# High-Pressure Liquid Chromatographic Assays for Clindamycin, Clindamycin Phosphate, and Clindamycin Palmitate

LEO W. BROWN

Received October 19, 1977, from *The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication January 5, 1978.

**Abstract** □ High-pressure liquid chromatographic procedures are described for clindamycin, clindamycin palmitate, and clindamycin phosphate as bulk drugs and in formulations. All three procedures utilize a $C_{18}$ reversed-phase chromatographic column with refractive index detection. The mobile phases are hydroalcoholic solutions containing dioctyl sodium sulfosuccinate or disodium ethylenediaminetetraacetate. Separations of related impurities or degradation products also are discussed. The relative standard deviations of the methods range from 0.8 to 1.8%.

**Keyphrases** □ Clindamycin—base, phosphate, and palmitate, high-pressure liquid chromatographic analyses in bulk drug and dosage forms □ High-pressure liquid chromatography—analyses, clindamycin base, phosphate, and palmitate in bulk drug and dosage forms □ Antibacterials—clindamycin base, phosphate, and palmitate, high-pressure liquid chromatographic analyses in bulk drug and dosage forms

Clindamycin[1] (I) is an antibiotic active against Gram-positive aerobes and very active against both Gram-positive and Gram-negative anaerobic pathogens (1). It is synthesized from lincomycin (II), an antibiotic produced by microbial fermentation. Clindamycin phosphate[1] (III), produced by chemical modification of clindamycin, is used in parenteral preparations. After injection, it is hydrolyzed to the active clindamycin. Clindamycin palmitate[1] (IV) is also produced by chemical modification of clindamycin. The presence of the palmitate group markedly lessens the bitter taste experienced with the parent compound, making it suitable for oral pediatric formulations. Although the ester is not biologically active, it is readily hydrolyzed to the active clindamycin *in vivo* (2).

Clindamycin, clindamycin phosphate, and clindamycin palmitate are presently assayed by GLC and microbiological procedures (3, 4). The GLC procedure for clindamycin involves derivatization with either trifluoroacetic anhydride or acetic anhydride. These procedures separate possible trace contaminants such as epilincomycin (V), epiclindamycin (VI), and clindamycin B (VII) from clindamycin. Clindamycin phosphate is assayed as clindamycin after hydrolysis using alkaline phosphatase. Clindamycin palmitate is derivatized with acetic anhydride and then chromatographed.

High-pressure liquid chromatographic (HPLC) assays for the three clindamycin antibiotics are presented here. Since no derivatization steps are involved, the HPLC assay is simpler and quicker than the GLC procedure.

## EXPERIMENTAL

**HPLC Conditions**—A liquid chromatograph[2] was used with a differential refractometer detector[3]. The column[4] was $C_{18}$ reversed-phase micro silica gel, 30 cm × 3.9 mm. Column pressure was maintained at 42.18 kg/cm$^2$ (1 ml/min), and a 10-μl injection volume was used with a loop injector[5]. Chart speed was 2.5 cm/5 min, and attenuation on the refractive index detector was $9.6 \times 10^{-5}$ unit full scale.

**Clindamycin Hydrochloride**—*Mobile Phase*—One gram of dioctyl sodium sulfosuccinate, 1 ml of formic acid, and 125 ml of water were added to a 500-ml volumetric flask. Methanol was added to volume.

*Internal Standard Solution*—A chloroform solution containing approximately 5 mg of testosterone propionate/ml was prepared.

*Reference Standard and Bulk Drug Preparation*—To each of two 4-ml shell vials was added 2 ml of internal standard solution, and the vials then were taken to dryness with a nitrogen stream. To one vial was added approximately 10 mg of clindamycin hydrochloride reference standard, and to the other was added approximately 10 mg of the bulk drug sample, both accurately weighed. Mobile phase, 1 ml, was added to each vial, which was swirled to dissolve the sample and internal standard.

*Procedure*—The sample and reference solutions were chromatographed using the same chromatographic conditions.

**Clindamycin Palmitate Flavored Granules**—*Mobile Phase*—One gram of dioctyl sodium sulfosuccinate, 0.77 g of ammonium acetate, 1.0 ml of acetic acid, and 37.5 ml of water were placed in a 500-ml volumetric flask. Methanol was added to volume.

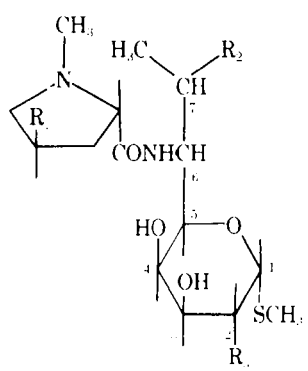
*Internal Standard Solution*—A chloroform solution was prepared containing approximately 12 mg of methyl palmitate/ml.

*Reference Standard Preparation*—Approximately 148 mg of clindamycin palmitate hydrochloride reference standard was accurately weighed and transferred to a 32-ml stoppered centrifuge tube. Water, 6 ml, was added to dissolve the sample.

*Sample Preparation*—Approximately 2.4 g of flavored granules was accurately weighed and transferred to a 32-ml stoppered centrifuge tube. To the tube was added 4.5 ml of water, and the tube was swirled to dissolve the sample.

*Procedure*—To the sample and reference tubes were added 10.0 ml of internal standard solution and 1.0 ml of 30% (w/v) sodium carbonate solution. The tubes were shaken vigorously for 15 min and then centrifuged at 2500 rpm for 10 min. The top aqueous layer was removed by suction, and a 3-ml aliquot of the chloroform layer was filtered and dried using 1 g of anhydrous sodium sulfate.

A 1-ml aliquot of the dried chloroform layer was transferred to a 4-ml shell vial and evaporated to dryness with a nitrogen stream. Mobile phase, 1 ml, was added to the glass-like residue. The vial was swirled and soni-



I: $R_1$ = *n*-propyl, $R_2$ = (*S*)-Cl, $R_3$ = OH
II: $R_1$ = *n*-propyl, $R_2$ = (*R*)-OH, $R_3$ = OH
III: $R_1$ = *n*-propyl, $R_2$ = (*S*)-Cl, $R_3$ = phosphate
IV: $R_1$ = *n*-propyl, $R_2$ = (*S*)-Cl, $R_3$ = palmitate
V: $R_1$ = *n*-propyl, $R_2$ = (*S*)-OH, $R_3$ = OH
VI: $R_1$ = *n*-propyl, $R_2$ = (*R*)-Cl, $R_3$ = OH
VII: $R_1$ = ethyl, $R_2$ = (*S*)-Cl, $R_3$ = OH

[1] The USAN name clindamycin phosphate refers to the 2-phosphate, and the generic name clindamycin palmitate refers to the 2-palmitate. Clindamycin is marketed as Cleocin by The Upjohn Co.

[2] DuPont model 830.

[3] Waters model R-401.

[4] Waters μBondapak.

[5] Valco.



0022-3549/78/0900-1254$01.00/0

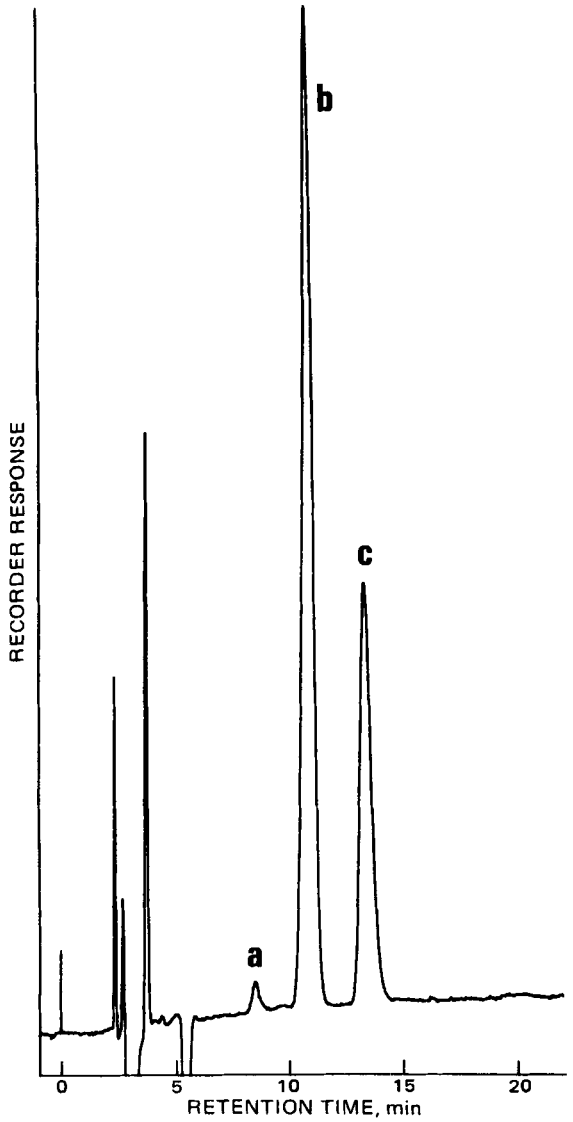



**Figure 1**—*Chromatogram of clindamycin B (a), clindamycin (b), and testosterone propionate used as the internal standard (c).*

cated to dissolve the residue, and the solution was chromatographed using the conditions already described.

**Clindamycin Phosphate Sterile Solution**—*Mobile Phase*—One gram of disodium ethylenediaminetetraacetate was added to approximately 200 ml of water in a beaker containing a magnetic stirring bar. The solution was adjusted to pH 5.8 with 5 *N* NaOH. Addition of base caused the disodium ethylenediaminetetraacetate to dissolve slowly. The solution was adjusted with water to 225 ml; then 275 ml of methanol was added, and the solution was mixed thoroughly.

*Internal Standard Solution*—A methanol–water (55:45) solution containing approximately 20 mg of propylparaben/ml was prepared.

*Reference Standard Preparation*—Approximately 20 mg of clindamycin phosphate reference was accurately weighed and transferred to a 4-ml shell vial. One milliliter of internal standard solution and 1 ml of methanol–water (55:45) were added and mixed.

*Sample Preparation*—Sterile solution clindamycin phosphate (3 ml, 150 mg base equivalents of clindamycin/ml) was added to a 25-ml volumetric flask and diluted to volume with methanol–water (55:45). Aliquots of 1 ml of this solution and 1 ml of internal standard solution were added to a 4-ml shell vial.

*Procedure*—Sample and reference solutions were chromatographed using the chromatographic conditions already described.

## RESULTS AND DISCUSSION

**Clindamycin Hydrochloride**—The assay of clindamycin hydrochloride bulk drug involved no extraction. The compound was simply dissolved in mobile phase containing internal standard and chromatographed. A relative standard deviation of 1.0% was obtained on six samples of various weights. A typical chromatogram for clindamycin is shown in Fig. 1. Formulations of clindamycin for which an extraction step is needed can be assayed by this procedure using the extraction shown under *Experimental* for clindamycin palmitate flavored granules.

Compounds II, V, and VII were separated from clindamycin by this procedure; but VI, a relatively insignificant impurity as determined previously by GLC, was not. Relative retention times of these impurities using the mobile phase for this assay are listed in Table I.

**Table I—Relative Retention Times (RRT) of Impurities and Degradation Products of Clindamycin Hydrochloride, Clindamycin Palmitate, and Clindamycin Phosphate Using the Mobile Phases in the *Experimental* Section**

| Compound | RRT |
|---|---|
| Clindamycin Hydrochloride | |
| Lincomycin B hydrochloride | 0.49 |
| Epilincomycin hydrochloride | 0.55 |
| Lincomycin hydrochloride | 0.55 |
| Clindamycin B hydrochloride | 0.79 |
| Clindamycin hydrochloride | 1.00 |
| Epiclindamycin hydrochloride | 1.00 |
| Testosterone propionate (internal standard) | 1.25 |
| Clindamycin Palmitate | |
| Clindamycin hydrochloride | 0.23 |
| Palmitic acid | 0.44 |
| Clindamycin 4-palmitate | 0.59 |
| Methyl palmitate (internal standard) | 0.70 |
| Clindamycin 3-palmitate | 0.75 |
| Clindamycin 2-palmitate | 0.82 |
| Clindamycin 2-palmitate | 1.00 |
| Clindamycin Phosphate | |
| Lincomycin 2-phosphate | 0.45 |
| Lincomycin hydrochloride | 0.61 |
| Clindamycin 2-phosphate | 0.71 |
| Clindamycin 3-phosphate | 0.90 |
| Clindamycin 4-phosphate | 0.92 |
| Clindamycin 2-phosphate | 1.00 |
| Propylparaben (internal standard) | 1.29 |
| Clindamycin hydrochloride | 1.94 |

**Clindamycin Palmitate Hydrochloride**—An extraction step was not necessary to assay clindamycin palmitate hydrochloride bulk drug. A relative standard deviation of 0.8% was obtained on eight samples of bulk drug covering a broad range of sample weights. The mobile phase used for clindamycin palmitate contained a larger percent of methanol than the mobile phase used for clindamycin. The palmitate ester group in clindamycin palmitate increased the retention time, necessitating the use of a less polar mobile phase to give a normal elution time. An extraction was used for the flavored granules formulation of clindamycin palmitate. With a chloroform–sodium carbonate solution, clindamycin palmitate was extracted into the chloroform layer, leaving formulation ingredients such as dye and flavoring agents in the alkaline aqueous layer.

Recovery results on clindamycin palmitate added to the flavored granules placebo formulation are shown in Table II. Recovery was complete, and a relative standard deviation of 1.8% was obtained. The amount of water added to the dry flavored granules in the sample preparation (*Experimental*) was approximately the same as would be present in reconstituted flavored granules, making the procedure applicable to samples before and after reconstitution. A chromatogram of clindamycin

**Table II—Recovery Results of Clindamycin Palmitate Added to Flavored Granule Excipients**

| Clindamycin Palmitate, mg | Excipients, g | Peak Height Ratio/ Weight, g | | Recovery, % |
|---|---|---|---|---|
| 149.85 | 2.309 | 6.084 | | 98.0 |
| 134.20 | 2.236 | 6.101 | | 98.3 |
| 145.50 | 2.251 | 5.999 | | 96.6 |
| 152.14 | 2.279 | 6.317 | | 101.8 |
| 131.12 | 2.232 | 6.153 | | 99.1 |
| 147.56 | 2.268 | 6.209 | | 100.0 |
| | | | Average | 99.0 |
| | | | *RSD* | 1.8% |

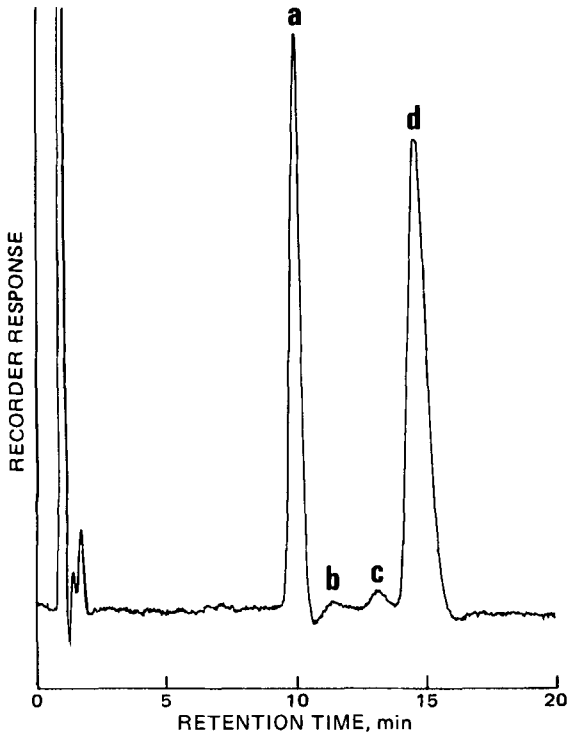



**Figure 2**—*Chromatogram of methyl palmitate used as the internal standard (a), clindamycin 2-palmitate (b), an unidentified peak (c), and clindamycin 2-palmitate extracted from flavored granules formulation (d).*

palmitate extracted from flavored granules is shown in Fig. 2. HPLC of placebo flavored granules showed no interfering peaks.

Since separation on reversed-phase columns varies from column to column, several columns were tried. Poor separation between methyl palmitate (internal standard) and clindamycin palmitate on a particular column was improved by increasing the water content of the mobile phase. A decrease in the amount of dioctyl sodium sulfosuccinate in the mobile phase decreased the separation. This behavior is due to differences in polarity of methyl palmitate and clindamycin palmitate.

Possible impurities or degradation products of clindamycin palmitate are clindamycin B 2-palmitate, clindamycin 3-palmitate, clindamycin 4-palmitate, epiclindamycin palmitate, clindamycin, and palmitic acid. Authentic samples were used to identify clindamycin B 2-palmitate, clindamycin, and palmitic acid chromatographically. A mixture of clindamycin 2-palmitate, clindamycin 3-palmitate, and clindamycin 4-palmitate was used to demonstrate the separation of these compounds (Fig. 3). The compounds were identified using the GLC procedure (3).

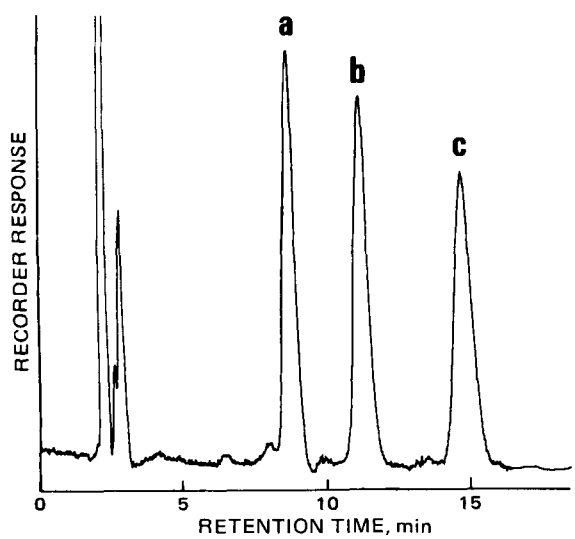



**Figure 3**—*Chromatogram of clindamycin 4-palmitate (a), clindamycin 3-palmitate (b), and clindamycin 2-palmitate (c).*

**Table III—Assay Results on Sterile Solution Clindamycin Phosphate (150 mg Base Equivalents/ml)**

| Clindamycin Phosphate, mg/ml | Peak Height Ratio | | Recovery, % |
|---|---|---|---|
| 112.5 | 0.7487 | | 102.1 |
| 127.5 | 0.8307 | | 99.9 |
| 142.5 | 0.9150 | | 98.5 |
| 157.5 | 1.0262 | | 99.9 |
| 172.5 | 1.1294 | | 97.6 |
| 187.5 | 1.2241 | | 100.1 |
| | | Average | 99.7 |
| | | *RSD* | 1.2% |

In Fig. 2, the unidentified peak eluting just prior to the clindamycin palmitate peak may be epiclindamycin palmitate. However, no authentic sample was available to confirm this finding. Although epiclindamycin is not separated from clindamycin (Table I) under conditions of the clindamycin assay, the decreased polarity of clindamycin palmitate may magnify differences in the molecule at the 7-position, thus producing separation. Table I shows the relative retention times of clindamycin palmitate impurities or degradation products using the mobile phase as described under *Experimental.*

Methyl palmitate, used as the internal standard, was chromatographed separately to check for possible impurities in this reagent, and a small amount of methyl stearate was detected in one lot. Methyl stearate has approximately the same retention time as clindamycin palmitate under the chromatographic conditions of this assay.

**Clindamycin Phosphate**—No extraction was necessary for the assay of sterile solution clindamycin phosphate. The sterile solution was diluted with water–methanol solvent, internal standard was added, and the solution was chromatographed. Assay results on six replicate samples of sterile solution clindamycin phosphate (150 mg clindamycin base equivalents/ml) are shown in Table III. An average recovery of 99.7% of theory was obtained with a relative standard deviation of 1.2%. Results showed linearity over the concentration range studied (75–125% of

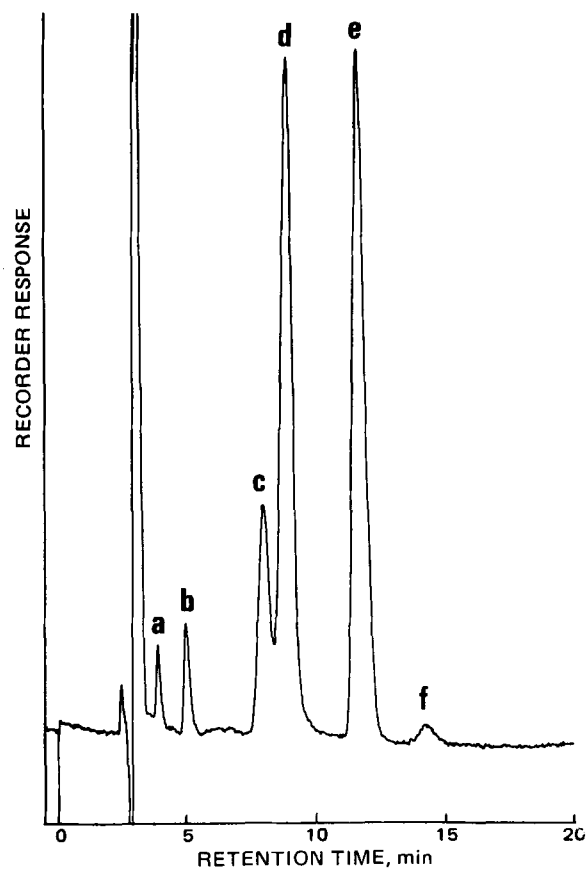



**Figure 4**—*Chromatogram of lincomycin 2-phosphate (a), benzyl alcohol (b), a mixture of clindamycin 3-phosphate and clindamycin 4-phosphate (c), clindamycin 2-phosphate (d), propylparaben used as the internal standard (e), and clindamycin (f).*

theory). Chromatography of the solution vehicle showed no interfering peaks. Benzyl alcohol, present in the sterile solution as a preservative, can be detected (Fig. 4) and quantitated.

Possible impurities or degradation products of clindamycin phosphate are free clindamycin, clindamycin 3-phosphate, clindamycin 4-phosphate, and clindamycin 2-phosphate. Since clindamycin is produced by chemical modification of lincomycin, the lincomyin analogs of the listed compounds are also possible impurities. Figure 4 shows a chromatogram of clindamycin phosphate with clindamycin, clindamycin 2-phosphate, clindamycin 3-phosphate, and clindamycin 4-phosphate present. Clindamycin 3-phosphate and clindamycin 4-phosphate are almost baseline separated from clindamycin 2-phosphate but are not separated from each other under the assay conditions.

The separation and elution order of the 2-, 3-, and 4-phosphate analogs of clindamycin are dependent on the pH and composition of the mobile phase. Mobile phase containing a greater percent of water causes clindamycin 4-phosphate to be eluted after clindamycin 2-phosphate while clindamycin 3-phosphate is eluted at the same time as clindamycin 2-phosphate. This modified mobile phase also shifts the clindamycin elution to a position before the internal standard peak.

Morozowich and Williams (5) chromatographed the clindamycin phosphate analogs on triethylaminoethylcellulose. The order of elution on the cellulose was 4-, 3-, and 2-phosphates. With the mobile phase of this assay, the relative retention times of possible impurities and degradation products are shown in Table I. If the mobile phase was adjusted to pH 6.0 or higher, the clindamycin phosphate peak tailed badly. If the pH was adjusted to 5.6 or less, the clindamycin phosphate peak broadened on the front side. Use of ammonium nitrate or sodium nitrate in the mobile phase did not improve the chromatography. To compensate for column-to-column variation, the amount of water in the mobile phases used for all three antibiotics was modified to give satisfactory chromatography for any particular column.

## REFERENCES


(1) A. A. Forist, R. M. DeHaan, and C. M. Metzler, *J. Pharmacokinet. Biopharm.*, **1**, 89 (1973).

(2) T. F. Brodasky and F. F. Sun, *J. Pharm. Sci.*, **63**, 360 (1974).

(3) L. W. Brown, *ibid.*, **63**, 1597 (1974).

(4) Code of Federal Regulations, Title 21, Part 453, 658 (Apr. 1, 1977).

(5) W. Morozowich and R. G. Williams, *J. Pharm. Sci.*, **64**, 313 (1975).


# pH–Solubility Profiles of Organic Carboxylic Acids and Their Salts


**Z. T. CHOWHAN**



Received October 18, 1977, from the *Institute of Pharmaceutical Sciences, Syntex Research, Palo Alto, CA 94304.* Accepted for publication January 6, 1978.



**Abstract** □ The solubilities of naproxen, 7-methylsulfinyl-2-xanthonecarboxylic acid, 7-methylthio-2-xanthonecarboxylic acid, and their sodium, potassium, calcium, and magnesium salts were determined as a function of pH. The results on the solubility of naproxen and its salts were in excellent agreement with the theoretical profiles describing the relationship between pH values of the solutions and the dissociation constant of the acid. The solubilities of the two xanthonecarboxylic acids were higher at higher pH values than the values calculated when complete dissociation in solution was assumed. The influence of the salt species on the solubility of organic carboxylic acids, at and above pH values of complete ionization, cannot be predicted even qualitatively from equations used for alkali and alkaline earth metal salts.



**Keyphrases** □ Solubility—naproxen, two xanthonecarboxylic acids, and various salts, as a function of pH □ Naproxen—and various salts, solubility as a function of pH □ Xanthonecarboxylic acids, substituted—and various salts, solubility as a function of pH □ Anti-inflammatory agents—naproxen and various salts, solubility as a function of pH


Organic carboxylic acids used as medicinal agents generally have poor water solubilities. Since solubility is an important factor in the overall drug absorption process, chemical stability, and formulation of dosage forms, salt-forming agents are used to increase the water solubility. Although salt formation results in an overall increase in solubility, no definitive quantitative methods of predicting the solubility of several salt species of the parent compound are available.

The influence of pH on the solubility of weak electrolytes was reported previously (1–3). The solubility interrelationships of the hydrochloride salt and free base of two amines were investigated extensively (4). Mathematical equations describing the total solubility at an arbitrary pH in terms of the independent solubilities of the hydrochloride and free base species and the dissociation constant of the salt were derived and fitted to the data with good results.

This paper discusses the solubility of three organic carboxylic acids as a function of pH and the salt species. The data were fitted to mathematical relationships similar to those used for organic hydrochlorides (4). The results of the solubility of naproxen and its salts were in excellent agreement with the theory. However, the solubilities of the xanthonecarboxylic acids and their salts were higher at higher pH values than those calculated when complete dissociation in solution was assumed. The effect of the salt species on the solubility of organic carboxylic acids, at and above pH values of complete ionization, cannot be predicted even qualitatively from equations used for alkali and alkaline earth metal salts.

## EXPERIMENTAL

**Materials**—Naproxen [*d*-2-(6-methoxy-2-naphthyl)propionic acid], 7-methylsulfinyl-2-xanthonecarboxylic acid, 7-methylthio-2-xanthonecarboxylic acid, and their sodium salts were at least 99% pure[1]. Other chemicals were analytical reagent grade unless otherwise indicated.

**Preparation of Naproxen Potassium**—Potassium bicarbonate, 21.7 g, was added to a 500-ml round-bottom flask and dissolved in 26 ml of distilled water. An equimolar quantity of naproxen was added slowly to the flask, and the contents were refluxed for about 2 hr or until all naproxen dissolved. Then the contents of the flask were poured into a petri dish and allowed to crystallize. The crystals were filtered, rinsed with

[1] Institute of Organic Chemistry, Syntex Research, Palo Alto, CA 94304.


0022-3549/78/0900-1257$01.00/0