Simplified Methods Development and Scale-up for Organic Synthesis using Segmented Column Technology

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Table 1: Flow Rate and Sample Load Increase Proportionally to Column Volume

Equation 1:

$$\frac{\text{Flow (prep)}}{\text{Flow (scaling)}} = \frac{\text{Volume (prep)}}{\text{Volume (scaling)}} = \frac{\text{Length (prep)} \bullet r^2\text{ (prep)}}{\text{Length (scaling)} \bullet r^2\text{ (scaling)}}$$

Equation 2:

$$\frac{\text{Mass (prep)}}{\text{Mass (scaling)}} = \frac{\text{Length (prep)} \bullet r^2\text{ (prep)}}{\text{Length (scaling)} \bullet r^2\text{ (scaling)}}$$

Table 2: Choice of Cartridge Columns Which Could be Used for the Separation of cis- and trans- isomers

Number of Cartridges	Column Volume (mls)	Flow Rate (ml/min)	Solvent Consumption (mls)	Load (mg)	Time of Analysis
1(8 x 100mm)	5.0	2.8	22.4	1.06	8.0
2 (8 x 100mm)	10.0	2.8	44.8	2.12	16.0
3 (8 x 100mm)	15.0	2.8	67.2	3.18	24.0
1 (25 x 100mm)	50.0	28.0	224.0	10.60	8.0
2 (25 x 100mm)	100.0	28.0	448.0	21.20	16.0
3 (25 x 100mm)	150.0	28.0	672.0	31.80	24.0
1 (40 x 100mm)	125.0	70.0	560.0	26.50	8.0
2 (40 x 100mm)	250.0	70.0	1120.0	52.88	16.0
3 (40 x 100mm)	375.0	70.0	1680.0	79.50	24.0

Shaded areas represent cartridge columns used in Figures 5&6. The sample load does not reflect the absolute capacity of the various cartridge columns.

Figure 1: *cis*- and *trans*- Isomers formed by Wadsworth-Emmons Reaction

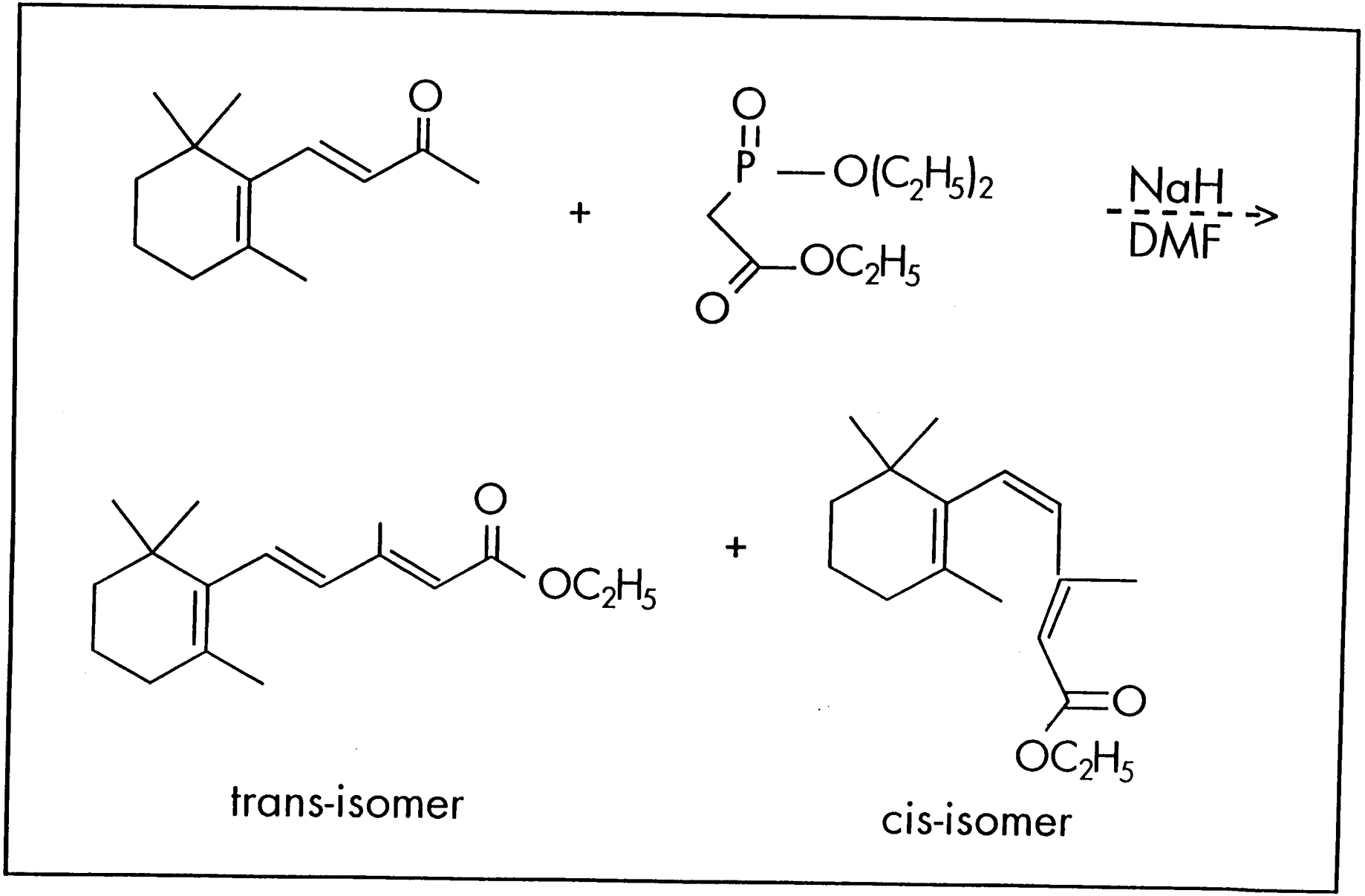


Figure 2: Analytical Separation of Crude Material

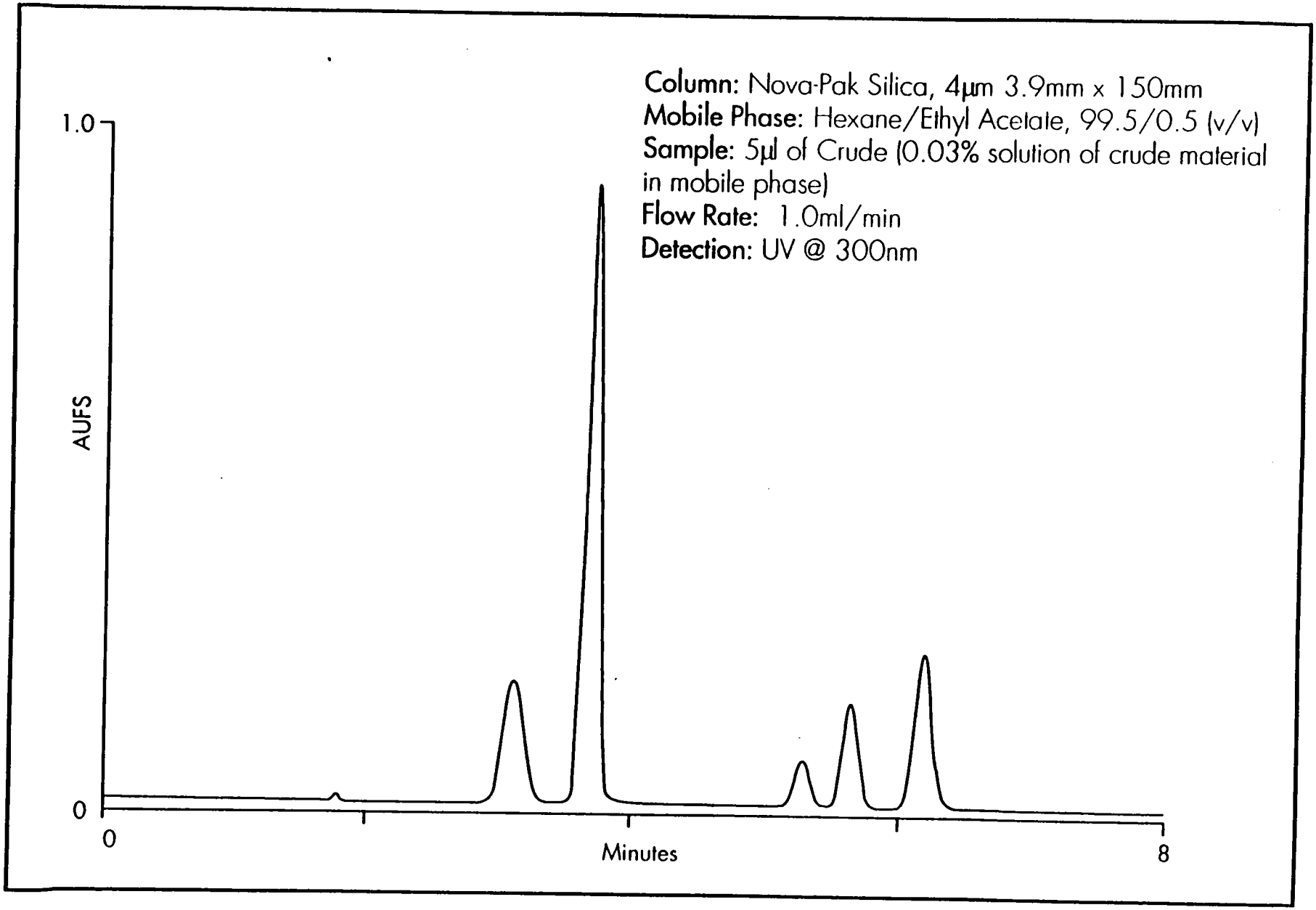


Figure 3: Comparison of Four Silica Packings

Mobile Phase: Hexane/Ethyl Acetate, 99.5/0.5 (v/v)
Sample: 2µl of Crude (20% solution of crude material in mobile phase)
Flow Rate: 2.8ml/min
Detection: UV @ 300nm

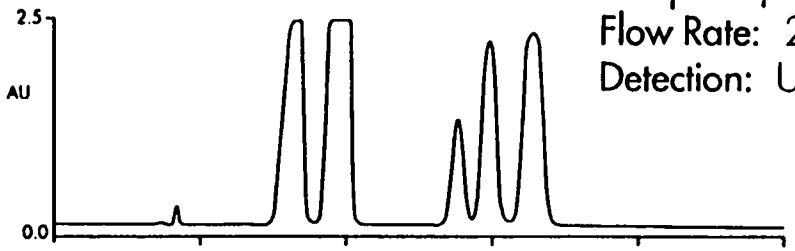


Figure 3A: Waters Nova-Pak Silica 6µm 8mm x 100mm Cartridge Column

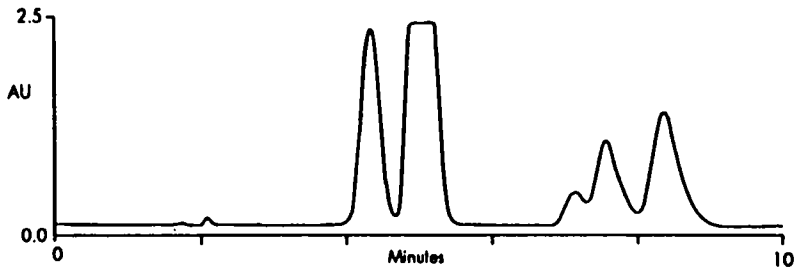


Figure 3B: Waters µPorasil™ 10µm 8mm x 100mm Cartridge Column

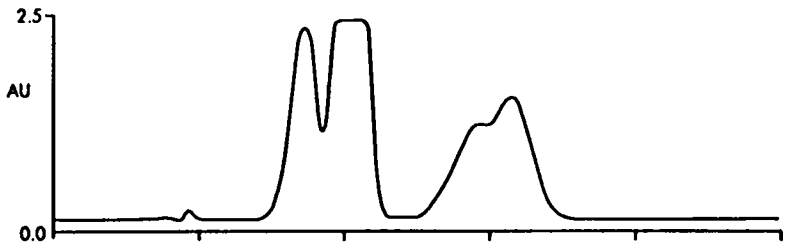


Figure 3C: Waters Porasil®15-20µm 8mm x 100mm Cartridge Column

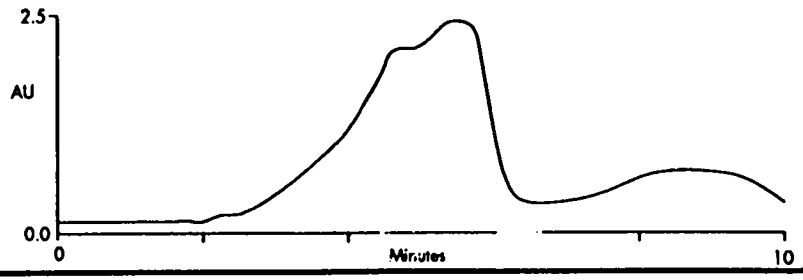


Figure 3D: Waters Porasil 37-55µm 8mm x 100mm Cartridge Column

Figure 4: Comparison of 100mm, 200mm and 300mm Cartridge Column Lengths

Mobile Phase: Hexane/Ethyl 99.5/0.5 (v/v)
Sample: 5µl of Crude (14.1 mg crude material/ml of mobile phase)
Flow Rate: 2.8 ml/min
Detection: UV @ 300nm

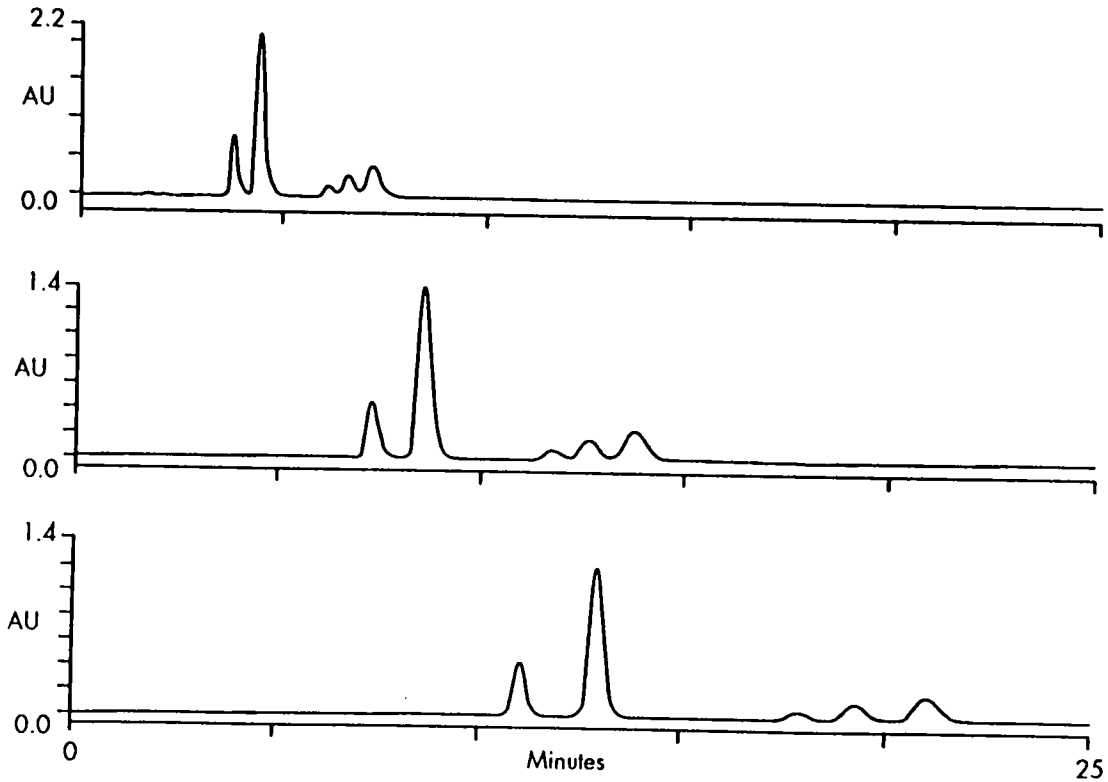


Figure 4A: Waters µPorasil 10µm, 8mm x 100mm Cartridge Column

Figure 4B: Waters µPorasil 10µm, 8mm x 200mm Cartridge Column

Figure 4C: Waters µPorasil 10µm, 8mm x 300mm Cartridge Column

Figure 5: Separation of Crude Material on a Scaling Column

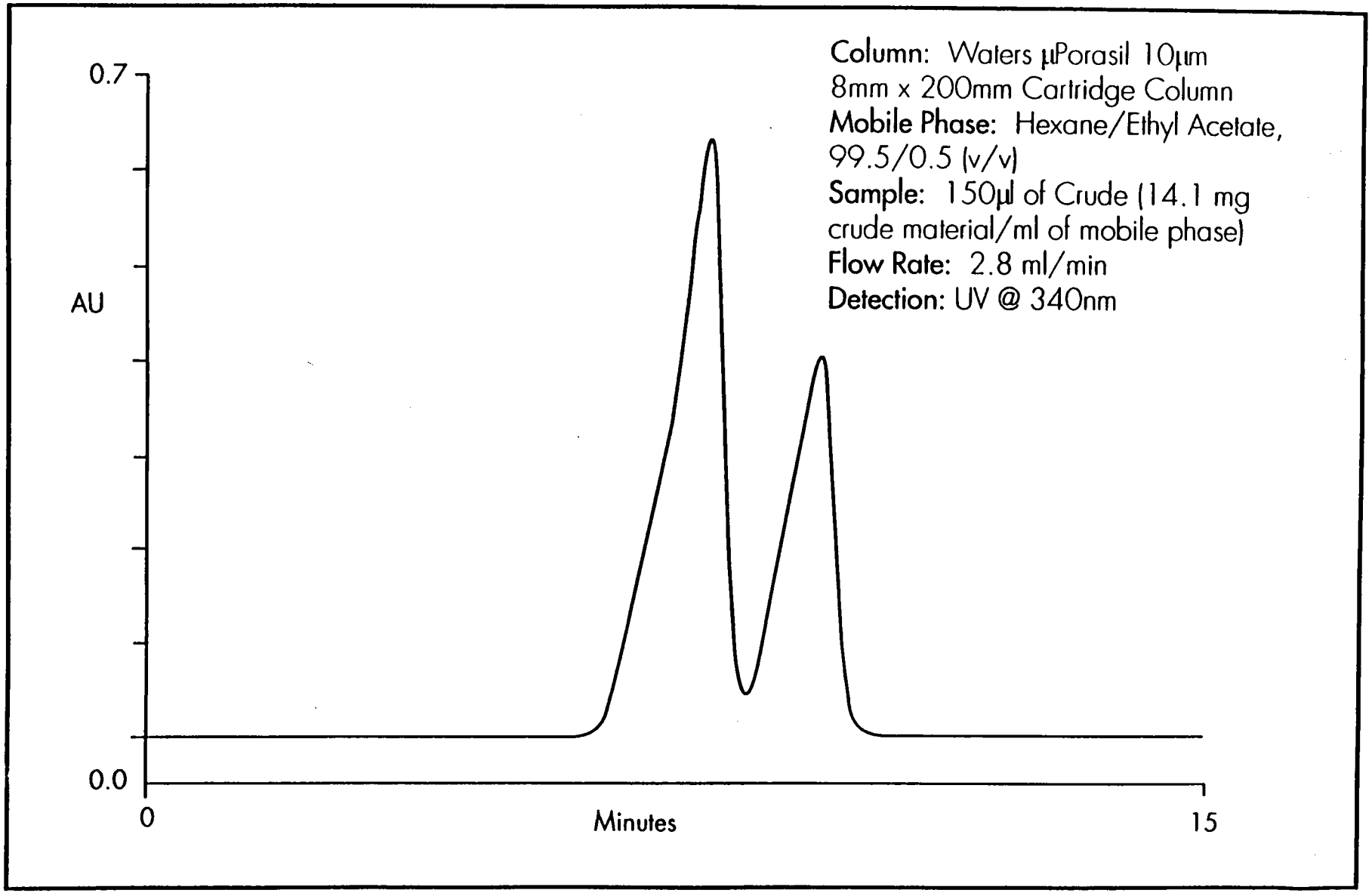


Figure 6: Preparative Separation of *cis* and *trans*-isomers

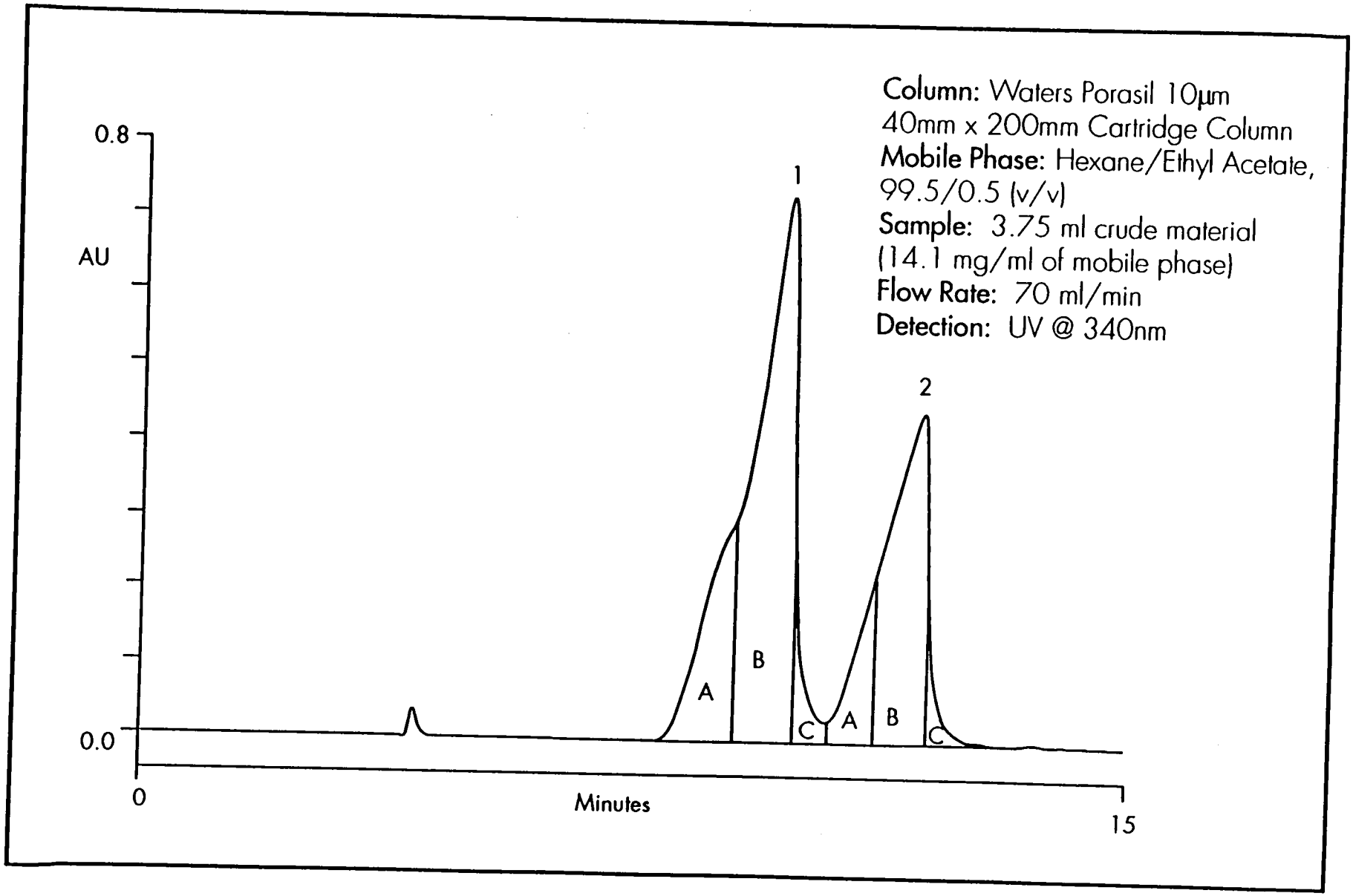
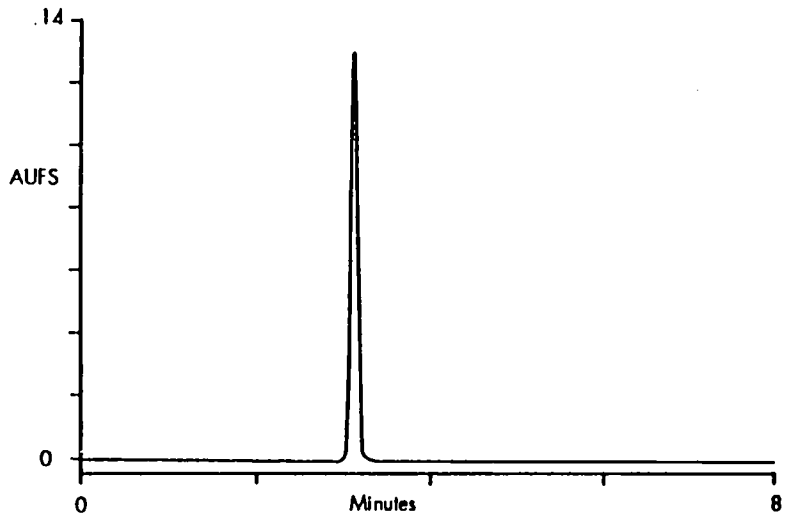


Figure 7: Analysis of Fractions from Preparative Separation



Column: Waters Nova-Pak Silica, 4µm, 3.9mm x 150mm Column
Mobile Phase: Hexane/Ethyl Acetate, 99.5/0.5 (v/v)
Sample: 5µl of collected fraction from preparative isolation
Flow Rate: 1.0ml/min
Detection: UV @ 300nm

Figure 7A: Fraction 1B from preparative separation

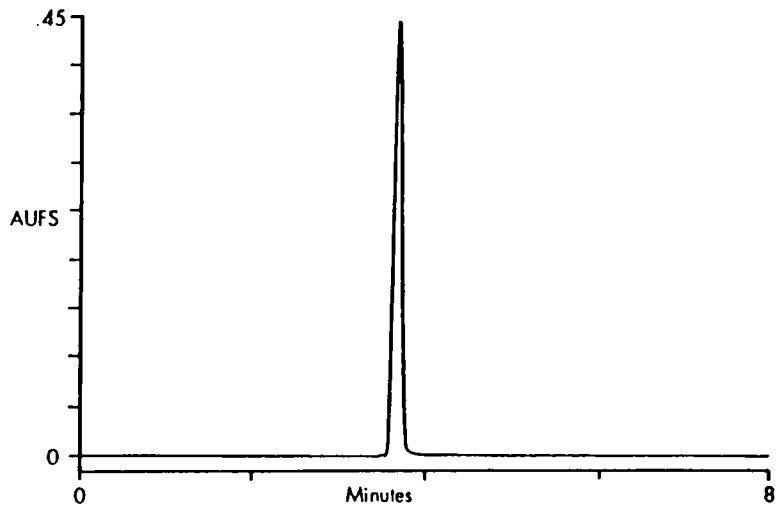


Figure 7B: Fraction 2B from preparative separation

Introduction

In order to increase the yield of a final product, many synthetic organic chemists need to purify reaction intermediates. The large scale purification of these synthetic intermediates is traditionally performed using flash chromatography. This methodology offers low resolution and can use excessive amounts of solvent. Presented here is an alternative methodology for the separation of reaction intermediates by HPLC using Segmented Column Technology (SCT).

SCT is a novel approach for preparative chromatography that permits column length and/or diameter to be varied in order to achieve increased resolution and/or capacity. Up to three 8mm, 25mm or 40mm I.D. cartridge columns or segments in 100mm lengths can be connected to optimize a separation and can conserve solvent. To demonstrate this simple and direct approach to the preparative isolation of reaction intermediates, the *cis*- and *trans*- products of a well characterized Wadsworth-Emmons reaction employed in the synthesis of many pharmaceutically interesting retenoids were purified using normal phase chromatography and SCT (Figure 1).

Analytical Separation

A separation was first developed on a high resolution Nova-Pak® Silica, 4µm, 3.9mm x 150mm analytical column, so that fractions ultimately collected from a preparative separation could be analyzed (Figure 2). The desired *cis*-isomer (peak 1) was well resolved from the *trans*- isomer (peak 2) and other reaction by-products (peaks 3-5). The analytical separation was then transferred to a preparative cartridge column. This transfer required the determination of which preparative silica packings should be used and the length and loading of the cartridge(s) required for this separation. In order to determine these parameters, 8mm I.D. scaling cartridges were utilized. Once the optimum conditions were determined, sample load and solvent consumption were decided by using several equations. The 8mm cartridge conditions could then be scaled to either 25mm or 40mm I.D. cartridges.

Preparative Silica Evaluation

Four different preparative silica packings, (6µm, 10µm, 15-20µm and 37-55µm), packed in 8mm x 100mm cartridges were evaluated to determine their ability to resolve the *cis*- and *trans*- isomers (Figure 3). Both the 6µm and the 10µm silica packings provided good resolution, whereas the 15-20µm and 37-55µm silica packings poorly resolved the pair for this length of cartridge column. The 10µm particle size silica was chosen to determine further scale-up parameters because it resolved the *cis*/*trans*-isomers and would provide a higher sample capacity than the 6µm silica.

Large Scale Purification

As predicted from Table 2, 52.88mg (3.75mls) could be loaded on the 40mm x 200mm cartridge column, resulting in the same chromatography (Figure 6). Fractions across the peaks of interest were collected, and aliquots were injected on the Nova-Pak Silica, 4µm, 3.9mm x 150mm analytical column to assess purity. Fractions 1A, 1B and 1C proved to be the *cis*-isomer while the remaining three were the *trans*-isomer (Figure 7).

Alternative Methodology

The pathway presented here represents only one way in which to perform this separation. The speed and capacity were optimized to offer a compromise between the two. Many other options exist when using Segmented Column Technology (SCT). For example, this separation could have been tailored to optimize speed or capacity. Using Equations 1 and 2 (Table 1) other options in column configuration can be employed (Table 2).

Determining Column Length: Optimizing the Separation to Suit Your Application

Injections were made on 100mm, 200mm and 300mm column lengths, using 8mm cartridge columns packed with 10µm silica to determine which would offer the largest sample capacity, resolution and fastest run time (Figure 4). The 100mm length offered a fast separation with low peak volumes but is limited in capacity. The 300mm length offered the greatest resolution and increased capacity but its long run time would use larger amounts of solvent and increase the peak volumes. The 200mm length was chosen for this application because it provided the best compromise between resolution of peaks of interest, separation time and solvent consumption.

Loading Study

A loading study was performed on the 8mm x 200mm cartridge column packed with 10µm silica to determine the maximum amount of crude sample the column could tolerate without compromising resolution of the *cis*- and *trans*- isomers. Once established, the flow rate and the maximum load on the scaling column could now be used to determine the conditions to be employed for any larger volume column packed with the same material. The sample load and flow rate were increased proportionally to the volume of the packed bed (Table 1). This results in identical chromatographic performance (retention times, peak widths and resolution) at the preparative scale as on the scaling cartridge column. The maximum sample load, on the scaling cartridge column, was 2.12 mg (Figure 5). Using equations 1 & 2, the flow rate and load were calculated for a 200mm length (two cartridge column configuration) with a 40mm diameter cartridge column.

Conclusion

The use of SCT offers the synthetic organic chemist a means to purify large amounts of material without the column defining the purification conditions. This separation was assessed by considering resolution, capacity, time and solvent consumption. The column length and packing material size were tailored to the separation and a capacity study was performed at the analytical scale. Having determined the optimum column length and capacity, the separation was scaled to a 40mm cartridge column with the same number of segments as in the scaling separation. Fractions collected from the preparative separation were analyzed by HPLC, confirming that pure products were obtained.

Acknowledgements

Sample courtesy of Hoffman LaRoche