Table II—Determination of Me_{50} Values for Glycopyrrolate (6.5 $\mu g/kg$ iv)

	Intraocular Pressure, mm Hg	Inhibition, %	Arterial Blood Pressure, mm Hg	Inhibition, %
Mean preantispasmodic	12.0		85.00	
Methacholine, 5.55 $\mu g/kg$ iv	4.0	66.66	35.02	58.80
Methacholine, 11.10 $\mu g/kg$ iv	6.0	50.00	49.98	41.20
Methacholine, 16.65 $\mu g/kg$ iv	7.5	37.50	57.46	32.40
Me_{50} value (from graphs)	10.08 $\mu g/kg$			8.10 $\mu g/kg$
Me_{50} mean	9.95 + 2.14 (n = 9)			5.79 ± 1.61 (n = 16)
t = 5.489				

with a resultant thrust of the iris in the direction of the cornea, decreasing the chamber volume and drainage angle, together with a (possible) block of the pupillary opening preventing the dissipation of increasing chamber pressure is responsible for the methacholine-induced rise in intraocular pressure. This rise probably occurs only after intravenous injection of methacholine when the rapid forward thrust of the iris by the thickening of the lens, which appears responsible for it, is able to overcome for a time the tendency of the drainage system to offset it, a tendency soon realized in the speedy return of pressure to baseline following the postinjection rise. Subsequent investigation might involve another route of methacholine administration in an effort to determine the influence of the administration route on the response. It should also attempt to determine whether a dose of methacholine that is threshold for blood pressure reduction has any effect on intraocular pressure or, conversely, the extent to which methacholine might reduce blood pressure before it effects an increase in intraocular pressure.

The significance of this study involves the use of the parameter of intraocular pressure to determine the liability of a neurotropic antispasmodic (parasympatholytic) agent to induce cycloplegia. If a direct relationship exists between intraocular pressure and lens thickness, as this study indicates, then the extent to which the neurotropic antispasmodic interferes with a cholinergic-induced rise in intraocular pressure is a measure of its tendency to produce cycloplegia or blurring of vision. Hence, neurotropic antispasmodic compounds with relatively high intraocular pressure Me_{50}'s (in relation to their antimotility or antisecretory Me_{50}'s) should have a high liability with respect to the side effect of cycloplegia.

The study reported is a facet of a comprehensive investigation conducted over years. Numerous determinations of the effect of neurotropic antispasmodic agents on the intraocular pressure response to methacholine in the dog were made, and the data reported here are representative.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 24, 1976, from the *Massachusetts College of Pharmacy, Boston, MA 02115.*

Accepted for publication September 16, 1976.

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Impurities in Drugs I: Imipramine, Desipramine, and Their Formulations

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Abstract □ Nineteen lots of imipramine tablets and four lots of desipramine tablets were examined for impurities by TLC. Iminodibenzyl, desipramine, and 10,11-dihydro-5-[3-(methylamino-3′-dimethylaminopropyl)propyl]-5*H*-dibenz[*b,f*]azepine dihydrobromide (I) were found in some imipramine tablets, and iminodibenzyl and imipramine were found in some desipramine tablets, all at levels of less than 0.3% of label claim of the drug. Except for I, the identity of the impurities was established by comparison with known standards; I was synthesized and its composition was established by elemental analysis. All impurities, including I, were characterized by TLC, GLC, and mass spectrometry.

Keyphrases □ Imipramine tablets—impurities, TLC, GLC, and mass spectral analysis □ Desipramine tablets—impurities, TLC, GLC, and mass spectral analysis □ Impurities—in imipramine and desipramine tablets, TLC, GLC, and mass spectral analysis □ Tablets—imipramine and desipramine, TLC, GLC, and mass spectral analysis of impurities □ Dosage forms—tablets, imipramine and desipramine, TLC, GLC, and mass spectral analysis of impurities □ Antidepressants—imipramine and desipramine tablets, TLC, GLC, and mass spectral analysis of impurities

Organic compounds found as impurities in drugs and drug formulations may be intermediates or by-products of the drug synthesis, products of drug or excipient degradation, products of drug–excipient interaction, or the result of contamination. The nature of the impurities in a drug may depend on the synthetic process and the source of materials used in manufacture or the nature and source of the excipients. Impurities may be toxic, and their

Table I—Chromatographic Characteristics

Compound	TLC R_f	TLC Detectability Limit[a], μg	GLC Retention Time, min
Iminodibenzyl	0.81	0.06	3
Imipramine[b]	0.67	0.03	6.5
I	0.26	0.03	23
Desipramine[c]	0.36	0.03	8

[a] Applied with 200 μg of the drug. [b] As an impurity in desipramine. [c] As an impurity in imipramine.

presence may be indicative of inadequately controlled manufacturing or storage conditions.

To obtain a broadly based perspective of impurity levels and the potential problems associated with them, drug products and formulations from many different sources should be examined. This paper, the first of a series reporting the occurrence of impurities in drugs, describes a study of imipramine and desipramine formulations.

Imipramine hydrochloride is official in USP XIX (1) and BP 1973 (2, 3). Limits for iminodibenzyl, one of the starting materials, are set at 0.1% by the USP and at 0.2% by the BP. Tablets and injections are described in the USP, and tablets are described in the BP, but only the latter specifies a limit for iminodibenzyl (0.3%) in tablet formulations.

Desipramine hydrochloride is official in NF XIV (4) and in BP 1973 (2). The limits specified for iminodibenzyl are 0.1 and 0.2%, respectively. Tablets and capsules are described in the NF, and tablets are described in the BP. The latter sets a limit of 0.3% for the content of iminodibenzyl in tablets.

EXPERIMENTAL

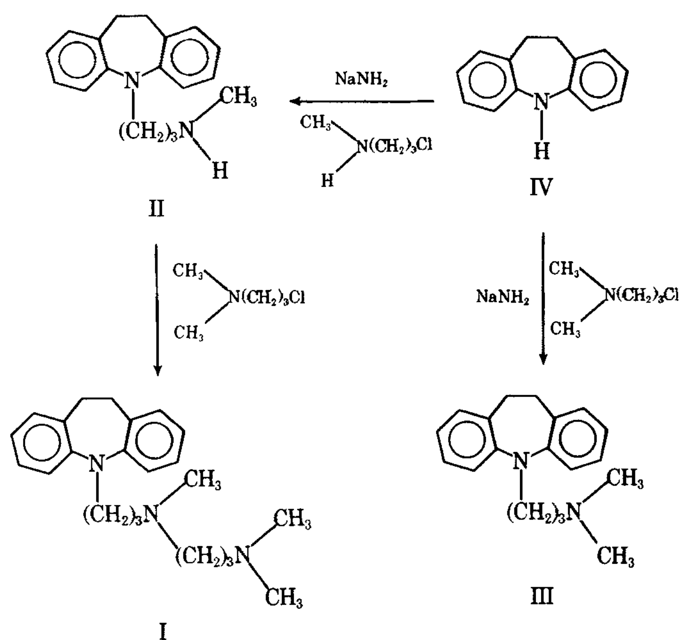
Materials—Imipramine hydrochloride and desipramine hydrochloride were the respective USP and NF reference standard materials. Nineteen lots of seven brands of imipramine tablets and four lots of two brands of desipramine tablets were obtained from the manufacturers. The other reagents used were redistilled chloroform[1], benzene[1], ethyl acetate[1], absolute ethanol[2], and dimethylaminopropyl chloride[3]. Macherey–Nagel TLC plates[4] were precoated with 0.25-mm silica gel G containing a 254-nm fluorescent indicator.

Standard Solutions—For imipramine studies, 56.5 mg of imipramine hydrochloride was dissolved in 2.0 ml of 0.1 *N* hydrochloric acid, made basic with 0.1 ml of concentrated ammonium hydroxide, and extracted by shaking into 5.0 ml of chloroform containing 0.15 mg of iminodibenzyl, 0.17 mg of desipramine hydrochloride, and 0.22 mg of 10,11-dihydro-5-[3-(methylamino-3′-dimethylaminopropyl)propyl]-5*H*-dibenz[*b*,*f*]-azepine dihydrobromide (I, Scheme I). The standard solution in chloroform was separated from the aqueous layer. The desipramine standard solution was prepared in a similar way by extracting 57.9 mg of desipramine hydrochloride into 5 ml of chloroform containing 0.15 mg of iminodibenzyl and 0.17 mg of imipramine hydrochloride.

Both reference standard drugs were shown, by the TLC system described in this paper, to be free of detectable impurities. Each standard solution contained 10 mg/ml of the free base form of the drug with the remaining compounds, also as the free bases, at 0.3% with respect to the drug.

TLC Solvent Systems—For imipramine, a system of benzene–ethyl acetate–absolute ethanol–concentrated ammonium hydroxide (50:50:15:3) was used. For desipramine, the proportions were 50:50:20:3, respectively. Both solutions were placed in filter paper-lined chromatographic tanks and allowed to equilibrate for 1 hr prior to use.

Tablet Extraction—Twenty tablets were weighed and finely powdered. An amount of drug equivalent to 50 mg of the free base was weighed into a 10-ml polytef-lined, screw-capped centrifuge tube. Then 2 ml of 0.1 *N* hydrochloric acid was added, and the tube was shaken vigorously for 15 min on a horizontal shaker. Concentrated ammonium hydroxide, 0.1 ml, and 5 ml of redistilled chloroform were added, and the tube was shaken for 20 min. A portion of the chloroform layer, containing the drug and impurities, was removed for analysis.



Scheme I

Screening for Impurities—The R_f of each drug and impurity in chloroform solution was determined in the appropriate TLC system. To check for possible decomposition, each compound was scraped from the plate and subjected to GLC and mass spectrometry. A portion of the eluate recovered from the gas chromatograph was rerun in the TLC system. The lower detection limit of each impurity was established by spotting serial dilutions of the appropriate stock solution.

Aliquots of 10 and 20 μl (100 and 200 μg) of the tablet extract in chloroform for each formulation were spotted beside 10- and 20-μl (100- and 200-μg) aliquots of the appropriate standard solution. Plates were developed, and the compounds were visualized using UV light at 254 nm and subsequently spraying with 0.5% (w/v) potassium dichromate in 20% (v/v) sulfuric acid. The concentration of impurities was approximated by comparison of the spot diameters and intensities with the corresponding spots due to the standard solutions.

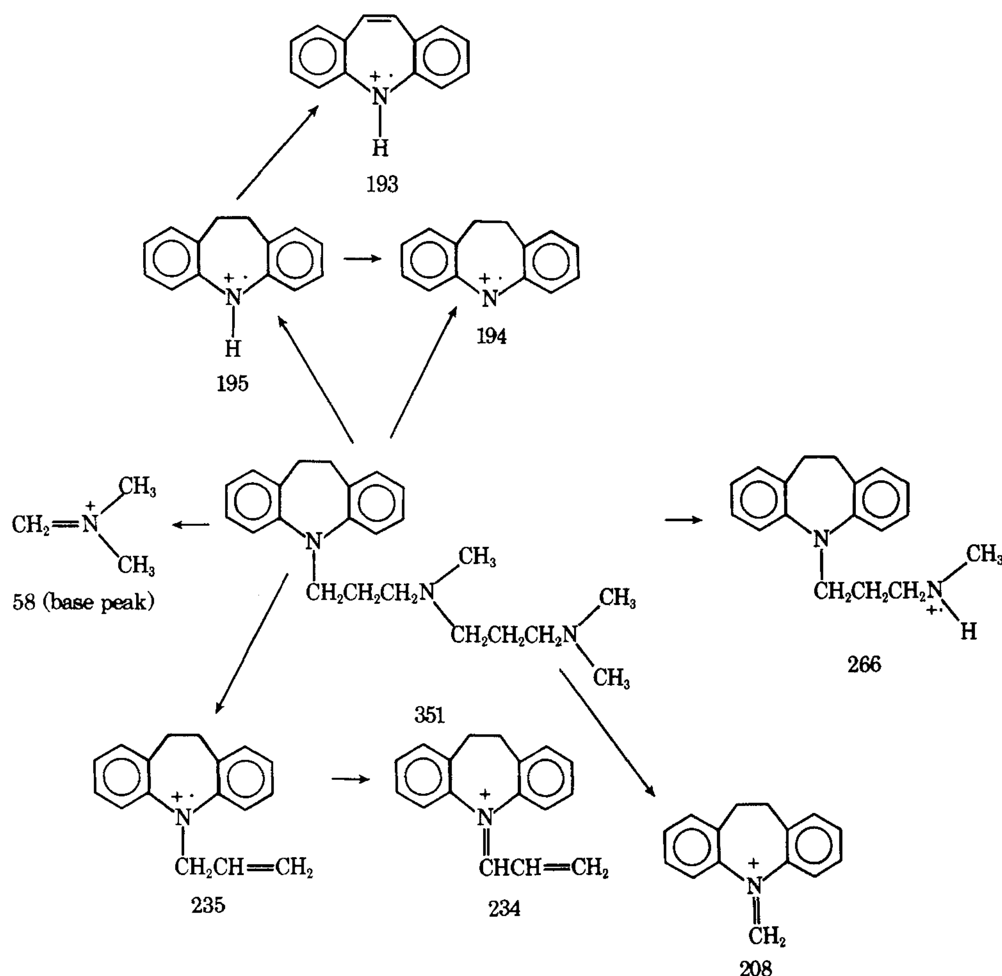
Isolation of Impurities—A tablet extract containing 40 mg of drug as the hydrochloride in 1.0 ml of chloroform was applied in a narrow band to a 0.25-mm TLC plate, 20 × 20 cm, and developed in the appropriate solvent system. Four such plates were prepared. Impurities were visualized by UV light at 254 nm and by spraying a 1-cm band along each side of the plate with 0.5% (w/v) potassium dichromate in 20% (v/v) sulfuric acid.

To reduce the possibility of decomposition during manipulation, the impurities were converted to the more stable hydrochloride form by spraying lightly with ethanolic hydrochloric acid, and the bands were scraped from the TLC plates. The scrapings were stirred with 25 ml of absolute ethanol for 60 min and then filtered, and the ethanol solution was evaporated to dryness on a rotary evaporator at room temperature. The purity of the isolated compounds was checked by TLC and GLC.

Samples of impurities for mass spectral analysis were isolated by GLC using a column of 5% OV-25 on 100–120-mesh Chromosorb W (HP). The injection port, detector block, and column temperatures were 250, 250, and 240°, respectively. The flow rates of nitrogen, hydrogen, and air were 40, 50, and 400 ml/min, respectively. Approximately 50 μg of drug from the TLC plate extract was injected into the chromatographic column.

Fractions were collected in dry ice-cooled capillary tubes held over the flame tip of the chromatograph. The hydrogen flame was extinguished just prior to elution of the required fraction and reignited after collection to burn off material condensed on the flame tip. TLC showed that each fraction consisted of a single component, was free from decomposition products, and was identical to the material originally removed from the TLC plate.

[1] Caledon Laboratories Ltd., Georgetown, Ontario, Canada.
[2] Consolidated Alcohols Ltd., Toronto, Ontario, Canada.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Brinkmann Instruments, Toronto, Ontario, Canada.



Scheme II

Synthesis—Dimethylaminopropyl chloride was obtained from its hydrochloride salt by triturating 16.0 g of the latter with 8.0 g of flaked sodium hydroxide (5). The free amine was decanted from the solid phase and distilled at 124–128°/160 mm Hg through a 5-cm vigreaux column.

Compound I was prepared by refluxing a mixture of 7.5 g of freshly distilled dimethylaminopropyl chloride and 0.83 g of desipramine (II) in 20.0 ml of dry acetone for 18 hr (Scheme I). The acetone and excess dimethylaminopropyl chloride were removed on a rotary evaporator. The residue was taken up in 10 ml of 0.1 *N* hydrochloric acid, washed twice with 50 ml of ether, made basic with 1.0 ml of concentrated ammonium hydroxide, and extracted with two 50-ml portions of chloroform. The chloroform was removed on a rotary evaporator at room temperature, and ethanolic hydrogen bromide was added to the residue. The resulting salt was crystallized by the addition of ether and dried at 56°/1 mm Hg. The crystalline melting point[5] was 218–221° (uncorrected).

Anal.—Calc. for $C_{23}H_{33}N_3 \cdot 2HBr$: C, 53.81; H, 6.87; N, 8.19. Found: C, 53.95; H, 6.97; N, 8.05.

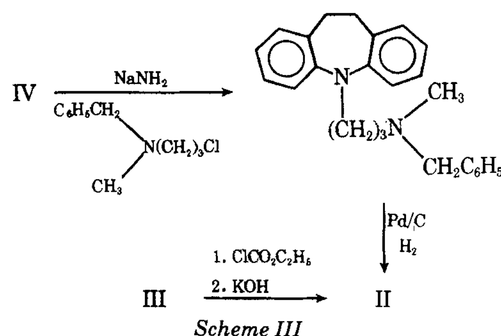
The PMR[6] spectrum (1% in deuterochloroform, 40°) exhibited distinctive signals downfield from trimethylsilane at 131 Hz due to the three-proton *N*-methyl singlet and at 138 Hz due to the six-proton *N,N*-dimethyl singlet, but the signal at 73.5 Hz due to the NH proton in desipramine was not observed. The mass spectrum[7] was obtained by direct probe in the electron-impact mode at 70 ev and 160°. Attempts to prepare the crystalline hydrochloride salt were unsuccessful.

RESULTS AND DISCUSSION

A single chloroform extraction was sufficient for complete removal of imipramine and desipramine from aqueous tablet powder mixtures. No drug was found in the chloroform fraction following a second extraction of the aqueous tablet powder mixture. In addition, the diameters and intensities of the TLC spots from tablet extracts were identical to those from standard solutions of the hydrochlorides at corresponding concentrations. No degradation of the drug or its impurities was observed when the free bases were applied at 0.5-hr intervals over 3 hr. The TLC solvent system was chosen to maximize separation of the impurities. The toluene–methanol (95:5) system described in BP 1973 (2) is adequate only for iminodibenzyl.

The identity of iminodibenzyl, imipramine, and desipramine found in tablet formulations was established by comparison of the TLC R_f values, GLC retention times, and mass spectral fragmentation patterns with those of known samples. Tablet impurities for GLC were recovered from TLC plates, and GLC eluates were collected for mass spectrometry and TLC, as previously described. The purity of the standard compounds was authenticated by subjecting them to the TLC, GLC, and mass spectral treatment.

The structure postulated for I is in agreement with the elemental analysis and the PMR spectrum. It is further supported by the mass spectral results, which conformed to the fragmentation scheme presented



Scheme III

[5] Thomas-Hoover capillary melting-point apparatus.
[6] Varian A-60A NMR spectrometer.
[7] Hitachi Perkin-Elmer RMU 6L mass spectrometer.

Table II—Impurities in Imipramine Tablets

Brand	Dose, mg	Impurities[a], %			
		Iminodibenzyl	Desipramine	I	Other
A	10	0.2	0.3	0.25	ND[b]
	25	0.05	0.1	0.10	ND
B	10	0.1	0.1	0.05	R_f 0.15; Tr[c]
	25	0.2	0.1	0.1	R_f 0.15; Tr
	50	0.2	0.2	0.2	ND
C	10	0.2	0.2	0.1	R_f 0.30; Tr
	25	0.05	0.05	0.05	ND
	50	0.05	0.1	0.1	R_f 0.30; 0.3%
	75	0.1	0.1	0.1	R_f 0.30; Tr
	150	0.2	0.1	ND	ND
D	25	ND	0.1	0.05	ND
	50	0.1	0.25	ND	ND
E	10	0.1	0.2	ND	R_f 0.30; 0.3%
	25	ND	0.2	ND	R_f 0.30; 0.2%
	50	0.1	0.15	ND	ND
F	10	0.1	0.2	0.2	ND
	25	0.1	0.2	0.2	ND
G	10	0.1	0.3	0.1	ND
	25	0.1	0.3	0.3	ND

[a] Expressed as percentage of the label claim of drug in the free base form. [b] None detected. [c] Trace.

in Scheme II. Detectability limits and R_f values by TLC and retention times by GLC for imipramine, desipramine, iminodibenzyl, and I are given in Table I.

Nineteen lots of imipramine tablets from seven manufacturers were tested for impurities (Table II). All lots contained desipramine at levels from 0.1 to 0.3%, and all but two contained iminodibenzyl at levels from 0.05 to 0.2%. Compound I was found in 14 lots in amounts to 0.3%. The occurrence of desipramine (II) and I in imipramine (III) formulations may be due to the presence of monomethylaminopropyl chloride in the dimethylaminopropyl chloride used as a starting material in at least one synthetic process (6) (Scheme I). Iminodibenzyl (IV) is used as a starting material, but its presence may also result from decomposition of the drug (7).

An impurity at R_f 0.30 was observed in five lots at levels from trace to 0.3%, on the basis of the sensitivity established for imipramine. This impurity may be the 10-hydroxy analog of imipramine suggested by Adank and Hammerschmidt (7), but attempts to isolate and identify the compound were unsuccessful. Trace amounts of an impurity at R_f 0.15 were observed in two lots, but no attempt at isolation and identification was made.

Each of four lots of two brands of desipramine contained iminodibenzyl, and three of the four contained imipramine, all at levels below 0.15% (Table III). Iminodibenzyl is a synthetic precursor (8) (Scheme III) and may also result from breakdown of the drug (9); the source of imipramine may be due to the residual material remaining if imipramine is demethylated *via* the chloroformate ester to produce desipramine (10). The level of iminodibenzyl in all formulations tested was below the limit of 0.3% specified for tablets in BP 1973, even though some formulations were manufactured to specifications other than those of the BP.

Table III—Impurities in Desipramine Tablets

Brand	Dose, mg	Impurities[a], %	
		Iminodibenzyl	Imipramine
A	10	0.15	0.15
A	25	0.15	0.15
A	50	0.15	0.15
B	25	0.1	ND[b]

[a] Expressed as a percentage of the label claim of drug in the free base form. [b] None detected.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 9, 1976, from the *Drug Research Laboratory, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada.*

Accepted for publication September 14, 1976.

The assistance of Mr. H. Avdovich and Dr. A. By in obtaining the PMR and mass spectra is gratefully acknowledged.

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