Sudan University of Science and Technology

College of Graduate Studies

Development and Validation of an Assay for Bisoprolol Fumarate and Hydrochlorothiazide Multicomponent Drug

تطوير وتحقق من طريقة لتحليل بيسوبرولول فومارات وهيدروكلوروثيازايد -عقار متعدد المواد

A Thesis submitted in fulfillment of the requirements for a master degree in chemistry

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الآيـــــــة

قال تعالـــــــي:

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آَمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِير ﴾

سورة المجادلة آية (١١)

Dedication

Dedicate

To

My Mother

My father

My wife and daughter

Acknowledgements

I would like to thank God for giving me health to accomplish this work. Many thanks also my supervisor Dr. Mohammed Suleiman Ali for this support, encouragement and assistance throughout the provider of research he has cheerfully answered my queries, provided me with materials, checked my result, assisted me in willingly in writing up and helpfully commented on the earlier drafts of this project.

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Many thanks also to Dr. babiker Yaqoob for moral support.

Abstract

A simple, precise, accurate and economical Reverse phase HPLC with a detection UV, method was developed and validated for the simultaneous determination of Bisoprolol fumarate and Hydrochlorothiazide in pharmaceutical formulations. Better separation was achieved using Cyanide column (250mm × 4.6mm, 5µm), maintained at 30 °C. The mobile phase was composed of buffer: methanol in the ratio of 82:18 (v/v), (the buffer was 1% solution of tetra butyl ammonium hydroxide, adjusted to pH = 5.0 by acetic acid). Isocratic elution was used with a flow rate of 0.9 ml/min, injection volume was 10µ, and effluents were monitored by UV (228nm). The retention time of Bisoprolol fumarate and Hydrochlorothiazide was 4.7min and 7.7min, respectively.

The method was tested for linearity over a concentration range of (40-160) µg/ml and (100-400) µg/ml, for Bisoprolol fumarate and hydrochlorothiazide, respectively; the correlation coefficient (R^2), was found to be 0.9998 and 0.999٩, respectively.

The method successfully passed all validation tests stipulated in the validation protocol of International Conference on Harmonization (ICH) and united State Pharmacopeia (USP).

مستخلص البحث

طريقه سهلة، دقيقة واقتصادية باستخدام كروماتوغرافيا السائل عالية الضغط مع مكشاف الاشعة فوق البنفسجية، تم تطويرها والتحقق منها لتحليل خليط عقار ثنائي مكون من بيسوبرولول فومارات وهيدروكلوروثيازايد. وقد تم الفصل بصورة جيدة باستخدام عمود السيانيد (٢٥٠مم * ٤,٦ مم* ٥ مايكرومتر) عند درجة حرارة ٣٠ درجة مئوي. الطور المتحرك يتكون من محلول منظم وميثانول بنسبة ١٨:٨٢ على التوالي (المحلول المنظم يحتوي على ١% محلول تترا بيوتيل امونيوم هيدروكسيد، تم ضبط الأس الهيدروجيني على ٥,٠ بواسطة حمض الخليك). وتم استخدام الازاحة احادية الطور المتحرك للفصل، وكان معدل سريان الطور المتحرك ٠,٩ مل لكل دقيقة، كمية حقن العينة ١٠مايكرولتر، وقد تم تقدير المادتين الفعالتين عند طول موجي ٢٢٨ نانوميتر وكان زمن استبقاء بيسوبرولول فومارات وهيدروكلوروثيازايد ٤,٧ دقيقة و٧,٧ دقيقة على التوالي.

تمت دراسة خطية العلاقة لمادة بيسوبرولول فومارات في مدي التراكيز (٤٠ مايكرو جرام/مل -١٦٠مايكرو جرام/مل) وهيدروكلوروثيازايد في مدي التراكيز (١٠٠ مايكرو جرام/مل -٤٠٠ مايكرو جرام/مل) فكان معامل الخطية لمادة بيسوبرولول فومارات ٠,٩٩٩٨ ولمادة هيدروكلوروثيازايد ٠,٩٩٩٩ على التوالي.

وقد اجتازت هذه الطريقة بنجاح جميع اختبارات التحقق المنصوص عليها ببروتوكول المؤتمر الدولي المعني بالمتطلبات التقنية لتسجيل الصيدلانيات الخاصة بالإنسان ودستور الولايات المتحدة الأمريكية للأدوية.

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List of Abbreviations

CHD	Coronary heart disease
CV	Cardiovascular
ALLAT	Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial
ACE	Angiotensin Converting Enzyme
RP-HPLC	Reverse phase - High Performance Liquid Chromatography
FDA	Food and Drug Administration
β	Beta
UPLC-MS	Ultra-Performance Liquid Chromatography - Mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
UPLC	Ultra-Performance Liquid Chromatography
HPLC-UV	High Performance Liquid Chromatography with Ultra violet detector
HPLC/TLC	High Performance thin-layer Chromatography
Rf	Retardation Factor
ICH	International Conference of Harmonization
USP	United State Pharmacopeia
LOD	Limit Of Detection
LOQ	Limit Of Quantitation
AVG	Average
STDEV	Standard Deviation
RSD	Relative Standard Deviation
RMSE	Root Mean Squire Error
nm	Nanometer
µm	Micrometer
mm	Millimeter
cm	Centimeter
ppm	Part Per Million
˚C	Celsius Scale

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Chapter one
Introduction and Literature Review

Chapter one
1. Introduction and Literature Review

1.1 Introduction

Hypertension is a major public health problem of worldwide distribution and is a major risk factor of cardiovascular disease morbidity and mortality. It is responsible for one-half of coronary heart disease (CHD) and about two thirds of cerebrovascular accidents. The relationship between blood pressure and risk of cardiovascular disease events is continuous, consistent and independent of other risk factors. The higher the blood pressure, the greater is the chance of myocardial infarction, heart failure, stroke and kidney disease. (Kavita, et al., 2013).

It is surprising that only about 50 years ago hypertension was considered an essential malady and not a treatable condition. Introduction of thiazide diuretics in late 50s made some headway in successful treatment of hypertension and ambitious multicenter VA co-operative study (phase 1 and 2) started in 1964 for diastolic hypertension ranging between 90 and 129 mmHg and completed by 1971 established for the first time that treating diastolic hypertension reduced CV events such as stroke and heart failure and improved mortality. In the next two decades, ALLHAT and other studies examined the comparability of outcomes with use of different classes and combinations of antihypertensive drugs. (Mohammad G. Saklayen and Neeraj V. Deshpande 2016).

Antihypertensive drugs d is a class of drugs that has an important place in the range of medicinal products currently used to treat cardiovascular diseases. Antihypertensive drugs combine several active ingredients with different mechanisms of action, but with synergistic action, have better tolerability and increased effectiveness. Although the first choice for reducing blood pressure is the lifestyle, expressed in diet and exercises, most patients also need drug therapy. The most commonly used antihypertensive drugs are diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin antagonists and calcium channel blockers, and in some cases, a combination of two or three of these needed. (Moisei et al., 2016).

Combining blood pressure-lowering drugs from different constituents, approximately, 5 times more effective than doubling the dose of one drug. It follows that to maximize efficacy combination therapy, preferably using low doses to minimize side effects, is substantially better than monotherapy and should be considered as routine initial therapy.

Obtaining the target blood pressure level by monotherapy can be currently challenging, especially for the patients who are suffering meanwhile from other diseases, It is demonstrated that a majority of hypertensive patients need two or more antihypertensive drugs to effectively lower their blood pressure. Consequently, fixed-dose that can be defined as that several active agents were combined in single pharmaceutical formulations appears to be a novel and underlying asset in overcoming the cardiovascular disease. Based on the analysis of some literature and relative data from FDA, the advantages of fixed-dose combination are elucidated, and formulations of common dual, triple combinations were summarized. Clinical practices proved that fixed-dose combinations had many benefits comparing with single drug and separate agents in terms of effects, convenience, compliance, and costs to a certain extent. From patients' perspective, fixed-dose combination therapy will be increasingly utilized in blood pressure control in the future. (Wald et al., 2009; Xinhuan Wan et al., 2014).

1.1.1 Bisoprolol Fumarate and Hydrochlorothiazide

i. Bisoprolol Fumarate

The molecular formula for Bisoprolol fumarate is: $(C_{18}H_{31}NO_4)_2 . C_4H_4O_4$, Molecular Weight 766.98 and IUPAC name is (±)-1-[4-[[2-(1-methylethoxy) ethoxy] methyl] phenoxy]-3-[(1-methylethyl) amino]-2-propanol (E) -2-butenedioate (2:1) and the chemical structure is shown in Figure1.1.

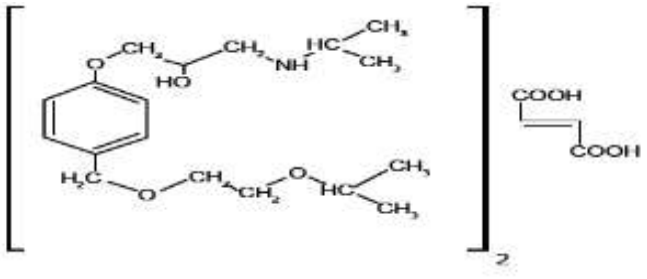


Figure 1.1: **Is Bisoprolol Fumarate structure. (USP 2016)**

Bisoprolol fumarate is a cardio selective β 1 -adrenergic blocker. It possesses an asymmetric carbon atom in its structure and is provided as a racemic mixture. The S (-) enantiomer is responsible for most of the beta-blocking activity. It is, almost completely, absorbed from the gastrointestinal tract and undergoes minimal first pass metabolism resulting in an oral bioavailability of about 90%. It is bound to plasma proteins at about 30%.

Bisoprolol has been used individually, and in combination with other antihypertensive agents for the treatment of hypertension, heart attacks, and kidney problems. (Savita's Yadav and Janhavirrao .2013; Bozal et al. 2013; Raju et al. 2016; Renuka et al. 2016).

ii. Hydrochlorothiazide

The molecular formula for Hydrochlorothiazide is $C_7H_8ClN_3O_4S_2$, Molecular Weight (297.74) and IUPAC name is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfomanide 1, 1-dioxide and the chemical structure is shown in Figure1.2.

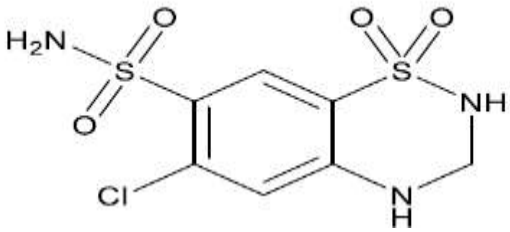


Figure 1.2: **Is Hydrochlorothiazide structure. (USP 2016)**

Hydrochlorothiazide is a diuretic/antihypertensive agent. is used mainly for treatment of mild to moderate hypertension of edema in people with congestive heart failure, cirrhosis of the liver, or kidney disorders and is, usually, administered with other drugs. Hydrochlorothiazide binds to and inhibits the enzyme carbonic anhydrase.

It is, frequently, used alone or in combination with other medications for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, hyperparathyroidism, and edema and prevention of kidney stones and used in the treatment of osteoporosis. (Savita's Yadav and Janhavirrao.2013; Renuka et al. 2016; Nidhal S. Mohammed and Ahmed J.Mohammed.2016).

1.1.2 Analytical procedure

The analytical procedure refers to the way of performing analysis. It describes, in details, the steps necessary to perform each ,analytical, test. This may include but is not limited to the sample, the reference standard and reagents preparation, use of the apparatus, generation of calibration curves, use of the formulae for the calculation, etc.

1.1.3 Methods validation

Methods are validated for System Suitability specificity, linearity, precision, accuracy and Robustness.

1.1.3.1 System Suitability

System suitability testing is an integral part of many analytical procedures. Tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

The parameters used in the system suitability tests report are as follows:

- Number of theoretical plates (Not less than 2000).
- Resolution (Not less than 2.0).
- Tailing factor (Not more than 2.0).
- Relative Standard Deviation (Not more than 2%).

1.1.3.2 Specificity

Specificity is the ability to assess, unequivocally, the analyte in the presence of components, which may be expected to be present. Typically, these include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure. This definition has the following implications:

Identification: to ensure the identity of an analyte.

Purity Tests: to ensure that all analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

- **Assay (content or potency)**

To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

1.1.3.3 Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, test data may need to be subjected to a mathematical transformation prior to regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

The range of the procedure is validated by verifying that the analytical procedure provides acceptable precision, accuracy, and linearity when applied to samples containing analyte at the extremes of the range as well as within the range.

1.1.3.4 Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not, necessarily quantitated as an exact value. The detection limit is, generally, expressed in the concentration of analyte (ppm) in the sample. A number of approaches are recommended by the ICH for determining the detection limit of sample, depending on instrument used for analysis, nature of analyte and suitability of the method. The acceptable approaches are:

- Visual evaluation.
- Signal-to-noise ratio.
- Standard deviation of the response.
- Standard deviation of the slope of linearity plot.

The formula for calculating LOD is:

$$LOD = 3.3\ \delta/S$$

Where δ = the standard deviation of the response

S = the slope of the calibration curve

1.1.3.5 Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte, in a sample, which can be, quantitatively, determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is particularly used for the determination of impurities and/or degradation products. Like LOD, ICH recommends the following four methods for estimation of LOQ.

The acceptable approaches are:

- Visual evaluation.
- Signal-to-noise ratio.
- Standard deviation of the response.
- Standard deviation of the slope of linearity plot.

The formula for calculating LOQ is:

$$LOQ = 10\ \delta/S$$

Where δ = the standard deviation of the response.

S = the slope of the calibration curve

1.1.3.6 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a, conventional, true value or as, an accepted, reference value, and the value found. This is sometimes termed trueness.

In the case of an assay of a drug substance, accuracy may be determined by application of an analytical procedure to an analyte of known purity (e.g., a Reference Standard) or by comparison, of the results of the procedure with those of a second, well-characterized procedure, the accuracy of which has been stated or defined.

In the case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical procedure to synthetic mixtures of the drug product components to which known amounts of analyte have been added within the range of the procedure. If it is not possible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product (i.e., "to spike") or to compare results with those of a second, well-established procedure, the accuracy of which has been stated or defined.

In the case of quantitative analysis of impurities, accuracy should be assessed on samples (of drug substance or drug product) spiked with known amounts of impurities. Where it is not possible to obtain samples of certain impurities or degradation products, results should be compared with those obtained by an independent procedure. In the absence of other information, it may be necessary to calculate the amount of an impurity based on comparison of its response to that of the drug substance; the ratio of the responses of equal amounts of the impurity and the drug substance (relative response factor) should be used, if known.

1.1.3.7 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially, prepared samples or a sample solution.

The precision of an analytical procedure is, usually, expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

The precision of an analytical procedure is determined by assaying a sufficient number of aliquots of a homogeneous sample to be statistically able to calculate valid estimates of standard deviation or relative standard deviation (coefficient of variation). Assays in this

context are, independent, analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result.

The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering a specified range of a procedure (i.e., three concentrations and three replicates of each concentration) or using a minimum of six determinations at 100% of the test concentration.

1.1.3.8 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be, suitably, controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness, should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that validity of the analytical procedure, is maintained whenever used. (ICH 2005; USP 2016).

1.2 Literature Review

1.2.1 HPLC methods

Patel et al. (2006); reported HPLC-UV method the determinate for bisoprolol Fumarate and Hydrochlorothiazide was developed and validated; using C_{18} column (20، x 4.6mm, 5µm) at ambient temperature, injection volume was 20µl. The mobile phase was water, acetonitrile and tetrahydrofuran in the ratio of 80:20:5 (v/v). Isocratic elution was used with 1ml/min flow rate, the eluents were UV monitored at 225nm. The Linearity ranges of Bisoprolol Fumarate and Hydrochlorothiazide were 10-150µg/ml and 1-90µg/ml; respectively. Limits of detection of Bisoprolol Fumarate and Hydrochlorothiazide were 3.5µg/ml and 0.4µg/ml, while limits of quantitation were 8.5µg/ml and 0.9µg/ml; respectively.

Qutab al. (2007); developed HPLC-UV method for determination of hydrochlorothiazide and candesartan Cilexetil using a Phenyl -2 column, mixture of 0.02M potassium dihydrogen phosphate, methanol and triethyl-amine, as mobile phase in the ratio of 25: 75: 0.2 (v/v), final pH 6.0 ± 0.1, as mobile phase. The flow rate was 1ml/min and eluents were monitored at 271nm and linearity ranges were 5–45µg/ml and 12–56µg/ml; respectively. The limits of detection were 0.08µg/ml and 0.13µg/ml while limits of quantitation were 0.19µg/ml, 0.22µg /ml, for hydrochlorothiazide and candesartan Cilexetil; respectively.

Havaldar and Vairal (2010); developed and validated a RP-HPLC method for the determination of atenolol, hydrochlorothiazide, losartan and valsartan. Separation was achieved with a Nucleolus 100 C_{18} column having 250 x 4.6mm i.d. with 5µm particle size and potassium dihydrogen phosphate buffer adjusted to pH 3.0 using diluted ortho phosphoric acid and acetonitrile (50:50, v/v) with isocratic program as eluent at a constant flow rate of 1.0/ml. UV detection was performed at 210nm for both.

Joshi et al. (2010); reported HPLC method was developed for simultaneous determination of bisoprolol and hydrochlorothiazide, using C_{18} column (250 × 4.6mm, 5µm). The mobile phase composed of 0.1M potassium dihydrogen phosphate buffer and acetonitrile (70:30, v/v); using isocratic elution with flow rate of 1ml/min, the eluents were monitored at

228nm. The Linearity ranges of Bisoprolol Fumarate and Hydrochlorothiazide were 2.5-50µg/ml and 6.25-125µg/ml; Limits of detection of Bisoprolol Fumarate and Hydrochlorothiazide were 0.01 µg/ml and 0.01µg/ml; while limits of quantitation were 0.03µg/ml, 0.05µg/ml respectively.

Kavitha, J. et al. (2011); developed and validated a RP-HPLC method for the analysis of Telmisartan and Hydrochlorothiazide. Princeton SPHER R C_{18} column was used, the mobile phase was composed of acetonitrile and potassium dihydrogen ortho phosphate (pH 3.5) in ratio 50:50 (v/v), an isocratic elution was used; flow rate was 1ml/min with a total run time of 10 minutes. Sulphadoxine was selected as internal standard. Detection of the multi compounds was carried out at 270nm.

Nalwade et al.(2011); developed and validated RP-HPLC method for the determination of Telmisartan, Amlodipine besylate and Hydrochlorothiazide using C18 column(100 × 2.1mm, 1.7µm) at 55◦C, using two mobile phases (A) 0.053M sodium perchlorate buffer pH 3.2 and acetonitrile (90:10, v/v) (B) was 0.053M sodium perchlorate buffer pH 3.2 and acetonitrile (20:80, v/v); using gradient elution with aflow rate of 0.6 ml/min. Eluents were monitored at 271nm for telmisartan and hydrochlorothiazide, 237nm for Amlodipine. Linearity ranges were 16.024–48.073 µg/ml, 2.02–6.02µg/ml and 5–15µg/ml for of Telmisartan, Amlodipine besylate and Hydrochlorothiazide; respectively.

Swamy et al. (2012); Reported an HPLC-UV method for determination of Aliskiren, Hydrochlorothiazide and amlodipine bisulfate using C_{18} column (250 × 4.6mm, 5µm) maintained at 25°C. injection volume was 10µl, mobile phase was Acetonitrile, methanol and phosphate buffer pH 3.0 in the ratio of 20:50:30 (v/v), isocratic elution was used, with 1ml/min flow rate, eluents were monitored at 239 nm. The linearity ranges of Aliskiren, Amlodipine besylate and hydrochlorothiazide were 75-450µg/ml, 5–30µg/ml and 12.5-75µg/ml, respectively. Limits of detections of Aliskiren, Amlodipine besylate and hydrochlorothiazide were 0.1521µg/ml, 0.2305µg/ml and 0.2132µg/ml, respectively; while limits of quantitation were 0.3015µg/ml, 0.2517µg/ml and 0.2615µg/ml, respectively.

Samya et al. (2012); developed a simultaneous HPLC-UV method for determination of amlodipine, hydrochlorothiazide and valsartan using C18 column (150 x4.6mm, 5µm) at 30°C, injection volume was 20µL, the mobile phase was phosphate buffer (pH 2.8): acetonitrile (60:40), isocratic elution with a flow rate of 0.8 ml/min. Eluents were monitored at 227 nm. The linearity ranges of amlodipine, hydrochlorothiazide and valsartan were 4µg/ml–28µg/ml, 1µg/ml–12µg/ml, and 5µg/ml–40µg/ml, limits of detection were 1.04µg/ml, 0.39µg/ml and 1.4µg/ml, while limits of quantitation were 3.16µg/ml, 0.81µg/ml and 4.3µg/ml, respectively.

Mamdouh et al. (2012); developed simultaneous HPLC-UV method for determination of valsartan and hydrochlorothiazide, using column C_{18} (150 x4.6 mm, 5µm), injection volume was 50 µ, the mobile phase was phosphate buffer pH 2.9 acetonitrile and methanol (50:40:10, v/v) using isocratic elution with flow rate 1.4 ml/min and eluents were monitored at 225 nm. Linearity ranges were 12-36 µg/ml and 2-9µg/ml for valsartan and hydrochlorothiazide, respectively; limits of detection and limits of quantitation were not recorded.

Rasha et al. (2013); developed a simultaneous HPLC-UV method for determination of amlodipine besylate, valsartan and hydrochlorothiazide, using C_8 Column (250 x 4.6mm, 5µ) at 25°C, injection volume was 20µl, the mobile phase was 0.025M phosphoric acid and acetonitrile using gradient elution with 1m/min flow rate. Eluents were monitored at ٢٣٨nm for amlodipine and 225nm for both valsartan and hydrochlorothiazide. The linearity ranges were 5–200µg/ml, 5–200µg/ml and 10–200µg/ml for amlodipine besylate, valsartan and hydrochlorothiazide; respectively. limits of detection were 0.26µg/ml, 0.24µg/ml and 0.12µg/ml, while limits of quantitation were 0.85µg/ml, 0.80µg/ml and 0.40µg/ml, for amlodipine besylate, valsartan and hydrochlorothiazide; respectively.

Vasanth et al. (2013); reported an HPLC-UV method for simultaneous determination of losartan potassium and hydrochlorothiazide, using C_{18} column (150 x 4.6mm, 5µm). The mobile phase was orthophosphric acid Buffer pH 3.0 and Acetonitrile in the ratio of 65:35 (v/v), isocratic elution with flow rate 1ml/min was applied, and eluents were monitored at 254nm. The linearity ranges of losartan potassium and hydrochlorothiazide were 25-

75µg/ml and 25-18.75µg/ml; respectively. Limits of detection were 1.779µg/ml and 0.375µg/ml; while limits of quantitation 5.393µg/ml and 1.138µg/ml for losartan potassium and hydrochlorothiazide, respectively.

Ritesh and Shyam Sunder (2012); developed a simultaneous HPLC-UV method for determination of valsartan, amlodipine and hydrochlorothiazide using C_{18} column (250 × 4.6mm, 5µm) at 35°C. injection volume was 40µL, formic acid buffer pH 3.50, Acetonitrile and Ammonium Formate as mobile phase using gradient elution with 1ml/min flow rate, eluents were monitored at 254. The linearity ranges of valsartan, amlodipine and hydrochlorothiazide were 50–4000ng/ml, 6–200ng/ml and 5-400ng/ml; respectively.

Kumaraswamy et al. (2014); developed a simultaneous HPLC-UV method for determination of Amlodipine, Atenolol and hydrochlorothiazide, using C_{18} column (250 x 4.6mm, 5µm), at 20 ±1°C, injection volume was 20µl. The mobile phase was Phosphate buffer pH 6.0, Acetonitrile and Methanol in the ratio of 30:20:50 (v/v) and using Isocratic elution with 1ml/min flow rate, eluents were monitored at 240nm. The linearity ranges of Amlodipine, Atenolol and hydrochlorothiazide were 2-12µg/ml, 10-60µg/ml and 2-12µg/ml; respectively. Limits of detection were 0.0677µg/ml, 0.1379µg/ml and 0.0478µg/ml, while limits of quantitation were 0.2051µg/ml, 0.4180µg/ml and 0.0145µg/ml, for Amlodipine, Atenolol and hydrochlorothiazide, respectively.

Ashutosh et al. (2014); developed RP-HPLC chromatographic method for simultaneous determination of hydrochlorothiazide, Amlodipine and Olmesartan using C_{18} column (150 x 4.5mm, 5µm) at ambient temperature, injection volume was 20µl, the mobile phase was triethylamine buffer pH 3.5 and Acetonitrile (40:60, v/v) an isocratic elution was applied, 0.8ml/min flow rate used, eluents were monitored at 230nm. The linearity ranges of hydrochlorothiazide, Amlodipine and Olmesartan were 25-62.5µg/ml, 10-25µg/ml and 10-100µg/ml; respectively. Limits of detection were 0.009µg/ml, 0.06µg/ml and 0.06µg/ml; while limits of quantitation 0.03µg/ml, 0.2µg/ml and 0.2µg/ml hydrochlorothiazide, Amlodipine and Olmesartan, respectively.

Ramachandran et al. (2014); a simultaneous HPLC-UV method for determination of Hydrochlorothiazide and Irbesartan was developed using C_{18} column (250 x 4.6mm, 5µm)

at ambient temperature, injection volume was 20µl, the mobile phase was Phosphate buffer pH 6.0 and Acetonitrile in the ratio of 60:40 (v/v), using isocratic elution with1ml/min flow rate, eluents were monitored at 235nm for both. The linearity ranges 90-210µg/ml and 7.5-17.5µg/ml for hydrochlorothiazide and Irbesartan; respectively. Limits of detection were 65.19µg/ml and 0.05µg/ml; while limits of quantitation were 197.56µg/ml and 0.15µg/ml for hydrochlorothiazide and Irbesartan; respectively.

Murali et al. (2014); developed simultaneous HPLC/UV method for determination of valsartan and hydrochlorothiazide using column C_{18} (150 x4.6mm, 5µm), injection volume was 20µl, the mobile phase was phosphate buffer (pH4) and acetonitrile (40:60), using isocratic elution with 1ml/min flow rate. Eluents were monitored at 225 nm. Linearity ranges were 30- 90µg/ml and 300-900µg/ml, limits of detection 2.903 and 2.941µg/ml and limit of quantitation 9.675 and 9.8µg/ml for amlodipine and losartan, respectively.

Renuka, et al. (2016); developed simultaneous HPLC-UV for determination of bisoprolol and hydrochlorothiazide, using C_{18} column (150 x 4.6mm, 5µm), injection volume was 10µl, and the mobile phase was phosphate buffer pH 2.5 and acetonitrile in the ratio of 80:20 v/v, was selected at flow rate of 1 ml/min. Eluents were monitored at 208nm. The linearity ranges of Bisoprolol and Hydrochlorothiazide were 2.5-75µg/ml, 3-90µg/ml and Limits of detection were 2µg/ml, 0.9µg/ml, while limits of quantitation were 6µg/ml, 1.8µg/ml for bisoprolol and hydrochlorothiazide; respectively.

Raju et al, (2016); validated HPLC-UV for the simultaneous determination of bisoprolol fumarate and hydrochlorothiazide using C_{18} column (250 x 4.6mm) Injection volume was 20µl, the mobile phase was Acetonitrile and phosphate buffer pH 3.0 in the ratio of 40:60 (v/v); flow rate of 1ml/min. Eluents were monitored at 228 nm. Linearity ranges were 20-100 µg/ml for both bisoprolol fumarate and hydrochlorothiazide. Limits of detection were 0.544 µg/ml, 0.658 µg/ml, while limits of quantitation were 1.64µg/ml, 1.99 µg/ml bisoprolol fumarate and Hydrochlorothiazide; respectively.

Ravi et al, (2016); reported that HPLC-UV method has been developed and validated for simultaneous determination of bisoprolol and hydrochlorothiazide in its bulk and combined tablet dosage form. Chromatographic separation was C_{18} column (250 × 4.6mm;

5µm particle size, maintained at a temperature of 30 ºC by a mobile phase consisted of 0.1% orthophosphoric acid and acetonitrile (55:45, v/v) with a flow rate of 1.0 ml/min. The detection wavelength was set at 259 nm for both. The linearity ranges of 40-120µg/ml for bisoprolol and 50-150µg/ml for hydrochlorothiazide. The limit of quantitation was 0.398 and 0.385µg/ml for bisoprolol and hydrochlorothiazide, respectively.

Shakya (2016); developed a simultaneous HPLC-UV method for determination of valsartan and hydrochlorothiazide, using column C18 (150 x 4.6mm, 5µm) maintained at 25◦C, injection volume was 20µL, the mobile phase was 0.25 ml/L triethylamine (pH 3.0), methanol and acetonitrile (50:38:37, v/v) using isocratic elution with 1.5 ml/min flow rate. Eluents were monitored at 265 nm. Linearity ranges were 1.25-64.00 µg/ml and 0.195-10.00 µg/ml, limits of detection were 0.253 and 0.0226 µg/ml, respectively; while limits of quantitation were 0.767 and 0.068 µg/ml for valsartan and hydrochlorothiazide, respectively.

Vatchavai et al. (2017); reported RP-HPLC method for the simultaneous determination of Telmisartan and hydrochlorothiazide in Pharmaceutical formulations. The separation of the drugs was achieved on a C_{18}, column 250×4.6mm, 5micron size column with a mobile phase consisting of acetonitrile and phosphate buffer pH 3.0 (60:40 v/v) and 1.0 ml/min flow rate, UV detection at 282 nm for both. Linearity was found to be 6-18µg/ml and 6-18µg/ml for Telmisartan and Hydrochlorothiazide; respectively. Limits of detection were 0.99µg/ml, 1.55µg/ml, while limits of quantitation were 3µg/ml, 4.7µg/ml for Telmisartan and hydrochlorothiazide; respectively.

1.2.2 HPLC/TLC methods

A HPLC/TLC chromatographic method for determination of losartan potassium and amlodipine was reported (Lakshmi and Lakshmi 2012); usimg chloroform, methanol, acetone and formic acid (7.5: 1.3: 0.5: 0.03, v/v) as mobile phase, eluents were monitored at 254 nm. The RF values amlodipine besylate, hydrochlorothiazide, and losartan potassium were 0.35, 0.57, and 0.74, respectively; linearity ranges were 500–3000 ng per spot for losartan potassium, amlodipine and hydrochlorothiazide.

Savita's Yadav and Janhavirrao (2013); a simultaneous high-performance thin-layer chromatographic method for determination bisoprolol fumarate and hydrochlorothiazide was developed, using precoated silica gel HPTLC aluminum plate 60 F254, ethyl acetate, methanol and ammonia in the ratio of 10:0.5:0.5, (v/v) as mobile phase. Detection was carried out densitometrically at 225nm. The RF value of bisoprolol fumarate and hydrochlorothiazide were 0.60 and 0.38; respectively; linearity ranges were 150-900ng/spot and 100-600ng/spot for bisoprolol fumarate hydrochlorothiazide; respectively.

1.2.3 A UV-Spectrophotometric methods

A UV-Spectrophotometric method has been developed for simultaneous estimation of amlodipine besylate and bisoprolol fumarate in combined dosage form was reported Kakde et al. (2008); The method employed simultaneous equation method for analysis using 10% methanol as a solvent. The two wavelengths 222 nm and 365 nm were selected for estimation of bisoprolol fumarate and amlodipine besylate; respectively. Linearity was observed in the concentration ranges of 5–100 μg/ml for both the drugs.

Wankhede et al. (2010); developed a simultaneous spectrophotometric method for the determination of amlodipine besilate, losartan potassium and hydrochlorothiazide using methanol as solvent; the detection wavelengths were 236.5, 254 and 271 nm, respectively, and linearities of procedure were within the concentration ranges 5-25 μg/ml, 10-50 μg/ml and 5-25 μg/ml, respectively. They developed also an HPLC method for determination of same drug using column C_{18} (250x4.6 mm, 5μm) at ambient temperature. The injection volume was 20 μl, the mobile phase was phosphate buffer (pH 3.7): Acetonitrile (57:43), with isocratic elution flow rate of 1.0 ml/min, and eluents were monitored at 232 nm. Linearity ranges were 2-14 μg/ml, 20-140μg/ml and 5-40μg/ml for amlodipine besylate, losartan potassium and hydrochlorothiazide, respectively. Limits of detection and limits of quantitation were not reported for both methods.

A UV-Spectrophotometric method developed for simultaneous estimation of losartan potassium, amlodipine besylate and hydrochlorothiazide in raw materials and in formulations are described was reported (Nagavalli et al. (2010); Calibrations were constructed using the absorption data matrix corresponding to the concentration data

matrix, with measurements in the range of 230.5nm–350.4 nm (id = 0.1 nm) in their zero order spectra. The linearity ranges were found to be 8–40 mg/ml, 1–5 mg/ml and 3–15 mg/ml for losartan potassium, amlodipine besylate and hydrochlorothiazide; respectively.

A UV-spectrophotometric method was developed for determination of amlodipine besylate, valsartan and hydrochlorothiazide were reported (Ananda et al. 2011); using methanol: water (1:1) as solvent, the selected wavelengths were 365 nm, 250 nm and 315 nm. The linear concentration ranges were 1µg/ml–32µg/ml, 4µg/ml-40µg/ml and 2µg/ml–20µg/ml, limits of detection were 0.2µg/ml, 0.3µg/ml and 0.25µg/ml, while limits of quantitation were 0.55µg/ml, 0.9µg/ml and 0.75µg/ml for amlodipine besylate, valsartan and hydrochlorothiazide respectively.

A UV-Spectrophotometric method was developed for simultaneous estimation of Telmesartan, amlodipine besylate and hydrochlorothiazide in bulk and in combined tablet dosage form was reported (Delhiraj et al. 2012); The method is based on the simultaneous equation method. Telmesartan, amlodipine besylate and hydrochlorothiazide has absorbance maxima at 292.8 nm, 238.5 nm and 271.2 nm; respectively. The linearity ranges of Amlodipine besylate, Telmesartan and hydrochlorothiazide were 4-24µg/ml, 4-24µg/ml and (4-24µg/ml); respectively.

Varsha et al. (2012); reported a simultaneous UV-Spectrophotometric method for determination of amlodipine besylate, hydrochlorothiazide and valsartan using methanol as solvent, the selected wavelengths were 359 nm, 317 nm and 250 nm, the linear concentration ranges were 5µg/ml -25µg/ml, 10µg/ml -50µg/ml and 5µg/ml -25µg/ml, limits of detection were 0.51µg/ml, 0.91µg/ml and 1.57µg/ml, while limits of quantitation were 1.68µg/ml, 3.02µg/ml and 4.77µg/ml respectively.

Sayyed et al. (2015); developed a simultaneous spectrophotometric method for determination of Amlodipine Besylate and Hydrochlorothiazide, using 0.1N sodium hydroxide as solvent and 238nm and 271nm UV detention respectively; linearity of procedure was within the concentration ranges were 5-30µg/ml, 2.5-15µg/ml Amlodipine Besylate and Hydrochlorothiazide, respectively. Limits of detection and limits of quantitation were not recorded.

Bobade and Ganorkar (2017); reported a simultaneous spectrophotometric method for determination of bisoprolol fumarate and Hydrochlorothiazide using 0.1N Sodium hydroxide as solvent. Detection wavelengths were 238.4 and 274 nm. The linarites of their procedure were within the concentration ranges of 8-96µg/ml, 4-48µg/ml for bisoprolol fumarate and hydrochlorothiazide; respectively. Limits of detection and limits of quantitation were not recorded.

1.2.4 UPLC methods

Anandkumar et al. (2015); a simultaneous UPLC-MS method was developed for determination of amlodipine besilate, losartan potassium and hydrochlorothiazide using C_{18} column (50 x 2.1 mm, 1.7µm) at ambient temperature, injection volume was 2µL, the mobile phase was 1% ammonium acetate (pH 2.6) and acetonitrile, gradient elution with 0.4 ml/min flow rate. Eluents were monitored at 254 nm. The linearity ranges of hydrochlorothiazide, amlodipine and losartan were 125ng/ml - 750ng/ml, 50ng/ml - 300ng/ml, and 500ng/ml - 3000ng/ml, limits of detection were 0.6ng/ml, 0.1ng/ml and 2ng/ml, while limits of quantitation were 1ng/ml, 1ng/ml and 5ng/ml, respectively.

Sevinc et al, (2014); validated UPLC/BE chromatographic method for the simultaneous determination of bisoprolol fumarate and hydrochlorothiazide in their combined dosage forms in spiked human urine samples. The separation was achieved on an Acquity UPLC BEH C_{18} 1.7µm (2.1 × 50 mm) column, at 40 °C with mobile phase of acetonitrile and phosphate buffer pH 3.0, a gradient elution at 225 nm. The linearity ranges 0.5–150µg/ml for hydrochlorothiazide and 0.5–250µg/ml for bisoprolol fumarate. Limit of detection and limit of quantitation for hydrochlorothiazide were 0.01µg/ml and 0.03µg/ml, respectively, and for bisoprolol fumarate were 0.07µg/ml and 0.21µg/ml, respectively.

1.2.5 Other methods

Bhoya et al. (2013); reported TLC-densitometry method for simultaneous determination of bisoprolol fumarate and hydrochlorothiazide using precoated silica gel HPTLC aluminum plate 60 F254, using chloroform, ethanol and glacial acetic acid in the ratio of 5:1.5:0.2 (v/v) as mobile phase. Detection was carried out densitometrically at 225nm. The R_f value were bisoprolol fumarate and Hydrochlorothiazide 0.62 and 0.40; respectively.

The linearity ranges were 200–1200ng/spot and 100-800ng/spot for bisoprolol fumarate and Hydrochlorothiazide; respectively.

Bozal et al. (2013); developed a simultaneous voltammetry, chromatographic, and spectrophotometric methods for determination of bisoprolol fumarate and hydrochlorothiazide Differential pulse and square wave voltammetry techniques were used to analyze bisoprolol fumarate and hydrochlorothiazide simultaneously by measuring at about 1400 mv and 1100 mv; respectively. RP-HPLC was the second method for simultaneous analysis of the compounds. The mixture of bisoprolol fumarate, hydrochlorothiazide and Moxifloxacin as an internal standard was separated on C_{18} column (150 × 4.6mm, 5μm) using acetonitrile and 15 mM phosphate in the ratio of 25: 75,) v/v) as mobile phase with 1ml/min flow rate. The third method was based on first derivative of the ratio spectra method obtained from the measurements of the amplitudes at 246nm, 257nm for bisoprolol fumarate and hydrochlorothiazide, respectively.

1.3 Objectives

- To develop methods for Bisoprolol fumarate and Hydrochlorothiazide.
- To determine System suitability, Specificity, Linearity, Accuracy and Robustness for the developed method.
- To compare the results with acceptance criteria of USP and ICH guidelines.

Chapter Two

Materials and Methods

Chapter Two
2. Materials and Methods

2.1 Chemicals

Bisoprolol fumarate (purity: 99.30%), Hydrochlorothiazide (purity: 99.10%) and Tetra butyl ammonium hydroxide 40% were obtained from Aurobindo, Unichem and Emplura India. Methanol (HPLC grade). All other chemicals used were of analytical grade. Purified water from Milli-Q-system (Millipore, Bangalore, India).

2.2 Instruments

- High Performance Liquid Chromatography HPLC

Type: HPLC prominence – i

Model: LC-2030C3D

Serial No: L21455300660AE

Company: Shimadzu Corporation

Origin: Japan

- **Analytical Balance**

Type: AY220

Serial No: O4328143000

Capacity: 220 g

Readability: 0.1 mg

Company: Shimadzu Corporation

Origin JAPAN

- **Ultrasonic**

Model: 621.05.003

Company: ISO Lab Laborgerate -GmbH

Origin: Germany

- **pH-meter**

Model: PHS-550

Origin: Romania.

- **Magnetic Stirrer**

Model: LMS, 1001

Serial No: 2016017862

Company: QAIHAN LAB TECH Co-LTD

Origin: Korea

2.3 Methods

2.3.1 Optimized chromatographic conditions

- Cyanide column (250 × 4.6mm, 5µm), and simple isocratic elusion, were used (one pump required) with flow-rate of 0.9ml/min, both, active, ingredients were detected at 228nm, injection volume was 10µl (universal loop) at 30˚C.
- Temperature Procedure was 30˚C.

2.3.2 Buffer Solution pH 5.0

In 1000 ml volumetric flask 980 ml of deionized water, 10 ml of tetra butyl ammonium hydroxide (40%) were mixed, was adjusted pH 5.0 with glacial acetic acid, and the volume was completed to the mark with deionized water.

2.3.3 Mobile Phase

Mixture of buffer and methanol were prepared in 82:18 v/v ratio. The mixture was shaken, filtered at pump through 0.45µm nylon membrane filter, transferred to solvent reservoir and sonicated for 5 min.

2.3.4 Standard Stock Solution

0.25g Hydrochlorothiazide and 0.1g bisoprolol fumarate were accurately weighed, transferred quantitatively to the same 100ml volumetric flask, 50ml Methanol were added and sonicated for 5 min, cooled to reach room temperature, and completed to volume with mobile phase.

2.3.5 System Suitability

Subsequent, dilutions were made from the stock solution with mobile phase to give concentrations of 250µg/ml hydrochlorothiazide and 100µg/ml Bisoprolol Fumarate. System suitability solution was injected six times.

2.3.6 Specificity

- **Standard**

The stock solution was diluted with mobile phase to give the concentrations of 250µg/ml hydrochlorothiazide and 100µg/ml bisoprolol fumarate. System suitability solution was injected six times.

- **Placebo**

A placebo equivalent to average weight of one tablet was transferred to 50-ml volumetric flask, the flask was half filled with mobile phase, sonicated for 10 minutes, cooled to room temperature, and the volume was completed to the mark with the same solvent. Subsequent, dilutions were made in mobile phase similar to those made for standard preparation.

- **Sample**

Five tablets were placed into a clean, dry 100 ml volumetric flask and dissolved with 10 ml methanol, sonicated for 10 min, cooled, then 50 ml mobile phase was added, sonicated for 20 min, cooled to reach room temperature, and then completed to volume with mobile phase. 5ml were diluted to volume with mobile phase in a 25ml volumetric flask, passed through 0.45µm pore filter.

- **Sample with fumaric acid Preparation**

10 mg fumaric acid were placed into clean dry 100 ml volumetric flask and dissolved in 10 ml methanol, sonicated for 10 min, cooled, then 50 ml mobile phase were added, sonicated for 20 min, cooled to reach room temperature, and then completed to volume with mobile phase. 1ml was diluted to volume with mobile phase in a 10 ml volumetric flask, passed through 0.45µm pore filter.

2.3.7 Linearity

The stock solution was diluted with mobile phase to give concentrations of 100, 150, 200, 250, 300, 350 and 400µg/ml hydrochlorothiazide and 40.60,80,100,120,140 and 160µg/ml bisoprolol fumarate. Each solution was injected three times and results were collected, LOD and LOQ were calculated from linear regression analysis.

2.3.8 Accuracy

- **Standard**

The stock solution was diluted with mobile phase to give the concentrations of 250µg/ml hydrochlorothiazide and 100µg/ml Bisoprolol Fumarate. System suitability solution was injected six times.

- **Preparation of Test Solution**

Three 100-ml volumetric flasks were labeled; a placebo equivalent to tablets weight was transferred to each flask. A volume of standard stock solution required to produce 50%, 100%, and 150% tablets content of hydrochlorothiazide and bisoprolol fumarate were placed to flasks. The flasks were half filled with mobile phase, sonicated for 10 minutes, cooled to reach room temperature and completed to the mark with the same solvent. Subsequent dilutions were made with mobile phase like those made for the standard preparation. Each solution was injected three times. The results were collected and subjected to statistical treatments.

2.3.9 Precision

- **Standard**

The stock solution was diluted with mobile phase to give the concentrations of 250µg/ml hydrochlorothiazide and 100µg/ml bisoprolol Fumarate. System suitability solution was injected six times.

- **Sample**

Five tablets were taken into clean and dry 100 ml volumetric flask dissolved with 10 ml methanol, sonicated for 10 min, cooled, then 50 ml mobile phase were added, sonicated for 20 min, cooled to reach room temperature, completed to volume with mobile phase. Then 5ml were diluted with mobile phase into 25ml volumetric flask, passed through 0.45µm pore filter.

2.3.10 Robustness

Accuracy sample solution of target concentration (100%) was used. a method remains unaffected by small, deliberate changes in method parameters like column temperature, flow rate and detection wavelength. Here the column temperature was varied ±5˚C, detection wavelength varied ±2nm and flow rate was varied ±0.1 ml/min.

Chapter Three

Results and Discussions

Chapter Three

3. Results and Discussions

3.1 Results

3.1.1 System Suitability

Shows Table (3.1) and Table (3.2). System suitability results for bisoprolol fumarate and hydrochlorothiazide; respectively

Table (3.1) System suitability results for bisoprolol fumarate

No	Area	Retention time	Tailing factor	Resolution	Theoretical plates
1	1476536	4.718	1.247	10.468	39604
2	1477324	4.71	1.241	10.438	39290
3	1475963	4.72	1.238	10.422	39390
4	1475068	4.722	1.236	10.421	39316
5	1470496	4.71	1.235	10.385	39211
6	1475461	4.719	1.234	10.358	39092
AVG	1475141.333	4.7165	1.2385	10.41533333	39317.16667
STDEV	2411.26321	0.005205766	0.00484768	0.038913579	173.3717586
RSD%	0.163459809	0.110373498	0.39141541	0.373618186	0.440956898

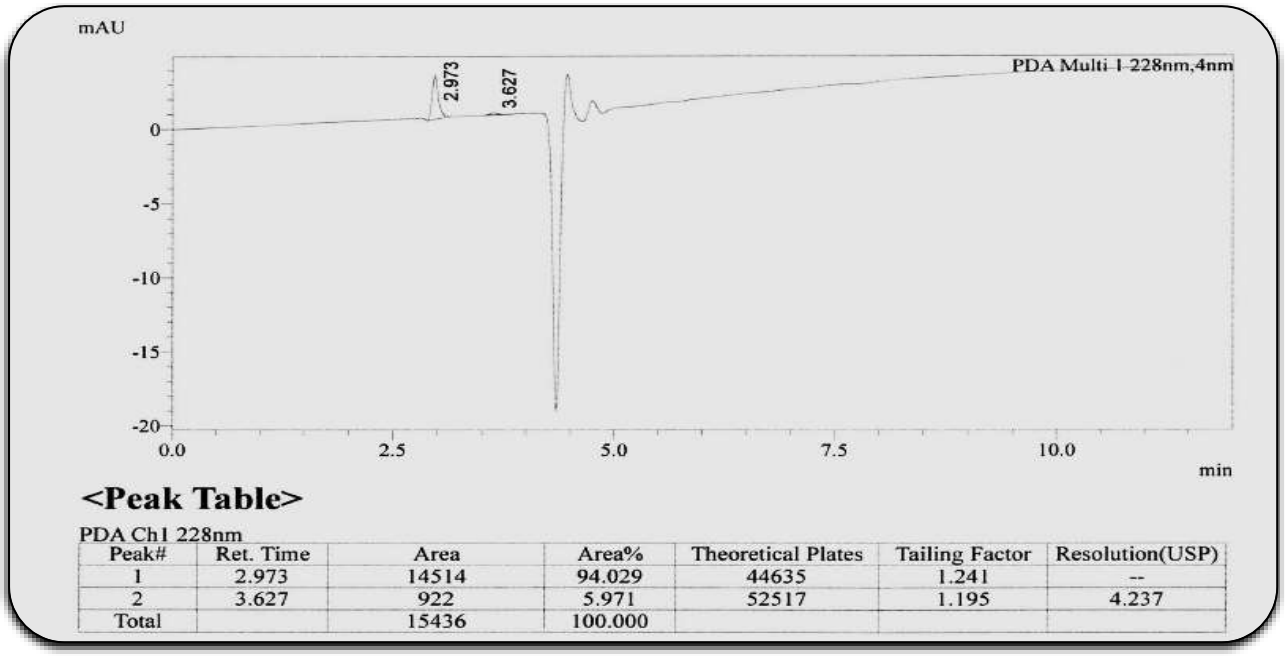
Table (3.2) System suitability results for hydrochlorothiazide

No	Area	Retention time	Tailing factor	Resolution	Theoretical plates
1	12358531	7.783	1.166	10.468	55637
2	12369606	7.775	1.161	10.438	55195
3	12365433	7.784	1.157	10.422	55143
4	12322841	7.785	1.154	10.421	55316
5	12323359	7.763	1.154	10.385	54845
6	12363664	7.779	1.151	10.358	54432
AVG	12350572.33	7.778166667	1.157166667	10.41533333	55094.66667
STDEV	21575.19108	0.008304617	0.005492419	0.038913579	413.9993559
RSD%	0.174689808	0.106768311	0.474643729	0.373618186	0.751432726

Shows Table (3.1) and (3.2) Tailing factors, resolution, theoretical plates and %RSD were satisfactory with to USP and ICH guidelines

3.1.2 Specificity

Figure 3.1, Figure 3.2, Figure 3.3 and Figure 3.4 shows the specificity chromatograms for placebo, sample, standard of bisoprolol fumarate, hydrochlorothiazide and Fumaric acid; respectively.



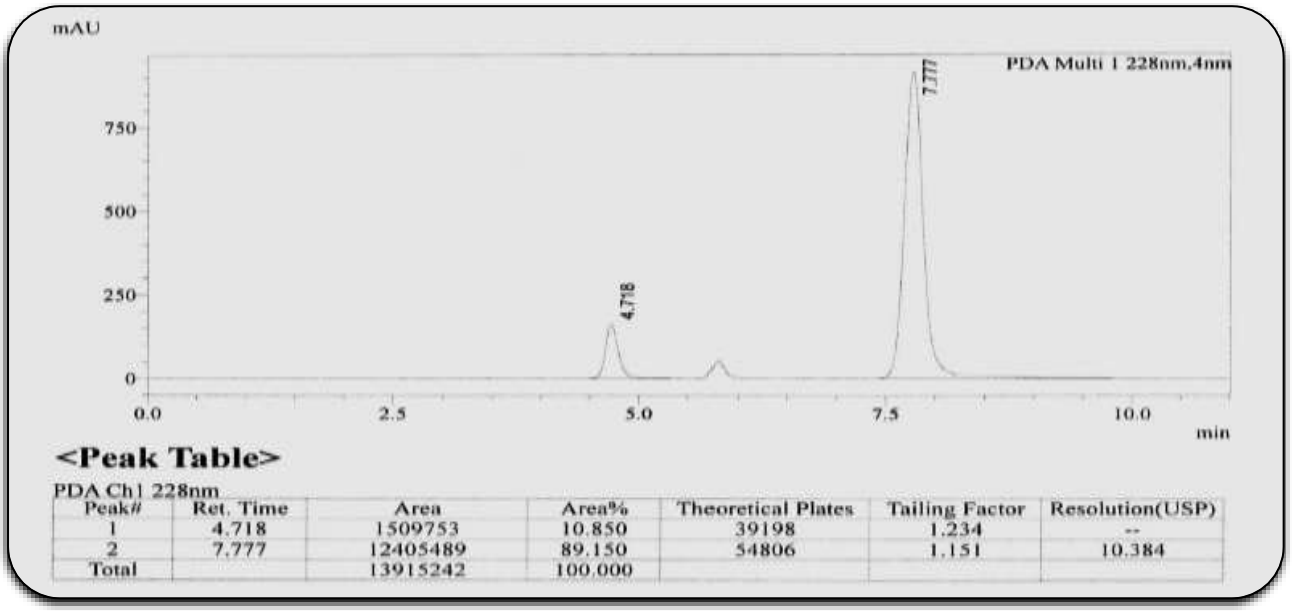
<Peak Table>

PDA Ch1 228nm

Peak#	Ret. Time	Area	Area%	Theoretical Plates	Tailing Factor	Resolution(USP)
1	2.973	14514	94.029	44635	1.241	--
2	3.627	922	5.971	52517	1.195	4.237
Total		15436	100.000			

Figure 3.1 **chromatogram for the Placebo of bisoprolol fumarate and hydrochlorothiazide**

none of placebo peaks had same retention time of active ingredients peaks, this indicates that the excipients used in the formulation did not interfere.

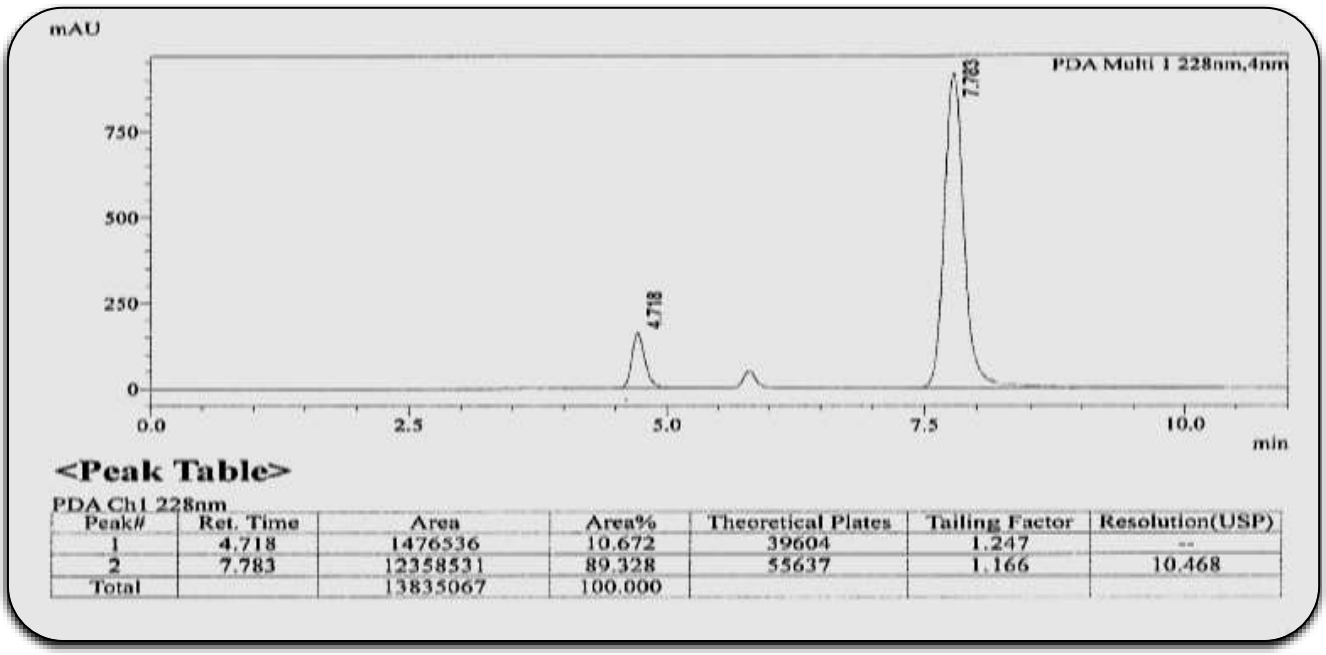


<Peak Table>

PDA Ch1 228nm

Peak#	Ret. Time	Area	Area%	Theoretical Plates	Tailing Factor	Resolution(USP)
1	4.718	1509753	10.850	39198	1.234	--
2	7.777	12405489	89.150	54806	1.151	10.384
Total		13915242	100.000			

Figure 3.2 **chromatogram for the sample of bisoprolol fumarate and hydrochlorothiazide**



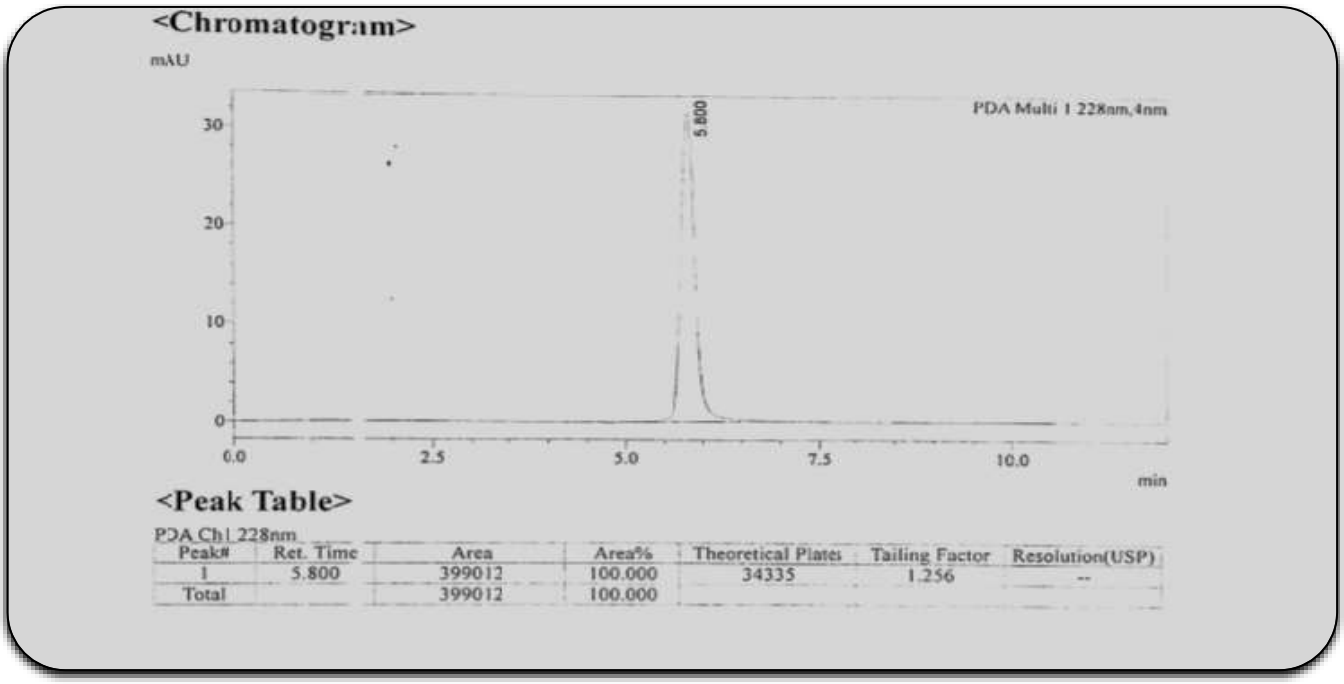
<Peak Table>

PDA Ch1 228nm

Peak#	Ret. Time	Area	Area%	Theoretical Plates	Tailing Factor	Resolution(USP)
1	4.718	1476536	10.672	39604	1.247	--
2	7.783	12358531	89.328	55637	1.166	10.468
Total		13835067	100.000			

Figure 3.3: **chromatogram for mixed standard of bisoprolol fumarate and hydrochlorothiazide**

Shows Figure 3.2 and Figure 3.3 The method was found to be specific to these two active ingredients.



<Peak Table>

PDA Ch1 228nm

Peak#	Ret. Time	Area	Area%	Theoretical Plates	Tailing Factor	Resolution(USP)
1	5.800	399012	100.000	34335	1.256	--
Total		399012	100.000			

Figure (3.4) **chromatogram for fumaric Acid**

This peak confirmed and given the same retention time as second peak the combination by injection of fumaric acid.

3.1.3 Linearity, LOD and LOQ

I. Bisoprolol fumarate

Shows Table (3.3) linearity results for bisoprolol fumarate

Table (3.3) linearity results for bisoprolol fumarate

%	40%	60%	80%	100%	120%	140%	160%
C. µg/ml	40	60	80	100	120	140	160
Area – 1	567911	856268	1123553	1395731	1691940	1973292	2229507
Area – 2	567682	855959	1123277	1395368	1691052	1968819	2229121
Area – 3	568128	855616	1121591	1394959	1690548	1977723	2248307
AVG Area	567907	855947.666	1122807	1395352.667	1691180	1973278	2235645
STDEV	223.0269	326.1477	1062.0904	386.2283	704.7723	4452.016	10967.312
RSD%	0.0393	0.0381	0.0946	0.0277	0.0417	0.2256	0.4906

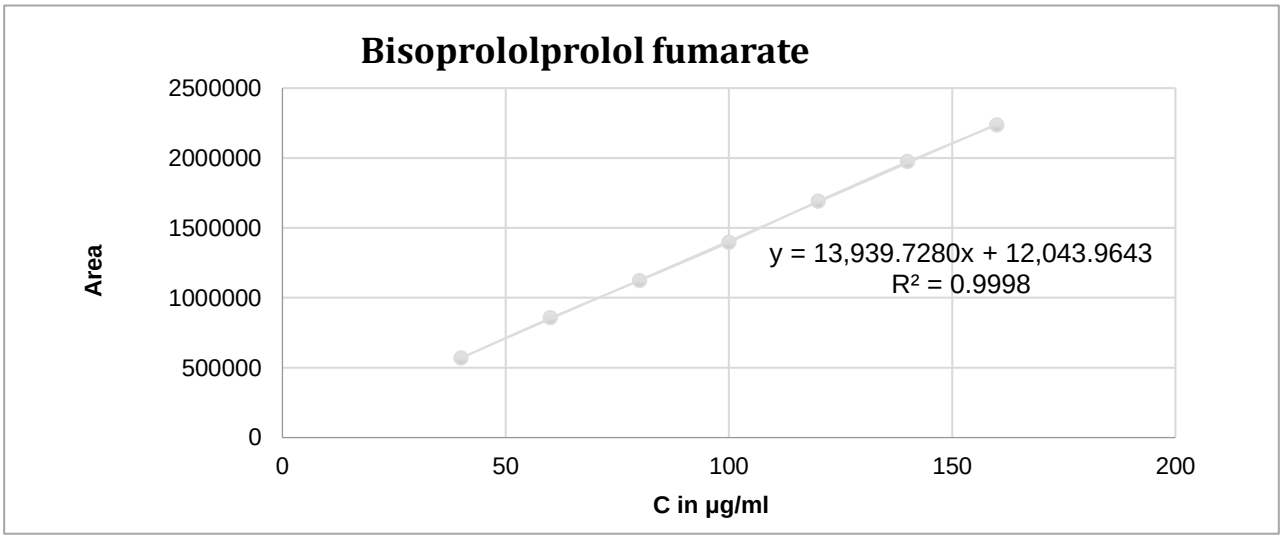


Figure 3.5: **Plot of average area versus concentration for bisoprolol fumarate**

Table (3.4) shows regression parameters for Bisoprolol fumarate

Table (3.4) Regression parameters for data Bisoprolol fumarate

Parameter	**Value**
Regression Coefficient R2	0.999829
Root Mean Squire Error(RME)	8631.25704
Slope (S)	13939.728
Intercept	12043.9643

- **Limit of detection and limit of quantitation for Bisoprolol fumarate**

LOD =3*RMSE/S

= 3* 8631.257/13939.728= 1.857µg/ml

LOQ=10*RMSE/S

=10*8631.257/13939.728=6.192µg/ml

II. Hydrochlorothiazide

Shows table (3.5) linearity results for Hydrochlorothiazide

Table (3.5) linearity result for Hydrochlorothiazide

%	**40%**	**60%**	**80%**	**100%**	**120%**	**140%**	**160%**
C. µg/ml	100	150	200	250	300	350	400
Area – 1	4671998	6863543	9069697	11397175	13575926	15737025	17890700
Area – 2	4646667	6862508	9086374	11395711	13581165	15679591	17893591
Area – 3	4670935	6863214	9045076	11367125	13551764	15716988	17865704
AVG Area	4663200	6863088	9067049	11386670	13569618	15711201	17883331
STDEV	14327.859	528.81975	20775.951	16942.57552	15682.62396	29150.9900	15334.2898
RSD%	0.3072538	0.0077052	0.2291369	0.148793063	0.115571592	0.18554271	0.08574627

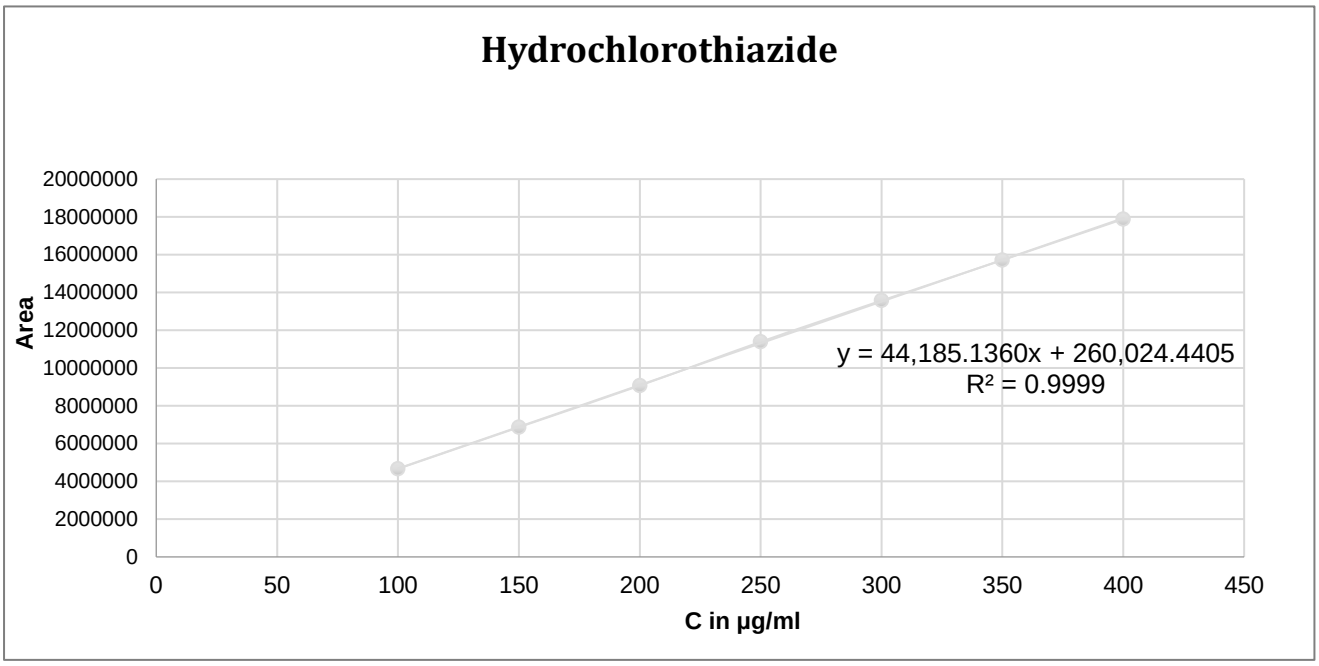


Figure 3.6: **Plot of average area versus concentration for hydrochlorothiazide**

The concentrations range was (40–160) µg/ml for bisoprolol fumarate and (100–400) µg/ml for hydrochlorothiazide. Each solution was injected in triplicate. Plot of average area versus prepared concentrations, correlation (R^2 =0.999) for both components indicates a very good linearity.

Shows Table (3.6) Regression parameters for Hydrochlorothiazide by excel program

Table (3.6) Regression parameters for Hydrochlorothiazide

Parameter	**Value**
Regression Coefficient (R^2)	0.9998984
Root Mean Squire Error(RME)	52699.89639
Slope (S)	44185.13595
Intercept	260024.4405

- **Limit of detection and limit of quantitation for Hydrochlorothiazide**

LOD =3*RMSE/S

= 3* 52699.89639/ 44185.13595= 3.578µg/ml

LOQ=10*RMSE/S

=10*52699.89639/44185.13595=11.927µg/ml

3.1.4 Accuracy

Table (3.7) shows the results of mixed standard of bisoprolol fumarate and hydrochlorothiazide while the accuracy results for bisoprolol fumarate and hydrochlorothiazide samples were shown in Table (3.8) and Table (3.9), respectively; summary of accuracy results for both components is shown in Table (3.10).

Table (3.7) Results of hydrochlorothiazide and bisoprolol fumarate standard for accuracy test

No	Bisoprolol fumarate	Hydrochlorothiazide
1	1412198	12000697
2	141312	12006732
3	1413927	12011592
4	1413575	12006457
5	1416091	12027056
6	1414754	12013025
AVG	1413945.17	12010926.5
STDEV	1350.53995	9024.636209
RSD%	0.096	0.075

Table (3.8) Accuracy results for bisoprolol fumarate

Content	50%	100%	150%
1	726795	1415612	2169404
2	726784	1416273	2141343
3	725474	1415666	2169404
AVG	726351	1415850	2160050
STDEV	759.524	367.035	16201.026
RSD%	0.10457	0.02592	0.75003
Recovery	51.371	100.135	152.768
Recovery%	102.741	100.135	101.845

Table (3.9) Accuracy results for hydrochlorothiazide

Content	**50%**	**100%**	**150%**
1	6044602	12026875	18223119
2	6024101	12033751	18290031
3	6045482	12028800	18316606
AVG	6038062	12029809	18276585
STDEV	12098.296	3547.238	48172.028
RSD%	0.20037	0.02949	0.26357
Recovery	50.271	100.157	152.166
Recovery%	100.543	100.157	101.444

Table (3.10) Summary of accuracy results for Recovery % bisoprolol fumarate and hydrochlorothiazide

Content%	**Recovery % of Bisoprolol fumarate**	**Recovery% of Hydrochlorothiazide**
50	102.74	100.54
100	100.14	100.16
150	101.85	101.44
AVG	101.57	100.71
STDEV	1.32402	0.66045
RSD%	1.30351	0.65576

The recovery percentage for bisoprolol fumarate at the above concentrations was found to be 102.7, 100.1 and 101.9, respectively; while for hydrochlorothiazide, it was 100.5, 100.1 and 101.4 respectively. The average of recovery percentage for bisoprolol fumarate and hydrochlorothiazide was 101.5% and 100.7%, respectively, (this results within limits) indicates that the proposed method is accurate.

3.1.5 Precision

I. Intraday Precision

Table (3.11) shows results of bisoprolol fumarate and hydrochlorothiazide mixed standard for intraday precision test.

Table (3.11) for the results of bisoprolol fumarate and hydrochlorothiazide mixed standard for intraday precision test

No	Bisoprolol fumarate	Hydrochlorothiazide
1	1476536	12358531
2	1477324	12369606
3	1475963	12365433
4	1475068	12322841
5	1470496	12323359
6	1475461	12363664
AVG	1475141.333	12350572
STDEV	2411.26321	21575.19
RSD%	0.16	0.17

Table (3.12) and Table (3.13) show intraday precision for bisoprolol fumarate and hydrochlorothiazide, respectively

Table (3.12) Intraday results for bisoprolol fumarate

NO	1st	2nd	3rd	4th
1st trial	1509753	1494563	1505483	1506264
2nd trial	1508969	1502898	1500187	1503023
3rd trial	1509348	1506247	1505875	1500863
AVG	1509357	1501236	1503848	1503383
STDEV	392.072	6016.697	3176.86	2718.47
RSD%	0.02598	0.400783	0.21125	0.180823
Recovery	102.3195	101.76896	101.9461	101.91453
Recovery%	102.3195	101.76896	101.9461	101.91453

Table (3.13) Intraday results for hydrochlorothiazide

NO	1st	2nd	3rd	4th
1st trial	12405489	12311249	12376373	12316188
2nd trial	12456555	12358094	12358972	12349591
3rd trial	12460101	12363895	12312891	12350422
AVG	12440715	12344412.67	12349412	12338733.67
STDEV	30558.1	28866.67	32802.99	19529.5
RSD%	0.24563	0.233844	0.265624	0.15828
Recovery	100.72987	99.950126	99.990605	99.904145
Recovery%	100.72987	99.950126	99.990605	99.904145

Table (3.14) shows Summary of intraday precession for bisoprolol fumarate and hydrochlorothiazide

Table (3.14) Summery intraday precession for bisoprolol fumarate and hydrochlorothiazide

Trial	Bisoprolol fumarate	Hydrochlorothiazide
1st	102.3195	100.7299
2nd	101.76896	99.95013
3rd	101.9461	99.99061
4th	101.9145	99.90414
AVG	101.987265	100.143695
STDEV	0.234539599	0.392396622
RSD%	0.229969496	0.391833577

Bisoprolol fumarate intraday precision, the RSD for the recovery percentage of assay repetitions was 0.025%, 0.40%, 0.211% and 0.18% respectively; whereas for hydrochlorothiazide RSD was 0.245%, 0.233%, 0.265% and 0.158%; respectively. The RSD values was found to be less than 2.0% so it is acceptable according to USP and ICH guidelines.

II. Interday Precision

Table (3.15) shows results of bisoprolol fumarate and hydrochlorothiazide mixed standard for interday precision test

Table (3.15) results of bisoprolol fumarate and hydrochlorothiazide mixed standard for interday precision test

No	Bisoprolol fumarate	hydrochlorothiazide
1	1476536	12358531
2	1477324	12369606
3	1475963	12365433
4	1475068	12322841
5	1470496	12323359
6	1475461	12363664
AVG	1475141.333	12350572
STDEV	2411.26321	21575.19
RSD%	0.16	0.17

Table (3.16) shows intraday precision for both components, respectively. Table (3.11) shows the summary of interday precision

Table (3.16) shows intraday precision for both components

Trial	Bisoprolol fumarate			Hydrochlorothiazide		
	Day1	Day2	Day3	Day1	Day2	Day3
1st trial	1508519	1505433	1505679	12447338	12353751	12332485
2nd trial	1508412	1505402	1503785	12458251	12353319	12290494
3rd trial	1505675	1504767	1504175	12461550	12352396	12334072
AVG	1507535.333	1505200.667	1504546.33	12455713	12353155.33	12319017
STDEV	1611.983974	375.8860643	1000.11266	7438.166374	692.1678505	24714.38425
RSD%	0.1069284	0.02497249	0.0664727	0.05971691	0.005603166	0.200619776
Recovery	102.195993	102.0377257	101.993368	100.851302	100.020914	99.74450307
Recovery%	102.195993	102.0377257	101.993368	100.851302	100.020914	99.74450307

Shows table (3.17) shows interday precision summary for both bisoprolol fumarate and hydrochlorothiazide

Table (3.17) interday precision summary for both bisoprolol fumarate and hydrochlorothiazide

NO	Bisoprolol fumarate	Hydrochlorothiazide
1st trial	102.195993	100.851302
2nd trial	102.0377257	100.020914
3rd trial	101.993368	99.74450307
AVG	102.0756956	100.205573
STDEV	0.106515284	0.576042693
RSD%	0.10434931	0.574860934

For the interday, the RSD for the recovery percentage of bisoprolol fumarate three assay repetitions was 0.106%, 0.024% and 0.066%; respectively, whereas for hydrochlorothiazide RSD was 0.059%, 0.005% and 0.20%, respectively. The RSD values was found to be less than 2.0% so it is acceptable according to USP and ICH.

3.1.6 Robustness

The robustness of the method was determined as per ICH guidelines under a variety of conditions like change in Temperature, wavelength and flow rate. The results obtained by deliberately variation in method parameters.

3.1.6.1 Robustness study of Bisoprolol fumarate

i) Optimized conditions

Shows table (3.18) results of bisoprolol fumarate sample at optimum conditions

Table (3.18) results of optimum conditions of bisoprolol fumarate

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1410282	4.935	41371	1.195	9.64
2	1410818	4.938	41649	1.194	9.648
3	1411423	4.937	41641	1.194	9.651
AVG	1410841	4.936667	41553.67	1.194333	9.646333
STDEV	570.8476154	0.001528	158.2445	0.000577	0.005686
RSD %	0.040461513	0.030942	0.38082	0.048341	0.058947

ii) 5 ˚C more

Shows table (3.19) results of bisoprolol fumarate at 35˚C column temperature

Table (3.19) results of bisoprolol fumarate at 35˚C column temperature

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1466463	4.846	43990	1.197	8.668
2	1465325	4.847	44157	1.198	8.663
3	1466023	4.847	44141	1.2	8.661
AVG	1465937	4.846667	44096	1.198333	8.664
STDEV	573.8536399	0.000577	92.14662	0.001528	0.003606
RSD %	0.03914586	0.011912	0.208968	0.127471	0.041615

iii) 5 ˚C less

Shows table (3.20) results of bisoprolol fumarate at 25˚C column temperature

Table (3.20) results of bisoprolol fumarate at 25˚C column temperature

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1409363	4.942	41291	1.194	9.702
2	1411298	4.935	41269	1.196	9.631
3	1412089	4.936	41256	1.197	9.619
AVG	1410916.667	4.937667	41272	1.195667	9.650667
STDEV	1402.437283	0.003786	17.69181	0.001528	0.044859
RSD %	0.099399016	0.076675	0.042866	0.127755	0.464828

iv) 10 % more Flow rate

Shows table (3.21) results of bisoprolol fumarate at 1ml/min flow rate

Table (3.21) results of bisoprolol fumarate at 1ml/min flow rate

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1269184	4.454	38704	1.193	9.299
2	1267406	4.455	38698	1.191	9.297
3	1267818	4.454	38715	1.193	9.297
AVG	1268136	4.454333	38705.67	1.192333	9.297667
STDEV	930.6793218	0.000577	8.621678	0.001155	0.001155
RSD %	0.073389551	0.012962	0.022275	0.096844	0.012419

v) 10 % less Flow rate

Shows table (3.22) results of bisoprolol fumarate at 0.8ml/min flow rate

Table (3.22) results of bisoprolol fumarate at 0.8ml/min flow rate

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1588774	5.549	44867	1.191	9.99
2	1588679	5.551	44957	1.192	9.997
3	1589747	5.553	45043	1.193	9.999
AVG	1589066.667	5.551	44955.67	1.192	9.995333
STDEV	591.0975667	0.002	88.00758	0.001	0.004726
RSD %	0.037197783	0.03603	0.195765	0.083893	0.04728

vi) 2nm more Wavelength

Shows table (3.23) results of bisoprolol fumarate at 230nm wavelength

Table (3.23) results of bisoprolol fumarate absorbance at 230nm wavelength

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1201242	4.935	41273	1.195	9.62
2	1201570	4.938	41554	1.194	9.628
3	1202095	4.937	41547	1.194	9.63
AVG	1201635.667	4.936667	41458	1.194333	9.626
STDEV	430.2747184	0.001528	160.2529	0.000577	0.005292
RSD %	0.035807419	0.030942	0.386543	0.048341	0.054971

vii) 2nm less Wavelength

Shows table (3.24) results of bisoprolol fumarate at 226nm wavelength

Table (3.24) results of bisoprolol fumarate absorbance at 226nm wavelength

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	1522691	4.935	41382	1.194	9.661
2	1523097	4.938	41671	1.193	9.669
3	1523809	4.937	41652	1.193	9.671
AVG	1523199	4.936667	41568.33	1.193333	9.667
STDEV	565.9363922	0.001528	161.6488	0.000577	0.005292
RSD %	0.037154462	0.030942	0.388875	0.048381	0.054738

3.1.6.2 Robustness study of hydrochlorothiazide

i) Optimized conditions

Shows table (3.25) results of hydrochlorothiazide sample at optimum conditions

Table (3.25) results of hydrochlorothiazide sample at optimum conditions

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	11957725	7.774	56244	1.153	9.64
2	11955607	7.776	56322	1.154	9.648
3	11959046	7.774	56397	1.154	9.651
AVG	11957459.33	7.7746667	56321	1.1536667	9.6463333
STDEV	1734.824006	0.0011547	76.504902	0.0005774	0.0056862
RSD %	0.014508299	0.0148521	0.1358373	0.0500448	0.0589472

ii) 5 ˚C more

Shows table (3.26) results of hydrochlorothiazide at 35˚C column temperature

Table (3.26) results of hydrochlorothiazide at 35˚C column temperature

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	11699930	7.242	56668	1.153	8.668
2	11693708	7.238	56676	1.153	8.663
3	11700910	7.238	56698	1.155	8.661
AVG	11698182.67	7.239333	56680.67	1.153667	8.664
STDEV	3906.031405	0.002309	15.53491	0.001155	0.003606
RSD %	0.03339007	0.031901	0.027408	0.10009	0.041615

iii) 5 ˚C less

Shows table (3.27) results of hydrochlorothiazide at 25˚C column temperature

Table (3.27) results of hydrochlorothiazide at 25˚C column temperature

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	11963304	7.809	56350	1.153	9.702
2	11964965	7.778	55976	1.153	9.631
3	11972469	7.777	55869	1.154	9.619
AVG	11966912.7	7.788	56065	1.1533333	9.6506667
STDEV	4883.06874	0.0181934	252.54901	0.0005774	0.044859
RSD %	0.0408047	0.2336082	0.4504575	0.0500593	0.4648284

iv) 10 % more flow rate

Shows table (3.28) results of hydrochlorothiazide at 1ml/min flow rate

Table (3.28) results of hydrochlorothiazide at 1ml/min flow rate

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	10800235	6.996	53330	1.156	9.299
2	10792695	6.996	53335	1.156	9.297
3	10790233	6.993	53386	1.155	9.297
AVG	10794387.67	6.995	53350.33	1.155667	9.297667
STDEV	5211.414523	0.001732	30.98925	0.000577	0.001155
RSD %	0.048278927	0.024761	0.058086	0.049958	0.012419

v) 10 % less flow rate

Shows table (3.29) results of hydrochlorothiazide at 0.8ml/min flow rate

Table (3.29) results of hydrochlorothiazide at 0.8ml/min flow rate

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	13409613	8.747	59746	1.15	9.99
2	13406495	8.748	59887	1.15	9.997
3	13413274	8.748	59982	1.15	9.999
AVG	13409794	8.747667	59871.67	1.15	9.995333
STDEV	3393.122603	0.000577	118.7448	0	0.004726
RSD %	0.025303316	0.0066	0.198332	0	0.04728

vi) 2nm more Wavelength

Shows table (3.30) results of hydrochlorothiazide absorbance at 230nm wavelength

Table (3.30 results of hydrochlorothiazide absorbance at 230nm wavelength

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	9667692	7.774	55928	1.156	9.62
2	9666205	7.775	56005	1.156	9.628
3	9669871	7.773	56085	1.157	9.63
AVG	9667922.66	7.774	56006	1.156333	9.626
STDEV	1843.8531	0.001	78.5047	0.0005	0.0052
RSD %	0.0190	0.0128	0.140	0.049	0.054

vii) 2nm less Wavelength

Shows table (3.31) results of hydrochlorothiazide absorbance at 226nm wavelength

Table (3.31) results of hydrochlorothiazide absorbance at 226nm wavelength

Trial No	Area	Retention Time	Theoretical plates	Tailing factor	Resolution
1	13503899	7.775	56647	1.15	9.661
2	13500076	7.776	56720	1.151	9.669
3	13503510	7.774	56796	1.151	9.671
AVG	13502495	7.775	56721	1.150667	9.667
STDEV	2103.925141	0.001	74.50503	0.000577	0.005292
RSD %	0.015	0.012	0.131	0.050	0.054

The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions such as column temperature (± 5°C), flow rate (± 0.1ml) and detection wavelength (±2 nm). RSD for the area at all different conditions for target indicates that the proposed method is robust.

3.2 Discussions

System suitability was carried out with six injections of solutions of 100% concentration having 100µg/ml of Bisoprolol Fumarate and 250µg/ml Hydrochlorothiazide of each in to the chromatographic system. Since the number of theoretical plates for the two drugs was above 2000, it indicates that the column was efficient in separating all the three drugs. Tailing factors, resolution and %RSD were satisfactory with to USP and ICH guidelines.

Linearity of this method was checked using seven solutions centered with the target concentration, the concentrations range was (40–160) µg/ml for bisoprolol fumarate and (100–400) µg/ml for hydrochlorothiazide. Each solution was injected in triplicate. Plot of average area versus prepared concentrations indicates a very good linearity correlation (R^2 =0.999) for both components. The limit of detection for bisoprolol fumarate and hydrochlorothiazide was found to be 1.8575µg/ml and 3.57811µg/ml; respectively, whereas the limit of quantitation was found to be 6.19184 µg/ml and 11.92µg/ml, respectively.

In specificity tests, none of placebo peaks had same retention time of active ingredients peaks, this indicates that the excipients used in the formulation did not interfere in the estimation when we used this method for assay in tablets. The second peak in Sample and standard was confirmed as fumarate by injection of fumaric acid alone and given the same retention time as the combination. Shows (Figure 3.2, 3.3 and Figure 3.4).

Accuracy was evaluated for bisoprolol fumarate and hydrochlorothiazide using three concentrations in content of 50%, 100%, and 150 of target concentration. The recovery percentage for bisoprolol fumarate at the above concentrations was found to be 102.741, 100.135 and 101.845, respectively; while for hydrochlorothiazide, it was 100.543, 100.157 and 101.444 respectively. The average of recovery percentage for bisoprolol fumarate and hydrochlorothiazide was 101.5736% and 100.7146%, respectively, (this results within limits) indicates that the proposed method is accurate.

The precision of the methods was examined by estimating the corresponding recovery percentages four times on the same day in intraday precision and three times at three different days for inter day precision. The concentrations used was 100% of target concentration as per ICH. For bisoprolol fumarate intraday precision, the RSD for the

recovery percentage of assay repetitions was 0.025%, 0.40%, 0.211% and 0.18% respectively; whereas for hydrochlorothiazide RSD was 0.245%, 0.233%, 0.265% and 0.158%; respectively. For the interlay, the RSD% for the recovery percentage of bisoprolol fumarate three assay repetitions was 0.106%, 0.024% and 0.066%; respectively, whereas for hydrochlorothiazide RSD was 0.059%, 0.005% and 0.20%, respectively. The RSD values was found to be less than 2.0% so it is acceptable according to USP and ICH. The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions such as column temperature (± 5°C), flow rate (± 0.1ml) and detection wavelength (±2 nm). RSD for the area at all different conditions for target indicates that the proposed method is robust.

3.3 Conclusions

- The proposed method is simple, sensitive and reproducible.
- Statistical analysis of the results has been carried out revealing high accuracy and good precision.
- The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement with USP and ICH guidelines.
- The developed method can be used for routine quantitative simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in multicomponent pharmaceutical preparation.

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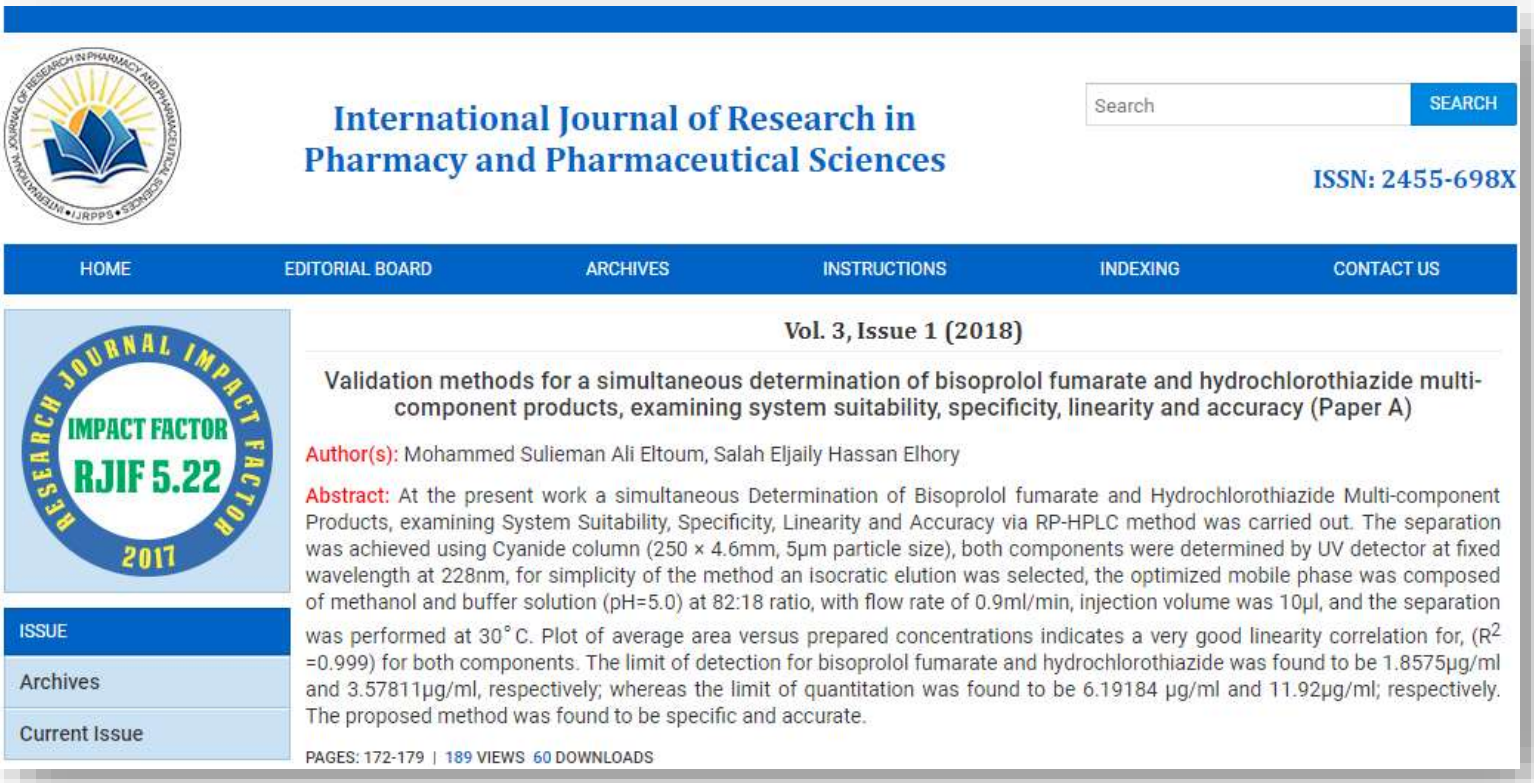
Appendixes

Published Papers

Eltoum M. S. A and Elhory, S.E.H. (2018), Validation methods for a simultaneous determination of bisoprolol fumarate and hydrochlorothiazide multi-component products, examining system suitability, specificity, linearity and accuracy. *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, **3**(1),172-179.

Eltoum M. S. A and Elhory, S.E.H. (2018), Validation methods for a simultaneous determination of bisoprolol fumarate and hydrochlorothiazide multi-component products, examining system suitability, precision and robustness. *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, **3**(1),236-243.

Paper NO.1



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Archives

Current Issue

Vol. 3, Issue 1 (2018)

Validation methods for a simultaneous determination of bisoprolol fumarate and hydrochlorothiazide multi-component products, examining system suitability, specificity, linearity and accuracy (Paper A)

Author(s): Mohammed Sulieman Ali Eltoum, Salah Eljaily Hassan Elhory

Abstract: At the present work a simultaneous Determination of Bisoprolol fumarate and Hydrochlorothiazide Multi-component Products, examining System Suitability, Specificity, Linearity and Accuracy via RP-HPLC method was carried out. The separation was achieved using Cyanide column (250 × 4.6mm, 5µm particle size), both components were determined by UV detector at fixed wavelength at 228nm, for simplicity of the method an isocratic elution was selected, the optimized mobile phase was composed of methanol and buffer solution (pH=5.0) at 82:18 ratio, with flow rate of 0.9ml/min, injection volume was 10µl, and the separation was performed at 30° C. Plot of average area versus prepared concentrations indicates a very good linearity correlation for, (R^2 =0.999) for both components. The limit of detection for bisoprolol fumarate and hydrochlorothiazide was found to be 1.8575µg/ml and 3.57811µg/ml, respectively; whereas the limit of quantitation was found to be 6.19184 µg/ml and 11.92µg/ml; respectively. The proposed method was found to be specific and accurate.

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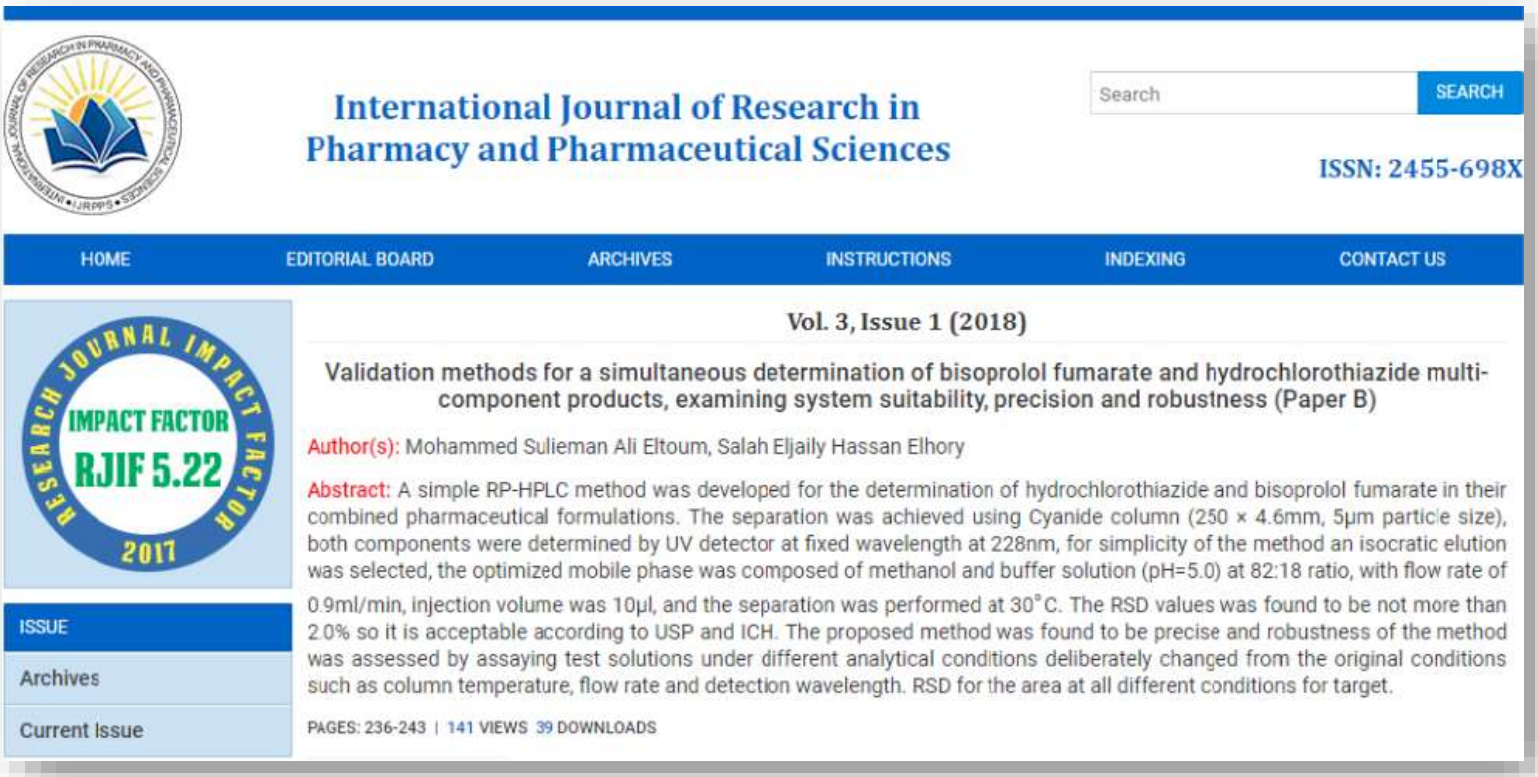

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Current Issue

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Validation methods for a simultaneous determination of bisoprolol fumarate and hydrochlorothiazide multi-component products, examining system suitability, precision and robustness (Paper B)

Author(s): Mohammed Sulieman Ali Eltoum, Salah Eljaily Hassan Elhory

Abstract: A simple RP-HPLC method was developed for the determination of hydrochlorothiazide and bisoprolol fumarate in their combined pharmaceutical formulations. The separation was achieved using Cyanide column (250 × 4.6mm, 5µm particle size), both components were determined by UV detector at fixed wavelength at 228nm, for simplicity of the method an isocratic elution was selected, the optimized mobile phase was composed of methanol and buffer solution (pH=5.0) at 82:18 ratio, with flow rate of 0.9ml/min, injection volume was 10µl, and the separation was performed at 30° C. The RSD values was found to be not more than 2.0% so it is acceptable according to USP and ICH. The proposed method was found to be precise and robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions such as column temperature, flow rate and detection wavelength. RSD for the area at all different conditions for target.

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