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DEVELOPMENT AND VALIDATION OF A REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF GLIMEPIRIDE IN BULK AND DOSAGE FORMS

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ABSTRACT

A novel method for the determination of Glimepiride in bulk and in dosage form by RP-HPLC has been developed and validated. This is simple, rapid, precise and accurate method. The method was developed on a cosmosil ODS (250×4.6mm, 5μ) column using ammonium acetate buffer (pH 5.1) and acetonitrile (40:60v/v) as mobile phase which was pumped at a flow rate of 1.0 ml/min and the detection was done at 230 nm. The retention time was found to be 9.05 min. The developed method was validated by performing studies related to linearity, accuracy, precision, specificity and robustness. The linearity was observed in the concentration range of 100-500μg/ml. The results of recovery studies indicated that the method was accurate. Hence the developed method was found to be suitable for the estimation of Glimepiride in bulk and in dosage form.

KEY WORDS

Glimepiride, RP-HPLC, determination in bulk & dosage forms, linearity.

INTRODUCTION

Glimepiride is a 3rd generation hypoglycemic (sulfonyl urea) agent which is used as an anti-diabetic (NIDDM TYPE-2)[1]. Chemically Glimepiride is 3-ethyl-4-methyl-N- (4- [N- ((1r ,4r) - 4 -methylcyclohexylcarbamoyl) sulfamoyl] phenethyl) -2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide It acts as an insulin secretagogue. It lowers blood sugars by stimulating the release of insulin by pancreatic beta cells and by including increased activity of intra cellular receptors. Glimepiride is insoluble in water, ethanol, n-butanol, iso-propanol, 0.1N HCl, and dilute NaOH. It is sparingly soluble in DMSO, slightly soluble in acetone, and very slightly soluble in ethyl acetate, methanol and acetonitrile. Glimepiride is an antidiabetic drug. Hence, it has to be used along with proper diet and exercise program to control high blood sugar. It is used in patients with type-2 diabetis (non- insulin dependent diabetis)

It works by stimulating the release of body's natural insulin controlling high blood sugars. It also helps to prevent kidney damage, blindness, nerve problems, loss of limbs and sexual dysfunctional problems. Proper control of diabetes may also lessen a risk of a heart attack or stroke.

Literature survey reveals that few spectrophotometric methods[2-4],HPLC methods[5-6] and simultaneous estimations[7-14] are available for the estimation of Glimepiride.So, the authors have proposed a highly effective, new validated, sensitive and reproducible HPLC method for the determination of Glimepiride in bulk and in pharmaceutical dosage forms.

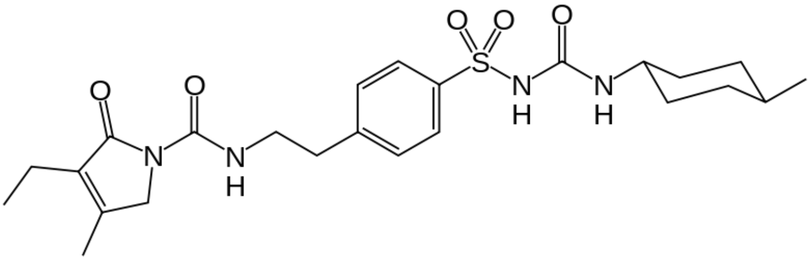

Figure 1: Structure of Glimepiride

EXPERIMENTAL

Instruments and reagents

Prominent isocratic Waters 2695 – separation module with a UV detector, Shimadzu UV-visible spectrophotometer – 1601 with UV – probe software and a Cosmosil C_{18} (4.6 X 250 mm, 5µ) column were used. A 20 µl Hamilton injection syringe was used for sample injection

Ammonium acetate buffer of GR grade, acetonitrile and methanol of HPLC grade and water of Milli-Q grade were used.

Chromatographic conditions

A Cosmosil C_{18} (4.6 X 250 mm, 5µ) column and a freshly prepared mobile phase comprising of ammonium acetate buffer (pH 5.1) and acetonitrile in the ratio of 40:60v/v was used. The solvents were filtered through 0.45 µ membrane filter and sonicated before use. The flow rate of mobile phase was maintained at 1.0ml/min. The column temperature was maintained at 25°C and the detection was carried out at 230nm.

Selection of analytical wavelength

From the Standard stock solution further dilutions were prepared using mobile phase and scanned over the range of 200-400nm and the spectrum was overlain. It was observed that 230 nm was lambda max of Glimepiride and it was preferred as suitable wavelength for detection.

Selection of column and mobile phase

During the development the method was tried with various columns such as Devlosil ODS C_{18} (250X 4.6 mm,5u), Inertsil ODS, C_{18} (250X 4.6 mm,5u), Cosmosil ODS C_{18} (250X 4.6 mm,5u), and various mobile phase compositions like methanol, water, ammonium acetate buffer (pH 5.1) and acetonitrile and finally Cosmosil ODS (250X 4.6 mm,5µ), was selected and ammonium acetate buffer (pH 5.1) and acetonitrile in the ratio of 40:60v/v was selected as mobile phase. 100% Purified Milli Q Water was selected as diluent.

Preparation of mobile phase

Dissolve 24.8g of ammonium acetate in 200ml of Milli-Q water. Add about 25ml of glacial acetic acid and make up to 500ml with water. Check the pH and adjusted to 5.1 with acetic acid added drop wise. Buffer and acetonitrile are mixed in the ratio of 40:60v/v. The solution was filtered through 0.45µ membrane filter, degassed with a helium purge for 20 min.

Preparation of stock and standard solution

100mg of Glimepiride working standard was weighed and transferred into 100ml volumetric flask and volume was made up to the mark with methanol. 50ml methanol was added and the flask was sonicated for 20min and the volume was made up with methanol. 2ml of this solution was transferred into 10ml volumetric flask and the volume was made up 10ml with methanol.

The blank solution was prepared and injected. The prepared standard solution was injected and the chromatogram was recorded. A typical chromatogram was shown in **Figure 2**.

Linearity and construction of calibration curve

Solutions containing 100, 200, 300, 400 and 500 µg / ml of Glimepiride were prepared from standard solution to determine the linearity range. Each one of these drug solutions (20 µl) was injected 5 times into the column by maintaining a flow rate of 1.0 ml/min. The detection was carried out at 230 nm. Chromatograms were recorded and peak area was recorded for all the injections. A calibration plot of concentration over the peak area was constructed and was shown in **Figure 3.**

Assay of Glimepiride in bulk and in dosage form

Weigh tablet powder equivalent to average weight of tablets and transfer into 100ml volumetric flask. Add 50ml methanol and sonicate for 20min and make up the volume with methanol. Filter solution through 0.45µ membrane filter. Centrifuge a portion of the sample at 3000rpm for 15minutes. The solution was further diluted with the mobile phase to obtain a sample of 200 µg / ml.

RESULTS AND DISCUSSIONS

Method development: In the present investigation we have developed a simple precise and accurate reverse phase high performance liquid chromatographic method for the determination of Glimepiride. The method was developed using mobile phase comprising a mixture of ammonium acetate buffer (pH 5.1): acetonitrile (40:60v/v) and a Cosmosil ODS C_{18} (4.6 X 250 mm, 5µ) column. The flow rate selected was 1.0 ml/min. The detection was carried out at 230nm. The developed method was found to be appropriate for the determination of Glimepiride in bulk and in dosage forms. A typical chromatogram obtained was shown in **Figure 2**.

Validation of the proposed method

Precision

Intra-day and inter-day precision studies were performed by injecting each concentration for six times and the % RSD was calculated and it was found to be within limits. The results of precision studies were shown in **Table 1**

Linearity

The linearity of the method was found to be in the range of 100-500µg/ml. The values of linearity studies were shown in **Table 1**. The regression coefficient was found to be 0.999 from the calibration curve. The linearity plot was shown in **Figure 2**.

Accuracy

The percentage recovery studies were carried out at three different concentration levels. The percentage recovery and the percentage RSD values were found within the limits. The results of accuracy studies were shown in **Table 3**.

Specificity

Chromatogram of blank did not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. No interference was observed from the excipients and degradation products of degradation studies. Hence the method was found to be specific and stable.

Robustness

The robustness studies were performed by changing the organic phase proportion of the mobile phase and buffer pH. The results of robustness were shown in **Table 4**.

CONCLUSION

The proposed HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of Glimepiride in bulk and in pharmaceutical dosage forms. Hence this method can be easily and conveniently adopted for routine analysis of Glimepiride in pure and pharmaceutical formulations.

Fig-2: A typical chromatogram of Glimepiride standard

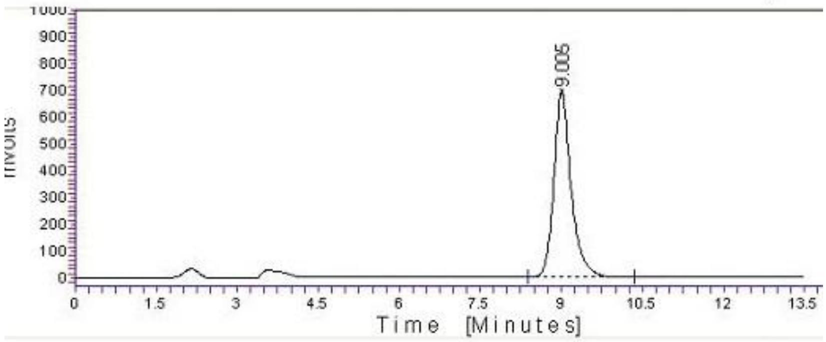

Table 1: Results of intra-day and inter-day precision

Concentration of Glimepiride (ppm)	Intra-day precision			Inter-day precision		
	Mean amount found	Percent amount found	Percent RSD	Mean amount found	Percent amount found	Percent RSD
50	49.90	99.8	0.501	49.80	99..60	0.268
100	99.78	99.87	0.274	99.76	99.77.	0.150
150	149.77	99.9	0.130	149.87	99.91	0.160

Table 2: Results of Linearity studies

S.No	Concentration (µg/ml)	Area
1	5	6129
2	10	8005
3	15	10002
4	20	12321
5	25	14740
6	30	17618

Figure 3: Standard curve of the proposed method

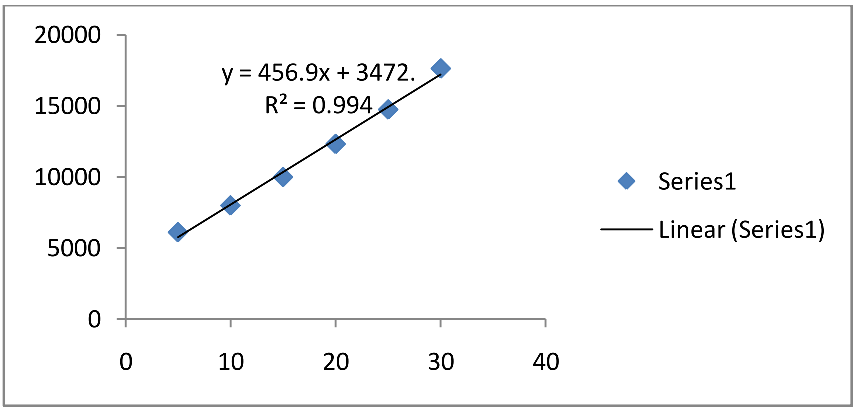

Table-3: Results of Accuracy studies

S.NO	Spike level	µg/mL added	µg/mL found	% recovery	Mean % recovery
1	50%	4.01	3.99	99.4	99.5
2	50%	4.01	3.99	99.3	
3	50%	4.01	4.00	99.7	
1	75%	6.02	6.00	99.7	99.7
2	75%	6.02	5.99	99.6	
3	75%	6.02	6.01	99.9	
1	100%	8.03	8.00	99.7	99.8
2	100%	8.03	8.02	99.9	
3	100%	8.03	8.01	99.7	
1	125%	10.03	10.04	100.0	100.1
2	125%	10.03	10.05	100.2	
3	125%	10.03	10.03	100.0	
1	150%	12.04	12.08	100.3	100.2
2	150%	12.04	12.06	100.1	
3	150%	12.04	12.05	100.1	

Table-4: Results of robustness studies

Result	Flow rate (ml/min)			pH of the buffer			Organic proportion of the phase (%)		
	0.8	1.0	1.2	2.8	3.0	3.2	50	55	60
Tailing factor	1.0	0.7	0.9	1.0	0.7	1.1	0.9	0.7	1.0
Theoretical plates	7856	8934	8125	7901	8934	8462	8211	8934	7995

Table-5: System suitability parameters

System suitability	Acceptance criteria	Observed value
Theoritical plates	Not less than 3000	8443
Tailing factor	Not more than 2.0	1.3
%RSD for replicated injections	Not more than 2.0	0.09

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