# Phase Solubility Analysis and PMR Study of Complexing Behavior of Dinoprostone with β-Cyclodextrin in Water

**SYLVAN G. FRANK** * **and MOO J. CHO** ‡x

Received May 11, 1977, from the *Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, Ohio State University, Columbus, OH 43210, and the ‡Pharmacy Research Unit, The Upjohn Company, Kalamazoo, MI 49001. Accepted for publication March 30, 1978.

**Abstract** □ The mechanism of inclusion compound formation by dinoprostone (prostaglandin $E_2$) with β-cyclodextrin was studied by phase solubility analysis and PMR spectroscopy. As indicated by the linear increase of aqueous solubility of dinoprostone with β-cyclodextrin concentration, some types of molecular interactions definitely exist between dinoprostone and the complexing ligands. The temperature dependence of a 1:1 complex formation constant yielded the following thermodynamic data at 20°: $\Delta G°$ = −4.11 kcal/mole, $\Delta H°$ = 7.20 kcal/mole, and $\Delta S°$ = 10.5 e.u. Since water was the solvent system, these parameters appear to be largely determined by solvent reorganization through hydrogen bonding rather than solely by the binding of desolvated free dinoprostone and β-cyclodextrin entities. PMR data indicate that dinoprostone is included within the cavity and also interacts with protons on the exterior of the β-cyclodextrin molecule. A model consisting of a 1:1 complex, in which a dinoprostone molecule is partially included within the cavity and the remainder of the molecule extends around the edge of the opening of the cavity to the exterior of the β-cyclodextrin molecule, is proposed as the most probable structure of this inclusion compound.

**Keyphrases** □ Dinoprostone—complexation with β-cyclodextrin, phase solubility and PMR analyses □ β-Cyclodextrin—complexation with dinoprostone, phase solubility and PMR analyses □ Complexation—dinoprostone with β-cyclodextrin, phase solubility and PMR analyses □ Phase solubility—analysis of complexation of dinoprostone with β-cyclodextrin □ PMR—analysis of complexation of dinoprostone with β-cyclodextrin□ Prostaglandins—dinoprostone, complexation with β-cyclodextrin, phase solubility and PMR analyses

Various molecules of pharmaceutical interest form inclusion compounds with cyclodextrins (1–4). The cyclodextrins, which are also known as Schardinger dextrins or cycloamyloses, are water-soluble, macrocyclic polymers that contain glucose units joined by α-1,4-linkages (5). The most common is β-cyclodextrin, which is formed by glucose units oriented in a ring, enclosing an internal cavity of about 8 Å diameter.

## BACKGROUND

The cyclodextrins readily form relatively insoluble inclusion compounds with many substances of sizes able to be held wholly or partially within the cavity (1, 2, 5–11). The degree of reactivity of the host and guest is dependent on the presence of a suitable group or ring capable of entering the cyclodextrin cavity.

The chemical stability of dinoprostone (prostaglandin $E_2$) can be improved in the form of solid molecular complexes with polyvinylpyrrolidone (12), β-cyclodextrin (13), polyalkyl ethers (14), and other similar complexing ligands (15). In a given solvent system, if the molecular interaction between dinoprostone and these ligands is strong enough to produce a molecular complex with a definite stoichiometric ratio, then the apparent physicochemical properties of dinoprostone will differ from its intrinsic properties. The magnitude of the property change that occurs in the presence of a complexing ligand will depend not only on the difference of the property between pure dinoprostone and the complex but also on the concentration of the ligand in the system, because it will determine the equilibrium position of the reaction.

Changes in various physical properties of a compound can be followed as a quantitative measure of its molecular interactions with other ligands. The apparent solubility is perhaps the most commonly measured property, mainly because of its high sensitivity toward the formation of a complex and the simplicity involved in the determination. In this study, the apparent aqueous solubility of dinoprostone was measured as a function of the concentration of β-cyclodextrin at 10, 20, and 30°. From the thermodynamic parameters associated with the molecular interactions, the nature of the intermolecular forces involved was evaluated.

Experimental evidence indicates that dinoprostone undergoes hydrogen bonding with various functional groups, particularly hydroxyl groups; that it has a relatively long retention time on a silica gel column; and that it has extremely high and low solubilities in alcohols and simple aliphatic hydrocarbons, respectively. Consequently, the hydrogen bonding ability of dinoprostone with a compound known to complex with it was examined specifically. Even though water can participate competitively in hydrogen bonding, it was chosen as the solvent system, because β-cyclodextrin is sufficiently soluble in water [up to 1.85% at room temperature (5)] to cover a wide range of ligand concentrations.

NMR and X-ray investigations established that the cyclodextrin glucose units were in the C-1 chair configuration (4, 16). This arrangement had primary and secondary hydroxyl groups around the opening of the cavity on both sides of the molecule, with H-3, H-5, and, possibly, H-6 located within the cavity and H-1, H-2, and H-4 on the exterior. Atoms H-3 and H-5 were shielded by guest molecule protons on formation of an inclusion compound with cyclodextrins (17, 18).

The technique in these studies (17, 18) was based on the shielding of the interior protons of the cyclodextrin ring as a result of the anisotropy of the guest aromatic moiety; increasing chemical shifts could be detected with increasing concentrations of the guest species up to the saturation concentration. In the present work, this NMR technique was used to investigate the mechanism of formation of the β-cyclodextrin–dinoprostone inclusion compound.

## EXPERIMENTAL

Into a series of 7-ml vials containing different amounts of β-cyclodextrin[1] in 5.0 ml of glass-distilled water, a large excess of dinoprostone was added. The vials were then shaken for 12 hr in a temperature-controlled water bath. At solubility equilibrium, each vial was left standing for 1 hr; then an aliquot of supernate was withdrawn and directly diluted with 1.0 *N* KOH. The absorbance was read[2] at 282 nm.

In calculating the dinoprostone concentration, an apparent absorptivity value of 52.0 $A_{282}$ ml/mg cm was used. β-Cyclodextrin solutions were transparent at 282 nm. The concentrations of β-cyclodextrin stock solutions were calculated on the basis of the weight of the solutes.

Samples for NMR spectroscopy were prepared by saturating a 2% (w/v) solution of β-cyclodextrin in deuterium oxide[3] with dinoprostone. The excess β-cyclodextrin inclusion compound was allowed to precipitate, and the supernate was decanted. PMR spectra[4] at 100 MHz were determined on the supernate of the inclusion compound at 34 ± 1° in standard 5-mm tubes.

## RESULTS AND DISCUSSION

**Phase Solubility Analysis**—As shown in Fig. 1, the apparent solubility of dinoprostone increased linearly with the concentration of β-cyclodextrin at all temperatures studied. For such a phase diagram, it is generally adopted that the complex formed is first order in ligand; the equilibrium constant for the formation of a 1:1 complex is given by:

$$K = \frac{\text{slope}}{S_0\,(1 - \text{slope})} \qquad \text{(Eq. 1)}$$

where $S_0$ is the solubility of the substrate in the absence of ligand, β-

[1] Nutritional Biochemicals, Inc. (used without further purification).
[2] Beckman model DB-G spectrophotometer.
[3] Bio-Rad Laboratories (99.85 mole % $D_2O$).
[4] Varian XL-100 NMR spectrometer.

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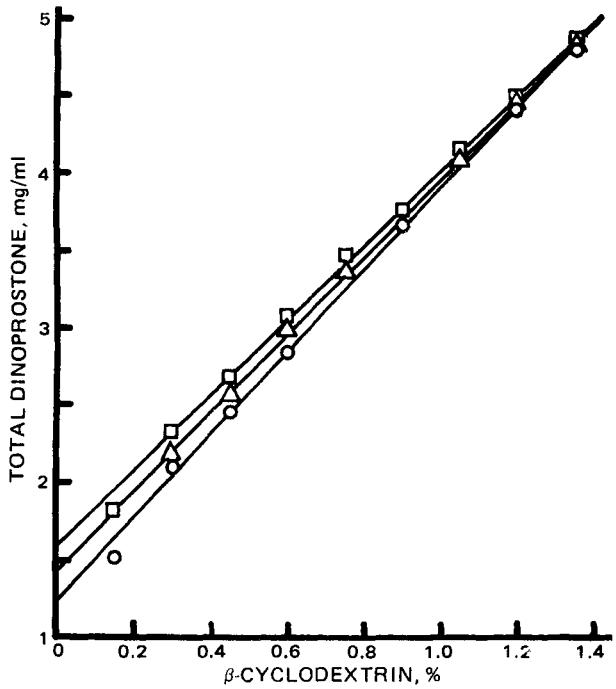


**Figure 1**—*Apparent aqueous solubility of dinoprostone as a function of the concentration of β-cyclodextrin at 10 (○), 20 (△), and 30° (□).*

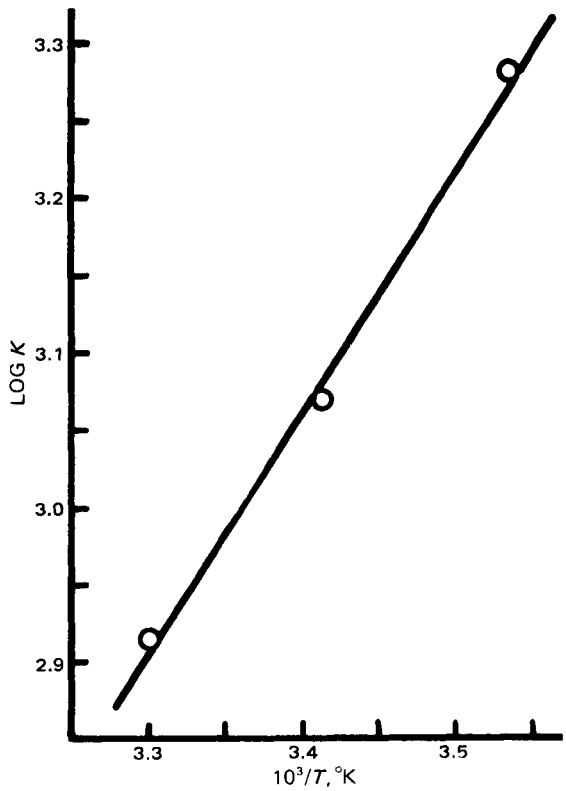


**Figure 2**—*Van't Hoff plot for the formation of a 1:1 complex of dinoprostone and β-cyclodextrin.*

cyclodextrin (19). The equilibrium constants calculated by Eq. 1 are listed in Table I. The key assumption was that the 1:1 complex was the only significant species present in the system besides free dinoprostone and β-cyclodextrin. All thermodynamic parameters (20) associated with the 1:1 complex formation are listed in Table I. As shown in Fig. 2, within experimental error, $\Delta H°$ appears to be independent over the temperature range of 10–30°.

Cyclodextrins bind a vast array of guest molecules equally well with little substrate specificity. The intermolecular forces involved can be hydrogen bonding (21), nonspecific van der Waals forces (21), hydrophobic interactions (22), or their combinations. As shown in Table I, the favorable enthalpy changes overcompensate for the unfavorable entropy changes, resulting in negative free energy changes at all temperatures. The magnitudes are very similar to those associated with the interaction of hydrocinnamic acid ($C_6C_5CH_2CH_2COOH$) with α-cyclodextrin [$(C_6H_{10}O_5)_6$], where, at 25°, $\Delta G° = -4.2$ kcal/mole, $\Delta H° = -7.5$ kcal/mole, and $\Delta S° = 11$ e.u. (23).

Since the size of the cavity provided by cyclodextrins plays the most important role in forming inclusion compounds, hydrocinnamic acid, being smaller than dinoprostone in size, conceivably fits better to α-cyclodextrin, whereas dinoprostone binds to β-cyclodextrin to the same extent. Hydrocinnamic acid does not form a complex with the β-form (23, 24), and the interaction of dinoprostone with the α-form is much less than its interaction with the β-form (13).

Since water, which may solvate dinoprostone and β-cyclodextrin in free entities through hydrogen bonding, was the solvent system employed, it is a very complicated task to interpret the thermodynamic data presented in Table I. However, if the mechanism involved in the interaction between hydrocinnamic acid and α-cyclodextrin is followed, then the heat gained on formation of a complex by the increased hydrogen bonding of water molecules released from the cyclodextrin cavity and the dinoprostone surroundings appears to be the major driving force of the interactions.

Even though dinoprostone is assumed to undergo hydrogen bonding actively with β-cyclodextrin, its contribution to the overall $\Delta H°$ reported in Table I may not be significant, simply because the number of hydrogen bonds involved must be smaller than that accompanied with the solvent reorganization. An unfavorable entropy change can result from the ordering effect of solvent reorganization as well as the reduced degrees of freedom of dinoprostone when it is confined in and around the cyclodextrin cavity.

**PMR Studies**—The PMR spectra of β-cyclodextrin and the β-cyclodextrin–dinoprostone inclusion compound in deuterium oxide are shown in Fig. 3. The β-cyclodextrin spectrum (Fig. 3B) is broken for ease in comparison of the two spectra; the large peak at approximately 4.6 ppm

**Table I—Thermodynamic Parameters Associated with the Reaction Dinoprostone + β-Cyclodextrin ⇌ 1:1 Complex**

| Temperature | $10^{-3}K$, liter/mole | $\Delta G°$, kcal/mole | $\Delta H°$, kcal/mole | $\Delta S°$, e.u. |
|---|---|---|---|---|
| 10° | 1.917 | −4.25 | −7.20 | −10.4 |
| 20° | 1.170 | −4.11 | −7.20 | −10.5 |
| 30° | 0.819 | −4.04 | −7.20 | −10.4 |

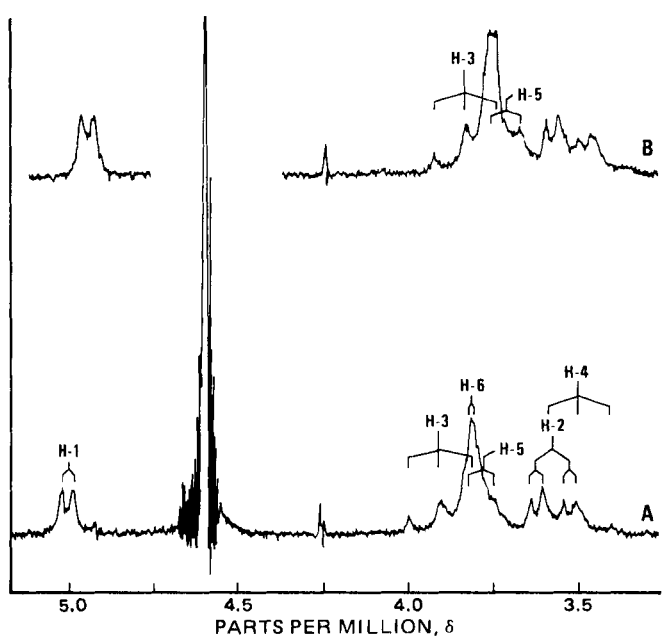


**Figure 3**—*PMR spectra of β-cyclodextrin (A) and the β-cyclodextrin–dinoprostone inclusion compound (B) in deuterium oxide.*

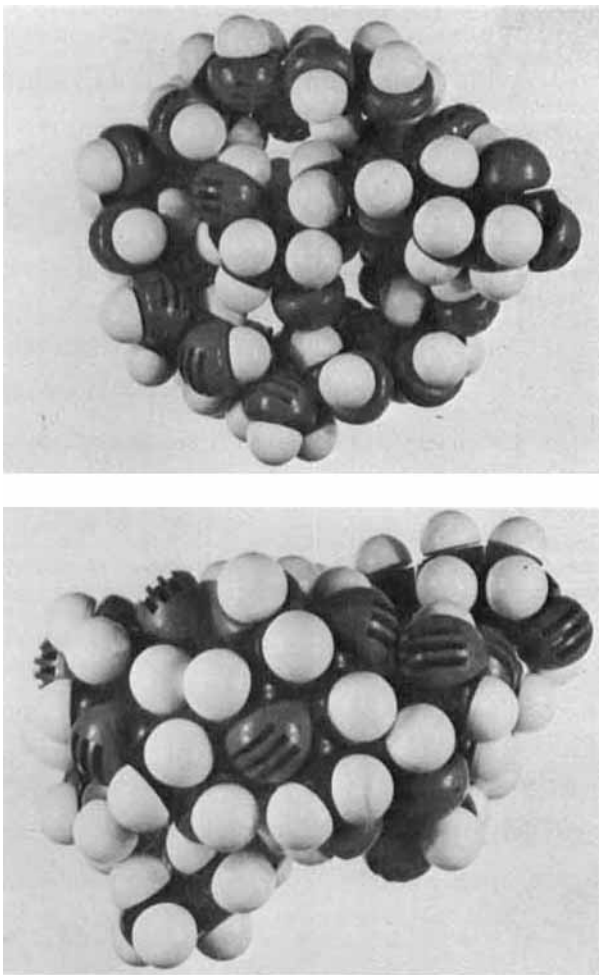

**Figure 4**—*CPK space-filling molecular model of the proposed dinoprostone–β-cyclodextrin (1:1) inclusion complex. Top: View from above with β-cyclodextrin in the plane of the page (the dinoprostone molecule extends from the right side of the β-cyclodextrin torus into the cavity). Bottom: View from the side with dinoprostone in the plane of the page, extending from upper right to lower left through the β-cyclodextrin cavity.*

due to small amounts of deuterium hydroxide and water as impurities is common to both spectra. No other peaks appear on the full 100-MHz spectrum; except for the deuterium hydroxide–water peak, all of the peaks observed in Fig. 3 are those of β-cyclodextrin.

In the presence of dinoprostone, the β-cyclodextrin spectrum is shifted upfield. The anomeric hydrogens, H-1, appear as a doublet at the farthest downfield position and are gauche, whereas the rest of the β-cyclodextrin protons are axial. Farther upfield is the large deuterium hydroxide–water peak. Due to its fixed position, this peak was used as an internal standard in the measurement of the chemical shifts of the portions of the β-cyclodextrin moiety in the presence of dinoprostone relative to their positions in the absence of dinoprostone. Still farther upfield is a side band, 4.25 and 4.80 ppm (Fig. 3A), followed by a group of peaks corresponding to the remainder of the β-cyclodextrin protons. The sideband appears only on the β-cyclodextrin spectrum.

From the chemical shift data in Table II, it appears that all of the protons are experiencing a shielding effect, suggesting that a portion of the dinoprostone molecule in the inclusion compound is proximate to each proton. Corey–Pauling–Koltan (CPK) space-filling molecular models indicate that the entire dinoprostone molecule cannot enter the β-cyclodextrin cavity from either side of the β-cyclodextrin torus, regardless of whether the approach is made by the ring portion or the combined, parallel-oriented chain portions of the molecule. However, if the dinoprostone chains are not parallel but oriented at angles to each other, tending toward a linear arrangement, one chain can enter the β-cyclodextrin cavity. The rather loose fit of the dinoprostone chain and the expected corresponding weaker anisotropic shielding effect on the

**Table II—Dinoprostone-Induced Chemical Shifts of β-Cyclodextrin Protons**

| Proton | Δδ, ppm |
|---|---|
| H-1 | 0.06 |
| H-2 | 0.05 |
| H-3 | 0.08 |
| H-4 | 0.03 |
| H-5 | 0.08 |
| H-6 | 0.07 |

interior protons of the β-cyclodextrin ring are reflected in the lower chemical shift values for these protons.

A possible molecular arrangement for the inclusion compound is that the molecule of dinoprostone wraps itself around the open end of the β-cyclodextrin ring in a configuration in which one chain is buried in the cavity, the five-membered ring is adjacent to the edge of the torus, and the other chain extends over the exterior of the β-cyclodextrin molecule. CPK space-filling molecular models indicate that the dinoprostone molecule is capable of such a configuration (Fig. 4). The virtually identical values of the chemical shift for H-3, H-5, and H-6 indicate that a single dinoprostone chain could enter from either direction on the PMR time scale. Since H-1 and H-4 are both located close to the equator of the exterior of the torus, the lower value of the chemical shift of H-4 as compared to that of H-1 may also indicate that the dinoprostone chain preferentially orients proximate to H-1 on the PMR time scale.

While clustering of dinoprostone molecules in and around the β-cyclodextrin torus could also possibly explain the PMR observations, such an arrangement can be expected to be thermodynamically unstable since molecular models indicate that only a portion of one dinoprostone molecule can occupy the cavity. Thus, the most probable arrangement of the complex is that in which the chain of the dinoprostone molecule bearing the carboxyl group extends over the exterior of the β-cyclodextrin molecule. This orientation, while admittedly speculative, would be consistent with the 1:1 complex suggested by phase solubility analysis.

Information on the solution process of dinoprostone in water was obtained. The solubility of dinoprostone in water is shown in Fig. 5 as a function of temperature. The apparent heat of solution calculated from the slope when a mole fraction unit was used for the solubility was 2.16 kcal/mole. In the mole fraction calculation, 55.5 moles/liter was used as the concentration of water. If the solution process is considered as the sum of two steps, fusion of solid dinoprostone to liquid and a subsequent mixing of liquid dinoprostone with water, then the second process should be exothermic in nature with $\Delta H°$ of about −6 kcal/mole since the heat of dinoprostone fusion is about 8 kcal/mole.

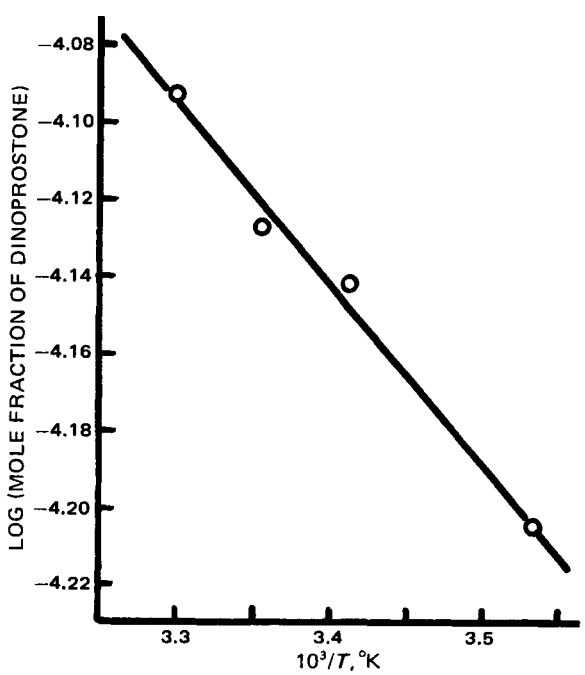


**Figure 5**—*Estimation of the apparent heat of solution of dinoprostone in water.*

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[5] After the present study was completed, the authors became aware of the work of K. Uekama *et al.* on a very similar subject [*J. Pharm. Sci.*, **66**, 706 (1977)]. The present results are in general agreement in that the equilibrium constants for the molecular interaction are in the order of $10^3$ mole$^{-1}$ at 25°.


## ACKNOWLEDGMENTS

Grateful appreciation is extended by S. G. Frank to The Upjohn Co. for a Summer Visiting Professorship in Pharmacy Research (1972), during which a portion of this project was completed.


# Simultaneous Determination of Pseudoephedrine and Chlorpheniramine in Pharmaceutical Dosage Forms


**AVRAHAM YACOBI ˣ, ZEE M. LOOK, and CHII-MING LAI**



Received October 17, 1977, from the *Department of Biopharmaceutical Sciences, Research and Medical Affairs, Arnar-Stone Laboratories, Inc., McGaw Park, IL 60085.* Accepted for publication March 23, 1978.



**Abstract** □ A simple and sensitive high-pressure liquid chromatographic (HPLC) determination of pseudoephedrine and chlorpheniramine in a pharmaceutical dosage form is described. Quantities of 1.5 μg of pseudoephedrine and 0.1 μg of chlorpheniramine are sufficient to determine concentrations in an aqueous solution. Small volume samples, without any extraction procedures, can be treated for direct drug concentration measurement with a high-pressure liquid chromatograph. The stability-indicating property and the accuracy of this method are comparable to those of an established GLC method. The HPLC method can be applied directly and successfully for dissolution studies. The latter application eliminates the need for volume replacement or subsequent mathematical corrections.



**Keyphrases** □ Pseudoephedrine—high-pressure liquid chromatographic analysis, simultaneously with chlorpheniramine, in dosage forms □ Chlorpheniramine—high-pressure liquid chromatographic analysis, simultaneously with pseudoephedrine, in dosage forms □ High-pressure liquid chromatography—simultaneous analyses, pseudoephedrine and chlorpheniramine in dosage forms □ Adrenergics—pseudoephedrine, high-pressure liquid chromatographic analysis, simultaneously with chlorpheniramine, in dosage forms □ Antihistaminics—chlorpheniramine, high-pressure liquid chromatographic analysis, simultaneously with pseudoephedrine, in dosage forms


Some cough–cold–allergy dosage forms contain both pseudoephedrine hydrochloride, a nasal decongestant, and chlorpheniramine maleate, an antihistamine. These compounds are usually determined individually by GLC and/or UV spectrophotometry following TLC for drug separation. These methods require tedious extraction and lengthy reaction procedures. High-pressure liquid chromatographic (HPLC) systems also have been utilized for the determination of pseudoephedrine in cough–cold mixtures (1) and of chlorpheniramine in combination with other antihistamines (2) and antitussive preparations (3) and for quantitation of other antihistamines including chlorpheniramine in cough syrups (4).

This paper describes a suitable HPLC method for the simultaneous determination of pseudoephedrine and chlorpheniramine in pharmaceutical dosage forms.

## EXPERIMENTAL

**Instrumentation**—A liquid chromatograph[1] was equipped with a UV detector operated at 254 nm and a nonpolar column[2] (30 cm long × 4 mm i.d.). The column was eluted with a mobile phase consisting of acetonitrile–methanol–sodium nitrate (35:40:25) and 1-heptanesulfonic acid[3] (0.001 *M* each), pH 5, at a flow rate of 2 ml/min. The output of the detector was recorded[4] at 10 mv.

A gas–liquid chromatograph[5] was equipped with a flame-ionization detector. A glass column (1.8 m long × 4 mm i.d.) was packed with 3% OV-17 on 100–120-mesh Chromosorb W-HP; helium was used as the carrier gas at a rate of 40 ml/min. The temperatures of the injection port, column, and detector were 225, 165, and 300°, respectively, for pseudoephedrine and 225, 235, and 300°, respectively, for chlorpheniramine.

[1] Model ALC/GPC 204, Waters Associates, Milford, Mass.
[2] μBondapak $C_{18}$, Waters Associates, Milford, Mass.
[3] Pic B-7 Waters Associates, Milford, Mass.
[4] Omniscribe recorder, Houston Instruments, Austin, Tex.
[5] Model 65, Beckman Instruments, Irvine, Calif.




*0022-3549/78/1200-1668$01.00/0*