HPLC Preparative Scaleup of Calcium Channel Blocker Pharmaceuticals

Application

Pharmaceuticals

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Abstract

Agilent Reversed Phase Prep columns demonstrated both good loadability and good scalability for calcium channel blockers using gradient conditions. The columns also displayed good resolution at all dimensions and allowed a linear scaleup from analytical to preparative dimensions.

Introduction

Calcium ions are essential in a variety of biological processes and are necessary for the electrical activity involved in the contraction of cardiac and smooth muscle and the conduction of nerve cells. Calcium channel blockers (also known as calcium antagonists, calcium entry-blockers and slow channel blockers) selectively inhibit calcium ion influx across cell membranes of both cardiac and vascular smooth muscle resulting in relaxation of blood vessel walls and cardiac muscle, allowing blood to flow more freely and lowering blood pressure. This action reduces oxygen demand in the heart and relieves anginal pain. Calcium channel blockers are used in the treatment of angina, cardiac arrhythmias, and hypertension.

Among the more important calcium channel blockers are those based on 1,4-dihydropyridine. Commercial versions of these compounds are similar in structure and are insoluble in water but soluble in acetonitrile; thus, they are good candidates for separation by reversed-phase chromatography. Figure 1 shows the structures of three of the more popular calcium antagonists manufactured by Bayer, Leverkusen, Germany.

Our goal in this study was to develop a preparative HPLC method to separate high milligram-to-gram quantities of the three calcium channel blocker compounds. Goals in preparative chromatography include maximizing throughput, purity, and yield. The scaleup process involves injecting larger amounts of material on column until the resolution becomes so poor that purity is compromised. There are two approaches to the scaleup process. The first is to scale up on an analytical column until resolution is barely acceptable (arbitrarily set at R_s <1.2); then, based on the preparative mass requirement, move to a suitably sized prep column; and adjust the conditions derived from the analytical scaleup to match the preparative column. The second approach is to choose the preparative column needed, then perform method development directly on this column until resolution and the resultant analyte purity is achieved. The first approach is preferred since it requires less solvent, sample, and time. If the preparative column has the same chemical composition as the analytical column, then the scaleup should be a linear process.

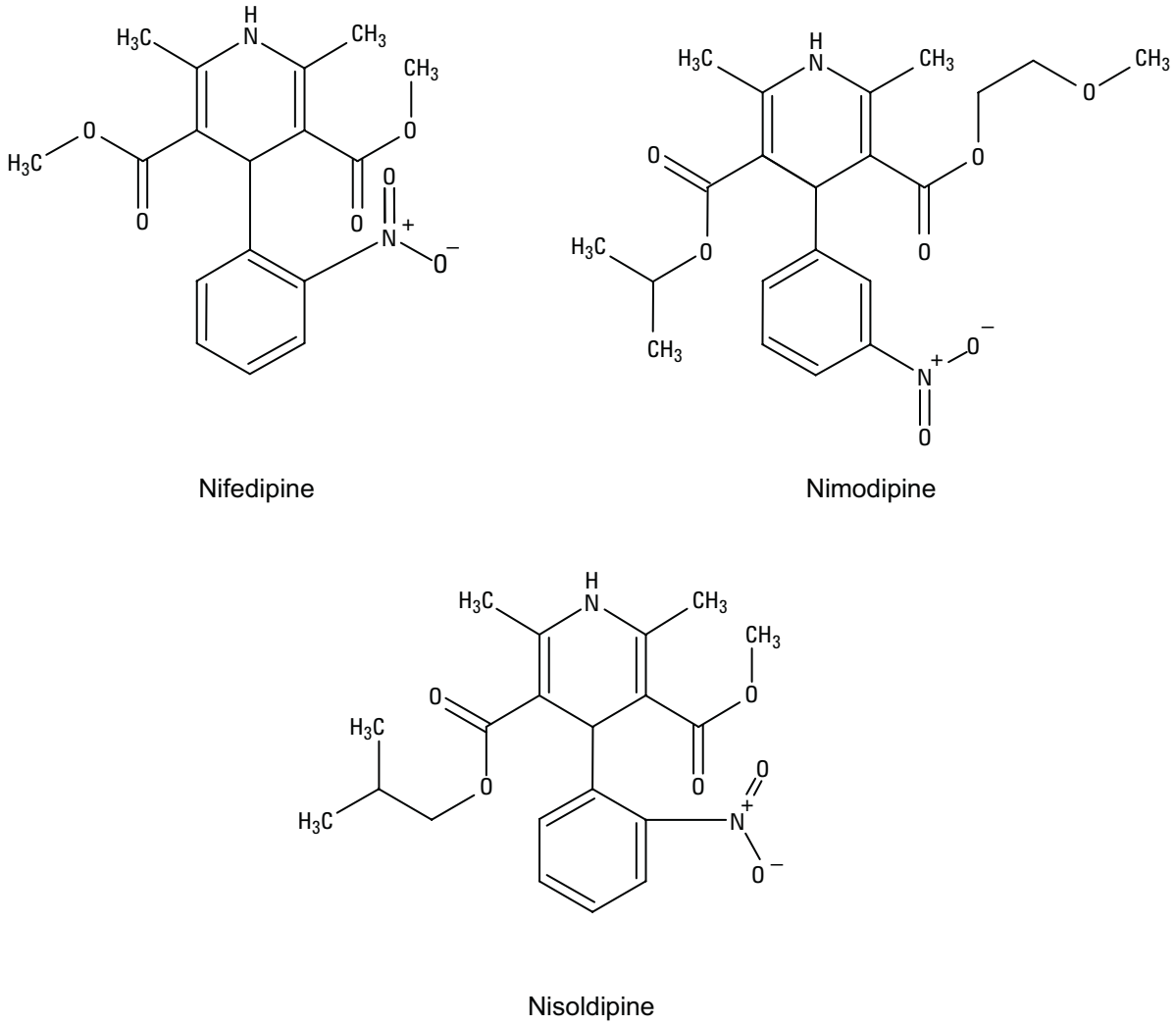

Figure 1. Structures of calcium channel blockers.

Our approach was to first develop a rapid analytical HPLC method on an optimized phase and then perform a linear scaleup of this method to a preparative column using the same packing material. For this study, we tested a number of reversed-phase analytical columns. We found that the Agilent Prep 5-µm Scalar column gave the best gradient separation within the time allotted for the separation (less than 10 minutes). The final analytical separation obtained on this column is depicted in Figure 2. The conditions for this experiment as well as the later experiments are depicted in Table 1. At analytical levels, resolution (R_s, determined by the ChemStation from the measurement of peak width at half height) of all three peaks was more than adequate, with the last two (critical pair) displaying an R_s value of 2.9. This resolution is adequate for a preparative scaleup since good resolution implies that a larger amount of sample may be injected before overloading occurs.

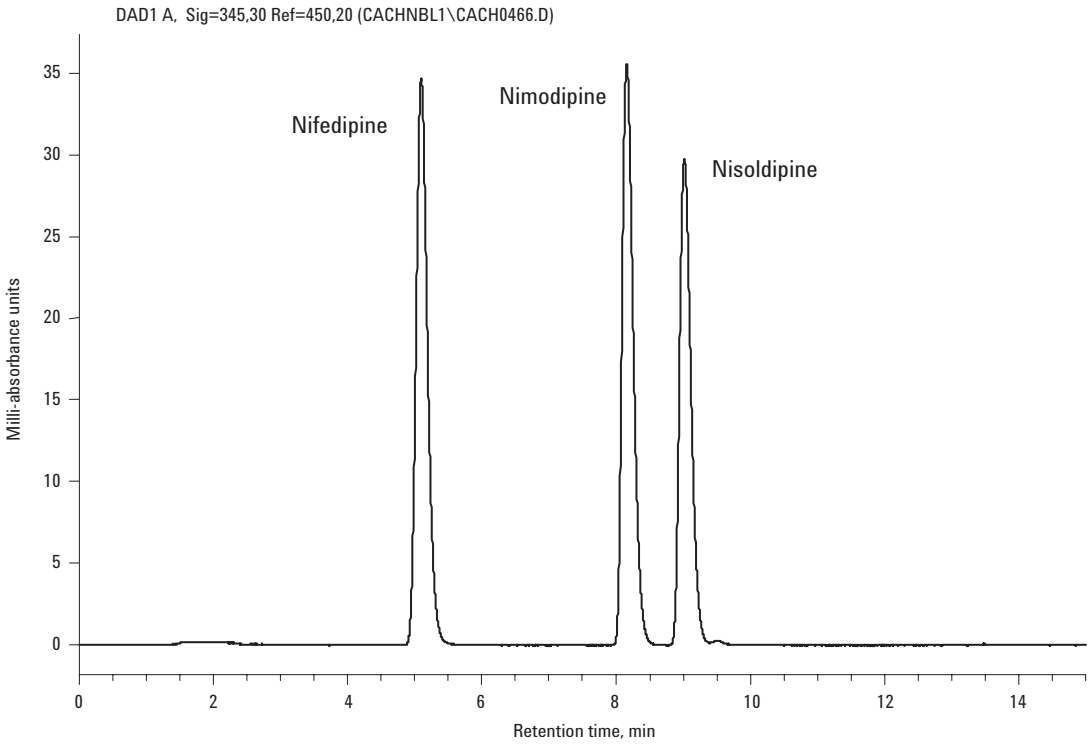

Figure 2. Analytical separation of calcium channel blockers on Agilent Scalar-C18 column, 5 µm, 4.6 × 100 mm.

Table 1. Agilent Prep-C18 Chromatographic Conditions for Calcium Channel Blockers

Mobile phase A:	Water + 0.1% formic acid (v/v)
Mobile phase B:	Acetonitrile + 0.1% formic acid (v/v)
Gradient:	Time 0 = 50% B 10 min = 75% B 12 min = 75% B 12.2 min = 50% B
Stop time:	15 min
Post time:	3 min
Flow:	0.7 mL/min, 4.6-mm id 14.87 mL/min, 21.2-mm id 29.74 mL/min, 30.0-mm id 85.7 mL/min, 50.8-mm id
Detector:	DAD: Signal = 345 nm, Bandwidth 30 Reference = 450 nm, Bandwidth 20
Flowcell:	0.06-mm pathlength
Injector loop:	4.6-mm id, no loop 21.2-mm id, 500-µL loop 30.0-mm id, 1500-µL loop 50.8-mm id, 5000-µL loop
Normal injection volume:	4.6-mm id, 25 µL 21.2-mm id, 500 µL 30.0-mm id, 1000 µL 50.8-mm id, 3000 µL

Injection volume for final (largest) amounts on column = 2× (Normal injection volume)

Sample: Nifedipine, Nimodipine, and Nisoldipine; 80 mg/mL each in neat DMSO

Theoretical Considerations in Preparative Scaleup

The sample capacity of an HPLC column is related to the mass of packing material, to the packing's surface area or bonded phase coverage, to the chromatographic conditions employed and to the chromatographic resolution of the critical analytes of interest. For a fixed chromatographic method with the same stationary phase and column length, the sample capacity is directly related to the mass of packing in a column as depicted in Equation 1.

$$x_2 = \frac{x_1 \bullet r_2^2 \bullet C_L}{r_1^2} \qquad (1)$$

where:
x_2 is the maximum amount (mass) of sample injected on column 2 (the larger id column)
x_1 is the maximum amount (mass) of sample injected onto column 1 (the smaller id column)
r_1 and r_2 are the radii of columns 1 and 2, respectively
C_L is the ratio of column lengths of column 2 to column 1 (in this case both lengths are the same)

It can easily be deduced that the sample mass that can be injected is directly proportional to the radius ratios squared. For a linear scaleup, the chromatographic flow conditions should be adjusted to reflect this ratio and maintain an equivalent linear velocity. For example, scaling up a separation done in a 4.6-mm id analytical column with a flow rate of 0.7 mL/min to an equivalent preparative column with a 21.2-mm id, the flow rate should be adjusted to $(21.2/4.6)^2 \times 0.7$ mL/min or to 14.87 mL/min to have the same separation time. In characterizing a column material for preparative possibilities there are two factors to consider:

- Loadability
- Scalability

These factors will be explained in the next two sections.

Column Loadability in the Preparative Separations of Calcium Antagonists

Loadability is defined as the maximum amount of analyte that can be injected onto a column that no longer permits the isolation of product at the desired level of purity or recovery levels, in other words, maximum sample capacity of a particular column for the analyte(s) of interest. In the reversed-phase mode for a column of fixed dimensions, loadability is affected mostly by bonded phase coverage, chromatographic resolution, and chromatographic conditions such as mobile phase, injection solvent, temperature, etc.

To test loadability in a preparative separation, progressively larger amounts of sample are injected onto a column until resolution becomes so poor that collection of pure analyte is rendered difficult. Typically, during loadability studies, column overloading eventually occurs. When overloading occurs peak shapes become skewed by fronting and/or tailing. Such behavior is typical of preparative separations and occurs even on high performance columns. To test the loadability of Agilent Prep columns, a stock solution mixture of 80-mg/mL of each pharmaceutical was prepared in dimethylsulfoxide (DMSO). DMSO is an ideal sample solvent for reversed-phase preparative chromatography since it readily dissolves most organic compounds, elutes from the column at/near the void volume and has low UV-absorbance [1]. A series of serial 1:1 dilutions of this stock solution was performed to prepare samples of known concentration. For each column tested, progressively larger amounts of each concentration was injected until resolution was impaired for the critical pair on analytes (arbitrarily set at R_s=1.2 for this study).

Figure 3 shows the loadability chromatograms for the 30.0 mm × 100-mm preparative column packed with 5-µm particles. Sample masses for total calcium antagonist from 2.0 mg to 0.5 g (0.66 mg to 170 mg each) are depicted. Only for the largest sample injection did noticeable peak skewing occur. The high sample capacity noted for the analytes is a result of a high C18 bonded phase coverage, good resolution and wide column diameter.

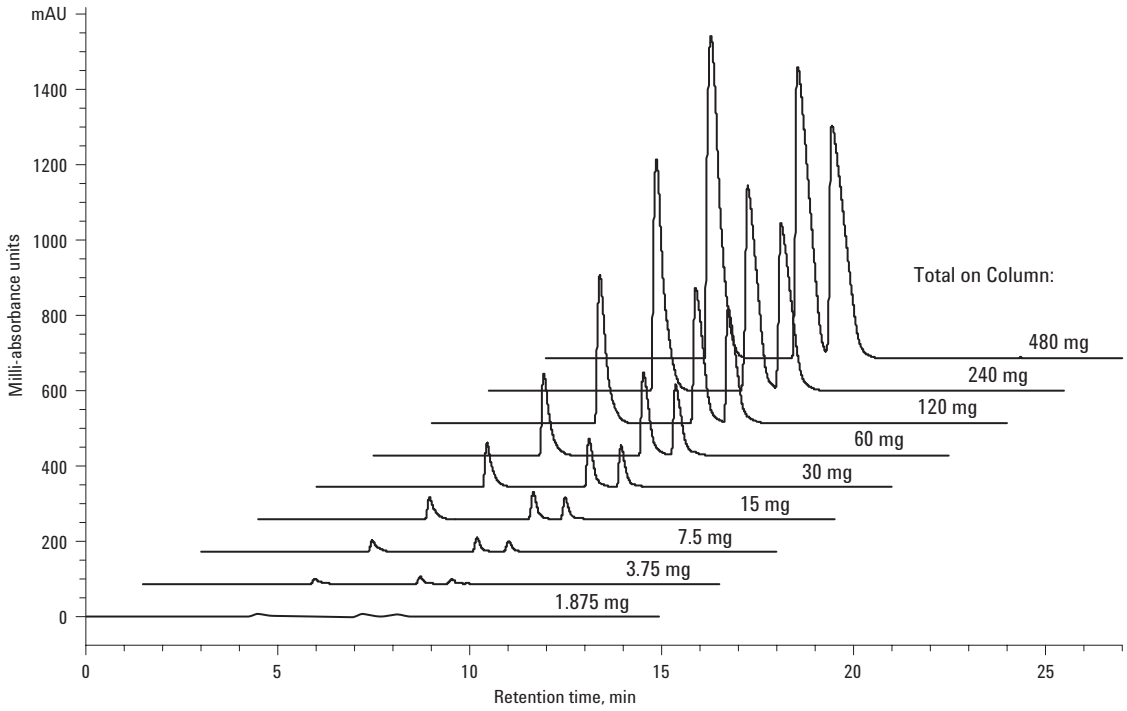

Figure 3. Loadability of calcium channel blockers on Agilent Prep-C18, 30 × 100 mm, 5 µm from 1.875 to 480 mg.

For the other column diameters, similar loadability profiles were obtained. To simplify the presentation of these results, Figure 4 shows a plot of resolution for peaks 2 and 3 (the critical pair) versus relative sample masses injected for the preparative columns (21.2-mm id and greater). These columns all had the same column length and the same chromatographic conditions were employed (for example, linear velocity, mobile phases, gradient, etc.).

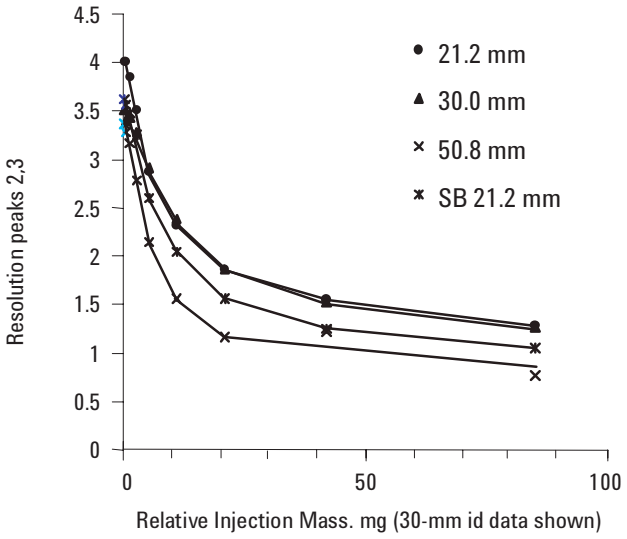

Figure 4. Resolution of critical pair for Agilent Prep* Columns as a function of loading.

*Also includes ZORBAX Stablebond (SB) C18 for comparison

Another stationary phase, Agilent ZORBAX StableBond (SB) C18 with a slightly different chemistry, was included for comparison. On the x-axis, only the masses injected onto the 30-mm id column is shown. For the other columns that mass per column injected was equivalent according to Equation 1.

As can be seen in Figure 4, as the sample size increases, resolution quickly decreases. Eventually around R_s = 1.2 peaks become severely overlapped so that individual peak purity is compromised. Obviously, even with poor resolution, use of heart-cutting techniques enables pure analyte to be obtained, but at the cost of yield. Note that the shape of all the resolution vs. loading curves is similar indicating that similar behavior of all column dimensions and phase types.

Scalability in Preparative Separations of Calcium Antagonists

The scalability of a chromatographic process is the reproducibility of results on columns of different internal diameters when using the same particle size and bonded phase. In order to assess scalability for the Agilent Prep columns, we determined the maximum loadability of total analyte for each column dimension; and then compared all four column dimensions to see if the performance was similar. Figure 5 shows the same relative injected masses, indicated on the chromatogram, on columns of 4.6-, 21.2-, 30- and 50.8-mm id, respectively, by 100-mm length. The curves indicate that up to 720 mg of total antagonist (that is, 240 mg each) could be injected onto the 50.8-mm id column and the R2,3 resolution was 1.22, still acceptable. At a higher loading, the resolution was less than 1, and the separation was deemed unacceptable.

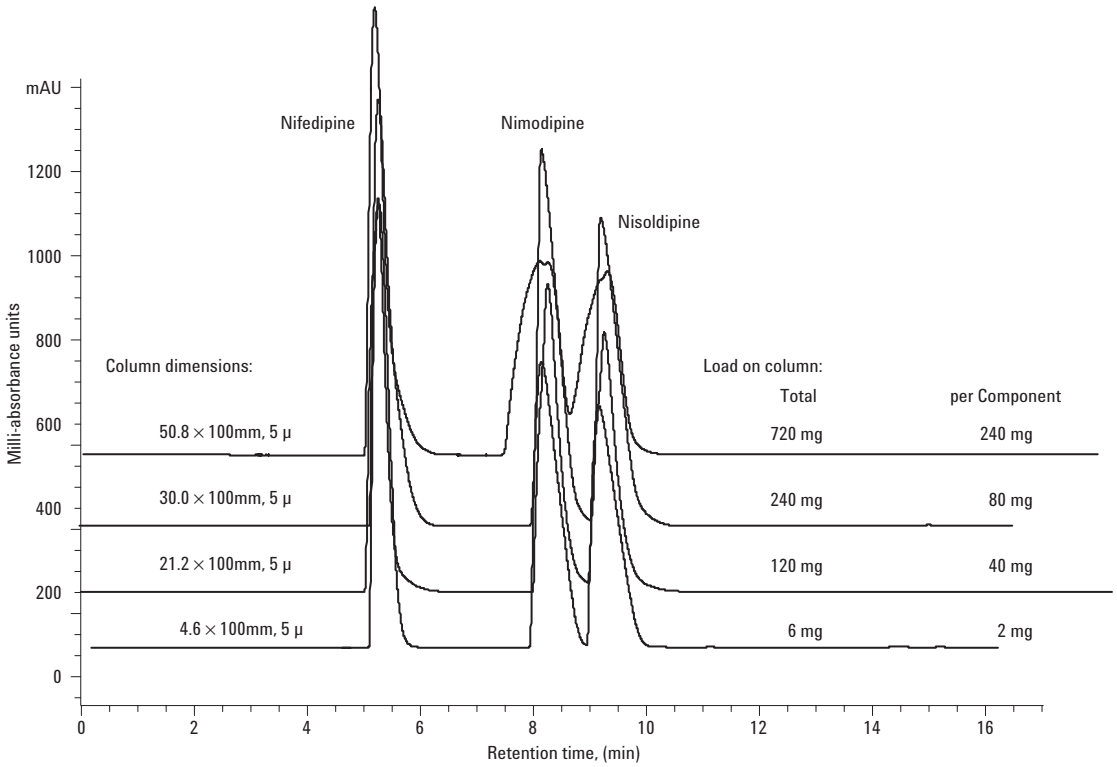

Figure 5. Scalability of Agilent Prep-C18 at high load for the analysis of calcium channel blockers.

Conclusion

It has been demonstrated that Agilent Reversed Phase Prep columns showed both good loadability and good scalability for calcium channel blockers using gradient conditions. The columns also displayed good resolution at all dimensions and allowed a linear scaleup from analytical to preparative dimensions.

Reference

1. A. Kaufmann and U. Jegle, (2005) "Using DMSO as an Injection Solvent to Increase Sample Load in Preparative LC", Agilent Technologies, publication number 5989-2485EN www.agilent.com/chem

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