Analytical Profiles of Drug Substances

Volume 6

Edited by

Klaus Florey

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PREFACE

Although the official compendia list tests and limits for drug substances related to identity, purity, and strength, they normally do not provide other physical or chemical data, nor do they list methods of synthesis or pathways of physical or biological degradation and metabolism. For drug substances important enough to be accorded monographs in the official compendia such supplemental information should also be made readily available. To this end the Pharmaceutical Analysis and Control Section, Academy of Pharmaceutical Sciences, has undertaken a cooperative venture to compile and publish Analytical Profiles of Drug Substances in a series of volumes of which this is the fifth.

The concept of analytical profiles is taking hold not only for compendial drugs but, increasingly, in the industrial research laboratories. Analytical profiles are being prepared and periodically updated to provide physicochemical and analytical information of new drug substances during the consecutive stages of research and development. Hopefully, then, in the not too distant future, the publication of an analytical profile will require a minimum of effort whenever a new drug substance is selected for compendial status.

The cooperative spirit of our contributors has made this venture possible. All those who have found the profiles useful are earnestly requested to contribute a monograph of their own. The editors stand ready to receive such contributions.

Klaus Florey

CONTENTS

AMPHOTERICIN B

Irvin M. Asher
George Schwartzman
and the
*USASRG**

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The USASRG was formed at the request of P. Weiss, the National Center for Antibiotic Analysis, FDA, and is presently coordinated by the Office of Science, FDA. Individual contributions are referenced where possible.

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1. DESCRIPTION

1.1 Drug Properties

Amphotericin B is a macrocyclic, polyene antibiotic produced by streptomycetes nodosus (M4-575). It was originally isolated from a soil culture from the Orinoco River region, Venezuela (1). Used topically as a cream, or parenterally as a Na-desoxycholate suspension (Fungizone), it is effective against a broad variety of fungi and yeasts, and some protozoans (1-3; see Section 8).

The possibility that Amphotericin B combines with

cholesterol to form ion-transporting channels across cell membranes is being widely investigated (4-6). The absence of membrane sterols would thus explain the inability of Amphotericin B to affect bacterial growth.

In canine experiments (7), orally administered Amphotericin B induced a 20-45% reduction in serum cholesterol, suggesting a possible future role as a hypocholesterolemic agent. Amphotericin B has also been used (8) to treat canine prostatic hyperplasia (~ 30% reduction in gland size). However, the toxicity of the bile salt complex (9,10) may discourage such applications in humans. Work on less toxic derivatives is underway (3). In mice, intraperitoneal LD_{50} is 280 mg/kg for Amphotericin B (3,11), 88 mg/kg for Fungizone and 1320 mg/kg for the methyl ester. The corresponding intravenous dosages are over an order of magnitude lower (3).

1.2 Chemical Properties

Amphotericin B is an amphoteric, macrocyclic heptaene with a mycosamine sugar head group. It yields a volatile base in concentrated NaOH and can bleach $KMnO_4$ or Br_2-CCl_4 (1). Its original separation was based on its solubility properties (1; see Section 6).

Amphotericin B is a particularly difficult antibiotic to characterize analytically. It is insoluble in many solvents (Section 2.3). Vibrator grinding dramatically affects X-ray powder diffraction patterns (Section 2.2) and infrared absorption spectra (Section 3.2).

pH dramatically affects ORD and specific rotation (Section 3.4). H_2O or CO_2 (or both) may be associated with the lattice (Section 1.4). Such contingencies have led to irreproducible results and conflicts in the literature. This report tries to analyze some of the pitfalls, but considerable caution (and often ingenuity) is still required for a meaningful analysis.

1.3 The U. S. Standard

The current U. S. antibiotic standard (Ampho. B-2; 11/27/74) was obtained from Squibb which markets the drug under the name Fungizone. The final stages of manufacture include precipitation from aqueous methanol (pH controlled by HCl then NaOH), washing with acetone, drying, and forcing through a sizing screen. The standard is stored in lots of 250 mg at -20°C, protected from light and moisture. Samples were dried for 3 hours at 60°C ($\leq$ 5 mm pressure) before measuring potency, ultraviolet absorption, or specific rotation. There is also an Amphotericin B-1 (Amphotericin B-2 further recrystallized with various solvents and salts) for which no U. S. standard exists; it is not further

considered here. There is also an international standard (WHO) for Amphotericin B (12).

1.4 Chemical Composition

1.41 Empirical Formula and Molecular Weight ($^{12}C = 12.000$)

(a) $C_{47}H_{73}NO_{17}$
MW = 923.62

in agreement with recent x-ray (13) and mass spectrometric (14) measurements; accepted by USP-XIX (15), supersedes:

(b) $C_{46}H_{73}NO_{20}$
MW = 959.62

reported in Reference (11,16).

1.42 Elemental Composition

(a) $C_{47}H_{73}NO_{17}$ requires:

C 61.12% H 7.96% N 1.52% O 29.45%

Reference 1 found:

C 60.40% H 8.38% N 1.62% --

with negative results for halogens, sulfur, and acetyl and methoxyl groups, for samples prepared by the methods of Reference 1.

(b) $C_{46}H_{73}NO_{20}$ requires:

C 57.58% H 7.67% N 1.46% O 33.34%

and Reference 17 found:

C 57.17% H 7.80% N 1.20% O 29.98%

for untreated U.S. standard Amphotericin B, consistent with the CHN results of References 18,19. (In the latter Amphotericin B was dried 3 hours at 80°C prior to analysis.) Other measurements (20) on dried samples of the U.S. standard (3 hours, 60°C) gave results (C 59.61%, H 8.32%, N 1.43%) closer to those of Reference 1.

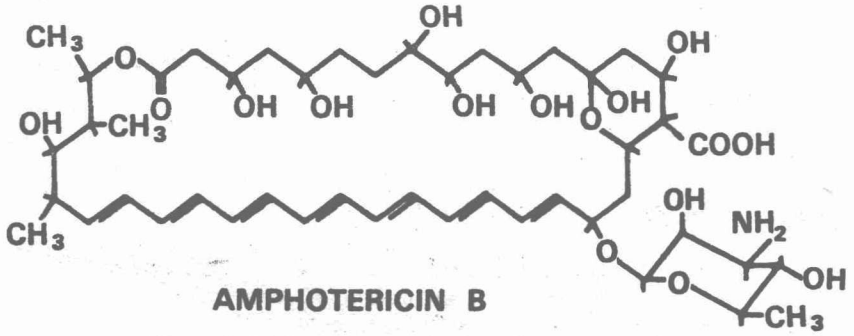
Notice that the oxygen content of Reference 17 is consistent with 1.41(a) rather than 1.41(b).

The full CHNO analysis of Reference 17 is consistent with the hydrochloride salt of 1.41(a) plus 1.5 waters of hydration. (Variation in water content alone can only partially resolve the discrepancies noted above.) However, tests (20) for Cl in the U.S. standard were negative ($\leq$ 0.11%).

(c) The Karl Fisher test gave 6.36% water content for the untreated U.S. standard (21). The standard exhibits a 4-5% loss on drying at 60°C under a vacuum. At atmospheric pressure, thermal gravimetric analysis (Section 2.12) indicates an $\sim$ 3.5% weight loss between 60-100°C. Although some of this water may be adsorbed, some appears to be incorporated into the lattice; the Amphotericin B derivative investigated in Reference 13 incorporated three tetrahydrofuran molecules and one water molecule per unit cell.

1.5 Structure

The following structure is based on x-ray crystallographic studies of N-iodoacetyl Amphotericin B, tri-tetrahydrofuran monohydrate crystal (13). It corresponds to formula 1.41(a).



AMPHOTERICIN B

The rigid heptaene chain elongates the macrocycle, such that one side (polyene) is hydrophobic, while the other side (aliphatic) is hydrophillic due to the presence of seven hydroxyl groups and an ester carbonyl group. This may account for its ability to act as an ion-channel in membranes (4-6). A mycosamine residue is attached to one end, providing a free amino group. There is an internal hemi-ketal ring. It has been suggested (14) that the ketal-form may be in equilibrium with an open keto-form in solution. However, recent ^{13}C-NMR results (22) confirm the presence of the ketal-form in DMSO solution (Section 4.2), and provide no evidence for a keto-form in that environment.

This structure supersedes an earlier, partial structure by Cope, et al., (23) which is incorrect in several details.

1.6 Physical Description

Bright yellow powder. Microscopic examination reveals prisms or needles for samples freshly recrystallized from dimethylformamide (11); but thin, irregular fragments (roughly 5-15 μ long, less than 0.3 μ thick) in the U.S. standard (25). The fragments tend to clump into large (~ 80 μ diameter) clusters. The grinding process used in drug manufacture may also convert some crystals to an amorphous form (24; Section 2.2). A typical photomicrograph of the standard is shown in Figure 1.

2. PHYSICAL PROPERTIES

2.1 Thermal Properties

2.11 Differential Thermal Analysis (DTA)

DTA scans (25) show a gradual, approximately linear decrease from 35 to 135°C with peaks near 157 and 209°C (Figure 2). The sample begins to decompose above 200°C, without melting. The 157°C transition is accompanied by a change in color from bright yellow to brown-orange which begins around 130°C, and increases progressively. This presumably reflects an endothermic chemical change involving the chromophore.

2.12 Thermal Gravimetric Analysis (TGA)

TGA scans (25) show an ~ 3.5% weight loss starting below 65°C which reaches completion near 90°C (Figure 2). A further reduction in weight begins near 180°C and levels off near 220°C, with maximum slope near 205°C. These changes may reflect loss of residual solvent and decomposition respectively.

2.13 Melting Point

We find no evidence of the melting in Amphotericin B up to 250°C, at which temperature the antibiotic has already decomposed. This is consistent with Reference (1), but perhaps not Reference (16,18). Vaporization is detected (26) above 250°C in a mass spectrometer (vacuum $< 10^{-5}$ torr). Trimethylsilyl-ether derivatives of Amphotericin B may vaporize as low as 180°C (26).

2.2 X-Ray Powder Diffraction

The X-ray powder diffraction pattern of "untreated" (unground, unheated) U.S. standard Amphotericin B demonstrates definite crystalline structure. The observed d-spacings are given in Table 1 and Figure 3 (solid curve). Unground samples heated 15 minutes at 158°C produce a pattern with less intense peaks, slightly shifted d-spacings and increased background (Figure 3, dotted curve). These

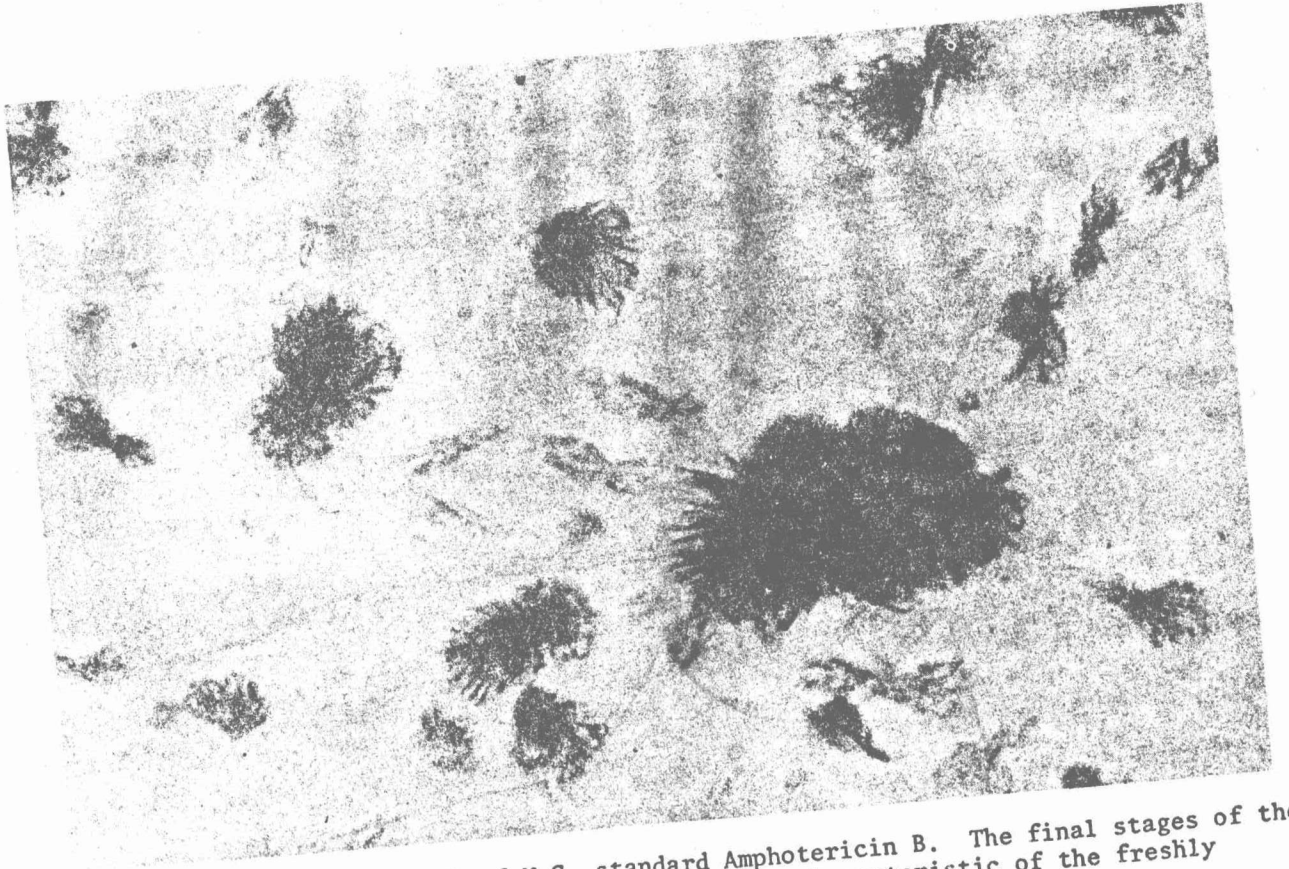


Figure 1. Photomicrograph (x100) of U.S. standard Amphotericin B. The final stages of the manufacturing process break the thin needles characteristic of the freshly recrystallized antibiotic.

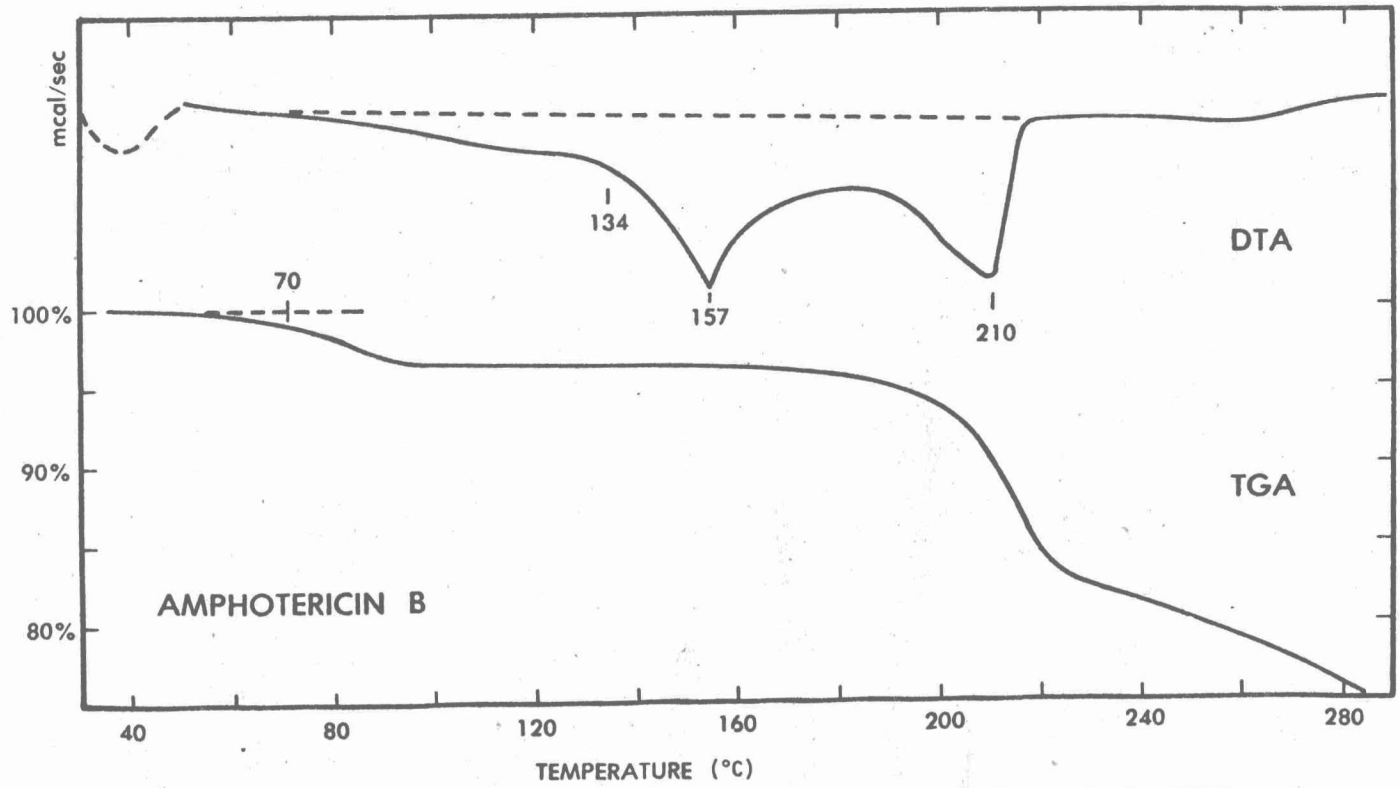


Figure 2. Differential thermal analysis (DTA) and thermal gravimetric analysis (TGA) scans of Amphotericin B.

TABLE 1
X-Ray Powder Diffraction Data
for Amphotericin B (Untreated Sample)

	d(Å)	I/I_o		d(Å)	I/I_o
	18.0	23		3.87	17
	9.30	6		3.79	16
	7.73	12		3.49	12
	7.42	10		3.33	16
*	6.30	91		3.22	13
	5.82	21		2.925 B	11
	5.14	33		2.775	9
	4.82	17		2.460 B	4
	4.65	7		2.370	4
	4.55	5		2.315 B	4
	4.27	46		2.240 B	11
*	4.15 T	90		2.040 B	7
*	4.11	100			

T = triplet
B = broad.
* = three most intense lines

TABLE 2
Solubility of Amphotericin B (MG/ML)

dimethyl sulfoxide (1)	30. - 40.
formamide	6.40
ethylene glycol	2.60
dimethyl formamide (1)	2. - 4.
acetic acid (1)	1. - 2.
propylene glycol (1)	1. - 2.
pyridine	1.75
methanol *	1.60
isoamyl alcohol	1.05
water	0.75
benzyl alcohol	0.75
1,4-dioxane	0.55
ethanol	0.50
ethyl ester	0.50
acetone	0.35
ethyl acetate	0.30
ethylene-Cl	0.30
isoamyl acetate	0.30
CS_2	0.24
methyl ethyl ketone	0.16
isopr. alcohol	0.11
$CHCl_3$	0.08
benzene	0.06
c-hexane	0.02
pet. ether	0.01
CCl_4	0.002
toluene	0.0
iso-octane	0.0

*0.2 - 0.4 mg/ml for anhydrous methanol in Reference 1.