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QUANTITATIVE ANALYSIS OF MIRABEGRON ER TABLETS: A COMPREHENSIVE RP-HPLC METHOD AND VALIDATION FOR THE IMPURITY DETERMINATION

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ABSTRACT

This research aimed to design as well as evaluate reverse-phase high-performance liquid chromatography (RP-HPLC) process to precise quantification for potential impurities in Mirabegron (MIRA) ER Tablets. The developed method utilized an Inertsil C8 - 3, 150 x 4.6 mm, 3µm column with a gradient elution program utilizing Buffer and Acetonitrile as mobile phases. The chromatographic separation achieved on a C8 stationary phase demonstrated robustness and reproducibility, with detection at a wavelength of 240 nm and a constant column temperature of 40°C. The research study was focused on MIRA and four impurities (MIR-1, Deshydroxy, Diamide-1, and Diamide-2). The method exhibited specificity, precision, linearity, accuracy, robustness, ruggedness, and stability indicating characteristics. Correlation coefficients exceeding 0.997 for MIRA and its foreign substances showcased the accuracy and sensitivity of the method. Low detection and quantization limits (ranging from 0.06ppm to 0.20ppm) highlighted its high sensitivity. Accuracy is finalized by rates of recovery as 96.1% to 100.3% for all foreign substances. The stability-indicating is validated by forced degradation studies, meeting peak purity circumstances also achieving mass balance. Robustness studies further established the method's reliability, demonstrating resilience to minor chromatographic variations. This proposed RP-HPLC method accommodates a dependable, reproducible, highly accurate, and more sensitive means to quantifying MIRA-related substances in MIRA ER Tablets, making it a valuable tool for pharmaceutical quality control.

Keywords: RP-HPLC, MIRA - ER Tablets, 0.06ppm to 0.20ppm, Reproducible, Recovery.

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INTRODUCTION

Overactive bladder (OAB) is common along with complex other symptoms, and may substantially influence the quality of life of patients.[1] Treatment of an overactive bladder gives a symptomatic relief and externally affects the emptied phase of the micturition cycle.[2] This is highly activated BAT in adults and rodents by β3-adrenoreceptor-mediated sympathetic activation.[3] MIRA is chemically 2-(2-amino-1,3-thiazol-4-yl)-N- [4-(2-{[(2R)2hydroxy-2-phenylethyl]amino}ethyl) phenyl] acetamide. MIRA is solid, white in color, hygroscopic in nature, and odorless but has a very bitter taste with a melting point of 138°C - 145°C [4.] This present work targeted MBN oral dosage form tablets strengths as 50 and 25 mg.[5] Naresh Konduru *et al,*[5] developed and validated in products of formulation, and validation was not performed for foreign substances. J. Patel et.al.,[6] developed a formulation containing MB and SFS. These are approved by the management of overactive bladder. These authors reported one analytical method to the simultaneous determination of these analytes. Spandana *et al*.,[7] used C18 G (250 x4.6 mm x 5 µm). Wavelength is identified at 246 nm. The authors do not report the separation of impurities. Sankar *et.al*.,[8] studied for assay of Mirabegron ER tablet formulation with column Eclipse XDB C18 (250 x

4.6mm, 5µm); at a wavelength of 251 nm, but the study did not evaluate foreign substances separation and quantification. Suryawanshi *et.al*.,[9] developed, and validated MIRA by Eclipse XDB C18 (250 x 4.6mm x 5µm). 243nm is the wavelength, but not discussed about the separation of foreign substances. Yehia *et al.*,[10] studied MIRA in the presence of degradation product, and separation is achieved by Agilent C18 (250x 4.6 mm x 5µm) at a wavelength of 250 nm, but not evaluated for separation of foreign substances and specificity not proved for foreign substances. B Mounika *et al.,*[11] estimated MIRA in tablets by column Waters Acquity HSS T-3 C18 (100 × 2.1mm, 1.7µm) at a wavelength of 243 nm. but not discussed for the separation of foreign substances. Jyothsna M *et.al.,*[12] used THERMO, C18, 250X4.6mm, 5µm at a wavelength of 248nm. This method discussed the estimation of MIRA only. Few other authors have[13] developed and validated methods using UV spectroscopy. S. Mishra et.al.,[14] used Shimpack Solar C18 column (250 × 4.5 mm, 5 µm). For this analysis authors are used acetonitrile as 90v/v and 5 mM ammonium acetate as 10% v/v . The rate of flow is identified at 1.20mL/min. B. Annapurna et.al.[15] used Acquity BEH C18 (50*3.0mm. 1.7m) for the separation. The mobile phase used is in the proportion of 70v/v for Potassium dihydrogen phosphate and 30v/v for Methanol. 254nma is wavelength detection. The observed linearity range is 50g/ml to 150g/ml and r2 value is 0.997. B.K.Suresh et.al.,[16] proposed this drug at a wavelength range of 249 nm. They use the solvent as 1N Hcl. 3 µg/ml to 15µg/ml is the linearity range. The correlation coefficient is 0.999. Rv. Teijlingen et.al.,[17] performed this chromatographic separation with the help of Phenomenex Synergi Fusion-RP C18 analytical column. Wavelength detection is done by a triple-quad mass spectrometer with his associated equipped by a Heated Electrospray Ionization interface. Eman Bahgat, H et.al.[18] got the ultra-sensitive results by a linearity range as (0.55 ng ml−1 to 100.20 ng ml^{-1} and a low LOD is 0.51 ng ml−1 and LOQ is 1.54 ng ml−1. pH is adjusted at 6.0 by using rate of scan at 100 mV s^{-1}. Validated[19-20] UV spectroscopy and LC-MS in bulk, tablet, and formulation is proposed by different authors. B. Hatwar et.al.,[21] used the microemulsion stability as well as haemolytic activity are done latter dilution in 5% dextrose solution for injection to 1 mg/mL ceftazidime. *In vitro* studies for haemolysis revealed that Ceftazidime microemulsions are well tolerated by erythrocytes. The foreign substances identified are represented in Fig.-1 for MIRA and foreign substances.

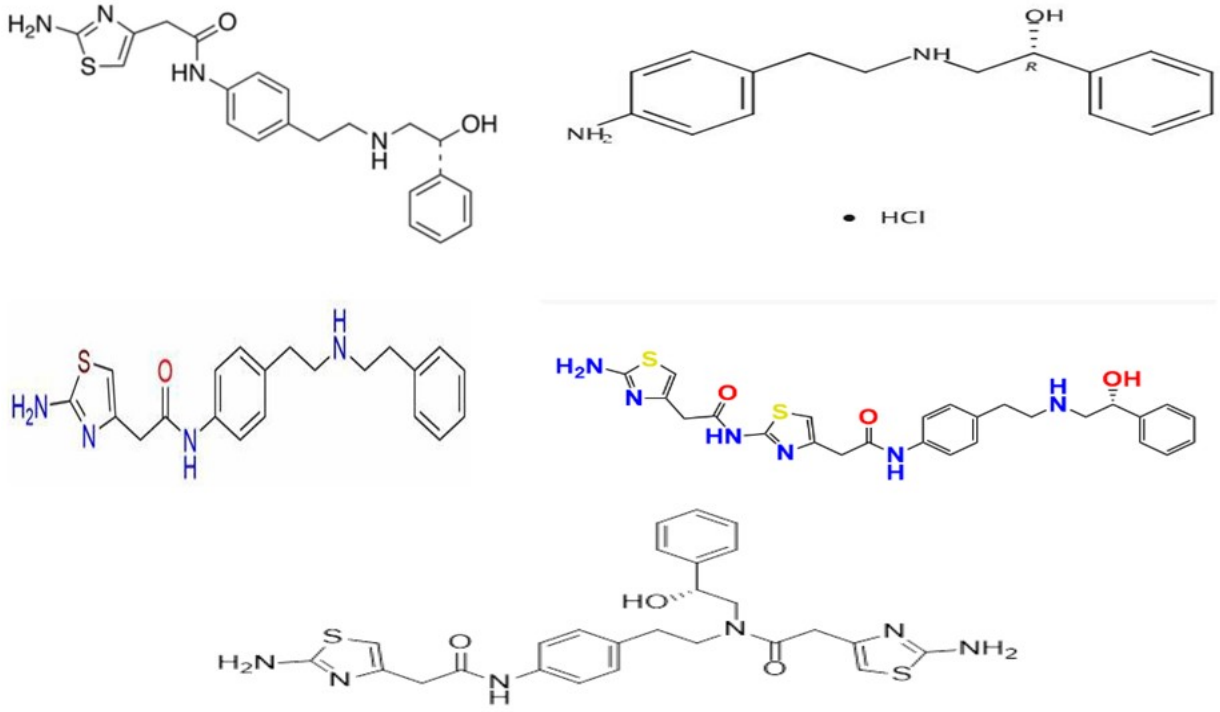

Fig.-1: MIRA, MIR1 Impurity, Deshydroxy Impurity, Diamide-2 Impurity, Diamide-1 Impurity

EXPERIMENTAL

Chemicals and Reagents

Chemicals such as Glacial Acetic acid, Ammonium Acetate, NaOH, HCL, and H_2O_2 are used in this present research work. These were procured by Merck, India. Acetonitrile as well as methanol are procured by Jt Baker, India for the preparation of diluent as well as mobile phase. Reference, standards of MIRA and all foreign substances are provided by Dr Reddy's Laboratory, Hyderabad, India, to conduct experiments required for specificity. The research is performed by utilizing Milli-Qwater which is prepared in-house.

Instruments and Software

The samples, chemicals as well as standards were subjected to a weighing process with the help of MAS225S-100-DA model sartorius semi-microbalance. The MIRA foreign substances are weighed using an MSA2.75-000-DM model Sartorius microbalance. Mobile phase buffer pH 6.0 is prepared and also verified by utilizing a pH meter which is made with Lab India. The analysis is performed over a Waters HPLC model Alliance 2695 by 2,998 PDA detectors as well as 2,489 UV detectors. Changes in the used instrument are done with the help of Waters Arc™ HPLC model with 2,998 PDA detectors as well as 2,489 UV detectors. A hot air oven is utilized for forced degradation study which is designed by Thermo lab. The drug photostability chamber Newtronic is utilized to photo preparation stress samples. The chromatography process was developed by an Inertsil C8 - 3, 150 x 4.6 mm, 3µm, this is procured from GL Sciences.

Analytical Solutions Preparation

Prepared diluent solution by taking water at 60v/v and acetonitrile at 40v/v are used. For this analysis, we prepared standard preparation as 1.5µg/ml, Related substance 25 mg and 50 mg (50µg/ml), foreign substances stock solutions preparation, prepared related substance 25 mg and 50 mg (50µg/ml), and Placebo solutions. Weighed and transferred placebo powder 310mg o into a dried 100ml standard. Then 70.00mL of methanol sonicate was for 30 minutes with intermediate shaking. Allowed the flasks on the bench top for not less than 10 minutes and diluted to the required volume using methanol. Centrifuged the quantity at 3500RPM in a time interval of 10 minutes. 5.00mL of above was centrifuged into a 10.00mL standard flask and diluted to volume with diluent and mixed well.

Chromatography Conditions

Mobile phase buffer 6.0pH was designed by combining 3.8000g of ammonium acetate in 1000.00mL of triple distilled water. pH is adjusted to 6.0 by diluted glacial acetic acid. Mobile phase A designed by mixing Buffer which pH is maintained at 6.0 as 90v/v and Acetonitrile as 10v/v. The mobile phase B is prepared by taking buffer as 20v/v and Acetonitrile as 80v/v. 240nm wavelength and connected with column Inertsil C8- 3, 150 x 4.6 mm, 3µm. 40°C is the column oven temperature. The rate of flow is concluded at 1.00mL/min. The prepared analytical solution was injected about 10µL with a run time of 70 minutes gradient program. The gradient program is represented in Table-1.

Table-1: Gradient Program

Time in minutes	Mobile phase-A as %	Mobile phase-B as %
0	95	5
15	85	15
40	60	40
50	50	50
51	10	90
60	10	90
61	95	5
70	95	5

RESULTS AND DISCUSSION

Validation of analytical process was done by considering the International Conference on Harmonization Q2 R1 and US Pharmacopeia general chapter 1,225.

System Suitability

Prepared standard solutions with the help of MIRA working standards depends upon the procedure. After no of replicate injections into HPLC system suitability is checked and finally identified that they are within the acceptable limit. The values obtained are represented in Table-2.

Specificity

An investigation is conducted to establish placebo interference. The related substances method is accomplished over a placebo of MIRA ER 25mg and 50mg tablets weight of placebo contemporary in a portion of preparation to test as per standard test process. For the placebo, the chromatograms identified revealed that there are no peaks in the Rt of MIRA as well as impurities. Results are denoted in Table-3 and related chromatograms in Fig.-2.

Table-2: Results of System Suitability

System suitability parameters	Checked value
Tailing factor for MIRA for standard preparation	1.0
% RSD to total five replicate injections of standard	0.4

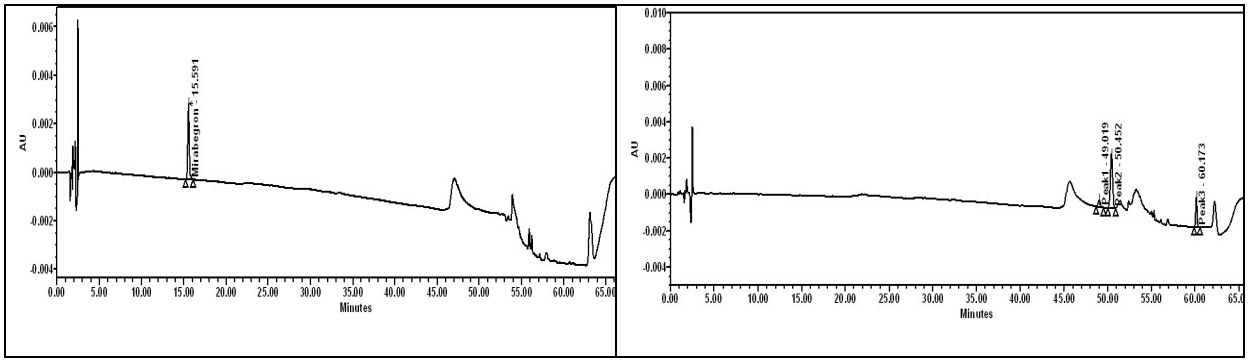

Fig.-2: MIRA Standard and Placebo Chromatogram

Table-3: Retention Time of MIRA and Impurities

S. No.	Impurity Name	Retention Time
1	MIR-1 Impurity	9.7
2	Deshydroxy Impurity	20.3
3	Diamide-2 Impurity	23.9
4	Diamide-1 Impurity	2.03
5	MIRA	30.7

Interference from Degradation Products

For this a study is performed to explain efficient estrangement of detestables by MIRA quantity for drug product as well as placebo was exposed for stress circumstances for inducing degradation. For this 5N HCl solution for about 1 hour at 60°C, 30 minutes at 60°C, 3% H_2O_2 for about 1 hour over a water bath, water in a time interval of 6 hours at 60°C, 5N NaOH solution in 30 minutes at 60°C, 3% Hydrogen peroxide (H_2O_2) for about 1hour, a UV light for 200 Watts hr/ square meter, 1.2 million lux in a time interval visible light, Dry heating is done in the vicinity of 80° C for in a time interval of 24 Hrs, to humidity at 90% RH at 25°C for 7 Days. Finally, total degradation peaks are resolved by chromatograms of MIRA peak in the total stressed samples. The chromatograms of stressed samples are done evaluation for purity in peak for MIRA with the help of software of water's empower networking. Mass balance was also calculated for all stress circumstances and concluded that they were within the limit. This reveals that there is no interference by the degradants in quantification MIRA present in MIRA ER Tablets. The results are tabulated in Table-4 and 5.

Table-4: Stress Study Results

S.No.	Conditions	%Net Degradation	% Mass Balance	PAG	PTH	Flag
1	As Such	NA	NA	0.072	0.296	No
2	Acid Stress: Stressed with 5N HCl solution for about 1 hour at 60°C in water bath	9.0474	97.9	0.093	0.357	No
3	Base Stress: Stressed with 5N NaOH solution for about 30 minutes at 60°C on a water bath	2.1438	95.8	0.117	0.326	No
4	Peroxide Stress: Stressed with 3% Hydrogen peroxide (H_2O_2) for about 1 hour at room temperature.	15.4265	100.7	0.257	0.345	No
5	Water Stress: Stressed with water for 6 hours at 60°C on a water bath	Nil	98.1	0.095	0.278	No
6	Thermal: Dry heating is done at 80° C for 24 Hrs.	0.0616	97.7	0.120	0.306	No
7	Photolytic degradation (200watt Hours/ Square meter in a time interval of 1.2 million lux hours	Nil	99.4	0.113	0.283	No
8	Humidity: 90% RH at 25°C in a time interval of 7 Days	0.0599	94.7	0.102	0.299	No

Table-5: Stress Study Results

Stress condition	% Impurity			
	MIR-1	Des-hydroxy	Diamide-2	Diamide-1
As Such	0.0150	0.0228	0.0693	0.0743
Stressed with 5N HCl solution for about 1 hour at 60°C in a water bath	9.1091	0.0309	0.0198	ND
Base Stress: Stressed with 5N NaOH solution for about 30 minutes at 60°C on a water bath	0.4256	0.0962	0.0658	ND
Stressed with 3% Hydrogen peroxide (H_2O_2) for about 1 hour at room temperature.	ND	0.3104	0.0711	12.2318
Stressed with water for 6 hours at 60°C in a water bath	ND	0.0468	0.0330	0.0542
Dry heating is done at 80° C for 24 Hrs.	ND	0.0704	0.0559	ND
Photolytic degradation (200-watt Hours/ Square meter and 1.2 million lux hours	ND	0.0370	0.0656	0.0805
90% RH at 25°C for 7 Days	ND	0.0560	0.0374	ND

Limit of Detection (LOD) and Limit of Quantification (LOQ)

In order to establish both LOD as well as LOQ for MIRA also their related substances are performed. LOD and LOQ are established depending on the signal-to-noise ratio process by spiking MIRA as well as its related compounds in a placebo individually by suitable precision as well as accuracy. Precision of MIRA along with foreign substances for LOQ is performed. A total of six test preparedness of MIRA ER tablets placebo, having MIRA, MIR-1, Deshydroxy, Diamide-2, and Diamide-1 foreign substances at the level of about LOQ are prepared after that injected into the system. % RSD to six replicate preparations are found within the limit. Accuracy of known foreign substances and MIRA at about LOQ level is performed. Three test preparations are prepared at the LOQ level by spiking known foreign substances in the test and also injected into the system. LOQ accuracy is calculated to MIRA (for unknown foreign substances) on a placebo. Percent Recovery of known foreign substances, MIRA, and found well within the acceptance. The obtained values are represented in Table-6.

Table-6: Table of results of LOD and LOQ

Impurity name	LOD (%)	LOQ (%)	LOQ	
			Precision (%RSD)	Accuracy (% Recovery)
MIRA	0.0119	0.0378	4.5	104.2
MIR-1	0.0114	0.0345	1.9	100.0
Deshydroxy	0.0128	0.0340	2.2	101.4
Diamide-2	0.0127	0.0366	3.8	107.2
Diamide-1	0.0139	0.0396	1.9	107.1

Linearity

This parameter is entrenched by drawing a graph by considering strength along with area response for MIRA measured by its related foreign substances also coefficient of correlation. A sequence of solutions for MIRA and its related compounds strengths range ranging about LOQ level to about 150% of the stability specification (0.4%) of Mira and its foreign substances are prepared and also injected in system of HPLC. Correlation coefficient value as well as % bias for 100% level, for all the components are identified that they are within the limit. Values so obtained are tabulated in Table-7.

Table-7: Table for Results of Linearity

Name of the impurity	Correlation coefficient	Slope	Intercept	100% Bias	Range µg/mL
MIRA	0.999001	30352.717583	-395.7056	-1.31	0.1785-1.4875
MIR-1	0.999660	18836.189505	-96.0314	-0.26	0.0765-1.9126
Deshydroxy	0.999610	25743.531035	108.7976	0.21	0.1793-2.9880
Diamide-2	0.999664	18548.256945	115.2344	0.31	0.1805-3.0088
Diamide-1	0.999741	20747.092847	-49.6524	-0.12	0.1786-2.9771

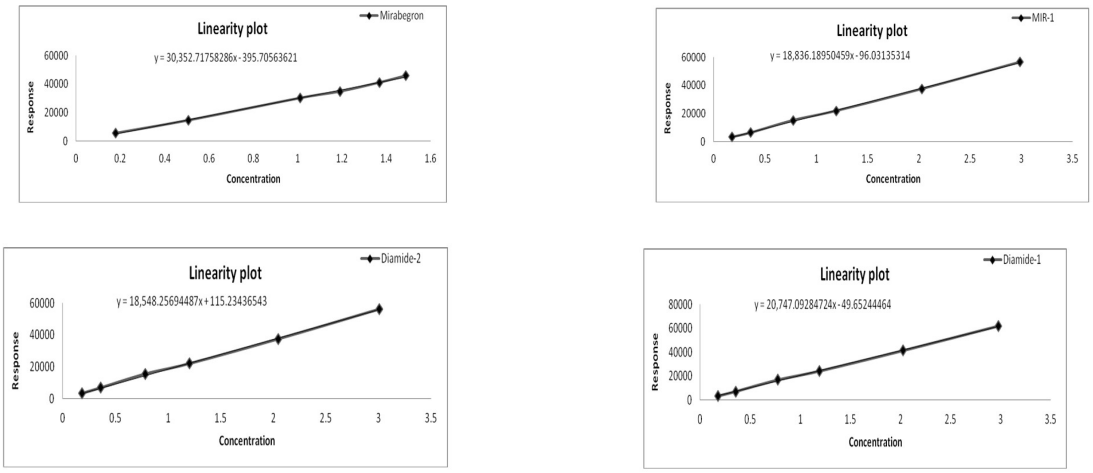

Fig.-3: Linearity Plots for MIRA and Its Related Impurities

Method Precision

This parameter for the test process is determined by passing a total of six samples that are prepared by spiking test preparation by MIRA foreign substances blend solution in order to identify 0.15% of MIR-1, Des-hydroxy, Diamide-2, and Diamide-1 foreign substances. Precision is performed by unknown solution by injecting a total of six samples by spiking with MIRA over placebo to get 0.4 % of test strength. Relative standard deviation is identified that they are within the limit to all above-mentioned foreign substances as well as MIRA. Similarly conducted intermediate precision by analyst-to-analyst variability by two different analysts, using different HPLC systems for system-to-system variability, different HPLC columns to check column-to-column variability on different days after passing a total six samples by preparation of spiking test solution with MIRA foreign substances blend solution and found that 0.15% of MIR-1, Des-hydroxy, Diamide-2 as well as Diamide-1 foreign substances.

Accuracy

To this parameter, a study of MIRA and their related compounds was done by spiking foreign substances over the preparation of the test solution. Samples are designed by spiking with MIRA foreign substances solutions to get LOQ to 3.0µg/mL strength of MIR-1, Des-hydroxy, Diamide-2, and Diamide-1 foreign substances. Performed accuracy study for MIRA by spiking API of MIRA over placebo and get the strength of LOQ as 1.5µg/mL. Prepared each and every sample as triplicate after spiking the prepared test solution by respective to levels of the target foreign substances strength. The obtained results are represented in Table-8.

Table-8: Table for Accuracy and Method Precision Results

Parameter	MIR-1	Des-hydroxy	Diamide-2	Diamide-1	MIRA
LOQ Accuracy (Mean,95% CF)	100.0, 99.5 and 100.5	101.4, 100.3 and 102.5	107.2, 105.7 and 108.7	107.1, 104.4 and 109.8	104.2, 102.8 and 105.6
50% Accuracy (Mean, 95% CF)	97.7, 95.2 and 100.2	100.4, 100.3 and 100.5	95.2, 94.1 and 96.3	98.1, 97.2 and 99.0	98.0, 96.8 and 99.2
100% Accuracy (Mean, 95% CF)	97.7, 96.6 and 98.8	96.8, 95.4 and 98.2	96.0, 95.4 and 96.6	102.7, 102.4 and 103.0	100.3, 98.8, 101.8
150% Accuracy (Mean, 95% CF)	96.6, 96.3 and 96.9	99.2, 98.9 and 99.5	97.3, 96.9 and 97.7	108.0, 107.7 and 108.3	99.3, 98.3 and 100.3
Repeatability (%RSD)	1.2	0.7	0.6	0.5	1.8
Intermediate precision (%RSD)	2.6	2.8	0.6	0.4	1.5
Cumulative (%RSD)	2.0	2.2	2.8	1.2	2.2

Ruggedness

Bench Top Stability of Standard and Test Solution

Established the stability of MIRA standard solution stability for a period of analogy factor to the preparation of standard is measured contrary to a freshly prepared standard solution. Similarity factors to the standard on days 1, 2, and 6 are found within the acceptable limits. Established test solution on bench top conducted in a time interval of 2 days. Samples of MIRA ER tablets are prepared in duplicate by spiking the 0.15% of each MIR-1, Des-hydroxy, Diamide-2, and Diamide-1 foreign substances in test solution as per procedure also injected into HPLC. The factor of these solutions is stored over a benchtop for a time interval of 6 days after that injected into the system of HPLC after 1 day, 2, and 6 days. % Individual foreign substances as well as total foreign substances were calculated against a freshly prepared standard solution. The % difference between initial and bench top stability samples for % of Individual, total foreign substances were found within acceptable limits in a time interval of 2 days. From this analysis, finalized that the standard solution is highly reliable in a time interval of 6 days on the bench top as well as sample solution is stable in 2 days at the bench top.

Bench Top Stability of Mobile phase

A study for establishing the stability of the mobile phase over bench top is performed in a time interval of about 6 days. Standard, test samples of MIRA tablets were prepared by spiking the 0.15% of MIR-1, Deshydroxy, Diamide-2, and Diamide-1 foreign substances (at Release specification) in the test solution as per test process after that passed into HPLC at initial after 1, 2 and 6 days. System suitability parameters are found within the acceptance criteria. % Individual foreign substances and total foreign substances are calculated against a freshly prepared standard solution. % difference among initial also samples for % of individual foreign substances and total foreign substances are found within acceptable limits. From this study, it is concluded that the mobile phase is highly reliable in a time interval of 6 days over bench top.

Robustness

As part of this parameter established allowable changes in the rate of flow 0.80mL/min - 1.20mL/min. 35°C - 45°C is the allowable change in column temperature. The allowable divergence in buffer pH (Buffer pH in mobile phase-A) is from pH 5.8 - 6.2, in mobile phase-B is from 90% - 110% of, and in acetonitrile composition in mobile phase-A is 90% - 110% of as such composition.

Filter Validation

Validation of filter is done in two various filters, 0.45µm PVDF along with 0.45µm Nylon by preparing test preparation in duplicate by spiking by known foreign substances at the specification level of foreign substances after that injected into the system of HPLC by using the test process. Centrifuged some portion of test solutions after filtering a small quantity portion of each and every test solution with twain filters after discarding the first 3.00mL of solution of the sample. %difference of foreign substances, total foreign substances among centrifuged samples, PVDF filtered samples, and Nylon filtered samples are within acceptance limit and within acceptance criteria. Hence finalized that PVDF and Nylon filters are suitable for filtration. The obtained chromatogram is represented in the Fig.-4.

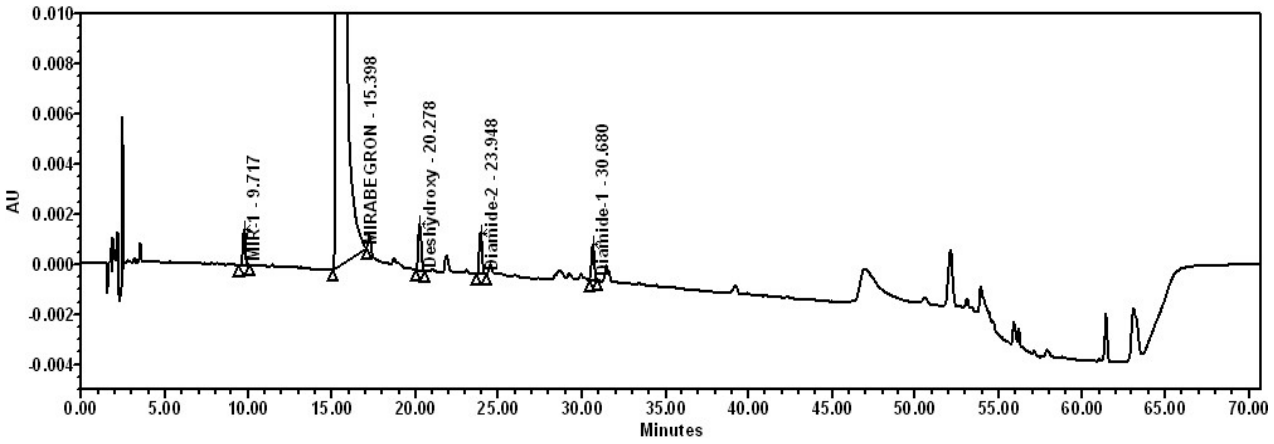

Fig.-4: Spiked Sample Chromatogram

CONCLUSION

HPLC method for MIRA ER Tablets validated for Des-hydroxy, Diamide-2 and Diamide-1 impurities was validated as per ICH Guidelines and concluded that this process subjected to the validation for system suitability, specificity (interference from placebo as well as degradation products), linearity, method precision, ruggedness (intermediate precision), accuracy, the establishment of LOD and LOQ, precision at LOQ, accuracy at LOQ, range (linearity, precision, and accuracy), stability of solutions (bench top stability solutions of standard as well as sample), stability in the mobile phase (on bench top) also robustness (rate of flow variation, column temperature variation, acetonitrile, mobile phase-B variation, variation of pH in mobile phase-A and Filter validation). The developed method is suitable to use Quality control routine analysis.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest among the authors.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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