Application Note

Biopharma/Pharma

Column Chemistry Scouting for Forced Degradation of a Small Molecule GLP-1 Drug

Author

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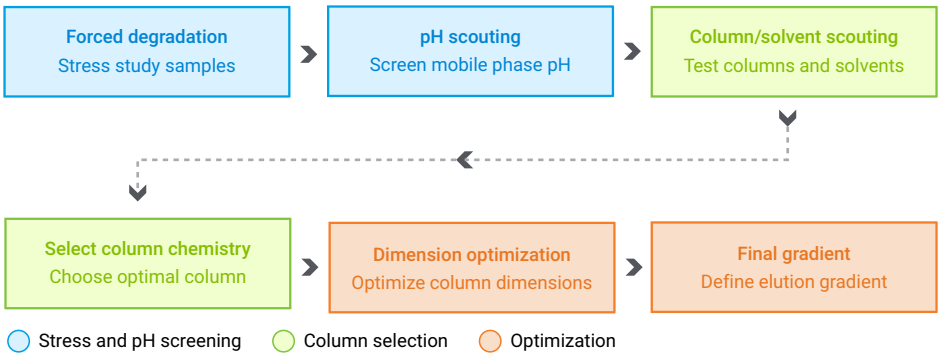
Abstract

Orforglipron is a recently approved oral, non-peptide glucagon-like peptide-1 (GLP-1) receptor agonist that represents a significant advancement over traditional injectable peptide-based GLP-1 therapies. To support impurity profiling and method development, forced degradation studies were performed to generate representative degradation products and challenge chromatographic separation conditions. A systematic liquid chromatography/mass spectrometry (LC/MS) method scouting study was conducted using six stationary phase chemistries, including C18, phenyl-based, pentafluorophenyl (PFP), and mixed-mode phases, evaluated with both acetonitrile and methanol mobile phases. Column performance was assessed based on the active pharmaceutical ingredient (API) retention, impurity separation, and the total number of impurity peaks detected. The Agilent InfinityLab Poroshell 120 EC-C18 column provided the best overall selectivity and impurity resolution. Further optimization using a 100 mm column with 1.9 µm particles and an extended 26-minute gradient significantly improved separation of degradation products and related impurities. The final method enabled effective UV and MS characterization of orforglipron and its impurities.

Systematic LC method scouting for separation of orforglipron forced-degradation products



Introduction

GLP-1 receptor agonists are an important class of therapeutics used for the treatment of type 2 diabetes and obesity. Historically, most GLP-1 therapies have been peptide-based drugs that exhibit poor oral bioavailability due to enzymatic degradation and limited absorption in the gastrointestinal tract, requiring administration by subcutaneous injection. Although oral semaglutide demonstrated that peptide-based GLP-1 therapies can be delivered orally, specialized formulations are required to overcome these challenges. The approval of orforglipron (Figure 1) represented a significant advancement in the field because it is a non-peptide, small-molecule GLP-1 receptor agonist that can be administered orally, potentially offering greater convenience and improved patient access compared with injectable therapies.

With the introduction of this new therapeutic, characterization and monitoring of potential impurities are critical to ensuring product quality and safety. Forced degradation studies provide valuable insight into the stability profile of the active pharmaceutical ingredient (API) by generating degradation products that may form during manufacturing, storage, or use. The resulting degradation mixtures can be used to challenge chromatographic methods and ensure adequate separation of the API from related impurities and degradants. In this study, a systematic LC/MS method scouting approach was employed to develop an impurity profiling method for orforglipron. Six stationary phase chemistries with distinct selectivity mechanisms were evaluated, including reversed-phase, aromatic-selective, and mixed-mode columns, prior to selection and optimization of the final chromatographic method.

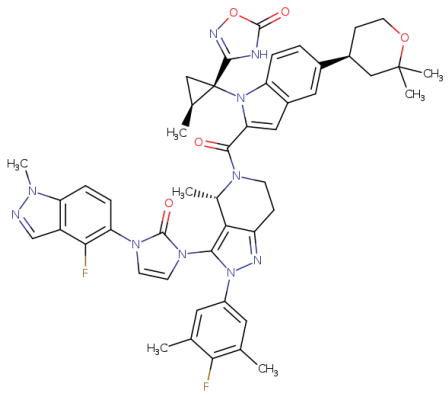


Figure 1. Orforglipron structure.

Experimental

Chemicals

Orforglipron was procured from Cayman Chemical (Ann Arbor, Michigan). LC/MS-grade ammonium hydroxide and formic acid were purchased from Sigma-Aldrich (St. Louis, Missouri). InfinityLab LC/MS-grade water, acetonitrile, and methanol were used as sample solvents and mobile phase components.

Instrumentation

An Agilent 1290 Infinity II binary pump (G7120B) was paired with an external valve drive and a 13-port solvent switching valve (G4235A) to facilitate mobile phase scouting. Samples were introduced by an Agilent multisampler (G7167B) into a thermostatted multicolumn compartment (G7116B) equipped with an 8-column switching valve (G4239C). Impurity detection was performed using a diode array detector configured according to the settings described in Table 1. The eluent was subsequently directed to the Agilent InfinityLab LC/MSD Pro iQ for mass spectrometric detection. MS operating parameters are summarized in Table 2. Acquisition and data analysis was performed with the Agilent OpenLab CDS, Version 2.8.

Table 1. DAD settings.

DAD	
Parameter	**Setting**
Signal	254 nm
Bandwidth	4
Data Rate	40 Hz
Spectrum	190 to 400 nm
Step	2 nm

Table 2. MS settings.

IQ Pro	
Parameter	**Setting**
Gas Temperature	325 °C
Gas Flow	11 L/min
Nebulizer	30 psi
Capillary Voltage	3,500 V
Acquisition Mode	
Scan (+)	70 to 1,000 *m/z*
SIM (+)	883.4 *m/z*

Sample preparation and forced degradation

Ten milligrams of orforglipron were dissolved in 4 mL of 60:40 (v/v) acetonitrile: water and sonicated until fully dissolved. Aliquots of 900 µL of this solution were transferred into three separate vials for forced degradation studies. Degradation was induced by adding 100 µL of 1 M hydrochloric acid (acidic degradation), 100 µL of 1 M sodium hydroxide (basic degradation), or 100 µL of 30% hydrogen peroxide (oxidative degradation).

The acidic and basic degradation samples were heated at 70 °C for 2 hours, while the oxidative degradation sample was heated at 70 °C for 1 hour. Following incubation, the three degraded samples were combined to quench further degradation reactions. The resulting mixture was transferred to an HPLC vial for analysis.

A solvent blank and an undegraded orforglipron reference sample were also prepared and analyzed for comparison.

Method scouting

In OpenLab, automated changes to method parameters can be achieved through modifications in the sequence panel. A selection of method override columns can be added to the sequence, allowing specific method settings to be overridden (Figure 2). For example, when an 8-column selection valve is installed, different column chemistries can be evaluated using the same method by advancing the valve position within the worklist and running a short equilibration method before sample analysis. Other parameters available for method override include column temperature, initial gradient composition, and flow rate.

Six columns, all configured as 2.1 × 50 mm, 2.7 um particles were selected for testing and are shown in Table 3.

Table 3. Agilent HPLC columns evaluated.

Chemistry (2.1 × 50 mm, 2.7 µm)	Part Number
InfinityLab Poroshell HPH-C18	699775-702
InfinityLab Poroshell 120 CS-C18	699775-942
InfinityLab Poroshell 120 EC-C18	699775-902
InfinityLab Poroshell 120 PFP	699775-408
InfinityLab Poroshell 120 Phenyl-Hexyl	699775-912
Altura Poroshell 120 PFAS	227205-007
Additional Dimensions	
Infinity Lab Poroshell EC-C18, 2.1 × 100, 2.7 µm	695775-902
Infinity lab Poroshell EC-C18, 2.1 × 100, 1.9 µm	695675-902

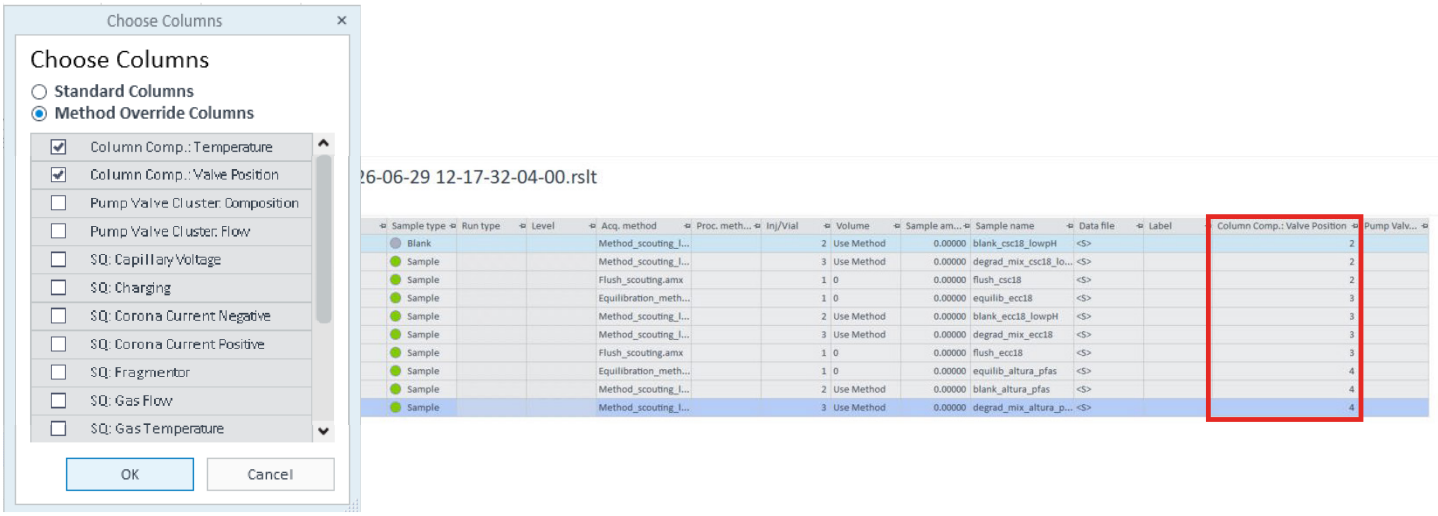


Figure 2. OpenLab Method Override columns available in the Sequence Panel.

Results and discussion

Method scouting results

Initial pH scouting was conducted using the Poroshell HPH-C18 column because it is compatible with a wide pH range. The aqueous mobile phase consisted of either water with 0.1% formic acid or 10 mM ammonium hydroxide and was paired with the corresponding modifier in the acetonitrile organic phase. After the low-pH conditions were found to provide better retention (Figure 3), a methanol-based organic mobile phase containing 0.1% formic acid was prepared and incorporated into the automated testing workflow by switching the 12-port solvent selection valve.

In the initial scouting gradient, the API and all impurities eluted within a one-minute window at a high organic solvent composition. A rising baseline beneath the peaks indicated significant coelution. To enable a more effective evaluation of different column chemistries, a shallow gradient beginning at a high percentage of organic solvent was developed (Figure 4) and used for testing the additional columns.

Poroshell EC-C18 was included because its surface and bonded-phase characteristics differ from those of Poroshell HPH-C18 and could therefore provide complementary retention and selectivity for orforglipron and its degradation products. The Poroshell CS-C18 column was included because its alternative C18 selectivity could potentially improve impurity separation, while the Poroshell PFP phase was evaluated due to its unique selectivity toward aromatic compounds such as orforglipron. The Poroshell Altura PFAS column was also included in the screening because its mixed-mode retention mechanism provides selectivity beyond traditional reversed-phase interactions and could offer advantages for resolving impurities that are not adequately separated on conventional C18 phases.

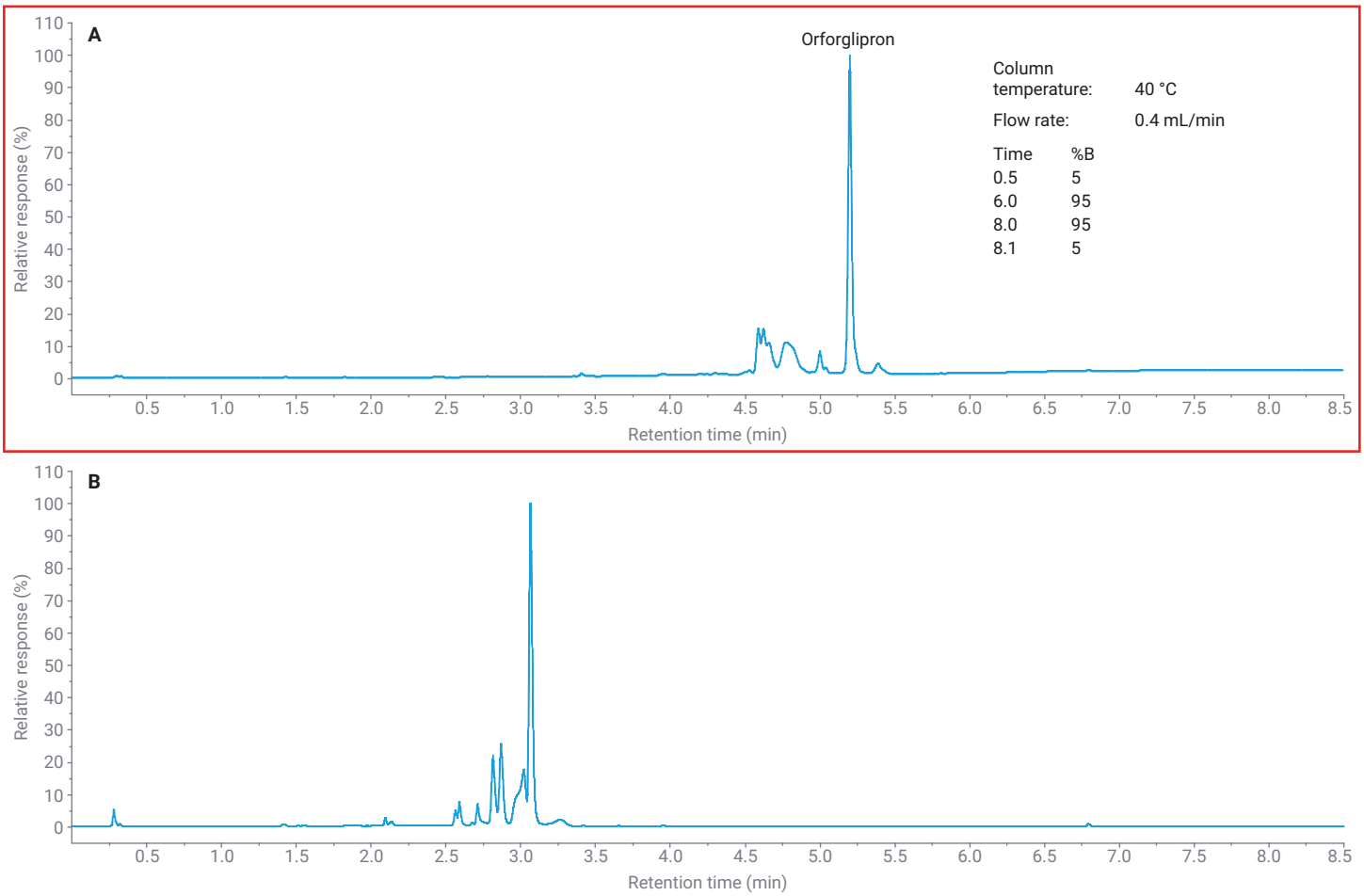


Figure 3. Degradation mixtures chromatograms with an Agilent Poroshell HPH-C18 column (A) formic acid gradient (B) ammonium hydroxide gradient.

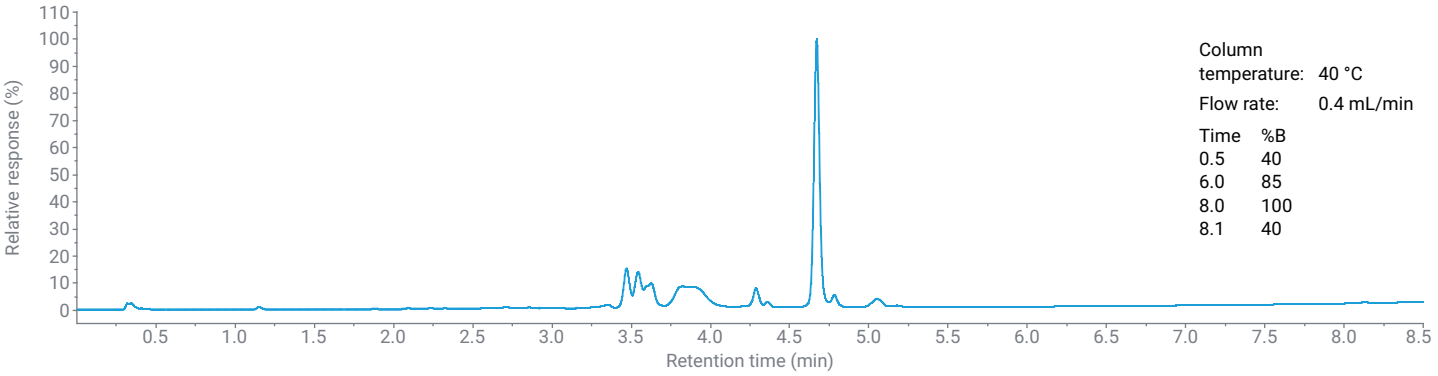


Figure 4. Degradation mix chromatogram with an Agilent Poroshell HPH-C18 column and low pH focused scouting gradient.

Figure 5 presents chromatograms from selected stationary. Column performance was assessed using three primary metrics: separation of impurities from the API, API retention, and the total number of impurity peaks detected. Based on these criteria, the Poroshell EC-C18 column provided the best overall performance.

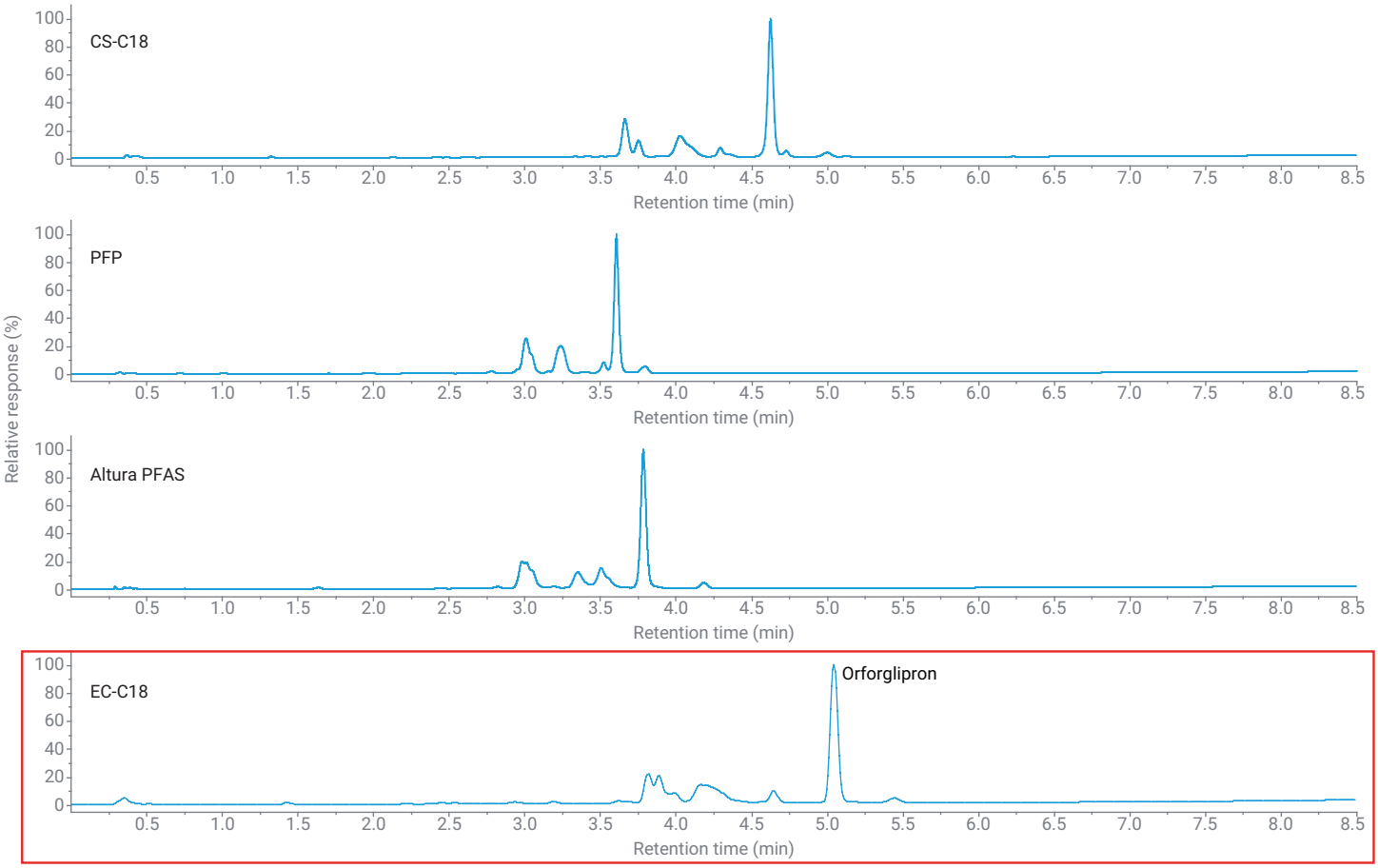


Figure 5. Degradation mixture chromatograms.

All columns were evaluated using both acetonitrile and methanol as organic modifiers. In most cases, acetonitrile provided superior performance, resulting in greater separation between the API and impurity peaks. The only exception was the PhenylHexyl column. Figure 6 compares separations obtained on the PhenylHexyl column using (A) acetonitrile and (B) methanol. This stationary phase was included because it can provide unique selectivity through π-π interactions with highly aromatic compounds such as orforglipron. Methanol is known to alter the selectivity of PhenylHexyl phases relative to acetonitrile, and in this case greater impurity separation was observed with methanol. However, neither PhenylHexyl condition outperformed the Poroshell EC-C18 column with respect to impurity separation from the API or the total number of impurity peaks detected.

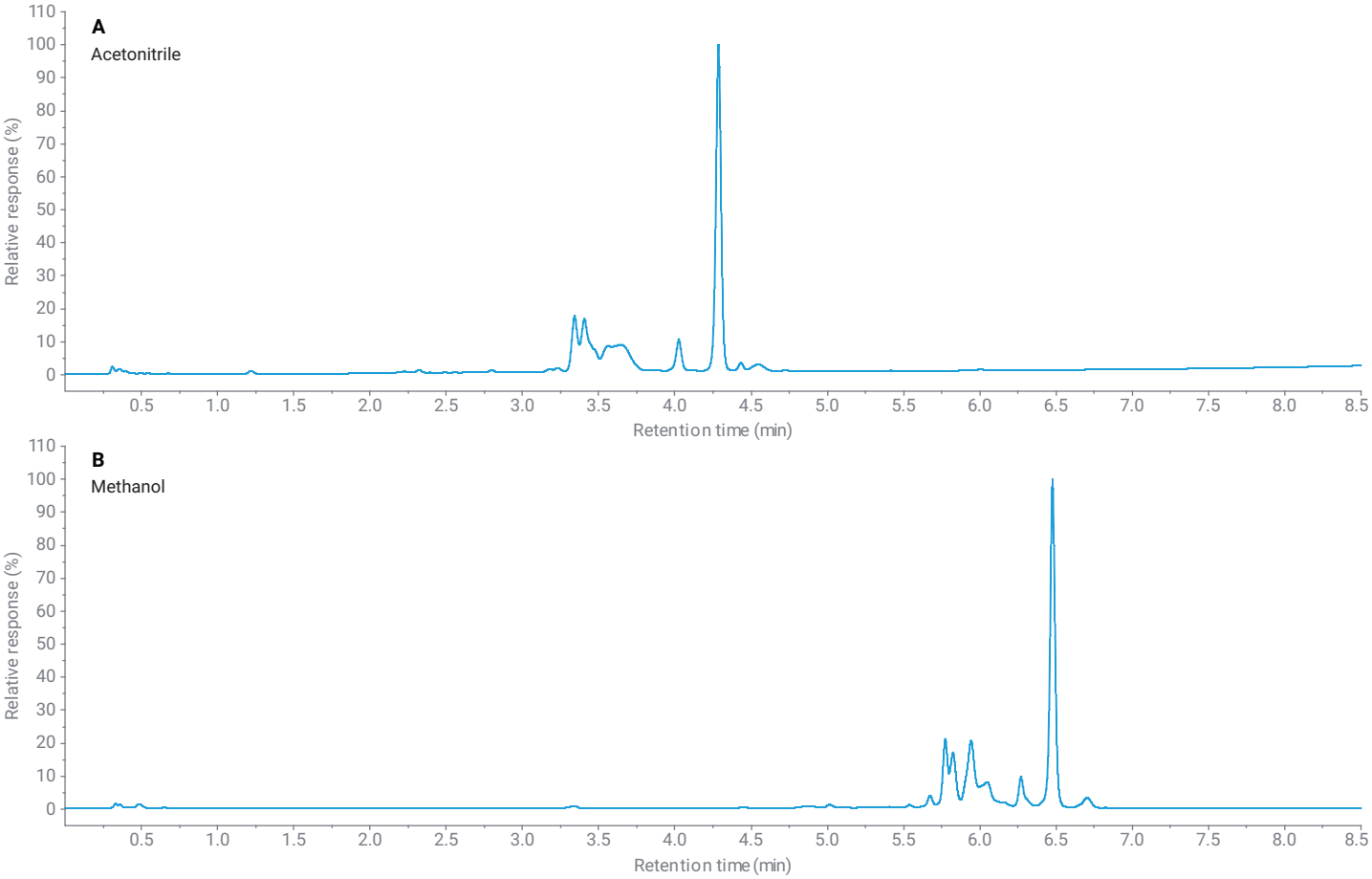


Figure 6. Degradation mixture chromatograms with an Agilent Poroshell PhenylHexyl column with (A) acetonitrile and (B) methanol as the organic mobile phase.

Poroshell EC-C18 separation optimization

Another approach for improving impurity resolution was to increase column length. The Poroshell EC-C18 column chemistry identified during the initial screening was therefore evaluated in a 100 mm format with both 2.7 and 1.9 µm particle sizes using an 8.5-minute gradient. The 100 mm × 1.9 µm column provided the best overall separation and was selected for further method development. In addition, the API mass spectrum showed a clean protonated molecular ion at *m/z* 883.4 along with an in-source fragment ion at *m/z* 797.3 resulting from a neutral loss (Figure 7).

Although the 8.5-minute gradient provided baseline separation of the major impurities from the API, a number of impurity peaks remained only partially resolved. To further improve resolution, a longer and fully optimized gradient was developed.

The final method employed a 26-minute gradient, which significantly improved impurity separation and enabled more complete characterization of the impurity profile (Table 4). The shallow initial gradient was necessary to fully separate the impurities. Representative UV and MS chromatograms obtained with the optimized method are shown in Figure 8. The enhanced resolution achieved with the longer gradient facilitated improved detection and characterization of individual impurity peaks while maintaining acceptable API peak shape and retention.

Table 4. Final optimized LC method for orforglipron impurity profiling.

Parameter	Final Condition
Column	Agilent InfinityLab Poroshell 120 EC-C18
Dimensions	2.1 × 100 mm, 1.9 µm
Mobile Phase A	Water with 0.1% formic acid
Mobile Phase B	Acetonitrile with 0.1% formic acid
Flow Rate	0.5 mL/min
Column Temperature	40 °C
Injection Volume	1 µL
Gradient Program	Time (min) %B 0.5 40 16 52 18.4 70 24 100

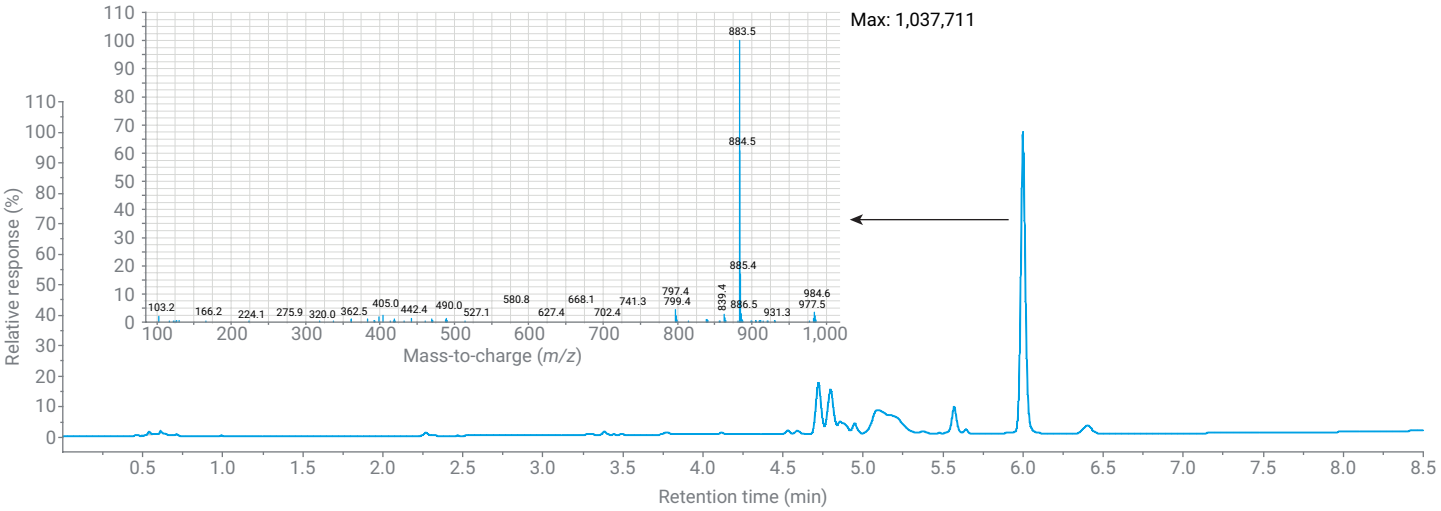
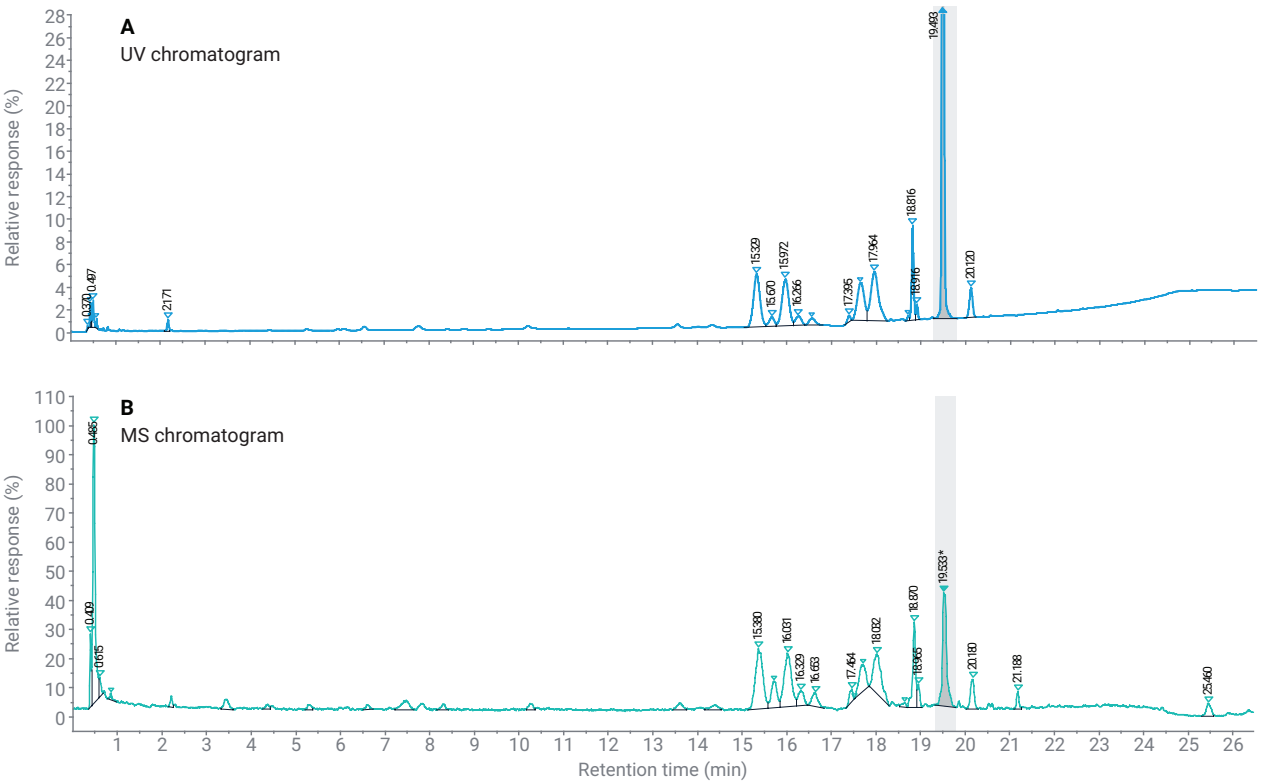


Figure 7. Degradation mixture UV chromatogram using an Agilent EC-C18 2.1 × 100 mm, 1.9 µm column and 8.5-minute gradient and MS spectra of API peak.



RT	*m/z*
0.496	130.1
15.329	917.4
15.670	696.3
15.972	917.4
16.266	917.4
16.568	917.4
17.395	859.5
17.657	951.4
17.964	951.4
18.816	887.4
18.916	887.4
19.493	883.4
20.120	917.5

Figure 8. Degradation mixture (A) UV chromatogram (B) MS chromatogram using the final gradient for the Agilent Poroshell EC-C18 column and MS table of all peak *m/z*.

Conclusion

Column screening identified the Agilent InfinityLab Poroshell 120 EC-C18 phase as the best overall option among the stationary phases evaluated for impurity profiling of orforglipron, providing superior API retention, impurity separation, and impurity peak detection. Acetonitrile generally produced better separations than methanol, although methanol improved selectivity on the Agilent InfinityLab Poroshell 120 PhenylHexyl column.

Further optimization demonstrated that increasing column length and reducing particle size enhanced resolution, with the 100 mm Agilent InfinityLab Poroshell 120 EC-C18 column packed with 1.9 µm particles providing the best performance. While an 8.5-minute gradient separated the major impurities from the API, extending the gradient to 26 minutes further improved resolution of the impurity profile. The final method enabled effective UV and MS analysis of orforglipron and its impurities, including detection of the protonated molecular ion at *m/z* 883.4 and was selected as the preferred approach for impurity analysis.

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