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Impact of normal-phase gradient elution in chiral chromatography: a novel, robust, efficient and rapid chiral screening procedure

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Abstract

Novel normal-phase gradient systems have been employed for fast high-throughput chiral analyses of Discovery compounds in our research laboratories in Eli Lilly and Company. In this report, we describe an automated screening approach based on gradient elution, in order to achieve accurate enantiomeric excess determinations, and chiral separations when needed, in the shortest possible timeframe. Baseline resolution of enantiomers has been obtained for over 85% of the samples so tested. For the remaining cases, complete enantioseparation by isocratic optimisation is generally achieved in a single shot. This technique has been proven to be robust and is now standard operating procedure at our analytical research laboratories.

Keywords: Screening; Enantiomeric excess; Enantiomer separation; Gradient elution

1. Introduction

Chiral separation of bulk drug substances is an important subject of interest in the pharmaceutical discovery process, especially in light of increased regulatory requirements and often enhanced activity for molecules with differing stereochemical orientations. The routine screening of both enantiomers as well as the racemate is necessary in the early phase cycle of drug development [1–3]. This has led to a significant increase in number of samples submitted for chiral analytical separations over recent years. Traditionally, methods were developed for racemic compounds by screening several isocratic methods on several single columns. This trial and error process proved to be time consuming as even small changes in structure required further method development. Due to the increasing number of compounds that requires chiral studies and in an attempt to shorten complete analysis cycle time, new screening approaches based on isocratic elution mode are continuously emerging [4]. Unfortunately, racemic mixtures in early drug discovery frequently contain other related substances from synthetic routes under development, and carry over of related substance frequently occurs when columns are not cleaned prior to the next sample injection. Chiral separation of enantiomers and separation of these enantiomers

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from these related substances require cleaning the column between injections to prevent contamination. To address this point by means of isocratic elution, the mobile phase composition needs to be carefully defined for each single compound, as either too long or too little retention may be observed dependent on the structural type and polarity. Therefore, a high level priority for our analytical groups has been to focus on first, automating chiral methodology to increase productivity in Drug Discovery and secondly, to possibly utilise a robust gradient screening approach as is normally adopted for reversed-phase LC–MS purity assessments [5,6]. Several publications have emerged recently showing that normal-phase gradient elution is a reproducible process with short re-equilibration times, mainly for solvents of low and moderate polarity such as hexane-DCM [7,8] and 2-propanol in *n*-heptane [9] gradients. Jandera et al. have, theoretically and experimentally, highlighted the robust application of this elution mode in normal-phase [10,11].

Automated normal-phase preparative high-performance liquid chromatography (HPLC) under gradient elution has been described as a substitute for flash chromatography in research laboratories [12]. A literature study has shown that packed column supercritical fluid chromatography gradients have been successfully applied to chiral stationary phases (CSPs) using methanol gradients containing either basic or acidic modifiers [13]. This suggested that a gradient approach for traditional HPLC chiral analysis could be a viable opportunity and an investigation was initiated. Gradient elution was also considered as a means of cleaning related substances from column prior to subsequent injection of a new sample**.** As Daicel® columns are worldwide recognized for being effective for separation of most racemic compounds, the four standard Chiralpak AD, Chiralpak AS, Chiralcel OD and Chiralcel OJ columns were selected for our studies. An initial gradient was investigated in our laboratories, using mixtures of hexane and an alcohol (either IPA or EtOH) in a linear 5 to 60% alcohol gradient. Based on the specifications defined by the manufacturers, the flow-rate was set at 0.5 ml min^{-1}. Each gradient run was 30 min long followed by 10 min re-equilibration step (Craig White, unpublished results). This methodology was successfully applied to the chiral analysis of Discovery compounds over 6 months, providing sufficient evidence that gradients could be used routinely in this mode without compromising the column lifetime. However, our analytical laboratories are confronted with the continuous increase in the demand for higher sample throughput, thus shorter analysis time. To address this issue, different chromatographic parameters defined in this first approach were optimised. The final gradient screen so developed is highlighted in the results and discussion part of this paper.

2. Experimental procedure

2.1. Chemicals

Model racemic compounds were selected on the basis of their differing structural properties. Suitable polarity for resolution under normal-phase conditions was taken into account. Those products included commercial drug compounds as well as investigational drug compounds internal of Ely Lilly Pharmaceutical Research and Development. α-Bromophenyl acetic acid (common synthetic starting material), (±) *e*-10-Hydroxylated nortryptiline (bioactive metabolite of the tryciclic antidepressant nortryptiline), Ibuprofen (non-steroidal anti-inflammatory drug), Propranolol (β blocker), Verapamil (calcium channel blocker) and Warfarin (anticoagulant) were the commercially available drugs included in this study. *Trans*-stilbene oxide was selected as standard for column performance studies. All of them were supplied by Sigma–Aldrich (Steinheim, Germany). The names and structures of Lilly compounds used to illustrate chromatographic separations cannot be disclosed due to proprietary reasons.

HPLC grade hexane and ethanol were purchased from Merck (Darmstandt, Germany), while isopropanol was supplied by LabScan (Dublin, Ireland). Spectrophotometric grade trifluoroacetic acid (TFA) and dimethylethylamine (DMEA) were also purchase from Sigma–Aldrich.

Sample solution of each racemate was prepared by dissolving 1 mg in 1 ml of a 1:1 v/v of hexane–ethanol mixture.

2.2. Instrumentation

All the analytical studies were performed on a HP series 1100 system (Agilent, Waldbronn, Germany) driven by ChemStation 8.03® software. This system was composed of a quaternary pump, an autosampler, a mobile phase degasser and a diode array detector. Connections from the autosampler to column inlet and from column outlet to detector were made with 0.005″ I.D. Polyether ether ketone (PEEK) connections were made as short as possible in order to minimise dead volume. The Cheminert® HPLC 8 column selector (VALCO) was controlled via remote logic level signal.

Chromatography was performed on the following polysaccharide CSPs: CHIRALPAK® AD [amylose tris (3,5-dimethyl-phenyl carbamate)], CHIRALPAK® AS [amylose tris ((*S*)-1-phenylethyl carbamate)], CHIRALCEL® OD [cellulose tris (3,5-dimethylphenyl carbamate)] and CHIRALCEL®OJ [cellulose tris (4-methyl benzoate)] columns. The columns dimensions were 250×4.6 mm with the enantioselective phase coated onto a 10 μm silica-gel substrate.

2.3. Chromatographic conditions

Experiments were carried out at room temperature. The wavelength of UV detection was monitored from 210 to 380 nm. Chromatograms were recorded at 215 and 254 nm signals. In order to confirm results, a minimum of two replicate injections was carried out for each experiment during the optimisation studies. Chromatographic conditions were as follows:

2.3.1. Backpressure studies

Analysis of this parameter was performed taking the backpressure limit recommended by manufactures, 50 bar, as standard reference. Runs were identical in respect to the initial and final mobile phase composition. The gradient started immediately upon injection. Mobile phase consisted on mixtures of hexane (A) and an alcohol (B; either IPA or EtOH). Elution was from 5 to 100% alcohol in a linear gradient. A 5 min reverse gradient followed by a 5 min isocratic equilibration step was used after the end of each experiment. With the change to a new mobile phase and/or column, there was a 30 min equilibration step under the new conditions to ensure that traces of the previous mobile phase had no influence on the new chromatographic experiment. For Chiralpak AD there is a restriction on the use of hexane–ethanol mixtures from 85/15 to 40/60 due to UV absorption and stability issues (polymer leakage), as is described by manufactures in the corresponding instruction sheet. This column was not included in the hexane–ethanol gradient studies.

2.3.2. Final standard gradient conditions

Mobile phase consisted on mixtures of hexane–0.05% TFA (A) and an alcohol (B; either IPA or EtOH). A range gradient from 20 to 70% alcohol over 20 min was investigated. This latter solvent composition was held for 2 min and the return to the initial conditions was performed within 5 min. After 5 min of isocratic equilibration, the next sample was injected. The flow-rate was held constant at 0.75 ml min^{-1} on all column types and gradients. After loading a new method, the selected new column was equilibrated for 10 min before injection. As aforementioned, Chiralpak AD was not included in the standard hexane–ethanol gradient studies. At the end of a complete chiral screen, all columns were flushed with the recommended 90:10 hexane–isopropanol storage solvent for 15 min to minimize interaction of the stationary phase with components in the mobile phase, thus preserving the column lifetime.

2.3.3. Column performance

Column performance was monitored monthly by injecting *trans*-stilbene oxide. Chromatographic parameters such as retention times, peak width, symmetry of peaks and selectivity were compared to those obtained when the column was used by the first time.

3. Results and discussion

The polysaccharide CSPs have been used extensively for chiral separations [14,15]. We chose the Chiralpak AD, Chiralpak AS, Chiralcel OD and Chiralcel OJ units as standard columns for screening samples. The selection of the mobile phase has a direct impact on chiral chromatography, as the polar

component affects the retention of the solutes in different ways. First by influencing the solvation in the mobile phase and secondly, by competing with the solute molecules for the specific adsorption sites on the stationary phase. Effects on resolution, column efficiency, separation factor (α) and elution order, among others, have been observed by changing from one alcohol to another one [16]. Mixtures of hexane and isopropanol or ethanol, proved to be the most suitable eluent for efficient separation of a wide variety of racemates on polysaccharide-based CSPs [17], were the selected mobile phases for our studies.

On the basis of these previous observations, two sets of columns were defined. Each one consisted of four columns (one of each CSP selected). A Hexane/IPA gradient (System I) was applied to the first set, while Hexane/EtOH (System II) gradient elution was performed in the second group. Chiralpak AD column was discarded from the studies under wide ethanol gradient conditions. However, a high polar gradient composition was defined for this unit, as discussed in Section 3.5.

In order to increase our capacity, full automation of the system was performed. An 8-port column-switching valve from Valco was permanently installed using the selected chiral columns.

3.1. Backpressure studies

In our opinion, one of the main arguments against the use of gradients in normal-phase chiral chromatography is the lack of in-depth studies on the parameters affecting the stability of the stationary chiral phases. Thereby, and prior to modifying any

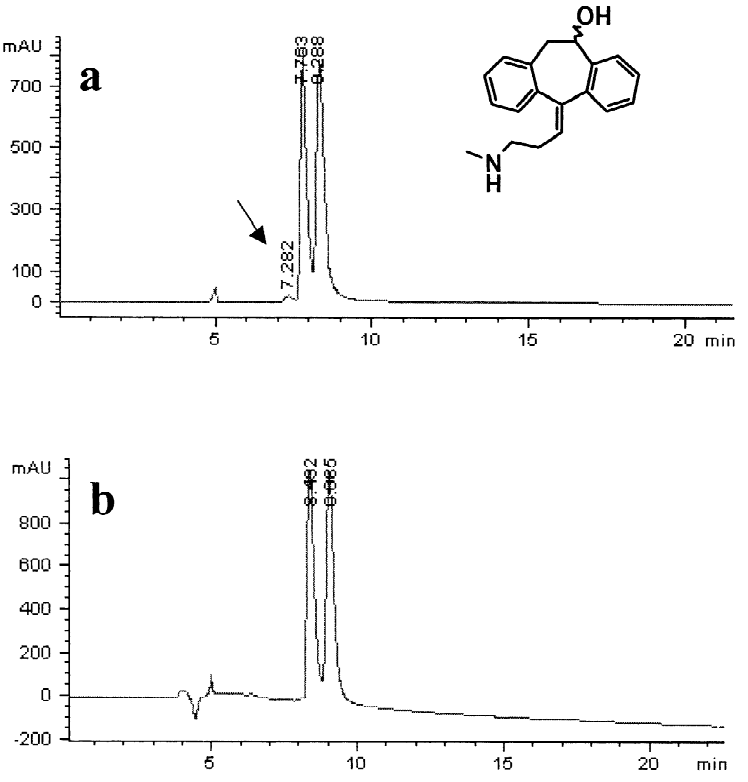

Fig. 1. Comparison of gradient enantioseparation under acidic (a) and basic (b) elution conditions. For the former, resolution of impurities is better achieved. For the latter, baseline drifting is clearly observed. Chromatographic conditions: column, Chiralpak AD; mobile phase, (a) Hexane–0.05%TFA (A)/IPA (B), (b) Hexane–0.2%DMEA (A)/IPA (B); gradient from 20 to 70%B in 20 min; flow-rate, 0.75 ml/min.; detection, 254 nm.

chromatographic parameter, we initiated an extensive analysis of the backpressure values by gradient elution under extreme conditions. The factors that seemed to show the greatest influence on this parameter included content of the organic modifier (alcohol) in the mobile phase, gradient step (run time) and flow-rate.

In order to evaluate the stability of columns, backpressure was measured over a wide range of extreme conditions:

1. The amount of alcohol present in the mobile phase was varied from the lowest 5% value to a maximum of 100%. It was obvious that this extremely high percentage of the polar modifier was not necessary for a complete elution of enantiomers. However, it was the best way to expose the columns to extreme conditions, thus maximising backpressure data.
2. Decreasing gradient time by increasing gradient step and/or by increasing flow-rate were the two common choices to reduce total cycle run time. Initially we set the analytical run time to 30 min and secondly to 20 min. In combination we also tested the flow-rate, which was increased stepwise from 0.5 ml min^{-1} to 0.75, 1.00 and 1.25 ml min^{-1}.

Analysis of all the results from the above experiments allowed a full comparison of backpressure values at different gradient time, mobile phase composition and flow-rate (M.L. de la Puente, unpublished results). Based on these observations, we were in the position to optimise the main chromatographic parameters to achieve accurate enantiomeric resolution of sample mixtures in a minimum time and without affecting either column performance or lifetime by overpressure effects.

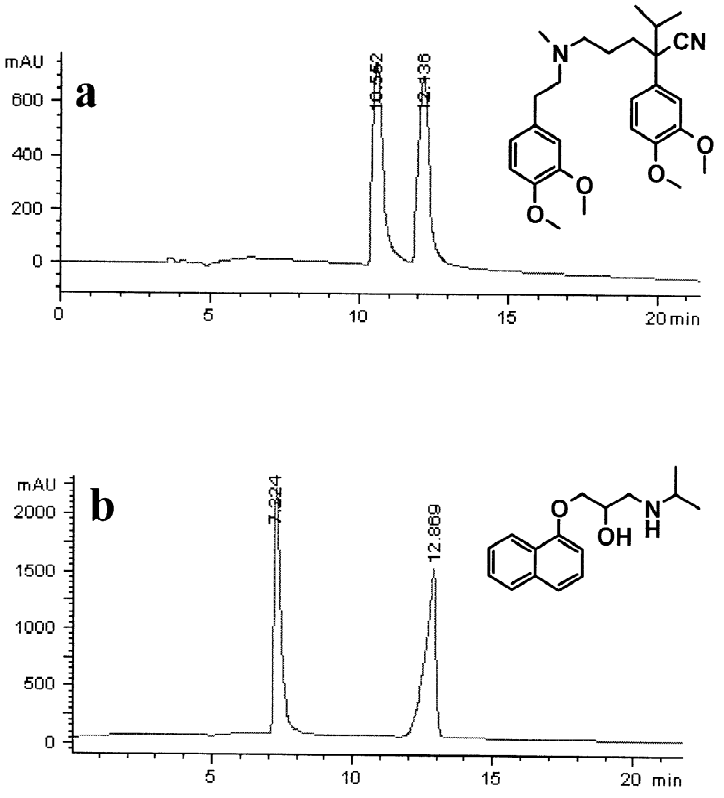

Fig. 2. Enantiomeric resolution of Verapamil (a) and Propranolol (b) on a Chiralcel OD column. Chromatographic conditions: mobile phase, Hexane–0.05%TFA (A)/EtOH (B); gradient elution, from 20 to 70% B in 20 min; flow-rate, 0.75 ml/min; detection, 215 nm.

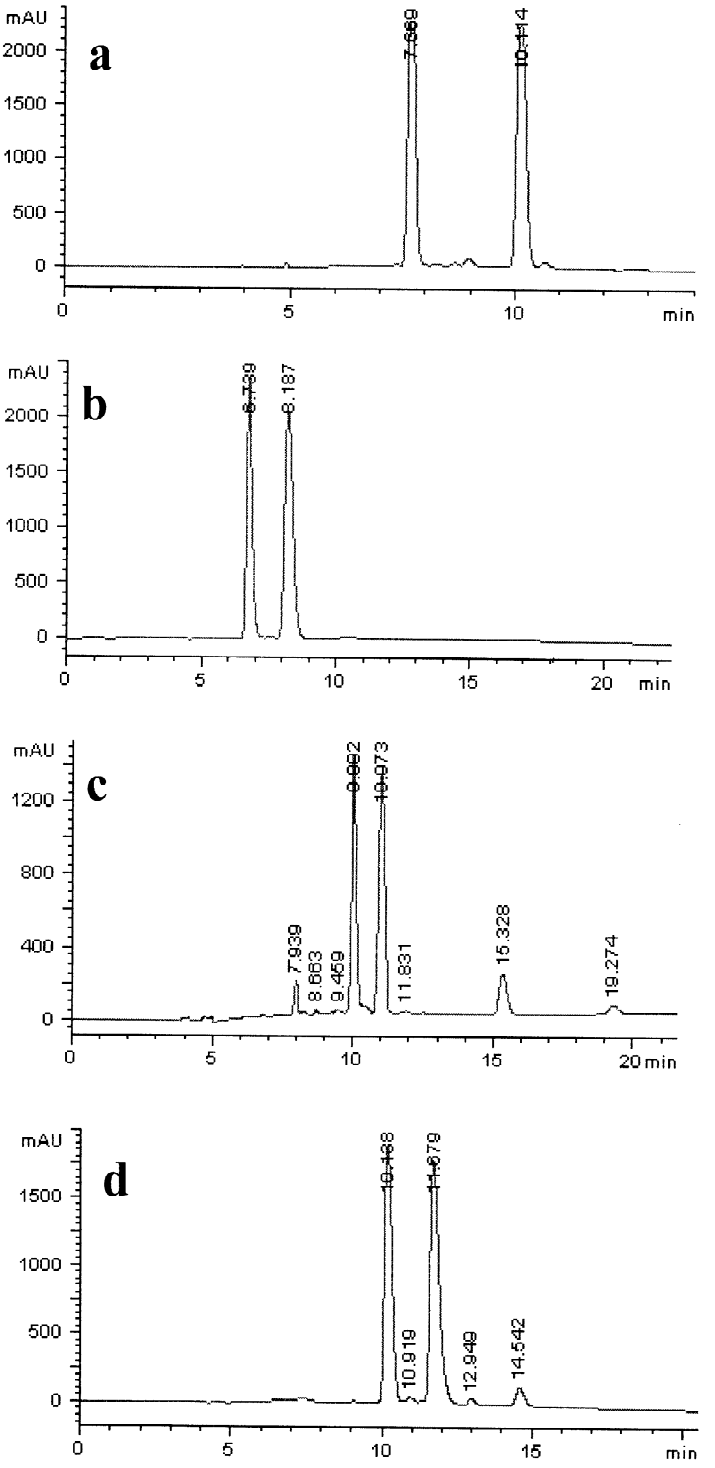

Fig. 3. Enantiomeric resolution of several pharmaceutical intermediates prepared in our research laboratories. Baseline resolution of peaks is achieved on either simple or complex mixtures. Chromatographic conditions: column and mobile phase, (a/c) Chiralpak AD and Hexane–0.05%TFA (A)/IPA (B), (b) Chiralpak AS and Hexane–0.05%TFA (A)/EtOH (B), (d) Chiralcel OJ and Hexane–0.05%TFA (A)/EtOH (B); gradient from 20 to 70% B in 20 min; flow-rate, 0.75 ml/min; detection, 215 nm.

3.2. Gradient range: mobile phase composition

The first step in the optimisation of our analytical gradient elution was focussed on the determination of the best initial and final mobile phase composition. In order to avoid cross-contamination between sequential injections, complete elution of compounds within the gradient time is an important issue for any automated screening. Although a clean up step at the end of a run is usually applied in reversed-phase chromatography without affecting analysis cycle time, chiral analysis shows a very different behaviour. The standard procedure for washing polysaccharide based chiral columns consists on flushing pure ethanol at 0.5 ml min^{-1} followed by slow re-equilibration of the column to the initial conditions, which has proved to be a long process. It was necessary to define a standard initial (X) and final (Y) alcohol composition that ensured complete resolution of peaks within the gradient time while avoiding any further clean-up step. Moreover, those conditions, including specific flow-rate, had to always maintain the column pressure below the recommended limits (50 bars). The final alcohol concentration was mainly determined by backpressure measured under IPA elution. This alcohol generates a higher backpressure value with respect to that afforded by ethanol, which is derived from its higher viscosity. It was considered that a standard final 70% alcohol composition (Y) could be easily reached, under elution with either IPA or EtOH, without observing overpressure provided that we controlled the flow-rate and gradient slope. It was assumed that such large ratio of alcohol should achieve elution of the undesirable more retained products, thus eliminating the need of any column-washing step (as it has been further supported by data from 18 months of daily work). The starting eluent composition needed to be defined next. There are many references in the literature dealing with the

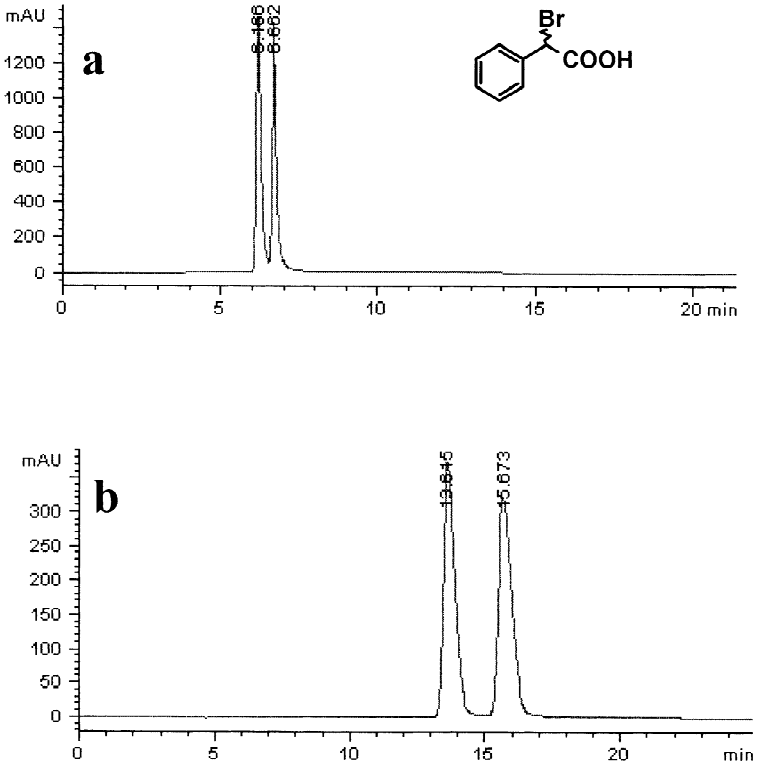

Fig. 4. Representative chromatograms for the resolution of α-Bromo-phenylacetic acid using gradient elution. Chromatographic conditions: column, (a) Chiralpak AD, (b) Chiralcel OJ; mobile phase, Hexane–0.05%TFA (A)/IPA (B); gradient from 20 to 70% B in 20 min; flow-rate, 0.75 ml/min; detection, 254 nm.

importance of selecting an appropriate concentration of the stronger mobile phase component at the start of a gradient, due to its influence on the reproducibility and resolution of the analysis [18,19]. This initial concentration (X) should yield maximum resolution and selectivity in the shortest time. With the final concentration of alcohol (Y) already pre-set in our analysis, different experimental parameters were taken into account to define the initial mobile phase composition. The following were observed:

- When the gradient range is decreased, the resolution of peaks increases.
- When X is increased, the retention volumes decrease (thus shortening the time for analysis)
- When X is increased, higher reproducibility is achieved, as 'solvent demixing effect' is minimised [18,19].
- Increasing X reduced the re-equilibration step dramatically.

Hence, we investigated the largest value of X to obtain an optimum gradient steepness, but also sufficiently low to avoid poor retention without any sign of chiral recognition. These investigations lead us to define a 20% alcohol initial composition (X) as the best first choice.

3.3. Gradient step: run time and flow-rate

Minimum run time necessary to achieve the desired resolution should be defined. With consideration of the mobile phase composition already defined, as well as the length and the internal diameter of the selected columns, the gradient time was preset at 20 min. In order to maintain the backpressure value below the recommended limit when the largest concentration of alcohol was reached at the end of the gradient, the flow-rate was adjusted to 0.75 ml min^{-1}.

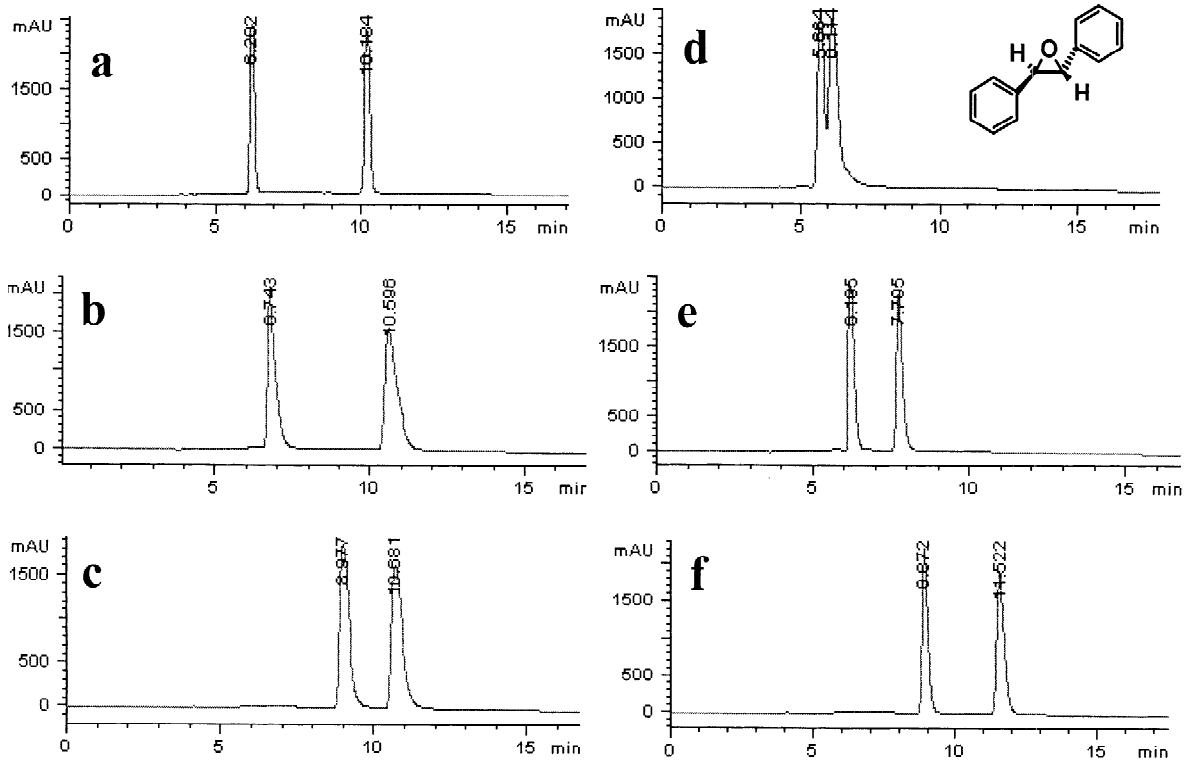

Fig. 5. Chiral gradient screen for *trans*-stilbene oxide. Chiralpak AS afforded partial resolution of peaks by elution under both mobile phase systems (only gradient with IPA is included in the figure). Chromatographic conditions: column and mobile phase, (a) Chiralpak AD and Hexane–0.05%TFA (A)/IPA (B), (b) Chiralcel OD and Hexane–0.05%TFA (A)/IPA (B), (c) Chiralcel OJ and Hexane–0.05%TFA (A)/IPA (B), (d) Chiralpak AS and Hexane–0.05%TFA (A)/IPA (B), (e) Chiralcel OD and Hexane–0.05%TFA (A)/EtOH (B), (f) Chiralcel OJ and Hexane–0.05%TFA (A)/EtOH (B); gradient from 20 to 70%B in 20 min; flow-rate, 0.75 ml/min; detection, 215 nm.

3.4. Mobile phase additives

The behaviour of the separation system depends not only on the mobile and stationary phase but also on the nature of the analytes. In order to fully optimise our screen we also explored the influence of mobile phase modifiers on retention and enantioselectivity. Those modifiers play important roles in any enantioseparation process. First, they can modify the structure of the chiral stationary phases by interaction with their carbamate moieties through hydrogen bonding. Secondly, they can compete with the analytes for the CSPs adsorption sites favouring their desorption, which results in a shorter analysis time. And finally, they may also compete with the analytes by interaction with the free silanols groups of the silica support, thus minimising the tailing of peaks. When an analyte contains a basic amino group, addition of a small amount of amine results in better separation without tailing of peaks [20–22]. On the other hand, many acidic compounds can be easily resolved by addition of a small amount of a strong acid [23–25]. After an extensive review on those modifiers compatible with our stationary chiral phases, TFA and DMEA were pre-selected for our chiral screening. Those components were added to hexane in standard concentrations of 0.05 and 0.2% v/v, respectively. It has been reported that the addition of amine additives (such as DMEA, DEA or TEA) to normal-phase mobile phases lead to a severe drifting of the baseline, compared to neutral or acidic chromatographic conditions [26,27]. This effect is derived from the presence of impurities that tend to reduce the usable UV range. As result, lower sensitivity at low wavelengths is observed. Due to this phenomenon, basic compounds were simultaneously analysed under both acidic and basic conditions. In most cases better baseline was achieved by using

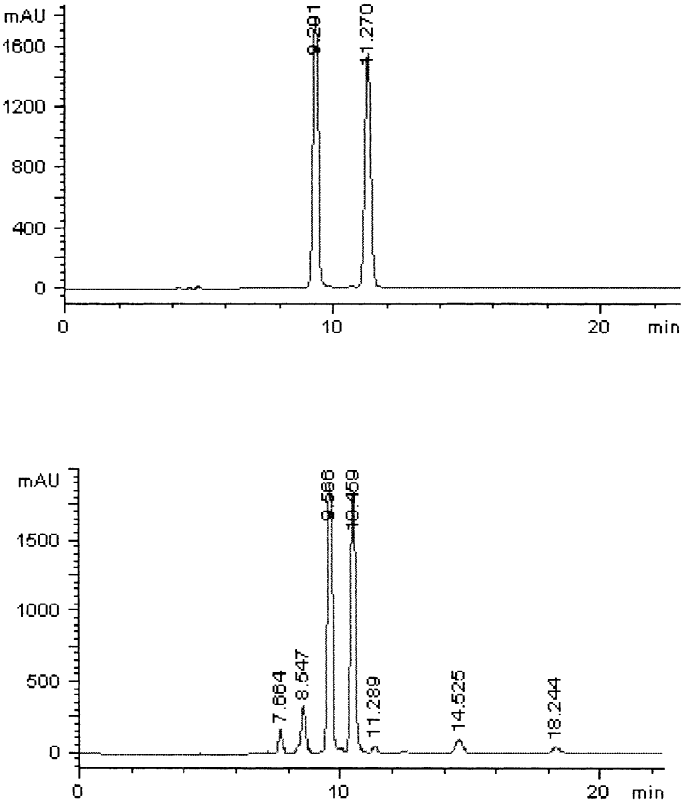

Fig. 6. Enantiomeric resolution of two pharmaceutical intermediates, prepared in our research laboratories, under neutral conditions. Chromatographic conditions: column Chiralpak AD; mobile phase, Hexane (A)/IPA (B); gradient elution, from 20 to 70%B in 20 min; flow-rate, 0.75 ml/min; detection, 215 nm. Baseline resolution of peaks is achieved on either simple or complex mixtures.

TFA as modifier. Fig. 1 shows a comparison of the two chromatograms obtained by analysis of (±) *e*-10-hydroxylated nortriptilyne under both gradient conditions.

With the new gradient elution conditions already pre-set, experiments with commercially available drugs were performed. Fig. 2 shows the results achieved by acidic chiral screening on the Calcium antagonist Verapamil and the β-blocker Propanolol. For the latter, accurate ee determination is performed with no dependence on its basic character. Baseline resolution of enantiomers, under acidic conditions, has been also observed on other components of the same β-blocker series such as Metoprolol, Pindolol and Oxprenolol.

As accurate resolution of peaks is one of the main targets of our gradient methodology, TFA was finally defined as standard modifier for routine analysis.

Over the last 18 months, hundreds of investigational drug compounds, selected from our internal Pharmaceutical Research and Development sources on the basis of their differing structural properties as well as their appropriate polarity for resolution under normal-phase conditions, have been successfully analysed under our gradient methodology. Four representative examples are shown in Fig. 3. Their names and structures are not disclosed for proprietary reasons.

Further on, resolution of the same racemate under different CSPs and gradients can be frequently observed, as it was observed for the α-Bromo-phenyl acetic acid and the standard *trans*-stilbene oxide (Figs. 4 and 5).

When acidic conditions may impact on compound stability, neutral or basic elution is applied Fig. 6 illustrates results achieved by analysis of two internal compounds. As above cited, their names and structures cannot be included due to proprietary reasons.

3.5. Gradient under high polar mode

Successful enantioseparations using polysaccharide CSPs in combination with pure polar organic mobile phases have been reported lately [28,29].

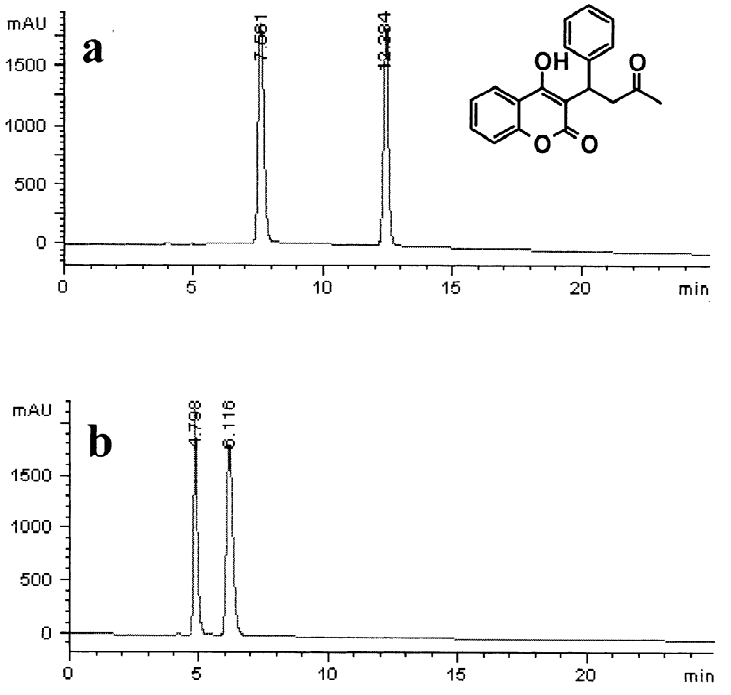

Fig. 7. Chromatograms for the enantioseparation of Warfarin by gradient elution under low (a) and high (b) polar modes on Chiralpak AD column. Chromatographic conditions: mobile phase, (a) Hexane–0.05%TFA (A)/IPA (B), gradient from 20 to 70% B in 20 min, (b) Hexane–0.05%TFA (A)/EtOH (B), gradient from 70 to 100% B in 20 min; flow-rate 0.75 ml/min; detection, 215 nm.

Pure polar organic mode offers an advantage of higher solubility of analytes as well as alternative chiral recognition mechanisms. Among all of the chiral columns, Chiralpak AD exhibited higher resolution compared to the cellulose derivatives Chiralcel OD and Chiralcel OJ. This fact makes it a choice for analytical studies. Hence, the second unit of the Chiralpak AD column, which had been set aside for our standard 20–70% gradient elution, was used to explore this elution mode. We designed a new gradient composition between 70 and 100% ethanol while keeping the rest of chromatographic parameters as above indicated. Due to the low viscosity of Ethanol, pure alcohol can be flushed through the column without observing overpressure effects. Our initial backpressure studies had already confirmed this observation.

Satisfactory and reproducible results have been achieved with this elution mode. Depending on the nature of the analyte, a low retention factor may be observed. In fact, by applying our methodology we have observed that resolution on Chiralpak AD columns under high polar mode, using ethanol, is usually accompanied by resolution in the standard low alcohol mode. Thus, accurate ee determination is always achieved although poor retention under high polar mode is seen. Fig. 7 shows results yielded by chiral screening of Warfarin under both elution modes.

We fully support the development of this elution mode in our standard screening as it has proven to be especially useful in preparative scale purifications.

3.6. Isocratic optimisation

One of the key points in the analysis of drugs by chiral chromatography is the accuracy in the qualitative/quantitative determination of the enantiomeric

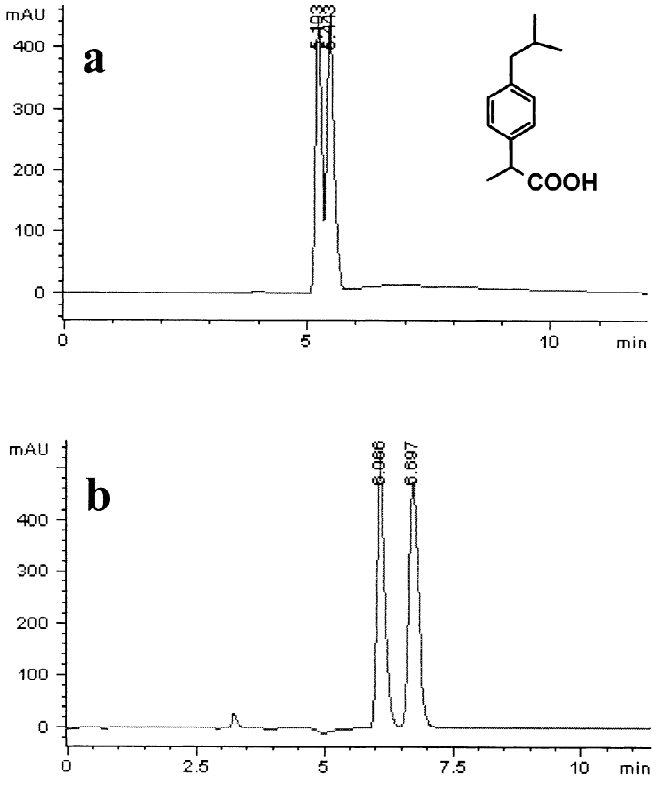

Fig. 8. Chiral HPLC analysis of Ibuprofen using a Hexane–TFA 0.05% (A)/EtOH (B) gradient on a Chiralcel OJ column. Elution under gradient conditions (a) requires a single isocratic test to achieve baseline resolution of peaks (b). Chromatographic conditions: (a) gradient from 20 to 70% B in 20 min; flow-rate 0.75 ml/min, (b) isocratic 5%B; flow-rate, 1 ml/min; detection, 215 nm.

Table 1
Analysis of retention data measured in gradient elution allows easy optimisation to isocratic conditions for either accurate ee determination or purification purposes

Compound	Gradient 0.75 ml/min *RT* (min)	Alcohol (%)	Isocratic 1 ml/min *RT* (min)
1	5.2/5.4	5	6.1/6.7
2	6.3/6.9	5	10.6/13.5
3*	**6.7/7.4**	**5**	**12.8/15.6**
4*	**6.2/7.8**	**10**	**5.9/8.3**
5	7.1/7.9	10	8.2/10
6	8.8/9.7	10	11.3/13.6
7*	**8.4/9.7**	**10**	**10.6/13.8**
8*	**8.4/9.7**	**15**	**7.8/9.7**
9	8.0/8.5	15	8.7/9.8
10	10.5/11.8	15	12.4/14.6
11*	**9.4/10.5**	**15**	**11.6/14.2**
12*	**9.4/10.5**	**20**	**8.4/10.1**
13	9.7/10.3	20	8.9/9.9
14	11.3/11.9	20	12.8/14.6
15*	**10.1/11.7**	**20**	**9.9/13.0**
16*	**10.1/11.7**	**25**	**7.8/9.9**
17	13.4/15.0	25	11.2/13.6
18*	**13.0/13.5**	**30**	**9.0/9.7**

Mobile phase and flow-rate can be adjusted to afford results in less than 12 min (**in bold***). The names and structures of the internal compounds **1–18** are not disclosed due to proprietary reasons.

ratio. Due to the physical properties of enantiomers, UV detection appears as a very precise technique. For that purpose, baseline resolution of the two species is required. The specific gradient conditions described in this paper achieve this goal over 85% cases. For the remained mixtures, signs of chiral recognition are detected in any of the gradient conditions included in our screen. Only for those latter cases, isocratic optimisation is required as is usually performed in non-chiral separations. Analysis of the experimental results so achieved on internal compounds has showed a good relation of the isocratic mobile phase composition, applied to afford complete resolution, with respect to the retention behaviour under gradient elution. Further on, we have observed that conversion from gradient to isocratic mode is not a function of compound type. Table 1 includes some of the aforementioned results as a quick guide for pre-selecting isocratic elution conditions, not only for complete enantioseparation when needed but also for purification purposes.

Fig. 8 shows isocratic optimisation step for the racemic mixture of Ibuprofen.

3.7. Column performance

Column performance has been monitored monthly by injecting the standard *t*-stilbene oxide (Fig. 9). We have not observed any damage to the columns over 18 months of daily work by using this methodology. Optimum stability and reproducibility of all the columns included in this study has been confirmed.

4. Conclusions

The standard screening herein proposed represents a simple but robust general scheme with a tremendous impact on Drug Discovery research, as conclusive results are usually achieved in 20–40 min. Applicable for a wide range of structural types, efficient, reproducible and easily automated, this novel methodology is capable of rapidly quantifying enantiomeric purity. Moreover, it can be advantageously employed in any laboratory where conventional HPLC systems are available, thus minimising investment costs.

In contrast to the traditional concept, monthly monitoring the column performance has confirmed the suitability of the gradients over 18 months of daily work.

To date, more than 800 compounds synthesised in our research laboratories have been successfully analysed under those conditions. Over 85% cases baseline resolution of peaks is achieved. In the remained mixtures, signs of chiral recognition permitted selection of the adequate chiral stationary phase to further develop the optimum isocratic conditions for complete resolution of peaks.

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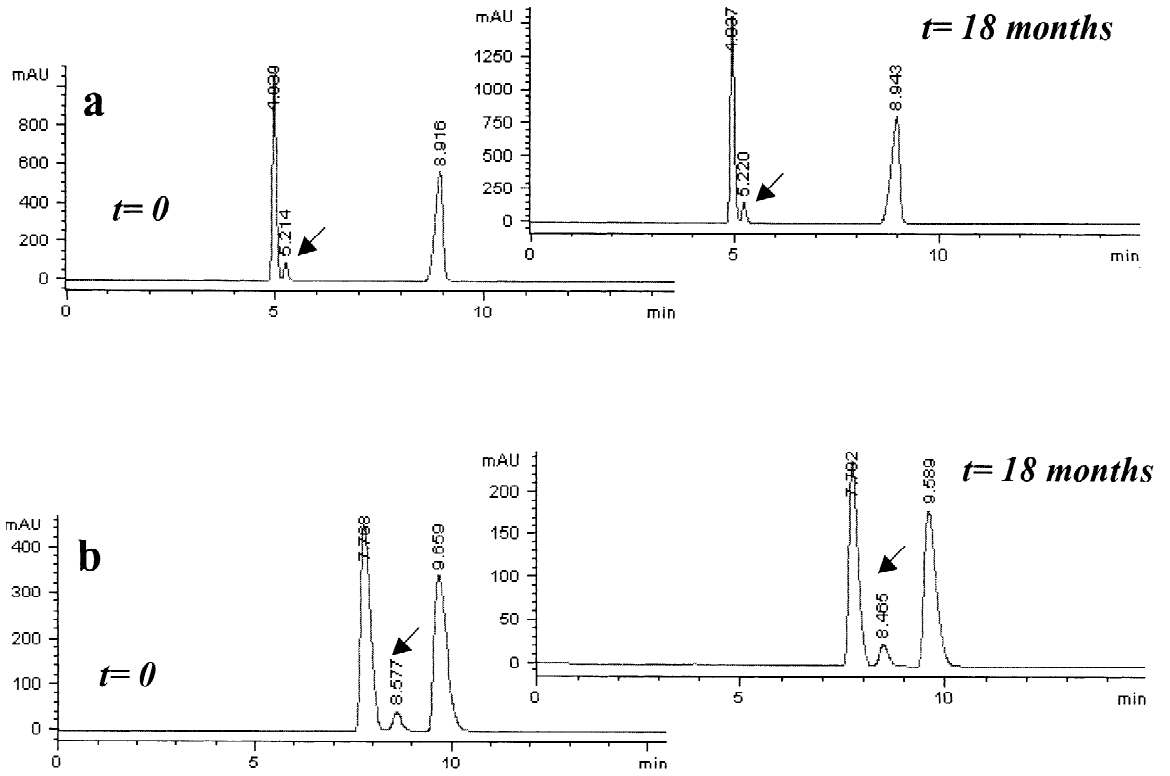

Fig. 9. Representative chromatograms of chiral resolution on *trans*-stilbene oxide as standard for column performance tests. Analyses were performed under isocratic conditions as described by manufactures. Chromatographic conditions: column and mobile phase, (a) Chiralpak AD, Hexane–TFA 0.05% (A)/IPA (B), isocratic 10%B, (b) Chiralcel OJ, Hexane–TFA 0.05% (A)/EtOH (B), isocratic 10%B; flow-rate, 1 ml/min; detection, 254 nm.

References

[1] J.R. Brown, in: G. Subramanian (Ed.), A Practical Approach to Chiral Separations by Liquid Chromatography, VCH, Weinheim, 1994, p. 57, Chapter 3.
[2] W.H. De Camp, J. Pharm. Biomed. Anal. 11 (1993) 1167.
[3] W.H. De Camp, Chirality 1 (1989) 2.
[4] C. Perrin, V.A. Vu, N. Matthijs, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 947 (2002) 69.
[5] J.W. Dolan, L.R. Synder, N.M. Djordjevic, D.W. Hill, T.J. Waeghe, J. Chromatogr. A 857 (1999) 1.
[6] L.A. Romanyshyn, P.R. Tiller, J. Chromatogr. A 928 (2001) 41.
[7] V.R. Meyer, J. Chromatogr. A 768 (1997) 315.
[8] S.D. Killops, J. HRC Chromatogr. Commun. 9 (1986) 302.
[9] P. Jandera, J. Chromatogr. A 797 (1998) 11.
[10] P. Jandera, J. Chromatogr. A 845 (1999) 133.
[11] P. Jandera, J. Chromatogr. A 965 (2002) 239.
[12] P. Renold, E. Madero, T. Maetzke, J. Chromatogr. A 908 (2001) 143.
[13] A. Medvedovici, P. Sandra, L. Toribio, F. David, J. Chromatogr. A 785 (1997) 159.
[14] E. Yashima, Y. Okamoto, Bull. Chem. Soc. Jpn. 68 (1995) 3289.
[15] Y. Okamoto, E. Yahima, Angew. Chem. Int. Ed. 37 (1998) 1021.
[16] B.-A. Persson, S. Andersson, J. Chromatogr. A 906 (2001) 195.
[17] E. Yashima, C. Yamamoto, Y. Okamoto, Synlett (1998) 344.
[18] P. Jandera, J. Chromatogr. A 797 (1998) 11.
[19] P. Jandera, M. Kucerová, J. Chromatogr. A 759 (1997) 13.
[20] Y. Okamoto, Y. Kaida, R. Aburatani, K. Hatada, J. Chromatogr. 477 (1989) 367.
[21] A.M. Krstulovi, M.H. Fouchet, J.T. Burke, G. Gillet, A. Duran, J. Chromatogr. 452 (1988) 477.
[22] C.B. Ching, B.G. Lim, E.J.D. Lee, S.C. Ng, Chirality 4 (1992) 174.
[23] Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, Chem. Lett. (1988) 1125.
[24] Y. Okamoto, R. Aburatani, K. Hatada, J. Chromatogr. 448 (1988) 454.

[25] Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume, M. Nakano, Chirality 1 (1989) 239.
[26] Y.K. Ye, B.S. Lord, L. Yin, R.W. Stringham, J. Chromatogr. A 945 (2002) 147.
[27] M. Reta, P.W. Carr, J. Chromatogr. A 855 (1999) 121.
[28] B. Chankvetadze, I. Kartozia, C. Yamamoto, Y. Okamoto, J. Pharm. Biomed. Anal. 27 (2002) 467.
[29] L. Miller, C. Orihuela, R. Fronek, J. Murphy, J. Chromatogr. A 865 (1999) 211.