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COMMUNICATION

# Normal-Phase TLC Separation of Enantiomers of 1.4-Dihydropyridine Derivatives

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## ABSTRACT

*A new TLC-based method was proposed for the separation of enantiomers and mixtures of racemic DHP derivatives differing in the kind of substituent in the phenyl ring. The conditions for the effective determination of the substances involved and the mechanism of their sorption were also studied. For the separation of felodipine, nilvadipine, and isradipine enantiomers, thin-layer chromatography was used, with a chiral stationary phase of the ligand exchange type, and developing phases of a different concentration of methanol (Φ) as an organic modifier. The retention coefficient values $k'$ were used to make the plots $\log k' = f(\log \Phi)$ and $\log k' = f(\Phi)$. The processes taking place in the chromatographic systems were shown to be described by the Snyder–Soczewiński equation.*

## INTRODUCTION

Recently much attention has been directed to the spatial configuration of the drug molecule determined by the existence of isomers, mainly because of the fact that the activity and effectiveness of the chiral molecules largely depend on their configuration.

Except for nifedipine and lacidipine, all other DHP calcium channel blockers currently approved for use in humans are chiral compounds and have been clinically evaluated as racemic mixtures of two enantiomers. It has been shown that particular optical isomers of DHP derivatives can substantially differ in the pharmacological effect despite the same chemical structure (1–7). The center of chirality of these compounds is the tetrahedral carbon atom $C_4$ in the DHP ring. The literature provides much information on different pharmacological activity of DHP enantiomers [10,11]. According to Mikus et al., [12] as well as to earlier observations by Soons [13,14], the level of the active stereoisomer of a DHP derivative in human blood may very significantly and depends on whether the patient has been given a single enantiomer or a racemate.

Results of the study on pharmacokinetics of FL enantiomers have indicated that isomers differed in absorption and metabolism. According to the results of the clinical tests, the isomer *S*(−)FL is approximately five times more active as a peripheral vasodilatator than is *R*(+)FL in oral administration [15].

In 1988 Tokuma et al. [16] first reported the stereoselective pharmacokinetics in humans of a DHP calcium antagonist, nilvadipine. It was shown that the plasma concentrations of the pharmacological more potent *S*-enantiomer were approximately three times higher than those of the *R*-enantiomer. Thereafter, the stereochemical aspects of the pharmacokinetics of nilvadipine and other DHP calcium antagonists were studied extensively.

Tokuma et al. [17,18] examined the pharmacokinetics of nilvadipine enantiomers in healthy subjects, dogs, and male and female rats who have been orally administered racemic nilvadipine, and the results showed that AUC and $C_{max}$ were higher for more potent *S*(+) nilvadipine in humans and in dogs.

The pharmacokinetics parameters of isradipine enantiomers and their activities were also found to differ. In vitro *S*(+) isradipine is approximately 160 times more active than is *R*(−) isradipine. A study on rabbits proved that *S*(+) isradipine was twice more active when given alone than when given as a racemic mixture, and *R*(−) isradipine activity is only 1/200 of that of *S*(+) enantiomer [19–21]. For many other dihydropyridines, stereoselective differences in pharmacokinetics have already been documented [22–28].

## MATERIALS AND METHODS

Felodipine (FL) (Cipla, Bombay India); felodipine *R*(+) and *S*(−) (Astra Hässle AB, Mölndal, Sweden); isradipine (IS) Schwarz Pharma AG, Monheim; nilvadipine (NV) (Klinge Pharma.); methanol and acetonitryl were of HPLC grade. Other reagents were of analytical grade.

### Thin-Layer Chromatography

Chromatographic plates were covered with 0.3 $\mu$L of solutions of FL, FL*R*(+), FL*S*(−), IS, and NV at a concentration of $3.039 \times 10^{-4}$ mol/dm$^3$. The plates were made by Macherey-Nagel (Chiralplate), 10 × 3 cm in size, and the thickness of the deposited layer was 0.2 mm. The plates were developed over a 9-cm section in a saturated classical chamber in the dark. The spots were visualised in the UV lamp radiation ($\lambda$ = 300–400 nm).

The plates were developed using two kinds of mobile phases, containing methanol as an organic modifier ($\Phi$): Phase 1, chloroform:25% ammonia:methanol (MtOH) = 10:0.02:$\Phi$; and Phase 2, acetonitrile (ACN):0.1% triethylamine (TEA):methanol = 5:3:$\Phi$.

The calculated values of $R_F$ were used for the determination of retention coefficients $k'$ of the DHP derivatives studied, Tables 1 and 2. The results were presented as a dependence of the log of $k'$ on the log of the molar fraction ($\Phi$) of the organic modificator in the mobile phase, so as a plot of the function $\log k' = f(\log \Phi)$, and the parameters describing the dependencies were collected in Table 3.

The mechanism of adsorption was studied assuming the validity of the Snyder–Soczewiński equation. The equation was derived for the situation of formation of reversible molecular complexes among the functional groups present on the surface of the adsorbent and the adsorbate. The basic form of the equation is:

$$\log k' = \text{const.} - m \cdot \log \Phi \quad (1)$$

where $\Phi$ is the molar fraction of methanol, $k'$ is retention coefficient, const. = $R_M$ (retention in methanol), and $m$ is the direction cosine of the line.

## RESULTS AND DISCUSSION

Chromatographic methods have been considered highly suitable for the selection of enantiomers. In this study, the so-called direct method of separation was applied, which involves dynamic formation of diastereoisomer derivatives during the HPLC analysis [23]. To enable the separation of a racemic mixture, the enantiomers were differentiated using the chiral selectors included in the stationary phase.

The separation of the enantiomers with the use of a stationary phase can be called the ligand exchange chromatography (LEC). In this system, the formation of diastereoisomeric complexes composed of a DHP derivative enantiomer, selector (enantiomer of aminoacid, propline), and $Cu^{2+}$ ions occurred. The separation of enantiomers by the LEC method is determined by the two mechanisms: the first is different stability of the complexes enantiomer-metal ion, selector, whereas the second is different adsorption of the three-component diastereoisomer complexes on a chemically bonded stationary phase.

The substances separated by the LEC method should contain two groups of different spatial arrangement, which would play the role of ligands complexing the metal ions, and an additional element most often sterical, which would allow differentiation among the enantiomers.

One of the most important problems in chromatography, including the thin-layer type, is the optimization of the process leading to a control of retention expressed as

***Table 1.***

*Retention Parameters of DHP Derivatives Versus the Amount of Methanol (Φ) in the Mobile Phase I ($CHCl_3$:$NH_4OH$:Φ)[a]*

| | Φ | | | |
|---|---|---|---|---|
| | $1.9570 \times 10^{-2}$ [b] | $2.9069 \times 10^{-2}$ [c] | $4.7528 \times 10^{-2}$ [d] | $9.0744 \times 10^{-2}$ [e] |
| NV | | | | |
| $R_F$ | 0.8020 | 0.8362 | 0.8980 | 0.940 |
| $k'$ | 0.2469 | 0.1959 | 0.1136 | 0.0638 |
| log $k'$ | −0.6075 | −0.7080 | −0.9447 | −1.1950 |
| FL | | | | |
| $R_F$ | 0.6550 | 0.8163 | 0.8570 | 0.9300 |
| $k'$ | 0.5267 | 0.2250 | 0.1669 | 0.0753 |
| log $k'$ | −0.2784 | −0.6477 | −0.7776 | −1.1233 |
| $FL_R$ | | | | |
| $R_F$ | 0.6200 | 0.8074 | 0.8430 | 0.9350 |
| $k'$ | 0.6229 | 0.2385 | 0.1862 | 0.0695 |
| log $k'$ | −0.2126 | −0.6224 | −0.7299 | −1.1579 |
| $FL_S$ | | | | |
| $R_F$ | 0.6400 | 0.8150 | 0.8509 | 0.9230 |
| $k'$ | 0.5625 | 0.2270 | 0.1752 | 0.0834 |
| log $k'$ | −0.2499 | −0.6440 | −0.7564 | −1.0787 |

[a] $CHCl_3$: Chloroform; $NH_4OH$: 25% Ammonia.
[b] log Φ = −1.7084; [c] log Φ = −1.5366; [d] logΦ = −1.3230; [e] log Φ = −1.0422.

***Table 2.***

*Retention Parameters of DHP Derivatives Versus the Amount of Methanol (Φ) in the Mobile Phase II (ACN:TEA:Φ)[a]*

| | Φ | | | | |
|---|---|---|---|---|---|
| | $1.11 \times 10^{-1}$ [b] | $2.0 \times 10^{-1}$ [c] | $3.8461 \times 10^{-1}$ [d] | $4.666 \times 10^{-1}$ [e] | $5.151 \times 10^{-1}$ [f] |
| NV | | | | | |
| $R_F$ | 0.3640 | 0.4040 | 0.5045 | 0.5769 | 0.6689 |
| $k'$ | 1.7473 | 1.4752 | 0.9822 | 0.7334 | 0.4950 |
| log $k'$ | 0.2423 | 0.1689 | −0.0078 | −0.1346 | −0.3054 |
| IS | | | | | |
| $R_F$ | 0.4125 | 0.4200 | 0.4877 | 0.5538 | 0.6054 |
| $k'$ | 1.4242 | 1.3809 | 1.0504 | 0.8057 | 0.6518 |
| log $k'$ | 0.1536 | 0.1402 | 0.0214 | −0.0938 | −0.1859 |
| FL | | | | | |
| $R_F$ | 0.3617 | 0.4205 | 0.4340 | 0.5032 | 0.4915 |
| $k'$ | 1.7647 | 1.3781 | 1.3041 | 0.9873 | 0.0346 |
| log $k'$ | 0.2467 | 0.1393 | 0.1153 | 0.0055 | 0.0147 |
| $FL_R$ | | | | | |
| $R_F$ | 0.3713 | 0.4000 | 0.4300 | 0.4942 | 0.4930 |
| $k'$ | 1.6932 | 1.5000 | 1.3256 | 1.0235 | 1.0284 |
| log $k'$ | 0.2287 | 0.1761 | 0.1224 | 0.0101 | 0.0122 |
| $FL_S$ | | | | | |
| $R_F$ | 0.3550 | 0.4000 | 0.4271 | 0.5006 | 0.5014 |
| $k'$ | 1.8169 | 1.5000 | 1.3414 | 0.9976 | 0.9944 |
| log $k'$ | 0.2593 | 0.1761 | 0.1275 | −0.0010 | −0.0024 |

[a] ACN: acetonitryl; TEA: triethyloamine.
[b] log Φ = −0.95430; [c] log Φ = −0.6990; [d] log Φ = −0.4150; [e] log Φ = −0.3910; [f] logΦ = 0.2880.

***Table 3.***

*Parameters of the Plots of log k′ Versus Increasing Fraction of Methanol (Φ) in the Mobile Phase for Selected 1,4-Dihydropyridine Derivatives*

| DHP Derivatives | $\log k' = f(\log \Phi)$ | | | |
|---|---|---|---|---|
| | Mobile Phase 1 | | Mobile Phase 2 | |
| | *m* | const. | *m* | const. |
| NV | −1.4075 | −2.7368 | −0.7576 | −0.4236 |
| FL | −1.4076 | −2.7369 | −0.3374 | −0.0811 |
| FL *R*(+) | −1.3196 | −2.5315 | −0.3280 | −0.0703 |
| FL *S*(−) | −1.1552 | −2.3025 | −0.3861 | −0.1002 |

*m*: the direction cosine of the line; const.: constant of the Snyder–Soczewiński equation (retention in methanol).

the value of $k'$. The control of the $k'$ value was achieved by adjustment of the qualitative and quantitative composition of the eluents applied. The effect of the mobile phase on the value of $k'$ depends on the mechanism of the separation taking place in the chromatographic system. Usually, two or more mechanisms act simultaneously. The most important effect of this assumption is the equation describing the dependence of the retention coefficient $k'$ of the chromatographed substance on the eluating force of the solvent used, known as the Snyder–Soczewiński equation. In the model, assumed adsorption of a molecule is a result of pushing one or a few molecules of the solvent from the surface of the adsorbent. The equation was derived for the adsorption model proposed by Soczewiński, assuming that the interactions in the mobile phase can be disregarded.

It is difficult to determine unambiguously the mechanism of retention taking place on the phases studied because it would be very hard to take into account all the factors (kind and concentration of the mobile phase, ionic strength, pH, $Cu^{2+}$ concentration) determining it.

The parameters of the chromatographic process suggest the retention mechanism typical of reversed phases. Therefore, the mobile phases composed of organic solvents with ammonia or triethylamine must have been more polar than the chemically bonded stationary phases.

The mechanism of adsorption of DHP derivative enantiomers was explained assuming the Snyder–Soczewiński equation. The results were presented as plots of $\log k' = f(\log \Phi)$, and the parameters of the lines suggested the mechanism of adsorption. The formation of complexes made of the enantiomer (adsorbent or solvent) adsorbent on the surface of the adsorbent as a result of pushing the molecules of the solvent out is manifested by the value of the direction cosine of the line $\log k' = f(\log \Phi)$. The agreement between the obtained experimental dependence and the corresponding plot of the Snyder–Soczewiński equation testifies to the fact that this equation well describes the chromatographic performance of the enantiomers studied. In many cases, a linear dependence of the log Φ on increasing amount of the modifier in the mobile phase, characterized by a good correlation coefficient (r), was obtained. As follows from the data in Table 3, for the DHP derivatives analyzed, the direction cosines m take fractional values. This fact can be explained by a mixed character of the mechanism of adsorption based on single and double bond. The character of interaction between the molecules of the adsorbate and the surface of the adsorbent depends on the number of stereoselective interactions, which are determined by the molecular structure of the adsorbent. It can be supposed that more than one molecule of the solvent are pushed out because of a relatively large size of FL and IS molecules. The value of direction cosine depends also on variable solvatation effect in the volume phase. The solvatation interactions blocking the active group of the molecule studied may be responsible for an increase of the direction cosine of the line $\log k' = f(\log \Phi)$.

As follows from the data in Table 3, much higher *m* values were obtained using a mobile phase containing chloroform. The direction cosine of the line $\log k' = f(\log \Phi)$ is a measure of the selectivity of the enantiomers separation. The differences in the direction cosines indicate that the selectivity $\Delta \log k'$ changes with the composition of the eluent. A quantitative measure of the selectivity is the separation coefficient $\beta = k_1/k_2 \geq 1$, determining the maximum difference $R_F$, which can be obtained in certain conditions. The selectivity is characteristic of a given system mobile phase/adsorbent and depends significantly on the structural difference between the separated compounds. For example the separation coefficients calculated for felodipine enantiomers were not much different from unity, $k_{FLR} = 1.32$, $k_{FLS} = 1.16$, so the separation coefficient is $\beta = 1.14$.

## CONCLUSION

The processes taking place in the chromatographic systems with the use of chiral stationary phase of the ligand exchange chromatography type can be described by the Snyder–Soczewinski equation.

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