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STABILITY INDICATING SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ENROFLOXACIN AND FLUMEQUINE

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ABSTRACT

Three simple and accurate spectrophotometric methods have been developed for the selective determination of enrofloxacin (EN) and flumequine (FL) in presence of their decarboxylated degradates. The first method is based on the chelation of the intact drugs with iron (II) in acetate buffer solution (pH5.6 ± 0.10) to form yellow colored chelates measured at 355 nm and 360 nm for EN and FL, respectively. Where good linearities were obtained in the range of 8-72 μg ml^{-1} for EN and 16-80 μg ml^{-1} for FL.

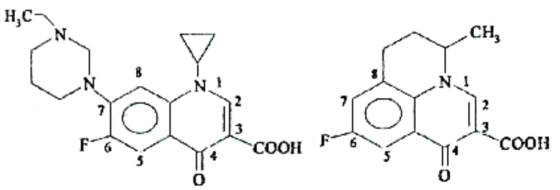
The second method depends on the measurement of the difference absorbance (ΔA) of each drug between 0.1M HCl and 0.1M NaOH solutions at 283 nm for EN and at 346 nm for FL. Beer's law was obeyed in the range of 2-16 μg ml^{-1} for EN and 4-32 μg ml^{-1} for FL. In the third one, EN and FL are determined using first derivative (^{1}D) and second derivative (^{2}D) spectrophotometric techniques at 288 nm and 263 nm in the ranges of 10-22 μg ml^{-1} and 8-24 μg ml^{-1}, respectively.

The intraday and interday precision (RSD %) were ranged between 0.16 and 2.45% for EN and FL. EN and FL can be determined in presence of up to 75% and 70% of their degradates via their iron (II) chelates, up to about 73% and 35%, respectively through the ΔA technique and up to about 65% by derivative spectrophotometry.

They were also successfully determined in their pharmaceutical formulations with mean percentage recoveries ± RSD ranged between 99.9-100.6±0.44-1.33% for EN and between 99.9-100.5±0.44-1.18% for FL.

INTRODUCTION

Enrofloxcin (EN) and flumequine (FL) are members of the fluoroquinolone antibiotics and are used in veterinary medicine[1].



Enrofloxcin (EN) Flumequine (FL)

EN has been extensively determined by HPLC in pharmaceutical formulations[2-5], in biological fluids[6,7] and animal tissues[8-11] Colorimetric[12,13], spectro-fluorimetric[14], TLC[15], voltammetric[16] and capillary electrophoretic[17] methods have also been published for its determination.

Different techniques have been reported for the analysis of FL including HPLC[18-22], TLC[23,24], HPTLC[25,26], electrophoresis[27], colorimetry[28,29] and polarography[30].

Since the carboxylic group in quinolones antibiotiotics is essential for their pharmacological activity[31], thus the decarboxylated degradates of EN and FL are of particular importance.

From the literature review, no methods were reported for the analysis of EN or FL in presence of their decarboxylated products.

The aim of this work is to develop simple and efficient methods for the selective determination of intact EN and FL in presence of their decarboxylated products.

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EXPERIMENTAL

1. Pure Sample

Reference standard enrofloxacin (EN) Batch No. 20020326 and flumequine (FL), Batch No. 20020735 (China Jiangsu, International Economic Technical Cooperation Corporation, China) were provided by ADWIA, 10th of Ramadan City-Egypt. Their purity was checked by HPLC methods adopted by ADWIA research and development laboratories[32] and found to be 99.30% and 99.62%, respectively.

2. Market Samples

- Enrotryl Oral Solution, product of ADWIA. Batch No. 990958; label claim for 100 ml was 10 gm enrofloxacin.
- SEF Oral Solution, product of SEDICO, 6th October City, Giza, Egypt, Batch No. 0400102; label claim for each ml was 100 mg enrofloxacin.
- Flumequine 20% Powder, product of El-Nasr Co.(ADWIC) Cairo, Egypt. Batches No. 2/836/2000 and 5/836/2000.

3. Standard Solutions

- Enrofloxacin (EN) solutions, 0.16 mg ml^{-1} and 5×10^{-4} M in aqueous acetate buffer (pH 5.6±0.1).
- Enrofloxacin solution, 0.2 mg ml^{-1} in ethanol.
- Flumequine (FL) solutions, 0.16 mg ml^{-1} and 5×10^{-4} M in methanol – acetate buffer (pH 5.6±0.1) mixture (8 - 2 v/v).
- Flumequine (FL) solutions, 0.2 mg ml^{-1} in methanol.

4. Chemicals and Reagents

All reagents used were of analytical grade, solvents were of spectroscopic grade, and water was freshly distilled.

Ethanol and methanol (Fisher, England). HCl (Prolabo, France), 0. 1 M, 2 M, and 4 M solutions; and NaOH (Prolabo, France), 0.1 M and 5 M solutions. $FeSO_4.5H_2O$(Prolabo, France); 3×10^{-3} M and

5×10^{-4} M solutions in water, and in mixture of methanol-water (8 : 2 v/v), sodium acetate – acetic acid (Prolabo) buffer solution, pH 5.6±0.1.

5. Apparatus

- Shimadzu, UV-Vis 1601 Spectrophotometer
- Jenco digital pH / temp. meter with double junction glass electrode
- Vector, IR (8201 Pc) Spectrometer.
- Varian Chemini, NMR (90 MHz) Spectrometer

6. Procedures

6.1. Stock degraded Solutions[12]

Pure EN or FL powder (0.4 g) were refluxed with 75 ml 2M HCl or 75 ml 4 M HCl solution, respectively, for 36 hours while protected from light. After cooling, solutions were adjusted to pH 7-8 with 5M NaOH solution and evaporated to dryness under vacuum. The residue was extracted three times, each with 70 ml of ethanol for EN or methanol for FL, then filtered into a 250 ml volumetric flask, and adjusted to volume with the appropriate solvent. These stocks when analyzed by iron(III) chelation procedures[12,13], 6% undegraded EN or 7% undegraded FL was found to be present, thus each 1 ml of it corresponds to the degradate from 1.504 mg of EN or 1.488 mg of FL

Preparation of Degradate Solutions for Chelation Procedure - A volume of 10 ml from the degraded EN stock solution was transferred into a 100-ml beaker, evaporated almost to dryness at about 70°C and dissolved in about 60ml of hot acetate buffer solutions of pH 5.6±0.1. Then transferred quantitatively into a 100- ml volumetric flask, cooled and adjusted to volume with the buffer solution. For FL, 10ml of its stock degraded solution was transferred into a 100ml volumetric flask, 20 ml of acetate buffer (pH 5.6±0.1) was added and completed to the mark with methanol. Each one ml of the obtained solutions corresponds to the decarboxylated degradate from 0.150 mg ml^{-1} EN plus 0.01 mg ml^{-1} intact EN or 0.149 mg ml^{-1} FL plus 0.011mg ml^{-1} intact drug to be used in the chelation procedure.

Preparation of Degradate Solutions for ΔA and derivative procedures - A volume of 12.5 ml of the stock degraded solutions was diluted to 100ml with ethanol or methanol for EN or FL, respectively. A solution containing 0.188 mg ml^{-1} degraded EN plus 0.012mg ml^{-1} intact drug or 0.186 mg ml^{-1} degraded FL plus 0.014 mg ml^{-1} intact FL, was obtained and used in the ΔA and derivative procedures.

6.2. Linearity

A. Chelation procedure

Aliquots of EN solution (0.16 mg ml^{-1}) in acetate buffer (pH 5.6±0.1) contain 0.08-0.72 mg of EN were transferred into a series of 10 ml volumetric flasks. One ml of 3×10^{-3} M aqueous $FeSO_4$ solution (equivalent to 840 µg $FeSO_4$) was added, then diluted to volume with the same buffer. For FL, different volumes of its standard solution (0.16 mg ml^{-1}) in methanol - acetate buffer mixture equivalent to 0.16-0.80 mg, were transferred into a series of 10 ml-volumetric flasks, one ml of 3×10^{-3} M $FeSO_4$solution in methanol- water mixture was added and volume was adjusted with methanol-acetate buffer (pH 5.6±0.1) mixture (8 : 2 v/v). After one hour, absorbance of each solution was measured at 355 nm or 360 nm for EN or FL, respectively. Blank was prepared similarly but omitting the metal ion solution.

Calibration curves relating the absorbance at 355nm or 360 nm to EN or FL concentrations were plotted.

B. ΔA procedure

Into two separate sets of 25-ml volumetric flasks, aliquots of the standard drug solution (0.2 mg ml^{-1}) equivalent to 0.05-0.40 mg of EN or equivalent to 0.1-0.8 mg of FL, were transferred. One set was diluted with 0.1M HCl and the second set was adjusted to volume with 0.1 M NaOH. ΔA of each concentration of EN was computed at 283 nm by placing acid solution in the sample beam and the alkaline one in the reference beam. For FL, ΔA was recorded at 346 nm taking the alkaline solution as sample and acid solution as reference. Calibration curves were constructed relating the absorbance difference (ΔA) values to the drug concentrations.

C. Derivative spectrophotometric procedure

Different volumes of standard drug solutions (0.2 mg ml^{-1}) containing 0.10-0.22 mg EN or 0.08 –0.24 mg FL, were introduced into a series of 10 ml volumetric flasks, then diluted to volume with ethanol for EN or methanol for FL. First derivative (^1D) spectra for EN or second derivative (^2D) spectra for FL were recorded against the respective solvent as blank at $\Delta\lambda$ equal 1 and ordinate values of (+0.01 and -0.015) or (+0.02 and -0.01) for EN or FL, respectively. The height of the trough at 288 nm for EN or the height of the amplitude at 263 nm for FL were measured in cm. Calibration curves of the measured heights in cm against drug concentrations in µg ml^{-1} were constructed.

6.3. Application to Pharmaceutical Formulations

a) **Determination of EN in Enrotryl and SEF Oral Solutions**-Enrotryl and SEF oral solutions, 0.4 ml or 0.5 ml (Equivalent to 40 mg or 50 mg EN) was transferred into a 250- ml volumetric flask and adjusted to volume with acetate buffer (pH 5.6) for chelation procedure or with ethanol for ΔA and ^1D-Spectrophotometric procedures, as described under linearity.

b) **Determination of FL in Flumequine 20% Powder** - Three preparations of Flumequine 20% powder were mixed. An amount of the powder equivalent to 16 mg FL was accurately weighed, shaked for 20 min with methanol - acetate buffer (pH 5.6) mixture (8:2) to make 100ml solution, then filtered and the filtrate was analysed by the chelation procedure. For ΔA and ^2D spectrophoto-

metric procedures an amount of the mixed powder equivalent to 20 mg FL was extracted with methanol by shaking for 20 min to make 100ml solution and filtered. Then filtrate was analyzed as described under linearity

RESULTS AND DISCUSSION

Degradation of EN and FL

As reported for norfloxacin, the two fluoroquinolones under study were expected to undergo decarboxylation reaction when heated with 2M HCl at 100°C[2,34] for 15 hours. However, under these conditions, only 50% decomposition was achieved. Maximum degradation, 94% for EN and 93% for FL, as analyzed by the iron(III) complexation procedure[12,29] was attained by boiling the acid solution for 36 hrs and using 2M HCl for EN or 4 M HCl for FL.

The obtained solutions were separated on silica gel $60F_{254}$ plates using a mobile phase of chloroform - methanol (4:9 v/v) or (3:9 v/v) for EN or FL from its degradation product, respectively. Separated spots were subjected to UV detection at 254 nm. R_f values for intact and degraded EN were 0.25 and 0.65, and were 0.17 and 0.82 for intact and degraded FL, respectively.

Confirmation of the degradation products

The obtained degradates were separated on preparative plates using the respective eluting system and extracted with chloroform, the extracts were evaporated to dryness and then confirmed in the residues by IR and H^1NMR. The IR spectra of both pure enrofloxacin and flumequine in KBr show a strong absorption vibrational band at 1700 cm^{-1} which characterizes the carbonyl moiety stretching of the carboxylic group. This band disappeared completely from the IR spectra of the degradation products, confirming decarboxylation. The ^{1}HNMR spectra of pure enrofloxacin and flumequine in $CDCl_3$ are characterized by a sharp singlet at $\simeq$ 8.5 ppm due to the CH proton in position 2. Whereas the ^{1}HNMR of the degradation products spectra revealed two new doublets at $\simeq$ 7.5 and 6.0 ppm which may be attributed to the CH proton at position 2 and the new CH proton in position 3 produced as a result of the decarboxylation.

Iron(II) chelation procedure

Enrofloxacin (EN) and flumequine (FL) were determined spectrophotometrically through their ability to complex metal ions. Decarboxylated products failed to give this reaction because they lack carboxylic group which is apart of the formed chelate. EN or FL reacted with iron (II) in acetate buffer (pH 5.6±0.1) or in methanol - acetate buffer (pH 5.6 ± 0.1) mixture (8:2 v/v) to form yellow colored chelates absorb at λ max 355nm or 360 nm, respectively. However, their degradates showed negligible peaks around 340 or 400 nm which may be referred to 6% or 7% undegraded EN or FL, respectively (Fig. 1-4).

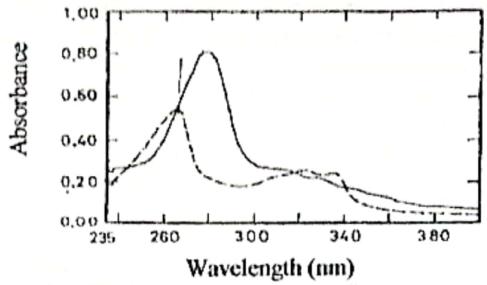


Fig.(1): UV-absorption spectra of:
— Enrofloxacin, 8 μg ml^{-1}
---- Decarboxylated degradate, 8 μg ml^{-1}
both in acetate buffer of pH 5.6

During the optimization of different reaction conditions, it was found that the reaction is pH dependent; maximum intensity of the yellow color was reached at pH 5.6 ± 0.1 using acetate buffer for EN and methanol-acetate buffer (pH 5.6 ± 0.1) mixture (8: 2, v/v) for FL.

A concentration of iron (II) starting from 0.5 ml of 3×10^{-3} M $FeSO_4$ solution per 10 ml (42 μg ml^{-1}) was enough to give maximum absorbance at 355 nm with 48 μg ml^{-1} of EN or at 360 nm with 40 μg ml^{-1} FL. Excess concentration of iron(II) up to 3 ml of 3×10^{-3} M $FeSO_4$ (252 μg ml^{-1}) did not affect the intensity and stability of the formed chelates. Thus, one ml of 3×10^{-3} M iron(II) per 10 ml reaction mixture (84 μg ml^{-1}) were used throughout the analysis.

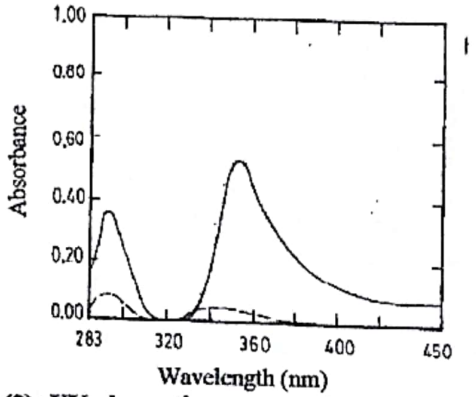
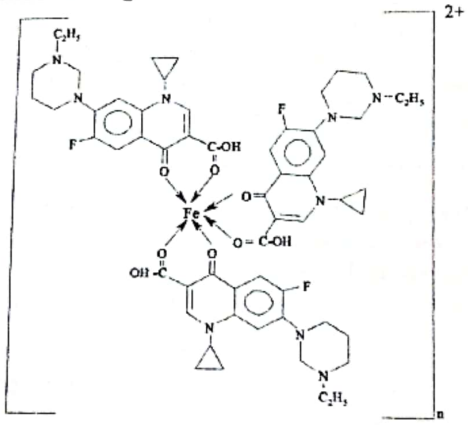


Fig. (2): UV-absorption spectra of:
— Enrofloxacin, 40μg ml^{-1}
---- Decarboxylated degradate, 40μg ml^{-1}
both in presence of 84 μg ml^{-1} iron in acetate buffer of pH 5.6

Maximum color intensity was attained after standing with iron(II) for one hr at room temperature for both drugs, it remained stable for further one hr. The stoichiometric ratio of drug to metal ion was determined using molar ratio method[35] using 5×10^{-4} M of each drug and iron II solutions. The ratio was found to be 3 : 1 for both drugs: metal ion. The logarithmic stability constant for EN - iron (II) or FL – iron(II) chelates were calculated[35] to be 13.4 or 13.5, respectively. Thus, EN and FL were suggested to

chelate iron(II) in quantitative reaction forming six-membered ring chelates, as indicated in Scheme (1).



Scheme (1): The proposed structure of EN–iron(II) chelate

Under the experimental conditions described above, linear correlations were obtained between absorbance at 355nm or 360 nm to EN or FL concentrations in the ranges of 8-72 μg ml^{-1} or 16-80 μg ml^{-1}, respectively the corresponding regression parameters are presented in Table (1).

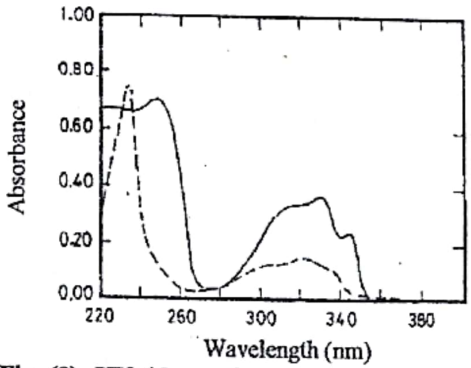


Fig. (3): UV-Absorption spectra of
—— Flumequine, 8 μg ml^{-1}
---- Decarboxylated degradate, 8 μg ml^{-1}
both in acetate buffer of pH 5.6

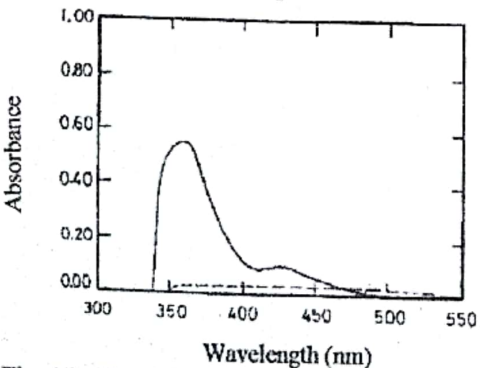


Fig. (4): UV-absorption spectra of:
—— flumequine, 48 μg ml^{-1}
---- decarboxylated degradate, 48 μg ml^{-1}
both in presence of 84 μg ml^{-1} iron in methanol- acetate buffer of pH 5.6 (8-2).

ΔA Procedure

ΔA measurements of intact EN and its degradate between 0.1M HCl and 0.1M NaOH at 283 revealed that interference due to the decarboxylated degradate was eliminated Fig. (5, 6). For flunequine, although the ΔA spectrum of its decarboxylated derivative showed a similar peak to that of the intact drug at 346 nm (Fig. 7 and 8), but it is of much lower sensitivity that allowed for the selective determination of intact drug in presence of up to 35% of its degradate. ΔA procedure was found to be sensitive in the range of 2-16 μg ml^{-1} and 4-32 μg ml^{-1} for EN and FL, respectively. The corresponding regression equations were computed and their parameters are illustrated in Table (1).

Derivative Spectrophotometric Procedure

A third technique for eliminating interference due to the degradation products was adopted by using first-derivative (^1D) or second-derivative (^2D) spectrophotometry for EN or FL, respectively. Comparing the zero-order absorption spectra of intact and degraded EN they were found to be extensively overlapped (Fig. 9a). Such overlapping was omitted by the first derivative scanning where, a well-defined separated trough at 288 nm was obtained for the intact drug (Fig. 9b)

The zero-order spectra of FL and its decarboxylated degradate in methanol have shown severe band overlappings (Fig. 10a). Although their ^1D spectra, showed a well separate trough at 260 nm for the intact drug, however in the mixtures it was dragged by the adjacent trough of the degradate at about 245nm (Fig 10 b) and failed to give accurate results. Their ^2D spectra revealed that pure FL has a typical amplitude at 263 nm at which the contribution of its decarboxylated form was zero (Fig. 10c).

Therefore, the trough of pure EN at 288 nm in its ^1D spectrum and the amplitude of FL at 263nm in its ^2D spectrum were adopted for the selective determination of pure drugs and found to be linear in the ranges of 10-22 μg ml^{-1} and 8-24 μg ml^{-1} for EN and FL, respectively. The corresponding regression parameters are shown in Table (1).

Table (1) also shows the spectral characteristics for the three proposed procedures, where the obtained correlation coefficients (0.9995-0.9999) indicate perfect linearities. The values of A (1%, 1cm) of the yellow chelates were calculated to be 127 and 111, while the ΔA (1%, 1cm) were 629 and 303 for EN and FL, respectively. The lower detection limit (LOD) ranged between 0.18 and 1.52 μg ml^{-1}.

The intraday and interday precision (RSD%) were ranged between 0.16 and 2.45% (Table 1) proving that the proposed procedures are accurate and precise.

The repeatability and reproducibility of the instrumental response (absorbance for the chelation and ΔA procedures and peak height for the derivative

procedure) were checked during methods development and they were assessed from five replicate determination of sample solutions of EN and FL at the analytical concentrations 16 μg ml^{-1} and 24 μg ml^{-1}, respectively. The RSD% for repeatability and reproducibility were less than 0.99 and 0.74, respectively.

The specificity of the procedures was tested by analyzing laboratory-prepared mixtures of each drug with its decarboxylated degradate. The results revealed that, EN and FL can be determined in presence of up to 75% and 70% of their degradates via their iron(II) chelates, up to about 73% and 35%, by the ΔA technique and up to about 65% and 64%, respectively through derivative spectrophotometry (Tables 2-4).

The proposed procedures were also applied for the determination of both drugs in their pharmaceutical formulations, results presented in Table (5) revealed that there is no interference from excipients or additives. In addition, the recoveries of the two drugs from their formulations were almost the same as the recoveries of the pure added when applying the standard addition technique. The mean percentage recoveries ± RSD of added were ranged between 99.5-100.7±0.26-1.43% for EN and between 99.6-100.2±0.54-1.25% for FL (Table 5).

In addition, the results obtained by the proposed procedures were statistically compared with those obtained by the reported methods for EN[12] or FL[29]. The calculate t and F values are less than the tabulated ones indicating no significant difference between the proposed and reported methods with respect to accuracy and precision at 95% confidence limit; Tables 6 and 7.

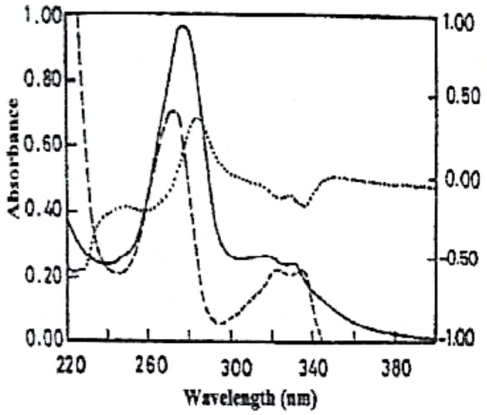


Fig. (5): UV Absorption spectra of pure —— enrofloxacin (8 μg ml^{-1}) in 0.1 M HCl, ---- 0.1 M NaOH and ..... their ΔA spectra.

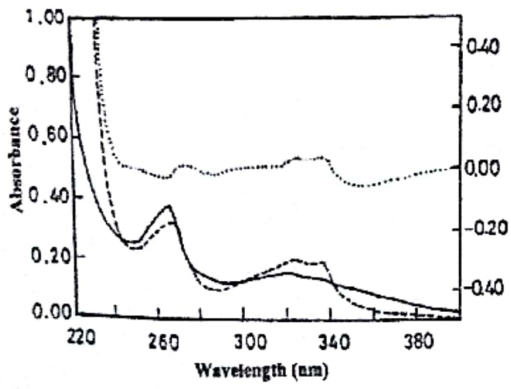


Fig. (6): UV absorption spectra of: Decarboxylated enrofloxacin (8 μg ml^{-1}) in —— 0.1 M HCl, ---- 0.1M NaOH and ..... their ΔA spectra.

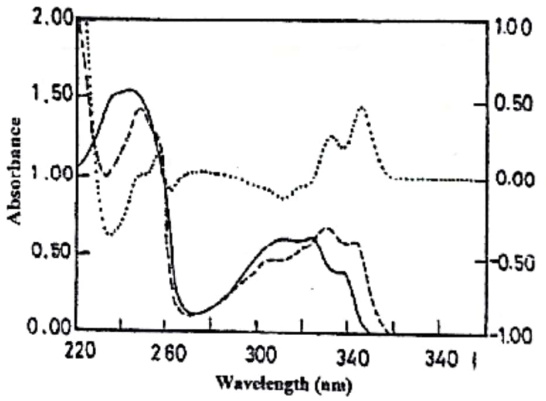


Fig. (7): UV absorption spectra of pure flumequine;16 μg ml^{-1} in 0.1M NaOH (–), 0.1M HCl (–) and their ΔA spectra (....)

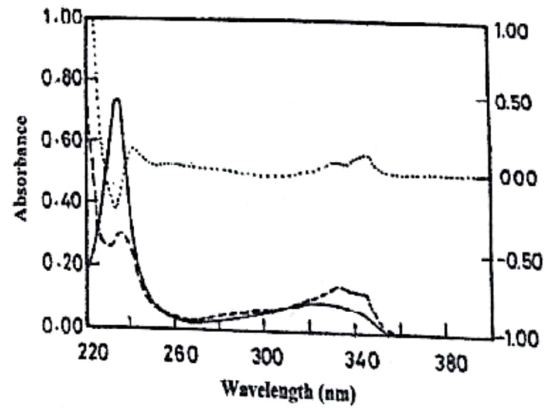


Fig. (8): UV absorption spectra of decarboxylated flumequine;16 μg ml^{-1} in 0.1M NaOH (–), 0.1M HCl (–) and their ΔA spectra (....)

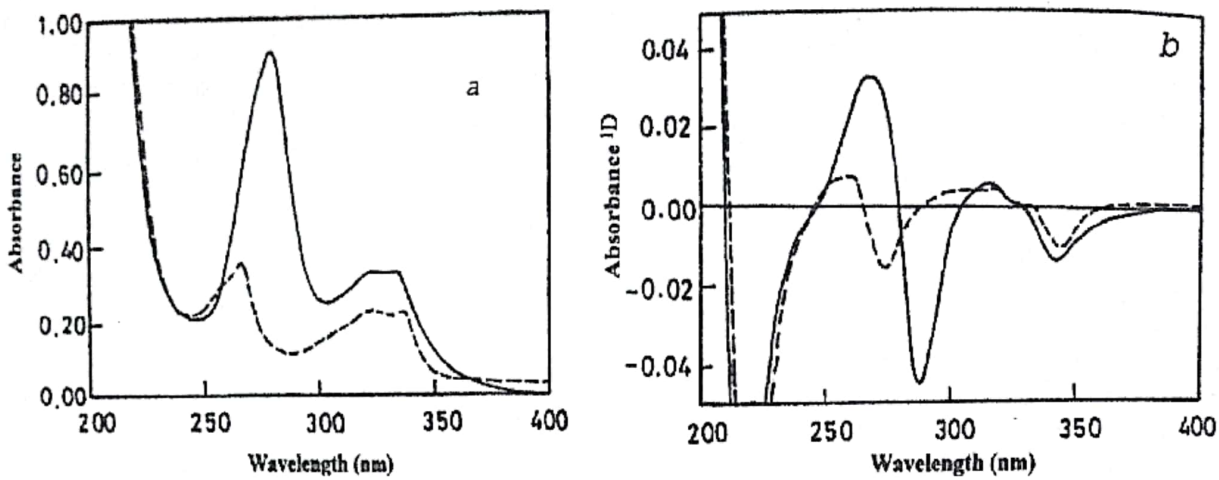


Fig. (9): The UV spectra of (a.zero order b. first derivative) of 8 μg ml^{-1} pure enrofloxacin (—) and its decarboxylated degradate (—) in ethanol

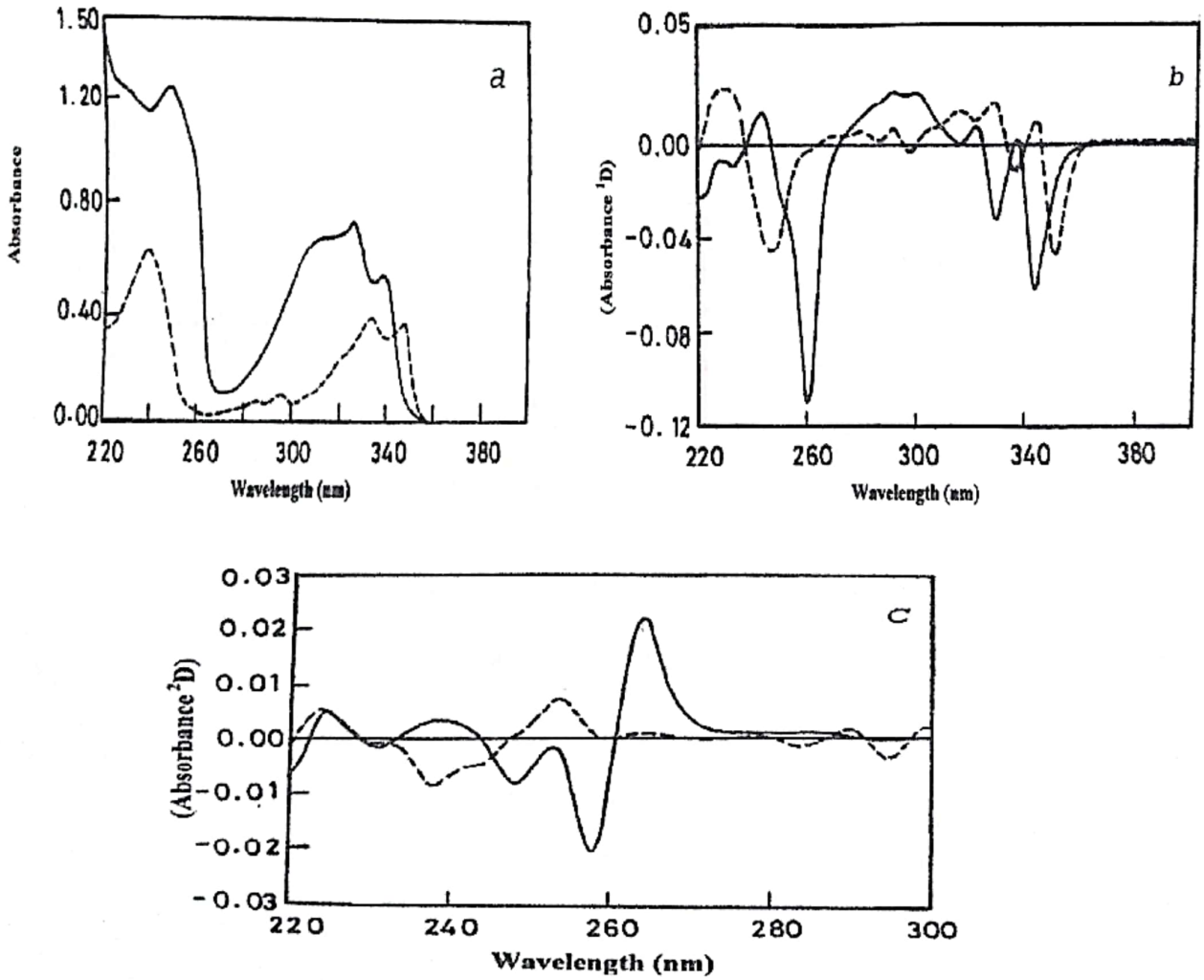


Fig. (10): UV absorption spectra of (a- zero order b- first derivative and c- Second derivative) of 16 μg ml^{-1} of pure flumequine (–) and its decarboxylated degradate (–) in methanol

Zagazig J. Pharm. Sci., June 2002
Vol.11, No. 1, pp. 1-10

Table (1):Selected Spectral Data of the Three Proposed Procedures for the Determination of EN and FL.

Parameters	Enrofloxacin			Flumequine		
	Iron(II)	Δ A	^{1}D	Iron(II)	ΔA	^{2}D
Linearity range ($\mu g ml^{-1}$)	8 - 72	2 - 16	10 - 22	16 - 80	4 - 32	8 - 24
LOD	0.72	0.18	1.12	1.52	0.42	0.83
Regression Parameters						
Slope ±SD (s_b)	0.0127±4.26E-05	0.0622±3.88E-0.3	0.3500±0.0050	0.0111±5.91E-0.5	0. 032±0.67 E-0.3	0.2033±0.0019
Intercept ± SD(s_a)	-0.0003±0.0019	0.0021±0.0039	-0.0285±0.0832	-0.0020±0.0030	0.0021±0.0017	-0.0422±0.0323
SD of residual(S_{xy})	0.0026	0.0050	0.0534	0.0036	0.0039	0.0298
Correlation Coefficient	0.9999	0.9998	0.9995	0.9999	0.9999	0.9996
Accuracy* Intraday R% Interday R%	101.6-102.8. 101.2-102.0	99.4- 101.5 99.8-102.0	98.1-101.3 98.0-101.1	99.5-102.1 99.4-102.1	100.7- 102.0 100.4- 101.1	98.4 – 103.6 98.5- 102.0
Precision* Intraday RSD Interday RSD	0.54-1.74 0.23-1.09	0.16-0.56 0.30.1.25	0.78-1.53 0.69-1.61	0.72-0.97 0.24-1.73	0.22-0.56 0.19-0.28	1.21-2.45 0.34-2.20

*n=4

Table (2): Determination of Enrofloxacin and Flumequine in Mixtures with Their Decorboxylated Degradates by the Proposed Chelation Procedure.

Enrofloxacin				Flumequine			
Intact* $\mu g ml^{-1}$	Degradate $\mu g\ ml^{-1}$	Degradate** %	Recovery of intact %	Intact* $\mu g\ ml^{-1}$	Degradate $\mu g\ ml^{-1}$	Degradate** %	Recovery of intact %
72.48	7.52	9.4	100.10	81.12	14.88	15.5	99.60
64.96	15.04	18.8	100.40	73.68	22.32	23.2	99.90
57.44	22.56	28.2	100.50	66.24	29.76	31.0	101.50
49.92	30.08	37.6	101.90	58.80	37.20	38.7	100.40
42.40	37.60	47.0	100.70	51.36	44.64	46.5	100.40
34.88	45.12	56.4	100.90	43.92	52.08	54.3	100.60
27.36	52.64	65.8	99.40	36.48	59.52	62.0	101.60
19.84	60.16	75.3	100.80	29.04	66.96	69.8	100.20
Mean± RSD%			100.6±71				100.5±0.70

*Taken + undegraded part in the hydrolysed solutions; 6% for EN and 7% for FL
** Of the total weight

Table (3): Determination of Enrofloxacin and Flumequine in Mixtures with Their Decarboxylated Degradates by the Proposed ΔA Procedure

Enrofloxacin				Flumequine			
Intact* $\mu g\ ml^{-1}$	Degradate $\mu g\ ml^{-1}$	Degradate** %	Recovery% of intact	Intact* $\mu g\ ml^{-1}$	Degradate $\mu g\ ml^{-1}$	Degradate** %	Recovery% of intact
16.12	1.88	10.4	99.40	30.14	1.86	5.81	97.89
14.24	3.76	20.9	98.70	28.28	3.72	11.62	100.08
12.36	5.64	31.3	100.20	26.42	5.58	17.43	101.85
10.48	7.52	41.8	99.00	24.56	7.44	23.25	102.34
8.60	9.40	52.2	98.40	22.70	9.30	9.06	101.58
6.72	11.28	62.7	99.90	20.84	11.16	34.87	101.25
4.84	13.16	73.1	99.00				
Mean ± RSD %			99.20±0.65				100.83+1.62

* Taken + undegraded part in the hydrolyzed solutions; 6% for EN and 7% for FL
** of the total weight.

Table (4): Determination of Enrofloxacin and Flumequine in Mixtures with Their Decarboxylated Degradate by the Proposed Derivative Spectrophotometric Procedures.

Enrofloxacin (^{1}D at 292 nm)				Flumequine (^{2}D at 266 nm)			
Intact* µg ml^{-1}	Degradate µg ml^{-1}	Degradate** %	Recovery of intact %	Intact* µg ml^{-1}	Degradate µg ml^{-1}	Degradate** %	Recovery of intact %
22.60	9.4	29.4	99.5	24.56	7.44	23.6	99.1
20.72	11.28	35.3	100.9	22.70	9.30	29.1	100.8
18.84	13.16	41.1	100.3	20.84	11.16	34.9	100.2
16.96	15.04	47.0	99.6	18.98	13.02	40.7	99.6
15.08	16.92	52.9	100.6	17.12	14.88	46.5	98.8
13.20	18.80	58.8	101.9	15.26	16.74	52.3	101.0
11.32	20.68	64.6	101.1	13.40	18.60	58.1	100.1
				11.54	20.46	63.9	99.0
Mean±RSD%			100.6±0.85				99.8±0.83

* Taken + undegraded part in the hydrolysed solutions; 6% for EN and 7% for FL
** Of the total weight

Table (5): Application of Standard Addition Technique for Determination of EN and FL in Their Pharmaceutical Formulations by the Three Proposed Procedures.

Formulation	Recovery ± RSD%*						
	Enrofloxacin			Flumequine			
	Chelation with Fe (II)	ΔA	^{1}D-spectrophotometry	Formulation	Chelation with Fe (II)	ΔA	^{2}D-spectrophotometry
Enrotryl Oral solution N.B. 990958	100.1± 0.55	99.9± 0.67	100.6±0.89	Flumequine 20% powder B.N. 2/836/2000	100.3±0.60	99.9±0.58	100.4±1.18
Standard addition	99.8±0.48	99.7±0.26	100.7±1.43	Standard addition	99.8±0.58	99.6±0.79	100.1±1.25
SEF oral Solution N.B. 0400102	100.2±0.44	100.1±0.53	99.9±1.33	Flumequine 20% powder B.N. 5/836/2000	100.2±0.44	100.5±0.56	99.9±1.06
Standard addition	99.9±0.29	99.5±0.29	100.60±0.75	Standard addition	100.2±0.77	99.7±0.54	100.10±0.69

* n=5.

Table (6): Statistical Analysis of the Results Obtained by the Three Proposed and Compendial[12] Procedures for the Determination of EN in Enrotryl and SEF Oral Solution.

Parameters	Enrotryl Oral solution				S E F Oral solution			
	Compendial* Procedure	Iron(II) chelation	ΔA	^{1}D Procedure	Compendial* Procedure	Iron (II) Chelation	ΔA	^{1}D Procedure
Concentration Range (µg ml^{-1})	25 - 125	8 - 72	2 -16	10 -22	25 - 125	8 -72	2 -16	10 - 22
N	5	5	5	5	5	5	5	5
Mean %	100.40	100.10	99.90	100.60	99.60	100.20	100.10	99.90
SD	0.61	0.55	0.67	1.89	0.79	0.44	0.53	1.33
Variance	0.37	0.30	0.45	0.79	0.62	0.19	0.28	1.77
t	-	0.82	1.48	0.42	-	1.49	1.18	0.43
F	-	1.23	1.22	2.14	-	3.26	2.21	2.85

The theoretical value of F= 6.39 and t=2.31 at (p = 0.05)
* EN was determined by chelation with iron(III) in water and the orange colored chelate was measured at 434 nm[12].

Table (7): Statistical Analysis of the Results Obtained by the Three Proposed and Compendial[29] Procedures for the Determination of FL in Flumequine 20% Powder.

Parameters	Flumequine 20% powder B.N. 2 / 836 / 2000				Flumequine 20% powder B.N. 5 / 836 / 2000			
	Compendial* Procedure	Iron (II) chelation	ΔA	^{2}D Procedure	Compendial* Procedure	Iron(II) chelation	ΔA	^{2}D Procedure
Concentration Range (μg ml^{-1})	60-130	16-80	4-32	10-22	60-130	16-80	4-32	10-22
N	5	5	5	5	5	5	5	5
Mean %	100.2	100.30	99.90	100.40	99.60	100.20	100.50	99.90
SD	0.54	0.60	0.58	1.18	0.78	0.44	0.56	1.06
Variance	0.29	0.36	0.34	1.39	0.61	0.19	0.31	1.12
t	-	0.27	0.84	0.35	-	1.50	1.86	0.52
F	-	1.24	1.17	4.79	-	3.21	1.97	1.84

The theoretical value of F= 6.39 and t=2.31 at (p = 0.05)

*FL was determined by chelation with iron(III) at pH 3 in dimethyl formamide medium, yellow complex was formed, measured at 384 nm[29].

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إستخدام ثلاث طرق طيفضوئية لتقييم ثباتية مادتى إنروفلوكساسين وفلومكين

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فى هذا البحث تم أستخدام ثلاث طرق لتقييم ثباتية مادتى انروفلوكساسين وفلومكين

الطـريقة الأولـى: تعتمد على تكوين مركب معقد اصفر اللون لهذه المركبات مع الحديد الثنائي فى وجود محلول الخلات المنظم عياريته ٥,٦

الطـريقة الثانـية: تعـتمد علـى قياس الفرق فى الأمتصاص الضوئى للمركب بين محلولين أحدهما فى حمض الهيدروكلوريك (٠,١ عيارى) والثانى هيدروكسيد الصوديوم (٠,١ عيارى) .

الطريقة الثالثة : تعتمد على إستخدام المشتق الأول للمقياس الطيفى لتعيين مادة إنروفلوكساسين واستخدام المشتق الثانى للمقياس الطيفى لتعيين مادة فلومكين.

وقـد أمكن أستخدام الطرق المستحدثة لتقييم المواد تحت البحث فى وجود نواتج تحللهم وبدون تداخل من هذه النواتج وكذلك فى مستحضراتها الصيدلية.