

## Application Note 162

# Separations of Paclitaxel and Its Metabolite 6 $\alpha$ -hydroxypaclitaxel by Reversed-Phase HPLC using Discovery Columns

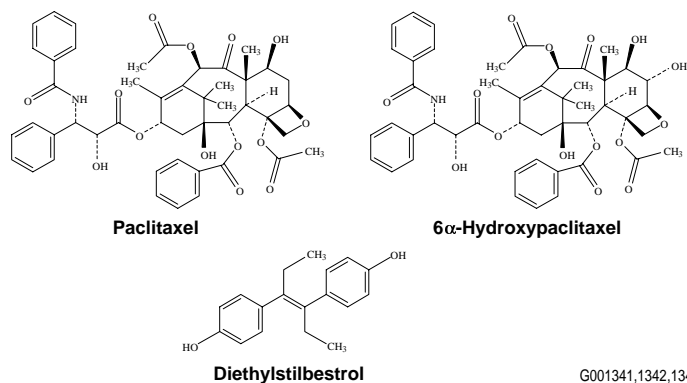
**A simple isocratic elution method was developed to separate paclitaxel, 6 $\alpha$ -hydroxypaclitaxel and an internal standard in real biological samples by reversed-phase HPLC using Discovery C18, Discovery C8, and Discovery RP-AmideC16 columns. These columns offer excellent resolution, stability, and unique selectivity.**

### Key Words:

paclitaxel • 6 $\alpha$ -hydroxypaclitaxel • Discovery HPLC columns

Paclitaxel is a novel anti-cancer drug originating from the bark of the Pacific yew tree (*Taxus brevifolia*) proven to be effective in the treatment of a variety of cancers, including ovarian, breast, lung, and leukemia<sup>2, 3, 4</sup>. 6 $\alpha$ -Hydroxypaclitaxel is the major paclitaxel metabolite produced by the P450 enzyme, CYP2C8. Quantitative determination of this metabolite in paclitaxel treated cancer patients is critical in pharmacology and toxicology<sup>5, 6</sup>. With the increased demand for testing, the requirement for more stable and reproducible analytical testing methods has also emerged.

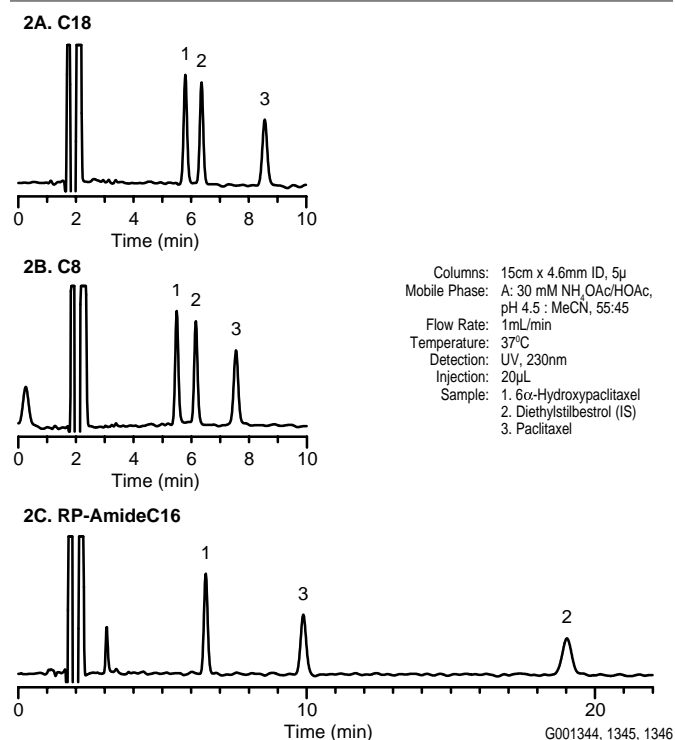
**Figure 1. Structures of paclitaxel, 6 $\alpha$ -hydroxypaclitaxel, and diethylstilbestrol.**



Paclitaxel, 6 $\alpha$ -hydroxypaclitaxel, and internal standard diethylstilbestrol were well separated by a reversed-phase isocratic elution (NH<sub>4</sub>Ac buffer : MeCN, 55 : 45) on three Discovery Columns: C18, C8 and RP-AmideC16 (Figure 2). On Discovery C18 and C8, less hydrophobic 6 $\alpha$ -hydroxypaclitaxel eluted first, followed by internal standard diethylstilbestrol and paclitaxel, respectively (Figures 2 A&B). A unique selectivity difference was observed on Discovery RP-AmideC16 column (Figure 2C). The elution order of diethylstilbestrol and paclitaxel was reversed while diethylstilbestrol was retained much longer than paclitaxel on this column. This phenomenon can be attributed to the hydrogen bonding between the aromatic hydroxyl

group on diethylstilbestrol and the amide functionality of RP-AmideC16. Differences in selectivity among Discovery columns provide us another dimension in method development when trying to improve chromatographic resolution.

**Figure 2. Chromatograms of a standard mixture of paclitaxel, 6 $\alpha$ -hydroxypaclitaxel, and internal standard diethylstilbestrol on Discovery C18, C8, and RP-AmideC16 columns.**

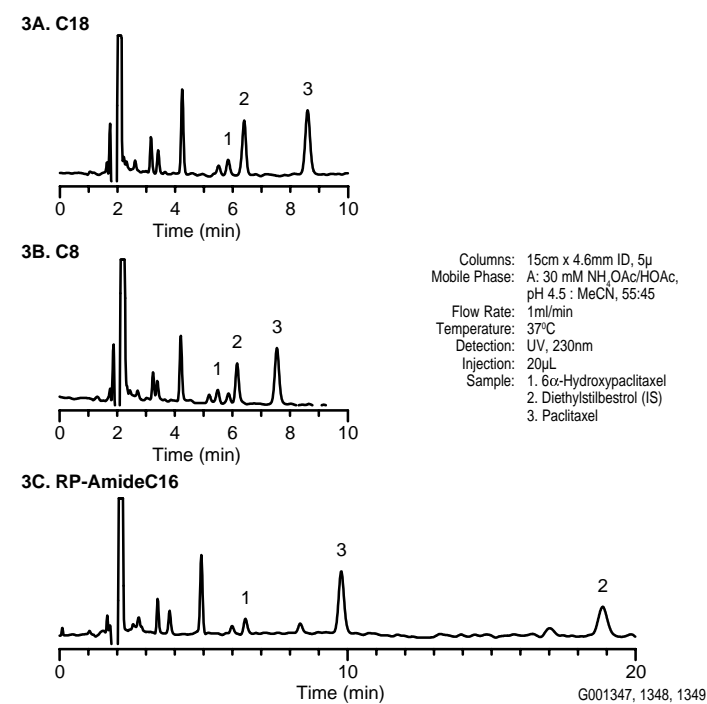


In the human body, paclitaxel can be metabolized enzymatically into several metabolites. One important metabolite is 6 $\alpha$ -hydroxypaclitaxel, which is generated through paclitaxel 6 $\alpha$ -hydroxylation when paclitaxel is incubated with enzyme CYP2C8 (one form of cytochrome P450 enzymes). One sample was paclitaxel incubated with enzyme CYP2C8, the other was paclitaxel incubated with human liver microsome with diethylstilbestrol as internal standard in both samples. After extraction, the dry sample residues were reconstituted with mobile phase solvent before injections. These samples were again run on Discovery C18, C8, and RP-AmideC16 columns (Figures 3&4). It is clear that the biological matrix introduces additional peaks, but all three Discovery columns gave good resolution without interfer-

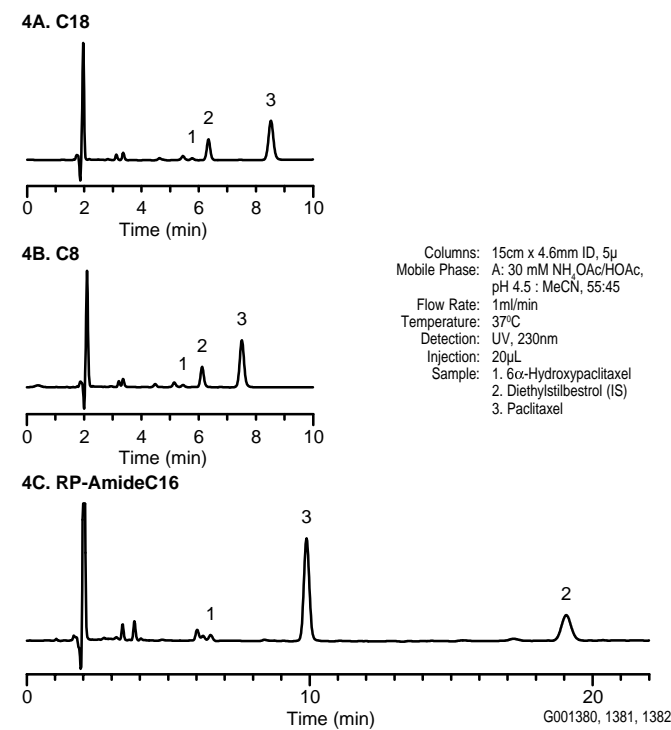
ence from the matrix peaks. Discovery RP-AmideC16 exhibits a selectivity and resolution that is particularly noteworthy, in that all matrix peaks are clearly baseline resolved from the three peaks of interest.

This simple isocratic elution method is well suited for qualitative and quantitative analysis of paclitaxel and its metabolite 6 $\alpha$ -hydroxypaclitaxel in real biological samples. The volatile ammonium acetate buffer used in this method also makes it attractive and convenient for LC-MS applications if desired.

**Figure 3. Isocratic elution of Paclitaxel sample incubated by enzyme CYP2C8 using Discovery C18, C8, and RP-AmideC16 columns.**



**Figure 4. Isocratic elution of Paclitaxel sample incubated with human liver microsome by reversed-phase HPLC on Discovery C18, C8, and RP-AmideC16 columns.**



### Ordering Information:

Description	Cat. No.
Discovery RP-AmideC16 15cm x 4.6mm	505013
Discovery C18 15cm x 4.6mm	504955
Discovery C8 15cm x 4.6mm	59353-U

### References

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