Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1100 (2005) 108–115
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Enhanced chromatographic resolution of amine enantiomers as carbobenzyloxy derivatives in high-performance liquid chromatography and supercritical fluid chromatography

Christina M. Kraml [a,*], Dahui Zhou [b], Neal Byrne [a], Oliver McConnell [a]

[a] *Discovery Analytical Chemistry, Wyeth Research, CN 8000, 865 Ridge Road, Princeton, NJ 08543, USA*
[b] *Discovery Medicinal Chemistry, Wyeth Research, CN 8000, 865 Ridge Road, Princeton, NJ 08543, USA*

Received 25 March 2005; received in revised form 30 August 2005; accepted 9 September 2005
Available online 28 September 2005

Abstract

The carbobenzyloxy (cbz) protecting group is evaluated for it's potential to enhance the resolution of chiral amine enantiomers using high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). A series of cbz derivatives of commercially available racemates was prepared and analyzed by enantioselective chromatography using a variety of mobile phases and polysaccharide and Pirkle-type chiral stationary phases (CSPs). The cbz-derivatized product consistently demonstrated enhanced chiral resolution under HPLC and SFC conditions. Improved selectivity and resolution combined with an automated preparative HPLC or SFC system can lead to the rapid generation of highly purified enantiomers of desirable starting materials, intermediates or final products.

Keywords: HPLC; SFC; Enantioselective chromatography; cbz derivatives; Chiral amines; Pharmaceuticals; Preparative separations

1. Introduction

The need for enantiomerically pure compounds in all stages of drug development is stimulating the demand for efficient processes to resolve racemic mixtures. At the discovery stages, semi-preparative quantities of the enantiomers of racemic mixtures are commonly obtained using chromatography on chiral stationary phases. The chromatographic method usually furnishes both enantiomers in high optical purity in a process that is rapid and generally applicable [1]. At later stages of drug development, there is a rising interest in the preparation of enantiomers on very large scales. Since the introduction of simulated moving bed (SMB) technology [2], as well as advances in supercritical fluid chromatography (SFC) technology, the production of large quantities of purified enantiomers is increasingly popular. It is clear that for large-scale separations, only optimized conditions must be used, as small increases in selectivity, resolution and loading capacity will yield major increases in productivity [3]. When gram-quantities of one or each enantiomer of a chiral target molecule are required, it is useful to consider each intermediate within a synthetic scheme for possible chiral separation. In many cases, the final product may not be the best choice for a large-scale separation. Small changes in compound structures as well as the addition of protecting groups can influence chromatographic resolution and may lead to great increases in productivity.

Chiral amines are common intermediates or final products of enantioselective syntheses in medicinal chemistry. In many cases, chiral amines are difficult to separate chromatographically. Chiral phases have been developed for their direct resolution using ligand exchange [4], chiral urea derivative phases [5], chiral crown ether stationary phases [6,7] and others [8,9]. However, in many cases, these phases are limited to analytical-scale applications due to the low saturation capacities of their stationary phases and/or impractical mobile phase compositions. To avoid such difficulties, one might consider the strategy described by Francotte [10], in which the solute is adapted to the stationary phase. For example, achiral derivatization was used as a means of improving the chromatographic resolution of racemic aliphatic and aromatic alcohols on benzoylcellulose chiral stationary phases (CSPs) [10]. In another case, phosphate and phosphonate derivatives of chiral alcohols were prepared

* Corresponding author. Tel.: +1 732 274 4480.
E-mail address: kramlc@wyeth.com (C.M. Kraml).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.09.017

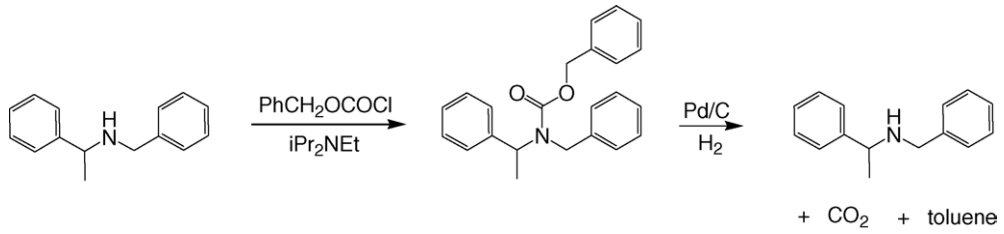

Fig. 1. Addition and removal of the cbz from *N*-benzyl-α-methylbenzylamine.

to enhance chromatographic resolution and consequently obtain appreciable quantities of the enantiopure alcohol following basic hydrolysis [11].

Although indirect chiral separation methods have certain drawbacks compared with direct separation methods, the importance of the flexibility and broad applicability of derivatization methods cannot be overestimated [12]. Derivatization of the amine function for chromatographic separation is a well-developed field whose reactions include the use of isothiocyanates, isocyanates, chloroformates, acid chlorides and aromatic anhydrides as non-chiral derivatizing agents or tags, such as the 3,5-dinitrobenzoyl tag [12]. Amino acid derivatization also includes the use of isothiocyanates to make phenyl and methyl thiohydantoin derivatives, acid chlorides using 9-fluorenylmethyl chloroformate (FMOC), orthophthaldialdehyde (OPA), chloroformates, as well as dansyl-derivatives and others [12].

To be useful for preparative chromatography, a derivatizing agent must be easily attached and removed. Although cbz-derivatization has been used for decades as a protecting strategy in synthesis and even to enable resolution of amino acids and certain primary and secondary amines, the extent of the usefulness of this derivative has not been demonstrated. A simple procedure for the derivatization of compounds with primary or secondary amines involves acylation of the amine with benzyl chloroformate [13]. Following separation, the amine is regenerated by catalytic hydrogenolysis using palladium on carbon; a reaction which is typically quantitative [13]. The product is isolated by simple filtration and evaporation of the solvent with the generation of toluene and carbon dioxide by-products [13] (Fig. 1). Cleavage can also be carried out under different reaction conditions depending on the nature of the amine. These methods have been well studied, and proceed without significant racemization [13,14]. When developing methods for scale-up, the analytical objective is to resolve the enantiomers with the greatest possible resolution factor (at least 2.0). If derivatization improves resolution, other considerations include solubility and ease of protection and deprotection. The cbz derivative appears to contribute positively and consistently to all of these areas. To demonstrate the benefits of carrying out this procedure, a diverse set of chiral primary and secondary amines (Fig. 2), was purchased, derivatized and analyzed chromatographi-

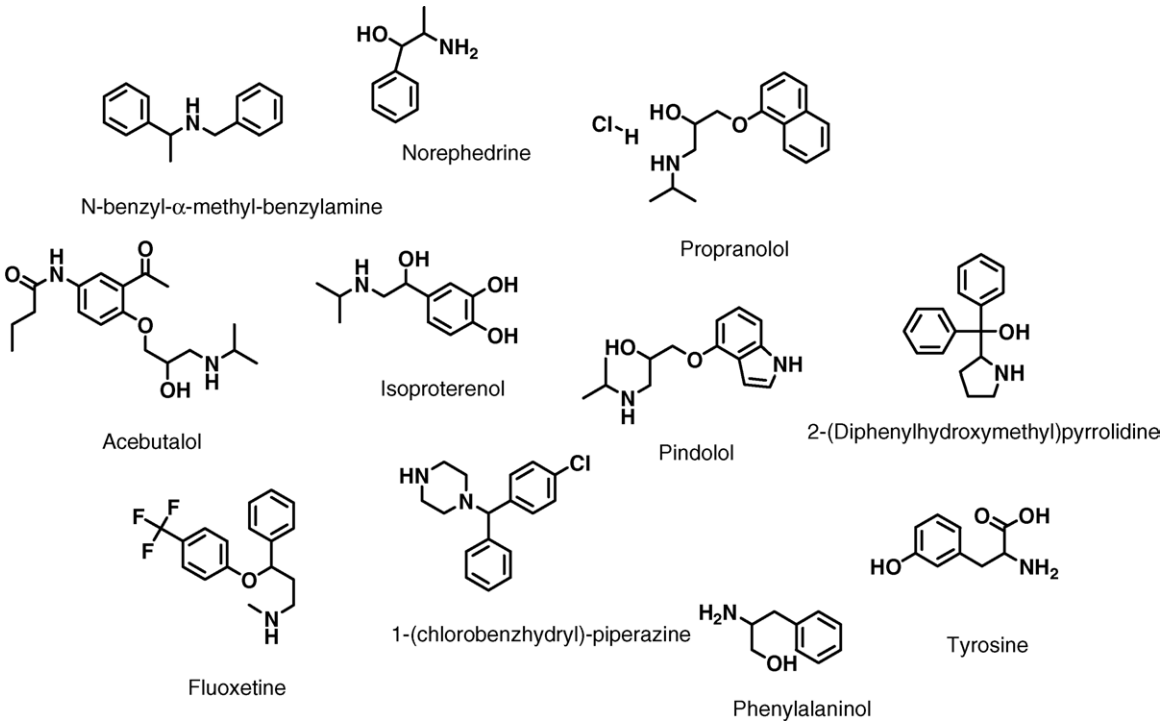

Fig. 2. Amines used in this study.

cally by high-performance liquid chromatography (HPLC) and SFC.

2. Experimental

2.1. Reagents and solvents

All compounds were obtained from Sigma–Aldrich (St. Louis, MO, USA). Structures are shown in Fig. 1. The cbz derivatives of each amine were synthesized (see Section 2.2). Carbon dioxide (SFC grade) was obtained from Air Gas (Radnor, PA, USA). All HPLC grade solvents were obtained from EMD Chemicals (Gibbstown, NJ, USA) except for ethanol, which was obtained from Mallinckrodt Chemicals (Phillipsburgh, NJ, USA) and 2-butanol obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation of cbz derivatives

The derivatives were prepared by one of two methods: (1) to a solution of 0.1–5 g of the chiral amine (1 mol-eq) in THF was added benzyl chloroformate (tech. 95%, 1.05 mol-eq) and *N*,*N*-diisopropylethylamine (99% pure, 3 mol-eq) at 0 °C. The resulting reaction mixture was stirred at 0 °C for a few hours until there was no starting material left. The reaction was quenched with water and extracted with methylene chloride. The sample was purified by HPLC (RediSep-Flash SiO_2 120 gm Teledyne ISCO column; 15–50% ethyl acetate in hexane gradient elution, sample was loaded onto the column in methylene chloride) to obtain the desired product as a clear oil; (2) chiral amines bearing several hydroxyl groups were prepared using the following procedure: to a solution of 0.1–5 g of chiral amine (1 mol-eq) in dioxane–water (1:1) was added benzyl chloroformate (tech. 95%; 1.05 mol-eq) and Na_2CO_3 (3 mol-eq) at 0 °C. The resulting reaction mixture was stirred at 0 °C for a few hours until there was no starting material left. Solvent was removed under vacuum affording the desired product, which was studied without further purification.

2.3. Chiral HPLC screen

All compounds were analyzed on five types of (25 cm × 0.46 cm) chiral columns: Chiralcel OD and OJ, Chiralpak AS and AD from Chiral Technologies (Exton, PA, USA) and a Whelk-O1 (S,S) column from Regis (Morton Grove, IL, USA). HPLC was carried out on two Agilent 1100 series HPLC equipped with quaternary pumps (model G13411A), autosamplers (model G1367A), diode-array detectors (model G1315B) from Agilent Technologies (CA, USA) and LC Spiderling Deluxe automated column selection systems (Chiralizer Services, Newton, PA, USA). Six isocratic mobile phases were used for this screen: ethanol, 1:1 hexane:ethanol, 8:2 hexane:2-propanol on one system and methanol, 5% water in methanol, and 10% water in acetonitrile on the second system. Diethylamine was added to each mobile phase at a concentration of 0.1% (v/v) for analysis of the free amines. Samples were run isocratically at a flow rate of 1 mL/min for 20 min and detection at 254, 280 and 230 nm. The cbz derivatives dissolved readily in ethanol and 10 μL of 5–10 mg/mL solutions were injected for each analysis. The free amines bearing hydroxyl groups were dissolved in dioxane:ethanol and heated, all other amines were dissolved in ethanol and 10 μL injections of 5–10 mg/mL solutions were made for each analysis.

2.4. Chiral SFC screen

All compounds were analyzed on five (25 cm × 0.46 cm) chiral columns: Chiralcel OD and OJ, Chiralpak AS and AD-H from Chiral Technologies (Exton, PA, USA) and a Whelk-O1 (S,S) column from Regis (Morton Grove, IL, USA). Samples were analyzed on a Berger Analytical SFC System equipped with a dual pump (FCM-1200), an auto sampler (ALS 3100), a column oven (TCM-200) and a diode-array detector (DAD-4100) (Berger Instruments, Newark, DE, USA). The screen was carried out under isocratic conditions using 20 and 40% of organic modifier in CO_2 at a backpressure of 100 bars with a flow rate of 2.5 mL/min, a temperature of 40 °C for 15 min and detection at 254, 280 and 230 nm. The organic modifiers used include methanol, ethanol, 2-propanol, 2-butanol and acetonitrile. The free amines were analyzed with the addition of 0.1% diethylamine (v/v) to the mobile phase. The cbz derivatives dissolved readily in ethanol and 10 μL of 5–10 mg/mL solutions were injected for each analysis. The free amines bearing hydroxyl groups were dissolved in dioxane:ethanol and heated, all other amines were dissolved in ethanol and 10 μL injections of 5–10 mg/mL solutions were made for each analysis.

2.5. Preparative SFC separation of cbz-N-benzyl-α-methylbenzylamine

A total of 20.2 g of the cbz derivative of *N*-benzyl-α-methylbenzylamine was prepared for chiral separation (as described in Section 2.2). The separation was carried out on a Berger Multigram III SFC equipped with two SD-1 Varian pumps, a Knauer K-2501 Spectrophotometer, 6-ton bulk CO_2 tank with built-in chiller and heater, and G700 compressor (Mettler-Toledo, Newark, DE, USA). The sample was dissolved to a volume of 30 mL in ethanol. Injection volume was 4.5 mL (3 g/injection) and sample was injected at intervals of 160 s onto a 25 cm × 5 cm ModCol spring-loaded column (5000 psi) packed with Whelk-01 (R,R), 10 μm, 100 Å packing material (Regis, Morton Grove, IL, USA). Sample peaks were collected using collection 'windows' at a wavelength of 254 nm. A total of 9.67 g of enantiomer 1 was collected in a volume of approximately 900 mL of 2-propanol with enantiomeric excess (ee) >99.9%. A total of 10.15 g of enantiomer 2 was collected in a volume of approximately 2 L of 2-propanol with ee = 99.4%. Total sample recovery was 98.1%.

3. Results and discussion

Primary and secondary chiral amines were derivatized for the purpose of developing an efficient process for their separa-

Table 1
Retention factors, selectivity and resolution of chiral amines and their cbz derivatives

Compound	Amine				cbz derivative of amine			
	k_1	k_2	α	R_s	k_1	k_2	α	R_s
1. *N*-Benzyl-α-methylbenzylamine	No separation				4.42	6.31	1.55	14.4
2. Acebutalol	No separation				4.20	5.28	1.34	7.26
3. Fluoxetine	No separation				5.60	6.23	1.14	4.34
4. Norephedrine	2.69	3.28	1.35	4.09	3.87	5.45	1.55	14.69
5. Isoproterenol	No separation				4.85	6.0	1.3	6.98
6. 1-(Chlorobenzhydryl)-piperazine	2.46	3.23	1.53	7.93	2.37	3.90	2.11	14.77
7. Propranolol	1.88	2.39	1.57	7.5	2.73	5.81	2.79	19.40
8. Pindolol	2.12	3.95	2.64	16.41	3.94	7.50	2.21	12.76
9. Phenylalaninol	No separation				7.37	8.46	1.17	5.59
10. 2-(Diphenylhydroxymethyl)-pyrrolidine	No separation				2.66	3.75	1.66	11.64
11. Tyrosine	No separation				1.32	1.95	3.0	8.20

Best results from SFC screening study. (1) *N*-benzyl-α-methyl-benzylamine, no separation and cbz-*N*-benzyl-α-methyl-benzylamine, see Fig. 3. (2) Acebutalol, no separation and cbz-acebutalol, AS, 20% 2-propanol (DEA)/CO_2. (3) Fluoxetine, no separation and cbz-fluoxetine, see Fig. 3. (4) Norephedrine, see Fig. 4 and cbz-norephedrine, see Fig. 4. (5) Isoproterenol, no separation and cbz-isoproterenol, see Fig. 4. (6) 1-(Chlorobenzhydryl)-piperazine, AD-H, 40% 2-propanol)/CO_2 and cbz-1-(chlorobenzhydryl)-piperazine, AD-H, 40% 2-butanol (DEA)/CO_2. (7) Propranolol, see Fig. 5 and cbz-propranolol, see Fig. 5. (8) Pindolol, OD, 40% methanol (DEA)/CO_2 and cbz-pindolol, AD-H, 20% ethanol (DEA)/CO_2. (9) Phenylalaninol, no separation and cbz-phenylalaninol, see Fig. 4. (10) 2-(Diphenylhydroxymethyl)-pyrrolidine, no separation and cbz-2-(diphenylhydroxymethyl)-pyrrolidine, OD, 40% 2-propanol (DEA)/CO_2. (11) Tyrosine, no separation and cbz-tyrosine, AD-H, 20% 2-propanol (DEA)/CO_2. See Section 2 for method details.

tion. For preparative separations, optimal throughput depends primarily on resolution, the loading capacity of the column, the flow-rate and the feed concentration [15]. When developing methods for scale-up, the analytical objective is to resolve the enantiomers with the greatest possible resolution factor, with a minimum of 2.0 [16]. Methods that can enhance chromatographic separation and increase solubility on CSP's bearing high saturation capacities are constantly being sought. Four types of polysaccharide-based CSPs (cellulose and amylose derivatives) Chiralpak AD, Chiralpak AS, Chiralcel OJ, Chiralcel OD, and a Pirkle Whelk-O1 (S,S) column were selected because of their high saturation capacities [17] and their ability to resolve more than 80% of the chiral drugs on the market [18]. In addition to numerous in-house examples, commercial compounds reported here, have been individually screened using 55 HPLC and SFC methods. The aim of our screening strategy was to analyze molecules with diverse structures and compare them to their corresponding cbz derivative using a minimal set of experimental conditions. The highest resolutions, selectivities and retention factors were tabulated in Tables 1 and 2.

Although optimal separation conditions were not developed for any sample, methods associated with these results constitute a good starting point for further optimization. Typically, if a separation is not achieved using our screening strategy, we proceed to look at specific changes that can be made and tailor our optimization according to the individual properties of the sample. Our screening strategy failed to produce adequate results for 5 of the 11 amines tested. Although studies

Table 2
Retention factors, selectivity and resolution of chiral amines and their cbz derivatives

Compound	Amine				cbz derivative of amine			
	k_1	k_2	α	R_s	k_1	k_2	α	R_s
1. *N*-benzyl-α-methylbenzylamine	No separation				2.22	2.65	1.36	5.15
2. Acebutalol	1.42	1.74	1.78	5.61	1.51	1.85	1.66	3.48
3. Fluoxetine	No separation				1.38	1.61	1.61	3.55
4. Norephedrine	1.21	1.48	2.27	7.64	1.41	1.82	1.98	8.06
5. Isoproterenol	No separation				No separation			
6. 1-(Chlorobenzhydryl)-piperazine	2.77	4.94	2.23	5.11	2.29	5.64	3.58	34.53
7. Propranolol	1.29	1.70	2.41	9.41	1.62	3.84	4.56	20.51
8. Pindolol	1.32	2.65	5.23	14.07	1.98	2.77	1.8	8.68
9. Phenylalaninol	No separation				3.90	5.66	1.61	12.06
10. 2-(Diphenylhydroxymethyl)-pyrrolidine	1.48	1.59	1.22	2.10	2.11	3.67	2.40	15.09
11. Tyrosine	No separation				1.49	3.26	4.58	16.33

Best results from HPLC screening study. (1) *N*-Benzyl-α-methyl-benzylamine, no separation and cbz-*N*-benzyl-α-methyl-benzylamine, AD, 5% water in methanol. (2) Acebutalol, OJ, 10% water in acetonitrile and cbz-acebutalol, AD, 5% water in methanol. (3) Fluoxetine, no separation and cbz-fluoxetine, AS, 8:2 hexane:2-propanol. (4) Norephedrine, OD, 5% water in methanol and cbz-norephedrine, AD, 5% water in methanol. (5) Isoproterenol, no separation and cbz-isoproterenol, no separation. (6) 1-(Chlorobenzhydryl)-piperazine, see Fig. 5 and cbz-1-(chlorobenzhydryl)-piperazine, see Fig. 5. (7) Propranolol, see Fig. 5 and cbz-propranolol, see Fig. 5. (8) Pindolol, OD, 1:1 hexane:ethanol and cbz-pindolol, AD, 5% water in methanol (DEA). (9) Phenylalaninol, no separation and cbz-phenylalaninol, OJ, 10% water in methanol (DEA). (10) 2-(Diphenylhydroxymethyl)-pyrrolidine, see Fig. 4 and cbz-2-(diphenylhydroxymethyl)-pyrrolidine, see Fig. 4. (11) Tyrosine, no separation and cbz-tyrosine, OJ, 8:2 hexane:2-propanol. See Section 2 for method details.

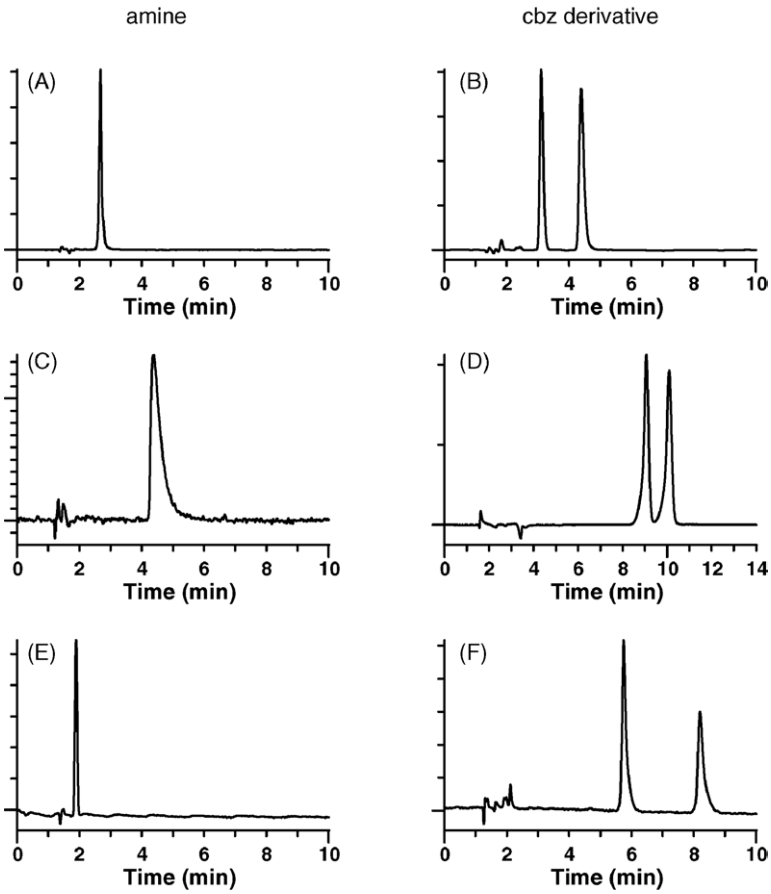

Fig. 3. SFC separations of the cbz derivatives of 2-(diphenylhydroxymethyl)pyrrolidine, fluoxetine and *N*-benzyl-α-methylbenzylamine: (A) 2-(diphenylhydroxymethyl)pyrrolidine, no separation; (B) cbz-2-(diphenylhydroxymethyl)pyrrolidine, OD, 40% 2-propanol (DEA)/CO_2; (C) fluoxetine, no separation; (D) cbz-fluoxetine, AD-H, 25% 2-propanol (DEA)/CO_2; (E) *N*-benzyl-α-methyl-benzylamine, no separation; (F) cbz-*N*-benzyl-α-methyl-benzylamine, Whelk-01, 20% 2-propanol (DEA)/CO_2. See Section 2 for method details.

of these amines appear in the literature, in only two cases do the authors report rugged methods exhibiting baseline separation of the enantiomers. One, using a mobile phase consisting of a 97.4:2.5:0.1 ratio of hexane:2-propanol:TFA [19] and the other using a mobile phase with the simultaneous addition of TFA and DEA additives to the mobile phase [20]. The use of small amounts of alcohol or a combination of DEA and TFA are useful in specific cases but cannot be employed in a screening strategy. In a study by Perrin et al. [21], it was shown that a significant decrease in resolution is observed for both acidic and basic compounds when DEA and TFA are added to the mobile phases simultaneously. This may lead the analyst to the wrong conclusion about the feasibility of a separation.

Our screening results indicate that in all cases but one, superior resolution was observed for the cbz-derivative compared to the amine. Retention factors for the later eluting peak of the derivatized compounds were higher than those obtained for the amines. In all cases, the k values resulting from the screening fall within the optimum k range, roughly $1 < k < 10$. For those amines for which baseline resolution was obtained, acebutalol and 2-(diphenylhydroxymethyl)pyrrolidine were resolved using LC only, while norephedrine, 1-(chlorobenzhydryl)piperazine, propranolol and pindolol gave varied selectivities for LC and SFC. These results are consistent with the view that the presence of polar functions, like primary or secondary hydroxyl or amine functions, can result in marked discrepancies in selectivity between LC and SFC [22] and that the techniques are complimentary. For all other amines, no method was obtained from the automated SFC and HPLC screens, whereas the corresponding cbz derivatives demonstrated selectivities ranging from 1.3 to 4.58 and resolutions of 3.48 to 34.53 (Tables 1 and 2). Examples include the SFC separations of the cbz derivatives of 2-(diphenylhydroxymethyl)-pyrrolidine, fluoxetine and *N*-benzyl-α-methylbenzylamine shown in Fig. 3. The difficulties involved in using both direct and indirect methods for the determination of fluoxetine enantiomers have been reported previously [23], however, the cbz derivative of the compound had not been studied. Without optimization, the cbz derivative of fluoxetine yielded a resolution of 4.34 and selectivity of 1.14 using SFC conditions (25% 2-propanol with 0.01% DEA in CO_2 at 2.3 mL/min on a 25 by 0.46-cm AD-H column) and a resolution of 3.55 and selectivity of 1.61 using HPLC conditions (9:1 acetonitrile:water with 0.01% DEA at 1 mL/min on a 25 by 0.46-cm Whelk-01 column).

In cases where a poor separation was achieved for the amine, the cbz-derivatized compound showed great improvement in selectivity and resolution. Examples are shown in Fig. 4. In cases where a good separation was achieved for the amines, 1-(chlorobenzhydryl)-piperazine and propranolol,

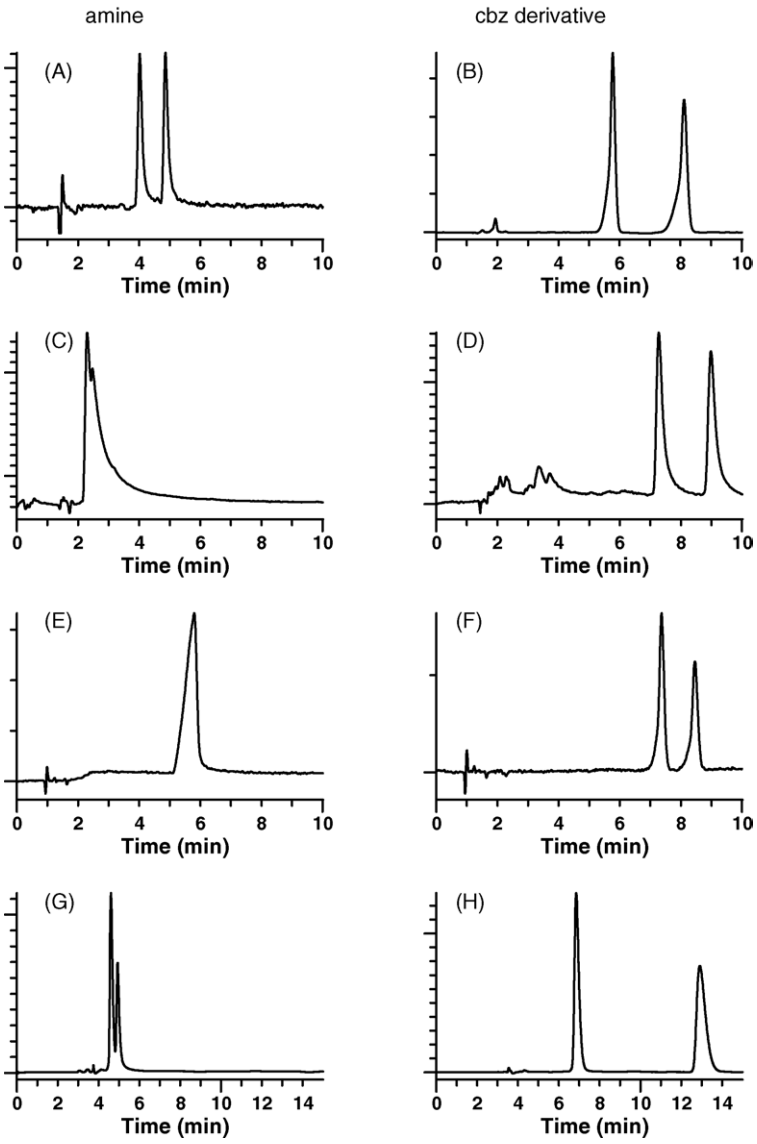

Fig. 4. SFC and HPLC separations of the cbz derivatives of norephedrine, isoproterenol, phenylalaninol and 2-(diphenylhydroxymethyl)pyrrolidine: (A) norephedrine, AD-H, 20% 2-propanol/CO_2; (B) cbz-norephedrine, AD-H, 20% methanol (DEA)/CO_2; (C) isoproterenol, AD-H, 20% methanol (DEA)/CO_2; (D) cbz-isoproterenol, AD-H, 20% methanol (DEA)/CO_2; (E) phenylalaninol, no separation; (F) cbz-phenylalaninol, AD-H, 20% 2-propanol (DEA)/CO_2; (G) 2-(diphenylhydroxymethyl)pyrrolidine, OJ, 1:1 hexane:ethanol (DEA); (H) cbz-2-(diphenylhydroxymethyl)pyrrolidine, OD, 8:2 hexane:2-propanol (DEA). See Section 2 for method details.

enhanced results were obtained for the corresponding cbz derivative with remarkable selectivities ranging from 3.58 to 4.56 and resolutions ranging from 20.51 to 34.53 (Fig. 5). Every compound studied gave favourable results for the derivatized amine. Although the methods that resulted in the highest resolutions and selectivities may be impractical for analytical purposes, the potential for optimization of such methods are obvious. Pindolol was the single case which gave slightly better results for the amine compared to the cbz derivative using both the SFC and HPLC screens; both compounds, however, displayed highly favourable results. Norephedrine gave comparable results for the amine and its derivative using the HPLC screen and enhanced resolution for the cbz derivative using the SFC screen. Based on these results, the cbz derivative of a desired amine appears to be a worthwhile pursuit when considering preparative separations.

Each cbz derivative analyzed resulted in highly favourable resolutions and selectivities on Chiralpak AD or Chiralpak AD-H columns, although baseline separation was not exclusive to these columns (Table 3). Chiralpak AS and Whelk-01, unable to resolve the amines even in the presence of diethylamine, were able to baseline resolve 5 of the 10 cbz-derivatized compounds. The larger selection of columns resulting in baseline separations of the cbz derivatives provides the chemist with many possibilities when considering preparative scale separations. Both SFC and HPLC should be explored when undertaking method development of a new sample. As in the case of isoproterenol, HPLC failed to produce a method using our screen whereas the SFC screen resulted in a method yielding a resolution of 6.98.

For preparative separations, the solubility of the solute is often the limiting factor in terms of throughput. Ideally the

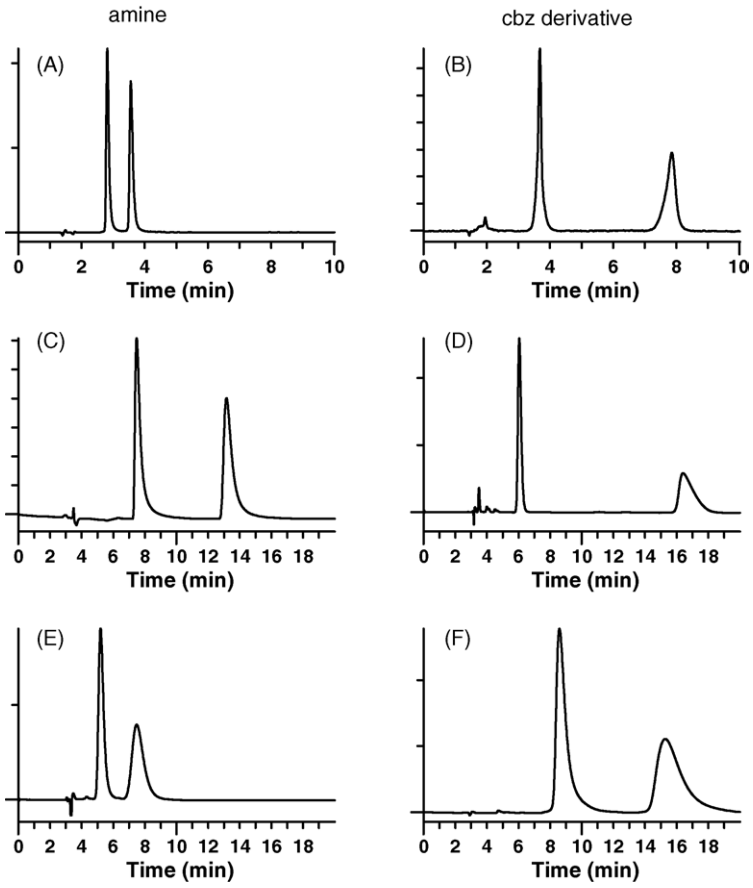

Fig. 5. Enhanced separation of the cbz derivatives of propranolol and 1-(chlorobenzhydryl)piperazine by HPLC and SFC: (A) propranol, OD, 8:2 hexane:2-propanol; (B) cbz-propranolol, AD, ethanol; (C) propranolonl, AD-H, 30% ethanol (DEA)/CO_2; (D) cbz-propranolol, AD-H, 40% isopropranolol (DEA)/CO_2; (E) 1-(chlorobenzhydryl)-piperazine, OJ, 1:1 hexane:ethanol (DEA); (F) cbz-1-(chlorobenzhydryl)-piperazine, AD, 8:2 hexane:ethanol (DEA). See Section 2 for method details.

best selectivity under high solubility conditions should be applied. The cbz-derivatized compounds were easily dissolved in ethanol. Experimental measurements of the actual solubility of all compounds were not carried out and hence we could not provide better evidence for our observations. The solubility of the cbz derivative of *N*-benzyl-α-methylbenzylamine was 670 mg/mL of ethanol. Fig. 6 demonstrates the baseline separation of 3 g of material in less than 5 min on a 25 cm × 5 cm Whelk-O1 (R,R) column. The separation of 20.2 g of material was accomplished in 21 min using injection intervals of 160 s (Fig. 7). Sample recovery was 98.1% and the ee for the first and second enantiomer was 99.9% and 99.4%, respectively. Under these conditions, the productivity for the isolation of the first

Table 3
Columns affording baseline separation by HPLC and/or SFC using a variety of mobile phases

Compound	Amine	cbz derivative of amine
N-Benzyl-α-methylbenzylamine	–	AD, OJ, Whelk-01
Acebutalol	OJ	AD, OD, Whelk-01, AS
Fluoxetine	–	AD, AS
Norephedrine	AD	AD, OD
Isoproterenol	–	AD
1-(Chlorobenzhydryl)-piperazine	AD, OJ	AD, OD, OJ, Whelk-01, AS
Propranolol	AD, OD	AD, OD, OJ, Whelk-01
Pindolol	AD, OD	AD, OD
Phenylalaninol	–	AD, AS
2-(Diphenylhydroxymethyl)-pyrrolidine	OJ	AD, AS
Tyrosine	–	AD, OJ

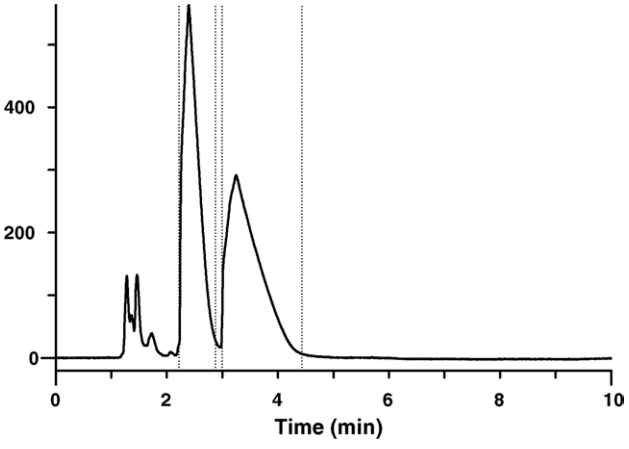

Fig. 6. Preparative separation of 3 g of cbz-*N*-benzyl-α-methylbenzylamine using SFC. Whelk-O1 (R,R) (5 cm × 25 cm) column, 43% 2-propanol in CO_2 (100 bar), 350 mL/min, 4.5 mL injection (670 mg/mL). Sample dissolved in ethanol.

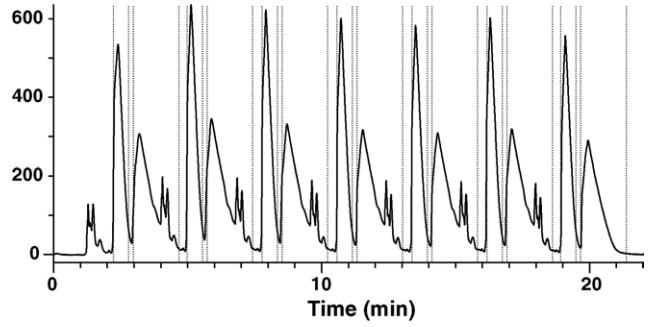

Fig. 7. Preparative separation of 20.2 g of material in 21 min. Whelk-O1 (R,R) (5 cm × 25 cm) column, 43% 2-propanol in CO_2 (100 bar), 350 mL/min, 4.35 mL average injection (670 mg/mL). Sample dissolved in ethanol.

enantiomer is 5.4 kg enantiomer/kg CSP/24 h. Solvent consumption is 156 L/kg of racemate and carbon dioxide consumption is 207 L/kg of racemate.

With the development of scalable technologies, such as SMB and SFC, chromatography is now regarded as technically and economically attractive for the preparation of enantiomerically pure compounds of interest. In contrast to the enantioselective synthetic approach, chromatography offers the advantage of reducing the number of reaction steps for preparing the racemate compared to those needed for the asymmetric synthetic route. Although derivatization adds two steps to the process, the benefits from increased selectivity, solubility and the ease of protecting and deprotecting the racemate should be considered. In some cases, cbz protection of the amine may already be a necessary step in the synthesis of the target molecule. In those cases, one should consider the opportunity to separate the enantiomers of the protected amine before proceeding to the next step in the synthetic route.

4. Conclusion

The enhanced resolution, selectivity and solubility of the cbz derivatives combined with the ease of their preparation and deprotection provide chromatographers with a general and effective alternative for the separation of chiral amine intermediates or final products. Method development should be carried out using both HPLC and SFC in complimentary fashion.

Acknowledgements

We would like to thank Jennifer Van Anda and Kimber Fogelman of Mettler-Toledo for their guidance in our investigation of the extended flow range of the Berger Multigram III SFC system. Also, we would like to thank Michael Kraml for his useful suggestions.

References

[1] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.
[2] M. Juza, M. Mazzotti, M. Morbidelli, Trends Biotechnol. 18 (3) (2000) 108.
[3] M. Schulte, in: G. Subramanian (Ed.), Chiral Separation Techniques: A Pratical Approach, Wiley-VCH, Weinheim (Federal Republic of Germany), 2001, pp. 199–201.
[4] V.A. Davankov, J. Chromatogr. A 1000 (2003) 891.
[5] N. Ôi, H. Kitahara, F. Aoki, J. Chromatogr. A 694 (1995) 129.
[6] M.H. Hyun, S.C. Han, B.H. Lipshutz, Y.-J. Shin, C.J. Welch, J. Chromatogr. A 959 (2002) 75.
[7] R.J. Steffeck, Y. Zelechonok, K.H. Gahm, J. Chromatogr. A 947 (2002) 301.
[8] Y.K. Ye, K.G. Lynam, R.W. Stringham, J. Chromatogr. A 1041 (2004) 211.
[9] R.W. Stringham, J. Chromatogr. A 1070 (2005) 163.
[10] E.R. Francotte, Chirality 10 (1998) 492.
[11] Y. Leblanc, C. Dufresne, R. Carson, L. Morency, C.J. Welch, Tetrahedron: Asymmetr. 12 (2001) 3063.
[12] M. Schulte, in: G. Subramanian (Ed.), Chiral Separation Techniques: A Practical Approach, Wiley-VCH, Weinheim, 2001, pp. 188–198.
[13] T.W. Greene, P.G.M. Wuts (Eds.), Protective Groups in Organic Synthesis, third ed., Wiley-Interscience, New York, 1999, pp. 531–537.
[14] P.J. Kocienski, Protecting Groups, Thieme Verlag, Stuttgart, 1994.
[15] E.R. Francotte, J. Chromatogr. A 906 (2001) 379.
[16] S. Ahuja, Chiral Separations by Chromatography, Oxford University Press, New York, 2000, p. 157.
[17] E. Francotte, J. Chromatogr. 576 (1992) 1.
[18] Y. Okamoto, Y. Kaida, J. Chromatogr. A 666 (1994) 403.
[19] K. Anton, J. Eppinger, L. Frederiksen, E. Francotte, T.A. Berger, W.H. Wilson, J. Chromatogr. A 666 (1994) 395.
[20] Y.K. Ye, R. Stringham, J. Chromatogr. A 927 (2001) 53.
[21] C. Perrin, V.A. Vu, N. Matthijs, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 947 (2002) 69.
[22] N. Bargmann-Leyder, A. Tambuté, M. Caude, Chirality 7 (1995) 311.
[23] B.A. Olsen, D.D. Wirth, J.S. Larew, J. Pharm. Biomed. Anal. 17 (1998) 623.