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ISOLATION OF 5-N-ALKYL, 5-N-ALKENYL- AND 5-N-ALKDIENYL-RESORCINOL HOMOLOGS FROM RYE GRAINS

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Key words: homologs of 5-n-alk(en)yl-resorcinols, rye grains, argentation chromatography.

For the isolation of pure individual saturated, mono and di unsaturated homologs of 5-n-alk(en)ylresorcinols from the acetone extracts of rye grains the chromatographic procedure was used. This procedure employed column chromatography on silica gel followed by reversed-phase high pressure liquid chromatography and silver nitrate impregnated silica gel. The yeld of the pure homologs after rechromatography by reversed-phase high pressure liquid chromatography was about 27 per cent.

INTRODUCTION

Derivatives of 1,3-dihydroxybenzene having an aliphatic chain attached in the position „5" with an odd number and length of 15 to 25 carbon atoms were shown to be common not only in plants from families Anacardiaceae, Ginkgoaceae or Proteaceae but also in Gramineae [2, 8, 17, 18].

The aliphatic chains of these coumpounds are predominantly saturated. However, the presence of double bonds was noted [7, 18].

The 5-n-alk(en)ylresorcinols present in rye grains are supposed to be responsible for the deleterious effect of rye when fed to young rats, swines [18] and chickens [10, 11]. The isolated alkylresorcinols and alkenylresorcinols induced significant changes in the permeability of liposome and erythrocyte membranes. Moreover, the alkenylresorcinols showed also a strong lytic activity [5]. Both alkyl- and alkenyl- resorcinols induced significant changes in the membrane bilayer structure showing also an interaction with membrane proteins [6].

In order to determine the biological activity of saturated and unsaturated 5-n-alk(en)ylresorcinols and the role of the chain length there is need for an appropriate amount of isolated individual homologs.

This work presents the procedure used for the isolation of individual 5-n-alkyl-, 5-n-alkenyl- and 5-n-alkdienyl-resorcinol homologs in the preparative scale.

MATERIALS AND METHODS

Rye of the „Dańkowskie złote" variety, seed generation — elita from the 1982 harvest was obtained from the Plant Breeding Station Rogaczewo. „Pentane oil", the starting material used for the preparation was obtained from rye grains acetone extracts as described earlier [9].

THIN LAYER CHROMATOGRAPHIC ANALYSIS

In all types of thin layer chromatography, the samples were dissolved in chloroform-acetone (1:1) and 10-20 μl of each were applied on a 10× ×10 cm plates in the form of a 1 cm long line. The chromatographies were run in natural solvent vapour saturation chambers at an 8 cm distance of solvent migration.

A. Normal-phase TLC

The samples were separated on high performance thin layer chromatography (HPTLC) silica gel plates in chloroform-acetone (85:15) solvent system.

B. Argentation TLC

HPTLC silica gel plates were impregnated by spraying with 20% silver nitrate solution in 50% ethanol and then dried at 105° for 20 min. The samples were separated on the impregnated plates using a chloroform--acetone (85:15) solvent system.

C. Reversed-phase TLC

The samples were separated on HPTLC silica gel with chemically bound octyl hydrocarbon residues to the gel surface (HPTLC RP 8).

The plates were developed in a methanol-water (955:45) mixture. The separated bands were visualized by deeping the dry chromatograms into 0.05% aqueous Fast Blue B solution for a few minutes. Isolation of crude

5-n-alk(en)ylresorcinols from „pentane oil" was performed by silica gel column chromatography.

The material (ca 2 g) was dissolved in chloroform-hexane (1:1) and placed on 3×60 cm column filled with 200 g of silica gel 60 suspended in the same solvent. Elution was run first with chloroform-hexane (1:1) then with chloroform at a flow rate of 5 ml/min. About 50 ml fractions were collected and analyzed by thin layer chromatography. Fractions containing 5-n-alk(en)ylresorcinols were pooled and the solvent was evaporated in vacuo.

ARGENTATION SILICA GEL COLUMN CHROMATOGRAPHY

Silica gel 60 (200 g) dried for 4 h in 120° was mixed with 0.5 l of 6% silver nitrate solution in 80% ethanol and sirred for 40 min. The solvent was then evaporated in vacuo first at 35° then at 45° and finally at 80° for 30 min. Dry gel was stored and protected from light. For packing the column the gel was suspended in chloroform-acetone (93:7). Alk(en)yl-resorcinols (0.8 g) dissolved in 4 ml of chloroform-acetone (93:7) were applied on the column (2×70 cm). Elution was run with the same solvent at a flow of 0.5 ml/min. The effluent was monitored with a differential refractometer. The 20 ml fractions (10 ml in peaks) were collected, analyzed by TLC and evaporated in vacuo.

REVERSED-PHASE HIGH PRESSURE COLUMN CHROMATOGRAPHY

The separation of 5-n-alk(en)ylresorcinol homologs according to their aliphatic chain length was performed using procedures employing hydrophobic phase silica gels. The separation on silica gel RP 2 (ethyl groups bound to the silica gel surface) was performed with ethyl acetate-methanol (2:1) containing 27% water [4] or methanol-water (825:175) as a solvent system. The separations on silica gel RP 8 (octyl groups bound to the silica gel surface) were performed in the conditions described below.

The solvent system degased under vacuum was delivered with a pump (PMRB 10E12, Milton Roy, USA) via an injection system equipped with 1 ml sample loop (Rheodyne, Berkeley, USA) on the stainless steel column (24×2 cm I.D. — Knauer, Berlin, FRG) filled with LiChrosorb Si 60 RP 8 (7 μm particle size) silica gel. The 5-n-alk(en)-ylresorcinols in the efluent were monitored with a differential refractometer (Knauer, Berlin, FRG) connected to a multi-range pen recorder (Linseis, Selb, FRG). For the separation of 5-n-alk(en)-ylresorcinols methanol-water (955:45) mixture was used at the working inlet pressure of 65 kPa (flow rate of 10 ml/min).

GAS-LIQUID CHROMATOGRAPHIC ANALYSIS

The length of the aliphatic chains in the obtained 5-n-alk(en)ylresorcinol homologs was determined in two ways. The first was based on the relation between carbon atom number in the aliphatic chain of homologs and their retention times [7]. In the second, 5-nal(en)ylresorcinols were converted to free fatty acids by permanganate oxidation [1]. The obtained fatty acids were methylated and identified using fatty acid methyl ester standards [3].

Reagents

Silica gel 60 (Polygosil 60-63100, Macherey-Nagel, Duren, FRG), HPTLC silica gel plates for nano-TLC, HPTLC RP 8 silica gel plates for nano-TLC, LiChrosorb Si 60 RP 2, LiChrosorb 60 RP 8 (Merck, Darmstadt, GFR), Fast Blue B (Chemapol, Brno, Czechoslovakia), 5-n-pentadecylresorcinol (Aldrich, Milwaukee, USA), fatty acid methyl esters (Serva, Heidelberg, FRG). The remaining reagents used were of analytical grade.

RESULTS AND DISCUSSION

The good separation of saturated and unsaturated 5-n-alk(en)ylresorcinol species by analytical thin layer chromatography on silver nitrate impregnated silica gel [7, 15] suggested the possibility of obtaining similar results also in preparative scale column chromatography. For the isolation of individual saturated and unsaturated 5-n-alk(en)ylresorcinol homologs two procedures are possible (Fig. 1). In general these procedures differ in their sequence of argentation and reversed-phase chromatographic separations.

The results of thin layer chromatographic analysis of the crude 5-n-alk(en)ylresorcinols obtained after silica gel chromatography of „pentane oil" are shown on Fig. 2. The application of smaller silica gel particles (HPTLC plates) and of a solvent containing higher amount of acetone allowed to show in the crude 5-n-alk(en)ylresorcinols also the presence of olefinic alkylresorcinol derivatives with two double bonds (Fig. 2B) not detected previously [7]. The composition of homologs according to their aliphatic chain length (Fig. 2C) was similar to the results obtained earlier [4, 7, 12, 14].

Application of the argentation silica gel column chromatography for preparative separation of the crude 5-n-alk(en)ylresorcinols (Procedure A) resulted in an uncomplete separation of saturated, monoolefinic and diolefinic derivatives (Fig. 3A). The obtained alkyl resorcinolic fractions

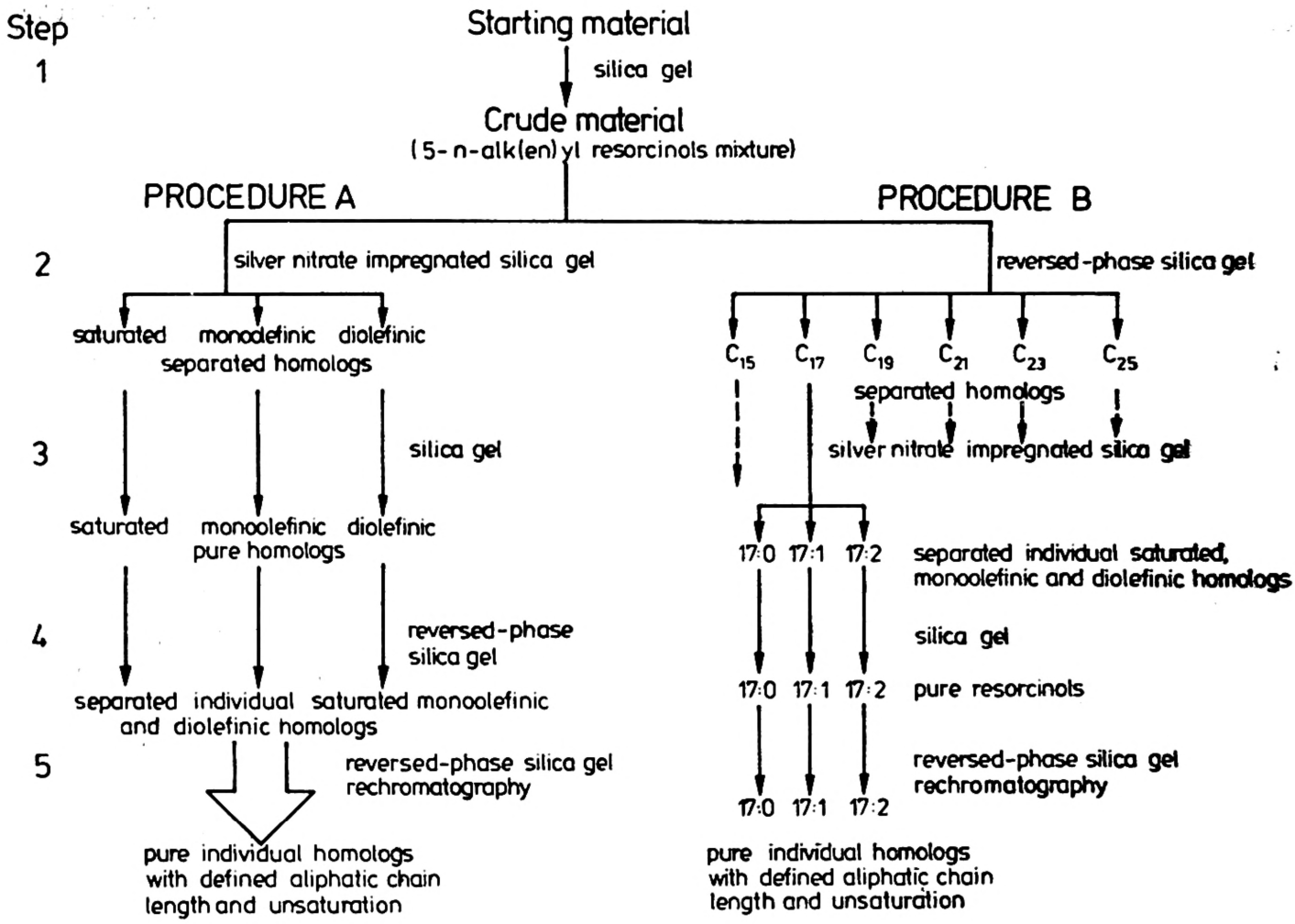

Fig. 1. Two schemes of chromatographic procedure for isolation of individual saturated, monoolefinic and diolefinic 5-n-alk(en)ylresorcinol homologs

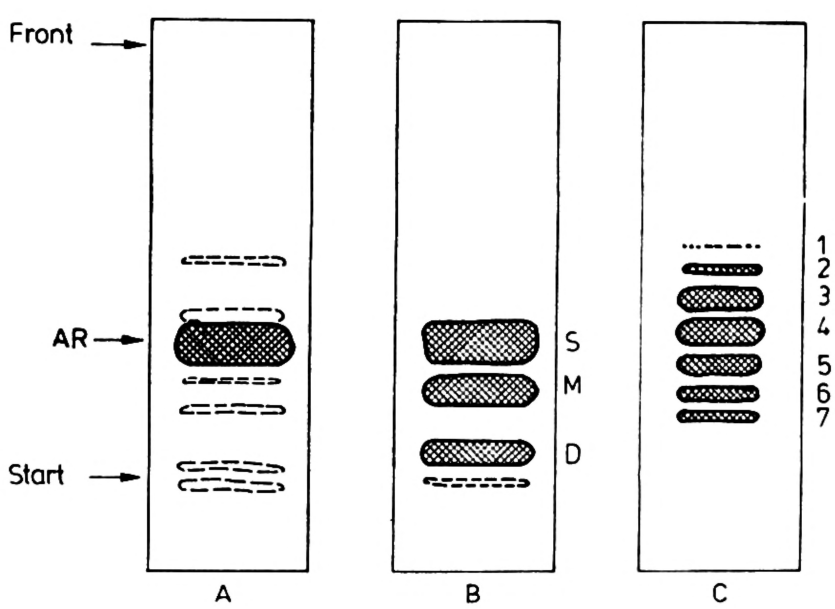

Fig. 2. Thin-layer chromatographic analysis of 5-n-alk(en)ylresorcinols mixture (crude material) obtained after silica gel chromatography of „pentane oil" (Step 1); A — silica gel, B — silver nitrate impregnated silica gel, C — reversed-phase (RP 8) silica gel, AR — 5-n-alk(en)ylresorcinols, S — saturated derivatives of 5-n-alk(en)ylresorcinols, M — monoolefinic derivatives of 5-n-alk(en)ylresorcinols, D — diolefinic derivatives of 5-n-alk(en)ylresorcinols, 1 — tridecylresorcinol, 2 — pentadecylresorcinol, 3 — heptadecylresorcinol, 4 — nonadecylresorcinol, 5 — heneicosylresorcinol, 6 — tricosylresorcinol, 7 — pentacosylresorcinol

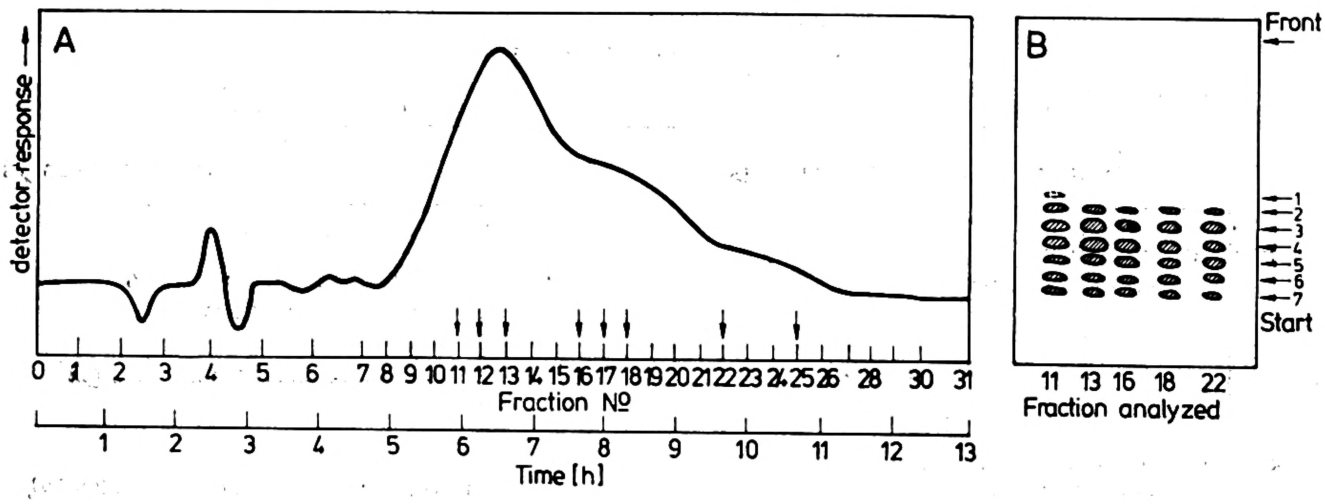

Fig. 3. The separation and TLC analysis of 5-n-alk(en)ylresorcinols mixture by silver nitrate impregnated silica gel column chromatography (Procedure A, Step 2); A. elution profile, B. reversed-phase (RP 8) silica gel TLC. Remaining abreviations as in Fig. 2.

were about 85% pure as shown by silica gel TLC but were cross-contaminated as shown by argentation silica gel TLC. This cross-contamination is supposed to be due to the tendency of homologs to separate during argentation chromatography also according to the differences in their aliphatic chain length which can be concluded from

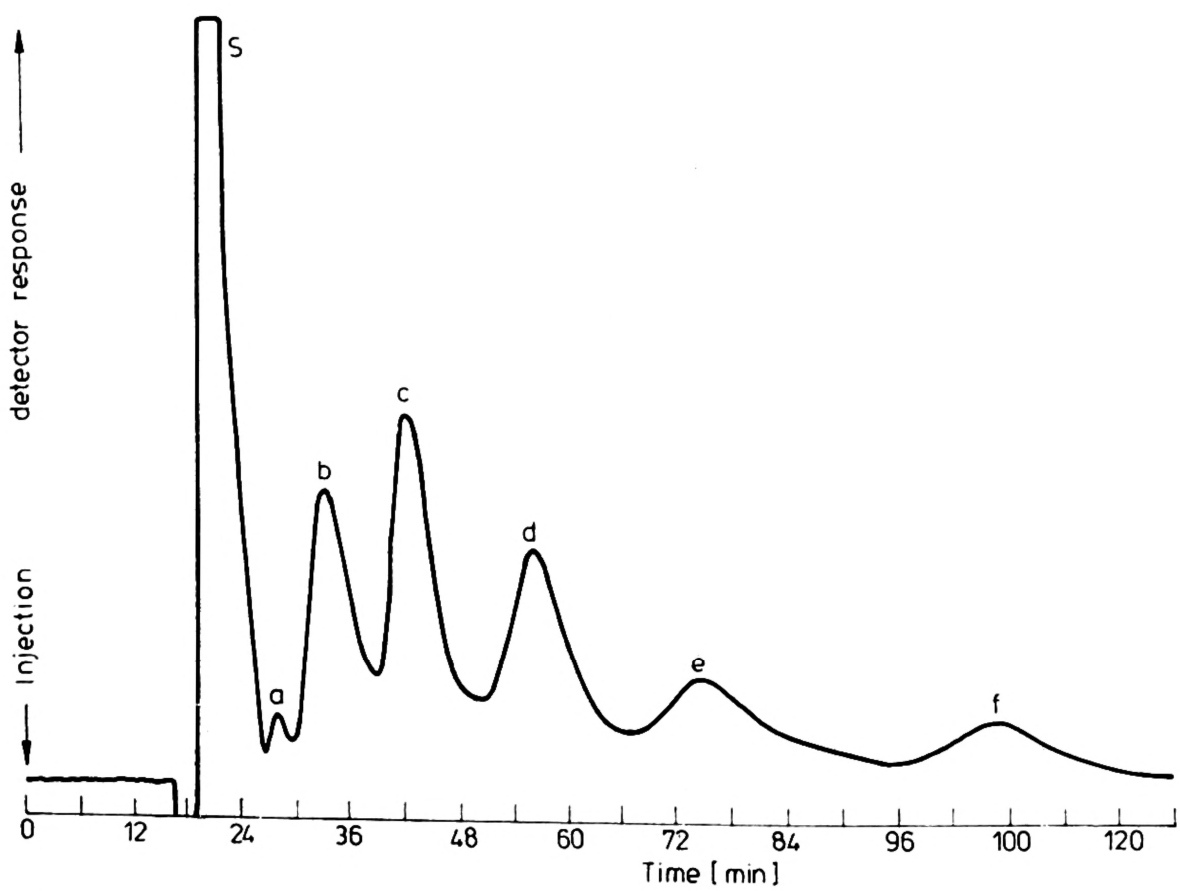

Fig. 4. Reversed-phase high performance liquid chromatographic separation of 5-n-alk(en)ylresorcinols on RP 2 (ethyl) silica gel. Solvent system — methanol:water (825:175), flow rate 2.3 ml/min, pressure 25 MPa, sample load — 50 mg in 1 ml solvent, refractometric detection; a — pentadecylresorcinol, b — heptadecylresorcinol, c — nonadecylresorcinol, d — heneicosylresorcinol, e — tricosylresorcinol, f — pentacosylresorcinol

the reversedphase TLC analysis (Fig. 3B). Similar observations were obtained during silica gel column chromatography of 5-n-alkylresorcinols [16]. For those reasons first the separation of homologs according to their chain length was employed (Procedure B).

The crude material was separated in the Step 2 on two different hydrophobic silica gels namely RP 2 and RP 8. The preparative separation of 5-n-alk(en)ylresorcinol homologs was achieved with both types of stationary phases (Fig. 4, Fig. 5A) although the separation on RP 2 silica

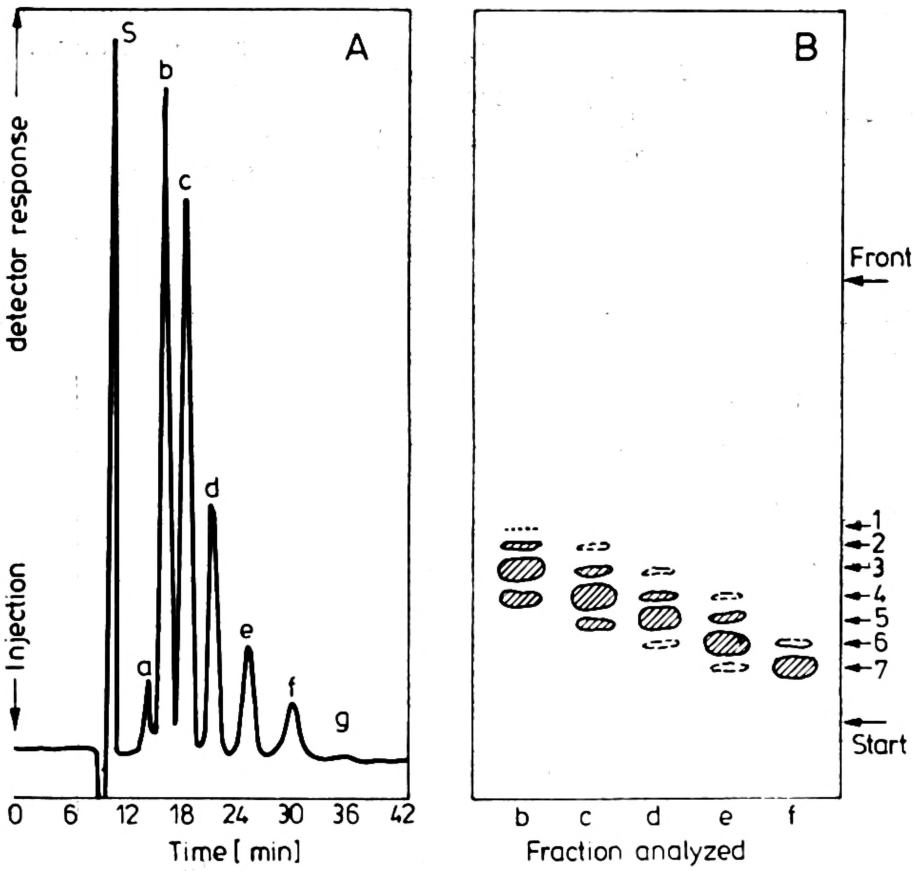

Fig. 5. Reversed-phase high performance liquid chromatographic separation of 5-n-alk(en)ylresorcinols on RP 8 (octyl) silica gel and TLC analysis of obtained fractions (Procedure B, Step 2). A. elution profile. Solvent system — methanol:water (955:45), flow rate 10 ml/min, pressure 6.5 MPa, sample load 120 mg in 1.2 ml solvent, refractometric detection; B. reversed-phase (RP 8) silica gel TLC. Remaining abreviations as in Fig. 2.

featured a few disadvantages over the separation on RP 8 silica. The separation on RP 2 silica was more time consuming than on RP 8 silica (about four times longer). Due to lower hydrophobicidity of RP 2 silica as high an amount of water in the solvent as 27% had to be used [4]. In the course of this work, the methanol-water (825:175) solvent system was found to give similar results of separation on RP 2 silica. On the other side, the high amount of water in the solvent systems used for RP 2 induced significant reduction of 5-n-alk(en)ylresorcinols solubility which caused a decrease of the sample load to 50 mg per run.

The separation of 5-n-alk(en)ylresorcinols on more hydrophobic silica gel required a significant decrease of water content in the solvent because of a stronger interaction of solute with hydrophobic support. For the

separation of 5-n-alk(en)ylresorcinol homologs on RP 8 silica 4.5% of water in the solvent system was sufficient (Fig. 5A). The small amount of water in the solvent system resulted in an increase of sample solubility and as a consequence in an increase of the sample load (up to 150 mg per run).

Table. An example of preparation of individual paraffinic and olefinic homologs of 5-n-alk/en/ /ylresorcinol with defined aliphatic chain length (Procedure B)

Step	Procedure	Material weight mg	Alk/en/yl resorcinols content mg	Purity of material %		Yield mg
				alk/en/yl resorcinols	impurities	
	Starting material — „pentane oil"*	1500	450**	30	70	100
1	silica gel chromotography (crude material)	430	345	80	20	76.6
2	reversed-phase chromotography	395	337	85	15	74.8
3	silver nitrate impregnated silica gel chromatography	146	132	90	10	29.3
4	second silica gel chromotography	129	129	100	0	28.8
5	second reversed-phase chromotography	123	123	100	0	27.4

* Pentane oil was obtained from rye grains with the procedure of Mejbaum-Katzenellenbogen et al [9].
**Alk/en/ylresorcinols content was determined with the colorimetric micromethod of Tłuścik et al [13].

The obtained separated homologs in which 5-n-heptadec(en)yl- and 5-n-nonadec(en)yl- recorcinols were the major constituents were approximately 85% pure as shown by silica gel TLC. The presence of saturated, monoolefinic and diolefinic alk(en)ylresorcinols in each homolog fraction was demonstrated by argentation silica gel TLC. Reversed-phase TLC analysis of separated peaks showed in each one the presence of small amounts of homologs with shorter and longer aliphatic chains (Fig. 5B). These results suggest that the presence of contaminating homologs may be due to the tendency of saturated and olefinic homologs for separation also during reversed-phase silica gel chromatography. That suggestion was supported with the results obtained from the separation of homologs with higher water content in the solvent system (Fig. 6). Similar

observation was obtained during separation of 5-n-alk(en)ylresorcinol homologs by aluminium oxide thin layer chromatography [14].

The saturated, monoolefinic and diolefinic derivatives of each homolog obtained from reversed-phase column were isolated by argentation silica gel column chromatography (Step 3). A typical elution profile obtained

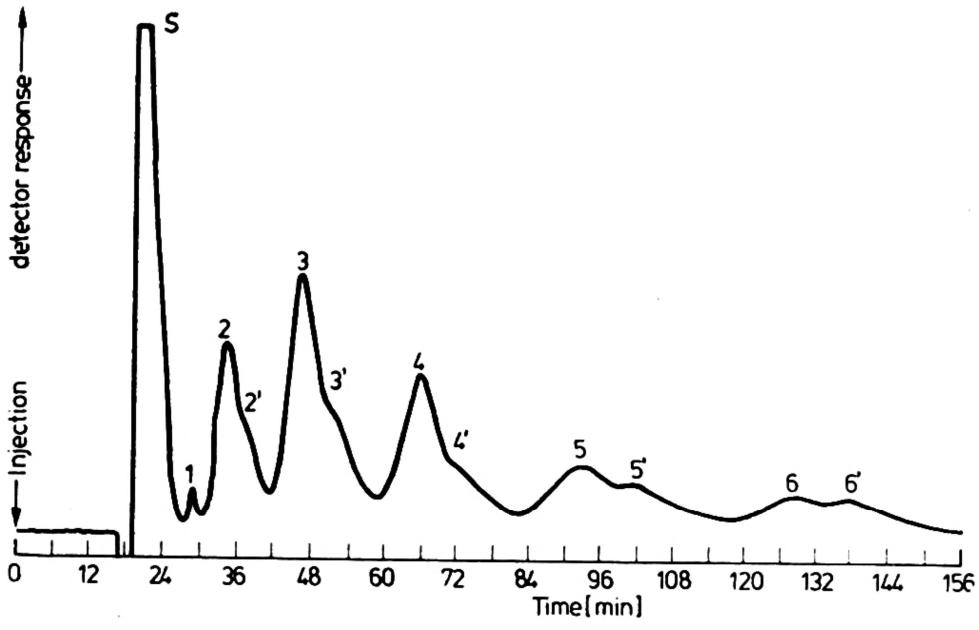

Fig. 6. Reversed-phase high performance liquid chromatographic separation of 5-n-alk(en)ylresorcinols on RP 2 silica gel in a more polar solvent system. Solvent system — methanol:water (80:20), flow rate 2.2 ml/min, pressure 25 MPa, sample load — 50 mg in 1 ml solvent, refractometric detection. Remaining abreviations as in Fig. 2.

is shown on Fig. 7A. The fractions containing only pure saturated, monoolefinic or diolefinic derivatives were pooled and used for further purification. The results of TLC analysis are shown on Fig. 7B, C.

The rechromatography of the materials obtained after argentation chromatography on silica gel column (Step 4) resulted in alkyl- alkenyl- or alkdienyl- resorcinols free of non resorcinolic contaminations but still crosscontaminated with the other homologs. As an example the results of TLC analysis of 5-n-heptadecenylresorcinol are shown on Fig. 8.

For removal of the longer and shorter chained contaminated homologs, each of the individual saturated or unsaturated homologs was rechromatographed on reversed-phase silica gel column (Step 5). The rechromatographic patterns of 5-n-heptadecenylresorcinol are shown as an example (Fig. 9). This step resulted in a practically pure individual saturated, monoolefinic and diolefinic homologs with defined aliphatic chain length which was showed both by TCL (Fig. 10) and GLC analysis (Fig. 11).

A typical record of the preparation procedure is showed in Table. In the course of the described procedure the mixture of saturated, monoolefinic and diolefinic alkylresorcinols with different chain length was purified (from 30% to 100%) and separated into individual homologs with the yield of 27%. It can be seen that argentation silica gel column

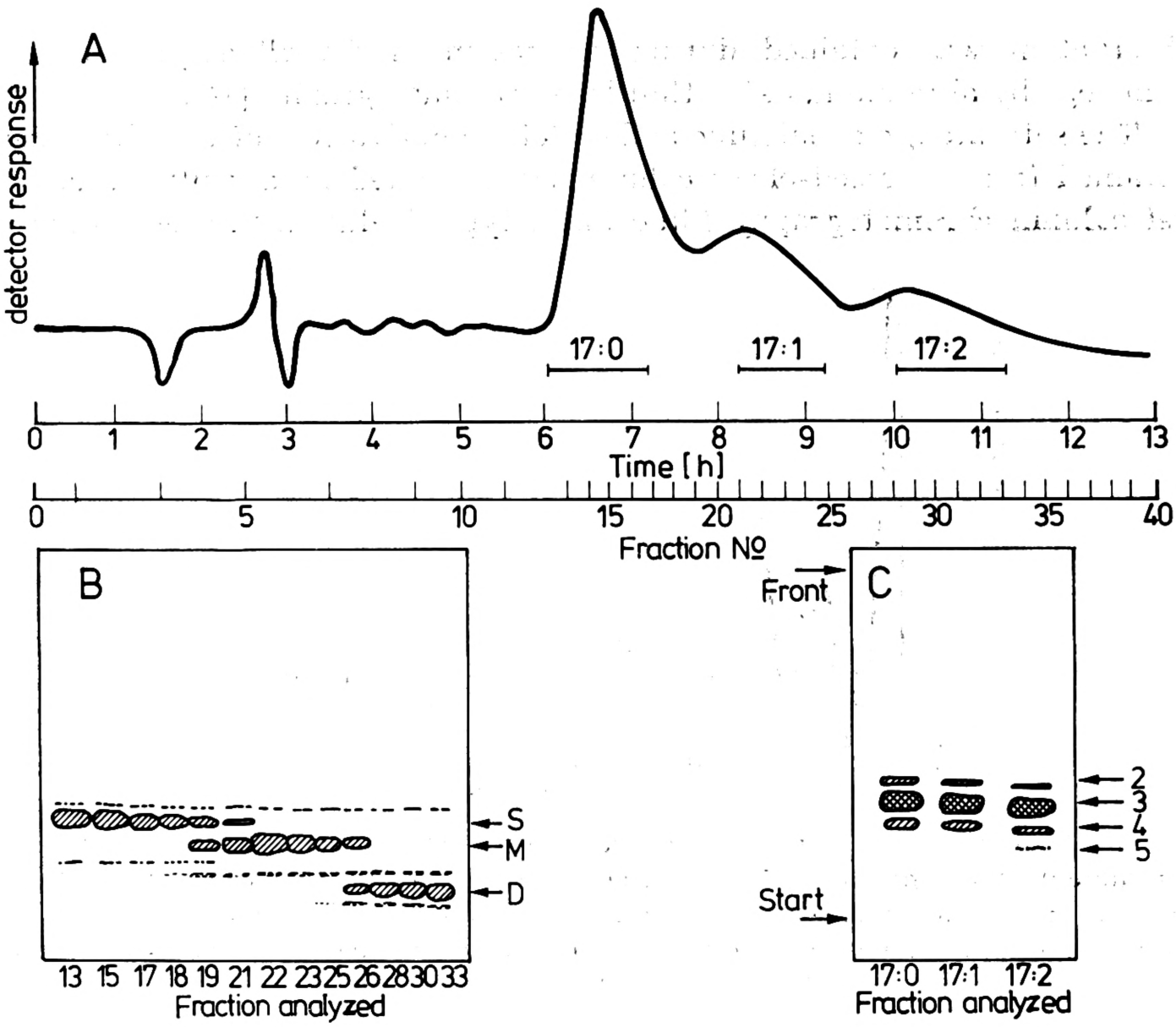

Fig. 7. Separation of 5-n-heptadec(en)ylresorcinol by silver nitrate impregnated silica gel column chromatography (Procedure B, Step 3); A. elution profile, B. silver nitrate impregnated silica gel TLC, C. reversed-phase (RP 8) silica gel TLC. Bars represent fractions conraining pure saturated, monoolefinic or diolefinic hologs which were pooled. Abreviations as in Fig. 2.

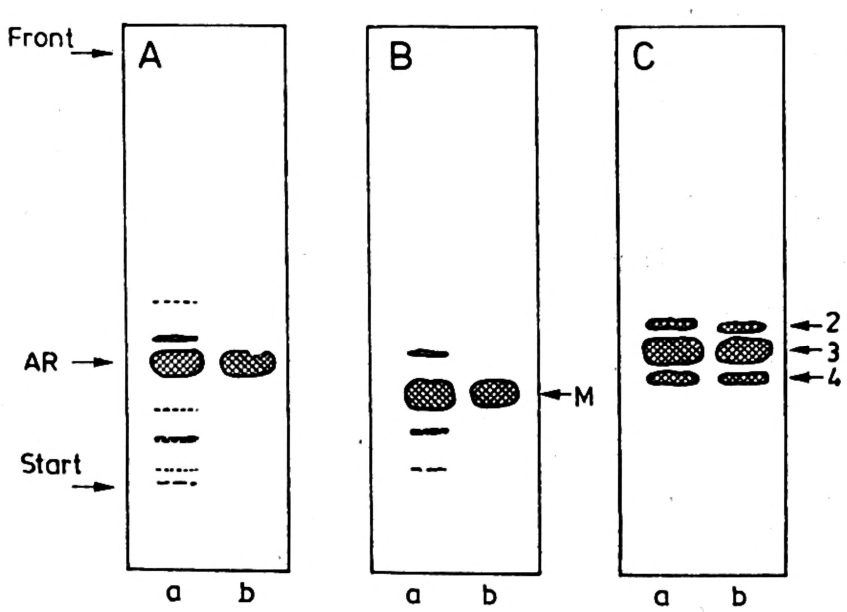

Fig. 8. Thin-layer chromatographic analysis of 5-n-heptadecenylresorcinol before and after purification by silica gel column chromatography (procedure B, Step. 4); A. silica gel TLC, B. silver nitrate impregnated silica gel TLC, C. reversed-phase (RP 8) silica gel TLC; a — before, b — after purification. Abreviations as in Fig. 2.

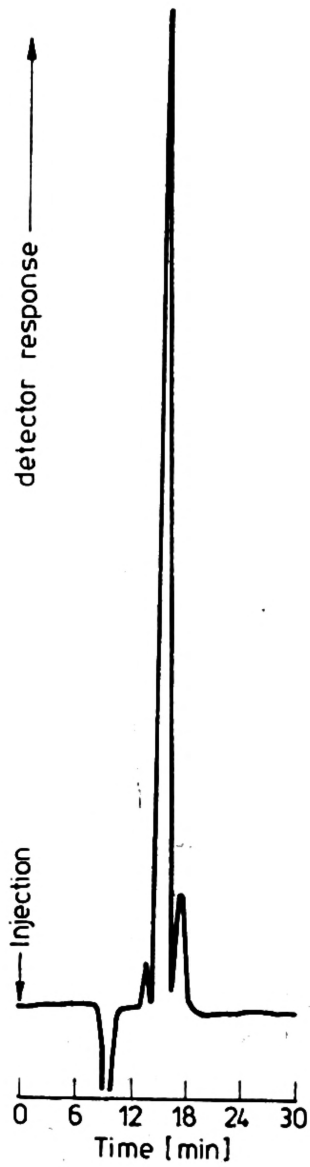

Fig. 9. Reversed-phase high performance liquid chromatographic rechromatography of 5-n-heptadecenylresorcinol on RP 8 (octyl) silica gel (Procedure B, Step 5); solvent system — methanol:water (955:45), flow rate 10 ml/min, pressure 6,5 MPa, sample load 100 mg in 1.2 ml solvent, refractometric detection.

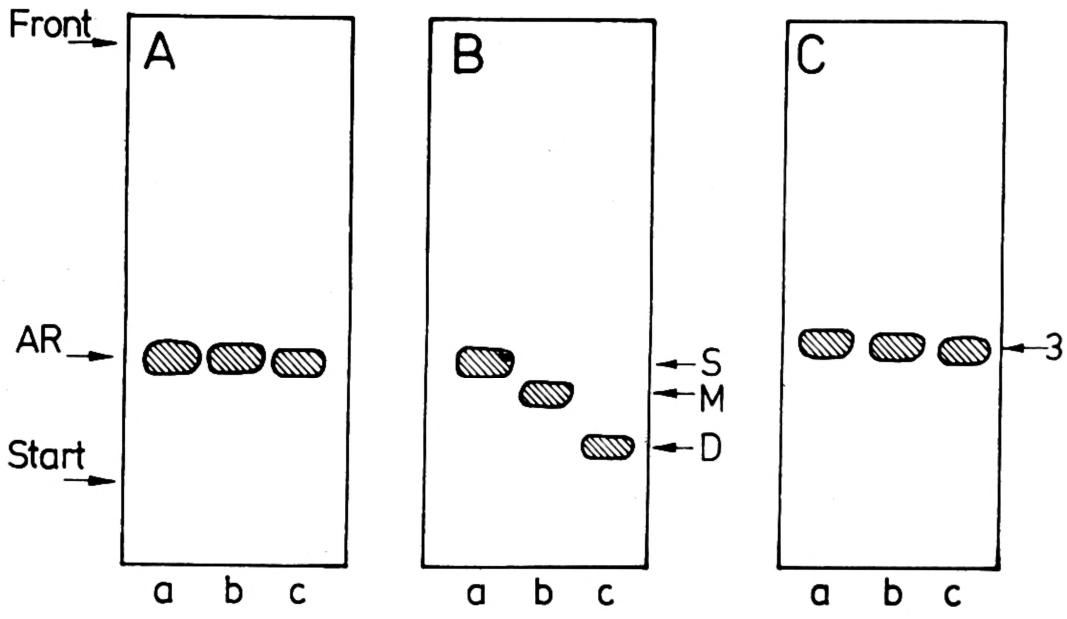

Fig. 10. Thin-layer chromatographic analysis of individual paraffinic and olefinic hologs of 5-n-heptadec(en)ylresorcinol obtained after rechromatography by reversed-phase column chromatography; A. silica gel TLC, B. silver nitrate impregnated silica gel TLC, C. reversed-phase (RP 8) silica gel TLC; a — 17:0 alkyl resorcinol, b — 17:1 alkyl resorcinol, c — 17:2 alkyl resorcinol. Remaining abreviations as in Fig. 2.

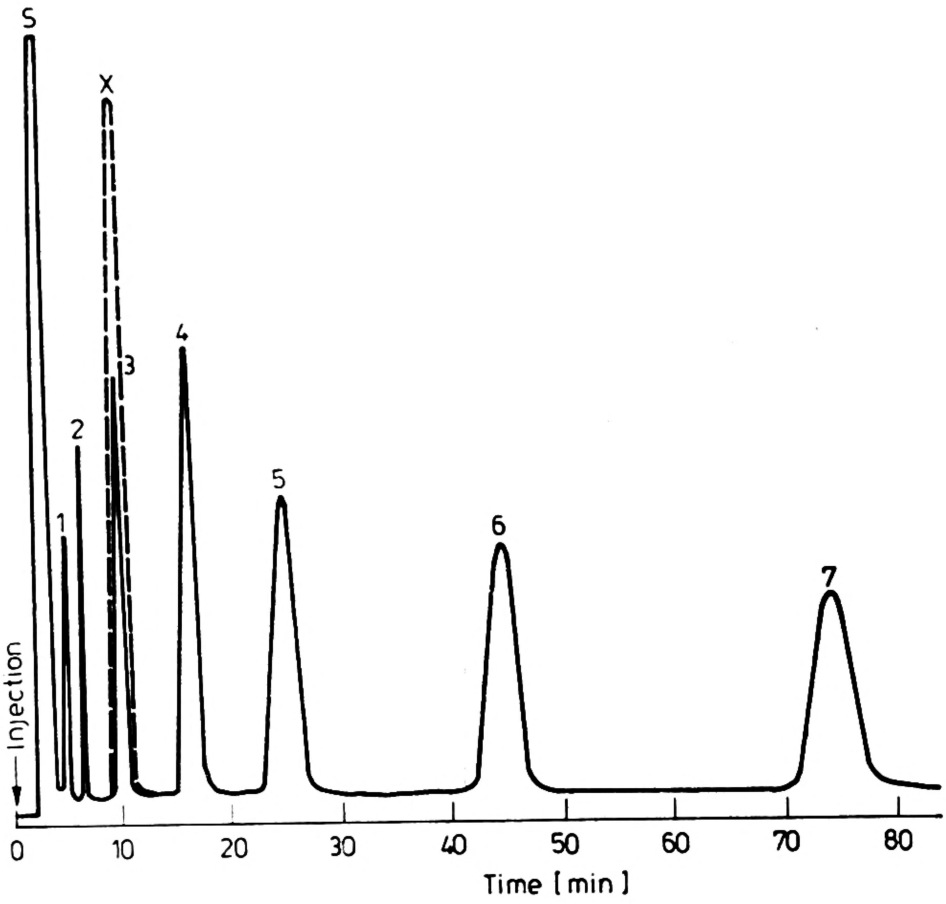

Fig. 11. Gas-liquid chromatographic analysis of fatty acid methyl esters obtained after permanganete oxidation of 5-n-heptadecylresorcinol. Conditions of separation are presented in the Materials and methods section; 1 — methyl myristate, 2 — methyl palmitate, 3 — methyl stearate, 4 — methyl arachidate, 5 — methyl behenate, 6 — methyl lignocerate, 7 — methyl cerotate, X — methyl ester of fatty acid obtained after oxidation of 5-n-heptadecylresorcinol, S — solvent

chromatography was the most limiting step of the yield of the procedure. The employment of high pressure argentation silica gel chromatography on smaller silica gel particles could improve the separation efficiency and decrease the separation time as done recently for the separation of urushiol diacetates (3-n-alk(en)ylcatechol diacetates) [19].

Isolated by the described procedure pure homologs were used for the study of the influence of the 5-n-alk(en)ylresorcinols on biological membranes. The results of the study on the perturbation of erythrocyte membrane osmotic properties induced by these compounds are to be published.

The procedure presented in this paper could have also a general application for isolation of plant phenolic materials which are usually highly heterogeneous because of differences in chain length and unsaturation of homologs.

CONCLUSIONS

1. Described procedure allows the preparative-scale isolation of pure saturated and unsaturated homologs of 5-n-alk(en)ylresorcinols with the defined aliphatic chain length from the cereal grains.

2. For the separation of 5-n-alk(en)ylresorcinol homologs both RP 2 and RP 8 silica gels can be used but RP 8 allowed the significant decrease of the separation time.

3. As the homologs tend to separate according to the differences in their aliphatic chain length during argentation chromatography, this separation step should be proceeded by separation of each homologues according to their chain length.

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IZOLACJA 5-N-ALKILOWYCH, 5-N-ALKENYLOWYCH ORAZ 5-N-ALKDIENYLOWYCH HOMOLOGÓW REZORCYNOLU Z ZIARNIAKÓW ŻYTA

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Streszczenie

W celu preparatywnej izolacji czystych nasyconych, mono oraz dwu nienasyconych homologów 5-n-alk(en)ylorezorcynolu o określonej długości łańcucha alifatycznego z ziarniaków żyta zastosowano postępowanie chromatograficzne. Stwierdzono, że mieszanina hologów 5-n-alk(en)yloretorcynolu wykazuje podczas rozkładu w kolumnowej chromatografii argentacyjnej tendencję do rozdziału nie tylko w zależności od stopnia nasycenia łańcucha alifatycznego, lecz także w zależności od różnic w długości tego łańcucha. W opracowanej procedurze zastosowano więc następującą kolejność rozdziałów chromatograficznych: chromatografia na żelu krzemionkowym, wysokociśnieniowa chromatografia na hydrofobowym żelu krzemionkowym, chromatografia argentacyjna na żelu krzemionkowym, rechromatografia na żelu krzemionkowym oraz hydrofobowym żelu krzemionkowym. Wykazano, że do rozdziału 5-n-alk(en)ylorezorcynoli w zależności od długości łańcucha alifatycznego korzystne jest użycie silnie hydrofobowego żelu krzemionkowego. Umożliwia to znaczne skrócenie czasu rozdziału, poprawia jego jakość oraz umożliwia zwiększenie wielkości próbki rozdzielanej jednorazowo do 150 mg (przy użyciu kolumny o wymiarach 24×2 cm). Podane postępowanie dało w wyniku poszczególne czyste nasycone i nienasycone homologi z wydajnością ok. 27%. Stwierdzono, że najbardziej ograniczającym wydajność preparacji krokiem była normalnociśnieniowa chromatografia argentacyjna, której wydajność była rzędu 25%.