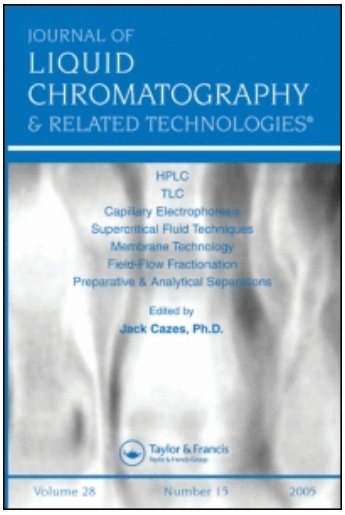

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Chromatographic Resolution of Chiral Drugs on Polyamides and Cellulose Triacetate

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CHROMATOGRAPHIC RESOLUTION OF CHIRAL DRUGS ON POLYAMIDES AND CELLULOSE TRIACETATE

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Abstract

Many chiral drugs are resolved very efficiently by chromatography on optically active polyamides and microcrystalline cellulose triacetate. These adsorbents having a wide range of applications can be used repeatedly on an analytical as well as on a preparative scale without losing their resolution efficiency.

Introduction

Enantiomers of drugs usually differ not only in their pharmacological action but also in their side effects. However, in many cases it is very difficult either to synthesize enantiomers starting from optically active compounds or to resolve the racemates via diastereoisomeric salts by fractional crystallisation or diastereoisomeric derivatives by silica gel chromatography. Meanwhile, the direct chromatographic resolution of racemic drugs has been developed in many cases into an economical process.

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Therapeutic agents are mostly polar compounds differing widely in their structural skeleton and substitution patterns. Thus, optically active adsorbents with a very wide range of application are necessary for their direct chromatographic resolution. Cross-linked polyacrylamides like the phenylalanine derivative 1 and the cyclohexylethylamine derivative 2 as well as microcrystalline cellulose triacetate (3) have proved to be very efficient adsorbents.

The polyamides 1-2 are not yet commercially available. However, they are easily prepared[1,2] (fig. 1): (S)-Phenylalanine ethylester is reacted with acrylic acid anhydride, (S)-cyclohexylethylamine with methacrylic acid anhydride in chloroform. On a larger scale, the acylation is accomplished with the corresponding acid chloride in the two-phase system chloroform/aqueous sodium carbonate. Both unsaturated, optically active amides are white, crystalline compounds.

The optically active adsorbents are then obtained by radical copolymerization with crosslinking agents like ethylenediacrylate as white bead polymers that swell in toluene, dioxane or tert-butylmethylether/tetrahydrofuran to give transparent gels. Because of the cross-linking the adsorbents are insoluble and mechanically stable; they can be dried and swelled again and enable high flow rates depending on the particle size. The following procedures for preparation of monomers, the polymers 1 and 2 and for resolution experiments are recommended.

Preparation of monomers

To a solution of 12.7 g (0.1 mol) (S)-cyclohexylethylamine (optical purity checked by derivatization with isopropylisocyanate to the urea derivative and analytical gas chromatography on a capillary column of Chirasil-Val[4]) in 250 ml of toluene, a

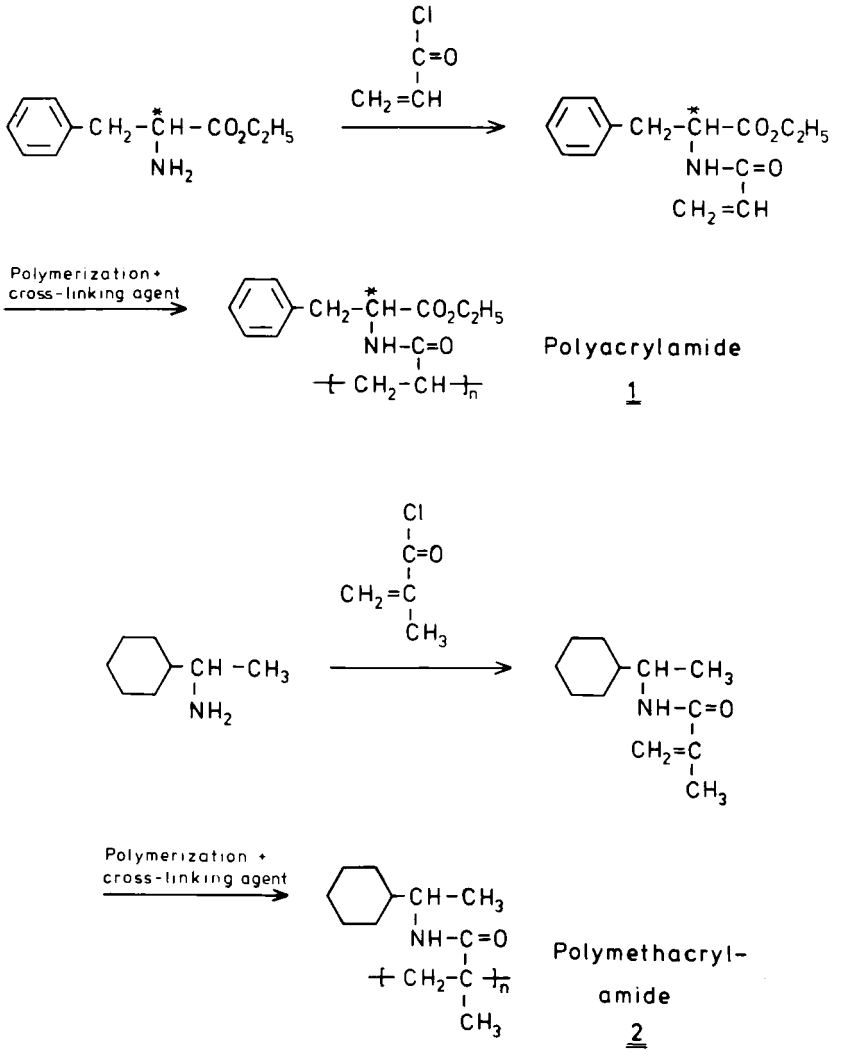

fig. 1: Synthesis of optically active polyamides 1 and 2.

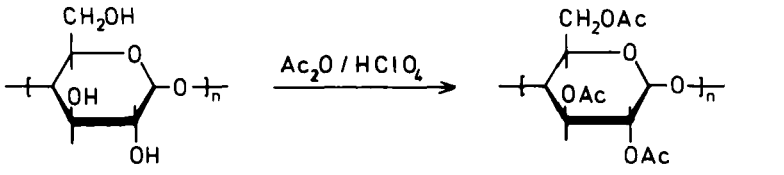

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fig. 2: Synthesis of microcrystalline cellulose triacetate (3).

saturated aqueous solution of 12.5 g (0.15 mol) sodium carbonate was added, and the reaction flask was cooled to 5°C. Under stirring, the solution of 10.5 g (0.1 mol) methacrylic acid chloride and 100 mg tert-butylpyrocatechol in 50 ml of toluene was added dropwise. After 1 hour of stirring at 5°C, the toluene layer was washed with 2N sulfuric acid, 2N sodium hydroxide and water, dried over sodium sulfate and evaporated in vacuo (rotatory evaporator). The oily residue (avoid heating otherwise the oil will polymerize!) was recrystallized from petrol ether (boiling point 30-40°C). The yield was 13.7 g (70 %) of long, white needles, $[\alpha]_D^{20}$ = -12.7 (c= 1.4, toluene). Accordingly, S-phenylalanine ethylester is acylated with acrylic acid chloride to give the amino acid derivative as white crystals, m.p. 42°C, $[\alpha]_D^{20}$ = -93.3 (c= 2.5, methanol).

Polymerization procedure[3)]

To the cold solution of 10.0 g (40.5 mmol) (S)-N-acryloylphenylalanine ethylester (optically active monomer), 0.69 g (4.05 mmol, 10 mol-%) 1,2-ethanedioldiacrylate (cross-linking agent) and 0.066 g (0.405 mmol, 1 mol-%) azoisobutyronitrile (radical starter) in 17 ml of toluene, 197 ml of cold 5 percent aqueous

polyvinylalcohol solution was added and stirred (metal stirrer, about 300 r.p.m.) under an atmosphere of nitrogen. Under stirring, the temperature was raised to 80°C and stirred at this temperature for 5 hours. After cooling, the organic phase was removed by steam distillation and the aqueous phase was decanted. The white bead polymers were thorougly washed with hot water, methanol, toluene and finally with petrol ether and dried in vacuo. The yield is almost quantitative. In the same manner, the adsorbent 2 is obtained, starting with N-methacryloyl-(S)-cyclohexylethylamine.

Chromatographic methods

The sieved polymer fractions with a particle size ranging between 50-100 µm was boiled under reflux for 10 minutes in the solvent system. After cooling, the solvent together with very fine particles was decanted, the beads suspended again in fresh solvent and the slurry filled into glass columns with teflon adapters (Pharmacia GmbH, F.R.G.). The column was packed using a low pressure pump under a solvent pressure of 2-3 atmospheres. The racemates, dissolved in the solvent, were injected via a loop. By this method, the pump does not need to be switched off during sample injection. The eluate was passed through the flow cell of the UV detector and a 80 µl flow cell of a polarimeter and then was collected in volume fractions.

Microcrystalline cellulose triacetate, which is commercially available with particle size of 15-25 and 25-40 µm[5], can be prepared by heterogenious acetylation of microcrystalline cellulose (Avicel^{R}) using acetic acid anhydride/perchloric acid according to Hesse and Hagel[6] (fig. 2). For column chromatography, this adsorbent is swollen by boiling in ethanol. The cooled suspension is decanted or filtered, stirred with fresh ethanol and degassed under vacuum. The slurry is filled into the column.

Further packing of the column is performed as described for the polyamides. A detailed procedure for preparation of the slurry and packing is also given in lit[7].

Resolution mechanisms

Both types of adsorbents, polyamides as well as microcrystalline cellulose triacetate, apparently do not resolve due to direct diastereoisomeric interactions between individual optically active residues of the chiral packings and the enantiomers. Instead, a more complex resolving mechanism has to be taken into consideration. In the case of optically active amides, copolymerization with cross-linking agents will give a three-dimensional polymeric network. It seems to be that chromatographic resolutions are due to an inclusion of the enantiomers into asymmetric cavities of this network. The different degrees of fit of both enantiomers determine their average residence time in the adsorbent's cavities. Hydrogen bondings between the polar groups of the enantiomers and the CO-NH-groups of the polymer are assumed to be the main adsorbing forces. The polyamides therefore are most suitable for the resolution of polar drugs, polar structural elements for example being amide and imide units[8].

As conclusion, retention mechanisms are very complex, and predictions regarding adsorption and separation are very difficult to make. This is also exemplified by the observation that some racemic drugs like chlortalidone (4) are resolved on the phenylalanine polymer 1 but not on 2, others like thalidomide (5a) on the cyclohexyl polymer 2 and but not on 1. Furthermore, a polyacrylamide on the one hand and a polymethacrylamide on the other hand substituted with the same optically active residues sometimes are differing entirely in their resolution efficiency.

Microcrystalline cellulose triacetate (3) is very useful for the separation of many classes of chiral drugs. By the heterogenious acetylation, the fibre structure of cellulose with crystalline areas remains unchanged. The lamellar arrangement in the manner of a crystalline lattice acts as a molecular sieve, making possible the inclusion of enantiomers. These are held in an environment which is asymmetric owing to the sterically fixed acetyl groups[6]. Therefore, also this adsorbent seems to resolve the enantiomers due to an inclusion into asymmetric cavities.

Neither chlortalidone or thalidomide are resolved on cellulose triacetate. Furthermore, charged or dissociating compounds like ammonium salts and carboxylic acids including amino acids are scarcely retained on this adsorbent and therefore are not resolved. However, many other polar compounds like amides, imides, esters, amines and ketones are frequently resolved. A phenyl or cycloalkyl group close to the center of chirality seems to improve the resolution. Unpolar racemates like hydrocarbons were resolved too, but this is not of interest in the field of drugs which are mostly compounds with polar functional groups. According to Hesse[6] "even with inclusion chromatography on cellulose esters, complete racemate resolution still remains a matter of luck. A measurable activation of chiral compounds, however, is nearly always obtained".

Applications of polyamides 1 and 2 and microcrystalline cellulose triacetate (3)

Several complete chromatographic resolutions of chiral drugs have been published and summarized[8]. As example for a preparative application, chlortalidone (4), a diuretic, is completely resolved[9] on the phenylalanine polymer 1. In this experiment, as shown in fig. 3, the column temperature was raised to 40°C after

the elution of (-)-chlortalidone. Thus, by increasing the elution rate of the more retained (+)-enantiomer, the time required for the resolution is shortened. The enantiomers of chlorthalidone were thus isolated for the first time and in a quantitative yield. Thalidomide (5a) is another excellent example[10] for the preparative application of column chromatography (fig. 4). Up to 500 mg of racemic 5a were completely resolved in one run on 65 g adsorbent 2, corresponding to a ratio racemate/adsorbent of 1:130. Although the peaks are broadened due to the overloaded column, a substance-free fraction is still left between the eluates of the (+)- and (-)-form[11]. These chromatographically isolated thalidomide enantiomers have been investigated[11] for their teratogenic activity. Only S-(-)-thalidomide displayed teratogenic properties on intraperitoneal administration.

Since also extremely small quantities can be separated without any losses on the same adsorbent, the optical purity of thalidomide samples was determined analytically by means of chromatography on 2. This low scale method also was used to examine the racemization rate of thalidomide in vivo[12]: C-14-labeled thalidomide enantiomers, obtained by chromatographic separation of the C-14-labeled racemate, were administered to mice. Radioactive thalidomide, reisolated from the animal's organs together with radioactive metabolites, was separated from the metabolites by TLC on silica gel and eluted from the silica gel layer. The radioactive eluate of the thalidomide spot containing less than 1 µg thalidomide was chromatographed again on a small column of the adsorbent 2. The ratio of enantiomers was easily and accurately deduced, because the count rates in the (-)- and (+)-fraction corresponded to the enantiomeric ratio of the reisolated thalidomide samples.

The separation efficiency of adsorbent 2 can be improved in some cases by using the solvent system tert-butylmethylether/tetrahydrofuran. For example, fig. 5 shows the chromatography of 5 mg of

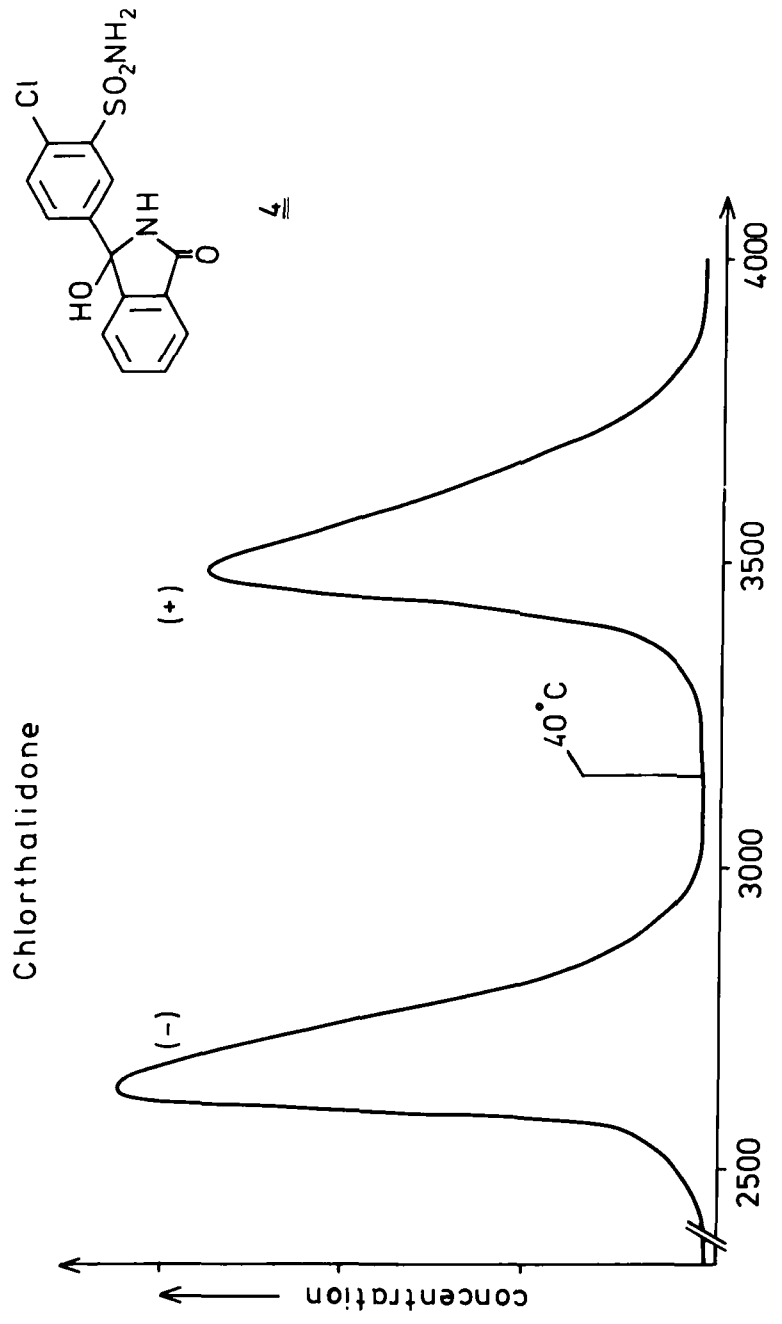
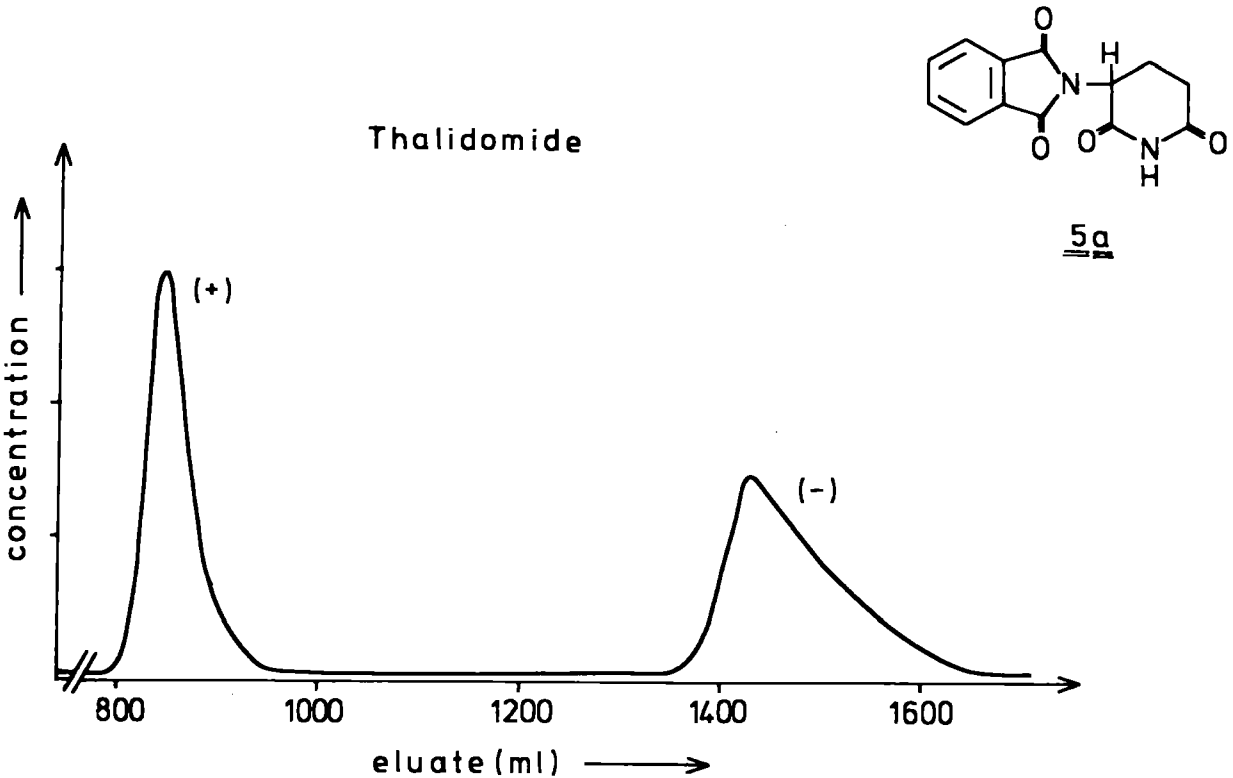


fig. 3: Chromatographic resolution of 530 mg of racemic chlortalidone (4) on 250 g of 1, column 36 x 3.2 cm, toluene/dioxane (1:1), pressure 1.2 bar.



5a

fig. 4: Chromatographic resolution of 52 mg of racemic thalidomide (5a) on 65 g of 2, column 80 x 2.3 cm, benzene/dioxane (4:1).

the racemic thalidomide analogue 5b on 2. Only a partial resolution of this C-methyl derivative 5b was obtained with toluene/dioxane as eluent.

Microcrystalline cellulose triacetate was used for the resolution of several racemic barbiturates as exemplified by the preparative resolution of methylcyclohexylethyl barbituric acid[13] (6)(fig. 6). A more detailed comparison of chromatographic resolutions of racemates including chiral drugs on microcrystalline cellulose triacetate (3) and the phenylalanine adsorbent 1 has been published recently[13].

New experimental results

Meanwhile, we have investigated the chromatographic resolution of a large number of commercially available chiral drugs[4,14,15]. Most of them are at least partially, some completely separated either on the phenylalanine polyamide 1, cyclohexylethylamine derivative 2 or cellulose triacetate (3). The fig. 8-10 and 12-15 are demonstrating some of these optical resolutions. For example, a number of racemic benzothiadiazine diuretics including 7a-f were resolved into their optical isomers on the phenylalanine adsorbent 1[15,16]. For these racemates lacking suitable functional groups, conventional resolving methods via diastereoisomeric salts or derivatives failed. Enantiomeric resolutions which were in some cases almost complete, also depended considerably upon the substitution of the heterocyclic moiety in the drug molecules. The optically pure enantiomers of penflutizide (7a) and bendroflumethiazide (7b) were obtained by repeated chromatography on a semipreparative scale. Bemetizide (7c/d) exists in four stereoisomeric forms due to the second chiral center in the side chain. A complete separation was obtained for the two diastereoisomers which were, in addition, partially resolved into the enantiomers. Partial enantiomeric resolution[17] was also observed with paraflutizide (7e) and its ortho isomer 7f which was detec-

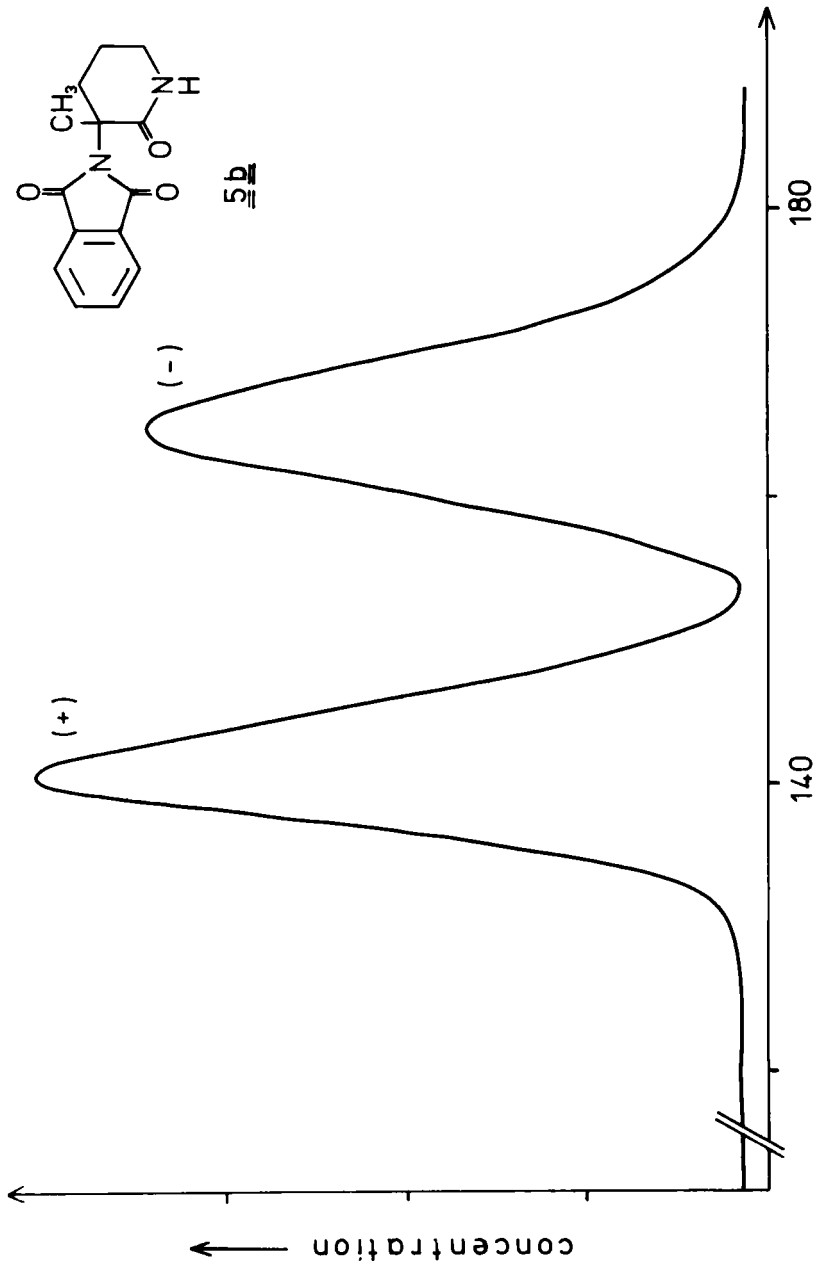


fig. 5: Chromatographic resolution of 5.0 mg of racemic 5b on 5.0 g of 2, column 37 x 1.0 cm, tert-butylmethylether/tetrahydrofuran (7:3).

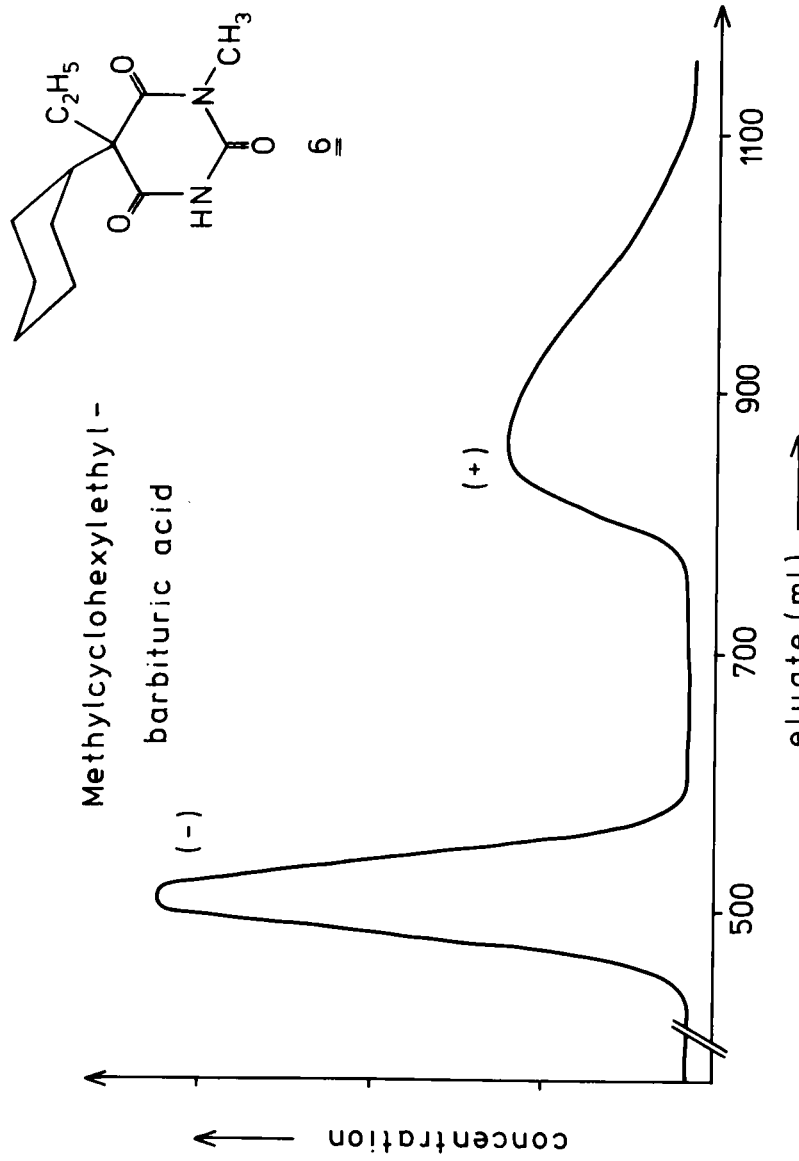
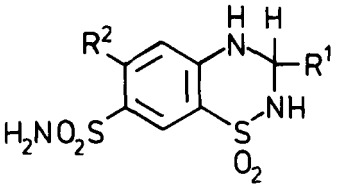


fig. 6: Chromatographic resolution of 205 mg of methylcyclohexylethyl barbituric acid (6) on 210 g of microcrystalline cellulose triacetate (3), column 85 x 2.5 cm, ethanol 96 %, pressure 1.2 bar, flow rate 50 ml/hour.



	R^1	R^2
7a	$CH_2(CH_2)_3CH_3$	CF_3
7b	CH_2-Ph	CF_3
7c/d	CH(CH$_3$)-Ph	Cl
7e	CH_2-Ph-F	Cl
7f	CH_2-Ph-F	Cl

fig. 7: Structures of chiral benzothiadiazine diuretics (7a-f).

ted as an impurity in 7e, the ortho isomer being eluted much faster than the para isomer.

Examples[14] for complete chromatographic preparative resolutions on cellulose triacetate are oxapadol (8) (fig. 8), ketamin (9) (fig. 9), mianserin (10) (fig. 10), praziquantel (11), and rolipram (12). Methaqualone[18] (13) and chlormezanon[14] (14) are almost completely resolved.

Using ethanol als eluent, in some cases the second enantiomer elutes from the cellulose triacetate column as a very broad peak. Changing the solvent from ethanol to methanol decreases the rentention time as exemplified in fig. 10 with mianserin[14,19].

The optical isomers of chlormezanon (14) were also isolated by chromatography on a preparative scale in gram quantities[14]. The

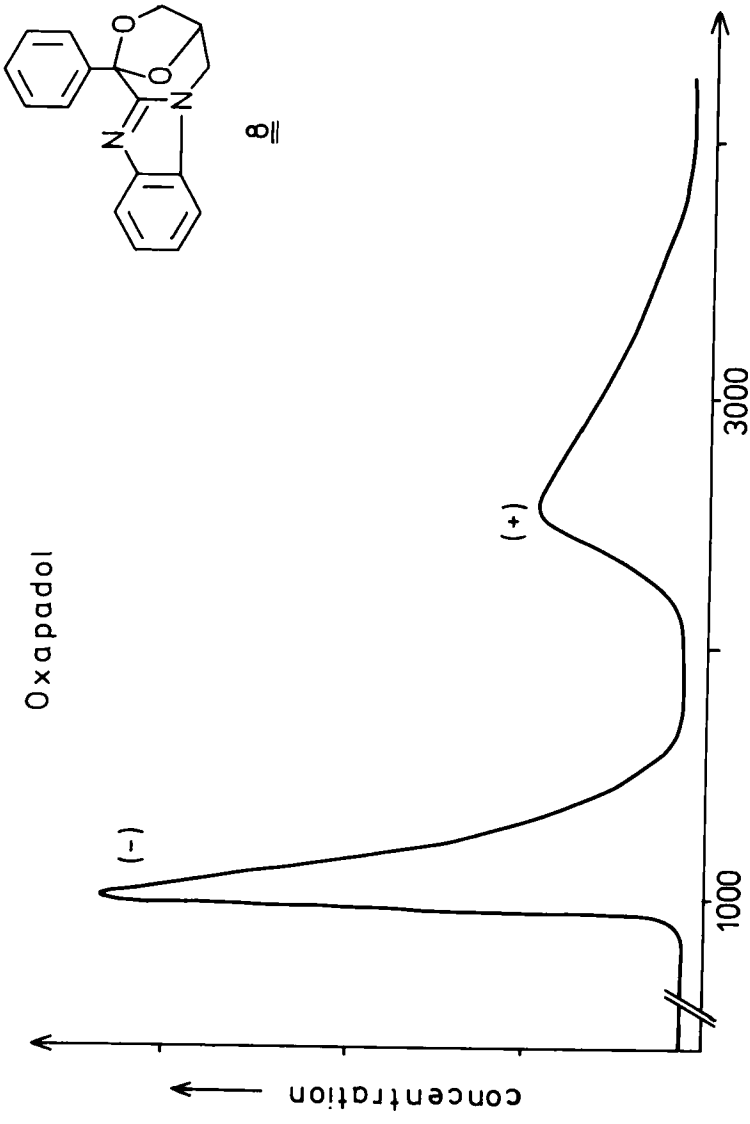


fig. 8: Chromatographic resolution of 2.1 g of oxapadol (8) on 380 g of microcrystalline cellulose triacetate (3), column 70 x 3.8 cm, ethanol 95 %, 2 bar, flow rate 90 ml/hour.

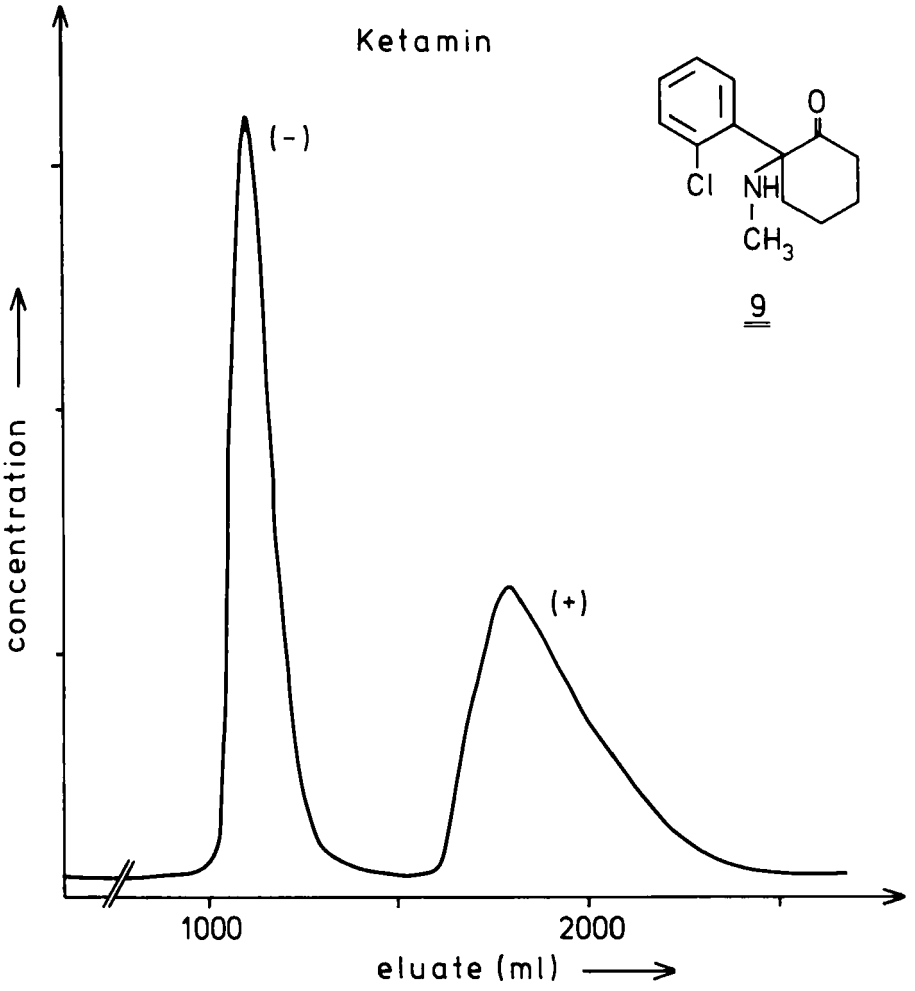


fig. 9: Chromatographic resolution of 450 mg of ketamin (9), column as in example of fig. 8.

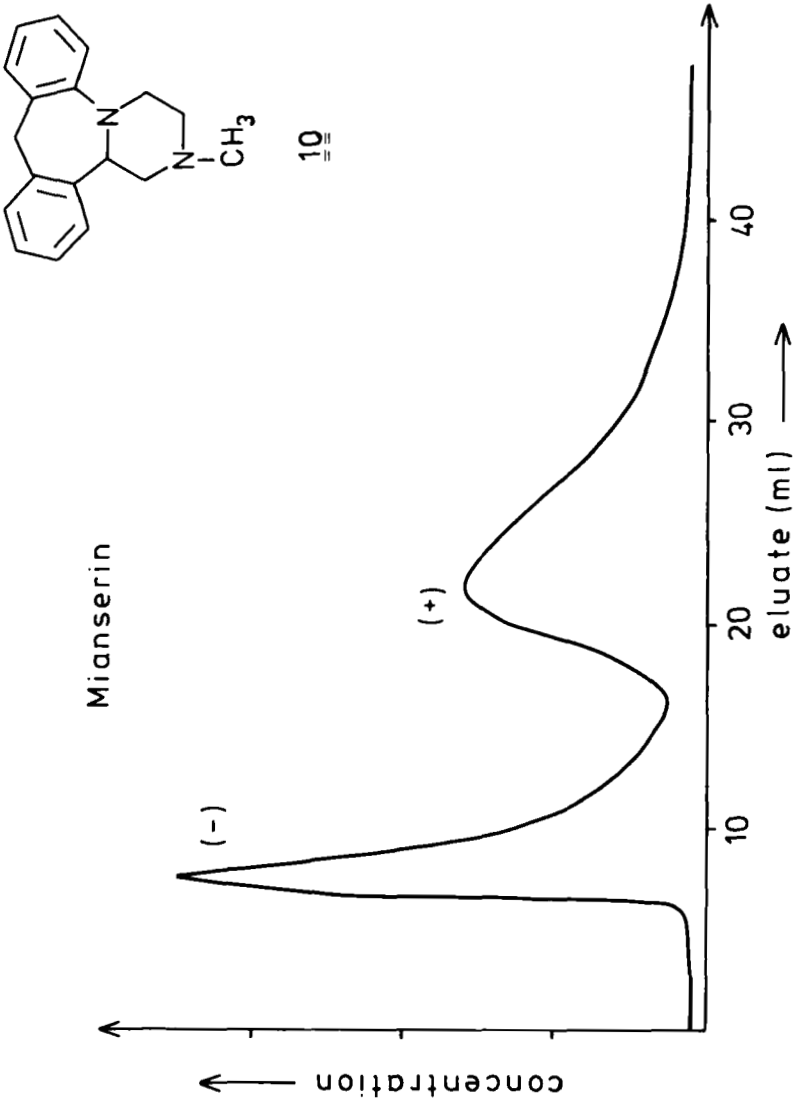
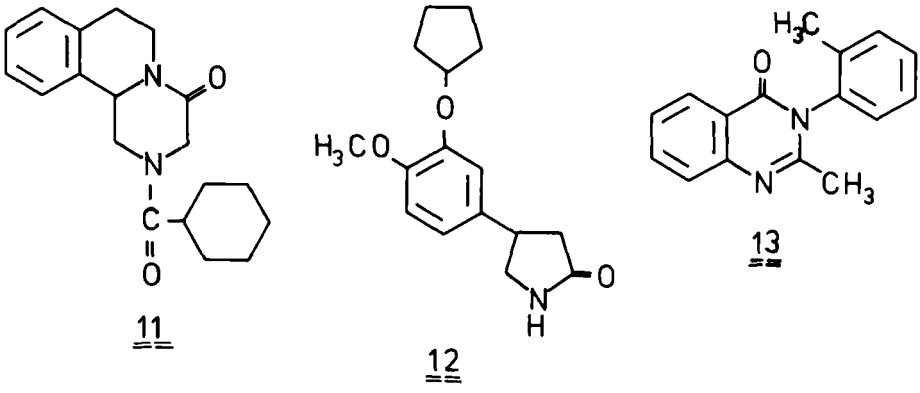


fig. 10: Resolution of 8 mg of mianserin (10) on 8 g of microcrystalline cellulose triacetate, column 20 x 1.5 cm, methanol as eluent, flow rate 24 ml/hour.



11 12 13

fig. 11: Structures of praziquantel (11), rolipram (12) and methaqualone (13).

enantiomers thus obtained for the first time have been tested for their pharmacological action[20]. Whereas chlormezanon is not resolved on the polyamide 1, the N-demethyl derivative 15 is resolved completely[15] on 1 (fig. 12).

The important anticancer drugs with the oxazaphosphorine skeleton are chiral, due to the unsymmetrically substituted phosphor atom. Some of these racemates like ifosfamide (16)[21] are resolved on adsorbent 1. This separation method was used for the preparation of optically pure radioactive labeled ifosfamide enantiomers (fig. 13)[22] as for the isolation of ifosfamide enantiomers in gram quantities: On a column with 630 g of adsorbent 1, more than 1.5 grams of racemic ifosfamide could be separated completely per day. Both enantiomers have been tested for their anticancer activity[23].

Compound 17 (fig. 14) is chiral with sulfur as center of chirality. On adsorbent 1, the racemate is nicely resolved. Addition of methanol to the solvent system gives a much faster elution with

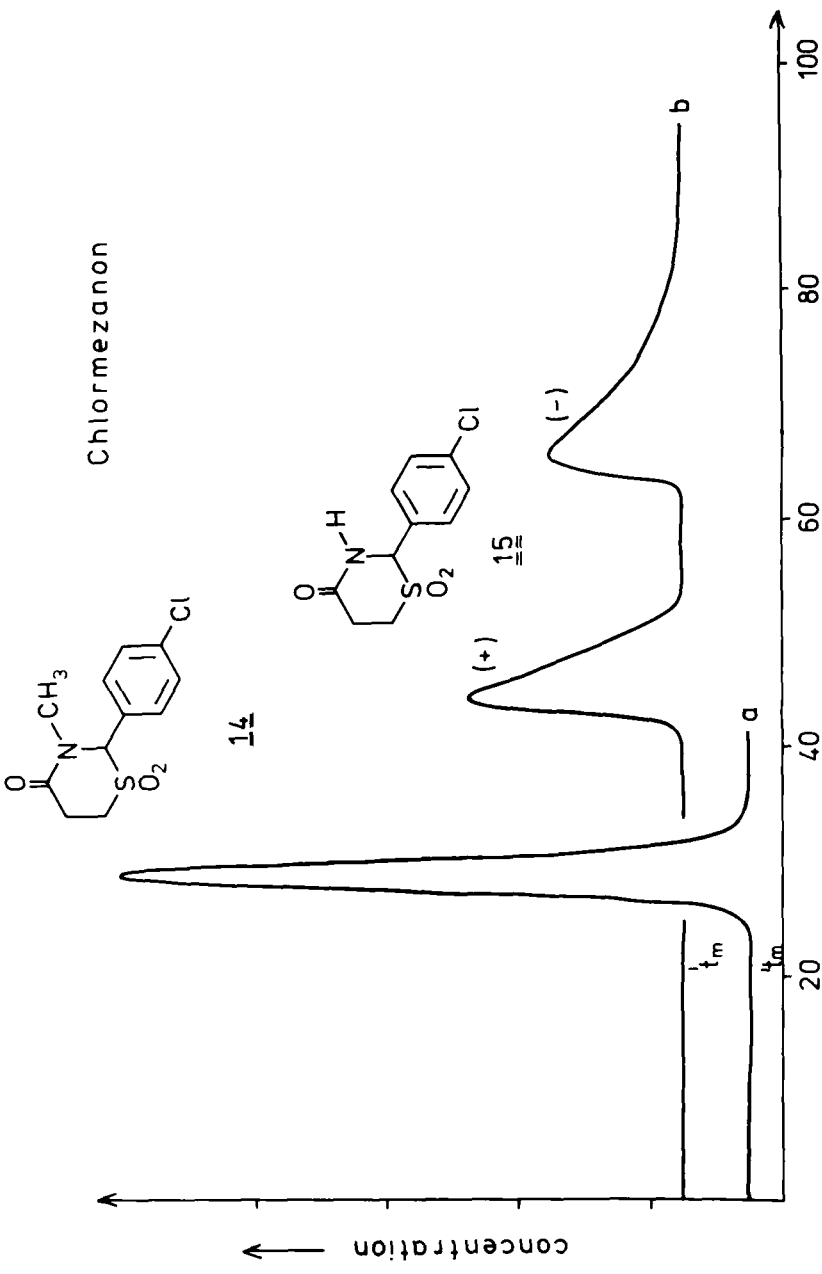


fig. 12: Resolution of 5 mg of chlormezanon (14, curve a) and 6 mg N-demethylchlormezanon (15, curve b) on 5 g of polyamide 1, column 29.5 x 1.0 cm, toluene/dioxane (1:1).

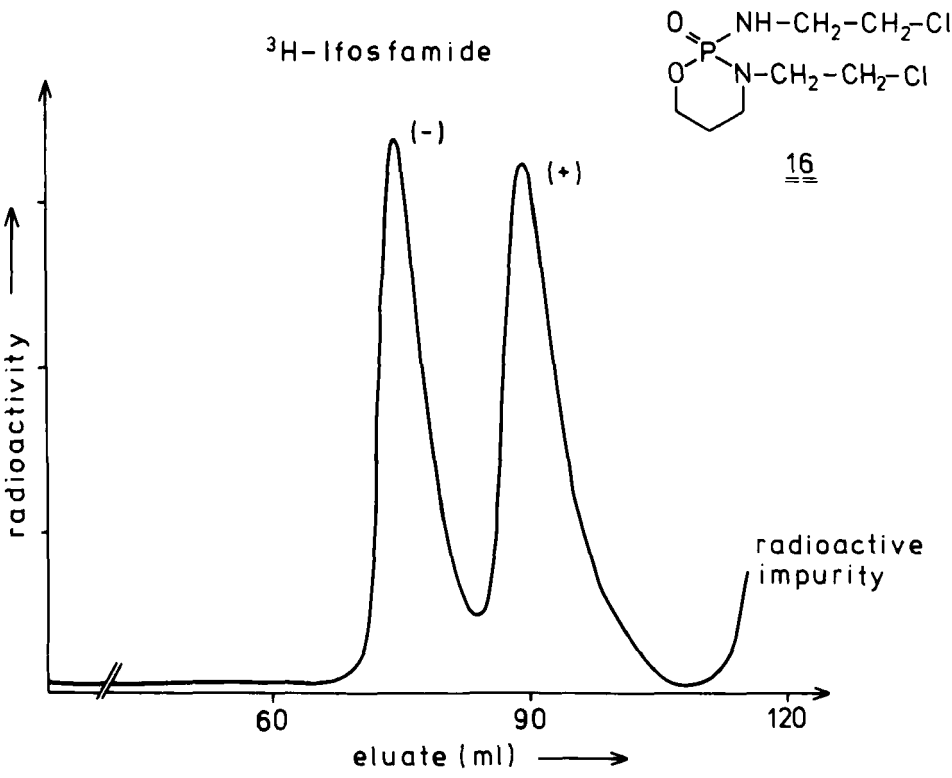


fig. 13: Resolution of 4 µCi of H-3-ifosfamide (16), specific activity 1.6 µCi/mg on 8 g amide 1, column 35 x 1.0 cm, toluene/dioxane (7:3), flow rate 3.4 ml/hour.

still complete separation[15]. Racemates of similar structure are also separated on cellulose triacetate, the α-values of the resolution being up to 4.6[14].

HPLC resolutions

Polyacrylamides like the non crosslinked polymer 1 when covalently bound to HPLC silica gel[24] enable fast separations on an analytical scale under high pressure conditions. Examples for these separations are given in fig. 15,16: The benzodiazepine

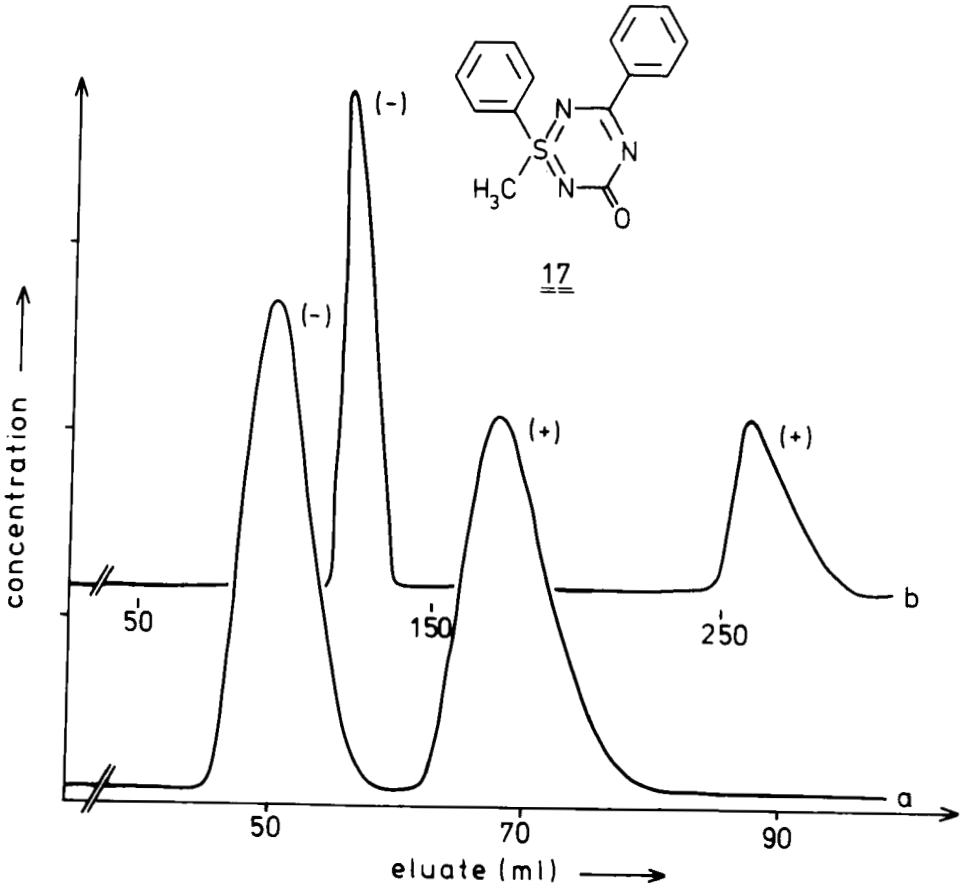


fig. 14: Resolution of 15 mg of racemate 17 on 6.2 g of 1 (column 24 x 1.0 cm) with toluene/dioxane/methanol (47:47:6, curve a); 2.0 mg of racemate 17 on 5.0 g of 1 (column 29.5 x 1.0 cm) with toluene/dioxane (1:1, curve b).

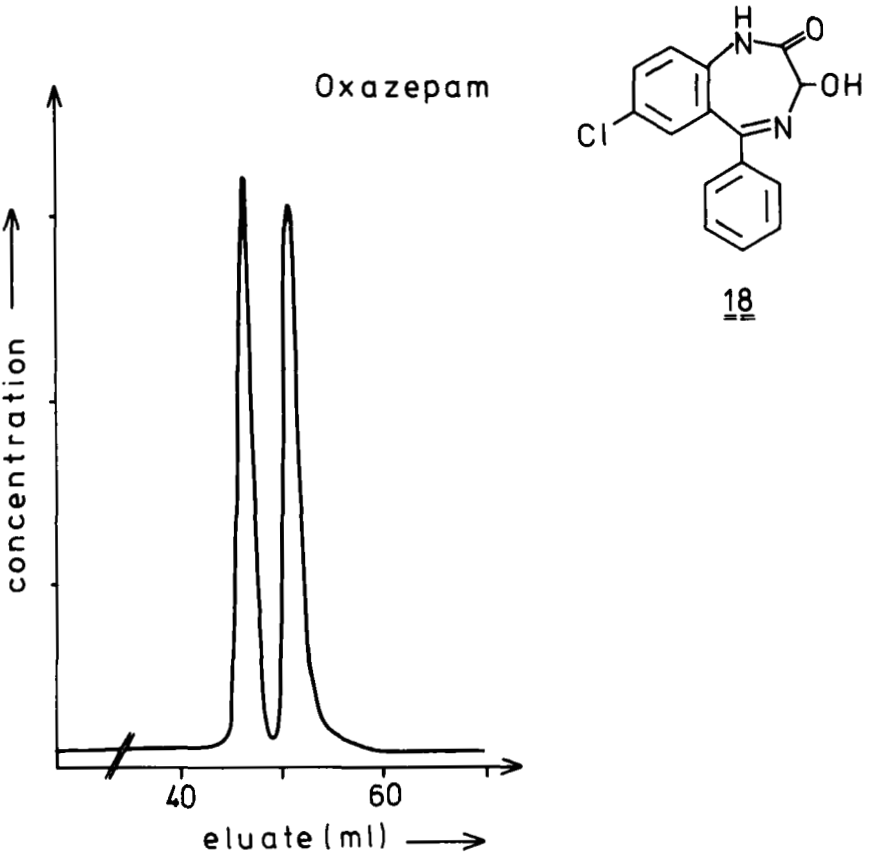
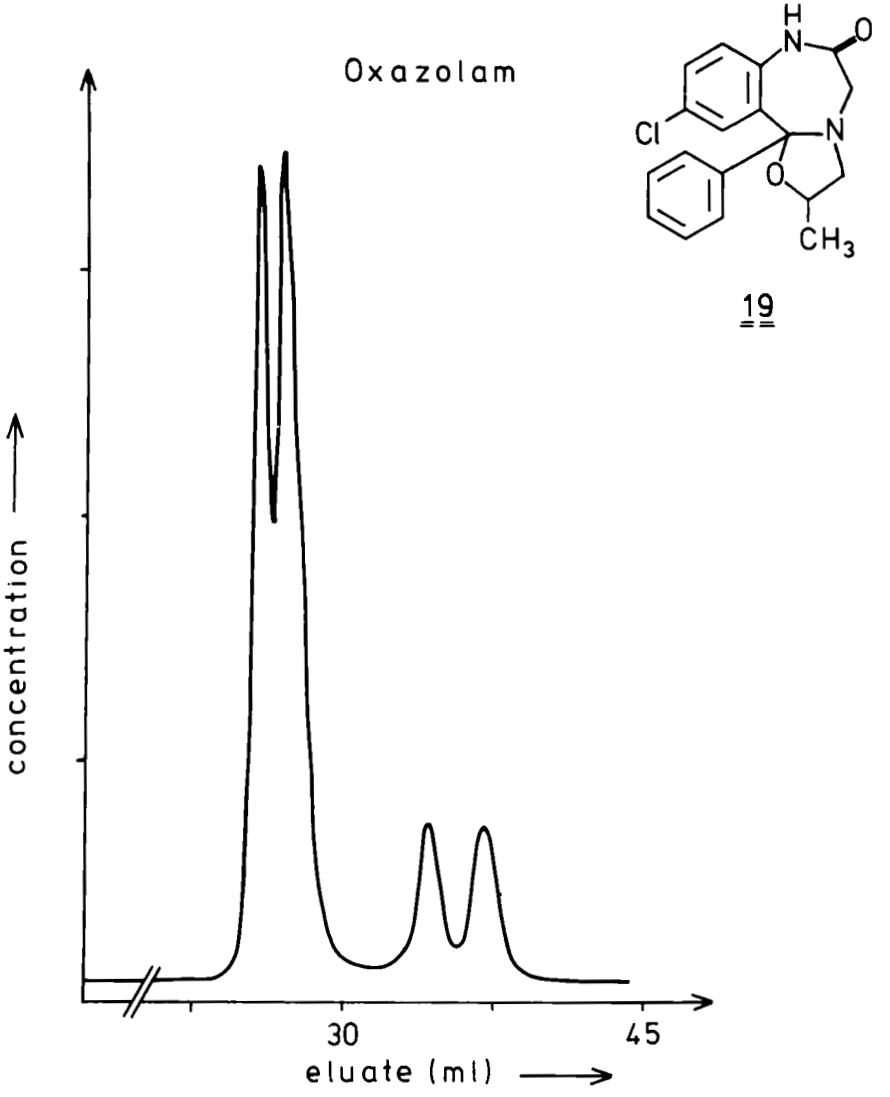


fig. 15: Resolution of 1 µg of oxazepam (18), 2.4 g adsorbent 30 x 0.4 cm, silica gel + polyacrylacryloyl S-phenylalanine ethylester), n-hexane/dioxane (65:35), pressure 62 bar, 1 ml/min.

oxazepam (18), which had been separated previously on cross-linked 1 on a preparative scale[9], is completely resolved (fig. 15). The diastereoisomeric benzodiazepine oxazolam (19) is completely separated to give the diasteroisomers. One of them is almost completely, the other one partially resolved giving the enantiomers. Therefore, four peaks are observed in the chromatogram of fig. 16.



19

fig. 16: Resolution of 5 µg of oxazolam (19), 2.2 g adsorbent (25 x 0.4 cm, silica gel + polyacryloyl S-phenylalanine ethylester), n-hexane/dioxane (8:2), pressure 24 bar, 1 ml/min.

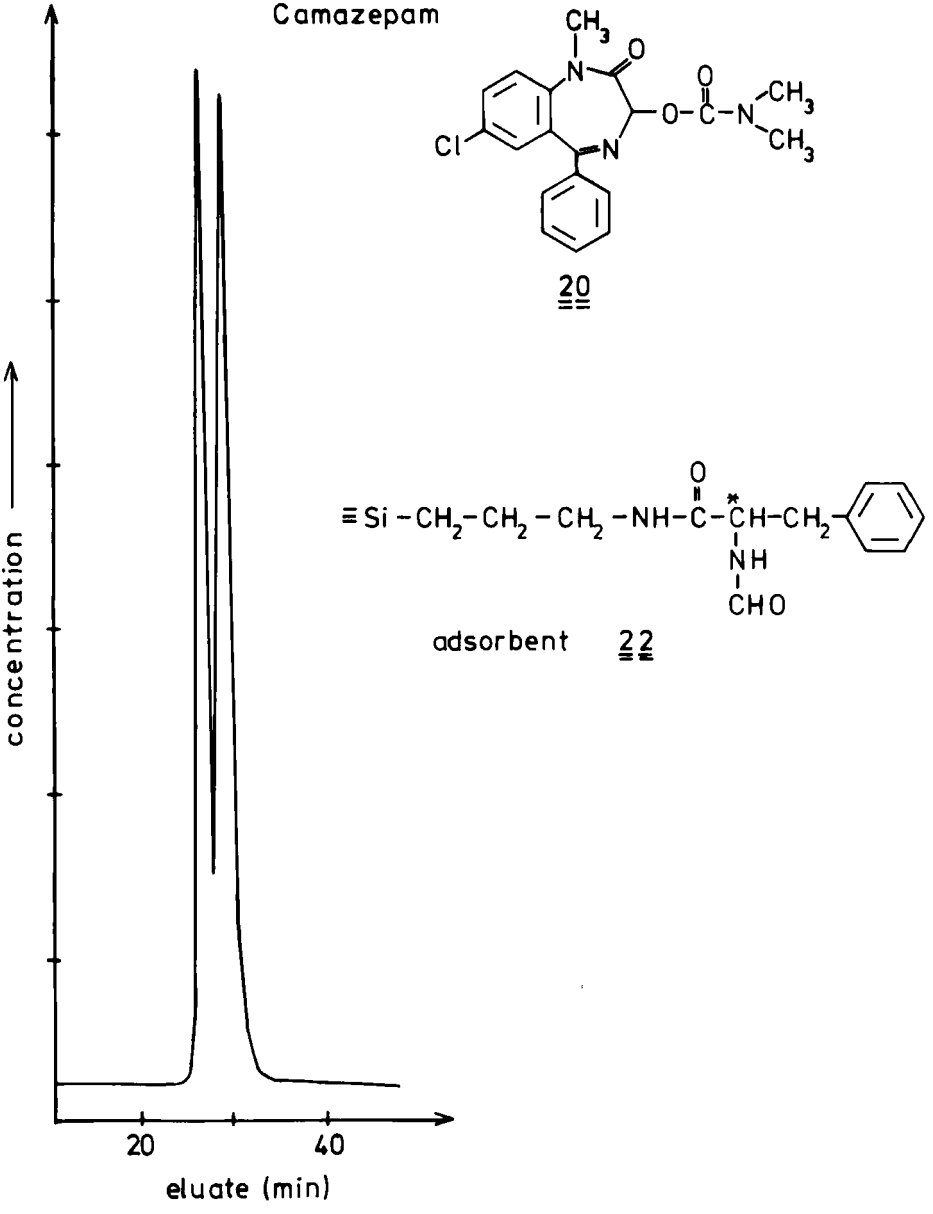


fig. 17: Resolution of 10 µg of camazepam (20) on 3.0 g of adsorbent 22, n-hexane/isopropanol (97:3), flow 1 ml/min.

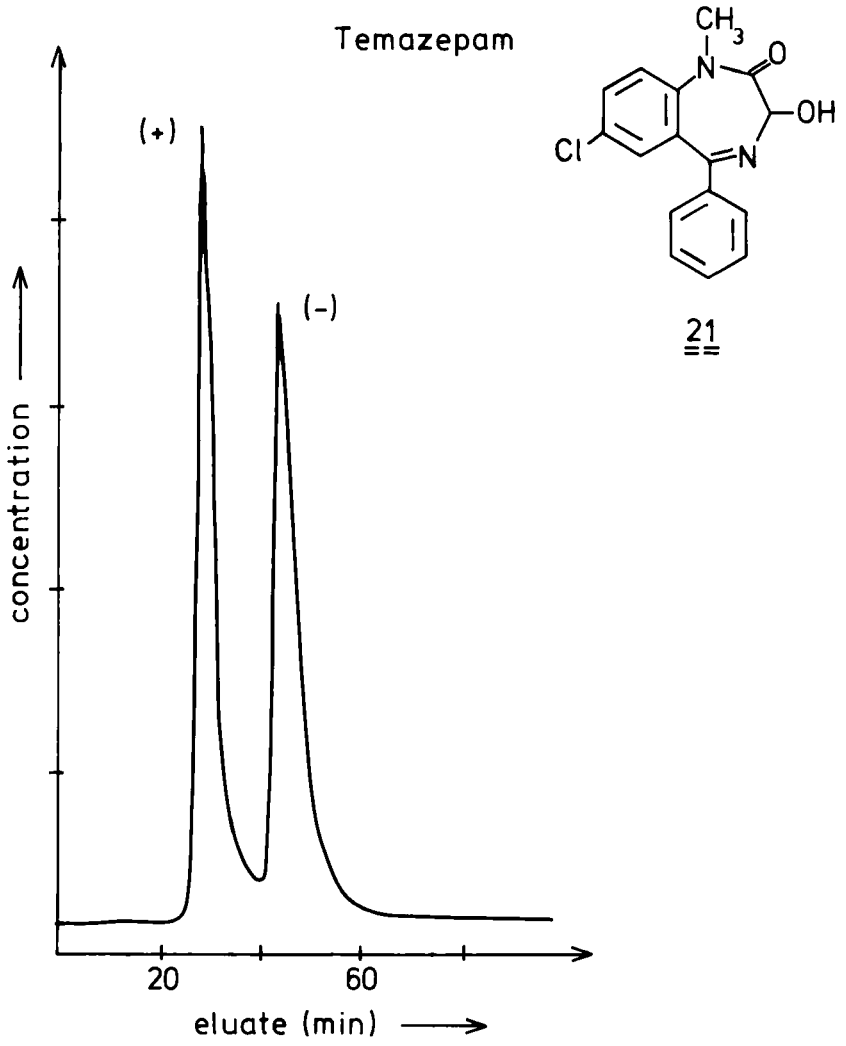


fig. 18: Resolution of 10 µg of temazepam (21), experimental conditions as in fig. 17.

Also cellulose triacetate[7,14] as well as commercially available[25] crosslinked microcrystalline cellulose triacetate[14,26] can be used in columns for HPLC. Cross-linked microcrystalline cellulose triacetate can also be used for low pressure liquid chromatography[14].

Furthermore, on silica gel substituted with optically active amino acids like N-formyl phenylalanine some drugs can be separated by HPLC. Examples are the benzodiazepines camazepam (20) and temazepam (21), which are resolved almoust completely on the adsorbent 22 (fig. 17,18).

Outlook

Within a short time, chromatographic resolutions of racemic drugs have matured into an efficient process, often permitting enantiomers to be obtained for the first time. Applications for this method will be not only be the isolation of enantiomers on a preparative scale, but also the determination of enantiomeric purities on an analytical scale.

Acknowledgements

I am indepted to my coworkers mentioned in this contribution, to Dr. Jürgen Maibaum on comments on the manuscript and to the Deutsche Forschungsgemeinschaft, the Fonds der Chemie and the Ministerium für Familie, Jugend und Gesundheit for financial support.

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