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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Alogliptin and Metformin HCl Drug from Bulk and Pharmaceutical Dosage Form

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ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method with subsequently validate as per ICH guidelines for the determination of Alogliptin benzoate and Metformin hydrochloride using mobile phase [mixture of Phosphate buffer- pH-3.6 and acetonitrile in the ratio of 65:35] as the solvent. The proposed method involves the measurement of Retention time at selected analytical wavelength. 235.0 nm was selected as the analytical wavelength. The retention time of ALO and MET was found to be 5.055 and 2.838 respectively. The linearity of the proposed method was investigated in the range of 1-5 µg/ml (r = 0.9998) for ALO and 10-50µg/ml (r = 0.9999) for MET respectively. The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD (Relative Standard Deviation) value indicating high grade of precision of the method.

Keywords: RP-HPLC METHOD, Alogliptin benzoate, Metformin hydrocholiride, Validation.

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INTRODUCTION:

GLP-1 and glucose-dependent insulinotropic polypeptide (also known as gastric inhibitory peptide) belong to the incretin class of gastrointestinal hormones. Incretins stimulate a decrease in blood glucose levels by causing increased postprandial insulin release from the beta cells of the pancreas[1]. GLP-1 also suppresses glucagon secretion and exhibits other glucoregulatory actions after secretion in the gut.[11] DPP-4 is an enzyme that rapidly degrades, and thereby inactivates, both GLP-1 and gastric inhibitory peptide. DPP-4 inhibitors prolong the endogenous plasma levels and hence the activity of both of these key hormones.[2] Alogliptin, a potent and highly selective DPP-4 inhibitor, is the fourth DPP-4 inhibitor to be introduced in Canada, following the approval of sitagliptin, saxagliptin, and Alogliptin.

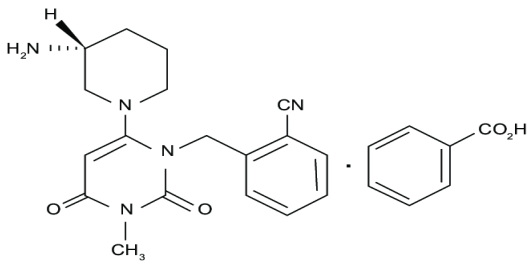

Fig 1: Structure of Alogliptin

Metformin is a biguanide oral hypoglycemic primarily for treating type 2 diabetes mellitus (T2D). Evidence suggests that, in addition to improving glycemic control, metformin is associated with improved all-cause and cardiovascular mortality[1] and decreased risk of some cancers (*eg*, breast cancer).[2] Despite the potential benefits, since metformin was introduced in the United States in the mid-1990s, clinicians have been advised to exercise caution in prescribing the drug to individuals with certain comorbidities due to perceived risks of serious side effects, including LA. Lactic acidosis (LA) is defined as blood lactate concentration >45mg/dl (5.0mEq/L), decreased blood pH, and electrolyte disturbances with an increased anion gap

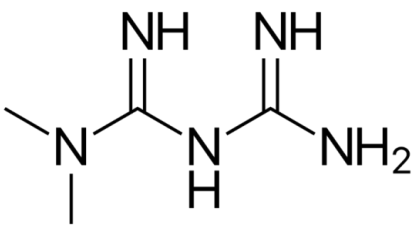

Fig 2: Structure of Metformin

MATERIALS AND METHODS:

Analytically pure sample of Alogliptin and Metformin with purities greater than 99% were obtained as gift samples. The tabulated information regarding the procurement of various samples are given below in table 1 and table 2 contains all the instrument list used for this work.

Table.No.1 List of chemicals and standards used

Sr.no	Chemical	Manufacture name	grade
1	Water	Merck	HPLC grade
2	Acetonitrile	Merck	HPLC grade
3	Methanol	Merck	HPLC grade
4	Ortho phosphoric acid	Merck	G.R
5	Triethyl amine	Merck	G.R
6	0. 22µ Nylon filter	Advanced lab	HPLC grade
7	0.45µ filter paper	Millipore	HPLC grade
8	Alogliptin And Metformin	Gift samples from Ajanta Pharma (aurangabad, India)	HPLC grade

Table.No.2. List of instruments used.

Weighing Balance	PGB 100
Ultra Sonicator	WUC-4L
UV-Spectrophotometer And Software	UV2450 UV Probe v 2:3:3
HPLC	HPLC 3000 Series P-3000-M Reciprocating (Binary pump) UV-3000-M (UV-Visible Detecter)

Selection of wavelength:

10 mg of Alogliptin and Metformin was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum of alogliptin and Metformin was obtained and the isobestic point of Alogliptin And Metformin showed absorbance's maxima at 254 nm. The spectrums are shown in Fig. 3

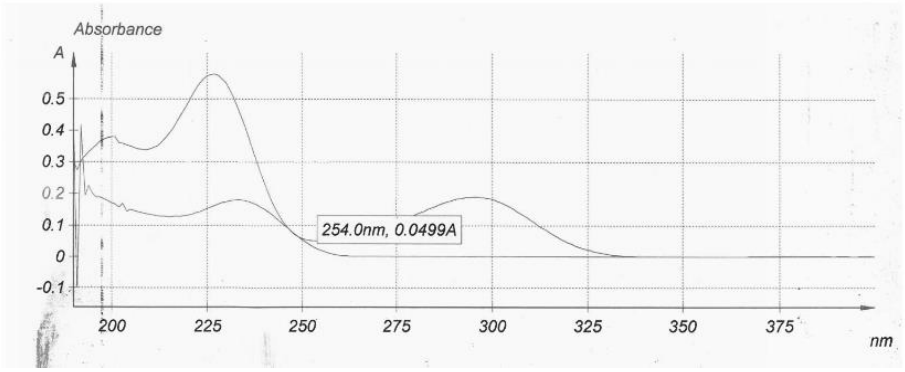

Fig.3. Spectrum showing overlapping spectrum of Alogliptin and Metformin

The chromatographic method development for the simultaneous estimation of Alogliptin and Metformin were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method was selected for the separation and quantification of Alogliptin and Metformin in API and pharmaceutical dosage form by RP-HPLC method.

The separation of the drugs was achieved on Water X bridge C18 column (4.6×150mm) 5µ particle size). The mobile phase consists of a mixture of Methanol: Acetonitrile pH 3.0 (130: 870 % v/v) at a flow rate of 1.0 ml/minute and the volume injected was 10 µl for every injection .the detection wavelength was set at 240 nm.

Preparation of Buffer solution: (Mobile phase A).

Add 0.25 g of Sodium 1-octane Sulfonate monohydrate in 1000 mL of water, mix and filterthe solution through 0.45µ Nylon membrane disc filter, to this add 2.0 mL of Triethylaminemix and adjust the pH 3.0 ± 0.1 with Ortho-phosphoric acid Mix and degas

1. Preparation of Mobile Phase B:

   Mix Acetonitrile and Methanol in the proportion of 870:130 v/v respectively and use as mobile phase B.

2. Preparation of Mobile Phase:

   Prepare the mixture of Mobile phase A: Mobile Phase B in the ratio of 85:15 v/v respectively.

3. Preparation of Diluent:

   Prepare mixture of Water and Acetonitrile in the ratio 85:15 v/v respectively, mix and degas.

4. Preparation of Standard stock solution:

Alogliptin Stock.

Weigh accurately about 25 mg of Alogliptin standard and transfer into 50 mL volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and make up to volume with diluent and mix.

Metformin HCl Stock.

Weigh accurately about 50 mg of Metformin HCl standard and transfer into 100 mL volumetric flask. Add about 70 mL of diluent, sonicate to dissolve and make up to volume with diluent and mix.

5. Preparation of Standard solution:

Further transfer 10 mL Metformin HCl Stock Solution and 20 mL ofAlogliptin Stock Solution into 50 mL volumetric flask and dilute up to the mark with diluent and mix.

6. Preparation of Sample Stock solution:

Weigh and transfer 10 tablets into 1000 mL volumetric flask. Add about 700 mL of diluent, sonicate for 30 minutes with intermittent shaking. Allow it to cool to room temperature and make up to volume with diluent and mix. Let the solution stand for 5 minutes.

Filter the sample solution through 0.45µ Nylon membrane syringe filter. Discard first 3 mL of filtrate.

RESULTS AND DISCUSSION

Method Development.

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. resolution factor (Rf) between peaks, tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Alogliptin at 6.2 min and Metformin at 2.6 min.

Figure 4 represents chromatograms of mixture of standard solutions. The total run time is 25 minutes with all system suitability parameters as ideal for the mixture of standard solutions.

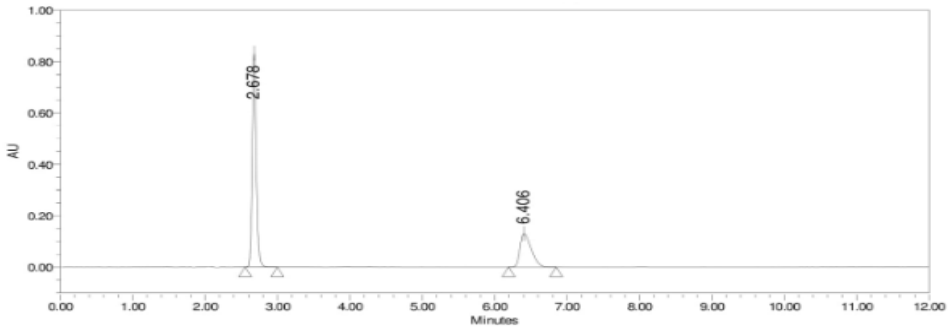

Fig 4: Typical chromatogram of the mixture of the standard sample.

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N), peak resolution (Rs) and peak Tailing factor (T) were evaluated for six replicate injections of the standards at working concentration. The results given in **Table 3** were within acceptable limits.

Table 3: System suitability studies results.

PARAMETERS*	REQUIRED LIMITS	Alogliptin	Metformin
Retention time (min)	% RSD < 1%	6.2	2.6
Number Of Theoretical plates (N)	Not less Than 2000	4423	6049
Tailing factor (T)	Not More Than 2	1.3	1.2

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. The RP-HPLC method developed was validated according to International Conference on Harmonization10 guidelines for validation of analytical procedures. The method was alidate for the parameters in terms of system suitability, selectivity, linearity, accuracy, precision, ruggedness, robustness, limit of detection(LOD) and limit of quantitation(LOQ).

Specificity.

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Fig.No.5.7.

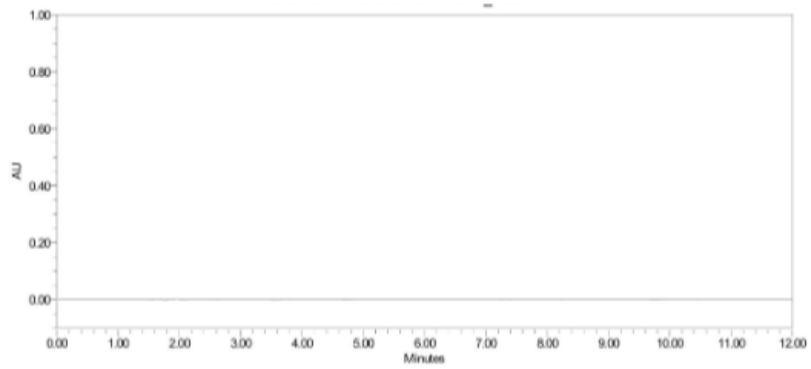

Fig.5.7 Chromatogram showing blank (mobile phase preparation)

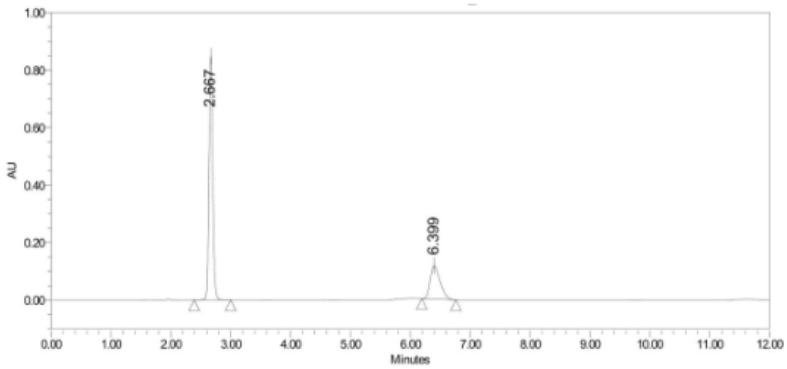

Fig.6. Chromatogram showing standard injection

Linearity.

The linearity study was performed for the concentration of 10 ppm to 50 ppm Alogliptin and 20 ppm to 100 ppm Metformin level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results aretabulated in Table.4-5. Calibration graph for Alogliptin and Metformin are shown in Fig.No.8 &Fig 9.

Table 4. Linearity Results for Alogliptin

Sr.No	Linearity level	Concentration	Area
1	1	50ppm	703249
2	2	80ppm	1119712
3	3	90ppm	1263844
4	4	100ppm	1399228
5	5	150ppm	2081515
		Correlation Coefficient	1.000

Table 5. Linearity Results for Metformin

Sr.no	Linearity level	Concentration	Area
1	1	50ppm	1639365
2	2	80ppm	2610330
3	3	90ppm	2955233
4	4	100ppm	3268898
5	5	150ppm	4865242
		Correlation Coefficient	1.000

The linearity study was performed for concentration range of 50µg -150µg Alogliptin and 50µg - 150 µg metformin and the correlation coefficient was found to be 1.000 and 1.000.(NLT 0.999)respectively.

Accuracy:

The accuracy study was performed for 50%, 100% and 150 % for Alogliptin and Metformin. Each levelwas injected in triplicate into chromatographic system. T he area of each level was used for calculation of % recovery. The results are tabulated in Table.No.6-7.

Table: 6. Accuracy results for Alogliptin.

%Concentration (at specification level)	Average area	% Recovery	Mean recovery
50%	1411902	98.4	98.8
100%	1400900	98.9	
150%	1404583	99.2	

Table.7. Accuracy results for Metformin.

%Concentration (at specification level)	Average area	% Recovery	Mean recovery
50%	3438212	102.2	100.5
100%	3409956	100.3	
150%	3408213	100.4	

The accuracy study was performed for % recovery of Alogliptin and Metformin. The % recovery was found to be 98.8 % and 100.5% respectively (NLT 98% and NMT 102%).

Precision.

1.system precision

2.Method precision

1.0 system precision.

Single injection of Blank (Diluent) and six replicate injections of standard solution were injected on the system. The data obtained is summarized in Table 9.

Table `9: System precision

	For Alogliptin	For Metformin HCl
Symmetry factor	1.3	1.2
Theoretical plates	4423	6049
S. No.	Area	Area
1	1398462	3408366
2	1399489	3418137
3	1401540	3407958
4	1399820	3415686
5	1401309	3423660
6	1400876	3416303
Mean	1400249.333	3415018.333
%RSD	0.09	0.18

6.5.2 Method Precision:

Six independent sample preparations were prepared and injected in duplicate on the HPLC. The data obtained is summarized in Table 10 and 11, 12.

For Alogliptin

	Response			
Sample no	1	2	3	% Assay
1	1323512	1324023	1323767.5	95.3
2	1336783	1336081	1336432	96.2
3	1319381	1325423	1322402	95.2
4	1364166	1363980	1364073	98.2
5	1363272	1359189	1361230.5	98.0
6	1336870	1335388	1336129	96.2
			mean	96.5
			%RSD	1.35

For Metformin.

Sample no	1	2	3	% Assay
1	3402226	3401364	3401795	98.5
2	3399658	3398478	3399068	98.4
3	3406175	3419096	3412635.5	98.8
4	3404064	3439805	3421934.5	99.0
5	3407303	3416774	3412038.5	98.8
6	3376031	3397298	3386664.5	98.0
			mean	98.6
			% RSD	0.37

Intermediate precision/Ruggedness.

Same procedure of method precision is followed by another Analyst by using same lot of Alogliptin and Metformin HCl Tablets on different instrument different column and on different day. The data obtained from Analyst-II are summarized in Table 13.

Table 13: System suitability

	For Alogliptin	For Metformin
The symmetry factor for Alogliptin peak	1.4	1.2
The EP theoretical plates for Alogliptin peak	6459	4281
Sr.no	Area	Area
1	1499519	3684107
2	1494289	3670036
3	1498845	3685519
4	1503497	3698331
5	1505602	3706061
6	1506060	3709982
Mean	1501302	3692339.333
% RSD	0.30	0.41

CONCLUSION:

The data shows that % assay difference of unfiltered standard solution and filtered standard solution with different filters is within the acceptance criteria. Hence Nylon filter, PVDF filters are suitable for standard solution. The data shows that % assay between centrifuged sample solution and filtered sample solution with 0.45µ PVDF filter, Nylon filter is within the acceptance criteria. Hence all tested filters except Teflon+Glass filter are suitable for sample solution filtration.

REFERENCES

1.Koduru S, Kalepu S, method development for the simultaneous estimation of alogliptin and metformin by using RP-HPLC,international journal of pharma research and health science,(2015) vol.3,page no.747-753

2 Shyam sundar P, tabassum I, development and validation of stability indicating RP-HPLC method for the simultaneous estimation of alogliptin and metformin in bulk and pharmaceutical dosage form. (2017) vol.5,page no.223-233.

3. Praveen Kumar A, Aruna G, Analytical Method Development And Validation Of Alogliptin And Metformin Hydrochloride Tablet Dosage Form By Rp-Hplc Method,(2013) Vol.3.Page No. 58-68.

4. http://www.drugbank.ca/drugs/DB0893

5. http://www.drugbank.ca/drugs/DB08864

6. .http://www.webmd.com

8. .http://www.drugsinfo.com

9. .http://www.update.com

10. neha sultana,Dr.iffath rizwana,development and validation of stability indicating RP- HPLC method for simultaneous estimation dosage form and alogliptin and metformin in bulk and tablet dosage form the pharma innonation journal(2018) vol.7.Page no.319-324

11.http://www.mediclnewstoday.com

12. http://www.thediabeticvoice.com