What are the **KEY FOCUS AREAS?**

in Pharma Lab

Pharma Lab Leaders Survey

Overall goals for the future in the quest to "*improve human health*"

650 **Laboratories** in **big pharma**, **biotech** and **contract research** organizations

07 **Countries** across Europe, Asia and the USA

3 types of senior lab leaders including laboratory manager, director and supervisor

Primary Concerns of Pharma Lab Leader are:-

55%

achieving quicker results

44%

superior quality

43%

data integrity

and

83%

find that their current workflow requires optimization

Achieving quicker results

is the number #1 concern for pharma lab leaders **(55%)**

65% say the most important type of laboratory innovations are those that **increase efficiency**

38% of those who work with generic medicines say one of the most common challenges is the **increased demand to get these medicines to market quickly**

Organization needs:-

- Increased capacity
- Shorter time to market
- Increased profitability

Laboratory needs:-

- Better use of resources
- Increase productivity
- Reduce costs

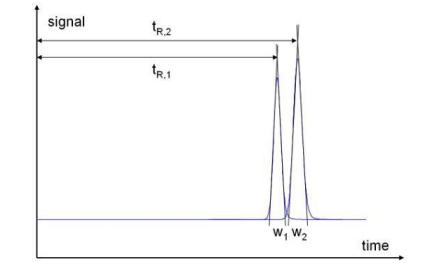
Achieving better results is more crucial than ever before with increased performance expectations placed on laboratory technologies and innovations

41% cite innovations that **improve instrument performance and sensitivity** as being one of the most important innovations for their labs

85% said that they are looking to buy **more sophisticated instruments with a greater degree of specificity,** in order to progress medicines through the pipeline more quickly

Translation in actions:-

- Maximize ALL the Instruments in the Lab
- Faster Analysis with Good Resolution
- More Analysis & Longer Column Lifetime



Impact of USP <621> Guidance on Laboratory Efficiency (HPLC)

What else do you need to know?

Wimut Sermsri
Business Development Specialist
Chemistries and Supplies Division
Agilent Technologies

DE56373591

Agilent
Trusted Answers

DE56373591

Understanding USP <621>

Increasing Productivity & Reducing Operational Cost

Achieving quicker results
is the number #1 concern for pharma lab leaders **(55%)**

65% say the most important type of laboratory innovations are those that **increase efficiency**

- Increase manpower
- Increase instrument
- Save cost

20 mins Runtime

Adjust

5 mins Runtime

What is Chapter <621> All About?

Chapter <621> provides the definition of allowable adjustments to chromatography systems in order to meet system suitability (SS) requirements.

USP-NF July 31, 2019

USP-NF to be updated Dec 1, 2022

⟨621⟩ CHROMATOGRAPHY

INTRODUCTION

Chromatographic separation techniques are multistage separation methods in which the components of a sample are distributed between two phases, of which one is stationary and the other is mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, distributed as a film, or applied by other techniques. The mobile phase may be in a gaseous or liquid form, or a supercritical fluid. The separation may be based on adsorption, mass distribution (partition), or ion exchange; or it may be based on differences among the physicochemical properties of the molecules, such as size, mass, and volume. This chapter contains general procedures, definitions, and calculations of common parameters and describes general requirements for system suitability. The types of chromatography useful in qualitative and quantitative analyses employed in *USP* procedures are column, gas (GC), paper, thin-layer (TLC) [including high-performance thin-layer chromatography (HPTLC)], and pressurized liquid chromatography [commonly called high-pressure or high-performance liquid chromatography (HPLC)].

200 usp The standard of trust

Concerns!

- The USP has specified the limits of chromatography allowable adjustments in the general chapter <621>
- Adjustments made to chromatography systems **within** the limits of chapter <621> **do not need method revalidation**
- After adjustments system suitability (SS) test rules still apply
- Modifications **outside these limits** are considered “changes” and **require revalidation**.
- Changes to the “L designation” of the column chemistry requires revalidation.

Why are adjustments allowed?

- Adjustments may be necessary to meet system suitability requirements
- Adjustment could be desirable due to the fact that not all methods in the USP are modern technology (hence, implementing modern technology to improve current chromatographic methods)

https://hmc.usp.org/system/files/general-chapters/GC-Pdfs_2019/_621_%20CHROMATOGRAPHY.pdf

Allowable Adjustments

What is NEW?

Variable	Isocratic	Gradient
Particle Size	Per constant L/dp or N; -25% to + 50%	No adjustments allowed
Column Length		
Flow Rate	Base on particle size and ±50%	No adjustments allowed
Column ID	Any allowed	No adjustments allowed
Injection Volume	Any allowed	Any allowed
Column Temperature	± 10°C	± 10°C
Mobile phase pH	± 0.2 units	± 0.2 units

- Adjustments to the composition of the MB in gradient elution may cause change of selectivity, hence is not recommended
- Change in column packing (maintain the same chemistry), duration of an initial isocratic hold (if present in the official method) and/or dwell time adjustments are allowed

New Allowable Adjustment

Gradient Elution is allowed.

Variable	Isocratic	Gradient
Particle Size	Per constant L/dp or N; - 25% to + 50%	L/dp: -25% to +50% or Ratio $(t_R/W_h)^2$ -25% to +50%
Column Length		
Flow Rate	Base on particle size and ±50%	Based on dp: $F_2=F_1\times[(dc_2^2\times dp_1)/(dc_1^2\times dp_2)]$ Additional adjustments: ±50%, provided N decreases ≤20%
Column ID	Any allowed	Flexible, w/ constant linear velocity
Injection Volume	Any allowed	Any allowed
Column Temperature	± 10°C	± 5°C
Mobile phase pH	± 0.2 units	± 0.2 units

New Allowable Adjustment (cont.)

Gradient Adjustment

- A change in column dimensions, and thus in column volume, impacts the gradient volume which controls selectivity. Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. This applies to every gradient segment volume.

$$t_{G2} = t_{G1} \times \left(F_1/F_2\right)\left[\left(L_2 \times {dc_2}^2\right)/\left(L_1 \times {dc_1}^2\right)\right]$$

- Thus, the change in conditions for gradient elution requires **three steps**:

  - Adjust the column length and particle size according to *L/dp*.
  - Adjust the flow rate for changes in particle size and column diameter.
  - Adjust the gradient time of each segment for changes in column length, diameter, and flow rate. The example below illustrates this process.

New Allowable Adjustment

Gradient Adjustment

1. An 11% increase within allowed *L/dp* change of -25% to +50%
2. Calculated using $F_2 = F_1 [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$
3. Calculated using $t_{G2} = t_{G1} \times (F_1/F_2) [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$

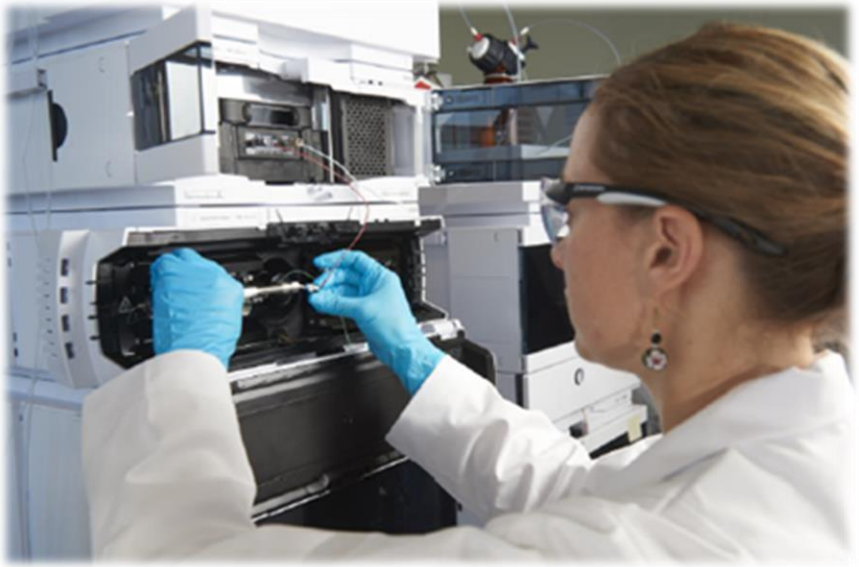
Column temperature: ±5°C, where the operating temperature is specified, unless otherwise prescribed
Further adjustment in procedure conditions (mobile phase, temperature, pH, etc.) may be required, within the permitted ranges described under System Suitability and Adjustment of Chromatographic Conditions in this Chapter.

Variable	Original Conditions	Adjusted Conditions	Comment
Column length (*L*), in mm	150	100	User's choice
Column diameter (*dc*), in mm	4.6	2.1	User's choice
Particle size (*dp*), in µm	5	3	User's choice
L/dp	30.0	33.3	(1)
Flow rate, in mL/min	2.0	0.7	(2)
Gradient adjustment factor (t_{G2}/t_{G1})	-	0.4	(3)
Gradient conditions	-	-	-
B(%)	Time (min)	Time (min)	
30	0	0	-
30	3	(3 x 0.4) = 1.2	-
70	13	[1.2 + (10 x 0.4)] = 5.2	-
30	16	[5.2 + (3 x 0.4)] = 6.4	-

Source: https://online.uspnf.com/uspnf/document/1_GUID-6C3DF8B8-D12E-4253-A0E7-6855670CDB7B_2_en-US

What are the column OPTIONS?

- **Smaller particle size**
  - Higher efficiency >> Shorter column >> Faster Method
  - Increase resolution
  - Better sensitivity
  - Consider pressure limit of instrument
- **Smaller diameter**
  - Solvent saving
  - Depends on instrument configuration and plumbing
- **Bonded phase**
  - Match USP designation
  - More robust column lifetime
  - Consider a different bonded phase?

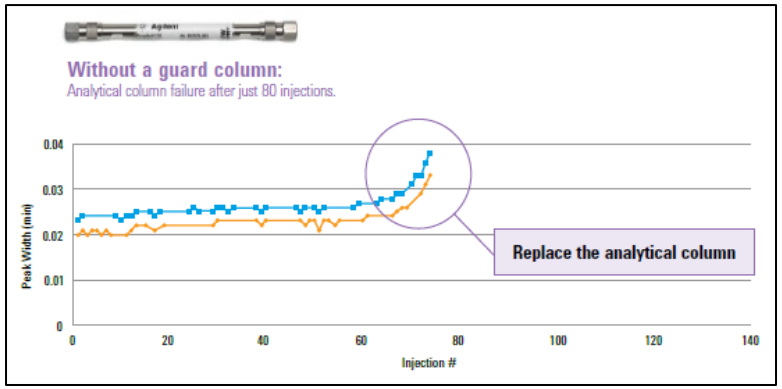


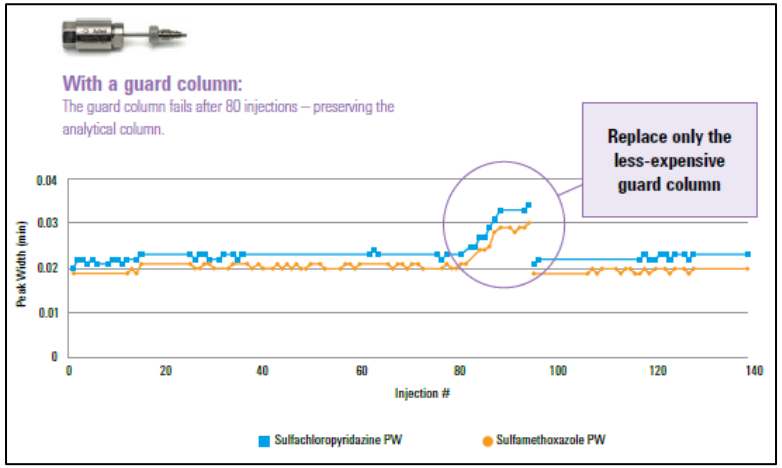
What's about guard column?

Allowable Adjustment: USP Chapter <162>

Unless otherwise indicated in the monograph, a ***guard column may be used***. The following requirements applies:

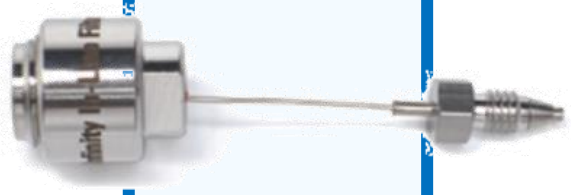
- All system suitability test specified in the monograph must be met with the guard installed
- The length of the guard column must be no more than 15% of the length of the analytical column
- The inner diameter must be the same or smaller than that of the analytical column
- The ***packing material should be the same as the analytical column*** and contain the same bonded phase

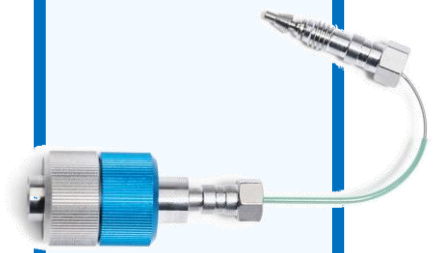




InfinityLab Quick Change In-line Filter

From





To

NEW
Launched Jan 2024

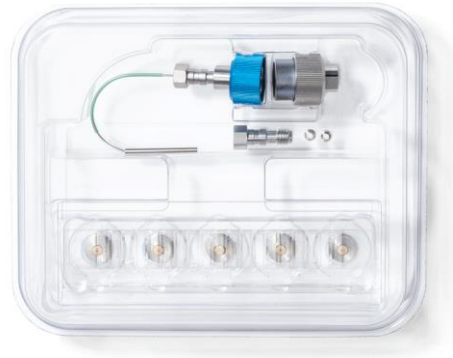
1. Insert a filter disk,
by using touchless packaging

2. Turn the housing unit
you hear a “CLICK”

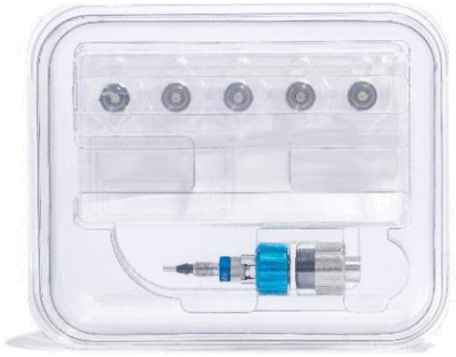
3. Disconnect the fitting connection
at your column

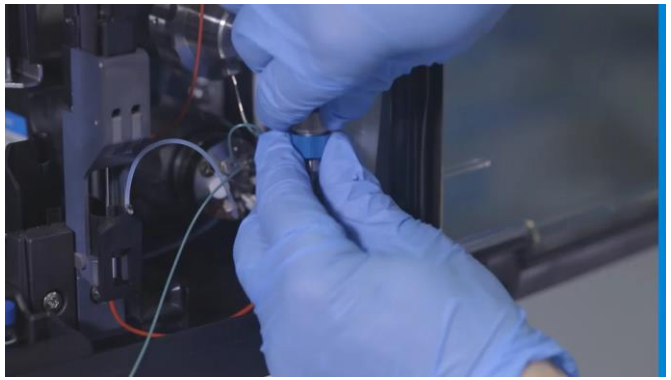
4. Connect the capillary onto
the female end of in-line filter

5. Turn on the flow until the eluent
come out then turn the in-line
filter onto your column



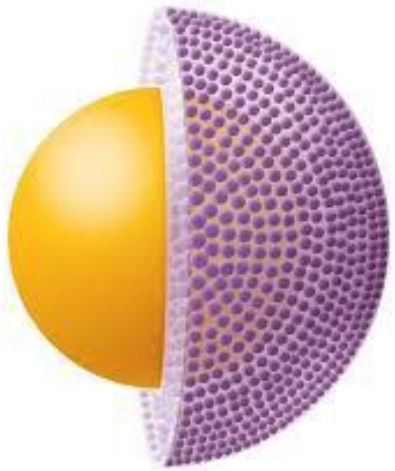
Assemblies Package





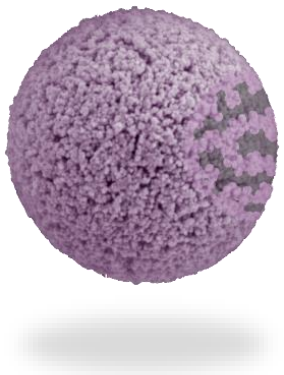
The Advantages of Superficially Porous Particle

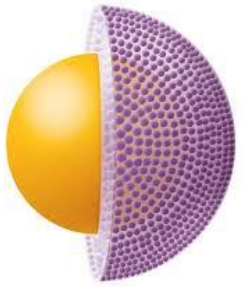
Poroshell



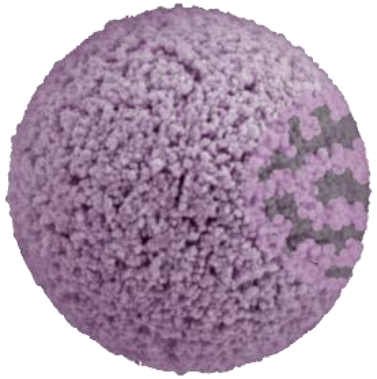
Agilent's Premium Column

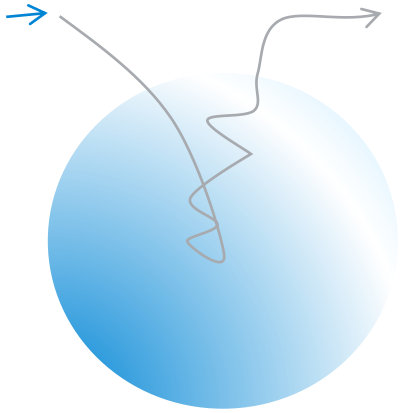
Brand/ Subject	Zorbax	Poroshell
Particle Technology	Totally porous particle	Superficially porous particle
Particle Size Available (μm)	5, 3.5, 1.8	4, 2.7, 1.9

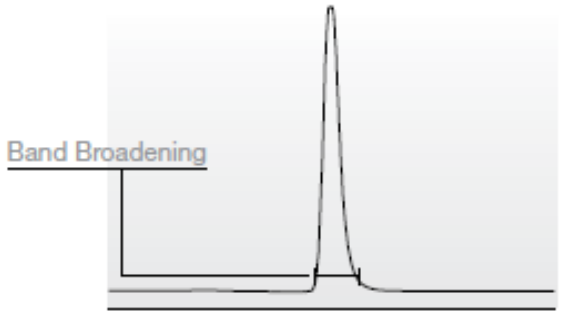


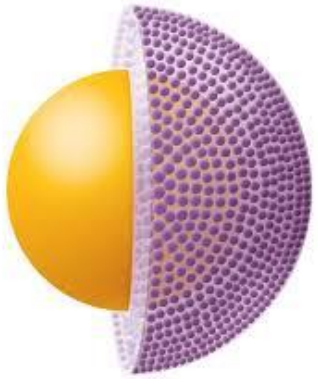


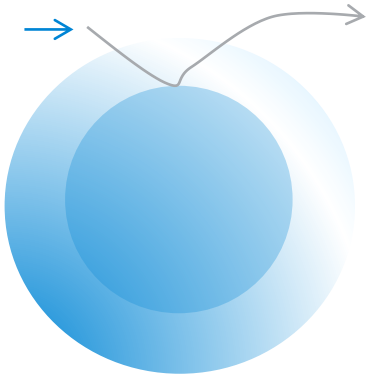
The Advantages of Superficially Porous Particle

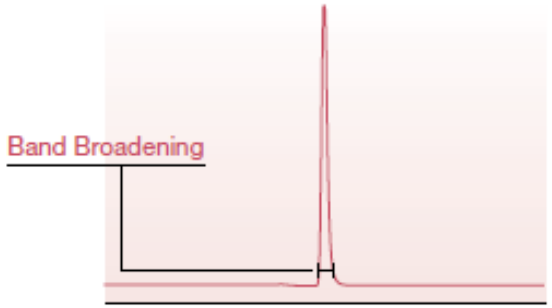












- Totally Porous Particles (Zorbax)
  - Diffusion throughout particle
- Superficially Porous Particles (Poroshell)
  - Diffusion limited to outer shell

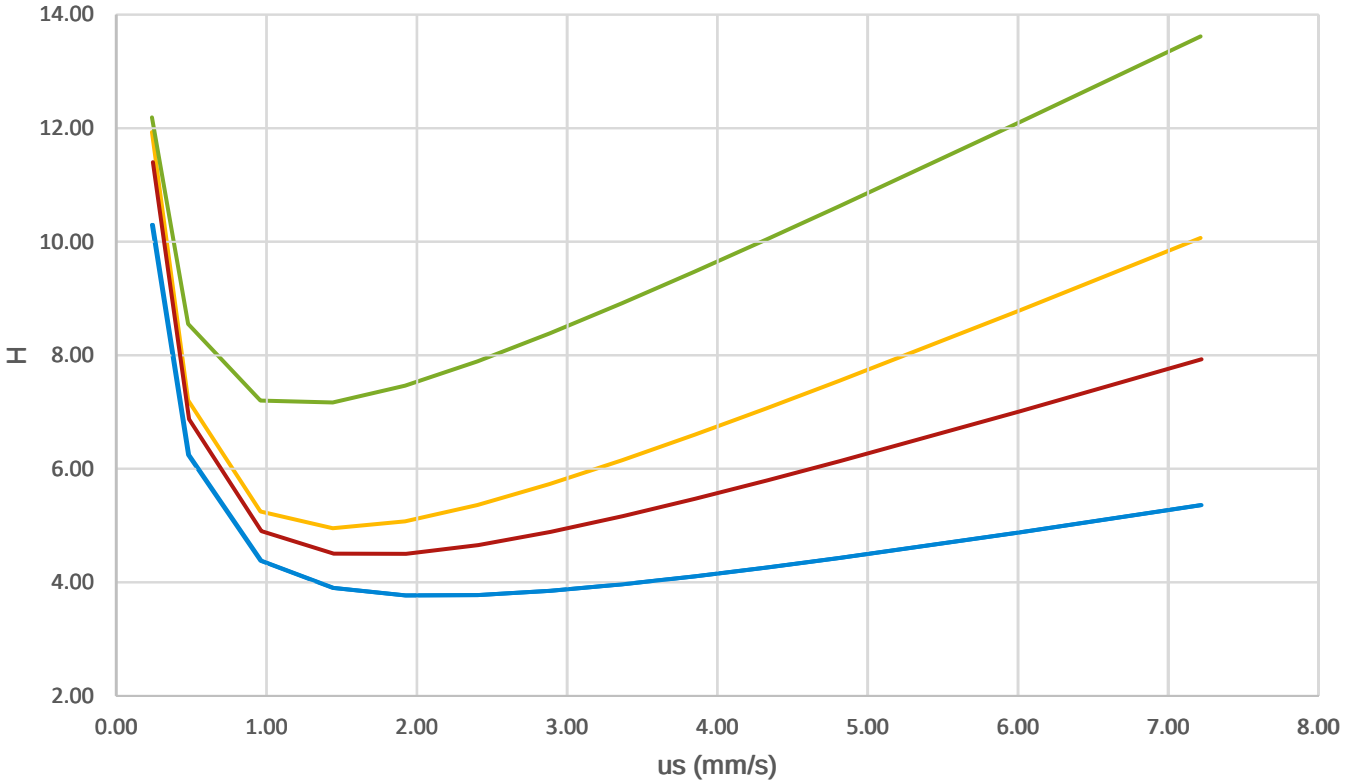
van Deemter equation:

$$h = A + B/\nu + C \cdot \nu$$

- Results:
  - Lower C term
  - Higher efficiency
- And
  - Higher flow rate with
  - Minimal impact on efficiency

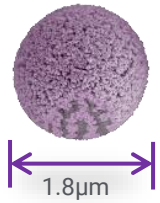
van Deemter Equation

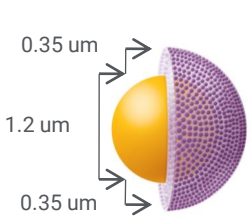
Let's compare TPP, SPP, and particle size!

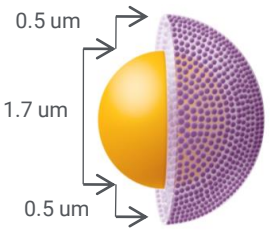


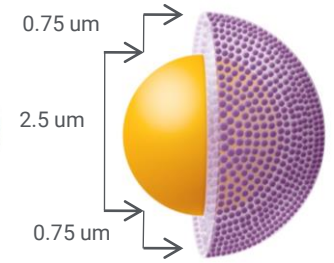
Particle	Pressure	Efficiency
1.9 um Poroshell	↑	↑
1.8 um Zorbax		
2.7 um Poroshell		
4 um Poroshell		

A = eddy diffusion
B = diffusion of analytes along column
C = Resistance to mass transfer







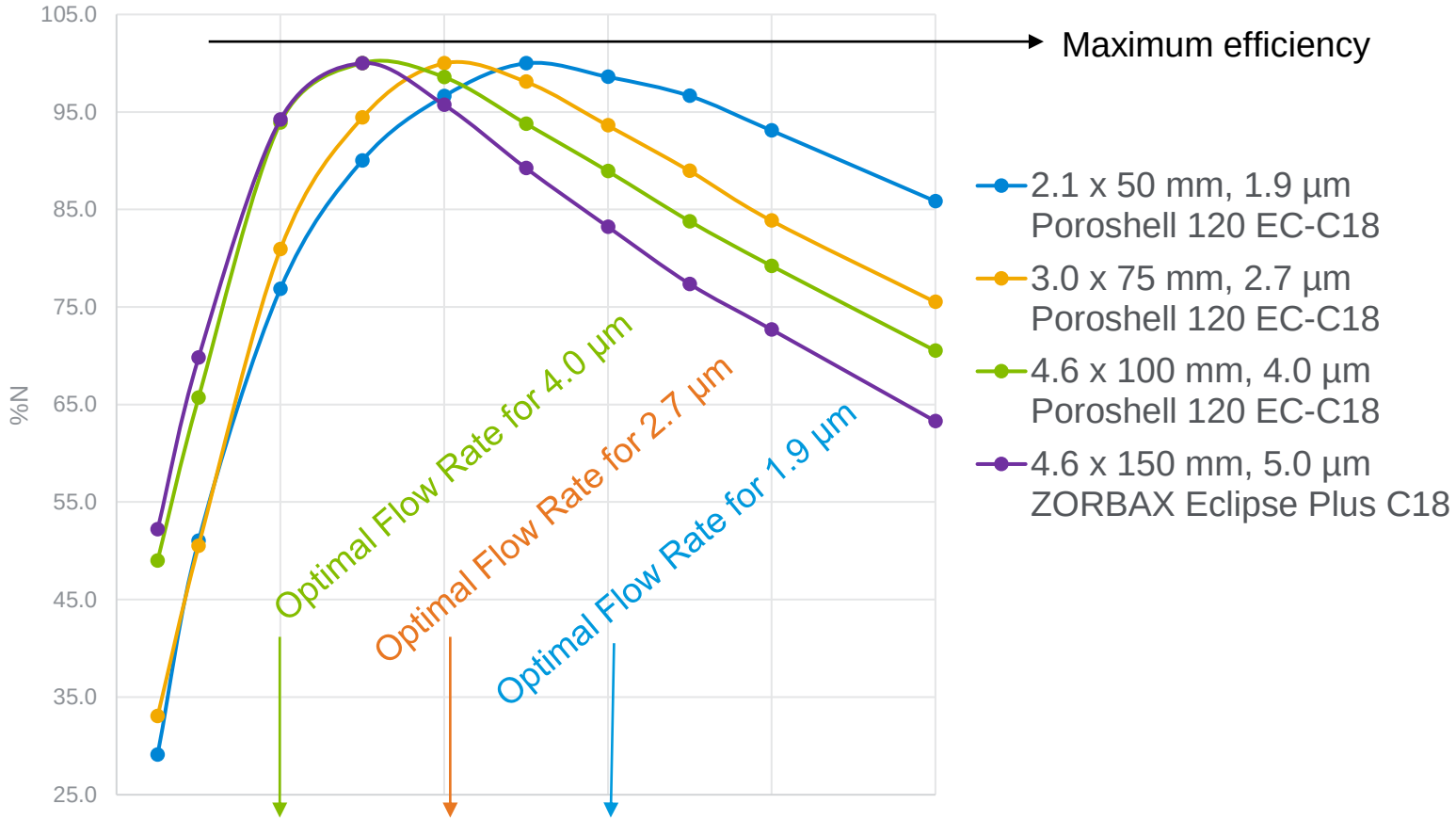


$$h = A + B/\nu + C \cdot \nu$$

van Deemter equation

Optimal Flow Rates For Agilent InfinityLab Poroshell 120 Columns

Smaller particles have higher optimal flow rates



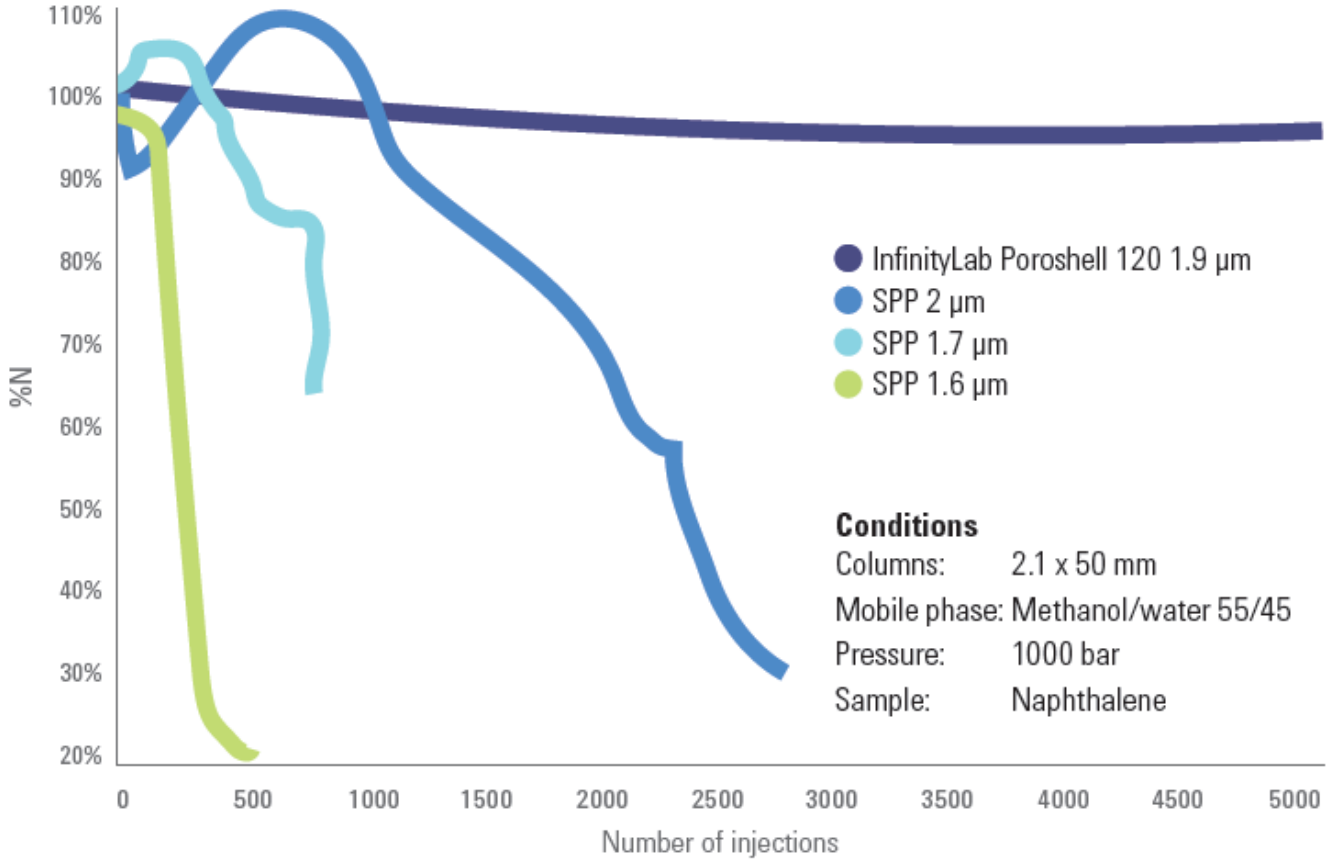
2.1 mm id columns	0.2	0.4	0.6	0.8	1.0
3.0 mm id columns	0.4	0.8	1.2	1.6	2.0
4.6 mm id columns	1.0	2.0	3.0	4.0	

Equivalent flow rates (mL/min) for different column internal diameters

- Operating below or above the column's optimal flow rate negatively impacts efficiency (and also resolution)
- When comparing column dimensions, flow rates should be geometrically scaled maintain a constant linear velocity

Cost of Short Column Lifetimes

- More columns to purchase
- Disruption to workflow
- Re-work required



Brand	Cost/ Column	# Column for 5,000 Injections	LC Column Cost for 5,000 Injections
Agilent	$ 750	1	$ 750 x 1 = $ 750
Brand H	$ 500	2	$ 500 x 2 = $ 1,000
Band P	$ 400	5	$ 400 x 5 = $ 2,000
Brand W	$ 800	10	$ 800 x 10 = $8,000

Concerns!

USP Allowed?
Superficially
Porous Particle

A change from totally porous particle (TPP) columns to superficially porous particle (SPP) columns ***is allowed***…

Liquid Chromatography: Gradient Elution

Adjustment of chromatographic conditions for gradient systems requires greater caution than for isocratic systems.

COLUMN PARAMETERS AND FLOW RATE

Stationary phase: No change of the identity of the substituent (e.g., no replacement of C18 by C8); the other physicochemical characteristics of the stationary phase i.e., chromatographic support, surface modification, and extent of chemical modification must be similar; a change from totally porous particle (TPP) columns to superficially porous particle (SPP) columns is allowed provided the above-mentioned requirements are met.

Summary of Allowable Adjustments per USP General Chapter <621>

Gradient Adjustment

Adjustments of the composition of the mobile phase and the ***gradient are acceptable*** provided that:

The ***system suitability*** criteria are ***fulfilled***.

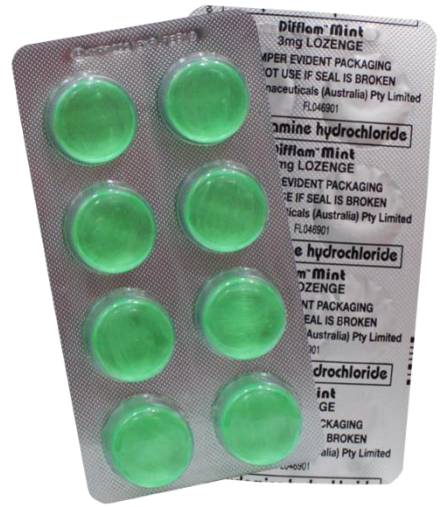
The principal peak(s) elute(s) ***within ±15%*** of the retention time(s) obtained with the original conditions; this requirement does not apply when the column dimensions are changed.

The composition of the mobile phase and the gradient are such that the first peaks are ***sufficiently retained*** and the ***last peaks are eluted***.

Method Parameters: Official USP Method Benzocaine Lozenges

An Example

Parameters	Values
Buffer	1 M monobasic potassium phosphate, adjusted to pH 3 with phosphoric acid
Mobile Phase	Acetonitrile: Water : Buffer (250:700:50)
Flow Rate	1.5 ml/min
Injection Volume	20 µl
Detector	280 nm, for Identification test A use a diode array detector
Diluent A	0.1 N Hydrochloric Acid
Diluent B	Acetonitrile & Water (1:1)
Standard 1	0.01 mg/ml Benzocaine RS in Diluent A
Standard 2	0.01 mg/ml Benzocaine RS in Diluent B
System Suitability	Standard Solutions A & B, Tailing Factor NMT 1.5, RSD NMT 2.0%



USP Benzocaine Lozenge Assay Method

Zorbax Eclipse Plus C8 ➔ Poroshell 120 EC-C8

➔

With superficially porous columns a second avenue to adjustment exist with the efficiency of the column adjustment range

N: -25% to +50%

In this case the efficiency of the proposed column is measured with the API of interest and compared with the efficiency the comparison column as listed in the method.

In this case the N=17078 and so a window exists where the efficiency is acceptable between $(\frac{100-25}{100})$ ≥ 17,078 ≤ $(\frac{100+50}{100})$ or between 12,808 and 25,617 based on L/dp column we can see that a shorter column could meet the N requirement, we need to prove it with the API in the method.

Based on this we have a solution to ***reduce*** the column ***length from 250 mm to 100mm or 75 mm***.
This is 40 or 30 % of the original column length using a standard product.
We will ***save up to 70% of time*** and ***solvent*** at the ***same flow rate***

USP Benzocaine Lozenge Assay Method

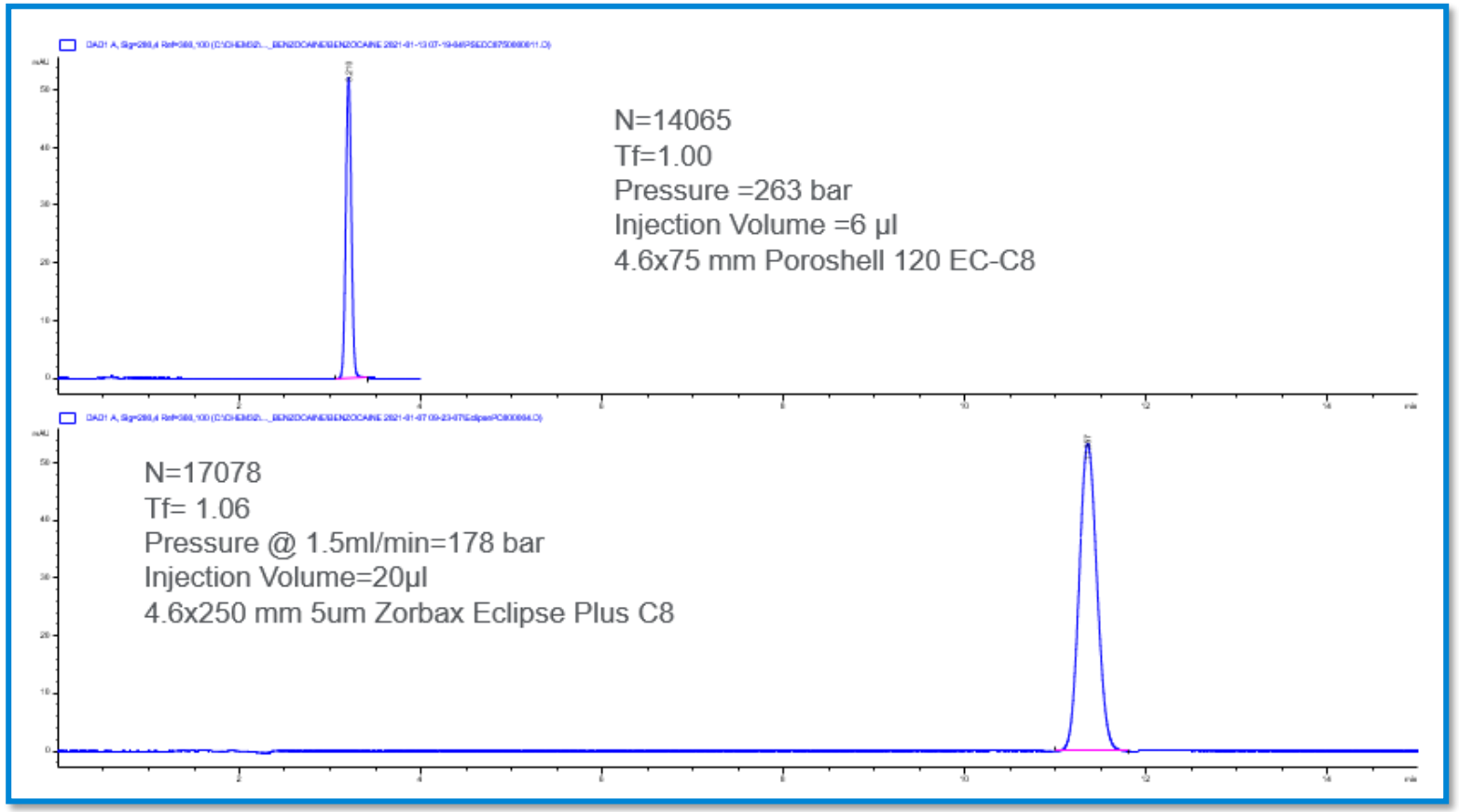
Zorbax Eclipse Plus C8 to Poroshell 120 EC-C8

Using N Rule:
We apply the 4.6 x 75 mm, 2.7 µm

Change Process:
➔ Change Column
➔ Scale Injection Volume
Proportionately to Column Volume

Outcome:
We have reduced the Run time, as observed

Results:
☺ Even Faster Analysis
☺ More Solvent Savings
☺ Still the Same Instrument



USP Allowable Adjustments Benzocaine Assay to

4.6x 150, 100 and 75 mm 2.7µm Poroshell 120 EC-C8

Column	Column Length (L, mm)	Particle Size (dp, µm)	L/dp Ratio	Allowable L/dp Range (-25%- +50 %)	N Benzocaine Standard	Allowable N Range (-25%- +50 %)	% Time Saving	Pressure (Bar)
Zorbax Eclipse Plus C8	250	5	50,000	37,500-75,000	17,078	12,808 - 25,617		176
Poroshell 120 EC-C8	150	4	37,500	Meets specification ☺☺☺	23,943	No need to check	40%	264
Poroshell 120 EC-C8	150	2.7	55,555	Meets specification ☺☺☺	14,174	No need to check	40%	421
Poroshell 120 EC-C8	100	2.7	37,037	Does not meet specification ☹☹☹	18,325	Meets Specification (+7%)	60%	314
Poroshell 120 EC-C8	75	2.7	27,778	Does not meet specification ☹☹☹	14,128	Meets Specification (-17%)	70%	287

No change in flow rate is made

Results of the System Suitability Tests

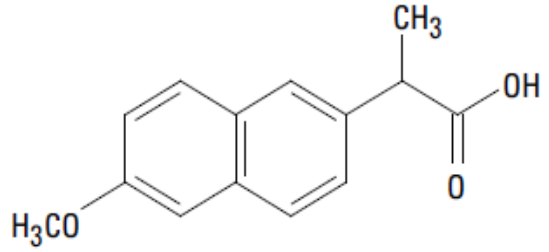
	System Suitability Requirements	Poroshell 120 EC-C8 4.6x75 mm 2.7um 1.5 ml/min Std A		Poroshell 120 EC-C8 4.6x75 mm 2.7um 1.5 ml/min Std B	
USP Tailing Factor	NMT 2.0	1.0		1.0	
Relative Standard Deviation (RSD)	NMT 2.0 %	Area	0.3 %	Area	0.3%
		Retention Time	0.3%	Retention Time	0.2%

NMT : (**N**ot **M**ore **T**han)

5 replicates were used.

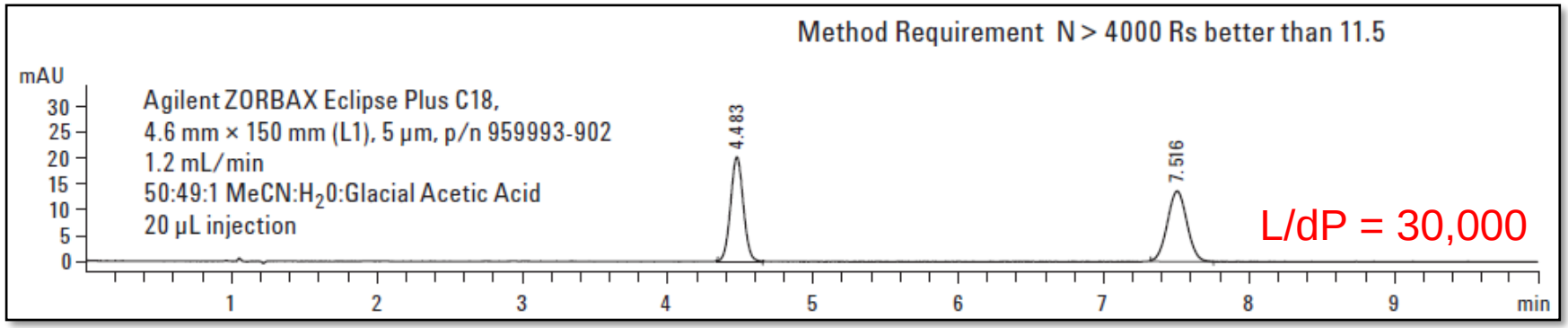
Agilent Application Note (5990-7456EN)

Analysis of Naproxen Using Poroshell 120 EC-C18



The chromatographic and performance requirements of the method are listed in the USP method. These are summarized below. [1]

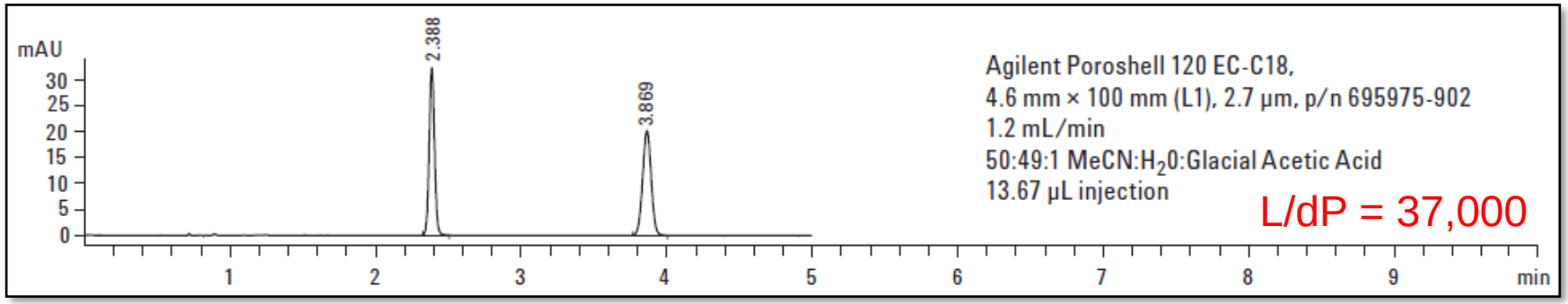
- 4.6 mm × 150 mm column, L1 column (C18)
- N of the analyte not less than 4000 plates
- Resolution between the analyte and internal standard peaks is not less than 11.5



Topic	Zorbax EC-C18 4.6x150mm 5µm	Poroshell EC-C18 4.6x100mm 2.7µm
Analysis Time (mins)	~9	5
Sample/ hr	7	12
Sample/ day	56	96
Sample/ week	280	480
Solvent Used (mL)	10.8	6

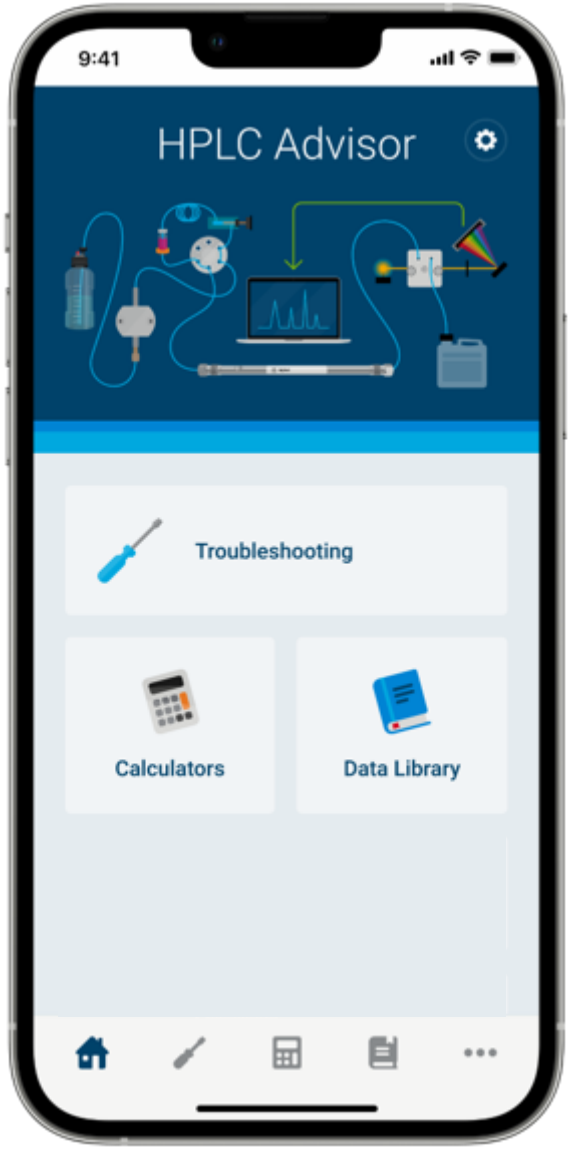
Productivity ~70% ↑

Solvent Used ~44% ↓



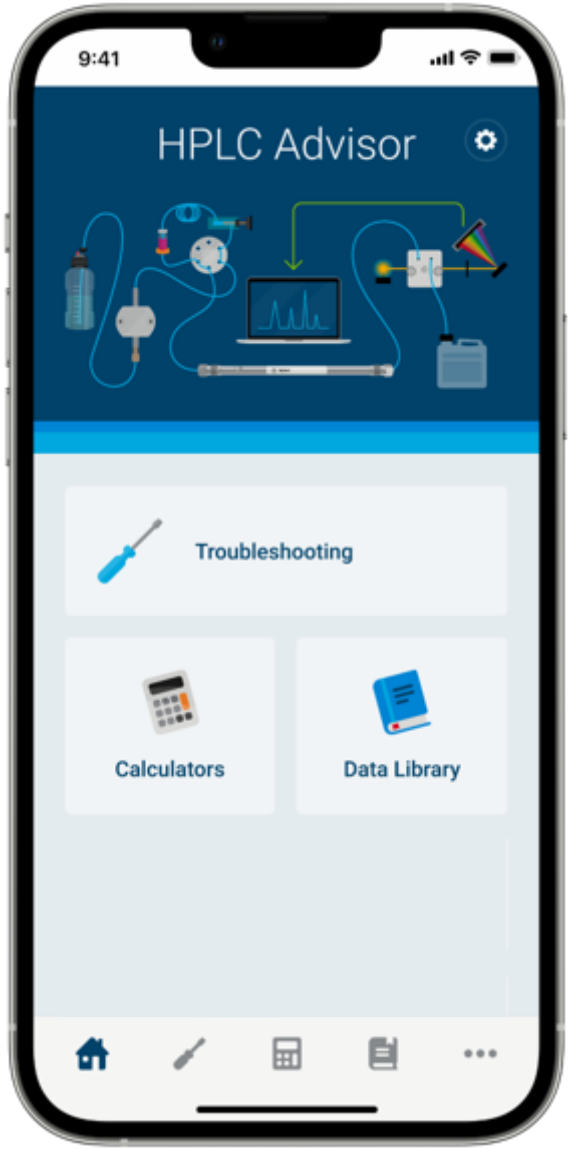
Agilent InfinityLab HPLC Advisor

HPLC Advisor Application



HPLC Advisor Application

Tool for Any Brand of HPLC



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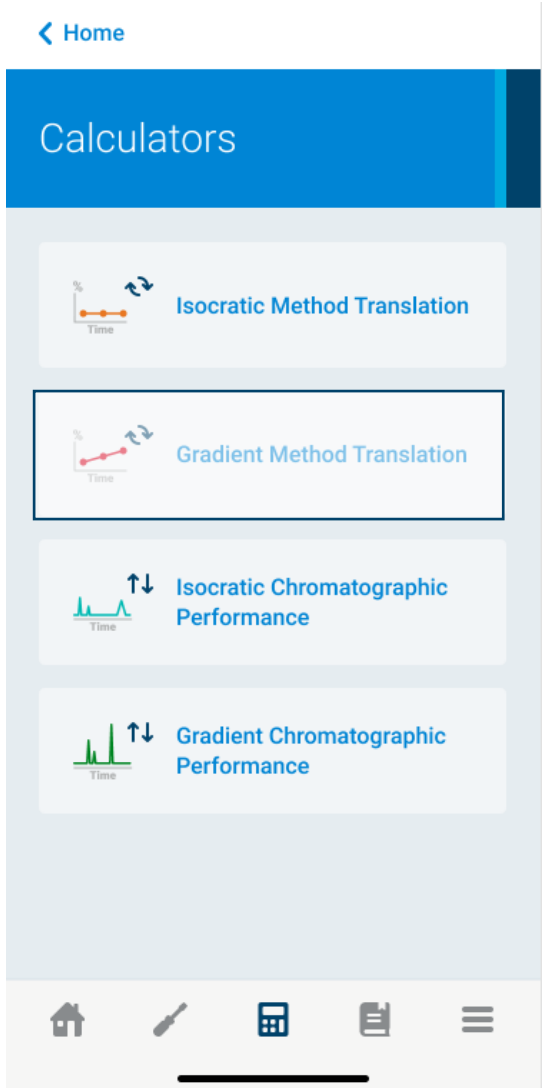
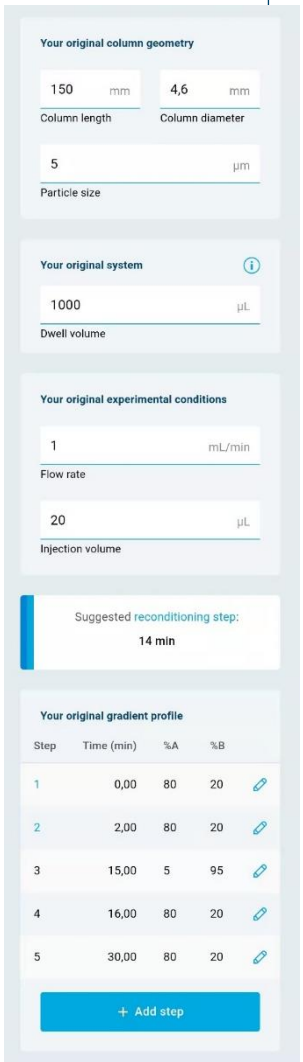
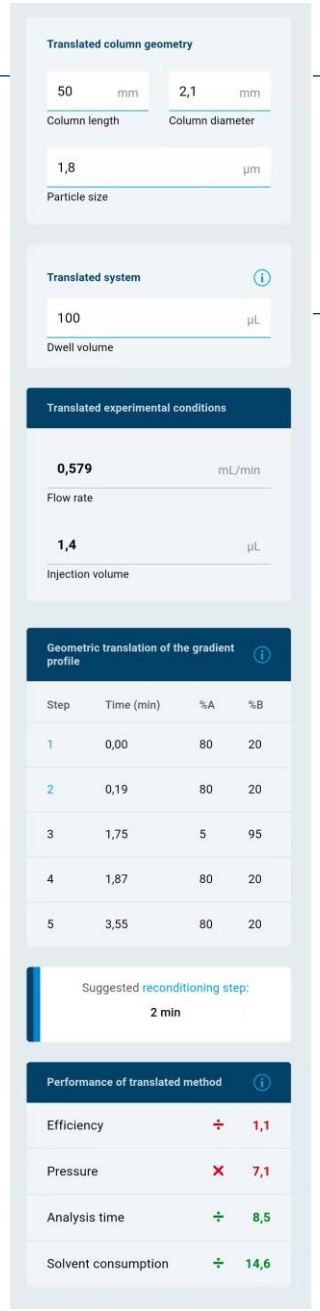
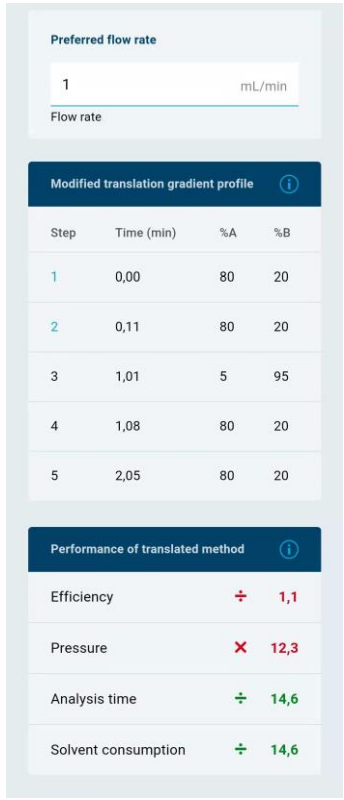
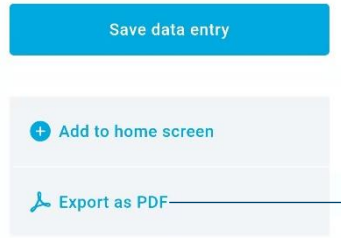
Method Conversion











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