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Simultaneous Estimation of Quinine and Ofloxacin in Bulk by Using UV-Spectroscopic Methods

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ABSTRACT

The simple, accurate, precise, reproducible and economic UV spectroscopic methods of quinine and ofloxacin have been developed for the simultaneous estimation. The method A-Dual wavelength, method B-Area under curve and method C-Absorption ratio method were developed by using JASCO double beam UV Spectrophotometer (model-V630). As simultaneous estimation was carried out, measurement of absorbance at two wavelengths 234nm and 244nm which show λ_{max} values of quinine and ofloxacin respectively in distilled water. Quinine and ofloxacin show linearity at selected wavelengths and obeys Beers law in the concentration range of 5-30 µg/ml with good correlation coefficient 0.992 and 0.998 respectively. For the area under the curve, the wavelength selected for quinine was from 228nm to 238nm and for ofloxacin 230nm to 257nm which gives peak area for quinine 0.5852 and for ofloxacin 6.5388. For the method C involved formation of isoabsorptive point at 242nm.

1. INTRODUCTION

Quinine and ofloxacin are the drugs used in the treatment of malaria and typhoid fever respectively. Quinine is cinchona alkaloid that acts as a blood schizonticidal and weak gametocyte against *Plasmodium vivax* and *Plasmodium malariae*. As an alkaloid, it is accumulated in the blood vacuoles of *Plasmodium* species. Quinine is less effective and more toxic as a blood schizonticidal agent than chloroquine. However, still it is very effective and widely used in the treatment of acute cases of severe *P. falciparum*. It is especially useful in areas where there is known to be a high level of resistance to chloroquine[(1)]. Ofloxacin (OFL) is a second generation fluoroquinolone acting as antimicrobial agent. Ofloxacin has *in-vitro* activity against a wide range of gram-negative and gram-positive microorganisms. The bactericidal action of ofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair and recombination[(2)].

Quinine sulphate is an antimalarial drug chemically described as cinchonan-9-ol, 6'methoxy-(8α, 9R)-sulphate (2:1) (salt), dihydrate with molecular formula of $(C_{20}H_{24}N_2O_2)2.H_2SO_4.2H_2O$ and a molecular weight of 782.96[(3)]. Ofloxacin chemically described as 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo [7.3.1.0 {5,13}]trideca 5(13),6,8,11-tetraene-11-carboxylic acid[(4-5)]. Its molecular formula is $C_{18}H_{20}FN_3O_4$ with molecular weight 361.368g/mol [(6)].

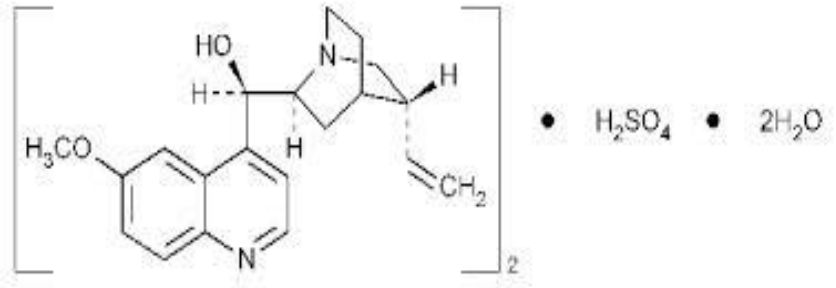


Figure 1. Structure of quinine sulphate

Figure 2. Structure of ofloxacin

There are so many methods described for the quinine and ofloxacin individually. However, there is no method reported for simultaneous estimation of both drugs.

2. MATERIALS AND METHODS

UV-visible double beam spectrophotometer Jasco Model V-630 with spectral bandwidth of 2nm, wavelength accuracy of 0.3nm and a pair of 10nm matched quartz cell was used. The API used as ofloxacin, quinine sulphate and chemicals as distilled water was obtained from the institute P.D.V.V.P. College of Pharmacy, Ahmednagar.

2.1. Preparation of Stock Solution:

Ofloxacin 10mg was weighed by using electronic balance (Model ShimadzuAUW-220D) and dissolves in a small amount of distilled water and then completely dissolved. After this, the solution was adjusted to 100 ml in volumetric flask so that the solution of 100 µg/ml was prepared. Like this, the stock solution of quinine sulphate was also prepared by dissolving 10mg of the drug into distilled water. For complete solubility, the solution was sonicated for 2 min by using ultrasonic bath sonicator (model 5.5L150H) and then adjusted the volume up to 100 ml in volumetric flask.

2.2. Preparation of Sample:

From the stock solution of ofloxacin and quinine sulphate, the standard solutions of both were prepared. For ofloxacin the standards were prepared as 5-35 µg/ml by taking 0.5 ml of stock solution adjusted to 10 ml by using distilled water to prepare 5 µg/ml, 1 ml of stock solution was

adjusted to 10 ml to get 10 µg/ml, 1.5 ml of stock solution was adjusted to 10 ml to get 15 µg/ml, 2 ml of stock solution was adjusted to 10 ml to get 20 µg/ml, 2.5 ml of stock solution was adjusted to 10 ml to get 25 µg/ml, 3 ml of stock solution was adjusted to 10 ml to get 30 µg/ml and 3.5 ml was adjusted to 10 ml to get 35 µg/ml. After this, the samples for quinine sulphate were prepared as 5-35 µg/ml. The dilutions were prepared as 0.5 ml from stock were taken and diluted up to 10 ml so as to get the solution of 5 µg/ml, 1 ml stock solution were adjusted to 10 ml to get the solution of 10 µg/ml, 1.5 ml stock solution were taken and adjusted to 10 ml to get 15 µg/ml, 2 ml of stock solution were taken and adjusted to 10 ml to get 20 µg/ml, 2.5 ml of stock solution were taken and adjusted to 10 ml to get 25 µg/ml, 3 ml of stock solution were taken and adjusted to 10 ml to get 30 µg/ml and 3.5 ml of stock solution were taken and adjusted to 10 ml to get 35 µg/ml.

2.3. Measurement of Readings:

After sample preparation readings were taken by using Jasco make double beam UV- visible spectrophotometer. For the measurement of samples, the blank readings were taken for correction of baseline. For measurement of λ_{max} 20 µg/ml samples of quinine sulphate and ofloxacin were taken. It shows the λ_{max} of quinine sulphate and ofloxacin as 234nm and 244nm respectively. And the sample readings were taken by adjusting fixed wavelength at 234nm and 244nm.

Method A. Dual Wavelength Method:

If a sample contains two absorbing drugs of each of which absorbs at the λ_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equations.

$$C_x = A_2\, ay_1 - A_1 ay_2 / ax_2\, ay_1 - ax_1\, ay_2 \quad (1)$$

$$C_y = A_1\, ax_2 - A_2\, ax_1 / ax_2\, ay_1 - ax_1\, ay_2 \quad (2)$$

Method B. Area under Curve:

For the simultaneous estimation using the area under curve method, suitable dilutions of the standard stock solutions (100 µg/ml) of quinine sulphate and ofloxacin were prepared separately in distilled water. The solutions of drugs were scanned in the range of 200-400nm. For area under the curve method, the sampling wavelength ranges selected for estimation of quinine

sulphate and ofloxacin were 228-238nm (λ_1-λ_2) and 230-257nm (λ_3-λ_4). Mixed standards were prepared and their area under the curve was measured at the selected wavelength ranges. Concentration of two drugs in mixed standard and the sample solution were calculated using equation (3) and equation (4).

$$C_x = A_2 ax_2 - A_1 ay_2 / ax_2 ay_1 - ax_1 ay_2 \qquad (3)$$

$$C_y = A_2 - ax_2 \times C_x / ay_2 \qquad (4)$$

Method C. Absorbance Ratio Method:

Absorbance ratio method is the uses of absorbance at two selected wavelengths; one of them is isoabsorptive point and second is λ_{max} of any of the drugs. From the overlain spectra of the two drugs quinine sulphate and ofloxacin show isoabsorptive point as 242nm. The second wavelength is 244nm which is the λ_{max} of ofloxacin. Working standard solutions having the concentration range of 5-35 µg/ml in distilled water. And the absorbance at isoabsorptive point and λ_{max} of ofloxacin were taken.

The concentrations of two drugs were measured by using the equations (5) and (6).

$$C_x = \{(Q_m - Q_y) / (Q_x - Q_y)\} \times A_1 / ax_1 \qquad (5)$$

$$C_y = \{(Q_m - Q_x) / (Q_y - Q_x)\} \times A_1 / ay_1 \qquad (6)$$

Where A_1 and A_2 are absorbance's of mixture at 242nm and 244nm, ax_1 and ay_1 are absorptivities of quinine and ofloxacin at 244nm, ax_2 and ay_2 are absorptivities of quinine and ofloxacin at 234nm, $Q_m = A_2/A_1$, $Q_x = ax_2/ax_1$, $Q_y = ay_2/ay_1$.

2.4. Validation of Proposed Methods

The proposed methods are validated according to the International Conference on Harmonization (ICH) guidelines [7].

2.4.1. Linearity

The calibration curves were taken for both quinine and ofloxacin at 234nm and 244nm. Both the drug shows linearity and obeys Beer's Law in the concentration range of 5-30 µg/ml. The correlation coefficient of calibration curves were 0.992 and 0.998.

2.4.2. Precision

Relative standard deviations (% R.S.D.) for intraday and interday were calculated as precision study.

2.4.3. Limit of Detection

The limit of detection was determined using the formula:

$$LOD = 3.3\sigma/S$$

Where, LOD is Limit of Detection, σ is the standard deviation, S is the slope of the calibration curve. LOD was found to be 0.3548 and 0.6600 for quinine and 0.5689 and 0.4852 for ofloxacin at the wavelength 234nm and 244nm.

2.4.4. Limit of Quantitation

The limit of quantitation was determined using the formula:

$$LOQ = 10\ \sigma/S$$

Where, LOQ is Limit of Quantitation, σ is the standard deviation, S is the slope of the calibration curve. LOQ was found to be 1.0750 and 2.0000 for quinine and 1.7241 and 1.4705 for ofloxacin at the wavelength 234nm and 244nm.

3. RESULTS AND DISCUSSION

The combination of quinine and ofloxacin used to treat two diseases, the patient suffering simultaneously. The present work gives a very novel, simple, accurate and economic method for the simultaneous estimation of both drugs. Both drugs show linearity at the wavelength selected 234nm for quinine and 244nm for ofloxacin and validated according to the ICH guidelines. Quinine and ofloxacin show linearity at selected wavelengths and obeys Beers law in the concentration range of 5-30 µg/ml with good correlation coefficient 0.992 and 0.998 respectively. For the area under the curve the wavelength selected for quinine was from 228nm to 238nm and for ofloxacin 230nm to 257nm which gives the peak area for quinine 0.5852 and for ofloxacin 6.5388. For the method C involved formation of isoabsorptive point at 242nm.

4. CONCLUSION

The developed UV- method was novel, specific, reproducible, eco-friendly, cost effective, fast and reproducible for simultaneous estimation of quinine sulphate and ofloxacin in bulk mixture. The method utilizes simple sample preparation, short analysis time. It is concluded that this method can be adopted by the industries and academic institutions for their combination drug estimation. It shows the novelty and utility of the overall work.

REFERENCES

1. Vugt M, Brockman A, Gemperli B, Luxemburger C, Gathmann I, Royce C, Slight T, Looareesuwan S, White N J, Nosten F. A randomised comparision of artemether-benflumetol and artesunate-mefloquine in the treatment of multidrug resistant falciparum malaria. Antimicrobe Agents Chemother, 1998, 135-139.
2. Drlica K, Zhao X; Zhao. "DNA gyrase, topoisomerase IV, and the 4-quinolones". *Microbiol Mol Biol Rev*. PMC 232616. PMID 9293187, 1997, 61 (3): 377–92.
3. Indian Pharmacopoeia, Government of India Ministry of Health and Family Welfare, Published by the Indian Pharmacopoeia Commission, GAZIABAD, Volume III, 2010:1092-1093.
4. Tripathi KD. Essentials of Medical Pharmacology, Jaypee Publisher, New Delhi. 6th Edn: 2008, 688, 798.
5. Indian Pharmacopoeia, Controller of Publication, Govt. of India Ministry of Health and Family Welfare, Published by the Indian Pharmacopoeia Commission, GAZIABAD, Volume III, 2010, 1808-11, 1823.
6. Nelson, JM.; Chiller, TM; Powers, JH.; Angulo, FJ. "Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: a public health success story". *Clin Infect Dis* 44 (7): doi:10.1086/512369. PMID 17342653, 2007, 977–80.
7. ICH, Q_2 (R_1) Validation of Analytical Procedure: Text and Methodology, International Conference on Harmonization, 2005.

Table No. 1. Optimized method parameters for dual-wavelength spectroscopy

Sr.No.	Method Parameters	Optimized Parameters
1.	Solvent	Distilled water
2.	Scanning Range	200nm to 400nm
3.	Scan Speed	Medium
4.	Analytical wavelength for determination of quinine	234nm and 244nm
5.	Analytical wavelength for determination of ofloxacin	234nm and 244nm

Table No. 2. Linearity

Quinine (Conc.µg/ml)	Absorbance		Ofloxacin (Conc.µg/ml)	Absorbance	
	234nm	244nm		234nm	244nm
5	0.5978	0.3316	5	0.3363	0.3812
10	0.9284	0.5162	10	0.6127	0.7090
15	1.4115	0.7977	15	0.9562	1.1081
20	1.9234	1.0824	20	1.2097	1.4020
25	2.3426	1.3151	25	1.5230	1.7552
30	2.6469	1.5156	30	1.7969	2.0873
35	2.7971	1.5305	35	2.0592	2.4091

Table No. 3. Intraday Precision studies

Sr. No.	Concentration of drug (µg/ml)	Absorbance difference		SD		%RSD	
		Quinine	Ofloxacin	Quinine	Ofloxacin	Quinine	Ofloxacin
1.	10 (n=3)	0.9283 0.9182 0.9080	0.7090 0.6989 0.6890	0.01	0.01	1.11	1.43
2.	15 (n=3)	1.4115 1.4012 1.4014	1.1081 1.1180 1.1079	0.0058	0.0057	0.42	0.52
3.	20 (n=3)	1.9233 1.9132 1.9232	1.4020 1.3919 1.4019	0.0058	0.0058	0.30	0.30

Limit: % RSD for area NMT 2.0%

Table No. 4. Interday precision studies

Day	Concentration of drug (µg/ml)	Absorbance difference		SD		%RSD	
		Quinine	Ofloxacin	Quinine	Ofloxacin	Quinine	Ofloxacin
1	10 (n=3)	0.9282 0.9180 0.9079	0.7088 0.6988 0.6889	0.01	0.009	1.11	1.42
2	15 (n=3)	1.4114 1.4011 1.4013	1.1080 1.1180 1.1078	0.005	0.005	0.42	0.52
3	20 (n=3)	1.9232 1.9133 1.9230	1.4019 1.3920 1.4018	0.005	0.005	0.29	0.41

Limit: % R.S.D. for area NMT 2.0%

Table No. 5. Limit of Detection and Limit of Quantification

Parameters	Quinine		Ofloxacin	
	At 234nm	At 244nm	At 234nm	At 244nm
LOD(µg/ml)	0.3548	0.6600	0.5689	0.4852
LOQ(µg/ml)	1.0750	2.0000	1.7241	1.4705

Table No. 6. Regression Characteristics

Parameters	At 234nm	At 244nm
Beer's Law Range	5-30 µg/ml	5-30 µg/ml
Regression Equation (y= mx + c)	y = 0.093x - 0.031	y = 0.068x +0.030
Slope (m)	0.093	0.068
Intercept (c)	-0.031	0.030
Correlation Coefficient (R^2)	R^2 = 0.992	R^2 = 0.998

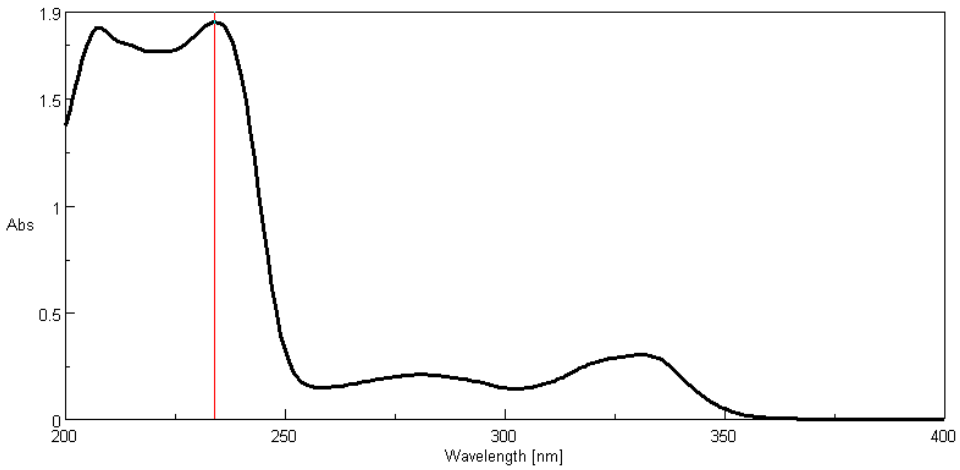


Figure 3. Spectra of quinine sulphate in distilled water

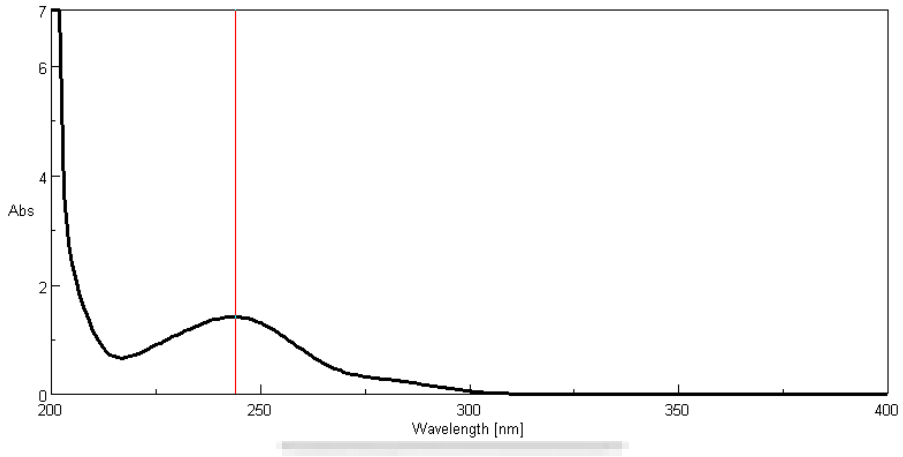


Figure 4. Spectra of ofloxacin in distilled water

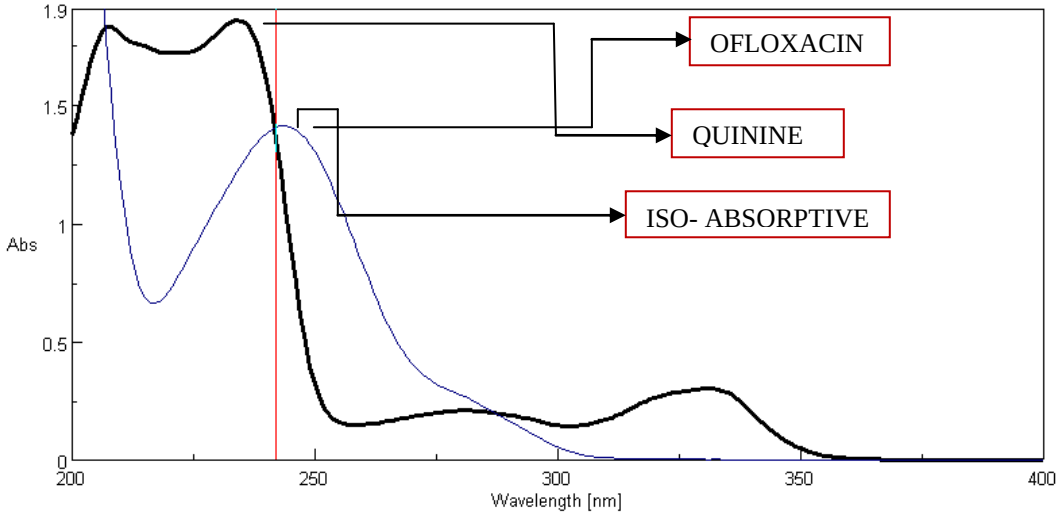
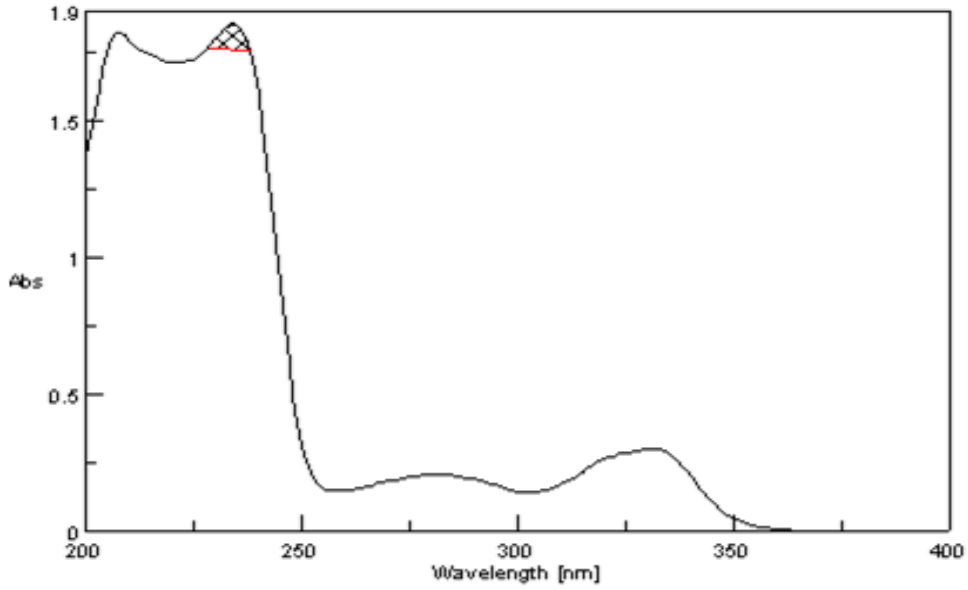


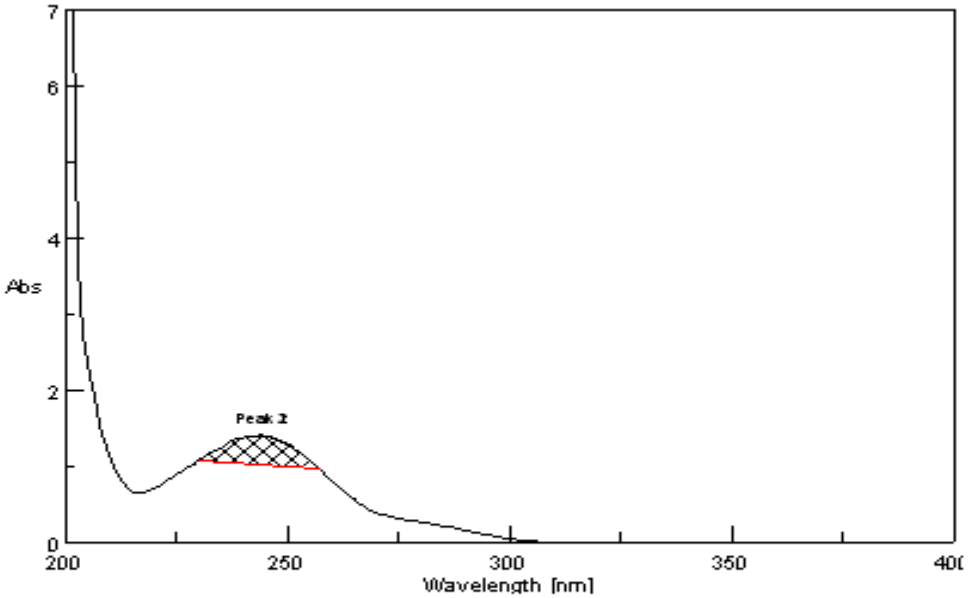
Figure 5. Overlain absorption spectra of quinine and ofloxacin showing isoabsorptive point (242nm) in distilled water



[Result of Peak Area Calculation]

	Calc Range		Base Range		Area	Ratio
Peak 1	----	----	228	238	0.58582	1
Peak 2	----	----	228	238	0.58582	1

Figure 6. Area under curve of quinine sulphate



[Result of Peak Area Calculation]

	Calc Range		Base Range		Area	Ratio
Peak 1	----	----	230	257	6.53883	1
Peak 2	----	----	230	257	6.53883	1

Figure 7. Area under curve of ofloxacin

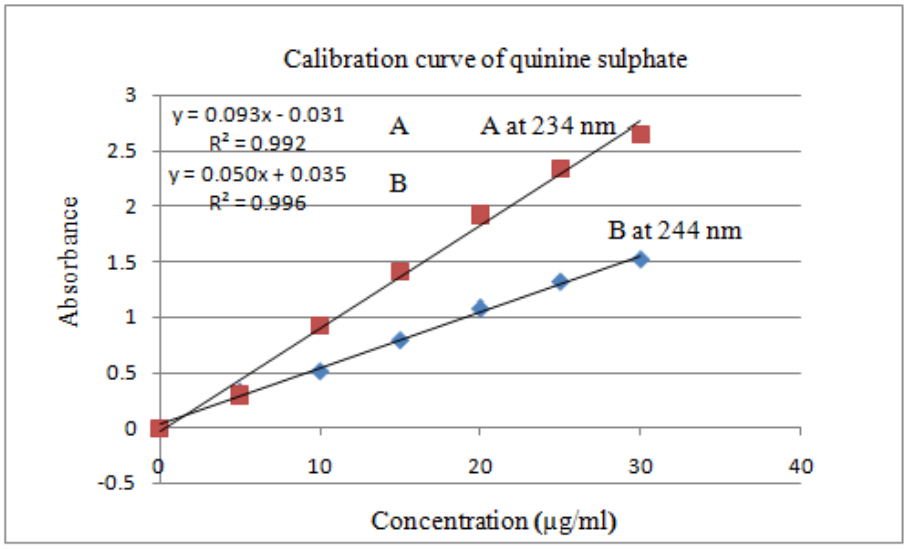


Figure 8. Standard calibration plot of quinine sulphate in distilled water

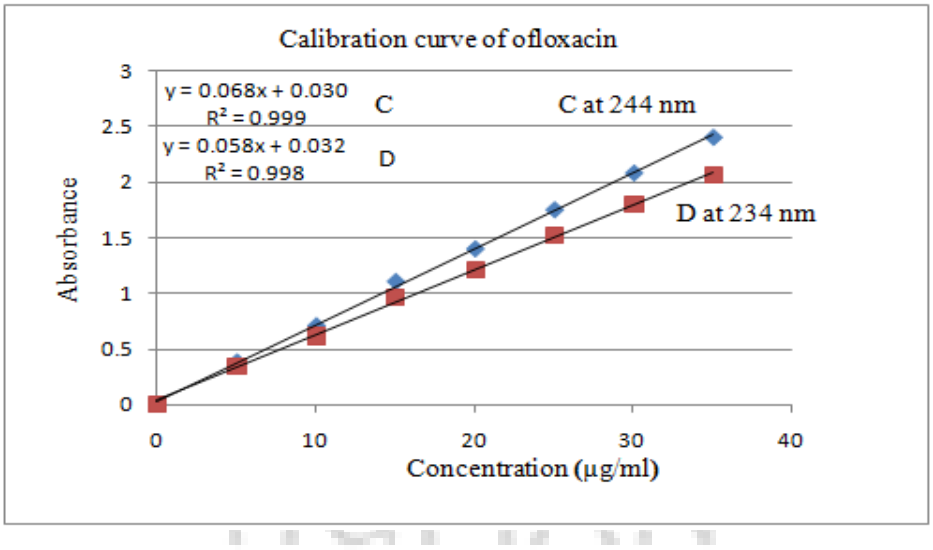


Figure 9. Standard calibration plot of ofloxacin in distilled water

Table No. 7. Peak areas of quinine sulphate and ofloxacin by AUC

Area under curve for quinine			
Peak	**Wavelength Range**	**Area**	**Area Ratio**
Peak 1	228-238	0.5858	1
Peak 2	228-238	0.5858	1
Area under curve for ofloxacin			
Peak 1	230-257	6.5388	1
Peak 2	230-257	6.5388	1