A FAST HPLC METHOD FOR DETERMINATION OF D-/L-AMINO ACIDS IN SEAWATER

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Abstract
The organic matter pool in the seawater is consisted of different classes of organic molecules with varying sizes and structures. The origin of these organic molecules could be the terrestrial processes or *in situ* marine production or degradation. Determination of D/L-amino acids in the seawater has been used in the last decades as one of the confirmatory tools to understand the origin of the organic matter. In this study, a fast and modified HPLC method was proposed for the analysis of amino acids in the seawater.

Keywords: Organic matter, Izmir Bay, Analytical methods

Introduction
Dissolved organic matter (DOM) can be classified as autochthonous (marine origin) and allochthonous (terrestrial origin) according to its source. However, there are vast amount of biomolecules at different structures and sizes that hardens their characterization and separation from each other. Among these biomolecules, most abundant ones found in DOM are amino acids, carbohydrates, lipids, fatty acids, sterols, humic acids, fulvic acids, and lignins. From these classes, amino acids are studied as a descriptive tool for prediction of the origin and transformation rate of DOM [1]. For example, D-amino acids are found in the cell walls of marine bacteria and the enantiomeric amino acid ratio in seawater gives important information about the origin of DOM [1,2]. However, total analysis time of the existing enantiomeric amino acid determination methods in HPLC are long and new rapid resolution columns could be used to decrease the analysis time [3,4]. In this study, a fast and modified HPLC method was proposed for the analysis of amino acids in the seawater.

Materials and Methods
The HPLC method used in this study is based on the fluorometric detection of chiral reaction products of amino acids according to Dittmar et al. 2009's method with modified mobile phase, HPLC column, column temperature and analysis time [3]. The derivatization was first achieved with o-phtaldialdehyde (OPA) and sequentially by N-isobutyryl-D/L-cysteine (IBLC/IBDC). Briefly, OPA was dissolved within 0.5 M borate buffer whose pH was adjusted to 9.5. IBLC and IBDC were first dissolved in methanol and after ultrapure water with the ratio of 4:6. For the derivatization reaction, 100 µL of sample was sequentially mixed with 2 µL of OPA and 2 µL of IBLC or IBDC in the autosampler whose temperature was adjusted to 4 °C. The injection volume was 10 µL. The separation was performed with Eclipse Plus C_{18} (4.6x150 mm, 3.5 µm) analytical HPLC column. The mobile phases A and B were 25 mM sodium acetate buffer (pH 6.0) and acetonitrile:methanol:water (45:45:10), respectively. The mobile phase conditions for the linear gradient elution were as follows: 0-2 min. 2-8% B, 2-20 min. 8-16% B, 20-23 min. 16-16.5% B, 23-35 min. 16.5-22.1% B, 35-67 min. 22.1-39.5% B, 67-68 min. 39.5-100% B, 68-75 min. hold at 100% B, 75-76 min. 100-2% B, 76-78 min. hold at 2% B. The flow rate was 1.1 mL/min and the column oven was kept at 40 °C. The fluorescence of the derivatized samples were recorded at excitation/emission wavelengths of 330/445 nm and 230/445 nm, respectively. The certified L-amino acid mix standards (including QNMR result) for the calibration were purchased from Sigma-Aldrich (Prod. No: 79248).

Discussion and Conclusion
In this study, total analysis time was reduced to 68 min. except the wash period which was applied for an extra 10 min (from 68 to 78 min.) (Figure 1). The method proposed in this study has high peak resolutions (minimum $R \geq 1.5$) and acceptable separation factors with minimum $\alpha \geq 1.03$. The amino acids that could be identified with this method are D/L-Asp, D/L-Glu, D/L-Ser, D/L-Thr, Gly, D/L-Arg, D/L-Ala, D/L-Tyr, D/L-Val, L-Ile, and D/L-Phe, respectively. With the method proposed in this study, it is possible to determine 20 D/L-amino acids in the seawater. However, in further studies, this method could be improved by using columns with smaller particle size or shorter columns and also by using different gradient elution programmes. The authors thank to TUBITAK for financial support of the project (113Y447). The authors also thank to project personnel and R/V K. Piri Reis crew for their support. Hakan Alyuruk thanks to TUBITAK-BIDEB 2211 for scholarship. This study was also a part of the Ph.D. thesis of Hakan Alyuruk.

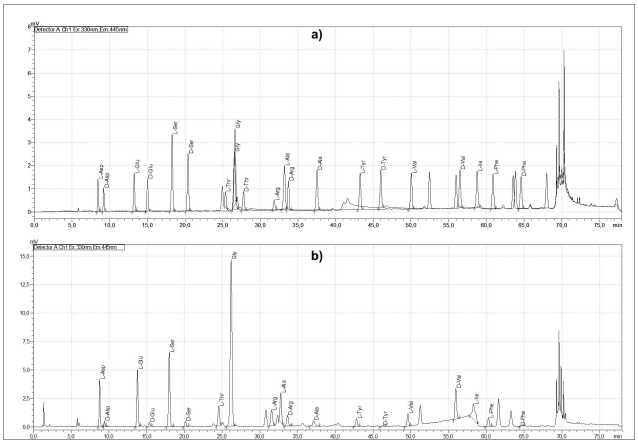

Fig. 1. The superimposed chromatograms for IBDC and IBLC methods using L-amino acid standards at 50 nM (a), and the chromatogram of a surface seawater sample (filtrated from GF/F filter) taken from the inner Izmir Bay, Aegean Sea, Autumn 2015 (38°27.216' N, 27°08.484' E) (b).

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