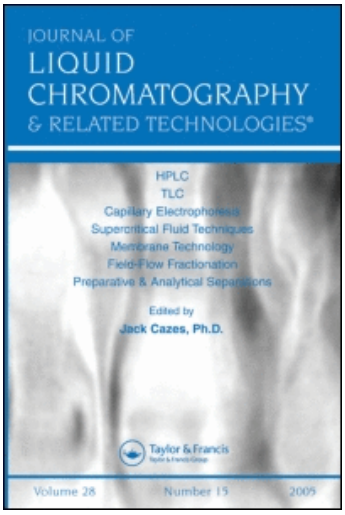


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Reversed-Phase Ion-Pair Chromatography of Nitrogen-Bridged Compounds

A. Shalaby[a]; Zs. Budvári-Bárány[a]; Gy. Szász[a]
[a] Institute of Pharmaceutical Chemistry Semmelweis Medical University, Budapest, Hungary

To cite this Article Shalaby, A. , Budvári-Bárány, Zs. and Szász, Gy.(1985) 'Reversed-Phase Ion-Pair Chromatography of Nitrogen-Bridged Compounds', Journal of Liquid Chromatography & Related Technologies, 8: 6, 1071 — 1091
To link to this Article: DOI: 10.1080/01483918508067129
URL: http://dx.doi.org/10.1080/01483918508067129

JOURNAL OF LIQUID CHROMATOGRAPHY, 8(6), 1071-1091 (1985)

REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF NITROGEN-BRIDGED COMPOUNDS

A. Shalaby, Zs. Budvári-Bárány, Gy. Szász
Institute of Pharmaceutical Chemistry
Semmelweis Medical University
Budapest, Hungary

ABSTRACT

The retention times and resolution factors of certain types of pharmacologically active nitrogen--bridged compounds have been investigated using reversed--phase operations of high-performance ion-pair chromatography. Factors studied included the effect of the pH of the mobile phase, the influence of the nature and concentrations of the mobile phase components, and the effects of various counter ions. Numerous examples of separations are presented.

INTRODUCTION

Reversed-phase ion-pair chromatography (RPIPC) is based on a liquid-liquid partition technique usually called ion-pair chromatography or ion-pair partition;

* Presented in Eastern Analytical Symposium 1984 New York, U.S.A.

 0148-3919/85/0806-1071$3.50/0

its use in classical LC and liquid-liquid extraction is considerably older. Ion-pair chromatography was rapidly accepted as a new HPLC method due to its unique advantages wide-ranging applicability, high selectivity, etc. Although ion-pair chromatography can be carried out in either normal or reversed phase, RPIPC is the more popular.

In this work the increase in selectivity for N-bridged compounds in the presence of an ion-pair forming agent will be shown. As a result of ion-pair formation, some pairs of very closely related compounds can be separated.

EXPERIMENTAL

Materials

All the model substances were synthetized in our laboratory /1,2/. Their identification and quality control were performed via melting point determination and chromatography.

All chemicals and solvent were of analytical grade (Merck), and were used without further purification.

Chromatography

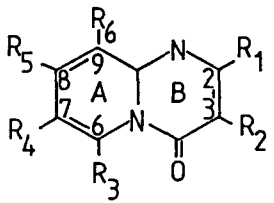
The HPLC apparatus was a LIQUOCHROM Model 2010 (Labor Mim, Budapest, Hungary). A variable wavelength detector was used, and the column effluent was monitored between 270 and 330 nm. The reversed-phase (Ultrasphere IP) C_{18} column measured 250 x 4.6 mm, and was prepacked with material with a particle size of 5 /um (Beckman). 20 /ul of

sample solution (0.1 mg/ml in methanol) was injected. Eluents of roughly equal elution strengths (methanol-water 70:30, acetonitrile-water 50:50 and terahydrofuran-water 40:60) were applied, with and without different concentrations of camphor sulphonic acid (CSA) and sodium lauryl sulphate (NaLS). The flow rate was 0.7 ml/min. All experiments were run at 25 °C.

RESULTS AND DISCUSSION

For about 40 compounds for structures, see Tables 1-4, two types of ion-pair forming agent (CSA and NaLS) were used at fix pH; different concentrations of each of the counter ions were applied. Figures 1 and 2 show that the optimum concentration was 0.005 M for both ion-pair forming agents. This optimum concentration was applied at different pH's. As expected, the ion-pairing was higher at low pH. Figures 3 and 4 show that (in the range pH 3-7) pH 3 is most suitable for the ion-pairing process as concerns the magnitude of the R_s values and the selectivity Table 5 lists the capacity factors (k^-), plate numbers (N) and column efficiencies (H) of the tested compounds, with and without the use of the counter ion, under the same conditions. The results (N and H) prove that NaLS is more suitable than CSA for these tested compounds. The same conclusion may be drawn following calculation of resolution factors (R_s) and selectivities (α) for some pairs of the tested compounds, with methanol

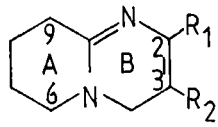
Table 1
STRUCTURE OF MODEL SUBSTANCE



Number of compd.	C_2	C_3	C_6	C_7	C_8	C_9
1	H	H	H	H	H	H
2	CH_3	H	H	H	H	H
3	H	CH_3	H	H	H	H
4	H	H	CH_3	H	H	H
5	H	H	H	CH_3	H	H

6	H	H	H	H	CH_3	H
7	H	H	H	H	H	CH_3
8	CH_3	CH_3	H	H	H	H
9	CH_3	H	CH_3	H	H	H
10	CH_3	H	H	H	H	CH_3
11	H	CH_3	CH_3	H	H	H
12	CH_3	H	C_2H_5	H	H	H
13	C_2H_5	H	CH_3	H	H	H
14	H	C_2H_5	CH_3	H	H	H
15	CH_3	C_2H_5	CH_3	H	H	H
16	CH_3	C_2H_5	CH_3	H	CH_3	H
17	CH_3	C_3H_7	H	H	H	H
18	CH_3	C_3H_7	CH_3	H	H	H
19	C_2H_5	CH_3	CH_3	H	H	H
20	C_3H_7	C_2H_5	CH_3	H	H	H

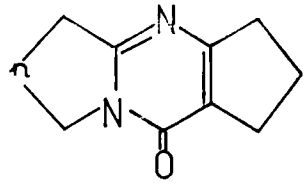
Table. 2.
STRUCTURE OF MODEL SUBSTANCES



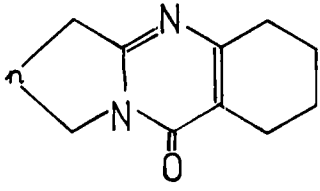
Number of compd.	C_2	C_3	C_6	C_9
21	H	H	H	H
22	CH_3	H	H	H
23	H	CH_3	H	H
24	H	H	CH_3	H
25	CH_3	CH_3	H	H
26	CH_3	H	CH_3	H
27	CH_3	H	H	CH_3
28	H	CH_3	CH_3	H
29	CH_3	C_2H_5	CH_3	H

Table 3
Structure of model substances

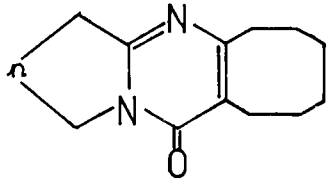
Number of compound	
30.	n = 1
31.	n = 2
32.	n = 3
33.	n = 4
31.a	C_6 Me
31.b	C_7 Me
31.c	C_8 Me
31.d	C_9 Me



34.	n = 1
35.	n = 2
36.	n = 3
37.	n = 4
34.a	C_6 Me
35.a	C_6 Me
35.b	C_7 Me
35.c	C_8 Me
35.d	C_9 Me



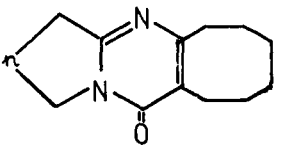
38.	n = 1
39.	n = 2
40.	n = 3
41.	n = 4



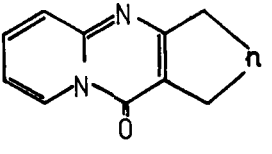
(continued)

Table 3 (continued)

Number of compound	
42.	n = 1
43.	n = 2
44.	n = 3
45.	n = 4



46.	n = 1
47.	n = 2
48.	n = 3
49.	n = 4
46.a	C_6 Me
46.b	C_7 Me
46.c	C_8 Me
46.d	C_9 Me
47.a	C_6 Me
47.b	C_7 Me
47.c	C_8 Me
47.d	C_9 Me



50.	n = 1
51.	n = 2
52.	n = 3
53.	n = 4
50.a	C_6 Me
50.b	C_{12}Me
52.a	C_{12}Me

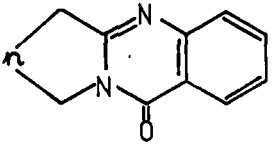
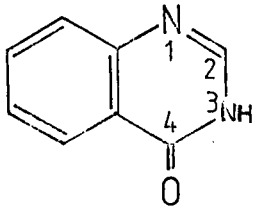


Table . 4
Structure of model substances



number of compd.	C_2	N_3
54.	H	H
55.	CH_3	H
56.	H	CH_3
57.	CH_3	CH_3
58.	CH_3	C_2H_5
59.	CH_3	C_3H_7
60.	CH_3	C_4H_9
61.	C_2H_5	CH_3
62.	C_2H_5	C_2H_5
63.	C_2H_5	C_3H_7
64.	C_2H_5	C_4H_9

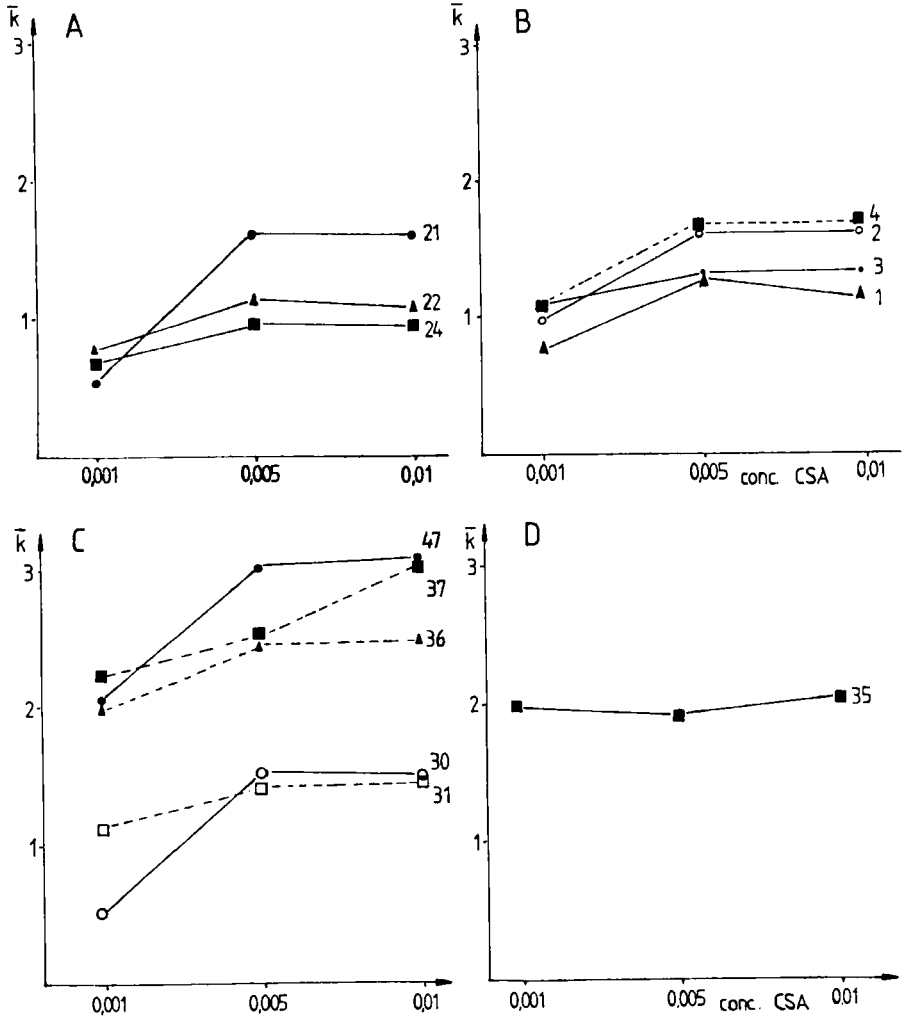


FIGURE 1. Relationship between k' and CSA Concentration (pH = 3)

A = Two Ring System with Saturated A Ring

B = Two Ring System with Unsaturated A Ring

C = Three Ring System with Saturated A Ring

D = Three Ring System with Unsaturated A Ring

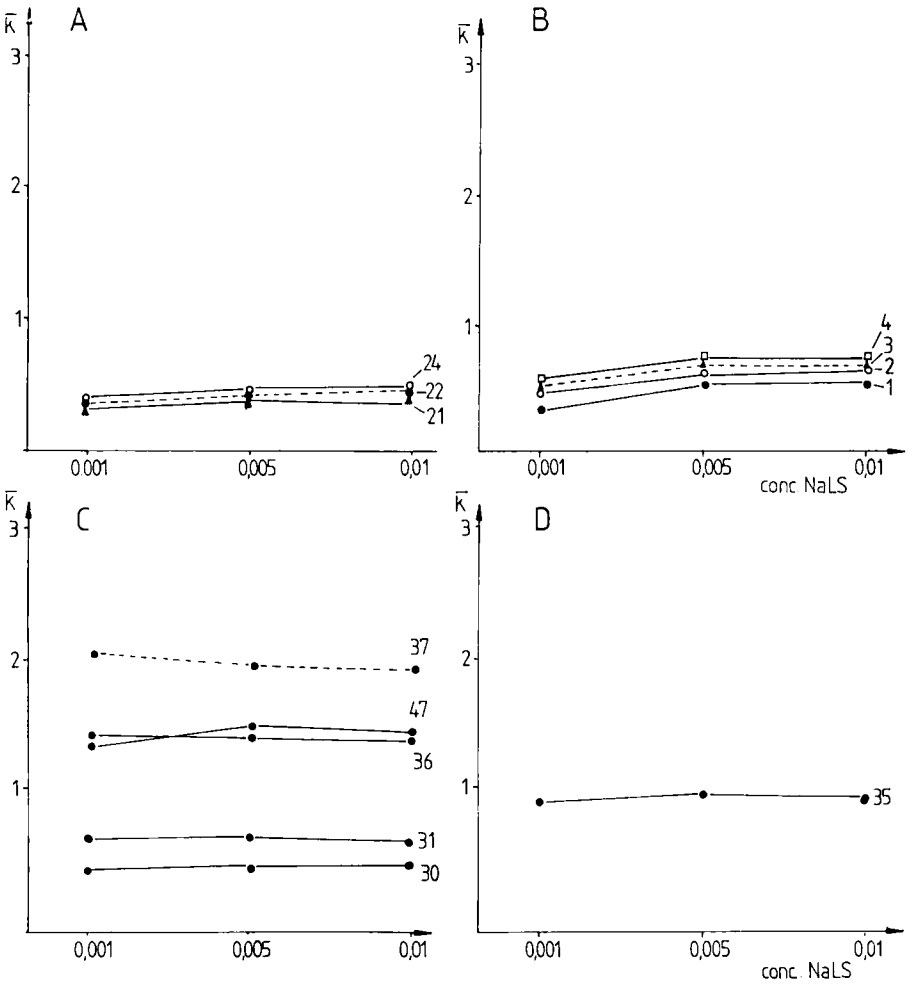


FIGURE 2. Relationship between k' and NaLS Concentration (pH = 6)

A = Two Ring System with Saturated A Ring

B = Two Ring System with Unsaturated A Ring

C = Three Ring System with Saturated A Ring

D = Three Ring System with Unsaturated A Ring

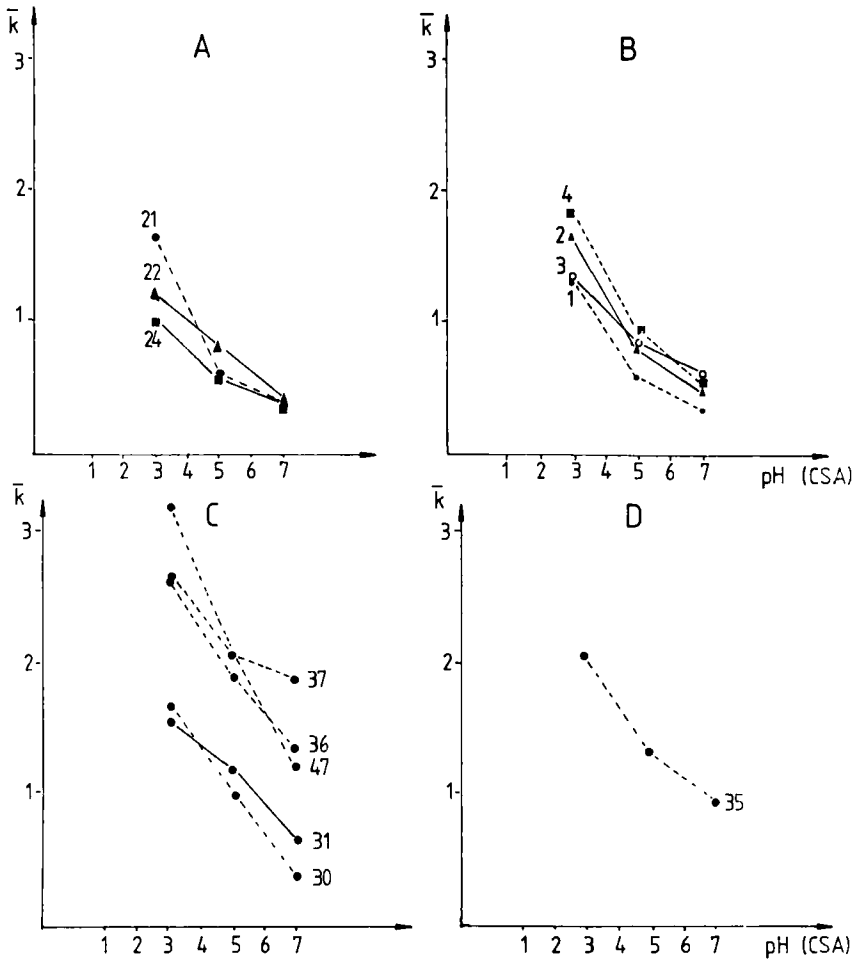


FIGURE 3. Relationship between k' and pH (wiht CSA)

A = Two Ring System with Saturated A Ring

B = Two Ring System with Unsaturated A Ring

C = Three Ring System with Saturated A Ring

D = Three Ring System with Unsaturated A Ring

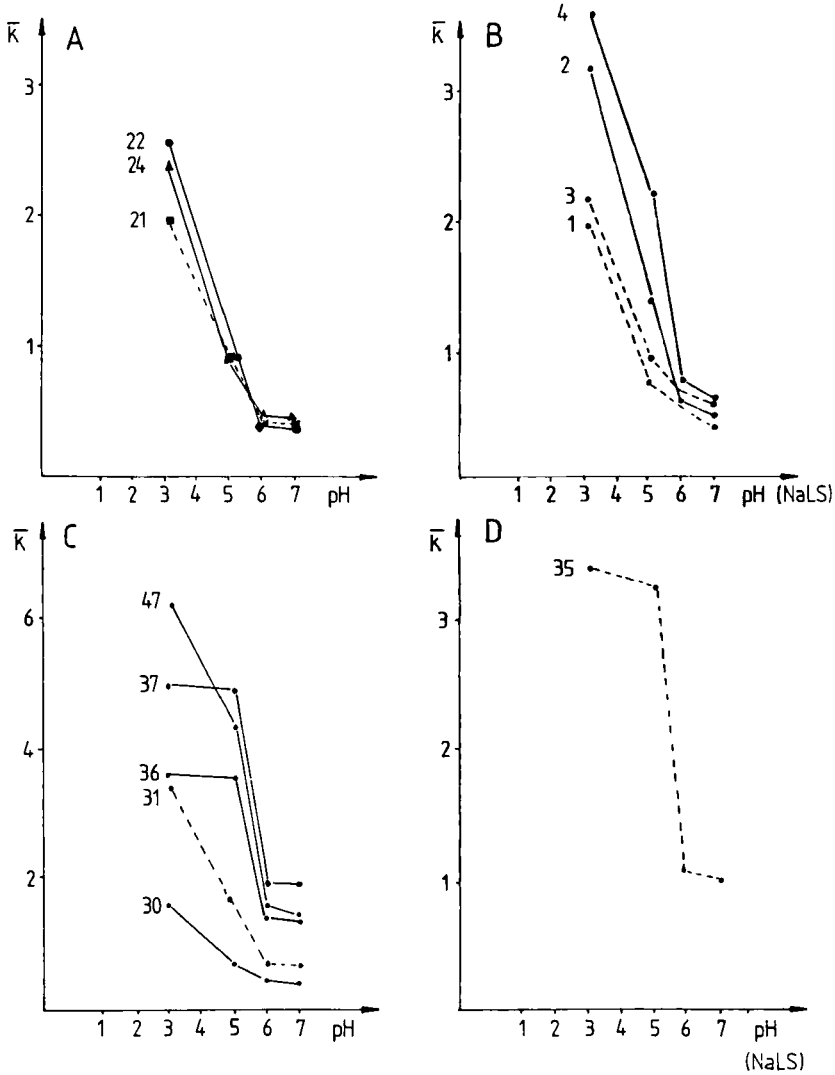


FIGURE 4. Relationship between k' and pH (with NaLS)

A = Two Ring System with Saturated A Ring

B = Two Ring System with Unsaturated A Ring

C = Three Ring System with Saturated A Ring

D = Three Ring System with Unsaturated A Ring

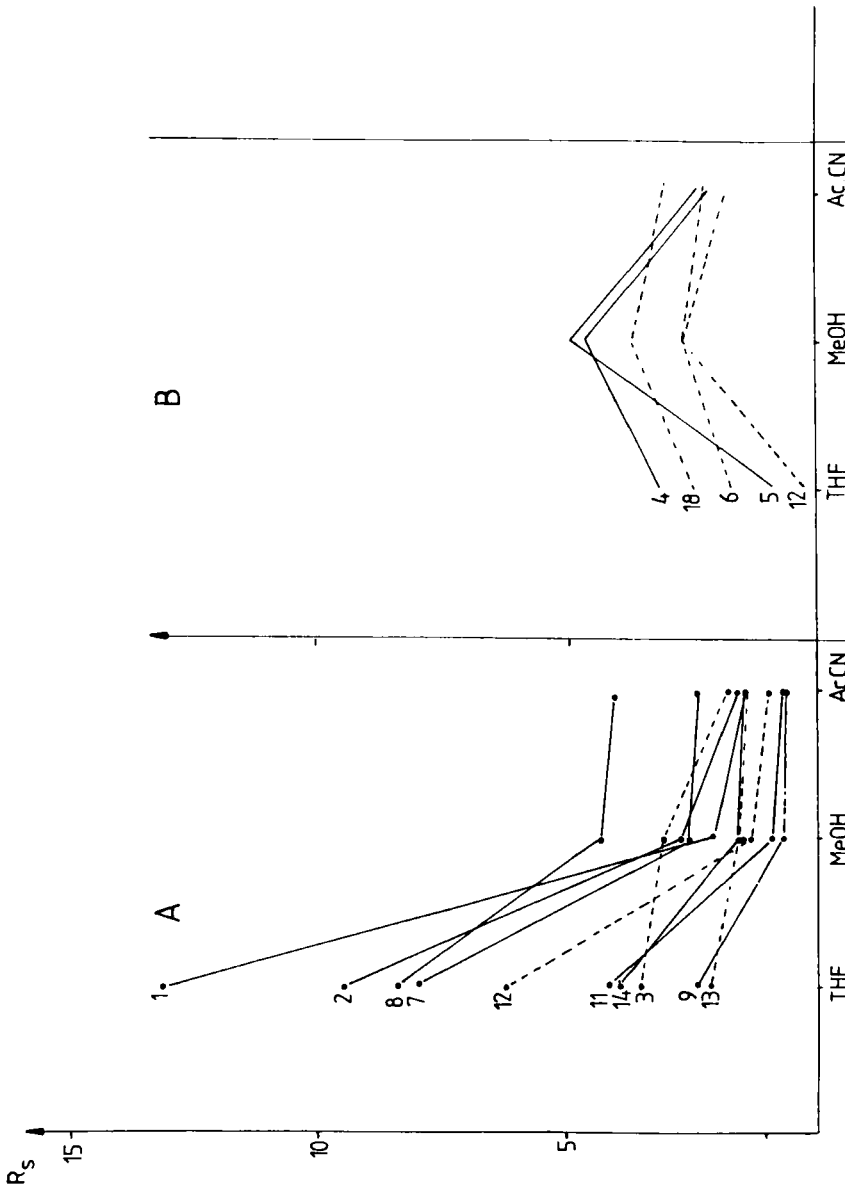


FIGURE 5. Resolution Factors in Different Eluents for Some Pairs of Compounds
A - Optimal Eluent: THF B - Optimal Eluent: Methanol

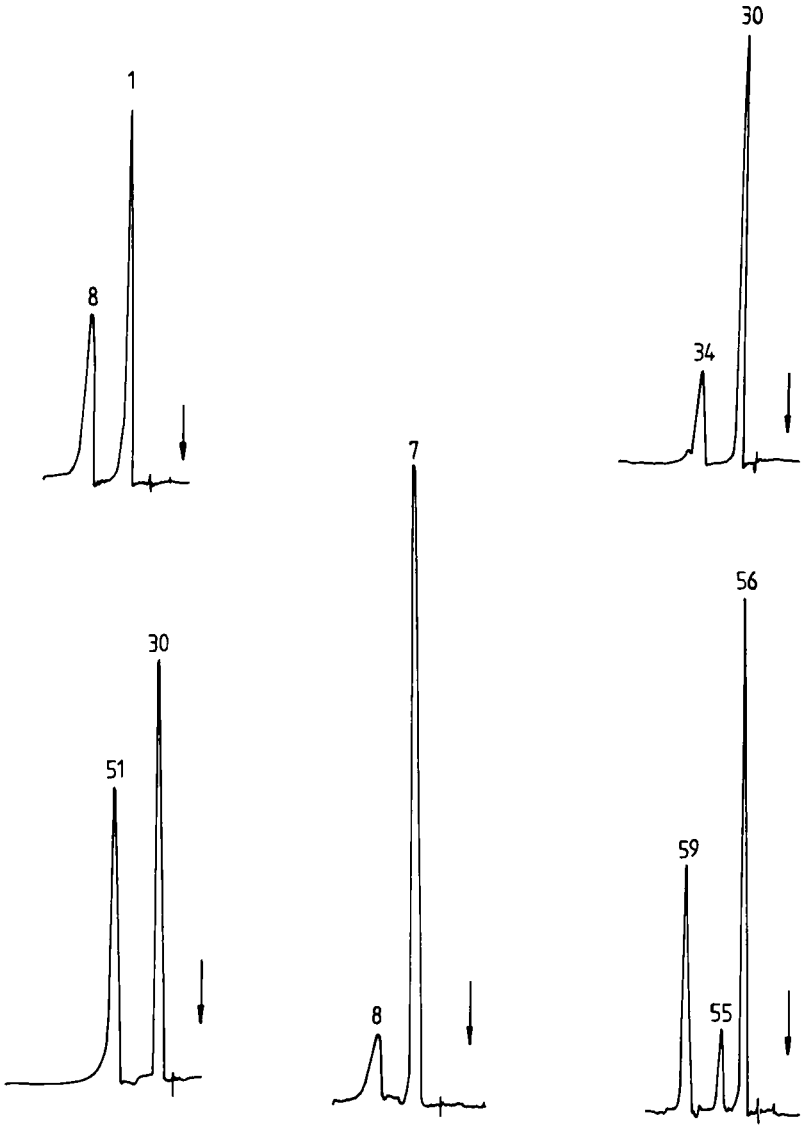


FIGURE 6. Separation of Some Pairs of Compounds by RPIPC
Stationary Phase: (Ultrasphere 1 P) 5 µm C_{18} Column
Mobile Phase: Methanol-Water 70:30 + 0,00r M NaLS
Flow Rate: 0,7 ml/min

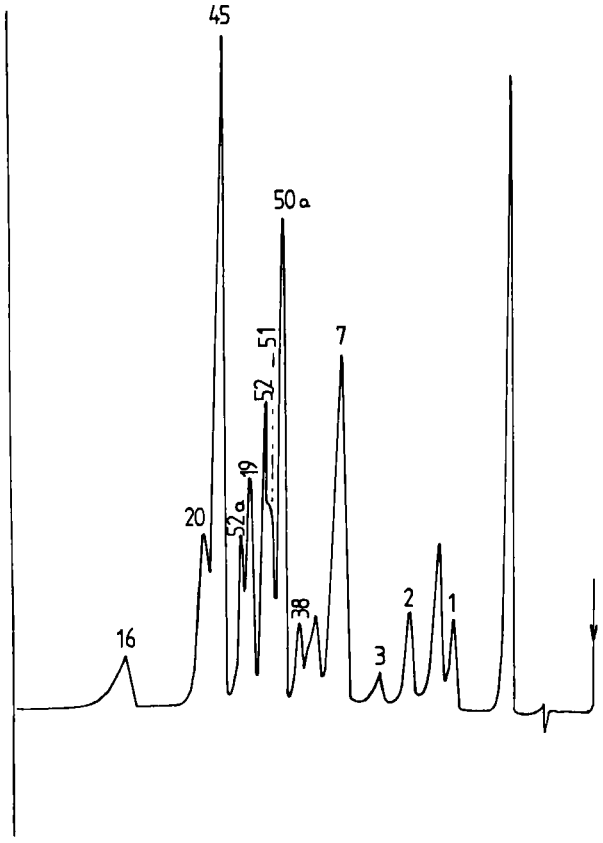


FIGURE 7. The Separation of the Mixture of Some N-Bridged Compounds by Gradient Linear Elution Technique

Stationary Phase: Nucleosil 5 SA/nm

Mobile Phase: a- 0,1 Mol KH_2PO_4 in Methanol-Water 40:60

b- 0,01 Mol KH_2PO_4 in Methanol-Water 40:60

Flow Rate: 1 ml/min

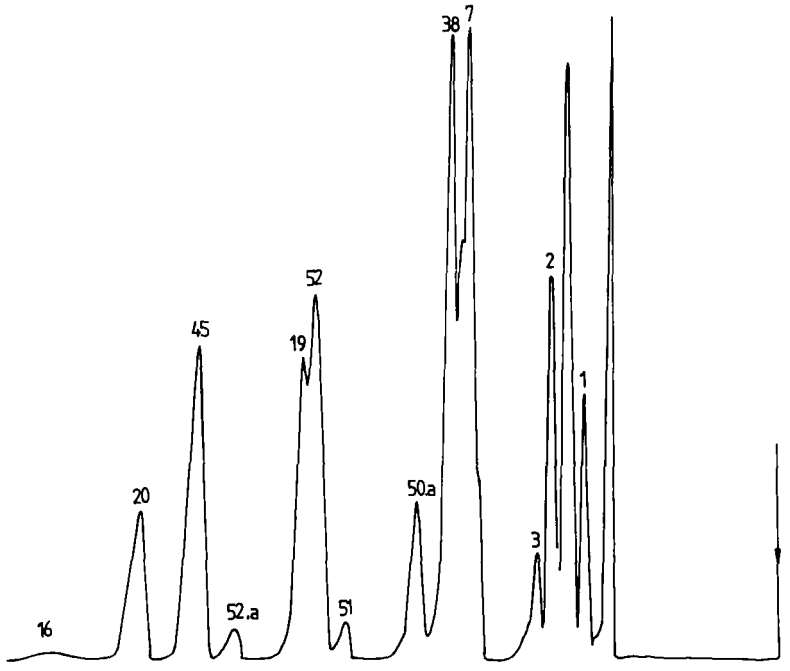


FIGURE 8. Separation of the Mixture of Some N-Bridged Compounds by RPIPC
Stationary Phase: (Ultrasphere 1P), 5 µm, C_{18} Column
Mobile Phase: THF-Water (40:60) + 0,005 M Na.L.S.
Flow Rate: 0,5 ml/min

as mobile phase at the same pH (Table 6). The resolution factors (R_s) for some pairs were larger when THF was used (Fig. 5A), while for other pairs they were larger for methanol (Fig. 5B), for each pair the lowest R_s was found with MeCN. Some chromatograms with methanol as mobile phase are presented in Fig. 6.

Figures 7 and 8 demonstrate that the efficiencies of separation in gradient elution /3/ and in RPIPC are very similar for a given mixture of compounds.

Table 5

Chromatographic data of tested compounds

Number of compound	CH_3OH-H_2O 70:30	CH_3OH-H_2O 70:30 + 0.005 M CSA			CH_3OH-H_2O 70:30 + 0.005 M NaLS		
	$\overline{K}$	$\overline{K}$	N	H	$\overline{K}$	N	H
1	0.318	1.333	1157	0.216	2.0	3600	0.069
2	0.454	1.666	1130	0.221	3.2	4096	0.061
3	0.591	1.333	1196	0.209	2.2	4096	0.061
4	0.5	1.7333	1186	0.211	3.6	4761	0.053
5	0.5	1.266	1128	0.222	2.8	3249	0.077
6	0.456	1.066	1154	0.217	2.13	3927	0.064
7	0.591	1.2	1355	0.184	1.8	3136	0.079
8	0.727	2.2	1304	0.192	4.4	4199	0.059
9	0.682	2.2	1369	0.1826	3.133	3844	0.065
10	1.000	1.666	1300	0.192	4.8	4844	0.052
21	0.272	1.666	1178	0.212	2.0	3600	0.069
22	0.318	1.20	1121	0.223	2.6	5184	0.048

24	0.363	1.000	1119	0.223	2.4	4624	0.054
25	0.5	1.000	1144	0.219	4.6	4515	0.055
26	0.409	1.2	1144	0.219	3.133	3844	0.065
27	0.636	1.4	1576	0.158	3.6	4761	0.053
30	0.363	1.666	1400	0.179	1.6	3893	0.064
31	0.591	1.533	1191	0.209	3.4	2787	0.089
34	0.636	1.86	1295	0.193	2.8	2152	0.116
35	0.909	2.066	1120	0.223	3.4	1089	0.229
35a	1.258	2.266	1187	0.211	5.8	2601	0.096
35b	1.3548	3.2	1282	0.195	6.133	2862	0.087
35c	1.1934	1.8	1970	0.127	5.4	3072	0.081
36	1.272	2.63	1276	0.196	3.6	2177	0.115
37	1.68	2.666	1400	0.179	5.0	2025	0.123
39	1.290	2.00	1810	0.138	6.06	2119	0.118
47	1.13636	3.2	1159	0.216	6.266	2346	0.1066
47a	1.6774	1.8	1870	0.134	7.133	2940	0.085
47b	1.5483	3.3	1202	0.208	4.2	3893	0.064
47c	1.4516	2.066	1117	0.224	3.533	2959	0.084
51	1.00	2.266	1384	0.181	4.00	2500	0.1
54	0.4	0.8666	1348	0.185	1.2	1936	0.129
55	0.56	1.0666	1427	0.175	2.4	4624	0.540
56	0.58	1.000	1576	0.159	1.2	2787	0.089
59	1.34	2.333	1205	0.207	4.6	4515	0.055

Table 6

The resolution factor and selectivity factor of some pairs of tested compounds at different mobile phase

Number of pairs	CH_3OH-H_2O 70:30		CH_3OH-H_2O 70:30 + 0.005 M CSA		CH_3OH-H_2O 70:30 + 0.005 M NaLS		AcCN-H_2O 50:50 + 0.005 M NaLS		THF-H_2O 40:60 + 0.005 M NaLS	
	R_s	α	R_s	α	R_s	α	R_s	α	R_s	α
1(4+24)	0.528	0.726	0.9	0.577	2.57	0.666	1.5	0.708	9.4	0.3410
2(10+27)	1.35	0.636	0.5	0.840	2.0	0.75	1.33	0.77	13.2	0.2644
3(5+25)	0.0	0.0	0.4	0.789	3.0	0.608	1.6	0.66	3.5	0.564
4(1+8)	0.667	0.4374	1.35	0.605	4.5	0.454	2.0	0.56	3.08	0.7706
5(7+8)	0.511	0.8129	2.1	0.545	4.87	0.409	2.25	0.52	0.63	0.9446
6(1+2)	0.42	0.700	0.55	0.800	2.57	0.625	1.71	0.8	1.58	0.840
7(56+59)	2.4	0.432	2.0	0.428	6.8	0.261	2.4	0.409	-	-
8(55+56)	0.132	0.962	0.14	0.937	3.27	0.5	1.125	0.75	_	_
9(35+47)	0.695	0.799	4.0	0.646	2.529	0.543	2.307	0.6	7.98	0.395
10(30+51)	2.111	0.363	2.37	0.735	4.23	0.4	3.93	0.4	8.365	0.3827
11(35+51)	0.333	0.909	0.333	0.911	0.64	0.85	0.625	0.857	2.08	0.808
12(30+31)	0.723	0.6142	1.333	0.92	3.6	0.471	3.00	0.466	2.29	0.657
13(34+35)	0.25	0.699	0.3	0.90	0.6	0.823	0.818	0.80	4.14	0.491
14(30+34)	0.9	0.571	1.3	0.893	2.53	0.571	2.14	0.58	0.164	0.973
15(36+37)	1.05	0.757	0.05	0.986	1.425	0.72	1.25	0.75	1.714	0.857
16(35+37)	2.2	0.541	0.818	0.775	1.5	0.68	1.25	0.75	3.91	0.678
17(34+36)	1.75	0.500	0.15	0.709	1.2	0.777	0.818	0.80	6.28	0.389

REFERENCES

1. Mészáros Z. et al.: Arzneim. Forsch. 22. 815 (1972)
2. Kökösi J. et al.: Heterocyclic. Chem. 19. 909 (1982)
3. Shalaby A. et al: J. of Liq. Chromatogr. 7. 1151 (1984)