

# Purity and Mass Determination by LC/MS Using Solid Core HPLC Columns: a Column Lifetime Study

Lucas Westling<sup>1</sup>, Kate Evans<sup>2</sup>

<sup>1</sup>Genomics Institute of the Novartis Research Foundation, San Diego, CA; <sup>2</sup>Thermo Fisher Scientific, San Diego, CA

## Key Words

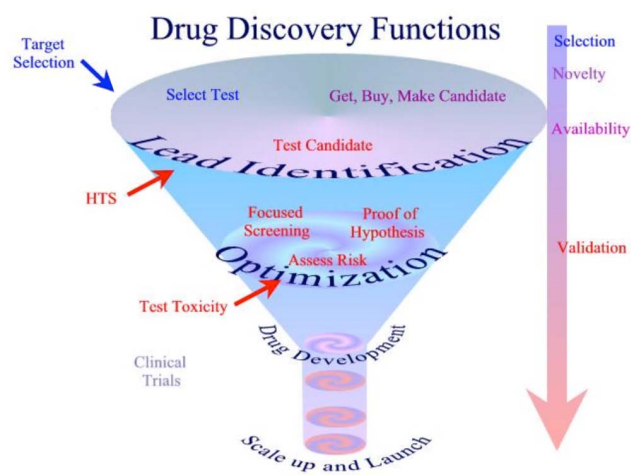
Accucore aQ 2.6  $\mu\text{m}$  HPLC column, drug discovery, small molecule, library screening, Core Enhanced Technology, solid core, QC screening (purity, identity)

## Abstract

A fast and reliable separation for quality control of small molecule drug candidates is demonstrated using Thermo Scientific™ Accucore™ aQ HPLC columns. The method run time was 3.25 minutes with each compound adequately resolved. The excellent peak shape was maintained for more than 11000 injections.

## Introduction

The drug discovery process begins with the identification of a medical need, including insight into the adequacy of existing therapies. From this analysis and current knowledge about the target disease come hypotheses on how to improve therapy. This may include efficacy, safety, or mechanistically novel improvements that will advance the method of drug treatment for the target disease. In the lead optimization process, it is imperative that high purity drug candidates are selected and isolated in a timely manner. Screening often involves thousands of compounds before a “hit” is identified. The process includes detecting relevant biological activity (a “hit”) for a structurally novel compound *in vitro* and then finding a related compound with *in vivo* activity in an appropriate animal model. These steps are followed by maximizing the biological activity through the preparation of analogous structures and finally **selecting one compound as the drug development candidate**. The chosen drug candidate then undergoes toxicological testing in animals. If the compound passes all of these tests, the accumulated research data are assembled and submitted as an Investigational New Drug Application (IND) to the Food and Drug Administration (FDA) before clinical trials are initiated.



Accucore aQ HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6  $\mu\text{m}$  diameter particles are not totally porous, but have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The polar functional group in the Accucore aQ HPLC column, which is a polar endcapped C18 phase, provides a controlled interaction mechanism by which polar compounds can be retained and resolved and enables the use of 100% aqueous mobile phases. The tightly controlled 2.6  $\mu\text{m}$  diameter of the Accucore particles results in much lower backpressures than typically seen with sub-2  $\mu\text{m}$  materials.

## Experimental Details

Consumables		Part Number
Mobile Phase Components:		
Solvent A:	Water (Millipore®)	
Solvent B:	Acetonitrile	
	Both solvents contain 0.05% TFA	
Chemical Standards:	Sulfanilamide	(Sigma-Aldrich® P/N S9251-100G)
	Sulfamerazine	(Alfa Aesar® P/N L04194)
	Sulfamethoxazole	(Sigma-Aldrich P/N S-7507)
	Hydrocortisone	(Sigma-Aldrich P/N H4001-10G)
	Ketoprofen	(Sigma-Aldrich P/N K1751-1G)

### Sample Preparation

Calibration standard preparation:	Standards were prepared in 3:1 water / acetonitrile.
Sample preparation:	Samples were prepared in DMSO.

Separation Conditions		Part Number
Instrumentation:	HPLC binary pump and DAD detector	
Columns:	Accucore aQ, 2.6 µm, 30 x 2.1 mm	17326-032130
Guard column:	C18, 4.0 x 2.0 mm	
Mobile Phase A:	Water + 0.05% TFA	
Mobile Phase B:	Acetonitrile + 0.05% TFA	
Gradient:	Time	%A      %B
	0.00	95.0      5.0
	0.10	95.0      5.0
	2.60	10.0      90.0
	2.61	0.0      100.0
	3.20	0.0      100.0
	3.22	95.0      5.0
	3.25	95.0      5.0
Flow rate:	1 mL/min	
Run time:	3.25 min	
Injection solvent:	Water / acetonitrile (3:1 v/v)	
UV detector wavelength:	214 nm to 400 nm at 2.5 Hz	

### Data Processing

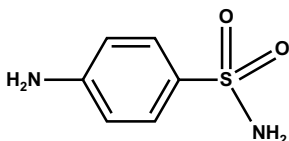
Software:	Chromatography Data System
-----------	----------------------------

## Results

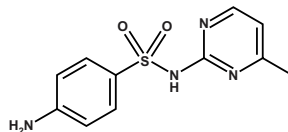
The Accucore aQ HPLC column was used to obtain small molecule screening data for an extended period of time. Performance was monitored for peak shape, resolution and retention time of 5 standards used in a test mix. More than 11,700 injections were obtained without loss of performance.

The chromatograms in Figures 1 through 5 are photodiode array (PDA) traces starting with injection 1 and ending with 11,759 injections. This application proves the robustness of the Accucore aQ HPLC column run under routine operating conditions for an extended period of time. There is no loss of performance, and excellent peak shape and retention time are maintained. The first eluting peak, at 9.6 s was evaluated and determined to be well retained with a capacity factor of approximately 2. The system was optimized for dead volume, flow rate, injection volume and gradient conditions.

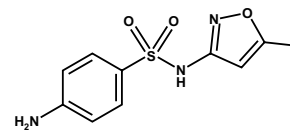
The following standards were used:



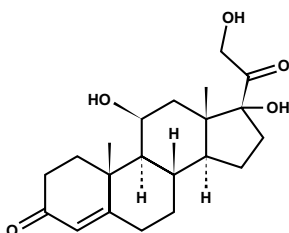
Peak 1- sulfanilamide



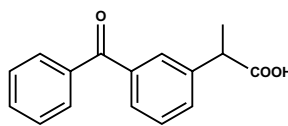
Peak 2- sulfamerazine



Peak 3- sulfamethoxazole



Peak 4- hydrocortisone



Peak 5- ketoprofen

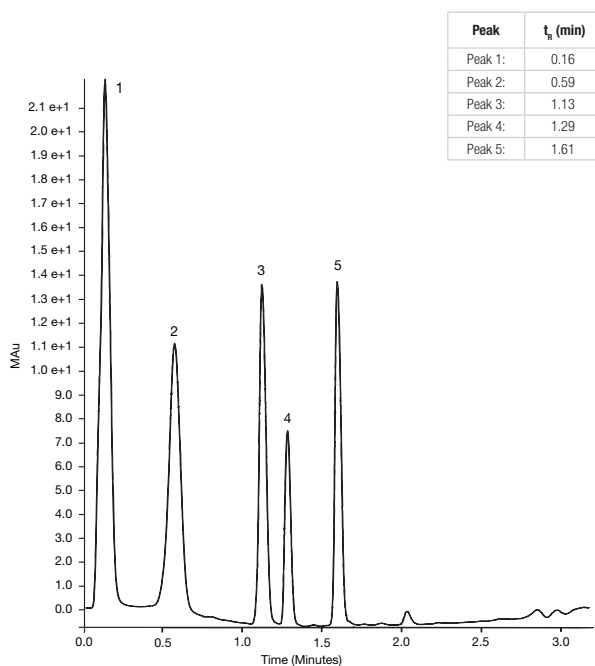


Figure 1: Injection 1

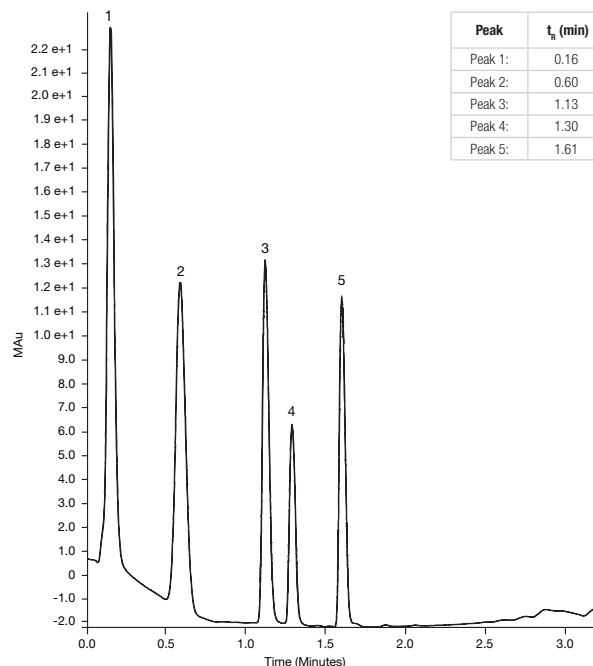


Figure 2: Injection 2,570

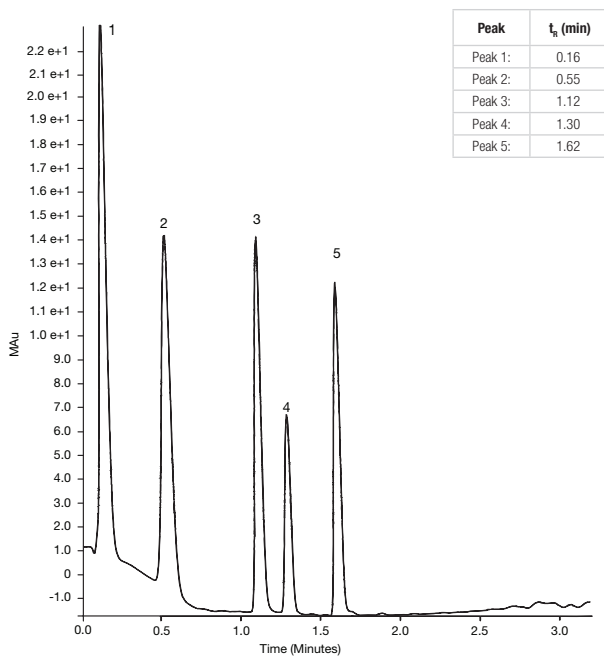


Figure 3: Injection 5,260

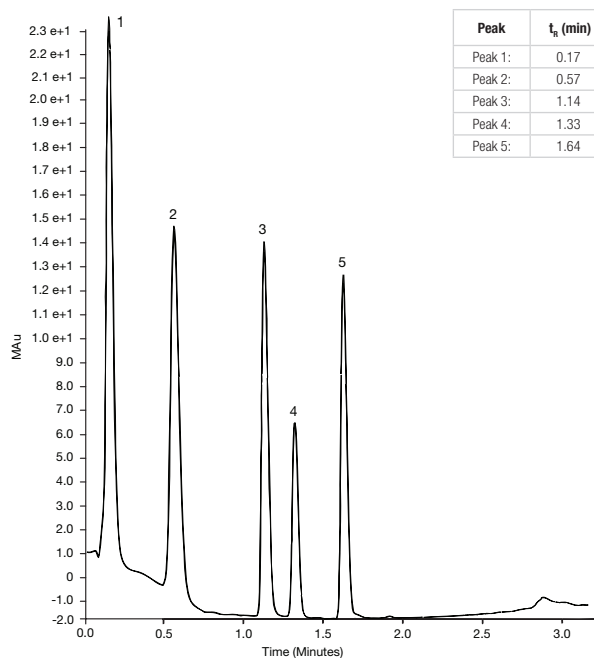


Figure 4: Injection 10,139

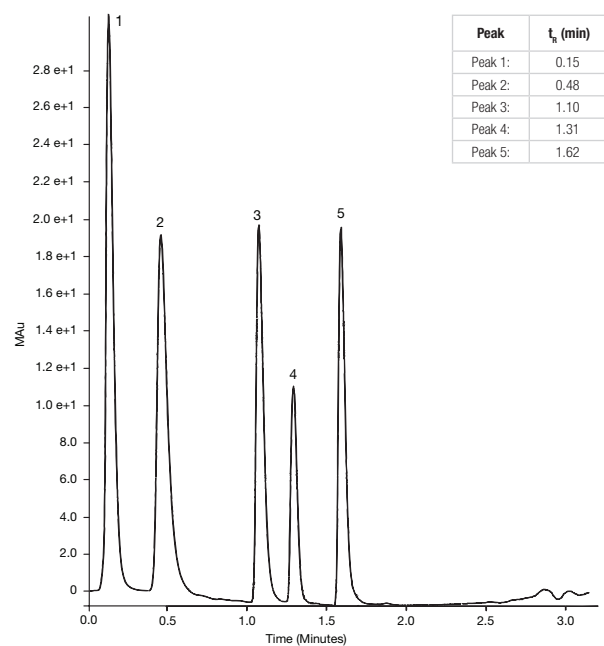


Figure 5: Injection 11,759

## Conclusion

- Drug discovery, lead optimization and general drug candidate screening are applicable to many biotechnology and pharmaceutical environments.
- Accucore aQ HPLC columns (2.6  $\mu\text{m}$ , 30 x 2.1 mm) demonstrated excellent performance under typical fast chromatography and generic mobile phase conditions for drug candidate screening purposes, in which samples to be analyzed are normally small molecules (generally between MW 100 and MW 1800) and contain a broad range of functional groups.
- Accucore aQ HPLC columns showed outstanding robustness, peak shape and retention time reproducibility. Long column lifetimes lead to more productivity and day-to-day confidence in results.

## Reference

Smith, A., Screening for drug discovery: The leading question. *Nature*, doi:10.1038/418453a.

## Acknowledgment

Front cover image: Drug discovery process, courtesy of [drugdiscovery.weebly.com/index.html](http://drugdiscovery.weebly.com/index.html)

[thermoscientific.com/chromatography](http://thermoscientific.com/chromatography)

© 2012 Thermo Fisher Scientific Inc. All rights reserved. Sigma-Aldrich is a registered trademark of Sigma-Aldrich Co. LLC. Millipore is a registered trademark of Merck KGaA. Alfa Aesar is a registered trademark of Alfa Aesar GmbH & Co KG. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

**USA and Canada** +1 800 332 3331  
**France** +33 (0)1 60 92 48 34  
**Germany** +49 (0) 2423 9431 20 or 21  
**United Kingdom** +44 (0)1928 534110  
**Japan** +81 3 5826 1615

**China** +86 21 68654588 +86 10 84193588  
 +86 20 83145199 800 810 5118  
**India** +91 22 6742 9494 +91 27 1766 2352  
**Australia** 1 300 735 292 (free call domestic)  
**New Zealand** 0800 933 966 (free call domestic)  
**All Other Enquiries** +44 (0) 1928 534 050

**Technical Support**  
**North America** +1 800 332 3331  
**Outside North America** +44 (0) 1928 534 440

**Thermo**  
 SCIENTIFIC

Part of Thermo Fisher Scientific