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Method of increasing the sensitivity of liquid chromatography–atmospheric pressure chemical ionization mass spectrometry using a semi-micro column

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Abstract

A method for increasing the signal intensity of liquid chromatography–atmospheric chemical ionization mass spectrometry (LC–APCI-MS) using a semi-micro column was studied. Prostaglandin E_1 was used as a model compound. However, this method was not effective for detection with APCI-MS without an improved APCI interface, in which the diameter of the micro-pipe on the APCI interface was decreased from 0.1 to 0.05 mm. The signal intensity of APCI-MS detection using a semi-micro column with an improved APCI interface was five times that obtained with use of a conventional column.

1. Introduction

Liquid chromatographic techniques are in common use in analytical, biological and other types of studies, and LC–MS in particular is widely used as a powerful analytical tool. However, LC–MS suffers from certain problems, including the inability to use non-volatile buffer solutions as the mobile phase and low sensitivity compared with GC–MS. Non-volatile buffer solutions for use as mobile phases have been reported with column-switching techniques [1,2] or with the use of suppressor in ion chromatography [3]. Derivatization methods have been reported for increasing the sensitivity of detection [4,5]. The use of a semi-micro or micro column technique for LC–MS to increase sensitivity has already been reported for fast atom bombardment ionization [6], electrospray ionization [7,8] and ionspray ionization [9] interfaces, but there has been no report of such use for an atmospheric pressure chemical ionization (APCI) interface. A semi-micro column leads to less band spreading of solutes and higher numbers of theoretical plates than does a conventional column, and therefore has been used to increase the signal intensity with ultraviolet, fluorescence and other types of detection for HPLC. In this study, we attempted to increase the signal intensity for prostaglandin E_1 as a

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model compound using a semi-micro column for LC–APCI-MS.

2. Experimental

2.1. Materials and reagents

Prostaglandin E_1 (PGE_1) was purchased from Funakoshi (Tokyo, Japan). A 100 μg/ml solution of PGE_1 was prepared in 10% (w/w) ethanol. The deionized water used in all experiments was obtained from a Milli-Q system (Waters, Milford, MA, USA). All other solvents and reagents were of analytical-reagent or HPLC grade.

2.2. LC–APCI-MS systems and conditions

A Model M-1000 LC–APCI-MS system connected with a Model L-6200 liquid chromatograph (Hitachi, Tokyo, Japan) was used. A 10-μl volume of sample solution was injected with a microsyringe through a Model 7125 loop injector with a 20-μl loop (Rheodyne, Cotati, CA, USA). Separation was performed on a Develosil ODS-5 column (particle size 5 μm; Nomura Chemical, Seto, Japan). The semi-micro column (150 × 2.0 mm I.D.) and the conventional column (150 × 4.6 mm I.D.) were stainless-steel tubes and the flow-rates were 0.2 and 1.0 ml/min, respectively. The tubes connected with the injector, the column and the mass spectrometer were polyether–ether–ketone (PEEK) tubes of 0.13 and 0.25 mm I.D. for the semi-micro column and the conventional column, respectively. The other operating conditions were as reported previously [10].

3. Results and discussion

In this study, PGE_1, the characteristics of which had already been studied with LC–APCI-MS using a conventional column [10], was used as a model compound, and had a base ion peak at m/z 337 ($[M + H - H_2O]^+$) in the APCI mass spectrum. When a semi-micro column was used as the separation column, the PGE_1 peak was very broad on LC–APCI-MS, and the signal intensity was about twice that with a conventional column (Fig. 1). These findings suggested the presence of diffusion of PGE_1 in the APCI interface. A block diagram of the APCI interface is shown in Fig. 2. The flow route was closed between the injector and the micro-pipe of the APCI interface, but was open between the micro-pipe and the first aperture. Therefore, it was thought that diffusion of mobile phase occurred between the micro-pipe and the first aperture, and PGE_1 was assigned the broad peak on LC–APCI-MS. In fact, the PGE_1 peak was sharp and exhibited a high intensity with UV detection. The pressure in the micro-pipe fell to about 4–5 kg/cm^2 when the semi-micro column was used from 20–30 kg/cm^2 when the conventional column was used, because the flow-rate changed to 0.2 ml/min from 1 ml/min, respectively.

This low pressure was also thought to be responsible for the broad peak on LC/APCI-MS. For that reason, suppression of diffusion of mobile phase and solute and increase in the pressure in the micro-pipe are required to obtain a sharp PGE_1 peak.

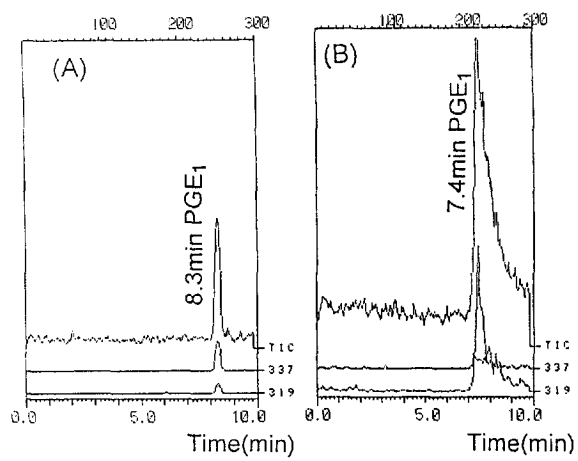

Fig. 1. Total ion current chromatograms (m/z = 200–500 with 2 s per scan) of 100 ng of PGE_1 on (A) conventional column and (B) semi-micro column.

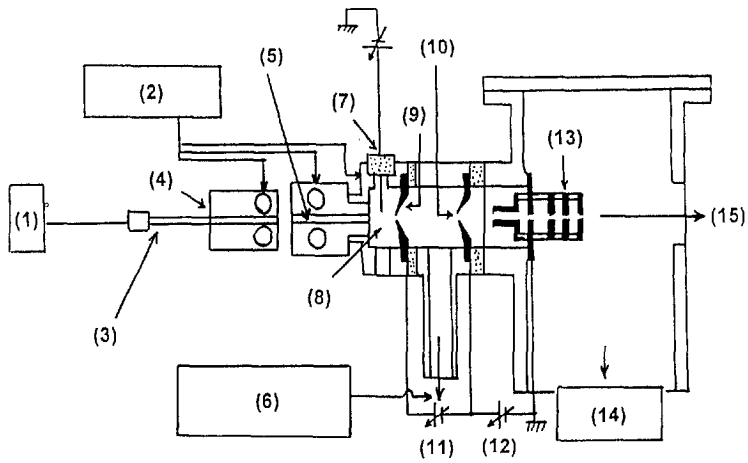

Fig. 2. Block diagram of the APCI interface: 1 = HPLC unit; 2 = electric source for heating; 3 = micro-pipe; 4 = vaporizer; 5 = desolvation; 6 = vacuum pump; 7 = needle electrode for corona discharge; 8 = APCI ion source housing; 9 = first aperture; 10 = second aperture; 11 = electric source for drift voltage; 12 = electric source for ion acceleration; 13 = quadruple lens; 14 = vacuum pump; 15 = mass MS spectrometer.

3.1. Adjustment of micro-pipe position

Initially, the micro-pipe position was adjusted to increase signal intensity and sharpen the PGE_1 peak. Normally, the micro-pipe was set on the vaporizer heating block in an appropriate position and the micro-pipe [Fig. 2 (3)] was moved toward the first aperture [Fig. 2 (9)], since it was thought that the PGE_1 peak shape was broad owing to the poor arrival of the mobile phase vapour and the solute at the needle electrode for corona discharge and the first aperture due to low pressure in the micro-pipe, but this method was not effective in increasing signal intensity or improving the peak shape even when the micro-pipe was moved 3 cm towards the first aperture. These findings suggested that increasing the pressure in the micro-pipe was necessary.

3.2. Postcolumn addition of solvent

Next, postcolumn addition of solvent was attempted. This method was aimed at increasing the pressure in the micro-pipe and suppressing diffusion of solutes in the column. The addition pump used was a Model 6010 (Hitachi), which was connected to a T-connector at the post-column. With addition of 0.8 ml/min of mobile phase, the peak intensity and the PGE_1 peak shape were the same as those obtained using the conventional column. These findings indicated there was diffusion of the solute after column separation. In order to suppress diffusion, hexane, which is not miscible with water, was used as an additional solvent. This method increased the sensitivity of APCI–MS to about 2–3 times that of the conventional column, but the peak shape was not markedly improved.

3.3. Improvement of micro-pipe

The inside diameter of the micro-pipe is 0.1 mm, and this diameter is justified with use of a conventional column. When a semi-micro column is used, this diameter decreases the pressure in the micro-pipe, and the correct diameter for use is about 0.05 mm. Therefore, improvement of the micro-pipe by using a 0.05 mm I.D. was attempted. The micro-pipe consisted of a heating stem block and stainless-steel tube (1/16 in., 0.1 mm I.D.), and the stainless-steel tube was removed from the heating stem block of the micro-pipe. Next, a stainless-steel tube of 0.5 mm I.D.

was inserted into the heating stem block as a guide-pipe. In addition, a tube of 0.05 mm I.D. prepared from Polisil Tubing (GL Sciences, Tokyo, Japan), which features a polymer coated on fused-silica tubing was inserted into the guide-pipe, and was fixed with a union and connectors.

This improved micro-pipe was set on the vaporizer block and chromatographic analyses were performed. A fivefold increase in peak intensity compared with that with a conventional column was obtained, along with improvement of the peak shape compared with that with a 0.1 mm I.D. micro-pipe (see Fig. 4).

Certain factors were considered as reasons for these findings. One was proper suppression of band spreading of solutes on the column. The vaporization area of the mobile phase was also thought to be such a factor, but the vaporization areas on the conventional and semi-microcolumns did not differ greatly (1.7 and 1.3 cm^2, respectively). These areas were measured from areas of change in colour of thermal paper that had been positioned at the first aperture position. The state of the mist of the vapour was thought to be another such factor, since the states of nebulized mist from 0.1 to 0.05 mm I.D. micro-pipes differed appropriately, whereas mist particles from the 0.05 mm I.D. micro-pipe were smaller than those from the 0.1 mm I.D. micro-pipe. Vapour mist composed of smaller particles yields a good efficiency of vaporization and collisions with the solvent, which is ionized by a corona discharge, and solute are increased, yielding a high signal intensity. However, a fivefold increase in intensity was obtained with the semi-micro column and this increase corresponded to the decrease in column cross-sectional area. Therefore, the factor responsible for sensitivity was suppression of band spreading of solutes in the semi-micro column, and improvement of the micro-pipe with a small diameter resulted in optimum mobile phase vapour conditions for analysis using a semi-micro column.

3.4. Mass spectra of PGE_1

PGE_1 mass spectra measured under several sets of conditions are shown in Fig. 3. The mass spectrum obtained using a conventional column had a base peak at m/z 337 $[(M + H - H_2O]^+$, Fig. 3A), but that obtained using a semi-micro column and a 0.1 mm I.D. micro-pipe had a base peak at m/z 319 ($[M + H - 2H_2O]^+$, Fig. 3B). A fragment ion peak at m/z 333 also appeared in the latter spectrum but not in the spectrum obtained using the conventional column. This peak at m/z 333 was thought to represent $(M + H - 3H_2O + MeOH)^+$. This difference in the mass spectra was due to the difference in the moving times of mobile phase in the vaporizer region; for the conventional and semi-micro columns with flow-rates of 1 and 0.2 ml/min, the moving times were about 20 and 100 ms, respectively. Thus, using the semi-micro column, PGE_1 was heated for a longer time than in the conventional column. When the micro-pipe position was slid 3 cm for the first aperture, the mass spectrum obtained was the same as that for the semi-micro column (Fig. 3C). When hexane was added to mobile phase postcolumn, the ratio of peaks with m/z 319 and 337 differed from that for the conventional column. The moving time of the mobile phase with the post-addition method was the same as that with the conventional column, but the heats of vaporization of the mobile phase and the mixture of mobile phase and hexane differed [11]. This difference in heats of vaporization influenced the mass spectra. When a micro-pipe of 0.05 mm I.D. was used and the flow-rate was set at 0.2 ml/min, the moving time in the vaporizer region was 25 ms, and this time and the heat of vaporization of mobile phase were the same as those for the conventional column. The mass spectrum obtained was the same as that for the conventional column (Fig. 3E). Thus, comparison of mass spectra indicated that the method using a micro-pipe of 0.05 mm I.D. was also effective.

3.5. Application

The semi-micro column method was used for the identification of a degradation product of PGE_1. The degraded sample used was a PGE_1 solution (100 μgl/ml, pH 7.0 phosphate buffer) stored for 1 h at 60°C. The residual PGE_1 level in this sample was about 80% (peak area per-

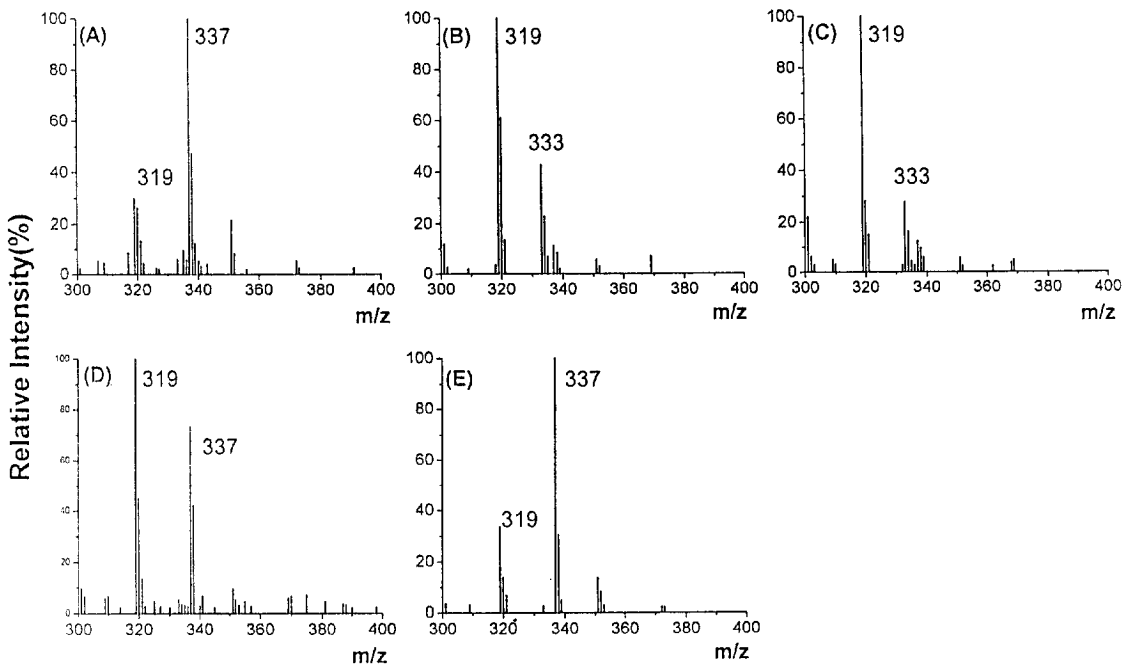

Fig. 3. PGE_1 mass spectra: (A) conventional column; (B) semi-micro column with normal micro-pipe; (C) semi-micro column with micro-pipe positioned forward (5 mm); (D) semi-micro column with postcolumn addition of hexane; (E) semi-micro column with improvement of micro-pipe.

centage with UV detection: 210 nm). Portions of 10 μl of this solution were subjected to LC–APCI-MS using a conventional column and a semi-micro column, and the results are shown in Fig. 4. The PGE1 peak appeared at a retention time of 6.8 min of on the conventional column and at 7.5 min on the semi-micro column, although both columns had a linear flow-rate of 6 cm/min. This difference in retention time is thought to be due to differences in the conditions of column packing and the lot numbers of the packing materials.

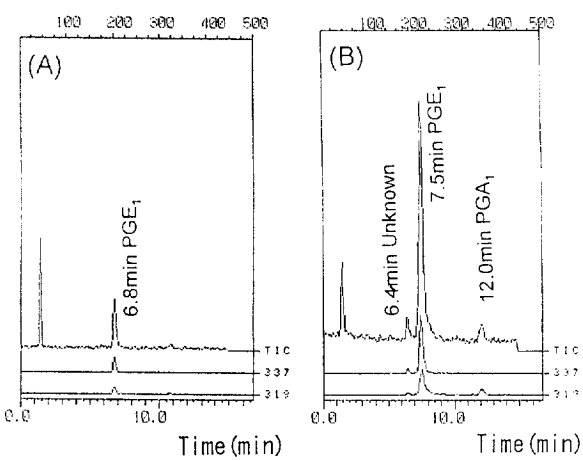

Fig. 4. Total ion current chromatograms (m/z = 200–500 with 2 s per scan) of 10 μl of degraded PGE_1 sample on (A) conventional column and (B) semi-micro column.

With use of the semi-micro column a degradation product was detected behind the PGE_1 peak (retention time 12.0 min), but with use of the conventional column the degradation product was not clearly detected in the same intensity range. The spectrum of this peak was the same as that previously reported for PGA_1 [10], and this degradation peak was in fact identified as that of PGA_1. In addition, a very small unknown peak was present on the LC–APCI-MS trace just before the PGE_1 peak (retention time 6.4 min). This peak could not be identified, but its spectrum was the same as that of PGE_1; it may therefore have been the 8-epimer of PGE_1.

These findings indicated when a small amount of a compound is to identified by LC–APCI-MS, the use of a semi-micro column as the analytical column is more advantageous than the use of a

conventional column if the same volume of sample for analysis is to be injected. We believe that our semi-micro column method will be effective for any analyte.

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