EVALUATION OF THE CHROMATOGRAPHIC PURITY OF ESTRADIOL USING SUPERCRITICAL FLUID CHROMATOGRAPHY

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INTRODUCTION

Supercritical Fluid Chromatography (SFC) is a normal phase separation technique that uses carbon dioxide as the main mobile phase and often employs the use of polar modifiers such as methanol. Since the principles of SFC are similar to those of HPLC, methods should be able to be converted to SFC providing reduced solvent usage and disposal which will lower cost per analysis cost while enhancing health, safety, environmental initiatives. Methods converted to a SFC solution must maintain data quality and must produce results that are equivalent to the current normal phase methods. SFC is generally considered a cost effective, sustainable and green technology, but widespread adoption of analytical SFC, particularly in the area of impurity analysis, has been limited by instrumentation which does not provide sensitivity levels similar to modern HPLC systems. Using a newly designed analytical supercritical fluid chromatography system, ACQUITY UPSFC® (figure 1), a method for the evaluation of the chromatographic purity of estradiol was developed. Results obtained from the UPSFC method were directly compared to results obtained for the current United States Pharmacopeia (USP) method for estradiol impurities. These results were similar with the UPSFC method showing enough sensitivity to detect impurities in estradiol similar to those obtained from the normal phase (NP-HPLC) USP method.

Figure 1. Waters ACQUITY UPSFC system.

METHODS

Currently, the (USP) method for the estimation of chromatographic purity of estradiol utilizes a 4.6-mm × 25 cm silica column that uses a mobile phase consisting 2,2,4-trimethylpentane, *n*-butyl chloride, and methanol (45:4:1) running at 2 mL/minute. This method is reliable and robust and generates acceptable results. On the downside, this method is relatively long with a 60 minute run time and is fairly costly using approximately $6.00 worth of solvent per run. Further, each run generates over 100 mL of waste solvent which, along with disposal costs, would not generally be considered environmentally friendly.

A sample of estradiol was prepared and analyzed using the current USP method (Figure 2). The results of this analysis were used to compare against the results obtained with the method developed on an ACQUITY UPSFC System (Figure 3) using the identical sample preparation. The UPSFC method conditions were as follows:

System:	ACQUITY UPSFC consisting of a UPSFC-Binary Solvent Manager, UPSFC-Sample Manager, UPSFC-Photo Diode Array detector, and a UPSFC Manager
Column:	VIRIDIS™ Hybrid, 2.1 X 150 mm 1.7 µm
Mobile Phase:	A=CO_2 B=Methanol/2-Propanol 1:1
Back Pressure	130 Bar/1880 psi
Temperature	45 °C
Detection	UV/PDA at 280 nm (compensated 500 – 600 nm)
Inj. Volume	2 µL
Data System	Empower™ II

Time (min)	Flow (mL/min)	%A	%B	Curve
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

UPSFC Gradient Table

RESULTS AND DISCUSSION

A comparison of results from the two methods is shown in Table 1. Both the NP-HPLC and UPSFC method detected at least 5 impurities below 0.1% (based on area). Signal to noise values for peaks in the range of 0.01% were all about 3:1 for both methods with the UPSFC results giving slightly higher values. The largest impurity (approximately 0.05% based on area) gave a signal to noise value of 16:1 for UPSFC and 9:1 for NP-HPLC. These results clearly show the UPSFC system has the sensitivity required to successfully analyze minor impurities from estradiol. Retention time reproducibility for Estradiol and the main impurity with the NP-HPLC method for were 0.8% RSD and 0.5% RSD respectively. The UPSFC method's retention time reproducibility was slightly better with %RSD < 0.15 for both estradiol and the main impurity. Although the USP method only calls for single wavelength UV data (280 nm) to be collected, full spectral data (200—400 nm) was collected with each method using the photodiode array detector. Spectra data from each method (normal phase and UPSFC) from the main impurity peak was compared and is shown in figure 4.

Compound	RT	Area%	S/N	RT	Area%	S/N
Unknown Impurity	2.26	0.012	3.4 : 1	6.24	0.006	2.9 : 1
Unknown Impurity	2.59	0.004	1.9 : 1	Not Found		
Unknown Impurity	3.34	0.010	3.1 : 1	10.86	0.010	2.7 : 1
Unknown Impurity	5.66	0.006	1.7 : 1	Not Found		
Unknown Impurity	6.15	0.016	5.5 : 1	20.85	0.018	3.0 : 1
Unknown Impurity	8.13	0.013	3.1 : 1	26.63	0.021	3.2 : 1
Estradiol	8.81	99.89	———	30.86	99.87	———
Main Impurity	9.99	0.046	16.0 : 1	36.81	0.077	9.2 : 1

Table 1. Comparison of results from a conventional NP-HPLC and UPSFC analysis of impurities of estradiol.

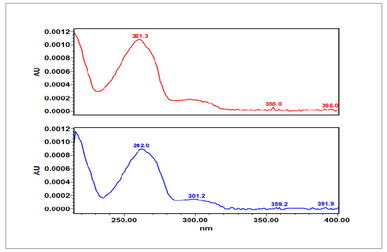

Figure 4 – Main Impurity UV Spectra for NP-HPLC (red, top) and UPSFC (blue, bottom).

The spectral data from both techniques are very similar and demonstrate that UPSFC PDA data can be used for PDA library matching to aid in peak identification. The bigger benefit of the UPSFC method can be seen in the cost benefit analysis. Run time of the UPSFC method is considerably shorter than the NP-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 NP-HPLC method was $5.89 compared to less than $0.05 per run using UPSFC. In total the NPLC method generated, as mixed chlorinated waste for disposal, 108 mL of 2,2,4-trimethylpentane, 9.6 mL of *n*-butyl chloride, and 2.4 mL of methanol. The UPSFC method generated disposal waste of 0.60 mL each of methanol and 2-propanol. The CO_2 used in the separation was vented through the laboratory exhaust. Waste disposal costs were reduced by more than 150 times using the UPSFC method. It can be clearly seen that moving from a normal phase method such as this to an UPSFC methods results in a significant cost savings and improves laboratory productivity with no compromise on the quality of the results produced.

CONCLUSION

- **Using an ACQUITY UPSFC system, a method for the estimation of the chromatographic purity of estradiol was developed.**
- **This UPSFC method was 3 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 UPSFC method with impurities as low as 0.01% of the main peaks being easily detected.**
- **The ACQUITY UPSFC system is an ideal choice for laboratories looking for an alternative to conventional normal phase chromatography.**

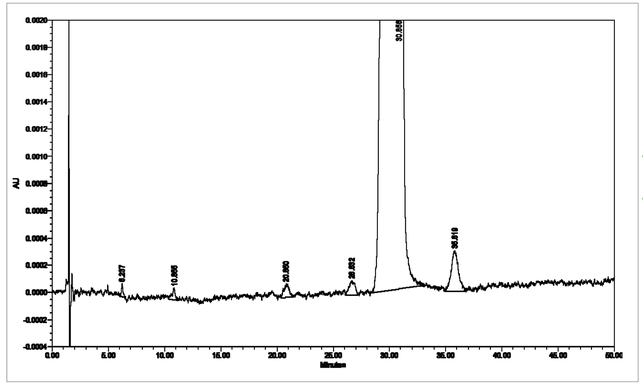

Figure 2 – Normal Phase HPLC separation of Estradiol and Impurities

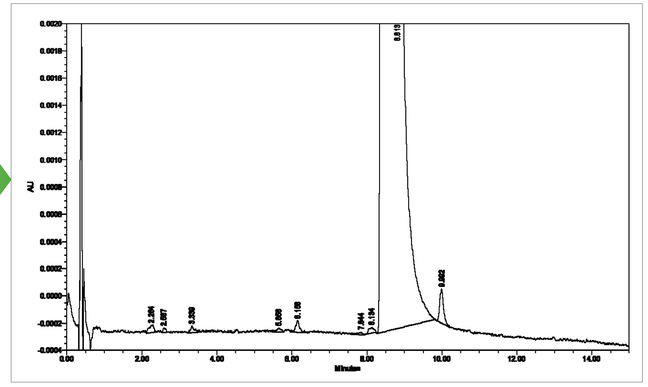

Figure 3 – ACQUITY UPSFC separation of Estradiol and Impurities

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