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GLC Determination of Methenamine in Tablets

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Abstract □ A rapid and sensitive GLC method was developed for the quantitative determination of methenamine in tablets. The method was shown to possess several advantages over the official NF assay. After dissolution of the whole tablet in absolute ethanol and addition of an internal standard (pentylenetetrazol), an aliquot was injected into the gas chromatograph for analysis. The sample was chromatographed using a stainless steel column packed with 10% OV-17 on Chromosorb W-HP. Quantitation was achieved by measuring peak heights. The simplicity, directness, extreme rapidity, and accuracy of the method represent an improvement over the official method and the other proposed assays.

Keyphrases □ Methenamine—GLC analysis, commercial tablets □ GLC—analysis, methenamine, commercial tablets □ Antibacterials, urinary—methenamine, GLC analysis, commercial tablets

Methenamine is a urinary antibacterial useful in the long-term therapy of chronic urinary tract infections. The method most often used for the determination of methenamine is based on the hydrolysis of methenamine to formaldehyde and ammonia in a strong acid solution. The formaldehyde may be volatilized by continued heating and the excess acid may be back-titrated, or the liberated formaldehyde may be determined by colorimetry. However, these assays do not differentiate between decomposed and unhydrolyzed methenamine. The NF XIII (1) method involves the back-titration procedure and may be subject to errors due to the uncertainty of complete hydrolysis.

The official NF XIV (2) method and other commonly used techniques (3–5) for the determination of methenamine are based on reaction of formaldehyde with chromotropic acid. Since the decomposition product, formaldehyde, and not the intact methenamine is the analytically available moiety, any formaldehyde released by decomposition and trapped in the tablet is determined with the drug.

Other proposed methods include complexation (6), fluorometry (7), NMR spectroscopy (8), and a 2-hydrazinobenzothiazole reaction (9). This report presents a direct method of analysis of the intact methenamine by GLC. The procedure is rapid, simple, and accurate.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] equipped with a dual flame-ionization detector was used. The chromatographic column was a 3-mm (i.d.) stainless steel coiled column, 1.83 m in length, packed with 10% OV-17 on 80–100-mesh Chromosorb W-HP. The operating temperatures were 250° for the injection port, 190° (isothermal) for the column oven, and 250° for the detector. Nitrogen, with a flow rate of 60 ml/min, was the carrier gas. The flow rates of hydrogen and compressed air were adjusted to optimum sensitivity. The electrometer range was 10^{-10} amp/mv.

Materials and Reagents—Methenamine[2] and pentylenetetrazol[3] were used as received. Methenamine tablets[4,5] from two different companies were purchased in a local pharmacy. Absolute alcohol[6] USP was also used.

Analytical Calibration Curve—Six samples of methenamine[2], 105.0, 210.0, 420.0, 634.0, 845.0, and 1680.0 mg, were weighed. Each sample was placed into a 100-ml volumetric flask, and 10.0 ml of pentylenetetrazol internal standard alcoholic solution (50 mg/ml) was added and diluted to volume with absolute alcohol. Three microliters of each solution was injected into the chromatograph. The calibration curve was then obtained by plotting the known concentrations of methenamine against the corresponding peak height ratios (methenamine–pentylenetetrazol).

Tablet Analysis—Randomly selected tablets were individually wrapped inside glassine weighing paper and crushed. The powder was transferred to a 25-ml volumetric flask containing about 10 ml of absolute alcohol, shaken until dissolved, and diluted to 25 ml with absolute alcohol. Methenamine tablets contain no insoluble excipients and are totally soluble in the alcohol. Aliquots of 4.0 ml each were removed, placed into 10-ml volumetric flasks containing 1.0 ml of pentylenetetrazol internal standard solution (50 mg/ml), and diluted to 10 ml with absolute alcohol. Three-microliter samples of each tablet solution were injected into the chromatograph.

Calculations—The ratio of the peak height of the sample to the internal standard for each tablet solution was determined. The amount of methenamine per tablet was calculated from:

$$\text{mg/tablet} = \frac{R_{sa}}{R_{st}} \times \text{standard weight} \times \text{dilution factor} \quad \text{(Eq. 1)}$$

where R_{sa} is the ratio of the methenamine peak height to the internal standard peak height for the sample solution, and R_{st} is the methenamine–pentylenetetrazol peak height ratio for the known standard solution (5 mg/ml in absolute alcohol).

[1] Varian Aerograph model 1830.
[2] Riker Laboratories, Northridge, CA 91324.
[3] Metrazol, Knoll Pharmaceutical Co., Whippany, NJ 07981.
[4] Eli Lilly and Co., Indianapolis, IN 46206.
[5] Uritone, Parke-Davis and Co., Detroit, MI 48232.
[6] U.S. Industrial Chemicals Co., New York, N.Y.

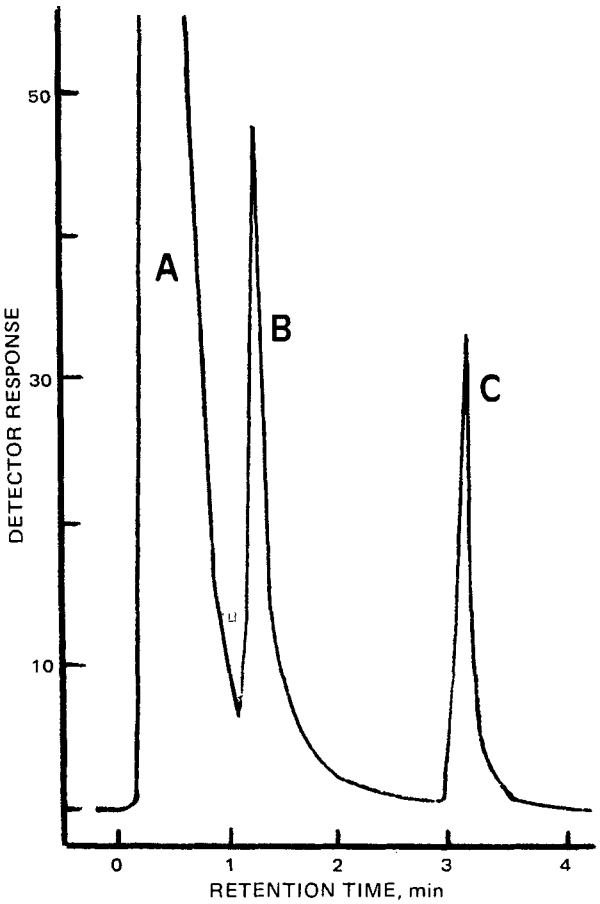

Figure 1—*GLC peaks. Key: A, solvent; B, methenamine; and C, pentylenetetrazol.*

RESULTS AND DISCUSSION

Figure 1 shows a typical gas chromatogram obtained for a tablet solution. Both the methenamine and the calibration marker, pentylenetetrazol, gave sharp, narrow, well-resolved peaks with no tailing. The retention time for each compound was relatively short, 1.2 min for methenamine and 3.0 min for pentylenetetrazol.

This analytical method was specific for intact methenamine. The retention time for formaldehyde, one of the decomposition products, was about 0.8 min. The flame-ionization detector did not respond to ammonia, the other decomposition product.

The linearity of the system was shown by analyzing various amounts of methenamine over the concentration range of 1.05–16.80 mg/ml with a constant amount of pentylenetetrazol (5 mg/ml). The correlation coefficient of the linear regression line was 0.998.

Results obtained by applying the proposed GLC method to known weights of methenamine are presented in Table I. The overall recovery for the five samples was 101.0 ± 1.85% (*SD*).

Six individual tablets from each commercial product were analyzed,

Table I—Assay Data for Known Amounts of Methenamine[a]

Amount Weighed, mg/ml	Amount Found, mg/ml	Recovery, %
3.10	3.12	100.6
3.75	3.85	102.6
4.80	4.92	102.5
5.30	5.20	98.1
5.60	5.68	101.4
	Mean recovery, %	101.1
	SD	1.85

[a] Crystalline powder, Merck & Co., Rahway, N.J.

Table II—Results of Analysis of Commercially Available Methenamine Tablets (325 mg/Tablet)

Tablet Number	Amount Found, mg/Tablet[a]	Percent of Claim
A 1	312.8	96.2
A 2	316.4	97.4
A 3	314.7	96.8
A 4	321.9	99.0
A 5	313.3	96.4
A 6	315.5	97.1
	Mean recovery, %	97.2
	SD	1.01
B 1	315.0	96.9
B 2	314.8	96.9
B 3	311.3	95.8
B 4	308.0	94.8
B 5	316.5	97.4
B 6	313.2	96.4
	Mean recovery, %	96.3
	SD	0.95

[a] Average of duplicate assays.

Table III—ANOVA Table for the Assayed Weight of Methenamine Tablets

Source	*df*	*SS*	*MS*	F_0
Companies	1	20.803	20.803	2.051
Error	10	101.427	10.143	—
Total	11	122.230	—	—

and the data are summarized in Table II. The ANOVA values for the assay of the tablets, based on a one-factor analysis of variance, are given in Table III. From the *F* table (10), $F_{1,10(0.05)}$ is 4.96. The statistical analysis of the data shows that there was no detectable difference in the means of the two different commercial tablets at the 5% level of significance.

The method presented represents an improvement over the official method and over other assays for methenamine. The GLC procedure is applicable to methenamine dosage form analysis and is simple, direct, and rapid.

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