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VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND OFLOXACIN

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Abstract:

A simple, Accurate, precise Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed for the simultaneous estimation of the omeprazole and Ofloxacin in Tablet dosage form. Chromatogram was run through ODS (150 X 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrile in the ratio of 45:55 was pumped through column at a flow rate of 0.8 ml/min. Buffer temperature was maintained at 30°C. Optimized wavelength for Omeprazole and Ofloxacin was 220nm. Retention time of Omeprazole and Ofloxacin were found to be 2.16 min and 3.39 min. %RSD of the Omeprazole and Ofloxacin were and found to be 0.62 and 0.74 respectively. %Recover was Obtained as 100.07% and 100.72% for Omeprazole and Ofloxacin. LOD, LOQ values were obtained from regression equations of Omeprazole and Ofloxacin were 0.27ppm, 0.37ppm and 0.83ppm, 1.13ppm respectively. Regression equation of Omeprazole is y = 11878x + 281.6, and of Ofloxacin is y = 14453x + 3910.2.

Key Words: *Omeprazole, Ofloxacin, RP-HPLC, LOD, LOQ*

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INTRODUCTION:

Omeprazole [1], (RS)-5-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl)Methylsulfinyl)-1H-benzo[d] imidazole, is a proton pump inhibitor, it suppresses gastric acid secretion by specific inhibition of the H+/K+-ATPase in the gastric parietal cell. By acting specifically on the proton pump, omeprazole blocks the final step in acid production, thus reducing gastric acidity. Few bio analytical methods by HPLC using human plasma and also spectrophotometric methods using pharmaceutical dosage forms have been reported for the estimation of Omeprazole.

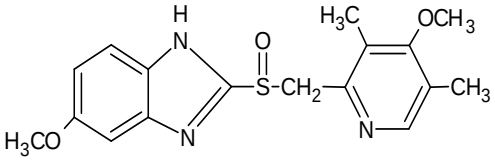

Fig.1: Structure of Omeprazole

Ofloxacin [2], Chemically (±)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-di]-1,4-benzoxazine-6-carboxylicacid, is a synthetic antibiotic of fluoro quinolone drug class considered to be a second-generation fluoro quinolone. Ofloxacin is a racemic mixture, which consists of 50% levofloxacin (the biologically active component) and 50% of its "mirror image" or enantiomer dextrofloxacin, its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian.

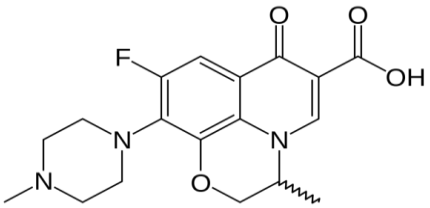

Fig.2: Structure of Ofloxacin

Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and bio analytical samples. In Pharmaceutical industry, spectrophotometric and chromatographic methods have gained the significance in recent studies**.** Hence a RP–HPLC method was developed and validated as per ICH guidelines [3]. The literature reveals that various methods for the determination of Omeprazole and Ofloxacin in pharmaceutical validations among these methods are LC-MS and LC-MS/MS [4-6], HPLC [7,8] method for title compounds, was reported. An attempt was made to develop a method which is precise, simple, robust and most economic method so far for their determination.

MATERIALS AND METHODS:

Materials: HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. Acetonitrile, Phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, triethylamine, ortho-phosphoric acid were analytical grade**.**

Methods: Preparation of 0.1% OPA buffer: 1ml of ortho phosphoric acid in a 1000ml of volumetric flask adds about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Standard Preparation: Accurately Weighed and transferred 25mg of Omeprazole and 10mg of Ofloxacin working Standards into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation: 5 ml was transferred into a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Method Development: There are many trials were done by changing columns and Mobile phases and were reported optimized method below**.** Drugs were eluted with good resolution, retention time all the parameters were within the limits.

Mobile phase : Buffer and Acetonitrile (45:55)
Flow rate : 0.8 ml/min
Column : ODS 150 X 4.6 mm, 5µ.
Detector wave length: 220nm
Column temperature: 30°C
Injection volume : 10µL
Run time : 6 min
Diluent: first dissolved in Methanol and made up with water

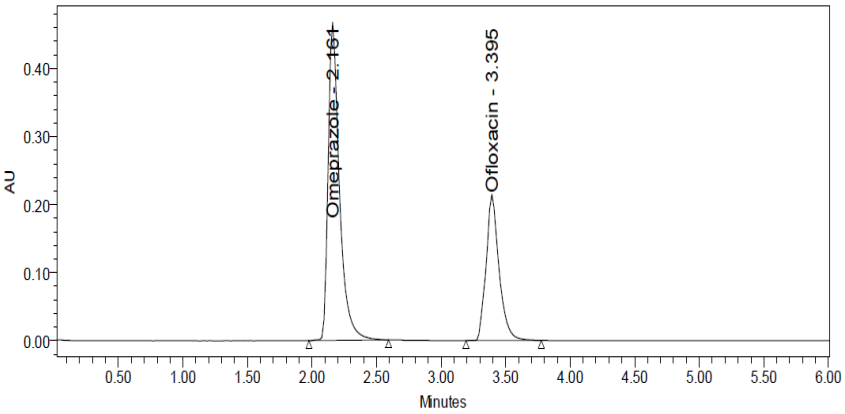

Fig.3: Optimized chromatogram

RESULTS AND DISCUSSION:

System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines, results were shown in table-1.

Table-1: System suitability studies

Property	Omeprazole	Ofloxacin
Retention time (t_R)	2.16± 0.3 min	3.39±0.3min
Theoretical plates (N)	3188 ± 163.48	5704±163.48
Tailing factor (T)	1.58 ± 0.117	1.35± 0.117
Peak area	282342	1503692

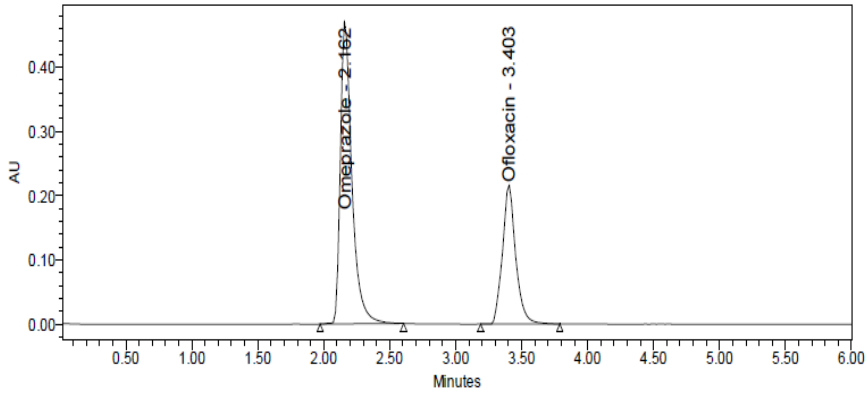

Fig.4: Typical chromatogram of Omeprazole and Ofloxacin.

Linearity: Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of Omeprazole and Ofloxacin are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 62.5ppm, 125ppm, 187.5ppm, 250ppm, 312.5ppm and 375ppm of Omeprazole and 25ppm, 50ppm, 75ppm 100ppm, 125ppm, 150ppm of Ofloxacin. Regression equation of the Omeprazole and Ofloxacin are found to be, y = 11878x + 281.6, and y = 14453x + 3910 and the regression co-efficient was 0.999. Linearity results were shown in table-2.

Table-2: Linearity Calibration data of Omeprazole and Ofloxacin.

S.No	Omeprazole Concentration (µg/ml)	Response	Ofloxacin Concentration (µg/ml)	Response
1	62.5	776358	25	379357
2	125	1500879	50	748453
3	187.5	2184123	75	1057693
4	250	2929388	100	1431042
5	312.5	3692826	125	1802617
6	375	4508415	150	2195967

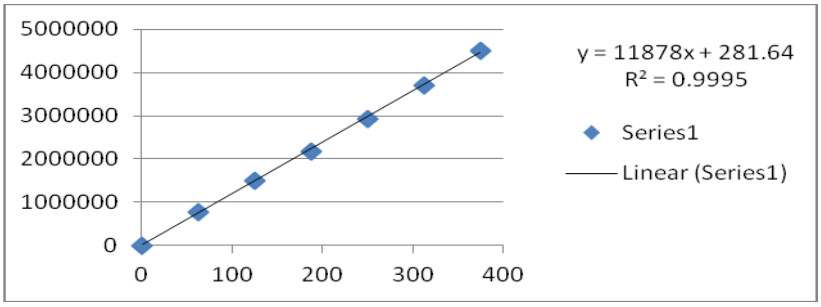

Fig 5: Calibration curve of Omeprazole

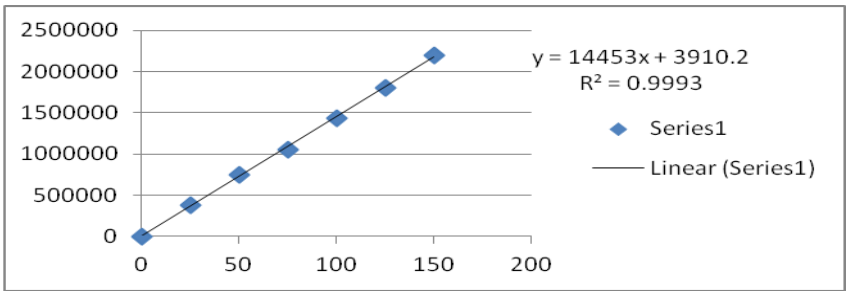

Fig 6: Calibration curve of Ofloxacin

Intraday precision: Intraday Precision was performed and % RSD for Omeprazole and Ofloxacin were found to be 0.62% and 0.74% respectively those are below 2. Results were shown in table-3.

Inter day precision: Inter day precision was performed with 24 hrs time lag and the % RSD Obtained for Omeprazole and Ofloxacin were 0.12% and 0.03%. Results were shown in table-3.

Table-3: Precision results

S.No	Inter day		Intraday	
	Omeprazole	Ofloxacin	Omeprazole	Ofloxacin
1	2849235	1502705	2823421	1503692
2	2868588	1490595	2851485	1501755
3	2848014	1497287	2876717	1529532
4	2833690	1503932	2858841	1521086
5	2851740	1508829	2844759	1507129
6	2845264	1501256	2858256	1519037
Mean	2847759	1500963	2852247	1513705
Std.Dev.	3528.46	414.36	17697.2	11132.9
%RSD	0.12	0.03	0.62	0.74

Accuracy: Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 4 and the recovery was within the range 98-102%.

Table-4: Accuracy results

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	% Recovery	% RSD
Omeprazole	125	124.62	99.7	0.60
	250	248.42	99.37	0.93
	375	373.87	99.70	1.44
Ofloxacin	50	50.31	100.62	0.46
	100	100.8	100.80	0.19
	150	149.25	99.5	0.22

LOD: Limit of detection was calculated by intercept Omeprazole and Ofloxacin method and LOD for Omeprazole was found to be 0.01 and Ofloxacin was 0.01 respectively.
LOQ: Limit of Quantification was calculated by intercept Omeprazole and Ofloxacin method and LOQ for Omeprazole and Ofloxacin were found to be 0.05 and 0.04 respectively.
Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range **%** RSD below 2 as per ICH Guide lines.

Table-5: Robustness data of Omeprazole and Ofloxacin.

S.No.	Robustness Condition	Omeprazole %RSD	Ofloxacin %RSD
1	Flow minus	0.40	0.27
2	Flow Plus	1.29	0.66
3	Mobile phase minus	0.3	0.4
4	Mobile phase Plus	1.29	1.36
5	Temperature minus	0.3	0.1
6	Temperature Plus	0.2	0.4

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.07% for Omeprazole and 100.72 for Ofloxacin.

Table-6: Assay Results

S.No	Omeprazole	Ofloxacin
1	99.06	100.06
2	100.04	99.93
3	100.93	101.78
4	100.30	101.22
5	99.81	100.29
6	100.28	101.08
Mean	100.07	100.72
Std.Dev.	0.6209	0.7408
%RSD	0.62	0.74

CONCLUSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Omeprazole and Ofloxacin in Tablet dosage form. Retention time of Omeprazole and Ofloxacin were found to be 2.16 min and 3.39 min. %RSD of the Omeprazole and Ofloxacin were and found to be 0.62 and 0.74 respectively. %Recovery was Obtained as 100.07% and 100.72% for Omeprazole and Ofloxacin respectively. LOD, LOQ values were obtained from the regression equations of Omeprazole and Ofloxacin were 0.27ppm, 0.037ppm and 0.083ppm, 1.13ppm respectively. Regression equation of Omeprazole is y = 11878x + 281.64, and of Ofloxacin is y = 14453x + 3910.2. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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