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Application Note

# Bridging the Performance Gap from Analytical to Preparative Chromatography: Efficient Target Isolation from a Complex Mixture

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## Abstract

This application note highlights the benifits of using SunFire Columns.

### Benefits

SunFire $C_{18}$ analytical and OBD preparative columns are designed to provide maximum loadability in simple mobile phase conditions, accurate scalability, and high peak capacity


## Introduction

Today's drug discovery environment demands the rapid isolation and purification of compounds with minimal chromatographic development. SunFire $C_{18}$ Columns are engineered with highly pure raw materials and a tightly controlled synthesis process. These columns provide high efficiencies, maximum loading, and symmetric peak shapes for the analysis of bases, neutrals, and acids. SunFire $C_{18}$ preparative columns are manufactured with the Optimum Bed Density (OBD) design to deliver consistent column-to-column performance, unmatched column lifetime with DMSO sample diluents, and accurate scalability.

## Experimental

### Experimental Conditions

| | |
|---|---|
| Columns: | SunFire $C_{18}$ 4.6 x 100 mm, 5 mm and 19 x 100 mm, 5 mm |
| Mobile phase A: | 0.1% trifluoroacetic acid in water |
| Mobile phase B: | 0.1% trifluoroacetic acid in acetonitrile |
| Flow rate: | 1.4 mL/min analytical, 23.9 mL/min preparative |

| | |
|---|---|
| Analytical gradient: | 10 min linear from 5% to 95% B, with 1 min initial hold time |
| Preparative gradient: | 10 min linear from 5% to 95% B, with 1.79 min initial hold time |
| Injection volume: | 60 mL (analytical) and 1020 mL (preparative) |
| Sample mixture: | 8-bromoguanosine (20 mg/mL), acetanilide (20 mg/mL), hydrocortisone (20 mg/mL), 6α-methyl-17α-hydroxyprogesterone (12.5 mg/mL), 3-aminofluoranthene (20 mg/mL), 2-bromofluorene (20 mg/mL), and perylene (1.3 mg/mL) prepared in DMSO |
| Total mass loading: | 6.9 mg (analytical), 115.7 mg (preparative) |
| Detection: | UV at 254nm |
| Instrument: | Waters AutoPurification System |

## Results and Discussion

The separation of the complex mixture on the analytical column is shown in Figure 1a. The total load is 6.9 mg. The flattened peak profiles reflect the saturation of the PDA detector under this high mass load. The separation was proportionally scaled-up and run on the preparative column as shown in Figure 1b. Note the direct scale up, excellent peak shapes and total mass load of 115.7 mg. In order to avoid saturation of the PDA detector under the preparative conditions, a 1/1000 dilution was employed on the preparative run.

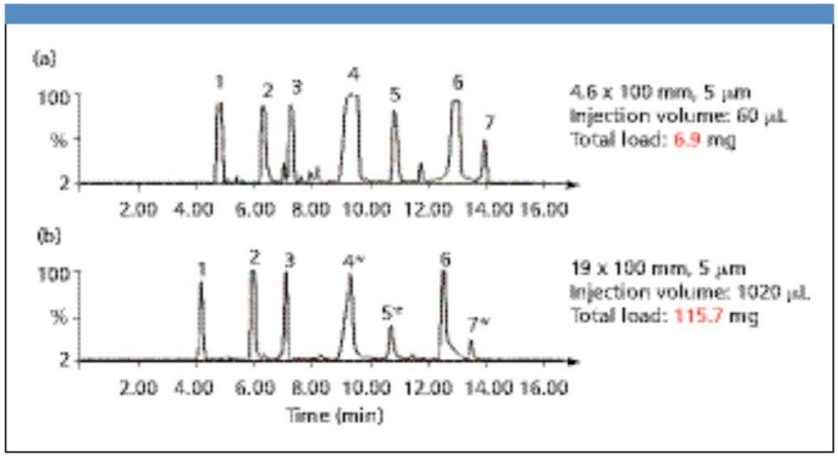


*Figure 1. Scale-up of the purification of the complex mixture from analytical to preparative SunFire $C_{18}$ columns. (a) SunFire $C_{18}$, 4.6 x 100 mm, 5 mm. (b) SunFire $C_{18}$ OBD, 19 x 100 mm, 5 mm. Analytes: 1) 8-bromoguanosine, 2) acetanilide, 3) hydrocortisone, 4) 6α-methyl-17α-hydroxyprogesterone, 5) 3-aminofluoranthene, 6) 2-bromofluorene, and 7) perylene. Note: The flat peak tops in (a) are due to the saturation response of PDA detector. The sharp peaks in (b) are due to the 1/1000 dilution employed on the preparative run.*

## Conclusion

Highly efficient isolation and direct scale-up are observed on both SunFire $C_{18}$ analytical and preparative columns. The SunFire Column chemistry ensures rapid target purifications with minimal chromatographic development.

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AutoPurification System <https://www.waters.com/10007147>

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