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Titrimetric and SpectrophotometricAssay of Diethylcarbamazine Citrate in Pharmaceuticals Using Permanganate as Oxidant

Nagib A. Qarah and K. Basavaiah.*

Department ofChemistry, University of Mysore, Manasagangothri, Mysore-570 006, India

Abstract : Two titrimetric and one spectrophotometric methods are described for the determination of diethylcarbamazine citrate in bulk drug and dosage forms using permanganate as an oxidimetric agent. In method A,the drug solution in H_2SO_4 is titrated directly at 80^0C to pink end point. DEC was treated with a measured excess of standard permanganate in H_2SO_4 medium, and after a contact time of 5 min, the residual oxidant back titrated with ammonium ferrous sulphate to a colorless end point (method B). Spectrophotometry is based on the measurement of the unreacted permanganate at 545 nm after the reaction between DEC and permanganate in H_2SO_4 medium is ensured to be complete (method C). In all methods, the amount of permanganate reacted was related to the amount/concentration of DEC. Experimental variables associated with the assay were carefully examined and optimized for better performance characteristics. Both the titrimetric methods are applicable for 1-10 mg range and the reaction follows 1:3 and 1:4 (DEC:$KMnO_4$) stoichiometry in direct and indirect methods, respectively. In spectrophotometry, Beer's law is obeyed in the inverse manner, and linearity is observed in the range 2.5-30 µg mL^{-1} with a molar absorptivity value of 8.03×10^3L mol^{-1} cm^{-1}. The limits of detection (LOD) and quantification (LOQ) were calculated to be 0.12 and 0.35 µg mL^{-1}, respectively. The methods were validated for precision and accuracy, robustness and ruggedness and selectivity. The methods were applied to the determination of DEC in tablets and syrup with satisfactory results. The accuracy of the methods was also assessed by recovery study via standard-addition procedure.

Keywords : Diethylcarbamazine citrate; assay; titrimetry; spectrophotometry; permanganate; pharmaceuticals..

Introduction

The World Health Organization (WHO) has called for an effort to eliminate Lymphatic Filariasis (LF) around the world[1]. A nematode worm (*wuchereria bancrofti*) is the cause of 90% of lymphatic filariasis cases globally. Mosquito bites transmit larval nematodes (microfilariae) present in the blood stream of infected persons, and although the adult nematodes are resistant to medical treatment, human transmission in endemic regions can be stopped by administering drugs, such as Diethylcarbamazine (DEC) (Fig. 1), that kill the microfilariae. DEC has had a long history of safe use in mass drug administration (MDA) LF eradication programs[2-4] and so far, *W. bancrofti*do not appear to have developed resistance to DEC[5,6]. A course of treatment of 6 mg/kg per day of DEC citrate for 12 days (daily dose around 300 mg) can significantly reduce the microfilariae count in an infected person. However, in regions where the disease is endemic, yearly drug administration to infected individuals must be continued over the adult worm lifetime of 4-6 years to eradicate the disease. As an alternative to pill-based MDA, DEC can be administered to local populations in the form of medicated cooking salt, with DEC citrate present at 0.2-0.4% w/w, which corresponds to a daily dose of 20-40 mg DEC citrate. Local production and distribution of medicated salt fortified with DEC has proved to be a particularly effective method[7,8] for eradicating LF from endemic regions[9,10].

In view of its pharmaceutical importance, considerable work has been done for its quantification in body fluids such as whole blood[11], plasma[12,13], serum[14] and urine[15].the drug in pharmaceuticals has been assayed by methods based on several techniques such as gas chromatography[16], liquid chromatography[17-21], DC-polarography[22], proton magnetic resonance (NMR) spectrometry[23], uv-spectrophotometry[24] and spectrofluorimetry[25].

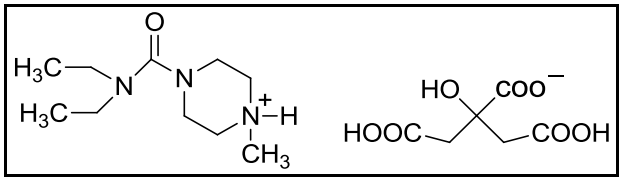

Fig. 1: Chemical structure of diethylcarbamazine citrate.

Determination of DEC by visible spectrophotometry has also been reported based on condensation reaction using malonic acid-acetic anhydride[25,26] and pyridine-acetic anhydride[27]; charge-transfer complexation reaction using iodine[28], chloranalic acid[29,30] and picric acid[31]; ion-pair complex formation using fast green FCF and orange[32], bromocresol green[33] and bromophenol blue[34]. In a method reported by Basu and Dutta [35], the solid ion-associate formed by DEC with ammonium reineckate in citric acid at pH 3.5 was filtered, dissolved in acetone and absorbance measured at 525 nm.

There are only three articles dealing with the titrimetric assay of DEC in pharmaceuticals. In a low tech method reported by Wearer et al.[36], the medicated salt dosed with DEC was treated with KIO_3 and liberated iodine was determined titrimetry. Using sodium tetraphenyl borate and acetous perchloric acid as titrants[37], the drug was determined by potentiometric titrimetry. Campbell et al.[38] employed ion-responsive electrode as an indicator electrode for the determination of DEC by potentiometric titration with sodium tetraphenyl borate as titrant.

No doubt, many instrumental methods cited above[16-25] are rapid, sensitive and selective, but they require cumbersome and time-consuming sample preparation, costly equipment and specialized training to handle them.Even the visible spectrophotometric methods[25-35] suffer from one as the disadvantage such as undesirable experimental variables such as heating[15,17], critical pH adjustment[32-35], measurement at shorter wavelength[8,15,28] and use of large amounts of organic solvents creating waste disposal problem[28-35], as shown in Table 1. The low tech titrimetric method[36] is limited to medicated salt and not applied to pharmaceuticals, and the method of Bhanumathi et al.[37] requires scrupulous anhydrous medium.The preparation of ion-responsive electrode[38] is cumbersome and the electrode response is critically dependent on several factors, including conditioning time, pH and ionic strength of the medium.

These limitations of the aforesaid reported methods necessiated the development of simple and cost-effective methods for the determination of DEC in pharmaceuticals. Though, titrimetric and spectrophotometric methods based on several chemistries have been reported, methods based on redox reaction were not found in the literature. Thus, the present study was devoted to explore the use permanganate as an oxidimetric agent for the titrimetric and spectrophotometric assay of DEC in bulk and dosage forms. The methods were found to have many advantages over the currently available methods with respect cost, ease of performance and facile experimental conditions.

Materials 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 all solutions.

Potassium permanganate0.01M

Prepared by dissolving about 0.395 g of the chemical (Merck, Mumbai, India) in water; the solution was boiled for 10 minutes to remove any residual manganese (IV) ions, cooled, filtered and diluted to 250 mL, and standardized using procedure as outlined in literature[39], and used in titrimetric assay. The stock solution was diluted to get 600 µg mL^{-1} concentrations for use in spectrophotometric method.

Ferrous ammonium sulphate

A 0.05M was prepared by dissolving 4.9 g of the salt (S.d. Fine Chem, Mumbai, India) in 50 mL of water containing 1 mL of concentrated H_2SO_4, and diluted to 250 mL with water.

Sulphuric acid

Concentrated sulphuric acid (Merck, Mumbai, India, Sp. gr. 1.18) was diluted appropriately with water to get 5 mol $L^{-1}$for all methods.

Pure diethylcarbamazine citrate

Certified to be 99.86% pure was procured from Inga Laboratories Pvt., Mumbai, India, and used as received. Banocide tablets (Glaxo Smith Kline Pharma. Ltd., Nashik, India) containing 50 mL and 100 mg DEC for tablet, Banocide syrup (Glaxo Smith Kline Pharma. Ltd., Bangaloe, India) containing 120 mg DEC/5mL and DECET tablets containing 150 mg DEC per tablet (RND Laboratories, Pvt. Ltd., India) were purchased from lcal commercial sources.

Standard drug solution

A 1mg mL^{-1} standard stock solution was prepared by dissoving 250 mg of pure DEC in water, and diluted to 250 mL in a calibrated flask and used in titrimetry (method A&B). This stock solution (1000 µg mL^{-1})was appropriately diluted stepwise with water to get a working concentration of 100 µg mL^{-1} for spectrophotometric investigation (method C)

General procedures

Direct Titration (method A)

A 10.0 mL aliquot of standard solution containing 1.0-10.0 mg of DEC was measured accurately and transferred into a 100 mL titration flask, 5 mL of 5 mol L^{-1} H2SO4 was added and the flask was kept on hot plate until the solution's temperature reached 80 0C, and titrated immediately against 0.01 mol L^{-1} $KMnO_4$ to the first appearance of pink color.

Indirect Titration (method B)

A 10.0 mL aliquot of pure drug solution containing 1.0-10.0 mg of DEC was measured accurately and transferred into a 100 mL titration flask. The solution was acidified by adding 3 mL 0f 5 mol L^{-1} H_2SO_4. Then 10 mL of 0.01 mol L^{-1} KMnO4 was added by means of a pipette and the flask was let stand for 5 min at room temperature and the unreacted KMnO4 was titrated immediately with 0.05 mol L^{-1} FAS to a colorless end point. A blank experiment was simultaneously performed.

The amount of DEC in the aliquot was computed from the formula:

$$\text{Amount (mg)} = \frac{V \times Mw \times S}{n}$$

where V = volume of titrant reacted.

M_w = relative molecular mass of DEC.

S = strength of titrant in mol L^{-1}.

n = number of moles of titrant reacting with per mole of DEC.

Spectrophotometry (method C)

Different aliquots (0.5,1.0, …… 3.0 mL,100 µg mL^{-1}) of standard drug solution were accurately measured into a set of 10 mL calibration flasks and the volume was brought to 3.0 mL with water. The solutions were acidified by adding 1 mL of 5M H_2SO_4, and to each flask was added 1 mL of 600 µg $mL^{-1}KMnO_4$. The contents were mixed and the flasks were set aside for 10 min with occasional shaking before diluting to the mark with water.The absorbance was measured at 550 nm againstwater blank.

Procedure for tablets

Twenty tablets were weighed accurately and finely powdered. A portion of the powder equivalent to 100 mg of DEC was transferred into a 100 mL calibrated flask, 60 mL of water was added and the flask was shaken for 15 min. Then, the volume was diluted to the mark with water, mixed well and the insoluble residue was filtered off using Whatman No.42 filter paper. First 10 mL of the filtrated was discarded and 5 mL of the subsequent portion was analyzed in five replicates following the recommended titrimetric procedure. The tablet extract (1000 µg mL^{-1} in DEC) was diluted to 100 µg $mL^{-1}$with water and 2.0 mL aliquot was subjected to analysis (n=5) following the spectrophotometric procedure.

Procedure for syrup

A 5 mL aliquot of the syrup containing 120 mg of DEC was accurately measured into a 100 mL calibrated flask, 60 mL of water added and shaken for 5 min before the volume was diluted to the mark with water, mixed well and filtered usingWhatman No.42 filter paper. Subsequently, the steps described under procedure for tablets were followed.

Procedure for placebo and synthetic mixture analyses

Inactive ingredients normally present in tablets, viz., starch (20 mg), talc (30 mg), calcium gluconate (20 mg) , methyl cellulose (10 mg), lactose (10 mg), sodium alginate (10 mg), magnesium stearate (10 mg) and gelatin (10 mg) were mixed to get a homogeneous mixutre. Fifty mg of placebo was transferred to a 100 mL calibrated flask and its aqueous extract was prepared as described under "procedure for tablets". Ten mL of the placebo extract was assayed by titrimetry, and 2 mL of the diluted extract was analyzed by spectrophotometry as described earlier. To 50 mg of the placebo was added 100 mg of pure DEC, and both were mixed well for uniform composition. The mixture was quantitatively transferred into a 100 mL calibrated flask and the steps described under "procedure for tablets" were followed.

Results and Discussion

The higher oxidation state of manganese (+7) in potassium permanganate leads to the strong oxidizing property and this property was not applied for diethylcarbamazine citrate. The innate intense purple color solution of permanganate absorbs in the vicinity of 550 nm. The Mn-containing products from redox reactions depend on the pH. In acid solutions, permanganate is reduced to the faintly pink Mn^{+2} as represented by the following equation:

$$MnO_4^- + 8H^+ + 5e^- \longrightarrow Mn^{+2} + 4H_2O$$

The standard potential in acid solution, E, has been calculated to be 1.51 volts, hence the permanganate ion in acid solution is a strong oxidizing agent. Sulphuric acid is the most suitable acid, as it has no action upon permanganate in dilute solution. In the titrimetric methods, DEC was found to react with $KMnO_4$ in 1:3 and 1:4 (DEC:$KMnO_4$) stoichiometry in direct and indirect methods, respectively. a possible reaction scheme is suggested as shown in Fig. 2.

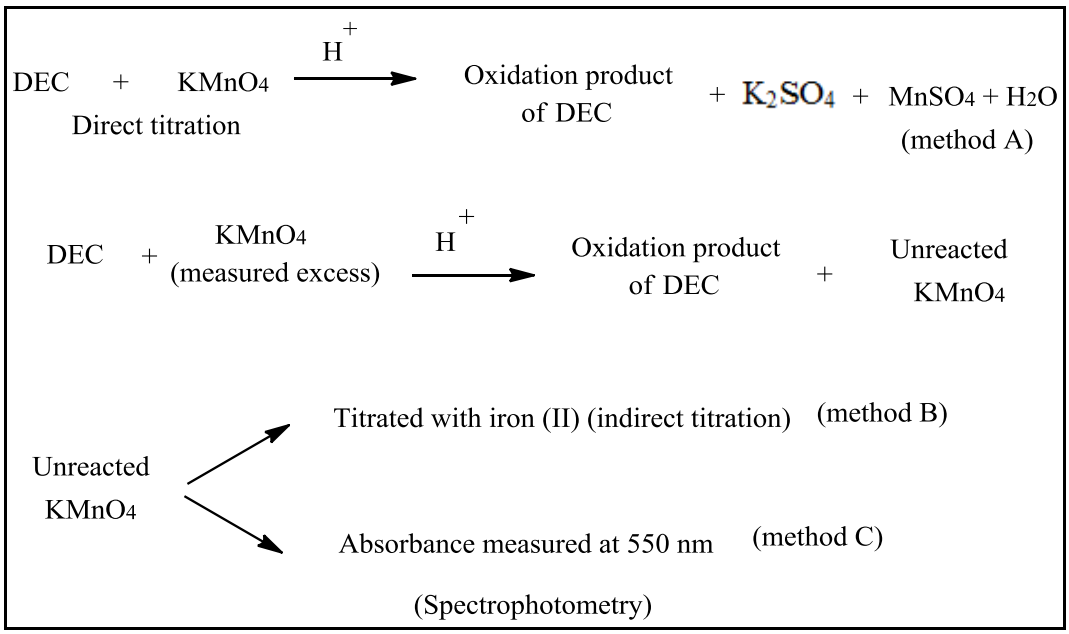

Fig. 2: Possible reaction path ways of the assay methods.

Method development (optimization of variables)

The experimental variables which provided accurate and precise results were optimized by keeping other variable constant and varying one parameter at a time. The influence of each variable involved in the methods was determined.

Titrimetry

The direct titration between DEC and $KMnO_4$ was slow at room temperature. This could be due to a series of slow reduction steps of Mn^{7+} to Mn^{2+}, while forming less stable intermediate product like Mn^{6+} and Mn^{4+}. In order to increase the reaction rate, the titration was performed at 80^0 C. The reaction stoichiometry wasfound with to be 1:3 (DEC: $KMnO_4$) and it did not change when the reaction temperature was maintained between 70 and 90 ^{0}C. In the absence of H_2SO_4 as a reaction medium, the reduction of Mn^{7+} to Mn^{4+} predominates, and the end point is the appearance of brown color which is difficult to detect. The effect of acid concentration on the reaction between DEC and $KMnO_4$was studied by varying the concentration of H_2SO_4 keeping the amount of drug the same. The reaction stoichiometry was found to be unaffected when 3-7 mL of H_2SO_4 was maintained. Hence, 5 mL of 5 mol $L^{-1}H_2SO_4$acid in a total volume of 15 mL in the beginning was required.In case of method B (indirect), measured excess of $KMnO_4$ was allowed to react with DEC in H_2SO_4medium and the unreacted $KMnO_4$ was subsequently determined by back titrating with FAS. In the presence of excess of $KMnO_4$, the reaction stiochiometry was found to be 1:4 (DEC: KMnO4) in the 1-10 mg range. The reaction between DEC and$KMnO_4$ was found to be complete and quantitative in 5 min in the presence of 3 mL of 5 mol $L^{-1}H_2SO_4$. Hence 5 min was fixed throughout the titrimetric method.

Spectrophotometry (method C)

Absorption spectra

When a fixed concentration of permanganate was reacted with increasing concentrations of DEC in H_2SO_4 acid medium, there occurred a concomitant fall in the concentration of permanganate as revealed by the decreasing absorbance at 550 nm (Fig 3), which served as the basis for quantification. A preliminary experiment showed that permanganate can be determined upto 60 µg mL^{-1} (Fig 4) at 550 nm under the optimum acidic conditions of assay. Hence, different concentrations of DEC were reacted with 1 mL of 600 µg mL^{-1} $KMnO_4$ to determine the concentration range over which DEC could be determined. To check the effect of acid concentration on the reaction, 0-5 mL of 5 mol L^{-1} H_2SO_4 was added to the fixed concentration of DEC and $KMnO_4$, and it was observed that maximum absorbance readings were obtained with 1.0 mL of acid beyond which absorbance slightly decreases. Hence 1.0 mL of 5 mol L^{-1} H_2SO_4 was used in a total volume of 10 mL. Effect of hydrochloric acid was not studied since KMnO4 being a strong oxidizing agent would react with HCl

to liberate chlorine. The reaction between DEC and KMnO4 in the acid concentration employed was complete in 10 min, and the absorbance of the measured unreacted $KMnO_4$ was found to be stable upto 20 min thereafter. The reagent blank consisting of acid and permanganate showed maximum absorbance (equal to the intercept).

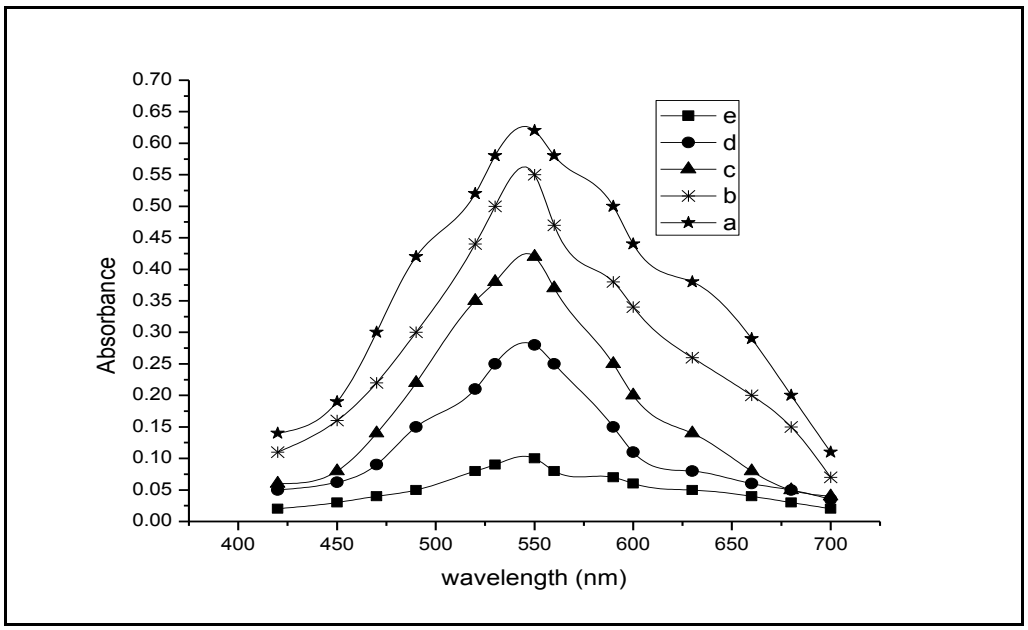

Fig. 3. Absorption spectra of $KMnO_4$ (60 µg mL^{-1}) after treating with: a. 0, b. 5, c. 10, d. 20 & e. 30 µg mL^{-1} DEC.

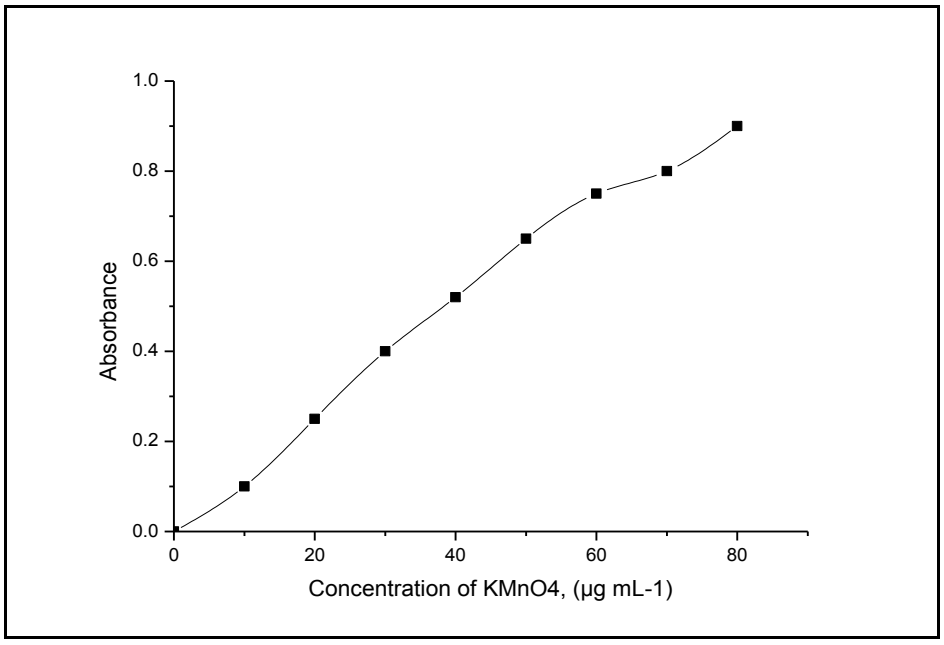

Fig. 4: Linear relation between absorbance at 550 nm and $KMnO_4$ concentration in H_2SO_4.

METHOD VALIDATION

Method validations were done according to the present ICH guidelines[40].

Analytical parameters of spectrophotometric methods

A linear correlation was found between absorbance at λ_{max} and concentration of DEC in the range given in Table 1. The graph is described by the regression equation:

$Y = a + bX$

(Where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in µg mL^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and sandell sensitivity values of both methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines[40] are also presented in Table 1.

Table 1: Sensitivity and Regression Parameters

Parameter	Method C
λ_{max}, nm	550
Colour stability	20 min
Linear range, µg mL^{-1}	2.5-30
Molar absorptivity (ε), L mol^{-1} cm^{-1}	8.03×10^3
Sandell sensitivity*, µg cm^{-2}	0.0487
Limit of detection (LOD), µg mL^{-1}	0.12
Limit of quantification (LOQ), µg mL^{-1}	0.35
Intercept (a)	0.62
Slope (b)	-0.018
Standard deviation of a (S_a)	0.0998
Standard deviation of b (S_b)	0.00316
Regression coefficient (r)	-0.9978

*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.

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$\text{LOD} = \frac{3S}{k} \qquad \text{and} \qquad \text{LOQ} = \frac{10S}{k}$$

where S is the standard deviation of five reagent blank determinations, and *k* is the slope of the calibration curve.

Assay precision and accuracy

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of DEC were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day is within 2.0 and inter-day is within 2.50 which showed that the precision was good. The accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for ENP and found to be within the RE (%) values of 2.80 (Intra-day accuracy) and 3.33 (Inter-day accuracy).

Method selectivity

Placebo analysis was carried out in order to find the interference. There was absolutely no interference from the placebo as shown by the titer values in titrimetry and absorbance value in spectrophotometry. In order to study the selectivity of the methods, a separate experiment was performed with synthetic mixture.The percent recoveries of DEC were 98.86, 101.9 and 102.5 for method A, method B, and method C, respectively. This confirms the selectivity of methods under the optimized conditions.

Robustness and ruggedness

For the evaluation of the method robustness, three important experimental variables such as reaction time, reaction temperature and H_2SO_4 concentration were slightly varied deliberately. The analysis was performed at the deliberately varied experimental conditions by taking three different concentrations of DEC

and found to remain unaffected as shown by the RSD values in the range of 0.65 to 3.14 %. Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using three different burettes in case of titrimetric procedures and two different spectrophotometers in case of spectrophotometry. The results are shown in Table2.

Table 2: 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	3.08	1.28	2.66	3.06	1.64	2.00
	6	5.88	1.33	1.98	5.80	2.32	1.13
	9	8.88	0.96	1.32	8.89	1.59	1.24
B	2	2.04	1.55	1.97	1.97	1.13	1.48
	4	4.09	1.36	2.18	4.11	1.24	2.68
	8	8.11	0.99	1.41	8.21	1.42	2.54
C	10	9.87	1.59	1.29	9.89	2.44	1.15
	20	20.3	1.48	2.17	20.5	1.38	2.48
	30	30.5	1.37	1.67	30.6	1.46	1.98

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

In methods A and B, DEC taken/found are in mg and they are µg mL^{-1} in method C.

Application to tablets and suspension (application to analysis of pharmaceutical formulation)

The proposed methods were applied for the determination of DEC in one brand each of tablets and syrup containing 100 mg and 120 mg respectively of active component and the results are presented in Table 4. The same batch tablet powder was subjected to the assay by the reference method, and results were statistically evaluated by applying Student's t- and variance ratio F-test. The evaluated t- and F-values did not exceed the tabulated values at the 95% confidence level for four degrees of freedom, indicating agreeing accuracy and precision between the proposed methods and the reference method.

Table 3: Method Robustness And Ruggedness Expressed As Intermediate Precision (% RSD)

Method	DEC taken*	Robustness (%RSD)			Ruggedness (%RSD)	
		Parameters altered			Inter-burette's[#] /inter instruments[$] (n=3)	Inter-analysis (n=3)
		Reaction temperature[**]/reaction time[***]	Acid concentration			
A	3	0.76	0.69		1.56	2.08
	6	0.83	1.88		1.82	1.89
	9	1.12	1.41		1.98	1.24
B	2	1.87	1.67		1.62	2.3
	4	0.94	1.55		1.42	2.2
	8	1.32	1.43		1.33	1.95
C	10	1.23	0.83		2.11	2.25
	20	1.41	1.07		1.97	2.01
	30	1.35	0.76		2.18	2.45

*mg in method A and method B; µg mL^{-1} in method C.

**In method A reaction temperature were 75,80 and 85 ^{0}C and H_2SO_4 volume used were 4.5,5.0 and 5.5 mL

***In method B, reaction time used were 4.5,5.0 and 5.5 min and H_2SO_4 volume used were 2.5,3.0 and 3.5 mL; In method C, reaction time used were 9,10 and 11 min, and H_2SO_4 volume used were 0.8,1.0 and 1.2 mL.#In the case of methods A and B.$In the case of method C.

Table 4: Results of Analysis of Tablets by the Proposed Methods and Statistical Comparison of the Results With the Official Method

Formulation analyzed	Nominal amount	Found* (% of nominal amount ± SD)			
		Official method	Proposed methods		
			Method A	Method B	Method C
Banocide forte tablets	100 mg per tablet	101.6±0.95	102.23±1.23 t =0.91 F= 1.68	101.4±1.65 t = 0.23 F = 3.02	102.3.±1.86 t = 0.75 F=3.83
Banocide syrup	120 mg per 5 mL	100.9±1.32	99.78±0.82 t =1.61 F=2.59	101.7±1.82 t = 0.8 F = 1.9	101.8±1.88 t = 0.88 F=2.03
Analysis Time, min Analysis Cost, $		38.0 1.80	4.0 1.0	5.0 0.8	17 0.5

*Mean value of five determinations.
(Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77).
(Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39).

Table 5: Results Of Recovery Experiment Through Standard-Addition Method.

Method	Tablet studied	DEC in Tablet, (mg/µg mL^{-1})	Pure DEC added, (mg/µg mL^{-1})	Total found, (mg/µg mL^{-1})	Pure DEC recovered Percent±SD*
A	Banocide forte tablets	4.09 4.09 4.09	2.00 4.00 6.00	6.15 8.22 10.3	100.99±0.81 101.6±0.54 102.08±0.88
	Banocide syrup	3.99 3.99 3.99	2.00 4.00 6.00	6.07 8.13 10.2	101.34±1.33 101.80±1.34 102.20±1.37
B	Banocide forte tablets	4.06 4.06 4.06	2.00 4.00 6.00	6.14 8.10 9.99	101.32±1.36 100.5±0.78 99.33±1.41
	Banocide syrup	4.07 4.07 4.07	2.00 4.00 6.00	5.97 7.98 9.98	98.35±2.61 98.88±1.42 99.11±0.89
C	Banocide forte tablets	10.23 10.23 10.23	5.0 10.0 15.0	15.31 20.14 25.22	100.54±1.76 99.57±1.33 99.96±1.44
	Banocide syrup	10.18 10.18 10.18	5.0 10.0 15.0	15.24 20.26 25.25	100.42±1.46 100.37±0.98 100.29±1.55

*Mean value of three determinations.

Recovery study

Pre-analyzed tablet powder was spiked with pure DEC at three levels and the total was found by the proposed methods. The determination each level was replicated thrice. The results of percent recovery of drug which are an indication of accuracy are summarized in Table 5, and demonstrate the methods' freedom from interference by the co-formulated substances in the tablets.

Conclusions

The results demonstrate that micro level determination of diethylcarbamazine citrate is possible by titrimetry which can be performed with ease, rapidly and by using inexpensive chemicals. The method has the advantage of being applicable over along range compared the narrow range offered by the only existing titrimetric method. Unlike most currently available spectrophotometric method, the present method is free from un welcome steps such ash eating or extraction and also from critical pH oracid/alkaline conditions. A significant advantage of the spectrophotometric method is the irremarkable sensitivity which is higher than that of the existing methods and is comparable to the sensitivity offered by some sophisticated techniques such as voltammetry, HPLC, HPTLC, densitometry and fluorimetry. Anadditional advantageis that the absorb an cemeasurement is madeat longer wavelength (490 nm)where the interference from tablet xcipientsis expected to be less compared to shorter wavelengths (about 400 nm)use dinmostavailable methods. These advantages coupled with afairly good accuracy and precision lendth emethod soptly suitable forroutin equality control. **Table 6.**

Table 6: Comparison of Performance Characteristics of the Present Method With the Existing Methods

SI. No.	Reagent used	Methodology	λmax (nm)	Linear range (µg mL^{-1})	Remarks	Ref.No.
1	*CAA	Measurement of purple color CT complex in dioxane-$CHCl_3$	540	10-400	Mixture of organic solvents used	29
2	Malonic acid-acetic anhydride	Measurement of absorption of condensation product	333	-	Heating step and longer contact time involved	25
3	HOAc-Ac_2O with pyridine	Absorbance of yellow color product measured	428	10-100	Heating step and longer contact time involved	27
4	Ammonium reineckate	Absorbance of red color product at pH=3.5 in acetone measured	525	-	Tedious and time consuming	35
5	Fast green FCF, Orange-II	Ion-pair complex extracted into $CHCl_3$ and measured	-	-	Tedious and time consuming extraction step and critical pH adjustment involved	32
6	BCG	Yellow ion-pair complex measured in $CHCl_3$	-	-	Tedious and time consuming extraction step and critical pH adjustment involved	33
7	BPB	Extracted ion-pair complex measured	-	-	Tedious and time consuming extraction step and critical pH adjustment involved	34
8	$KMnO_4$	Measurement of absorption of product	550	2.5-30	Rapid, no extraction step involved, selective	Present work

*CCA-chloranilic acid, BCG-bromocresol green, BPB-bromophenol blue.

Conflict of interest:

Both the authors declare no conflict interest.

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