APPLICATION NOTE

#Appn_SC006-0

SiliaChrom® Plus

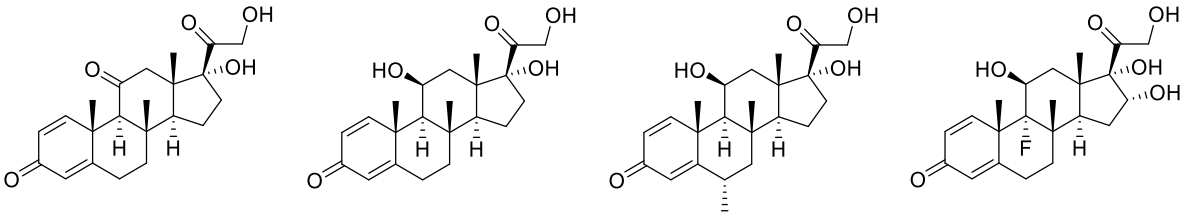
Scalability of SiliaChrom Plus C18 for Corticosteroids Separations

Corticosteroids are anti-inflammatory compounds prescribed to treat a wide range of afflictions, including arthritis, allergies, and skin diseases. Prednisone, prednisolone, methylprednisolone, and triamcinolone are some of the commonly used corticosteroids. This Application Note presents the HPLC method scalability for the separation of a mixture of these corticosteroids using SiliaChrom Plus columns.

LEARN MORE about SiliaChrom Plus in our brochure "*Solutions for Analytical & Preparative Chromatography*".

HPLC chromatography is an efficient method for organic compounds purification such as corticosteroids. Prednisone, prednisolone, methylprednisone, and triamcinolone are commonly prescribed corticosteroids (*Figure 1*) as they are inexpensive and fast acting.

Figure 1: Chemical structures of the four corticosteroids



a) Prednisone b) Prednisolone c) Methylprednisolone d) Triamcinolone

Their similar chemical structures make them challenging to separate, especially on a larger scale. This Application Note details the development of a method for the separation of these four corticosteroids and its transfer from analytical to semi-preparative scale using Silia*Chrom* Plus C18 columns.

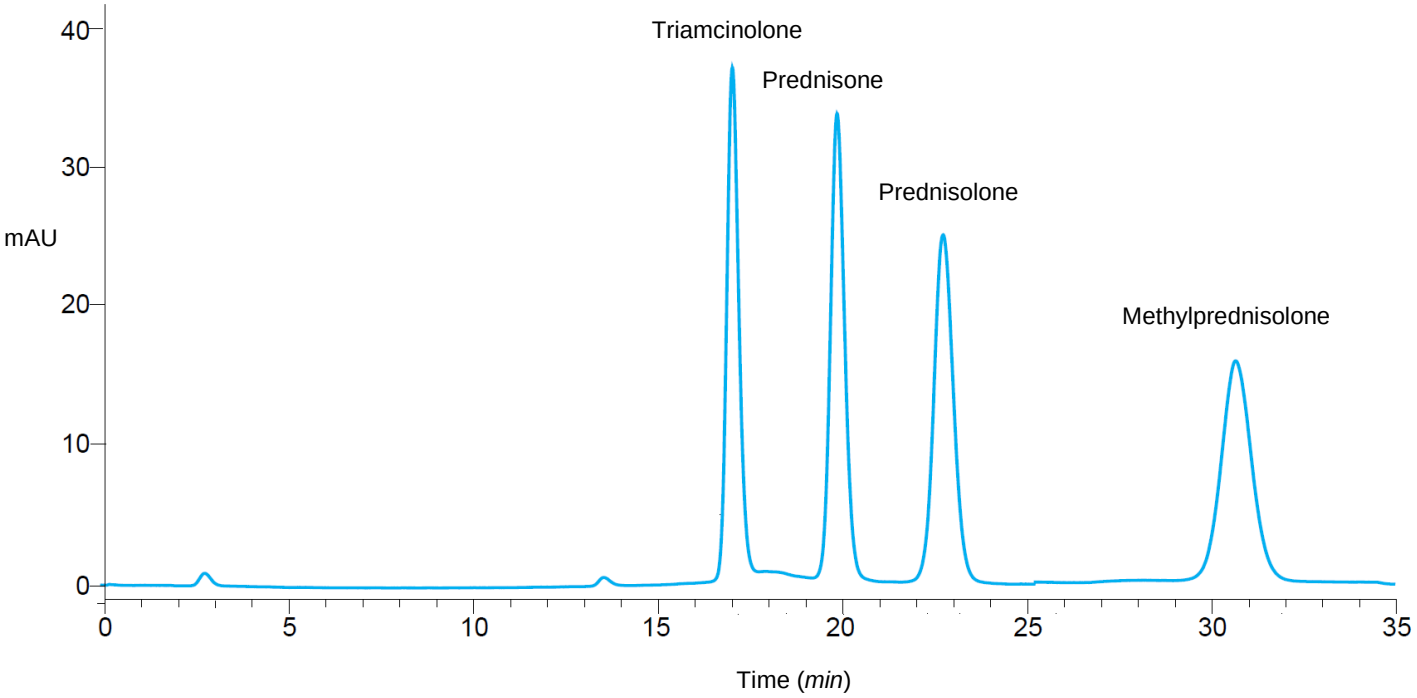
ANALYTICAL SCALE (*4.6 mm ID COLUMN*)

The conditions for separation were first optimized on a 4.6 x 250 mm analytical column (*Table 1*). These conditions yielded sufficient separation of the four corticosteroids, as shown on the chromatogram in Figure 2.

Table 1: Analytical chromatographic conditions

Analytical Chromatographic Conditions	
Parameter	**Value**
COLUMN	Silia*Chrom* Plus HPLC Column, C18, 4.6 x 250 mm, 10 µm, 100 Å
PART NUMBER	HPL-S03207E-A-N250
MOBILE PHASE	Gradient Mobile phase A: water Mobile phase B: methanol 1. 80 % A to 50 % A (*15 minutes*) 2. 50 % A (*17 minutes*) 3. 80 % A (*3 minutes*)
FLOW RATE	2.0 mL/min
DETECTOR	245 nm
INJECTION	0.25 mg in 5 µL of DMSO

Figure 2: Separation of the corticosteroids mixture with a Silia*Chrom* Plus C18 analytical column



SCALING-UP

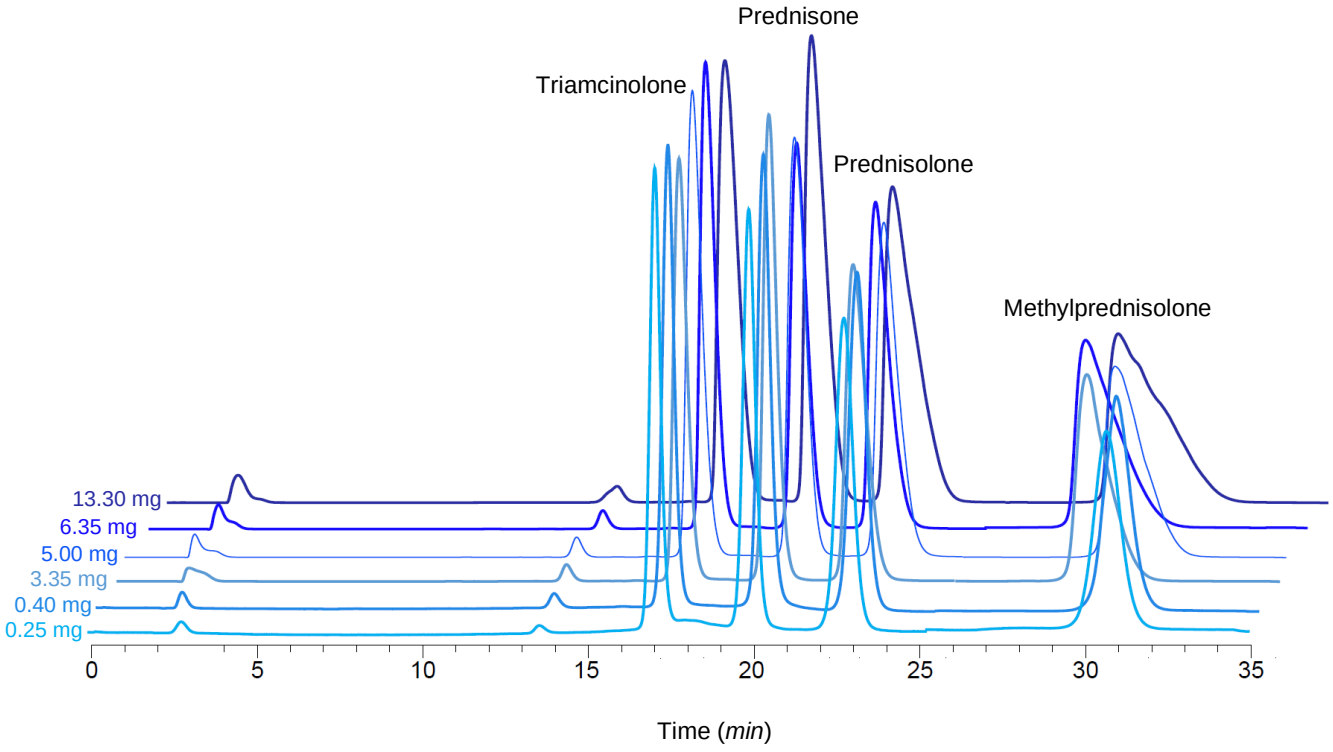
To scale-up an HPLC method, once elution conditions are found on the analytical scale, the loading is increased progressively on the same analytical column by injecting more sample mass in the same volume. This is done until the resolution of the separation reaches a point where it is still acceptable, but whereby further increasing the loading would make it unacceptable.

Six increasing sample loads were injected on the analytical column (*Table 2*), until such a resolution was reached (*13.30 mg for the present study*). Chromatograms are shown in Figure 3.

Table 2: Increasing sample loads - Analytical scale

Increasing Sample Loads - Analytical Scale	
Parameter	**Value**
COLUMN	Silia*Chrom* Plus HPLC Column, C18, 4.6 x 250 mm, 10 µm, 100 Å
PART NUMBER	HPL-S03207E-A-N250
MOBILE PHASE	Gradient Mobile phase A: water Mobile phase B: methanol 1. 80 % A to 50 % A (*15 minutes*) 2. 50 % A (*17 minutes*) 3. 80 % A (*3 minutes*)
FLOW RATE	2.0 mL/min
DETECTOR	245 nm
INJECTIONS (*in DMSO*)	1. 0.25 mg in 5 µL 2. 0.40 mg in 8 µL 3. 3.35 mg in 67 µL 4. 5.00 mg in 100 µL 5. 6.35 mg in 133 µL 6. 13.30 mg in 266 µL

Figure 3: Increasing sample loads with a Silia*Chrom* Plus C18 analytical column



The conditions on the analytical column with the largest possible loading were then transferred to the semi-preparative column.

DETERMINATION OF THE SEMI-PREPARATIVE PARAMETERS

The semi-preparative column selected was of the same length as the analytical one (*250 mm*). This ensures that the retention time and resolution obtained for prednisone and prednisolone could be compared in terms of scalability between the two columns with different internal diameters.

The flow rate on the semi-preparative column (*10 mm ID*) was determined using Equation 1.

Equation 1: Determination of the semi-preparative flow rate

$$V_2 = \frac{V_1(r_2)^2}{(r_1)^2}$$

Where: V_2 = Flow rate on the semi-preparative column
V_1 = Flow rate on the analytical column
r_2 = Radius of the semi-preparative column
r_1 = Radius of the analytical column

The flow rate thereby calculated for the semi-preparative column was 9.45 mL/min.

Then, to determine the sample loads to use on the semi-preparative column, Equation 2 was used.

Equation 2: Determination of the semi-preparative sample load

$$X_2 = \frac{X_1(r_2)^2 L_2}{(r_1)^2 L_1}$$

Where: X_2 = Sample load on the semi-preparative column
X_1 = Sample load on the analytical column
r_2 = Radius of the semi-preparative column
r_1 = Radius of the analytical column
L2 = Length of the semi-preparative column
L1 = Length of the analytical column

The sample loads thus calculated for the semi-preparative column were 1.18, 1.89, 15.83, 23.63, 31.43, and 62.85 mg.

DMSO was chosen as the injection solvent due to its excellent ability to dissolve many organic compounds, especially at the higher sample concentrations needed for semi-preparative chromatography. Moreover, DMSO elutes rapidly, ensuring that it does not overlap or interfere with the peaks of interest.

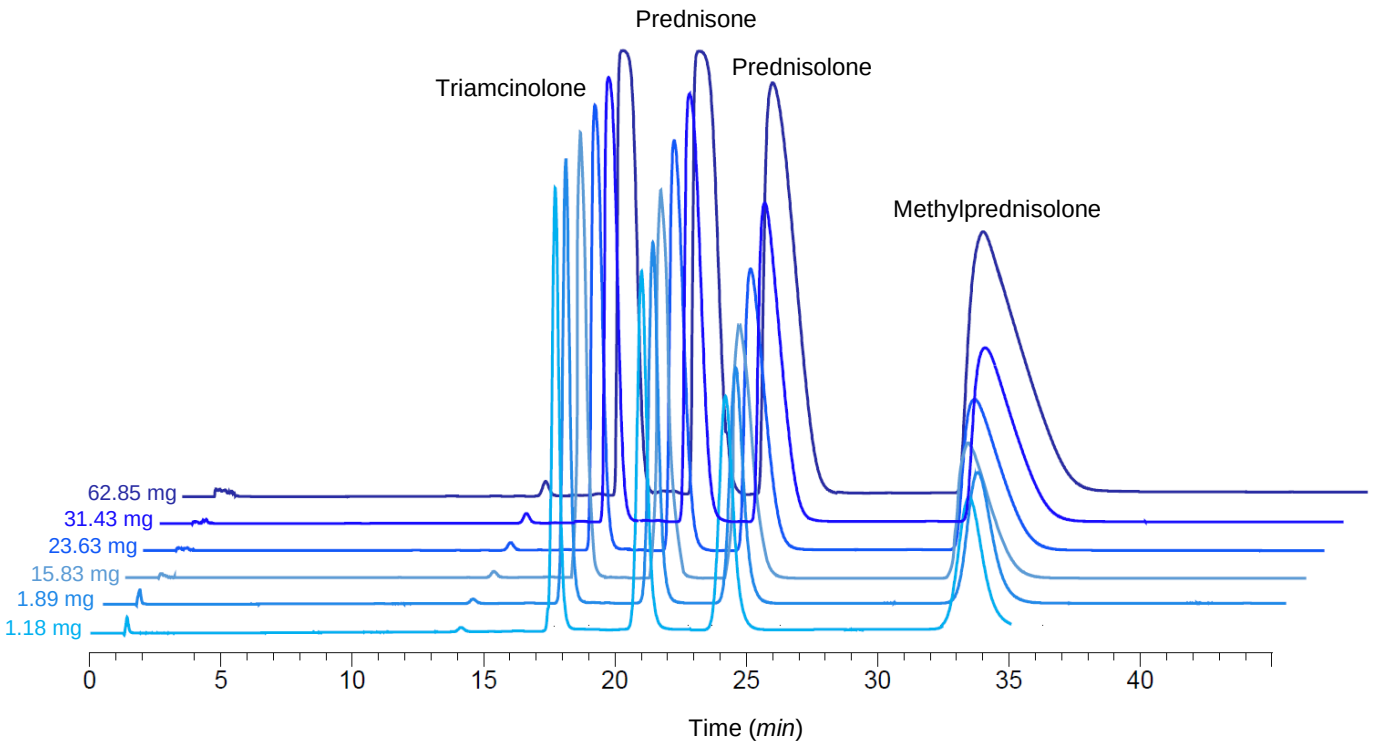
SEMI-PREPARATIVE SCALE (*10 mm ID COLUMN*)

The chromatographic conditions are presented in Table 3. The chromatograms obtained are presented in Figure 4.

Table 3: Semi-preparative chromatographic conditions

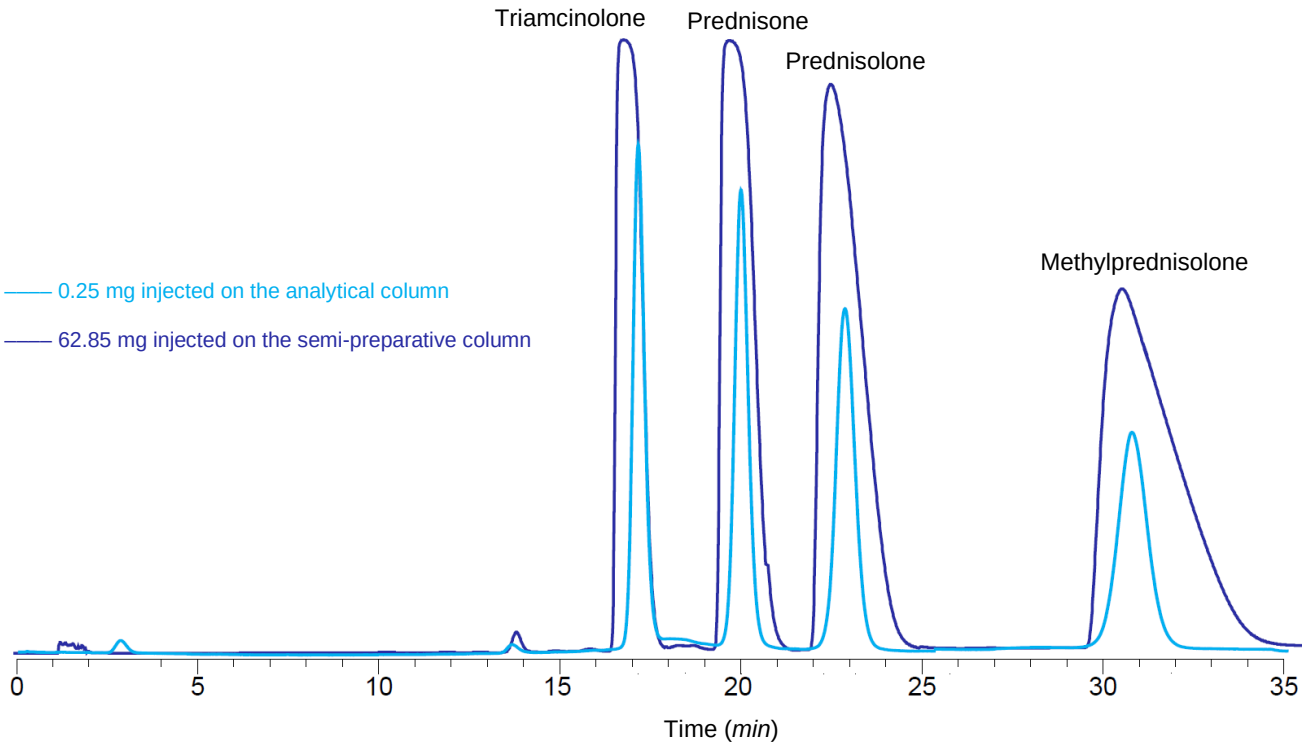
Semi-Preparative Chromatographic Conditions	
Parameter	**Value**
COLUMN	Silia*Chrom* Plus HPLC Column, C18, 10 x 250 mm, 10 µm, 100 Å
PART NUMBER	HPL-S03207E-A-Q250
MOBILE PHASE	Gradient Mobile phase A: water Mobile phase B: methanol 1. 80 % A to 50 % A (*15 minutes*) 2. 50 % A (*17 minutes*) 3. 80 % A (8 *minutes*)
FLOW RATE	9.45 mL/min
DETECTOR	245 nm
INJECTIONS (*in DMSO*)	1. 1.18 mg in 20 µL 2. 1.89 mg in 40 µL 3. 15.83 mg in 320 µL 4. 23.63 mg in 470 µL 5. 31.43 mg in 630 µL 6. 62.85 mg in 1,260 µL

Figure 4: Separation of the corticosteroids mixture with a Silia*Chrom* Plus C18 semi-preparative column



The retention times obtained for the corticosteroids on semi-preparative scale are similar to the retention times obtained on analytical scale. The differences can be explained by the different void volumes of the instruments used and different pumps (*quaternary pump for analytical and binary pump for the semi-preparative*). The similarities in retention time shows the scalability between Silia*Chrom* Plus HPLC columns of different internal diameters, where scale-up was nearly linear for the gradient separation (*Figure 5*).

Figure 5: Comparison of the separation of the corticosteroids mixture in analytical and semi-preparative formats



CONCLUSION

A mixture of corticosteroids was separated using a Silia*Chrom* Plus C18 analytical column and scaled-up on a Silia*Chrom* Plus C18 semi-preparative column. Similar retention times were obtained on the semi-preparative column, showing the nearly linear scalability of the HPLC columns.

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