AccuTOF™

Convert AccuTOF™ LC into Nano-LC/MS System: An Application of Peptide Analysis

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Introduction

Nano-LC/MS has demonstrated many advantages, including lower sample consumption, higher mass sensitivity and less matrix effect. Here, we introduce a very simple method to convert regular AccuTOF™ LC system into a nano-LC/MS system without high cost. An application of peptide analysis was used as an example to test the nano-LC/MS system. The RSD of retention time for gradient elution is less than 1 %.

What you need

- AccuTOF time-of-flight mass spectrometer (JEOL)
- Agilent 1100 HPLC with binary pump (JEOL or Agilent)
- NanoESI source (JEOL)
- 6 port external loop sample injector (Valco, part number C2-0006)
- Peek tee (Upchurch, part number P-727)
- Peek tubing, 1/16'' OD x .0025" ID (Upchurch, part number 1560)
- Microtight sleeve, .0155 x .025 (Upchurch, part number F-185X)
- Microtight union (Upchurch, part number P-720)
- SilicaTip™ Emitter
- (New Objective, part number FS360-50-30-CE-5-C15 for the flow rate of 300 – 1000 nL/min; part number FS360-75-15-CE-5-C15 for the flow rate of 200 – 500 nL/min)
- Integrafrit column (recommend New Objective Proteopep II™ C_{18} column, part number IFC75-PP2-10)

How to connect

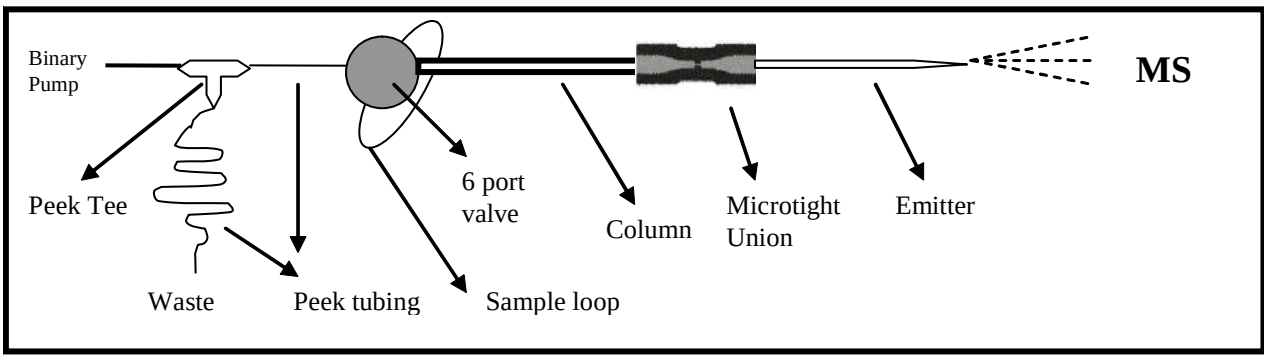

Figure 1. Diagram of nano-LC/MS system

1. Cut Peek tubing to an appropriate length for waste line.
2. Cut Peek tubing to an appropriate length to make a sample loop with desired volume.
3. Choose a nano-LC column and an emitter based on your desired flow rate.
4. Connect all parts according to the diagram in Figure 1.

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Experimental

Binary pump flow rate:	0.35 mL/min
Waste line:	Peak tubing, 1/16" OD x .0025" ID (65 µm), length 160 cm
Sample loop:	Peak tubing, 1/16" OD x 65 µm ID, length 10 cm, volume approximately 332 nL.
Column:	Proteopep II™ C_{18} (10 cm x 75 µm), flow rate approximately 650 nL/min
Emitter:	SilicaTip™ (FS360-50-30-CE-C15)
Orifice 1 voltage:	55 V
Orifice 2 voltage:	5 V
Ring lens voltage:	15 V
Ion guide peak voltage:	2500 V
Orifice 1 temperature:	80 ºC
Curtain gas:	Nitrogen 1.0 unit
Needle voltage:	2000 V
Sample:	Peptide standard mixture
Mobile phase:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Gradient:	2% of B for 2 min and then increase to 60% of B in 16 min and hold for 12 min

Results

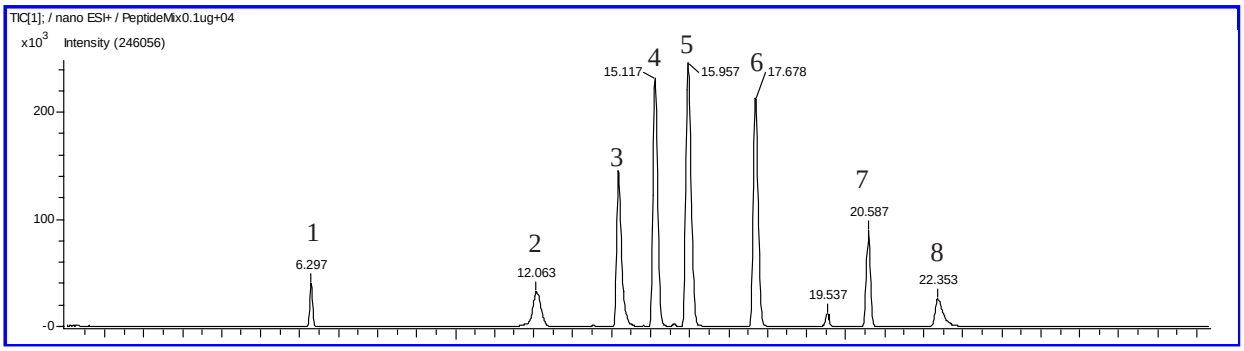

Figure 2. The total ion chromatogram of peptide standard mixture. 1 – RASG-1; 2 – Angiotensin frag 1-7; 3 – Bradykinin; 4 – Angiotensin II; 5 – Angiotensin I; 6 – Renin substrate; 7 – Enolase T35; 8 – Melittin.

Figure 1 shows the total ion chromatogram of peptide standard sample. All peptides are very well separated. The sample was injected three times in order to examine the reproducibility for gradient elution. The results are listed in Table 1.

Peptide	RASG-1	Angiotensin frag	Bradykinin	Angiotensin II	Angiotensin I	Renin substrate	Enolase T35	Melittin
Retention	6.338	12.148	14.164	15.102	15.94	17.672	20.554	22.282
	6.297	12.077	14.174	15.117	15.957	17.678	20.587	22.353
time (min)	6.207	12.062	14.116	15.075	15.928	17.696	20.558	22.281
RSD (%)	1.067	0.380	0.219	0.141	0.091	0.071	0.088	0.185

Table 1. Reproducibility of retention time for each peptide in sample

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