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Validated chiral high performance liquid chromatography separation method and simulation studies of dipeptides on amylose chiral column

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ABSTRACT

Chiral resolution of DL-alanine-DL-tyrosine and DL-leucine-DL-phenylalanine dipeptides was achieved on AmyCoat-RP column. The mobile phase used for DL-alanine-DL-tyrosine was acetonitrile-ammonium acetate (10 mM, pH 6.0) [50:50, v/v]. It was acetonitrile–methanol–ammonium acetate (10 mM; pH adjusted to 4.5 with glacial acetic acid) [50:20:30, v/v] for DL-leucine-DL-phenylalanine. The flow rate of the mobile phases was 0.8 mL/min with UV detection at 275 nm. The values of retention factors for LL-, DD-, DL- and LD-stereomers of DL-alanine-DL-tyrosine were 1.71, 2.86, 5.43 and 9.42, respectively. The values of separation and resolution factors were 1.67, 1.90 and 1.73 and 2.88, 6.43 and 7.90, respectively. Similarly, these values for DL-leucine-DL-phenylalanine stereomers were 1.50, 2.88, 3.50 and 4.07 (retention factors), 1.92, 1.22 and 1.62 (separation factors) and 2.67, 1.55 and 2.30 (resolution factors). The limits of detections and quantitation were ranged from 2.03 to 6.40 and 6.79 to 21.30 μg/mL, respectively. The modeling studies were in agreement with the elution orders. The mechanism of chiral recognition was established by modeling and chromatographic studies. It was observed that hydrogen bondings and π–π interactions are the major forces for chiral separation.

1. Introduction

The demand of chiral resolution is growing incessantly due to the different pharmaceutical activities of the enantiomers/stereomers. It is well known that one of the enantiomers/stereomers may be biologically active while the other may be inactive or toxic or ballast. The other stereomer may create various side effects and problems in human body [1]. This is due to the different stereoselective metabolisms, distribution rates, excretions and clearances of stereomers in human body. Therefore, the scientists, clinicians, academicians, industrialists and Government authorities are asking data on the chiral resolution of biologically important molecules. United States Food and Drug Administration (US FDA), European Committee for Proprietary Medicinal Products, Health Canada, Pharmaceutical and Medical Devices Agencies of Japan formulated the guidelines for the production and control of racemic drugs to ensure their safety [2,3]. Dipeptides are important molecules because of their involvement in many metabolic processes. The most important biological actions of dipeptides are protein synthesis, fertility, neurotransmission, control of inflammation process, vital activities of pathogenic microbes and some other functions. These features are responsible for the uses of dipeptides in drugs development [4–7] and biological markers [8]. Besides, dipeptides are also important from the food and nutrition point of views. For example, diet coca cola soft drink, Muller light cherry yoghurt, asparmate, hydrolysed whey protein, *etc.* are foods and food supplements containing various dipeptides. The dipeptides are chiral molecules having two asymmetric centers. Four stereomers are possible of a dipeptide having both amino acids in DL-configurations. It is interesting to mention here that the enzymatic actions of dipeptides are stereospecific in nature. Therefore, a single dipeptide may behave in four different ways. Aromatic amino acids are important for their roles in protein and nucleic acid recognition. The proteins and nucleic acids are chiral molecules and, consequently, dipeptides of aromatic amino acids are very important molecules. A search of literature [9] indicates

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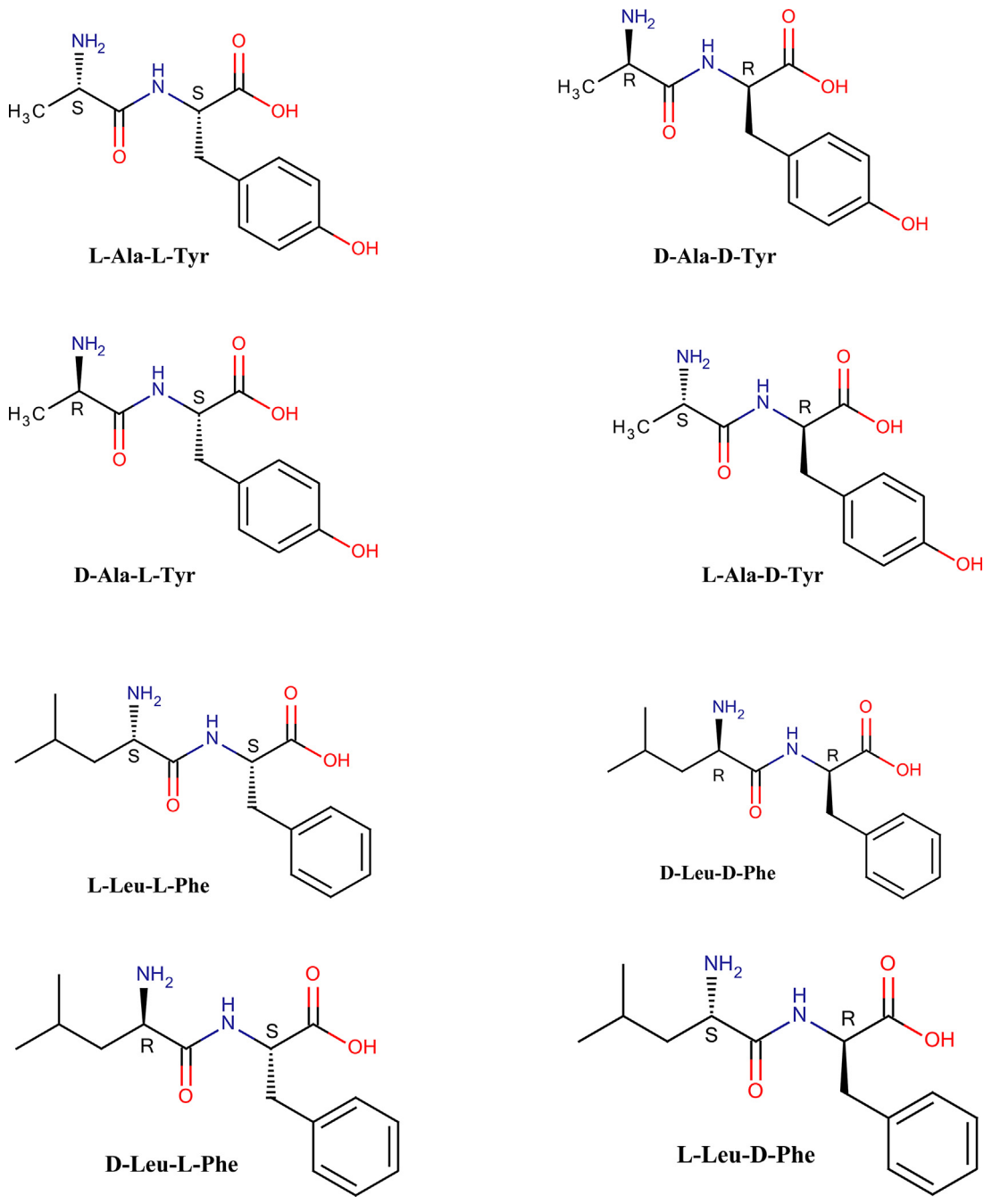

Fig. 1. The structures of stereomers of DL-alanine-DL-tryrosine and DL-leucine-DL-phenylalanine dipeptides.

that there are few papers describing chiral resolution of aromatic derivatized dipeptides. Moreover, no paper is available for chiral resolution of underivatized aromatic dipeptides having four stereomers. In view of these facts, the efforts were made to resolve the stereomers of two important dipeptides *i.e.* DL-alanine-DL-tyrosine and DL-leucine-DL-phenylalanine. Four stereomers of each dipeptide (Fig. 1) were resolved successfully on Amycoat-RP column. The results of these findings are reported herein.

2. Experimental

2.1. Chemicals and reagents

DL-Alanine-DL-tyrosine, DL-leucine-DL-phenylalanine dipeptides and their optically active pure stereomers were purchased from Sean Fisher Peptide 2.0, Inc., Chantilly, VA, USA. Methanol and acetonitrile of HPLC grade, glacial acetic acid and ammonium acetate of AR grade were supplied by Merck, Mumbai, India. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., U.S.A.) water purification system. The solutions (100 μg/mL) of DL-alanine-DL-tyrosine, DL-leucine-DL-phenylalanine and corresponding optically active pure stereomers were prepared in their respective mobile phases.

2.2. Instrumentation

The experiments were carried out on an HPLC system of Shimadzu, Japan consisting of solvent delivery pump (LC-10 AT VP), manual injector, UV-Visb. detector (SPD-10A) and Class-VP software. Column used was AmyCoat-RP [tris-(3,5-dimethylphenyl