An Isocratic High-Pressure Liquid Chromatographic Determination of Naproxen and Desmethylnaproxen in Human Plasma

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Abstract □ An isocratic high-pressure liquid chromatographic method for the determination of naproxen and its desmethyl metabolite in human plasma is presented. A reversed-phase octadecylsilane column was utilized with a mobile phase consisting of 55% methanol and 45% 0.10 *M* acetate buffer, pH 5.0. A spectrofluorometric detector with an excitation wavelength of 253 nm and a band pass filter provided high sensitivity with no interference from normal plasma constituents. The reproducibility and precision of the method were shown by analysis of spiked samples containing 2.5–70 μg/ml of plasma.

Keyphrases □ Naproxen—determination by isocratic high-pressure liquid chromatography, human plasma □ High-pressure liquid chromatography—isocratic, determination of naproxen and desmethylnaproxen in human plasma □ Metabolites—determination of naproxen and its desmethyl metabolite in human plasma, high-pressure liquid chromatography

Naproxen is a nonsteroidal anti-inflammatory drug commonly used for the treatment of arthritis, dysmenorrhea, and for the relief of mild to moderate pain (1). Drugs in this class share many side effects such as GI (2, 3) and renal toxicity (4). Due to the high incidence of side effects, there is interest in the use of patient monitoring as a method of minimizing adverse reactions.

A direct spectrophotometric method lacking specificity due to interference from metabolites and salicylic acid has been reported (5). Several GLC methods also have been reported, all of which require derivation and a minimum 0.5-ml plasma sample (6–10). Quantitation has been performed by high-pressure liquid chromatography (HPLC) (11–14), and methods have been described involving precipitation of plasma protein followed by injection of the supernate (11) and the direct injection of diluted samples (12). The use of ion-pairing has also been described (12). An HPLC method for screening solid dosage forms using dual UV detectors was reported recently (15). Methods capable of determining the metabolite require a 0.5-ml plasma sample (10, 13).

The present report describes a method for the rapid determination of naproxen and its desmethyl metabolite using an isocratic HPLC separation and fluorometric detection. Conditions for the extraction, separation, and detection of naproxen and desmethylnaproxen in a sample size of 0.1-ml plasma are discussed. The applicability of this model was demonstrated by the analysis of plasma from patients receiving oral naproxen.

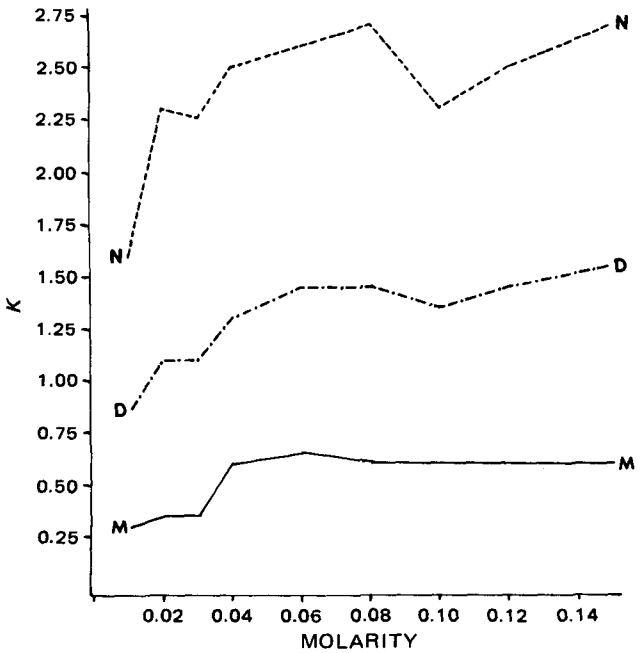

Figure 1—*Effect of molarity of the mobile phase at pH 5 on capacity factor, K'. Key: N, naproxen; M, desmethyl metabolite; and D, diphenylacetic acid (internal standard).*

EXPERIMENTAL

Instrumentation—A high-pressure liquid chromatograph[1] was equipped with a fluorometric detector[2] and an octadecylsilane column[3]

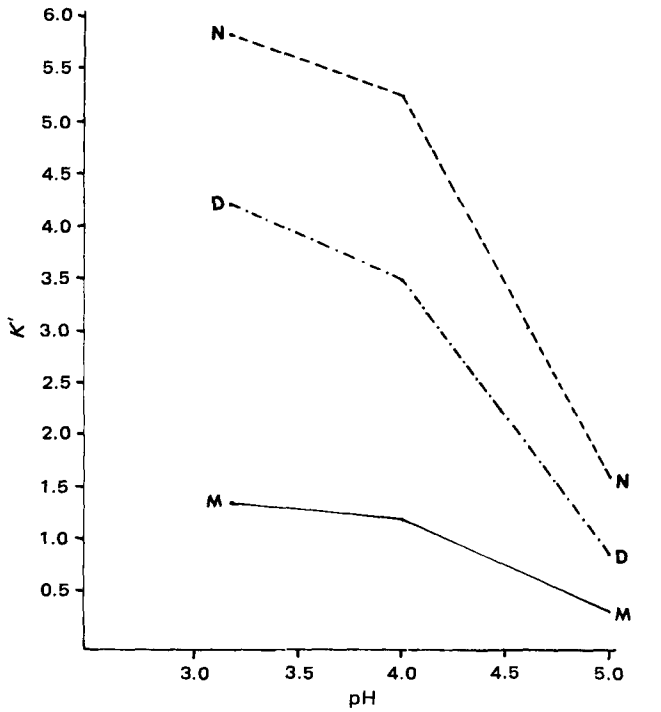

Figure 2—*Effect of pH on the mobile phase at 0.01* M *on capacity factor, K'. Key: N, naproxen; M, desmethyl metabolite; and D, diphenylacetic acid (internal standard).*

[1] Model 202 chromatograph, M6000 pump, and U6K Universal injector, Waters Associates, Milford, Mass.
[2] FS-970, Schoeffel, Westwood, N.J.
[3] Zorbax, Dupont, Wilmington, Del.

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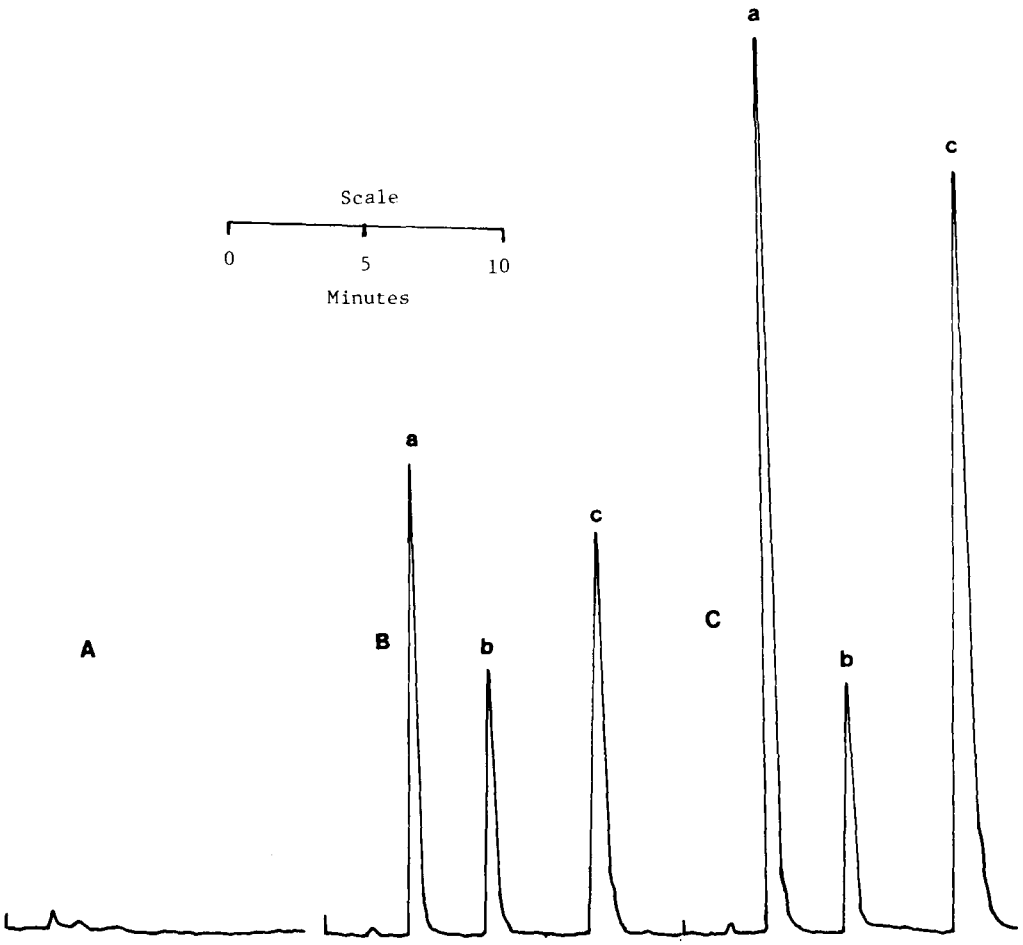

Figure 3—*Typical chromatograms of desmethylnaproxen (a), diphenylacetic acid (b), and naproxen (c) from plasma. Key: A, plasma blank; B, 15 μg drug and metabolite/1.0 ml plasma; and C, 30 μg drug and metabolite/1.0 ml plasma.*

(25 cm × 4.6-mm i.d.). The degassed mobile phase was pumped through the column at 1.5 ml/min (3200–3400 psi) at ambient temperature until a stable baseline was obtained. The fluorometer was set at an excitation wavelength of 253 nm and a band pass filter (230–420 nm) was used.

Chemicals and Reagents—Sodium acetate, sodium phosphate, acetic acid, phosphoric acid, methylene chloride, hexane, chloroform, ethyl acetate, ether, isopropanol, and diphenylacetic acid were reagent grade. HPLC grade methanol was used. Naproxen and desmethylnaproxen were obtained from the manufacturer[4].

Mobile Phases—To study the effect of ionic concentration and pH on retention time, methanol with pH 4 and 5 sodium acetate buffers (0.01, 0.02, 0.03, 0.04, 0.06, 0.08, 0.10, 0.12, and 0.15 *M*) or acetic acid solutions (0.01, 0.02, and 0.3 *M*) were used as mobile phases. All mobile phases were degassed under vacuum.

Extraction Conditions—Plasma containing naproxen and desmethylnaproxen was acidified with one of the following: 1.0 *M* phosphoric acid, 1.0 *M* sulfuric acid, 0.1 *M* phosphate buffer (pH 4), 0.1 *M* phosphate buffer (pH 3), or 0.1 *M* acetate buffer (pH 4). The acidified plasma was extracted with hexane, methylene chloride, chloroform containing 5% isopropanol, ether, or ethyl acetate.

Standard Stock Solution—A solution containing 10 mg each of naproxen and its desmethyl metabolite in 10 ml of methanol was prepared.

Extraction Solution—A solution of 120 mg of the internal standard, diphenylacetic acid, in 250 ml of methylene chloride was prepared (2.4 mg/5 ml).

Analytical Procedure—Spiked plasma standards were prepared by adding an appropriate aliquot of the standard stock solution to 1 ml of heparinized plasma. The spiked plasma was vortexed and 0.1-ml portions were placed in 15-ml screw-capped centrifuge tubes. The plasma was acidified with 0.3 ml of 1.0 *M* phosphoric acid, and 5-ml extraction solution containing the internal standard was added. The tubes were vortexed for 10 sec and centrifuged for 5 min at 3500 rpm. A 4-ml volume of the organic phase was transferred to a concentration tube[5] and evaporated to dryness at ambient temperature under a gentle nitrogen stream. The residue was dissolved in 0.5 ml of methanol and 10 μl was injected[6]. The mobile phase used for the analysis was 55:45 methanol and acetate buffer (pH 5, 0.10 *M*).

Quantitation—A standard curve was constructed by injecting plasma extracts simulating concentrations of the drug and metabolite. The chromatograms were recorded[7] at a chart speed of 0.5 cm/min. The ratios of peak heights (drug or metabolite to internal standard) were calculated and plotted *versus* the concentration in micrograms per milliliter plasma.

Interference—The possible interference of normal plasma constituents was tested by the analysis of blank plasma. The interference of other drugs was tested by direct injection of methanolic drug solution or by the analysis of extracts of plasma samples containing therapeutic concentrations.

Recovery—For the recovery study, plasma standards were prepared as described previously. After evaporation the residue was dissolved in 0.5 ml of methanol containing 360 ng of naproxen or desmethylnaproxen. A 10-μl aliquot containing 7.2 ng was injected onto the column.

Patient Sample Preparation and Analysis—Heparinized plasma samples from patients receiving oral naproxen were processed in duplicate as described previously. The amount of drug and metabolite was calculated by comparison with standards prepared daily.

RESULTS AND DISCUSSION

Increasing buffer molarity had a complex effect on the capacity factors of naproxen, desmethylnaproxen, and the internal standard (Fig. 1). The capacity factor was increased by decreasing pH (Fig. 2). Moreover, at low pH the compounds have a high capacity factor causing very long retention times and band broadening.

[4] Syntex, Palo Alto, Calif.

[5] Concentratube, Laboratory Research Co., Los Angeles, Calif.
[6] Hamilton Co., Reno, Nev.
[7] Model 56, Perkin-Elmer, Norwalk, Conn.

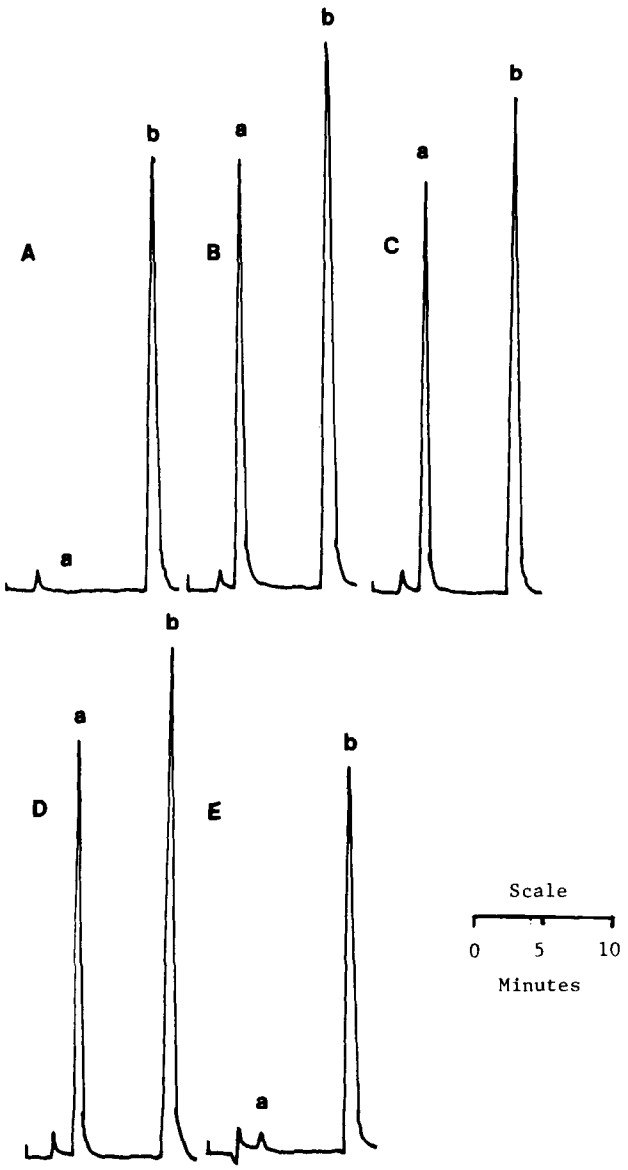

Figure 4—*Chromatograms of plasma extracts containing desmethylnaproxen (40 μg/ml) (a) and naproxen (40 μg/ml) (b). Key: A, hexane; B, methylene dichloride; C, chloroform containing 5% isopropanol; D, ether; and E, ethyl acetate.*

The choice of diphenylacetic acid as internal standard was based on coextractability with the drug and metabolite and an ideal relative retention time. A mobile phase of 55% methanol and 45% acetate buffer (pH 5, 0.10 *M*) gave well resolved, sharp peaks for naproxen, its metabolite and the internal standard, with retention times of 10.0, 3.3, and 5.9 min, respectively (Fig. 3). Under the described conditions 0.5 μg of naproxen or its desmethyl metabolite per milliliter of plasma could be quantitated.

Naproxen can be extracted readily from acidified plasma using organic solvents with a wide range of polarities; however, its desmethyl metabolite cannot be extracted with hexane or ethyl acetate (Fig. 4). The choice of acid or buffer for acidification of plasma can be used to optimize recovery and to minimize interference from plasma constituents, but due to the small sample size required and the small aliquot injected onto the column,

Table I—Statistical Analysis of Linear Regression

Parameter	Naproxen	Metabolite
Range of standards, μg/ml plasma	2.5–70	2.5–70
Correlation coefficient	0.9913	0.9922
Slope	0.1021 ± 0.0029	0.1247 ± 0.0046
Intercept	−0.0327 ± 0.0480	−0.0714 ± 0.0760

Table II—Assay Precision[a]

Theoretical, μg/ml plasma	Naproxen % Found	Naproxen *SE*	Metabolite % Found	Metabolite *SE*
2.5	113.6	0.09	113.6	0.22
5	101.0	0.15	101.8	0.11
10	101.3	0.52	100.3	0.21
15	101.3	0.56	103.6	0.26
20	90.6	0.26	89.8	0.25
30	103.4	0.82	103.4	0.99
40	96.5	0.96	98.7	0.98
50	104.2	0.99	101.5	0.12
70	99.0	1.41	99.6	1.72

[a] $N = 5$.

Table III—Possible Interferences Under Assay Conditions

Drug or Metabolite	Fluorescence at Assay Conditions	Retention Time, min
Acetaminophen	No	—
Caffeine	No	—
Carbamazepine	No	—
Fenoprofen	Yes	>15
Ibuprofen	Yes	>15
Indomethacin	No	—
Des(chlorobenzoyl) metabolite	Yes	Solvent front
Desmethyl metabolite	No	—
Phenobarbital	No	—
Phenytoin	No	—
Primidone	No	—
Quinidine	No	—
Salicylic acid	Yes	Solvent front
Sulindac	No	—
Sulfide metabolite	No	—
Sulfone metabolite	No	—
Theophylline	No	—
Tolmetin	No	—
Metabolite	No	—
Valproic acid	No	—

there was no interference from plasma constituents with any of the acids or buffers tested. Optimum recovery of naproxen (66.6%) and desmethylnaproxen (62.4%) from spiked plasma samples was obtained using 1.0 *M* phosphoric acid to acidify plasma prior to extraction with methylene chloride.

The ratio of peak height of naproxin or its metabolite to the peak height of the internal standard was plotted *versus* concentration. Statistical analysis indicated excellent linearity (Table I) and reproducibility (Table II).

Plasma levels of patients receiving 250-mg doses, two or three times daily were 21.5–65.1 μg/ml. The metabolite was not present in significant amounts since it is not expected to significantly accumulate unless the patient's renal function is compromised (12).

Aspirin and other commonly used drugs did not interfere with the analysis (Table III).

The described method is simple and rapid. Both naproxen and its desmethyl metabolite can be determined in a single isocratic assay with no interference from many commonly used drugs. This method can be recommended for routine patient monitoring and for pharmacokinetic studies.

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Extended Hildebrand Solubility Approach: Solubility of Tolbutamide, Acetohexamide, and Sulfisomidine in Binary Solvent Mixtures

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Received March 12, 1981, from the *Drug Dynamics Institute, College of Pharmacy, University of Texas, Austin, TX 78712.* Accepted for publication August 6, 1981.

Abstract □ The extended Hildebrand approach for predicting solubilities of crystalline compounds in solvent mixtures was tested using tolbutamide, acetohexamide, and sulfisomidine in mixed solvents consisting of hexane–absolute ethanol and 95% (v/v) ethyl alcohol–aqueous buffer. The solubility of these drugs was determined at 25 ± 0.2° and then back-calculated using the adhesive energy term, W, to account for solute–solvent interaction. Solubilities were predicted within 13% for tolbutamide, 31% for acetohexamide, and 43% for sulfisomidine, and with considerably better accuracy in most solvent mixtures.

Keyphrases □ Hildebrand solubility approach, extended—tolbutamide, acetohexamide, and sulfisomidine in binary solvent mixtures □ Tolbutamide—solubility in binary solvent mixtures, extended Hildebrand approach □ Acetohexamide—solubility in binary solvent mixtures, extended Hildebrand approach □ Sulfisomidine—solubility in binary solvent mixtures, extended Hildebrand approach

The Hildebrand–Scatchard theory (1) for crystalline solids in regular solution is expressed by:

$$-\log X_2 \simeq \frac{\Delta H_m^f}{2.303\,RT}\left(\frac{T_m - T}{T_m}\right) + \frac{V_2\,(\phi_1)^2}{2.303\,RT}(\delta_1 - \delta_2)^2 \quad \text{(Eq. 1)}$$

where X_2 is the mole fraction solubility, ΔH_m^f is the heat of fusion, T_m is the melting point of the solute expressed in absolute degrees, T is the absolute temperature of the solution, R is the gas constant expressed in cal/°Kmole, V_2 is the molar volume of the solute as a hypothetical supercooled liquid, δ_1 and δ_2 are the solubility parameters of the solvent and the solute, respectively, and ϕ_1 is the volume fraction of the solvent.

An approach (2) was suggested recently to extend regular solution theory to semipolar drugs in pure solvents and in polar binary solvent mixtures (2–4). The extended Hildebrand solubility equation may be written as:

$$-\log X_2 = -\log X_2^i + \frac{V_2\,(\phi_1)^2}{2.303\,RT}(\delta_1^2 + \delta_2^2 - 2W_{calc}) \quad \text{(Eq. 2)}$$

where X_2^i is the ideal solubility of the solute expressed in mole fraction, W_{calc} is the potential energy of solute–solvent interaction, and all other terms are identical with those in Eq. 1. The square of the solubility parameters are referred to as cohesive energy densities, and W may be referred to as an adhesive energy density since it involves both solute and solvent. The units of energy densities are calories per cubic centimeter (cal/cm^3) and for solubility parameters they are the square root of the same unit [(cal/cm^3)$^{1/2}$]. The ideal solubility term, $-\log X_2^i$, constitutes the first right-hand term of Eq. 1. Log X_2^i may be taken as roughly equal to $-\Delta H_m^f/2.303\,RT\,[(T_m - T)/T_m]$ as seen in Eq. 1, or as $\Delta S_m^f/R\,[\log (T/T_m)]$ as used in earlier studies (2–4). Although it has not been established which is more correct, either form provides satisfactory results

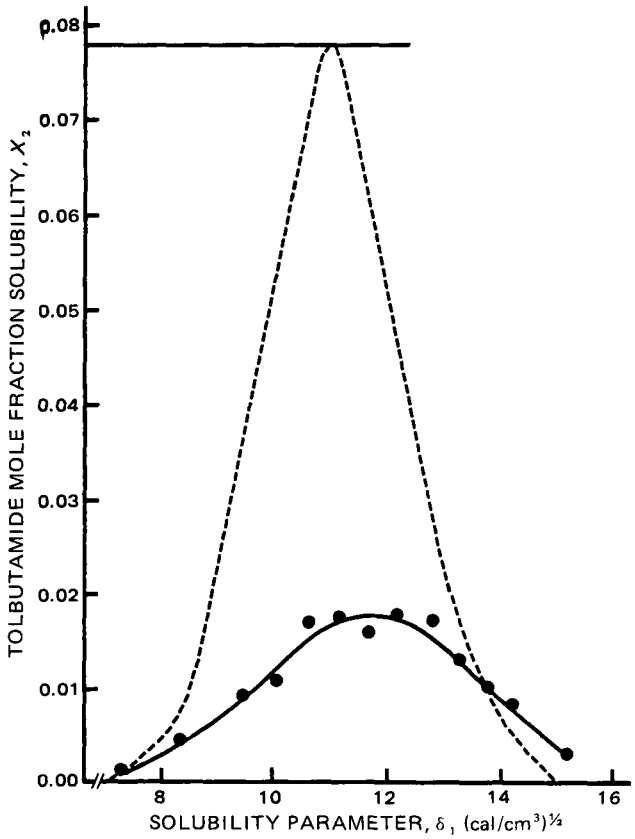

Figure 1—*Solubility profile of tolbutamide in* n*-hexane–absolute ethanol and 95% ethanol–aqueous buffer systems at 25°; δ_2 = 10.98, X_2^i = 0.07218. Key: (- - -) regular solution (Eq. 1); (—) calculated solubility (Eqs. 3*a *and* b*). The horizontal line intersecting the regular solution curve at its peak is the ideal solubility, X_2^i = 0.07218.*

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