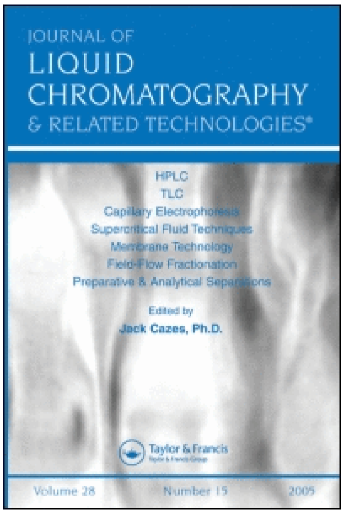

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HPLC OF NITROGEN-BRIDGED COMPOUNDS

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ABSTRACT

In the area of structure-activity relationships of nitrogen bridged compounds, certain structure-coherent physical properties with ion exhange HPLC behaviour has been studied. This paper illustrates the results in finding the best conditions to separate the various structural types of model compounds. For this purpose, ion exhange HPLC technique has proved as highly advantageous.

INTRODUCTION

In our previous work[1], we reported the results we achieved by the same chromatographic technique C_{18} reversed phase HPLC for the same model compounds[2], pyridopyrimidines with unsaturated and saturated A-ring Table 1, Table 2 and three ring systems with different ring size Table 3 .

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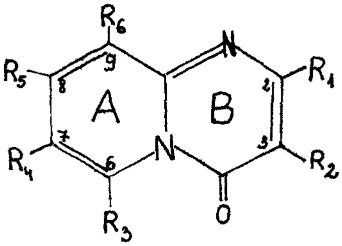
Table 1.

Structure of model substances

pyridopyrimidines with unsaturated "A"-ring

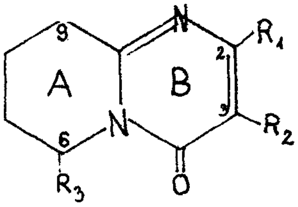


Nr	Substituents on C_2	C_3	C_6	C_7	C_8	C_9
1	H	H	H	H	H	H
2	CH_3	H	H	H	H	H
3	H	CH_3	H	H	H	H
4	H	H	CH_3	H	H	H
5	H	H	H	CH_3	H	H
6	H	H	H	H	CH_3	H
7	H	H	H	H	H	CH_3
8	CH_3	CH_3	H	H	H	H
9	CH_3	H	CH_3	H	H	H
10	CH_3	H	H	H	H	CH_3
11	H	CH_3	CH_3	H	H	H
12	CH_3	H	C_2H_5	H	H	H
13	H	C_2H_5	CH_3	H	H	H
14	CH_3	C_2H_5	CH_3	H	H	H
15	CH_3	C_2H_5	CH_3	H	CH_3	H
16	C_2H_5	CH_3	CH_3	H	H	H
17	C_3H_7	C_2H_5	CH_3	H	H	H

Table 2

Structure of model substances

pyridopyrimidines with saturated "A"-ring

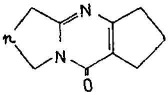


Nr	Substituents on C_2	C_3	C_6	C_9
18	H	H	H	H
19	CH_3	H	H	H
20	H	H	CH_3	H
21	CH_3	CH_3	H	H
22	CH_3	H	CH_3	H
23	H	CH_3	CH_3	H
24	CH_3	C_2H_5	CH_3	H
25	CH_3	H	H	CH_3

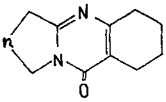
Table 3. Structure of model substances

Three ring systems with different ring size

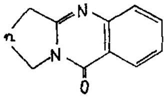
Number of compounds	
26.	n = 1
27.	n = 2
28.	n = 3
29.	n = 4



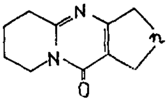
Number of compounds	
30.	n = 1
31.	n = 2
32.	n = 3
33.	n = 4



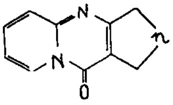
Number of compounds	
34.	n = 1
35.	n = 2
36.	n = 3
37.	n = 4
34.a	C_8 Me
34.b	C_3 Me
36.a	C_3 Me



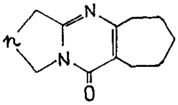
Number of compounds	
50.	n = 1
51.=31.	n = 2
52.	n = 3
53.	n = 4



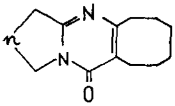
Number of compounds	
54.	n = 1
55.	n = 2
56.	n = 3
57.	n = 4



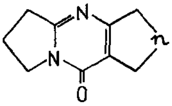
Number of compounds	
38.	n = 1
39.	n = 2
40.	n = 3
41.	n = 4



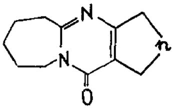
Number of compounds	
42.	n = 1
43.	n = 2
44.	n = 3
45.	n = 4



Number of compounds	
46.=26.	n = 1
47.	n = 2
48.	n = 3
49.	n = 4
47.a	C_8 Me



Number of compounds	
58	n = 1
59.	n = 2
60.	n = 3
61.	n = 4



Number of compounds	
62.	n = 1
63.	n = 2
64.	n = 3
65.	n = 4

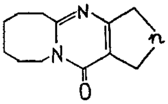


Table 4

The capacity factor and resolution factor of the HPLC system for some pairs

The pair of compounds	K						R_s					
	40%			6,7 pH			40 %			6,7 pH		
	4.7	5.5	6.7	30 %	40 %	50 %	4,7	5,5	6,7	30 %	40 %	50 %
1 18	1.421 1.578	1.421 0.895	1.105 1.105	1.631 1.526	1.105 1.105	0.421 0.421	2,5	2,4	0.0	0.19	0.0	0.0
2 4	2.473 3.947	1.210 1.684	1.473 1.842	2.263 2.842	1.473 1.842	0.578 0.526	1.8	1.8	1.8	0.69	1.8	0.27
4 9	3.947 6.736	1.684 2.368	1.842 2.474	2.842 3.947	1.842 2.474	0.526 0.578	2.7	2.2	2.2	1.08	2.2	0.25
9 12	6.736 8.368	2.368 2.895	2.474 3.368	3.947 5.821	2.474 3.368	0.578 0.684	1.59	1.4	2.5	1.03	2.5	0.5
18 19	1.578 1.842	0.895 1.033	1.105 1.210	1.526 1.105	1.105 1.210	0.421 0.421	0.46	0.7	0.44	0.95	0.44	0.0
20 22	1.421 2.263	0.789 1.053	1.00 1.368	1.589 2.263	1.00 1.368	0.368 0.368	1.7	1.1	1.5	1.5	1.5	0.0
26 27	1.736 3.315	1.210 1.474	1.631 2.474	2.526 4.157	1.631 2.474	0.578 0.593	2.02	0.9	2.6	1.9	2.6	0.5
30 31	3.105 8.578	1.947 3.263	2.526 3.789	4.316 6.578	2.526 3.789	0.736 0.842	5.2	3.3	2.6	2.3	2.5	0.5
34 35	2.789 6.00	1.263 2.842	2.47 3.94	4.894 7.315	2.474 3.947	0.631 0.842	2.93	0.6	1.25	1.15	1.25	0.2
38 28	4.157 3.315	2.105 2.105	2.842 2.789	5.00 4.736	2.842 2.789	0.632 0.632	0.9	0.0	0.12	0.25	0.12	0.0

Stationary phase: Nucleosil 5 SA /MN/
Mobile phase: Methanol-Water /KH_2PO_4 / solvent mixture
Methanol content : 30 %, 40 %, 50 %, pH of the mixture 4.7-5.5-6.7 respectively

Table 4.a

	$\bar{k}$ at different pH using 40 % CH_3OH			$\bar{k}$ at different concentration of $\bar{e}$ CH_3OH $\bar{e}$ constant pH 6.7		
Compounds number	4.7	5.5	6.7	30 %	40 %	50 %
1	1.421	1.421	1.105	1.631	1.105	0.421
2	2.473	1.210	1.473	2.263	1.473	0.578
3	2.578	1.316	1.736	2.631	1.736	0.526
4	3.947	1.684	1.842	2.842	1.842	0.526
5	2.473	1.526	1.684	2.684	1.684	0.526
6	2.894	1.447	1.736	2.578	1.736	0.526

7	1.789	1.105	2.157	2.210	1.789	0.421
8	5.736	2.105	2.421	3.947	2.421	0.631
9	6.736	2.368	2.474	3.947	2.474	0.578
10	2.631	1.474	1.947	3.210	1.947	0.473
11	7.421	2.632	2.263	4.737	2.263	0.684
12	8.368	2.895	3.368	5.821	3.368	0.684
13	35.105	3.842	4.210	10.052	4.210	0.789
14	19.315	6.789	5.263	10.210	5.263	0.921
15		18.695	12.631		12.631	1.368
16	33.42	5.895	5.109	18.046	5.105	0.947
17		16.211	10.579		10.579	1.210

Table 4.b

Compounds number	$\bar{k}$ at different pH using 40 % CH_3OH			$\bar{k}$ at different concentration of CH_3OH $\bar{c}$ constant pH 6.7		
	4.7	5.5	6.7	30 %	40 %	50 %
18	1.578	0.895	1.105	1.526	1.105	0.421
19	1.842	1.033	1.210	1.105	1.210	0.421
20	1.421	0.789	1.000	1.589	1.000	0.368
21	4.684	1.694	1.815	3.158	1.815	0.578
22	2.263	1.053	1.368	2.263	1.368	0.368
23	2.894	1.316	1.632	2.684	1.632	0.447
24	2.157	0.895	1.211	2.00	1.211	0.368
25	6.578	2.605	2.789	5.158	2.789	0.526

Table 4.c

	$\bar{k}$ at different pH using 40 % CH_3OH			$\bar{k}$ at different concentration CH OH e constant pH 6.7		
Compound's number	4.7	5.5	6.7	30 %	40 %	50 %
26	1.736	1.210	1.631	2.526	1.631	0.578
28	3.315	2.105	2.789	4.736	2.789	0.632
30	3.105	1.947	2.526	4.316	2.526	0.736
31	8.578	3.263	3.789	6.578	3.789	0.842
38	4.157	2.105	2.842	5.00	2.842	0.632
47.d	16.578	2.211	2.894	5.210	2.894	0.684
48	4.157	2.105	2.842	5.00	2.842	0.632
49	4.157	2.421	3.421	6.263	3.421	0.684
50	3.315	1.474	2.474	4.157	2.474	0.684
51	8.578	3.263	3.789	6.578	3.789	0.842
59	8.157	3.211	3.263	8.263	3.263	0.789
60	9.421	3.579	4.842	9.526	4.842	0.842
63	10.369	3.947	5.263	10.684	5.263	0.842
65		6.263	8.894	21.631	8.894	1.105

Table 4.d

Compounds number	$\bar{k}$ at different pH using 40 % CH_3OH			$\bar{k}$ at different concentration of CH_3OH $\bar{e}$ constant pH 6.7		
	4.7	5.5	6.7	30 %	40 %	50 %
34	2.789	1.263	2.474	4.894	2.474	0.631
35	6.00	2.842	3.94	7.315	3.947	0.842
36	6.052	2.578	4.631	9.00	4.631	0.789
34.a	3.526	2.158	3.00	5.684	3.000	0.631
34.b	4.789	2.316	4.263	8.210	4.263	0.789
36.a	11.473	6.211	8.526	20.894	8.526	1.053

To find the optimum conditions for the separation of the previous compounds, the capacity factor has been calculated in various pH's and concentrations of methyl alcohol (see Table 4, a,b,c,d); also, the resolution factor for some pairs of these compounds have been calculated.

EXPERIMENTAL

1. Materials

All model substances have been synthesed at our laboratory; the identification and control of these compounds was made by melting point determination and chromatography.

All other chemicals were analytical grade Merck, West Germany and used without further purification.

The HPLC grade of the solvents (Merck, West Germany) was used without further purification.

2. Apparatus

Biotronic UV Detector BT 3030

Biotronic HPLC pump BT 3020

3. Chromatographic procedure

A 250 mm x 4,6 mm Nucleosil column 5 SA/mn was used.

10 ul sample solutions 0.1 ug/ml in methanol were injected.

Experiments were run at room temperature using a mobile phase flow rate 1 ml/min.

Results and discussion

Table 4 shows the retention parameters and resolution factors for some pairs of homologues and structural isomers.

The pK values of these weak bases fall within a relatively narrow range (3.2-4) ; therefore at, pH 4.7, the compounds may be taken as partially protonated, while at pH 6.7 only the base form is assumed to exist. Consequently the chromatographic process is governed by the following equilibria

I $R - SO_3H + BH^+X^-$ $R - SO_3BH + HX$

At pH 4.7 (ion exchange)

II $R - SO_3H + B$ $R - SO_3H^{(+)} \ldots\ldots B^-$

At pH 6.7 (partition)

As may be seen from the R_s values, the selected pairs of various homologous compounds are separated very sharply by ion exchange liquid chromatography with a mobile phase containing 40 % methylalcohol at pH 4.7 (see R_s values of compounds 1-2, 9-12, 9-4, etc.).

This chromatographic system obviously offers optimal conditions for HPLC separation of our pyridopyrimidine model substances (Table 1,2).

Essentially the same conclusions may be drawn for the three-ring homologous with different ring sizes (see R_s values of compounds 34-35, 26-27).

That the higher homologous with C_2, C_6 methyl substituents have higher k' values may be a consequence of the hyperconjugational effect of the methyl group, i.e., the strong Lewis base character of the compound Nr 9, 2, 4. The lack of hyperconjugational effect in the methyl derivatives with a saturated A-ring causes reduced retention (smaller k' values) of this type of compound (Nr 18, 19, 20, 22).

As a proof of the partition mechanism (see equ. 2), the k' value for the same compound are smaller when mobile phase containing 40 % methanol and pH 6.7 was used compared with the k' values are determined using acidic developing solvents when ion exchange mechanism works.

At pH 6.7 increasing the methanol concentration causes the decrease of k' values. In general the resolution is poorer than in case of the ion exchange version.

As shown in Table 5, the log P values of the investigated series of compounds correlate rather well with log k' values, which were generated at pH = 6.7. Much poorer correlation was obtained with the more acidic solvent mixture (pH 5.5, 4.7). This may be taken as another proof of the partition character of the chromatographic process when the base form of the compounds exist.

By contrast, the correlation was found definitely better with the log k' values obtained with the acidic developing mixture when pK values were used as variables (Table 6). This experience gives strong support to the assumption that the protonated form of the compounds moves with an ion exchange mechanism and the retention is controlled mainly by the strength of the base (i.e. the acidity of the ammonium cation).

As shown in Fig. 1, the applied chromatographic system indicates rather high selectivity; the small structural differences are reflected much more by the HPLC

Table 5

Relationship between logK' and logP values

$\log K'_{pH=4,7} = 0{,}513 \log P - 0{,}218 \quad r = 0{,}671 \quad n = 15$ /Table 1/

$\log K'_{pH=6,7} = 0{,}409 \log P - 0{,}051 \quad r = 0{,}967 \quad n = 17$ /Table 1/

$\log K'_{pH=4,7} = 0{,}397 \log P = 0{,}184 \quad r = 0{,}823 \quad n = 8$ /Table 2/

$\log K'_{pH=6,7} = 0{,}254 \log P = 0{,}015 \quad r = 0{,}849 \quad n = 8$ /Table 2/

$\log K'_{pH=4,7} = 0{,}362 \log P + 0{,}244 \quad r = 0{,}772 \quad n = 12$ /Table 3, saturated rings/

$\log K'_{pH=6,7} = 0{,}250 \log P + 0{,}174 \quad r = 0{,}950 \quad n = 12$ /Table 3, saturated rings/

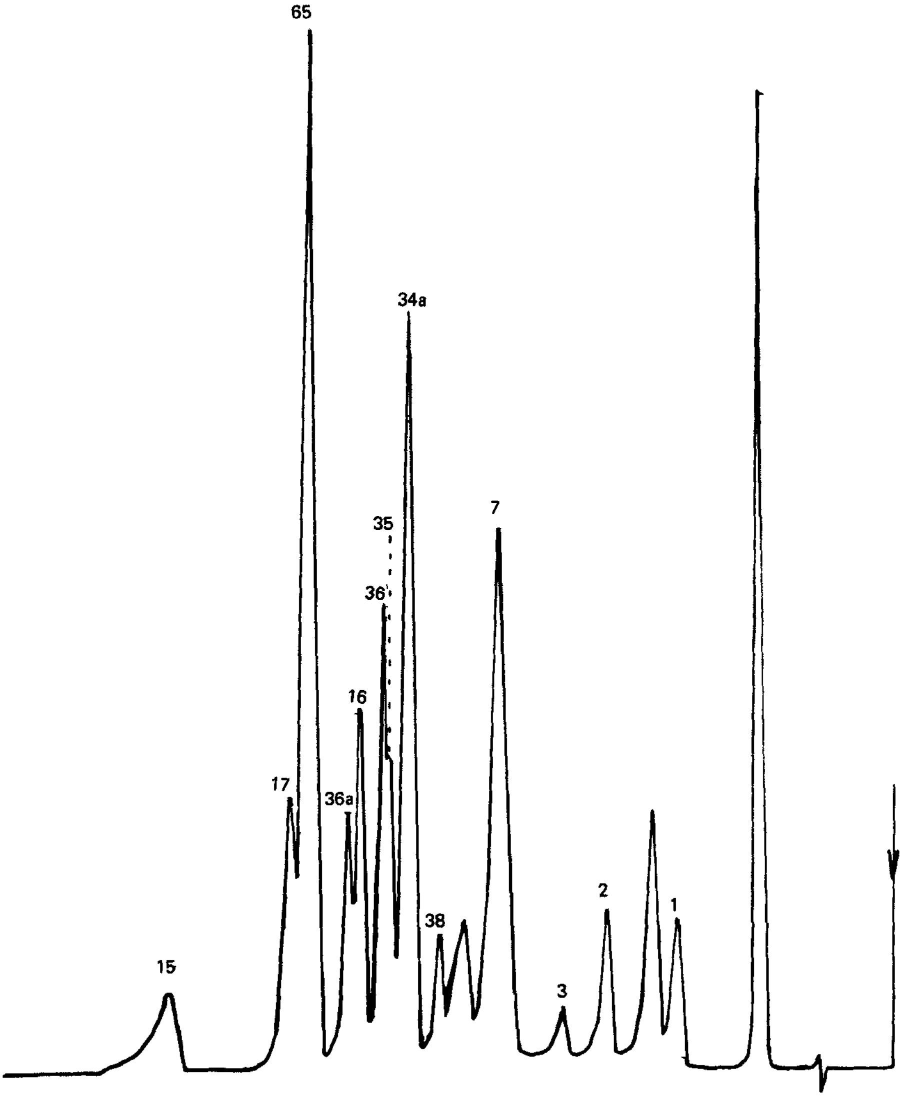


Fig. 1. The separation of mixture of some nitrogen bridged compounds by gradient linear elution technique. /Stationary phase nucleosil 5 SA/mn, mobile phase a 0.1 Mol KH_2PO_4, - b 0.01 Mol KH_2PO_4 in methanol--water 40:60, pH from 3 to 5.5, flow rate 1 ml/min./

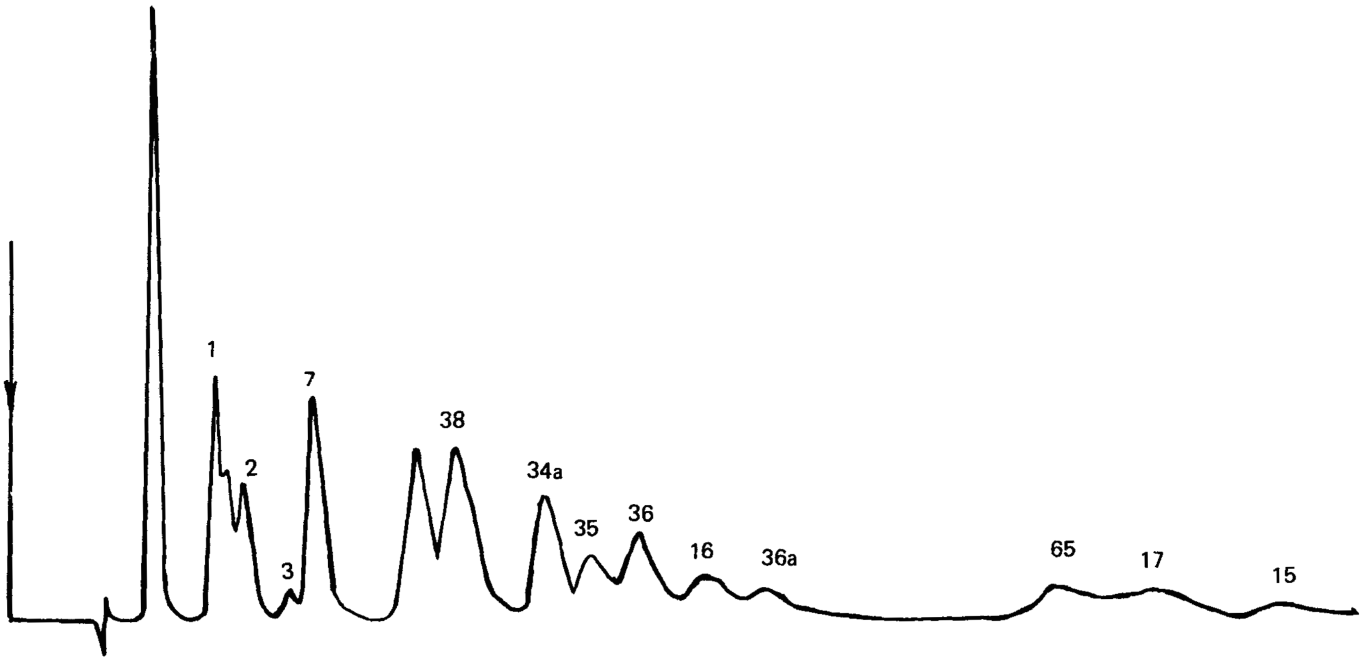


Fig. 2. The separation of mixture of some nitrogen bridged compounds with isocratic elution technique /stationary phase nucleosil 5 SA/mn, mobile phase methanol-water 40:60, pH 5.5, flow rate 1 ml/min./

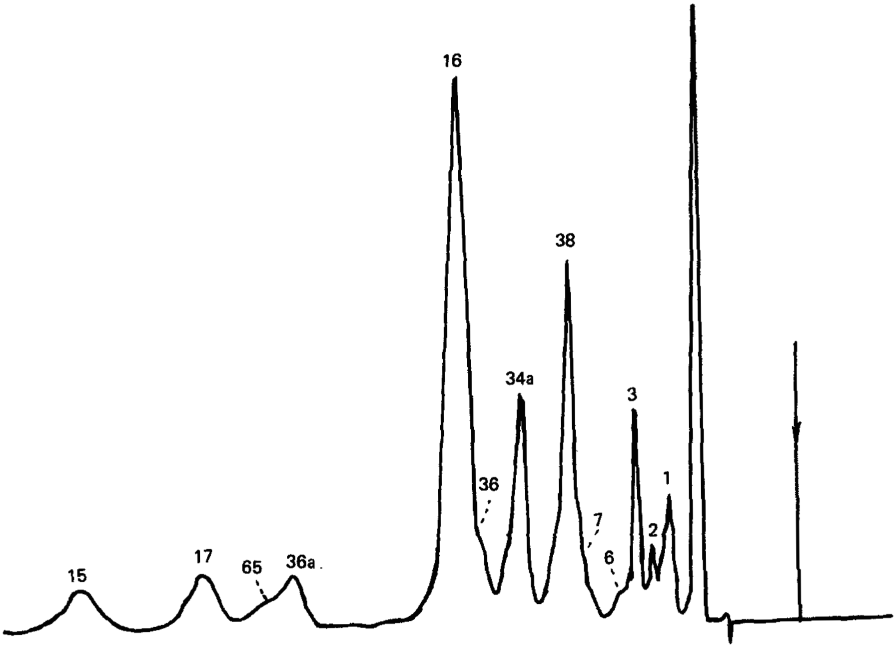


Fig. 3. The separation of mixture of some nitrogen bridged compounds with isocratic elution technique /stationary phase nucleosil 5 SA/mn, mobile phase methanol-water 40:60, pH 6.7, flow rate 1 ml/min./

Table 6

Relationship between logK' and pK values

$\log K'_{pH=6,7} = 0{,}234\ pK - 0{,}301$ r = 0,759 n = 11 /Table 3/

$\log K'_{pH=4,7} = 0{,}312\ pK - 0{,}435$ r = 0,909 n = 11 /Table 3/

behaviour when the gradient elution technique is used (compare the Figures 1 and 2 and 3).

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