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New changes to the US Pharmacopeia: Reduce time and solvent usage with newer technology columns

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Introduction

Setting up a new USP method in your lab is often associated with standard HPLC columns from prior generations. Methods were developed on then state-of-the-art 10 or 5 µm particle size and long columns. The methods were robust and steady and worked well for their purpose. However, as we progress further into the 21st century, “slow and steady” is associated with “costly”, and in the new world of climate change and the need for less environmental impact, using large volumes of solvents may be a less popular feature.

Major steps have been taken by the US Pharmacopeia to allow labs to improve upon their methods, effectively saving time and money as well as reducing solvent consumption. As of December 1, 2022, the US Pharmacopeia will allow for changes within particle size, length, and inner diameter, for both isocratic and gradient methods, to improve the original method to new standards. The European Pharmacopoeia and Japanese Pharmacopoeia will follow suit with their newest editions, implemented from January 1, 2023. We will take you through what this transition from an original US Pharmacopeia method to its improved version on newer technology columns will look like. For the sake of simplicity, we provide this example on a specific method—USP standard operating procedure for determining organic impurities of pramipexole dihydrochloride.

Experimental

Materials and reagents

Name	CAS	Supplier	Part number
Potassium dihydrogen phosphate	7778-77-0	Sigma-Aldrich	P0662
Sodium 1-octanesulfonate monohydrate	207596-29-0	Sigma-Aldrich	74885
Phosphoric acid	7664-38-2	Sigma-Aldrich	695017
Acetonitrile	75-05-8	Fisher Chemicals	A998-4
Pramipexole for system suitability	–	European Directorate for the Quality of Medicines & HealthCare	Y0001187
Screw top vials	–	Thermo Fisher Scientific	6AK92W

Mobile phase A	9.1 g of potassium dihydrogen phosphate and 5.0 g of sodium 1-octanesulfonate monohydrate were digested in 1 L of water. The solution was adjusted with phosphoric acid to a pH of 3.0.
Mobile phase B	Acetonitrile and mobile phase A (50:50)
Diluent	Acetonitrile and mobile phase A (20:80)
System suitability solution	7.5 mg of pramipexole for system suitability standard was dissolved in 5 mL of the diluent solution.
Suitability requirements	For system suitability solution, resolution should be not less than 6.0 between pramipexole related compound A. Also tailing factor should be not more than 2.0 for pramipexole peak.

Method

For the method of Pramipexole dihydrochloride, a standard L1 column is required in the dimensions 5 µm particle, 4.6 mm inner diameter and 150 mm length. Flow is suggested to be at 1.5 mL/min, which is standard for a column of these dimensions. The most important criteria for this application are the asymmetry of the main peak and the resolution between the main peak and its compound A, which are to be <2.0 and >6.0, respectively. The USP suggests the Thermo Scientific™ BetaSil™ C18 column amongst Thermo Fisher Scientific's column portfolio as the most adequate column for the original method, so this column was selected as our starting point (Figure 1).

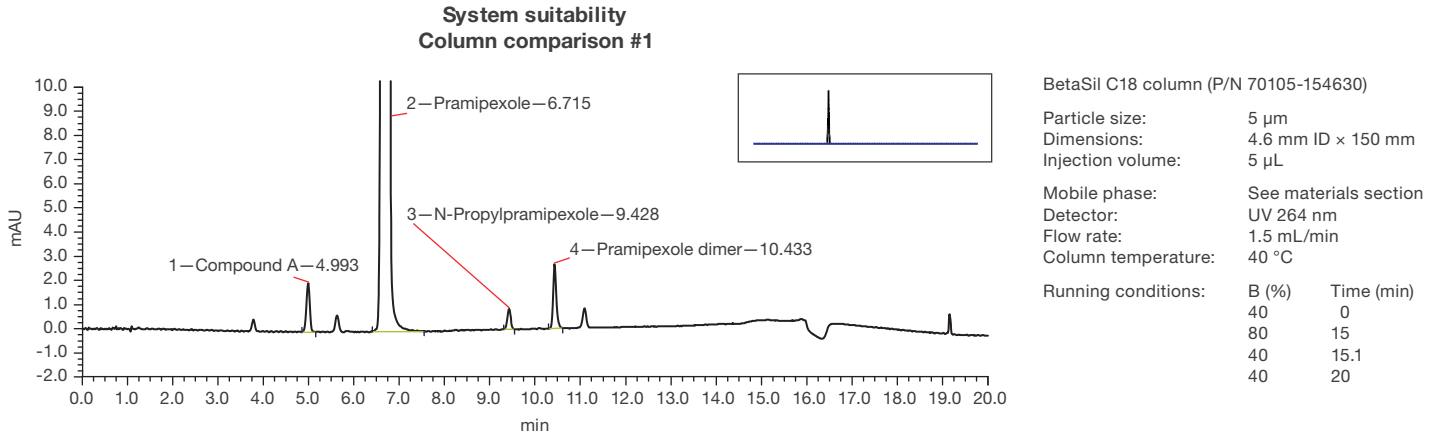

Figure 1. USP method for organic impurities of pramipexole dihydrochloride on a BetaSil C18 column

Although the runtime of the method is 20 minutes, the column itself performs to the requirements of the original method with great margin. Table 1 displays the data from six subsequence runs and their calculated average.

Table 1. Results from six runs of the USP method for organic impurities of pramipexole dihydrochloride on a BetaSil C18 column

No.	Peak name	Retention time (min)	Area (mAU*min)	Height (mAU)	Asymmetry (EP)	Resolution (EP)
1	Pramipexole	6.78	130.45	1354.983	0.87	18.5
2	Pramipexole	6.788	130.271	1350.845	0.86	18.42
3	Pramipexole	6.722	130.351	1404.847	0.86	18.57
4	Pramipexole	6.715	130.435	1355.159	0.86	18.51
5	Pramipexole	6.718	130.38	1357.106	0.86	18.46
6	Pramipexole	6.717	130.412	1359.875	0.87	18.33
Average		6.74	130.38	1363.80	0.86	18.47
Standard deviation		0.03	0.07	20.32	0.01	0.08
% RSD		**0.51**	**0.05**	**1.49**	**0.60**	**0.45**

We attempted to improve both runtime and solvent consumption of this method by using USP General Chapter <621>, officially active on December 1st, 2022. According to the new revision, no changes are allowed on the stationary phase. This means that the stationary phase will have to remain as a L1 selectivity, an octadecyl chain commonly called C18. However, the following changes are allowed:

Column dimensions (particle size, length)	**L/dp: -25% to +50%**
Adjustments from totally porous to superficially porous particles	For the application of particle-size adjustment from totally porous to superficially porous particles, other combinations of *L* and *dp* can be used provided that the ratio $(tR/Wh)^2$ is within -25% to +50%, relative to the prescribed column ***for all peaks used to determine the system suitability parameters***. These changes are acceptable provided system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.
Internal diameter	Can be adjusted
Column temperature	±5 °C

We attempted to modernize the method by changing particle size, length, and inner diameter for the columns. Each step is explained carefully in the following sections to ease the process of modernization.

How to modernize your method

L/dp

As mentioned above, as long as the ratio between the column length, *L*, and the particle size, *dp*, is maintained within -25% and +50%, the change in column is allowed. The BetaSil C18 column ratio is 150/5 = 30.

$$\frac{150}{5} = 30$$

Selecting a column with smaller particle size, the Thermo Scientific™ Hypersil GOLD™ C18 3 µm column with a length of 100 mm and an inner diameter of 2.1 mm, gives the following ratio:

$$\frac{100}{3} = 33.3$$

The percentage difference is calculated by subtracting the ratio of the new column from the old, then dividing this result by the ratio for the old column. To allow for a percent result, the result is multiplied by 100.

$$\%\ deviation = \frac{(Ratio_{New} - Ratio_{Old})}{Ratio_{Old}} \times 100$$

In this case, it becomes:

$$\%\ deviation = \frac{(33.3 - 30)}{30} \times 100 = 11\%$$

This is within the -25% to +50% limit.

Flow

When changing the particle size and ID of your column, you must adjust for flow. The new flow is calculated by this equation:

$$F_2 = F_1 \times \left[\frac{(dc_2^{\,2} \times dp_1)}{(dc_1^{\,2} \times dp_2)}\right]$$

For our selected column for modernization, the Hypersil GOLD C18 column (3 µm, 100 mm × 2.1 mm ID), this is:

$$F_2 = 1.5\ \frac{mL}{min} \times \left[\frac{(2.1\ mm\ ID^2 \times 5\ \mu m)}{(4.6\ mm\ ID^2 \times 3\ \mu m)}\right] = 0.52\ mL/min$$

F_1 = Old flow rate
F_2 = New flow rate
dc_1 = Inner diameter of column originally used
dc_2 = Inner diameter of used column
dp_1 = Particle size used in original method
dp_2 = Particle size used in modernized method

Gradient

When modernizing your method, it is important to adjust the gradient to the new run time of the method. This way the separation remains as required.

Gradient steps remain the same, only the time changes. However, calculating the time steps requires some mathematical skill. First, the gradient time change factor t_{G2} is calculated. As t_{G1} always equals 1 (as the original gradient time is set to 1), the following equation is used to calculate t_{G2}:

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

This gives

$$t_{G2} = 1 \times \left(\frac{1.5\ mL/min}{0.52\ mL/min}\right) \times \left[\frac{(100\ mm \times 2.1\ mm\ ID^2)}{(150\ mm \times 4.6\ mm\ ID^2)}\right] = 0.4$$

t_{G1} = 1
t_{G2} = New gradient time
F_1 = Old flow rate
F_2 = New flow rate
L_1 = Length of column originally used
L_2 = Length of used column
dc_1 = Inner diameter of column originally used
dc_2 = Inner diameter of used column

With t_{G2}, the new times for the gradient can be calculated.

Each new time is calculated by taking multiplying t_{G2} with the delta time for each step and then adding the time of the prior step. Delta time is calculated by subtracting the current time from the prior time.

For the monograph, the time for the gradient steps and calculations of delta *t* can be found in Table 2, along with calculations of new times for gradient steps.

Table 2. Calculating gradient steps for the Hypersil GOLD column

B (%)	Time (min)	Delta *t*	New time (min)
40	0	–	0
80	15	15 - 0 = 15	Prior time + Delta $t \times t_{G2}$ = 0 + 15 × 0.4 = 6
40	15.1	15.1 - 15 = 0.1	Prior time + Delta $t \times t_{G2}$ = 6 + 0.1 × 0.4 = 6.04
40	20	20 - 15.1 = 4.9	Prior time + Delta $t \times t_{G2}$ = 6.04 + 4.9 × 0.4 = 8

Injection volume

Injection volume can be adjusted as the original method had a larger particle and larger surface area, but this is optional according to the US Pharmacopeia. The injection volume was not adjusted for this study, but if it were to be, the following calculation would be used, according to the USP equation.

$$V_{Inj2} = V_{Inj1} \times \left(\frac{L_2 \times dc_2^{\,2}}{L_1 \times dc_1^{\,2}}\right)$$

$$V_{Inj2} = 5\ \mu L \times \left(\frac{100\ mm \times 2.1\ mm\ ID^2}{150\ mm \times 4.6\ mm\ ID^2}\right) = 0.7\ \mu L$$

V_{Inj1} = Injection volume for original method
V_{Inj2} = New injection volume
L_1 = Length of column originally used
L_2 = Length of used column
dc_1 = Inner diameter of column originally used
dc_2 = Inner diameter of used column

Hypersil GOLD column—3 µm, 2.1 mm ID × 100 mm

A chromatogram of the modernized method is shown in Figure 2. Table 3 shows average asymmetry was measured at 0.71, well below the required 2.0 limit, and resolution between impurity peak A and the main peak was measured at 10.92, well above the required 6.0. Furthermore, the method run time is reduced from 20 minutes to just under 8 minutes.

New method parameters
Hypersil GOLD C18 (P/N 25003-102130)

Particle size:	3 µm
Dimensions:	2.1 mm ID × 100 mm
Injection volume:	5 µL
Mobile phase:	See materials section
Detector:	UV 264 nm
Flow rate:	0.52 mL/min
Column temperature:	40 °C

Running conditions:	B (%)	Time (min)
	40	0
	80	6
	40	6.04
	40	8

Original method parameters
BetaSil C18 column (P/N 70105-154630)

Particle size:	5 µm
Dimensions:	4.6 mm ID × 150 mm
Injection volume:	5 µL
Mobile phase:	See materials section
Detector:	UV 264 nm
Flow rate:	1.5 mL/min
Column temperature:	40 °C

Running conditions:	B (%)	Time (min)
	40	0
	80	15
	40	15.1
	40	20

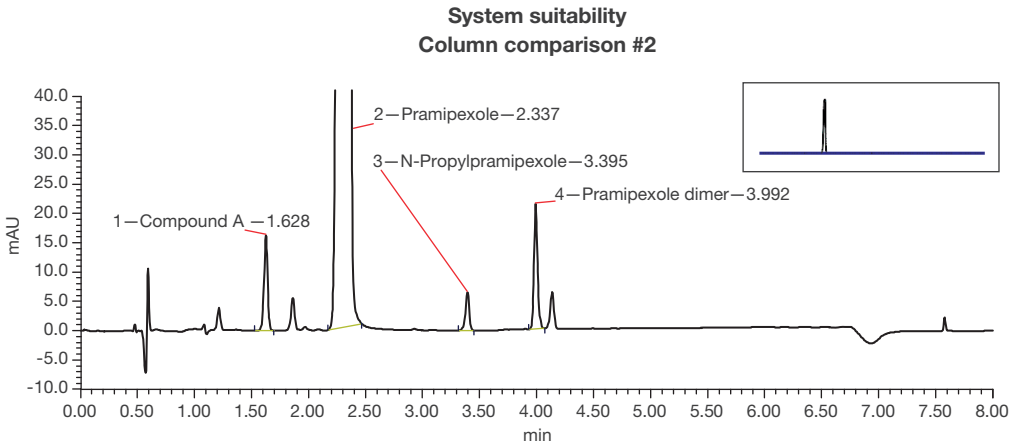

Figure 2. Modernized USP method for organic impurities of pramipexole dihydrochloride on a Hypersil GOLD C18, 3 µm column, 2.1 mm ID × 100 mm length

Table 3. Results from six runs of the USP method for organic impurities of pramipexole dihydrochloride on a Hypersil GOLD 3 µm column and calculations

No.	Peak name	Retention time (min)	Area (mAU*min)	Height (mAU)	Relative area	Asymmetry (EP)	Resolution (EP)
1	Pramipexole	2.335	280.51	3407.14	99.43	0.71	10.92
2	Pramipexole	2.332	279.557	3396.432	99.43	0.7	10.92
3	Pramipexole	2.335	278.388	3403.559	99.43	0.69	10.94
4	Pramipexole	2.332	279.232	3403.197	99.43	0.73	10.96
5	Pramipexole	2.332	280.057	3402.733	99.43	0.71	10.93
6	Pramipexole	2.337	281.965	3411.857	99.43	0.7	10.84
Average		2.33	279.95	3404.15	99.43	0.71	10.92
Standard deviation		0.00	1.22	5.12	0.00	0.01	0.04
% RSD		**0.09**	**0.44**	**0.15**	**0.00**	**1.93**	**0.38**

Hypersil GOLD column—1.9 µm, 2.1 mm ID × 50 mm

For an example of how this method will look modernized to a UHPLC platform, see Figure 3. Similar calculations were done for a Hypersil GOLD 1.9 µm column, as were for the Hypersil GOLD 3 µm column (calculations are not shown here). Average asymmetry was measured at 0.79, as shown in Table 4, well below the required 2.0 limit. The resolution between impurity peak A and the main peak was measured at 8.94, well above the required 6.0. Furthermore, the method run time is reduced from 20 minutes to just below 3 minutes.

New method parameters
Hypersil GOLD C18 column (P/N 25002-052130-V)

Particle size:	1.9 µm
Dimensions:	2.1 mm ID × 50 mm
Injection volume:	5 µL
Mobile phase:	See materials section
Detector:	UV 264 nm
Flow rate:	0.823 mL/min
Column temperature:	40 °C

Running conditions:	B (%)	Time (min)
	40	0
	80	1.95
	40	1.963
	40	2.6

Original method parameters
BetaSil C18 column (P/N 70105-154630)

Particle size:	5 µm
Dimensions:	4.6 mm ID × 150 mm
Injection volume:	5 µL
Mobile phase:	See materials section
Detector:	UV 264 nm
Flow rate:	1.5 mL/min
Column temperature:	40 °C

Running conditions:	B (%)	Time (min)
	40	0
	80	15
	40	15.1
	40	20

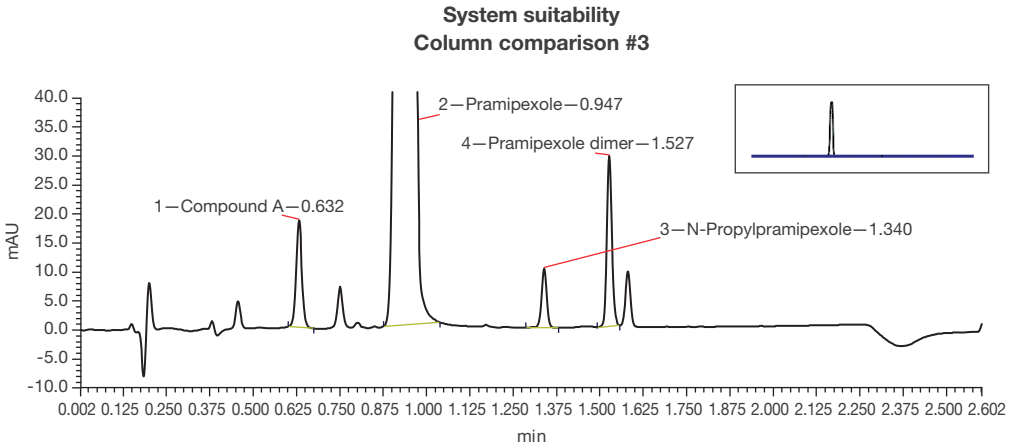

Figure 3. Modernized USP method for organic impurities of pramipexole dihydrochloride on a Hypersil GOLD C18 column, 1.9 µm, 2.1 mm ID × 50 mm length

Table 4. Results from six runs of the USP method for organic impurities of pramipexole dihydrochloride on a Hypersil GOLD 1.9 µm column and calculations

No.	Peak name	Retention time (min)	Area (mAU*min)	Height (mAU)	Relative area	Asymmetry (EP)	Resolution (EP)
1	Pramipexole	0.948	129.765	3425.102	99.22	0.78	8.91
2	Pramipexole	0.947	130.383	3432.773	99.22	0.81	8.92
3	Pramipexole	0.947	130.017	3431.818	99.22	0.81	8.94
4	Pramipexole	0.947	130.098	3418.733	99.22	0.8	8.92
5	Pramipexole	0.947	129.912	3431.064	99.22	0.78	8.95
6	Pramipexole	0.947	129.47	3430.997	99.22	0.77	8.97
Average		0.95	129.94	3428.41	99.22	0.79	8.94
Standard deviation		0.00	0.31	5.46	0.00	0.02	0.02
% RSD		**0.04**	**0.24**	**0.16**	**0.00**	**2.18**	**0.25**

Time and solvent reduction

Goals for modernizing the USP monograph method with the Hypersil columns instead of the older technology BetaSil column included reducing time and solvent consumption. By modernizing to the Hypersil GOLD 3 µm column, 60% of the analysis time can be saved. By optimizing UHPLC and the Hypersil GOLD 1.9 µm column, up to 87% of the analysis time can be saved. See Figure 4 for the visualization of the three methods side by side.

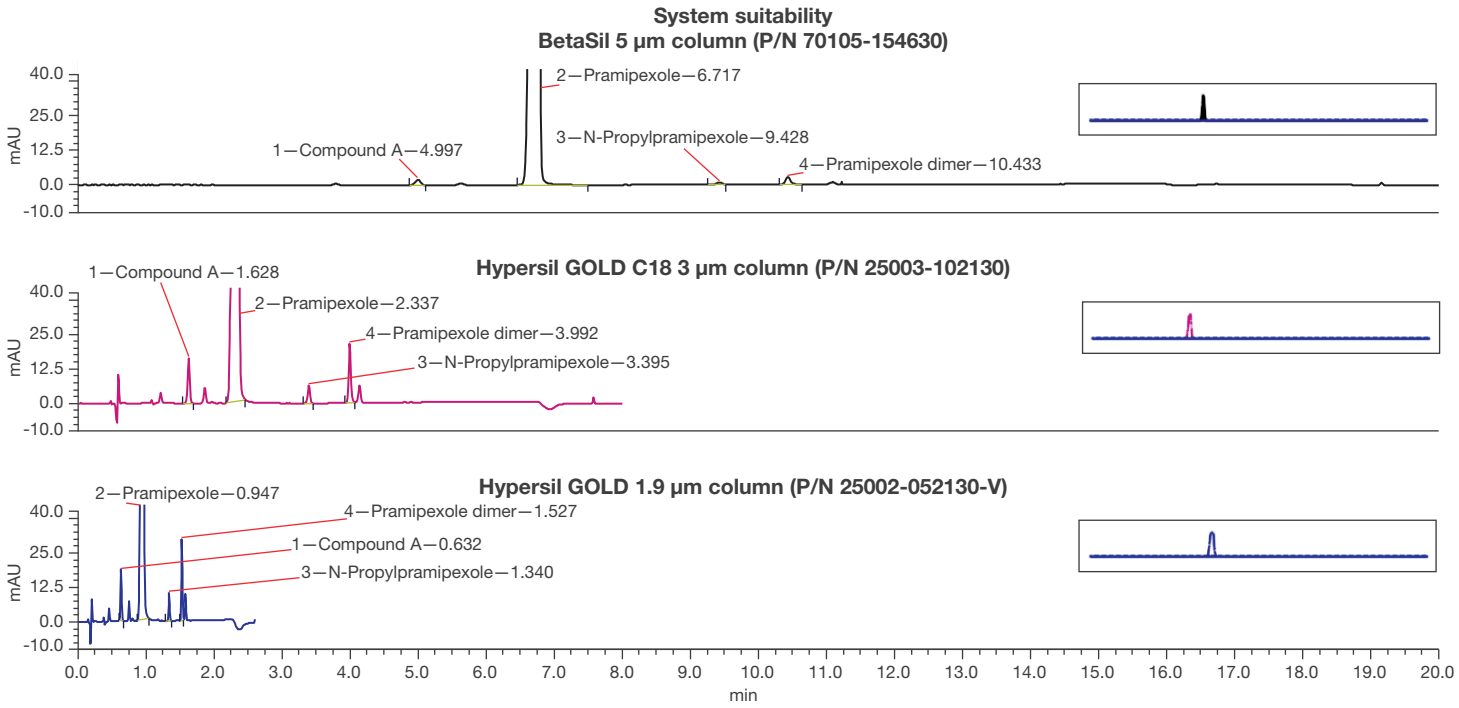

Figure 4. The three chromatograms of the USP method for organic impurities of pramipexole dihydrochloride on a BetaSil 5 µm column, Hypersil GOLD C18 3 µm column, and Hypersil GOLD 1.9 µm column

Modernization also lowers solvent consumption, which makes the method more environmentally friendly and reduces costs. The calculations for solvent consumption for 100 injections, for the three columns presented in this article, are shown in Figure 5. For the original monograph, 100 injections consume about 1,800 mL of mobile phase A (buffer) and about 1,200 mL of mobile phase B (50% acetonitrile and 50% buffer). With the Hypersil GOLD 3 µm column, reagent use can be reduced by 86%; with the Hypersil GOLD 1.9 µm column, this can be reduced by over 92%.

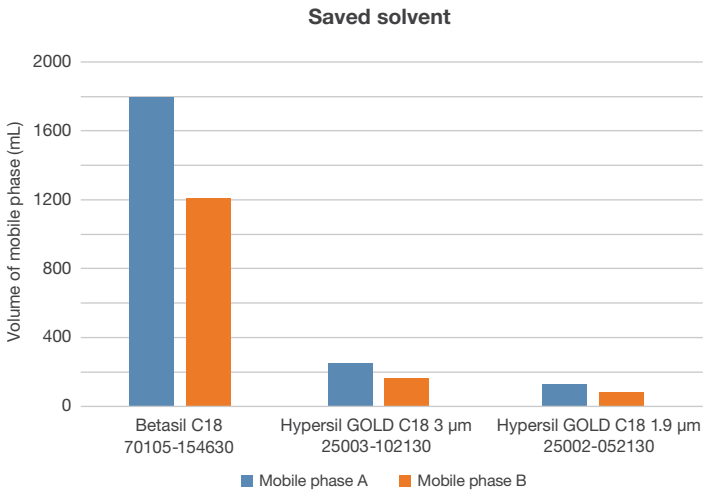

Figure 5. Saved solvent per 100 injections for the three methods

Conclusion

By modernizing an older USP monograph after the regulation provided in USP General Chapter <621>, the run time can be reduced by 60% and solvent consumption can be lowered by almost 86% by simply migrating to a 3 µm column. Moving to a UHPLC column further increases the time and solvent savings to 87% and 92%, respectively, all while staying well within the regulation provided by the US Pharmacopeia.

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