

HPLC Column Offers Longer Lifetime and Improved Resolution of Taxanes

Interest in taxol, a taxane in the bark of the Pacific yew tree, is growing because of the compound's antitumor properties. However, analysis of taxol by traditional reversed phase HPLC is difficult – the complex plant cell extract shortens column lifetimes and is poorly resolved. Improved selectivity for taxol and related taxanes offered by SUPELCOSIL LC-F columns allows analysis of taxol in complex extracts or in relatively pure drug formulations. Independent evaluators report the column is stable over 2000-3000 injections of taxol mixture. (ChromFax: 394015)

Key Words:

taxol • taxanes • antitumor agents

Taxol (Figure A, I) is a chemically and pharmacologically unique taxane diterpene amide produced by the Pacific yew tree, *Taxus brevifolia*. Recently approved by the US Food and Drug Administration for treatment of ovarian cancer, this natural antitumor agent shows promise for treating other cancers, including leukemia, breast cancer, and lung cancer. Taxol interferes with microtubules – the fine, hollow organelles in the cell cytoplasm that play a role in cell division (1).

Analysis of taxol in crude bark extracts is challenging, due to the array of indigenous compounds present. Furthermore, taxol is only one of a series of taxane compounds in *T. brevifolia* extracts. Cephalomannine (Figure A, III), another taxane present in significant quantities, is difficult to separate from taxol. Another closely eluting taxane, pretaxol or 7-epi-10-deacetyltaxol (Figure A, II), is often ignored in published methods (2-4), but potentially can interfere with the analysis. Concern about overharvesting the tree has spurred interest in the needles, but large amounts of photosynthetic pigments and cuticular waxes make analyses of foliage extracts more difficult than analyses of bark extracts. Attempts at tissue culture continue (2), but reproducible success is elusive.

Traditional C18, phenyl, and cyano reversed phase columns have been used to resolve the extracts (5,6), but the analyses are characterized by long separation times, poor resolution, and short column lifetime because of the complex matrix involved. Recently, commercial pentafluorophenyl phase (PFP) columns were developed for this analysis (7,8). The work summarized here introduces the SUPELCOSIL™ LC-F column and compares its performance to that of other columns. The crude taxol mixture we used to evaluate column performance was provided by Mr. Steve Richeimer of Hauser Chemical Research.

Figure A. Taxol and Related Taxanes

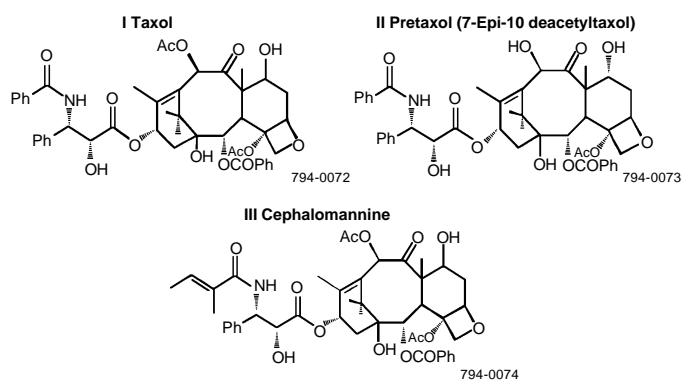


Figure B. Tetrahydrofuran Concentration Affects Taxol Analysis on a SUPELCOSIL LC-F Column

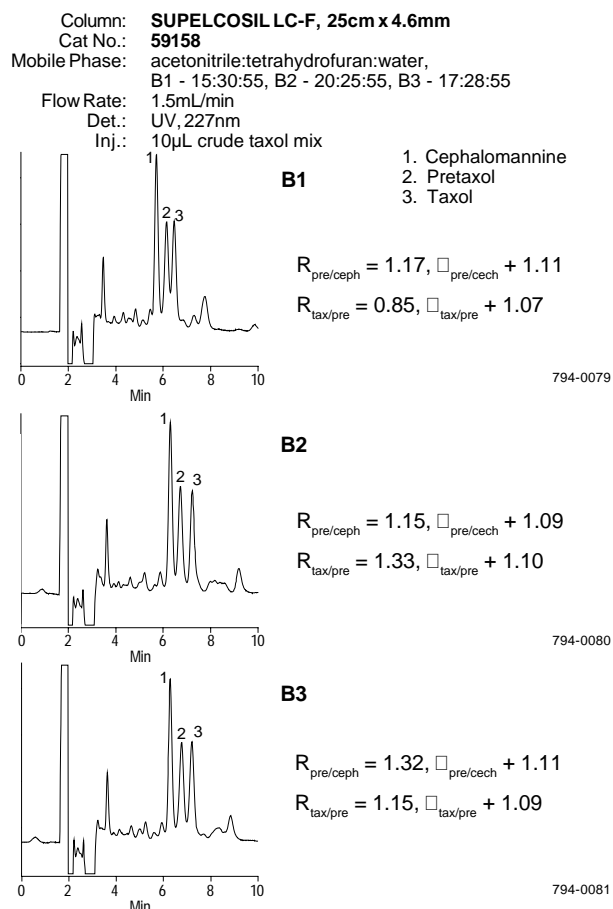
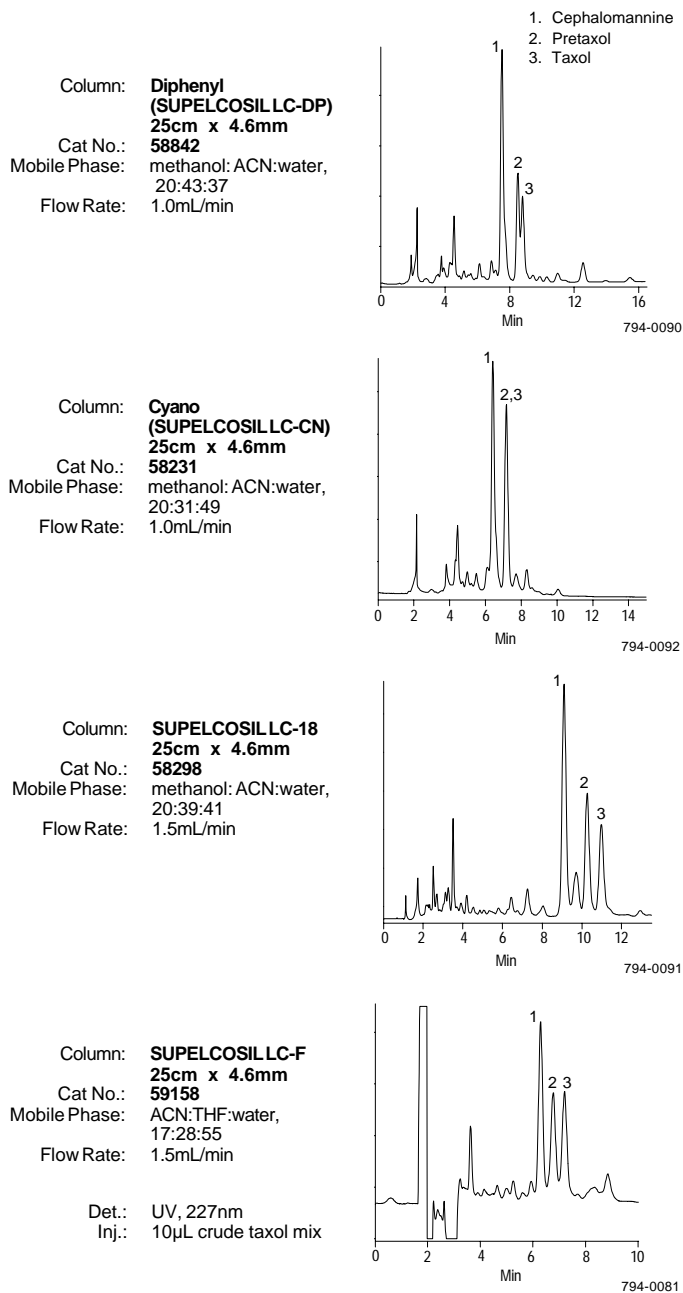


Figure C. Taxol Analysis: Phase Comparison



Our evaluations indicate that a three-component mobile phase consisting of acetonitrile, tetrahydrofuran, and water, 17:28:55, and the conditions shown in Figure B provide the best taxanes separations on SUPELCO SILLC-F columns. Selectivity (S) and resolution (R) are sensitive to changes in the tetrahydrofuran content of the mobile phase, as Figure B shows. Because as little as a 2% change in THF content affects the analysis, you can adjust the mobile phase for the specific composition of your samples.

Once suitable conditions are established, SUPELCO SILLC-F columns show consistent performance for the analysis. For 13 columns, selectivity values for pretaxol/cephalomannine and taxol/pretaxol separations were 1.10-1.12 and 1.08-1.09, respectively. Each batch of SUPELCO SILLC-F packing is tested with a crude taxol mixture, to ensure separation of the three taxanes. Every column is tested to guarantee high efficiency and good peak symmetry.

Figure C compares taxanes analyses on traditional reversed phase columns, using conditions described in the literature (2-8). Of the columns tested, SUPELCO SILLC-F and SUPELCO SILLC-18 columns exhibited the best combinations of resolution, selectivity, and analysis time. However, all traditional reversed phase columns, including SUPELCO SILLC-18 columns, foul quickly when used with complex taxol-containing extracts. In contrast, SUPELCO SILLC-F columns are both durable and capable of selective, reproducible analyses. Customer evaluators report that, when protected by a guard column, SUPELCO SILLC-F columns are stable over 2000-3000 injections of taxol mixture; only 1/3-1/2 as many injections can be made on diphenyl or PFP columns. The unique selectivity of SUPELCO SILLC-F columns also affords flexibility in tuning the separation.

Ordering Information:

SUPELCO SILLC-F Column	25cm x 4.6mm	59158
Supelguard™ Guard Column Kit	2cm cartridge guard column, holder, connecting hardware	59520
Guard Columns	package of 2	59521

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