Improved Throughput, Productivity, and Performance for the USP Assay for Norethindrone and Mestranol using Kinetex® 2.6 µm C8 Core-Shell Columns

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The ultra-high efficiency provided by Kinetex 2.6 µm C8 core-shell columns is utilized to reduce analysis times and increase productivity for the USP assay for norethindrone and mestranol. Several alternatives to shorter column lengths and adjustments to flow rate were used to illustrate how the dramatic increase in efficiency can be leveraged to provide a balance between increasing productivity and reducing solvent usage.

Introduction

The introduction of Kinetex core-shell columns has brought dramatic benefits to chromatographers. The ability to obtain ultra-high chromatographic separations on conventional HPLC systems with significant reductions in sample analysis time has been especially beneficial for laboratories tasked with the routine analysis of drug products. These laboratories typically have limited resources and can benefit greatly from faster separations and higher sample throughput.

Mestranol and norethindrone tablets contain a combination of female hormones that prevent ovulation and are effective as oral contraceptives to prevent pregnancy. These products combine an estrogen (mestranol) and progestin (norethindrone) that are similar to the natural sex hormones (estrogen and progesterone) produced in a woman's body. In general, a combination of estrogen and progestin has been determined to work better than a single-ingredient product.

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. HPLC grade acetonitrile and water were purchased from Honeywell, Burdick & Jackson (Muskegon, MI). USP norethindrone reference standard (RS), mestranol RS, and progesterone were purchased from US Pharmacopeia (Rockville, MD).

Equipment and Materials

Columns Used:
A fully porous 3 µm C8 150 x 4.6 mm column (as specified by the monograph) was compared with Kinetex 2.6 µm C8 150 x 4.6 mm, 100 x 4.6 mm, and 75 x 4.6 mm columns.

Instrumentation:
Agilent® 1100 Series HPLC (Agilent Technologies Inc., Santa Clara, CA), equipped with quaternary gradient pump, autosampler, column oven, and variable wavelength detector.

Mobile Phase Preparation:
A 50:50 mixture of acetonitrile and water is filtered and degassed.

Standard Solution Preparation:
A solution containing 0.055 mg/L of mestranol, 0.055 mg/L of norethindrone, and 0.28 mg/mL of progesterone was prepared by diluting accurately weighed quantities of USP Mestranol RS, USP Norethindrone RS, and progesterone in acetonitrile.

Chromatographic Method:
10 µL of sample was injected with isocratic chromatographic separation using 50:50 acetonitrile/water as the mobile phase at a flow rate of 1.0 mL/min. The column was maintained at 25 °C with UV detection at 200 nm.

Conditions:
Column: Kinetex 2.6 µm C8 100 Å
Fully Porous 3 µm C8
Dimensions: Kinetex: 150 x 4.6 mm, 100 x 4.6 mm, and 75 x 4.6 mm
Fully Porous: 150 x 4.6 mm
Part No.: 00F-4497-E0, 00D-4497-E0, and 00C-4497-E0
Mobile Phase: Acetonitrile/Water (50:50)
Flow Rate: 1.0 mL/min or 1.5 mL/min
Inj. Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 200 nm
Instrument: Agilent® 1100
Sample: 1. Norethindrone
2. Progesterone (IS)
3. Mestranol

Results and Discussion

The ultra-high efficiency Kinetex columns are used for the USP assay of norethindrone and mestranol to highlight the improvements in throughput and productivity achievable using core-shell technology columns. Analyzing the standard solution containing norethindrone, progesterone, and mestranol using the HPLC column and conditions specified in the USP monograph for assay of norethindrone and mestranol tablets yields the expected chromatogram **(Figure 1)**. The system suitability requirements for this assay specify that the efficiency determined for mestranol (peak 3) must not be less than 6,000 theoretical plates, and resolution between progesterone and mestranol must not be less than 5.0. The chromatographic result shown in **Figure 1** demonstrates that these requirements are easily met using the fully porous 3 µm 150 x 4.6 mm C8 column, with efficiency of 13,256 plates/column and resolution of 12.29, and analysis time just under 25 minutes.

Figure 2 shows the separation of the standard solution on the Kinetex 2.6 µm C8 150 x 4.6 mm column under the same mobile phase conditions and flow rate. The high efficiency expressed by the Kinetex core-shell particle results in significantly narrower peaks. Efficiency measured for mestranol is 27,397, and resolution between progesterone and mestranol is 18.03, easily meeting the system suitability requirements. The peak heights are also significantly greater than on the fully porous 3 µm column. Overall, the separation is accomplished in less than half the time.

Using a smaller particle (as much as 50 % smaller) can provide the benefits of increased efficiency and resolution, but comes with a price of significant increases in back pressure that may not be palatable to some chromatographers. However, the dramatic increase in chromatographic efficiency provided by the core-shell particle technology can be leveraged further by using a shorter length column, as much as 70 % shorter per USP General Chapter <621>. Since pressure is directly proportional to column length, using a shorter column can significantly offset increases in pressure attributable to the smaller core-shell particle diameter.

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Figures 3a and 3b show the chromatographic separation obtained on a Kinetex® 2.6 µm C8 100 x 4.6 mm and 75 x 4.6 mm column, respectively, for the standard solution using the mobile phase conditions and flow rate specified in the monograph. Although the efficiency for mestranol on the shorter columns (19,298 on the 100 x 4.6 mm, and 15,913 on the 75 x 4.6 mm) is less than that obtained with the longer 150 x 4.6 mm Kinetex C8 column, it still easily meets the requirement for system suitability specified in the monograph. Resolution between progesterone and mestranol (15.43 and 14.54, respectively, on the 100 and 75 mm columns) is also well above the minimum of 5.0 required for system suitability. When using the 100 x 4.6 mm Kinetex C8 column, the separation occurs in less than eight minutes with observed backpressure of 200 bar (~2900 psi); and the 75 x 4.6 mm core-shell column requires just under 6 minutes with observed pressure of about 150 bar (<2180 psi), which is a much more comfortable operating pressure for laboratories performing repetitive sample analysis on conventional HPLC systems.

The shorter 75 mm Kinetex C8 column separates the mixture containing norethindrone, progesterone, and mestranol in about one-third of the time required on the fully porous 3 µm column specified in the monograph while maintaining the required efficiency and resolution of the monograph method. This faster separation results in greater than three-fold improvement in productivity and reduces mobile phase consumption by almost 75 %. These are significant benefits for laboratories with limited resources, helping to improve laboratory productivity and reducing costs, and represent an additional benefit provided by the Kinetex core-shell particle technology.

Some laboratories that analyze high volumes of samples constantly search for ways to reduce analysis times in order to make better use of the analytical instrumentation on hand and to defer, or avoid, additional capital investment in new instruments for as long as possible. For these laboratories, solvent savings may be a secondary benefit, and increasing the mobile phase flow rate with shorter length, ultra-high efficiency columns can provide the desired increase in sample throughput without compromising data quality. USP <621> allows for as much as a 50 % increase in flow rate.

Figures 4a and 4b show the chromatograms obtained with 100 x 4.6 mm and 75 x 4.6 mm Kinetex 2.6 µm C8 columns, respectively, for the standard solution using the monograph mobile phase conditions at a flow rate of 1.5 mL/min. With the 100 x 4.6 mm column, the faster flow rate reduces analysis time to less than 5 minutes, which is just over two minutes faster than using a 1.0 mL/min flow rate on the same column. Perhaps of greater significance is the increase in backpressure to >290 bar, which is almost 50 % greater than the 200 bar observed at a flow rate of 1.0 mL/min, and might raise concerns for laboratories that are more sensitive to operating at pressures above 250-300 bar on conventional HPLC systems. A similar effect is observed for the 75 x 4.6 mm column, where the analysis time is reduced from six to four

Figure 1.
Chromatogram for Norethindrone, Progesterone, and Mestranol Standard Solution on a Fully Porous 3 µm C8 150 x 4.6 mm Column, as Specified in the USP Monograph for Norethindrone and Mestranol Tablets

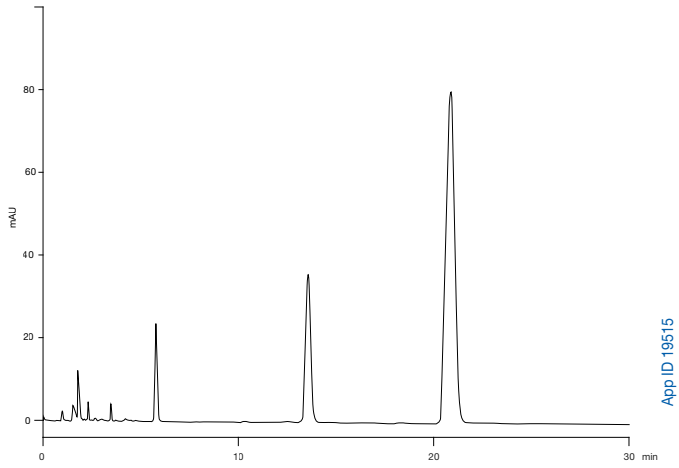


Figure 2.
Chromatogram for Norethindrone, Progesterone, and Mestranol Standard Solution on a Kinetex 2.6 µm C8 150 x 4.6 mm Column, as Specified in the USP Monograph for Norethindrone and Mestranol Tablets

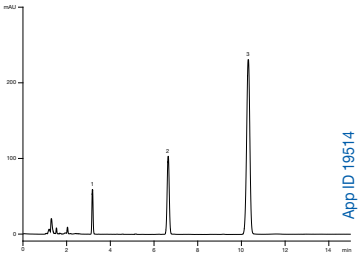
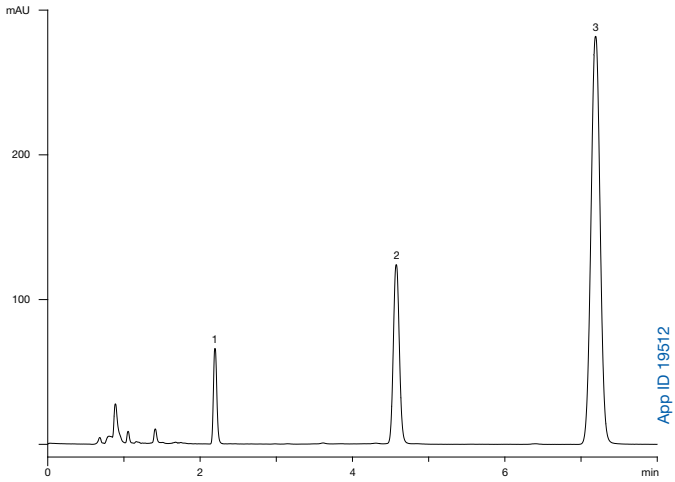


Table 1.
Summary of Results

Column	System Suitability	Fully porous 3 µm C8	Kinetex 2.6 µm C8				
Length (mm)	-	150	150	100		75	
Flow Rate (mL/min)	-	1.0	1.0	1.0	1.5	1.0	1.5
Efficiency	NLT 6000	13256	27397	19298	19557	15913	16850
Resolution	NLT 5.0	12.29	18.03	15.43	15.31	14.54	14.59

Figure 3.
Chromatogram for Norethindrone, Progesterone, and Mestranol Standard Solution at 1.0 mL/min on a Kinetex® 2.6 µm C8 Column, as Specified in the USP Monograph for Norethindrone and Mestranol Tablets

a) 100 x 4.6 mm



b) 75 x 4.6 mm

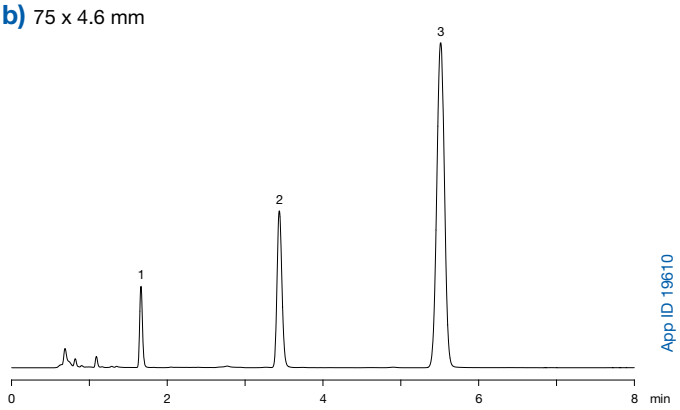
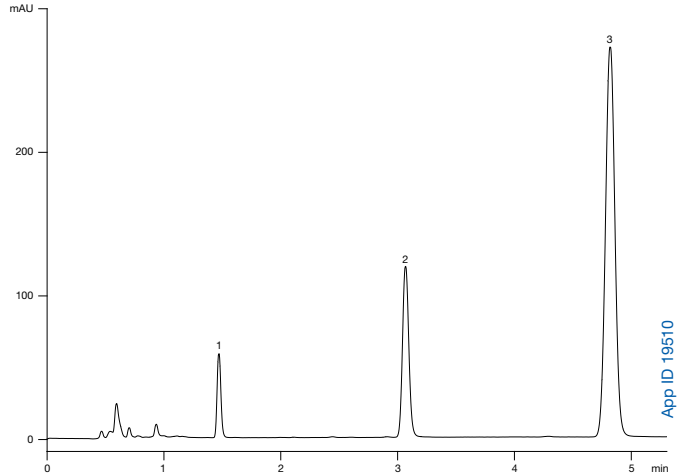
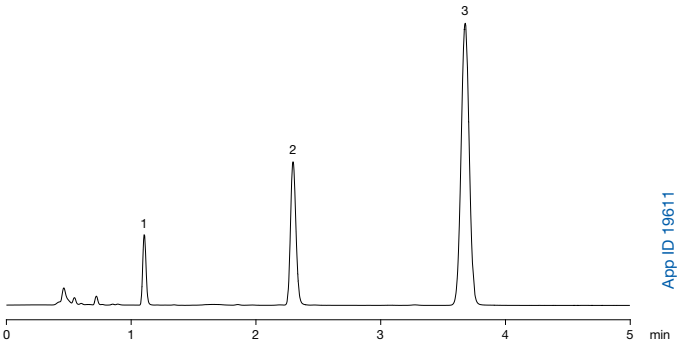


Figure 4.
Chromatogram for Norethindrone, Progesterone, and Mestranol Standard Solution at 1.5 mL/min on a Kinetex 2.6 µm C8 Column, as Specified in the USP Monograph for Norethindrone and Mestranol Tablets

a) 100 x 4.6 mm



b) 75 x 4.6 mm



minutes with an increase in pressure from 150 to 225 bar (~3265 psi), which is just over 50 % of the maximum operating pressure for conventional HPLC systems. While a 50 % increase in flow rate represents the maximum allowable adjustment, increasing flow rate less than the maximum allowed can still provide benefits for laboratories looking to improve productivity with shorter, high efficiency core-shell columns.

With the combination of faster flow rate and shorter column length, the Kinetex 2.6 µm C8 100 x 4.6 and 75 x 4.6 mm columns also meet the system suitability requirements for this monograph method. The measured efficiency and resolution for each column at each flow rate are summarized in **Table 1.**

Conclusions

The USP assay for norethindrone and mestranol on Kinetex C8 columns of different lengths at different flow rates illustrates the many benefits provided by high efficiency core-shell particle technology. These columns provide extremely high chromatographic efficiency, which can be leveraged to help laboratories reduce analysis times, increase productivity, and/or reduce solvent usage in ways that meet their needs.

References

1. USP Monograph for Norethindrone and Mestranol Tablets, USP33-NF28, 2010.
2. USP General Chapter <621>, USP33-NF28, 2010.

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Kinetex® Ordering Information

2.6 µm Analytical Columns (mm)

	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard™ Ultra Cartridges‡ /3pk	KrudKatcher™ Ultra In-Line Filter* /3pk
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768	AF0-8497
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768	AF0-8497
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJ0-8770	AF0-8497
PFP	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0	AJ0-8773	AF0-8497
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AJ0-8772	AF0-8497

for 4.6 mm ID

‡SecurityGuard Ultra cartridges require holder, Part No.: AJ0-9000. Check for availability in your country.
*KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.

2.6 µm MidBore™ Columns (mm)

	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard Ultra Cartridges‡ /3pk	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	—	00B-4496-Y0	—	00D-4496-Y0	—	AJ0-8775	AF0-8497
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775	AF0-8497
C8	—	00B-4497-Y0	—	00D-4497-Y0	—	AJ0-8777	AF0-8497
PFP	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0	AJ0-8780	AF0-8497
HILIC	—	—	—	—	00F-4461-Y0	AJ0-8779	AF0-8497

for 3.0 mm ID

2.6 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard Ultra Cartridges‡ /3pk	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782	AF0-8497
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782	AF0-8497
C8	00A-4497-AN	00B-4497-AN	00D-4497-AN	00F-4497-AN	AJ0-8784	AF0-8497
PFP	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN	AJ0-8787	AF0-8497
HILIC	—	00B-4461-AN	00D-4461-AN	00F-4461-AN	AJ0-8786	AF0-8497

for 2.1 mm ID

1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard Ultra Cartridges‡ /3pk	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	00B-4498-AN	00D-4498-AN	—	AJ0-8782	AF0-8497
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782	AF0-8497
C8	00B-4499-AN	00D-4499-AN	—	AJ0-8784	AF0-8497
PFP	00B-4476-AN	00D-4476-AN	00F-4476-AN	AJ0-8787	AF0-8497
HILIC	00B-4474-AN	—	—	AJ0-8786	AF0-8497

for 2.1 mm ID

guarantee

If Kinetex core-shell technology does not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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