

Quantitative Determination of Ecdysteroids in Insects by HPLC

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Ecdysteroids are one of the main insect hormones, which involve in regulation of the important physiological processes.

We developed the simple, fast and sensitive method for sample preparation and quantification of ecdysone and 20-hydroxyecdysone titers in insect hemolymph and whole body homogenates by RP-HPLC. The procedure of sample preparation includes extraction by MeOH, delipidization, concentration by solid-phase extraction with Diapak-C16 cartridge. Samples were analyzed by HPLC-UV. The chromatographic system Agilent 1100 consisted of quaternary pump with vacuum degasser, thermostatted column compartment, variable wavelength detector and analytical column Diasorb C16T (BioChemMac, Russia), the mobile phase was acetonitrile:water (1:5 v/v). The limits of detection for ecdysone, 20-hydroxyecdysone and inokosterone (internal standard) were measured. The method allows quantifying of the ecdysteroids in insects with titers more than 10ng/ml of hemolymph or ng/g of body weight.