HPLC Analysis of Indomethacin and Its Impurities in Capsule and Suppository Formulations

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Abstract □ Indomethacin and its impurities in suppository and capsule formulations were quantitatively determined by HPLC using a reversed-phase, octadecyl column and a mobile phase of methanol–water–acetonitrile–acetic acid (55:35:10:1). Analysis of the suppository formulations provided a mean potency for indomethacin of 103.8%. The same formulation was found to contain 4-chlorobenzoic acid (0.02%), 5-methoxy-2-methyl-3-indoleacetic acid (0.07%), 4-chlorobenzoic acid-α-monoglyceride (0.39%), and indomethacin-α-monoglyceride (0.9%) as impurities. The latter two impurities were a result of the interaction of indomethacin and 4-chlorobenzoic acid with glycerin used in the suppository base. Capsule formulations were likewise assayed with an average potency of 99.9 and 101.5% for 25- and 50-mg dosage forms, respectively. Only one of the two capsule formulations examined contained detectable quantities of 4-chlorobenzoic acid (0.05%).

Keyphrases □ Indomethacin—HPLC analysis, impurities in capsule and suppository formulations □ HPLC—indomethacin, impurities in capsule and suppository formulations □ Formulations—indomethacin, impurities in capsule and suppositories, HPLC analysis

Indomethacin is an anti-inflammatory and analgesic agent used in the treatment of rheumatoid arthritis, gout, degenerative joint disease, and other inflammatory conditions that do not respond to salicylates. Indomethacin may be synthesized by several routes (1–5), but these are generally modifications of the original method (1). The USP XX (6) contains monographs for the drug substance and capsule formulations, and the BP 1973 (7) includes monographs for these, as well as suppository formulations. The official pharmacopeias do not specify tests for impurities, and the hydrolytic, titrimetric assay procedure would not be indicative of purity if substances with structural similarities were present.

It has been reported that indomethacin is subject to hydrolysis in basic solutions (8) and in the presence of polysorbates (9). However, in only one instance has the presence of impurities in untreated capsule and suppository formulations been noted (10). Previous HPLC assay methods reported for the analysis of indomethacin in plasma (11) or capsule formulations (12) were not concerned with the detection or determination of impurities. The present method offers a rapid and simple HPLC procedure for the simultaneous quantitation of indomethacin and its major impurities in capsule and suppository formulations.

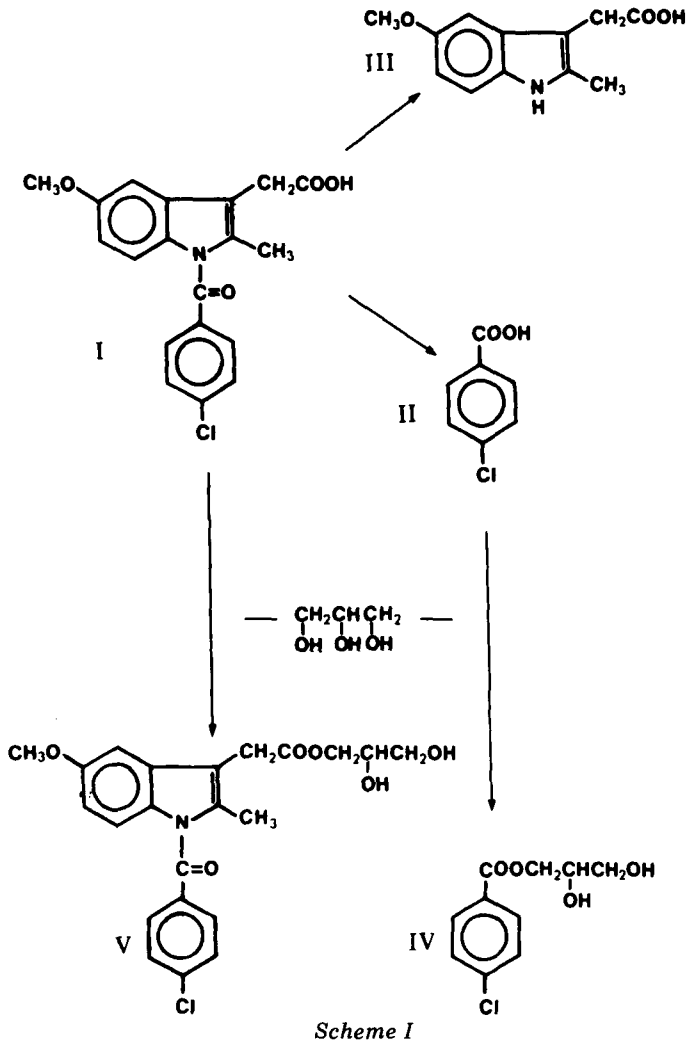
EXPERIMENTAL

Materials—All indomethacin (I) capsule and suppository formulations were obtained directly from the manufacturer[1]. Testosterone[2], 4-chlorobenzoic acid[3] (II), 5-methoxy-2-methyl-3-indoleacetic acid[4] (III), glycerin[5], and I drug substance[2] were used without further purification.

Methanol, water, and acetonitrile were HPLC quality[6], and acetic acid[7] and ether[8] were reagent grade. TLC plates[9] were precoated with silica gel G-25 UV (254) (20 × 20 cm, 0.25 mm). Solvents used for TLC were reagent grade[8].

HPLC System—An isocratic HPLC[10] equipped with a single piston pump[11], 20-μl loop injector[12], fixed wavelength detector[13] (254 nm), electronic data system printer/plotter[14], and a 5-μ reversed-phase (C-18) column (4.6 mm × 25 cm)[15] were used throughout. The mobile phase consisted of methanol–water–acetonitrile–acetic acid (55:35:10:1) and was pumped at 1 ml/min.

Synthesis of 4-Chlorobenzoic acid-α-monoglyceride (IV)—In a



Scheme I

[1] Merck Sharp & Dohme, Kirkland, Quebec, Canada.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] ICN Pharmaceuticals, Inc., Plainview, N.Y.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] J. T. Baker Chemical Co., Phillipsburg, N.J.
[6] Fisher Scientific Co. Ltd., Vancouver, B.C., Canada.
[7] Canadian Industries Ltd., Vancouver, B.C., Canada.
[8] Caledon, Georgetown, Ontario, Canada.
[9] Machercy-Nagel and Co., Distributed by Brinkmann Instruments Inc., Westbury, N.Y.
[10] Altex Scientific Inc., Berkeley, Calif.
[11] Model 110A, Altex Scientific, Inc.
[12] Model 7125, Rheodyne Inc., Berkeley, Calif.
[13] Model 153, Altex Scientific Inc.
[14] Model C-R1A, Shimadzu Corp., Kyoto, Japan.
[15] Ultrasphere, Altex Scientific Inc.

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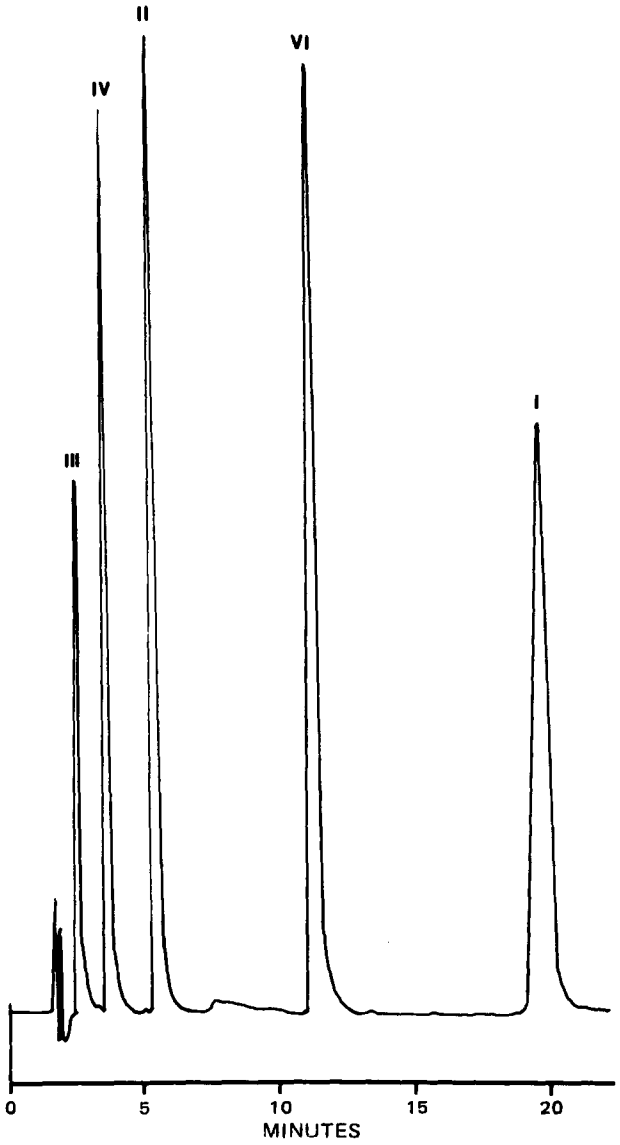

Figure 1—*HPLC of synthetic mixture of indomethacin and its impurities. Peaks: (I) indomethacin; (II) 4-chlorobenzoic acid; (III) 5-methoxy-2-methyl-3-indoleacetic acid; (IV) 4-chlorobenzoic acid-α-monoglyceride; (VI) the internal standard, testosterone.*

50-ml screw-capped culture tube, 1 g of II was heated with 30 ml of glycerin at 60° for 96 hr. During this period II went into solution. The glycerin solution was dissolved in water (50 ml) and extracted with three 50-ml portions of ether. The combined ether extracts were evaporated under vacuum and the residue was dissolved in a minimum volume of ether, diluted with petroleum ether (bp 60–80°)[5] and left at 4° until crystals separated. The crystalline material was recrystallized from ether–petroleum ether to a constant melting point (88–89°). The product was analyzed by HPLC to determine the absence of starting material or other impurities, and its structure was confirmed by PMR, IR, and mass spectrometric analysis. The compound exhibited identical characteristics to that reported earlier (10).

Table I—Slope of the Calibration Curves for Indomethacin and its Impurities Determined at 254 nm

Compound	Slope	Intercept	Correlation Coefficient[a]
Indomethacin (I)	1.05	0.17	0.999
4-Chlorobenzoic Acid (II)	1.008	0.0	0.999
5-Methoxy-2-methyl-3-indoleacetic acid (III)	0.380	−0.008	0.999
4-Chlorobenzoic acid-α-monoglyceride (IV)	0.834	−0.003	0.996

[a] $n = 4$.

Table II—Recovery of Indomethacin from Capsule and Suppository Formulations

Form and Dosage	Drug Weight in Formulation, mg	Amount of Drug Added, mg	Recovery mg	Recovery (%)
Suppository, 100 mg	50.06	20.12	69.52	99.1
Capsule, 50 mg	49.15	21.58	68.81	98.1
Capsule, 25 mg	25.08	15.20	40.10	99.6

Isolation and Identification of Indomethacin-α-monoglyceride (V)—A 100-mg suppository was crushed, suspended in 5 ml of water, and extracted with three 50-ml portions of ether. The combined ether extracts were evaporated under vacuum, the residue was taken up in 4 ml of ether, and applied in a narrow band to four TLC plates precoated with silica gel G-25 UV. The plates were eluted with ether–acetic acid (100:1) for a distance of 15 cm. The major impurity band corresponding to V (R_f 0.25) was removed from the four plates and extracted from the silica gel with 100 ml of chloroform. The subsequent identity of this material was confirmed by PMR, IR, and mass spectrometric analysis and was identical to that reported earlier (10).

Standard Solutions—Stock solutions of I (175 mg/50 ml), II (0.5 mg/100 ml), III (1 mg/100 ml), and IV (2 mg/25 ml) were prepared in methanol. Two working solutions of the internal standard, testosterone, were prepared in ether (50 mg/50 ml) and methanol (50 mg/50 ml).

Determination of Linearity and Calibration Curves—*Impurities*—To four 10-ml screw-capped culture tubes were added 0.25, 0.50, 1.0, and 1.5 ml of each of the stock solutions of II, III, and IV, along with 1 ml of the internal standard in methanol. The volume of each tube was brought to 10 ml with methanol and 20 μl was used for analysis of each sample.

Indomethacin—To four 10-ml screw-capped culture tubes were added 0.5, 1, 2, 3, 4, and 8 ml of the stock solutions of I, along with 1 ml of the internal standard in methanol. The volume of each tube was brought to 10 ml with methanol, and 20 μl was used for analysis of each sample.

Extraction of Capsule and Suppository Formulations—*Capsules*—The contents of four capsules (25- or 50-mg dose) were removed, weighed, and combined. Two aliquots equivalent to 50 mg of I were transferred to two 50-ml screw-capped culture tubes. A 3-ml aliquot of the internal standard in ether was added to each tube along with 15 ml of water and 25 ml of ether. The tubes were tightly capped and tumbled for 20 min. The ether layer was removed, dried, and evaporated under a stream of clean, dry nitrogen. The residue was dissolved in 30 ml of methanol and 20 μl was used for analysis.

Suppositories—Four suppositories (100 mg of I/dose) were weighed and crushed. Five aliquots equivalent to 50 mg were placed in five 50-ml screw-capped culture tubes and treated as described previously.

Recovery of Indomethacin from Formulations—An amount of I, equivalent to half the amount of I contained in a suppository formulation (~50 mg), was placed in a screw-capped tube. An aliquot of 3 ml of the internal standard in ether, 15 ml of water, and 25 ml of ether were added, and the mixture was treated as described previously. A similar recovery experiment was conducted on capsule formulations.

RESULTS AND DISCUSSION

A previous publication (10) reported the degradation of indomethacin and its interaction with glycerin used in a suppository base (Scheme I). To more precisely analyze indomethacin and its impurities, an HPLC

Table III—Quantitation of Indomethacin and its Impurities in Capsule and Suppository Formulations

Compound	Dosage Forms[a], % 100-mg[b] Suppository	50-mg[c] Capsule	25-mg[c] Capsule
Indomethacin (I)	103.800 ± 2.160	101.50	99.85
4-Chlorobenzoic Acid (II)	0.020 ± 0.002	0.05	None
5-Methoxy-2-methyl-3-indole-acetic acid (III)	0.070 ± 0.007		
4-Chlorobenzoic acid-α-monoglyceride (IV)	0.390 ± 0.023		
Indomethacin-α-mono-glyceride (V)	0.919 ± 0.067		

[a] All dosage forms were obtained from the same manufacturer. [b] Values are the mean of five determinations and are presented as the percentage based on labeled claim of indomethacin (±SD). [c] Values for capsules were taken from the average of two determinations.

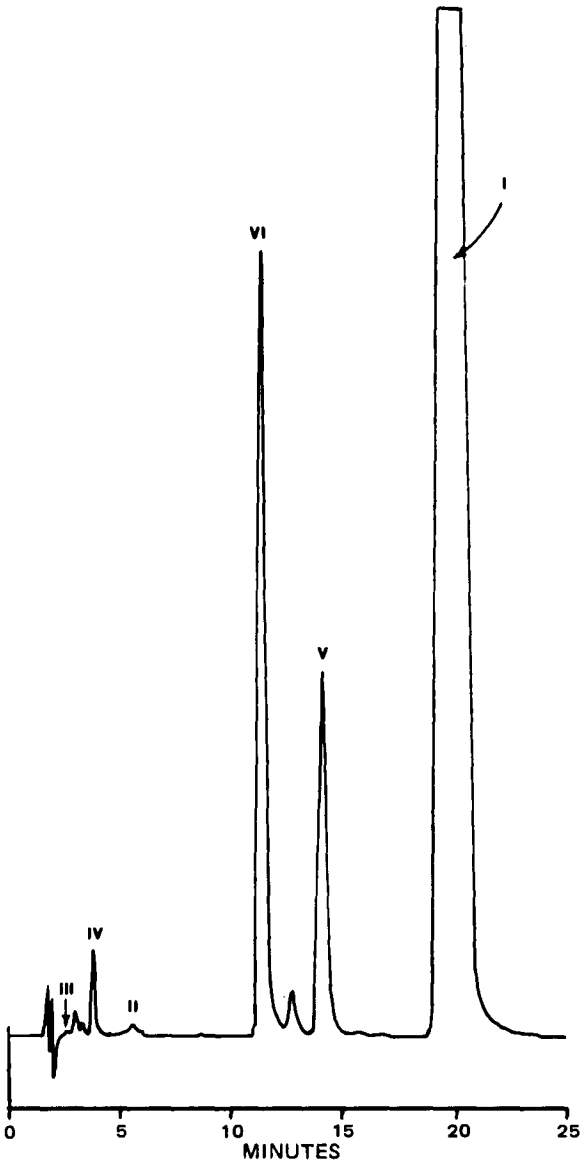

Figure 2—*HPLC of extract of indomethacin suppository formulation. Peaks: (I) indomethacin; (II) 4-chlorobenzoic acid; (III) 5-methoxy-2-methyl-3-indoleacetic acid; (IV) 4-chlorobenzoic acid-α-monoglyceride; (V) indomethacin-α-monoglyceride; (VI) the internal standard, testosterone.*

method was investigated. The chromatogram shown in Fig. 1 was obtained for a standard mixture of I and its impurities II–IV. Impurity V could not be included in this chromatogram due to lack of sufficient quantities of pure material. Calibration curves for I and the impurities II–IV were determined by dissolution in methanol containing the internal standard, testosterone. It was not possible to isolate a sufficiently pure crystalline form of V; therefore, the response factor for this impurity was equated to that of I. This was considered sufficiently accurate, as little difference in the UV absorptivity at 254 nm would be expected for these two compounds, due to the identical nature of the basic chromophore portion of both molecules. To establish the reliability of the calibration curves determined by dissolution in methanol, a single mixture of I and the impurities II–IV were likewise partitioned between the water–ether mixture used to extract the formulations. The recoveries of the drug and impurities from the aqueous phase into ether were quantitative. The response factors and correlation coefficients for I–IV are given in Table I.

The extraction of I from capsule and suppository formulations was determined by analysis of an aliquot of the formulation to which a quantity of indomethacin, equivalent to half of the theoretical amount of indomethacin, was added. The extraction efficiencies thus determined are summarized in Table II. The apparent high recovery indicates that the formulation excipients do not interfere with such a simple, single extraction.

The results obtained for the analysis of I and its impurities II–V in both capsule and suppository formulations are given in Table III. A representative chromatogram for a suppository formulation is shown in Fig. 2. Impurities II–V account for a total of 1.4% of the amount of indomethacin in the suppository formulation, while only small quantities of 4-chlorobenzoic acid were detected in one of the two capsule formulations.

Examination of the chromatogram of a suppository formulation (Fig. 2) reveals an impurity at 12.5 min following the internal standard. Several attempts to isolate and identify this material were unsuccessful. No attempt was made to isolate the minor components appearing between impurities III and IV. However, the remaining impurities II–V were confirmed by comparison of their retention times with authentic samples.

All formulations analyzed by the HPLC procedure described fall within the limits specified for capsule and suppository formulations in the USP (6) and BP (7). The presence of impurities, particularly in the suppository formulations represents a distinct example of the interaction of a drug with an excipient, glycerin. Although the amount of the α-monoglyceride impurities were less than 1.4% of the amount of indomethacin, these levels could be increased several-fold by holding the suppository at its melting point. Since all formulations were obtained fresh from the manufacturers, the rate of this reaction under normal storage conditions could not be determined, and accelerated stability studies were not considered due to the relatively low softening temperature of the suppository base.

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