

# ABSTRACT

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Name of diploma thesis: Optimization of separation conditions for HPCCC of paclitaxel

The aim of this work is a feasibility study of natural substances isolation (in my case it is paclitaxel and its intermediate 10-deacetylbaccatin) using modern technique called High performance countercurrent chromatography (HPCCC) available in Teva in Opava. For this basic research it was necessary to study the literature and test various solvent systems. Chosen solvent systems were then tested together with natural substance in HPCCC, results were processed and discussed.

Countercurrent chromatography is considered a modern separation technique based on two immiscible liquids. One of them is called a stationary phase and is held inside the column by centrifugal force, the second liquid is called a mobile phase and is pumped through the column. Then the injected liquid sample is also pumped through the column and depending on distribution coefficients the sample components are either held in the stationary phase or pass through the column and detector into fraction collector.

Advantages of this method are e.g. total sample recovery, high mass and volume injection loadings, high flow rates connected with reduced analysis time or suitability for separation of crude natural materials. The disadvantage can be bigger cost of equipment.

This work consists of series of test tube experiments and calculation of distribution coefficients that was necessary to perform for selection of convenient solvent systems. Other target was to optimize the HPCCC instrument and reduce the stationary phase loss which is caused by the centrifugal force and the pressure of mobile phase being pushed through the column. For that reason I tested different rotation speed, flow rates and distribution coefficient concentration dependence.

The summary of distribution coefficients in tested solvent systems is a valuable groundwork for further experiments. Three solvent systems were selected and separation conditions were set to 1200 rpm and 5 ml/min. For most experiments dual mode was used allowing us to separate compounds presenting different polarity (different distribution coefficients) in shorter time.

**Key words: paclitaxel, 10-deacetylbaccatin, high performance countercurrent chromatography, dual mode, solvent system, distribution coefficient**