

Sensitive and Selective Extractive Spectrophotometric Method for the Determination of Hydroxyzine Dihydrochloride in Pharmaceuticals

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Abstract. Hydroxyzine dihydrochloride (HDH), a piperazine H_1 -receptor antagonist and antihistamine, is a rapid acting anxiolytic used principally as an anti-emetic. A sensitive, selective, and precise and accurate spectrophotometric method based on the formation of an ion-pair with orange II (ORG II) as ion-pair complexing agent was developed and validated for the determination of HDH in pharmaceuticals. The chloroform-extractable ion-pair complex exhibited an absorption maximum at 480 nm. Optimization of different experimental conditions is described. Beer's law is obeyed in the range of $1.5\text{--}15\text{ }\mu\text{g mL}^{-1}$ with an apparent molar absorptivity value of $2.07 \times 10^4\text{ L mol}^{-1}\text{ cm}^{-1}$ and Sandell's sensitivity value of $0.0216\text{ }\mu\text{g cm}^{-2}$. The limit of detection (LOD) and limit of quantification (LOQ) are 0.14 and $0.41\text{ }\mu\text{g mL}^{-1}$, respectively. A Job's plot of absorbance *versus* molar ratio of HDH to ORG II indicated (1:2) stoichiometric ratio. Within- and between-day relative standard deviations at three different concentration levels were $< 3\%$. The developed method was successfully applied to commercial tablets. The results obtained were in good agreement with those obtained using the official method. No interference was encountered from co-formulated substances. Recoveries were 96-109 %.

Keywords: Spectrophotometry, hydroxyzine, orange II, pharmaceuticals.

Resumen. El dihidrocloruro de hidroxicina (HDH), una piperazina antagonista del receptor H_1 y un antihistamínico, es un ansiolítico de acción rápida usado principalmente como anti-emético. Se desarrolló y validó un método espectrofotométrico sensible, selectivo, preciso y exacto basado en la formación de un par iónico con naranja II (ORG II) como un agente complejante para la determinación de HDH en productos farmacéuticos. El par iónico extraído en cloroformo exhibió un máximo de absorción a 480 nm. Se describió la optimización de las diferentes condiciones experimentales. La ley de Beer se obedeció en el intervalo de $1.5\text{--}15\text{ }\mu\text{g mL}^{-1}$ con un valor de absorptividad molar aparente de $2.07 \times 10^4\text{ L mol}^{-1}\text{ cm}^{-1}$ y un valor de sensibilidad de Sandell de $0.0216\text{ }\mu\text{g cm}^{-2}$. El límite de detección (LD) y el límite de cuantificación (LC) son 0.14 y $0.41\text{ }\mu\text{g mL}^{-1}$, respectivamente. Una gráfica de Job de absorbancia *versus* relación molar de HDH y ORG II indicó una relación estequiométrica (1:2). Las desviaciones estándar relativas dentro y entre días, a tres diferentes niveles de concentración, fueron $< 3\%$. El método desarrollado fue aplicado exitosamente a tabletas comerciales. Los resultados obtenidos estuvieron en buena concordancia con los obtenidos con el método oficial. No se encontró ninguna interferencia de parte de las sustancias de la formulación. Las recuperaciones fueron de 96-109 %.

Palabras clave: Espectrofotometría, hidroxicina, naranja II, productos farmacéuticos.

Introduction

Hydroxyzine dihydrochloride (HDH), chemically known as 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]ethanol, dihydrochloride, is a piperazine H_1 -antagonist with sedative properties. It is widely used in the control of anxiety [1] and also used principally as an anti-emetic [2, 3].

A variety of analytical techniques such as high-performance liquid chromatography [4-8], gas chromatography [9], thin layer chromatography [10], micellar liquid chromatography [11], capillary zone electrophoresis [3], voltammetry [12], LC-MS [13], potentiometry [14, 15], gravimetry [16] and titrimetry [17-22] have been reported for the determination of HDH in biological fluids and pharmaceuticals. The official USP method available for the assay of the drug in tablets employs a chromatographic system equipped with a UV-detector, where HDH can be detected at 232 nm [23].

In the literature, four visible spectrophotometric methods [18, 24-26] are found for the quantitative determination of HDH in pharmaceuticals. In the method reported by Basavaiah and Charan [18], to a fixed concentration of Hg(II)-diphenylcarbazone complex different amounts of drug were added and the

decrease in the absorbance of Hg(II)-diphenylcarbazone complex, consequent to the replacement of diphenylcarbazone of the complex by the chloride of the drug, was measured at 540 nm and the method is applicable in the range $0\text{--}60\text{ }\mu\text{g mL}^{-1}$. Sane et al [24] have used 3 dyes for the determination of the drug content in pharmaceuticals. Kurzawa et al [25] employed the spectrophotometric procedure for HDH by using reinecke salt. One of the present authors has also reported a method based on the charge transfer complex formation reaction with chloranilic acid [26] in acetonitrile.

The reported visible spectrophotometric methods though seem simple, they are less sensitive [18, 26], some are critically dependent on pH [24] and the reaction products either require long time to form or less stable [25] (Table 1).

The present work describes a simple, sensitive and selective spectrophotometric method for the determination of HDH in pharmaceuticals. The method is based on the measurement of the red coloured chloroform extractable ion-pair complex formed between HDH and ORG II in H_2SO_4 medium. The HDH-ORG II ion-pair complex is highly stable and the ion-pair formed and extracted in acid medium without requiring rigid pH control.

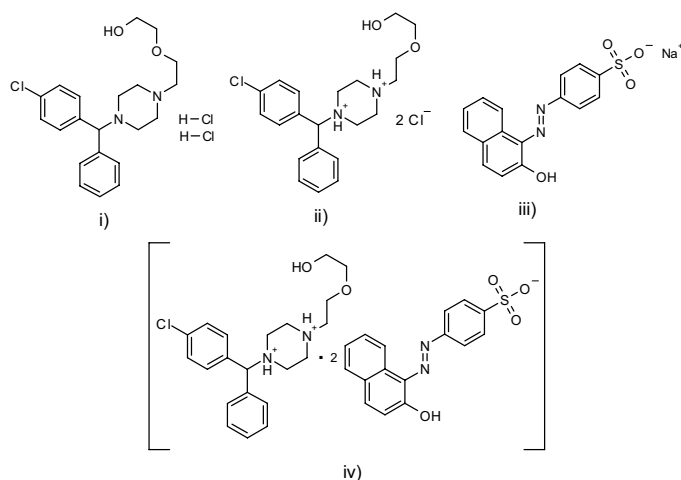
Table 1. Comparison of the performance characteristics of the proposed method with reported visible spectrophotometric methods.

Sí. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range ($\mu\text{g mL}^{-1}$) (ϵ in $\text{L mol}^{-1} \text{cm}^{-1}$)	LOD ($\mu\text{g mL}^{-1}$)	Remarks	Ref
1	Hg(II)-diphenylcarbazone	Decrease in the absorbance of Hg(II)-diphenylcarbazone complex measured	540	0-60 (6.62×10^3)	NA	Less sensitive	18
2	Three dyes	Measurement of absorbance of ion-pair complex	410	—	NA	Ion-pair formation and extraction entirely depends on pH	24
3	Reinecke salt	Absorbance of hydroxyzine-reineckate complex measured	525.5	30-525 (1.27×10^5)	NA	The procedure tedious and time consuming in which the drug is precipitated as Reinecke salt, filtered, dried at 60°C for 6 h and then dissolved in acetone before measuring the absorbance	25
4	Chloranilic acid-acetonitrile, ammonia	Absorbance of charge-transfer complex measured	535	25-150 (1.37×10^3)	1.25	Less sensitive	26
5	ORG II- CHCl_3	Absorbance of HDH-ORG II ion-pair complex measured	480	1.5-15 (2.07×10^4)	0.14	Highly sensitive, applicable for wide linear dynamic range, Highly stable coloured product	Present work

ϵ . Molar absorptivity; NA. Not available.

Results and discussions

Ion-pair extractive spectrophotometry has gained great attention for the pharmaceutically important compounds [27-31]. Due to the sulfonate anionic group present in the dye, ORG II, there is a strong tendency to form the complex with the counter cations as ion-pair complex.



Scheme 1. Structures of i) HDH; ii) protonated HDH; iii) ORG II and iv) HDH-ORG II ion-pair complex.

Hydroxyzine, as a positively charged amino compound in acid medium, forms chloroform extractable red colored ion-pair complex with the anionic dye ORG II and the resulting red colored species shows an absorption maxima at 480 nm (Fig 1). The structures of the protonated HDH, ORG II and HDH-ORG II ion-pair complex are shown in scheme 1.

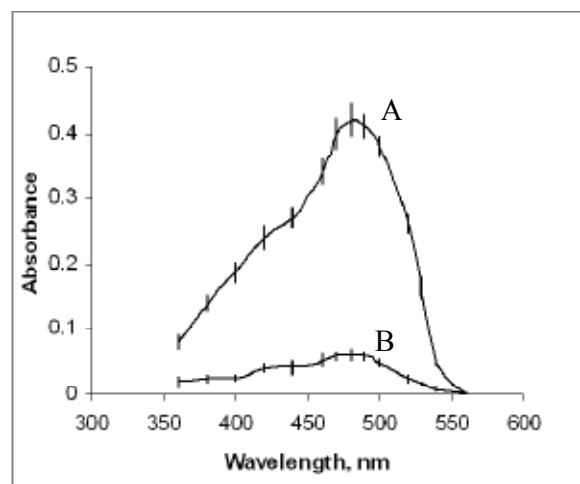


Fig. 1. Absorption spectra of: A. ion-pair complex ($9.0 \mu\text{g mL}^{-1}$ HDH); B. blank.

In order to establish optimum conditions necessary for rapid and quantitative formation of coloured product with maximum stability and sensitivity, control experiments were performed by measuring the absorbance at 480 nm by varying one and fixing the other parameters.

Method development: Optimization of experimental variables

Effect of concentration of sulphuric acid. Effect of H_2SO_4 concentration of the aqueous solution on the formation and extraction of the HDH-ORG II ion-pair was studied by varying the concentration of the acid in the range 0.0333-0.333 M (1-5 mL of 2 M acid in a total volume of 30 mL) keeping all other variables constant (5 mL of $30\ \mu\text{g mL}^{-1}$ HDH, 20 mL water and 5 mL dye solution), and it was found that maximum and constant absorbance readings were obtained in the range studied (fig 2), but the absence of H_2SO_4 resulted in lower absorbance. When the acid concentration of the aqueous phase was 0.0333 M, (1 mL of 2 M), blank showed negligible absorbance, but showed increasing trend with increasing concentration of the acid. So, 1 mL of 2 M H_2SO_4 in a total volume of 30 mL (0.0333 M) was used throughout the investigation.

Effect of ORG II. The effect of ORG II concentration on the formation of ion-pair was investigated by varying the volume of dye solution, and using a fixed concentration of drug. The results revealed that the complex formation and its extraction were unaffected in the range of 2.0 to 10.0 mL of 0.1% ORG II solution. Hence, 5 mL of 0.1 % ORG II solution was fixed as optimum in a total volume of 30 mL of aqueous phase.

Effect of ratio of aqueous to organic phases. In order to obtain the maximum absorbance, 3 mL HDH solution ($30\ \mu\text{g mL}^{-1}$) and 1 mL of 2 M H_2SO_4 were diluted to 5, 10, 15, 20, 25 and 30 mL with water before mixing with 5 mL of the dye

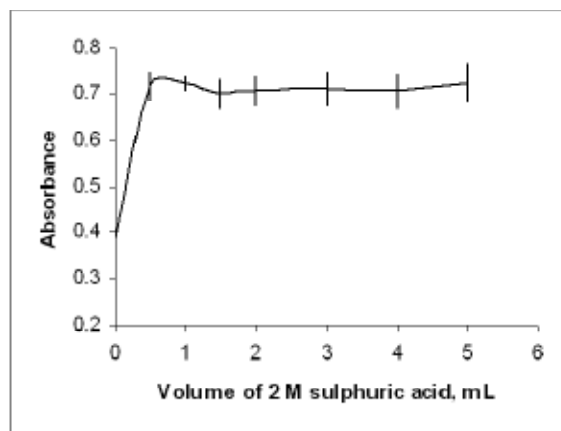


Fig. 2. Effect of sulphuric acid concentration on the absorbance of the drug-dye ion-pair complex obtained for $15\ \mu\text{g mL}^{-1}$ HDH, 5 mL ORG II dye (0.1%), 30 mL aqueous phase and 10 mL CHCl_3 (organic phase).

solution and the ion-pair was extracted with 10 mL of CHCl_3 . The study revealed that, minimum blank absorbance and maximum complex absorbance values were obtained with clear separation of organic and aqueous layers when the volume of water in the aqueous phase was maintained at > 20 mL. Hence, an aqueous phase volume of 30 mL was used in all subsequent work.

Choice of organic solvent. It was found that the ion-pair complex was readily and quantitatively extractable in chloroform. In chloroform reproducible and precise results were obtained. Therefore chloroform was used as extracting solvent.

Reaction time. After the addition of dye, the effect of standing time was studied in the time range 5-30 min before extraction. After a contact time of 5 min, measured absorbance of the complex after extraction into chloroform, showed almost constant from 5-30 min. So a contact time of 5 min was adequate for the complex to form.

Effect of shaking time. The effect of the shaking time on the extraction of the HDH-ORG II ion-pair was studied. Shaking times ranging from 30 to 180 s produced no change in absorbance, by maintaining all other parameters constant. So a 30 sec shaking time was fixed.

Number of extractions. To obtain a quantitative extraction of the HDH-ORG II ion-pair, under optimum conditions, the drug-dye ion-pair in the aqueous phase was extracted with three 10 mL portions of CHCl_3 successively and absorbance was measured each time. After the second extraction, the absorbance of the organic layer was negligibly small. Hence, a single extraction with 10 mL of CHCl_3 was used in subsequent work.

Equilibration time and stability of the coloured complexes. The organic and aqueous phases were clearly separated in less than 1 min. The drug-dye ion-pair complex was stable for more than 72 h at laboratory temperature ($30 \pm 2^\circ\text{C}$).

Effect of order of addition of reactants. The sequence of order of addition of the reactants prior to extraction had small change in the absorbance values. So the order of addition of reactants should be in the described manner.

Study of the complexing ratio of the drug and dye. The complexing ratio of HDH and ORG II was examined by Job's continuous variations method [32]. Aqueous HDH and dye solutions of 5.1×10^{-4} M each were mixed in various molar ratios (with a total volume of 6 mL). After adding 1 mL H_2SO_4 , the total volume was diluted to 30 mL with water. The extraction was performed using 10 mL of CHCl_3 and the absorbance was measured at 480 nm. The graph of the results obtained (fig. 3) gave a maximum at a molar ratio of $X_{\text{max}} = 0.333$ which indicated the formation of a 1:2 (HDH:ORG II) complex.

Method Validation

Linearity, sensitivity, limits of detection and quantification.

Calibration graph was constructed from six points covering the concentration range 1.5-15.0 $\mu\text{g mL}^{-1}$. Regression analysis of the Beer's law data indicated a linear relationship between absorbance and concentration, (Table 2) which is corroborated by high value (close to unity) of the correlation coefficient. A plot of \log absorbance and \log concentration, yielded straight line with slope equal to 1.002, further establishing the linear relation between the two variables. The calculated molar absorptivity and Sandell sensitivity [33] values are summarized in Table 2. The limits of detection (LOD) and quantification (LOQ) were calculated according to the ICH guidelines [34] using the formulae:

$\text{LOD} = 3.3 S/b$ and $\text{LOQ} = 10 S/b$, (where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot). The values are also summarized in Table 2. The high value of ϵ and low value of Sandell sensitivity and LOD indicate the high sensitivity of the proposed method.

Precision and accuracy. The assays described under "General Procedures" were repeated seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision) of the method. These assays were performed for three levels of analyte. The results of this study are summarized in Table 3. The percentage relative standard deviation (%RSD) values were $\leq 2.6\%$ (intra-day) and $\leq 3\%$ (inter-day) indicating high precision of the method. The accuracy of the method was determined by the percent mean deviation from known concentration, bias % = [(concentration found - known concentration) \times 100 / known concentration]. Bias was calculated at each concentration and these results are also presented in Table 3. Percent relative error (%RE) values $\leq 2\%$ demonstrate the high accuracy of the proposed method.

Recovery study. To further assess the accuracy of the method, recovery experiments were performed by applying the

standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done 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 method. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 98.33 and 104.4 with standard deviation in the range 1.42-2.98%. Closeness of the results to 100 % showed the fairly good accuracy of the method. The results are shown in Table 4.

Selectivity. A systematic study was performed to determine the effect of matrix on the performance of the method by analyzing the placebo blank and synthetic mixture containing HDH. A placebo blank of the composition: starch (10 mg), acacia (15

Table 2. Sensitivity and regression parameters.

Parameter	Value
λ_{max} , nm	480
Color stability	>72 h
Linear range, $\mu\text{g mL}^{-1}$	1.5-15
Molar absorptivity(ϵ), $\text{L mol}^{-1} \text{cm}^{-1}$	2.07×10^4
Sandell sensitivity*, $\mu\text{g cm}^{-2}$	0.0216
Limit of detection (LOD), $\mu\text{g mL}^{-1}$	0.14
Limit of quantification (LOQ), $\mu\text{g mL}^{-1}$	0.41
Regression equation, Y^{**}	
Intercept (a)	-0.0260
Slope (b)	0.05
Standard deviation of a (S_a)	0.013
Standard deviation of b (S_b)	0.0014
Variance (S_a^2)	1.69×10^{-4}
Regression coefficient (r)	0.9990

*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 $l = 1 \text{ cm}$. ** $Y = a + bX$, where Y is the absorbance, X is concentration in $\mu\text{g mL}^{-1}$, a is intercept, b is slope.

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

HDH taken, $\mu\text{g mL}^{-1}$	Intra-day accuracy and precision ($n = 7$)			Inter-day accuracy and precision ($n = 5$)		
	HDH found*, $\mu\text{g mL}^{-1}$	%RE	%RSD	HDH found*, $\mu\text{g mL}^{-1}$	%RE	%RSD
3.0	3.06	2.0	2.60	2.96	1.33	2.99
9.0	8.85	1.67	1.40	9.08	0.89	1.86
15.0	15.06	0.40	1.70	15.16	1.07	2.36

%RE. Percent relative error, SD. Standard deviation, %RSD. relative standard deviation.

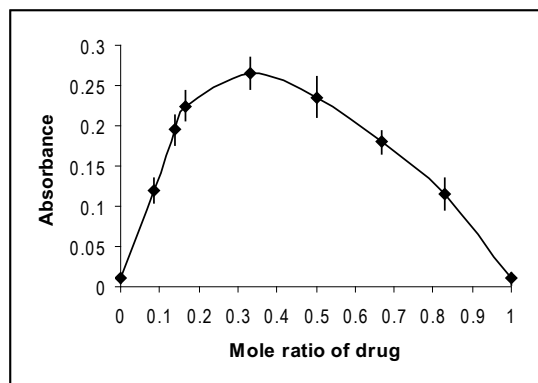


Fig. 3. Job's method of equimolar solutions for the HDH-ORG II complex in CHCl_3 .

mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was obtained and its solution was prepared as described under 'tablets', and then subjected to analysis. The absorbance of the placebo solution almost equal to the absorbance of the blank which revealed no interference from the above substances. To assess the role of the inactive ingredients on the assay of HDH, a synthetic mixture was separately prepared by adding 10 mg of HDH to the placebo mentioned above. The drug was extracted and solution prepared as described under the general procedure for tablets. The solution after appropriate dilution was analyzed following the recommended procedure. The absorbance of the coloured product resulting from the extract containing $10 \mu\text{g mL}^{-1}$ HDH was nearly the same as that obtained for pure HDH solution of identical concentration. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of HDH. Further, the slope of the calibration plot prepared from the synthetic mixture solution was about the same as that prepared from pure drug solution.

Robustness and ruggedness. The robustness of the method was evaluated by making small incremental changes in volume of H_2SO_4 and contact time and the effect of the changes was studied on the absorbance of the coloured systems. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD ($\leq 1.68\%$).

Method ruggedness was demonstrated by having the analysis done by four analysts, and also by a single analyst performing analysis on four different instruments in the same laboratory. Intermediate precision values (%RSD) in both instances were in the range 1.78-3.58 % indicating acceptable ruggedness. The results are presented in Table 5.

Application

The proposed method was applied for the quantification of HDH in commercial tablets. The results were compared with these obtained by the official US Pharmacopoeial method [23]. Statistical analysis of the results did not detect any significant

difference in performance between the proposed method and reference method with respect to accuracy and precision as revealed by the Students t-value and variance ratio F-value [35]. The results of assay are given in Table 6.

Conclusion

The ion-pair extractive spectrophotometric method for the determination of HDH in bulk drug and tablets was developed and validated for matrix effects, selectivity, linearity, sensitivity, precision and accuracy. The sensitivity offered by the proposed method surpasses that of the existing spectrophotometric methods in terms of linear range and quantification limits. The method is quite selective as the drug contains basic moiety which preferentially interacts with orange II, and the drug-dye ion-pair is extracted into the organic solvent before measurement. Simplicity of pretreatment and measurement, and use of non-rigid experimental conditions could make the proposed method as effective tool to analyze accurately and precisely HDH in tablets using simple instrumentation and low-cost materials.

Experimental

Apparatus. A Systronics model 106 digital spectrophotometer with 1 cm path length quartz cells was used for absorbance measurements.

Chromatographic analyses were carried out using Alliance Waters HPLC system equipped with Alliances 2657 series low pressure quaternary pump, a programmable variable wavelength UV-visible detector, Waters 2996 photodiode array detector and auto sampler. Data were collected and processed using Waters Empower 2.0 software.

Chemicals and reagents. All chemicals used were of analytical reagent grade. Chloroform (spectroscopic grade) was purchased from Merck, Mumbai, India. Distilled water was used throughout the investigation.

Table 4. Results of recovery study via standard-addition method.

Tablets studied	HDH in tablet, $\mu\text{g mL}^{-1}$	Pure HDH added, $\mu\text{g mL}^{-1}$	Total HDH found \pm SD*, $\mu\text{g mL}^{-1}$	% HDH recovered
Atarax 25	6.01	3.0	9.08 ± 0.18	102.3
	6.01	6.0	12.21 ± 0.23	103.3
	6.01	9.0	15.41 ± 0.42	104.4
Atarax 10	6.0	3.0	8.95 ± 0.22	98.33
	6.0	6.0	11.99 ± 0.17	99.83
	6.0	9.0	15.15 ± 0.24	101.7

*Mean value of three determinations.

Table 5. Method robustness and ruggedness expressed as intermediate precision (% RSD).

HDH taken, $\mu\text{g mL}^{-1}$	Robustness		Ruggedness	
	Parameters altered		Inter-analysts (%RSD), (n = 4)	Inter-instruments (%RSD), (n = 4)
	Volume of H_2SO_4 *	Reaction time**		
3	1.68	0.96	2.64	3.58
9	0.84	1.03	1.78	2.86
15	0.78	0.93	2.36	3.14

*The volumes of H_2SO_4 used were 0.8, 1.0 and 1.2 mL.

**The reaction times were 4, 5 and 6 min.

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

Tablet brand name ^ψ	HDH taken	HDH found \pm SD*		Student's <i>t</i> -value	Variance <i>F</i> -value
		Reference method	Proposed method		
Atarax 25 ^a	6.0	6.07 \pm 0.08	5.91 \pm 0.16	2.11	4.00
	9.0	9.05 \pm 0.18	9.04 \pm 0.15	0.10	1.44
Atarax 10 ^a	6.0	5.99 \pm 0.05	6.03 \pm 0.08	0.97	2.56
	9.0	8.98 \pm 0.12	8.97 \pm 0.23	0.10	3.67

*Mean value of 5 determinations.

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

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

^ψMarketed by: ^aUCB Pharma Ltd, Mumbai, India.

Sulphuric acid (2 M): Concentrated acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.84) was appropriately diluted with water to get 2 M.

Sodium hydroxide (2 M): Prepared by dissolving 8 g of the pure NaOH (Merck, Mumbai, India) in water and diluting to 100 mL with water.

Orange II (ORG II), (0.1 %): Accurately weighed 500 mg of the dye (S.D. Fine Chem, Mumbai, India) was dissolved in water with the aid of heat, filtered through Whatman No.42 filter paper and diluted to 500 mL with water.

Preparation of standard drug solution (proposed method).

Pharmaceutical grade HDH was procured from UCB Pharma Ltd, Mumbai, India, as a gift, and was used as received. A stock standard solution of HDH (750 $\mu\text{g mL}^{-1}$) was prepared by dissolving accurately weighed 75 mg of pure drug in water and diluting to the mark in a 100 mL graduated flask. The solution was diluted stepwise with water to get 30 $\mu\text{g mL}^{-1}$ HDH solution and used.

Preparation of sample (Reference HPLC method). Pure HDH solution.

A 100 $\mu\text{g mL}^{-1}$ standard HDH solution was prepared in methanol by dissolving accurately weighed 10 mg of pure drug in a 100 mL volumetric flask. The stock solution was diluted to 6 and 9 $\mu\text{g mL}^{-1}$ with methanol to get working solutions.

Tablet. Atarax 25 and Atarax 10 (UCB Pharma Ltd, Mumbai, India)-both tablets, were used in the investigation.

Twenty tablets were accurately weighed, finely pulverized and mixed using a mortar and pestle. An amount of tablet powder equivalent to 10 mg HDH was weighed and transferred into a 100 mL volumetric flask, 50 mL of methanol was added and was sonicated for 20 min in an ultrasonic bath to complete dissolution of the HDH, and the mixture was then diluted to the mark with methanol, mixed well and filtered. The obtained 100 $\mu\text{g mL}^{-1}$ solution was diluted with methanol to get 6 and 9 $\mu\text{g mL}^{-1}$ HDH.

Chromatographic conditions. The analysis was carried out on a chromatopack column (250 mm \times 4.6 mm i.d., 5 μm particle size). A solution containing a mixture of methanol and monobasic potassium phosphate (50:50) was used as a mobile phase. The flow rate was 2 mL min^{-1} , the detector wavelength was set at 232 nm and the injection volume was 20 μL .

General procedures for pure drug. Proposed method.

Varying volumes, 0.5, 1.0, 1.5, ... 5.0 mL; of standard HDH solution (30 $\mu\text{g mL}^{-1}$ equivalent to 1.5-15 $\mu\text{g mL}^{-1}$ HDH) were transferred into a series of 125 mL separating funnels and the total volume was brought to 25 mL by adding water. Then, to each funnel were added 1 mL of 2 M H_2SO_4 and 5 mL of 0.1 % ORG II dye solution. Content was mixed well and kept aside for 5 min. The drug-dye ion-pair was next extracted with 10 mL of chloroform by shaking for 30 sec and the layers were allowed to separate. The organic layer was then passed over anhydrous sodium sulphate and the absorbance was measured at 480 nm against the reagent blank.

A standard graph was prepared by plotting the absorbance vs drug concentration, and the concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the absorbance-concentration data.

Reference method

An aliquot of a 20 μL sample solution (6 or 9 $\mu\text{g mL}^{-1}$) was injected (in triplicate) and eluted with the mobile phase under the described chromatographic condition. The average peak area was calculated for each concentration.

Assay procedures for tablets. Proposed method.

Twenty tablets were weighed and powdered. An accurate amount of the powder equivalent to 30 mg of HDH was transferred into a 100 mL calibrated flask and 70 mL of water was added. The mixture was shaken for 20 min and, the volume was brought up to the mark with water. The solution was then filtered through Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded and 10 mL of the filtrate was diluted to 100 mL to get a working HDH solution of 30 $\mu\text{g mL}^{-1}$ and a suitable aliquot (say 3 mL) was used for assay following the procedure described above.

Reference method. An aliquot of 20 μL of tablet extract (100 $\mu\text{g mL}^{-1}$ HDH) prepared above was injected (triplicate) and eluted with the mobile phase under the reported chromatographic conditions. By means of the average peak area, the quantity of HDH was calculated by using the following formula:

$$\text{mg HDH} = LC/D \cdot r_u/r_s$$

where *L* is the labeled quantity, in mg, of hydroxyzine hydrochloride in each tablet; *D* is the concentration, in $\mu\text{g mL}^{-1}$ of

hydroxyzine hydrochloride in the *assay preparation* on the basis of the labeled quantity in each tablet and the extent of dilution; C is the concentration, in $\mu\text{g mL}^{-1}$, of pure HDH solution; and r_u and r_s are the average peak areas obtained from the tablet and pure drug solution, respectively.

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