

Retention time reproducibility of a Flux nano LC/MS system

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Introduction:

Two different ways to generate nL/min flows for LC are currently on the market. Splitless systems (mainly syringe drive pumps) can deliver such flow rates. Advantages of such systems are the direct control and monitoring of the flow through the column. Disadvantages include a limited flow range (usually not more than a few $\mu\text{L}/\text{min}$) and high price. A second approach is to use conventional LC pumps in combination with a flow split. The latter approach has the advantage of being cost effective, robust and far more versatile since on the same system flow rates from nL to mL/min can be achieved. The main disadvantage is that the flow through the column is essentially unknown. However, with the Flux Instruments S-AFM nanoflow generator used in this experiment nL/min flow rates through the column are readily monitored and logged.

Objective:

This application note evaluates the reproducibility of retention times for a Flux Rheos nano-LC system with S-AFM nanoflow generator for the analysis of tryptic digests in proteomics.

Setup:

Flux Instruments Rheos 2200 quaternary micro LC pump

Flux Instruments S-AFM nanoflow generator

CTC HTC-PAL

Thermo LTQ

All of these components were controlled by Thermo's Xcalibur

LC conditions:

Eluent A: H₂O w. 0.1% formic acid (v/v), eluent B: MeCN w. 0.1% formic acid (v/v)

Flow rate on column 500 nL/min, generated and monitored by S-AFM

Gradient

t (min)	%A	%B
0	95	5
45	10	90
52	10	90
53	95	10
60	95	5

Sample: Human serum albumin, tryptic digest (reduced and alkylated), 2 μ L @ 10fmol/mL

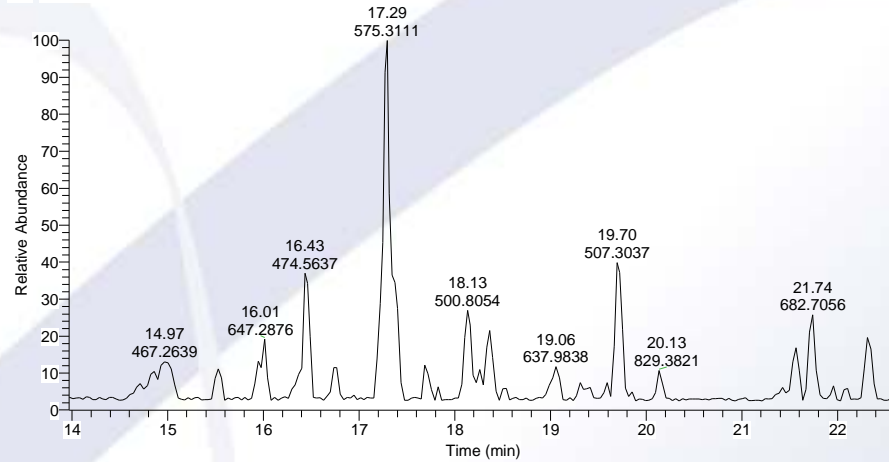
Results:

D:\DATA\...hSA_20fmol_repro01

20.12.2005 06:34:13

hSA 10fmol/ul 2ul injected

RT: 13.97 - 22.62



NL: 4.68E6
Base Peak F:
FTMS + c NSI Full
ms [
450.00-1600.00]
MS
hSA_20fmol_repro0
1

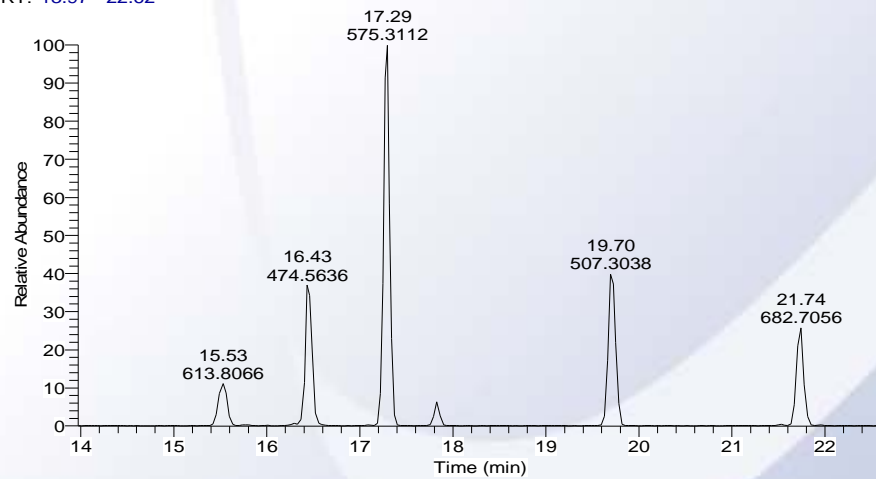
BPC for the tryptic HSA digest

D:\DATA\...hSA_20fmol_repro01

20.12.2005 06:34:13

hSA 10fmol/ul 2ul injected

RT: 13.97 - 22.62



NL: 4.68E6
Base Peak m/z=
474.1000-475.1000+506.8000-507.8000+
574.8000-575.8000+613.3000-614.3000+
682.2000-683.2000 F: FTMS + c NSI Full ms
[450.00-1600.00] MS hSA_20fmol_repro01

XICs for 5 selected HSA peptides

Results:

Five different peptides (SIM traces see above) that were used to monitor the system showed that an excellent the reproducibility of the retention time has been achieved over 10 runs with an average CV of 0.47%.

10 Runs	m/z				
	613.81	474.56	575.31	507.30	682.70
m	15.64	16.55	17.35	19.76	21.83
s	0.07	0.08	0.09	0.09	0.08
CV (%)	0.47	0.47	0.55	0.47	0.39

Conclusion:

The setup consisting of a Rheos 2200 with an S-AFM is a cost effective, robust and reliable solution for nano LC.