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On-line recovery of trimeprazine enantiomers following chiral separation by reversed-phase high-performance liquid chromatography using a β-cyclodextrin-containing mobile phase*

A.D. COOPER and T.M. JEFFERIES†

School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK

Abstract: A procedure is described which allows the on-line recovery of enantiomers following semi-preparative chiral separation by RP–HPLC using β-cyclodextrin in the mobile phase. By this method, the phenothiazine antihistamine trimeprazine (I) was resolved into its antipodes at greater than 95% optical purity at a throughput of more than 1 mg of each enantiomer per hour, using 4 mm i.d. columns. The recovered trimeprazine was found to be free from cyclodextrin.

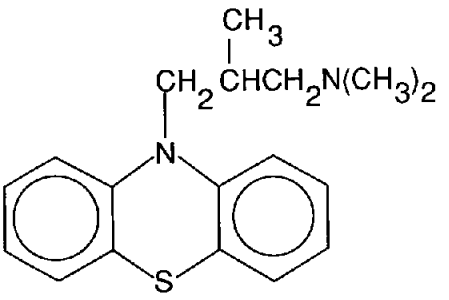
Keywords: *Chiral separation; cyclodextrin; semi-preparative HPLC; trimeprazine.*

Introduction

Chiral chromatographic separations is a field of study which has expanded rapidly over the last few years, and is of particular importance to the pharmaceutical industry due to the emphasis increasingly being placed on the implications of chirality by the regulatory authorities [1].

A variety of HPLC chiral separation methods have been developed in recent years, some of which have proved suitable for preparative applications. "Indirect" methods, involving diastereomer formation with a chiral derivatizing agent have been widely used preparatively [2]. However, such methods have notable disadvantages. The purity of the chiral derivatizing reagent is critical in determining optical purity of the final product, racemization may occur during derivative formation, and recovery of the starting material from the derivative following resolution may prove difficult.

"Direct" chiral chromatographic methods, involving either chiral stationary phases (CSPs) or chiral mobile phase additives (CMAs) avoid the disadvantages of indirect methods noted above, and also have found application to preparative enantiomer resolution. A wide range of CSPs are available, based on chiral



(I)

moieties including cellulose, starch, crown ethers, cyclodextrins, amino acids, and synthetic polymers [3]. Whilst many large-scale separations have been achieved, no one CSP has been found to be effective for a wide range of racemates, and their capital cost is often high.

Chiral mobile phases, employing such additives as chiral ion-pairing agents, cyclodextrins, and metal ion–amino acid complexes have found application to analytical-scale chiral HPLC on achiral columns [4]. However, they have been little applied to larger-scale separations, due to cost considerations, and

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†Author to whom correspondence should be addressed.

the problem of recovering the resolved enantiomers free from the eluent additive [5].

Cyclodextrins have proved to be among the more useful chiral selectors in analytical-scale chiral HPLC, as they exhibit enantioselectivity for a wide range of solutes. They have been incorporated into CSPs such as the Cyclobond range developed by Armstrong and his co-workers [6], or used as mobile phase additives [7]. β-Cyclodextrin polymer gels have been used as CSPs for the mg-scale resolution of racemates such as methyl mandelate [8] and indole alkaloids [9]. Analogous separations of mandelic acid and warfarin on Sephadex gel via β-cyclodextrin complexation also have been reported [10]. In these cases, the throughputs achieved were low owing to the low flow rates used. Semi-preparative HPLC chiral separations using cyclodextrins are as yet unreported.

We report herein the results of investigations into the potential use of β-cyclodextrin as a mobile phase additive to achieve semi-preparative chiral separations. The problem of recovering the resolved enantiomers from the additive is overcome by the use of a column switching procedure.

Experimental

Materials and apparatus

The chromatograph consisted of an LDC Constametric IIIG pump, with a Promis 2 autosampler with integrated stream switching (ISS) facility (on loan from LDC Analytical, Stone, Staffs, UK). The detectors used were an LDC Spectromonitor 3000 UV detector, and a DuPont UV spectrophotometer with low-volume flow-cell.

SGE-100GLC4-C8-30/5 columns were loaned by Scientific Glass Engineering (Milton Keynes, UK). Two columns (100 × 4.6 mm i.d.) were slurry packed in acetone with Lichroprep RP18 25–40 μm material (obtained from BDH, Poole, UK).

Acetonitrile (HPLC grade), chloroform (HPLC grade), and glacial acetic acid (SLR grade) were obtained from FSA (Loughborough, UK). Triethylamine (Spectrosol grade) was obtained from BDH (Poole, UK). Beta-cyclodextrin (purum) was obtained from Fluka (Glossop, Derbys, UK), and was used as received. Trifluoroacetic acid (99%) was obtained from Aldrich (Gillingham, Dorset, UK) and was distilled before use. Trimeprazine hemi(+) tartrate was obtained from Sigma (Poole, UK), and 1-methotrimeprazine hydrochloride was a gift from Rhone-Poulenc (Dagenham, UK). Water was distilled before use. All mobile phases were filtered through 0.45-μm membrane filters and degassed by sparging with helium before use.

^{1}H-NMR spectra were measured using a JEOL GX-270 MHz spectrometer, in D_2O, with DSS (sodium 2,2-dimethyl-2-silapentane-5-sulphonate) as an external reference (0.00 ppm).

Preparation of ±-trimeprazine trifluoroacetate. ±-Trimeprazine hemi-(+)-tartrate (0.5 g) was dissolved in distilled water (20 ml). Trifluoroacetic acid was added dropwise until trimeprazine trifluoroacetate was produced as a gelatinous white precipitate. This was extracted into chloroform (2 × 10 ml), and the organic phase was dried with $MgSO_4$, before evaporation. After drying over P_2O_5 at reduced pressure, a viscous green oil resulted which showed no tendency to crystallize. The crude trifluoroacetate was used for NMR comparison with chromatographically resolved material.

Resolution of trimeprazine enantiomers

Trimeprazine enantiomers were separated on an SGE-100GLC4-C8-30/5 column (5 μm octylsilica; 300 Å pore size; column dimensions 100 × 4 mm i.d.). The mobile phase was prepared by mixing 900 ml of an aqueous solution containing 0.8% (v/v) triethylamine and adjusted to pH 4 with glacial acetic acid with 100 ml of acetonitrile, and adding 9 g of β-cyclodextrin.

Following separation, the enantiomers were switched onto two "recovery" columns (Lichroprep RP18, 25–40 μm, 100 × 4.6 mm i.d.) using the dual-valve integrated stream switching (ISS) facility on the LDC Promis 2 autosampler. When the capacity of one of the recovery columns was reached, they were flushed with 2.5 ml water, followed by 5 ml acetonitrile–water (10:90, v/v), to remove cyclodextrin and buffer components. The trimeprazine enantiomers were then eluted in turn (and collected) with acetonitrile–0.14% aqueous trifluoroacetic acid (70:30, v/v), from which they could be recovered (as the trifluoroacetate salt) by evaporation of solvent.

The switching valve arrangement is illustrated in Fig. 1. The two Rheodyne 7000 valves

that form the ISS facility on the Promis 2 allowed the switching of the solvent stream to recovery column 1 (for retention of the first enantiomer), to column 2 (for retention of the second enantiomer), or directly to waste (before and between the two peaks). A third Rheodyne 7000 valve, operated manually, allowed the by-passing of the separation column during the flushing and elution stages of the procedure, thus reducing equilibration time.

Two UV detectors were placed in-line. The first monitored the effluent from the separating column (in order to determine the valve switching times), and the second monitored the effluent from the recovery system (in order to assess when loading, flushing, or elution were complete).

The eluted enantiomer fractions were assayed for recovery and optical purity by re-injection into the HPLC system described above, using levo-methotrimeprazine as an internal standard. Their chemical purity was assessed by ^{1}H-NMR spectroscopy.

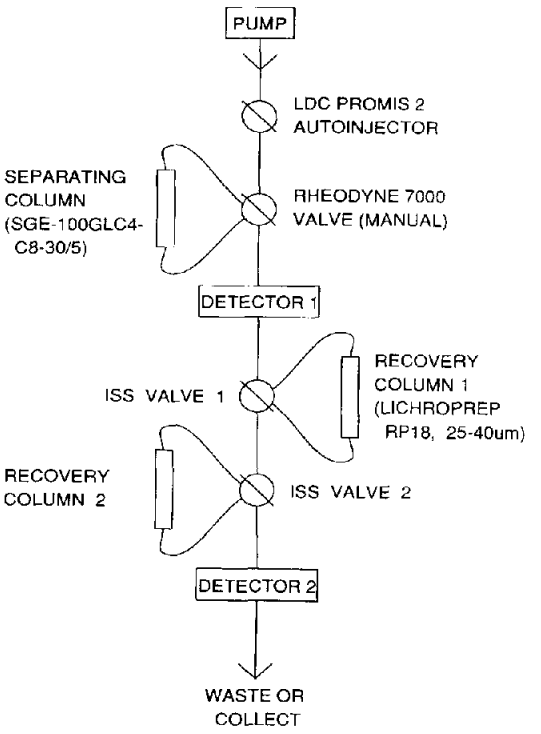

Figure 1
Column switching system for on-line recovery of enantiomers.

Results

The separation of trimeprazine enantiomers on an analytical scale using a β-cyclodextrin-containing mobile phase has been reported by Mularz [11]. As shown in Fig. 2, baseline resolution of enantiomers could readily be achieved at analytical loading, in an analysis time of under 15 min. This separation was considered an ideal candidate for "scaling-up". The largest loading of ±-trimeprazine hemi-(+)-tartrate achievable, consistent with good resolution, was found to be 1 mg per injection. The optimum injection volume was found to be 50 μl. Smaller injection volumes containing 1 mg were found to lead to loss of resolution.

The effect of mobile phase composition was investigated. The presence of acetonitrile was found to decrease enantioselectivity (by interfering with complexation), but to increase column efficiency.

Optimal resolution was achieved using an eluent containing 10% (v/v) acetonitrile. The optimum cyclodextrin concentration was found to be 9 mg ml^{-1}, i.e. well below its maximum solubility. This cyclodextrin concentration was found to be sufficient to give near-maximal selectivity at an appropriate retention time (k' = 5–10). The presence of triethylamine

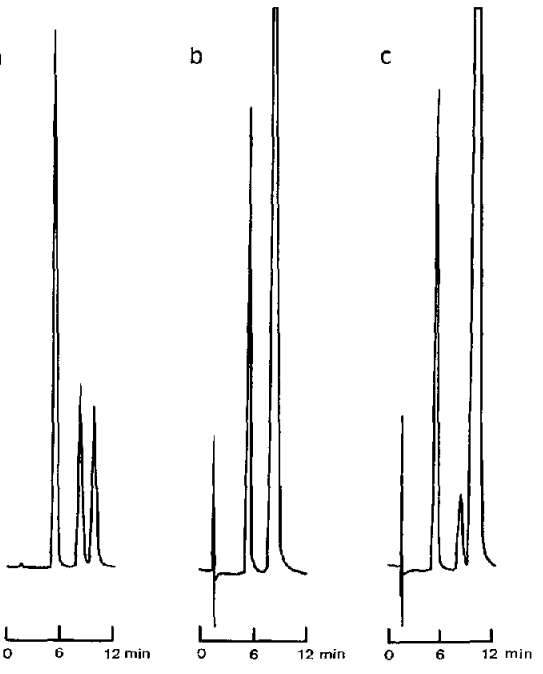

Figure 2
Analytical resolution of trimeprazine enantiomers. Conditions as text. 0.25 μg int. std. on col. Detection UV 300 nm. (a) ±-Trimeprazine tartrate 0.25 μg on col. (b) Collected trimeprazine peak 1 (optical purity >99%). (c) Collected trimeprazine peak 2 (optical purity 96%).

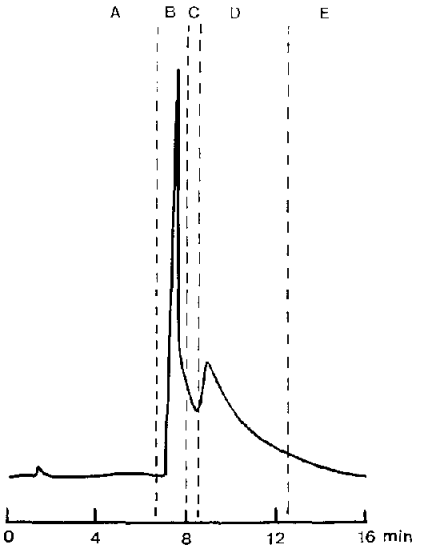

Figure 3
Semi-preparative resolution of trimeprazine (1 mg on col.), showing valve switching times. A, C and E = to waste; B = collect peak 1; D = collect peak 2.

was found to improve peak shape, presumably due to silanol masking. Buffering at pH 4 ensured complete protonation of the basic drug molecule.

The above conditions gave satisfactory resolution in 14 min per injection, as shown in Fig. 3. The capacity of recovery column 2 was reached first, at 8 mg loading. Thus 16 injections of racemate were made before the recovery columns were flushed and the enantiomers eluted. The optical purity, recovery, and throughput values obtained for each enantiomer are shown in Table 1. The high optical purity of the recovered fractions is illustrated in Fig. 2.

The procedure was found to be robust and reproducible. Over the 4 h of a 16-injection "run" it was not necessary to change the valve switching times, despite slight variations in laboratory temperature. The system could therefore be left to run unattended during the "loading" part of the procedure. Slight day-to-day variations in retention times were observed, presumably due to batch-to-batch mobile phase changes. These variations did not adversely affect the resolution, and could readily be allowed for by appropriate choice of switching times.

Table 1
Summary of semi-prep results

Peak	Recovery (%)	Optical purity (%)	Throughput (mg h^{-1})
1	92	>99	1.6
2	85	96	1.5

NMR analysis of the collected antipodal fractions, as shown in Fig. 4, revealed them to be almost completely free of β-cyclodextrin (giving no appreciable signal at 5.1 ppm), but showed the presence of significant quantities of triethylamine (giving a characteristic triplet at 1.25 ppm).

Discussion

The procedure described herein shows promise as a means by which the enantioselectivity of β-cyclodextrin may be exploited to achieve mg-scale chiral separations. In the experiments reported here, more than 1 mg h^{-1} of each trimeprazine enantiomer was prepared at high optical purity. This throughput could be markedly increased by the use of larger columns. To this end, 10 mm i.d. columns are currently being prepared for use in subsequent studies.

The column switching procedure proved to be effective at producing resolved enantiomers free from cyclodextrin. The triethylamine contamination may be removed by a more effective flushing procedure, or by changing the recovery column stationary phase. Studies into this problem are continuing.

This method is shown to be a viable alternative to CSPs for mg-scale chiral separations. It takes advantage of the high capacity and low capital cost of conventional (achiral) stationary phases, and utilizes a relatively cheap and selective chiral agent in β-cyclodextrin. The suitability of this procedure for the resolution of a given racemate will depend on the degree of chiral discrimination imparted by the cyclodextrin. Future studies will be focusing on the development of predictive strategies to allow the rapid evaluation of the applicability of this approach, and facile method optimization.

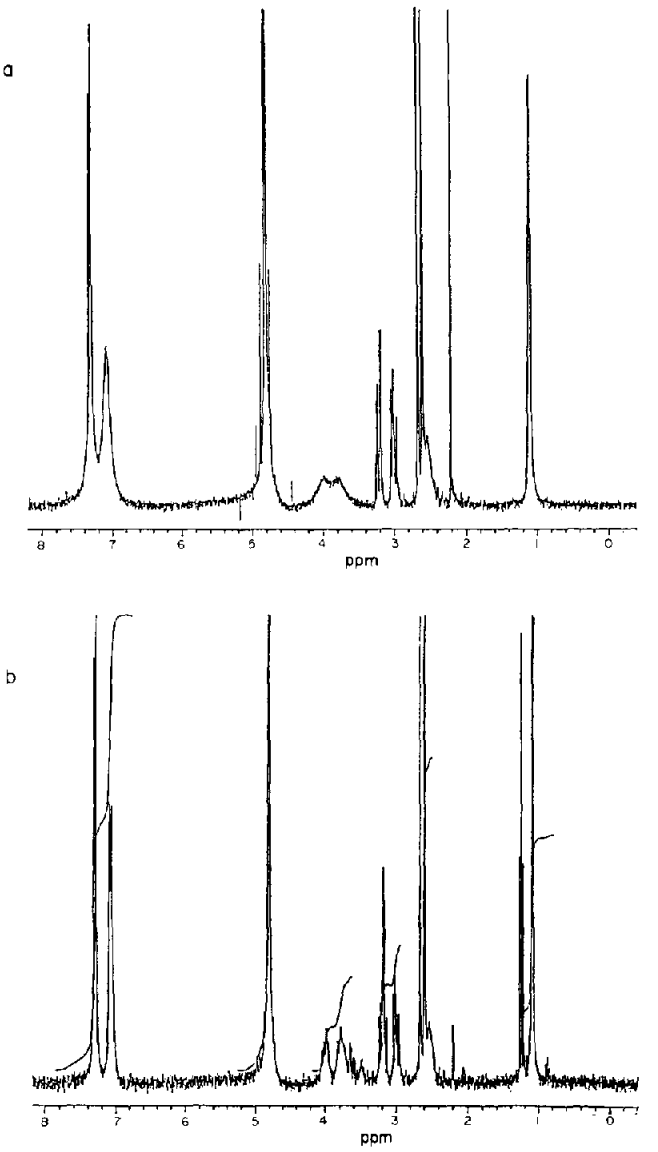

Figure 4
^{1}H-NMR spectra of trimeprazine trifluoroacetate. (a) Racemate (prepared from trimeprazine tartrate, as described). (b) Recovered peak 1 from semi-prep system, showing absence of cyclodextrin.

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References

[1] W. Lindner, *Chromatographia* **24**, 97–107 (1988).
[2] W.H. Pirkle and J. Finn, in *Assymetric Synthesis* (J.D. Morrison, Ed.), Vol. 1, pp. 87–124. Academic Press, New York (1983).
[3] W.H. Pirkle and B.C. Hamper, in *Preparative Liquid Chromatography* (B.A. Bidlingmeyer, Ed.), pp. 237–240. Elsevier, Amsterdam (1987).
[4] B.J. Clark, *Chromatogr. Anal.* 5–7 (June 1989).
[5] W.H. Pirkle and D.L. Sikkenga, *J. Chromatogr.* **123**, 400–404 (1976).
[6] D.W. Armstrong, T.J. Ward, R.D. Armstrong and T.E. Beesley, *Science* **30**, 1132–1135 (1986).
[7] D. Sybilska, *A.C.S. Symp. Ser. (Ordered Media Chem. Sepn.)* **342**, 218–234 (1987).
[8] A. Harada, M. Furue and S. Nozakura, *J. Polym. Sci: Polym. Chem. Ed.* **16**, 189–196 (1978).
[9] B. Zsadon, L. Decsei, M. Szilasi, F. Tudos and J. Szejtli, *J. Chromatogr.* **270**, 127–134 (1983).
[10] Y. Sato and Y. Suzuki, *Chem. Pharm. Bull.* **33**, 4606–4609 (1985).
[11] E.A. Mularz, Ph.D. dissertation, Seton Hall Univ., N.J. (1988).

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