STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF MARBOFLOXACIN IN BULK & THEIR DOSAGE FORM BY USING SPECTRO PHOTOMETRY

B. Lakshmi Kalyani*[1], Dr. B. Chandra Sekhara Rao[1]
J. Raga swetha[1] MohdSiddiq Pasha[2], Heena Qureshi[3], inamul haque[4],
Ashwaq Ahmed[5], AbuSamad[6].

[1-6]Assistant Professor, Department of Analysis, ChilkurBalaji College Of Pharmacy, Aziz nagar,Moinabad,Telangana, India.

Corresponding author: B.Lakshmi Kalyani

ABSTRACT:

Marbofloxacin is a veterinary fluoroquinolone drug used for the treatment of bacterial infections in animals. In this study, a stability-indicating UV-visible spectrophotometric method was developed and validated for the estimation of marbofloxacin in bulk and their dosage form. The method involved the use of a UV-visible spectrophotometer to measure the absorbance of marbofloxacin at its maximum wavelength of 301 nm. The method was validated for linearity, accuracy, specificity, precision, and robustness as per ICH guidelines. The linearity range was found to be 5-50 µg/mL, with a correlation coefficient of 0.9996. The percentage recovery was found to be within the limit of 98-102%, indicating the accuracy of the method. The method was found to be specific, as there was no interference from the excipients or degradation products of marbofloxacin. The precision of the method was evaluated by repeatability and intermediate precision, and the results showed RSD less than 2%. The method was found to be robust, and small deliberate changes were made to the analytical variables, which did not significantly affect the method's performance. The validated method was successfully applied for the estimation of marbofloxacin in bulk and pharmaceutical dosage form. The proposed method is a simple, accurate, precise, and stability-indicating UV-visible spectrophotometric method for the determination of marbofloxacin in bulk and pharmaceutical formulations.

KEYWORD: Bulk, Dosage Form,Estimation,Marbofloxacin, Stability Indicating Method, Spectrophotometry, Validation.

INTRODUCTION:

Marbofloxacin is a carboxylic corrosive subordinate, third era Fluoroquinolone anti-infection. It is utilized as a part of veterinary drug. A detailing of Marbofloxacin joined with (Clotrimazole + Dexamethasone) is accessible under the name Auriol. The Molecular equation is ($C_{17}H_{19}FN_4O$) and the molecular weight is 362.356. The structure formula is

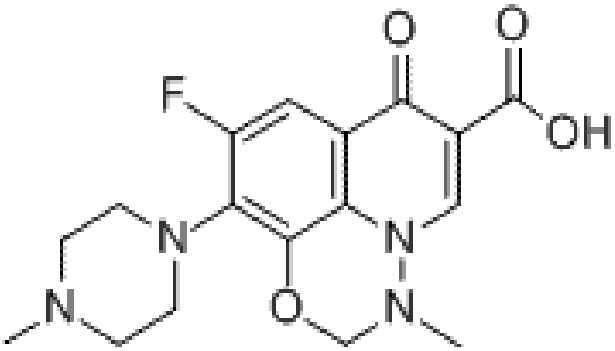
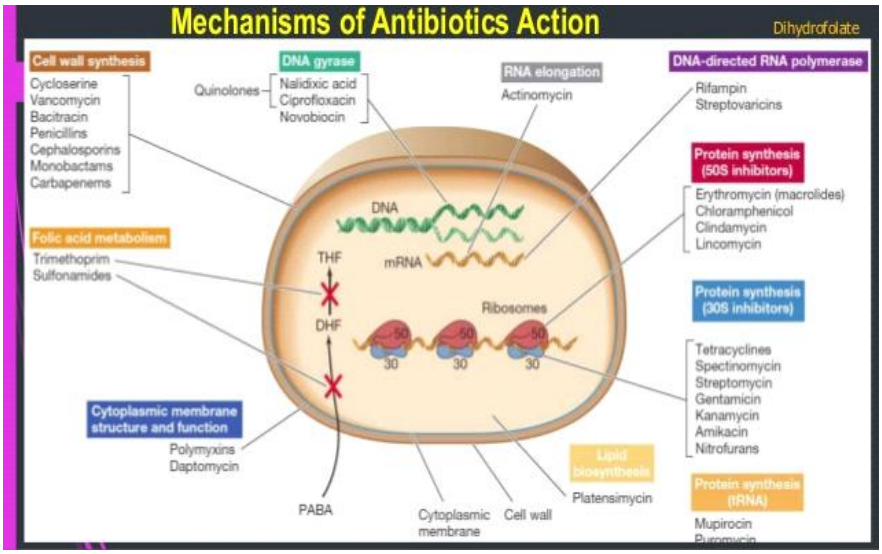


Fig No.1: Structure of Marbofloxacin

Marbofloxacin is a veterinary antibiotic that belongs to the fluoroquinolone class of drugs. It is primarily used in animals for the treatment of bacterial infections, particularly those affecting the respiratory and urinary systems. Marbofloxacin works by inhibiting the activity of bacterial enzymes that are essential for DNA replication and repair. This action helps to halt the growth and spread of bacteria, ultimately leading to their elimination. Marbofloxacin is known for its broad-spectrum activity, meaning it is effective against a wide range of bacteria. It is commonly available in tablet or injectable form, and its use should be under the guidance of a veterinarian.



FigNo.2: Mechanism of Action

Fluoro quinolone Anti biotic target Bacterial DNA-gyrase, an Enzyme which lessens DNA Strain amid replication, since DNA-gyrase is required amid DNA-replication, ensuing DNA union and at last Cell division is repressed. Marbofloxacin can be utilized both topically and orally. It's especially utilized for contaminations of the skin, respiratory system and mammary organs in cats and dogs. Pseudomonas aeruginosa (0.5µg/ml.)Staphylococcus aurous (0.25µg/ml.) For mutts dosage ranges from (2.75-5.5mg/kg) once every day. Minimum duration of treatment no less than five days. Most extreme duration of treatment is 30 days.

METHOD DEVELOPMENT

The present work is aimed at the analytical method development and validation for the selected drug by UV- VISIBLE SPECTROSCOPIC METHOD.

Preparation of standard stock solutions:

Marbofloxacin (pure form): Marbofloxacin pure10 mg having 99.89 % purity was weighed on Schimazdu ATY224 having 0.1 mg sensitive nature which is pre calibrated and transferred to a 10 ml of borosilicate class A volumetric flask and dissolved in methanol and sonicate it if necessary for complete dissolution it would be approximately may equal to 1000 µg/ ml. This is treated as primary stock solution.

The second dilution were made by pipetting the 1ml with help of pipette and transferred to another 10 ml volumetric flask and made to final volume with solvent selected for the first dilution. Now this would become as 100 µg/ ml which is used for scanning in the UV spectrometer as a standard Marbofloxacin.

The third dilution were made by pipetting the 0.4ml with help of micro pipette and transferred to another 10 ml volumetric flask and made to final volume with solvent selected for the first dilution. Now this would become as 4 µg/ ml which is used for scanning in the UV spectrometer as a standard Marbofloxacin.

6.2.2. Preparation of sample stock solution:

Marbofloxacin(marbomet) dosage form: Formulation of Marbofloxacin (marbomet) 48.32 mg having equivalent of 10 mg of Marbofloxacin Pure and was weighed on Schimazdu ATY224 having 0.1 mg sensitive nature which is pre calibrated and transferred to a 10 ml of borosilicate class A volumetric flask and dissolved in methanoland sonicate it if necessary for complete dissolution it would be approximately may equal to 1000 µg/ ml. This is treated as primary stock solution.

The second dilution were made by pipetting the 1 ml with help of pipette and transferred to another 10 ml volumetric flask and made to final volume with solvent selected for the first dilution. Now this would become as 100 µg/ ml which is used for scanning in the UV spectrometer as a sample Marbofloxacin (marbomet).

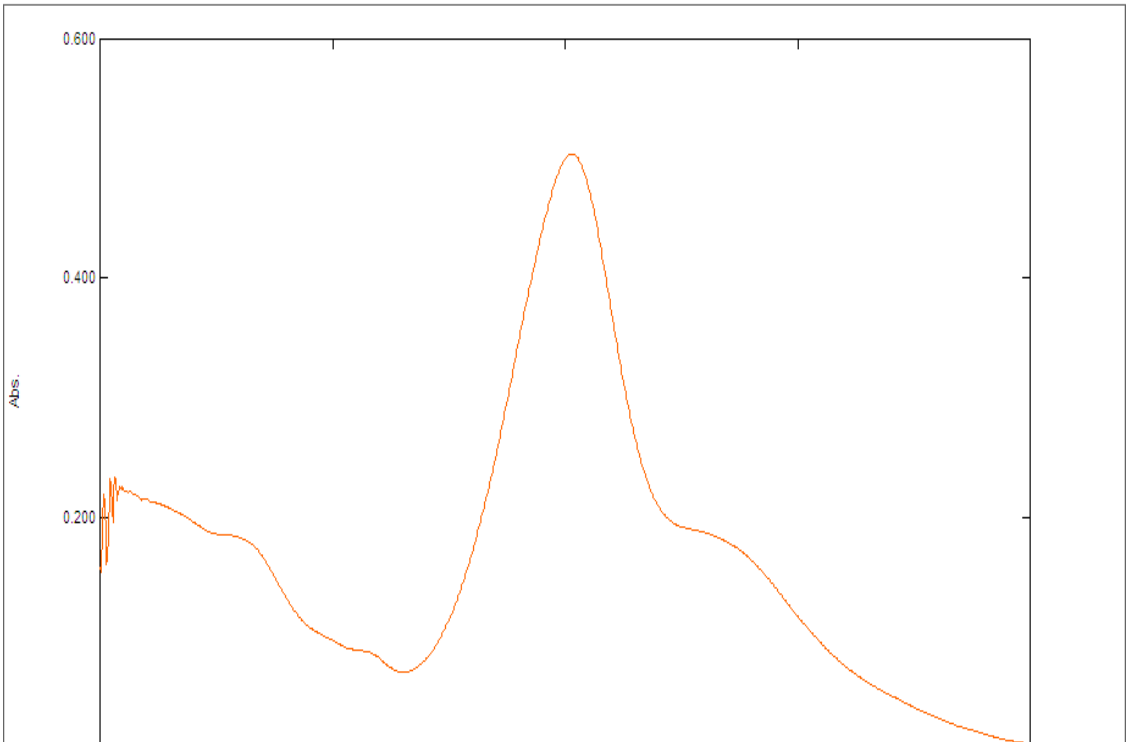
The third dilution were made by pipetting the 0.4 ml with help of pipette and transferred to another 10 ml volumetric flask and made to final volume with solvent selected for the first dilution. Now this would become as 4 µg/ ml which is used for scanning in the UV spectrometer as a sample Marbofloxacin (marbomet).

Marbofloxacin:

Selection of absorption maximum: The sensitivity of the UV-Visible spectroscopic method depends upon the proper selection of absorption maximum.

Marbofloxacine (API)

Marbofloxacine (ure form)	
Instrument	SHIMAZDU UV - 1800
Cuvette	Matched cells 1cm^2
Solvent	Methanol
Scanning range	200-400
Wavelength	301.00nm
Absorbance	0.504



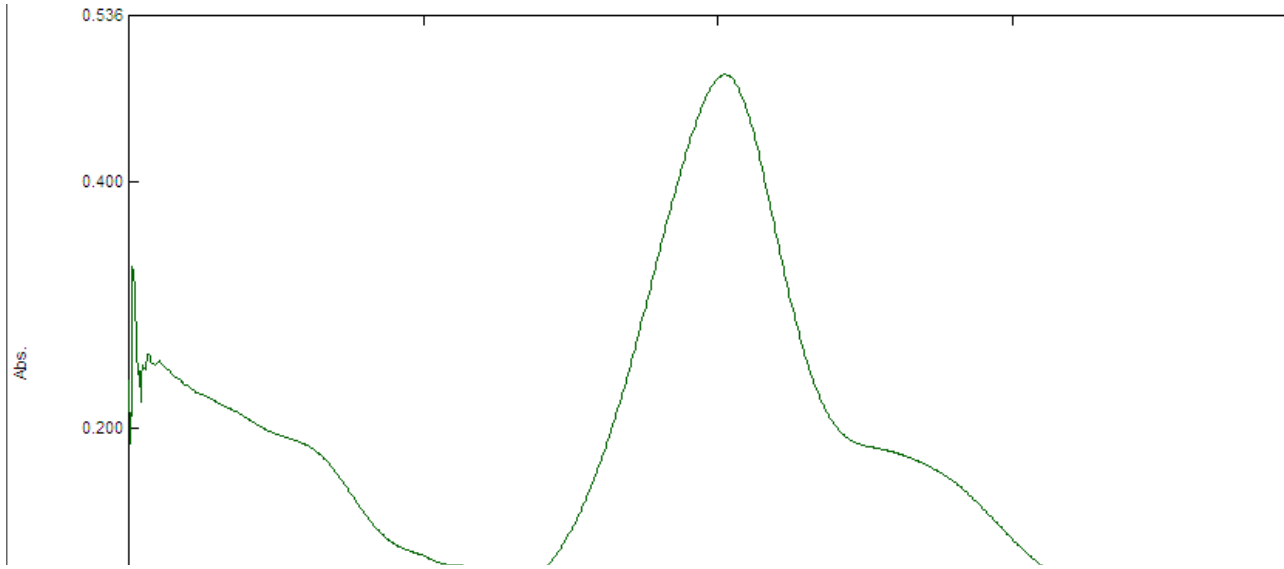
Spectrum of Marbofloxacin (API)

Discussion: A standard solution of marbofloxacin about 4 mcg per ml were prepared and checked for the UV absorbance against the blank solution and the lambda max or the drug was seen at 301.00 nm with Absorbance of 0.504.

Marbofloxacine (Marbomet) Dosage form:

Marbofloxacin (Dosage form)	
Instrument	SHIMAZDU UV - 1800
Cuvette	Matched cells 1cm^2
Solvent	Methanol

Scanning range	200-400
Wavelength	301.00nm
Absorbance	0.502



Spectrum of Marbofloxacin (dosage form)

Discussion: A sample solution of Marbofloxacin about 4 mcg per ml were prepared and checked for the UV absorbance against the blank solution and the lambda max or the drug was seen at 301.00 nm with Absorbance of 0.502.

7.1.1. Calibration Curve (Linearity)

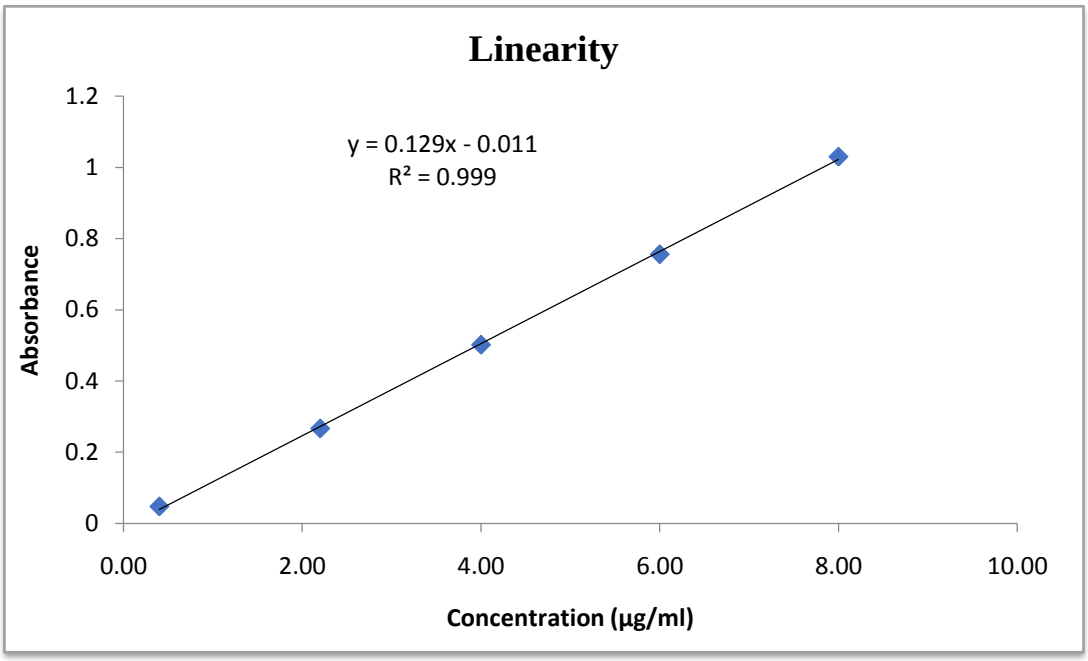
Procedure:

From the above concentration's that is 0.4, 2.2, 4, 6, 8 µg/ml samples calculate absorbance at 301 nm by taking balnk as methanol, plot the graph with resulted absorbance by taking absorbance and wave length on y and x axis and regression equation is calculated and as shown in the table no.7.3.

Acceptance criteria: Correlation coefficient should not be < 0.999.

Linearity of Marbofloxacin

S. No	Linearity Level	Concentration	Absorbance
1	10	0.40	0.048
2	50	2.20	0.267
3	100	4.00	0.502
4	150	6.00	0.756
5	200	8.00	1.03



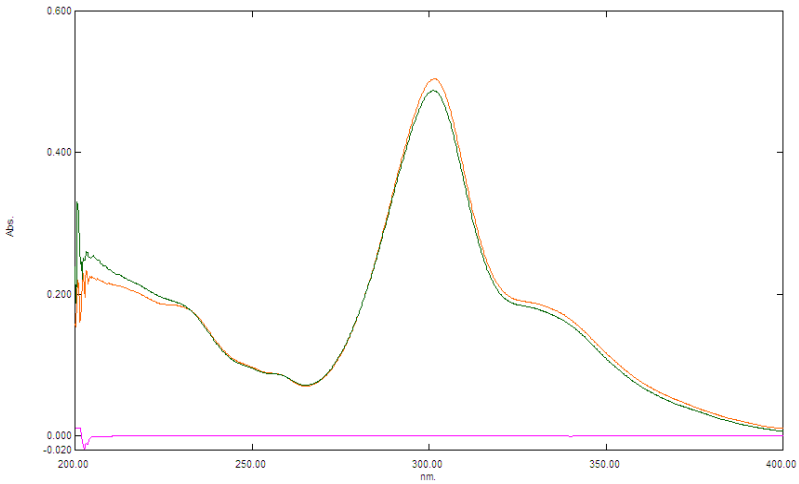
Linearity of Marbofloxacin

Discussion: The various concentrations of Marbofloxacin were prepared as hypothetical knowledge. To check the linearity of the method the marbofloxacin were observed the absorbance at various concentrations. The plot will be drawn with help of concentration versus absorbance on two different axis (X&Y). The correlation was found at 0.999 and the slope has got 0.1292X.

The method is set for its linearity because the correlations between the two values are fit with in the 1.0. Hence the method is linear.

7.1.2. Specificity:

S.No	Type	Wavelength(nm)	Absorbance
1	Standard	301.00	0.503
2	Sample	301.00	0.501



Spectrum for specificity (marbofloxacin)
Sample and Standard Observance of marbofloxacin

Discussion: A blank graph is taken with blank solution to observe the any interference peak in the solution of blank. Then standard and sample solutions of 4mcg per ml were taken for the spectrum and it would be found no interference will be seen except sample and standard marbofloxacin peaks. Hence the method is suitable or specific for the estimation of marbofloxacin in bulk and sample dosage forms. The sample and standard are observed for at the selected wave length for their specificity. The mean, standard deviation and relative standard deviation of the precision values are lyed with in the specified ICH guidelines.

Precision:

The precision of an analytical method is the degree of agreement among individual test results, when the method is applied repeatedly to multiple samplings ofhomogenous samples. It provides an indication of random error results and was expressed as %RSD.

The intra & inter-day precision was evaluated by analyzing six sample solutions (n = 6), at the final concentration of analyses (4µg/ml) ofMarbofloxacin. The Marbofloxacin concentrations were determined and the relative standard deviations (RSD) were calculated. % RSD was calculated and as shown in the table no.7.5 and 7.6.

Acceptance criteria: The %RSD responses of six samples should not be > 2.

Results of Intraday precision of marbofloxacin

S. No	9.30 AM		1.30 PM		5.30 PM	
	Absorbance	% Assay	Absorbance	% Assay	Absorbance	% Assay
1	0.503	99.81	0.500	99.21	0.498	98.82
2	0.499	99.02	0.501	99.41	0.499	99.02
3	0.502	99.61	0.496	98.42	0.498	98.82
4	0.495	98.22	0.493	97.83	0.500	99.21
5	0.503	99.81	0.497	98.62	0.499	99.02
6	0.501	99.41	0.499	99.02	0.501	99.41
Average	0.500	99.31	0.500	98.75	0.500	99.05
STDEV	0.001	0.61	0.002	0.58	0.001	0.23
% RSD	0.62	0.62	0.59	0.59	0.23	0.23

Discussion: The day precision will be carried out by using the same solution at definite time intervals to observe the absorbance of the sample and they are assayed. The precision is done with 6 replicate samples are observed at same wave length in photometric mode of UV- Vis spectroscopy. In the same day it is observed that the sample will be in the range of 99 – 102%. The mean, standard deviation and relative standard deviation of the precision values are lyed with in the specified ICH guidelines. Hence the method is precised for the selected drugs.

Results of Intermediate Precision of marbofloxacin

S. No	Day 1		Day 2		Day 3	
	Absorbance	% Assay	Absorbance	% Assay	Absorbance	% Assay
1	0.500	99.21	0.498	98.82	0.499	99.02
2	0.501	99.41	0.498	98.82	0.499	99.02
3	0.499	99.02	0.495	98.22	0.501	99.41
4	0.501	99.41	0.501	99.41	0.500	99.21
5	0.502	99.61	0.501	99.41	0.500	99.21
6	0.501	99.41	0.500	99.21	0.502	99.61
Average	0.500	99.35	0.500	98.98	0.500	99.25
STDEV	0.003	0.20	0.003	0.46	0.001	0.23
% RSD	0.21	0.21	0.46	0.46	0.23	0.23

Discussion: The intermediate precision is also called ruggedness; it can be done the same experiment with same solutions on different days or two subsequent success days. The experiment is carried out like precision and the mean, standard deviation and relative standard deviations of the absorbance are found and they fit as per the guidelines of ICH and theylyed less than 2% of RSD and on each day the assay will not be less than 98.98 % and not more than 99.35%.

Accuracy:

The % Mean recovery and its %RSD were calculated.

Formula: Amount Recovered = Amount found – Amount add

% Recovery = Amount recovered ÷ Amount added × 100

Acceptance criteria: The %Recovery and the mean %Recovery should be in the range from 98 – 102.0%

%RSD should not be > 2.

Results of Accuracy of marbofloxacin

S. No	Accuracy Level	Wt. of Sample	Absorbance	Amount Added	Amount Found	% Recovery	Mean % Recovery
1	80%	38.656	0.403	2.37	2.39	100.84	100.63
2		38.656	0.402	2.37	2.39	100.84	
3		38.656	0.400	2.37	2.38	100.42	
4		38.656	0.400	2.37	2.38	100.42	
5		38.656	0.404	2.37	2.40	101.27	
6		38.656	0.399	2.37	2.37	100.00	
7	100%	48.32	0.501	3.96	3.97	100.25	100.34
8		48.32	0.500	3.96	3.96	100.00	
9		48.32	0.503	3.96	3.99	100.76	
10	120%	86.976	0.603	5.70	5.74	100.70	100.76
11		86.976	0.605	5.70	5.76	101.05	
12		86.976	0.600	5.70	5.71	100.18	
13		86.976	0.604	5.70	5.75	100.88	
14		86.976	0.604	5.70	5.75	100.88	
15		86.976	0.604	5.70	5.75	100.88	

Discussion: The accuracy for the marbofloxacin was conducted by taking three, six, three replicate absorbance for three different concentrations. Hence a total fifteen absorbance will be seen in the accuracy. The percentage recovery will be determined by spike level added to the sample solution. The mean recovery will be in the range of 98.00 – 102.00%. Hence the method is validated as per the guidelines of the ICH and should be found the values are within the limit as they specified.

Limit of detection (LOD) and Limit of quantification (LOQ)

Results of LOD & LOQ of marbofloxacin

Parameter	Result
Slope	0.1292
STDEV	0.001
LOD (µg/ml)	0.02
LOQ (µg/ml)	0.08

Discussion: The sensitivity of the procedure will be declared by determining the LOD and LOQ of the standard solution by noise to signal ratio and could be conducted by taking standard deviation of the system suitability and slope from the linearity. The formula given by the tripartite committee and they were found to be 0.02 and 0.08 ppm for the marbofloxacin as LOD and LOQ respectively.

Robustness:

Results of Robustness of marbofloxacin

S. No	Parameter	Condition	Absorbance	% Assay
1	Wavelength	299.00	0.500	99.21
2		301.00	0.501	99.41
3		303.00	0.499	99.02
4	Solvent (methanol)	301.00	0.503	99.81

Discussion: The robusted method is always given that any method is changed to its optimized experimental conditions that the procedure or method should not be effect after a slight change in the procedure and the value for the assay should not degrade as 99.81%. Hence the method is robusted when a change in wave length and its solvent composition and the values are lyed with in the specified limit of the ICH.

Degradation Studies:

Stability studies or Forced degradation studies:

The ability of the drug towards forced external factors which can degrade the drug

- Addition of Acid
- Addition of base
- Addition of Oxidising agent

- Exposure to Ultra violet rays
- Exposure to sunglight
- Heating

- **Addition of Acid (1N HCL):**Prepare a standard solution 4µg/ml into 10 ml volumetric flask and add 1ml 0.1N HCL and room temperature at 30^0C for 1hr, and neutralize by adding 1ml of 1N NaOH and calculate at maximum absorbance .
- **Addition of Base(1N NaOH):**Prepare a standard solution 4µg/ml into 10 ml volumetric flask and add 1ml 0.1N NaOH and room temperature at 30^0C for 1hr, and neutralize by adding 1ml of 1N HCL and calculate at maximum wavelength.
- **Addition of OxidisingAgent:**To the standard preparation add 2-3 drops of (Hydrogen peroxide)Oxidising agent and heat for 15min.,and calculate the absorbance.
- **Exposure to Ultra violet rays:**Expose the standard preparation 4µg/ml to ultra violet rays in uv cabinet for 5 hrs, and absorbance is calculated.
- **Exposure to Sunlight:**Expose the standard preparation 4µg/ml to direct Sunlight for 5 hrs, and absorbance is calculated
- **Exposure to heat:**Heat the standard preparation 4µg/ml for 15min., and absorbance is calculated and this all are shown in the table no. 7.10.

Stability studies of marbofloxacin

S. No	Condition	Absorbance	% Assay	% Degradation
1	Acid (0.1 HCl)	0.462	91.67	8.33
2	Base (1N NaOH)	0.459	91.08	8.92
3	H2O2 (2-3 drops)	0.46	91.28	8.72
4	UV (5hours)	0.455	90.29	9.71
5	Heat	0.454	90.09	9.91

Discussion: The method is subjected to the forced degradation studies to achieve the stability of the solution at normal conditions. The acid, alkali, peroxide chemicals are used for the forced degradation of the products and there is also conducted the thermal and photolytic degradation to know the effect of these conditions on the experimental procedure and drug present in it. The results were analyzed at photometric mode of spectrometer.
We have achieved or justified our title by doing forced degradation studied as to state its stability in various chemicals and conditions.

Parameters	**Marbofloxacin**	**ICH guidelines**
	UV	
Range	0.4 - 8	NA
Wavelength (nm)	301.00nm	NA
Solvent phase	Methanol	NA
Regression equation (Y)	y = 0.1292x	NA
Intercept (c)	0.00	NA
Correlation coefficient	$R^2 = 0.999$	PASS
Acid Hydrolysis	91.67	PASS
Alkali Hydrolysis	91.08	PASS
Thermal Degradation	90.90	PASS
Photolytic Degradation	90.29	PASS
Peroxidation	91.28	PASS
LOD (µg / ml)	0.02	PASS
LOQ (µg / ml)	0.08	PASS
% Mean Recovery	100.57	PASS
Intraday Precision	0.48	PASS
Intermediate Precision	0.30	PASS
Assay Purity	99.06	PASS

SUMMARY&CONCLUSION

The method selected for the Marbofloxacin was successfully made with methanol. was successfully made with 50:50 ratios of methanol and water. The selected method is most suited for the analysis of drugs in the bulk and pharmaceutical dosage forms. The method is optimized with making a solvent selected and UV- 1800 shimazdu the methods was developed by trial and error. The method selected is validated as per the guidelines of the ICH to its selectivity, Linearity, Accuracy, Precision, Sensitivity and others as per the Q2(R1).All the parameters are successfully procedure as per guidelines and the results were noticed in each parameters and observed that the method is validated.The developed method is suitable for the routine analysis of Marbofloxacin by UV visible spectroscopy in Pharmaceutical analysis laboratory and Industry.

BIBLIOGRAPHY

- *https://www.slideshare.net/derivative-spectroscopy*by- mohammed-abdulwahhab-hasseni-university-of-duhok 14 june 2015.
- AdnanH.MahmoodabGregoryA.MedleyaJeffreyE.GriceaXinLiuaMichaelS.Robertsac Determination of trovafloxacin and marbofloxacin in sheep plasma samples by HPLC using UV detection"*Journal of pharmaceutical and biomedical analysis* 62:220 3 · March 2012.
- Afzal, G, Nawaz, M, Saleem, B. *Veterinary Journal* 1982; 2:57–60.
- Attimarad MV, Harsha NS, Setty RS. *Liquid Chromatography of Related Technology* 2012; 35(6):768–77.
- Baeyens, V.; Varesio, E.; Veuthey, J. L.; Gurny, R.; *Journal of Chromatography& bio analytical techniques of life sciences*1997, 692, 222.
- Bakshi M, Singh S (2002) *Journal of Pharmaceutical and Biomedical Analysis* 28(6):1011–1040.
- Bhoir SI, Gaikwad PV, Parab LS, Shringarpure RN, Savant SS, Verma PJ. *Journal of Chromatography Sciences* 2011; 49(1):84–7.
- Blum, RA. *American Journal of Medicine*: 1992; 92:18S–21S.
- Boothe, D.M., W. B. Saunders Co.,Philadelphia, PA. (2001) Antimicrobial drugs. In Small Animal ClinicalPharmacology and Therapeutics, pp. 150–173.
- British Pharmacopoeia – 2000.