GUIDE TO ULTRA-FAST HPLC

Quick Tips for Converting Conventional Reversed-Phase HPLC Separations to Ultra-Fast Separations

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There has been enormous interest recently in so-called "ultra-fast" HPLC columns that can reduce run times by 70% or more. These ultra-fast columns are typically packed with particles considerably smaller than what is packed in conventional columns, giving them the advantage of equivalent separating power in much shorter length columns as well as the advantage that they maintain their separating power at higher mobile phase flow rates. The ability to use shorter columns and higher flow rates offers an opportunity to reduce analysis time and increase sample throughput significantly by substituting an ultra-fast column for a conventional column in an established method.

To facilitate converting conventional reversed-phase separations to ultra-fast separations, we have created this quick tips guide. It is intended to assist you in selecting an ultra-fast column and modifying conditions for a faster run time. In addition, this guide will help you estimate how the new ultra-fast conditions will affect run time, resolution, and back pressure.

First, one qualification: An important chromatographic parameter that seems to be ignored in most discussions of ultra-fast HPLC is selectivity. Although selectivity is beyond the scope of this guide, you should be aware that converting from a conventional column to an ultra-fast column will sometimes be accompanied by a change in selectivity for one or more peak pairs in your chromatogram. This may be true if you continue to use the same bonded phase chemistry, such as C18, and even true if the ultra-fast column you choose is the same brand as the conventional column that it is replacing. However, in most cases the change in selectivity will be minor enough that the estimation models used in this guide will still be useful.

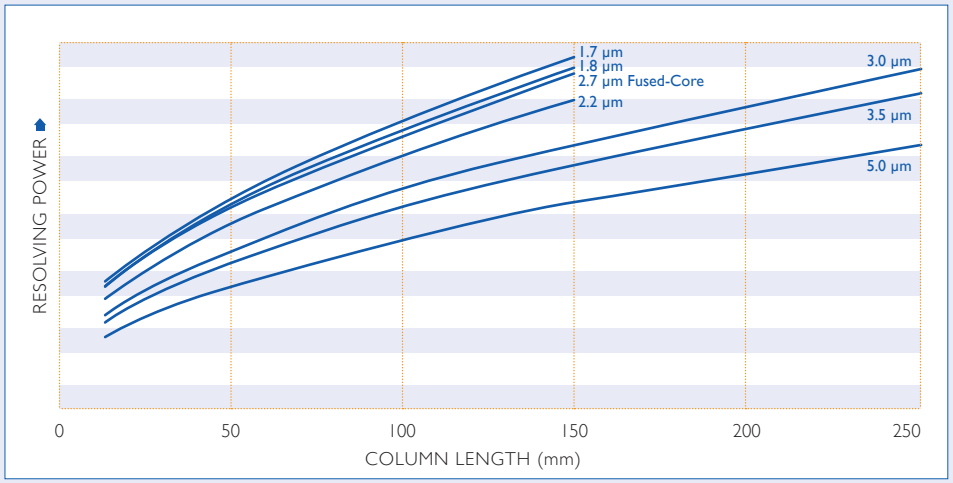
Suggested Steps for Converting a Conventional Reversed-Phase Separation to an Ultra-Fast Separation

1. Select the shortest ultra-fast column that can provide resolution equivalent to or better than the conventional column. *See Figure A.*

2. Estimate the back pressure for the selected "ultra-fast" column. *See Figure B.* If the pressure exceeds the maximum acceptable pressure for your system, select an alternate column with lower back pressure, most likely one packed with larger particles. You could elect to operate at a lower flow rate to keep the pressure acceptable, but this would also increase the run time, negating the purpose of converting to an ultra-fast column.

Figure A: Resolving Power as a Function of Particle Size and Column Length

Instructions: This chart plots Resolving Power (the ability of a column to separate components in a mixture) versus column length for 7 different column packings. Three of the packings are conventional particles (5.0 µm, 3.5 µm, and 3.0 µm) and four are ultra-fast particles (2.2 µm, 2.7 µm Fused-Core™, 1.8 µm, and 1.7 µm). As column length increases, so does Resolving Power, but run time also increases. Notice that the columns packed with ultra-fast particles provide greater Resolving Power in much shorter column lengths compared to the columns packed with conventional particles. When converting a conventional separation to an ultra-fast separation, choose the shortest ultra-fast column that provides Resolving Power equal to or better than the conventional column it is replacing. This will allow you to minimize run time and maintain acceptable resolution.



Example: A 100 mm ultra-fast column packed with 2.7 µm Fused-Core particles meets the criteria of providing equal or better Resolving Power compared to a 250 mm column packed with conventional 5 µm particles. This ultra-fast column is an appropriate choice for replacing the 250 mm length conventional column in an ultra-fast method.

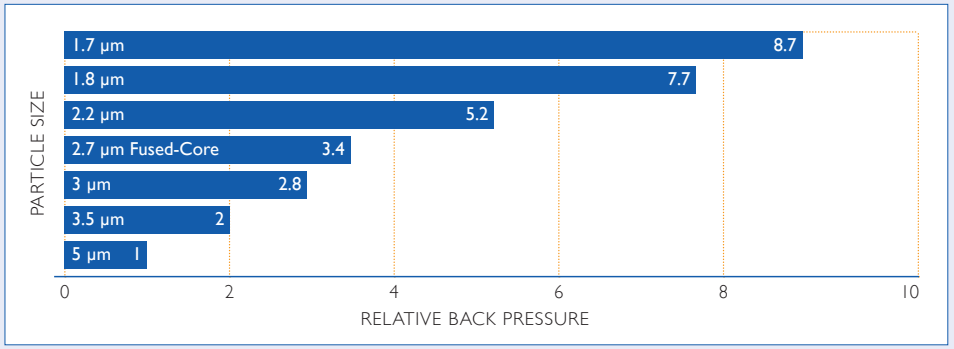
Figure B: Relative Back Pressure versus Particle Size

Instructions: For the ultra-fast column configuration selected in Step 1 (length, particle size), estimate the expected back pressure on this column by multiplying the pressure observed on the conventional column by the ratio of the "Relative Pressure" of the ultra-fast column to the conventional column and then by the ratio of the column lengths.

Note: This calculation assumes that the mobile phase velocity is the same for both the conventional column and the ultra-fast column.

$$P_2 = P_1 \times \frac{RP_2}{RP_1} \times \frac{L_2}{L_1}$$

P_2: Estimated back pressure of the ultra-fast column
RP_2: Relative back pressure of the ultra-fast column
L_2: Length of the ultra-fast column
P_1: Measured back pressure of conventional column
RP_1: Relative back pressure of the conventional column
L_1: Length of the conventional column



Example: A 100 mm ultra-fast column packed with 1.8 µm particles will generate approximately 3 times the back pressure of a 250 mm conventional column packed with 5 µm particles.

$$P_{\text{ULTRA-FAST COLUMN}} = P_1 \times \frac{7.7}{1} \times \frac{100\text{ mm}}{250\text{ mm}} = 3.08 \times P_{\text{CONVENTIONAL COLUMN}}$$

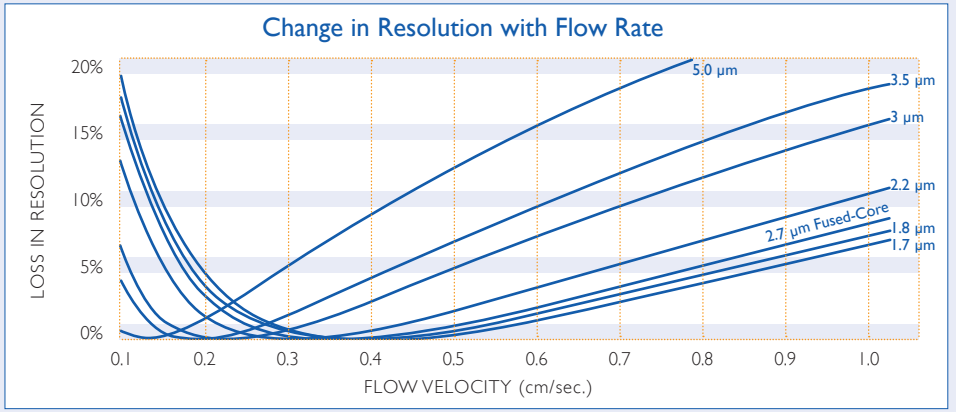
3. Confirm that the selectivity and resolution of the ultra-fast column is adequate. Since the selectivity of the ultra-fast column may be different from the selectivity of the conventional column (this may be true even if both the conventional column and the ultra-fast column are the same brand), run your separation with the ultra-fast column and calculate resolution. If the resolution does not meet the minimum required resolution, you may have to choose a longer column, or possibly even a different brand of ultra-fast column (with a different selectivity) to achieve acceptable resolution. If the resolution exceeds the required resolution, you may be able to use an even shorter ultra-fast column, or at least operate at a higher mobile phase flow rate to reduce the run time even further.

4. Once a column has been selected that provides acceptable resolution and pressure, increase the flow rate to minimize the run time while maintaining acceptable resolution and pressure. Considerable time savings and greater sample throughput can be achieved by operating at higher flow rates with an ultra-fast column, as long as you don't exceed your system's maximum back pressure. *See Figure C.*

Figure C: Resolution versus Mobile Phase Flow Rate

Instructions: : If the resolution on the selected ultra-fast column exceeds the minimum required resolution for the separation and does not exceed the pressure limit, you will be able to reduce analysis time further by increasing the flow rate. Since the optimum flow velocity (for maximum resolution) of an ultra-fast column is 3 to 4 times faster than for a conventional column, you may actually be able to both reduce the run time and increase resolution by operating at a higher flow rate.

This chart estimates change in Resolution with changes in mobile phase velocity. Not only do ultra-fast columns have their optimum efficiency at higher mobile phase velocities, they also sacrifice less of their efficiency as mobile phase velocity is increased beyond their optimum.



Example: An ultra-fast column packed with 2.7 µm Fused-Core particles can be operated at a relatively fast mobile phase velocity of 0.7 cm/sec and still retain over 96% of its resolving power. A conventional column packed with 5 µm particles run at the same flow velocity would retain only about 82% of its resolving power.

Converting Mobile Phase Velocity (cm/sec) to Column Flow Rate (ml/min)

Mobile Phase Velocity	Column ID (mm)			
	1.0	2.1	3.0	4.6
0.1	0.030	0.13	0.27	0.63
0.2	0.059	0.26	0.53	1.3
0.3	0.089	0.39	0.80	1.9
0.4	0.12	0.52	1.1	2.5
0.5	0.15	0.65	1.3	3.1

Mobile Phase Velocity	Column ID (mm)			
	1.0	2.1	3.0	4.6
0.6	0.18	0.79	1.6	3.8
0.7	0.21	0.92	1.9	4.4
0.8	0.24	1.0	2.1	5.0
0.9	0.27	1.2	2.4	5.7
1.0	0.30	1.3	2.7	6.3

5. If the separation uses gradient elution, you will need to adjust the gradient time (t_G) to the volume of the ultra-fast column and for any changes in flow rate. *See Figure D.*

Figure D: Adjusting Gradient Time (t_G) for Changes in Column Volume and Flow Rate

$$t_{G2} = t_{G1} \times \frac{V_{m2}}{V_{m1}} \times \frac{F_1}{F_2}$$

t_{G2}: Gradient time for the ultra-fast separation
V_{m2}: Column volume of the ultra-fast column (see Table 1)
F_2: Flow rate for the ultra-fast separation
t_{G1}: Gradient time for the conventional separation
V_{m1}: Column volume of the conventional column (see Table 1)
F_1: Flow rate for the conventional separation

See Table on page 12 for estimates of column volumes for most commercially available column dimensions.

Important Note: The system dwell volume (gradient mixing volume) can have a significant effect on the chromatography when using gradients because it adds an isocratic hold to the beginning of the gradient. The time of this "hold" is equal to the dwell volume divided by the flow rate. When the flow rate is changed, this isocratic hold will also change. This change in gradient hold will generally have more of an effect on early eluting peaks, but it will also affect all peaks in the chromatogram to some extent. To minimize the effect on your separation, keep the dwell volume as small as possible by using micro gradient mixers and keeping the tubing volume in the system to a minimum.

Example: A conventional method uses a column 4.6 x 150 mm (1.57 ml), a flow rate of 1.0 ml/min, and a gradient of 15% B to 35% B in 20.0 minutes. The gradient time for an ultra-fast method that uses a column 4.6 x 50 mm (2.7 µm Fused-Core, 0.42 ml) and a flow rate of 2.0 ml/min is:

$$t_{G\ ULTRA\text{-}FAST} = 20 \text{ minutes} \times \frac{0.42\text{ml}}{1.57\text{ml}} \times \frac{1.0\text{ml/min}}{2.0\text{ml/min}} = 2.7 \text{ min.}$$

6. Adjust the injection volume to the ultra-fast column's volume. *See Figure E.*

Figure E: Adjust Sample Injection Volume for Changes in Column Volume

$$S_{v2} = S_{v1} \times \frac{V_{m2}}{V_{m1}}$$

S_{v2}: Injected sample volume for the ultra-fast column
S_{v1}: Injected sample volume for the conventional column
V_{m2}: Volume of the ultra-fast column (see Table 1)
V_{m1}: Volume of the conventional column (see Table 1)

Example: A conventional method uses a sample injection volume of 20 µl on a column 4.6 x 150 mm. The sample volume that should be injected on to a 4.6 x 50 mm ultra-fast column (2.7 µm Fused-Core) is:

$$S_{v\ ULTRA\text{-}FAST} = 20\ \mu l \times \frac{0.42\ ml}{1.57\ ml} = 5\ \mu l$$

An Example of Converting a Conventional Separation to an Ultra-Fast Separation.

Conventional HPLC Separation Conditions

COLUMN: 4.6 x 250 mm, 5 µm
FLOW RATE: 1.5 ml/min
MOBILE PHASE: Isocratic
RUN TIME: 10 minutes
PRESSURE: 1,580 psi, 109 bar
Maximum acceptable pressure = 4,000 psi, 275 bar
RESOLUTION: 3.0
SAMPLE INJECTION VOLUME: 20 µl

Converting to Ultra-Fast Separation Conditions

1. Select the shortest ultra-fast column that provides resolution equivalent to or better than the conventional column. ***(See chart in Figure A.)***

A column 4.6 x 100 mm packed with 1.7 µm particles is selected for further investigation.

2. Estimate back pressure. ***(See relative back pressure table in Figure B.)***

$$P_{\text{Ultra-Fast Column}} = 1{,}580 \text{ psi} \times \frac{8.7}{1} \times \frac{100 \text{ mm}}{250 \text{ mm}} = 5{,}498 \text{ psi}$$

Since this ultra-fast column exceeds our maximum acceptable back pressure (4,000 psi), a different ultra-fast column is selected for investigation.

The alternative ultra-fast column selected is a 4.6 x 100 mm packed with 2.7 µm Fused-Core particles (HALO HPLC column). The back pressure on this column is:

$$P_{\text{Ultra-Fast Column}} = 1{,}580 \text{ psi} \times \frac{3.4}{1} \times \frac{100 \text{ mm}}{250 \text{ mm}} = 2{,}149 \text{ psi}$$

This ultra-fast column provides both acceptable resolution and acceptable back pressure for our method.

3. Confirm that the selectivity and resolution of the ultra-fast column is adequate.

For simplicity, we will assume that the selectivity of this ultra-fast column is almost identical to the selectivity of the conventional column and, therefore, the resolution is adequate.

4. Optimize flow rate to minimize run time. ***(See Figure C to estimate changes in resolution with changes in flow rate.)***

We can further reduce run time by operating the ultra-fast column at a higher flow rate. We just have to make sure we stay within the requirements of minimum resolution and maximum pressure. The ultra-fast column we selected has low enough back pressure that we can operate at a flow rate of 2.5 ml/min and still stay within our defined limits of pressure and resolution.

$$P_{\text{at 2.5 ml/min}} = 2{,}149 \text{ psi} \times \frac{2.5 \text{ ml/min}}{1.5 \text{ ml/min}} = 3{,}582 \text{ psi}$$

5. Adjust the gradient time.

This is an isocratic separation, so no adjustment to gradient time is required.

6. Adjust the injection volume. ***(See Figure E for calculations and table on page 12 with estimated column volumes.)***

$$\text{Sample volume} = 20 \text{ µl} \times \frac{0.84 \text{ ml}}{2.62 \text{ ml}} = 6.4 \text{µl}$$

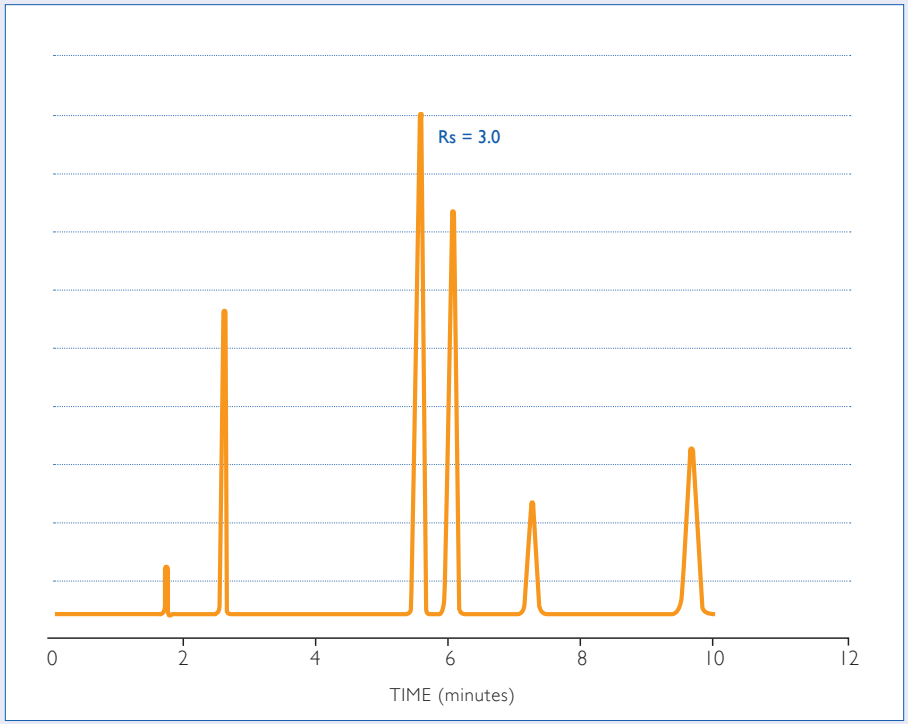
Ultra-Fast Conditions

COLUMN: 4.6 x 100 mm, 2.7 µm Fused-Core (HALO)
FLOW RATE: 2.5 ml/min
RUN TIME: $10 \text{ min} \times \frac{0.84 \text{ ml}}{2.62 \text{ ml}} \times \frac{1.5 \text{ ml/min}}{2.5 \text{ ml/min}} = 1.9 \text{ min}^*$
RESOLUTION: 3.1
PRESSURE: 3,582 psi
SAMPLE INJECTION VOLUME: 6.4 µl

* Run time for the ultra-fast separation can be estimated by multiplying the run time on the conventional column by the ratio of the volumes of ultra-fast column to the conventional column and then by the inverse ration of the flow rates on the two columns. (See page 12)

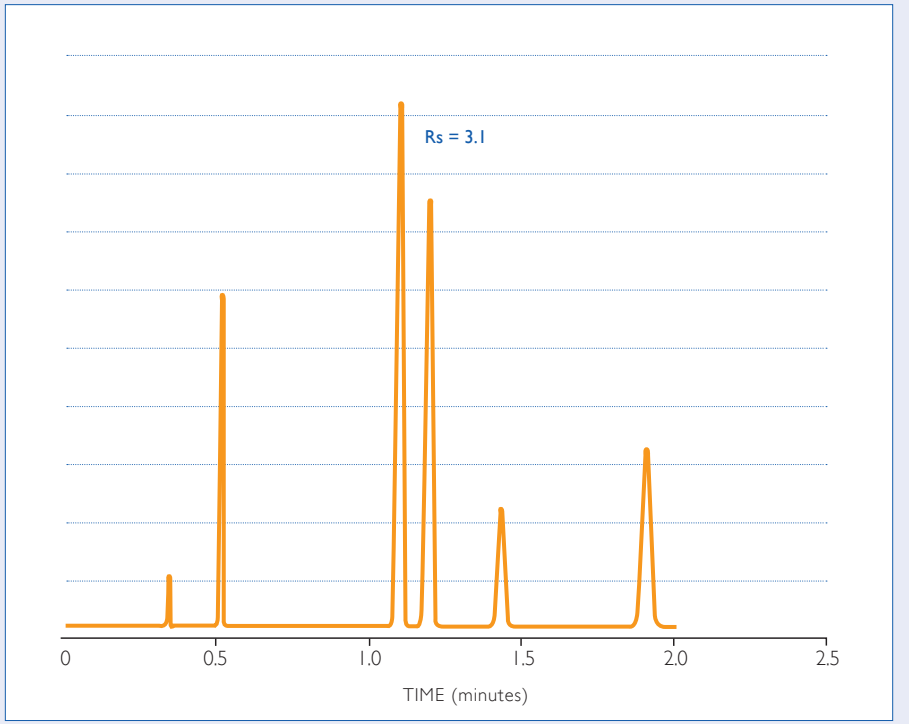
Conventional Separation

COLUMN: 4.6 x 250 mm, 5 µm, C18
FLOW RATE: 1.5 ml/min
RESOLUTION: 3.0
PRESSURE: 1,580 psi
RUN TIME: 10 minutes
SAMPLE INJECTION VOLUME: 20 µl



Ultra-Fast Separation

COLUMN: 4.6 x 100 mm, 2.7 µm Fused-Core, C18 (HALO™ HPLC column)
FLOW RATE: 2.5 ml/min
RESOLUTION: 3.1
PRESSURE: 3,582 psi
RUN TIME: 1.9 minutes
SAMPLE INJECTION VOLUME: 6.4 µl



These are simulated chromatograms generated from theoretical data. Differences in selectivity between a conventional column and an ultra-fast column or changes in secondary retention interactions will yield different chromatographic results from what is predicted here.

Reference Tables and Equations for Quick Estimates

Table 1: Estimated volume, V_m, for a variety of available column dimensions

ID (mm)	Length (mm)	V_m (ml)	V_m Fused-Core (ml)	ID (mm)	Length (mm)	V_m (ml)	V_m Fused-Core (ml)
1.0	20	0.010	0.008	3.0	20	0.089	0.071
1.0	30	0.015	0.012	3.0	30	0.134	0.107
1.0	50	0.025	0.020	3.0	50	0.223	0.178
1.0	75	0.037	0.030	3.0	75	0.334	0.267
1.0	100	0.050	0.040	3.0	100	0.445	0.356
1.0	150	0.074	0.059	3.0	150	0.668	0.534
1.0	250	0.124	0.099	3.0	250	1.11	0.89
2.1	20	0.044	0.035	4.6	20	0.209	0.168
2.1	30	0.066	0.052	4.6	30	0.314	0.251
2.1	50	0.109	0.087	4.6	50	0.524	0.419
2.1	75	0.164	0.131	4.6	75	0.785	0.628
2.1	100	0.218	0.175	4.6	100	1.05	0.84
2.1	150	0.327	0.262	4.6	150	1.57	1.26
2.1	250	0.546	0.436	4.6	250	2.62	2.09

Note: Column volumes listed here are estimates only. However, most commercial columns can be expected to have volumes within about 5% of what is reported here. Columns packed with Fused-Core particles are an exception and, therefore, are listed separately.

Table 2: Column plate number, N, for columns packed with different size/type particles

Particle	N per cm of column length	Particle	N per cm of column length
5 µm	800	2.7 µm Fused-Core	2200
3.5 µm	1140	1.8 µm	2220
3 µm	1330	1.7 µm	2300
2.2 µm	1800		

Note: Estimates in this Table are for near ideal conditions. Column plate number is dependent on many factors including the solute, mobile phase viscosity and flow velocity and it is not unusual under "real-world" conditions for column plate numbers to be over 20% lower than what is reported here.

Estimating changes in run time with changes in column volume and flow rate

$$RT_{ultra\text{-}fast} = RT_{conventional} \times \frac{V_{ultra\text{-}fast}}{V_{conventional}} \times \frac{F_{ultra\text{-}fast}}{F_{conventional}}$$

RT = Run Time
V = Column Volume
F = Flow rate (If column ID changes, mobile phase velocity should be used instead of flow rate.)

Estimating changes in Resolution with changes in column plate number

$$Rs_{ultra\text{-}fast} = Rs_{conventional} \times \sqrt{\frac{N_{ultra\text{-}fast}}{N_{conventional}}}$$

Rs = Resolution
N = Column plate number

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