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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND ALOGLIPTIN BY USING RP-HPLC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The main aim of present analytical research is to develop a simple, accurate, rapid and economical RP-HPLC method for the simultaneous estimation of Metformin and Alogliptin in bulk and pharmaceutical dosage forms. The drug analysis plays an important role in the development, manufacture and therapeutic use of drug. To validate the method according to ICH guidelines.

KEYWORDS: Metformin, Alogliptin, Methanol, Buffer.

Plan of work: An attempt was made in an stepwise manner to develop a simple, rapid, selective method using high performance liquid chromatography (RP) for Metformin and Alogliptin. The following stepwise manner protocol was followed.

• An attempt was made in a step wise manner to develop a simple, rapid, selective method using high performance liquid chromatography (RP) for Metformin and Alogliptin combination.
• The following stepwise protocol was followed.
• As a startup, literature survey has done for the parameters like solubility chemical structure, pka value and analytical profile.
• From the data obtained by literature & practically, UV spectroscopic method has been chosen for the detection of λmax using the selective solvent and validated.
• Later several trails were done in RP-HPLC using a different combinations of mobile with available columns to optimize the method.

• After optimization of HPLC method validation of analytical method was done as per ICH guidelines.

Literature Review

1. Praveen Kumar, et al., In the present work, RP-HPLC method for two drugs have been developed and validated for simultaneous determination of Alogliptin and Metformin Hydrochloride in Tablet dosage form. In this method, 0.2% TEA pH adjusted with OPA to 6.0 was used as buffer mobile phase was prepared by adjusting the ratio of buffer and Methanol as 30:70 v/v + 0.2% Triethylamine and a flow rate of 1.0 ml/min. The optimum wavelength for detection was 254 nm and a run time of 10 minutes was used. The column used was Agilent C18 with dimension 250 mm length, 4.6 mm i.d., 5µ particle size. The method was validated for its linearity, accuracy, precision, specificity, robustness. The system suitability parameters passed and linearity was observed in the range of 25-150 µg/ml for Alogliptin and Metformin Hydrochloride respectively and the correlation coefficient of Alogliptin and Metformin Hydrochloride were 0.9995 and 0.9996. The accuracy was performed and the % recovery was observed as 99.96% and 99.83% for Alogliptin and Metformin Hydrochloride respectively. Thus a novel, sensitive, accurate, specific, precise method was developed for the Simultaneous determination of Alogliptin and Metformin Hydrochloride in tablet dosage form by RP-HPLC Method.[25]

Ashutosh KS et al., A validated new stability indicating RP-HPLC method for the quantitative determination of metformin and alogliptin in human plasma was developed as per US-FDA guidelines. The drug was spiked in the plasma and extracted with mobile phase by precipitation method. The extracted analyte was injected into X-Terra C18 (4.6 × 150 mm, 3.5 µm, Make: ACE) or equivalent, maintained at 25°C temperature and effluent was monitored at 235 nm. The mobile phase was consisted of sodium dihydrogen ortho phosphate [pH 4.0]: acetonitrile [HPLC Grade] (70:30 v/v). The flow rate was maintained at 1.0 mL/min. The calibration curve for metformin and alogliptin was linear from 300.0 to 700.0 µg/mL (r2=0.997) and 7.5 to 17.5 µg/mL (r2=0.998) respectively.

The inter-day and intra-day precision was found to be within limits. The Lower limit of quantification (LLOQ) for metformin and alogliptin were 5.936 and 1.983 µg/mL respectively. The average % recovery for metformin and alogliptin were 100.17 and 99.40-99.55% respectively and reproducibility was found to be satisfactory. This RP-HPLC method is suitable for determining the concentration of metformin and alogliptin in human plasma

and it can applied for routine analysis for determination of the metformin and alogliptin from dosage form during pharmacokinetic study.

MATERIALS AND METHODS

Instruments

- HPLC –Waters Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS).
- Sonicator(FAST CLEAN).

Chemicals

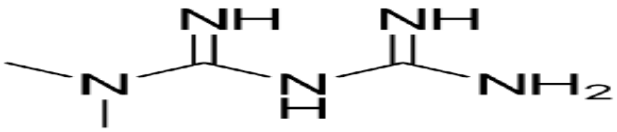
- Methanol HPLC Grade.
- Buffer (KH2PO4) HPLC Grade.

Raw Material

Metformin and Alogliptin Working Standards.

Drug Profile

Metformin



Chemical Formula **: C4H11N5**

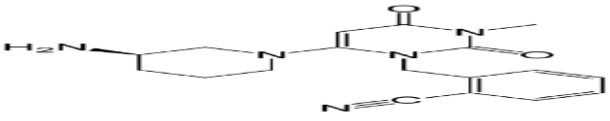
Molecular Weight : 129.1636 g/mol

IUPAC name : 1-carbamimidamido-N,N-dimethylmethanimidamide

Description : Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptakeandutilization.

Category : antihyperglycemic agents

Alogliptin



Chemical formula **: C18H21N5O2**

Molecular Weight **:** 339.391g/mol

IUPAC name **:** 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl}methyl)benzonitrile**.**

Description : Alogliptin inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1 (GLP-1).

Category : antihyperglycemic agents

Method Development Trials

Trial. 1: Chromatographic conditions.

Column : C18Symmetry

Flow rate : 1.0 mL/ min

Wavelength : 254 nm

Mobile phase : Methanol : Water (60:40)

Injection volume : 20 µL

Column Temperature : Ambient

Retention time : 10 min

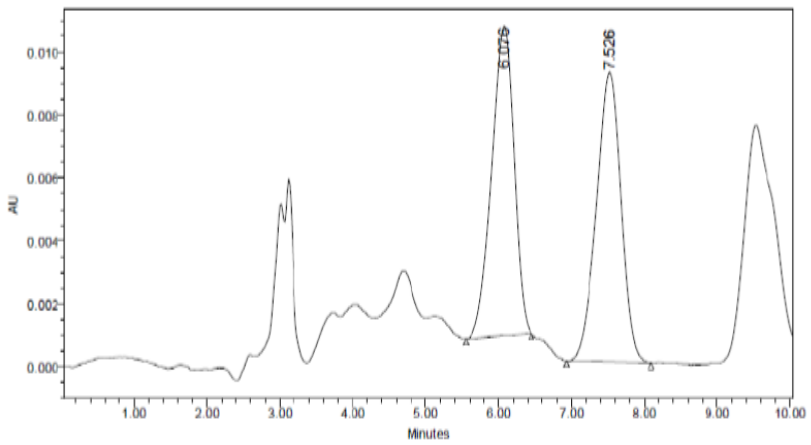


Fig. 1. Chromatogram for Trial-1.

Observation: Peaks are not eluted clearly and some impurities were observed.

Trial. 2: Chromatographic conditions

Column : Symmetry C18 (4.6 x 150mm, 5□m, Make: Waters)
Flow rate : 1.0 mL/min
Wavelength : 254 nm
Mobile phase : pH 3.5 phospate buffer : methanol(40:60)
Injection volume : 20 µL
Column Temperature : Ambient
Retention time :10 min

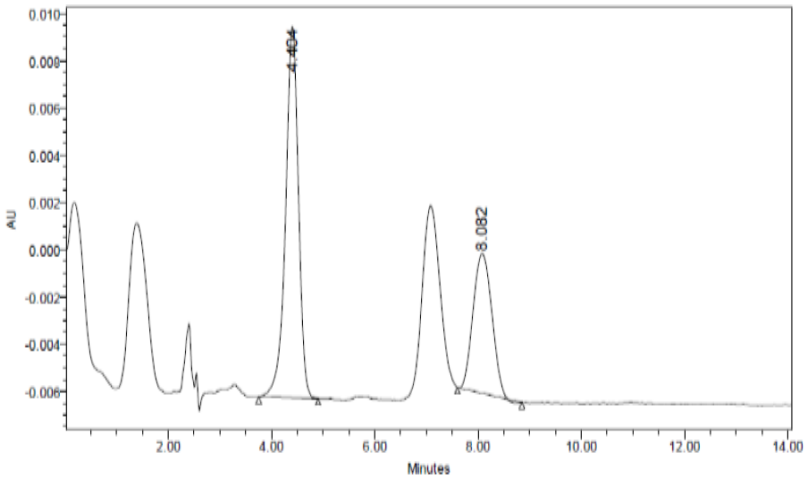


Fig. 2: Chromatogram for Trial 2.

Observation: peaks are not eluted clearly and some impurities were observed

Trial. 3: **Chromatographic conditions.**

Column : Symmetry C18 (4.6 x 150mm, 5□m, Make: Waters)
Flow rate : 1.0 mL/min
Wavelength : 254 nm
Mobile phase : Water : Acetonitrile (20:80)
Injection volume : 20 µL
Column Temperature : Ambient
Retention time : 10 min

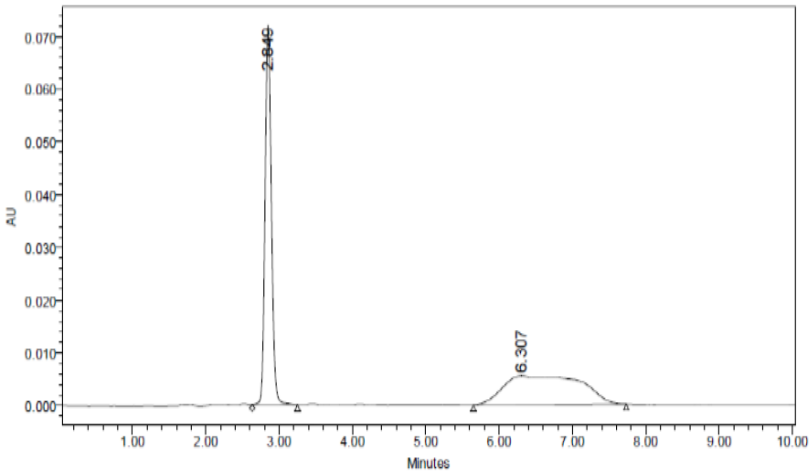


Fig. 3: Chromatogramfor Trial 3.

Observation: Metformin peak eluted clearly and some impurities were observed.

Optimized Chromatographic Conditions

Preparation of (KH2PO4 0.1M) buffer: Weight 3.8954g of di-sodium hydrogen phosphate and 3.4023 of potassium dihydrogen phosphate in to a beaker containing 1000ml of distilled water and dissolve completely. Then ph is adjusted with orthophosphoric acid and then filtered through 0.45µm membrane filter.

Preparation of standard stock solution: Accurately weighed about 100 mg of Metformin and 10 mg of Alogliptin were weighed and transferred into separate 100 ml volumetric flasks. To them about 70 ml of mobile phase was added and sonicated for 20min to dissolve it completely. Then the volume was made up to the mark with the mobile phase. Further working standard solution of mixer of Metformin and Alogliptin (100µg/ml and 10µg/ml) was prepared with mobile phase.

Preparation of sample solution: Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 100 mg of Metformin and 10mg of Alogliptin was weighed and dissolved in 80 ml of methanol. Then it is sonicated for 30 min and solution was filtered through 0.45 µ membrane filter into a 100 ml volumetric flask. Filter paper was washed with the solvent, adding washings to the volumetric flask and volume was made up to mark. Further working sample solutions of 100µg/ml and 10µg/ml of Metformin and Alogliptin was prepared with mobile phase respectively.

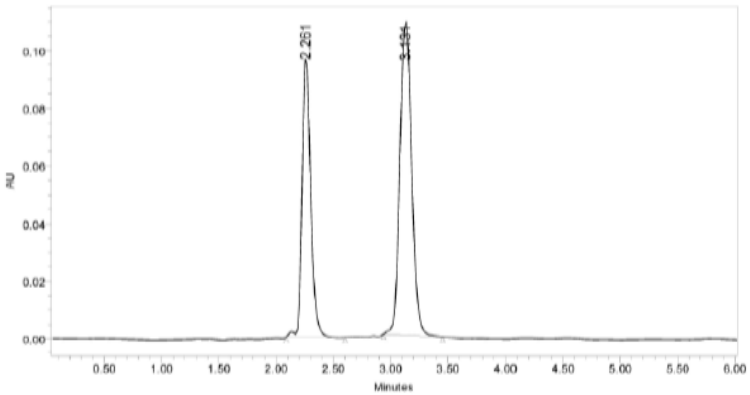
Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Symmetry C18
Mobile Phase	Methanol and Buffer in the ratio of 80:20 V/V.
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	254 nm
Drug RT (min)	10 min

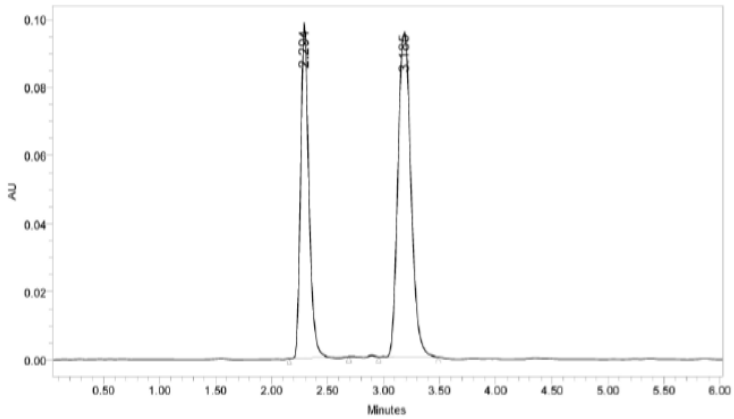
Observation: There is no tailing and fronting good peaks are observed.

Accuracy

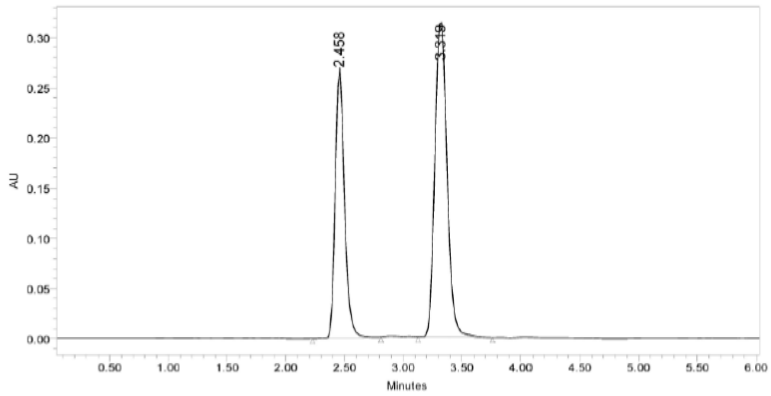
Accuracy at 50%



Accuracy at 100%



Accuracy at 150%



Precision

System Precision

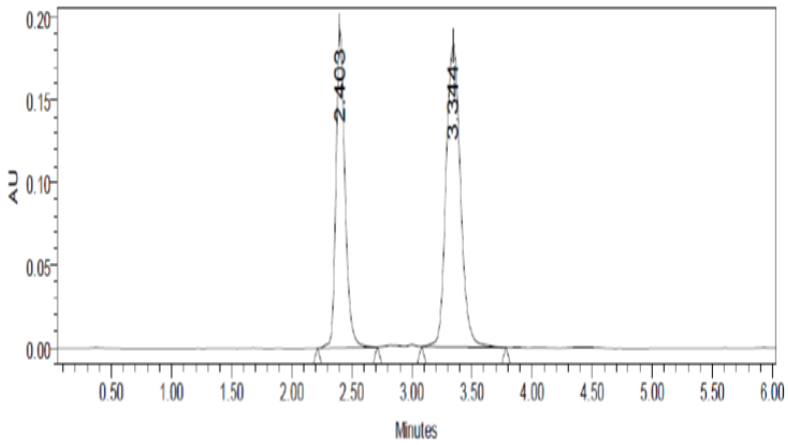


Fig. 5: Chromatogram for System Precision.

Method Precision

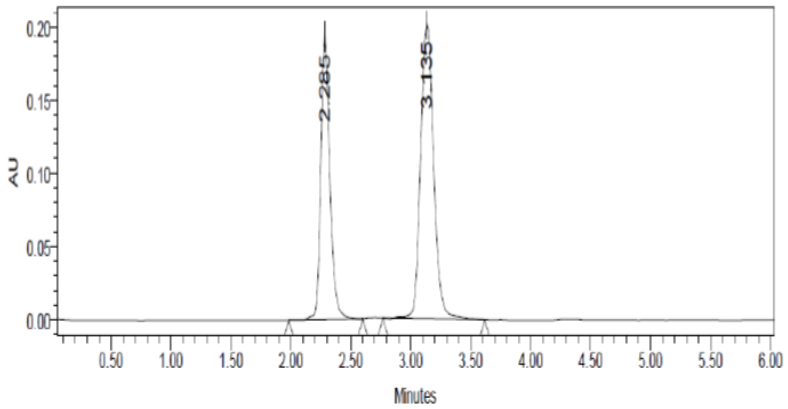
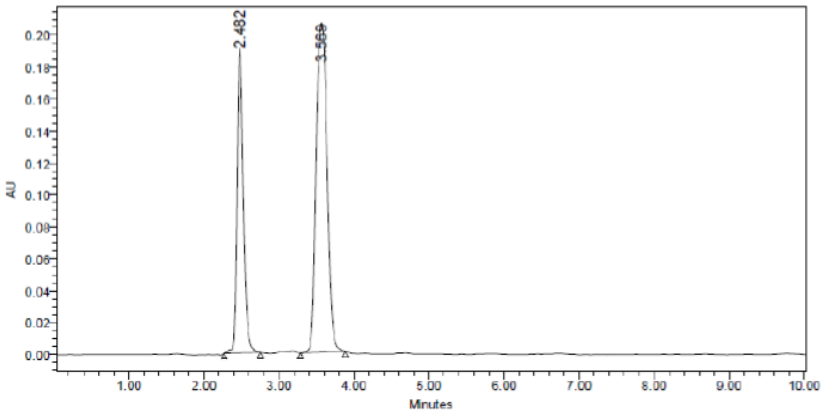


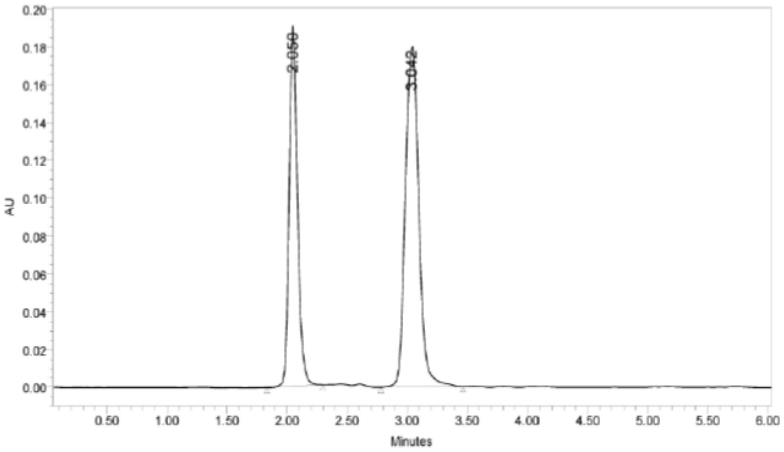
Fig. 6: Chromatogram for Method Precision.

Robustness

With Flow Rate

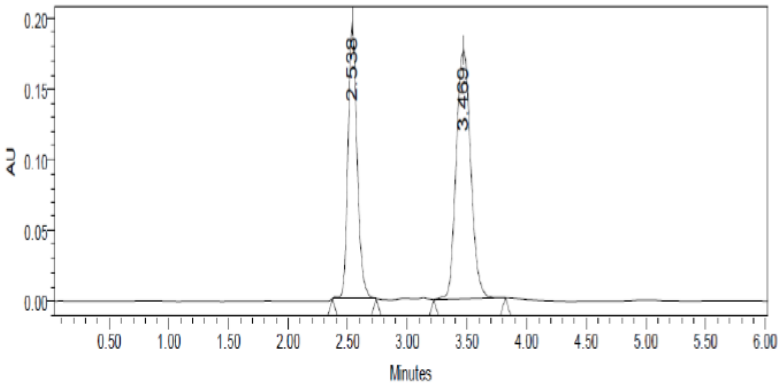


Robustnes studies For Less flow



Robustnes studies for More flow

Ruggedness



Ruggedness of Metformin and Alogliptin

Validation Parameters

Table. 2: Validation Studies for Metformin & Alogliptin.

S. No	Parameter	Results for Metformin	Results for Alogliptin
1	**Precision**	% RSD= 1.6	% RSD= 0.5
2	**Method precision**	% RSD= 0.9	% RSD= 0.7
3	**Accuracy**	%Recovery= 98.0 to 102.0%	% Recovery= 98.0 to 102.0%.
4	**Linearity**	R2= 0.999	R2= 0.999
5	**Limit of detection**	3.01 µg/mL	2.96 µg/mL
6	**Limit of quantification**	1.74 µg/mL	1.69 µg/mL
7	**Robustness**	Deliberate change	Deliberate change
8	**Ruggedness**	% RSD= 0.12	% RSD= 0.56
9	**Specificity**	Degradation was Observed	Degradation was Observed

CONCLUSION

➢ The results indicating that the proposed methods are precise, accurate, specific and simple. These methods were developed and validated according to the ICH guidelines. So the developed methods can be easily applied for routine analysis.

➢ The results of recovery studies were in good agreement with the respective label claim of the formulation. Thus the method is less time consuming and can be employed for routine batch analysis of Metformin and Alogliptin.

➢ It is clear from the present study that the RP-HPLC method for the determination of Metformin and Alogliptin is simple, accurate, specific and precise. This method was validated statistically.

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