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NOVEL ASSAY OF VARDENAFIL. HCL IN PHARMACEUTICAL PREPARATIONS AND ENVIRONMENTAL WATER SAMPLES

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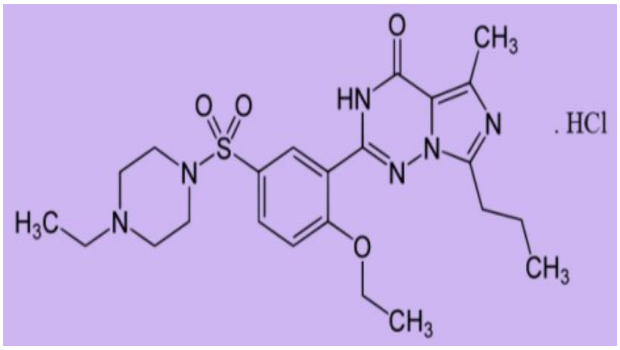
ABSTRACT

A simple, rapid, accurate and sensitive spectrophotometric method for determination of vardenafil hydrochloride has been developed. The proposed method is based on the reaction between chloride ion and mercuric thiocyanate, formation of a colored complex by the reaction between released thiocyanate and ferric ions to form red soluble product with maximum absorption at 460 nm. Beer's law is obeyed over the concentration range of 4-40µg/ml, with molar absorptivity of $0.819x10^4$ l/mol.cm. The present method is considered to be simple because it does not need either heating or hydrolysis or solvent extraction steps. The ingredients often formulated with vardenafil hydrochloride have been shown not to interfere, and the proposed method is suitable for the routine determination of vardenafil hydrochloride. The method has been successfully applied for the determination of vardenafil hydrochloride in pure form, pharmaceutical formulations (Tablets) and environmental water sample.

KEYWORD: Vardenafil.HCl, Mercuric thiocyanate, Environmental water, Pharmaceutical Formulations.

INTRODUCTION

Vardenafil (VAR) is chemically **(1**-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propyli-midazo [5,1-f][1,2,4]triazin-2-yl)-4-ethoxyphenyl]sulfonyl]-4-ethylpipera-zine,mono hydrochloride (Figure.1). Vardenafil hydrochloride is not official in any Pharmacopoeia, used to treat erectile dysfunction. Vardenafil inhibit phosphor diesterase type 5 (PDE-5) enzyme, which in turn maintains higher levels of cyclic guanosine monophosphate, which relaxes smooth muscles, promotes penile blood flow and enhances erectile function[1-3]



$C_{23}H_{32}N_6O_4S$,HCl = 525.1

Figure 1: Chemical Structure of Vardenafil hydrochloride.

Several methods for the determination of Vardenafil hydrochloride have been described in the literature, including spectrophotometric methods[4-8], capillary electrophoretic methods[9], stability indicating LC method[10], High performance liquid chromatographic methods[11-15], and atomic emission and atomic absorption spectrometry method.[16] The present work describes a new, simple spectrophotometric method for the determination of Vardenafil hydrochloride in pure form, pharmaceutical formulations and in industrial wastewater samples. The method is based on reaction between chloride ion and mercuric thiocyanate, formation of a colored complex by the reaction between released thiocyanate and ferric ion.

EXPERIMENTAL

Apparatus

Shimadzu UV- 1700 pharma spec (double beam) spectrophotometer with 1.0 cm quartz cells was used for absorption measurements.

Reagents

All chemical used were of analytical or pharmaceutical grade and Vardenafil hydrochloride standard material was provided from (PIONER), company for pharmaceutical industries -Iraq.

Vardenafil hydrochloride standard solution: **0.01%**
This solution was prepared by dissolve 0.01g of Vardenafil hydrochloride in 100 mL of distilled water in volumetric flask.

Ferric ammonium sulfate solution: 4%
4g of ferric ammonium sulfate [$FeNH_4(SO4)_2.12H_2O$] was dissolved in 50 ml double distilled water and 20ml of concentrated nitric acid was added and diluted with double distilled water to 100ml.

Mercuric thiocyanate solution: 0.5%
0.5g of mercuric thiocyanate was dissolved and diluted to 100 ml with ethanol. Mixed and filtered through filter paper.

General procedure
Different aliquots of standard Vardenafil hydrochloride solution equivalent 100-1000 µg(1-10 ml) were transferred into a series of 25ml volumetric flasks, and 2.5mL of ferric ammonium sulfate solution were added and 2ml of saturated solution of mercuric thiocyanate were added to each flask and mixed well with occasional shaking. This was diluted to 25ml with double distilled water and mixed well. Let stand for 5 min, the absorbance of each solution was measured at 460 nm against a reagent blank.

Procedures for pharmaceutical preparations (Tablets)
To minimize a possible variation in the composition of the tablets (containing 20mg of Vardenafil hydrochloride tablet were provided from(PIONER), company for pharmaceutical industries –Iraq. The mixed content of 4 tablets were weighed and grounded, then the powder equivalent to 10 mg of Vardenafil hydrochloride in about 70 ml of distilled water was stirred well for 30 min and then filtered through whatman No. 42 filter paper and the filtrate solution was diluted to 100ml by distilled water and different volume of this solution was treated as described above under general procedure.

Procedure for distilled water samples
To demonstrate the practical applicability of the proposed method, real distilled water samples were analyzed by spiked with the concentrations ranging from 4-40 µg /ml of Vardenafil hydrochloride and aliquot of this solution was treated as described above under general procedure.

RESULT AND DISCUSSION
The method depends upon the displacement of thiocyanate ion from mercury (I1) thiocyanate by chloride ion in the vardenafil hydrochloride. The free thiocyanate then complexes with Fe III at room temperature resulting in formation of red colored complex which absorbed at 465nm (Figure. 2).and the intensity of its color is proportional to the original chloride ion].[17,18]

$$2Cl^- + Hg(SCN)_2 + 2Fe^{3+} \longrightarrow HgCl_2 + 2[Fe(SCN)]_2^+$$

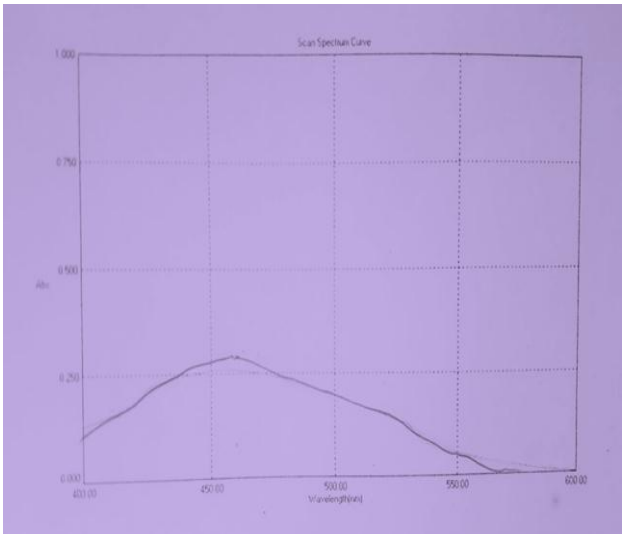


Figure 2: Absorption spectra of 20µg /ml of Vardenafil hydrochloride.

The various experimental affecting the development and stability of the reaction product was optimized by changing each variable in turn while keeping all other variables constant.

Effect of ferric ammonium sulfate solution
The amount of ferric ammonium sulfate solution (4%) for maximal color intensity was examined the maximum constant intensity was reached at 1.5 ml of reagent solution and remained constant up to 6ml. However 2.5 ml of the reagent solution was selected for the subsequent work.

Effect of mercuric thiocyanate solution
The amount of mercuric thiocyanate solution (0.5%) for maximal color intensity was examined the maximum constant intensity was reached at 1 ml of reagent solution and remained constant up to 5ml. However 2 ml of the reagent solution was selected for the subsequent work.

Effect of temperature and time
The results obtained indicated that complete color formation occurred immediately and not affected by temperature therefore, room temperature was selected as suitable temperature. The absorbance remained constant for 6 hours at least, and 5 min was selected as a suitable time.

Effect of order of addition
To test the effect of order of the addition of the reagents on the absorbance of the product, different order was tested. The selected order was sample solution, mercuric thiocyanate solution followed by ferric ammonium sulfate which was gave high absorbance value.

Calibration graph
Employing the conditions described in the general procedure a linear calibration graph of Vardenafil hydrochloride which obeys Beer's law in the concentration range of 4-40µg/ml (Figure.3). Linear

regression equation: Y=0.0156X-0.0051 (r=0.9988). Where Y is the absorbance and X is concentration in µg/ml. The apparent molar absorptivity was $0.819x10^4$ $l.mol^{-1}.cm^{-1}$ and sandell's sensitivity was $0.064\mu g.cm^{-2}$.The limit of detection and quantification were evaluated as.[19,20]

$$LOD = 3.3\frac{S_0}{b} \quad LOQ = 3LOD$$

Where b is the stop and S_0 is the standard deviation of the regression line. The limit of detection was 0.3µg. ml^{-1} and the limit of quantification as the lowest standard concentration which could be determine with acceptable accuracy, and precision was 0.9 $\mu g.ml^{-1}$. The applied method can be used routinely for the estimation of pure drug salts through their chloride concentration.

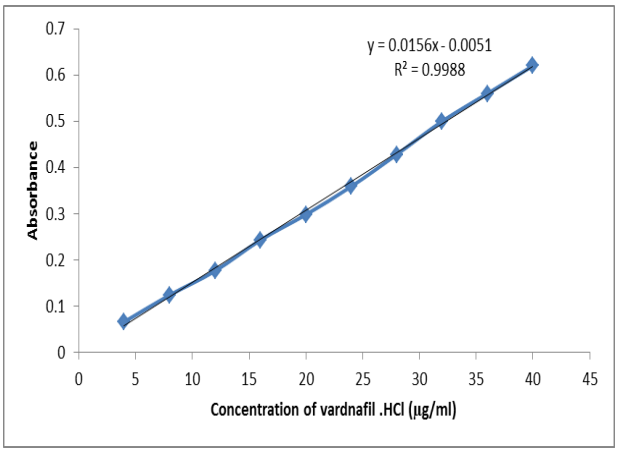


Figure 3: Calibration curve of Vardenafil hydrochloride.

Accuracy and precision

The accuracy and precision of the method were established by analyzing the pure drug solution at three different levels. The average recovery which is a measure of accuracy is 100 ±0.99% revealing high accuracy of the method. The relative standard deviation (RSD),which is an indicator of precision, is less than 2%, the result are compiled in (Table.1).

Table [1]: optical characteristics and statistical data for regression equation of the proposed method.

Parameters	Value
λ max (nm)	460
Beer's law limits ($\mu g.ml^{-1}$)	4-40
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	0.819×10^4
Limit of detection ($\mu g.ml^{-1}$)	0.3
Limit of quantification ($\mu g.ml^{-1}$)	0.9
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.064
Correlation coefficient (r)	0.9988
Regression equation (Y=C+bX)	
Intercept (C)	-0.0051
Slope (b)	0.0156
Recovery	100±0.99
Relative standard deviation (%)	< 2

Application of the proposed method

The proposed method was successfully applied to the analysis of Vardenafil hydrochloride in tablets and distilled water samples. The result of analysis for pharmaceutical formulations revels that there is close agreement between the results obtained by the proposed method and the label claim(table. 2), And the results of water samples (table.3) show that the recovery values obtained were close to 100%.

Table 2: Assay of Vardenafil hydrochloride in pharmaceutical formulations.

Pharmaceutical formulation supplied by PIONER	Amount of Vardenafil hydrochloride* Proposed method	Label claim	% Recovery
Horse man tablet 20mg	20.04	20 mg	100.2

*Mean of ten determinations.

Table 3: Determination of Vardenafil HCL in spiked distilled water samples.

	Vardenafil hydrochloride (µg/ml) *		Recovery %
Water samples	Found -	**Taken -**	
Distilled water	**4.0 20 40**	**4.0 20.05 40.3**	**100.0 100.25 100.75**

*Mean of ten determinations.

Application of the proposed method to content uniformity[21]

Content uniformity or the Uniformity of dosage unit was defined as the degree of uniformity in the amount of active substance among dosage units. The risk assessment strategy underlying content uniformity testing is the assumption that some pre-specified limits exist where safety and efficacy outcomes may change if content uniformity fails. The proposed method proved to be suitable for the content uniformity test, where a great number of assays on individual tablets are required. Data presented in (table 4) indicate that the proposed method cans accurately and precisely quantitative Vardenafil hydrochloride in its commercially available tablets. The mean percentage (with RSD) of the labeled claim found in ten tablets was 100.1(0.1176%) which fall within the content uniformity limits specified by the Japanese Pharmacopoeia.[22]

Table 4: Content uniformity testing of Vardenafil hydrochloride tablets by Proposed method.

Parameter	% of the label claim
Tablet No.1	100.3
Tablet No.2	99.9
Tablet No.3	100.2
Tablet No.4	100.2
Tablet No.5	99.96
Tablet No.6	100.1
Tablet No.7	99.98
Tablet No.8	100.2
Tablet No.9	100,21
Tablet N0.10	99.98
Mean(X)	100.103
%RSD	0.363
Max. allowed unit value[22]	±15%

CONCLUSIONS

The applied method was simple, rapid, accurate, precise, sensitive and low economical cost. Furthermore, the proposed method which doesn't require elaboration of procedures, which are usually associated with chromatographic methods. The proposed method could be applied successfully for determination of Vardenafil Hydrochloride in environmental water samples, and pure form as well as in tablet dosage forms.

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