

Automated Large and Extra-Large Volume Injections in Nanoscale HPLC

INTRODUCTION

Miniaturized LC columns with typical I.D. from 50 μm to 1.0 mm provide increased sensitivity compared to conventional sized columns due to lower chromatographic dilution of the sample.

For 300 μm Capillary LC columns and 1.0 mm Micro LC columns this can easily be achieved with conventional six-port valves equipped with appropriate sample loops (sample focusing). The applied flow rates are usually high enough to prevent too long delay times as a result of sample loading.

RESULTS AND DISCUSSION

The delay times as a function of the column I.D and the flow rate for a 5 μL sample are given in Table 1. The delays obtained in Nano LC are neither practical nor acceptable.

To increase sample throughput and extend lifetime of Nano LC columns, 5 μL of a tryptic digested cytochrome C sample was applied on a 300 μm I.D. x 5 mm precolumn at a flow rate of 10 $\mu\text{L}/\text{min}$, reducing the

Table 1. Overview of Relation of Column I.D., Flow Rate and Sample Loading Times for 5 μL Injections

Name	Column I.D.	Flow rate	Sample loading time (min)	
			Without Preconcentration	With FAMOS
Conventional HPLC	4.6 mm	1 mL/min	< 0.1	< 0.1
Micro LC	1.0 mm	40 $\mu\text{L}/\text{min}$	< 0.1	< 0.1
Capillary LC	300 μm	5 $\mu\text{L}/\text{min}$	1	0.1
Nano LC	75 μm	200 nL/min	25	0.5

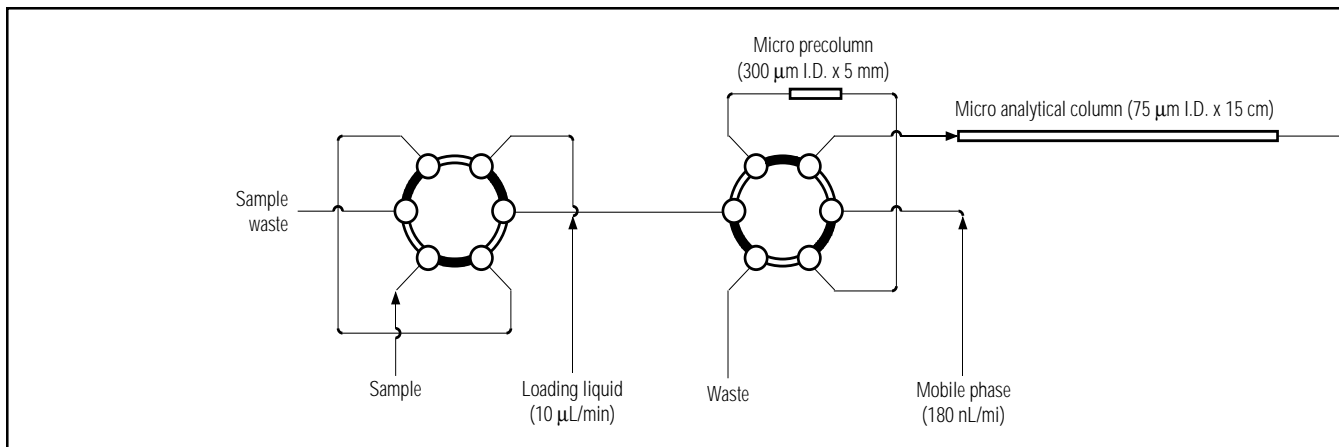


Figure 1. Set-up of FAMOS micro autosampler for automated sample loading and preconcentration prior to Nano LC analysis.

sample loading time to only 30 seconds (Figure 2). The separation of the cytochrome C digest was performed on a 75 µm I.D. x 15 cm nano LC column at a flow rate of 180 nL/min. Automated preconcentration was performed with a FAMOS™ Micro Autosampler.

The sensitivity of nano LC with 75 µm I.D. columns compared to conventional HPLC with 4.6 mm I.D. columns can—according to the theoretical down-scale factor—increase approximately 3700 times.

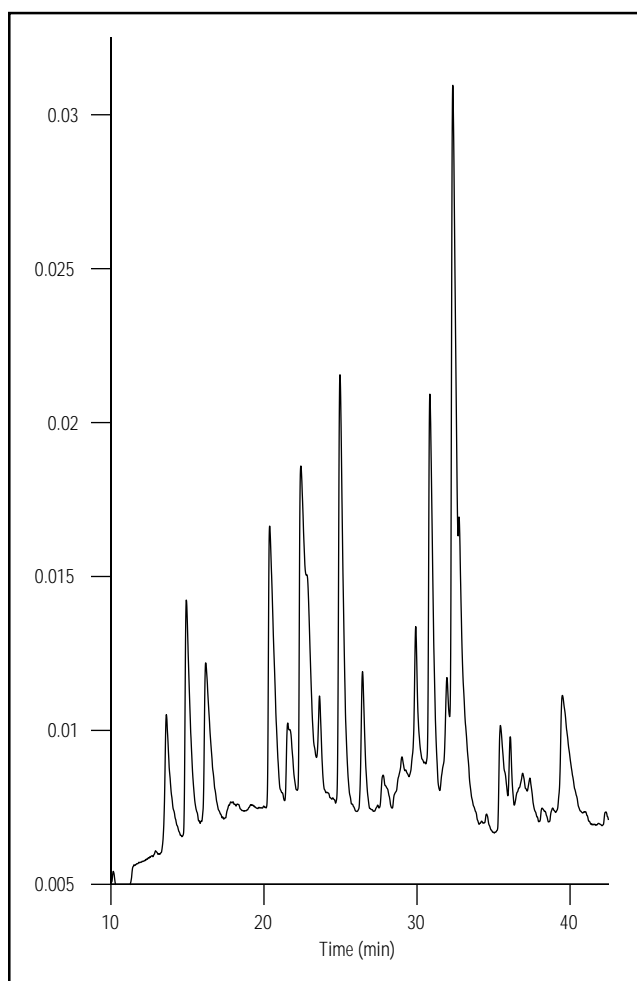


Figure 2. Chromatogram of cytochrome C digest on 75 µm x 15 cm Nano LC column packed with C18.

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* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.



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