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APPLICATION NOTE

# High-Temperature HPLC Analysis of Cyclosporine on NanoPak-C All-Carbon Columns

**Background**. Cyclosporine (also known as cyclosporine A or CyA) is an immunosuppressant that has been a drug of choice for the prevention of graft rejection in organ transplant patients since the 1980s [1]. CyA is a cyclic undecapeptide analyzed via RP-HPLC (typically C18 columns) under high-temperature conditions to minimize peak broadening caused by conformational isomerism. Cyclosporine analysis under pharmacopoeial conditions is commonly performed at 80°C, but conventional silica C18 columns can show limited lifetime under these aggressive operating conditions [2], prompting evaluation of alternative stationary phases.

NanoPak-C All-Carbon microbeads are a new class of porous graphitic media that can withstand temperatures up to 200◦C [3]. This application note summarizes the evaluation of NanoPak-C All-Carbon columns as an alternative for high-temperature cyclosporine analysis, using European Pharmacopoeia (EP)/ United States Pharmacopeia (USP)/ Compounded Preparation or Pharmacopeial (CP)-like solvent systems and conditions.

The goal of the study was to assess whether NanoPak-C All-Carbon could provide suitable chromatographic performance and repeatability at the same elevated temperature while maintaining a retention profile aligned with reference methods.

| **Table 1. Experimental Conditions** | |
|---|---|
| ***Cyclosporine solution*** | 1 mg/mL purified cyclosporine in acetonitrile: water (50:50 v/v) solution |
| ***Columns*** | 250 mm x 4.6 mm, 6 µm<br>150 mm x 4.6 mm, 5 µm<br>50 mm x 4.6 mm, 6 µm |
| ***Mobile phase*** | Phosphoric acid: TBME: Acetonitrile: Water (**1: 48: 447: 504**, v/v/v/v) |
| ***Column*** | NanoPak-C 250 mm x 4.6 I.D, 6um |
| ***Flow rate*** | 1.9 mL/min |
| ***Column oven temp.*** | 80°C |
| ***Wavelength*** | 210 nm |
| ***Injection volume*** | 20uL |
| ***Syringe rinse solvent*** | Acetonitrile |

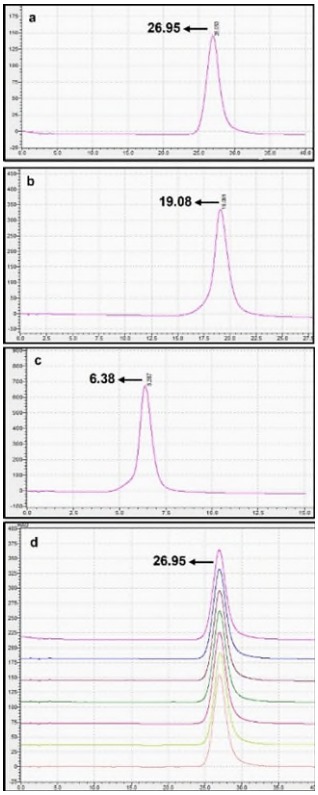


***Figure 1. Representative chromatogram of cyclosporine on (a) 250 X 4.6 mm, (b) 150 X 4.6 mm, (c) 50 X 4.6 mm NanoPak-C All-Carbon columns. (d) Offset chromatograms of seven consecutive injections on the 250 X 4.6 mm column.***

## Results

On the 250 mm NanoPak-C column, initial retention was 32.980 min at 1.8 mL/min, and after optimization of the acetonitrile/ water composition, the cyclosporine peak eluted at 26.953 min, within the customer's target retention window of 25 to 30 min.

**Column stability at 80°C** was assessed over seven consecutive injections. Across the sequence, retention time remained near 27.0 min, tailing factor remained in the range of 1.252 to 1.287, and peak area remained consistent. No significant drift in retention time or peak area was observed across the seven injections, indicating good repeatability and stable performance under the optimized high-temperature conditions.

Taken together, the results reproduced the target pharmacopoeial retention window while maintaining acceptable peak symmetry under 80°C operation.

**Shorter columns reduced retention time** to 19.081 min on the 150 mm format and 6.387 min on the 50 mm format, supporting their use for faster analyses where monograph-style retention is not required. These formats can therefore be positioned as faster alternatives for in-house or higher-throughput applications. The 250 mm format remains the closest to the reference monograph style.

***The data support NanoPak-C All-Carbon HPLC columns as a suitable alternative for routine high-temperature cyclosporine analysis, especially in workflows where reduced lifetime has been observed with conventional silica C18 columns.***

**Summary**

- Matches pharmacopoeial retention window at 80 °C on NanoPak-C All-Carbon 250 × 4.6 mm column.
- Demonstrates stable retention time, peak symmetry, and area over seven injections.
- Shorter column formats enable faster cyclosporine analyses with predictable retention.

**Column Selection Guidance**

| **Table 2. NanoPak-C All-Carbon Column selection guidance for cyclosporine analysis at 80 °C** | | |
|---|---|---|
| ***Column format*** | ***Observed retention time*** | ***Positioning*** |
| 250 × 4.6 mm, 6 µm | 26.95 min | Closest to reference monograph-style method |
| 150 × 4.6 mm, 5 µm | 19.08 min | Shorter runtime for faster analysis |
| 50 × 4.6 mm, 6 µm | 6.38 min | Very fast screening or in-house high throughput methods |

**References**

[1] N. Talal, Cyclosporine As An Immunosuppressive Agent For Autoimmune Disease: Theoretical Concepts And Therapeutic Strategies, Transplant Proc 20(3 Suppl 4) (1988) 11-5.

[2] H.A. Claessens, M.A. Van Straten, Review On The Chemical And Thermal Stability Of Stationary Phases For Reversed-Phase Liquid Chromatography, Journal Of Chromatography A 1060(1) (2004) 23-41.

[3] M.J. Parente, B. Sitharaman, Synthesis And Characterization Of Carbon Microbeads, Acs Omega 8(37) (2023) 34034-34043.