IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 2, February 2024

UV Spectrophotometry and Validation of Diphenhydramine HCl.

Wavhal Payal Rajendra, Chaughule Rumana, Neherkar Madhuri, Chaudhary Mohini, Hinge Sakshi
Prof. Mr. Sagar E. Tambe
Samarth Institute of Pharmacy Belhe, Maharashtra, India

Abstract: *Simple, accurate, cost effective and reproducible spectrophotometric method has been developed for the estimation of Diphenhydramine Hydrochloride in soft gelatin capsule dosage form. For UV spectrophotometric method, maximum absorption was found at Amax 258nm. The percentage recovery of Diphenhydramine Hydrochloride ranged from (98.97 ± 0.2989) in capsule dosage form. The developed method was validated as per ICH guidelines with respect to linearity, accuracy (recovery), precision and specificity. Beers law was obeyed in the concentration range of 10-100µg/ml having line equation y 0.016x 0.018 with correlation coefficient of 0.9934. By treating the data statistically and by recovery study, results of study were validated.*

Keywords: Spectroscopy, Validation, Diphenhydramine Hydrochloride, etc.

I. INTRODUCTION

The molecular formula of diphenhydramine hydrochloride (DPH) is CHNO. The chemical formula of HCL is 2-(diphenylmethoxy)-N,N-dimethylethyl-1-amine hydrogen chloride (1/1). Diphenhydramine hydrochloride is an antihistamine used with sleep benefits in the treatment of sinusitis and other pyramidal disorders. It is used to reduce allergy symptoms. For urticaria and rhinitis, it is described as an antiemetic and is used to prevent vomiting during chemotherapy.

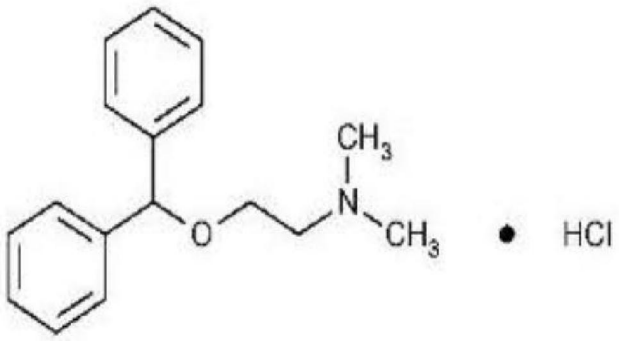

Fig. Structure of Diphenhydramine Hydrochloride.

FT-Raman spectroscopy and HPLC methods for liquid formulations are also reported in reference 9. Analytical chemistry has been important since the early days of chemistry and has provided a way to determine what elements and chemicals are present in the world around us. Modern analytical chemistry relies mostly on measurement. Many analysts specialize in a type of measurement such as UV spectrophotometry, which leads to the development of analytical methods to have good insurance and routine analysis in well-designed and established laboratories. Although Santon G. also reported second derivative UV spectroscopy for the prediction of diphenhydramine hydrochloride and naphazoline. et al, but did not report an estimate of 10 for this drug in a soft gelatin formulation. Therefore, the aim of this study is to develop and use a simple, sensitive, accurate, precise and reproducible UV method for diphenhydramine hydrochloride. DPH is soluble in 0.1 N HCl, water and 0.1 N sodium hydroxide. λmax was found to be 258 nm.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 2, February 2024

II. MATERIAL AND METHODS

Apparatus: Instruments used were UV-Visible double beam spectrophotometer (UV-1800, SHIMADZU Limited, Japan) with 1cm matched quartz cells, Micropipette of Variable volume 10-1000 µL (Gene Pete Co.) and Digital balance.

Reagents and Chemicals : Diphenhydramine Hydrochloride pure drug was obtained from RPG Life science Ltd., Mumbai as gift sample with 99.99% w/w assay value and was used without further purification. Methanol, Potassium dihydrogen phosphate, Hydrochloric acid, Sodium hydroxides were purchased from CDH (P) Ltd. New Delhi. All chemicals and reagents used were of analytical grade. Soft gelatin capsules of DPH were purchased from local market of Moradabad.

Method:

Preparation of the preparation: Weigh 100 mg of pure substance, transfer to a 100 ml volumetric flask, add 50 ml of 0.1 N HCl to the top of the container, melt and adjust to fill with 0.1 N HCl.

Preparation of drug sample: Weigh 10 tablets (one DPH tablet equals 25 mg) and 4 soft gelatin tablets (about 100 mg), dissolve them in 0.1N HCL and determine the weight between soft gelatin capsules. Shake frequently and adjust to the 100 ml mark with 0.1 N HCl. The solution was filtered through Whattman filter paper #41. The filtrate was appropriately diluted with 0.1 N HCl to obtain a solution with a concentration of 50 µg/ml. Six replicate tests were performed and samples were graded individually. Method validation 11, 12, 13: Various methods were used to analyze DPH in gelatin capsules and the method was validated according to ICH Q2B guidelines.

Linearity: The aliquots of concentration ranging 10-140 µg/mL were prepared in triplicate, but linearity was found to be between 10-100µg/ml concentrations. The linearity was calculated by the least square regression method. Precision and Accuracy: The precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on two different days and the results were presented as the standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from triplicate trials over two different concentration levels (50µg/mL and 60µg/mL) covering the linearity range were obtained. LOD and LOQ: LOD (k= 3.3) and LOQ (k= 10) of the method were established according to ICH definitions. LOD and LOQ of method are reported in Table no 1. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$$LOD = 3.3\ S/M;\ LOQ = 10\ S/M$$

Where S is the standard deviation of the absorbance of the sample and M is the slope of the calibrations curve. The limit of quantification (LOQ) is defined as the lowest concentration of analyte that can be estimated with an acceptable limit of accuracy and precision. The values of LOD and LOQ are given in Table 1. Stability: The stability of DPH in 0.1N HCL solution was studied by the developed method. Sample solutions (50µg/mL) were prepared in triplicate and heated to maintain 50OC and 60°C for 60min. The absorbance data of these samples reveled information about the stability of DPH. RESULTS AND DISCUSSION: In the start of the method development for this drug, different solvents were tested such as water, methanol, 0.1N HCl, 0.1N NaOH and Phosphate buffer (pH7.4). Due to greater solubility and reproducible readings of maximum absorbance in 0.1N HCL, it was selected for further study. By serial dilution of standard stock of DPH (100µg/ml), the different dilutions of standard drug with concentration 10, 20, 30, 40, 50, 60, 70, 80, 90, 100µg/ml were prepared and calibration curve was prepared by plotting graph between absorbance and concentration (µg/ml) (Fig. 2). The results of linearity are presented in table 1

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 2, February 2024

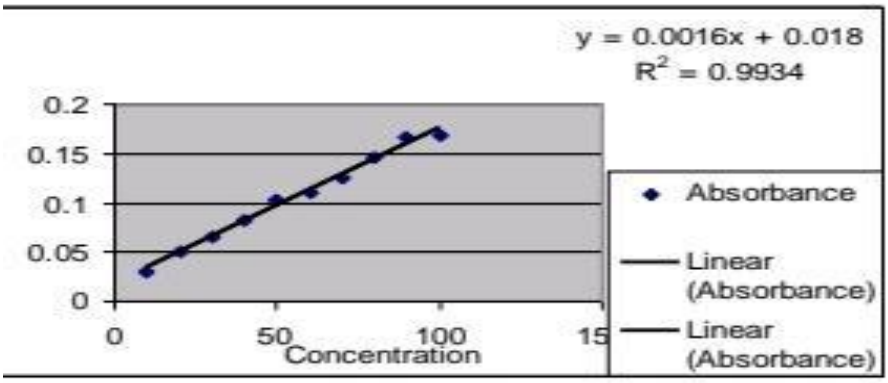

Fig. Calibrations curve of Diphenhydramine Hydrochloride .

The data was statistically validated by means of least square regression method. Calibration curve data were constructed in the range of the concentrations of 10µg/ml to 100µg/ml. Beer's law was obeyed over this concentration range. The regression equation was found to be Y =0.0016x +0.016. The correlation coefficient (r2) of the standard curve was found to be 0.9934. The stock solutions and working standards were made in 0.1N HCL.

Table 1. Analytical Validation

Parameters :	**Result :**
Absorption Maxima	**258nm**
Linearity range (ug/ml)	**10-100**
Standard regration equation	**Y=0.016x-0.018**
Correlation Coefficient ®	**0.9934**
Accuracy (% recovery ±SD)	**98.97±0.2989**
Precission (%)	**1.0280±0.131**
LOD (µg/ml)	**3.130**
LOQ (µg/ml)	**9.401**
%Drug found in Soft Gelatin Capsule	**99.06**

Conclusion

The developed UV spectrophotometric method is simple, précised, accurate and reproducible for the estimation of DPH in soft gelatin capsule formulation. Limit of detection was found to be 3.130 and the Limit of quantification to be 9.401. The calibration curves showed linearity between the absorbance and concentration (10-100µg/ml) and correlation coefficient was found to be 0.9934. The percentage recovery was found to be 98.97±0.2989and the sample solution was stable for up to 60minutes at 40°C. The proposed method will be suitable for the analysis of DPH in Soft gelatin capsule pharmaceutical formulation**.**

Method:

Chemicals (Preparation stock solution of Diphenhydramine HCI) :

Prepare 1000µg. ml of standard DPH by dissolving 0.1g of it in 100ml of distilled water and prepare a diluted solution towork.

General procedures :

After selecting the best conditions, the color output is set of the following:

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 2, February 2024

Coupling the diphenhydramine HCl with the Thymol blue reagent:

In a 50ml separation funneladded 0.5ml of 100ug/ml. of standard DPH add 0.5 ml. of Buffer phosphate at pH 4.0 and which coupling with 0.5 ml. of the 2.143 x 10 Thymol blue reagent. The dye formed is treated with 4.0 ml. of chloroform in order to separate it. Then added 0.5ml distilled water to complete the final volume to 6.0 ml., and shake the solution for 4.0 min. to separate the yellow dye. The product dye was measured at a wavelength 400nm.

Linearity and absorption spectra :

The calibration curve was done after achieve to the optimum conditions for determination of diphenhydramine HCl. The calibration curve completing by using an increasing concentration of drug range within 10-50 µg/ml., a calibration curve was obtained as shown in (Fig. 8& 9). It was All parameters of the analytical data were calculated such asdetection limit (DI.), quantification limit (QL) and relative standard deviation (RSD) as shown In table.

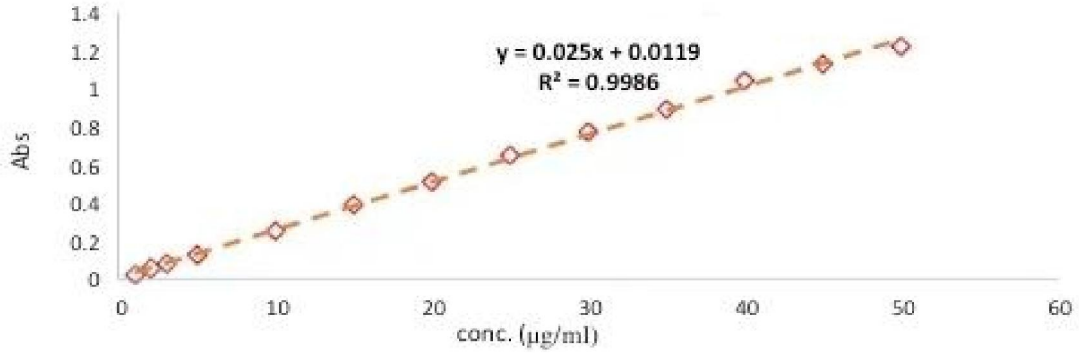

Fig. Spectrum UV-Visible absorption of the final compound of diphenhydramine hydrochloride with thymol blue reagent at wavelength 400 nm.

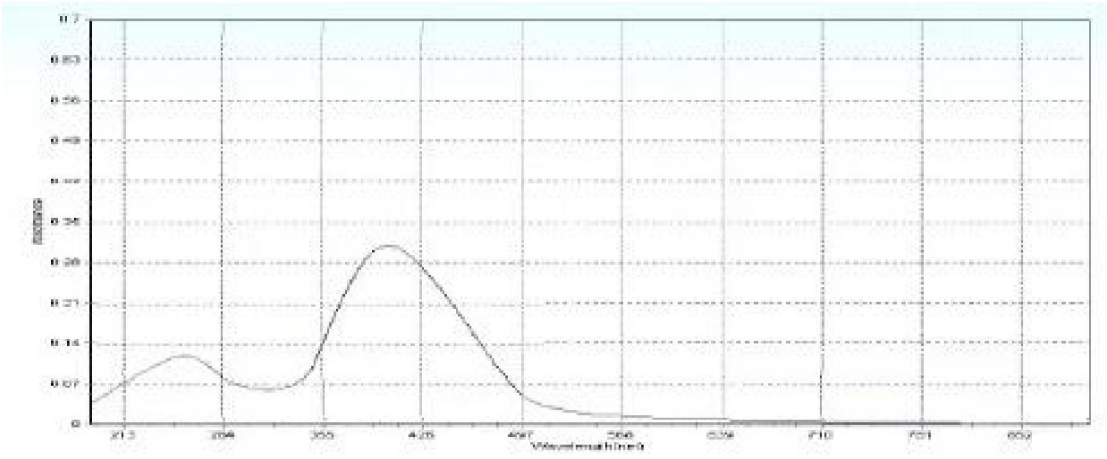

Fig. The calibration curve for final complex dye resulting by coupling diphenhydramine hydrochloride with thymol blue reagent.

Parameters :	**Result**:
Colour	Yellow
Lambda (nm)	400
Linearity range uz ml.	10-50
Molar absorptivity (4)	10x18
Regression equation	Y=0.0254 +0.0119
Slope	0.0250

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 2, February 2024

Intercept	0.0119
Sandell's sensitivity S (ug. Cm)	0.0399
Coefficient of determination R	0.9986
Limit of detection LOD µg ml.	0.0786
Limit of quantification pg. nl.	0.2358
Mean RSD%	0.211
Distribution coefficient (D)	17.118

III. CONCLUSION

The proposed method isaccurate, simple and expensive for the assay of diphenhydramine HCl and without interference for common pharmaceutical askditives. The proposed method is higher sensitive. This study is successfully for estimation diphenhydramine HCI drug in pure and commercial preparations by Improve experimental conditions.

REFERENCES

[1] LL. Schroeck, J. Ford. EL. Conway, K.E. Kurtzhalts, M.E. Gee, K.A. Vollmer, K.A. Mergenhagen, Review of Safety and Efficacy of Sleep Medicines in Older Adults, Clinical Therapeutics, 2016,38 2340-2372. https://doi.org/10.1016/j.clinthera. 2016.09.010.

[2] YF. Hao, J-G. hang, Origin and evolution of china pharmacopoeia and its implication for traditional medicines, Mini Reviews in Medicinal Chemistry. 2015.15 595-603.

[3] B. Pharmacopoeia, British pharmacopoeia, 2016

[4] C. Southan, JL.. Sharman, H.E. Benson, E. Faccenda, AJ, Pawson, S.P.H. Alexander, O.P. Buneman, A.P. Davenport, J.C. McGrath, JA. Peters, others, The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands, Nucleic Acids Research.2015.44 D1054-D1068.

[5] S. ZIERLER. D. PUROHIT, Prenatal antihistamine exposure and retrolental fibroplasia, American Journal of Epidemiology, 1986,123 192-196.

[6] HE. Brown, How to stabilize an acutely psychotic patient, 2012

[7] Product specification, Building https://doi.org/10.1080/09613219308727250, Research & Information. 2017.21 21-22.

[8] H.A. Merey. N.K. Ramadan, S.S. Diah, A.A. Moustafa, Validated UPLC method for the determination of guaiphenesin, oxeladin citrate, diphenhydramine, and sodium benzoate in their quaternary mixture used in treatment of cough, in the presence of guaiphenesin related substance (guaiacol), Chemical Papers, 2018, https://doi.org/10.1007/s11696-018-0454-2.

[9] N.M. Njuguna, K.O. Abuga, FN. Kamau, G.N. Thoithi, a liquid chromatography method for simultaneous determination of diphenhydramine, promethazine, chlorpheniramine and ephedrine in cold-cough syrups, 2017.51 153-158. https://doi.org/10.1007%11094-017-1574-7.

[10] P. Taylor, S.M. Kishk, 1. Salama, S. Mostafa, M. El-sadek, Journal of Liquid Chromatography & Related stability-indicating chromatographic method for the determination of benzonatate, diphenhydramine, guaifenesin and phenylephrine, n.d.

[11] D.D. Rao, P.V, Rao, S.S. Sait, K. Mukkanti, D. Chakole, Simultaneous Determination of Ibuprofen and Diphenhydramine Citrate in Tablets by https://doi.org/10.1365-10337-009-0977-3. Validated LC. 2009. 1133-1136.

[12] D.D. Rao, S.S. Sait, K. Mukkanti, Development and Validation of an UPLC Method for Rapid Determination of Ibuprofen and Diphenhydramine Citrate in the Presence of Impurities in Combined Dosage Form. 2011.49.281-286.

[13] K.A. Mirzaeva, U.G. Gamzaeva, ARTICLES A Molibdenum (VI) Gallein Diphenhydramine lon Associate and Its Application to the Extraction-Photometric Determination of Diphenhydramine, 2018,73 661-666. https://doi.org/10.1134/S1061934818070110,

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 2, February 2024

[14] A.M. El-didaniony, M.A. Moustafa, Spectrophotometric determination of diphenhydramine hydrochloride in pharmaceutical preparations and biological fluids via ion-pair formation, Arabian Journal of Chemistry, 2010,3 265-270. https://doi.org/10.1016/j.arabjc.2010.06.014.
[15] S. Tatar, F. Tuncel, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy Spectrophotometric method for the determination, validation, spectroscopic and thermal analysis of diphenhydramine in pharmaceutical preparation, https://doi.org/10.1016/j.saa. 2010.05.031 2010.77 324-329
[16] R.P. Bhole, S.S. Shinde, S.S. Chitlange, S.B. Wankhede, A High-Performance Thin Layer Chromatography (HPTLC) Method for Simultaneous Determination of Diphenhydramine Hydrochloride and Naproxen Sodium in Tablets, 2015, 47-51. https://doi.org/10.4137/ACLS31506.
[17] C.Z. Hao, X.C. Hai, S.T. Ao, L.I. Minghua. K.J. lao, Selective Determination of Diphenhydramine in Compound Pharmaceutical Containing Ephedrine by Flow-Injection Electrochemiluminescence, 2008,24 535-538.
[18] C. Wang. G. Fan, M. Lin, Y. Chen, W. Zhan, Y. Wu, Development and validation of a liquid chromatography/ tandem mass spectrometry assay for the simultaneous determination of d amphetamine and diphenhydramine in heagle dog plasına and its application to a pharmacokinetic study, 2007,854 48-56, https://doi.org/10.1016/j.jchromb.2007.03.038,
[19] Spectrophotometric Determination of Ketotifen Fumarate in Pure and Pharmaceutical Preperations by Bromophenol Blue Reagent Abstract, 2011, 24.
[20] Z.J. Azar, M. Afshar, DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING ION- PAIR RP-HPLC METHOD FOR DETERMINATION OF BETHANECHOL WITH UV DETECTION APPLICATION, 2019,53 376 382 https://doi.org/10.1007/s11094-019-02008-w.
[21] Y. Hamid, A fast and green preconcentration method based on surfactant ion pair-switchable solvent dispersive liquid liquid microextraction for determination of phenazopyridine in pharmaceutical and biological samples, Journal of the Iranian Chemical Society, 2018,https://doi.org/10.1007/13738-018- 1378-z
[22]https://ijpsr.com/bft-article/development-and-validation-of-uv-spectrophotometric-method-for-estimation-of-diphenhydramine-hydrochloride-in-soft-gelatin-capsule/
[23]https://www.researchgate.net/publication/251570591_DEVELOPMENT_AND_VALIDATION_OF_UV_SPECTROPHOTOMETRIC_METHOD_FOR_ESTIMATION_OF_DIPHENHYDRAMINE_HYDROCHLORIDE_IN_SOFT_GELATIN_CAPSULE/
[24]https://scholar.google.co.in/scholar?q=review+paper+on+uv.+spectroscopy+of+diphenhydramine+hcl&hl=en&as_sdt=0&as_vis=1&oi=scholart#d=gs_qabs&t=1705983356952&u=%23p%3DArQuCz2SCHAJ
[25]https://scholar.google.co.in/scholar?q=review+paper+on+uv.+spectroscopy+of+diphenhydramine+hcl&hl=en&as_sdt=0&as_vis=1&oi=scholart#d=gs_qabs&t=1705983322268&u=%23p%3DvrImcmTH22cJ
[26]https://scholar.google.co.in/scholar?q=review+paper+on+uv.+spectroscopy+of+diphenhydramine+hcl&hl=en&as_sdt=0&as_vis=1&oi=scholart#d=gs_qabs&t=1705983287127&u=%23p%3DFUn73CM5OpoJ
[27]https://scholar.google.co.in/scholar?q=review+paper+on+uv.+spectroscopy+of+diphenhydramine+hcl&hl=en&as_sdt=0&as_vis=1&oi=scholart#d=gs_qabs&t=1705983257342&u=%23p%3DRJmhRxqp_L0J