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ANALYSIS

THIN–LAYER CHROMATOGRAPHIC ANALYSIS OF NALIDIXIC ACID, OXOLINIC ACID AND CINOXACIN

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Abstract: DNA–gyrase inhibitors: cinoxacin, nalidixic acid and oxolinic acid, were separated by TLC method on normal and reversed–phase plates by ascending and horizontal techniques using suitable mobile phases. The substances were identified by UV irradiation and by the dyeing reagents. The TLC method is simple, sensitive and it was used to identify nalidixic and oxolinic acids in tablets.

Keywords: DNA inhibitors, TLC separation, identification, tablets.

Quinolones, similar to sulfonamides, are totally synthetic chemical compounds used to combat infections.

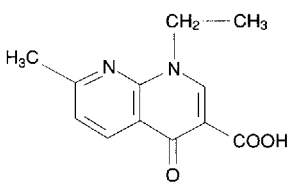
Nalidixic acid (1–ethyl–1,4–dihydro–7–methyl–4–oxo–1,8–naphthyridine–3–carboxylic acid), oxolinic acid (1–ethyl–1,4–dihydro–6,7–methylenedioxy–4–oxoquinoline–3–carboxylic acid) and cinoxacin (1–ethyl–1,4–dihydro–4–oxo[1,3]dioxolo[4,5–g]–cinnoline–3–carboxylic acid) (Figure 1) constitute the first generation of 4–quinolones and have been studied extensively as to their chemical properties, mechanism of action and resistance. This drugs are antibacterial agents of the 4–quinolone group with the spectrum of greater activity against gram–negative than against gram–positive organisms. The first generation of the quinolones are still used as drugs for the treatment of urinary tract infections.

Nalidixic acid and all its analogs act by selectively inhibiting bacterial DNA–gyrase (1, 2).

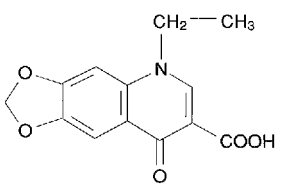
Many reaction and various techniques have been developed for the determination of nalidixic acid and its analogs in pharmaceutical preparations. High performance thin–layer procedure combined with densitometry, was developed for monitoring nalidixic acid and its photodegradation products (3).

The determinations by HPLC method of oxolinic acid and other quinolones in fish tissues (4, 5, 6), marine sediments (7, 8), and nalidixic acid in tablets (9) are described. Horie *et al.* determined oxolinic acid, nalidixic acid and piromidic acid in fish by the HPLC–mass spectrometry method (10).

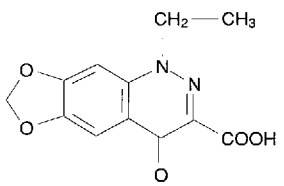
The spectrophotometric methods were developed for the determination of nalidixic acid with $Fe(NO_3)_3$ (11) and norfloxacin, ciprofloxacin, cinoxacin and nalidixic acid with $FeCl_3$ in DMSO–MeOH medium (12) and nalidixic acid was converted into its hydroxamic acid which form a yellow stable complex with $FeCl_3$ solution (13). The spectrophotometric determination of nalidixic acid with



NALIDIXIC ACID



OXOLINIC ACID



CINOXACIN

Figure 1. The structural formulae of DNA–gyrase inhibitors.

Cd(II) ion (14) and nalidixic acid, flumequine and izoniazyde through ternary complex formation with Cd(II) ion and rose bengal was described (15). The synthesis and characterization of Cd(II) (16) complexes and Ni(II), Zn(II) (17) complexes with cinoxacin were investigated. The fluorescence spectrophotometry was used for direct determination of nalidixic acid in urine (18). The characteristics of host–quest complexation between nalidixic acid and γ-cyclodextrin was investigated by fluorescence spectrophotometry (19). The capillary electrophoresis separation of antibacterial quinolones was described (20).

This work describes separation and identification of nalidixic acid, oxolinic acid and cinoxacin by thin–layer chromatography in the pure form and in pharmaceutical preparations.

EXPERIMENTAL

Chemicals, apparatus and materials

Nalidixic acid and oxolinic acid substances from Chinoin S.A., Budapest (Hungary), Nevigramon and Gramurin tablets from local chemist's, Cinoxacin substance from Eli Lilly (Italy) were applied. All reagents and solvents were analytical grade or chromatography–grade. Solutions were fresh prepared or used within 1–5 days.
TLC pre–coated silica gel $60F_{254}$ and $RP–18F_{254}$ plates (5×20 cm) were purchased from Merck (Germany). Classical chambers with glass ground lids from Camag (Switzerland), DS–horizontal chambers from Chromdes Lublin (Poland) and a microsyringe 25 µl (accuracy 0,5) from Hamilton (Switzerland) were used.

Initial tests

In a preliminary study the experimental conditions were established such as:

- the solubility of the examined drugs in different organic solvents and in inorganic solvents,
- time needed for chamber saturation by mobile phase vapours,
- the sensitivity of reagents used for the detection of three analysed substances.

Normal–phase chromatographic analysis

The working solutions were prepared by dissolving of 10 mg of nalidixic and oxolinic acids in 1 ml of 0,1 mol/l NaOH solution and made up with methanol to a volume of 10 ml. Two µl volumes of each substance solution (corresponding to 2 µg) were spotted on TLC plates.

The development distance was 10 cm from the baseline, using the ascending technique in the chambers saturated with solvent vapours or horizontal technique in unsaturated chambers. The plates were dried at room temperature, viewed under UV (at λ=254 nm) and then sprayed with acidified 15% $FeCl_3$ solution or iodine reagent.

Chromatographic analysis of nalidixic acid and oxolinic acid in tablets

The tablets were weighed and triturated to a fine powder. The powdered tablets were weighed in amounts needed to obtain the working solutions of the concentration of 1 mg/ml.

Extraction from tablets was carried out with 0.1 mol/l NaOH solution, the solutions obtained were filtered, and 1 ml of these filtrates was pipetted to 10 ml volumetric flasks and made up with methanol to the mark.

Two µl volumes were spotted on TLC plates as well as 2 µl volumes of adequate standard solutions.

Chromatograms were developed by using the best solvent system (N° 10, see Table 1).

Reversed–phase chromatographic analysis

Two µl of analysed solutions (corresponding to 2µg) were applied on RP–18 TLC plates. The chromatograms were developed on the distance of 10 cm by using the horizontal technique, dried, viewed under UV (at λ=254 nm) and then sprayed with acidified 15% $FeCl_3$ solution or iodine reagent.

The influence of the composition of the mobile phase and of pH of the phosphate buffer in the eluent mixture (in the range from 2.47 to 7.10) on separation of analysed drugs in reversed–phase technique was investigated.

RESULTS AND DISCUSSION

In this paper, some DNA–gyrase inhibitors: nalidixic acid, oxolinic acid and cinoxacin were analysed together by TLC technique.

In the initial tests we found that pre–coated silica gel plates could be sufficiently activated by staying at room temperature for 10 h before applying the tested substances.

The 55 chromatographic systems were examined with regard to their separation efficiency and developing time. The Rf values of the analysed substances in 20 proposed TLC solvent systems are shown in Table 1.
The best separation was achieved using methanol–butanol–25%NH_3 (5:5:2), ethyl acetate–dichloromethane–methanol–25%NH_3 (3:3:5:2) and dichloromethane–triethylamine (10:1) as the mobile phases.

Figure 2 presents the chromatogram of separation of tested substances achieved by development with the chosen solvent system (N° 10, see Table 1).

The developing time was usually from 20 to 90 min in the ascending technique and from 40 to 120 min in the horizontal technique. The time of development for the distance of 10 cm, by using finally chosen eluent mixture, was 25 minutes.

The separation of examined drugs in the ascending and the horizontal techniques was satisfactory, but the spots of the substances were more symmetrical in the ascending technique.

Table 1. Some chromatographic systems and R_f values of TLC analysis of DNA-gyrase inhibitors

Number of system	The composition of mobile phase (v/v)	R_f x 100		
		Nalidixic acid	Oxolinic acid	Cinoxacin
1.	Ethanol-isobutyl alcohol-water-triethylamine (20:10:5:5)	45	37	47
2.	Dichloromethane-ethyl acetate-methanol-acetic acid (5:10:5:2)	74	61	46
3.	Acetone-methanol-acetic acid (10:2:2)	10	93	80
4.	Ethylmethylketone-methanol-25% NH_3 (20:10:1)	15	17	23
5.	Dichloromethane-ethyl acetate-acetic acid (4:10:2)	52	24	19
6.	Isopropyl alcohol-water-acetic acid (10:5:0,5)	63	53	45
7.	Ethyl acetate-dioxane-acetic acid (15:10:2)	64	48	40
8.	Ethyl acetate-acetic acid (10:3)	58	31	23
9.	Ethyl acetate-triethylamine (10:1)	9	13	0
10.	Methanol-n-butyl alcohol-25% NH_3 (5:5:2)	53	46	60
11.	Ethyl acetate-dichloromethane-methanol-25% NH_3 (3:3:5:2)	51	58	62
12.	Dichloromethane-triethylamine (10:1)	12	18	5
13.	Ethyl acetate-n-butyl alcohol-water-acetic acid (10:5:1,5:0,15)	72	53	30
14.	Ethyl acetate-acetone-acetic acid (10:5:2)	68	53	40
15.	Ethyl acetate-n-butyl alcohol-water (10:5:1)	60	50	20
16.	Ethyl acetate-benzene (5:10)	8	3	0
17.	Ethyl acetate-benzene-acetic acid (5:10:2)	40	15	7
18.	Dichlorometane-acetic acid (10:3)	92	61	47
19.	Dichloromethane-methanol-triethylamine (10:2:3)	45	30	55
20.	N-butyl alcohol-acetic acid (6:1)	20	33	42

Table 2. Compoposition of the mobile phase on RP–18 TLC

DNA–gyrase inhibitors	Acetonitrile in buffer % vol.					THF in buffer % vol.				
	10	30	50	70	100	10	30	50	60	100
	R_f×100									
Nalidixic acid	0	3	31	54	0	2	18	40	43	80
Oxolinic acid	0	5	45	55	0	2	13	37	45	80
Cinoxacin	0	6	51	70	0	5	11	34	48	83

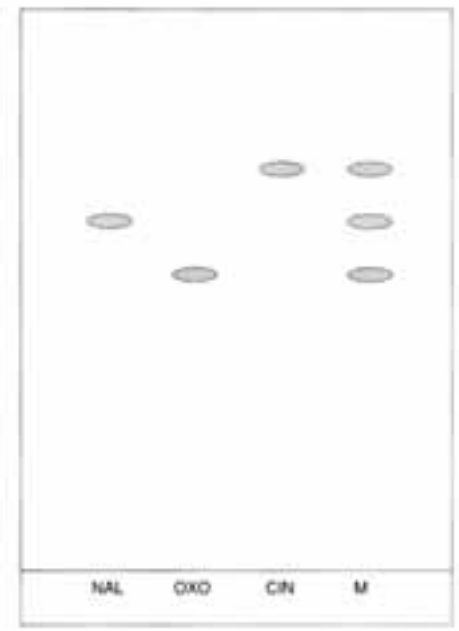


Figure 2. Exemplary chromatogram of separation of analysed quinolones [silica gel 60 F254 plates, mobile phase: methanol – n–butyl alcohol – 25% NH3 (5:5:2, v/v)]; OXO – oxolidic acid, NAL – nalidixic acid, CIN – cinoxacin, M – mixture of three tested drugs.

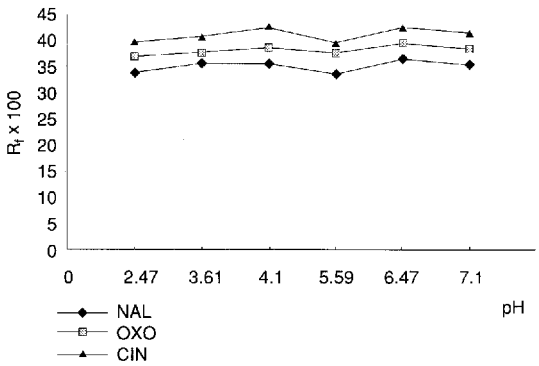


Figure 3. Comparison of R_f values of analysed drugs obtained at various pH of the buffer. The mobile phase: THF – phosphate buffer (5:5).

It was also found that the greatest sensitivity of detection of each drug (0.1 μg) was obtained by using UV irradiation. Satisfactory results were also obtained by spraying the plates with iodine reagent or acidified 15% $FeCl_3$ solution. In these conditions the lowest detectable amount of each drug was 0.5 μg.

The mobile phase used on reversed–phase TLC plates were composed of the following solvents: acetonitrile, methanol and tetrahydrofuran (THF) in a phosphate buffer.

R_f values of analytes were significantly dependent on the composition of the mobile phase (Table 2).

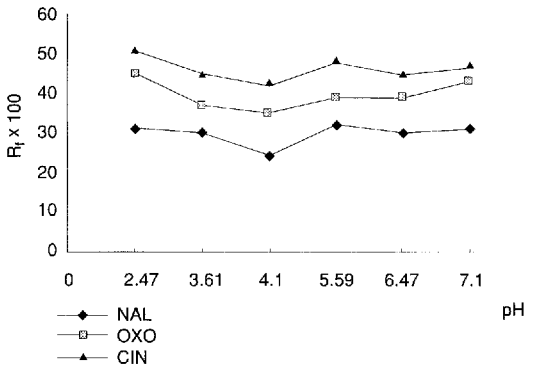


Figure 4. Comparison of R_f values of analysed drugs obtained at various pH of the buffer. The mobile phase: Acetonitrile – phosphate buffer (5:5).

The separation of drugs on reversed–phase TLC was independent on pH values in the range from 2.47 to 7.10 of the phosphate buffer solution, used in the mobile phase composed of THF or acetonitrile–phosphate buffer (5:5) (Figure 3 and Figure 4).

The TLC method, first carried out with the pure substances, subsequently was used to identify of nalidixic and oxolinic acids in tablets.

Summing up, we conclude that the elaborated TLC method is simple, rapid and sufficiently selective for the routine analysis of examined DNA–gyrase inhibitors in tablets.

REFERENCES

1. Grinquaz A.: Introduction to medical chemistry, p. 265, Viley–VCH, New York (1997).
2. Quinolone Anti–bacterials, Kuhlmann J., Dalhoff A., Zeiler H.J. Eds., Springer Verlag, Berlin Heidelberg 1998.
3. Solomies H., Koski S.: J. Planar Chromatogr. 9, 103 (1996).
4. Sonavio E., Angeletti R., Piro R.: Boll. Chim. Ig. Parte Sci. 47, 139 (1996); after Chem. Abstr. 126, 59035p (1997).
5. Horie M., Saito K., Nose N., Nakazawa H.: Shokuhin Eiseigaku Zassahi 36, 62 (1995); after Chem. Abstr. 122, 237993b (1995).
6. Degroodt J.M., Wyhowski De Bukanski B., Srebrnik S.: J. Liq. Chromatogr. 17, 1785 (1994).
7. Pouliquen H., Le Bris H., Pinault L.: Quim. Anal. 13, 109 (1994).
8. Pouliquen H., Le Bris H., Pinault L.: J. Liq. Chromatogr. 17, 929 (1994).
9. Foda N.H.: J. Liq. Chromatogr. 18, 4135 (1995).
10. Horie M., Saito K., Nose N., Tera M., Nakazawa H.: J. Liq. Chromatogr. 16, 1463 (1993).
11. Chowdary K.P.R., Rao P.N.: Indian Drugs 28, 569 (1991).
12. Bhowal S.K., Das T.K.: Anal. Lett. 24, 25 (1991).
13. Sandhyavali M.S., Thampi P.P., Thampi C.S.: East. Pharm. 34, 129 (1991).
14. Djurdjevic P., Jelic R., Stankov M., Vaselinovic D.S., Stankov D.: J. Serb. Chem. Soc. 60, 403 (1995).
15. Amer S.M., El Sheril Z.: J. Pharm. Sci. (Egipt) 35, 627 (1994).
16. Ruiz M., Ortiz R., Perello L., Garcia–Granda S., Diaz M.R.: Inorg. Chim. Acta 217, 149 (1994).
17. Ruiz M., Ortiz R., Perello L., Castineiras A., Quiros M.: Inorg. Chim. Acta 211, 133 (1993).
18. Antonio J., Pulgarin M., Molina A.A., Lopez P.F.: Talanta 43, 431 (1996).
19. Duran–Meras I., Muneoz de la Pena A., Salinos F.: Analyst 119, 1215 (1994).
20. Sun S.W., Chen L.Y.: J. Chromatogr. 766, 215 (1997).

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