Colorimetric Determination of Phenylephrine hydrochloride Drug by Diazotization Reaction

Hajir S. Alteemi1, Kassim H. Kadim2

1,2 Chemistry Department/ College of science/ University of Babylon/Iraq

Corresponding author:

Hajir S. Alteemi

hajaralteemii@gmail.com

Abstract:

By diazotization coupling reaction is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution as a method for estimation drug of Phenylephrine hydrochloride by reliant on azo-coupling reaction between (PHE)HCl and Clonazepam as a reagent. To reach to the aim of obtaining the colored product with a maximum absorbance 466nm. The linearity range for Phenylephrine hydrochloride was (0.5-9µg/ml) while the detections limit 0.006and quantification limit 0.02µg/ml The Molar Absorptivity$2.5457 \times 10^4$L/mol.cm with Sandal’s sensitivity 0.01µg/cm2. Eventually these methods were successfully completed to determination of PHE.HCl in pure and commercial form.

Keywords: Spectrophotometric Determination, Phenylephrine hydrochloride, Azo-coupling Reaction.

Introduction:

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. The majority of compounds of pharmaceutical interest are colorless and hence studies of their light absorbance characteristics, the method developed were naturally only qualitative (or at best semi-quantitative)
procedure for detection compounds [1]. UV-Vis spectroscopy is a widely used analytical technique in quality assurance/quality control and pharmaceutical research. UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes[2], such as transition metal ions, highly conjugated organic compounds, and biological macromolecules[3, 4].

Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied. The development of the instrumental analysis had an important effect to improve the methods of analyzing drug compounds, as well as in the standards used by the manufacturers of drugs. The most important of these methods are[5] include: Chromatography[6] which is most frequently used in pharmaceutical analysis by its ability to achieve high-resolution separation and it is include: chromatography[7], Column Ion-exchange chromatography Gel-permeation (molecular sieve) chromatography[8] Affinity chromatography[9], Paper chromatography[10], Thin-layer chromatography[11], Gas chromatography (GS)[12], Dye-ligand chromatography[13], Hydrophobic interaction chromatography[14], Pseudo affinity chromatography[15], High-pressure liquid chromatography (HPLC)[16]. These methods required to use very accurate concentrations and high purity. In other hand the application of electrochemical techniques[17] in the analysis of drugs and pharmaceuticals has increased greatly over the last few years. An azo coupling[4] is an organic reaction between a diazonium compounds and another aromatic compound that produces an azo compound. In this electrophilic aromatic substitution reaction, the aryldiazonium cation is the electrophile and the activated arene is a nucleophile. In most cases, including the examples below, the diazonium compound is also aromatic. The process of conversion of primary aromatic amines into its diazonium salt is called Diazotization[18]. Diazonium salts are important synthetic intermediates that can undergo coupling reaction to form azo dyes and electrophilic substitution reaction to introduce functional groups. Phenylephrine hydrochloride (PHP), [(R)-1-(3-hydroxyphenyl)-2-(methylamino) ethanol hydrochloride], is a white crystalline powder, and belongs to the group of medicines called sympathomimetics[19, 20]. It acts stimulating the alpha receptors in certain areas of the body. It is used locally, as decongestant, for nonspecific and allergic conjunctivitis, sinusitis and nasopharyngitis[21]. PHP nasal drops[22] are used for treating symptoms such as runny nose, sneezing, itching of the nose and throat. Phenylephrine Hydrochloride has molecular weight equal to 203.66 g/mol shown in figure(1)

![Phenylephrine Hydrochloride](image)
Figur(1): Structure of Phenylephrine Hydrochloride

Experimental Apparatus
- Each the absorption are measurement T-8 .UV-Visible Spectrophotometric (P.G) Instrumental (I.T.D) and used (1.0) cm quartz cells.
- Heating -cooling water bath (Hakk,F)
- Sartourious B.P-3015-Germany
- Oven (B.S) size two

MATERIALS
Each chemical materials are used in this research highly purity from They were prepared as following method:
- Clonazepam reduced (CZP): It obtainable from State the company of the Drug Industry with the Medical Apparatus; (S.D.I) / (Iraq). The stock solution of reduced clonazepam (CZP) at 0.001M prepared from taken 0.0315 gm of (CZP )and then dissolved it 25 ml of ethanol with 2ml from the distilled water and 2ml from concentrated HCl (~11.64) M and add1.89gm from zinc powder, leave he mixture a bout (15 min) after that filtered by filter paper in a volumetric flask, and completed volume with distilled water, as a stock solution which taking away from the light[23]
- Phenylephrine hydrochloride: Solution 100ppm Prepare by dissolve 0.005 gm of PHP in distilled water and then diluted to mark the volume with distilled water to obtain (100) ppm of PHP
- Sodium nitrite (NaNO2) [0.1]M: It supplied from (BDH-Chemical I.T.D) by dissolve (0.1725gm) as a pure material in (25 ml) Distilled water. this solution prepared daily
- Sulfamic acid [0.2 ]M: Prepared by dissolving (0.485gm) from the substance in 25 ml distilled water
- Hydrochloric acid 1M: it was provided by from (GCC) at 98% prepared by diluting appropriate concentration hydrochloric acid at 25ml distilled water.

Procedure:
In the series of calibrated flask of 10ml aliquots 0.2ml from HCl (1M) with 0.6ml of (0.1M) Sodium Nitrite, add 1ml from Clonazepam drug (0.001)M as a reagent and then add 0.6ml from Sodium Nitrate. Wait for (10min) in order to complete the (azo-coupling) reaction successively after that 1.2ml from ammoniumhydroxide (4M) and 0.5ml from PHE.HCl and complete with distilled water to the mark of calibrated flask. The obtained solutions can be sitting stable for (60 min) at room temperature and absorbance 466nm was measured versus blank reagent.
Procedure for Pharmaceutical Preparations

Nasal drop (Nasophrine): Take 4ml from Nasophrine contain 10ml with concentration (0.25g/100ml) and dilute volume in volumetric flask 100ml to the volume mark.

RESULTS AND DISCUSSION

In the procedure, effects of different parameters on color characteristics for azo dye by gain optimum conditions reaction.

Effect of Acid Volume

To be sure through experiment of existence effect acid on the absorbance product, using hydrochloric1M with series different volumes (0.1-1ml).0.2ml from acid give the perfect absorbance. Figure (2)

![The effect of acid HCl](image)

Fig. 2: Effect of Volume of Hydrochloric Acid

Effect of Sodium Nitrite Volume

Series of different volume (0.2-1.2 ml) from Sodium Nitrite (0.1M) and found that the perfect absorbance obtain at 0.6ml of Sodium Nitrite while the best time (5min) that enough the result colored intenseness have been for complete diazotization of PHE.HCl.
Effect of Reagent (CZP):

Volume Series of different concentration from reagent (0.2-1.4ml) effect on color intense and the absorbance measured and found (1ml) was the optimum volume as shown in Fig (4)

Effect of Ammonium hydroxide Volume:

Series of different concentration from Ammonium hydroxide (0.2-1.2ml) effect on color intense and the absorbance measured and found (1.2ml) was the optimum volume as shown in Fig (5)
Fig (5): Effect of Ammonium hydroxide Volume

**Effect of Reaction Time**

To be sure effect on the stable intense colored prepared phenylephrine hydrochloride with Clonazepam under the perfect conditions and measured at different intervals time approach at 60 min the result obtain refer to that the color intensity improve and stay constant at least 60 min [24].as shown in Fig (6)

Fig. 6: Effect of Reaction Time

**Absorption Spectra**

The reaction of Phenylephrine hydrochloride with Clonazepam under optimum conditions give orange intense color as product at maximum wave length 466nm while solution blank colorless absorption as shown in Fig (7)
Fig. 7: Spectrum of Phenylephrine hydrochloride with Clonazepam

Calibration Curve

By benefit from the optimum conditions. The linearity range for Phenylephrine hydrochloride was on the range (0.5_9µg/ml) Fig. (8) with slope 0.1011L.mg⁻¹ and Correlation Coefficient 0.9975 an intercept was 0.0207 . The Molar Absorptive2.0366×10⁴ L/mol.cm⁻¹ with Sandal’s sensitivity (0.01µg.cm²) detections limit (0.006) and quantification limit (0.02µg.ml). The characteristics of the spectrophotometric method improved are as shown in Table (1)
Table 1: Characteristics of the Method Developed for Determination PHE. HCl

| Parameters          | Value                                  |
|---------------------|----------------------------------------|
| Regression equation | $Y=0.1011+0.0207$                      |
| Slope               | 0.1011                                 |
| Correlation Coefficient | 0.9975                              |
| Linearity range     | 0.05-9 mg/ml                           |
| Molar Absorptivity  | $2.0366 \times 10^4$                   |
| Sandal’s sensitivity| 0.01 µg/cm²                            |
| L.O.D               | 0.006 µg/ml                            |
| L.O.Q               | 0.02 µg/ml                             |

**Precision and Accuracy**

To determinate the precision and accuracy that checked by measuring three solution of PHE.HCl and repeated test three times. The result values give a good satisfactory accuracy and precision show in Table (2).

Table 2: Precision and Accuracy

| Concentration of PHE.HCL | %E | Rec* | %RSD  |
|--------------------------|----|------|-------|
| Taken | Found |      |       |
| 1     | 1.020 | 2    | 102   | 1.76  |
| 3     | 2.401 | 2.5  | 102.5 | 0.63  |
| 5     | 5     | 0    | 100   | 0.579 |

*Average of three determinations
Application

The suggested method is applying for determination Phenylrphrine hydrochloride in Nasophrine (Nasal drop) the results explain in Table (1) show values of a good recovery obtainable, The proposed method scored more successfully and effectively for the standard method as appear in Table(3).

Table 3: Results Show the Comparison between Standard and Proposed Method

| Phenylephrine(Nasophrine) | %E | Rec* | %RSD |
|---------------------------|----|------|------|
| Taken | Found |
| 1 | 1.02 | 3 | 103 | 1.65 |
| 3 | 2.919 | 0.1 | 100.1 | 1.05 |
| 5 | 4.976 | -2.4 | 97.6 | 0.54 |

*Average of three determinations

CONCLUSION

A precise, simple and accurate spectrophotometric method have been improved investigation of trace an amount of PHE. HCl adopted on (diazonitization - coupling) reaction with Clonazepam with present of sodium nitrate (0.01M) ,(1M) HCl and Ammonium hydroxide in pure and doses forms.

Acknowledgment

This work dedicated to my supervisor prof. Dr. Kassim H. Kadim “mercy and peace for his pure spirt.

Reference

1. Gorog, S., Ultraviolet-visible spectrophotometry in pharmaceutical analysis. 2018: CRC press.
2. Aljeboree, A.M., Adsorption and Removal of pharmaceutical Riboflavin (RF) by Rice husks Activated Carbon. International Journal of Pharmaceutical Research 2019. 11( 2): p. 255-261.
3. Aljeboree, A.M. and A.N. Alshirifi, Oxidative coupling of Amoxicillin using 4-Aminoantipyrine: Stability and higher sensitivity. Journal of Physics: Conference Series, 2019. 1294(5): p. 052001.
4. Smith, M.B., March’s advanced organic chemistry: reactions, mechanisms, and structure. 2020: John Wiley & Sons.
5. Willard, H.H., et al., Instrumental methods of analysis. 1988.
6. Watson, D.G. and R. Edrada-Ebel, *Pharmaceutical Analysis, A Textbook for Pharmacy Students and Pharmaceutical Chemists, 3: Pharmaceutical Analysis*. 2012: Elsevier Health Sciences.

7. Rezk, M.R., et al., *Chromatographic determination of cyclopentolate hydrochloride and phenylephrine hydrochloride in the presence of their potential degradation products*. Journal of AOAC International, 2017. 100(2): p. 434-444.

8. Winzor, D.J., *Analytical exclusion chromatography*. Journal of biochemical and biophysical methods, 2003. 56(1-3): p. 15-52.

9. Mallik, R. and D.S. Hage, *Affinity monolith chromatography*. Journal of separation science, 2006. 29(12): p. 1686-1704.

10. Block, R.J., E.L. Durrum, and G. Zweig, *A manual of paper chromatography and paper electrophoresis*. 2016: Elsevier.

11. Zlatkis, A. and R.E. Kaiser, *HPTLC-high performance thin-layer chromatography*. 2011: Elsevier.

12. Jennings, W., *Gas chromatography with glass capillary columns*. 2012: Elsevier.

13. Labrou, N., K. Mazitsos, and Y. Clonis, *Dye-ligand and biomimetic affinity chromatography*. Handbook of affinity chromatography. New York: Marcel Dekker, Inc, 2005: p. 231-55.

14. Alpert, A.J., *Electrostatic repulsion hydrophilic interaction chromatography for isocratic separation of charged solutes and selective isolation of phosphopeptides*. Analytical chemistry, 2008. 80(1): p. 62-76.

15. Coskun, O., *Separation techniques: chromatography*. Northern clinics of Istanbul, 2016. 3(2): p. 156.

16. Wu, N. and A.M. Clausen, *Fundamental and practical aspects of ultrahigh pressure liquid chromatography for fast separations*. Journal of separation science, 2007. 30(8): p. 1167-1182.

17. Gadhari, N.S., B.J. Sanghavi, and A.K. Srivastava, *Potentiometric stripping analysis of antimony based on carbon paste electrode modified with hexathia crown ether and rice husk*. Analytica chimica acta, 2011. 703(1): p. 31-40.

18. El-Desoky, H.S., M.M. Ghoneim, and N.M. Zidan, *Decolorization and degradation of Ponceau S azo-dye in aqueous solutions by the electrochemical advanced Fenton oxidation*. Desalination, 2010. 264(1-2): p. 143-150.

19. Gupta, P.S., *Imperialism and the British Labour Movement, 1914-1964*. 2002: SAGE Publications India.

20. Aljeboree, A.M. and A.N. Alshirifi, *Colorimetric Determination of phenylephrine hydrochloride drug Using 4-Aminoantipyrine: Stability and higher sensitivity*. Journal of Pharmaceutical Sciences and Research, 2018. 10(7): p. 1774-1779.

21. Al-Abachi, M.Q. and S. Subhi, *Flow injection-spectrophotometric determination of phenylephrine hydrochloride and amoxicillin trihydrate in pharmaceutical preparations*. Al-Nahrain Journal of Science, 2013. 16(1): p. 42-52.
22. Soleimani, G., M. Akbarpour, and M. Mohammadi, *Safety and efficacy of phenylephrine nasal drops in bronchiolitis*. Iranian journal of pediatrics, 2014. 24(5): p. 593.

23. Al-Abachi, M.Q., H. Hadi, and F.J. Yousef, *Flow Injection-Spectrophotometric Determination of Vancomycin Hydrochloride in Pharmaceutical Preparations Using Diazotized Metoclopramide*. Al-Nahrain Journal of Science, 2015. 18(1): p. 9-19.

24. Aljeboree , A.M. and A.N. ALSHIRIFI, *Spectrophotometric Determination of phenylephrine hydrochloride drug in the existence of 4- Aminoantipyrine: Statistical Study*. International Journal of Pharmaceutical Research, 2018. 10(4)