High-Resolution Analysis of Taxanes Using Rapid Resolution HT (1.8 µm) Agilent Eclipse Plus Phenyl-Hexyl Columns

Application

Pharmaceutical

Authors

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Abstract

Thirteen taxane derivatives were separated using an Agilent ZORBAX Rapid Resolution High Throuhput (RRHT) Eclipse Plus Phenyl-Hexyl column. Using an easily modified gradient separation, high resolution (Rs = 2) is maintained for all peaks. No significant loss in chromatographic resolution is noted when an Agilent G1956B Single Quadrupole Mass Spectrometer is installed in series after the G1315SL diode array detector. Eclipse Plus Phenyl-Hexyl characteristics such as improved silica, endcapping, and bonding technology, as well as the molecular interactions of the phenyl-hexyl moiety with the conjugation within the taxane derivatives, make the Eclipse Plus Phenyl-Hexyl column an ideal choice for this RRHT taxane method.

Introduction

Taxanes are diterpenes that include paclitaxel and docetaxel, and are used to treat cancer. The principal mechanism of the taxane class of drugs is the disruption of microtubule function. Microtubules are cellular structures essential to cell division, and taxanes stop the mitosis step. In normal cell growth, microtubules are formed when a cell starts dividing. After the cell divides, the microtubules are broken down. Taxanes stop the microtubules from breaking down; cancer cells become so clogged with microtubules that they cannot grow and divide. [1,2]

Paclitaxel was originally derived from the Pacific yew tree (*Taxus brevifolia*). The isolation of 10 g of paclitaxel required over 1,200 kg of bark. Shortages of Pacific yews led to semisynthetic taxanes derived from other trees, namely the Himalayan yew tree. Today most commercial taxane is produced using plant cell fermentation. [2,3]

Different taxane derivatives possess varied medicinal properties. In attempts to improve the effectiveness of treatments, pharmaceutical companies continue to investigate and develop newer taxane variants. In this work, paclitaxel and more than 12 variants are successfully separated on an Eclipse Plus Phenyl-Hexyl column.

Experimental

Taxane analysis was performed with the Agilent 1200 Rapid Resolution LC (RRLC) system:

- G1312B binary pump SL with mobile phase A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile. Flow rate was 1 mL/min. The gradient was 30% B initial composition, ramping to 45% B over 15 minutes, then ramping to 60% B over an additional 5 minutes.

- G1376C automatic liquid sampler SL (ALS); injection volume was 5.0 µL.
- G1316B Thermally Controlled Column Compartment (TCC) SL; temperature was 25 °C.
- G1316C Diode Array Detector (DAD) Wavelength used was 254,4 nm Ref = off, with a G1315-60024 microflow cell (3-mm path, 2-µL volume)
- G1956 MSD/SL Single Quadrupole mass spectrometer connected in series from the DAD microflow cell outlet to the mass spectrometer inlet.

ZORBAX Columns:

- Eclipse Plus Phenyl-Hexyl 4.6 x 50 mm, 1.8 µm, Part Number 959941-912
- Eclipse Plus C18 4.6 x 50 mm, 1.8 µm, Part Number 959941-902

Chemicals

HPLC-grade acetonitrile was from Burdick & Jackson (Muskegon, MI). 18 MΩ Milli-Q Water was produced onsite. Formic acid was from Sigma Aldrich (Bellfonte, PA). The "13-mix" taxane mixture was purchased from IB Hauser Chemical (Boulder, CO) and consisted of approximately 50 µg/mL each of the taxane alkaloids, presented in the order of elution in Table 1. Standards of paclitaxel and cephalomannine were purchased from Sigma Aldrich. These two compounds were dissolved in acetonitrile at 1 mg/mL, diluted with water, and diluted to a final 100 µg/mL each for use in peak confirmation in addition to mass spectral analysis.

Results and Discussion

The structure of paclitaxel is shown in Figure 1A. Other related compounds found in this mixture are subtle variants on this structure. An example of the subtle substitutions in this series is cephalomannine, shown in Figure 1B, with the dissimilarity highlighted in the boxes.

The large number of analytes in similar structures indicate that a gradient method would likely be preferred over an isocratic method. The heavy conjugation and multiple aromatic rings suggest that a phenyl column be tried in addition to a C18 column. Because many drug discovery labs use mass spectroscopy for additional information, a volatile acidic mobile phase additive was chosen (formic acid). This allows for good column life, limits the effects of residual silanols, and provides

Table 1. Summary of Chromatographic Experiments on Eclipse Plus Phenyl-Hexyl

Peak	Compound	Molecular Weight	tr	Rs	k'
1	10-deacetylbaccatin III	544	1.79	NA	2.6
	Impurity	Not determined	2.75	8.42	4.5
2	Baccatin III	586	3.81	6.98	6.6
3	7-xylosyl-10-deacetyltaxol B	921	5.86	11.73	10.8
4	Taxinine M	703	6.34	2.37	11.7
5	7-xylosyl-10-deacetyltaxol	943	7.1	3.59	13.2
6	7-xylosyl-10-deacetyltaxol C	937	7.93	4.02	14.9
7	10-deacetyltaxol	811	9.01	4.7	17.1
8	7-xylosyltaxol	985	9.55	2.27	18.1
9	Cephalomannine (Taxol B)	831	11.53	7.85	22.1
10	7-epi-10-deacetyltaxol	811	12.22	2.42	23.5
11	Paclitaxel	853	13.12	3.11	25.3
12	Taxol C	847	14.24	3.81	27.6
13	7-epitaxol	853	16.82	9.21	32.7

good ionization of compounds for MS. Short rapid-resolution high-throughput (RRHT) columns were chosen because of their high efficiency, fast analysis, and short equilibration times, as well as their low pressure even on typical 400-bar HPLC systems. [4]

Previous work published by Theodoris et al. evaluated the same Hauser 13 taxane mix using several C18 and phenyl columns. The separations required

Paclitaxel

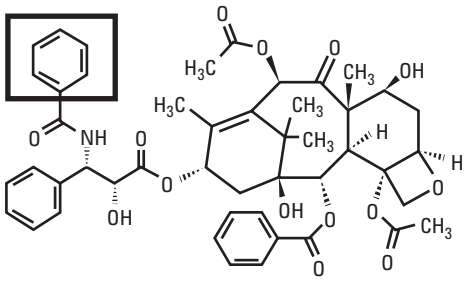

Figure 1A.

Cephalomannine

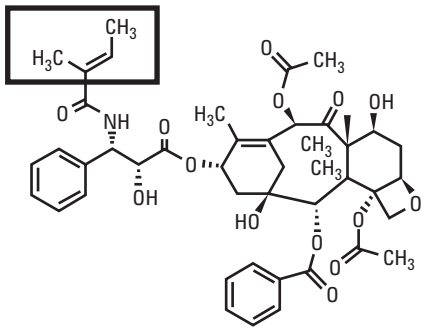

Figure 1B.

between 30 and 70 minutes with an additional 5 to 10 minutes for equilibration (3 to 5 column volumes). In most of these cases, the separations were very good but did not fully resolve all compounds and preserve the separation in the MS. [5]

In this investigation, final separations were faster and the resulting resolution improved. Figure 2 shows a chromatographic overlay of the taxanes separated on an Eclipse Plus Phenyl-Hexyl column and on an Eclipse Plus C18 column. The methods are identical except for the different columns (stationary phases). The Eclipse Plus Phenyl-Hexyl column has better selectivity for taxanes than the Eclipse Plus C18. Note that peaks 6 and 7 are not fully resolved on the C18 column. Better Rs is likely due to π to π interactions of the analytes with the phenyl hexyl group.

Figure 3 shows both the UV and the MS total ion chromatogram from the phenyl-hexyl column. The critical pair is the 10-deacetyltaxol and 7-xylosyltaxol (peaks 7 and 8), both clearly baseline resolved. These peaks remain baseline resolved even after transfer to the MS. During the course of experimentation, an additional peak, a degradation not identified, appeared. It too was separated on this gradient. It was also found in the original sample mix from Hauser, albeit at much lower concentrations.

MS was used to verify the elution order of the analytes on the Eclipse Plus Phenyl-Hexyl column. Table 1 lists the molecular weight of the compounds of interest; pseudomolecular ions with an *m/z* value of M+1 were found for all compounds listed. No significant loss of resolution was found between the DAD and MS detector, as can be seen in Figure 3. The maximum pressure reached during this analysis was approximately 240 bar with the pulse damper used. This indicates that a faster flow rate is possible. Analysis time could be shortened proproportional to the flow rate, while maintaining k' using the formula:

Gradient time adjustment

$$t_{G2} = t_{G1} \times \frac{V_2}{V_1} \times \frac{F_1}{F_2}$$

t gradient times
V respective column void volumes
F respective flow rates

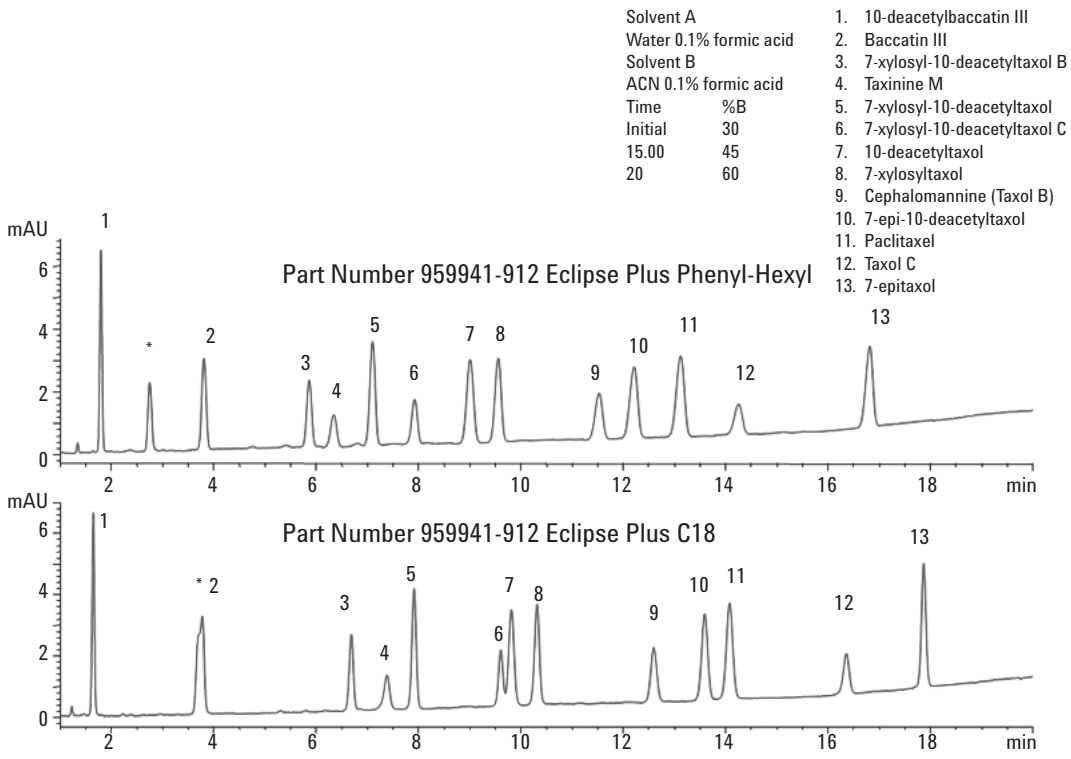

Figure 2. Separation of taxane mixtures on Agilent ZORBAX Eclipse Plus Phenyl-Hexyl and C18 columns.

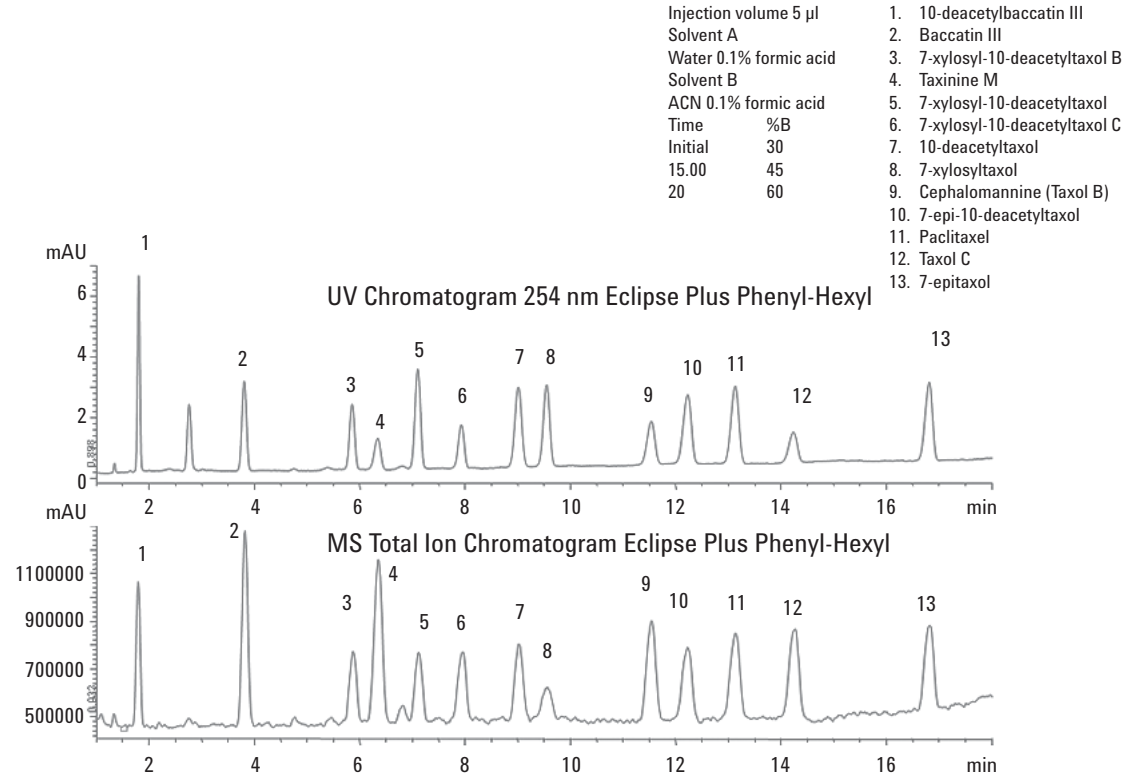

Figure 3. Overlay of UV and MS chromatograms of taxane mix on Agilent ZORBAX Eclipse Plus Phenyl-Hexyl columns.

Conclusions

The RRHT Eclipse Plus Phenyl-Hexyl column provides an excellent separation of a mixture of 13 taxane compounds. The results with this column were superior to those with the corresponding C18 column because the method as shown here can provide researchers opportunities to maximize separations based on π to π and phenyl interactions. It is easily implemented and requires no complicated mobile phase compositions. The separation shown on an Eclipse Phenyl-Hexyl column can be performed on most commercial HPLC systems, as the pressure is substantially lower than 400 bar.

Taxanes remain a mainstay in chemotherapy treatment of advanced-stage cancer, reflected by the fact that most patients with advanced cancer receive a taxane at some point during treatment. While paclitaxel and docetaxel lead this market, benefit awaits companies (and patients) following the development of novel taxanes that demonstrate superior efficacy over that of existing taxanes.

Eclipse Plus Phenyl-Hexyl column characteristics, such as improved silica, endcapping, and bonding technology, as well as the molecular interactions of the phenyl hexyl moiety with the conjugation within the taxane derivatives make the Eclipse Plus Phenyl-Hexyl column a good choice for this RRHT taxane method.

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