USP Analysis of Norethindrone and Mestranol Tablets with Agilent Poroshell 120 EC-CN and EC-C8 Columns

Application Note

Pharmaceuticals

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Abstract

Norethindrone, mestranol, and progesterone were analyzed according to the United States Pharmacopeia (USP) analysis for norethindrone and mestranol tablets. The dissolution and assay analyses were improved by using superficially porous Agilent Poroshell 120 columns as compared to the USP-suggested 5 µm columns. Each method was adjusted within the guidelines in USP Chapter 621 to allow for time and solvent savings with the Poroshell 120 columns. All chromatographic system requirements were met with the improved superficially porous column.

Introduction

There is significant interest in transferring LC methods to superficially porous particles from larger 5 µm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2 µm totally porous particles. This is attributed primarily to a shorter mass transfer distance and a narrower particle size distribution. Furthermore, the larger particle size results in lower backpressure, allowing for these columns to be implemented in methods on virtually any LC system. The benefits of transferring from larger particle columns are very significant time and cost savings, because superficially porous particles are optimally run at faster flow rates and achieve similar resolution with a much shorter column length [1,2].

This application note describes two methods from the USP for the analysis of norethindrone and mestranol tablets that were transferred from suggested 5 µm columns to shorter 2.7 µm superficially porous Agilent Poroshell 120 columns. Each analysis was compared against the USP chromatographic system requirements to ensure column suitability for the analysis. All method modifications are allowable within USP Chapter 621.

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Materials and Methods

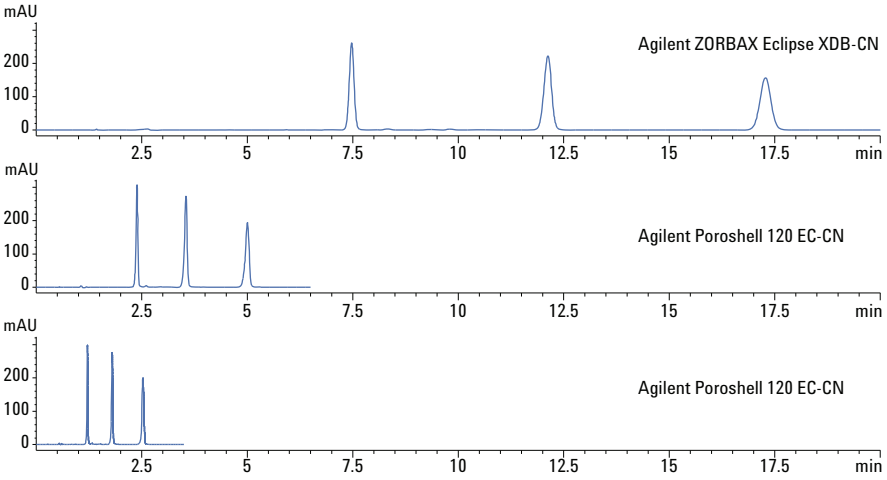
The instrument setup was optimized for lowest possible extra column volume with short 0.075 mm id capillaries found in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [3].

Norethindrone, mestranol, and progesterone were purchased from Sigma-Aldrich Corp. Acetonitrile was purchased from Honeywell International Inc. Water was 18 Mohm.cm Milli-Q.

Results and Discussion

USP dissolution analysis of norethindrone and mestranol tablets

Figure 1 shows the USP dissolution analysis for norethindrone and mestranol tablets, with progesterone as an internal standard. The top chromatogram shows the analysis performed as specified by the USP with a 4.6 × 250 mm, 5 µm column with L10 packing, which in this case was an Agilent ZORBAX Eclipse XDB-CN column. The three compounds were easily separated in approximately 18 minutes.



Conditions

Columns:	Agilent Poroshell 120 EC-CN, 3.0 × 100 mm, 2.7 µm (p/n 695975-305) Agilent ZORBAX Eclipse XDB-CN, 4.6 × 250 mm, 5 µm (p/n 990967-905)
Samples:	Norethindrone, mestranol, progesterone
Eluent:	A = H_2O B = CH_3CN
Injection volume:	5 µL for 4.6 × 250 mm column, 1 µL for 3.0 × 100 mm column
Flow rate:	1 mL/min for 4.6 × 250 mm column, 0.43 mL/min or 0.85 mL/min for 3.0 × 100 mm column
Isocratic:	40% B
Temperature:	25 °C
Detector:	205 nm
Instrument:	Agilent 1290 Infinity LC

Figure 1. USP dissolution analysis of norethindrone and mestranol tablets using Agilent ZORBAX Eclipse XDB-CN and Agilent Poroshell 120 EC-CN columns. The bottom chromatogram shows the Poroshell 120 EC-CN column used at its optimal flow rate.

Elution order

1. Norethindrone (0.5 mg/mL)
2. Progesterone (0.5 mg/mL)
3. Mestranol (0.05 mg/mL)

According to the guidelines in USP Chapter 621, the 4.6 × 250 mm, 5 µm analysis can be transferred to a 3.0 × 100 mm, 2.7 µm Poroshell 120 EC-CN column, shown in the middle chromatogram of Figure 1. The bottom chromatogram shows the same Poroshell 120 EC-CN column used at its optimal flow rate, which accomplished the desired separation in 2.5 minutes.

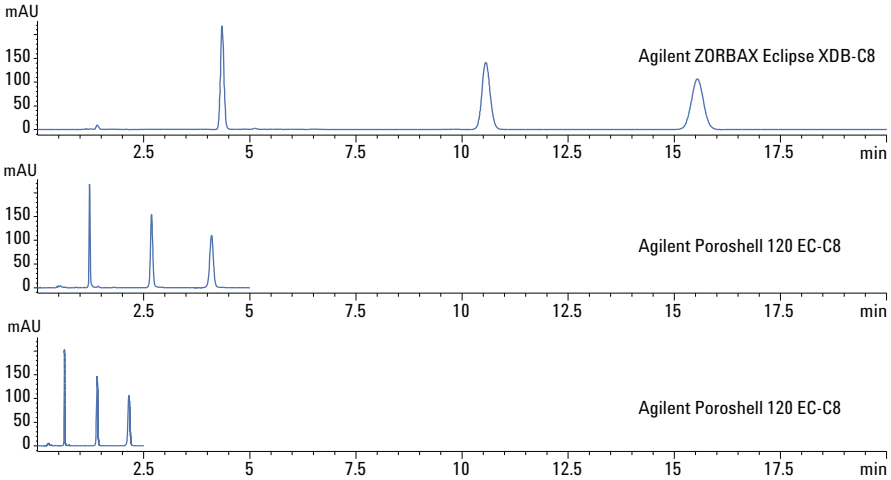
Table 1 lists the USP chromatographic system requirements and the measured values for each of the three chromatograms found in Figure 1. Not only was the 5 µm column suitable for this analysis, but so was the 2.7 µm Poroshell 120 column. Additionally, the Poroshell 120 column, when used at its optimal flow rate, saved significant time and solvent compared to the original analysis, allowing for increased productivity as well as substantial cost savings.

Table 1. USP chromatographic system requirements and measurements for the dissolution analysis for norethindrone and mestranol tablets (N denotes measured values for norethindrone, P for progesterone, and M for mestranol)

USP chromatographic system requirements	**5 µm (2 mL/min)**	**2.7 µm (0.43 mL/min)**	**2.7 µm (0.85 mL/min)**
Relative standard deviation for replicate injections is not greater than 3.0%	N: 0.26% P: 0.24% M: 0.23%	N: 0.92% P: 0.56% M: 0.39%	N: 0.51% P: 0.56% M: 0.39%
Minimum number of theoretical plates for mestranol is 4,000	M: 20304	M: 14790	M: 16297
Tailing factors for norethindrone and mestranol peaks do not exceed 1.5	N: 1.01 M: 1.00	N: 0.87 M: 0.83	N: 0.92 M: 0.86
The relative retention times are approximately 0.4 for norethindrone and 1.0 for mestranol	N: 0.43 M: 1.0	N: 0.48 M: 1.0	N: 0.48 M: 1.0

USP assay analysis of norethindrone and mestranol tablets

Figure 2 shows the USP assay analysis for norethindrone and mestranol tablets, with progesterone as an internal standard (elution order as before). The top chromatogram was generated using the USP-specified 4.6 × 150 mm, 5 µm L7 (ZORBAX Eclipse XDB-C8) column. The middle chromatogram is a direct transfer to a 4.6 × 50 mm, 2.7 µm Poroshell 120 EC-C8 column. The bottom chromatogram shows the Poroshell 120 column used at its optimal flow rate. Analysis time for these theree compounds was reduced from 16 minutes to less than 2.5 minutes with the Poroshell 120 column.



Conditions

Columns:	Agilent Poroshell 120 EC-C8, 4.6 × 50 mm, 2.7 µm (p/n 699975-906) Agilent ZORBAX Eclipse XDB-C8, 4.6 × 150 mm, 5 µm (p/n 993967-906)
Samples:	Norethindrone, mestranol, progesterone
Eluent:	A = H_2O B = CH_3CN
Injection volume:	3 µL for 4.6 × 150 mm column, 1 µL for 4.6 × 50 mm column
Flow rate:	1 mL/min for 4.6 × 150 mm column, 1 mL/min or 2 mL/min for 4.6 × 50 mm column
Isocratic:	50% B
Temperature:	25 °C
Detector:	200 nm
Instrument:	Agilent 1290 Infinity LC

Figure 2. USP assay analysis of norethindrone and mestranol tablets using Agilent ZORBAX Eclipse XDB-C8 and Agilent Poroshell 120 EC-C8 columns. The bottom chromatogram shows the Agilent Poroshell 120 EC-CN column used at its optimal flow rate.

Table 2 shows that all chromatograms met the USP chromatographic system requirements. The superficially porous Poroshell 120 column can easily be substituted for the traditional 5 µm column to save costs and improve productivity.

Table 2. USP chromatographic system requirements and measurements for the assay analysis of norethindrone and mestranol tablets (N denotes measured values for norethindrone, P for progesterone, and M for mestranol)

USP chromatographic system requirements	5 µm (1 mL/min)	2.7 µm (1 mL/min)	2.7 µm (2 mL/min)
Column efficiency determined from the mestranol peak is not less than 6,000 theoretical plates	M: 13571	M: 10718	M: 10480
Resolution between progesterone and mestranol is not less than 5.0	Rs (P,M): 11.1	Rs (P,M): 10.9	Rs (P,M): 10.8
Relative standard deviation for replicate injections is not greater than 2.0%	N: 0.26% P: 0.29% M: 0.28%	N: 0.91% P: 0.78% M: 0.59%	N: 1.4% P: 0.95% M: 0.71%
The relative retention times are about 2.5 for mestranol and 1.0 for norethindrone	M: 3.6 N: 1.0	M: 3.3 N: 1.0	M: 3.4 N: 1.0

Conclusions

Superficially porous Agilent Poroshell 120 columns were successfully substituted for traditional 5 µm columns for the USP dissolution and assay analyses of norethindrone and mestranol tablets. Smaller dimension Poroshell 120 columns could be used to improve productivity and to save time and money over larger 5 µm columns, while meeting all USP requirements for the chromatographic system.

References

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Printed in the USA
January 7, 2013
5991-1688EN

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