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ESTIMATION OF CIPROFLOXACIN AND ORNIDAZOLE BY UV- SPECTROSCOPY, RP-HPLC AND THEIR APPLICATION IN PHARMACOKINETIC STUDIES

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Abstract

To develop a simple method for estimation of ciprofloxcin and ornidazole by UV spectroscopy.

Estimation was done in aqueous stock solutions of ciprofloxcin and ornidazole at λmax of 271 and 321nm respectively. The isobestic point was found to be at 294nm.

The linearity of both drugs was plotted at 1-7 and 2-14µg/ml in aqueous stock solution with regression coefficient of 0.994 and 0.997 respectively. Assay results show from the above method was found to be 100.05%and 100.16% comparatively. Pharmacokinetic study showed 0-48hr time interval window with $t^{1/2}$ of 8hr and various parameters.

The above method was performed based on the root principles of ICH Guidelines and found prudent to be used in routine QC analysis.

Keywords: Ciprofloxcin, ornidazole, UV spectroscopy, pharmacokinetic study

Introduction

Ultraviolet and visible spectrometers have been in general use for the last 3-decades and over this period have become the most important analytical instrument in the modern day laboratory. In many applications other techniques could be employed but none rival UV-Visible spectrometry for its simplicity, versatility, speed, accuracy and cost-effectiveness.

Beer-Lambert Law

The Beer-Lambert Law states that the concentration of a substance in solution is directly proportional to the absorbance of the respective compound.

$$A \infty C$$

Where A- Absorbance of the compound

C- Concentration of the compound

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and excipients.

Accuracy

Accuracy is the closeness of the test results obtained by the analytical method to the true value. The percentages of recoveries were calculated.

Linearity

Linearity test solutions for the related substance method were prepared by diluting stock solutions to the required concentrations. Correlation coefficient, value for the slope, Y-intercept and % bias of the calibration curve was calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolutions between all peaks were recorded; and system suitability parameters were recorded. The variable evaluated in the study was pH of the mobile phase (±0.2), column temperature (±5°C) and flow rate (±0.2).

Precision

The precision of the related substances method was checked by evaluating six individual preparations of standard solution of bulk. Calculations were performed with respect to respective analyte concentration. %Relative Standard Deviation of area (HPLC) and difference in absorbance (UV)for each solution was calculated.`

Limits of Detection and Quantification

The LOD and LOQ for analyte were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

LOD=3.3 (SD/S)

LOQ=10 (SD/S)

SD-Standard Deviation

S - Slope

Drug profile

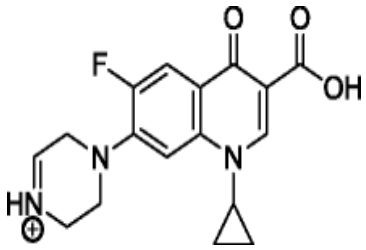

Fig.1. Chemical structure of Ciprofloxacin.

Chemical name: 1 - cyclopropyl - 6 - fluoro - 1, 4 - dihydro - 4 - oxo - 7 - (1- piperazinyl) - 3 - quinoline-carboxylic acid. Molecular formula: $C_{17}H_{18}FN_3O_3$.Molecular weight: 331.4 Category: broad spectrum antibiotic. Description: pale yellow, slightly hygroscopic, crystalline powder. Solubility: soluble in water, very slightly soluble in alcohol,insoluble in acetone and in ethyl acetate. Melting point: 255-257°C. Absorbance: 271nm. Bioavilability: 69%.Dosage For adults 3000 mg / kg and for children 60 mg / kg body weight. Half-life: 4 hours . pka: 6.09. Storage: Ciprofloxacin should be stored at room temperature.

Mechanism of Action: The bactericidal action of ciprofloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination.

Uses: Urinary tract infections, Respiratory infections, Otitis, Anthrax, Cervicitis.

Side effects: The most common side effects are dizziness, fainting, fast or pounding heartbeat; sudden pain or swelling near joints (especially in arm or ankle); diarrhea that is watery or bloody; confusion.

Drug Interactions: Administration of ciprofloxacin with theophylline lead to elevated serum concentrations of theophylline and prolongation of its elimination half-life. This may result in increased risk of theophylline related adverse reactions. Ciprofloxacin, have also been shown to interfere with the metabolism of caffeine this may lead to reduced clearance of caffeine and a prolongation of its serum half-life.

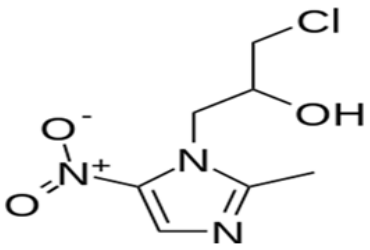

Fig.2. Chemical structure of Ornidazole.

Structural Formula: 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol. Molecular formula: $C_7H_{10}ClN_3O_3$. Molecular weight:219.625. Category: Anti protozoal. Description: solid Solubility: Soluble in Chloroform and Methanol. Melting point: 77-78°CpKa: 2.4 ± 0.1 Absorbance: 319 nm Bioavilability: 85%Half-life:12 Hours. Storage: Store at room temperature

Dosage: 500mg to 1500mg daily for 1 to 7 days

Mechanism of action: Ornidazole is a 5-nitroimidazole derivative active against protozoa and anaerobic bacteria. It is converted to reduction products that interact with DNA to cause destruction of helical DNA structure and strand leading to a protein synthesis inhibition and cell death in susceptible organisms.

Uses: Treatment of parasitic infections, Amebiasis, Giardiasis, Trichomonas vaginalis

Side effects: Somnolence, headache, nausea, vomiting, dizziness, tremor, rigidity, poor coordination, seizures, tiredness, vertigo, temporary loss of consciousness and signs of sensory or mixed peripheral neuropathy, taste disturbances, abnormal LFTs, skin reactions.

Drug interactions: Potentiates effect of coumarin-type oral anticoagulants. Prolongs the muscle-relaxant effect of vecuronium bromide.

Solvents and Chemicals

Double Distilled Water was prepared in college premises, Sodium Hydroxide, methanol, analytical grade was from Finar (Ahmedabad, India), hcl from ranbaxy(newdelhi) and Formulation was obtained from the local market.

Equipment

A double beam Spectrophotometer from shimadzu with 1.7 version and sample cells of 1cm pathlength cuvettes made of quartz. Isocratic RP-HPLC was performed using a cyber lab chromatograph, equipped with high-pressure isocratic pump (type LC- 100), a Rheodyne model injector (sample loop 20µL) and LC-UV100 UV Detector (operated at 254nm) controlled by HCL PC Pentium D computer .The analytical column was a cap cell pak C-18, 4.6 mm × 250 mm I.D (type:MG, col.no: AKAD05185, *shiseido*).

The temperature was maintained at 25^{o}c and the data were analyzed using the cyber lab- WS-100 chromatograph workstation V4.0. Identification was based on retention times and UV–VIS spectra by comparison with commercial standards. Pci sonicator (India), eppendorf vials and borosilicate glassware were also used.

Spectroscopy conditions:

The solvent used was double distilled water and the instrument was adjusted to blanks with water.

Chromatographic conditions:

The mobile phase consisted of water. The chromatograph was operated in the isocratic mode starting at a mobile phase of water. Eluent was delivered at a flow rate of 0.8ml/min. Absorbance was monitored at λmax = 296nm.

Standard solutions

Standard stock solution of ciprofloxacin and ornidazole was prepared by dissolving 10 mg of ciprofloxacin and ornidazole in water yielding a solution of 1mg/ml of stock solution. Series of dilutions were prepared by aliquoting 1ml from stock solution and diluted with the mobile phase to Yield 10mL of standard solutions containing 1 to 15 µg/ml respectively.

Sample solutions

Twenty five its capsules of each solid oral dosage forms were weighed and powdered in a mortar and pestle. A mass of powder equivalent to one t was weighed and 25 ml of mobile phase was added. The mixture was sonicated for a period of 15 minutes.

Pharmacokinetic study:

The pharmacokinetic study was conducted in six male Wistar rats (body weight 200–250 g), with the permission from the Institutional Animal Ethics Committee (IAEC), Vaagdevi Institute of Pharmaceutical Sciences, Kishanpura, Hanamkonda, Telangana, India. Before starting the experiment, Wistar rats were kept in an environmentally controlled room for one week and fed with standard laboratory food and water *ad libitum*.

The rats fasted overnight before the experiment. Rats were given the ciprofloxcin and ornidazole suspension (prepared by using 1% sodium carboxy methyl cellulose) orally at doses of 10 mgkg–1 body weight. Blood samples (0.5 mL) were collected sublingually at the intervals of 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0h.

All blood samples were allowed to clot and were centrifuged (REMI COOLING, India) for 5 min. The Plasma was separated and transferred into clean microcentrifuge tubes and stored at −20°C until UV And HPLC analysis. Physiological saline (0.5 mL) was administered to compensate for the blood loss after each blood draw. As per the single compartmental pharmacokinetic model, different pharmacokinetic parameters like peak plasma concentration (*C*Max),

time to reach peak concentration (*T*Max), area under the curve (AUC0-t and AUC0-∞), half life (*t*1/2), elimination rate constant (K), and volume distribution (Vd) were calculated by using the software Kinetica(Version 5.2).

Further, these plasma concentration *vs* time profile data were exposed to the noncompartmental model (statistical moment theory), and then the area under the moment curve (AUMC0-t and AUMC0-∞), and mean residence time (MRT) were determined.

Results and Discussion

Determination of absorbance maxima (λ_{max})

Small amount of drug is dissolved in the mobile Phase and the absorbance maxima was determined in Shimadzu UV spectrophotometer using mobile phase as blank.

The isobestic point was found to be at was found to be 296 nm.

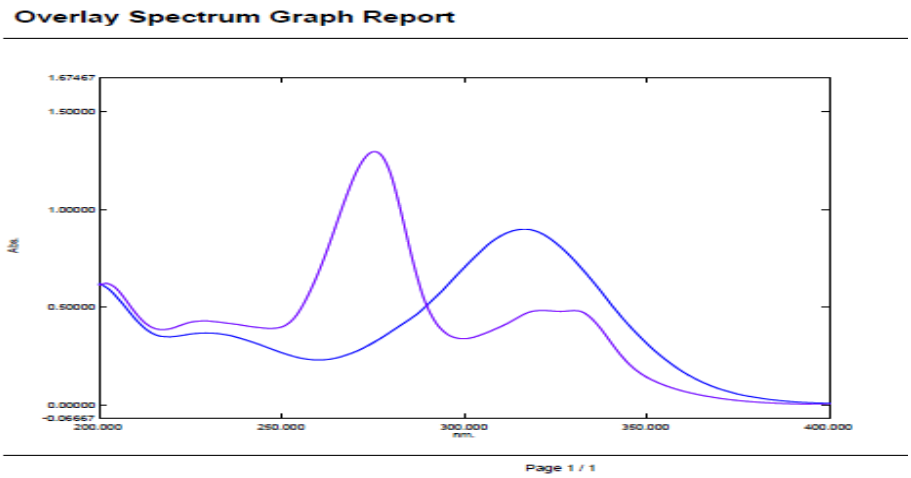

Fig:3. Overlay Spectra of ciprofloxacin and ornidazole.

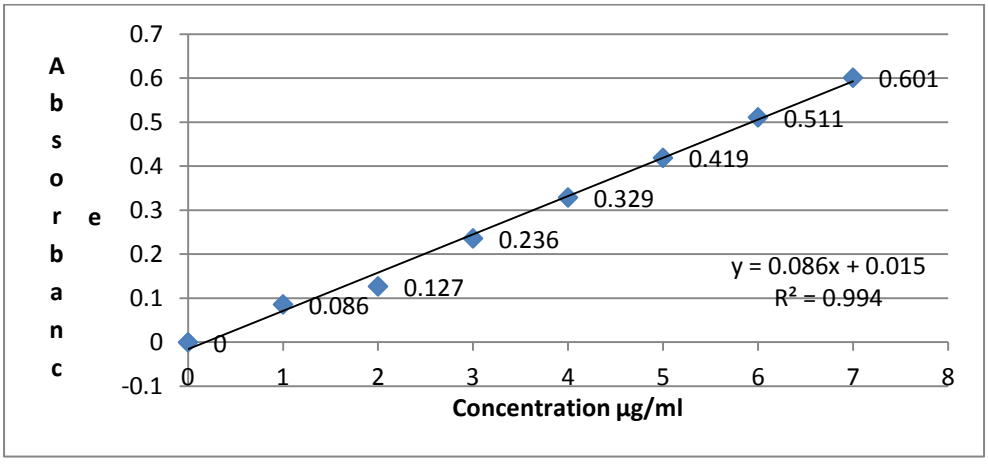

Fig:4. Linearity of ciprofloxcin.

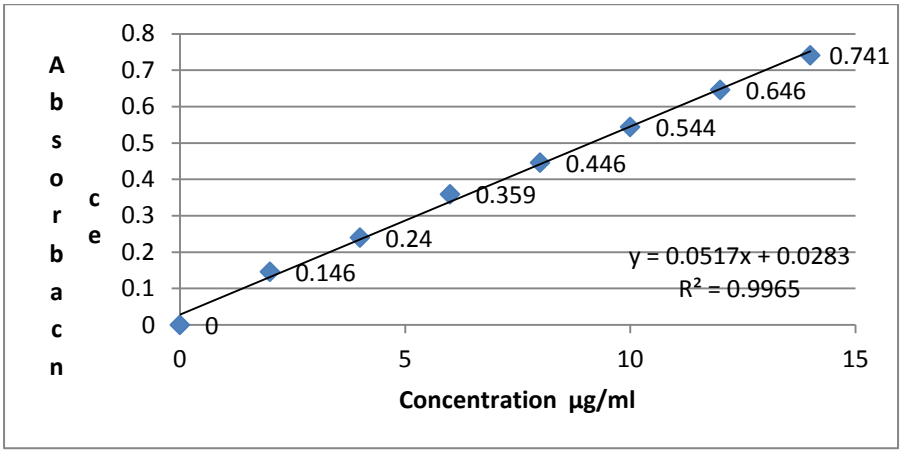

Fig:5. Linearity of ornidazole.

Table.1: Precision: Ciprofloxcin Interday.

Conc	Set 1	Set 2	Set 3	Mean	Std
1	0.089	0.089	0.086	0.088	0.0017
2	0.129	0.128	0.126	0.128	0.00058
3	0.241	0.24	0.236	0.240	0.00404

Table.2: Precision: Ciprofloxcin Intraday.

Conc	Set 1	Set 2	Set 3	Mean	Std
1	0.091	0.091	0.086	0.088	0.00289
2	0.126	0.126	0.123	0.125	0.00058
3	0.245	0.245	0.243	0.244	0.00115

Table.3: Precision: Ornidazole Interday.

Conc	Set 1	Set 2	Set 3	Mean	Std
2	0.176	0.176	0.173	0.174	0.00346
4	0.263	0.263	0.259	0.261	0.00231
6	0.364	0.364	0.357	0.365	0.00379

Table.4: Precision: Ornidazole Intra day

Conc	Set 1	Set 2	Set 3	Mean	Std
2	0.176	0.176	0.173	0.174	0.00346
4	0.263	0.263	0.259	0.261	0.00231
6	0.364	0.364	0.357	0.365	0.00379

Table.5: Recovery of Ciprofloxacin

Sample	Amount Taken (µg/ml)	Amount Added(µg/ml)	Found (%)
80%	5	4	100.06
100%	5	5	100.02
120%	5	6	100.13

Table.6: Recovery of Ornidazole

Sample	Amount Taken (µg/ml)	Amount Added(µg/ml)	Found (%)
80%	5	4	100.14
100%	5	5	100.10
120%	5	6	100.21

Table.7: L OD&LOQ:

Sample	Ciprofloxacin (µg/ml)	Ornidazole (µg/ml)
LOD	0.642	1.0
LOQ	0.936	1.3

Pharmacokinetic Study:

A simple and specific UV SPECTROSCOPY method for the determination of Ciprofloxcin and Ornidazole in Wistar rat Plasma was developed and successively applied to an in vivo kinetic study in rats. This method, it allowed us to investigate the pharmacokinetics of Ciprofloxcin and Ornidazole in rats after oral administration of standard drugs at a dose of 10 mg kg^{-1} and 20mg kg^{-1} body weight. This investigation contributes not only to the determination Ciprofloxcin

and Ornidazole in rat Plasma by UV Spectroscopy and RP-HPLC, but also to our understanding of the linear pharmacokinetic characteristics of Ciprofloxcin and Ornidazole over the dose studied in rats after oral administration

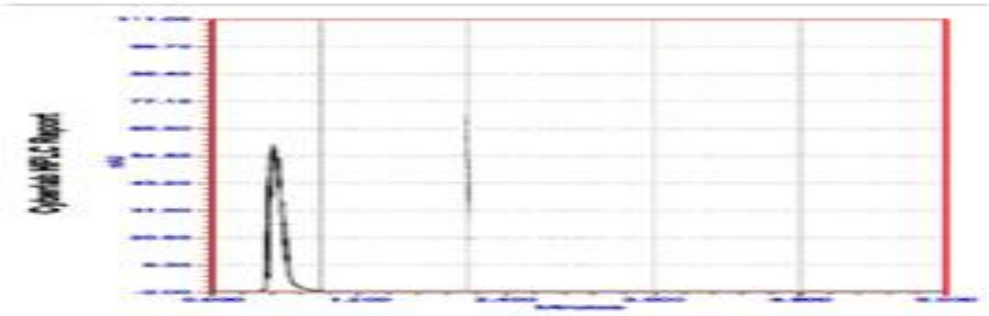

Fig:6. The Blank Rat Plasma Chromatogram.

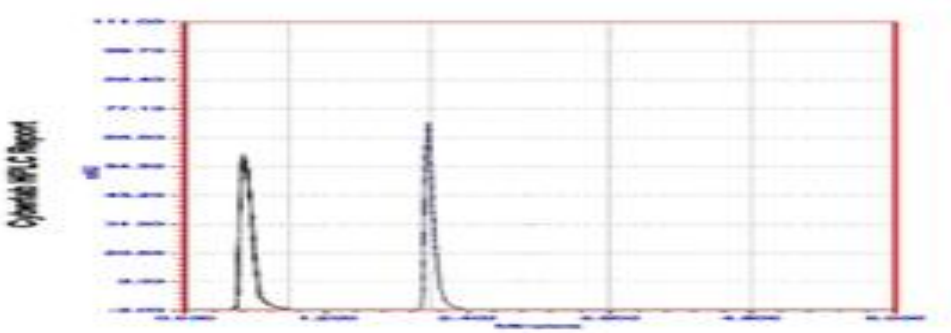

Fig:7. Chromatogram of ciprofloxacin with 10µg/ml spiked in plasma.

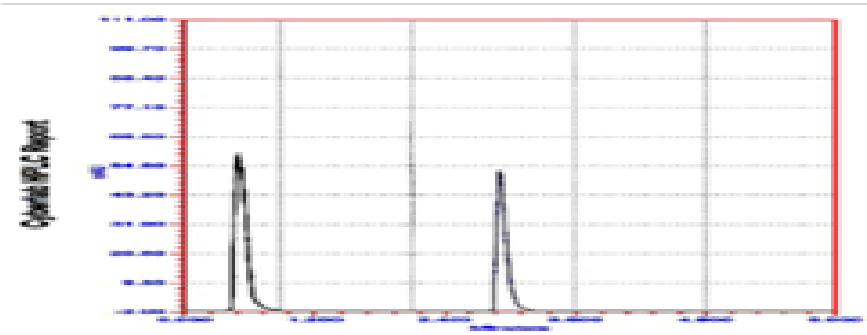

Fig:8. Chromatogram of ornidazole with 20µg/ml spiked in plasma.

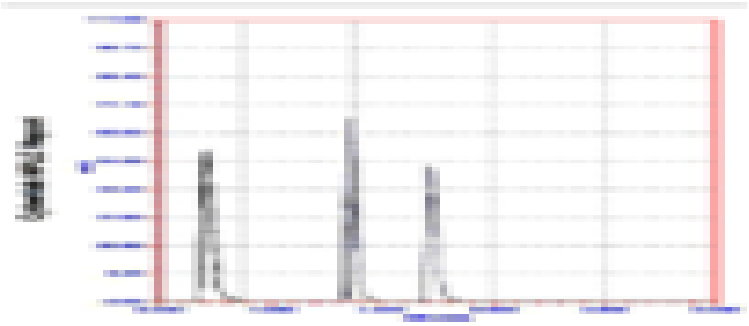

Fig:9. Chromatogram of ciprofloxacin 10µg/ml and ornidazole20µg/ml spiked in plasma.

Table.8: Ciprofloxcin: Pharmacokinetic results of ciprofloxacin

Parameter	Group1	Group2	Group3	Group4	Group5	Group6	mean	std
AUC_{-o-n}(µg/mL*h)	112.28	103.74	110.71	107.47	107.33	100.56	107.015	4.333
AUC_{total}(µg/mL*h)	112.78	104.24	111.21	108.25	107.83	101.05	107.56	4.34
CL_F(mL/h)	4.82	4.05	4.77	4.14	4.32	4.98	4.51	0.39
C_{max}(µg/mL)	20.64	19.62	18.101	16.9	19.28	18.601	18.85	1.29
$t_{1/2}$ (h)	6.201	6.33	6.36	6.29	6.3	6.39	6.31	0.06
tmax(h)	4.2	4.8	4.7	4.4	4.2	4.5	4.46	0.25
MRT(h)	6.21	6.204	6.38	6.58	6.16	6.17	6.28	0.16

Table.9: Ornidazole: Pharmacokinetic results of ornidazole

Parameter	Group1	Group2	Group3	Group4	Group5	Group6	mean	std
AUC_{-o-n}(µg/mL*	254.24	254.48	259.14	273.31	263.91	242.81	257.98	10.27
AUC_{total}(µg/mL*	256.94	258.05	285.9	296.7	265.29	244.16	267.84	19.72
CL_F(mL/h)	5.72	5.54	5.87	5.98	5.87	5.42	5.73	0.21
C_{max}(µg/mL)	21.49	16.94	15.59	22.33	23.86	16.91	19.52	3.45
$t_{1/2}$ (h)	7.02	7.504	7.32	7.12	7.5	7.04	7.25	0.22
t_{max}(h)	1.4	1.6	1.5	1.2	1.1	1.2	1.33	0.19
MRT(h)	11.5	13.44	19.22	17.88	11.3	11.89	14.205	3.47

Table.10: Standard combination: Pharmacokinetic results of ciprofloxacin and ornidazole.

Parameter	Group1	Group2	Group3	Group4	Group5	Group6	mean	std
AUC-o-n(µg/mL*h)	370.05	363.21	372.19	383.26	377	348.79	369.0833	12.004
AUCtotal(µg/mL*h)	373.58	367.77	392.15	404.07	379.85	351.54	378.16	18.47
CL_F(mL/hr)	9.56	9.42	9.8	10.08	9.8	9.06	9.62	0.357
Cmax(µg/mL)	33.83	31.79	32.81	32.49	34.69	32.13	32.95	1.1
$t_{1/2}$ (h)	6.9	6.43	6.77	6.97	6.88	6.8	6.79	0.191
tmax(h)	2.4	2.6	2.1	2.54	2.8	2.6	2.5	0.237
MRT(h)	9.99	11.53	14.43	14.33	10.14	10.52	11.82	2.05

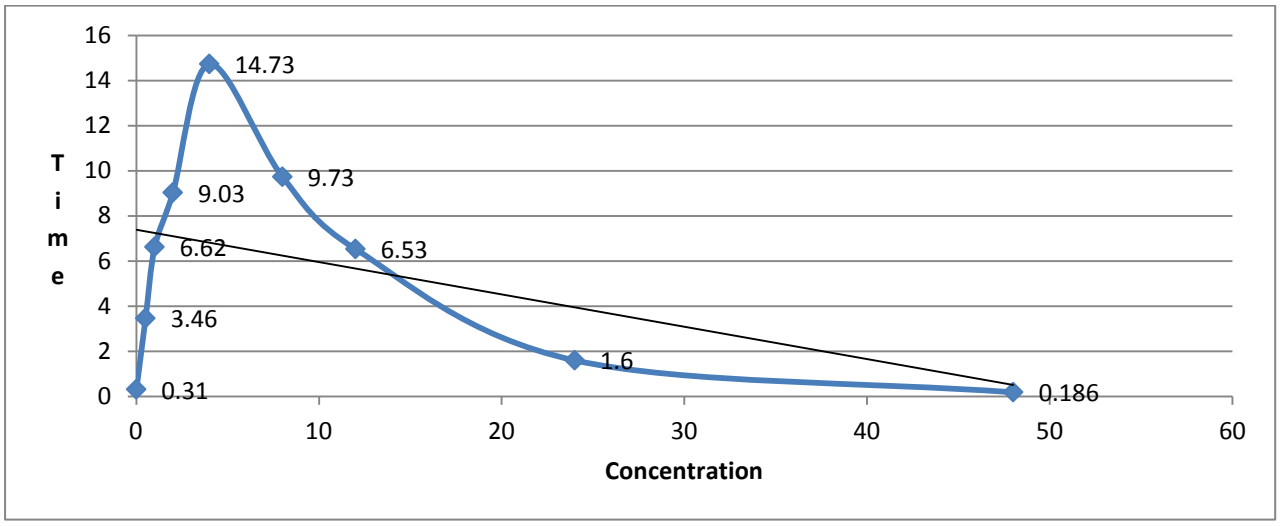

Fig:10. Time v/s concentration of ciprofloxacin.

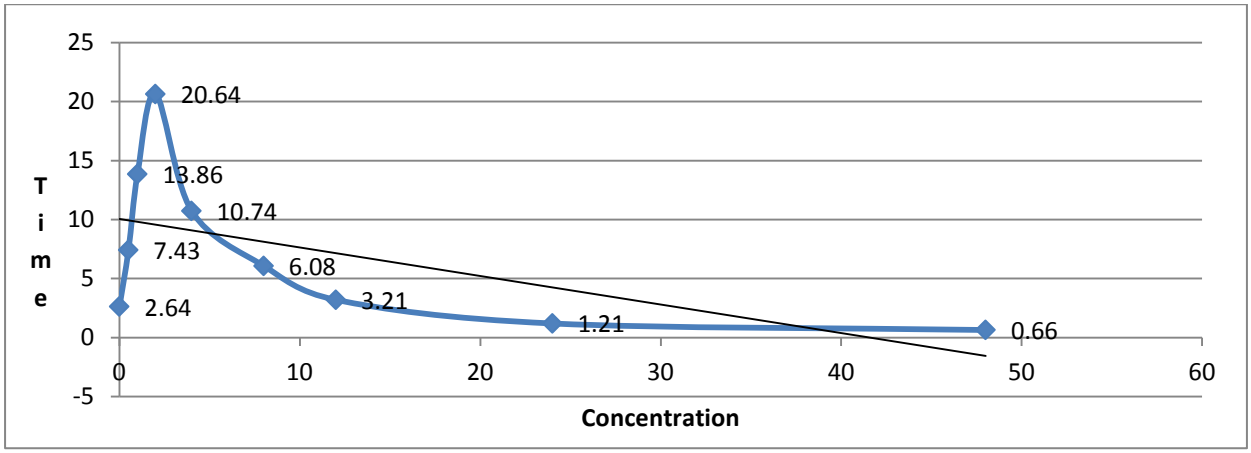

Fig:11. Time v/s concentration of Ornidazole.

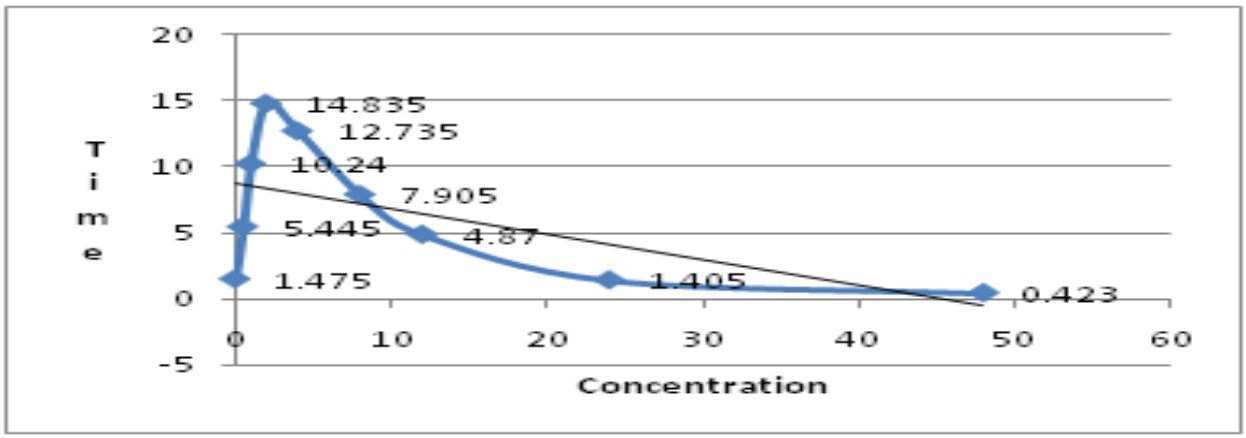

Fig:12. Time v/s concentration of ciprofloxacin and ornidazole.

Summary and Conclusion

Table.11: Ciprofloxacin Summary

Solvent	Distilled Water
λ max	271 nm
Linearity	1-7 µg/ml.
Correlation	0.994
y=mx+c	0.086x+0.015
Interday	0.128±0.00058
Intraday	0.125±0.00058
LOD	0.642 µg/ml.
LOQ	0.936 µg/ml.

Table.12: Ornidazole Summary

Solvent	Distilled Water
λ max	320 nm
Linearity	2-14 µg/ml.
Correlation	0.996
y=mx+c	0.048x+0.079
Interday	0.261±0.00231
Intraday	0.174±0.00346
LOD	1.0 µg/ml.
LOQ	1.3 µg/ml.

Conclusion:

- A simple method was estimated according to ICH guidelines for ciprofloxacin and ornidazole by UV-spectroscopy and pharmacokinetic study was estimated by RP-HPLC.
- From the above studies, it is obvious that plasma concentrations of ciprofloxacin and ornidazole increased in combination above the maximum effective concentration in both single and multiple dosage forms.
- In single and multiple dosage groups of ciprofloxacin and ornidazole AUC increased.
- The study clearly indicates a potential interaction between ciprofloxacin and ornidazole. The interaction leads to an increase in AUC.

A new simple and sensitive method was developed, optimized the following parameters were validated according to ICH guidelines. From the above results, I conclude that the developed UV spectrophotometric method represented an excellent technique for determination of ciprofloxacin and Ornidazole with good sensitivity, precision, reproducibility and including with Pharmacokinetic study by using UV-Spectroscopy (y=mx+c) and RP-HPLC. The sample preparation involving liquid-liquid extraction is very simple and cost effective.

Acknowledgements

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