Spectrophotometric Determination of Bromhexine Hydrochloride by Diazotization and Coupling Method in Its Pharmaceutical Preparations

Saad Hasani Sultan*1, Zainab Walid Majed2
1Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq
2Department of New and Renewable Energies, College of Science, University of Mosul, Mosul, Iraq

Received: 10/10/2019 Accepted: 16/4/2020

Abstract
A simple, fast, and sensitive spectrophotometric method was suggested for the determination of Bromhexine Hydrochloride (BHH) in its pharmaceutical formulations. The method depends on the diazotization of BHH by sodium nitrite in acidic medium to produce the corresponding diazonium salt. The latter is coupled with phloroglucinol reagent in alkali medium to form a yellow water soluble azo-dye which has a maximum absorption at 405 nm with a molar absorptivity of 2.7×10^4 L.mol^{-1}.cm^{-1} and Sandell’s sensitivity of 0.01517 µg.cm^{-1}. Beer’s low is obeyed within a concentration range of 0.25-15 µg.mL^{-1} of BHH. The LOD and LOQ values of the proposed method were 0.087 µg.mL^{-1} and 0.293 µg.mL^{-1}, respectively. The proposed method was validated with standard methods and successfully applied to the determination of Bromhexine in its pharmaceutical formulations as tablets, syrup, and injections.

Keywords: Bromhexine hydrochloride, diazotization and coupling, phloroglucinol, spectrometry.

Introduction
Primary aromatic amines react with nitrous acid to yield diazonium salts which are coupled with...
phenols in alkali medium to produce a colored azo-dyes; this is one of the most important reactions in organic chemistry [1]. From the point of view of analytical chemistry, this reaction is considered as highly sensitive because of the intense colors of the formed azo dyes. Thus, this reaction was used widely to assay the primary aromatic drugs [2-6] and for the synthesis of indicators [7].

Bromhexine Hydrochloride (BHH) (Fig.1.) is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus [8]. It exhibits its action by increasing bronchial secretion and reducing their viscosity. Also, this agent was recently recommended as a new therapy for pathological conditions, such as alcoholic chronic pancreatitis where increased viscosity is involved [9]. It was developed in the research laboratory of Boehringer Ingelheim in the late 1950s as an active ingredient for pharmaceutical use. It was then introduced in 1963 under the trademark of Bisolvon® [10]. BHH is chemically known as N-(2-Amino-3,5-dibromobenzyl)-N-methylcyclohexanamine hydrochloride, and it is a white or almost white crystalline powder [11].

![Figure 1-Bromhexine Hydrochloride](image)

Many methods have been reported for the estimation of BHH in its pure form or pharmaceutical preparations. These methods include spectrophotometry [12-14], chromatography [15-18], volumetry [19,20] and capillary electrophoresis [21]. However, spectrophotometric methods are still more spread than other techniques due to their simplicity and inexpensive equipment. In this work, we developed an easy and sensitive spectrophotometric method for assaying BHH in its pharmaceutical formations by converting the amino group in the drug to a diazonium salt, which is coupled with phloogluclin to produce a colored azo dye, the concentration of which is related with the original concentration of the studied drug.

**Experimental Part**

**Instruments**
The spectrophotometric measurements were performed on CE CILL 2700 UV-Vis spectrophotometer using 1.0 cm plastic cell.

**Chemicals and samples.** All chemicals used in this work were of analytical grade.

**Bromhexine hydrochloride solution (100 µg.mL⁻¹)**

Pure Bromhexine hydrochloride (0.0100 g, State Company for drug industries and medical appliances, Samarra, Iraq, SDI) was dissolved in 100 mL of distilled water with gentle heating.

**Hydrochloric acid solution (1N)**

This solution was prepared by diluting 8.6 mL of the concentrated acid (11.6 N, (Thomas Baker) to 100 mL by adding distilled water.

**Sodium nitrite solution (10000 µg.mL⁻¹)**

of sodium nitrite (1.0 g, BDH) was dissolved in a sufficient amount of water and the volume was completed to 100 mL.

**Sulphamic acid solution (30000 µg.mL⁻¹)**

This solution was prepared by dissolving 3.0 g of sulphamic acid (BDH) in 100 mL distilled water.

**Phlorogluclin solution (1000 µg.mL⁻¹)**

A weight of 0.1 g of this reagent (Fluka) was dissolved in 100 mL distilled water.

**Sodium hydroxide solution (1 M)**

This solution was prepared by the appropriate dilution of the concentrated solution of 10 M (BDH) with distilled water to 1 L using volumetric flask.

**SDS (1×10⁻³ M)**

This solution was prepared by dissolving 0.0288 g of sodium dodecyl sulfate in 100 mL distilled water.

**CPC (1×10⁻³ M)**
This solution was prepared by dissolving 0.0339 g of cetylpyridinium chloride in 100 mL distilled water.

**Triton X-100 (1.5×10^{-2} M)**

This solution was prepared by dissolving 1.0 g of pure reagent in 100 mL distilled water.

**Tablets solution (100 µg.mL^{-1})**

Ten tablets (each tablet contains 8 mg of BHH) were crushed well and an accurate weight of the powder (equivalent to 0.01 g of BHH) was dissolved in a sufficient amount of water with gentle heating. The residue was filtered into 100 mL volumetric flask and the volume was completed to the mark by repeated washing with distilled water.

**Syrup solution (100 µg.mL^{-1})**

A volume of 12.5 mL of syrup solution (each 5mL contains 4 mg of BHH) was transferred into 100 mL volumetric flask and the volume was completed to the mark with distilled water.

**Injections solution (100 µg.mL^{-1})**

The content of three ampoules (each 2 mL ampoule contains 8 mg of BHH) was mixed, 2.5 mL of the resulting solution was transferred into 100 mL volumetric flask, and the volume was made up to the mark with distilled water.

**Preliminary Investigations**

A volume of 1.0 mL of the standard BHH solution (100 µg.ml^{-1}) was added to a 20 mL volumetric flask, followed by 2.0 mL of 1 N HCl solution and 1.0 mL sodium nitrite solution (10000 µg.ml^{-1}). The contents were left for one min, then 1.0 mL of sulphamic acid solution (30000 µg.ml^{-1}) was added with occasional shaking for one min. After that, 1.0 mL of phloroglucinol reagent (1000 µg.ml^{-1}) and 3.0 mL of 1.0 M sodium hydroxide were added and the volume was diluted to the mark by distilled water. A yellow colored product was obtained with λ_{max} 405 nm against the blank solution.

**Results and Discussion**

100 µg of BHH with 20 mL as a final volume was used for the next investigations.

**Principle of the method**

The first step in the proposed method involves diazotization of BHH in aqueous solution to form the corresponding diazonium salt, which is coupled with phloroglucinol in basic medium to produce a colored azo dye, as follows.

Optimization of reaction circumstances

The influence of different parameters on the absorption intensity of the produced azo dye was studied. These included the quality and quantity of acids, the amount of sodium nitrite used for diazotization process, the amount of sulphamic acid needed for destruction of excess sodium nitrite, the amount of phloroglucinol reagent, and finally the quality and quantity of suitable bases that showed maximum color development.

**Effect of acids used**

The influences of the amount and type of acids on the diazotization process were investigated by adding various volumes of acids. The results listed in the Table-1 and Figure-2 demonstrate that 2.5 mL of 1N HCl is the typical volume since it showed the highest absorbance.
Table 1-Effect of acids on absorbance

| Acid type | 1 N | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
|-----------|-----|-----|-----|-----|-----|-----|
| HCl       | 0.376 | 0.396 | 0.407 | 0.415 | 0.414 |
| HNO₃      | 0.366 | 0.375 | 0.371 | 0.386 | 0.413 |
| H₂SO₄     | 0.409 | 0.401 | 0.319 | 0.339 | 0.347 |
| CH₃COOH   | 0.070 | 0.061 | 0.053 | 0.058 | 0.057 |

Figure 2-Effects of acids on absorbance

Effect of sodium nitrite amount and time reaction

The quantity and the reaction time of sodium nitrate was examined by adding different amounts of sodium nitrite solution for different times. The obtained results in Table- 2 indicate that 250 µg.ml⁻¹ of NaNO₂ solution for 2 min was sufficient for complete diazotization of BHH and, therefore, it was selected for next experiments.

Table 2-Effects of sodium nitrite quantity and time reaction

| NaNO₂ (µg.mL⁻¹) sol. | 1 | 2 | 3 | 5 |
|----------------------|---|---|---|---|
| 50                   | 0.301 | 0.331 | 0.347 | 0.312 |
| 125                  | 0.404 | 0.411 | 0.410 | 0.340 |
| 250                  | 0.421 | 0.425 | 0.422 | 0.355 |
| 375                  | 0.420 | 0.399 | 0.418 | 0.363 |
| 500                  | 0.415 | 0.389 | 0.412 | 0.301 |

Effects of sulphamic acid

The excess of sodium nitrite should be removed due to its undesirable reactions [22]. According to previous studies, the most suitable agent for destruction of sodium nitrite is sulphamic acid. Therefore, the influence of sulphamic acid quantity and time reaction was investigated and the results listed in Table- 3 indicate that 1125 µg.ml⁻¹ of sulphamic acid solution with 3 min time reaction was typical for the complete removal of excess sodium nitrite.
Effects of coupling agent amount

The effects of phloroglucinol solution amount on the absorbance of the produced azo dye was studied by adding various amounts of the coupling agent to increase the amount of BHH. The results in Table-4 show that the concentration of 50 µg.mL⁻¹ of phloroglucinol solution was suitable for high sensitivity and, therefore, it was chosen for next experiments.

Table 4-Effects of coupling agent amount

| Phl. (µg.mL⁻¹) sol. | 25  | 50  | 100 | 150 | R²  |
|----------------------|-----|-----|-----|-----|-----|
| 12.5                 | 0.170 | 0.201 | 0.442 | 0.629 | 0.9835 |
| 25                   | 0.194 | 0.266 | 0.452 | 0.668 | 0.9952 |
| 50                   | 0.190 | 0.273 | 0.445 | 0.651 | 0.9975 |
| 75                   | 0.198 | 0.286 | 0.450 | 0.661 | 0.9964 |
| 100                  | 0.225 | 0.283 | 0.535 | 0.658 | 0.9843 |

Effect of base

The preliminary experiments revealed that the produced azo dye had the maximum color development in basic medium. Thus, the effects of different bases on color intensity of the formed azo dye was studied. The results in Table-5 indicate that 3 ml of 1 M NaOH solution showed a high absorbance and, therefore, it was selected for the next experiments.

Table 5-Effects of type and amount of base

| Type of media (1M) | Variable | mL of base added |
|-------------------|----------|------------------|
|                   |          | 1.0  | 1.5  | 2.0  | 2.5  | 3.0  | 3.5  |
| NaOH              | Abs.     | 0.278 | 0.290 | 0.300 | 0.417 | 0.448 | 0.442 |
|                   | λ_max (nm) | 417   | 407   | 405   | 408   | 405   | 402   |
|                   | pH       | 1.46  | 1.57  | 1.71  | 12.15 | 12.45 | 12.75 |
| KOH               | Abs.     | 0.290 | 0.336 | 0.344 | 0.381 | 0.403 | 0.411 |
|                   | λ_max (nm) | 4019  | 413   | 409   | 410   | 409   | 406   |
|                   | pH       | 1.58  | 1.63  | 1.67  | 7.05  | 8.57  | 12.14 |
| Na₂CO₃            | λ        | 0.238 | 0.273 | 0.296 | 0.353 | 0.375 | 0.384 |
|                   | λ_max (nm) | 419   | 413   | 409   | 410   | 409   | 406   |
|                   | pH       | 5.47  | 7.16  | 8.45  | 9.12  | 9.50  | 10.03 |
| NaHCO₃            | Abs.     | 0.270 | 0.275 | 0.279 | 0.281 | 0.283 | 0.296 |
|                   | λ_max (nm) | 427   | 421   | 419   | 417   | 416   | 412   |
|                   | pH       | 1.68  | 2.33  | 5.77  | 6.16  | 6.47  | 6.99  |
Effects of surfactants

The effects of 3 types of surfactants was investigated by the addition of 2 ml of sodium dodecyl sulphate (SDS), cetylpyridinium chloride (CPC) and Triton X-100 with various orders to the medium reaction. The obtained results showed that there is no improvement in absorbance and, thus, the studied surfactants were excluded in the subsequent experiments.

Effects of reaction time and stability

The influence of the reaction time on the formation and stability of the colored product was investigated and the results in Table-6 reveal that the colored azo dye was formed as soon as the base was added and that it stayed stable for minimum 2 hrs.

Table 6-Effects of reaction time and stability of the azo dye

| Time(min.) | After addition | 10  | 20  | 30  | 40  | 50  | 1 hrs. | 2 hrs. |
|------------|----------------|-----|-----|-----|-----|-----|--------|--------|
| 25         | 0.120          | 0.118| 0.118| 0.116| 0.118| 0.117| 0.115  |
| 200        | 0.665          | 0.648| 0.646| 0.641| 0.636| 0.635| 0.641  |

Final absorption spectra

The final absorption spectra of the colored azo dye, formed from the reaction between diazotized bromhexine and phloroglusinol reagent in basic medium, versus its reagent blank revealed a maximum absorption at 405 nm, in contrast to the phloroglusinol reagent blank which showed a slight absorption at the same wavelength (Figure-3).

![Final absorption spectra of 100 µg of BHH/20 mL according to the optimum conditions, measured against: (A) blank (B) distilled water (C) blank against distilled water](image)

General procedure and calibration curve

Increasing volumes of BHH covering concentrations from 5 to 300 µg were transferred to a series of 20 ml calibrated flasks, followed by the addition of 2.5 ml of 1M HCl and 0.5 mL of 10000 µg.ml⁻¹ sodium nitrite solution. The solution was left for a period of two min, then a 0.75 mL of 30000 µg.ml⁻¹ sulphamic acid solution was added with discontinuous shaking for 3 min to destroy the excess sodium nitrite. After that, 1 ml of 1000 µg.ml⁻¹ phloroglucinol and 3 ml of 1 M sodium hydroxide were added and the volumes were completed with distilled water. The absorbance was measured at 405 nm versus the reagent blank solution. Beer’s law was obeyed within concentrations ranging from 5 to 300 µg of bromhexine hydrochloride in final a volume of 20 mL (0.25-15 µg.ml⁻¹), as shown in Figure-4. The molar absorptivity and Sandell’s sensitivity were $2.7 \times 10^4$ l.mol⁻¹.cm⁻¹ and 0.01517 µg.cm⁻¹, respectively.
Nature of the produced azo dye

To establish the composition of the produced azo dye, Job’s and mole-ratio methods were adopted. The obtained results indicated that the dye has a composition of 1:1 of diazotized bromhexine to phloroglucinol. Figures (5 and 6), revealing that a mono azo dye was formed.

Hence, the structure of the produced azo dye may be drawn as follows:
The effects of interferences
The effects of some foreign compounds which are expected to exist in pharmaceutical formulation of bromhexine were studied by adding various amounts of additives using the recommended procedure. The results in Table-7 show that there is no significant interference in the determination of BHH in the presence of these additives.

Table 7- Study the effect of interferences

| Additives   | Recovery (%) of 100 µg BHH/µg of added compound |
|------------|-----------------------------------------------|
|            | 100 | 500 | 1000 |
| Glucose    | 96.3 | 95.8 | 95.5 |
| Lactose    | 100.2 | 96.7 | 95.8 |
| Starch     | 100.0 | 98.5 | 96.7 |
| Arabic gum | 98.7 | 99.2 | 96.3 |

Accuracy and precision of the proposed method
Under the optimum conditions, the accuracy and precision of the proposed method were investigated. The results are listed in Table 8 and indicated good accuracy and precision.

Table 8- Accuracy and precision

| Amount taken (µg) | Amount measured (µg) | Recovery (%) | Relative error (%c) | Relative standard deviation (%) |
|-------------------|----------------------|--------------|---------------------|--------------------------------|
| 50                | 49.98                | 99.96        | -0.04               | ±2.05                          |
| 100               | 100.84               | 100.84       | +0.84               | ±0.47                          |

* Average of five determinations

Application of the proposed method
To test the applicability of the suggested method, it was performed to test BHH in its pharmaceutical preparations. The results in Table-9 reveal that satisfactory results were obtained for the applied pharmaceutical forms which were in a good agreement with the label claims.

Table 9- Application of the proposed method

| Pharmaceutical preparation | Amount taken (µg) | Present Method | Standard addition Method |
|----------------------------|------------------|---------------|--------------------------|
|                            | Amount measured(µg) | Recovery (%) | Amount measured(µg) | Recovery (%) |
| Tablet(Bisolvon), Nile Co., Egypt | 50 | 49.0 | 98.0 | 49.3 | 98.6 |
| Syrup(Solvodin), SDI, Iraq   | 50 | 47.6 | 95.2 | 48.0 | 96.0 |
| Ampules(Bisolvon), Nile Co., Egypt | 50 | 49.8 | 99.6 | 50.1 | 100.2 |
|                             | 100 | 99.2 | 99.2 | 102.5 | 102.5 |

*Average of five determinations

Also, the performance of the proposed method was evaluated by t-test and F-test compared with the standard method (British Pharmacopoeia, 2013) for a 95% confidence level and eight degrees of
freedom. The results listed in Table-10 indicate no significant differences between the suggested and standard methods for BHH analysis.

Table 10-t-test analysis of the proposed method

| Pharmaceutical preparation                  | Recovery*, % | t-test | F-test |
|--------------------------------------------|--------------|--------|--------|
| Tablet (Bisolvon), Nile Co., Egypt          | 98.1         | ± 0.54 | 3.06   |
| Syrup (Solvodin), SDI, Iraq                 | 95.6         | ± 0.79 | 4.51   |
| Ampules (Bisolvon), Nile Co., Egypt         | 99.5         | ± 0.37 | 2.33   |

Comparison of the proposed method

To make a comparison between the present method and the reported spectrophotometric method, some analytical variables were listed in Table 11, which indicate that the present method has the highest sensitivity, in addition to the fact that the proposed method was applied for three forms of pharmaceutical drugs.

Table 11-Comparison with another spectrophotometric method

| Analytical parameters                  | Present method       | Literature method [5] |
|----------------------------------------|----------------------|-----------------------|
| **Reagent**                            | Phloroglucinol       | Chromotropic acid     |
| **pH**                                 | 12.45                | Acidic                |
| **Temperature(C°)**                    | R.T                  | R.T                   |
| **Development time(min.)**             | After dilution       | 10                    |
| **λ max(nm)**                          | 405                  | 507                   |
| **Beer’s law range(µg.ml⁻¹)**          | 0.25-15              | 2-60                  |
| **Molar absorptivity (L.mol⁻¹.cm⁻¹)**  | 2.7×10⁴              | 1.5×10⁴               |
| **Stability of azo-dye(hr.)**          | 2                    | 2                     |
| **Color of the dye**                   | Yellow               | Red                   |
| **Nature of the dye**                  | 1:1                  | 1:1                   |
| **Application of the method**          | Tablets, Syrup and Injection | Syrup                 |

Conclusions

A simple, rapid, and sensitive spectrophotometric method was described for the estimation of BHH in aqueous solution. The proposed method depends on the diazonization of the studied drug by sodium nitrite in the presence of hydrochloric acid and coupling with phloroglucinol reagent in basic medium to produce a colored azo dye, which is stable for at least 2 hrs. The proposed method has simple a procedure and was applied successfully for testing BHH in three forms of pharmaceutical preparations: tablets, syrup and injections.

Acknowledgment

The authors are very grateful to the University of Mosul/College of Science/Department of Chemistry for providing their facilities, which helped to improve the quality of this work.

References

1. Morrison, R.T. and Boyd, R.N. 1992. Organic Chemistry. 6th ed. New Jersey. Prentice-Hall.
2. Israa, M. J. and Sadeem, S. A. 2018. FIA– Spectrophotometric methods for the determination of Naringenin in supplements and urine samples using diazotization coupling reactions. Iraqi J. Sci., 59(2): 635-644.
3. Al-Abachi, M.Q. and Al-Nedawi, Z.A. 2015. Batch and Flow Injection Spectrophotometric Determination of Tetracycline Hydrochloride and Doxycycline Hyclate in Pharmaceutical Preparations. Iraqi J. Sci. 56(2): 909-920.
4. Abed, S.S. and Hussein, O.T. 2015. Spectrophotometric Determination of Vancomycin Hydrochloride (Batch and Flow-Injection) Using O-Nitroaniline as diazotized Chromogenic Reagent. *Iraqi J. Sci.* 56(4): 3025-3035.

5. Saadiyjah, A. D. Noor J. M. and Shetha F. N. 2015. Spectrophotometric Determination of Carbofuran with Diazotized Benzidine in Environmental Water Samples. *Baghdad Sci.J.*, 13: 498-510.

6. Al-Abachi, M. Q. and Suad S. M. 2015. The Spectrophotometric Determination of Famotidine Drug via Coupling with Diazotized Metachlopramide Hydrochloride. *Baghdad Sci. J.*, 12: 730-739.

7. Al-Majidi, S.M. and Al-Khuzaie, M.G. 2019. Synthesis and Characterization of New Azo Compounds Linked to 1, 8- Naphthalimide and Studying Their Ability as Acid-Base Indicators. *Iraqi J.Sci.*, 60(11): 2341-2352.

8. Rele, R.V. 2015. Simultaneous UV-spectrophotometric estimation of bromhexine hydrochloride and salbutamol sulphate by area under curve method in combined dosage form. *Der Pharmacia Sinica*, 6: 8-14.

9. Ziad, A.N. 2007. Formulation and stability evaluation of 1% w/v oral solution of bromhexine hydrochloride for veterinary use. *Islam. Univ. J.*, 15: 13-22.

10. Bhaga, A. and Rachana. 2018. Bromhexine: A comprehensive review. *Int. J. Biol. Med. Res.*, 9: 6455-6459.

11. British Pharmacopoeia. 2013. *CD-ROM*, System Simulation Ltd., The Stationary Office, London, pp. 186.

12. Al-ward, H.S. 2011. Spectrophotometric method for the determination of bromhexine hydrochloride in pure and pharmaceutical preparations. *Iraqi J.Sci.*, 52: 400-407.

13. Narayana, A., Rao, C.N. and Sivakumar, K. 2015. Spectrophotometric determination of bromhexine using charge transfer complex reaction. *Indian J.Advan.Chem.Sci.*, 3: 128-132.

14. Siddappa, K. and Hanamshetty, P.C. 2016. Spectrophotometric quantitative determination of bromhexine hydrochloride in bulk and pharmaceutical dosage form using p-nitrobenzaldehyde reagent. *Int. J. Pharm. Sci. Rev. Res.*, 39: 260-265.

15. Jain, V. and Sharma, M.C. 2016. Validated RP-HPLC method for determining the levels of bromhexine HCl, chlorpheniramine maleate, dextromethorphan HBr and guaiphenesin in their pharmaceutical dosage forms. *J. Taibah Univ. Sci.*, 10: 38–45.

16. Rao, N. and Gawde, K. D. 2018. Method development and force degradation studies for simultaneous estimation of salbutamol sulfate, etofylline and bromhexine hydrochloride in pharmaceutical dosage form using reversed-phase high-performance liquid chromatography method. *Asian J Pharm Clin Res.*, 11: 378-382.

17. Joshi, H.V. Ujash S., Patel, J.K. and Patel, S.M. 2017. Development and validation of analytical method for simultaneous estimation of bromhexin HCl and enrofloxacine in combined pharmaceutical dosage form. *Eurasian J. Anal. Chem.*, 12: 1631-1638.

18. Obeid, M., Allous Land Hirbali, J. 2016. validated PR-HPLC method for determination of bromhexine hydrochloride,terbutaline sulfate and guaiphenesin in pharmaceutical dosage forms. *Asian J. Pharm. Anal. Med. Chem.*, 4: 69-73.

19. Kong, D., Han L., Wang, Z., Jiang, L., Zhang, Q., Wu, Q., Su, J. Lu, C. and Chen, G. 2019. An electrochemical sensor based on poly (procaterol hydrochloride /carboxyl multiwalled carbon nanotube for the determination of bromhexine hydrochloride. *RSC Adv.*, 9: 11901-11911.

20. Mika, J., Moreira, J.C., Nemeckova, A., Zema, J., Barek, J. and Dejmekov H. 2015. Determination of bromhexine at a glassy carbon paste electrode using differential pulse voltammetry and flow injection analysis with amperometric detection. *Monatshefte für Chemie*, 146: 1211–1215.

21. Oliva, D.C., Velez, K.T. and Vazquez, A.L.R. 2011. Simultaneous determination of bromhexine and amoxicilline in pharmaceutical formulations by capillary electrophoresis. *J. mex. Chem. Soc.*, 55: 79-83.

22. Clayden, J., Greeves, N., Warren, S. and Wothers, P. 2001. *Organic Chemistry*. 2ndEdn., Oxford University Press, London.