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The study of enantioseparation of zolmitriptan on vancomycin-bonded chiral stationary phase

In this study, the chiral stationary phase was prepared by bonding vancomycin to 5 μm spherical silica gel according to "one-pot" synthetic strategies, and used to separate the enantiomers of zolmitriptan under polar ionic mode. The influences of mobile phase composition, such as the concentration and ratio of glacial acetic acid (HOAc) and triethylamine (TEA), on the enantioseparation were investigated, and the chiral recognition mechanism is discussed. It was found experimentally that the retention factors were increased with the increase of the HOAc/TEA concentration in a certain extent, and the ionic interactions, hydrogen bondings, and steric interactions may play key role together. The method is suitable for baseline separation of zolmitriptan enantiomers.

Key Words: Chiral recognition mechanism; Chiral stationary phase; Enantioseparation; Vancomycin; Zolmitriptan

Received: June 7, 2005; revised: July 22, 2005; accepted: July 25, 2005

DOI 10.1002/jssc.200500238

1 Introduction

The separation of enantiomers with HPLC has proven to be a most useful method for the analysis of numerous different chiral substances. The enantiomers of a chiral drug may have different pharmacological activities, *i. e.*, both pharmacokinetic and pharmacodynamic effects, and the administration of only a single enantiomer in high enantiomeric purity is the major goal of the pharmaceutical industry [1]. Zolmitriptan (Fig. 1.), a single enantiomer

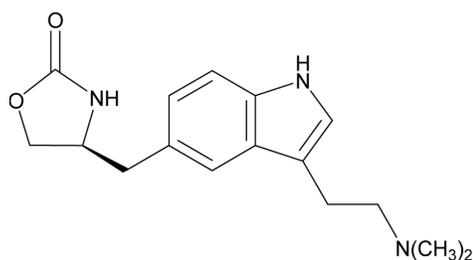


Figure 1. Chemical structure of zolmitriptan.

(4*S*)-4-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]-methyl]-2-oxazolidinone has recently been used in acute treatment of severe migraine and related vascular headaches [2]. The chiral separation of zolmitriptan is helpful both to study their biological activities and to control the enantio-

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Abbreviation: CSP, chiral stationary phase; HOAc, glacial acetic acid; TEA, triethylamine

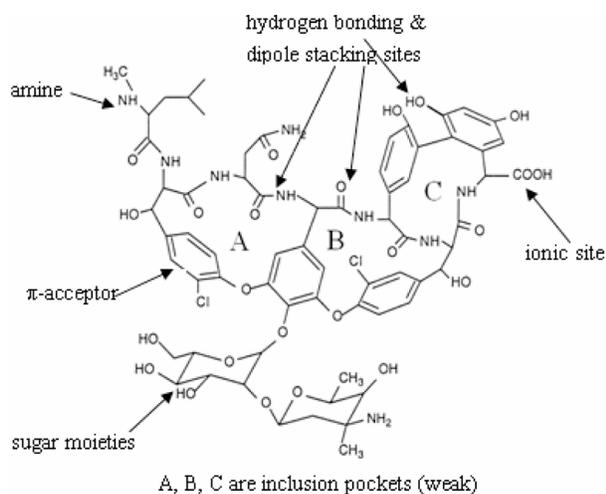


Figure 2. Proposed structure of vancomycin and enantioselective interactions [12].

meric purity of pharmaceutical formulations. However, as far as we know, only a few papers were reported in the literature for the enantiomeric separation of zolmitriptan using CE with β -CD as chiral additives or using HPLC with Chiralpak AD-H and Norvancomycin-bonded chiral stationary phase (CSP) [3–7], and the mobile phase used here is simpler than used in literature.

In this study, vancomycin (Fig. 2.), a macrocyclic glycopeptide which was introduced by Armstrong *et al.* [8] in 1994, was prepared according to "one-pot" synthetic strategies using 1,6-diisocyanatohexane as spacer [9]. Structure of vancomycin is very complex: it contains 18 chiral centers with various functional groups surrounding its

three pockets or cavities, it contains ionizable groups, *i. e.*, carboxylic acid moiety ($pK_a = 2.9$), glycopeptide alcoholic or phenolic hydroxyls ($pK_a = 7.2, 8.6, \text{ and } 9.6$), and amino groups ($pK_a = 10.4 \text{ and } 11.7$), the *pI* is 7.2 [10, 11]. During the synthesis the free amino group of vancomycin reacted with isocyanate, and additional linkages between glycopeptide alcoholic or phenolic hydroxyls may also be linked with isocyanate groups, the left ionizable groups may differ from the commercial one Chirobiotic V, and this may lead to different chromatographic behavior. The polar ionic mode is applicable to all molecules with at least one ionizable group on or near the chiral center and one additional functional group anywhere in the structure, and usually basic compounds demonstrate more selectivity in this mobile phase [10]. To the best of our knowledge, vancomycin CSP has not been applied to separate enantiomers of zolmitriptan. In this paper, the influences of glacial acetic acid (HOAc) and triethylamine (TEA) were studied, and the possible enantiodiscrimination mechanism is discussed.

2 Experimental

2.1 Chemicals and reagents

R,S-Zolmitriptan and *S*-zolmitriptan were obtained from Hangzhou Hehe Bio-Chem Technology. Vancomycin was obtained from Zhejiang Medicine, Xinchang Pharmaceutical Factory (P.R. China). (3-Aminopropyl)triethoxysilane (purity >99%), 1,6-diisocyanatohexane (purity >99) were purchased from Acros Organics (New Jersey, USA). Kromasil Si 100 silica gel (5 μm) was kindly provided by Professor Dr. Joachim N. Kinkel in Georg-Simon-Ohm University of Applied Science (Nürnberg, Germany). Methanol was HPLC grade and all other chemicals were analytical or chemical grade reagents produced in P.R. China.

2.2 Apparatus

Enantioseparations were performed using Waters 2690 Separations Module equipped with a Waters 996 Photodiode Array Detector and Waters Millennium³² System (Waters, Milford, MA, USA). Haskel Air Driven Fluid Pump (Haskel, Burbank, USA) was used for column packing.

2.3 Preparation of vancomycin CSP

Vancomycin-bonded CSP was prepared according to the method described by D'Acquarica [9]. Briefly: After silanization with 3-aminopropyltriethoxysilane, 3 g of dry 3-aminopropyl silica was stirred in 50 mL dry toluene using an ice-bath cooled under nitrogen, and 2.5 mL of 1,6-diisocyanatohexane was added. The mixture was heated at 70°C for 2 h; after cooling to room temperature, the liquid phase was removed by suction filtration through a filter under a nitrogen atmosphere. The excess reactant was removed by adding another 10 mL dry toluene. A suspen-

sion of 1.0 g vancomycin in 100 mL dry pyridine was added to the activated silica, and then the mixture was heated at 70°C for 12 h with continuous stirring. After cooling, the CSP was filtrated and washed with 50 mL portions of pyridine, water, methanol, dichloromethane, and dried under vacuum. Anal. found: for 3-aminopropyl Kromasil Si 100, C 5.08; H 1.22; N 1.66; for vancomycin-CSP, C 15.18; H 2.47; N 3.95.

The CSP was packed into a 250 mm \times 4.6 mm stainless-steel column using a conventional slurry method at 6000 psi. A 1:1 mixture of acetone and chloroform was used as slurring agent and ethanol was used as pressurizing agent. The dead time (t_0) was determined by injecting 100% ethanol. The theoretical plate numbers (N/m) of vancomycin was 7986 for acetophenone with *n*-hexane/ethanol (90:10, 1.0 mL/min) as eluent at room temperature.

2.4 Chromatography

The mobile phase compositions were methanol with different percentage of HOAc and/or TEA. The samples were dissolved in ethanol. All solvents and mobile phase were filtered by 0.45 μm filter membrane. The flow rate was 1.0 mL/min. The column temperature was 25°C. UV detection was performed at 286 nm. The results of chiral separation are summarized in Tables 1, 2, and a representative chromatogram is shown in Fig. 3. The elution order was determined by injecting *S*-zolmitriptan.

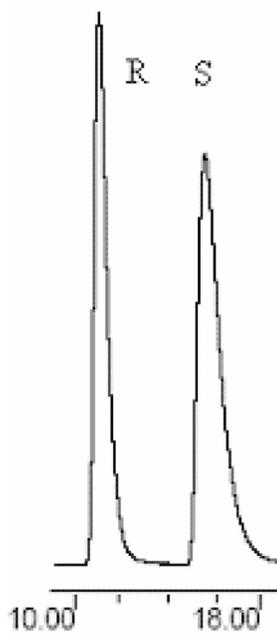


Figure 3. Chromatography of zolmitriptan on chiral stationary phases. Mobile phase: 100:0.2:0.2 (MeOH/HOAc/TEA, v/v/v); flow rate, 1.0 mL/min; detection wavelength, 286 nm; and temperature, 25°C.

Table 1. Influence of TEA concentration on the enantioseparation of zolmitriptan

$V_{(\text{MeOH})}/V_{(\text{TEA})}$	k_1	α	R_s
100:0.00	4.89	1.48	2.40
100:0.01	4.75	1.46	2.91
100:0.02	4.58	1.47	3.18
100:0.05	3.49	1.48	3.00
100:0.10	2.67	1.47	2.99
100:0.20	2.05	1.46	2.97

Flow rate: 1.0 mL/min; detection wavelength: 286 nm; and temperature: 25°C.

Table 2. Influence of HOAc/TEA concentration and ratio on the enantioseparation of zolmitriptan

$V_{(\text{MeOH})}/V_{(\text{HOAc})}/V_{(\text{TEA})}$	k_1	α	R_s
100:0.001:0.001	2.42	1.43	2.25
100:0.01:0.01	2.75	1.50	2.22
100:0.05:0.05	4.11	1.51	2.77
100:0.08:0.08	4.17	1.49	2.90
100:0.10:0.10	4.32	1.49	2.99
100:0.15:0.15	4.68	1.53	3.14
100:0.20:0.20	4.63	1.50	3.36
100:0.50:0.50	3.29	1.49	3.25
100:0.20:0.10	3.86	1.49	2.90
100:0.10:0.20	4.51	1.53	3.31

Flow rate: 1.0 mL/min; detection wavelength: 286 nm; and temperature: 25°C.

3 Results and discussion

Zolmitriptan has one ionizable group (carbamate) near the chiral center and other functional groups (1H-indole and nitrogen in tertiary form) in the molecule. Under polar ionic mode, the most important bindings involved are hydrogen bonding, dipole interactions, steric interactions, and anionic and cationic bindings, and the use of acid and base is to protonate the basic analyte and CSP, and the ratio was adjusted so as not to have more acid than is necessary for the protonation of the solutes [10].

Initially, ACN, an aprotic solvent, was used as mobile phase, and the solutes were not eluted out after 100 min; then methanol was chosen as mobile phase, and the solutes eluted earlier and achieved baseline separation. Methanol is a protic solvent and could set off hydrogen bonds which lead to earlier elution of the solutes. It was also observed in the absence of TEA when the mobile phase consisted of methanol/HOAc (100:0.001, v/v), the solutes eluted near dead volume with worse peak shape and poor enantiomeric separation; this could be explained

on the basis of strong repulsive effects between the protonated amino groups of the solutes and of the CSP. Therefore methanol and TEA with or without HOAc were chosen as mobile phase for the following studies.

From Table 1, it was found that the retention factors (k') were decreased with the increasing of TEA concentration in the mobile phase, and the separation factors (α) were nearly unchanged, while the resolutions (R_s) were increased with the TEA concentration varied from 0.0 to 0.02, and decreased with the continuous increase of TEA concentration. The TEA is a strong base, and can compete with solutes for hydrogen bonding sites and dipole sites which decreased the interactions between solutes and CSP.

As seen from Table 2, the concentration (ratio) of HOAc/TEA had a substantial influence on the chiral separation; the retention factors and resolutions were increased with the increasing of HOAc/TEA concentration from 0.001:0.001 to 0.15:0.15, and decreased with further increasing of HOAc/TEA concentration to 0.5:0.5. This phenomenon was different from Chirobiotic V described in handbook [10], which reported that the analyte is eluting faster with the increasing of acid/base concentration. The retention factors and resolutions at 1:1 ratio were bigger than that at 2:1 and 1:2 (HOAc/TEA, v/v) ratio, while the separation factors were nearly unchanged. Due to the differences in molecular weight and density, there is more than a two-fold molar excess of acid at 1:1 ratio, the excess acid is to protonate analyte and CSP. With the increase of acid concentration, the hydroxyls and carboxylic acid residue of vancomycin will be less charged, and may only serve as hydrogen bonding sites, and with the continued increase of HOAc concentration, the repulsive ionic interaction of protonated amino groups may have played a major role. The ion pair formed between acid anion and protonated analyte may enhance the hydrogen bondings and dipolar interactions between analyte and CSP; hence, the retention factors were increased with the increase of HOAc/TEA concentration till there is too much acid than needed.

The elution order of *R*-enantiomer before *S*-form was observed whatever the chromatographic conditions tested. This could be due to the steric effect, the inclusion baskets and the other functional moieties provide more suitable chiral sites for *S*-enantiomer which results in the chiral discrimination between zolmitriptan enantiomers.

4 Concluding remarks

The self-prepared vancomycin CSP is suited for the baseline separation of zolmitriptan enantiomers under polar ionic mode, and the ratio (concentration) of HOAc and/or TEA can influence ionic interaction and hydrogen interaction between analyte and CSP; the ionic interactions,

hydrogen bondings, and steric interactions may have played key role together.

We are grateful to Professor Dr. Joachim N. Kinkel in Georg-Simon-Ohm University of Applied Science (Nürnberg, Germany) for providing kromasil 5 μm (10 nm) silica gel.

5 References

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