HIGHLY SENSITIVE SIMULTANEOUS DETERMINATION OF PROSTAGLANDINS BY USING HPLC/LASER INDUCED FLUORESCENCE (LIF)

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Abstract - We have developed a laser induced fluorometric (LIF) detection method combined with HPLC and an analytical procedure for prostaglandins (PGs) which allows analysis of practical samples without interference from admixtures and residual reagents. We were able to derivatize PGs with 9-anthryldiazomethane (ADAM) at 4°C in the dark after 12 hours. This method was applied to analysis of human urine (20 μ L). Clear separation and detection of PGs at fmole levels could be achieved. The lower detection limit was 1.0 fmole for PGF₁ α .

Key words prostaglandins, laser induced fluorometry, trace analysis, human urine

It is well known that PGs play many important roles in various pathophysiological processes such as thrombus formation, and inflammatory and allergic reactions. Human health is kept by balancing these metabolites. If balancing of PG mutabilities in human body is measured, it is possible to get disease symptoms with clinical inspections. This requires development of a PG determination method of at least the 10 fmole level. Nowadays, PGs are mainly determined by using radio immunoassay (RIA). However, as RIA determines one kind of PG, it is difficult to measure other PG metabolites present. On the other hand, the simultaneous determination method of PGs by using HPLC has been studied, however. HPLC can not be used to determine ultratrace PGs, because its sensitivity is insufficient.

In this paper, we combined HPLC/LIF in which the latter employed, as the excitation source, the third harmonic of a pulse YAG laser (355nm, 0.7mW). Although the LIF method has provided ultrahigh sensitivity, for practical samples, the ultratrace analysis are difficult due to large and unstable background caused by admixtures and residual reagents. Then in the present study, we also examined a sample preparation procedure suitable for the practical biological materials which had no interference from those.

EXPERIMENTAL

The laser fluorometry system was laboratory constructed. A YAG laser was chosen as the excitation source because of its convenient wavelength (355nm) for ADAM fluorescence excitation. The UV laser radiation (laser power: 0.7mW, pulse repetition: 1kHz, pulse width: 60 ps) was incidented into a flow cell in which an optical fiber was directly inserted to collect the fluorescence [1]. The size of the sample channel in the flow cell was 1mm and its volume was about 10 μ L.

PGs were extracted by the modified Poweel method [2]. A 1 mL portion of human urine was collected in a test tube. PGs were extracted by using an ODS column (Sep-Pak C18, Waters Ltd.) PGs could be separated from biological admixtures by washing with water. The extracted PGs were derivatized with 20 μ L of 0.2 % ADAM at 4°C in the dark for 12 hours. The derivatized PGs were separated from the unreacted reagents by Sep-Pak Silica. The sample solution was diluted to 1ml with distilled water for the isocratic phase HPLC, and 20 - 100 μ L portions of those solutions were injected into the HPLC system.

RESULTS AND DISCUSSION

It has been reported that fatty acids including PGs could be derivatized with ADAM at room temperature for 3 hours without a catalyst [3]. At room temperature, however, many kinds of PGs were deteriorated. We derivatized with ADAM at around 4 °C. At first, 10 μ L of 0.2 % ADAM were added to the solution which contained 400 nmole of PGF₁ α . We measured fluorescence intensity of derivatized PGF₁ α for derivatization periods of 3, 6 and 12 hours in the dark. Table 1 shows those results.

Fluorescence intensity of PGF₁ α derivatized at 20 °C for 3 hours is also given for comparison. PGs were completely derivatized with ADAM at 4 °C after 6 or more hours. We set the derivatization conditions as in the dark at 4 °C for 12 hours.

The derivatized PGs were determined by this method. At first, 25 to 125 fmole of standard PGF₁ α was derivatized with ADAM as above. The determination line of the PGF₁ α showed a good linearity, and detection limit was 1.0 fmole (S/N=2).

This method was applied to analyze PGs in human urine. A typical chromatogram is shown in Fig. 1. There was clear separation and highly sensitive detection at fmole levels of PGs. The base line was very low and stable because of effective separation of the admixture and unreacted ADAM. Reduction of the ADAM reagent quantity also contributed to decreased the background level. Admixture in human urine was prevented from being derivatized with ADAM under the low temperature reaction condition.

The ADAM reagent has been applied to HPLC separation and fluorometric detection of PGs. However, this reagent has been considered to be unsuitable to practical analysis of them, because of the high and unstable background. In the present experiment, we changed the reaction temperature and minimized the reagent quantity, utilizing the ultrahigh sensitivity of the detector, and as a result lowered the background and improved the detection limit, about two to three orders of magnitude. This method was superior for simultaneous quantitative analysis to other methods and is expected to be applied to various practical biological samples.

TABLE 1. Conditions for derivatization and fluorescence intensity

Temperature (°C)	Period (h)	Fluorescence intensity (Arb. U.)
20	3	1.0
4	3	0.63
4	6	1.0
4	12	1.0

Fig. 1. Chromatogram of PGs

- (A) Chromatogram of Standard PGs
 1 : PGE₂ 2.5fmol ; 2 : PGD₂ 2.5fmol ;
 3 : 13,14-Dihydro-15-dehydro-PGE₂ 2.5fmol
 (B) Chromatogram of PGs in Human Urine (20 μ l)

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