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Research Paper

Simple, Sensitive and Selective Spectrophotometric Methods for the Determination of Methdilazine in Pharmaceuticals through Charge Transfer Complex Formation Reaction

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Three rapid, selective and sensitive spectrophotometric methods are described for the quantitative determination of methdilazine hydrochloride (MDH) in pure form as well as in its pharmaceutical formulations. The methods are based on charge transfer complexation reaction of base form of MDH as n-electron donor with 2, 3-Dichloro-5, 6-dicyano-1,4-benzoquinone (DDQ), 2,4-Dinitrophenol (DNP) and 2,4, 6-Trinitrophenol (picric acid; PA) as π-acceptors to give highly coloured radical anion species. The coloured products were quantified spectrophotometrically at 585 nm with DDQ (method A), at 420 nm with DNP (method B) and at 425 nm with PA (method C). Under the optimized experimental conditions, Beer's law is obeyed over the concentration ranges of 4-64, 2.5-40 and 2-32 μg ml^{-1} MDZ for method A, method B and method C, respectively. The molar absorptivity, Sandell sensitivity, limits of detection and quantification are also reported. The effects of reaction medium, reaction time and reagent concentration on the sensitivity and stability of the complexes formed have been examined. The proposed methods were successfully applied to the determination of MDH in pure form and commercial tablets and syrup with good accuracy and precision. Statistical comparison of the results was performed using Student's t-test and F-ratio at 95% confidence level and the results showed no significant difference between the reference and proposed methods with regard to accuracy and precision. Further, the accuracy and reliability of the methods were confirmed by recovery studies *via* standard addition technique.

Key Words: Methdilazine; Determination; Charge-transfer Complexes; Spectrophotometry; Pharmaceuticals

Introduction

Methdilazine hydrochloride (MDH), chemically known as (10-[(1-Methyl-3 pyrrolidinyl) methyl] phenothiazine monohydrochloride) [1] (Fig. 1), is a synthetic analogue of phenothizone derivative used as an antihistamine and it is also found to possess anti-pruritic action [2].

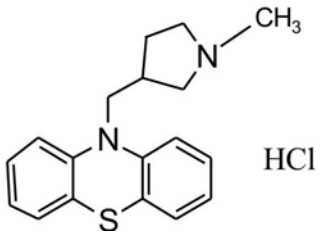

Fig. 1: Structure of MDH

The drug is official in United States Pharmacoepia [3], which describes UV-spectrophotometric assay in aqueous medium. Literature survey revealed availability of few methods for the assay of MDH in pharmaceuticals. Quantification of MDH has been achieved by high performance liquid chromatography (HPLC) [4, 5], reverse phase and ion-exchange chromatography [6], liquid chromatography [7], spectrofluorimetry [8], differential fluorimetry and differential UV-spectrophotometry [9]. Some of these methods have

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sufficient sensitivity to determine lower concentrations of the drug. However, these methods involve several manipulation steps which are not simple for routine analysis of pharmaceutical formulations and require sophisticated instruments.

Visible spectrophotometry may serve as an useful alternative to many of the aforesaid sophisticated techniques because of its cost-effectiveness, ease of operation, sensitivity, fair accuracy and precision and wide applicability. A few spectrophotometric methods have earlier been reported for MDH. Van Urk reagent [10] as a chromogenic reagent has been reported for the determination of MDH in tablets and syrup, but the method is poorly sensitive with a narrow linear range 10-24 μg ml^{-1}. Another method, based on the reaction of MDH with sodium cobaltinitrite in 85% H_3PO_4 medium [11] where the reaction mixture was boiled for 15 min before measuring the absorbance at 372 nm, has also been reported. Sastry *et al*. [12] have devised a method involving hematin formed *in situ* from haematoxylin and chloramine-T at pH 7.0 and MDH at 70°C leading to the formation of pink colored chromogen measurable at 555 nm. The same authors [13] have reported three procedures based on oxidative coupling reaction involving MDH, MBTH and iron (III), persulphate or hypochlorite. A few indirect methods are also found in the literature. In one method reported by Basavaiah and Charan [14] MDH was reacted with measured excess of vanadate, in H_2SO_4 medium and the unreacted oxidant was determined by treating it with H_2O_2 and measuring the resulting complex at 460 nm. In a related method by the same authors [15], the unreacted vanadate was determined by reacting it with chromotropic acid in the presence of hydroxylamine chloride, and measuring the absorbance at 420 nm. Using KIO_3 as the oxidimetric reagent, the same authors [16] have reported three methods for MDH. In the first method, MDH was treated with a measured excess of KIO_3, and the unreacted oxidant was reacted with variamine blue, and the resulting colour was measured at 540 nm. In another method, the drug was reacted with a large excess of iodate in the presence of chloride ions, the ICl_2^- generated was used to iodinate 2',7'-dichlorofluorescein, and the red colour of the iodinated dye was measured at 525 nm. The third method involved the extraction of the liberated iodine with CCl_4 and measurement of the absorbance at 520 nm. Chloranilic acid has been used for the assay of MDH based on charge-transfer reaction [17].

Apart from the above, quite a few extractive spectrophotometric methods based on ion-pair formation reaction of MDH with dyes have also been reported. Gowda *et al*. [18] have reported a method based on the formation of chloroform-soluble ion-associate complex formed by the interaction of drug with brilliant blue G in neutral medium and measurement at 614 nm. The same authors developed two extractive spectrophotometric methods based on similar reaction with bromopyrogallol red and bromothymol blue [19]. The drug has also been determined spectrophotometrically based on ion-pair complex formation with fast green FCF [20] at pH 5.0 followed by extraction into chloroform and measurement at 620 nm. Basavaiah and Charan have also developed an extractive spectrophotometric method for the assay of MDH using bromophenol blue, the absorbance being measured at 420 nm [21]. Based on the same reaction turbidimetric method where the absorbance of the ion-pair was measured at 650 nm, has also been reported [21]. Sastry *et al*. suggested another procedure based on extraction of MDH-cobaltthiocyanate ion associate complex [22] and measurement at 620 nm.

The visible spectrophotometric methods currently available suffer from one or the other disadvantage critically like dependence on pH, poor sensitivity, labour-intensive, tedious and time-consuming liquid-liquid extraction step, use of large amount of organic solvents as indicated in Table 1.

The present work describes three simple, sensitive, selective, rapid, direct, accurate and precise spectrophotometric methods for the determination of MDH in pure form, tablets and in syrup using three reagents viz., 2,3-dichloro-5,6-dicyano-1, 4-benzoquinone (DDQ), 2,4-dinitrophenol (DNP) and 2,4,6-trinitrophenol (Picric acid; PA) as charge-transfer reagents. The cited π-acceptors have earlier been employed for the assay of several drug substances based on similar reaction [23-27].

Table 1: Comparison of the performance characteristics of the present methods with the published methods

S.No.	Reagents used	Methodology	λ_{max} (nm)	Linear range (µg/ml) (ε = L/mol/cm)	Remarks	Ref
1	Van Urk	Complex formed measured	515	10-24	Low sensitivity	10
2	Sodium cobaltinitrite	Measured radical cation	372	2-16	Heating steps	11
3	Chloramine-T, Haematoxylin	Redox reaction	555	4-32 5.1×10^3	Requires critical pH control, time consuming	12
4	MBTH- iron (III)					13
5	Sodium metavanadate, H_2O_2	Unreacted metavanadate measured	460	0-250	Low sensitivity	14
6	a) Metavanadate, Chromotropic acid	Unreacted metavanadate measured		0-75 2.95×10^4	reaction time is high, moderately sensitive	15
	b) Vanadium (IV), Ferrin	Ferroin measured	510	5-25 5.31×10^4		
7	a) KIO_3, Variamine blue	Unreacted iodate measured	540	1.25-8.75 25.26×10^3	Requires heating, tedious pH control	16
	b) KIO_3, NaCl, dichloroflurescein	Iodinated dye measured	525	5.0-60.0 2.65×10^3	Requires critical pH control	
	c) KIO_3, CCl_4	Liberated iodine extracted to CCl_4 measured	520	50-600 2.19×10^2	Requires extraction step, less sensitive	
8	Chlaranilic acid	Charge-transfer complex was measured	520	25-125 1.48×10^3	Less sensitive	17
9	Brilliant blue G	Chloroform extractable ion-pair complex measured	614	0.1-6.0 3.14×10^4	Tedious extraction step, pH dependent	18
10	Bromopyrogollal red	Chloroform extractable ion-pair complex measured	485	2-35 1.07×10^4	Requires critical pH, liquid-liquid extraction	19
	Bromothymol blue		420	1-18 2.13×10^4		
11	Fast green FCF	Chloroform extractable ion-pair complex measured	620	-	Requires critical pH, liquid-liquid extraction	20
12	Bromophenol blue	Chloroform extractable ion-pair complex measured	420	2-16	Extraction step, tedious pH control	21
		Turbidity of suspension measured	650	10-70		
13	Cobaltthiocyanate	Benzene extractable ion-pair complex measured	620	50-500	Requires critical pH control	22
14	a) DDQ	Measurement of red colored radical anion	585	4-64.0 4.9×10^3	Moderately/highly sensitive with wide linear dynamic ranges, no heating or extraction step, no pH-adjustment, single step reaction and inexpensive instrumental setup	Present methods
	b) DNP	Measurement of yellow colored charge transfer complex	420	2.5-40.0 1.0×10^4		
	c) PA		425	2-32.0 1.0×10^4		

Experimental

Instrument

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) provided with 1 cm matched quartz cells was used for all absorbance measurements.

Materials

Chemicals used were of analytical reagent grade. Methidilazine hydrochloride certified to be 99.85% pure was obtained from Glaxo Laboratories, Mumbai, India, and used as received. Dilosyn 8 mg tablet and Dilosyn syrup (Glaxosmithkline Pharmaceuticals Ltd) were purchased from local commercial sources.

All reagents used were of analytical reagent grade and HPLC grade organic solvents were used throughout the investigation. Dichloromethane and acetone were purchased from Merck Mumbai, India. Solutions of 0.1% DDQ in 1,4-dioxan, 0.1% DNP and 0.1% PA in dichloromethane were prepared separately.

Standard Drug Solution in Free Base Form (MDZ)

Into a 125 ml separating funnel, an accurately weighed 22.57 mg of pure MDH was transferred and dissolved in about 20 ml of water and the solution was rendered alkaline to litmus paper with 6 N ammonia solution and 1 ml was added in excess, shaken for 5 min. The free base (MDZ) formed was extracted with four 20 ml portions of dichloromethane, the extract was passed over anhydrous sodium sulphate and collected in a 100 ml volumetric flask. The volume was made up to mark with dichloromethane and the resulting solution (200 μg ml^{-1} MDZ) was diluted with dichloromethane to get a working concentration of 50 μg ml^{-1} MDZ for method B, 40 μg ml^{-1} for method C. In method A, the extract was evaporated to dryness, the residue was dissolved in acetonitrile and used for the assay.

Preparation of Calibration Curves

Method A (Using DDQ)

Varying aliquots (0.25-4.0 ml) of a standard MDZ solution (80 μg ml^{-1}) were accurately transferred into a series of 5 ml calibrated flasks using a micro burette and the total volume in each flask was brought to 4 ml by adding adequate quantity of acetonitrile. To each flask, 1 ml of 0.1% DDQ solution was added, the content was mixed well and the absorbance was measured at 585 nm against a reagent blank similarly prepared without adding MDZ solution.

Method B (Using DNP)

Different aliquots (0.25-4.0 ml) of standard MDZ solution (50 μg ml^{-1}) were accurately transferred into a series of 5 ml calibration flasks as described above. One ml of 0.1% DNP solution was added to each flask and diluted to volume with dichloromethane. The content was mixed well and the absorbance was measured at 420 nm against a reagent blank.

Method C (Using PA)

Aliquots (0.25-4.0 ml) of a standard MDZ (40 μg ml^{-1}) solution were accurately transferred into a series of 5 ml calibration flasks and the total volume was brought to 4 ml by adding dichloromethane. To each flask, 1 ml of 0.1% PA solution was added and the solution made up to volume with dichloromethane. The content was mixed well and the absorbance was measured at 425 nm against a reagent blank.

Standard graph was prepared by plotting the absorbance versus MDZ concentration, and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation.

Procedure for Tablet

Twenty tablets (Dilosyn 8 mg) were weighed and pulverized. The amount of tablet powder equivalent to 22.57 mg of MDH was transferred into a 100 ml volumetric flask containing 60 ml of water. The content was shaken well for 20 min and diluted to the mark with water. The resulting solution was filtered through Whatmann No. 42 filter paper, the filtrate and washings were collected in to a 125 ml separating funnel. The MDH was converted to free base as described earlier, MDZ solutions of required concentrations were prepared as described under the general procedure for pure drug and a suitable aliquot

was used for assay by applying procedures described earlier.

Procedure for Syrup

The content of two dilosyn syrup (5 ml of dilosyn syrup equivalent to 4 mg of MDH) bottles (100 ml per bottle) were quantitatively transferred into a separating funnel. The bottles were washed with water and the washing was also transferred into the separator. The content was rendered alkaline to litmus paper with 6 N ammonia solution and 1 ml was added in excess. The contents were then extracted with 4 x 20 ml portions of dichloromethane, the extract was passed over anhydrous sodium sulphate and collected in a 100 mL volumetric flask, the volume was made upto mark with dichloromethane. The resulting solution was diluted appropriately to their respective concentration and assayed using a convenient aliquot in all the three methods.

Placebo Blank Analysis

A placebo blank of the composition: talc (35 mg), starch (25 mg), acacia (25 mg), methyl cellulose (30 mg), sodium citrate (25 mg), magnesium stearate (25 mg) and sodium alginate (20 mg) was made and its solution was prepared as described under the procedure for tablets, and then analysed using the procedures described above.

Procedure for the Determination of MDZ in Synthetic Mixture

To 20 mg of the placebo blank of the composition described above, 11.28 mg of pure MDH was added, homogenized and transferred to 100 ml volumetric flask and the solution was prepared as described under procedure for tablets. The extract (100 μg ml^{-1} in MDZ) was diluted appropriately with dichloromethane and then subjected to analysis by the procedures described above. This analysis was performed to study the interference by excipients usually present in tablet.

Results and Discussion

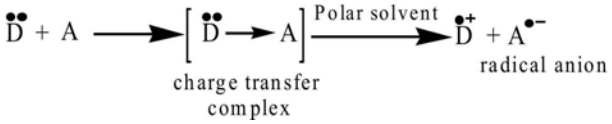
Absorption Spectra

The reaction of methdilazine base (MDZ) as n-electron donor) and the π-acceptors such as DDQ, DNP and PA results in the formation of C-T complexes. The absorption spectra of MDZ-DDQ charge-transfer complex resulted in the formation of an intense reddish violet color which exhibited three maxima at 585, 550 and 460 nm [28] (Fig. 2a). These bands can be attributed to the formation of DDQ radical anions arising from the complete transfer of n-electrons from donor to acceptor moieties in acetonitrile. The absorption band at 585 nm was selected as analytical wavelength keeping in view the sensitivity of the reaction product and blank absorbance. Similarly, the absorption spectra of MDZ-DNP and MDZ-PA C-T complex resulted in the formation of intense yellow products which exhibit absorption maxima at 420 and 425 nm, respectively (Figs. 2b, 2c).

Reaction Mechanism

Charge-transfer complex formation is characterized by electronic transition to an excited state where the transfer of electronic charge from donor to acceptor moiety takes place partially. As a result, the resonance in this excitation energy occurs very frequently in the visible region of the electro-magnetic spectrum [29]. This produces the usually intense colour characteristic for these complexes. Therefore, MDZ, a nitrogenous base acting as n-donor was made to react with DDQ (π-acceptor) to produce coloured charge transfer complexes in 1,4-dioxan-acetonitrile solvent system.

MDZ, being an n-electron donor, reacts with π-acceptors (A) to form CT complexes of n-π type which dissociate to give the coloured free radical anions of the acceptors according to the following equation:



The chemistry used in method A is based on the reaction of the basic nitrogen of MDZ as n-donor with DDQ as π-acceptor to form charge transfer complex which subsequently dissociates into radical

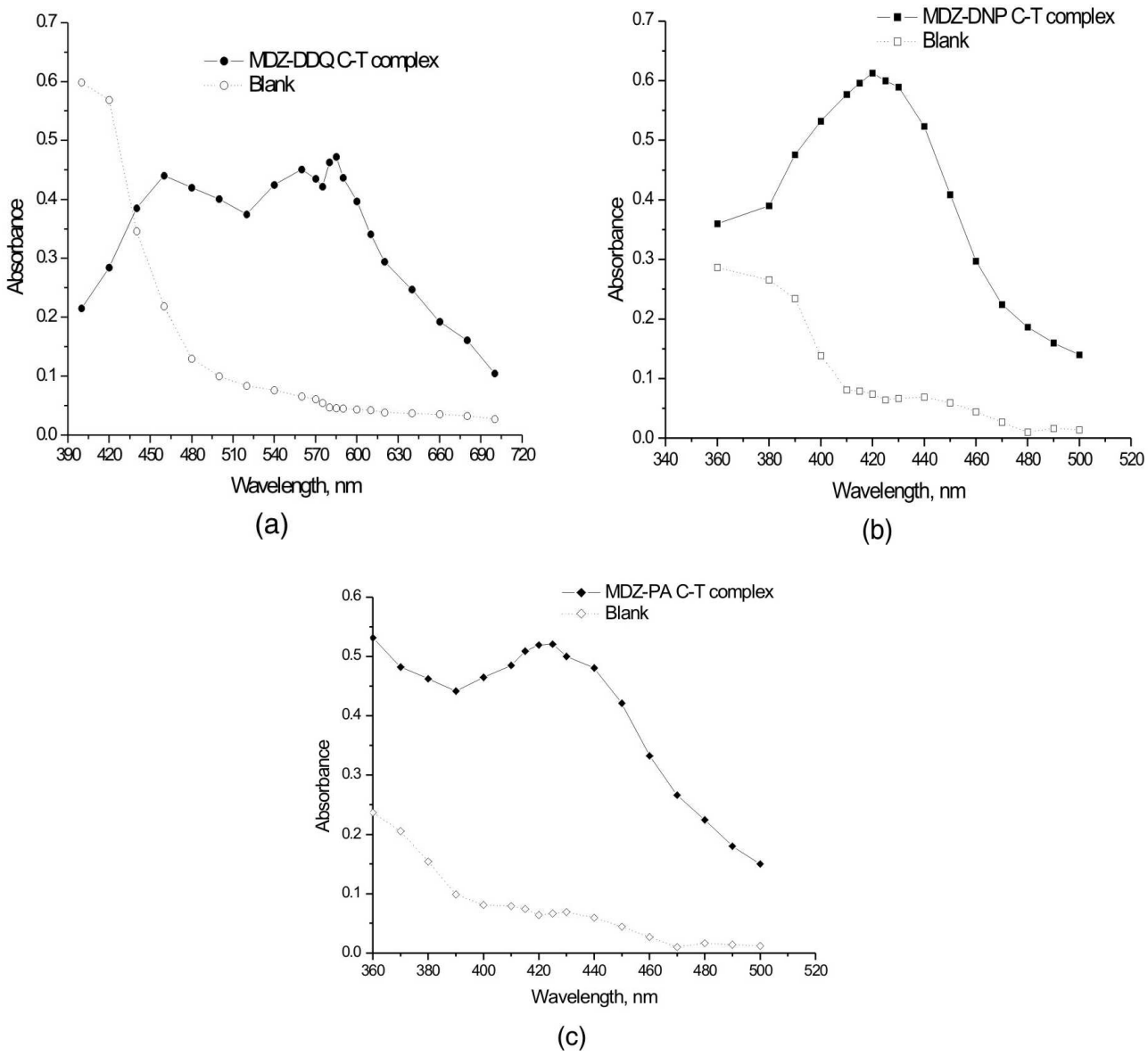
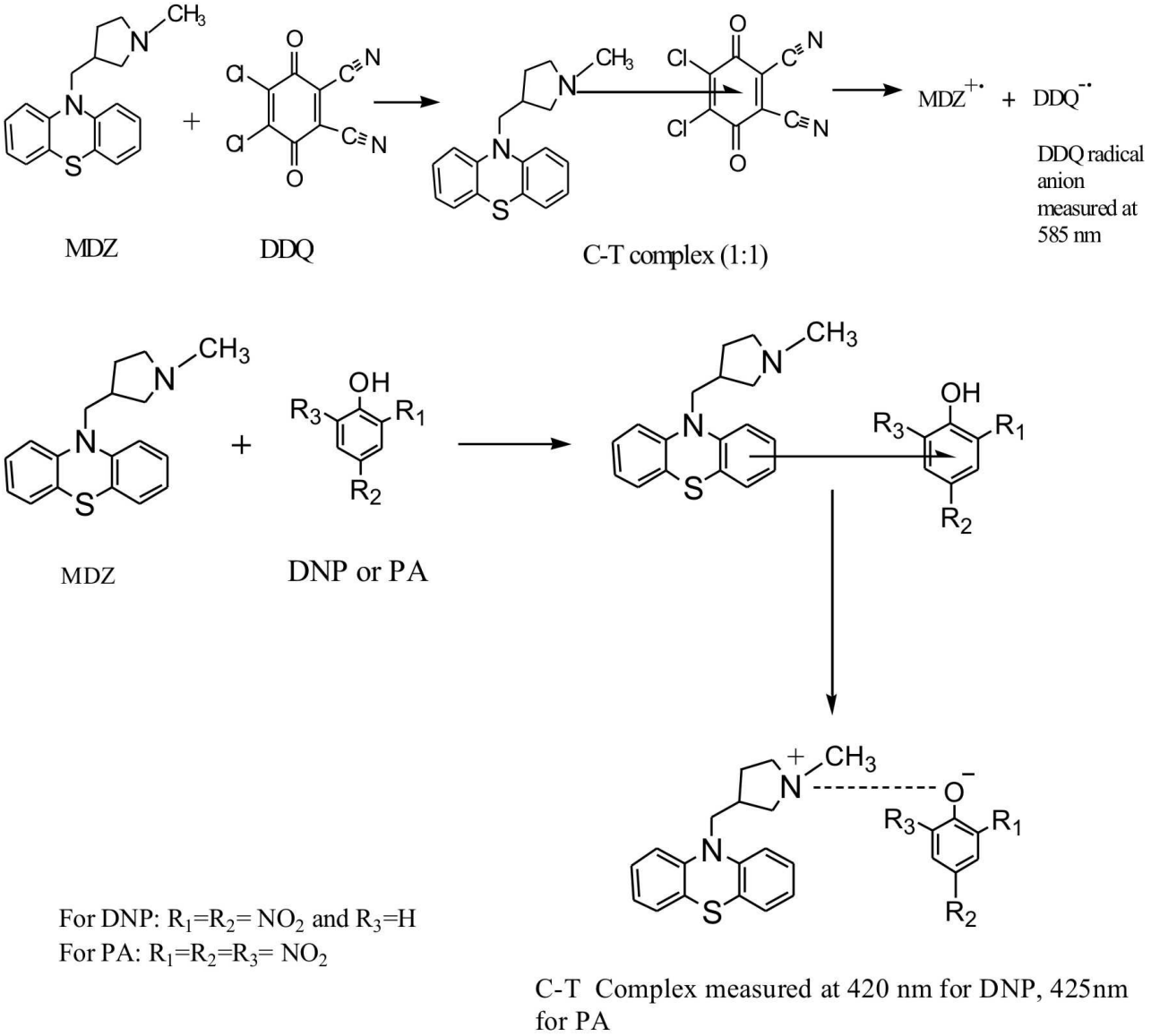


Fig. 2: Absorption spectra of: (a) MDZ-DDQ complex (method A), (b) MDZ-DNP complex (method B), and (c) MDZ-PA (method C)

anions depending on the polarity of the solvent used [30]. In polar solvents, such as acetonitrile, complete electron transfer from the donor to the acceptor moiety takes place with the formation of red chromogen with absorption maxima at 585 nm due to the formation of radical anion [31].

When an aromatic amine is combined with a polynitrophenol, one type of force field produces an acid-base interaction, and the other, an electron donor-acceptor interaction. The former interaction leads to the formation of true phenolate by proton-transfer, and the latter, to a true molecular compound by charge-transfer [32]. Based on this, the mechanism for method B and method C can be discussed in terms of transfer of electronic charge from the benzene ring of MDZ, an electron-rich molecule (a Lewis-base donor), to the ring of DNP or PA, an electron-deficient molecule (a Lewis-acid acceptor), and at the same time the proton of the hydroxyl group of DNP or PA will transfer to the secondary amine of MDZ. The explanation for the produced colour in method B and

method C lies in the formation of complexes between the pairs of molecules MDZ-DNP and MDZ-PA, and this complex formation leads to the production of two new molecular orbitals and, consequently, to a new electronic transition [33]. The tentative reaction pathways of all the three methods are given in Scheme 1.



Scheme 1: Tentative reaction pathway for the formation of C-T complex between drug (MDZ) reagents (DDQ, DNP and PA)

Optimization of Experimental Variables

Many experimental variables which are found to affect the colour intensity and stability of the resulting complexes were optimized to achieve maximum sensitivity and adherence to Beer's law.

Effect of Reagent Concentration

The optimum concentration of the reagent required to achieve maximum sensitivity for the colour developed in each method was ascertained by adding different amounts of the reagent DDQ, DNP or PA to a fixed concentration of MDZ. The results showed that 1.0 ml each of 0.1% DDQ, DNP and PA solution

was optimum for the production of maximum and reproducible color intensity. The results are shown in Fig. 3.

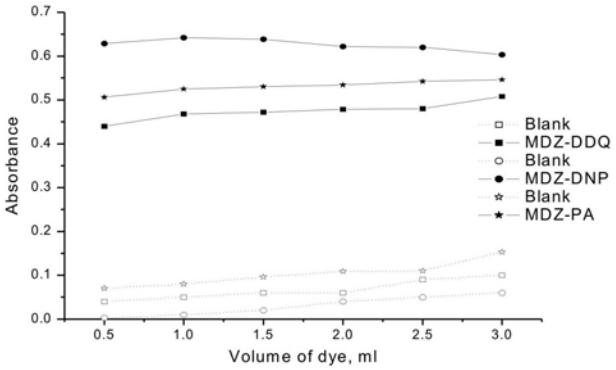


Fig. 3: Effect of reagent concentration on the formation of (MDZ-DDQ complex, 32 µg ml^{-1} MDZ), (MDZ-DNP complex, 20 µg ml^{-1} MDZ) and (MDZ-PA complex, 16 µg ml^{-1} MDZ)

Effect of Solvent

Several organic solvents such as chloroform, dichloromethane, 1,2-dichloroethane were tried for the extraction of base form of the methdilazine. Only dichloromethane favoured the extraction of the drug to its base form. In order to select a suitable solvent for preparation of the reagent solutions used in the study, the reagents were prepared separately in different solvents such as 1,4-dioxane, chloroform, acetonitrile, acetone, 2-propanol and dichloromethane, and the reaction of MDZ with DDQ, DNP or PA was followed. In method A, 1,4-dioxane was best suited for preparation of DDQ solution. The dichloromethane solvent was found to be the ideal solvent for preparation of both DNP and PA for method B and method C, respectively. Similarly, the effect of the diluting solvent was studied for all methods and the results showed that the ideal diluting solvent to achieve maximum sensitivity was acetonitrile in method A and dichloromethane in method B and method C.

Effect of Reaction Time and Stability of the C-T Complexes

The optimum reaction times were determined by measuring the absorbance of the complex formed upon the addition of reagent solution to MDZ solution at room temperature. The reaction of MDZ with DDQ in method A and DNP in method B was instantaneous while complete colour development was attained after 5 min with PA. The absorbance of the resulting C-T complexes remained stable for at least 1 h for method A, 2 h in method B and more than 3 h in method C (Fig. 4).

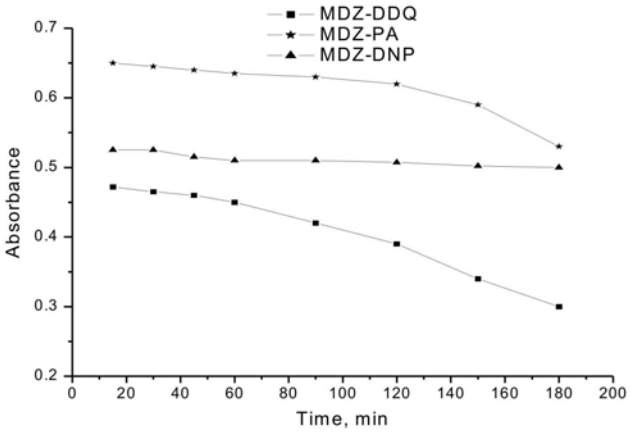


Fig. 4: Effect of time on formed complex between drug and dyes. (MDZ-DDQ complex, 32 µg ml^{-1} MDZ, Method A), (MDZ-DNP complex, 20 µg ml^{-1} MDZ, Method B) and (MDZ-PA complex, 16 µg ml^{-1} MDZ, Method C)

Composition of the C-T Complexes

The composition of the C-T complex was established by Job's method of continuous variations [34] using equimolar concentrations of the drug (base form) and reagents (3.48 x 10^{-4} M in method A, 2.68 x 10^{-4} M in method B and 2.26 x 10^{-4} M in method C). The results indicated that 1:1 (drug:reagent) complex is formed in all methods. Five solutions containing MDZ and the reagent (DDQ, DNP or PA) in various molar ratios, with a total volume of 5 mL in all methods were prepared. The absorbance of solutions was subsequently measured at 585 nm in method A, 420 in method B and 425 nm in method C. The graphs of the results obtained (Fig. 5) gave a maximum at a molar ratio of x_{max} = 0.5 in all the methods which indicated the formation of a 1:1 C-T complex between MDZ and reagent (DDQ, DNP or PA).

Method Validation

The proposed methods were validated for linearity, sensitivity, selectivity, accuracy, precision,

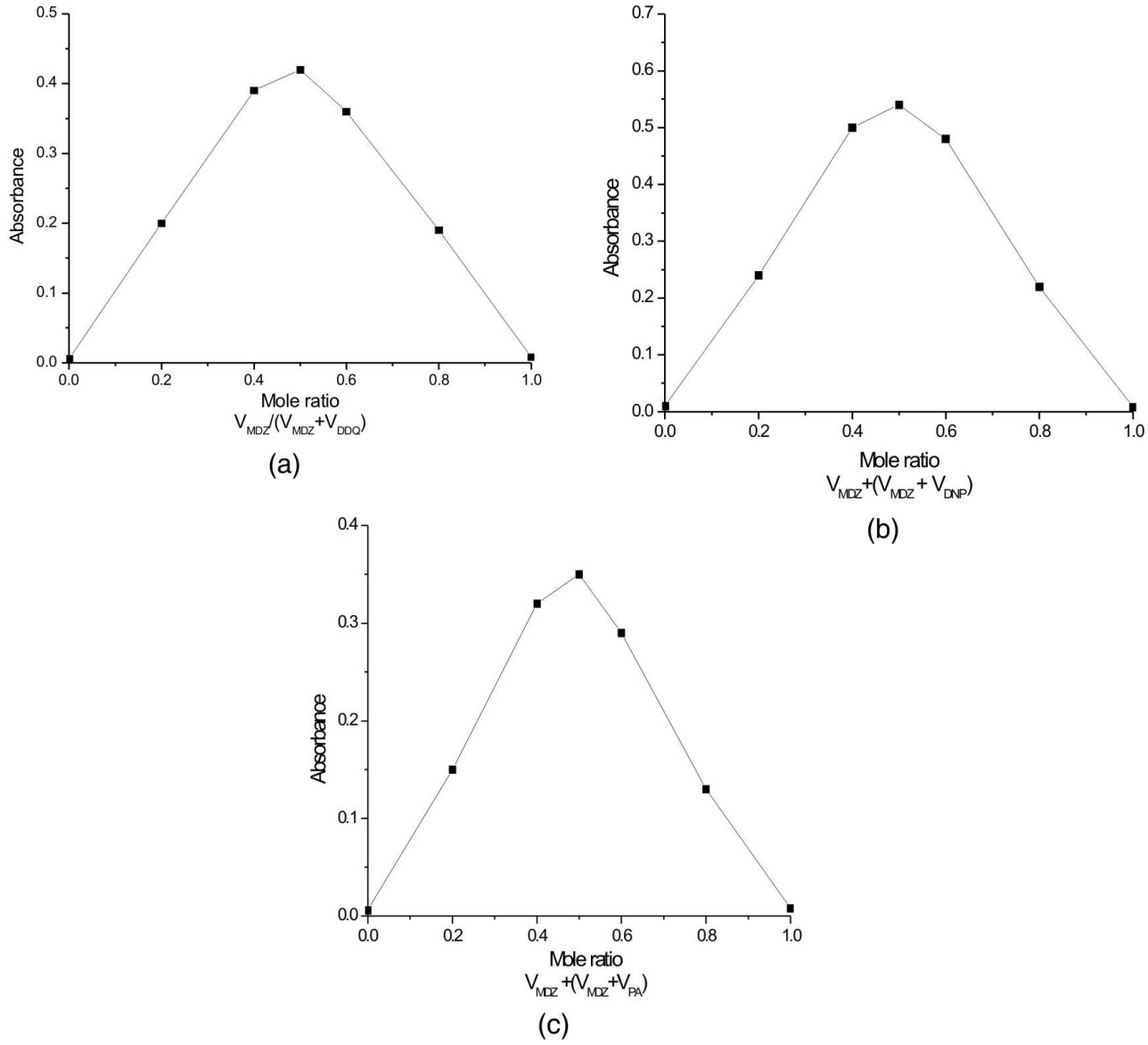


Fig. 5: Job's plots obtained for (a) 3.48 x 10^{-4} M MDZ and DDQ C-T complex; (b) 2.68 x 10^{-4} M MDZ and DNP C-T complex and (C) 2.26 x 10^{-4} M MDZ and PA C-T complex

robustness, ruggedness and recovery according to the current ICH guidelines [35].

Linearity and Sensitivity

Under the optimum conditions a linear relation was obtained between absorbance and concentration of MDZ in the ranges given in Table 2 (Fig. 6). 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 2. 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 [34] and compiled in Table 2. LOD and LOQ were calculated according to the same guidelines using the following formulae:

$$LOD = \frac{3.3 \times \sigma}{S} \text{ and } LOQ = \frac{10 \times \sigma}{S}$$

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

working range) were prepared and analyzed in seven replicates during the same day (intra-day precision) and on five consecutive days (inter-day precision) and the results are presented in Table 3. The percentage relative error (RE%) was ≤ 2.85 which indicates that the accuracy of the methods is

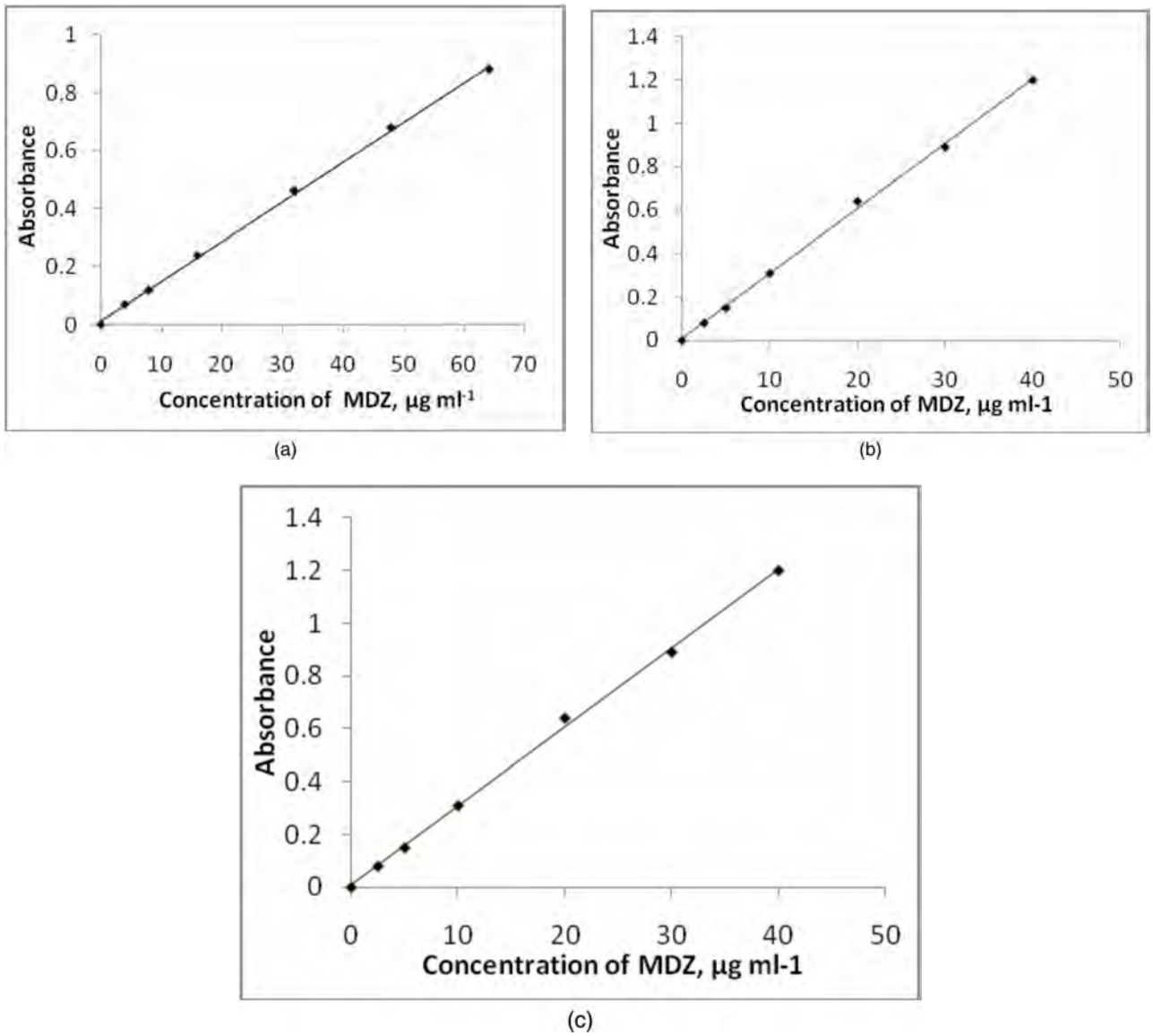


Fig. 6: Calibration curves (a): Method A, (b): Method B and (c) Method C

Accuracy and Precision

In order to determine the accuracy and precision of the proposed methods, pure drug (MDZ) solution at three different concentration levels (within the

satisfactory. Percentage relative standard deviation (RSD %) for intra-day was ≤ 1.23 and for inter-day it was ≤ 2.01 indicating repeatability and usefulness of the proposed methods in the routine analysis.

Table 2: Sensitivity and regression parameters

Parameter	Method A	Method B	Method C
λ_{max}, nm	585	420	425
Linear range, μg ml^{-1}	4-64	2.5-40	2-32
Color stability, hrs	1	2	3
Molar absorptivity (ε), l mol^{-1} cm^{-1}	4.9×10^3	1.0×10^4	1.0×10^4
Sandell sensitivity[a], μg cm^{-2}	0.0668	0.0325	0.0324
Limit of detection (LOD), mg ml^{-1}	1.62	0.53	0.61
Limit of quantification (LOQ), mg ml^{-1}	3.62	1.59	1.85
Regression equation, Y^b			
Intercept (*a*)	0.0018	0.0106	0.0215
Slope (*b*)	0.0822	0.0298	0.0352
Standard deviation of intercept (S_a)	0.01078	0.01050	0.09599
Standard deviation of slope (S_b)	0.00870	0.00745	0.00445
Regression coefficient (*r*)	0.9997	0.9992	0.9989

[a]Limit of determination as the weight in μg per ml 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; [b]$Y = a + bX$, where, Y is the absorbance, X is concentration in μg ml^{-1}.

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

Method	MDZ taken μg ml^{-1}	Intra-day accuracy and precision (*n*=5)			Inter-day accuracy and precision (*n*=5)		
		MDZ found μg ml^{-1}	%RE	%RSD	MDZ found μg ml^{-1}	%RE	%RSD
A	16.0	16.24	1.78	1.25	16.24	1.89	1.75
	32.0	31.78	0.36	0.96	31.78	0.87	1.36
	48.0	48.39	1.31	1.14	48.39	1.52	1.42
B	10.0	10.23	2.32	1.18	10.23	2.45	1.26
	20.0	20.38	1.93	1.97	20.38	2.08	2.07
	30.0	30.28	0.95	1.57	30.28	1.25	1.67
C	8.0	7.94	0.73	1.35	7.94	0.97	1.13
	16.0	15.70	1.86	1.47	15.70	1.95	1.89
	24.0	23.59	1.66	1.74	23.59	2.06	1.94

RE-Relative error and RSD-Relative standard deviation

Selectivity

The selectivity of the proposed methods for the analysis of MDZ was evaluated by placebo blank and synthetic mixture analyses. The recommended procedures were applied to the analysis of placebo blank and the resulting absorbance readings in all methods were the same as that of the reagent blank, confirming that there is no interference from the placebo. The analysis of synthetic mixture solution prepared as described earlier yielded percent recoveries of 98.3±2.13, 99.1±1.76 and 98.9±1.91 (n = 5) for method A, method B and method C, respectively. The results of this study showed that the inactive ingredients did not interfere in the assay indicating the high selectivity of the proposed method and applicability to use for routine determination in pure and in tablet form.

Robustness and Ruggedness

To evaluate the robustness of the methods, two important experimental variables, *viz*., volume of reagent in all the methods and reaction time in method C, were altered incrementally and the effect of this change on the absorbance of the C-T complexes was studied. The results of this study are presented in Table 4 and they indicated that the proposed methods

Table 4: Robustness and ruggedness expressed as intermediate precision (%RSD)

Method	MDZ taken,	Method robustness Parameters altered μg ml^{-1}		Method ruggedness	
		Dye, ml[a] RSD, % (*n*=3)	Time, min[b] RSD, % (*n*=3)	Inter-analysists' RSD, % (*n*=4)	Inter-instruments' RSD, % (*n*=3)
A	16.0	1.30		1.46	1.79
	32.0	1.49	-	1.72	2.02
	48.0	1.26		1.39	2.27
B	10.0	1.56		1.71	1.92
	20.0	1.29	-	1.68	2.24
	30.0	1.08		1.28	1.58
	8.0	1.49	1.82	1.84	2.16
C	16.0	1.04	1.12	1.16	1.79
	24.0	1.54	1.64	1.59	2.52

[a]Dye (DDQ, DNP and PA) volumes used were 0.8, 1.0 and 1.2 ml; [b]Reaction time were 4.0, 5.0 and 6.0 min

are robust. Method ruggedness was evaluated by performing the analysis following the recommended procedures by three different analysts and on three different spectrophotometers by the same analyst. From the %RSD values presented in Table 4, one can conclude that the proposed methods are rugged.

Application to Analysis of Tablets

The proposed methods were successfully applied to the determination of MDZ in tablets and syrup and the results are compiled in Table 5. The results obtained were statistically compared with those obtained by the reference method [3] by applying the Student's t-test for accuracy and F-test for precision at 95% confidence level. The reference method consisted of measurement of the absorbance of aqueous solution of MDH at 252 nm. As can be seen from the Table 5, the calculated t- and F-values at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, for four degrees

Table 5: Results of analysis of tablets by the proposed methods

Tablet/syrup Brand name	Label claim*	Found[a] (Percent of label claim ±SD)			
		Reference method	Proposed methods		
			A	B	C
Dilosyn tablet[b]	8	100.17±0.61	100.42±1.22 **t** =0.82 **F**=4.0	101.04±1.13 **t** =1.51 **F** =3.41	99.52±0.99 **t** =1.83 **F** =2.63
Dilosyn syrup[b]	4	98.58±0.87	99.34±1.37 **t**=1.14 **F**=2.47	98.66±1.28 **t** =0.89 **F**=2.16	99.16±1.31 **t** =1.74 **F** =2.26

*mg/tablet in tablets, mg/5 ml in syrup; [a] Mean value of five determinations; [b]Glaxo-Smithkline Pharmaceuticals Ltd.; The value of t and F (tabulated) at 95 % confidence level and for four degrees of freedom are 2.77 and 6.39, respectively

Table 6: Results of recovery study for tablets and injection by standard addition method

Method	Tablet (T)/syrup(S) studied	MDZ in tablet µg ml^{-1}	Pure MDZ added µg ml^{-1}	Total found µg ml^{-1}	Pure MDZ recovered* Percent ± SD
A		24.04	12.0	35.93	99.16 ± 1.78
	Dilosyn T	24.04	24.0	48.41	101.55 ± 1.31
		24.04	36.0	61.01	102.69± 1.24
		23.95	12.0	35.97	101.53 ± 2.26
	Dolosyn S	23.95	24.0	47.71	101.89 ±1.55
		23.95	36.0	60.56	102.71 ± 0.62
B		14.98	7.5	22.57	101.26 ± 1.42
	Dilosyn T	14.98	15.0	29.87	99.28 ± 1.18
		14.98	22.5	37.20	98.76 ± 0.85
		14.94	7.5	22.43	99.93 ± 1.41
	Dolosyn S	14.94	15.0	29.79	99.03 ±0.44
		14.94	22.5	37.01	98.10± 1.63
C		12.04	6.0	18.05	100.20 ± 2.34
	Dilosyn T	12.04	12.0	24.31	102.26 ± 1.60
		12.04	18.0	29.95	99.50 ± 0.82
		11.93	6.0	17.88	99.25 ± 2.31
	Dolosyn S	11.93	12.0	23.82	99.12 ± 0.74
		11.93	18.0	29.66	98.52 ± 2.12

*Mean value of three determinations.

of freedom. This indicates that there are no significant differences between the proposed methods and the reference method with respect to accuracy and precision.

Recovery Study

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet and syrup was spiked with pure MDZ at three concentration levels (50, 100 and 150 % of that in tablet/syrup) and the total was analyzed by the proposed methods. The results of this study are presented in Table 6 and indicate that the excipients present in the tablets did not interfere in the assay.

Conclusions

This paper presents three visible spectrophotometric methods for the quantitative determination of methdilazine in pure drug and its formulations. The proposed methods are based on charge-transfer complexation reaction, and have the advantages of simplicity, speed, accuracy and precision, and use of inexpensive equipment compared to the reported methods. The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Whereas most of the reported methods rely on the use of multiple reagents/reactions, the proposed methods employ a single reagent/reaction, with minimal manipulation which results in considerably increased precision. The DDQ method is less sensitive than both DNP and PA methods as can be seen from the lower molar absorptivity value. The methods are characterized by wide linear dynamic ranges and high sensitivity compared to many existing spectrophotometric methods. Moreover, the proposed methods are free from the usual analytical complications like heating or extraction steps and can be performed at room temperature. Thus, the proposed methods are useful for the quality control and routine analysis of MDH in pharmaceuticals since there is no interference from the common excipients that are usually found in commercial tablets.

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