of nystatin. The latter has been shown to possess spectral characteristics nearly identical to that of amphotericin A[16,17].

The paired t test was used to compare bioassay results from the manufacturer with those from the HPLC method. No significant statistical difference was found at the 95% confidence level. It would be difficult and impractical at this juncture to make further comparisons among sample results because of the poor precision of microbiological assays for antifungal substances.

It can, therefore, be concluded that the commercial bulk material is predominantly amphotericin B containing a limited amount of the A congener and several heptaenes. The potency of type I amphotericin B may measure >900 μg/mg with ~6% amphotericin X, whereas type II shows a potency of ~800 μg/mg with ~11-14% amphotericin X.

REFERENCES

(1) I. M. Asher, G. Schwartzman, and the USASRG (U.S. Antibiotics Standards Research Group), "Analytical Profiles of Drug Substances," vol. 6, Klaus Florey, Ed., Academic, New York, N.Y., 1977, pp. 1-42.

(2) A. H. Thomas, *Analyst (London)*, **101**, 321 (1976).

(3) "Code of Federal Regulations," Food and Drugs, Title 21, part 449.4, U.S. Government Printing Office, Washington, D.C., 1981.

(4) J. M. T. Hamilton-Miller, *J. Pharm. Pharmacol.*, **25**, 401 (1973).

(5) S. C. Cheung, G. Medoff, D. Schlessinger, and G. S. Kobayashi, *Antimicrob. Agents Chemother.*, **8**, 426 (1975).

(6) W. Mechlinski and C. P. Schaffner, *J. Chromatogr.*, **99**, 619 (1974).

(7) I. Nillson-Ehle, T. T. Yoshikawa, J. E. Edwards, M. Schotz, and L. B. Guze, *J. Infect. Dis.*, **135**, 414 (1977).

(8) J. L. DiCesare, M. W. Dong, and L. S. Ettre, *Chromatographia*, **14**(5), 257 (1981).

(9) M. Margosis, *J. Chromatogr.*, **236**, 469 (1982).

[16] V. Walton, Food and Drug Administration, 1979, unpublished results. FDA semiannual report available from M. Margosis, Pharmaceutical Research and Testing, FDA, HFN-178, 200 C St., S.W., Washington, DC 20204.

[17] A. Aszalos, J. D. Weber, E. P. Mazzola, M. J. Hayden, and T. Alexander, Food and Drug Administration, 1981, unpublished results. FDA semiannual report available from M. Margosis, Pharmaceutical Research and Testing, FDA, HFN-178, 200 C St., S.W., Washington, DC 20204.

ACKNOWLEDGMENTS

Presented at the American Pharmaceutical Association, Academy of Pharmaceutical Sciences annual meeting, Orlando, Fla., November 1981.

The authors express their gratitude to David G. Skiados of the Perkin-Elmer Corporation for graciously providing the use of the scanning system in the Rockville, Md., laboratories and to Alice Marcotte of the Technical Editing Section, Food and Drug Administration, for editorial assistance.

In Vitro Stability of Sodium Nitroprusside Solutions for Intravenous Administration

CHERYL MAHONY *x, JAMES E. BROWN, W. WAYNE STARGEL, CHACKO P. VERGHESE, and THORIR D. BJORNSSON

Received August 27, 1982, from the *Divisions of Clinical Pharmacology and Cardiology, Departments of Pharmacology and Medicine, Duke University Medical Center, Durham, NC 27710.* Accepted for publication March 21, 1983. *Present address: Division of Cardiology, College of Medicine, University of Kentucky Medical Center, Lexington, KY 40536.

Abstract □ A sensitive high-performance liquid chromatographic assay for nitroprusside using an ion-exchange column and UV detection was developed to evaluate the stability of aqueous solutions of sodium nitroprusside in light-protected glass and plastic containers and during simulated infusions. The results showed that sodium nitroprusside is stable in 5% dextrose, normal saline, and lactated Ringer's solutions in light-protected glass or plastic containers. In addition, there was no decrease in the delivered potency of sodium nitroprusside solutions during simulated infusions lasting up to 24 h.

Keyphrases □ Sodium nitroprusside—*in vitro* stability, solutions, intravenous administration, HPLC □ Stability—*in vitro*, sodium nitroprusside solutions, intravenous administration, HPLC

Sodium nitroprusside [$Na_2Fe(CN)_5NO{\cdot}2H_2O$] is a valuable agent in the treatment of congestive heart failure (1), cardiogenic shock complicating acute myocardial infarction (2), and hypertensive crisis (3). This drug is also used for the production of intraoperative hypotension (4). However, there are several problems associated with the clinical use of sodium nitroprusside, including tolerance (5), tachyphylaxis (6), and the toxicity of its metabolites, cyanide and thiocyanate (7). Some of these problems might be alleviated by an understanding of the pharmacokinetics of nitroprusside in humans. To derive pharmacokinetic parameters from data collected during a constant infusion, the amount of sodium nitroprusside delivered must be known. The purpose of this study was to investigate the stability of sodium nitroprusside in various intravenous solutions and during simulated infusions.

EXPERIMENTAL

Reagents and Chemicals—Sodium nitroprusside[1] was used as received. Analytical-grade KH_2PO_4 and H_3PO_4 and deionized water were used to prepare the mobile phase. All glassware used for sampling or storage of stock solutions was silanized[2].

Instruments and Chromatographic Conditions—An anion-exchange column[3] and a 0.5 M KH_2PO_4 buffer (pH 3.0) with H_3PO_4, were used to achieve the chromatographic separation. The high-performance liquid chromatography (HPLC) was performed with a solvent pumping system[4], a variable-wavelength UV detector[5], and an injector equipped with a 50-μL sample loop[6]. The absorbance was measured at 230 nm with a 0.1 AUFS deflection and was recorded on a three-pen recorder[7]. The flow rate was 1.4 mL/min, and the mobile phase was filtered and deaerated prior to use.

Stability Studies—Sodium nitroprusside (50 mg) was dissolved in 5 mL of water and then added to 500 or 1000 mL of a test solution, resulting in a final concentration of 100 and 50 μg/mL, respectively. The stability of sodium nitroprusside was tested in 5% dextrose, normal saline (0.9% NaCl), and lactated Ringer's solutions[8] in both glass[8] and plastic[8] containers. After the addition of the sodium nitroprusside concentrate, the test solution was mixed and a sample was withdrawn for use in the construction of a standard curve. The bottle or plastic bag was wrapped in aluminum foil and left exposed to laboratory (fluorescent) light for 48 h. Sodium nitroprusside standards were protected from light and stored at 4°C. The stability of the standard solutions was verified by comparing their concentrations with freshly prepared 100-

[1] Roche Laboratories, Nutley, N.J.
[2] Prosil-28; PCR Research Chemicals, Gainesville, Fla.
[3] Partisil 10-SAX; Whatman Inc., Clifton, N.J.
[4] Constametric I; Laboratory Data Control, Riviera Beach, Fla.
[5] Spectromonitor III; Laboratory Data Control, Riviera Beach, Fla.
[6] Model 7120; Rheodyne Inc., Berkeley, Calif.
[7] Linear Instruments, Irvine, Calif.
[8] Abbott Laboratories, North Chicago, Ill.

0022-3549/84/0600-0838$01.00/0

Table I—Sodium Nitroprusside Stability in Lactated Ringer's, Normal Saline, and 5% Dextrose over 48 h in Glass and Plastic Containers [a]

Concentration, μg/mL	Lactated Ringer's		Normal Saline		5% Dextrose	
	Glass 50	Plastic 100	Glass 100	Plastic 50	Glass 50	Plastic 100
Time, h						
2	104.0	100.7	100.4	100.4	101.0	100
4	103.7	100.7	100.6	100.4	103.4	101.0
8	101.7	99.4	100.2	100.9	98.7	100.9
24	98.2	99.4	104.0	101.0	100.7	101.7
32	102.7	101.9	98.2	100.4	98.8	101.8
48	103.1	100.3	101.3	100.7	100.0	104.2

[a] Each value represents the mean of two experiments and is expressed as percent of control.

or 50-μg/mL sodium nitroprusside standard solutions; no deterioration in the refrigerated and light-protected stock solutions was noted over a 48-h period.

Infusion Studies—A volumetric pump[9] and infusion set[8] were employed. The plastic tubing made of polyvinyl chloride was left exposed to light. The infusion set tubing was 3 m long and held a volume of 20 mL. Flow rates of 10 and 50 mL/h for 24 and 8 h, respectively, were studied. Samples were taken from both the bottle and directly from the end of the tubing at each sampling interval.

Standard Curves—The standard solution of sodium nitroprusside, serially diluted with water to yield concentrations of 6.25, 12.5, 25, and 50 μg/mL, was injected directly onto the column with measurement of peak heights to generate a calibration curve. Each sample was injected in duplicate. The coefficients of variation for the standard curves ranged from 1 to 3%. The unknown samples were analyzed immediately after withdrawal from the container or infusion set at 0, 2, 4, 8, 24, 32, and 48 h after preparation. All experiments were performed in duplicate.

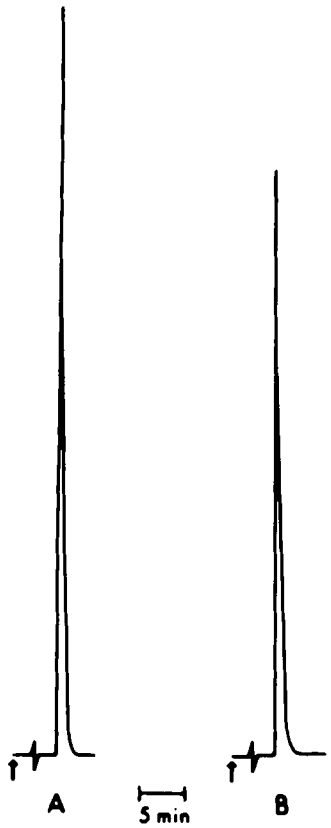

Figure 1—*Chromatogram of 5 μg of sodium nitroprusside in 5% dextrose before (A) and after exposure to light for 4 h (B). The arrow denotes the time of injection.*

[9] Life Care Pump; Abbott Laboratories, North Chicago, Ill.

Table II—Sodium Nitroprusside Stability in Intravenous Infusion Sets at Flow Rates of 10 and 50 mL/h [a]

Time, h	10 mL/h	50 mL/h
2	99.6	101.8
4	100.9	101.8
8	100.9	103.2
24	100.3	—

[a] Values represent the mean of two experiments and are expressed as percent of control.

RESULTS AND DISCUSSION

A chromatogram of sodium nitroprusside, 100 μg/mL in 5% dextrose, is shown in Fig. 1. Using the previously described chromatographic conditions, the retention time is ~5 min. Also shown in Fig. 1 is a chromatogram of the same solution of sodium nitroprusside after exposure to light for 4 h. At 230 nm, the degradation of sodium nitroprusside is manifested as a decrease in the peak height; none of the breakdown products are visible on the chromatogram. Beer's law is obeyed over the sodium nitroprusside concentration range of 1–200 μg/mL. The precision of the assay was evaluated by injecting four replicate samples of 12.5, 25, 50, and 100 μg/mL of sodium nitroprusside; the coefficient of variation was <1%.

The results of the stability studies of sodium nitroprusside in 5% dextrose, lactated Ringer's, and normal saline are shown in Table I. When a glass or plastic container is wrapped in aluminum foil and then left exposed to light for 48 h, there is no appreciable breakdown of sodium nitroprusside at a concentration of either 50 or 100 μg/mL. Other studies have found that sodium nitroprusside is stable in 5% dextrose in glass (8, 9) and plastic (10) containers when protected from light, and this study extends those observations to include normal saline and lactated Ringer's solution. There appears to be no factual basis for the current recommendation that sodium nitroprusside be administered solely in 5% dextrose.

Table II shows the effect of light exposure on sodium nitroprusside solutions while traversing plastic intravenous infusion sets. Duplicate determinations in either 5% dextrose, normal saline, or lactated Ringer's solution at flow rates of 10 or 50 mL/h showed no deterioration in the delivered concentration of the sodium nitroprusside solution over 24 and 8 h, respectively. This finding contrasts with that of Baaske *et al.* (8) who noted a small (3.5%), but consistent, decrease in the potency of a nitroprusside solution delivered from an intravenous infusion set exposed to light for 5 h. This discrepancy may possibly be explained by differences in the composition, and therefore the light diffusion characteristics, of the infusion sets.

REFERENCES

(1) N. H. Guiha, J. N. Cohn, E. Mikulic, J. A. Franciosa, and C. J. Limas, *N. Engl. J. Med.*, **291**, 587 (1974).

(2) K. Chatterjee, W. W. Parmley, W. Ganz, J. Forrester, P. Walinsky, C. Crexells, and H. J. C. Swan, *Circulation*, **48**, 1183 (1973).

(3) I. Tuzel, R. Limjuco, and B. Kahn, *Curr. Ther. Res. Clin. Exp.*, **17**, 95 (1975).

(4) T. H. Taylor, M. Styles, and A. J. Lamming, *Br. J. Anaesth.*, **42**, 859 (1970).

(5) P. P. Moraca, E. M. Bitte, D. E. Hale, C. E. Wasmuth, and E. F. Poutasse, *Anesthesiology*, **23**, 193 (1962).

(6) L. Amaranath and W. F. Kellermeyer, Jr., *Anesthesiology*, **44**, 345 (1976).

(7) J. E. Cottrell, P. Casthely, J. D. Brodie, K. Patel, A. Klein, and H. Turndorf, *N. Engl. J. Med.*, **298**, 809 (1978).

(8) D. M. Baaske, M. D. Smith, N. Karnatz, and J. E. Carter, *J. Chromatogr.*, **212**, 339 (1981).

(9) M. J. Frank, J. B. Johnson, and S. H. Rubin, *J. Pharm. Sci.*, **65**, 44 (1976).

(10) R. A. Anderson and W. Rae, *Aust. J. Pharm. Sci.*, **NS1**(2), 45 (1972).

ACKNOWLEDGMENTS

Cheryl Mahony is the recipient of a Pharmaceutical Manufacturers Association Foundation Award for Cancers in Clinical Pharmacology. Thorir D. Bjornsson is the recipient of a Pharmaceutical Manufacturers Association Foundation Faculty Development Award in Clinical Pharmacology.