Separation of Warfarin Enantiomers by Capillary Gas Chromatography with Chiral Stationary Phase

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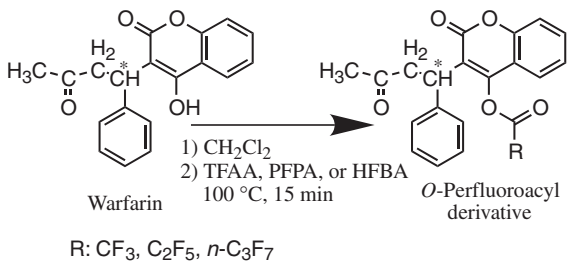
Warfarin was converted into *O*-perfluoroacyl derivatives and separated into enantiomers by capillary GC with chiral stationary phase of polydimethylsiloxane anchored with *S*(−)-*t*-leucine-*S*(−)-1-phenylethylamide. All the derivatives showed almost complete separation. Especially in the case of *O*-heptafluorobutyryl derivative, baseline separation could be observed.

Warfarin is a representative anticoagulation pharmaceutical that has been prescribed frequently for the treatment of various types of thromboembolic disease. Warfarin has one asymmetric center. Enantiomeric properties are of utmost importance in the activity of pharmaceuticals. In case of warfarin, the *S*(−)-enantiomer is said to be more effective and metabolized faster than the *R*(+)-enantiomer.[1]

Usually, enantiomeric analysis of pharmaceuticals is carried out by the HPLC method.[2] However, contrary to the HPLC method, we have been engaged in the GC method for separation of pharmaceutical enantiomers.[3] The principal merits of the GC over HPLC method are (1) the possibility of employing widely spread GC–MS which can easily identify the objective compounds present in complex matrices, (2) the lower capital and operating costs of GC than those of HPLC. Pharmaceuticals which can be provided for GC analysis are limited by the vapor pressure, that is, the larger the molecular size, the lower the vapor pressure. The thermal stability of the pharmaceuticals also limits the use of GC method. In case of enantiomer separation, enantioselectivity of the column also plays a dominant factor. However, the enantioselectivity of the column decreased thermodynamically with the increase of the column temperature, and at the column temperature of over 200 °C, the apparent enantioselectivity has become almost lost in most cases.

In previous papers, we have described the preparation of chiral stationary phases.[4] These phases like once called Chirasil–Val, but greatly improved the thermal stability and enantioselectivity by more statistically distributing the chiral selectors along the polymer chain. The phases have been prepared by



Scheme 1. Derivatization of warfarin. Asterisk denotes an asymmetric center.

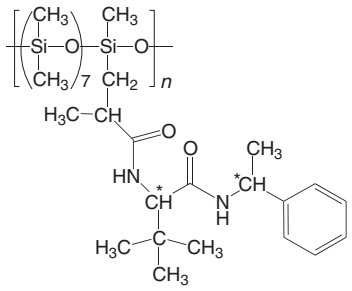


Figure 1. Structure of the polydimethylsiloxane anchored with *S*(−)-*t*-leucine-*S*(−)-1-phenylethylamide. Asterisk denotes an asymmetric center.

block condensation of 1,5-bis(diethylamino)-hexamethyltrisiloxane and 2′,2′,2′-trifluoroethyl (3-dichloromethylsilyl)-2-methylpropionate followed by nucleophilic displacement of the functionalized ester with various chiral selectors. Chiral phases prepared by this method were found to be efficient for the separation of pharmaceutical enantiomers.[5] Propranolol (MW: 259) and Pindolol (MW: 248), in spite of their low volatilities, could be separated into their enantiomeric pair by converting them into *N*,*O*-perfluoroacyl derivatives up to the column temperature of 180 °C.

Considering the effectiveness of the phases for the separation of pharmaceutical enantiomers, we have developed a novel approach to the separation of less volatile warfarin (MW: 308) enantiomers at higher column temperature.

The experimental procedure is as follows:

Standard warfarin solution of 50-μL aliquot containing *RS*- or *R*-warfarin to a concentration of 0.5 w/v % in methanol was pipetted into a Reacti-Vial (1-mL volume). The vial was evaporated to dryness with nitrogen stream at 100 °C. After standing the vial for about 5 min under room temperature, 50 μL of dichloromethane and 50 μL of trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), or heptafluorobutyric anhydride (HFBA) were added and tightly capped. The vial was then heated at 100 °C for 15 min. After the vial was cooled down to room temperature, the cap was opened, and excess reagents were evaporated to dryness with a gentle stream of nitrogen by heating at 100 °C. The resulting warfarin derivative was dissolved in 30 μL of ethyl acetate, and 1–2 μL of the solution was injected into the GC. The derivatization process is shown in Scheme 1.

Fused silica capillary (10 m × 0.25 mm) was first deactivated and then coated with a stationary phase of polydimethylsiloxane anchored with chiral selector of *S*(−)-*t*-leucine-*S*(−)-1-phenylethylamide through amide bonding.[5] The structure of the phase is shown in Figure 1.[6] The column was conditioned

Table 1. Separation data of the *O*-perfluoroacyl warfarin enantiomers

Derivative	RT of the *R*-enantiomer /min	RT of the *S*-enantiomer /min	α	Rs
O-TFA	16.26	16.80	1.0349	1.37
O-PFP	17.99	18.63	1.0374	1.52
O-HFB	17.74	18.37	1.0367	1.59

RT: retention time; α: separation factor; Rs: resolution; Column temp: 210 °C; Retention time of the unretained peak:[7] 0.675 min; For other conditions, see Figure 2.

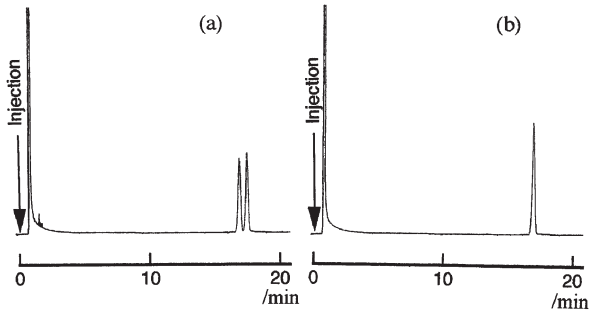


Figure 2. Gas chromatograms of *O*-heptafluorobutyryl derivatives of warfarin enantiomers prepared from (a) *RS*-warfarin and (b) *R*-warfarin. Column: Fused silica capillary (10 m × 0.25 mm) coated with polydimethylsiloxane anchored with *S*(−)-*t*-leucine-*S*(−)-1-phenylethylamide; Film thickness: 0.15 μm; Column temp: 210 °C, isothermal; Injection temp: 260 °C; Carrier gas: He, 1.0 kg/cm^2; Detector: FID; Split ratio: 1:40. The *R*-enantiomer elutes prior to the *S*-enantiomer.

at 240 °C overnight before use.

Table 1 represents retention times (RT), separation factors (α), and resolutions (Rs) of the 3 derivatives of warfarin enantiomers, respectively. In comparison of the 3 derivatives, RT and α increased either in order of *O*-TFA, *O*-HFB, and *O*-PFP derivatives. Rs increased in order of *O*-TFA, *O*-PFP, and *O*-HFB derivatives. As a result, warfarin enantiomeric isomer as its *O*-HFB derivative was found to give maximum Rs value. Figure 2 shows a typical GC of *O*-HFB derivatives of warfarin enantiomers prepared from (a) *RS*-warfarin and (b) *R*-warfarin. As can be seen from the Figure 2a, the *RS*-warfarin was clearly separated into enantiomers. Moreover, no racemization was involved in the course of this experiment as recognized from the Figure 2b.

The separation mechanism is not yet fully understood. The enantioselectivity of the diamide selector towards the racemic warfarin is presumed to result from the spatial requirement of the hydrogen bonding enantioselective associate between selector and selectand.

We also tried the separation of *RS*-warfarin as its non-derivatized form on the same capillary column. However, the separation remained unsuccessful.

In conclusion, chiral GC stationary phase of polydimethylsiloxane anchored with *S*(−)-*t*-leucine-*S*(−)-1-phenylethylamide was found to give high enantioselectivity even at high temperature of over 200 °C. This method suggests the applicability of chiral capillary GC for separation of enantiomeric isomers to a wide range of racemic compounds containing functional group(s) capable of hydrogen bonding between sample and the phase as their non-derivatized or derivatized forms, such as amino acids, amines, aminoalcohols, peptides, hydroxy acids, etc.

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