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Original Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND OMEPRAZOLE IN BULK DRUG

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Abstract: Ofloxacin (OFL) and Omeprazole (OMP) are synthetic drugs active against *H. Pylori*. UV-Spectrophotometric analytical method was validated to assay Ofloxacin and Omeprazole in tablet. Measurements were taken at 298 and 302 nm using methanol and water as solvent. This new method was developed and validated in accordance with ICH requirements, which include linearity, precision, accuracy, specificity, detection and quantitation limits. The simultaneous estimation method demonstrated good linearity over the range of 5-30 µg/mL. The mean percentage recoveries were 101.11, 100.86 of OMP and OFL. In Derivative spectroscopy, percentage recovery of OMP and OFL were 101.13, 100.86. In Zero crossing method, values found were 101.73, 101.53 OMP and OFL respectively. The repeatability values were found 0.0045, 0.0053 for OMP, 0.0058 and 0.0054 for OFL in simultaneous method. In first order derivative spectroscopy, values were found 0.0045, 0.0053 for OMP, 0.0058 and 0.0054 for OFL at 225 and 235 nm. In simultaneous estimation method, LOD values were found to be 2.29, 1.56 and LOQ values were 2.86, 2.72 for OFL and OMP respectively, in first order derivatives, LOD values were found to be 2.65 and 2.08. LOQ values were 4.75, 3.92 for OMP and OFL respectively, In Q Analysis method, LOD values were found to be 1.97 and 2.64. LOQ values were 3.32, 3.84 for OMP and OFL respectively. In house Formulation by simultaneous equation method showed that the percentages 101.98, 101.21 and 102.31, 102.01 for OMP and OFL. In first order derivative spectroscopy method, values were 102.31, 102.09 for OMP and 102.12, 101.96 for OFL, In Q-Analysis method, values were found 102.65, 102.41 for OMP and 102.32, 101.78 for OFL. The proposed method might be applied in routine quality control in the pharmaceutical industries since it is precise, accurate, simple, and economic, produces very low amounts of solvents and residues.

.**Keywords:** Simultaneous quantitative determination, UV, HPLC and Ofloxacin and Omeprazole.

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Introduction: Spectrophotometric measurements are applied in number of different ways such as in organic, analytical and pharmaceutical chemistry; some of the most significant application of UV

spectroscopy is to study the extent of configuration, distinction between conjugated and non-conjugated compound, detection and identification of chromophores.

Organic compounds and drugs can be identified and qualitatively analysed by spectrophotometer, since absorption spectra of compounds in particular solvents are unique with respect to their shape, $\lambda_{max,}$ ratio of absorption and absorptivity at different wavelengths. Quantitative analysis by UV-spectrophotometer may either be one component analysis or multicomponent analysis.

Analytical techniques that are generally used for drug analysis are spectral methods, chromatographic methods, electro analytical techniques, biological and microbiological methods, radioactive methods, physical methods and miscellaneous techniques like conventional titrimetric, gravimetric and polar metric methods.

Peptic ulcer occurs in the parts of the gastrointestinal tract which exposed to gastric acid and pepsin, i.e. the stomach and duodenum. The etiology of peptic ulcer is not clearly known. It result probably due to an imbalance between the aggressive (acid, pepsin, bile and H.pylori) and the defensive (gastric mucous and bicarbonate secretion, prostaglandin, nitric oxide, innate resistance of the mucosal cells) factors. A variety of psychosomatic, humoral and vascular derangement has been implicated and the importance of Helicobacter pylori infection as a contributor to ulcer formation and recurrence has been recognised. In gastric ulcer, generally acid secretion is normal or low. In duodenal ulcer, acid secretion is high in half of the patients but normal in the rest.

Materials and Method: LABINDIA UV-3000+, Standard Ofloxacin and Omeprazole purchase from Yarrow Chemical Mumbai.

Simultaneous equation method, First order derivative and Q-Analysis method and validation.

Experimental: (For Research Articles Only)- Simultaneous Equation Method

Selection of solvent: Selection of solvent was done on the basis of Solubility of drugs in different solvents. Ethanol, methanol, alcohol, chloroform and distilled water were selected for solubility study. Among all selected solvents both the drugs showed good and acceptable solubility in methanol and distilled water (20: 80). So, methanol and distilled water (20:80) was selected for further analysis.

Selection of analytical wavelengths: Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. OMP and OFL showed absorbance maxima at 302 nm and 289 nm respectively.

Preparation of stock solution

Omeprazole (OMP) standard stock solution: Standard OMP 100.0 mg was weighed and transferred to a 100 mL volumetric flask and dissolved in methanol and distilled water (20:80).The flask was shaken and volume was made up to the mark with methanol and distilled water (20:80)to give a solution containing 1000 µg/mL OMP.

Ofloxacin (OFL) standard stock solution: Standard OFL100.0 mg was weighed and transferred to a100 mL volumetric flask and dissolved in methanol and distilled water (20:80).The flask was shaken and volume was made up to the mark with methanol and distilled water (20:80) to give a solution containing 1000 µg/mL OFL.

Selection of analytical concentration ranges: From the standard stock solution of OMP, appropriate aliquots were pipette out in to 10 ml volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations 5, 10, 15, 20, 25 and 30µg/mL. Absorbance's for these solutions were measured at 302 nm. A calibration curve of absorbance against concentration was plotted.

Similarly, a series of standard solutions of concentration 5, 10, 15, 20, 25 and 30 µg/ml were prepared for OFL and their absorbance were measured at 289 nm. A standard calibration curve of absorbance against concentration was plotted. Both drugs followed the Beer Lamberts law in the

range of 5 - 30µg/mL and 5-30µg/mL for OMP and OFL respectively.

Calibration curve for the OMP (5 - 30 µg/mL)

Appropriate volume of aliquots, from standard OMP stock solution were transferred to different volumetric flasks of 10 mL capacity. The volume was adjusted to the mark with methanol and distilled water (20:80) to obtain concentrations of 5, 10, 15, 20, 25 and 30µg/ml. Absorbance spectra of each solution against methanol and distilled water (20:80) as blank were measured at 302 nm and 289 nm and the graph of absorbance against concentration was plotted. The regression equation and correlation coefficient was determined in each case.

Calibration curve for the OFL (5– 30 µg/mL)

Appropriate volume of aliquots, from standard OFL stock solution were transferred to different volumetric flasks of 10 mL capacity. The volume was adjusted to the mark with methanol and distilled water (20:80) to obtain concentrations of 5, 10, 15, 20, 25 and 30µg/ml. Absorbance spectra of each solution against methanol and distilled water (20:80) as blank were measured at 302 nm and 289 nm and the graph of absorbance against concentration was plotted. The regression equation and correlation coefficient was determined in each case.

Sample preparation for determination of OMP and OFL from combined dosage form

Twenty tablets were weighed and finely powdered. The powder equivalent to 20 mg OMP and 200 mg OFL was accurately weighed and transferred to volumetric flask of 100Ml capacity containing 25mLof the methanol and distilled water (20:80) and sonicate for 5 min. The flask was shaken and volume was made up to the mark with methanol and distilled water (20:80) to give a solution of 200 µg/mL OMP and 2000 µg/mL OFL. The above solution was carefully filtered through Whatmann filter paper (No.41 mm). 1 mL from this solution was diluted to 100 mL with methanol and distilled water (20:80) and used for the estimation of OMP and OFL.

Estimation of Omeprazole and Ofloxacin in combined dosage form

Absorbance spectra of each solution against the methanol and distilled water (20:80) were measured at 302 nm and 289 nm. The absorbance of each solution was substituted in the simultaneous equation to calculate the amount of the drug present.

Assay method

Assay was performed by using the formula

$$\text{Assay} = \frac{\text{sample area}}{\text{standard area}} \times \frac{\text{standard weight}}{\text{dilution}} \times \frac{\text{dilution}}{\text{sample weight}} \times \text{purity of standard} \times \text{average weight of tablet}$$

Validation of Spectrophotometric method

Accuracy: Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy, 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels i.e. 80%, 100% and 120% of the actual amount taking in to consideration percentage purity of added bulk drug samples.

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 of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV).

Precision study was conducted by preparing replicates of both the drugs simultaneously and were analysed at different time intervals. Six replicates of OMP of 35 µg/mL and six replicates of OFL of 35 µg/mL were prepared and their respective absorbance was measured at 302 nm and 289nm respectively, first initially, after 1, 2 and3 hr.

Repeatability: Six dilutions of 35 µg/mL of OMP were prepared and absorbance was measured at 301 nm and 289 nm taking the methanol and distilled water (20:80) as the blank. The absorbance of the same concentration solution was measured three times and standard deviation was calculated. In a similar manner 6 solutions of OFL of 35µg/mL were prepared and absorbance was

measured at 301 nm and 289 nm taking the methanol and distilled water (20:80) as the blank. The procedure was repeated six times and standard deviation was calculated.

Intra-day and inter-day precision: Variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed. Intra-day precision was determined by analyzing OMP and OFL individually for three times in the same day at 302 nm and 289 nm. Inter-day precision was determined by analyzing both the drugs individually daily once for two days at 302 nm and 289 nm.

Intra-day study was performed by preparing dilutions of 5 – 60 µg/mL of OFL and OMP taking their respective absorbance at 301 nm and 289 nm respectively, first initially, after 1, 2 and 3 hr.

Inter-day study was performed by preparing dilutions of 5 – 60 µg/mL of OFL and OMP and taking their respective absorbance at 302 nm and 289 nm respectively, on first day and on second day.

Reproducibility: The absorbance's was measured by another analyst and the values obtained were evaluated using t- test to verify their reproducibility.

Linearity and Range: The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower level so analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Dilutions of 5 – 30 µg/mL OMP and OFL were prepared and absorbance was taken of each dilution at 302 and 289 nm respectively.

Results and Discussions:- The overlain spectra of OFL and OMP at different concentrations revealed that different concentration of OFL possess maximum absorbance at 289 nm whereas OMP possess significant absorbance. In a similar manner, different concentrations of OMP possess maximum absorbance at 302 nm whereas OFL possesses significant absorbance (Fig. 1.1, 1.2). Considering above facts, wavelength 289 nm and 302 nm were selected for the estimation of OFL and OMP by spectrophotometer.

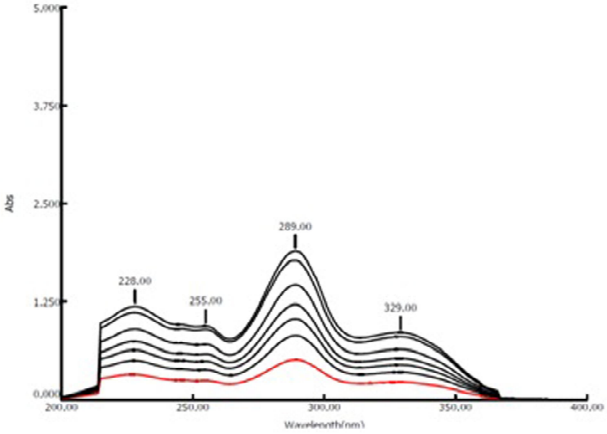


Fig1.1. Overlain spectra of ofl 5-30 µg/ml in methanol and distilled water (20:80)

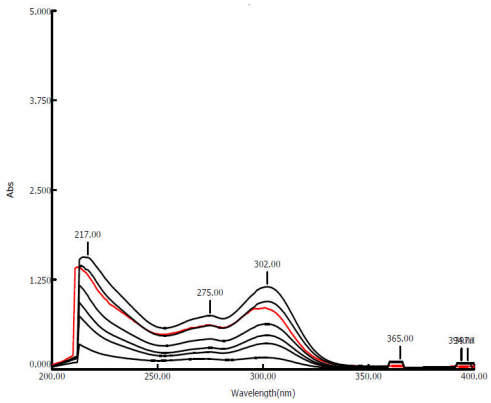


Fig: 1.2. Overlain spectra of omp 5-30µg/ml in methanol and distilled water (20:80).

Table: 1.1. Result of calibration curve for OFL at 289 and 302 nm in methanol: distilled water (20:80) by simultaneous equation method

Conc. µg/ml	OFL (289 nm)		OFL (302 nm)	
	Absorbance Mean ± Std. Deviation (n=6)	% CV	Absorbance Mean ±Std. Deviation (n=6)	% CV
5	0.239± 0.0008	0.37	0.296 ± 0.0014	0.4970
10	0.399 ± 0.0011	0.29	0.395 ± 0.0020	0.5234
15	0.552 ± 0.0010	0.18	0.497 ± 0.0024	0.4877
20	0.691± 0.0019	0.27	0.594 ± 0.0036	0.6064
25	0.818 ± 0.0016	0.19	0.705 ± 0.0015	0.0015
30	0.939± 0.0019	0.20	0.897 ± 0.0022	0.2510

Table: 1.2. Result of calibration curve for OMP at 289 and 302 nm in methanol: distilled water (20:80) by simultaneous equation method

Conc. µg/ml	OMP (289 nm)		OMP (302 nm)	
	Absorbance Mean ± Std. Deviation (n=6)	% CV	Absorbance Mean ±Std. Deviation (n=6)	% CV
5	0.290 ± 0.0007	0.25	0.240 ± 0.0008	0.34
10	0.397 ± 0.0010	0.26	0.404 ± 0.0008	0.20
15	0.496 ± 0.0007	0.15	0.562 ± 0.0016	0.28
20	0.590 ± 0.0008	0.13	0.690 ± 0.0009	0.14
25	0.697 ± 0.0015	0.21	0.818 ± 0.0044	0.53
30	0.796 ± 0.0008	0.10	0.940 ± 0.0008	0.08

Calibration data for OFL and OMP are shown in Table 1.1, 1.2. Calibration curves for OFL and OMP were plotted between absorbance and concentration. The following equations for straight line were obtained for OFL and OMP.

Linear equation for OFL at 289 nm, y = 0.0274x (r^2 = 0.9972) and y = 0.1363x (r^2 = 0.9981) at 302 nm. And OMP at 289 nm, y = 0.02x (r^2 = 0.9997) and y = 0.0201x (r^2 = 0.9996) at 302 nm.

Table: 1.3 Assay results of In house Formulation by simultaneous equation method

Formulation	Actual Concentration (mg)		% OMP	% OFL
	OMP	OFL		
Tablet 1	20	200	101.98	102.31
Tablet 2	20	200	101.21	102.01

Table: 1.4 Summary of validation parameters of spectrophotometry by simultaneous equation method

Parameter	OMP	OFL
Linear Range (µg/ml)	5-30	5-30
Slope	0.0274x	0.0201x
Standard deviation of slope	0.01904	0.0095
Limit of Detection (µg/ml)	2.29	1.56
Limit of Quantification (µg/ml)	2.86	2.72
Molar absorbtivity	1.4×10^4	3.2×10^4
Sandell's sensitivity	0.3513	0.4231
% Recovery		
Tablet 1	100.12 – 100.89	100.89 – 101.11
Tablet 2	100.11 – 101.01	100.57 – 101.67
Repeatability SD (n=6)		
At 289 nm	0.00580	0.00246
At 302 nm	0.00456	0.00472
Precision (% CV)		
At 289 nm		
Inter-day (n=6)	1.30-1.82	1.23-1.52
Intra-day (n=6)	0.95-1.60	1.13-1.52
At 302 nm		
Inter-day (n=6)	1.07 – 3.96	0.93-3.06
Intra-day (n=6)	0.40 - 2.37	1.62-3.41

Derivative Spectroscopy: First order derivative spectra of standard OFL and OMP, with a derivative interval of 1 nm. Zero crossing point of Ofloxacin was found at 289 nm and hence selected for estimation of Omeprazole. Zero crossing point of Omeprazole was found at 302 nm and hence selected for the estimation of Ofloxacin.

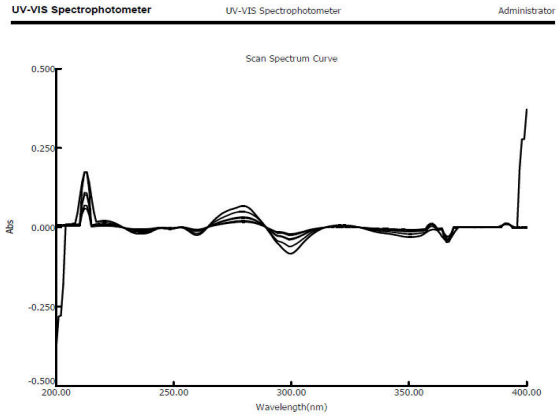
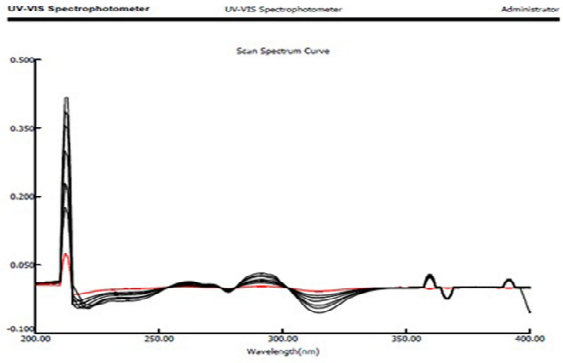


Fig: 1.3. First order spectra of OFL 5- 30 µg/mL in methanol and distilled water (20:80).



Calibration curves for OFL and OMP were plotted between absorbance and concentration. The following equations for straight line were obtained for OFL and omp. Linear equation for ofl at 289 nm, y = 0.0188x (r^2 = 0.9947) and OMP at 302 nm, y = -0.0016x (r^2 = 0.9899)

Table: 1.5 ruggedness results for OMP at 302 nm by first order derivative spectroscopy

Analyst 1	Analyst 2	Result of t-test*	Inference
-0.008 ± 0.0006	-0.007 ± 0.0006	0.9025	No significant difference

Table: 1.6 Assay results of in house formulation by first order derivative spectroscopy

Formulation	Actual concentration (mg)		% OMP	% OFL
	OFL	OMP		
Tablet 1	20	200	102.31	102.12
Tablet 2	20	200	102.09	101.96

Table: 1.7 Summary of validation parameters of spectrophotometer by first order derivative spectroscopy

Parameter	OFL	OMP
Linear range (µg/ml)	5-30	5-30
Slope	-0.0016x	-0.0018x
Standard deviation of slope	0.003848	0.007574
Limit of detection (µg/ml)	2.65	2.08
Limit of quantification (µg/ml)	4.75	3.92
Molar absorbitivity	2.76 x 10^4	3.85 x 10^4
Sandell's sensitivity	2.753	1.940
% recovery Tablet 1 Tablet 2	 100.06 – 100.11 100.11 – 100.86	 100.79 – 101.13 100.45 – 101.11
Repeatability sd (n=6) At 289 nm At 302 nm	 -------- 0.00076	 0.00114 --------
Precision % cv At 289 nm Inter-day (n=6) Intra-day (n=6) At 302 nm Inter-day (n=6) Intra-day (n=6)	 -------- -------- 1.33 – 1.53 1.34 – 1.87	 0.41 – 1.68 0.67 – 1.76 -------- --------

Q analysis OR Zero crossing method

For estimation of OFL and OMP using spectrophotometer, by q analysis method two wavelengths are required. One wavelength is selected at which either of drug shows maximum absorbance, second wavelength is selected where both the drugs shows same absorbance i.e. Isoabsorption point. Two isoabsorption points were observed in overlain spectra of OFL and OMP 289 nm and 302 nm. Using 297 nm as isoabsorption point significant results were obtained. Considering above fact wavelength 289 nm and 297 nm were selected for the estimation of OFL and OMP by spectrophotometer.

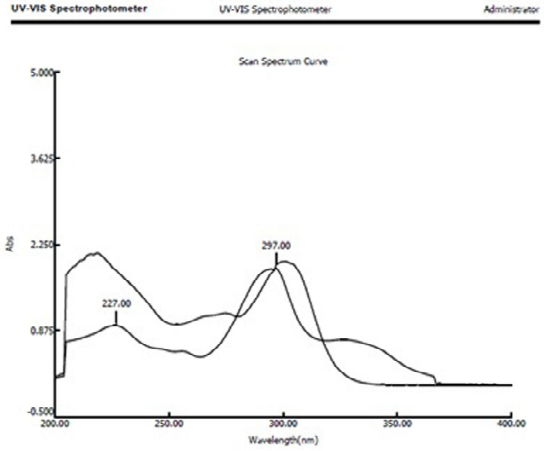


Fig: 1.4. Overlain spectra of OFL 50 µg/ml and OMP 50 µg/ml in methanol and distilled water (20:80).

Table: 1.8 result of calibration curve for ofl at 289 and 297 nm in methanol and distilled water by q-analysis method.

conc. µg/ml	OFL 289 nm		OFL 297 nm	
	Absorbancemean ± std. Deviation (n=6)	% cv	Absorbancemean ± std. Deviation (n=6)	% cv
5	0.193 ± 0.1933	0.29	0.200 ± 0.0005	0.28
10	0.297 ± 0.0015	0.51	0.381 ± 0.0005	0.15
15	0.481 ± 0.0010	0.20	0.565 ± 0.0080	1.42
20	0.798 ± 0.0005	0.07	0.754 ± 0.0005	0.37
25	0.997 ± 0.0005	0.05	0.932 ± 0.0011	1.12

Table: 1.9 result of calibration curve for OMP at 289 and 297 nm in methanol and distilled water by q-analysis method.

conc. µg/ml	OMP 289 nm		OMP 297 nm	
	Absorbancemean ± std. Deviation (n=6)	% cv	Absorbancemean ± std. Deviation (n=6)	% cv
5	0.084 ± 0.0005	0.68	0.178 ± 0.0005	0.32
10	0.172 ± 0.0011	0.66	0.332 ± 0.0020	0.62
15	0.249 ± 0.0005	0.23	0.499 ± 0.0005	0.11
20	0.328 ± 0.0005	0.01	0.655 ± 0.0005	0.48
25	0.411 ± 0.0011	0.28	0.815 ± 0.0005	0.67

Calibration data for ofl and omp are shown in table 1.27 and 1.28. Calibration curves for ofl

and omp were plotted between absorbance and concentration. The following equations for straight line were obtained for ofl and omp.

Linear equation for OFL: at 289 nm, y = 0.0328x (r^2 = 0.9911); at 297 nm, y = 0.0367x (r^2 = 0.9999) and OMP at 302 nm, y = 0.0162x (r^2 = 0.9996); at 297 nm, y = 0.0319x (r^2 = 0.9999).

Table: 1.10 reproducibility results for OFL and OMP by q-analysis method:

Drugs	Analyst 1	Analyst 2	Result of T-test*	Inference
Ofl 289 nm	0.193 ± 0.0035	0.189 ± 0.0020	0.9792	No significant difference
Ofl 296 nm	0.085 ± 0.0035	0.091 ± 0.0020	0.9449	No significant difference
Omp 289 nm	0.200 ± 0.0025	0.194 ± 0.00331	0.9772	No significant difference
Omp 296 nm	0.178 ± 0.0070	0.175 ± 0.0040	0.9709	No significant difference

Where *n=6 at 95% confidence level.

Table: 1.11 Assay results of in house formulation by q-analysis method:

Formulation	Actual concentration (mg)		% OMP	% OFL
	Ofl	Omp		
Tablet 1	20	200	102.65	102.32
Tablet 2	20	200	102.41	101.78

Table: 1.12 summary of validation parameters of spectrophotometry by q-analysis method:

Parameter	OFL		OMP	
	289	296	289	296
Linear range (µg/ml)	5-25	5-25	5-25	5-25
Slope	0.032x	0.016x	0.031	0.032x
Standard deviation of slope	0.000153	0.000135	0.0000	0.000153
Limit of detection (µg/ml)	1.97	1.75	2.64	1.18
Limit of quantification (µg/ml)	3.32	2.53	3.84	2.96
Molar absorbtivity	2.53×10^3	1.09×10^4	3.84×10^4	1.64×10^3
Sandell's sensitivity	0.7647	1.7317	0.6493	1.9631
% recovery Tablet 1 Tablet 2	 100.03 – 100.45 100.16 - 101.53		 100.97 – 101.16 100.97 – 101.73	
Repeatability				
sd (n=6) At 225 nm At 235.5 nm	0.0058 0.0054		0.0045 0.0053	
Precision % cv at 225 nm Inter-day (n=6) Intra-day (n=6) At 235.5 nm Inter-day (n=6) intra-day (n=6)	 1.03 – 1.82 0.95 – 1.60 1.60 – 2.11 1.23 – 2.01		 1.23 – 1.52 1.13 – 1.52 1.30 – 2.93 1.67 – 2.55	

Discussion

Linearity: The simultaneous estimation method demonstrated good linearity over the range of 5-30 µg/mL with a correlation coefficient for Ofloxacin at 289 nm (r^2 = 0.9972) and (r^2 = 0.9981) at 302 nm and Omeprazole at 289 nm (r^2 = 0.9997) and (r^2 = 0.9996) at 302 nm. In Derivative spectroscopy, Linearity for Ofloxacin at 289 nm (r^2 = 0.9911) and (r^2 = 0.9999) at 297 nm and Omeprazole at 302 nm (r^2 = 0.9996) and (r^2 = 0.9999) at 297 nm. In Zero crossing method, Linearity for Ofloxacin at 289 nm (r^2 = 0.9911) and (r^2 = 0.9999) at 297 nm and Omeprazole at 302 nm (r^2 = 0.9996) at 297 nm (r^2 = 0.9999) as shown.

Accuracy: Simultaneous estimation method, accuracy of the proposed method was assessed by determining the average recoveries of samples using the standard addition method. the mean percentage recovery of omeprazole and ofloxacin was 101.11, 100.86 % respectively. In Derivative spectroscopy, percentage recovery of omeprazole and ofloxacin was 101.13, 100.86 % respectively., In Zero crossing method , percentage recovery of omeprazole and ofloxacin was 101.73, 101.53 % respectively. The accuracy value of the current method was excellent.

Precision: Simultaneous estimation method, precision (% CV) for omeprazole, 1.30-182 (Inter day) 0.95-160 (Intraday) at 289 and 1.07-3.96 (Inter day), 0.40-2.37 (Intraday) at 302 nm, for ofloxacin precision in derivative spectroscopy was found 1.23-1.52 (Inter day) 1.13-1.52 (Intraday) at 289 and 0.93-3.06 (Inter day), 1.62-3.41 (Intraday) at 302 nm. In first order derivative spectroscopy, omeprazole was found 0.41-1.68

(Inter day) 0.67-1.76 (Intraday) at 289, for ofloxacin, 1.33-1.53 (Inter day), 1.34-1.87 (Intraday) at 302 nm. In Q-Analysis method, Omeprazole shown at 225 and 1.30-2.93 (Inter day), 1.67-2.55 (Intraday) at 235 nm, for Ofloxacin 1.03-1.82 (Inter day) 0.95-160 (Intraday) at 225 and 1.60-
2.11 (Inter day), 1.23-2.01 (Intraday) at 235 nm.

Repeatability: Simultaneous estimation method, repeatability for omeprazole 0.0045 at 225 and 0.0053 at 235 nm, for Ofloxacin 0.0058 at 225 and 0.0054 at 235 nm. In first order derivative spectroscopy, repeqtibility for omeprazole was found 0.0045 at 225 and 0.0053 at 235 nm, for Ofloxacin 0.0058 at 225 and 0.0054 at 235 nm.

In Q-Analysis method, Omeprazole shown 0.0045 at 225 and 0.0053 at 235 nm, for Ofloxacin 0.0058 at 225 and 0.0054 at 235 nm shown.

LOD and LOQ: In simultaneous estimation method, LOD values were found to be 2.29 and 1.56. LOQ values were 2.86, 2.72 for OFL and OMP respectively, in first order derivatives, LOD values were found to be 2.65 and 2.08. LOQ values were 4.75, 3.92 for OMP and OFL respectively, In Q Analysis method, LOD values were found to be 1.97 and 2.64. LOQ values were 3.32, 3.84 for OMP and OFL respectively.

Assay: The validated method was applied to the determination of Omeprazole and Ofloxacin were analyzed. The results, expressed as the percentage drug as related to the content label claim, are shown. In house Formulation by simultaneous equation method showed that the percentages were found 101.98, 101.21 of OMP and 102.31, 102.01 of OFL. In first order derivative spectroscopy method, percentages were found 102.31, 102.09 of OMP and 102.12, 101.96 of OFL, In Q-Analysis method, percentages were found 102.65, 102.41 of OMP and 102.32, 101.78 of OFL shown.

Conclusion: Ofloxacin is intermediate between ciprofloxacin and norfloxacin in the activity against gram- negative bacteria, but it is comparable to or more potent than ciprofloxacin for gram-positive organisms and certain anaerobes. Good activity against *chlamydia* and *mycoplasma* has been noted: it is an alternative drug for non-specific urethritis, cervicitis and atypical pneumonia. It also inhibits m. Tuberculosis; can be used in place of ciprofloxacin. It is highly active against *m. Leprae* is being used in alternative multidrug therapy regimens

Out of the three UV methods, The Simultaneous Equation Method involves only measurement of absorbance's at selected wavelengths and solving of simultaneous equation, the first order derivative method has the advantage that it eliminates the spectral interference from one of the two drugs while estimating the other drug by selecting zero crossing point on the derivative spectra of each drug at selected wavelength. As the Q-Analysis method employs the measurement of ratio of absorbances at isoabsorption point and wavelength maxima of the one drug, the error involved are minimized.

Three simple, sensitive, accurate and precise spectrophotometric methods via simultaneous equation, first order derivative and Q-Analysis methods have been developed for the purpose. In addition to positive requirements for analytical methods, the striking advantage of all the presently developed methods is that they are economical. These methods are validated in terms of sensitivity, accuracy and precision.

Conflict of Interest: It is hereby declared that this paper does not have any conflict of interest.

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