Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, No. 6, pp. 383–404, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00

Review

The chiral chromatographic separation of β-adrenoceptor blocking drugs

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Abstract: Over 100 chromatographic procedures for the separation of β-blocker enantiomers are reviewed including a large number for the analysis of biological samples. All the principal chiral chromatographic procedures have found use, namely Chiral Mobile Phase Additives (CMPA), Chiral Derivatization Agents (CDA) and Chiral Stationary Phases (CSP). Chiral Mobile Phase Additives are less frequently employed than the other two procedures and many of the earlier methods were based on the use of CDAs. However, the recent development of sophisticated custom-made CSPs has allowed the separation of native (underivatized) analytes and this approach appears to be gaining in popularity. The β-blockers are an extensive group of drugs and stereoselective separations have been reported for 40 different structures.

Keywords: *Review; β-blockers; chromatographic separation; chiral separation; enantiomers; optical isomers.*

Introduction

The chiral perspective in pharmaceutical development

The pharmacological significance of molecular chirality has long been appreciated although it is only comparatively recently that it has been given full consideration in initial stages of drug design. The increased understanding of the stereospecific pharmacology of optical isomers has been aided by the development of enantioselective analytical methodology. Such methods have also facilitated the development and understanding of stereoselective synthetic processes. A consequence of this heightened awareness has been increased pressure from regulatory agencies for the development of the 'single-enantiomer' drug. The reader is referred to ref. 1, a particularly useful review of the enantioselective aspects of drug action.

Many techniques have been used for analysing optical isomers, e.g. crystallization, NMR, chromatography, polarimetry, circular dichroism, enzymatic methods, etc. Enantiomeric separation techniques based on chromatography have undergone tremendous development over the past decade. They have been applied in particular to drug compounds to investigate both the pharmacokinetic/pharmacodynamic aspects of drug action [2–7] and also to elucidate the mechanism of chiral chromatographic separation.

One such extensively investigated group is the β-adrenoceptor blocking drugs. These are amongst the most widely prescribed (and therefore therapeutically important) drugs in the world having over 20 members based on the aryloxypropanolamine backbone (see Fig. 1). A measure of their importance is reflected in the fact that there have been over 100 procedures reported for the separation of their enantiomers over the past decade. The chromatographic separation of β-blockers (including their enantiomers) has been reviewed by Davies in 1990 [8] whilst procedures for their determination in biological materials have been twice reviewed [9, 10] in the recent past. However, such is the pace of development in this field that many novel separations have since been reported and a further, comprehensive review of the topic is merited. This article therefore considers the chromatographic strategies which have been reported for the enantioselective separation of β-blockers. Whilst a considerable amount of effort is being expended on developing non-separatory procedures (e.g. NMR, ORD spectroscopy, etc.) these are considered to be outside the scope of this article.

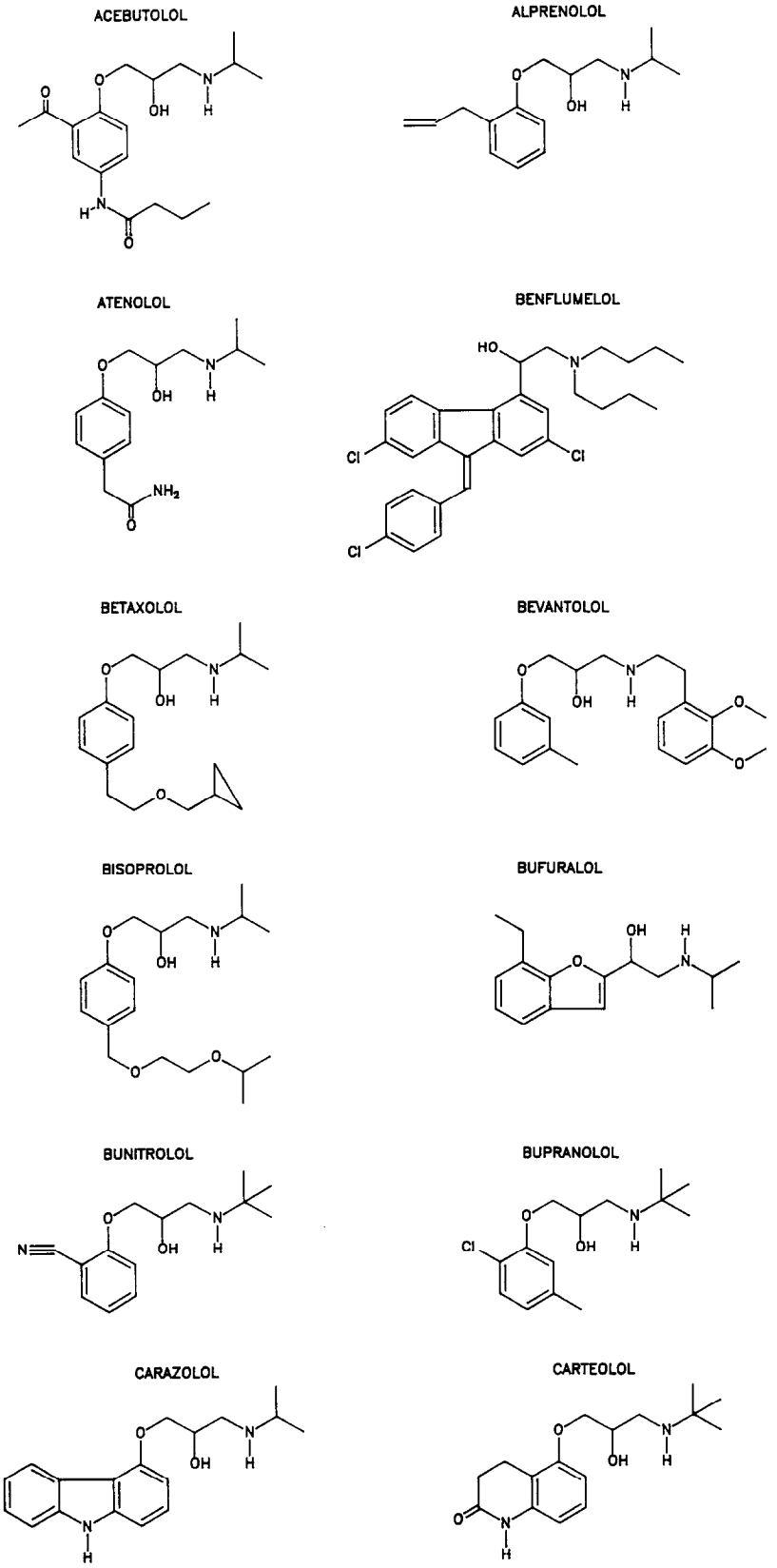
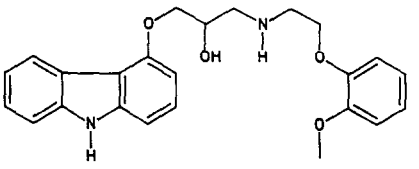


Figure 1
Structures of β-blockers.

CARVEDILOL



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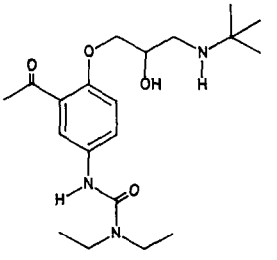
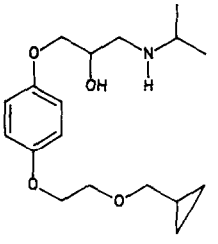
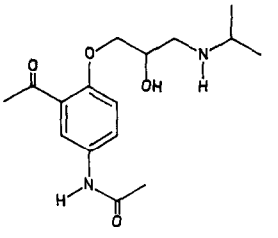


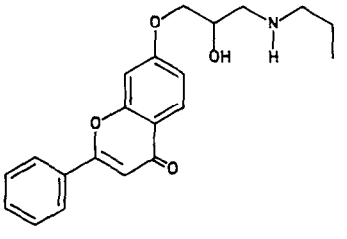
CICLOPROLOL



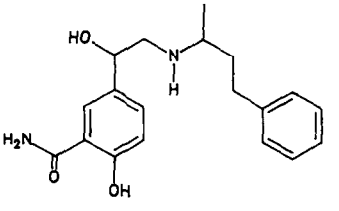
DIACETOLOL



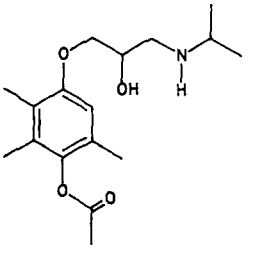
FLAVODILOL



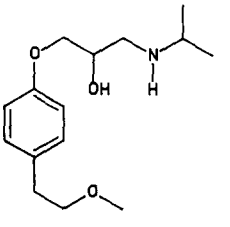
LABETALOL



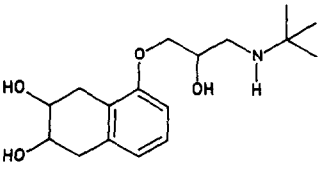
METIPRANOLOL



METOPROLOL



NADOLOL



NIFENALOL

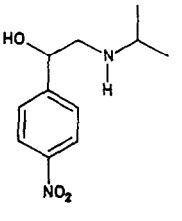


Figure 1
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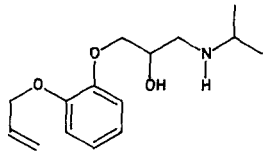
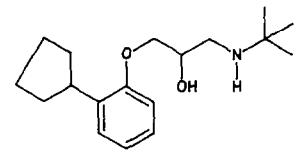
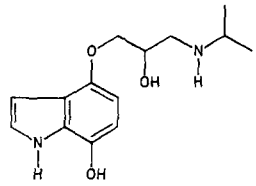
OXPRENOLOL



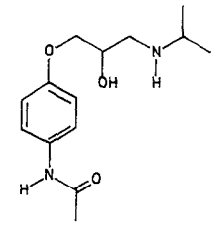
PENBUTOLOL



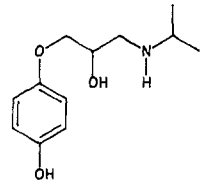
PINDOLOL



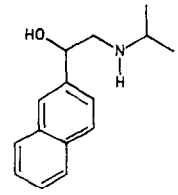
PRACTOLOL



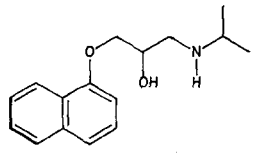
PRENALTEROL



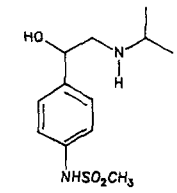
PRONETHALOL



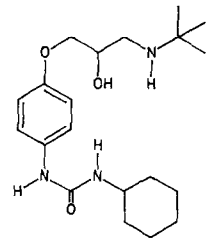
PROPRANOLOL



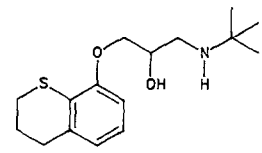
SOTALOL



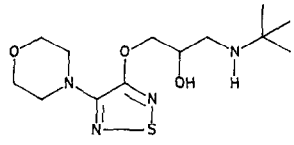
TALINOLOL



TERTATOLOL



TIMOLOL



TOLIPROLOL

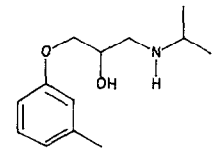


Figure 1
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Chiral chromatographic separation strategies

Chiral separations using both gas and liquid chromatographic procedures have been achieved and such separations may be classified as direct or indirect. In indirect separation the enantiomeric analytes are reacted with a homochiral derivatizing reagent (CDA) to form a mixture of diastereomers. These differ in their physicochemical properties and can thus be separated by conventional achiral chromatography. Direct separations rely on the formation of transient diastereomeric complexes between the enantiomeric analytes and a chiral selector in the system. Where the selector is incorporated in the eluent the separation is termed as one based on a 'Chiral Mobile Phase Additive' (CMPA). Where it is bound to the chromatographic sorbent support the system is considered as containing a 'Chiral Stationary Phase' (CSP).

Several excellent reviews have been published which discuss chiral chromatographic separation strategies in terms of diastereomer formation, CMPAs and CSPs [11–13]. Wainer has reviewed [14] the types of CSP in use which he characterized into five categories and this classification has become accepted and found widespread use. No similar classification of chiral GC stationary phases has been established nor have CMPAs been classified.

Gas Chromatographic Procedures

Early GC procedures relied upon the indirect separation of diastereomers formed by reaction of the analyte with a chiral derivatizing reagent. Thompson *et al.* [15] resolved propranolol enantiomers following derivatization with (*R*)-(+)-phenylethyl isocyanate on column packed with OV-22 using temperature programming.

Caccia *et al.* [16, 17] compared the performance of the reagents *N*-trifluoroacetyl-*l*-prolyl chloride (TPC) and *N*-heptafluorobutyryl-*l*-prolyl chloride (HPC) on both capillary and packed columns of OV-225. The HPC derivatives gave superior resolution for alprenalol, oxprenolol, atenolol, pindolol and propranolol whilst the TPC derivatives gave the better separation for nifenalol and pronethalol. The capillary column (SCOT, 60 m × 0.2 mm i.d.) showed significantly better separation than the packed column (3% OV-255 on Chromosorb W, 2 m × 4 mm i.d.) with oxprenolol, atenolol and pindolol failing to elute from the latter even at its maximum operating temperature. The HFB derivatives of propranolol in biological samples were separated on the packed column using a procedure in which the minimum detectable amount was 25 ng per sample.

The direct separation of β-blockers has been pioneered by Konig and co-workers [18–22] using a capillary glass column coated with the chiral selector XE-60-1-valine-(*R*)-α-phenylethylamine. The analytes were converted to their oxazolidin-2-one or heptafluorobutyryl derivatives to improve their volatility using achiral reagents. Resolution of both isopropylamino- (e.g. oxprenolol, propranolol) and *t*-butylamino-structures (e.g. penbutolol, metoprolol) was achieved with the (*R*)-(+)-enantiomers eluting ahead of their (*S*)-antipodes (see Fig. 2).

Thin-layer Chromatographic Separations

Despite a recent resurgence in the popularity of instrumental TLC there are relatively few reported chiral TLC separations. In 1984 Gubitz and Mihellyes [23] separated a number of β-blockers as their diastereomeric (*R*)-(−)-1-(1-naphthyl) ethylureas. The derivatives were separated on HPTLC silica gel plates using an eluent of benzene–ether–acetone (88:10:5, v/v). Potential quantitative applications are discussed but not verified. In 1987 Pflugmann *et al.* [24] also used diastereomer formation as a means of indirect separation. The reagent (*S*)-(+)-benoxaprofen chloride was used to form fluorescent derivatives of propranolol, metoprolol or oxprenolol, extracted from urine. Metabolites were seen not to interfere in the procedure which was able to detect enantiomers at a level of 100 ng ml^{-1} in urine. The procedure was linear up to 30 μg ml^{-1} (Oxp, Pro) or 40 μg ml^{-1} (Met).

Chiral ion-pair reagents have long been used for separation of amino acid enantiomers although they have had relatively few applications for other analyte types. In 1989 Tivert and Backman [25] reported the use of the chiral reagent *N*-benzoxycarbonyl-glycyl-*l*-proline (ZGP) to resolve alprenolol and propranolol enantiomers on HPTLC–DIOL plates with dichloromethane as eluent (see Fig. 3). Ethanolamine was included in the eluent to reduce tailing which can often be a problem with amine analytes.

Duncan *et al.* [26] investigated a wider range

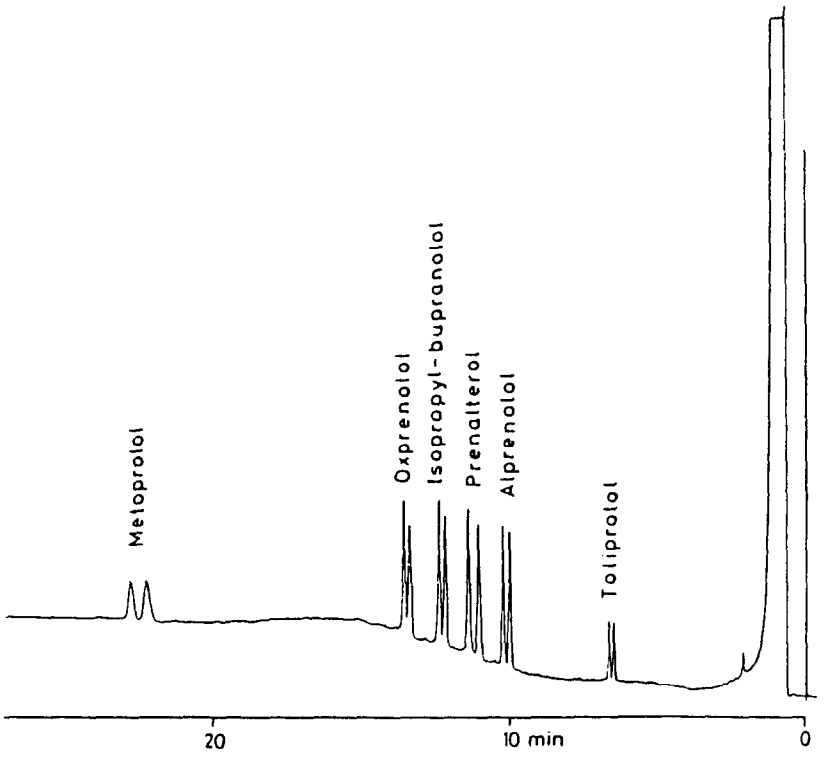

Figure 2
Capillary GC separation on a GC–CSP. (Reproduced from ref. 19 with permission from Elsevier Science Publishers.)

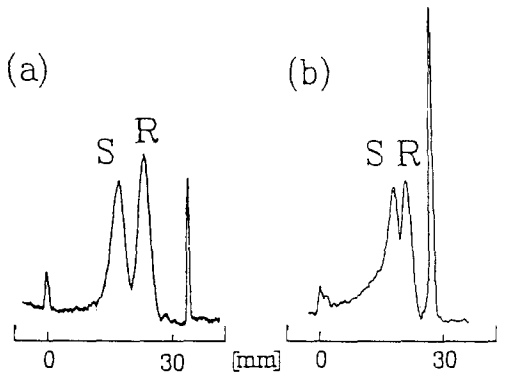

Figure 3
HPTLC separation of enantiomers of (a) propranolol and (b) alprenolol using ZGP as a CMPA. (Reproduced with permission from Dr Alfred Huethig Publishers, taken from A.M. Tivert and A. Backman, *J. Planar Chromatogr.* **2**, 472–473.

of chiral ion-pair reagents and stationary phases. They noted the importance of using dry solvents and plates to achieve enantiomeric separation. Alumina and cellulose plates showed little movement of spots whilst ethyl plates displayed streaking. The diol and silica plates gave effective, reproducible enantiomeric separation. ZGP was shown to be the most versatile reagent, resolving a wide range of analytes.

Wall [27] reported the first direct separation of β-blockers on ionic or covalent Pirkle-type plates. Plates were prepared by reacting HPTLC-amino plates with DNB-phe or DNB-leu. These plates were light sensitive, with the ionic phase darkening more noticeably than the covalent phases over several days. The darkening of the plates could be prevented by protecting them from light although the darkened plates still function acceptably. The enantiomeric elution order was seen to be determined by the nature of the chiral selector rather than the type of link between selector and support, an observation which has also been made for Pirkle-type HPLC columns (see ref. 57).

Column Liquid Chromatographic Procedures

Chiral Mobile Phase Additive (CMPA) procedures

In 1981 Pettersson and Schill [28] first reported the use of a mobile phase containing the homochiral reagent (+)-10-camphor sulphonic acid (CSA) to separate enantiomers of alprenolol, metoprolol, oxprenolol and propranolol. Stereoselective association between

the analyte enantiomers and the counter-ion leads to the formation of transient diastereomeric ion-pairs which can be resolved on adsorption-based columns such as silica. They used a low polarity organic eluent whose composition was varied to assess the effect of solvent polarity on efficiency of separation. As with other workers, they noted the destructive effect of water on weakly polar chiral equilibria and recommended dry solvents and reagents.

Leeman, Dayer *et al.* [29–31] also used CSA as the chiral counter-ion in the separation of metoprolol or bufuralol enantiomers in plasma. Eluents consisting of dichloromethane were modified with propranolol or methanol and separation took place on a Lichrosorb-DIOL 5 μ column. Fluorescence detection allowed sensitivity of 5 ng ml^{-1} sample with a linear response extending over two orders of magnitude.

However, the stereoselectivity of CSA is not very great, due in part of its rigid structure allowing only two points of interaction [32]. The chiral counter-ion ZGP is more flexible and, having several polar functions which allow more points of interaction. Leeman and co-workers investigated the usefulness of this material for the separation of enantiomers of metoprolol and its metabolites in biological samples [33]. They noted that ZGP brought '. . . a clear improvement in selectivity, retention times and sensitivity over *D*-(+)-10-camphorsulphonic acid'.

Pettersson and Josefsson [34] achieved separations using ZGP and a Lichrosorb-DIOL column. They studied the effect of the concentration of both the counter-ion and modifiers such as triethylamine and water on the separations. Using a series of metoprolol-related structures they evaluated the effect of analyte structure on separation. They also used analogues of ZGP to investigate the effect of the chiral counter-ion structure on separation of (*R*)- and (*S*)-alprenolol. They concluded that the carboxylic function of ZGP was vital for enantioselectivity and that the carbonyl group should be free from steric interference. Increasing the concentration of ZGP in the mobile phase from 0.1 to 5 mM resulted in an increase of selectivity (α) of separation of (±)-alprenolol from 0.93 to 1.32. Increasing the concentration of the modifier triethylamine from 0.01 to 0.2 mM resulted in little loss of resolution but a significant reduction in capacity factor. TEA is therefore well suited for adjustment of retention time. ZGP has also found use in SFC separations where Steuer *et al.* [35] evaluated the role of parameters such as operating temperature and pressure as well as the nature and concentration of the ZGP modifier.

Mama *et al.* [36] also used metoprolol as a model analyte, however they used β-cyclodextrin as the CMPA and achieved separation on a porous graphitic carbon column (PGC). The robustness of PGC and relative freedom from tailing-effects with amines were cited as potential advantages of these columns. Fanali also employed cyclodextrin in conjunction with capillary zone electrophoresis [37]. He evaluated the effect of the structure and concentration of this additive in the supporting electrolyte on the separation of propranolol enantiomers. There appeared to be significant advantages of chiral CZE over conventional CMPA-LC.

The effect of temperature, solvent viscosity and solute structure was studied by Petterson *et al.* [38]. They observed separation of oxprenolol, atenolol and propranolol enantiomers on Lichrosorb-DIOL or Nucleosil-CN (atenolol) columns with ZGP as the counter-ion and an eluent of dichloromethane modified with triethylamine (see Fig. 4). The systematic error resulting from the contamination of ZGP with its antipode benzoxyglycyl-*d*-proline was considered. The effects of the impurity on retention and stereoselectivity were described mathematically and conditions for reducing the

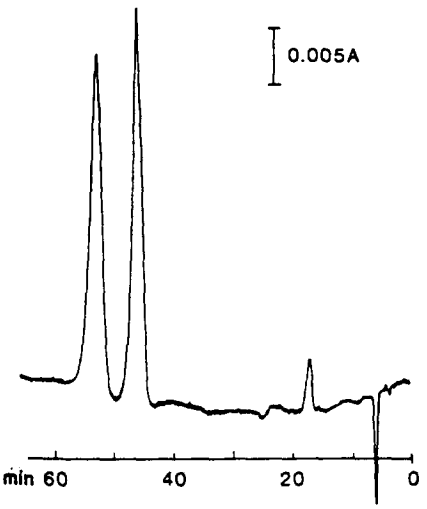

Figure 4
Separation of oxprenolol enantiomers on a Lichrosorb-DIOL column using ZGP as a CMPA. (Reproduced from ref. 38 with permission from Elsevier Science Publishers.)

error discussed. They extended the procedure to the separation of propranolol enantiomers in plasma [39] on a Lichrosorb–DIOL column. The method was sensitive to 0.2–0.3 ng of each enantiomer per millilitre of plasma. System peaks arose as a result of the sample having a different solvent to the eluent although ways of overcoming the interference were suggested.

The use of chiral counter-ion strategies in preparative applications are potentially very desirable. Gaskell and Crooks [40] used 0.01 M (+)-tartaric acid in propan-2-ol to resolve propranolol, atenolol and practolol enantiomers on a normal-phase system. Separations on silica were seen to be superior to those on a Lichrosorb-DIOL column as the former has more adsorption sites leading to a more efficient mass transfer. Separations employed pyridine rather than triethylamine as a competing base in the ratio ion-pair reagent:base 5:1.

Another group also looked at tartaric acid derivatives as chiral complexing agents for use in both reversed-phase and normal-phase separations [41]. Both systems were liquid–liquid (partition-type) separations as the normal-phase columns were coated with a liquid stationary phase consisting of an aqueous solution of a phosphate buffer. In both normal- and reversed-phase system the eluent consisted of the tartaric acid derivative in *n*-hexane, dichloroethane or dichloromethane. Porous graphitic carbon gave a higher stereoselectivity than modified silica and di-*n*-butyltartrate gave very high separations ($\alpha = 1.3$) in some normal-phase systems.

Separations on Chiral Stationary Phases (CSPs)

1. Attractive–interaction phases (Pirkle phases). The development by Pirkle *et al.* [42] of the first commercially available chiral stationary phase DNB–Phe (3,5-dinitrobenzoyl-α-phenylglycine grafted onto amionpropyl-derivatized silica) lead to a number of direct separations of enantiomeric drugs, including β-blockers. Wainer *et al.* [43] separated propranolol enantiomers derived from human serum on DNB–Phe. Calibration curves were linear over the range 0.5–100 ng ml^{-1} and the procedure was capable of quantitating 0.5% of one isomer in the presence of the other. A characteristic of separation of β-blockers on such phases is the need to reduce strong interactions between analyte and selector, thus derivatization is undertaken with achiral reagents to mask the reactive amino and/or alcohol functions. Wainer converted propranolol to its oxazolidin-2-one by reaction with phosgene whilst others have formed naphthamide derivatives of the β-blockers prior to their separation on a DNB–Phe column [44]. The 2-naphthamide derivative of propranolol exhibited longer retention and better resolution than the 1-naphthamide derivative. This was attributed to the more favourable steric orientation of the 2-naphthamide ring allowing a stronger solute–selector complex to form.

Yang and co-workers [45–47] reported the facile formation of urea derivatives of β-blockers as an alternative, more rapid masking derivatization. They used α-naphthylisocyanate to form the urea derivative of the side-chain amino function. They achieved separations of propranolol, oxprenolol, alprenolol, metoprolol and timolol on DNB–Phe CSP. Derivatization with α-naphthylisocyanate has also been employed in the direct single-step resolution of all four stereoisomers of nadolol [48].

In addition to masking strongly polar functions, the achiral derivatization can be used to introduce into the analyte molecule groups which can enhance the chiral recognition process. Dyas *et al.* [49] assessed the effect of derivative group function on stereoselectivity using the urea derivative as a model. A series of aliphatic, alicyclic and aromatic isocyanates were reacted with propranolol to form urea derivatives which were chromatographed on a number of Pirkle-type CSPs. The different resolutions achieved cast some light on the separation mechanism involved. The use of isothiocyanate reagents yielded thiourea derivatives which were superior to their urea analogues on certain phases. A similar approach was adopted by researchers who evaluated the separation of *t*-butyl isocyanate derivative of propranolol on 12 different urea-based CSPs [50]. The phases were urea derivatives of substituted amino acids and the separations achieved were related to interactions such as hydrogen bonding, dipole stacking and in particular π–π overlap. They were able to resolve alprenalol and oxprenolol enantiomers using such phases.

The crucial influence of structure on separation lead to the design of CSPs having more elaborate functions in an attempt to enhance enantio-selectivity. Pirkle's observations on

the reciprocal aspect of chiral recognition were fundamental to this development. He noted that analytes containing naphthyl functions were often well separated on DNB-derived CSPs and so postulated that the converse would probably also be true, i.e. DNB-derived enantiomeric analytes might be resolved on naphthyl-based CSPs. This hypothesis was borne out when he reported good separations of a number of β-blockers on novel CSPs based on *N*-acyl-α-arylalkylamines [51, 52].

Further evaluation of such CSPs has continued with two groups [53, 54] using β-blocker derivatives as model compounds. The continued rational development of CSPs eventually lead to the development by Pirkle and Burke of phases capable of resolving β-blocker enantiomers without prior derivatization [55]. Such phases contain weakly polar functions such as carboxylic or phosphonic acids and were useful in the separation of metoprolol, pronethalol, propranolol, pindolol and bufuralol although not oxprenolol (see Fig. 5).

The influence of the selector-support bond was investigated by Dyas *et al.* [56, 57] using a range of urea and thiourea derivatives of propranolol. Various ionic- and covalent-bonded CSPs were used and there appeared to be a systematic relationship between the derivative type and the type of column (covalent or ionic). The enantiomeric elution order was generally seen to be dependant on the structure of the chiral selector rather than on the derivative structure or the selector-support bond.

Gasparrini *et al.* [58] developed a multifunctional CSP which was based on *N*, *N'*-di-(3,5-dinitrobenzoyl)diaminocyclohexane (DACH) grafted onto silica which separated the oxazolidin-2-one derivatives of a large number of β-blockers. DACH is a diastereomeric material and Gasparrini demonstrated the possibility of inverting the elution order of propranolol enantiomers by switching from (*S*,*S*)-DACH to (-*R*,*R*)-DACH. By this means it was possible to detect (*R*)-propranolol in the presence of a 99.98% enantiomeric excess of the (*S*)-antipode.

The usefulness of ZGP as a chiral mobile phase additive lead Ohwa *et al.* [59] to synthesize a CSP comprising glycyl-*l*-proline grafted onto silica gel. They reported separation of propranolol, pindolol, carteolol, metoprolol and atenolol on this material. They evaluated the effect of mobile phase components (halogenated hydrocarbon, alcohol and amine) on chromatographic performance in order to optimize the separation. Dyas *et al.* [60] also evaluated solvent effects using a different model separation of propranolol (as phenylurea derivative) on a phenethylpropylurea CSP. The nature of the hydrocarbon selector as the main solvent was seen to have a significant effect on separation and a tentative relationship between solvent viscosity and separation was identified.

The successful transposition from CMPA to CSP does not always follow, as the grafting stage itself can detrimentally modify the chiral selector structure. Whilst CSA has been used successfully as a CMPA for separations, Coors and Matusch [61] were unable to achieve any separation of the enantiomers of oxprenolol or propranolol using a CSA–CSP.

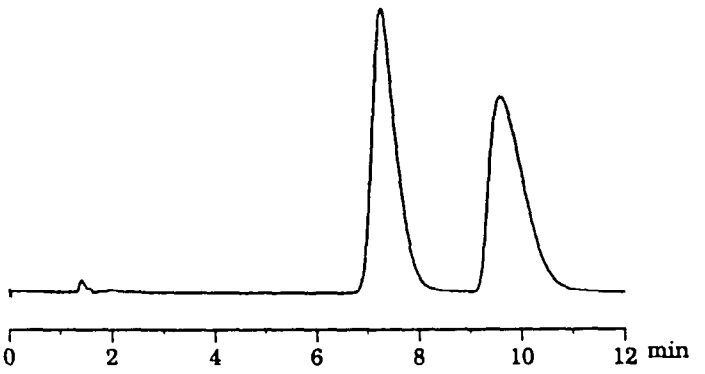

Figure 5
Direct separation of underivatized propranolol enantiomers on a selectively designed Pirkle CSP. (Reproduced from ref. 55 with permission from Elsevier Science Publishers.)

2. Inclusion–interaction phases. In the early 1980s Okamoto *et al.* pioneered the development of a CSP based on substituted cellulose derivatives adsorbed onto silica gel. In 1986 [62] they undertook an extensive evaluation of the separation of β-blockers on cellulose triphenylcarbamate derivatives. Propranolol and pindolol were chromatographed on each of 13 different phases from which it emerged that 4-nitrophenyl and 4-methoxyphenyl functions lead to a loss of stereo-selectivity.

Disubstituted carbamates showed themselves to be particularly useful with cellulose tris-(3,5-dimethylphenyl)carbamate yielding good resolution of alprenolol, oxprenolol, propranolol and pindolol without prior derivatization. It proved to be a versatile phase capable of resolving a variety of enantiomeric drugs. Aboul-enein and co-workers reported resolutions of carazolol [63], penbutolol [64] (see Fig. 6), and timolol [65] on it. They evaluated the effect of polar modifiers such as alcohols and amines on the separation as well as demonstrating that the separations were very sensitive to changes in temperature. It is notable that in the separation of carazolol the (*S*)-(−)-enantiomer eluted first whilst the converse was true for the separations of penbutolol and timolol. Okamoto in his earlier separations also observed that the (*R*)-(+)-enantiomer eluted first, making carazolol atypical.

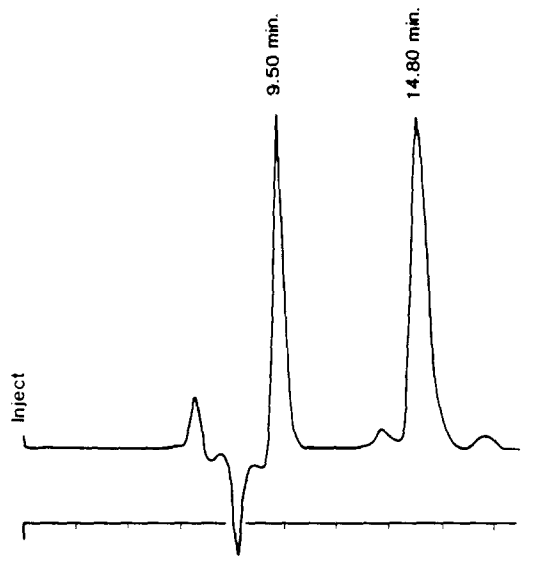

Figure 6
Direct separation of penbutolol enantiomers on a cellulose tris-(3,5-dimethylphenyl) carbamate CSP. (Reprinted by permission of Wiley–Liss, copyright © 1989 Wiley–Liss, taken from H.X. Abohl-enein and M.R. Islam, *Chirality* **1**, 301–304.)

Gaskell and Crooks [66] also investigated the effect of the polar modifier in order to improve the separation of hydrophilic β-blockers.

They noted that relatively hydrophobic β-blockers (octanol–water partition coefficient $P > 2.2$) are often resolved as free bases using base-modified organic eluents. However, separation of more hydrophilic moieties ($P < 2.2$) are precluded by their low organic solubility. Use of eluents modified with carboxylic acids (e.g. trichloroacetic acid) allowed formation of organic-soluble ion-pairs which resulted in significant improvement in chromatographic performance.

Cellulose tris-(3,5-dimethyl phenyl) carbamate has found application in the determination of β-blocker enantiomers in biological fluids. Metoprolol has received particular attention with four methods being published to date [67–70]. All groups used the commercially available column Chiralcel OD (Daicel Chemical Industries Ltd) as well as fluorescence detection. Limits of detection ranging from 3 to 6 ng ml^{-1} were achieved with resolutions as high as 5.5 [67]. The procedure given in ref. 68 is notable as it uses a racemic internal standard which is itself resolved, i.e. yielding two I.S. peaks.

Chiralcel OD was also used by Straka *et al.* [71] to resolve propranolol enantiomers in serum using, as they had in ref. 69, (±) verapamil as internal standard. They used dimethyloctylamine rather than octylamine as the mobile phase modifier and achieved a limit of detection of 7.5 ng ml^{-1} with fluorescence detection. Hartman *et al.* [72] used dimethylamine as the modifier in the separation of celiprolol extracted from plasma and urine. Again internal standardization and fluorescence detection were employed in a procedure which was capable of detecting 1.5 ng (plasma) or 2.5 ng (urine) per millilitre of sample.

Krstulovic *et al.* [73] reported a method for the separation of betaxolol enantiomers in human plasma using Chiralcel OD which was also of value in the determination of enantiomeric purity of the bulk drug. The procedure was capable of detecting 5 ng ml^{-1} of each isomer in rat hepatocyte suspension or down to 0.5% (w/w) of (*R*)-(+)-betaxolol in a bulk sample of the (*S*)-(−)-antipode. They also investigated the use of sub-critical fluid chromatography (Sub-FC) using this phase. Sub-

FC eluents yielded improved separation and reduced retention over conventional normal-phase eluents [74]. Enantiomeric separations were reported for betaxolol, pindolol, metoprolol, propranolol and cicloprolol whilst nadolol could only be resolved into its diastereomers.

Whilst the tris-phenyl derivatives have provided the majority of the separations, other cellulose-based separations have been reported. Isaksson and Lamm [75] used the triacetyl-derivative of microcrystalline cellulose to resolve enantiomers of propranolol and of metoprolol. They converted the analytes to their cyclic carbamate (oxazolidin-2-one) derivative prior to elution with 95% ethanol. The procedure was used semi-preparatively to separate up to 10 mg of sample with the parent β-blocker being liberated by hydrolysis of the eluate. It is noteworthy that the cyclic-derivatives exhibited enhanced stereoselective separation over the parent enantiomers, an observation which the authors used to rationalize chiral recognition on this type of phase.

Cellulose is not the only polymer which has found use as an inclusion–interaction-type CSP. Okamoto and co-workers [76] compared the performance of CSPs based on cellulose, amylose and xylan. They reported the enantiomeric separation of a wide range of drugs including the β-blockers propranolol, alprenolol, atenolol, pindolol, oxprenolol and acebutolol. Sotalol enantiomers could not be separated on a cellulose column but were resolved on amylose tris-(3,5-dimethylphenyl) carbamate.

Schulze and co-workers also looked at alternative polymer supports, reporting separations based on covalently bound monosaccharides [77–79]. They resolved enantiomers (as their phenylurethane or oxazolidin-2-one derivatives) of the β-blockers toliprolol, penbutolol, bupranolol (and analogues), metoprolol, propranolol, alprenolol, bisoprolol, carazolol and betaxolol.

3. Cavity-inclusion stationary phases. β-blocker separations on this group of CSP are limited to a single published example. Armstrong *et al.* [80] have separated enantiomers of propranolol and metoprolol, a system comprising two sequential 25 cm β-cyclodextrin columns. There have been no other reported separations using cyclodextrin CSPs or other cavity-inclusion CSPs such as bound crown ethers. This is quite significant as cyclodextrin CSPs are finding great utility for a wide range of drugs. It would appear that the aryloxypropanolamines do not satisfy the structural criteria for chiral recognition on these selector types.

4. Ligand-exchange columns. Ligand-exchange separation of enantiomers were first reported in the 1960s when Davankov reported the chromatographic resolution of amino acid enantiomers. Amino acids have remained the principal models for this type of separation as they easily satisfy the functional requirements for stereoselective complexation. Kicinski and Kettrup [81] have achieved separation of bupranolol enantiomers using an (*R*,*R*)-tartaric acid-modified silica apparently operating in LEC mode. The stationary phase comprised *N*-(3-(trimethyloxy-silylpropyl))-(*R*,*R*)-*O*,*O*-diacetyl tartaric acid bound to silica gel whilst the mobile phase used was a solution of Cu^{2+} in a buffer. The metal ions are co-ordinated by the bound selector yielding a complex which is responsible for the further stereoselective complexation with bupranolol enantiomers. The stability of the stereoselective interaction is affected by the organic solvent concentration in the mobile phase and by the pH of the buffer. The separation was remarkable in that pH 7.5 acetate buffer eluted the (*l*)-bupranolol enantiomer first whilst pH 4.5 phosphate buffer eluted the (*d*)-antipode first.

5. Protein stationary phases. The first commercially available protein phase, based on α_1-acid glycoprotein (AGP), was the Enantiopac column pioneered by Hermansson in the early 1980s. Hermansson achieved separation of a wide variety of drugs on this column including the β-blockers [82] alprenolol, oxprenolol, metoprolol, pindolol and propranolol. The β-blockers were chromatographed as their oxazolidin-2-one derivatives and some correlation between the structure of the aromatic portion of the molecules and retention/resolution was made. AGP showed itself to be a very versatile selector, although efficiency of the early columns was at times low. In an attempt to optimize such separations, Schill and co-workers [83, 84] evaluated the effect on model separations of variables such as temperature, flow rate, pH and

composition of the eluent as well as sample loading. Enantiomeric resolutions were achieved for labetalol, nadolol and metoprolol under a wide range of conditions. Le Garrec and colleagues developed separations of (*d*)- and (*l*)-sotalol on this column [85, 86]. There were problems of robustness with the Enantiopac columns which were apparently overcome by changing the manner in which the α_1-acid glycoprotein was bound to the silica gel. The new type of column, called Chiral-AGP, was critically compared to enantiopac by Balmer *et al.* [87] using metoprolol as a model compound. They evaluated the effect of such variables as pH, temperature, gradient conditions and organic solvent on the separation, demonstrating the superior performance of Chiral-AGP. They did not compare the ruggedness of the two columns although the fragility of the Enantiopac column has since become apparent. The nature and performance of the immobilized protein was examined in some depth by Enquist and Hermansson [88] using as models the β-blockers atenolol, metoprolol, pindolol, propranolol, oxprenolol and alprenolol. Enantiomeric separations of each of these agents were achieved with selectivities (α) ranging from 1.2 to 1.8. The same authors went on to develop the separation of (*R*)- and (*S*)-atenolol in samples of human plasma and urine on the AGP column [89]. The atenolol enantiomers were acetylated to allow them to pass through the reversed-phase guard columns employed to overcome interference from endogenous compounds. However, the derivatization was seen also to enhance enantioselectivity significantly. The effect of mobile phase pH and of washing the column regularly was investigated as this had a bearing on the robustness of the procedure. The method was capable of detecting 6 ng ml^{-1} of each enantiomer and was linear over the range 6–502 ng ml^{-1} plasma and 0.4–45.2 μg ml^{-1} urine.

Persson and co-workers determined of β-blocker enantiomers in plasma [90] using this type of phase. They separated (*R*)- and (*S*)-metoprolol using Chiral-AGP, gradient elution and fluorescence detection demonstrating the sensitivity of the separation to temperature. They obtained R_s values varying from 3.4 to 2.6 over the temperature range 7–28°C. As with reference [87] earlier, they demonstrated the superior performance of Chiral-AGP over Enantiopac. The procedure was sensitive to about 0.5 ng ml^{-1} of each enantiomer under ideal conditions.

Walhagen and Edholm also used Chiral-AGP for the quantitation of metoprolol enantiomers in plasma [91], however they employed coupled-column technology to enhance sensitivity. The enantiomers were resolved on the chiral column and the peaks then separately heart-cut onto achiral columns. This caused peak compression so that the new sharper bands could finally be switched through a fourth (achiral) column and detected as sharper peaks. The column switching allowed 100% transfer and detection in the region of 10 pmol of each enantiomer. The same group also adapted the column-switching method to allow liquid chromatographic–tandem mass spectrometric analysis of the resolved enantiomers derived from plasma samples [92] using deuterium-labelled metoprolol as an internal standard. Use of selected ion monitoring at $m/z = 268$ also increased selectivity and removed baseline disturbances caused by valve switching. The method was capable of quantitating 70 ng ml^{-1} of racemic metoprolol. The β-blockers labetalol and nadolol each consist of four stereoisomers and Chiral-AGP has been used for the full enantiomeric separation of each drug [74, 93].

Whilst AGP is the most popular protein CSP, it is not the only one to have found commercial utility. Bovine Serum Albumin (BSA), pioneered by Allenmark, has also been widely used. However, it is generally accepted that BSA is of most value in the resolution of chiral anionic drugs and is thus little used for β-blockers. Kusters and Giron [94] achieved some success in the separation of pindolol enantiomers on Resolvosil BSA7. They first derivatized the two secondary amino functions of the analytes with isopropyl isocyanate before resolving the enantiomer derivatives with a mobile phase comprising of phosphate buffer and propan-2-ol.

Columns employing bound ovomucoid have received much attention as this protein has excellent chiral recognition properties, appears more stable to external influence than AGP and is both readily available and relatively inexpensive. Kirkland and co-workers [95] critically compared OVM and AGP phases, concluding that ovomucoid '. . . showed generally higher resolution, greater flexibility in operating parameters, and better long-term stability than the acid glycoprotein column'.

Table 1
Comparison of the performance of Chiral-AGP and an ovomucoid column. The chromatographic parameters are calculated using the first eluting enantiomer. (For details see ref. 95)

	K'		N		α		R_s	
	AGP	OVM	AGP	OVM	AGP	OVM	AGP	OVM
Atenolol	6.9	—	550	—	1.2	1.0	1.2	—
Propranolol	29.2	3.9	1639	—	1.1	1.1	1.1	0.8
Pindolol	6.1	2.5	3005	992	1.2	1.6	1.9	2.3

However, whilst this may generally hold true for the wide range of drugs he evaluated, it was not particularly evident for the β-blocker models chosen (see Table 1).

The good separation on ovomucoid seen by Kirkland for pindolol was also seen by Miwa *et al.* [96] whilst Arai and Kuroda [97] achieved separation of carvedilol on an Ultron ES-OVM column (see Fig. 7). Miwa *et al.* [98] went on to develop a column based on the chicken egg-white protein, Avidin. This also failed to achieve separations of β-blocker enantiomers (pindolol and oxprenol) despite good results for acidic drug groups.

Indirect separations using chiral derivatization agents (CDAs)

The indirect determination of enantiomers as their diastereomers formed by reaction with a homochiral derivatizing reagent has long been established. Indeed the *O*-glucuronide diastereomers of propranolol are formed naturally during the metabolism and may be separated easily on an achiral sorbent [99, 100].

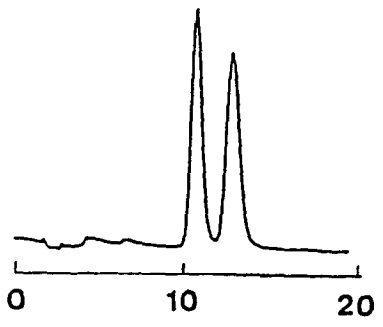

Figure 7
Direct separation of carvedilol enantiomers on an ovomucoid-derived CSP. (Reproduced with permission from ref. 97.)

The two target functional groups of β-blockers which are of use in diastereomer formation are the amino and alcohol groups, although the latter is less widely used. Derivatization reagents which have found use include acid anhydrides, acid chlorides, cyanides or isocyanates/isothiocyanate, as given in Fig. 8.

1. Acid anhydrides. Anhydrides of amino acids and of tartaric acid derivatives have found use as CDAs. Hermansson has quantitated alprenolol, metoprolol and propranolol in plasma using symmetrical anhydrides of *tert*-butoxycarbonyl-*l*-leucine (*t*-BOC-Leu) or *t*-BOC-alanine [101, 102]. The plasma extracts are reacted with the BOC-*l*-Ala or BOC-*l*-Leu reagents in the presence of triethylamine as a catalyst. After completion of the reaction, the protective BOC function is cleaved off with trifluoroacetic acid. Fluorescence detection was employed which allowed the detection of 0.2–1.1 ng ml^{-1} depending on the analyte. BOC-*l*-Leu was used by Guttendorf *et al.* [103] for the quantitation of propranolol enantiomers in rat plasma. They worked with 100 μl samples and showed excellent inter- and intra-day precision at levels down to 25 ng ml^{-1}.

In contrast to the *t*-BOC reagents, the substituted tartaric acid anhydride reagents couple with the side-chain secondary alcohol. This results in a diastereomeric tartaric acid monoester of the alkanolamine which may assume the structure of an intramolecular zwitterionic ring according to Lindner *et al.* [104]. They achieved separation of 15 different β-blockers using *O,O*-dibenzoyl tartaric acid anhydrides as derivatizing reagent as well as investigating the effect of the reagent substituent groups on the separation achieved for celiprolol. Lindner, Uray and colleagues extended this work to the enantioselective

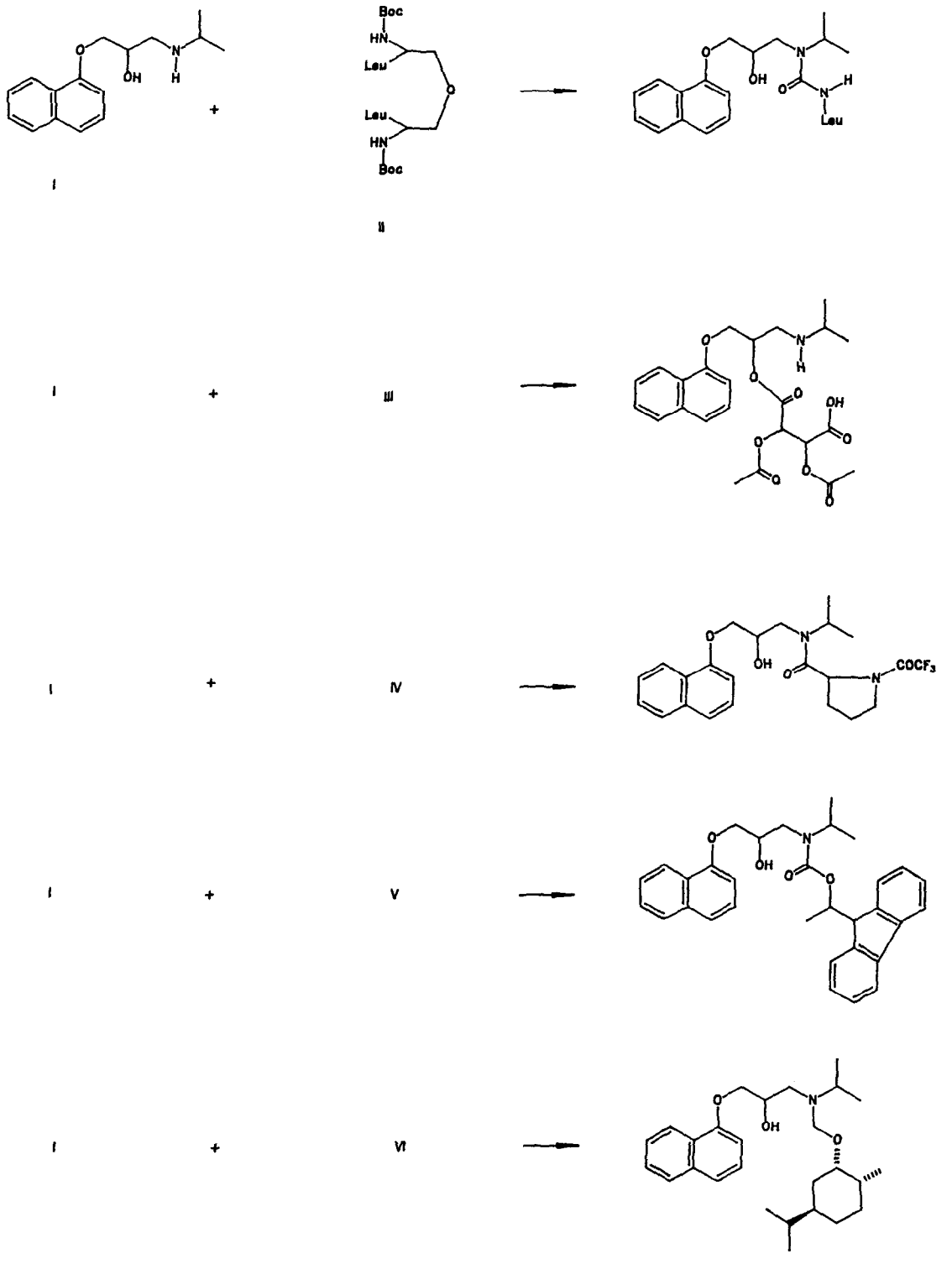

Figure 8
Formation of diastereomers by reaction between propranolol (I) and the CDAs *t*-BOC-Leu (II), diacetyltartaric acid anhydride (III), TPC (IV), FLEC (V), MCF (VI), MBNCC (VII) and PEIC (VIII).

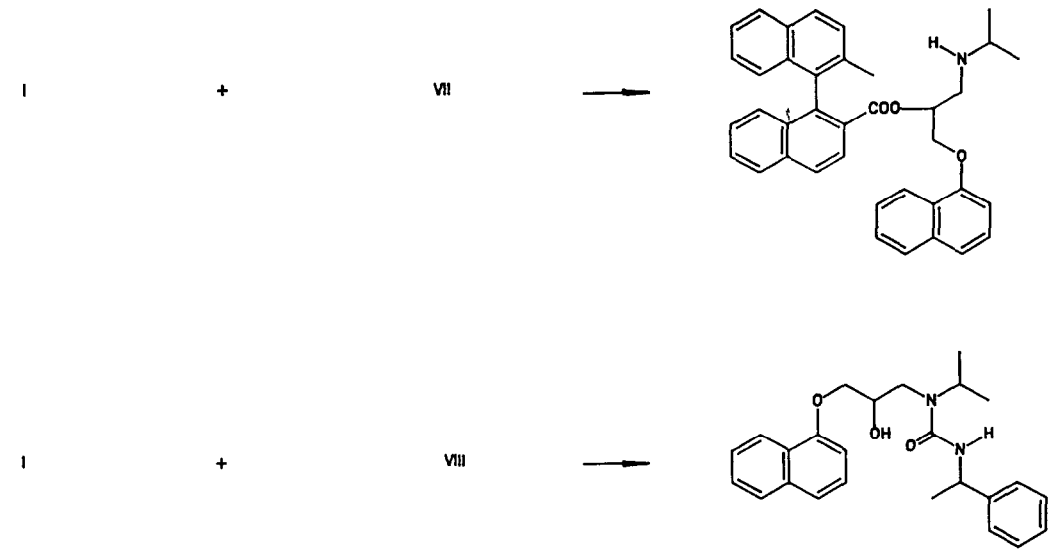

Figure 8
Continued

determination of propranolol in human plasma using (*R,R*)-*O,O*-diacetyltartaric acid anhydride as derivatizing reagent [105]. They achieved a sensitivity of approximately 1 ng ml^{-1} of each isomer and, more importantly, were able to detect traces of one isomer in the presence of a 200-fold greater concentration of the other. Wilson *et al.* [106] used the di-*p*-toluoyl substituted tartaric acid anhydride to resolve atenolol enantiomers on a preparative scale. They observed it was necessary to protect the secondary amine group from derivatization by ion-pair blocking with trifluoroacetic acid. The recovery of the individual isomers was subject to problems which appear related to the structure of atenolol, and specifically its primary amide function which dehydrated to form the nitrile in the presence of excess trifluoroacetic acid. Takahashi and co-workers used the free di-*p*-toluoyl tartaric acid rather than the anhydride for the resolution of propranolol enantiomers in biological fluids [107]. They used racemic penbutolol as an internal standard in a procedure which was sensitive to 3 ng ml^{-1} of each enantiomer. Yost and Holtzman [108] also used di-*p*-toluoyl tartaric acid for preparative resolution, in this instance of atenolol. The diastereomers were separated by crystallization and the enantiomerically pure free bases liberated by decomposition with sodium hydroxide solution.

2. *Acid chlorides.* Two types of acid chloride have found use in the indirect resolution of β-blockers. *N*-trifluoroacetyl-*l*-prolyl chloride (TPC) was one of the earliest reagents to be used, whilst chiral chloroformates made up the other, diverse group of reagents.

Hermansson and von Bahr [109] first reported the use of TPC for the determination of propranolol enantiomers in human plasma in 1980 although this was followed quickly by a procedure also reported in 1980 by Silber and Riegelman [110]. Both groups employed fluorescence detection and C_{18} stationary phases. Hermansson compared the performance of μBondapak C_{18}, Lichrosorb RP-8 and Lichrosorb RP-18 and selected the RP-18 phase whilst Silber adopted Ultrasphere ODS. Both groups used mass spectrometry to confirm the structure of the derivatives and some discussion of the importance of the optical purity of the derivatizing reagent was made. Silber and Riegelman cited a limit of detection of 1.5 ng ml^{-1} of plasma and whilst Hermansson and von Bahr did not give a sensitivity limit, they demonstrated linearity of the standard curve over the range 1–50 ng ml^{-1}.

Enantiomers of acebutolol and its metabolite diacetolol have been separated and quantitated in urine and plasma samples by Sankey *et al.* using TPC [111]. They separated the diastereomers on a 3 μ Hypersil ODS column and employed fluorescence detection to

achieve maximum sensitivities of 0.05 μg ml^{-1} (plasma) and less than 1 μg ml^{-1} (urine).

In 1987 Einarsson and co-workers employed pre-column derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate [FLEC] to separate diastereomer derivatives of (*R*)- and (*S*)-metoprolol [112]. They achieved almost baseline resolution of the enantiomers in under 10 min on a 5 μ Spherisorb C_8 column (see Fig. 9). This reagent has also been used for the determination of propranolol [113] and of atenolol [114] in plasma on a C_{18} column. In the latter case the internal standard employed was racemic practolol which also resolved into its diastereomers yielding chromatograms with two analyte and two I.S. peaks.

In 1989 a number of procedures based on the use of (−)-menthylchloro-formate (MCF) as derivatizing reagent were reported. Schmitthenner *et al.* [115] and Mehvar [116] used MCF for optical purity determinations of a range of pure β-blockers. Schmitthenner achieved separation of propranolol enantiomers and concluded MCF was superior in performance to phenylethyl isocyanate (PEIC) for the separation of flavadilol isomers. Mehvar achieved separation of six chiral β-blockers as their MCF derivatives and developed a strategy for dealing with the excess reagent which precipitates when mixed with water. He went on to develop a procedure for the analysis of atenolol enantiomers in plasma and urine based on MCF-derivatization [117]. Again a racemic internal standard (methoxamine) was employed which separated into its diastereomers upon reaction to yield a chromatogram with two analyte and two I.S. peaks. The method was sensitive to 2.5 ng ml^{-1} of each enantiomer. Prakash *et al.* [118] also used MCF for the analysis of drugs in body fluids, quantitating propranolol enantiomers down to 1 ng ml^{-1}. They employed a homochiral I.S., (+)-flecainide and confirmed the attachment was via the secondary amine rather than the alcohol by ^1H NMR.

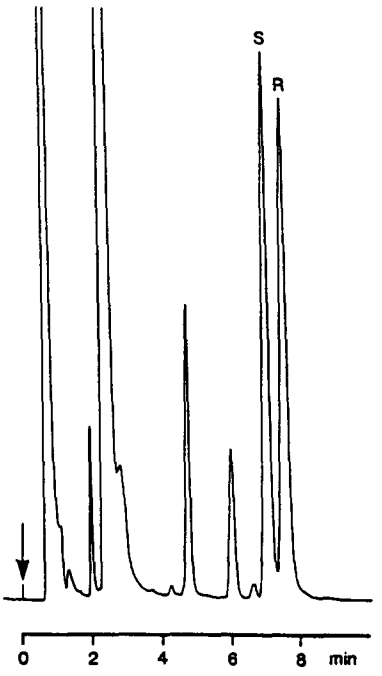

Figure 9
Achiral separation of the diastereomeric derivatives formed by reaction of metoprolol with FLEC. (Reprinted with permission from S. Einarsson *et al.*, *Anal. Chem.* **59**, 1191–1195. © 1987 American Chemical Society.)

Ahnoff and co-workers used metoprolol as a model for the investigation of the usefulness of 11 different chloroformates which included MCF [119]. The isosorbide- and isomannide-chloroformates are available in high enantiomeric purity and, having rigid structures, can yield very good separations. A number of 3-(chloroformoxy)butyrate derivatives were included in the evaluation and appeared very useful as they could have appreciable water solubility. This would allow the direct reaction of enantiomers in their biological matrices without extraction.

3. Cyanides. A group from Tohoko University developed a cyanide reagent which reacts with the β-blocker secondary alcohol to yield fluorescent diastereomeric derivatives [120, 121]. The chiral axis reagent (+)-2-methyl-1,1′-binaphthalene-2′-carbonyl cyanide formed diastereomeric esters with propranolol, penbutolol and bufuralol which could be resolved on silica using normal-phase solvents.

When applied to the determination of propranolol in plasma the procedure was modified to include the internal standard (+)-bufuralol [122]. The sample was cleaned up on a sep-pak C_{18} cartridge prior to derivatization and separation on an ion-exchange gel. The limit of detection of each enantiomer was cited as 100 pg ml^{-1}.

4. Isocyanates/isothiocyanates. Isocyanates react with primary and secondary amines to form the urea compounds and isothiocyanates form the corresponding thioureas. Whilst isocyanates also react with secondary alcohols (to form carbamates) the reaction with amines occurs far more readily under conventional

conditions (non-reacting solvents, ambient temperature) and is the preferred mechanism. A comparative evaluation of certain chiral isocyanates and chiral acid chlorides as derivatization agents has been reported. (*S*)-(−)-phenylethyl isocyanate (SPEIC) was compared to both the isocyanate and the acid chloride of (*S*)-(+)-flunoxaprofen for the separation of propranolol enantiomers in biological samples [123]. Limits of detection in the order 1–2 ng ml^{-1} were reported for a procedure which employed racemic pronethalol as an I.S. As before, the I.S. itself was well resolved. The chromatographic performance of amide and urea derivatives of propranolol were seen to be comparable although the authors stated they favoured the use of SPEIC due to its good reactivity and the potential for interfering peaks which accompanies the use of the fluorescent agent flunoxaprofen.

One of the first reported applications of isocyanates to the chiral separation β-blockers was made in 1987 by Tsuru *et al.* [124]. They resolved propranolol enantiomers in human serum using (*R*)-(+)-methylbenzyl isocyanate (or *R*-α-methylbenzyl isocyanate, *R*-AMBI). This reagent is also referred to as (*R*)-(+)-1-phenylethyl isocyanate (*R*-PEIC). They compared it favourably to TPC, and were able to detect 0.2 ng of each enantiomer using fluorescence detection. Several other groups [125–127] have also used *R*-PEIC to resolve enantiomers of propranolol and its metabolite hydroxypropranolol (4-HOP) in plasma and/or urine. Laganiere and co-workers reported on-column limits of detection of 100 pg per enantiomer. Wilson and Walle observed that two sets of diastereomers of 4-hydroxypropranolol were formed due to reaction at both the secondary amino and 4-phenolic functions. They developed separations based on both reversed-phase (5 μ C_{18}) and normal-phase (10 μ Si) columns although separation of 4-HOP was achieved only on the latter.

Gulaid and co-workers [128] followed the metabolism of acebutolol using *R*-PEIC as the chiral derivatizing reagent. They asserted that even in the presence of 1000-fold excess of derivatizing reagent none of the carbamate derivative was formed. They showed a linearity of response over the range of 0.05–15 μg ml^{-1} of each of the enantiomers acebutolol and its metabolite diacetolol in plasma.

Spahn *et al.* [129] used radio receptor assay (RRA) to validate a chromatographic procedure for the determination of metoprolol enantiomers in plasma. Metoprolol enantiomers were derivatized with *R*-PEIC and the resultant diastereomers separated by reversed-phase LC with fluorescent detection. Approximately 2 ng ml^{-1} of each enantiomer in plasma could be detected and a close agreement between HPLC and RRA was observed indicating active metabolites were not present to any significant extent.

The (*S*)-antipode (*S*)-(−)-1-phenylethyl isocyanate (*S*-PEIC) has also found use. When diastereomers were formed using *R*-PEIC the (*S*)-enantiomers of the β-blockers eluted ahead of the (*R*)-enantiomers. But when *S*-PEIC was used the opposite case was observed and the *R*-enantiomers eluted first. Dieterle and Faigle [130] separated oxprenolol enantiomers using *S*-PEIC and observed the (*R*)-(+) enantiomer eluted first. Normal-phase LC and UV detection was employed and the procedure was validated using labelled oxprenolol in an isotope-dilution assay.

Hsyu and Giacomini [131] demonstrated the separation of penbutolol enantiomers (as *S*-PEIC diastereomers) in both urine and plasma using reversed-phase LC and fluorescence detection. Extensive validation was reported for this method which was capable of detecting 2 ng ml^{-1} of each enantiomer in plasma. The procedure was also shown to be capable of separating atenolol and penbutolol enantiomers. Other groups have used similar approaches to the separation of metoprolol [132] and atenolol [133] enantiomers in body fluids.

All the groups thus far used the isocyanate reagent. In 1984 Gal and Sedman [134] reported the separation of propranolol enantiomers using *R*-1-phenylethyl isothiocyanate (*R*-PETC) to form the thiourea diastereomers. Whilst this was not the first use of an isothiocyanate for diastereomer formation, it was the first application of a commercially available material. The advantages of isothiocyanates over isocyanates, such as improved chemical stability, were discussed. D.M. Desai, in his Ph.D thesis [135] undertook an extensive evaluation of the usefulness of a number of isocyanates/isothiocyanates in the chiral separation of a variety of drugs including the β-blockers propranolol and labetalol.

The modification of the chiral reagent to include a naphthyl rather than a phenyl substituent was shown to improve the stereo-

selectivity by Jira *et al.* [136]. They compared separations of β-blockers using *R*-PEIC and *R*-(−)-1-(1-naphthyl)ethyl isocyanate (*R*-NEIC) and showed an increase in α from 1.12 to 1.20 for propranolol. Separations were also achieved with NEIC for pindolol and talinolol enantiomers which were unsuccessful with PEIC. Separations of atenolol and metipranolol enantiomers (as *R*-NEIC diastereomers) were also reported. All used reversed-phase LC with UV detection.

A number of groups have employed the opposite antipode, (*S*)-(+)-1-(1-naphthyl) ethyl isocyanate. Piquette-Miller and co-workers [137, 138] determined the enantiomers of acebutolol and its metabolite diacetolol in human plasma and urine down to levels of 1 ng ml^{-1}. Carr *et al.* [139] developed a quantitative procedure for sotalol in plasma in which the diastereomers were separated using normal-phase chromatography. A sensitivity was reported in the region of 20–50 ng ml^{-1} of each enantiomer. Lave and co-workers [140] measured the novel β-blocker tertatolol in urine and plasma using *S*-NEIC which they reported gave better separation than GITC and better sensitivity than PEIC. Using fluorescence detection and a 3 μm ODS column they achieved limits of detection of 6 ng ml^{-1}.

Two groups used *R*-NEIC to study betaxolol pharmacokinetics. Darman and Thenot [141] achieved sensitivity to 0.5 ng ml^{-1} of each enantiomer due to the improved fluorescence properties of the naphthyl reagent over its phenyl homologue. They noted a reduced recovery if the reaction mixture was heated to 60°C (normal conditions are based on ambient temperature) which they postulated was due to the formation of a late-eluting di-derivative. Stagni and co-workers [142] also reported separation of betaxolol enantiomers as their NEIC diasteromers, their paper stated that the analyte enantiomers were derivatized with *R*-NEIC or *S*-NEIC although they go on to show only the separations achieved with *R*-NEIC.

Whilst the aromatic reagents found use as isocyanates or isothiocyanates the saccharides have been used exclusively as isothiocyanates. In 1983 Sedman and Gal [143] compared the performance of two candidate reagents 2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl isothiocyanate (AITC) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC). Diastereomers were formed with a wide range of β-blockers and separations were carried out using reversed-phase LC with UV detection. GITC gave superior stereoselectivity to AITC although the order of elution of the enantiomers was reagent-dependent. The (*S*)-(−) enantiomers eluted first when used as their GITC diasteromers, whilst the (*R*)-(+) enantiomers were the first to elute with AITC. Thus problems of determining a minor enantiomer on the tail of an excess of the opposite antipode could be overcome by switching reagent to elute the enantiomer of interest first. Several other groups have tried GITC for the enantiomeric separation of β-blockers such as pindolol [144, 145] propranolol [144] or its metabolite 4-HOP [146], atenolol [147], carvedilol [148], metoprolol [149], bevantolol [150], oxprenolol [151] and timolol [151] in biological matrices.

Kushiya *et al.* [144] optimized the pindolol separation by adjusting both the pH of the eluent and its organic solvent composition. Schuster [149] ascertained that reducing the concentration of GITC employed did not affect the extent to which the reaction proceeded, but did improve sensitivity by reducing extraneous peaks.

An interesting variation was the use by Martin and co-workers [152] of fluorescent isocyanate derivatives of the drugs (*S*)-(−)-flunoxaprofen and (*S*)-(−)-naproxen. These reagents were synthesized and used to derivatize a range of racemic drugs including propranolol and metoprolol. Whilst the reagents did display some problems (e.g. chemical stability, optical purity) they were successfully used for optical purity determinations. Their fluorophors also rendered them potentially very useful for separations requiring sensitive detection.

Conclusions

There have been extensive investigations into methods for the enantiomeric separation of the therapeutically important β-blocker group of drugs. Success has been achieved using most of the accepted chiral separation techniques although the published literature indicates certain approaches are more favoured than others. The use of chiral derivatization agents to form diastereomeric derivatives which can be separated on conventional, achiral columns was the most popular technique in the early evolution of chiral separation. Thus, although there are a comparable number of reported separations based on

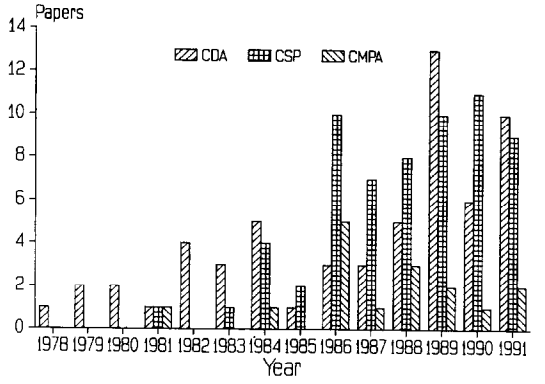

Figure 10
Procedures reported since 1978 for the chromatographic separation of β-blocker enantiomers by year of publication.

CDAs (59) and CSPs (63) this does not reflect the recent trend. The number of published procedures per annum for β-blocker separations are represented back as far as 1978 in Fig. 10. This reveals that most of CSP papers have appeared over the past 5 years whilst the CDA methods have accumulated over the full 13 years. The understanding of chiral recognition process is increasing as is the development of novel, more readily available chiral stationary phases. Focus has been placed on the direct separation of the enantiomers without prior derivatization using techniques which are sufficiently sensitive to allow their determination at low levels, for example in biological samples and as impurities in enantiomerically 'pure' bulk drugs.

Acknowledgements — I should like to thank Mr John O'Boyle and Mrs Hazel Taylor for their assistance with the literature search and Dr Malcolm Robinson for his constructive comments during the preparation of this manuscript.

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[Received for review 18 December 1991; revised manuscript received 5 February 1992]

Appendix

1. Glossary of terms

AGP	α-1-Acid glycoprotein
AITC	2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl isothiocyanate
BSA	Bovine serum albumen
CDA	Chiral derivatization agent
CMPA	Chiral mobile phase additive
CSP	Chiral stationary phase
DACH	(*R*,*R*)-*N*,*N*′-di(3,5-dinitrobenzoyl)diaminocyclohexane
DNB–Phe	(*R*)-(+)-*N*-(3,5-dinitrobenzoyl)–α-phenylglycine
DNB–Leu	(*R*)-(+)-*N*-(3,5-dinitrobenzoyl)–*l*-leucine
FLEC	(+)-1-(9-fluorenyl)ethyl chloroformate
GITC	2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate
I.S.	Internal standard
MBNCC	(+)-2-methyl-1,1′-binaphthalene-2′-carbonyl cyanide
MCF	Menthyl chloroformate
R-PEIC	(*R*)-(+)-1-phenylethyl isocyanate
R-PETC	(*R*)-(+)-1-phenylethyl isothiocyanate
R-NEIC	(*R*)-(−)-1-(1-naphthyl)ethyl isocyanate
S-NEIC	(*S*)-(+)-1-(1-naphthyl)ethyl isocyanate
S-PEIC	(*S*)-(−)-1-phenylethyl isocyanate
Sub-FC	Sub-critical fluid chromatography
t-BOC-Leu	*tert*-butoxycarbonyl-*l*-leucine
t-BOC-Ala	*tert*-butoxycarbonyl-*l*-alanine
TPC	*N*-trifluoroacetyl-*l*-prolyl-chloride
ZGP	*N*-benzoxycarbonylglycyl-*l*-proline

2. Index of chiral chromatographic separations of β-blockers. Underlined references indicate procedures reported for biological samples

Drug	Reference		
	CDA	CMPA	CSP
Acebutolol	104, 111, 128, 137, 138		58, 76
Alprenolol	16, 102, 104, 143	25, 28, 34, 41	19, 21, 32, 44, 46, 48, 50, 51, 53, 58, 62, 76, 79, 82, 88
Atenolol	16, 104, 106, 113, 115, 117, 133, 136, 143, 147	38, 40	27, 58, 59, 62, 76, 88, 89, 95
Benflumelol			45
Betaxolol	141, 142		58, 73, 74, 79
Bevantolol	150		
Bisoprolol			79
Bufuralol	121	32	
Bunitralol	23, 104		22
Bupranolol	104, 143		18, 21, 79, 81
Carazolol	104		63, 79
Carteolol			59
Carvedilol	148		97
Celiprolol	104		72
Cicloprolol	141		74
Diacetolol	111, 128, 138		
Falvodilol	115		
Labetalol	104		83
Metipranolol	136		
Metoprolol	23, 24, 102, 104, 112, 116, 117, 129, 132, 143, 149, 152	236, 28, 29, 31, 33, 34, 35, 41	18, 19, 21, 22, 27, 36, 45, 51, 53, 55, 58, 59, 67, 68, 69, 70, 74, 78, 79, 80, 82, 83, 84, 87, 90, 91, 92
Nadolol			48, 74
Nifenalol	16, 104		
Oxprenolol	16, 23, 24, 104, 130, 151	35, 38	19, 21, 44, 46, 50, 51, 53, 55, 58, 60, 62, 76, 82, 88, 98
Penbutolol	120, 121		18, 21, 64, 78, 79
Pindolol	104, 131, 136, 143, 144, 145	26, 35	19, 54, 55, 58, 59, 62, 74, 76, 82, 88, 94, 95, 96, 98
Practolol	104	42	
Prenalterol	146		19, 20
Pronethalol	16, 123, 143		46, 55, 58
Propranolol	15, 17, 23, 24, 99, 100, 101, 103, 104, 105, 109, 110, 113, 115, 116, 120, 121, 122, 123, 124, 125, 126, 127, 134, 136, 143, 144, 152	25, 26, 28, 34, 35, 37, 38, 39, 40, 41	19, 21, 27, 44, 45, 46, 49, 50, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 70, 74, 76, 79, 80, 82, 95
Sotalol	116, 139, 143		
Talinolol	136		
Tertatolol	140		46, 65
Timolol	104, 151	26	19, 21, 77, 78, 79
Toliprolol	116		
Number of papers	59	16	63