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Direct high-performance liquid chromatographic separation of an enantiomeric peptidoleukotriene antagonist and its homologues

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ABSTRACT

Racemic SK&F 106203 and its homologues can be directly separated on Chiralcel OD, Chiralpak AD and AS columns. The corresponding dimethyl esters, however, can only be resolved on the Chiralcel OD column. Hydrogen bonding between the carboxylic acid proton of the analyte and the chiral stationary phase appears critical for chiral recognition on Chiralpak AS column for these carboxylic acid compounds. Substitution at the 2 position of the parent phenyl ring appeared to aid chiral separation on Chiralcel OD column. The elution order of SK&F 106203 obtained on a Chiral OD column can also be reversed on a Chiralpak AS column.

INTRODUCTION

Leukotriene D_4 (LTD_4), formed from the metabolism of arachidonic acid by 5′-lipoxygenase, is considered an important receptor–mediator of human bronchial asthma [1]. Considerable efforts are ongoing in the pharmaceutical industry in pursuing specific leukotriene LTD_4 receptor antagonists as potential therapeutics for the treatment of 'asthma. Among compounds of this class are ICI-204, 219, SK&F 104353, SK&F 106203 [2] and ONO-1078 [3].

With the current emphasis on enantiospecific pharmacological evaluations in drug development [4,5], chiral chromatography has become increasingly important. Where significant pharmacological differences exist between two enantiomers, the eutomer should be developed, and the other enantiomer may be regarded as an impurity. SK&F *S*-106203 {3(*S*)-[2-(carboxyethyl)thio]-3-[2-(phenyloctyl)phenyl]propanoic acid (**1**)} is a potent and selective LTD_4 receptor antagonist that is currently in clinical trials. The two enantiomers of this LTD_4 receptor antagonist showed markedly different pharmacological activities. We have described the direct enantiospecific separation of **1** and reported on the utility of this chiral HPLC method in assigning absolute stereochemistry to the disodium salt of **1**, a non-crystalline amorphous compound which

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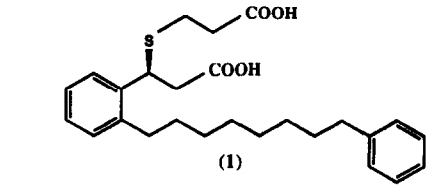

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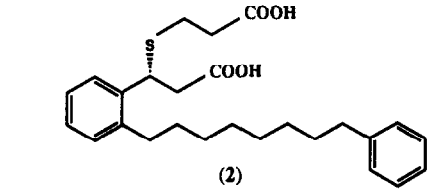

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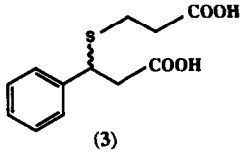

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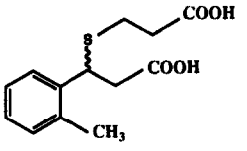

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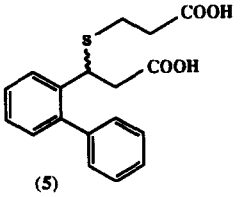

(5)

is not amenable to single crystal X-ray analysis [6]. In this communication, we report on the chiral HPLC of **1** and its homologues on cellulose Chiralcel OD, amylose Chiralpak AD and amylose Chiralpak AS columns.

EXPERIMENTAL

Apparatus

The liquid chromatograph system consisted of a Beckman System Gold HPLC pump connected to an HP 1050 series autosampler and variable-wavelength UV detector. Chiralcel OD, Chiralpak AD and Chiralpak AS column (all three 250 × 4.6 mm) were obtained from Chiral Technologies (Exton, PA, USA). Data were acquired and processed using a Waters 860 networking computer system.

Chemicals

Trifluoroacetic acid (Baker Analyzed HPLC reagent-grade ampoules) and HPLC-grade isopropanol and *n*-hexane were obtained from J.T. Baker (Phillipsburg, NJ, USA). All compounds used in this investigation were synthesized in our laboratory, and were fully characterized by NMR, IR, MS, elemental analysis and impurity profile by HPLC.

Chromatographic conditions

All experiments were performed at ambient temperature and with UV detection at 215 nm. Mobile phase compositions optimized for the separation of **1** and **2** were: for Chiralcel OD, *n*-hexane–isopropanol–trifluoroacetic acid (96.5:3.5:0.1), flow-rate, 2 ml/min; for Chiralpak AD, *n*-hexane–isopropanol–trifluoroacetic acid (94:6:0.1), flow-rate, 0.6 ml/min; for Chiralpak AS, *n*-hexane–isopropanol–trifluoroacetic acid (88:12:0.1), flow-rate, 0.6 ml/min.

RESULTS AND DISCUSSION

In order to evaluate the toxicological profile of the single SK&F *R*-106203 enantiomer (**2**), we required an enantiospecific HPLC method to measure its chiral purity. On cellulose Chiralcel OD column, the *R*-enantiomer, SK&F *R*-106203 eluted first, followed by the *S*-enantiomer, SK&F *S*-106203 (**1**) [6]. For low detection, it is advantageous to have the undesired enantiomer eluted first so that it will not be affected by the tailing of the major component. Since amylose-based Chiralpak AS column has a chiral center in the *S*-configuration at the phenylethyl group which can serve as an additional chiral selector [7] (Fig. 1), we attempted the chiral HPLC of racemic SK&F 106203 on a Chiralpak AS column. As shown in Fig. 2, the elution order observed on Chiral OD column for racemic SK&F 106203 [6] can be successfully reversed on an amylose Chiralpak AS column. The differences in the backbone structure of either stationary phase, *i.e.*, liquid crystal-like leading to a rigid and linear structure on the surface of the silica gel support for cellulose Chiralcel OD column [8], and pseudo-helical for amylose Chiralpak AS column, apparently, were not critical

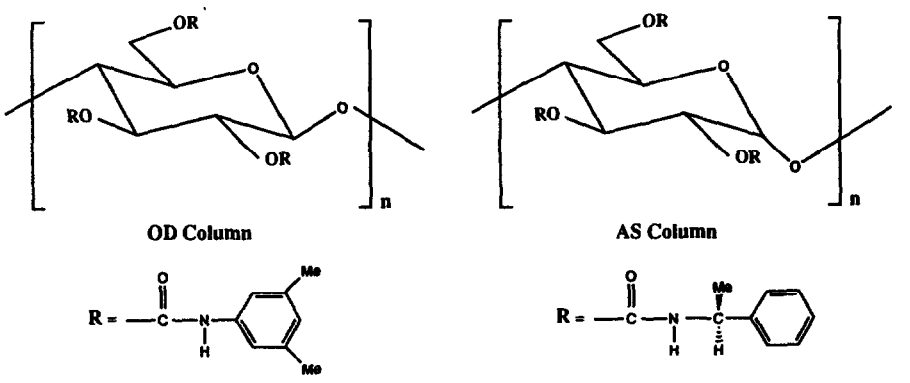

Fig. 1. Structures of cellulose Chiralcel OD and amylose Chiralpak AS columns.

for chiral separation. To the best of our knowledge, this is the first observation of reversal of elution order between a cellulose Chiralcel OD and an amylose Chiralpak AS column. Reversal of elution order between a Chiralpak AR and a Chiralpak AS column in the fashion of the well documented elution order reversal obtained on (*R*) and (*S*) Pirkle-type dipeptide columns, has been reported [8]. However, Chiralpak AR column also has limited resolving power compared to Chiralpak AS or Chiralcel OD columns [8]. Previous applications of Chiralpak AS column has primarily been on heterocyclic compounds possessing the chiral center on the ring, *e.g.*, diazapine and β-lactam [7].

Since chemically related compounds differing only in the length of the carbon chain are known to exhibit different physical properties and elicit different biological responses [9], we also examined the chromatographic behaviour of SK&F 106203 (**1**) and its simple homologues, namely, the unsubstituted (**3**), the methyl (**4**) and phenyl (**5**) derivatives on Chiralcel OD, Chiralpak AD and Chiralpak AS columns. As the retention and

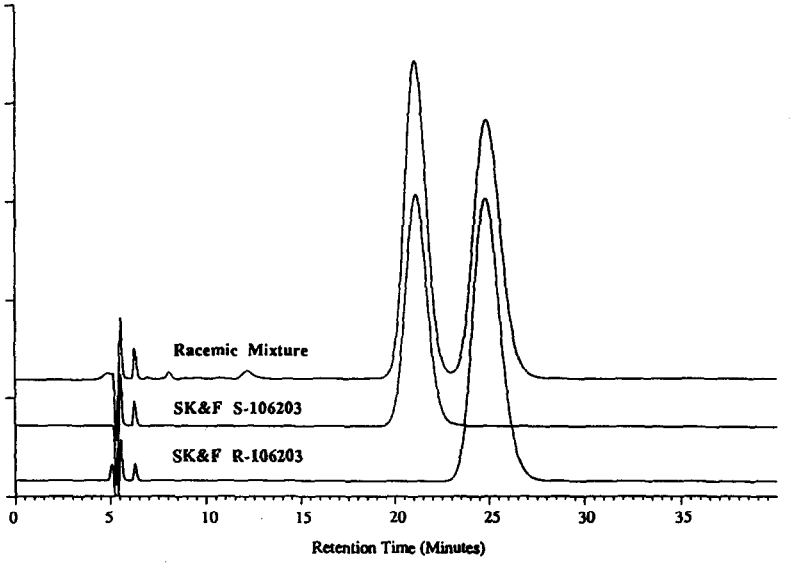

Fig. 2. Chiral HPLC chromatograms of (top trace) the racemic mixture of **1** and **2**, (middle trace) **1**, and (bottom trace) **2** on Chiralpak AS column. The HPLC conditions are: mobile phase flow-rate 0.6 ml/min, UV detection at 215 nm.

separation characteristic of these columns were different, and the respective polarity of the analogues was varied, we could not perform the chromatography on all three columns using identical mobile phase and flow-rate. To compare the separation of racemic mixture of **1** and **2**, **3**, **4** as well as **5** on these columns, we used a mobile phase and flow-rate on each column that was optimized for the separation of racemic mixture of **1** and **2**. The results are shown in Figs. 3, 4 and 5, and the separation factors are presented in Table I. Under these conditions, in all cases, the presence of 0.1% (v/v) trifluoroacetic acid in the mobile phase was required for good peak shape and resolution. As shown in Fig. 3, the *ortho*-substituted analogues are better separated than the unsubstituted analogue on Chiralcel OD column. *ortho*-Substitution therefore appeared to aid separation on the OD column for this class of compounds. However, no such clear trend exists for the amylose-based AD and AS column.

The results also showed that the phenyl analogue (**5**) was well separated on all three chiral columns. Thus, for this class of compounds, contribution from the *ortho*-phenyl substituent to the $\pi-\pi$ interaction between the analyte and the chiral stationary phase appeared to facilitate chiral recognition.

Whereas the unsubstituted analogue (**3**) was not well resolved on the cellulose OD column, **3** was well separated on both amylose AD and AS columns under similar conditions. The methyl analogue (**4**), on the other hand, was well resolved on the OD column but not on either the AD or AS columns. Since the steric hindrance of the phenyloctyl side chain in **1** is very similar to the methyl substituent in **4** because of its steric flexibility, it was anticipated that on both AD and AS columns under the same conditions where the methyl analogue **3** was barely baseline-resolved, the separation of racemic mixture of **1** and **2** would also be problematic. However, as shown in Table I and Figs. 4 and 5, although the racemic mixture of **1** and **2**, as expected, was not well resolved on the AS column (resolution factor: 1.6), it was nonetheless well separated on the AD column (resolution factor: 4.0). More data are therefore needed in order to decipher a clear trend of predictive value for either the AD or AS column based on the relative steric hindrance of the ortho-substituted alkyl side chain for this class of compounds.

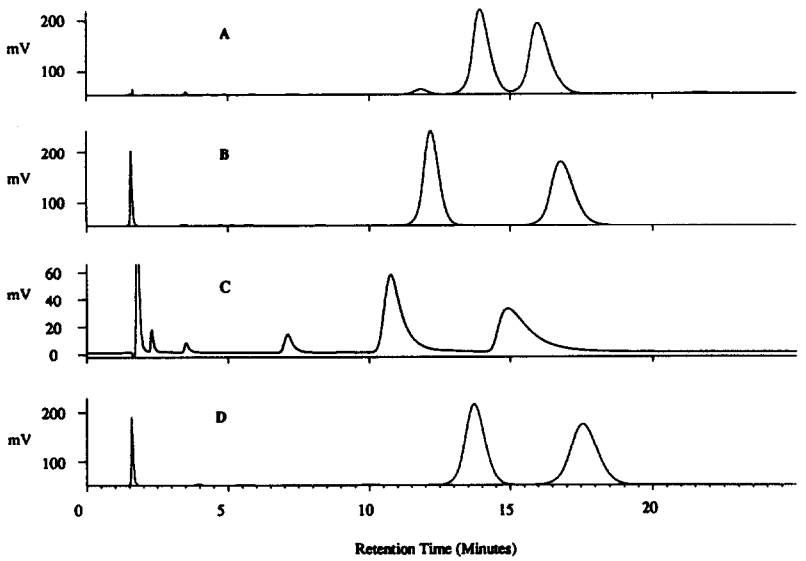

Fig. 3. Chiral HPLC chromatograms of the racemic mixture of (A) **3**, (B) **4**, (C) **5** and (D) **1** and **2** on Chiralcel OD column. The HPLC conditions are: mobile phase *n*-hexane–isopropanol–trifluoroacetic acid (96.5:3.5:0.1), flow-rate 2 ml/min, UV detection at 215 nm.

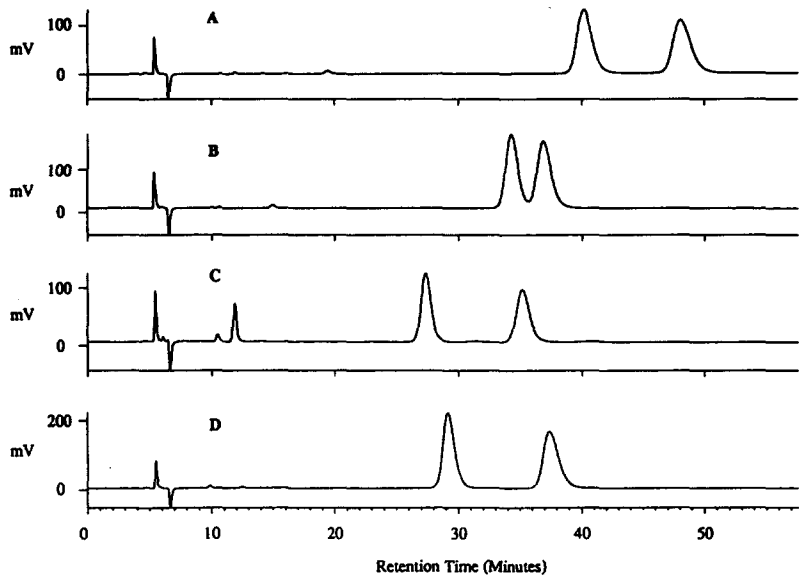

Fig. 4. Chiral HPLC chromatograms of the racemic mixture of (A) **3**, (B) **4**, (C) **5** and (D) **1** and **2** on Chiralcel AD column. The HPLC conditions are: mobile phase *n*-hexane–isopropanol–trifluoroacetic acid (94:6:0.1), flow-rate 0.6 ml/min, UV detection at 215 nm.

The separations of the dimethyl esters of racemic mixture of **1** and **2**, **3** as well as **4** on the OD, AD and AS columns are shown in Figs. 6, 7 and 8 and Table II. As expected, methylation of the carboxylic acids reduced the polarity of these compounds, resulting in weaker retention on the

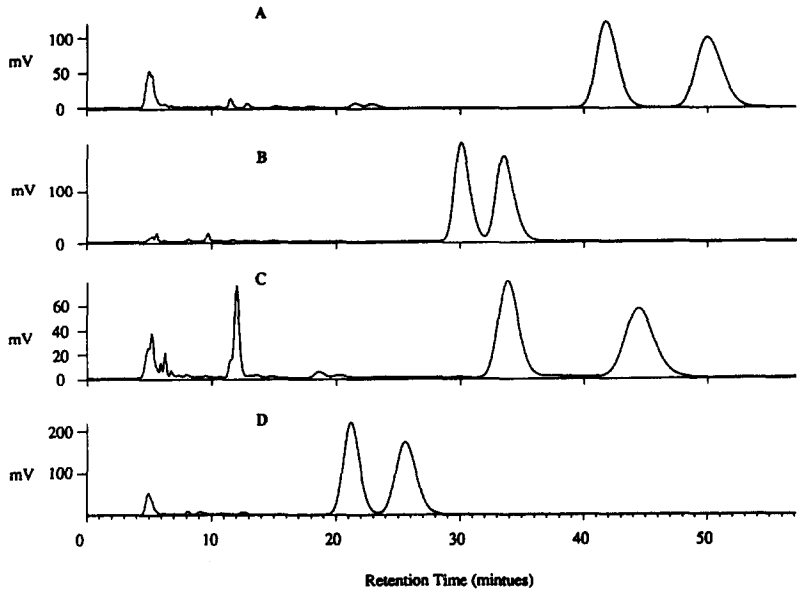

Fig. 5. Chiral HPLC chromatograms of the racemic mixture of (A) **3**, (B) **4**, (C) **5** and (D) **1** and **2** on Chiralpak AS column. The HPLC conditions are: mobile phase *n*-hexane–isopropanol–trifluoroacetic acid (88:12:0.1), flow-rate 0.6 ml/min, UV detection at 215 nm.

TABLE I

CHIRAL RESOLUTION OF RACEMIC MIXTURES OF 1 AND 2, 3, 4 AND 5 ON CHIRALCEL OD, AD AND CHIRALPAK AS COLUMNS

k'_1 = Capacity factor of the first eluted isomer; R_s = Resolution factor = 2 (difference of retention times of (+) and (−) isomers/the band widths of the two peaks).

	OD Column		AD Column		AS Column	
	k'_1	R_s	k'_1	R_s	k'_1	R_s
1 and 2	8.1	2.5	5.5	4.0	4.1	1.6
3	8.3	1.6	7.9	3.2	8.9	2.3
4	7.1	3.7	6.6	1.3	6.2	1.4
5	6.2	2.9	5.1	4.4	7.1	2.8

cellulosic columns. Accordingly, the mobile phase flow rate on OD column was reduced by half. Methylation of the carboxylic acids in **1** and **2** also significantly affected the resolution of the two enantiomers on OD column, resulting in a shift of resolution factor from 2.5 for the free diacids to 5.4 for the dimethyl ester. Although the free diacids **1** and **2**, **3** as well as **4** were successfully separated on the OD column, the corresponding dimethyl esters could not be resolved on the AS column. Hydrogen bonding between the acid proton of the analyte and the chiral stationary phase, therefore, appeared critical for chiral recognition on the AS column. The capacity factor of these dimethyl esters on the AS column (Table II) also showed an interesting trend, with the capacity factor for the side chain substitution: unsubstituted > methyl > phenyl-octyl. The greater hydrophorbicity of the side chain resulted in weaker retention on the AS column. No clear trend was observed on the OD column.

Separation of other leukotriene antagonists, e.g., Merck's MK-571 required prior derivatization with a chiral derivatizating agent [10]. Merck's L-699392, on the other hand, was separated by normal-phase chromatography on a diol column using a mobile phase consisting of methylene chloride and *n*-propanol containing quinine as the chiral selector [11]. Lily's close structural analogue to **1** [12] was separated on a chiral α_1-acid glycoprotein column. Our attempts to separate SK&F 107310, the methoxy sulfonyl derivative that is structurally related to **1** on all commercially available cellulosic columns, were also unsuccessful.

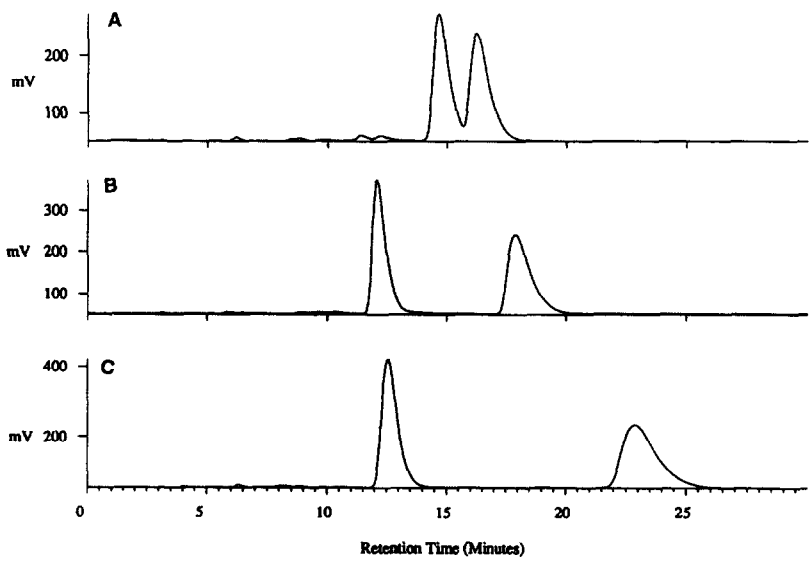

Fig. 6. Chiral HPLC chromatograms of the dimethyl ester of (A) **3**, (B) **4** and (C) racemic mixture of **1** and **2** on Chiralcel OD column. The HPLC conditions are: mobile phase *n*-hexane–isopropanol–trifluoroacetic acid (96.5:3.5:0.1), flow-rate 1 ml/min, UV detection at 215 nm.

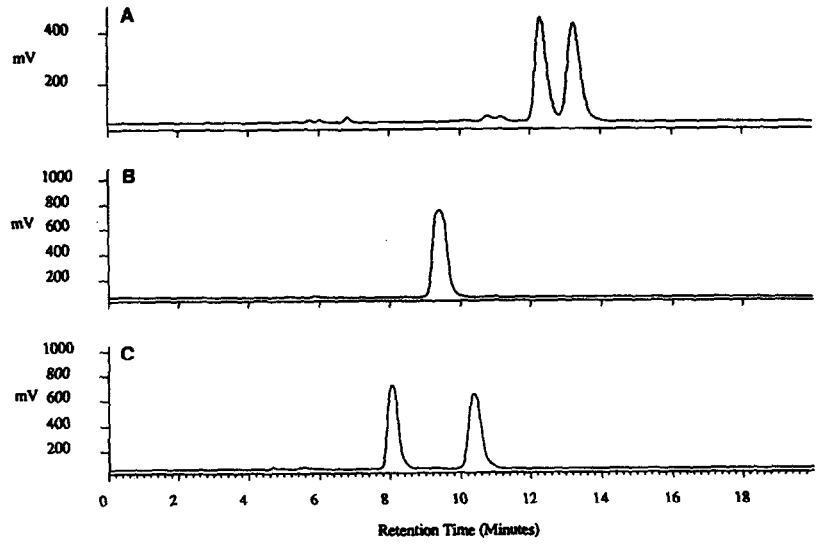

Fig. 7. Chiral HPLC chromatograms of the dimethyl esters of (A) **3**, (B) **4** and (C) racemic mixture of **1** and **2** on Chiralcel AD column. The HPLC conditions are: mobile phase: *n*-hexane–isopropanol–trifluoroacetic acid (96.5:3.5:0.1), flow-rate: 1 ml/min, UV detection at 215 nm.

CONCLUSIONS

SK&F 106203 (**1**) and its homologues can be directly separated on Chiralcel OD, Chiralpak AD and AS column without prior derivatization. Although well separated on the Chiralcel OD column, the dimethyl esters of **1** and its homologues could not be separated on an Chiralpak AS column. Hydrogen bonding between the acid proton of the analyte and the chiral stationary

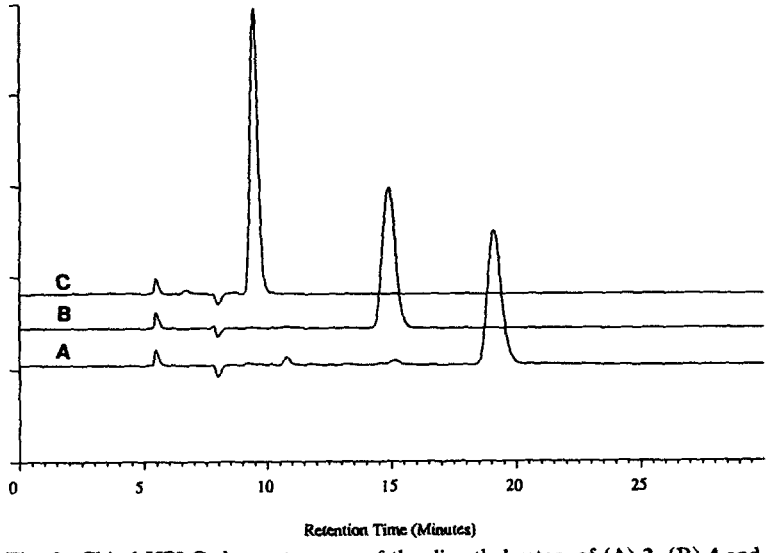

Fig. 8. Chiral HPLC chromatograms of the dimethyl esters of (A) **3**, (B) **4** and (C) racemic mixture of **1** and **2** on Chiralcel AS column. The HPLC conditions are: mobile phase *n*-hexane–isopropanol–trifluoroacetic acid (88.2:11.8:0.1), flow-rate 0.6 ml/min, UV detection at 215 nm.

TABLE II

CHIRAL RESOLUTION OF THE DIMETHYL ESTERS OF RACEMIC **1** AND **2**, **3** AND **4** ON CHIRALCEL OD, AD AND CHIRALPAK AS COLUMNS

– = No separation.

	OD Column		AD Column		AS Column	
	k'_1	R_s	k'_1	R_s	k'_1	R_s
1 and **2**	4.5	5.4	2.1	4.1	1.3	–
3	5.4	4.2	3.6	1.5	2.5	–
4	4.3	1.2	2.6	–	3.6	–

phase appeared critical for chiral recognition on the AS column. The elution order of **1** on Chiralcel OD column can be reversed by performing the chromatography on an Chiralpak AS column. Further work is in progress to probe the generality of this elution order reversal, and the results will be reported in a future communication.

ACKNOWLEDGEMENTS

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