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COLORIMETRIC ASSAY OF HEXAMETHYLENETETRAMINE

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Two photometric procedures are described for the determination of hexamethylenetetramine, free and in pharmaceutical preparations. The two methods are based on color formation by the ammonia released after the hydrolysis of hexamethylenetetramine. In the first method sodium-1,2 naphthoquinone-4-sulphonate reagent was added to the hydrolyzed hexamine sample. after adjustment of the pH of the solution to 9.3. When excess of reagent was eliminated, pink color appeared that was measured at 495 nm ($\varepsilon = 8.6 \times 10^3$).

Ninhydrin was applied, in the second proposed method, to the hydrolyzed sample of hexamethylenetetramine after adjustment of the solution pH to 6.5. The color produced had its maximum absorbance at 565 nm with high intensity ($\varepsilon = 1.2 \times 10^4$).

Beer's law was obeyed in the range of concentration of 2-20 mcg of hexamine per ml with standard deviation of ± 0.66 and 2-30 mcg/ml with SD ± 0.85 in the case of the first and the second methods respectively. The two methods were applied successfully to the analysis of commercially available hexamethylenetetramine tablets.

Hexamethylenetetramine, methenamine is urinary antibacterial agent. It is useful in the long term therapy of chronic urinary tract infections, beside its veterinary uses.

Methenamine gives its action in acid medium, thus it is usually adminstered in tablets with sodium biphosphate or cholic acid, or is prescribed as mandelate salt.

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The method most often used for the determination of methenamine is based upon its hydrolysis to formaldehyde and ammonia in strong acid standard solution. The formaldehyde is expelled and the excess acid is then titrated (3, 12, 17). Alternatively, the liberated formaldehyde may be determined colorimetricaly.

The NF XlV, and some other commonly used techniques for the analysis of methenamine, are based on the reaction of formaldehyde with chromotropic acid(2, 8, 10).

Such colorimetric procedures as those based on picrate formation or 2-hydrazinobenzothiazole reaction[19], complexometric, precepitimetric[14], non-aqueous titrimetric[6] or iodimetric[7].

The present work provides two new accurate colorimetric methods for the analysis of methenamine and for its dosage. These methods are based on the reaction of ammonia with sodium 1,2-naphthoquinone-4- sulphonate and with ninhydrin.

EXPERIMENTAL

Apparatus:

Spectra were made on Spectromom 203-ultraviolet and visible range(Mon, Budapest, Hungary). pH measurements were made using Titri pH-meter Type OP-401/2 with its glass and saturated calomel electrodes.

Materials:

Pharmaceutical grade methenamine was used as a working standard after the accurate determination of its percentage content by a compendial method[1]. All other chemicals were analytical grade. Solvents used were spectrograde.

Dosage forms:

The following commercial preparations were subjected

to the analytical procedure:-

1- Methenamine- cholic acid tablets(Felamine Tablets, Swisspharma, Cairo, Egypt): contain 225 mg methenamine per tablet.

2- Methenamine acid phosphate tablets (Methenamine -Potassium acid phosphate Tablets NF-X) prepared according to the compendial directions to contain 300 or 500 mg methenamine per tablet.

3- Methenamine Tablets:(USP XV111) Prepared according to the compendial directions to contain 300 or 500 mg methenamine per tablet using avicel, gum acacia, lactose and starch as diluents.

Reagents for Colorimetric Method-I:

a- Sodium 1,2- naphthoquinone -4- sulphonate solution (color Reagent I):- A 0.5% solution of the compound (Prolabo, Paris, France) in distilled water. The reagent was found to be stable for 24 hours at 0^{o}, protected against light.

b- Sodium Hydroxide solution:- 0.1 N in water.

c- Buffer solution (pH 9.3): A 0.01 M solution of borax in freshly boiled and cooled distilled water. The pH was standardized to 9.3 against glass and calomel electrodes.

d- Mixture of Acetic acid, Hydrochloric Acid and Formalin:

Three parts by volume of 1.5 N hydrochloric acid was mixed with one part glacial acetic acid and four parts of formalin solution.

e- Sodium Acid sulphite solution: 0.1 M solution of sodium hydrogen sulphite in distilled water.

Reagents for Colorimetric Method-II:

a- Ninhydrin solution (color Reagent II): A 0.5% of ninhydrin(E.Merck, Darmstadt) reagent was prepared in n-butanol. This reagent solution was stable for two weeks, when kept in a dark stoppered bottle at 4°C.

b- Buffer solution (pH 6.5): Ten ml of 0.5 M disodium phosphate solution was mixed with 15 ml of 0.5 M mono-sodium phosphate solution in 100-ml volumetric flask and completed to volume with freshly boiled and cooled distilled water. The pH was calibrated to 6.5 against the glass and calomel electrodes.

c- Ethanol- Acetone Mixture: Equal volumes of ethanol and acetone were mixed together.

Sample Preparation:

a- Standard Methenamine: The working standard(250 mg) was accurately weighed and dissolved in 25 ml distilled water.

b- Tablets: From a composite of ten finely powdered tablets, an amount equivalent to 250 mg methenamine was accurately weighed and extracted with three 10 -ml portions of distilled water. This extract was subjected to the hydrolysis procedure.

Hydrolysis of Methenamine Sample:

The prepared sample solution was transferred into a 50-ml round-bottom flask containing 2.5 ml concentrated hydrochloric acid. The mixture was then heated gently under a reflux condenser for 15 min, cooled and the condenser inner walls were washed into the flask using small amount of distilled water. The contents of the flask were quantitatively transferred to a 250-ml vol. flask and completed to volume with distilled water. Further dilutions were then made to obtain solutions containing 20-200 ug methenamine per ml.

One milliliter of this solution was used for color formation with either color Reagent, I or II.

Colorimetric Method I:

One milliliter of the prepared sample solution was transferred into a 10-ml volumetric flask and neutralized to phenolphthalein with 0.1 N sodium hydroxide. One milliliter of borate buffer and I ml of the color Reagent -I were added, and the solution was mixed and placed in boiling water-bath for 10 min. After cooling in an ice-bath for 5 min one milliliter of the mixture of acids and formalin was added followed by 1 ml 0.1 N sodium bisulfite.

The mixture was stirred and allowed to stand for-10-30 min. and the absorbance was measured at 495 nm.

Colorimetric Method II:

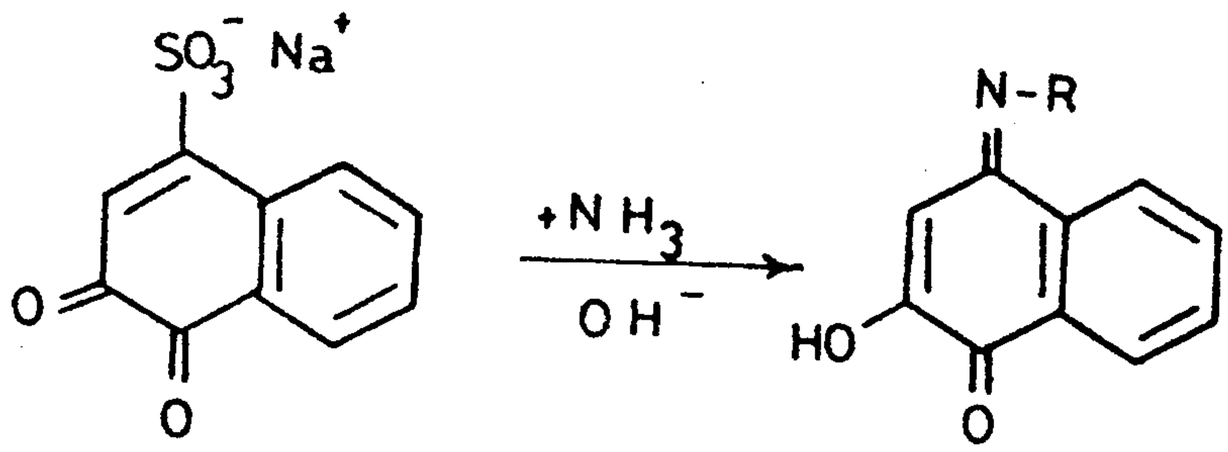
One milliliter of the prepared sample solution was transferred into a 10-ml volumetric flask containing 1 ml of ethanol-acetone mixture. The pH of the solution was adjusted to 6.5 by phosphate buffer (ca. 0.1 ml), then 2 ml of color Reagent-II was added. The solution was stirred and heated on a boiling water- bath for 20 min, then cooled in an ica bath for 5 min. The volume was finally completed to the mark by methanol, filtered if necessary, and the absorbance was measured at 565 nm. against a reagent blank.

RESULTS AND DISCUSSION

Method I:

Reaction Involved:

In the method presented it is assumed that the ammonia released from methenamine hydrolysis interacted with the O-quinone(1,2-naphthoquinone -4- sulphonate, Folin-Wu Reagent Yeilding a measurable chromogen[5]; (λ_{max} 295 and ε_{max} 8,600) in alkaline medium (Scheme 1)



Scheme 1

The chromogen formed belongs to the class of 4-amino naphthoquinones and it has been used by many authors for for the determination of free ammonia, as well as for aliphatic and aromatic amines(4,5,11,13,16).

In the previous reports excess reagent was decolorized with sodium thiosulfate, in presence of sodium lauryl-sulfate and formaldehyde. Formaldehyde was included to delay the decomposition of sodium thiosulphate in acid medium[4]. In the presented assay method, a mixture of sodium bisulfate and formaldehyde was used instead.

With pure ammonium chloride, identical absorption spectrum was obtained, suggesting the same basic interacting chemical species. This allows the use of ammonium chloride as a working standard in place of hydrolized methenamine.

Accuracy, Precision and Sensitivity:

High degrees of accuracy and prècision were obtained by analyzing aliquots of different concentrations of standard methenamine(Table I). Confermance to Beer's law was observed in the range of 2-20 µg/ml final dilution of methenamine.

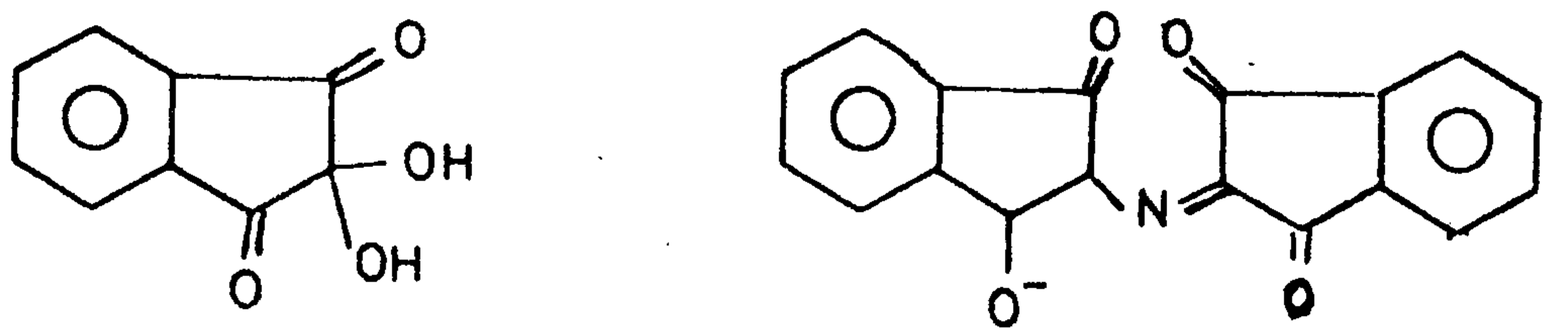
Application to Dosage Forms:

The applicability of the method to dosage forms was checked by analyzing two simple preparations containing methenamine and the results are shown in Table I. The amount of the drug in the prepatation was calculated by reference to the calibration curve.

Method II:

Reaction Involved:

The reaction of ninhydrin (I, Scheme2) with amino acids, primary or secondary amines has been used for the colorimetric determination of these compounds[18]. The reaction with amino acids has been explained to be due to oxidative deamination of the amino acid to carbon dioxide, ammonia and an aldehyde containing one less carbon atom than the parent amino acid. The reaction proceeds with the simultaneous reduction of ninhydrin to hydrindantin. Condensation of this latter with the liberated ammonia then yields the colored product(II, Scheme 2).



I II

Scheme 2

Selection of Assay Procedure:

Many assay procedures have been suggested for the amino acid determination using ninhydrin reagent(15.18).

One of these techniques involves the use of pyridine to intensify the color formation. This procedure has also been used for the analysis of oral hypoglycemic agents[1]. It has been reported that the presence of formaldehyde prevents the color formation. On applying this pyridine procedure for the analysis of methenamine, it was found that the formalin produced during the acid hydrolysis, though not preventing the color formation completely, it interfered with the intensity and the reproducibility of the formed color. Therefore, several trials were made in an attempt to

prevent the interference caused by formaldehyde in this pyridine procedure. These trials included:

a- Expelling formaldehyde as a gas by applying direct heat until the odour of formaldehyde was undetectable. This technique was found to be time consuming, in addition to the difficulty of detecting the last traces of formaldehyde.

b- Addition of sodium bisulphite aiming to form a non-reactive additive compound. However, the product was found insoluble in the reaction medium rendering the method impractical.

c- Use of a cyanide buffer[15], which was expected to form a weakly dissociable complex with formaldehyde. However, non-reproducible results were obtained.

Consequently, the pyridine procedure was rejected and another assay procedure was tried. This second technique was reported for the assay of the amino acids at pH 6.5. On application of this method successful results were obtained. This leads to suggestion that at this pH the interference of formaldehyde was minimal, possibly because this pH is not too acidic to inhibit color formation and not too basic to give chance for the reversible reaction to occur, i.e, reformation of methenamine;

$$6\ HCHO + 4\ NH_3 \underset{\text{acidic pH}}{\overset{\text{alkaline pH}}{\rightleftharpoons}} (CH_2)_6\ N_4 + 6\ H_2O$$

or to allow secondary undesired side reations of HCHO in alkaline medium to occur. Accordingly, this weekly acid procedure was adopted for the determination of methenamine.

Peak Position and Intensity of Chromogen:

The chromogen produced from the interaction of hydrolyzed methenamine and ninhydrin reagent exhibits an absorption maxium at 565 nm and molar absorptivity of 8,400.

With pure ammonium chloride, identical absoption spectrum was obtained, suggesting the same basic interacting chemical species. This also allows the use of ammonium chloride as a working standard in place of hydrolyzed methenamine.

On applying the assay procedure to analyze unhydrolyzed methenamine, similar color was formed but having lower absorbance readings amounting to about 20% of the readings for the hydrolyzed compound suggesting the formation of the chromogen without prior hydrolysis. This result may be explained on basis of partial hydrolysis at the acidic pH of the procedure.

Sensitivity, Accuracy and Precision:

Beer's law is valid at 565 nm over a concentration range of 2-16 µg/ml of methenamine. Accuracy and precision of the presented method were tested by analyzing aliquots of different concentrations of standard methenamine solutions. Average recovery of 99. 1% and SD of1.47 were obtained on applying the presented method (Table II)

Application to Dosage Forms:

The applicability of the method to dosage forms was checked by analyzing two simple preparations containing methenamine and the results are in Table-II. The amount of the drug in the preparation was calculated by reference to calibration curve.

It is clear from this report that the provided two colorimetric methods for the analysis of methenamine, free and in its dosage forms, are convenient and accurate. They offer greater versatility over other colorimetric methods depending on the aldehyde part, where other aldehydes and sugars may interfere with the determination.

Table I- Analysis of Bulk Drug and Dosage Forms of Methenamine by Method I.

Sample or Tablet[a]	Methenamine Sample or Tablet			Standard Methenamine		
	Taken, mg or mg/ Tablet	Recovery[b] %	SD ±	Added, mg	Recovery %	SD ±
Bulk drug	2	97.7		0.21		
2	5	101.1		0.36		
3	10	99.1		0.60		
Methenamine	300	67.9	0.66	300	100.5	1.35
tablets NF X	500	97.4	0.45	500	100.2	1.20
Felamine Tablet	225	95.8	0.52	225	99.2	0.82

a- Detailed composition under experimental,

b- Average of three determinations.

Table II- Analysis of Bulk Drug and Dosage Forms of Methenamine by Method II

Sample or Tablet[a]	Methenamine Sample or Tablet			Standard Methenamine		
	Taken, mg or mg/ Tablet	Recovery[b] %	SD ±	Added, mg	Recovery %	SD ±
Bulk Drug	4	98.5	1.81			
2	8	100.3	0.85			
3	12	98.6	1.76			
Methenamine Tablet NF X	300	99.4	1.25	300	99.5	0.27
	500	100.5	0.48	500	99.9	1.61
Felamine	225	101.2	1.25	225	100.7	1.47

(a) Detailed composition under experimental.

(b) Average of three determination.

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تعيين الهكسامين بطـرق لونيـــــة

على محمود طـه ـ نـوال على الرباط ـ فـردوس عبد الفـتاح
كلية الصيدلة ـ جامعة اسيوط ـ ج ٠ م ٠ ع

أن الطرق المعروفة لتعيين الهكسامين ـ المادة المستعملة لتطهير المجارى البوليـــة تعتمد على تحليل المركب فى وجود حمض قوى ثم تعيين كمية الفورمالدهيد المتصاعد او تعييـن كمية الحامض المتبقى بعد تمام التحليل ٠

اما الطريقة الحالية فتختص بتعيين الهكسامين باستعمال المتفاعلات التى تكون الوان مع النوشـادر عادة فى تعيين الامينـات الاوليـة

من هذه التفاعلات اختير المركب (١) ـ ١ ر٢ـ نافتوكينون ـ ٤ سلفونيت الصوديوم والمركـب يستخدم عادة فى تعيين الامينات الاوليـة

والمركب الثانى (٢) واسمه النينهدرين يستخدم فى تعيين الاحماض الامينية ٠ ولقـــد تعرض البحث الحالى لدراسة تاثير حموضة المحلول على كل من الالوان التى امكن الحصـول عليها باضافة كل من المتفاعلين المذكورين نتيجة لذلك تم التوصل للاتى :

المتفاعل (١) عند اضافته لمحلول الهكسامين المتحلل بحمض قوى وتمت معالجته بالبورات ثم بخليط من حمض الخليك والايدروكلوريك والفورمالين وامكن الحصول على لون احمر قيس عنـد ٤٩٠ نم بدرجة امتصاص جزئ بلغت ٨٦٠٠

المتفاعل (٢) باضافته لمحلول هكسامين متحلل بحمض قوى ثم ضبطت درجة حرارته ودرجـة حموضته بملح الفوسفات القلوى ـ تم ظهور لون بنفسجى امكن قياسه عند ٥٦٥ نم بامتصـاص جزئ يقـدر بـ ١٢٠٠٠

تمتاز هاتان الطريقتان بالدقة اذ امكن تعيين الهكسامين فى مدى تركيز من ٢ ـ ٣ ميكروجرام/مللى وبدرجة دقة جيدة من ٩٨ر٥ ـ ١٠١ر٥٪ خطأ ـ وحياد قياسى وصـــل الى ± ٠٠٥ر٠

ولقد امكن تطبيق الطريقتين بنجاح فى تعيين الهكسامين منفردا وفى الاقراص فى وجـــود حمض الكوليك وفوسفات الصوديوم الحمضى دون تداخل اى منهما فى التعيين ٠