Waters Lab Highlights

An Internal Communication of Applications and Techniques

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Scale Up Methods on the Delta-Prep™ 3000 Preparative Chromatography System

SCALE UP METHODOLOGY. Scaling up to large scale separations can be done successfully if performed in a logical sequence. To minimize solvent, sample, and time consumption the separation conditions are developed on a small scale and then transferred to the large scale separation. When identical packing materials are used, resolution can be maintained if; the linear velocities are equivalent, there are no decreases in the efficiency of the system, sample size is proportional to column volume, and in gradient elution, the separation occurs over the same number of void volume changes.

Linear velocity can be determined by measuring the time required for an unretained compound to traverse the length of the column. In scaling up, one uses the ratio of the column diameters squared to quickly get in the range of the correct flow rate. If exact equivalent linear velocities are required, a flow rate adjustment can be made (See FLOW RATE below).

Prediction of retention time on the large scale separation can be accomplished using the k' from the small scale methods development. In calculating k' it is important to consider any instrumental delay time.

APPLICATION. A synthetic peptide was separated by reverse phase techniques utilizing radial compression technology in the RCM-100® Radial Compression Module and the 1000 PrepPak® Module, and using the scale-up capabilities of Waters Delta Prep™ 3000 Preparative Chromatography system.

FLOW RATE. The flow rate for the large scale separation was calculated using the ratio of the column diameters squared to approximate the 76 mm/min. linear velocity on the small scale separation.

$$2.2 \text{ mL/min.} \times (57 \text{ cm}/8 \text{ cm})^2 = 112 \text{ mL/min.}$$

The 112 mL/min. flow rate gave a linear velocity of 84 mm/min. An adjustment was made to achieve the 76 mm/min. linear velocity desired.

$$112 \text{ mL/min.} \times 76 \text{ mm/min.}/84\text{mm/min.} = 101 \text{ mL/min.}$$

GRADIENT. The small scale gradient volume of 30 mL represented 10.2 void volume changes. An equivalent number of void volume changes on the large scale separation was accomplished with a 41 minute gradient duration at the 101 mL/min. preparative flow rate.

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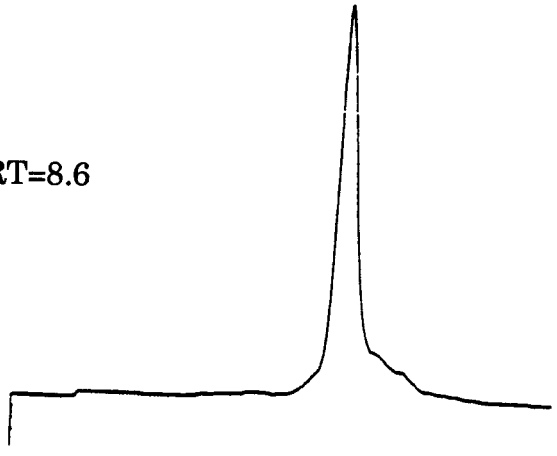
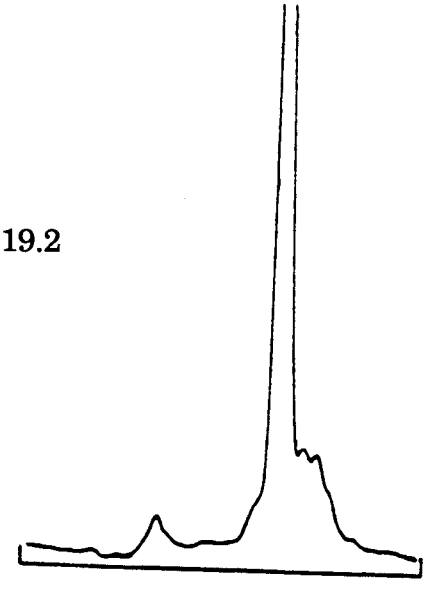
RETENTION TIME. After subtraction of the instrument delay time from the retention time of the major peak of LHRH in Figure 1, the k' was determined and used to predict the retention time of 19.3 minutes for the LHRH in the large scale separtion of Figure 2.

Figure 1. Small Scale Methods Development



Sample:	LHRH
Column:	Radial-Pak™ Cartridge custom packed with preparative C_{18} (55-105μ, 8 mm x 10 cm)
Eluent:	A) Water with 0.05% TFA B) ACN with 0.05% TFA
Gradient:	0-100% B, linear, 13.5 min.
Flow Rate:	2.2 mL/min.
Detector:	M441 @ 214 nm with semi-prep cell
Injection:	125 μg in 100 μL of A
Instrument:	Delta Prep™ 3000

Figure 2. Scaled-Up Separation



Sample:	LHRH
Column:	Prep-Pak® 500C_{18}Cartridge (55-105μ, 8 mm x 30 cm)
Eluent:	A) Water with 0.05% TFA B) ACN with 0.05% TFA
Gradient:	0-100% B, linear, 41 min.
Flow Rate:	101 mL/min.
Detector:	M441 @ 214 nm with semi-prep cell
Injection:	20 mg in 10 mL of A
Instrument:	Delta Prep™ 3000

In this case Figure 2 has better resolution since it has 3x plates (30 cm vs. 10 cm in Figure 1).

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