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

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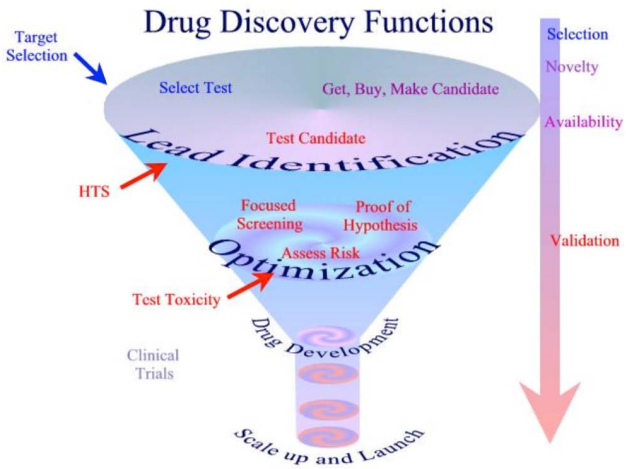
Key Words
Accucore aQ 2.6 µm HPLC column, drug discovery, small molecule, library screening, Core Enhanced Technology, solid core, QC screening (purity, identity)

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 µm 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 µm diameter of the Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

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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			

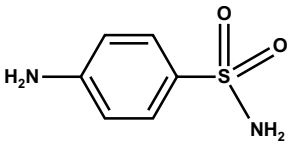
Data Processing	
Software:	Chromatography Data System

Results

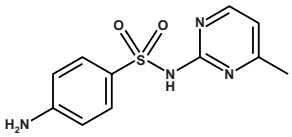
The Accucore aQ HPLC column was used to obtain small molecule screening data for an extended period of time. Performance was monitored for peak shape, resolution and retention time of 5 standards used in a test mix. More than 11,700 injections were obtained without loss of performance.

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.

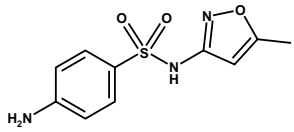
The following standards were used:



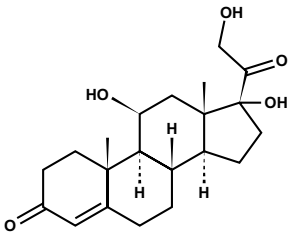
Peak 1- sulfanilamide



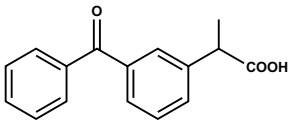
Peak 2- sulfamerazine



Peak 3- sulfamethoxazole



Peak 4- hydrocortisone



Peak 5- ketoprofen

Peak	t_R (min)
Peak 1:	0.16
Peak 2:	0.59
Peak 3:	1.13
Peak 4:	1.29
Peak 5:	1.61

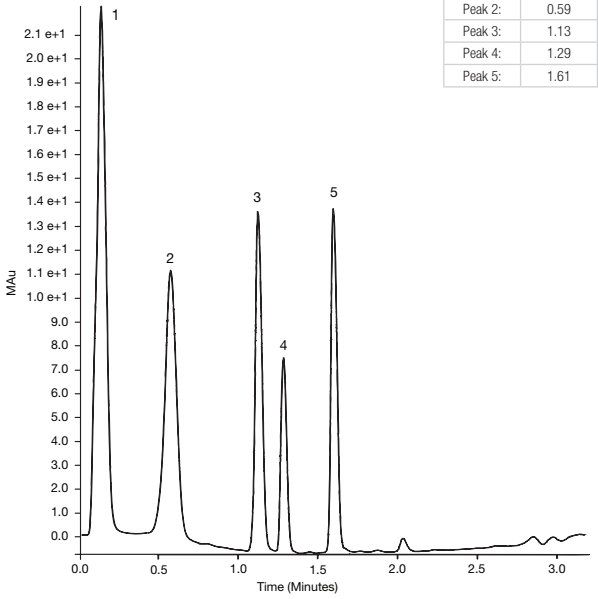


Figure 1: Injection 1

Peak	t_R (min)
Peak 1:	0.16
Peak 2:	0.60
Peak 3:	1.13
Peak 4:	1.30
Peak 5:	1.61

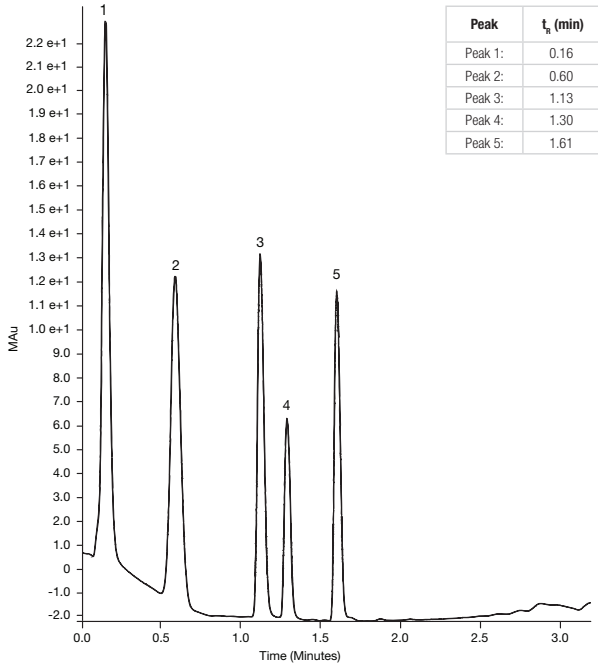
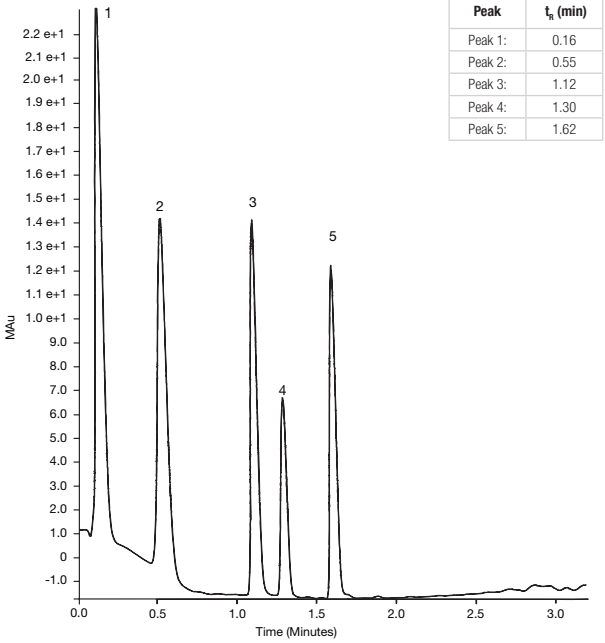
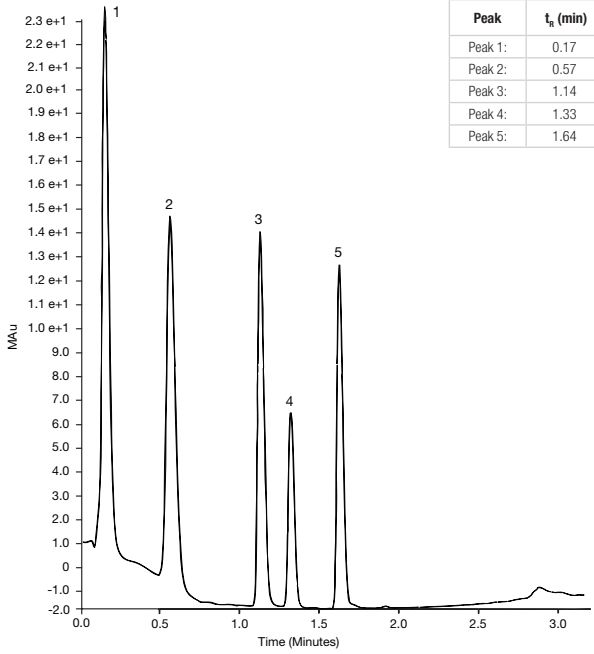


Figure 2: Injection 2,570



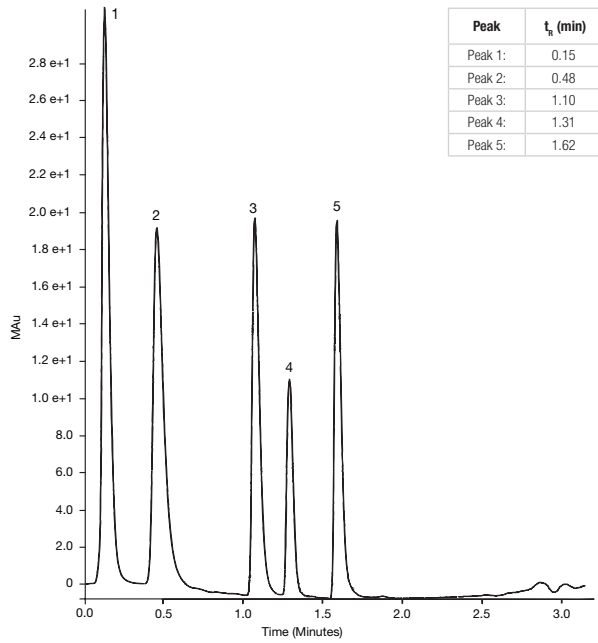
Peak	t_R (min)
Peak 1:	0.16
Peak 2:	0.55
Peak 3:	1.12
Peak 4:	1.30
Peak 5:	1.62

Figure 3: Injection 5,260



Peak	t_R (min)
Peak 1:	0.17
Peak 2:	0.57
Peak 3:	1.14
Peak 4:	1.33
Peak 5:	1.64

Figure 4: Injection 10,139



Peak	t_R (min)
Peak 1:	0.15
Peak 2:	0.48
Peak 3:	1.10
Peak 4:	1.31
Peak 5:	1.62

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 µm, 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

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