
HEART-CUTTING VS COMPREHENSIVE MULTI-DIMENSIONAL LIQUID CHROMATOGRAPHY FOR ANALYSIS OF CYCLOTIDES IN VIOLA IGNOBILIS

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Cyclotides are a unique class of head-to-tail cyclic cysteine rich microproteins up to 37 amino acids in length that exhibit a wide range of biological properties, ranging from anti-HIV to insecticidal activity [1]. Due to the complexity of cyclotide matrixes and their different isomers, suitable separation could not be obtained by electrophoresis and high performance liquid chromatography (HPLC) [2]. The aim of this study is introduction of a two-dimensional column chromatography method to obtain good resolutions of cyclotides. The sample of cyclotides was extracted from *Viola ignobilis*. Cyclotides contain both hydrophilic and hydrophobic portions and they also have similar hydrophobic groups [3]. Thus, a reversed-phase HPLC (RP-HPLC) is not able to separate them properly, especially the cyclotides which show more hydrophilic characteristics. Therefore, it is very suitable if we can use a special technique that involves the separation of cyclotides on the basis of various hydrophobicities. Two-dimensional liquid chromatography (2D-HPLC) is an ideal method for separation of mixtures which have different hydrophobicities according to the use of two columns having two orthogonality. Multi-dimensional separations can be either heart-cutting or comprehensive in approach. In heart-cutting separation a region of interest is excised from the first-dimension separation, and subjected to a further separation in the second-dimension. Three types of 2D-HPLC, C8 (250×4.6 mm, 5 μm), C18 (250×4.6 mm, 5 μm), C18 -C8 and C8 -CN (250×4 mm, 5 μm) were investigated. The optimal mobile phase and flow rate were obtained based on peak capacity and shape of peaks. In comprehensive mode, C8 columns have been connected to CN column and results compared with heart-cutting data. UV detector at 218 nm was used for all analyses. Fourier transform mass spectrometry (FT-MS) was used to confirm the cyclotides structures.

Keywords: Cyclotide, *Viola ignobilis*, Two dimension liquid chromatography

References

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