Xilobam: Analysis, Determination of Decomposition Products, and Assessment of Stability

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Abstract □ A stability-indicating UV assay was developed for xilobam, a member of a new class of CNS agents. The method was specific, precise, and accurate. TLC and high-pressure liquid chromatography were used to support method specificity. Xilobam is sensitive to heat, moisture, and basic conditions. The degradation products were identified as *N*-methylpyrrolidone, 2,6-dimethylaniline, and *N*,*N*′-bis(2,6-dimethylphenyl)urea. At high temperatures, the incorporation of molecular sieves into glass bottles of xilobam tablets was effective in preventing decomposition caused by moisture or volatile decomposition products.

Keyphrases □ Xilobam—analysis, determination of decomposition products, stability assessment □ CNS agents—xilobam, analysis, determination of decomposition products, stability assessment □ Decomposition products—xilobam, analysis, stability assessment □ Muscle relaxants—xilobam, analysis, determination of decomposition products, stability assessment

Xilobam, *N*-(2,6-dimethylphenyl)-*N*′-(1-methyl-2-pyrrolidinylidene)urea, a new class of central nervous system agents, is being evaluated clinically as a skeletal muscle relaxant (1, 2). Xilobam is a sparingly soluble base with a spectrophotometrically determined pKa of 6.1. A stability-indicating assay was required to evaluate drug safety supplies and clinical dosage forms. To develop the method, it was necessary to know the various degradation products of xilobam. The isolation and identification of these degradation products and the development of a rapid spectrophotometric assay for xilobam are described. Preliminary stability data and the identification of degradation products are given for a tablet dosage form.

EXPERIMENTAL

Apparatus—UV spectra were obtained using 1-cm cells on a double-beam spectrophotometer[1]. TLC plates (5 × 20 cm) were silica gel[2]. The liquid chromatograph[3] was equipped with a UV detector (254 nm), a 6000-psi pump, and a loop injector. The column was 25 cm × 2 mm i.d. stainless steel, slurry-packed with a reversed-phase C_{18} packing[4]. The mobile phase was 0.1% ammonium carbonate–acetonitrile (60:40) with a 1.0-ml/min flow rate.

Chemicals—Solvents were analytical reagent grade. Reference standards of the degradation products were obtained[5]. The acetonitrile[6] was distilled-in-glass quality. The molecular sieves were type 4A pellets[7] (a synthesized sodium alumino silicate).

Xilobam Degradation—Xilobam was dissolved or mixed separately in 1 *N* HCl, 1 *N* NaOH, and water at a concentration of 10 mg/ml. The samples were heated at 80 ± 2° for 24 hr.

The tablet degradation products were extracted with methanol, chromatographed on TLC plates, isolated, and identified by IR and NMR spectroscopy and mass spectrometry.

Intermediate Identification—A 12.5-mg/ml solution of xilobam in methanol was prepared. Aliquots (4 ml) of the solution were diluted separately to 50 ml with pH 4.0 and 7.0 citrate–phosphate buffers. The solutions were equilibrated with 50 ml of toluene at 80° for 4 hr. The toluene layers were separated and washed with 0.1 *N* HCl to remove residual xilobam and any 2,6-dimethylaniline.

The toluene layers were dried over magnesium sulfate and then analyzed by TLC and UV and IR spectroscopy. *m*-Chloroaniline was added to the toluene layers in excess, and the mixtures were allowed to stand for 2 days. The precipitate was filtered, dried, and assayed by electron-impact mass spectrometry. A control of 2,6-dimethylphenyl isocyanate in toluene was run simultaneously.

TLC—Xilobam was dissolved in methanol, and the degradation compounds were dissolved in methanol or methanol–chloroform (1:1). The TLC chambers were 2-liter, wide-mouth Mason jars fitted with standard lids. The developing solvent was 0.2 *M* sodium acetate buffer (adjusted to pH 4.7)–methanol–chloroform–ethyl acetate (2.5:17.5:53:27). Each plate was developed until the solvent front was ~2.5 cm from the top of the plate. The plates were air dried and viewed under short wavelength UV light or stained with iodine vapor.

Stability-Indicating Assay—A sample equivalent to 100 mg of xilobam was transferred to a 120-ml screw-capped bottle to which were added exactly 50 ml of chloroform and 25 ml of 0.1 *N* NaOH. The bottle was shaken mechanically for 30 min and centrifuged, and the aqueous layer was aspirated and discarded. The chloroform layer was transferred to a clean container. A 15-ml aliquot of the chloroform layer was pipetted to a 100-ml volumetric flask, diluted to volume with chloroform, and mixed.

A 5-ml aliquot of the diluted chloroform solution was pipetted into a 60-ml screw-capped bottle containing exactly 8 ml of cyclohexane. A 50-ml aliquot of 0.1 *N* HCl was pipetted into the bottle, and the bottle was capped, shaken mechanically for 30 min, and centrifuged. The acid layer was decanted to a clean container.

A 6-ml aliquot of the acid layer was pipetted into a 25-ml volumetric flask, diluted to volume with 0.1 *N* NaOH, and mixed. The UV absorbance of the sodium hydroxide solution was recorded in 1-cm cells *versus* a solvent blank of 0.1 *N* NaOH.

A 100-mg sample of xilobam reference standard was treated as were the test samples, and its spectrum was recorded at the same time as the test samples.

The calculation was:

$$\text{percent of label amount} = \frac{A_\mu C_s}{A_s C_\mu} \times 100 \qquad \text{(Eq. 1)}$$

where A_μ and A_s are the absorbance of the sample and standard, respectively, at the maximum near 245 nm and C_μ and C_s are the concentrations in milligrams per milliliter of the sample and standard, respectively. The molar absorptivity at the maximum is ~23,500. A minimum occurs at 225 nm with a molar absorptivity of ~9100.

Specificity Study—All possible degradation products (Table I) were taken through the UV assay at a molar concentration equivalent to 100 mg of xilobam. The various tablet excipients that might be present also were assayed.

Precision, Accuracy, and Recovery—A stock solution of xilobam (25.27 mg/ml in methanol) was prepared. A 5-ml aliquot was added to a 120-ml screw-capped bottle, evaporated to dryness under a nitrogen stream, and assayed.

While the test samples were being assayed, a 4-ml aliquot of the stock solution was diluted to the same final concentration and solvent composition as the test samples. To assess recovery, the absorbances of these solutions were recorded and compared to the test sample absorbances.

In addition, samples of 20 tablets, each containing 200 mg of xilobam, were ground to a fine powder. Triplicate samples were weighed and assayed, and the mean and standard deviation were calculated.

High-Pressure Liquid Chromatography (HPLC) Analysis—

[1] Beckman Acta V.
[2] GF, Analtech, Newark, Del.
[3] Waters Associates, Milford, Mass.
[4] LiChrosorb RP-18, MCB Manufacturing Chemists.
[5] Chemical Research Department, McNeil Pharmaceuticals.
[6] Burdick & Jackson Laboratories, Muskegon, Mich.
[7] Linde, Union Carbide Corp., South Plainfield, N.J.

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Table I—TLC R_f Values and HPLC Retention Times for Xilobam and Degradation Products

Compound	TLC, R_f	HPLC Retention Times, min
Xilobam	0.61	2.1
1-Methyl-2-iminopyrrolidine fluoroborate	0.0	—
N,N'-Bis(2,6-dimethylphenyl)urea	0.34	1.2
2,6-Dimethylaniline	0.77	3.3
N-Methylpyrrolidone	0.55	—
N-(2,6-Dimethylphenyl)methyl carbamate[a]	0.74	2.6
N-(2,6-Dimethylphenyl)ethyl carbamate[a]	0.75	2.6

[a] The carbamates are not degradation products but result from reaction of 2,6-dimethylphenyl isocyanate with methanol and ethanol, respectively.

Table II—Results of Precision and Accuracy Study

Analyst	Percent of Label Amount	
	Solution	Tablets
1	96.8, 96.5	96.1, 95.5
	95.3, 95.4	95.6
2	98.7, 99.4	99.2, 99.1
	99.3	98.9
3	98.1, 98.0	97.6, 97.6
	97.6	97.5
Mean	97.5	97.5
SD	1.48	1.46

Table III—Recovery Data for Xilobam through the Assay

Analyst	Absorbance at Maximum near 245 nm	
	Through Assay	Diluted Directly
1	0.715	0.700
	0.713	0.704
	0.704	0.709
	0.705	0.709
2	0.709	0.712
	0.714	0.707
	0.713	0.709
3	0.712	0.695
	0.711	0.700
	0.708	0.703
Mean	0.710	0.705
SD	0.004	0.005

Liquid samples were diluted with methanol to a concentration of ~1 mg of xilobam/ml. A 5-ml aliquot was pipetted into a 10-ml volumetric flask, 4 ml of the internal standard solution was added, and methanol was added to volume. A 10-μl sample was injected into the chromatograph.

The internal standard was *N*-(3-chlorophenyl)-*N'*-(1-methyl-2-pyrrolidylidene)urea at a concentration of 2 mg/ml. Standard curves were obtained by plotting the peak height ratio *versus* concentration.

pH Stability Profile—A stock solution of xilobam (5 mg/ml in methanol) was prepared, and 4 ml was diluted to 0.1 mg/ml with buffers of varying pH. Potassium chloride–hydrochloric acid solutions were used for pH 1.0, citrate–phosphate buffers were used for pH 2.2–7.0, and glycine–sodium hydroxide buffers were used for pH 9.0–11.0. The solutions were stored at 60, 70, and 80° and were assayed for xilobam by the UV method.

RESULTS AND DISCUSSION

Solution Degradation Product Identification—The acidic, basic, and aqueous hydrolysis solutions were assayed by HPLC, and assay values of 90, 0, and 0% of xilobam were obtained, respectively. By HPLC, the acidic solution gave a very large peak, which corresponded to 2,6-dimethylaniline, and two minor peaks close to the solvent peak. Sufficient solution was collected by semipreparative HPLC of the major peak, evaporated to dryness, and recrystallized; the residue was determined to be 2,6-dimethylaniline by its IR spectrum.

The basic and aqueous solutions both contained a precipitate. The precipitates were identified as *N,N'*-bis(2,6-dimethylphenyl)urea by IR and mass spectrometric analyses. The solution was chromatographed by HPLC, and the major peak was collected and purified as described. The material was identified as 2,6-dimethylaniline by IR spectrophotometry.

Intermediate Identification—The intermediate in xilobam decomposition was postulated to be 2,6-dimethylphenyl isocyanate. To prove this assumption, the intermediate was trapped by partitioning into toluene while xilobam was decomposed in the pH 4.0 and 7.0 solutions. The pH values represented hydrogen-ion concentrations on either side of the pKa of xilobam, which was determined spectrophotometrically to be 6.1. If different decomposition pathways occurred, it was anticipated that the different intermediates would be isolated.

The UV and IR spectra of the dried toluene layer were identical to those of 2,6-dimethylphenyl isocyanate treated similarly. TLC of the toluene layers, including the control, gave one spot each with an R_f value of ~0.75, corresponding to the carbamate, formed by the *in situ* reaction with the alcohol in the mobile phase. Table I lists the R_f values of the various degradation products separated from xilobam. The 2,6-dimethylphenyl isocyanate readily forms a methyl carbamate when treated with methanol. To test definitely for the isocyanate, 3-chloroaniline was added to the toluene layer. The precipitate was identified as 2,6-dimethylphenyl-3-chlorophenylurea. This compound could be formed only if the isocyanate was present in the toluene layer.

UV Assay Specificity—To verify assay specificity, each degradation product listed in Table I was assayed. There was no interference from any degradation product. Assay of the common tablet excipients also showed no UV interference.

Precision, Accuracy, and Recovery—Three analysts, to whom the stock solution concentration was unknown, assayed the solutions (Table II). The data show that the method is precise, since the standard deviation was ±1.48, and accurate, since the mean value was 97.5% of the theoretical value. A precision study also was conducted on a composite sample obtained by grinding 20 tablets. The mean from nine assays was 97.5% of the label amount with a standard deviation of 1.46.

The efficiency of the extraction was demonstrated by assaying portions of the stock solution and comparing the resulting absorbances to other portions simultaneously diluted to the same final concentration and solvent composition. The data are compared in Table III and indicate complete xilobam extraction.

Drug Substance Stability—Crystalline xilobam was stable for up to 3 months at 40, 60, and 40° at 80% relative humidity (RH) when stored in tight containers. Xilobam decomposed completely and rapidly at 80°. The major degradation product was identified as *N,N'*-bis(2,6-dimethylphenyl)urea.

pH Stability Profile—Decomposition of xilobam in solution followed first-order decomposition. The rate constants in reciprocal hours determined at 25, 60, 70, and 80° are given in Table IV. Higher pH values led to greater instability. The most stable pH range was 1–4. The calculated time in days for 10% decomposition at 25° is given in Table V.

Dosage Form Stability—Since xilobam hydrolyzes easily in aqueous solution, a tablet with minimum exposure to water was developed and

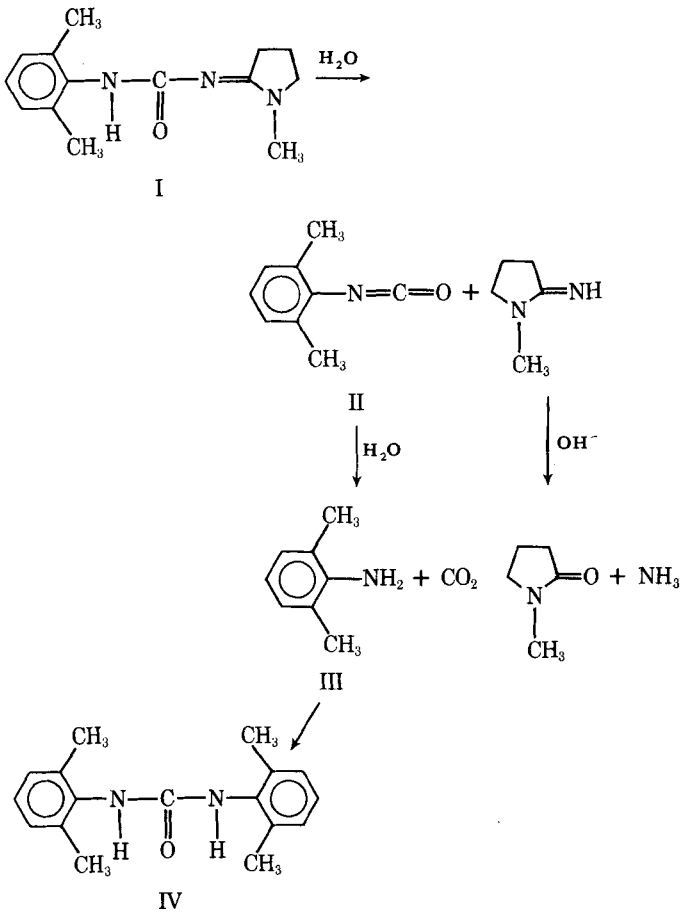
Table V—Estimated Time for 10% Decomposition at Room Temperature for Xilobam Solutions

pH	Days
2.2	16.1
7.0	8.7
11.0	7.2

Table IV—Decomposition Rate Constants of Xilobam[a]

Temperature	pH							
	1.0	2.2	3.0	4.0	6.0	7.0	9.0	11.0
80°	0.123	0.140	0.161	0.239	1.17	1.32	1.32	1.37
70°	—	0.0551	0.0670	0.0938	0.346	0.415	0.427	0.445
60°	0.0148	0.0181	0.0204	0.0259	0.0980	0.101	0.109	0.110
25° (calculated)	—	0.00027	0.00029	0.00027	0.00057	0.00050	0.00063	0.00060

[a] Values are in reciprocal hours.



Scheme I—Proposed decomposition pathway for xilobam.

placed on stability. The tablets were stable at room temperature and 40° but decomposed at 40° and 80% RH and at 60° and decomposed rapidly at 80°. An odor of ammonia was noted from the decomposed samples. Tablets then were stored at 70° for 2 days in containers with and without closures. The tablets stored without closures were white and assayed satisfactorily. Tablets stored with closures were badly discolored, and

Table VI—Xilobam Tablet Stability

	Percent of Label Amount (200 mg/tablet)				
Days	25°	40°	60°	80°	With Molecular Sieves (80°)
Initial	98.7	98.7	98.7	98.7	98.7
19	—	—	—	—	96.1
39	98.4	97.9	24.5	5.4	—
109	98.1	96.2	—	—	77.4

the assays showed extensive degradation. The experiment was repeated, and the headspace was analyzed by GLC–mass spectrometry. The mass spectroscopic data showed the presence of ammonia.

Molecular sieves that absorb both water and ammonia vapors were added to bottles of xilobam tablets. The tablets in amber glass bottles with standard closures were stored at 80° for 109 days. Tablets stored with molecular sieves were much more stable at 80° (Table VI). Without molecular sieves, complete degradation was observed.

The tablets that were degraded extensively were ground, extracted with methanol, and chromatographed by TLC. The separated spots were scraped from the plates; sufficient quantities were obtained to run IR, NMR, and mass spectrometric spectra. The degradation products were *N*-methylpyrrolidone, 2,6-dimethylaniline, and *N,N'*-bis(2,6-dimethylphenyl)urea. The proposed degradation pathway is given in Scheme I.

Xilobam tablets were studied for up to 2 years at 25° (room temperature) and 40° in amber glass containers. No differences were observed at these conditions for tablets stored without molecular sieves compared to those stored with molecular sieves. However, at higher temperatures, the tablets with molecular sieves were much more stable than those without them.

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Studies on Drug Metabolism by Use of Isotopes XXVI: Determination of Urinary Metabolites of Rutin in Humans

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Abstract □ Determination of urinary metabolites of orally administered rutin and rutin-2′,5′,6′-d_3 in humans was carried out by TLC and GLC–mass spectrometry. In human urine, 3-hydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxytoluene, and β-*m*-hydroxyphenylhydracrylic acid were identified as rutin metabolites. Unchanged rutin and quercetin were not present in the urine.

Keyphrases □ Rutin—GLC–mass spectrometric analysis, urinary metabolites in humans □ GLC–mass spectrometry—analysis, urinary metabolites of rutin in humans □ Metabolites, urinary—of rutin, GLC–mass spectrometric analysis, humans

Rutin, a flavonol glycoside, has been used to treat disease states characterized by capillary bleeding associated with increased capillary fragility.

The metabolic fate of rutin has been studied extensively in animals (1–4), but there have been only a few studies in humans. Some investigators (5, 6) reported that unchanged

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