CHAPTER (3)

RESULTS AND DISCUSSION

3.1. Spectrophotometric studies of ion pair complexes in solution

3.1.1. Absorption spectra of ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR) and enrofloxacin (ENR) with rose bengal reagent (RB)

In order to investigate the optimum conditions of drug-reagent complex formation, the following studies should be taken in consideration:

3.1.1.1. *Effect of pH*

The effect of pH on the complex formation between CIP, LEV, NOR and ENR with RB was studied in universal buffer solutions of pH range 2-14. A portion (0.5 ml) of 1 x 10^{-3} M RB, 0.5 ml of 100 μg ml^{-1} drugs and 4 mL buffer of different pH values were mixed well. The volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug at the same pH value. The optimum pH values giving maximum absorption recommended for subsequent studies of drug-reagent complexes, is 4, 4, 4.5 and 4 on using CIP, LEV, NOR and ENR respectively.

3.1.1.2. *Effect of buffer concentration*

To study the effect of buffer concentration on the complex formation with last pH value 4, 4, 4.5 and 4 for CIP, LEV, NOR and ENR respectively, the concentration of drugs and reagent were kept constant at 100 μg ml^{-1} and $1x10^{-3}$ M respectively while that of buffer was regularly varied, the volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug. Illustrative spectra are shown in Figure (10) for the effect of buffer concentration, inspection of these figure shows that the optimum ml added of buffer giving maximum absorption recommended for subsequent studies of drug-reagent complexes, are 1 ,3 ,3 and 2 ml for CIP, LEV, NOR and ENR respectively.

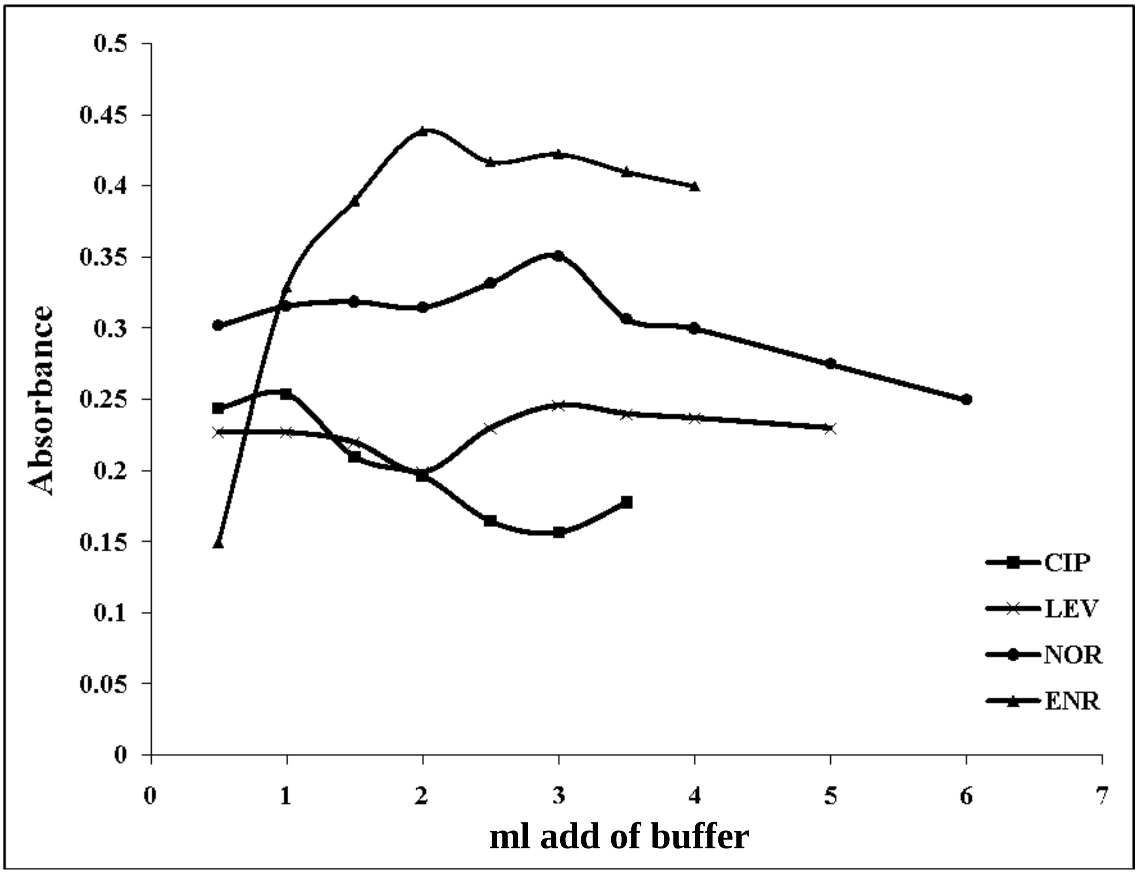

Figure (10). Effect of buffer concentration on absorption rang of 1 X10^{-3} M RB with 100 μg ml^{-1} of studied drugs

3.1.1.3. Determination of λ_{max} of the complex species

For determine the value of λ_{max} at which the complex species possesses the maximum absorption, the following spectra must be recorded:

A- Spectrum of pure drugs 1 ml of 100 μg ml^{-1} at optimum pH value using buffer solution at the recommended pH value as a blank.

B- Spectrum of pure reagent 1 ml of 1 x 10^{-3} at optimum pH value using the same buffer as a blank.

C- Spectrum of mixture of drugs (A) and reagent (B) at the optimum pH value using reagent at the same pH as a blank.

D- Spectrum of solution (C) against (B) as a blank.

The absorption spectra show that the formed complex absorbed maximally at 573nm for RB with drugs CIP, LEV, NOR and ENR as shown in Figure (11). This optimal wavelength is chosen for further investigations.

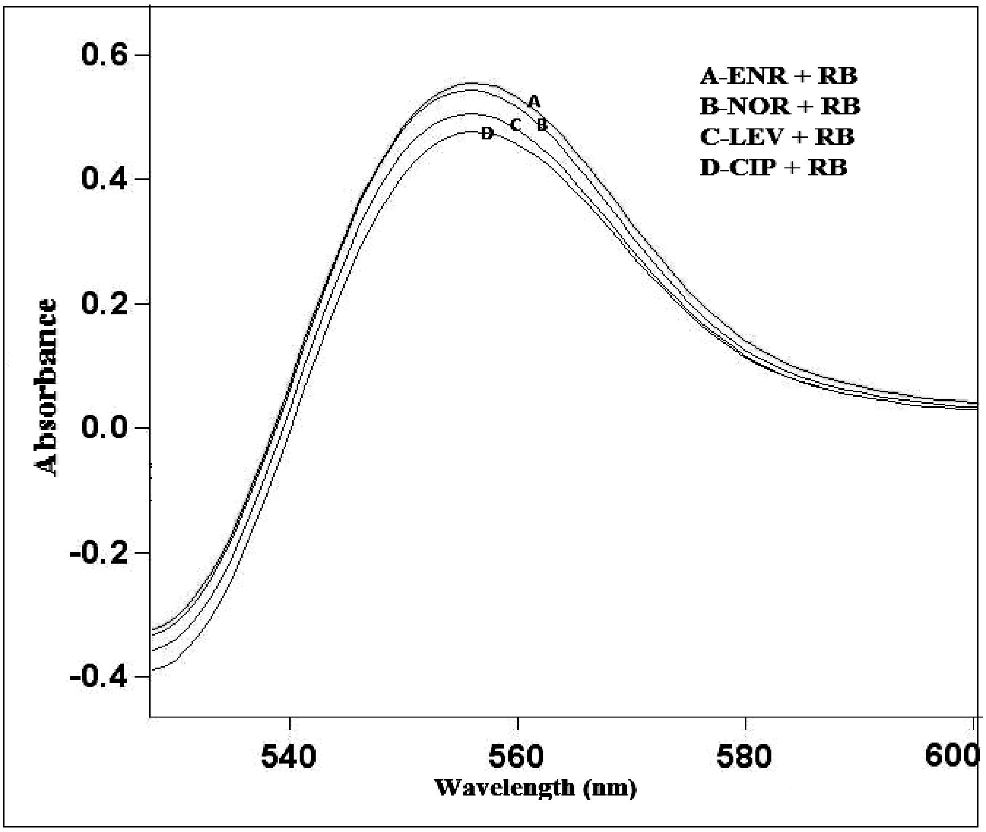

Figure (11). Absorption spectra of ion pair complex of 1 X10^{-3} M RB with 100 µg ml^{-1} of studied drugs

3.1.1.4. *Effect of sequence of addition*

The Effect of sequence of addition on complex formation was studied by measuring the absorption of solutions prepared using different sequences of addition against blank solution prepared in the same manner. The experiments showed that the best sequence of addition is drug - buffer - reagent - water.

3.1.1.5. ***Effect of time and temperature***

The Effect of time on complex formation was studied by measuring the absorbance of complex at optimum pH against a blank solution of the same pH at various time intervals. Also, the effect of temperature was studied for the same solution by incubation the sample and blank in water bath at different temperatures (25-45 ºC) the absorption was measured after cooling to room temperature.

The experiments showed that complexes are formed simultaneously after mixing drug and reagent and remain stable for about two hours. Also, it was found that, increasing the temperature up to 45 ºC has slight effect on the absorbance above which the color began to fade slowly.

3.1.1.6. ***Effect of reagent concentration***

To study the effect of reagent concentration on the complex formation with optimum pH value concentration of buffer for CIP, LEV, NOR and ENR respectively, the concentration of drugs were kept constant at 100 µg ml^{-1} while that of reagent was regularly varied, the volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug. Illustrative spectra are shown in Figure (12) for the effect of reagent concentration, inspection of these figure shows that the optimum ml added of reagent giving maximum absorption recommended for subsequent studies of drug-reagent complexes, are 1 ,2 ,2 and 1.5 ml for CIP, LEV, NOR and ENR respectively.

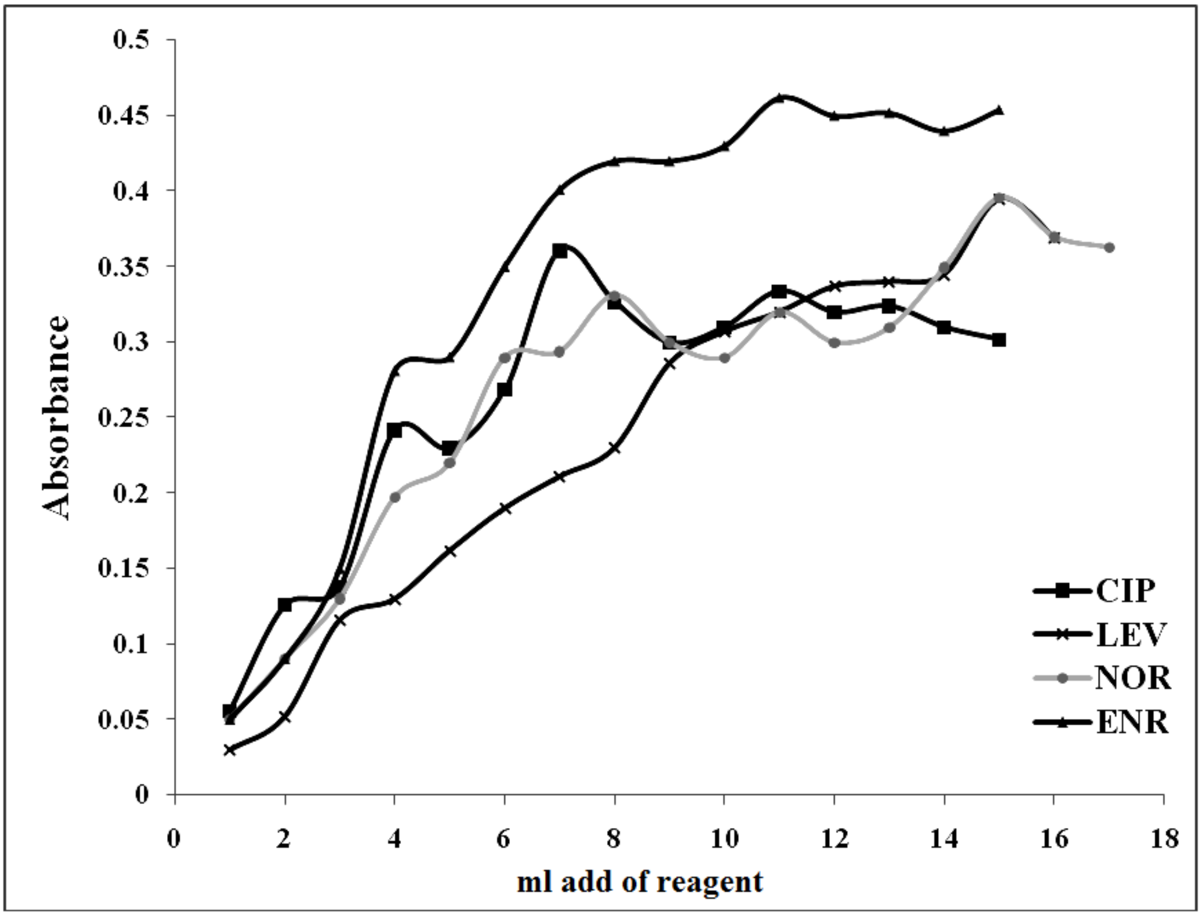

Figure (12). Effect of reagent concentration on absorption rang of 1 X10^{-3} M RB with 100 µg ml^{-1} of studied drugs

3.1.1.7. *Molar ratio method*

In order to investigate the molecular ratio of the complexes formed between the drugs under investigation and RB at the selected conditions, the molar ratio and continues variation methods were carried out. Experimental results revealed that the complexes formed between the drugs and RB have 1: 1 stoichiometric ratio as shown in Figure (13) and (14).

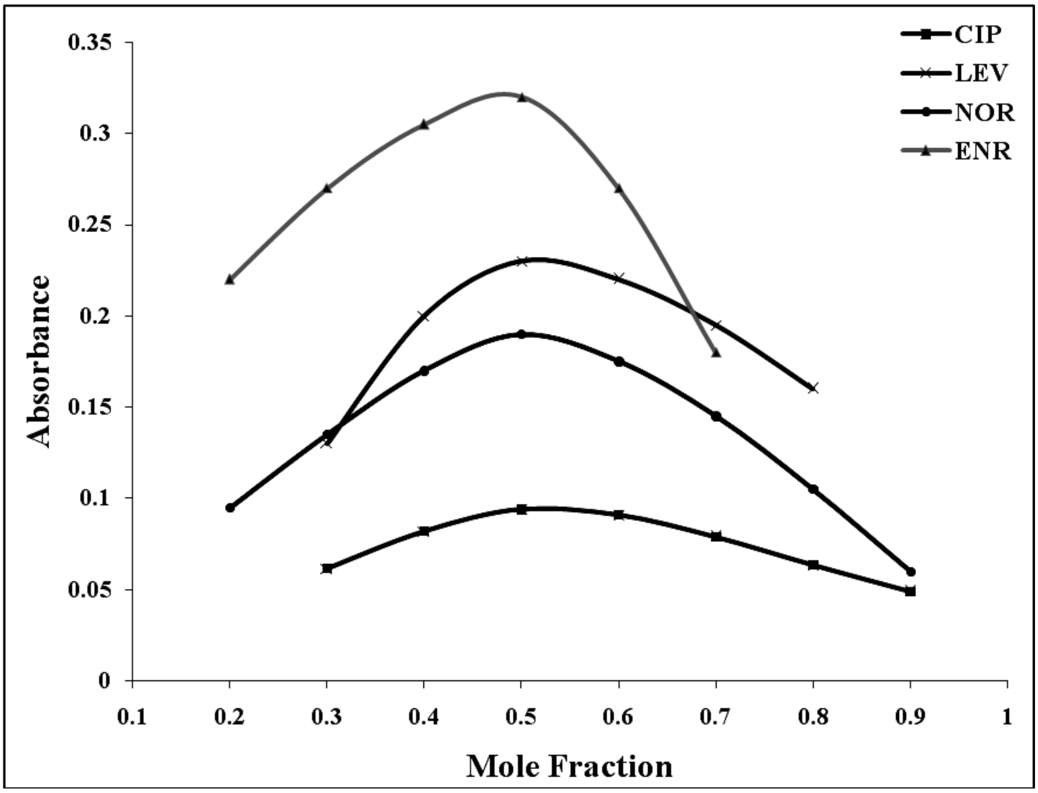

Figure (13). Continuous variation for 1 X10^{-3} M RB with 100 µg ml^{-1} of studied drugs

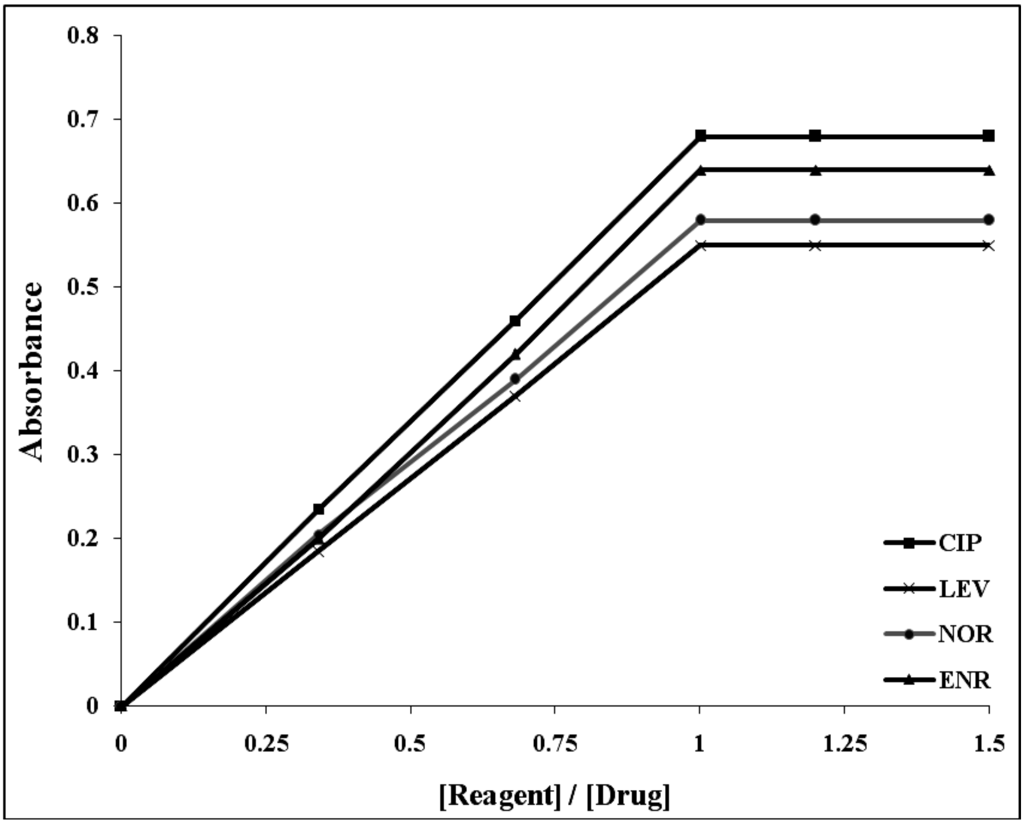

Figure (14). Mole ratio for 1 X10^{-3} M RB with 100 µg ml^{-1} of studied drugs

3.1.1.8. *Stability constant of the complexes*

Spectrophotometric methods can be applied for the determination of the stability constant of the complexes. The calculated stability constants are listed in Table (1), from which it is found that the RB forms the most stable complex with NOR then ENR then LEV and CIP drug.

3.1.1.9. *Validation of the method*

Method validation is the process of providing that the analytical method is acceptable, there is various validation studies developed and recorded below.

3.1.1.9.1. Linearity

under optimum conditions of pH, reagent concentration, sequence of additions, time and temperature for each of the different complexes, different concentrations of drug (μg ml^{-1}) were transferred into 10 ml measuring flask 1, 2, 2 and 1.5 ml of reagent (1×10^{-3} M) with CIP, LEV, NOR and ENR respectively, 1 ,3 ,3 and 2 mL of the optimum pH value buffer with CIP, LEV, NOR and ENR respectively were added and then completed to volume with bidistilled water. The absorbance was measured at optimum λ_{max}, and then plotted against drug concentration as shown in Figure (15). Limits of beer' s law, the molar absorbability (ε; L $mol^{-1}cm^{-1}$) and ***Sandell*** (76) sensitivity results are listed in Table (1), indicating high sensitivity in the microdetermination of the drug.

For more accurate analysis, ***Ringbom*** (77) optimum concentration rang was determined by calculating the transmittance percent from the following equation:

$$T\ \% = 10^{-A} \times 100$$

Where: T %: transmittance percent,

A: the absorbance of the complex

By plotting logarithm of drug concentration (log [C_D]) in µg mL^{-1} against T %, the linear portion of the S-shaped curve gave an accurate range of analysis as shown in Figure (16); Results are listed in Table (2).

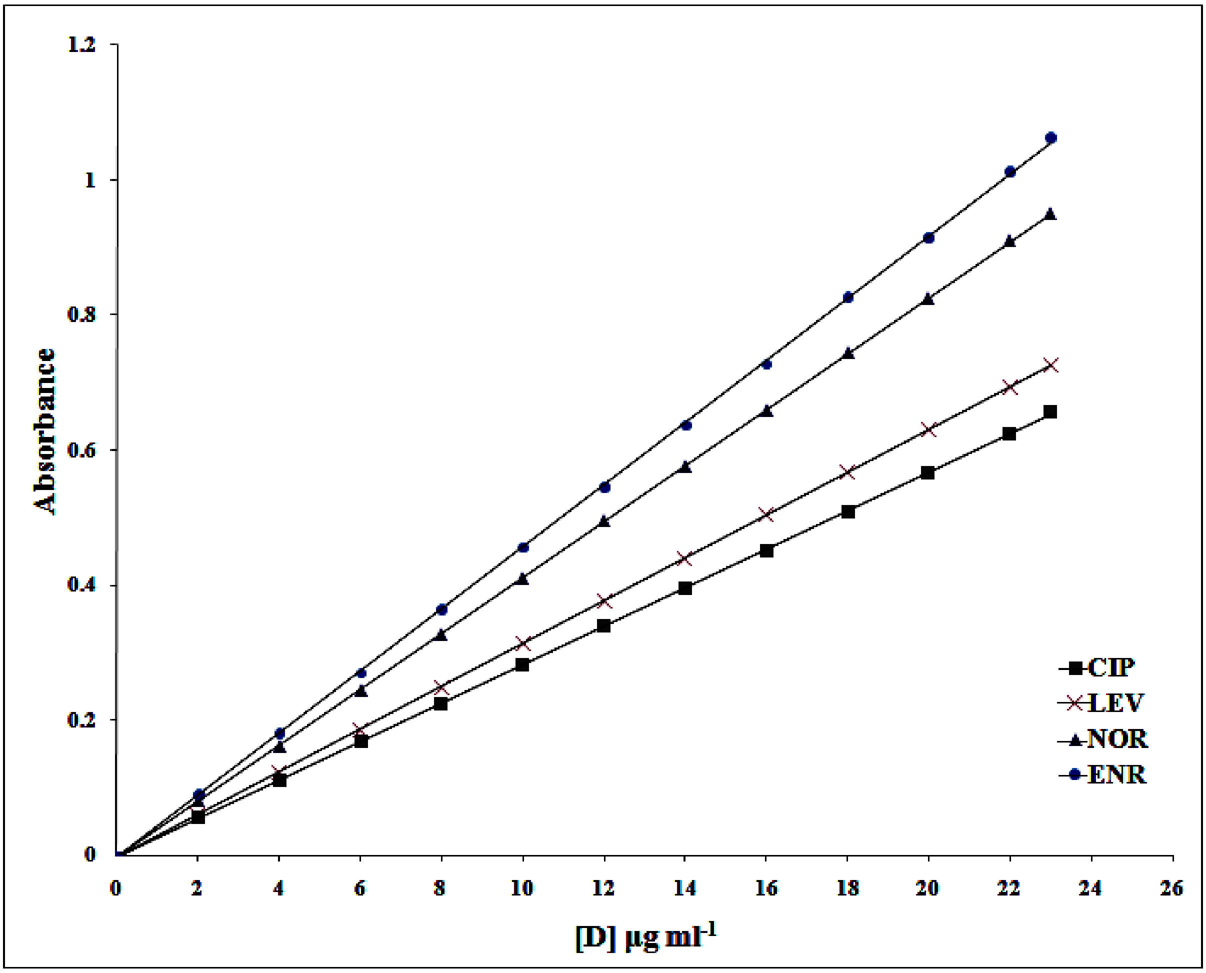

Figure (15). Applications of Beer's law for 100 µg ml^{-1} studied drugs using the optimum volume of 1 $X10^{-3}$ M RB.

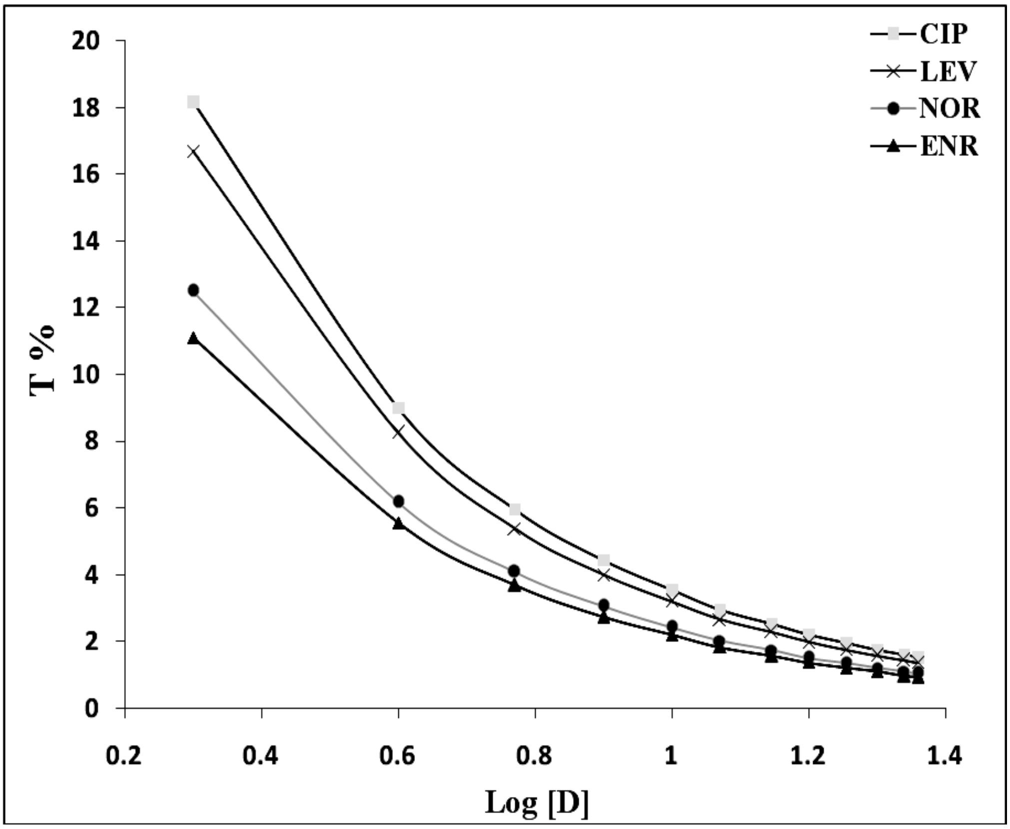

Figure (16). Ringbom plots for 100 μg ml^{-1} of the studied drugs using 1 X10^{-3} M RB

3.1.1.9.2. Range

The range of an analytical method is the concentration interval over which acceptable accuracy, linearity and precision are obtained. In practice the range is determined using data from the linearity and accuracy studies. The calibration range of the method is given in Table (1).

3.1.1.9.3. Detection and quantitation limits

Detection limit of the method is the lowest analyte concentration that produces a response detectable above the noise level of the system, where the quantitation limit is the lowest level of the analyte that can be accurately and precisely measured. Detection and quantitation limits are listed in Table (1).

Table (1). Optical and regression characteristics of RB with different drugs

Drugs / Parameters	CIP	LEV	NOR	ENR
λ_{max} (nm)	555	555	555	555
Bear's law limits ($\mu g\ ml^{-1}$)	2-23	2-23	2-23	2-23
Ringbom limits ($\mu g\ ml^{-1}$)	4-20	4-20	4-20	4-20
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	9.67×10^3	10.62×10^3	28.15×10^3	16.5×10^3
Sandell sensitivity ($ng\ cm^{-2}$)	34.20	31.09	11.34	21.74
Detection limits ($\mu g\ ml^{-1}$)	0.090	0.123	0.111	0.078
Quantitation limits ($\mu g\ ml^{-1}$)	0.30	0.41	0.37	0.26
Regression equation*: Slope (b)	0.0292	0.03216	0.08816	0.046
Intercept (a)	1.73×10^{-2}	1.34×10^{-2}	1.63×10^{-2}	0.36×10^{-2}
Correlation coefficient (r)	0.99980	0.99980	0.99996	0.99993
RSD%**	0.952	0.832	0.655	0.721
Stoichiometric R: D	1 : 1	1 : 1	1 : 1	1 : 1

* With respect to $A = a + b\ C$ where C is concentration of drug in $\mu g\ ml^{-1}$ and A is absorbance.

** Relative standard deviation for six determinations

3.1.1.9.4. Interference

the effect of the presence of co-exiting additives and excipients such as sodium acetate, bicarbonate, magnesium citrate, talc powder, starch, glucose, fructose, sucrose and lactose was studied by adding an excess amount of each of them to a solution containing 100 $\mu g\ ml^{-1}$ of CIP, LEV, NOR and ENR drug. Study of the effect of interfering species showed that such ingredients; up to 10 %, do not interfere in the determination of CIP, LEV, NOR and ENR drug indicating that complexation does not occur with those additives under reaction conditions.

3.1.1.9.5. Accuracy and precision

The accuracy and precision of the method is the closeness of the measured value to the true value for the sample. Accuracy of the proposed methods when applied to pharmaceutical preparation is evaluated by applying standard addition technique. In which variable amounts of the drugs under investigation were added to the previously analysed portion of pharmaceutical preparations. The results shown in Tables (2 – 5), confirmed that the proposed method is not liable to interference by fillers (lactose, monohydrated, microcrystalline cellulose, talc powder, sucrose, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, hydroxyl ethyl cellulose, flavors and magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (2). Determination of CIP, in its pharmaceutical dosage forms applying the standard addition technique using RB

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Ciprofloxacin tablets	3.00	2.00	5.02	100.40
		4.00	6.97	99.57
		6.00	8.95	99.44
Rancif tablets	3.00	2.00	4.99	99.80
		4.00	6.95	99.28
		6.00	9.01	100.10
Ciprofar tablets	3.00	2.00	4.97	99.40
		4.00	7.03	100.43
		6.00	8.98	99.78
Mifoxin tablets	3.00	2.00	4.98	99.60
		4.00	6.99	99.85
		6.00	8.97	99.67

*: average of six determinations.

Table (3). Determination of LEV, in its pharmaceutical dosage forms applying the standard addition technique using RB

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Levoxin tablets	3.00	2.00	4.99	99.90
		4.00	6.98	99.71
		6.00	9.02	100.17
Unibiotic tablets	3.00	2.00	4.97	99.30
		4.00	6.98	99.68
		6.00	8.96	99.53
Lee-flox tablets	3.00	2.00	4.98	99.60
		4.00	7.02	100.21
		6.00	8.98	99.78
Tavanic tablets	3.00	2.00	4.98	99.78
		4.00	6.99	99.93
		6.00	8.99	99.83

*: average of six determinations.

Table (4). Determination of NOR in its pharmaceutical dosage forms applying the standard addition technique using RB

Dosage forms	**Taken** **μg ml^{-1}**	**Added** **μg ml^{-1}**	**Found*** **μg ml^{-1}**	**Recovery (%)**
Epinor tablets	3.00	2.00	4.98	99.78
		4.00	6.99	99.86
		6.00	8.97	99.67
Noracin tablets	3.00	2.00	5.01	100.24
		4.00	6.98	99.73
		6.00	8.96	99.56
Norbactin tablets	3.00	2.00	4.97	99.30
		4.00	7.01	100.07
		6.00	8.99	99.89

*: average of six determinations.

Table (5). Determination of ENR, in its pharmaceutical dosage forms applying the standard addition technique using RB

Dosage forms	**Taken** µg ml^{-1}	**Added** µg ml^{-1}	**Found *** µg ml^{-1}	**Recovery (%)**
Enro-Flox sterile solution	3.00	2.00	4.98	99.78
		4.00	6.99	99.86
		6.00	8.97	99.67
Enroxin solution	3.00	2.00	5.01	100.24
		4.00	6.98	99.73
		6.00	8.96	99.56

*: average of six determinations.

3.1.1.9.6. Analytical application

Results from the analysis of RB with pharmaceutical preparations of different drugs are in a good agreement with those of the British pharmacopoeia. The relative standard deviation (six determinations) and the percentage recoveries of the proposed methods and pharmacopoeia procedure are recorded. The results obtained were compared statistically by the student's t-test and the variance ratio f-test with those obtained by pharmacopoeia method on samples of the same batch Tables (6 – 7). The student's t-test values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value, indicating no significant difference between the methods compared.

Table (6). Evaluation of the accuracy and precision of the proposed procedure of RB with different drugs

Drug	Taken µg ml^{-1}	Found µg ml^{-1}	Recovery %	RSD$^{(a)}$ %	RE$^{(b)}$ %	Confidence limits$^{(c)}$
CIP	5	5.090	101.8	0.59	0.62	5.09±0.039
	10	9.820	98.2	0.31	0.32	9.82±0.056
	15	15.150	101.0	0.20	0.21	15.15±0.049
LEV	5	5.040	100.8	0.81	0.85	5.04±0.065
	10	9.980	99.8	0.41	0.43	9.98±0.084
	15	15.075	100.5	0.27	0.29	15.08±0.077
NOR	5	5.025	100.5	0.74	0.77	5.03±0.044
	10	9.920	99.2	0.37	0.39	9.92±0.057
	15	15.090	100.6	0.25	0.26	15.09±0.053
ENR	5	4.985	99.7	0.52	0.55	4.99±0.056
	10	9.870	98.7	0.26	0.28	9.87±0.083
	15	15.225	101.5	0.17	0.18	15.23±0.069

(a) Relative standard deviation for six determinations

(b) Relative error

(c) 95% confidence limits and five degrees of freedom

Table (7). Evaluation of the accuracy and precision of the proposed and official methods for determination of the studied drugs in different pharmaceutical forms using RB

Drug	Official method			Proposed method				
	Taken µg	Found * µg	Recovery (%)	Taken µg	Found * µg	Recovery (%)	t ** value	f** value
Ciprofloxacin tablets	10	9.96	99.60	10	9.88	98.80	0.290	1.05
Rancif tablets	10	9.98	99.80	10	9.95	99.50	0.260	1.50
Ciprofar tablets	10	9.94	99.40	10	9.92	99.20	0.170	1.80
Mifoxin tablets	10	9.95	99.50	10	9.89	98.90	0.110	1.36
Levoxin tablets	10	9.88	98.80	10	9.94	99.40	0.196	1.35
Unibiotic tablets	10	9.92	99.20	10	9.83	98.30	0.228	1.09
Lee-flox tablets	10	9.85	98.50	10	9.96	99.60	0.228	1.70
Tavanic tablets	10	9.84	98.40	10	9.98	99.80	0.259	2.05
Epinor tablets	10	9.90	99.00	10	10.01	100.10	0.127	1.15
Noracin tablets	10	9.87	98.70	10	9.97	99.70	0.178	0.95
Norbactin tablets	10	9.89	98.90	10	9.99	99.90	0.177	1.55
Enro-Flox sterile solution	10	9.86	98.60	10	9.15	91.50	0.183	1.65
Enroxin solution	10	9.91	99.10	10	9.85	98.50	0.160	2.15

*: average of six determinations.

**: theoretical values for t-value and F-test values for the five degree of freedom and 95% confidence limits.

3.1.2. Absorption spectra of ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR) and enrofloxacin (ENR) with bromocresol purple reagent (BCP)

In order to investigate the optimum conditions of drug-reagent complex formation, the following studies should be taken in consideration:

3.1.2.1. *Effect of acid concentration*

The effect of acid concentration on the complex formation between CIP, LEV, NOR and ENR with BCP. The concentration of drugs and reagent were kept constant at 100 µg ml^{-1} and 1x 10^{-3} M respectively while that the volume of 1 x10^{-2} M hydrochloric acid with all drugs except NOR is 1 x10^{-3} M was regularly varied, the volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug. Illustrative spectra are shown in Figure (17) for the effect of acid concentration, inspection of these figure shows that the optimum ml added of acid giving maximum absorption recommended for subsequent studies of drug-reagent complexes, are 0.2 ,0.1 ,0.1 and 0.8 ml for CIP, LEV, NOR and ENR respectively.

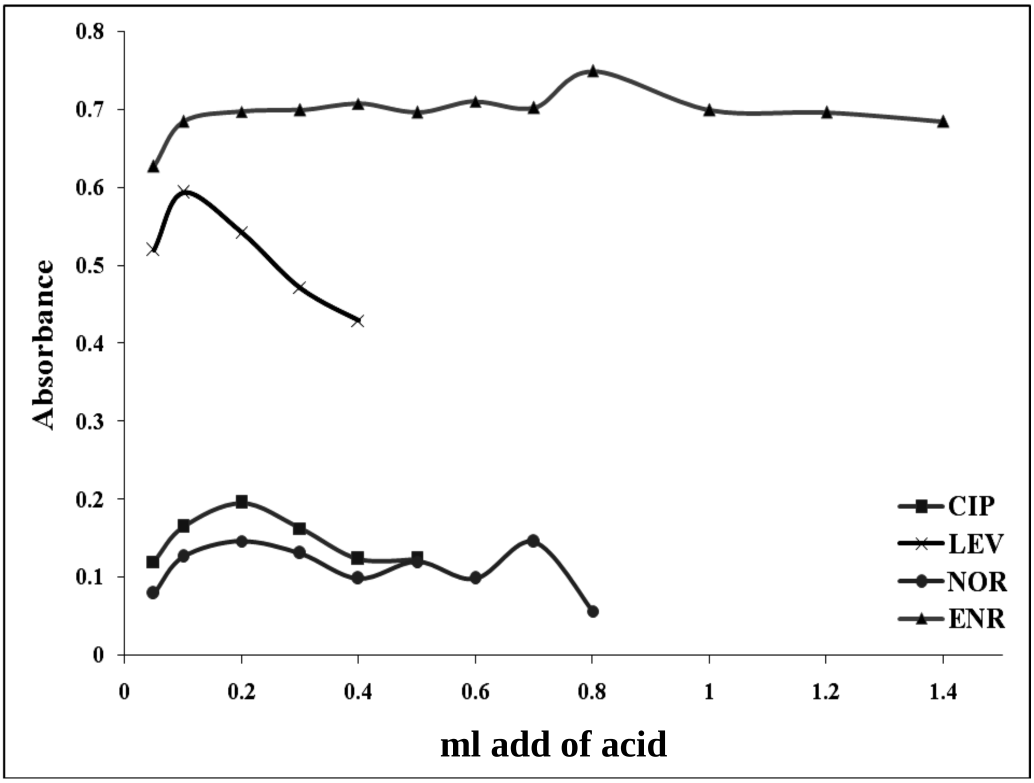

Figure (17). Effect of ml added of acid (1 x10^{-2} M HCl) and (1 x10^{-3} M HCl) on the absorbance of the studied drugs using 1X10^{-3} M BCP.

3.1.2.2. ***Determination of λ_{max} of the complex species***

To determine the value of λ_{max} at which the complex species possesses the maximum absorption, the following spectra must be recorded:

A- Spectrum of pure drugs 1 ml of 100 μg ml^{-1} at optimum acid value using acid solution at the recommended acid value as a blank.

B- Spectrum of pure BCP 1 ml of 1 x 10^{-3} at optimum acid value using the same acid as a blank.

C- Spectrum of mixture of drugs (A) and reagent (B) at the optimum acid value using reagent at the same acid as a blank.

D- Spectrum of solution (C) against (B) as a blank.

The absorption spectra show that the formed complex absorbed maximally at 564 nm for BCP with CIP, LEV, NOR and ENR drugs as shown in Figure (18). This optimal wavelength is chosen for further investigations.

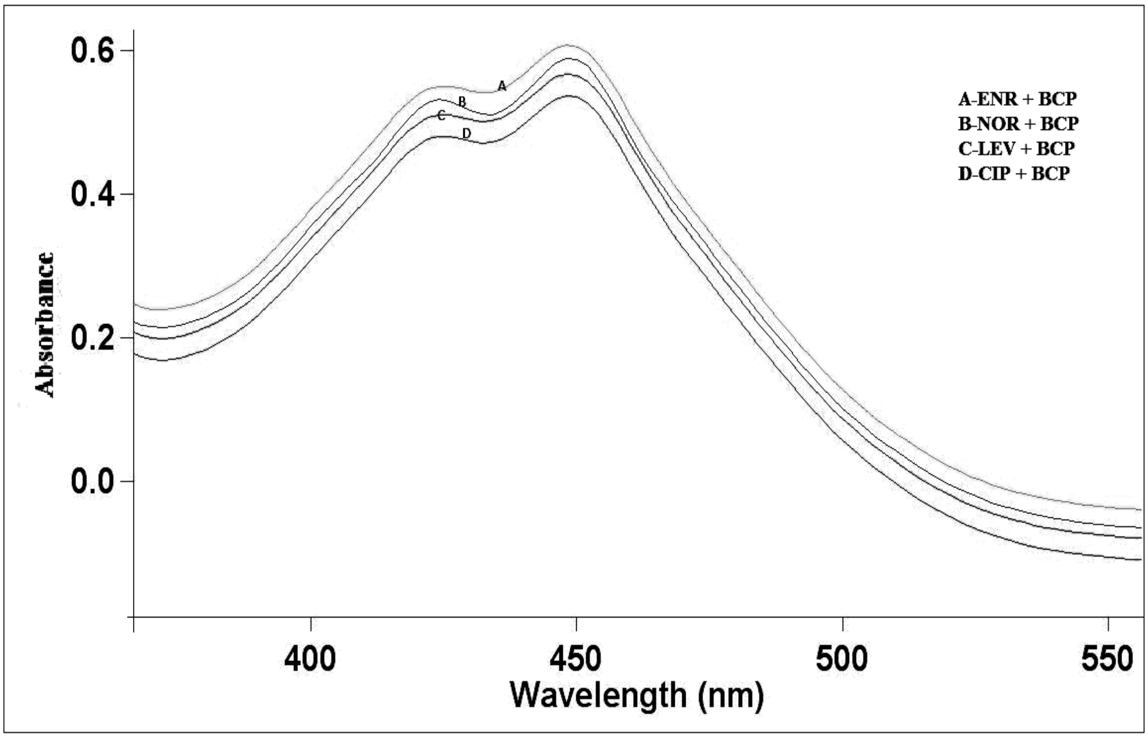

Figure (18). Absorption spectra of ion pair complex of 1 X10^{-3} M BCP with 100 μg ml^{-1} of studied drugs.

3.1.2.3. ***Effect of sequence of addition***

The Effect of sequence of addition on complex formation was studied by measuring the absorption of solutions prepared using different sequences of addition against blank solution prepared in the same manner. The experiments showed that the best sequence of addition is drug –acid – reagent – water.

3.1.2.4. ***Effect of time and temperature***

The Effect of time on complex formation was studied by measuring the absorbance of complex at optimum acid concentration against a blank solution of the same acid concentration at various time intervals. Also, the effect of temperature was studied for the same solution by incubation the sample and blank in water bath at different temperatures (25-45 ºC) the absorption was measured after cooling to room temperature.

The experiments showed that complexes are formed simultaneously after mixing drug and metal chloride and remain stable for about two hours. Also, it was found that, increasing the temperature up to 45 ºC has slight effect on the absorbance above which the color began to fade slowly.

3.1.2.5. ***Effect of reagent concentration***

To study the effect of BCP concentration on the complex formation with optimum acid value concentration for CIP, LEV, NOR and ENR respectively, the concentration of drugs were kept constant at 100 µg ml^{-1} while that of BCP was regularly varied, the volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug. Illustrative spectra are shown in Figure (19). For the effect of reagent concentration, inspection of these figure shows that the optimum ml added of reagent giving maximum absorption recommended for subsequent studies of drug-reagent complexes, are 1.7 ,1 ,0.6 and 0.7 ml for CIP, LEV, NOR and ENR respectively.

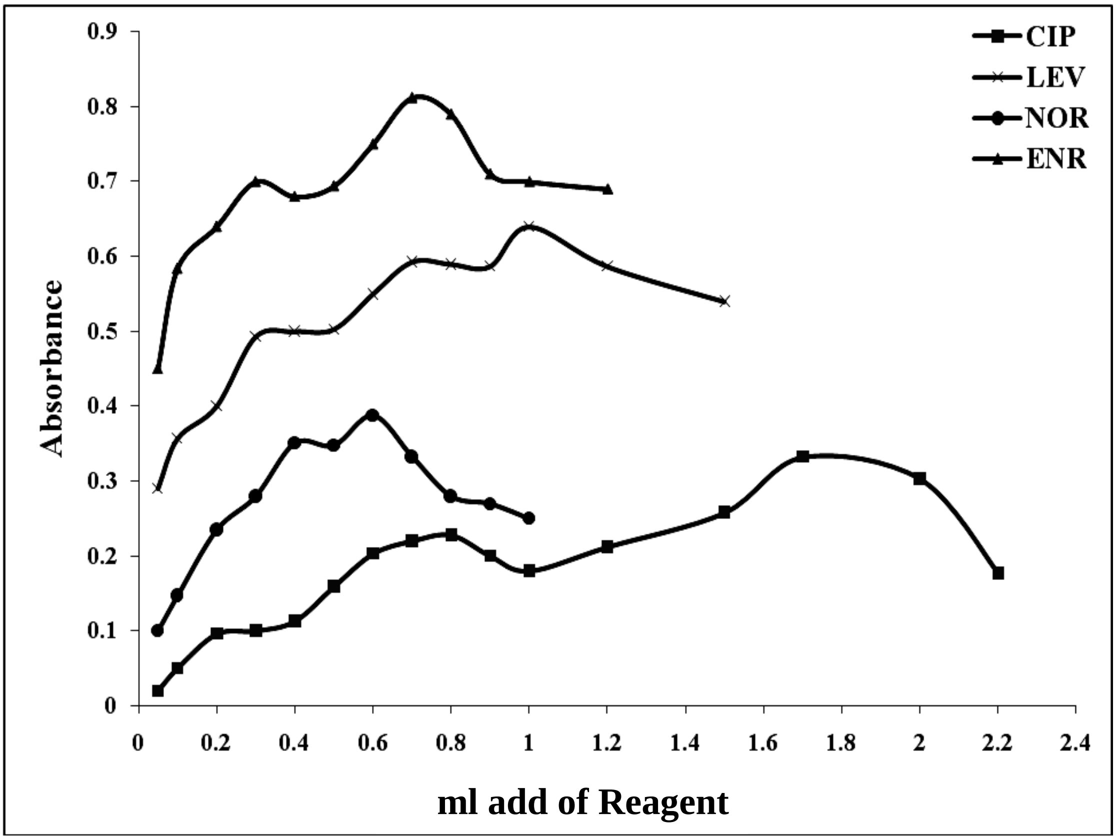

Figure (19). Effect of reagent concentration on absorption rang of 1 X10^{-3} M BCP with 100 µg ml^{-1} of studied drugs

3.1.2.6. *Molar ratio method*

In order to investigate the molecular ratio of the complexes formed between the drugs under investigation and BCP at the selected conditions, the molar ratio and continues variation methods were carried out. Experimental results revealed that the complexes formed between the drugs and BCP have 1: 1 stoichiometric ratio as shown in Figures (20 and 21).

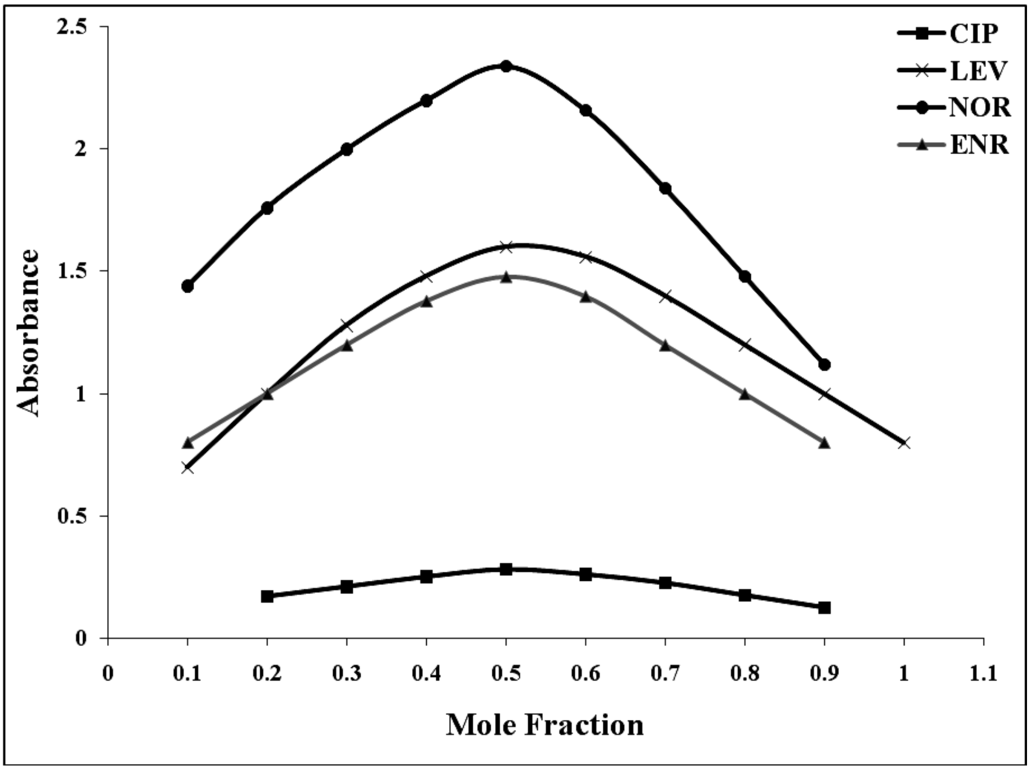

Figure (20). Continuous variation for (1 X10^{-3} M) reagent (II) with 100 μg ml^{-1} of studied drugs

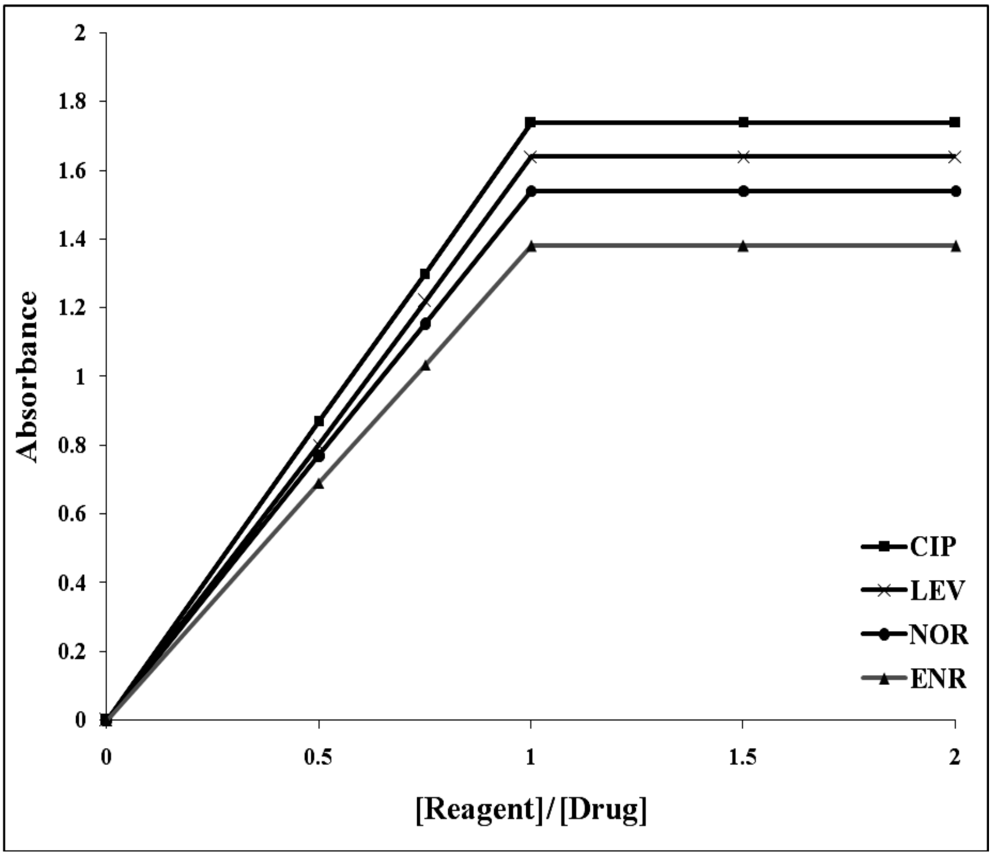

Figure (21). Mole ratio for 1 X10^{-3} M BCP with 100 μg ml^{-1} of studied drugs

3.1.2.7. *Stability constant of the complexes*

Spectrophotometric methods can be applied for the determination of the stability constant of the complexes. The calculated stability constants are listed in Table (8), from which it is found that BCP forms the most stable complex with NOR then ENR then LEV and CIP drug.

3.1.2.8. *Validation of the method*

Method validation is the process of providing that the analytical method is acceptable, there is various validation studies developed and recorded below.

3.1.2.8.1. Linearity

Under optimum conditions of pH, reagent concentration, sequence of additions, time and temperature for each of the different complexes, different concentrations of drug (μg ml^{-1}) were transferred into 10 ml measuring flask. 1.7, 1, 0.6 and 0.7 of reagent (1 x 10^{-3} M) with CIP, LEV, NOR and ENR respectively, 0.2, 0.1, 0.1 and 0.8 ml of the optimum pH value acid with CIP, LEV, NOR and ENR respectively were added and then completed to volume with bidistilled water. The absorbance was measured at optimum λ_{max}, and then plotted against drug concentration as shown in Figure (22). Limits of beer' s law, the molar absorbability (ε; L mol^{-1}cm^{-1}) and ***Sandell*** (76) sensitivity results are listed in Table (8), indicating high sensitivity in the microdetermination of the drug.

For more accurate analysis, ***Ringbom*** (77) optimum concentration rang was determined by calculating the transmittance percent from the following equation:

$$T\ \% = 10^{-A} \times 100$$

Where: T %: transmittance percent,

A: the absorbance of the complex

By plotting logarithm of drug concentration (log [C_D]) in μg mL^{-1} against T %, the linear portion of the S-shaped curve gave an accurate range of analysis as shown in Figure (23).Results are listed in Table (9).

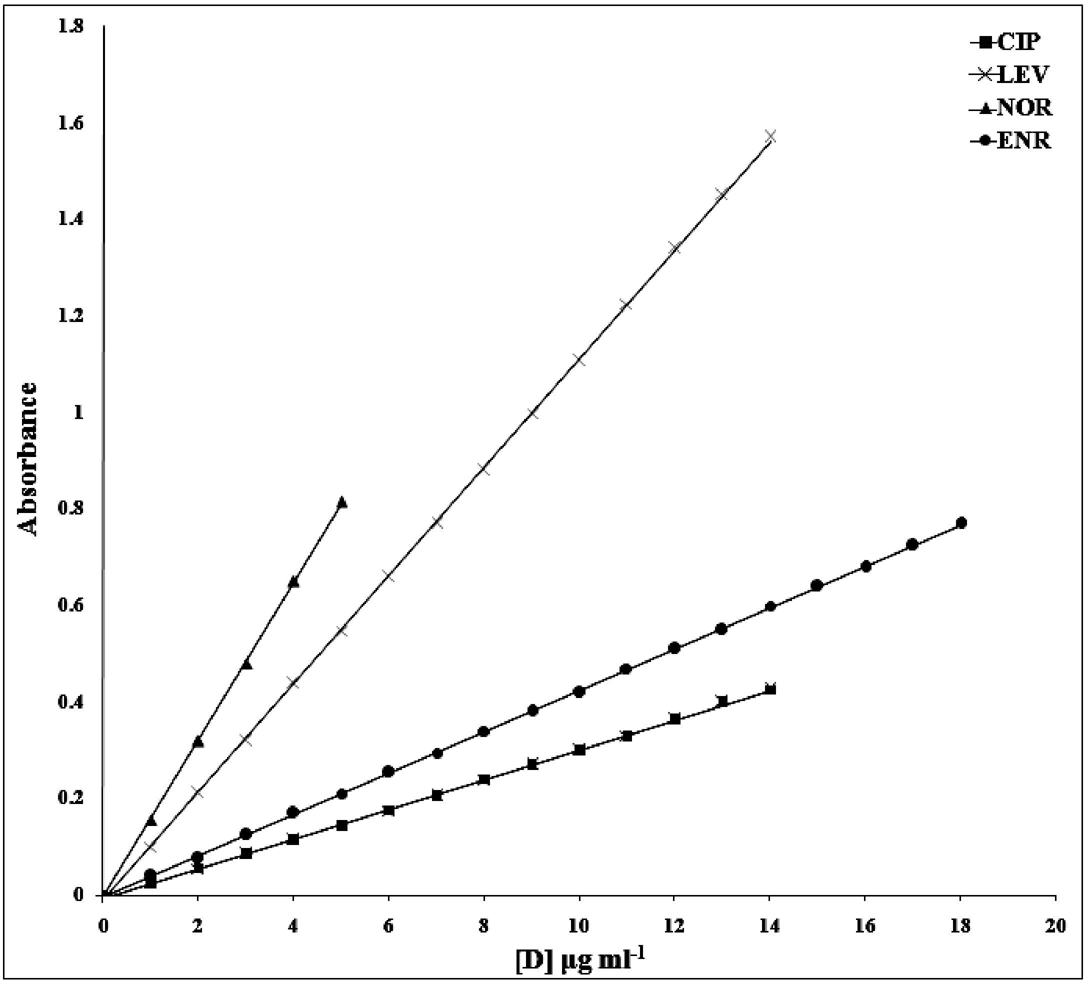

Figure (22). Applications of Beer's law for the studied drugs (100 μg ml^{-1}) using the optimum volume of 1 $X10^{-3}$ M BCP

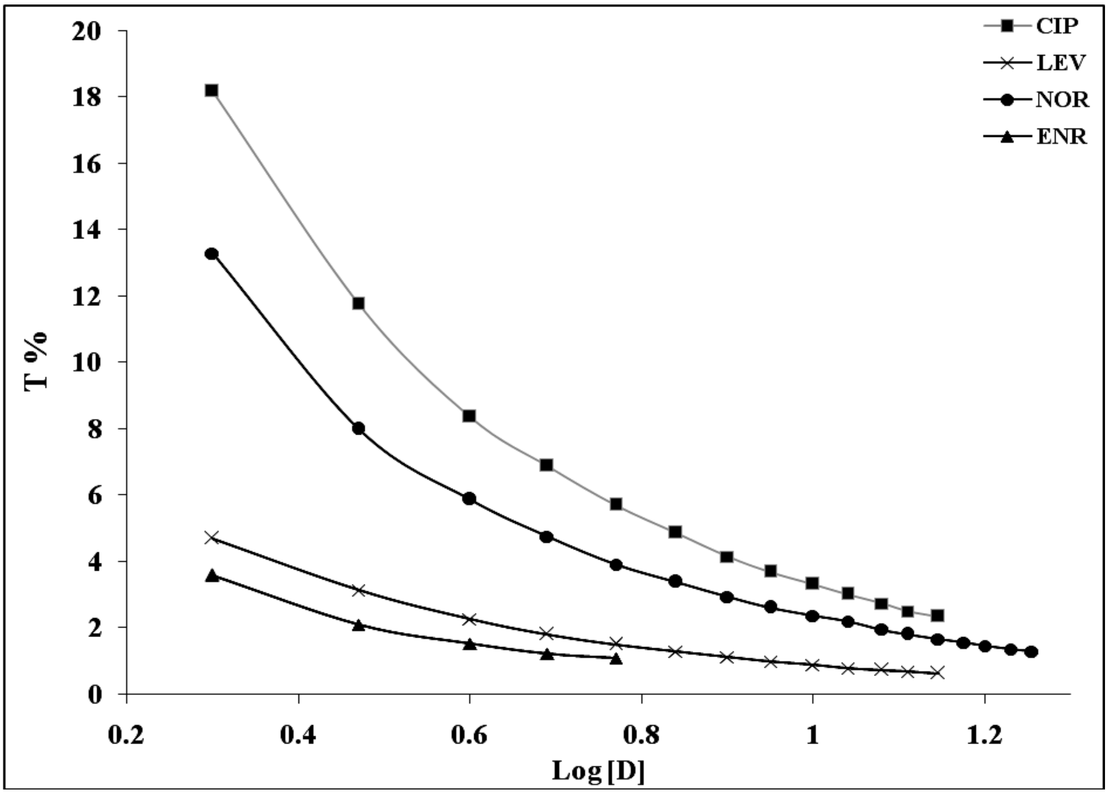

Figure (23). Ringbom plots for the studied drugs (100 μg ml^{-1}) using 1 X10^{-3} M BCP

3.1.2.8.2. Range

The range of an analytical method is the concentration interval over which acceptable accuracy, linearity and precision are obtained. In practice the range is determined using data from the linearity and accuracy studies. The calibration range of the method is given in Table (8).

3.1.2.8.3. Detection and quantitation limits

Detection limit of the method is the lowest analyte concentration that produces a response detectable above the noise level of the system, where the quantitation limit is the lowest level of the analyte that can be accurately and precisely measured. Detection and quantitation limits are listed in Table (8).

Table (8). Optical and regression characteristics of BCP with different drugs

Drugs / Parameters	CIP	LEV	NOR	ENR
λ_{max} (nm)	564	564	564	564
Bear's law limits (µg ml^{-1})	1-14	1-14	1-17	1-5
Ringbom limits (µg ml^{-1})	2-12	2-12	2-14	2-4
Molar absorptivity (L mol^{-1} cm^{-1})	$10.27x\ 10^3$	$41.2\ x\ 10^3$	$13.5\ x\ 10^3$	$58.6\ x\ 10^3$
Sandell sensitivity (ng cm^{-2})	32.20	8.76	23.60	6.13
Detection limits (µg ml^{-1})	0.090	0.123	0.111	0.078
Quantitation limits (µg ml^{-1})	0.30	0.41	0.37	0.26
Regression equation*: Slope (b)	0.03100	0.11410	0.04236	0.16300
Intercept (a)	$1.13\ x\ 10^{-2}$	$3.60\ x\ 10^{-2}$	$0.45\ x\ 10^{-2}$	$0.80\ x\ 10^{-2}$
Correlation coefficient (r)	0.9993	0.9996	0.9930	0.9949
RSD** %	0.655	0.732	0.812	0.692
Stoichiometric R : D	1 : 1	1 : 1	1 : 1	1 : 1

* With respect to $A = a + b\ C$ where C is concentration of drug in µg ml^{-1} and A is absorbance.

** Relative standard deviation for six determinations

3.1.2.8.4. Interference

the effect of the presence of co-exiting additives and excipients such as sodium acetate, bicarbonate, magnesium citrate, talc powder, starch, glucose, fructose, sucrose and lactose was studied by adding an excess amount of each of them to a solution containing 100 µg ml^{-1} of CIP, LEV, NOR and ENR drug.

Study of the effect of interfering species showed that such ingredients; up to 10 %, do not interfere in the determination of CIP, LEV, NOR and ENR drug indicating that complexation does not occur with those additives under reaction conditions.

3.1.2.8.5. Accuracy and precision

The accuracy and precision of the method is the closeness of the measured value to the true value for the sample. Accuracy of the proposed method when applied to pharmaceutical preparation is evaluated by applying standard addition technique. In which variable amounts of the drugs under investigation were added to the previously analysed portion of pharmaceutical preparations. The results shown in Tables (9 –12), confirmed that the proposed method is not liable to interference by fillers (lactose, monohydrated, microcrystalline cellulose, talc powder, sucrose, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, hydroxyl ethyl cellulose, flavors and magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (9). Determination of CIP in its pharmaceutical dosage forms applying the standard addition technique using BCP

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Ciprofloxacin tablets	3.00	2.00	4.94	98.80
		4.00	6.90	98.57
		6.00	8.96	99.56
Rancif tablets	3.00	2.00	4.93	98.50
		4.00	7.01	100.14
		6.00	8.95	99.44
Ciprofar tablets	3.00	2.00	4.92	98.40
		4.00	6.98	99.70
		6.00	8.93	99.22
Mifoxin tablets	3.00	2.00	4.95	99.00
		4.00	6.90	99.57
		6.00	8.88	98.67

*: average of six determinations

Table (10). Determination of LEV in its pharmaceutical dosage forms applying the standard addition technique using BCP

Dosage forms	Taken $\mu g\ ml^{-1}$	Added $\mu g\ ml^{-1}$	Found * $\mu g\ ml^{-1}$	Recovery (%)
Levoxin tablets	3.00	2.00	4.92	98.40
		4.00	6.97	99.57
		6.00	8.93	99.24
Unibiotic tablets	3.00	2.00	4.96	99.20
		4.00	6.99	99.86
		6.00	8.95	99.44
Lee-flox tablets	3.00	2.00	4.94	98.70
		4.00	6.93	99.00
		6.00	8.92	99.10
Tavanic tablets	3.00	2.00	4.93	98.50
		4.00	6.91	98.71
		6.00	8.94	99.35

*: average of six determinations

Table (11). Determination of NOR in its pharmaceutical dosage forms applying the standard addition technique using BCP

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Epinor tablets	3.00	2.00	4.96	99.24
		4.00	6.93	99.01
		6.00	8.91	99.00
Noracin tablets	3.00	2.00	4.96	99.18
		4.00	6.96	99.24
		6.00	8.94	99.33
Norbactin tablets	3.00	2.00	4.91	99.12
		4.00	6.92	98.85
		6.00	8.99	99.88

*: average of six determinations

Table (12). Determination of ENR in its pharmaceutical dosage forms applying the standard addition technique using BCP

Dosage forms	**Taken** µg ml^{-1}	**Added** µg ml^{-1}	**Found *** µg ml^{-1}	**Recovery (%)**
Enro-Flox sterile solution	3.00	2.00	4.96	99.22
		4.00	6.93	99.02
		6.00	8.93	99.17
Enroxin solution	3.00	2.00	4.91	98.18
		4.00	6.92	98.86
		6.00	8.90	98.88

*: average of six determinations

3.1.2.8.6. Analytical application

Results from the analysis of BCP with pharmaceutical preparations of different drugs are in a good agreement with those of the British pharmacopoeia. The relative standard deviation (six determinations) and the percentage recoveries of the proposed methods and pharmacopoeia procedure are recorded. The results obtained were compared statistically by the student's t-test and the variance ratio f-test with those obtained by pharmacopoeia method on samples of the same batch Tables (13 and 14). The student's t-test values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value, indicating no significant difference between the methods compared.

Table (13). Evaluation of the accuracy and precision of the proposed procedure of BCP with different drugs

Drug	Taken µg ml^{-1}	Found µg ml^{-1}	Recovery %	RSD[(a)] %	RE[(b)] %	Confidence limits[(c)]
CIP	5	4.90	98.00	0.649	0.75	4.9±0.045
	10	9.94	99.40	0.367	0.45	9.94±0.032
	15	15.07	100.46	0.254	0.34	15.07±0.062
LEV	5	4.96	99.20	0.867	0.98	4.96±0.071
	10	10.01	100.10	0.468	0.56	10.01±0.042
	15	14.96	99.73	0.326	0.42	14.96±0.12
NOR	5	5.01	100.20	0.795	0.90	5.01±0.051
	10	9.96	99.60	0.427	0.52	9.96±0.077
	15	14.98	99.86	0.309	0.39	14.98±0.036
ENR	5	4.97	99.50	0.577	0.68	4.975±0.047
	10	9.90	99.00	0.318	0.41	9.9±0.092
	15	14.93	99.53	0.226	0.32	14.93±0.2

(a) Relative standard deviation for six determinations

(b) Relative error

(c) 95% confidence limits and five degrees of freedom

Table (14). Evaluation of the accuracy and precision of the proposed and official methods for determination of the studied drugs in different pharmaceutical forms using BCP

Drug	**Official method**			**Proposed method**				
	Taken µg	**Found * µg**	**Recovery** (%)	**Taken µg**	**Found * µg**	**Recovery** (%)	**t **** value	**f **** value
Ciprofloxacin tablets	10	9.83	98.3	10	9.91	99.05	0.88	2.97
Rancif tablets	10	9.94	99.4	10	10.03	100.30	0.67	1.54
Ciprofar tablets	10	9.86	98.6	10	9.88	98.80	0.7	1.23
Mifoxin tablets	10	9.91	99.1	10	10.01	100.10	0.58	2.6
Levoxin tablets	10	10.06	100.6	10	10.02	100.20	0.29	2.48
Unibiotic tablets	10	98.80	98.8	10	9.96	99.60	0.39	3.39
Lee-flox tablets	10	9.91	99.1	10	9.93	99.30	0.29	3.02
Tavanic tablets	10	98.20	98.2	10	9.97	99.70	0.85	2.55
Epinor tablets	10	9.92	99.2	10	9.95	99.50	0.36	2.66
Noracin tablets	10	9.85	98.5	10	10.01	100.10	0.633	1.77
Norbactin tablets	10	9.90	99.0	10	9.96	99.60	0.76	1.28
Enro-Flox sterile solution	10	9.88	98.8	10	9.94	99.40	0.66	1.64
Enroxin solution	10	9.91	99.1	10	9.98	99.80	0.25	2.06

*: average of six determinations

**: theoretical values for t-value and F-test values for the five degree of freedom and 95% confidence limits

3.1.3. Absorption spectra of ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR) and enrofloxacin (ENR) with Erythrosin reagent (ERY)

In order to investigate the optimum conditions of drug-reagent complex formation, the following studies should be taken in consideration:

3.1.3.1. *Effect of pH*

The effect of pH on the complex formation between CIP, LEV, NOR and ENR with ERY was studied in universal buffer solutions of pH range 2-14. A portion (0.5 ml) of 1 x 10^{-3} M ERY, 0.5 ml of (100 μg mL^{-1}) drug and 4 ml buffer of different pH values were mixed well. The volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug at the same pH value. The optimum pH values giving maximum absorption recommended for subsequent studies of drug-reagent complexes, is 4 on using CIP, LEV, NOR and ENR.

3.1.3.2. *Effect of buffer concentration*

To study the effect of buffer concentration on the complex formation with last pH value 4 for CIP, LEV, NOR and ENR, the concentration of drugs and reagent were kept constant at 100 μg ml^{-1} and 1 x 10^{-3} M respectively while that of buffer was regularly varied the volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug. Illustrative spectra are shown in Figure (24) for the effect of buffer concentration, inspection of these figure shows that the optimum ml added of buffer giving maximum absorption recommended for subsequent studies of drug-reagent complexes, are 1 ,2.5 ,1.5 and 1.5 ml for CIP, LEV, NOR and ENR respectively.

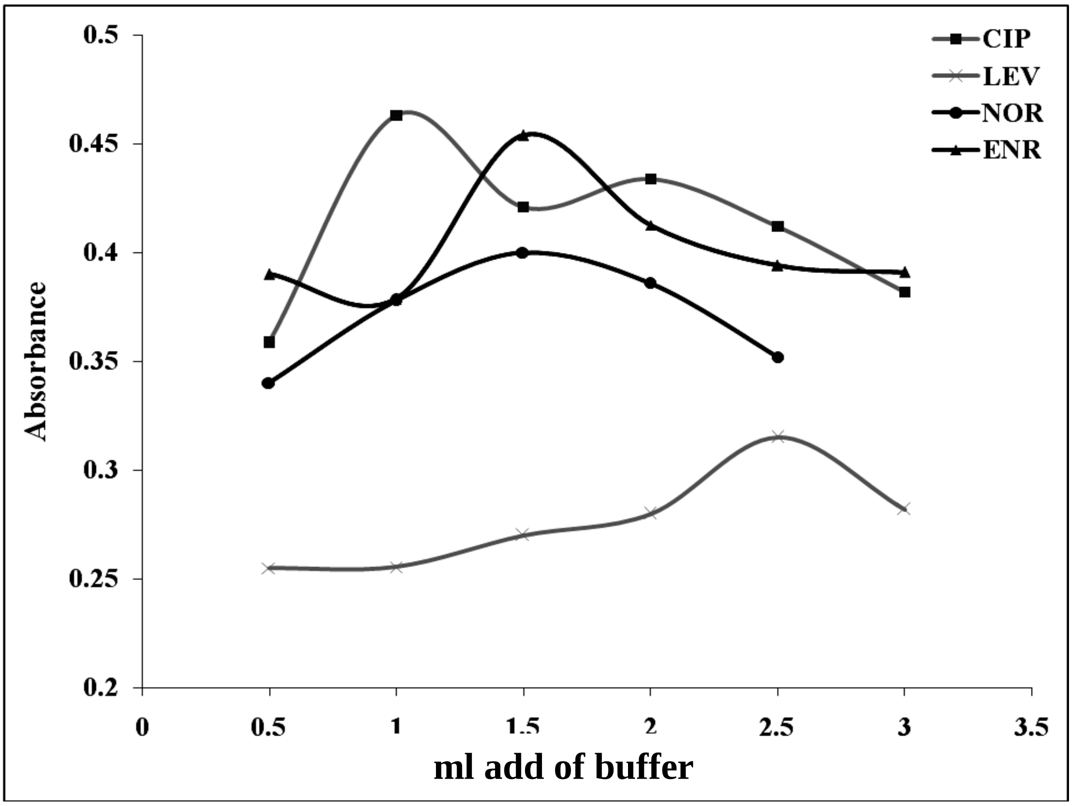

Figure (24). Effect of buffer concentration on absorption rang of 1 X10^{-3} M ERY with 100 µg ml^{-1} of studied drugs

3.1.3.3. ***Determination of λ_{max} of the complex species***

For determine the value of λ_{max} at which the complex species possesses the maximum absorption, the following spectra must be recorded:

A- Spectrum of pure drugs 1 ml of 100 µg ml^{-1} at optimum pH value using buffer solution at the recommended pH value as a blank.

B- Spectrum of pure reagent 1 ml of 1 x 10^{-3} at optimum pH value using the same buffer as a blank.

C- Spectrum of mixture of drugs (A) and reagent (B) at the optimum pH value using reagent at the same pH as a blank.

D- Spectrum of solution (C) against (B) as a blank.

The absorption spectra show that the formed complex absorbed maximally at 573 nm for ERY with drugs CIP, LEV, NOR and ENR as shown in Figure (25). This optimal wavelength is chosen for further investigations.

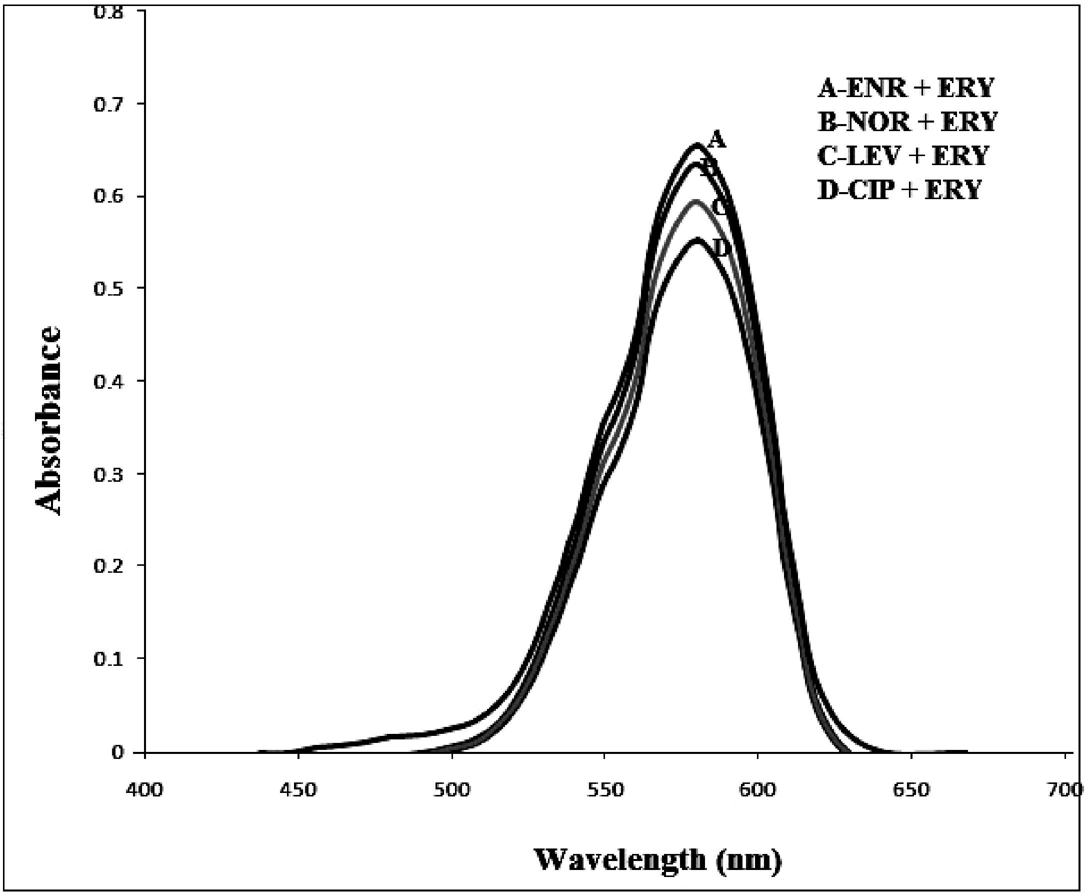

Figure (25). Absorption spectra of ion pair complex of 1 X10^{-3} M ERY with 100 μg ml^{-1} of studied drugs

3.1.3.4. *Effect of sequence of addition*

The Effect of sequence of addition on complex formation was studied by measuring the absorption of solutions prepared using different sequences of addition against blank solution prepared in the same manner without drug. The experiments showed that the best sequence of addition is drug - buffer or acid – reagent - water.

3.1.3.5. *Effect of time and temperature*

The Effect of time on complex formation was studied by measuring the absorbance of complex at optimum pH against a blank solution of the same pH at various time intervals. Also, the effect of temperature was studied for the same solution by incubation the sample and blank in water bath at different temperatures (25-45 ºC) the absorption was measured after cooling to room temperature.

The experiments showed that complexes are formed simultaneously after mixing drug and reagent and remain stable for about two hours. Also, it was found that, increasing the temperature up to 45 ºC has slight effect on the absorbance above which the color began to fade slowly.

3.1.3.6. *Effect of reagent concentration*

To study the effect of reagent concentration on the complex formation with optimum pH value concentration of buffer for CIP, LEV, NOR and ENR respectively, the concentration of drugs were kept constant at 100 µg ml^{-1} while that of reagent was regularly varied, the volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug. Illustrative spectra are shown in Figure (26) for the effect of reagent concentration, inspection of these figure shows that the optimum ml added of reagent giving maximum absorption recommended for subsequent studies of drug-reagent complexes, are 1.7 ,1.5 ,1.5 and 1.2 ml for CIP, LEV, NOR and ENR respectively.

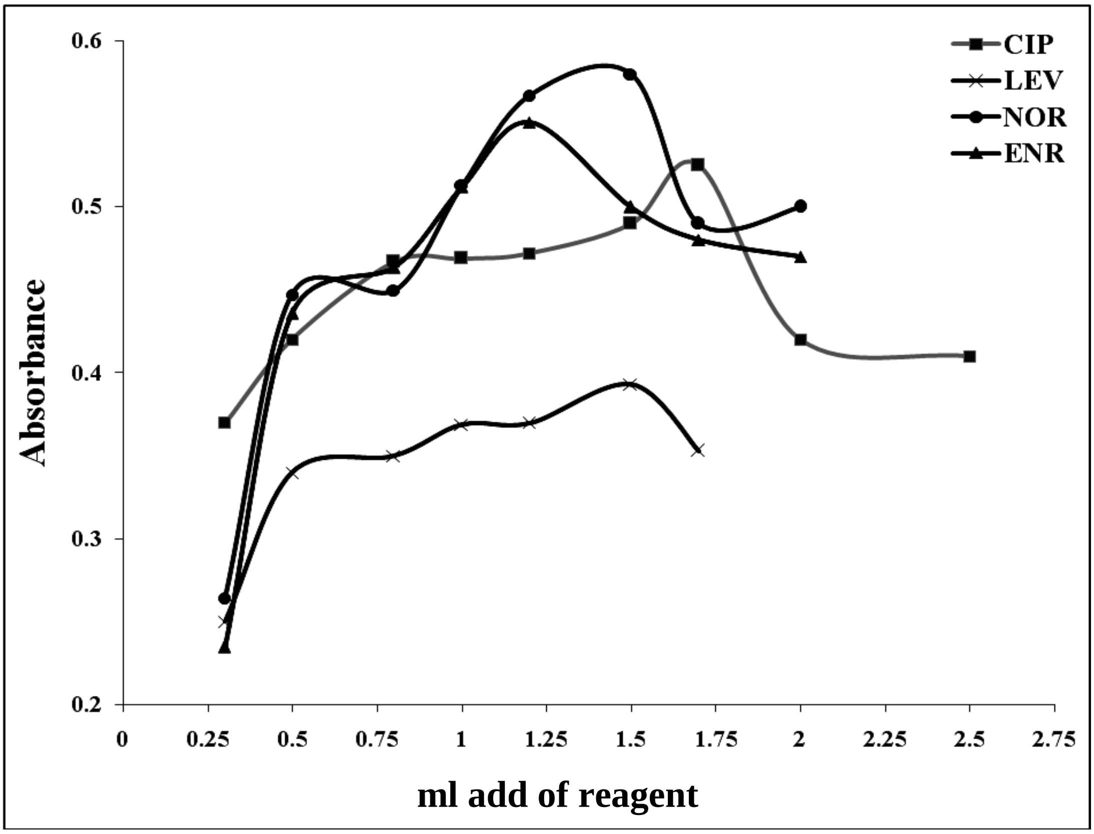

Figure (26). Effect of reagent concentration on absorption rang of 1 X10^{-3} M ERY with 100 µg ml^{-1} of studied drugs

3.1.3.7. *Molar ratio method*

In order to investigate the molecular ratio of the complexes formed between the drugs under investigation and ERY at the selected conditions, the molar ratio and continues variation methods were carried out. Experimental results revealed that the complexes formed between the drugs and ERY have 1:1 stoichiometric ratio as shown in Figures (27 and 28).

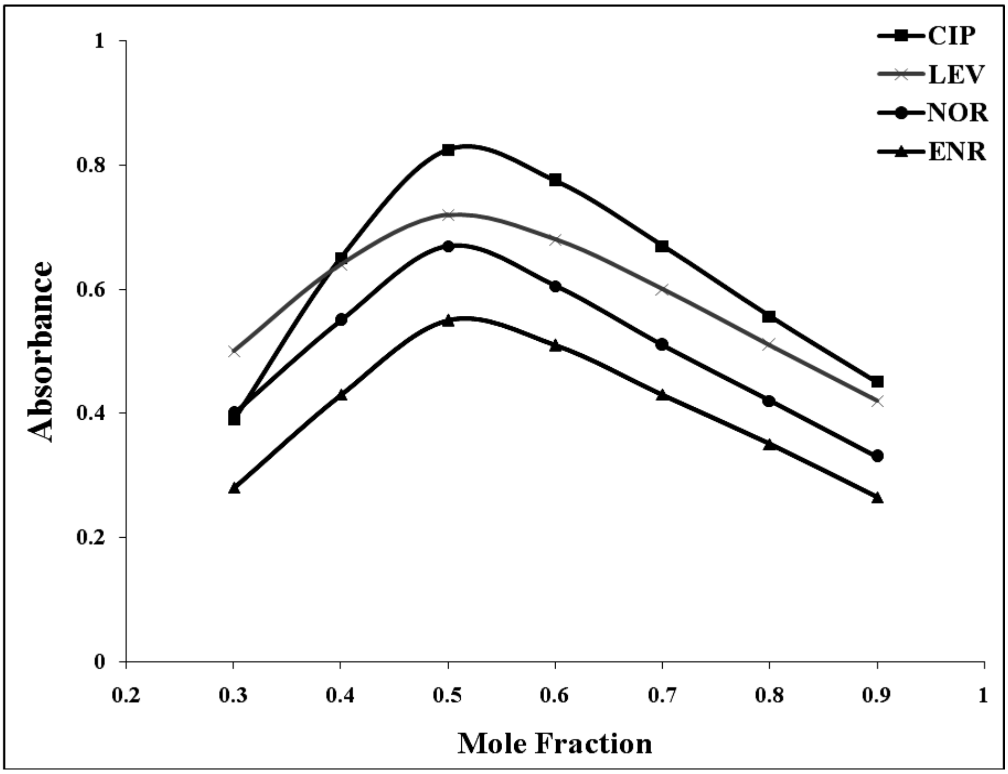

Figure (27). Continuous variation for (1 X10^{-3} M) ERY with 100 µg ml^{-1} of studied drugs

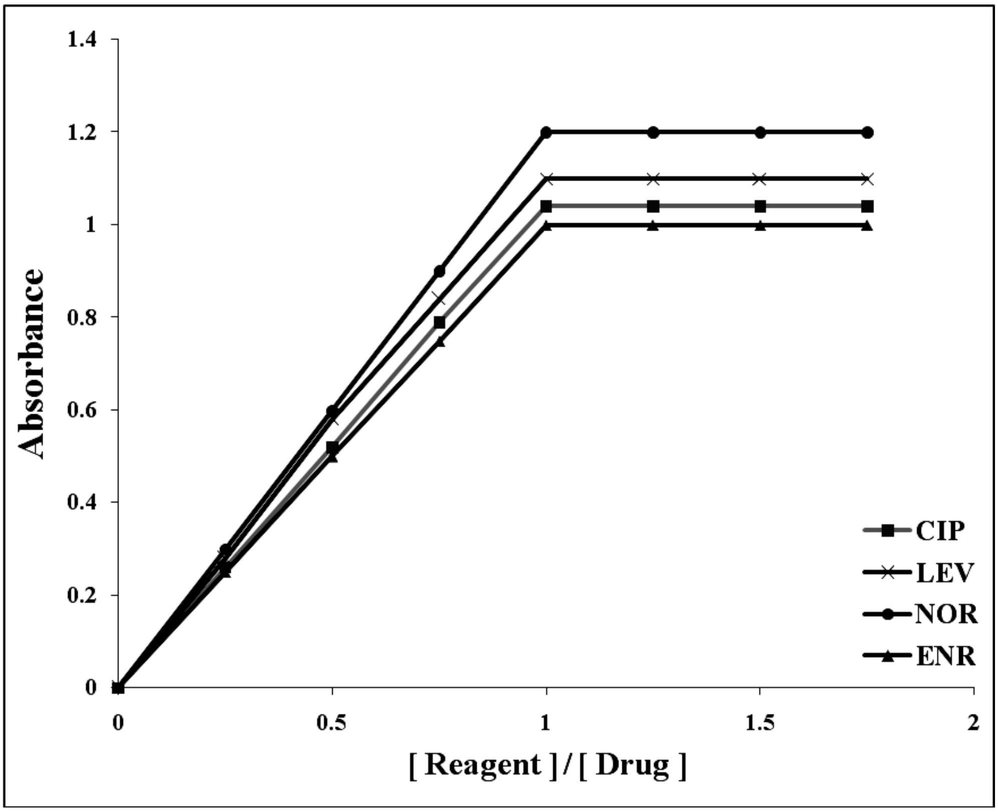

Figure (28). Mole ratio for 1 X10^{-3} M ERY with 100 µg ml^{-1} of studied drugs

3.1.3.8. *Stability constant of the complexes*

Spectrophotometric methods can be applied for the determination of the stability constant of the complexes. The calculated stability constants are listed in Table (15), from which it is found that the BCP forms the most stable complex with NOR then ENR then LEV and CIP drug

3.1.3.9. *Validation of the method*

Method validation is the process of providing that the analytical method is acceptable, there is various validation studies developed and recorded below.

3.1.3.9.1. Linearity

under optimum conditions of pH, reagent concentration, sequence of additions, time and temperature for each of the different complexes, different concentrations of drug (μg ml^{-1}) were transferred into 10 ml measuring flask. 1.7 ,1.5 ,1.5 and 1.2 ml of reagent (1 x 10^{-3}) with CIP, LEV, NOR and ENR respectively, 1 ,2.5 ,1.5 and 1.5 ml of the optimum pH value buffer with CIP, LEV, NOR and ENR respectively were added and then completed to volume with bidistilled water. The absorbance was measured at optimum λ_{max}, and then plotted against drug concentration as shown in Figure (29). Limits of beer' s law, the molar absorbability (ε; L $mol^{-1}cm^{-1}$) and ***Sandell*** (76) sensitivity results are listed in Table (15) indicating high sensitivity in the microdetermination of the drug.

For more accurate analysis, ***Ringbom*** (77) optimum concentration rang was determined by calculating the transmittance percent from the following equation:

$$T \% = 10^{-A} \times 100$$

Where: T %: transmittance percent,

A: the absorbance of the complex

By plotting logarithm of drug concentration (log [C_D]) in μg mL^{-1} against T %, the linear portion of the S-shaped curve gave an accurate range of analysis as shown in Figure (30); Results are listed in Table (15).

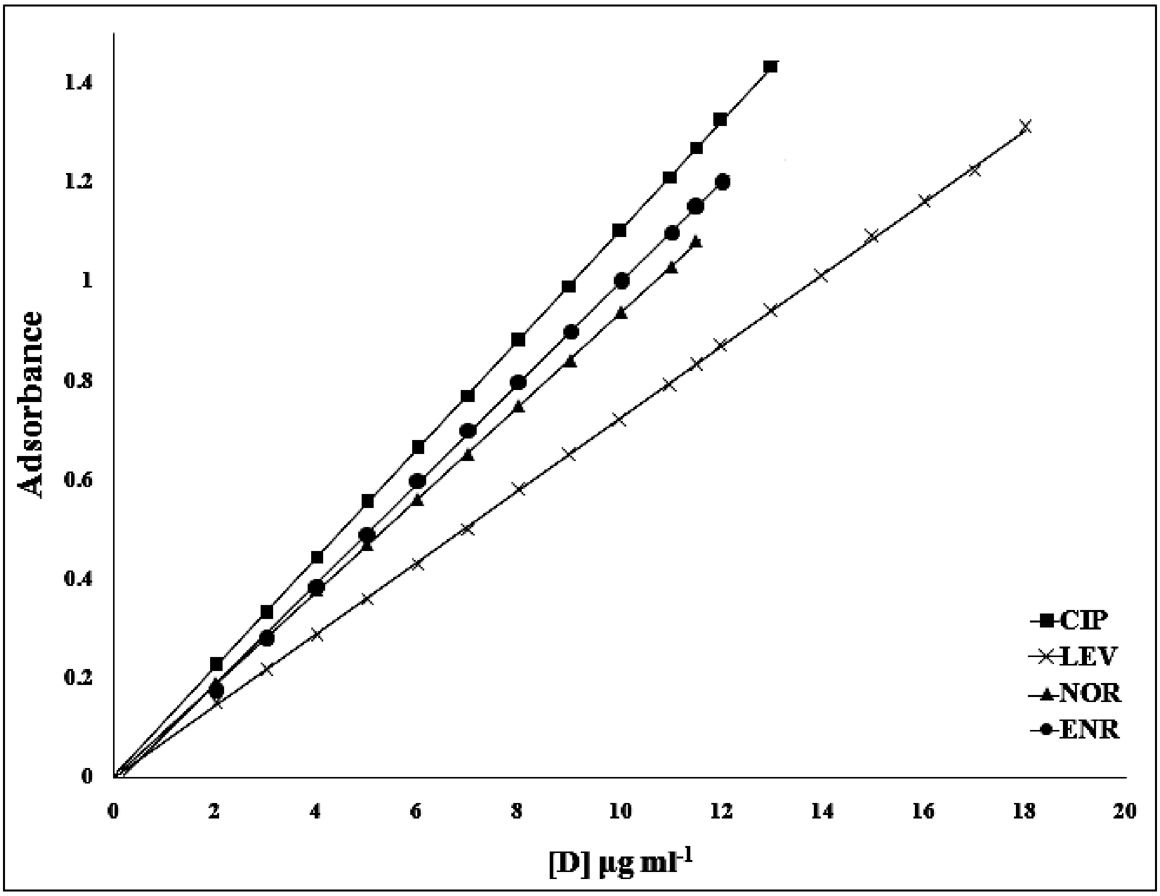

Figure (29). Applications of Beer's law for the studied drugs (100 μg ml^{-1}) using the optimum volume of (1 X10^{-3} M) ERY

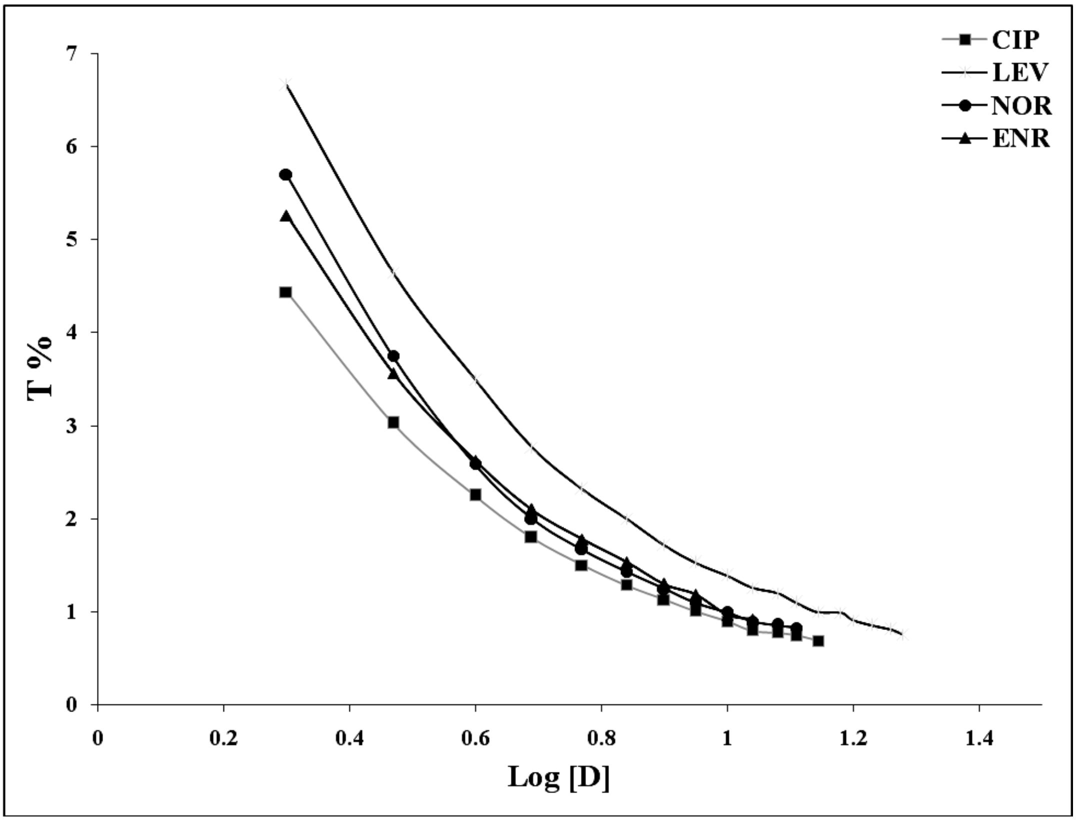

Figure (30). Ringbom plots for the studied drugs (100 μg ml^{-1}) using 1 X10^{-3} M ERY

3.1.3.9.2. Range

The range of an analytical method is the concentration interval over which acceptable accuracy, linearity and precision are obtained. In practice the range is determined using data from the linearity and accuracy studies. The calibration range of the method is given in Table (15).

3.1.3.9.3. Detection and quantitation limits

Detection limit of the method is the lowest analyte concentration that produces a response detectable above the noise level of the system, where the quantitation limit is the lowest level of the analyte that can be accurately and precisely measured. Detection and quantitation limits are listed in Table (15).

Table (15). Optical and regression characteristics of ERY with different drugs

Parameters \ Drugs	CIP	LEV	NOR	ENR
λ_{max} (nm)	573	573	573	573
Bear's law limits (µg ml^{-1})	2-13	2-18	2-12	2-11.5
Ringbom limits (µg ml^{-1})	4-12	4-16	4-10	4-10
Molar absorptivity (L mol^{-1} cm^{-1})	0.99×10^3	2.39×10^3	9.58×10^3	3.59×10^3
Sandell sensitivity (ng cm^{-2})	333.30	15.15	33.03	100.00
Detection limits (µg ml^{-1})	0.096	0.138	0.117	0.072
Quantitation limits (µg ml^{-1})	0.32	0.46	0.39	0.24
Regression equation*: Slope (b)	0.0030	0.0066	0.0300	0.0100
Intercept (a)	1.10×10^{-2}	7.30×10^{-2}	1.05×10^{-2}	9.50×10^{-2}
Correlation coefficient (r)	0.99980	0.99980	0.99996	0.99993
RSD %**	0.86	0.95	0.77	0.54
Stoichiometric R: D	1 :1	1 :1	1 :1	1 :1

* With respect to $A = a + b\,C$ where C is concentration of drug in µg ml^{-1} and A is absorbance.

** Relative standard deviation for six determinations

3.1.3.9.4. Interference

the effect of the presence of co-exiting additives and excipients such as sodium acetate, bicarbonate, magnesium citrate, talc powder, starch, glucose, fructose, sucrose and lactose was studied by adding an excess amount of each of them to a solution containing 100 µg ml^{-1} of CIP, LEV, NOR and ENR drugs. Study of the effect of interfering species showed that such ingredients; up to 10

%, do not interfere in the determination of CIP, LEV, NOR and ENR drugs indicating that complexation does not occur with those additives under reaction conditions.

3.1.3.9.5. Accuracy and precision

The accuracy and precision of the method is the closeness of the measured value to the true value for the sample. Accuracy of the proposed method when applied to pharmaceutical preparation is evaluated by applying standard addition technique. In which variable amounts of the drugs under investigation were added to the previously analysed portion of pharmaceutical preparations. The results shown in Tables (16 –19), confirmed that the proposed method is not liable to interference by fillers (lactose, monohydrated, microcrystalline cellulose, talc powder, sucrose, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, hydroxyl ethyl cellulose, flavors and magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (16). Determination of CIP in its pharmaceutical dosage forms applying the standard addition technique using ERY

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Ciprofloxacin tablets	3.00	2.00	4.99	99.80
		4.00	7.02	100.28
		6.00	8.92	99.11
Rancif tablets	3.00	2.00	5.01	100.20
		4.00	6.94	99.14
		6.00	8.97	99.67
Ciprofar tablets	3.00	2.00	4.95	99.00
		4.00	6.97	99.63
		6.00	8.92	99.11
Mifoxin tablets	3.00	2.00	4.97	99.40
		4.00	6.96	99.42
		6.00	8.94	99.33

*: average of six determinations

Table (17). Determination of LEV in its pharmaceutical dosage forms applying the standard addition technique using ERY

Dosage forms	Taken μg ml^{-1}	Added μg ml^{-1}	Found * μg ml^{-1}	Recovery (%)
Levoxin tablets	3.00	2.00	4.917	98.34
		4.00	6.925	98.92
		6.00	8.915	99.10
Unibiotic tablets	3.00	2.00	4.948	98.96
		4.00	6.956	99.37
		6.00	8.930	99.22
Lee-flox tablets	3.00	2.00	4.970	99.40
		4.00	6.940	99.14
		6.00	8.980	99.78
Tavanic tablets	3.00	2.00	4.910	98.20
		4.00	6.945	99.21
		6.00	9.020	100.20

*: average of six determinations

Table (18). Determination of NOR in its pharmaceutical dosage forms applying the standard addition technique using ERY

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Epinor tablets	3.00	2.00	4.913	98.26
		4.00	6.954	99.34
		6.00	8.970	99.67
Noracin tablets	3.00	2.00	4.980	99.60
		4.00	6.950	99.29
		6.00	8.930	99.22
Norbactin tablets	3.00	2.00	4.942	98.42
		4.00	6.910	98.71
		6.00	8.890	98.78

*: average of six determinations

Table (19). Determination of ENR in its pharmaceutical dosage forms applying the standard addition technique using ERY

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Enro-Flox sterile solution	3.00	2.00	4.932	98.64
		4.00	6.955	99.36
		6.00	8.890	98.78
Enroxin solution	3.00	2.00	4.927	98.54
		4.00	6.938	99.13
		6.00	8.922	99.11

*: average of six determinations

3.1.3.9.6. *Analytical application*

Results from the analysis of ERY with pharmaceutical preparations of different drugs are in a good agreement with those of the British pharmacopoeia. The relative standard deviation (six determinations) and the percentage recoveries of the proposed methods and pharmacopoeia procedure are recorded. The results obtained were compared statistically by the student's t-test and the variance ratio f-test with those obtained by pharmacopoeia method on samples of the same batch Tables (20 and 21). The student's t-test values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value, indicating no significant difference between the methods compared.

Table (20). Evaluation of the accuracy and precision of the proposed procedure of ERY with different drugs

Drug	Taken µg ml^{-1}	Found µg ml^{-1}	Recovery %	RSD$^{(a)}$ %	RE$^{(b)}$ %	Confidence limits$^{(c)}$
CIP	5	5.02	100.40	0.530	0.56	5.02±0.036
	10	9.85	98.50	0.240	0.26	9.85±0.064
	15	15.07	100.40	0.140	0.16	15.07±0.072
LEV	5	4.97	99.40	0.750	0.79	4.97±0.082
	10	9.91	99.10	0.350	0.37	9.91±0.22
	15	15.01	100.06	0.210	0.23	15.01±0.03
NOR	5	4.95	99.10	0.670	0.71	4.95±0.065
	10	9.98	99.80	0.310	0.33	9.98±0.04
	15	15.02	100.10	0.190	0.20	15.02±0.052
ENR	5	4.92	98.30	0.457	0.49	4.92±0.1
	10	9.80	98.00	0.197	0.22	9.8±0.09
	15	15.15	101.00	0.150	0.12	15.15±0.075

(a) Relative standard deviation for six determinations

(b) Relative error

(c) 95% confidence limits and five degrees of freedom

Table (21). Evaluation of the accuracy and precision of the proposed and official methods for determination of the studied drugs in different pharmaceutical forms using ERY

Drug	Official method			Proposed method				
	Taken µg	**Found * µg**	**Recovery (%)**	**Taken µg**	**Found * µg**	**Recovery (%)**	**t** ** value	**f** ** value
Ciprofloxacin tablets	10	9.84	98.4	10	9.93	99.3	0.32	1.54
Rancif tablets	10	9.89	98.9	10	9.97	99.7	0.55	1.64
Ciprofar tablets	10	9.93	99.3	10	10.02	100.2	0.19	1.29
Mifoxin tablets	10	9.88	98.8	10	9.89	98.9	0.52	2.41
Levoxin tablets	10	9.91	99.1	10	9.92	99.2	0.36	2.11
Unibiotic tablets	10	9.93	99.3	10	9.94	99.4	0.24	1.77
Lee-flox tablets	10	9.99	99.9	10	9.96	99.6	1.01	2.38
Tavanic tablets	10	9.94	99.4	10	9.98	99.8	0.25	1.99
Epinor tablets	10	9.87	98.7	10	10.01	100.1	0.64	2.97
Noracin tablets	10	9.92	99.2	10	9.98	99.8	0.21	1.76
Norbactin tablets	10	10.02	100.2	10	10.03	100.3	1.02	3.08
Enro-Flox sterile solution	10	9.98	99.8	10	9.87	98.7	0.88	2.77
Enroxin solution	10	9.86	98.6	10	9.91	99.1	0.66	2.30

*: average of six determinations

**: theoretical values for t-value and F-test values for the five degree of freedom and 95% confidence limits

3.1.4. Absorption spectra of ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR) and enrofloxacin (ENR) with ferric chloride ($FeCl_3$)

In order to investigate the optimum conditions of drug-Metal complex formation, the following studies should be taken in consideration:

3.1.4.1. *Effect of acid concentration*

The effect of acid concentration on the complex formation between CIP, LEV, NOR and ENR with $FeCl_3$- ferric chloride hexa hydrate $FeCl_3.6H_2O$ - the concentration of drugs and reagent were kept constant at 800 µg ml^{-1} and 7x 10^{-3} M respectively while that the volume of hydrochloric acid (1 x10^{-2} M) was regularly varied, the volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug. Illustrative spectra are shown in Figure (31) for the effect of acid concentration, inspection of these figure shows that the optimum ml added of acid giving maximum absorption recommended for subsequent studies of drug-metal complexes, are 0.5 ,0.5 ,0.7 and 0.2 ml for CIP, LEV, NOR and ENR respectively.

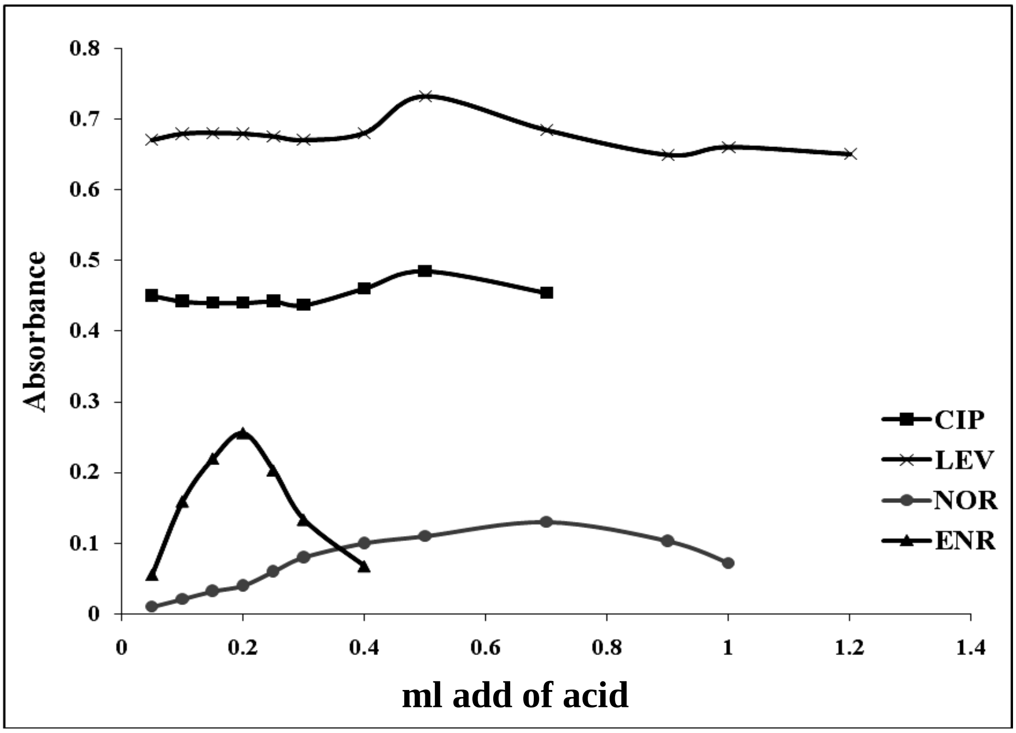

Figure (31). Effect of acid concentration on absorption rang of 7 X10^{-3} M $FeCl_3$ with 800 µg ml^{-1} of studied drugs

3.1.4.2. ***Determination of λ_{max} of the complex species***

For determine the value of λ_{max} at which the complex species possesses the maximum absorption, the following spectra must be recorded:

A- Spectrum of pure drugs 1 ml of 800 µg ml^{-1} at optimum pH value using buffer solution at the recommended pH value as a blank.

B- Spectrum of pure $FeCl_3$, 1 ml of 7 x 10^{-3} M at optimum pH value using the same buffer as a blank.

C- Spectrum of mixture of drugs (A) and $FeCl_3$ (B) at the optimum pH value using reagent at the same pH as a blank.

D- Spectrum of solution (C) against (B) as a blank.

The absorption spectra show that the formed complex absorbed maximally at 444 for $FeCl_3$ with drugs CIP, LEV, NOR and ENR as shown in Figure (32). These optimal wavelengths are chosen for further investigations.

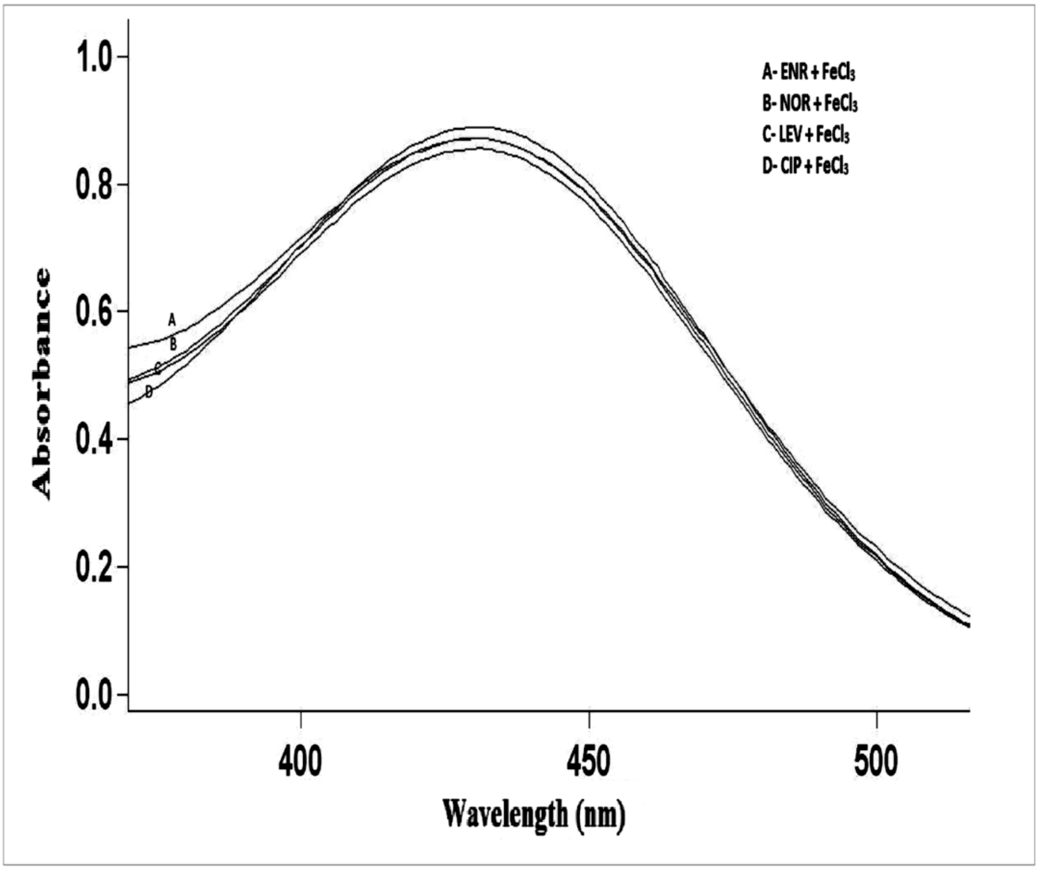

Figure (32). Absorption spectra of ion pair complex of 7 X10^{-3} M $FeCl_3$ with 800 µg ml^{-1} of studied drugs.

3.1.4.3. *Effect of sequence of addition*

The Effect of sequence of addition on complex formation was studied by measuring the absorption of solutions prepared using different sequences of addition against blank solution prepared in the same manner. The experiments showed that the best sequence of addition is drug - buffer or acid – metal chloride - water.

3.1.4.4. *Effect of time and temperature*

The Effect of time on complex formation was studied by measuring the absorbance of complex at optimum acid concentration against a blank solution of the same acid concentration at various time intervals. Also, the effect of temperature was studied for the same solution by incubation the sample and blank in water bath at different temperatures (25 - 45 °C) the absorption was measured after cooling to room temperature.

The experiments showed that complexes are formed simultaneously after mixing drug and metal chloride and remain stable for about two hours. Also, it was found that, increasing the temperature up to 45 °C has slight effect on the absorbance above which the color began to fade slowly.

3.1.4.5. *Effect of $FeCl_3$ concentration*

To study the effect of $FeCl_3$ concentration on the complex formation with optimum acid value concentration for CIP, LEV, NOR and ENR respectively, the concentration of drugs were kept constant at 800 μg ml^{-1} while that of $FeCl_3$ was regularly varied, the volume was completed to 10 ml with bidistilled water. The absorption spectra were recorded using a blank solution prepared in the same way without drug. Illustrative spectra are shown in Figure (33) for the effect of $FeCl_3$ concentration, inspection of these figure shows that the optimum ml added of reagent giving maximum absorption recommended for subsequent studies of drug-reagent complexes, are 1.5 ,1.2 ,2.5 and 0.9 ml for CIP, LEV, NOR and ENR respectively.

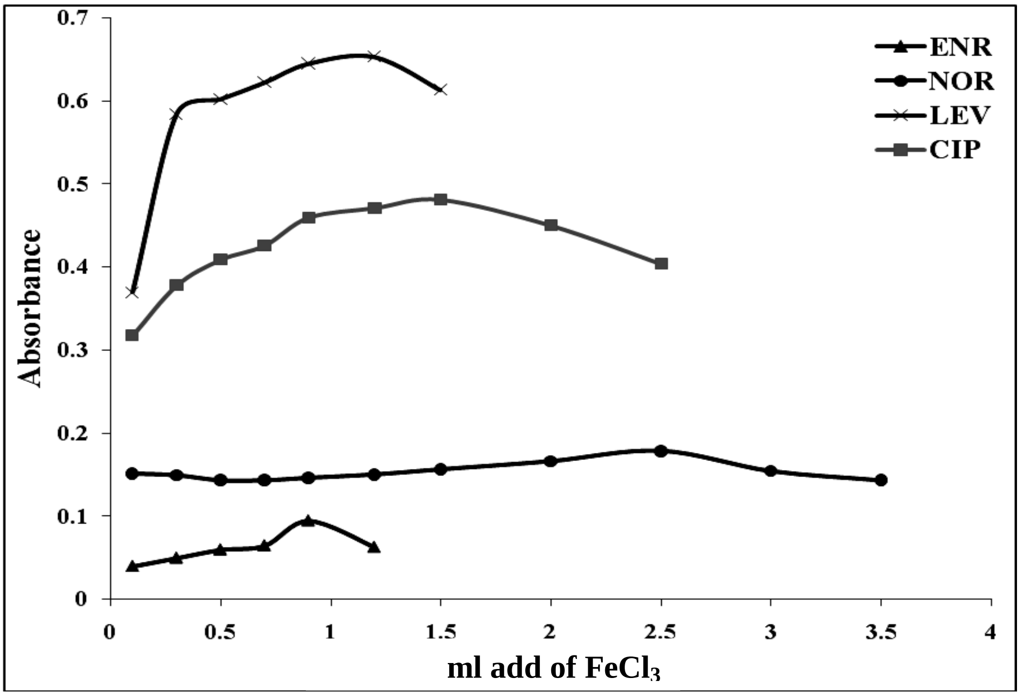

Figure (33). Effect of $FeCl_3$ concentration on absorption rang of 7 X10^{-3} M $FeCl_3$ with 800 μg ml^{-1} of studied drugs

3.1.4.6. ***Molar ratio method***

In order to investigate the molecular ratio of the complexes formed between the drugs under investigation and $FeCl_3$ at the selected conditions, the molar ratio and continues variation methods were carried out. Experimental results revealed that the complexes formed between the drugs and $FeCl_3$ have 2:1 stoichiometric ratio as shown in Figures (34 and 35).

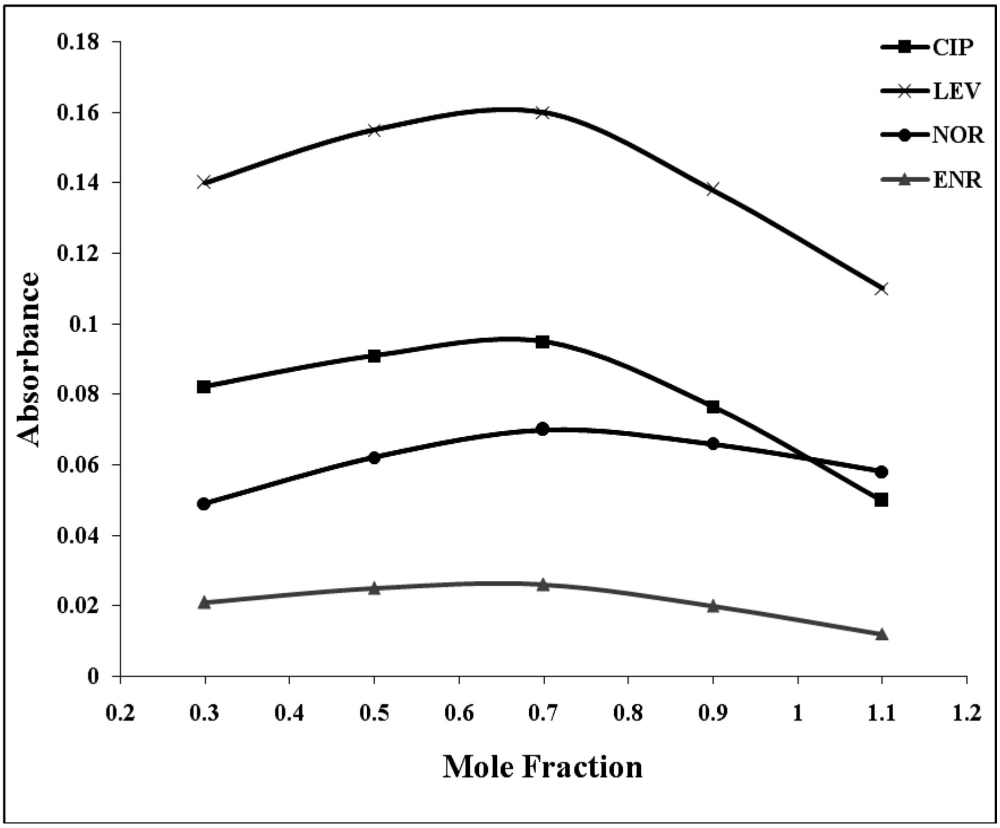

Figure (34). Continuous variation for 7 X10^{-3} M $FeCl_3$ with 800 μg ml^{-1} of studied drugs

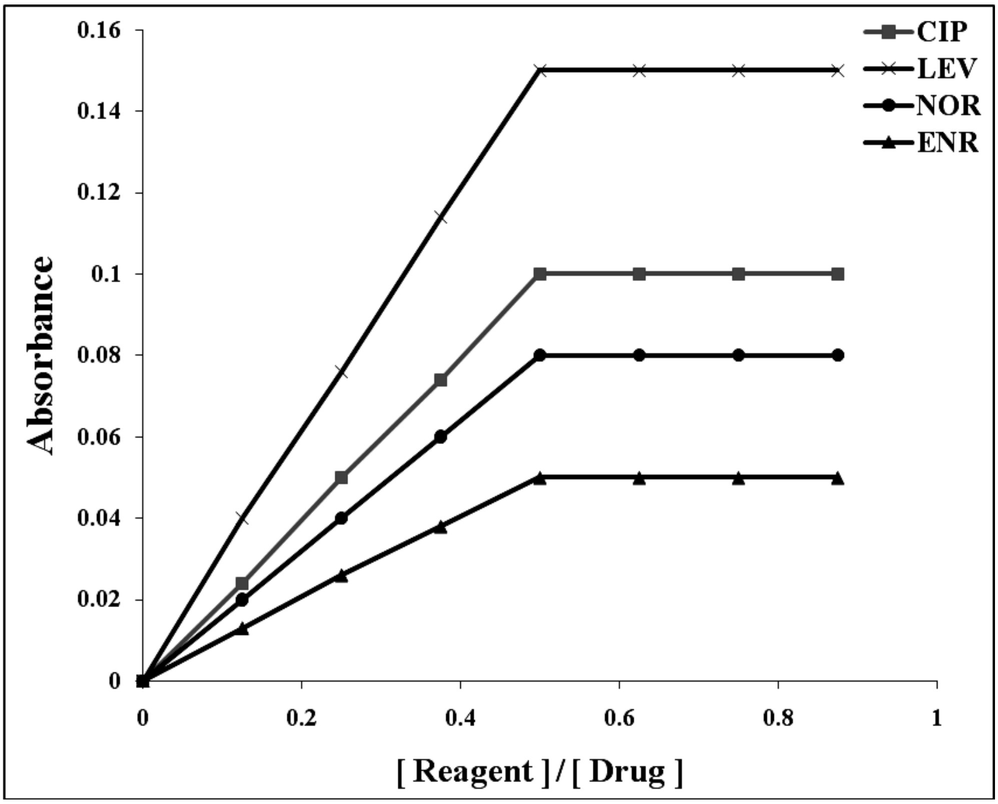

Figure (35). Mole ratio for 7 X10^{-3} M $FeCl_3$ with 800 μg ml^{-1} of studied drugs

3.1.4.7. ***Stability constant of the complexes***

Spectrophotometric methods can be applied for the determination of the stability constant of the complexes. The calculated stability constants are listed in Table (22), from which it is found that the $FeCl_3$ forms the most stable complex with NOR then ENR then LEV and CIP drug.

3.1.4.8. ***Validation of the method***

Method validation is the process of providing that the analytical method is acceptable, there is various validation studies developed and recorded below.

3.1.4.8.1. Linearity

under optimum conditions of pH, reagent concentration, sequence of additions, time and temperature for each of the different complexes, different concentrations of drug (μg ml^{-1}) were transferred into 10 ml measuring flask. 1.5, 1.2, 2.5 and 0.9 ml of $FeCl_3$ 7 x 10^{-3} with CIP, LEV, NOR and ENR respectively, are 0.5, 0.5, 0.7 and 0.2 ml of the optimum pH value acid with CIP, LEV, NOR and ENR respectively were added and then completed to volume with bidistilled water. The absorbance was measured at optimum λ_{max} then plotted against drug concentration as shown in Figure (36). Limits of beer' s law, the molar absorbability (ε; L $mol^{-1}cm^{-1}$) and ***Sandell*** (76) sensitivity results are listed in Table (22), indicating high sensitivity in the microdetermination of the drug.

For more accurate analysis, ***Ringbom*** (77) optimum concentration rang was determined by calculating the transmittance percent from the following equation:

$$T\ \% = 10^{-A} \times 100$$

Where: T %: transmittance percent,

A: the absorbance of the complex

By plotting logarithm of drug concentration (log [C_D]) in µg mL^{-1} against T %, the linear portion of the S-shaped curve gave an accurate range of analysis as shown in Figure 37; Results are listed in Table 22.

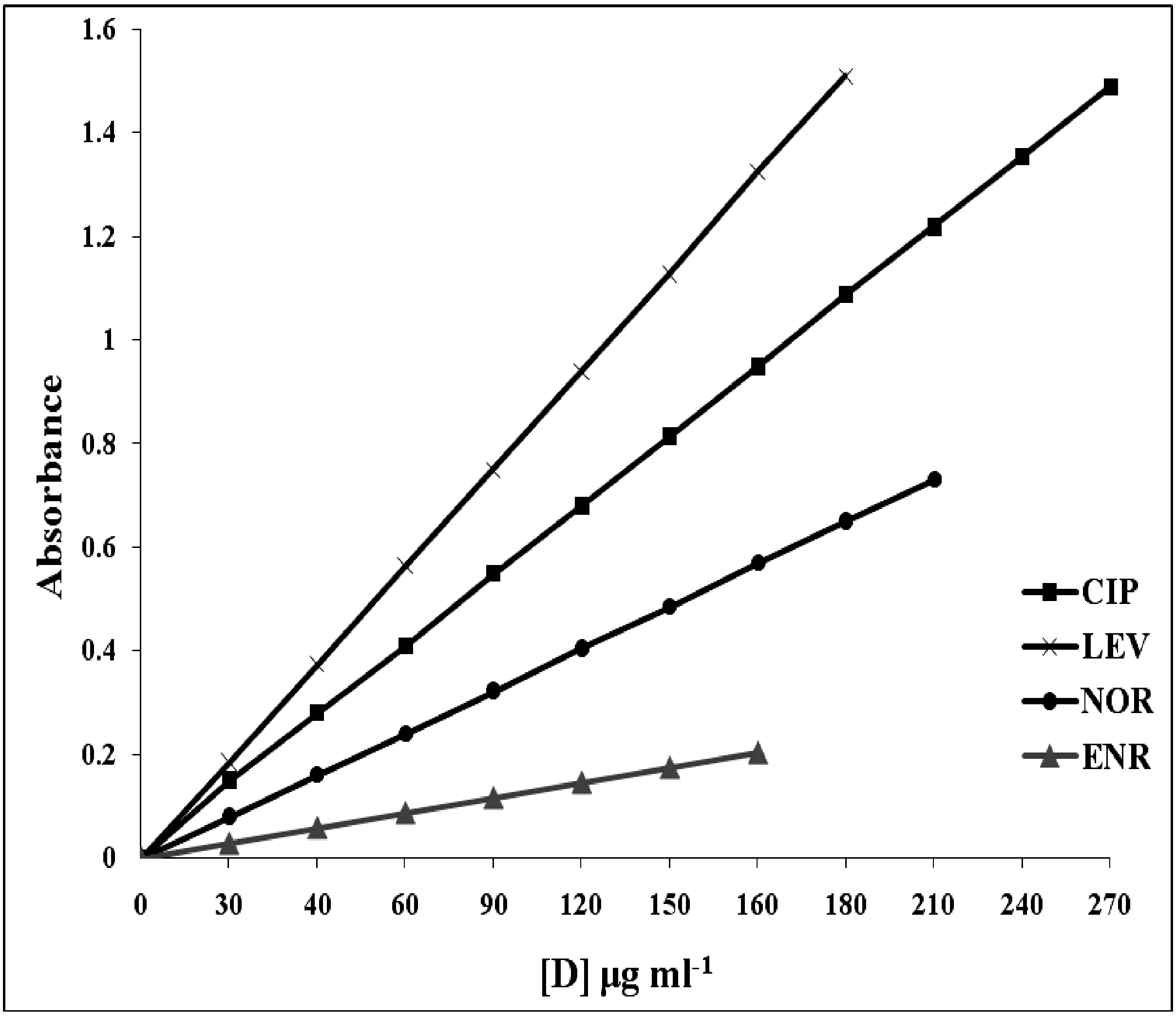

Figure (36). Applications of Beer's law for the studied drugs (800 µg ml^{-1}) using the optimum volume of 7 $X10^{-3}$ M $FeCl_3$

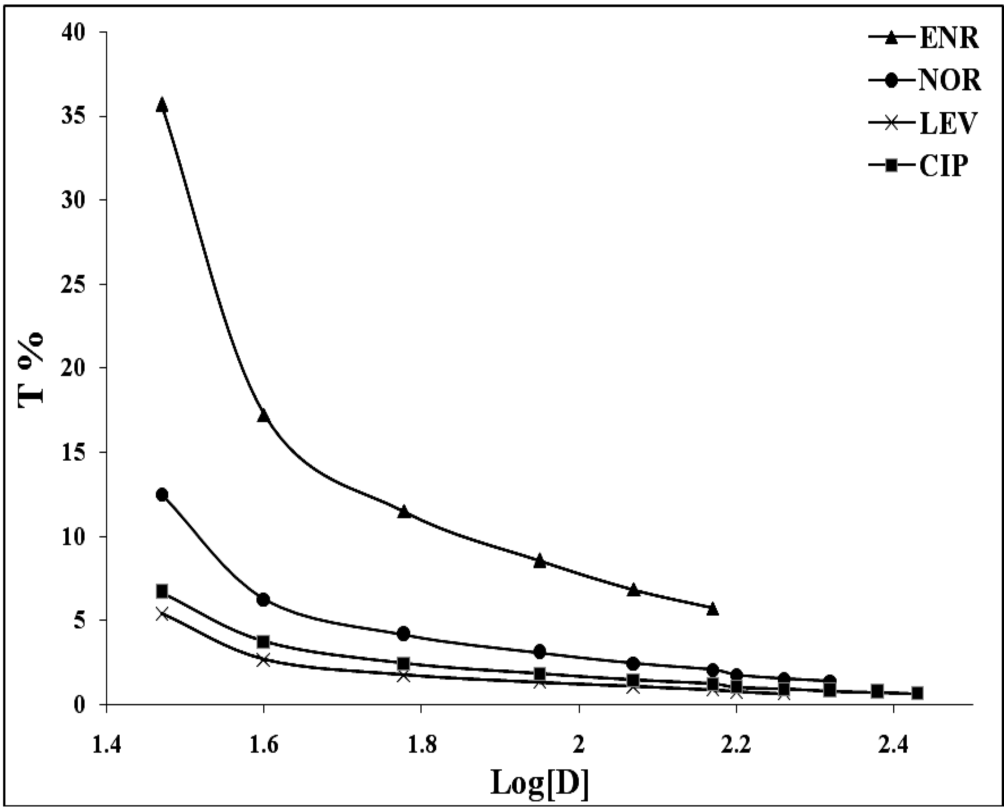

Figure (37). Ringbom plots for the studied drugs (800 μg ml^{-1}) using 7 X10^{-3} M $FeCl_3$

3.1.4.8.2. Range

The range of an analytical method is the concentration interval over which acceptable accuracy, linearity and precision are obtained. In practice the range is determined using data from the linearity and accuracy studies. The calibration range of the method is given in Table (22).

3.1.4.8.3. Detection and quantitation limits

Detection limit of the method is the lowest analyte concentration that produces a response detectable above the noise level of the system, where the quantitation limit is the lowest level of the analyte that can be accurately and precisely measured. Detection and quantitation limits are listed in Table (22).

Table (22). Optical and regression characteristics of $FeCl_3$ with different drugs

Parameters \ Drugs	CIP	LEV	NOR	ENR
λ max (nm)	444	444	444	444
Bear's law limits (µg ml^{-1})	60-276	20-180	40-210	40-160
Ringbom limits (µg ml^{-1})	70-260	25-160	45-200	45-150
Molar absorptivity (L mol^{-1} cm^{-1})	1.88×10^3	3.04×10^3	1.27×10^3	0.47×10^3
Sandell sensitivity (ng cm^{-2})	176.70	119.00	729.20	21.74
Detection limits (µg ml^{-1})	0.0126	0.0156	0.0138	0.0114
Quantitation limits (µg ml^{-1})	0.042	0.052	0.046	0.038
Regression equation*: Slope (b)	0.00566	0.0084	0.00397	0.0013
Intercept (a)	3.0×10^{-2}	3.2×10^{-2}	3.3×10^{-2}	1.3×10^{-2}
Correlation coefficient (r)	0.9960	0.9920	0.9980	0.9948
RSD %**	0.92	1.02	0.87	1.10
Stoichiometric R : D	2 : 1	2 : 1	2 : 1	2 : 1

* With respect to $A = a + b\,C$ where C is concentration of drug in µg ml^{-1} and A is absorbance.

** Relative standard deviation for six determinations

3.1.3.8.4. Interference

the effect of the presence of co-exiting additives and excipients such as sodium acetate, bicarbonate, magnesium citrate, talc powder, starch, glucose, fructose, sucrose and lactose was studied by adding an excess amount of each of them to a solution containing 800 µg ml^{-1} of CIP, LEV, NOR and ENR drug.

Study of the effect of interfering species showed that such ingredients; up to 10 %, do not interfere in the determination of CIP, LEV, NOR and ENR drug indicating that complexation does not occur with those additives under reaction conditions.

3.1.3.8.5. Accuracy and precision

The accuracy and precision of the method is the closeness of the measured value to the true value for the sample. Accuracy of the proposed method when applied to pharmaceutical preparation is evaluated by applying standard addition technique. In which variable amounts of the drugs under investigation were added to the previously analysed portion of pharmaceutical preparations. The results shown in Tables (23 – 26), confirmed that the proposed method is not liable to interference by fillers (lactose, monohydrated, microcrystalline cellulose, talc powder, sucrose, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, hydroxyl ethyl cellulose, flavors and magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (23). Determination of CIP in its pharmaceutical dosage forms applying the standard addition technique using $FeCl_3$

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Ciprofloxacin tablets	3.00	2.00	5.02	100.40
		4.00	6.94	99.10
		6.00	8.97	99.67
Rancif tablets	3.00	2.00	4.96	99.20
		4.00	6.93	99.00
		6.00	9.03	100.27
Ciprofar tablets	3.00	2.00	4.94	98.80
		4.00	6.92	98.86
		6.00	8.98	99.78
Mifoxin tablets	3.00	2.00	4.93	98.60
		4.00	6.94	99.14
		6.00	8.92	99.11

*: average of six determinations

Table (24). Determination of LEV in its pharmaceutical dosage forms applying the standard addition technique using $FeCl_3$

Dosage forms	**Taken** µg ml^{-1}	**Added** µg ml^{-1}	**Found *** µg ml^{-1}	**Recovery (%)**
Levoxin tablets	3.00	2.00	4.95	99.00
		4.00	6.95	99.29
		6.00	8.96	99.56
Unibiotic tablets	3.00	2.00	4.99	99.90
		4.00	6.91	98.70
		6.00	8.88	98.67
Lee-flox tablets	3.00	2.00	4.91	98.20
		4.00	6.97	99.57
		6.00	8.96	99.50
Tavanic tablets	3.00	2.00	4.94	98.80
		4.00	6.93	99.00
		6.00	8.94	99.33

*: average of six determinations

Table (25). Determination of NOR in its pharmaceutical dosage forms applying the standard addition technique using $FeCl_3$

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Epinor tablets	3.00	2.00	4.94	98.70
		4.00	6.98	99.64
		6.00	8.96	99.55
Noracin tablets	3.00	2.00	4.94	98.80
		4.00	6.95	99.26
		6.00	8.92	99.13
Norbactin tablets	3.00	2.00	4.92	98.30
		4.00	6.94	99.07
		6.00	8.92	99.11

*: average of six determinations

Table (26). Determination of ENR in its pharmaceutical dosage forms applying the standard addition technique using $FeCl_3$

Dosage forms	Taken µg ml^{-1}	Added µg ml^{-1}	Found * µg ml^{-1}	Recovery (%)
Enro-Flox sterile solution	3	2	4.98	99.60
		4	6.95	99.29
		6	8.93	99.22
Enroxin solution	3	2	4.94	98.80
		4	6.96	99.43
		6	8.94	99.33

*: average of six determinations

3.1.2.8.6. Analytical application

Results from the analysis of $FeCl_3$ with pharmaceutical preparations of different drugs are in a good agreement with those of the British pharmacopoeia. The relative standard deviation (six determinations) and the percentage recoveries of the proposed methods and pharmacopoeia procedure are recorded. The results obtained were compared statistically by the student's t-test and the variance ratio f-test with those obtained by pharmacopoeia method on samples of the same batch Tables (27 and 28). The student's t-test values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value, indicating no significant difference between the methods compared.

Table (27). Evaluation of the accuracy and precision of the proposed procedure of $FeCl_3$ with different drugs

Drug	Taken µg ml^{-1}	Found µg ml^{-1}	Recovery %	RSD[(a)] %	RE[(b)] %	Confidence limits[(c)]
CIP	5	4.93	98.60	0.69	0.80	4.93±0.09
	10	9.94	99.35	0.41	0.49	9.94±0.72
	15	14.97	99.80	0.30	0.38	14.97±0.065
LEV	5	5.02	100.40	0.91	0.96	5.02±0.03
	10	9.96	99.60	0.52	0.61	9.96±0.052
	15	14.98	99.86	0.37	0.47	14.98±0.027
NOR	5	4.99	99.80	0.84	0.93	4.99±0.023
	10	9.86	98.60	0.47	0.57	9.86±0.16
	15	14.86	99.10	0.36	0.44	14.86±0.22
ENR	5	4.98	99.50	0.62	0.73	4.98±0.082
	10	9.95	99.45	0.37	0.46	9.95±0.059
	15	14.95	99.67	0.27	0.37	14.95±0..043

(a) Relative standard deviation for six determinations

(b) Relative error

(c) 95% confidence limits and five degrees of freedom

Table (28). Evaluation of the accuracy and precision of the proposed and official methods for determination of the studied drugs in different pharmaceutical forms using $FeCl_3$

Drug	Official method			Proposed method				
	Taken µg	**Found * µg**	**Recovery (%)**	**Taken µg**	**Found * µg**	**Recovery (%)**	**t **** value	**f **** value
Ciprofloxacin tablets	10	9.87	98.7	10	10.03	100.3	0.57	1.89
Rancif tablets	10	9.97	99.7	10	9.92	99.2	0.79	1.59
Ciprofar tablets	10	9.94	99.4	10	9.89	98.9	0.43	2.44
Mifoxin tablets	10	9.95	99.5	10	9.98	99.8	1.09	3.08
Levoxin tablets	10	10.03	100.3	10	9.95	99.5	0.46	3.55
Unibiotic tablets	10	9.89	98.9	10	10.04	100.4	0.84	2.33
Lee-flox tablets	10	10.02	100.2	10	9.96	99.6	0.68	2.34
Tavanic tablets	10	9.93	99.3	10	10.05	100.5	0.45	2.09
Epinor tablets	10	10.01	100.1	10	9.97	99.7	0.19	3.14
Noracin tablets	10	9.94	99.4	10	9.86	98.6	0.24	1.45
Norbactin tablets	10	10.03	100.3	10	9.95	99.5	0.74	1.58
Enro-Flox sterile solution	10	9.96	99.6	10	10.01	100.1	0.91	2.35
Enroxin solution	10	9.95	99.5	10	9.93	99.3	0.34	1.58

*: average of six determinations

**: theoretical values for t-value and F-test values for the five degree of freedom and 95% confidence limits

3.2. Determination of the cited drugs via metal chelate complex formation.

3.2.1. Elemental analyses of the complexes

The results of elemental analyses listed in Table (29), suggest that the complexes are formed in 1 : 2 [metal] : [Drug] ratio with iron and they proposed to have the general formulae $[Fe(D)_2(H_2O)_2]Cl_2 \cdot 6H_2O$ and the complexes are formed in 1 : 3 [metal] : [Drug] ratio with Uranium and they proposed to have the general formulae $[UO_2(D)_3](NO_3)_2 \cdot 4H_2O$, D is the drugs under investigations.

Table (29). Analytical Data for the complexes

Complex	Analysis Found (Calc.) %				
	M	C	H	N	O
$[Fe(CIP)_2(H_2O)_2]Cl_2 \cdot 6H_2O$	5.99 (6.01)	43.95 (43.7)	5.28 (5.57)	8.7 (8.99)	23.01 (23.90)
$[Fe(LEV)_2(H_2O)_2]Cl_2 \cdot 6H_2O$	5.9 (6.15)	42.75 (42.35)	5.01 (5.55)	9.11 (9.26)	21.95 (22.50)
$[Fe(NOR)_2(H_2O)_2]Cl_2 \cdot 6H_2O$	5.83 (6.14)	42.6 (42.25)	5.45 (5.76)	9.77 (9.24)	24.20 (24.61)
$[Fe(ENR)_2(H_2O)_2]Cl_2 \cdot 6H_2O$	5.20 (5.64)	46.55 (46.12)	6.01 (6.11)	8.32 (8.49)	22.42 (22.61)
$[UO_2(CIP)_3](NO_3)_2 \cdot 4H_2O$	16.14 (16.12)	42.16 (42.00)	4.55 (4.26)	10.87 (10.57)	17.26 (17.29)
$[UO_2(LEV)_3](NO_3)_2 \cdot 4H_2O$	15.40 (15.19)	41.85 (41.88)	4.98 (4.39)	9.99 (9.95)	16.50 (16.29)
$[UO_2(NOR)_3](NO_3)_2 \cdot 4H_2O$	16.55 (16.73)	40.90 (40.48)	4.75 (4.91)	10.96 (10.82)	17.85 (17.7)
$[UO_2(ENR)_3](NO_3)_2 \cdot 4H_2O$	15.33 (15.25)	44.75 (44.39)	4.95 (4.80)	9.75 (9.99)	16.11 (16.35)

3.2.2. Infrared spectral data

The infrared spectral data and their assignment are listed in Table (30). Comparing the main IR frequencies free drugs in Figure (38), with that of metal complexes in Figures (39.a- 39.g), the following was found:

1- There was three strong absorption peaks in the spectrum of the drug, two of them in the rang 1760-1690 cm^{-1} (C=O) stretch for carboxylic acid and in the range of 1320-1000 cm^{-1} (C-O) stretch for carboxylic acid and the other one in the rang 850-550 cm^{-1} (C-Cl) stretch, these beaks was shifting in the complexes with metals.

2- Different from the spectrum of the drugs, the bands in the range 3300-2500 cm^{-1} (O-H) stretch for carboxylic acid and in the range 950-910 cm^{-1} (O-H) broad for carboxylic acid for all the complexes completely vanished. So we proposed that the ligand (drugs) interaction with the metal ions through bidentate chelating. This was consistent with the disappearance of the strong absorption at around 1720 cm^{-1} in the spectra of complexes. The averaging effect took place between the single and double bonds on the carbonyl group owing to the coordination.

3- New vibrating absorptions were observed from 617 - 698 cm^{-1}, which was characterized as the absorption of M-O bonds.

Table (30). The main IR frequencies for the drugs and the complexes

	Assignments					
Drug / Complex	C=O (S) Carboxylic acid	O-H (S) Carboxylic acid	C-O (S) Carboxylic acid	O-H (B) Carboxylic acid	M-O	C-Cl (S)
CIP	1704	2925	1025	942	-	803
$[Fe(CIP)_2(H_2O)_2]Cl_2\cdot 6H_2O$	1628	-	1063	-	617	815
$[UO_2(CIP)_2](NO_3)_2\cdot 4H_2O$	1626	-	1051	-	675	854
LEV	1724	2847	1090	927	-	802
$[Fe(LEV)_2(H_2O)_2]Cl_2\cdot 6H_2O$	1623	-	1064	-	633	894
$[UO_2(LEV)_3](NO_3)_2\cdot 4H_2O$	1620	-	1050	-	688	814
NOR	1727	2849	1029	930	-	825
$[Fe(NOR)_2(H_2O)_2]Cl_2\cdot 6H_2O$	1625	-	1067	-	698	852
$[UO_2(NOR)_3](NO_3)_2\cdot 4H_2O$	1627	-	1053	-	684	864
ENR	1736	2825	1022	953	-	830
$[Fe(ENR)_2(H_2O)_2]Cl_2\cdot 6H_2O$	1625	-	1068	-	694	852
$[UO_2(ENR)_3](NO_3)_2\cdot 4H_2O$	1626	-	1052	-	681	862

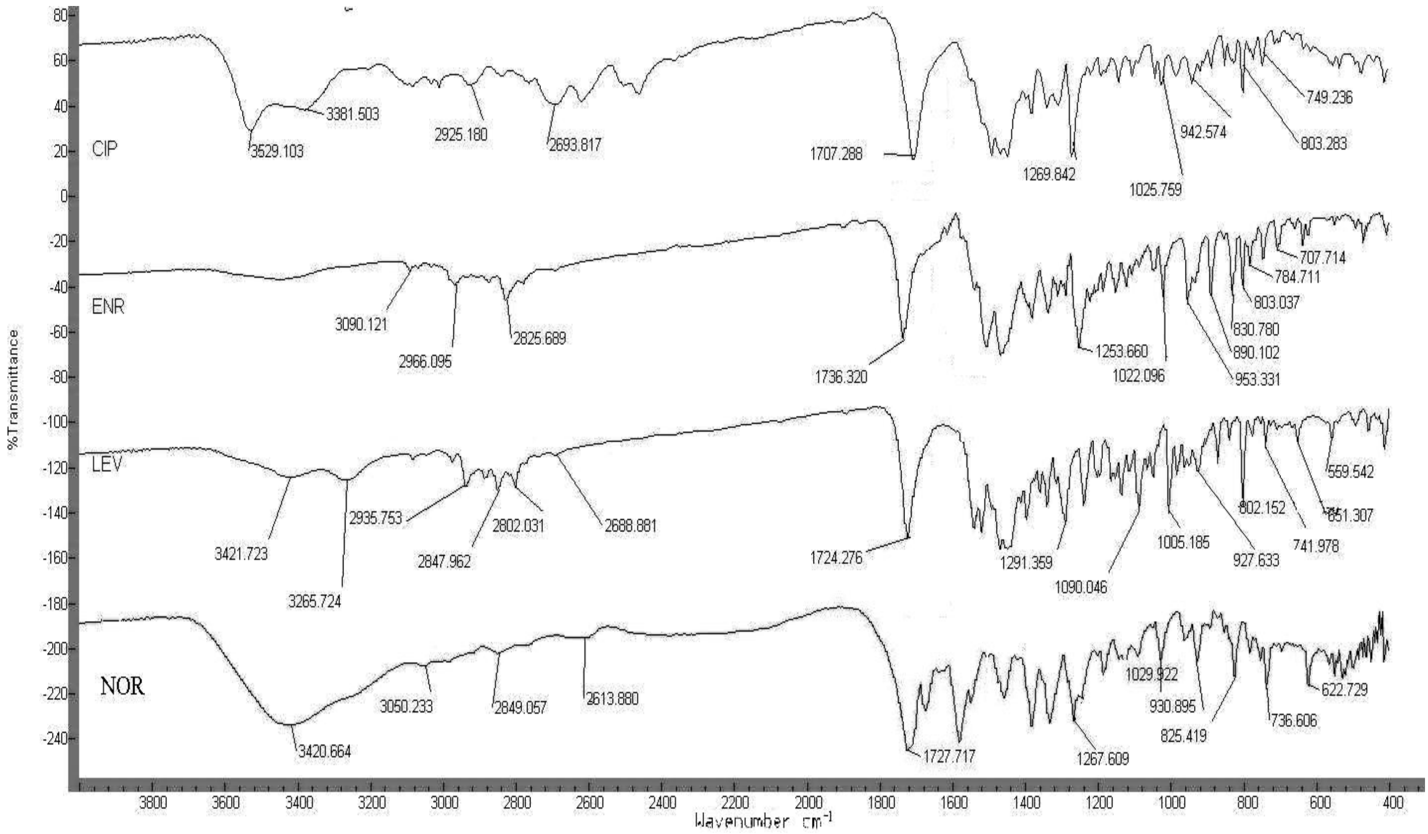

Figure (38). IR spectra of free drugs under study

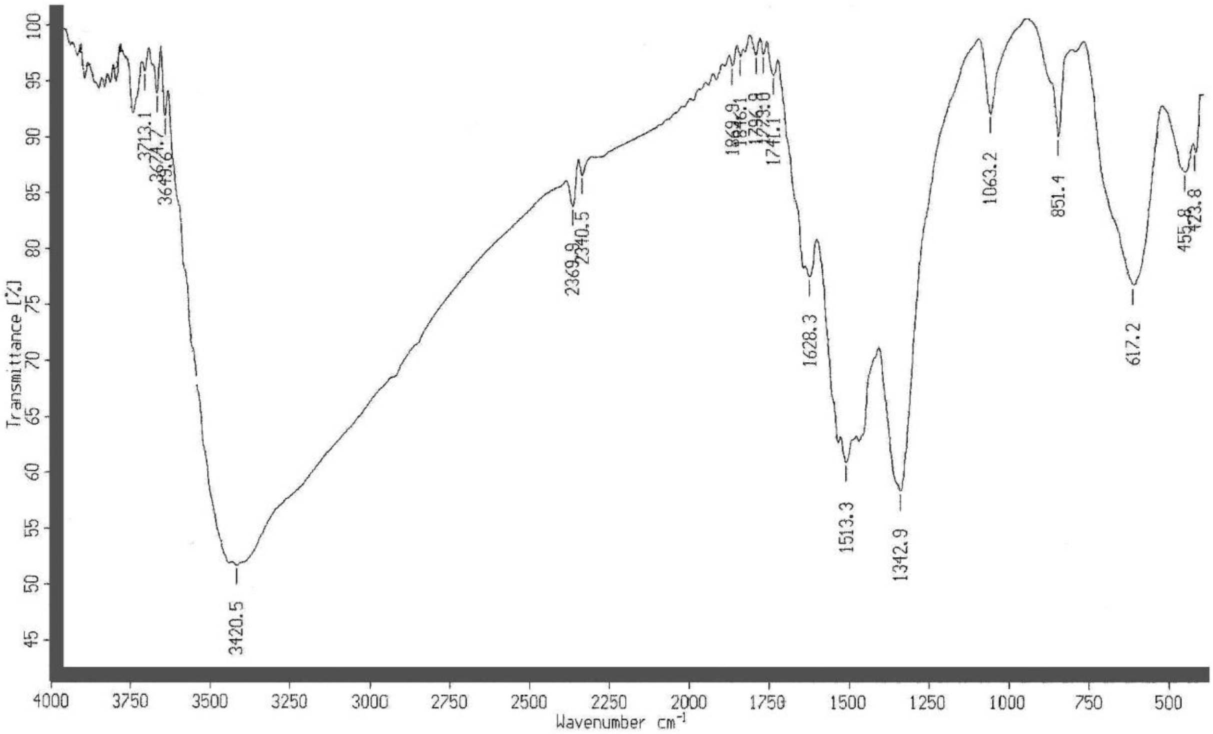

Figure (39.a). IR spectra of Fe (III) complex with CIP

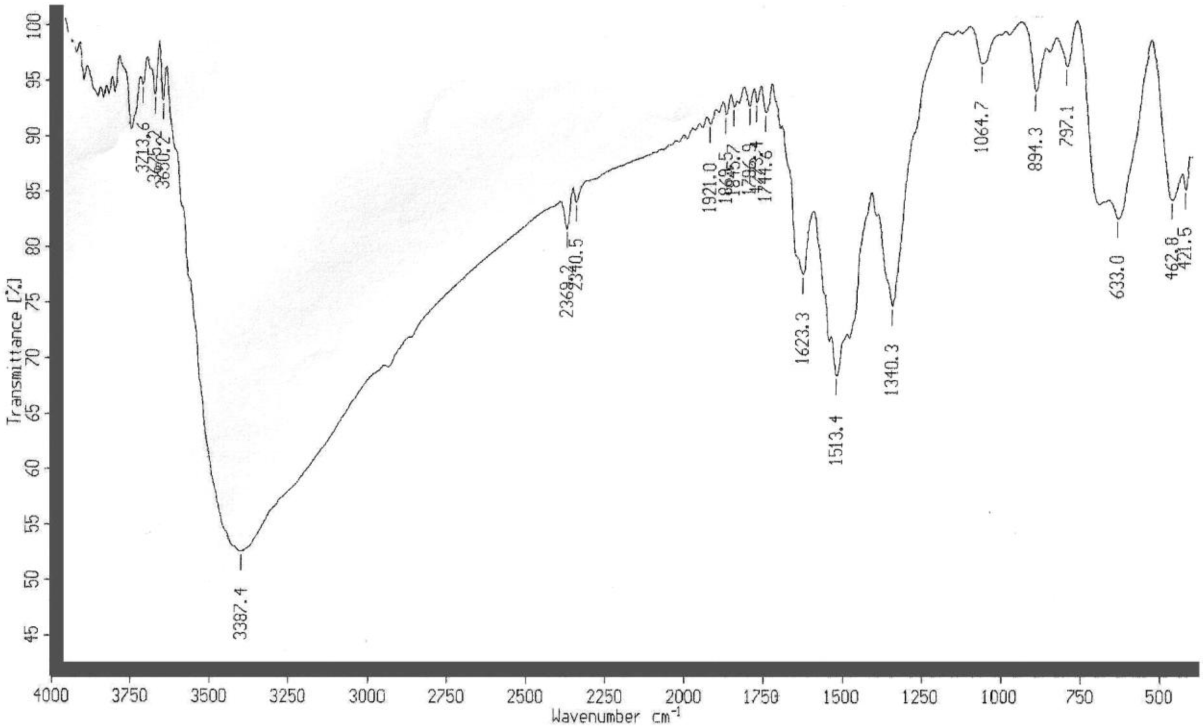

Figure (39.b). IR spectra of Fe (III) complex with LEV

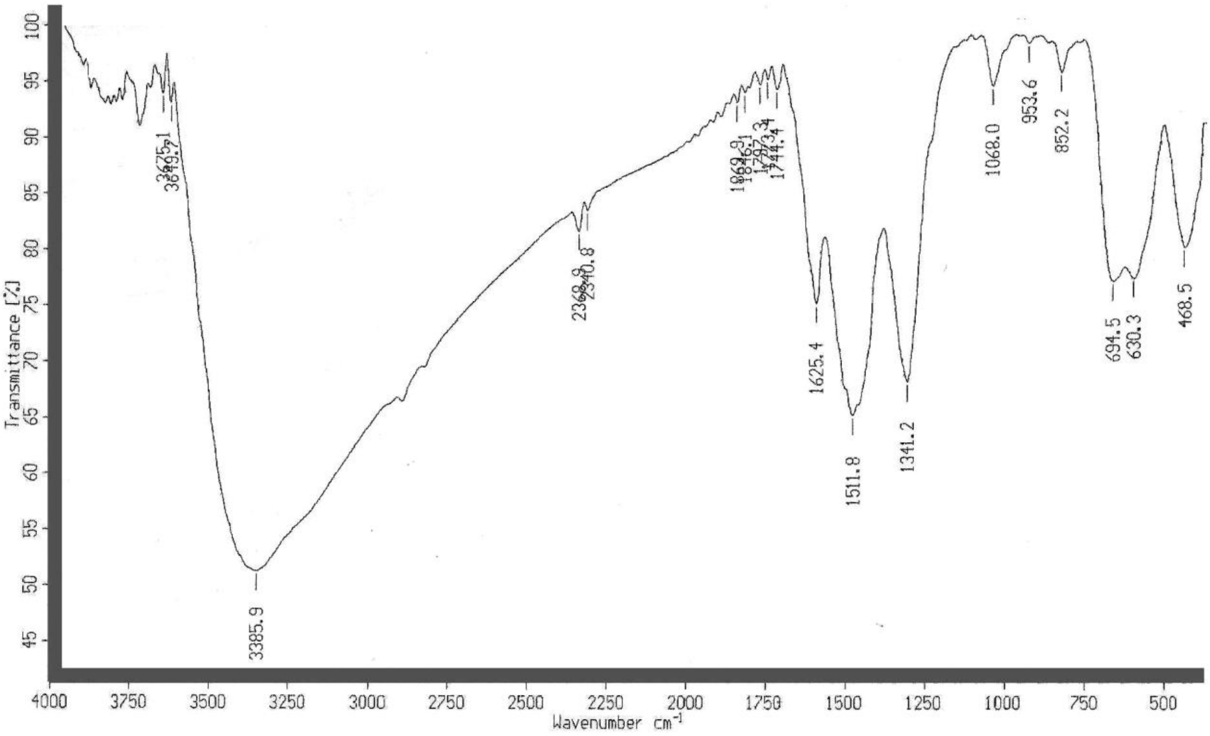

Figure (39.c). IR spectra of Fe (III) complex with NOR

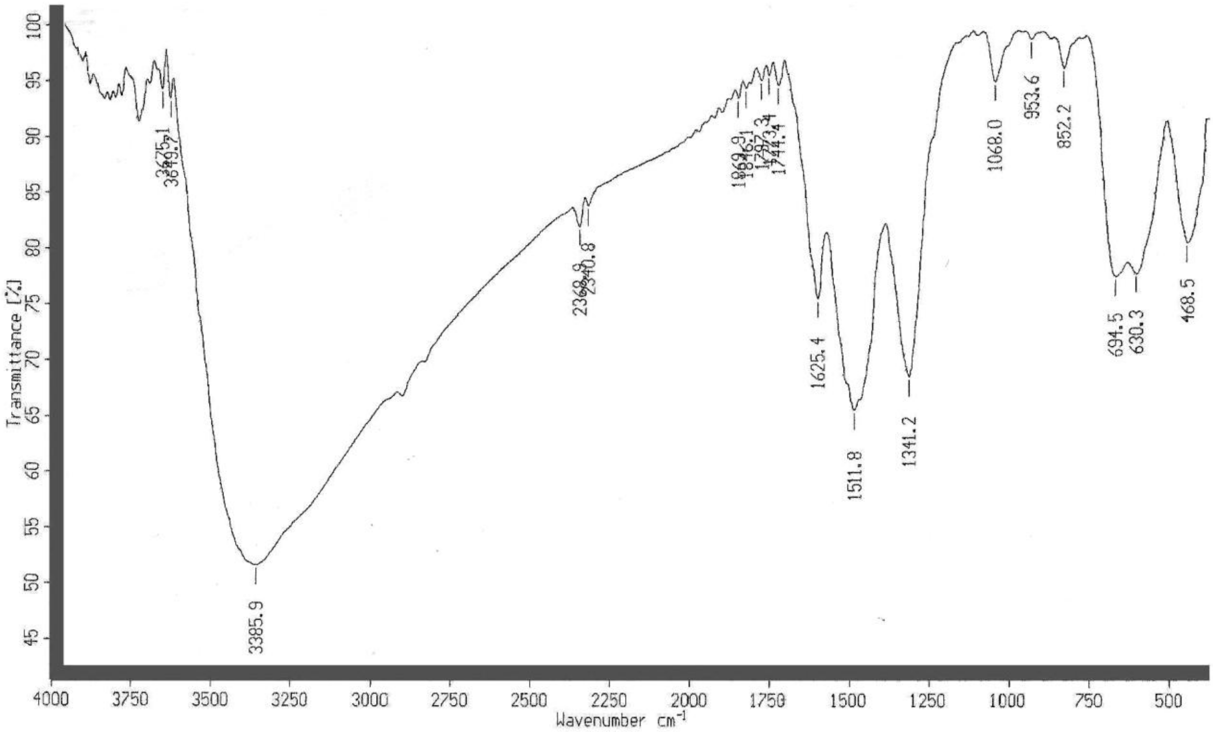

Figure (39.d.) IR spectra of Fe (III) complex with ENR

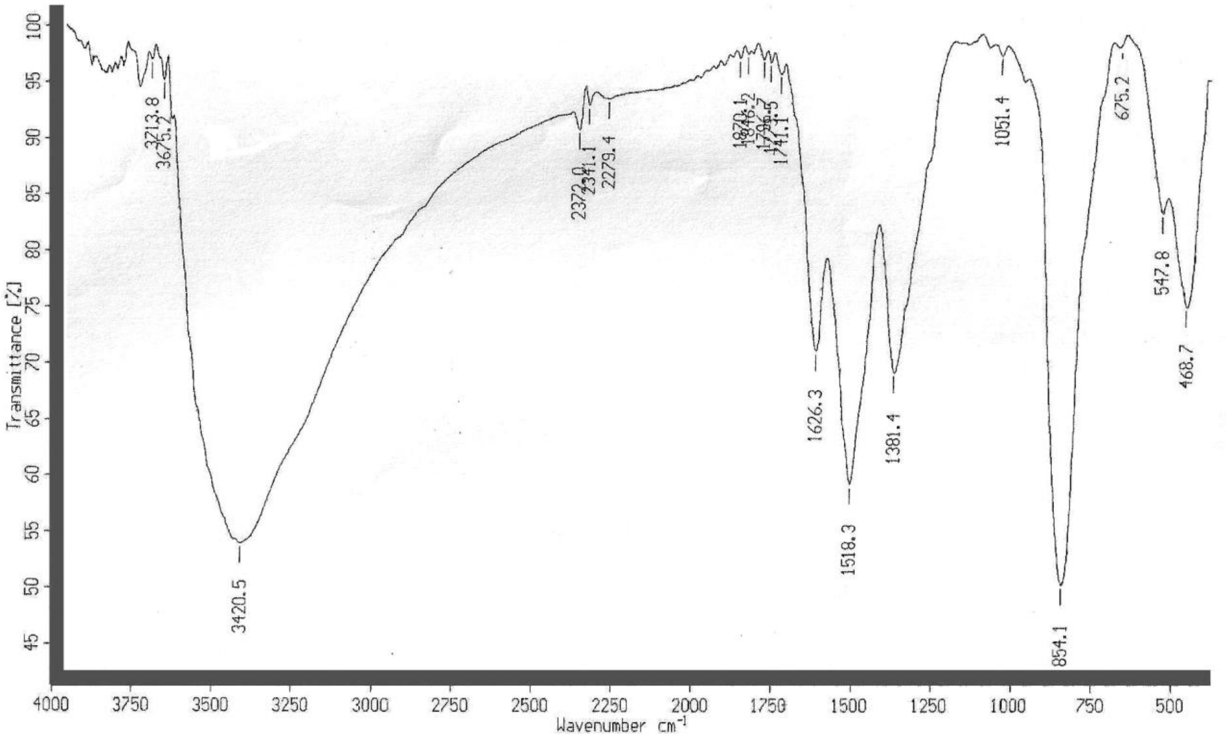

Figure (39.e). IR spectra of U(IV) complex with CIP

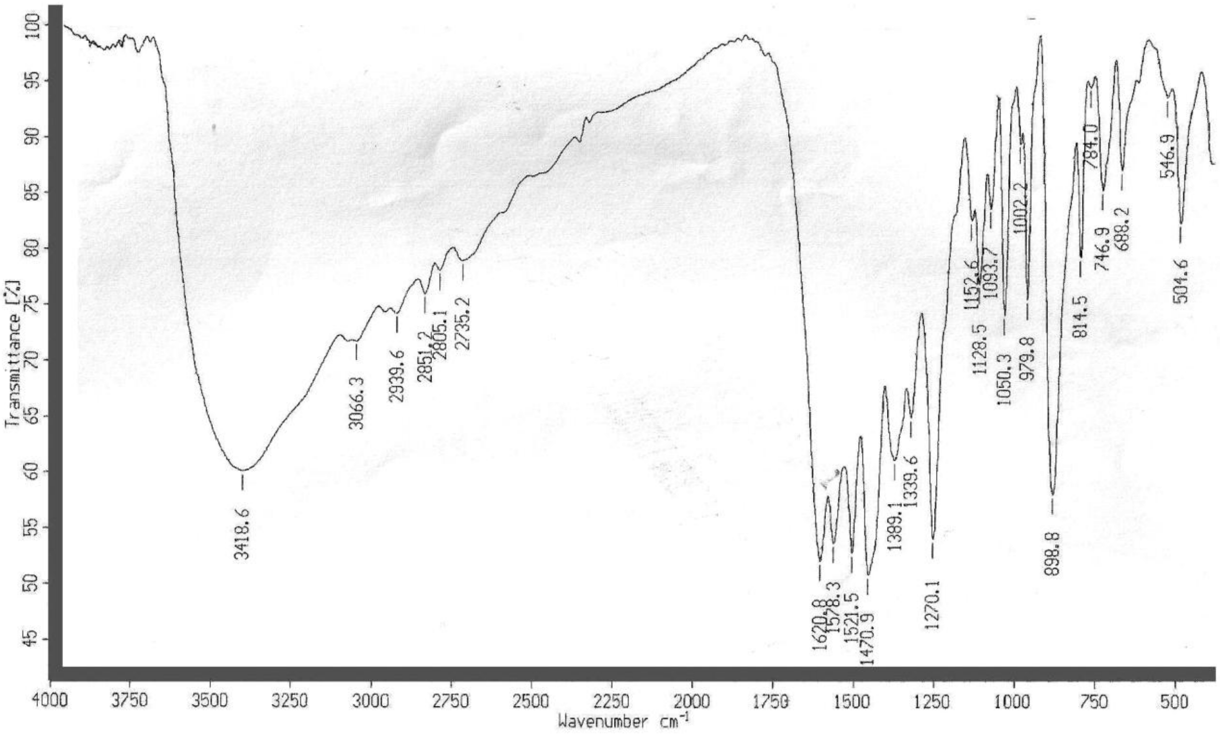

Figure (39.f). IR spectra of U (IV) complex with LEV

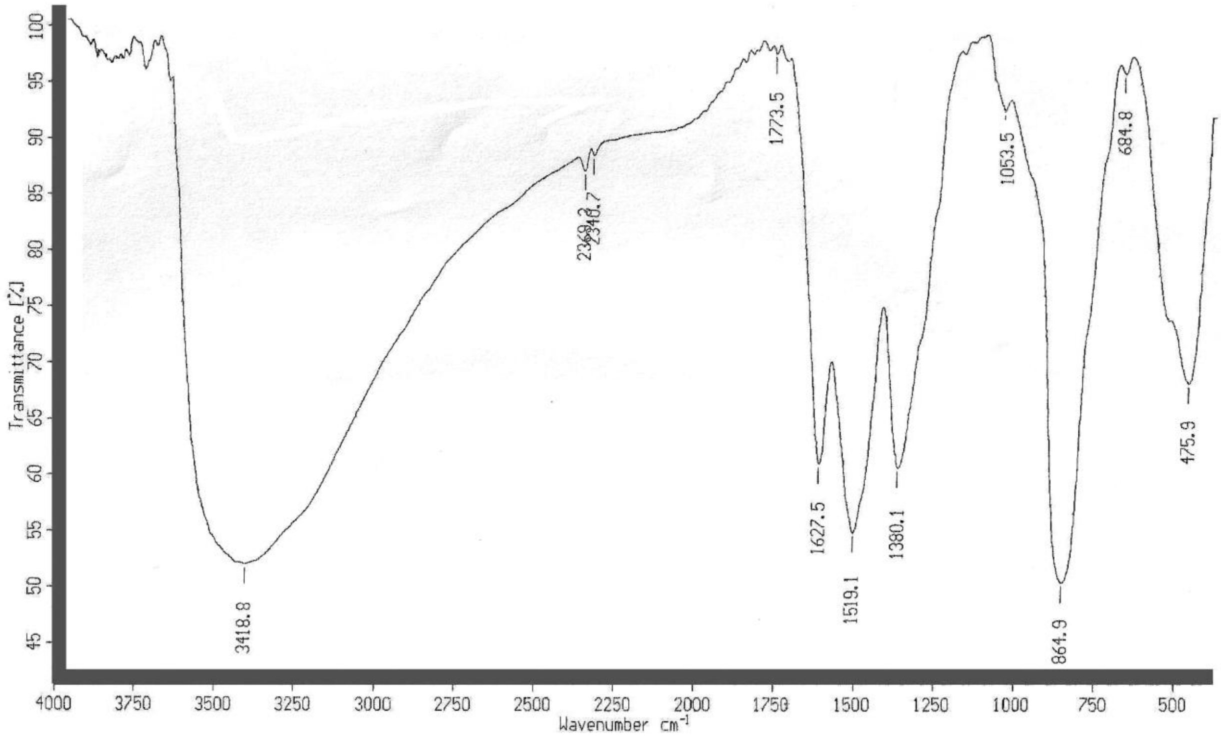

Figure (39.g). IR spectra of U (IV) complex with NOR

3.2.3. Thermal analysis

In this study we have chosen only one drug (CIP) for thermogravemetric analyses because of all drugs have the same function groups and structure. For the complex between Fe(III) and CIP drug as shown in Figure (40.a) was decomposed in three degradation steps within temperature range 50–1000 ºC. The first decomposition occurs between 50 and 250 ºC with a mass loss of 20.791 % and the second decomposition starts at 250 ºC and ends at 350 ºC with a 10.859% mass loss. The next third decomposition step occurs in the range 350–1000 ºC with an 8.321% mass loss. Refers to the DTA curve, the decomposition steps located at 115 and 303 ºC, which, were attributed to the liberation 2CIP organic moieties.

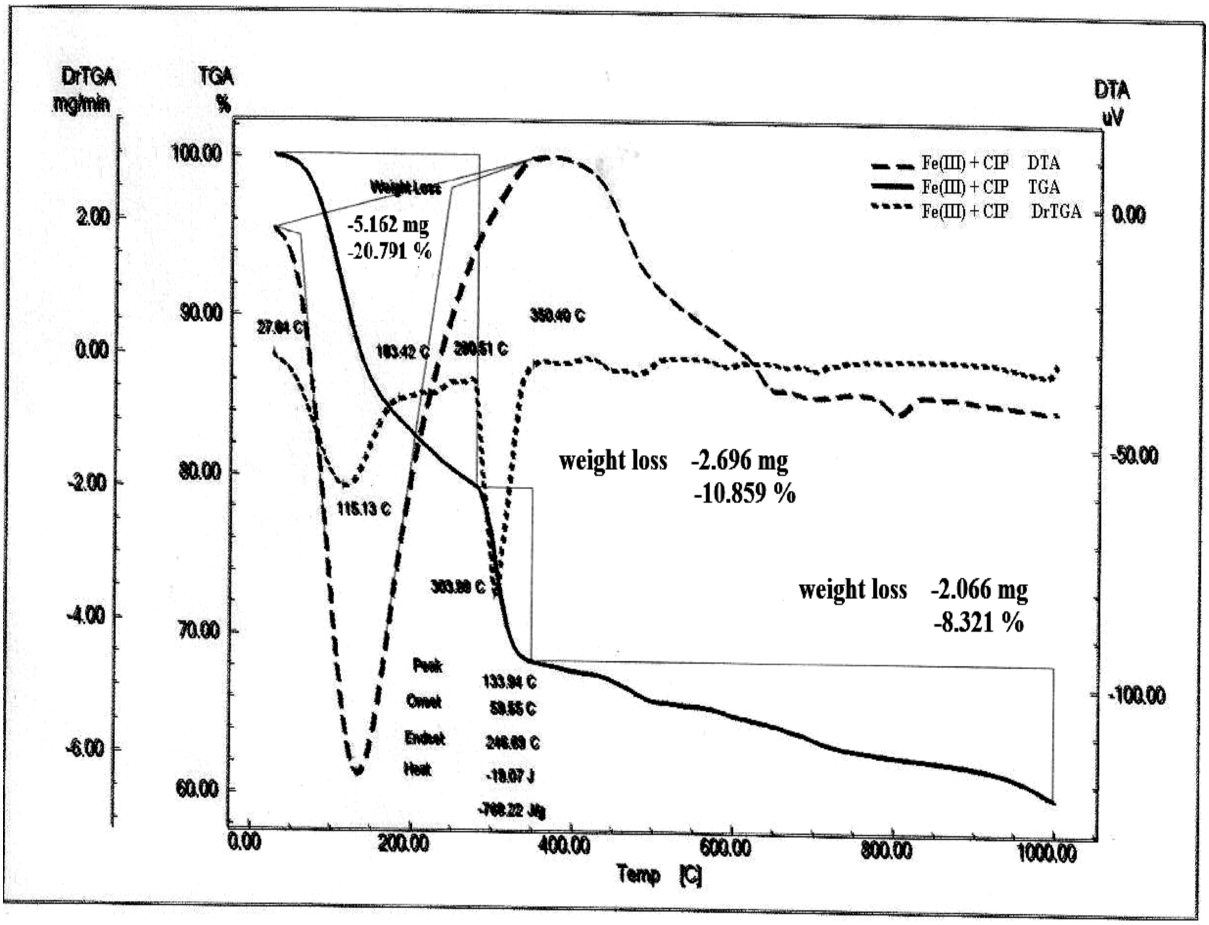

Figure (40.a). TGA and DTA diagrams of complex between Fe (III) and CIP drug

For the complex between U(IV) and CIP drug as shown in Figure (41.b). was decomposed in three degradation steps within temperature range 50–1000 ◦C. The first decomposition occurs between 50 and 250 °C with a mass loss of 11.586 % and the second decomposition starts at 250 °C and ends at 500 °C with 26.660 % mass loss. The next third decomposition step occurs in the range 500–1000 °C with 17.869% mass loss. Refers to the DTA curve, the decomposition steps located at 91 and 374 °C, which, were attributed to the liberation 2CIP organic moieties.

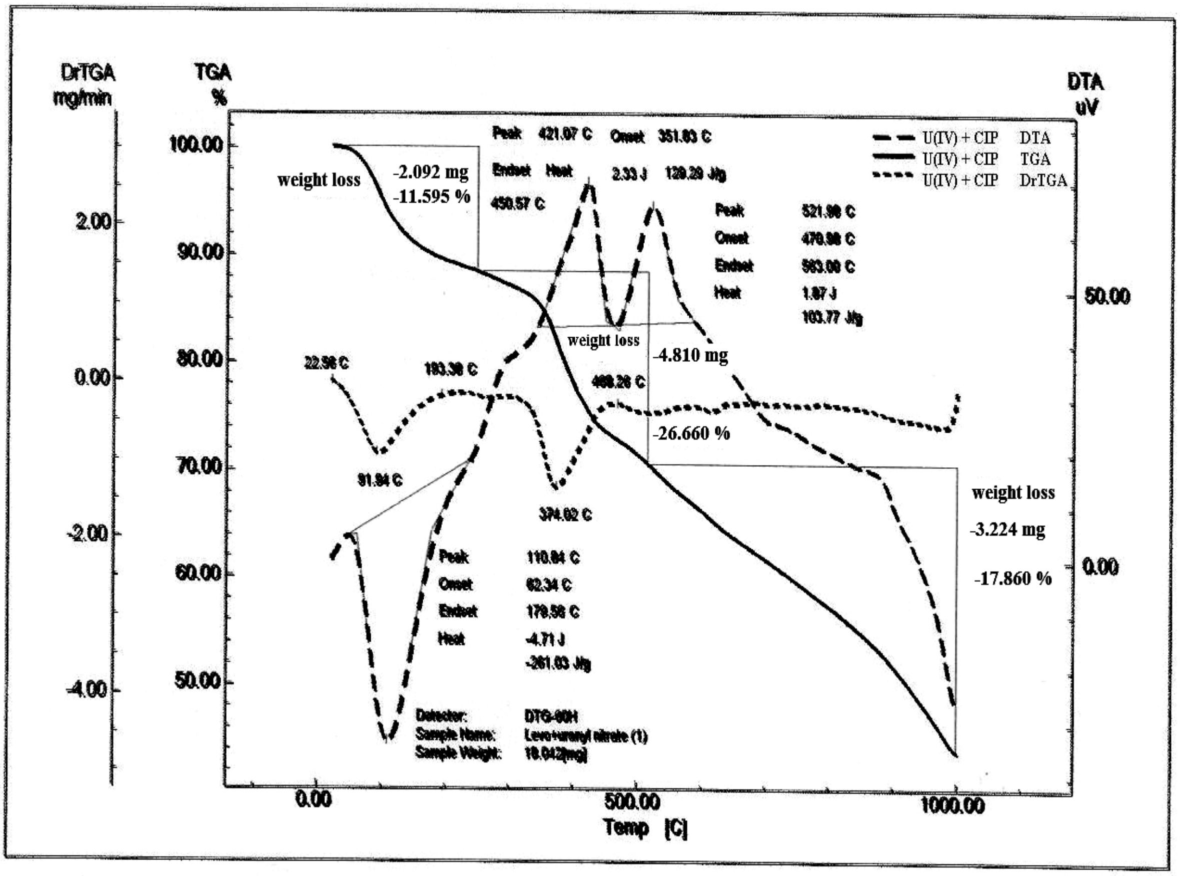

Figure (41.b). TGA and DTA diagrams of complex between U (IV) and CIP drug