Analytical Method Development for USP Related Compounds in Paclitaxel Using an Agilent Poroshell 120 PFP

Application Note

Clinical Research

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Abstract

An analytical method for the analysis of USP related compounds in paclitaxel was run on a superficially porous Agilent Poroshell 120 PFP 4.6 × 250 mm, 4 µm column. The method repeated the United States Pharmacopeia test for related compounds in paclitaxel. The analytical method was then transferred to a 3.0 × 100 mm, 2.7 µm Poroshell 120 PFP with significant solvent and time saving. Both columns met all system suitability requirements.

Introduction

Paclitaxel, a drug also known as Taxol and Onxol, was first isolated from the bark of the Pacific yew tree, *Taxus brevifolia* in 1967. After the 1990s, synthetic methods and plant cell fermentation technology were used for its production. United States Pharmacopeia (USP) includes three different HPLC analytical methods for related compounds, based on the source of the paclitaxel. The method for the related compounds analysis in paclitaxel from natural sources uses a PFP column for the analysis [1].

The Agilent Poroshell 120 PFP (pentafluorophenyl) stationary phase can give extra retention and selectivity for positional isomers of halogenated compounds. These PFP columns can also be used for selective analysis of nonhalogenated compounds, such as polar compounds containing hydroxyl, carboxyl, nitro, or other polar groups. This selectivity is enhanced when the functional groups are on an aromatic or other rigid ring system [2].

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We describe a method developed for the USP related compounds analysis with a 4 µm Poroshell 120 PFP column transferred to a 2.7 µm Poroshell 120 PFP column. This approach delivered significant time and solvent savings.

Materials and Methods

All reagents and solvents were HPLC or analytical grade. The standards were purchased from USP. Glacial acetic acid, methanol, and acetonitrile were purchased from J&K Scientific Ltd, Beijing. The standard and assay solutions were prepared according to the USP monograph for paclitaxel. The HPLC analysis was performed with an Agilent 1290 Infinity LC system, including an:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A).

Columns

- Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 689975-902)
- Agilent Poroshell 120 PFP, 3.0 × 100 mm, 2.7 µm (p/n 695975-308)
- Agilent Poroshell 120 PFP, 4.6 × 250 mm, 4 µm (p/n 690970-408).

Results and Discussion

Selectivity comparison

The Poroshell 120 PFP stationary phase can give extra retention and selectivity for positional isomers of halogenated compounds. The column successfully separated the isomers in lapatinib in a previous note [4]. PFP columns can also be used for selective analysis of nonhalogenated compounds, especially for functional groups on an aromatic or other rigid ring system. Paclitaxel and its impurities, as examples of such types of compound, were separated on Poroshell 120 PFP and EC-C18 columns (Figure 1). Both columns showed different selectivity. The PFP column had short retention, but with good resolution between the impurities and paclitaxel. However, EC-C18 had long retention, but did not fully resolve impurity B and paclitaxel.

Conditions

Columns:	Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 689975-902) Agilent Poroshell 120 PFP, 3.0 × 100 mm, 2.7 µm (p/n 695975-308)
Sample:	Paclitaxel, impurity A and impurity B in methanol containing 0.5% acetic acid
Mobile phase:	Water:acetonitrile (55:45)
Temp:	30 °C
Flow rate:	1.5 mL/min for 4.6 × 100 mm column, 0.64 mL/min for 3.0 × 100 mm column
Inj vol:	4 µL for 4.6 × 100 mm column, 2 µL for 3.0 × 100 mm column
Detection:	UV, 227 nm

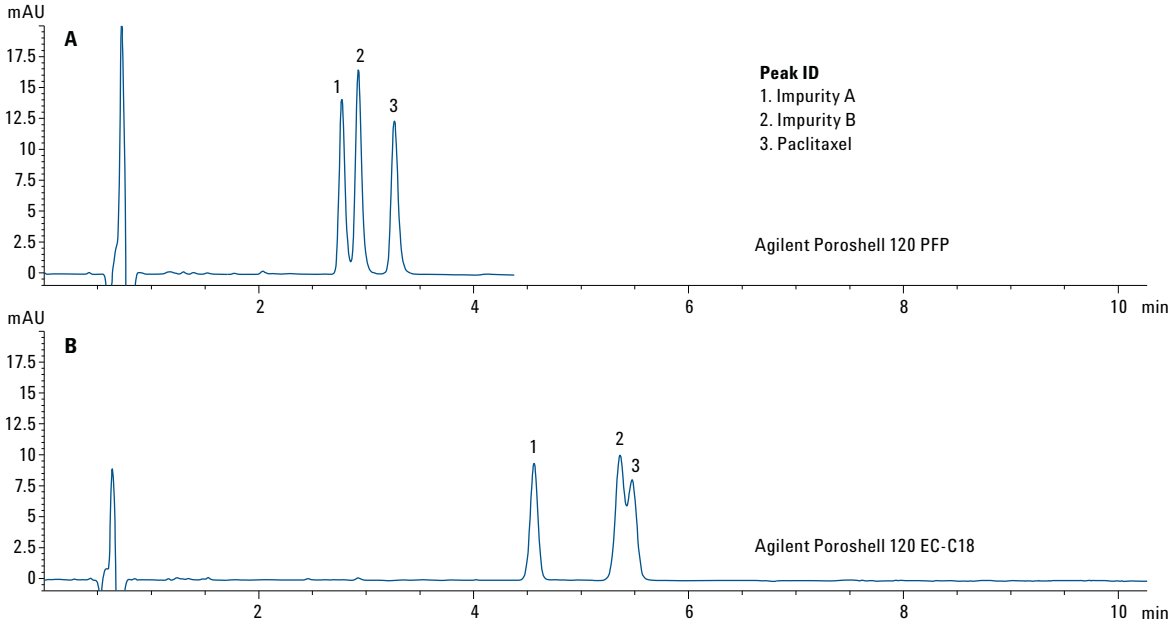

Figure 1. Selectivity comparison for separating paclitaxel and its impurities on Agilent Poroshell 120 PFP and EC-C18 columns.

System suitability test

The USP includes test 1 for paclitaxel labeled as isolated from natural sources. The chromatographic conditions for related compounds in paclitaxel require that "The liquid chromatograph is equipped with a 227-nm detector and a 4.6 mm × 25 cm column that contains 5-µm packing L43" [1].

In this trial, we first used a Poroshell 120 PFP, 4.6 × 250 mm, 4 µm column under LC conditions specified in USP methods. Figure 2 shows the system suitability analysis for the related compounds analysis on the column. The chromatograms show that the resolution between impurities A and B, impurity B and paclitaxel were good enough to meet the system suitability (shown in Table 1).

Conditions

Columns:	Agilent Poroshell 120 PFP, 4.6 × 250 mm, 4 µm (p/n 690970-408)
Sample:	Paclitaxel, impurity A and impurity B in methanol containing 0.5% acetic acid
Mobile phase:	A: water B: acetonitrile; 0-35 min, 35% A; 35-60 min, 35% A-80% A; 60-70 min, 80% A- 35% A; 70-80 min, 35% A
Temp:	30 °C
Flow rate:	2.6 mL/min
Inj vol:	10 µL
Detection:	UV, 227 nm

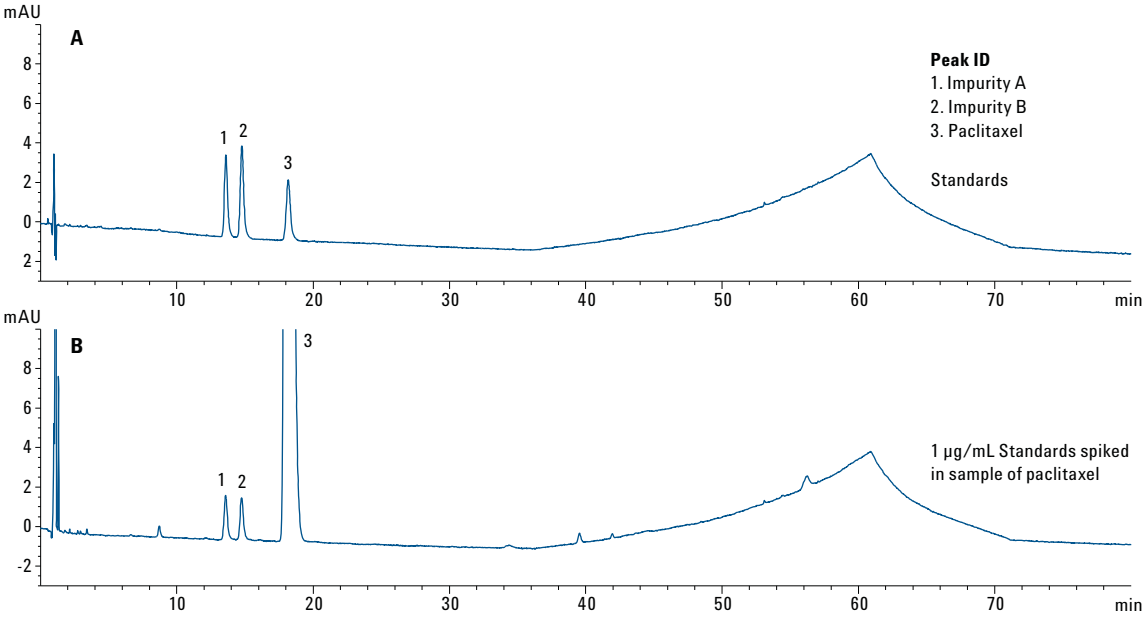

Figure 2. Chromatograms of standards and spiked sample of paclitaxel on an Agilent Poroshell 120 PFP, 4.6 × 250 mm, 4 µm column.

Table 1. USP chromatographic system suitability requirements and measured values for related compounds in paclitaxel.

USP requirements	Agilent Poroshell 120 PFP, 4.6 × 254 mm, 4 µm	Agilent Poroshell 120 PFP, 3.0 × 100 mm, 2.7 µm
The relative retention times are about 0.78 for paclitaxel-related compound A and 0.86 for paclitaxel-related compound B.	T_RA = 0.75 T_RB = 0.81	T_RA = 0.75 T_RB = 0.81
The resolution, R, between paclitaxel-related compound A and paclitaxel-related compound B is not less than 1.0.	$Rs_{1,2}$ = 2.7	$Rs_{1,2}$ = 2.2
The relative standard deviation for replicate injections is not more than 2.0%.	RSD = 0.78%	RSD1 = 0.59%

Method transfer

The analytical method was then transferred to a Poroshell 120 PFP, 3.0 × 100 mm, 2.7 µm column (Figure 3). The analysis was performed in 32 minutes, down from 80 minutes on the original column. The resolution of impurity A and impurity B was 2.2, compared to 2.7 on the 4.6 × 250 mm column. The narrow-bore column significantly saved time and solvents, and still produced results that met the USP system suitability requirements. The USP chromatographic system suitability requirements were all measured according to the USP related compounds analysis in paclitaxel on both columns. Table 1 lists the USP system requirements and measured values on the columns. The methods on the columns met all the USP chromatographic system requirements.

Conditions

Column:	Agilent Poroshell 120 PFP, 3.0 × 100 mm, 2.7 µm (p/n 695975-308)
Sample:	Paclitaxel, impurity A and impurity B in methanol containing 0.5% acetic acid
Mobile phase:	A: water B: acetonitrile; 0-14 min, 35% A; 14-24 min, 35% A- 80% A; 24-28 min, 80% A- 35% A; 28-32 min, 35% A
Temp:	30 °C
Flow rate:	1.1 mL/min
Inj vol:	2 µL
Detection:	UV, 227 nm

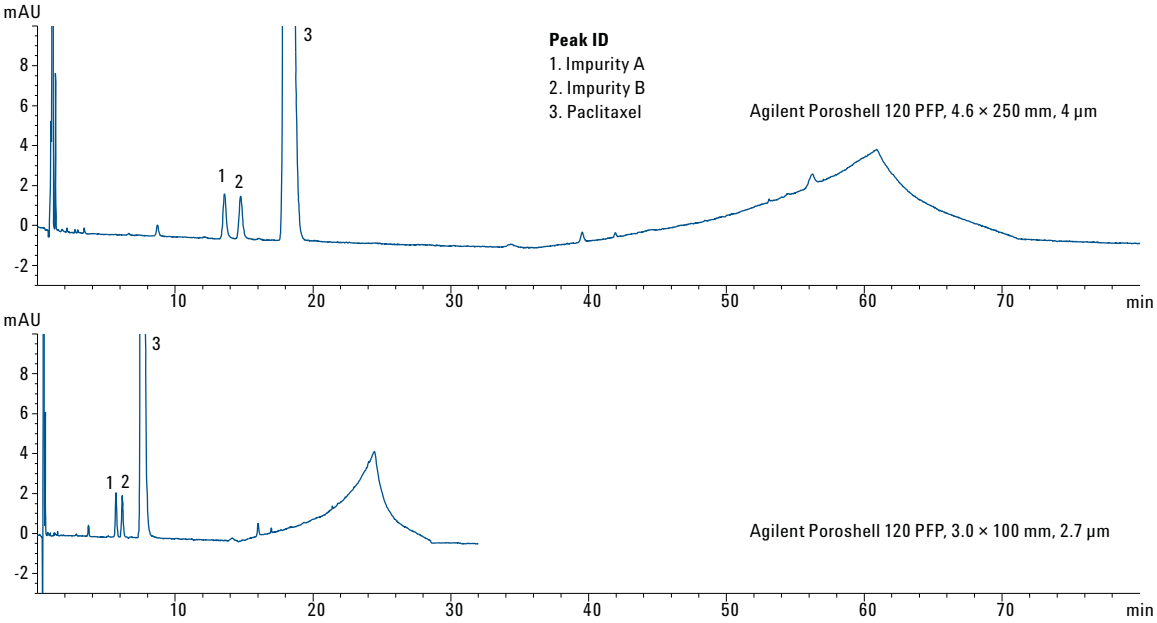

Figure 3. Chromatograms of a spiked sample for paclitaxel on Agilent Poroshell 120 PFP, 4.6 × 250 mm, 4 µm column and 3.0 × 100 mm, 2.7 µm columns.

According to USP37 NF32S1 guidelines after 1 August 2014, changes in length, column inner diameter, and particle size for gradient separations are not allowed. Therefore, to realize the benefits of speed and resolution offered by the Poroshell 120 PFP columns some method development will be required. Several experimental parameters must be tested. These include robustness, linearity, accuracy, precision, limit of detection, limit of quantitation, analytical specificity/selectivity, range, and ruggedness. An Agilent application note describes a step-by-step approach to method development [4].

Conclusions

This application shows that the Agilent Poroshell 120 PFP, 4 µm column is suitable for USP related compounds analysis of paclitaxel using the USP method conditions. With changes to the method, a narrow bore 3.0 × 100 mm column decreases analysis time by 60% with significant solvent saving.

References

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