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Application of derivative UV spectrophotometry for the determination of enoxacin and nalidixic acid in tablets

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First-, second-, third- and forth-order derivative spectrophotometric methods, using "peak-zero" (P-O) and "peak-peak" (P-P) techniques of measurement have been developed for the determination of enoxacin and nalidixic acid in tablets. The calibration curves were linear in the concentration range of 2.0–12.0 μg ml^{-1} for the analysed quinolones. The procedure was simple, rapid and the results were reliable.

1. Introduction

The first quinolone of commercial importance, nalidixic acid was prepared in 1962s by Lesher [1]. It was introduced into therapy in 1964. Chemical modification led to new analogues, some of which have significantly improved effectiveness. Most of the highly active fluoroquinolones have a fluorine atom at C_6 which causes an increase of lipophilicity and facilitates penetration into tissues and cells. Many analogues have the piperazine group at atom C_7 which broadens the antibacterial spectrum, especially against gram-negative organisms like *Pseudomonas aeruginosa*. Important members of this new group of antibacterial agents are ciprofloxacin, norfloxacin, ofloxacin, enoxacin.

The fluorinated quinolone antibiotics were used as drugs for the treatment of urinary, respiratory tract infections, gastrointestinal infections, osteomyelitis, skin infections and sexually transmitted diseases [2, 3].

Spectrophotometric methods to determine enoxacin and nalidixic acid are limited in number. An UV spectrophotometric determination of nalidixic acid in pure form and preparations [4] and in liver, kidney, stomac, intestinal wall from human cadaver [5] was used.

Simultaneous spectrophotometric determinations of nalidixic acid and metronidazol in tablets [6] and nalidixic acid with phenazopyridine [7] were developed. A second-derivative spectrophotometric method for the determination of enoxacin concentrations in urine was presented [8].

The spectrophotometric technique is based on the principle that, for any given wavelenght and concentration interval, the fulfilment of the Lambert-Beer law for the n-th derivative is governed by the following equation:

$$\frac{d^n A}{d\lambda^n} = \frac{d^n \varepsilon}{d\lambda^n} \cdot c \cdot l$$

where A represents the absorbance, ε is the molar absorptivity (1 mol^{-1} cm^{-1}), c denotes the concentration (mol · l^{-1}) and l is the pathlenght (cm) of the cell. This technique offers an alternative approach to the enhancement of sensitivity and specificity in drugs analysis.

This paper describes the application of derivative UV spectrophotometry to the determination of enoxacin and nalidixic acid in pharmaceutical preparations.

Table 1: Statistical evaluation of the elaborated method for enoxacin

Derivative	λ (nm)	Technique	Regression equation	Correlation coefficient
D1	355.2	P–O	Y = 0.46032 (±0.00619) x + 0.21953 (±0.04825)	0.9996
D1	323.2	P–O	Y = 0.26068 (±0.00297) x + 0.13686 (±0.02313)	0.9997
D1	278.0	P–O	Y = 1.61969 (±0.02012) x + 0.92020 (±0.15673)	0.9997
D1	254.0	P–O	Y = 1.01686 (±0.01153) x + 0.61466 (±0.08988)	0.9997
D1	224.0	P–O	Y = 0.30275 (±0.00779) x + 0.11586 (±0.06068)	0.9998
D1	355.2–323.2	P–P	Y = 0.72101 (±0.00908) x + 0.35640 (±0.07074)	0.9997
D1	278.0–254.0	P–P	Y = 2.63669 (±0.03159) x + 1.53387 (±0.24609)	0.9997
D2	362.8	P–O	Y = 0.03014 (±0.00027) x + 0.00233 (±0.00217)	0.9998
D2	336.0	P–O	Y = 0.04137 (±0.00038) x + 0.01006 (±0.00300)	0.9998
D2	282.8	P–O	Y = 0.17585 (±0.00143) x + 0.02266 (±0.01118)	0.9999
D2	272.8	P–O	Y = 0.16898 (±0.00132) x + 0.01393 (±0.01035)	0.9999
D2	230.0	P–O	Y = 0.07395 (±0.00069) x + 0.00913 (±0.00540)	0.9998
D2	282.8–272.8	P–P	Y = 0.34484 (±0.00273) x + 0.03660 (±0.02130)	0.9999
D3	355.2	P–O	Y = 0.00570 (±0.00020) x + 0.00160 (±0.00155)	0.9976
D3	329.6	P–O	Y = 0.00401 (±0.00005) x + 0.00506 (±0.00042)	0.9996
D3	286.4	P–O	Y = 0.01872 (±0.00042) x + 0.00406 (±0.00159)	0.9998
D3	277.2	P–O	Y = 0.04905 (±0.00033) x + 0.00760 (±0.00262)	0.9999
D3	257.2	P–O	Y = 0.01845 (±0.00012) x + 0.00213 (±0.00100)	0.9999
D3	224.0	P–O	Y = 0.01534 (±0.000075) x + 0.0016 (±0.00061)	0.9999
D3	286.4–277.2	P–P	Y = 0.06772 (±0.00048) x + 0.01206 (±0.00377)	0.9999
D4	290.4	P–O	Y = 0.02085 (±0.00006) x + 0.00023 (±0.00047)	0.9983
D4	281.6	P–O	Y = 0.00968 (±0.00019) x + 0.00154 (±0.00152)	0.9992
D4	273.6	P–O	Y = 0.00859 (±0.00016) x + 0.00087 (±0.00131)	0.9992
D4	261.2	P–O	Y = 0.00306 (±0.00006) x + 0.00018 (±0.00045)	0.9993
D4	251.6	P–O	Y = 0.00191 (±0.00004) x − 0.000006 (±0.00030)	0.9992
D4	228.4	P–O	Y = 0.00281 (±0.00004) x + 0.00030 (±0.00034)	0.9996
D4	281.6–273.6	P–P	Y = 0.01827 (±0.00036) x + 0.00246 (±0.00281)	0.9993

(standard solutions − n = 6)

2. Investigations, results and discussion

In a preliminary work the influence of 0.1 mol · l^{-1} NaOH, 0.1 mol · l^{-1} HCl and CH_3OH on the absorption spectra of enoxacin and nalidixic acid were studied. From the results, it is evident that the highest absorbances were obtained using 0.1 mol · l^{-1} HCl for the determination of enoxacin and 0.1 mol · l^{-1} NaOH for the determination of nalidixic acid.

In order to determine the value of spectra derivatives a spectrophotometer interfaced with a microcomputer enabling to store the spectra derivatives in its computer memory and to read the value of a given derivative at any point marked on the recorded spectrum has been used.

The derivative values were recorded by means of two graphic measurements – "peak-zero" and "peak-peak".

In the "peak-to-peak" technique, the determination was carried out by measuring the amplitude (from the maximum to minimum of the curve). In the "baseline-to-peak" technique the measurement was carried out from the maximum to the zero line or from the minimum to the zero line.

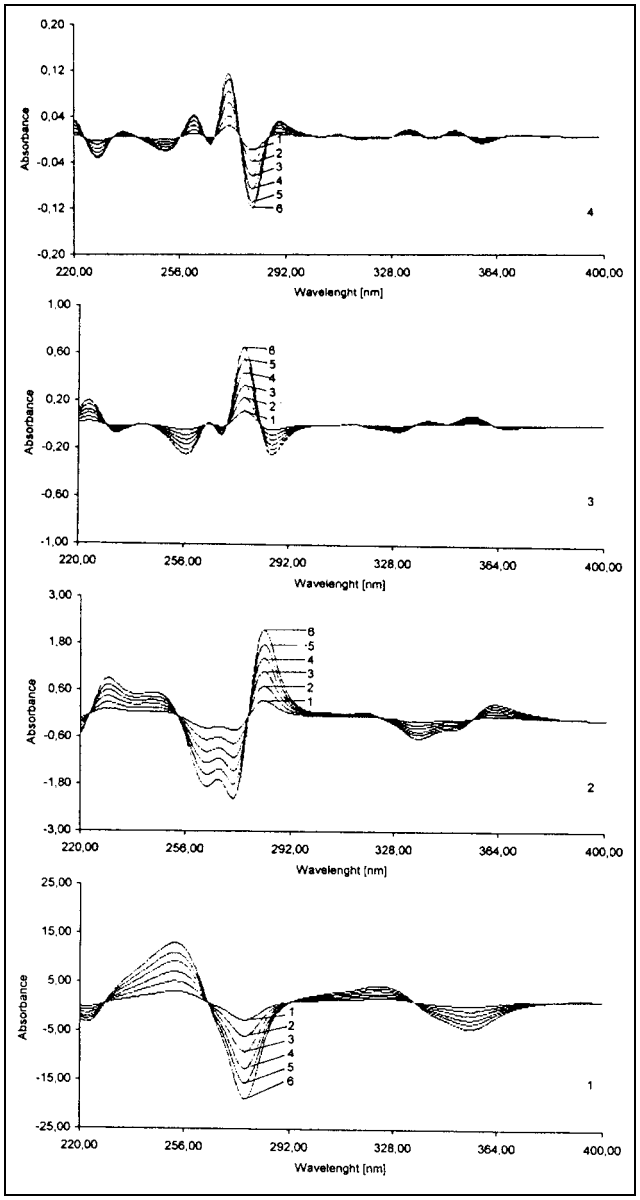

Fig. 1: First (1), second (2), third (3) and fourth (4) derivative spectra of enoxacin in 0.1 mol · l^{-1} HCl Spectra 1,2,3,4,5,6-concentrations: 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 μg · ml^{-1}, respectively

The calibration curves were constructed by plotting the graphically measured (mm) amplitudes of first-, second-, third- and fourth-order derivative spectra vs. the corresponding concentrations of the examined drugs.

Fig. 1 shows first-, second-, third- and fourth-order derivative spectra of enoxacin in 0.1 mol · l^{-1} HCl, recorded in the range of 220–400 nm for the concentrations of 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 μg · ml^{-1} of enoxacin.

Fig. 2 shows first-, second-, third- and fourth-order derivative spectra of nalidixic acid in 0.1 mol · l^{-1} NaOH, recorded in the range of 220–400 nm for the concentrations of 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 μg · ml^{-1} of nalidixic acid.

The linear equations obtained throught regression analysis of data of enoxacin and nalidixic acid are shown in Tables 1 and 3, respectively. Tables 2 and 4 illustrate data on the determination of the investigated quinolones in tablets with statistical evaluation of the results.

The best result for determining of enoxacin in Enoxor® tablets was obtained with the "peak-zero" technique for the fourth derivative spectrum at a wavelenght of 251.6 nm (SD – $1.16905 \cdot 10^{-4}$, RSD – 0.05%).

The best result for determining of nalidixic acid in Nevigramon® tablets were obtained with "peak-zero" techni-

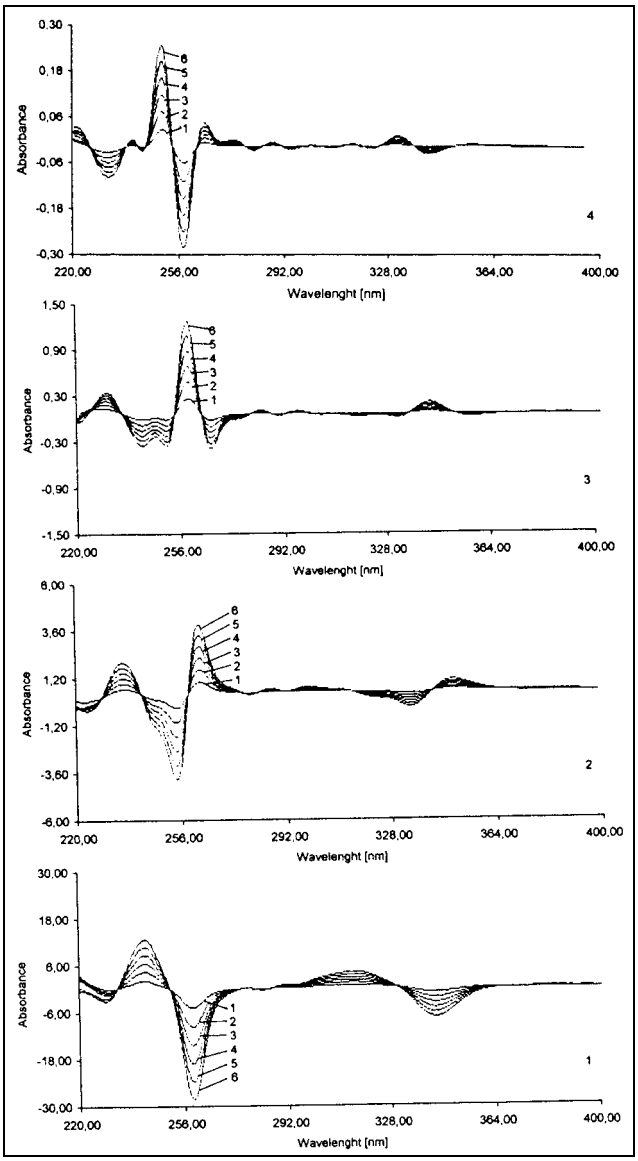

Fig. 2: First (1), second (2), third (3) and fourth (4) derivative spectra of nalidixic acid in 0.1 mol · l^{-1} NaOH Spectra 1,2,3,4,5,6-concentrations: 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 μg · ml^{-1}, respectively.

Table 2: Statistical analysis of the determination of enoxacin in Enoxor® tablets (n = 6)

Derivative	λ (nm)	Technique	Content of enoxacin (g)	Standard deviation (SD)	Variance	Correlation coefficient (RSD)	Confidence interval (95%)
D1	355.2	P–O	0.19791	5.9429×10^{-3}	3.5318×10^{-4}	3.00	0.1916–0.2041
D1	323.2	P–O	0.1998	6.4018×10^{-3}	4.0983×10^{-5}	3.23	0.1930–0.2064
D1	278.0	P–O	0.1969	6.3635×10^{-3}	4.0517×10^{-5}	3.16	0.1902–0.2036
D1	254.0	P–O	0.1966	6.1614×10^{-3}	3.7963×10^{-5}	3.13	0.1901–0.2030
D1	224.0	P–O	0.2087	9.1696×10^{-3}	8.4083×10^{-5}	4.39	0.1991–0.2183
D1	355.2–323.2	P–P	0.1986	5.9985×10^{-3}	3.5983×10^{-5}	3.02	0.1923–0.2048
D1	278.0–254.0	P–P	0.1968	6.2805×10^{-3}	3.9445×10^{-5}	3.19	0.1902–0.2034
D2	362.8	P–O	0.2059	0.01072	1.1500×10^{-4}	5.20	0.1947–0.2172
D2	336.0	P–O	0.2058	9.5484×10^{-3}	9.1173×10^{-5}	4.63	0.1957–0.2158
D2	282.8	P–O	0.20566	0.01033	1.0680×10^{-4}	5.02	0.1948–0.2165
D2	272.8	P–O	0.2057	0.010461	1.0945×10^{-4}	5.08	0.1947–0.2166
D2	230.0	P–O	0.17511	9.7624×10^{-3}	9.5305×10^{-5}	5.57	0.1648–0.1853
D2	282.8–272.8	P–P	0.20765	0.10168	1.0340×10^{-4}	4.89	0.1969–0.2183
D3	355.2	P–O	0.1947	8.1085×10^{-3}	6.5748×10^{-5}	4.16	0.1861–0.2032
D3	329.6	P–O	0.19446	9.9961×10^{-3}	9.9922×10^{-5}	5.14	0.1839–0.2049
D3	286.4	P–O	0.19323	6.1461×10^{-3}	3.7774×10^{-5}	3.18	0.1867–0.1996
D3	277.2	P–O	0.19298	6.0966×10^{-3}	3.7169×10^{-5}	3.15	0.1865–0.1993
D3	257.2	P–O	0.20175	5.6479×10^{-3}	3.1899×10^{-5}	2.79	0.1958–0.2076
D3	224.0	P–O	0.19246	5.2217×10^{-3}	2.7266×10^{-5}	2.71	0.1869–0.1979
D3	286.4–277.2	P–P	0.19741	6.1045×10^{-3}	3.7265×10^{-5}	3.09	0.1910–0.2038
D4	290.4	P–O	0.20025	6.8169×10^{-3}	4.6471×10^{-5}	3.04	0.1930–0.2074
D4	281.6	P–O	0.19805	5.8490×10^{-3}	3.4211×10^{-5}	2.95	0.1919–0.2041
D4	273.6	P–O	0.1966	5.8542×10^{-3}	3.4272×10^{-5}	2.97	0.1904–0.2027
D4	261.2	P–O	0.2066	4.8940×10^{-3}	2.3952×10^{-5}	2.36	0.2014–0.2117
D4	251.6	P–O	0.21281	1.1690×10^{-4}	1.3666×10^{-8}	0.05	0.2045–0.2097
D4	228.4	P–O	0.1979	7.3069×10^{-3}	5.3392×10^{-5}	3.69	0.1902–0.2055
D4	281.6–273.6	P–P	0.1973	5.8275×10^{-3}	3.3960×10^{-5}	2.95	0.1911–0.2034

Table 3: Statistical evaluation of the elaborated method for nalidixic acid

Derivative	λ (nm)	Technique	Regression equation	Correlation coefficient
D1	343.2	P–O	Y = 0.58238 (±0.00035) x + 0.08264 (±0.02745)	0.9999
D1	315.2	P–O	Y = 0.03065 (±0.00024) x + 0.044213 (±0.0019)	0.9998
D1	259.2	P–O	Y = 2.13687 (±0.00133) x + 0.302747 (±0.0104)	0.9999
D1	243.6	P–O	Y = 0.99562 (±0.00052) x + 0.202267 (±0.0041)	0.9999
D1	343.2–315.2	P–P	Y = 0.88786 (±0.00058) x + 0.138047 (±0.0045)	0.9999
D1	259.2–243.6	P–P	Y = 3.05886 (±0.08277) x + 1.24068 (±0.64476)	0.9986
D2	350.4	P–O	Y = 0.04212 (±0.00002) x + 0.0060 (±0.000204)	0.9999
D2	335.6	P–O	Y = 0.05814 (±0.00004) x + 0.01908 (±0.00033)	0.9999
D2	263.6	P–O	Y = 0.26762 (±0.00017) x + 0.04726 (±0.00139)	0.9999
D2	255.2	P–O	Y = 0.33345 (±0.00021) x + 0.03842 (±0.00164)	0.9999
D2	237.2	P–O	Y = 0.12507 (±0.00005) x − 0.01494 (±0.00041)	0.9999
D2	350.4–335.6	P–P	Y = 0.10026 (±0.00006) x + 0.02512 (±0.00053)	0.9999
D2	263.6–255.2	P–P	Y = 0.60230 (±0.00036) x + 0.07229 (±0.00283)	0.9999
D3	342.0	P–O	Y = 0.0104 (±0.000005) x + 0.00176 (±0.00004)	0.9999
D3	266.4	P–O	Y = 0.03302 (±0.000021) x + 0.00817 (±0.00016)	0.9999
D3	258.8	P–O	Y = 0.09044 (±0.00005) x + 0.01548 (±0.00039)	0.9999
D3	243.2	P–O	Y = 0.03104 (±0.00001) x + 0.00298 (±0.00014)	0.9999
D3	231.2	P–O	Y = 0.01868 (±0.00037) x + 0.04953 (±0.00288)	0.9993
D3	266.4–258.8	P–P	Y = 0.12347 (±0.00007) x + 0.02366 (±0.00054)	0.9999
D3	243.2–231.2	P–P	Y = 0.05031 (±0.00004) x + 0.039953 (±0.0003)	0.9998
D4	347.6	P–O	Y = 0.0015 (±0.000001) x − 0.00008 (±0.00001)	0.9998
D4	336.4	P–O	Y = 0.0019 (±0.000001) x + 0.0002 (±0.000008)	0.9999
D4	270.0	P–O	Y = 0.00441 (±0.000003) x + 0.0011 (±0.00002)	0.9999
D4	262.4	P–O	Y = 0.02065 (±0.000116) x − 0.0080 (±0.00090)	0.9994
D4	255.2	P–O	Y = 0.01938 (±0.000012) x + 0.0024 (±0.00009)	0.9999
D4	237.2	P–O	Y = 0.00567 (±0.000005) x + 0.0037 (±0.00004)	0.9998
D4	347.6–336.4	P–P	Y = 0.003407 (±0.000004) x + 0.0005 (±0.00003)	0.9997
D4	262.4–255.2	P–P	Y = 0.04103 (±0.00022) x − 0.01652 (±0.00178)	0.9994

(standard solutions – n = 6)

que for the fourth derivative spectrum at wavelenght 336.4 nm (SD – $2.86217 \cdot 10^{-4}$, RSD – 0.05%).

In summary, the proposed analytical procedure based on the first-, second-, third- and fourth-order UV derivative spectroscopy permits a simple, rapid, sensitive and direct determination of analysed drugs.

3. Experimental

3.1. Reagents and materials

Enoxacin substance and Enoxor-tablets (0.200 g of enoxacin) from Pierre Fabre Médicament (France), nalidixic acid substance from Chinoin (Hungary) and Nevigramon-tablets (0.500 g of nalidixic acid) from a local drug store were used.

Table 4: Statistical analysis of the determination of nalidixic acid in tablets Nevigramon (n = 6)

Derivative	λ (nm)	Technique	Content of nalidixic acid (g)	Standard deviation (SD)	Variance	Correlation coefficient (RSD)	Confidence interval (95%)
D1	343.2	P–O	0.50501	2.6776×10^{-3}	1.3860×10^{-5}	0.73	0.5003–0.5096
D1	315.2	P–O	0.49825	5.4814×10^{-3}	3.0046×10^{-5}	1.1	0.4914–0.5050
D1	259.2	P–O	0.49378	2.6776×10^{-3}	7.1700×10^{-6}	0.54	0.4904–0.4971
D1	243.6	P–O	0.50308	0.01003	1.0159×10^{-4}	2.00	0.4905–0.5155
D1	343.2–315.2	P–P	0.49724	2.8104×10^{-3}	7.8988×10^{-6}	0.56	0.4937–0.5007
D1	259.2–243.6	P–P	0.49366	3.1473×10^{-3}	9.9056×10^{-6}	0.63	0.4897–0.4975
D2	350.4	P–O	0.50002	3.7772×10^{-3}	1.4267×10^{-5}	0.75	0.4953–0.5047
D2	335.6	P–O	0.49498	3.3742×10^{-3}	1.1385×10^{-5}	0.68	0.4907–0.4991
D2	263.6	P–O	0.49819	3.9674×10^{-3}	1.5740×10^{-5}	0.79	0.4932–0.5031
D2	255.2	P–O	0.49704	2.6576×10^{-3}	7.0631×10^{-6}	0.53	0.4933–0.5003
D2	237.2	P–O	0.53364	0.01050	1.1034×10^{-4}	1.96	0.5209–0.5466
D2	350.4–335.6	P–P	0.49709	3.4854×10^{-3}	1.2148×10^{-5}	0.70	0.4926–0.5014
D2	263.6–255.2	P–P	0.49792	3.0203×10^{-3}	9.1224×10^{-6}	0.60	0.4947–0.5016
D3	342.0	P–O	0.50273	4.4273×10^{-3}	1.9601×10^{-5}	0.88	0.4973–0.5082
D3	266.4	P–O	0.49892	4.5301×10^{-3}	2.0522×10^{-5}	0.90	0.4939–0.5045
D3	258.8	P–O	0.49786	2.9492×10^{-3}	8.6983×10^{-3}	0.59	0.4949–0.5015
D3	243.2	P–O	0.44966	4.9654×10^{-3}	2.4655×10^{-5}	1.10	0.4439–0.4558
D3	231.2	P–O	0.39494	9.3545×10^{-3}	8.7508×10^{-5}	2.36	0.3832–0.4065
D3	266.4–258.8	P–P	0.49815	3.3455×10^{-3}	1.1192×10^{-5}	0.67	0.4939–0.5023
D3	243.2–231.2	P–P	0.46408	6.2760×10^{-3}	3.9389×10^{-5}	1.35	0.4562–0.4718
D4	347.6	P–O	0.50195	0.022092	4.8485×10^{-4}	4.38	0.4788–0.5250
D4	336.4	P–O	0.50584	2.8621×10^{-4}	8.1920×10^{-8}	0.05	0.5054–0.5061
D4	270.0	P–O	0.50479	6.5737×10^{-3}	4.3214×10^{-5}	1.30	0.4966–0.5129
D4	262.4	P–O	0.50782	3.5452×10^{-3}	1.2568×10^{-3}	0.69	0.5034–0.5122
D4	255.2	P–O	0.49764	3.7599×10^{-3}	1.4137×10^{-5}	0.75	0.4929–0.5023
D4	237.2	P–O	0.47218	5.9062×10^{-3}	3.4883×10^{-5}	1.25	0.4648–0.4795
D4	347.6–336.4	P–P	0.49709	0.01025	1.0518×10^{-4}	2.06	0.4843–0.5098
D4	262.4–255.2	P–P	0.50796	3.5671×10^{-3}	1.2724×10^{-5}	0.70	0.5029–0.5118

Hydrochloric acid, sodium hydroxide were of analytical-reagent grade and double distilled water was used throughout to prepare the solutions.

3.2. Apparatus

A Perkin-Elmer Lambda 15 double-beam UV-visible spectrophotometr, with the capability of applying the derivative mode, was used. The optimized operating conditions for recording the first-, second-, third-, fourth-order derivative spectra were: scan speed 240 nm min^{-1}, response time 2s, spectral slit width 2 nm, delta wavelenght 6 nm. All measurements were carried out in an 1.0 cm quartz cuvette.

3.3. Calibration curves

3.3.1. Calibration curve for enoxacin

Working standard solutions of enoxacin in 0.1 mol · l^{-1}HCl solution (containing increasing concentrations of enoxacin ranging from 2.0 to 12.0 μg ml^{-1}) were prepared daily from stock solution of enoxacin (1.0 mg · ml^{-1}) in 0.1 mol · l^{-1} HCl. The first-, second-, third- and fourth-order derivative spectra of these solutions were recorded over the wavelenght range 220–400 nm, against 0.1 mol · l^{-1} HCl as blank and the amplitudes of the maximum and minimum were recorded.

3.3.2. Calibration curve for nalidixic acid

Working standard solutions of nalidixic acid in 0.1 mol · l^{-1} NaOH solution (containing increasing concentrations of nalidixic acid ranging from 2.0 to 12.0 μg · ml^{-1}) were prepared daily from stock solution of nalidixic acid (1.0 mg · l^{-1}) in 0.1 mol · l^{-1} NaOH.
The first-, second-, third- and fourth-order derivative spectra of these solutions were recorded over the wavelenght range 220–400 nm, against 0.1 mol · l^{-1} NaOH as blank and the amplitudes of the maximum and minimum were recorded.

3.4. Determination of the quinolones in pharmaceutical preparations

3.4.1. Determination of enoxacin in tablets

Ten tablets of Enoxor (mean mass of a tablet: 0.5435 g; containing 0.200 g of enoxacin) were weighed and powdered. An accurately weighed amount of the homogenized powder equivalent to 50 mg of enoxacin was transferred into a 50 ml volumetric flask containing approx. 25 ml of 0.1 mol · l^{-1} HCl. The mixture was extracted by shaking for 30 min, then the volume was brought to 50 ml with 0.1 mol · l^{-1} HCl and the obtained solution was filtered. This resulting clear solution (1 ml) was diluted to 10 ml with 0.1 mol · l^{-1} HCl. Then 0.8 ml of this solution was placed in a 10 ml volumetric flask and diluted to the mark with 0.1 mol · l^{-1} HCl. The first-, second-, third- and fourth-order derivative spectra of this solution were recorded. The amplitudes of the minimum and maximum were graphically determined and the concentration of enoxacin in the sample solution was obtained by interpolating the corresponding calibration curve. This procedure was repeated six times.

3.4.2. Determination of nalidixic acid in tablets

Ten tablets was weighed and powdered. An accurately weighed portion of the powder equivalent to 50 mg of nalidixic acid (mean mass of a tablet: 0.5218 g; containing 0.500 g of nalidixic acid according to a declaration) was extracted with 25 ml of 0.1 mol · l^{-1} NaOH in a 50 ml flask by shaking for 30 min and the solution was diluted to the mark with the same solvent. After filtration, the extract of 1.0 ml-volume was transferred into a 10 ml flask and diluted to 10 ml with 0.1 mol · l^{-1} NaOH. Then 0.8 ml of this solution was placed in 10 ml volumetric flask and was brought to 10 ml with 0.1 mol · l^{-1} NaOH. The first-, second-, third- and fourth-order derivative spectra of this solution were recorded. The amplitudes of the minimum and maximum were graphically determined and the concentration of nalidixic acid in the sample solution was obtained by interpolating the corresponding calibration curve. This procedure was repeated six times.

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Received September 13, 1999
Accepted November 15, 1999

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