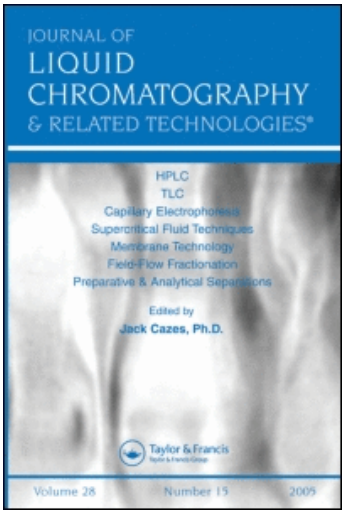


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# HPLC Separation of Taxol and Cephalomannine

John H. Cardellina II[a]
[a] Laboratory of Drug Discovery Research, Development National Cancer Institute, Frederick, Maryland

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# HPLC SEPARATION OF TAXOL AND CEPHALOMANNINE

**JOHN H. CARDELLINA II**
*Laboratory of Drug Discovery*
*Research & Development*
*National Cancer Institute*
*Building 1052, Room 121*
*Frederick, Maryland 21702-1201*



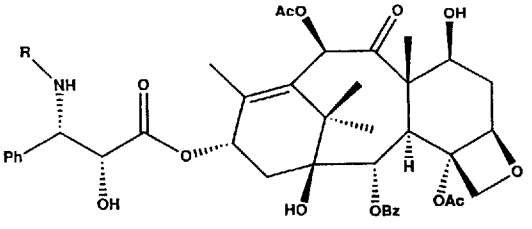
## ABSTRACT

Taxol [1], a highly functionalized antimitotic diterpene which occurs as a minor component of extracts of the bark from trees of the genus Taxus, is required in increasingly larger quantities for clinical trials. Final purification of taxol requires separation from cephalomannine [2]. An efficient separation of those closely related analogs has been achieved by normal phase HPLC on a cyanopropyl column.

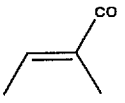
## INTRODUCTION

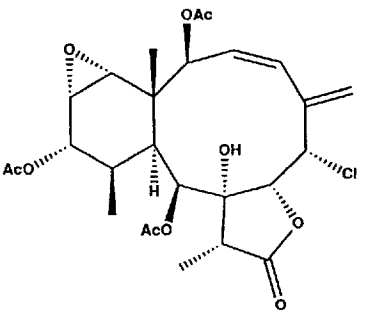
Discovered by Wall's group in 1967 (1), taxol [1] is a chemically and pharmacologically unique diterpene. The highly functionalized taxane diterpenes are known only from various species of Taxus and a few members of the Taxodiaceae (2). Unlike vinblastine and colchicine, taxol enhances, rather than inhibits tubulin polymerization (3,4). Now in Phase II clinical trials sponsored by the U.S. National Cancer Institute, taxol exhibits antitumor activity in a number of animal solid tumor and leukemia cell lines (1,5,6); more recently, taxol has shown interesting clinical activity (7).

Substantial quantities of taxol are required for continuing clinical evaluations and further development. Production by chemical synthesis has not, to date, proven feasible. The current approach to large scale isolation consists of methanol extraction of the bark of T. brevifolia, a $CH_2Cl_2$-$H_2O$ partition, a precipitation step and several silica and Florisil chromatographies. One of the difficulties in the currently




1 R = PhCO

2 R = 


3

employed isolation scheme is the separation of taxol and cephalomannine [2]. Normal phase silica HPLC, with 1-3% isopropanol or butanol in dichloromethane, has been required to effect the separation of these two compounds. A number of analytical HPLC methods exist, including the use of silica or $C_{18}$ phases (8) and reverse phase separations on phenyl and cyano bonded silica (9), but backpressure, solubility and scale-up considerations make these approaches less efficient for large scale applications. Herein we report an efficient, normal phase separation of these closely related compounds on a cyano bonded phase column.

## EXPERIMENTAL

General. Thin layer chromatography was conducted on cyano bonded TLC plates developed with hexane-isopropanol (3:2); detection with vanillin/sulfuric acid/ethanol revealed taxol and cephalomannine as dark blue spots. Sephadex LH-20 chromatography was monitored with an ISCO UA-5 detector set at 254 nm. HPLC separations employed Rainin Dynamax-Cyano$^R$ bonded phase columns of varying size -1 x 25 cm, 2.1 x 25 cm and 4.14 x 25 cm. Exact conditions are indicated below and in Figures 1 and 2.

Analysis of Taxol/Cephalomannine. Samples of taxol and cephalomannine/taxol mixtures were applied to a Rainin Dynamax-Cyano$^R$ column (2.1 x 25 cm) in $CH_2Cl_2$ and eluted with hexane-iPrOH (2:1) as indicated in Figure 1. Cephalomannine eluted after 38.1 minutes, taxol after 45.3 minutes.

Isolation of Taxol [1]. A sample of about 28 g of methanol extract of the bark of Taxus brevifolia was dissolved in 1000 mL MeOH-$H_2O$ (9:1) and extracted with hexane (3 x 400 mL). The polar phase was adjusted to 25% water and extracted with $CCl_4$ (3 x 400 mL). The upper phase was then adjusted to 35% water and extracted with $CHCl_3$ (3 x 400 mL). The methanol was removed from the polar phase at reduced pressure and the residual aqueous suspension was extracted with EtOAc (3 x 400 mL). Finally, the aqueous phase was lyophilized and the organic phases were reduced, in vacuo, to

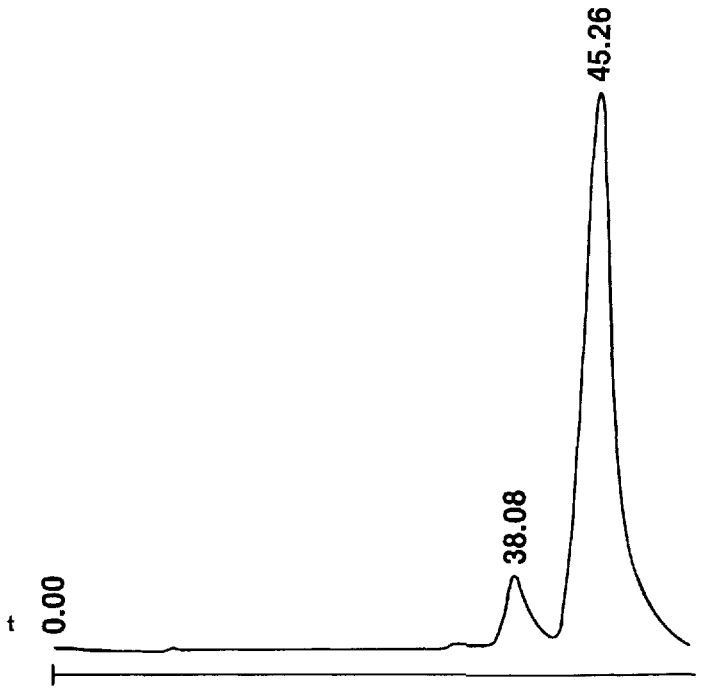



**Figure 1.** HPLC separation of taxol and cephalomannine. Conditions: Rainin Dynamax-Cyano column (2.1 x 25 cm); Elution, hexane-isopropanol (2:1); Flow, 9 ml/min; Detection, 270 nm. Retention times - cephalomannine [2], 38.1 minutes; taxol [1], 45.3 minutes, 20 mg injected.



dryness. The yields were: hexane - 0.567 g; $CCl_4$ - 0.450 g; $CHCl_3$ - 4.269 g; EtOAc - 6.89 g; and $H_2O$ - 16.24 g.

After TLC and $^1$H-NMR analyses indicated the $CCl_4$ phase as the locus of taxol, that portion of the extract was permeated through Sephadex LH-20 (column 1.5 x 150 cm) with $CH_2Cl_2$-MeOH (1:1). Nine fractions were obtained; TLC and $^1$H-NMR suggested that fraction 3 contained taxol. This fraction was applied to a semi-preparative HPLC column (Rainin Dynamax-Cyano$^R$, 2.1 x 25 cm) and eluted with a gradient program as detailed in Figure 2. Taxol eluted in about 33 minutes.

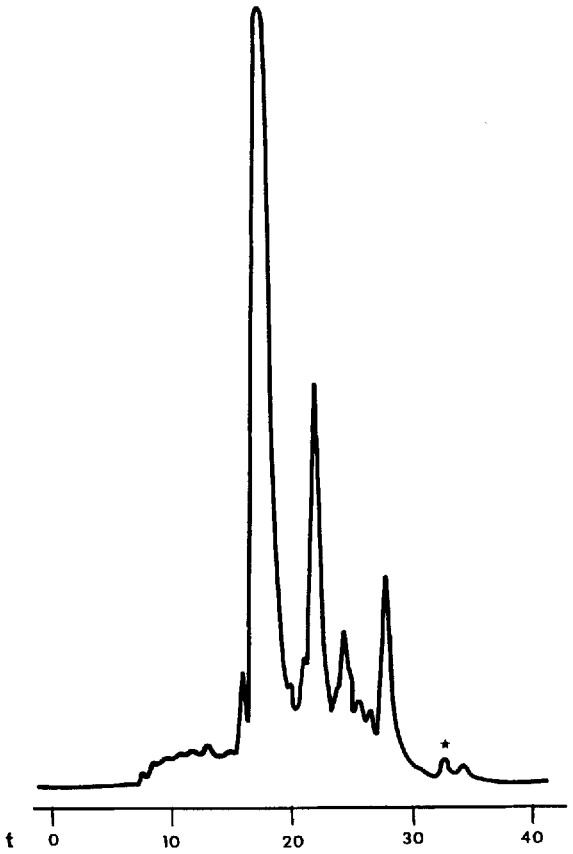



**Figure 2.** HPLC separation of taxol from methanol extracts of Taxus brevifolia. Conditions: Rainin Dynamax-Cyano column (2.1 x 25 cm); Elution, isocratic with hexane-isopropanol (7:3) for 7 minutes, gradient via curve #7 (Waters) to hexane-isopropanol (11:9) over 14 minutes, isocratic for 4 minutes, then gradient via curve #4 (Waters) to hexane-isopropanol (7:13); Flow, 9 ml/min; Detection, 270 nm; taxol (*) retention time, 33.3 minutes.

## RESULTS AND DISCUSSION

Previous success (10-12) in applying cyano bonded phase HPLC to the purification of a variety of briaran diterpenes, represented by brianthein Z [3], suggested that this methodology might prove useful as a final step in the isolation of taxol and other taxanes. TLC and analytical HPLC scouting indeed indicated that taxol and cephalomannine could be resolved on a cyano bonded phase column. As illustrated in Figure 1, complete separation of **1** and **2** was achieved on a semi-preparative scale (20-40 mg injections). These same conditions were applied to partially purified mixtures of **1** and **2**, with traces of other taxane diterpenes, obtained from extracts of T. brevifolia. In each case, any other constituents present eluted ahead of **1** and **2**.



Next, an evaluation was made of the potential applicability of the method to the isolation of taxol from crude extracts. The methanolic extract of T. brevifolia bark was subjected to a four-step solvent partitioning scheme (10). Taxol was concentrated primarily in the $CCl_4$ phase; this material was permeated through Sephadex LH-20 to give a taxol enriched fraction. Isocratic HPLC analysis of this material revealed that it was still comprised of a complex mixture. Modifying the elution conditions to a hexane-isopropanol gradient (Figure 2) did pinpoint taxol and application of this approach provided 2.2 mg of pure taxol (unoptimized). No analysis of the $CHCl_3$ soluble fraction was made.

In summary, a new efficient method has been developed, then, for the resolution of mixtures of taxol and cephalomannine. This approach offers the advantages of mild conditions and use of cheaper (and easier to dispose of) solvents. The HPLC conditions can be readily adapted for purity checks on drug samples and quantitative methods for analysis of taxol in physiological media for clinical and metabolic studies. The method can also be adapted to larger scale isolation of taxol from partially purified extracts. The cyano bonded phase column is quite probably amenable to the separation of other taxane diterpenes as well.

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