

Spectrophotometric Determination of Histidine Hydrochloride Monohydrate in Pharmaceutical Formulations

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ABSTRACT: Rapid, accurate, precise and specific colorimetric method was developed for the determination of histidine hydrochloride monohydrate from the mixture of amino acids. This method is based on the formation of yellow colour as a result of reaction between histidine and diazotized sulfanilic acid. The absorbance was measured at 405 nm and the calibration curve was linear in the concentration range of 33 µg/ 15 ml – 77 µg/ 15 ml. The molar absorptivity was found to be $3.169 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$ and Sandell's sensitivity of 0.1988 per 0.001 absorbance unit. The colour is stable for 6-7 minutes after the reaction. The proposed method was successfully extended to pharmaceutical preparations-capsules.

KEY WORDS: Histidine hydrochloride monohydrate, Spectrophotometry, Method Validation, Pharmaceutical formulation

INTRODUCTION

Histidine hydrochloride monohydrate (HHM) ¹, (s)-2-amino-3-(imidazole-4-yl) propanoic acid, is a non essential amino acid necessary for proper growth and development of infants. Metabolism of histidine^{2,3} in the body produces histamine, which is involved in the inflammatory response and the production of hydrochloric acid by the stomach.

British Pharmacopeia⁴ and USP⁵ suggest TLC method for the estimation of HHM in bulk. The other analytical methods that have been reported for its determination in bulk include HPLC^{6,7,8}, Thin layer chromatography⁹, Gas chromatography^{10,11}, High performance capillary electrophoresis¹² etc. However, no spectrophotometric method is reported till date for the determination of HHM in combination with other amino acids, like L-lysine, glycine etc. with which it is generally available. Hence, it was thought to develop a spectrophotometric method for the same.

In the present study, a colorimetric method for the determination of HHM in bulk and in pharmaceutical

formulation in presence of other amino acids is described. The method is based on yellow colour formation as a result of reaction between HHM with diazotized sulfanilic acid. The absorbance measurement was made at the maxima of 405 nm at sharp 6th minute of reaction. This method is simple, rapid, accurate, precise and easy to apply in routine usage and does not need any costly instrumentation.

MATERIALS AND METHODS

Apparatus

Absorbance measurements were made on Shimadzu UV-1601 UV/Visible Spectrophotometer with 10 mm matched quartz cells.

Reagents and solutions

All chemicals were of analytical reagent grade and solutions were prepared with purified water of I.P.¹³ grade. Pharmaceutical grade of HHM was gifted by Elysium Pharmaceuticals Ltd., Baroda, India and

certified to contain 99.5% of HHM. It was used without further purification.

Sodium Hydroxide (1 M)

Sodium hydroxide solution was prepared by dissolving 40 mg of sodium hydroxide (S.D.Fine chemicals, Boisar, India) in purified water and making the volume to 1000 ml with purified water.

Coloring reagent

Into a 100 ml volumetric flask, 200 mg of sodium nitrite was taken and dissolved in 60 ml of distilled water. One ml of hydrochloric acid was added and allowed to stand for one hour. Sulfanilic acid, 500 mg, was then added and the final volume was made up to the mark with purified water. This reagent was used after 30 minutes of preparation.

HHM stock solution (220 µg/ml)

Into a 250 ml volumetric flask containing 60 ml of 1 M sodium hydroxide, 55 mg of HHM was dissolved and the volume was adjusted with 1 M sodium hydroxide.

Optimization of Parameters

All the optimization parameters are estimated at room temperature. HHM was found to yield clear yellow color with coloring reagent, showing absorbance maxima at 405 nm. The color product may be due to the reaction of imidazole ring with diazotized sulfanilic acid. Therefore, investigations were carried out to establish the most favorable conditions for the formation of colored product. The influence of the concentration as well as volume of reagent on the reaction has been studied. Different concentrations and different volumes were tried for all the reagents, by varying one parameter at a time. The optimum concentration of sulfanilic acid was 500 mg and of sodium nitrite was 200 mg, similarly optimum volume of coloring reagent was 10 ml. The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. Also the color stability of newly formed complex was measured and the color was found to be stable between 6th and 7th minute. Graphical presentations indicating stability of color of colored complex is given in figure 1.

Procedure for calibration curve

Suitable aliquots of the drug solution (3 to 7 ml) were taken in a series of 100 ml volumetric flasks and the volume was adjusted with 1 M sodium hydroxide solution. Aliquot of 5 ml from each solution was taken into separate test tubes of 50 ml capacity. Ten ml of coloring reagent was added in each test tube simultaneously taking the absorbance at 405 nm exactly at the 6th minute against 1M sodium hydroxide as a blank. The absorbance were plotted against the respective concentrations to obtain the calibration curve.

Estimation of HHM in Pharmaceutical capsules

Twenty capsules were emptied, weighed accurately and the contents were mixed thoroughly. An accurately weighed quantity of powdered sample equivalent to 4.4 mg of HHM was dissolved in 1 M sodium hydroxide with the aid of ultrasonics for 15 minutes. Then it was filtered through Whatman filter paper no. 42 into a calibrated 100 ml volumetric flask. Filter paper was

rinsed twice with 5 ml of 1 M sodium hydroxide and the volume was made up to 100 ml with 1 M sodium hydroxide. Twenty five ml of this solution was then diluted to 100 ml with 1 M sodium hydroxide solution. An appropriate aliquot was then taken in such a way that the final concentration in 15 ml lies within the range tested. The assay was completed as described in procedure for calibration curve.

Method Validation

The developed methods were validated for its accuracy, precision, reproducibility and selectivity. The accuracy of the method was determined by performing recovery studies on capsule formulation and for prepared solutions containing known amount of drug by standard addition method in which preanalyzed samples were taken and standard drug was added at five different levels. The results are shown in table 2. Also, the experiment was repeated three times in a day to determine intra-day precision and on three different days to determine inter-day precision. The percent relative standard deviation (% RSD) was calculated at each concentration level and the results are given in table 3. The reproducibility was confirmed by repeating the method by three different analysts and the % RSD was calculated. The selectivity of the method was checked by monitoring a standard solution of HHM in presence of other compounds of capsules, (amino acids like L. Lysine, vitamins like Folic acid, Choline bitartrate and iron supplements like Ferrous glycine sulphate,) and excipients at the same concentration levels as used in capsules using the method described in the procedure for calibration curve in pharmaceutical capsules.

RESULTS AND DISCUSSION

The proposed method is simple, rapid and precise and it does not suffer from any interference due to other amino acids and common excipients of capsule. The coloring agent was prepared by diazotization reaction with sulfanilic acid. It contains an amino group which reacts with sodium nitrite in the presence of hydrochloric acid to give a diazonium salt of sulfanilic acid. It is proposed that this coloring agent then reacts with the carboxylic group on HHM to form a yellow colored complex. The stability of colored complex was estimated with respect to time and it is evident from figure 1 that color was stable between sixth and seventh minute so, it is recommended that the readings should be taken exactly at the sixth minute of reaction.

The proposed colorimetric method was found to be linear in the range of 33 to 77 µg/15ml with correlation coefficient (r^2) 0.9997, slope 0.006 and intercept 0.1203 as shown in table 1. The method was validated in terms of accuracy, precision, reproducibility and the results are recorded in tables 2 and 3. The accuracy of the method was determined by performing recovery studies by standard addition method in which preanalyzed samples were taken and standard drug was added at five different levels. Values of recovery \pm SD greater than 99.0% indicate that the proposed method is accurate for the

analysis of drug. The precision of the proposed method was estimated in terms of inter-day precision and intra-day precision wherein the method was repeated on three different days and repeated for three different time periods in the same day respectively. The results shown in table 3 indicating % RSD of less than 1% at each level clearly indicate that the proposed method is precise enough for the analysis of drug. The reproducibility of the method was confirmed by performing the proposed method by three different analysts. The values of % RSD less than 1% indicate that the proposed method is reproducible for the analysis of HHM. The selectivity of the method was checked by monitoring a standard solution of HHM in presence of other compounds of capsules. The absorbance values of solution of other amino acids alone were also measured at 405 nm showing no significant difference from the baseline. The excipients too did not show any effect on the estimation of HHM. Hence, the determination of HHM in the capsule is considered to be free from interference due to other amino acids and excipients. Rigorous analysis of

the results indicate that the presence of excipients in capsule formulation did not interfere with the final determination of the active component. This reveals the potential utility of this developed method for the routine analysis of HHM in pharmaceutical preparations.

CONCLUSION

A new, simple and selective spectrophotometric method was developed for the analysis of HHM in bulk and in pharmaceutical formulation. In this method the color of newly formed complex was measured at the maxima of 405 nm. The developed method was also validated and from the statistical data, it was found that method was accurate, precise, reproducible and can be successfully applied to the pharmaceutical formulation without interference of excipients.

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Table 1 Optimum conditions, optical characteristics and statistical data of the regression equations for color development with HHM

Parameters	Data
Drug Aliquot (ml)	3 – 7
Amount of coloring reagent (ml)	10
Absorption maxima (nm)	405
Beer's law limit ($\mu\text{g}/15 \text{ ml}$)	33 – 77
Apparent molar absorptivity ($1 \text{ mol}^{-1} \text{ cm}^{-1}$)	2.63618×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-1}$ per 0.001 A)	1.9877×10^{-1}
Regression equation ^a	$Y = 0.006 x + 0.1203$
Intercept (a)	0.1203
Slope (b)	0.6×10^{-2}
Correlation coefficient (r^2)	0.9997

^a n = 15

Table 2 Recovery Study Data

Level of Standard Addition (%)	% Recovery \pm SD*	
	A	B
80	101 \pm 0.30	99.51 \pm 0.58
90	99.66 \pm 0.26	99.77 \pm 0.82
100	100.75 \pm 0.46	100.87 \pm 0.43
110	99.77 \pm 0.52	99.68 \pm 0.91
120	99.60 \pm 0.22	99.31 \pm 0.73

A = From Capsule

B = From standard solution

*Average of three determinations

Table 3 Inter-day and Intra-day precision

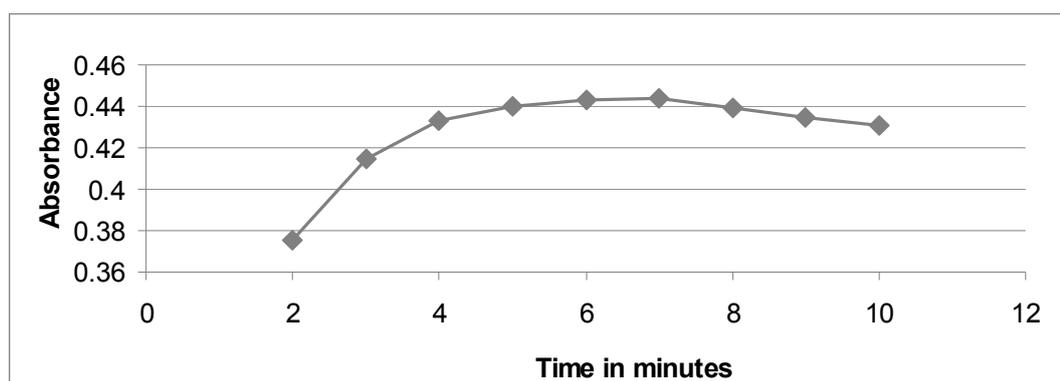
Inter-day			Intra-day		
Amount taken*	Amount found \pm SD **	% RSD	Amount taken*	Amount found \pm SD **	% RSD
2	1.98 \pm 0.01	0.51	2	1.95 \pm 0.01	0.30
3	2.98 \pm 0.03	0.84	3	2.88 \pm 0.03	0.92
4	4.01 \pm 0.04	0.90	4	3.98 \pm 0.02	0.38
4.5	4.50 \pm 0.03	0.68	4.5	4.49 \pm 0.01	0.26
5	5.00 \pm 0.02	0.31	5	5.06 \pm 0.05	0.93

*Concentration in μ g

** Average of three determinations

Table 4 Determination of HHM in commercial Capsules

Formulation	Label claim ^a	Amount Found (mg)	%Recovery \pm SD ^b	%RSD
Feel Plus Capsule	4.4	4.52	102.72 \pm 0.020	0.442

^a Amount in mg^b Mean \pm Standard Deviation for three determinations**Figure 1: Effect of time on the stability of yellow color product****REFERENCES**

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