Chromatography

Technical Note

Development of an Automated Sample Injection System for Pillar Array Columns

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Abstract

A pillar array column with a perfectly ordered internal structure has a higher separation efficiency than a particle-packed column used in conventional high-performance liquid chromatography. However, applying the pillar array column technology to quantitative analysis is challenging as the injection volume of the sample varies for each injection. This occurs because the sample volumes are in the order of nanoliters and the sample is injected manually. In this study, an automated sample injection system was developed to solve this problem. The system was composed of two pumps (one for the sample and another for the mobile phase), a six-way valve that can be controlled by a PC, and an autosampler. The peak height deviation, which is more than 20% in the conventional manual injection system, was improved to 1.2% and 0.4% for the two coumarin dye samples. This result indicated that the automated sample injection method developed in this study could be applied to pillar array columns to allow for quantitative analysis.

Keywords: Microchip; Liquid chromatography; Automation

1. Introduction

High-performance liquid chromatography (HPLC) is reproducible and is an invaluable tool for quantitative analysis. It has become one of the most important analytical tools in various fields such as life sciences, the food industry, and clinical research [1-5]. Particle-packed columns with micrometer-sized spherical particles are widely used in HPLC, and it is well known that there is a limit in their separation efficiency since it is impossible to pack the particles uniformly [6,7].

To overcome the problem of nonuniformity in the internal structure of particle-packed columns, pillar array columns with a completely uniform internal structure have recently been developed [8-13]. The use of semiconductor microfabrication technology enables the uniform arrangement of pillars of the same size in a silicon substrate. Pillar array columns can significantly reduce eddy diffusion owing to its uniform internal structure. They also have a better resolution compared with that of particle-packed columns.

In order to employ pillar array column in practical applications, such as the analysis of biological samples, it is necessary to secure a certain degree of flow path on a small chip. Low-dispersion turns that prevent sample diffusion have been developed, and pillar array columns with long channels were created while suppressing sample diffusion [14,15]. These long-channel pillar array columns have excellent resolution, allowing for the analysis of complex samples. However, the analysis using pillar array columns requires the samples to be manually injected on the chip. The volume of these samples is approximately 1 nL [16,17]. Due to the small volume, the injected amounts of the sample can vary for each injection, which prevents pillar array columns from being used for quantitative analysis.

A solution to this problem would be to use the internal standard method. This has been used successfully in the quantitative analysis of branched-chain amino acids in biological samples using pillar array columns [17]. However, it is sometimes not easy to find an appropriate internal standard. In this study, we have developed a system.
that can provide a constant sample injection volume by automating the sample injection step.

2. Experimental

2.1. Chemicals

Coumarin dyes, Coumarin 525 and Coumarin 545, were purchased from Exciton (Dayton, OH). Dimethyloctadecylchlorosilane was purchased from Tokyo Chemical Industry (Tokyo, Japan). Acetonitrile (HPLC grade) was provided by Merck KGaA (Darmstadt, Germany). A Milli-Q system (Merck, Darmstadt, Germany) was used for water purification.

2.2. Microchip fabrication and modification

Microchip was fabricated by multistep ultraviolet photolithography and deep reactive ion etching. A pillar array column with a total length of 110 mm was fabricated on a 20 x 20 mm² microchip (Fig. 1). The channel width was 400 µm in the straight part and 110 µm in the turn part. Further, the depth of the pillar array column was 30 µm, and 60 µm in the injection part. The pillar size was 3 µm on a side, and the inter-pillar distance was 2 µm. The fabricated pillar array column was C₁₈ modified by dimethyloctadecylchlorosilane for reversed-phase separation. Details of the fabrication and modification of pillar array columns were given in our previous papers [14,18].

2.3. Chromatographic conditions

Mobile phase was a water/acetonitrile (65/35, v/v) mixture at a flow rate of 1 µL/min (LC-20ADnano, Shimadzu, Kyoto, Japan). A pump (LC-20AD, Shimadzu) was used to carry 50 µL of the sample injected by an autosampler (SIL-30AC, Shimadzu) to the chip. Switching flow path was performed by a high-pressure six-way valve (FCV-20AH, Shimadzu). Fluorescence excitation was performed by a metal halide lamp. The filter cube was composed of an excitation filter (BP460-490, Olympus, Tokyo, Japan), a dichroic mirror (505DRLP, Omega Optical, Brattleboro, VT), and emission filter (HQ 535/50m, Chroma Technology, Rockingham, VT). An IX70 inverted microscope system (Olympus) and electron-multiplying charged-coupled device camera (iXon3, Andor Technologies, South Windsor, CT) were used for observing fluorescence images. Detection was performed near the outlet of the pillar array column.

3. Results and discussion

3.1. Manual sample injection system

Figure 2 depicts the configuration of the conventional manual sample injection system. This system consisted of a pump, a syringe, and a valve. The syringe was filled with the sample, and the valve was connected to the sample channel of the chip. Therefore, it was possible to control the opening and closing of the sample channel using the valve. When the valve was switched to Position A, the sample was injected into the separation channel. In this injection system, the stronger the pushing force on the syringe, and the more time the valve spent in Position B, the more sample was injected onto the chip. As the amount of injected sample was about 1 nL, the injection volume was not deemed to be accurate.

The analysis of coumarin dyes, Coumarin 525 (C525) and Coumarin 545 (C545), were performed by manual injection. Both C525 and C545 had a relative standard deviation (RSD) of more than 20% for the peak heights (n=3). This result confirmed that the manual injection system is not applicable to quantitative analysis as a constant sample injection volume cannot be achieved using the manual injection system.

3.2. Automated sample injection system

In this study, an automated sample injection system was developed to keep the sample injection volume constant (Fig. 3). The manually operated valve was changed such
that it could be controlled by a PC, and an autosampler was used instead of a syringe. In addition, another pump (Pump II) was added to carry the sample to the chip.

The valve was initially set to position X, and the mobile phase were carried from Pump I and Pump II. For the injection of the sample by autosampler, the valve was changed to position Y, and the sample was carried to the chip by Pump II. When the sample reached the chip, the valve was switched to Position X, and a part of the sample was injected into the separation channel by Pump I. In this system, the sample introduction and the switching of the valve could be performed automatically. Since the injection conditions could be reproduced via the autosampler, a constant sample injection volume can be achieved.

3.3. Optimization of injection conditions

The injection conditions, including the valve switching time and the flow rate of Pump II, were optimized using coumarin dyes (C525 and C545). First, we examined the relation of the valve switching time and the peak heights of coumarin dyes. Good linearity was obtained between 0.6 and 3.0 sec (Fig. 4). Next, flow rate of Pump II was examined. Variance of peak heights at several flow rates of Pump II was evaluated. As shown in Fig. 5, the dispersion was found to be the lowest at 10 µL/min. A possible reason is that the sample took longer to reach the chip, and the sample dilution could have occurred prior to the injection. Under the optimized conditions of the automated sample injection system (valve switching time: 1.2 sec, flow rate of pump II: 10 µL/min), the RSD values of the peak heights of C525 and C545 were improved to 1.2% and 0.4%, respectively (Fig. 5, n=3). Fig. 6 shows chromatograms obtained by analyzing coumarin dyes in triplicate by using the automated sample injection system. The chromatograms obtained for the three analyses compared well to each other. These results indicate that the automated sample injection system developed has the ability to control the sample volume.

4. Conclusion

In this study, an automated sample injection system for pillar array columns was developed to maintain a constant sample injection volume. The evaluation of coumarin dyes
Chromatography showed a variation of more than 20% in the peak heights obtained using the conventional manual injection system. The RSD values of the peak heights were improved to 1.2% and 0.4% for C525 and C545, respectively, using the automated sample injection system. The developed automated sample injection system could be advantageous for the quantitative analysis of biological compounds using pillar array columns as it provides a highly reproducible sample injection volume.

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Fig. 6. Chromatogram of Coumarin dyes (Coumarin 525 and 545). The analysis was repeated three times by the automated injection system. Peaks: 1 Coumarin 525, 2 Coumarin 545.