Spectrophotometric Determination of Methenamine and Its Salts by 2-Hydrazinobenzothiazole

JAMES K. LIM▲ and CONNIE C. C. CHEN

Abstract □ A rapid and sensitive method is described for the assay of the pure powder form and the tablet dosage form of methenamine and its salts. The procedure is shown to be capable of measuring as little as 0.005 μmole of material and is highly reproducible. The proposed method is based on the color development of formaldehyde released from the acid hydrolysis of methenamine with a dye, 2-hydrazinobenzothiazole. Beer's law is obeyed in the concentration range of 1–8 μ*M* of methenamine at 510-nm. wavelength. This method offers greater versatility over the official assay methods, which have been designed primarily for determining specifically either methenamine or its mandelate salt. Data comparing the assays obtained by the official methods with this new technique are presented.

Keyphrases □ Methenamine and salts in powders and tablets—colorimetric analysis using 2-hydrazinobenzothiazole, compared to compendial methods □ 2-Hydrazinobenzothiazole—used as color-forming agent in spectrophotometric analysis of methenamine and its salts □ Colorimetry—analysis, methenamine and salts in powders and tablets

The NF XIII (1) assay for methenamine involves its acid hydrolysis by reflux until the "odor of formaldehyde is no longer perceptible," followed by back-titration with an alkali. The procedure proved to be not only tedious but also subject to inaccuracies arising from human error in obtaining the "perceptible end-point," *i.e.*, absence of formaldehyde odor. The USP XVIII (2) method for determining methenamine mandelate, on the other hand, represents an indirect method of analysis. It essentially involves nesslerization followed by an iodometric titration of the mandelic acid moiety; therefore, this method cannot be employed for determining other salts of methenamine.

Other methods also have been developed for the quantitation of this compound, including those based on chromotropic acid reactions (3–5), the Hantzsch reaction between acetylacetone, ammonia, and formaldehyde (6), and fluorometric techniques (7). This report presents a method for determining methenamine and its salts through the formation of xanthylinum dicationic dyes with formaldehyde and formaldehyde-releasing compounds. Measurements based on such a reaction are reported (8, 9) to be more sensitive than those by chromotropic acid.

EXPERIMENTAL

Apparatus—Absorbance measurements were made on a spectrophotometer[1].

Reagents and Chemicals—2-Hydrazinobenzothiazole[2] was the dye-forming reagent used. Powdered samples of methenamine[3–5], methenamine mandelate[6], and methenamine hippurate[7] were used as received. All other chemicals and reagents used were USP or the highest grade of commercial materials. Several commercial brands of either the plain or enteric-coated tablets of methenamine, methenamine mandelate, and methenamine hippurate were employed for the tests comparing the official assays with the proposed method. The dye reagent consists of a 0.5% solution of 2-hydrazinobenzothiazole in 10% hydrochloric acid. It is stable for 2 weeks.

Assay Procedure—Aqueous solutions of methenamine or the methenamine salt in the concentration range of 0.01–0.08 μmole/ml. were made up in 100-ml. volumetric flasks. A 0.5-ml. volume of each solution was placed in a test tube and mixed with 0.5 ml. of 10 *N* sulfuric acid. The samples were refluxed in a boiling water bath for 15 min. to obtain complete hydrolysis and were allowed to cool to room temperature before the addition of 0.5 ml. of the 2-hydrazinobenzothiazole reagent. After 5 min., 0.5 ml. of a 1% aqueous solution of potassium ferricyanide was added and this reaction was allowed to proceed another 20 min. before the final admixture of 3 ml. of dimethylformamide solvent. The color thus formed was allowed to stabilize for 20 min. before absorbance readings were made at a 510-nm. wavelength against a reagent blank.

Table I—Absorbance by Different Methenamine Concentrations

Determination Number	Concentration, μmole/5 ml. 0.005	0.01	0.02	0.04
1	0.075	0.141	0.297	0.598
2	0.084	0.161	0.290	0.598
3	0.084	0.161	0.276	0.584
Average	0.081	0.154	0.288	0.593
± *SD*	0.004	0.009	0.008	0.007

Table II—Assay of Commercial Methenamine Powder by the 2-Hydrazinobenzothiazole and NF Methods

Powder	Percent Found—2-Hydrazinobenzothiazole Method	NF Method
A	98.5[a] ± 1.08[b]	98.8
B	96.3 ± 2.07	93.5

[a] Average of three determinations. [b] Standard deviation.

RESULTS AND DISCUSSION

Absorbance of the color obtained of known standards in the concentration range of 0.005–0.04 μmole/5 ml. of methenamine was seen to obey Beer's law. The calculated linear correlation coefficients were: methenamine, 0.9996; and methenamine mandelate and methenamine hippurate, 0.9969 and 0.9975, respectively. Initial studies showed that the slopes might vary from time to time, probably due to aging of the reagents employed with the 2-hydrazinobenzothiazole reaction. However, the experimental values were reproducible within the normal analysis period (Table I). It is, therefore, recommended that a fresh standard curve be prepared with each analysis.

Table II compares the percentages of methenamine ($C_6H_{12}N_4$) found in two different commercial brands of methenamine powder as assayed by the 2-hydrazinobenzothiazole and the NF methods.

[1] Bausch and Lomb Spectronic 70.
[2] Eastman Organic Chemicals.
[3] Baker Chemical Co.
[4] Amend Drug Chemical Co.
[5] Fisher Scientific Co.
[6] K & K Laboratories, Inc.
[7] Riker Laboratories, Inc.

Table III—Assay of Commercial Methenamine Products by the 2-Hydrazinobenzothiazole Method

Determination Number	Percent Found: Powders: Methenamine Mandelate	Powders: Methenamine Hippurate	Tablets: Methenamine	Tablets: Methenamine Hippurate	Tablets: Methenamine Mandelate, Enteric Coated
1	96.6	96.6	103.8	96.6	95.2
2	96.6	101.4	99.0	101.4	91.0
3	96.6	99.0	99.0	99.0	91.0
Average	96.6	99.0	100.6	99.0	92.4
± *SD*	0.0	2.0	2.3	2.0	2.0

Only 0.02 μmole of material was needed per determination in this proposed procedure compared to approximately 1 g. or 7.1 mmoles in the NF method. Table III presents the results of assays on methenamine mandelate and methenamine hippurate powders and those on their tablet dosage forms. Tablets were crushed, dissolved, and filtered, if necessary, before an aliquot representing approximately 0.02 μmole was withdrawn for the determination. There appeared to be no significant interference in absorbance where color was formulated in the tablets because of the high dilutions involved in obtaining the extremely small amount of material.

It is clear from these data that the proposed 2-hydrazinobenzothiazole procedure possesses several distinct advantages over the present official methods for the analysis of methenamine or methenamine mandelate. It offers the convenience of a single method for the analysis of either the free base or salts. It has been found to be simple, sensitive, and less time consuming. It does not require prior standardization of a number of titrants, as for nessleriza-tion with the official assay of methenamine mandelate. Furthermore, the proposed method can be used for the assay of other salts of methenamine, unlike the USP procedure. With respect to the NF method, the lengthy period needed for the acid hydrolysis of methenamine using 1 *N* sulfuric acid as well as the uncertain end-point of the hydrolysis can be eliminated with the 2-hydrazinobenzothiazole procedure.

REFERENCES

(1) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 433.

(2) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 415.

(3) D. A. MacFayden, *J. Biol. Chem.*, **158**, 107(1945).

(4) C. E. Bricker and H. R. Johnson, *Anal. Chem.*, **17**, 400 (1945).

(5) V. Knight, J. W. Drapper, E. A. Brady, and C. A. Attmore, *Antibiot. Chemother.*, **2**, 615(1952).

(6) T. Nash, *Biochem. J.*, **55**, 416(1953).

(7) S. Belman, *Anal. Chim. Acta*, **29**, 120(1963).

(8) E. Sawicki, T. R. Hauser, and S. McPherson, *Anal. Chem.*, **34**, 1460(1962).

(9) P. W. West and B. Sen, *Z. Anal. Chem.*, **153**, 177(1956).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 16, 1973, from the *School of Pharmacy, West Virginia University, Morgantown, WV 26506*

Accepted for publication April 4, 1973.

The authors acknowledge the encouragement and support of Dr. R. O. Bachmann, the late Dean of the School of Pharmacy, in this work.

▲ To whom inquiries should be directed.

Chromatographic Separation and Assay of Morphine in Injectables

I. J. HOLCOMB, R. B. LUERS, Jr., and S. A. FUSARI▲

Abstract □ A method specific for the determination of morphine sulfate in injectables was developed. Morphine is separated from preservatives, pseudomorphine, and other degradation products by a column chromatographic method. The eluate containing morphine is extracted into an acid solution and determined spectrophotometrically. Recovery from standard solutions is better than 99%. The method presented is more specific for the separation and determination of morphine in injectables than present official methods.

Keyphrases □ Morphine sulfate injection—separation from preservatives and degradation products, UV analysis □ Column chromatography—separation, morphine from preservatives and degradation products, morphine sulfate injection □ UV spectrophotometry—analysis, morphine sulfate injection

Morphine has been well established as an effective analgesic and sedative since its isolation in 1805 (1, 2). Due to the limited solubility of morphine base in aqueous media, the more soluble hydrochloride and sulfate salts have been used in pharmaceutical preparations. The dosage form commonly employed since 1941 has been morphine sulfate as an injectable aqueous solution, since it is the most stable of the morphine salts in solution (3). When stored for a long time or exposed to heat, morphine sulfate injections develop a light-brown color.

The discoloration is not desirable, since decomposition of the dosage form is suggested and is, in fact, taking place; however, the extent of the discoloration is not an indication of the extent of decomposition. The cause has been attributed to the decomposition of morphine sulfate to pseudomorphine and further oxidation products (4–8). Foster *et al.* (5) and later Lach and Yeh (9–11) demonstrated that morphine injections decomposed first to pseudomorphine and then to oxidation products of pseudomorphine. Foster *et al.* (5) also showed that rubber closures contributed to the discoloration of the morphine injection and possibly to the decomposition of morphine sulfate.

Numerous assay methods have been developed for