

Reversed phase C-18 chromatography: From analytical scale to preparative scale

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Abstract

The ability to convert an analytical-scale reversed phase C-18 method to a preparative method is often perceived as a daunting task by synthetic organic chemists.

Recent work at Teledyne Isco for scaling a reversed phase C-18 analytical method to a preparative method on Teledyne Isco's *CombiFlash*[®] *Companion*[®] using *RediSep*[®] reversed phase C-18 media will be discussed.

Background

Reversed phase chromatography is most commonly performed on analytical instrumentation such as high performance liquid chromatography (HPLC) systems for the analytical separation of various organic compound mixtures. Sample load per injection on such analytical instruments is typically in the concentration range of parts per million. For the purification of larger quantities, an analytical separation is first performed and the resulting method is transposed to a large scale method on a preparative scale system. However, expensive instrumentation such as a preparative HPLC system and preparative columns often require multiple injections thereby decreasing the amount of sample recovered. Though the transposed large scale method is a good starting point, at times the preparative method may need to be modified for improved efficiency. As a result, large scale reversed phase separation on a preparative HPLC system can require more of the chemist's time to determine efficient loading capacity and to perform multiple injections.

Teledyne Isco's automated flash chromatography instrument — the *CombiFlash Companion* — and *RediSep* reversed phase C-18 flash columns offer chemists an easier and efficient alternative to preparative HPLC separations. The *Companion's* PeakTrak[®] software provides the chemist with convenient default C-18 separation methods or the flexibility to adjust the method parameters during the separation in an effort to further increase resolution. The reusable *RediSep* reversed phase C-18 columns are capable of separating sample quantities ranging from milligrams to grams in a single injection. Such conveniences decrease the number of runs needed to develop an optimal preparative method and eliminate the need for multiple injections. It has been reported that reversed phase separations on a *CombiFlash Companion* with *RediSep's* C-18 reversed phase columns have increased sample recovery by up to 30% as compared to multiple injections on a preparative HPLC system, thus increasing the chemist's productivity.

Objective

Our objective is to show that the conversion of an analytical reversed phase C-18 separation to a preparative method on the *CombiFlash* Companion requires minimal time and effort. In addition, examples for the separation of several grams of sample requiring only one injection will be given while demonstrating consistent resolution when scaling a method from milligrams to a multi-gram scale.

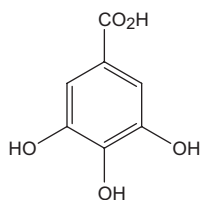
Experimental and Results

Method development or method transposition from an analytical system to a preparative system is not always consistent due to the differences in performance of high pressure systems, column vendors, column size, and media size and type. The ease of method development for C-18 separations on the Companion was tested against four HPLC C-18 separations, performed on three C-18 columns from different vendors with different media size. Converting an analytical method to a preparative method for the *CombiFlash* Companion is as simple as loading PeakTrak's default method for a given reversed phase RediSep column size or editing the default gradient profile to match the gradient profile used in the analytical separation.

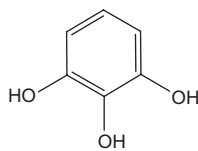
Galic Acid and Pyrogallol

Refer to Figures 1 and 2 on pages 10 and 11.

For the separation of gallic acid (1) and pyrogallol (2), the default preparative gradient profile was changed to match the HPLC gradient profile, which resulted in separation of the two components of the mixture.



Galic acid
(1)

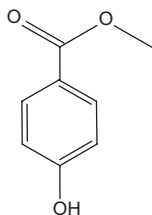


Pyrogallol
(2)

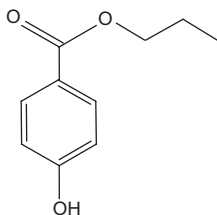
Methyl paraben and Propyl paraben

Refer to Figures 3 and 4 on pages 12 and 13.

In the separation of methyl paraben (3) and propyl paraben (4), the default preparative method was changed to match the HPLC gradient profile. Purity for this separation is high, based on the consistent absorbance ratio of $\lambda_{\text{det}}/\lambda_{\text{mon}}$ as indicated by the linearity of the green trace. Although separation occurs it should be noted that the unmodified default method for reversed phase separation achieved baseline separation.



Methyl paraben
(3)

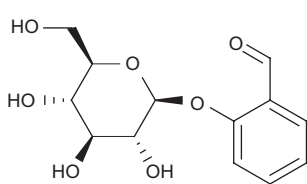


Propyl paraben
(4)

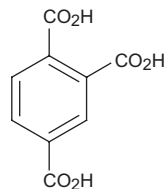
Helicin and 1,2,4-Benzenetricarboxylic Acid

Refer to Figures 5 and 6 on pages 14 and 15.

In the separation of helicin (5) and 1,2,4-benzenetricarboxylic acid (6) an initial preparative method was developed by changing the default gradient profile to match the HPLC gradient profile, resulting in partial separation. An enhanced method was developed by modifying the initial gradient profile during the course of the separation which allowed for improved sample resolution. In this example, upon detection of the first peak, an isocratic hold was placed in the gradient profile further increasing resolution between the two peaks.



Helicin
(5)

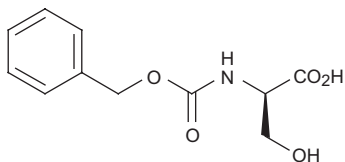


1,2,4-Benzenetricarboxylic
acid (6)

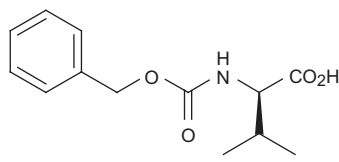
CBz-ser-OH and CBz-val-OH

Refer to Figures 7 and 8 on pages 16 and 17.

Analysis of the analytical separation of CBz-ser-OH (7) and CBz-val-OH (8) showed very low absorbing peaks at the detection wavelength of 250 nm. Upon application of the sample mixture on the Companion, it was observed that a higher absorption could be obtained at $\lambda_{\text{obs}} = 215 \text{ nm}$. In the initial separation, the *CombiFlash* Companion system was set to detect at 250 nm (red trace), consistent with the analytical HPLC method and monitored at 215 nm (purple trace). The preparative method for CBz-ser-OH (7) and CBz-val-OH (8) was developed by editing the default gradient profile to match the HPLC gradient profile. As a result, baseline separation occurred, though not observed at the detecting wavelength as used in the analytical method. Use of the dual wavelength feature made it apparent collection should be based on the absorbance detected at 215 nm instead of 250 nm. For subsequent scale-up runs from 260 mg to more than 7 grams of this mixture, the preparative methods were developed by editing the column's default C-18 gradient profile to match the HPLC gradient profile with the detection wavelength set at 215 nm.

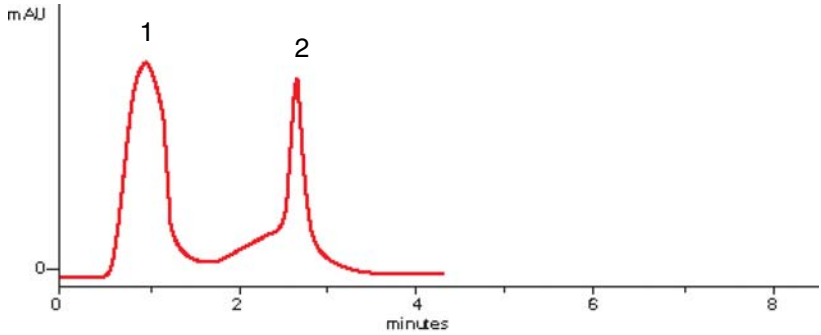


CBz-ser-OH (7)



CBz-val-OH (8)

Figure 1. Gallic Acid and Pyrogallol
on Polaris C-18 3×100mm



Instrument: Agilent 1100 HPLC

Column: Polaris C18, 3 μ m, 3×100mm

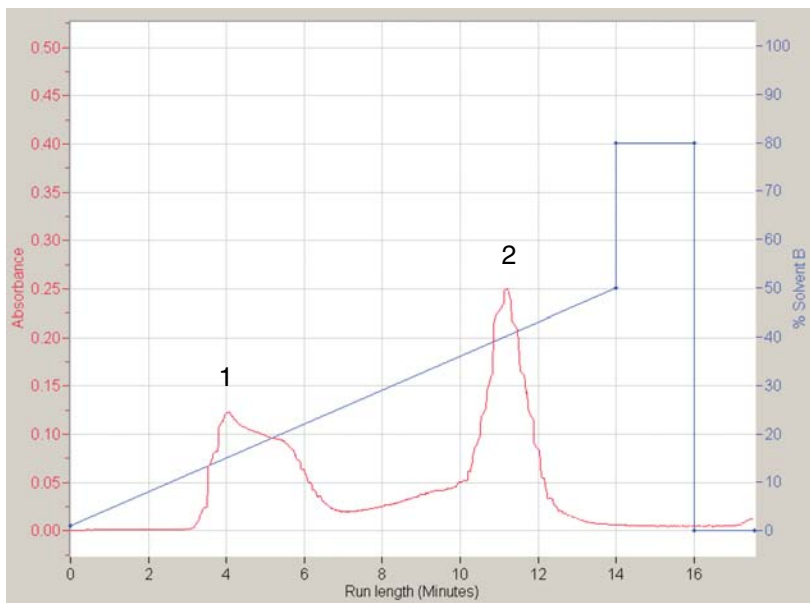
Mobile Phase: A: H₂O
B: CH₃CN

Gradient: 1%B to 50% B in 10 min

Flow Rate: 0.5 ml/min

Detection Wavelength: 215 nm

**Figure 2. Gallic Acid and Pyrogallol
on RediSep C-18**



Instrument: CombiFlash Companion

Column: 4.3 g RediSep C-18 Reversed Phase,
40–63 μ m

Sample Load: 10 mg

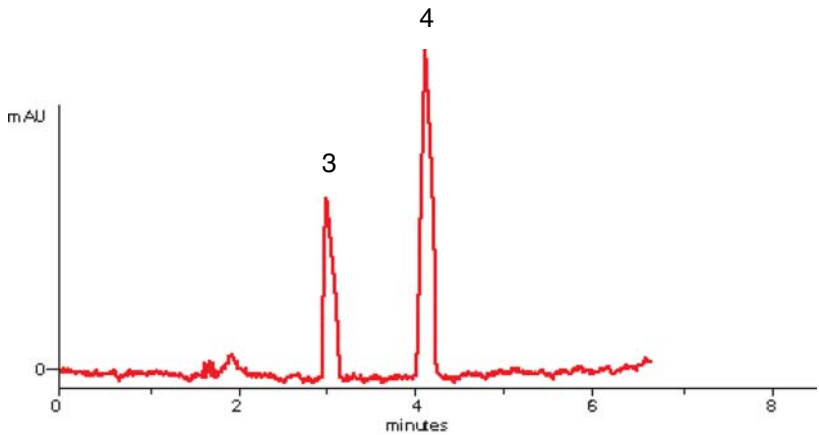
Mobile Phase: A: H₂O
B: CH₃CN

Gradient: Shown on chromatogram

Flow Rate: 16 ml/min

Detection Wavelength: 215 nm

Figure 3. Methyl paraben and Propyl paraben on Adsorbosphere C-18 4.6×250mm



Instrument: Agilent 1100 HPLC

Column: Adsorbosphere C18, 5 μ m, 4.6×250mm

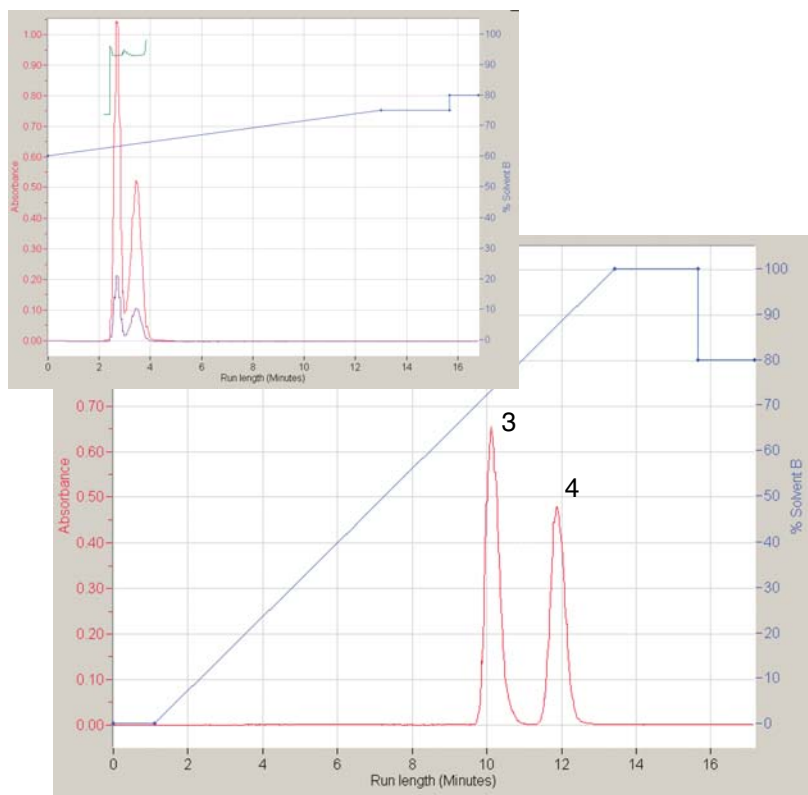
Mobile Phase: A: H₂O
B: CH₃CN

Gradient: 60 to 75%B in 7 minutes

Flow Rate: 1.0 ml/min

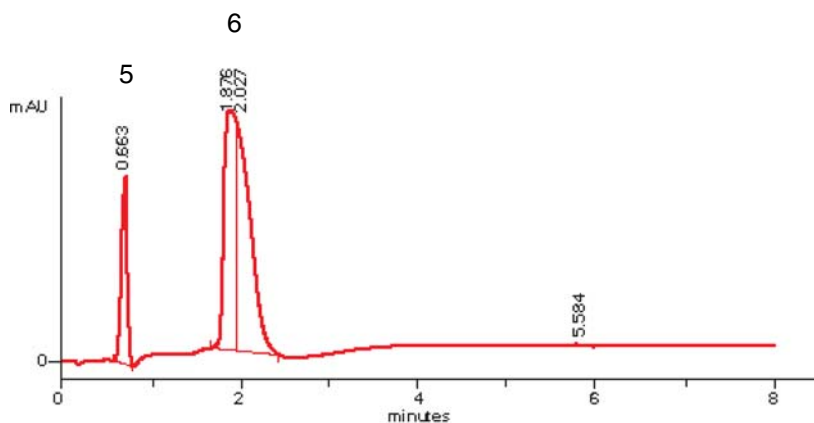
Detection Wavelength: 500 ELSD with LTA

Figure 4. Methyl paraben and Propyl paraben on RediSep C-18



Instrument: CombiFlash Companion
Column: 4.3 g RediSep C-18 Reversed Phase, 40–63 μ m
Sample Load: 10 mg
Mobile Phase: A: H₂O
B: CH₃CN
Gradient: Shown on chromatogram
Flow Rate: 16 ml/min
Wavelength: Detection 254 nm (red), Monitor 280 nm (purple)

Figure 5. Helicin and 1,2,4-Benzenetricarboxylic Acid on Phenomenex Luna C-18 2×50mm



Instrument: Agilent 1100 HPLC

Column: Phenomenex Luna C18, 5 μ m, 2×50mm

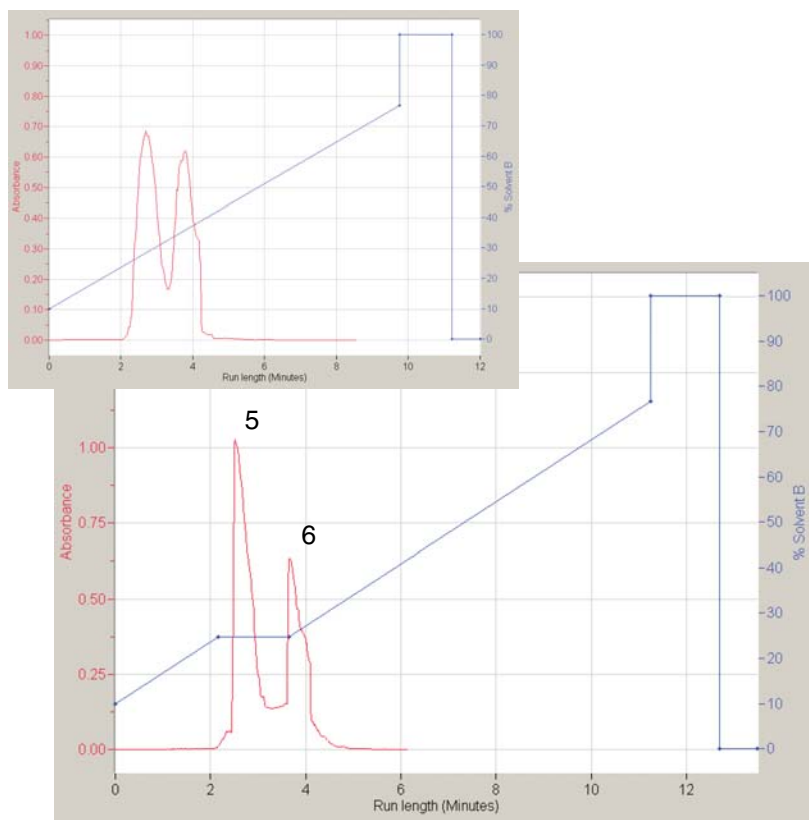
Mobile Phase: A: H₂O with 0.05% HCO₂H
B: CH₃CN with 0.05% HCO₂H

Gradient: 10%B to 100%B in 6 min,
hold at 100%B for 4 min

Flow Rate: 0.3 ml/min

Detection Wavelength: 215 nm

Figure 6. Helicin and 1,2,4-Benzenetricarboxylic Acid on RediSep C-18



Instrument: CombiFlash Companion

Column: 4.3 g RediSep C-18 Reversed Phase,
40–63 μ m

Sample Load: 10 mg

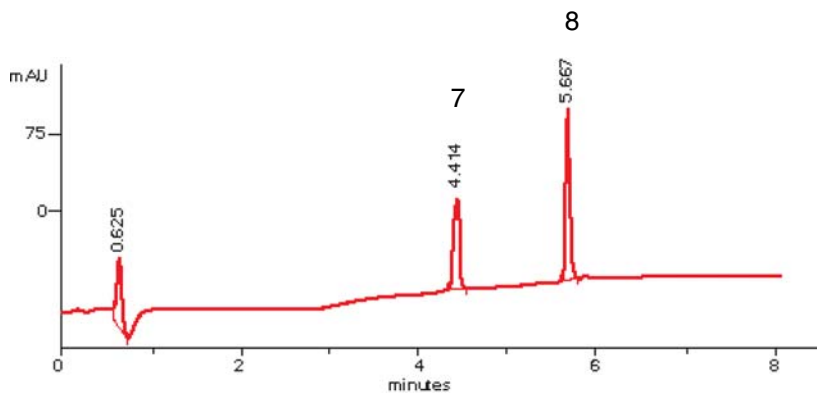
Mobile Phase: A: H₂O with 0.05% HCO₂H
B: CH₃CN with 0.05% HCO₂H

Gradient: Shown on chromatogram

Flow Rate: 16 ml/min

Detection Wavelength: 215 nm

Figure 7. CBz-ser-OH and CBz-val-OH on Phenomenex Luna C-18 2×50mm



Instrument: Agilent 1100 HPLC

Column: Phenomenex Luna C18, 5 μ m, 2×50mm

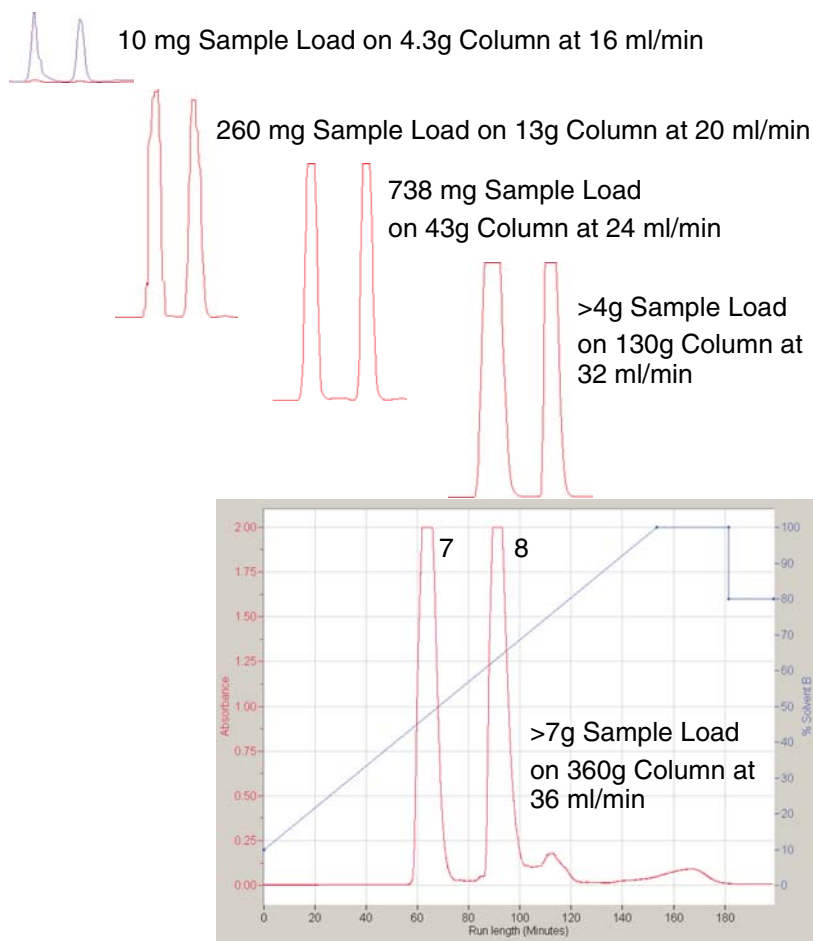
Mobile Phase: A: H₂O with 0.05% HCO₂H
B: CH₃CN with 0.05% HCO₂H

Gradient: 10%B to 100%B in 6 min,
hold at 100%B for 4 min

Flow Rate: 0.3 ml/min

Detection Wavelength: 250 nm

Figure 8. CBz-ser-OH and CBz-val-OH on RediSep C-18



Instrument: *CombiFlash Companion*

Column: *RediSep C-18* Reversed Phase, 40–63 μ m

Mobile Phase: A: H₂O with 0.05% HCO₂H
B: CH₃CN with 0.05% HCO₂H

Wavelength: 4.3 g column: detection (red) 250 nm,
monitor (purple) 215 nm

All other runs: detection 215 nm

Summary

Method development for preparative reversed phase C-18 separations on the *CombiFlash Companion* using *RediSep C-18* columns (Figure 9) required minimal time and effort. In addition, separations requiring only one injection were obtained for sample sizes ranging from 10 mg to over 7 grams. Consistent resolution was observed in scaling a method for milligrams to several grams on various *RediSep C-18* column sizes. It was noted that regardless of the column size or media vendor, if the separation between two components is ≥ 1.5 minutes on an analytical method, the probability for achieving baseline resolution on the *CombiFlash Companion* is high. Minimal method development makes it easy for the chemist to scale the method to any size column to accommodate varying amounts of sample. Additionally, use of the Companion system's built-in features, such as click and drag gradient editing, dual wavelength detection, and standard C-18 default methods provide the chemist with a fast, convenient, and successful approach for converting an analytical-scale reversed phase C-18 method to a preparative scale method on a *CombiFlash Companion*.

Figure 9. CombiFlash Companion



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