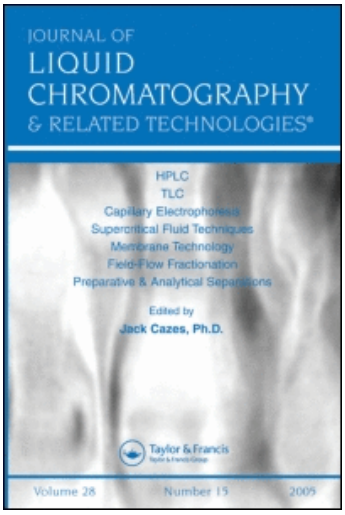

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CHIRAL RESOLUTION OF SEVERAL PHENOTHIAZINE COMPOUNDS AND TRIMIPRAMINE, A DIBENZAZEPINE DRUG ON CHIRALCEL OJ-R

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ABSTRACT

The Chiralcel OJ-R phase was tested on its discriminative properties towards six phenothiazine compounds, namely promethazine, oxamemazine, thiazinamium, ethopropazine, trimeprazine, and dixyrazine, besides one dibenzazepine compound, trimipramine. The effect of changes of the mobile phase upon the resolution of the enantiomers was investigated.

Acetonitrile supports a symmetrical peak shape while methanol promotes chiral interactions of the analytes on the stationary phase. Consequently the highest resolutions were mostly found for analyses with ternary mixtures of acetonitrile, an alcohol and aqueous sodium perchlorate solutions. Only the drugs with small substituents on side-chain nitrogen showed generally a satisfactory chiral interaction; promethazine and thiazinamium, the two most resembling analytes, could be baseline separated.

INTRODUCTION

The Chiralcel OJ-R column is a recently developed member of the successful family of polysaccharide polymer phases.[1] Racemic phenothiazine drugs are a group of medicines that belong to different pharmacological classes. Promethazine, oxamemazine, trimeprazine, and thiazinamium have antihistaminic properties; dixyrazine is an antipsychotic and ethopropazine an anticholinergic drug. Trimipramine, a dibenzazepine compound, is an antidepressant. The chemical structures of these drugs are given in Fig. 1.

HPLC analysis on different chiral stationary phases has already been envisaged for most of these drugs. Direct chiral resolution was successfully accomplished for trimipramine with α_1-glycoprotein added to the mobile phase[2] or immobilised on silica, described for several phenothiazine-related drugs.[3-5] β-Cyclodextrin stationary phases have proved to be also appropriate for this type of compounds.[6,7]

Ponder *et al.* examined the resolution of promethazine, ethopropazine, trimeprazine and trimipramine enantiomers on a variety of columns, including the Chiralcel OJ phase using n-hexane mixed with ethanol or isopropanol.[8]

This study investigates whether this similar tris(4-methylbenzoyl) ester of cellulose (Fig. 2) that is to be used under reversed phase conditions, allows the chiral discrimination of the cited drugs. The Chiralcel OJ-R phase can be applied with a greater variety of eluents.

For the aqueous portion, sodium perchlorate was dissolved up to a concentration range of 0.1 M to 1.0 M. Other components of the mobile phase that were tested included acetonitrile, methanol, ethanol, propanol and isopropanol.

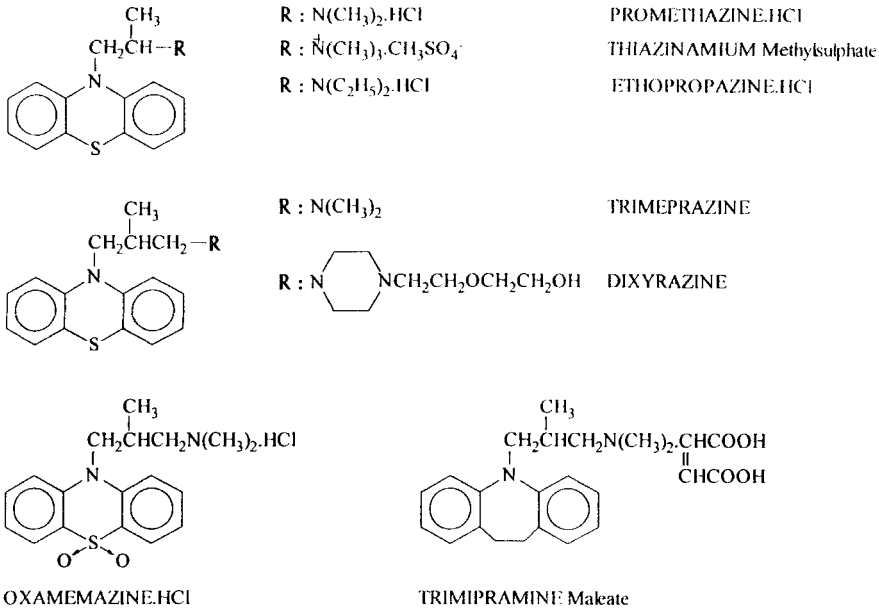

Figure 1. Chemical structures of the chiral compounds used in this study.

MATERIALS

Chemicals

The racemic drugs promethazine hydrochloride, trimipramine maleate, oxamemazine hydrochloride and thiazinamium methylsulphate were provided by SPECIA (Paris, France). Dixyrazine was obtained by extraction from Esucos® tablets (UCB Pharma, Belgium). Ethopropazine hydrochloride and trimeprazine hemi-tartrate were purchased from Sigma-Aldrich (Bornem, Belgium).

The latter drug was treated to set free the base form. It was also transformed into its hydrochloric salt but no significant difference was observed

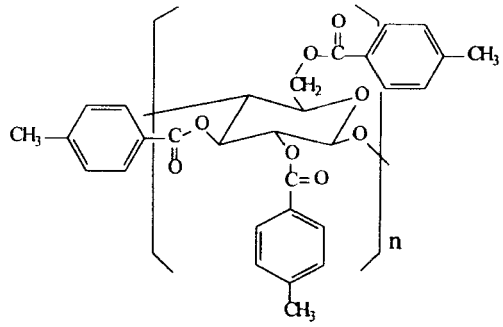

Figure 2. Monomer unit of the Chiralcel OJ-R cellulose derivative, coated on a silica support.

between the chromatograms of the base form and the salt under several conditions tested.

All solvents were of analytical or HPLC quality; sodium perchlorate was purchased from Merck (Darmstadt, Germany). Deionised and distilled water was used throughout.

Apparatus and Chromatographic Conditions

Chromatography was performed on a 15 cm x 4.6 mm I.D. Chiralcel OJ-R column (Daicel Co., Tokyo, Japan) at ambient temperature. The different constituents of the mobile phase were mixed instantly in the appropriate proportions by a Varian 9010 SDS pump (Varian Associates Inc., Walnut Creek, CA, USA) and pumped at a constant flow of 0.5 mL min^{-1}. Analytes were dissolved in either acetonitrile:water 30:70 or in methanol, since pure acetonitrile as a solvent gave rise to bad peak shapes (fronting due to its high elution strength) and the use of methanol generally resulted in comparable peak shapes, as when taking the mobile phase as solvent. The solutions contained 100 μg mL^{-1} of racemic drug and were kept in the dark. The volume injected was 20 μl (Rheodyne injector).

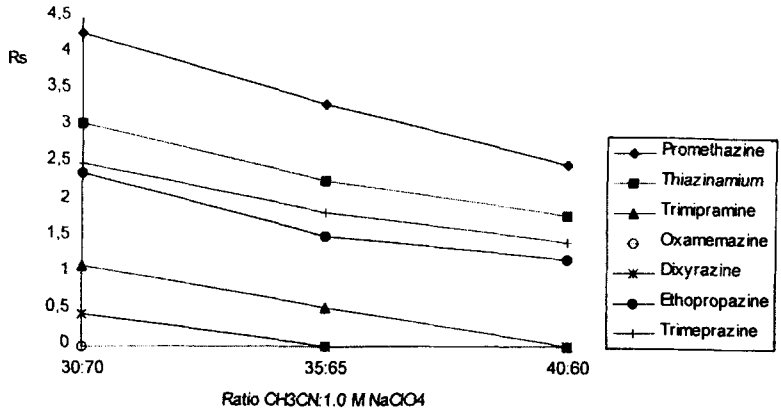

Figure 3. Influence of the change in volume ratio CH_3CN:1.0 M $NaClO_4$ of the mobile phase upon the resolution.

Detection was achieved at two wavelengths simultaneously (230 and 254 nm) with a Hewlett Packard 1050 Diode Array Detector (Waldbronn, Germany). Integration of the more intense chromatogram was made with the Hewlett Packard software package (1990), being at 254 nm for all analytes except for oxamemazine. The following parameters were measured:

k'1 : capacity factor of the first eluted enantiomer: $(t_1-t_0) / t_0$.

k'2 : capacity factor of the second eluted enantiomer: $(t_2-t_0) / t_0$.

The elution time of methanol was used to determine the t_0-value on Chiralcel OJ-R.

α : selectivity factor: k'2 / k'1.

Rs : resolution factor: $Rs = 1.18 (t_2-t_1) / (w_1+w_2)$; w is the width at half-height of the peak based on peak area and height.

Rp : Kaiser's peak separation index: the ratio of valley height between two peaks and the peak height, which rises to 1 for perfectly separated peaks. This factor was therefore preferred to Rs factors for enantioms that were not completely resolved.

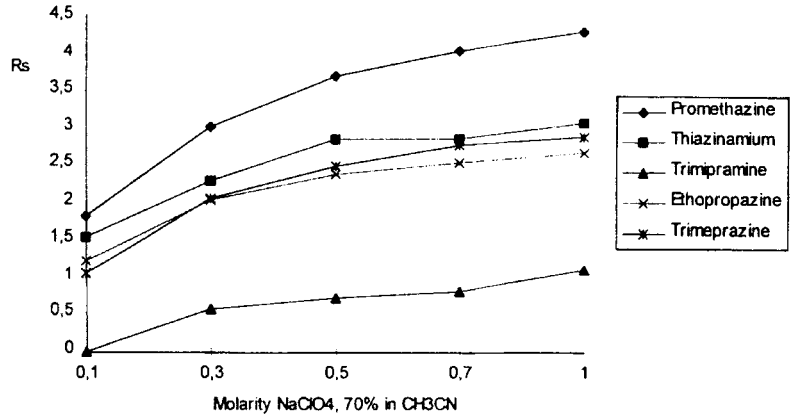

Figure 4. Influence of the molarity of the $NaClO_4$ solution mixed with acetonitrile as mobile phase upon the resolution.

RESULTS

At first stage, a binary mixture of acetonitrile and aqueous $NaClO_4$ solutions were applied since this mobile phase has proved very useful in other RP-Chiralcel systems. Similarly, as seen with the commonly used reversed phase HPLC systems, rendering the mobile phase more polar enhances the retention times of the analytes on the cellulose-based stationary phase; therefore the greater the portion of acetonitrile the quicker the compounds elute. By increasing the aqueous portion, better resolutions can be obtained at the expense of higher retention times. Substitution of 5% acetonitrile by aqueous fraction, nearly doubles elution times. In Fig. 3, the effect of different ratios of acetonitrile:1.0 M $NaClO_4$ upon the resolution is displayed.

Higher molarity of sodium perchlorate slightly enhanced retention times of the compounds and clearly improved chiral interactions. The salt concentration of sodium perchlorate was increased from 0.1 M to 1.0 M and the effect on the resolution examined (Fig. 4). Oxamemazine could not be chirally separated and dixyrazine enantiomers were only slightly resolved (Rs = 0.5). Further analyses were performed with 1.0 M salt solution. The use of perchlorate buffer solutions (acidified with concentrated perchloric acid to pH 2) had merely a small effect on the peak shape; peak broadening was reduced a little.

Table 1

Chiral Resolution on the Chiralcel OJ-R Column using Ternary Mixtures as Mobile Phase

Drug	Mobile Phase Composiiton (v/v)					Rs(Rp)*	k'_1	α
	CH_3CN	$NaClO_4$ 1.0M (aq)	CH_3OH	C_2H_5OH	C_3H_7OH			
Promethazine	20	40	40			3.18	2.04	1.98
	15	40	45			3. 7	4.49	2.20
	15	50		35		1.67(0.98)	2.95	1.64
	15	65			20	1.81(0.98)	2.40	1.52
Thiazinamium	20	40	40			1.93	2.47	1.86
	15	40	45			2.98	5.49	1.98
	15	50		35		1..30(0.93)	2.52	1.78
	15	65			20	1.29(0.91)	1.90	1.54
Ethopropazine	20	40	40			1.30(0.84)	2.44	1.33
	15	40	45			1.47(8.89)	2.90	1.38
	15	50		35		1.06(0.64)	2.64	1.34
	15	65			20	0	0.38	1
Trimeprazine	20	40	40			1.35(0.87)	3.54	1.32
	15	40	45			1.50(0.86)	4.68	1.34
	15	50		35		0.91(0.40)	3.85	1.23
	15	65			20	0	0.36	1
Trimipramine	20	40	40			0	2.22	1
	15	40	45			0.89(0.30)	5.02	1.20
	15	50		35		0	2.98	1
	15	65			20	0	2.45	1
Dixyrazine	20	40	40			0	3.71	1
	15	40	45			1.02(0.48)	9.33	1.36
	15	50		35		0	4.11	1
	15	65			20	0	2.81	1

* The Kaiser peak resolution factor is given between brackets for the not completely baseline separated enantiomers.

The addition of methanol as a third constituent of the mobile phase had an advantageous influence on the chiral interaction. The resulting resolutions however were not as high as could be expected from the improved selectivity factors due to peak broadening when using alcohols. Table 1 lists the chromatographic parameters for various ternary mixtures using different primary alcohols. Oxamemazine is not included as its enantiomers could not

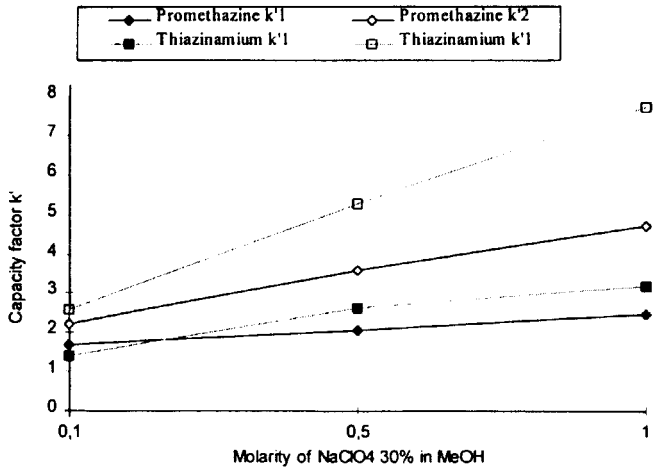

Figure 5. Influence of the molarity of the $NaClO_4$ solution mixed with methanol as mobile phaseupon the capacity factors of promethazine and thiazinamium.

be separated under any of the applied conditions. Dixyrazine and trimipramine only showed chiral interaction if the elution time of the less retained enantiomer was prolonged up to at least 20 minutes. Substitution of 1-propanol by 2-propanol interfered with the chiral interaction of the analytes. No chiral discrimination was observed using this secondary alcohol.

From Table 1, it is clear that only promethazine and thiazinamium, the two most similar compounds tested, interact satisfactorily with the Chiralcel OJ-R column and that methanol is the preferred alcohol. Therefore methanol was added in various proportions to 1.0 M $NaClO_4$. The peak broadening effect prevented easy baseline separations despite the enhanced selectivity factors compared to acetonitrile composed eluents. Plate numbers however amount to less than 10 % for methanol compared to acetonitrile composed mobile phases. No chiral discrimination was observed for trimipramine, oxamemazine and dixyrazine. The influence of the concentration of sodium perchlorate was striking in combination with methanol as well. This is represented in Figure 5 showing the improved separations for the promethazine and thiazinamium enantiomers.

Figure 6 illustrates the similar effect of mobile phase modification on the chromatographic behaviour of promethazine and thiazinamium. Figure 7 shows chromatograms of the analyses of ethopropazine and trimeprazine and Figure 8 gives an idea of the poor chiral interaction of trimipramine and dixyrazine on the Chiralcel OJ-R column.

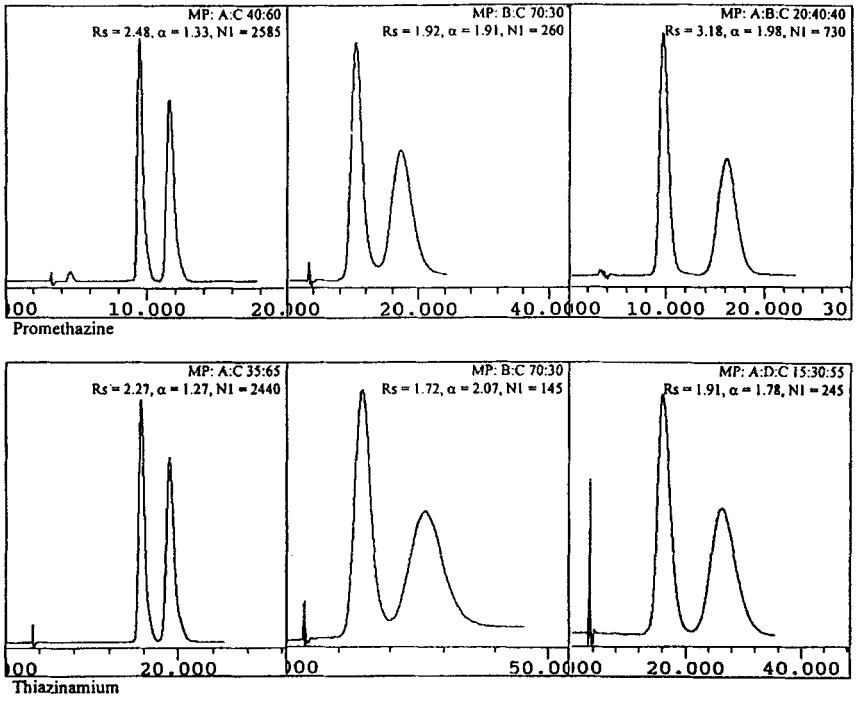

Figure 6. Chiral resolution of promethazine and thiazinamium using varied mobile phase compositions.
MP: mobile phase; A: acetonitrile; B: methanol; C: 1.0 M sodium perchlorate solution; D: ethanol. Other parameters: cf. Materials section

DISCUSSION

Cellulose tris (4-methylbenzoate) is a synthetic polymer which exists as a β-polymeric chain of derivatized D-(+) glucose residues in β-1,4-linkage. These chains lie side by side and possess a certain degree of rigidity and assume an extended helical structure into which the enantiomers of the analytes can interact stereospecifically. The chiral recognition process may thus proceed through interactive forces such as H-bonding, dipole, and π–π interactions between the analytes, and the 4-methylbenzoate moieties of the cellulose polymer, but is also determined by the formation of appropriate interstrand and intrastrand inclusion complexes between the chiral cavities in these cellulosic polymers and the cyclic groups in the drugs investigated in this study. A detailed adsorption mechanism of the chiral solutes versus the polymer has not been established in detail thus far.[9-11]

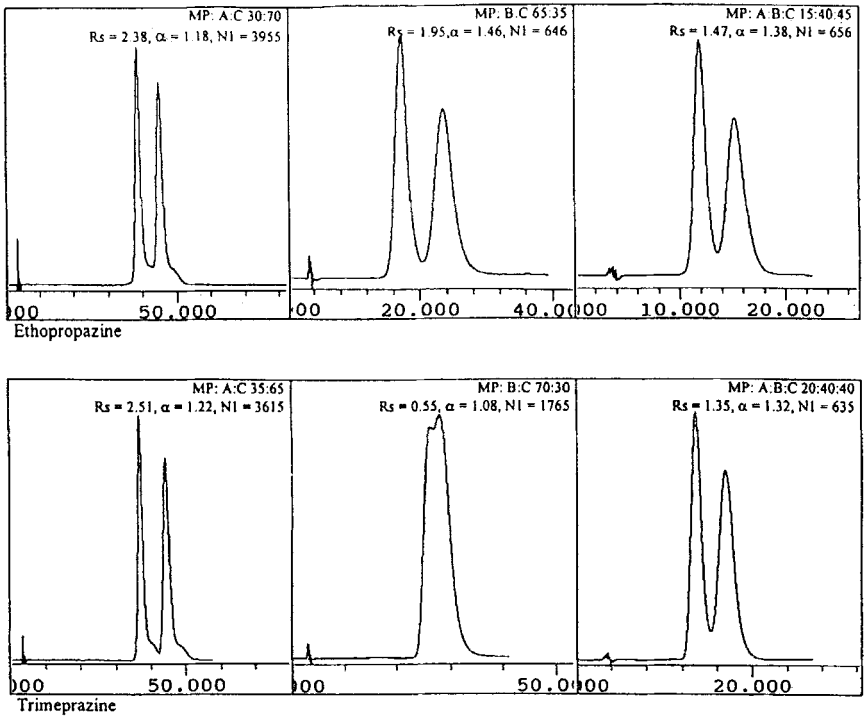

Figure 7. Chiral resolution of ethopropazine and trimeprazine using varied mobile phase compositions.
MP: mobile phase; A: acetonitrile; B: methanol; C: 1.0 M sodium perchlorate solution. Other parameters: cf. Materials section

Francotte *et al.*[12] postulated that the chiral cavities on the cellulose polymer have a high affinity for the aromatic groups. Aromatic compounds would accordingly interact with the substituted derivatized cellulose, the 4-methylbenzoate group in this case, through π–π interactions. Therefore, a suitable sterical fit of (part of) the enantiomer into these cavities plays an essential role in effective resolution of the drug racemates. These multiple interactions, along with solvent effects, contribute to the resolution process for a chiral CSP of this type.

Strict stereospecific requirements are set for a sufficient selective interaction of both enantiomers, which is illustrated by the analyses of this limited group of compounds. Promethazine and thiazinamium only differ in the nature of the nitrogen in the side chain.

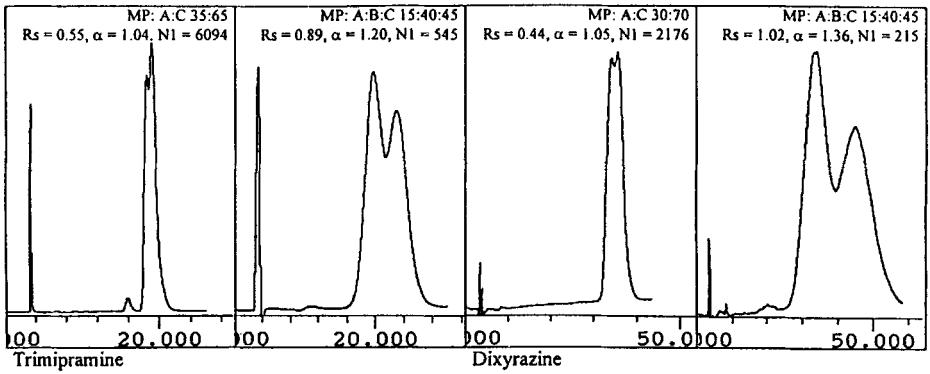

Figure 8. Chiral resolution of trimipramine and dixyrazine using varied mobile phase compositions.
MP: mobile phase; A: acetonitrile; B: methanol; C: 1.0 M sodium perchlorate solution. Other parameters: cf. Materials section

The chiral interaction got only slightly worse due to the additional methyl substituent on the nitrogen. If both methyl groups on this very nitrogen moiety of promethazine are replaced by ethyl groups (rendering it more lipophilic) as in ethopropazine, chiral interaction is greatly negatively affected.

While the latter three drugs have the hydrogen bonding site (-N-) in α-position to the chiral centre, in all the other compounds this site is distanced by one methylene group more (β). Although one could conclude out of the similar results obtained for promethazine and thiazinamium, that the hydrogen binding possibility of that nitrogen can be considered of minor importance, the additional length of one of the substituents on the chiral carbon atom interferes with adequate chiral interaction sites on the polymer; for the results are quite less satisfactory for trimeprazine compared to promethazine.

Moreover, any chiral discrimination is prevented when the sulfur moiety is oxidized (trimeprazine vs. oxamemazine). This indicates that the sulfur moiety with its free electron pair plays an essential role in the interaction phenomena towards the cellulose polymer. The negative effect of large substituents of the nitrogen moiety is confirmed comparing the results for trimeprazine and dixyrazine.

Replacing the sulphur bridge of trimeprazine by an ethylene group as in trimipramine will result in an increasing degree of non-planarity of the two aromatic rings i.e. the two aromatic rings in trimipramine become more folded along the central azepine heterocyclic ring. An enhanced degree of twisting of the aromatic ring thus probably prevents an advantageous stereopositioning of the tricyclic system towards the cellulose cavities.

Compared to the results on the Chiralcel OJ column as published by Ponder *et al.*,[8] the best chiral resolutions were also noted for promethazine, and near baseline separation was found for ethopropazine as well as for trimeprazine. The latter therefore clearly interacted more favourably on the normal phase analogue of the cellulose ester.

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