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Note

Separation of *cis–trans* isomers of prostaglandins with a cyclodextrin bonded column

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Cyclodextrins are chiral, toroidal shaped molecules with a hydrophobic cavity and hydrophilic exterior faces. On one edge of the cone are the secondary hydroxyl groups and on the other edge the primary hydroxyl groups. The inner diameter on the cone is 7.8 Å for β-cyclodextrin. The use of cyclodextrins to control solubility, reactivity and bioavailability through inclusion complexes has led to their use in chromatographic systems. Several groups have developed methods to attach the cyclodextrin molecule to silica[1–3] and cyclodextrins have also been used as mobile phase modifiers[4,5].

The formation of the inclusion complex depends on hydrophobic and hydrogen-bonding interactions and the displacement of solvent in the cavity. The binding strength of the analyte in the cavity depends on the physical size of the analyte as well as the polarity of the molecule. For some molecules only certain side-chains will be able to effectively penetrate into the cavity. To take advantage of the hydrophobic cavity, the use of polar solvents is necessary for formation of the inclusion complex[3,6,7]. The use of methanol–water mobile phases for the development of a separation has been recommended[3,6,7] because methanol provides greater selectivity. The pH of the mobile phase is recommended to be between 4 and 7.5[6,7]; the lower end of the range being limited by the stability of the cyclodextrin and the upper end by the stability of the silica. Mobile phase development is essentially the same as for other reversed-phase systems except that the time to change mobile phases is longer. The equivalent of 40 dead-volumes is to be passed through the column when changing mobile phases[6]. Some examples of the types of separations that have been reported for cyclodextrins include separating the enantiomers of mandelic acid[5], enantiomers of dansylated amino acids[7], propranolol[1], *o,m,p*-isomers of cresol, nitroaniline or aminobenzoic acid[1,2], *cis–trans* isomers of stilbene, and separation of benzo[*e*]pyrene from benzo[*a*]pyrene[5]. Use of these columns is reviewed in refs. 3, 6 and 7.

The determination of trace levels of the *trans* isomer in the presence of the "natural" *cis* isomer of the prostaglandin is often analytically challenging. Examples of methods used for the resolution of *cis–trans* isomers of prostaglandins high-performance liquid chromatography (HPLC) include: (i) reversed-phase separation of *p*-bromophenacyl esters[8], (ii) reversed-phase separation of the underivatized molecule[9], (iii) normal-phase separation of underivatized molecule[10] or as naphthacyl esters[11,12], (iv) use of silver chloride as a mobile phase modifier with silica[13] and

ion-exchange[14] columns, and (v) the use of cyclodextrin as a modifier in the mobile phase[15]. For the normal-phase methods, the use of *p*-nitrophenacyl or naphthacyl ester may be necessary for separation as well as enhanced detection[12]. The reversed-phase methods tend to give less resolution than the normal-phase methods[13]. The use of the silver chloride loaded on an ion-exchange column is broadly applicable but is lower in efficiency than other methods. As the upper and lower chains of prostaglandins as well as the ring could be envisioned to be capable of interacting with the cavity of the cyclodextrin, some unique specificity in separations might be expected. Previous work by Uekama *et al.* showed that inclusion complexes are formed with E, A and B type prostaglandins[15]. The cyclodextrin bonded column method described here provides a broadly applicable selective method that operates in a reversed-phase system and that also has a moderate degree of efficiency.

EXPERIMENTAL

Instrumentation

The high-performance liquid chromatograph used for the separation consisted of a microprocessor-controlled unit (Varian Model 5000, Walnut, Creek, CA, U.S.A.) comprised of three solvent reservoirs, a variable-wavelength detector (Laboratory Data Control, Model III) used at 200 nm, an autosampler (Varian Series 8000) and a recorder (Houston Model Omniscribe). The flow-rate was 1–1.5 ml/min. The injection volume was 20 μl. The detector and recorder were set for 0.05 absorbance units full scale. Integration was completed on an in-house computer system.

Column

The columns were β-cyclodextrin and γ-cyclodextrin (7 and 8 glucose units, respectively) chemically bonded to a 5-μm silica gel (Cyclobond I, Astec, Whippany, NJ, U.S.A.). The column dimensions were 250 × 4.6 mm. The method used for bonding the cyclodextrin to silica was developed by Armstrong[3].

Mobile phases

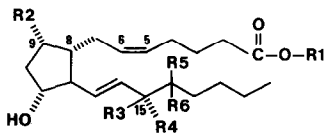
All solvents used were distilled in glass (Burdick and Jackson).

Samples

All samples were from the research laboratories of The Upjohn Company. For injection, samples were dissolved in mobile phase. Where trace analysis was desired for minor components, the concentration of the dissolved prostaglandin was approximately 0.5 mg/ml.

RESULTS AND DISCUSSION

The prostaglandins used to evaluate the cyclodextrin columns in this study are shown in Fig. 1. All except one has a free carboxylic acid group. Several different types of prostaglandins are represented, *i.e.*, PGE_2, $PGF_{2\alpha}$, PGI_2. For these prostaglandins, the *cis–trans* or *Z–E* isomer of concern involves the double bond at C-5. For ciprostene, an additional five-carbon ring is present in which C-6 in Fig. 1 connects to C-9 through a methylene group. Also, a methyl group is attached to C-9.



Name	R1	R2	R3	R4	R5	R6
Carboprost	H	OH	CH_3	OH	H	H
Dinoprostone	H	=O	H	OH	H	H
Arbaprostil	H	=O	OH	CH_3	H	H
Methyl Carboprost	CH_3	OH	CH_3	OH	H	H
Ciprostene	H	a	H	OH	H	H
Metenprost	H	$=CH_2$	H	OH	CH_3	CH_3

Fig. 1. Structures of prostaglandins. See text for description.

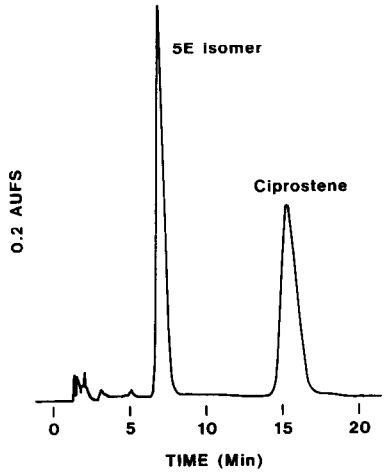
The general approach taken to development of the mobile phase was to use methanol as a modifier and to keep the mobile phase as aqueous as possible because Armstrong and DeMond[5] and Beesely[7] have reported that this gives the highest selectivity. Because ionization of the carboxylic acid group was predicted to have a strong effect on retention, a pH near the upper end tolerated by the column was used initially, *i.e.*, pH 7 and was attained with a 0.02 *M* phosphate buffer. The pH used in this report refers to the pH of aqueous part of the mobile phase prior to mixing. For the prostaglandins in Fig. 1, the percent methanol in the mobile phase needed to keep the capacity factor (k') in the range 3–9 was 20–45%. With this mobile phase system, resolution of the *cis–trans* isomers was easily attained. As shown in Table I, resolution factors of 1–6 were attained for the separation of the *cis–trans* isomers for all except meteneprost where essentially no resolution was attained. Figs. 2 and 3 show the separation of the *Z* and *E* isomers of ciprostene and the *cis–trans* isomers of methyl carboprost, respectively. The effect of methanol concentration on retention and resolution is shown for methyl carboprost and ciprostene in Tables II and III. Over the range of methanol concentrations studied, no unusual effects were observed between retention or resolution and changes in methanol concentration. The theoretical plate

TABLE I

RETENTION AND RESOLUTION OF *cis–trans* ISOMERS OF SEVERAL PROSTAGLANDINS

Compound	*Methanol** (%)	t_R (min)		*Resolution*
		5,6-cis	*5,6-trans*	
Carboprost	20	12.4	8.9	1.6
Dinoprostone	20	13.4	9.3	3.2
Arbaprostil	20	13.9	12.2	0.9
Methyl carboprost	35	10.8	13.1	2.2
Ciprostene	40	12.5	6.4	5.65
Meteneprost	40	15.3	15.3	–

* Remainder of mobile phase is 0.02 *M* pH 7 phosphate buffer.



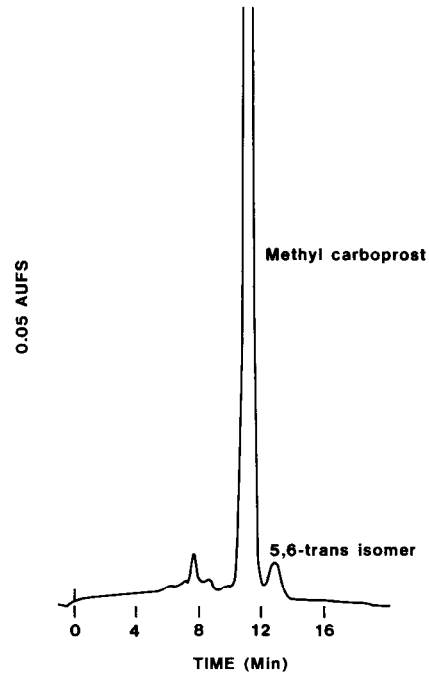


Fig. 2. Chromatogram of *Z* and *E* isomers of ciprostene with a β-cyclodextrin column and methanol–0.02 *M* pH 7 phosphate buffer (40:60) as mobile phase.

Fig. 3. Chromatogram of *cis–trans* isomers of methyl carboprost with a β-cyclodextrin column and methanol–0.02 *M* pH 7 phosphate buffer (35:65) as mobile phase.

count for the separations was approximately 7000. The effect of pH on the retention of ciprostene was as expected *i.e.*, large increases in retention were observed as pH was decreased from pH 7. Loss of resolution of the *cis–trans* isomers was not observed upon decreases in pH and subsequent increase in methanol concentration. For example, with ciprostene and methyl carboprost, excellent resolution was still attained with a mobile phase of pH 4 (the lowest recommended pH) and a mobile phase of 50% methanol.

TABLE II

EFFECT OF METHANOL CONCENTRATION ON RETENTION AND RESOLUTION OF *cis–trans* ISOMERS OF METHYL CARBOPROST

Methanol (%)	t_R *(min)*		*Resolution*
	5,6-cis	*5,6-trans*	
35	10.8	13.7	5.65
40	8.75	10.16	3.5
45	7.19	8.07	1.25
50	6.04	6.56	–

TABLE III

EFFECT OF METHANOL CONCENTRATION ON RETENTION AND RESOLUTION OF *cis–trans* ISOMERS OF CIPROSTENE

Methanol (%)	t_R *(min)*		*Resolution*
	5,6-cis	*5,6-trans*	
40	12.5	7.3	7.3
44	9.7	6.4	6.4
48	7.3	5.7	5.7
52	5.5	3.5	3.5

The γ-cyclodextrin column had lower retention than the β-cyclodextrin column. Figs. 2 and 4 show the resolution of the *Z* and *E* isomers of ciprostene using a β- and γ-cyclodextrin column, respectively. For the γ-cyclodextrin column, the mobile phase was 25% methanol instead of 40% and equivalent separations were not attained. Experiments with the other prostaglandins also showed that less retention was observed for the γ-cyclodextrin column. These experiments confirm that the physical size of the hydrophobic cavity is important in the separation of the isomers.

In an inclusion complex with a prostaglandin, the prostaglandin molecule would tend to keep the polar functional groups away from the hydrophobic interior and/or allow hydrogen-bonding with the hydroxyl groups on the edges of the cavity. Molecular models of the prostaglandins show that for such a conformation, the

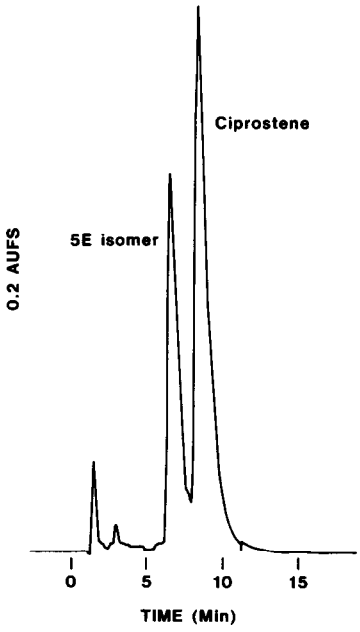


Fig. 4. Chromatogram of *Z* and *E* isomers of ciprostene with a γ-cyclodextrin column and methanol–0.02 *M* pH 7 phosphate buffer (25:75) as mobile phase.

cis-isomer tends to be more compact. As the *trans*-isomer elutes prior to the *cis*-isomer for all the prostaglandins except the one that is the methyl ester, the more compact conformation allowed by the *cis*-isomer apparently gives a stronger complex. This is also supported by the comparison of the β- and the γ-cyclodextrin column, one glucose unit larger, in that larger retention is observed with the beta column. The lack of resolution observed with meteneprost could be due to a reduction in interactions that depend on the orientation of the C-5 double bond because the replacement of one of the ring hydroxyls with a methylene group would allow interactions with the hydrophobic cavity not favorable with the hydroxyl present. The presence of the 16,16-dimethyl group could also be important. The importance of the polar groups in the nature of the interaction is also shown with methyl carboprost which shows a reversal in order of retention in comparison to carboprost which has a free acid group. Thus, the cyclodextrin-bonded column provides additional geometric constraints on interactions with the analyte that provides for additional selectivity.

Compared to reported methods for the separation of *cis–trans* isomers of prostaglandins, this method appears to be more universal in application. For example, methyl carboprost[10], carboprost[12], and arbaprostil[13] are not separated from their *trans*-isomers with reversed-phase mobile phases but ciprostene[9] and meteneprost[16] are separated. Methyl carboprost and carboprost are separated from their *trans*-isomers with nortmal-phase mobile phases but arbaprostil requires an silver chloride-modified system[13].

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