RESEARCH ARTICLE

Simple, Sensitive and Eco-Friendly Methods for the Determination of Methdilazine in Tablets and Syrup Using Cerium (IV)

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Simple, Sensitive and Eco-Friendly Methods for the Determination of Methdilazine in Tablets and Syrup Using Cerium (IV)

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Abstract
One titrimetric and two spectrophotometric methods, which are simple, rapid, cost-effective and eco-friendly, are described for the determination of methdilazine hydrochloride (MDH) in bulk drug, tablet and syrup formulations based on the oxidation of MDH by Cerium (Ce) (IV). In titrimetry (method A), the acidified solution of MDH is titrated directly with Ce(IV) using ferroin as indicator. The spectrophotometric methods are based on oxidation-reduction reaction involving MDH and Ce(IV), and the resulting Ce(III) is complexed with either arsenazo(III) at pH 7.8±1.0 and absorbance measured at 620 nm (method B) or chromotrope 2R at pH 2.5±0.8 and absorbance measured at 530 nm (method C). Under optimized experimental conditions, titrimetric procedure is applicable over the range of 3-15 mg of MDH, and the reaction stoichiometry is found to be 1:2 (MDH:Ce(IV)). The spectrophotometric methods are applicable over the ranges of 0.4-10.0 µg mL^{-1} (method B) and 0.4-12 µg mL^{-1} (method C). The molar absorptivities are calculated to be 3.4×10^4 and 2.8×10^4 L $mol^{-1}cm^{-1}$ for method B and method C, respectively, and the corresponding Sandell sensitivity values are 0.0096 and 0.0118 µg cm^{-2}. The limits of detection are calculated and found to be 0.12 and 0.38 µg mL^{-1} for method B and method C, respectively, with corresponding limits of quantification of 0.09 and 0.71. The methods were applied to the determination of MDH in tablets and syrup, and the results were compared statistically with those of a reference method.

Keywords: Methdilazine; determination; titrimetry; spectrophotometry; Ce(IV); Ce(III); pharmaceuticals.

1. Introduction

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

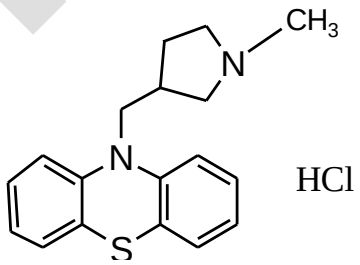


Figure 1: Structure of MDH.

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 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 a 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^{o}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 reaction 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 color 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 color 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].

Two reports were found in the literature for the assay of MDH using titrimetry. Basavaiah and Charan reported two methods in which MDH was treated with a known excess of vanadate in acid medium and the unreacted oxidant was back titrated with iron (II) using N-phanylanthranilic acid indicator. In a slightly different procedure, vanadate (IV) produced in the redox reaction was titrated with Ce(IV) using ferroin indicator. The same authors reported another method, involving the oxidation of MDH by a known excess KIO_3 followed by the determination of unreacted oxidant by iodometric back titration [16].

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 titrimetric methods reported earlier [14, 16] are indirect and time-consuming since they require a standing time of 15 min. The reported spectrophotometric methods also suffer from one or the other disadvantage as narrow linear range, poor sensitivity, dependence on critical experimental variables, tedious and time-consuming extraction/heating step, and/or use of expensive reagent or large amounts of organic solvents as indicated in Table 1.

The present work is aimed at developing and validating simple, rapid, sensitive and selective titrimetric and spectrophotometric methods employing Ce(IV) as the oxidimetric agent, Arsenazo (III) and chromotrope 2R as chromogenic agents. In titrimetry (method A), the acidified solution of MDH is titrated directly with Ce(IV) to a visual end point, and the spectrophotometric methods involve the addition of a known excess of Ce(IV) MDH followed by the determination of the resulting Ce(III) by complexing with either ARS(III) and measuring the absorbance at 530 nm (method B) or C2R and measuring absorbance at 620 nm (method C). The cited dyes have earlier been employed for the assay of several drug substances [23-25] and they are widely used for the determination of elements by complexation reactions [26, 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 step	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 b) Vanadium (IV), Ferrin	Unreacted metavanadate measured Ferroin measured	 510	0-75 ($.95\times10^{4}$) 5-25 (5.31×10^{4})	Longer contact time, moderately sensitive	15
7	a) KIO_3,Variamine blue b) KIO_3, NaCl, dichloroflurescein c) KIO_3, CCl_4	Unreacted iodate measured Iodinated dye measured Liberated iodine extracted to CCl_4 measured	540 525 520	1.25-8.75 (25.26×10^{3}) 5.0-60.0 (2.65×10^{3}) 50-600 (2.19×10^{2})	Requires heating, tedious pH control Requires critical pH control Requires extraction step, less sensitive	16
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 Bromothymol blue	Chloroform extractable ion-pair complex measured	485 420	2-35 (1.07×10^{4}) 1-18 (0.13×10^{4})	Requires critical pH, liquid-liquid extraction	19
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 Turbidity of suspension measured	420 650	2-16 10-70	Extraction step, tedious pH control	21
13	Cobaltthiocyanate	Benzene extractable ion-pair complex measured	620	50-500	Requires critical pH control	22
14	a) Ce (IV)-Arsenazo (III) b) Ce (IV-Chromotrope 2R	Measurement of Ce(III)-Arsenazo (III) complex Measurement of Ce(III)-Chromotrope 2R) complex	620 530	0.4-10.0 (3.4×10^{4}) 0.8-12.0 (2.8×10^{4})	Highly sensitive with wide linear dynamic ranges, stable, selective, no heating or extraction and inexpensive instrumental setup.	This work

2. Methods

2.1. Instrumentation

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements. An Elico 120 digital pH meter was used to measure pH.

2.2. Materials and reagents

All the reagents used were of analytical-reagent grade and distilled water was used throughout the investigation. Methdilazine hydrochloride certified to be 99.85% pure was obtained from Glaxo Laboratories, Mumbai, India. Dilosyn 8 mg tablet and Dilosyn syrup (GlaxoSmithkline Pharmaceuticals Ltd.) were purchased from local commercial sources.

2.2.1. Ce(IV) sulphate (0.01M)

The solution was prepared by dissolving 2.02 g of the chemical (Loba Chemie, Mumbai, India, assay 99.9%) in 15 mL of conc. H_2SO_4 and diluting to volume in a 500 mL calibrated flask with water, mixed well, filtered using glass wool, standardized [28] and used in titrimetric work and diluted appropriately with 0.5 M sulphuric acid to yield 1000 µg ml^{-1} Ce(IV) solution for use in both spectrophotometric methods.

Sulphuric acid (5 M): Prepared by appropriate dilution of the conc. H_2SO_4 (Merck, Mumbai, India; sp. gr. 1.84) with water.

2.2.2. Ferroin indicator

Prepared by dissolving 0.742 g of 1,10-phenanthroline monohydrate in 50 mL of 0.025 M ferrous sulphate solution (0.348 g of ferrous sulphate heptahydrate in 50 mL water).

2.2.3. Arsenazo(III) (0.025%)

Prepared by dissolving 0.025 g of dye (S.D. Fine Chem, Mumbai, India) in 50 ml water of water, shaken for 15 min and made upto mark in 100 ml volumetric flask with water.

2.2.4. Chromotrope 2R (0.02%)

Prepared by dissolving 0.023 g of dye (Loba Chemie, Mumbai, India, assay 85%) in water and made upto mark in 100 ml volumetric flask with water.

2.2.5. Borax 0.1 M

An amount (3.81g) of the chemical (S.D. Fine Chem, Mumbai, India) was dissolved in water, transferred into a 100 ml calibrated flask and diluted to the mark with water.

2.2.6. Walpole buffer of pH 2.5

A buffer solution of pH 2.5 was prepared by mixing 50 ml of 0.1 M sodium acetate (Merck, Mumbai, India) and 50 ml of 0.1 M HCl (Merck, Mumbai, India; sp. gr. 1.18) and the pH was adjusted with a pH meter using these solutions.

2.2.7. Preparation of stock solution

A stock standard solution equivalent to 1.5 mg mL^{-1} of MDH was prepared by dissolving accurately weighed 150 mg of pure drug in water, and diluted to the mark in a 100 mL calibrated flask and used in titrimetric work (method A). Another stock solution equivalent to 200 µg mL^{-1} of MDH was prepared by dissolving accurately weighed 20 mg of pure drug in water and diluting to the mark in a 100 ml calibrated flask. This was diluted appropriately with water to get working concentration 20 µg mL^{-1} MDH for use in both spectrophotometric methods (method B and method C).

2.3. Procedures

2.3.1. Method A (Titrimetry)

A 10 mL aliquot of pure drug solution containing 3–15 mg of MDH was accurately measured and transferred into a 100 mL titration flask, 5 mL of 5 M H_2SO_4 were added, and titrated with 0.01 M Ce(IV) sulphate using 1 drop of ferroin as indicator until the sky blue color appeared. A blank titration was performed and necessary volume corrections were made. The amount of the drug in the measured aliquot was calculated from

$$Amount\ (mg) = VM_wR/n$$

where V = volume of Ce(IV) consumed, mL; M_w = relative molecular mass of the drug; and R = molarity of Ce(IV) and n = number of moles of Ce(IV) reacting with each mole of MDH.

2.3.2. Spectrophotometric Method B using arsenazo(III)

Different aliquots (0.2, 0.5, 1.0, ----5.0 mL) of a standard 20 μg mL^{-1} MDH solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 5 mL by adding adequate quantity of water. To each flask were added 1 mL of 1000 μg mL^{-1} Ce(IV) solution followed by 1.0 mL of 0.1M borax. Finally, 1 mL of 0.025% Ars(III) dye was added and the volume was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 620 nm against a reagent blank after 5 min.

2.3.3. Spectrophotometric Method C (using chromotrope 2R)

Varying aliquots (0.2, 0.5, 1.0, ----6.0 mL) of a standard 20 μg mL^{-1} MDH solution were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 6 mL by adding water. To each flask were added 1 mL 1000 μg mL^{-1} Ce(IV) solution followed by 1 mL of buffer of pH 2.5. Finally, 1 mL of 0.02% C2R dye was added and the volume was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 530 nm against a reagent blank after 5 min.

In both methods, a standard graph was prepared by plotting the increasing absorbance values *vs* concentration of MDH. The concentration of the unknown was read from the standard graph or computed from the respective regression equation derived using the Beer's law data.

2.3.4. Procedure for tablet

Thirty tablets (Dilosyn 8 mg) were weighed and pulverized. An amount of the powder equivalent to 150 mg of MDH was accurately weighed into a 100 mL volumetric flask, 60 mL water was added and content shaken thoroughly for about 20 min. The volume was diluted to the mark with water, mixed well and filtered using Whatman No. 42 filter paper. First 10 mL portion of the filtrate was rejected and a convenient aliquot of filtrate (containing 1.5 mg mL^{-1} MDH) was taken for assay by titrimetric procedure. The tablet extract was diluted stepwise to get 20 μg mL^{-1} MDH concentration for use in both spectrophotometric methods. A suitable aliquot was then subjected to analysis following the procedures described earlier.

2.3.5. 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) equivalent to 150 mg MDH were quantitatively transferred into a separating funnel. The content was rendered alkaline to litmus paper with 6N 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 evaporated to dryness. The residue was dissolved in HCl and made upto mark with water in 100 ml volumetric flask. The convenient aliquot of the resulting solution was taken for the assay in titrimetric method and diluted appropriately to their respective concentration and assayed using a convenient aliquot in both spectrophotometric methods.

2.3.6. Placebo blank analysis

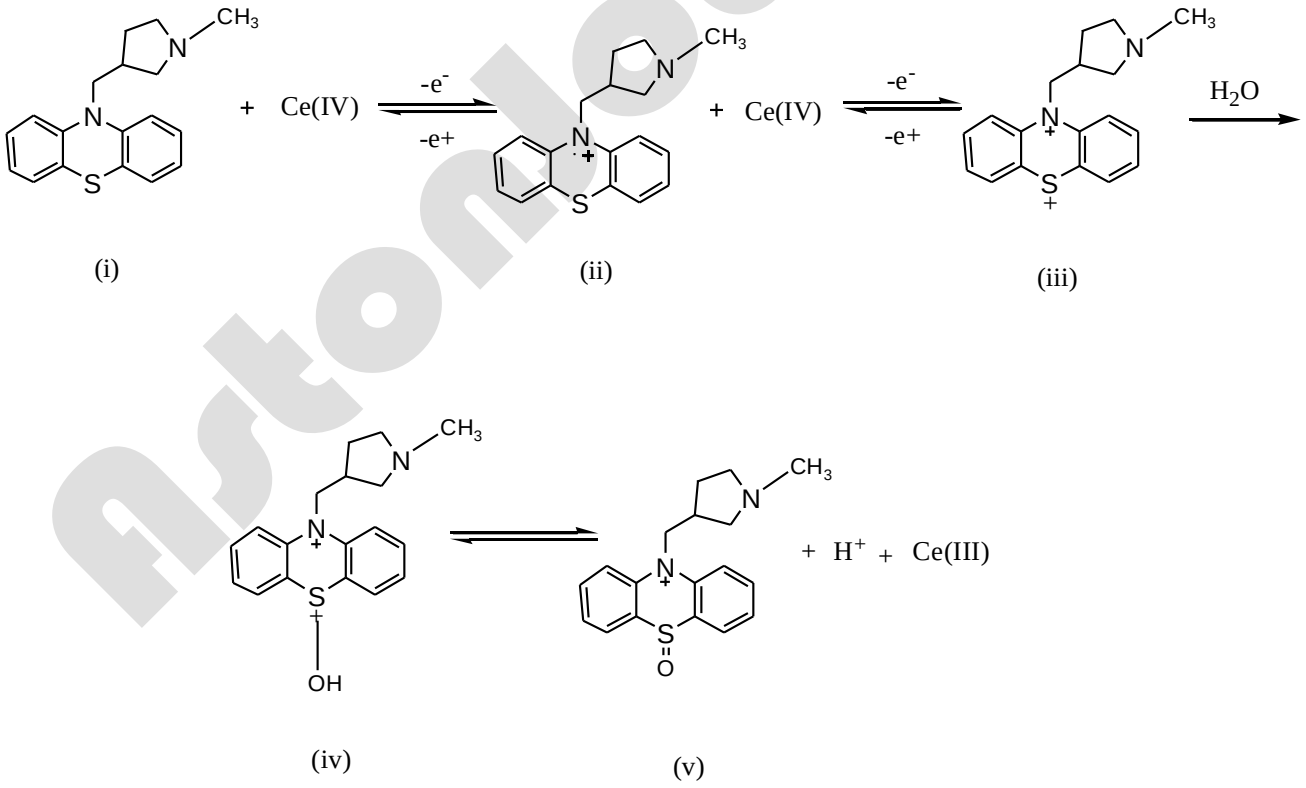
A placebo blank of the composition: talc (35 mg), starch (25 mg), acacia (25 mg), methyl cellulose (30 mg), sodium citrate (25 mg), magnesium stearate (25 mg) and sodium alginate (20 mg) was prepared and its solution prepared by taking 20 mg as described under the procedure for tablets, and then analysed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

2.3.7. Procedure for the determination of MDH in synthetic mixture

To 100 mg the placebo blank of the composition described above, 150 mg of MDH was added homogenized, and transferred to a 100 mL calibration flask, and solution was prepared as described under tablets. The solution was mixed well and filtered using a Whatman no. 42 filter paper. The resulting solution was assayed (n = 5) by titrimetry according to the procedure described above. The synthetic mixture solution (1.5 mg mL^{-1} MDH) was then diluted stepwise with water to obtain working concentrations of 20 µg mL^{-1} MDH for spectrophotometric methods. A convenient aliquot was then subjected to analysis by using the procedures described above.

3. Results and Discussion

Methdilazine is reported to undergo oxidation with different oxidizing reagents such as metavanadate [14, 15] and KIO_3 [16]. MDH being a N-substituted phenothiazine derivative undergoes two step oxidation by Ce(IV) in sulphuric acid medium to a colorless sulphoxide [29]. Also, Ce(IV) has been used as an oxidimetric reagent for the assay of many oxidizable pharmaceutical substances [30-33]. In addition, Ce(III) has been reported to produce colored complex with ARS(III) and C2R at particular pH [34, 35]. These observations have been used to develop three methods using titrimetric and spectrophotometric techniques for the determination of MDH in tablets and syrup based on the oxidation of MDH by Ce(IV) in acid medium in titrimetry. In spectrophotometry, Ce(III), the reduced form of Ce(IV), was reacted with ARS(III) in method B , and C2R in method C at selected pH to form highly colored complexes having absorption maxima at 620 and 530 nm, respectively. Scheme 1 represents the most probable detailed mechanism of MDH (i) oxidation process. The first is the reversible reaction involving the loss of an electron giving a colored semiquinoid free radical (ii). This free radical loses another electron giving the colorless phenothiazonium ion (iii). Compound (iii) is hydrolysed to phenothizone derivative (iv) and sulphoxide (v) and Ce(IV) is reduced to Ce(III). The resulting Ce(III) is utilized to form colored complex with the cited dyes in spectrophotometric methods.



Scheme 1: Probable reaction pathway for reaction between MDH and Ce(IV) and reduction of Ce(IV) to Ce(III).

3.1. Titrimetry

MDH was found to react with Ce(IV) in sulphuric acid medium. H_2SO_4 medium was favored over to HCl and $HClO_4$ medium since the reaction was found to yield a regular stoichiometry in the concentration range studied. Reproducible and regular stoichiometry was obtained when 1.15–2.05 M H_2SO_4 concentration was maintained. Hence, 5 mL of 5 M H_2SO_4 solution in a total volume of 15 mL (1.66 M H_2SO_4 overall) was found to be the most suitable concentration for the quantitative reaction between MDH and Ce(IV). Under the optimized reaction condition, there was found to be a definite reaction stoichiometry of 1:2 between MDH and Ce(IV) within the range of 1.5-15 mg of MDH.

3.2. Spectrophotometry

Methdilazine, a phenothiozine derivative, has been used as a reducing agent for the determination of Se(IV) [36] and a fluorophore for the assay of propranolol hydrochloride and piroxicam [37]. In the present work, re-dox reaction of MDH with Ce(IV) results in the formation of Ce(III) which in turn reacts with arsenazo(III) or chromotrope 2R to yield colored complexes.

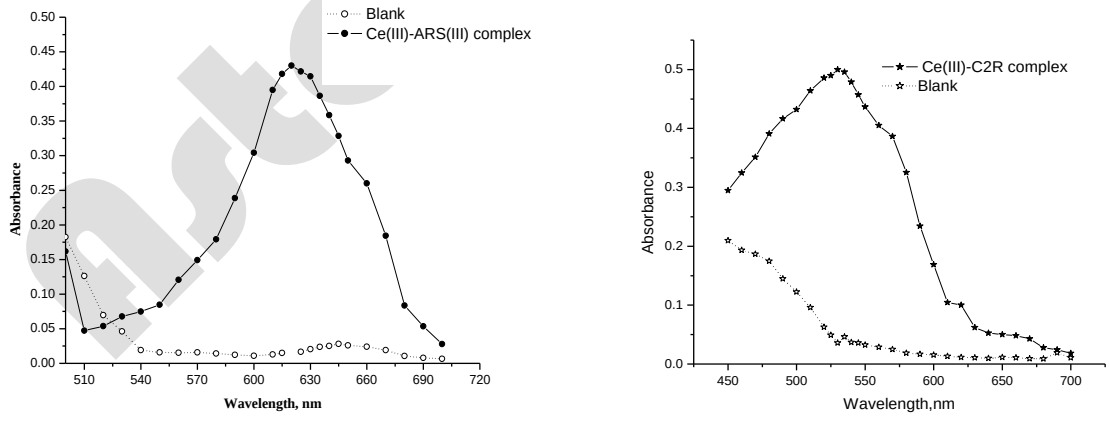
MDH undergoes oxidation with Ce(IV) under acidic condition to form red colored radical cation then to colorless sulphoxide and Ce(III) formed was determined by formation of greenish-blue colored complex with arsenazo(III) at pH 7.8±1.0 having absorption maxima at 620 nm in method B. In method C, Chromotrope 2R was used as an chromogenic agent to form complex with Ce(III) at pH 2.5±0.8 giving reddish-pink color which absorbs maximally at 530 nm. The amount of Ce(III) formed was found to be proportional to the amount of MDH serving as basis for its quantification.

MDH, when added in increasing concentrations, consumes Ce(IV) proportionally and consequently there will be concomitant increase in Ce(III) concentration. This is observed as a proportional increase in the absorbance of the coloured species with increasing concentration of MDH and fixed concentration of reagent. The increasing absorbance values at 620 nm in method B and at 530 nm in method C were plotted against the concentration of MDH to obtain the calibration graph.

3.3. Method development: Optimization of experimental variables

3.3.1. Absorption spectra

Absorption spectrum of the greenish-blue colored complex formed by Ce(III) with arsenazo(III) at pH 7.8±1.0 *vs* reagent blank shows maximum absorption at 620 nm in method B as can be seen from figure 2a. The reddish-pink complex formed between Ce(III) and Chromotrope 2R at pH 2.5±0.8 in method C absorbs maximally at 530 nm against its corresponding reagent (blank) as can be seen in Figure 2b.



(a) (b)

Figure 2: Absorption spectra of formed complex between: a) Ce(III)-ARS(III) (6 μg/mL MDH) and b) Ce(III)-C2R(6 μg/mL MDH).

3.3.2. Effect of pH

The reaction between Ce(III) and the cited dyes is found to be pH dependent. The effect of pH on the absorbance of colored products was investigated by carrying out the reaction in buffer solutions of different pH. In method B,

when the pH of the buffer used was ≤ 6.8, the colored product formed was found to be unstable with continuous decrease in the absorbance and increase in blank solution absorption after some time. When buffer of pH 7.8±1.0 used, the blank solution showed negligible absorbance, and the absorbance of the colored product was stable. In method C, when the pH of the buffer used was ≤ 1.7, the colored species formed was unstable but gave stable colored product at pH 2.5±0.8. In order to achieve high sensitivity for assay of MDH, buffers of pH 7.8 and 2.5 was selected as the optimal experimental condition in method B and method C, respectively.

3.3.3. Effect of type of buffer solution

In method B, the effect of different buffers of pH 7.8 such as KH_2PO_4/NaOH, Kolthoff buffer (KH_2PO_4/borax), KH_2PO_4/Na_2HPO_4 and 0.1 M borax was studied. We found that the KH_2PO_4/NaOH buffer was not suitable as it resulted in least absorbance of the colored species compared with all buffers tested making the method less sensitive. The remaining buffers used were found suitable for the assay but the color stability and sensitivity of the measured species were more with 0.1 M borax. In method C, Na_2HPO_4/citric acid, Walpole buffer (CH_3COONa/HCl) and potassium biphthalate/HCl were studied. Among them, CH_3COONa/HCl buffer was found suitable for the assay with high stability and sensitivity of the complex. Therefore, 0.1 M borax and CH_3COONa/HCl buffer of pH 2.5 was selected and used for the assay in method B and method C, respectively.

3.3.4. Effect of volume of buffer solution

The effect of amount of buffer solution on the absorbance of the measured species (6 µg ml^{-1} MDH in both methods) was studied using different volumes (0.0, 0.25, ... 2.0 ml) of 0.1 M borax in method B, and CH_3COONa/HCl buffer of pH 2.5 in method C. We found that in both the methods, the absorbance of the colored products increased with increasing volume of buffer from 0.25-1.00 ml (Figure 3) and any extra volume of the buffer (1.00 ml < volume < 2.00 ml) had no effect on the absorbance of the measured species. So, 1.0 ml of 0.1 M borax and CH_3COONa/HCl buffer of pH 2.5 was fixed and used for the assay of MDH in both methods B and C, respectively.

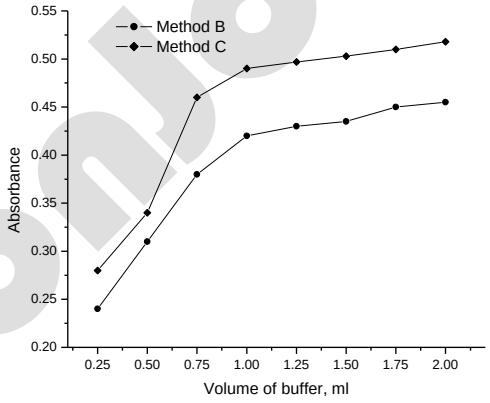


Figure 3: Effect of volume of buffer on the complex formation.

3.3.5. Effect of concentration of Ce(IV) on oxidation of MDH

Following the assay procedure for both methods, the absorbance of the reaction product of a fixed concentration of MDH (6 µg ml^{-1} MDH in both methods) with different volumes (0.5, 1.0, …… and 3.0 ml) of 1000 µg ml^{-1} was measured against the corresponding blank. It was found that the absorbance of the colored reaction product showed maximum with 0.5-2.0 ml of 1000 µg ml^{-1} Ce(IV) ion. Therefore, 1.0 ml of 1000 µg ml^{-1} Ce(IV) ion in a total volume of 10.0 ml was selected as the optimum to oxidize MDH and subsequent formation of Ce(III).

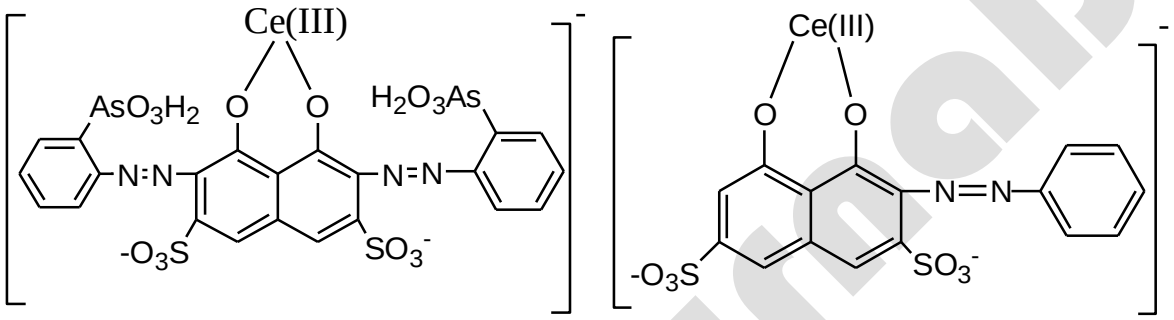
3.3.6. Effect of volume of dye, and reaction time, and stability of the complex

In order to determine the optimum amount of dye required to obtain maximum absorbance, experiments were performed separately by measuring the absorbance of the final solution resulting from the reaction mixture

containing a fixed concentration of MDH, Ce(IV) and various amounts of the dye. It was found that 1 ml of dye solution (0.025% arsenazo(III) in method B, 0.02% Chromotrope 2R in method C) was sufficient to produce maximum and reproducible absorbance. Oxidation of MDH as well as formation of complex between Ce(III) and dyes was instantaneous, and absorbance of the resulting complexes was found to be stable for at least 6 h in method B and 3 h in method C at room temperature (28±2 °C).

3.3.7. Reaction stoichiometry

From the titrimetric method, the reaction between MDH and Ce(IV) is found to have the stoichiometry ratio of 1:2 (drug:Ce(IV)). In spectrophotometric methods, the reaction between Ce(III) and the cited dyes follows a stoichiometric ratio of 1:1 as can be seen from the literature [23, 38, 39]. On this basis probable structure of the formed complex between Ce(III) and arsenazo(III)/Chromotrope 2R is shown in Scheme 2.



(a) (b)

Scheme 2: Probable structure of the complex: a) Ce(III)-ARS(III) and b) Ce(III)-C2R.

3.4. Validation of the proposed methods

The proposed methods were validated for linearity, sensitivity, selectivity, precision, accuracy, robustness and ruggedness and recovery.

3.4.1. Linearity

A linear relation is found between absorbance and concentration of MDH within the Beer's law range given in Table 2. The calibration graphs are described by the equation:

$$Y = a + bX$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in µg/mL) obtained by the method of least squares. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values and the limits of detection and quantification are calculated as per the current ICH guidelines [40] which are compiled in Table 2 that speaks of the excellent sensitivity of the proposed method. Limits of detection (LOD) and quantification (LOQ) were calculated from the following equations.

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

where σ is the standard deviation of "n" reagent blank determinations and S is the slope of the calibration curve.

3.4.2. Accuracy and precision

In order to study the precision and accuracy of the proposed methods, three amounts/concentrations of pure MDH within the linearity range were analyzed, each determination being repeated seven times (intra-day precision) on the same day and one time each for five days (inter-day precision). The percentage relative standard deviation (%RSD) was ≤ 1.73% (intra-day) and ≤ 2.76% (inter-day). In addition, the accuracy of the proposed method was measured by calculating the percentage relative error (%RE), which varied between 0.68% and 2.95%. The results of this study compiled in Table 3 indicate the high accuracy and precision of the proposed methods.

Table 2: Sensitivity and regression parameters.

Parameter	Method B	Method C
λ_{max}, nm	620	530
Color stability, hr	6	3
Linear range, µg/mL	0.4-10	0.4-12
Molar absorptivity(ε), L/mol·cm	3.4×10^4	2.8×10^4
Sandell sensitivity*, µg cm^{-2}	0.0096	0.0118
Limit of detection (LOD), µg/mL	0.12	0.24
Limit of quantification (LOQ), µg/mL	0.38	0.71
Regression equation, Y**		
Intercept (a)	0.0082	0.0050
Slope (b)	0.1006	0.0828
Standard deviation of intercept (S_a)	0.3758	0.2879
Standard deviation of slope (S_b)	0.0767	0.0645
Regression coefficient (r)	0.9997	0.9995

*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. **Y=a+bX, where Y is the absorbance, X is concentration in µg/ml.

3.4.3. Robustness and ruggedness

To evaluate the robustness of the methods, two important experimental variables, *viz*., the amount of acid (in titrimetry) and volume of reagent and buffer (in spectrophotometry), were slightly varied, and the capacity of all the methods was found to remain unaffected by small deliberate variations. The results of this study are presented in Table 4 and indicate that the proposed methods are robust. Method ruggedness is expressed as %RSD of the same procedure applied by three analysts and using three different burettes in titrimetry and cuvettes in spectrophotometry by the same analyst. The inter-analysts' and inter-cuvettes/burettes' RSD values were ≤ 3.28% indicating ruggedness of the proposed methods. The results of this study are presented in Table 4.

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

Method	MDH taken*, mg/µg/mL	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
		MDH found, µg/mL	%RE	%RSD	MDH found, mg/µg/mL	%RE	%RSD
A	4.5	4.56	1.33	1.24	4.60	2.22	1.93
	9.0	9.21	2.34	1.64	9.27	2.95	2.63
	13.5	13.85	2.56	1.78	13.89	2.89	2.83
B	2.0	2.02	1.06	0.93	2.03	1.51	2.04
	4.0	4.04	1.04	1.17	4.06	1.53	2.37
	6.0	5.95	0.68	0.63	5.92	1.35	1.96
C	4.0	3.97	0.70	1.22	3.91	2.25	2.76
	6.0	5.91	1.44	1.38	5.88	2.02	2.68
	8.0	8.06	0.78	1.29	8.08	1.13	2.29

%RE: Percent relative error, %RSD: Relative standard deviation, n = Number of measurements.
*mg in method A, and µg/mL in method B and method C.

3.4.4. Selectivity

In the present methods, interference by the excipients often used in pharmaceutical formulations or as possible co-active substances was studied. Selectivity was evaluated by both placebo blank and synthetic mixture analyses. The placebo blank, consisting of the composition as mentioned under "Analysis of Placebo blank" was prepared

and analyzed as described under the recommended procedures. The resulting absorbance readings for the methods were same as the reagent blank, inferring no interference from the placebo. The selectivity of the methods was further confirmed by carrying out recovery study from synthetic mixture. The percent recoveries of MDH were 102.1±1.35 for method A, 98.7±1.18 and 101.4±1.63 for method B and method C, respectively. This confirms the selectivity of the proposed methods in the presence of the commonly employed tablet excipients.

Table 4: Method robustness and ruggedness expressed as intermediate precision (%RSD).

Method	MDH taken*	Robustness (%RSD)	Ruggedness	
			Inter-analysts (%RSD), (n=4)	Inter-cuvettes/burettes (%RSD), (n=4)
Titrimetry (method A)	4.5	1.65	1.84	2.78
	9.0	2.15	2.47	3.28
	13.5	1.98	2.72	2.67
Spectrophotometric (method B)	2.0	0.85	1.28	2.58
	4.0	1.06	1.10	1.96
	6.0	1.28	1.42	2.85
Spectrophotometric (method C)	4.0	1.34	1.58	3.15
	6.0	0.92	1.76	2.74
	8.0	0.66	1.32	3.08

In titrimetry, volume of H_2SO_4 added was 5±0.5 mL.
The volume of reagent added was 1±0.1 mL and buffer 1±0.1 mL.
*mg in titrimetry and µg mL^{-1} in spectrophotometry.

3.4.5. Application to analysis of tablets and syrup

The proposed methods were successfully applied to the determination of MDH in tablets and syrup. The results presented in Table 5 showed that there was a close agreement between the results obtained by the proposed methods and the label claim. The results were also compared with those of the reference method [3] statistically by a Student's *t*- test for accuracy and variance ratio *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. The calculated *t*- and *F*-values indicate that there is no significant difference between the proposed methods and the reference method with respect to accuracy and precision.

Table 5: Results of analysis of tablets and syrup by the proposed methods and statistical comparison of the results with the reference method.

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	101.13± 1.22 t=2.37 F=5.26	101.04± 1.13 t=1.78 F=4.57	99.52± 0.99 t=2.64 F=3.9
Dilosyn syrup[b]	4	98.58±1.37	98.34±1.37 t=2.4 F=4.74	98.66 ±1.28 t=2.5 F=5.76	99.16 ± 1.31 t=1.8 F=4.78

*mg/tablet in tablet, mg/5mL 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.

3.4.6. Recovery studies

To further ascertain the accuracy of the proposed methods, a standard addition technique was followed. A fixed amount of drug from pre-analyzed tablet powder/syrup was taken and pure drug at three different levels (50, 100 and 150 % of that in tablet powder/syrup content) was added. The total was found by the proposed methods. The determination at each level was repeated three times and the percent recovery of the added standard was calculated. Results of this study presented in Table 6 reveal that the accuracy of methods was unaffected by the various excipients present in the formulations.

4. Conclusion

This paper presents one titrimetric and two spectrophotometric methods for the assay of methdilazine hydrochloride in bulk and in its pharmaceutical formulations. The assay results demonstrate that it is possible to use Ce(IV) in titrimetry and spectrophotometry, which is stable enough and need no special precautions during storage or use, as a reagent for determination of MDH in bulk drug and pharmaceuticals. The proposed titrimetric method is direct, simple, rapid, free from critical experimental variables and is superior the reported titrimetric methods [14, 16] which involve the indirect titrimetric assay which is time consuming and multi-step reaction. The proposed spectrophotometric methods are free from the usual analytical complications like heating or extraction steps. Moreover, the methods are accurate, reproducible, adequately sensitive and free from interference by common additives and excipients. The proposed methods rely on the use of simple, eco-friendly, cheap, and readily available chemicals and techniques and they have been demonstrated to be free from interference by common tablet excipients and additives.

Table 6: Results of recovery study *via* standard-addition method with tablet/injection.

Method	Tablet studied	MDH in tablet mg/µg/mL	Pure MDH added mg/ µg/mL	Total found µg/mL	Pure MDH recovered* Percent ± SD
A	Dilosyn tablet	4.55	2.25	6.96	102.35±1.65
		4.55	4.50	9.28	102.54±1.35
		4.55	6.75	11.63	102.92±1.57
	Dilosyn Syrup	4.42	2.25	6.53	97.90±1.24
		4.42	4.50	8.75	98.09±1.38
		4.42	6.75	10.96	98..03±1.16
B	Dilosyn tablet	3.04	1.5	4.55	101.25 ± 0.48
		3.04	3.0	6.02	99.53 ± 1.57
		3.04	4.5	7.57	100.81 ± 1.17
	Dilosyn Syrup	2.96	1.5	4.43	98.55 ± 0.81
		2.96	3.0	5.93	99.26± 0.58
		2.96	4.5	7.50	100.91 ± 0.84
C	Dilosyn tablet	4.03	2.0	6.01	99.13 ± 1.73
		4.03	4.0	8.05	100.73 ± 0.94
		4.03	6.0	10.11	101.42 ± 1.25
	Dilosyn Syrup	3.97	2.0	5.98	100.60 ± 2.19
		3.97	4.0	7.94	99.38 ± 0.92
		3.97	6.0	9.90	9.186± 0.67

*Mean value of three determinations.

Competing Interests

None declared.

Authors' Contributions

Both authors contributed equally to this work.

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