

Nota de aplicación

Assay of the Drug Substance Anthralin Using the ACQUITY UPC² System

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

Abstract

A compendial normal phase HPLC method for the analysis of anthralin was successfully converted to a supercritical fluid chromatography method using the Waters ACQUITY UPC² System.

Benefits

A USP compendial HPLC method was successfully converted to a high quality UPC² method at a cost of \$0.05 per run (compared to \$0.90) and was 1.6 times faster.

Introduction

Currently, the United States Pharmacopeia (USP) assay for the drug substance anthralin, (9(10H)-Anthracenone, 1,8-dihydroxy-1,8- Dihydroxy-9-anthrone [CAS #1143-38-0] is a normal phase HPLC method. The isocratic separation is done using a 4.6 x 250 mm silica column (L3) with a mobile phase that consists of a mixture of 82:12:6 n-hexane, dichloromethane, and glacial acetic acid at a flow rate of 2 mL/minute as shown in Figure 1. The run time of this current compendial method is approximately 10 minutes (2X of the last major peak). Although this method is rugged and reliable, many laboratories have a desire to decrease the use of typical normal phase chromatography solvents (such as hexane and dichloromethane) for health, safety, environmental, and cost reasons.

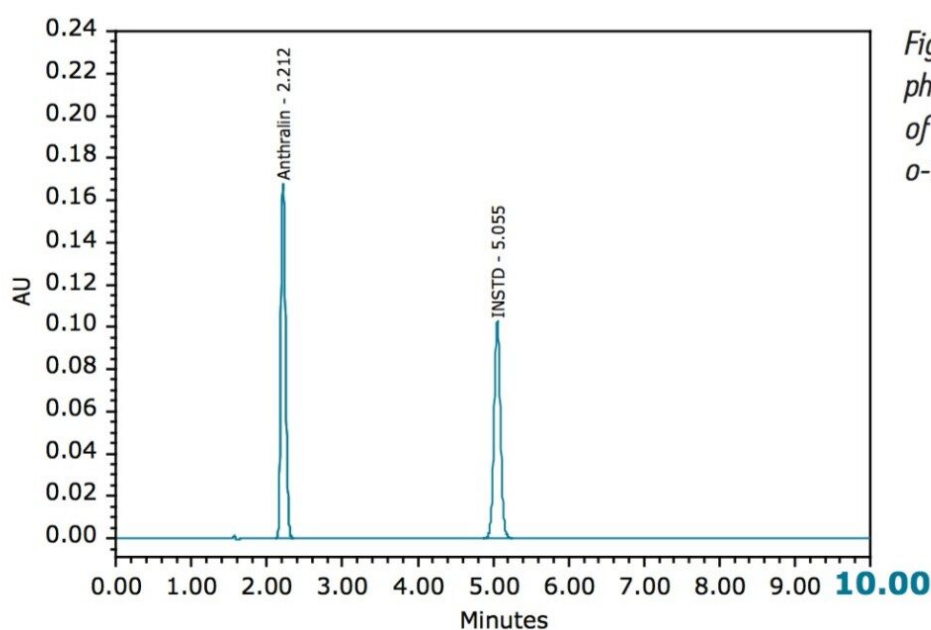


Figure 1. Normal phase HPLC separation of anthralin and o-nitroaniline (INSTD).

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 while enhancing health, safety, and environmental initiatives.

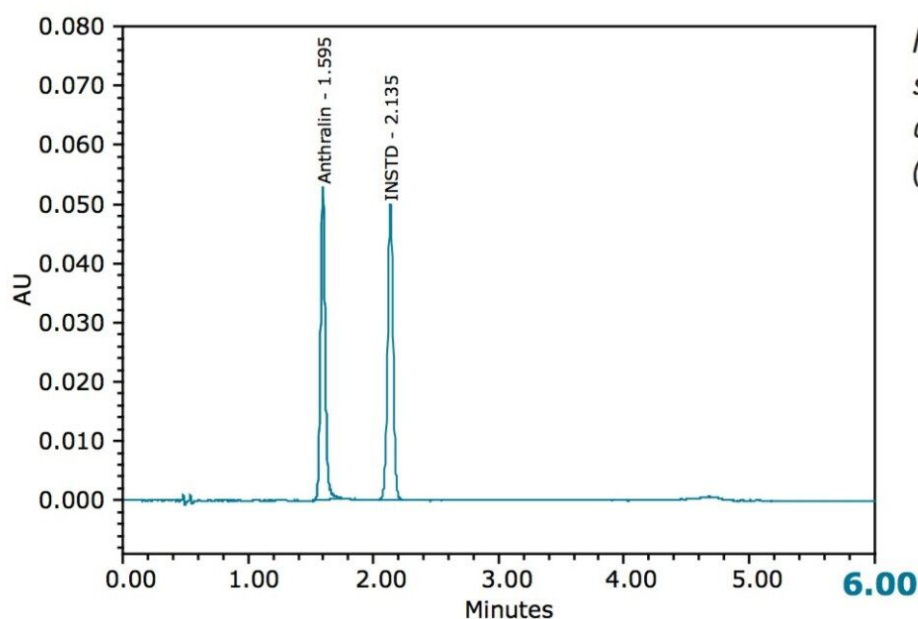


Figure 2. UPC² separation of anthralin and o-nitroaniline (INSTD).

Methods converted to an SFC solution must maintain data quality (retention time reproducibility, resolution between compounds of interest and other components in the sample) and must produce results that are equivalent to the current normal phase method.

Experimental

A sample of anthralin was prepared and analyzed using the current USP method (this sample was also used for the UPC² analysis). The results of this analysis were used to compare the results obtained with the method developed on an ACQUITY UPC² System. The UltraPerformance Convergence Chromatography (UPC²) method conditions were as follows:

Column:	Viridis Silica 2-Ethylpyridine, 4.6 x 150 mm, 5 µm
Temp.:	40 °C
Mobile phase:	95% Carbon dioxide: 5% methanol containing 0.25% glacial acetic acid
Flow rate:	3.5 mL/min
Back pressure:	150 bar
Detection:	UV /PDA at 351 nm

Results and Discussion

A comparison of key suitability parameters are shown in Table 1. In all cases, the results from the converted UPC² method easily met or exceeded the required USP suitability values and were quite favorably comparable to the values from the normal phase method. Interestingly, there was a selectivity change between the two components of the suitability mix (anthralin and danthron) which did not negatively impact the results of the method conversion. The results of the analysis of an unknown purity anthralin sample were in good agreement between the two methods. The assayed anthralin sample contained 94.3% anthralin when analyzed using the normal phase HPLC method and 94.6% when analyzed by UPC².

		USP Required	Normal Phase	UPC ²
Retention time	Anthralin	Not Defined	2.10	1.64
	Danthron	Not Defined	2.52	1.08
	INSTD	Not Defined	5.04	2.17
USP Resolution	Suitability Soln	>1.3	3.00	8.90
USP Resolution	Standard Soln	Not Defined	20.00	6.60
USP Tailing	Anthralin	<1.5	1.23	1.05
	Danthron	Not Defined	1.07	1.05
	INSTD	Not Defined	1.02	0.97
Retention time	Anthralin	Not Defined	0.07	0.60
Reproducibility	Danthron	Not Defined	0.02	0.50
	INSTD	Not Defined	0.01	0.25
Area count	Anthralin	<2.00	0.10	0.40
Reproducibility	Danthron	Not Defined	0.76	0.90
	INSTD	Not Defined	0.08	0.77

Table 1. Key suitability parameters.

In this example, each normal phase HPLC run used 16.4 mL of hexane and 1.2 mL of dichloromethane. In contrast, the UPC² method used only 1.05 mL of methanol. This demonstrates the significant reduction in organic solvent use that can be achieved by moving normal phase methods to UPC². Based on current solvent prices, each normal phase HPLC run costs roughly \$0.90 per run compared to \$0.05 for each UPC² run.

Conclusion

Using the ACQUITY UPC² System, a USP compendial HPLC method was successfully converted to a UPC² method. This new UPC² method produced data of equal or better quality than the current HPLC method, was 1.6 times faster, and consumed less solvent. When high quality results are produced faster, laboratory productivity increases and cost per sample decreases. The ACQUITY UPC² System is an ideal solution for laboratories converting their current normal phase HPLC methods to more efficient and cost effective UPC² methods while enhancing health, safety, and environmental concerns.

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ACQUITY UPC2 System <<https://www.waters.com/134658367>>

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