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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN AND MIGLITOL IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: The aim of the present study was the development and validation of a simple, precise, rapid, accurate and specific RP-HPLC method in which the peaks will be appeared with high resolution and short period of retention time as per ICH Guidelines.

Methods: Simple, sensitive, specific, accurate reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of Metformin and Miglitol in bulk drug and in combined dosage forms. RP-HPLC separation was achieved on a kromasilC_{18} (4.6 x 150mm, 5 µ) under an Isocratic Mode. The mobile phase composed of Phosphate Buffer (60%) (whose pH was adjusted to 5.3 by using potassium hydroxide) & Methanol (40%) [HPLC Grade]. The flow rate was monitored at 0.9 ml per min. The wavelength selected for the detection was 238 nm.

Results: The retention times of Metformin and Miglitol were found to be 3.86min and 7.56min respectively. Linearity was established for Metformin and Miglitol in the range of 200-500µg/ml and 20-50µg/ml, respectively. The percentage recoveries for Metformin and Miglitol were found to be in the range of 98.12-101.53% and 98.06-101.8% respectively. This method can be successfully employed for simultaneous quantitative analysis of Metformin and Miglitol in bulk drugs and formulations.

Conclusion: A new, simple, accurate, precise, linear and rapid RP-HPLC method was developed and validated for the simultaneous estimation of Metformin and Miglitol in bulk drugs and formulations as per ICH guidelines. Hence the method can be used for the routine and stability analysis in various pharmaceutical industries in bulk drugs and formulations.

Keywords: RP-HPLC, Metformin, Miglitol.

INTRODUCTION

Metformin [**figure1**] is an oral antidiabetic drug in the biguanide class. It is most widely prescribed antidiabetic drug in the world used to treat type 2 diabetes. Metformin helps to control the amount of glucose (sugar) in blood. It decreases the amount of glucose and also increases body's response to insulin, a natural substance that controls the amount of glucose in the blood. It is not used to treat type 1 diabetes[1]. It is also used for treatment of gestational diabetes, polycystic ovary syndrome (PCOS)[1]. It works by decreasing hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis). It helps to reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain. Metformin comes as a liquid, as a tablet, and as an extended-release (long-acting) tablet taken orally. It is used alone or with other medications . Very rare but serious side effect with Metformin is lactic acidosis. Other than that common side effect are gastrointestinal irritations, including diarrhea, cramps, nausea, vomiting and increased flatulence. Literature survey revealed

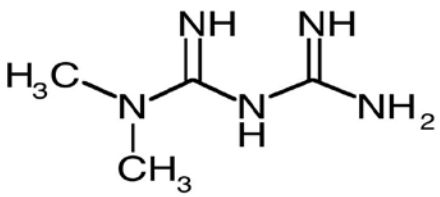

Fig. 1: Structure of Metformin

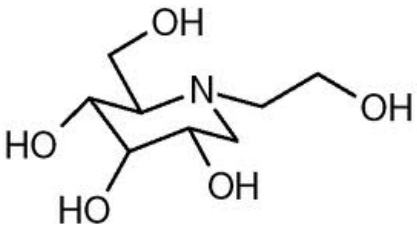

Fig. 2: Structure of Miglitol

The HPLC methods for estimation of metformin in Bulk, human plasma and pharmaceutical dosage forms [2–7]. LC-MS-MS method was reported for the determination of Metformin in human plasma [8]. Literature survey reveals several Analytical and Bioanalytical methods for the analysis of Metformin. These methods reported with Metformin alone or in combination with other drug. These include, HPLC [9-11] and spectrophotometric analysis of Metformin in tablets [12-14].

Miglitol [**figure2**] belongs to a class of drug called alpha-glucosidase inhibitors used to control blood glucose (sugar) levels in type 2 diabetes (non-insulin-dependent diabetes). It is approved by FDA in December 1996. Miglitol inhibits glycoside hydrolase enzymes called alpha-glucosidases thereby slowing the appearance of sugar in the blood after meal. It works by slowing down the absorption of carbohydrates from diet, so that blood sugar does not rise as much after meal. Alpha-glucosidase inhibitors are used to help control blood sugar levels that are not controlled by diet and exercise alone. It is believed that strict control of blood sugar in people with diabetes decreases the risk of eye, kidney and nerve damage.

Controlling high blood sugar helps to decreases the risk of eye, kidney, nerve damage, loss of limbs and sexual function problems. Recent study on rats by shrivastva et al showed that Miglitol has antioxidant effect and hypocholesterolemic effect.[15] It is used alone or in combination with a sulfonylurea such as glyburide (Diabeta). It is an oral administrative drug available in form of tablet. The most common side effects of miglitol are Gastrointestinal symptoms such as abdominal pain, diarrhea, flatulence and skin rash. Rare but possible side effects include low serum iron. Literature survey revealed that several Analytical and Bioanalytical methods for its estimation using Reversed Phase-High Performance Liquid Chromatography [RP-HPLC] with UV detection, HPLC - electrospray tandem mass spectrometry, LC-MS, liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry and RP-HPLC method[16-20]. The developed method has various advantages over the above mentioned methods, as it is simple, economical, faster, precise, accurate and specific for quantitative determination of Miglitol in pharmaceutical dosage form. As per our detailed literature survey as on date, there are very few reports [21] using UV & RP-HPLC for the simultaneous quantitative estimation of Metformin and Migllitol in Bulk & Pharmaceutical dosage forms. We here in reported a new, simple, sensitive, precise, accurate, linear and isocratic RP-HPLC method for the simultaneous quantitative estimation of Metformin and Migllitol in bulk & Formulation as per ICH Guilences [22]

MATERIALS AND METHODS

Chemicals and reagents

Metformin and Miglitol standard was obtained from reputed companies, formulation tablets were purchased from local pharmacy. HPLC grade Methanol, Water and Acetonitrile were purchased from Merck specialties Pvt. limited, Mumbai. 0.45µm nylon membrane filter papers were obtained from Pall Life Sciences, Mumbai. A combined dosage tablet MIGNAR MF was purchased from local market.

Instruments

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump, Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Chromatographic conditions

Separation of the drugs was achieved on a reverse phase C18 column, kromosil C18 (250 X4.6mm; 5µ). The mobile phase consists of a mixture methanol and potassium dihydrogen orthophosphate buffer (0.01M, pH adjusted to 5.3 using potassium hydroxide) in ratio of 40:60, v/v. The mobile phase was set at a flow rate of 0.9 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 238 nm.

Mobile phase Preparation

The mobile phase was prepared by mixing Methanol and buffer in the ratio of 40:60, v/v and later sonicated for 10 minutes for the removal of air bubbles.

Buffer Preparation

The buffer solution was prepared by weighing 1.368g of potassium dihydrogen orthophosphate (KH_2PO_4) and transferring to 1000 ml of HPLC grade water to get 0.01M buffer strength, which was adjusted to pH 5.3 using 30% w/v of potassium hydroxide. Later the buffer was filtered through 0.45 µm nylon membrane filter.

Preparation of stock and working standard solution

10mg of standard drug weighed accurately into 10ml volumetric flask diluted upto mark with diluent. Standard stock solutions of concentration 1000µg/ml of Metformin and 1000µg/ml of Miglitol were prepared separately using methanol. The stock solution was stored at 2-8 °C and protected from light.

Preparation of Sample Solution

MIGNAR MF Tablet containing 500mg of Metformin and 50 mg of Miglitol. Twenty tablets were weighed separately and their average weight was determined. The sample solution prepared by powder weighed equivalent to 50mg of Metformin and transferred in to 50ml clean and dried volumetric flask and add about 25ml diluent and sonicated for 15min. Then volume was made up to 50 ml with diluent and filtered through 0.45mm nylon membrane filter paper. The final sample solution was diluted properly to prepare a concentration of 350µg /ml of Metformin and 35µg/ml of Miglitol.

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 Metformin at 3.86min, Miglitol at 7.56min . Figures 3 and 4 represent chromatograms of blank solution and mixture of working standard solutions respectively. The total run time is 12 minutes with all system suitability parameters meeting acceptable criteria for the mixture of standard solutions.

System Suitability

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 mixture of standards at working concentration. The results were given in Table 1 within acceptable limits. In order to test the applicability of this developed method to a commercial formulation, 'MIGNAR MF ' was chromatographed at a concentration equivalent to working standards concentration and it is shown in Figure 6. The sample peaks were identified by comparing the relative retention times with the standard drugs mixture (Figure 4). System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and each drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible quantification of the two drugs with error less than 10%, which is the standard level in any pharmaceutical quality control.

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. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [20] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD) and limit of quantitiation (LOQ)

Table 1: System suitability studies results

Drug	Retention time (min)	Resolution	USP Plate Count	USP Tailing
Metformin	3.86	11.18	2047.63	1.35
Miglitol	7.56		8905.47	1.21

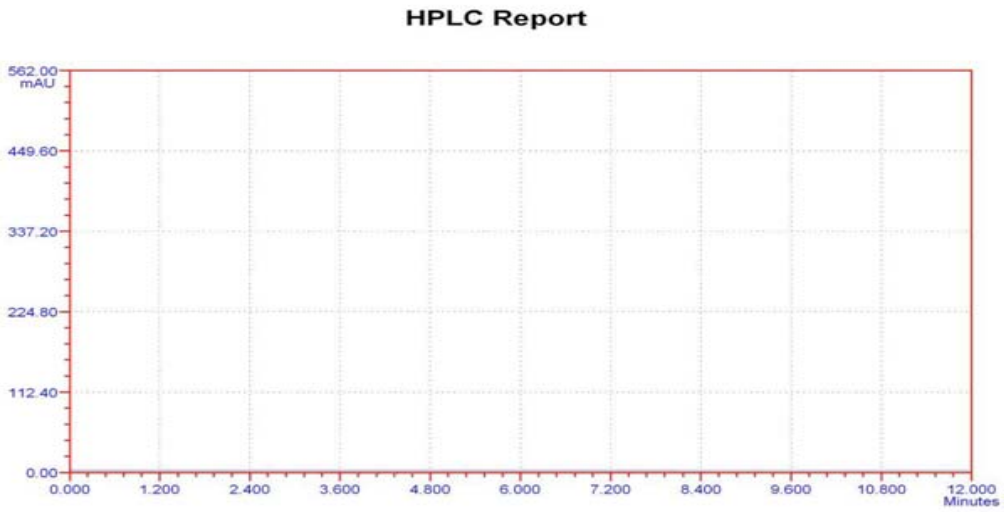

Fig. 3: Typical Chromatogram of Blank solution

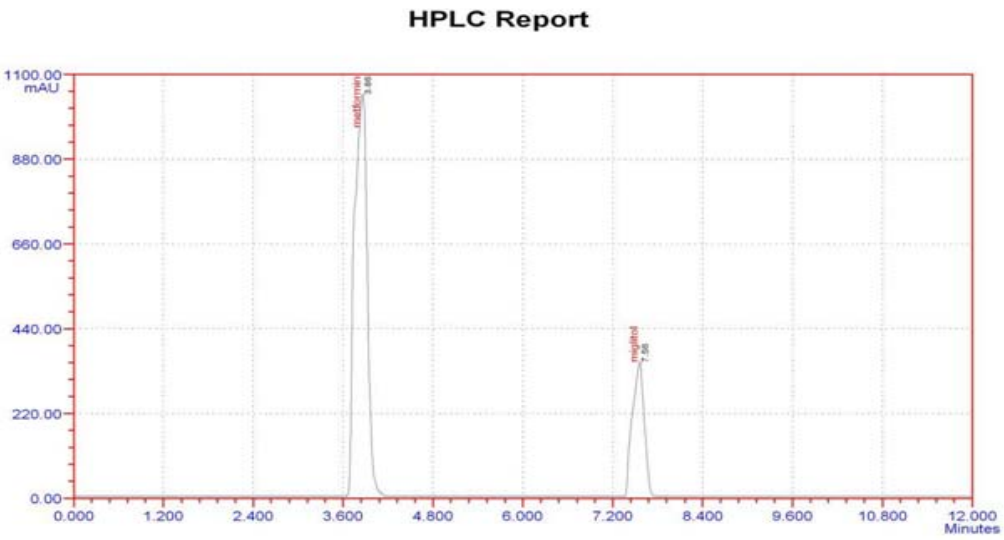

Fig. 4: Standard chromatogram of Metformin and Miglitol

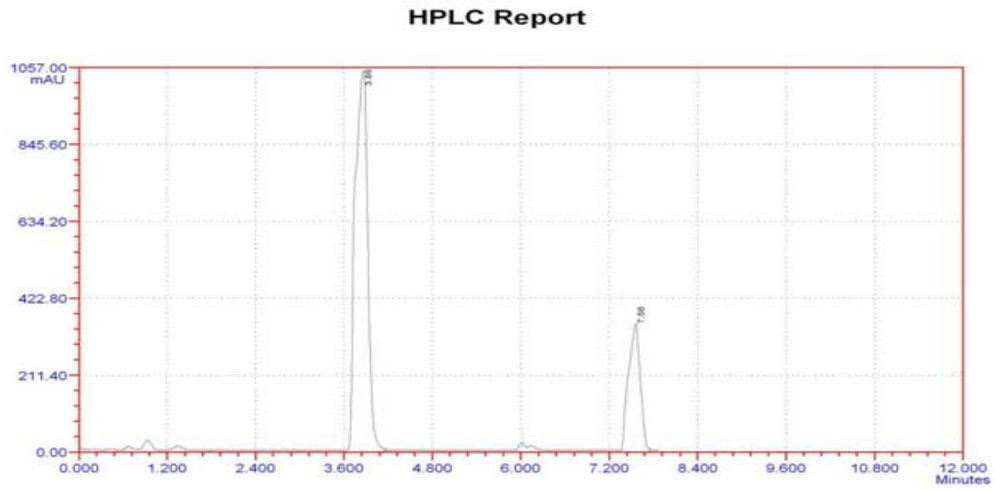

Fig. 5: Typical chromatogram for the sample (tablet).

Specificity

Figures 3-5 of blank, mixture of standard drug solution and sample chromatogram reveal that the peaks generated in mixture of standard solution and sample solution at working concentrations are only because of the drugs as blank has no peaks at the retention times Metformin, and Miglitol . Hence the method developed is said to be specific.

Precision

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits. [Table 2]

Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits. (Table 3).

Table 2: Precision result for metformin and migliton

S.NO	Metformin (area)	Miglitol (area)
1	1271782	401202
2	1274409	392181
3	1271736	405955
4	1253883	396136
5	1271272	405985
6	1258271	392166
RSD	0.67	1.60

Table 3: Intermediate precision /Ruggedness result for metformin and migliton

S.No	Metformin	Miglitol	Metformin	Miglitol
	Intraday precision		**Interday precision**	
1	1246671	422272	1263128	408605
2	1265362	416426	1264069	419058
3	1274087	409154	1257582	417750
4	1251158	421129	1264484	405698
5	1271272	415950	1265198	407533
6	1273745	407132	1250284	417847
RSD	0.94	1.47	0.46	1.47

Table 4: Accuracy results of Metformin

S.NO	%Recovery	Concentration in µg/ml			Amount	%	RSD
		Target	Spiked	Total	Found	recovery	
1	50%	200	100	300	294.55	98.19	
2		200	100	300	298.42	99.47	0.65
3		200	100	300	296.65	98.88	
4	100%	200	200	400	399.83	99.95	
5		200	200	400	394.96	98.74	0.78
6		200	200	400	394.08	98.52	
7	150%	200	300	500	507.67	101.53	
8		200	300	500	490.63	98.12	1.71
9		200	300	500	497.87	99.57	

Table 5: Accuracy results of Miglitol

S.NO	%Recovery	Concentration in µg/ml			Amount	%	RSD
		Target	Spiked	Total	Found	recovery	
1	50%	20	10	30	29.64	98.81	
2		20	10	30	30.55	101.85	1.59
3		20	10	30	30.35	101.18	
4	100%	20	20	40	39.22	98.06	
5		20	20	40	40.55	101.38	1.73
6		20	20	40	39.55	98.87	
7	150%	20	30	50	49.45	98.91	
8		20	30	50	49.40	98.80	0.21
9		20	30	50	49.25	98.50	

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The standard solution with Accuracy 50%, Accuracy 100% and Accuracy 150% were injected into chromatographic system and calculated the amount found and amount added for Metformin and Miglitol and further calculated the individual recovery values. (Table 4 & 5).

Linearity

It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope or regression line. It is determined by series of three to six injections of five of more standards. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The calibration curve was represented in fig. 6 & 7. (Table 6 & 7).

Table 6: Results for linearity of Metformin

S.No	Concentration(µg/ml)	Peak area
1	200	353685
2	250	649899
3	300	986165
4	350	1266430
5	400	1589142
6	450	1882921
7	500	2111201

Table 7: Results for linearity of Miglitol:

S.No	Concentration(µg/ml)	Peak area
1	20	31013
2	25	145333
3	30	281478
4	35	400719
5	40	516167
6	45	642131
7	50	788939

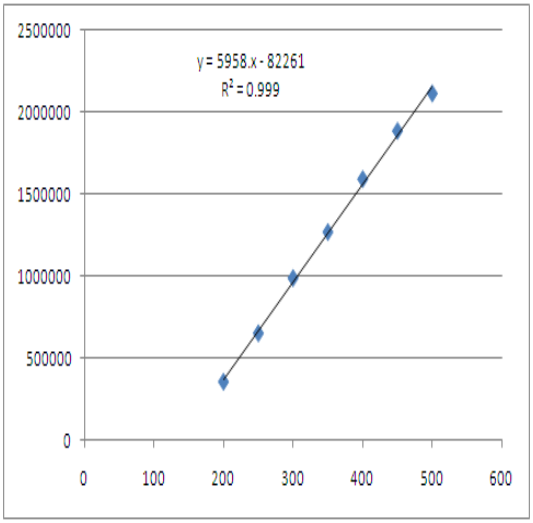

Fig. 6: Linearity curve for the drug Metformin

Detection and quantification limit (LOD &LOQ)

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected. It may be expressed as a concentration that gives a signal to noise ratio of approximately 3:1. While the Quantification limit or LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy with a signal to noise ratio of approximately 10:1 can be taken as LOQ of the analyte. Our method showed the (LOD) for Metforman and Miglitol were found to be 30ng/ml and 100ng/ml respectively and the LOQ values for Metforman and Miglitol were found to be 0.25µg/ml and 0.82 µg/ml respectively

Robustness

The robustness of assay method was studied by incorporating small but deliberate changes in the analytical method (variations in flow rate, column temperature, mobile phase composition, pH of buffer,) and also by observing the stability of the drugs for 24 hours at room temperature in the dilution solvent. In all the varied chromatographic conditions, there was no significant change in chromatographic parameters. Result were given in Table 8 & 9.

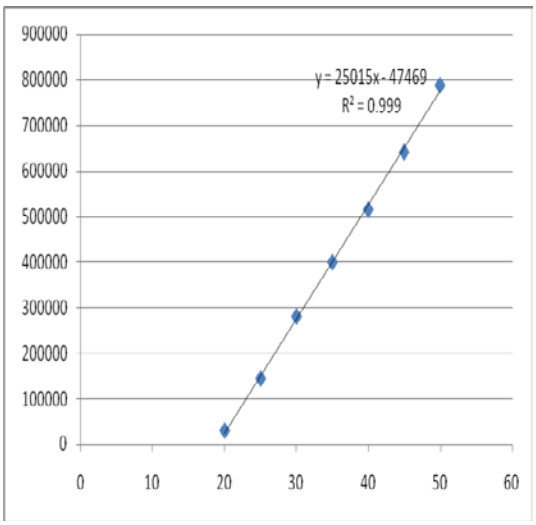

Fig. 7: Linearity curve for the drug Miglitol

Formulation

The prepared concentration of the tablet solution was injected into the HPLC. The resulting peak areas were compared with the standard peak areas and the assay was calculated for the method. % assay was found to be 99.51% for Metformin and 99.80 % for Miglitol. High % assay that was more than 99.5% was found for the both drugs. Hence the method can successfully apply for the simultaneous estimation of Metformin and Miglitol in pharmaceutical formulation. Results of the formulation analysis were shown in Table 10.

Table 8: Results for robustness of the Metformin and Miglitol.

S.No.	Condition	Change	Metformin		Miglitol	
			Area	% Change	Area	% Change
1	Standard	……..	1266430	……..	400719	…….
2	MP 1	45:55(v/v)	1281012	1.15	408003	1.81
3	MP 2	35:65(v/v)	1250273	1.28	394823	1.47
4	WL 1	240nm	1282037	1.23	406449	1.43
5	WL 2	236nm	1280197	1.08	398855	0.46
6	pH 1	5.2	1274709	0.65	407932	1.80
7	pH 2	5.4	1255694	0.84	406811	1.52

Table 9: Results for stability

Time in hrs	Area		% Assay	
	Metformin	for Miglitol	Metformin	Miglitol
0	1266430	400718	100	100
1	384333	398745	99.60	99.50
2	389250	399548	99.92	99.70
4	386839	398547	99.77	99.45
6	385058	395325	99.78	98.65
12	385657	397145	98.83	99.10
18	389880	396231	98.70	98.90
24	367220	394988	98.62	98.56
26	367220	389547	97.61	97.21
28	356541	385478	95.97	96.20

Table 10: Result for formulation of Metformin and Miglitol

S.No.	Drug	Brand	Dosage	Amount Prepared	Amount Found	%Assay
1	Metformin	MIGNAR MF	500mg	350	348.317	99.51
2	Miglitol		50mg	35	34.930	99.80

CONCLUSION

A reverse phase HPLC isocratic method developed and has been validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, robustness, limit of detection and limit of quantitiation for the simultaneous quantitative estimation of Metformin and Miglitol. The correlation coefficients were greater than 0.999 for both the drugs. The inter day and intraday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries after spiking experiments were between 98 and 101%, an indicative of accurate method. Accordingly it can be concluded that the developed reverse phase HPLC method is accurate, precise, linear and robust and therefore the method can be used for the routine analysis of Metformin and Miglitol in tablets. Based on this evidence the method can be stated as highly economical and it is recommended for routine use in quality control laboratories and stability studies.

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