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Indirect Spectrophotometric Assay of Hydroxyurea with its Pharmaceutical Application as Capsules

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ABSTRACT

The oxidation–reduction reaction between hydroxyurea and ferric ions has been utilised for spectrophotometric assay of hydroxyurea. The ferrous ions that are produced from the redox reaction were complexed with 2,2′-bipyridyl reagent to form a pink-red tris-chelate which has an absorption maxima at 522 nm. Beer's law agreed with the range of 5-150 µg of hydroxyurea per 20 ml, i.e., 0.25-7.5 ppm, with a corresponding average molar absorptivity of $1.65x10^4$ $l.mol^{-1}.cm^{-1}$, Sandell sensitivity index is 0.0044 $\mu g.cm^{-2}$, limit of detection 0.01$\mu g.ml^{-1}$ and limit of quantitation of 0.03 $\mu g.ml^{-1}$. The present method has been applied successfully to the determination of hydroxyurea in its pharmaceutical preparation as capsule.

Keywords: Hydroxyurea, 2,2′-bipyridyl, spectrophotometry.

التقدير الطيفي غير المباشر للهيدروكسي يوريا وتطبيقاته في مستحضر صيدلاني على شكل كبسول

الملخص

تم في هذا البحث تقدير الهيدروكسي يوريا في المستحضر الصيدلاني عن طريق تفاعلات الاكسدة والاختزال وتعتمد الطريقة على اختزال ايون الحديد الثلاثي بوساطة الهيدروكسي يوريا الى ايون الحديد الثنائي ثم مفاعلة الحديد الثنائي مع الكاشف 2,2′- ثنائي البيريديل لينتج معقداً كليتياً مستقراً ذا لون احمر-وردي وأعطى أعلى امتصاص عند طول موجي قدره 522 نانوميتر. وكانت حدود قانون بير للطريقة المقترحة في مدى التراكيز 5-150 مايكروغرام/20 مل أي (0.25-7.5) جزء لكل مليون، أما قيمة معامل الامتصاص المولاري فكانت 1.65×10^{-4} لتر.مول$^{-1}$.سم$^{-1}$ ، ودلالة ساندل كانت 0.0044 مايكروغرام. سم$^{-2}$ أما حد الكشف للطريقة فكان 0.01 مايكروغرام. مل$^{-1}$ وحد التقدير 0.03 مايكروغرام.مل$^{-1}$. تم تطبيق الطريقة بنجاح على المستحضر الصيدلاني للهيدروكسي يوريا على شكل كبسول.

INTRODUCTION

Hydroxyurea, $H_2NCONHOH$, is considered to be the derivative of hydroxylamine (Kovacic, 2011) or of the symmetrical urea molecule (Donehower, 1990). Since hydroxyurea blocks DNA synthesis by inhibiting ribonucleotide reductase (Koç *et al*., 2004), it therefore represents an effective treatment for sickle cell anemia and a number of cancers especially leukemias (Huang *et al*., 2002; Zhou *et al*., 2002 ; Rodriguez *et al*., 1998; Sommers *et al*., 2001; Kwon *et al*., 1991). Furthermore, it can serve as a source for the production of NO which plays an important role in the maintenance of normal blood pressure flow (Rupon *et al*., 2000). Such activity has led to the suggestion that hydroxyurea could be a possible HIV therapy (Frank *et al*., 2004). Hydroxyurea has also recently emerged as a new approved treatment for sickle cell disease (Lanzkron *et al*., 2008; Silva *et al*., 2011). The beneficial effects of hydroxyurea treatment to the sickle cell patient appear to result from an increase in the production of fetal hemoglobin (Platt, 2008), a genetically distinct hemoglobin that prevents the polymerisation of deoxy sickle cell hemoglobin (Steinberg *et al*., 2011).

To the best of our knowledge, the only visible spectrophotometric method for the determination of hydroxyurea is that based on its oxidation with excess iodine to form nitrite. The latter was allowed to diazotize sulfanilic acid to form the corresponding diazonium salt which is subsequently coupled with N-(1-naphthyl)ethylenediamine to form a purple azo dye which has absorption maxima at 540 nm. The method has been used to find the stability of hydroxyurea in oral solution (Heeney *et al*., 2004).

The present study is devoted to describe the development of a spectrophotometric method which is based on the color intensity of the pink tris-chelate of ferrous-bipyridyl system as a measure of original amount of hydroxyurea present in solution.

EXPERIMENTAL

Apparatus

Spectrophotometric measurements were performed on, using a Shimadzu UV-160 double-beam recording spectrophotometer with 1-cm quartz cells.

pH readings were performed using HANNA pH 211 Microprocessor pH meter with a combined glass electrode.

Reagents

All chemicals used were of the highest available purity.

Hydroxyurea stock solution (1000 µg.ml^{-1}). This solution was prepared by dissolving 0.025 g of the compound (NDI, Iraq) in distilled water; the resulting solution was diluted to 25 ml.

Hydroxyurea working solution (50 µg. ml^{-1}). This solution was prepared by diluting 5 ml of the stock hydroxyurea solution to 100 ml with distilled water. This solution is stable at least for 15 days.

Buffer solution (pH 2.7). This solution was prepared by mixing 50 ml of 0.1 M glycine solution with 12.1 ml of 0.2 M HCl solution and diluting with distilled water to 100 ml in a volumetric flask (Perrin and Dempsy, 1974).

Ferric ion solution (1x10^{-2} M). This solution was prepared by dissolving 0.121g of $Fe(NH_4)(SO_4)_2.12H_2O$ (Riedel-De Haen AG) in distilled water that contains two drops of concentrated sulfuric acid and diluting the over all solution to 25 ml.

2,2´-Bipyridyl reagent solution ($1x10^{-2}$ M). This solution was prepared by dissolving 0.156 g of the compound (ABCR GMbH and Co.KG) in 4 ml of ethanol then diluting the volume to 100-ml with distilled water.

Foreign compound solutions (1000 µg.ml^{-1}). These solutions were prepared either by dissolution in distilled water or in some ethanol and diluting to the mark with distilled water in a 100- ml volumetric flask.

Capsule solution (500 mg hydroxyurea). This solution was prepared by dissolving an amount of capsule powder content (0.05g) (Filaxis, Argentina) and diluting to volume (100 ml) with distilled water.

Recommended procedure and calibration graph

To a number of 20-ml volumetric flasks, 5 to 150 µg of hydroxyurea, 2 ml of ferric ion solution and 5 ml of glycine buffer solution were added. The solution was left to stand for 5 minutes, then 3 ml of the chromogenic reagent solution (2,2´-bipyridyl), and finally distilled water was added to the mark. After 5 minutes, the absorbances against the corresponding reagent blank were measured at 522 nm. The equation of the linear regression is $A=0.2176\ C^{f}_{ppm} + 0.0143$ (R^2=0.9992, n=12) [Fig. (1)]. Beer's law was agreed with the range of 5-150 µg of hydroxyurea per 20 ml (over this range will be negative deviation). The limit of detection and limit of quantitation are 0.01 µg ml^{-1} and 0.03 µg ml^{-1}, respectively (Valcarcel, 2000). The average molar absorptivity, with respect to hydroxyurea, at the wavelength of maximum absorption was $1.65x10^4$ l. mol^{-1}.cm^{-1} and the sensitivity index was 0.0044 µg.cm^{-2}.

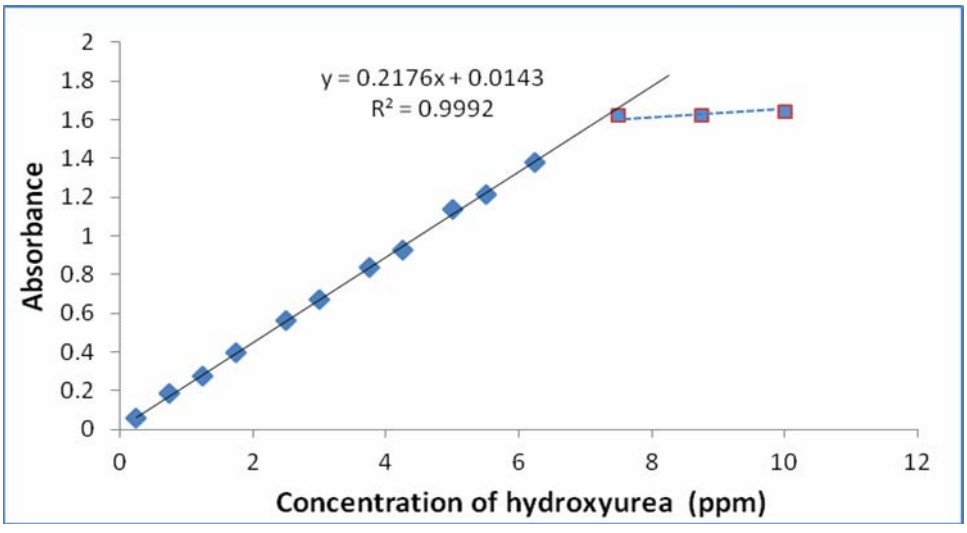

Fig. 1: Calibration graph for determination of hydroxyurea depending on the reaction between ferrous ion with 2,2´-bipyridyl reagent.

RESULTS AND DISCUSSION

Principle of the method

Hydroxyurea reduces ferric ions to the ferrous state which are subsequently complexed with 2,2´-bipyridyl (chromogenic reagent) to form the well-known tris-chelate of a red-pink color with absorption maxima at 522 nm. This wavelength was maintained for absorbance measurements.

For the subsequent experiments, 50 µg hydroxyurea was taken and final volumes were 20 ml.

Optimisation of experimental conditions

The various parameters affecting and related to the color intensity had been examined and optimized.

Effect of pH

The effect of pH on the color intensity was tested through the addition of (0.1-1.0) ml of 0.1M H_2SO_4 to the reaction mixture, then measuring the absorbance of the final (20 ml) solution against the blank solution, the results are shown in Table 1.

Table 1: Effect of pH on absorbance.

ml of 0.1 M H_2SO_4	Absorbance	Final of pH
0	0.500	3.67
0.1	0.546	2.93
0.2	0.552	2.75
0.3	0.556	2.67
0.5	0.557	2.47
0.7	0.568	2.31
0.8	0.542	2.27
0.9	0.493	2.22
1.0	0.491	2.15

The experimental data revealed that a pH of 2.93 to 2.27 gave a maximum intensity and a lower blank value. A series of buffers was prepared and tested for maximum color intensity. A glycine-HCl, KH-phthalate, tartaric acid-NaOH, and citric acid-NaOH buffers of pH 2.67 were examined for maximum color intensity. Useful results were given by glycine-HCl and KH-phthalate. One-third of the intensity was given by tartaric acid-NaOH and citric acid-NaOH buffers due to their complexing ability of ferric ions which then resist reduction. A 1-7 ml of glycine-HCl buffer gave maximum color intensity and 5 ml of the buffer was selected for the subsequent experiments.

Effect of Oxidant

The effect of various oxidant amounts (1-5 ml of 10^{-2} M) of ferric ion oxidant on the intensity of the color produced was next investigated, the results are shown in Table 2 .

Table 2: The effect of oxidant amount on absorbance.

ml of oxidant (1×10^{-2} M)	Absorbance / µg of hydroxyurea in 20 ml				
	5	25	50	100	R^2
1	0.060	0.285	0.537	0.965	0.9961
2	0.102	0.281	0.552	1.106	0.9994
3	0.061	0.286	0.564	1.128	1.00
5	0.062	0.281	0.571	1.077	0.9991

The results shown in Table 2 indicate that volume of 2 ml of 10^{-2} M solution gives a good determination coefficient and lower blank value (0.015) while replacing ferric ammonium sulphate by ferric chloride gave higher blank values. Also, ferric ammonium sulphate can be obtained in a more pure state than ferric chloride.

Effect of reaction time

The time needed to oxidize hydroxyurea quantitatively is 5 minutes. Also, it was found that extending oxidation time upto 30 minutes did not affect results.

Effect of chromogenic reagent amount

The effect of various amounts (1-7 ml of 10^{-2} M) of 2,2′-bipyridyl reagent was tested and the results are shown in Table 3.

Table 3: Effect of reagent amount on absorbance.

ml of reagent (1×10^{-2} M) solution	Absorbance / µg of hydroxyurea					
	1	5	10	20	Blank	R^2
1	0.057	0.197	0.302	0.487	0.009	0.9795
3	0.072	0.291	0.571	1.111	0.018	0.9999
5	0.070	0.285	0.548	1.099	0.026	0.9999
7	0.070	0.292	0.531	1.077	0.028	0.9994

From the above results, a 3 ml of 10^{-2} M 2,2′-bipyridyl chromogenic reagent solution gave maximum absorbance with a determination coefficient (R^2) = 0.9999 and the lower blank value (0.018).

Effect of surfactansts

The effect of various kinds of surfactants (cationic, CTAB; anionic, SDS ; and neutral, Triton X-100) on the color intensity of the chelate produced was investigated and the results are shown in Table 4.

Table 4: The effect of surfactant.

Surfactant solution 3ml	Absorbance / order of addition				
	I	II	III	IV	V
CTAB 1×10^{-3}M	0.565	0.556	0.569	0.560	0.555
Triton X-100 1%	0.557	0.566	0.550	0.566	0.559
SDS 1×10^{-3}M	Turbid solutions				

* I= Hydroxyurea (Hu)+ Buffer (B) + Oxidant (O) + Reagent (R) .
II=Hu + Surfactant (S) + B + O +5 min + R .
III=Hu + B + S + O + 5 min + R .
IV=Hu + B + O + 5 min + S + R .
V=Hu + B + O + 5 min + R +S .

The results in Table 4 indicate that all types of surfactants do not have an effect on the intensity of absorbance, while SDS (5 ml of 10^{-3}) formed turbid reaction solution.

Effect of order of reagents addition

Table 5 shows the order of addition on absorbance.

Table 5: Order of addition of reagents.

	Order	Abs. sample vs blank	Abs. blank vs water
I	Hu + Fe + B +R	0.575	0.016
II	Hu+ B + Fe + R	0.594	0.016
III	Hu + Fe + R + B	0.579	0.008

HU= Hydroxyurea , B=Buffer , R=Reagent.

From Table 5, the second order was the best choice because it gives the highest absorbance

Effect of time

The effect of time on the development and stability of coloured complex for different amounts of hydroxyurea is investigated under the optimum experimental conditions established, the results are shown in Table 6.

Table 6: The effect of time on the absorbance of complex.

Time (min.)	Absorbance* / µg Hydroxyurea		
	25	50	70
0	0.293	0.545	0.771
5	0.292	0.550	0.796
10	0.291	0.551	0.797
15	0.292	0.551	0.795
20	0.292	0.551	0.795
25	0.291	0.551	0.794
30	0.291	0.551	0.794
35	0.291	0.551	0.795
40	0.291	0.551	0.795
Abs/Blank	0.018	0.020	0.019

*The solution was left for five minutes after dilution to get the optimum stable absorbance readings.

From the above results, the complex was stable for at least 35 minutes only.

Final absorption spectra

Under the above established optimum conditions, the absorption spectra of standard treated according to the recommended conditions, versus the corresponding blank and distilled water and of blank versus distilled water are shown in Fig. (2). The absorption maxima was shown at 522 nm which was kept for subsequent absorbance measurements.

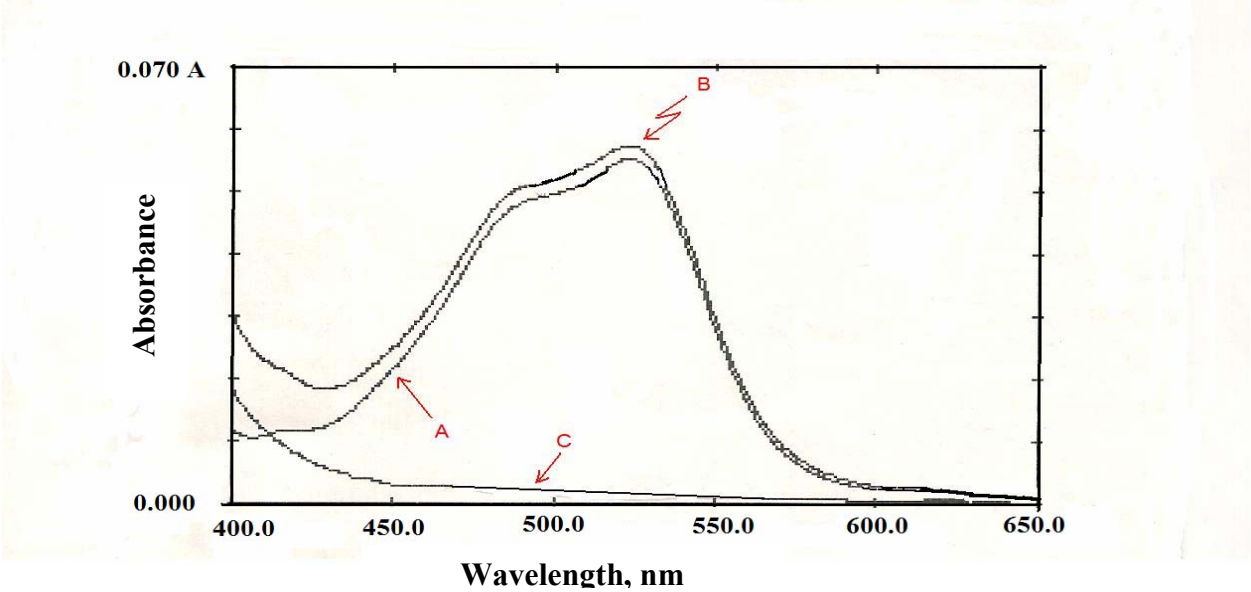

Fig. 2: Absorption spectra for final colored product versus the corresponding blank (A), absorbance spectrum versus distilled water (B) blank versus distilled water (C).

Nature of the reaction

Job's method of continuous variations (Fig. 3) was used to establish the stoichiometry (Delevie,1997) of the reaction between hydroxyrea and ferric ions. The obtained results indicated a 1:4 molar ratio of hydroxyurea to ferric ions so we propose the following reaction:

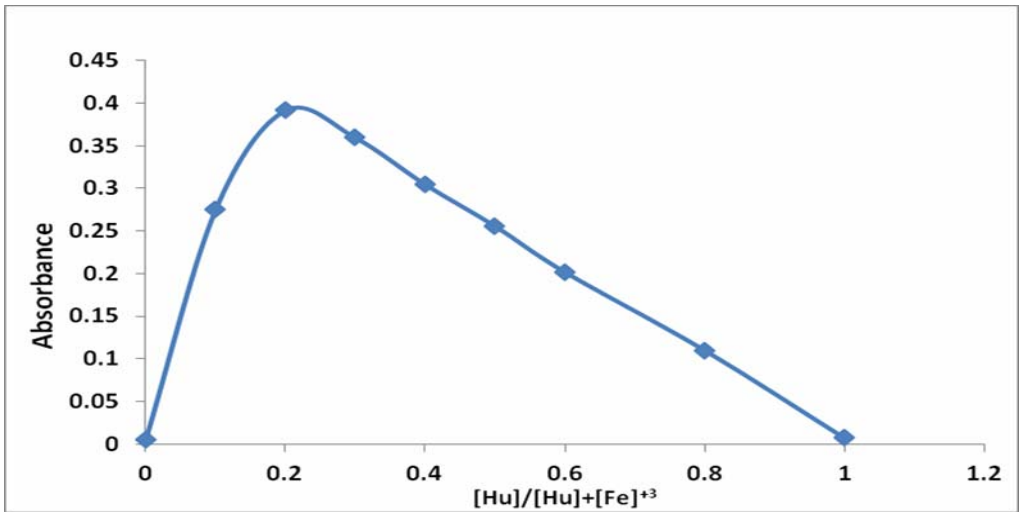

Fig. 3: Job's plot for hydroxyurea – Fe III .

$H_2NCONHOH + 4\ Fe^{+3} \longrightarrow N_2 + CO_2 + 4Fe^{+2} + 4H^+$

When the mole-ratio method was applied a 1:5 to 1:6 molar ratio were obtained and we postulate the following reactions:

$H_2NCONHOH + 5Fe^{+3} + 0.5\ H_2O \longrightarrow 1/2\ N_2O + CO_2 + 5Fe^{+2} + 5H^+$

$H_2NCONHOH + 6Fe^{+3} + H_2O \longrightarrow N_2O + CO_2 + 6Fe^{+2} + 6H^+$

Whereas the molar absorptivity value of $17x10^3$ l. mol^{-1} cm^{-1} indicates a 1:2 molar ratio since the original value of $8.7x10^3$ l. $mol^{-1}.cm^{-1}$, with respect to ferrous iron, so we suggest the following reaction:

$H_2NCONHOH + 2Fe^{+3.} \longrightarrow H_2NCONO + 2Fe^{+2} + 2H^+$

The iron in the ferrous state is complexed with 2,2\-bipyridyl resulting in the formation of the characteristic pink-red tris-chelate which was stable and soluble in aqueous solution. On the other hand, ferric ions did not give this characteristic chelate in aqueous medium.

Effect of organic solvents

The reaction mixtures were diluted with some organic solvents instead of water in order to study their effects on the optical properties of the colored solution, the results are shown in Table 7.

Table 7: The effect of organic solvents.

Solvent	Abs	λ_{max}(nm)	ε ($l.mol^{-1}.cm^{-1}$)
n-propanol	0.624	522	$1.9x10^4$
Ethanol	0.638	522	$1.9x10^4$
Methanol	Turbid	----	----
Acetone	0.648	522	$1.9x10^4$
DMF	----	----	----
Acetic acid	0.726	522	$2.2x10^4$
Formic acid	0.540	522	$1.6x10^4$
Water	0.595	522	$1.8x10^4$

It was found from Table 7 that acetic acid gave the highest molar absorptivity followed by acetone, ethanol and propanol and the least formic acid while when we use DMF gave a blank with a colour similar to that of the standard, which means that both ferric and ferrous ions will react with reagent together . A new fact that is yet discovered.

Effect of interferences

In order to assess the applicability of the developed method, the effect of various compounds at various levels on the determination of 50 µg hydroxyurea was carried out. The results are given in Table 8.

Table 8: Effect of foreign compounds on the determination of hydroxyurea.

Foreign compound	Recovery % of 50 µg hydroxyurea/µg foreign compound present			
	25 µg	50 µg	100 µg	500 µg
Ascorbic acid	122.3	134.4	175.7	435.2
Citric acid	17.3	10.5	9.8	6.7
Calcium (as sulphate)	98.3	98.3	101.2	99.6
Cadmium (as acetate)	96.6	98.1	98.3	18.7
Copper (as sulphate)	100.9	102.7	99.8	99.4
Glucose	100.5	97.2	97.2	97.0
Gum acacia	101.6	101.2	104.7	100.9
Hydrazine sulfate	99.4	103.0	102.0	103.6
Hydroxylamine hydrochloride	124.8	149.5	192.8	435.8
Lactose	98.4	96.4	99.3	98.5
Lead (as nitrate)	98.3	102.6	101.7	99.5
Magnesium stearate	103.5	turbid	turbid	turbid
Paracetamol	101.7	110.0	115.4	153.5
Povidone	99.1	96.4	99.1	99.1
Sodium dihydrogen phosphate	101.9	102.3	101.9	96.2
Sodium dodecyl sulfate	95.5	96.4	97.5	98.5
Sorbitol	99.1	102.6	99.5	99.1
Starch	97.6	96.6	96.0	96.0
Urea	99.0	97.2	97.4	97.8
Thiourea	101.2	98.7	100.5	115.6

The above results indicate that ascorbic acid, hydroxylamine, paracetamol and citric acid interfere to various extents. But fortunately these compounds are not present with hydroxyurea except citric acid which is present with hydroxyurea as additives. But we can reduce this interference by taking the smallest amount of sample possible.

Application of the method

The proposed method was successfully applied to the determination of hydroxyurea in capsule. The results which are shown in Table 9 indicate that good recoveries were obtained.

Table 9: Analytical applications.

Pharmaceutical preparation	µg of Hydroxyurea present / 20 ml	Recovery * %
Hydroxyurea capsule (500 mg) (Filaxis, Argentina)	10	101.9
	50	101.2
	75	101.5

*Average of five determinations.

The capsule, supplied (Filxas, Argentina), is labeled to contain 500 mg per capsule was assayed by the present method and found to contain (506.5 ± 3.7) mg and by the titrimetric British Pharmacopeia method (British Pharmacopeia, 1980) to contain (489 ± 2.7) mg. The tabulated F value (at 95% confidence interval) at 4 and 4 degrees of freedom is 6.3 which is larger than the calculated 1.2 value, indicating that there is no significant difference between the two methods.

Comparison of the methods

Table 10 shows the comparison between the present method and the standard method.

Table 10: Comparison of the methods

Analytical parameter	Present method	Standard method*
pH	2.6	------
Temperature	R.T	R.T
$\lambda_{max,}$nm	522	------
Reagents used	3 reagents	3 reagents
Principle reagent	2,2/-bipyridyl	iodine
Time of analysis	10 min.	20-25 min.
Determination range	0.25-7.5 ppm	------
Molar absorpitivity,l.mol^{-1},cm^{-1}	1.65 x 10^4	------
reaction stoichiometry	1:4	------
Recovery, %	101.3	97.8
RSD, %	± 0.9	± 0.7
Application	Has been applied to the assay of hydroxyurea in capsule	Has been applied to the assay of hydroxyurea in tablets

*Britishpharmacopia, 1980

The results indicate that the proposed method has a good sensitivity and has a wide application part in determination of the drug under investigation in its pharmaceutical preparation (capsule).

CONCLUSION

The proposed method for the spectrophotometric determination of hydroxyurea in capsule is simple, sensitive, rapid, accurate, and precise. The method is based on oxidation-reduction reaction between hydroxyurea and ferric ion, then the subsequent reaction of ferrious ion with 2,2′-bipyridyl reagent in acidic medium to produce pink-red complex which is stable, water soluble and has a maximum absorption at 522 nm. The relative standard deviation (RSD) was ± 0.9 % depending on the concentration level. This means the proposed method has been applied successfully to the determination of the intended compound in capsule.

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