Lot-to-Lot Reproducibility Methodology—a Quality Tool for Column Characterization

Yury Agroskin, Harjit Gill, and Iqbal Hundal, Dionex Corporation, Sunnyvale, CA, USA

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ABSTRACT

The term lot-to-lot reproducibility is broadly used by customers and vendors to describe the quality of chromatographic columns. This reproducibility is particularly important in the pharmaceutical and environmental industries to develop robust analytical methods and to ensure product quality.

However, there is a lack of common understanding of the measurement and interpretation of this parameter. Conceptually, lot-to-lot reproducibility is easy to comprehend but a number of factors need to be defined. Here, we discuss a methodology for the calculation of this term based on a statistical analysis approach (ANOVA), and interpretation of the results in light of a column performance characterization. We demonstrate how customers can combine information about a column's specification and lot-to-lot reproducibility for the intelligent evaluation of the column's applicability in their applications.

INTRODUCTION

Column Reproducibility

Column Reproducibility (σ_0) is a part of any column quality characterization.

Column-to-column reproducibility (σ_{col}) represents:

- Column packing processes with separation media
- Hardware supplies

Lot-to-lot reproducibility (σ_{lot}) represents:

- Polymerization processes
- Raw material supplies
- Separation media properties
  - – Physical/chemical properties of bed support
  - – Chemical properties of particles' surface or latex

The total column reproducibility can be calculated by using Formula 1 below:

$$\sigma_0^2 = \sigma_{col}^2 + \sigma_{lot}^2 + \sigma_{er}^2 \qquad (1)$$

where σ_{er} is the measurement/experimental error.

Reproducibility Characterization

Tools for reproducibility characterization should be divided between column-to-column reproducibility and lot-to-lot reproducibility.

Column-to-Column Reproducibility

- Shelf life column stability
- IQC of column hardware (dimensional control)
- Functional chromatographic test:
  - – Backpressure (Pr_{iso})
  - – Efficiency (N_{iso})
  - – Asymmetry (As_{iso})
  - – Retention time (RT_{iso})

Lot-to-Lot Reproducibility

- In-process control
- IQC of raw materials
- Physical characterization of separation media:
  - – Particle size distribution
  - – Pore size distribution (for porous stationary phases)
- Functional chromatographic test:
  - – Backpressure (Pr_{grd})
  - – Efficiency (N_{grd})
  - – Resolution (R_{grd})
  - – Retention time (RT_{grd})

METHODOLOGY

Lot-to-lot reproducibility is defined as a variance component of the total column reproducibility derived from ANOVA analysis for each specification parameter. Two-way classification is applied for this purpose (see Table 1).

ANOVA is required for design of experiment (DOE) planning, or historical data can be used.

Table 1. ANOVA Analysis	
Source of Variance	**Random Effects**
Lot-to-Lot	$\sigma_{er}^2 + m^*\sigma_{lot}^2$ (2)
Column-to-Column	$\sigma_{er}^2 + n^*\sigma_{col}^2$ (3)
Interaction (Column × Lot)	none
Error	σ_{er}^2

- Two sources of variance are considered:
  - Lot effect (DF = n, where n is number of lots under evaluation)
  - Column effect (DF = m, where m is number of columns per given lot)
- All effects are considered random.
- Each specification parameter is treated separately.
- Only columns that have passed specification requirements should be used in the study.

LOT-TO-LOT REPRODUCIBILITY DETERMINATION FOR THE PROPAC WCX-10 COLUMN

Here, we propose a method for the determination of lot-to-lot reproducibility by using the Dionex ProPac® WCX-10 column. The following conditions and tools were applied for this study:

- MiniTab 15 statistical software
- 9 years manufacturing data for ProPac WCX-10 columns
- Data pool: 97 lots, 343 columns
- All columns went through a vigorous QC process (Figure 1) and met the specification requirements for the resin qualification.

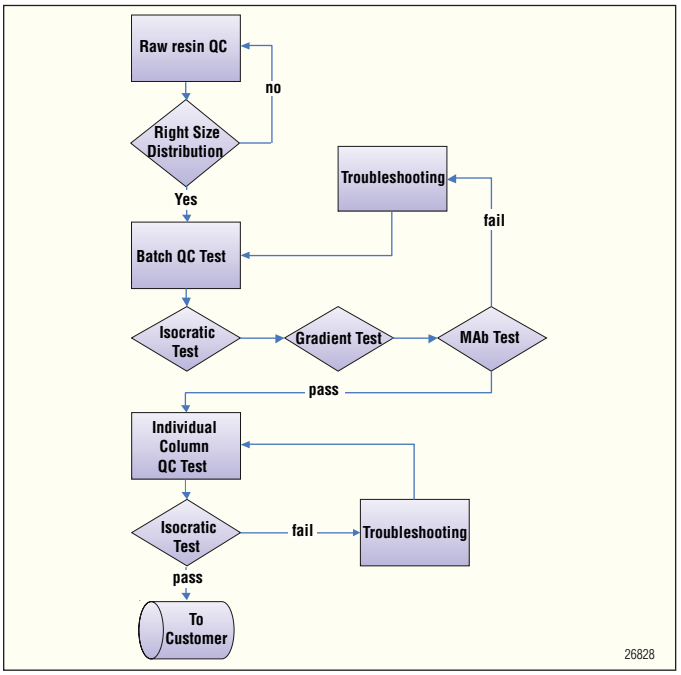

Figure 1. Dionex QC process.

Chromatographic Tests

Two chromatographic tests were conducted to ensure the column quality of the ProPac WCX-10 column:

Isocratic Test (Figure 2)

- Prime purpose: to control resin packing process.
- Parameters of interest: backpressure, retention time, and efficiency.
- Sample: cytochrome C.

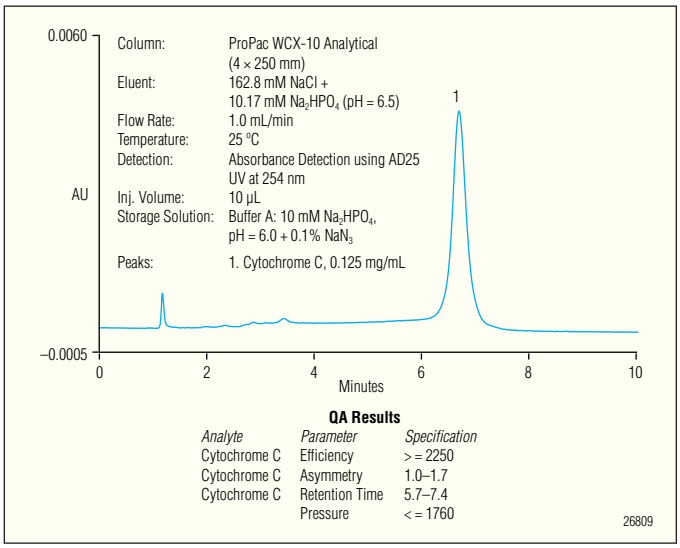

Figure 2. An isocratic test for the ProPac WCX-10 column.

Gradient Test (Figure 3)

- Prime purpose: to control resin chemistry and polymerization process.
- Parameters of interest: resolution and retention time.
- Sample: protein mixture of cytochrome C, ribonuclease A, and lysozyme.

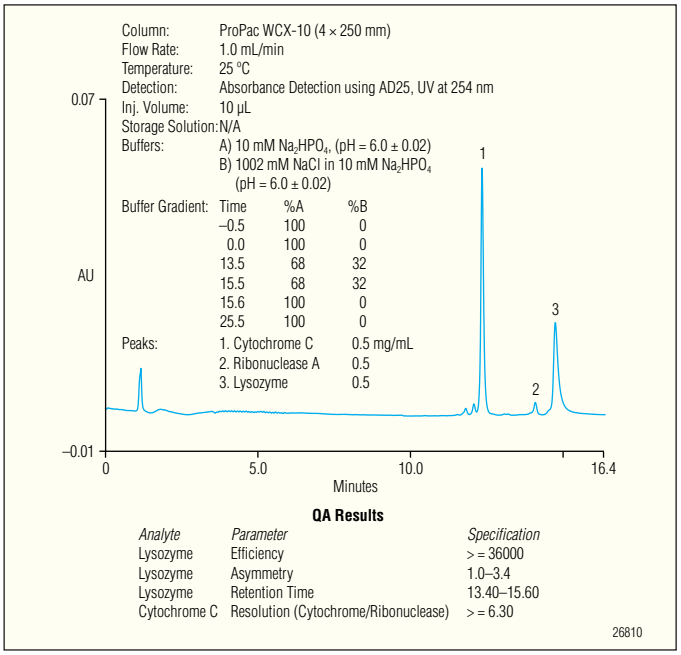

Figure 3. A gradient test for the ProPac WCX-10 column.

Parameters of the functional chromatographic tests described below are used for reproducibility calculation:

- Backpressure (Pr_{iso})
- Efficiency (N_{iso})
- Retention time (RT_{grd})
- Retention time (RT_{iso})
- Resolution (R_{grd})

CONTROL CHARTS: PROPAC WCX-10 MANUFACTURING

A useful tool for visual evaluation of variation patterns in manufacturing is a control chart. Figures 4–8 show the control charts for five specification parameters of the ProPac WCX-10 column. The control charts represent an average parameter value for a given lot over a period of time. Each data point is an average of the test results of 3–5 columns.

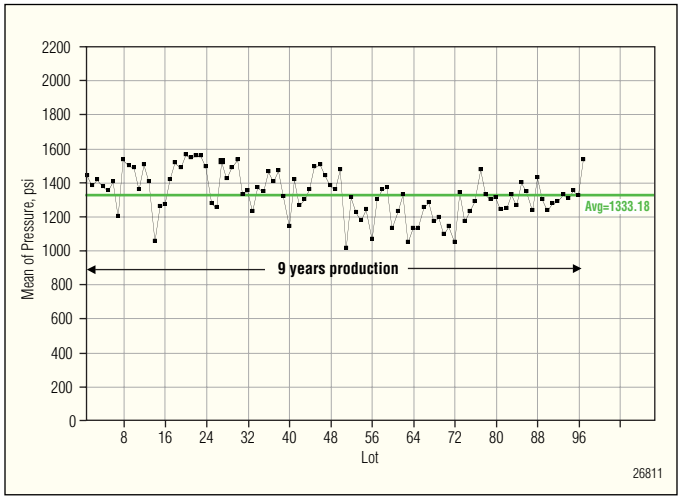

Figure 4. Control chart of backpressure: isocratic test of ProPac WCX-10 column.

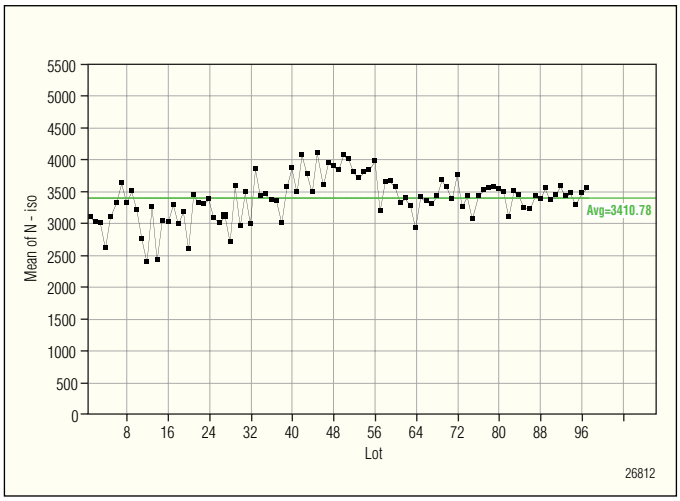

Figure 5. Control chart of cytochrome C efficiency: isocratic test of the ProPac WCX-10 column.

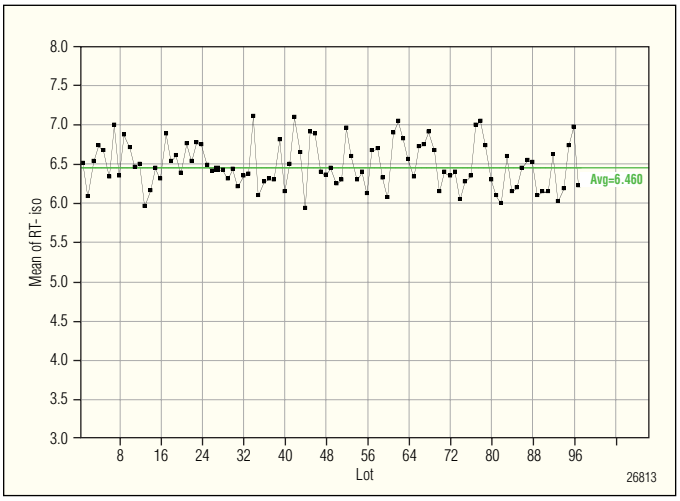

Figure 6. Control chart of cytochrome C retention time: isocratic test of ProPac WCX-10 column.

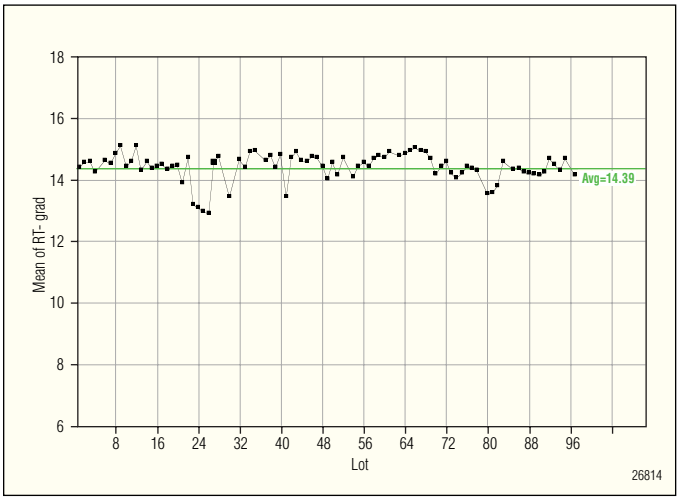

Figure 7. Control chart of lysozyme retention time: gradient test of ProPac WCX-10 column.

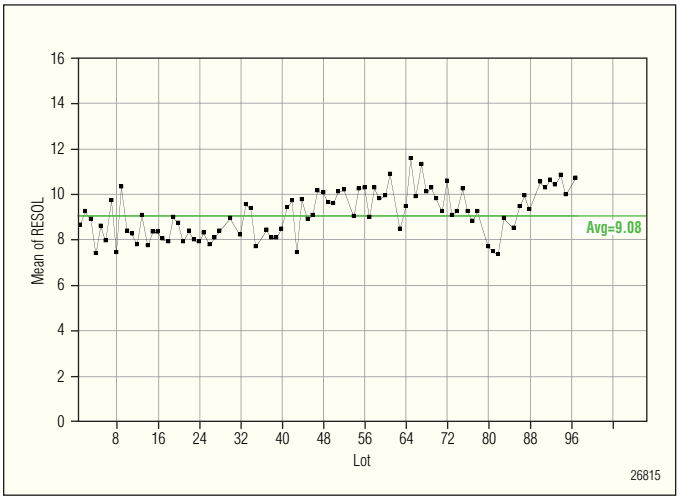

Figure 8. Control chart of resolution: gradient test of ProPac WCX-10 column.

CALCULATION OF LOT-TO-LOT REPRODUCIBILITY

Here, we present an example of the calculation of lot-to-lot reproducibility for the isocratic retention time (CytC) - RT_{iso}. The calculation is based on historical data and ANOVA analysis.

Relative standard deviation (RSD) is a convenient way to describe lot-to-lot reproducibility:

$$RSD_{lot} = (SD_{lot} / Mean) * 100, \% \quad (4)$$

where $SD_{lot} = (\sigma_{col}^2)^{1/2}$

The mean can be calculated based on a statistical distribution (Figure 9) for a given specification parameter → Mean(RT_{iso}) = 6.46 min.

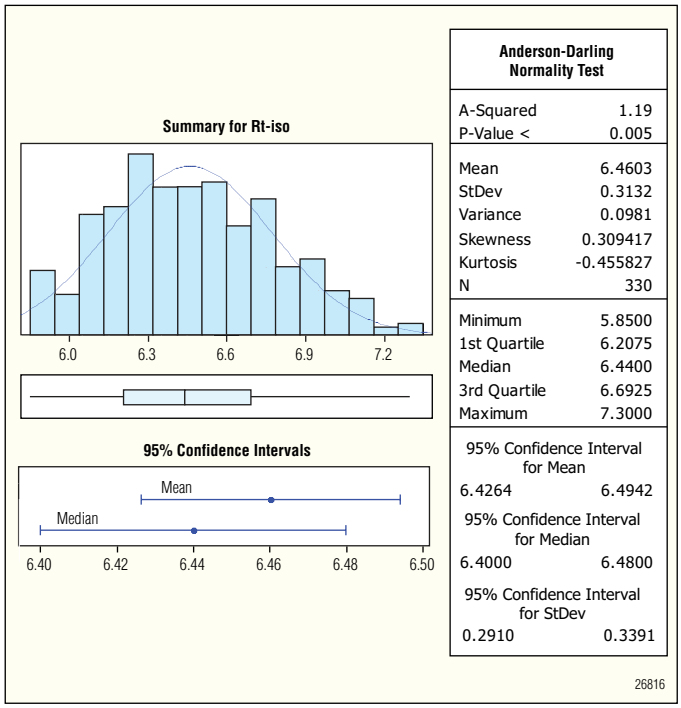

Figure 9. Statistical distribution: retention time (isocratic).

To define SD_{lot}, a two-way classification ANOVA should be applied (Table 2) → SD_{lot} = 0.26 min.

Table 2. Two-Way Classification ANOVA for Isocratic Retention Time			
Source of Variance	**Variance Component**	**% of Total**	**SD**
Lot-to-Lot	0.07	71.4	0.26
Column-to-Column	0.014	14.3	0.12
Error	0.014	14.3	0.12
Total	0.098		0.31

Using Formula 4: RSD_{lot} (RT_{iso}) = (0.26 / 6.46) * 100 = 4.1%

Table 3 summarizes ProPac WCX-10 lot-to-lot reproducibility for all five chromatographic parameters. As observed, RSD values are in a narrow range, indicating high-quality manufacturing.

Table 3. Lot-to-Lot Reproducibility for ProPac WCX-10 Column			
Parameter	**Mean**	**SD**	**RSD%**
Backpressure (Pr_{iso})	1334	130	9.8
Retention time (RT_{iso})	6.46	0.265	4.1
Efficiency (N_{iso})	3410	345	10.1
Retention time (RT_{grd})	14.4	0.33	2.3
Resolution (R_{grd})	9.13	0.96	10.5

CONSIDERATIONS FOR METHOD DEVELOPMENT

Once lot-to-lot reproducibility for any column product is calculated, it is important to evaluate the total column effect on analytical method development. Some considerations should be implemented in the development process:

- Any method development should be based on a column's manufacturer specification and not on a particular column's performance.
- If a relationship between the method characteristics (y_j) and the column's specification parameters (x_i) could be established, Formula 5 can be written as follows:

$$y_j = F\ (x_1, x_2, \ldots\ldots x_n) \qquad (5)$$

In this case, the method's variance is defined as a function of Formula 5 below:

$$\sigma_y^2 = \sum (\partial\ F/\partial\ x_i)^2 \qquad (6)$$

- If the relationship (Formula 5) could not be established, it is advisable to expect that the method's variance (σ_y^2) is equal to or higher than the column's reproducibility as shown below:

$$\sigma_y^2 \geq max\ [\sigma_{xi}^2] \qquad (7)$$

CONCLUSIONS

- The methodology of column quality, in general, and lot-to-lot reproducibility, in particular, is discussed.
- Lot-to-lot reproducibility for a chromatographic column is defined in precise quantitative terms.
- An easy method to calculate column variance is demonstrated based on ANOVA analysis.
- An example of the determination of lot-to-lot reproducibility is presented for the ProPac WCX-10 column.
- Important considerations for chromatographic method development are discussed in light of column characteristics.

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America
U.S./Canada (847) 295-7500

South America
Brazil (55) 11 3731 5140

Europe
Austria (43) 1 616 51 25 Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific
Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

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