GLC Method for Iminodibenzyl and Desipramine Impurities in Imipramine Hydrochloride and Its Formulated Products

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Abstract □ A GLC method is described for the determination of iminodibenzyl and desipramine impurities in imipramine hydrochloride and its formulated products. These impurities were extracted from an alkaline solution with a mixture of 30% methylene chloride in hexane for chromatography on a 3% OV-17 GLC column. Iminodibenzyl was determined using anthracene as an internal standard and desipramine was determined (after derivatization) using nortriptyline as an internal standard. Based on spiked excipient mixtures typically used to compound imipramine tablets, recoveries were 93–109% for iminodibenzyl and 93–107% for desipramine at 0.2–0.4% of the labeled claim of imipramine. Minimum detection levels were ~0.02% for each impurity, and procedural standards gave coefficients of variation of <1% for each impurity. The method was linear in the 0.05–0.5 μg range and typically gave correlation coefficients ≥0.999.

Keyphrases □ Imipramine—GLC method for determining iminodibenzyl and desipramine impurities, drug substance and formulated products □ GLC—determination of iminodibenzyl and desipramine impurities in imipramine and its formulated products □ Antidepressants—GLC determination of iminodibenzyl and desipramine impurities in imipramine and its formulated products □ Desipramine—as an impurity in imipramine, determined by GLC

A determination of the kinds and amounts of organic impurities in drugs and drug formulations is a measure of both product stability and good manufacturing practices. Impurities may be present as by-products of synthesis, inadequate purification after synthesis, or from decomposition due to improper storage and handling.

It would be highly impractical to analyze for all of the possible organic impurities that might be present in imipramine and its formulated products. The present study determined which imipramine impurities might be indicators of both product stability and good manufacturing practices and developed an analytical system capable of measuring one or more of these impurities.

Several investigators have reported analytical procedures for imipramine and its related compounds using techniques such as GLC (1–6), GC–mass spectrometry (7–10), TLC (11–15), HPLC (16–20), and spectrophotometric methods (21–24). However, these reports primarily applied to the analysis of imipramine and its biological metabolites rather than the analysis of impurities in the drug substance.

Other investigators have reported impurities in imipramine and its formulated products by TLC. Adank and Hammerschmidt (25) reported the presence of eight impurities in commercial imipramine drug substance with total impurities < 0.2% and no single entity > 0.05%. The presence of similar amounts and kinds of impurities in commercial clomipramine (a compound structurally related to imipramine) was also reported (26). McErlane *et al.* (27) performed a study on impurities in imipramine, desipramine, and their formulations. When 19 lots of imipramine tablets from seven manufacturers were tested, five impurities were detected. Two of the major impurities were iminodibenzyl and desipramine at levels of up to 0.3% of the labeled claim of the drug.

A recent study (28) reported relatively high levels of impurities in imipramine samples taken from a hospital pharmacy in Richmond, Virginia. Iminodibenzyl levels of up to 2.8% and desipramine levels of up to 3.2% by GC–mass spectrometry techniques were reported. These results appear high when compared with this and previous studies (25–27).

The present report describes a GLC procedure for the quantitative determination of two major imipramine impurities, iminodibenzyl and desipramine, which appear to be indicators of product stability and good manufacturing practices.

EXPERIMENTAL

Materials—Desipramine hydrochloride, nortriptyline hydrochloride, and iminodibenzyl were the respective USP or NF reference standards. Reagent grade anthracene[1] was used as received. All other reagents and solvents were analytical reagent grade.

Apparatus—Analyses were performed on a gas chromatograph[2] equipped with a flame ionization detector and a strip chart recorder[3]. Air and hydrogen flow rates were set to maximize the detector response. The amplifier sensitivity settings were generally 3–6 × 10^{-10} amps full scale.

Column—A 1.8-m (6 ft) × 2-mm glass column packed with 3% OV-17 on 100–120 mesh Gas Chrom Q was conditioned overnight at 260° with nitrogen flow. The column temperature was maintained at 190° for the iminodibenzyl determination and 240° for the desipramine determination. The injection port and detector temperatures were held at 250°. Nitrogen was used as the carrier gas at 40 ml/min.

Edetate Disodium Solution—Four grams of ACS edetate disodium was dissolved in 50 ml of 1.5 *N* NaOH.

Internal Standard Solution—Approximately 4 mg of USP nortriptyline hydrochloride reference standard and ~2.5 mg of reagent grade anthracene were dissolved in 60 ml of methylene chloride. This was diluted to 200 ml with hexane and mixed thoroughly.

Standard Preparation—Approximately 2.5 mg each of USP reference iminodibenzyl and desipramine standards (accurately weighed) were dissolved in methanol and diluted to volume in a 5-ml volumetric flask (prepare fresh daily). Aliquots (100, 200, and 300 μl) of the standard preparation were then transferred to separate 15-ml test tubes fitted with polytef-lined screw caps. Each was evaporated to dryness using a nitrogen stream and gentle heat.

Procedure—A portion of imipramine hydrochloride drug substance, tablets or injection, equivalent to ~50 mg of imipramine hydrochloride was accurately weighed and transferred to a 15-ml test tube with a polytef-lined screw cap. The internal standard solution (5 ml) and 5 ml of the edetate disodium solution were added (for the injection, substitute 2 ml of 10% NaOH plus 400 mg of edetate disodium). The solution was shaken vigorously for 5 min and centrifuged until the upper organic layer was clear. A 4–4.5 ml portion of the upper layer was transferred to a tapered 15-ml centrifuge tube and evaporated to 0.5–1 ml using nitrogen and gentle heat. To equilibrate the system, 1–2 μl of this sample extract was injected into the gas chromatograph three or more times prior to quantitation.

[1] Eastman Kodak Co., Rochester, N.Y.
[2] Perkin-Elmer Model 900.
[3] Perkin-Elmer Model 56.

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Table I—Tabulation of Recoveries on Synthetic Tablet Formulations [a]

Manufacturer/ Number		μg, Iminodibenzyl Added per 50 mg Imipramine	%, Iminodibenzyl Recovered	μg, Desipramine Added per 50 mg Imipramine	%, Desipramine Recovered
I	1	100	102	100	93
	2	100	99	100	104
	3	150	104	150	94
	4	200	100	200	100
	5	200	104	200	104
II	1	150	102	150	102
	2	150	102	150	104
	3	150	97	150	104
	4	150	109	150	104
	5	150	101	150	105
III	1	100	94	100	104
	2	100	97	100	105
	3	150	98	150	107
	4	200	97	200	105
	5	200	98	200	107
IV	1	100	93	100	101
	2	100	98	100	102
	3	150	102	150	101
	4	200	99	200	102
	5	200	98	200	106
V	1	100	99	100	105
	2	100	102	100	100
	3	150	102	150	101
	4	200	99	200	103
	5	200	99	200	104
Mean			99.8		102.7
SD			3.4		3.4

[a] Excipient mixtures typically used to compound imipramine tablets.

The peak height ratios of iminodibenzyl to anthracene internal standard *versus* concentration for the calibration curve was plotted.

After the iminodibenzyl determination, 0.5 ml of methylene chloride and 5–7 drops of reagent grade acetic anhydride were added to the sample and standard extracts. This solution was mixed thoroughly and allowed to stand at room temperature for ~5 min. Then, 0.5 ml of methanol was added, mixed, and evaporated to dryness using a nitrogen stream and moderate heat (steam bath). The residue was dissolved in ~1 ml of 30% methylene chloride in hexane.

The GC column was maintained at 240° for the desipramine calibration curve, as previously described for iminodibenzyl, using peak height ratios of desipramine to nortriptyline internal standard.

RESULTS AND DISCUSSION

The TLC systems reported previously for imipramine and its impurities (25–27) showed good separations and adequate sensitivities for all the compounds when applied to an examination of imipramine drug substance and some commercial products. Eight impurities were detected at levels of ≤0.3% based on spot size and intensity. The predominant impurities were shown to be iminodibenzyl and desipramine. Additional examination of some commercial products by TLC, GLC, and GC–mass spectrometry in this laboratory did not reveal any impurities other than those reported previously.

Preliminary decomposition studies were conducted to determine the conditions necessary for the formation of iminodibenzyl and desipramine impurities in imipramine drug substance. Imipramine was heated at 100° in 0.1 *N* HCl for 3 hr with a current of air flowing over the surface. This treatment was followed by a 15-min exposure to long-wave UV light. TLC examination of the treated imipramine drug substance, compared to a nontreated sample, revealed a significant increase in both iminodibenzyl and desipramine content based on R_f values and spot intensity. The chromatogram of the treated sample was similar to that of a nontreated commercial imipramine tablet in that at least eight different known decomposition products were observed.

In another decomposition study, pure (by TLC) imipramine free base was exposed to normal laboratory fluorescent lighting and/or air for 72 hr on a silica gel TLC plate. At the end of this exposure period, the imipramine was extracted with methanol to be rechromatographed. The

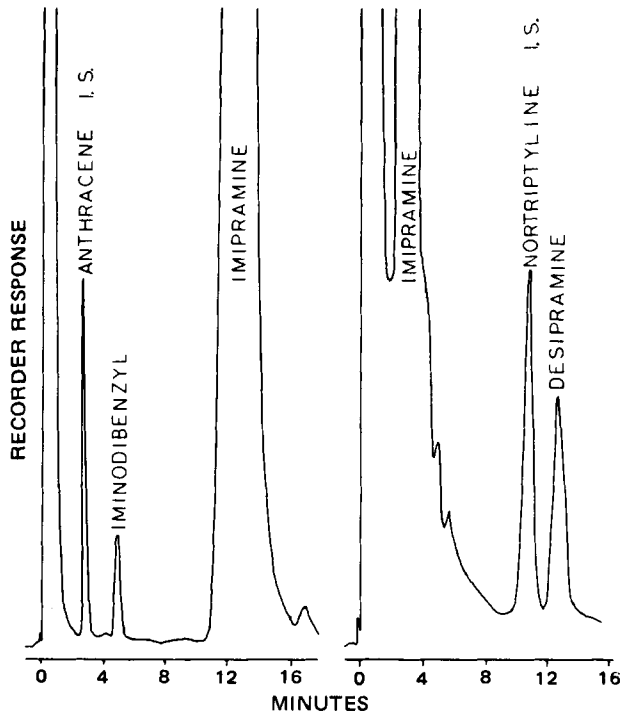

Figure 1—*Gas chromatogram of a commercial imipramine hydrochloride tablet extract at a column temperature of 190° prior to derivatization (left). The chromatogram on the right is the same extract at a column temperature of 240° after derivatization.*

imipramine exposed only to fluorescent light showed no discoloration and indicated only a trace of an impurity at the same R_f value as that of desipramine. The imipramine exposed to both the fluorescent lighting and air showed considerable discoloration and five additional spots on TLC. The predominant spots were those corresponding in R_f value to iminodibenzyl and desipramine. Although the presence of both iminodibenzyl and desipramine was confirmed by GC–mass spectrometry, the predominant compound present at the R_f value for iminodibenzyl had a molecular weight of 240 and is currently unidentified. Because chromatographically pure imipramine free base was used, it would appear that the unidentified compound is definitely related to imipramine. Interestingly, no compound with a molecular weight of 240 was detected in any of the commercial products examined in this study.

The decomposition studies indicated that there is a probability of some type of decomposition occurring in imipramine if adequate control of heat, light, air, and moisture is not used during commercial product formulation. In addition, iminodibenzyl and possibly desipramine may be present as part of these decomposition products.

The GLC procedure used offers comparable sensitivity to TLC (~0.02–0.04% impurities based on the labeled claim of imipramine) and considerable improvement over TLC in accuracy and precision. The method is linear in the 0.05–0.5 μg range and standard curves typically give correlation coefficients of ≥0.999. Procedural standards gave coefficients of variation of <1% for each impurity. The uncertainty of quantitation of impurities by TLC using spot size and intensity was estimated to be ±30%.

Two commercial tablet products were encountered which consistently gave low iminodibenzyl and desipramine recoveries. Further study revealed that calcium salts were present as high percentage excipients in the two products in question. Additional recovery studies using only iminodibenzyl and desipramine standards and the calcium salts revealed that calcium was indeed interfering with the assay. Several techniques such as multiple extractions, pH adjustments, and salting out were all used without success. Edetate disodium was finally added to complex the calcium and this provided successful assay results.

Excipient mixtures used to compound imipramine tablets from five different manufacturers were spiked with iminodibenzyl and desipramine at three concentration levels (Table I) and assayed in quintuplicate as a measure of precision and accuracy. Recoveries for iminodibenzyl ranged from 93 to 109% with a mean of 99.8% and a standard deviation of 3.4%. Recoveries for desipramine ranged from 93 to 107% with a mean of 102.7% and a standard deviation of 3.4%. A typical chromatogram of a com-

Table II—Impurities in Imipramine Tablets, Injection, and Drug Substance [a]

Brand	Product	Strength	Iminodibenzyl, %	Desipramine, %
A	a	50 mg	0.47	0.13
	a	25 mg	0.35	0.15
	a	10 mg	013	0.13
B	a	50 mg	0.05	0.05
	a	25 mg	0.05	0.09
	a	10 mg	0.18	0.13
C	a	50 mg	0.08	0.16
	a	25 mg	0.10	0.09
	a	10 mg	0.22	0.11
D	a	50 mg	0.04	0.11
	a	25 mg	0.07	0.09
E	a	50 mg	0.08	0.07
	a	25 mg	0.05	0.06
F	a	50 mg	0.08	0.06
	a	25 mg	0.04	0.05
	a	10 mg	0.07	0.09
G	a	50 mg	0.37	0.12
H	b	12.5 mg/ml	0.08	0.07
I	c	N/A	0.06	0.10
J	c	N/A	0.03	0.08

[a] Product a, tablet; product b, injection; and product c, drug substance.

mercial tablet extract shown in Fig. 1 demonstrates that adequate separation is achieved for all compounds. The increased retention time of the desipramine derivative is apparent from the relative locations of imipramine in each chromatogram.

The reactions of desipramine and nortriptyline with acetic anhydride to form their respective derivatives appear to be quantitative within 5 min based on the absence of underivatized desipramine and nortriptyline peaks in the gas chromatogram. Iminodibenzyl does not form the derivative under the conditions used in the method, nor with moderate heat (~100°).

No interfering GLC peaks were detected in any commercial products at the retention times of the anthracene and nortriptyline internal standards.

A tabulation of iminodibenzyl and desipramine levels found in some commercial products is shown in Table II. Wide variations in impurity levels were found for the various products and manufacturers. There are currently no USP limits for either iminodibenzyl or desipramine in tablets or injections and only a limit of 0.1% of iminodibenzyl in imipramine drug substance USP. Since imipramine USP was used in the manufacturing of the products studied, some decomposition must have occurred either in the manufacturing process or because of instability of the finished product. Interestingly, product C was found to contain more iminodibenzyl (0.37%) 3 years prior to its expiration date, whereas product A (50 mg) contained only a small amount of iminodibenzyl (0.08%) within 3 months of its expiration date.

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