



**DETERMINATION OF BISOPROLOL FUMARATE AND ITS METABOLITES IN
BIOLOGICAL, PHARMACEUTICAL AND ENVIRONMENTAL SAMPLES BY
DIFFERENT ANALYTICAL TECHNIQUES**

ALAM F², JUDDER MI^{1*}, ISLAM M², RAHMAN R¹, PATHAK B¹ AND RAHMAN Z³

1: Royal School of Pharmacy, The Assam Royal Global University, Betkuchi, Guwahati, Assam, INDIA,
781035

2: Faculty of Pharmaceutical Science, Assam Down Town University, Panikhaiti, Guwahati, Assam,
INDIA, 781026

3: NEF College of Pharmaceutical Education and Research, Nagaon Assam, INDIA, 782001

***Corresponding Author: Mr. Moidul Islam Judder: E Mail: moonzodder@gmail.com**

Received 26th July 2022; Revised 19th Sept. 2022; Accepted 11th Jan. 2023; Available online 1st Sept. 2023

<https://doi.org/10.31032/IJBPAS/2023/12.9.7433>

ABSTRACT

Purpose: Bisoprolol fumarate is considered to be one of the most crucial beta-blockers that are needed for biological preparation and pharmaceutical fluids. Now in recent times, there are multiple analytic techniques developed for the determination of bisoprolol activity.

Data collection: The collection of data has been done by information searching from Google scholar and Proust consisting of peer-reviewed articles. Pharmaceutical preparation, bisoprolol are the important keywords that have searched for the proper technique of analyzing the study.

Results: Results of the study is observed to be maintained by some methods which are favourable for the quantification in this scenario. Moreover, it focused on spectrophotometric, HPLC and some other methods that are beneficial for determining BSL quantification. In the case of the quantification of the raw materials, the HPLC method is considered to be one of the best analytical techniques because it has a high range of productivity.

Conclusion: Quantification and the technique of limit detection is the first and foremost crucial technique that can be applied under the HPLC method for the best outcome. Regulation and limitation of pH are also important in this scenario because they generate a high range of productivity.

Keywords: Bisoprolol, spectrophotometry, HPLC, analytical techniques, BSL quantification, limit detection

INTRODUCTION

In analytical chemistry, BSL or Bisoprolol fumarate or Zebeta is potentially an adrenoceptor blocking agent that primarily acts as a beta selective way. In general, it is a hypersensitive drug that is beneficial for treating the level of hypertension. In other words, it reduces artery wall pressure by treating a range of fluid retention techniques. Different analytical techniques have been analyzed in environmental, biological and pharmacological samples for the determination of BSL. This study discusses an array of understanding analytical methods of determining the level of bisoprolol fumarate for understanding anti hypersensitive actions

of this drug. Moreover, this article also focuses on the determination from different samples and activity of BSL that become influenced by HPLC, spectrophotometric and some other methods.

Overview and role of bisoprolol fumarate

BSL is a drug for the blocker class that exhibits a bioavailability of more than 80% as a white crystalline powder. It is soluble in water and methanol as well as glacial acetic acid. The molecular formula of BSL is “(C₁₈H₃₁NO₄).2C₄H₄O₄” and it can be orally absorbed by from the tract of gastro intestine.

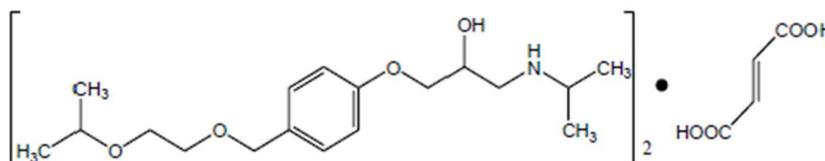


Figure 1: Structure of bisoprolol fumarate
(Source: Elshaprawy *et al* 2020) [1]

BSL has a long elimination half-life that is indicated by 10-11 hours. In the case of the biological samples, such as kidney, liver and some other organs, blood flow remain enhanced in this scenario for proceeding with the best outcome. As per the view of Berillo *et al* (2021) [2], it consists of an array of drug metabolite enzyme that enhances the activity of pharmacodynamic responses. In order to discuss the metabolites of BSL, it has been observed that 50% of single BSL has been metabolized by the CYP3A4 enzyme to the

form of inactive metabolites. About a range of 50% of an oral dose is observed to be excreted unchanged in the urine with a reminder of inactive form. Another metabolite of BSL is Cimetidine which is subjected to liver enzyme inhibition. Different analytical methods for Bisoprolol fumarate determination (pharmacy and environment) several methods are reported for analysis of BSL and its metabolites in the pharmaceutical and environmental samples for proceeding with the best result. Some methods that are popular in this regard which is

depending on the level of alkali tested chromatogram analysis.

1. Validation of linearity method

A method of linearity is considered to be one of the most crucial analytical techniques that

are used to determine the level of BSL in the pharmacy and environmental samples. It relies on adherence of Beer's law that consists of a range of concentrations of about 5-25 µg/ml of BSL.

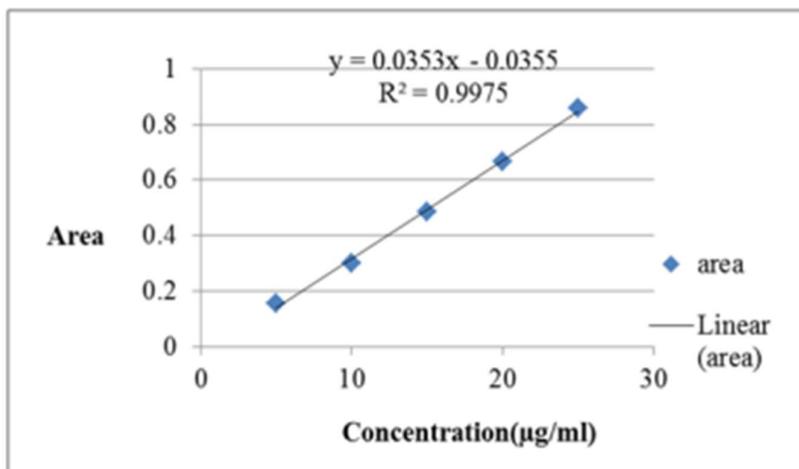


Figure 2: Linearity graph for Bisoprolol fumarate (Source: MS excel)

The above image shows different parameters for determining the level of BSL with the aid of regression data in the case of the biological and plant sample. Moreover, the data also indicate that the level of precision of intraday and inter-day studies are beneficial for understanding the level of different concentrations of BSL with the aid of linearity change. The process of linearity change is directly proportional with the form of inter-day variation and triplicate formulation of RSD.

2. Spectrophotometric method

It is one of the best and most pivotal analytical techniques for determining the level of pure pharmaceutical forms that are abundant in environmental samples. This method is simple, fast and sensitive that it needed to be

determined by the process of BTB or bromothymol blue [1]. A solution, containing HCL- KCL buffer consisting of a pH of 2.2 is able to form an ion-pair complex that can be extracted and measured spectrophotometrically by the inclusion of the wavelength of about 412 nm. It is one of the best quantifying methods of determination in pharmaceutical samples for example- a standard stock solution of 1.0×10^{-2} mol L⁻¹ for both drug AMD and BSL. This determination and formation are based on the formation of a reaction complex with the inclusion of methyl orange at a variety of pH values. A variety of pH values is important in this scenario for proceeding with the best outcome.

3. High-performance method of liquid chromatography

It is considered to be a minute but useful analytical technique for the determination of BSL in environmental as well as in the pharmaceutical aspects. In the case of biological samples, it is also observed to be a

helpful method [3]. According to the ideas of Elgendy *et al* (2021) [4], a process of liquid-liquid extraction technique with the inclusion of diethyl ether is the first and foremost crucial aspect that is needed for the synthesis of BSL to an ample extent.

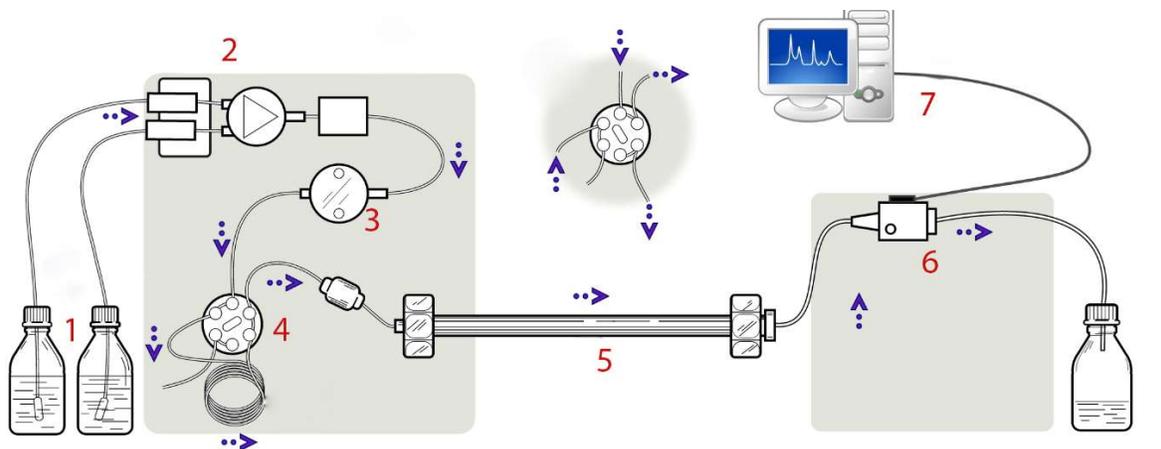


Figure 3: Schematic presentation of HPLC system
(Source: Cacabelos *et al* 2021) [5]

Formation of a linear curve of calibration is become obtained in this scenario with an average recovery of 72%. The process of chromatography becomes performed in this scenario with the inclusion of an octadecyl silica gel column. The separation method becomes performed by the process of normal phase chromatography with the inclusion of a reverse phase technique of chromatography. The chromatographic method is described in this scenario for proceeding with the determination of it in the human plasma [6]. Contrarily, [7] indicate that application of potentiometric sensors is important for analyzing the concentration of different drug molecules in this scenario. Therefore, this

point is about determination in the biological samples. Under this process, a detector of fluorescence has been used under the rate flow of about 525 and 458 nm [8]. It is also found to be validated by the technique of limit detection, quantification and precision with the aid of system suitability. The concentration range of this method of extraction is found to be changed at the level of 10-2000 mg/ml of the concentration. The separation technique with the inclusion of chromatography indicates that BSL determination can only be achieved by an isocratic elution of flow rate with the aid of 1.2 ml/ minute. The formation of a reverse phased column is considered to be beneficial in this scenario because this phase involves the

inclusion of a mobile phase that includes a methanol-water formulation. Methanol water formulation is used in this context because BSL is observed to be diluted in these two solvents. In pharmaceutical samples, such as pharmacokinetic study, it is beneficial to optimize with borate buffer for producing a yellowish fluorescent colour in this scenario. According to the opinion of Herrera-Chacón *et al* (2021) [9], the formation of this fluorescent colour is dependent on the pH, because it is the major index of acidity and alkalinity of a solution. On the contrary, Gholve *et al* (2021) [10] indicates that the measurement of spectra is also beneficial in this scenario for understanding different ranges of experimental parameters. However, the study indicates that the formation of pH becomes 9.5 at a range of IS-NBD spectrum of 457 nm. HPLC is considered to be one of the most suitable methods that can be applied in the pure culture and can be carried out for routine analysis of drugs and a range of biological fluid exemplified as human plasma.

4. Tandem mass spectrometry

It is also one of the most powerful tools for analyzing the array of organic compounds. Selectivity and chromatographic integrity are one of the main themes in this scenario that is beneficial for understanding it in the internal standard. 10 mg bisoprolol coated tablets are used in this context for analyzing the antibiotic formulation by the inclusion of liquid chromatographic technique (Wohlfart *et al*

2021) [11]. Moreover, a technique for protein precipitation is used in this scenario that is further used by an analytic technique of acetonitrile that is further dissolved into the mobile phase. A Reverse-phase high-performance process of liquid chromatography has also been performed in this scenario for proceeding with the best outcome (Logoyda *et al* 2020) [12]. This technique takes 2.5 minutes time for complete and the linearity range is maintained by 0.5-100 ng/ml with LOQ or limit of quantification technique.

5. Electrospray method of ionization

It is a technique for Mars colonization that has been developed by the determination of Bsl in human plasma. The alkalization process of sodium hydroxide is performed in this scenario that has been extracted by ethyl acetate and separated by the HPLC method. Performance of HPLC method with 10 mm ammonium acetate buffer created the mobile phase that contains 0.1 % formic acid methanol [13]. Less than 5 minutes are there located time for chromatographic separation which shows a concentration range of 0.05 to 120 mg per ml. Standard deviation is observed to be less than 3.8 and the percentage becomes 7.5 with good sensitivity. Linearity has no significant disturbance to the matrix which is caused by a process of the compound with the indigenous mixture.

MATERIAL AND METHOD

I. Concentration determination of bisoprolol and perindopril

There are two rapid processes of indicated and selectivity-maintained processes that are developed by a concurrent and validated determination for proceeding with the best result. The capillary electrophoresis method is one of the most convenient methods for understanding the degradation of biological products by enough rate and mass spectrometry that obtained a mixture of perindopril and bisoprolol. Pharmacopoeia is considered to be impurity is present in drugs that have been observed by the linearity graph of 1 to 20 µg/mL [14]. A technique has been confirmed in this scenario that is validated for a proposed technique of international conference of harmonization. Quantitative determination of degradation of product can be understood in this scenario for sufficing with the best outcome. A combined pharmaceutical combination has been maintained in this context which is able to count impurities regarding perindopril.

II. Analysis of bisoprolol by an enantiomeric mixture

High-performance liquid chromatography with an enantiomeric selective method has been developed to determine the level of BSL which is achieved by baseline resolution. A baseline resolution process uses the stationary phase with CSP and macrocyclic antibiotic teicoplanin with the polar ionic mobile phase. A polarizing mobile phase consisting of glacial triethylamine and methanol acetic acid that has a flow rate of 1.5 ml per minute consists of a fluorescent detection technique with a wavelength of 275 nm [14]. C18 cartridge is included for this study that father carried out at room temperature and involves an extraction that is based on a solid phase prior to HPLC analysis. Consumers can be recovered by a c18 cartridge which is validated between 20 to 200 mg per ml.

III. A mixture of rosuvastatin and bisoprolol

It is used in intensity studies with the emission wavelength of 297 to 485 NM; excitation indicates that the emission spectrum of the drug needs to be started from zero-emission in comparison to the other drug.

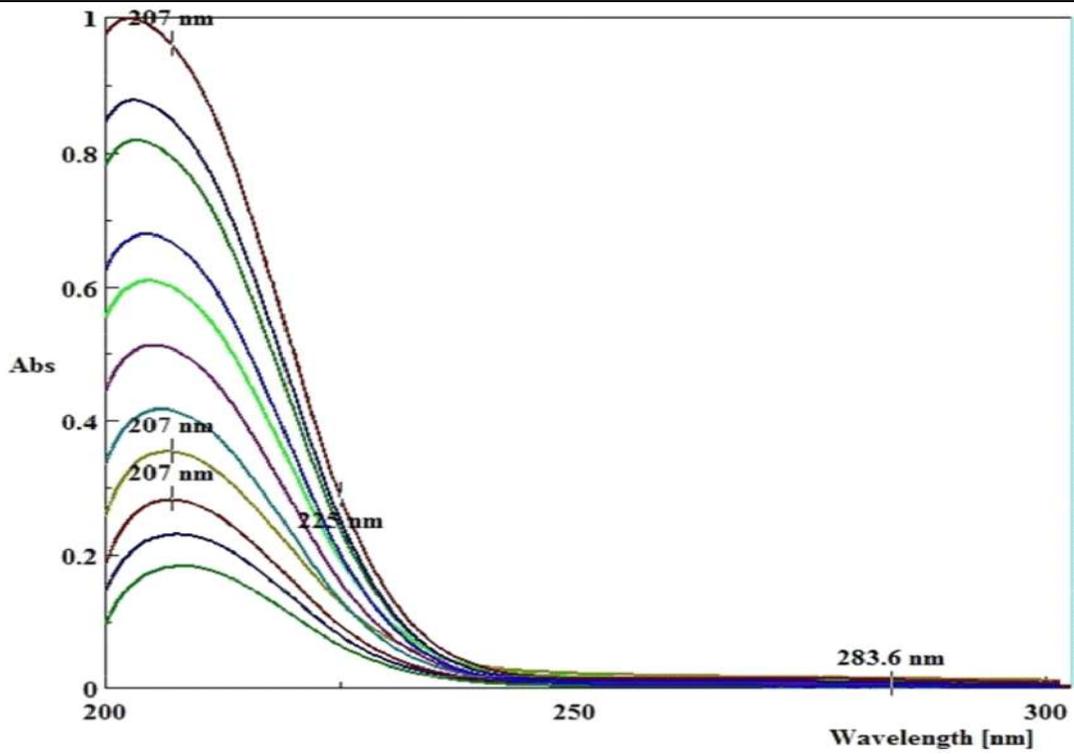


Figure 4: Zero emission spectra of BSL
(Source: Gholve et al 2021) [10]

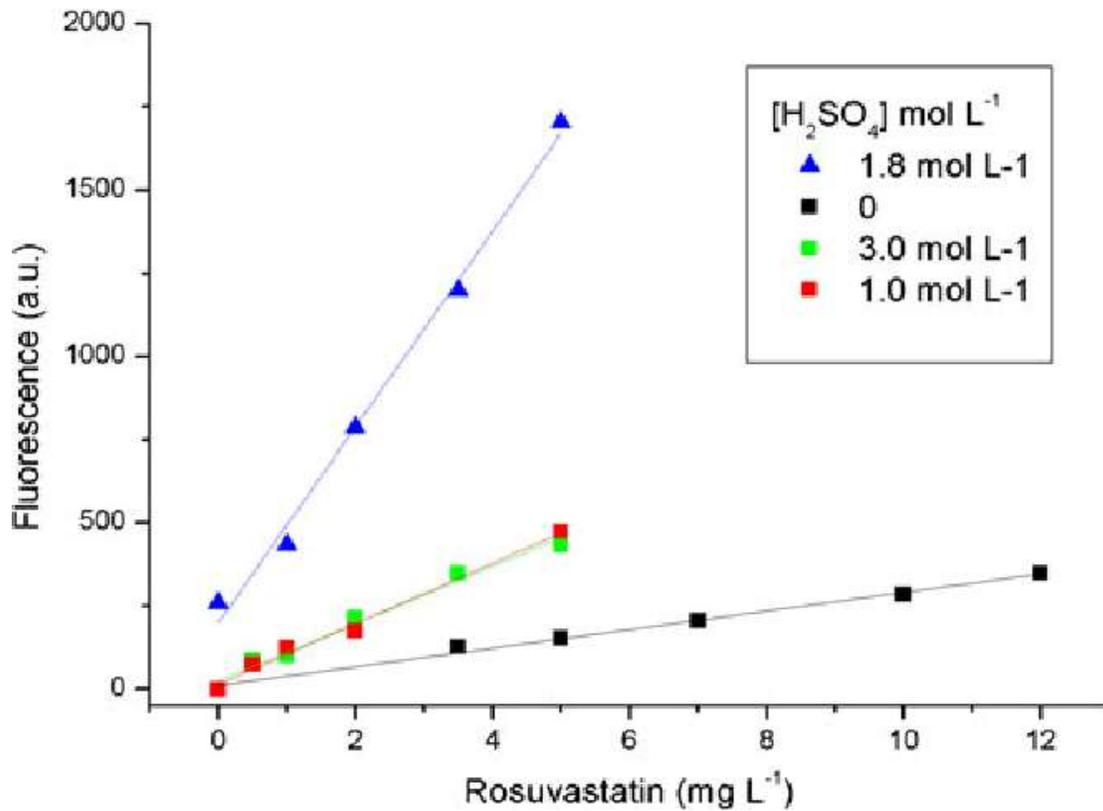


Figure 5: Liquid- Liquid extraction technique by measuring linearity
(Source: Gholve et al 2021) [10]

The emission spectrum of each drug consists of tedious derivatization steps with the aid of excellent linearity for rosuvastatin. The high sensitivity of these methods consists of a motivation application with analysis of drugs that are quoted in plasma of the human body. International conference of harmonization guidelines has followed this method which consists of an analytical and bioanalytical method of validation with the minimum difference.

RESULTS DISCUSSION

Robustness for the changes regarding chromatographic parameters is considered to be one of the main themes in this scenario that regulates flow rate, buffer phase and pH of acceptance criteria. Acceptance criteria are

further followed by the formation of USP resolution between BSL and AMD peaks. Results obtained from [10] indicate that the standard solution stability is high in the case of the second standard solution than that of the first standard solution. A total range of two days has been given in this context for understanding the level of BSL and AMD with the aid of fresh and stored standards. In the case of BSL, the response curve is high room temperature is indicated by 12,597.53. Retention time is considered to be one of the most important determinations of bsl that varies in the buffer phase and ph. Buffer phase and quantity determination technique has also been followed in this scenario for proceeding with the best outcome.

Table 1: Chromatographic condition with the variation of retention time

Variation of chromatographic condition	Retention time of BSL
Flow rate with 0.1 ml per minute	2.785
10% of buffer in negative form	2.316
10% of buffer in positive form	2.673
PDP quantity at 10% negative	2.446
PDP quantity at 10% positive	2.563

Table 1 discusses the variation of chromatographic condition with the inclusion of retention time. PDP quantity and buffer of 10% at positive form has been critically described in this scenario which indicates the values of 2.446 and 2.563 in both negative and positive form.

The extraction efficiency of BSL is higher in the case of the biological sample than that of pharmaceutical components that have been cleared from this study. In the case of liquid microextraction technique determination of

DSL can be performed by a method of ultraviolet detection which has been absent in this study.

A process of quantitative determination is performed with the aid of a liquid phase microextraction method that father compiled with high-performance liquid chromatography. Therefore, it can be said that solid-phase extraction is considered to be one of the most important extraction techniques that are used to separate BSL from the plasma samples. The recovery rate can be successfully understood

for BSL from the biological samples. For example, in the case of arterial hypertension, BSL is used for limiting the excess pressure in the artery wall by the recovery technique [16]. The complex composition of biological fluids is observed to be present before pretreatment therefore BSL quantitative assay with HPLC method is beneficial for determining potential inferences of environmental samples with impurities.

It has been observed by the multifarious study that hypertension is one of the most common diseases that can be restricted by the activity of BSL which acts as selective beta-blockers. The study of selective beta-blockers is also important for the quantitative determination of protein precipitation which is needed for the preparation of biological samples. The technique of protein precipitation is one of the most solubilize techniques characterized by universality and simplicity. Therefore, all of these analytic techniques are beneficial in this scenario that consists of a low value of pH and reduction of water solubility.

CONCLUSION

After completing this study, it has been observed that the HPLC method is abundant in this scenario because it used precision, quantification and limit detection. It can be considered as a potential method for the best outcome. It can be recommended for upcoming lab procedures that, BSL fumarate can be determined by maintaining proper procedure of pharmaceutical norms that do not

affect anyone. Maintenance of pH range is beneficial in this scenario for proceeding with the best outcome. Without managing the level of pH it is very cumbersome for an individual to enhance the level of efficiency of BSL. All method of analytical techniques has been determined by the inclusion of raw materials, biological fluids and pharmaceutical preparation technique. The spectrophotometric process of analysis is simple for this study whereas is HPLC method is beneficial but complex in nature.

ACKNOWLEDGMENT

Authors are thankful to Royal School of Pharmacy, The Assam Royal Global University for providing necessary facilities like internet surfing, library and other technical support to carry out the research.

CONFLICT OF INTEREST

The authors have no conflict of interest for publication of this paper.

REFERENCES

- [1] Elshaprawy, D.S., Elmosallmy, M.F. and Elgendy, K., 2020. Spectrophotometric Determination of Bisoprolol Fumarate and Levobunolol Hydrochloride in Bulk and Pharmaceutical Forms. *Eurasian Journal of Analytical Chemistry*, 15(1), pp.20-31.
- [2] Berillo, D., Zharkinbekov, Z., Kim, Y., Raziyeva, K., Temirkhanova, K. & Saparov, A. 2021, "Stimuli-Responsive Polymers for Transdermal,

- Transmucosal and Ocular Drug Delivery", *Pharmaceutics*, vol. 13, no. 12, pp. 2050.
- [3] Teaima, M.H., Mohamed, M.A.A., Abd El Rehem, R.T., Tayel, S.A., El-Nabarawi, M.A. and Fouad, S.A., 2021. Enhanced transdermal delivery of bisoprolol hemifumarate via combined effect of iontophoresis and chemical enhancers: ex vivo permeation/in vivo pharmacokinetic studies. *Pharmaceutics*, 13(5), p.682.
- [4] Elgendy, K., Elmosallamy, M.A., Soltan, M.K., Amin, A.S. and Elshaprawy, D.S., 2021. Novel potentiometric methods for the estimation of bisoprolol and alverine in pharmaceutical forms and human serum. *Reviews in Analytical Chemistry*, 40(1), pp.127-135.
- [5] Cacabelos, R., Naidoo, V., Corzo, L., Cacabelos, N. & Carril, J.C. 2021, "Genophenotypic Factors and Pharmacogenomics in Adverse Drug Reactions", *International Journal of Molecular Sciences*, vol. 22, no. 24, pp. 13302.
- [6] Mourad, R., El badry Mohamed, M., Frag, E.Y., El-Boraey, H.A. and EL-Sanafery, S.S., 2021. A novel molecularly imprinted potentiometric sensor for the fast determination of bisoprolol fumarate in biological samples. *Electroanalysis*, 33(1), pp.66-74.
- [7] Özbek, O., Berkel, C. and Isildak, Ö., 2020. Applications of potentiometric sensors for the determination of drug molecules in biological samples. *Critical Reviews in Analytical Chemistry*, pp.1-12.
- [8] Mohamed, M.E.B., Frag, E.Y. and Marzouk, H.A., 2021. A validated potentiometric method for the rapid determination of chromium ions content in food samples using a portable graphite sensor. *Food Chemistry*, 363, p.130324.
- [9] Herrera-Chacón, A., Cetó, X. and Del Valle, M., 2021. Molecularly imprinted polymers-towards electrochemical sensors and electronic tongues. *Analytical and Bioanalytical Chemistry*, 413(24), pp.6117-6140.
- [10] Gholve, R.B., Pekamwar, S.S. and Kalyankar, T.M., 2021. Stability-indicating RP-HPLC method development and validation for simultaneous estimation of bisoprolol fumarate and amlodipine besylate in bulk and in tablet dosage form. *Journal of Applied Pharmaceutical Science*, 11(12), pp.121-134.
- [11] Wohlfart, J., Jäckel, E., Scherf-Clavel, O., Jung, D., Kinzig, M., Sörgel, F. and Holzgrabe, U., 2021.

- Impurity profiling of bisoprolol fumarate by liquid chromatography-high-resolution mass spectrometry: A combination of targeted and untargeted approaches using a synthesis reaction matrix and general unknown comparative screening. *Journal of Chromatography Open*, 1, p.100012.
- [12] Logoyda, L., Kovalenko, S., Gaafar, M., Abdel-Megied, A.M. and Elbarbry, F.A., 2020. A validated LC-MS/MS method for the quantification of amlodipine, bisoprolol, enalapril and enalaprilat—Application to pharmacokinetic study in healthy volunteers. *Microchemical Journal*, 155, p.104700.
- [13] Telavane, S.A., Kothari, S. and Lokhande, M.V., 2021. Method of validation for residual solvents in bisoprolol fumarate by GC technique. *Asian Journal of Pharmaceutical Research*, 11(3), pp.147-155.
- [14] Bawane, S., Telrandhe, R. and Pande, S.D., 2018. Formulation and evaluation of oral fast dissolving film of bisoprolol fumarate. *International Journal of Pharmaceutics and Drug Analysis*, pp.105-115.
- [15] Piponski, M., Balkanov, T. and Logoyda, L., 2021. Development and validation of a fast and simple HPLC method for the simultaneous determination of bisoprolol and enalapril in dosage form. *Pharmacia*, 68, p.69.
- [16] Rajput, S.J., Sathe, M.A. and Patel, S.D., 2018. Development and validation of a HPLC-based bioanalytical method for lorcaserin using solid phase extraction and application to a pharmacokinetic study in rats. *Indian journal of pharmaceutical sciences*, 80(2), pp.235-241.