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Spectrophotometric Assay of Diethylcarbamazine Citrate in Pharmaceuticals and Human Urine via Ion-Pair Reaction Using Methyl Orange Dye

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Abstract

Two simple and moderately selective spectrophotometric methods are described for the determination of Diethylcarbamazine Citrate (DEC) in bulk drug, dosage forms and spiked human urine. The first method (method A) is based on the formation of yellow colored ion-pair complex between DEC and Methyl Orange (MO) dye, at pH 4.95 ± 0.05, which was extracted into chloroform and measured at 420 nm. The second method (method B) involved the breaking of yellow ion-pair complex in acid medium followed by the measurement of the free dye at 520 nm. Experimental parameters influencing the formation and extraction of the ion-pair complex in method A and breaking of the complex in method B were scrupulously examined and optimized. Beer's law is obeyed over the concentration ranges of 10-90 and 5-100 µg mL^{-1} DEC with corresponding molar absorptivity values of 2.90×10^3 and 3.54×10^3 L mol^{-1} cm^{-1} for method A and method B, respectively. The Sandell's sensitivity values were 0.1351 and 0.1106 µg cm^{-2} for method A and method B, respectively. The Limits of Detection (LOD) and Quantification (LOQ) were calculated to be 0.36 and 1.09 µg mL^{-1} (method A) and 0.34 and 1.02 µg mL^{-1} (method B). The composition of drug-dye ion-pair complex used in method A was found to be 1:1 by Job's method of continuous variations. The proposed methods were validated for robustness, ruggedness and selectivity, and applied to the determination of DEC in tablet, syrup formulations and spiked human urine samples. The results demonstrated that the proposed methods are as accurate and precise as the reference method. The accuracy of the methods was further ascertained by recovery study via standard-addition method.

Keywords: Diethylcarbamazine citrate; Assay; Spectrophotometry; Ion-pair; Methyl orange; Pharmaceuticals

Introduction

Diethylcarbamazine Citrate (DEC) is chemically known as N,N-diethyl-4-methylpiperazine-1-carboxamide dihydrogen citrate [1]. It is a piperazine anthelmintic agent indicated for the treatment of individual patients with lymphatic filariasis, tropical pulmonary eosinophilia and loiasis. It acts by inhibiting arachidonic acid metabolism (Figure 1) [2].

Due to its therapeutic importance, DEC has been assayed in body fluids by Proton Magnetic Resonance (PMR) spectrometry [3], gas chromatography [4-6] and liquid chromatography-mass spectrometry [7]. Methods based on non-aqueous titrimetry and gravimetry [8], neutralization titrimetry [9], ion-responsive electrode-titrimetry [10], gas chromatography [11], High Performance Liquid Chromatography (HPLC) [12-16], DC-polarography [17], Proton Magnetic Resonance (PMR) spectrometry [18] and UV-spectrophotometry [19] have been described for the assay of DEC in pharmaceuticals.

Visible spectrophotometry continuous to be used in industrial quality and clinical laboratories due to its inherent simplicity, sensitivity, selectivity, speed and easily availability of the instrument. Few methods based on color reactions like condensation [20] and charge-transfer complexation [21] are found in the literature for the determination of DEC in bulk drug and dosage forms. Extractive-spectrophotometry is widely used in pharmaceutical analysis [22-30] because of its sensitivity and selectivity; the technique also finds use in the assay of DEC in pharmaceuticals. The charge-transfer complex formed by the interaction between the chloranilic acid and DEC [31] was adopted for the assay of the drug in pure powder and in tablets. The yellow colored condensation product formed by an acetous solution of DEC with acetic anhydride pyridine mixture [32] was measured at 428 nm and used for the assay of DEC in pharmaceutical formulation.

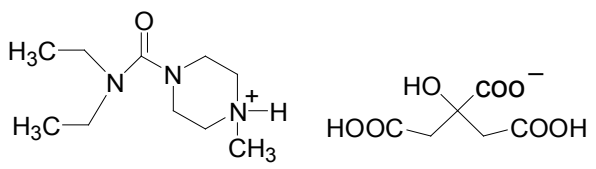

Figure 1: Chemical structure of diethylcarbamazine citrate.

In this paper, we describe the application of Methyl Orange (MO) dye as an ion-pair agent for the spectrophotometric assay of DEC in pharmaceutical formulations and spiked human urine. The methods are based on either the extraction of the formed DEC-MO ion-pair complex, in buffer of pH 4.95, into chloroform and measuring its absorbance at 420 nm or breaking the formed ion-pair complex with alcoholic H_2SO_4 followed by measurement of the free dye at 520 nm. The methods were applied to the determination of DEC in commercial formulations and spiked urine samples and the results demonstrated that the co-formulated substances seldom interfered in the assays.

Experimental Apparatus

A Systronics model 166 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) equipped with matched 1 cm quartz cells

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was used for absorbance measurements. A digital pH meter Model Elico L1 120 was used for pH measurements.

Materials

Pure Diethylcarbamazine Citrate (DEC), certified to be 99.86% pure was procured from Inga Laboratories Pvt. Ltd., Mumbai, India and used as received. Banocide forte tablets (Glaxo SmithKline Pharma. Ltd., Nashik, India) containing 50 and 100 mg DEC per tablet, Banocide syrup (Glaxo SmithKline Pharma. Ltd., Bangalore, India) containing 120 mg DEC per 5 mL and Decet tablets containing 150 mg DEC per tablet (from RND Laboratories Pvt. Ltd., India) were purchased from local commercial sources.

Reagents and chemicals

All reagents and solvents used were of analytical reagent grade. Double distilled water was used to prepare aqueous solutions wherever required.

1. Walpole buffer of pH 4.95 was prepared by mixing 1.0 M solutions of sodium acetate (Merck Pvt. Ltd., Mumbai, India) and hydrochloric acid (Merck Pvt. Ltd., Mumbai, India, sp. gr. 1.18) and the pH was adjusted using pH meter.
2. A 0.05% solution of Methyl Orange (MO) (S.D. Fine Chem., Mumbai, India) was prepared by dissolving 50 mg of the dye in water.
3. A 1.0% (v/v) solution of sulphuric acid (Merck, Mumbai, India, sp. gr. 1.84) was prepared in ethanol.

Standard stock solution

A stock standard solution (100 μg mL^{-1} DEC) was prepared by dissolving accurately weighed 10 mg of pure DEC in water and diluted to volume with the same solvent in a 100 mL calibrated flask.

General Procedures

Method A

To a set of 125 mL separating funnels, appropriate aliquots (1.0-9.0 mL) of the standard working solution (100 μg mL^{-1}), were transferred to obtain concentrations in the range of 10-90 μg mL^{-1} DEC, and the solutions were diluted to 10 mL with water. To each separating flask were added 2 mL of buffer solution of pH 4.95, followed by 6 mL of 0.05% MO solution and made up to 20 mL with water and mixed well. A 10 mL portion (accurately measured) of chloroform was added to each separating funnel and the contents were shaken for 1 min. The two layers were allowed to separate for one min and the absorbance of the chloroform layer was measured at 420 nm against a reagent blank after drying over anhydrous sodium sulphate.

Method B

Aliquots of 0.25-5 mL drug-dye ion-pair complex solution, equivalent to 200 μg mL^{-1} drug, were accurately transferred into a set of 10 mL calibrated flasks and made up to 5 mL with chloroform. To each flask was added 1.0 mL 1% alcoholic H_2SO_4 solution, and diluted to the mark with ethanol, and mixed well. The absorbance of each solution was measured at 520 nm against a reagent blank.

In each method, the calibration graph was prepared, and the concentration of the unknown was computed from the regression equation derived using the Beer's law data.

Procedure for formulations

Procedure for the assay of DEC in tablets: Twenty tablets were weighed and powdered. An amount of powder equivalent to 10 mg of DEC was shaken with ~50 mL of water in a 100 mL calibrated flask for 20 min and the content was diluted to the mark with water and mixed well. The insoluble matter was filtered off using a quantitative filter paper. A 5 mL aliquot of tablet extract was taken for analysis in five replicates, as described under the procedure for bulk drug (method A). In method B, 1 mL aliquot of ion-pair complex (200 μg mL^{-1}) was taken in five replicates, and the general procedure was followed.

Procedure for the assay of DEC in 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 was added and shaken for 5 min before the volume was diluted to the mark with water, mixed well and filtered using Whatman No. 42 filter paper. Subsequently, the steps described under procedure for the assay of DEC in tablets were followed.

Procedure for placebo and synthetic mixture analyses: A placebo blank of the composition of talc (25 mg), starch (25 mg), acacia (20 mg), methyl cellulose (10 mg), sodium citrate (15 mg), magnesium stearate (20 mg) and sodium alginate (15 mg) was prepared by uniform mixing. Ten milligrams of placebo was shaken with water and its extract prepared as described under 'procedure for the assay of DEC in tablets'. 5 ml of the extract was subjected to analysis as described earlier. To 10 mg of the placebo, 10 mg of pure drug was added, homogenized, and its extract prepared as described under 'procedure for the assay of DEC in tablets'. After dilution to 100 μg mL^{-1}, different aliquots were taken for analysis (n=5).

Procedure for spiked human urine sample: To 5 mL of urine taken in a 50 mL calibrated flask, accurately weighed 5 mg of DEC was added, and diluted to mark with water, and mixed well to ensure the dissolution of the drug. The spiked-urine solution (100 μg mL^{-1} in DEC) was subjected to analysis using method A in five replicates by taking 1.0 mL aliquots. A 0.5 mL of DEC-MO ion-pair complex (100 μg mL^{-1} in DEC; prepared as for spiked urine in method A) was transferred into a 10 mL standard flask and the procedure was then applied as under method B. The nominal content of DEC was calculated using the corresponding regression equation.

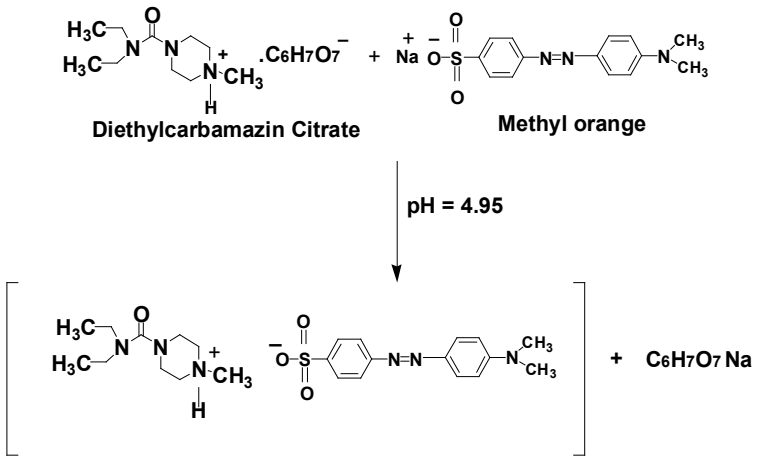
Results and Discussion

Absorbance spectra

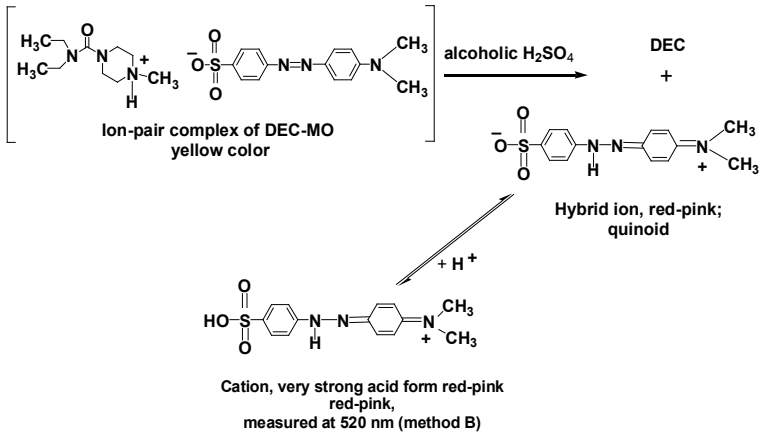
DEC reacted with MO in buffer solution (pH= 4.95) to form a yellow-colored ion-pair complex, which was extracted into chloroform and measured at 420 nm, where the reagent blank had negligible absorbance. The drug-dye complex is broken in alcoholic H_2SO_4 medium, and free cationic form of the dye (Schemes 1 and 2) showed its maximum absorption at 520 nm as shown in Figure 2.

Optimization of experimental variables: The optimization of the methods was carefully studies to achieve complete reaction, quantitative extraction of the ion-pair complex, and highest sensitivity. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH, type of organic solvent, volumes of the dye, and extant of shaking time for the extraction of ion-pair complex. In method B, alcoholic sulphuric acid concentration required for complete breaking of the ion-pair complex was optimized.

Effect of pH on the ion-pair formation: In order to establish the optimum pH range, DEC was mixed with MO in aqueous solutions



Scheme 1: The probable reaction pathway for DEC-MO complex formation (method A).



Scheme 2: Breaking of DEC-MO ion-pair complex (method B).

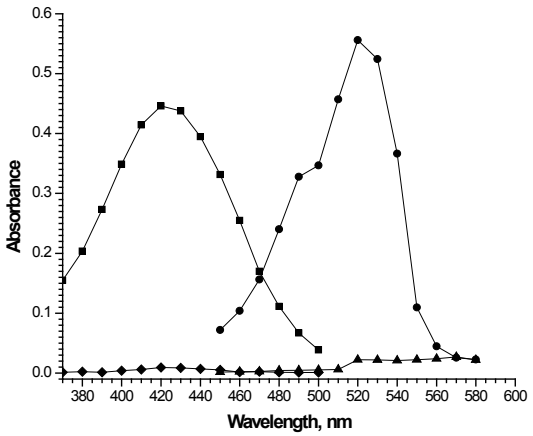


Figure 2: Absorption spectra of: DEC-MO ion-pair complex (-■-, method A); acid form of the dye (-●-, method B) and blanks (♦, ▲) (DEC: 50 µg mL^{-1} in method A and 70 µg mL^{-1} in method B).

buffered to pH 3.09, 4.19, 4.58, 4.95 and 5.2, and the absorbance of ion-pair complex was measured. Figure 3 shows that constant absorbances were obtained over the pH range 4.58-5.2 in Walpole buffer; hence a pH of 4.95 was selected as the optimal.

Effect of buffer system: Different buffer systems of pH 4.95 were tried, but Walpole buffer was preferred due to higher sensitivity and stability of the complex formed. The effect of volume of Walpole buffer of pH 4.95 was studied by adding (0-5 ml) and the results showed that the optimum volume of buffer was found to be 2 mL in a 20 mL total volume of aqueous phase (Figures 4 and 5).

Effect of methyl orange, shaking time and alcoholic H_2SO_4 concentration: A 6 mL portion of 0.05% MO solution was found to be optimum as shown in Figure 6. Constant absorbance was obtained for shaking period of 1 to 4 min; hence a shaking time of 2 min was selected. Clear separation of the two layers occurred in 1 min. For method B, the effect of alcoholic sulphuric acid concentration required to break the ion-pair complex and formation of the acidic form of the dye was studied by measuring the absorbance of the solutions containing

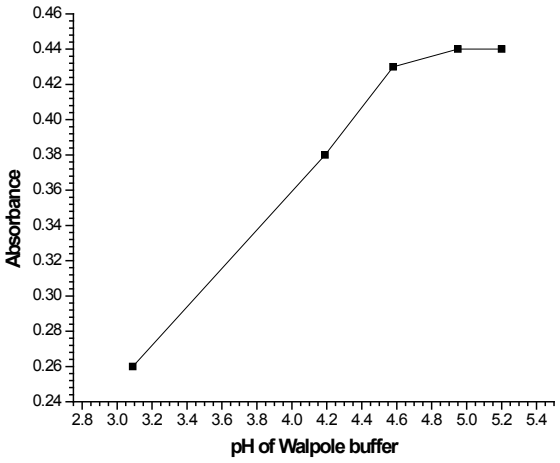


Figure 3: Effect of pH ion-pair complex formation in method A (DEC: 50 µg mL^{-1}).

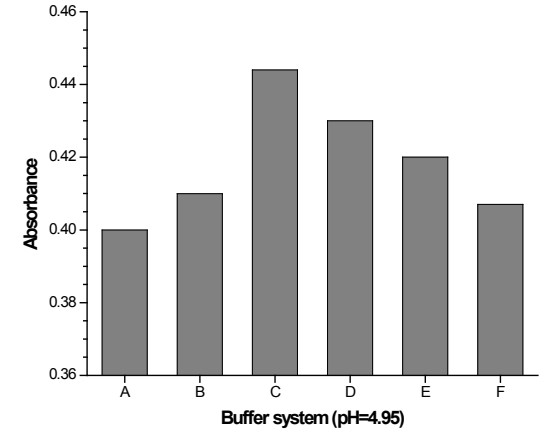


Figure 4: Effect of different buffers: A. Mc-Ilvaine; B. Clark and Lubs 1; C. Walpole; D. Kolthoff; E. Sorenson; F. Clark and Lubs 2 in method A (DEC: 50 µg mL^{-1}).

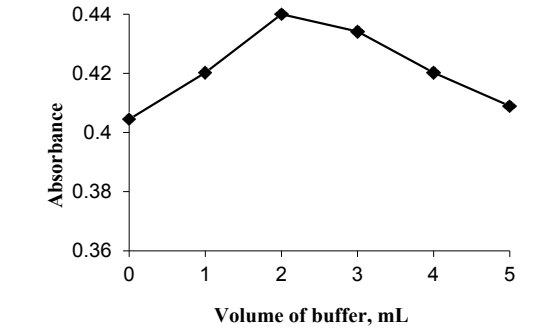


Figure 5: Effect of volume of buffer in method A (DEC: 50 µg mL^{-1}).

a fixed concentration of ion-pair complex (50 µg mL^{-1}; in DEC) and different volumes of alcoholic H_2SO_4. It was found that 1 mL of 1.0% alcoholic H_2SO_4 was adequate to break the ion-pair complex and to yield a maximum absorbance at 520 nm. Although larger volumes of alcoholic H_2SO_4 had no pronounced effect on the absorbance of the measured species.

Effect of selecting of the extracting solvent: The effect of different solvents, such as dichloromethane, chloroform, 1,2-dichloroethane, ethyl acetate and benzene, as extracting solvent was studied and the results are showed in Table 1. Chloroform found to be the best extracting solvent because of its extraction efficiency of the drug-dye ion-pair complex from the aqueous phase. A ratio of 2:1 of aqueous to chloroform phases was required for efficient extraction of the colored species. One extraction with 10 mL portion of chloroform was adequate for quantitative extraction of the complex.

Composition of the ion-pair complex: The composition of the ion-pair complex formed in method A between DEC and MO was established by applying Job's method of continuous variations. In this method, 4×10^{-4} M solutions of DEC and MO were used and mixed in varying volume ratios in such a way that the total volume of the drug and dye was kept at 10 mL. The absorbance of extracted ion-pair in each instance was measured and potted against the mole fraction of the drug (Figure 7). The plot reached a maximum

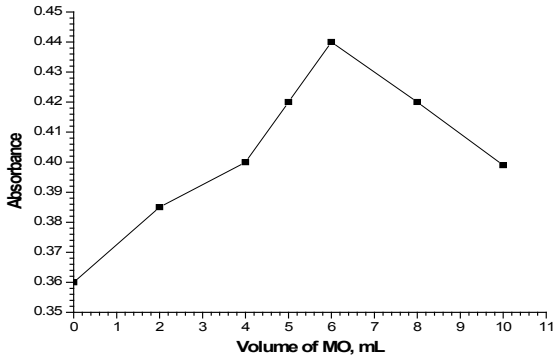


Figure 6: Effect of volume of MO dye solution in method A (DEC: 50 µg mL^{-1}).

Solvent	$A_{ion-pair}$	A_{blank}
Dichloromethane	0.277	0.019
Chloroform	0.395	0.016
1,2-dichloroethane	0.245	0.018
Ethyl acetate	0.112	0.072
Benzene	0.003	0.005

Table 1: Effect of the extracting solvent on absorbance of DEC-MO ion-pair (50 µg mL^{-1} DEC).

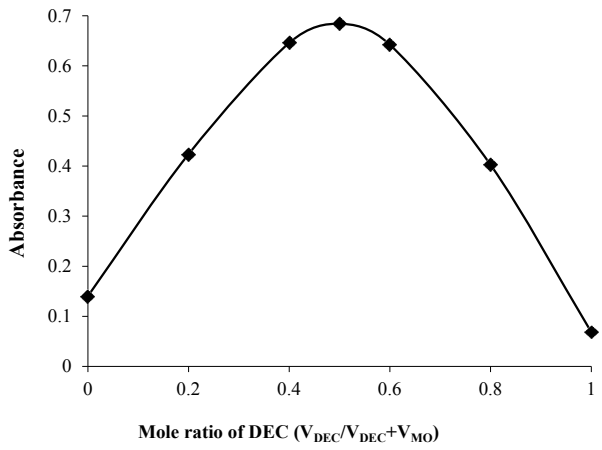


Figure 7: Job's continuous-variations plot, [DEC]=[MO]=4.0 × 10^{-4} M.

absorbance reading at a mole fraction of 0.5 which showed that a 1:1 (DEC:MO) ion-pair complex is formed between positive protonated DEC and MO anion.

Method validation: The proposed methods were validated for linearity, sensitivity, precision, accuracy, robustness, ruggedness, selectivity and recovery according to the International Conference on Harmonization (1996) guidelines.

Linearity and sensitivity: Under the described experimental conditions, calibration graphs were constructed by plotting absorbance *vs.* concentration. The linear ranges, regression equations, slopes and intercepts and their standard deviations of both methods are given in Table 2. The molar absorptivity and Sandell's sensitivity values of each method were also calculated and are presented in Table 2.

The Limits of Detection (LOD) and quantification (LOQ) were calculated using the formulae:

$$\text{LOD} = \frac{3s}{b} \text{ and } \text{LOQ} = \frac{10s}{b}$$

where 's' is the standard deviation of the replicate measurements of the absorbances of the reagent blank, and '*b*' is the sensitivity of the method, i.e., the slope of the calibration graph. The calculated values are given in Table 2.

Accuracy and precision: Pure drug solution and drug-dye complex at three concentration levels were subjected to analysis on intra-day and inter-day basis. The %RSD as a measure of precision and %RE as a measure of accuracy were calculated. The results of this study are presented in Table 3 and indicate excellent repeatability and reproducibility of the methods.

Selectivity: The placebo blank extract when analyzed using the described procedures yielded absorbances close to the reagent blanks implying the selectivity of the method. Analysis of the synthetic mixture yielded satisfactory percent recoveries as shown in Table 4. The results indicate that excipients and additives which often accompany in tablets (starch, lactose, talc, alginate, stearate, methyl cellulose, etc.) did not interfere in the proposed methods.

Robustness and ruggedness: For evaluation of the method robustness, some experimental variables (volume of buffer and dye in method A, and volume of alcoholic H_2SO_4 in method B) were slightly altered and their effect on performance of the methods was studied. To determine the ruggedness, the assay was done by one analyst using three different cuvettes as well as performing the analysis by three analysts on the same cuvette. The results of this study expressed as intermediate precision (%RSD) were presented in Table 5.

Parameter	Method A	Method B
λ_{max}, nm	420	520
Linear range, µg mL^{-1}	10-90	5-100
Molar absorptivity (ε), L mol^{-1} cm^{-1}	2.90×10^3	3.54×10^3
Sandell sensitivity*, µg cm^{-2}	0.1351	0.1106
Limit of detection (LOD), µg mL^{-1}	0.36	0.34
Limit of quantification (LOQ), µg mL^{-1}	1.09	1.02
Regression equation, Y**		
Intercept (a)	0.0021	0.0153
Slope (b)	0.0075	0.0080
Standard deviation of a (S_a)	9.98×10^{-2}	9.98×10^{-2}
Standard deviation of b (S_b)	1.31×10^{-3}	1.12×10^{-3}
Regression coefficient (r)	0.9999	0.9993

*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

Table 2: Sensitivity and regression parameters.

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	25.0	25.55	1.81	2.21	25.33	1.65	1.32
	50.0	50.82	2.75	1.64	50.93	1.92	1.86
	75.0	76.31	1.95	1.75	76.44	2.01	1.92
B	30.0	30.66	2.03	2.20	30.49	1.23	1.63
	60.0	60.92	0.76	1.53	60.89	0.87	1.48
	90.0	91.83	1.25	2.03	91.45	1.12	1.61

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

Table 3: Evaluation of intra-day and inter-day accuracy and precision.

DEC concentration, µg mL^{-1}	Method A	Method B
	Percent recovery ± SD (n=5)	Percent recovery ± SD (n=5)
20.0	97.73 ± 1.40	-
40.0	98.38 ± 0.86	-
60.0	96.38 ± 1.45	-
30.0	-	101.5 ± 0.88
60.0	-	98.87 ± 0.86
90.0	-	99.78 ± 0.68

Table 4: Results of analysis of synthetic mixture.

Applications to analysis of pharmaceutical formulations: Both methods were applied to quantitate DEC in two brands of tablet and syrup and the results are presented in Table 6. Same batch tablets were also analyzed by the reference method [33] for comparison, and the statistical tests applied to these results overwhelmingly support that no significant difference exists between the proposed methods and the reference method with respect to accuracy and precision.

To ascertain the accuracy of the proposed methods, recovery experiment was performed *via* standard addition technique. To a fixed and known amount of DEC in tablet powder (pre-analyzed), pure DEC was added at three levels 50, 100 and 150% of the level present in the tablet and the total was found by the proposed methods. Results of this study are presented in Table 7. In both methods, the percent found ranged from 97.12 to 104.52 with standard deviation values in the range 1.08 to 1.89 and indicate that the co-formulated substances did not interfere in the assay.

Applications to analysis of spiked urine sample: The proposed methods were applied to spiked human urine samples and the results are presented in Table 8. The results are satisfactory since no complicated extraction procedure was used to prepare the sample for the assay.

Conclusion

The present work is intended to demonstrate the potential of ion-pair based spectrophotometry to achieve fast and very efficient assay of DEC in pharmaceuticals and spiked human urine.

High reproducibility, extraction efficiency and low cost are the advantages of the proposed methods. Besides, a major advantage of the extractive spectrophotometric methods is its high selectivity in the presence of multi component mixture. The proposed methods are based on well characterized ion-pair complexation reaction, and have the advantages of sensitivity, selectivity, speed, accuracy and precision, and use of inexpensive instrument compared to the reported HPLC and GC methods. Comparison of the proposed methods with those of the reported methods revealed that the present methods offer a high sensitivity than RP-HPLC [16], derivative UV spectrophotometric [19] and visible spectrophotometric [31] methods as shown in Table 9. Moreover, the proposed methods can be performed at room temperature and are practically robust in variations of experimental conditions such as pH and reagent concentration. The highlight of the proposed methods is their ability to quantitate the drug in urine samples without involving complicated liquid-liquid extraction or solid phase extraction. Thus, the methods are useful for the analysis of DEC in quality control laboratories of under developed and developing countries.

Method	DEC taken (µg mL^{-1})	Robustness (%RSD) Parameters altered			Ruggedness	
		Volume of buffer*	Volume of MO*	Volume of alcoholic H_2SO_4*	Inter-analysts (%RSD), (n=3)	Inter-cuvettes (%RSD), (n=3)
A	50.0	1.42	1.62	-	0.96	0.88
B	60.0	-	-	1.81	1.76	0.94

*In method A, the volumes of buffer and MO added were 1, 2 and 3 mL; 5, 6 and 7 mL respectively, and in method B the volumes of alcoholic H_2SO_4 added were 0.8, 1.0 and 1.2 mL.

Table 5: Results of robustness and ruggedness study.

Formulation analyzed	Nominal amount	Found* (% of nominal amount ± SD)		
		Reference method	Proposed methods	
			Method A	Method B
Banocide forte tablets	50 mg per tablet	99.94 ± 1.23	100.42 ± 1.05 t=0.64 F=1.58	101.65 ± 1.27 t=2.08 F=1.08
Banocide forte tablets	100 mg per tablet	101.8 ± 0.91	101.33 ± 1.14 t=0.72 F=1.57	101.41. ± 1.11 t=0.61 F=1.49
Decet tablets	150 mg per tablet	101.6 ± 1.07	102.83 ± 1.31 t=1.62 F=1.50	99.98 ± 1.62 t=1.94 F=2.29
Banocide syrup	120 mg per 5 mL	101.3 ± 1.06	99.87 ± 1.15 t=2.04 F=1.18	99.92 ± 1.09 t=2.02 F=1.06

*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 6: Results of analysis of tablets by the proposed methods and statistical comparison of the results with the reference method.

Acknowledgements

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Method	Formulation studied	DEC in Formulation,	Pure DEC added,	Total found,	Pure DEC recovered Percent ± SD*
A	Banocide forte Tablets (100 mg)	20.27 20.27 20.27	20 40 60	38.99 61.95 83.88	97.12 ± 1.44 102.82 ± 1.66 104.52 ± 1.52
	Banocide syrup	19.97 19.97 19.97	20 40 60	41.04 62.95 82.09	102.72 ± 1.89 103.88 ± 1.62 102.65 ± 1.18
B	Banocide forte Tablets (100 mg)	20.28 20.28 20.28	20 40 60	41.24 62.32 83.25	102.41 ± 1.64 103.43 ± 1.52 103.69 ± 1.74
	Banocide syrup	19.98 19.98 19.98	20 40 60	41.52 61.89 81.88	104.21 ± 1.36 102.97 ± 1.08 102.38 ± 1.39

*Mean value of three determinations

Table 7: Accuracy by recovery study.

Method	Spiked concentration (µg mL^{-1})	Found ± SD*	% Recovery ± SD*
A	10.0	9.48 ± 0.85	95.13 ± 0.87
B	5.0	5.27 ± 0.68	104.94 ± 1.53

*Mean value of five determinations

Table 8: Results of %recovery from spiked urine samples.

S. No.	Technique	Methodology	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	Remarks	Ref. No.
1	HPLC	Analysis performance on a phenomenex C_{18} column using mixed phosphate buffer adjusted to pH 3.0–acetonitrile (40:60 v/v) as mobile phase at a flow rate of 1 mL/min and UV detection at 216 nm	24-144	0.62	0.105	Less sensitive	16
2	UV spectrophotometry	Absorbance of in 0.01 N NaOH measured at 216 nm	10-50	2.28	6.91	Narrow linear range, less sensitive	19
3	Visible spectrophotometry	Purple colored charge transfer complex between DEC and CAA in dioxane-$CHCl_3$ measured at 540 nm	10-400	NR	NR	Mixture of organic solvents used	31
4	Visible spectrophotometry	Yellow colored DEC-MO ion-pair complex measured at 420 nm Breaking the DEC-MO ion-pair complex in alcoholic H_2SO_4 and formation of acidic form of the dye which measured at 520 nm	10-90 5-100	0.36 0.34	1.09 1.02	Facile experimental conditions involved, wide linear dynamic, sensitive	Present methods

CAA: Chloranilic Acid; NR: Not Reported

Table 9: Comparison of performance characteristics of the present method with some existing methods.

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