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Packed column supercritical fluid chromatography of a peroxysome proliferator-activating receptor agonist drug Achiral and chiral purity of substance, formulation assay and its enantiomeric purity☆

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Abstract

This paper describes packed column supercritical fluid chromatography (SFC) for the analysis of a peroxysome proliferator-activating γ-receptor agonist that is a carboxylic acid. Evaluation of conditions for the separation of this candidate drug and related compounds in bulk substance is described. A Chiralcel OD column was used for this purpose due to its high selective retention of related substances and relative inertness, though the enantioselectivity was negligible, with methanol as polar modifier. A high enantioselectivity was obtained on Chiralpak AD and it was possible to determine the enantiomeric purity within 10 min on a 5 cm short column. Both the achiral and the chiral systems were run without acid additive in the mobile phase and the level of detection of impurities by area was about 0.1%. For the analysis of samples dissolved in water, without any isolation step, 2-propanol was used as modifier. Due to the column surface activity, evidently generated by injected water, citric acid 1 mM was included as additive in the 2-propanol in order to maintain symmetric and undistorted peak shape. The detection limit for the assay was 21 μg mL^{-1} (50 nmol mL^{-1}) for 5 μL injected (R.S.D. 6.4 %, $n = 8$). A 5 cm short Chiralcel OD column was used. Determination of enantiomeric purity of the drug in aqueous samples required increased sensitivity. The sample was acidified and extracted into a small volume of 1-pentanol, out of which 25 μl was analyzed by SFC. The minor enantiomer at the 3% (w/w) level added could be confirmed. Its ratio remained constant during the procedure as measured relative to a reference solution in organic media.

Keywords: Supercritical fluid chromatography; Enantiomer separation; Pharmaceutical analysis; Peroxysome proliferator-activating receptor agonist

1. Introduction

Packed column supercritical fluid chromatography (SFC) with alcohol modified carbon dioxide offers chromatographic separation selectivity that is often similar to that observed with normal-phase liquid chromatography. This is an attractive feature that complements commonly used reversed-phase LC systems, which today is by far the preferred LC mode. SFC can either be used in order to confirm results obtained by LC, or to verify selectivity shown with LC. Preparative SFC is also gaining interest in the pharmaceutical field. The SFC literature is covered on a regular basis in *Analytical Chemistry*; the latest reviews have appeared in 2000 and 2002 [1,2]. The field has also been the topic of a couple of monographs [3,4] and a chapter included in a book on a more general analytical treatment of drug impurities [5]. Smith has written a thorough and personal review of the ups and downs of supercritical fluids in separation science over the years [6]. In our laboratories we have developed and published a number of methods for the analysis of a handful of different bulk drug substances by packed column SFC [7–10].

Separation of enantiomers has been an important side of SFC since Macaudière et al. showed a large number of separations on various chiral columns in the late 1980s [11]. Many enantioselective normal-phase LC columns have been used for SFC except protein based ones [12]. Some of the most popular columns are based on 3,5-dimethylphenylcarbamate derivatized cellulose,

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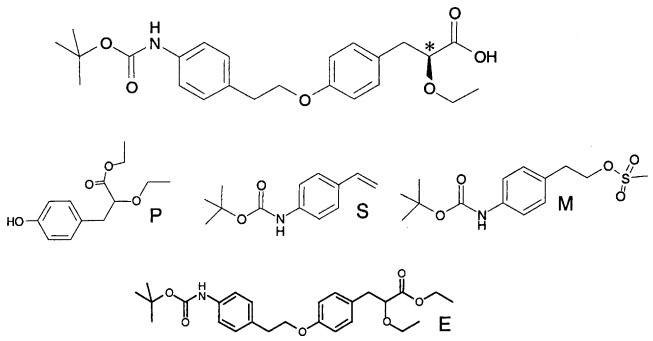

Fig. 1. Structure of drug and related substances. Identification of related substances: S, a styrene; P, a phenol; M, a mesylate; E, an ethyl ester.

or amylose, precipitated on silica gel. Besides π–π and hydrogen-bonding interaction possibilities between selector and analyte, these columns, Chiralcel OD and Chiralpak AD being the most common, have cavities that can enhance the enantioselective retention based on molecular shape. Sandra and co-workers ranks AD before OD regarding the rate of success for achieving enantio-resolution of unknown racemic mixtures, among useful enantioselective columns for SFC [13,14]. A short but fairly recent review on enantio-separations was published by Terfloth [15]. This topic is also well covered in the biannual reviews on SFC in *Analytical Chemistry* mentioned above [1,2].

The carboxylic acid being in focus in this work (Fig. 1) is pharmacologically active as a PPAR (peroxysome proliferator-activating receptor) agonist. Its pK_a is about 3.5 and there are some related compounds and possible impurities available (Fig. 1).

The purpose of this work was to develop methods based on packed column SFC for different analytical needs regarding this drug as a complement to liquid chromatographic methods. Screening of various columns was vital for the selective analysis of impurities. Development of a method for enantiomeric purity of bulk substance was fairly straightforward. Assay of the aqueous formulation, without any pre-treatment of the sample, was based on knowledge about what conditions to select in SFC in order to prevent irregularities in the chromatographic system due to water [16–18]. This information was not applicable, however, when the sensitivity had to be increased at least a 10-fold for the simultaneous estimation of the minor enantiomer ("impurity") in the aqueous formulation. In this case a simple extraction step into a small volume of organic solvent was used in order to enhance the sensitivity.

2. Experimental

2.1. SFC instrumentation

The instrument for packed column SFC was a Model G1205A from Hewlett-Packard, Little Falls Site (DE, USA) configured in the downstream mode. It was equipped with a variable-wavelength detector (VWD). The original HP-injector had been replaced with an automatic liquid sampler ALS 3100 from Berger Instruments (Newark, DE, USA) with an electrically actuated Valco injector (5 μL loop). This injector is manufactured as Model 718 Series by Alcott Chromatography (Norcross, GA, USA). The VWD was set to monitor 220 nm except when indicated. Backpressure was kept at 150 bar throughout. Control of the instrument was by the BI-SFC ChemStation version 3.6.6 software.

2.2. Reagents, chemicals and columns

All solvents and reagents were of analytical-regent quality or better. The candidate drug and related compounds were from the Department of Medicinal Chemistry, AstraZeneca R & D Mölndal, Sweden. Carbon dioxide was supplied in cylinders with dipper tubes by AGA (Lidingö, Sweden).

The Chiralcel OD and Chiralpak AD columns were manufactured by Daicel (Tokyo, Japan) and were 250 mm × 4.6 mm and 50 mm × 4.6 mm i.d., respectively. The Kromasil KR100-5CHI-TBB and -DMB columns were 250 mm × 4.6 mm i.d. from Eka Chemicals (Bohus, Sweden) (TBB: *tert*-butylbenzoyl and DMB: dimethylbenzoyl). A Bakerbond dinitrobenzylphenylglycine column (DNBPG) 250 mm × 4.6 mm i.d. was from J.T. Baker (Phillipsburg, NJ, USA).

The Hypersil bare silica column was 200 mm × 4.6 mm i.d. from Hypersil (formerly Shandon, Astmoor, UK).

2.3. Methods

Chromatographic data were calculated by standard procedures. Reported data are in general the mean of at least two injections.

2.3.1. Bulk drug substance purity

Sample solutions (10 mg mL^{-1}) were prepared in dichloromethane and analyzed on a 25 cm long Chiralcel OD column. Available minor impurities were also added in order to confirm their retention and possible presence. Samples were then diluted 1:1 in order to confirm the linearity of the detector.

2.3.2. Enantiomeric purity of bulk drug substance

Samples were prepared in methanol (2 mg mL^{-1}) and analyzed on a 5 cm long Chiralpak AD column. They were then diluted 1:1 in order to confirm the linearity of the detector.

2.3.3. Assay of drug in aqueous solution

The mobile phase modifier was 2-propanol containing 1 mM citric acid as additive and the column temperature was 50 °C, after optimization. The samples were injected neat (5 μL) without any pretreatment.

2.4. Enantiomeric purity of drug in aqueous solution

The extraction and concentrating step was as follows: 10.0 mL of aqueous buffer pH 9.5, 70 μL of 1 M sulfuric acid and 1.00 mL of 1-pentanol were equilibrated for 10 min and centrifuged. The organic phase was transferred (∼0.4 mL × 2) using a Finnpipette (Labsystems, Helsinki, Finland) to 1.5 mL sample vials.

The method was tested using a stock solution of 1-pentanol containing 2.18 mg and 64.8 μg of the minor enantiomer diluted to 10.00 mL. The latter amount was obtained by taking 300 μL of a 216 μg mL^{-1} solution of the opposite enantiomer. Then 1.0 mL samples of the organic stock solution was submitted to the described procedure above and compared with the stock solution as reference. Injection volume was 25 μL and the UV detector set to monitor 234 nm. Separation was performed on a 25 cm TBB and a 5 cm Chiralpak AD in series with 1.5 mL min^{-1} of 25% of methanol in carbon dioxide as mobile phase at 40 °C.

3. Results and discussion

3.1. Selection of column for achiral analysis of drug substance purity

The aim was to find a suitable column that showed good selectivity towards known impurities and that gave acceptable symmetry of the peaks. Though the analyte in question (Fig. 1) is a moderately strong acid (pK_a 3.5) we had a preference not to use acid additives in the system if possible. Since the compound is a single enantiomer we investigated enantioselective columns partly because some of them have shown that it is not necessary to use an acid additive in order to get nice symmetric peaks of carboxylic acids like profen acids on certain such columns [19,20]. In most normal-phase LC enantio-separations of chiral acids some strong such as trifluoroacetic acid is used as additive in order to improve peak shape. Such additives can have a detrimental effect on the stability of the stationary phase as well as increase the base line noise when low wavelengths are used for UV detection.

On a tartaric acid network column derivatized with 3,5-dimethylbenzoyl groups [21] the target analyte chromatographed nicely with good symmetry and column efficiency. Furthermore, fine-tuning of the separation of some of the minor impurities was possible. This could be accomplished either through changing the percentage of methanol modifier as illustrated in Fig. 2a or by changing the temperature as in Fig. 2b. At a low temperature as 30 °C, or a high percentage of modifier, the styrene and the phenol compounds co-elutes. In packed column SFC one can often see such selectivity changes related to the column temperature [9,10,22,23], which is in line with the normal-phase LC like behavior of packed column SFC. In Fig. 2a one can also observe a minute peak ahead of the phenol at 12% of methanol as modifier. This peak is seen just after the co-eluting pair at 16%.

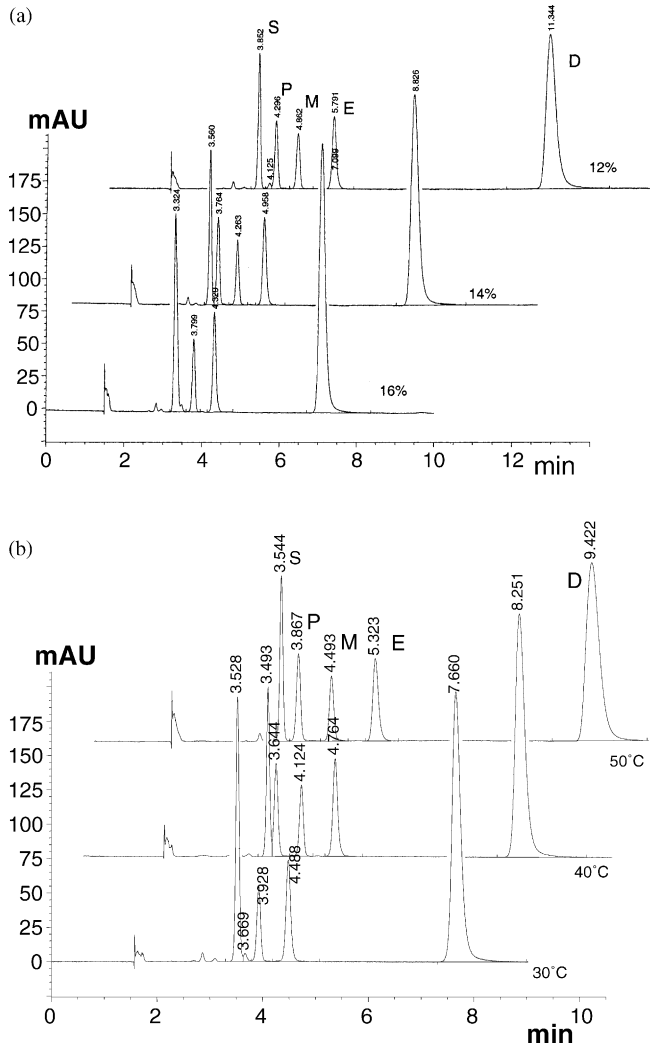

Fig. 2. Selectivity tuning on a tartaric acid network DMB column. (a) Variation of methanol *modifier* percentage at 40 °C. (b) Variation of column *temperature* at 14.5% of methanol modifier. Conditions: Flow rate, 1.5 mL min^{-1}, 25 cm column. Peak identification: S, a styrene; P, a phenol; M, a mesylate; E, an ethyl ester; D, the drug. Structures in Fig. 1.

On the DNBPG column the drug was also eluted as a symmetric peak but required 35 % of methanol in order to give a retention factor <10. With these conditions the available impurities eluted early and near the solvent front. It is likely that the π–π interactions of this support with the analyte drug are a bit on the strong side compared to those of the other columns investigated. At 10% of methanol as modifier the four related low molecular-mass substances were baseline resolved within 6 min (k_{max} <2).

Chiralcel OD was finally chosen as column for this kind of analysis due to its good overall selectivity, though the column efficiency is not outstanding and has a marked flow-rate dependence also in SFC. Compared to the DMB column the retentivity was slightly higher (Table 1). The selectivity was roughly the same as with the DMB column (Table 1) though

Table 1
Comparison of selectivity factors α towards available possible by-products from the synthesis on Chiralcel OD, DMB and DNBPG, respectively

Column	MeOH (%)	Styrene	Phenol	Mesyl	Ethyl ester	**Drug**	"0.4"	*k* drug	t_R min
Chiralcel OD	30	0.08	0.13	0.26	0.44	**1.00**	1.66	4	9.7
DMB	10	0.21	0.26	0.32	0.42	**1.00**	1.56	8	15
DNBPG	35	[a]	[a]	[a]	[a]	**1.00**	n.m.	8.9	19

"0.4" unknown peak that elutes after the drug, ~0.4% by a area. Conditions: Flow rate: 1.5 mL min^{-1}; column temperature: 40 °C. Other details in Section 2.

[a] Not resolved, close to the front.

it required a higher concentration of methanol in order to elute the drug within a reasonable time. On the other hand, the trailing edge of the main peak was less pronounced with this column which was more evident with real samples and higher sample loads. The separation in Fig. 3 was done on a similar test mixture as in Fig. 2. In this case there is also a tendency of the racemic ethyl ester to be resolved.

Some separations were also investigated on a more traditional support Hypersil bare silica. Here, however, the support gave rather pronounced tailing of the drug peak. Since additives to the mobile phase at low-wavelength UV detection is not attractive this support was not pursued any further, though for a similar carboxylic acid drug, sufficient selectivity was obtained towards related compounds.

3.2. Analysis of related compounds in bulk drug substance

Fig. 4 shows an example with a chromatogram from the analysis of a batch of bulk drug substance. The chromatogram has three significant peaks beside the main peak. They range from 0.1 to 0.4 relative area%. The last two in the chromatogram have extra phenyl groups attached. For completeness of the figure the unscaled main peak has also been inserted in this chromatogram. In general, this column worked well for this kind of analysis though occasionally some column bleed was observed, which might depend on the nature or age of the column. One can also expect the occasional loss of aromatic groups from the cellulose backbone to be responsible for noise. Dual injections of each sample confirmed if such small peaks were from the column, or from the sample itself. The detector linearity was confirmed by diluting the sample 1:1 and repeating the analysis. From the insert in Fig. 4, there is no sign from the shape of the peak that it is outside the linearity of the detector at this wavelength. Compared to LC most of the related compounds have shorter retention times in SFC than the parent drug itself. Dichloromethane was used as solvent since with methanol some minor spontaneous methylation was observed, which could not be avoided. The ethyl ester is a possible contaminant from the synthesis or, more likely, clean up step of the drug. Precision on determination of impurities were not evaluated in the present work but has been investigated in detail for clevidipine and related compounds [9]. In the cited study it was better than 10% R.S.D. at the 0.1% (w/w) added level, which is adequate for such studies. Likewise, no evaluation of column-to-column variations concerning selectivity or retention factors has been done. The cost of these columns deters such a study without a stronger need for such validation work. Over time several batches have been analyzed and only small adjustments in the percentage of methanol modifier has been needed in order to maintain the retention and resolution.

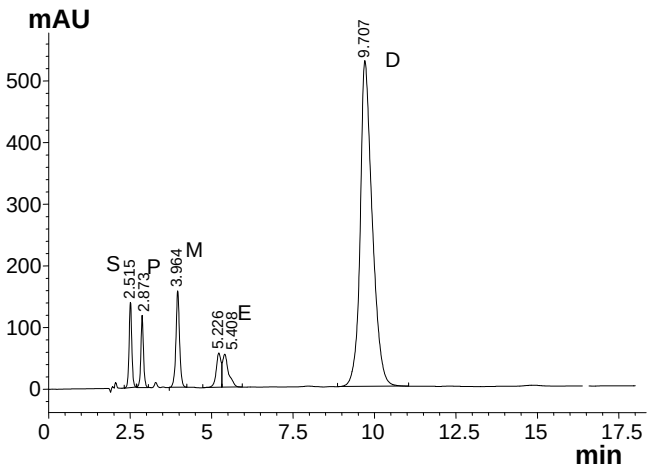

Fig. 3. SFC of a test mixture on Chiralcel OD. Conditions: Flow rate, 1.5 mL min^{-1}; 30% methanol, 40 °C, 25 cm column. Peak identification: S, a styrene; P, a phenol; M, a mesylate; E, an ethyl ester; D, the drug.

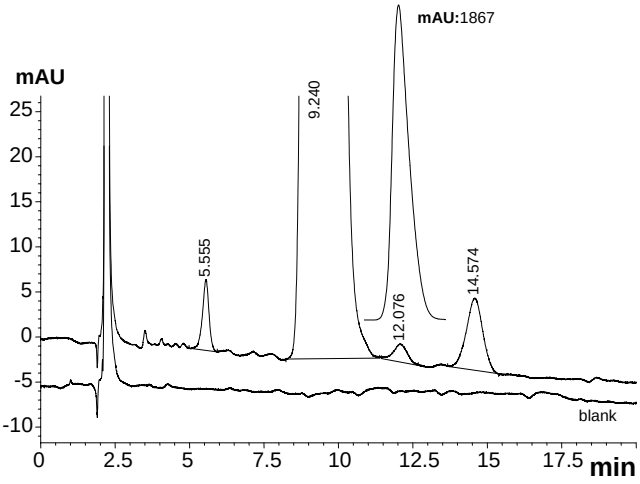

Fig. 4. SFC of a bulk drug substance batch on Chiralcel OD. Conditions: As in Fig. 3, sample concentration 10 mg mL^{-1}. Lower trace dichloromethane blank injection. Insert unscaled peak.

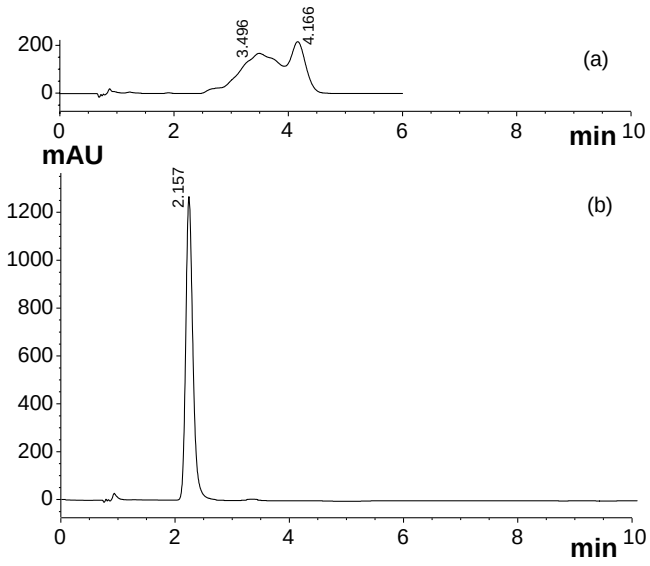

Fig. 5. Restoration of a Chiracel OD column that had been maltreated with samples dissolved in water. SFC of the drug (a) after a series of injections of samples dissolved in water and (b) after the introduction of 1 mM citric acid as additive in the modifier. Conditions: 5 cm column at 30°, 1.0 mL min^{-1}, 30% methanol. Sample: 1 mg mL^{-1} of drug in 2-propanol.

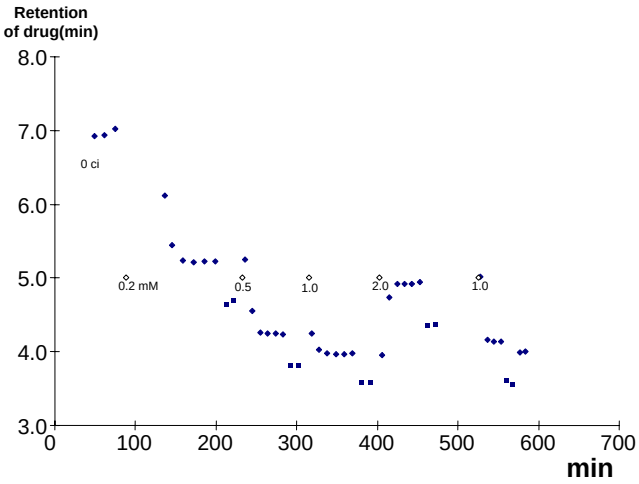

Fig. 6. Time course for the equilibration of various concentrations of citric acid as additive in the 2-propanol modifier. Conditions: 5 cm Chiralcel OD column at 30 °C, 1.5 mL min^{-1}, 20% 2-propanol. Symbols: open diamonds (◇) indicate the time when changes in citric acid concentration were made (and the new concentration), full diamonds (◆) sample dissolved in 2-propanol, and full squares (■) sample dissolved in water.

3.3. Analysis of drug in water solution

Since packed column SFC is similar to normal-phase system, the use of water as sample medium would normally be completely discouraged. However, using a rather high percentage of alcohol modifier in the mobile phase, the moderate volumes loaded are not so detrimental. Furthermore, with 2-propanol as modifier we have shown that samples dissolved in water can be compatible with the chromatographic medium [16–18]. Furthermore, by tuning the water generated system peak into co-elution with the analyte, peak compression of the analyte can take place, and extremely high apparent plate numbers can be obtained [18]. On a 10 mm i.d. Hypersil column we were able to load 0.2 mg quantities of clevidipine dissolved in as much as 150 μl of water–2-propanol (80:20) [18].

Direct injection of the drug dissolved in water on a system based on a short Chiral OD column as support and 35% of ethanol looked promising at first, and separation times were short. But a closer look at the chromatograms revealed skewed peak shapes. With methanol replacing ethanol as modifier the analysis of samples dissolved in a friendly solvent, like 2-propanol, discouraging peak shape was revealed (Fig. 5a). The column was thought to have been destroyed by the injection of water samples, and it was regarded as a loss. But from detailed work on a dihydropyridine substituted acid, formed from hydrolysis of clevidipine, we knew that a low concentration of citric acid has a beneficial effect on bare silica regarding column efficiency, but not on the peak symmetry [9]. Also in this situation, as shown in Fig. 5b, citric acid showed favorable effect on the analyte peak. The concentration of citric acid needed, and the time required for reaching steady state with each new concentration of citric acid, is illustrated in Fig. 6. Column volumes are more correct than time to plot, but since the dead time recorded varied with modifier composition that mode was not used in this presentation. The time required to reach a new equilibrium of the system with each new concentration was short, especially when the dead volume of the modifier tubing is taken into account which is about 12.5 min. There is a marked decrease in retention with the presence of even a small concentration such as 0.2 mM of citric acid (Fig. 6). About 2 min decrease was observed with 0.2 mM, which translates into a shift in k from 16 to 12. Citric acid 1.0 mM of was chosen since with 2.0 mM the retention increased and aqueous samples showed pronounced broadening of the analyte peak. Perhaps a saturation level has been reached and the last adsorbed citric acid molecules interact with the analyte that contributes to bandbroadening. Fig. 6 also shows that depending on whether the analyte was dissolved in water or 2-propanol there was also a significant difference in retention time, about 0.4–0.5 min. Moreover, the plate number differed to the favor of organic solvent but with increasing column temperature from 30 °C this difference leveled out and was negligible at 50 °C (Table 2). For the samples in 2-propanol there was no change in plate number over this temperature range. The manufacturer recommends that the column is not to be used at temperatures above 40 °C. Still 50 °C was selected and no detrimental effects were observed during short term use at least. At elevated column temperature bandbroadening can be caused by incomplete mixing with the mobile phase. The exact chemistry behind the beneficial effect of citric acid is not known at present. It is assumed, however, that water in the sample creates active sites on the surface of the silica support of the column, that

Table 2
Optimization of the column temperature for drug in water samples

Temperature (°C)	Solvent	Retention time (min)	*N*
30	2-Propanol	3.96	1050
	Water	3.58	710
40	2-Propanol	4.06	1010
	Water	3.66	900
50	2-Propanol	4.30	1045
	Water	3.90	1080

Conditions: Chiralcel OD 5 cm column, 1.5 mL min^{-1} carbon dioxide with 20% of 2-propanol containing 1 mM of citric acid.

have been protected by some simple means in the manufacturing process or by the derivatized polysaccharide itself by precipitation/adsorption, and citric acid protects these sites in a reversible manner. In the previously cited example sufficient deactivation remained on the column, even when the citric acid was no longer present in the modifier [9]. On the other hand, in that case the analyte was a much weaker acid, its pK_a was only 7 [9]. In the present system the persistence of the positive effect on the retention was much weaker. After some 500 column volumes the retention time was close to that observed before the acid was added to the chromatographic system. Shorter retention of the analyte drug dissolved in water relative an alcohol as sample media, may be explained by the classical phenomenon that samples dissolved in a more strongly eluting solvent than the mobile phase show shorter retention times. Ye et al. reported strong persistence of positive effects from sulfonic acid additives in normal-phase LC on Chiralpak AD [24,25]. After 3000 min, i.e. 50 h, the beneficial effect on k and α for racemic amino acid derivatives was still pronounced [24,25]. A closer comparison is perhaps not possible since the analytes are far from identical.

The optimized system was now investigated further by analyzing six known samples in the range 50–1100 nmol mL^{-1} and plotting peak area versus concentration. The equation of the standard curve showed a minute positive intercept and a correlation coefficient of 0.9997. The precisions at a few selected concentrations are given in Table 3 and a representative chromatogram at the lowest level is shown in Fig. 7 together with an injection of a blank. Note the rather prominent system peak due to the injection of water. Finally seven samples ranging from 0.05 to 1 μmol mL^{-1} were analyzed by this method and compared with the results obtained by corresponding reversed phase LC method. The equation of the line for LC values versus SFC ones was $y = 0.979x - 0.0048$ with a correlation coefficient of 0.9994. The conclusion is that the SFC method is sufficiently precise and accurate for the determination of samples of this drug dissolved in water.

Table 3
Precision data for the direct analysis of drug dissolved in aqueous buffer pH 9.5

Drug (μmol mL^{-1})	R.S.D. (%)	*n*
0.60	0.7	8
0.15	1.8	8
0.05	6.4	8
0.05	4.4	7

Conditions: as in previous table. 0.05 μmol mL^{-1} corresponds to 21 μg mL^{-1} or 105 ng loaded on column. Last row – one injection rejected.

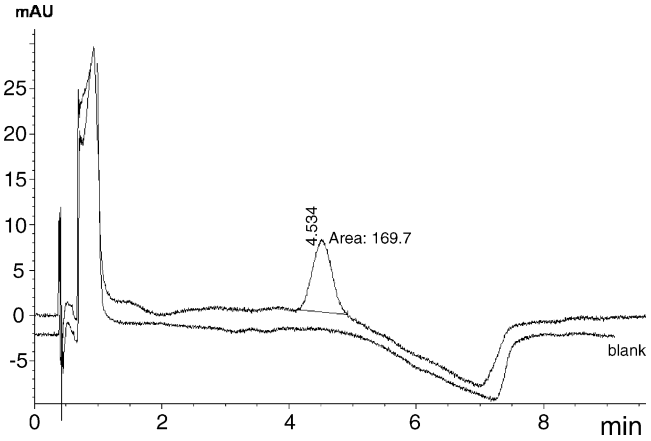

Fig. 7. Direct SFC of water sample with 21 μg mL^{-1} of drug on Chiralcel OD. Conditions: 5 cm column at 50 °C, 1.5 mL min^{-1}, 20% 2-propanol with 1 mM of citric acid. Lower trace buffer pH 9.5 injected (blank).

3.4. Analysis of enantiomeric purity in bulk drug substance

On Chiralpak AD the enantioselectivity was so good that a 5 cm column was adequate and gave baseline resolution of the minor peak within 10 min (α 1.3, 25% methanol at 40 °C). A larger plate number can still have a certain interest since in the early development stage a 25 cm long column resolved a small impurity that would otherwise have co-eluted with the minor enantiomer. Later batches of the drug were cleaner and the shorter column could be used for faster analysis and higher sample throughput. Fig. 8 shows a

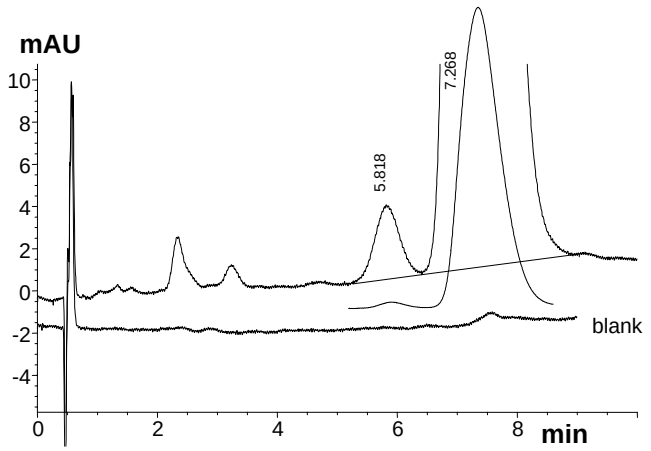

Fig. 8. SFC for the determination of the enantiomeric purity of drug on Chiralpak AD. Conditions: 5 cm column at 40 °C, 1.5 mL min^{-1}, 25% methanol. Concentration: 1 mg mL^{-1}.

Table 4
Effect on column efficiency on increased injection volumes with 2-propanol as sample media

Loop volume (μL)	*N*	Area (mAU s)	Drug (μg mL^{-1})
5	4580	6220	500
10	4340	5980	250
20	4460	5530	125
50	1630	5740	50[a]

Conditions: Chiralpak AD 25 cm, 1.5 mL min^{-1} 35% 2-propanol with citric acid 1 mM, 50 °C.
[a] Sample dissolved in 1-pentanol.

chromatogram from an actual analysis. Also inserted in this figure is the full-scale chromatogram. The minor enantiomer was 1.2 relative area% in this case. The limit of detection of the minor enantiomer is about 0.05 area%.

3.5. Enantioselective analysis of drug in water samples

On Chiralpak AD the system peak generated by injected water interfered and made it impossible to quantitate the minor isomer with adequate precision. The problem was even more pronounced with larger sample volumes injected that would be required for sufficient sensitivity of the minor enantiomer, even if the level required is relatively high, i.e. a few percent (w/w). Perhaps the lower wavelength used contributed to the difficulties encountered. Therefore a different approach was pursued, based on acidification of the aqueous sample and extraction of the analytes into a small volume of an organic solvent, at the expense of convenience. About a 10-fold improvement in detectability could thus be obtained. Combination of this step with a five times larger sample injection loop and the use of a more optimal wavelength increased the sensitivity sufficiently. Table 4 shows the results with increasing volume injected on the area and efficiency. The short Chiralpak AD column was combined with a regular length TBB one, since the system with a 25 cm AD only did not behave satisfactorily. The former provided the required enantioselectivity and the latter TBB column a good plate number without being to retentive as compared to Chiralpak AD. A representative chromatogram is shown in Fig. 8. The selectivity factor on the TBB column alone is low, about 1.06 at 30 °C using 10% of 2-propanol as mobile phase.

The preservation of the enantiomeric ratio during the extraction conditions was investigated together with any possible losses of analyte (Fig. 9). No formulation of the drug in water was available at this stage of development but an organic sample was prepared and equilibrated with a 10-fold larger volume of buffer after acidification. As shown in Table 5 there is a negligible difference in the enantiomeric ratio of the sample average and that of the reference. Concerning any losses this could not be proven since the absolute areas of the samples were on average 24% higher than that of the corresponding non-equilibrated samples (Table 5). This cannot be caused by a change in volume of the organic phase since experimentally this change is small, <5%. The actual fraction of the minor enantiomer was 2.89% (w/w), and its inherent presence in the major one <0.05% in this batch. The absence of drug in the remaining aqueous phase was also confirmed by taking a 5.0 ml aliquot to a second extraction with 1.0 ml of 1-pentanol. No significant amounts could be detected in the organic phase. Thus, the isolation and concentration step should work satisfactorily for its purpose. A relatively large injection port wash was necessary due to the fact that the large volume of gaseous carbon dioxide liberated from the liquid carbon dioxide in the 25 μl loop splashed back into the sampler media tubing, which in turn did not work properly afterwards, and consequently the precision of the method was reduced.

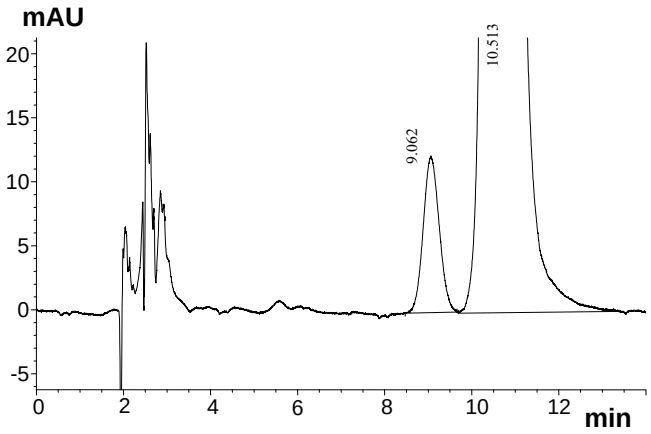

Fig. 9. SFC for the determination of enantiomeric composition of formulation of drug in buffer pH 9 with opposite enantiomer added to 3% (w/w). Conditions: see Table 5. Preparation of sample: see Section 2. Columns: 25 cm TBB and 5 cm Chiralcel OD in series. UV detection at 234 nm.

3.6. SFC–MS for the identification of impurities

The set up was simple and consisted basically of a Swagelock T through which a fused silica tubing was led from the outlet screen of the column directly into the ion source of the MS instrument [5,26]. Atmospheric pressure chemical ionization (APCI) gave poor generation of ions, whereas in the presence of ammonium acetate in the mobile phase a good signal-to-noise ratio was obtained of the drug. This made it possible to detect impurities below 1% by area in a 1 mg mL^{-1} solution. More details and results will be presented in a separate work [27].

3.7. Concluding remarks

The carboxylic acid drug investigated in this work was chromatographed on Chiralcel OD for possible impurities with good selectivity and the possibility to detect most of these related compounds. A high enantioselectivity was shown with Chiralpak AD, and a 5 cm column was adequate for the determination of the minor enantiomer down to the

Table 5
Determination of enantiomeric purity of drug and recovery after equilibration with a 10-fold excess of buffer pH 9.5

Sample	Area% of minor enantiomer	R.S.D. (%) (three injections)	Area (mAU s)	R.S.D. (%) (three injections)
Reference 1	2.923	3.9	8644	0.6
Reference 2	2.922	1.2	8618	1.5
Reference 3	2.980	1.9	8667	1.6
Sample 1	2.882	1.6	10634	1.4
Sample 2	2.907	1.0	10651	1.1
Sample 3	2.930	2.9	10993	0.8
Sample 4	2.879	1.4	10678	0.7
Average references	2.942		8624	
Average samples	2.900	−1.4 (decrease)	10739	24 (increase)

Conditions: 25 cm TBB and 5 cm Chiralpak AD columns at 40 °C, 25% of methanol with 1 mM citric acid in the carbon dioxide 1.5 mL min^{-1}. The 25 μL loop of the injector was loaded with 40 μL of sample. A 10 μL bubble of air was used as divider to the sampler solvent; ethanol. An injection port wash was done after each injection. The sample preparation is fully described in Section 2.

0.1% area level. For theses SFC systems no acidic additive to the mobile phase was required.

The aqueous formulation of the drug in buffer pH 9.5 can be assayed directly on a short OD column with 2-propanol as modifier containing citric acid. For corresponding enantio-separation a pre-concentration extraction step to small volume of 1-pentanol was performed.

The results show that packed column SFC can be a useful analytical technique for the analysis of various samples of this lipophilic drug.

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