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Chiral separation of selected proline derivatives using a polysaccharide type stationary phase by high-performance liquid chromatography

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Abstract

Proline derivatives, such as Boc-proline, Boc-2-methylproline, Boc-2-methylproline benzyl ester and Boc-2-methyl-4-hydroxy-proline benzyl ester, have been widely used as a building block leading to a variety of pharmaceutical compounds. Therefore, there is a wide interest in the chiral separation of these compounds. High-performance liquid chromatography (HPLC) methods were developed using a Chiralpak AD-H column to separate enantiomers of these proline derivatives. The effect of mobile phase composition and column temperature was studied. For the proline derivatives studied in this work, good resolution was achieved using a mobile phase composition of hexane, ethanol and 0.1% TFA. For prolines containing carboxyl or hydroxy group, resolution was changed dramatically corresponding to changes as little as 1% of ethanol in the mobile phase, suggesting that the dominant chiral recognition is from hydrogen bonding interactions. On the other hand, for prolines containing a benzyl ester instead of hydroxy group next to the chiral center, resolution was not affected as significantly with the changes of ethanol content in the mobile phase, indicating a different leading chiral recognition mechanism, such as inclusion, steric effect, or possible π–π interaction. Linearity, precision and limit of detection were also measured for Boc-2-methylproline and Boc-2-methylproline benzyl ester.

Keywords: Chiral separation; Proline derivatives; Chiralpak AD-H

1. Introduction

It is well known that enantiomers can exhibit completely different physiological and biological activities, as well as pharmacodynamic and pharmacokinetic characteristics [1–3]. Therefore, the U.S. Food and Drug Administration has been issuing guidelines for drug development requiring the analysis and control of the enantiomeric purity of drug substances [4].

Proline derivatives, such as Boc-proline, Boc-2-methylproline (boc acid), Boc-2-methylproline benzyl ester (boc ester) and Boc-2-methyl-4-hydroxy-proline benzyl ester (boc hydroxy ester) (see Fig. 1), are important intermediates for the development of peptides or pharmaceutical compounds [5–9]. It is advantageous to control the enantiomeric purity of starting materials and intermediates in order to control the enantiomeric purity of the final drug substance. Therefore, there is a wide interest in the chiral separation of these compounds.

Studies on chiral separations of Boc-proline by high-performance liquid chromatography (HPLC) have been reported using a urea-linked cinchona-calixarene hybrid type of chiral stationary phase (CSP) [10,11] and a teicoplanin-based CSP [12]. Capillary electrophoresis was also utilized for the separation of Boc-proline [13]. Resolution of the enantiomers reported in each study varied, from baseline resolution to no separation. Chiral separation of Boc-2-methylproline (boc acid) has been conducted using a chiral derivatizing agent [14] and a direct HPLC enantioseparation on a quinine-derived chiral anion-exchanger stationary phase [15]. Chiral separation of Boc-2-methylproline benzyl ester (boc ester) and Boc-2-methyl-4-hydroxy-proline benzyl ester (boc hydroxy ester) has not been reported to the best of our knowledge.

In the work presented here, the chiral separation of these proline derivatives is conducted on the commercially available polysaccharide type of chiral stationary phase, Chiralpak AD-H column. Baseline or near baseline resolution was achieved using the AD-H column for each proline derivative under its optimized chromatographic condition. The effect of mobile phase composition and column temperature was also studied.

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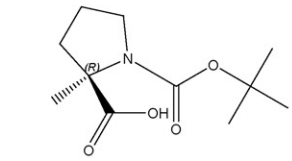
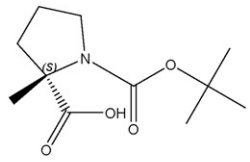


(R)-Boc-2-methyl-proline (boc acid)



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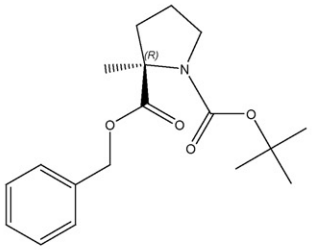
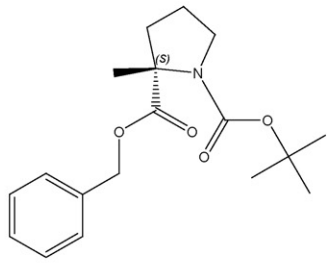
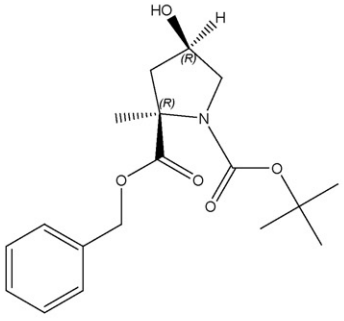


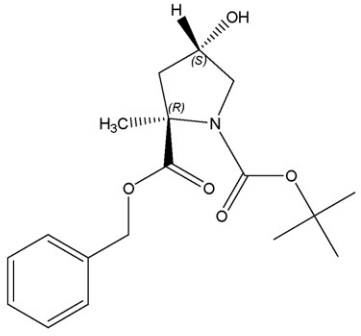
(R)-Boc-2-methylproline benzyl ester (boc ester)



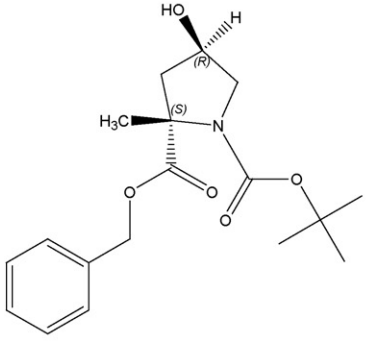
(S)-Boc-2-methylproline benzyl ester (boc ester)



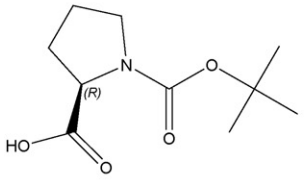
(2R, 4R)-Boc-2-methyl-4-hydroxy-proline benzyl ester (hydroxy ester)



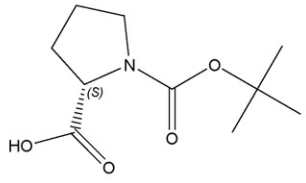
(2R, 4S)-Boc-2-methyl-4-hydroxy-proline benzyl ester (hydroxy ester)



(2S, 4R)-Boc-2-methyl-4-hydroxy-proline benzyl ester (hydroxy ester)



(R)-Boc-proline or D-Boc-proline



(S)-Boc-proline or L-Boc-proline

Fig. 1. Structures of proline derivatives.