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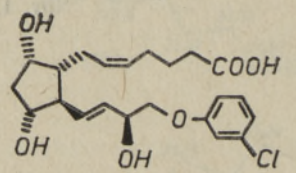
ADSORPTION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PROSTANOIDS, USE OF WATER-CONTAINING MOBILE PHASES FOR SEPARATION OF 15*R*/*S*-ISOMERS OF CLOPROSTENOL, AND PROSTAGLANDINS E_1 AND E_2

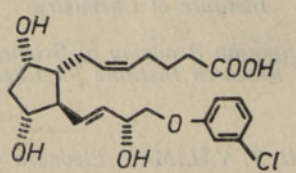
Reversed-phase high-performance liquid chromatography (HPLC) has proved a very useful tool for separating prostaglandins (PG) and their analogs [1–4]. Nevertheless, for the resolution of many PG-isomers the hydrophobic stationary phase offers no sufficient selectivity. If silica gel is used for HPLC resolutions, derivation of the acid moiety is necessary prior to chromatography [5–7]. Moreover, acidic modifiers in the mobile phase or silicaic acid columns are used in the separation of nonderived PGs to avoid peak tailing and to improve resolution efficiency [8, 9]. These methods hardly meet the requirements for preparative-scale separations due to the difficulty in recovering pure initial components and to the instability of some PGs (e.g. the E-series) under acidic conditions [10].

The improvement in the resolution by deactivating strong adsorption sites with water as a mobile phase component has been shown by several authors [11–14], but the separation of carboxylic acids as well as PGs has not yet been performed by this method. We demonstrate here the improvement in the separation efficiency using a trace amount of water as a mobile phase component for several solvent systems in separating 17,18,19,20-tetranor-16(3-chloro)-phenoxy-$PGF_{2\alpha}$ (cloprostenol (2)) from its 15*R*-isomer (1) and PGE_1 (4) from PGE_2 (3) (Fig. 1).



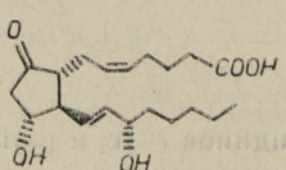
(15R)–17,18,19,20–tetranor–16(3-chloro)–phenoxy–$PGF_{2\alpha}$

(1)



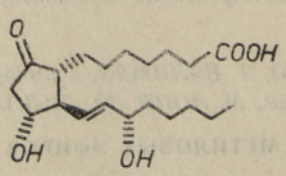
17,18,19,20–tetranor–16–(3-chloro)–phenoxy–$PGF_{2\alpha}$ (cloprostenol)

(2)



PGE_2

(3)



PGE_1

(4)

Fig. 1. Structures of the compounds studied.

Experimental

A Du Pont HPLC system N 8845 with UV-spectrophotometrical and refractive index (RI) detectors was used. The Zorbax SIL column (150×4.6 mm I.D.) theoretical plate count was 9700. The column compartment operated at 35 °C. The eluent flow rate was 0.6 ml/min. 15*R*/*S*-isomers of cloprostenol were detected spectrophotometrically at 269 or 280 nm (when the solvent system contained benzene) or refractometrically (when the solvent system contained acetone). PGE_1 and PGE_2 were detected refractometrically and spectrophotometrically at 211 nm (when hexane—isopropanol—water was used). The two-pen strip chart recorder operated at a speed of 10 or 20 cm/h.

Solvents. All solvents (analytical grade) were purchased from Reakhim (USSR). Hexane, ethanol and 1,4-dioxane were used without purification. Methanol was redistilled. Benzene and acetone were redistilled over P_2O_5. Chloroform was washed with H_2O, dried over $CaCl_2$ and redistilled over P_2O_5. Ethyl acetate was washed with 5% aqueous Na_2CO_3 and saturated $CaCl_2$, dried over K_2CO_3 and redistilled over P_2O_5. Acetonitrile and isopropanol were rectified. Bidistilled water was used. The water content in the solvents was checked on a Chrom-5 gas chromatograph equipped with a katharometer. 1.5 m glass columns with Teparon-18 and Separon CHN stationary phases were used. The solvent responses were calibrated vs. water response. The water content in ethanol (4.2% v), acetone (0.2% v) and dioxane (1.2% v) was taken into consideration in calculating solvent composition. All the other solvents contained less than 0.1% of water before mixing. The water content in the mobile phases varied from 0 to 100% of its saturation at ambient temperature. In all cases, the ternary eluting solutions used were transparent and there were no water droplets on the reservoir walls. The columns were equilibrated by pumping 20 ml of each eluting mixture through column before injections.

Samples. Cloprostenol was synthesized by the authors and identified by ^{13}C NMR spectroscopy.* Biosynthetical natural prostaglandins E_1 and E_2 were purchased from the Pilot Production Plant of Organic Synthesis and Biopreparations (Institute of Chemistry, Tallinn). Samples were dissolved in chloroform at a concentration of 10—20 mg/ml. When UV-detection was used, a 20—100 μg sample was injected, in case of RI-detection 100—500 μg.

Calculations. The capacity factors (k'), resolution factors (α), plate counts ($N_{5\sigma}$) and peak asymmetry factors were calculated, and the resolution function (R_s) was estimated according to L. R. Snyder and J. J. Kirkland[14]. The parameters were the arithmetic means of three measurements. The column void volume was measured as elution volume of toluene by using the eluting system hexane—isopropanol=75/25 (v/v) which was found to be 1.89 ml.

Results and discussion

It is well known that the 15 *R*- and *S*-isomers of prostaglandins are preparatively separable on silica gel[15]. We have found, however, that the use of carefully dried solvents leads to very broad and unresolved peaks of these compounds on the Zorbax SIL column (Fig. 2). If water was added to the eluting mixture, the peak shape improved drastically, and the column efficiency increased (Table 1). The best resolution was

* The authors are indebted to T. Välimäe for interpreting the ^{13}C NMR data.

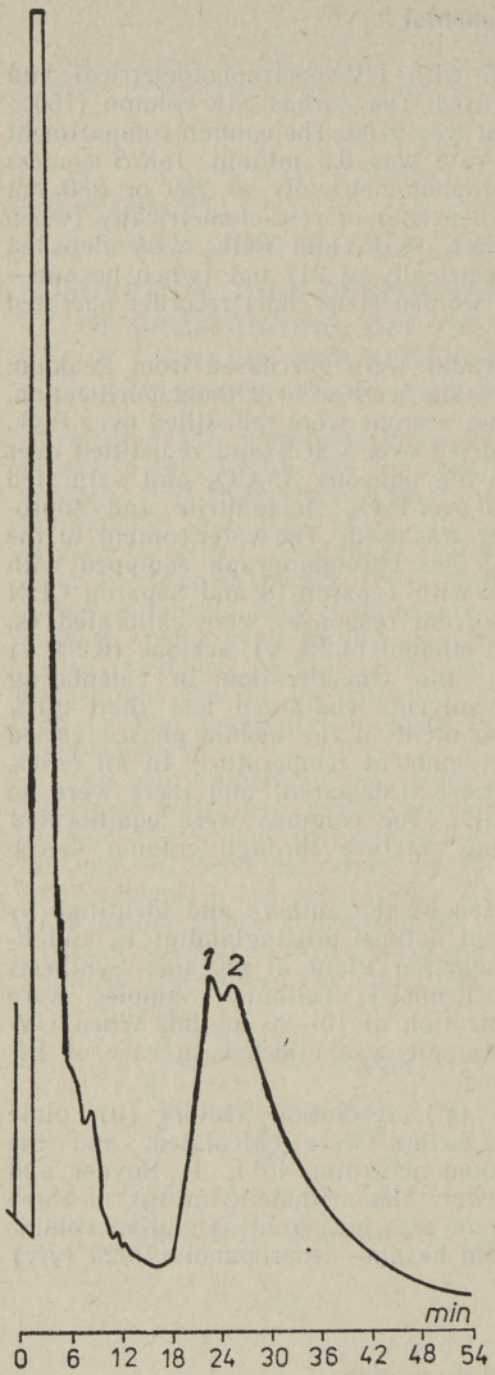
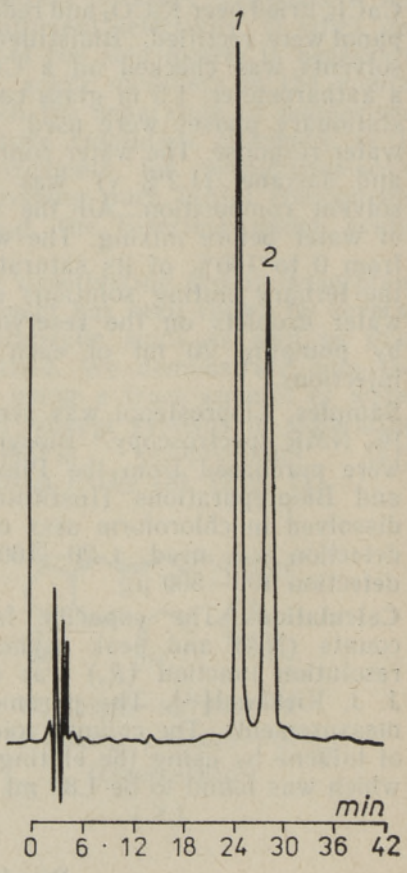


Fig. 2. Separation of cloprostenol from its 15*R*-isomer. Column Zorbax SIL (4.6×150 mm); mobile phase — hexane—isopropanol = 80:20 v/v; flow rate — 0.6 ml/min; UV-spectrophotometer — 269 nm; absorbance — 0.08 AUFS; column temperature — 35 °C; ~ 100 µg injected; peaks, see Fig. 1.

Fig. 3. Separation of cloprostenol from its 15*R*-isomer. Column Zorbax SIL (4.6×150 mm); mobile phase — hexane—isopropanol—water = 85:14.25:0.75 v/v/v; flow rate — 0.6ml/min; UV-sepctrophotometer — 269 nm; absorbance — 0.08 AUFS; column temperature — 35°; ~20 µg injected; peaks, see Fig. 1.



achieved with a maximum water content in the eluting mixture (Fig. 3), and therefore it was subsequently used in each case. It is notable that benzene as a less polar component of the mobile phase offers a better resolution of compounds (1) and (2) than hexane (Table 2). When comparing different ternary eluting systems it can be seen that benzene—acetonitrile—water and benzene—acetone—water provide the highest selectivity in resolution, but the former (Fig. 4) is more advantageous due to UV transmission problems. It can also be supposed that methanol

Table 1

Dependence of peak asymmetry factor and plate count on the water content in hexane—isopropanol and benzene—isopropanol mixtures

Sample: 17,18,19,20-tetranor-16(3-chloro)-phenoxy-$PGF_{2\alpha}$

Eluent composition, v/v/v	k'	Peak asymmetry factor	$N_{5\sigma}$
Hexane—isopropanol—water			
80:20 :0	10.0	4.0	20
80:19.9 :0.1	5.9	2.8	45
80:19.8 :0.2	5.2	2.5	65
80:19.6 :0.4	4.0	1.9	250
80:19.4 :0.6	3.6	1.3	850
80:19.2 :0.8	3.5	1.1	1800
80:19 :1.0	3.5	1.0	4200
80:18.8 :1.2	3.5	1.0	5700
85:14.25:0.75	8.1	1.0	4100
Benzene—isopropanol—water			
90:10 :0	12.7	4.0	20
90: 9.95:0.05	9.4	3.8	60
90: 9.9 :0.1	7.0	3.5	70
90: 9.8 :0.2	5.8	3.2	150
90: 9.7 :0.3	5.4	3.0	580
90: 9.6 :0.4	4.4	2.3	1000
90: 9.5 :0.5	3.8	1.3	2400
95: 4.8 :0.2	3.1	2.0	1250
80:18.6:1.4	1.2	1.3	6900

Table 2

Mobile phase selection for resolving 15*S*- and 15*R*-isomers of 17,18,19,20-tetranor-16(3-chloro)-phenoxy-$PGF_{2\alpha}$

k' values are given for 15*S*-isomer; α values are expressed as the ratio of k' value of 15*S*-isomer to k' of 15*R*-isomer; $N_{5\sigma}$ and the peak asymmetry factor are arithmetic means of both isomers

Eluent composition, v/v/v	k'	α	Peaks asymmetry factor	$N_{5\delta}$
Hexane—isopropanol—water				
80 :19 :1	3.5	1.13	1	4200
Benzene—isopropanol—water				
90 : 9.5 :0.5	3.8	1.36	1.3	2400
Benzene—ethanol—water				
93 : 6.64:0.36	5.9	1.35	1	6000
Benzene—methanol—water				
95.5: 4.27:0.23	9.6	1.31	1	9800
Hexane—dioxane—water				
60 :39.2:0.8	8.8	1.20	1	6200
Benzene—dioxane—water				
70 :29.32:0.68	4.1	1.42	1.4	2000
Benzene—acetone—water				
70 :29.40:0.60	5.5	1.48	1	5000
Benzene—acetonitrile—water				
60 :38.8 :1.2	4.5	1.48	1	6800
Benzene—acetonitrile				
60 :40	12.8	1.51	4.0	300
Benzene—acetonitrile—methanol				
60 :38.8 :1.2	5.5	1.54	2.0	1500

and ethanol support the blocking of strong adsorption sites of silica gel by water, and therefore they increase the plate count (Table 2).

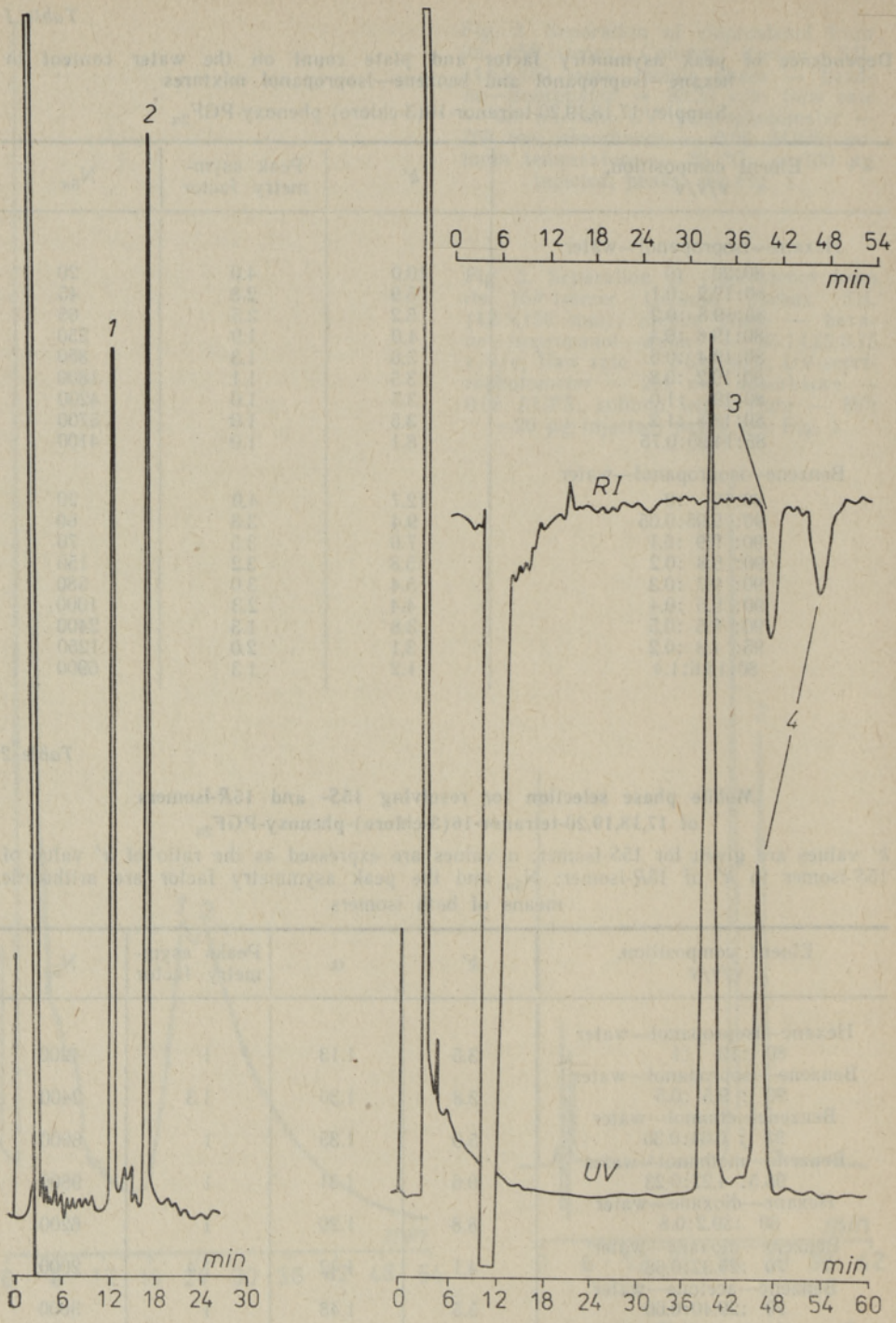


Fig. 4. Separation of cloprostenol from its 15*R*-isomer. Column Zorbax SIL (4.6×150 mm); mobile phase — benzene—acetonitrile—water = 60:38.8:1.2 v/v/v; flow rate— 0.6 ml/min; UV-spectrophotometer — 280 nm; absorbance — 0.16 AUFS; column temperature — 35°; ~30 μg injected; peaks, see Fig. 1.

Fig. 5. Separation of PGE_2 from PGE_1. Column Zorbax SIL (4.6×150 mm); mobile phase — hexane—isopropanol—water = 90:9.6:0.4 v/v/v; flow rate — 0.6 ml/min; UV-spectrophotometer — 211 nm; absorbance — 0.64 AUFS; RI-detector — 0.05×10^3 RI units full scale; column temperature — 35°; 310 μg of PGE_2 and 250 μg of PGE_1 injected; peaks, see Fig. 1.

Table 3

Data about selecting mobile phases for resolution of PGE_1 and PGE_2

k' is given for PGE_1; α is expressed as the ratio the k' value of PGE_1 to the k' value of PGE_2

Eluent composition, v/v/v	k'	α	R_s
Benzene—methanol—water 95.5: 4.32:0.18	8.7	1.21	large
Benzene—ethanol—water 94 : 5.7 :0.3	8.3	1.20	large
Benzene—isopropanol—water 92 : 7.68:0.32	4.2	1.18	1.25
Hexane—isopropanol—water 92 : 7.7 :0.3	8.6	1.17	large
Chloroform—methanol—water 96 : 3.84:0.16	7.8	1.11	0.95
Hexane—ethylacetate—water 40 : 5.94:0.6	6.6	1.11	0.95
Benzene—acetone—water 80 :19.65:0.35	9.5	1.09	0.8
Chloroform—ethanol—water 94 : 5.8 :0.2	5.4	1.07	0.8
Chloroform—isopropanol—water 92 : 7.72:0.28	4.8	1.06	0.7
Benzene—acetonitrile—water 65 :34.2 :0.8	4.3	1.05	0.6
Hexane—dioxane—water 65 :34.62:0.38	9.4	1.05	0.7
Chloroform—acetone—water 80 :19.57:0.43	12.5	1.04	07.5
Chloroform—acetonitrile—water 65 :33.95:1.05	4.5	~1.0	
Chloroform—ethylacetate—water 45 :54.26:0.74	5.3	~1.0	
Benzene—dioxane—water 80 :19.76:0.24	4.8	~1.0	
Chloroform—dioxane—water 80 :19.74:0.26	6.5	0.96	0.6

Selecting a suitable mobile phase for the resolution of PGE_1 and PGE_2 as free acids, we found that the eluting mixtures containing solvents of extremely different polarity yield the best selectivity in resolution (Table 3). It is noteworthy that a relatively high column load (Fig. 5) may be achieved with no significant decrease in column efficiency. This enables to resolve preparatively the above-mentioned compounds.

Plate counts $N_{5\sigma}$ for PGE_1 and PGE_2 were on the average 1.5 times lower than for *R/S*-isomers of cloprostenol. This was probably due to the lower water content in the mobile phase capable of producing acceptable k' values in the PGE-series (a strong solvent content in the eluting mixture should be lower than for the PGF-series).

In conclusion it may be said that further investigation is needed to elucidate the conditions of the water-deactivation of silica gels in resolving different prostaglandins and their analogs.

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PROSTANOIDIDE KÕRGEFEKTIIVNE VEDELIKU-ADSORPTSIOONKROMATOGRAAFIA. VETT SISALDAVATE LIIKUVATE FAASIDE KASUTAMINE KLOPROSTENOOLI 15*R*/*S*-ISOMEERIDE JA PROSTAGLANDIINIDE E_1 JA E_2 LAHUTAMISEKS

On näidatud kolonni efektiivsuse tõus ja piigi sümmeetrilisuse paranemine vee sisalduse kasvamisel solventsüsteemides heksaan—isopropanool ja benseen—isopropanool. Määrati 8 ja 16 vett sisaldava solventsüsteemi selektiivsus vastavalt kloprostenooli 15*R*- ja 15 *S*-isomeeride lahutamisel ja prostaglandiinide E_1 ja E_2 lahutamisel.

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АДСОРБЦИОННАЯ ВЫСОКОЭФФЕКТИВНАЯ ЖИДКОСТНАЯ ХРОМАТОГРАФИЯ ПРОСТАНОИДОВ И ИСПОЛЬЗОВАНИЕ ВОДОСОДЕРЖАЩИХ ПОДВИЖНЫХ ФАЗ ДЛЯ РАЗДЕЛЕНИЯ 15*R*/*S*-ИЗОМЕРОВ КЛОПРОСТЕНОЛА И ПРОСТАГЛАНДИНОВ E_1 И E_2

Показано, что с повышением содержания воды в сольвентных системах гексан—изопропанол и бензол—изопропанол возрастают эффективность колонки и четкость разделения пиков. Определена селективность 8 и 16 водосодержащих сольвентных систем для разделения 15*R*/*S*-изомеров клопростенола и простагландинов E_1 и E_2 соответственно.