Application Note

Pharmaceuticals

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Overcoming Strong Solvent Effects in the Analysis of Vepdegestrant

Using feed injection with the Agilent 1260 Infinity III hybrid multisampler

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Abstract

Vepdegestrant (ARV-471) is a targeted protein degradation (TPD) therapeutic utilized as a proteolysis-targeting chimera (PROTAC). It contains multiple aromatic rings, making it prone to recrystallization in solution. To prevent recrystallization for high-performance liquid chromatography (HPLC) analysis, dissolution in polar organic solvents such as dimethyl sulfoxide (DMSO) or N,N-dimethylformamide (DMF), or dilution to low concentration, is typically required. However, the use of highly polar organic solvents often introduces strong solvent effects, which limit allowable injection volumes and compromise chromatographic performance.

The Agilent 1260 Infinity III hybrid multisampler addresses this limitation through feed injection, enabling effective mitigation of strong solvent effects. In this application note, a DMF-based vepdegestrant solution was analyzed using feed injection to minimize the chromatographic impact of DMF. Under a 20 µL injection condition, excellent linearity of the calibration curve was achieved, and both the main peak and impurity peaks exhibited good peak shape and chromatographic performance.

Introduction

TPD is a therapeutic strategy that selectively eliminates proteins of interest by hijacking the intracellular protein quality control system. PROTACs represent a major class of TPD modalities, with vepdegestrant (ARV-471) being one of the most well-characterized pharmaceutical candidates. Structurally, PROTACs consist of a ligand that binds the protein of interest (POI) and a ligand that recruits an E3 ubiquitin ligase, connected through a chemical linker. Upon formation of the ternary complex, PROTACs induce ubiquitination of the target protein, leading to its degradation by the proteasome.[1]

Unlike conventional small-molecule inhibitors that require sustained occupancy of the active site to suppress protein function, PROTACs operate through a catalytic mechanism. This enables effective target degradation at low concentrations and allows prolonged pharmacological activity through maintained systemic exposure.[2] Furthermore, because target protein degradation can be achieved solely by recruitment of the PROTAC molecule, this approach expands the druggable space to proteins that have historically been considered challenging for traditional inhibition-based therapeutics.[3]

Despite these advantages, PROTAC molecules are typically rich in aromatic rings (Figure 1), which promotes intermolecular π–π stacking and increases the propensity for crystallization. In addition, most PROTACs violate Lipinski's Rule of Five and exhibit extremely low aqueous solubility.[4] To overcome these limitations, polar organic solvents such as DMSO or DMF are commonly used to prepare stock solutions, and careful consideration of diluent composition and concentration is required during sample dilution. Among these solvents, DMSO and DMF provide excellent solubilization and long-term solution stability for PROTAC compounds. From a thermal stability perspective, DMF, with a melting point of −61 °C, is particularly suitable as a diluent for maintaining sample solutions in the autosampler at 4 °C.

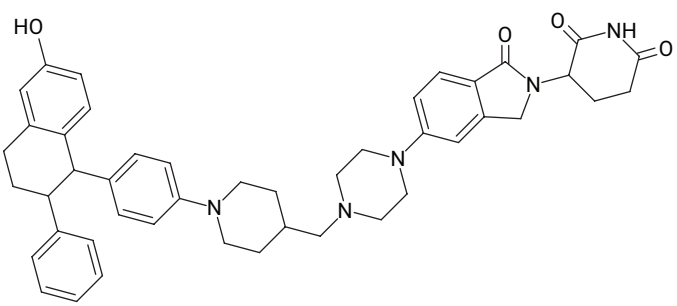

Figure 1. Chemical structure of vepdegestrant.

Although DMF is frequently employed as a solubilizing cosolvent for HPLC analysis, its strong solvent strength often leads to pronounced strong solvent effects, necessitating dilution with weaker solvents or minimization of injection volume. In contrast to conventional flow-through injection, the 1260 Infinity III hybrid multisampler utilizes feed injection to effectively mitigate the strong solvent effects associated with DMF (Figure 2). By overcoming solvent-induced breakthrough and poor peak shape, feed injection enables robust PROTAC analysis without compromising injection volume or inducing recrystallization caused by excessive dilution.

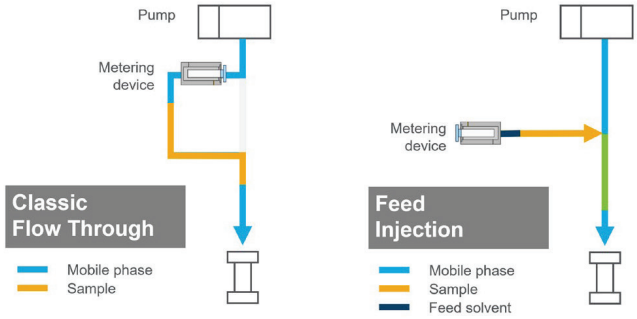

Figure 2. Schematic comparison of classic flow-through injection and feed injection.

In this application note, vepdegestrant and TPD samples prepared in DMF were analyzed using the 1260 Infinity III hybrid multisampler. The feed injection approach provided excellent peak shape, good calibration linearity, and reliable detection even at low analyte concentrations.

Experimental

Instrumentation

The following instrumentation was used in this study:

- Agilent 1260 Infinity III flexible pump (part number G7104C)
- Agilent 1260 Infinity III Hybrid Multisampler (part number G7167C) with sample thermostat
- Agilent 1290 Infinity III Multicolumn Thermostat (part number G7116B) with Agilent InfinityLab Quick Connect heat exchanger, standard flow (part number G7116-60015)
- Agilent 1290 Infinity III Diode Array Detector (product number G7117B) with Agilent InfinityLab Max-Light cartridge cell, 10 mm (part number G4212-60008)

Standards and reagents

Vepdegestrant was purchased from MedChemExpress. Acetonitrile (ACN) and methanol (MeOH) were obtained from B&J, and DMF was purchased from Fisher. Trifluoroacetic acid (TFA) was obtained from Sigma-Aldrich.

Samples

The TPD sample was donated by a local customer.

Preparation of standard and sample solutions

Vepdegestrant and the TPD sample were dissolved in DMF at 10 mg/mL and then diluted with DMF to the desired concentrations. To visually assess solubility and recrystallization, the TPD sample was further diluted to 1 mg/mL in MeOH, ACN, and water, and stored at 4 °C for three days.

Column

The column used was an Agilent InfinityLab Poroshell CS-C18 column, 4.6 × 150 mm, 2.7 µm (part number 693975-942).

Methods

Table 1. Agilent 1260 Infinity III Prime LC method parameters.

Parameter	Details
Mobile Phase	A) 0.1% TFA in water B) 0.1% TFA in ACN
Injection Volume	20 µL
Flow Rate	1 mL/min
Sampler Temperature	4 °C
Column Temperature	40 °C
UV Detection	286 nm
Gradient	Time (min) %A %B 0 80 20 1 80 20 10 40 60 10.1 65 20 15 59 20
Flow-Through Injection	– Draw speed: 100 µL/min – Outer wash program: Standard Outer wash: S1 – Water:MeOH:ACN = 1:1:1/3s
Feed Injection	– Feed speed: 10% of flow (adaptive) – Automatic overfeed volume – Flush-out solvent: 20% ACN – Inner wash program: Standard Inner wash: S1 – Water:MeOH:ACN = 1:1:1/150 µL Reconditioning: S2 – 20% ACN – Outer wash program: Standard Outer wash: S1 – Water:MeOH:ACN = 1:1:1/3s

Software

The software used in this application note was Agilent OpenLab CDS software, version 2.8 with feature pack 2.

Results and discussion

The TPD sample was dissolved in DMF at a concentration of 10 mg/mL and subsequently diluted to 1 mg/mL using DMF, MeOH, ACN, and water. After storage at 4 °C for three days, crystal formation was observed in the MeOH and ACN solutions, while immediate precipitation occurred upon dilution with water, as shown in Figure 3. In contrast, the sample remained stable and fully dissolved in DMF under the same storage conditions.

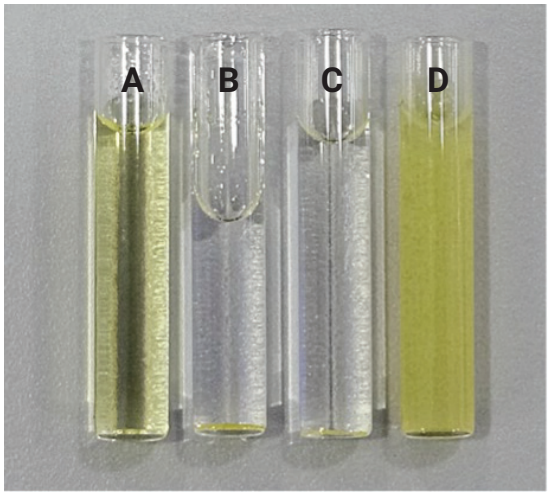

Figure 3. Solution stability of the TPD sample stored at 4 °C for three days in: (A) 100% DMF, (B) 10% DMF in 90% MeOH, (C) 10% DMF in 90% ACN, and (D) 10% DMF in 90% water.

When a 100 µg/mL TPD sample prepared in DMF was analyzed using flow-through injection and feed injection, distinct differences in chromatographic performance were observed (Figure 4). Flow-through injection resulted in pronounced breakthrough and poor peak shape, whereas feed injection provided clear separation of the main peak as well as individual impurity peaks.

Feed speed is a critical parameter for reducing strong solvent effects in feed injection. It is defined relative to the pump flow rate and utilizes the three-way channel of the injection valve to introduce the sample solution into the column together with the mobile phase while maintaining continuous flow. Based on the results shown in Figure 5, feed speeds of 20% or lower were required under the conditions listed in Table 1. However, excessively long feeding times can lead to peak broadening due to sample diffusion. Therefore, in this application, the feed speed was set to 10% of the pump flow rate to achieve an optimal balance between solvent effect suppression and chromatographic efficiency.

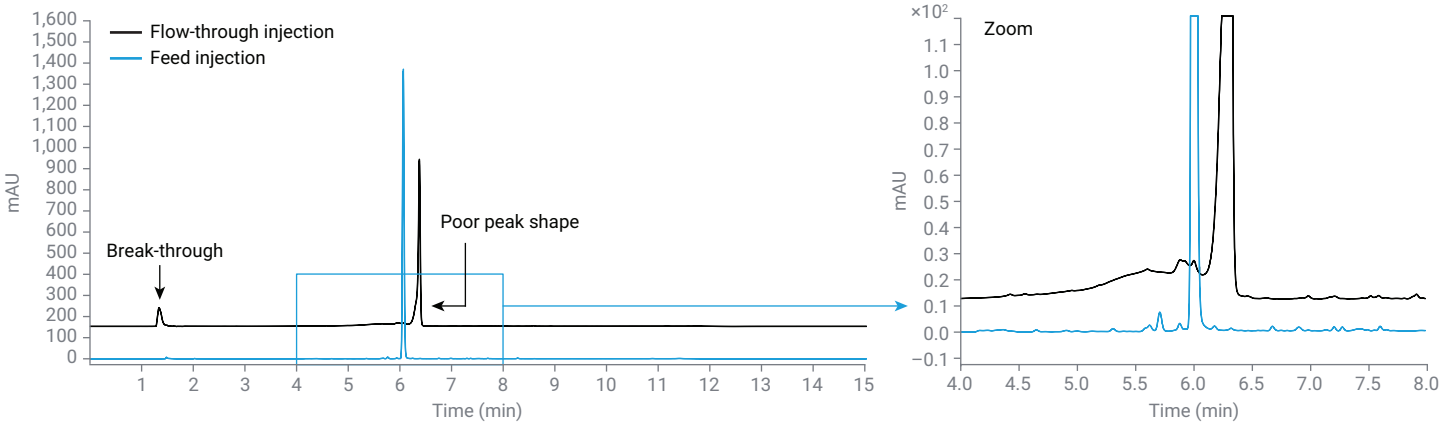

Figure 4. Chromatograms of the TPD sample obtained using flow-through injection (black) and feed injection (blue).

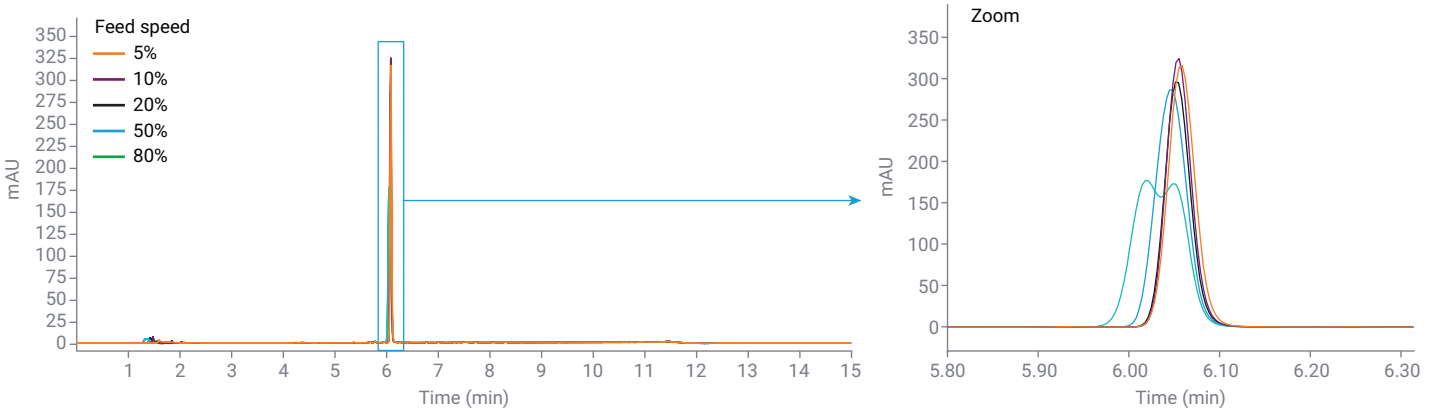

Figure 5. Effect of feed speed on the chromatographic performance of the TPD sample. Feed speeds were 5% (orange); 10% (purple); 20% (black); 50% (blue); and 80% (green).

TPD sample and vepdegestrant solutions prepared in 100% DMF at various concentrations were analyzed under the conditions summarized in Table 1. Consistent retention times and symmetrical peak shapes were observed across all concentration levels (Figure 6). Notably, no breakthrough associated with the strong solvent was detected, even with an injection volume of 20 µL.

Using feed injection with the 1260 Infinity III hybrid multisampler, a calibration curve for vepdegestrant over the concentration range of 0.01 to 100 µg/mL was obtained, as shown in Figure 7. The coefficient of determination (R^2) was 0.99994, demonstrating excellent linearity across the evaluated concentration range.

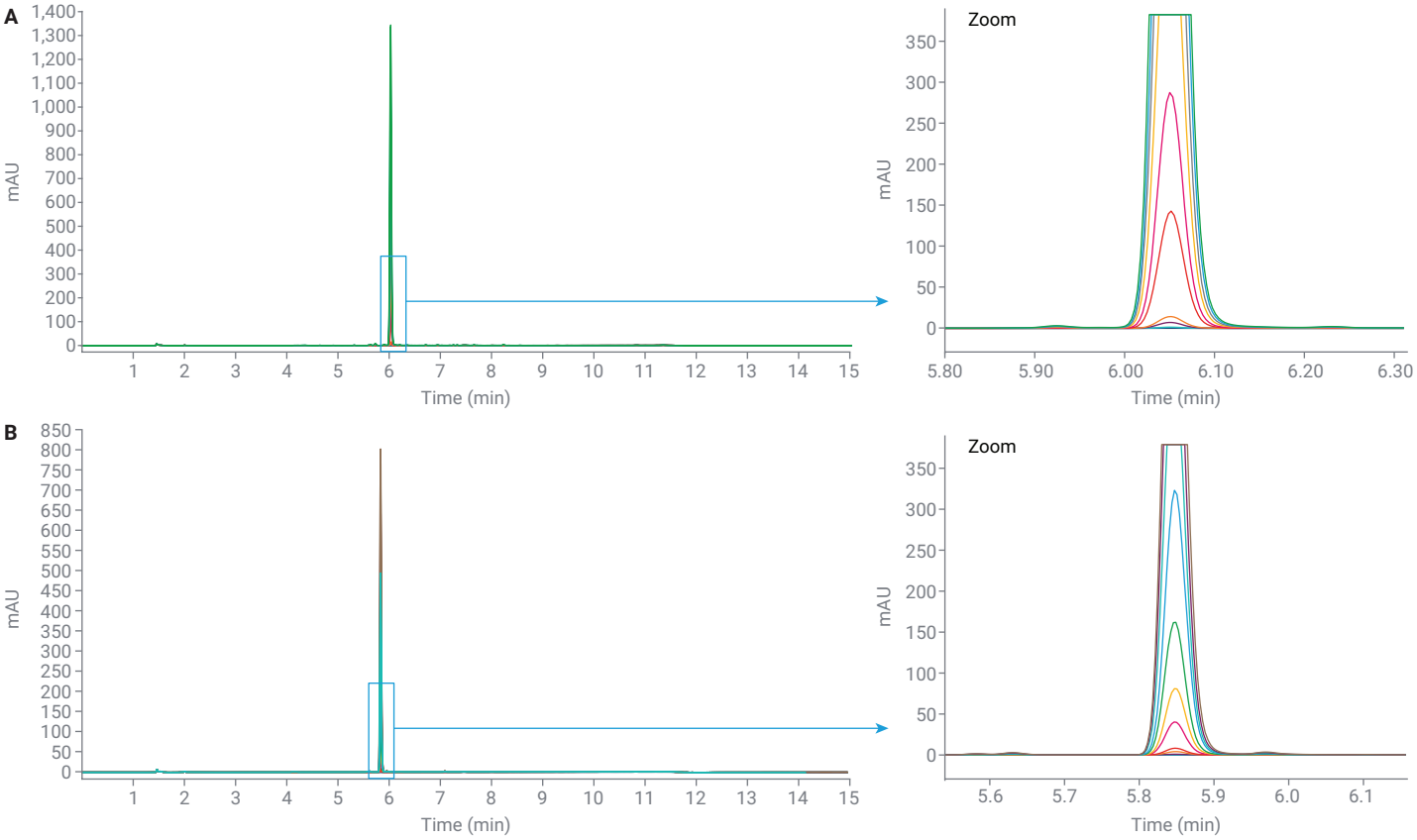

Figure 6. Overlaid chromatograms of TPD samples and vepdegestrant solutions at different concentrations: (A) TPD sample, 0.05 to 100 µg/mL; (B) vepdegestrant, 0.01 to 100 µg/mL.

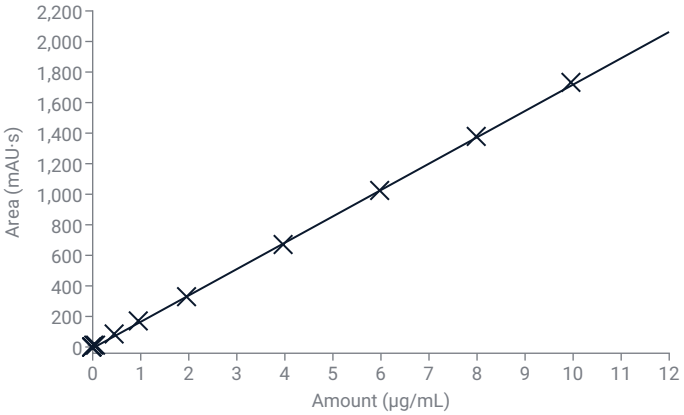

Figure 7. Calibration curve of vepdegestrant over the concentration range of 0.01 to 100 µg/mL.

Figure 8 shows overlaid chromatograms of a blank and vepdegestrant solutions at concentrations of 0.01, 0.05, and 100 µg/mL. The signal-to-noise ratio of a peak corresponding to 0.01% of the 100 µg/mL vepdegestrant concentration was 14.0, while a signal-to-noise ratio of 69.2 was observed for the 0.05% level.

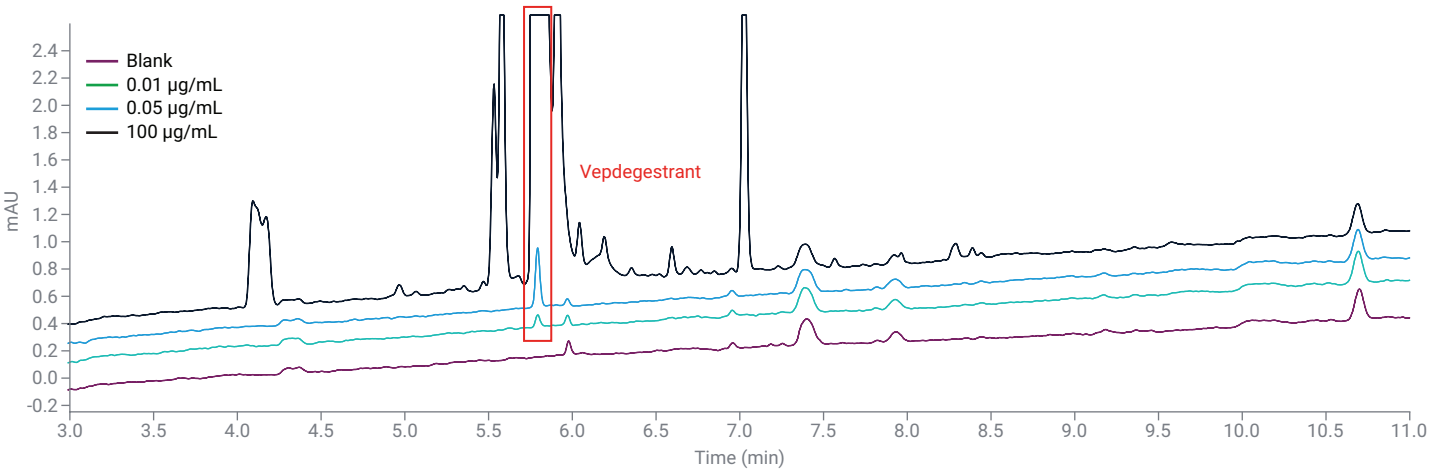

Figure 8. Overlaid chromatograms of vepdegestrant solutions at different concentrations: blank (purple); 0.01 µg/mL (green); 0.05 µg/mL (blue); and 100 µg/mL (black).

Conclusion

HPLC method development for TPDs must be carefully designed with consideration of their physicochemical properties. In particular, the use of polar organic solvents such as DMSO or DMF, which are commonly required to prevent recrystallization of TPD compounds, introduces strong solvent effects that can significantly limit the applicable analytical window.

Feed injection using the Agilent 1260 Infinity III hybrid multisampler effectively overcomes these limitations without the need for additional dilution. Analysis of vepdegestrant dissolved in 100% DMF demonstrated that a 20 µL injection volume could be employed without solvent-induced breakthrough or poor peak shape. Both the target compound and impurity peaks were clearly resolved, and excellent linearity was achieved over the concentration range of 0.01 to 100 µg/mL with a coefficient of determination of 0.99994. These results indicate that the 1260 Infinity III hybrid multisampler enables robust HPLC analysis of TPD compounds while eliminating concerns related to recrystallization and injection volume constraints.

References

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