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DEVELOPMENT AND VALIDATION OF DIFFERENCE UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF DONEPEZIL HYDROCHLORIDE IN BULK AND IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The aim of this research is to develop and validate difference UV spectrophotometric method for the estimation of Donepezil Hydrochloride in bulk and in pharmaceutical dosage forms. The method was based on measuring of the difference of absorbance of two solution of Donepezil Hydrochloride of equal concentration in 0.1MHCL and 0.01MNOH at λmax 270nm. The method was validated as per ICH guidelines. Plot of absorbance difference against concentration showed a linear relationship (R^2=0.999) in the range of 10-50µg/ml. The limit of detection and limit of quantification were 0.747µg/mL and 2.265µg/mL respectively. RSD%s were1.8, 1.8 and 1.17 for repeatability, intraday and interday respectively. The recovery% (n=3) were 98.9, 99.2 and 100 for level of 50%,100% and150% of test concentration respectively. The method was successfully used for determination of the content percent of Donepezil Hydrochloride in the tablet dosage forms.

KEYWORDS: Donepezil Hydrochloride, Difference UV Spectrophotometric Method, Pharmaceutical dosage forms, Estimation, Content percent.

INTRODUCTION

Donepezil hydrochloride (DH) fig.1, 2,3-dihydro-5,6-dimethoxy-2-[{1-(phenylmethyl)-4-piperidinyl}-methyl]-1H-inden-1-one hydrochloride, can reversibly inhibits acetyl cholinesterase. It is approved by the FDA for the treatment of the Alzheimer's disease. Due to the presence N-benzylpiperidine and an indanone moiety, DH was demonstrated to have a potent and selective inhibition of brain acetylcholinesterase with less side effects compared to physostigmine and tacrine.[1, 2] DH is now marketed in many countries under the trade name of aricept.[3]

Difference spectrophotometry is modified spectrophotometric method for the determination of certain compounds in the presence of absorbing substance that interfere in a direct spectrophotometric assay. The basis of a difference spectrophotometric method is that an absorbance difference (ΔA) is measured between two equimolar solutions of the absorbing analyte, in the presence of different reagent that reproducibly alter the spectral properties of the analyte. Provided that the absorbance of the other absorbing interferences is not affected by the reagents, their ΔA is zero and their contribution to the measured ΔA of the sample is eliminated. This is particularly useful in eliminating specific interference in pharmaceutical analysis, from degradation products and co-formulated drugs, and also non-specific irrelevant absorption from the formulation matrix.[4]

A literature survey revealed that there are various methods available for quantitative determination of Donepezil hydrochloride in pharmaceutical dosage forms. These methods include: , UV-spectrophotometric (5-7), HPLC(8-11). These methods for determination are laborious, expensive and need skilled trained staff. Development of difference UV Spectrophotometric method for determination of Donepezil hydrochloride in bulk and tablet dosage forms was reported

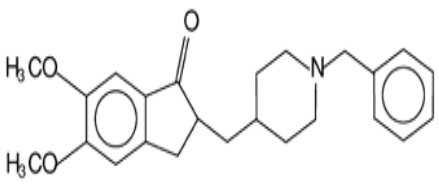

Figure 1: Chemical structure of Donepezil hydrochloride.

MATERIALS AND METHODS

2.1. Materials

Drugs

Donepezil HCl working standard [99.6%] obtained as a gift from Tabuk Pharmaceutical Company Ltd. Khartoum, Sudan.

DonepeziloFiximе 5mg (brand) tablets manufactured in West pharma – prodções de Especialidades Farmacêuticas S. A(Potugal)

Chemicals

Sodium Hydroxide pallets (NaOH) manufactured in SDFCL (India.

Hydrochloric acid 37% (HCl), Manufactured in Chem-lab (Belgium.

Instruments

Schimadzu single beam 7315 UV-Visible Spectrophotometer model 1cm matched quartz cell.

Methods

Solvents preparation

Preparation of 0.1M hydrochloric acid (HCl)

Hydrochloric acid 0.1 M was prepared by diluting 8.4 mL of 37% hydrochloric acid to 1000 mL by distilled water.

Preparation of 0.01M Sodium hydroxide (NaOH)

Sodium hydroxide pellets (0.4g) was weighed and transferred to 1000 mL volumetric flask then the volume was completed to the mark by distilled water and stirred for 10 minutes.

Preparation of Donepezil HCl standard solutions in 0.1M HCl and 0.01M NaOH

Ten milligrams of standard Donepezil HCl was weighed and transferred to 100 mL volumetric flask then it was dissolved with 0.1M HCL and 0.01M NaOH separately, the volumes were completed to the mark then solutions stirred for 15min.to obtain solution of concentration (100µg/mL**)** solution A1 and A2 respectively.

Estimation of λ_{max}

From solutions A1 and A2test concentrations of 10µg/mL were prepared by diluting 10 mL of above standard solutions to 100 mL with 0.1M HCL and 0.01M NaOH respectively. The prepared solutions were scanned over the range of 200-400nm against their respective reagents as blanks.

Construction of the Calibration Curve

Five concentrations (10, 20, 30, 40 and 50 µg/mL) from standard were prepared, then the absorbance's of each concentration were measured in0.1M HCl and 0.01M NaOH separately. Then the differences in absorbance's, were plotted versus concentrations and calibration curve was constructed.

linearity: Serial volumes of a A1 and A2(2.5,5.0,7.5,10,12.5)ml were transferred to 100ml. Each series was completed with respective solventto obtain final concentrations (2.5,5.0,7.5,20,10,12.50 µg/ml). The absorbance of all solution were measured at 307nm using the respective solvent blank. The calibration curve was constructed by plotting the absorbance difference against concentration.

Precision

Repeatability

Two solutions (3 ml each) from solution A1 and A2 each was transferred to 100ml volumetric flask and completed with the respective solvent to obtain 30µg/ml /ml. The absorbance of each solution was measured at 230six timesusing the respective solvent blank. RSD% of the absorbance difference was calculated.

Intraday Precision

Two solutions (3 ml each) from solution A1 and A2 each was transferred to 100ml volumetric flask and was completed with the respective solvent to obtain 30µg/ml. The absorbance of each solution was measured at 230three times in the same day using the respective solvent blank. RSD% of the absorbance difference was calculated.

Interday precision

Two solutions (3 ml each) from solution A1 and A2 each was transferred to 100ml volumetric flask and completed with the respective solvent to obtain 30µg/ml /ml. The absorbance of each solution was measured at 230 in three different days using the respective solvent blank. RSD% of the absorbance difference was calculated.

Accuracy

Accuracy of the method is determined by using standard addition method at 3 levels. Standard quantity equivalent of 50%, 100% and 150% were added to pre-analyzed Donepezil HCl tablets sample.

Determination of content % of Donepezil HCl in tablet brand

Ten tables of Donepezil HCl150mg were weighed and grinded; weight that equivalent to 10 mg of Donepezil HCl was taken and transferred into 100ml volumetric flask with 0.01M NaOH and 0.1M HCl separately then the two solutions were put in magnetic stirred for 15 min then in sonication for 30 min and the volumes were completed to 100 mL after that solutions were filtered then 2mL of each solution was taken and completed to 10 mL to give final concentrations of 20µg/mL. The absorbance of each solution was measured at 270nmusing the respective solvent blank.. The absorbance difference was calculated and substituted in regression equation to obtain the actual concentration of DonepezilHCl in the tablet powder and the content % of DonepezilHCl was calculated using the relationship:

Actual concentration/theoretical concentrationx100% the method using the equation of the standard calibration curve. The result of sample solution, 20µg/mL in 0.1M HCL and0.01M NaOH were 0.536 and 0.1955 respectively, the absorbance difference was 0.3405, from the calibration curve equation: Y = mx+c

Y=0.0234x-0.1612

Y=absorbance, x = concentration, m= slope, c = intercept

Y=0.3405

So x = 21.44µg/mL, which is the actual concentration.

The content percent = actual conc./theoretical conc.*100

21.44/20*100 = 107.2%

RESULTS AND DISCUSSION

Donepezil hydrochloride drug used for treatment of mild to moderate Alzheimer's disease. It is a new class of Acetylcholine inhibitor having an N-benzylpiperidine and an indanone moiety which shows longer and more selective action.

The aim of present work is to develop a simple, accurate precise, and an economical difference UV spectrophotometric method for the determination of Donepezil Hydrochloride in bulk and in Pharmaceutical dosage form. The method was based on measurement of the difference of absorbance of two solutions of Donepezil Hydrochloride in e of equal concentration at 0.1 MNAOH, 0.1MHCL. The method was validated as per ICH guidelines.

Estimation of wave length of maximum absorbance

The solution A1 and solution A2 were scanned between 200-400nm to select the wave length of maximum absorbance λmax. The two solution λmax showed at 307nm respectively fig2&3

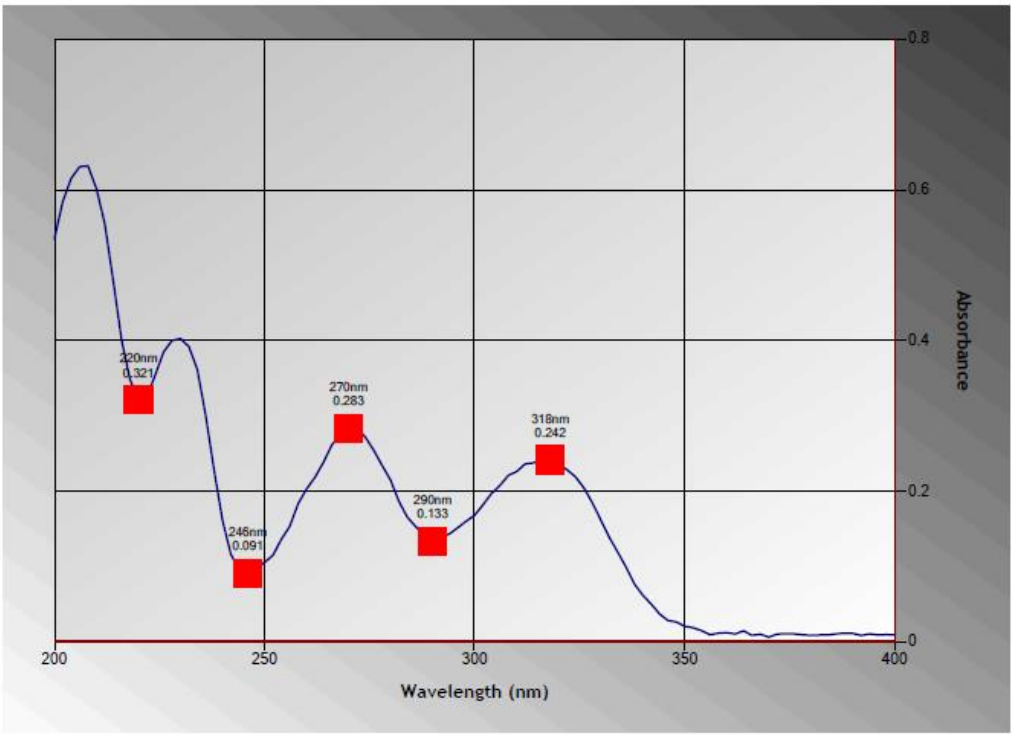

Figure 2: UV spectrum of Donepezil Hydrochloride in 0.1M HCl (10µg/ml).

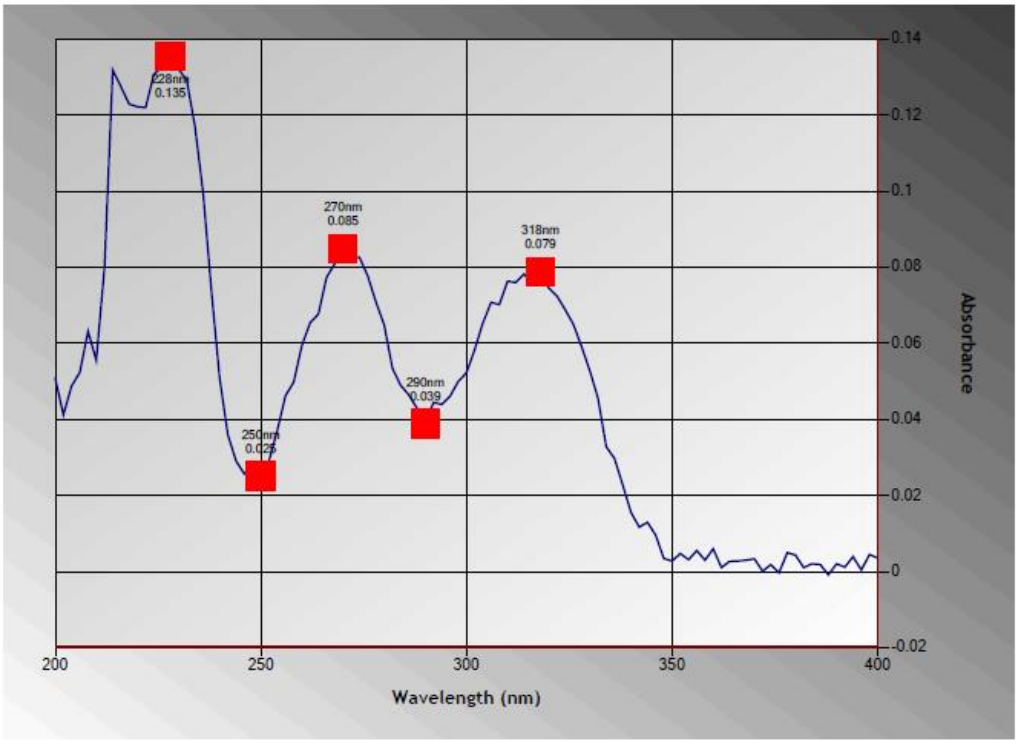

Figure 3: UV spectrum of Donepezil Hydrochloride in 0.1M NaOH (10µg/ml).

Linearity

Plot of absorbance difference against concentration showed a linear relationship (R^2=0.999) in the range of 10-50µg/ml., regression equation y= 0.023x+0.161 where y is absorbance difference and x is concentration. The limit of detection and limit of quantification were 0.747µg/mL and 2.265µg/mL respectively. These results indicate linearity and sensitivity of the developed method.

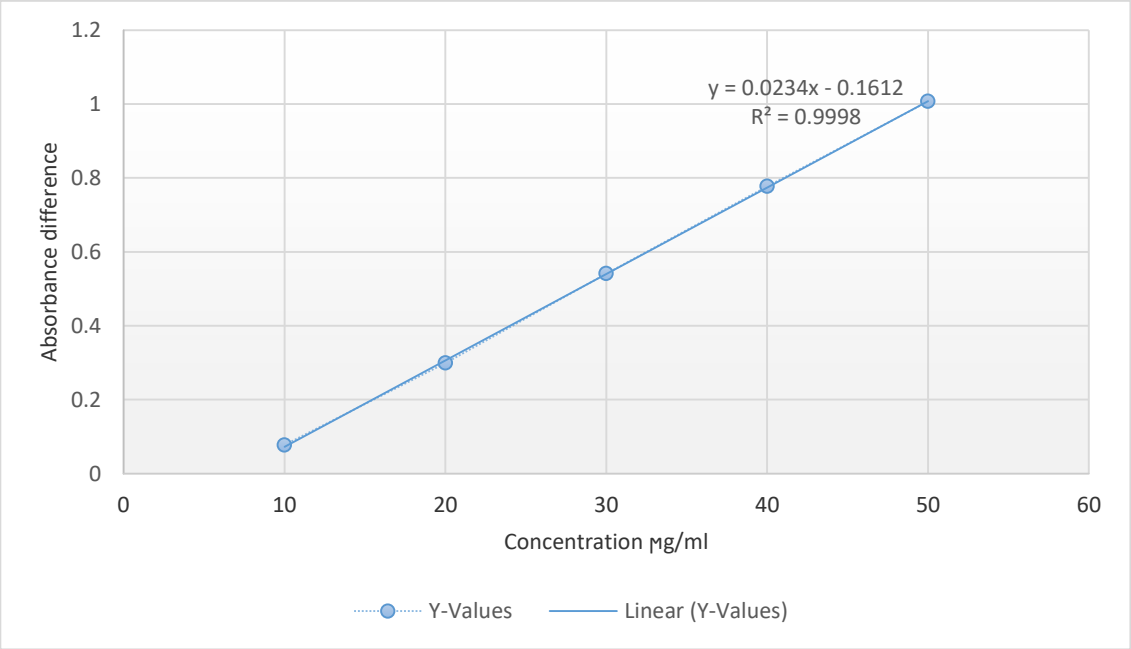

Figure 4: The calibration curve of Donepezil Hydrochloride in 0.1M HCl and 0.01M NaOH at 270 nm.

Table1: Regression analysis parameters of the method.

Equation	y=0.0234x-0.1612
Correlation coefficient	0.9998
*Slope ± tsb	0.0234± 0.34
**Intercept ± tsa	0.1612± 0.0045
LOD	0.747µg/mL
LOQ	2.265µg/mL
Range	10-50µg/mL

*Standard error of slope calculated at 95% confidence limit for n- 2 degrees of freedom.

**Standard error of intercept calculated at 95% confidence limit for n-2 degrees of freedom

Precision

The precession of the method was assessed with respect repeatability, interaday and interday precision. The RSD% values were1.8, 1.85 and 1.1 for repeatability, interaday and interday

precision respectively. The low RSD values(>2) of all levels of precision indicate the precision of the proposed method.

Accuracy

Accuracy of the method was studied by calculating the added recovery by standard addition method. The drug sample were spiked level of 50%,100% and 150% of test concentration. The added recovery% was calculated for each level. The recovery%(n=3) were 98.98%, 99.62% and 100% for level of 50%,100% and150% of test concentration respectively, table 2. These result indicate the accuracy of the method and its freedom of interference of excipients.

Table 2: Results of accuracy study of the method.

Concentration	Absorbance in 0.1M HCl	Absorbance in 0.01 M NaOH	Difference in absorbance	Recovery %
20µg/mL sample	0.525	0.169	0.356	
20µg/mL sample+50%STD	0.768	0.314	0.454	98.98
20µg/mL sample+100%STD	0.996	0.375	0.621	99.62
20µg/mL sample+150% STD	1.301	0.437	0.864	100

Assay of Donepezil Hydrochloride commercial brand

The content percent of Donepezil Hydrochloride in the tablet 5mg dosage form manufactured in West pharma –prodções de EspecialidadesFarmacêuticas S.A(Potugal) was assayed using the proposed method and illustrated in table.3.The content percent determined by the method(107.2) which falls in the limit of the IP [90-110%].

Table 3: The content percent of Donepezil Hydrochloride in the tablet dosage form.

Theoretical concentration of the sample (µg/ml)	Difference in absorbance of sample	Content percent
20	0.3405	107.2

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