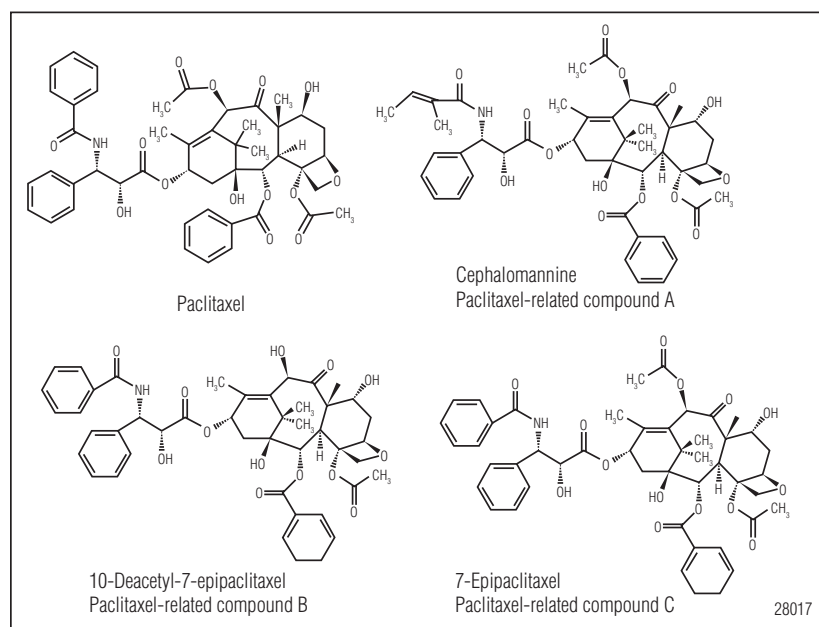


Rapid Separation of Paclitaxel and Related Compounds in Paclitaxel Injection

INTRODUCTION

Paclitaxel was approved as Taxol® by the National Cancer Institute (NCI) in 1991 for the treatment of ovarian cancer. An analysis of paclitaxel and related compounds—cephalomannine (related compound A), 10-deacetyl-7-epipaclitaxel (related compound B), and 7-epipaclitaxel (related compound C) (structures shown in Figure 1)—by reversed-phase (RP) HPLC was published by both the United States Pharmacopeia (USP) and the Chinese Pharmacopoeia (CP). These methods each required over 70 min.^{1,2}

There are several RP-HPLC assays for paclitaxel in the literature;³⁻⁶ however, all of them are also relatively long (15 to 35 min). Therefore, researchers interested in the rapid separation of paclitaxel and related compounds developed a UHPLC method,⁷ but the separation did not include paclitaxel-related compound B, which is required by the USP. Use of an UltiMate® 3000 RSLC system and an Acclaim® RSLC C18 column packed with smaller particles is an easy way to increase speed and peak capacity. Therefore, the authors used this system to create an UHPLC method for the analysis of paclitaxel and related compounds including related compound B.



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Figure 1. Structures of paclitaxel and related compounds.

The work shown here describes a rapid and efficient UHPLC method to separate paclitaxel and related compounds in a Paclitaxel Injection sample. The method uses an Acclaim RSLC 120 C18 column (2.1×100 mm, $2.2 \mu\text{m}$) with a water/acetonitrile/methanol gradient mobile phase (Table 1) at a flow rate of 0.42 mL/min , and a detection wavelength of 227 nm . Figure 2 shows an overlay of a chromatogram of a Paclitaxel Injection sample on that of a mixture of standards.

This improved UHPLC method achieves a baseline separation within 6 min. The resolution values ($R_s > 2.2$) were greater than those required in the USP and CP methods, (not less than [NLT] 1.2 for paclitaxel and paclitaxel-related compound B, and NLT 1.0 for paclitaxel-related compound A and paclitaxel-related compound B).

One difference worth noting is the order of elution for paclitaxel and related compound B. Initial experiments using a larger Acclaim 120 C18 column (4.6×250 mm, $5 \mu\text{m}$) with a water/acetonitrile binary gradient, as described in the USP and CP methods, showed related compound B eluting before paclitaxel. However, with the smaller Acclaim 120 C18 RSLC column (2.1×100 mm, $2.2 \mu\text{m}$) and water/acetonitrile/methanol ternary gradient, related compound B elutes after paclitaxel.

The peak area relative standard deviation (RSD) for five replicate injections is $\leq 0.45\%$, demonstrating good performance for the rapid separation of paclitaxel and related compounds using an UltiMate 3000 RSLC system and an Acclaim C18 column packed with smaller particles.

With this improved UHPLC method, it was found that the methanol/acetonitrile/water ternary gradient dramatically reduced the analysis time by an order of magnitude while still meeting USP and CP requirements for peak resolution and peak area reproducibility.

Table 1. Gradient for the Separation of Paclitaxel and Related Compounds

Time (min)	Flow Rate (mL/min)	H ₂ O (%)	Acetonitrile/Methanol (40:60, v/v) (%)
-2.0	0.42	40	60
0.0		40	60
4.4		28	72
4.5		20	80
6.0		20	80

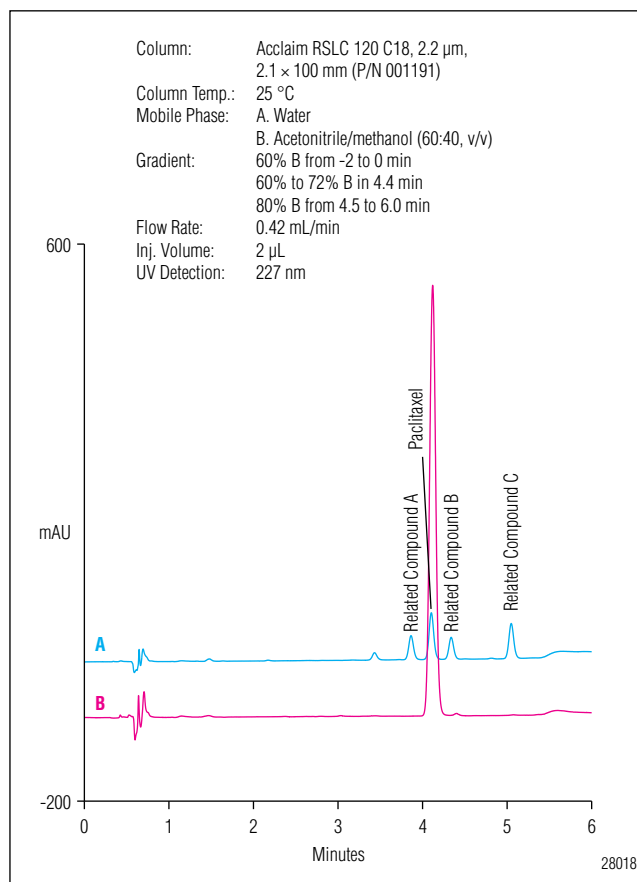


Figure 2. Overlay of chromatograms of (A) mixture of paclitaxel and related compounds standards ($5 \mu\text{g/mL}$ for each) and (B) Paclitaxel Injection sample.

EQUIPMENT

Dionex UltiMate 3000 RSLC system including:

HPG-3400RS Pump

WPS-3000RS Autosampler

TCC-3000RS Thermostatted Column Compartment

DAD-3000RS UV-vis Detector

Chromeleon[®] Chromatography Data System (CDS)

software version 6.80 SR9

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