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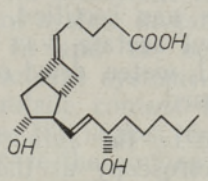
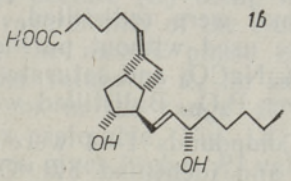
RESOLUTION OF *E*- AND *Z*-ISOMERS OF PROSTACYCLIN CARBA-ANALOGS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Since 1976, when prostacyclin was discovered [1], its remarkable biological properties have stimulated intense research into the preparation of stable synthetic analogs for therapeutic use [2, 3]. Among them, the carba-analogs of prostacyclin are of considerable importance [4]. As both the *E*- and *Z*-isomers of different biological activity are formed in the Wittig olefination, one of the key steps in carbacyclin synthesis [3], methods for their separation are highly needed. Up to now, only R. F. Newton and A. H. Wadsworth [5] have used the HPLC method for determining the ratio of *E*/*Z*-isomers of some prostacyclin carba-analogs.



1a

(5E)-9-desoxy-Δ^5-6,9α-cyclo-PGF_1



1b

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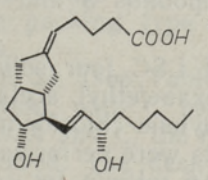

2a

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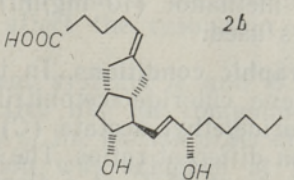
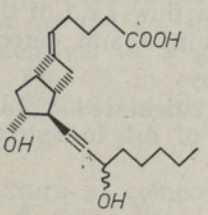


2b

(5E)-9-desoxy-6,9α-methano-Δ^5-PGF_1



3a

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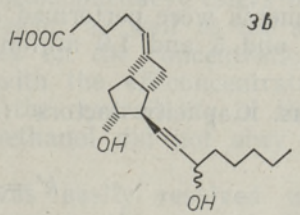
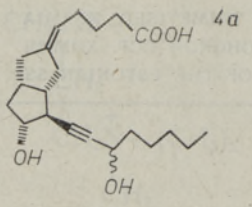
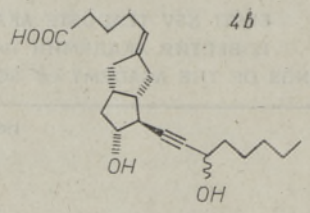


3b

(5Z)-9-desoxy-13,14-didehydro-15α/β-Δ^5-6,9α-cyclo-PGF_1



4a



4b

(5Z)-9-desoxy-13,14-didehydro-15α/β-6,9α-methano-Δ^5-PGF_1

(5E)-9-desoxy-13,14-didehydro-15α/β-6,9α-methano-Δ^5-PGF_1

We have studied the possibilities of separating the *E*/*Z*-isomers of four different carba-analogs (1—4) of prostacyclin by liquid-solid (LSC) and reverse-phase mode bonded phase (BPC) chromatography.

Materials and methods

Apparatus. A DuPont HPLC system N 8845 with a UV spectrophotometrical detector was used.

Columns. Zorbax SIL, 4.6×250 mm (DuPont) **1**; Zorbax SIL, 6.0×150 mm **2** and Silasorb 600 (5 μm), 6.0×150 mm **3** packed in the Special Designing Bureau (SDB), Tallinn, were used in the LSC studies.

Zorbax ODS, 4.6×250 mm (DuPont) **4**; Zorbax C-8, 4.6×250 mm (DuPont) **5** and Zorbax ODS, 6.0×150 mm **6** packed in the SDB were used in the BPC studies.

Solvents. All solvents were supplied by Reakhim. Methylene chloride and methanol were redistilled, acetonitrile was rectified. Hexane and H_3PO_4 were used without purification. Ethyl acetate was washed with aqueous 5% $NaCO_3$ and saturated with $CaCl_2$ water, dried on K_2CO_3 and distilled over P_2O_5. Bidistilled water was used.

Samples. Compounds 1—4 were synthesized by the authors of the present work and identified by ^{13}C-NMR spectroscopy at the Institute of Chemical Physics and Biophysics, Tallinn. By LSC, samples were converted into *p*-bromophenacyl esters according to [6, 7] and injected in methylene chloride (1 mg/ml). In the case of BPC the samples were injected in methanol (10 mg/ml). For compounds 3 and 4 the 15α/β-mixture was used.

Chromatographic conditions. In the case of LSC four solvent systems, viz. methylene chloride/acetonitrile (*A*), hexane/ethyl acetate (*B*), methylene chloride/ethyl acetate (*C*) and methylene chloride/methanol (*D*) were used in different ratios. The experiments were performed at ambient temperature or at 35 °C at a flow rate of 0.6 ml/min for column **1** and 1.0 ml/min for columns **2** and **3** and detected at 260 nm.

In the case of BPC the varied content of acetonitrile or methanol in aqueous 0.017 M H_3PO_4 was used (solvent system *E* and *F*, respectively). The experiments were performed at 35° at a flow rate of 0.8 ml/min for columns **4** and **5** and 1.0 ml/min for column **6** and detected at 208—210 nm.

Calculations. Capacity factors (k') were calculated according to the formula

$$k' = \frac{t_R - t_0}{t_0},$$

where t_R — retention time (measured from the chromatogram, nm), t_0 — retention time of the unretained compound (mm). t_0 was calculated from

operational values of the void volume of column (V_0), chart speed and flow rate. For reverse phase columns V_0 was determined as the elution volume of KNO_3 (for **4**, **5** and **6** it was 2.04, 1.85 and 2.08 ml, respectively). For the column **1** pore volume was calculated as 2.41 ml according to [8]. The elution volume of the first eluted nonpolar compound was 2.68 ml. It means that the «true» void volume $2.41 < V_0 \leqslant 2.68$. Therefore, V_0 was taken as 2.68 ml, and V_0 for **2** and **3** as 2.74 ml in the same way.

The resolution factors (α) were calculated according to the formula:

$$\alpha = k'_b / k'_a ,$$

where k'_b — capacity factor of the compound *b*, k'_a — capacity factor of the respective compound *a*. The resolution function (R_s) was estimated according to [8].

Results and discussion

The resolution of *E*/*Z*-isomers of prostacyclin analogs as their *p*-bromophenacyl ester is a complicated problem due to their very similar behaviour on silica gel. It means that the differences in specific interactions between *E*/*Z*-isomers, solvents and the adsorbent are very small.

Compounds 1 and 2 were chosen as model compounds in the elucidation of the possibilities of separating the *E*/*Z*-isomers of prostacyclin analogs. The results for 1*a* and 1*b* *p*-bromophenacyl esters in the case of LSC are given in Table 1. According to these results 1*a* and 1*b* are fairly separable in methylene chloride-acetonitrile (*A*) and hexane-ethyl acetate (*B*), with methylene chloride-ethyl acetate (*C*) being ineffective. For each solvent system investigated the reversibility of the elution order of compound 1*a* and 1*b* occurs when the mobile phase content is changed. For solvent system *A*, the proper values of R_s lie in the area with an acetonitrile content below 12% or in the range of 50—70%. In the first case the higher temperature is preferable (Fig. 1*A*), whereas in the second case the ambient temperature gives higher R_s values (Fig. 1*B*). For solvent system *B* a higher temperature (35°) decreases peak tailing and results in lower k' values (shorter analysis time) and better resolution (Fig. 1*C*).

The same α values were obtained for column 3. We conclude that a change in the packing type does not affect the resolution selectivity of *E*/*Z*-isomers.

For 2*a* and 2*b* (see Table 2), solvent systems *A* and *B* gave the desired resolution, with *C* being ineffective like for compounds 1. For solvent systems *A* and *C*, the reversibility of the elution order of 2*a* and 2*b* was also observed. The desired resolution was achieved at the acetonitrile content below 20% (Fig. 2*A*), but the solvent system *B* is preferable at a higher temperature (35°) due to shorter elution time (Fig. 2*B*).

Consequently, in the resolution of *p*-bromophenacyl esters of 1*a* and 1*b*, and also of 2*a* and 2*b*, the elution order as well as the selectivity of resolution depends to a great degree on the concentration of the mobile phases. This is in accordance with the «*B*-concentration rule» [8], but only for the solvent system *D* the reversibility of the elution order in the range of 1.5 to 5% of methanol did not obey this rule (Table 3).

The *E*/*Z*-mixture of compounds 3 was easily resolved using the solvent system *A* (separation on 15α/β-isomers also occurs). Conditions for compounds 3: column **1**; methylene chloride/acetonitrile=90/10 v/v; $k'_{3a}=4.8$ and 5.1; $k'_{3b}=5.2$ and 5.5 (15α/β-isomers of 3 and 4 were not

Table 1

Dependence of the resolution factor (α), resolution function (R_s) of compounds 1*a* and 1*b* *p*-bromophenacyl esters and capacity factor (k'_{1b}) of compound 1*b* *p*-bromophenacyl ester on the mobile phase content (*A* — acetonitrile in methylene chloride, *B* — ethyl acetate in hexane, *C* — ethyl acetate in methylene chloride, %) by LSC.
Column Zorbax SIL (4.6×250 mm)

Mobile phase	Column temperature	Calculated parameter	Mobile phase content, %													
			100	90	80	70	60	50	45	40	35	30	25	20	15	12	11
		k'_{1b}		0.83	0.91	0.97	1.15	1.76	1.94	2.4	3.0	3.9	5.1	7.2	16.6	22	
A	ambient																
		α		0.91	0.91	0.91	0.91	0.91	0.93	0.94	0.96	0.97	~1	~1	1.02	1.06	
		R_s		0.95	1.00	1.00	1.05	1.20	1.00	0.90	0.85	0.70			0.70	1.00	
		k'_{1b}	0.60	0.63	0.68	0.81	0.97	1.28		2.0		3.3	4.4	6.0	10.1		16.7
A	35 °																
		α	0.91	0.91	0.91	0.92	0.92	0.93		0.94		0.96	~1	~1	1.04		1.06
		R_s	0.85	0.90	0.95	1.00	1.05	1.1		0.95		0.70			0.75		1.15
		k'_{1b}	0.91	1.15	1.69	2.5	4.6	8.3	11.4	17.1							
B	ambient																
		α	0.91	0.94	~1	~1	1.06	1.10	1.12	1.17							
		R_s	0.70	0.60			0.65	0.90	1.0	1.1							
		k'_{1b}	0.75		1.43		3.0	5.4	7.6	11.5	17.3						
B	35 °																
		α	~1		~1		1.04	1.09	1.12	1.15	1.16						
		R_s					0.75	1.00	1.25	1.40	1.70						
		k'_{1b}	0.75	0.85	1.06	1.42	1.89	2.8		4.1		7.0		14.6			
C	35 °																
		α	~1	0.94	0.95	0.95	0.96	~1		~1		~1		1.06			
		R_s		0.70	0.70	0.70	0.65							0.80			

identified); R_s values between these peaks are 1.05, 0.70 and 1.00, respectively. Conditions for compounds 4: column 2; methylene chloride/acetonitri-

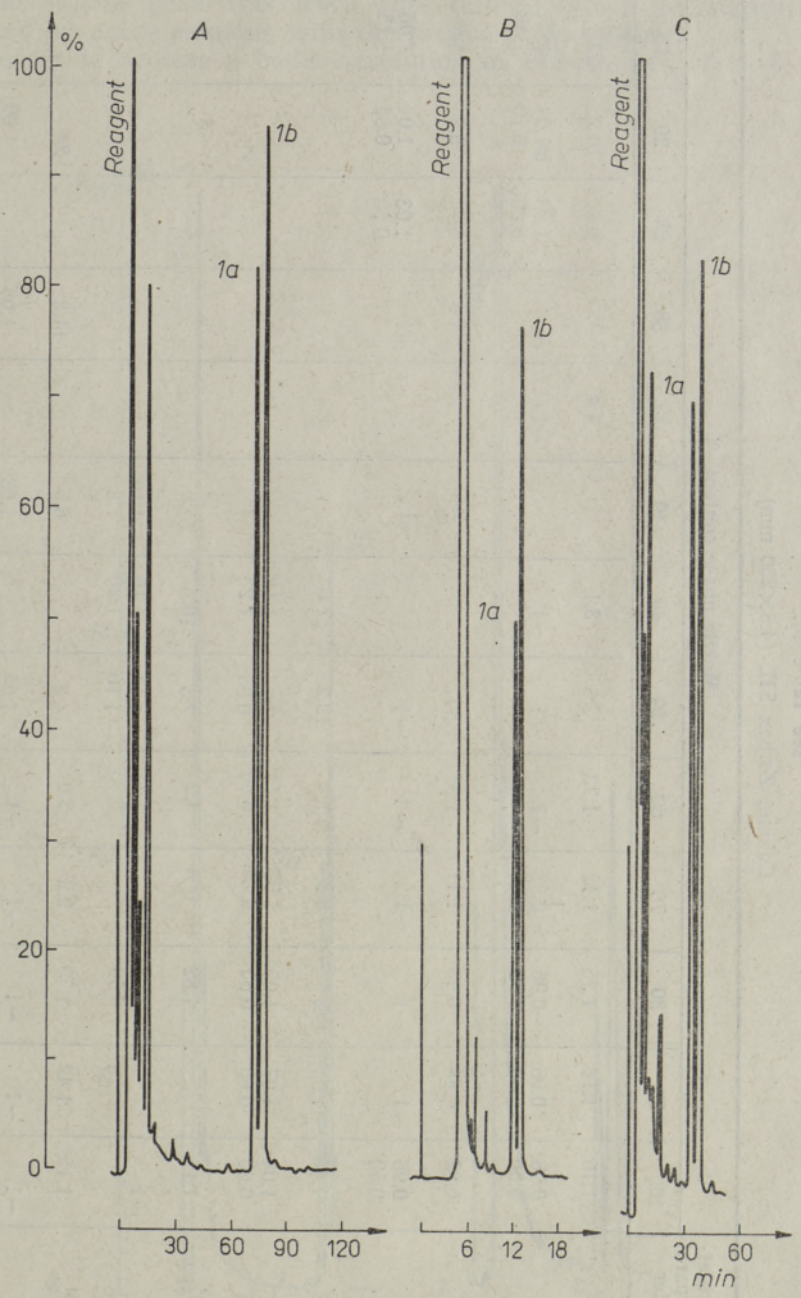


Fig. 1. Chromatograms of the separation of compounds 1*a* and 1*b* p-bromophenacyl esters.

Conditions: Column — Zorbax SIL (4.6×250 mm); flow rate — 0.6 ml/min; detection — UV 260 nm.

A-mobile phase — methylene chloride/acetonitrile=89/11; column temperature — 35 °C; absorbance — 0.32 AUFS; chart speed — 2.5 cm/h; ~8 μg injected.

B-mobile phase — methylene chloride/acetonitrile=50/50; column temperature —ambient; absorbance — 0.64 AUFS; chart speed — 10 cm/h; ~4 μg injected.

C-mobile phase — hexane/ethyl acetate=55/45; column temperature — 35 °C; absorbance — 0.32 AUFS; chart speed — 2.5 cm/h; ~ 4 μg injected.

Table 2

Dependence of the calculated parameters (see Table 1) of compounds 2*a* and 2*b* *p*-bromophenacyl esters on the mobile phase content (*A*, *B* and *C* see Table 1).

Column Zorbax SIL (4.6×250 mm)

Mobile phase	Column temperature	Calculated parameter	Mobile phase content, %													
			100	90	80	70	60	50	45	40	35	30	25	20	15	14
A	ambient	k'_{2b}	1.10	1.16	1.25	1.42	1.73	2.3	3.1		4.4		9.3	10.2	23	
		α	0.96	0.96	0.96	0.97	~1	~1	~1		~1		1.03	1.04	1.06	
		R_s	0.50	0.50	0.50	0.50							0.60	0.70	1.50	
A	35 °	k'_{2b}	0.82	0.88	0.95	1.11	1.37	1.80		2.6		4.5	6.4	9.2	15.6	17.4
		α	0.96	~1	~1	~1	~1	~1		~1		~1	1.03	1.04	1.06	1.06
		R_s	0.50										0.50	0.85	1.15	1.3
B	ambient	k'_{2b}	1.55	1.94	2.7	3.8	7.3	12.2	17.0							
		α	1.04	1.07	1.05	1.07	1.11	1.15	1.18							
		R_s	0.60	0.60	0.60	0.70	0.80	0.95	1.20							
B	35 °	k'_{2b}	1.15		1.82		4.3	7.3	10.5	15.8						
		α	~1		1.06		1.11	1.15	1.16	1.19						
		R_s			0.50		0.85	1.10	1.30	1.8						
C	35 °	k'_{2b}	1.15	1.42	1.70	2.2	3.0	4.2		6.5		11.3		24		
		α	~1	~1	~1	~1	~1	1.03		1.03		1.03		1.09		
		R_s						0.50		0.55		0.80		0.90		

le=85/15 v/v; k'_{4a}=6.8 and 7.5; k'_{4b}=7.4 and 7.6; R_s between the first and the second peak was 0.90. This resolution can be significantly improved by using columns with the proper plate number.

In order to achieve a better resolution of *E*/*Z*-isomers of 1—4, BPC

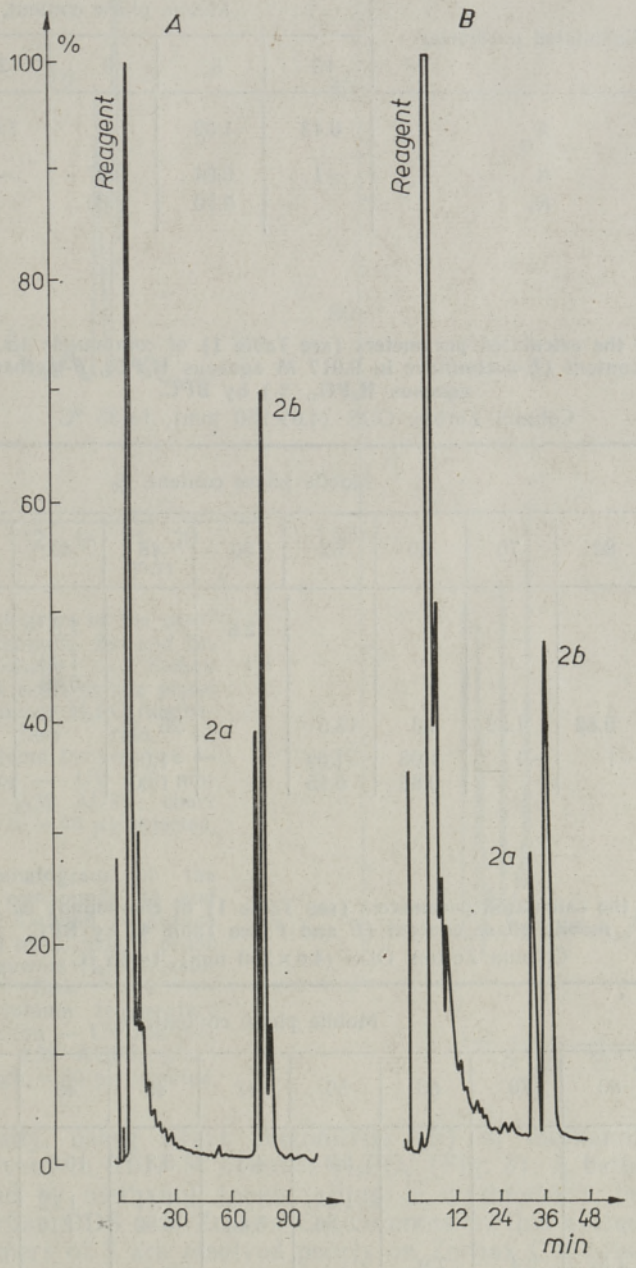


Fig. 2. Chromatograms of the separation of compounds 2*a* and 2b p-bromophenacyl esters.

Conditions: Column — Zorbax SIL (4.6×250 mm); flow rate — 0.6 ml/min; column temperature — 35 °C; detection — UV 260 nm; absorbance — 0.32 AUFS.

A-mobile phase — methylene chloride/acetonitrile=86/14; chart speed — 2.5 cm/h; ~12 μg injected.

B-mobile phase — hexane/ethyl acetate=50/50; chart speed — 5 cm/h; ~10 μg injected.

Table 3

Dependence of the calculated parameters (see Table 1) of compounds 1*a* and 1*b* *p*-bromophenacyl esters on the mobile phase content (*D*—methanol in methylene chloride, %) by LSC.

Column Zorbax SIL (6.0×150 mm), t=35 °C

Mobile phase	Calculated parameter	Mobile phase content, %				
		10	5	3	2	1.5
D	k'_{1b}	0.43	1.02	3.3	7.6	14.1
	α	~1	0.94	0.97	~1	1.02
	R_s		0.70	0.60		0.55

Table 4

Dependence of the calculated parameters (see Table 1) of compounds 1*a* and 1*b* on the mobile phase content (*E*-acetonitrile in 0.017 M aqueous H_3PO_4, *F*-methanol in 0.017 M aqueous H_3PO_4, %) by BPC.

Column Zorbax ODS (4.6×250 mm), t=35 °C

Mobile phase	Calculated parameter	Mobile phase content, %								
		80	70	60	52	50	48	40	36	32
E	k'_{1b}					2.6		7.5	11.4	25
	α					~1		1.05	1.07	1.07
	R_s							0.80	0.90	1.00
F	k'_{1b}	0.82	1.22	5.3	15.5		26			
	α	~1	~1	1.06	1.09		1.09			
	R_s			0.85	0.95		1.00			

Table 5

Dependence of the calculated parameters (see Table 1) of compounds 2*a* and 2*b* on the mobile phase content (*E* and *F* see Table 4) by BPC.

Column Zorbax ODS (4.6×250 mm), t=35 °C

Mobile phase	Calculated parameter	Mobile phase content, %								
		80	70	60	50	47	45	40	36	34
E	k'_{2b}				3.3	4.3	5.3	10.0	15.6	24
	α				1.03	1.05	1.11	1.12	1.14	1.15
	R_s				0.60	0.80	0.90	1.10	2.20	2.50
F	k'_{2b}	1.51	2.4	7.9						
	α	1.08	1.08	1.15						
	R_s	0.50	0.90	1.50						

was studied, using 1 and 2 as model compounds. These data are given in Tables 4 and 5, respectively. It is rather difficult to obtain acceptable resolution of 1*a* and 1*b* on columns Zorbax ODS, but 2*a* and 2*b* are

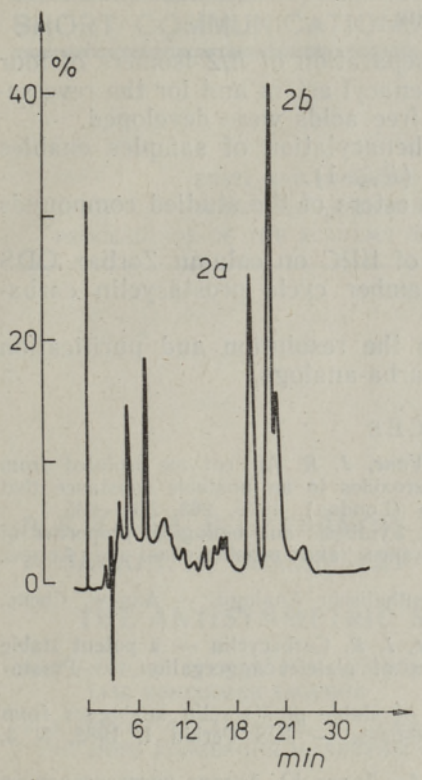


Fig. 3. Chromatogram of the separation of compounds 2*a* and 2*b*. Conditions: Column — Zorbax ODS (4.6×250 mm); mobile phase — 0.017 M aqueous H_3PO_4/methanole=40/60; flow rate — 0.8 ml/min; column temperature — 35 °C; detection — UV 210 nm; absorbance — 0.32 AUFS; chart speed — 10 cm/h; ~40 μg injected.

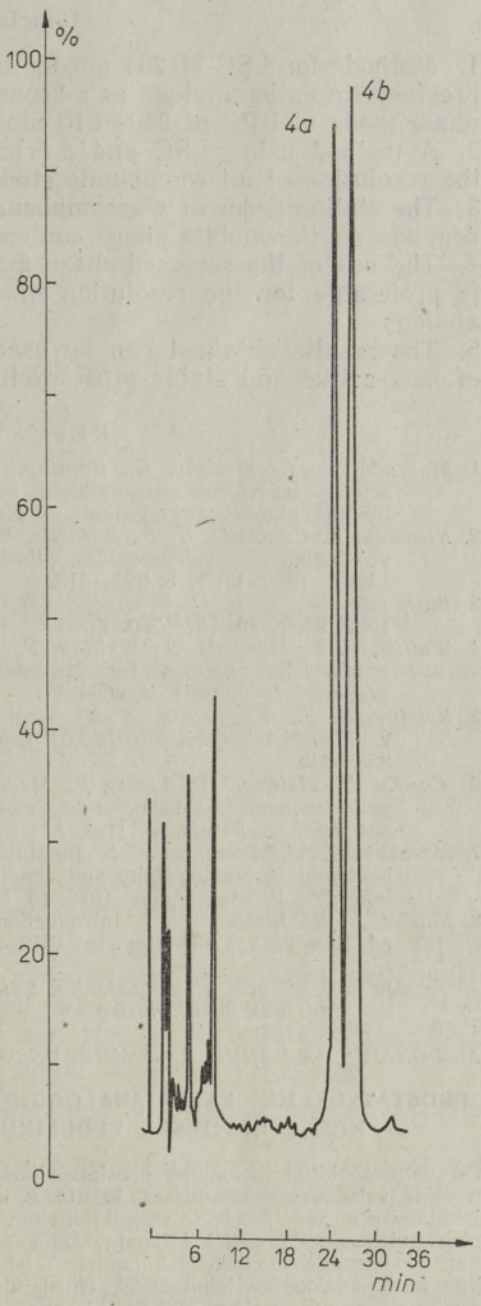


Fig. 4. Chromatogram of the separation of compounds 4*a* and 4*b*. Conditions: Column — Zorbax ODS (6.0×150 mm); mobile phase — 0.017 M aqueous H_3PO_4/acetonitrile=60/40; flow rate — 1.0 ml/min; column temperature — 35 °C; detection — UV 208 nm; absorbance — 0.32 AUFS; chart speed — 10 cm/h; ~80 μg injected.

resolved easily, using either acetonitrile (*E*) or methanol (*F*) as a «strong» solvent in 0.017 M aqueous H_3PO_4 (Fig. 3). A better resolution was achieved by methanol (peak tailing is diminished), but the «lifetime» of Zorbax ODS and Zorbax C-8 columns in this system is shorter. The *E*/*Z*-isomers of 3 are resolved poorly on Zorbax C-8 column (column 5; 0.017 M aqueous H_3PO_4/acetonitrile=68/32 v/v; $k'_{3a}=22$; $k'_{3b}=21$; $R_s=0.95$) and similarly to 1 on Zorbax ODS column. Likewise, the resolution of 5-member cycle carba-analogs 4*a* and 4*b* is easily obtained on Zorbax ODS column (see conditions in Fig. 4). Zorbax C-8 column gives poorer results. The 15α/β-isomers of these compounds were inseparable.

Conclusions

1. Methods for LSC at 254 nm for the separation of *E*/*Z*-isomers of four prostacyclin carba-analogs as *p*-bromophenacyl esters and for the reverse-phase mode of BPC at 200—210 nm as free acids were developed.
2. A method using LSC and *p*-bromophenacylation of samples enables the resolution of all compounds studied ($R_s \geqslant 1$).
3. The elution order of *p*-bromophenacyl esters of the studied compounds depends on the mobile phase content.
4. The use of the reversed-phase mode of BPC on column Zorbax ODS is preferable for the resolution of 5-member cycle prostacyclin carba-analogs.
5. The results obtained can be used in the resolution and purification of new active and stable prostacyclin carba-analogs.

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PROSTATSÜKLIINI KARBA-ANALOOGIDE *E*- ja *Z*-ISOMEERIDE LAHUTAMINE KÕRGEFEKTIIVSEL VEDELIKUKROMATOGRAAFIAMEETODIL

On välja töötatud metoodika prostatsükliini nelja erineva karba-analoogi *E*- ja *Z*-isomeeride vahekorra määramiseks, kasutades nende isomeeride 1) *p*-bromofenatsüülestrite lahutamiseks vedelik-adsorbtsioonikromatograafia ja 2) vabade hapete lahutamiseks pööratud seotud faasilist kromatograafia meetodit. Esimene meetod võimaldas kõigi uuritud ühendite täielikku lahutamist. Teist meetodit tuleb eelistada 5-liikmelise tsükliga karba-analoogide lahutamisel. On leitud, et uuritud isomeeride *p*-bromofenatsüülestrite elueerumisjärjekord silikageelil sõltub liikuva faasi koosseisust.

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РАЗДЕЛЕНИЕ *E*- И *Z*-ИЗОМЕРОВ КАРБА-АНАЛОГОВ ПРОСТАЦИКЛИНА МЕТОДОМ ВЫСОКОЭФФЕКТИВНОЙ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ

Разработана методика для анализа смесей *E*- и *Z*-изомеров четырех карба-аналогов простациклина, заключающаяся в разделении их в виде *п*-бромофенациловых эфиров с помощью жидкостно-адсорбционной хроматографии и в виде свободных кислот с помощью химически связанной обращенно-фазовой хроматографии. Первый способ обеспечил полное разделение всех изученных соединений. Второй способ предпочтительнее для разделения карба-аналогов с 5-членным циклом. Найдено, что порядок элюирования *п*-бромофенациловых эфиров исследованных изомеров на силикагеле зависит от состава подвижной фазы.