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R E S E A E C H A R T I C L E

Develop and Validate a Method for the Estimation of Marbofloxacin in Pure and Pharmaceutical Dosage Form by Photo Fluorimetry

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A B S T R A C T

A simple and sensitive spectro fluorometric method was developed for the determination of Marbofloxacin in pure and its pharmaceutical dosage forms. The method is based on the solubilization of Marbofloxacin in 5:5 of ethanol& distilled water. The fluorescence produced by Marbofloxacin was measured at 486 nm after excitation at 360 nm. The different validation parameters like system suitability, accuracy, precision, LOQ, LOD, recovery studies, specificity, robustness, linearity, range, ruggedness were carefully studied and optimized. The method is applicable over the concentration range of 30-120 µg/ml with correlation coefficient of 0.999 for both tablet and injectable formulations. The detection limit (LOD) of Marbofloxacin was 0.303 & 0.306µg/ml for tablet and injectable dosage form and quantitation limit (LOQ) was 1.01& 1.02µg/ml for tablet and injectable dosage form respectively. The developed method is suitable for the determination of Marbofloxacin in pharmaceutical preparations with mean recoveries of 99.74-100.03 and 99.34-100.04 for Marbofloxacin tablets and injections respectively. The results were showed good precision, robustness. The method was applied for stress conditions of acid, base, peroxide, UV-light, hydrolytic conditions. This method was used for the routine analysis of marketed formulations by using photo fluorometer.

Keywords: Marbofloxacin, Photo fluorometer, Ultra-Violet

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Marbofloxacin is a synthetic, third generation fluoroquinolone developed for veterinary use only. It shows potent activity against several bacteria and its frequent use in the treatment of both gram negative and gram positive bacteria[1,2]. Marbofloxacin inhibits the bacterial DNA gyrase. In addition the increasing cross-over between antibiotics used for veterinary and human purposes has given rise to concerns over the development of antibiotic resistance in humans[3,4,].High-performance liquid chromatography (LC) with spectro photometric ultraviolet (UV) detection is currently the most used technique for the development of stability indicating methods of analysis.[5,6] Chemically the Marbofloxacin is 0-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-;7h-pyrido (3,2,1-ij) (4,1,2) benzoxadiazine-6-carboxylicacid, 2, 3-dihydro-9-fluor;8-Fluoro-3-methyl-9-(4-methyl-piperazin-1-yl)-6-oxo-2, 3-dihydro-1H,6H-1,3,3a-triaza-phenalene-5-carboxylic acid and molecular formula is $C_{17}H_{19}FN_4O_4$[7,8].Different methods are developed for the estimation of Marbofloxacin. Anew photo Fluorometric [9,10,11]method was developed for the estimation of Marbofloxacin in tablet and injectable dosage form. The drug structure of Marbofloxacin is given below.

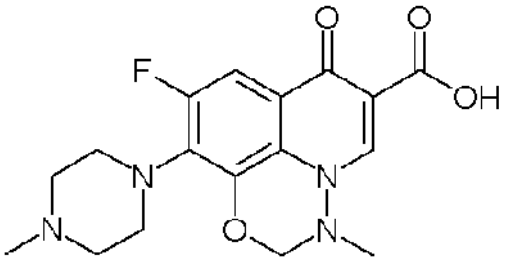

Fig: 1 Structure of Marbofloxacin

2. Materials and Methods

The chemicals and reagents were of analytical grade. Water was double distilled and filtered with a membrane filter. Ethanol (50:50) used as solvent. Pharmaceutical grade standard drug viz., Marbofloxacin was kindly gifted by Granuls pharma, Hyderabad, India. The tablet formulation contains 50 mg and injectable dosage is 100 mg of Marofloxacin purchased from local market of Kurnool. The development of method by using single beam photo fluorometer 152, (systronics) with 1cm matched.

Preparation of solutions

Standard stock solution:

Weigh accurately a quantity of 10mg of Marbofloxacin is taken into 10ml volumetric flask and it is made up to volume with the diluent. Then 1ml of above the solution is taken into a 100ml volumetric flask and it is made up to volume with dilution.

Working standard solution: Take 5ml of above the solution is taken into a 10ml volumetric flask and it is made up to volume with the diluents and give a final concentration of 5 µg/ml of Marbofloxacin.

Stock solution for tablet dosage form:

Weigh and powder 20 tablets and calculate the average weight of Marbofloxacin. Weigh accurately a quantity of powder equivalent 10mg of Marbofloxacin is taken into 10ml volumetric flask and it is made up to volume with the diluent. Then 1ml of above the solution is taken into a 100ml volumetric flask and it is made up to volume with dilution.

Working sample solution:

Take 5ml of above the solution is taken into a 10ml volumetric flask and it is made up to volume with the diluents and give a final concentration of 5 µg/ml of Marbofloxacin sample.

Stock solution of injectable dosage form:

Pipette out 0.1ml of Marbofloxacin solution from injection vials (10% w/v) into 10 ml volumetric flask and made upto volume with the diluent. Then 1ml of above the solution is taken into a 100ml volumetric flask and it is made up to volume with diluent.

Working sample solution:

Take 5ml of above the solution is taken into a 10ml volumetric flask and it is made up to volume with the diluents and give a final concentration of 5 µg/ml of Marbofloxacin sample.

Method Validation: The method validation was performed according to International Council for Harmonization guidelines. The following method validation parameters resembling specificity, precision, accuracy, linearity, robustness, ruggedness limit of detection and limit of quantification[13].

1. System Suitability Test:

The purpose of system suitability is to ensure that the complete testing including instrument, method and analyst is suitable for the intended application. This test was performed with 100% standard solution.

Table No 1: Results of system suitability

S. No	Concentration (µg/ml)	Fluorescence Intensity
1	1	6
2	2	10
3	3	14
4	4	18
5	5	22
6	6	26
7	7	30
8	8	34
9	9	39

2. Precision

The precision was studied through intermediate precision and intraday precision. The repeatability of the method was studied by measuring the 100% concentration of both tablet and injectable dosage form. The %RSD values were obtained 0.97% for standard, tablet, injectable dosage form. The results were showed good intra-day precision and intermediate precision. The results were tabulated in table no 2,3,4. The % RSD should not more than 2.0.

3. Accuracy: The accuracy of the method was performed by spiking standard solution with sample solution at three concentration levels 50%, 100%, 150%. The recovery

studies were studied under optimized conditions in replicate. The results were showed in table no 5,6. The accuracy should between 98%-102%. The % RSD value should not more than 2.0.

4. Linearity:

The linearity of the method was obtained through calibration curve (peak area vs concentration).The solutions were checked in the concentration range of 7-104 µg/ml for pure and table dosage form and 8-103 µg/ml for injecctable dosage form. The calibration curve was showed linear over concentration range and r^2 values were found to be 0.999 for Morbofloxacin. The linearity graphs were constructed between peak area and concentration. The data of graphs were showed in figures 2&3. The Correlation coefficient should be not less than 0.999.

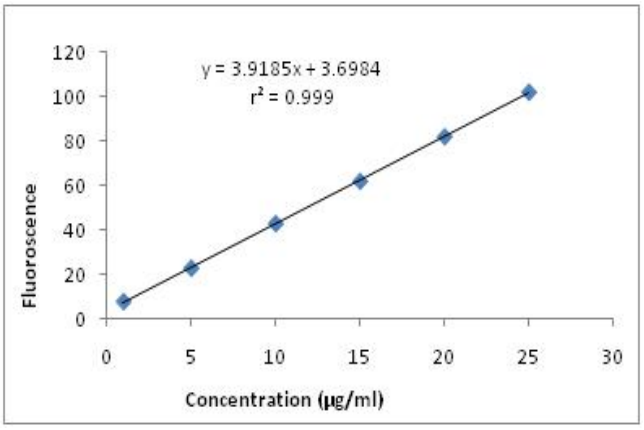

Fig 2 Graph for Tablet dosage form

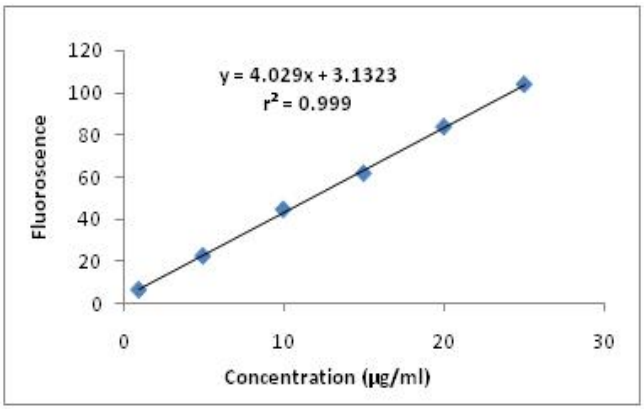

Fig:3 Graph for Injectable dosage dosage form.

5. Limit of Detection (LOD) and Limit of Quantification (LOD): The limit of detection and limit of quantification were studied through signal to noise ratio 3:1 and 10:1 ratio. The LOD of morbofloxacin was estimated 0.303 & 0.306 µg/ml for tablet and injectable dosage form respectively and LOQ was found to be 1.02 µg/ml-1.03µg/ml for both tablet and injectable dosage forms.

6. Robustness:

The method was unaffected with deliberate change with respected to change the concentration ranges water: ethanol and performed at 100% test concentration. The method was robust to the above mentioned condition. The results tabulated in table no 8. The % assay was found to be 99.98% & 99.99% for tablet and injectable dosage form.

7. Ruggedness:

The method was performed with different analysits and performed at 100% test concentration. The results tabulated in table no 9. The % assay was found to be 99.98% & 99.99% for tablet and injectable dosage form.

8. Assay of Morbofloxacin:

Twenty tablets of marketed formulation was powdered. Accurately weigh equivalent quantity of tablet powder of Morbofloxacin. The final concentration of was 5µg/ml. Injectable dosage form also prepared into 5µg/ml and measured. Results were tabulated in table no 10.

9. Stress indicated studies:

The stress indicated studies were performed to the morbofloxacin. There was no interference of degradants and blank, the developed spectro florometric method prove the capability of stability indicating method for the analysis of morbofloxacin. Different stress indicating studies were conducted like acid (0.1 N HCl, refluxed for 1 H at 60ºC), Base (0.1 N NaOH refluxed for 3H at 60ºC), H_2O_2 (3% H_2O_2 Stored at room temperature for 3H), hydrolytic 5H at 60ºC and UV light (near UV ≥200 for 5 days). The degradation conditions were optimized to obtain target degradation between 10 to 30% as per ICH guidelines. The results were summarized in table no 11.

3. Results and Discussions

The photo fluorometric method was developed and validated for the estimation of Morbofloxacin in tablet and injectable formulations. The system suitability test was performed for tablet and injectable dosage forms and results were found to be 6-39 of fluorescence intensity and concentration ranging from 1-9 µg/ml. The precision of the method was performed intraday precision and intermediate precision. The %RSD was obtained 0.976 for both tablet and injectable dosage forms. The method shows good system precision and method precision. The accuracy of the method was performed at 3 concentration levels 50%, 100%, 150%. The % recovery of Morbofloxacin was found to be 99.74-100.30% & 99.34-100.40% for tablet and injectable dosage form. The linearity study of Marbofloxacin was found to be 1-25 µg/ml. The fluorescence intensities of tablet & injectable dosage form were obtained 103 & 104 respectively. The R^2 was found to be 0.999 for tablet and injectable dosage forms. The LOD was found to be 0.303 & 0.306 µg/ml for tablet and injectable dosage form and LOQ were found to be 1.02 µg/ml-1.03µg/ml for both tablet and injectable dosage forms. The robustness of the method was found to be 99.98-99.99% for tablet and injectable dosage form. The method was not effected with change the proportions of solvent system. The ruggedness of the method was found to be 99.98-99.99% for tablet and injectable dosage form. The method was performed with different analysts and method shows good ruggedness. The analysis of tablet & injectable dosage form was found to be 99.84%. This method was used for routine analysis of pharmaceutical dosage forms by using photo fluorometry. The stress indicated studies were

performed with HCl(0.1N1H at 60ºC), NaOH(0.1N3H at 60ºC),H_2O_2(3H at room temperature), UV(≥200 for 5 days), Hydrolytic (5H at 60ºC). The drug was shown more stability in the hydrolytic conditions.

Table No 2: Results of precision for standard drug

S. No	Concentration (5µg/ml)	Fluorescence Intensity			
		Day-1	Day-2	Day-3	Repeatability studies
1	1	22.8	23	22.8	23
2	2	22.8	23	22.8	23
3	3	23.3	23.3	23.3	23.3
4	4	23.3	22.3	23.3	22.3
5	5	23	22.8	23	22.8
6	6	23	22.8	23	22.8
Average		23.03	23.03	23.03	23.03
%Assay		99.98	99.98	99.98	99.98
SD		0.225	0.225	0.225	0.225
%RSD		0.976	0.976	0.976	0.976

Table No: 3 Tablet Dosage Form:

S. No	Concentration (5µg/ml)	Fluorescence Intensity			
		Day-1	Day-2	Day-3	Repeatability studies
1	1	22.8	23.3	22.8	23
2	2	22	23	22.8	23
3	3	23.3	23.3	23.3	23.3
4	4	23.8	22	23.3	22.3
5	5	23	22.8	23	22.8
6	6	23	22.8	23	22.8
Average		23.03	23.03	23.03	23.03
%Assay		99.98	99.98	99.98	99.98
SD		0.225	0.225	0.225	0.225
%RSD		0.976	0.976	0.976	0.976

Table No: 4 Injection Dosage Form:

S. No	Concentration (5µg/ml)	Fluorescence Intensity			
		Day-1	Day-2	Day-3	Repeatability studies
1	1	22.8	23	22.8	23
2	2	22.8	23	22.8	23
3	3	23.3	23.3	23.3	23.3
4	4	23.3	22.3	23.3	22.3
5	5	23	22.8	23	22.8
6	6	23	22.8	23	22.8
Average		23.03	23.03	23.03	23.03
%Assay		99.99	99.99	99.99	99.99
SD		0.225	0.225	0.225	0.225
%RSD		0.976	0.976	0.976	0.976

Table No: 5 Results of Tablet Dosage Form:

S.No	% Spike level	Samples	Amount added	Amount found	% Recovery	% Average
1	50	11.3	2.47	2.45	99.45	100.30
2		11.5	2.47	2.5	101.2	
3		11.3	2.47	2.45	99.45	
1	100	22.5	4.94	4.89	99.01	99.74
2		22.5	4.94	4.89	99.01	
3		23	4.94	5	101.2	
1	150	34	7.42	7.39	99.61	100.70
2		34.5	7.42	7.5	101	
3		34	7.42	7.39	99.61	

Table No: 6 Results of Injectable Dosage Form

S.No	% Spike level	Samples	Amount added	Amount found	% Recovery	% Average
1	50	11.6	2.47	2.52	100.80	100.40
2		11.5	2.47	2.50	100.60	
3		11.3	2.47	2.45	99.50	
1	100	22.4	4.94	4.86	98.17	99.34
2		22.6	4.94	4.91	99.50	
3		23	4.94	4.86	100.08	
1	150	34	7.42	7.31	99.30	99.62
2		34.5	7.42	7.50	100.80	
3		33.8	7.42	7.34	98.76	

Table No 7: Results of Linearity

S. No	Concentration (µg/ml)	Fluorescence Intensity		
		API Dosage Form	Tablet Dosage Form	Injection Dosage Form
1	1	7	7	8
2	5	23	23	23
3	10	45	45	44
4	15	62	62	64
5	20	84	84	85
6	25	104	104	103

Table No 8: Results of Robustness

S. No	Diluent Concentration (Water: Ethanol)	Sample concentration (5µg/ml)	Fluorescence intensity	
			Tablet Dosage Form	Injection Dosage Form
1	4:6	5	22.8	22.8
2		5	23	23
3		5	23.3	23.2
1	5:5	5	23	23
2		5	23.3	23.2
3		5	22.8	22.8
1	6:4	5	23.3	23
2		5	22.8	22.8
3		5	23	23.2
Average			23.03	23.03
%Assay			99.98	99.99

Table No: 9 Results of Ruggedness

S. No	Analysts	Sample concentration (5µg/ml)	Fluorescence intensity	
			Tablet Dosage Form	Injection Dosage Form
1	I	5	22.8	22.8
2		5	23	23
3		5	23.3	23.2
1	II	5	23	23
2		5	23.3	23.2
3		5	22.8	22.8
1	III	5	23.3	23
2		5	22.8	22.8
3		5	23	23.2
Average			23.03	23.03
%Assay			99.98	99.99

Table No: 10 Results of Assay

S. No	Dosage form	Concentration (5µg/ml)	Fluorescence Intensity	% Assay
1	Pure form	5	23	99.84
2	Tablet form	5	23	99.84
3	Injection form	5	23	99.84

4. Conclusion

The developed method is simple, economic, precise, accurate method for the estimation of marbofloxacin using photo fluorimeter. The method was used for routine analysis tablet and injectable formulations.

5.Acknowledgement

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