Efficiency test of ReadyToProcess columns

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Key words: Efficiency test, Theoretical plate, Asymmetry factor, Process-scale chromatography

Abstract

ReadyToProcess™ columns are prepacked, pre-qualified, and intended for immediate use. Every individual column is qualified by efficiency testing, which comprises analysis of theoretical plates per m packed bed (N/m) and asymmetry factor (As). Acceptance limits have been established for efficiency testing at 100 cm/h, which is a higher liquid velocity than that usually recommended in order to obtain the maximal calculated plate number/m packed bed. This application note compares the results obtained for different columns and sizes at 30 cm/h and 100 cm/h using a BioProcess™ system with a peristaltic pump.

Although the values obtained at 100 cm/h are lower than values obtained at 30 cm/h, the difference is predictable. The values obtained at 100 cm/h are directly comparable with those given in the certificates accompanying ReadyToProcess columns, and better reflect the efficiency during operation.

Introduction

ReadyToProcess columns are prepacked, pre-qualified, and pre-sanitized process chromatography columns suited for purification of biopharmaceuticals (e.g., proteins, vaccines, plasmids, and viruses) for clinical phase I and II studies. ReadyToProcess columns are available with several media at different volumes (2.5, 10, and 20 l).

The quality of packed columns is frequently expressed in terms of number of theoretical plates/m packed bed (N/m) and asymmetry factor (A_s). These values are calculated as follows:

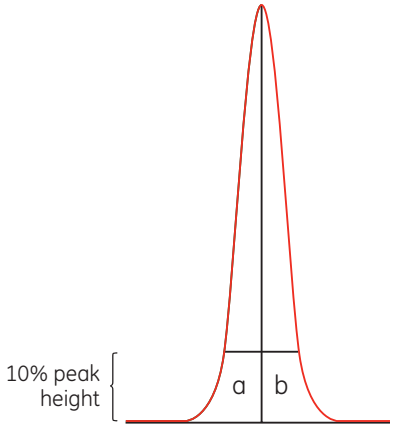

Fig 1. Typical test chromatogram showing asymmetry factor (A_s) value calculations.

Number of theoretical plates, N

$N = 5.54 \times (V_R/w_h)^2$, assuming a Gaussian peak

where

N = number of theoretical plates

V_R = peak retention (elution) volume or time

w_h = peak width at half height expressed in the same units as V_R

Asmmetry factor $A_s = b/a$

where

a is partial peak width, measured at 10% of the peak height for the leading part of the peak (Fig 1) and

b is partial peak width, measured at 10% of the peak height for the tailing part of the peak.

Experimentally, both values are easily determined in an efficiency test, by applying a tracer (sample) such as acetone. The calculated theoretical plates/m varies depending on the test conditions and should therefore be regarded as a reference value. To achieve comparable results, conditions and equipment should be kept as constant as possible since changes in buffers, sample volume, liquid velocities, liquid pathway, temperature, etc. will influence the result. For more information of principles for efficiency testing in general, see Reference 1.

To ensure a well-packed column, each individual ReadyToProcess column is qualified by analysis of theoretical plates/m packed bed and asymmetry factor as a part of the production procedure. Acceptance limits have been established for efficiency testing at 100 cm/h and the analysis results are given in a Certificate of Analysis accompanying each column. This is a higher liquid velocity than that normally recommended for optimal determination of theoretical plates/m packed bed (10–50 cm/h), and is a balance between better reflecting the running conditions used during separation, and the possibility to obtain reliable values. The values will differ between media due to the effect of the particle size; larger particles give a lower plate number.

This application note describes a reliable and reproducible efficiency test that can be employed by the user of ReadyToProcess columns.

Material and methods

The column to be tested was attached to a BioProcess system with a peristaltic pump and UNICORN™ software 5.11. The system configuration is described in Figure 2 and the method used is shown in Figure 3. The columns are designed for upwards flow. Three repeated runs were made on each column. Theoretical plates/m packed bed and asymmetry factor were calculated by using the peak integration function in the software evaluation mode.

Results and discussion

Theoretical plates/m packed bed and asymmetry factor values have been determined for ReadyToProcess columns packed with different separation media and with various volumes. The results obtained at a liquid velocity of 100 cm/h

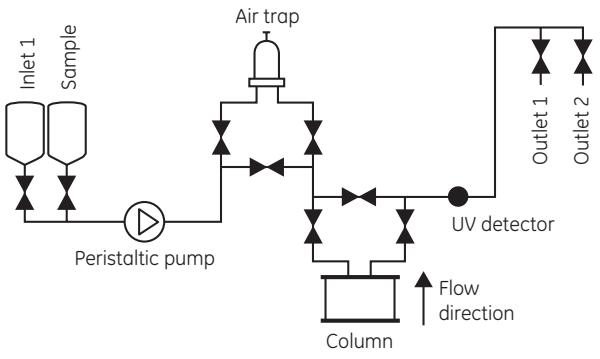

Fig 2. Schematic drawing of the system configuration.

Running conditions

Sample (Inlet Sample):	2% acetone (v/v) in distilled water
Eluent (Inlet 1):	Distilled water
Liquid velocity:	100 cm/h (upflow)
Column:	Equilibrated with distilled water

Running method

Step 1. Priming the system with sample

Inlet:	Sample
Outlet:	Waste
Air trap:	Bypass
Column:	Bypass
Duration:	Fill the system with sample (check UV signal)

Step 2. Apply sample, 1% of column volume, to column

Inlet:	Sample
Outlet:	Waste
Air trap:	Bypass
Column:	Inline
Duration:	1% of CV
Method base:	Time

Step 3. Wash out sample, fill the system with eluent

Inlet:	1
Outlet:	Waste
Air trap:	Bypass
Column:	Bypass
Duration:	Fill the system with eluent (check UV signal)

Step 4. Elute sample

Inlet:	1
Outlet:	Waste
Air trap:	Inline
Column:	Inline
Duration:	1.3 CV

Fig 3. Running conditions recommended for efficiency tests of ReadyToProcess columns.

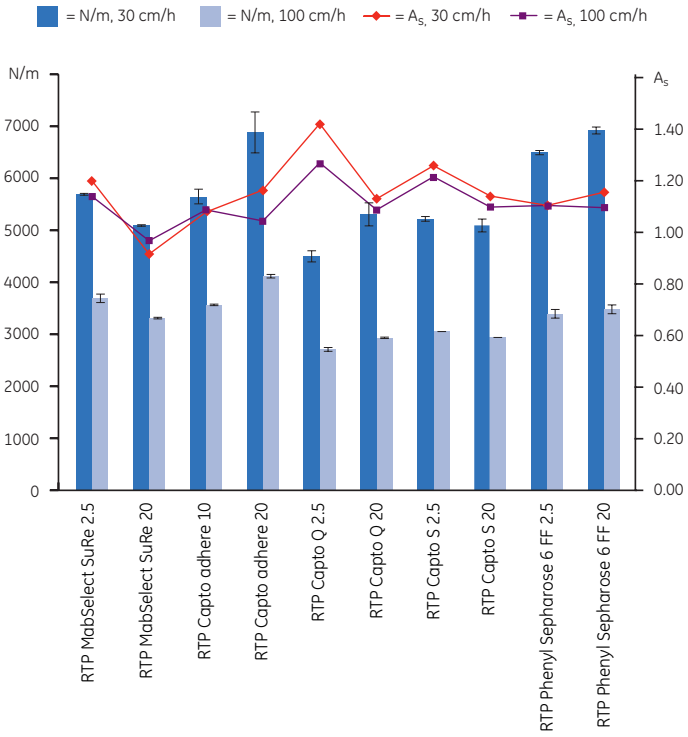

Fig 4. Average theoretical plates/m packed bed and asymmetry factor calculated for repeated runs performed on 10 ReadyToProcess columns at a liquid velocity of 30 and 100 cm/h respectively. The bars represent different column volumes, media, and natural variation in packing quality within specification. The standard deviation of asymmetry factor was lower than 0.06.

Table 1. Theoretical plates/m packed bed and asymmetry factor calculated for RTP Capto S 20

Liquid velocity (cm/h)	Theoretical plates/m packed bed (N/m)	Asymmetry factor (A_s)
30	5432	1.13
100	3371	1.08

have been compared with values obtained from equivalent experiments run at 30 cm/h, which is a normal liquid velocity for efficiency tests (Fig 4, Table 1).

When the test was performed at a liquid velocity of 100 cm/h, an increase in band broadening was seen when compared to the same test performed at 30 cm/h (Fig 5). The difference depends on mass transfer effects between the mobile phase and the stationary phase. The asymmetry factor is often improved at higher liquid velocities. This is probably due to a better liquid distribution over the packed bed and a symmetric broadening of the peak. It was found that the asymmetry factor is larger for small columns than for large ones. The system contributes with some asymmetry and the total asymmetry is proportionally larger for small columns. A larger sample volume, 2.5% of the column volume, will decrease the asymmetry factor, and is recommended for efficiency testing.

As expected, the plate numbers achieved at 100 cm/h differ from the plate numbers obtained at 30 cm/h. The values obtained at 100 cm/h were between 50% and 65% of values obtained at the lower liquid velocity (Fig 4, Table 2). This result is consistent for many separation media and column sizes.

Column: RTP Capto™ S 20, bed height 20 cm
Equilibration solvent: Distilled water
Sample: 2% acetone in distilled water
Sample load: 200 ml
Eluent: Distilled water
Liquid velocity: 30 or 100 cm/h
System: BioProcess system with peristaltic pump

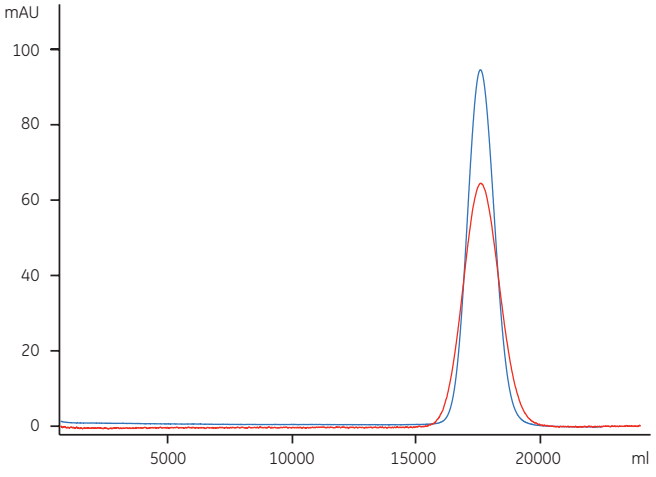

Fig 5. Sample peak with a liquid velocity of 30 cm/h (blue curve) and 100 cm/h (red curve).

Table 2. Ratio of theoretical plates/m packed bed obtained at 100 cm/h and 30 cm/h, respectively

Column	Ratio 100:30 cm/h flow rates (%)
RTP MabSelect SuRe™ 2.5	65
RTP MabSelect SuRe 20	65
RTP Phenyl Sepharose™ 6 FF (low sub) 2.5	52
RTP Phenyl Sepharose 6 FF (low sub) 20	50
RTP Capto Q 2.5	60
RTP Capto Q 20	56
RTP Capto S 2.5	58
RTP Capto S 20	59
RTP Capto adhere 10	63
RTP Capto adhere 20	60

Conclusions

This application note compares the results from efficiency testing of different ReadyToProcess columns and sizes obtained at 30 cm/h and 100 cm/h, respectively, using a BioProcess™ system with a peristaltic pump. The values obtained for theoretical plates/m at 100 cm/h are lower than the corresponding values obtained at 30 cm/h, but the variation is predictable. Generally, 100 cm/h gives better asymmetry (closer to 1.0). The data for 100 cm/h are representative for the values obtained in the column certificates for ReadyToProcess columns. Since the system contribution to the asymmetry factor value is diminished at higher sample volumes, production testing is performed with a larger sample volume, 2.5% and it is recommended to use this sample volume for similar measurements.

Reference

1. Sofer, G. and Hagel, L. Eds., *Handbook of process chromatography: A guide to optimization, scale-up and validation.* Academic Press, San Diego (1997).

Ordering information

Product	Column size	Code No.
RTP Capto Q 2.5	2.5 l	28-9017-23
RTP Capto Q 10	10 l	28-9017-24
RTP Capto Q 20	20 l	28-9017-25
RTP Capto S 2.5	2.5 l	28-9017-29
RTP Capto S 10	10 l	28-9017-30
RTP Capto S 20	20 l	28-9017-31
RTP Capto adhere 2.5	2.5 l	28-9017-14
RTP Capto adhere 10	10 l	28-9017-15
RTP Capto adhere 20	20 l	28-9017-16
RTP MabSelect SuRe 2.5	2.5 l	28-9017-17
RTP MabSelect SuRe 10	10 l	28-9017-18
RTP MabSelect SuRe 20	20 l	28-9017-19
RTP Phenyl Sepharose 6 FF (low sub) 2.5	2.5 l	28-9017-35
RTP Phenyl Sepharose 6 FF (low sub) 10	10 l	28-9017-36
RTP Phenyl Sepharose 6 FF (low sub) 20	20 l	28-9017-37

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