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Two-phase Titrimetric Assay of Hydroxyzine Dihydrochloride in Pharmaceuticals

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Abstract: two-phase titrimetric method for the determination of hydroxyzine dihydrochloride (HDH) in bulk drug and in tablets has been developed and validated. Sodium lauryl sulphate is used as the titrant and dimethyl yellow as the indicator in the presence of chloroform and sulphuric acid. The proposed procedure gives sharp end points as the colour of organic phase changes from yellow to intense pink. The method is applicable over the range of 1-9 mg HDH. The procedure was also applied for the determination of HDH in its tablets and the results were found to be in a good agreement with those obtained by the reference method. The precision results, expressed by intra-day and inter-day relative standard deviation values, were satisfactory (RSD ≤ 2.0 %). The accuracy was satisfactory as well (RE ≤ 3 %). Excipients used as additives in pharmaceutical formulations did not interfere in the proposed procedures as shown by the recovery study *via* standard addition technique with percentage recoveries in the range 99.5-103.8% with a standard deviation values 0.86-1.6%.

Key words: Hydroxyzine hydrochloride, Two-phase Titration; Sodium lauryl sulphate; Methyl yellow; Pharmaceutical preparations

INTRODUCTION

Hydroxyzine dihydrochloride (HDH), chemically known as 2-[2-[4-[(4-chlorophenyl)phenymethyl]-1-piperazinyl]ethoxy]ethanol, dihydrochloride (Fig 1), is a first generation antihistamine (H_1 receptor antagonist), anxiolytic and sedative. It is used in the treatment of branchial asthma, anxiety and agitation or tension[1-3].

A number of analytical methods for the quantitative determination of HDH in pharmaceuticals are known; and include United States Pharmacopoeia (USP)[4] describes a HPLC method with uv-detection at 232 nm, high-performance liquid chromatography[5-9], gas chromatography[10], thin layer chromatography[11], micellar liquid chromatography[12], capillary zone electrophoresis[13], voltammetry[14], LC-MS[15], potentiometry[16,17], gravimetry[18] and visible spectrophotometry[19-24].

A review of literature revealed that four titrimetric procedures have been reported for the estimation of HDH in pharmaceuticals. Basavaiah and Charan proposed two titrimetric procedures for the determination of HDH. One approach[21] involves the precipitation of chloride with $AgNO_3$, filtration of the precipitate and titration of the residual $AgNO_3$ with thiocyanate using Fe(III) as indicator. The method[22] uses mercury(II) as titrant and diphenylcarbazone-bromothymol blue as indicator. The method by Sanrick and Janik[25] also involves the precipitation of the drug with sodium tetraphenyl borate, filtration, dissolution of the precipitate in acetone and potentiometric titration with $AgNO_3$. The complexometric determination of HDH[26] also involves the precipitation of the drug with cadmium nitrate, filtration of the precipitate, and titration of residual cadmium with EDTA.

Except the direct titrimetric procedure[22], the remaining involve precipitation, filtration and titration of the residual precipitant, steps that are complex, tedious and time-consuming. This lengthens the analysis times and may cause a decrease in the accuracy of the method.

This paper proposes a two-phase titration of HDH in bulk drug and in tablets that allows a quick determination with high accuracy and precision and without any interference from tablets. In the method, an aqueous solution of the drug was titrated with sodium lauryl sulphate using (di)methyl yellow as indicator in the presence of chloroform and H_2SO_4. This procedure gives sharp end-points, as the organic layer changes from yellow to intense pink and there is less likelihood of error.

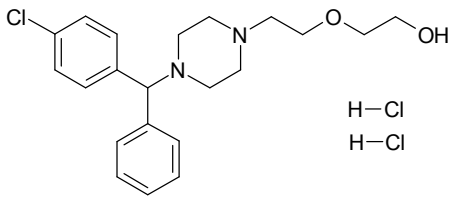


Figure 1. Chemical structure of hydroxyzine dihydrochloride.

EXPERIMENTAL

Reagents and Solutions

All chemicals used were of analytical reagent grade. Chloroform used was spectroscopic in grade. Distilled water was used through out the investigation.

Sodium Lauryl (dodecyl) sulphate (SLS): A 4.2 x 10^{-3} M aqueous solution was prepared by dissolving required amount of the specially pure SLS (Loba Chemie, Mumbai, India, Purity 99.0%) powder and standardized[27].

Methyl yellow (MY) indicator solution (0.01%): Ten mg of the pure MY (Rolex Laboratory) was dissolved in 100 mL alcohol.

Sulphuric acid (2 M): Prepared by successive dilution of appropriate volume of concentrated acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.84) with water.

Standard drug solution

A standard solution containing 1 mg mL^{-1} drug was prepared by dissolving the required amount of HDH (UCB Pharma Ltd, Mumbai, India) in water.

Procedure for pure drug

An aliquot of the standard drug solution containing 1.0-9.0 mg of HDH was measured accurately and transferred into a clean 100 mL beaker and the total volume was brought to 20 mL with water. Five mL of 2 M H_2SO4, 10 mL of chloroform and 1 mL of MY (0.01%) solution were added. The content was stirred magnetically and titrated against standard (4.2 x 10^{-3} M) SLS to the appearance of intense pink colour in the organic phase.

A blank titration was also performed and the necessary volume corrections were made. The amount of the drug in the measured aliquot was calculated from:

$$Amount(mg) = \frac{VM_wR}{n}$$

where V = volume of SLS, mL; M_w = relative molecular mass of the drug; and R = molarity of the SLS and n = number of moles of SLS reacting with each mole of HDH.

Procedure for tablets

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

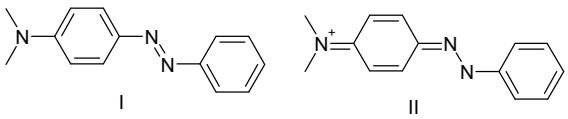
Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 100 mg of HDH was weighed accurately into 100 mL calibrated flask, 70 mL of water was added and shaken for about 20. Then the volume was made up to the mark with water, mixed well and filtered using Whatmann No 42 filter paper. The first 10 mL portion of the filtrate was discarded. A suitable aliquot was next subjected to analysis as described earlier.

RESULTS AND DISCUSSIONS

Various reports[28-31] have appeared regarding the two-phase titration of quaternary ammonium salts with dyes as indicators. End point detection is based on movement of the dye from one phase to the other, but accurate detection is difficult.

In the two-phase titration proposed here, sodium lauryl sulphate is used as titrant with methyl yellow as indicator in the presence of chloroform. After treating tertiary amine ($HDZH_2$) with H_2SO_4, the resulting protonated amine ($HDZH_2^{++}$) was titrated with sodium lauryl sulphate using MY. Methyl yellow does not possess any hydrophilic group. Therefore, color change occurs only in the organic phase. The azoid (I) form of MY is yellow in colour and the acid form of MY (quinoid (II)) is pink in colour[32]. The salts of both the forms of MY with SLS are soluble in chloroform and the end-point is indicated by a pink color in the organic phase only. The aqueous phase remains colorless throughout the titration.

When the aqueous phase is sufficiently acidic, MY, present in the organic phase, takes on yellow colour (free form), a pink colour for complexed form with SLS.



I II

Scheme 1. Azoid (I) (MY) and quinoid (II) (HMY^+) form of methyl yellow.

The possible reaction pathway at the equivalence point between the protonated drug ($HDZH_2^{++}$) and lauryl sulphate (LS^-) is believed to be as follows[33].

$$HDZH_2^{++}{}_{(aq)} + 2LS^-_{(aq)} \rightleftharpoons \left(HDZH_2^{++}.\,2LS^-\right)_{(org)}$$

Beyond the equivalence point, the excess LS^- forms complex with indicator in the following manner.

$$LS^-_{(aq)} + HMY^+_{(org)} \rightleftharpoons MY.LS_{(org)} + H^+_{(aq)}$$

The above titration reaction produced a stoichiometry of 1:2 (drug:titrant) which served as the basis for calculation. Using 4.2 x 10^{-3} M SLS, 1.0-9.0 mg of HDH was conveniently determined. The relationship between the drug amount and the titration end point was examined. The linearity between two parameters is apparent from the correlation coefficient of 0.9986 obtained by the method of least squares. From this it is implied that the reaction between HDH and SLS proceeds stoichiometrically in the ratio 1:2 in the range studied.

Method Optimisation

In order to obtain the optimum conditions necessary for the quantitative determination of HDH, stoichometric amount of drug was calculated by titrating a fixed amount of the drug and varying other parameters.

After a series of studies, 5 mL of 2 M H_2SO_4 in a total volume of 25 mL aqueous phase was sufficient and the same volume of acid was used throughout the investigation. There was clear indication of the end-point when the amount of indicator varied from 0.5 mL to 1.5 mL. There was a better separation of the organic and aqueous layers and clear indication of the end point when the volume of chloroform was maintained at more than 10 mL in a total volume of 35 mL. Therefore, 1 mL indicator and 10 mL of chloroform were used in the assay.

Method Validation

Intra-day and inter-day accuracy and Precision

The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different amounts of HDH within the range of study were analyzed in seven replicates, during the same day (intra-day precision) and five consecutive days (inter-day precision). For inter-day precision, each day analysis was performed in triplicate and pooled-standard deviation was calculated. The percentage relative standard deviation (%RSD) values were < 2 % (intra-day) and < 2.19 % (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) x 100 / known concentration]. Bias was calculated at each concentration. Percent relative error (%RE) values of < 3 % demonstrate the high accuracy of the method. Results of this study were presented in table I.

Table I. Intra-day and inter-day accuracy and precision data.

HDH taken, mg	Intra-day accuracy and precision			Inter-day accuracy and precision		
	HDH found*, mg	RE, %	RSD, %	HDH found*, mg	RE, %	RSD, %
3.0	3.02	0.67	1.85	3.05	1.67	2.19
6.0	5.86	2.33	1.35	6.10	1.67	1.89
9.0	8.74	2.90	0.52	9.08	0.89	0.65

*Mean value of seven determinations. RE.relative error, RSD. relative standard deviation.

Selectivity

A systematic study was performed to determine the effect of matrix by analyzing the synthetic mixture containing HDH. A placebo blank of the composition: starch (20 mg), acacia (30 mg), hydroxyl cellulose (20 mg), sodium citrate (20 mg), talc (40 mg), magnesium stearate (30 mg) and sodium alginate (20 mg) was made. To assess the role of the inactive ingredients on the assay of HDH, a synthetic mixture was separately prepared by adding 50 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 was analyzed following the recommended procedure. The amount of HDH found was stoichiometric and same as those obtained for pure HDH solutions of identical concentrations. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of HDH.

Robustness and ruggedness of the methods

The robustness of the methods was evaluated by making small incremental changes in volumes of H_2SO_4 (5±1 mL) and $CHCl_3$ (10±1 mL) and the effect of the changes was studied by calculating the RSD values. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD. The values were lying in the range 1.85-3.0 %.

Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using four different burettes. The inter-analysts RSD were within 2.5 % whereas the inter-burettes RSD for the same HDH amount were ranged from1.6 to 3.3 % suggesting that the developed method was rugged.

Application

The described titrimetric procedure was successfully applied for the determination of HDH in its pharmaceutical formulations (Atarax tablets of 10 and 25 mg HDH/tablet). The obtained results (table II) were statistically compared with those obtained by the official chromatographic method[4]. The reference method was consisted that chromatographic detection of HDH using UV-detector at 232 nm. 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[34]. The results of assay are given in table II.

Table II. Results of assay in tablets and comparison with official method.

Brand name	Nominal amount, mg/tablet	Found* (Percent of label claim ± SD)	
		Official method	Proposed method
Atarax 25	25	99.1±1.39	101.2±1.85 **t** =2.05 **F** =1.77
Atarax 10	10	98.7±1.55	100.5±1.36 **t** =1.95 **F** =1.30

*Average of five determinations.,Tabulated t value at the 95% confidence level is 2.77.
Tabulated F value at the 95% confidence level is 6.39.

Table III. Results of recovery study *via* standard addition method.

Tablet studied	HDH in tablet extract, mg	Pure HDH added, mg	Total HDH found, mg	Pure HDH recovered±SD* %
Atarax 25	2.0	2.0	3.99	99.5±1.08
	2.0	4.0	6.05	100.5±0.86
	2.0	6.0	7.97	102.6±1.6
Atarax 10	2.0	2.0	4.07	103.6±1.79
	2.0	4.0	6.01	100.2±1.92
	2.0	6.0	7.99	99.86±1.02

*Mean value of three determination.

Recovery Study

Accuracy and the reliability of the methods were further ascertained by performing recovery experiments. To a fixed amount of drug in formulation (pre-analyzed): pure drug at three different levels was added, and the total was found by the proposed methods. Each test was repeated three times. The recoveries were in the range from 99.5 to 103.6 % with standard deviation in the range 0.86-1.9 % indicating that commonly added excipients to tablets did not interfere in the determination and closeness of the results to 100 % showed the fairly good accuracy of the methods. These results are shown in table III.

CONCLUSIONS

A two-phase titrimetric procedure for the determination of hydroxyzine hydrochloride employing sodium lauryl sulphate as titrant and methyl yellow as indicator in the presence of chloroform has been developed and validated. The most striking feature of this method is its simplicity and rapidity, low cost per analysis, without the need for complex and time-consuming steps found in the reported titrimetric assay. The procedure therefore is suitable for the determination of HDH in pharmaceuticals as a routine method since it is free from interference from tablet additives.

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REFERENCES

1. Reynolds, J.E.F.; Martindale.; The Extra Pharmacopoeia, 30th ed.; The Pharmaceutical Press, London, 1993.
2. Khalid-Khan, S.; Rynn, M.; Rickels K.; Generalised Anxiety Disorder, Martin Dunitz, UK, 2002, p.125.
3. Argyropoulos, S.V.; Sandford, J.J.; Nutt, D. The psychobiology of anxiolytic drug. Part 2: Pharmacological treatments of anxiety. *J. Pharmacol. Ther,* 2000, 88, 213-227.
4. The US Pharmacopeia (USP 28); The National Formulary (NF 23).; US Pharmacopeial Convention Inc, 2005, p.982.
5. Roberts, S.E.; Delany, M.F.; Simultaneous determination of ephedrine sulfate, hydroxyzine hydrochloride and theophylline in tablets by reversed-phase high-performance liquid chromatography. *J. Chromatogr,* 1982, 242(2), 364-368.
6. Menon G.N.; Norris B.J.; Simultaneous determination of hydroxyzine hydrochloride and benzyl alcohol in injection solutions by high-performance liquid chromatography. *J. Pharm. Sci,* 1981, 70(6), 697-698.
7. Papas, A.N.; Marchise, S.M.; Delancy, M.F.; Determination of hydroxyzine hydrochloride in dosage form by liquid chromatography. *Liq. Chromatogr,* 1984, 2, 120–121.
8. Dragana, B-B.; Rodulovic, D.; Ivanovic, D.; Ristic, P.; Simultaneous assay of ephedrine hydrochloride, theophylline, papaverine hydrochloride and hydroxyzine hydrochloride in tablets using RP-LC. *J. Pharm. Biomed. Anal,* 1999, 21(1), 15-22.
9. Pehoursq, F.; A simple high-performance liquid chromatographic method for detection

of hydroxyzine in human plasma after overdose. *J. Pharm. Tox. Meth,* 2004, 50(1), 41-44.
10. Kintz, P.; Godelar, B.; Mangin, P.; Gas chromatographic identification and quantification of hydroxyzine: Application in a fatal self-poisoning. *Forensic, Sci. Int,* 1990, 48(2), 139-143.
11. Ackermann, H.; Kretzschmann, F.; Kruger, S.; Lexow, B. TLC method for semiquantitative determination of Hydroxyzinrückstanden in animal material. *Nahrung-Food,* 1977, 21(7), 603-610.
12. Martinez-Algaba, C.; Bermudez-Saldana, J.M.; Villanueva-Camanas, R.M.; Sagrado, S.; Medina-Hernandez, M.J.; Analysis of pharmaceutical preparations containing antihistamine drugs by micellar liquid chromatography. *J. Pharm. Biomed. Anal,* 2006, 40(2), 312-321.
13. Capella-Peiro, M.E.; Bossi, A.; Esteve-Romero.; Optimization by factorial design of a capillary zone electrophoresis method for the simultaneous separation of antihistamines. *J. Anal. Biochem,* 2006, 352(1), 41-49.
14. Beltagi, A.M.; Abdallah, O.M.; Ghoneim, M.M.; Development of a voltammetric procedure for assay of the antihistamine drug hydroxyzine at a glassy carbon electrode: Quantification and pharmacokinetic studies. *Talanta,* 2008, 74(4), 851-859
15. Neng Zhou.; Liang Yi-Zeng.; Chen Ben-Mei.; Ping Wang.; Xian Chen.; Liu Feng-Ping.; Development and Validation of LC–MS Method for the Determination of Hydroxyzine Hydrochloride in Human Plasma and Subsequent Application in a Bioequivalence Study. *Chromatographia,* 2007, 66(7-8), 481-486.
16. Bouklouze, A.; Elbouzekraoui, M.; Cherrah, Y.; Hassar, M.; Kauffmann, J.M.; Potentiometric Sensor for Hydroxyzine Determination. *Electroanalysis,* 2002, 14(19-20), 1369-1374.
17. Ciaccio, L.L.; Missan, S.R.; McMullen, W.H.; Grenfeel, T.C.; Nonaqueous Titration of 1,4-Disubstituted Piperazines**.** *Anal. Chem,* 1957, 29 (11), 1670–1673.
18. Pasich, J.; Stasiewska, K.; Simple gravimetric determination of hydroxyzine, diprophylline, chlorpromazine and ethionamide in suppositories. *Acta. Pol. Pharm,* 1962, 19, 181–182.
19. Sane, R.T.; Vaidya, U.M.; Nayak, V.G.; Dhamankar, A.Y.; Joshi, S.K.; Doshi, V.J.; Sawant, S.V.; Malkar, V.B.; Pandit, V.R.; Sathe, A.Y.; Jukar, S.; Nadakarni A.D.; Application of 3 new dyes in extractive photometric determination of 15 drugs. *Indian drugs,* 1982, 19, 398–403.
20. Marzanna, K.; Brunon, D.; Aleksandra, S-C.; Pat, S., Spectrophotometric determination of imipramine hydrochloride, doxepin hydrochloride and hydroxyzine dihydrochloride with reinecke salt. *Acta. Pol. Pharma,,* 1999, 56(6), 415-417.
21. Basavaiah, K.; Charan, V.S.; Determination of embramine and hydroxyzine applying precipitation and complexation reactions. *Acta Ciencia Indica,* 2001, 27(3), 91-96.
22. Basaviah, K.; Charan, V.S.; Titrimetric and spectrophotometric assay of some antihistamines through the determination of the chloride of their hydrochlorides. *Il Farmaco,* 2002, 57, 9–17.
23. Basaviah, K.; Charan, V.S.; Spectrophotometric determination of two antihistamines by charge- transfer complex formation with chloranilic acid. *Ind. J. Pharm Sci,* 2003, 65(6), 660-662.
24. Nacea, V.; Murgu, L.; Detection of hydroxyzine in blood by reaction with copper(II) chloride. *Rev. Chem. (Bucharest),* 1978, 29, 577–579.
25. Sanrick, B.; Janik, B.; Determination of hydroxyzine with sodium tetraphenylborate. *Acta. Pol. Pharm,* 1966, 23, 573–575.
26. Dembinski, B.; Complexometric determination of hydroxyzine hydrochloride. *Chem. Anal. (Warsaw),* 1993, 38, 183–187.
27. A Text Book of Pharmaceutical Analysis, Edited by Higuchi T and Einar B-H, Ist Indian Edition, p. 399-400, 1997.
28. Epton, S R.; A Rapid Method of Analysis for Certain Surface-Active Agents. *Nature*, 1947, 160, 795-796.
29. Mukerjee, P.; Use of ionic dyes in the analysis of ionic surfactants and other ionic organic compounds. *Anal Chem*, 1956, 28, 870-873.
30. Cross, J. T.; The identification and determination of cationic surface-active agents with sodium tetraphenylboron. *Analyst,* 1965, 90, 315.
31. Cooper, P. J.; Hammond, P. W.; The assay of certain organic bases in aqueous eye-drops. *Analyst,* 1967, 92, 180.
32. A Text Book of Cationic Surfactants, Analytical and Biological Evaluation, Surfactant Science Series, vol 53, Edited by John Cross, Edward J Singer, Madison Avenue, New York, 1994, p.152.

33. A Text Book of Anionic Surfactants, Analytical Chemistry, Surfactant Science Series, vol 73, 2nd ed, Edited by John Cross, Madison Avenue, New York, 1998, p.47-48.

34. Inczedy, J.; Lengyel, T.; Ure, A.M.; IUPAC Compendium of Analytical Nomenclature: Definitive Rules, Blackwell Science Inc, Boston 1998.

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