Research Article

Validated Spectrophotometric Methods for Determination of Donepezil Hydrochloride in Pharmaceutical Formulations Based on Redox Reaction with Cerric (IV) Ammonium Sulphate

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ABSTRACT

Three simple, sensitive and accurate spectrophotometric methods have been developed for the determination of donepezil hydrochloride (DNP) in pure form and pharmaceutical formulations. The proposed methods are based on the oxidation of donepezil hydrochloride by a known excess of cerric(IV) ammonium sulphate (CAS) in acid medium followed by determination of unreacted CAS by adding a fixed amount of amaranth (AM), methylene blue (MB), and indigo carmine (IC) dyes followed by measuring the absorbance at 520, 664 and 610 nm, respectively. The experimental conditions affecting the reaction were studied and optimized. The beer's law was obeyed in the concentration ranges of 1.0-10, 0.5-8.0 and 1.0-12 µg mL-1 using AM, MB and IC methods, respectively with a correlation coefficient ≥ 0.9994. The calculated molar absorptivity values are 2.7364 × 104, 1.9845 × 104 and 1.3746 × 104 L mol-1 cm-1 using AM, MB and IC methods, respectively. The limits of detection and quantification are also reported. Intra-day and inter-day precision and accuracy of the methods have been evaluated. The methods were successfully applied to the assay of donepezil hydrochloride in tablets and the results were statistically compared with those of the reference method by applying Student's t-test and F-test. No interference was observed from the common tablet excipients. The accuracy and reliability of the methods was further ascertained by performing recovery studies using standard-addition method.

Keywords: Spectrophotometry; Donepezil hydrochloride; Cerric(IV) ammonium sulphate; dyes; pharmaceutical formulations.

INTRODUCTION

Donepezil hydrochloride (DNP) is chemically known as 2,3- dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1-H-inden-1-one hydrochloride (Figure 1). DNP is acetylcholinesterase inhibitor used in the treatment of Alzheimer's disease [1-3].

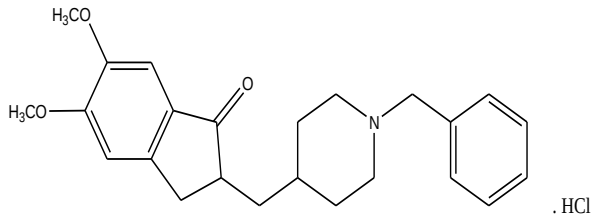

Figure 1: Chemical structure of donepezil hydrochloride (DNP).

The literature survey has revealed some methods have been described for the analysis of DNP including high performance liquid chromatography[4-10], capillary electrophoresis[10, 11], potentiometry[12] and spectrofluorimetric[13]. However, these methods are expensive, not simple for routine analysis, time consuming and not available at most laboratories.

The spectrophotometric technique continues to be the most preferred method for the assay of different classes of drugs in pure and pharmaceutical formulations, due to its simplicity and reasonable sensitivity with significant economic advantages. There are several methods for the determination of DNP in pharmaceutical dosage forms using spectrophotometric technique [14-23] (Table 1). These methods were associated with some major drawbacks such as decreased selectivity due to measurement in ultraviolet region, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid–liquid extraction step and/or some other methods have a relatively narrow dynamic linear range, involve a heating step, and/or use of expensive reagent or large amounts of organic solvents. For these reasons, it was worthwhile to develop a new, simple, cost effective, accurate and selective spectrophotometric method for the determination of DNP in pure form and pharmaceutical dosage forms.

Cerric(IV) ammonium sulphate (CAS) has been widely used as an effective analytical reagent in spectrophotometric methods for the determination of many pharmaceutical compounds [24-28]. CAS is a strong oxidant, and it has not been applied for the assay of aripiprazole in pure form and tablets.

This paper describes for the first time the application of acidic CAS to the spectrophotometric determination of DNP using amaranth (AM), methylene blue (MB) and indigo carmine (IC) as chromogenic agents. The proposed methods have the advantages of simplicity, sensitivity and rapidity besides being accurate precise and validated spectrophotometric method for the estimation of DNP in pure and dosage forms and can be adopted by the pharmaceutical laboratories for industrial quality control.

MATERIALS AND METHODS

Apparatus

Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10 mm quartz cell was used for absorbance measurements. It has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm.

Materials and Reagents

All chemicals and reagents used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Materials

Pure material of DNP was kindly supplied by Pfizer-Egypt S.A.E Cairo, A.R.E and was certified to have a purity of 99.60 ± 1.22%. Aricept tablet labeled to contain (5.0 mg DNP per tablet) (Pfizer-Egypt S.A.E Cairo, A.R.E under authority of Pfizer INC., U.S.A); alzepizil tablets labeled to contain (5.0 mg DNP per tablet) (Global Napi Pharmaceuticals Company (GNP), Egypt) and donepezil tablets labeled to contain (5.0 mg DNP per tablet) (Delta Pharma, Egypt) were purchased from local commercial markets.

Standard DNP solution

A stock standard solution of DNP (100 µg mL^{-1}) was prepared by dissolving an exact weight (10 mg) of pure DNP in bidistilled water and diluted to 100 mL with bidistilled water in a 100 mL measuring flask. The working solution (20 µg mL^{-1}) of DNP was obtained by dilution the stock solution 5.0-fold. The standard solution was found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use.

Reagents

A stock solution of 5.0 × 10^{-3} mol L^{-1} CAS (E-Merk, Darmstadt, Germany) was freshly prepared by dissolving 316.2 mg CAS in the least amount of H_2SO_4 (2.0 mol L^{-1}) then completed to the mark in a 100 mL calibrated flask with the same acid and kept in a dark bottle and a refrigerator when not in use.

A stock solution of 2.0 mol L^{-1} H_2SO_4 was prepared by adding 10.8 mL of concentrated acid (Merck, Darmstadt, Germany, 98%, Sp. Gr. 1.84) to bidistilled water, cooled to room temperature, transfer to 100 mL with measuring flask, diluted to the mark and standardized as recorded [29].

A stock solution (1000 µg mL^{-1}) AM, MB and IC dyes were first prepared by dissolving accurately weighed 112 mg of each dye (Sigma-aldrish, 90 % dye content) in bidistilled water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 10-fold to get the working concentration of 100 µg mL^{-1} for all dyes.

Recommended general procedures

Different aliquots (0.2-2.0 mL), (0.1-1.6 mL) and (0.2-2.4 mL) of a standard 50 µg mL^{-1} DNP solution using AM, MB and IC methods, respectively were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 5.0 mL by adding adequate quantity of water. To each flask 2.0 mL of 2.0 mol L^{-1} H_2SO_4 and 1.5 mL of (5.0 × 10^{-3} mol L^{-1}) CAS solution were added, respectively. The flasks were stoppered, contents were mixed well, and the flasks were kept aside for 5.0 min with occasional shaking. Finally, 1.0 mL of (100 µg mL^{-1}) AM, MB and IC dye solution was added to each flask and mixed well, and then the volume was diluted to the mark with bidistilled water. The color intensity of dyes was measured after 5.0 min against reagent blank solution treated similarly omitting the drug, at their corresponding λ_{max} 520, 664 and 610 nm, respectively. The concentration of unknown was determined in each case from calibration graph or computed from the regression equation derived using Beer's law data.

Procedure for pharmaceutical formulations

The contents of ten tablets were crushed, finely powdered, weight out and the average weight of one tablet was determined for each drug. An accurate weight equivalent to 5.0 mg DNP was transferred into a 50 mL calibrated flask, dissolved in bidistilled water with shaking for 5.0 min and filtered through a sintered glass crucible (G_4). The filtrate was diluted to 50 mL with bidistilled water in a 50 ml measuring flask to give 100 µg mL^{-1} stock solutions. Aliquot of the cited solutions was taken and analyzed as described under the above recommended procedures for construction of calibration curves. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph. The method of standard addition was used for the accurate determination of DNP content.

RESULTS AND DISCUSSION

Absorption spectra

The proposed spectrophotometric methods for the determination of DNP involves two steps namely:

1. Oxidation of DNP with a known excess of CAS in acidic medium at room temperature (25 ±2 °C).

2. Determination of the residual CAS by reacting it with a fixed amount of AM, MB, and IC dyes and measuring the increase in absorbance at λ_{max} 520, 664, and 610 nm, respectively (Scheme 1).

Table 1: Comparison between the reported spectrophotometric methods for determination of DNP.

Method	λ_{max} (nm)	Beer's law (µg mL^{-1})	LOD (µg mL^{-1})	Molar absorpitivity ($Lmol^{-1}cm^{-1}$)	References
$KMnO_4$ /K_2CO_3	547	1.0- 30	-	$1.1084X10^4$	14
Eriochrome Black T	510	5.0- 20	0.4	3.277×10^4	15
UV method	230.8	5.0 – 40	0.2	1.38×10^5	
Methyl orange	425	2.0-14	0.064	$2.425x10^4$	16
UV method	231	5.0-40	-	$1.38x10^5$	17
2, 4-dinitrophenyl hydrazine	454	10-60	-	$3.077x10^4$	
Bromocresol purple (BCP)	410	2.0-14	0.077	$2.7785 x10^4$	18
Alizarin red S	430	2.5-12.5	0.2082	$1.10x10^4$	19
Tropaoline (TPooo)	480	2.5-12.5	0.08174	$4.86x10^4$	
Orange G	482	5.0-35	-	-	20
Citric acid / acetic anhydride	580	8.0-24	-	-	21
Cobalt thiocyanate	620	16-48	-	-	
BCG	420	1.0-12	0.16	$3.4613 x10^4$	22
BCP	409	1.0-12	0.24	$2.428 x10^4$	
BTB	413	1.0-10	0.19	$3.0722 x10^4$	
BPB	415	1.0-10	0.25	$1.8631 x10^4$	
CAS/AM	520	1.0-10	0.25	$2.7364 x10^4$	The proposed methods
CAS/MB	664	0.5-8.0	0.15	$1.9845 x10^4$	
CAS/IC	610	1.0-12	0.28	$1.3746 x10^4$	

Table 2: Analytical parameters of the proposed methods for determination of DNP.

Parameters	AM	MB	IC
Wavelength, nm	520	664	610
Beer's law limits, µg mL^{-1}	1.0-10	0.5-8.0	1.0-12
Ringboom limits, µg mL^{-1}	2.0-8.0	2.0-6.0	2.0-10
Molar absorptivity, x 10^4 (L mol^{-1} cm^{-1})	2.7364	1.9845	1.3746
Sandell sensitivity, ng cm^{-2}	15.20	20.96	30.26
Regression equation [a]			
Intercept (a)	0.0063	0.0002	-0.0002
SD of intercept (S_a)	0.028	0.008	0.006
Slope (b)	0.0612	0.105	0.033
SD of slope (S_b)	0.024	0.01	0.03
Correlation coefficient, (r)	0.9993	0.9994	0.9997
Mean ± SD	99.50±0.76	99.10±0.85	99.70±1.10
Relative standard deviation (RSD%)	0.76	0.86	1.10
Relative error (RE%)	0.80	0.90	1.16
Limit of detection (LOD), µg mL^{-1}	0.25	0.15	0.28
Limit of quantification (LOQ), µg mL^{-1}	0.83	0.50	0.93
Calculated *t*-value [b]	0.21	0.52	0.48
Calculated *F*-value [b]	1.47	1.17	1.43

[a] $A = a + bC$, where C is the concentration in µg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope.

[b] *The theoretical values of* t *and* F *are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (*p*= 0.05).*

Table 3: Results of intra-day and inter-day accuracy and precision study for DNP obtained by the proposed methods.

Method	Taken (µg mL^{-1})	Intra-day				Inter-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
AM	3.0	99.10	0.40	-0.90	2.973 ±0.012	99.00	0.60	-1.00	2.97 ±0.019
	6.0	99.30	0.56	-0.70	5.958 ±0.035	99.10	0.68	-0.90	5.946 ±0.042
	9.0	100.60	0.90	0.60	9.054 ± 0.086	99.50	1.20	-0.50	8.955 ± 0.113
MB	2.0	100.20	0.60	0.20	2.004 ±0.013	99.50	0.50	-0.50	1.99 ±0.01
	4.0	99.20	0.80	-0.80	3.968 ± 0.033	99.80	1.10	-0.20	3.9992 ± 0.046
	6.0	99.60	1.30	-0.40	5.976 ± 0.082	99.30	1.40	-0.70	5.958 ± 0.088
IC	3.0	99.70	0.48	-0.30	2.991 ±0.015	99.30	0.30	0.30	2.979 ±0.009
	6.0	99.40	0.70	-0.60	5.964 ±0.044	100.50	0.45	0.50	6.03 ±0.028
	9.0	100.40	1.50	0.40	9.036 ± 0.142	99.10	0.80	-0.90	8.919 ± 0.075

[a] RSD%, percentage relative standard deviation; RE%, percentage relative error; [b] Mean ± standard error.

Table 4: Results of method robustness and ruggedness.

Methods	Nominal amount concentration (µg mL^{-1})	RSD%			
		Robustness		Ruggedness	
		Variable alerted [a]			
		Reagent volume (n=3)	Reaction time (n=3)	Different analysts (n=3)	Different instruments (n=3)
AM	3.0	1.05	0.95	0.60	0.50
	6.0	1.40	1.30	1.70	1.70
	9.0	1.80	2.10	2.20	2.40
MB	2.0	0.90	1.15	1.25	0.85
	4.0	1.30	1.60	1.50	1.40
	6.0	2.20	1.80	1.90	1.70
IC	3.0	0.75	0.80	0.90	1.0
	6.0	1.50	1.40	1.10	1.50
	9.0	2.50	2.60	1.30	2.30

[a] Volume of (2.0 mol L^{-1}) H_2SO_4 is (2.0±0.2 mL) and reaction time is (5.0±2.0 min) after adding CAS were used.

Table 5: Results of recovery experiments by standard addition method for the determination of DNP in tablets using the proposed methods.

Method	Taken drug (µg mL^{-1})	Pure drug Added (µg mL^{-1})	Arecipt tablets		Alzepizil tablets		Donepezil tablets	
			Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD
AM	2.0	2.0	3.956	98.90±0.32	3.964	99.10±0.22	3.976	99.40±0.45
		4.0	5.952	99.20±0.36	5.976	99.60±0.45	5.952	99.20±0.60
		6.0	7.96	99.50±0.60	7.96	99.50±0.55	7.984	99.80±0.50
MB	2.0	2.0	3.968	99.20±0.28	4.016	100.40±0.34	3.976	99.40±0.30
		4.0	6.018	100.30±0.50	5.982	99.70±0.80	6.042	100.70±1.20
		6.0	7.944	99.30±0.80	7.96	99.50±1.10	7.944	99.30±0.50
IC	2.0	2.0	4.008	100.20±0.40	3.972	99.30±0.37	3.96	99.00±0.40
		4.0	5.946	99.10±0.60	6.024	100.40±0.70	5.97	99.50±0.60
		6.0	7.936	99.20±0.90	7.928	99.10±0.95	8.008	100.10±1.20

[a] Average of six determinations.

Table 6: Results of analysis of tablets by the proposed methods for the determination of DNP and statistical comparison with the reported method.

Samples	Recovery [a] (%) ± SD			
	Proposed Methods			Reported method [22]
	AM	MB	IC	
Arecipt tablets	99.50 ± 0.56	99.40 ± 0.80	99.80 ± 0.70	99.66 ± 0.62
t-value [b]	0.43	0.57	0.29	
F-value [b]	1.23	1.66	1.27	
Alzepizil tablets	99.45 ± 0.74	99.60 ± 0.42	99.70 ± 0.65	99.84 ± 0.56
t-value [b]	0.94	0.77	0.36	
F-value [b]	1.75	1.78	1.35	
Donepezil tablets	99.50 ± 0.65	99.90 ± 0.85	99.60 ± 0.90	99.74 ± 0.76
t-value [b]	0.54	0.29	0.27	
F-value [b]	1.37	1.25	1.40	

[a] Average of six determinations.

[b] The theoretical values of *t* and *F* are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

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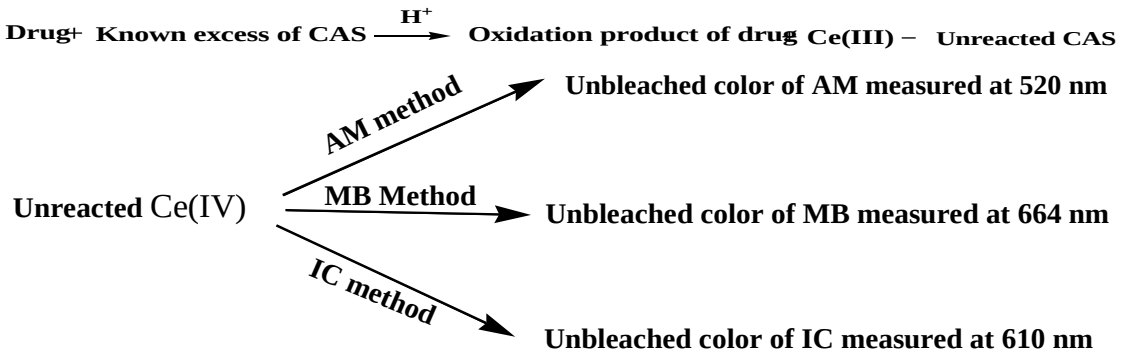

Scheme 1: The suggested reaction pathway for the proposed spectrophotometric methods for determination of DNP using CAS and dyes.

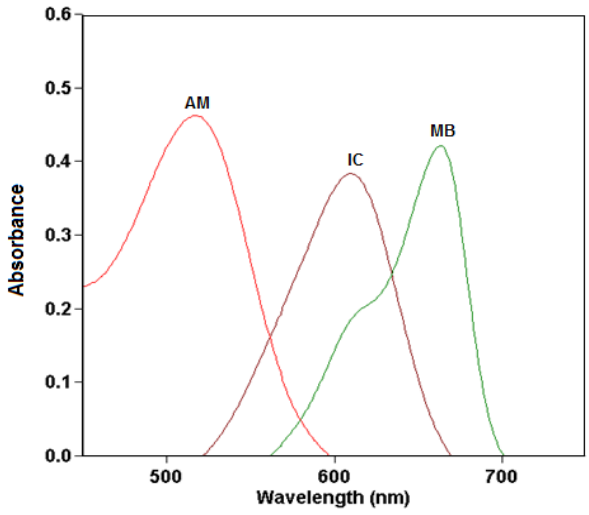

Figure 2: Absorption spectra for the unreacted CAS oxidant that determined by reacting with a fixed amount of AM, MB and IC dyes and measuring the absorbance at 520, 664 and 610 nm for AM, MB and IC methods, respectively.

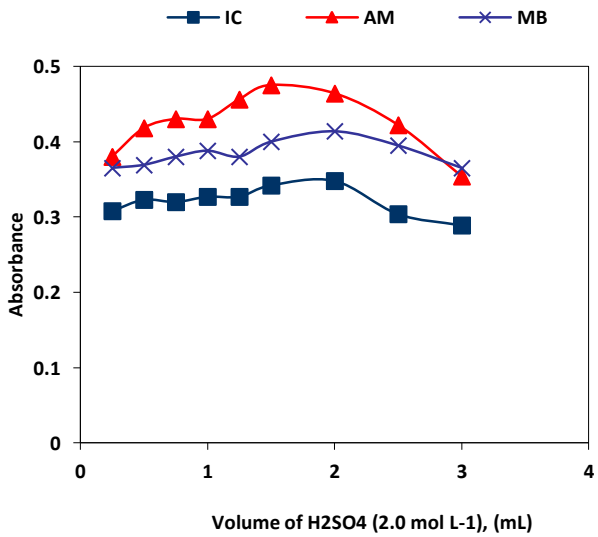

Figure 3: Effect of volume of H_2SO_4 (2.0 mol L^{-1}) on the absorbance of DNP (8.0 µg mL^{-1}) with CAS (5.0 x 10^{-3} mol L^{-1}) and dyes.

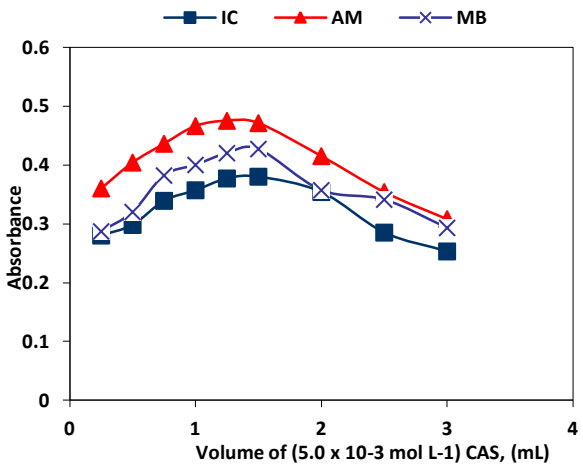

Figure 4: Effect of volume of CAS (5.0 x 10^{-3} mol L^{-1}) on the reaction product of DNP (8.0 µg mL^{-1}) with CAS and dyes in H_2SO_4 medium.

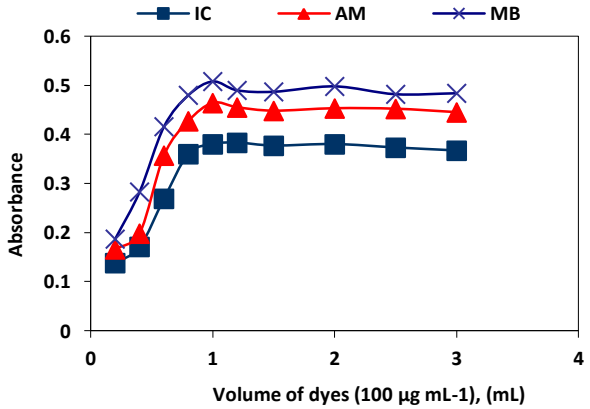

Figure 5: Effect of volume of dyes on the reaction product of DNP (8.0 µg mL^{-1}) with CAS and dyes in H_2SO_4 medium.

Optimization of the reaction conditions

The optimum conditions for the assay procedures and color development for each method have been established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

Effect of acid type and concentration

To investigate the effect of acid concentration, different types of acids were examined (HCl, H_2SO_4, H_3PO_4, HNO_3 and CH_3COOH) to achieve maximum yield of redox reactions. The results indicated that the sulphuric acid (H_2SO_4) (2.0 mol L^{-1}) was the most suitable acid with CAS as oxidant. Moreover, different volumes (0.2–3.0 mL) of 2.0 mol L^{-1} H_2SO_4 were tested and found to be a constant absorbance was obtained with 1.5–2.5 mL of H_2SO_4 (2.0 mol L^{-1}), so 2.0 mL of H_2SO_4 (2.0 mol L^{-1}) was the optimum volume for subsequent studies (Figure 3).

Effect of CAS concentration

The influence of the concentration of CAS on the absorbance of the colored products was investigated using different volumes of 5.0 × 10^{-3} mol L^{-1} CAS solution from (0.25-3.0 mL). The results indicate that the maximum and constant absorbance was obtained using 1.5 mL of 5.0 × 10^{-3} mol L^{-1} CAS solution and the color intensity decreased above the upper limits. Therefore, 1.5 mL of 5.0 × 10^{-3} mol L^{-1} CAS was taken as the optimum concentration for all measurements (Figure 4).

Effect of dye concentration

The effect of dye concentration on the intensity of the color developed was carried out to obtain the optimum concentration of dyes that produces the maximum and reproducible color intensity by reducing the residual of CAS. The effect dye concentration was studied using different volumes (0.25–3.0 mL) of the studied dyes (100 µg mL^{-1}). It was observed that maximum color intensity of the oxidation products was achieved with 1.0 mL of each dye solution. (Figure 5). The color was found to be stable up to 24 h.

Effect of temperature and mixing time

The effect of temperature was studied by heating a series of sample and blank solutions at different temperatures ranging from 20 to 60 °C in water bath. It was found that raising the temperature does not accelerate the oxidation process and does not give reproducible results, so maximum color intensity was obtained at room temperature (25±2 °C). The effect of mixing time required completing oxidation of aripiprazole and for reducing the excess oxidant was studied by measuring the absorbance of sample solution against blank solution prepared similarly at various time intervals 2.0–20 min. It was found that the contact times gave constant and reproducible absorbance values at 5.0 min. After oxidation process, 5.0 min standing time was found necessary for the complete bleaching of the dye color by the residual CAS and the absorbance of the unreacted dye was stable for at least 24 h, thereafter.

Effect of sequence of addition

After optimizing all other experimental variables, further experiments were performed to ascertain the influence of sequence of addition of reactants on the color development by measuring the absorbance. The optimum sequence of addition was DNP–H_2SO_4–CAS–dye. Other sequences gave lower absorbance values under the same experimental conditions.

Method validation

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and sensitivity

Under the optimum conditions a linear correlation was found between absorbance at λ_{max} and the concentration of DNP in the ranges of 1.0-10, 0.5-8.0 and 1.0-12 µg mL^{-1} using AM, MB and IC methods, respectively. The calibration graph is described by the equation:

$$A = a + b\,C \qquad (1)$$

Where A= absorbance, a= intercept, b= slope and C= concentration in µg mL^{-1}, obtained by the method of least squares. Correlation coefficient, intercept and slope of the calibration data are summarized in Table 2. For accurate determination, Ringbom concentration range [30] was calculated by plotting log concentration of drug in µg mL^{-1} against transmittance % from which the linear portion of the curve gives an accurate range of microdetermination of DNP and represented in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines [31] and illustrated in Table 2. The high molar absorptivity and lower Sandell's sensitivity values reflect the good and high sensitivity of the proposed methods. The validity of the proposed methods was evaluated by statistical analysis [32] between the results achieved from the proposed methods and that of the reported method [22]. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 2), there is no significant difference between the proposed and reported methods regarding accuracy and precision.

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas [31, 32]:

$$LOD=3.3\sigma/s \quad \text{and} \quad LOQ=10\sigma/s \qquad (2)$$

Where σ is the standard deviation of five reagent blank determinations, and s is the slope of the calibration curve.

Accuracy and precision

To evaluate the precision of the proposed methods, solutions containing three different concentrations of DNP were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 3. Lower values of the relative standard deviation (RSD%) and percentage relative error (RE%) indicate the precision and accuracy of the proposed methods. The percentage relative error is calculated using the following equation:

$$\%\ R.E. = \left[\frac{found - taken}{taken} \right] \times 100 \qquad (3)$$

The assay procedure was repeated six times, and percentage relative standard deviation (RSD%) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision). For the same concentrations of drug inter- and intra-day accuracy of the proposed methods were also evaluated. The percentage recovery values with respect to found concentrations of each drug were evaluated to ascertain the accuracy of the methods. The recovery values close to 100% as compiled in Table 3 shows that the proposed methods are very accurate.

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation of method variables, including concentration of analytical reagent and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. The analysis was performed with altered conditions by taking three different concentrations of DNP and it was found that small variation of method variables did not significantly affect the procedures as shown by the RSD values in the ranges of 0.75-2.60%. This provided an indication for the reliability of the proposed methods during its routine application for the analysis of DNP and so the proposed methods are considered robust. Ruggedness was expressed as the RSD and was also tested by applying the proposed methods to the assay of DNP using the same operational conditions but using three different instruments as well as three different anaysts. The inter-analysts RSD were in the ranges 0.60-2.20%, whereas the inter-instruments RSD ranged from 0.50-2.40% suggesting that the developed methods were rugged. The results are shown in Table 4.

Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure aripiprazole (50, 100 and 150% of the level present in the tablet) to a fixed amount of drug in tablet powder (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\%\ \text{Recovery} = \frac{[C_F - C_T]}{C_p} \times 100 \qquad (4)$$

Where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drug) added to tablets preparations. The results of this study presented in Table 5 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets which did not interfere in the assay.

Application of pharmaceutical formulations (tablets)

The proposed methods were applied to the determination of DNP in tablets. The results in Table 6 showed that the methods are successful for the determination of DNP and that the excipients in the dosage forms do not interfere. A statistical comparison of the results obtained from the assay of DNP by the

proposed methods and the reported method [22] by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values for five degrees of freedom [32] (Table 6). Hence, no significant difference between the proposed methods and the reported method at the 95 % confidence level with respect to accuracy and precision.

CONCLUSIONS

A new, simple, rapid, useful and cost-effective spectrophotometric methods have been developed for determination of DNP in pure form and tablets using CAS as oxidizing agent and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity of operation, high selectivity, comparable sensitivity, low-cost instrument, they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many of the previous methods reported for aripiprazole. The proposed methods have some additional advantages involve less stringent control of experimental parameters such as the stability of the colored system, accuracy, reproducibility, time of analysis, temperature independence and cheaper chemicals. These advantages encourage the application of the proposed methods in routine quality control analysis of DNP in pure and dosage forms.

REFERENCES

1. Sweetman S, Martindale, The Complete Drug Reference, 37th Edition, The Pharmaceutical Press; London; 2011; Electronic version. 32, 2002, 1417.
2. Bryson M, Benfield P, Donepezil. New Drug Profile. Drugs and Aging, 10, 1997, 234-239.
3. Winblad B, Kilander L, Eriksson S, Minthon L, Batsman S, Wetterholm AL, Jansson- Blixt C, Haglund A, Donepezil in patients with severe Alzheimer's disease: Double-blind, parallel-group, placebo-controlled study, Lancet, 367, 2006, 1057.
4. Nakashima K, Itoh K, Kono M, Nakashima MN, Wada M, Determination of donepezil hydrochloride in human and rat plasma, blood and brain microdialysates by HPLC with a short C 30 column, J. Pharma. Biomed. Anal., 41, 2006, 201-206.
5. Iordachescu A, Silvestro L, Tudoroniu A, Rizea Savu S, Ciuca V, LC–MS–MS method for the simultaneous determination of donepezil enantiomers in plasma, Chromatographia., 75, 2012, 857-864.
6. Mallu UR, Reddy KH, Bobbarala V, Penumajji S, RP-HPLC method development for the determination of assay of donepezil hydrochloride, Int. J. Chem. Anal. Sci., 1, 2010, 35-36.
7. Liew KB, Peh KK, Fung Tan YT, RP-HPLC analytical method development and optimization for quantification of donepezil hydrochloride in orally disintegrating tablet, Pak. J. Pharm. Sci., 26, 2013, 961-966.
8. Abonassif MA, Hefnawy MM, Kassem MG, Mostafa GA, Determination of donepezil hydrochloride in human plasma and pharmaceutical formulations by HPLC with fluorescence detection, Acta Pharm, 61, 2011, 403-413.
9. Kafkala S, Matthaiou S, Alexaki P, Abatzis M, Bartzeliotis A, Katsiabani M, New gradient high-performance liquid chromatography method for determination of donepezil hydrochloride assay and impurities content in oral pharmaceutical formulation, J. Chromatogr. A., 1189, 2008, 392-327.
10. Mr. Prakash Kumar B, Sharath HN, Development and validation for estimation of donepezil HCl from bulk and marketed dosage forms by using RP-HPLC, Int. J. Pharm. Pharm. Res., 7, 2016, 343-349.
11. Gotti R, Cavrini V, Pomponio R, Andrisano V. Analysis and enantioresolution of donepezil by capillary electrophoresis, J. Pharma. Biomed. Anal., 24, 2001, 863-870.
12. Mostafa GA, Hefnawy M, Al-Majed A, Membrane sensors for the selective determination of donepezil hydrochloride, J. AOAC Int., 93, 2010, 549-555.
13. Patel C., Patel N., Kothar C, Quantitative determination of donepezil hydrochloride by a simple and accurate synchronous spectrofluorimetric method in human plasma, J. Young Pharma., 6, 2014, 47-50.
14. Jayanna BK, Nagendrappa G, Ninge Gowda A, A facile spectrophotometric method for the determination of donepezil hydrochloride in tablets formulation using potassium permanganate, Asian J. Pharm. Biol. Res., 2, 2012, 216-218.
15. Ravi Kumar V, Chaitanya S, Sambasiva Rao A, Sreekiran M, Estimation of donepezil hydrochloride by ion complex extractive spectrometry, Int. J. Res. Pharm. Chem., 1, 2011, 512-518.
16. Shirwaikar A, Devi S, Rajagopal PL, Kiron SS, Sreejith KR, Ion pair extraction method for determination of donepezil hydrochloride in pure and dosage forms, Int. J. Res. Pharm. Biomed Sci., 4, 2013, 1386-1393.
17. Sangsetti JN, Mahaparale PR, Paramane S, Sinde DB, Spectrophotometric estimation of donepezil hydrochloride in bulk and tablet formulation, Trends in Appl. Sci. Res., 3, 2008, 109-112.
18. Shirwaikar A, Devi S, Rajagopal PL, Kiron SS, Sreejith KR, Ion pair extraction method for determination of donepezil hydrochloride in pure and dosage forms, Asian J. Pharm. Clin. Res. Sci., 7, 2014, 149-153.
19. Venkatrao1 SV, Malleswararao1 NNV, Sivaramakrishna1 V, Neeharika T, Rambabu C, Simple and sensitive extractive spectrophotometric method for the assay of donepezil HCl in pure and pharmaceutical formulations, J. Pharm. Res. 5, 2012, 3527-3529.
20. Pillai S, Singhvi I, Application of orange dye for quantitation of donepezil hydrochloride from tablet formulation, Indian J. Pharm. Sci., 68, 2006, 682-684.
21. Raghubabu K, Santhi Swarup L, Kalyana Ramu B, Rupakumari G, Narayanarao M, Ramdas C, Development and validated visible spectrophotometric methods for the assay of donepezil hydrochloride in pharmaceutical preparations, Res. J. Pharm. Tech., 5, 2012, 228-232.

22. El Sheikh R, Gouda AA, Soliman H, Sensitive spectrophotometric determination of acetylcholine esterase inhibitor donepezil hydrochloride in pure form and pharmaceutical formulations using sulphonphthalin dyes, Int. J. Pharm. Pharm. Sci., 7, 2015, 274-281.

23. Patel JA, Chavhan VD, Deulgaonkar YB, Mahajan MP, Spectrophotometric determination of donepezil hydrochloride in bulk & tableted Form by absorption maxima, first order derivative spectroscopy and area under the curve, Indo. Amer. J. Pharm. Res., 3, 2013, 3760-3766.

24. Abdellatef HA, El-Henawee MM, El-Sayed HM, Ayad MM, Spectrophotometric and spectrofluorimetric methods for analysis of acyclovir and acebutolol hydrochloride, Spectrochim. Acta A., 65, 2006, 997-999.

25. Krebs A, Starczewska B, Puzanowska-Tarasiewicz H, Sledz J. Spectrophotometric determination of olanzapine by its oxidation with N-bromosuccinimide and cerium(IV) sulfate, Anal. Sci., 22, 2006, 829-833.

26. El-Didamony AM, Erfan EAH, Utilization of oxidation reactions for the spectrophotometric determination of captopril using brominating agents, Spectrochim. Acta. A., 75, 2010, 1138-1145.

27. El Shiekh R, Amin AS, Hafez EM, Gouda AA, Spectrophotometric estimation of vardenafil HCl and tadalafil in pure forms and tablets using cerium(IV) ammonium sulphate, Der Pharm. Lett., 8, 2016, 153-165.

28. El-Didamony AM, Hassan WS, Spectrophotometric and fluorimetric methods for determination of naltrexone in urine, serum and tablets by oxidation with cerium (IV), J. Chil. Chem. Soc., 57, 2012, 1404-1408.

29. Jeffery GH, Bassett J, Mendham J, Denney RC, Titrimetric analysis. In Vogel's a text book of quantitative inorganic analysis, 5th Ed., ELBS: London, 1989; 308.

30. Ringbom A. "Accuracy of calorimetric determinations", Z Anal. Chem., 115, 1939, 332-343.

31. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology, London, November 2005.

32. Miller JN, Miller JC, "Statistics and chemometrics for analytical chemistry" 5th ed., Prentice Hall, England, 2005.

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