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Article

Titrimetric and spectrophotometric assay of diethylcarbamazine citrate in formulations using iodate and iodide mixture as reagents

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One titrimetric and two spectrophotometric methods are proposed for the determination of diethylcarbamazine citrate (DEC) in bulk drug and in formulations using potassium iodate and potassium iodide as reagent. The methods employ the well-known analytical reaction between iodate and iodide in the presence of acid. In titrimetry (method A), the drug was treated with a measured excess of thiosulfate in the presence of unmeasured excess of iodate-iodide mixture and after a standing time of 10 min, the surplus thiosulfate was determined by back titration with iodine towards starch end point. Titrimetric assay is based on a 1:3 reaction stoichiometry between DEC and iodine and the method is applicable over 2.0–10.0 mg range. The liberated iodine is measured spectrophotometrically at 370 nm (method B) or the iodine-starch complex measured at 570 nm (method C). In both methods, the absorbance is found to be linearly dependent on the concentration of iodine, which in turn is related to DEC concentration. The calibration curves are linear over 2.5-50 and 2.5-30 μg mL^{-1} DEC for method B and method C, respectively. The calculated molar absorptivity and Sandell sensitivity values were 6.48×10^3 L mol^{-1} cm^{-1} and 0.0604 μg cm^{-2}, respectively, for method B, and their respective values for method C are 9.96×10^3 L mol^{-1} cm^{-1} and 0.0393 μg cm^{-2}. The intra-day and inter-day accuracy and precision studies were carried out according to the *ICH* guidelines. The methods were successfully applied to the analysis of DEC formulations.

Uniterms: Diethylcarbamazine citrate/determination. Titrimetry/quantitative analysis. Spectrophotometry/quantitative analysis. Pharmaceutical formulations/analysis.

Propõem-se titulação e dois métodos espectrofotométricos para a determinação de citrato de dietilcarbamazina (DEC) a granel e em suas formulações, usando iodato de potássio e iodeto de potássio como reagente. Os métodos utilizam a reação analítica conhecida entre iodato e iodeto, na presença de ácido. Na titulometria (Método A), o fármaco foi tratado com excesso medido de tiossulfato, na presença de excesso não medido de mistura iodato-iodeto e, depois de um tempo de repouso de 10 min, o excesso de tiossulfato foi determinado por titulação de retorno com iodo até o ponto final com amido. A titulação é baseada em reação com estequiometria 1:3 entre DEC e iodo e o método é aplicável na faixa de 2.0-10.0 mg. O iodo liberado é medido espectrofotometricamente a 370 nm (método B) ou o complexo de iodo-amido medido a 570 nm (método C). Em ambos os métodos, a absorvância é considerada linearmente dependente da concentração de iodo, a qual, por sua vez, está relacionada à concentração de DEC. As curvas de calibração são lineares para concentrações de DEC de 2.5-50 e 2.5-30 mg mL^{-1} para o método B e para o método C, respectivamente. A absortividade molar calculada e os valores de sensibilidade Sandel foram 6.48×10^3 L mol^{-1} cm^{-1} e 0.0604 ug cm^{-2}, respectivamente, para o método B, e os seus respectivos valores para o método C são 9.96×10^3 L mol^{-1} cm^{-1} e 0.0393 mg cm^{-2}. Os estudos de exatidão e precisão intra-dia e inter-dia foram realizados de acordo com as diretrizes da *ICH*. Os métodos foram aplicados com sucesso na análise de formulações de DEC.

Unitermos: Citrato de dietilcarbamazina/determinação. Titulometria/análise quantitativa. Espectrofotometria/análise quantitativa. Formulações farmacêuticas/análise.

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INTRODUCTION

Diethylcarbamazine citrate (DEC) [Figure 1], 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 (Swinyard, 1990). It is also the alternative drug of choice in *Onchocerca volvulus* infections and tropical eosinophilia (Adkiwu, Ofokansi, Attama, 1999). The drug is official in British Pharmacopoeia [BP] (1988), which describes a non-aqueous titration method for its determination and also official in the United States Pharmacopoeia [USP] (United States Pharmacopoeia, Monographs), which uses a liquid chromatographic technique with phosphate buffer system for the assay.

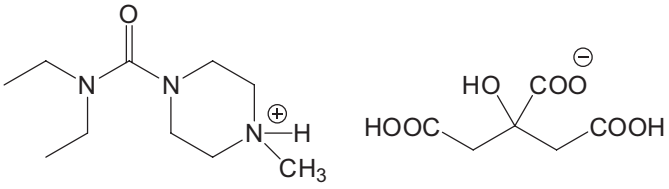

FIGURE 1 - Structure of diethylcarbamazine citrate.

Other than these official methods, a variety of techniques have been reported for the determination of DEC in pharmaceutical dosage forms, and include gas chromatography [GC] (Ramana, Raghuveer, Khadgapathi, 1986; Nene, Anjaneyalu, Rajagopalan, 1984; Pfaff, Gayral, Mahuzier, 1978; Joseph, Lawrence, 2001), high performance liquid chromatography [HPLC] (Mathew, Kalyanasundaram, 2001; Reddy *et al.,* 2011; Krishna Vamsi *et al.,* 2012), proton magnetic resonance [PMR] spectroscopy (El-Obeid, 1984, Jezzy *et al.,* 1996), DC polarography analysis (Walash *et al.,* 1985), ion selective electrode potentiometry (Campbell *et al.,* 1980) and titrimetry (Abigail *et al.,* 2011; Bhanumathi *et al.,* 1981). 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, El-Obeid, Gad-Kariem (El-Obeid, Gad-Kariem, 1986), for the assay of 1-6 μg mL^{-1} DEC in tablets. Chloranilic acid has been employed by two groups of workers (Rizk, Walash, Ibrahim, 1984; Michael, Kenneth, Anthony, 1999) 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 (1961), 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 (Refaat *et al.,* 1987) 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 (Bulbule, Kasture, Wadodkar, 1981), 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 (Bhuee *et al.,* 1981) 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 (1970) 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 (Vadodaria, Vora, Mukherji, 1968) 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 M HCl were successfully employed by Sastry *et al.* (1988), 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 (Michael, Kenneth, Anthony, 1999), tedious and time-consuming steps like precipitation, filtration and washing (Basu, Dutta, 1961), heating (Refaat *et al.,* 1987; Bulbule, Kasture, Wadodkar, 1981; Bhuee *et al.,* 1981). The extraction methods (Rao, Subrahmanyam, 1970; Vadodaria, Vora, Mukherji, 1968; Sastry *et al.,* 1988) though sensitive suffer from disadvantages like laborious liquid-liquid extraction step, critical pH adjustment, critical dependence on pH of the aqueous phase and the aqueous-organic phase's ratio. Additionally, incomplete extraction of the analyte may lead to erratic results. Hence, there is a need for developing a method free from such disadvantages.

The aim of the present study was directed at developing and validating simple, rapid, sensitive, cost-effective titrimetric and two spectrophotometric methods

using iodate and iodide as reagents. Iodide of the mixture present is oxidized by iodate in an amount equivalent to the COOH group present in citrate in DEC to iodine and the liberated iodine is determined. The methods were successfully applied to quantify DEC in pharmaceutical formulations.

MATERIAL AND METHODS

Apparatus

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

Reagents and standards

All chemicals used were of analytical reagent grade and distilled water was used to prepare solutions.

Potassium iodate

A high purity grade of the chemical (Merck, Mumbai, India) was used. A saturated solution of potassium iodate was prepared by stirring approximately 20 g of the chemical in a beaker containing 100 mL water with the help of magnetic stirrer for 60 minutes. The solution was decanted and filtered using quantitative filter paper.

Potassium iodide

A saturated solution of potassium iodide (Merck, Mumbai, India) was prepared just before use in order to prevent atmospheric oxidation to iodide.

Sodium thiosulphate

A 0.01 N sodium thiosulphate (S.D. Fine Chem., Mumbai, India) was prepared by dissolving 1.25 g in 500 mL water and standardized against 0.01N potassium dichromate (Vogel, 1961).

Iodine solution

A 0.01 N iodine solution (Merck, Mumbai, India) was prepared by dissolving 5 g of iodate-free potassium iodide in 30-40 mL of water in a glass stoppered 500 mL calibrated flask. Weighed out 0.63 g of analytical reagent (AR) grade or resublimed iodine on a watch glass and transferred into a flask containing concentrated potassium iodide by means of a small dry funnel, inserted the glass stopper into the flask, and shaken well in the cold condition until all the iodine has dissolved. Allowed the solution to acquire room temperature, and made up to mark with distilled water in a 500 mL calibrated flask (Vogel, 1961).

Saturated Borax

Approximately 30 g of borax (S.D. Fine Chem., Mumbai, India) were dissolved in 100 mL water and stirred with the help of magnetic stirrer for 15 minutes. The solution was decanted and filtered. The pH of the solution was between 8 and 9.

1% starch

Made a paste of 1 g of the soluble starch (Potato starch, Loba Chemie, Mumbai, India) with a little water, and poured the paste with constant stirring, into 100 mL of boiling water, and boiled for 1 min., allowed the solution to cool before use and prepared afresh every day (Vogel, 1961).

Standard drug solution

Pharmaceutical grade DEC (99.7 per cent pure) was procured from Inga Laboratories Pvt. Ltd., Mumbai, India, and was used as received. Banocide forte tablets (Glaxo Smith Kline Pharma. Ltd., Nashik, India) and Banocide syrup (Glaxo Smith Kline Pharma. Ltd., Bangalore, India) both were purchased from local commercial stores.

A stock standard solution equivalent to 1 mg mL^{-1} DEC for method A was prepared by dissolving 250 mg of pure drug in water and diluting to 250 mL in calibrated flask with water. The stock solution was diluted appropriately to get working concentration of 100 µg mL^{-1} with water for spectrophotometric methods.

Recommended procedures

Method A

A 10 mL aliquot of pure DEC solution containing 2-10 mg of DEC was taken in an Erlenmeyer flask. Five mL each of saturated solutions of KIO_3 and KI followed by 10 mL thiosulfate (accurately measured) were added and the flask was stoppered and let stand for 10 min with occasional swirling. Finally, 1 mL of 1% starch indicator was added and unreacted thiosulfate was titrated against standard iodine solution until the decoloration of blue color.

The amount of DEC was calculated from the following formula:

$$\text{Amount (mg)} = \frac{\{(B - S) \times M_W \times C\}}{n}$$

where, B= volume of iodine consumed in the blank titration, mL; S= volume of iodine consumed in the sample titration, mL; Mw= relative molecular mass of DEC; C= molar concentration of thiosulfat; n= number of moles of iodine reacting with each mole of DEC.

Method B

Varying aliquots (0.25-5.0 mL) of 100 μg mL^{-1} DEC was added in to a series of 10 mL calibrated flasks by means of micro burette. To each flask, 1.5 mL of KIO_3 and 1 mL of KI were added flasks stoppered, content mixed and let stand for 15 min. Then 1 mL of saturated borax was added and made up to the mark with water. Absorbance of each solution was measured at 370 nm against reagent blank**.**

Method C

Different volumes (0.2-3.0 mL) of 100 μg mL^{-1} DEC were taken in a series of 10 mL calibrated flasks. One mL each of saturated KIO_3 and KI solutions were added, flasks were stoppered and content mixed. The flasks were let stand for 15 min before adding 1 mL of saturated borax and 1 mL of 1% starch to each flask and finally made up to 10 mL with water. Absorbance of each solution was measured at 570 nm against reagent blank.

Standard graph was prepared by plotting the absorbance *versus* drug concentration, and the concentration of the unknown was computed from the respective regression equation.

Procedure for tablets

Twenty tablets were weighed accurately and ground into a fine powder. An accurately weighed amount of the powdered tablet equivalent to 100 mg of DEC was transferred into a 100 mL calibrated flask. Sixty mL water was added and the content was shaken thoroughly for 15-20 min to extract the drug into the liquid phase; the volume was finally diluted to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. An aliquot of the filtrate (1 mg mL^{-1} DEC) was used for method A and diluted to required concentrations and used for the assay in method B and method C as described above.

Procedure for syrup

The content of five 100 mL syrup bottles was pooled and 5 mL syrup containing 120 mg DEC was taken and its solution prepared as described in 'procedure for tablets'. This 1.2 mg mL^{-1} DEC syrup extract was further diluted to get working standard solution of 100 μg mL^{-1} for method B and method C with water and analyzed by taking an appropriate volume in all the methods.

Procedure for the analysis of placebo blank and synthetic mixture

A placebo blank containing starch (45 mg), acacia (35 mg), sodium citrate (40 mg), hydroxyl cellulose (45 mg), magnesium stearate (50 mg), talc (40 mg) and sodium alginate (35 mg) was prepared by mixing all the components into a homogeneous mixture. A 100 mg of the placebo blank was accurately weighed and its solution was prepared as described under 'tablets', and then subjected to analysis by following the general procedures.

To 50 mg of the placebo blank of the composition described above, 100 mg of DEC was added and homogenized, transferred to a 100 mL calibrated flask and the solution was prepared as described under "Procedure for tablets", and then subjected to analysis by the procedure described above. This analysis was performed to study the interference by excipients normally present in tablet preparation.

RESULTS AND DISCUSSION

Preliminary experiments showed that DEC is sufficiently acidic to release iodine from iodate-iodide mixture allowing the titrimetric and spectrophotometric determination of drug. Iodide present is oxidized by iodate in an amount equivalent to the COOH group present in citrate in DEC to iodine and the liberated iodine is determined. In titrimetry, the liberated iodine was reacted with a measured excess of thiosulphate and residual reductant was titrated with iodine and the end point being located visually with starch, while in spectrophotometry, it was determined by two different color reactions.

Method development

Method A

The quantitative nature of the reaction between DEC and iodate-iodide reagent was checked by treating 2.0-10.0 mg of drug with an excess of reagent and determining the iodine released. For the range studied (2.0-10.0 mg), 5.0 mL each of saturated solution of iodate and iodide and reaction time of 10 min were found adequate. The end point was located visually using starch. The reaction stoichiometry is 1:3 (drug: liberated iodine), the COOH group in the citrate is acidic enough to liberate iodine.

$$IO_3^- + 5I^- + 6H^+ \text{ (DEC)} \rightarrow 3I_2 + 3H_2O$$

$$I_2 + 3Na_2S_2O_3 \text{ (excess)} \rightarrow 2NaI + Na_2S_4O_6 + \text{unreacted } Na_2S_2O_3$$

$$\text{unreacted } Na_2S_2O_3 + \text{starch} > < \text{Iodine}$$

Method B and method C

Absorbance of the liberated iodine or starch-iodine complex was measured at 370 or 570 nm as deduced from the absorption spectra of the colored species (Figure 2a and 2b). In both methods, the reaction was relatively fast

in the beginning and iodine continued to be liberated even after 15 min. Since most of the iodine was liberated within 15 min, the reaction was stopped by adding borax to the reaction mixture after a standing time of 15 min. The absorbance remained constant for 25 and 45 min in method B and method C, respectively. Attempts to hasten the reaction by heating were unsuccessful owing to the volatility of iodine and dissociation of iodine-starch complex at elevated temperature.

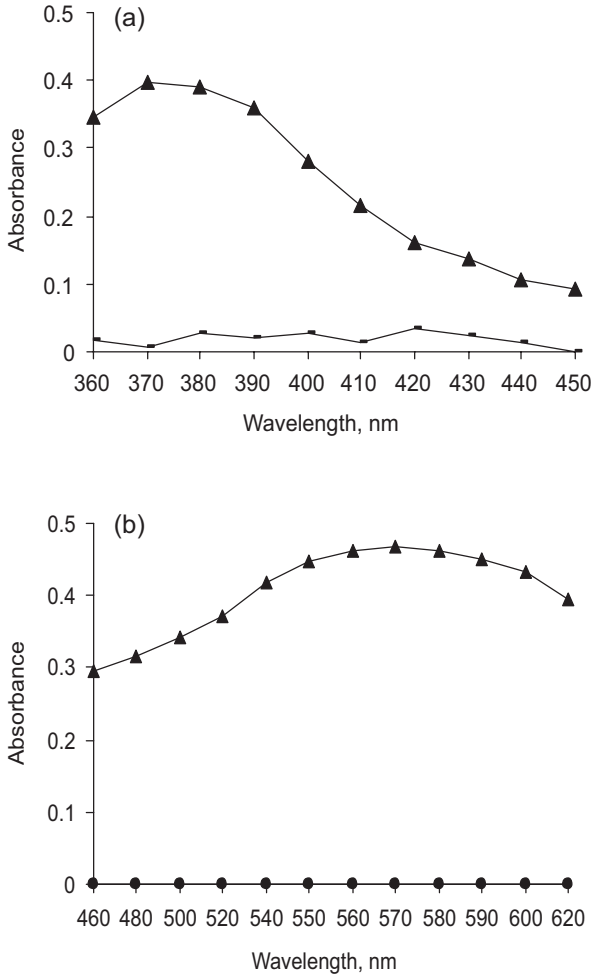

FIGURE 2- (a) Absorption spectra of DEC-iodide complex (▲) [20 µg mL^{-1} DEC] and blank (-); **(b)** Absorption spectra of DEC-iodine-starch complex (▲) [20 µg mL^{-1} DEC] and blank (●)

The possible reaction schemes responsible for change in absorbance as a function of DEC concentration are represented below:

$$IO_3^- + 5I^- + 6H^+ (DEC) \rightarrow 3I_2 + 3H_2O$$
(Method B: measured at 370 nm)
$$I_2 + \text{Starch} \rightarrow I_2\text{-Starch complex}$$
(Method C: measured at 570 nm)

VALIDATION PROTOCOL

Linearity, detection and quantification limits

Under the optimum conditions, a linear relation was obtained between absorbance and concentration of DEC in the ranges given in Table I. The calibration graph in each instance is described by the equation:

$$Y = a + b\,X$$

where Y = absorbance, a = intercept, b = slope and X = concentration in µg mL^{-1}). The correlation coefficient, intercept and slope for the calibration data are summarized in Table I. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection (LOD) and quantification (LOQ) are calculated as per the current *ICH* guidelines (*ICH* guidelines, 1996) and compiled in Table I. LOD and LOQ were calculated according to the same guidelines using the following formulae:

$$LOD = \frac{3.3 \times \sigma}{S} \quad \& \quad LOQ = \frac{10 \times \sigma}{S}$$

where σ is the standard deviation of six reagent blank determinations and s is the slope of the calibration curve, also molar absorptivity (ε) and Sandell sensitivity (SS) were calculated using the relation;

$$\varepsilon = \frac{\Delta A \times M_W \times 10^3}{\text{ppm}} \quad \& \quad SS = \frac{0.001 \times M_W \times 10^9}{\varepsilon \times 1000}$$

where ΔA is the change in absorbance, Mw is the molecular weight of DEC and ppm is the concentration of DEC.

Selectivity

The results obtained from placebo blank and synthetic mixture analyses revealed that inactive ingredients used in the tablet preparation did not interfere in the assay of active ingredient. The absorbance values obtained from the placebo blank solution were almost equal to the absorbance of the blank which revealed no interference from the adjuvants. To study the role of additives added to the synthetic sample, the analysis of synthetic mixture solution prepared as described earlier yielded percent recoveries of 98.9±2.13, 99.2±1.36 and 102.31±1.29 (n=5) for method A, method B, and method C, respectively. This demonstrates the accuracy as well as the precision of the proposed methods and complements the findings of the placebo blank analysis with respect to selectivity.

Precision

The precision of the method was calculated in terms

TABLE I - Sensitivity and regression parameters of spectrophotometric methods

Parameter	Method B	Method C
λ_{max}, nm	370	570
Color stability, min.	≥ 20	≥ 60
Linear range, µg mL^{-1}	2.5–50	2.5–30
Molar absorptivity (ε), L mol^{-1} cm^{-1}	6.48×10^{3}	9.96×10^{3}
Sandell sensitivity*, µg cm^{-2}	0.0604	0.0393
Limit of detection (LOD), µg mL^{-1}	0.25	0.11
Limit of quantification (LOQ), µg mL^{-1}	0.75	0.33
Regression equation, Y**		
Intercept (a)	0.0126	0.0786
Slope (b)	0.0169	0.0337
Standard deviation of a (S_a)	9.98×10^{-2}	9.98×10^{-2}
Standard deviation of b (S_b)	2.24×10^{-3}	3.98×10^{-3}
Regression coefficient (r)	0.9974	0.9939

*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.

of intermediate precision (intra-day and inter-day) (*ICH* guidelines, 1996). Three different concentration of DEC were analyzed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed that the precision was good (Table II).

Accuracy

The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for DEC. The results obtained are compiled in Table II and show that the accuracy is satisfactory for all the methods.

Application to analysis of tablets and syrup

The proposed methods were applied to determine DEC in one brand each of tablets and syrup. The results were statically compared with those obtained by the official

TABLE II - Evaluation of Intra-day and inter-day accuracy and precision

Method	*DEC Taken	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=7)		
		*DEC Found[a]	RSD[b] %	RE[c] %	*DEC found	RSD[b] %	RE[c] %
A	3.0	2.95	1.02	1.67	2.93	1.54	2.33
	6.0	5.89	1.76	1.73	5.81	2.58	3.26
	9.0	8.89	0.95	1.11	9.11	1.29	1.22
B	10	9.83	1.69	1.73	9.78	2.46	2.76
	20	19.71	1.44	0.95	19.65	1.74	3.54
	30	29.43	1.88	1.54	29.14	2.85	3.26
C	15	14.79	1.54	1.40	14.71	1.49	1.94
	20	19.69	0.97	1.55	19.63	2.13	1.85
	25	24.72	1.29	1.12	24.69	1.81	1.24

DEC-diethylcarbamazine citrate. *mg in method A and µg mL^{-1} in methods B and C; [a] Mean value of 7 determinations; [b] Relative standard deviation (%); [c] Relative error (%).

British Pharmacopoeia (1988) method, which describes a non-aqueous titration method for its determination for accuracy and precision by applying the Student's *t*-test and variance ratio *F*-test. Statistical analysis of the results using Student's *t*-test for accuracy and *F*-test for precision revealed no significant difference between the proposed methods and the official method at the 95% confidence level with respect to accuracy and precision (Table III).

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure DEC at three concentration levels (50,100 and 150 % of that in tablet powder) and the total was found by the proposed methods. In all cases, the added DEC recovery percentage values ranged 98.9-102.7 with a standard deviation of 0.79-1.82. The results of this study given in Table IV indicated that the recovery was good, and that the co-formulated substances did not interfere in the determination.

TABLE III - 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	Method C
Banocide forte tablets	100 mg per tablet	98.2±1.63	99.1±1.32 *t* = 0.96 *F* = 1.52	98.9±1.25 *t* = 0.76 *F* = 1.7	98.6±1.65 *t* = 0.39 *F* = 1.02
Banocide syrup	120 mg per 5 mL	101.2±1.29	102.7±1.31 *t* = 1.82 *F* = 1.03	101.6±1.63 *t* = 0.43 *F* = 1.6	101.9±1.7 *t* = 0.73 *F* = 1.74

*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 IV - Results of recovery study *via* standard addition technique

Method	Formulation studied	*DEC in tablet	*Pure DEC added	Total found	*Pure DEC recovered[a] Percent±SD
A	Banocide forte tablets	2.97	1.5	4.17	99.2±1.34
		2.97	3.0	5.73	100.6±1.29
		2.97	4.5	7.30	101.4±1.18
	Banocide syrup	3.08	1.5	4.63	101.2±1.27
		3.08	3.0	6.23	102.5±0.93
		3.08	4.5	7.70	101.6±1.17
B	Banocide forte tablets	9.89	5.0	15.15	101.8±1.36
		9.89	10.0	19.67	98.9±1.63
		9.89	15.0	24.79	99.6±1.54
	Banocide syrup	10.16	5.0	15.48	102.1±1.81
		10.16	10.0	20.54	101.9±0.99
		10.16	15.0	25.46	101.2±1.36
C	Banocide forte tablets	9.86	5.0	15.06	101.4±1.45
		9.86	10.0	19.78	99.6±1.29
		9.86	15.0	24.96	100.4±1.54
	Banocide syrup	10.19	5.0	15.60	102.7±1.82
		10.19	10.0	20.57	101.9±0.79
		10.19	15.0	25.52	101.3±1.59

*mg in method A and µg mL^{-1} in methods B and C; [a]Mean value of three determinations.

CONCLUSIONS

Three methods have been developed for determination of DEC in bulk drug and in its dosage forms and validated as per the current *ICH* guidelines. The methods use cheap and readily available chemicals, compared to the lone titrimetric method [official method], the presented methods are rather simple and sensitive. The reported methods suffer from such draw backs as high cost, and several clean-up steps. They are time-consuming and often poorly reproducible, some require toxic organic solvents. Any method chosen for routine analysis should be reasonably simple, used materials should be readily available in the laboratory or readily obtainable, and require a minimum amount of equipment. The methods are selective as none of the common tablet excipients contain acidic groups to interfere with the present proposed methods. The proposed spectrophotometric methods are free from rigid experimental variables such as pH control, heating or extraction step and/or use of organic solvents. They are characterized by high selectivity and comparable sensitivity with respect to the existing methods (Table V).

TABLE V - Comparison of performance characteristics of the present methods with the existing methods

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range ($\mu g/mL^{-1}$) 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	Michael, Kenneth, Anthony, 1999
2	Ammonium reineckate	Absorbance of red color product at pH=3.5 in acetone measured	525	-	Tedious and time consuming, Pptn washing and dissolution steps involved	Basu, Dutta, 1961
3	Malonic acid-acetic anhydride	Measurement of absorption of condensation product	333	-	Heating step and longer contact time involved	Refaat *et al.*, 1987
4	Malonic acid-acetic anhydride	Measurement of absorption of condensation product	334	0-4	Heating step, longer contact time and ethanol as diluent	Bulbule, Kasture, Wadodkar,1981
5	HOAc-Ac_2O and pyridine	Absorbance of yellow color product measured	428	10-110	Heating step and longer contact time involved	Bhuee *et al.*, 1981
6	*BPB	Extracted ion-pair complex measured	-	-	Tedious and time consuming extraction step and critical pH adjustment involved	Rao, Subrahmanyam, 1970
7	*BCG	Yellow ion-pair complex measured in chloroform	-	-	Tedious and time consuming extraction step and critical pH adjustment involved	Vadodaria, Vora, Mukherji, 1968
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	Sastry *et al.*, 1988
9	a) KI-KIO_3	Measurement of liberated iodine	370	2.5–50 6.48×10^3	Rapid, no heating or extraction step involved, selective, sensitive with wide linear dynamic range, used aqueous medium	Present work
	b) KI-KIO_3 and starch	Absorbance of blue color iodine-starch complex measured	570	2.5–30 9.96×10^3		

* CAA-chloranilic acid, BCG-bromocresol green, BPB-bromophenol blue, BTB-bromothymol blue

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