Analysis of Positional Isomers of Lapatinib with Agilent Poroshell 120 PFP Columns

Application Note

Small Molecule Pharmaceuticals

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Abstract

The Agilent Poroshell 120 PFP (pentafluorophenyl) stationary phase provides extra retention and unique selectivity for positional isomers of halogenated compounds. These PFP columns can also be used for selective analysis of entities such as polar compounds that contain hydroxyl, carboxyl, nitro, or other polar groups. This selectivity is enhanced when the functional groups are located on an aromatic or other rigid ring system [1].

In this application note, three positional isomers of lapatinib and its two impurities were well separated using an Agilent Poroshell 120 PFP column. The method was then optimized to allow separation of the two impurities from the main lapatinib component.

Agilent Technologies

Introduction

Lapatinib is a small molecule and a member of the 4-anilinoquinazoline class of kinase inhibitors. It is present as the monohydrate of the ditosylate salt, with chemical name N-(3-chloro-4-{[(3-fluorophenyl)methyl]oxy}phenyl)-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furanyl]-4-quinazolinamine bis(4-methylbenzenesulfonate) monohydrate. It has the molecular formula $C_{29}H_{26}ClFN_4O_4S$ $(C_7H_8O_3S)_2$ H_2O and a molecular weight of 943.5 [2]. The two positional isomers shown in Figure 1 may be present during synthesis of lapatinib, and so a limit for them should be set for quality control purposes.

In a previous study, neither C18 nor Phenyl-Hexyl phases resolved all three compounds. However, when analyzed on a PFP stationary phase, the compounds show unique selectivity. In this application note, a gradient method with acetate buffer and methanol or acetonitrile was developed to determine a 0.2% limit in the main peak.

The 2.7 µm superficially porous particles of Poroshell have high efficiency, similar to that of sub-2 µm totally porous particles. This is attributed primarily to a shorter mass transfer distance and a narrower particle size distribution. Furthermore, the larger particle size results in lower backpressure, allowing these columns to be used on virtually any LC system.

Materials and methods

All reagents and solvents were HPLC or analytical grade. The standards were provided by Zhejiang Jinxing Pharma in China. Acetonitrile, methanol, ammonium acetate, and glacial acetic acid were purchased from J&K Scientific Ltd, Beijing.

The HPLC analysis was performed with an Agilent 1290 Infinity LC, including an Agilent 1290 Infinity Binary Pump (G4220A), Agilent 1290 Infinity Autosampler (G4226A), Agilent 1290 Infinity Thermostatted Column Compartment (G1316C), and Agilent 1290 Infinity Diode Array Detector (G4212A).

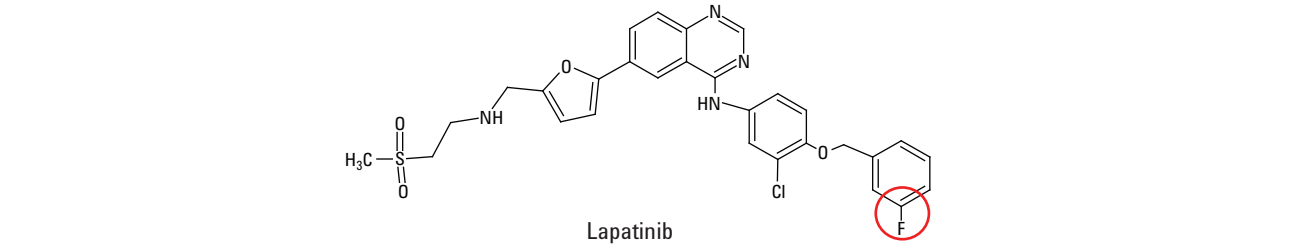

Figure 1. Structures of lapatinib and its two isomer impurities.

Conditions

Columns: Agilent Poroshell 120 PFP, 4.6 × 100 mm, 2.7 µm (p/n 695975-408)
Agilent Poroshell 120 Phenyl-Hexyl, 4.6 × 100 mm, 2.7 µm (p/n 695975-912)
Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902)
Agilent Poroshell 120 SB-C18, 4.6 × 100 mm, 2.7 µm (p/n 685975-902)

Mobile phase: A, acetate buffer (3.85 g ammonium acetate in 990 mL pure water, adjust with glacial acetic acid to pH 4.5 ± 0.05 and then add water to 1,000 mL)
B, acetonitrile or methanol

Gradient 1 for acetonitrile organic phase

Time (min)	% B
0	40
5	40
13	58
17	90
19	90
19.1	40
21	40

Gradient 2 for methanol organic phase

Time (min)	% B
0	60
2	60
17	70
19	90
21	90
21.1	60
23	60

Temperature: 40 °C
Flow rate: 1.5 mL/min
Injection volume: 7 µL
Detection: UV, 261 nm

Results and Discussion

The sample to assess system suitability was resolved on various stationary phases, as shown in Figure 2. This separation first used acetonitrile as organic phase. Both EC-C18 and SB-C18 were unable to resolve the three peaks. In addition, peaks 2 and 3 coeluted on the Phenyl-Hexyl column. However, all three compounds were baseline separated on the Poroshell 120 PFP column.

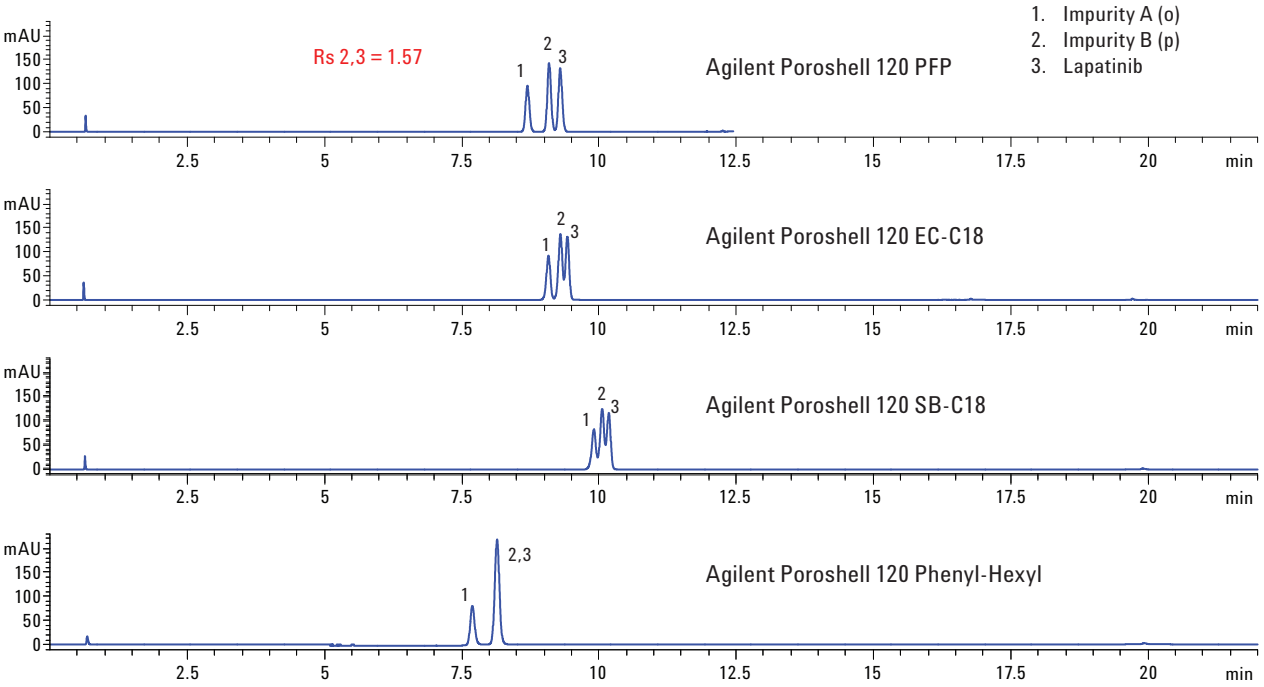

Figure 2. System suitability for lapatinib isomers with different Agilent Poroshell 120 phases using acetonitrile as organic phase.

The resolution of peaks 2 and 3 was 1.57. This is normally good enough for quantitative analysis. Nonetheless, in a real sample, lapatinib is present at a much higher concentration than impurity B (peak 2). The content of impurity A and B is around 0.2% in lapatinib. It is very difficult for the impurities to be distinguished from a high concentration of lapatinib. A real sample of lapatinib spiked with impurity A and B at 0.2% each in lapatinib was analyzed using acetonitrile as organic phase (upper chromatogram in Figure 4). Peak 2 (impurity B) was not well separated from the high concentration of lapatinib.

Methanol organic phase usually provides different selectivity to acetonitrile, and so we modified the gradient to optimize the separation with methanol. The system suitability sample was also separated on different stationary phases, as shown in Figure 3. It appears that the resolution of peaks 2 and 3 was improved on the PFP column, from 1.57 to 2.79. The improved resolution makes it possible for the low level of impurity B to be distinguished from lapatinib.

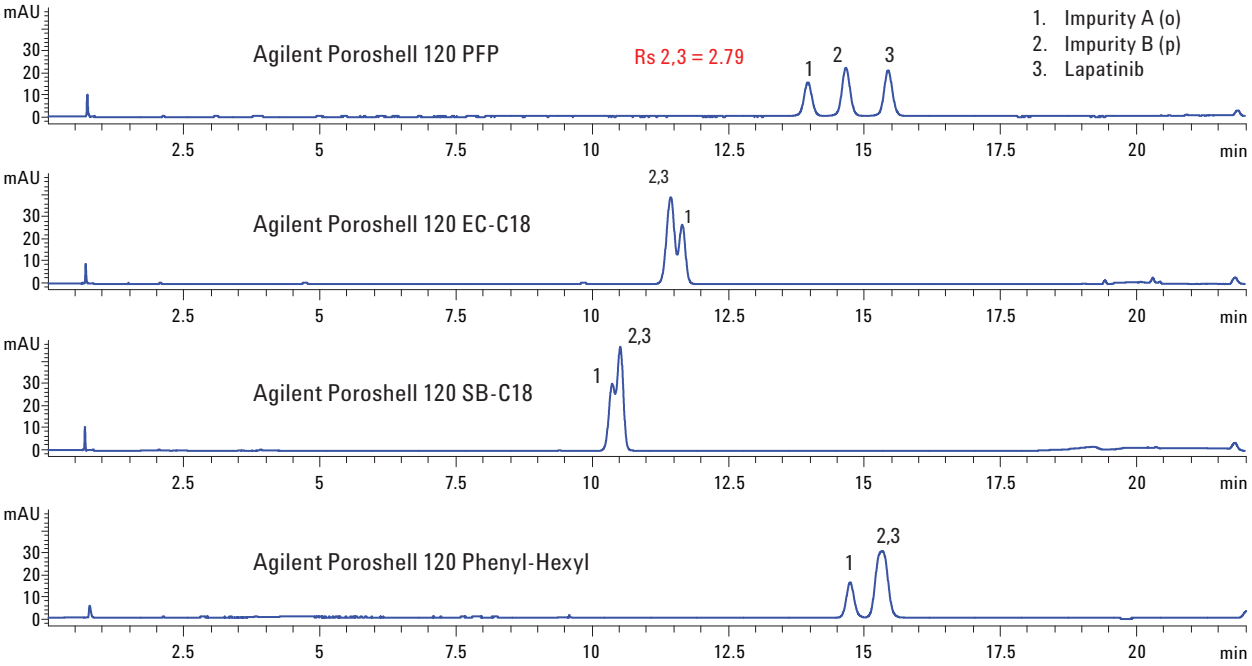

Figure 3. System suitability for lapatinib isomers with different Agilent Poroshell 120 phases using methanol as organic phase.

The real sample was run with the optimized method on the PFP column, as shown in the lower chromatogram in Figure 4. Here, the impurities A and B were baseline separated from lapatinib, and the method could be used for their determination.

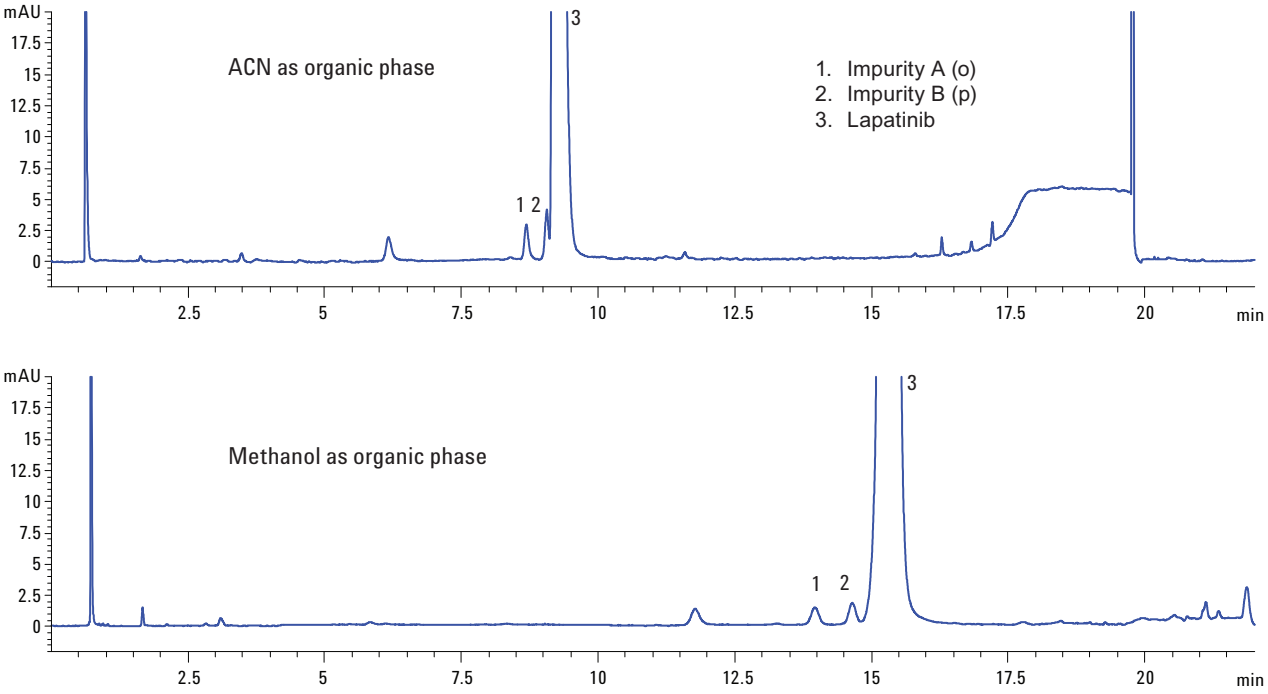

Figure 4. Chromatograms of samples of lapatinib spiked with 0.2% impurities using acetonitrile and methanol as mobile phases

Conclusions

The Agilent Poroshell 120 PFP column provides unique selectivity for lapatinib and its two positional isomers. The method developed on the PFP columns using methanol as organic phase can easily determinate isomer impurities in lapatinib.

References

1. Long, W. *Analysis of Positional Isomers with Agilent Poroshell 120 PFP Columns*; Agilent Technologies, Inc. Application Note, publication number 5991-4373 EN, **2014**.
2. http://www.rxlist.com/tykerb-drug.htm

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