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## Note

# Separation of prostaglandins $E_1$, $E_2$, $F_{1\alpha}$ and $F_{2\alpha}$ by reversed-phase high-performance liquid chromatography*

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Prostaglandin (PG) levels have been determined by bioassay[1], radioimmunoassay[2], enzymatic assay[3], gas chromatography–mass spectrometry[4] and high-performance liquid chromatography (HPLC)[5]. Although many reports have been published on the separation of classical PGs by HPLC[6–8], nothing has appeared on the separation of the $PG_1$ from the $PG_2$ group by reversed-phase HPLC. In this paper, we describe a rapid method for the separation of $PGE_1$, $PGE_2$, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ by reversed-phase HPLC.

## EXPERIMENTAL

### *Reagents and chemicals*

Acetonitrile (HPLC grade) and potassium dihydrogen orthophosphate were obtained from Katayama (Osaka, Japan), and methanol (HPLC grade) from Wako (Osaka, Japan). Standard $PGE_1$, $PGE_2$, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were supplied by the Central Research Institute, Ono Pharmaceutical (Osaka, Japan). Prednisolone, used as an internal standard, was obtained from Nippon Merck-Banyu (Tokyo, Japan).

### *Apparatus*

A Model FLC-350 high-performance liquid chromatograph [Japan Spectroscopic Co. (JASCO), Tokyo, Japan] with a Model UVIDEC 100 ultraviolet spectrophotometer (JASCO) and a Model VL-611 variable loop injector (JASCO) was utilized. Chromatograms were recorded with a Hitachi single-pen recorder.

### *Columns*

A SC-02 reversed-phase column (JASCO) of dimensions 25 cm × 4.6 mm I.D. was used for separations.

* *Editor's note:* See also the article by A. R. Whorton, K. Carr, M. Smigel, L. Walker, K. Ellis and J. A. Oates, *J. Chromatogr.*, 163 (1979) 64–71, which had not appeared when this note was submitted.

*Chromatographic conditions*

The mobile phase was 0.02 M potassium dihydrogen orthophosphate–acetonitrile (3:2). The flow-rate was 1.4 ml/min and the chromatographic separation was monitored with a UV spectrophotometer set at 215 nm and 0.04 a.u.f.s.

Chromatography was performed in triplicate at ambient temperature. A 3-$\mu$l volume of standard PGs in methanol and the 2 $\mu$l of prednisolone in methanol were injected into the three columns (each 25 cm long and joined in series), through a 5-cm long pre-column and eluted as described above.

## RESULTS AND DISCUSSION

To separate $PGE_1$, $PGE_2$, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ by reversed-phase HPLC, distilled water–acetonitrile mixtures were tried as the solvent system. These mixtures separated the PGE group from the PGF group, but separations of the $PG_1$ from the $PG_2$ group were unsatisfactory. When potassium dihydrogen orthophosphate was added to this system, further separation between $PG_1$ and $PG_2$ was obtained. This is probably due to diminished ionization of the carboxyl group in the PGs. The separation results are shown in Table I and Fig. 1.

TABLE I

RETENTION TIMES OF PROSTAGLANDINS

| *Prostaglandin* | *Retention time (min)* |
|---|---|
| $PGF_{2\alpha}$ | 18.0 |
| $PGF_{1\alpha}$ | 19.2 |
| $PGE_2$ | 20.6 |
| $PGE_1$ | 22.6 |

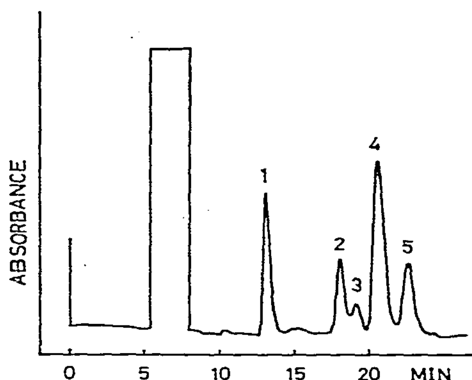


Fig. 1. Chromatogram of standard PGs. Column: SC-02 (three 25-cm columns and a 5-cm pre-column). Solvent: 0.02 *M* $KH_2PO_4$–acetonitrile (3:2). Flow-rate: 1.4 ml/min. Absorption measured at 215 nm (0.04 a.u.f.s.). Peaks: 1 = prednisolone; 2 = $PGF_{2\alpha}$; 3 = $PGF_{1\alpha}$; 4 = $PGE_2$; 5 = $PGE_1$.

The calibration graph for standard PGs was measured in triplicate. The data presented in Fig. 2 clearly indicate that the relationship between PG concentration and peak-height ratio is linear.

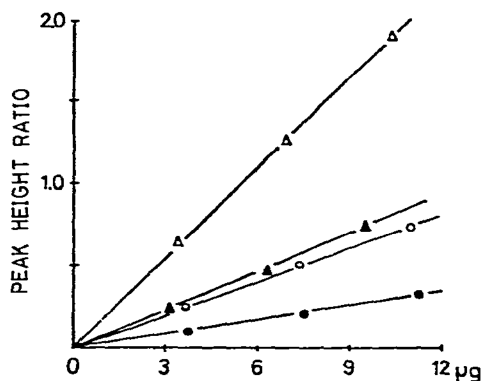


Fig. 2. Calibration graph for standard PGs. The internal standard was prednisolone (200 μg/ml). ▲, $PGE_1$; △, $PGE_2$; ●, $PGF_{1\alpha}$; ○, $PGF_{2\alpha}$.

No labelling with a UV absorbent or fluorescent substance was necessary for the detection of PGs by measuring the absorption at 215 nm, although this modification could improve the sensitivity of the method.

## ACKNOWLEDGEMENTS

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