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Method Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Metformin and Miglitol in Pharmaceutical Dosage Form

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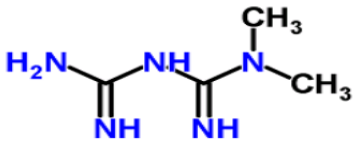
ABSTRACT

The data obtained from result and discussion it showed that the RP-HPLC method for estimation of pharmaceutical dosage form was developed successfully. The RP-HPLC analysis was performed on the PRIMESIL C_{18},(250*4.6)mm, 5µm particle size in the isocratic mode, at Ambient column oven temperature using Acetonitrile: Water (80:20 v/v) as mobile phase; flow rate was adjusted to 0.7 ml/min. The detection was carried out at 236nm. The average retention time for candidate drugs was found to be 3.7333 and 8.5833 min. Linearity was observed in the concentration range of 20-100 µg/ml (r^2= 0.9987 and r^2=0.9984). The method has been successively applied for the determination of candidate drug in tablet formulation. There was no interference from the excipients present in the tablet. The drug content was found to be 101.63% for candidate drug. Accuracy of the method was studied by the recovery studies at three different levels 80%, 100%, 120%. The % recovery was found to be within the limit of the acceptance criteria with average recovery of 98-102. According to USP, system suitability tests are integral part of chromatographic methods. They are used to reproducibility of the chromatographic system.

I. INTRODUCTION

Metformin, marketed under the trade name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in treatment of polycystic ovary syndrome. Limited evidence suggests metformin may prevent the cardiovascular disease and cancer complications of diabetes. Chemical name: N,N-Dimethyllimidodicarbonimidic diamide. Empirical formula: $C_4H_{11}N_5$. Molecular weight: 129.164. Description: Metformin is an orally administered biguanide derivative used to lower blood glucose. M.P: 224 °C. Solubility: It is soluble in Acetonitrile; sparingly soluble in water. Bioavailability: Metformin, 50–60%. Half–life: 4-8.7 hours. Volume of distribution. 654 ± 358 L. Clearance: renal clearance (CLr) is 510±120 ml/min. Active tubular secretion in the kidney is the principal route of metformin elimination. Protein binding. plasma protein binding of metformin is negligible Dose. 1.25 to 5 mg daily with a maximum of 10 mg daily.

Structure:

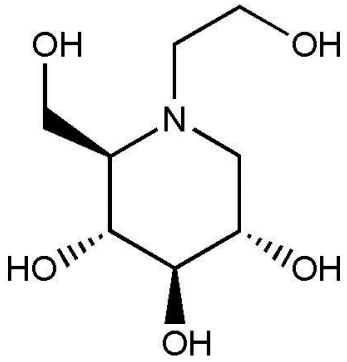


Metformin

Miglitol is an oral anti-diabetic drug that acts by inhibiting the ability of the patient to break down complex carbohydrates into glucose. It is primarily used in diabetes mellitus type 2 for establishing greater glycemic control by preventing the digestion of carbohydrates (such as disaccharides, oligosaccharides, and polysaccharides) into monosaccharides which can be absorbed by the body. Chemical name: (2R,3R,4R,5S)-1-(2-Hydroxyethyl)-2-(hydroxymethyl)-3,4,5-piperidinetriol. Empirical formula: C8H17NO5.

Molecular weight: 207.22. Description: Miglitol is an oral anti-diabetic drug that acts by inhibiting the ability of the patient to breakdown complex. M.p. 146°. Toxicity. Symptoms ofoverdose are transient increase in flatu-lence, diarrhea, and abdominal discomfort. Bioavailability: 50-70%. Half–life: 2 hours.Volume of distribution. 0.18 L/kg. Clearance. Systemic plasma clearance, about 0.8 L/min. Protein binding. The protein binding of miglitol is negligible (<4.0%). Dose. An initial dose of 5 mg daily is given followed by a usual maintenance dose of 2.5 to 10 mg. Elderly and patients with impaired liver function: initial dose of 2.5 mg daily.

Structure:



II. MATERIALS AND METHOD

A. **Materials :** Acetonitrile, Distilled Water, Metformin, and Miglitol.

Table 1. Instrument Used

INSTRUMENT	SPECIFICATIONS
HPLC SYSTEM • **Pump** • **Detector** • **Data Processor** • **Column**	Younglin HPLC System SP930D Solvent delivery system. UV Detector Autochrom 3000 C_{18} (5 µm ,4.6 mm x 250 mm)

1. Selection of Chromatographic Parameters

a) Selection of Stationary Phase : Primesil C_{18} (4.6 mm x250 mm) with Particle Size 5 µm Was Selected.

b) Selection of Solvent: Acetonitrile and Water was selected as a solvent for Metformin and Miglitol.

B.METHOD

1. Preparation of Stock Standard Solution

Stock Standard Solution was Prepared by dissolving 10 mg Metformin and 10 mg of Miglitol in 10 ml Methanol that give concentration of 1000 µgm/ml Metformin 1000 µgm/mL Miglitol.

2. Optimization of Chromatographic Parameters

Optimization in HPLC is the process of finding as set of condition that adequately separate and enable the quantification of the analytes from the endogeneous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

3. Optimization of Mobile Phase Strength

The mobile phase was chosen after several trials with Acetonitrile and Water in various proportions. A mobile Phase consisted of Acetonitrile and Water (80:20v/v) was selected to achieve symmetrical peak. The effects of flow rates in the ranges of 0.5 to 1ml/min. were examined. A flow rate of 0.7ml/ min. gave good results, system suitability parameter and reasonable retention time. The retention time of Metformin and Miglitol Was observed 3.7333 and 8.5833 min at 236nm wavelength. Finalized chromatographic conditions are shown in table 2.

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table 2. Chromatographic condition

Chromatographic condition	
HPLC Software Column Particle size packing Stationary phase Mobile phase Detection Wavelength Flow rate Temperature Sample size	Younglin (S.K.)Gradient system. UV Detector Autochro -3000 4.6 x 250 mm 5 µm C_{18} (primesil) Acetonitrile:Water (80:20) 236 nm 0.7 ML/min. Ambient 20µl.

Linearity studies

From stock standard solution, aliquots of 0.2, 0.4, 0.6, 0.8, 0.10 ml were taken in 10 ml volumetric flasks and

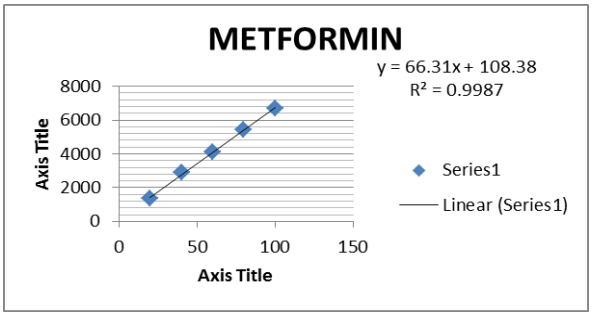
diluted up to the mark with methanol such that to obtained concentration of Metformin and Miglitol in the range 20-100 µl/ml. Volume of 20µL of each sample was injected with the help of Hemilton syringe. All measurements were repeated two time for each concentration and curve was constructed by plotting the peak area versus the drug concentration. The observation and calibration curve is shown in table 3.

Table 3. Data showing Linearity study of Metformin.

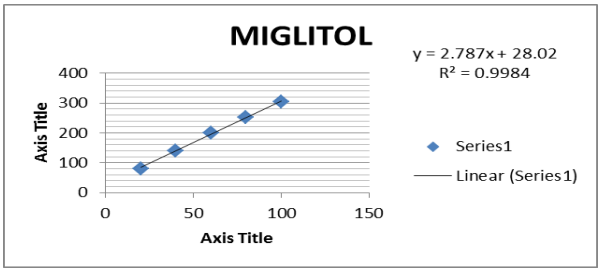
Sr. No.	Concentration of Ramipril (µg/mL)	Mean peak area	SD	%RSD
1	20	1344.83	9.86	0.73
2	40	2882.78	7.44	0.26
3	60	4088.74	84.34	1.34
4	80	5402.78	57.57	1.07
5	100	6715.85	33.11	0.49

Table 4. Data showing Linearity study of Miglitol.

Sr. No.	**Concentration of Felodipine (µg/mL)**	**Mean peak area**	**SD**	**%RSD**
1	20	80.715	1.12	1.39
2	40	139.705	0.60	0.43
3	60	200.97	3.45	1.72
4	80	251.07	2.36	0.94
5	100	303.735	5.48	1.80



Graph 1. Linearity of Metformin



Graph 2. Linearity of Miglitol

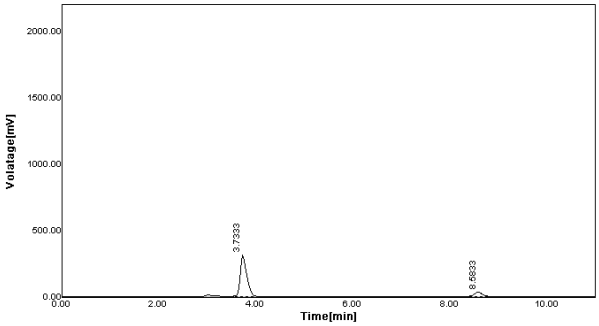


Figure 1. Chromatogram of standard of Metformin and Miglitol. (10 µg/mL)

The Trial Experimental work was carried out for linearity parameter and the graphs has obtained has shown in the Figure 3,4,5,6,7,8, as follows

Trial 1. of Metformin and Miglitol with Acetonitrile: water (90:10) at 235 nm with flow rate 0.7ml

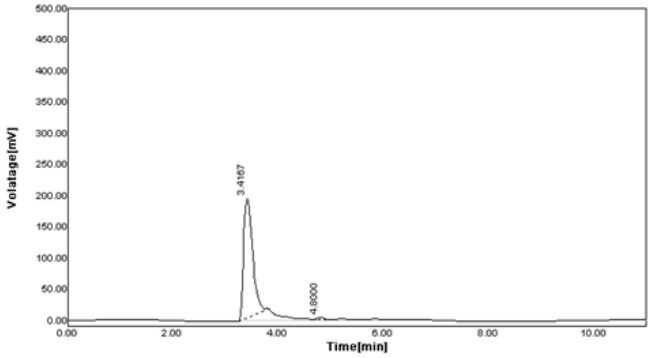


Figure 2. Chromatogram of Metformin and Miglitol

Table 5. Trial 1 of Metformin and Miglitol

No.	**RT[min]**	**Area[mV*s]**	**Area %**	**TP**	**TF**	**Resolution**
1	3.4167	2279.5659	98.82	1618.3	1.3889	0.0000
2	4.8000	27.2441	1.18	7186.6	1.1429	4.1500
Sum		**2306.8101**				

Trial 2. Metformin and Miglitol with Acetonitrile: water (80:20) at 235 nm with flow rate 0.7ml.

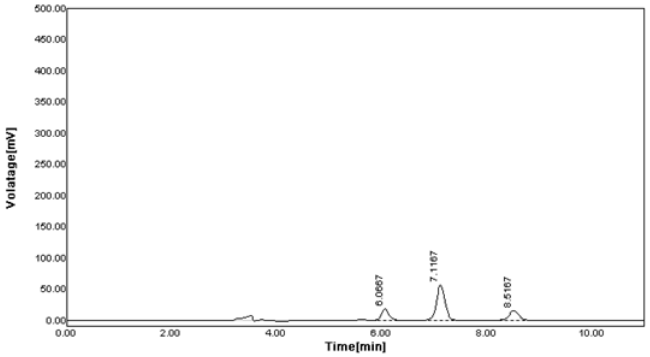


Figure 3. Chromatogram of Metformin and Miglitol

Table 6. Trial 2 of Metformin and Miglitol

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	3.8000	191.5861	16.67	8007.3	0.7917	0.0000
2	4.0833	366.3116	31.87	1027.3	1.7143	0.2394
3	4.4667	102.8074	8.94	398278.4	1.2000	1.2105
4	4.7500	156.6841	13.63	3127.8	1.0714	1.3077
5	5.1833	113.9153	9.91	6621.4	1.0625	1.1818
6	5.3833	54.6425	4.75	4938.7	3.2500	0.6667
7	5.6667	163.6040	14.23	134.6	1.3333	0.2957
Sum		1149.5511				

Trial. 3. Metformin and Miglitol with Acetonitrile: water (80:20) at 240 nm with flow rate0.7ml.

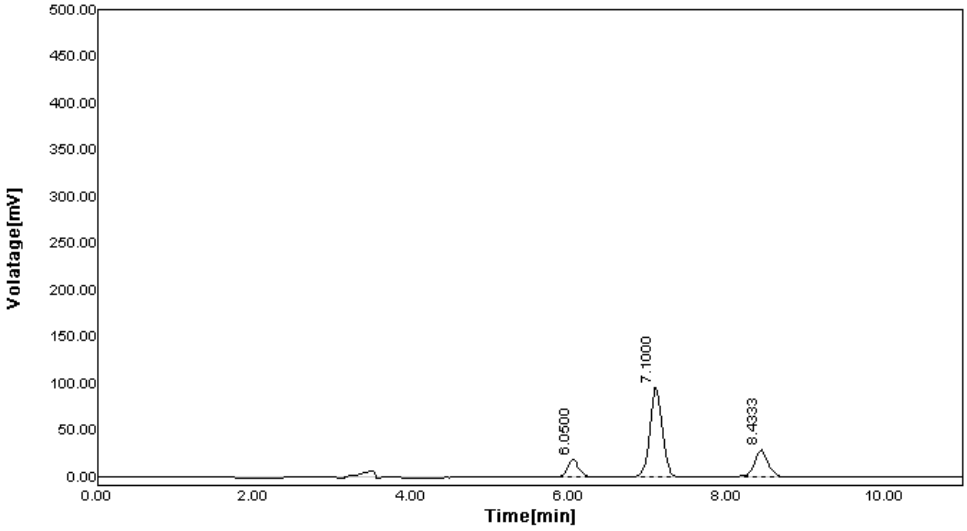


Figure 4. Chromatogram of Metformin and Miglitol.

Table 7. Trial 3 of Metformin and Miglitol

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	6.0667	178.9879	17.32	7347.2	1.1111	0.0000
2	7.1167	653.7641	63.24	8355.8	1.0417	3.0000
3	8.5167	200.9586	19.44	10055.3	0.9667	3.6522
Sum		1033.7106				

Trial 4. Metformin and Miglitol with Methanol:water (60:40) at 238nm with Flow rate 0.7.

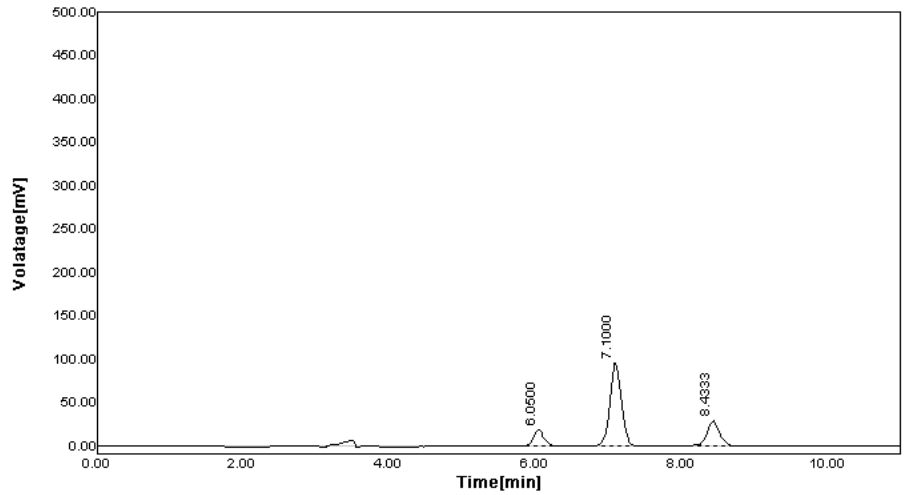


Figure 5. Chromatogram of Metformin and Miglitol.

Table 8. Trial 4 of Metformin and Miglitol

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	6.0500	181.1603	11.19	7306.9	1.0500	0.0000
2	7.1000	1058.1221	65.37	8316.7	1.0000	3.0000
3	8.4333	379.2775	23.43	9859.5	1.0000	3.4783
Sum		1618.5598				

Trial 5. Metformin and Miglitol with Methanol:water (80:20) at 238nm with Flow rate 0.7

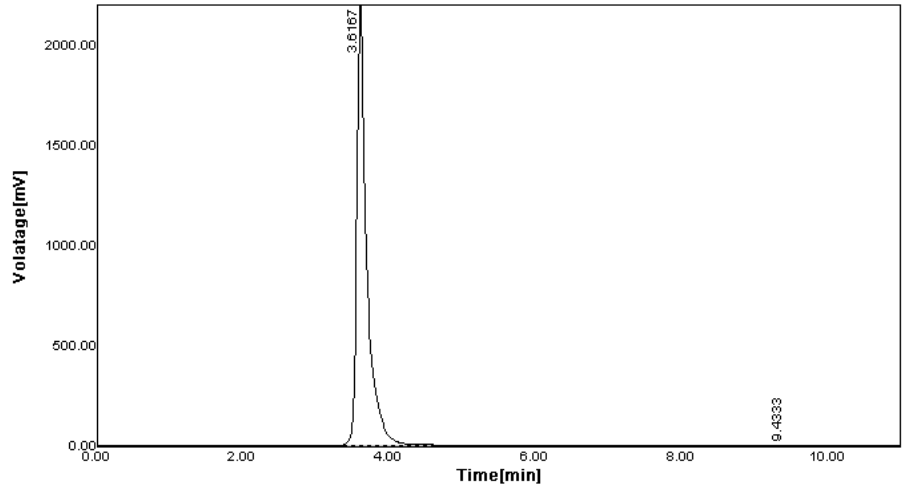


Figure 6. Chromatogram of Metformin and Miglitol.

Table 9. Trial 5 of Metformin and Miglitol

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	3.6000	56907.8906	99.89	2587.2	2.3333	0.0000
2	9.3167	65.5109	0.11	8840.7	1.1538	14.2917
Sum		56973.4023				

Trial 6. Metformin and Miglitol with Acetonitrile: water (80:20) at 236 nm with flow rate 0.7ml

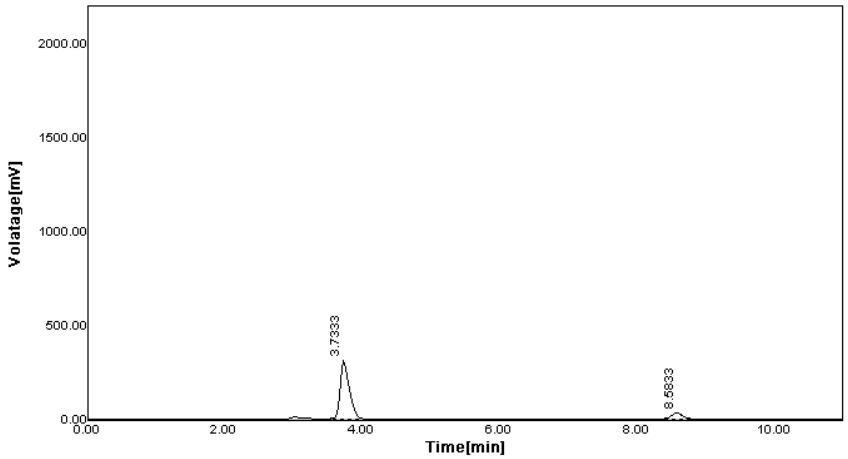


Figure 7. Chromatogram of Metformin and Miglitol

Table 10. Trial 6 of Metformin and Miglitol

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	3.7333	2947.3479	88.55	2782.4	1.4286	0.0000
2	8.5833	381.0026	11.45	12154.8	0.9615	13.8571
Sum		3328.3506				

III. RESULTS AND DISCUSSION

1 Determination of λmax of Metformin and Miglitol

The λ-max was determined on Shimadzu UV-Visible spectrophotometer in the range 200-400 nm using methanol as blank. Spectrophotometric study of Metformin and Miglitol was carried out in methanol. Optimum solubility of the drugs was obtained in the methanol. Spectral study showed that the λmax for the Metformin and Miglitol at 236 nm. The solution of exhibited maxima at about 236 nm .

The primary target in developing this stability indicating RP-HPLC method is to achieve resolution of drug and its degradation product. We use a stationary phase C-18.

A mobile phase consisting of different buffers ratio and column, but peak shape and retention time of Metformin and Miglitol broad compared to water, acetonitrile composition as mobile phase, pH was adjust 3.2±0.05 with orthophosphoric acid. Acetonitrile : Water ratio was selected at proportion of 80:20. This shows good resolution chromatogram with symmetrical peaks.

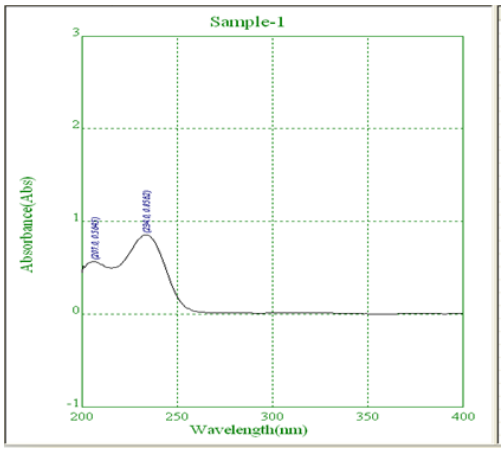


Figure 8. UV Curve of Metformin

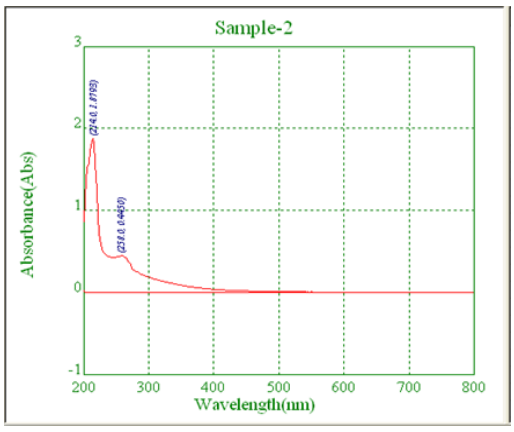


Figure 9. UV Curve of Miglitol

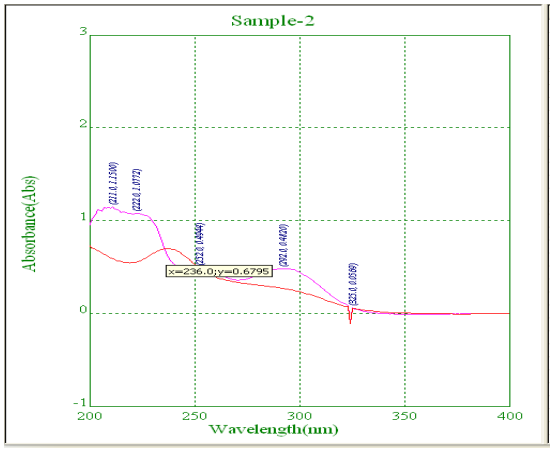


Figure 10. Isobestic point of Metformin and Miglitol

IR ANALYSIS

IR ANALYSIS OF METFORMIN:

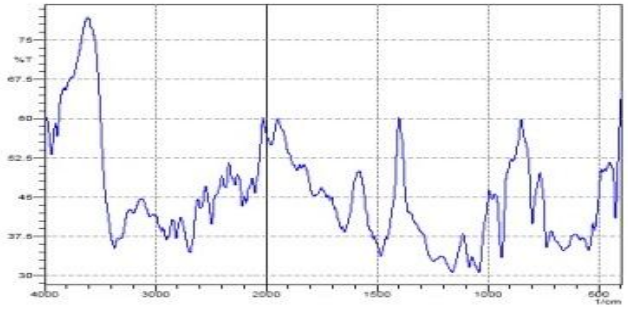


Figure 11. IR Interpretation of Metformin

Table 11. Interpretation of Metformin

Sr No	Observation	Peak Region
1	Primary N-H Stretching	3400-3200
2	Secondary N-H	3300-3100
3	Aliphatic C-H Stretching	2900-2700
4	C=N Stretching	1700-1600
5	C-N Stretching	1300-1000

IR ANALYSIS OF MIGLITOL:

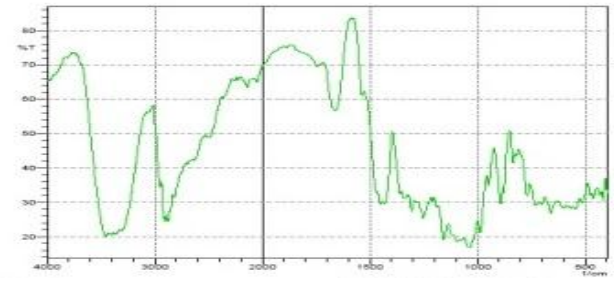


Figure 12. IR Interpretation of Miglitol

Table 12. Interpretation of Metformin

Sr No	Observation	Peak Region
1	Primary O-H Stretching	3400-3200
2	Aliphatic C-H Stretching	2900-2700
3	C-N Stretching	1300-1000
4	O-H Bending	1000-900

IR ANALYSIS OF METFORMIN AND MIGLITOL:

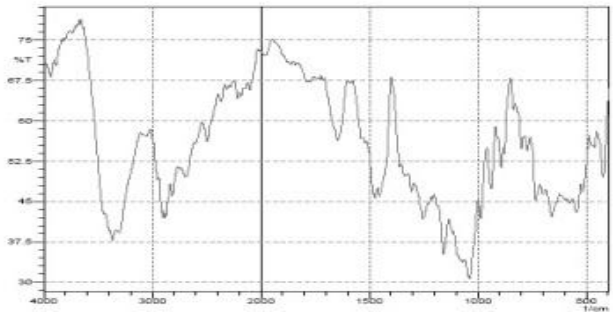


Figure 13. IR Interpretation of Metformin andMiglitol

Table 13. Interpretation of Metformin and Miglitol

Sr No	Observation	Peak Region
1	Primary O-H Stretching	3400-3200
2	Primary N-H Stretching	3400-3200
3	Secondary N-H Stretching	3300-3100
4	Aliphatic C-H Stretching	2900-2700
5	C=N Stretching	1700-1600
6	C-N Stretching	1300-1000
7	O-H Bending	1000-900

Tablet Assay for Metformin and Miglitol:

Table 14. Data Showing Tablet Assay

Conc	Area	Amt Found	% Label Claim
40.00	2803.15	40.65	101.63
40.00	2831.26	41.06	102.65
Mean	2817.21	39.67	102.14
SD	19.88	0.29	0.07
%RSD	0.71	0.73	0.07

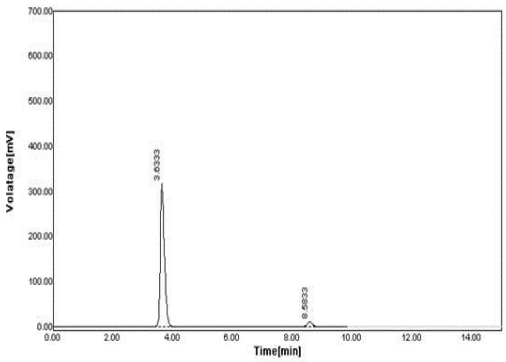


Figure 14. Chromatogram of Tablet Assay

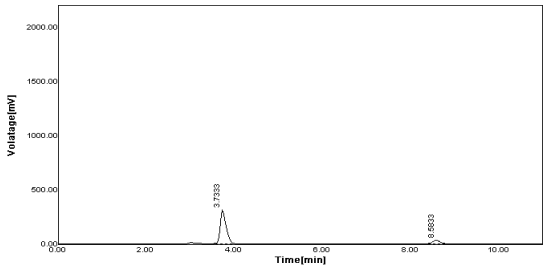


Figure 15. Chromatogram Of Standard Solution

Acceptance criteria:

The % assay should be within 98102%.

The results of the assay indicate that the method is selective for the analysis of Metformin and Miglitol without interference from the excipients used to formulate and produce these tablets.

Validation of RP-HPLC Method For Metformin and Miglitol

Method of Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions precision may be considered as three levels repeatability, intermediate precision and reproducibility. The %RSD should not be more than 2%.

Table 15. Precision of Metformin.

Sample no.	Conc.	Area I	Area II	Mean	SD	%RSD
1	40	2902.96	2956.85	2929.91	38.11	1.30
2	60	4178.32	4255.2	4216.76	54.36	1.29
3	80	5523.28	5497.22	5510.25	18.43	0.33

Table 16. Precision of Miglitol.

Sample no.	Conc.	Area I	Area II	Mean	SD	%RSD
1	40	139.86	136.42	138.14	2.43	1.76
2	60	201.96	199.1	200.53	2.02	1.01
3	80	249.36	251.72	250.54	1.67	0.67

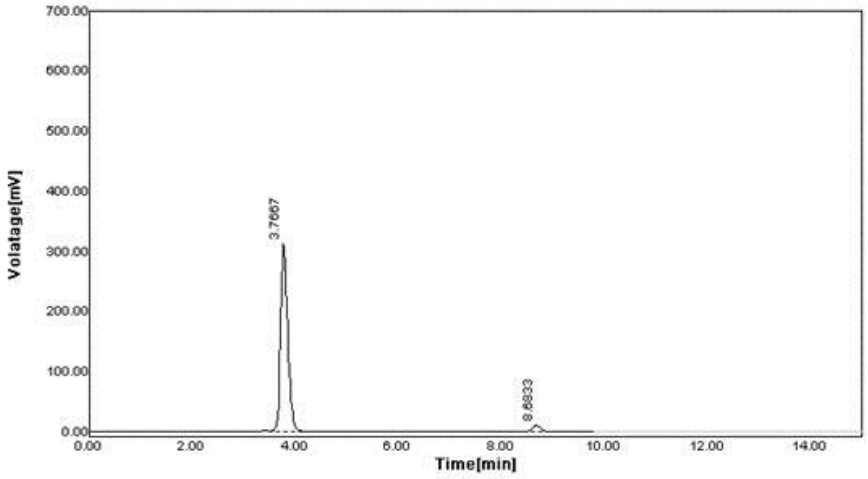


Figure 16. Chromatogram of Precision at 40 µg/ml

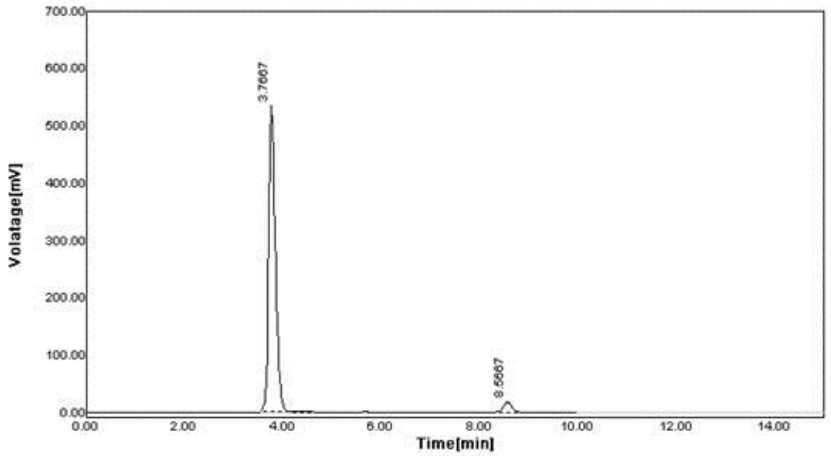


Figure 17. Chomatogram of precision by 60 µg/ml.

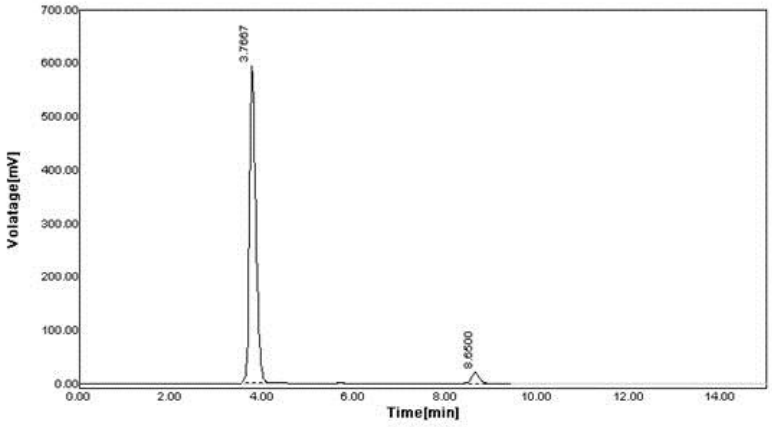


Figure 18. Chomatogram of precision by 80 µg/ml.

Acceptance criteria: % RSD NMT 2% for test results.

a) Repeatability (Intra-Assay precision): Repeatability express the precision under the same operating condition over a short intervals of time. Repeatability is also termed as intra-assay precision.

Table 17. Repeatability of Metformin and Miglitol.

Reno.	Conc.	Peak Area	Amt. Found	% Amt. Found
1	60	4221.32	60.91	101.52
2	60	202.92	61.62	102.70
		Mean	61.26	102.11
		SD	0.51	0.84
		%RSD	0.83	0.82

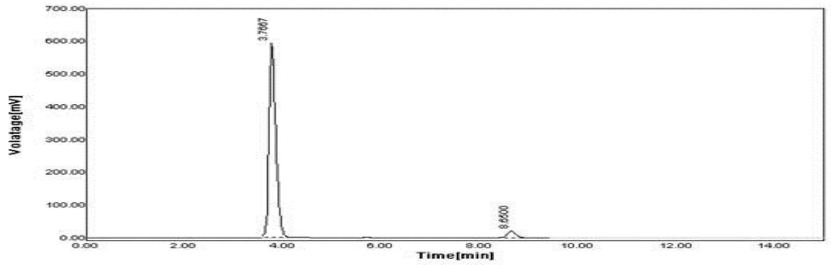


Figure 19. Chromatogram of Repeatability by 20 µg/ml. Metformin and Miglitol.

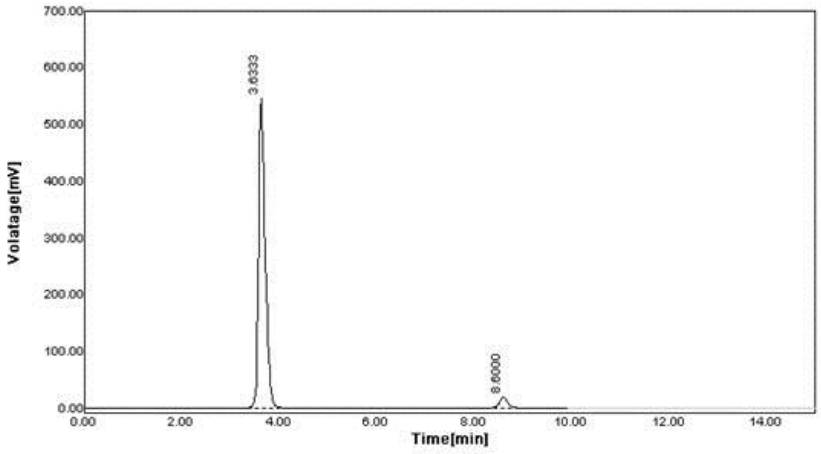


Figure 20. Chromatogram of Repeatability by 20 µg/ml. Metformin and Miglitol.

Acceptance criteria:

% RSD of Two standard injections should not be more than 2.0. Results obtained lies well within the acceptance criteria.

b) Intermediate precision: Intermediate precision expresses within laboratory variations different day, different analyst or equipment etc. Intermediate precision is usually demonstrated by repeated measurement of the sample used in therapeatability experiment within the same laboratory

c) Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology). Repeatability is usually demonstrated by means of an inter laboratory trial.

Specificity:

Specificity is ability to asses unequivocally the analyte in the presence of components that may be expected to be present. Typically these might be include impurities, degradents, matrix etc.

Specificity may often be expressed as the degree of bias of test results obtained by analysis of samples containing added impurities, degradation products, related chemical compounds, or placebo ingredients when compared to test results without added substances.No peak is observed and the result shows that the method is specific for estimation.

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as convention true value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometime termed trueness. Accuracy should be established across the specified range of analytical procedure. Accuracy is usually demonstrated by adding known amounts of analyses to the sample matrix and determined the measured result using the analytical procedure. Result shows that the Method is accurate and precise.

Table 18. Accuracy of Metformin.

Metformin Sample No.	**80% % Recovery**	**100% % Recovery**	**120% % Recovery**
1	97.99	100.70	101.94
2	97.02	99.35	101.40
Mean	97.51	100.03	101.58
SD	0.69	0.95	0.38
%RSD	0.70	0.95	0.38

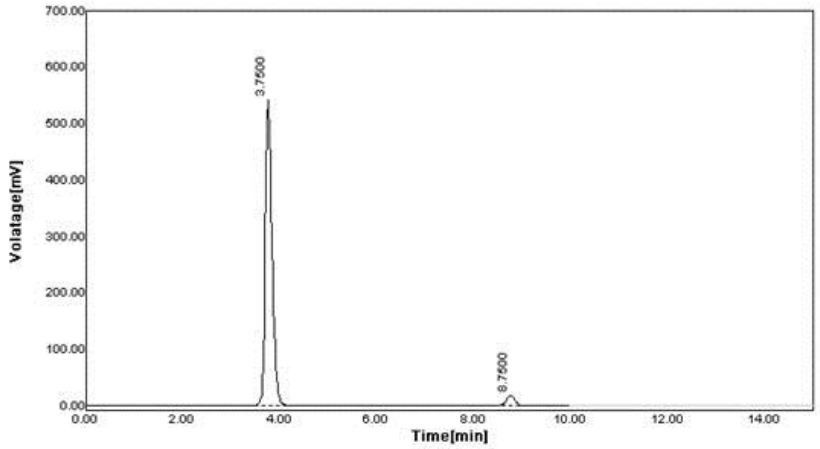


Figure 21. Chromatogram Accuracy of Metformin.

Table 19. Accuracy of Miglitol.

Miglitol Sample No.	80% % Recovery	100% % Recovery	120% % Recovery
1	99.31	102.26	97.96
2	101.29	101.73	99.08
Mean	100.30	102.00	101.58
SD	1.40	0.37	0.79
%RSD	1.40	0.37	0.78

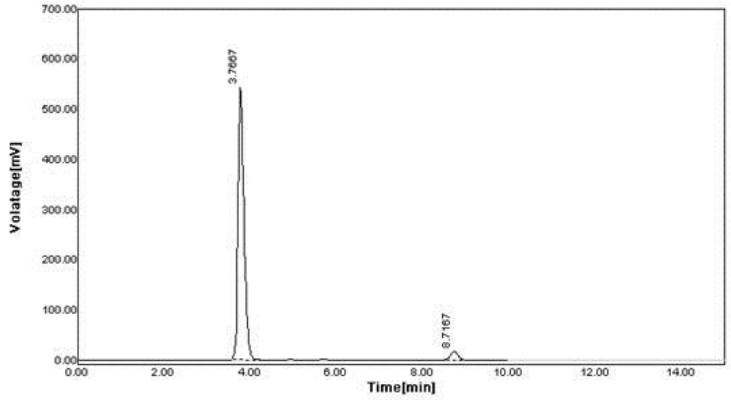


Figure 22. Chromatogram Accuracy of Miglitol.

Acceptance Criteria:

Recovery at each level and % mean recovery should be between 98-102 with % RSD should not be more than 2.0%.

The % recovery at each level, % mean recovery, and % RSD at each level meets the established acceptance criteria. Hence the method is accurate in the specified range.

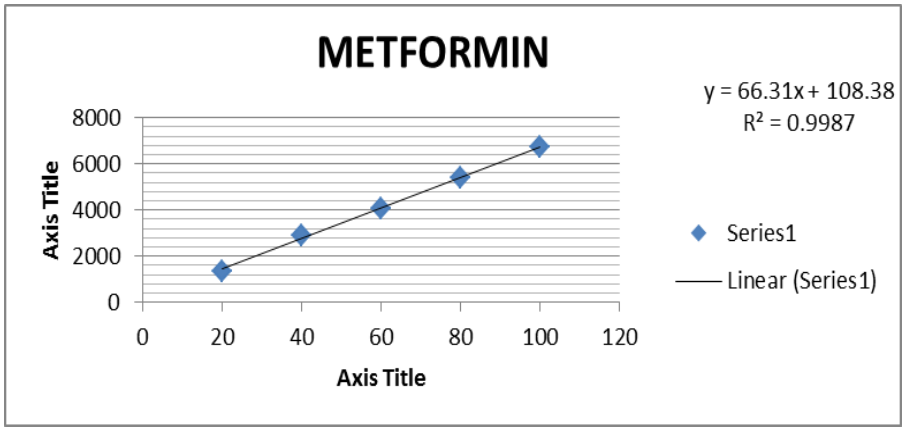
Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample linearity is usually demonstrated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of regression line by the method of list squares. In some cases to obtain linearity between assay and sample concentration, the tesdat may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be healpful to provide mathematical estimates of the degree of linearity.

The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be calculated. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.
A minimum of five concentrations is recommended other approaches should be justified.

Table 20. Linearity of Metformin.

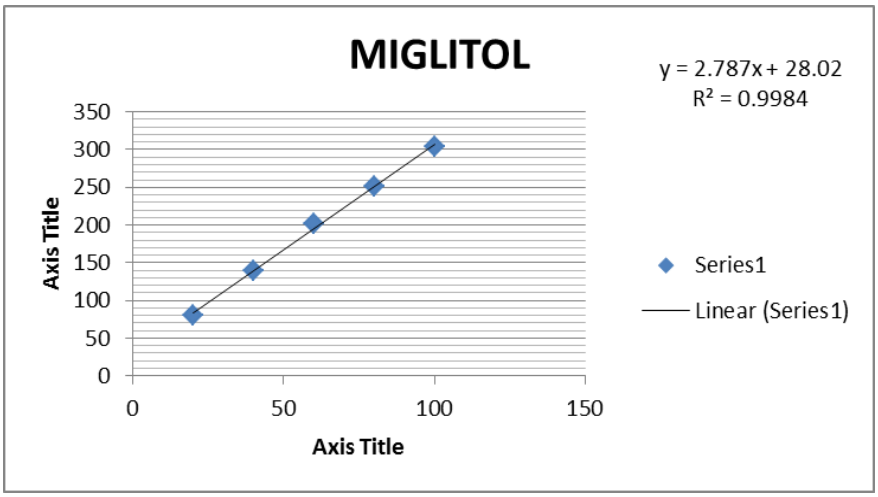
Conc.	Mean	SD	%RSD
20	1344.83	9.86	0.73
40	2882.78	7.44	0.26
60	4088.74	84.34	1.34
80	5402.78	57.57	1.07
100	6715.85	33.11	0.49



Graph 3. Linearity of Metformin.

Table 21. Linearity of Miglitol.

Conc.	Mean	SD	%RSD
20	80.715	1.12	1.39
40	139.705	0.60	0.43
60	200.97	3.45	1.72
80	251.07	2.36	0.94
100	303.735	5.48	1.80



Graph 4. Linearity of Miglitol.

Acceptance criteria:

The correlation coefficient value should not be less than 0.995. The areas obtained are directly proportional to the concentration of analyte in the sample. The method is linear in the specified range.

Robustness:

The procedure for the commencement of method validation was followed. The result were obtain are as follows.

Changing of mobile phase by ±1 ml.

Table 22. Changing of mobile phase by (81-19)

Metformin	Mobile phase (81-19)	Miglitol	(Mobile phase:81-19)
1	1389.56	1	59.12
2	1370.99	2	59.3
Average	1380.28	Average	59.2
SD	13.13	SD	0.13
% RSD	0.95	% RSD	0.21

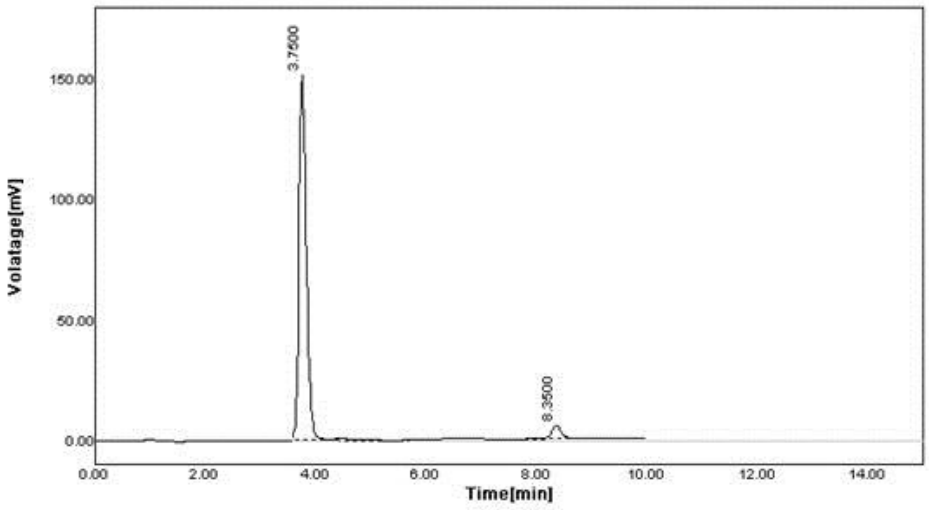


Figure 23. Chromatogram of Robustness Changing of mobile phase by (81-19)

Table 23. Changing mobile phase by (79-21)

Metformin	Mobile Phase (79-21)	Miglitol	Mobile Phase (79-21)
Sample no.	Robustness	Sample no.	Robustness
1	1348.26	1	57.25
2	1366.59	2	56.99
Average	1357.4	Average	57.12
SD	12.96	SD	0.18
%RSD	0.95	%RSD	0.32

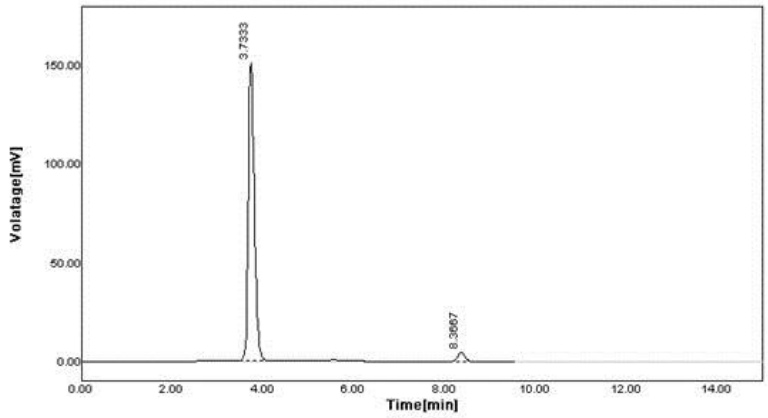


Figure 24. Chromatogram of Robustness Changing mobile phase by (79-21)

Changing Flow rate ± 0.1 ml.

Table 24. Changing Flow rate 0.8 ml.

Metformin	**Flow rate :0.8 ml**	**Miglitol**	**Flow rate: 0.8ml**
Sample no.	**Robustness**	**Sample no.**	**Robustness**
1	1185.1	1	51.07
2	1174.91	2	49.67
Average	1180.01	Average	50.37
SD	7.21	SD	0.99
%RSD	0.61	%RSD	1.97

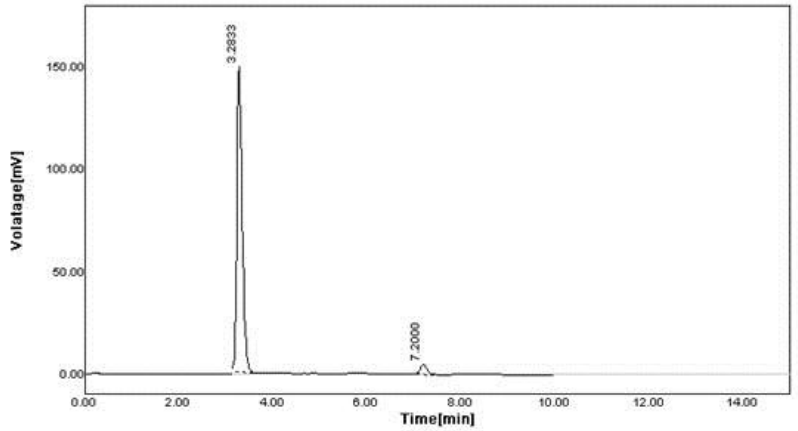


Figure 25. Chromatogram of Robustness Changing Flow rate 0.8 ml.

Table 25. Changing Flow rate 0.6 ml.

Metformin	**Flow rate 0.6 ml**	**Miglitol**	**Flow rate 0.6 ml**
Sample no.	**Roubstness**	**Sample no.**	**Roubstness**
1	1540.21	1	59.39
2	1538.11	2	61.06
Average	1539.16	Average	60.23
SD	1.48	SD	1.18
%RSD	0.10	%RSD	1.96

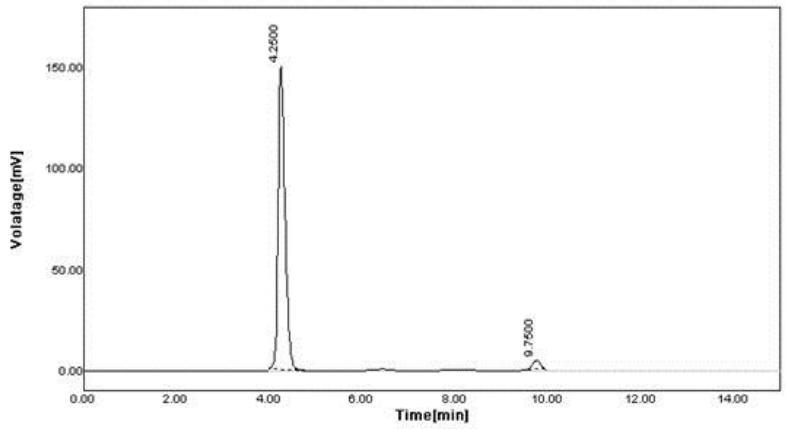


Figure 26. Chromatogram of Robustness Changing Flow rate 0.6 ml.

Changing Wavelength by ±1nm.

Table 26. Changing wavelength by +237 nm.

Metformin	**Wavelength 237nm**	**Miglitol**	**Wavelength 237nm**
Sample no.	**Roubstness**	**Sample no.**	**Roubstness**
1	1312.77	1	51
2	1310.3	2	52.07
Average	1311.5	Average	51.5
SD	1.75	SD	0.76
%RSD	0.13	%RSD	1.47

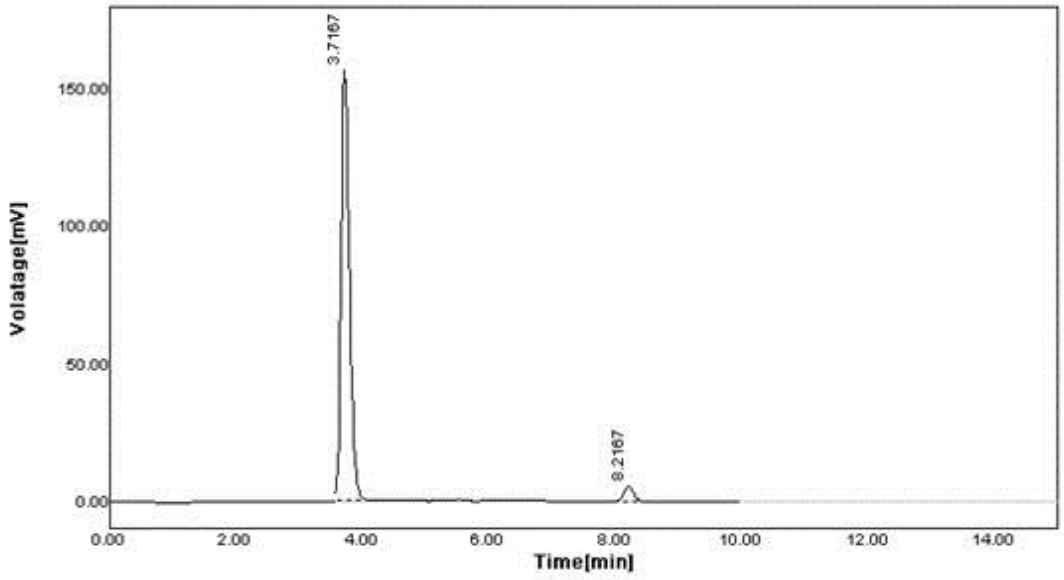


Figure 27. Chromatogram of Robustness Changing wavelength by +237 nm.

Table 27. Changing wavelength by - 235 nm.

Metformin	Wavelength 235 nm	Miglitol	Wavelength 235 nm
Sample no.	**Roubstness**	**Sample no.**	**Roubstness**
1	1364.92	1	51.99
2	1397.32	2	53.01
Average	1381.12	Average	52.50
SD	22.91	SD	0.72
%RSD	1.66	%RSD	1.37

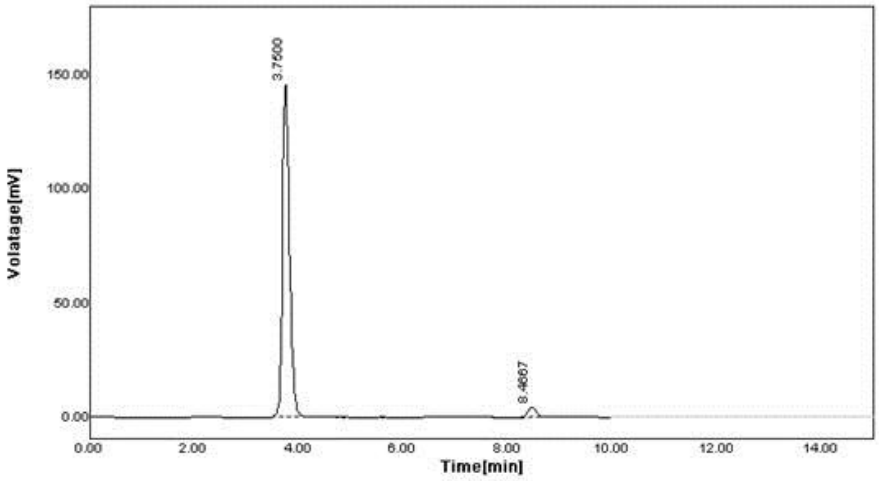


Figure 28. Chromatogram of robustness changing wavelength by - 235 nm.

STABILITY

Acid Degradation

Table 28. Data Showing Stability For Acid Degradation (0.1 N HCL) (After 1 Hr)

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	DEG 1	3.1000	1816.6869	35.56	2997.5	1.2727	0.0000
2	MET	3.7333	3031.1052	59.33	1932.2	0.9231	1.9000
3	DEG 2	4.5167	88.0248	1.72	3365.7	1.1250	2.0435
4	DEG 3	5.4667	31.5897	0.62	2330.4	0.9063	2.1111
5	MIG	8.3333	141.5015	2.77	9627.1	1.0294	6.1429
Sum			5108.9082				

DEG 1= Degradation, MET= Metformin, DEG 2= Degradation, DEG 3= Degradation, MIG= Miglitol.

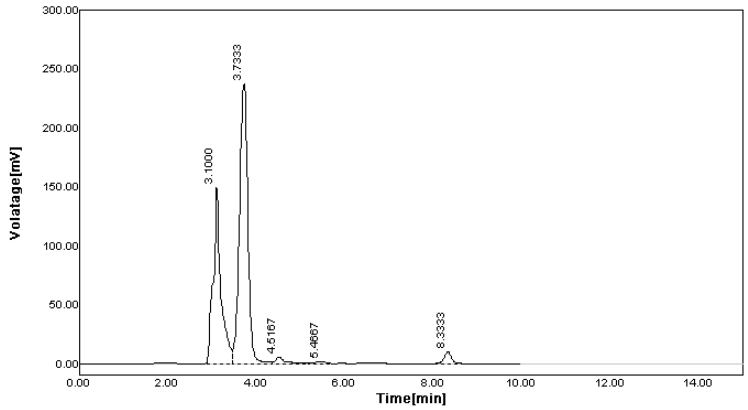


Figure 29. Chromatogram of stability for Acid Degradation (0.1 N Hcl) (After 1 Hr)

Base Degradation

For 0.1N NaoH

Table 29. Data showing stability for base degradation (0.1 N NaoH) (After 1 Hr)

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	DEG 1	3.1833	49.3753	1.26	5619.3	0.9000	0.0000
2	DEG 2	3.3167	15.7109	0.40	8783.8	1.1667	0.7273
3	MET	3.8333	3728.2952	95.40	1303.7	0.7895	1.5500
4	MIG	8.3333	114.7569	2.94	9627.1	1.0000	10.0000
Sum			3908.1382				

DEG 1= Degradation, DEG 2= Degradation, MET= Metformin, MIG= Miglitol.

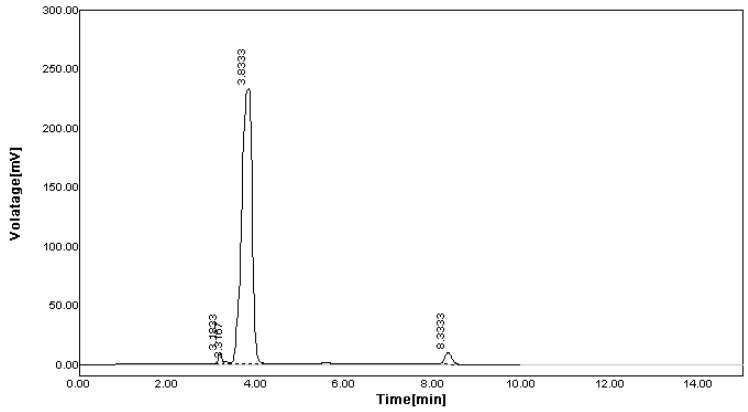


Figure 30. Chromatogram of stability for Base Degradation of 0.1N NaoH (After 1 Hr)

Oxidative/ Peroxide Degradation:

Table 30. Data Showing Stability For 3% H2O2 (After 1 Hr)

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	DEG 1	3.0833	30.8480	0.49	1897.8	1.0000	0.0000
2	MET	3.9000	6026.0122	96.68	841.1	0.6852	1.6897
3	DEG 2	5.7167	26.7433	0.43	1479.3	0.8889	2.7250
4	MIG	8.7500	149.5588	2.40	10613.9	1.0833	5.5152
Sum			6233.1621				

DEG 1= Degradation, MET= Metformin, DEG 2= Degradation, MIG= Miglitol.

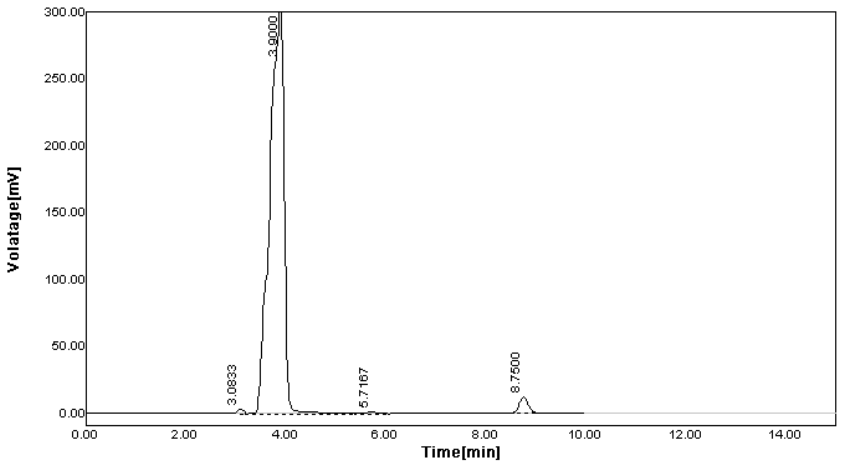


Figure 31. Chromatogram of stability for of 3% H_2O_2 (After 1Hr)

Neutral Degradation After 1 Hr min.

Table 31. Data showing stability for H_2O (After 1 Hr)

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	DEG 1	3.1167	52.9534	1.63	1346.6	1.1000	0.0000
2	MET	3.8333	3049.0105	94.01	1496.6	0.9333	1.6538
3	DEG 2	5.7167	21.0371	0.65	4530.5	0.8947	4.3462
4	MIG	8.7167	120.2059	3.71	10533.1	1.0000	7.5000
Sum			3243.2068				

DEG 1= Degradation, MET= Metformin, DEG 2= Degradation, MIG= Miglitol.

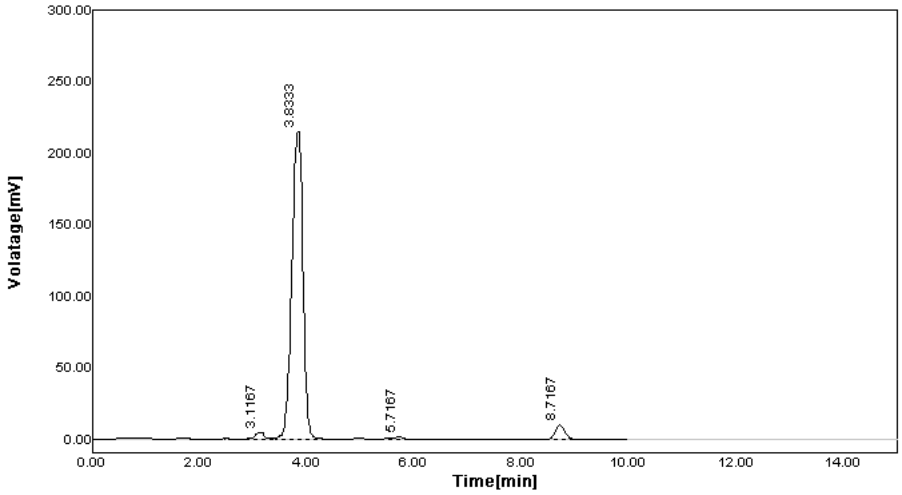


Figure 32. Chromatogram of Stability For Neutral Degradation of H_2O (After 1Hr.)

For 1N NaOH

Table 32. Data Showing Stability For Base Degradation (1 N NaOH) (After 1Hr)

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	DEG 1	3.2000	67.8441	1.38	3194.0	0.8571	0.0000
2	MET	3.7333	3543.6804	71.94	2782.4	0.6400	1.7778
3	DEG 2	3.8833	1113.6755	22.61	2488.0	5.5000	0.4286
4	DEG 3	4.3333	71.8982	1.46	81.1	5.2143	0.3418
5	MIG	8.3500	128.7018	2.61	9665.6	0.9000	3.0125
Sum			4925.8003				

DEG 1= Degradation, MET= Metformin, DEG 2= Degradation, DEG 3= Degradation, MIG= Miglitol.

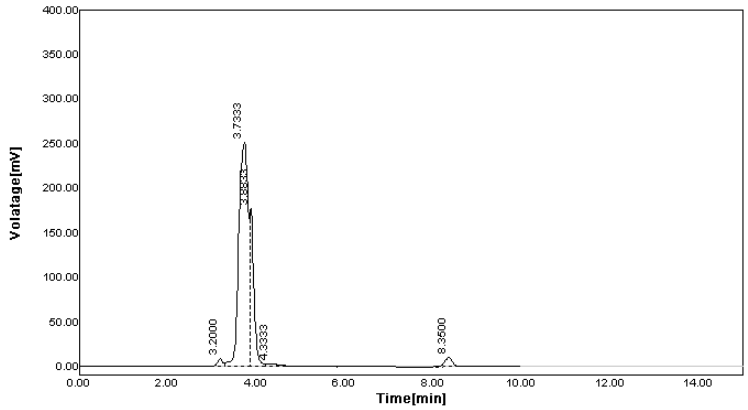


Figure 33. Chromatogram of stability for Base Degradation of 1N NaOH (After 1 Hr)

For 5% H_2O_2

Table 33. Data showing Stability For 5% H_2O_2 (After 1 Hr)

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	DEG 1	3.0167	44.5053	0.88	1816.7	0.5690	0.0000
2	MET	3.6500	4863.0430	96.40	1846.9	0.8250	1.7273
3	DEG 2	5.5167	21.7153	0.43	7500.5	0.8824	5.3333
4	MIG	8.4500	115.1934	2.28	11780.1	1.0000	8.8000
Sum			5044.4570				

DEG 1= Degradation, MET= Metformin, DEG 2= Degradation, MIG= Miglitol.

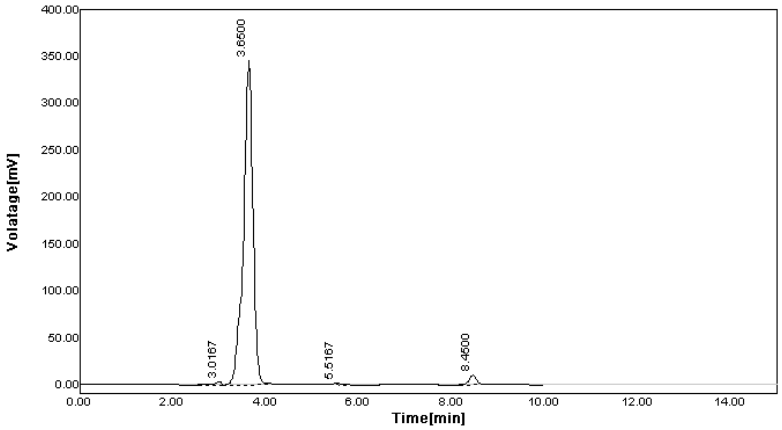


Figure 34. Chromatogram of Stability For of 5% H_2O_2 (After 1 Hr)

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