Waters™

응용 자료

Modifying Waters AMDS for *IS* Method Development

Michael D. Jones, Kate Evans

Waters Corporation

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

Abstract

This application brief explains the procedures and configuration changes needed to use Waters AMDS to develop methods with *IS* Columns, eliminating the need to first develop methods on analytical column dimensions before converting to *IS* Columns.

Introduction

The Waters Automated Method Development System (AMDS) provides an alternative to traditional, inefficient, manual, and often unreliable trial-and-error processes typically used to develop and optimize HPLC methods. AMDS combines the precision, accuracy and reliability of the Alliance HPLC System with the ease and flexibility of Empower Software configured with the automated method development decision manager. The System provides an automated and efficient way of developing, optimizing and transferring high quality, robust

HPLC methods. The possibility of combining AMDS with the latest Waters Intelligent Speed (*IS*) Column technology, provides even greater opportunities to further optimize chromatographic separation speed, thus increasing productivity.

AMDS is currently configured with standard analytical column dimension algorithms embedded in the internal logic that calculates chromatographic starting conditions. With the increasing popularity of *IS* technology, more scientists want to incorporate *IS* Columns into their methods to leverage the separation speed for productivity gains. Proper modifications must be made within the AMDS Software, and the Alliance 2695[1] and 2996 PDA[2] must be optimized to achieve the lowest system volume possible. This application brief will explain the procedures and configuration changes needed to use Waters AMDS to develop methods with *IS* Columns, eliminating the need to first develop methods on analytical column dimensions before converting to *IS* Columns.

Experimental

In order implement *IS* Column technology, we must first understand how AMDS calculates flow rates and gradient run times. AMDS version 1.2 determines flow rate as follows:

F = (1/L) * (Dc^2) * (Dp^2) * (Pmax/1000) * Pf *Sf

Where:

F = flow rate in mL/min

L = column length in cm

Dc = column id in cm

Dp = packing particle size in microns

Pmax = maximum pressure in psi

Pf = pressure factor

Sf = solvent factor: (Water = 2.0, ACN = 3.75, MeOH = 2.0)

The pressure factor is predetermined experimentally with conventional analytical column dimensions. Currently, this value is 0.5 for both Waters XTerra and Symmetry Columns. This pressure factor considers column backpressure, but not system backpressure. Observed HPLC system backpressure is negligible at optimum flow rates for conventional analytical columns. The optimal flow rate for *IS* Columns is much higher (approximately 3.0–4.0 mL), and therefore system backpressure is a factor. Currently, AMDS does not take this pressure into account when proposing starting conditions. Thepotential increase of 1000 psi contributed by system backpressure must be compensated for in the AMDS proposal screen. The flexibility of AMDS allows the user to make these flow rate modifications in the "Propose Data Run" screen as depicted in Table 1. Note the "User" modifications are documented with a dot (▪) to signify a change to the AMDS proposed starting conditions.

Step: Propose data runs			
Condition	Step	Proposed value	User
Low temperature	All	35 °C	
High temperature	All	50 °C	
Injection Volume	All	20 µL	
Maximum run time	All	5 min	
Minimum resolution	All	1.5	
Maximum pressure	All	3000 psi	
A Solvent	All	10 mM ammonium bicarbonate, pH 9	
B Solvent	All	acetonitrile	
Flow rate	ACN	3.0 ml/min	▪
Short gradient time	ACN	1.5 min	▪
Long gradient time	ACN	3.0 min	▪
Low %B	ACN	5 %	
High %B	ACN	85 %	

Table 1. Typical "Proposal Screen" modifications when using IS Columns. This is a table taken from the AMDS message log file. Modifications are highlight in red.

The other starting conditions that may have to be modified are the short and long gradient times, where gradient

time is depicted in Drylab as tG (time of Gradient). The long gradient is advised to be approximately a factor of 3 greater than the short gradient in order to achieve an optimal Drylab resolution plot. The calculation for tG is as follows:

tG = 0.01 * (k*) * (Max %B - Min %B) * (0.5 *(column length) * (Column diameter)^2) * s-value/flow rate

Where:

k* for short gradient = 2 (defaults in the AMDS configuration screen)

k* for long gradient = 6 (defaults in the AMDS configuration screen)

S-value = 5 (a value related to molecular weight of the samples. 5 is suitable for small molecules)

tG = gradient time

One can see that flow rate is an important factor in the equation. Since the flow rate (F) has been modified the gradient time (tG) also needs to be modified. As a rule of thumb, it is best to choose short and long gradient that are a factor of 3 apart, and allow for gradient profiles that provide enough time to collect data for Drylab's predictions.

References

1. Jenkins, T., *et al* "Alliance 2695 Separations Module: Optimization and Performance with 4.6mm I.D. Intelligent Speed (*IS*) Columns." Waters Technical Note – 720000723EN.
2. Jenkins, T., *et al* "2996 Photodiode Array Detector: Optimization for Intelligent Speed (*IS*) Columns." Waters Technical Note – 720000722EN.

Featured Products

Alliance HPLC <https://www.waters.com/514248>

Empower Chromatography Data System <https://www.waters.com/513188>

720001045, November 2004