

DE Application Note - Paclitaxel Purification

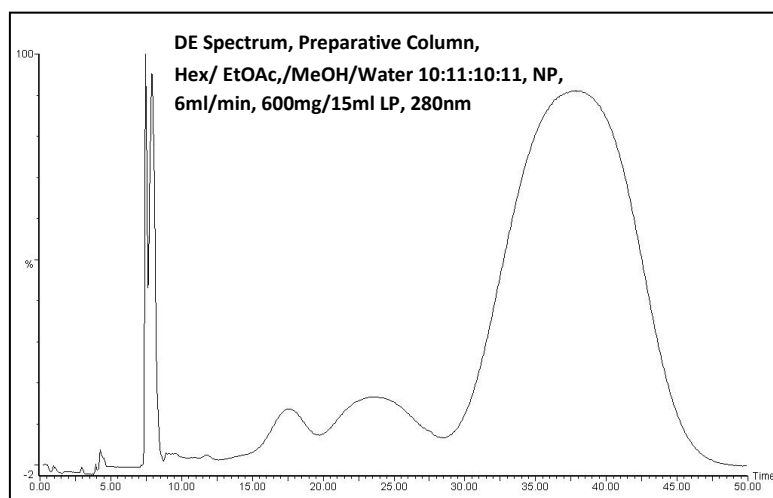
Introduction

Paclitaxel is a cytotoxic, chemo-therapeutic agent used in the treatment of ovarian, breast and non-small cell lung cancer. The commercial sources of Paclitaxel and a related compound which can be synthetically modified to yield Paclitaxel, are the leaves, bark and roots of various Yew species. The commercial purification process of one producer of Paclitaxel uses High Speed Counter Current Chromatography (HSCCC).

This application note describes the successful purification of Paclitaxel by High Performance Counter Current Chromatography (HPCCC).

Experimental

The separation was performed on a Dynamic Extractions Spectrum HPCCC instrument. The separation was developed and loading capacity studies were performed on the analytical scale column. The chromatogram shown was obtained by using the preparative scale column. The Solvent System used was Hexane/Ethyl Acetate/ Methanol /Water (HEMWat) in the proportions 10:11:10:11 respectively. For the chromatogram shown, a 600mg sample of 67% crude Paclitaxel was dissolved in 15ml of the lower stationary phase. The separation was performed in Normal Phase mode (upper, mainly organic phase was used as mobile phase), at 240g and with a flow rate of 6ml/minute. Stationary Phase retention was 90%.



Following the preparative scale chromatography, fractions between 32 and 43 minutes were combined and concentrated *in vacuo* to yield an 89% recovery of >99% purity, Paclitaxel.

The separation was scaled up to a loading of 1.4g on the same column, with similar results.

Conclusions

None of the runs took more than one hour to perform and a good recovery of high purity Paclitaxel was obtained. The 1.4g loading would scale up linearly to 10g on a Dynamic Extractions Midi HPCCC instrument with a 940ml column volume and with the separation still performed in less than one hour with the same recovery and purity.

The table to the right shows the performance values of HSCCC and HPCCC for the Paclitaxel purification. Of particular note is the potentially 150-fold improvement in productivity gained when the purification is conducted using HPCCC instrument instead of HSCCC machine.

Instrument & Characteristic	HSCCC	Spectrum HPCCC	Midi HPCCC
	NP	NP	NP
Column volume (ml)	260	134	950
Flow rate (ml/min)	2 (max)	6	42
Typical SP retention		85 - 90	85 - 90
Cycle time (min)	150	50	50
Runs per day	2	6	6
Sample loading per run (g)*	0.2	1.4	9.8
Solvent usage per cycle (ml/g)	1600	600	600
Throughput per day (g)	0.4	8.4	59
Quality of chromatogram	low	good	

* Feeds: HSCCC 92% Paclitaxel, HPCCC 67% Paclitaxel