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Voltammetric analysis of certain 4-quinolones in pharmaceuticals and biological fluids

M. Rizk *, F. Belal, F. Ibrahim, S. Ahmed, N.M. EL-Enany

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt

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Abstract

The voltammetric behaviour of Enrofloxacin (I), Sparfloxacin (II) and Fleroxacin (III) was studied using direct current (DC_t), differential pulse (DPP) and alternating current (AC_t). All the drugs manifest cathodic waves in Britton–Robinson buffer over the pH range of 4.0–11.98. The waves were characterized as being irreversible, diffusion-controlled with limited adsorption properties. The diffusion current–concentration relationships were found to be rectilinear over the ranges 4×10^{-5}–5×10^{-4} M, 1×10^{-5}–2×10^{-4} M, 1×10^{-5}–4×10^{-4} M using DC_t mode for I, II and III, respectively and 1×10^{-6}–4×10^{-5} M, 1×10^{-6}–1×10^{-4} M, and 2×10^{-6}–8×10^{-5} M, using DPP mode for I, II and III respectively, with minimum detectability ($S/N = 3$) of 1×10^{-7} M for I, II and 2×10^{-7} M for III. The proposed method was successfully applied to the determination of the studied compounds either per se or in formulations and biological fluids. The results obtained were concordant to those given using reference methods.

Keywords: Enrofloxacin; Sparfloxacin; Fleroxacin; Biological fluids; Dosage forms

1. Introduction

Enrofloxacin, sparfloxacin and fleroxacin are fluorinated 4-Quinolone derivatives, they have a broad spectrum of antibacterial activity against both gram negative and gram positive bacteria through inhibition of their DNA gyrase [1,2].

A good guide to the work published for these compounds is found in the review written by Belal et al. [3]. The literature is enriched with hundreds of reports on the determination of the 4-Quinolone antibacterials. The more recent techniques include, spectrophotometry [4], potentiometry [5] and fluorimetry [6]. The reported microbiological methods offer adequate sensitivity but they suffer from the inability to determine individual antibiotic in their combined formulation, and the long time of the incubation period. Although chromatographic methods offer high degree of specificity, yet, sample clean up and the instrument limitations preclude their use in routine clinical studies. The voltammetric techniques offer another possibility for the estimation of these compounds. Review of the literature revealed that, up to the present time, nothing has

* Corresponding author. Tel.: +20-50-347496; fax: +20-50-347496.

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been published concerning electrochemical behaviour of these compounds. Molecular structure of the studied compounds characterized by the presence of an electroactive carbonyl group adjacent to carboxylic group initiated the present study. The results obtained were promising.

2. Experimental

2.1. Apparatus

The polarographic study and DPP measurements were carried out using the polarecord E 506 Metrohm (Herisau, Switzerland). The drop time of 1 s was electronically controlled using a 663 VA stand from the company. The polarograms were recorded using a potential scan of 10 mV/s. A three-electrode system composed of a Dropping Mercury Electrode (DME), Ag/AgCl reference electrode, and a graphite rod as the auxiliary elctrode was used. Phase selective AC_t polarograms were recorded using the same instrument; the superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°. The effect of mercury height was studied using a 506 VA stand of the same company with Ag°/AgCl reference electrode and a platinum wire as an auxiliary electrode.

2.2. Materials and reagents

Pure drugs were kindly provided by pharmaceutical firms and were used as received; enrofloxacin was kindly provided by Bayer–Drugofa, Abt. Drugofa, Germany. Tablets each containing 100 mg of sparfloxacin was supplied by Rhone-Poulenc Pharma, Germany. Fleroxacin was provided by Hoffman-La Roche, (Nutley, NJ) USA. Tablets containing the studed drugs were obtained from commercial sources. Plasma was obtained from Mansoura University Hospital. Urine was obtained from healthy volunteers.

2.3. Reagents

- Britton Robinson buffers (0.08 M) covering the pH range 4.0–11.98 [7].
- Methanol: AR grade (Aldrich) USA.
- Acetonitrile: AR grade (Aldrich) USA.
- Chloroform
- dichloromethane
- phosphoric acid (85%) AR grade (Aldrich).

Stock solutions 2.5×10^{-3} M, 1×10^{-3}M and 2.5×10^{-3} M of I, II and III respectively were prepared in methanol for (I, II) and in acetonitrile for (III) and were further diluted with the same solvent to give the appropriate concentrations. The methanol concentration in the polarographic cell was kept always at 20%. The solutions were purged with pure nitrogen for 5 min, then polarographed at ambient temperature.

2.3.1. Procedure for tablets

Weigh and pulverize 10 tablets. Transfer a weighed quantity of the powder equivalent to 20.0 mg of the studied compounds into a conical flask, extract the drug three times each with 30 ml methanol for (I and II) or acetonitrile for III. Filter the extract into a 100 ml volumetric flask. Wash the conical flask with few mls of methanol or acetonitirle and pass the washings into the volumetric flask, complete to volume with the same solvent. Transfer aliquot volumes of the studied compounds covering the working range into 25 ml volumetric flask. Complete to the mark with BRb of pH 8.0, 7.0 or 6.0 for compounds I, II and III, respectively, pour into the polarographic cell and pass nitorgen gas for 5 min Record the DC_t and DPP polarograms. Calculate the nominal content of the tablets using either calibration graph or the corresponding regression equation (Table 4).

2.4. Assay of 4-quinolones in biological fluids

2.4.1. Urine

Transfer 5 ml of spiked urine into a separating funnel. Add 2 ml of phosphate buffer (pH 7.0), shake well and extract with 3×20 ml of dichloromethane–chloroform (1:1), then allow to separate into two layers. Filter the orgainc layer over anhydrous sodium sulphate. Evaporate the combined extracts under nitrogen gas. Dissolve

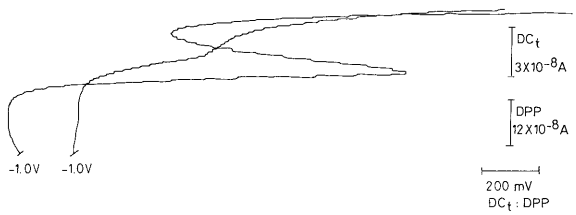


Fig. 1. Typical Polargram of Sparfloxacin (2×10^{-4} M) in BRb of pH 7.0 containing 20% methanol.

the residue in 25 ml of methanol or acetonitrile, then proceed as described above.

2.4.2. Plasma

Transfer 1 ml of spiked plasma into a centrifuge tube. Extract with 3×5 ml of acetonitrile for deproteination [8] and shake well on a vortex mixer for 30 s then centrifuge for 5 min at 12000 rpm in a microcentrifuge. Transfer the protein-free supernatant into a 25 ml standard flask and dilute to the volume with acetonitrile. Filter if necessary, then proceed as described above.

3. Results and discussion

Fig. 1 shows a typical DC_t and DPP polarogram of sparfloxacin in BRb of pH 7.0 containing 20% methanol. Methanol was added as a solubilizer for sparfloxacin, meanwhile, it decreases the adsorption phenomena likely to occur on the surface of DME. Reduction of the studied compounds at the DME was found to be pH dependant as the $E_{1/2}$ values were shifted to more negative values upon increasing the pH. Fig. 2 shows the behaviour of sparfloxacin as a model example. A plot of $E_{1/2}$ versus pH for sparfloxacin gave two straight lines with one break at pH 8.6 (Fig. 3). A plot of $E_{1/2}$ versus pH gave two straight lines with one break at pH 8.3 for compound I and a break at pH 8 for compound III.

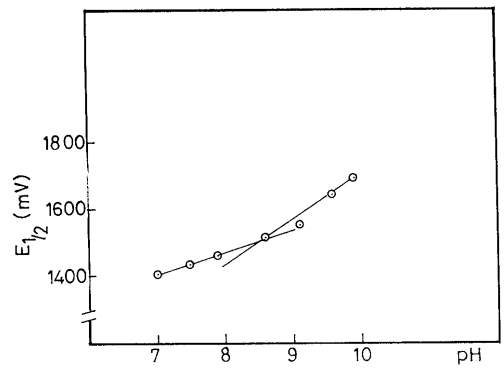


Fig. 3. The relationship between pH and $E_{1/2}$ (mV) values of sparfloxacin (2×10^{-4} M).

The relation between $E_{1/2}$ values and the pH of the solution is represented by the following equation:

1. For Enrofloxacin:

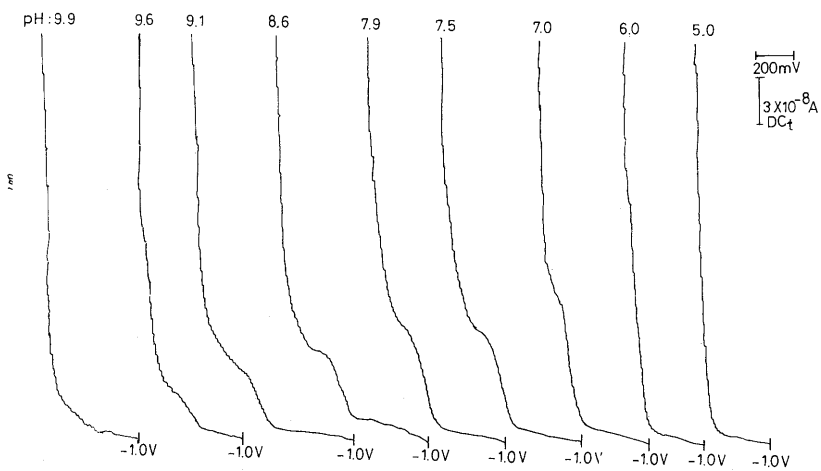


Fig. 2. Effect of pH on the development of polargraphic waves of Sparfloxacin (2×10^{-4} M).

Table 1
Effect of pH on the development of the polarographic waves of sparfloxacin [a]

PH	$E_{1/2}$ (V)	$\Delta E_{1/2}/\Delta$pH	Half-peak width ($W_{1/2}$) mV	Number of protons (Z)	αn_a
7.0	−1.41		11	1.094	1.076
		−60			
7.5	−1.44		15	0.69	0.82
		−50			
7.9	−1.46		13	1.16	0.80
		−85.7			
8.6	−1.52		14	0.97	0.717
		−80			
9.1	−1.56		15	1.89	0.620
		−180			
9.6	−1.65		Broad	1.68	0.60
		−166			
9.9	−1.7		Broad	–	0.58

[a] $W_{1/2}$: is the half-peak width in DPP mode.

$E_{1/2} = -1.04 - 0.04$ pH $(R = 0.9895)$

over the pH range 5.0–8.0 and

$E_{1/2} = -0.27 - 0.14$ pH $(R = 0.9753)$

over the pH range 8.5–11.2

2. For Sparfloxacin:

$E_{1/2} = -1.06 - 0.05$ pH $(R = 0.9933)$

over the pH range 7–7.9 and

$E_{1/2} = -0.29 - 0.14$ pH $(R = 0.9861)$

over the pH range 8.6–9.9

3. For Fleroxacin:

$E_{1/2} = -1.05 - 0.04$ pH $(R = 0.9931)$

over the pH range 4–7.5 and

$E_{1/2} = -0.37 - 0.12$ pH $(R = 0.9979)$

over the pH range 8–9.9.

The number of protons, Z., consumed in the electrode reaction is given by the following equation [9]

$$\Delta E_{1/2}/\Delta\text{pH} = \frac{0.059Z}{\alpha n_{\alpha}},$$

where α is the transfer coefficient. The value of n_a was calculated from the following equation $E = E_{1/2} - (0.059/\alpha n_a)\ \log[(i/\text{id} - i)]$ where id is the diffusion current. At pH 7.0 for compound (II) Z was found to be 1.1 i.e, two protons are probably consumed in the electrode reaction.

Logarithmic analysis of the reduction waves obtained in BRb of different pH values resulted in straight lines. The αn_a values were calculated using the treatment of Meites and Israel [10]. The results for compound II are shown in Table 1. Assuming that the rate determining step involves the transfer of two electrons (a free radical, one-electron transfer is not likely to occur), the values of the slopes suggest that the reduction process is irreversible in nature. For compound I. It is noticed that, the degree of reversibility increased as the pH is raised up to pH 8.0, then it began to decrease.

3.1. Study of the wave characteristics

Increasing the mercury height (h) resulted in a corresponding increase in the waveheight (w); a plot of $\sqrt{h}$ vs the waveheight gave a straight line. A plot of log $h$vs log w gave a straight line, the slope of which was 0.5 for I, II and 0.48 for III. Changing the buffer concentration over the range 0.006–0.06 M resulted in a negligible decrease in the waveheight. The waveheight showed a linear correlation with increase in the temperature. The temperature coefficient calculated according to Meites [11] was 2.36%/°C over the range 23–40°C. these characteristics point to a diffusion

Table 2
Correlation between the concentration of sparfloxacin and the diffusion current in the DC_t mode[a]

No	Concentration (mM)	Current (μA)	id/C (μA/mM)	Id = id/C $m^{2/3}$ $t^{1/6}$
1	0.02	0.156	7.813	5.621
2	0.04	0.313	7.813	5.621
3	0.06	0.477	7.943	5.714
4	0.10	0.789	7.875	5.676
5	0.20	1.575	7.875	5.665
$\bar{X}$				5.659
±S.D.				0.03

[a] Each result is the average of three separate determinations.

Table 3
Analytical parameters for the polarographic determination of the studied compounds

Parameter	Enrofloxacin		Sparfloxacin		Fleroxacin	
	DC_t mode	DPP mode	DC_t mode	DPP mode	DC_t mode	DPP mode
pH	8	8	7	7	6	6
$E_{1/2}$ (mV)	1400	1400	1410	1410	1260	1260
Id/C (μA/mM)	1.7 ± 0.01		7.87 ± 0.05		4.21 ± 0.04	
Linearity	4×10^{-5} – 5×10^{-4} M	1×10^{-6} – 4×10^{-5} M	1×10^{-5}–2×10^{-4} M	1×10^{-6} – 1×10^{-4} M	1×10^{-5} – 4×10^{-4} M	2×10^{-6} – 8×10^{-5} M
Minimum detectability		1×10^{-7} M		1×10^{-7} M		2×10^{-7} M
Linear regression equation	$C = 0.001$ + 1.696 id	$C = 0.0001$ + 2.12 id	$C = -0.0001$ + 7.89 id	$C = 0.0001$ + 7.81 id	$C = 0.0003$ + 4.19 id	$C = 0.0002$ + 5.23 id
Sy/x[a]	3.228×10^{-3}	1.059×10^{-4}	3.717×10^{-3}	4.623×10^{-3}	5.402×10^{-3}	1.127×10^{-3}
S_a[b]	0.011	1.322×10^{-3}	0.018	0.035	0.047	9.789×10^{-3}
S_b[c]	7.353×10^{-3}	3.115×10^{-3}	0.022	0.044	0.032	0.015
% Error[d]	0.33	0.30	0.27	0.11	0.28	0.25

[a] Sy/x = Standard deviation of residuals.
[b] S_a = Standard deviation of intercept of regression line.
[c] S_b = Standard deviation of slope of regression line.
[d] % Error = RS.D%/$\sqrt{n}$.

controlled process. These three characters point out to the diffusion controlled nature of the wave (Tables 2 and 3).

The alternating current-behaviour (AC_t) of sparfloxacin was studied using a phase-selective angle of 90°. In BRb of pH 7 and 8., the summit potentials (Es) were shifted to more negative values of 110, 80 mV than the corresponding $E_{1/2}$ values respectively. Fig. 4 demonstrates that no adsoption neither of the depolarizer nor its reaction product, takes place.

The diffusion coefficient (D) of sparfloxacin was calculated using Ilkovic equation [12] and was found to be 2.17×10^{-6} $cm^2 s^{-1} \pm 0.03$ in BRb of pH 7.0. This small value is attributed to the bulky nature of the compound.

Solutions of the studied 4-Quinolones in BRb of pH 8.0, 7.0 and 6.0 for I, II and III respectively, were found to be stable for more than 2 h.

The relation between the diffusion current i_d (μA) and concentration, C (M) was found to be rectilinear over the concentration ranges of $4 \times$

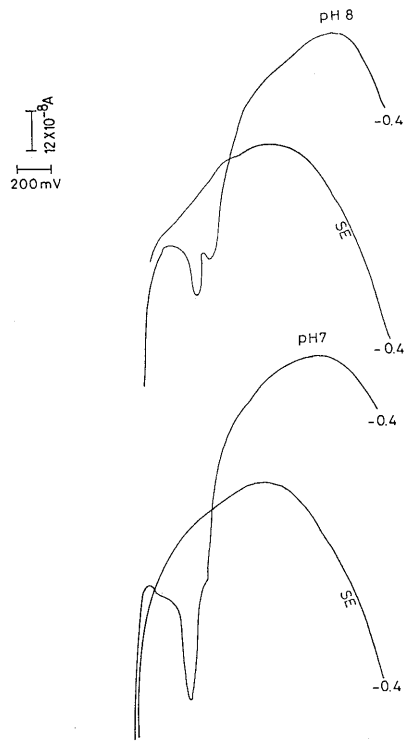


Fig. 4. Alternating current behaviour of Sparfloxacin (2×10^{-4} M) in BRb of different pH values; superimposed alternating voltage: 15 mV; Frequency: 75 Hz; phase angle: 90° (S.E.: Supporting electrolyte).

10^{-5} to 5×10^{-4} M, 1×10^{-5} to 2×10^{-4} M, 1×10^{-5} to 4×10^{-4} M using DC_t mode for I, II, III respectively, and 1×10^{-6} to 4×10^{-5} M, 1×10^{-6} to 1×10^{-4} M and 2×10^{-6} to 8×10^{-5} M using DPP mode for I, II, III, respectively, with a minimum detectability ($S/N = 3$) of 1×10^{-7} M for I, II and 2×10^{-7} M for III.

Linear regression analysis of the data gave the following equations.

1. For Enrofloxacin:

   $C = -0.001 + 1.70$ id $\quad (R = 0.9999)$

   using DC_t mode and

   $C = -0.0001 + 2.12$ id $\quad (R = 0.9999)$

   using DPP mode.
2. For Sparfloxacin:

   $C = -0.0001 + 7.89$ id $\quad (R = 0.9999)$

   using DC_t mode and

   $C = 0.0001 + 7.81$ id $\quad (R = 0.9999)$

   using DPP mode.
3. For Fleroxacin:

   $C = 0.0003 + 4.19$ id $\quad (R = 0.9999)$

   DC_t mode and

   $C = 0.0002 + 5.23$ id $\quad (R = 0.9999)$

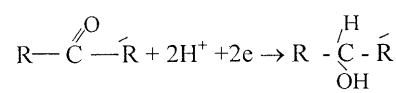
   using DPP mode.

Where C is the concentration in mM and id is the current in μA.

The diffusion current constant [$Id = id/ C m^{2/3} t^{1/6}$] was calculated at 25°C and was found to be 1.23 ± 0.008 for I, 5.66 ± 0.03 for II and 3.03 ± 0.028.

3.2. The number of electrons involved in the electrode reaction

The number of electrons consumed during the reaction was accomplished through comparison of the waveheight of I with that obtained from an equimolar solution of a closely related compound i.e. ciprofloxacin HCL [13]. In BRb of pH 8.0 both compounds gave one peak of the same height, and thus pointing out to a two-electron transfer process. It can be concluded that, only the carbonyl group is involved in the reduction process according to the following equation.



3.3. Analytical applications

Polarogram of the studied 4-Quinolones in BRb of pH 8.0, 7.0, 6.0 for I, II, III respectively exhibit well-defined cathodic waves. The current is diffusion-controlled and proportional to the concentration of the depolarizers over a convenient range of concentration. Both DC_t and DPP modes were successfully applied to the assay of the studied 4-Quinolones either per se, in pharmaceutical dosage forms or in spiked biological fluids.

Table 4
Polarographic determination of the studied compounds in dosage forms[a]

Preparations	% Recovery			Reference Methods
	DC_t mode	DPP mode		
I- Byatrile tablets* (250 mg enrofloxacin/ tablet)	100.73 ± 0.83	101.01 ± 0.43		100.89 ± 0.64
Student's t-value	1.29	0.34	(2.37)	
Variance ratio F-test	1.68	2.22	(5.79)	
II- Spar tablets** (100 mg of sparfloxacin / tablet).	98.99 ± 0.68	99.67 ± 0.86		100.08 ± 0.58
Student's t-value	2.17	0.98	(2.23)	
Variance ratio F-test	2.10	2.19	(4.35)	
III- Fleroxacin tablets (400 mg of Fleroxacin/ tablet).	100.05 ± 0.96	100.80 ± 0.45		100.62 ± 1.07
Student's t-value	0.79	0.45	(2.45)	
Variance ratio F-test	1.24	4.96	(6.94)	

[a] The results are the average of six separate determinations. Figures in parenthesis are the tabulated t and F values, respectively, at $P = 0.05$ [17].

The percentage recoveries for the studied compounds in tablets are, 100.73 ± 0.83, 98.99 ± 0.68, 100.05 ± 0.96 for I, II and III respectively using DC_t mode and 101.01 ± 0.43, 99.67 ± 0.86, 100.80 ± 0.45 for I, II and III respectively using DPP mode.

The results obtained for the studied compounds were favourably compared with reference methods [14–16]. For enrofloxacin [14], acid dye technique was utilized using supracene violet 3B and glycine buffer and extracting with chloroform, the absorbance was measured at 575 nm. For sparfloxacin and fleroxacin, the absorbance of the solution was measured in 0.1 M sodium hydroxide at 280 nm [15,16].

Statistical analysis [17] of the results by both methods using the student t-test and variance ratio F-test, shows no significant difference between the performance of the two methods regarding the accuracy and precision, respectively Table 4.

Table 5
Polarographic determination of sparfloxacin in spiked urine and plasma using DPP mode[a]

Sample	Added amount (μg)	Amount found (μg)	% Recovery
Urine	3.20	3.122	97.56
	8.0	7.778	97.23
	16.0	15.851	99.07
	22.40	22.008	98.25
	$\bar{X}$		98.03
	±S.D.		0.70
Plasma	3.2	3.022	94.44
	4.8	4.673	97.35
	8.0	7.682	96.03
	16.0	15.3840	96.15
	24.0	23.1432	96.43
	$\bar{X}$		96.08
	±S.D.		0.94

[a] Each result is the average of three separate determinations. Figures in parenthesis are the tabulated t and F values, respectively, at $P = 0.05$ [17].

3.3.1. Biological analysis

The DPP mode could be successfully applied to the determination of the studied compounds in spiked urine and plasma, over the specific concentration range for each compound. The results are abridge in Table 5. The percentage recoveries for the studied compounds in spiked urine are, 97.63 ± 0.68, 98.03 ± 0.70 and 97.88 ± 0.83 for I, II, and III respectively the percentage recoveries in spiked plasma are 92.74 ± 0.83, 96.08 ± 0.94 and 97.14 ± 1.16 for I, II and III, respectively using DPP mode.

Sparfloxacin is readily absorbed following oral administration, it binds weakly to plasma protein and exhibits excellent tissue distribution [18–20]. The oral dose of sparfloxacin is 100 mg, this dose gives a final plasma concentration of 2 ug ml^{-1}, which lies within the working concentration range of the proposed method. Preliminary human pharmacokinetic studies of fleroxacin indicated that it had a longer half-life than other quinolone antibacterial agents [21]. After oral administration of a 400 mg dose of fleroxacin to human subjects, the mean peak level in serum was 5–6 ug ml^{-1}, which was attained 0.7–1.3 h after administration, and the elimination half-life was about 12 h [22]. Thus, the proposed method proved to be satisfactory for the pharmcokinetic studies and routine estimation of the drugs in human urine and plasma. For serum only a deproteination process was carried out using acetonitrile as sample pretreatment. As for urine sample an extraction procedure was necessary.

3.3.2. Precision

The withen-day precision was evaluated through replicate analysis of urine sample spiked with enrofloxacin at different concentration levels. The percentage recoveries based on the average of six separate determinations were 97.03 ± 0.68, thus indicating the high precision of the method.

The inter-day precision was evaluated through replicate analysis of urine sample spiked with fleroxacin (8.00 ug ml^{-1}). The percentage recoveries based on the average of three separate determinations are 100.21 ± 1.35, thus indicating the high accuracy of the method.

On conclusion, a simple, rapid and highly sensitive method is reported for the determination of three 4-Quinolone antibacterials. The method proved to be suitable for the determination of the studied compounds in dosage forms and biological fluids. It can be considered as a promissing substitute for HPLC.

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