An Improved HPLC Method for the Separation of Norethindrone and Mestranol with Progesterone as Internal Standard

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Key Words

Syncronis, progesterone, mestranol, norethindrone, HPLC, C8, USP code L7

Abstract

This work demonstrates the use of a Thermo Scientific™ Syncronis™ 3 µm C8 column to produce effective separation of norethindrone, progesterone and mestranol giving equivalent results to those required from the USP method but in less time.

Introduction

One of the key goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained. The Syncronis column range has been engineered to provide exceptional reproducibility due to its highly pure, high surface area silica.

One area where this is important is in the manufacture and testing of pharmaceutical products in order to maintain product quality and consistency in the manufacturing process. A number of companies use standard USP methods to support quality testing but traditionally these have been developed on older column technologies. The development of new robust columns such as Syncronis and an increased acceptance of the use of smaller particle sizes present an opportunity to update such methods to take advantage of the superior reproducibility and robustness that can be achieved when using these columns.

This approach is illustrated in this work demonstrating the analysis of three compounds used in some brands of birth-control medication. Norethindrone and mestranol are the active components; progesterone is used as an internal standard in the original USP method.

Experimental Details

Consumables	Part Number
Norethindrone, progesterone and mestranol standards were obtained from commercial suppliers.	
Fisher Scientific HPLC grade acetonitrile	A/0626/17
18 MΩ HPLC grade water from Thermo Scientific Smart2Pure™ system	13217449

Sample Handling	Part Number
Premium 8mm thread, 2 mL clear screw vial, seal, cap (convenience pack)	60180-600

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Sample Preparation
Prepare working standards to 200 µg/mL in water:acetonitrile (1:1, v:v)

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Dionex™ UltiMate™ 3000 RS system consisting of LPG-3600-RS dual-ternary pump, WPS-3000 RS thermostatted split-loop autosampler, TCC-3000 RS column thermostat, DAD-3000 RS diode array detector	
Column:	Syncronis C8, 3.0 µm, 100 x 3 mm	97203-103030
Mobile phase:	water : acetonitrile 40:60 (v:v)	
Flow rate:	1000 µL/min	
Column temperature:	40 °C	
Injection details:	5 µL partial loop	
Injection wash solvent:	water / acetonitrile 1:1 (v:v)	
UV detector wavelength:	200 nm	
Backpressure:	207 bar at 1000 µL/min	

Results

Good separation of all three components was achieved under isocratic method conditions. Six replicate injections showed excellent inter-sample reproducibility with RSD values of less than 1% for retention time, peak area and resolution. Peak shape was excellent as demonstrated by asymmetry values of less than 1.25 overall with the mestranol peak having an average value of 1.06 calculated by the USP criteria. This data is presented below in Table 1.

	Norethindrone (1)			Progesterone (2)				Mestranol (3)			
	t_R (min)	Peak area	A_s	t_R (min)	Peak area	$R_{s\ 1,2}$	A_s	t_R (min)	Peak area	$R_{s\ 2,3}$	A_s
Mean	1.22	936859	1.22	2.48	1320688	19.6	1.14	3.17	4350982	7.3	1.06
CV %	0	0.87	1.63	0.19	0.81	0.24	0.94	0.24	0.73	0.79	0.45

Table 1: Data from six replicate analyses of a mixture of norethindrone, progesterone and mestranol
Retention time (t_R), peak resolution (R_s), peak asymmetry (A_s)

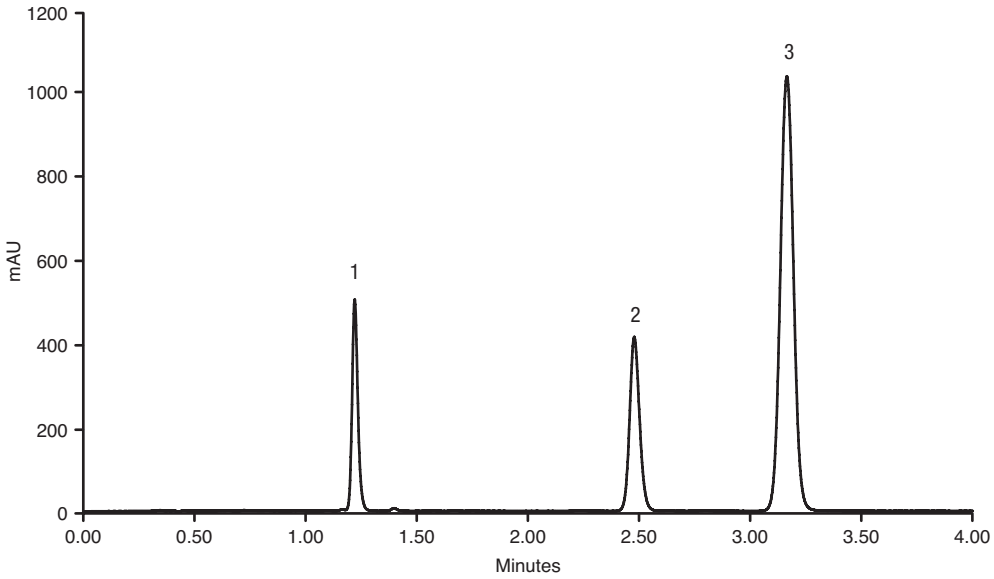

Figure 1: UV chromatogram (200 nm) showing separation of three analytes, norethindrone (1), progesterone (2) and mestranol (3)

Column efficiency and peak resolution meet the USP criteria as shown in Table 2.

USP method criteria	Average result obtained from this method
Column efficiency of more than 6000 theoretical plates, determined from the mestranol peak	14 010 theoretical plates
Resolution between progesterone and mestranol peaks greater than 5.0	Resolution = 7.3

Table 2: Comparison of USP method criteria and results obtained from this application. Average values obtained from six replicate injections.

Conclusion

This work demonstrates that improved chromatography, that exceeds the criteria set in established USP methods, can be achieved when using smaller particle size columns. This is achieved without resorting to high backpressure UHPLC solutions when this hardware is not available. In this instance the run time is reduced to 4 minutes compared to the defined USP method where run times in excess of 20 minutes can be observed. The reduction in run time offers manufacturing benefits including faster release of product, quicker release of manufacturing equipment following cleaning validation, increased sample capacity per instrument and consequential benefits of reduced solvent consumption and reduced waste disposal costs.

The excellent reproducibility of the data supports better consistency of peak identification, quantitation and data processing including improved precision when using such data in system control charts and other process monitoring tools.

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