SPECTROPHOTOMETRIC DETERMINATION OF GLUCOSAMINE AND MANNITOL WITH 4-AMINO-5-HYDRAZINO-4H[1,2,4]-TRIAZOLE-3-THIOL IN PHARMACEUTICAL FORMULATIONS

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تستخدم ترايازولات المتماثلة وذلك لإنتاج كواشف قادرة على التفاعل مع الأدوية المحتوية على مجموعة كاربونيليه وكذلك مع الأدوية الحساسة للأكسدة بواسطة الحمض فوق اليودي periodic acid وذلك لإنتاج مركبات محتوية على مجموعة كربونيلية مثل دايولات diols وأمينات كحولية. وفي هذه الدراسة تم تحليل مادتي جلوكوزامين ومانيتول، من خلال الأكسدة بالحمض فوق اليودي، للحصول على مادة فورمالدهيد والتي تركت لتكثف مع مادة 4-أمينو-5-هيدرازينو-4 يد[4,2,1] – ترايازول -3-ثيول. وتم أكسدة المكثف الناتج للحصول على مركب أرجواني اللون له أقصى امتصاص عند 550 نانومتر. وقد امتثل لقانون "بير" في مدى التركيز 12.5 - 125 مكغ/مل لمادة جلوكوزامين و 25 – 125 مكغ/مل لمادة مانيتول. وقد تم تقدير الدوائين أيضاً بنجاح في صيغهما الصيدلانية بنسبة استرداد ± انحراف معياري نسبي بين 100.69–101.15% ± 1.57–0.44 لمادة جلوكوزامين و 100.06% ±1.08 لمادة مانيتول.

s-Triazoles have been utilized to produce reagents that can react with drugs containing carbonyl group and drugs that are susceptible to oxidation with periodic acid to produce carbonyl function such as diols and amino alcohols. In this study, glucosamine and mannitol were analyzed through oxidation with periodic acid to give formaldehyde which was allowed to condense with 4-Amino-5-hydrazino-4H[1,2,4]-triazole-3-thiol (AHTT) (I). The condensation product was further oxidized to yield a purple colored compound (II) with maximum absorption at 550 nm. Beer's law was obeyed in the range of 12.5-125 μg ml^{-1} for glucosamine and 25-150 μg ml^{-1} for mannitol. Both drugs were also successfully determined in their pharmaceutical formulations with mean percentage recoveries ± RSD ranged between 100.69-101.51% ±1.57-0.44 for glucosamine and 100.06 % ±1.08 for mannitol.

Key words: 4-Amino-5-hydrazino-4H[1,2,4]–triazole-3-thiol, drug analysis, glucosamine, mannitol, colorimetry.

Introduction

Glucosamine (GL) or chitosamine is 2-amino sugar compound found in chitin, in mucoproteins and in mucopolysaccharides (1). Glucosamine was isolated from chitin or prepared synthetically. It has been used in the treatment of rheumatic disorders including orthoarthritis (2). Mannitol (MT) is a sugar alcohol. It is useful as a medicinal agent acting as osmotic diuretic and it is indicated as an irrigating solution in trans-urethral prostatic resection (3). GL has been reported to be determined by different techniques including radiometry (4), potentiometry (5), spectrophotometry that involves the reactions of glucosamine with 0.2% 4-dimethylaminobenzaldehyde solution (6), the first derivative UV spectrophoto-metry (7), or with palladium(II)-*o*-hydroxyhydroquinonephthalein-hexadecyltri-methylammonium complex (8). GL has been also extensively

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determined by HPLC (9-14). Different techniques have been reported for the analysis of MT including polarography (15), colorimetric assay using a mixture of acetyl acetone, ammonium acetate and sodium-thiosulfate and measuring the absorbance at 412 nm (16). Also HPLC (17-21), GC (22-24) and capillary electrophoresis (25) techniques were applied for determination of MT.

The aim of the present work is to develop a simple and accurate method for the determination of glucosamine and mannitol that permits their analysis in their dosage forms without interference from their additives, excipients and other co-formulated drugs.

Experimental

1. Materials:

a. 4-Amino-5-hydrazino-4H[1,2,4]-triazole-3-thiol reagent (AHTT), it was synthesized according to a reported procedure (26).
b. Periodic acid (Winlab, U.K.).
c. Authentic samples:
Reference standard glucosamine sulfate (GL) provided by EVA Pharma for pharmaceutical and medical appliances, Egypt. Mannitol (MT) provided by the Nile Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt.
d. Pharmaceutical preparations: Joflex® capsules were obtained from Global Napi Pharmaceutical for Ema Pharm. Company. Batch No. 37506; label claim for each capsules were 500 mg glucosamine sulphate. Glucosamine® capsule, was obtained from EVA Pharma; Batch No. 308424; label claim for each capsule was 500 mg glucosamine sulfate. Mannitol®10% intravenous infusion was provided from Otsuka Pharmaceutical Company, S.A.E 10th of Ramadan City, Egypt; Batch No. 2B752B.
e. All other chemicals used were of analytical grade and water was freshly distilled.

2. Reagents and standard solutions:

a. AHTT solution: 0.5% in 0.5N hydrochloric acid.
b. Periodic acid solution: 1 mg ml^{-1} solution of periodic acid in 0.2N potassium hydroxide.
c. Standard solutions:
- Glucosamine (GL) standard solutions: one mg ml^{-1} solution of glucosamine was prepared in distilled water. Aliquots of this solution were diluted to produce working solutions of 12.5, 25, 50, 75, 100 and 125 µg ml^{-1} of GL. The solutions were stable at room temperature for at least two weeks.
- Mannitol (MT) standard solutions: one mg ml^{-1} solution of mannitol was prepared in distilled water. Aliquots of this solution were diluted to produce working solutions of 25, 50, 75, 100, 125, and 150 µg ml^{-1} of MT. The solutions were stable at room temperature for at least two weeks.

3. *Apparatus*:
Shimadzu UV/VIS 1601 spectrophotometer.

4. Procedure:

4.1. Assay of Glucosamine and Mannitol:

One ml of each working and assay solutions of both drugs were transferred in a test tube then 1.0 ml periodic acid was added. The mixture was left at room temperature for 15 minutes for GL and 20 minutes for MT, 0.5 ml 5N KOH solution was then added followed by 1 ml of AHTT solution. The mixture was shaken and allowed to stand for about 10 minutes for both drugs. Absorbance of the resulting solution was measured at 550 nm, against blank experiment. Calibration curves relating the absorbance at 550 nm to GL or MT concentrations were plotted and regression analysis of the results was computed.

4.2. Assay Glucosamine and Mannitol in dosage forms:

- In **Joflex®** capsules: The well mixed powdered content of 10 capsules was used in the assay. An amount equivalent to 100 mg of GL was transferred into 100 ml volumetric flask, dissolved in distilled water then adjusted to volume and treated as previously mentioned.
- In **Glucosamine®** capsules: The contents of 10 capsules were mixed well and amount equivalent to 100 mg GL was dissolved in distilled water and filtered. The filtrate was diluted to 100 ml with distilled water and treated as previously mentioned.
- In **Mannitol®** 10% I.V. infusion: One ml of the preparation was transferred into 100 ml volumetric flask then diluted to volume with distilled water and treated as previously mentioned.

Results and Discussion

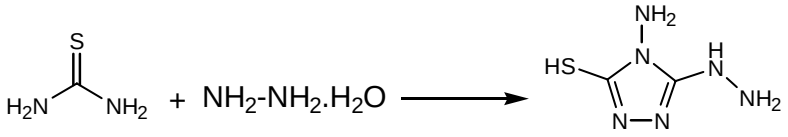
In the present work, the selective oxidizing effect of periodic acid on glucosamine and mannitol was utilized to convert them into formaldehyde and the corresponding carboxylic acids. This reaction was taken as a base in order to develop a colorimetric method for their determination.

The liberated aldehyde was allowed to react with 4-Amino-5-hydrazino-4H[1,2,4]-triazole-3-thiol, which is a specific reagent for aldehydic group[27]. AHTT was synthesized by reaction of hydrazine with thiourea following a reported procedure[26] as shown in Scheme (1).

When AHTT was allowed to condenses with aldehydes followed by treatment with periodic acid and alkalinization, it produces a colored product, [1,2,4]triazolo-[4,3-b][1,2,4,5]tetrazine-3-thiol as shown in (Scheme 2).

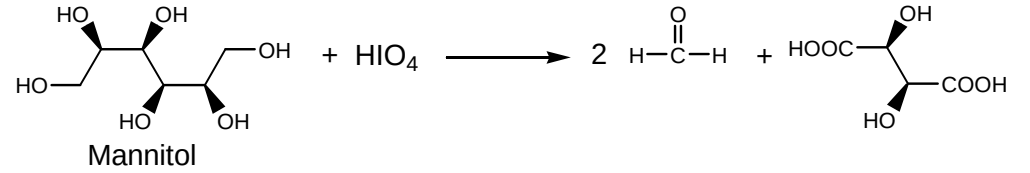
As reported by Jacobsen and Dickinson (27), the reaction involved addition of an alkaline solution of AHTT to aldehyde solution and treating the reaction mixture to give the purple colored product. Recently Mimura *et al.* (28), modified the procedure of color development by the use of periodic acid as oxidizing agent instead of aeration.

In the present study, periodic acid has a dual function. It acts as a selective oxidizing agent for polyhydroxy compounds to convert them to formaldehyde and corresponding carboxylic acids. The development of the purple colored product was obtained according to Mimura *et al.* (28) modification. It is important to emphasize that Jacobsen and Dickinson (27) used alkaline solution of AHTT (1% in 1N NaOH) for the color development with aldehydes. However this procedure was modified by using acidic solution of AHTT (0.5% in 0.5N HCl). This offers two advantages, the first was the use of lower concentration of AHTT, and the second was the higher stability of AHTT solution as mentioned by Mimura *et al.*(28).

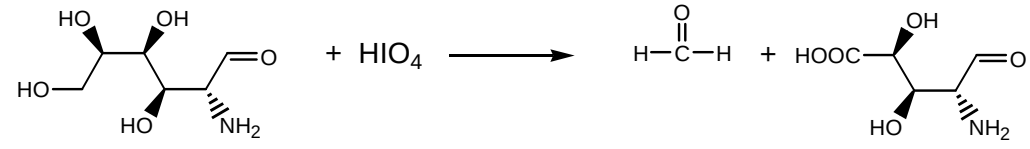


(I)

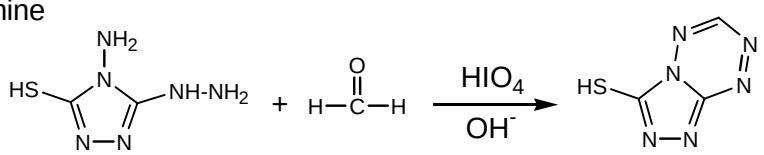
Scheme (1): Synthesis of AHTT (I)



Mannitol



Glucosamine



AHTT (I) colored product(II)

Scheme (2): Reactions involved in analysis of Mannitol and Glucosamine

As reported for colorimetric determination of some diol-containing drugs (29) and certain amino-glycosides (30), solution of glucosamine and mannitol in the present study were left with periodic acid solution for sometime, then 5N KOH and AHTT solutions were added whereby a purple color was developed with maximum absorption at 550 nm (Figures 1 and 2). Maximum color intensity was obtained when periodic acid solution was made to react with glucosamine for 15 minutes and mannitol for 20 minutes (Figure 3).

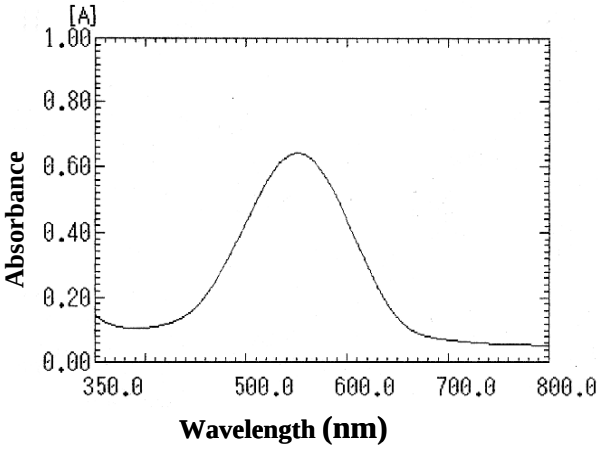


Figure 1. Absorption spectrum of the colored product produced from the reaction between AHTT and 75 µg ml^{-1} glucosamine.

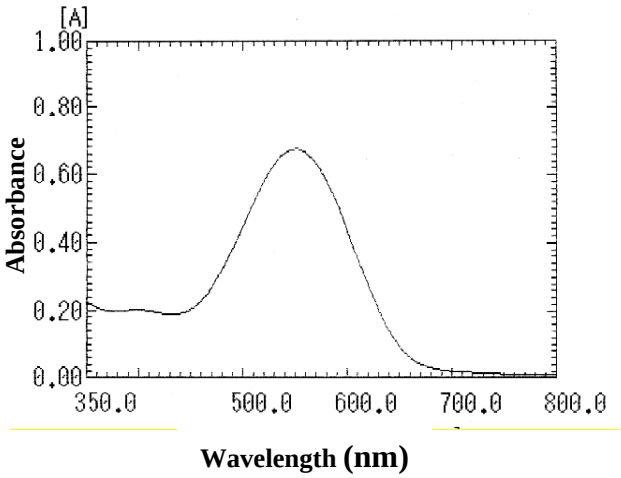


Figure 2. Absorption spectrum of the colored product produced from the reaction between AHTT and 100 µg ml^{-1} mannitol.

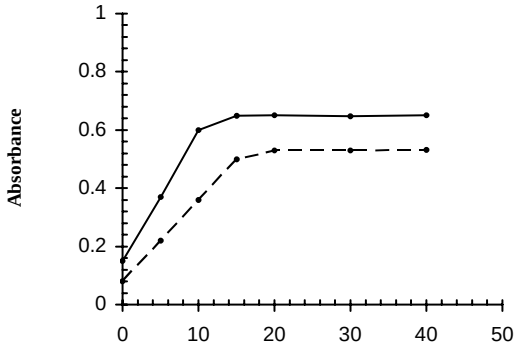


Figure 3. Effect of time of reaction of 75 µg ml^{-1} GL (—) and 75 µg ml^{-1} MT (---) with periodic acid on the intensity of the color.

The effect of periodic acid concentration was also studied and was found to be critical; the use of 1 mg/ml^{-1} solution of periodic acid in 0.2 N KOH produces maximum color intensity. Excess periodic acid solution causes a great decrease of the intensity of the produced color which is attributed to the strong oxidizing effect of periodic acid on both drugs which may proceed to give further oxidation products.

The effect of AHTT concentration was also studied where maximum intensity was obtained upon using AHTT solution of 0.5% in 0.5N HCl. Shaking of the reaction mixture for 4-5 minutes was essential after the addition of AHTT solution and waiting period of 10 minutes produced maximum color intensity. The obtained color remained stable for about 40 minutes.

Linearity was obtained in a concentration range of 12.5-125 µg.ml^{-1} of GL solution and 25-150 µg ml^{-1} of MT solution. The corresponding regression parameters are shown in Table (1), which also shows the spectral characteristics for both drugs where the obtained correlation coefficients (0.9991-0.9998) indicate perfect linearities. The values of A(1%,1cm) of the obtained purple color were found to be 86 and 69 for GL and MT respectively.

Intraday and inter-day precision (RSD%) were ranged between 0.22 and 2.45 (Table 1), providing that the proposed procedure is accurate and precise. The repeatability and reproducibility of the instrumental response (absorbance of the formed color) were checked during method development and they were assessed from five replicate determinations of sample solutions of GL and MT at

the concentration 50 μg ml^{-1}. The RSD% for repeatability and reproducibility were less than 0.99 and 0.74 respectively.

The proposed procedure was also applied for the determination of both drugs in their pharmaceutical formulations; however, the procedure could not be used as a stability-indicating assay as the degradation product still contains a diol function. The results presented in Table (2) revealed that there is no interference from excipients, additives or co-formulated drugs such as ascorbic acid present in glucosamine capsules. In addition the recoveries of the two drugs from their formulations were almost the same as the recoveries of the pure added when applying the standard addition technique. The mean percentage recoveries ± RSD of added were ranged between 100.06-100.22% ± 0.60-0.86 for GL and 99.63% ± 1.04 for MT (Table 2).

In addition, the results obtained by the proposed procedure were statistically compared with those obtained from the official methods for GL[31] and MT[32]. The calculated ***t*** and ***F*** values are less than the tabulated ones indicating no significant difference between the proposed and official methods with respect to accuracy and precision at 95% confidence limit (Table 3).

Conclusion

The proposed procedure is selective for polyhydroxy aliphatic compounds, simple and rapid as it consumes from 15 to 20 minutes for the sample to be ready for measuring. It is useful in routine analysis of glucosamine and mannitol in their pharmaceutical formulations and in-process quality control.

Table 1: Selected spectral data of the proposed procedure for the determination of GL and MT.

Parameters	Glucosamine	Mannitol
Linearity range (μg ml^{-1})	12.5-125	25-150
Regression Parameters		
Slope ± SD(s_b)	0.0083 ± 4.305	0.0069 ±1.03 E-04
Intercept ± SD(s_a)	0.0134 ± 0.0032	-0.0049 ±0.010
SD of Residual (S_{xy})	7.04 E-05	4.67 E-04
Correlation coefficient	0.9998	0.9991
Accuracy		
Intraday R%	101.6-102.8	98.0-101.1
Interday R%	101.2-102.0	98.1-101.3
Precision		
Intraday R%	0.78-1.55	0.22-0.56
Interday R%	0.25-1.73	1.21-2.45

* n = 4

Table 2: Application of standard addition technique for determination of GL and MT in their pharmaceutical formulation by the proposed procedure.

Recovery ± RSD %.			
Glucosamine Formulation		**Mannitol Formulation**	
Joflex ® capsules B.N 37506	100.69 ± 1.57	***Mannitol*** 10% I.V infusion B.N 2B 75.2B	100.06 ± 1.08
Standard addition	100.06 ± 0.60	Standard addition	99.63 ± 1.04
Glucosamine® capsules B.N 308424	101.51 ± 0.44		
Standard addition	100.22 ± 0.86		

Table 3: Statistical analysis of the results obtained by the proposed and reported procedures for the determination of GL and MT in their pharmaceutical formulations.

Parameters	Joflex® capsule	Reference [31]	Glucosamine® capsule	Reference [31]	Mannitol 10% I.V infusion	Official BP Method [32]
N	4	4	4	4	4	4
Mean %	100.69	99.69	101.51	99.81	100.06	99.63
SD	1.57	1.57	0.44	1.26	1.08	1.01
Variance	2.46	2.46	0.19	1.58	1.16	1.02
t	0.90	-	1.78	-	0.58	-
F	1.00	-	8.35	-	1.14	-

The theoretical value of $\boldsymbol{F}$ = 9.28 and $\boldsymbol{t}$ = 1.94 at (P = 0.05).

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