ABSTRACT

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Title of Thesis: HPLC Analysis of Podophyllotoxin

The diploma thesis is focused on optimalization of HPLC method for the analysis of podophyllotoxin. The method was developed for the analysis of *Juniperus virginiana* leaves extract. Various stationary phases, mobile phases, temperatures, ultrasonic extraction times were tested. Following conditions were chosen as optimal: 30 minutes ultrasonic extraction time, LiChrospher 100 RP-18 250-4 column, 0,1% CH_3COOH:ACN 45:55 mobile phase, temperature 40 °C, flow rate 0,8 ml/min, sample volume 3 µl, fluorescence detection λ_{ex} 240 nm, λ_{em} 320 nm, time of analysis 9 minutes. The method was also developed for the analysis of plant tissue cultures of *Juniperus virginiana*, for which gradient elution was chosen. This method had following conditions: LiChrospher 100 RP-18 250-4 column, mobile phase 0,1% CH_3COOH:ACN, change of acetic acid ratio: 0–8 min 70–10 %, 8–9 min 10 %, 9–10 min 10–70 %, 10–12 min 70 %, temperature 40 °C, flow rate 0,8 ml/min, sample volume 3 µl, fluorescence detection λ_{ex} 240 nm, λ_{em} 320 nm, time of analysis 12 minutes. Subsequently, both methods were validated. Evaluated parameters included precision, accuracy, linearity, selectivity, limit of detection and limit of quantification.