Rapid Determination of Chlorpromazine Hydrochloride and Two Oxidation Products in Various Pharmaceutical Samples Using High-Performance Liquid Chromatography and Fluorescence Detection

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Abstract □ A rapid, simple, and accurate high-performance liquid chromatographic procedure using an amino-bonded microparticulate column is reported for the determination of chlorpromazine hydrochloride (I) and its two oxidation products, the sulfoxide (II) and the sulfone (III), in commercially available pharmaceutical dosage preparations. Simultaneous identification and quantitation of II and III in the presence of at least 100 times the amount of parent compound were achieved with selective fluorometric detection. Identification and quantitation of I were achieved by changing the fluorescence detection mode. A typical chromatographic run was completed within 15 min. The described procedure is simpler, more sensitive, and more accurate than the USP XIX methods, particularly for the analysis of II in injectables. Quantitation of II and III at levels as low as 0.1 μg, representing 0.1% contamination, was demonstrated.

Keyphrases □ Chlorpromazine hydrochloride—and oxidation products, high-performance liquid chromatography and fluorescence detection, dosage forms □ High-performance liquid chromatography—rapid determination of chlorpromazine hydrochloride and two oxidation products, dosage forms □ Phenothiazines—chlorpromazine hydrochloride and two oxidation products, rapid determination by high-performance liquid chromatography and fluorescence detection, dosage forms

Tricyclic antidepressants of the phenothiazine type are important psychopharmacological agents in modern psychotherapy and have continuously attracted interest since their discovery. One clinically useful member of this class is chlorpromazine hydrochloride (I). It readily undergoes sulfoxidation at the sulfur atom in position 5 to form the sulfoxide (II), which may be further oxidized to the sulfone (III).

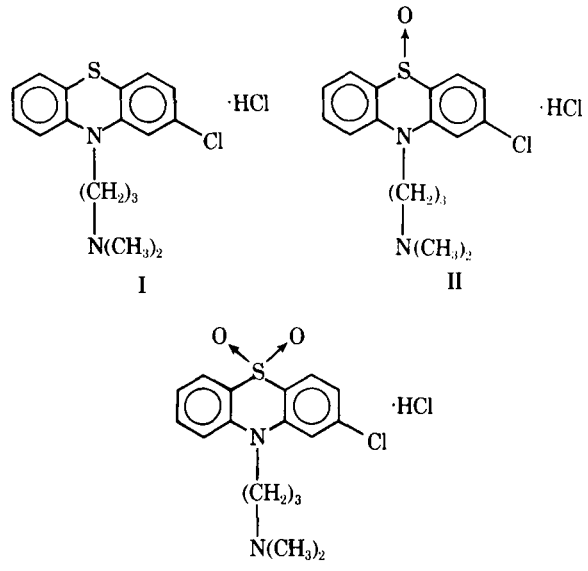
BACKGROUND

The USP XIX monographs (1) specify limits for certain impurities and degradation products in various dosage formulations of chlorpromazine and its hydrochloride salt. For instance, a maximum of 0.5% for "other alkylated phenothiazines" is allowed. Although the widespread occurrence of both alkylated and nonalkylated compounds was recently demonstrated in various tablet formulations, many of the impurities found are also used as therapeutic agents and thus were not considered as a health hazard (2). However, the presence of these artifacts deserves attention during manufacturing quality control procedures (2) as an index to adherence with recognized Current Good Manufacturing Practices.

The USP XIX permits II in injectables up to a maximum of 5%, but the TLC identification test used is only semiquantitative at best. Furthermore, this test lacks the specificity desired since the comparison is made with the TLC spot of the USP chlorpromazine hydrochloride reference standard and not with that of the sulfoxide reference material. Similar TLC identification tests are also used for the other alkylated phenothiazines. One is never certain whether II, III, the other alkylated or nonalkylated phenothiazines, or other degradation products are being determined.

The quantitative determination of chlorpromazine and its hydrochloride salt in dosage preparations is based on the differential UV absorption of the drug using two absorption maxima (254 and 277 nm). Pure drug substances are assayed by perchloric acid titration.

In general, it is inherently more desirable to use simpler and more accurate techniques and to avoid complex and nonspecific analytical methods. Thus, there is a need for developing a suitable analytical method to identify and quantitate I and its degradation products rapidly (especially II).



Many procedures have been reported for the determination of I and its metabolites. Previously reported fluorescence properties of phenothiazine drugs (3–6) indicate spectrofluorometry to be useful for qualitative and quantitative analyses of these compounds. The differences in the fluorescence spectra of I and its *S*-oxidation products may be particularly useful in the development of a rapid and simultaneous determination. However, it has been virtually impossible to differentiate between II and III by conventional spectrofluorometry when present in a mixture (6).

In recent years, high-performance liquid chromatography (HPLC) has been employed in the determination of I and its derivatives (7–11). In general, UV detection has been used. However, a method for the simultaneous determination of I–III has not been previously reported. This article describes the development of such a method, combining both the HPLC separation and fluorescence detection techniques for determining these three compounds in mixtures with other constituents normally present in commercial pharmaceutical preparations.

EXPERIMENTAL

Reagents and Chemicals—Compounds I[1], II[2], and III[2] were used as received. All other reagents were analytical reagent grade except *d*-araboascorbic acid[3]. All solvents were glass distilled[4].

Apparatus—The liquid chromatograph[5] was equipped with reciprocating dual-piston constant-flow rate pumps, a rotary valve injector[6],

[1] United States Pharmacopeial Convention, Rockville, Md.
[2] Smith Kline and French Laboratories, Philadelphia, Pa.
[3] Eastman Kodak Co., Rochester, N.Y.
[4] Burdick & Jackson Laboratories, Muskegon, Mich.
[5] Model 3500B, Spectra-Physics, Santa Clara, Calif.
[6] Six-port rotary valve (7000-psi capacity) with a 100-μl external loop, Valco Instrument Co., Houston, Tex.

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a filter fluorescence detector[7], a variable-wavelength UV detector[8], a computing integrator[9], and a recorder[10]. The HPLC columns were: (*a*) a 25-cm × 4.6-mm i.d. stainless steel column commercially packed with 10-μm amino-bonded microparticle packing[11] and (*b*) a 30-cm × 4-mm i.d. stainless steel column commercially packed with 10-μm amino-bonded microparticle packing[12]. Ambient temperature was used throughout these experiments.

Mobile Phase—The mobile phase consisted of acetonitrile–benzene–water (16:4:1), with the water containing 0.01% each of sodium metabisulfite and *d*-araboascorbic acid as antioxidants. The prepared mobile phase was filtered[13], degassed, and stored in the solvent reservoir of the instrument.

Standard Solutions—All standard materials were dissolved in and made to volume with acetonitrile. All solutions were protected from light.

Linearity Standards—Five standard solutions, ranging in concentration from 1.00 to 50.0 μg/ml, were individually prepared in acetonitrile for II or III. The I standard solutions (10.0–500 μg/ml) also were prepared in acetonitrile. Duplicate 100-μl aliquots of each solution were chromatographed.

Daily Calibration Standard Mixture—A mixture of the three standard materials in acetonitrile was made fresh each day and contained 10.0 μg/ml each of II and III and 1000 μg/ml of I.

Analysis of Pharmaceutical Dosage Preparations—For II or III determination, sample solutions were accurately prepared as indicated so that the final concentration of I, based on the declared potency, was 1 mg/ml. For I determination, sample solutions were accurately prepared so that the final concentration of I, based on the declared potency, was 100–250 μg/ml.

Injectables—A sample aliquot was diluted and made to volume with acetonitrile.

Tablets—Tablets were powdered, weighed, warmed over a steam bath in acetonitrile, and shaken vigorously for 1 min. The solution was allowed to cool and then was filtered and made to volume with acetonitrile.

Concentrates—An aliquot was diluted and made to volume with acetonitrile.

Syrup—An aliquot was mixed with acetonitrile, warmed over a steam bath, and shaken vigorously for 1 min. The solution was allowed to cool and then was filtered and concentrated under nitrogen to volume before injection.

Suppositories—A sample was warmed over a steam bath in acetonitrile until dissolution was complete. After cooling, the sample solution was filtered and made to volume with acetonitrile.

Detection—The HPLC eluates were monitored by the UV absorption at 345 nm and by fluorometry with excitation at 280 nm and emission at 385 nm for II and III or at 450 nm for I. All three compounds in approximately equal amounts can be simultaneously detected by fluorometry if the emission detection is set at 400 nm. Peak height was used for quantitation.

RESULTS AND DISCUSSION

HPLC Column—Polar bonded phases used in the normal mode frequently produce separations similar, but often more efficient, to those obtained on silica gel packings. One highly polar hydrogen bonded phase that is readily available commercially is a chemically bonded aminosilane. It is stable toward hydrolysis, can be used for normal or reversed-phase chromatography, and is less sensitive to water adsorption than is silica gel.

This material performed well in the normal phase HPLC separation of I and its two oxidation products, II and III (Fig. 1). Its unique selectivity and speed were amply demonstrated. Since these materials eluted rapidly, the expenditure of time and expensive organic solvents was greatly minimized.

HPLC Mobile Phase—Various isocratic and gradient mobile phase systems were tried before acetonitrile–benzene–water (16:4:1) was selected. Water-free mobile phases were undesirable since the elution of II was delayed considerably. They also resulted in a badly tailing peak. With the addition of water to the mobile phase, II rapidly eluted in a sharp and symmetrical peak. The amount of water added was carefully controlled as soon as it was optimized since varying the amount of water produced changes in the retention time of II. However, unlike silica gel, the amino-bonded packing was less sensitive to water adsorption, and lengthy equilibration was not required as the water content of the mobile phase was varied.

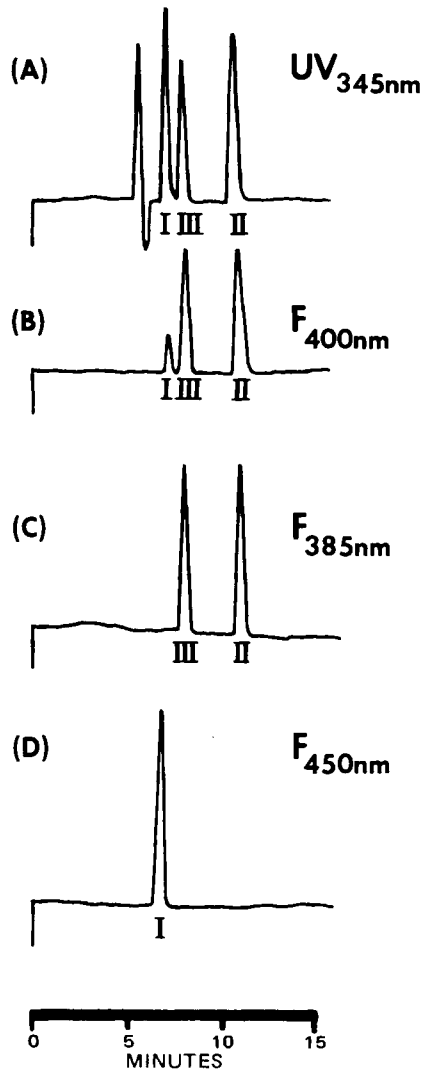

Figure 1—*Chromatograms of standard mixtures of chlorpromazine hydrochloride (I) and its two S-oxidation products, sulfoxide (II) and sulfone (III) (5 μg each). UV absorption was at 345 nm (A) and fluorescence emission was at 400 (B), 385 (C), or 450 (D) nm. The HPLC column was μBondapak NH_2, the pressure was 140 psi, and the flow rate was 1.0 ml/min.*

The inclusion of benzene in the mobile phase also was desirable to stabilize the retention time of II. Without benzene, II always eluted a little slower when injected in a mixture with I (a 100:1 ratio) and III. When the amount of benzene in the mobile phase was increased, the resolution of the three compounds greatly improved, although it took longer for a complete chromatographic run (*e.g.*, 12 *versus* 30 min). However, an increase in the amount of benzene limited the amount of water that could be mixed into the mobile phase. For instance, when benzene was increased from 20 to 50%, water had to be reduced to ~1% to keep the mobile phase homogeneous.

Selection of Fluorescence Detection Mode—Compound I displayed three UV maxima while II and III each had four UV maxima (12, 13). Since benzene was used as a component of the HPLC system, the absorbance below 280 nm of these phenothiazine derivatives was lost. However, this loss did not present problems since the compounds were monitored at wavelengths longer than 280 nm.

Simultaneous quantitation of I and its two oxidation products was possible with the aid of a UV detector only when all three compounds were present in approximately equal amounts and were completely free

[7] Fluorichrom with deuterium lamp source, Varian Associates, Palo Alto, Calif.
[8] Model 837 spectrophotometer, DuPont Instruments, Wilmington, Del.
[9] Model 3385A, Hewlett-Packard Co., Palo Alto, Calif.
[10] Model 285, Linear Instrument Corp., Irvine, Calif.
[11] Lichrosorb NH_2, Altex Scientific, Berkeley, Calif.
[12] μBondapak NH_2, Waters Associates, Milford, Mass.
[13] A 5.0-μm type LS filter, Millipore Corp., Bedford, Mass.

Table I—Analysis of Pharmaceutical Dosage Preparations [a]

Dosage Preparation	Declared Potency of Chlorpromazine Hydrochloride	Number of Samples Analyzed	Chlorpromazine Hydrochloride Content (Average), %	Chlorpromazine Sulfoxide Content, %
Tablet	10 mg/tablet	2	100	ND[b]
Suppository[c]	25 mg/suppository	4	99	ND
Syrup	10 mg/5 ml	4	95	ND
Injection, single dose (1 ml)	25 mg/ml	10	103	ND
Injection, multiple dose (10 ml)	25 mg/ml	2	102	ND
Concentrate (120 ml)	30 mg/ml	4	102	1.03, 0.69, 0.73, 0.90

[a] Chlorpromazine sulfone was not detected in any pharmaceutical dosage preparation tested. [b] ND = none detected. [c] Contained chlorpromazine.

from other UV-absorbing interferences. When I was present in a 100-fold excess, III was completely obscured by I. Furthermore, when such a large amount of I was chromatographed, other strong UV-absorbing impurities, although present in small amounts relative to the parent compound, became detectable and interfered with the determination of II.

Fluorescence detection is more selective and, therefore, better suited for this type of analysis. The differences in the fluorescence characteristics of I, II, and III were useful in developing this rapid HPLC procedure for their determination. To determine II and III, the fluorescence emission signal of I was completely eliminated by selectively choosing the emission signals of the oxidation products at 385 nm while I was similarly determined by switching to an emission wavelength of 450 nm. The impurities in I observed in its UV chromatogram were completely eliminated in its fluorescence chromatogram when the emission signal was set at 385 nm.

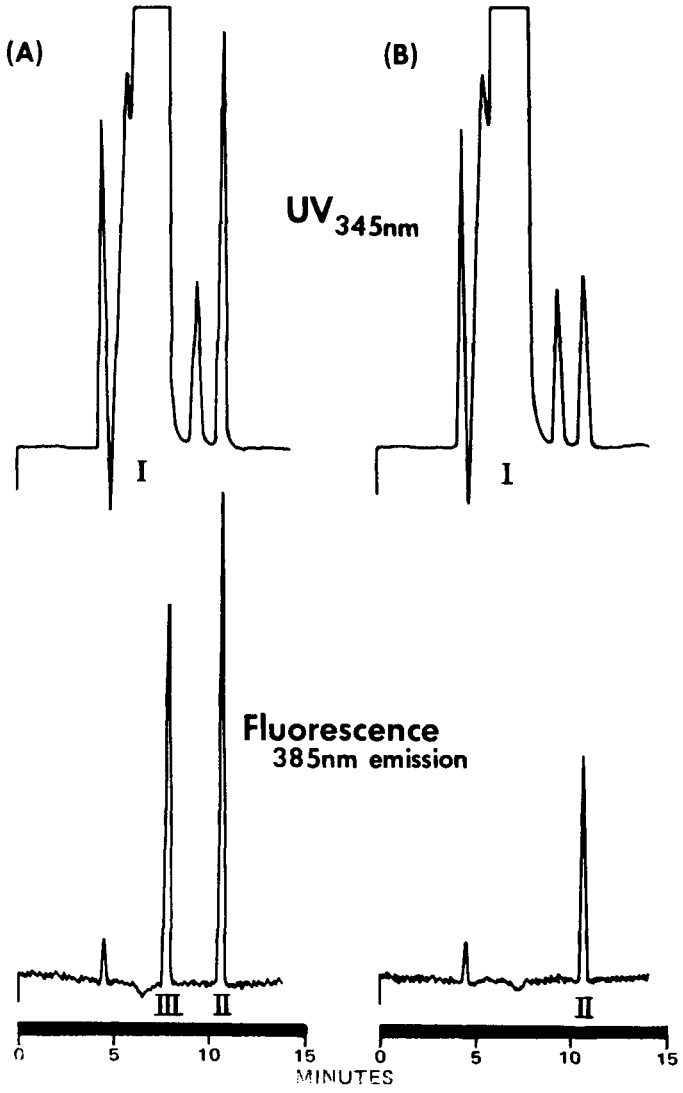

Figure 2—*Typical chromatograms of chlorpromazine hydrochloride (I) concentrate. Key: A, sample spiked with sulfoxide (II) and sulfone (III) at 1% (1 μg) level; and B, unspiked sample. The HPLC column was Lichrosorb NH_2, the pressure was 110 psi, and the flow rate was 1.0 ml/min.*

An alternative means of quickly and simultaneously identifying these three compounds was to determine selectively their fluorescence emission at 400 nm. At this wavelength, all three compounds could be detected if they were present in approximately equal amounts.

One problem encountered in the quantitative fluorescence determination of very low concentrations of phenothiazines is the interference from Raman scattering (3). This interference becomes particularly critical in the determination of II (6) or III. In the HPLC procedure described, a deuterium lamp source was used at 280 nm to lessen the Raman scatter influence. It also provided the stronger energy available at this wavelength.

The detection limit for UV and fluorescence modes remained about the same for I. For II and III, fluorescence was relatively more sensitive. However, unlike UV photometric detection, fluorescence detection is generally free from interferences caused by pump pulses and changes in the refractive index due to solvent peaks. Fluorescence detection also provides a convenient option of selectively eliminating all unwanted signals. All peaks obtained were well defined, sharp, and symmetrical and eluted within 15 min.

Linearity, Reproducibility, and Sensitivity—Fluorescence response curves of I–III were all linear in the ranges examined (1–50 μg for I and 0.1–5 μg for both II and III).

Good reproducibility of the described HPLC procedure using fluorescence detection was demonstrated when six 100-μl aliquots of a standard mixture containing 1.0 μg each of II and III in the presence of 100 μg of I were chromatographed. The relative standard deviations were 1.5 and 2.1% for II and III, respectively. This standard mixture was then diluted with acetonitrile so that each 100-μl injection represented 25 μg of I. The reproducibility of the method for I was determined by chromatographing six 100-μl diluted aliquots. The relative standard deviation was 1.2%.

The minimum detectable limit of I–III under the fluorescence conditions described was 0.05 μg for all three compounds. Under the sample conditions described, this limit represents, in most cases, a minimum detectable contamination level of 0.05% for II and III with respect to the parent compound.

Sample Analysis and Recovery—Various I formulations (Table I) were analyzed by this HPLC procedure using fluorescence detection. The UV detection mode was useless for III since it was completely obscured by the 100-fold amount of the parent drug. Furthermore, UV-absorbing impurities of the parent drug interfered with the determination of II. In addition, the UV detection mode was sensitive to changes in the refractive index of the solvent passing through it.

Table I indicates that, in almost all samples analyzed, neither II nor III was detected at the detection limit of 0.05%, even though the samples assayed were a few years old and were not representative of current formulations. (All samples were obtained during December 1975 and stored intact in the dark at room temperature. Samples were analyzed during November 1977.) The notable exceptions were the four samples of I concentrate that contained 0.7–1.0% of II. Figure 2 shows a typical chromatogram of I concentrate containing a low level of II.

All samples were also analyzed for I; the diluted aliquots of the sample solutions prepared for II and III assays were used. The same HPLC parameter was used throughout, except that the fluorometric detection was monitored at the 450-nm emission. All pharmacopeial samples were

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.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975.

(2) W. N. French, F. Matsui, D. L. Robertson, and S. J. Smith, *Can. J. Pharm. Sci.*, **10**, 27 (1975).

(3) S. Udenfriend, D. E. Duggan, B. M. Vasta, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **120**, 26 (1957).

(4) T. J. Mellinger and C. E. Keeler, *Anal. Chem.*, **35**, 554 (1963).

(5) *Ibid.*, **36**, 1840 (1964).

(6) J. B. Ragland and V. J. Kinross-Wright, *Anal. Chem.*, **36**, 1356 (1964).

(7) D. H. Rogers, *J. Chromatogr. Sci.*, **12**, 742 (1974).

(8) M. Caude, J. P. Lefebre, and R. Rosset, *Chromatographia*, **8**, 217 (1975).

(9) M. Caude, L. X. Phan, B. Terlain, and J. P. Thomas, *J. Chromatogr. Sci.*, **13**, 390 (1975).

(10) P. J. Twitchett and A. C. Moffat, *J. Chromatogr.*, **111**, 149 (1975).

(11) C. Gonnet and J. L. Rocca, *ibid.*, **120**, 419 (1976).

(12) R. J. Warren, I. B. Eisdorfer, W. E. Thompson, and J. E. Zarembo, *J. Pharm. Sci.*, **55**, 144 (1966).

(13) A. De Leenheer, *J. Assoc. Offic. Anal. Chem.*, **56**, 105 (1973).

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Impurities in Drugs IV: Indomethacin

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Received July 27, 1979, from the *Pharmaceutical Chemistry Division, Drug Research Laboratories, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada.* Accepted for publication September 20, 1979. *Present address: Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada.

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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