Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 79 (2015) 30660-30663

ISSN: 2229-712X

Development and validation of a reversed-phase HPLC method for simultaneous determination of metformin and gliclazide in tablet dosage forms

Gandla Kumara Swamy[1,*], N.Ravindra[2] and Ch.Anjali[2]

[1]Pharmaceutical Analysis , Jawaharlal Nehru Technological University Kakinada, Kakinada -533003.A.P.

[2]Department of Pharmaceutical Analysis, Chilkur Balaji College of Pharmacy, Aziz Nagar –Hyderabad. 500075, Telangana (State). India.

ARTICLE INFO

Article history:

Received: 9 September 2014;

Received in revised form:

15 February 2015;

Accepted: 24 February 2015;

Keywords

Metformin, Gliclazide,

RP-HPLC Method,

Tablet Dosage forms.

ABSTRACT

A simple, accurate, rapid and precise isocratic reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of Metformin and Gliclazide in tablets. The chromatographic separation was carried out on an Cosmosil ODS analytical column (250×4.6 mm; 5µm) with a mixture of Methanol: Hplc grade water pH 6 adjusted with Orthophosphoric acid(70:30, v/v) as mobile phase; at a flow rate of 1 ml/min. UV detection was performed at 235 nm. The retention times were 2.50 and 6.02 min. for Metformin and Gliclazide, respectively. Calibration plots were linear (r^2>0.998) over the concentration range 5-30µg/ml for Metformin and 1-6µg/ml Gliclazide. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Metformin and Gliclazide in bulk drug and tablets dosage form.

Introduction

Metformin is a biguanide class of anti-diabetic drug, chemically is N,N-dimethylimidodicarbonimidicdiamide hydrochloride. It is an oral anti-diabetic drug from the biguanide class. Molecular Formula –C4H11N5, Molecular Weight – 163.3 Solubility - Slightly soluble in methanol and in isopropyl alcohol, sparingly soluble in dehydrated alcohol, freely soluble in methanol.

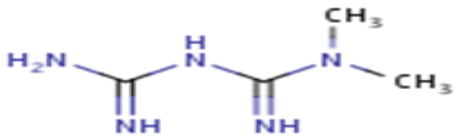


Fig.No.1.Chemical structure of Gliclazide

Chemical name : Benzenesulfonamide, N-[[(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)amino]carbonyl]-4-methyl

Molecular formula:$C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$ and **Molecular weight:** 323

Solubility :chloroform, methanol, acetone.

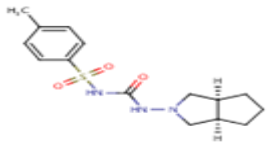


Fig.No.2.Chemical structure of Metformin

From the literature survey it was found that many methods are available for determination of Metformin and Gliclazide individually and few methods in combination with other drugs. However, no stability indicating HPLC has been reported for simultaneous determination metformin and Gliclazidein combination.

In the proposed study an attempt will be made to develop a stability indicating HPLC method for simultaneous estimation of Metformin and Gliclazidein pharmaceutical formulation (tablets).

Pharmaceutical grade of Metformin and Gliclazidewere kindly supplied as gift samples by Dr. Reddy's laboratories, Hyderabad, India, certified to contain > 99% (w/w) on dried basis. Commercially available Reclimet (Dr. Reddy's laboratories Ltd., Hyderabad, India) tablets claimed to contain 500 mg metformin and 80mg of Gliclazidehave been utilized in the present work. All chemicals and reagents used were of HPLC grade and were purchased from Agenta Chemicals, Hyderabad, India.

Chromatographic system and conditions:

The HPLC system (Analytical Technologies Gujarat, India) consisted of pump. The Analytical column a CosmosilC_{18} (250mm x 4.6mmi.d., 5µ particle size)was operated at ambient temperature (20 + 1°c). Isocratic elution with Methanol: Hplc grade water (70:30 v/v pH 6) was used at flow rate at 1ml/min, column (150×4.6 mm; 5µm). Before analysis the mobile phase was filtered through a 0.2µm membrane and degassed by ultrasonification. Detection was monitored at 235 nm and injection volume was 20µl. All the experiments were performed at ambient temperature. Pharmaceutical grade of Metformin and Gliclazidewere kindly supplied as gift samples by Dr. Reddy's laboratories, Hyderabad, India, certified to contain > 99% (w/w) on dried basis. Commercially available Reclimet (Dr. Reddy's laboratories. Ltd, Hyderabad, India), tablets claimed to contain 500 mg of metformin and 80mg of Gliclazide have been utilized

Tele:

E-mail addresses: kumaraswamy.gandla@gmail.com

in the present work. All chemicals and reagents used were of HPLC grade and were purchased from AgentaChemicals, India.

Standard solutions and calibration graphs for chromatographic measurement:

Stock standard solutions were prepared by dissolving separately 50 mg of Metformin and Gliclazidein 50 ml methanol (1000µg/ml). The standard calibration solutions were prepared by appropriate dilution of the stock solution with methanol to reach a concentration range of 5-30 µg/ml for metformin and 1-6µg/ml for Gliclazide. Triplicate 20 µl injections were made for each concentration and chromatographed under the optimized conditions described above. The peak area were plotted against the corresponding concentrations to obtain the calibration graphs.

Sample preparation:

Twenty tablets contents were accurately weighed, their mean weight was determined and they were mixed and finely powdered. A portion equivalent to about one tablet was accurately weighed and transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 15 min and diluted to 100 ml with methanol. The resulting solution was centrifuged at 100 rpm for 15 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 µ filter (Millipore, Milford, MA). The original stock solution was further diluted to get sample solution of drug concentration of 100µg/ml Metformin and 10µg/mlGliclazide. A 20µl volume of sample solution was injected into HPLC, six times. The peak areas for the drugs were measured at 230 nm and amounts of Metformin and Gliclazide were determined using the related linear regression equations.

Method validation:

The developed method was validated according to the ICH guidelines [24]. The system suitability was evaluated by six replicate analyses of Metformin and Gliclazidemixture at a concentration of 100µg/ml Metformin and 100µg/mlGliclazide. The acceptance criteria were a R.S.D. of peak areas and retention times less than 2%, Theoretical plate numbers (N) at least 2500 for each peak and tailing factors (T) less than 1% for Metformin and Gliclazide.

Standard calibration curves were prepared in the mobile phase with six concentrations ranging from 5-30µg/ml for MET and 1-6µg/ml forGliclazideinto the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. To study the reliability and suitability of the developed method, recovery experiments were carried out at three levels 50, 100 and 150%. Known concentrations of commercial tablets were spiked with known amounts of Metformin and Gliclazide. At each level of the amount six determinations were performed and the results obtained were compared with expected results. Recovery for pharmaceutical formulations should be within the range 100±5%. The percent R.S.D. of individual measurements was also determined. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) for 2 consecutive days. Two different concentrations of metformin and Gliclazidewere analyzed in six independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision). The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of percent RSD.

All chromatograms were examined to determine if compounds of interest co-eluted with each other or with any additional excipients peaks. Marketed formulations were analyzed to determine the specificity of the optimized method in the presence of common capsule excipients. Limit of detection (LOD) and limit of quantitation (LOQ) were estimated from the signal-to-noise ratio. LOD and LOQ were calculated using 3.3σ/s and 10σ/s formulae, respectively, where, σ is the standard deviation of the peak areas and *s* is the slope of the corresponding calibration curve. To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included variation of flow rate, percentage of buffer in the mobile phase, and pH of mobile phase.

Results And Discussion

During the optimization of HPLC method, columns (Cosmosil C18 5µm; 250 mm × 4.6 mm), two organic solvents (acetonitrile and methanol), two buffers (acetate and phosphate) at three different pH values (4, 5 and 6) were tested. Initially methanol: water; acetonitrile: water: methanol; methanol: phosphate buffer were tried in different ratios at pH 4, 5 and 6. Metformin was eluted with the tried mobile phases, but Gliclazide was retained. Then, with Methanol: Hplc grade waterall the two drugs eluted. The mobile phase conditions were optimized so the peak from the first-eluting compound did not interfere with those from the solvent, excipients. Other criteria, *viz*. time required for analysis, appropriate k range ($1<k<10$) for eluted peaks, assay sensitivity, solvent noise were also considered. Finally a mobile phase consisting of a mixture of methanol: hplc grade water pH 6 adjusted with Ortho phosphoric acid in ratio (70:30 (v/v), was selected as mobile phase to achieve maximum separation and sensitivity. Flow rates between 0.5 to 1.2 ml/min were studied. A flow rate of 1 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed phase C18 column, the retention times for Metformin and Gliclazidewere observed to be 2.502 and 6.024 min respectively. Total time of analysis was less than 10 min. The chromatogram at 230 nm showed a complete resolution of all peaks (fig. 2 Representative chromatograms of standard solutions (a) Standard solution of Metformin (100µg/ml); (b) standard solution of Gliclazide (10µg/ml) and (c) a standard solution containing 100µg/ml Metformin and 50µg/ml Gliclazide .Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability test.

All critical parameters tested met the acceptance criteria on all days. As shown in the chromatogram, all two analytes are eluted by forming symmetrical single peaks well separated from the solvent front Excellent linearity was obtained for all the two drugs in the range of 5-30µg/ml for Metformin and1-6µg/ml Gliclazide. The correlation coefficients (r^2) were found to be greater than 0.999 (n=6) in all instances. The results of calibration studies are summarized in Table 1. The proposed method afforded high recoveries for Aspirin, Atenolol and Amlodipine capsules. Results obtained from recovery studies presented in Table 2, indicate that this assay procedure can be used for routine quality control analysis of this ternary mixture in capsules. Precision of the analytical method was found to be reliable based on % RSD (< 2%) corresponding to the peak areas and retention times. The % RSD values were less than 2, for intra-day and inter-day precision. Hence, the method was found to be precise for all the three drugs.

The chromatograms were checked for the appearance of any extra peaks. It was observed that single peak for Metformin (R_t±SD, 2.502±0.01) and Gliclazide(R_t± SD, 6.024±0.01) were obtained under optimized conditions, showing no interference from common tablets excipients and impurities. Also the peak areas were compared with the standard and % purity calculated

was found to be within the limits. These results demonstrate the specificity of the method

Table 1. Linearity Parameters For The Simultaneous Estimation Of And Amlodipine (*N*=6)

PARAMETERS	METFORMIN	GLICLAZIDE
λ_{max} (nm)	240	235
Beers law limit (µg/ml)	5-30	1 - 6
Correlation coefficient (r)	0.999032	0.999072
Regressionequation (y=mx+c)	y= 0.772151x + 2424786	y=0.326938 x + 542963.5
Slope (m)	0.772151	0.326938
Intercept (c)	2424786	542963.5
LOD (µg/ml)	0.1379	0.0677
LOQ (µg/ml)	0.4180	0.2051
Standard Error	0.001295	85052.77

Table No.2. Recovery Analysis of Formulation (Ato-Gaurd) By Rp - Hplc

Drug	Percentage	%recovery	S.d	%rsd	S.e
Metformin	50% 100% 150%	97.94 98.71 99.22	0.6433	0.6530	0.3714
Gliclazide	50% 100% 150%	100.10 99.75 99.16	0.8438	0.8406	0.4871

Table 3. System suitability parameters for the optimized chromatogram by RP - HPLC

PARAMETERS	METFORMIN	GLICLAZIDE
Tailing factor	1.24	1.21
Asymmetrical factor	1.80	1.54
Theoretical plates	5694	6489
Capacity factor	21.507	26.660
Theoretical plate per unit length	206.19	242.05
Resolution	Between MET and GLI 1.61	

Fig. 4. Calibration curve of metformin

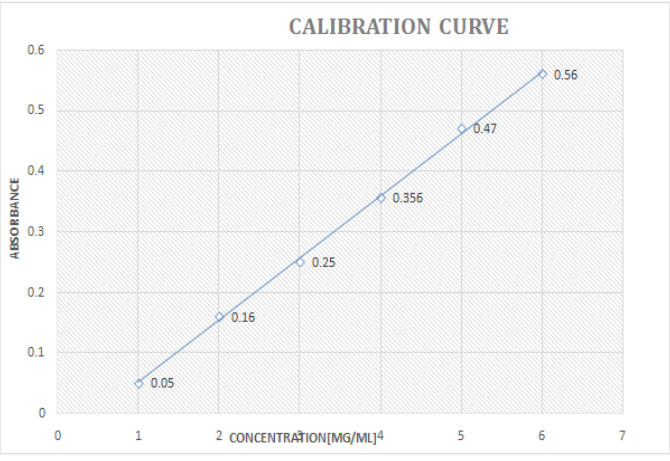
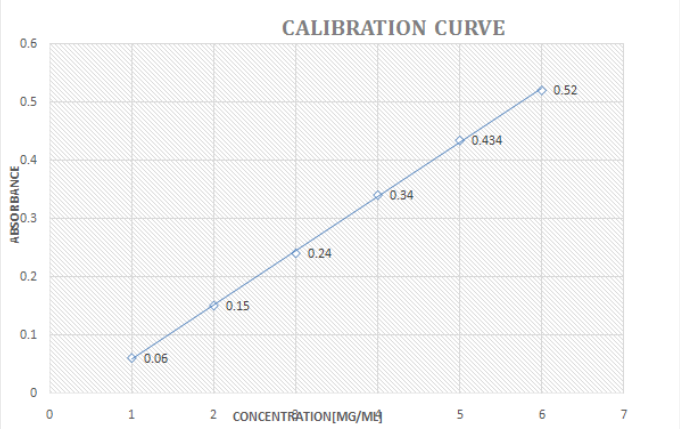
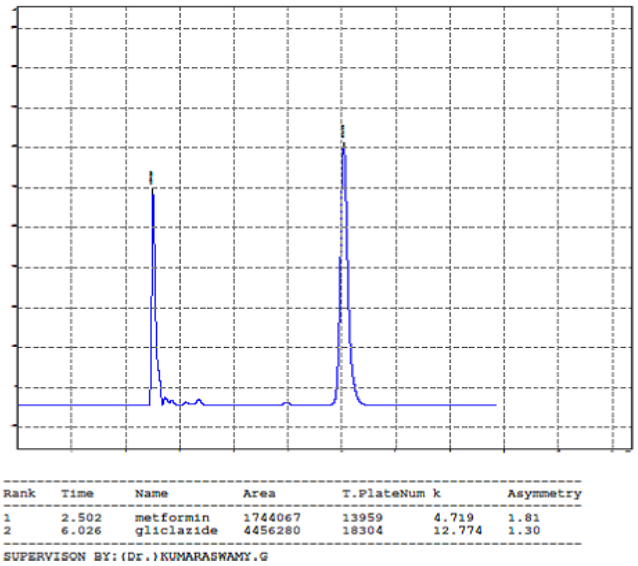


Fig. 4. Calibration Curve of Gliclazide





Rank	Time	Name	Area	T.PlateNum	k	Asymmetry
1	2.502	metformin	1744067	13959	4.719	1.81
2	6.026	gliclazide	4456280	18304	12.774	1.30

SUPERVISON BY:(Dr.)KUMARASWAMY.G

Fig. 5.Typical chromatogram of mixture of Metformin and Gliclizide

LOD and LOQ were found to be 0.1378µg/ml and 0.4180µg/ml for Metformin and 0.0677µg/ml and 0.2051µg/ml for Gliclazide. In all deliberately varied conditions, the SD of retention times of Metformin and Gliclazide were found to be well within the acceptable limit. The tailing factor for all the three peaks was found to be < 1.5 (Table 3). The validated method was used in the analysis of marketed conventional tablets Reclimet with a label claim: 500 mg of metformin and 80mg of Gliclazide per tablet. Representative chromatogram is shown in (fig. 4). The results for the drugs assay show a good agreement with the label claims.

The developed HPLC method is simple, specific, accurate and precise for the simultaneous determination of Metformin and Gliclazide from tablets. The developed method provides good resolution between metformin and Gliclazide. It was successfully validated in terms of system suitability, linearity, range, precision, accuracy, specificity, LOD, LOQ and robustness in accordance with ICH guidelines. Thus, the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

Acknowledge: The authors would like to Thanks to Dr. Reddy's Laboratories, Hyderabad, Indian for providing a samples of Metformin and Gliclazide. The authors are also thank full to Principal and Management of ChilkurBalaji college of pharmacy –Hyderabad for providing all necessary facilities

References:

1. Bhagyashri V.et.al., A Validated RP- HPLC Method for Simultaneous estimation of Gliclazide and Metformin in their dosage form.
2. Available from:htpp//www.en.wikipedia.org/wiki/Metformin and Gliclazide.
3. Available from:htpp//www.Pubmed Pharmacol.com /Metformin and Gliclazide.
4. Available from:htpp//www.en.Rxlist.com /Metformin and Gliclazide.
5. ICH Q2A Validation of Analytical procedure: Methodology International Conference on Harmonization, Geneva October 1994
6. ICH Q2B Validation of Analytical procedure: Methodology International Conference on Harmonization, Geneva March 1996.
7. K. Srilekha, Rajiv dinakar B. Method development and validation of Metformin and Gliclazide by RP-HPLC-PDA method. International Journal of Inventions In Pharmaceutical Sciences 1(4)2013;319-328.
8. P.N.Dhabale, C.R.Seervi Simultaneous UV Spectrophotometric Method for Estimation of Gliclazide and Metformin hydrochloride In Tablet Dosage Form. International journal of ChemTech research 2010;4{1}:1.20
9. Willard Merritt, Dean and Settle. Instrumental Methods of Analysis. 7th edition, CBS Publishers and Distributors, New Delhi, **1986**, 1, 592, 622-628.
10. Anonymous. www.amazon.com/che/al
11. Anonymous. Remington. The Science and Practice of Pharmacy. 21st edition Wolters Kluwer Health (India) Pvt. Ltd., New Delhi, **2007,** (I), 633-642
12. Code Q2A, Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 27 October, **1994**, 1 - 5.
13. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 6th November, **1996**, 1 - 8.
14. KanijFatema , Md. Zakiur Rahman reported "**Development and validation of a simple method for simultaneous estimation of Gliclazide in tablets by using RP-HPLC**
15. B. Udaykumar Rao and Anna PratimaNikalje, " Determination of Gliclazide in a tablet dosage form in the presence of metformin hydrochloride by ion pair –reversed phase liquid chromatographic technique," African Journal of Pharmacy and Pharmacology Vol. 5(11), pp. 1331-1337, 22 September, 2011