CHAPTER –1
INTRODUCTION

1.1Drug:

A drug may be defined as a substance meant for diagnosis, cure mitigation, prevention or treatment of diseases in human beings or animals or for alteringany structure or any function of the body.

Drugs play a vital role in the progress of human civilization by curing diseases. Today majority of the drug used are of synthetic origin. These are produced in the bulk and used for their therapeutic effects in pharmaceutical formulations. There are biologically active chemical substances generally formulated in to convenient dosage forms such as tablets, capsules, ointments and injectableets, these formulations deliver the drug substance in stable, on-toxic and acceptable form, ensuring its bioavailability and therapeutic activity.

1.2 Quality, safety and efficacy of drugs:

Safety and efficacy are two fundamental issues of importance in drug therapy. The safety of a drug is determined by its pharmacological- toxicological profile as well as the adverse effects caused by the impurities in bulk and dosage forms. The impurities in drugs often possess unwanted pharmacological or toxicological effect by which any benefit from their administration may be outweighed.

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles, journals and specific relating to individual drug and published in the form of book called as pharmacopoeias.

- Indian pharmacopoeia –IP
- British pharmacopoeia-BP
- United states pharmacopoeia-USP

- European pharmacopoeia-EP

Now a days, drug introduced in to the market have been increasing at a rapid rate. These drugs may be either new entities or partial structural modifications of the existing drugs and which are included in one or few pharmacopoeias only, after time lag because of several reasons. Under these conditions, standards and analytical procedures for these drugs may not be inaccessible. Hence it becomes necessary to develop newer analytical for such drugs

The quality and safety of the drugs is generally assured by monitoring and controlling the release of drug and level of impurities effectively. Thus the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis.

1.3 Introduction of pharmaceutical analysis:

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipients) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.

By now it should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product, safety and efficacy studies required that drug substance and drug product meet two critical requirements.

CHAPTER - 2

AIM AND OBJECTIVE

2.1 Aim:

From the literature survey conducted, it was found that there are few analytical methods reported for estimation of Glimepiride by using HPLC.

A comprehensive, validated and simple analytical method for assay of Glimepiride tablets and degradation products is therefore crucial. HPLC with PDA detector is a good selection as PDA detector is available in most laboratories. Therefore, in proposed project a successful attempt has been made to develop simple, accurate and economic methods for analysis of Glimepiride tablets and validated.

2.2. Objective:

The objectives of the present work are to development and validate a HPLC method with PDA detector for the assay of Glimepiride tablets to be employed in routine and stability tests.

In the method development of Glimepiride I have decided to carry out my project work by incorporating the reverse phase high performance liquid chromatography (HPLC).

Finally the developed method will be validated according to ICH guidelines for its various parameters.

CHAPTER – 3

DRUG PROFILE

3.1 Drug profile of Glimepiride:

3.1.1Structure of Glimepiride:

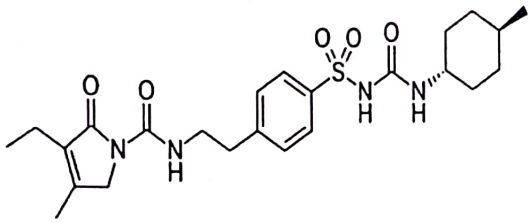

Figure No 12: Structure of Glimepiride

3.1.2 IUPAC Name: 3-Ethyl-4-methyl-*N*-[2-(4-{[(*trans*-4-methylcyclohexyl)carbamoyl]sulfamoyl}phenyl)ethyl]-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide

3.1.3 Molecular weight : 490.617 g/mol

3.1.4 Molecular formula : $C_{24}H_{34}N_4O_5S$

3.1.5 Solubility: Poorly soluble in water. slightly soluble in ethanol and sparingly soluble in methylene chloride.

3.1.6 Category : Type 2 diabetes

3.1.7 Mechanism of Action of Glimepiride:

The mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin.

CHAPTER -4

LITERATURE REVIEW

1. **Wankhede SB, Raka KC* et.al., Stability indicating method development for simultaneous to determination of Glimepiride in dosage form by RP-HPLC** Simple, specific, economical and precise high performance liquid chromatographic method for the simultaneous determination of Glimepiride in API (active pharmaceutical ingredient) and formulation has been developed and validated. Chromatography was carried out at 30˚C on a prepacked Zorbax SB C18 (5 mm, 250 x 4.6 mm) column with the 0.02 M potassium dihydrogen phosphate: Acetonitrile: OPA (43:57, v/v/v) was used as the mobile phase. The UV detection was carried at 232 nm. The results obtained showed good agreement with the declared contents. Glimepiride separated in less than 10 mins with good resolution and minimal tailing and without interference of excipients. The retention times of Glimepiride were 5.7 min and 9.1 min, respectively. The method was linear in the range of 5–50 µg/ml for concentration with a correlation co-efficient 0.9992 and the recovery was 99-102%. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The proposed method can be used for quantitative determination of Glimepiride combination from API and formulation.

2. **Md Arif Hosse, Md Ahsanul et.al., In the present study, a simple, sensitive and specific liquid chromatography (RP-HPLC) method of Glimepiride in tablet dosage form.** A simple, precise, accurate, and rapid HPLC method has been developed, and validated for the determination of Glimepiride, in combined tablet dosage form. The mobile phase used was a mixture of M phosphoric acid solution:acetonitrile pH 2.9 (60:40% v/v). The detection of Glimepiride was carried out on dual g absorbance detector at 240 nm and 254 nm, respectively. Results of the analysis were validated statistically, and by recovery studies. The proposed method can be successfully used to determine the drug contents of marketed formulation.

CHAPTER -5

PLAN OF WORK

In order to develop a simple, reliable and accurate method for the assay of Glimepiride tablet dosage form by reverse phase HPLC and validate the method for its repeatability and reproducibility.

The plan work includes:

- ✓ Procurement of raw materials.
- ✓ Establishment of system suitability parameters.
- ✓ Trials for the method development for Glimepiride.
- ✓ Setting the optimized method.
- ✓ Validation of optimized methods for Glimepiride.

Validation parameters like:

- ❖ System suitability and system precision.
- ❖ Specificity
- ❖ Linearity and range.
- ❖ Accuracy.
- ❖ Precision.
  - Method precision
  - System precision
  - Intermediate precision.
- ❖ Robustness
- ❖ Force Degradation

CHAPTER -6

MATERIALS AND EQUIPMENTS

6.1 CHEMICALS AND REAGENTS:

S.NO	NAME OF REAGENT	MAKE	GRADE
1	Water	Rankem	HPLC Grade
2	Ortho Phospharic acid	Rankem	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Hydrochloric acid	Merck	Pure
5	Sodium hydroxide	Merck	Pure
6	Hydrogen peroxide	Merck	Pure
7	Sodium bisulphate	Merck	Pure

Table No 6: Chemicals and Reagents

6.2 STANDARDS AND SAMPLES:

S.NO	NAME OF THE DRUG	MANUFACTURER OR SUPPLIER
1	Glimepiride working standard.	Cipla Pharmaceuticals .Ltd
2	Glimepiride drug substance.	Cipla Pharmaceuticals .Ltd
3	Glimepiride tablets 4 mg.	Cipla Pharma .Ltd

Table No 7: Standards and Samples

6.3 INSTRUMENTS OR EQUIPMENTS DETAILS:

S.NO	INSTRUMENT NAME	MAKE AND MODEL
1	HPLC	Waters(alliance 2695)
2	Ultra sonicator	Unichrome
3	Electronic balance	Ohaus
4	PH meter	Eutech
5	HPLC Column	Luna C18, 250mm x 4.6mm, 5µm.
6	Centrifuge	Remi
7	Refrigerator	Whirlpool

CHAPTER -7
EXPERIMENTAL DETAILS

7.1 Analytical method development:

In proposed project a successful attempt has been made to develop a simple, accurate for analysis of Glimepiride tablets (2 mg) by RP-HPLC.

7.2 Method development parameters:

Selection of following parameters is very important in method development.

- Mode of chromatography.
- Wave length.
- Column.
- Mobile phase composition and buffer P^H.
- Column temperature.
- Solvent delivery system.
- Flow rate.
- Injection volume.

7.2.1 Selection of Mode of chromatography:

Selected mode of chromatography: Reverse phase

Basis of selection : polarity of the molecule

Reason for selection : As Glimepiride is a polar molecule it elutes at faster rates along with the mobile phase.

7.2.2 Selection of detector wave length:

Selection of detector wavelength is a critical step in finalization of the analytical method. To determine exact wave length standard Glimepiride is prepared and injected into chromatographic system with PDA detector and the wave length which gives higher response for the compound.

CHAPTER -8

RESULTS AND DISCUSSIONS

8.1 PDA spectrum:

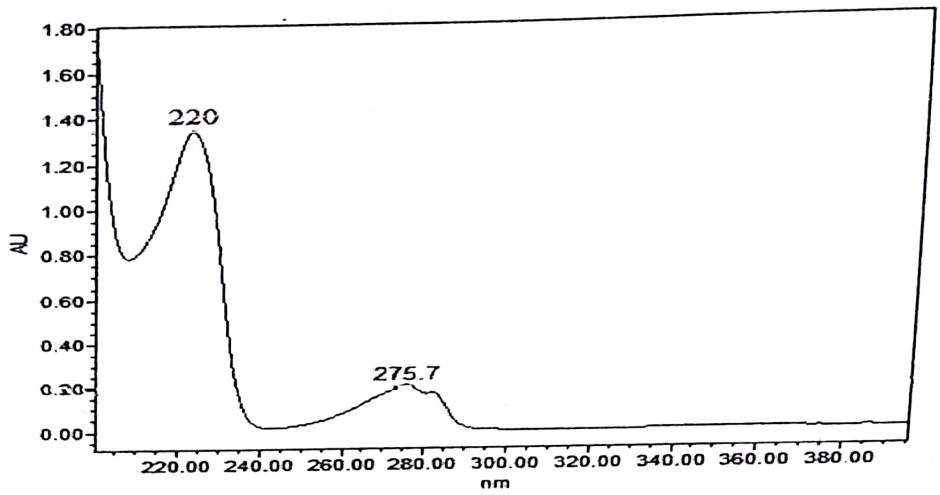

Figure No 14: PDA spectrum

S.NO	WAVELENGTH
1.	220 nm

8.2 ANALYTICAL METHOD DEVELOPMENT OF GLIMEPIRIDE BY RP-HPLC METHOD

TRAIL CHROMATOGRAMS

TRAIL -1

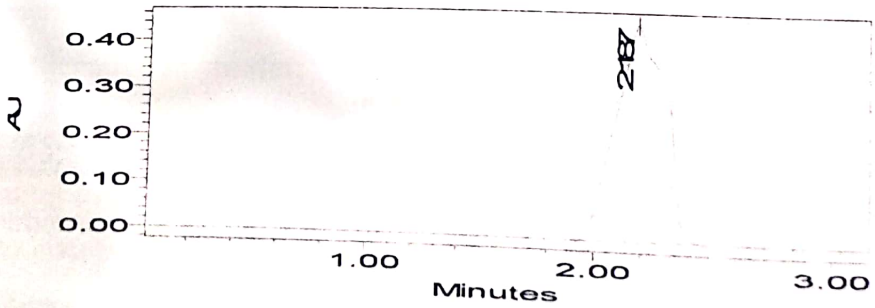

Figure No 14: - Typical Chromatogram Trail 1

	Name	Retention Time	Area	% Area	USP Tailing	USP Plate Count
1		2.187	6429928	100.00	3.58	1452

CHAPTER -9

SUMMARY OF RESULTS

S.NO	PARAMETER	RESULT
1.	System suitability	Relative standard deviation for area of Glimepiride peak for six replicate injections of standard solution 0.48 Tailing factor of drug peak is 1.12 Theoretical plates for Glimepiride peaks are 5895
2.	Specificity Blank interferences Forced degradation Acid degradation Base degradation Peroxide degradation Thermal degradation UV degradation	No peaks are observed in the blank chromatogram at the retention time of the Glimepiride peaks. % degradation of the of Glimepiride peak in all stressed samples is found to be with in the acceptance criteria.
3	Linearity	The square of correlation coefficient value for of Glimepiride is 0.999
4	Accuracy	Individual % recovery for of Glimepiride is found to be in the rage of 95.0 to 105%. Mean % recovery for of Glimepiride is found to be in the range of 95.0 to 105%. %RSD for of Glimepiride is found to be in the range of 0.4 vb5 For linearity of test method, the squared correlation coefficient derived from the least square fit of the data for Glimepiride is 0.99.

CHAPTER -10

CONCLUSION

Development and validation of RP-HPLC method for the estimation of Glimepiride 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 Glimepiride the bulk and 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 Glimepiride tablet dosage form.

Several analytical procedures have been proposed for the quantitative estimation of Glimepiride separately and in combination with other drugs.

So attempt was taken to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of Glimepiride in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.