within the USP XIX monograph limits. The I content of nonpharmacopeial concentrate was also satisfactory.

For a recovery study, five samples (tablet, concentrate, suppository, single-dose injectable, and multiple-dose injectable) were each spiked at 1% levels of II and III and analyzed. The mean recoveries ($n = 5$) were 98.3 and 97.5% with relative standard deviations of 1.8 and 2.4% for II and III, respectively. A syrup sample was spiked at 5% levels of II and III; 104 and 103% recoveries were obtained, respectively.

USP Reference Standard—Analysis of freshly obtained USP I reference standard (200 μg) by the UV absorption mode revealed 0.18% of "apparent" II. However, this peak was totally absent in the fluorescence chromatogram, proving that no II was present in the reference standard. The minimum detection level in this case was at 0.025%. When this standard solution was again analyzed 10 days later, its fluorescence chromatogram demonstrated the presence of II (0.03%). This change appeared to be due to the autoxidation of I. Although the solution was stored in the dark when not in use, dissolved oxygen was not purged and the solution was under a normal atmosphere at room temperature during its storage.

CONCLUSIONS

A novel approach of using an amino-bonded HPLC packing for the rapid and quantitative determination of I and two of its oxidation products was presented. Unlike the UV photometric determination, the selective fluorescence detection approach permitted a fast, qualitative, and quantitative determination of a mixture of trace components, II and III, in the presence of at least a 100-fold excess of parent drug. It also permitted a fast and selective determination of the major component, I. Interferences from other impurities were not encountered. A typical HPLC analysis was completed within 15 min. For all three compounds, the fluorescence response was linear in the ranges tested, and the minimum detectable amount was 0.05 μg.

The described HPLC procedure allows the injection of diluted raw samples directly into the HPLC system. The recoveries of II and III from commercial samples at 1% contamination levels were satisfactory. The described method is also simpler, more sensitive, and more accurate than the USP XIX methods for determining I or II.

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ACKNOWLEDGMENTS

The author thanks Smith Kline and French Laboratories, Philadelphia, Pa., for supplying 50 mg each of II and III. He also thanks Harvey K. Hundley, Research Coordinator, San Francisco District, Food and Drug Administration, and Neal Castagnoli, Professor of Chemistry, University of California, San Francisco, for helpful discussions during this work and for reviewing this paper.

Impurities in Drugs IV: Indomethacin

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Abstract □ One lot of indomethacin raw material, five lots of capsule preparations, and three lots of suppository formulations were screened for impurities by TLC. Only the suppository products exhibited impurities above trace levels. The two main impurities were present at levels estimated at ~0.5 and 2%. After isolation from preparative TLC plates, they were identified by NMR, IR, and mass spectroscopy as the α-substituted monoglyceryl esters of 4-chlorobenzoic acid and indomethacin, respectively.

Keyphrases □ Indomethacin—impurities, detection by NMR, IR, and mass spectroscopy □ Anti-inflammatory agents—indomethacin, detection of impurities by NMR, IR, and mass spectroscopy

Indomethacin is an analgesic and anti-inflammatory agent used to treat rheumatoid arthritis and other joint diseases. The NF XIV (1) contains monograph specifications for the drug substance and capsules, and BP 1973 (2) includes monograph specifications for the drug substance, capsule, and suppository. However, the compendia do not contain tests and limits for impurities in indomethacin formulations, nor are there publications dealing with their occurrence in commercial preparations. Indomethacin may be synthesized by several routes (3–7). However, these methods are generally modifications of the original procedure described by Shen *et al.* (3).

This paper deals with impurities observed in a routine TLC screening program of indomethacin drug substances and formulations. The main impurities found in suppository preparations were isolated and identified and serve to underline a classical instance of drug–excipient interaction.

EXPERIMENTAL

Materials—All drug substances (one sample) and formulations (five capsule and three suppository samples) were obtained directly from the manufacturers. Indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid] (I) was the NF reference standard material. 4-Chlorobenzoic acid[1] (II), 4-chlorobenzoyl chloride[1], 5-methoxy-2-

[1] Aldrich Chemical Co., Milwaukee, Wis.

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Table I—TLC Characteristics of Indomethacin and Its Impurities

Compound	R_f[a]	R_f[b]	Detection Limit, μg
Indomethacin (I)	0.80	0.40	0.1
4-Chlorobenzoic acid (II)	0.90	0.50	0.2
5-Methoxy-2-methyl-3-indoleacetic acid (III)	0.75	0.38	0.1
Indomethacin-α-monoglyceride (IV)	0.24	0.33	0.1
4-Chlorobenzoic acid-α-monoglyceride (V)	0.35	0.28	0.2
VI	0.64	0.53	—
VII	0.70	0.55	—

[a] In ether–acetic acid (100:3). [b] In benzene–methanol–acetic acid (90:10:3).

methyl-3-indoleacetic acid, (III), glycerin[1] (ACS grade), pyridine[1] (ACS grade), ether[2] (USP grade), acetic acid[3] (ACS grade), benzene[4] (ACS grade), methanol[4] (ACS grade), anhydrous sodium sulfate[4] (ACS grade), and sodium hydrogen carbonate[4] (analytical reagent) were obtained commercially.

TLC plates were precoated with silica gel 60 F-25[4] (20 × 20 cm, 0.25 mm) or silica gel GF[5] (20 × 20 cm, 1.0 mm).

Standard Solutions—Ether solutions of the following were prepared: I, 20 mg/ml; II, 0.25 mg/ml; and III, 0.25 mg/ml.

TLC Systems—Two developing systems were used: A, ether–acetic acid (100:3); and B, benzene–methanol–acetic acid (90:10:3). Both solutions were placed in filter paper-lined chromatographic tanks and allowed to equilibrate for 1 hr prior to use. Spots were visualized with UV light at 254 nm.

Drug Substance, Capsule, and Suppository Extraction—About 100 mg of indomethacin drug substance, accurately weighed, was dissolved in 5 ml of ether. The solution was analyzed by TLC.

The contents of 10 capsules were emptied and weighed. An amount of powder equivalent to about 100 mg of indomethacin was accurately weighed into a screw-capped tube, 10 ml of ether–water (1:1) was added, and the mixture was shaken vigorously for 15 min and then centrifuged. A portion of the ether layer was used for TLC analysis.

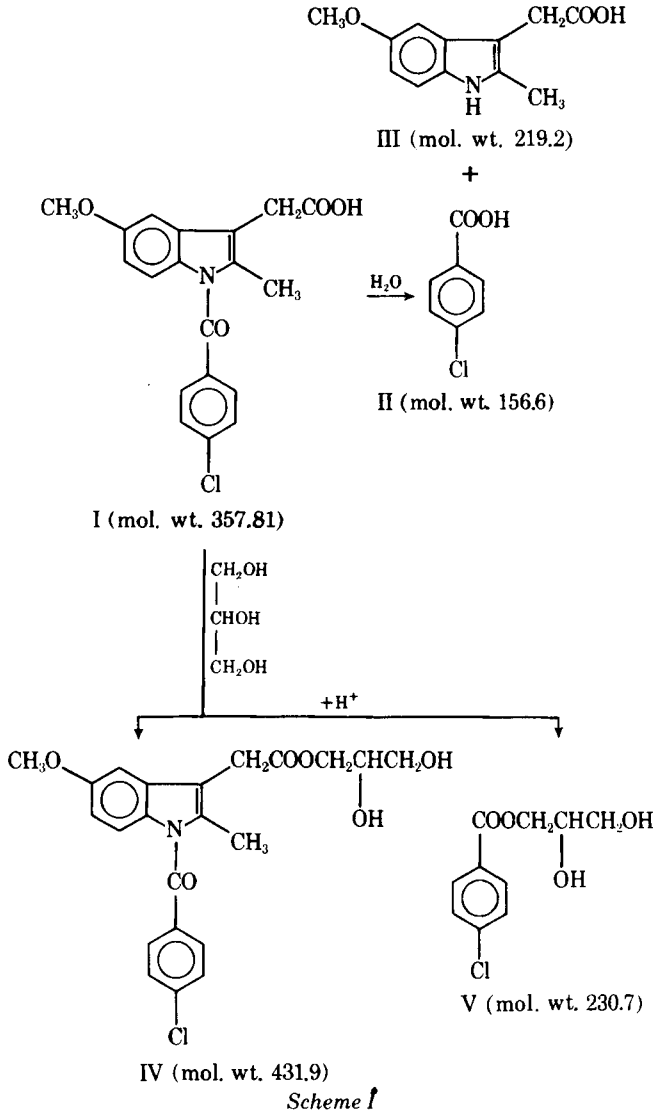
Five suppositories, equivalent to 500 mg of drug, were stirred vigorously in a stoppered flask for 30 min in 50 ml of ether–water (1:1). The heterogeneous solution was transferred to a screw-capped tube and centrifuged. The ether layer was used for TLC analysis.

Screening for Impurities—The R_f values of the drug and each impurity were determined in both TLC systems, and the lower limits of detectability of I, II, III, IV (indomethacin-α-monoglyceride), and V (4-chlorobenzoic acid-α-monoglyceride) were established (Table I). Aliquots of 1, 10, and 25 μl (20, 200, and 500 μg of I) of the ether extracts of each formulation and drug substance were spotted (0.25-mm plate) beside 1-, 10-, and 25-μl (20-, 200-, and 500-μg) aliquots of the standard solution of I and 1-, 5-, 10-, and 20-μl (0.25-, 1.25-, 2.5-, and 5-μg) portions of each standard solution of II and III.

The concentrations of impurities in the formulations (Table II) were estimated by comparison of the spot diameters and intensities with the corresponding spots of the standard solutions.

Isolation of Impurities—Impurities IV and V (Tables I and II) were isolated from Suppository Formulation A using the following procedure. Ten suppositories were shaken vigorously for 30 min in 100 ml of ether–water (1:1) in a separator. The ether layer was transferred to an erlenmeyer flask and dried over anhydrous sodium sulfate for 15 min. The mixture was filtered, and 1 ml of the filtrate was applied to a silica gel GF (1.0-mm) plate using a commercial TLC sample streaker[6]. The streaking operation was repeated on several plates. Each plate was developed in System A until the solvent front reached the top of the plate.

After development and hot air drying of each plate, the bands corresponding to IV and V were scraped off the plate into separate flasks; the total scrapings of each of these impurities were stirred in 75 ml of ether for 2 hr. The mixtures were filtered, and the filtrates were dried over anhydrous sodium sulfate and filtered again. The plate extract solutions of impurities IV and V were brought to dryness under a stream of pure dry nitrogen, and the separate residues were dried *in vacuo* at 60° overnight. Pale-yellow oils were obtained. The purity of each compound as isolated was checked by TLC in both solvent systems.



Scheme I

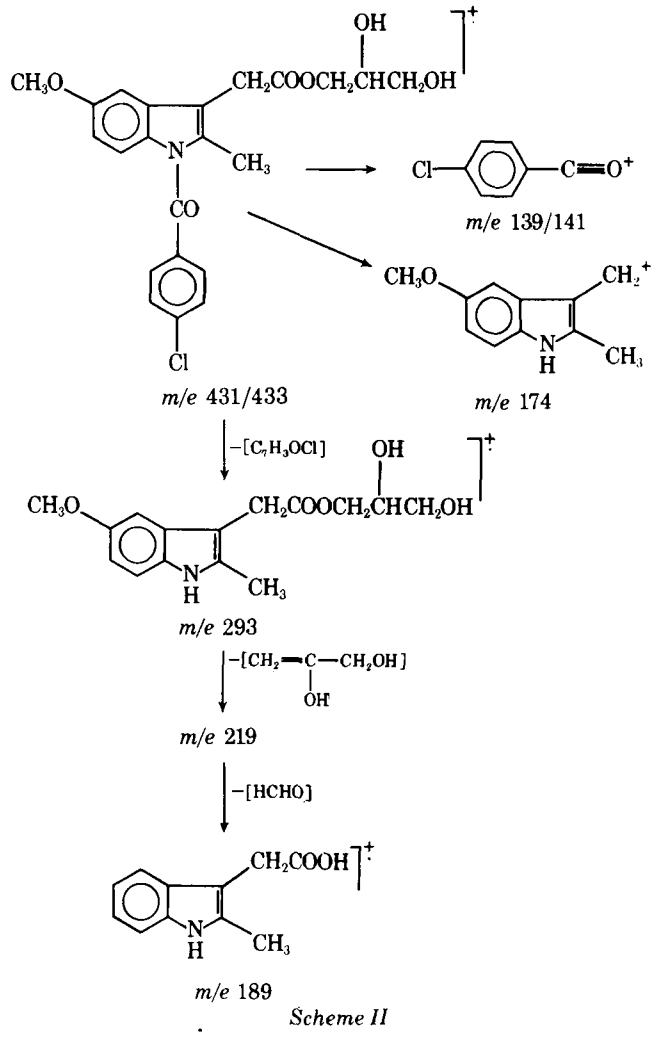
Synthesis of 4-Chlorobenzoic Acid-α-monoglyceride (V in Scheme I)—4-Chlorobenzoyl chloride (3.5 g, 0.02 mole) was slowly added dropwise with constant stirring to a solution of glycerin (4.0 g, 0.04 mole) in dry pyridine (10 ml) at 0°. Then the solution was allowed to warm to ambient temperature for 2 hr. The reaction mixture was diluted to 30 ml with water and extracted with three 25-ml portions of ether. The ether extracts were combined and shaken with four 15-ml portions of 5% aqueous sodium bicarbonate solution followed by two 10-ml portions of water. The ether layer was dried for 30 min over anhydrous sodium sulfate, the mixture was filtered, and the filtrate was evaporated to dryness under a stream of pure dry nitrogen. The oily residue was dried overnight *in vacuo* over phosphorus pentoxide at 50°. Recrystallization from benzene afforded white crystals (mp 88–89°).

The product was checked by TLC in both solvent systems against impurity V isolated from the plates and was subjected to NMR[7], IR[8], and mass spectral[9] analyses.

Characterization and Elucidation of IV and V Structures—*Indomethacin-α-monoglyceride (IV)*—IR (liquid film): 3200 (OH, br), 1725 (ester carbonyl), 1670 (amide carbonyl), and 1500 cm^{-1}; NMR[10] ($CDCl_3$): δ 7.69 [d, 2H, ArH (2′,6′), J = ~9 Hz], 7.47 [d, 2H, ArH (3′,5′), J = ~9 Hz], 6.97 [d, 1H, ArH (4), J = ~2.5 Hz], 6.90 [d, 1H, ArH (7), J = ~9 Hz], 6.67 [d, d, 1H, ArH (6), J = ~9 and 2.5 Hz], 4.22 (d, 2H, $COOCH_2$, J = ~5 Hz), 3.84 (s, 3H, OCH_3), 3.72 (s, 2H, CH_2COO), 3.62–3.53 (m, 3H, $OCHCH_2O$), 2.39 (s, 3H, CH_3), and 2.05 (s, 2H, OH, disappears on D_2O exchange) ppm;

[2] Mallinckrodt Canada Ltd., Toronto, Ontario, Canada.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] BDH Chemicals Ltd., Toronto, Ontario, Canada.
[5] Uniplates, Mandel Scientific Co. Ltd., Ville St-Pierre, Quebec, Canada.
[6] Applied Science Laboratories, State College, Pa.

[7] Brüker WP80 Fourier transform spectrometer.
[8] Perkin-Elmer model 621 grating IR spectrophotometer.
[9] Hewlett-Packard GLC–MS system 5985 with ionization potential of 70 ev.
[10] The aromatic protons on the indole ring are designated as 4, 6, and 7 while the aromatic protons on the 4-chlorobenzoyl ring are designated as *ortho* (2′ and 6′) and *meta* (3′ and 5′) to the carbonyl group.



Scheme II

mass spectrum: electron-impact mode, *m/e* 431/433 (M^+, 100/36%), 293 (16), 219 (1.8), 189 (23), 174 (35), and 139/141 (82/33). The fragmentation pattern of this impurity is illustrated in Scheme II.

4-Chlorobenzoic Acid-α-monoglyceride (V) (TLC Isolate and Synthesized Product)—IR (liquid film): 3200 (OH, br) and 1725 (ester carbonyl) cm^{-1}; NMR[11] ($CDCl_3$): δ 8.01 [d, 2H, ArH (2, 6), J = ~9 Hz], 7.43 [d, 2H, ArH (3, 5), J = ~9 Hz], 4.44 (d, 2H, $COOCH_2$, J = ~5 Hz), 4.09–3.48 (m, 3H, $OCHCH_2O$), and 2.05 (s, 2H, OH, disappears on D_2O exchange) ppm; mass spectrum: chemical-ionization mode (CH_4), *m/e* 231/233 ($M^+ + 1$, 100/35%) and 213/215 ($M^+ + 1 - H_2O$, 93/31).

RESULTS AND DISCUSSION

The ethereal indomethacin solutions used were saturated and, as such, were prone to precipitation if not kept well capped. Alternatively, more dilute solutions could be prepared by increasing the amount of ether for dissolution or extraction by 50%. However, for application of the same amounts of drug to the TLC plates, the larger sample volumes required resulted in somewhat more diffuse spots and bands.

[11] The aromatic protons are designated as *ortho* (2 and 6) and *meta* (3 and 5) to the carbonyl group.

Table II—Impurity Levels in Indomethacin Drug Substance and Formulations

Form	Dosage Level, mg/unit	Impurities[a], % II	III	IV	V	VI	VII
Bulk drug	—	Tr[b]	Tr	ND[c]	ND	ND	ND
Capsule	40	Tr	Tr	ND	ND	ND	ND
Capsule	25	Tr	Tr	ND	ND	ND	ND
Capsule	25	Tr	Tr	ND	ND	ND	ND
Capsule	25	Tr	Tr	ND	ND	ND	ND
Capsule	50	Tr	Tr	ND	ND	ND	ND
Suppository	100	Tr	Tr	2	0.5	Tr	Tr
Suppository	100	Tr	Tr	2	0.5	Tr	Tr
Suppository	100	Tr	Tr	2	0.5	Tr	Tr

[a] Estimated by TLC and expressed as a percentage of the label claim of drug. [b] Trace (<0.05%). [c] None detected.

The data given in Table II indicate that only the suppository products exhibited impurities (IV and V) above trace levels. Compounds IV and V were estimated by TLC to be present at levels of ~2 and 0.5%, respectively. Both impurities are formed through chemical interaction of the drug with glycerin present in the suppository. Indomethacin produced these materials in significant amounts when heated with glycerin in the presence of trace mineral acid at 80° for 24 hr. Moreover, when reacted with glycerin under the same conditions, 4-chlorobenzoic acid gave appreciable amounts of V. Reaction of III with glycerin gave rise to an oily product (R_f 0.19, System A), which did not correspond to any of the observed impurities but which was consistent (NMR and IR) with an α-monoglyceride structure. In addition to IV and V and trace levels of II and III, two other impurities (VI and VII) were observed in the suppositories. Since only traces of these impurities appeared to be present, they were not investigated further.

The bulk drug sample and the capsule formulations gave traces of II and III, confirmed by comparison of R_f values in the two solvent systems with authentic specimens of these compounds. No other impurities were observed in the bulk drug and capsule preparations.

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ACKNOWLEDGMENTS

The authors thank Dr. M. A. Poirier and Dr. A. By for help in interpreting the spectral data and Mr. R. J. Wood for technical assistance.