Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Original Article

SENSITIVE SPECTROPHOTOMETRIC DETERMINATION OF ACETYLCHOLINESTRASE INHIBITOR DONEPEZIL HYDROCHLORIDE IN PURE FORM AND PHARMACEUTICAL FORMULATIONS USING SULPHONPHETHALIN DYES

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Received: 31 Mar 2015 Revised and Accepted: 26 Jun 2015

ABSTRACT

Objective: Four sensitive, selective, rapid, validated and easily reproducible spectrophotometric methods have been developed for the determination of acetylcholinesterase inhibitor donepezil hydrochloride (DNP) in pure form and in pharmaceutical formulations

Methods: The proposed methods are based on ion-pair complex formation between donepezil hydrochloride with four acidic (sulphonphthalein) dyes; namely bromocresol green (BCG), bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol purple (BCP) which extracted into dichloromethane followed by the measurement of the yellow colored ion-pair complexes at 420, 413, 415 and 409 nm for DNP-BCG, DNP-BTB, DNP-BPB and DNP-BCP complexes, respectively.

Results: Beer's law was obeyed in the concentration ranges of 1.0-12 and 1.0-10 µg $ml^{-1}$for (BCG or BCP) and (BTB or BPB) methods, respectively with limits of detection (LOD) of 0.16, 0.24, 0.19 and 0.25 µg/ml using BCG, BCP, BTB and BPB methods, respectively. The stoichiometry of the ion-pair complex formed between the dug and dye found to be (1:1) was determined by Job's method of continuous variations. Various analytical parameters have been evaluated and the results have been validated by statistical data.

Conclusion: The proposed methods were validated in accordance with ICH guidelines and successfully applied to the determination of donepezil hydrochloride in pure and dosage forms. Statistical comparison of the results obtained by applying the proposed methods with those of the reported method revealed good agreement and proved that there was no significant difference in the accuracy and precision between the results.

Keywords: Donepezil hydrochloride, Spectrophotometry, Ion-pair complex, Sulphonphthalein 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 (fig. 1). DNP is acetyl cholinesterase inhibitor used in the treatment of Alzheimer's disease [1-3].

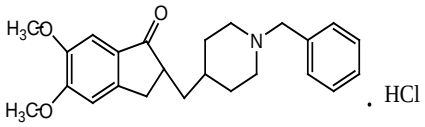


Fig. 1: Chemical structure of donepezil HCl (DNP)

A review of the literature showed that few analytical methods have been reported for the quantitative determination of DNP in pharmaceutical preparations, including high-performance liquid chromatography (HPLC) methods [4-9], capillary electrophoresis [10, 11], potentiometry [12] and spectro fluorimetric [13]. Most of these reported methods are not simple for routine analysis, cumbersome, time consuming and expensive.

While few spectrophotometric methods [14-22] has been reported for the determination of DNP in dosage forms. However, most of the reported visible spectrophotometric methods suffer from one or the other disadvantage like narrow range of determination, poor sensitivity, use of heating, strict pH control, etc. Hence, it is always required to develop and validate a simple, sensitive, fast, cost effectiveness, accurate and easy access in most quality control laboratories ion pair spectrophotometric methods for the determination of DNP in its dosage form. Spectrophotometric procedures through ion pair extraction technique are popular for their sensitivity in the assay of drugs [23–46].

In the present work, two simple, accurate and sensitive spectrophotometric methods with extraction and free from extraction based on ion-pair formation reaction between DNP and acid dyes (BCG, BTB, BCP or BPB) are proposed for the determination of DNP in pure form and pharmaceutical preparations. Also, the proposed methods are rapid and have the very low detection limit which cannot be reached by most other methods.

MATERIALS AND METHODS

Apparatus

All absorption spectra were made using Kontron Unikon 930 (UV-Visible) spectrophotometer (German) with a scanning speed of 200 nm/min. and a bandwidth of 2.0 nm, equipped with 10 mm matched quartz cells. The pH values of different buffer solutions were checked using a Hanna pH-meter instrument (pH 211) (Romania) equipped with a combined glass-calomel electrode.

Materials and reagents

All chemicals and reagents were of analytical grade and used without further purification and all solutions were prepared fresh daily. Doubly distilled water was used in experiments.

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%.

Pharmaceutical formulations

All pharmaceutical preparations were obtained from commercial sources in the local markets.

-Aricept tablet labeled to contain (5.0 mg DNP per tablet) was purchased from 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) were purchased from Global Napi Pharmaceuticals Company (GNP), Egypt.

- Donepezil tablets labeled to contain (5.0 mg DNP per tablet) were purchased from Delta Pharma, Egypt.

Preparation of stock standard solution

A stock standard DNP solution (100 µg/ml) and (1.0×10^{-3} mol/l) was prepared by dissolving an exact weight 10 and 42 mg of pure drug in 100 ml bidistilled water. The standard solutions were found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator at 4 °C. Serial dilution with the same solvent was performed to obtain the appropriate concentration range.

Reagents

All reagents and solvents used were of analytical-reagent grade. Bromocresol green (BCG), bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol purple (BCP) (BDH Chemicals LTD, Poole, England) and used without further purification. Stock solutions (1.0×10^{-3} mol/l) of reagents were prepared by dissolving the appropriate weight of each reagent in 5.0 ml of 96% ethanol and diluted to 100 ml in a calibrated flask with bidistilled water. These solutions were stable for at least one week at 4 °C.

Series of buffer solutions of NaOAc–HCl (pH=2.0-5.0), NaOAc–AcOH (pH=3.0-5.6) and potassium hydrogen phthalate–HCl (pH=2.0-7.0) were prepared by following the standard methods [32]. The pH of each solution was adjusted by addition of 0.2 mol/l hydrochloric acid or sodium hydroxide. Freshly prepared solutions were always employed. Chloroform and dichloromethane (BDH), anhydrous sodium sulfate (Prolabo), ethanol (BDH).

General procedures

Accurately measured aliquots (0.1–1.2 ml) of the standard DNP solution (100 µg/ml) were transferred to 10 ml measuring flasks. A volume of 2.0 ml acetate buffer with pH 3.0 and 3.5 was added for (BCG or BTB) and (BCP or BPB), respectively, then 2.0 ml of 1.0×10^{-3} mol/l reagent solution was added. The total volume of each solution was completed to 10 ml with bidistilled water. The formed ion associate complexes were extracted with 10 ml dichloromethane by shaking for 2.0 min, and then allowed to stand for clear separation of the two phases and the dichloromethane layer was passed through anhydrous sodium sulfate. The absorbance of the yellow colored ion-pair complexes were measured at 420, 413, 415 and 409 nm for BCG, BTB, BPB and BCP, respectively against corresponding reagent blank prepared similarly. All measurements were made at room temperature (25±2 °C). In both methods, a standard curve was prepared by plotting the absorbance values versus concentrations of drug. A linear equation for the standard curve was calculated by linear regression.

Applications to 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 bi distilled water with shaking for 5.0 min and filtered through a sintered glass crucible (G_4). The filtrate was diluted to 50 ml with bi distilled water in a 50 ml measuring flask to give 100 µg/ml stock solutions. Aliquot of the cited solutions was taken and analyzed as described under the above recommended procedures for construction of calibration curves. For the proposed methods, the content of the tablets was calculated using the corresponding regression equation of the appropriate calibration graph. The method of standard addition was used for the accurate determination of DNP content.

Stoichiometric relationship

The stoichiometric ratios of the ion-associates formed between DNP and the reagents were determined by applying the continuous variation method [33] at the optimum wavelengths of maximum absorbance. In continuous variation method, equimolar solutions were employed: a 1.0×10^{-3} mol/l standard solution of DNP and 1.0×10^{-3} mol/l solution of dye were used. A series of solutions was prepared in which the total volume of DNP and the dye was kept at 2.0 ml. The drug and reagent were mixed in various complementary proportions (0.2:1.8, 0.4:1.6, 0.6:1.4, 0.8:1.2, 1.0:1.0, 1.2:0.8, 1.4:0.6, 1.6:0.4, 1.8:0.2) and completed to volume in a 10 ml calibrated flask with the appropriate solvent for extraction following the above mentioned procedure.

RESULTS

Absorption spectra

The nitrogenous drugs are present in positively charged protonate forms and anionic dyes of sulphonpthalein group present mainly in anionic form at pH ≥ 2.5. So when treated with an acid dye at a pH range (2.5-5.0) of acidic buffers solutions, a yellow ion-pair complex which is extracted with dichloromethane is formed. The absorption spectra of the ion-pair complexes, which were formed between DNP and reagents, were measured in the range 350–550 nm against the blank solution. The maximum absorbance of ion-pair complexes shows at 420, 413, 415 and 409 nm for BCG, BTB, BPB and BCP, respectively (fig. 2 and 3).

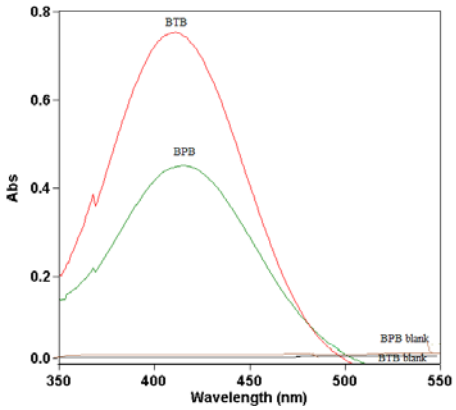


Fig. 2: Absorption spectrum of ion-pair complexes of (10 µg/ml) DNP and (1.0×10^{-3} mol/l) BTB and BPB reagents against reagent blank

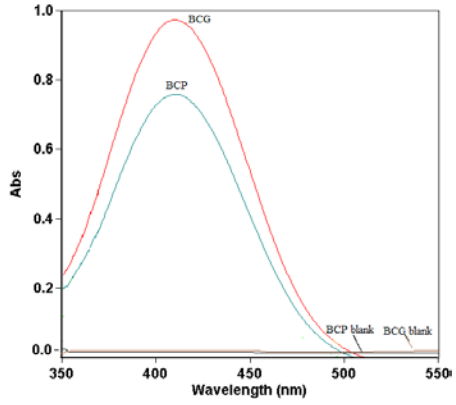


Fig. 3: Absorption spectrum of ion-pair complexes of (10 µg/ml) DNP and (1.0×10^{-3} mol/l) BCG and BCP reagents against reagent blank

Optimum reaction conditions for complex formation

The optimization of the methods was carefully studied to achieve complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH of the buffer, the type of organic solvent, volumes of the dye, reaction time and temperature for the extraction of ion-pair complexes.

Effects of pH on ion-pair formation

The effect of pH on the drug–reagent complex formation was studied by extracting the colored complexes in the presence of various buffers. It was noticed that the maximum color intensity and highest absorbance value were observed in NaOAc–AcOH buffer of pH 3.0 and 3.5 using (BCG or BTB) and (BCP or BPB) method, respectively (fig. 4). Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-4.0 ml). The higher absorbance value and reproducible results was obtained at using 2.0 ml of buffer solutions.

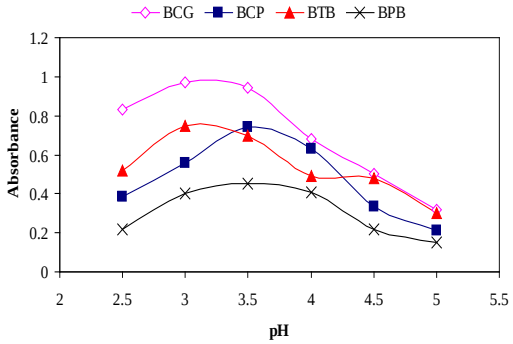


Fig. 4: Effect of pH of buffer solution on ion pair complex formation between (10 µg/ml) DNP and (1.0 x 10^{-3} mol/l) reagents

Effects of reagent concentration

The effect of the reagent was studied by measuring the absorbance of solutions containing a fixed concentration of DNP and varied amounts of the respective (1.0×10^{-3} mol/l) reagent from 0.5–4.0 ml. The maximum color intensity of the complex was achieved with 2.0 ml of each reagent (1.0 × 10^{-3} mol/l) solution. After this volume, the absorbance remains constant by increasing the volume of the reagents (fig. 5). So an excess of reagents has no effect on the determination of the drug.

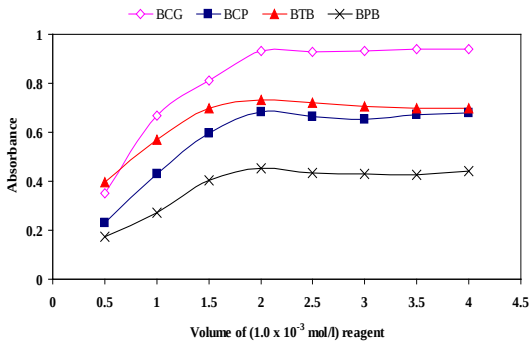


Fig. 5: Effect of volume of (1.0 ×10^{-3} mol/l) reagent on the ion pair complex formation with (10 µg/ml) DNP

Effect of extracting solvent

Different organic solvents as dichloromethane, carbon tetrachloride, chloroform, dichloroethane and ether were tested as extractive solvents for the proposed methods. Dichloromethane was preferred to other solvents for its selectivity and to obtain the highest absorbance. It was also observed that only one extraction with total volume 10 ml solvent was adequate to achieve a quantitative recovery of the complexes, maximum absorbance intensity and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.

Effect of shaking time and temperature

The optimum shaking time was investigated by shaking from 0.5-5.0 min. Maximum and constant absorbance values were obtained when extracted after shaking for 2.0 min. Therefore, shaking time of 2.0 min was maintained throughout the experiment. The effect of temperature on colored complexes was studied by measuring the absorbance values over the temperature range 20-35 °C. It was found that the absorbance of the colored ion pair complex was constantly up to 30 °C. At higher temperatures, the drug concentration was found to increase due to the volatile nature of the dichloromethane. Therefore, room temperature (25±2 °C) was chosen as the best temperature for micro-determination of DNP in pure and pharmaceutical formulations. The absorbance of both complexes remains stable for at least 12-24 hrs at room temperature.

Stoichiometric relationship

The molar ratio between DNP and dyes in the ion-pair complexes was determined by Job's method of continuous variation [33]. Continuous variation method of equimolar solutions was employed: a 1.0 × 10^{-3} mol/l standard solution of drug base and 1.0 × 10^{-3} mol/l solution of dye were used. A series solution was prepared in which the total volume of drug and reagent was kept at 2.0 ml in the total volume of 10 ml of the aqueous layer. The absorbance of extracting ion-pair in each instance was measured at the optimum wavelength and plotted against the mole fraction of the drug. The results indicated that the molar ratio of (drug: dye) is (1:1) complex was formed through the electrostatic attraction between the positive charged DNP^{+}ions and negatively charged BCG^{-}, BCP^{-}, $BTB^{-}$and BPB^{-} dye, (D^{-}) ions (fig. 6).

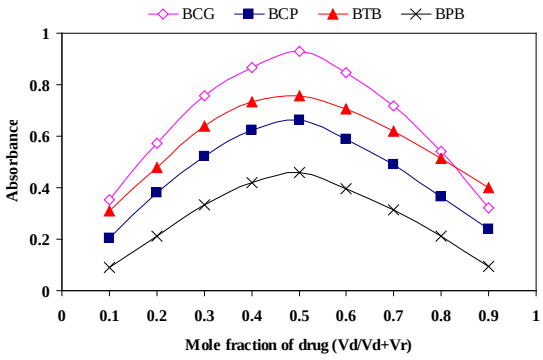
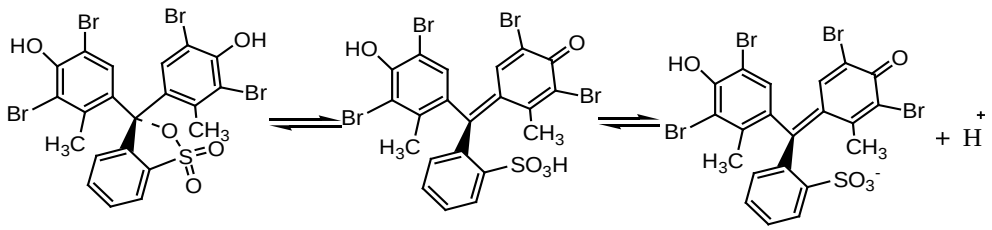
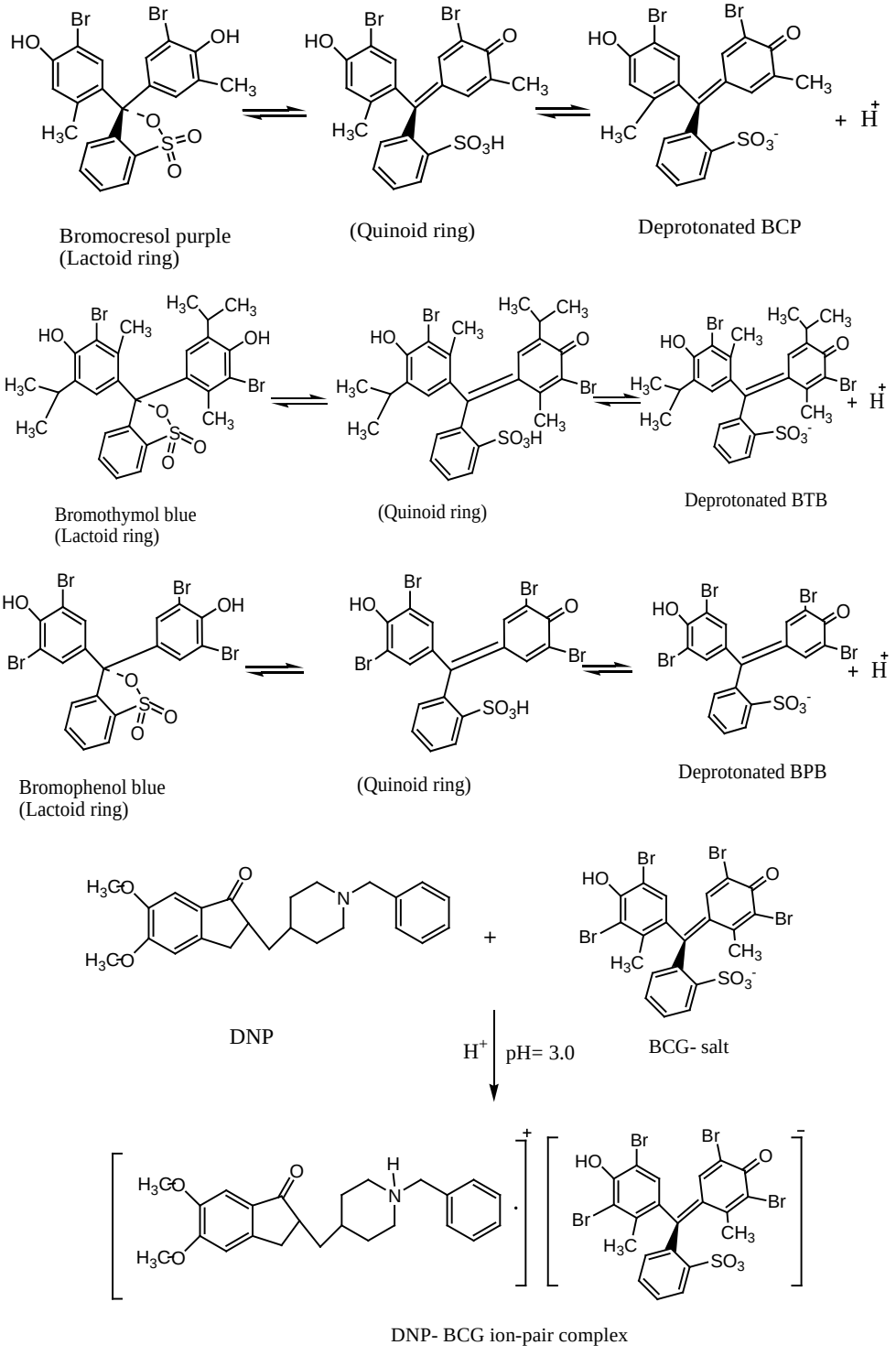


Fig. 6: Job's method of continuous variation graph for the reaction of DNP with the studied dyes, [drug] = [dye] = (1.0×10^{-3} mol/l)



Bromocresol green (Lactoid ring) (Quinoid ring) Deprotonated BCG



Scheme 1: Proposed reaction mechanism for the ion pair complex formation between DNP and BCG

Method of validation

Linearity

At described experimental conditions for DNP determination, standard calibration curves with reagents were constructed by plotting absorbance vs. Concentration of DNP. The statistical parameters were given in the regression equation calculated from the calibration graphs A = aC+b, where A is the absorbance and C is concentration in µg/ml. The linearity of calibration graphs was proved by the high values of the correlation coefficient (*r*) and the small values of the *y*-intercepts of the regression equations. Beer's law was obeyed in the concentration range of 1.0-12 and 1.0-10 µg/ml for (BCG or BCP) and (BTB or BPB) methods, respectively (fig. 7).

The apparent molar absorptivity of the resulting colored ion-pair complexes and relative standard deviation of response factors for each proposed spectrophotometric method were also calculated and recorded in table 1. The molar absorptivity of BCG>BTB>BCP>BPB ion-pair complexes with DNP.

Table 1: Statistical analysis of calibration graphs and analytical data in the determination of DNP using the proposed methods

Parameters	BCG	BCP	BTB	BPB
Wavelengths λ_{max} (nm)	420	409	413	415
Beer's law limits (µg/ml)	1.0-12	1.0-12	1.0-10	1.0-10
Molar absorptivity ε, (l/mol. cm) x 10^4	3.4613	2.428	3.0722	1.8631
Sandell·s sensitivity (ng/cm)	12.07	17.13	13.54	22.33
Regression equation [a]				
Intercept (*a*)	0.0071	0.0049	0.0076	-0.0017
Slope (b)	0.0798	0.0567	0.0704	0.0452
Correlation coefficient (*r*)	0.9994	0.9996	0.9993	0.9995
LOD (µg/ml)[b]	0.16	0.24	0.19	0.25
LOQ (µg/ml)[b]	0.53	0.80	0.63	0.83
mean±SD[b]	99.75±1.26	99.33±0.81	99.40±1.09	99.39±0.92
SE[b]	0.47	0.30	0.44	0.38
Variance	1.58	0.65	1.17	0.85
RSD%[b]	1.26	0.80	1.08	0.91
RE%[b]	1.32	0.84	1.14	0.96
t-test [c]	0.16	1.17	0.82	0.95
F-test[c]	4.88	2.02	3.66	2.61

[a]A = a+bC, where C is the concentration in µg/ml, A is the absorbance units, [b]LOD, limit of detection; LOQ, limit of quantification; SD, standard deviation; SE, standard error; RSD%, relative standard deviation; RE%, relative error, [c]The theoretical values of t and F at P= 0.05 are 2.57 and 5.05, respectively.

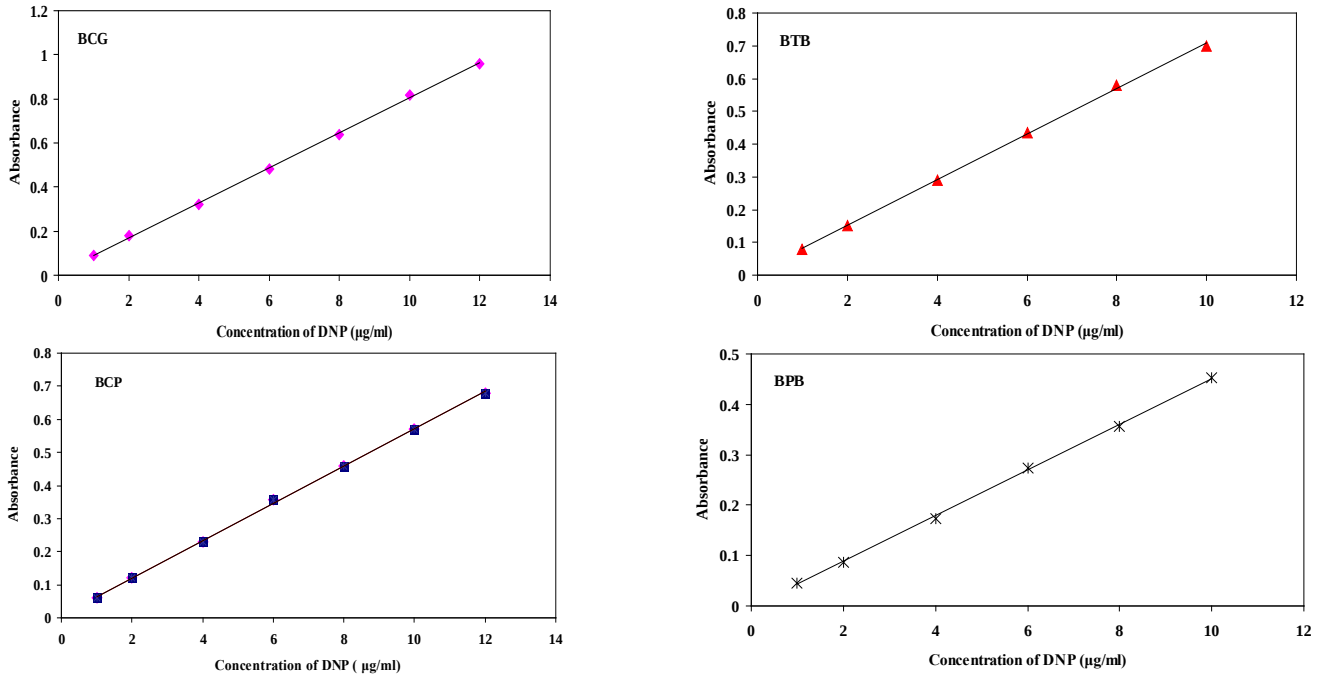


Fig. 7: Calibration curves for determination of DNP (1.0–12 µg/ml) with (BCG or BCP) and DNP (1.0–10 µg/ml) with (BTB or BPB)

Sensitivity

The limits of detection (LOD) and quantitations (LOQ) for the proposed methods were calculated using the following equation [34, 35]:

LOD = *3s/k* and LOQ = *10 s/k*

Where *s* is the standard deviation of the response of the blank or the standard deviation of intercepts of regression lines and *k* is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the limit of detection was found to be 0.16, 0.21, 0.19 and 0.25 µg/ml for BCG, BCP, BTB and BPB methods, respectively. The limit of quantitations was found to be 0.53, 0.80, 0.63 and 0.83 µg/ml for BCG, BCP, BTB and BPB methods, respectively (table 1).

Accuracy and precision

In order to evaluate the accuracy and precision of the proposed methods, solutions containing three different concentrations of DNP were prepared and the assay procedure was analyzed in six replicates, and percentage relative standard deviation (% R. S. D) 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).

The analytical results of intra-day and inter-day precision and accuracy were summarized in table 2. The low values of percentage relative standard deviation (R. S. D %) as precision and percentage relative error (RE %) as accuracy of the proposed methods was calculated. The percentage relative error was calculated using the following equation

RE % = [(founded–added)/added] x 100

These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility,

Table 2: Intra-day and Inter-day precision and accuracy data for DNP obtained by the proposed methods

Method	Added (µg/ml)	Intra-day				Inter-day			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]
BCG	2.0	99.10	0.40	-0.90	1.980± 0.008	99.30	0.39	-0.70	1.986± 0.008
	6.0	99.40	0.57	-0.60	5.964± 0.034	99.80	0.61	-0.20	5.988± 0.037
	10	100.40	0.80	0.40	10.040± 0.08	100.70	1.21	0.70	10.070±0.122
BCP	2.0	99.20	0.35	-0.80	1.984± 0.007	99.00	0.42	-1.00	1.980± 0.008
	6.0	100.60	0.64	0.60	6.036± 0.039	99.90	0.78	-0.30	5.994±0.047
	10	99.40	0.94	-0.60	9.940± 0.093	99.70	1.17	0.70	9.970± 0.117
BTB	2.0	99.40	0.46	-0.60	1.988± 0.091	99.40	0.58	-0.40	1.988± 0.012
	4.0	99.70	0.72	-0.30	3.988± 0.029	99.00	0.65	-1.00	3.960± 0.026
	8.0	100.30	1.06	0.30	8.024± 0.085	99.50	0.97	0.50	7.960± 0.077
BPB	2.0	99.10	0.50	-0.90	1.982± 0.010	99.70	0.43	-0.30	1.994± 0.009
	4.0	100.40	0.83	0.40	4.016± 0.033	99.90	0.71	-0.10	3.996±0.028
	8.0	99.40	1.14	-0.60	7.952± 0.091	100.20	1.34	0.20	8.016± 0.107

[a]Mean of six determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.

[b]Confidence limit at 95% confidence level and five degrees of freedom (t = 2.571).

Robustness and ruggedness

The robustness of the method was evaluated by making small incremental changes in the volume of dye, pH and shaking time, and the effect of these changes on the absorbance of the colored systems was studied. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as RSD (≤ 3.0 %). Method ruggedness was demonstrated by having the analysis done by two analysts, and also by a single analyst performing analysis on two different instruments in the same laboratory.

The results showed no statistical differences between different analysts and instruments suggesting that the developed methods were robust and rugged. Intermediate precision values (RSD) of this study were ≤ 3.0 % indicating acceptable ruggedness (table 3).

Table 3: Robustness and ruggedness of the proposed methods

Method	Taken (µg/ml)	Method robustness		Method ruggedness	
		Parameters altered Reagent volume, ml[a] RSD, % (n=3)	Reaction time [b] RSD, % (n=3)	Inter-analysts RSD, % (n=4)	Inter-cuvettes RSD, % (n=4)
BCG	2.0	0.97	1.40	1.42	1.24
	6.0	1.05	1.52	1.60	1.58
	10	1.40	1.60	1.40	1.19
BCP	2.0	1.02	1.35	1.84	1.32
	6.0	0.60	1.60	1.63	1.53
	10	1.20	1.94	1.20	1.07
BTB	2.0	0.80	1.46	1.76	1.38
	4.0	0.76	1.72	1.31	1.72
	8.0	1.10	1.06	1.50	1.10
BPB	2.0	1.24	1.50	1.32	1.36
	4.0	1.05	1.83	1.45	1.82
	8.0	0.86	1.14	1.60	1.29

[a]In all methods the volume of reagent 1.8, 2.0 and 2.2 ml., [b]The reaction time 1.5, 2.0 and 2.5 min for all methods, RSD%, percentage relative standard deviation.

Effects of interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany DNP in its dosage forms (tablets) (starch, lactose, glucose, succarose, talc, sodium chloride, titanium dioxide, and magnesium stearate) was studied. The results indicated that there is no interference from excipients and additives, indicating a high selectivity for determining the studied DNP in its dosage forms.

Applications of pharmaceutical formulations

The proposed methods have been successfully applied to the determination of DNP in dosage forms (Aricept tablet, Alzepizil

tablets, Donepezil tablets). Six replicate determinations were made. Moreover, to check the validity of the proposed methods, dosage forms were tested for possible interference with the standard addition method (table 4). There was no significant difference between slopes of calibration curves and the standard addition method. Therefore it is concluded that the excipients in pharmaceutical preparations of DNP did not cause any interference in the analysis of DNP. The results were compared with those obtained using the reported method for DNP [16]. Statistical analysis of the results did not detect any significant difference between the proposed methods and the reported method [16] in pharmaceutical formulations with respect to accuracy and precision as revealed by the Student's t-value and variance ratio F-value at 95% confidence level [36]. The results show that the Student's t-and F-values at 95 % confidence level did not exceed the theoretical values which confirmed that there is a good agreement between the results obtained by the proposed methods and the reported method [16] with respect to accuracy and precision (table 4).

Table 4: Application of the standard addition technique for the determination of DNP in dosage forms using the proposed methods

Samples	Taken (µg/ml)	Added (µg/ml)	Recovery[a] (%)				
			BCG	BCP	BTB	BPB	Reported method [16]
Aricept tablets	2.0	-	99.10	99.50	99.20	99.70	
		2.0	99.40	100.50	99.60	100.40	
		4.0	100.60	99.00	99.90	100.10	
		6.0	99.10	99.70	100.50	99.20	
		8.0	98.90	99.30	100.30	98.90	
mean±SD[a]			99.42±0.683	99.60±0.566	99.90±0.524	99.66±0.619	99.68±0.72
R. S. D%			0.679	0.564	0.523	0.617	0.720
V			0.467	0.32	0.275	0.383	0.520
t-value[b]			0.59	0.20	0.55	0.05	
F-value[b]			1.11	1.63	1.89	1.36	
Alzepizil tablets	4.0	-	99.40	100.15	99.50	99.00	
		2.0	100.40	99.80	99.00	99.70	
		4.0	99.20	100.30	100.30	99.90	
		6.0	99.50	99.20	100.80	100.50	
		8.0	100.40	99.10	99.40	100.10	
mean±SD			99.78±0.576	99.71±0.544	99.80±0.731	99.84±0.555	99.87±0.630
R. S. D%			0.575	0.542	0.712	0.554	0.630
V			0.332	0.295	0.535	0.308	0.400
t-value[b]			0.24	0.43	0.16	0.08	
F-value[b]			1.20	1.36	1.34	1.30	
Donepezil tablets	4.0	-	99.50	99.05	99.40	99.60	
		2.0	99.90	98.80	99.50	99.80	
		4.0	99.20	100.70	99.00	99.20	
		6.0	98.70	99.50	100.50	101.00	
		8.0	100.40	99.40	100.20	99.10	
mean±SD			99.54±0.650	99.49±0.732	99.72±0.614	99.74±0.760	99.50±0.860
R. S. D%			0.647	0.728	0.612	0.758	0.860
V			0.423	0.536	0.377	0.578	0.740
t-value[b]			0.08	0.02	0.47	0.47	
F-value[b]			1.75	1.38	1.96	1.28	

[a]Average of six determinations. SD, standard deviation; V, variance; RSD, relative standard deviation, [b]The theoretical values of *t* and *F* are 2.571 and 5.05, respectively at the confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

DISCUSSION

The proposed methods are based on the reactivity of tertiary amine group of DNP with four acid dyes (BCG, BTB, BPB and BCP). DNP forms an ion-association complex with acid dyes which is extractable into dichloromethane from an aqueous phase. The protonated nitrogen (positive charge) of DNP as hydrochloride is expected to attract the oppositely charged part (negative charge) of the dye in acidic buffer solution at pH ≥ 2.5 and behave as a single unit being held together by electrostatic attraction and a yellow ion-pair complex which is extracted with organic solvent is formed. The absorption spectra of the yellow ion-pair complexes, which were formed between DNP and BCG, BCP, BTB or BPB reagents and show maximum absorbance at 420, 409, 413 and 415 nm, respectively against the blank solution.

The results indicate that the molar ratio of (drug: dye) is (1:1) complex was formed through the electrostatic attraction between the positive charged DNP^{+}ions and negatively charged dye, D^{-} ions. The extraction equilibrium can be represented as follows:

$$ENP^{+}_{(aq)} + D^{-}_{(aq)} \longleftrightarrow ENP^{+}D^{-}_{(aq)} \longleftrightarrow ENP^{+}D^{-}_{(org)}$$

Where DNP^{+} and D^{-} represent the protonated drug and the anion of the dye (BCG^{-}, BCP^{-}, BTB^{-} or BPB^{-}), respectively, and the subcript (aq) and (org) refer to the aqueous and organic phases, respectively.

CONCLUSION

This paper describes the application of extractive ion–pair complexation reaction with acid dyes for the quantification of DNP in pure and dosage forms. Compared with the existing spectrophotometric methods, the proposed methods are relatively simple, rapid, cost-effective, free from auxiliary reagents and more sensitive for determination of DNP in pure and dosage forms. Moreover, the proposed methods are free from tedious experimental steps such as heating unlike the previously reported methods. The most attractive feature of these methods is its relative freedom from interference by the usual diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. Therefore, the validated methods could be useful for routine quality control assay of DNP in pure and dosage forms.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests with the company name used in the paper.

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