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Simultaneous spectrophotometric estimation of Levofloxacin and Ornidazole using DDQ and p-CA as analytical reagents

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Abstract

A new concept of area under curve (AUC) is proposed for the development of two sensitive and precise spectrophotometric methods for the simultaneous determination of Levofloxacin and Ornidazole in pure mixture and in pharmaceutical binary dosage forms. Method A involves the use of DDQ (2,3-Dichloro-5,6-dicyano-1,4-benzoquinone) as analytical reagent and the AUC between 390nm and 690nm for DDQ was used for determination. Method B involves the use of *p*-CA (*p*-Chloranilic acid: 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone) as an analytical reagent and the AUC between 400nm and 700nm for *p*-CA was used for determination. The methods developed and construction of calibration curves using two analytical reagents viz., DDQ and *p*-CA are described. Optical and analytical parameters for the individual and simultaneous determination of Levofloxacin and Ornidazole using AUC are tabulated. The methods have been validated and compared with HPLC methods in terms of standard deviation, t-test and F-test.

Keywords: Spectrophotometry, Simultaneous estimation, AUC, Levofloxacin, Ornidazole Lecom-OZ tablet, DDQ, *p*-CA, CT Complex, Validation

INTRODUCTION

In continuation of work on the simultaneous estimation of drugs in their binary dosage forms[1], the present study was aimed at the development of two simple and sensitive spectrophotometric methods for the simultaneous estimation of Levofloxacin and Ornidazole in pure mixture and pharmaceutical binary dosage forms using π-acceptors, p-CA and DDQ as analytical reagents.

Levofloxacin

Chemically, levofloxacin (Figure 1) is (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10- (4-methyl-1-piperazinyl) - 7-oxo - 7H—Pyrido [1,2,3-de]-1,4-benzox-azine- 6-carboxylic acid, hemihydrate. Levofloxacin (LEV) belongs to quinolone group of antibacterials and very active against several types of pathogens[2-5]. It is also used in the treatment of MDR (multi-drug resistant) tuberculosis[6]. It is greatly effective against both gram-negative and gram-positive bacteria[7-9] and it is the L-isomer of ofloxacin commercially obtained as hemihydrate.

Various analytical methods were reported for the quantification of LEV in bulk and pharmaceutical formulations and also in biological fluid systems. These methods include: UV Spectrophotometry[10], spectrophotometry involving ion-pair complexation[11,12], colorimetric spectrophotometry[13], vibrational spectroscopy[14], spectrofluorimetry[15], and HPLC-UV detection technique[16]. Da Silva et al[17] reported spectrofluorimetric determination of levofloxacin in pharmaceuticals and in human urine.

Ornidazole

Ornidazole (Figure 2), chemically known as 1-chloro-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl) -2-propanol. It is a 5-Nitroimidazole derivative and is used in the treatment of susceptible protozoal and anaerobic bacterial infections. Ornidazole (ORN) is also used for the treatment of duodenal ulcers, amebic liver abscesses, intestinal lambliasis, giardiasis and vaginitis[18-21].

ORN is an antibiotic used for curing *Helicobacter pylori* infection. It has been successfully used in patients with active Crohn's disease. Ornidazole has also been preferred for surgical prophylaxis because of its longer elimination half life and excellent penetration into lipidic tissues compare to other nitroimidazole derivatives[22,23].

The methods available in the literature for the quantification of ornidazole have been reviewed. The quantitative techniques including spectrophotometric methods[24,25], HPLC[26], voltammetry[27], adsorptive stripping voltammetry[28], chemiluminescence[29] for its determination in dosage forms and biological fluids systems are available in the literature. A method based on the reduction of the nitro group to amino group for the determination of ORN has been reported[30].

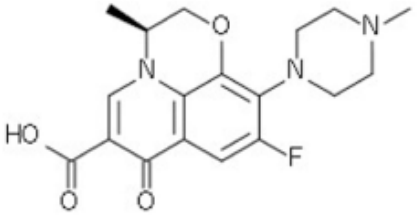

Figure 1: Structure of Levofloxacin

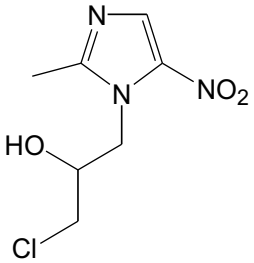

Figure 2: Structure of Ornidazole

Simultaneous estimation of levofloxacin and ornidazole by UV spectrophotometry by a mixed hydrotropy solubilization approach was reported[31]. Method development and validation for the simultaneous estimation of ofloxacin and ornidazole in tablet dosage form by RP-HPLC[32,33] are also available in the literature.

MATERIALS AND METHODS

Instruments

The UV-Vis Charge Transfer spectra of the study have been recorded on SHIMADZU 140 double beam spectrophotometer and also on ELICO SL 210 UV-Visible double beam spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 *p*H meter was used for *p*H measurement

Materials

DDQ (2,3-Dichloro-5,6-dicyano-p-benzoquinone) was obtained from SD Fine Chemicals. It was recrystallized twice from 3:1 mixture of chloroform and benzene. p-CA (P-Chloranilic acid) supplied by Rolex, Mumbai was used without further purification. HPLC grade acetonitrile was used throughout the work. The drugs Levofloxacin, Ornidazole and drug mixture analysed were procured from Dr. Reddy's laboratories, Hetero Drugs Private Ltd, Kekule Pharma Limited, Srini Pharmaceuticals Ltd. and Symed Laboratories Ltd. as gift samples. All these firms are located in and around Hydeabad, Telangana.

Methods and Calibration

Method A

This method is developed for the simultaneous estimation of drugs in a binary mixture using DDQ (2,3-Dichloro-5,6-dicyano-1,4-benzoquinone) as an analytical reagent. Into a series of 10ml of flasks, different aliquots (1-9ml) of Levofloxacin were taken and 1ml of DDQ was added, remaining volume was made up with solvent (Acetonitrile). The contents were shaken well and UV–Vis spectra were recorded. The OD at 480, 540 and 580nm for DDQ anion were noted. The areas under the curve (AUC) between 390nm and 690nm for DDQ were determined from the spectra. AUC_x was plotted against concentration of Levofloxacin. From the slope of the plot K_x was determined. Similarly, analogous experiments were repeated for determination of K_y for Ornidazole.

Stock solution of mixture of Levofloxacin and Ornidazole was prepared with same ratio as in tablet formulations. From the stock, 1-9 ml of mixture of drugs were taken into series of standard flasks and 1ml of reagent DDQ was added. Remaining volume was made up with solvent (Acetonitrile). The contents were shaken well. UV-Vis spectra were recorded. The OD at 480,540 & 580 for DDQ anion were noted. AUC_{mix} was plotted against either Cx or Cy for calibration.

Method B

This method is developed for the simultaneous estimation of drugs in a binary mixture using *p*-CA (*p*-Chloranilic acid: 2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone) as an analytical reagent. Into a series of 10ml of flasks, different aliquots (1-9ml) of Levofloxacin were taken and 1ml of *p*-CA was added, remaining volume was made up with solvent (Acetonitrile). The contents were shaken well and UV–Vis spectra were recorded. The OD at 540nm for *p*-CA anion were noted. The areas under the curve (AUC) between 400nm and 700nm for *p*-CA were determined from the spectra. AUC_x is plotted against the concentration of drug. From the slope of the plot K_x was determined. Similarly, analogous experiments were repeated for determination of K_y for Ornidazole

Stock solution of mixture of Levofloxacin and Ornidazole was prepared with same ratio as in tablet formulations. From the stock, 1-9 ml of mixture of drugs were taken into series of standard flasks and 1ml of reagent, *p*-CA was added. Remaining volume was made up with solvent (Acetonitrile). The contents were shaken well. UV-Visible spectra were recorded. The OD at 540nm for *p*-CA anion was noted. AUC_{mix} was plotted against either Cx or Cy for calibration.

RESULTS AND DISCUSSION

p-CA for example, is an analytical reagent and produces a band at 540nm for *p*-CA anion and is independent of the drug. It is also expected to interact with both the drugs in mixture and exhibits band at 540 nm. As the extent of interaction is different in mixture, it is possible to analyze the concentration of each although the analytical wavelength is same. This prompted the author to give a thought in these lines. For the quantification, generally optical density at λ_{max} is measured against concentration of drug for calibration purpose. The authors thought area under curve (AUC) is more appropriate than the optical density. The author proposes to measure the area under the curve for individual drugs as well as the mixture in a constant ratio of concentration as in the formulations.

AUC (Area under curve in mixture) = $AUC_X + AUC_Y$

where X and Y are two drugs in the binary mixture

but AUC of X α C_X

and AUC of Y α C_Y

$AUC_X = K_X C_X$

$AUC_Y = K_Y C_Y$

$AUC_{mix} = K_X C_X + K_Y C_Y$ …… (1)

Dividing both sides of equation by $K_X C_X$

$$\frac{AUC_{mix}}{K_x C_x} = 1 + \frac{K_Y C_Y}{K_x C_x}$$

$$\text{But } \frac{K_Y C_Y}{K_x C_x} = K \text{ (Constant)}$$

$$\frac{AUC_{mix}}{K_x C_x} = 1 + K$$

$AUC_{mix} = (1 + K)K_X C_X$

$AUC_{mix} = (K_X + K, K_X)C_X$ (2)

Similarly

$AUC_{mix} = K_X C_X + K_Y C_Y$

Dividing both sides with $K_Y C_Y$

$$\frac{AUC_{mix}}{K_Y C_Y} = 1 + \frac{K_x C_x}{K_Y C_Y}$$

$$\frac{K_x C_x}{K_Y C_Y} = K \text{ (Constant)}$$

$AUC_{mix} = (1 + K)K_Y C_Y$ .....(3)

$AUC_{mix} = (K_Y + K. K_Y)C_Y$ .....(4)

The equations 2 and 4 imply that AUC_{mix} is either proportional to C_x or C_Y

By determining the AUC_{mix} for a mixture of drugs having constant ratio it is possible to construct the calibrations to find the individual concentrations of drugs in a binary mixture.

Into a series of 10ml of flasks, different aliquots (1-9ml) of drug Levofloxacin were taken and 1ml of DDQ or p-CA was added, remaining volume was made up with solvent acetonitrile. The contents were shaken well and UV–Vis spectra were recorded. The OD at 540nm for *p*-CA anion and 480, 540 and 580nm for DDQ anion were noted. The area under the curve (AUC) between 390nm and 650nm for DDQ and between 400nm and 700nm for *p*-CA were determined from the spectra (Figures 3 and 4).

The plots of AUC_x vs concentration of Levofloxacin and with DDQ and *p*-CA are shown in Figures 5 and 6. From the slope of the plots K_x was determined. In the same way, analogous experiments were repeated for determination of K_y for Ornidazole (Figures 7, 8, 9 and 10).

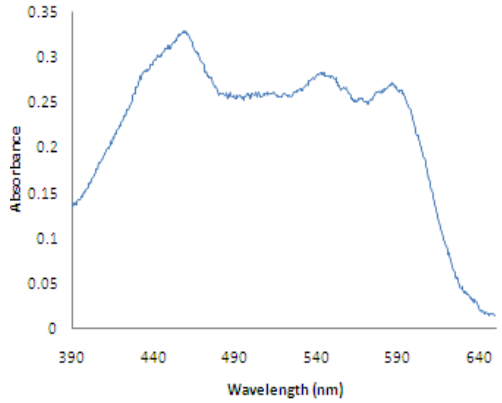

Figure 3: Charge transfer spectrum of Levofloxacin with DDQ

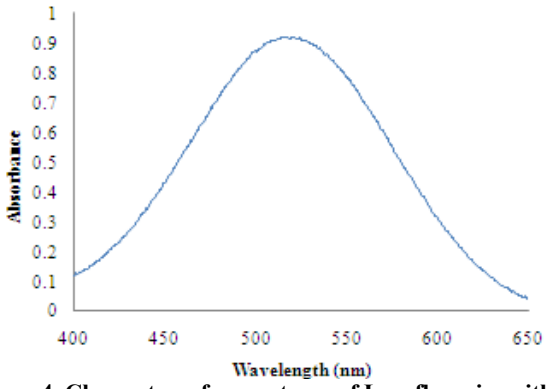

Figure 4. Charge transfer spectrum of Levofloxacin with p-CA

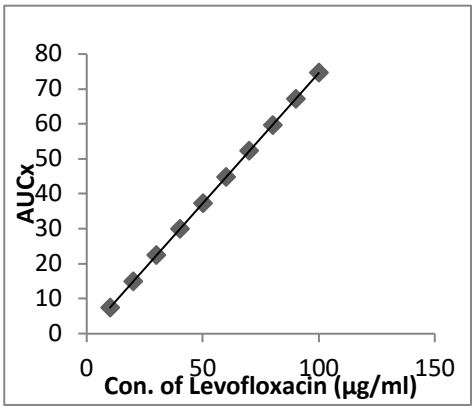

Figure 5: Plot of AUC vs Concentration of Levofloxacin-DDQ

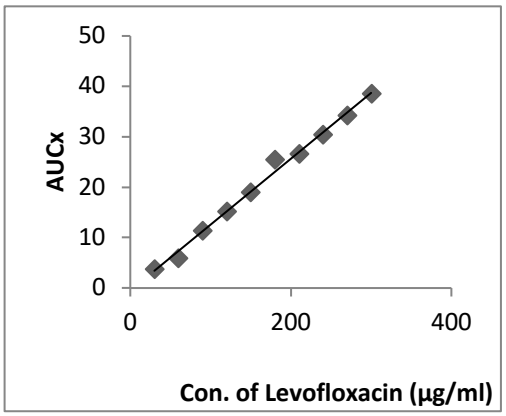

Figure 6: Plot of AUC vs Concentration of Levofloxacin -p-CA

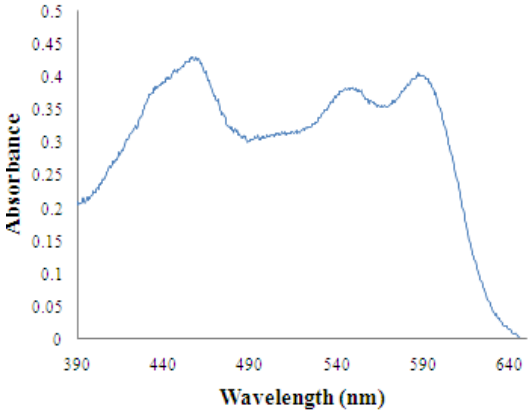

Figure 7: Charge transfer spectrum of Ornidazole with DDQ

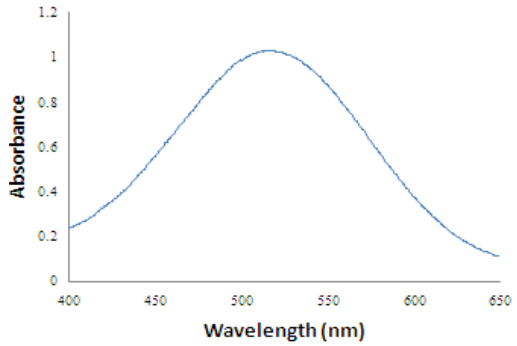

Figure 8: Charge transfer spectrum of Ornidazole with p-CA

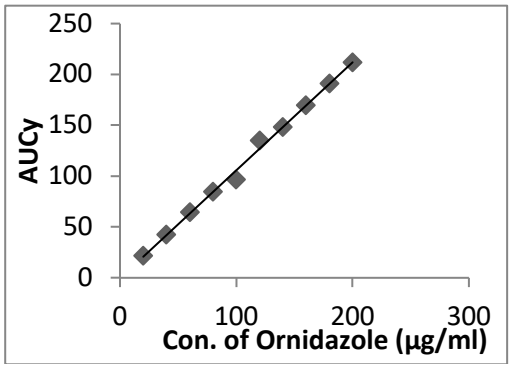

Figure 9: Plot of AUC vs Concentration of Ornidazole – DDQ

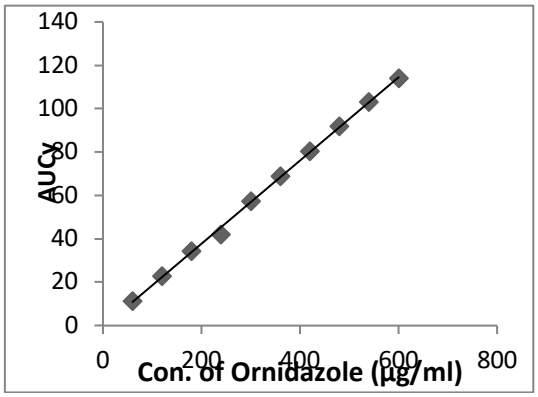

Figure 10: Plot of AUC vs Concentration of Ornidazole - p-CA

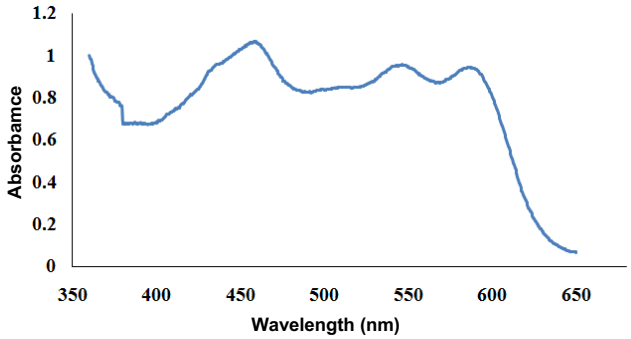

Figure 11: Charge transfer spectrum of LEV + ORN with DDQ in pure form

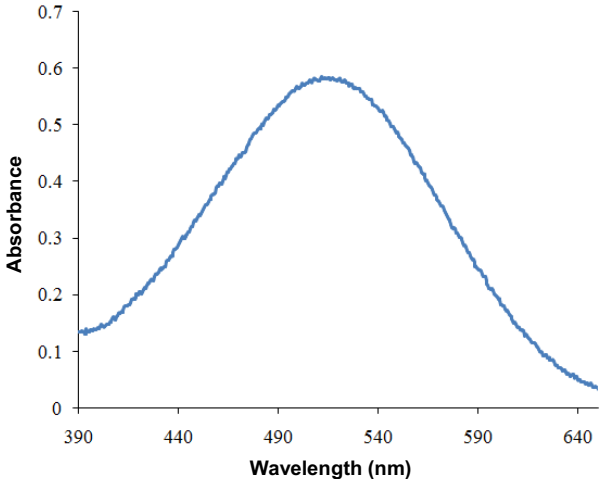

Figure 12: Charge transfer spectrum of LEV + ORN with p-CA in pure form

Stock solution of mixture of drugs was prepared with same ratio as in tablet formulations. From the stock 1-9 ml of mixture of drugs were taken into series of standard flasks and 1ml of reagent DDQ or *p*-CA was added. Remaining volume was made up with solvent (Acetonitrile). The contents were shaken well. UV-Visible spectra were recorded (Figures 11 and 12). The OD at 540nm for *p*-CA anion and 480, 540 & 580 for DDQ anion were noted. AUC_{mix} was plotted either Cx or Cy (Figures 13 and 14). The optical characteristics and statistical data for the regression equation of the proposed method for the individual estimation of levofloxacin and ornidazole are presented Table 1. Optical and analytical parameters for the simultaneous estimation of levofloxacin and ornidazole in synthetic mixture in the ratio as in tablet formulations are presented in Table 2.

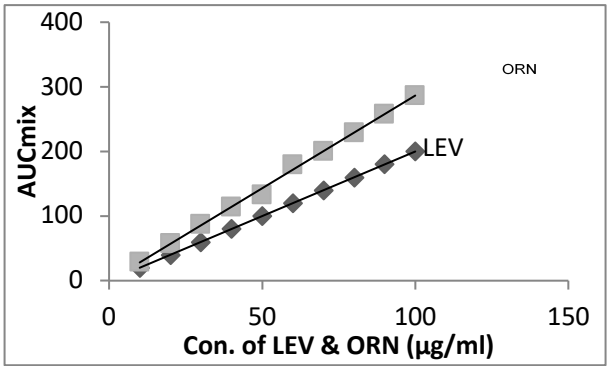

Figure 13: Plot of AUCmix vs Concentration of LEV & ORN with DDQ in pure form

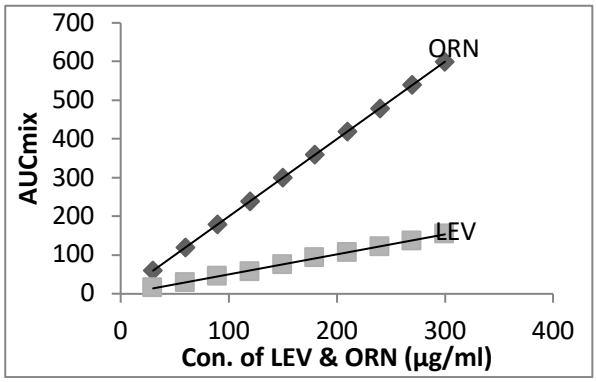

Figure 14: Plot of AUCmix vs Concentration of LEV & ORN with p-CA in pure form

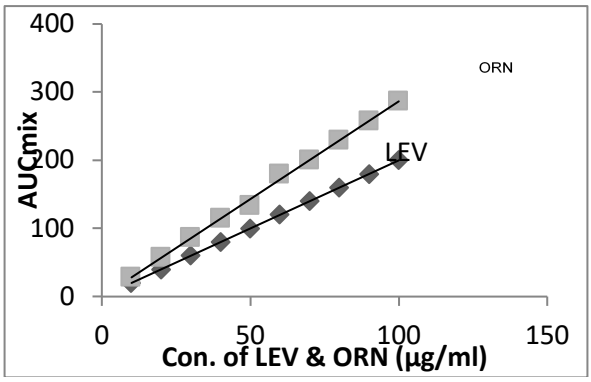

Figure 15: Plot of AUCmix vs Concentration of LEV & ORN with DDQ in dosage form

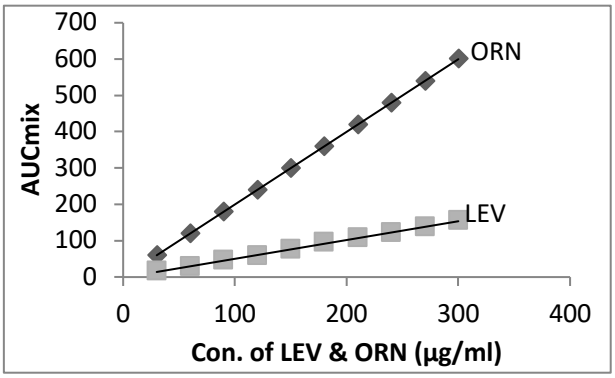

Figure 16: Plot of AUCmix vs Concentration of LEV & ORN with p-CA in dosage form

Five different solutions of pure drug mixture of levofloxacin and ornidazole prepared in the ratio as in the tablet formulation were chosen in the range of calibration curve and the recovery experiments were performed. The recoveries and their relative standard deviations are tabulated in Table 3.

Similarly, different solutions of Lecom-OZ tablets (ratio of levofloxacin and ornidazole as 1:2) in the range of calibration curve were selected and the assay was estimated using the calibration curve (Figures 15 and 16). The results of the recovery experiments are tabulated in Table 4.

Table 1: Optical and analytical parameters for the individual estimation of Levofloxacin and Ornidazole using area under curve

Parameters	DDQ		*p*-CA	
λ Lower and λ Higher for AUC (nm)	390-650		400-700	
Range of concentrations of drugs (µgmL^{-1})	Levofloxacin	Ornidazole	Levofloxacin	Ornidazole
	10-100	20-200	30-300	60-600
Slope	0.752	1.034	0.129	0.201
Intercept	0.097	1.491	-0.302	3.004
Correlation coefficient	0.998	0.997	0.998	0.992
Residual intercept	0.2278	0.6266	0.1172	0.3654
LOD	1	2	3	6
LOQ	3.3	6.6	9.9	19.8

Table 2: Optical and analytical parameters for the simultaneous estimation of Levofloxacin and Ornidazole in synthetic mixture in the ratio of 1:2 of drugs as in tablet using area under curve

Parameters	DDQ		*p*-CA	
λ Lower and λ Higher for AUC	390-650		400-700	
Range of concentrations of drugs (µgmL^{-1})	Levofloxacin	Ornidazole	Levofloxacin	Ornidazole
	10-200	10-200	30-600	30-600
Slope	2.009	2.884	1.987	0.512
Intercept	-0.946	-1.834	0.624	-1.082
Correlation coefficient	0.998	0.995	0.999	0.998
Residual intercept	0.6087	0.8739	1.8063	0.4654
LOD	1	1	3	3
LOQ	3.3	3.3	9.9	9.9

Table 3: Application of proposed methods for the simultaneous estimation of Levofloxacin and Ornidazole in the mixture in the ratio of 1:2 of drugs in pure form using area under curve

Taken (µg mL^{-1})				Found (µg mL^{-1})				Recovery (%)			
Levofloxacin		Ornidazole		Levofloxacin		Ornidazole		Levofloxacin		Ornidazole	
DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA
10	30	20	60	10.23	30.12	20.24	60.54	102.30	100.41	101.20	100.90
20	60	40	120	20.52	60.05	40.13	119.42	102.60	100.08	100.32	99.51
30	90	60	180	29.64	90.17	59.18	180.24	98.80	100.18	98.63	100.13
40	120	80	240	40.18	119.68	80.16	240.62	100.45	99.73	100.20	100.25
50	150	100	300	50.34	149.64	99.92	300.42	100.68	99.76	99.92	100.14
60	180	120	360	60.26	180.76	120.72	360.17	100.43	100.43	100.60	100.04

SD Proposed method				SD method				Reference
Levofloxacin		Ornidazole		Levofloxacin		Ornidazole		
DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	
1.3948	0.3047	0.8601	0.4451	0.8684	0.2465	0.5646	0.3215	

t-Test				F-test			
Levofloxacin		Ornidazole		Levofloxacin		Ornidazole	
DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA
0.6728	0.3187	0.6056	0.4805	0.3876	0.6544	0.4310	0.5210

Table 4: Application of proposed methods for the simultaneous estimation of Levofloxacin and Ornidazole in the mixture in the ratio of 1:2 of drugs in pharmacutical form (Lecom-oz tablets) using area under curve

Taken (μg mL^{-1})				Found (μg mL^{-1})				Recovery (%)			
Levofloxacin		Ornidazole		Levofloxacin		Ornidazole		Levofloxacin		Ornidazole	
DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA	DDQ	*p*-CA
10	30	20	60	10.12	29.64	20.05	60.19	101.20	98.80	100.25	100.31
20	60	40	120	20.06	60.52	40.42	120.08	100.30	100.86	101.05	100.06
30	90	60	180	29.64	90.07	59.16	180.26	98.81	100.07	98.61	100.15
40	120	80	240	40.62	120.72	79.92	239.12	101.55	100.60	99.90	99.63
50	150	100	300	49.46	150.21	100.12	300.06	98.92	100.14	100.12	100.02
60	180	120	360	60.21	179.92	119.87	360.21	100.35	99.95	99.89	100.05

SD Proposed method				SD Reference method			
Levofloxacin		Ornidazole		Levofloxacin		Ornidazole	
DDQ	p-CA	DDQ	p-CA	DDQ	p-CA	DDQ	p-CA
1.0179	0.7123	0.7929	0.2253	0.8584	0.8212	0.8930	0.1732

t-Test				F-test			
Levofloxacin		Ornidazole		Levofloxacin		Ornidazole	
DDQ	p-CA	DDQ	p-CA	DDQ	p-CA	DDQ	p-CA
0.2607	0.2226	0.1858	0.3917	0.7086	1.3291	1.2684	0.5909

CONCLUSION

A new way of analysis of mixed dosage forms using DDQ (Method A) and *p*-CA (Method B) involving the concept of area under curve is proposed, These methods are tested and validated and applied to the mixture of levofloxacin and ornidazole.

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