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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 performance (note peak tailing) and the need for additives in the mobile phase.

Other examples may use different columns (such as μ BondaPak®), 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.

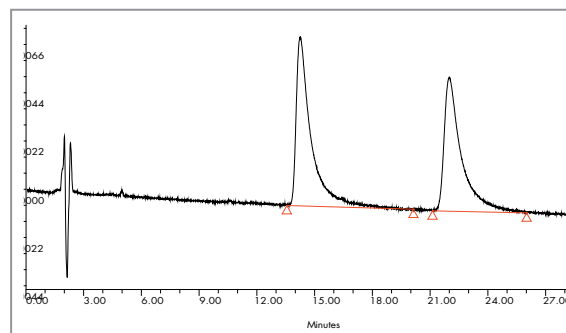


Figure 1: Analysis of desacetyl diltiazem (14.243 min) and related compound diltiazem (21.996 min) using USP diltiazem method. Conditions: Column: Nova-Pak® C₁₈, 300 x 3.9 mm; 4 μ m; 60 Å; Buffer: 0.1 M sodium acetate buffer with 1.16 g d-10-camphorsulphonic acid; Mobile Phase: 50:25:25 (Buffer: ACN: MeOH); Flow Rate: 1.0 mL/min; Injection Volume: 10 μ L; 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. Starting 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 t ₀): 2 times the void (t ₀)
Pressure <3000psi	Sample Information: Bases
210-400 nm MAXplot Column switch activated	UV compatible

Table 1. Method goals and preliminary information entered into AMDS.

AMDS suggested starting conditions of pH 9, 10 mM ammonium bicarbonate and an XTerra® MS C₁₈ 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).

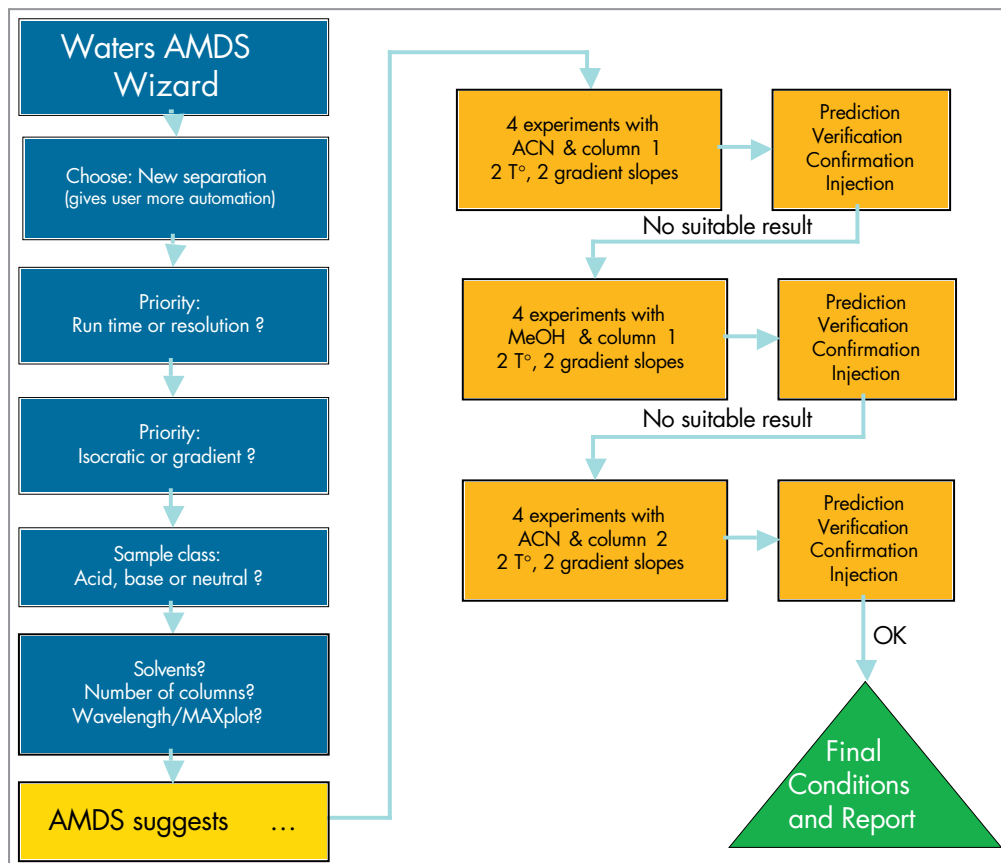


Figure 2: General AMDS decision manager flow path.

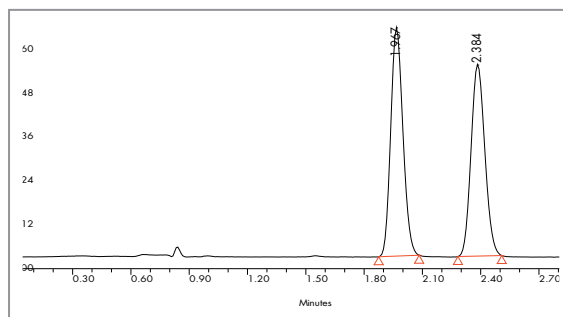


Figure 3: AMDS optimized results for the separation of desacetyl diltiazem diltiazem (1.966 min) and desacetyl diltiazem diltiazem (2.384 min).

Conditions: Column: XTerra RP18 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 @ 240nm

Results

Using the AMDS-suggested starting conditions, a 3.0 minute separation, Figure 3, was obtained that exceeded 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.

Conclusions

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 capabilities 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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