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*Short communication*

SHORT COMMUNICATION

# Optimized and validated spectrophotometric methods for the determination of hydroxyzine hydrochloride in pharmaceuticals and urine using iodine and picric acid

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*Abstract*: Two simple, rapid, cost-effective and sensitive spectrophotometric procedures are proposed for the determination of hydroxyzine dihydrochloride (HDH) in pharmaceuticals and in spiked human urine. The methods are based on the charge transfer complexation reaction of the drug with either iodine ($I_2$) as a σ-acceptor (method A) in dichloromethane or picric acid (PA) as a π--acceptor (method B) in chloroform. The coloured products exhibit absorption maxima at 380 and 400 nm for $I_2$ and PA, respectively. The Beer Law was obeyed over the concentration ranges of 1.25–15 and 3.75–45 μg $mL^{-1}$ for method A and B, respectively. The molar absorptivity values, Sandell sensitivities, limits of detection (*LOD*) and quantification (*LOQ*) are reported. The accuracy and precision of the methods were evaluated on intra-day and inter-day basis. The proposed methods were successfully applied for the determination of HDH in tablets and spiked human urine.

*Keywords*: hydroxyzine dihydrochloride; determination; spectrophotometry; charge-transfer complexation; pharmaceuticals; spiked urine.

## INTRODUCTION

Hydroxyzine dihydrochloride (HDH), chemically known as 2-[2-[4-[(4-chlorophenyl)phenymethyl]-1-piperazinyl]ethoxy]ethanol, dihydrochloride (Fig. 1), is an $H_1$ receptor antagonist. It is widely used as an anxiolytic agent to control anxiety[1] and anti-emetic.[2]

The therapeutic importance of HDH initiated several reports on its determination both in pharmaceutical formulations and in biological fluids, *viz*. high-performance liquid chromatography,[3,4] gas chromatography,[5] micellar liquid chromatography,[6] capillary zone electrophoresis,[7] voltammetry,[8] LC–MS,[9] potentio-

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metry[10] and titrimetry.[11–14] The United States Pharmacopoeia (USP)[15] describes an HPLC method for the determination of HDH in tablets in which HDH is detected at 232 nm with a UV-detector.

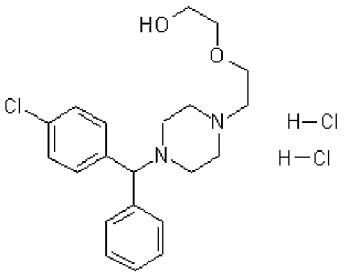


Fig. 1. Structure of hydroxyzine dihydrochloride.

Few visible spectrophotometric methods[12,16–18] have been reported for the determination of HDH in pharmaceuticals. In the method reported by Basavaiah and Charan,[12] different concentrations of the drug are added to a fixed concentration of Hg(II)-diphenylcarbazone complex and the decrease in the absorbance of the Hg(II)-diphenylcarbazone complex, as a consequence of the replacement of diphenylcarbazone in the complex by chloride from the drug, was measured at 540 nm and the method is reported to be applicable in the range 0–60 μg $mL^{-1}$. Sane *et al.*[16] used three dyes for the determination of the drug content in pharmaceuticals, the method being based on ion-pair formation. Marzanna *et al.*[17] employed Reinecke salt for the assay of the drug. A method based on the charge transfer complex formation reaction with chloranilic acid[18] in acetonitrile was also reported. The method was less sensitive and HDH could be determined only in the concentration range 25–150 μg $mL^{-1}$.

The aim of the present study was directed at developing and validating two simple, rapid, sensitive and cost-effective spectrophotometric methods based on charge-transfer (C-T) complexation reactions using iodine and picric acid as σ- and π-acceptors, respectively. In the first method (method A), the drug is reacted with iodine in dichloromethane to form a lemon yellow coloured C-T complex with an absorption maximum peaking at 380 nm. The second method (method B) employs picric acid as a π-acceptor to form a yellow C-T complex with the drug in chloroform and the absorbance of the complex is measured at 400 nm. The methods were successfully applied to quantify HDH in pharmaceutical formulations and in spiked human urine. The results obtained were satisfactorily precise and accurate.

## EXPERIMENTAL

### *Apparatus*

All absorption measurements were realised using a Systronics Model 106 digital spectrophotometer with 1 cm path length quartz cells.

*Materials*

Pharmaceutical grade HDH was procured from UCB Pharma Ltd., Mumbai, India, as a gift, and was used as received. Tablets containing HDH, Atarax 25 and Atarax 10 (UCB Pharma Ltd., Mumbai, India) were purchased from the local market and used in the investigation. The urine sample was collected from a healthy volunteer (Male, around 27-year-old) and kept frozen until use after gentle thawing. Chloroform and dichloromethane (spectroscopic grade) were purchased from Merck, Mumbai, India. Distilled water was used wherever required. All other employed chemicals were of analytical reagent grade.

*Reagents*

A 0.5 % iodine solution was prepared by dissolving 1.25 g of pure resublimed iodine (S.D. Fine Chemicals Ltd., Mumbai, India) in 250 mL of dichloromethane and used after 30 min. Picric acid (0.2 %) was prepared by dissolving 500 mg of pure substance (S.D. Fine Chemicals Ltd., Mumbai, India) in 250 mL of chloroform. To prepare 0.5 mol $L^{-1}$ sodium hydroxide solution, an accurately weighed 2 g of pure NaOH (Merck, India) was dissolved in water, the solution was made up to 100 mL with water.

*Standard drug solution*

A 250-μg $mL^{-1}$ solution of HDH was prepared by dissolving accurately weighed 25 mg of pure HDH in water and the volume was brought to 100 mL with water and mixed well.

*General recommended procedures*

*Preparation of HDH base (HDN) solution.* Method A: HDH solution (10 mL, 250 μg $mL^{-1}$) was transferred to a 125 mL separating funnel containing 10 mL of a 0.5 mol $L^{-1}$ NaOH solution and the content was mixed well. The hydroxyzine base was extracted with three 15 mL portion of dichloromethane, the extract was passed over anhydrous sodium sulphate and collected in a 100 mL volumetric flask, the volume was made up to the mark with dichloromethane and the resulting solution (25 μg $mL^{-1}$ HDN) was used for the assay in method A.

Method B: HDN solution (75 μg $mL^{-1}$) was prepared by repeating the above procedure for 30 mL of pure HDH solution (250 μg $mL^{-1}$) but chloroform was used instead of dichloromethane for extraction. The resulting solution was used for the assay in method B.

*Construction of the calibration curves.* Method A: Varying aliquots of standard HDN solution equivalent to 1.25–15 μg $mL^{-1}$ (0.25 – 3.0 mL of 25 μg $mL^{-1}$) were accurately measured and transferred into a series of 5 mL calibrated flasks and 2 mL of 0.5 % iodine solution was added to each flask, the content was mixed well and the flasks were allowed to stand at room temperature (30±2 °C) for 15 min. The volume was brought to the mark with dichloromethane and the absorbance was measured at 380 nm against a reagent blank similarly prepared but without the addition of HDN base solution.

Method B. Into a series of 5 mL calibration flasks, aliquots (0.25–3.0 mL) of standard HDN solution (75 μg $mL^{-1}$) equivalent to 3.75–45 μg $mL^{-1}$ HDN were accurately transferred, and to each flask 1 mL of 0.2 % picric acid solution was added and the mixture was diluted to 5 mL with chloroform. After 5 minutes, the absorbance of the yellow coloured C-T complex was measured at 400 nm against the reference blank similarly prepared.

*Procedure for commercial dosage forms*

Twenty tablets were weighed and pulverized. The amount of tablet powder equivalent to 25 mg of HDH was transferred into a 100 mL volumetric flask. The content was shaken well with about 50 mL of water for 20 min, diluted to the mark with water and filtered through Whatmann No. 42 filter paper. The first 10 mL portion of the filtrate was discarded. HDN so-

lutions of concentrations 25 and 75 μg $mL^{-1}$ for method A and B, respectively, were prepared as described under the general procedures for pure drug and a suitable aliquot was used for assay by applying the procedures described earlier.

*Procedure for spiked human urine*

A spiked urine sample was prepared by adding 20 mg of pure HDH to 10 mL of urine in a separating funnel. Twenty mL of water was added, followed by 10 mL of 0.1 M NaOH solution and the mixture was extracted with three 15 mL portions of dichloromethane. The organic layer was passed over anhydrous sodium sulphate and collected in a 50 mL volumetric flask. The solution was made to the mark with dichloromethane and mixed well. The obtained solution was diluted appropriately to obtain a working concentration of 25 μg $mL^{-1}$ HDN. A 1–2 mL portion of the solution was then subjected to analysis as described for the pure drug in method A. For method B, the required volume of the base solution in dichloromethane was evaporated to dryness. The residue was dissolved in chloroform and diluted with chloroform to a specific volume to obtain a working concentration of 75 μg $mL^{-1}$ HDN. A portion of the solution (*e.g.*, 2 mL) was used for the analysis by following the procedure described earlier.

## RESULTS AND DISCUSSION

Molecular interactions between electron donors and electron acceptors are generally associated with the formation of intensely coloured charge-transfer complexes, which absorb radiation in the visible region.[19] Charge transfer complexation reactions have been extensively utilized for the determination of electron-donating basic nitrogenous compounds using either an σ-acceptor (iodine)[20,21] or a π-acceptor (picric acid).[19,22–27] The application of picric acid for the quantitative estimation of orphendrine citrate and phentolamine mesylate injections listed in the USP.[28]

*Spectral characteristics*

HDN, an *n*-donor (D), forms a lemon yellow-coloured C-T complex with iodine ($I_2$) (σ-acceptor) in dichloromethane and the resulting coloured species was found to absorb maximally at 380 nm (Fig. 2). The colour of iodine in dichloromethane is violet showing absorption maximum ($\lambda_{max}$) at 500 nm. This colour changed into lemon yellow when the iodine was mixed with the drug, and showed an absorption peak at 380 nm (Fig. 2). HDN reacts with picric acid in chloroform medium to yield a yellow-coloured C-T complex peaking at 400 nm (Fig. 3). The interactions between HDN and acceptors to form charge transfer complexes follows according to Schemes 1 and 2.

*Optimization of the reaction conditions*

Optimum conditions were established by measuring the absorbance of C-T complexes at 380 and 400 nm, for method A and B, respectively, by varying one parameter at a time and keeping the others constant.

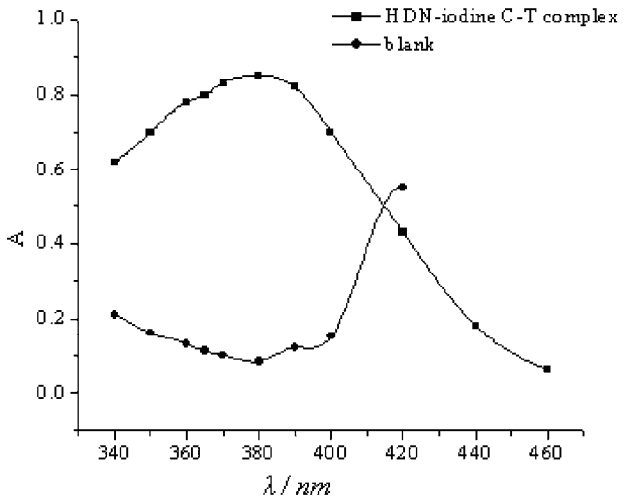


Fig. 2. Absorption spectra of the HDN–$I_2$ C-T complex (15 μg $mL^{-1}$ HDN) and the reagent blank.

$$D + I_2 \rightleftharpoons \underset{\text{Outer complex}}{D\text{-}I^+\,I^-} \rightleftharpoons \underset{\text{Inner complex}}{[D\text{-}I^+]^- + I^+} \overset{+I_2}{\rightleftharpoons} \underset{\text{Tri-iodide ion-pair}}{I_3^-}$$

Scheme 1. Reaction pathway for the formation of the C-T complex between HDN and $I_2$ in dichloromethane

$$D^{\cdot} + A \rightleftharpoons \underset{\text{Complex}}{(D\text{-}A)} \rightleftharpoons \underset{\text{radical ions}}{D^{\cdot+} + A^{\cdot-}}$$

Scheme 2. Reaction pathway for the formation of the electron donor–acceptor complex and radical ions between HDN and picric acid.

*Effect of reagent concentration*. To establish optimum amounts of the reagents for the sensitive and rapid formation of the HDN charge transfer complexes, the drug (HDN) was allowed to react with different volumes of the reagents (1–4 mL of 0.5 % iodine and 1–3 mL of 0.2 % picric acid in method A and B, respectively). At 2 mL of iodine and 1 mL of picric acid, maximum and minimum absorbance values with respect to the sample and blank were obtained for method A and B, respectively. Therefore, 2 mL of 0.5 % iodine and 1 mL of 0.2 % picric acid in a total volume of 5 mL were used throughout the following investigations.

*Choice of solvent*. Acetonitrile, chloroform, dichloromethane, ethanol, 2-propanol, methanol, benzene and 1,4-dioxane were the evaluated solvents. Dichloromethane was found to be the ideal solvent for $I_2$ and showed superior properties compared to the other solvents, since it is favourable for the formation of the tri-

iodide ion-pair (inner complex). With $I_2$, the other solvents produced lower absorption readings for the sample and higher absorption values for the blanks. In method B, chloroform afforded the maximum sensitivity when compared with all the investigated solvents. Hence, dichloromethane and chloroform were used as solvents for method A and B, respectively.

*Reaction time and stability*. Complete colour development of the C-T complex was attained after 15 and 5 min in method A and B, respectively, and the coloured species were stable for at least 1 and 16 h, respectively.

*Composition of the C-T complexes*

The composition of the C-T complex was established by the Job method of continuous variations[29] using equimolar concentrations of the drug (base form) and reagents ($4.28\times10^{-4}$ mol $L^{-1}$ in method A and $2.43\times10^{-4}$ mol $L^{-1}$ in method B). The results indicated that a 1:1 (drug:reagent) complex is formed in both cases.

*Method validation*

A linear correlation was found between the absorbance at $\lambda_{max}$ and the concentration of HDH in the ranges given in Table I. Regression analysis of the Beer Law data using the least squares method was performed to evaluate the slope (*b*), intercept (*a*) and the correlation coefficient (*r*) for each system and the values are presented in Table I. The optical characteristics such as the limits of the Beer Law, molar absorptivity and the Sandell sensitivity values of both methods are also given in Table I. In addition, the limits of detection (*LOD*) and quantification (*LOQ*), calculated according to ICH guidelines,[30] are also presented in Table I.

TABLE I. Sensitivity and regression parameters

| Parameter | Method A | Method B |
|---|---|---|
| $\lambda_{max}$ / nm | 380 | 400 |
| Colour stability, h | 1 | 16 |
| Linear range, μg $mL^{-1}$ | 1.25–15 | 3.75–45 |
| Molar absorptivity (ε), L $mol^{-1}$ $cm^{-1}$ | $2.59 \times 10^4$ | $8.06 \times 10^3$ |
| Sandell sensitivity, μg $cm^{-2}$ | 0.0172 | 0.0556 |
| *LOD* / μg $mL^{-1}$ | 0.13 | 0.62 |
| *LOQ* / μg $mL^{-1}$ | 0.39 | 1.88 |
| Regression equation, $Y^a$ | | |
| Intercept (*a*) | 0.0093 | 0.0113 |
| Slope (*b*) | 0.0565 | 0.0174 |
| Standard deviation of *a* ($S_a$) | 0.0998 | 0.0998 |
| Standard deviation of *b* ($S_b$) | 0.0095 | 0.0030 |
| Regression coefficient (*r*) | 0.9993 | 0.9995 |

[a] $Y = a + bX$, where *Y* is the absorbance, *X* is concentration in μg $mL^{-1}$, *a* is the intercept and *b* is the slope

*Precision and accuracy*

For three levels of analyte, the assays were repeated seven times within a day to determine the intra-day precision and five times on different days to determine the inter-day precision of the methods. The percentage relative standard deviation (*RSD*) values were ≤2.29 % (intra-day) and ≤2.98 % (inter-day), indicating the high precision of the methods. The accuracy was evaluated as the percentage relative error (*RE*) between the measured mean concentrations and the taken concentrations for HDN. The RE values of ≤2.40 % demonstrate the high accuracy of the proposed methods.

*Robustness and ruggedness*

The robustness of the methods was evaluated by making small incremental changes in the volume of reagent (2±0.2 mL of $I_2$; 1±0.2 mL of PA) and contact time (5±2 min in method B), and the effect of the changes was studied on the absorbance of the complex systems. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % *RSD* (≤3 %). Method ruggedness was demonstrated by having the analysis performed by four analysts, and by a single analyst performing the analysis on four different instruments in the same laboratory. The intermediate precision values (*RSD*) in both instances were in the range 1.62–3.16 %, indicating acceptable ruggedness.

*Applications*

*Commercial tablets*. The proposed methods were applied for the quantification of HDH in commercial tablets. The results were compared with those obtained by the US Pharmacopoeial method.[15] Statistical analysis of the results did not detect any significant difference between the performance of the proposed methods and reference method with respect to accuracy and precision as revealed by the Student's *t*-value and variance ratio *F*-value. The results of the assay are given in Table II.

TABLE II. Results of analysis of tablets by the proposed methods and statistical comparison of the results with the reference method

| Tablet brand name | Nominal amount mg/tablet | Found[a] (Percent of label claim ± *SD*) | | |
|---|---|---|---|---|
| | | Reference method | Method A | Method B |
| Atarax 25 | 25 | 99.16±0.88 | 100.6±1.36<br>$t$ = 2.03[b]<br>$F$ = 2.39[c] | 101.3±1.56<br>$t$ = 2.77<br>$F$ = 3.14 |
| Atarax 10 | 10 | 101.6±1.2 | 100.6±0.96<br>$t$ = 1.46<br>$F$ = 1.56 | 99.62±1.09<br>$t$ = 2.73<br>$F$ = 1.21 |

[a]Mean value of 5 determinations; [b]tabulated *t*-value at the 95 % confidence level and for four degrees of freedom is 2.77; [c]tabulated *F*-value at the 95 % confidence level and for four degrees of freedom is 6.39

*Recovery from spiked human urine*. As another application of the proposed methods, recovery from human urine samples was performed. A prior extraction step, according to Mehran *et al*.[31] was used. The recovery studies were realised with samples containing various amounts of HDN. The results of recovery studies revealed that the other constituents present in the urine did not interfere in the method. The recovery values were in the range 97.2–108.6 %.

*Recovery from tablet powder*. The recovery test was performed by spiking the pre-analyzed tablet powder with pure HDH at three different levels (50, 100 and 150 % of the content present in the tablet powder (taken) and the total was found by the proposed methods. Each test was repeated three times. In all the cases, the recovery values ranged between 98.40 and 105 %, with a standard deviation in the range 1.04–1.85 %. The closeness of the results to 100 % showed the fairly good accuracy of the methods.

## CONCLUSIONS

Two simple, sensitive, extraction-free, rapid and cost-effective spectrophotometric methods based on charge transfer complex formation reactions for the determination of HDH were developed and validated. The suggested methods utilize a single step reaction and single solvent. No substantial differences among the proposed methods arose from analysis of the experimental results. The methods are free from interferences from the common excipients and additives. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. These methods, which can be used as general methods for the determination of HDH in bulk powder, dosage forms and spiked human urine, have many advantages over the separation techniques, *e.g*., HPLC, such as reduced cost, and speed with high accuracy. Hence, the methods can be used in the routine analysis of drugs in quality control laboratories.

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## ИЗВОД

## ОПТИМИЗОВАНА И ВАЛИДИРАНА МЕТОДА ЗА СПЕКТРОФОТОМЕТРИЈСКО ОДРЕЂИВАЊЕ ХИДРОКСИЗИН-ХИДРОХЛОРИДА У ФАРМАЦЕУТСКИМ ПРЕПАРАТИМА И УРИНУ УЗ КОРИШЋЕЊЕ ЈОДА И ПИКРИНСКЕ КИСЕЛИНЕ

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Предложене су две економичне и осетљиве спектрофотометриске процедуре за одређивање хидроксизин-дихидрохлорида (HDH) у фармацеутским препаратима и оптерећеном урину. Метода се заснива на трансферу наелектрисдања комплексометријске реакције у леку са јодом као σ-акцептором (метода А) у дихлор-метану и са пикринском киселином као π-ак-

цептором (метода Б) у хлороформу. Обојени производи имају апсорпционе максимуме на 380 and 400 nm за $I_2$ и пикринску киселину. Сагласност са Beer-овим законом је постигнута у опсегу концентрација 1,25–15 и 3,75–45 μg $mL^{-1}$, за обе методе. Дате су вредности моларне апсорптивности, Санделовог индекса, граница детекције и квантификације и евалуирана тачност и прецизност методе. Предложене методе су успешно примењене за одређивање HDH у таблетама и оптерећеном хуманом урину.

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