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Journal of Chromatography A, 755 (1996) 299–307

JOURNAL OF CHROMATOGRAPHY A

Short Communication

Enantiomer separation on a Chirasil-Dex-polymer-coated stationary phase by conventional and micro-packed high-performance liquid chromatography

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Received 6 September 1995; revised 3 July 1996; accepted 5 July 1996

Abstract

Polymer-coating of Chirasil-Dex (a polysiloxane-anchored permethyl-β-cyclodextrin) on Nucleosil 300-5 followed by thermal immobilization furnishes a chiral stationary phase for enantiomer separation under reversed-phase conditions by conventional and micro-packed HPLC.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Drugs; Pesticides; Organochlorine compounds

1. Introduction

For enantiomer separation by HPLC, cyclodextrins (CDs) and their derivatives are employed both as chiral mobile phase additives [1] and as chiral stationary phases (CSPs) in the reversed-phase [2–5], normal-phase [6] or polar-organic-phase mode [7].

CDs and their derivatives used as CSPs in HPLC are directly bonded to macroporous silica through a spacer. Polymer-coating of silica with various polysiloxanes represents a complementary approach to link a selector to a silica support and this strategy is employed for achiral [8] and chiral [9,10] separations in HPLC. The linkage of chiral selectors to polysiloxanes with the potential of cross-linking leads to chemically inert gum-like chiral stationary phases suitable for immobilization onto non-porous (glass, fused-silica) or porous (silica) surfaces. The polysiloxane backbone creates a hydrophobic environment on silica and it effectively blocks free silanol groups.

Chirasil-Dex consists of a permethylated β-cyclodextrin covalently linked via a single octamethylene spacer to a dimethylpolysiloxane containing residual Si–H moieties which contribute to the cross-linking on the support surface (Fig. 1) [11].

The immobilization of Chirasil-Dex on a fused-silica surface [12] is the prerequisite of its use for enantiomer separation by supercritical fluid chromatography (SFC) [11], capillary electrochromatography (CEC) [13,14], open-tubular liquid chromatography (OT-LC) [15] and a unified approach thereof including gas chromatography (GC) [16,17]. Moreover, Chirasil-Dex can be immobilized thermally onto macroporous silica [18]. Thus, it has previously

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0021-9673/96/$15.00
PII S0021-9673(96)00618-8

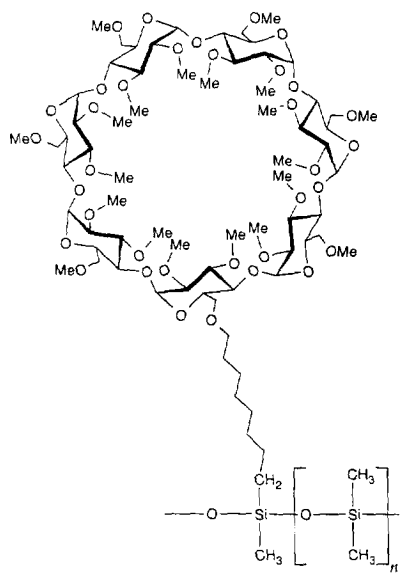


Fig. 1. Chirasil-Dex (polysiloxane anchored permethyl-β-cyclodextrin) [11].

been found that polymer-coated Nucleosil 300-5 containing Chirasil-Dex yields a hydrophobic (lipophilic) hydrolytically stable chiral stationary phase suitable for enantiomer separation by HPLC [16–19].

Here we describe in detail the preparation and liquid chromatographic performance of this polymer-coated chiral stationary phase under reversed-phase conditions, including the improvement of immobilization by applying an acidic polysiloxane copolymer [20,21]. A series of chiral analytes including hypnotic sedatives, anticoagulants, herbicides and organochlorines were separated on the present chiral stationary phase by normal and micro-packed HPLC [19].

2. Experimental

2.1. Materials

The synthesis of Chirasil-Dex has been carried out according to refs. [22,23]. In the present work, a 40% (w/w) mixture of mono-6-(oct-7-enyl)-permethyl-β-cyclodextrin and polydimethyl-methylhydro-siloxane (molecular mass ~3000) was subjected to platinum-catalyzed hydrosilylation. The polydimethylsiloxane, containing an average of 10% Si–H groups, was obtained by a 3:7 mixing of a polydimethylsiloxane with 20% Si–H groups and a polydimethylsiloxane with 7% Si–H groups, respectively [23]. The Chirasil-Dex thus obtained showed a specific rotation of $[\alpha]_D^{20}$ 54.5 (c=0.8, dichloromethane). The cyclodextrin content (~35%, w/w) of Chirasil-Dex was calculated by comparing the specific rotation of the pure chiral selector, i.e. permethylated-6-O-mono-(oct-7-enyl)-β-cyclodextrin: $[\alpha]_D^{20}$ 149 (c=0.8, dichloromethane) according to Ref. [23]. Spherical Nucleosil (4 g) (Macherey–Nagel, Düren, Germany) of 300 Å pore diameter and 5 μm mean particle diameter were dried by azeotrope distillation with toluene and then coated with 1 g chiral stationary phase (20%, w/w) consisting of a 10:1 mixture of Chirasil-Dex and an acidic copolymer in dichloromethane solution (approx. 15 ml). The copolymer, a precursor of Chirasil-Val, was prepared from poly[(2-methyl-propanoic acid)-methylsiloxane], octamethylcyclotetrasiloxane (1:2) and hexamethyldisiloxane (for end-capping) according to Ref. [21] and was employed in order to improve the immobilization on silica. After removal of the solvent with reduced pressure at room temperature, the coated material was submitted to a thermal immobilization procedure performed at high vacuum and 190°C for 24 h. The non-immobilized portion of Chirasil-Dex and traces of unreacted β-cyclodextrin, if any, were removed by washing the beads successively with methanol, dichloromethane and diethyl ether. The carbon analysis of the packing material (%C=6.42) indicated an 80% degree of immobilization. An approximate film thickness of 2.5 nm was calculated taking into account the 80% degree of immobilization, the silica specific surface of 100 m^2/g and assuming that the density of Chirasil-Dex is 1 g/cm^3.

The solvents methanol, acetonitrile and water were gradient grade for chromatography (LiChrosolv, Merck, Darmstadt, Germany). The analytes were dissolved in the same solvent (approx. 1 mg/ml) used as starting eluent, and were filtered through a 0.45-μm Chromafil filter (Macherey–Nagel).

Table 1
Chromatographic conditions and parameters for the separation of enantiomers on Chirasil-Dex-polymer-coated Nucleosil 300-5 by HPLC

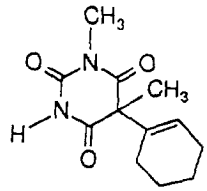
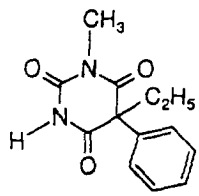
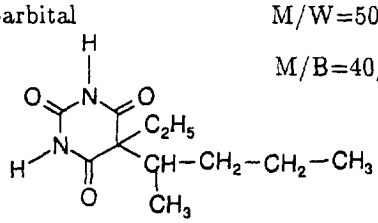
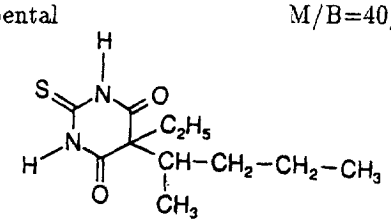
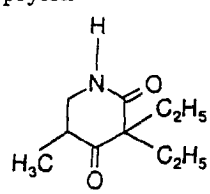
Compound	Mobile phase[a] (v/v)	Flow-rate (ml/min)	UV (nm)	t_{R2}[b] (min)	k_2[b]	α	R_s
Hexobarbital 	M/W=50/50	0.4	240	14.6	1.43	1.69	3.8
	M/B=50/50	0.4	240	14.1	1.34	1.67	3.3
Mephobarbital 	M/W=50/50	0.4	240	17.4	1.90	1.73	4.5
	M/B=50/50	0.4	240	16.6	1.77	1.71	4.1
Pentobarbital 	M/W=50/50	0.3	240	23.5	2.35	1.11	1.0
	M/B=40/60	0.4	240	32.8	4.47	1.13	1.4
Thiopental 	M/B=40/60	0.4	240	31.2	4.21	1.14	1.4
Methyprylon 	M/W=50/50	0.5	240	17.2	2.45	1.70	4.3
	M/B=50/50	0.5	240	12.8	1.55	1.74	4.1

Table 1. Continued

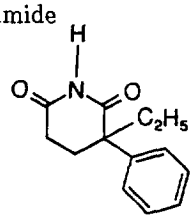
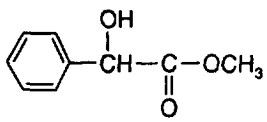
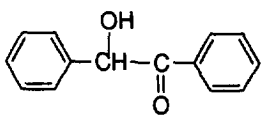
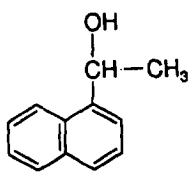
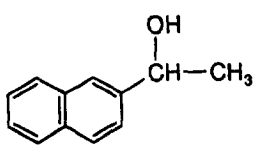
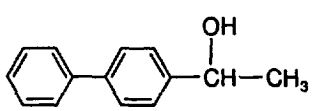
Compound	Mobile phase[a] (v/v)	Flow-rate (ml/min)	UV (nm)	t_{R2}[b] (min)	k_2[b]	α	R_s
Glutethimide 	M/W=50/50	0.4	240	14.9	1.50	1.20	1.4
	M/B=40/60	0.4	240	27.3	3.56	1.25	2.3
Methyl mandelate 	M/B=25/75	0.5	254	12.9	1.59	1.25	1.7
Benzoin 	M/B=30/70	1	225	15.3	5.13	1.26	1.5
1-(1-Naphthyl)ethanol 	M/B=35/65	1	254	28.5	10.39	1.16	1.7
1-(2-Naphthyl)ethanol 	M/B=30/70	1	254	44.3	16.74	1.12	1.4
1-(4-Biphenyl)ethanol 	M/B=45/55	1	254	36.2	3.48	1.12	1.5

Table 1. Continued

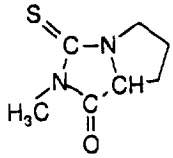
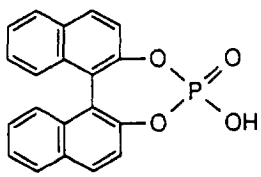
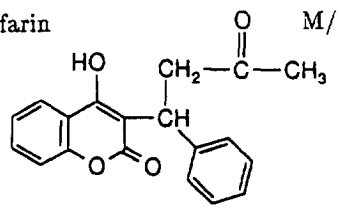
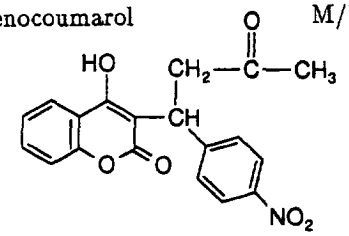
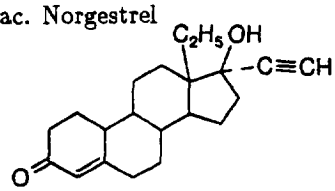
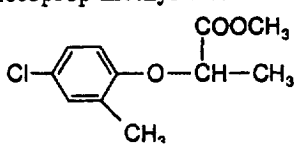
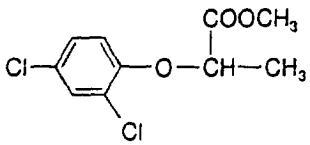
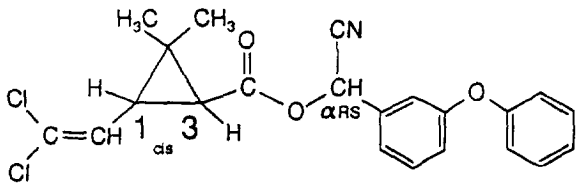
Compound	Mobile phase[a] (v/v)	Flow-rate (ml/min)	UV (nm)	t_{R2}[b] (min)	k_2[b]	α	R_s
MTH-Proline 	M/B=30/70	0.5	220	15.9	2.20	1.52	2.3
1,1'-Binaphthyl-2,2'-diyl hydrogen phosphate 	M/B=20/80	1.2	300	83.5	40.76	1.07	1.3
Warfarin 	M/B=30/70	1	225	26.6	9.63	1.18	1.4
Acenocoumarol 	M/B=30/70	1	225	36.7	13.69	1.19	1.3
rac. Norgestrel 	M/B=45/55	1	254	21.1	7.43	1.19	1.5
Mecoprop methyl ester 	M/B=50/50	0.2	254	24.5	2.06	1.46	2.1

Table 1. Continued

Compound	Mobile phase[a] (v/v)	Flow-rate (ml/min)	UV (nm)	t_{R2}[b] (min)	k_2[b]	α	R_s
Dichlorprop methyl ester 	M/B=50/50	0.2	254	13.5	0.68	1.39	1.3
Alphacypermethrin 	M/W=80/20	1	220	11.1	2.42	1.06	1.0

[a] The mobile phases used were mixtures: M/W–methanol/water and M/B–methanol/phosphate buffer, pH=4.5.
[b] Second eluted enantiomer.

2.2. Chromatographic conditions

2.2.1. Conventional packed columns

Columns were prepared by slurry packing of the above described material at constant pressure into 200×4.6 mm I.D. stainless steel tubes. For this purpose a Shandon solvent delivery system (Shandon Labortechnik, Frankfurt am Main, Germany) was employed. The packing material was suspended by ultrasonication in the slurry solvent methanol. The packing solvent, methanol, was pushed through the column at a packing pressure of 450 bar. The standard chromatographic set-up comprised a Chrompack CP ISOS GRAS Model HPLC pump (Chrompack, Middelburg, Netherlands), a Rheodyne 7125 Model syringe loading sample injector having a 20-μl internal volume (Rheodyne, Cotati, CA, USA) and a Chrompack UV-var detector. Data acquisition and evaluation was performed using a Chrompack Control and Integration System (PCI, Chrompack).

2.2.2. Packed capillary columns

For packing the capillaries the same Chrompack CP ISOS GRAS Model pump was used. The untreated fused-silica capillary (150×0.25 mm I.D.) was end-connected to a Valco fitting (1/16 in.; 1 in.=2.54 cm) (Valco Instruments, Houston, TX, USA), containing inside a very thin Valco stainless steel sieve (pores 2 μm), and was tightened by using a 3-cm piece of a PEEK-sleeve. The other end was installed into the packing cartridge, a 40×2 mm I.D. stainless steel column. The pressure was raised to 300 bar for 30 min by using a 0.3 ml/min flow-rate and then slowly decreased during 3–4 h under permanent ultrasonication. After total release of the pressure, the column was removed from the reser-

voir, checked for complete filling with the packing material, and installed into the injector for conditioning and testing, by using a second Valco fitting. The flow of the pump was split before injection (split=1:70) permitting the work under recycle conditions. A replaceable in-line filter, employed for protecting the column against possible impurities from solvents or samples, was installed after the split unit. In order to minimize the peak broadening caused by injection and detection in the capillary LC system, a 60-nl internal loop Valco injection valve and a fused-silica capillary bubble-type detector flow cell (tubing 50 μm I.D., bubble 250 μm I.D., Grom Analytik, Herrenberg, Germany) were employed, respectively.

Results and discussion

By employing a 200×4.6 mm I.D. stainless steel column packed with Chirasil-Dex, polymer-coated on Nucleosil, the enantiomers of various chiral compounds such as anticonvulsants and hypnotic sedatives, anticoagulants, insecticides and herbicides can be separated by HPLC under reversed-phase conditions. Chromatographic data are summarized in Table 1 and representative examples are shown in Fig. 2.

In Fig. 3, the influence of the type and amount of the organic modifier on the enantiomer separation of hexobarbital on the Chirasil-Dex-polymer-coated Nucleosil is demonstrated. The retention time (t_R) and the peak resolution (R_s) decrease with increasing amounts of the modifier, as shown for methanol, and strongly decrease in the order methanol<ethanol< acetonitrile<2-propanol. Therefore, methanol has been used as organic modifier for all subsequent investigations.

For each compound the separation was optimized either by varying the amount of methanol or by replacing water by phosphate buffer (pH 4.5) in the mobile phase (Table 1). For hexobarbital the retention factor k, separation factor α and resolution R_s increase with decreasing the temperature ($\alpha=1.22$ at 30°C, $\alpha=1.45$ at 10°C and $R_s=1.6$ at 30°C, $R_s=3.2$ at 10°C).

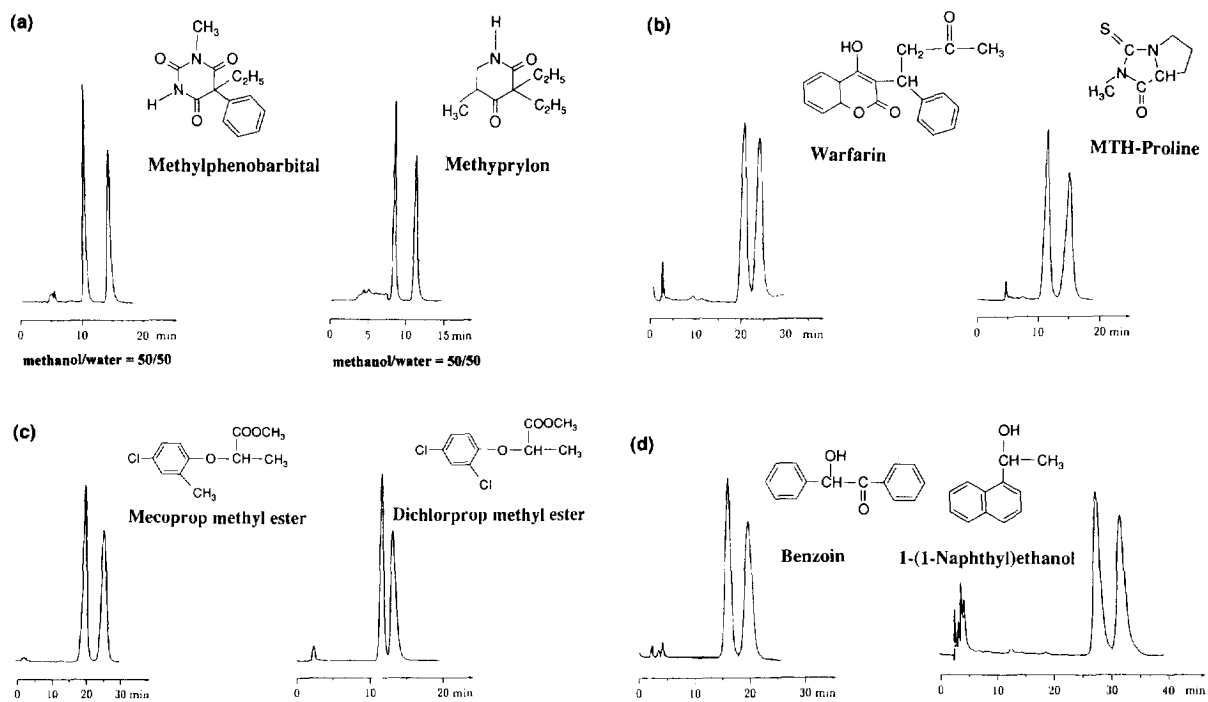


Fig. 2. Examples of enantiomer separations on a 200×4.6 mm I.D. stainless steel column packed with Chirasil-Dex-polymer-coated Nucleosil by HPLC. Chromatographic conditions as in Table 1.

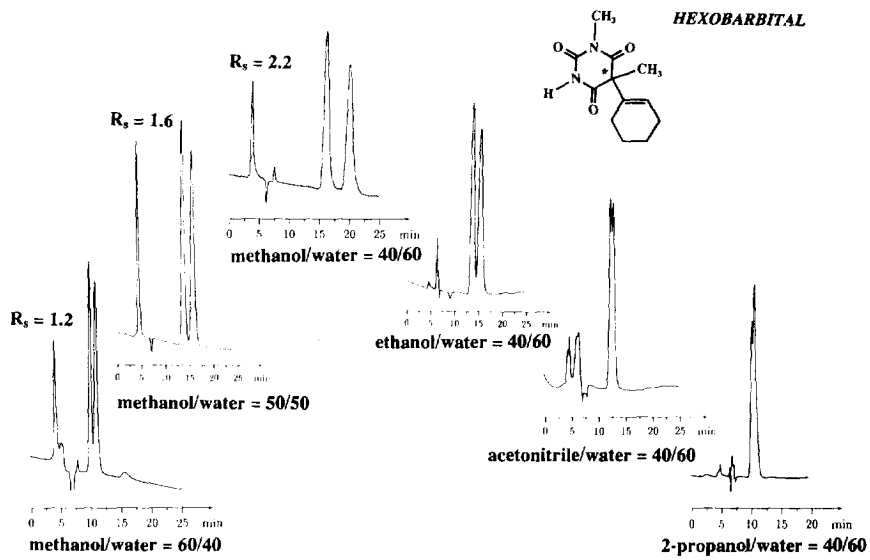


Fig. 3. Influence of the type and amount of the organic modifier on the enantiomer separation of hexobarbital on Chirasil-Dex-polymer-coated Nucleosil by HPLC. Conditions: 200×4.6 mm I.D. stainless steel column, 30°C, 0.3 ml/min.

The atropisomers of 2,2′,3,3′,4,6′-hexachlorobiphenyl, PCB 132, have been separated both semi-preparatively, allowing the determination of the enantiomerization barrier [24], and analytically (Fig. 4) on Chirasil-Dex by HPLC.

Miniaturization is an attractive trend in HPLC [25–28]. Advantages are (i) improvement in mass sensitivity for concentration-dependent detectors such as the UV detector in the analysis of biological samples [29], (ii) low flow-rates facilitating the use of refined detection systems, i.e. in the electrochemical [30], voltammetric [31], mass spectrometric detection mode [32] and (iii) economic use of chiral stationary phases and of expensive and/or toxic solvents. Previously, chiral micro-packed capillary HPLC has been described for chemically bonded β-cyclodextrins [25,33]. The approach can also be extended to polymer-coated silica. Thus, the enantiomer separation of glutethimide and rac. norgestrel was achieved with a micro-packed fused-silica capillary column (150×0.25 mm I.D.) packed with Chirasil-Dex-polymer-coated Nucleosil (Fig. 5).

In our hands, micro-packed HPLC exhibits a slight decrease in efficiency as compared to conventional HPLC when comparable retention factors are invoked at the arbitrary column dimensions selected (Fig. 5).

Acknowledgments

Support of this work by Deutsche Forschungsgemeinschaft and Fonds der chemischen Industie is gratefully acknowledged.

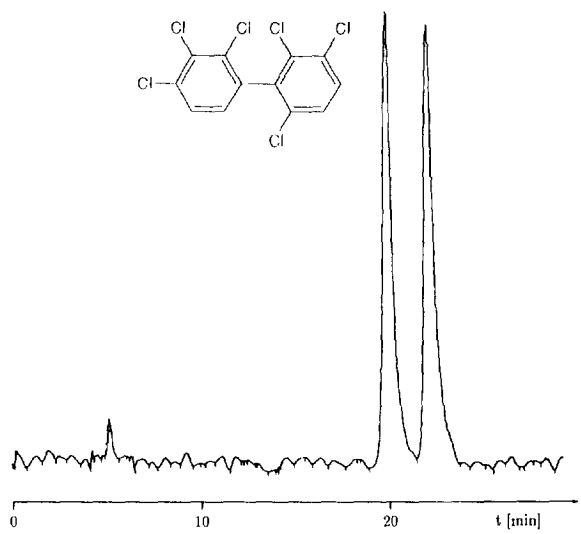


Fig. 4. Enantiomer separation of atropisomeric PCB 132 on Chirasil-Dex-polymer-coated Nucleosil by HPLC. Conditions: 250×4.6 mm I.D. stainless steel column, 23°C, 0.7 ml/min methanol–water (75:25, v/v), UV detection at 230 nm.

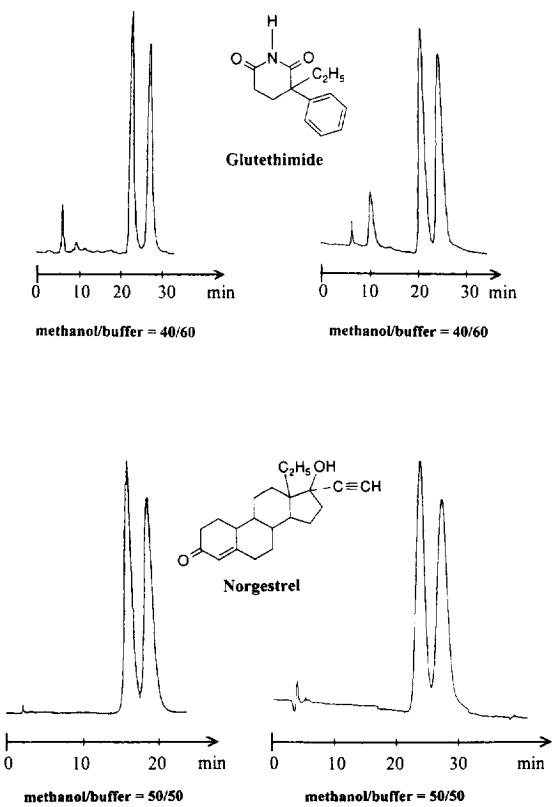


Fig. 5. Enantiomer separation of glutethimide (top) and rac. norgestrel (bottom) on a conventional HPLC column (200×4.6 mm I.D.) (left) and on a micro-packed fused-silica capillary column (150×0.25 mm I.D.) (right) both packed with Chirasil-Dex-polymer-coated Nucleosil. Chromatographic conditions as in Table 1.

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