

Application Note 174

Improving on the USP Method: LC/MS Analysis of Paclitaxel and Related Taxanes on Discovery HS F5

The high flow rates and absence of mobile phase ions in the USP 26 method for paclitaxel make it not readily amenable to analysis by LC/MS. We sought to modify the method to make it more LC/MS compatible, while still maintaining adequate resolution of all ten compounds. A Discovery HS F5 column in narrowbore dimensions was chosen for the study.

The Analysis of Paclitaxel

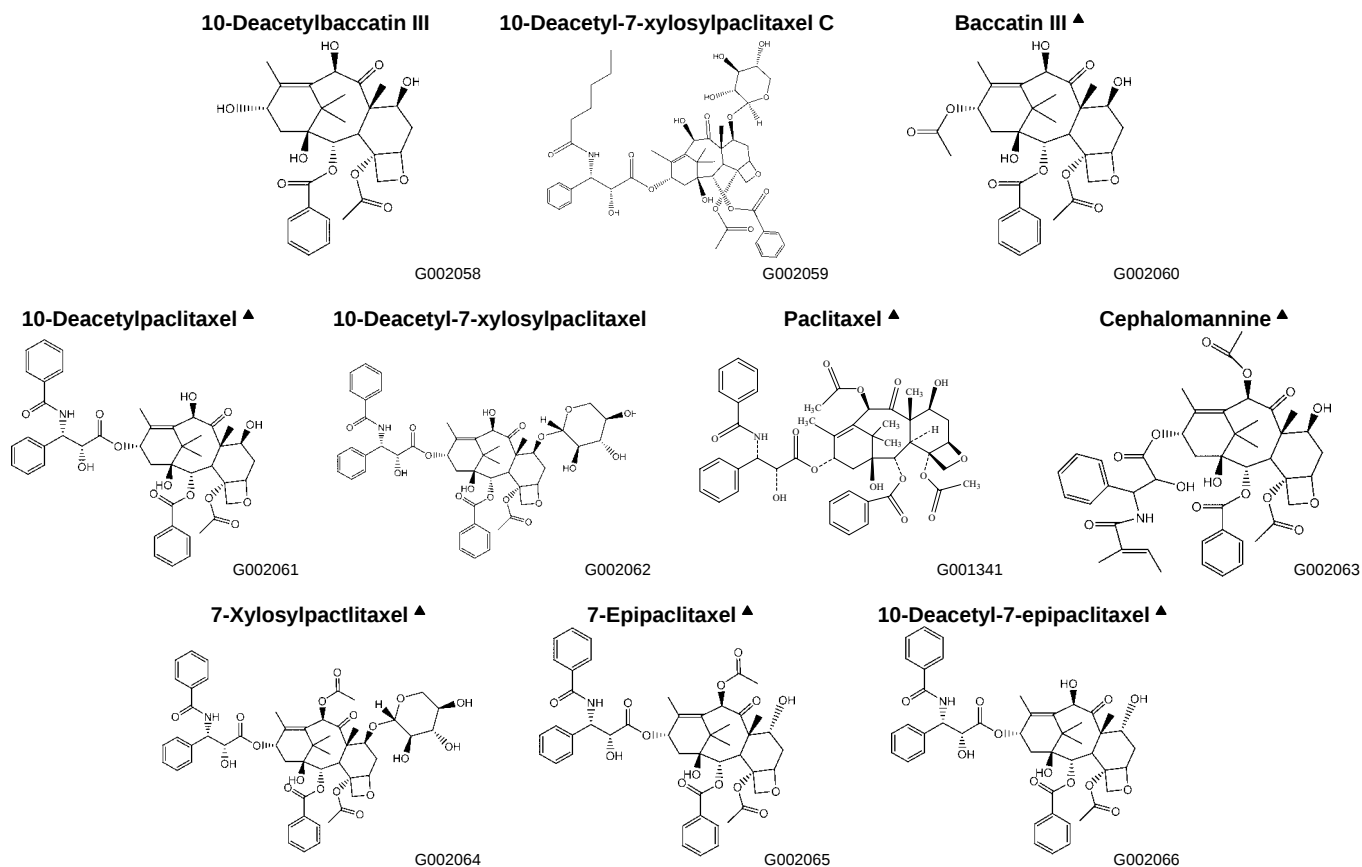
Paclitaxel, more commonly referred to by the trade name Taxol®, is a member of the larger family of compounds known as taxanes. Currently, paclitaxel is used in the treatment of breast, ovarian, certain non-small-cell lung cancers, and Kaposi's

sarcoma. The structures of paclitaxel and related taxanes are presented in Figure A.

High-performance liquid chromatography (HPLC) in conjunction with ultraviolet (UV) detection is most commonly used for paclitaxel analysis. The USP 26 method employs a pentafluorophenyl HPLC column (L43) and a gradient of acetonitrile in water to separate ten of the most common taxanes. Detection is via UV.

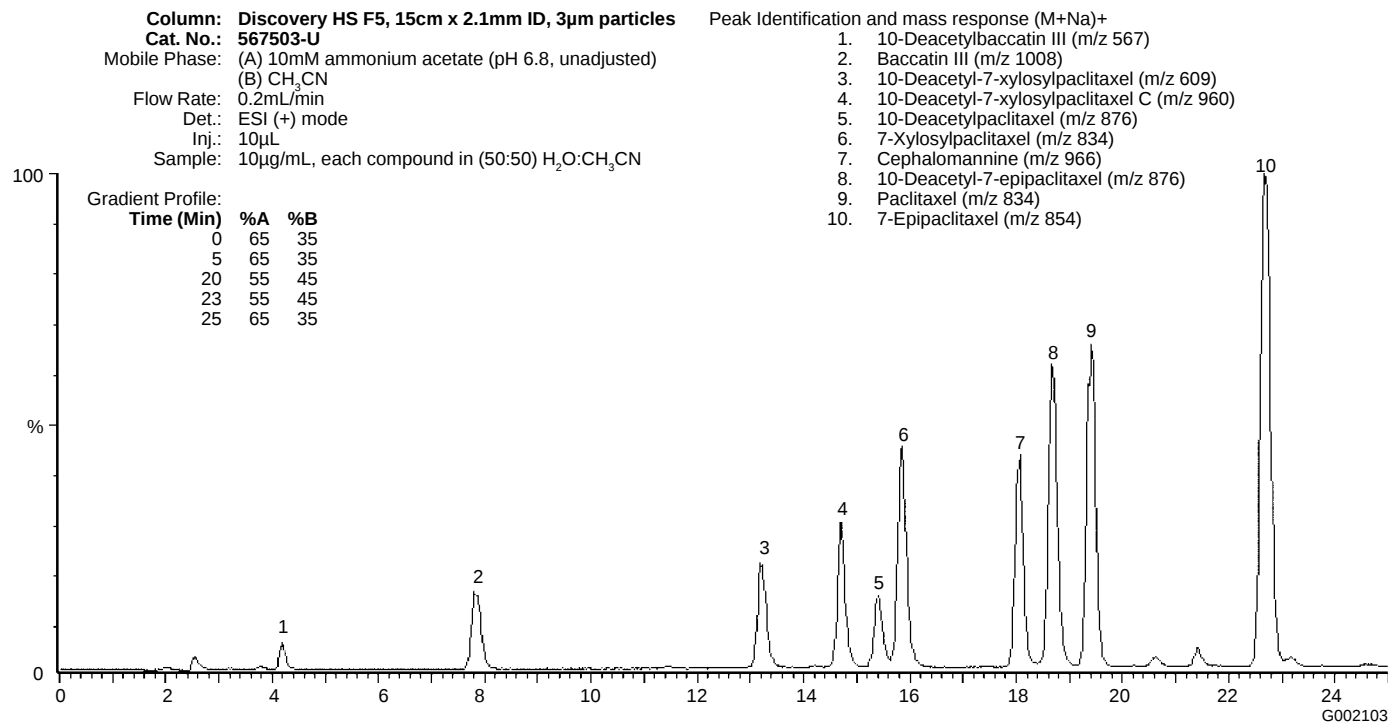
Taxane samples are often plant extracts that contain a complex mixture of closely-related compounds. Additionally, the activity of taxanes is high; compounds that exist at very low levels must be identified and investigated. The complexity of the sample and the need for low-level detection makes LC/MS an ideal method for taxane analysis due to the selectivity and sensitivity of the technique.

Figure A. Structure of Paclitaxel and Taxanes



▲ Listed in USP 26 method for paclitaxel

Figure B. LC/MS Separation of Taxanes on Discovery HS F5



LC/MS Analysis of Taxanes: Improvement over the USP Method

The objective of this application was to develop conditions suitable for the LC/MS analysis of paclitaxel and related taxanes. The current USP 26 method for taxanes is not readily amenable for LC/MS analysis due to the high flow rates and the absence of ionization control in the mobile phase. Two improvements were made in this study: a volatile buffer was added to the mobile phase to promote stable ionization, and a 2.1mm ID narrowbore Discovery HS F5 column was used. The narrow ID allowed the use of lower flow rates, while also increasing peak response (sensitivity).

Figure B shows baseline resolution of paclitaxel and nine related compounds on a 2.1mm ID Discovery HS F5 column using conditions suitable for LC/MS. The mixture of taxanes shown in Figure A and separated in Figure B contains all but three of the taxane compounds listed in the USP 26 method for paclitaxel. These three compounds were not commercially available. The following three commercially available taxanes were used in their place: 10-deacetylbaecatin III, 10-deacetyl-7-xylosylpaclitaxel, and 10-deacetyl-7-xylosylpaclitaxel. Under these conditions the predominant response for all analytes is the sodium adduct (M+Na)⁺. Note that this analysis used a total of 5mL of mobile phase (25 minutes at 0.2mL/min), while the USP method uses 200mL (80 minutes at 2.5mL/min).

Conclusion

A simple LC/MS method for the separation and identification of paclitaxel and nine related taxanes was developed on a 2.1mm ID Discovery HS F5 column. The use of MS detection promises to facilitate identification of the analytes in complex mixtures and should provide enhanced sensitivity over UV-based systems.

Trademarks

Discovery - Sigma-Aldrich Co.

Taxol - Bristol-Myers Squibb Company

Ordering Information

Other dimensions and Discovery phases are available. Please call or visit our web site.

Description	Cat. No.
Discovery HS F5, 3µm particles	
15cm x 2.1mm ID	567503-U
2cm x 2.1mm ID Supelguard kit*	567571-U
2cm x 2.1mm ID Supelguard cartridges, pk. of 2	567570-U

* Kit includes one cartridge, a stand-alone holder, a piece of 1/16" ID tubing, and 2 nuts and ferrules.

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