

# Paclitaxel

On DAVISIL® Chromatographic Silica Media

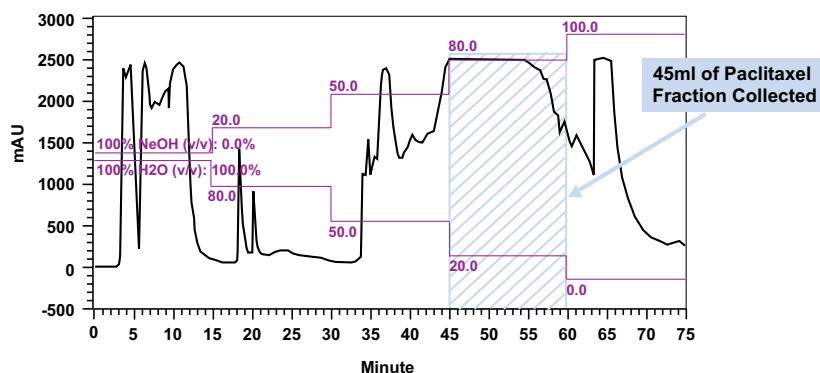


## 90% of Polar Impurities Removed from Paclitaxel Extract using Davisil® C18 Silica

### Introduction

Isolation of natural products on Davisil® C18 silica (CAT# 633NC18E) is a very useful and cost effective clean up method for removing impurities from target natural compounds. Davisil® reversed phase silica's high 17% carbon load, uniform 60Å pores, and tight 50 micron particle size distribution, provides excellent chemical and structural properties for low pressure purification of natural compounds soluble under aqueous conditions.

Paclitaxel, which is sold by the brand name, Taxol®, is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. Paclitaxel, a plant alkaloid, is used to treat breast, ovarian, lung, bladder, prostate, melanoma, esophageal, as well as other types of solid tumor cancers. It has also been used in Kaposi's sarcoma. Paclitaxel is a natural product isolated from the Canadian yew tree bark. Removing impurities from Paclitaxel is particularly challenging, since the molecular structures in the extracted mixture are very similar. The crude solvent extract contains Paclitaxel and large quantities of highly polar taxane impurities.



**Column:** 10mm i.d. x 200mm  
**Packing:** 10g Davisil® C18 silica (cat# 633NC18E)  
**Mobile Phase:** see chromatogram  
**Loading Volume:** 2mL  
**Detection:** UV@230nm

Figure 1. Preparative clean-up of Yew bark extract using Grace Davisil® C18, 60Å, 50µm silica.

## Experimental

### Methanol solvent extraction

10 grams of powdered Yew bark mixed with 500ml of methanol and rolled for 12 hours at room temperature. 400ml of bark extract was then concentrated to 200ml.

### Liquid-liquid extraction

90ml of the concentrated extract mixed with 100ml methylene chloride and 100ml water for 2 hours. The equilibrated mixture was then placed in a separatory funnel for 12 hours. The extracted solvent was collected and concentrated to a 2ml injection volume.

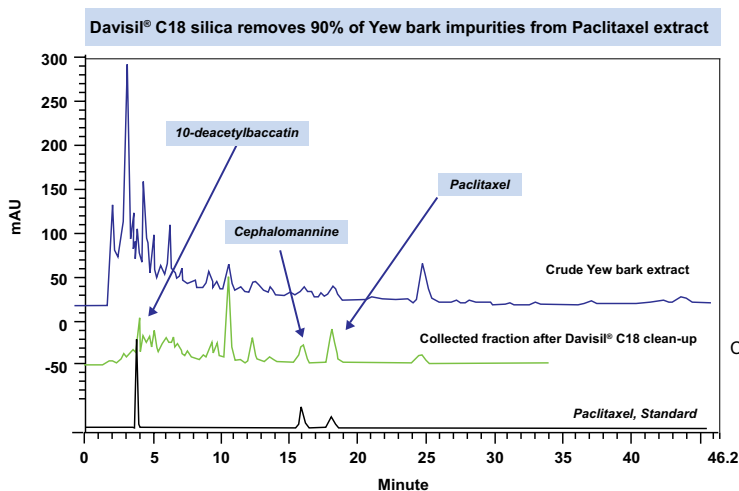
### Chromatography

10 grams of 633N C18 was slurred with 20ml of isopropanol to pack a low pressure 10mm i.d. x 200mm length column for purification. A methanol/water step gradient was run with the desired compound eluting from the column in the 80/20 methanol/water fraction (fig. 1). This fraction was analyzed with a Grace C18 analytical column (Denali® C18 column 5µm, 4.6mm i.d. x 250mm). The resolution of Cephalomannine/Paclitaxel is 2.78 and the Paclitaxel peak was identified with the standard reference (fig. 2).



## Results and Discussion

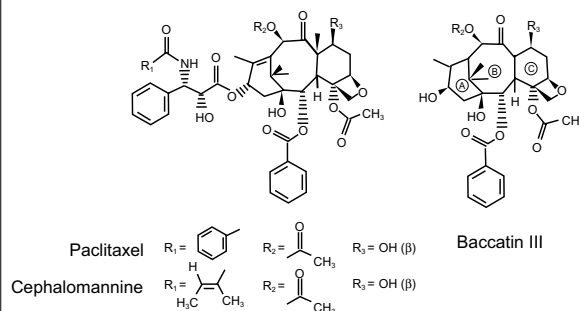
Grace Davisil® C18 silica effectively purified Paclitaxel from 90% of the Yew bark extract impurities using a methanol/water step gradient with methylene chloride. The analytical Grace Vydac® 238DE54 column separated the Paclitaxel from its neighboring peaks, cephalomannine and 10-deacetylbaccatin. The traditional stationary phase for crude separations has been silica gel, but the Davisil® C18 silica bonded phase was introduced to provide a material that separates solutes by dispersive interactions and some semi-polar interactions. The superior bonding technology and purity of the Davisil® C18 silica effectively retained and enriched the moderately polar target molecule, Paclitaxel while removing the majority of crude impurities from the extraction sample. Easy identification of the retained Paclitaxel and Cephalomannine peaks was performed on the Denali® C18 reversed phase analytical column due to its high retentivity, high efficiency and excellent peak symmetry. Paclitaxel and similar hydrophilic polar substances separate well on C18 reversed-phase Davisil® silica from their crude extraction mixtures in aqueous mobile phases with moderate organic content. High efficiencies and good resolution were obtained for these substances showing the benefit for crude separation on Davisil® high purity media.



**Analytical Column:** Denali® C18, 5µm  
**Eluent A:** 100% Acetonitrile  
**Eluent B:** 100% Water  
**Eluent C:** 100% Methanol

**Isocratic:** 30% A, 42% B and 20% C  
**Flow:** 1.000 mL/min  
**Detection:** UV@230  
**Injection Volume:** 10µl

Figure 2. Analytical analysis of the collected fraction purified using Davisil® C18 silica compared to the crude Yew bark extract and a Paclitaxel standard (Sigma Aldrich).



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