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Validation of reverse phase high performance liquid chromatography (HPLC) method for analysis of Camptothecin (CPT) and determination of CPT levels in *Ophiorrhiza rugosa*

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Camptothecin is an alkaloid present in *Ophiorrhiza rugosa* (family Rubiaceae) which is a clinically important anti-cancer drug. The present study was carried out to validate a simple HPLC method and to determine camptothecin levels in different parts of the plant using various solvents. An isocratic HPLC procedure was developed for quantitative determination of CPT. Separation of secondary metabolites of *Ophiorrhiza rugosa* was performed on a Phenyl column (150 × 2.1 mm). Acetonitrile: water (35:65) was used as the mobile phase at a flow rate of 0.8 mL/ min and carbamazepine was used as the Internal standard. The eluted substances were detected at 254 nm. The method was validated for linearity, accuracy, precision, interday and intraday variation, limit of detection and limit of quantification. Retention times obtained for CPT and carbamazepine were 3.0 and 4.2 min respectively. No interfering peaks were observed with the endogenous substances of the plant. The accuracy of the method exceeded 95%. The relative standard deviation of repeat measurements of the three quality control (QC) levels (10, 20 and 35 µg/mL) ranged from 0.58 – 1.16%. Limit of detection and limit of quantification of CPT were 0.10 µg/mL and 0.32 µg/mL respectively. Calibration curves were linear (R^2> 0.99) over a concentration range of 2.5 to 35 µg/mL. The mean recoveries were 99.79 ± 4.73, 100.73 ± 4.25 and 99.69 ± 1.72% for CPT at concentrations of 10, 20 and 35 µg/mL respectively. CPT levels present in leaves, stems, roots and flowers of *Ophiorrhiza rugosa* were quantified after extraction into chloroform, methanol, de-ionized water, and methanol: ethanol (9:1) mixture, 70% methanol and ethyl acetate separately. The extraction capacity of CPT from different parts of the plant was highest, when using the methanol: ethanol (9:1) mixture or methanol and the values in descending order were leaves > flowers > root > stem.

Keywords:Camptothecin, HPLC, *Ophiorrhiza rugosa,* validation, accuracy, LOD, LOQ, linearity