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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF SODIUM NITROPRUSSIDE

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SUMMARY

A rapid high-performance liquid chromatographic method for the determination of nitroprusside in commercial lyophilized products or in intravenous admixture solutions is described. The method is stability-indicating. Reversed-phase liquid chromatography was performed using a microparticulate (10 μm) phenyl column with a mobile phase acetonitrile–phosphate/tetrabutylammonium hydroxide buffer (pH 7.1) (30:70) and detection at 210 nm. A coefficient of variation of less than 3.1% was achieved over the concentration range studied (10–50 μg/ml). Total analysis time was 9 min.

This method was used to show that there is a small loss of nitroprusside due to photodegradation during intravenous infusion, even when the admixture container is wrapped in foil as recommended and used expeditiously.

INTRODUCTION

Sodium nitroprusside is hydrated sodium nitrosyl pentacyanoferrate $Na_2Fe(CN)_5NO \cdot 2H_2O$. It is a potent, rapid-acting hypotensive agent when administered intravenously. Sterile sodium nitroprusside (U.S.A. Pharmacopoeia, USP) is lyophilized sodium nitroprusside. The USP assay for this product is a polarographic determination of the ferric ion at a dropping mercury electrode. Polarographic methods for nitroprusside[1–3] require specialized instrumentation not available in many laboratories and are not suitable for automation for routine analysis. Colorimetric[4,5] and spectrophotometric[1,6] methods have been described for the determination of sodium nitroprusside and its photodegradation products. However, they are either

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not stability-indicating or they rely on unproven assumptions about the underlying chemistry of the photodegradation. Coulometric, gravimetric and titrimetric methods have also been described[7] but they also are time-consuming or require unusual instrumentation.

High-performance liquid chromography (HPLC) has become the method of choice for the determination of stability of organic pharmaceuticals, but has had little application in the quantitation of inorganic or quasi-inorganic compounds. A method for the determination of nitrate and nitrite by HPLC with ultraviolet (UV) detection was recently described[8]. This paper reports a method using ion-pair reversed-phase HPLC with UV detection for the rapid direct measurement of nitroprusside in vials or diluted intravenous solutions, and which provides the potential for measuring breakdown products. In the presence of light, sodium nitroprusside rapidly undergoes a wide variety of reactions to yield numerous products depending on the characteristics of the impinging light[1]. Therefore its stability in an infusion set was questionable.

EXPERIMENTAL

Materials

Sodium nitroprusside (Fisher Scientific, Fairlawn, NJ, U.S.A.) was used as the standard. Sterile sodium nitroprusside, USP (Roche Labs., Nutley, NJ, U.S.A.) and dextrose (5%) injection, USP (American McGaw, Irvine, CA, U.S.A.) were used to prepare admixtures. Distilled-in-glass acetonitrile (Burdick & Jackson, Muskegon, MI, U.S.A.), tetrabutylammonium hydroxide (Eastman, Rochester, NY, U.S.A.) sodium nitrate, sodium nitrite, potassium ferricyanide, potassium ferrocyanide and potassium phosphate monobasic (Mallinckrodt, St. Louis, MO, U.S.A.) were used without further purification. Purified water was further purified (Milli-Q water purification system, Millipore, Bedford, MA, U.S.A.) prior to use.

Apparatus

The liquid chromatographic system consisted of a solvent pumping system, an automatic sampler equipped with a 50-μl sample loop, a variable-wavelength detector (detection wavelength 210 nm) and a 10-mV recorder (Perkin-Elmer, Norwalk, CT). A 30 cm × 3.9 mm I.D. column packed with phenyl bonded to 10-μm silica gel (Water Assoc., Milford, MA, U.S.A.) was employed.

Mobile phase

The mobile phase was prepared fresh daily by thoroughly mixing 300 ml of acetonitrile and 700 ml of buffer. The buffer was 0.01 *M* potassium phosphate monobasic and 0.005 *M* tetrabutylammonium hydroxide adjusted to a final pH of 7.1 with phosphoric acid. The mobile phase was filtered through a 0.5-μm filter (Millipore) prior to use and pumped at a constant flow-rate of 2 ml/min ($<$2000 p.s.i.).

Standards

Sodium nitroprusside stock standard solutions were prepared at 1 mg/ml in 5% dextrose in water USP (D_5W). The flask was wrapped in foil to protect it from light.

The stock standard nitroprusside solution was diluted with D_5W to yield standards of 50, 40, 25 and 10 μg/ml, which were also wrapped in foil for protection from light.

A 1.5-ml volume of each standard and each sample was placed in autosampler vials (which had been made opaque with interior latex paint), capped and injected in duplicate by the autosampler. Peak heights were used to construct calibration curves and for sample analysis. Peak areas may also be used.

Analysis of unit dosage

The lyophilized sodium nitroprusside vials were reconstituted with exactly 5.0 ml of D_5W. The vial was sealed and shaken to dissolve the sodium nitroprusside. Exactly 1.0 ml of this solution was quantitatively transferred to a 200-ml volumetric flask and brought to volume with D_5W. The flask was wrapped with foil to protect it from light. Quadruplicate samples (50 μg/ml) were prepared from each vial.

Infusion set study

Admixture solutions were prepared to contain sodium nitroprusside 100 μg/ml as per package insert instructions. Solutions were immediately wrapped in foil. Prior to the attachment of the infusion set a control sample was taken from the admixture. Nitroprusside degradation while traversing the infusion set was defined in relation to this control, which was taken as 100% of theoretical. The infusion set spike was inserted into the IV bottle which was then inverted and hung. The tubing was filled by gravity flow upon release of the roller clamp. Flow-rate regulation was accomplished by measuring delivered volume over time at 10 drops/min. Flow-rates were monitored throughout the test period. An initial 3-ml sample was taken from each set. Additional samples were taken at 30, 60, 90, 120, 180, 240 and 300 min. The samples were stored in foil-wrapped glass vials with rubber stoppers at 10°C until assayed. These samples were diluted 1:1 with D_5W prior to analysis.

Light studies

Admixtures were prepared as described above in label free bottles and stored in a high intensity light cabinet (1400–2000 foot-candles), in sunlight and under 24 h/day flourescent lights. A control wrapped in foil was stored in the dark. These samples were diluted 1:1 with D_5W prior to analysis.

RESULTS AND DISCUSSION

Chromatography

Using ion-pair chromatography with tetrabutylammonium hydroxide (TBA, 0.005 *M*) as the ion-pairing agent, the effect of the eluent composition on the retention time of sodium nitroprusside and its major degradation products as well as potassium ferricyanide and ferrocyanide was investigated. Potassium ferricyanide and ferrocyanide were used to determine whether this HPLC system could differentiate ferrocyanide, ferricyanide and nitroprusside.

As the pH of the aqueous phase was increased, the retention time for nitroprusside, its major unidentified photodegradation product, ferrocyanide and ferricyanide increased as shown in Fig. 1. At the pH values studied, nitroprusside deg-

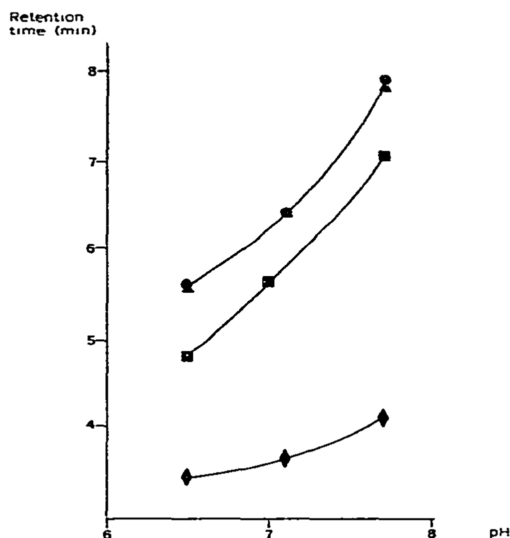


Fig. 1. Effect of the pH of the mobile phase on the retention times of nitroprusside, its major photodegradation product, ferricyanide and ferrocyanide. ●, Nitroprusside; ■, ferrocyanide; ▲, ferricyanide; ◆, degradation product.

radation products eluted first, then ferrocyanide, ferricyanide and finally nitroprusside. Ferricyanide and nitroprusside eluted at about the same retention times at all pH values.

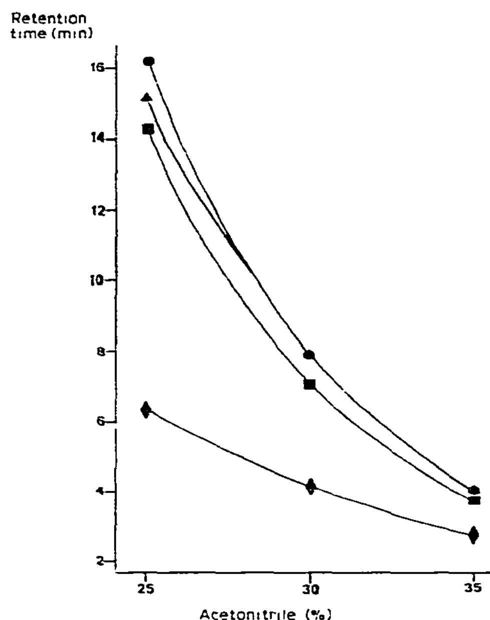
A pH of 7.1 was chosen for the chromatographic procedure because at this pH the greatest separation of degradation products and nitroprusside occurs and the integrity of the column can be maintained.

As the percentage of acetonitrile in the eluent decreases, the retention times for nitroprusside, its major photodegradation products, ferrocyanide and ferricyanide increased as shown in Fig. 2. A mobile phase composition of acetonitrile–buffer (30:70) was chosen for the cnromatographic procedure. Under these conditions nitroprusside is resolved from its photodegradation products and sample time is minimized for routine analysis.

The eluent used in the HPLC procedure for the determination of sodium nitroprusside in the commercial lyophilized product, admixture solutions, infusion set studies and photodegradation studies was acetonitrile–0.005 M tetrabutylammonium hydroxide, 0.01 M potassium phosphate (pH = 7.1) (30:70). Chromatograms of a sample of nitroprusside admixture solution, reference standard, infusion set sample and photodegraded sample under the above chromatographic conditions are given in Fig. 3. Several extra peaks near the solvent front, presumably degradation products, are present in the photodegraded sample. The retention times of these degradation products are similar to those of nitrite and nitrate.

Precision

Four replicate nitroprusside samples (50, 40, 25 and 10 μg/ml) were automatically injected, and their peak heights were measured (Table I). A correlation coef-



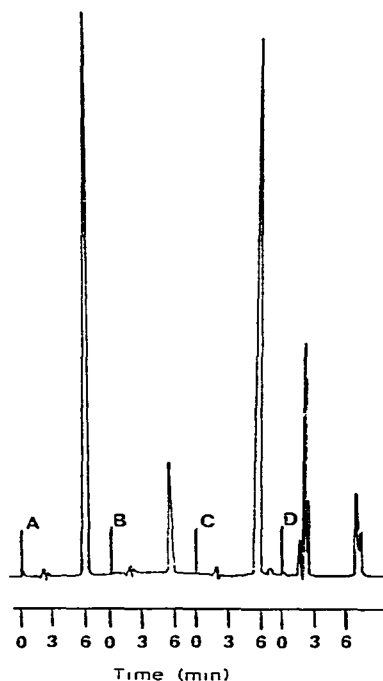


Fig. 2. Effect of acetonitrile content of the mobile phase on the retention times of nitrop· usside. Its major photodegradation product, ferricyanide and ferrocyanide. ●, Nitroprusside; ■, ferrocyanide; ▲, ferricyanide; ◆, degradation product.

Fig. 3. Actual chromatograms of nitroprusside admixtures. (A) Sodium nitroprusside admixture in dextrose (5%); nominal concentration is 50 μg ml. (B) Sodium nitroprusside standard at a nominal concentration of 10 μg/ml. (C) Sodium nitroprusside sample at a nominal concentration of 50 μg/ml after passage through an infusion set exposed to normal room light. (D) Sodium nitroprusside admixture after 3 days in a 1400–2000 foot-candle light cabinet. Nominal initial concentration of 50 μg/ml.

ficient of greater than 0.999, a coefficient of variation (C.V.) of less than 3.1% and an accuracy of greater than 98.2% were consistently obtained. Although similar results were obtained using peak areas, for consistency peak heights were employed for all analyses.

TABLE I

PRECISION DATA FOR SODIUM NITROPRUSSIDE EMPLOYING PEAK HEIGHTS

Nitroprusside concentration (μg/ml)	*Mean (n = 4) peak height (mm)*	*Adjusted group mean*	*S.D.*
50	182.25	96.6	2.4
40	144.25	100.7	2.1
25	91.00	99.7	1.2
10	36.38	99.7	1.1
Overall	—	99.9	1.8

Potency study

Four vials from two commercial lots of lyophilized sodium nitroprusside were analyzed (Table II). A C.V. between different vials of the same lot of less than 1 % was found. The pooled C.V. was less than 1.4%.

TABLE II

POTENCY OF SODIUM NITROPRUSSIDE VIALS IN MILLIGRAMS

Label claim is 50 mg.

	Lot A	*Lot B*		
		Vial A	*Vial B*	*Vial C*
Mean (n = 4)	48.16	48.04	48.92	50.02
Standard deviation	0.652	0.477	0.376	0.286

Admixture infusion set study

An admixture of sodium nitroprusside was connected to an infusion set and kept in the dark, which protected the nitroprusside from the effects of light but permitted gas exchange to occur. Duplicate experiments gave no evidence of nitroprusside degradation or adsorption.

Admixture solutions of sodium nitroprusside protected from light with aluminium foil were connected to administration sets and exposed to normal room light. Duplicate experiments were performed. Every delivered potency was equal to, or less than, the bottle control. This is the opposite of what is seen in the light-protected sets. A comparison of the light-exposed and -protected sets (Table III) shows that the light-exposed sets have a lower delivered potency over the entire time span of the experiment. This supports the theory that the loss of nitroprusside potency seen during i.v. infusion is due to light instability and not adsorption to plastic. Although the maximum difference is only 3.5%, an examination of the chromatograms of samples exposed to light shows very small peaks at approximately the retention times of the light-degradation products. While the peaks are too small to be definitive, they also indicate photodegradation.

TABLE III

POTENCIES DELIVERED FROM i.v. INFUSION SETS

Nominal concentration 100 μg/ml (10 drops/min)

Time (min)	*Sets protected from light (% bottle control)*	*Sets exposed to light (% bottle control)*
0	102.5	100.0
30	101.5	98.8
60	101.3	98.7
90	101.3	98.0
120	101.1	98.0
180	101.4	97.9
240	101.4	98.5
300	101.4	98.4

Because photodegradation would be a function of exposure time, and the exposure time is constant at a uniform drip-rate, each data point can be thought of as a replicate sample. Performing a T-test on the two data sets shows that while the nitroprusside loss due to light degradation is small it is statistically significant.

Photodegradation study

Sodium nitroprusside admixtures were exposed to various types and intensities of light to help assess their stability (Table IV). When properly protected from light the admixtures show good stability. Even when exposed to normal fluorescent light generally acceptable stability was seen for the first few hours. After 6 h on a laboratory bench with the room lights on the sample still exhibited 93% of its initial potency. However, since potentially toxic degradation products may form it would always be wise to follow package insert directions to wrap the infusion bottle with foil and use the admixture quickly after preparation. It is particularly interesting to note that direct sunlight was the most potent source of photodegradation tested, causing a 32% potency loss in only one hour. This ubiquitous potential source of degradation must be considered during all phases of admixture preparation, transportation and use. Finally, a high-intensity light cabinet was found to provide an effective, convenient model system for studying the photodegradation of sodium nitroprusside admixtures as all light sources gave similar chromatographically detected breakdown patterns.

TABLE IV

SODIUM NITROPRUSSIDE POTENCY (μg/ml) IN DEXTROSE (5%) ADMIXTURES STORED UNDER LIGHT CONDITIONS

Time (h)	*Control foil-wrapped dark cabinet*	*Room light, fluorescent light, 24 h/day*	*Window light eastern window*	*High-intensity light cabinet, 1400–2000 foot-candles*
Initial	50.54	52.12	50.54	50.76
1	50.46	51.97	34.44	45.26
2	52.39	52.52	27.56	38.38
4	51.04	50.55	22.51*	28.78
6	49.81	48.46	14.89	23.74*
7	48.83	46.74	11.94	21.53
24	49.39	43.30	11.87	9.68
31	49.22	41.45	8.49	7.65
48	51.47	38.57	6.76	4.09
60	47.62	31.66	3.78	1.18
144	49.07	23.63*	1.73	0.69

* From this time the solution was blue.

CONCLUSION

An analytical method has been developed which allows quantitation of sodium nitroprusside in admixture solutions, in commercially available lyophilized vials and during photodegradation studies. This HPLC method was used to show that while

there is no adsorption to the infusion set there is a small loss of nitroprusside due to photodegradation even when the infusion bottle is wrapped in foil.

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