Abastract

In this work, a new, simple and fast Dispersive liquid-liquid microextraction (DLLME) coupled with reversed-phase high performance liquid chromatography (RP-HPLC) was developed for the determination of betulinic acid in plant sample. The method was based on the rapid extraction of betulinic acid from a methanol sample solution into 90 µL chloroform, as an extraction solvent. After addition of water into the mixture, the extracting solvent phase immediately formed a distinct water-immiscible phase under the vial, which could easily be separated, evaporated and re-dissolved in 0.5 mL of acetonitrile for further HPLC analysis. The effects of various experimental parameters in extraction step were also studied using response surface methodology. Three independent variables were volume of extracting solvent (A: 30-90 µL), time (B: 1-10 min) and volume of water (C: 1-10 mL). statistical analysis showed the independent variable A and the interaction between B and C had significant effects on the peak area of betulinic acid ($p<0.05$). The optimized conditions were 90 µL of extracting solvent volume, an extraction time of 4.3 min and 5.2 mL of water volume. Under these conditions detection (LOD) and enrichment factor were 1.8 $\mu g.L^{-1}$ and 110, respectively. Furthermore, the relative standard deviation of the ten replicate was <3.9%. The developed procedure was then applied to the extraction and determination of betulinic acid in the plant samples.

Keywords: Betulinic acid, Dispersive Liquid-Liquid Microextraction, Response Surface Methodology, High Performance Liquid Chrmotography.

University of Zabol

Graduate School

Faculty of Science

Department of Chemistry

The Thesis Submitted for the Degree of Master of Science

(In the field of Analytical Chemistry)

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February 2013