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NOTES

Limit Test for Determination of Free Ferricyanide and Ferrocyanide in Sodium Nitroferricyanide

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Abstract □ A simple and rapid procedure for the isolation and determination of free ferricyanide and ferrocyanide in sodium nitroferricyanide (nitroprusside) was developed. The method, employing aluminum oxide column chromatography, was sensitive enough to detect ferricyanide and ferrocyanide at the 0.05% level.

Keyphrases □ Sodium nitroferricyanide (nitroprusside)—prepared solutions, chromatographic separation and spectrophotometric analyses of free ferricyanide and ferrocyanide □ Ferricyanide and ferrocyanide, free—chromatographic separation and spectrophotometric analyses in prepared solutions of sodium nitroferricyanide (nitroprusside) □ Antihypertensives—sodium nitroferricyanide (nitroprusside), prepared solutions, chromatographic separation and spectrophotometric analyses of free ferricyanide and ferrocyanide

Sodium nitroferricyanide (nitroprusside) (I) has been used extensively as an analytical reagent, primarily for the detection of organic compounds such as ketones and aldehydes, and recently it has gained stature as a potent antihypertensive in acute hypertensive crises (1–6). Since the mode of administration is intravenous infusion, a high quality substance is required. As a result, analytical methodology for the determination of purity and potency of I in pharmaceutical preparations is needed. Methodology and specifications are available for reagent grade material (7–9), but these methods are not suitable for a parenteral grade pharmaceutical product.

Two potential contaminants in I are free ferricyanide and ferrocyanide. Existing methods for the analysis of these two impurities are limit tests in which a faint color or turbidity is detected in the solution if the contaminants are present (7, 9). However, at levels near and below 0.05% ferricyanide and ferrocyanide, it is extremely difficult to detect these subtle changes in the sample solution. This paper reports a simple and rapid procedure for the isolation and determination of ferricyanide and ferrocyanide in I at the 0.05% level. The method involves the chromatographic separation of ferricyanide and ferrocyanide from I on an alumina column by a series of elutions of increasing acid strengths. Ferricyanide and ferrocyanide are determined spectrophotometrically at 415 nm.

The limit of 0.05% is realistic because of the relatively lower toxicity of ferricyanide and ferrocyanide *versus* I. The oral LD_{50} in rats for ferricyanide and ferrocyanide is 1600 mg/kg; for I, it is 20 mg/kg (10, 11).

EXPERIMENTAL

Reagents—Neutral aluminum oxide[1], activity grade I, was used as received. All other chemicals were ACS reagent grade or equivalent and were used without further purification.

Column Preparation—Amber glass columns, 1.45 cm i.d., fitted with polytef stopcocks, were each dry filled with 7 g of aluminum oxide.

Standard Solution—A solution containing 15.5 mg of potassium ferricyanide and 19.9 mg of potassium ferrocyanide trihydrate in 100 ml of 1 *N* HNO_3 was prepared.

Sample Solutions—Solutions of sodium nitroferricyanide at a concentration of 200 mg/ml were prepared in 1 *N* HNO_3.

Procedure—Aliquots of 2.0 ml of sample solutions and 2.0 ml of standard solution were placed on individual columns. The columns were eluted with 35 ml of 0.5 *N* HNO_3, which was discarded. Ferricyanide was then eluted with 25 ml of 2 *N* HNO_3, and ferrocyanide was eluted with 25 ml of 6 *N* HNO_3. The absorbances of the solutions were measured in 1-cm cells at 415 nm. If the sample absorbances were lower than the standard absorbances, the sample contained less than 0.05% each of ferricyanide and ferrocyanide.

RESULTS AND DISCUSSION

Complete separation of ferricyanide $[Fe(CN)_6^{3-}]$ and ferrocyanide $[Fe(CN)_6^{4-}]$ from I $[Na_2Fe(CN)_5NO]$ was achieved by column chromatography with aluminum oxide as the adsorbent. Determination at the 0.05% level was achieved by comparing the sample absorbances at 415 nm to those of the standards.

The dependence of ferricyanide and ferrocyanide recovery on the acid strength of the final elution was investigated. To each of several columns, 0.2 mg of ferricyanide was applied and the columns were eluted as pre-

[1] Woelm, ICN Pharmaceuticals.

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Table I—Addition of Ferricyanide and Ferrocyanide to I

Amount Added, %			Number of
Ferricyanide	Ferrocyanide	Recovery, %	Runs
0.05	—	0.047 ± 0.004	7
—	0.05	0.047 ± 0.005	7
0.04	—	0.037 ± 0.008	5
—	0.04	0.041 ± 0.014	5
0.03	—	0.031 ± 0.005	6
—	0.03	0.024 ± 0.004	6

viously described with the exception of the final wash. Here the nitric acid concentration was varied on individual columns from 1.0 to 2.5 *N*, and 25.0 ml of eluate was collected. A similar experiment using ferrocyanide was carried out using nitric acid strengths up to 6 *N*.

At nitric acid concentrations of 1.0 *N* and below, no ferricyanide was eluted; full recovery was obtained at an acid strength of 1.8 *N* and above. Ferrocyanide started eluting when the nitric acid concentration reached 2.5 *N*. At nitric acid strengths of 4.0 *N*, there was evidence that elution was still incomplete. An acid strength of 6.0 *N* eluted ferrocyanide completely. As a result of these data, nitric acid concentrations of 2.0 and 6.0 *N* were chosen for the ferricyanide and ferrocyanide elutions, respectively.

To verify the column results, a TLC separation of I, ferricyanide, and ferrocyanide was performed on aluminum oxide plates with 2.0 *N* HNO_3 as the developer. As with the column separation, I and ferricyanide traveled to the solvent front while ferrocyanide remained at the origin.

The linearity of the spectrophotometric response to changes in ferricyanide and ferrocyanide concentrations was evaluated. Known amounts of potassium ferricyanide and potassium ferrocyanide trihydrate were subjected to chromatography, and the absorbances of the solutions were determined spectrophotometrically. A plot of absorbance *versus* concentration was linear from 2 to 30 μg of ferricyanide and ferrocyanide/ml. Since a sample size of 400 mg was chosen to assure nonoverloading of the column, 8 μg each of ferricyanide and ferrocyanide/ml represented 0.05% of the I content. This concentration is well within the region of linear response.

To determine whether ferricyanide and ferrocyanide could be determined accurately at levels below 0.05%, known concentrations of each were added to I and the solutions were analyzed according to the procedure (Table I). The data indicate that the precision and accuracy of the method are lower than those normally acceptable for a quantitative determination. However, its use as a limit test is justified since the recoveries of both ferricyanide and ferrocyanide are at least semiquantitative when added to I.

Compared to the limit tests currently available for the determination of ferricyanide and ferrocyanide in I, this procedure offers the following advantages:

1. Semiquantitation of the contaminants, if more than a limit test is required, is possible.
2. The results are reproducible.
3. The preparation of reagents, samples, and standards is simple.
4. The procedure can be carried out rapidly.

Since I is now accepted and used as a pharmaceutical preparation, this improved procedure for controlling two possible contaminants will help in maintaining and ensuring the purity and integrity of the product.

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Determination of Carbocysteine from Human Plasma

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Abstract □ A fast and accurate GLC procedure for determining carbocysteine, using a sulfur photometric detector, is described. Interferences from nonsulfur constituents are eliminated. The method is linear from 2 to 15 μg. Results from the application of this method to a bioavailability study in humans are given.

Keyphrases □ Carbocysteine—GLC analysis in plasma □ GLC—analysis, carbocysteine in plasma □ Mucolytic agents—carbocysteine, GLC analysis in plasma

Carbocysteine, *S*-(carboxymethyl)cysteine, has been undergoing clinical evaluation as a mucolytic agent. Therefore, it was of interest to determine the pharmacokinetic parameters of this drug, and an analytical method was needed.

Carbocysteine has properties typical of amino acids and has been determined in biological materials by ion-exchange chromatography (1). That method is time consuming and did not appear practical. GLC procedures are more rapid and have varying degrees of success using trifluoroacetic anhydride derivatization with flame-ionization detection (2–8). In general, these methods require temperature programming, which is undesirable.

Since carbocysteine contains a sulfur atom, the use of a flame photometric detector in the sulfur mode appeared logical. Concentration of the drug through extraction into an organic solvent would also help in derivatization. Such a method was found to be of value by adopting Maynard's (9) procedure for extracting piperazine from an aqueous solution. That this method may be applicable to the analysis of other amino acids was demonstrated by

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