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Nanoflow gradient generator for capillary high-performance liquid chromatography–nanoelectrospray mass spectrometry

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Abstract

A novel nanoflow gradient generator using a 10-port switching valve with two injection loops installed, which is referred to here as the "Asymptotic-Trace-10-Port-Valve" (AT10PV) nanoGR generator, has been applied to capillary high-performance liquid chromatography (HPLC)–microelectrospray (microESI) or nanoelectrospray (nanoESI) time-of-flight mass spectrometry (TOF MS). In this study, performance of this capillary HPLC-micro/nanoESI-MS system was tested at a flow rate of 200 nl/min by using three typical peptides (angiotensins I, II, and III: 50 fmol each). The result demonstrated that this system provides reasonably good repeatability of peak retention times (R.S.D. of less than 0.5%). Sequential runs of a series of sample injections were performed in the same manner as conventional analysis at microflow rates.

Keywords: Mass spectrometry; Gradient elution; Instrumentation; Angiotensins; Peptides

1. Introduction

Capillary (or micro-) HPLC systems using small bore columns with internal diameters (i.d.) of less than 1 mm were initiated by several research groups during the late 1970s [1–6]. They were an important topic in the field of analytical chemistry, especially when combining the versatile separation capabilities of micro-HPLC with the power of mass spectrometers. Many types of ionization interfaces have been developed and modified to realize this hyphenated instrument [i.e., a capillary (or micro-) HPLC–MS]. At present, electrospray ionization (ESI), developed by Yamashita and Fenn [7–9] and Aleksandrov et al. [10], is the standard ionization interface in capillary HPLC-MS [11]. MicroESI (Emmett and Caprioli [12,13]) and nanoESI (Wilm and Mann [14,15]) introduced a new stage of ESI-MS. This technique demands a nanoflow capillary HPLC system, in particular a stable, reliable nanoflow gradient generator. To meet this new demand, several methods have been developed and classified as: (1) flow splitting [16–23], (2) miniaturization of high-pressure gradient pumps [24,25], (3) exponential (sigmoidal) gradient formation [26–30], and (4) off-line formation and storage of gradient solvents in injection loop(s) or preformed-gradient loop(s) [31–33].

In addition to these methods, we have recently proposed and developed a new method consisting of a standard semi-micro gradient delivery system and a 10-port switching valve with two injection loops installed, which was called the "Asymptotic-Trace-10-Port-Valve (AT10PV)" nanoGR generator [34]. An important aspect of this method is that any nanoflow-gradient profile can be easily and smoothly generated by simply shortening the switching period of the 10-port switching valve, without concern for gradient solvent mixing at nanoflow rates. In a previous report [34], the repeatability of gradient profiles and peak retention times of chromatograms was tested using the AT10PV nanoGR generator and a UV detector in the flow rate range of 50–500 nl/min. In this study, the generator is coupled with a micro/nanoESI time-of-flight (TOF) MS at a flow rate of 200 nl/min, and its performance is investigated in the analysis of mixtures of angiotensins I, II, III.

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2. Experimental

Fig. 1 shows a schematic diagram of a capillary HPLC(UV) or capillary HPLC–micro/nanoESI-TOF-MS system. The AT10PV nanoflow GR generator is indicated by the broken line. The major instrument components of the system and experimental conditions are described below.

2.1. Instrumentation

The AT10PV nanoflow GR generator is composed of a conventional gradient pump with low-pressure gradient capability at a microflow rate (μl/min) (i.e., microflow GR pump), an isocratic pump capable of delivering one solvent at a nanoflow rate (nl/min) (i.e., nanoflow isocratic pump), a 10-port switching valve with two injection loops, a back-pressure coil or column after the 10-port switching valve, and a controller to control the pumps and the 10-port switching valve. We used a Hitachi L-2100 LaChrome Elite pump (Hitachi High-Technologies, Tokyo, Japan) with low-pressure gradient capability for the microflow GR pump, a modified Hitachi L-2100 LaChrome Elite pump for the nanoflow-isocratic pump, and an Upchurch 10-port NanoPeak valve (Upchurch Scientific, Oak Harbor, WA, USA) with two homemade 1 μl injection loops, i.e., fused-silica capillary tubes (500 mm × 0.05 mm i.d. × 0.36 mm o.d.). Because the microflow GR pump has a remote control function operable by relay, switching of the 10-port switching valve was controlled by this pump. The back-pressure coil was made from a fused silica tube (200 mm × 0.05 mm i.d. × 0.36 mm o.d.), which was purchased from GL Science (Tokyo, Japan). The sample injector was an Upchurch M-435 microinjection valve (Upchurch Scientific). The UV detector was a Linear UVIS-205 with a flow cell volume of 250 nl obtained from Micro-Tech Scientific (Vista, CA). The ESI-TOF-MS detector was an AB Mariner (Foster City, CA, USA). Operation of the AT10PV nanoflow GR generator is briefly described in Section 3.

2.2. Chemicals, samples, and columns

Methanol (HPLC grade), acetonitrile (HPLC grade), water (HPLC grade), acetone, trifluoroacetic acid (TFA), formic acid, acetic acid, ammonium acetate, and ammonium hydrogencarbonate were purchased from Wako (Tokyo, Japan). Ethyl-, propyl-, and butylparabens were also obtained from Wako Chemical (Tokyo, Japan). The concentration of each of these parabens in the mixed sample was prepared to 10 ppm by diluting them with a solution of 10% methanol in water. The separation column used for these alkylparabens was a VC-5-C_{18}W-150 (50 mm × 0.15 mm i.d.) of Micro-Tech Scientific (Vista, CA, USA). Angiotensins I, II, and III were purchased from Peptide Institute (Osaka, Japan). Each concentration of these peptides in the mixed sample was prepared to 1 μmol/L by dissolving them with water. The separation column used for the peptide sample was a ProteoPep C_{18} column (50 mm × 0.075 mm i.d.) with a 15 μm tip from New Objective (Woburn, MA, USA).

2.3. Analytical conditions

In the capillary HPLC(UV) system, 0.5 μl of a mixture of ethyl-, propyl-, and butylparaben (each concentration: 10 ppm) was injected into the capillary silica-ODS column (VC-5-C_{18}W-150, 50 mm × 0.15 mm i.d.). Solvents A and B were water and acetonitrile–water (80:20 (v/v)), respectively. The flow rate of the nanoflow isocratic pump was 500 nl/min and the 10-port switching valve was switched once per minute. The detection wavelength was 250 nm.

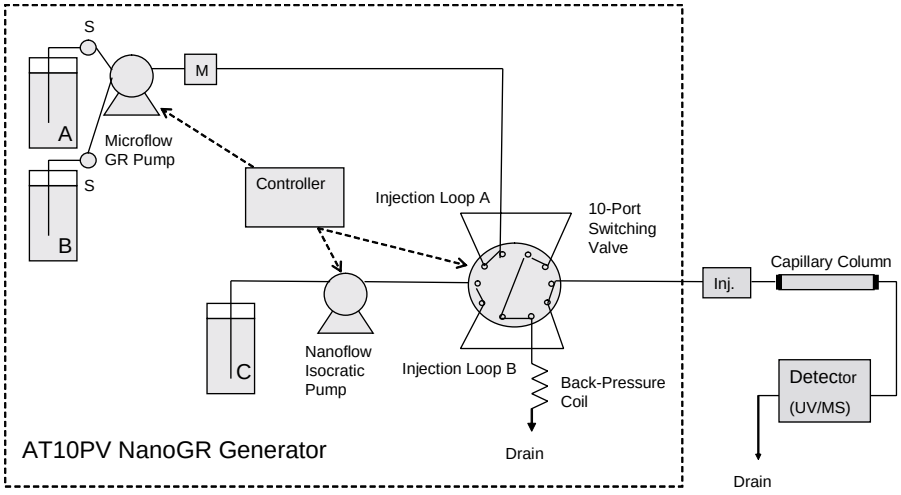

Fig. 1. Schematic diagram of capillary HPLC–micro/nanoESI-MS system based on the AT10PV nanoflow GR generator. The part within the broken-line is the AT10PV nanoflow GR generator, which consists of an conventional microflow GR pump with low-pressure GR capability and a 200 μl mixer (M), a nanoflow isocratic pump, a 10-port switching valve with two 1 μl injection loops, A and B. Flow rate of the microflow GR pump: 100 μl/min and flow rate of nanoflow isocratic pump: 50–500 nl/min. Period of 10-port valve switching: 1 min. Capillary column: ProteoPep C_{18} column (50 mm × 0.075 mm i.d.).

In the capillary HPLC-MS system, 50 nl of the mixture of angiotensins I, II, and III (concentration of each: 50 fmol) was injected into the ProteoPep C_{18} column (50 mm × 0.075 mm i.d.) with a 15 μm tip. The microGR pump delivered solvents A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 100 μl/min. The following gradient profile was used: solvent B was 0% for 0–4 min and then linearly increased up to 50% between 4 and 29 min. The flow rate of the nanoflow isocratic pump was 200 nl/min and the 10-port switching valve was switched once per minute. The settings for the electrospray (ESI) were: ESI voltage was 1.8–2.0 kV and the ESI nozzle temperature 180 °C. Curtain gas was not used in this study. The scan mass range was 200–2000 *m/z*.

3. Results and discussion

Here, we first briefly describe how the AT10PV nanoflow GR generator, shown in Fig. 1, works to generate a nanoflow gradient elution. The microflow GR pump creates an original gradient profile mixing the reservoir solvents A and B by controlling the corresponding solenoid valves (S). The well-mixed solvent is delivered at a flow rate, e.g., 100 μl/min, into either injection loop A or B of the 10-port switching valve, and drained after flowing through the backpressure coil (or column). Here, it should be noted that when injection loop A is being filled, the solvent loaded in injection loop B is being delivered to the capillary column at a nanoflow rate (nl/min) by the nanoflow isocratic pump. When the 10-port switching valve is switched, the roles of injection loops A and B are also switched, i.e., the solvent loaded into injection loop A is delivered and injection loop B starts to be filled. This valve switching is performed once per minute by a signal (on/off or pulse) sent from the microflow GR pump. Thus, the original microflow GR profile can be traced once per minute in a stepwise manner. An important feature of this gradient generator is that any type of nanoflow GR profile can be generated to asymptotically trace the original microflow GR profile by simply shortening the 10-port valve switching period (e.g., 0.25 min), without concern for gradient solvent mixing at nanoflow rates [34].

Fig. 2 compares the original microflow GR profile and the nanoflow GR profiles. Solvents A and B were water and 0.1% acetone in water, respectively. The flow rate of the microflow GR pump was 100 μl/min. The gradient program of solvent B was linearly increased from 0% at 0.0 min to 100% at 40 min, kept at 100% until 80 min, then returned to the initial condition of 0%. The original microflow GR profile (A) was monitored by the UV detector at 250 nm. The nanoflow GR profiles (B and C), which were created by activating the 10-port switching valve once per minute and by running the nanoflow isocratic pump at flow rates of 500 and 200 nl/min, were monitored by the UV detector in the same manner. The sample injector and the capillary column,

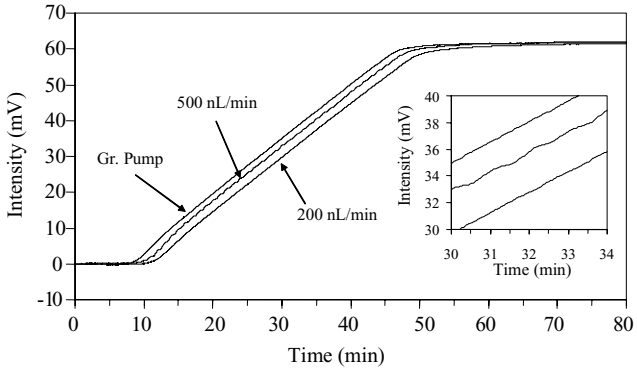

Fig. 2. Comparison between the original microflow gradient profile at a flow rate of 100 μl/min (A) and the created nanoflow gradient profiles at flow rates of 500 nl/min (B) and 200 nl/min (C). Solvent A: water; solvent B: 0.2% acetone in water. Gradient program of solvent B: 0% (0.0 min) → 100% (40 min) → 100% (80 min) → 0% (80.1 min) → 0% (105 min). UV detector wavelength: 250 nm. 10-port valve switching period: 1 min.

shown in Fig. 1, were removed in this experiment. Although the gradient-dwell times do not coincide exactly, it can be seen that the gradient profiles are very similar. "Steps" still remain at the flow rate of 500 nl/min (B) (see the figure insert). However, they can be easily eliminated by changing the 10-port valve switching period from 1 to 0.5 min, as discussed in the previous study [34], and disappear in the system with a column. The gradient profile of the 200 nl/min rate is smoother than that of the 500 nl/min rate. This is due to the diffusion effect at the lower flow rate.

Fig. 3 shows the chromatograms obtained from the nano-HPLC(UV) system shown in Fig. 1. Solvents A and B used were water and 80% acetonitrile in water, respectively. The flow rate of the nanoflow isocratic pump was 500 nl/min. The composition of solvent B started at 10%

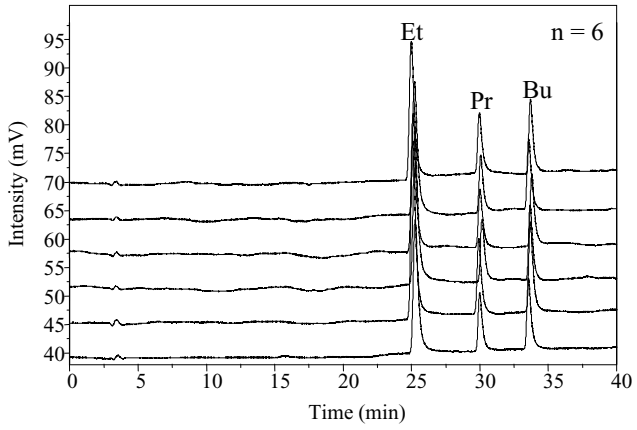

Fig. 3. Repeatability of UV chromatograms at a flow rate of 500 nl/min. Column: VC-5-C_{18}W-150 (50 mm × 0.15 mm i.d.). Sample: mixture of ethyl- (Et), propyl- (Pr), and butyl- (Bu) parabens (concentration: 10 ppm each). Injection volume: 0.5 μl. Solvent A: water and solvent B: 80% acetonitrile in water. Flow rate: 500 nl/min. Gradient program of solvent B: 10% (0–10 min) → 100% (45–75 min). UV detector wavelength: 250 nm. 10-port valve switching period: 1 min.

Table 1
Repeatability of retention times of alkyl-parabens

Run number	t_R (min)			Area		
	Et	Pr	Bu	Et	Pt	Bu
1	25.09	29.91	33.69	875 397	421 185	658 505
2	25.27	30.16	33.67	993 915	413 359	671 214
3	25.11	30.00	33.80	952 712	387 081	668 174
4	25.22	30.06	33.57	983 285	405 997	664 187
5	24.99	29.97	33.70	914 132	384 670	694 257
6	25.16	30.02	33.67	926 934	391 815	671 256
Average	25.16	30.02	33.67	941 063	400 685	671 266
R.S.D. (%)	0.45	0.29	0.27	4.7	3.8	1.8

Table 2
Repeatability of retention times of angiotensin I, II and III.

Run number	t_R (min)			Area		
	III	II	I	III	II	I
1	19.9	20.9	22.4	100 569	44 691	117 276
2	20.0	21.0	22.5	163 632	68 643	157 681
3	19.8	20.9	22.4	223 171	100 355	197 306
4	19.8	20.8	22.3	242 263	110 873	240 516
5	20.0	21.0	22.4	337 710	119 405	317 531
Average	19.9	20.9	22.4	213 469	88 793	206 062
R.S.D. (%)	0.50	0.40	0.32	41.6	35.2	37.5

and stayed constant for 10 min, and was then linearly increased to 100% at 70 min. The sample was a mixture of ethyl-, propyl-, and butylparaben (concentration: 10 ppm), and 0.5 μl was injected by the manual microinjection valve into the capillary silica-ODS column (VC-5-C_{18}W-150, 50 mm × 0.15 mm i.d.). The chromatograms were acquired six times and overlaid. Table 1 summarizes the retention times (RT) of the three component peaks in the chromatograms and some statistical data. The relative standard deviations (R.S.D.s) of the retention times and peak areas in this experiment were less than 0.5 and 5%, respectively. The repeatability of this capillary HPLC(UV) system is very good.

Fig. 4 shows the total ion chromatogram (TIC) of Angiotensins I, II, and III (50 fmol each) taken by five continuous runs at a flow rate of 200 nl/min using the nano-HPLC-MS system shown in Fig. 1. A ProteoPep C_{18} column (50 mm × 0.075 mm i.d.) with a 15 μm tip was used in this experiment. The initial value of solvent B was 0% and kept constant for 4 min, and was then linearly increased to 50% from 4 to 29 min. The repeatability of the obtained retention times (t_R) and areas of the three peaks are summarized in Table 2. The R.S.D.s of the peak t_R values were less than 0.5%, which is the same level of the previous result at the flow rate 500 nl/min. The peak intensities varied and the R.S.D.s of the peak areas were 100-fold higher than those of t_R values. As the peak t_R values are stable, it is due to an instability of electrospray at lower flow rates.

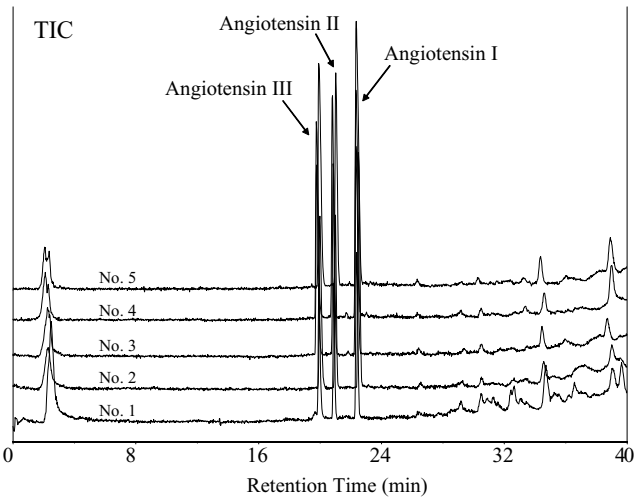

Fig. 4. Repeatability of total ion chromatograms (TIC) at a flow rate of 200 nl/min. Sample: mixture of angiotensins I, II, and III (0.1 μmol/L each) and injection volume: 50 nl. Capillary column: ProteoPep C_{18} column (50 mm × 0.075 mm I.D.). Solvent A: 0.1% formic acid in water and solvent B: 0.1% formic acid in acetonitrile. Gradient profile of solvent B: 0% (0–4 min) → 50% (29 min).

4. Conclusion

In this study, a novel nanoflow gradient generator, called the AT10PV nanoGR generator, was combined with a capillary HPLC(UV) system and a capillary

HPLC–ESI-TOF-MS system. Its performance was investigated by testing the repeatability of peak retention times and peak areas in the chromatograms acquired by both systems. The results showed that the R.S.D.s of the peak retention times were less than 0.5% at flow rates of 200–500 nl/min, while the R.S.D.s of peak areas were about 40%, which is 100-fold higher than that of the peak retention times.

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