Using DMSO as an Injection Solvent to Increase Sample Load in Preparative LC

Application

Pharmaceuticals

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Abstract

In preparative HPLC, peak splitting can be caused by incomplete sample solubility in the injection solvent. It was found that this can be avoided by using DMSO as the injection solvent, thereby increasing sample solubility and sample load. Preparative samples injected in DMSO eluted well-resolved in the mobile phase when using ZORBAX® Bonus-RP, Eclipse XDB, StableBond, and Extend HPLC Columns. It was shown that ZORBAX Bonus-RP is an ideal column for the analytical or preparative separation of secondary amines.

Introduction

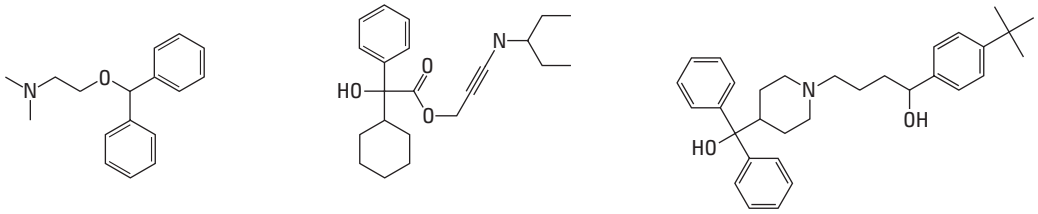
After optimizing a separation on an analytical HPLC column, the preparative chemist directly scales these separation conditions to a larger diameter column (assuming that the packing material in the analytical and preparative columns is identical). However, increasing injection size or maximizing sample load on a preparative scale may uncover unexpected solubility problems, for example, peak broadening or peak splitting. This problem may be resolved by adding the organic solvent DMSO (dimethylsulfoxide) to the injection solvent thereby improving sample solubility and re-establishing good chromatography, that is, symmetrical peaks.

Results

Some basic compounds, such as tertiary amines (see Figure 1), are difficult to separate and isolate by HPLC because they tail badly on many silica-based HPLC columns. However, the ZORBAX Bonus-RP HPLC column incorporates an amide linkage into the hydrocarbon bonded phase itself, shielding the secondary amines from unreacted silanols on the silica surface thus enabling these compounds to elute as sharp, symmetrical peaks. In the small chromatogram (upper right) in Figure 2, the analytical separation of three tertiary amines, diphenhydramine, oxybutynin, and terfenadine (structures shown in Figure 1), is optimized for resolution and analysis time on a 4.6 × 75 mm, 3.5-µm ZORBAX Rapid Resolution Bonus-RP column using a low pH, volatile mobile phase. The separation conditions are then scaled for preparative chromatography using a larger diameter column packed with the same bonded-phase.

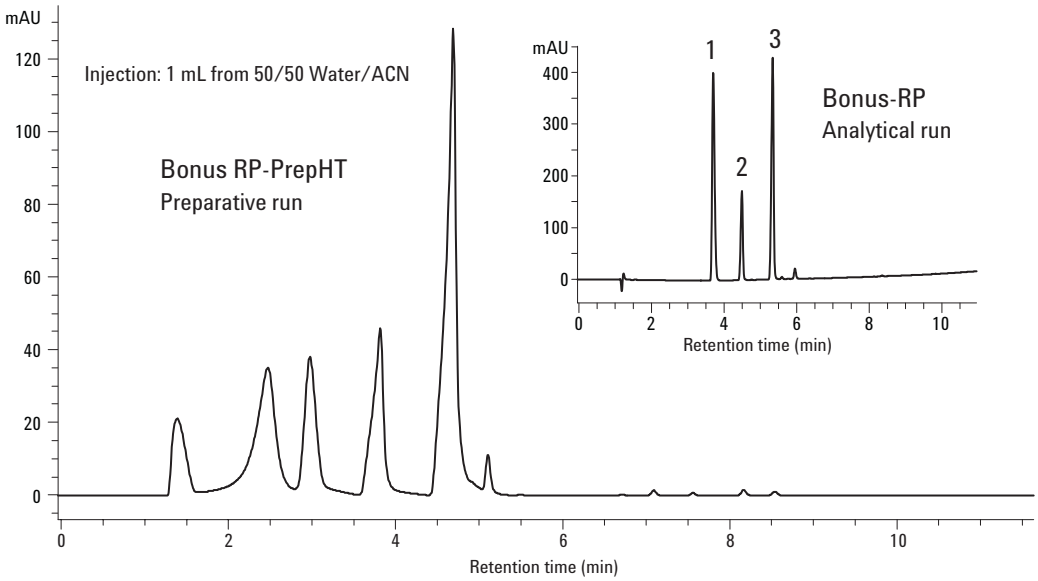
In order to maximize sample load, a 1000-µL injection of a sample containing 30 mg each secondary amine in a 50/50 (v/v) acetonitrile/water solvent with 0.1% formic acid was injected onto a 21.2 × 100-mm, PrepHT column packed with 5-µm ZORBAX Bonus-RP giving an unexpected result (left chromatogram in Figure 2). Instead of the three sharp peaks observed in the analytical run,

five distinct, but misshapen, peaks eluted from the Bonus-RP preparative column. In addition, the separation was not reproducible from one injection to another. Incomplete solubility of the sample in the injection solvent was suspected.



Diphenhydramine (1) Oxybutynin (2) Terfenadine (3)

Figure 1. Structures of tertiary amines used in this study.



Analytical Separation Conditions

Column:	ZORBAX Rapid Resolution Bonus-RP, 4.6 mm × 75 mm, 3.5 µm
Mobile phase:	Solvent A: Water with 0.1% formic acid; Solvent B: Acetonitrile with 0.1% formic acid
Gradient elution:	10% B to 90% B in 9 min; 90% B for 1 min; 90% B to 10% B in 1 min
Detector:	UV, 254 nm, 4 nm
Flow rate:	0.8 mL/min.
Injection volume:	5 µL
Injection solvent:	1:1 Water-Acetonitrile with 0.1% formic acid
Sample concentration:	10 mg/mL each analyte
Sample:	Diphenhydramine, oxybutynin, terfenadine

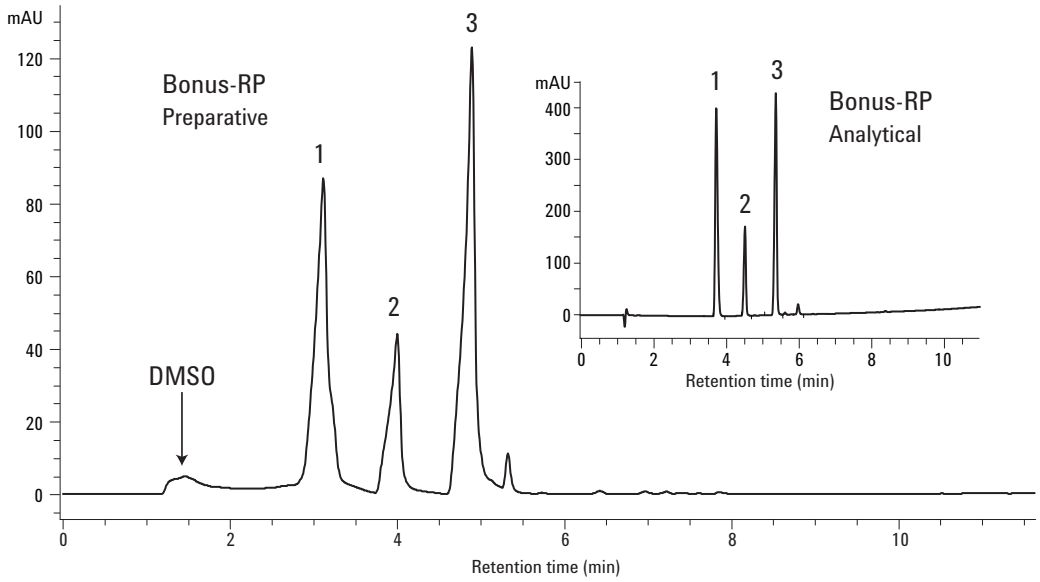
Preparative Separation Conditions

Column:	ZORBAX PrepHT Bonus-RP, 21.2 x 100 mm, 5 µm
Mobile phase:	Solvent A: Water with 0.1% formic acid; Solvent B: Acetonitrile with 0.1% formic acid
Gradient elution:	10% B to 90% B in 9 min; 90% B for 1 min; 90% B to 10% B in 1 min
Flow rate:	20 mL/min.
Detector:	UV, 254 nm, 4 nm
Injection volume:	1000 µL
Injection solvent:	50% Water/50% acetonitrile with 0.1% formic acid
Sample concentration:	30 mg/mL each analyte
Sample:	Diphenhydramine, oxybutynin, terfenadine

Figure 2. Optimized analytical separation of three tertiary amines on ZORBAX Bonus-RP (upper-right) and poor preparative chromatography due to sample solubility problems (left chromatogram).

Using DMSO to Increase Sample Solubility and Sample Load

The organic solvent DMSO can be used to increase sample solubility yet their inherent solvent strength in reversed-phase chromatography systems can also broaden the peaks of interest. Here we will illustrate the use of DMSO to improve sample loading in preparative chromatography. As can be seen in Figure 3, for this separation, changing the injection solvent from water/ACN (50/50) to 100% DMSO eliminated the peak splitting initially observed in Figure 2 for this sample loading and induced a small but acceptable amount of peak broadening to still achieve a good separation with an increased sample load.



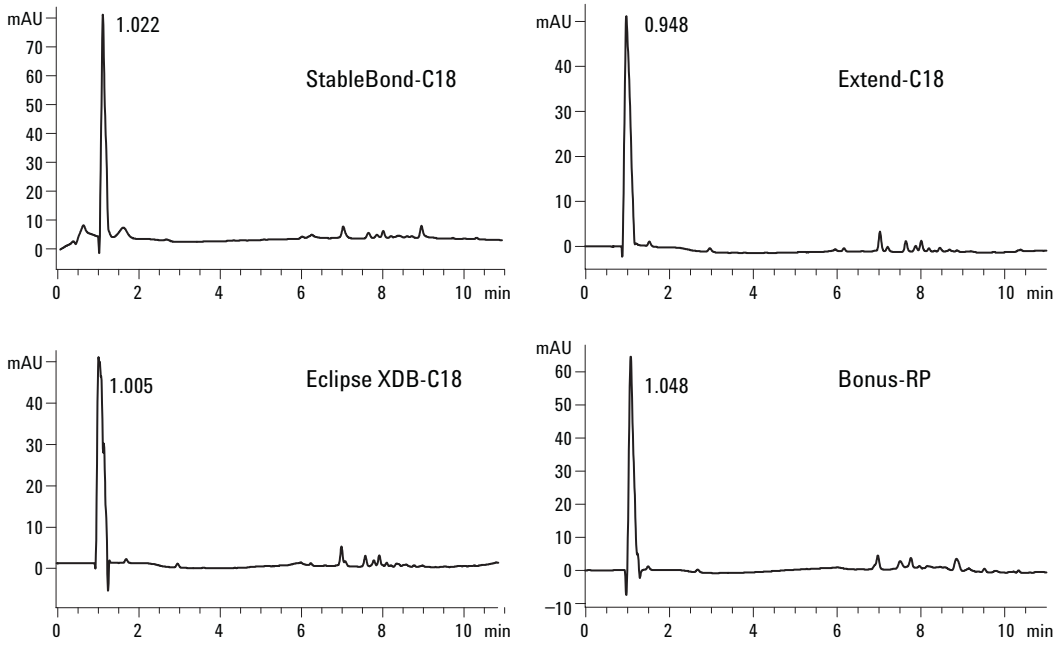
Preparative Separation Conditions

Column:	ZORBAX Bonus-RP, 21.2 mm × 100 mm, 5 µm
Mobile phase:	Solvent A: Water with 0.1% formic acid; Solvent B: Acetonitrile with 0.1% formic acid
Gradient conditions:	10% B to 90% B in 9 min; 90% B for 1 min; 90% B to 10% B in 1 min
Flow rate:	20 mL/min.
Detector:	UV, 254 nm, 4 nm
Injection volume:	1000 µL
Injection solvent:	DMSO
Sample concentration:	30 mg/mL each analyte
Sample:	Diphenhydramine, oxybutynin, terfenadine

Figure 3. Successful preparative separation of three tertiary amines (Diphenhydramine [1], Oxybutynin [2], and Terfenadine [3]) with DMSO as injection solvent.

DMSO as an Injection Solvent

In order to show that DMSO did not interfere with the peaks of interest, a blank injection of pure DMSO was performed for several analytical columns including ZORBAX Bonus-RP column used in this study. All of the DMSO eluted at or near the void volume, instead of bleeding slowly off the column as observed with other silica-based columns. Thus, compounds of interest are collected in mobile phase, not in DMSO, which is typically more difficult to evaporate than the pure mobile phase and would possibly interfere with secondary testing. These findings are also observed with ZORBAX StableBond, Eclipse XDB, and Extend HPLC Columns, as noted in Figure 4, indicating that DMSO may be a useful injection solvent for these phases also. The DMSO peak is larger in Figure 4 than in Figure 3 since the analytical path length of the flow cell was greater and there was less peak dispersion due to the smaller column volume.



Analytical Separation Conditions

Columns:	ZORBAX Rapid Resolution Columns, 4.6 mm × 75 mm, 3.5 µm a) StableBond-C18 b) Extend-C18 c) Eclipse XDB-C18 d) Bonus-RP
Mobile phase:	Solvent A: Water with 0.1% formic acid; Solvent B: Acetonitrile with 0.1% formic acid
Gradient elution:	10% B to 90% B in 9 min; 90% B for 1 min; 90% B to 10% B in 1 min
Detector:	UV, 254 nm, 4 nm
Flow rate:	0.8 mL/min.
Injection volume:	5 µL
Injection solvent:	100% DMSO

Figure 4. DMSO elutes in column void volume using all ZORBAX reversed-phase HPLC columns.

Conclusions

- ZORBAX Bonus-RP is an excellent choice for the analytical and preparative separation of difficult-to-separate basic compounds like secondary amines.
- Using DMSO as the injection solvent can increase sample solubility and sample load while avoiding peak splitting, although some peak broadening is likely.
- When injected onto a ZORBAX Bonus-RP, Eclipse XDB, StableBond, or Extend reversed-phase HPLC column, DMSO elutes at or near the void volume, thereby allowing preparative sample collection in pure mobile phase.
- If the solubilizing power of DMSO is exceeded, then precipitation and peak splitting may return.

Instrumentation

All analytical work was performed using an Agilent 1100 LC equipped with a solvent degasser, binary pump, auto-sampler, heated column compartment, and an ultraviolet detector. For preparative work, the Agilent 1100 Series Purification System was used.

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