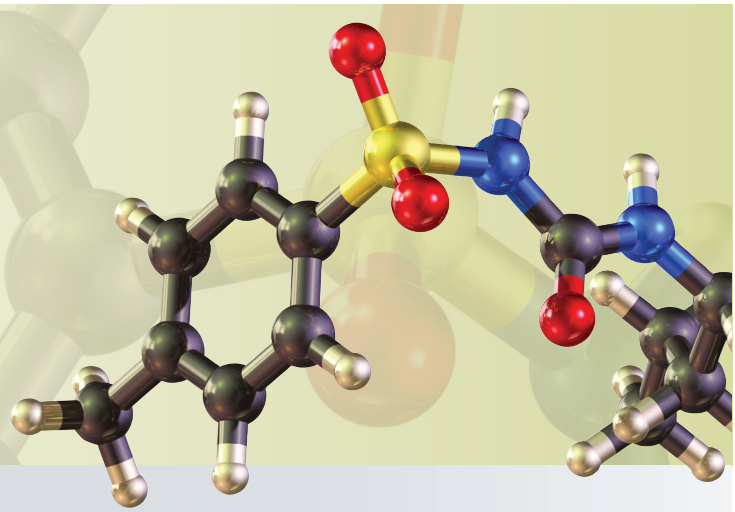
[TECHNOLOGY BRIEF]

Chromatographic Assay of Tolbutamide Using the ACQUITY UPC^2 System



GOAL

To successfully convert the compendial normal phase HPLC method for the assay of the drug substance tolbutamide to a supercritical fluid chromatography method using the Waters® ACQUITY UPC^2™ System.

This new UltraPerformance Convergence Chromatography™ (UPC^2™) method produced data of equal or better quality than the current HPLC method, was 10 times faster, and consumed less solvent.

BACKGROUND

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 reducing solvent usage and disposal which will lower cost per analysis while enhancing green initiatives. Methods converted to an SFC solution must maintain data quality and must produce results that are equivalent to the current normal phase method. Currently, the United States Pharmacopeia (USP) specifies a normal phase HPLC method for the assay of the drug substance tolbutamide (benzenesulfonamide, CAS# 64-77-7). This isocratic separation is done at 1.5 mL/min using a 4.0 x 300 mm, silica column (L3) with a mobile phase that consists of 475:475:20:15:9 hexane, water-saturated-hexane, tetrahydrofuran, alcohol, and glacial acetic acid with a run time of approximately 20 minutes. Like most compendial methods, this method is proven and reliable. It does, however, use a complicated mobile phase mixture that contains hexane and THF, solvents that many labs would like to eliminate in favor of green initiatives and reduced costs.

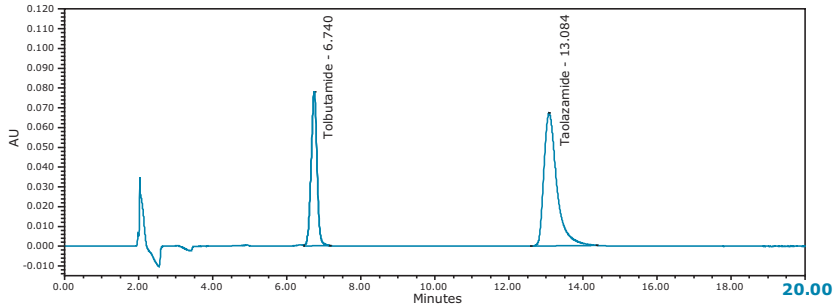


Figure 1. Normal phase HPLC analysis of tolbutamide and tolazamide.

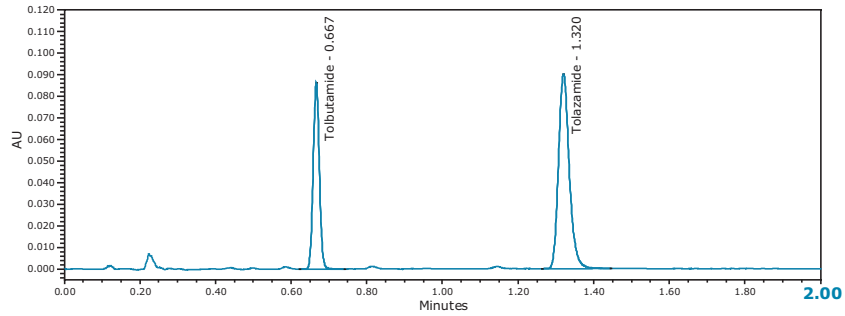


Figure 2. ACQUITY UPC^2 analysis of tolbutamide and tolazamide.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

THE SOLUTION

Samples were prepared and analyzed using the current USP method and contained tolbutamide along with the internal standard tolazamide. The results of this analysis were used to compare the results obtained with the method developed on an ACQUITY UPC^2 System. The UPC^2 method conditions were as follows:

Column:	ACQUITY UPC^2 BEH, 3.0 x100 mm, 1.7 µm
Temperature:	50 °C
Mobile phase:	95% Carbon dioxide: 5% methanol/IPA (1:1) containing 0.2% TFA
Flow rate:	2.5 mL/min
Back pressure:	120 Bar/1740 psi
Detection:	UV /PDA at 254 nm

The current normal phase HPLC method shown in Figure 1 generated acceptable chromatography, although it was noted that there was significant peak tailing of the internal standard peak (tailing factor of 1.65). No effort was made to improve the method as it passed the listed suitability criteria (the relative standard deviation for replicate injections is not more than 2.0%, and the resolution, R, between tolbutamide and tolazamide is not less than 2.0).

Results from the newly developed UPC^2 method also met required USP suitability values (1.2% and 0.9% retention time RSD for tolbutamide and tolazamide respectively and <0.90% RSD for area for both compounds, n=6) and had a significantly faster run time (2.0 min) while maintaining a similar resolution between the 2 compounds of interest (R = ~15). Tailing of the internal standard, tolazamide, was significantly improved (tailing factor of 1.2). It is also interesting to note that a number of small peaks were resolved and detected from the mixture using UPC,^{2} demonstrating the high separation efficiency of this technique. In this example, each normal phase HPLC run used approximately 29 mL of hexane and less than one mL each of THF and ethanol. In contrast, each injection with the UPC^2 method used roughly 0.25 mL each of methanol and IPA. This shows the significant reduction in organic solvent use that can be achieved by moving normal phase methods to UPC.^{2} Based on current solvent prices, each normal phase HPLC run costs roughly $1.40 per run compared to $0.01 for each UPC^2 run indicating the significant cost savings that can be achieved by moving traditional normal phase HPLC methods to UPC.^{2}

SUMMARY

A USP compendial HPLC method was successfully converted to a UPC^2 method, using the ACQUITY UPC^2 System. This new UPC^2 method produced data of equal or better quality than the current HPLC method, was 10 times faster, and consumed less solvent. When high quality results are produced faster, laboratory productivity increases and cost per sample decreases. The ACQUITY UPC^2 System is an ideal solution for laboratories wanting to convert their current normal phase HPLC methods to more efficient and cost effective methods while enhancing health, safety, and environmental concerns.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

February 2012 720004247EN LL-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com