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RP-HPLC Method Development of Metformin in Pharmaceutical Dosage Form

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Research Article

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ABSTRACT

The existing available literature reports do not show any stability indicative analytical method for the estimation of metformin in dosage form. Hence there is a need to develop and validate an analytical method to estimate metformin. Hence the present study aims is to develop and validate a suitable high precision and accurate analytical method for the simultaneous estimation of metformin in dosage form, by reverse phase high performance liquid chromatography (RP-HPLC). The main objective of the present work is to develop and validate a suitable high precision and accurate analytical method for the estimation of metformin in dosage form by reverse phase high performance liquid chromatography (RP-HPLC).

INTRODUCTION

Quantitative measurement was performed on a quaternary HPLC AGILENT series and HPLC pumps, with a 20 µl sample injection loop (automatic), and UV-Visible absorbance detector and photo-diode array detector. The output signal was monitored and integrated using the output signal was monitored and integrated using EZ-Chrome software C-18 column was used for the separation. [1,2]

Reagents Used for the Study

Ortho phosphoric acid: AR grade

Acetonitrile: HPLC grade

Water: Milli-Q grade

Methanol: HPLC grade

Diammonium hydrogen phosphate: ARgrade

METHOD DEVELOPMENT

The objective of this experiment was to optimize the assay method for metformin based on the literature survey made. [3,4].

The retension time was too long and the peak was non symmetric and they do not pass tailing factor in all the above 3 trails and the 4th trail was optimized.

Optimized Method

Chromatographic conditions

Flow rate: 1.0 mL per min

Column: Thermosil C18

Detector wave length: 232 nm

Column temperature: 25°C

Injection volume: 20 µl

Run time: 6.0 min

Diluent: mobile phase

Mobile phase: 40:60 (water:acetonitrile)

The retention time was found to be 2.064

It shows the good peak with good symmetry. Hence this method was finalized for the development of metformin.

Optimized Chromatographic Conditions for the Estimation of Metformin (Table 1 and Figure 1).

Preparation of Buffer

Weight 2.72 gm of potassium dihydrogen ortho phosphate in 1000 ml hplc water; adjust to pH 2.8 with ortho phosphoric acid.

Table 1. Conditions for the estimation of Metformin.

S.NO	Parameter	Method
1	Mobile Phase	Water:acetonitrile(40:60)
2	Column	C18 column
3	Elution mode	Isocratic
4	Flow rate	1.0 ml/min
5	Injection Volume	20 µl
6	Retention Time	3.25
7	Temperature	25°C
8	Wavelength	232 nm

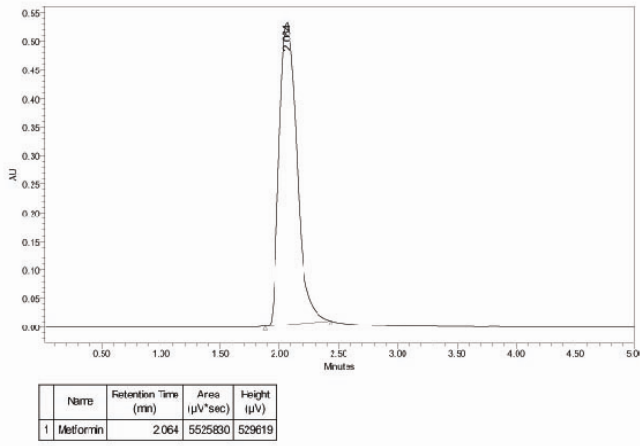


Figure 1. Standard graph for estimation of metformin

Mobile Phase

Take HPLC Water 400 mL (40%) and 600 mL of Acetonitrile HPLC (60%) and degas in ultrasonic water bath and mix for 5 minutes and then filter through 0.45 µ under vacuum filtration.

Test Solution Preparation

Weigh 10 mg of metformin and transfer it in to 10 ml of volumetric flask and then add 5 ml of diluent and sonicate dissolve it completely and make up the volume to the mark with the same solvent (Stock solution). Pipette out the 0.4 ml of the above solution and transfer in to volumetric flask make up the solution by adding diluent. Mix the solution thoroughly and filter through by using 0.45 µ filter.

Test Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the area for the metformin peak and calculate the%Assay by using the formulae.

System Suitability Requirements

Tailing factor: should not be more than 2.0%. Theoretical plates: should not be less than 2000.

RESULTS AND DISCUSSION

System suitability

For system suitability, two replicates of standard sample were injected and studied the parameters like plate number (N), HETP, peak symmetry of samples, %RSD of areas of reference solution

Evaluated the following system suitability parameters.

Theoretical plates & USP Tailing factor for standard

Acceptance criteria

1. The number of theoretical plates (N) for metformin peaks is NLT 2000.

2. The Tailing factor (T) for metformin peaks is NMT 2.0% **(Table 2 and Figure 2).**

Table 2. System suitability acceptance criteria.

System suitalibility				
	RT	**Peak area**	**Theoritical plates**	**USP plate count**
Std	2.004	2098288	2679.5	1.8
	2.006	2098252	2682.7	1.8
Mean	2.005	2098270	2681.1	1.8

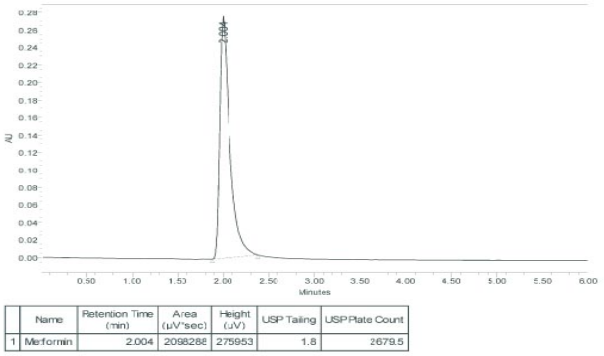


Figure 2. System Suitability graph.

Precision

Preparation of stock solution

Weigh the 10 mg of metformin and transfer in to 10 ml of volumetric flask and add 7 ml of diluent dissolve it completely. By using same solvent make up the volume up to the mark.

Preparation of 40 µg/ml solution

Pipette out 0.4 ml of the above stock solution and transfer into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix thoroughly and then filter through 0.45 µm filter.

Procedure

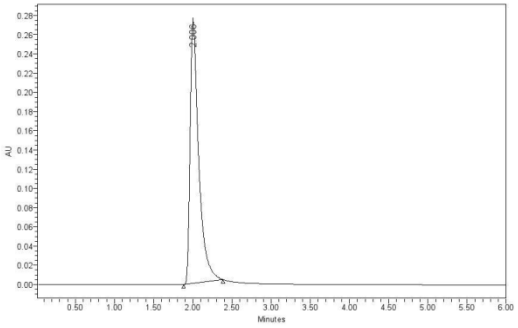
The standard solution was injected and measured the area in HPLC. This process is repeated to five times. The %RSD for the area of five injections was obtained to be within the specified limits **(Table 3).**

Table 3. %RSD for the area of injected samples.

Injection	**Area**	**RT**
Injection-1	2109526	2.005
Injection-2	2123059	2.006
Injection-3	2104546	2.006
Injection-4	2107149	2.006
Injection-5	2121405	2.005
Injection-6	2126913	2.005
Average	2115433	
Standard Deviation	9462.0	
%RSD	0.45	

Acceptance criteria

The% RSD for the area of five standard injections results should not be more than 2% **(Figures 3a-3e).**



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Metformin	2.006	2098252	276782	1.8	2682.7

Figure 3: Precession acceptance criteria graphs: **(a):** graph for channel 1 and Injection 1.

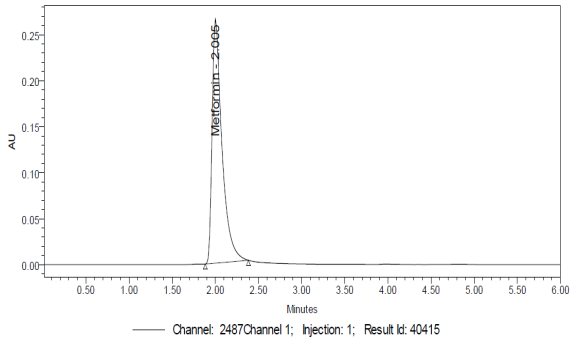


Figure 3(b). Graph for channel 1 Injection 2.

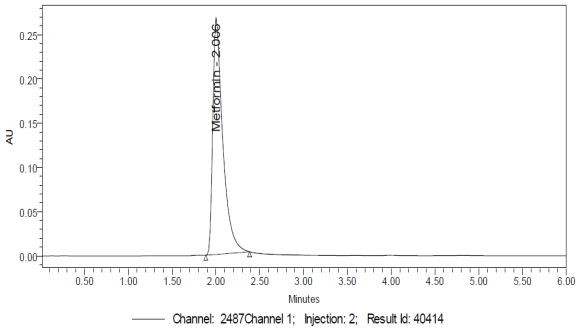


Figure 3(c). Graph for channel 1 Injection 3.

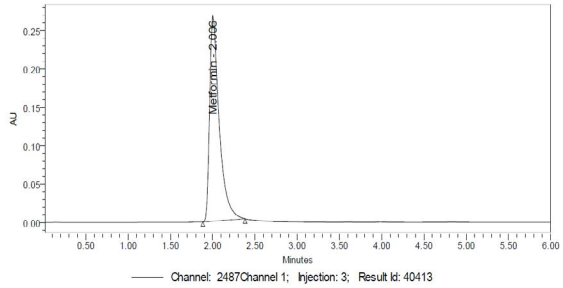


Figure 3(d). Graph for channel 1 Injection 4.

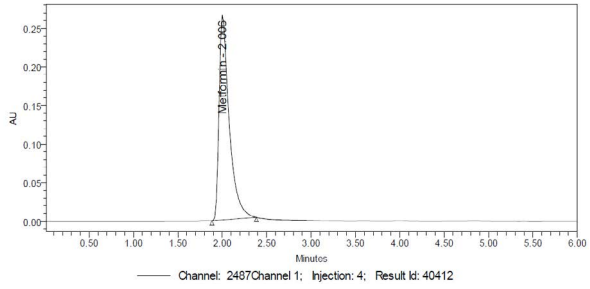


Figure 3(e). Graph for channel 1 Injection 5.

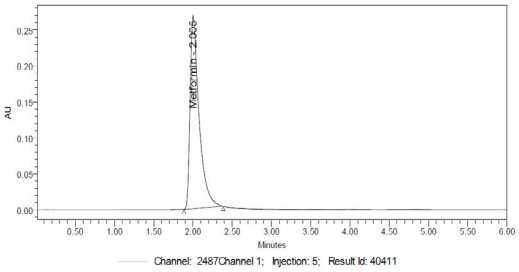


Figure 3(f). Graph for channel 1 Injection 6.

Accuracy [5,6]

Preparation of stock solution

Weigh the 10 mg of metformin and transfer in to 10 ml of volumetric flask and add 7 ml of diluent and dissolve it completely. By using same solvent make up the volume up to the mark.

Preparation of 40 µg/ml solution

Pipette out 0.4 ml of the above stock solution and transfer into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix thoroughly and then filter through 0.45 µm filter.

Preparation sample solutions

For preparation of 50% solution (With respect to Assay concentration)

Weigh the 05 mg of METFORMIN API and transfer in to 10 ml of volumetric flask and add 7 ml of diluent and dissolve it completely. By using same solvent make up the volume up to the mark and use it as stock solution. (with respect to Assay concentration).

Weigh the 10 mg of METFORMIN API and transfer in to 10 ml of volumetric flask and add 7 ml of diluent and dissolve it completely. By using same solvent make up the volume up to the mark and use it as stock solution.

Pipette out 0.4 ml of the above stock solution and transfer into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix thoroughly and then filter through 0.45 µm filter.

For preparation of 150% solution (With respect to target Assay concentration)

Weigh the 15 mg of METFORMIN API and transfer in to 10 ml of volumetric flask and add 7 ml of diluent and dissolve it completely. By using same solvent make up the volume up to the mark and use it as stock solution.

Pipette out 0.4 ml of the above stock solution and transfer into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix thoroughly and then filter through 0.45 µm filter

Procedure

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the obtained Amount and added Amount for METFORMIN and also calculate the individual and mean recovery values **(Table 4).**

Table 4. Individual calculations and mean recovery values.

%Concentration (at specification Level)	Avg Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2159388	5.0	5.08	101.7%	100.3%
100%	4236167	10.0	9.97	99.7%	
150%	6339656	15.0	14.9	99.5%	

Acceptance criteria

The% Recovery for each level should be between 98.0 to 102.0% **(Figures 4).**

Linearity: [7,8]

Preparation of stock solution

Accurately weigh and transfer 10 mg of METFORMINAPI sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent **(Table 5).** (Stock solution)

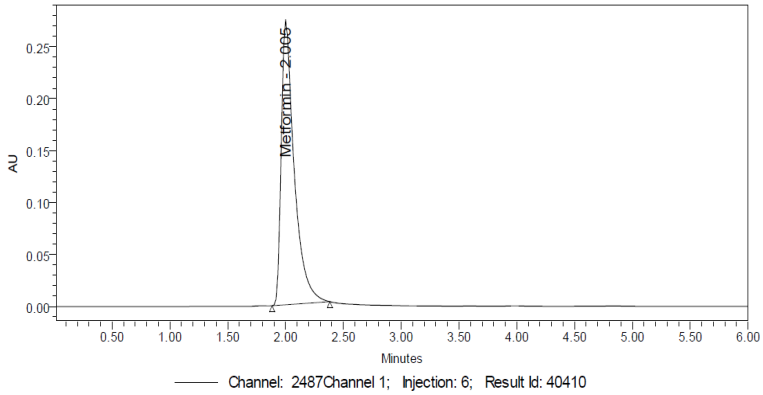


Figure 4. Graph shows accuracy acceptance criteria.

Table 5. Linearity correlation co efficient calculations.

S.No	Linearity Level	Concentration	Area	RT
1	I	20 µg/ml	1028416	2.002
2	II	30 µg/ml	1588963	2.006
3	III	40 µg/ml	2083770	2.009
4	IV	50 µg/ml	2580653	2.009
5	V	60 µg/ml	3187945	2.009
Correlation Coefficient			0.999	

Preparation of Level – I (20 µg/ml):

0.2 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluents

Preparation of Level – I (30 µg/ml):

0.3 ml of stock solution has taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (40 µg/ml):

0.4 ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (50 µg/ml):

0.5 ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (60 µg/ml):

0.6 ml of stock solution taken in 10 ml of volumetric flask dilute up to the mark with diluent.

Procedure

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient **(Table 6).**

Table 6. The flow rate analysis in robustness.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.7	2654.0	1.9
2	0.8	2679	1.8
3	0.9	2487	1.8

Acceptance criteria

Correlation coefficient should be not less than 0.999 **(Figures 5 and 6).**

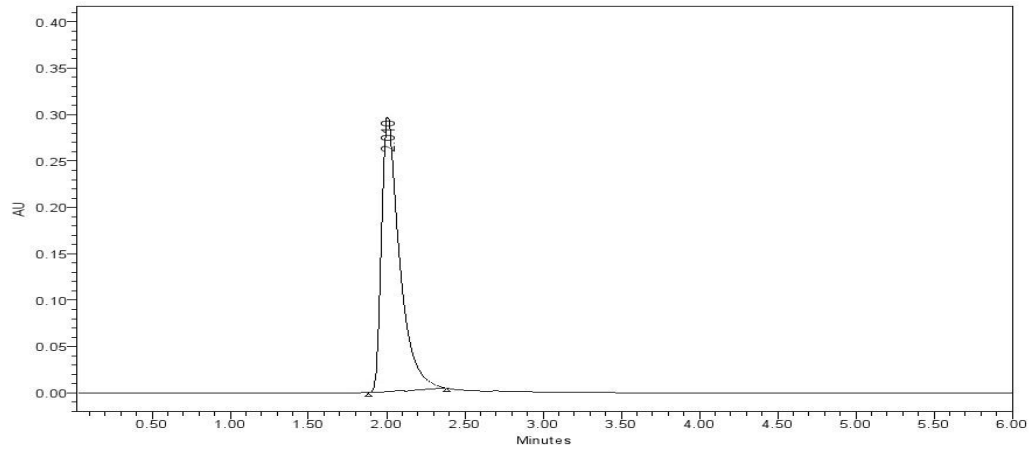
Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied.

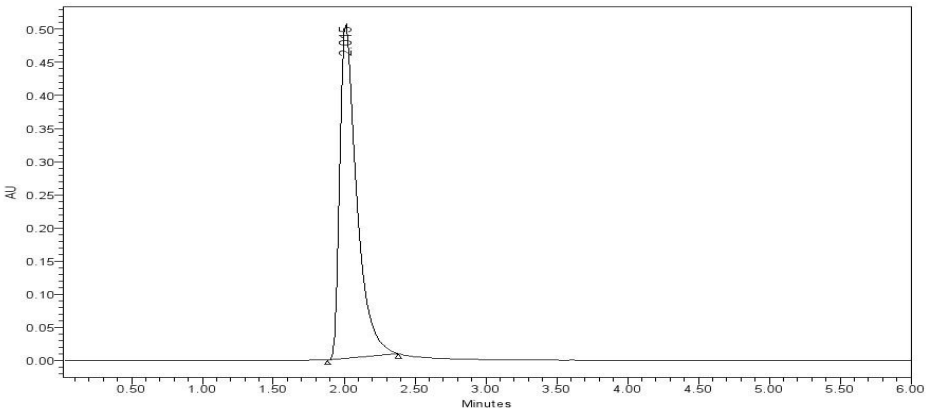
Standard solution 30 µg/ml was prepared and analysed using the varied flow rates along with method flow rate **(Table 7).**

More flow **(Figure 7)**



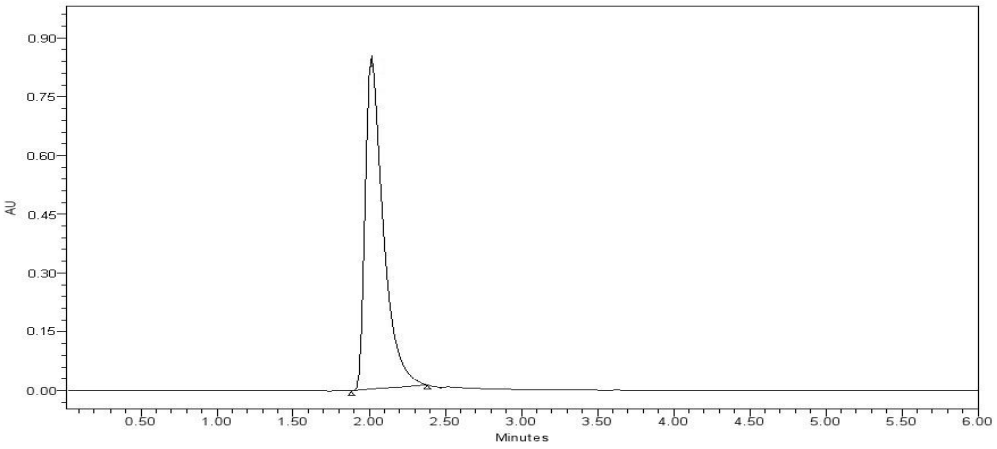
5(a)

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Metformin	2.010	2156821	284507



5(b)

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Metformin	2.015	4229774	557952



5(C)

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Metformin	2.019	6356323	838467

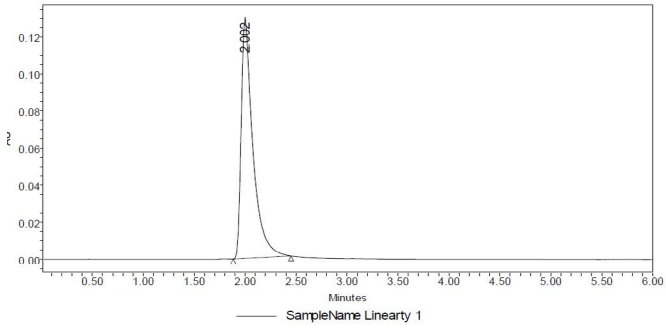
Figure 5. Linearity correlation coefficient for different samples.

Less flow **(Figure 8)**

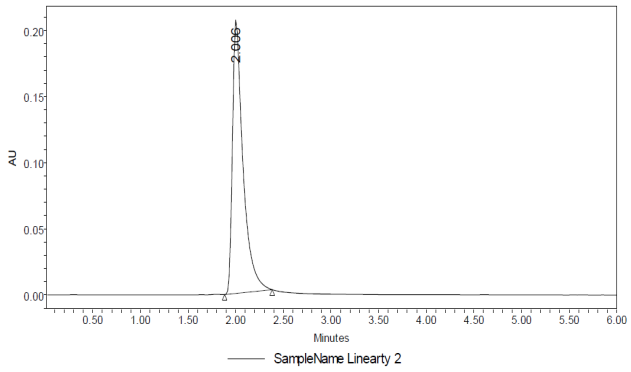
The Organic composition in the Mobile phase was varied.

Standard solution 30 µg/ml was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method **(Table 8 and Figure 9).**

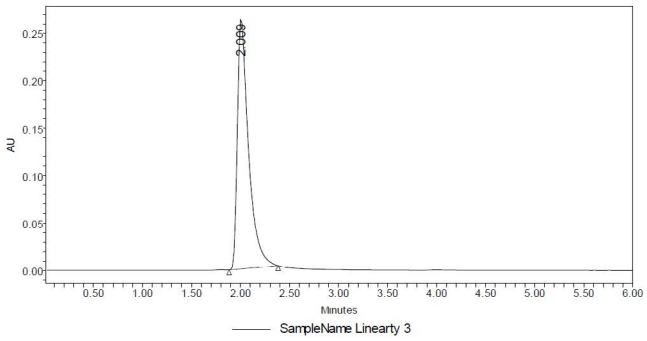
Assay Results (Table 8)



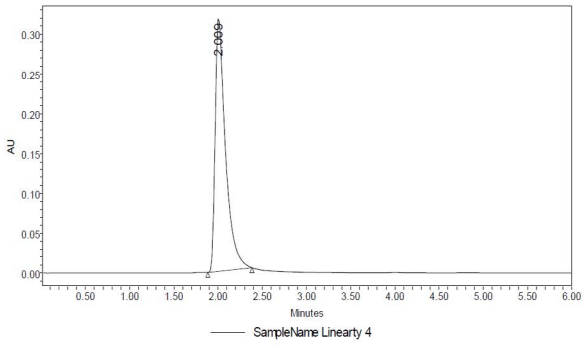
Sample 1



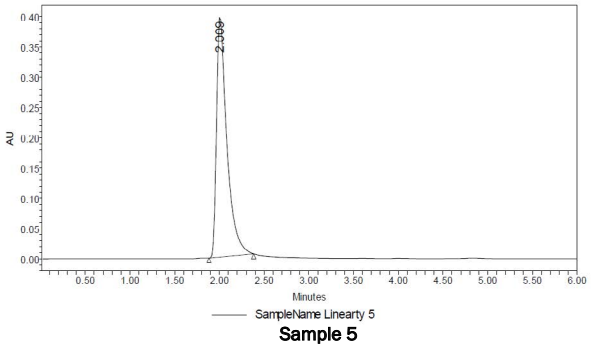
Sample 2



Sample 3



Sample 4



Sample 5

Figure 6. Linearity graph between the area and the concentration.

Table 7. Use of organic composition in the mobile phase.

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP plate count	USP tailing
1	10% less	2406	1.8
2	Actual	2679	1.8
3	10% more	2525	1.8

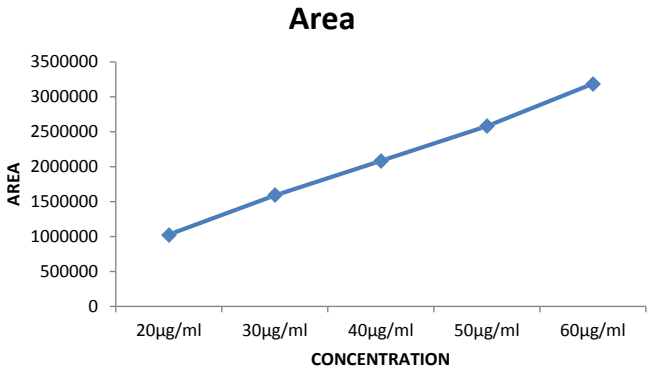
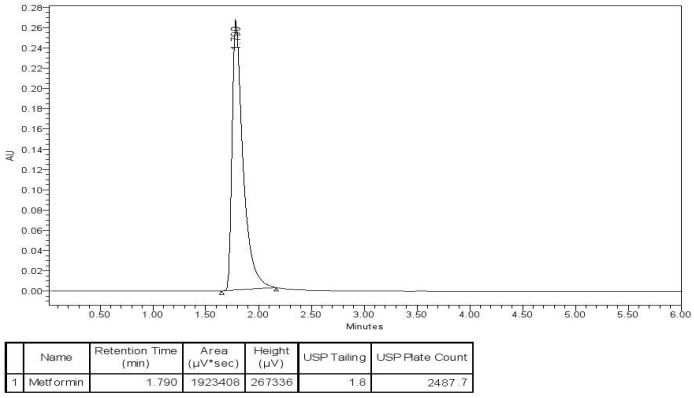


Figure 7. In robustness at more flow rate the graph show.

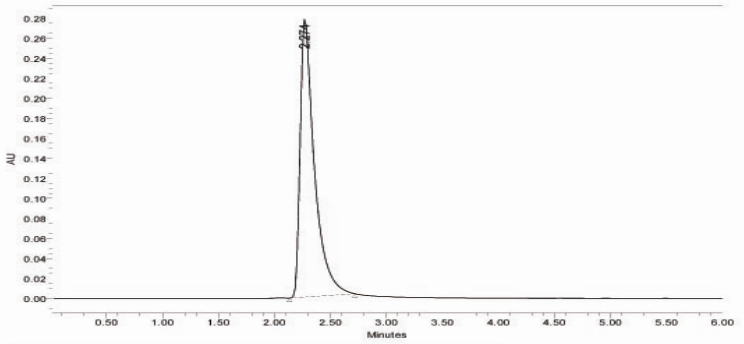


	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Metformin	1.790	1923408	267336	1.8	2487.7

Figure 8. In robustness at less flow rate the graph show.

Table 8. Assay results for metformin drug.

Name	S.NO	Peak area of metformin
Standard	STD-1	2098288
	STD-2	22098252
	Average	2098270
Sample	SMPL-1	2131459
	SMPL-2	2135119
	SMPL-3	2141703
	Average	2136094
%Assay		101.70%



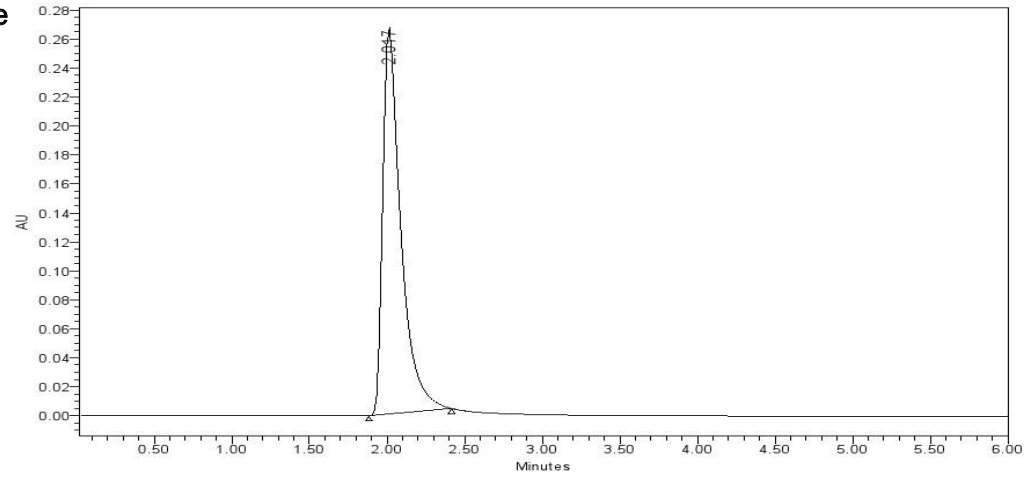
	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Metformin	2.274	2446862	279669	1.9	2654.6

Figure 9: In robustness changing the organic phase compositions.

Acceptance criteriai

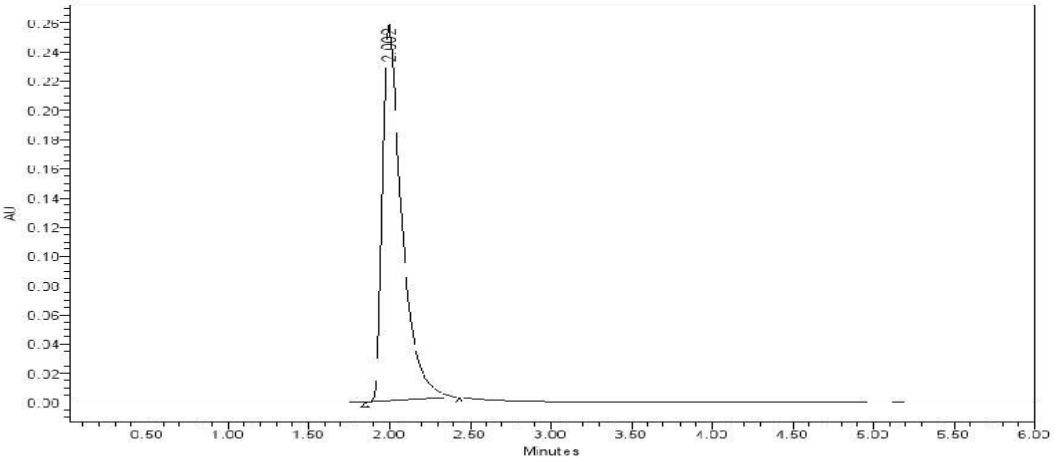
The acceptance criterion for an assay was in between 97% to 103% **(Figures 10a, 10b and 11a-11c).**

10(a): 10% more



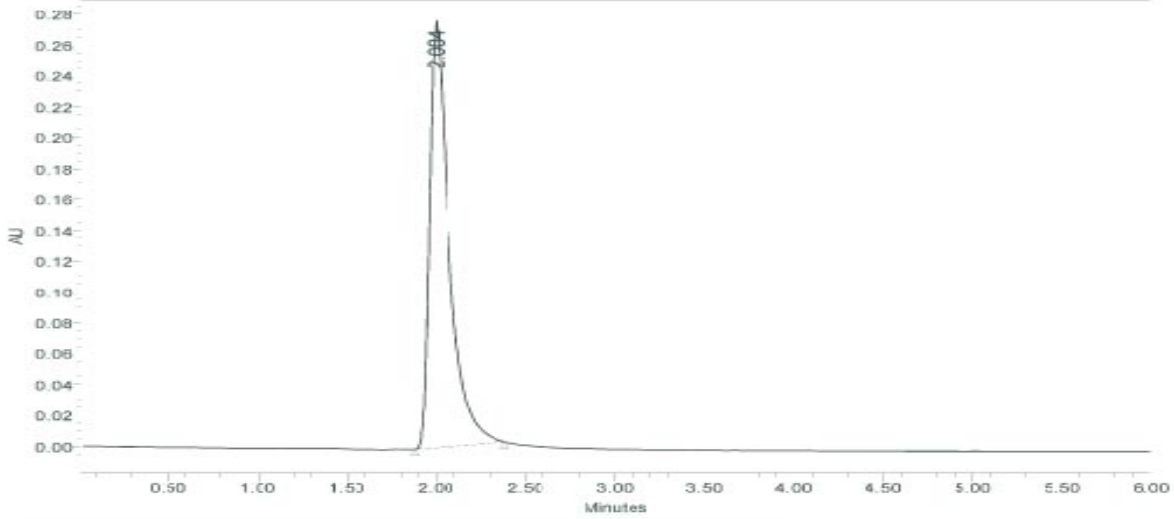
	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Metformin	2.017	2162988	265994	1.8	2525.7

10(b): 10% less



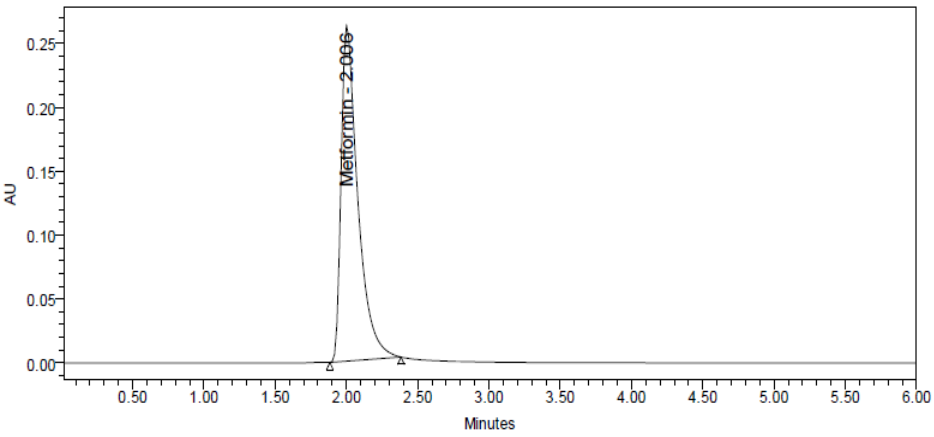
	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Metformin	2.002	2154647	257469	1.8	2406.0

10(c): Without any change in organic phase

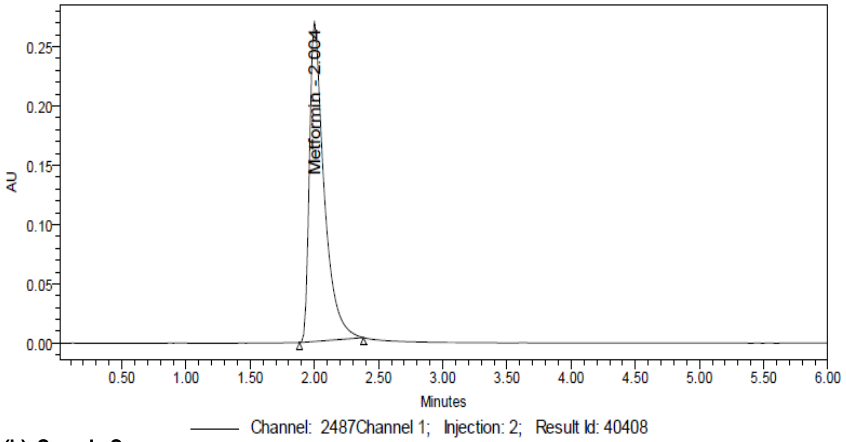


	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Metformin	2.004	2098288	275953	1.8	2679.5

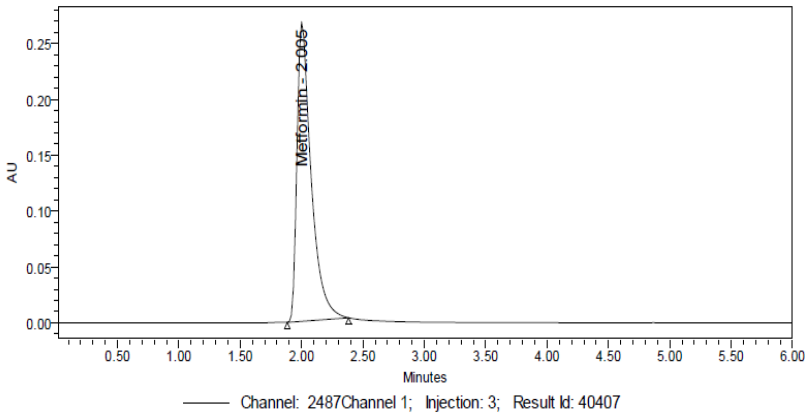
Figure 10. Standard graph for metformin drug.



11(a): Sample 1



11(b): Sample 2



11(c): Sample 3

Figure 11. Graphs for metformin drug by using samples.

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