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New micro-flow pumping system for liquid chromatography

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Abstract

This paper deals with the development of practical approaches to a new liquid-delivery system for capillary liquid chromatography. Under different chromatographic conditions, the factors affecting liquid-delivery performance are theoretically described, and the new liquid-delivery system without any splitter is evaluated with its flow-rate accuracy and precision using a variety of solvents. The experimental results demonstrate that the liquid-delivery system is capable of generating accurate, reproducible and conditions-independent micro- and nano-flows.

Keywords: Pumps; Instrumentation; Micro liquid chromatography; Flow-rate

1. Introduction

High-performance liquid chromatography (HPLC) based on micro-columns has become a powerful tool for analysis of a wide variety of samples, especially for enhanced sensitivity, less solvent consumption, minimal sample and easy direct interfacing with a mass spectrometer [1–12]. Recently, the micro-bore columns packed with small particles (1~3 μm) were increasingly used in HPLC [13,14] to reduce analysis time and provide larger numbers of theoretical plates with a short column length [14]. However, there were several unavoidable problems with application of a conventional pumping system such as the commercially available reciprocating piston and syringe pumps in micro-HPLC systems because their flow-rates are basically dependent on the operating conditions and they suffer from insufficient precision, inaccuracy, large pulsation at micro flow-rates. In addition, the combination of the conventional pumping system with a flow splitter has also been reported for capillary HPLC [3–9,11,13]. The existing flow splitters are based on capillary tubes of different lengths or internal diameters to obtain a certain flow split ratio and, therefore, have several disadvantages fundamentally. First, the flow-rate ratio varies with pressure-drop of column, mobile phase composition (especially during gradient runs) and operating temperature. Second, the difference in delay volume of the two capillaries can result in different solvent compositions in each capillary. Finally, the flow split technique does not reduce solvent consumption.

The purpose of this study is to develop a new liquid-delivery system which is capable of generating highly accurate and reproducible micro-flows and nano-flows for capillary HPLC without the use of any flow splitter. With the system, the effects of different parameters such as operating pressure, viscosity and compressibility of solvents and mobile phase composition on the accuracy and precision of

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flow-rate were evaluated. Moreover, the system was applied in gradient elution in capillary HPLC.

2. Experimental

2.1. New liquid-delivery system

Fig. 1 shows a schematic diagram of the micro-flow liquid-delivery system. The system was electrically driven with a micro stepping motor (50 000 steps/rev.), and the motor's rotary was converted into linear motion via a highly precise ball screw. The linear actuator was allowed to drive piston with a resolution of 0.3 nl/step and generate highly accurate and reproducible micro- and nano-flows (0.01–200 μl/min) without the need of any splitter.

In a case using dual pistons, the micro-flow liquid-delivery system had two independent drive systems to generate a continuous and constant flow. One of the pistons was used to pre-compress the solvent in piston chamber up to or close to a pressure at pump outlet during another piston performs delivery stroke. The compression process was monitored with the pressure sensors built in the piston chambers. Consequentially, the flow of each pistons can be resumed without pressure fluctuations and inconsistent flow when either of pistons started an intake stroke after completing its delivery stroke.

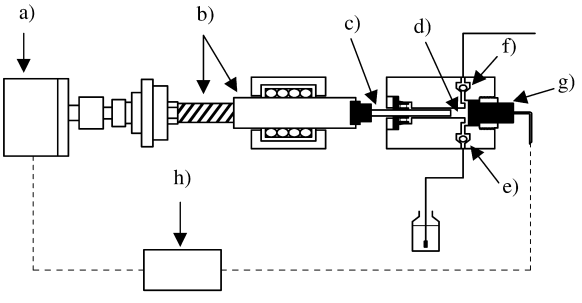

Fig. 1. Micro-flow liquid-delivery system. (a) Micro stepping motor, (b) highly precise ball screw, (c) piston, (d) piston chamber, (e) inlet check valve, (f) outlet check valve, (g) pressure sensor, and (h) system controller.

2.2. Flow-rate measurement

Flow-rates of pure solvents and mixtures were measured with both volumetrical and gravimetrical methods. A series of 5-, 10-, 25-, 50-, 100-, 250-μl graduated micro-syringes (Hamilton, NV, USA), which was previously calibrated, was used to measure different volumetric flow-rates. Gravimetrical flow-rates were performed using an analytical balance (Shimadzu, Kyoto, Japan), and the measured results were compared with those obtained by the micro-syringes. The experimental results are shown only with the measured volumetrical flow-rates because the agreement between volumetrical and gravimetrical methods was satisfactory.

Methanol (MeOH), acetonitrile (MeCN), tetrahydrofuran (THF), *n*-hexane and water were all of HPLC grade and obtained from Kishida (Osaka, Japan).

2.3. High-pressure gradient apparatus

Fig. 2 depicts the high-pressure gradient system for both the evaluation of basic performances and the chromatographic analysis. In the system, fused-silica capillary tubes with inner diameters of 0.05 and 0.075 mm were used to reduce line volume. For evaluating, one of two micro-flow liquid-delivery systems (micro-flow pump A) was used to deliver MeOH, and the other (micro-flow pump B) was to deliver 0.1% or 0.3% acetone in MeOH. These were to be connected together in a micro-volume tee and further mixed in a micro-volume static mixer (Analytical Scientific Instruments, CA, USA), and the mixture was pumped through a Model SPD-10AD vp

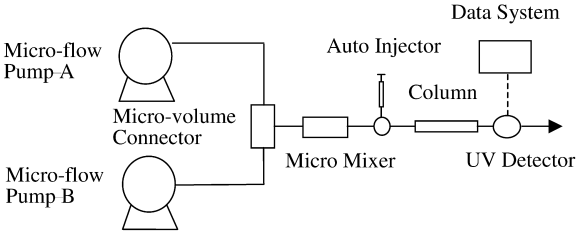

Fig. 2. High-pressure gradient system for the evaluation of basic performance and the chromatographic analysis.

UV–Vis detector (Shimadzu, Kyoto, Japan) fitted with a capillary flow cell (LC Packings, Amsterdam, The Netherlands). Ultraviolet detection was performed at 254 nm, and the signal from the detector was sent to a VSation data acquisition system (GL Sciences, Tokyo, Japan). Based on the programmed flow-rates of each pump as a function of time, different types of gradient would be possible according to analytical needs.

For the gradient analyses, the ODS columns with different inner diameters were applied in the high-pressure gradient apparatus. The ODS column of 150 mm×0.32 mm I.D. (3 μm particles) was purchased from LC Packings, and other ODS columns (5 μm particles) were obtained from GL Sciences. The columns were kept at a temperature of 30°C together the static micro-volume mixers using a Model CO630 column oven (GL Sciences). The typical gradient conditions for the analysis of peptides from a tryptic digest of cytochrome *c* were started at solvent B (0.1% trifluoroacetic acid and 10% water in acetonitrile)–solvent A (0.1% trifluoroacetic acid and 2% acetonitrile in water) (10:90), and went to solvent B–solvent A (60:40) within a interval of 50 min. The UV–Vis detector was set at a wavelength of 214 nm.

3. Results and discussion

3.1. Delay time of a delivery stroke due to liquid compressibility

Many liquid-delivery systems used in HPLC, in general, exhibit pressure-dependent flow-rates and large deviations in the flow-rate from the set value. Also, when different mobile phases are delivered, the more compressible solvents can lead to larger flow-rate errors. Foley et al. evaluated flow-rate accuracy of high-performance liquid chromatographic solvent-delivery systems using three common hydro–organic mobile phases (i.e., MeOH–water, MeCN–water and THF–water), and pointed out the sources of flow-rate errors in HPLC, one of which is non-ideal mobile phase behavior [14]. However, they never dealt with that the mobile phase compressibility must result in instrumental imperfections and mechanical limitations of the commercially available liquid-delivery systems, although discussed that the net effect of mobile phase compressibility is an increase in flow-rate of the column outlet relative to that of the column inlet. Martin et al. investigated that because of the liquid compressibility, a steady-state flow is achieved only after a long period of 15 to 60 min when using a syringe-type pump in HPLC system [15]. The syringe-type pump is very difficult for obtaining a continuous flow.

In the paper, we desired that the effect of mobile phase compressibility on constant and continuous liquid-delivery when using a dual-piston pump in HPLC system. Although the conventional dual-piston pumps are capable of generating a constant and continuous flow over a range of flow-rates larger than 0.1 ml/min, they suffer from insufficient precision, and inaccuracy at low-microliter flow-rates due to both the liquid compressibility and the mechanical limitation. The liquid-delivery processes of a reciprocating dual-piston pump can be broadly classified into three steps: (1) intake, (2) compression and (3) delivery. On the intake stroke, one of the dual pistons withdraws from the pump head, and the inlet check valve ball is pulled away from its seat when the pressure inside piston chamber decreases to that lower than atmospheric pressure. On the compression process, the piston then enters forward travel and compresses mobile phase in the piston chamber, and the inlet check valve is pushed closed against its seat; however, outlet check valve is still closed due to high pressure at the pump outlet (i.e., generally, same or close to pressure at the column inlet). When the mobile phase in piston chamber is pressurized to be equal to a pressure at the column inlet during the compression process, the outlet check valve is allowed to open and the mobile phase is delivered from the pump outlet. The compression process, therefore, leads to a delay-time of the delivery stroke and inconstant flow.

Although the compressibility of a liquid varies somewhat over a sufficiently wide range of pressure, the assumption of a constant compressibility results in a negligible error for pressures cased by a general HPLC column. Thus, the delay-time (T) of delivery stroke can be calculated using the following equation:

$$T = (V_0/F)[1 - \exp\ \beta(P_0 - P)] \quad (1)$$

where V_0 is volume of the piston chamber at start point of the compression process, P_0 and P are pressures at the start and end points of the compression process (P_0 is usually equal to atmospheric pressure), respectively, and F is a flow-rate set and β is the compressibility of a liquid. In the equation, V_0 is also assumed to be constant during the compression process. The delay times calculated with Eq. (1) and measured using a pressure sensor built in the piston chamber are shown in Table 1. As the results, the measured data were larger than those calculated by Eq. (1). One of the reasons for this is that changes in both V_0 and β during the compression process led to a calculation error. The deviation in the calculated and measured valves, however, was mainly cased by dilation of the piston chamber due to the extrusion of pump seal as discussed in next section.

Mobile phase in piston chamber included a delivered volume (V_s) per stroke and the volume (V_d) of residual mobile phase in a dead space of the piston chamber that did not be delivered during the delivery stroke. Therefore, we can change Eq. (1), and yield:

$$T = (V_s/F)[1 - \exp\ \beta(P_0 - P)] + (V_d/F)[1 - \exp\ \beta(P_0 - P)] \quad (2)$$

$$T_1 = (V_s/F)[1 - \exp\ \beta(P_0 - P)] \quad (3)$$

$$T_2 = (V_d/F)[1 - \exp\ \beta(P_0 - P)] \quad (4)$$

Because the intake process and the measurement of flow-rate at column outlet were carried out under atmospheric pressure, the compression for a portion V_s of the mobile phase delivered led to the delay time T_1 which cased pulsation only; however, the delay time T_2, which was cased by compressing mobile phase in dead space of the piston chamber, resulted in an decrease in flow-rate relative to the set valve.

3.2. Dilatation of piston chamber due to extrusion of pump seal

The dilatation of a well designed piston chamber, generally, can be negligible when the pump is used under maximum operating pressure (e.g., 40 MPa) and the pump head is made using a stainless steel. Table 1 seems to have suggested that other source of the increase in delay-time of the delivery stroke remained expecting the compressibility of liquid. When the compression process was monitored using the pressure sensor built in pump head, it was found that the pressure inside the piston chamber exhibited a transitory steady-state period before reached a pressure at column inlet, as shown in Fig. 3. Under the transitory period, the pump seal which is made using the more compressible polymer than the solvents used in HPLC has a tendency to slide back and extruded through an opening space. Thus, an increase in volume of the piston chamber may occur at high pressure during forward actuation of the piston, and a rise in pressure is stopped although the mobile phase in the piston chamber is compressed.

Table 1
Compressibility and delay-time of delivery stroke

Solvent	Compressibility[a] (10/atm)	Delay time[b] (s)			
		Calculated		Measured	
		20 MPa	30 MPa	20 MPa	30 MPa
Water	46.3	11	16	41	47
THF	70	17	25	58	65
Acetonitrile	74	18	26	61	68
Methanol	127	30	45	63	68
n-Hexane	161	38	57	75	83

[a] The compressibility are given under the conditions of 25°C and atmospheric pressure, and obtained from Refs. [14] and [15]. 1 atm = 101 325 Pa.

[b] The conditions calculated and measured for delay-time of the delivery stroke were set as follows. Volume of the piston chamber, $V_0 = 200$ μl; flow-rate, $F = 10$ μl/min; pressure at end point of the compression process, $P = 20$ or 30 MPa; pressure at start point of the compression process, $P_0 = 0$ MPa (namely, atmospheric pressure).

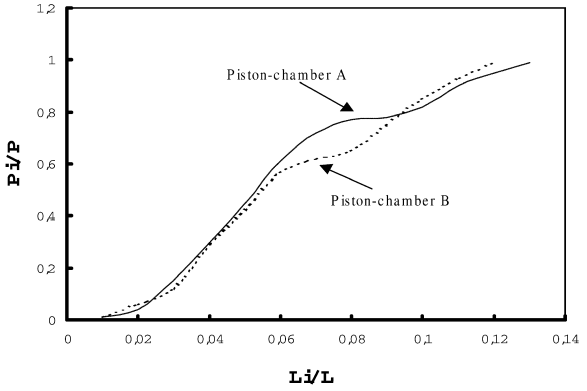

Fig. 3. Monitoring results of the piston-chamber pressure during compression process. P_i = Internal pressure of the piston-chamber, P = pressure at pump outlet (at column inlet), L_i = distance of piston travel during compression process, and L = stroke length.

This phenomenon led to a large delay time, pulsation and error in flow-rate. This type of the flow-rate errors cannot virtually be corrected using a correction factor or function, because it is very difficult to eliminate mechanical tolerances and minor difference in geometries of pump heads or pump seals.

3.3. Theoretical and practical approaches of micro-flow liquid-delivery system

Recently, many studies dealt with micro-flow liquid-delivery system in which the conventional HPLC pump was combined with a flow splitter to be used in capillary HPLC. Based on the experimental results related to the non-ideal behavior of mobile phase and the mechanism of both pulsation and error in flow-rate, we developed a new liquid-delivery system without using any flow splitter.

According to Eq. (1), we can find that the delay-time of the delivery stroke can be completely eliminated when the pressures at start and end points of the compression process are equal (i.e., $P = P_0$). It may be required to offer a solvent pressured at pump inlet; therefore, this leads to an expensive instrument. In the case of dual-piston pump, if one of the pistons can complete its compression process during delivery stroke of other piston, the problems can be easily overcame. Consequentially, the mechanical system with two independent drive units, monitoring of the compression process by the pressure sensors built in the piston chambers, and the systematic controller were employed to improve mechanical limitations and achieve constant and continuous liquid delivery.

3.4. Conditions-independent flow-rate accuracy and gradient profiles

As discussed in Section 3.1, when delivering different solvents, the conventional HPLC pumps, in general, provide different instrumental contributions to flow-rate error and pulsation due to the fact that the delay time of the delivery stroke is directly dependent on the compressibility of solvents. For a certain pure solvent, the systematic errors in flow-rate may be estimated using an appropriate correction factor or function which can be experimentally obtained; however, there is a large number of solvents applied in HPLC, and it is virtually impossible to correct especially for the mixtures under

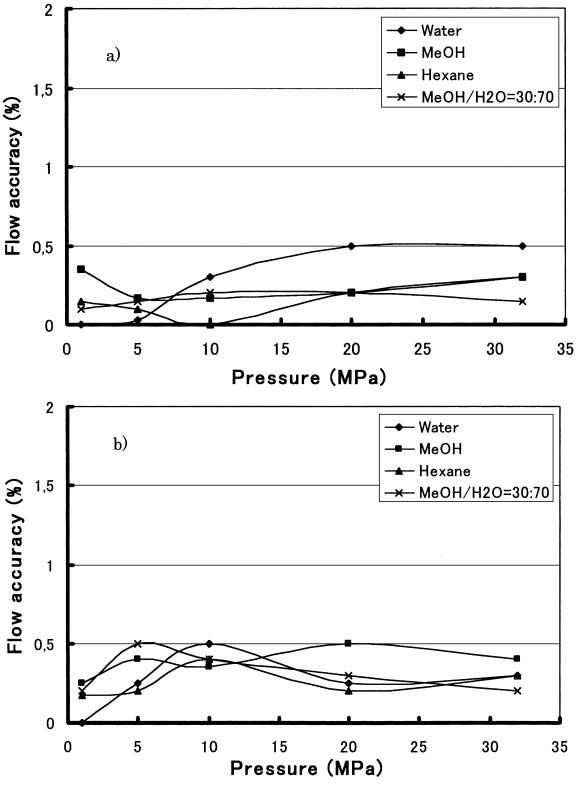

Fig. 4. Flow-rate accuracy of solvents with different compressibility. The set valves of flow-rate were (a) 5 μl/min and (b) 50 μl/min.

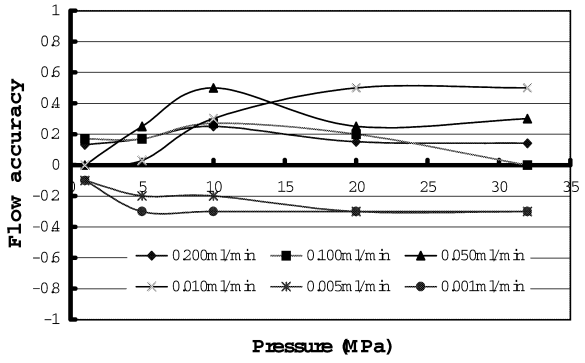

Fig. 5. Pressure-independent flow-rate accuracy. Liquid-delivery conditions: water, 20°C.

gradient conditions. As shown in Fig. 4a and b, the micro-flow liquid-delivery system exhibited excellent flow-rate accuracy (less than 0.5%) which was independent on the compressibility of liquid.

The pressure-dependent flow-rate accuracy is also an unavoidable problem with application of the commercially available reciprocating piston and syringe pumps in capillary HPLC. Under the gradient conditions, the system pressure varies with mobile phase compositions, and hence a steady-state flow cannot be achieved especially when the variation of the column pressure drop due to clogging. With application of the pre-compression methodology, we attempted to provide a pressure-independent flow-rate accuracy without using any correction factor or

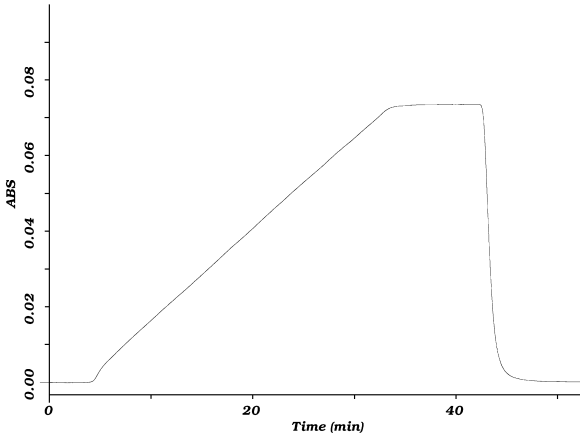

Fig. 6. Linear gradient profile obtained with two micro-flow liquid-delivery systems. Gradient conditions: total flow-rate, 100 μl/min; solvent A, MeOH; solvent B, 0.1% acetone (in MeOH); flow cell, inner volume of 1.2 μl; static mixer, 150 μl; started at 2.0 min, 0→100% B in 30 min, and hold in 10 min.

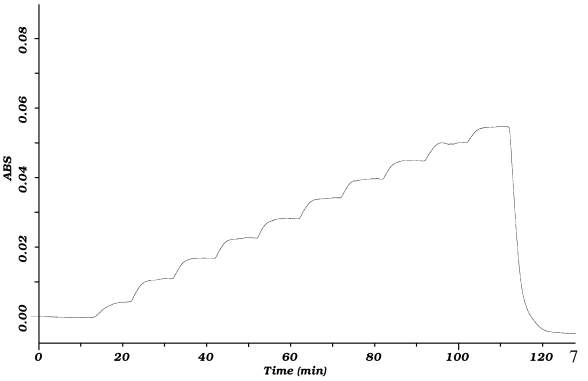

Fig. 7. Step gradient profile. Gradient conditions: total flow-rate, 10 μl/min; solvent A, MeOH; solvent B, 0.3% acetone (in MeOH); flow cell, inner volume of 35 nl; static mixer, 10 μl; from 0 to 100% B; step, 10% every 10 min.

function. Over a range of 1–200 μl/min, the deviations in flow-rate from the set value were examined. The experimental results conformed that the flow-rates of the micro-flow liquid-delivery system did not

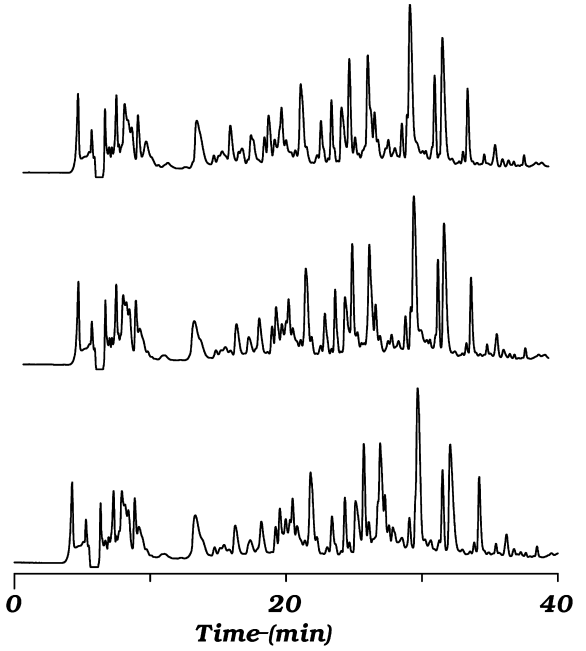

Fig. 8. Reproducibility of gradient analyses. Conditions: total flow-rate, 5 μl/min; eluent A, water–MeCN–trifluoroacetic acid (98:2:0.1); eluent B, MeCN–water–trifluoroacetic acid (90:10:0.1) gradient, 10% B to 60% B in 50 min; column, Inertsil ODS-3 (3 μm) 150 mm×0.32 mm I.D.; detection, UV 214 nm; sample, tryptic digest of cytochrome *c*; injection, 2 μl; column temperature, 30°C.

decrease with increasing pressure and appeared to be independent on the system pressure as shown in Fig. 5.

In addition, we evaluated performance of the micro-flow liquid-delivery system with the linear and step gradient profiles. Fig. 6 shows gradient accuracy in term of linear profile for the system. It can be observed that the gradient program can be accurately followed, and good linearity, rapid response and smooth baseline can be obtained because the system is capable of generating accurate and reproducible flow and allowed to eliminate the dilatable and compressible volumes such as pulse dampening unit. In other results shown in Fig. 7, step gradient profile with a high resolution can be soon achieved.

3.5. Chromatographic evaluation using gradient elution

For sensitive monitoring of gradient reproducibility, the tryptic digest of cytochrome *c* was used as a test sample. This complex sample is ideally suitable to evaluate performance of a gradient system because the retention behavior of tryptic fragments is strongly dependent on the mobile phase composition and minor changes in flow-rates of each pump result in obvious variations of retention time. As shown in Figs. 8 and 9, the high-pressure gradient system used with two micro-flow pumps exhibited excellent reproducibility.

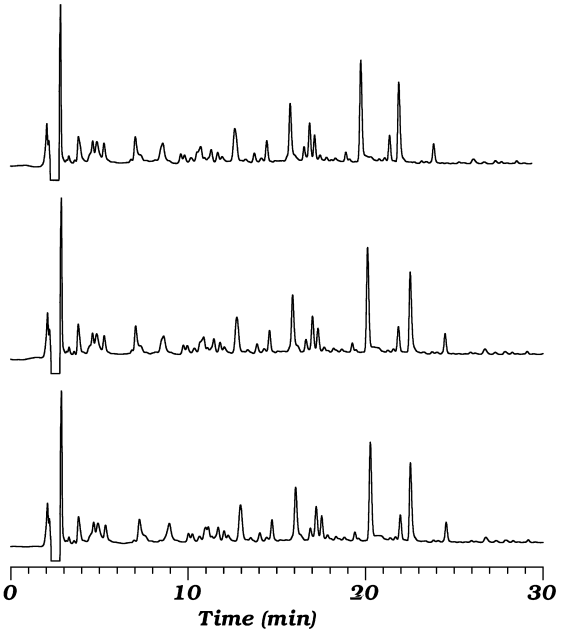

Fig. 9. Reproducibility of gradient analyses. Conditions: total flow-rate, 50 μl/min; eluents and gradient as in Fig. 8; column, Inertsil ODS-3 (5 μm) 150 mm I.D.×1.0 mm I.D.; detection, UV 214 nm; sample, tryptic digest of cytochrome *c*; injection, 2 μl; column temperature, 30°C.

4. Conclusions

Different instrumental contributions to flow-rate error and pulsation were theoretically and practically investigated. In conventional pumping systems, there were a several unavoidable problems caused by compressibility and other physical properties of the mobile phase. The experimental results conformed that the new micro-flow liquid-delivery system could overcome mechanical limitations and imperfections of the conventional piston and syringe pumps, and provide conditions-independent flow-rate accuracy and a powerful tool for highly reproducible gradient analyses in micro- and capillary-scale HPLC.

References

[1] H.E. Schwartz, B.L. Karger, Anal. Chem. 55 (1983) 1752.
[2] H. Poppe, Analusis 22 (1994) M22.
[3] D.B. Kassel, B. Shushan, T. Sakuma, J.-P. Salzmann, Anal. Chem. 66 (1994) 236.
[4] S.K. Chowdhury, J. Eshraghi, H. Wolfe, D. Forde, A.G. Hlavac, D. Johnston, Anal. Chem. 67 (1995) 390.
[5] R.C. Simpson, J. Chromatogr. A 691 (1995) 163.
[6] J.P.C. Vissers, A.H. de Ru, M. Ursem, J.-P. Chervet, J. Chromatogr. A 746 (1996) 1.
[7] J. Cai, J. Henion, Anal. Chem. 68 (1996) 72.
[8] J.P. Chervet, M. Ursem, Anal. Chem. 68 (1996) 1507.
[9] R. Grimm, M. Serwe, J.-P. Chervet, LC–GC 15 (1997) 960.
[10] Z. Zhang, A.G. Marshall, J. High Resolut. Chromatogr. 21 (1998) 291.
[11] R. Subramanian, W.P. Kelley, P.D. Floyd, Z.J. Tan, A.G. Webb, J.V. Sweedler, Anal. Chem. 71 (1999) 5335.
[12] J.E. MacNair, K.C. Lewis, Anal. Chem. 69 (1997) 983.
[13] J.E. MacNair, K.D. Patel, J.W. Jorgenson, Anal. Chem. 71 (1999) 700.
[14] J.P. Foley, J.A. Crow, B.A. Thomas, M. Zamora, J. Chromatogr. 478 (1989) 287.
[15] M. Martin, G. Blu, C. Eon, G. Guiochon, J. Chromatogr. 112 (1975) 399.