Quim. Nova, Vol. 38, No. 5, 715-722, 2015 http://dx.doi.org/10.5935/0100-4042.20150061

Nota Técnica

SIMPLE AND SELECTIVE SPECTROPHOTOMETRIC ASSAY OF DIETHYLCARBAMAZINE CITRATE USING 2,3-DICHLORO-5,6-DICYANO-*p*-BENZOQUINONE AND 2,4-DINITRO PHENOL

Nagaraju Swamy and Kanakapura Basavaiah*
Department of Chemistry, Manasagangothri, University of Mysore, Mysore-570 006, Karnataka, India

Recebido em 09/10/2014; aceito em 02/02/2015; publicado na web em 14/04/2015

Two simple, rapid and inexpensive spectrophotometric methods are described for the determination of diethylcarbamazine citrate (DEC) in bulk drug and formulations. The methods are based on the charge–transfer (CT) complexation reaction involving DEC as the n- donor and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone [DDQ] (method A) and 2,4-dinitro phenol [DNP] (method B) as π-acceptors in chloroform. The absorbance of CT complexes was measured at 480 nm for method A, and 420 nm for method B. Under optimum conditions, Beer's law was obeyed over the concentration ranges 4-90 and 4-100 μg mL^{-1} for methods A and B, respectively.

Keywords: diethylcarbamazine citrate; spectrophotometry; charge-transfer complexes.

INTRODUCTION

Diethylcarbamazine citrate (DEC), chemically known as [N, N-diethyl-4-methyl-1-piperazinecarboxamide citrate] is an anthelmintic agent used in the treatment of filarial infections caused by a host of organisms commonly found in the tropics.[1] It is also the alternative drug of choice in *Onchocerca volvulus* infections and tropical eosinophilia.[2] The drug is official in British Pharmacopoeia (BP),[3] which describes a non-aqueous titration method for its determination and also in the United States Pharmacopoeia (USP)[4] which uses a liquid chromatographic technique with phosphate buffer-methanol system as the mobile phase for the assay with uv detection at 220 nm.

Other than these official methods, several methods have been reported for the determination of DEC in pharmaceutical dosage forms, and include gas chromatography (GC),[5-8] high performance liquid chromatography (HPLC),[9-11] proton magnetic resonance (PMR) spectroscopy,[12,13] DC polarography,[14] ion selective electrode potentiometry[15] and titrimetry.[16,17] However, many of the reported methods for DEC, particularly, the chromatographic methods are complex, requiring expensive instrumental set up and skilled operator which are not always found in laboratories of developing and under developed countries. Thus, the need for a simple, selective and low-cost method is obvious, especially for routine quality control analysis of pharmaceuticals containing DEC.

Several spectrophotometric methods based on diverse chemical reactions are found in the literature for DEC. Charge-transfer complex formed with iodine was used by Wahbi *et al.*[18] for the assay of 1-6 μg mL^{-1} DEC in tablets. Chloranilic acid has been employed by two groups of workers[19,20] whereas picric acid was used by Ramachandran[21] as CT complexing agent for the assay of drug in pharmaceuticals based on the same type of reaction. In a method reported by Basu and Dutta,[22] the ion associate formed by DEC with ammonium reineckate at pH 3.5 was filtered, dissolved in acetone and absorbance measured at 525 nm. The colored condensation product[23] formed by malonic acid with acetic anhydride in the presence of DEC was measured at 333 nm facilitating the assay of the drug in dosage forms. In a similar method,[24] the base form of the drug was reacted with malonic acid and acetic anhydride at 80 °C for 30 min and the resulting condensation product was measured at 334 nm. The yellow colored condensation product[25] formed by an acetous solution of DEC with acetic anhydride-pyridine mixture was measured at 428 nm and used for the determination of DEC in 10-100 μg mL^{-1} range in commercial tablets.

There are three reports on the use of ion-pair complexation reactions for the spectrophotometric assay of DEC. Rao and Subramanyam[26] employed bromophenol blue at acidic pH as the ion pair complexing agent for the determination of the drug in tablets and biological fluids. The drug in tablets, syrups and parenterals was determined by extracting the ion-pair complex formed with bromocresol green[27] at pH 4.6 with chloroform. The colored complexes of the drug with Fast green FCF at pH 5.0 and orange II in 0.1 mol L^{-1} HCl were successfully employed by Sastry *et al.*,[28] for the determination of DEC in bulk drug and pharmaceutical preparations by extractive spectrophotometry.

The reported spectrophotometric methods suffer from one or the other disadvantage such as poor sensitivity and narrow linear range,[18-20] use of critical pH condition,[22-28] heating,[23-25] tedious and time consuming steps like precipitation, filtration and washing,[23] and extraction.[26-28] Hence, there is a need for developing a method free from such disadvantages.

The aim of the present study was to develop and validate two simple, rapid, sensitive, cost-effective and extraction-free spectrophotometric methods based on charge-transfer (CT) complexation reactions using DDQ and DNP as π-acceptors. In both the methods, drug is reacted with DDQ and DNP in chloroform to form orange/yellow colored CT complexes with absorption maxima peaking at 480 and 420 nm, respectively. The methods were successfully applied to quantify DEC in pharmaceutical formulations.

EXPERIMENTAL

Apparatus

A Systronics model 166 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) equipped with 1-cm matched quartz cells was used for all absorbance measurements.

Materials

Pharmaceutical grade DEC (99.7% pure) was procured from Inga Laboratories Pvt. Ltd., Mumbai, India, and was used as received. Banocide Forte tablets (Glaxo Smith Kline Pharma. Ltd.,

*e-mail: kanakapurabasavaiah@gmail.com

Nashik, India) and Banocide syrup (Glaxo Smith Kline Pharma. Ltd., Bangalore, India) both were purchased from local commercial stores.

Reagents and chemicals

All reagents used were of analytical reagent grade and spectroscopic grade organic solvents were used throughout the investigation.

A 0.1% DDQ (Avra Syntheis Pvt. Ltd., Hyderabad, India), and 0.4% DNP (S.D. Fine Chem., Mumbai, India) solutions were prepared separately in chloroform ($CHCl_3$) (Merck Ltd., Mumbai, India) for method A, and method B, respectively.

Standard DEC base solution [200 μg mL^{-1}]

An amount of the powdered salt equivalent to 10 mg DEC base was accurately weighed and dissolved in 10 mL water in a 125 mL separatory funnel. The solution was made alkaline by adding 10 mL of 1 mol L^{-1} NaOH and shaken successively for 2 min with three 10 mL portions of $CHCl_3$, each extract was pooled in a beaker and passed through 2 g of anhydrous sodium sulphate supported on a quantitative filter paper using small funnel in to a 50 mL calibrated flask and completed to the mark with $CHCl_3$.

ASSAY PROCEDURES

Method A (Using DDQ)

Different aliquots of 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, and 2.25 mL of standard DEC base solution (200 μg mL^{-1}) were accurately transferred to 5 mL calibrated flasks using a micro burette, and the total volume was adjusted to 2.5 mL by adding adequate quantity of $CHCl_3$ and 0.75 mL of 0.1% DDQ was added to each flask and the mixture was diluted to the volume with $CHCl_3$ and mixed well. The absorbance of each solution was measured at 480 nm against a reagent blank within 10 min.

Method B (Using DNP)

Varying aliquots of 0.1, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mL of standard DEC base solution (200 μg mL^{-1}) were accurately added to 5 mL standard flasks using a micro burette, and the total volume was adjusted to 2.5 mL with $CHCl_3$. One mL of 0.4% DNP was added to each flask and the mixture was diluted to the volume with $CHCl_3$ and mixed well. After 5 min, the absorbance of each solution was measured at 420 nm against a reagent blank prepared simultaneously.

A standard graph was constructed by plotting absorbance against concentration, and the unknown concentration was computed from the regression equation derived using Beer's law data.

Procedure for tablets

Ten tablets each containing 100 mg DEC was weighed accurately and ground into a fine powder. An accurately weighed quantity equivalent to 10 mg of DEC base was dissolved in 10 mL water in a beaker and filtered to remove the insoluble matter. The residue was washed with water, the washings along with the filtrate were transferred to a 125 mL separatory funnel and the base solution was prepared as described earlier. Appropriate aliquots were then subjected to analysis following the above procedures.

Procedure for syrup

The content of five 100 mL bottle syrup was pooled and 5 mL syrup was diluted with water to 50 mL, and 5 mL of the resulting dilute syrup was pipetted out into a 125 mL separating funnel and mixed with 10 mL water. Shaken for 2 minutes with 10 mL 1 mol L^{-1} NaOH and three 10 mL portions of chloroform, and the base were extracted as described under 'standard DEC base solution'. This 240 μg mL^{-1} syrup extract was further diluted to get working standard solution of 200 μg mL^{-1} with $CHCl_3$ and used in the assay.

RESULTS AND DISCUSSION

Charge-transfer complexes arise from a donor-acceptor mechanism of Lewis acid-base reaction between two or more different chemical constituents. The formation of electron donor acceptor complexes can be assessed for their validity as a simple quantitative analytical method for many drug substances which can act as electron donors. DDQ and DNP as π- acceptors have been successfully employed in the determination of a variety of electron donating basic compounds of pharmaceutical importance.[29-38]

Absorption spectra

The reaction of DDQ or DNP with DEC base, results in the formation of intense orange or yellow colored product. The absorption of colored products was recorded at 380-500 nm against the corresponding reagent blanks. The resulting colored charge-transfer complexes showed maximum absorbance at 480 nm and 420 nm for DEC base-DDQ (Figure 1) and DEC base-DNP (Figure 2), respectively.

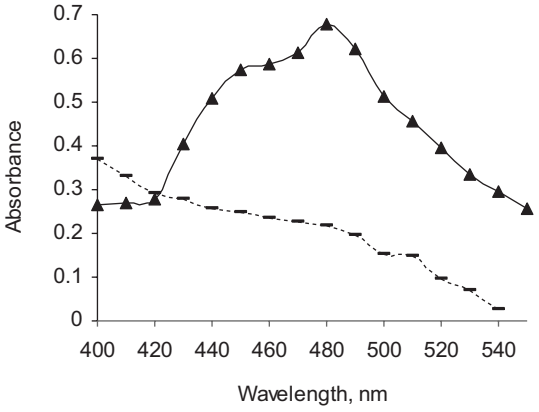


Figure 1. *Absorption spectra of DEC base-DDQ complex (- ▲) [40 μg mL^{-1} DEC] and blank (- ▬ -)*

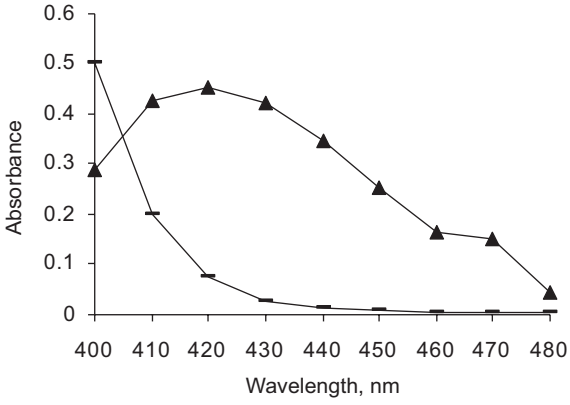
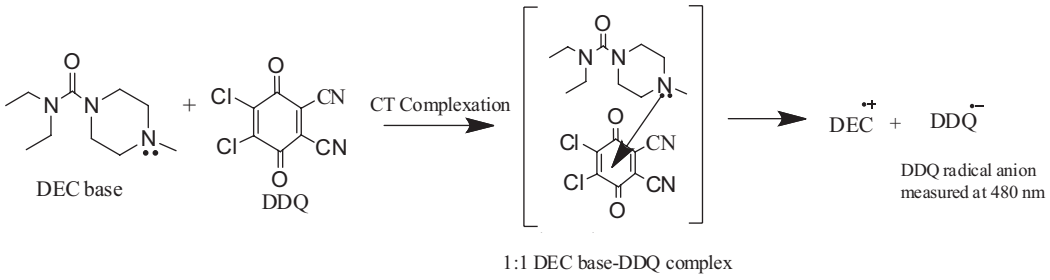
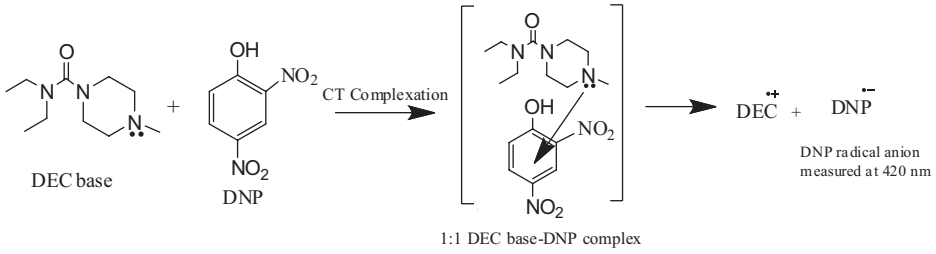


Figure 2. *Absorption spectra of DEC base-DNP complex (- ▲) [80 μg mL^{-1} DEC] and blank (- ▬ -)*



Scheme 1. *Reaction mechanism for the formation of DEC base-DDQ CT complex (method A)*



Scheme 2. *Reaction mechanism for the formation of DEC base-DNP CT complex (method B)*

Reaction mechanism

The reaction involved in the present methods is the charge-transfer complex formed by the electronic transition to an excited state where the transfer of electronic charge from donor to acceptor moiety takes place partially. As a result, the transition in this excitation energy occurs very frequently in the visible region of the electromagnetic spectrum.[39] DEC base reacts with DDQ or DNP yielding orange or yellow colored chromogen that exhibits a maximum absorption at 480 nm or 420 nm in chloroform. This can be attributed to the formation of charge-transfer complex between DEC base (as n-donor), DDQ and DNP (as π-acceptors) followed by the formation of radical anion due to the dissociation of DEC base-DDQ/DNP complex in chloroform. The interaction between the donor and acceptor occurs according to the Schemes 1 and 2.

Choice of solvent

DEC is soluble in water, hot alcohol and practically insoluble in most organic solvents, and hence the drug was converted into base form using sodium hydroxide and extracted into $CHCl_3$ before reacting with DDQ or DNP. For preparation of the reagent solutions, attempts were made to use different solvents such as 1,4-dioxan, $CHCl_3$, acetonitrile, acetone, *t*-butanol, 2-propanol and dichloromethane, and the reaction of DEC base with DDQ or DNP was followed. In both the methods, $CHCl_3$ was best suited for preparation of DEC base, and DDQ and DNP solutions. Similarly, the effect of the diluting solvent was studied and the results showed that the ideal diluting solvent to achieve maximum sensitivity was $CHCl_3$.

Effect of reagent concentration

Though, 0.5 mL of 0.1% DDQ was found adequate in method A (Figure 3), in order to achieve greater stability of the colored species, 0.75 mL of 0.1% DDQ was used; and 1.0 mL of 0.4% DNP gave maximum absorbance in method B (Figure 4). Larger volumes were unsuitable because of increasing blank absorbance values.

Reaction time and stability

In method A, although the complex (DEC base:DDQ) was formed instantaneously, constant absorbance readings were obtained after not less than 60 min of standing time at ambient temperature (28±2 ºC) and remained constant for at least 24 h (Figure 5), hence,

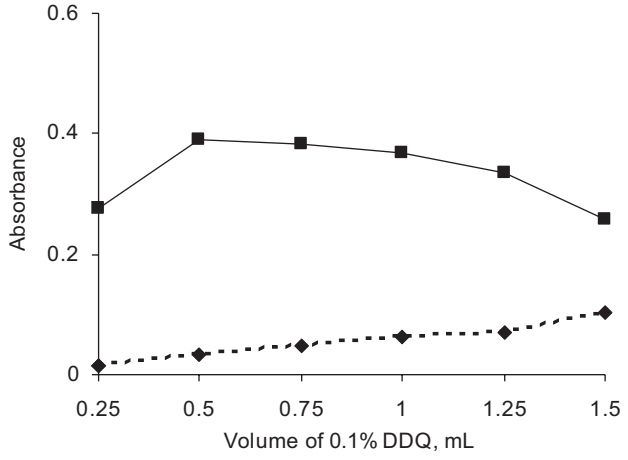


Figure 3. *Effect of volume of 0.1% DDQ (Method A)*

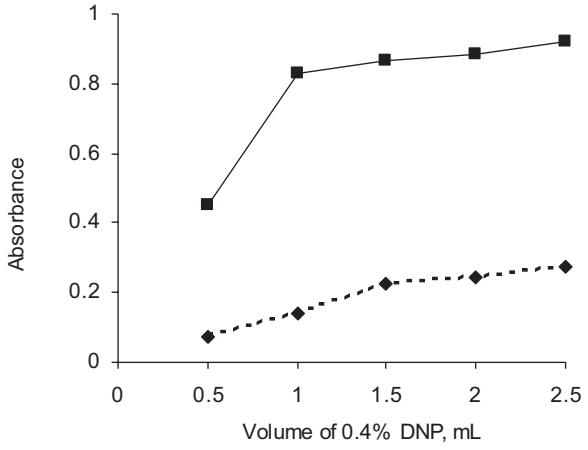


Figure 4. *Effect of volume of 0.4% DNP (Method B)*

in order to avoid a standing time of 60 min, absorbance were measured within 5 min.

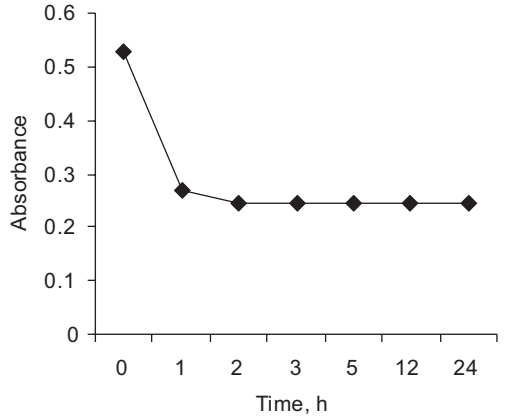


Figure 5. *Stability of DEC base-DDQ complex in method A*

Complete color development of the DEC base:DNP complex was attained immediately in method B, and the colored species were stable for at least 24 h (Figure 6).

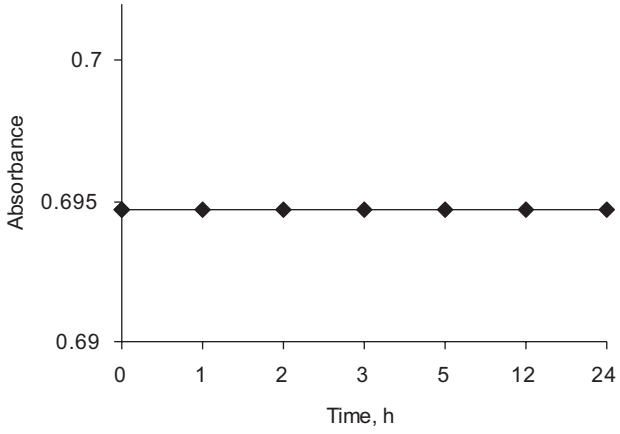


Figure 6. *Stability of DEC base-DNP complex in method B*

Composition of the CT complexes

The composition of the CT complexes was established by the Job's method of continuous variations[40] using equimolar concentrations of the drug (base form) and reagents (1.003×10^{-3} mol L^{-1} in both methods). The results indicated that a 1:1 (drug: dye) complex is formed in both methods, shown in Figure 7 and Figure 8.

METHOD VALIDATION

A linear correlation was found between the absorbance at λ_{max} and the concentration of DEC (Figure 9) in the ranges given in Table 1. Regression analysis of the Beer's Law data using the least squares method was performed to evaluate the slope (*b*), intercept (*a*) and the correlation coefficient (*r*) for each system and the values are presented in Table 1. The optical characteristics such as the limits of the Beer Law, molar absorptivity and the Sandell sensitivity values of both methods are also given. In addition, the limits of detection (*LOD*) and quantification (*LOQ*), calculated according to ICH guidelines[41] are also compiled in Table 1. As can be seen from Table 1, the values of slope indicate moderate sensitivity of the methods, and low values of intercept imply nearly perfect adherence to the Beer's law. Further,

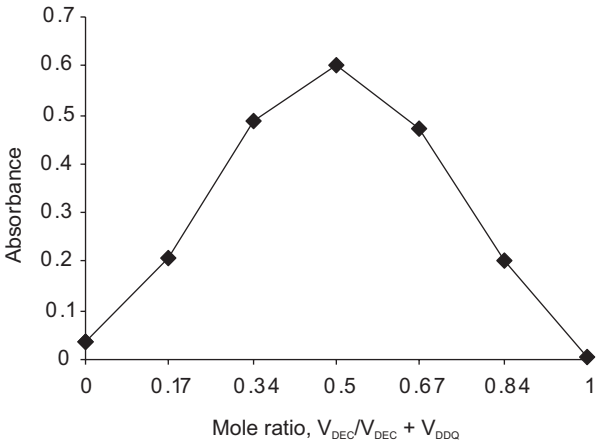


Figure 7. *Job's plot for DEC base-DDQ complex (1.003×10^{-3} M solutions of DEC base and DDQ in method A)*

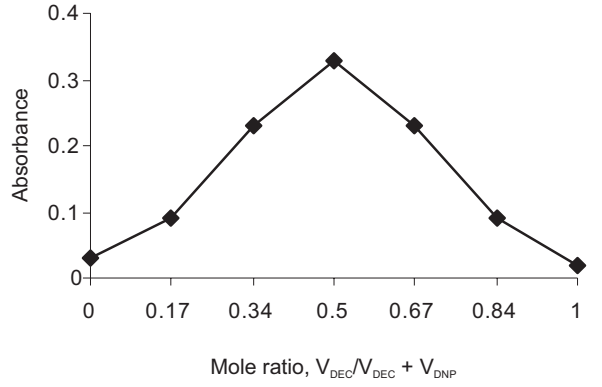


Figure 8. *Job's plot for DEC base-DNP complex (1.003×10^{-3} M solutions of DEC base and DNP in method B)*

low values of standard deviation of slope and intercept indicate that the points tend to be very close to the expected values.

Precision and accuracy

For three levels of analyte, the assays were repeated seven times within the day to determine the intra-day precision and five times on different days to determine the inter-day precision of the methods. The percentage relative standard deviation (*RSD*) values were ≤1.54% (intra-day) and ≤2.13% (inter-day), indicating the high precision of the methods. The accuracy was evaluated as the percentage relative error (*RE*) between the measured mean concentrations and the taken concentrations for DEC. The RE values of ≤2.25% demonstrate the high accuracy of the proposed methods as shown in Table 2.

Selectivity study

Selectivity was determined by using placebo blank and synthetic mixture analysis. A placebo blank, consisting of starch (20 mg), acacia (15 mg), hydroxyl cellulose (25 mg), sodium citrate (30 mg), talc (20 mg), magnesium stearate (25 mg) and sodium alginate (25 mg) was prepared by thorough mixing and its solution was prepared as described under "procedure for tablets", and then subjected to analysis. The absorbance of the placebo blank was almost equal to the absorbance of the reagent blank suggesting no interference.

A synthetic mixture was prepared by adding 10 mg of pure DEC to about 12 mg of the above mentioned placebo blank, and the mixture was homogenized. Following the same procedure for tablets, the synthetic mixture solution was prepared, and a suitable aliquot

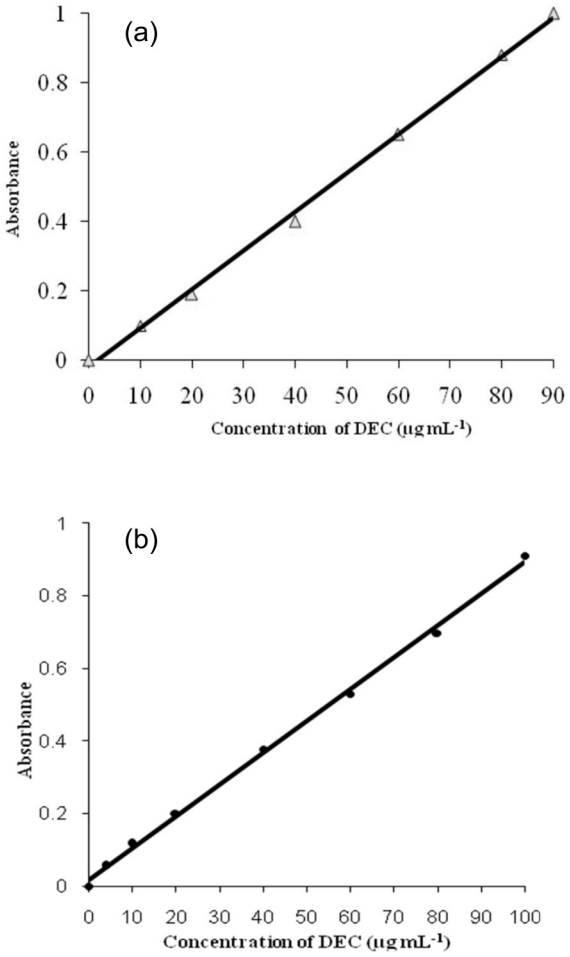


Figure 9. *Calibration plot: (a) Method A and (b) Method B*

was subjected to analysis by both methods (n=5), after appropriate dilution. The percent recoveries of DEC were 98.1±1.5 and 101.4±2.1 for methods A and B respectively. This confirms the selectivity of the methods in presence of common excipients.

Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of reagent (0.75±0.2 mL of DDQ; 1±0.2 mL of DNP) and contact time (1±0.2 min in both methods A and B), and the effect of the changes was studied on the absorbance of the complex systems. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as *RSD* % (≤ 3%). Method ruggedness was demonstrated by having the analysis performed by four analysts, and by a single analyst performing the analysis on four different instruments in the same laboratory. The intermediate precision values (*RSD*) in both instances were in the range 0.93-2.98%, indicating acceptable ruggedness as indicated in Table 3.

Table 1. Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	480	420
Color stability, hr.	> 24	> 24
Linear range, µg mL^{-1}	4–90	4–100
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.88×10^3	2.05×10^3
Sandell sensitivity*, µg cm^{-2}	0.1061	0.0975
Limit of detection (LOD), µg mL^{-1}	0.21	0.22
Limit of quantification (LOQ), µg mL^{-1}	0.63	0.67
Regression equation, Y**		
Intercept (a)	**(7.5±0.94)×10^{-2}**	**(2.4±0.91)×10^{-2}**
Slope (b)	**(12±0.16)×10^{-3}**	**(8.6±0.16)×10^{-3}**
Regression coefficient (r)	0.9996	0.9991

*Limit of determination as the weight in µg mL^{-1} of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. **Y=a+bX, Where Y is the absorbance, X is concentration in µg mL^{-1}, a is intercept and b is slope.

Applications

The proposed methods were applied for the determination of DEC in commercial formulations. The results were compared with those obtained by the US Pharmacopoeia method.[5] Statistical analysis of the results did not detect any significant difference between the performance of the proposed methods and official method with respect to accuracy and precision as revealed by the Student's *t*- and variance ratio *F*-values as shown in Table 4.

Recovery studies

The recovery test was performed by spiking the pre-analyzed commercial formulations with pure DEC at three different levels (50, 100 and 150% of the content taken) and the total was found by the proposed methods. Each test was repeated three times. In both the cases, the recovery values ranged between 98.40 and 102.8%, with a standard deviation in the range 0.59–1.84%. The closeness of the results to 100% showed the fairly good accuracy of the methods (Table 5).

Table 2. Evaluation of intra-day and inter-da-y accuracy and precision

Method	DEC taken (µg mL^{-1})	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=7)		
		DEC found[a] (µg mL^{-1})	RSD[b] %	RE[c] %	DEC found (µg mL^{-1})	RSD[b] %	RE[c] %
A	40.0	40.7	1.45	1.70	40.9	1.68	2.25
	60.0	59.2	1.18	1.34	61.2	1.43	2.01
	80.0	81.2	0.95	1.50	81.4	1.29	1.75
B	40.0	39.46	1.54	1.36	40.6	1.59	1.62
	60.0	60.91	0.96	1.52	60.93	2.13	1.55
	80.0	79.10	1.42	1.12	81.1	1.81	1.37

[a]Mean value of 7 determinations; [b]Relative standard deviation (%); [c]Relative error (%).

Table 3. Method robustness and ruggedness expressed as intermediate precision

Method	DEC taken (μg mL^{-1})	Method robustness		Method ruggedness	
		Parameters altered		Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
		Volume of DDQ / DNP mL[a] %RSD (n=3)	Reaction time[b] %RSD (n=3)		
A	40.0	1.26	1.12	1.35	1.63
	60.0	1.78	1.25	2.05	1.84
	80.0	2.09	1.13	2.12	2.98
B	40.0	0.93	1.27	1.12	1.36
	60.0	1.85	1.08	0.96	1.21
	80.0	1.52	2.09	1.89	2.35

[a]In method A, the volumes of reagent were 0.75±0.2 mL and in method B, the volumes were 1±0.2. [b]The reaction times were 1±0.2 min in both methods.

Table 4. Results of analysis of formulations by the proposed methods

Formulation analyzed	Label claim	Found* (Percent of label claim±SD)		
		Official method	Proposed methods	
			Method A	Method B
Banocide Forte tablets	100 mg per tablet	99.3±1.27	100.9±0.93	101.6±1.54
			t = 2.28	t = 2.58
			F = 1.86	F = 1.47
Banocide syrup	120 mg per 5 mL	102.4±1.09	103.5±1.13	102.9±1.74
			t = 1.57	t = 0.79
			F = 1.07	F = 1.12

*Mean value of five determinations. Tabulated *t*-value at the 95% confidence level is 2.77. Tabulated *F*-value at the 95% confidence level is 6.39.

Table 5. Results of recovery study *via* standard addition technique

Method	Formulation studied	DEC in formulation, μg mL^{-1}	Pure DEC added, μg mL^{-1}	Total found, μg mL^{-1}	Pure DEC recovered* Percent±SD
A	Banocide Forte tablets	20.18	10	30.09	99.7±1.54
		20.18	20	40.26	100.2±0.98
		20.18	30	50.48	100.6±1.59
	Banocide syrup	20.70	10	31.40	102.3±1.52
		20.70	20	41.47	101.9±0.97
		20.70	30	52.32	103.2±0.59
B	Banocide Forte tablets	20.32	10	29.84	98.4±1.49
		20.32	20	40.76	101.1±1.19
		20.32	30	49.91	99.2±0.54
	Banocide syrup	20.58	10	30.98	101.3±1.84
		20.58	20	41.72	102.8±0.89
		20.58	30	52.40	103.6±154

*Mean value of three determinations.

CONCLUSIONS

The proposed methods make use of simple reagents and instrument which an ordinary analytical laboratory can afford; the methods are rapid, extraction-free, cost-effective and do not involve any critical reaction conditions. The suggested methods utilize a single step reaction and single solvent. The proposed methods are accurate and precise, and free from interference caused by the excipients expected to be present in dosage forms. The method was demonstrated to be both robust and rugged. The methods offer several advantages over the existing methods in terms of sensitivity, selectivity, linear dynamic range and mild optimum conditions as indicated in Table 6 and also many advantages over the other techniques, such as HPLC, GC and PMR such as reduced cost, and speed with high accuracy. Hence, the methods can be used in the routine analysis of drugs in quality control laboratories.

ACKNOWLEDGEMENTS

Authors wish to thank the quality control manager, Inga Laboratories Pvt. Ltd., Mumbai, India, for gift sample of pure diethylcarbamazine citrate. Prof K. Basavaiah thanks the UGC New Delhi, for the award UGC-BSR faculty fellowship. One of the authors (NS) thanks the authorities of the University of Mysore, Mysore, for providing research facilities and for awarding a UGC NON-NET Ph.D fellowship.

Table 6. Comparison of performance of the present methods with the existing methods

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range (μg mL^{-1}) ε (L $mol^{-1}cm^{-1}$)	Remarks	Ref. No.
1	*CAA	Measurement of purple color CT complex in dioxane-$CHCl_3$	540	10-400	Mixture of organic solvents used	21
2	Picric acid	Yellow color CT complex measured in alcohol	-	-	-	22
3	Ammonium reineckate	Absorbance of red color product at pH=3.5 in acetone measured	525	-	Tedious & time consuming, Pptn washing and dissolution steps involved	23
4	Malonic acid-acetic anhydride	Measurement of absorption of condensation product	333	-	Heating step and longer contact time involved	25
5	HOAc-Ac_2O and pyridine	Absorbance of yellow color product measured	428	10-110	Heating step and longer contact time involved	26
6	*BPB	Extracted ion-pair complex measured	-	-	Tedious and time consuming extraction step and critical pH adjustment involved	27
7	*BCG	Yellow ion-pair complex measured in chloroform	-	-	Tedious and time consuming extraction step and critical pH adjustment involved	28
8	a) Fast green FCF b) Orange-II	Ion-pair complex extracted into chloroform and measured	-	-	Tedious and time consuming extraction step and critical pH adjustment involved	29
9	a) *DDQ	Measurement of CT complex in chloroform	480	4–90 1.88×10^3	Rapid, no heating or extraction step involved, sensitive, wide linear dynamic range	Present work
	b) *DNP	Absorbance of yellow color CT complex measured	420	4–100 2.05×10^3		

*CAA-chloranilic acid, BCG-bromocresol green, BPB-bromophenol blue, DDQ-2,3-dichloro, 5,6-dicyano-*p*-benzoquinone and DNP-2,4 -dinitro phenol.

REFERENCES

1. Swinyard, E. A.; *Remington's Pharmaceutical Sciences*, Mack Publishing Company: Easton, 1990, p. 1242.
2. Rollo, L. M.; *The Pharmacological Basis of Therapeutics*, Macmillan: New York, **1985**, p. 1010.
3. *The British Pharmacopoeia*, Her Majesty's Stationery Office, London, England, 1988, p. 190.
4. *United States Pharmacopoeia*, Monographs, (USP30-NF25 p.1929), Pharmacopoeial forum, Vol. 28, p. 1098.
5. Rao, G. R.; Raghuveer, S.; Khadgapathi, P.; *Indian Drugs* **1986**, *24*, 37.
6. Nene, S.; Anjaneyulu, B.; Rajagopalan, T. G.; *J. Chromatogr., Biomed. Appl.* **1984**, *308*, 334.
7. Pfaff, M. C.; Gayral, P.; Mahuzier, G.; *J. Chromatogr.* **1978**, *150*, 155.
8. Joseph, R. M. J.; Lawrence, F.; *J. Pharm. Biomed. Anal.* **2001**, *26*, 665.
9. Mathew, N.; Kalyanasundaram, M.; *Acta Trop.* **2001**, *80*, 97.
10. Mahesh Reddy, J.; Jeyaprakash, M. R.; Madhuri, K.; Meyyanathan, S. N.; Elango, K.; *Ind. J. Pharm. Sci.* **2011**, *73*, 320.
11. Krishna Vamsi, M.; Jayalakshmi, B.; Vijay, A. R.; Sandeep, C.; *Int. J. Pharm. Sci. Res.* **2012**, *3*, 3347.
12. El-Obeid, H. A.; *Spectrosc. Lett.* **1984**, *17*, 361.
13. Jezzy, W. J.; Dina B.; Frank, A. S.; Paol, E. S.; Mette, K. A.; *J. Pharm. Biomed. Anal.* **1996**, *14*, 543.
14. Walash, M. I.; Rizk, M. S.; Ibrahim, F. A.; *J. AOAC Int.* **1985**, *68*, 532.
15. Campbell, M. J. M.; Demetriou, B.; Jones, R.; *Analyst* **1980**, *105*, 605.
16. Abigail, W.; Patrick, B.; Shannon, H.; Marco, M.; Brennan, E. B.; Dominique, M.; Thomas, G. S.; Marya L.; *PLoS Neglected Trop. Dis.* **2011**, *5*, e1005.
17. Bhanumathi, L.; Wadodkar, S. G.; Kasture, A. V.; *Indian Drugs* **1981**, *18*, 204.
18. Wahbi, A. M.; el-Obeid, H. A.; Gad-Kariem, E. A.; *Farmaco Prat.* **1986**, *41*, 210.
19. Rizk, M.; Walash, M. I.; Ibrahim, F.; *Spectrosc. Lett.* **1984**, *17*, 423.
20. Michael, U. A.; Kenneth, C. O.; Anthony, A. A.; *Chem. Pharm. Bull.* **1999**, *47*, 463.
21. Ramachandran, M.; *Curr. Sci.* **1972**, *41*, 890.
22. Basu, K.; Dutta, B. N.; *Ind. J. Pharm.* **1961**, *23*, 326.
23. Refaat, I. H.; El-Kommos, M. E.; Farag, H. H.; El-Rabat, N. A.; *Bull. Pharm. Sci., Assiut Univ.* **1987**, *10*, 85.
24. Bulbule, M. V.; Kasture, A. V.; Wadodkar, S. G.; *Indian Drugs* **1981**, *19*, 27.
25. Bhuee, G. S.; Rastogi, S. N.; Jetley, U. K.; Singh, J.; *East. Pharm.* **1981**, *24*, 19.
26. Rao, K. N.; Subrahmanyam, D.; *Ind. J. Med. Res.* **1970**, *58*, 746.
27. Vadodaria, D. J.; Vora, M. N.; Mukherji, S. P.; *Ind. J. Pharm.* **1968**, *30*, 41.
28. Sastry, C. S. P.; Aruna, M.; Reddy, M. N.; Sankar, D. G.; *Ind. J. Pharm. Sci.* **1988**, *50*, 140.
29. Prashanth, K. N.; Basavaiah, K.; *Acta Pol. Pharm.* **2012**, *69*, 213.
30. Basavaiah, K.; Kalsang T.; Vinay, K. B.; *Croat. Chem. Acta* **2010**, *83*, 415.
31. Elmorsy, K.; *Talanta,* **2008**, *75,* 1167.
32. Sameer, A. M. A.; Basavaiah, K.; *Int. J. Anal. Chem.* **2011**, *2011*, 1, Article ID 619310.
33. Prashanth, K. N.; Basavaiah, K.; *Chem. Sci. J.* **2012**, CSJ-71.
34. El-Mammli, M. Y.; *Spectrochim. Acta* **2003**, *59*, 771.

35. Raghu, M. S.; Basavaiah K.; Vinay, K. B.; *Bull. Chem. Soc. Ethiop.* **2012**, *26*, 319.
36. Basavaiah K.; Abdulrahman S. A. M.; *Thai J. Pharm. Sci.* **2010**, *34*, 134.
37. Cijo, M. X.; Basavaiah, K.; Sameer, A. M. A.; Vinay, K. B.; *Chem. Ind. Chem. Eng. Q.* **2011**, *17*, 469.
38. Raghu, M. S.; Basavaiah, K.; *J. Assoc. Arab Univ. Basic Appl. Sci.* **2012**, *12*, 33.
39. Abou A. F. M.; *Il Farmaco* **2000**, *59*, 771.
40. Douglas, A. S.; Donald, M. W.; *Principles of Instrumental Analysis*, Holt Rinehart & Winston: New York, **1971**, p. 104.
41. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), Complementary Guideline on Methodology, dated 06 November **1996**, incorporated in November **2005**, London.