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Evaluation of generic chiral liquid chromatography screens for pharmaceutical analysis

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Abstract

Two different automated generic liquid chromatography screens for the separation of chiral compounds of pharmaceutical interest have been evaluated. The test set comprised 53 chemically diverse chiral compounds involving 55 enantiomeric pairs from the pharmaceutical industry (i.e. starting materials, synthetic intermediates and drug substances). The first screen utilised four polysaccharide-based columns with five mobile phases and showed enantioselectivity for 87% of the test compounds. The second screen employed three macrocyclic glycopeptide columns with two mobile phases and showed enantioselectivity for 65% of the test compounds. Merging of the two screening procedures resulted in an enantioselectivity for 96% of the chiral compounds. It is anticipated that the systematic use of the automated chiral HPLC screens described in this report will substantially reduce the necessary time for method development of pharmaceutically related chiral analytical methods.
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1. Introduction

It has long been acknowledged that enantiomers of drug substances may have distinct biological interactions and consequently different pharmacokinetic, pharmacological or toxicological activities, and thus enantiomers are considered as distinctly different compounds [1]. In light of increased regulatory requirements resulting from considerations of clinical efficacy and consumer safety, the pharmacological evaluation of all stereoisomers in addition to the racemate is an integral part of drug development [2,3]. Accordingly, the enantiomeric purity of drug substances is an important issue in the drug discovery process and this has led to a significant increase in demand for sensitive chiral analytical methods over recent years. Furthermore, the number of new chemical entities entering into development within the pharmaceutical industry has increased significantly and now fast method development is also a key requirement. Drug substance process research

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and development involves innovative chemistry focused on novel synthetic routes that can be scaled-up and developed into commercially viable chemical processes that are capable of producing up to several hundred tonnes of drug substance per annum. The stereochemistry in these chemical processes is controlled by development of appropriate analytical methods for starting materials, chiral reagents, synthetic intermediates and drug substances.

The most widely used analytical method is the direct separation of enantiomers using high performance liquid chromatography (HPLC) and chiral stationary phases (CSPs) owing to its simplicity and accuracy. Since the late 1970s considerable research effort has been invested in the development of chiral stationary phases and the search for chiral selectors capable of exhibiting enantioselectivity for a broad range of chiral compounds has been a continuous process [4]. To-date the main classes of commercially available CSPs are based on a number of different chiral selectors including: proteins, polysaccharides, cyclodextrins, Pirkle type, ligand exchange, crown ethers, macrocyclic antibiotics and chiral polymers [5]. A number of these classes of CSPs show analogous or complementary enantioselectivity and in order to be certain of obtaining enantioselectivity it would be desirable to evaluate all these classes of CSPs during method development. However, where rapid method development is required it is impossible to evaluate all classes of CSPs. Other possible approaches to method development include stepwise trial and error evaluation based on detailed consideration of the mechanism of the chiral selector with the chiral analyte, the analyst using their practical experience or consultation of literature and databases. All these approaches are time consuming and often unsuccessful owing to the fact that achieving enantioresolution is often purely empirical and the prediction of enantioresolution is thus far from perfect. As a result, evaluation of a smaller set of CSPs that offer broad-spectrum enantioselectivity and a high probability of success is required. Of the commercially available CSPs, the macrocyclic antibiotics and polysaccharides appear to be broad-spectrum chiral selectors and should offer the possibility of achieving success for a wide range of chemically diverse compounds.

Polysaccharide CSPs are derived from cellulose or amylose polymers. Cellulose contains β-glycosidic linkages and forms a linear structure, whereas amylose contains α-glycosidic linkages and adopts a helical structure. The most successful CSPs are based on benzoates or phenyl carbamate derivatives. The chiral recognition and resolving ability of the derivatives depend on the polysaccharide linkages and hence three-dimensional structure, and also on the substituents introduced on the phenyl moieties [6]. These substituents can interact with racemates through hydrogen bonding, dipolar interactions, pi–pi interactions and steric interactions to achieve chiral discrimination. Numerous polysaccharide derivatives exist and the most effective CSPs that offer complementary properties are the Chiralpak AD (amylose tris (3,5-dimethylphenylcarbamate)), Chiralcel OD (cellulose tris (3,5-dimethylphenylcarbamate)), Chiralpak AS (amylose tris [(*S*)-α-methylbenzylcarbamate]) and Chiralcel OJ (cellulose tris (4-methylbenzoate)). Typically, polysaccharide CSPs are operated in the normal-phase mode, although more recently new derivatives operated in the reversed-phase [7,8] and polar organic mode [9–11] have increasingly been used. In the normal-phase mode, mobile phases are typically based on hexane containing an alcohol modifier, such as 2-propanol or ethanol. The choice of modifier can have a dramatic effect on the chiral separation obtained [12], and in fact changing the alcohol modifier type can sometimes reverse the elution order [13]. Basic and acidic mobile phase additives are often required to improve separations and peak shape [14–19].

In the past few years macrocyclic antibiotic CSPs have been shown to be an important class of CSPs and a 50% increase in publications using antibiotic-based CSPs from January 2000 to March 2002 has been reported [20]. Macrocyclic antibiotic CSPs also offer broad-spectrum applicability and can be operated in normal-phase, reversed-phase and polar organic modes, with the latter two modes reportedly showing the broadest applicability [21]. The phases are based on the macrocyclic glycopeptides Vancomycin (Chirobiotic V), Teicoplanin (Chirobiotic T), and Ristocetin A (Chirobiotic R) that are covalently bonded to silica through multiple ether linkages. All three glycopeptides contain numerous chiral centres, several aromatic ring structures, a peptide core, one or more neutral sugar moieties and

one or more aminosaccharide moieties, with readily available hydrogen donor and acceptor sites nearby. Chiral discrimination depends upon the mode of operation and is achieved through pi–pi interactions, inclusion complexation, hydrogen bonding interactions, charge–charge interactions, hydrophobic interactions, dipolar interactions, steric interactions and through peptide as well as carbohydrate binding sites. The number and relative strength of these potential interactions varies in the three glycopeptides owing to their different physicochemical properties. This leads to a series of phases that exhibit both analogous and complementary enantioselectivity for a broad spectrum of analytes.

As both the polysaccharide and macrocyclic antibiotic CSPs offer broad-spectrum enantioselectivity and a high probability of success, this has led to the use of screening strategies being commonly employed in order to reduce method development times. In fact during the undertaking of this research several publications have appeared showing the successful application of screening approaches in chiral separations for a wide range of compounds using polysaccharide columns in normal-phase [19], reversed-phase modes [7] and very recently using gradient normal-phase systems [22]. Astec has produced a commercially available method development kit with protocols that utilise the screening of antibiotic columns in series [21,23]. Additionally, a number of chiral screening strategies employing capillary electrophoresis (CE) and cyclodextrins have been reported to be very efficient and have also shown high success rate for enantiomeric separations [24,25]. However, since our groups are also interested in semi and process scale preparative applicability of chiral separations, HPLC is often our preferred technique.

The aim of this study was to evaluate the use of a combination of complementary generic chiral method development protocols to offer a high probability of success for a diverse range of chiral compounds of pharmaceutical interest. The generic strategies employ short screening columns for the identification of suitable conditions to enable extrapolating to 250-mm analytical columns for further method optimisation to achieve baseline resolution. The final proposed screen employed a normal-phase and a polar organic mode screen with four polysaccharide columns alongside a screen with three macrocyclic glycopeptide columns utilising reversed-phase and polar organic modes of operation.

2. Experimental

2.1. Instrumentation

The liquid chromatographic system was comprised of a gradient pump (type P580 LPG), an autosampler (type GINA50) and a diode-array UV-detector (type UVD340S), all from Gynkotek (Germering, Germany). A thermostatic oven model Cool Pocket from Keystone Scientific Inc (Bellefonte, PA, USA) was used to maintain the temperature at a fixed level of 22 °C. A multiposition valve actuator for six columns (VICI, Valco International, Schenkon, Switzerland) was used to automate the screening procedures.

Two different sets of columns were used. The first set of columns had stationary phases based on polysaccharides and were obtained from Daicel Chemical Industries (Tokyo, Japan). It was comprised of a Chiralcel OD, a Chiralcel OJ, a Chiralpak AD and a Chiralpak AS column packed with the polysaccharide derivative coated on 10-μm silica particles. The column dimensions were 50×4.6 mm. The second set of columns was based on macrocyclic glycopeptides covalently bonded to 5-μm silica particles and were obtained from Astec (Whippany, NJ, USA). These were comprised of a Chirobiotic R column based on Ristocetin A, a Chirobiotic V column based on Vancomycin and a Chirobiotic T column based on Teicoplanin. The column dimensions were 100×4.6 mm.

2.2. Chemicals

The test set used in the screening procedures consisted of 53 different chiral compounds (Figs. 1–3), involving 55 enantiomeric pairs. The substances were obtained from various commercial sources or produced in-house by AstraZeneca (Södertälje, Sweden).

The solvents methanol, 2-propanol and isohexane were of HPLC grade from Merck (Darmstadt, Germany). Ethanol of spectroscopic grade, 99.5% was purchased from Kemetyl (Haninge, Sweden). Di-
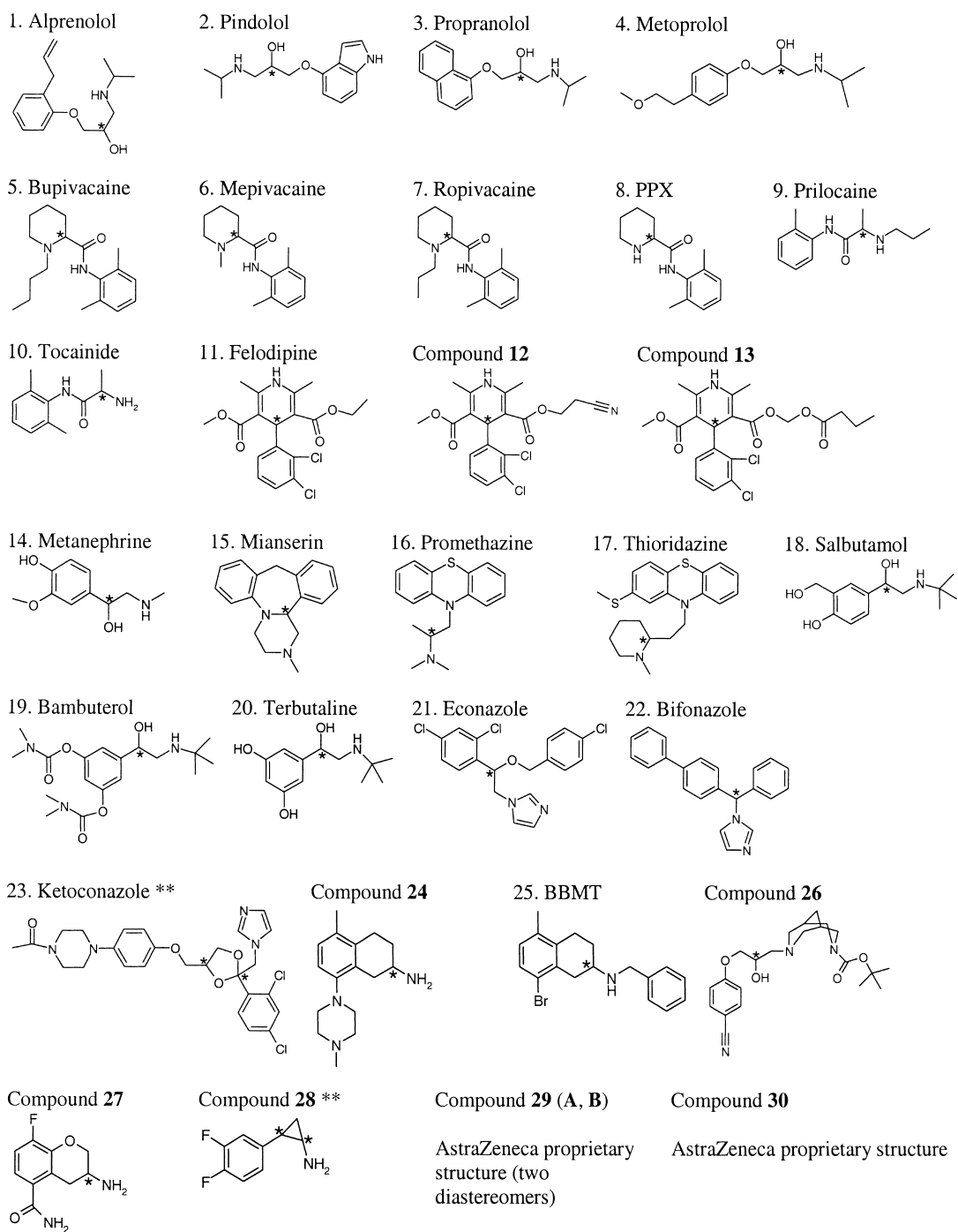
Fig. 1. Structures of the basic compounds. *Marks the chiral centre; **test substance only contained one of the diastereomers.
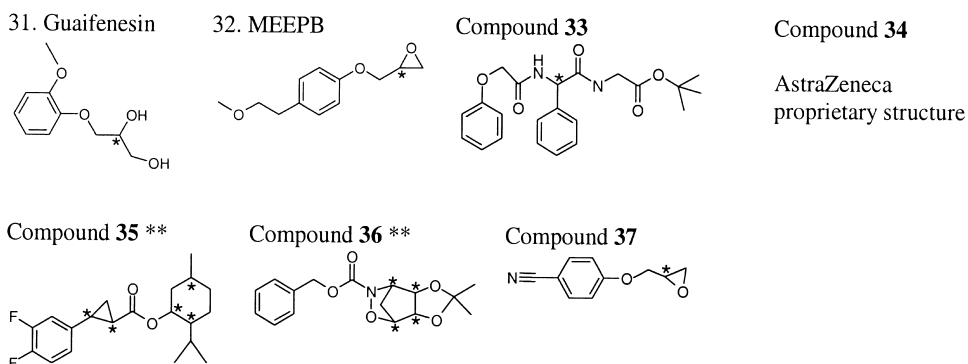
31. Guaifenesin

32. MEEPB

Compound **33**

Compound **34**

AstraZeneca
proprietary structure

Compound **35** **

Compound **36** **

Compound **37**

Fig. 2. Structures of the neutral compounds. *Marks the chiral centre; **test substance only contained one of the diastereomers.

Compound **38**

39. Metolazone

40. Bendroflumethiazide

41. Flurbiprofen

42. Ketoprofen

43. Naproxen

44. Ibuprofen

45. Ketorolac

46. Omeprazole

47. Lansoprazole

Compound **48**

Compound **49**

Compound **50**

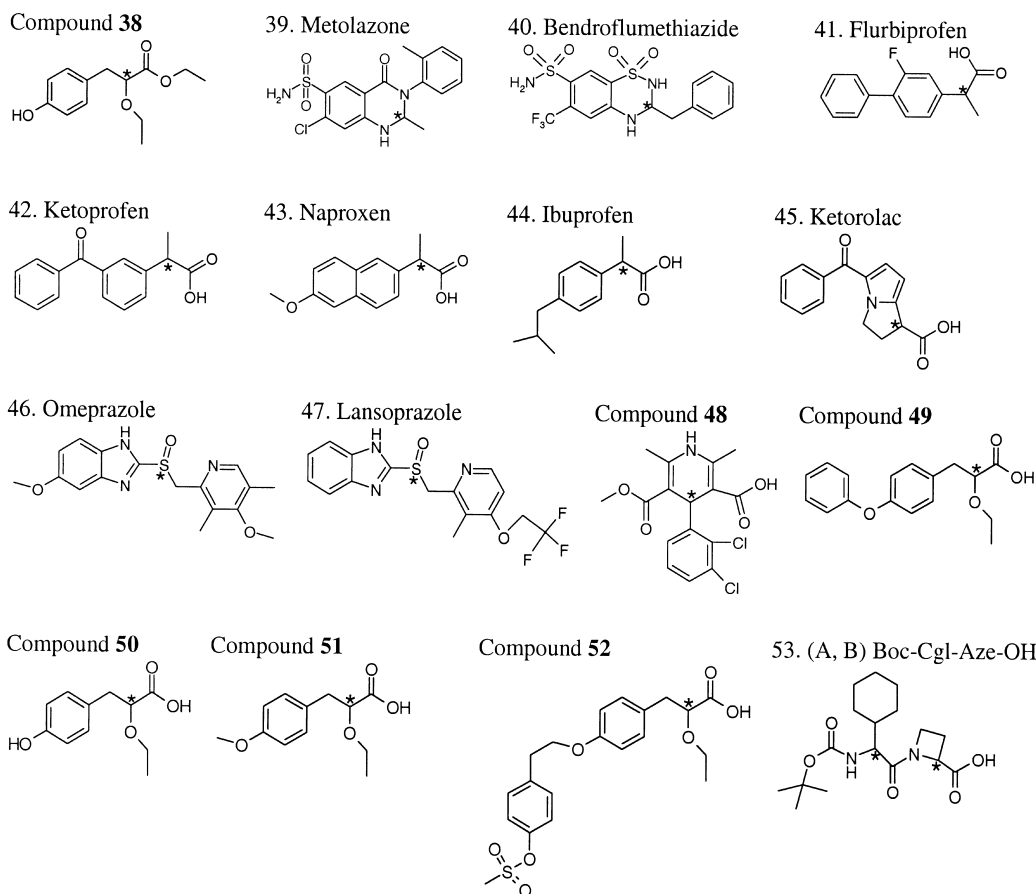Compound **51**

Compound **52**

53. (A, B) Boc-Cgl-Aze-OH

Fig. 3. Structures of the acidic compounds. *Marks the chiral centre.

ethylamine (DEA), triethylamine (TEA), acetic acid (HAc) and trifluoroacetic acid (TFA) were of pro analysi grade or better from BDH, Jansen Chimica, Merck and Sigma, respectively.

2.3. Chromatographic conditions

Enantioseparations were performed isocratically at a flow-rate of 1 ml/min for the polysaccharide columns and at 1–2 ml/min for the macrocyclic glycopeptide columns, depending on which mode the separations were performed in (i.e. normal-phase mode, polar organic mode or reversed-phase mode).

All the samples were dissolved to yield a concentration of approximately 0.2 mg/ml in ethanol, except for compound 34 that had to be dissolved in isohexane due to degradation in the presence of alcohols or water. Consequently, this substance was not evaluated in the reversed-phase mode. There were no problems with solubility in ethanol for any of the substances even though some of them were present in the form of inorganic salts such as sulfates and hydrochlorides or salts with potassium or sodium. The injection volume was 5 μl and detection of the enantiomers was carried out at 220 or at 205 nm for the weakly UV-absorbing compounds 34 and 53.

2.4. Calculations

Analytical data were acquired and treated with Chromeleon LC software package from Dionex (Germering, Germany). Resolution values (R_s) were calculated according to the United States Pharmacopoeia (USP):

$$R_{s,USP} = \frac{2((t_R)_B - (t_R)_A)}{(w_b)_B + (w_b)_A} \tag{1}$$

where $(t_R)_A$ and $(t_R)_B$ are the retention time of the first and last eluting peak, respectively (in min), and $(w_b)_A$ and $(w_b)_B$ are the base widths of peaks A and B (in min). The selectivity factor, α, was calculated with the following equation:

$$\alpha = \frac{(t_R)_B - t_0}{(t_R)_A - t_0} = \frac{k_B}{k_A} \tag{2}$$

where t_0 is the time required for an unretained

analyte to pass through the column, k_A and k_B are the retention factors for A and B, respectively. t_0 was determined by the first baseline disturbance when a solvent of different composition compared with the mobile phase was injected.

From the results on the 50- and 100-mm columns, predictions of resolution and retention times for 250-mm columns using identical chromatographic conditions were calculated according to the following relationships:

$$N = \frac{L}{H} \tag{3}$$

where N is the number of theoretical plates, L is the length of the column packing and H is the height of a theoretical plate.

$$N = 16R_s^2 \left(\frac{\alpha}{\alpha - 1}\right)^2 \left(\frac{1 + k_B}{k_B}\right)^2 \tag{4}$$

$$(t_R)_B = \frac{16R_s^2 H}{u} \left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1 + k_B)^3}{(k_B)^2} \tag{5}$$

where u is the linear velocity of the mobile phase.

From Eqs. (3) to (5) the following relationships can be obtained:

$$R_s \propto \sqrt{L} \tag{6}$$

$$(t_R)_B \propto R_s^2 \tag{7}$$

Eqs. (8) and (9) can be derived from Eqs. (6) and (7):

$$\frac{R_{s,1}}{R_{s,2}} = \sqrt{\frac{L_1}{L_2}} \Rightarrow R_{s,2} = R_{s,1} \cdot \sqrt{\frac{L_2}{L_1}} \tag{8}$$

$$\frac{(t_R)_{B,1}}{(t_R)_{B,2}} = \frac{R_{s,1}^2}{R_{s,2}^2} \Rightarrow (t_R)_{B,2} = (t_R)_{B,1} \cdot \frac{R_{s,2}^2}{R_{s,1}^2} \tag{9}$$

where 1 indicates the shorter column and 2 the longer one.

3. Results and discussion

3.1. Evaluation of polysaccharide columns

Polysaccharide CSPs based on cellulose and amylose derivatives are generally recognised for having

broad and complementary properties for chiral separations of pharmaceutically related compounds. Consequently, we decided to evaluate the Chiralpak AD, Chiralpak AS, Chiralcel OD and Chiralcel OJ columns in our first chiral screen.

3.1.1. Normal-phase mode

Cellulose and amylose derivative CSPs are mostly used in the normal-phase mode with hydrocarbon based mobile phases containing some alcohol as polar modifier. The hydrocarbon used is often *n*-hexane, but in this study isohexane was used instead due to potential toxicity issues with *n*-hexane. The type of alcohol greatly affects selectivity, elution order and retention. Ethanol and 2-propanol are the most commonly used modifiers reported in the literature. Gradient conditions were considered but based on the literature and our own experience, isocratic conditions using mobile phases with alcohol/isohexane in the ratio 15:85 (v/v) were selected. These would give suitable elution strength for the pharmaceutical compounds of interest in this study whilst gradients may be more appropriate for a more diverse collection of compounds.

Since optimal use of polysaccharide columns require that substances are analysed in their neutral form, additives had to be present in the mobile phases for the basic and acidic compounds. When the analytes contain a basic functional group, diethylamine is often added to the mobile phase in order to reduce peak tailing by masking the residual silanol groups of the CSP [14]. Trifluoroacetic acid is usually added to the mobile phase to attenuate the binding of acidic analytes, which are often extensively retained under normal-phase conditions with polysaccharide CSPs [15]. Addition of acidic and basic additives have been reported to produce significant enhancements in selectivity and resolution of ionic analytes [16–18]. Neutral compounds have been shown to be relatively unaffected by the addition of acid or base to the mobile phase [19]. Therefore, the test substances were divided into two groups, depending on their acid/base functionality, and trifluoroacetic acid (TFA) or diethylamine (DEA) was added to the mobile phases at a concentration of 0.1% (v/v). Neutral substances are unaffected by the additive and can be chromatographed with either TFA or DEA and were run together with the acids for simplicity.

The results from this screen and the success rate in obtaining enantioselectivity (i.e. $\alpha > 1.0$) on each of the columns and with each alcohol modifier is shown in Fig. 4. It is evident that Chiralpak AD was especially useful and this column also usually provided the highest number of theoretical plates as well as symmetrical peaks, which resulted in high resolution. Fig. 5 shows chromatograms for one of the compounds PPX on the four columns, with the two polar modifiers, exemplifying typical results obtained. As can be seen both the column type and the type of alcohol affects the enantioselectivity. In most cases enantioselectivity was observed with both alcohols but for several compounds enantioselectivity was only seen with one of the alcohols.

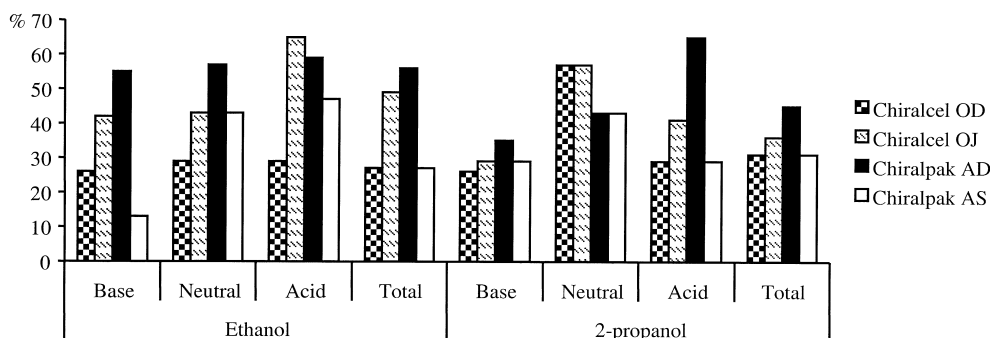In total, the four columns showed enantioselectivity for 85% of the compounds and the analysis

Fig. 4. Results from the screen with the four polysaccharide columns in normal-phase mode showing the extent of observed enantioselectivity for each combination of column and mobile phase. Mobile phase: alcohol/isohexane/additive (15:85:0.1, by vol.).
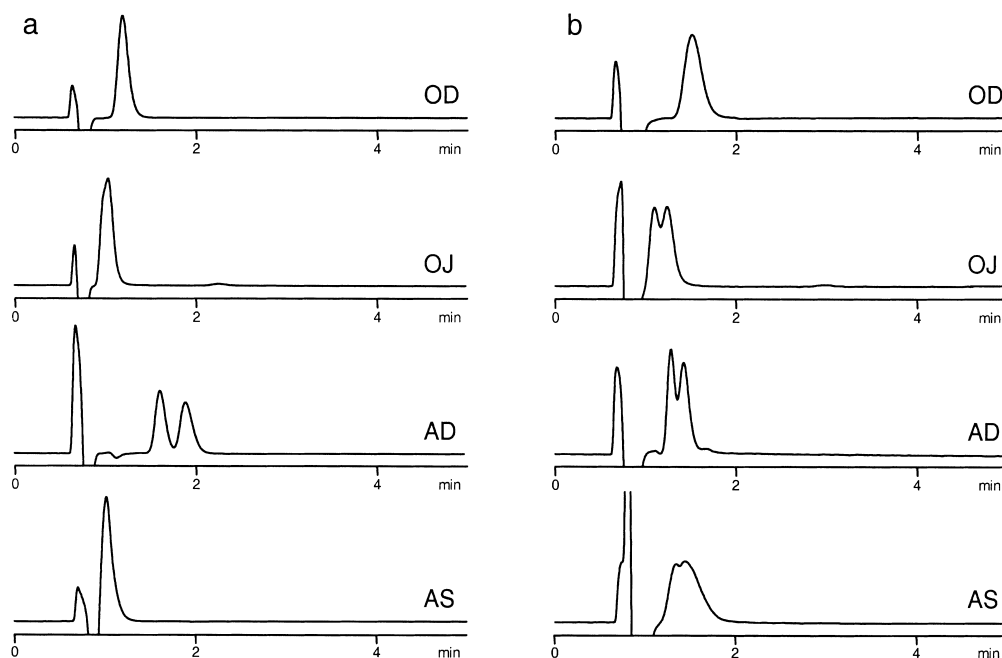
Fig. 5. Typical chromatograms of compound 8, PPX from the polysaccharide screen in the normal-phase mode. (a) Mobile phase: ethanol/isohexane/DEA (15:85:0.1, by vol.); (b) mobile phase: 2-propanol/isohexane/DEA (15:85:0.1, by vol.).

times were usually less than 10 min. As noticed previously [19], Chiralcel OJ was very good for separation of acids, especially with ethanol as modifier. Substances that did not show enantioselectivity on any of these columns in the normal-phase mode were typically poorly retained compounds, such as the local anaesthetics bupivacaine, mepivacaine and ropivacaine, as well as felodipine and compound 35. However, compound 30 and the bronchodilators salbutamol and terbutaline, which were sufficiently well retained under the chromatographic conditions, also displayed no indication of enantioresolution. In general, no pronounced compound class selectivity was observed with the polysaccharide columns except for the β-blockers where enantioresolution was observed on the AD and OD columns as well as for the NSAIDs on the OJ column.

3.1.2. Polar organic mode

The successful use of polar organic mobile phases with polysaccharide columns has been reported during the last few years [9–11]. Often improved solubility, increased resolution and short analysis times were obtained compared to the normal-phase

mode. However, very few suggestions as to when the polar organic mode is feasible and whether acid/base additives are necessary for good chromatography, have been reported.

Acetonitrile based mobile phases offer different properties compared to alcohols and were considered but excluded due to potential compatibility problems incorporating these mobile phases in the same screen as alkane/alcohol mobile phases. The substances were run with a mobile phase consisting of methanol/ethanol (50:50, v/v) with no other additive present. The results showed some limitations in the usefulness of this mode since many compounds either eluted in the solvent front or, like for most of the acids, as distorted peaks. For several of the compounds good resolution was observed, although the least retained enantiomer was almost unretained. This indicates that it might be difficult to obtain satisfying analytical methods from these types of mobile phases, since the possibility of affecting the elution strength by changing the composition of the mobile phase is rather limited. This could result in problems with correct quantification of the less retained enantiomer due to baseline disturbances.

However, for some compounds the polar organic mode was found to be complementary to the normal-phase screen. The best results were generally obtained for test substances that were either neutral or very weakly basic/acidic and which were moderately or well retained in the normal-phase mode such as ketoconazole, omeprazole, compound 13, compound 30 and compound 36. An exception to this was mianserin, which is a rather strong base that was well separated without base additive in the polar organic mode on the AD column. Due to the high elution strength of this mobile phase this should allow for elution of compounds that are extensively retained in the normal-phase mode.

3.1.3. Combined results for the polysaccharide columns

In Table 1, the compiled best results from the screen with the polysaccharide columns, suitable for further optimisation, is presented. The best results

Table 1
Combined best results suitable for further optimisation from screen with polysaccharide columns in normal-phase and polar organic mode

Compound	AT (min)	R_s	α	MP/Col	Compound	AT (min)	R_s	α	MP/Col
Bases					*Neutrals*				
1. Alprenolol	2.3	4.04	4.58	B/OD	31. Guaifenesin	4.9	4.15	2.29	C/OD
2. Pindolol	2.4	1.07	1.30	A/AD	32. MEEPB	4.9	1.13	1.15	D/OJ
3. Propranolol	2.6	1.43	1.48	A/OD	33. Compound 33	3.8	2.58	1.70	D/AD
4. Metoprolol	1.5	1.77	1.71	A/OD	34. Compound 34	2.7	3.50	1.86	C/AD
5. Bupivacaine	n.a.	0.00	1.00	n.a.	35. Compound 35	n.a.	0.00	1.00	n.a.
6. Mepivacaine	n.a.	0.00	1.00	n.a.	36. Compound 36	2.9	1.02	1.23	E/OJ
7. Ropivacaine	n.a.	0.00	1.00	n.a.	37. Compound 37	7.8	1.17	1.14	D/OJ
8. PPX	2.3	1.19	1.32	A/AD					
9. Prilocaine	1.7	0.67	1.40	B/OD					
10. Tocainide	2.3	0.51	1.29	A/OD	*Acids*				
11. Felodipine	n.a.	0.00	1.00	n.a.	38. Compound 38	2.6	2.20	1.60	C/OJ
12. Compound 12	2.5	1.50	1.46	A/AD	39. Metolazone	7.1	0.66	1.42	C/AD
13. Compound 13	3.8	2.21	7.54	E/OJ	40. Bendroflumethiazide	5.2	1.11	1.29	D/AD
14. Metanephrine	3.1	2.25	1.72	A/AD	41. Flurbiprofen	1.6	1.79	1.81	C/AD
15. Mianserin	1.6	2.21	2.78	E/AD	42. Ketoprofen	3.5	2.24	1.55	D/OJ
16. Promethazine	2.3	1.99	1.75	A/OJ	43. Naproxen	7.9	1.47	1.27	D/OJ
17. Thioridazine	2.2	0.27	1.18	A/OJ	44. Ibuprofen	1.4	0.11	1.21	C/OJ
18. Salbutamol	n.a.	0.00	1.00	n.a.	45. Ketorolac	5.9	0.98	1.16	C/AD
19. Bambuterol	3.6	2.91	1.81	B/AD	46. Omeprazole	3.3	3.12	2.29	E/AD
20. Terbutaline	n.a.	0.00	1.00	n.a.	47. Lansoprazole	5.6	0.94	1.41	C/OD
21. Econazole	2.2	1.24	1.61	A/AS	48. Compound 48	2.3	2.59	2.02	D/AD
22. Bifonazole	4.4	3.26	1.74	A/AD	49. Compound 49	4.5	0.81	1.16	C/OJ
23. Ketoconazole	4.5	3.21	2.16	E/AD	50. Compound 50	2.5	0.19	1.07	C/OJ
24. Compound 24	1.7	0.84	1.56	A/OJ	51. Compound 51	1.5	0.10	1.07	D/AS
25. BBMT	1.4	1.78	1.87	B/AD	52. Compound 52	7.7	2.41	1.46	D/AD
26. Compound 26	3.3	1.17	1.23	B/AD	53A. Boc-Cgl-Aze-OH[a]	2.3	0.82	1.45	C/AD
27. Compound 27	14.7	1.50	1.43	A/AS	53B. Boc-Cgl-Aze-OH[b]	2.3	2.00	2.37	C/AD
28. Compound 28	1.8	0.28	1.20	B/AS					
29A. Compound 29A[a]	16.7	9.20	4.41	A/AD					
29B. Compound 29B[b]	9.3	0.80	1.14	A/AD					
30. Compound 30	4.5	1.92	1.47	E/AD					

AT, total analysis time; R_s, resolution; α, selectivity factor; n.a., not applicable, no separation obtained. MP/Col, refers to the combination of mobile phase and column; A, ethanol/isohexane/DEA (15:85:0.1, by vol.); B, 2-propanol/isohexane/DEA (15:85:0.1, by vol.); C, ethanol/isohexane/TFA (15:85:0.1, by vol.); D, 2-propanol/isohexane/TFA (15:85:0.1, by vol.), E, methanol/ethanol (50:50, v/v). OD, OJ, AD and AS designate the four different polysaccharide columns.

[a] First diastereomer.
[b] Second diastereomer.

were determined by weighing together factors such as total analysis time, peak shape and resolution by visual inspection of the chromatograms, i.e. cases with very high resolution but with very retained second enantiomer were not considered as the best results. No enantioselectivity was observed for only seven out of the 55 enantiomeric pairs (or 13%) and these compounds were generally poorly retained in the mobile phases used in the screen.

3.1.4. Effect of additives in the mobile phase for the normal-phase mode

The acidic benzimidazoles, omeprazole and lanso-prazole, sometimes eluted as broad distorted peaks when TFA was used as additive in the mobile phase. When these compounds were chromatographed with 0.1% HAc instead, peak shapes and resolutions were improved as can be seen in Fig. 6. For most of the other stronger acidic compounds, TFA was required for obtaining good peak shapes. Some of these compounds were strongly retained and eluted as broad peaks with no or poor resolution when run on the OD column with a mobile phase containing HAc instead. In Fig. 7, it can be seen that naproxen and ketorolac display a difference in enantioselectivity

depending on the acid additive. However, when run on the OJ column, with ethanol as modifier, the type of acid had almost no effect on the enantioselectivity. This indicates that the choice of acid used to protonate the acidic compounds can have a major effect on the observed enantioresolution but that the resolution also depends on which column and type of alcohol that is used. Thus, it is not merely an effect of the pK_a of the additive, such that the stronger acids would always require TFA to be present in the unionised form and elute with good peak shapes and selectivity, but that in fact the combination of several different factors contribute to the overall performance. As a good compromise for a generic screen, TFA was considered to be the most suitable acid.

When using the same set of columns both with DEA and TFA as additives it is important that the additives do not remain in the column, which could possibly lead to irreproducible results. The manufacturer of the columns recommends that columns should be dedicated to either acid or base additive. The memory effect of acid and base additives on a Chiralpak AD column for separation of carboxyl protected amino acids and β-blockers has been investigated by Ye et al. [26]. These authors found
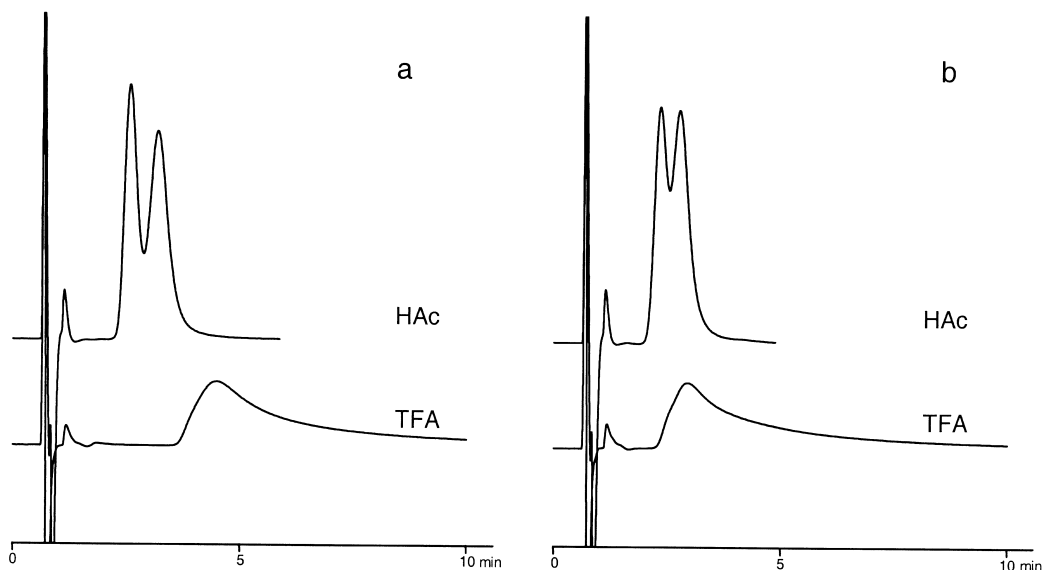

Fig. 6. Chromatograms showing the effect on peak shape of different acids in the mobile phase for omeprazole (a) and lansoprazole (b). Mobile phase: 2-propanol/isohexane/additive (15:85:0.1, by vol.) with Chiralcel OJ.
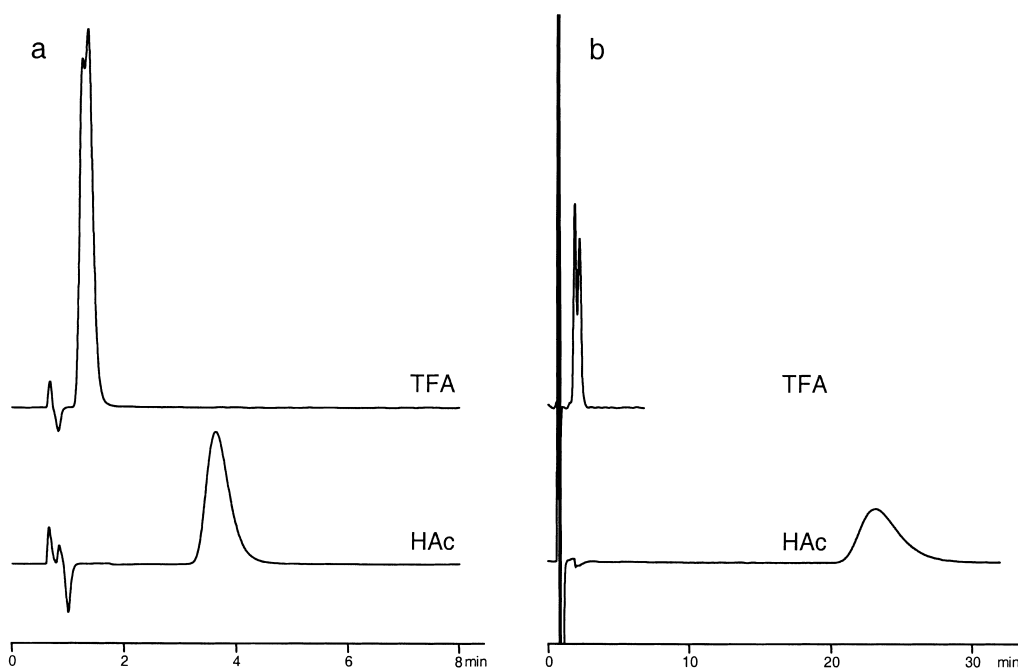
Fig. 7. Chromatograms showing the effect on peak shape of different acids in the mobile phase for naproxen (a) and ketorolac (b). Mobile phase: 2-propanol/isohexane/additive (15:85:0.1, by vol.) with Chiralcel OD.

that acid additives such as TFA affected the selectivity for some of the studied compounds but that the acid could be sufficiently removed by flushing with a mobile phase with base additive. We investigated this for some selected bases on the Chiralcel OD and OJ columns and the result is shown in Table 2. It is evident that the addition of TFA, probably working as an ion pairing reagent in the mobile phase, both affected the selectivity and retention for, e.g. pindolol, propranolol, tocainide, thioridazine and bifonazole but also that the effect was effectively removed after flushing with a mobile phase containing DEA. Only for tocainide and bifonazole on the OD column some remaining effect of altered selectivity persisted with a reduction of the α-value by approximately 10% after using TFA in the mobile phase. Thus, the alternating use of TFA and DEA does not seem to create too great a problem with irreproducibility and can be tolerated for a screening procedure. However, for the long term use of an analyte specific method, a specific column may be advisable.

3.2. Evaluation of macrocyclic glycopeptide columns

However effective the polysaccharide based columns proved to be in showing enantioselectivity for the studied substances, there were some compounds which remained unresolved by this screen. Furthermore, since there may be solubility problems using normal-phase eluents for very polar or ionic substances, the need for complementary screens is obvious. The normal-phase mode has recently been shown to be applicable even to very polar compounds such as unprotected amino acids [17,18] and the polysaccharide columns have also been found to be highly versatile in the reversed-phase mode [7,8], which greatly extends the applicability of these columns. However, these approaches are somewhat limited since the polysaccharide derivatives, which have no ionic groups present, require that substances are chromatographed as their neutral form either by ion pairing or by forcing the acid/base equilibrium to the unionised form of the compound by addition

Table 2
Investigation of memory effect of TFA used as additive in the mobile phase for separation of bases

Chiralcel OD						Chiralcel OJ					
Compound	Additive	t_1 (min)	t_2 (min)	R_s	α	Compound	Additive	t_1 (min)	t_2 (min)	R_s	α
3. Propranolol	DEA[a]	1.62	2.06	1.43	1.48	2. Pindolol	DEA[a]	3.16	3.56	0.76	1.17
3. Propranolol	TFA[b]	1.67	5.72	4.32	5.21	2. Pindolol	TFA[b]	4.74	4.97	0.16	1.06
3. Propranolol	DEA[c]	1.64	2.11	1.40	1.51	2. Pindolol	DEA[c]	3.13	3.54	0.82	1.17
10. Tocainide	DEA[a]	1.54	1.78	0.51	1.29	11. Felodipine	DEA[a]	1.70		0.00	1.00
10. Tocainide	TFA[b]	0.98		0.00	1.00	11. Felodipine	TFA[b]	1.64		0.00	1.00
10. Tocainide	DEA[c]	1.42	1.56	0.21	1.19	11. Felodipine	DEA[c]	1.67		0.00	1.00
13. Compound 13	DEA[a]	1.31		0.00	1.00	14. Metanephrine	DEA[a]	1.93		0.00	1.00
13. Compound 13	TFA[b]	1.26		0.00	1.00	14. Metanephrine	TFA[b]	1.86		0.00	1.00
13. Compound 13	DEA[c]	1.28		0.00	1.00	14. Metanephrine	DEA[c]	2.06		0.00	1.00
22. Bifonazole	DEA[a]	2.42	2.99	1.19	1.33	17. Thioridazine	DEA[a]	1.48	1.62	0.27	1.18
22. Bifonazole	TFA[b]	2.19	2.86	1.07	1.45	17. Thioridazine	TFA[b]	3.99	5.43	1.01	1.44
22. Bifonazole	DEA[c]	2.31	2.66	0.68	1.22	17. Thioridazine	DEA[c]	1.46	1.60	0.26	1.17
26. Compound 26	DEA[a]	1.47		0.00	1.00	22. Bifonazole	DEA[a]	5.29	6.68	1.49	1.30
26. Compound 26	TFA[b]	2.35		0.00	1.00	22. Bifonazole	TFA[b]	7.34	10.5	1.66	1.47
26. Compound 26	DEA[c]	1.40		0.00	1.00	22. Bifonazole	DEA[c]	5.12	6.53	1.65	1.32

Mobile phase: ethanol/isohexane/additive (15:85:0.1, by. vol.)

[a] Mobile phase with DEA run on a column with no previous exposure to other additives.

[b] Mobile phase with TFA. Experiment performed after washing with 90 column volumes of 2-propanol and equilibration with 40 column volumes of mobile phase containing TFA.

[c] Mobile phase with DEA. Experiment performed after using mobile phase with TFA followed by equilibration with 40 column volumes of mobile phase containing DEA.

of acids or bases to the mobile phase. Thus it is more likely that some other type of CSP, such as the macrocyclic glycopeptides, which have the possibility of ionic interactions, could provide complementary selectivity to the polysaccharide screen.

3.2.1. Columns connected in series

The feasibility of screening for chiral selectivity by using macrocyclic glycopeptide columns connected in series has been reported by Wang et al. [23]. A screening procedure using three different 100×4.6-mm columns and three mobile phases was investigated for a number of compounds. These authors found this coupled column approach very rapid and stated that since the different stationary phases usually give the same elution order, a low risk of missing a potentially successful separation was anticipated. Astec has now made this approach commercially available and three mobile phases in three different modes are suggested [21]. The columns are connected in the order of increasing

polarity with the least polar Chirobiotic R first, then Chirobiotic V and the Chirobiotic T column last.

In the normal-phase mode it was suggested that a mobile phase consisting of ethanol/isohexane (60:40, v/v) should be used and that the flow-rate should be set at 1.5 ml/min. It was furthermore suggested that only two of the columns (Chirobiotic V and Chirobiotic T) should be used in this mode. When applying this approach to the 55 enantiomeric pairs in this study, only 11% of the compounds showed enantioselectivity in the normal-phase mode with these columns. The best results were obtained for neutral or weakly basic/acidic compounds. From these results it could be concluded that for the screening of compounds of pharmaceutical interest the normal-phase mode was limited in usefulness since no modifier was present, which resulted in severe peak distortions for charged compounds. Addition of acid/base, similarly to the normal-phase eluents used for the polysaccharides, would probably increase the success rate for several of the compounds, but this was not investigated further.

In the polar organic mode a mobile phase consisting of methanol and a low concentration of acid/base is used to control the ionic interactions between the stationary phase and the analyte. For the polar organic mode to work properly, the compound must contain at least two functional groups capable of interacting with the stationary phase, primarily by hydrogen bonding and ionic interactions, and at least one of them must be on or near the stereogenic centre. In this mode, retention is controlled by the amount of acid/base in the mobile phase, with increased retention with lowered amount of additive, and the selectivity is altered by the ratio of acid/base. The compounds were run on three columns connected in the order R, V, T with a mobile phase composed of methanol/HAc/TEA (100:0.02:0.01, by vol.) at a flow-rate of 2.0 ml/min since the methanol phase only creates a very moderate backpressure and since the resolution deteriorates only marginally by an increase in flow-rate in the polar organic mode. The results from this mode were better and for 44% of the compounds some enantioselectivity was observed. However, there was a large predominance in favour of the basic compounds and for these the success rate was 58%, whereas none of the neutrals and merely 35% of the acids showed some enantioselectivity. Several of the acids and all of the neutrals were poorly retained in this mode, which can probably explain the lower success rate.

In the reversed-phase mode a mobile phase consisting of methanol and 0.1% triethylammonium acetate buffer, pH 6.0 in the ratio 25:75 (v/v) was used. In order to avoid high backpressure and also to allow more time for mass transfer limited cavity inclusion interactions, which are more important in this mode, the flow-rate was set at 1.0 ml/min. The results from the reversed-phase mode indicated that this mode was highly suitable for acids as selectivity was seen for 76% of the acidic compounds. Also, some of the local anaesthetics (bupivacaine, mepivacaine and ropivacaine) displayed some enantioresolution but at very high retention times and with tailing peaks. In fact, the pronounced retention, with only minor enantioselectivity, was generally observed for the basic compounds. The neutral compounds, which were unretained in the polar organic mode, were better resolved in the reversed-

phase mode with two compounds (33 and 37) showing good resolution.

3.2.2. Separate columns

Since the aim of our investigation for a screening strategy was to automate the procedure by using a column switching device, the possibility of screening columns sequentially for selectivity somewhat diminishes the advantages of columns connected in series. Due to the broad applicability of the macrocyclic glycopeptide columns there is a high probability that enantioselectivity is observed using the coupled column approach and then there is still the need to disconnect them and repeat the runs on separate columns in order to determine which column that gave the best selectivity. It is also interesting to evaluate to what extent the coupled column approach results in erroneous conclusions regarding observed enantioselectivity and the possibility of developing satisfying analytical methods based on the obtained results.

From the coupled column screen it could be concluded that the normal-phase mode was limited in applicability and it was decided not to investigate this any further. Also, it was concluded that the polar organic mode was only suitable for compounds with acid/base functionality and that the reversed-phase mode should primarily be applied to acids and
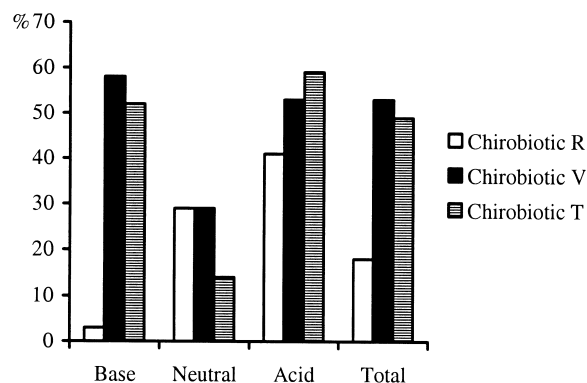

Fig. 8. Results from the screen with the three macrocyclic glycopeptide columns in both polar organic and reversed-phase mode showing the extent of observed enantioselectivity for each column. Mobile phases: methanol/0.1% triethylammonium acetate buffer pH 6.0 (25:75, v/v) and methanol/HAc/TEA (100:0.02:0.01, by vol.), 1 ml/min.

neutrals. Thus, the compounds were reanalysed on separate columns based on the findings of the coupled column screen and the results can be seen in Fig. 8. It is obvious that the Chirobiotic V and T columns were the most versatile columns for the investigated substances. This is primarily due to the fact that these columns were highly suitable for the bases, which dominated the test set, while Chirobiotic R mainly separated acids. In total, the macrocyclic glycopeptide CSPs showed enantio-

selectivity for 65% of the chiral compounds. In Table 3 the best separations from this screen are presented. The criteria for best results were the same as for the polysaccharide screen.

On the macrocyclic glycopeptide columns there was a clearly discernible compound class selectivity difference for the three CSPs. This is clearly observed in the polar organic mode for many basic compounds. For example, all the β-blockers (alprenolol, pindolol, propranolol and metoprolol) as

Table 3
Combined best results suitable for further optimisation from screen with separate macrocyclic glycopeptide columns in polar organic and reversed-phase mode

Compound	AT (min)	R_s	α	MP/Col	Compound	AT (min)	R_s	α	MP/Col
Bases					*Neutrals*				
1. Alprenolol	7.2	1.36	1.10	F/V	31. Guaifenesin	n.a	0.00	1.00	n.a
2. Pindolol	8.3	1.22	1.09	F/V	32. MEEPB	n.a	0.00	1.00	n.a
3. Propranolol	9.2	1.53	1.12	F/V	33. Compound 33	8.4	1.24	1.20	G/V
4. Metoprolol	8.3	1.21	1.09	F/V	34. Compound 34	n.a	0.00	1.00	n.a
5. Bupivacaine	5.3	1.73	1.18	F/V	35. Compound 35	n.a	0.00	1.00	n.a
6. Mepivacaine	12.5	0.98	1.08	F/T	36. Compound 36	n.a	0.00	1.00	n.a
7. Ropivacaine	5.6	1.72	1.17	F/V	37. Compound 37	3.6	1.00	1.12	G/V
8. PPX	9.9	1.73	1.14	F/V					
9. Prilocaine	6.3	0.20	1.03	F/V	*Acids*				
10. Tocainide	7.4	0.76	1.06	F/V	38. Compound 38	n.a	0.00	1.00	n.a
11. Felodipine	n.a	0.00	1.00	n.a.	39. Metolazone	7.7	1.31	1.30	G/V
12. Compound 12	n.a	0.00	1.00	n.a	40. Bendroflumethiazide	6.4	0.93	1.14	G/V
13. Compound 13	n.a	0.00	1.00	n.a	41. Flurbiprofen	4.7	0.93	1.18	G/R
14. Metanephrine	11.5	0.31	1.04	F/V	42. Ketoprofen	6.3	2.85	1.63	G/R
15. Mianserin	14.6	3.25	1.66	F/V	43. Naproxen	8.5	2.06	1.48	G/R
16. Promethazine	19.4	2.62	1.20	F/V	44. Ibuprofen	n.a	0.00	1.00	n.a.
17. Thioridazine	n.a	0.00	1.00	n.a.	45. Ketorolac	4.9	1.06	1.20	G/R
18. Salbutamol	6.6	1.54	1.13	F/V	46. Omeprazole	13.9	1.83	1.37	G/R
19. Bambuterol	21.9	3.82	1.28	F/T	47. Lansoprazole	7.7	0.69	1.13	G/R
20. Terbutaline	6.5	0.86	1.08	F/V	48. Compound 48	5.7	0.95	1.21	G/T
21. Econazole	n.a	0.00	1.00	n.a	49. Compound 49	2.3	1.47	1.61	G/T
22. Bifonazole	n.a	0.00	1.00	n.a	50. Compound 50	1.5	0.21	1.65	G/T
23. Ketoconazole	n.a	0.00	1.00	n.a	51. Compound 51	1.5	1.16	1.95	G/T
24. Compound 24	n.a	0.00	1.00	n.a	52. Compound 52	3.9	1.70	1.65	G/T
25. BBMT	11.2	3.20	1.28	F/V	53A. Boc-Cgl-Aze-OH[a]	n.a	0.00	1.00	n.a.
26. Compound 26	7.8	0.81	1.10	F/V	53B. Boc-Cgl-Aze-OH[b]	1.9	0.12	1.32	F/V
27. Compound 27	15.1	1.07	1.09	F/V					
28. Compound 28	31.6	3.62	1.26	F/T					
29A. Compound 29A[a]	n.a	0.00	1.00	n.a					
29B. Compound 29B[b]	n.a	0.00	1.00	n.a					
30. Compound 30	n.a	0.00	1.00	n.a					

AT, total analysis time; R_s, resolution; α, selectivity factor; n.a., not applicable, no separation obtained. MP/Col, refers to the combination of mobile phase and column; F, methanol/HAc/TEA (100:0.02:0.01, by vol.); G, methanol/0.1% TEAA pH 6.0 (25:75 v/v). R, V and T designates the three different macrocyclic glycopeptide columns.

[a] First diastereomer.
[b] Second diastereomer.

well as four of the local anaesthetics (bupivacaine, mepivacaine, ropivacaine and PPX) separated on Chirobiotic V and T, all the bronchodilators (salbutamol, bambuterol and terbutaline) on Chirobiotic T and two of the latter (salbutamol and terbutaline) on Chirobiotic V as well. In the reversed-phase mode, all but one of the NSAIDs (flurbiprofen, ketoprofen, naproxen and ketorolac) separated on the Chirobiotic R and all the β-ethoxycarboxylic acids (compounds 49–52) on Chirobiotic T. These results are in good agreement with the proposed method development protocol reported by Aboul-Enein et al. for various substances [27], which suggests that bases should be run on Chirobiotic V and T in the polar organic mode and that acids should be run on Chirobiotic T and R primarily in the reversed-phase mode but also in polar organic mode for, e.g. carboxylic acids.

Fig. 9 shows some examples where the connected column approach gave misleading results due to reversal of elution order for the compounds on the different CSPs. In recent articles the enantiomeric elution order for a series of chiral sulfoxides [28] and oxazolidiones as well as dansyl amino acids [29] showed a great deal of consistency for any single CSP and mobile phase. However, reversing the enantiomeric elution order was found to be possible by changing the CSP in the same mobile phase mode. These findings contradict previous results [23] and we found the coupled column approach not so useful for screening purposes when the final objective is to develop an analytical method where there is a large excess of one of the enantiomers. Developing a satisfying analytical method always requires that factors such as peak shape and retention order are taken into consideration when evaluating the results from a screen for enantioselectivity. Also, the examples of reversal of elution order on the three columns, which can lead to inaccurate conclusions about the feasibility of a separation, seems somewhat unsatisfactory. Accordingly, this potential for reversal of elution order should be evaluated already during the screening process in order to be fully exploited during the subsequent optimisation of the method.

3.3. Combined results from the two final screens

Based on the evaluations, two final automated

screens, utilising a column switcher, were set up. The first screen employed four polysaccharide based columns (Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS) and five mobile phases. Acidic compounds are analysed with two different mobile phases consisting of ethanol/isohexane/TFA (15:85:0.1, by vol.) and 2-propanol/isohexane/TFA (15:85:0.1, by vol). Basic compounds are analysed with three mobile phases consisting of ethanol/isohexane/DEA (15:85:0.1, by vol.), 2-propanol/isohexane/DEA (15/85/0.1, by vol.) and methanol/ethanol (50:50, v/v). A mixture of 2-propanol/isohexane (10:90, v/v) is used to wash the columns after the screens. Neutral compounds are analysed with the same mobile phases as for the basic compounds, but DEA can be replaced with TFA without any noticeable change in the results (the neutrals can even be run without any additives). The second screen utilises three macrocyclic glycopeptide columns (Chirobiotic R, Chirobiotic V and Chirobiotic T) and two mobile phases. Basic compounds are analysed in the polar organic mode with methanol/HAc/TEA (100:0.02:0.01, by vol.). Neutral compounds are analysed in the reversed-phase mode with methanol/TEAA (0.1%, pH 6) (25:75, v/v). Acidic compounds are analysed in both modes. 2-Propanol is used to wash the columns after the screens.

In Fig. 10 it can be seen that the polysaccharide columns showed enantioselectivity for 87% of the chiral compounds, while the macrocyclic glycopeptide columns showed enantioselectivity for 65% of them. When both screens were evaluated together they showed enantioselectivity for 96% of the chiral compounds.

The combined best results from the two screens are presented in Table 4 with predicted analysis times and resolution values for 250-mm analytical columns. The predicted analysis time is based on the elution time for the second enantiomer on the short column recalculated to a 250-mm column length and with an addition of 5 min to allow for complete elution of the peak. As can be seen, the combined chiral screens succeeded in showing enantioselectivity for 53 of the 55 enantiomeric pairs tested. Using 250-mm-long columns, baseline separation ($R_s \geq 1.5$) was predicted for 48 of the 55 enantiomeric pairs. It is likely that the separation of the
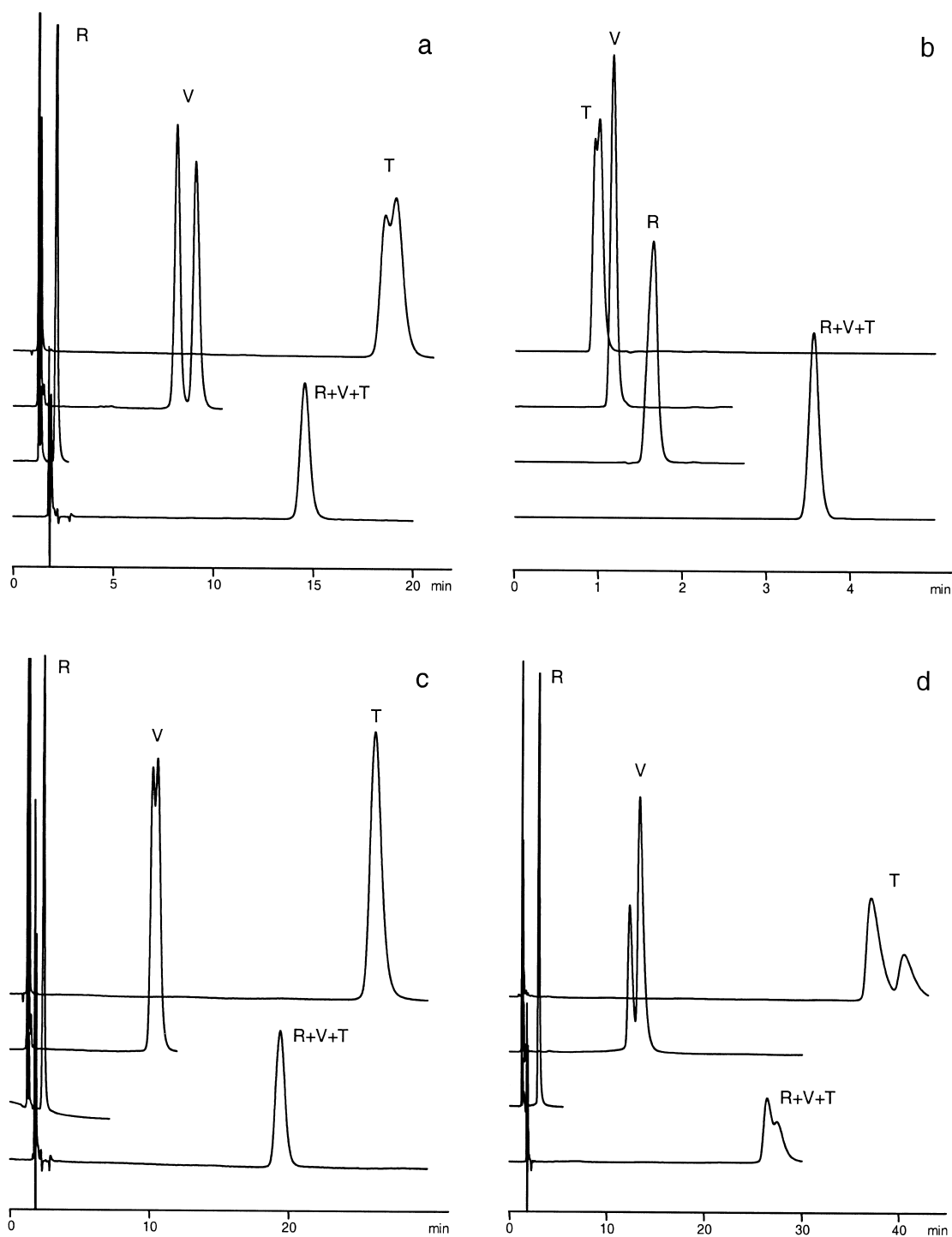
Fig. 9. Chromatograms showing some examples where the enantioselectivity of different macrocyclic glycopeptide columns counteracted each other when connected in series. Mobile phases: (a, c, d) methanol/HAc/TEA (100:0.02:0.01, by vol.) and (b) methanol/0.1% triethylammonium acetate buffer, pH 6.0 (25:75, v/v). Compounds: (a) PPX, (b) compound 50, (c) metanephrine, (d) compound 27.
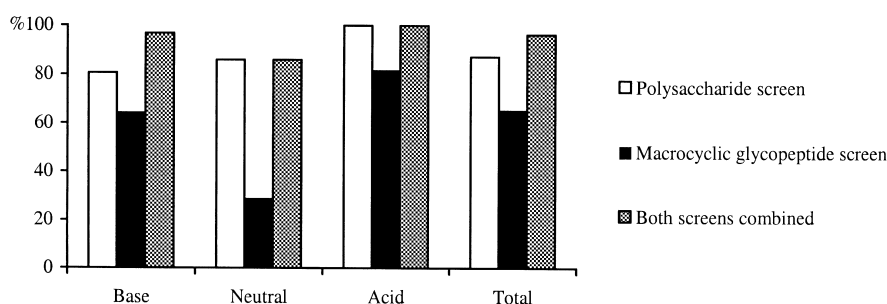
Fig. 10. Overview of the results from the two different screens, both separately and combined showing the extent of observed enantioselectivity and the complementary use of the two screens.

Table 4
The combined best results from both screening procedures, when recalculated from 50- or 100-mm-long columns to 250-mm-long columns

Compound	AT (min)	R_s	MP/Col	Compound	AT (min)	R_s	MP/Col
Bases				*Neutrals*			
1. Alprenolol	14.5	9.03	B/OD	31. Guaifenesin	25.0	9.28	C/OD
2. Pindolol	15.0	2.39	A/AD	32. MEEPB	25.1	2.53	D/OJ
3. Propranolol	15.3	3.20	A/OD	33. Compound 33	21.2	5.77	D/AD
4. Metoprolol	11.6	3.96	A/OD	34. Compound 34	16.0	7.83	C/AD
5. Bupivacaine	16.8	2.74	F/V	35. Compound 35	n.a.	0.00	n.a.
6. Mepivacaine	32.7	1.55	F/T	36. Compound 36	16.7	2.28	E/OJ
7. Ropivacaine	17.2	2.72	F/V	37. Compound 37	12.8	1.58	G/V
8. PPX	14.4	2.66	A/AD				
9. Prilocaine	11.5	1.50	B/OD	*Acids*			
10. Tocainide	13.9	1.14	A/OD	38. Compound 38	15.9	4.92	C/OJ
11. Felodipine	n.a.	0.00	n.a.	39. Metolazone	21.1	2.07	G/V
12 Compound 12	15.1	3.35	A/AD	40. Bendroflumethiazide	25.8	2.48	D/AD
13 Compound 13	17.3	4.94	E/OJ	41. Flurbiprofen	11.7	4.00	C/AD
14. Metanephrine	17.8	5.03	A/AD	42. Ketoprofen	19.4	5.01	D/OJ
15. Mianserin	11.0	4.94	E/AD	43. Naproxen	21.3	3.26	G/R
16. Promethazine	13.8	4.45	A/OJ	44. Ibuprofen	10.0	0.25	C/OJ
17. Thioridazine	13.1	0.60	A/OJ	45. Ketorolac	15.4	1.68	G/R
18. Salbutamol	20.0	2.43	F/V	46. Omeprazole	18.5	6.98	E/AD
19. Bambuterol	20.5	6.51	B/AD	47. Lansoprazole	24.8	2.10	C/OD
20. Terbutaline	19.1	1.36	F/V	48.Compound 48	14.7	5.79	D/AD
21. Econazole	12.9	2.77	A/AS	49.Compound 49	9.6	2.32	G/T
22. Bifonazole	23.9	7.29	A/AD	50. Compound 50	7.5	0.34	G/T
23. Ketoconazole	24.0	7.18	E/AD	51. Compound 51	8.0	1.83	G/T
24 Compound 24	10.9	1.88	A/OJ	52. Compound 52	12.0	2.69	G/T
25. BBMT	10.9	3.98	B/AD	53A. Boc-Cgl-Aze-OH[a]	12.8	1.83	C/AD
26. Compound 26	19.1	2.62	B/AD	53B. Boc-Cgl-Aze-OH[b]	13.4	4.47	C/AD
27. Compound 27	38.3	1.69	F/V				
28. Compound 28	77.8	5.72	F/T				
29A. Compound 29A[a]	79.2	20.6	A/AD				
29B. Compound 29B[b]	44.7	1.79	A/AD				
30. Compound 30	23.5	4.29	E/AD				

AT, total analysis time; R_s, resolution; n.a., not applicable, no separation obtained. MP/Col, refers to the combination of mobile phase and column. Same designation of mobile phases and columns as in Tables 1 and 3.

[a] First diastereomer.
[b] Second diastereomer.

remaining five enantiomeric pairs, which showed enantioselectivity but not baseline separation, could be improved by optimising the chromatographic conditions of that particular separation. The Chiralpak AS column was the least successful column used in the screens since it only showed best enantioselectivity for one of the test compounds. It is also evident that the Chiralpak AD column was superior to the other columns as it showed the best enantioselectivity for 38% of the test compounds.

The described generic chiral screens provide an excellent starting point for further method development to provide final analytical methods capable of reliable and robust determination of the minor enantiomer at and below levels required by the authorities. Since the screens offer very broad overall enantioselectivity, it is likely that one of the used combinations of column and mobile phase will produce favourable conditions for efficient enantioresolution. Indeed, as can be seen from Table 4, high enantioresolution was obtained for a majority of the tested compounds. Hence, it should be possible to develop optimised methods with detection levels even below 0.05% for most compounds even if the advantage of predictable reversal of enantioselectivity cannot be obtained on these stationary phases as is the case, e.g. for many Pirkle-type phases. Furthermore, all of the used stationary phases are available for semi and process scale preparative chromatography that can be utilised for isolation and production of chiral intermediates and drug substances during process research and development.

4. Conclusions

Four polysaccharide based CSPs were evaluated and compared with three macrocyclic glycopeptide based CSPs for rapid HPLC screening for the separation of chiral compounds of pharmaceutical interest.

The polysaccharide based columns were employed in the normal-phase and polar organic modes and showed overall enantioselectivity for 87% of the compounds tested. The four polysaccharide CSPs were found to have complementary properties with the Chiralpak AD and the Chiralpak AS being the most and least successful columns, respectively. The

Chiralcel OJ was found to be particularly successful for acidic analytes. The complementary properties of different alcohol mobile phase modifiers were also demonstrated and knowledge of the acid/base properties of the analytes is desirable as the use of additives was shown to improve peak shape and resolution of acidic and basic compounds.

The macrocyclic glycopeptide columns were employed in the reversed-phase and polar organic modes of operation and showed enantioselectivity for 65% of the analytes. Although the overall success rate is lower than for the polysaccharide based CSPs, these columns offer complementary enantioselectivity and increased compound class selectivity for several families of compounds. The Chirobiotic V and Chirobiotic T columns were the most successful, especially for basic analytes while the Chirobiotic R was particularly successful for acidic analytes. A proposed screen with the three macrocyclic glycopeptide columns coupled in series was discarded in favour of testing the columns separately, primarily due to the possibility of reversal of elution order on two columns cancelling out the overall enantioresolution.

When the results from both the polysaccharide and the macrocyclic glycopeptide screen were combined they showed enantioselectivity for 53 out of the tested 55 enantiomeric pairs (or 96%). This leads to the conclusion that the two screens have complementary properties, i.e. both types of columns should be included to achieve the highest probability of success. The screens were automated using a column switcher allowing for the different combinations of CSPs and mobile phases to be tested overnight. By extrapolating the results from the 50 and 100-mm columns used in the screen to 250-mm columns, it was possible to obtain baseline separation for 48 of the tested 55 enantiomeric pairs with a method development time not exceeding 24 h per sample.

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