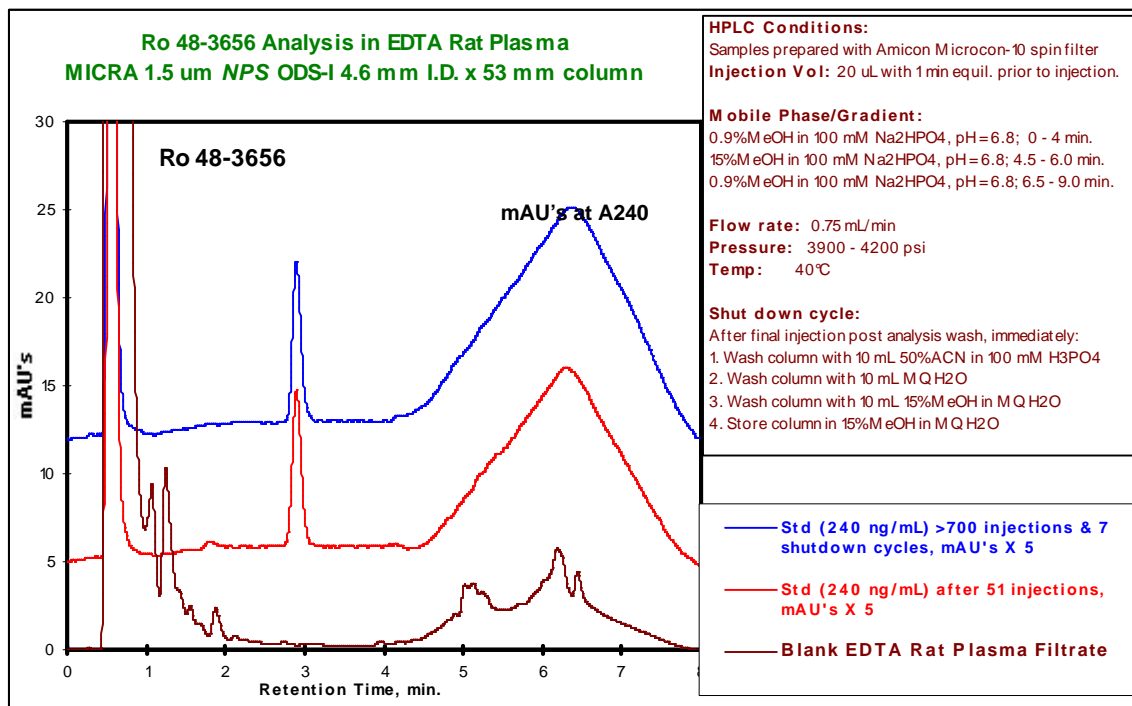


Metabolite Assay in EDTA Rat Plasma

MICRA NPS[®], a breakthrough in fast HPLC, is a highly uniform, non-porous silica based chromatographic support for use in HPLC columns. This revolutionary silica provides HPLC users with dramatic productivity improvements and significant cost savings through faster, reproducible, high sensitivity analyses, improved utilization of equipment and laboratory personnel and a real reduction in laboratory waste.

Separations involving analytes in plasma matrices represent some of the most challenging separations in HPLC. Matrix components (lipids, proteins, cell debris, etc.) that can be found make column ruggedness and robustness a critical issue. The absence of porosity in MICRA NPS HPLC supports makes them ideally suited for minimizing the problems these types of matrices present. Proper sample filtration and column equilibration are crucial for obtaining large numbers of injections with high reproducibility in retention time. The following data details a real example of a fast, stable and reproducible protocol developed at Genentech¹ for monitoring the metabolite Ro 48-3656 (see below) in EDTA Rat plasma. Suitable sample filtration and a post analysis column wash provides for a stable assay to well above 700 injections using a 1.5 μ MICRA NPS ODS-I 4.6 X 53 mm column. Day-to-day RT stability was maintained using an acidic shutdown cycle.

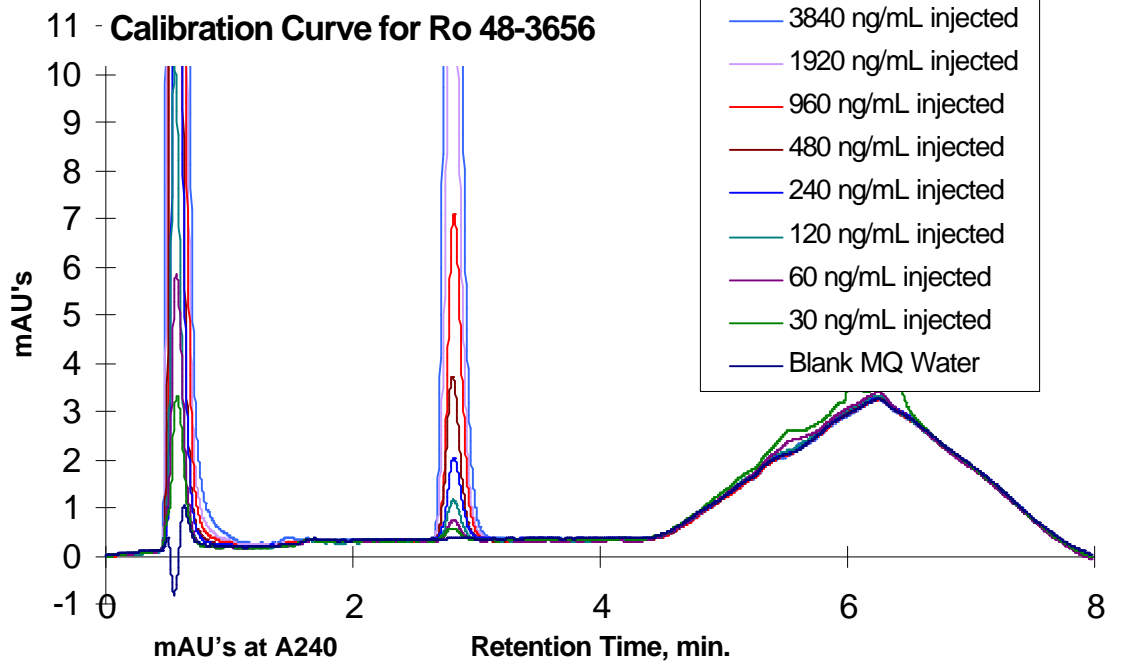
The doubly protected pro-drug metabolite, La Roche #: Ro 48-3656, is the precursor metabolite of a fibrinogen receptor antagonist being developed by Hoffmann-LaRoche and Genentech for secondary prevention of arterial thrombosis².



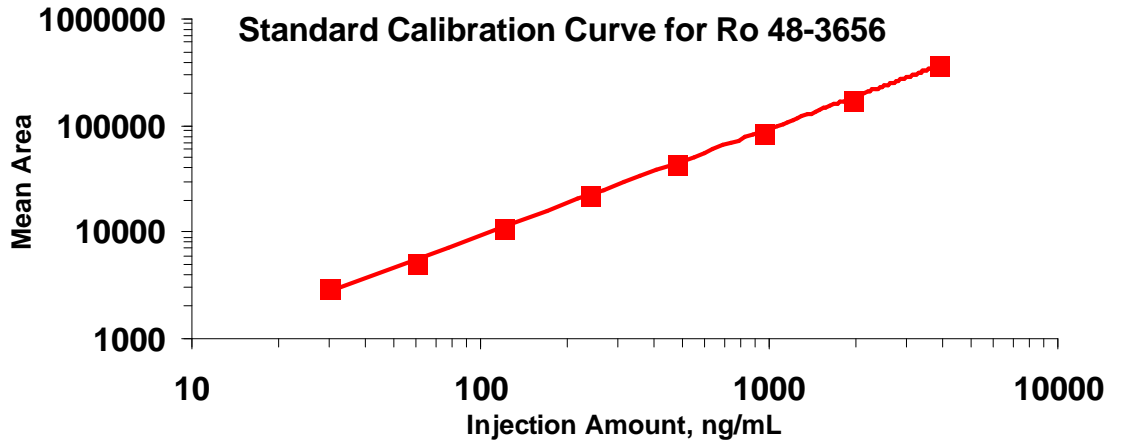
Ref 1. B. D. Paasch, Y.S. Lin, S. Porter, N. B. Modi, T. J. Barder; reprinted from *Journal of Chromatography*, B 704 (1997) 231-242.

Ref 2. T. Weller, A.M. Beresini, B. Blackburn, S. Bunting, P. Hadvary, M.H. Müller, D. Knopp, B. Levet-Trafit, M. T. Lipari, M. B. Modi, M. Müller, C. J. Refino, M. Schmitt, P. Schönholzer, S. Weiss, B. Steiner; *Journal of Medicinal Chemistry*, 39 (1996), 3139.

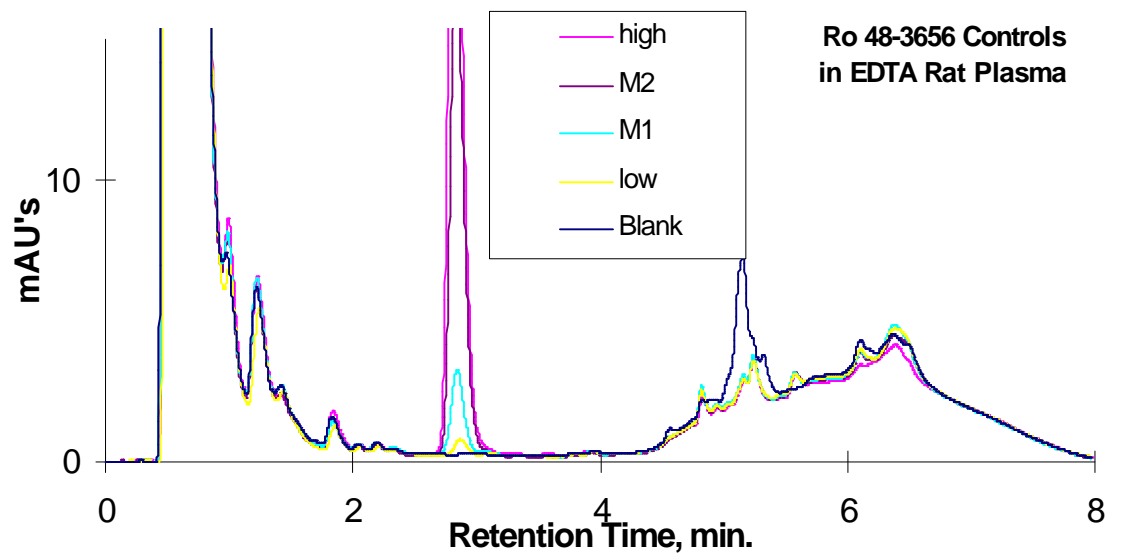
System Suitability checks prior to each run set verify the absence of run-to-run carryover of matrix components and analytes.



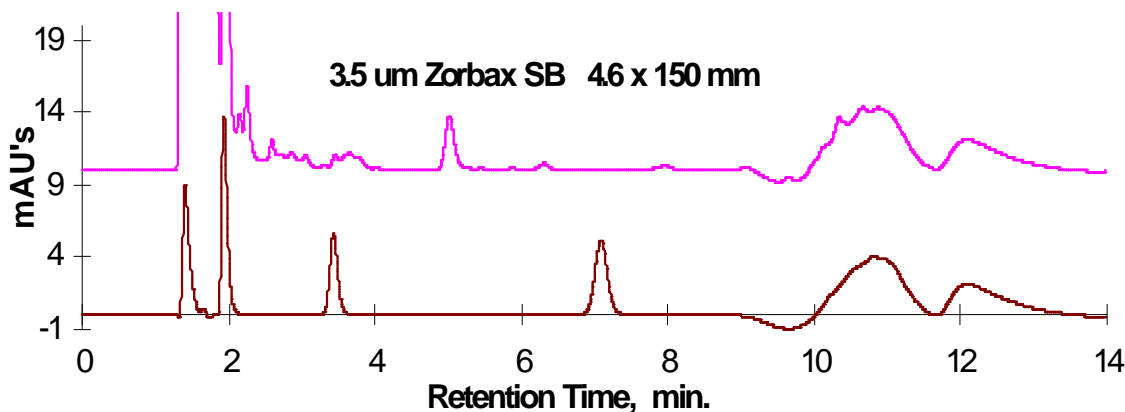
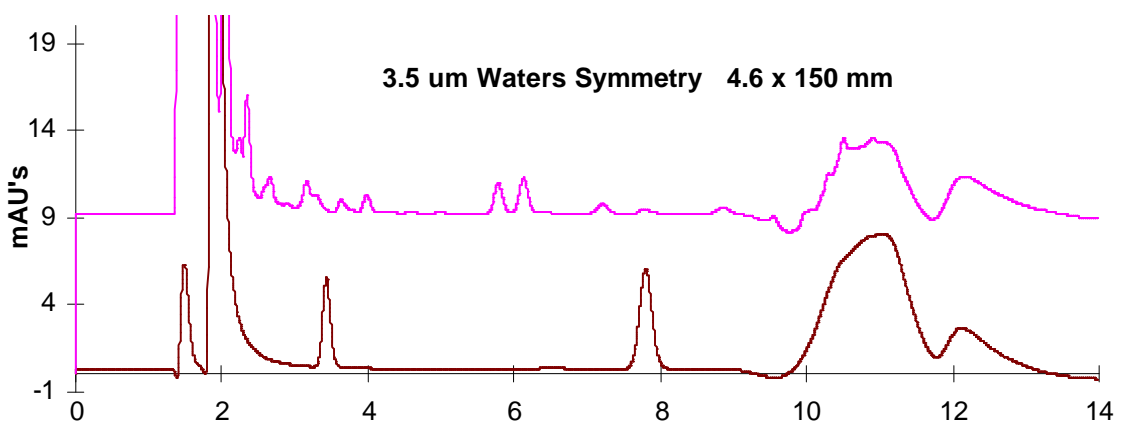
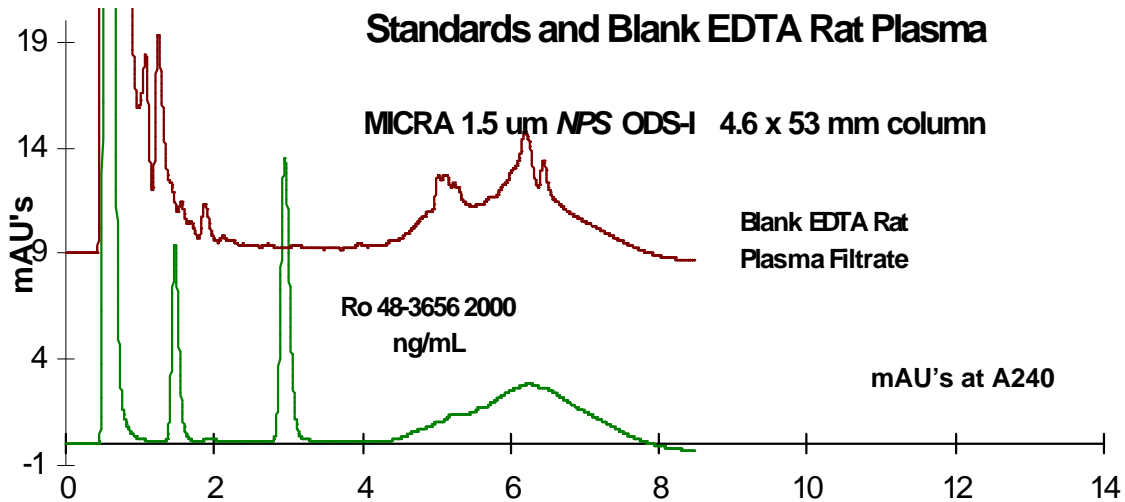
A cubic fit of mean peak areas vs. expected concentrations show good consistency for standards.



EDTA Rat Plasma controls spanned the standard calibration range to ensure method stability.



Standards and Blank EDTA Rat Plasma



MICRA NPS columns offer noticeable improvements in HPLC productivity.

Compared to conventional porous columns for this assay, MICRA NPS columns provide the user with:

- A 40% reduction in total analysis time
- A >50% increase in mass sensitivity.
- A 2 fold reduction in Mobile Phase usage.
- Reduced modifier Usage.
- A significant reduction in the interference of plasma matrix components in the HPLC assay.

	MICRA NPS	Zorbax [®] SB [®]	Waters [™] Symmetry
Total Run Time, min.	10.25	16.25	16.25
k - Ro 48-3656	5.87	4.46	5.00
Mobile Phase Usage, mL	7.69	16.25	16.25
% MeOH isocratic step	0.9	15	14
% MeOH strip step	15	50	50
Peak area, 2000 ng/mL/20 uL injection	156,293	101,431	106,293

Think small

Think fast

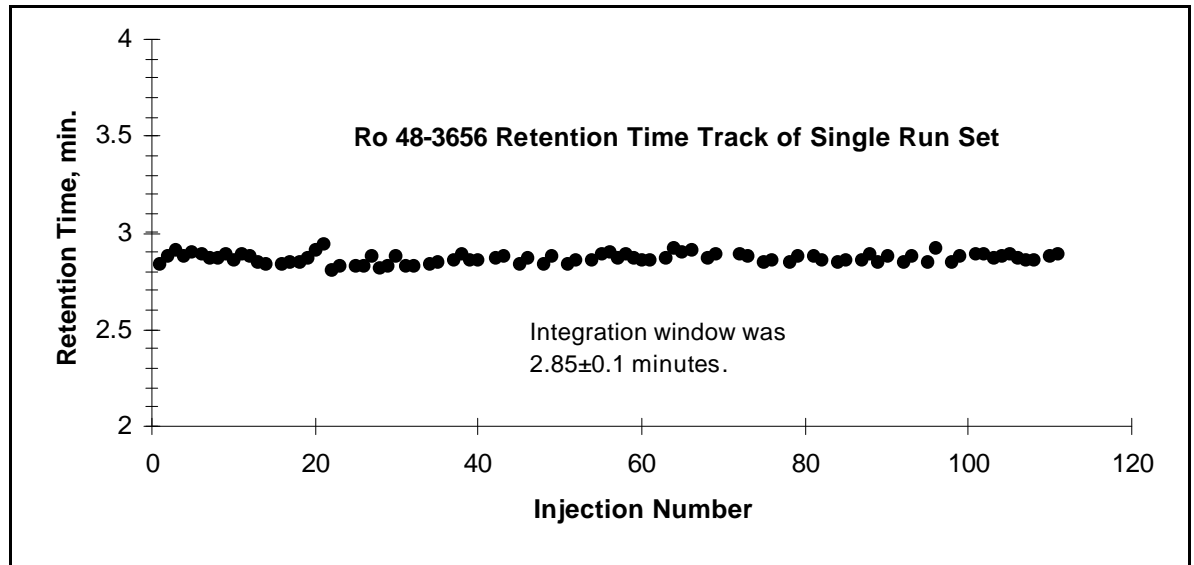
Think **NPS**[®]

20µL injections were routinely used for this assay. The lower limit of Quantitation of Ro 48-3656 was determined to be 40 ng/mL for this injection volume.

The addition of a MeOH strip step gave excellent stability of the retention time within a run set. The addition of an acidic wash-down step after each run set maintained the run set to run set retention time stability as well as the column stability to >800 injections.

Column to column stability was also demonstrated for the NPS columns. Two different lots of 1.5 µm NPS and 3 different lots of bonded batches over a period of one year gave reproducible retention times within 1% of the mean.

Limit of Quantification							
Final run set after >800 injections							
		EDTA Rat Plasma			MQ Water		
	Blank	40 ng/mL	50 ng/mL	80 ng/mL	40 ng/mL	50 ng/mL	80 ng/mL
mean	-	34.20	46.67	75.58	33.64	52.49	82.32
std. dev.	-	3.10	2.29	2.11	2.85	2.75	3.91
%CV	-	9.1%	4.9%	2.8%	8.5%	5.2%	4.8%
n	0	8	9	10	9	10	10
total n	10	10	10	10	10	10	10
	% ratio pls/water	101.7%	88.9%	91.8%			



MICRA NPS Column Reproducibility			
Column #	Silica Lot #	ODS-I Lot #	Ro 48-3656 mean retention time, min.
13016F08	M011295	0196/95c	2.95
08066F07	M011295	0196/95c	2.93
08066F05	M011295	0196/95f	3.01
28076F05	M081595	0228/96c	2.96

Eprogen
8205 S. Cass Avenue
Suite #106
Darien, Illinois 60561
USA
Phone: (630) 963-1481
Fax: (630) 963-6432
E-mail: info@eprogen.com
www.eprogen.com