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Development and Validation of RP-HPLC Method for Estimation of Anti Diabetic Drug in Bulk and Pharmaceutical Dosage Form Patil Aparna Prakashrao

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ABSTRACT :
A simple rapid, precise and reliable reverse phase HPLC method was developed for the seperation and estimation of glimepiride in bulk drug and pharmaceutical dosage Formrun time of glimepiride is 5 min.- the method was validated as a Final verification. Of method development with respect to precision. Linearity accuracy, ruggedness, robustnessthe validated method was successfully applied to the commercially available pharmaceutical dosage Form, yielding very good and reproducible result. A simple, rapid, precise and reliable. reverse phaseHPLC method was developed For the seperation and estimation of drugs in bulk and Pharmaceutical dosage Form; it was validated according to ICH and FDA guidelines.Pharmaceutical company For the treatment OF Type-2 diabetes; it potentiates the effect of incretin harmones through the inhibition of their degradation-Glimepiride is an antidiabetic medication within the Sulfonylureas class, primarily prescribed For the management of type 2 diabetes. It is regarded as a second-line option compared to metformin, due to metformin's well-established safety and efficacy - Duration of Action : 24 hrs pregnancy category: AU: Bioavailability - 100%. protein binding->99.5%Routes of administration: By Mouth Metabolism complete liver. (1st stage through CYP2C9)
Alogliptin benzoate, a member of dipeptidyl peptidase-4 inhibitors, is a recent drug developed by Takeda Pharmaceutical Company for the treatment of Type 2 diabetes; it potentiates the effect of incretin hormones through the inhibition of their degradation. Alogliptin can be used alone or in combination therapy. A new sensitive and rapid HPLC method was developed for the determination of alogliptin benzoate in bulk and pharmaceutical dosage forms; it was validated according to ICH and FDA guidelines. The HPLC analysis was performed on the Agilent 1200 system equipped with a Hypersil Gold Thermo Scientific C18 (250 cm × 4.6 mm) 5 µm column, with a mixture of acetonitrile and ammonium carbonate buffer in the ratio of 55 : 45 v/v as the mobile phase, at the flow rate of 1.0 mL/min. The detection was performed at the wavelength (λ) of 277, and the retention time of alogliptin benzoate was around 4min

KEYWORDS :RP-HPLC method, antidiabetic drug, Glimepiride - Type 2 diabetes alogliptin benzoate

I. INTRODUCTION :

Chromatography : Is a process for separating components of a mixture to get the process started the mixture in dissolved in a substance called the mobile phase which carries it through a second substance called the stationary phaseEx : liquid chromatography
TYPES OF CHROMATOGRAPHY : Gas chromatography Size exclusion chromatography Ion chromatographyPaper chromatography Affinity chromatography Reverse phase High performance liquid chromatography Thin layer chromatography Reverse phase High performance liquid chromatography (Rp-HPLC) involves the separation of molecule on the basis of HydrophobicityPRINCIPLE :Separation of molecule on the basis of Hydrophobicity the separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase
ADVANTAGES : Lower cost When compared with the other HPLC method Lower toxicity of the solvent Reduced sample evaporation
DISADVANTAGES : High cost of solvent and additives Skilled person required for operation
ANTIDIABETIC DRUGS :Drugs used in the diabetes treat diabetes mellitus by altering the glucose level in the blood These are the those drugs

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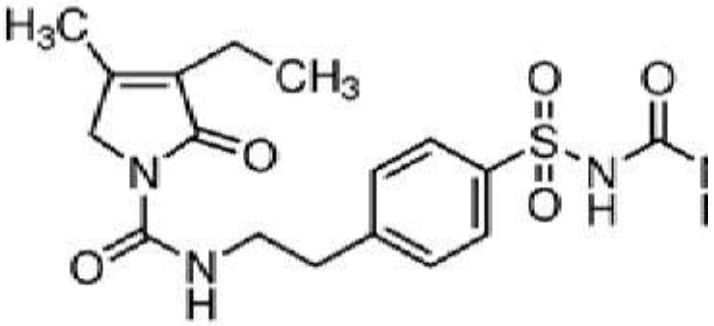
which are used in the treatment of diabetes mellitus this drugs is called antidiabetic drugs.
EXAMPLES :Glimepiride, Pioglitazon, Metformine
ADVERSE EFFECTS : Hypoglycemia-they can cause hypos but these are less common than with sulfonyl ureas Nausea the first evidence about a known case of diabetes mellitus nearly 3000 years ago and despite the great deal of research that has been done recently, diabetes mellitus is still a wide spread serious disease that affect the life quality of millions of people worldwide. It is estimated that the number of patients with diabetes mellitus will rise to about 592 millions by the year 2035 [1, 2]. These harmones includes glucagon-like peptide 1 and glucose dependent insulin tropic polypeptide [5, 6]. Dipeptidyl peptidase-4 is an enzyme found in the human body that helps inactivate the incretin hormones, thus terminating their hypoglycemic effect [2]. Alogliptina member of dipeptidyl peptidase-4 inhibitors is a recent drug developed in 2010 by Takeda Pharmaceutical Company [2, 7], which is used for the treatment of Type 2 diabetes, and it potentiates the effect of incretin hormones through inhibition of their degradationby the dipeptidyl peptidase-4 enzyme [2, 4]. Alogliptin Glimepiride: Molecular formula:- C24H34N405S, Molecular weight :-490.617g/mol, IUPAC:-4-ethyl-3-methyl-N-[2-[4-[(4 methylcyclohexyl carbamoylsulfamoyl)]phenyl]ethyl]-5-oxo-2H-pyrrole-1carboxamide Solubility :at very low water soluble, Appearance :white to yellowish – white, Category :type 2 diabetes, Dose :Adults 1-2mg once a day



Structure of Glimepiride

RESEARCH AIM :

The research Aim refers to the overarching goal or purpose of a research study, stating what the researcher intends to achieve or investigateIt provides direction and focus to the researchGuiding the selection of research questions,methods, and data analysis to address specific objectiveNeeds to contribute To existing knowledge in the field In this study New sensitive and rapid HPLC method Was develop to determine metformin hydrochloride and Evogliptin Tartarate in pharmaceutical dosage form

OBJECTIVE :

Research objective are specific measurable goals that outline what the researcher will accomplish within a study They are derived from the research Aim and serve as the building blocks for the research design, helping to define the scope, identify the variables to be studied and guide data collection and analysis to ultimately fulfill the research Aim

PLAN OF WORK :

The work is Planned to carry out As given below :Collection of relevant literature. To undertake solubility study Optimisation of initial chromatograpic Condition Gliclazide (Water insoluble acidic drug) Metformin (Water soluble basic drugl Simultaneous HPLC method for binary mixture in challenging Metformin is too polar to retain with gliclazide on the same chromatogram Usual attempts to retain metformin results in delayed, broad and asymmetric gliclazide peakSeparation on Altima CN (250 X4.6 mm X 5p) using 20 mM ammonium formate buffer (pH 3.5) and Acetonitrile (45:55)

STEPS OF HPLC METHOD DEVELOPMENT :

Information on sample, Define separation goals, Special procdure requirement, Detector selection and setting, Separation Condition optimization, Check for problem or special procedure requirements Recovery of purified material, Quantitative calibration/qualitative

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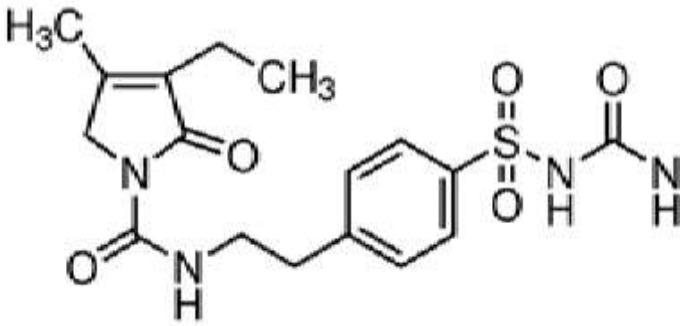
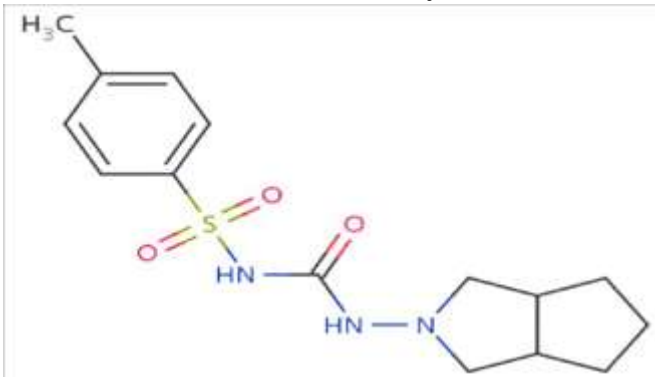
method, Method validation for release to laboratories

DRUG PROFILE :
DRUG NAME : Glimepiride
Molecular formula:- C24H34N405S
Molecular weight :-490.617g/mol
IUPAC:-4-ethyl-3-methyl-N-[2-[4-[(4 methylcyclohexyl carbamoylsulfamoyl)]phenyl]ethyl]-5-oxo-2H-pyrrole-1carboxamide
Solubility :at very low water soluble
Appearance :white to yellowish – white
Category :type 2 diabetes
Dose :Adults 1-2mg once a day



Structure of Glimepiride

DRUG NAME: Gliclazide Hel
Molecular Formula: C15H21N303S
Molecular weight: 323.4
IUPAC Name: 1-(3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]
pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea.
Solubility: Insoluble in water. Soluble in dichloromethane,
chloroform, methanol, DMSO.
Category: Type 2 diabetes mellitus.
Dose: The Appearance White Crystal Powder
total dailydose mayvary from 40to 320 mgtaken orally.



Structure of Gliclazide Hel

DRUG NAME: Alogliptin benzoate
Molecular Formula: C25H27N5O4 |
IUPAC Name: 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-
1,2,3,4-tetrahydropyrimidin-
1yl}methyl)benzonitrile]
pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea.
Solubility : The solubility of alogliptin (benzoate salt) in these solvents I
s approximately 0.1 mg/ml. Alogliptin (benzoate salt) is also slightly soluble in ethanol.
Category: DPP-4 inhibitor (gliptin) class.
Dose: Usual dose: 25 mg orally once a day When used in combination
with insulin or insulin secretagogues such as sulfonylureas, a lower
dose of insulin or the insulin secretagogue may be required to
minimize the risk of hypoglycemia.

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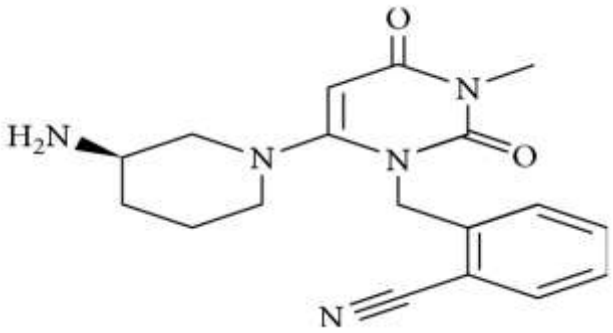

Structure of Alogliptin benzoate

II. MATERIAL AND METHOD :

Chemicals and Reagents A pharmaceutical grade sample of alogliptin benzoate (assigned purity 99.4%) was obtained as gift from Jordan Hikma Pharmaceuticals (Amman, Jordan). NESINA tablets containing 8.5 mg alogliptin benzoate were purchased from the local market. Acetonitrile HPLC grade and ammonium carbonate were purchased from Merck (Merck Serono Amman, Jordan). The double distilled water was obtained from a local pharmaceutical company.

Instrumentation

Agilent 1200 HPLC system was used for liquid chromatography method development and validation (Santa Clara, USA), equipped with a pump (model G1312A), an auto sampler (ALS) (model G1329A), and a Hypersil Gold Thermo Scientific C18 (250 cm × 4.6 mm) 5 µm column (Paisley, UK), and the detector consisted of UV/VIS operated at 277 nm. Chemstation Software (Version Rev B.04.03 (16)) was used for data processing and evaluation.

Chromatographic Conditions

The mobile phase was prepared by dissolving 1.0 gm ammonium carbonate in 1000 ml water. From the previous solution, 450 ml was mixed with 550 ml of acetonitrile. Prior to use the mobile phase was filtered through 0.45 µm membrane filters and degassed by sonication for 10 min. The analysis was carried out on an Agilent 1200 series HPLC system. The analytes were conducted on an analytical column C18, 5 µm, 250 × 4.6 mm with a detection wavelength of 277 nm. The operating temperature of the column was set at 30°C. The injection volume was 10 µL, and the flow rate was maintained at 1.0 mL/min. The run time was 6 minutes.

Preparation of Standard Solution

A standard solution of alogliptin benzoate was prepared by dissolving an accurately weighed amount of alogliptin benzoate (42.5 mg, which is equivalent to 31.25 mg alogliptin) in 50 ml of the mobile phase, and then 5 mL of the resulting solution was diluted to 25 mL by the same solvent to obtain a standard solution of alogliptin benzoate (170 µg/ml).

Preparation of Sample Solution

Twenty alogliptin tablets were weighed, triturated in porcelain mortar, and mixed, and the average weight of tablet was calculated. Accurately weighed amount of powder equivalent to 25 mg of alogliptin (34 mg alogliptin benzoate) was transferred completely to a 200 mL volumetric flask, and 150 mL of the mobile phase was added and sonicated for 30 minutes. The volume was completed to mark by the same solvent to obtain a solution of alogliptin benzoate with a concentration of 170 µg/ml. The prepared solution was filtered through 0.45 µm membrane filters.

Method Validation

The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy

Method precision

A method precision is evaluated by using measuring the height reaction for sixreplicate injection of six distinctive weigh of pattern solution organized as in stepwith proposed technique. The %RSD is calculated and it ought to no longer bemore than 2%.

Willpower

The precision of an analytical approach is decided through assaying asufficient wide variety of aliquots of a homogenous pattern so as to

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calculate statistically valid estimates of fashionable deviation or relative well-known deviation.(22)

ICH requirements

The ICH files recommended that repeatability must be assessed using a minimum quantity of 9 determinations masking the specified variety of the procedure (I, e., 3 concentrations and there replicates of each concentrations or the use of not less than six determinations at one hundred% of the test awareness). The ICH documents encouraged that accuracy should be assessed using at the very least nine determinations over at least three awareness ranges, covering the specified range (i.e., three awareness and three replicates of each concentration).

LINEARITY (25)

Definition: The linearity of an analytical technique is its capability (with in a givenvariety) to achieve the check results which might be at once proportional to theawareness (amount) of analyte inside the pattern.Dedication: Linearity of an analytical method is mounted minimum of five concentrations. it is established to start with by means of visual exam of plot of signals as a function of analyte awareness of content material .If there appears to be a linear dating ,take a look at outcomes are hooked up via suitable statistical methods(i.e., by calculation of the regression line with the aid of the technique of least squares).

ROBUSTNESS

Definition

The robustness of an analytical technique is a measure of its capability to stay unchanged through small however deliberately versions in technique parametersand presents an illustration of its reliability in the course of regular utilization.

Method Development and Optimization

Several physical and chemical properties of alogliptin benzoate were obtained from the literature. The analytical method was developed to select preliminary reversed phase HPLC-UV chromatographic conditions, including detection wavelength, mobile phase, stationary phase, and sample preparation procedure. For this purpose, a series of trials were performed by varying the ratio of acetonitrile and ammonium carbonate buffer and optimizing the chromatographic conditions on the Hypersil Gold Thermo Scientific C18 (250 cm × 4.6 mm) 5 µm column. The results of method optimization are summarized in

Table 1

Results of method optimization.

Column used	Mobile phase	Flow rate	Wavelength	Observation	Result
Restek C18, 125 × 4.0 mm i.d., 5 µm	(Buffer : methanol) (45 : 55) v/v	1.0 ml/min	216 nm	Poor resolution 1.4	Method rejected
Thermo Scientific C18, 250 × 4.6 mm i.d., 5 µm	(Buffer : acetonitrile) (25 : 75) v/v	1.0 ml/min	277 nm	Poor resolution 1.6	Method rejected
Thermo Scientific C18, 250 × 4.6 mm i.d., 5 µm	(Buffer : acetonitrile) (45 : 55) v/v	1.0 ml/min	277 nm	Good resolution 2.4	Method accepted

The mobile phase consisting of acetonitrile and ammonium carbonate buffer in the ratio 55 : 45 v/v with a flow rate of 1 mL/min, injection volume 10 µl, run time 6 min, and column temperature 30°C at wavelength (λ) 277 was optimized as the best chromatographic conditions for the entire study where alogliptin benzoate was eluted forming symmetrical peak shape, resolution and suitable analysis time with retention time about 4 min

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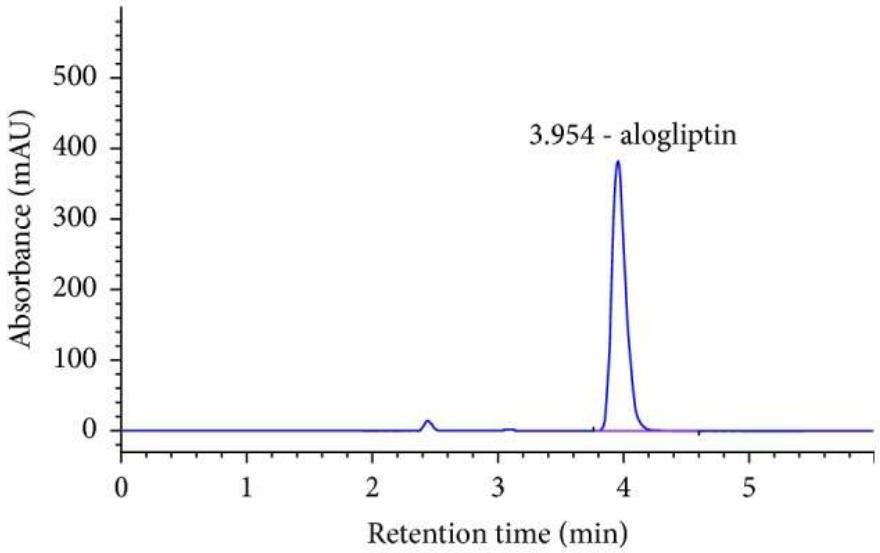
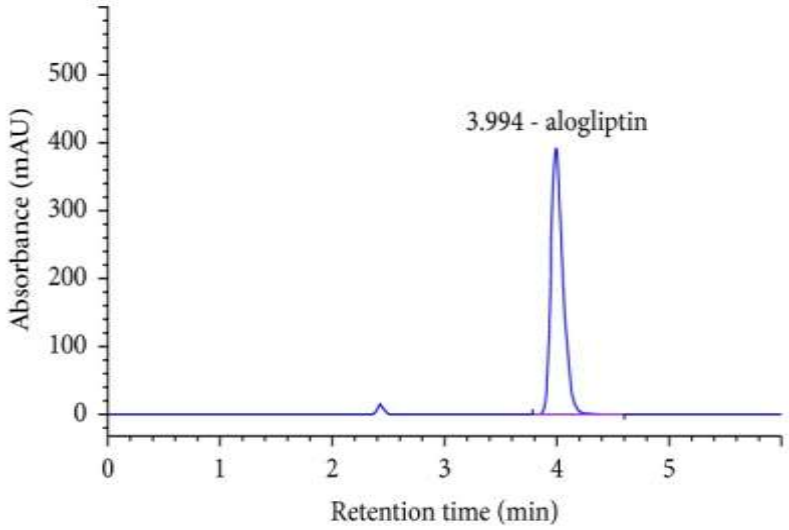


Chromatogram of alogliptin standard solution.

Method Validation

Specificity was evaluated by comparing the chromatograms of mobile phase blank, placebo solution, standard solution, and sample solution (alogliptin 170 µg/ml). For this purpose, 10 µl from solutions mobile phase blank, standard solution, and sample solution were injected into the HPLC system separately, and the chromatogram results are shown in Figures



Chromatogram of alogliptin sample solution.

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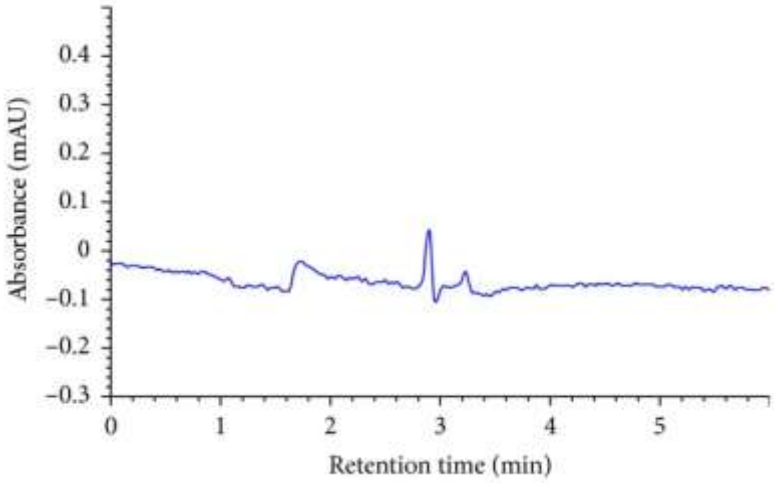
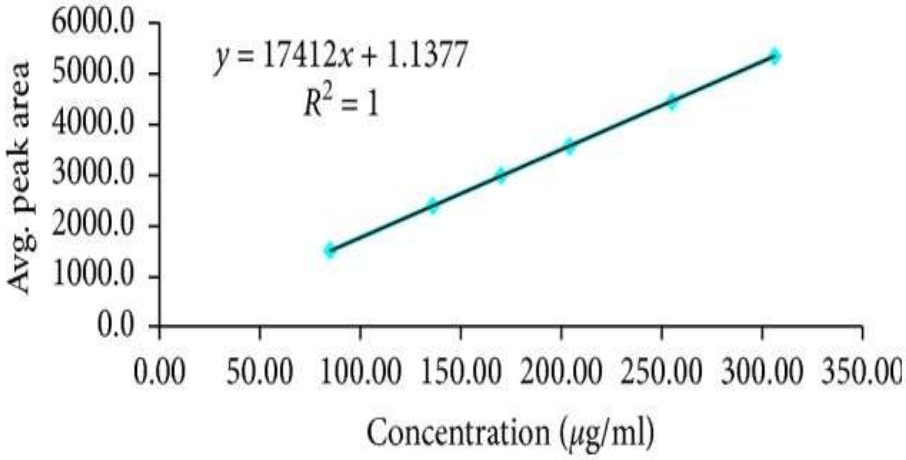


Chromatogram of blank solution

Linearity and Range

Analytical method linearity is defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The mean peak area obtained from the HPLC was plotted against corresponding concentrations to obtain the calibration graph.



Standard calibration curve of alogliptin benzoate.

Limit of Detection and Limit of Quantification (LOD and LOQ) The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, while the limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision

Accuracy The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy showed percentage recovery at all three levels in the range of 99.4–101.9%, and %RDS values were in the range of 0.06–0.43% as shown

III. RESULT AND CONCLUSION

In the present research, a fast, simple, accurate, precise, and linear stability-indicating HPLC method has been developed and validated for alogliptin benzoate, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for alogliptin benzoate. In addition, the main features of the

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developed method are short run time and retention time around 4 min. The method was validated in accordance with ICH guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions.

QUANTITATION

Samples obtained from local market. Gliclazide HCL -500mg, Sitagliptin-50mg Preparation of Dipotassium hydrogen phosphate buffer pH 4.5 Prepare about 0.02M dipotassium hydrogen phosphate in a suitable conical flask and adjust the pH to 4.5 with orthophosphoric acid.(0.02M of di potassiumhydrogen phosphate is prepared by taking1.3601mg of dipotassiumhydrogen phosphate in a volumetric flask , and make up to 1L with water).

Preparation of mobile phase:

Prepare a bath mixture of 4.5 pH, and acetonitrile at a rate of 55:45 filter with a membrane 0.45µ filter and remove Diluent Adjustment: Buffer 4.5 pH, with acetonitrile in a ratio of 55:45

Standard preparation

Accurately measure approximately 50mg of Gliclazide, 50mg of active Sitagliptin up to 100ml of volumetric flask. Complete it completely and sonicate it. Make up to 100ml mobile category. Take 3ml in the upper flask and make up to 50ml per cellphone.

Sample preparation:

Accurately weigh 20 tablets equivalent to 92.4mg to 100ml volumetric flask.Cellular phase to completely disperse and sonicate for 10 minuteswith medium movement Make up to 100ml per cellular phase and filter with 0.45 GHP filter µ. Mix again 3ml with 50ml cellular phase.

Calculation

Determine the % amount of Gliclazide HCL and Sitagliptin in tablets according to the following formula. AT ×W R × 3 × 100 × 50 × PR × Average Weight %Assay = × 100 AR × 100 × 50 × WT × 3 × 100 × LA There, AT = Location in the test solution AR = Location in the standard solution WR = Standard solution weight (mg) WT = Sample weight in test preparation (mg) PR = Performance purity (%)

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