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应用纪要

Take Advantage of New Column Technology: Update USP Methods Using AMDS

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Abstract

This application note illustrates how AMDS can be used to develop faster, more robust methods within existing USP method guidelines.

Benefits

Improved run time, peak symmetry, and robustness

Introduction

Lengthy and sometimes problematic USP methods can be updated to incorporate new advances in column technology while still maintaining the stated USP requirements. In this example, the Waters Automated Method Development System (AMDS) was used to redevelop a USP method for the analysis of diltiazem and related compounds. This illustrates how AMDS can be used to develop faster, more robust methods within existing USP method guidelines.

Experimental

Step 1: Establish a reference point

The USP diltiazem method was reproduced as a starting point for this example. Note the column dimensions, mobile phase and resulting chromatogram in Figure 1. Like most small molecule pharmaceutical products, the diltiazem mixture consists mainly of basic components, which accounts for the poor chromatographic per formance (note peak tailing) and the need for additives in the mobile phase.

Other examples may use different columns (such as µBondaPak), but there is a common thread within many QC laboratories: SOP methods may be largely based on existing methods that use older column chemistry technology. Even though the peak tailing in these methods is excessive by today’ s standards, the USP parameters are met: resolution is greater than 3.0 and the theoretical

plates are greater than 1200. However, this tailing produces reproducibility issue, which can lead to downtime in a QC lab, or worse, cause faulty out of specification (OOS) results.

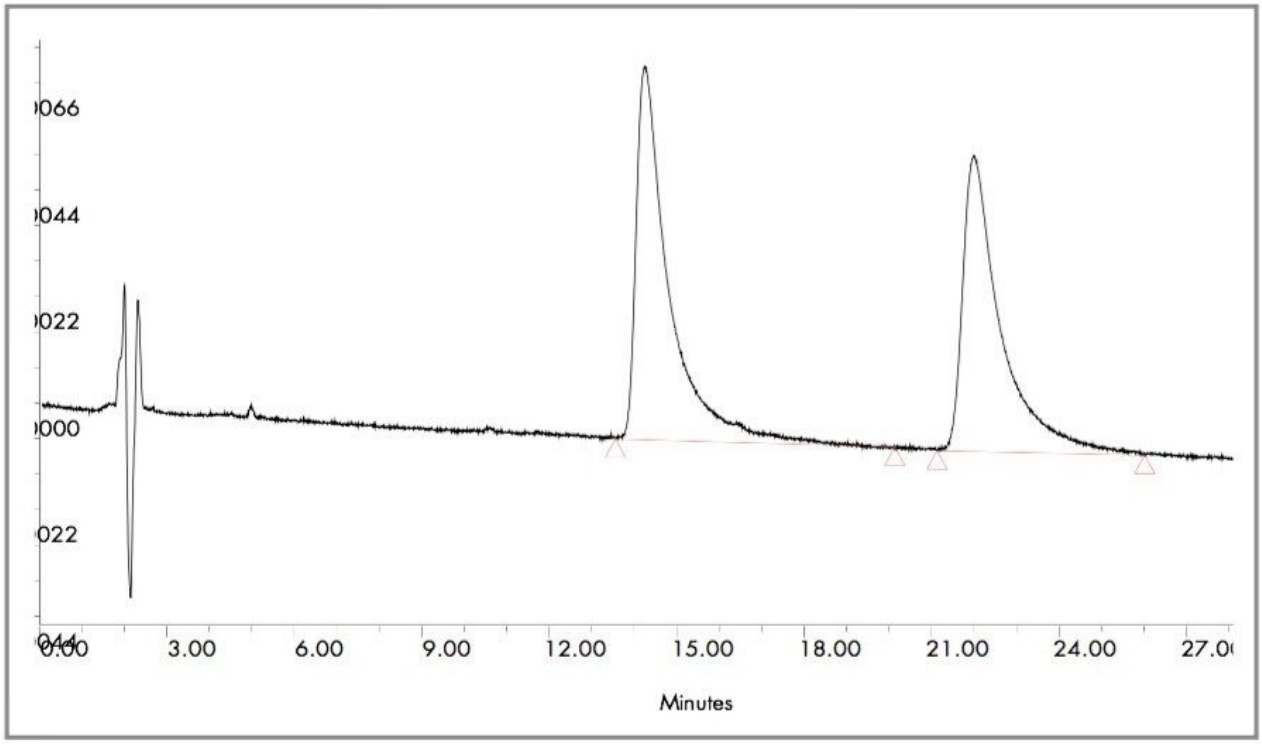

Figure 1. Analysis of desacetyl diltiazem (14.243 min) and related compound diltiazem (21.996 min) using USP diltiazem method. Conditions: Column: Nova-Pak C_{18}, 300 x 3.9 mm; 4 µm; 60 Å; Buffer: 0.1 M sodium acetate buffer with 1.16 g d-10- camphorsuphonic acid; Mobile Phase: 50:25:25 (Buffer: ACN: MeOH); Flow Rate: 1.0 mL/min; Injection Volume: 10 µL; Temperature: 30˚C; Detection: UV @ 240 nm; Instrument: Alliance 2695/2996 PDA.

Step 2: Redevelop the method with Waters Automated Method Development System (AMDS)

Method development should be approached methodically, avoiding trial, and error. Using AMDS, an efficient, SOP-like approach is employed that streamlines the process, saving considerable time. Star ting conditions are determined based on sample characteristics; AMDS automatically re-develops the method based on parameters entered by the user.

AMDS was set up according to the following protocol in response to a wizard-driven interview and user requirements:

Run time <5 minutes	Resolution >3.0 (based on USP requirements)
Isocratic preferred	First elution (min t0): 2 times the void (t0)
Pressure <3000psi	Sample Information: Bases
210-400 nm MAXplot Column switch activated	UV compatible

AMDS suggested starting conditions of pH 9, 10 mM ammonium bicarbonate and an XTerra MS C_{18} 100 x 4.6 mm, 3.5 µm Column. A series of automated chromatographic experiments are held in the AMDS logic queue in case these conditions do not meet the analyst requirements as depicted in the decision tree (Figure 2).

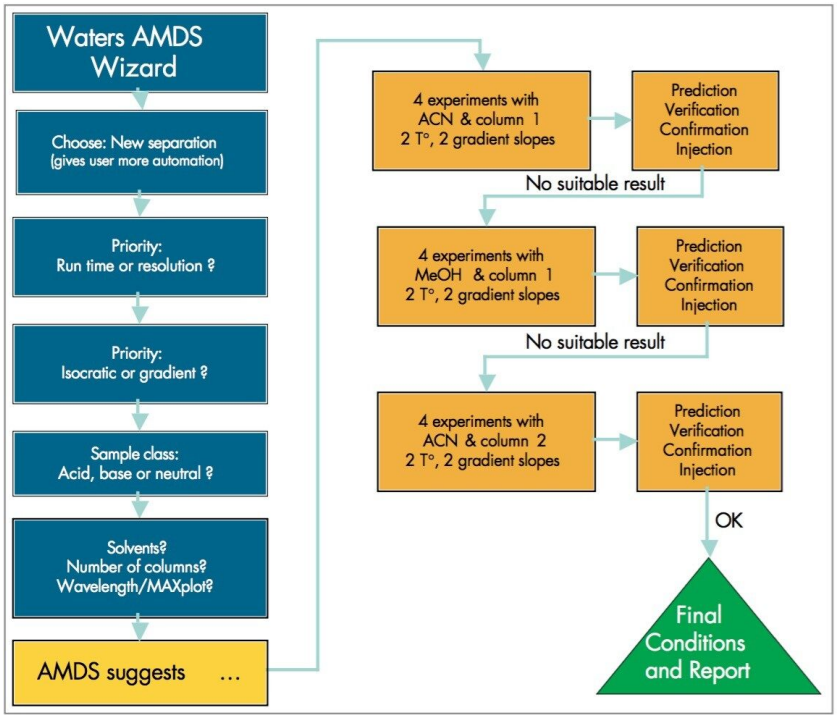

Figure 2. General AMDS decision manager flow path.

Results and Discussion

Using the AMDS-suggested starting conditions, a 3.0 minute separation, Figure 3, was obtained that exceded all of the USP requirements and improved peak shape. Actual results yielded a resolution of 3.2 with over 4000 theoretical plates. The higher pH used in the analysis allows the separation to be performed in a more robust pH zone. In addition, the improved peak symmetry enables more reproducible peak integration.

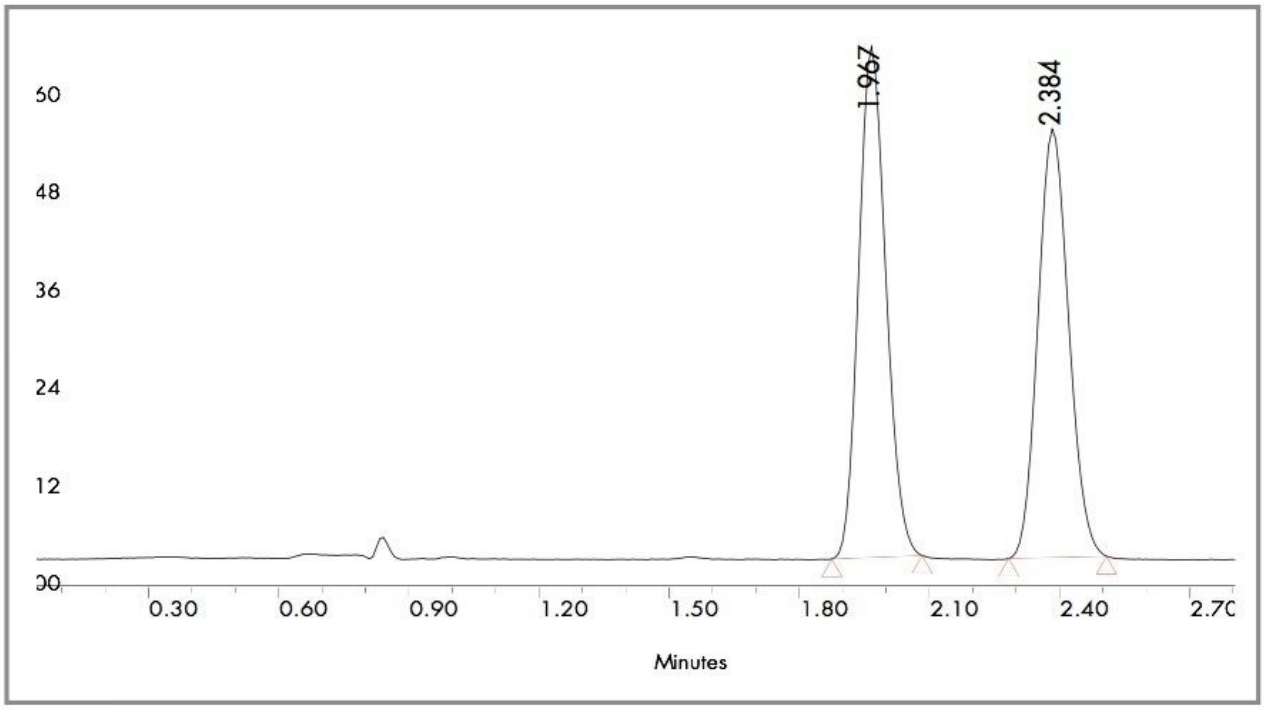

Figure 3. AMDS optimized results for the separation of desacetyl diltiazem diltiazem (1.966 min) and desacetyl diltiazem diltiazem (2.384 min). Conditions: Column: XTerra RP_{18} 100 x 4.6 mm; 3.5 µm; Mobile Phase: 50% A; 50% B, A: pH 9.8 Ammonium Bicarbonate: B: Acetonitrile; Flow Rate: 1.5 mL/min; Injection Volume: 10 µL; Temperature: 30 ˚C; Detection: UV @ 240 nm.

Conclusion

USP methods can be efficiently updated and optimized using the Waters Automated Method

Development System. With AMDS, a new method for the analysis of diltiazem and a related compound was developed that shows improved run time (3 versus 50 minutes), peak symmetry and robustness. The automated capabilites of the AMDS allowed the method development process to run unattended, freeing up the analysts' time to work on other projects.

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