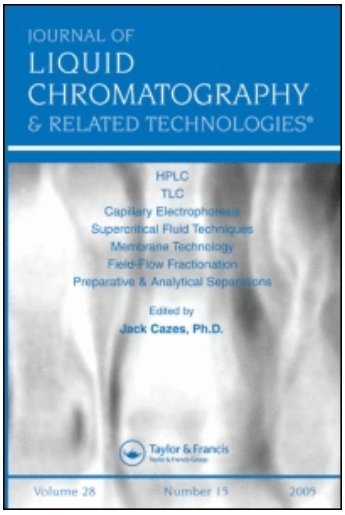

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SELF-NORMALIZED ANALYSIS OF LIPASE-CATALYZED CONVERSION OF NAPROXEN ENANTIOMERS

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ABSTRACT

Improved equations were derived to determine the conversion of Naproxen enantiomers or racemate by lipase-catalyzed esterification in an organic solvent. From the resultant ratio of peak areas for Naproxen and Naproxen ester, an accurate quantification of the conversion was obtained by a commercially available HPLC chiral stationary phase (CSP).

INTRODUCTION

2-Arylpropionic acids (profens) are an important group of non-steroidal anti-inflammatory drugs (NSAIDs) which have a chiral center within the propionic acid moiety. Past studies on pharmacological properties of these acids showed that S-isomers often have higher activities than R-isomers. Although FDA is still in a position that drug companies may sell chiral drugs as racemates or as single enantiomers, the vigorous justification for FDA approval of the former would push the companies to develop only the latter.[1] Now as the only member of these important acids to be sold as a single enantiomer, Naproxen has

received much attention from the researchers of asymmertric synthesis and resolution method.[2]

Various strategies have been proposed to resolve the racemic mixture of Naproxen, e.g. through the selective diastereoisomer or preferential crystallization, or by the chromatographic separation.[3,4] Recently enzymatic hydrolytic resolution of S-isomer of Naproxen esters by *Candida cylindracea* lipase (triacylglycerol ester hydrolases, E. C. 3.1.1.3) was addressed.[5,6] In order to fit the kinetic constants of reaction from experimental data[7], it is convenient to monitor all substrate and product concentrations varied with time by HPLC using CSP. Usually an internal standard with similar structure to the compounds of interest is added to compensate for various systematic errors in injection volume, extraction efficiency and other quantitative problems. Since R-isomer of the substrate has slower reaction rate than S-isomer at the initial stage, it is difficult to accurately determine the product concentration of R-isomer with time. Therefore herein is described an improved equation for the determination of conversion of each enantiomer or racemate by HPLC. Esterification of Naproxen by the lipase in an organic solvent was applied to test the validity of the resultant equation.

BACKGROUND

Assume all peaks are baseline resolved and the peak area A_{jk} for each species is proportionally related to the concentration M_{jk} as follows:

$$A_{jk} = f_j M_{jk} \qquad j = e, n \quad ; k = R, S \tag{1}$$

where the subscripts e, n, R and S represent Naproxen ester,

Naproxen, R-isomer and S-isomer respectively. Thus M_{eR} means the concentration of R-Naproxen ester. When UV detector is applied, the proportional constant f_j will be independent of each enantiomer of a racemate. From the definition of conversion for each enantiomer C_k, we may obtain

$$C_k = M_{ek}(M_{nk} + M_{ek})^{-1} = A_{ek}(A_{ek} + A_{nk}b^{-1})^{-1} \quad (2)$$

in which " b " is the ratio of $f_n f_e^{-1}$. Above equation may be further rearranged as

$$C_k^{-1} - 1 = (A_{nk}A_{ek}^{-1})b^{-1} \quad (3)$$

Consequently once the constant " b " has been determined, one may use this equation to find the conversion C_k from the data of area ratio $A_{nk}A_{ek}^{-1}$, and then the concentrations M_{ek} and M_{nk} from equation (2) and the conservation for the enantiomers of Naproxen and Naproxen ester. Moreover when A_{nk} and A_{ek} are replaced by $(A_{nR} + A_{nS})$ and $(A_{eR} + A_{eS})$ respectively, the conversion of racemate (C) is obtained from this equation by dropping out the subscript of C_k.

When an internal standard is applied, One may also calculate the conversion of racemate from

$$C = 1 - ((A_{nR} + A_{nS})A_i^{-1})/((A_{nR} + A_{nS})A_i^{-1})_o \quad (4)$$

where A_i is the peak area of the internal standard and the subscript " o " is for the initial condition. Of course it is easy to derive the conversion of each enantiomer as follows:

$$C_k = 1 - (A_{nk}A_i^{-1})/(A_{nk}A_i^{-1})_o \quad (5)$$

Consequently the advantage of using equation (3) but not equation (5) for quantification is evident, because the change of the

ratio $A_{nk}A_{ek}^{-1}$ is more sensitive to that of $A_{nk}A_i^{-1}$ or $A_{ek}A_i^{-1}$ to calculate the conversion C_k for low product concentrations.

MATERIALS AND METHODS

Materials

Optical pure S-Naproxen is the product of Alfa Chemical Co.. Lipase of type VII from *Candida cylindracea* (1010 U mg^{-1}) was purchased from Sigma Chemical Co.. Other reagents and solvents include sodium hydroxide (Wako Pure Chemical Ind.), n-Propanol, cyclehexanol, n-octanol (Hayashi Pure Chemical Ind.), Naphthalene (Jansson Chimica), anhyrous acetic acid, chloroform (Merck), 3-chloro-propanol (Sigma), and n-hexane, isooctane, isopropanol (Alps Chemical Co., Taiwan).

Apparatus

Chromatographic analysis was performed using a Alcott 760 HPLC pump, a Soma S-3702 variable-wavelength UV detector, and a Sic Chromatocorder 12 integrator. The analytical column (Chiralcel OD) was obtained from Daicel Chemical Ind. LTD.. The mobile phase was a v/v mixture of 97% n-hexane, 3% isopropanol and 1% acetic acid, at a flow rate of 0.4 ml min^{-1}. UV detection at 270 nm was used for quantification at the ambient temperature.

Calibration Line with Adding an Internal Standard

The S-isomer of Naproxen was racemized at 140 ^{o}C in ethylene glycol with sodium hydroxide for 4 hours and successively added distilled water and acidified to pH 4.0, giving a precipitate of Naproxen racemate. After drying by vacuum and dissolving in chloroform, the optical rotation of the racemate was measured as zero. Complete racemization was also checked from giving two peaks of the same area of the racemate by the above HPLC system.

A calibration line for Naproxen racemate with naphthalene as the internal standard was prepared at the ambient temperature. Thus we obtained the ratio of peak areas, $(A_{nR} + A_{nS})A_i^{-1}$, against the ratio of concentrations, $(M_{nR} + M_{nS})M_i^{-1}$, with M_i to be the naphthalene concentration.

Determination of the ratio " b "

To 0.05 mM of Naproxen racemate and 100 mM of various alcohols, dissolved in isooctane saturated with 0.1 N phosphate buffer (pH 7.0), was added 20 mg of crude lipase. The reaction mixture was stirred at 37 ^{o}C, and the progress was monitored by injecting the sample to the above HPLC system for different periods of time. Equation (4) was applied to find the conversion of racemate from the measurement of $(A_{nR} + A_{nS})A_i^{-1}$. Then using the resultant conversion against the data of $(A_{nR} + A_{nS})(A_{eR} + A_{eS})^{-1}$, we may find the ratio " b " via a linear regression technique.

RESULTS AND DISCUSSION

Typical chromatograms of naphthalene, Naproxen, Naproxen n-propyl and 3-chloropropyl esters are shown in Figure 1. It can readily be seen that resolution is sufficient for precise enantiomeric purity determination, as shown in Figure 1 (a). No improved resolution was found for Naproxen esters derived from primary, secondary or tertiary alcohols, unless an electron-withdrawing group such as a chlorine atom is present in the alcohol moiety as shown in Figure 1 (b). One may attribute this improvement of chiral recognition to the additional hydrogen bond and dipole-dipole interactions between the chlorine atom of Naproxen ester and the carbamate group of CSP.[8]

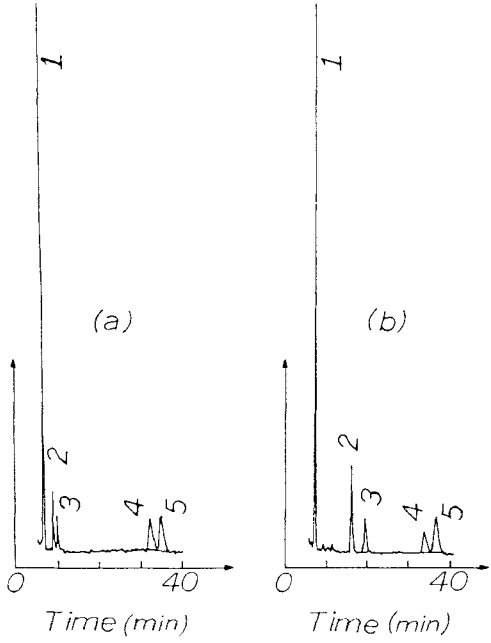

Figure 1: Separation of (a) Naproxen and n-propyl Naproxen ester, (b) Naproxen and 3-chloropropyl Naproxen ester with naphthalene as internal standard: (1) naphthalene, (2) R-isomer of ester, (3) S-isomer of ester, (4) R-Naproxen enantiomer, (5) S-Naproxen enantiomer.

Figure 2 shows the variation of the ratio of peak areas, i.e. $(A_{nR} + A_{nS})A_i^{-1}$, against the ratio of concentration $(M_{nR} + M_{nS})M_i^{-1}$. A straight line with the slope of one shows that the absorbance at 270 nm is due to the naphthalene group. Thus one may anticipate that both Naproxen and Naproxen alkyl (or cyclic) ester will have the same absorbance. Hence the ratio " b " should be equal to one.

Figure 3 shows the change of the ratio $(A_{nR} + A_{nS})(A_{eR} + A_{eS})^{-1}$ against the term $(C^{-1} - 1)$ for various Naproxen

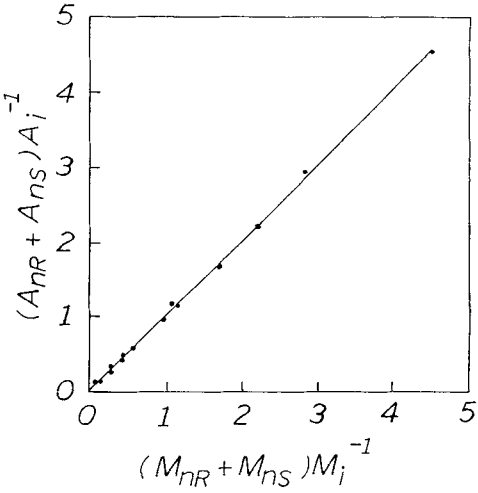

Figure 2: Calibration line of $(A_{nR} + A_{nS})A_i^{-1}$ against $(M_{nR} + M_{nS})M_i^{-1}$ for Naproxen racemate with naphthalene as internal standard.

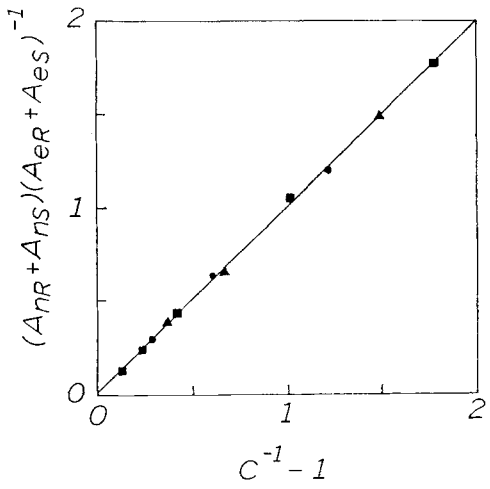

Figure 3: Variation of the ratio $(A_{nR} + A_{nS})(A_{eR} + A_{eS})^{-1}$ against the term $(C^{-1} - 1)$ for some Naproxen esters derived from: (■) n-propanol, (●) cyclehexanol, (▲) n-octanol.

derivatives. A linear regression to the experimental data gave a line with the slope of one through the origin. Thus one obtained the ratio " b " to be one, as expected. The present analysis has also been extended to other Naproxen esters derived from the alkyl alcohols such as methanol, ethonol, isopropnol, n-butanol, isobutanol, t-butanol and n-hexanol, although the data are not shown here. Thus a further investigation on the determination of this ratio for the Naproxen ester with an aryl group in the alcohol moiety should be studied. We also expect that the present result may be extended to other propionic acids.

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