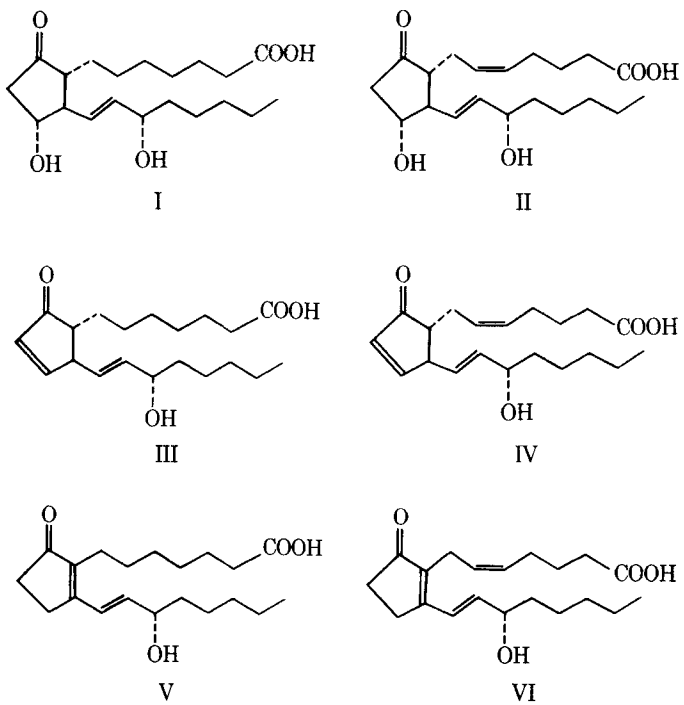
Utilization of Cyclodextrin Complexation for Separation of E, A, and B Prostaglandins by Ion-Exchange Liquid Chromatography

K. UEKAMA [x], F. HIRAYAMA, K. IKEDA *, and K. INABA [‡]

Abstract □ Application of cyclodextrin complexation to the separation of E-, A-, and B-type prostaglandins by ion-exchange liquid chromatography is demonstrated. The addition of α- or β-cyclodextrin into the mobile phase on an anion-exchange support decreased the retention times of the prostaglandins significantly because of soluble complex formation. Chromatographic separation behavior is discussed on the basis of the stability of the inclusion complex. A rapid and sensitive method for the separation and quantification of the prostaglandins, using β-cyclodextrin in the mobile phase, is described.

Keyphrases □ Prostaglandins—ion-exchange liquid chromatographic analysis after cyclodextrin complexation, prepared samples □ Cyclodextrin complexation—with various prostaglandins, ion-exchange liquid chromatographic analysis, prepared samples □ Ion-exchange liquid chromatography—analysis, various prostaglandins after cyclodextrin complexation, prepared samples □ Complexes—various prostaglandins with cyclodextrins, ion-exchange liquid chromatographic analysis, prepared samples

The prostaglandins are essentially long chain unsaturated fatty acids containing a substituted cyclopentane ring system. The β-hydroxyketo moiety of E-type prostaglandins (I and II[1]) is susceptible to dehydration in acidic or alkaline conditions to form A-type prostaglandins (III and IV[2]), which are isomerized consecutively to form the corresponding B-type prostaglandins (V and VI[3]) in alkaline conditions (1, 2). The A- and B-type prostaglandins are isomeric compounds, differing only in the position of the double bond in the cyclopentanone ring.



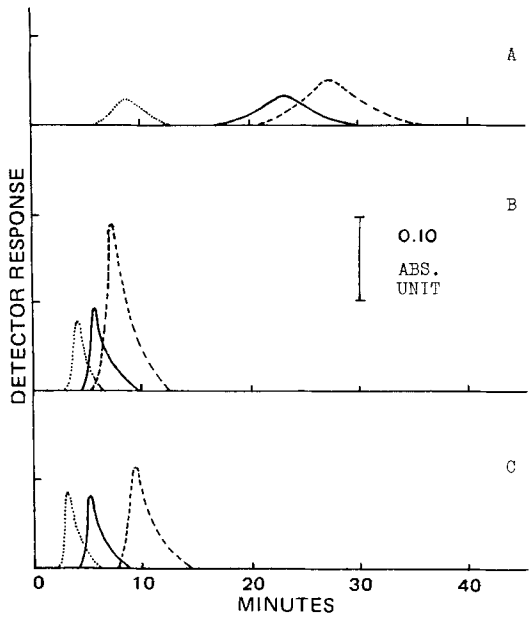


Figure 1—*Liquid chromatograms of I (20 μg), III (2.67 μg), and V (3.32 μg) on an anion-exchange column in the absence and presence of cyclodextrin in the mobile phase. Chromatographic conditions were: mobile phase, 0.1 M sodium phosphate buffer (pH 6.0, ionic strength 0.2); flow rate, 0.5 ml/min; and detection, 210, 221, and 282 nm for I, III, and V, respectively. Key: A, without cyclodextrin; B, with 0.5% α-cyclodextrin; C, with 0.5% β-cyclodextrin; ·····, I; —, III; and - - - -, V.*

High-performance liquid chromatography (HPLC) allows the direct analysis of closely related prostaglandins without protective derivatization (3). However, because the prostaglandins are hydrophobic, they generally exhibit long retention times with undesirable separation behavior on anion-exchange supports using an aqueous mobile phase. Therefore, a more rapid and sensitive HPLC method is required for the separation and detection of the prostaglandin isomers.

α- and β-Cyclodextrins form inclusion complexes with various drug molecules in water (4–9). The relatively hydrophobic environment of the cyclodextrin cavity is expected to attract the prostaglandins. A preliminary study indicated that I, III, and V formed soluble complexes with α- and β-cyclodextrins in water, which were stable in the neutral pH region (10).

The present paper deals with the application of cyclo-

[1] I is prostaglandin E_1 [(11α,13*E*,15*S*)-11,15-dihydroxy-9-oxoprost-13-en-1-oic acid], and II is dinoprostone [(5*Z*,11α,13*E*,15*S*)-11,15-dihydroxy-9-oxoprosta-5,13-dien-1-oic acid].

[2] III is prostaglandin A_1 [(13*E*,15*S*)-15-hydroxy-9-oxoprosta-10,13-dien-1-oic acid], and IV is prostaglandin A_2 [(5*Z*,13*E*,15*S*)-15-hydroxy-9-oxoprosta-5,10,13-trien-1-oic acid].

[3] V is prostaglandin B_1 [(13*E*,15*S*)-15-hydroxy-9-oxoprosta-8(12),13-dien-1-oic acid], and VI is prostaglandin B_2 [(5*Z*,13*E*,15*S*)-15-hydroxy-9-oxoprosta-5,8(12),13-trien-1-oic acid].

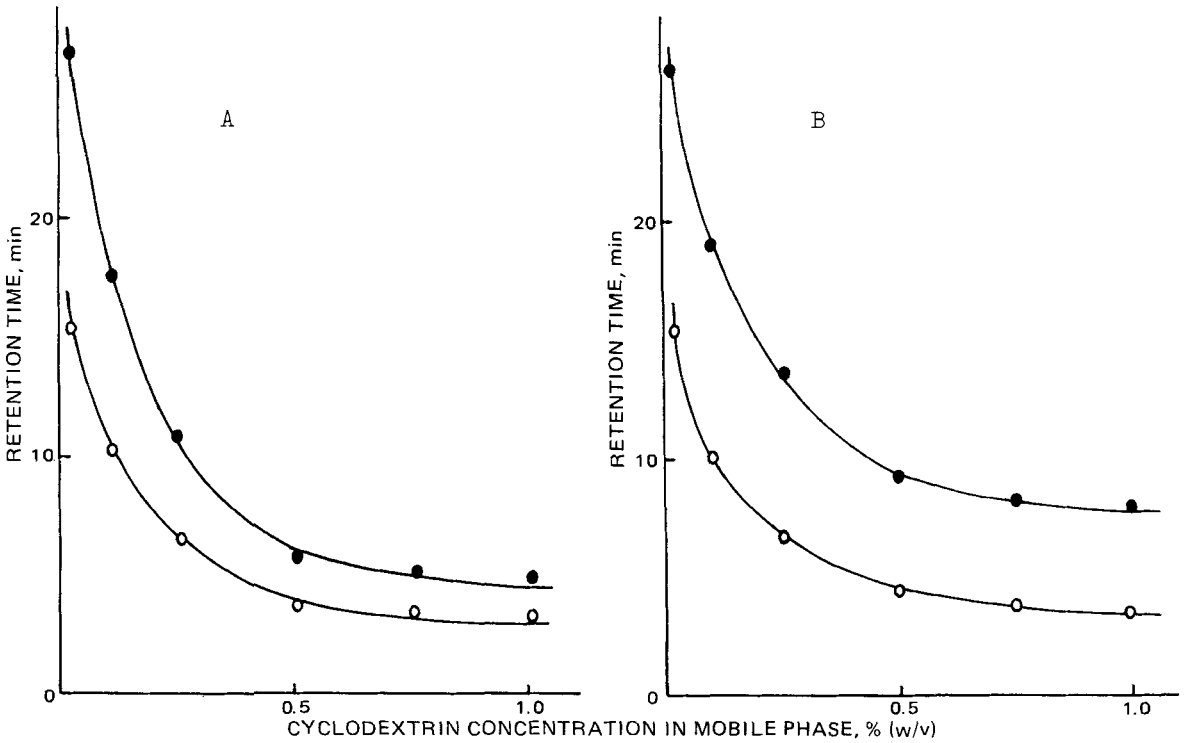


Figure 2—*Effects of cyclodextrin concentration on the retention times of III and V. Chromatographic conditions were the same as in Fig. 1. Key: A, α-cyclodextrin system; B, β-cyclodextrin system;* ○, *III; and* ●, *V.*

dextrin complexation to the separation of these prostaglandins by ion-exchange HPLC. α- or β-Cyclodextrin was added to the mobile phase on a strong anion-exchange support. To increase the detection sensitivity, A and B prostaglandins were monitored at their UV maximum wavelengths by a spectrophotometer equipped with a multiwavelength scanner. Furthermore, the concentration of cyclodextrin, pH, ionic strength, and solvent composition in the mobile phase were investigated to optimize resolution. The developed method is rapid, sensitive, and precise for the separation and quantification of the prostaglandins.

EXPERIMENTAL

Apparatus—The liquid chromatograph was equipped with a pump[4] (700 kg/cm^2 maximum), a double-beam spectrophotometric detector with a programmable multiwavelength scanner[5], an injection port[6], a microflowcell unit[7], and a strip-chart four-pen recorder[8].

Chromatographic Conditions—A 500 × 2.3-mm i.d. stainless steel column, packed with a strong anion-exchange pellicular support[9], was operated at a flow rate of 0.5 ml/min (about 1100 psi), unless otherwise stated. The pH and ionic strength of the mobile phase, phosphate buffer, were adjusted with 0.1 *M* phosphoric acid, 0.1 *M* sodium hydroxide, and sodium chloride.

Quantitative Analysis—Stock solutions of E, A, and B prostaglandins were prepared at concentrations of 6.63, 2.13, and 1.53 mg/ml in ethanol, respectively. A 2-μl aliquot of the sample was injected quantitatively with a 10-μl syringe[10]. The column temperature was ambient, and the chart speed of the recorder was 10 mm/min at a range of 0.2 absorbance unit full scale (aufs). Quantification was accomplished by the measurement of peak height. The retention time was measured as the elapsed time between injection and attainment of the chromatographic peak maximum.

Materials—α- and β-Cyclodextrins[11] and prostaglandins I–VI[12] were used as supplied. All other materials and solvents were reagent grade, and deionized, double-distilled water was used.

Solubility Studies—Solubility studies were carried out according to Higuchi and Lach (11). Excess amounts of the prostaglandins were added to aqueous α- or β-cyclodextrin solutions and were shaken at 25 ± 0.1°. After equilibration was attained, an aliquot was centrifuged and pipetted through a cotton filter. A 0.5-ml aliquot of the sample solution was extracted with 5 ml of ether from acidified media, and 2 ml of the ether phase was evaporated to dryness.

With I and III, the residues were converted to V by the treatment of 0.1 *M* sodium hydroxide solution at 80° for 1 hr (12). After cooling to room temperature, the resultant V was diluted suitably with 0.1 *M* sodium phosphate buffer (pH 7.0) and then subjected to UV spectrophotometry at 282 nm. With V, the residue was dissolved directly in a suitable amount of 0.1 *M* sodium phosphate buffer (pH 7.0) and subjected to UV spectrophotometry at 282 nm.

No appreciable degradations of I and III were confirmed during equilibration. The presence of cyclodextrin did not interfere in the spectrophotometric assay.

The stability constants for complexes were calculated from the phase diagrams obtained according to Higuchi and Lach (11).

Apparent Partition Coefficients—Solutions of E, A, and B prostaglandins (6.0×10^{-5} *M*) were prepared in chloroform, previously saturated with 0.1 *M* phosphate buffer (pH 6.0). These solutions were added to buffer solutions in suitable volume ratios and shaken at 25 ± 0.1° for 1 hr. After separation by centrifugation, an aliquot of the aqueous phase was assayed by the procedure described under *Solubility Studies*. The partition coefficient was defined as the ratio of the equilibrium concentration in the organic phase to that in the aqueous phase.

RESULTS AND DISCUSSION

Effects of Cyclodextrins on Retention Times of Prostaglandins—Figure 1 shows typical liquid chromatograms of I, III, and V on an anion-exchange support in the absence and presence of α- or β-cyclodextrin. The aqueous mobile phase was sodium phosphate buffer, since phosphate anions do not interfere with the inclusion complexation of cyclodextrins (13). A simple phosphate buffer gave long retention times

[4] Model FLC-A700, Jasco, Tokyo, Japan.
[5] Model UVIDEC-1M, Jasco, Tokyo, Japan.
[6] Model JP-350, Jasco, Tokyo, Japan.
[7] Jasco, Tokyo, Japan.
[8] Model D4R-1M, Ohkura Electric Co. Ltd., Tokyo, Japan.
[9] AV-02-500 (Vidac Anion), Jasco, Tokyo, Japan.
[10] Type MS-G10, Termo Co., Tokyo, Japan.
[11] Teijin Ltd., Tokyo, Japan.
[12] Ono Pharmaceutical Co. Ltd., Osaka, Japan.

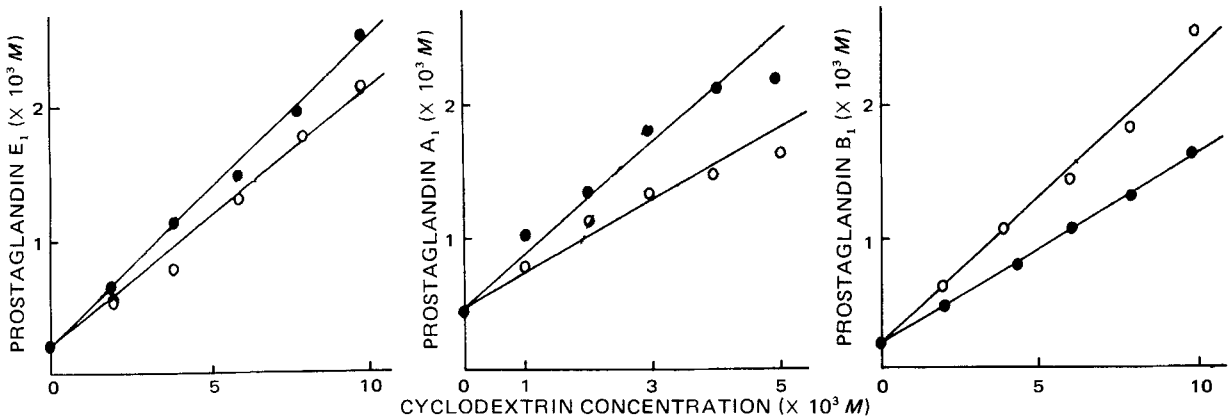


Figure 3—*Solubilities of prostaglandins as a function of α- or β-cyclodextrin concentration in water at 25°. Key: ○, α-cyclodextrin system; and ●, β-cyclodextrin system.*

with marked tailing, particularly for A and B prostaglandins. This result may have been due to the highly hydrophobic nature of the prostaglandins, as expected from their partition coefficients (Table I). When α- or β-cyclodextrin was added to the phosphate buffer, the retention times of the prostaglandins decreased significantly. The resolution of II, IV, and VI was somewhat better than that of I, III, and V (Table I).

Figure 2 shows that an increase in α- or β-cyclodextrin concentrations shortened the retention times of III and V. This result may be ascribed to the increase in the solubility of prostaglandins by complexation with cyclodextrins.

Solubility studies clearly indicated that prostaglandins form soluble complexes with α- and β-cyclodextrins in water (Fig. 3). Stability constants were calculated on the basis of 1:1 complexation from the straight-line portion of solubility diagrams (Table II).

Complexation of the prostaglandins with cyclodextrins was also studied by UV, circular dichroism, and ^{13}C-NMR spectrometry (10), where the importance of the spatial relationship between the guest and host molecules was suggested. For example, the small spectral changes observed for the β-cyclodextrin–V system may indicate that the inclusion of V in β-cyclodextrin of a larger cavity is loose compared to that of α-cyclodextrin and this is responsible for the magnitude of the stability constant in Table II.

The magnitude of the stability constants corresponds to the change in the retention times of the prostaglandins due to the presence of cyclodextrins. β-Cyclodextrin gave better resolution of the prostaglandin peaks compared to α-cyclodextrin, particularly for A and B prostaglandins. The insufficient separation of the peaks observed for α-cyclodextrin systems may reflect the smaller difference in the stability constants of A and B prostaglandins as compared to β-cyclodextrin systems. Use of β-cyclodextrin in the mobile phase (concentration of more than 0.5% w/v) yielded rapid separation of the A and B prostaglandins in anion-exchange HPLC (Fig. 2B).

Separation and Detection of A and B Prostaglandins—Morozowich (3) employed ion-exchange HPLC for the separation of IV and VI, followed by UV (254 nm) detection. The detector response of these

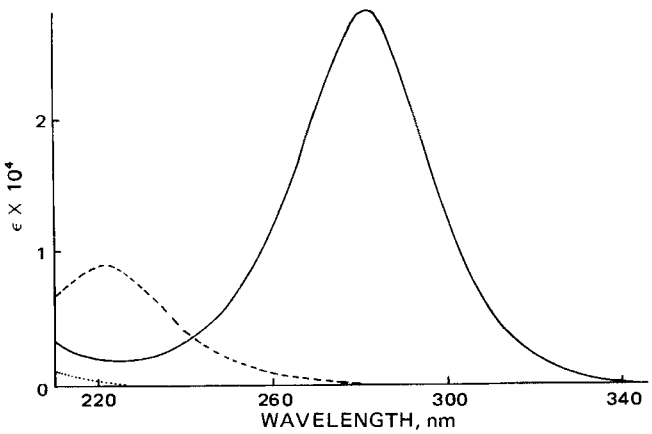


Figure 4—*UV absorption spectra of prostaglandins in 0.1 M sodium phosphate buffer at pH 6.0. Key: ·····, I; - - - -, III; and —, V.*

Table I—Retention Times[a] and Partition Coefficients[b] of Prostaglandins

Prostaglandin	Retention Time, min: Without Cyclodextrin	Retention Time, min: α-Cyclodextrin	Retention Time, min: β-Cyclodextrin	Partition Coefficient
I	8.63	4.15	3.81	1.14
II	7.42	3.93	3.51	0.638
III	23.0	5.63	5.39	80.8
IV	16.3	6.03	4.28	69.4
V	27.3	6.69	9.53	220
VI	22.5	7.67	8.49	192

[a] Chromatographic conditions were the same as in Fig. 1. [b] See text.

Table II—Stability Constants of Prostaglandin–Cyclodextrin Complexes in Water at 25°

Prostaglandin	Stability Constant, M^{-1}: α-Cyclodextrin	Stability Constant, M^{-1}: β-Cyclodextrin
I	1430	1700
III	1300	1400
V	1200	780

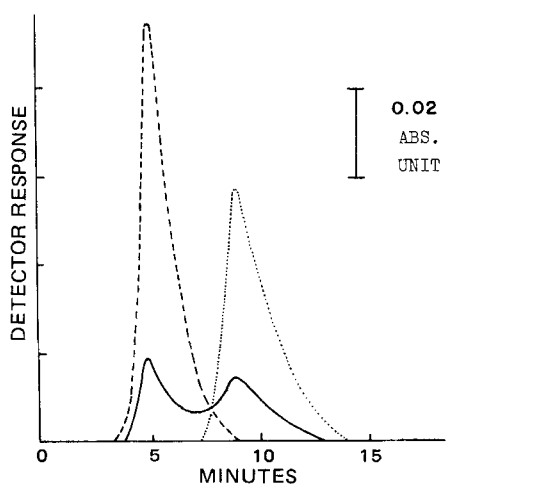


Figure 5—*Separation and detection of III (2.67 μg) and V (1.10 μg) by anion-exchange liquid chromatography using a multiwavelength scanner as the UV detector. Chromatographic conditions were: mobile phase, 0.1 M sodium phosphate buffer with 0.5% β-cyclodextrin (pH 6.0, ionic strength 0.2); and flow rate, 0.5 ml/min. The broken (- - - -), solid (—), and dotted (····) lines were monitored at 221, 254, and 282 nm, respectively.*

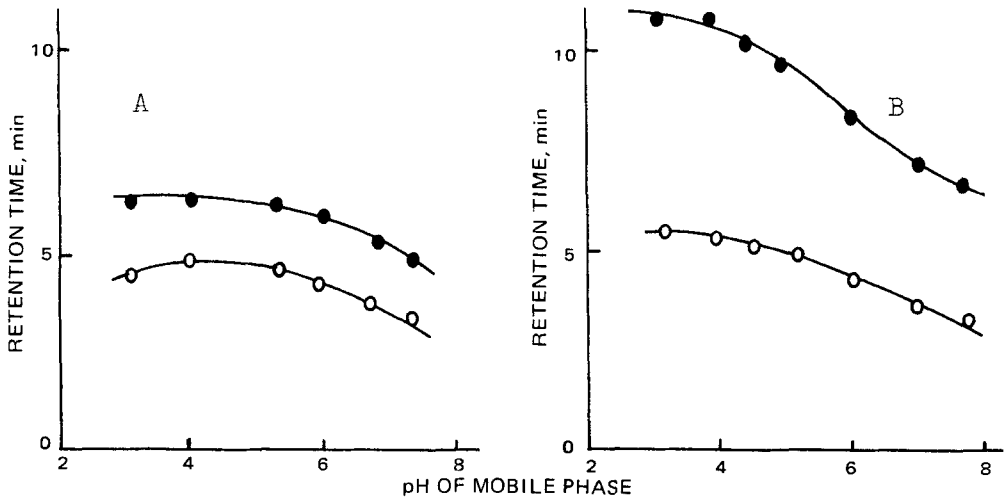


Figure 6—*Effects of pH on the retention times of III and V. Chromatographic conditions were: mobile phase, 0.1* M *sodium phosphate buffer with 0.5% cyclodextrin (ionic strength 0.2); flow rate, 0.5 ml/min; and detection, 221 and 282 nm for III and V, respectively. Key: A, α-cyclodextrin system; B, β-cyclodextrin system;* ○, *III; and* ●, *V.*

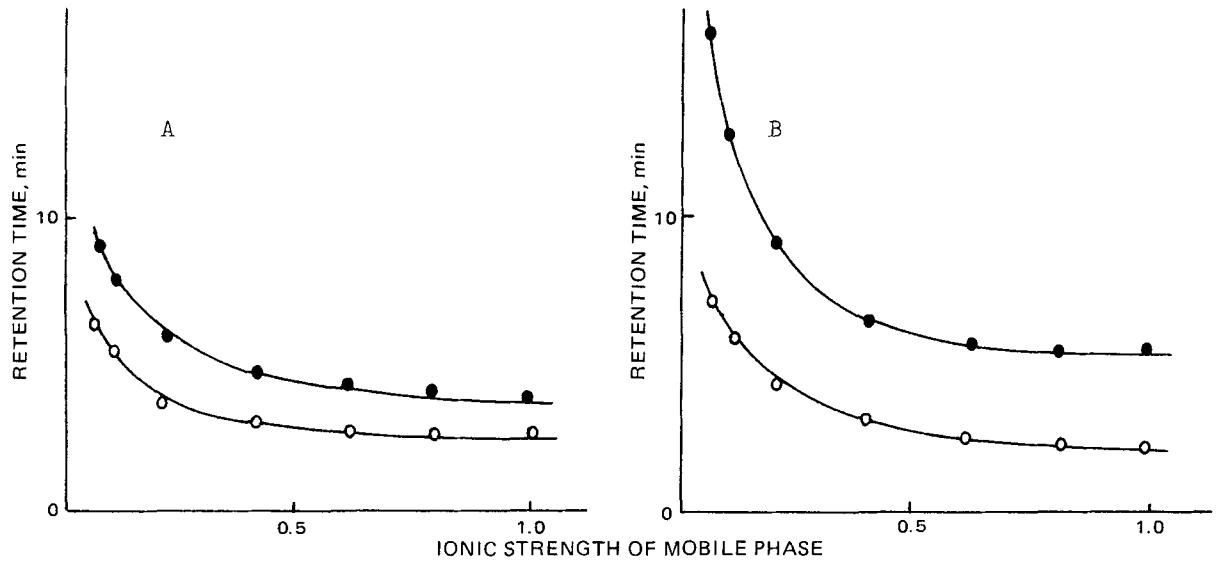


Figure 7—*Effect of ionic strength on the retention times of III and V. Chromatographic conditions were: mobile phase, 0.1* M *sodium phosphate buffer with 0.5% cyclodextrin (pH 6.0); flow rate, 0.5 ml/min; and detection, 221 and 282 nm for III and V, respectively. Key: A, α-cyclodextrin system; B, β-cyclodextrin system;* ○, *III; and* ●, *V.*

compounds, however, is expected to be quite low at 254 nm, as predicted from their molar absorption coefficients (Fig. 4).

The suitable selection of the detection wavelength would provide optimum resolution with higher sensitivity. In this study, A and B prostaglandins were monitored at their UV maximum wavelengths, using a programmable multiwavelength scanner connected to a double-beam spectrophotometer and multipen recorder. The multiwavelength scanner[13] was mapped continuously through a given set of detection wavelengths, and UV absorption data were taken at the desired wavelengths throughout the entire chromatogram.

Figure 5 shows liquid chromatograms of the mixtures of III and V, where their absorbances were monitored at 221, 254, and 282 nm. This method was also applicable to the separation and detection of IV and VI, using the β-cyclodextrin solution as the mobile phase; the resolution was somewhat better than that of III and V. These results indicate that use of the multiwavelength scanner as the detection system is a significant improvement in the separation and quantification of lower levels of prostaglandins compared to ordinary 254 nm detection.

Variations in pH, ionic strength, and solvent composition in the mobile phase were investigated to determine the best operating conditions. Figure 6 shows the effect of pH on the retention times of III and V, where the concentration of cyclodextrin (0.5%) and ionic strength (0.2) were fixed. No attempts were made at pH > 8, since III is susceptible to isomerization at room temperature. The pH–retention time profile showed the general chromatographic behavior of weak acids: the higher the pH, the faster the elution because of ionization of the acids. A lower pH of the eluent gave somewhat better resolution, but this advantage was offset by the longer retention time.

Figure 7 shows the effects of the variations of ionic strength on the retention times of III and V, where the pH and concentration of cyclodextrin were fixed. The mobility of the prostaglandins was increased significantly with an increasing sodium chloride concentration owing to a salting-out effect (14). These results indicate that use of higher pH and greater electrolyte concentration is preferable for the faster elution of prostaglandins.

Retention time also decreased with increasing concentrations of organic solvents such as methanol, ethanol, and acetonitrile in the mobile phase. The presence of higher organic solvent concentrations (about 10% v/v), however, led to undesirable separation behavior, particularly for the α-cyclodextrin systems. This result may be due to the fact that the effective concentration of cyclodextrin decreases with increasing organic solvent because of the interference of inclusion complexation with prostaglandins. The smaller size of the α-cyclodextrin cavity may be particularly susceptible to competitive complexation by solvent as compared with β-cyclodextrin. Combination of β-cyclodextrin with a small amount of organic solvent (less than 5%) as the mobile phase is suggested for the elution of the prostaglandins.

Quantification of Prostaglandins—Linearity of the chromatographic conditions was evaluated by running standard mixtures of various concentrations against their corresponding peak heights. The mobile phase was 0.1 *M* sodium phosphate buffer (pH 6.0 and ionic strength 0.2) containing 0.5% β-cyclodextrin. A linear response was obtained for E, A,

[13] The scanning rate and time interval between data points were 100 nm/sec and 2 sec, respectively.

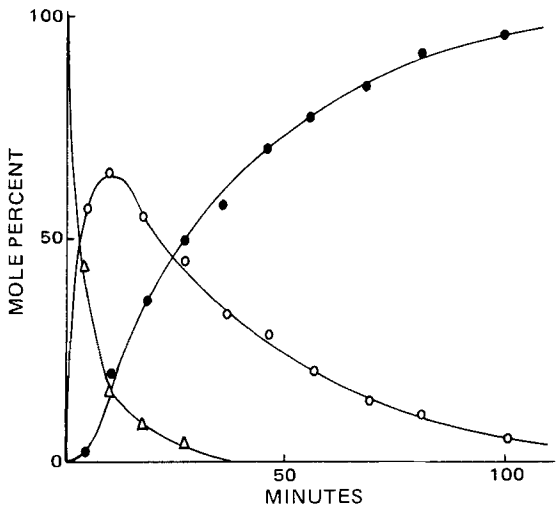


Figure 8—*Reaction profile of base-catalyzed conversion of I to III and V. A solution of I (0.863 mg/ml) in 0.05* M *sodium hydroxide at 30° under a nitrogen stream was periodically subjected to HPLC by direct injection of the alkaline mixture (pH 12.7) sample (4 μl) onto the column. Chromatographic conditions were the same as in Fig. 5. Key: △, I; ○, III; and ●, V.*

and B prostaglandins over concentration ranges of 0–30, 0–5, and 0–3 μg, respectively. In all cases, the correlation coefficient for the linear regression lines was 0.999. The minimum detection quantities of E, A, and B prostaglandins (followed at 210, 221, and 282 nm, respectively) were 150, 30, and 25 ng, respectively, when injected at a recorder range setting of 0.05 aufs.

The developed HPLC system is expected to have great utility in kinetic studies of the degradation of E and A prostaglandins. For example, Fig. 8 shows the reaction profile of base-catalyzed conversion of I to III and V. The rate constants of dehydration (0.267 min^{-1}) and isomerization (0.027 min^{-1}) were obtained by the direct analysis of the reaction species.

The described results were more rapid and sensitive than literature methods.

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Determination of Meclizine Hydrochloride by Ion-Pair Extraction with Methyl Orange

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Abstract □ A method, based on ion-pair extraction, is described for the quantification of meclizine hydrochloride in various pharmaceutical dosage forms, for content uniformity determination, and for concentration monitoring in dissolution and bioavailability studies. Methyl orange, dissolved in pH 2.8 MacIlvaine buffer, gave excellent recovery of meclizine after its isolation from aqueous solutions of gelatin, urine, and blood serum. The chloroform-extracted molecular species appeared to be a 1:1 ion-pair. Beer's law was obeyed for a wide concentration range. Because the extracted species seemed well defined and stable and since a surface or an interphase adsorption phenomenon was not a problem, the reported method is considered sensitive, accurate, precise, rapid, and simple.

Keyphrases □ Meclizine hydrochloride—spectrophotometric analysis using ion-pair extraction with methyl orange, pharmaceutical dosage forms and biological fluids □ Ion-pairs—meclizine hydrochloride–methyl orange, spectrophotometric analysis, pharmaceutical dosage forms and biological fluids □ Spectrophotometry—analysis, meclizine hydrochloride using ion-pair extraction with methyl orange, pharmaceutical dosage forms and biological fluids □ Antiemetics—meclizine hydrochloride, spectrophotometric analysis using ion-pair extraction with methyl orange, pharmaceutical dosage forms and biological fluids

Meclizine hydrochloride USP (1), an antiemetic, has been used in the management of motion sickness for many years. All available methods cited by Wong *et al.* (2), including the official ones (1), that are used for the determination of meclizine are not suitable or convenient for the rapid assay of numerous small quantities of the drug