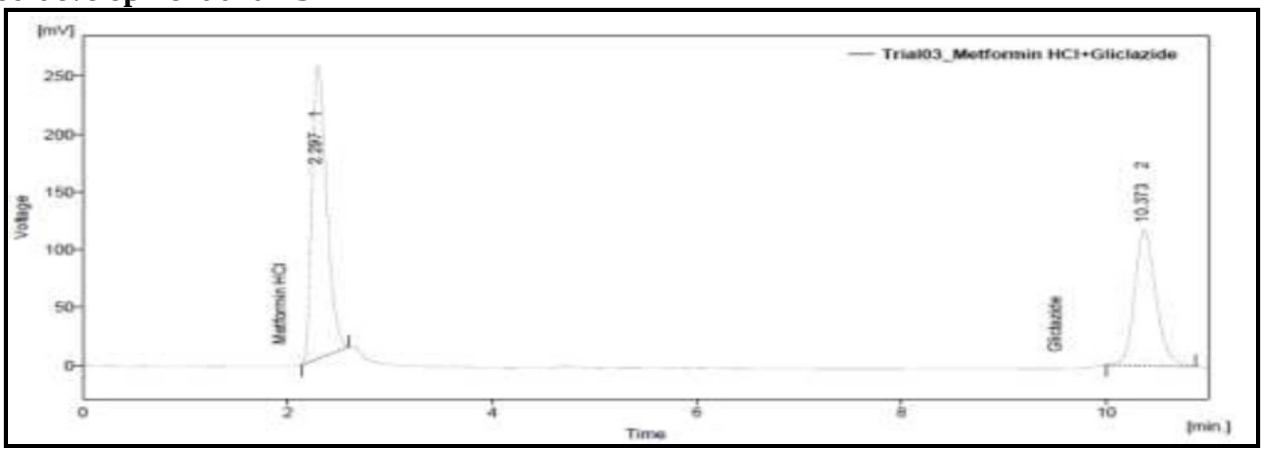

Figure No.6: Chromatogram of trial-3 by using mobile phase

Method development trial -4

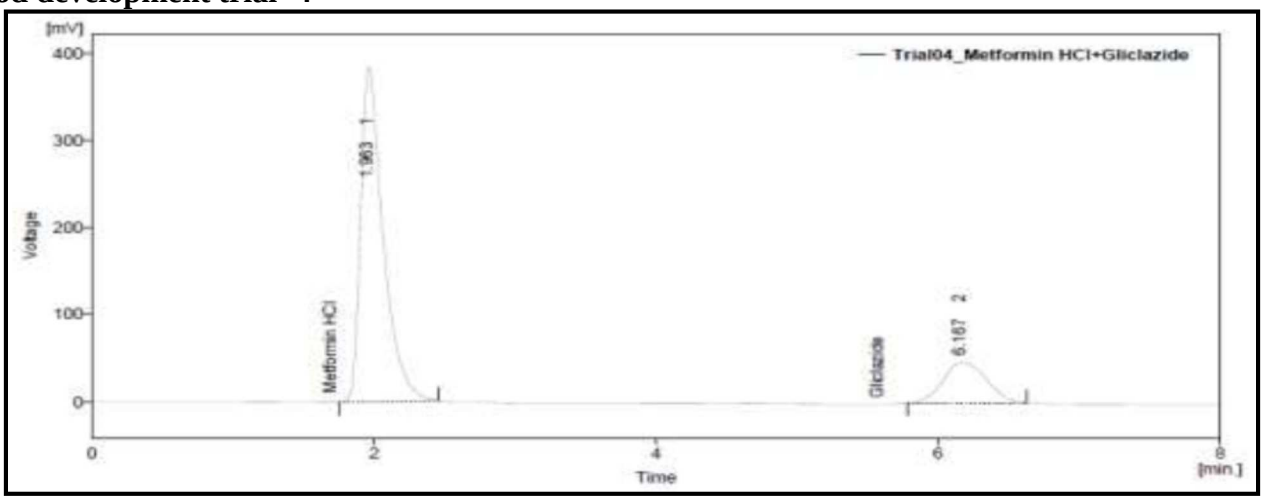

Figure No.7: Chromatogram of trial-4 by using mobile phase

Method development trial -5

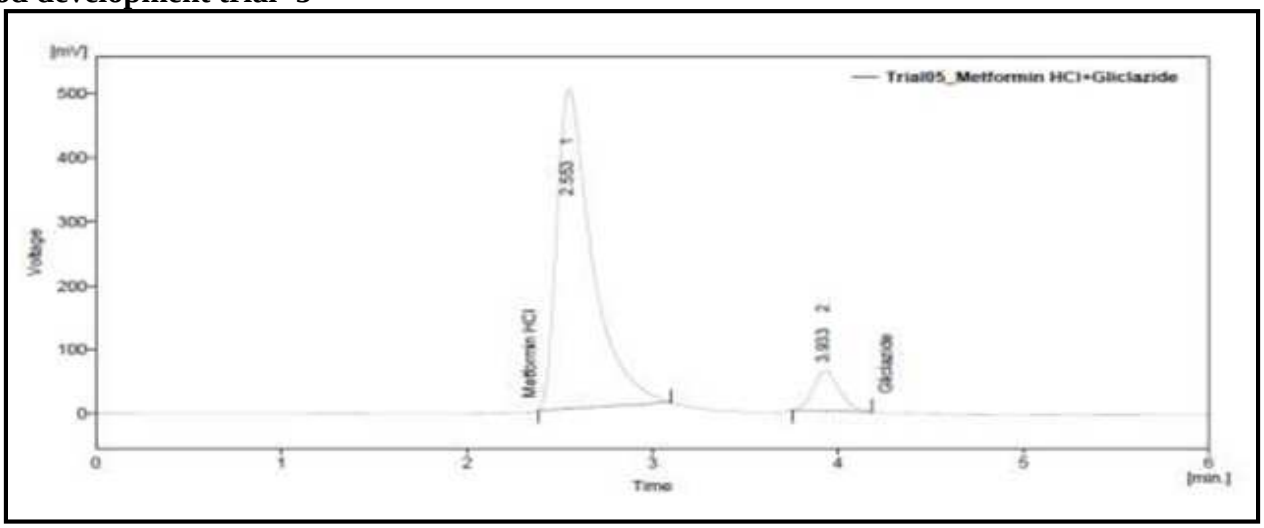

Figure No.8: Chromatogram of trial-5 by using mobile phase

Method development trial -6

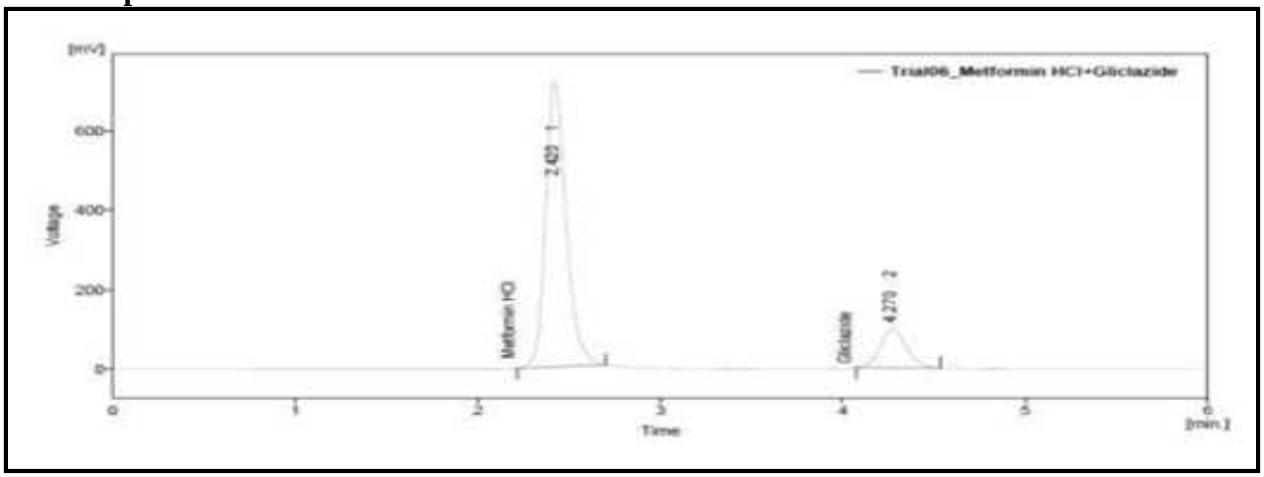

Figure No.9: Chromatogram of trial-6 by using mobile phase

CONCLUSION

The Objective of present work is to develop new validated RP-HPLC Method for Selected Pharmaceutical Dosage Form. In The Present Work The RP-HPLC Method Development was done for Metformin Hcl and Gliclazide using Mobile Phase Mixed Phosphate Buffer : Acetonitrile In The Ratio Of 55:45 And Detection Was Performed At 248nm With A Retention Time Of 2.420 min And 4.270 min. The Method Was Validated For All Validation Parameters As Per ICH Guidelines. The Linearity Values Of Given Method With Respect To R2 Value Is 0.999, Found Was Within Acceptable Limits. The %RSD For Precision Was < 2%. The Accuracy Of Method Was Validated By Recovery Studies And Was Found To Be Significant And Under Specification Limits, Within Acceptable Range 98-102%. The Method Also Passes The Specifications For Robustness Parameters. Hence It Can Be Used For The Routine Analysis Of Metformin HCl And Gliclazide, in Their Combine Dosage Form In Quality Control Laboratory And Stability Studies. Development and validation of RP-HPLC method for the simultaneous estimation of Metformin HCl and Gliclazide in bulk and Pharmaceutical dosage forms" with the facilities and the results are incorporated in this thesis. In conclusion a validated RP-HPLC method has been developed for determination of Metformin HCl and Gliclazide in their bulk and combined tablet dosage forms. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of both drugs in combined tablet dosage form. In the future, this method may be applied for routine analysis of both the drugs in API and in tablet formulation.

ACKNOWLEDGEMENT

The School of Pharmacy, Jawaharlal Nehru Technological University, Kakinada supports for doing this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Yoshiko Arikawa, Basic education in analytical chemistry, *Analytical sciences*, 17(4), 2001, 571-573.
2. Miller K E, Synovec R E. Review of analytical next term measurements facilitated by drop formation technology, *Talanta*, 51(5), 2000, 921-933.
3. Keith D. Bartle, Peter Myers. History of gas Chromatography, *TrAC Trends in Analytical Chemistry*, 21(9-10), 2002, 547-557.
4. Snyder L R and Kirkland J J. Practical HPLC Method development, *Wiley inter science publications, New York.* 1997, 1-9, 685-712.
5. Jeffery G H, Denney R C, Bassett J, Mendham J. *Vogel's Text Book of Quantitative Chemical Analysis*, person education, 6th Edition, 2003, 2-7, 216-227.
6. Munson J W. Pharmaceutical analysis, *part - B, Marcel Dekkar, New York*, 2nd edition, 1994, 87-135.
7. Validation of analytical procedures / methodology, *ICH harmonized triplicate guideline*, 1996, 1-8.
8. Syed Imtiaz Haider. Validation standard operating procedures, 2002.
9. Gurdeep R. Chatwal, Sham K. Anand. *Instrumental methods of chemical analysis*, 2008, 2.566-2.587.
10. Gennaro A R, Remington. The sciences and practice of pharmacy, Luppincott, Williams and Wilkins, *Baltimore, Maryland. USA*, 28th Edition, 2000, 534-549.
11. Indian Pharmacopoeia, *Published by controller of publications, New Delhi*, 2(1), 1996, A65-A68.
12. Connors K A. A text book of pharmaceutical analysis, *Wiley - interscience publication, New York*, 3rd Edition, 1982, 638-639.
13. Sankar D, Gowri; Kumar B. Anil; Babu P, Joy; Latha and Madhavi P V.
14. Gupta A, Mishra P and Shah K. Simple UV Spectrometric determination of Rosuvastatin calcium in pure form and pharmaceutical

formulations, *E-Journal of Chemistry*, 6(1), 2009, 89-92.

15. Rajput S J and Raj H A. Simultaneous estimation of ezetimibe and rosuvastatin in drug mixture by first derivative spectroscopic method, *International Journal of Chemical Sciences*, 7(4), 2009, 2354-2362.
16. Sane R T, Kamat S S, Menon S N, Inamdar S R, Mote M R. Determination of rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high-performance thin-layer chromatography, *Journal of Planar Chromatography Modern TLC*, 18(103), 2005, 194-198.
17. Sharma M C, Sharma S, Kohli D V and Sharma A D. A validated HPTLC method for determination of simultaneous estimation Rosuvastatin Calcium and Ezetimibe in pharmaceutical solid dosage form, *Archives of Applied Science Research*, 2(1), 2010, 1-7.
18. Sankar D G, Babu P J, Kumar B A, Krishna M V. *Acta Ciencia Indica, Chemistry*, 33(1), 2007, 1-4.
19. Gomes F P, Garcia P, Alves J, Singh A, Kedor-Hackmann E and Santoro M. Development and Validation of Stability-Indicating HPLC Methods for Quantitative Determination of Pravastatin, Fluvastatin, Atorvastatin, and Rosuvastatin in Pharmaceuticals, *Analytical Letters*, 42(12), 2009, 1784-1804.
20. Sharma B K. HPLC, Instrumental methods of chemical analysis, *Goel pubkishers*, 24th Edition, 2005, 286-300.
21. Gurudeep R, Chatwal, Sharm, K. Anand. HPLC Instrumental methods of chemical analysis, 2(4), 2010, 624-639.
22. ICH, Text on Validation of Analytical Procedures, ICH – Q2A, International Conference on Harmonization, IFPMA, Geneva, 2-3, 1995, A–1-A–3.

Please cite this article in press as: Vegesna Swetha *et al.* Method development and validation for the simultaneous estimation of Metformin Hcl and Gliclazide by RP-HPLC in bulk and tablet dosage forms, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 5(1), 2017, 13-22.