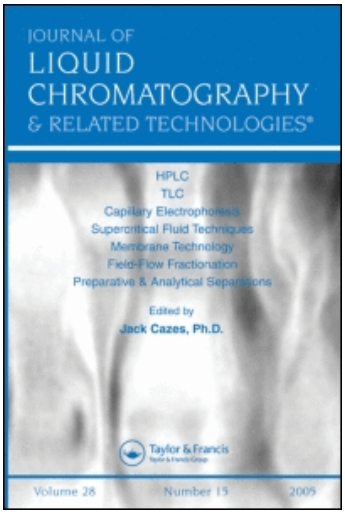


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC Investigation of 11-Amino Undecanoic Acid's Ion Pairing Ability on Fluoroquinolone Gyrase Inhibitors

Zs. Budvári Bárány[a]; A. Lõre[a]; Gy. Szász[a]; K. Takács-Novák[a]; I. Hermecz[b]
[a] Institute of Pharmaceutical Chemistry, Semmelweis University of Medicine, Budapest, Hungary [b] Chinoin Pharmaceutical Works, Budapest, Hungary

To cite this Article Bárány, Zs. Budvári , Lõre, A. , Szász, Gy. , Takács-Novák, K. and Hermecz, I.(1994) 'HPLC Investigation of 11-Amino Undecanoic Acid's Ion Pairing Ability on Fluoroquinolone Gyrase Inhibitors', Journal of Liquid Chromatography & Related Technologies, 17: 9, 2031 — 2044
To link to this Article: DOI: 10.1080/10826079408013477
URL: http://dx.doi.org/10.1080/10826079408013477

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(9), 2031-2044 (1994)

HPLC INVESTIGATION OF 11-AMINO UNDECANOIC ACID'S ION PAIRING ABILITY ON FLUOROQUINOLONE GYRASE INHIBITORS

ZS. BUDVÁRI BÁRÁNY[1], A. LŐRE[1], GY. SZÁSZ[1], K. TAKÁCS-NOVÁK[1], AND I. HERMECZ[2]
[1]*Institute of Pharmaceutical Chemistry*
Semmelweis University of Medicine
Budapest, Hungary
[2]*Chinoin Pharmaceutical Works*
Budapest, Hungary

ABSTRACT

The bonding of 11-aminoundecanoic (11-AA), 8-amino octanoic and 6-aminohexanoic acids as representatives of Zwitterionic ion-pairing agents, was determined on C_{18} column in the pH range 3-8. The break through curve of 11-AA has shown minimum in the vicinity of the isoelectric point. In the same pH range the chromatographic behaviour of fluoroquinolone gyrase inhibitor derivatives was studied in 0.002 M 11-AA containing methanol-phosphate buffer (1:1) eluent. The retention of the fluoroquinolones has shown a maximum close to the pH of the isoelectric points. As explanation the adsorption of the neutral form is suggested by the authors. The reverse chromatographic behaviour of 11-AA and the fluoroquinolone lomefloxacin harmonizes well with the results of pH dependent species distribution.

INTRODUCTION

The idea of Zwitterion-pair chromatography has been emerged in earlier works of Knox and Jurand (1,2), when they used 11-amino undecanoic acid (11-AA) as Zwitterionic pairing agent in a reversed phase HPLC (RP HPLC) system. The examined purine-, pyridine- and pyrimidine-nucleotides have shown

a characteristic, pH and ion-pairing agent concentration dependent behaviour. Beside of the usually occuring bipolar ion-pair products Knox and Jurand suggest the formation of "quadrupolar" ionpair species in a reaction between the amphoteric solute (S) and pairing agent (P)

$$S^{\pm} + P^{-}_{+} \rightleftarrows [S^{\pm}_{-}{}^{-}_{+}P].$$

Although, according to the mentioned authors the method ("Zwitterionic pairing HPLC) seemed to promise a great versatility and flexibility we found no data about the further application. Therefore, a more detailed study of binding and ion pairing properties of 11-AA in RP HPLC systems appeared reasonable. The investigation was extended to 8-amino octanoic- and 6-amino hexanoic acid. Eight fluoroquinolone gyrase inhibitor derivatives were applied as model substances in a RP HPLC system containing Zwitterionic pairing agent.

EXPERIMENTAL

Chromatography

The HPLC apparatus was comprised in an ISCO pump, Model 2350 (USA) combined with a Valco injector unit. An ISCO variable wavelength (230-800 nm) absorbance detector was used. In case of fluoroquinolone derivatives the effluent was monitored at 254 nm.

For plotting of the break through curves of the alkanecarboxylic acids a Waters Differential Refractometer, Model R 401 was employed. For the break through determination in case of lomefloxacin the UV detector was used. These equipment units subsequent to the pump were thermostatted at 25° ±0.1°. (Ultrathermostat MLW Type U2C, Freital, Germany). The chromatograms were recorded and the retention data were collected by a Hewlett-Packard integrator, Model 3396 ser.II. The sorbent Chromsil-6 C_{18} (6 μm particle size, Labor MIM Budapest) and LiChrosorb RP-18 (5 μm, Merck) were packed in stainless steel columns (250x4.6 mm I.D.). As mobile phase mixtures of methanol and aqueous phosphate buffer solutions (pH 3-8) containing 0.002 M of amino alkanecarboxylic acid were applied. Each retention data was calculated as an

average of three parallel runs. The column void time was signalled by the solvent peak of methanol. Following testing, the columns, were brought to their initial state by two hours elution with methanol-water (1:1) mixture and finally with methanol. Flow rate 1.0 ml/min.

Adsorption isotherms of the three alkanecarboxylic acids were measured by using of the break through method. The equipment used for the break through measurements (Fig. 1) is a modified version of the one was described in a previous work (3).

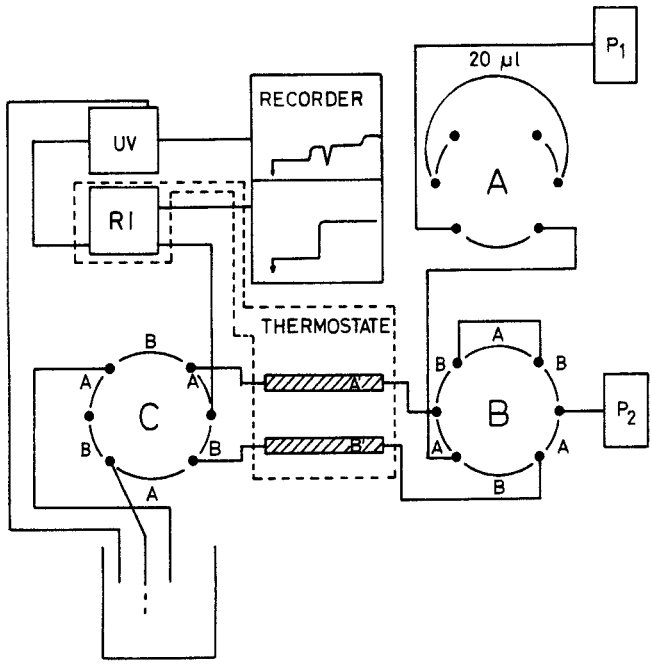
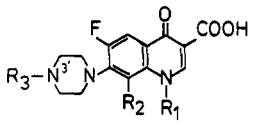


Fig. 1
Diagram of the equipment for plotting of the break through curves.

P_1 : pump (eluent delivery to the detector)
P_2 : pump (for washing)
A,B,C : injectors
A', B' : columns
UV : UV detector
RI : RI detector



	R_1	R_2	R_3
1. Norfloxacin	$-C_2H_5$	-H	-H
2. 8-F-Norfloxacin	$-C_2H_5$	-F	-H
3. Pefloxacin	$-C_2H_5$	-H	$-CH_3$
4. 8-F-Pefloxacin	$-C_2H_5$	-F	$-CH_3$
5. Lomefloxacin	$-C_2H_5$	-F	-H ($3'-CH_3$)
6. Ciprofloxacin	CH_2 / CH_2 >CH-	-H	-H
7. Amifloxacin	$-NHCH_3$	-H	$-CH_3$
8. Ofloxacin			

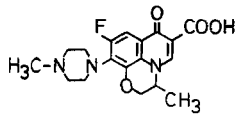


Fig. 2
Structures for the investigated fluoroquinolones

Materials

The fluoroquinolone model substances (**Fig. 2**) were synthetized at Chinoin Pharmaceutical Works (Budapest) and used without further purification.

11-amino undecanoic acid 99%, 8-amino octanoic acid, 6-amino hexanoic acid 98% (Aldrich).

Buffer solutions in the pH range 3-8 were prepared by mixing the proper volumes of 0.067 M aqueous solutions of potassium dihydrogenphosphate and disodium hydrogenphosphate (KH_2-PO_4, $Na_2HPO_4.2H_2O$, anal. grade, Reanal, Budapest). The pH of the solutions was tested by potentiometry with an accuracy ±0.02 unit.

Methanol, HPLC grade (Chemolab, Budapest).

RESULTS AND DISCUSSION

The binding of 11-amino undecanoic acid.

Figure 3 demonstrates the effect of temperature and sorbent quality on the binding of 11-amino undecanoic acid (11-AA) was calculated by the evaluation of the break through curves. Since the pH of the mobile phase must have a great influence on the measure of binding, a more detailed study seemed reasonable. **Figure 4** shows the pH dependence of 11-AA adsorption in the range 3-8. As it can be seen, the adsorption process may be described by a minimum curve. The amount of 11-AA was adsorbed from the eluent containing-30% of methanol, is comparable with the finding of Knox et al. (**1**) working with an eluent with 10% methanol content. As it is expected, the amount of adsorbed 11-AA strongly decreases with the increase of methanol content in the eluent. The pH of minimum binding coincides rather well with the isoelectric point of 11-AA : 7.65 (pK_{a1} : 10.74, pK_{a2} : 4.56)*. This experience indicates the highly polar character of 11-AA Zwitterion, since the C_{10}-chain allows no interaction between the Zwitterionic poles..

8-amino octanoic acid (8-AA) was adsorbed by the C_{18} column, in a very small extent even from a 30% methanol containing eluent:

pH	3	5	6	7	8
adsorbed 8-AA μM/g	2.2	1.1	0.8	0.4	0.5

6-amino hexanoic acid practically was not binded by the C_{18} sorbent.

The behaviour of fluoroquinolones.

Table 1 and 2 involve the retention data of the investigated fluoroquinolone derivatives in eluents with different pH, 50% and 30% methanol and 0.002 M 11-AA content.

---

* The macroscopic dissociation constants of 11/AA were determined by the potentiometric method was described previously (4).

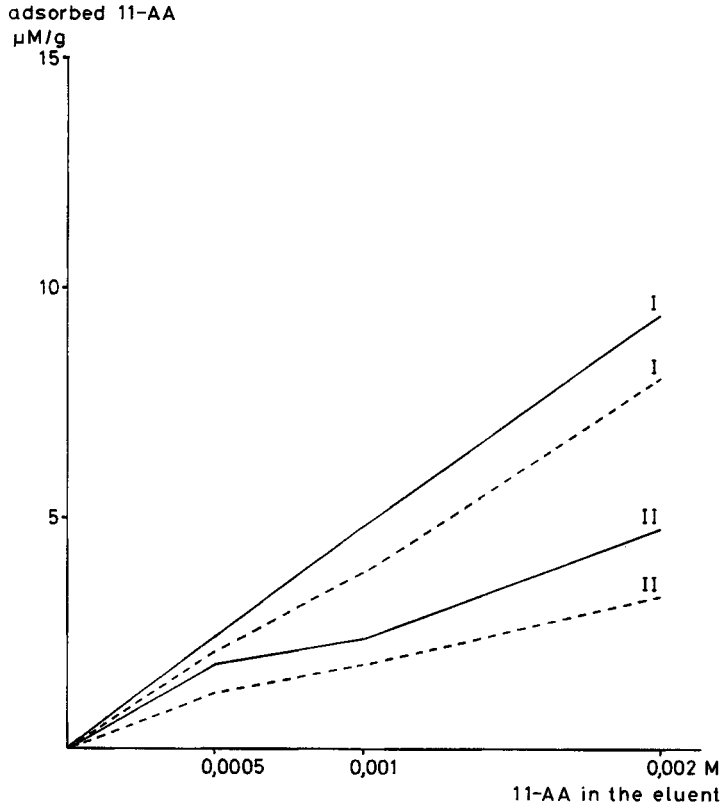


Fig. 3
The effect of temperature and sorbent quality on the adsorption of 11-AA.

Column: I. Chromsil C_{18} (6 μm), II. Lichrosorb RP-18 (5 μm)
Mobile phase: Methanol-phosphate buffer 50:50 (pH = 6)
——— 25 °C
--------- 40 °C

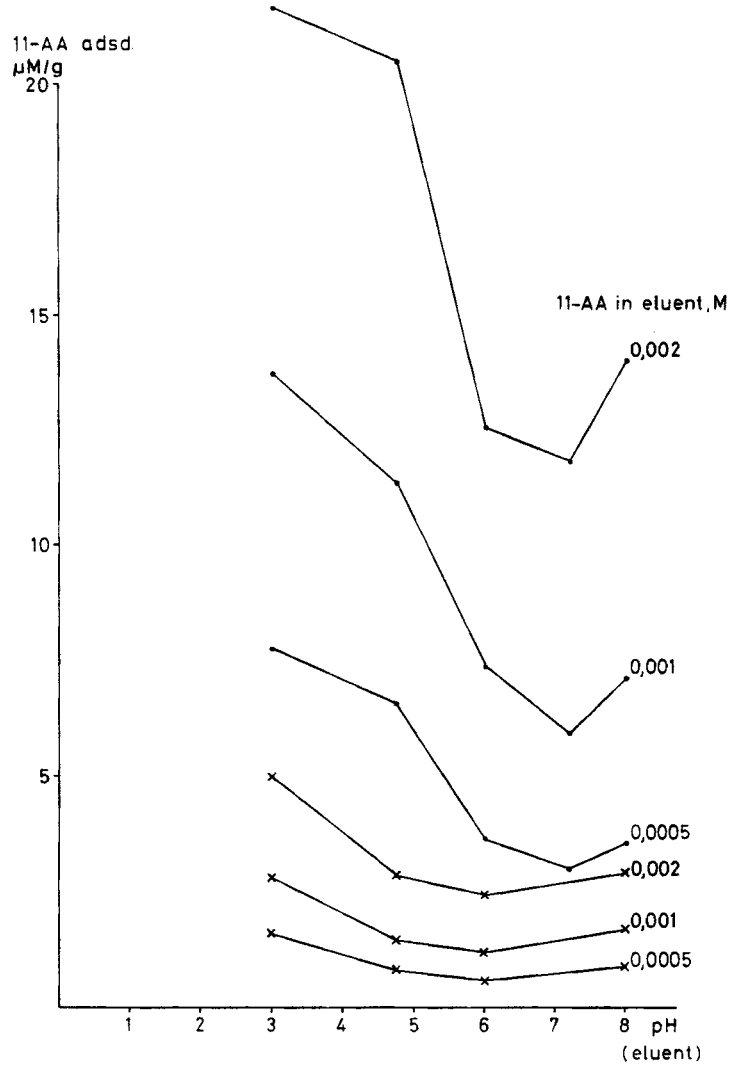


Fig. 4
The pH-influence on the binding of 11-AA.

Column: Chromsil-C^{18} (6 μm)
Mobile phase: methanol-phosphate buffer in ratio 50:50, or 30:70 v/v
• MeOH 30%
x MeOH 50%

Table 1.

pH dependence of the retention

COMPOUND	RETENTION DATA													
	pH*													
	3.0		5.0		5.5		6.0		6.5		7.0		7.5	
(i. e. p.)	k'_0	k'_{AA}	k'_0	k'_{AA}	k'_0	k'_{AA}	k'_0	k'_{AA}	k'_0	k'_{AA}	k'_0	k'_{AA}	k'_0	k'_{AA}
1. (7.37)	0.76	1.46	2.69	4.23	3.75	4.60	4.11	4.63	3.77	4.14	2.28	3.22	1.17	1.66
2. (7.44)	0.52	1.13	1.86	2.54	1.99	2.70	1.95	2.17	1.44	1.59	0.99	1.15	0.70	0.90
3. (6.91)	1.17	3.56	9.40	13.90	13.26	15.42	15.43	15.08	11.49	12.15	5.76	7.90	2.40	3.23
4. (6.73)	0.69	2.54	6.50	7.62	7.81	9.02	8.38	8.97	6.73	6.73	3.75	3.76	1.81	2.14
5. (7.14)	0.61	1.15	1.88	2.47	2.11	2.79	2.24	2.54	1.76	1.87	1.21	1.81	0.93	1.25
6. (7.50)	0.82	1.55	2.87	3.95	3.78	4.78	3.88	4.35	3.29	3.55	2.01	2.25	1.01	1.33
7. (6.50)	0.83	2.18	6.92	9.05	9.24	10.34	10.48	10.54	7.05	7.48	3.23	4.17	1.42	1.79
8. (7.00)	0.81	2.17	4.67	6.83	6.72	8.07	7.06	8.37	5.91	8.07	3.33	4.31	1.74	2.08

k'_0 mobile phase: methanol - phosphate buffer (0.067 M) 50 : 50
k'_{AA} mobile phase: methanol - phosphate buffer (0.067 M) 50 : 50 + 0.002 M 11-AA

* pH values measured in aqueous buffer solutions increased with approximately 1 pH unit after the mixing with methanol

Table 2.

pH dependence of the retention

COMPOUND	RETENTION DATA									
	pH*									
	3.0		5.0		6.0		7.0		8.0	
	k'_0	k'_{AA}	k'_0	k'_{AA}	k'_0	k'_{AA}	k'_0	k'_{AA}	k'_0	k'_{AA}
1.	8.97	3.20	13.58	7.69	15.26	12.30	7.40	9.10	4.69	4.18
2.	6.90	2.35	10.99	5.63	6.58	6.18	3.10	3.57	3.77	3.31
3.	14.42	5.02	34.18	26.85	∞		25.37	39.30	12.06	11.87
4.	11.04	4.80	24.15	15.99	∞		12.21	19.10	11.57	12.49
5.	8.45	3.097	12.79	7.34	7.70	8.23	4.50	5.33	5.34	5.02
6.	9.84	3.35	14.81	8.37	14.52	12.72	5.92	8.39	4.58	4.30
7.	9.67	3.41	23.88	16.69	∞		11.96	22.24	6.64	6.85
8.	11.71	3.84	19.57	15.09	26.00	25.20	14.69	19.43	8.28	8.59

k'_0 mobile phase: methanol - phosphate buffer (0.067 M) 30 : 70
k'_{AA} mobile phase: methanol - phosphate buffer (0.067 M) 30 : 70 + 0.002 M 11-AA

* for explanation see Table 1.

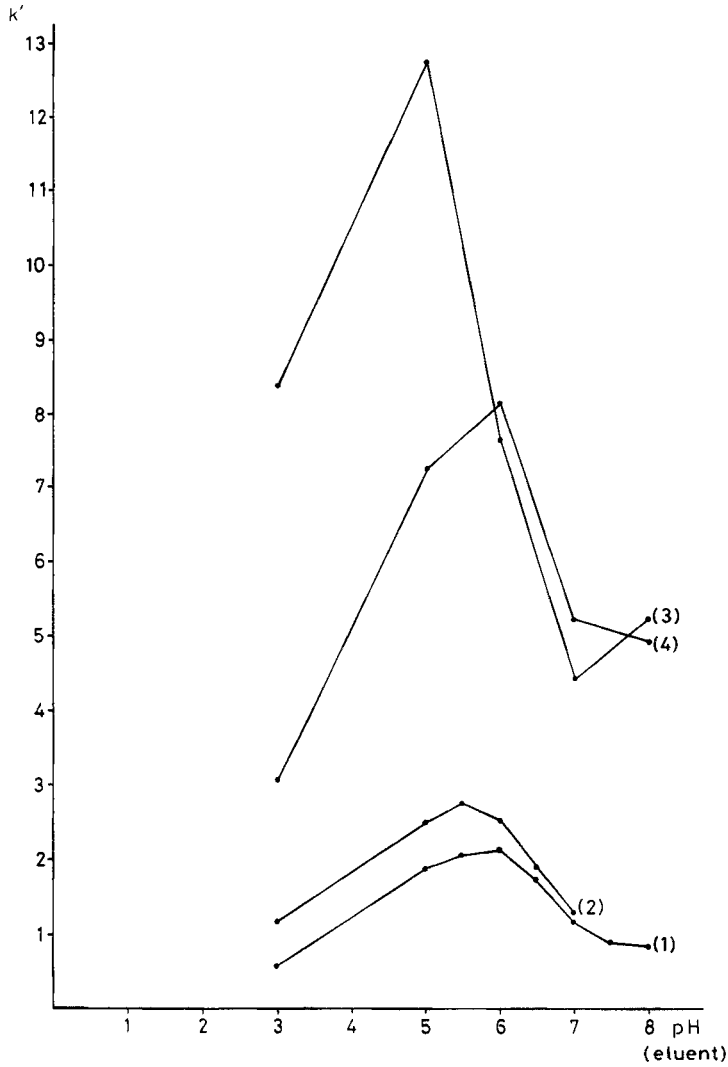


Fig. 5
The effect of 11-AA on the retention of lomefloxacin in eluent with 30% and 50% methanol content.

(1) Methanol 50%-phosphate buffer solution (0.067 M) 50%
(2) As (1), 0.002 M of 11-AA is added
(3) Methanol 30%-phosphate buffer solution (0.067 M) 70%
(4) As (3), 0.002 M of 11-AA is added
Column: Chromsil-6 C_{18}

Table 3

The effect of phosphate, hexansulfonic acid, cetrimide and 11-AA on the retention of floxacins

Compound	1	2	3	4	5	6	7
Norfloxacin	0.43	0.76	1.46	1.46	1.17	1.19	1.66
8-F-Norfloxacin	0.38	0.52	1.15	1.13	0.70	0.96	0.90
Pefloxacin	0.46	1.17	1.85	3.56	2.40	2.42	3.23
Ciprofloxacin	0.49	0.82	1.54	1.55	1.01	1.21	1.33

1. MeOH - Ph (0.025 M pH = 3) 50 : 50
2. MeOH - Ph (0.067 M pH = 3) 50 : 50
3. MeOH - Ph (0.067 M pH = 3) 50 : 50 + 0.01 M hexansulfonate
4. MeOH - Ph (0.067 M pH = 3) 50 : 50 + 0.002 M 11- AA
5. MeOH - Ph (0.067 M pH = 7.5) 50 : 50
6. MeOH - Ph (0.067 M pH = 7.5) 50 : 50 + 0.01 M cetrimide
7. MeOH - Ph (0.067 M pH = 7.5) 50 : 50 + 0.002 M 11-AA

(Ph: phosphate buffer solution)

As it is shown the retention reaches maximum at a pH value close to the isoelectric point of the compounds (the pH-shift by methanol is to be considered, see **ref 5**). **Figure 5** clearly illustrates the similarities and differences between the *retention curve-pairs gained in 11-AA containing and noncontaining eluents. The* similar shape of the curve-pairs indicates the ion pair forming effect of the phosphate ion itself. The quite different effect of 11-AA on the retention in 30% and 50% methanol containing systems may be explained by the different contribution of ion pair formation and substitution in the development of the final retention values. The dominancy of the substitution effect appears to result in great retention depression by 11-AA in the case of eluent with 30% methanol content.

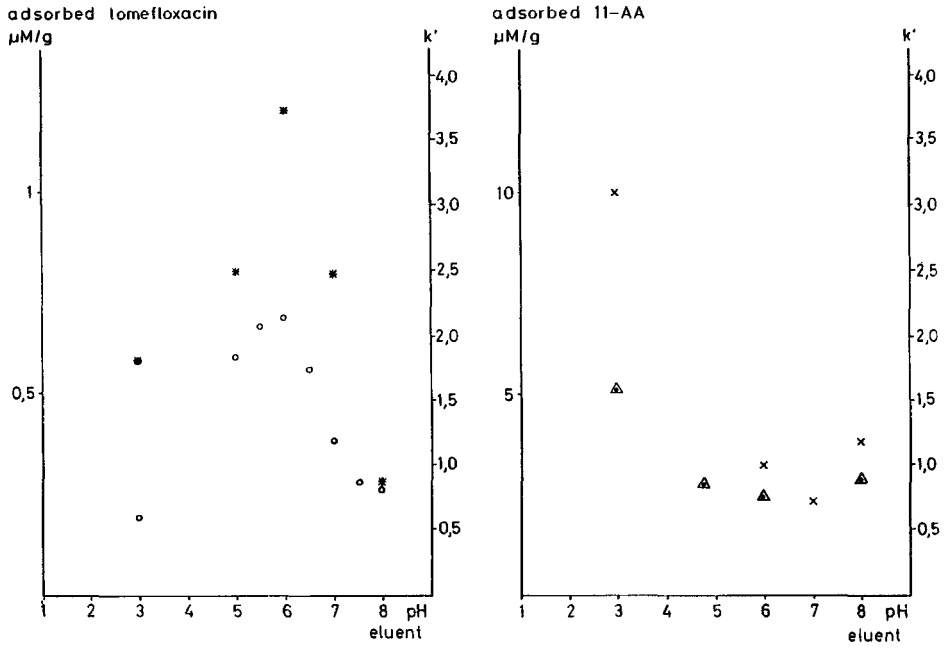


Fig. 6
Comparing of adsorption and retention curves of lomefloxacin and 11-AA

lomefloxacin: o Retention (k') (MeOH-Ph 50:50)
* Adsorption (MeOH-Ph 50:50 + 0,0005 M lomefloxacin)

11-AA: x Retention (k') (MeOH-Ph 50:50)
Δ Adsorption (MeOH-Ph 50:50 + 0.002 M 11-AA)

Ph: phosphate buffer solution (0.067 M), pH = 3-8,
Column: Chromsil C_{18} (6 μm)

Table 3 shows the effect of phosphate, hexanesulfonate, cetrimide and 11-AA on the retention of fluoroquinolone derivatives. A comparison of the respective data suggests that phosphate ion functions as an anionic ion pairing agent, while 11-AA, depending upon the pH of the medium can act as a dual (cationic or anionic) ion pairing reagent. To the formation of "quadrupolar ion pairs" (1) can not be concluded in case of the investigated fluoroquinolones.

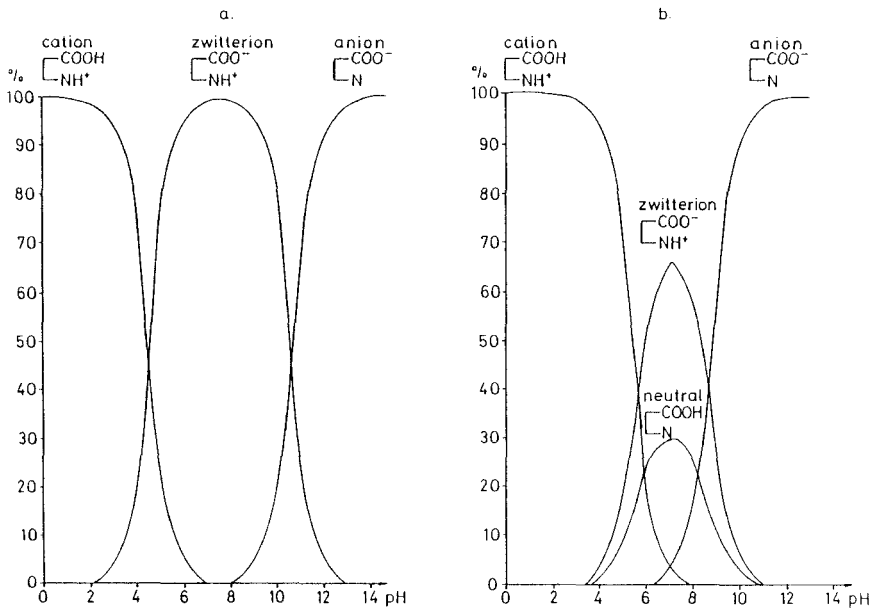


Fig. 7
The microspeciation diagram for 11-AA (a) and lomefloxacin (b)

The quite reversed (i.e. the minimum-curve and the maximum curve character of the adsorption and the retention curves of 11-AA and lomefloxacin) reflects the presence of the most polar and the most apolar species at the isoelectric point respectively (**Fig. 6**). This finding becomes understandable by **Fig. 7**. At the pH of the isoelectric point the 11-AA solution contains exclusively Zwitterionic species, while a significant amount of the neutral form is present in lomefloxacin solution. It appears, that in the retention of lomefloxacin the adsorption of the neutral species plays a dominant role. This assumption seems to confirm the conclusion was drawn by Takács-Novák et al. on the pH dependence of log P among fluoroquinolones (**4**).

ACKNOWLEDGEMENT

The authors are greatful to I. Kovács-Derzsi and E. Asztalos-Túróczy for the excellent technical assistance.

REFERENCES

1. Knox, J.H., Jurand, J.: J. Chromatogr. 203, 85 (1981)
2. ibid: 186, 763 (1979)
3. Lőre, A., Budvári-Bárány, Zs., Szász, Gy., Shalaby, A.: J. Liq. Chrom. 14, 2065 (1991)
4. Takács-Novák, K., Noszál, B., Hermecz, I., Keresztúri, G., Podányi, B., Szász, Gy.: J. Pharm. Sci. 79, 1023 (1990)
5. Budvári-Bárány, Zs., Szász, Gy., Takács-Novák, K., Hermecz, I., Lőre, A.: J. Liq. Chrom. 14, 3411 (1991)

Received: December 3, 1993
Accepted: December 9, 1993