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Validated Method Development for Estimation of Ketamine HCl as API and in Pharmaceutical Dosage Forms By UV-Spectroscopy.

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ABSTRACT

Ketamine HCl is a white crystalline powder and anaesthetic drug. It is a noncompetitive NMDA receptor (NMDAR) antagonist. A method for the determination of Ketamine HCl in API and Pharmaceutical dosage form was developed in UV-Spectroscopy, which was found to be simple, sensitive, cost effective, reproducible, validated & economic. The maximum absorbance of Ketamine HCl was at 220 nm. The Beer's Lambert law was obeyed successfully. The linearity was from 5 to 40µg/ml and effective range was found to be from 12 to 28 µg/ml in calibration curve. The correlation coefficient was found to be 0.999. The precision, accuracy, repeatability, specificity, linearity was done and all the parameters were found within the acceptable range. All the procedure were followed according to ICH guidelines.

Keywords: UV-Spectroscopy, , Kitamine HCL, Validation, ICH guidelines.

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INTRODUCTION

Ketamine HCl is a white crystalline powder. It is a (*RS*)-2-(2-chlorophenyl)-2-methylaminocyclohexanone hydrochloride structure was shown in figure 1. Molecular Formula of Ketamine HCl is $C_{13}H_{16}C_lNO$. HCl & Mol. Weight is 274.2. It is freely soluble in water and methanol., Ketamine is a noncompetitive NMDA receptor (NMDAR) antagonist. Some UV Spectroscopic [1,2], HPLC [3-8], HPLC-MS/MS [5], LC/LCMS [1,7,9,10, 11] methods are present for the determination of Ketamine HCl. The aim of present study is to develop a simple, sensitive, cost effective, reproducible, validated and reliable UV-spectroscopy method [12,13].

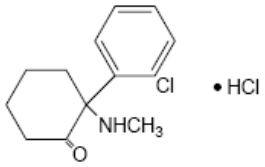

Figure 1: Structure of Ketamine HCL.

Chemicals and Reagents

Ketamine HCl is a gift sample from Alchem International Ltd. The solvents used were of Analytical grade. The marketed formulation which contained Ketamine HCl was purchased from local market.

Instrumentation

The experiment was carried out using UV Spectrophotometer (My 13510001). The powder was weighed by using Digital Weighing Balance GR (200). FT-IR Parkin Almar (8400S).

Preparation of standard stock solution of Ketamine HCl

Accurately 50 mg of the drug was weighed with the help of digital balance and transferred to 50 ml of volumetric flask. It was dissolved in about 30 ml of distilled water and mixed properly and then the volume was made up to the mark. This is stock solution. Then 1ml of this solution was pipette out and transferred to 10 ml of volumetric flask and diluted up to 10 ml with distilled water. This solution contained 100 µg of drug per ml.

Method development

Determination of wavelength of maximum absorbance (λ_{max})

1ml of the solution from standard stock solution was pipette out and transferred to 10 ml of the volumetric flask and diluted up to 10 ml with distilled water. This solution contained 10 µg/ml of the drug. The absorbance of this solution was scanned in the UV range of 200 to

400 nm against water as blank. The maximum absorbance of Ketamine HCl was obtained at 220 nm as shown in the Figure2 .

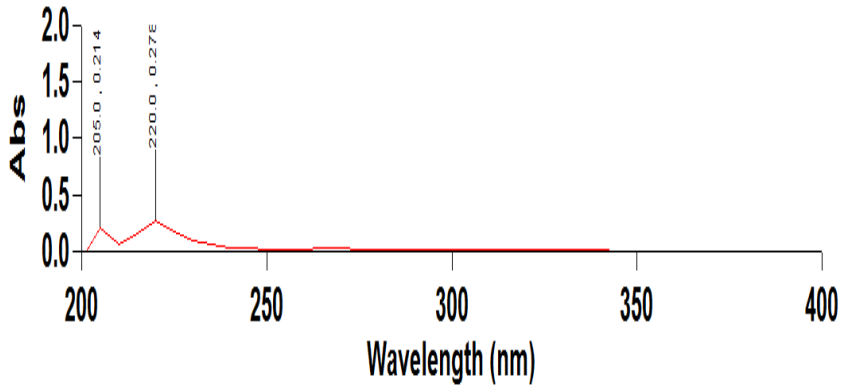

Figure 2: Scan of Ketamine HCl in the range of 200 to 400 nm.

Preparation of calibration curve for Ketamine HCl

2.5ml of stock solution was pipette out and transferred to 50 ml of volumetric flask and diluted up to 10 ml with distilled water. This solution contained 50 µgm of drug per ml of the solution. 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml and 8ml of the solution from this solution were pipette out into a series of 10 ml volumetric flask. The volumes were made up to the mark with distilled water and mixed to obtain solutions in the concentration range of 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml of the drug. The absorbance of these resultant solutions were measured at 220 nm against distilled water as blank and the graph was plotted between absorbance obtained and the concentrations of solutions. The Beer's Lambert law was obeyed in concentration range of 5 to 40 µg/ml at 220 nm as shown in the table1 and figure3.

Tabel 1: Data for calibration curve of Ketamine HCl

Concentration(µg/ml)	Absorbance*
5	0.1642
10	0.2620
15	0.3734
20	0.4782
25	0.5866
30	0.6729
35	0.7707
40	0.8737

*average of three

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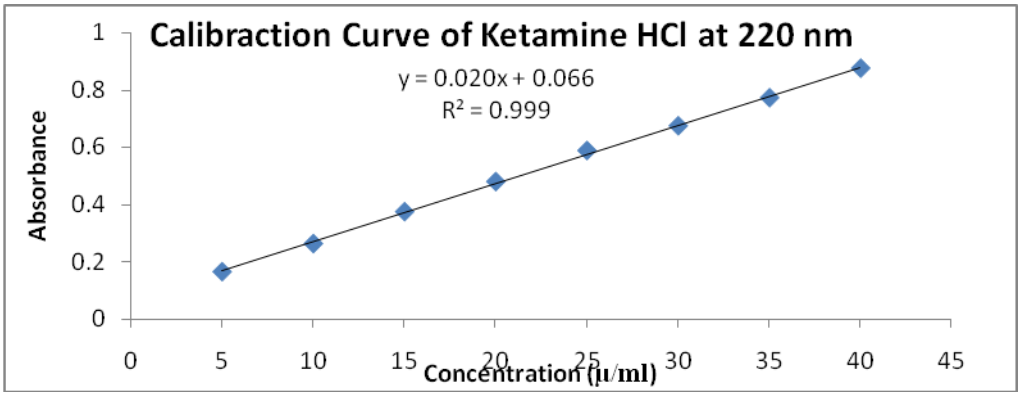

Figure 3: Calibration curve of Ketamine HCl at 220 nm.

Determination of optical parameters

The molecular absorptivity and Sandell's sensitivity were calculated as:

Molecular absorptivity (Є) = AM/cl

A= absorbance
M= molecular weight
c = concentration±
l = path length

Sandell's sensitivity = M/ Є

M= molecular weight
Є= molecular absorptivity

Other optical parameters i.e. Beer's limit, slope, intercept and correlation coefficient were calculated from calibration curve. The results are shown in the table 2.

Validation of the proposed method according to I.C.H guidelines:

Specificity

1ml of the solution from standard stock solution was pipette out and transferred to 10 ml of six volumetric flasks and diluted up to 10 ml with distilled water. This solution contained 10 µg/ml of the drug. The absorbance of these solutions were measured and recorded and about 2 ml of 2 µg/ml solution of each excipients was added to them and the volume was made up to the mark with water. The absorbances was measured and recorded. The concentration of the solution was determined and % interferences were calculated. The results are shown in the table 2.

Linearity and Range

A linear relationship was obtained between absorbance and concentration in the range was found to be 12 to 28 µg/ml of the drug in the solution as shown in the figure. A correlation coefficient (r) of 0.999 was observed as shown in the Table 2.

Accuracy

The accuracy of the developed method was determined by a recovery study carried on Ketamine HCl. 40 µg/ml sample solution of Ketamine HCl was added with solutions of 18, 20 and 22 µg/ml of Ketamine HCl standard solution. The absorbances were measured and the % recovery was calculated. The percentage recovery results are shown in table 2. (Acceptance criteria: The percentage recovery should be in the range 98-102%)

Precision

Repeatability

20µg/ml solutions of the drug were made by taking 4ml of the drug solution from the second stock solution (50 µg/ml) in 10 ml volumetric flask and volume was made up to the mark. The absorbance of this solution was measured six times and recorded. The results are shown in the table 2. (Acceptance Criteria: RSD should be less than 2 %)

Intra-day precision

3ml, 4ml and 5ml of the drug solution was pipette out from the standard stock solution and transferred to 10 ml volumetric flask and the volume was made up with distilled water to obtain the concentrations of 15, 20 and 25 µg/ml respectively. The absorbance of these solutions were measured individually thrice within a day and recorded. The results are shown in the Table 2. (Acceptance criteria: RSD should be less than 2%)

Inter-day Precision

3ml, 4ml and 5ml of the drug solution was pipette out from standard stock solution and transferred to 10 ml volumetric flask and the volume was made up with distilled water to obtain the concentrations of 15, 20 and 25 µg/ml respectively. The absorbance of these solutions were measured individually thrice in three days and recorded. The results are shown in the Table 2.

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Table 2: Optical parameters and Regression characterstics of ketamine HCl in distilled water.

Parameters	Observations
Linearaty (µg/ml)	5-40 (µg/ml)
Range	12-28 (µg/ml)
Molar absorptivity ($1\ mole^{-1}\ cm^{-1}$)	6.8×10^{-3}
Sandell's sensitivity ($\mu g/cm^2$/0.001 absorbance unit)	2.47×10^{-1}
Regression equation(y=a+bc) Slope(b) Intercept(a)	 0.020 0.066
Correlation coefficient (r)	0.999
Accuracy (% recovery)	100.82±0.5267
Specificity (% interference)	0.75
Repeatability (RSD)	0.5098
Precision (RSD) Inter-day precision Intra-day precision	0.56 0.4611

Estimation of Ketamine HCl in Aneket (Neon Laboraties Ltd.).

Ketamine injection of (Aneket) brand contains 100 mg in 2 ml. 0.5 ml of the injection was pipette out and volume was made up to 50 ml. This solution contains 500 µg/ml. 2 ml of this solution was pipette out and volume was made up to 25 ml. This solution contained 40 µg/ml. From this solution 4 ml of the drug was pipette out thrice and taken in 10 ml volumetric flask and the volume was made up to the mark. The absorbance of this solution was measured and recorded. The concentration was then determined from the calibration curve. The results are shown in Table 3.

Table 3: Results for estimation of Ketamined HCl Injection.

Brand	Label claim (mg/ml)	Theorotical Conc. (µg/ml)	Amount found (mg/ml)	% Assay	Mean % Assay ± SD
Aneket	50	16	16.01 16.00 16.05	100.06 100.00 100.31	100.12±0.00252

RESULTS AND DISCUSSION

The Ketamine HCl showed well defined peak at the absorbance wavelength 220 nm. Linear response was obtained in the concentration range of 12-28 µg/ml with correlation coefficient 0.999, recovery of the drug was found to be 100.82 % and relative atandard deviation was found to be less than 2% for precision studies. The proposed method is simple, sensitive, accurate and precise and can be successfully employed for the routine analysis of the Ketamine HCl in bulk drug, & in Injection formulation.

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CONCLUSION

The proposed method was validated by studying parameter such as accuracy, precision, linearity, specificity, repeatability. The accuracy of methods was greater than 98% and RSD not more than 2%.

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