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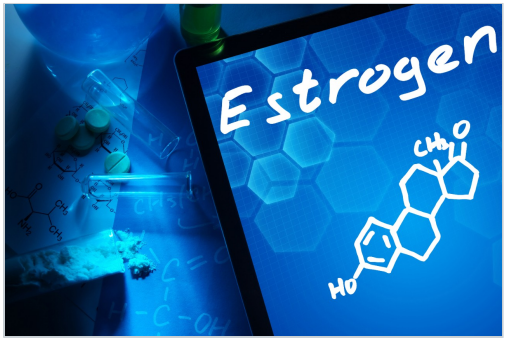
Application Note

Chromatographic Purity of Estradiol Using the ACQUITY UPC^2 System

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrate the results obtained from the impurity analysis of estradiol with the ACQUITY UPC^2 System are equal to or better than those achieved using the current USP method.

Benefits

The UPC^2 method used for the estimation of the chromatographic purity of estradiol was three times faster than the current normal phase HPLC method and reduced cost per analysis by more than 100 times.

Introduction

Currently, the United States Pharmacopeia (USP) method for the estimation of chromatographic purity of estradiol utilizes a 4.6 x 250 mm silica column and a mobile phase consisting of 2,2,4-trimethylpentane, *n*-butyl chloride, and methanol 45:4:1 at 2 mL/minute. Since many laboratories have a desire to limit the use of aliphatic hydrocarbons and chlorinated solvents, alternative chromatographic techniques, like supercritical fluid chromatography (SFC) must be evaluated.

The Waters ACQUITY UPC^2 System was used to develop a method for the evaluation of the chromatographic purity of estradiol. Results obtained from the UltraPerformance Convergence Chromatography (UPC^2) method were directly compared to results obtained for the current USP method to detect estradiol impurities. The results from both techniques were similar with the UPC^2 method showing adequate sensitivity to detect impurities in estradiol equal to those obtained from the normal phase HPLC USP method. In addition, when using UPC^2, sample run time is reduced and overall cost per analysis (based on solvent usage and waste disposal costs) is significantly reduced.

Experimental

The UPC^2 method conditions were as follows:

Column:	ACQUITY UPC^2 BEH, 2.1 x 150 mm, 1.7 µm
Mobile phase:	A=CO_2 B=1:1 Methanol/2-Propanol
Back pressure:	130 bar/1880 psi
Temp.:	45 °C
Detection:	UV/PDA at 280 nm

Gradient:

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	1.2	97	3	–
15.0	1.2	93	7	8
15.1	1.2	97	3	6
20.0	1.2	97	3	6

Results and Discussion

A sample of estradiol was prepared and analyzed using the current USP method as shown in Figure 1. The results of this analysis were used to compare with the results obtained in the method developed on an ACQUITY UPC^2 System as seen in Figure 2 using the identical sample preparation.

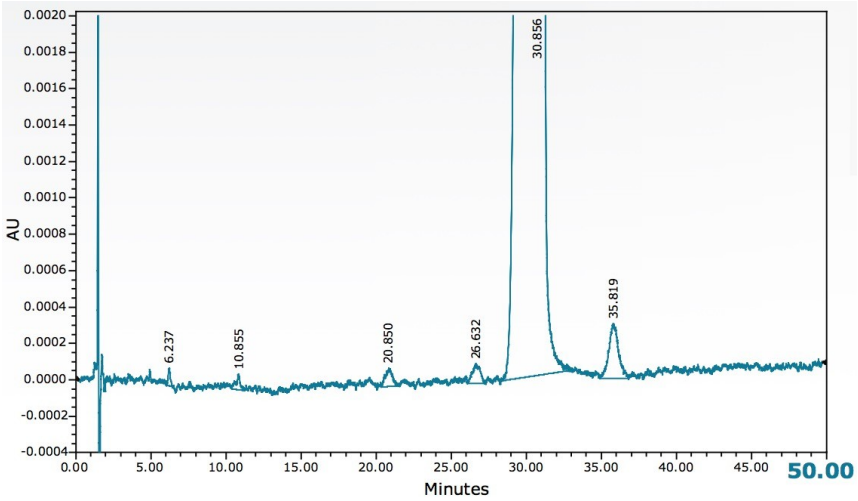


Figure 1. Normal phase HPLC separation of estradiol and impurities.

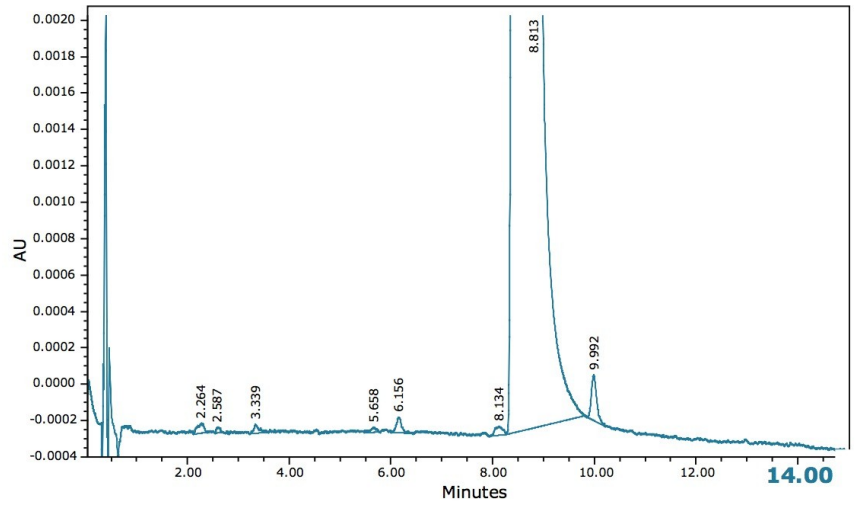


Figure 2. ACQUITY UPC^2 separation of estradiol and impurities.

A comparison of results from the two methods is shown in Table 1. Both the normal phase HPLC and UPC^2 methods detected at least five impurities below 0.1% (based on area). Signal to noise values for peaks in the range of 0.01% were all approximately 3:1 for both methods with the UPC^2 results giving slightly higher values. The largest impurity (approximately 0.05% based on area) gave a signal to noise value of 16:1 for UPC^2 and 9:1 for normal phase HPLC. These results clearly show

that the ACQUITY UPC^2 System can be used to successfully analyze minor impurities from estradiol. The run time of the UPC^2 method was considerably shorter than the normal phase HPLC method (20 minutes compared to 60 minutes) resulting in an increase in lab productivity. An analysis of cost per run showed that the cost of solvent for the normal phase HPLC

Compound	UPC^2 RT	%Area	S/N	Normal phase HPLC RT	%Area	S/N
Unk. impurity	2.26	0.012	3.4	6.24	0.006	2.9
Unk. impurity	2.59	0.004	1.9	Not Found		
Unk. impurity	3.34	0.010	3.1	10.86	0.010	2.7
Unk. impurity	5.66	0.006	1.7	Not Found		
Unk. impurity	6.15	0.016	5.5	20.85	0.018	3.0
Unk. impurity	8.13	0.013	3.1	26.63	0.021	3.2
Estradiol	8.81	99.890	–	30.86	99.87	–
Main impurity	9.99	0.046	16.0	36.81	0.077	9.2

Table 1. Comparison of estradiol impurity detection using UPC^2 vs. normal phase HPLC.

Conclusion

A method for the estimation of the chromatographic purity of estradiol was developed, using the ACQUITY UPC^2 System. This UPC^2 method was three times faster than the current normal phase method from the USP. In addition to speed, this method reduced the cost per analysis by more than 100 times, primarily by reducing the need for aliphatic hydrocarbons and chlorinated solvents. Required sensitivity levels were achieved in the UPC^2 method with impurities as low as 0.01% of the main peaks being easily detected. The ACQUITY UPC^2 System is an ideal choice for laboratories looking for an alternative to conventional normal phase HPLC.

Featured Products

ACQUITY UPC2 System

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