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KS Nataraj
Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, West Godavari, Andhra Pradesh, India

A Srinivasa Rao
Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, West Godavari, Andhra Pradesh, India

KV Nagamani
Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, West Godavari, Andhra Pradesh, India

P Divya
Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, West Godavari, Andhra Pradesh, India

B Renuka Chandrasekhar
Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, West Godavari, Andhra Pradesh, India

Correspondence
KS Nataraj
Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram, West Godavari, Andhra Pradesh, India

Analytical method development & validation of metformin, pioglitazone & glimepiride by RP-HPLC in tablet dosage forms

KS Nataraj, A Srinivasa Rao, KV Nagamani, P Divya and B Renukachandrasekhar

Abstract
A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of bulk and pharmaceutical formulations. Separation of Metformin, Pioglitazone and Glimepiride was successfully achieve WATERS, C18, 25cmx4.6mm, 5µm or equivalent in an isocratic mode utilizing OPA(ortho phosphoric acid) : Methanol (60:40) at a flow rate of 1.0mL/min and elute was monitored at 273nm, with a retention time of 4.7 minutes for Metformin, 6.4 minutes for Pioglitazone and 9.7 minutes Glimepiride. The method was validated for linearity, accuracy, precision, detection limit, quantification limit, specificity, system suitability and solution stability. Results of all validation parameters were within the limits as per ICH guidelines.

Keywords: Metformin**,** pioglitazone & glimepiride

Introduction
Metformin is used to treat high blood sugar levels that are caused by a type of diabetes mellitus or sugar diabetes called type 2 diabetes. With this type of diabetes, insulin produced by the pancreas is not able to get sugar into the cells of the body where it can work properly.
Pioglitazone is a thiazolidinedione that depends on the presence of insulin for its mechanism of action. ACTOS decreases insulin resistance in the periphery and in the liver resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. Pioglitazone is not an insulin secret a gogue
Glimepiride is only found in individuals that have used or taken this drug. It is the first III generation sulphonyl urea it is a very potent sulphonyl urea with long duration of action. 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

Materials and Methods
Instrumentation: High performance liquid chromatography (Shemadzu) with pump Auto injector, Primesil $C_{18,}$ (150 × 4.6 mm, 3.5µ) column detection of drug carried by PDA detector at data processing was carried out by LC solutions software, weighing balance (Mettler Toledo), pH meter (Thermo scientific), ultra sonicator (trans- o- sonic).

Reagents and Chemicals
Ortho Phosphoric acid (HPLC grade), Acetonitrile (HPLC grade), Methanol, (HPLC grade), Water (HPLC grade).The solvents were filtered through 0.45µ PVDF membrane filter.

Chromatographic Conditions
Column: THERMO, C18, 25cmx4.6mm, 5µm, Flow rate: 1.0 mL /min, Run time: 15min (for Sample, Blank & Placebo) 10min (for Standard), Wavelength: 273nm, Column temperature: 45°C, Injection Volume: 10 µL.

Solution Preparation
Preparation of Standard solution
Accurately weighed and transfer of 500mg Metformin 15mg Pioglitazone and 10 mg Glimepiride into 100ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or)

shake 5min and make with water.
Transfers the above solution into 1ml into 10ml volumetric flask dilute to volume with water.

Preparartion of sample solution
20 tablets were weighed and powdered the powdered equivalent to the 620 mg of Metformin, Pioglitazone and Glimepiride of active ingredients w ere transfer into a 100ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with water.
Transfers above solution 1ml into 10ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

Results and Discussion
1) System suitability
The main purpose of the system suitability is to ensure the system including instrument, analyst, chemicals and electronics are suitable to the intended application. One Blank, Sample & Placebo injections and Six replicative Standard injections were injected and the chromatograms were recorded for the drugs and the chromatogram were shown in Fig No.1 and result were shown in table No: 1, 2, 3

2) Specificity
2.1) Blank interference
Blank was prepared and injected as per test method. It was observed that no blank peaks were interfering with analytical peaks.

2.2) Placebo interference
Placebo solutions were prepared in duplicate and injected as per test method. It was observed that no placebo peaks were interfering with analytical peaks.

2.3) Impurity interference
All known impurities solution was prepared at about 1% of the test concentration and analyzed as per test method. It was observed that no co elution of the all known impurities peaks with analytical peaks. Prepared sample and spiked sample solutions by spiking all known impurities at about 1% of the target test concentration in triplicate and analyzed as per test method. Chromatogram was shown in fig No 2 and table No 4

3) Accuracy/Recovery
A series of solutions were prepared by spiking the placebo and API in the range of about 50% to 150% of test concentration in triplicate and injected into HPLC system and analyzed as per the test method. Calculated individual mean recovery and % RSD at each level and the results were found to be within the acceptable limits.
Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount.
% Recovery was 100.00% for Metformin, 100.00% for Pioglitazone and100.00% for Glimepiride. All the results indicate that the method is highly accurate and was shown in fig No: 3,4 and was shown in table No 5,6&7

4) Precision
Preparation of sample
Transfer the 200.5mg of sample into a 100ml of volume at flask and add 10ml of water and 10ml of Methanol and sonicate 20min and makeup with water. Transfer the above solution into 5ml into 50ml volume metric flask dilute to the volume with water.
The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injections.

Acceptance criteria: The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention times is not more than 2.0 and was shown in fig No:5
Results of variability was summarized and % RSD of peak areas was calculated for various runs.% RSD was found to be less than 2% which proves that method is precise.

5) Linearity & Range
Weighed accurately about 80 mg of Oxybutynin HCl working standard in to a 100 ml volumetric flask added 50ml Diluent-01, to this added required amount of Impurity-A & Impurity-D sonicated to dissolve and made up the volume with ACN. Centrifuge the solution for 3min at 300rpm further transfer 5ml of above Centrifuged solution into 10ml of volumetric flask and make up to the volume with Diluent -2 then filter through PVDF syringe filters.
A linear relationship between peak areas versus concentrations was observed for Metformin, Pioglitazone and Glimepiride in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for Metformin, Pioglitazone and Glimepiride which prove that the method is linear in the range of 50% to 150% were shown in Fig No 6, 7&8

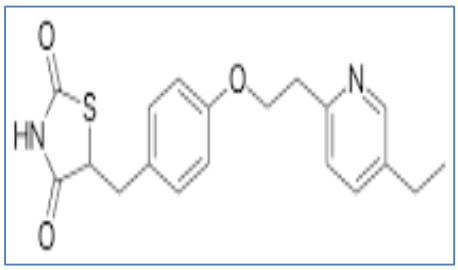
6) Robustness
6.1) Effect of variation in flow rate
Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates. Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked.

6.2) Effect of variation in wavelength: Prepare the system suitability solution as per the test method and injected into the HPLC with ± 2nm variation in wavelength. Evaluate the system suitability values as required by the test method for both wavelengths.
The results of Robustness of the present method had shown that changes made in the Flow and Temperature did not produce significant changes in analytical results which were presented in the above table. As the changes are not significant we can say that the method is Robust and were shown in table No 8,9&10 and fig No 9&10

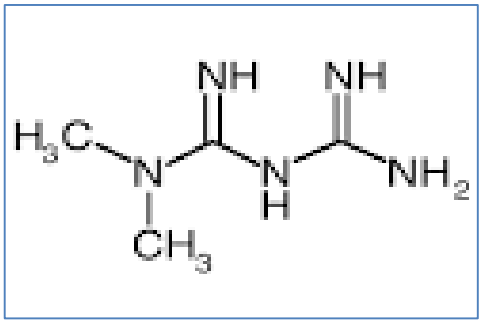
7) LOD & LOQ
Limit of detection (LOD)
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. 3.3mL from the 10% solution was taken in a 10mL volumetric flask and made up to the volume with Diluent-02. It was injected to the system and Signal-to-noise ratio was observed. Results were shown in fig no: 11 and table No 11

Limit of Quantification (LOQ)

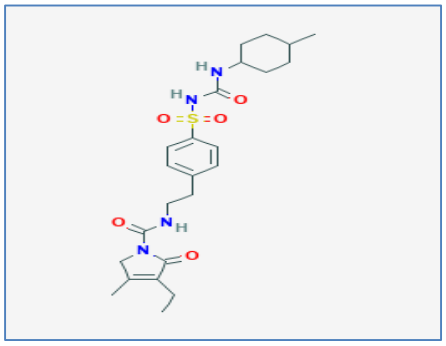
Weighed accurately about 80 mg of Oxybutynin HCl working standard in to a 100 ml volumetric flask added 50ml Diluent-01, to this added 0.2ml of Impurity-A&0.4ml Impurity-D sonicated to dissolve and made up the volume with ACN. Centrifuge the solution for 3min at 300rpm further transfer 5ml of above Centrifuged solution into 10ml of volumetric flask and make up to the volume with Diluent -2 then filter through PVDF syringe filters. Results were shown in fig no: 12 and table No 12



Metformin



Pioglitazone



Glimeprimid

Table 1: System suitability Result Metformin

Peak Name: METFORMIN

	SampleName	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	STD2	METFORMIN	4.754	1251347	6369	1.15
2	STD2	METFORMIN	4.753	1259724	6268	1.15
3	STD2	METFORMIN	4.784	1257745	6297	1.15
4	STD2	METFORMIN	4.822	1243760	6233	1.15
5	STD2	METFORMIN	4.832	1250775	6236	1.14
Mean				1252670.2		
% RSD				0.5		

Table 2: System suitability Result, Pioglitazone

Peak Name: PIOGLITAZONE

	SampleName	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	STD2	PIOGLITAZONE	6.390	1297119	6827	5.87	1.12
2	STD2	PIOGLITAZONE	6.387	1309620	6814	5.84	1.12
3	STD2	PIOGLITAZONE	6.420	1307211	6756	5.81	1.11
4	STD2	PIOGLITAZONE	6.463	1291601	6713	5.75	1.11
5	STD2	PIOGLITAZONE	6.468	1301272	6743	5.74	1.11
Mean				1301364.7			
% RSD				0.6			

Table No 3: System suitability Result Glimepiride

Peak Name: GLIMEPIRIDE

	SampleName	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	STD2	GLIMEPIRIDE	9.948	1734637	5150	8.13	1.32
2	STD2	GLIMEPIRIDE	9.980	1745200	5148	8.16	1.32
3	STD2	GLIMEPIRIDE	10.174	1740071	5114	8.39	1.31
4	STD2	GLIMEPIRIDE	10.313	1717214	5258	8.58	1.31
5	STD2	GLIMEPIRIDE	10.300	1728236	5218	8.52	1.30
Mean				1733071.7			
% RSD				0.6			

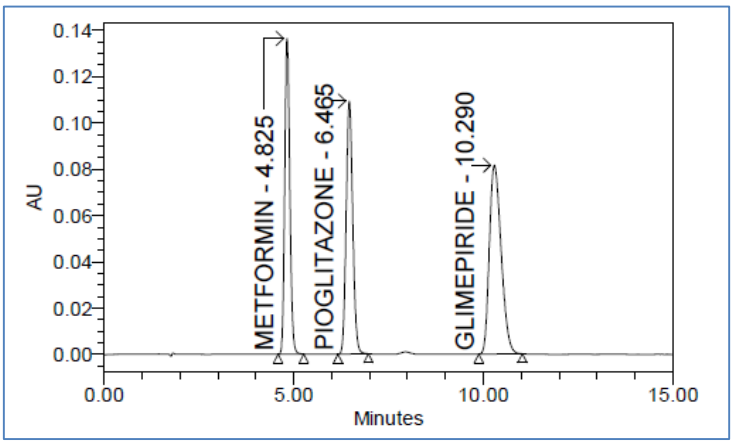


Fig 1: System suitability chromatography of Metformin, Pioglitazone and Glimepiride.

Specificity

Table 4: Specificity data for Metformin, Pioglitazone and Glimepiride

S No.	Sample Name	Metformin Area	Rt	Pioglitazone Area	Rt	Glimepiride Area	Rt
1	Standard	3162736	2.937	4809123	3.461	1739935	9.969
2	Sample	3164640	2.943	4804294	3.467	1731157	10.290
3	Blank	-	-	-	-	-	-
4	Placebo	-	-	-	-	-	-

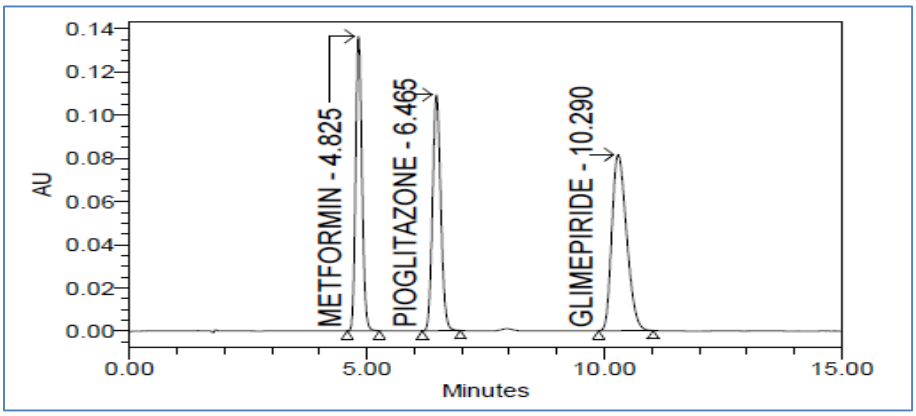


Fig 2: Chromatogram Representing Specificity of Sample

Table 5: Accuracy data for Metformin

S. No	Accuracy Level	Injection	Sample area	RT
1	50%	1	626019	4.767
		2	626377	4.757
		3	626610	4.761
2	100%	1	1253091	4.756
		2	1259598	4.766
		3	1258774	4.766
3	150%	1	1873617	4.757
		2	1875618	4.755
		3	1876049	4.769

Table 6: Accuracy data for Pioglitazone

S. No	Accuracy Level	Injection	Sample area	RT
1	50%	1	650885	6.403
		2	650808	6.390
		3	650544	6.390
2	100%	1	1300013	6.389
		2	1303825	6.383
		3	1303838	6.408
3	150%	1	1954601	6.395
		2	1957285	6.398
		3	1957599	6.415

Table 7: Accuracy data for Glimepiride

S. No	Accuracy Level	Injection	Sample area	RT
1	50%	1	866062	10.020
		2	866798	9.969
		3	866935	9.949
2	100%	1	1739248	9.870
		2	1736469	9.754
		3	1735279	9.763
3	150%	1	2592778	9.678
		2	2593081	9.675
		3	2593764	9.744

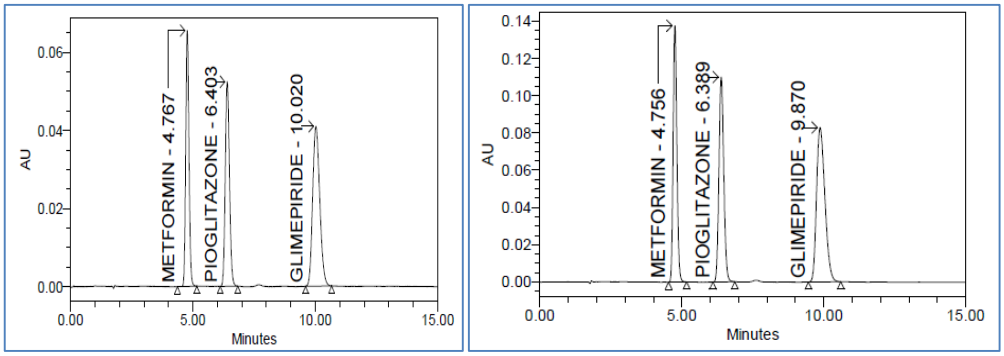


Fig 3: Typical chromatogram for Accuracy 50% & 100%

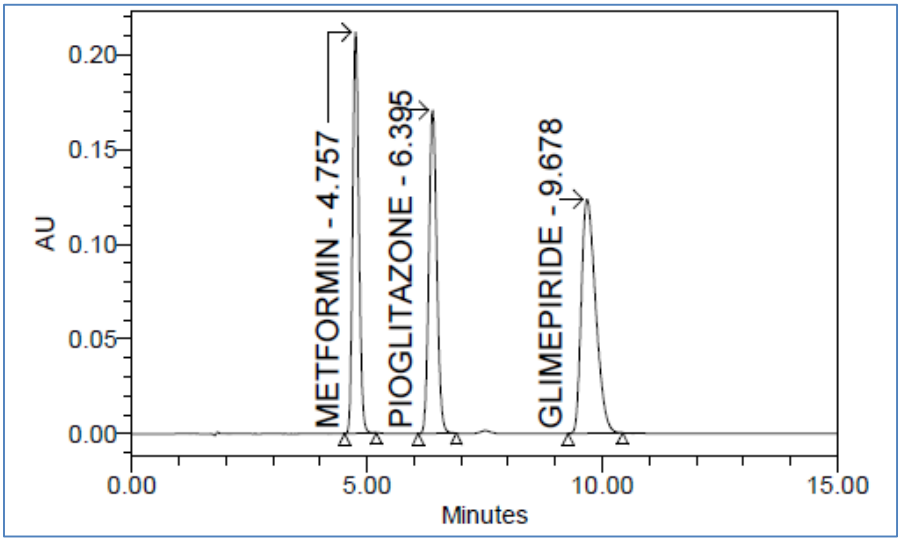


Fig 4: Typical chromatogram for Accuracy 150%

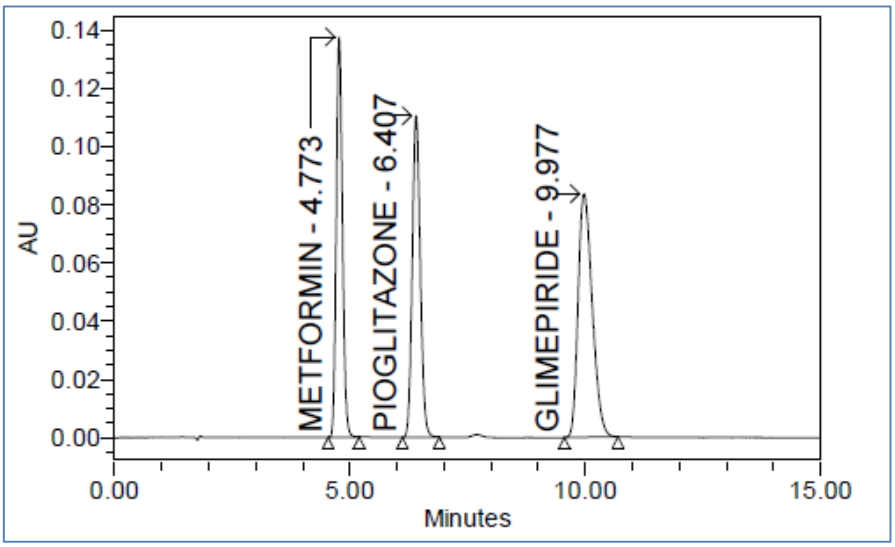


Fig 5: Chromatogram for precision

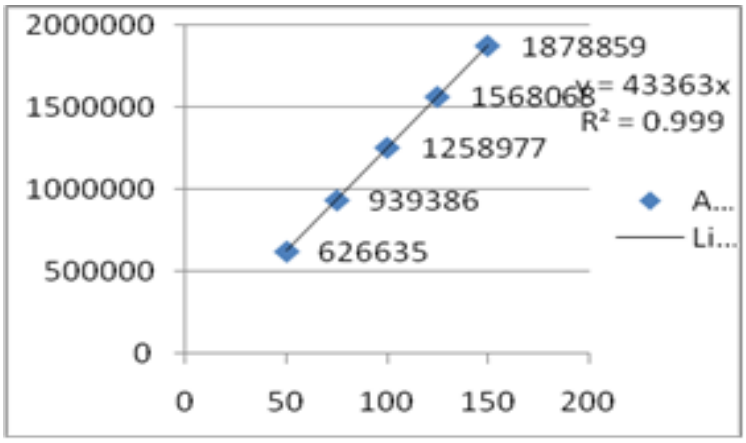


Fig 6: Linearity plot of Metformin

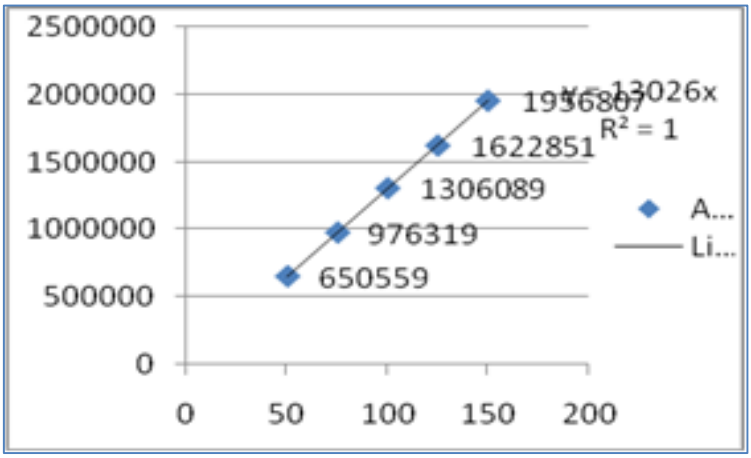


Fig 7: Linearity plot of Pioglitazone

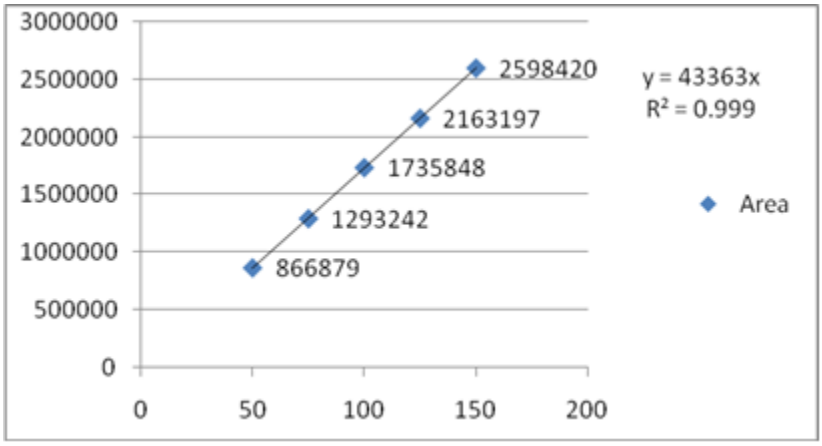


Fig 8: Linearity plot of Glimepiride

Table 8: Robustness data for Metformin

Parameter	RT	Theoretical plates	Tailing Factor
Decreased flow rate (0.8ml/min)	4.782	6288	1.15
Increased flow rate (1.2ml/min)	4.776	6319	1.15
Decreased temperature (20^0c)	4.779	6295	1.14
Increased temperature (30^0c)	4.790	6322	1.15

Table 9: Robustness data for Pioglitazone

Parameter	RT	Theoretical plates	Tailing factor
Decreased flow rate (0.8ml/min)	6.427	6844	1.11
Increased flow rate (1.2ml/min)	6.419	6798	1.11
Decreased temperature(20^0c)	6.423	6723	1.11
Increased temperature(30^0c)	6.437	6731	1.11

Table 10: Robustness data for Glimepiride

Parameter	RT	Theoretical plates	Tailing Factor
Decreased flow rate (0.8ml/min)	9.841	5148	1.31
Increased flow rate (1.2ml/min)	9.822	5213	1.31
Decreased temperature 20^0c)	9.841	5241	1.30
Increased temperature (30^0c)	9.857	5161	1.30

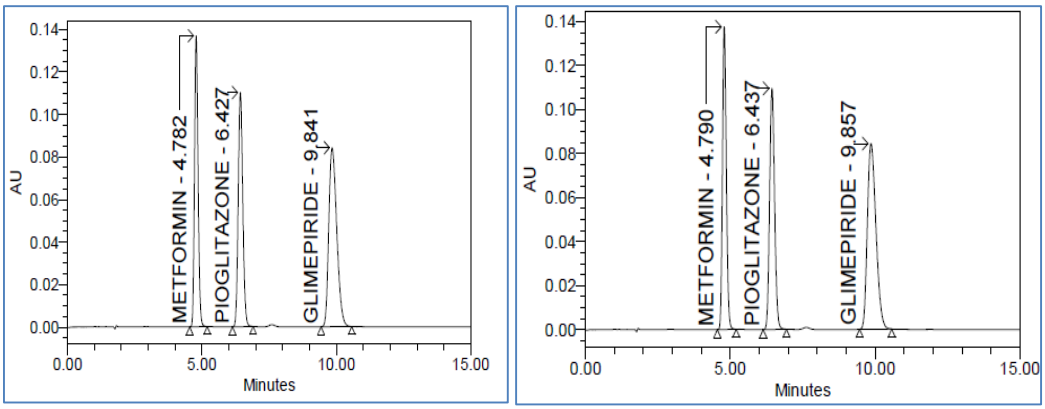


Fig 9: Chromatogram for decreased, increased flow rate

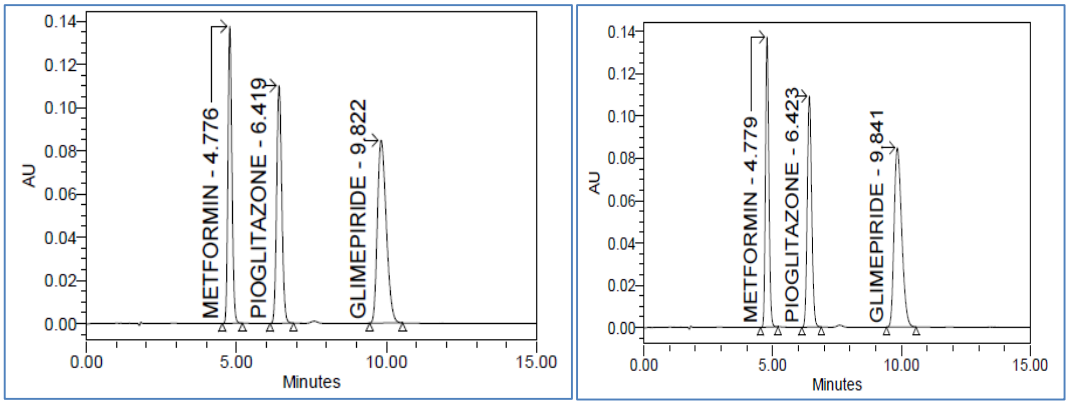


Fig 10: Chromatogram for decreased, increased temperature

Table 11: LOD data for Metformin, Pioglitazone and Glimepiride

S. No	Sample name	RT	Area
1	Metformin	4.761	115156
2	Pioglitazone	6.399	11153
3	Glimepiride	9.885	158459

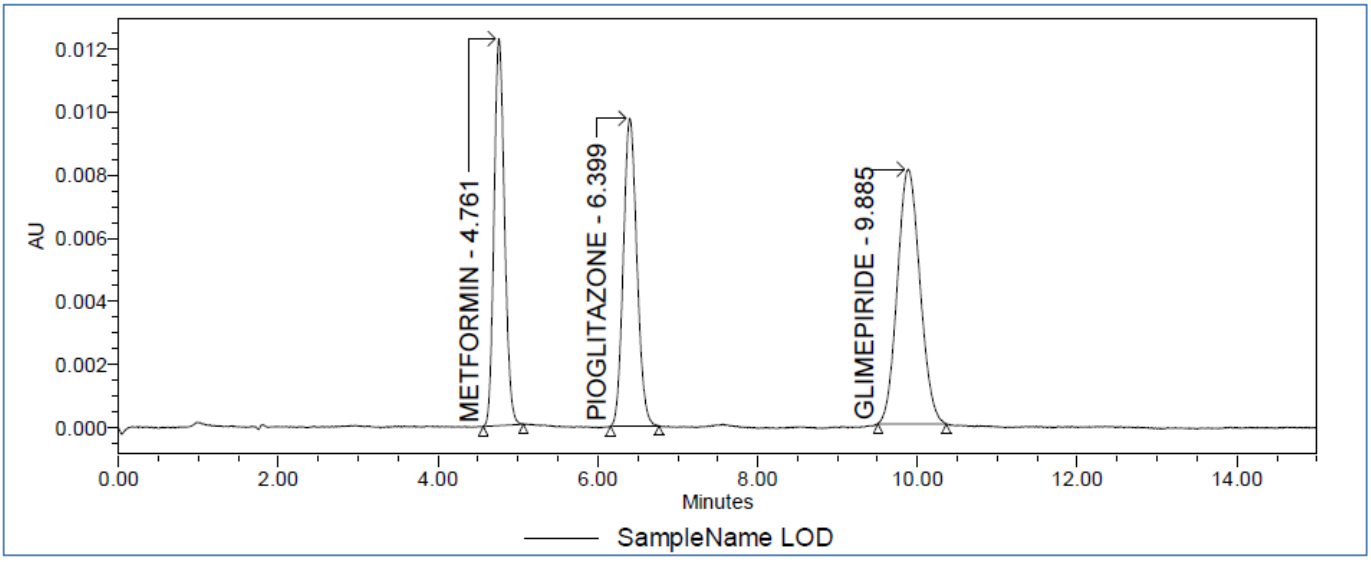


Fig 11: Chromatogram for LOD

Table 12: LOQ data for Metformin, Pioglitazone and Glimepiride

S. No	Sample name	RT	Area
1	Metformin	4.774	376301
2	Pioglitazone	6.418	468550
3	Glimepiride	9.876	816935

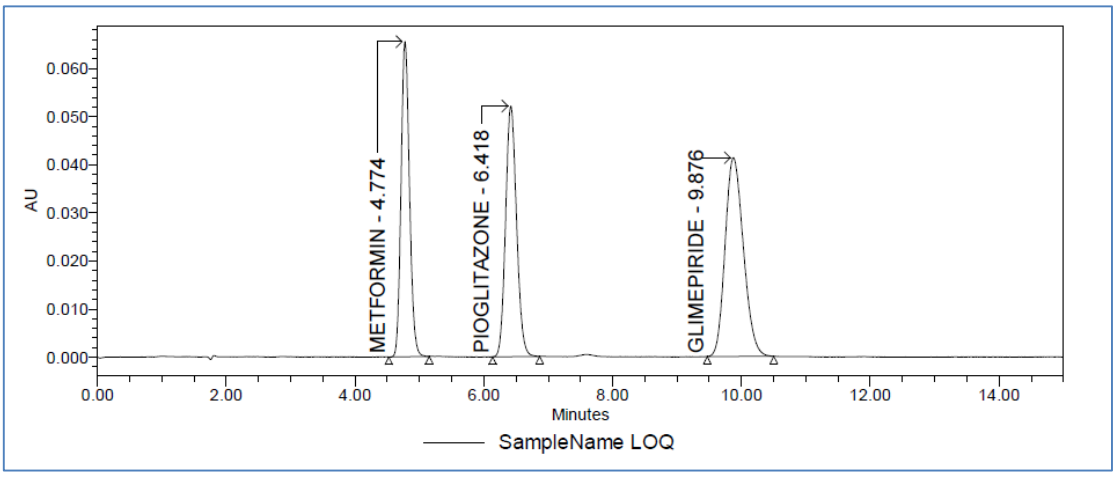


Fig 12: Chromatogram for LOQ

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