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A SIMPLE ION-ASSOCIATION COMPLEX FORMATION BASED SPECTROPHOTOMETRIC STUDY OF DI[R, S]-3-[4-(2-METHOXYETHYL)PHENOXY-1-(ISOPROPYL AMINO)PROPAN-2-OL] TARTRATE (METOPROLOL TARTRATE) BY APPLYING AN AZO DYE, TROPAEOLIN OOO

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ABSTRACT

Based on the drug's complexation with Tropaeolin OOO (TPOOO), a novel, sensitive, accurate, and simple visible spectrophotometric approach has been established for metoprolol tartrate (MPT) assessment in this research study. A quick and simple technique to measure MPT quantitatively in biological matrices would be helpful for both therapeutic monitoring and cases of intoxication. A validated UV-visible spectrophotometric quantitative assessment method of MPT in caplet as well as in bulk formulation at a molar absorptivity of 1.746×10^4 L. mole^{-1} cm^{-1} and λ_{max} of 485 nm is presented in this research. Targeted for validation were the following parameters: precision, accuracy, sensitivity, and linearity. The basis for this advocated visible spectrophotometric approach is the secondary amine group present in the MPT molecule. It has been demonstrated that the chromogenic reagent, TPOOO is very effective in the identification and quantification of compounds containing amino groups. MPT and TPOOO co-ordination complex, which can be extracted into chloroform from an aqueous solution, is the colorful species that results. The current work is based on the complex formed having a blue color that forms when the drug, MPT is treated with TPOOO as it contains a secondary amine group. The method presented here is more sensitive, selective, rapid, cheap, easily reproducible, and simple. These factors make it suitable for routine examination, including the measurement of MPT in biological samples and pharmaceutical formulations.

Keywords: Metoprolol, Cardiovascular, Method Validation, Spectrophotometric, Tropaeolin OOO, B-Blockers.
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INTRODUCTION

Globally, cardiovascular disease is one of the most prevalent causes of death, accounting for one in three fatalities.[1–3] A cardio specific β1 adrenergic receptor antagonist, metoprolol tartrate (MPT) is primarily used to treat myocardial infarction, angina pectoris, ventricular tachycardia, cardiac arrhythmia, supraventricular tachycardia, and to prevent migraine headaches.[4-7] Table-1 and 2 show the official status of the medication and the structural characteristics of metoprolol as tartrate salt, respectively. It is a white powder, crystalline with a molar mass of 684.82 and a formula of $(C_{15}H_{25}NO_3)_2\ C_4H_6O_6$. In water, it dissolves readily with an elimination half-life of 3-7 hours.[8-10]

MPT works by lessening the heart's agonistic response to catecholamines, which are produced during conditions of both physical and emotional stress. This indicates that the typical rise in blood pressure, cardiac output, heart rate, and cardiac contractility brought on by an abrupt catecholamine surge is diminished.[11] This medication, even in small dosages, is highly sensitive; and significantly inhibits β1adreno receptors.[12,13] Nevertheless, some global organizations like International Olympic Committee, (IOC) have included β-blockers in the list of prohibited substances because of their abuse as doping agents in sports.[14] Thus, it is extremely important to create this type of analytical approach for determining MPT. The medication is formally included in Martindale's The Extra Pharmacopoeia.[15] The British Pharmacopoeia lists the drug's approved test and provides instructions for a potentiometric titration technique.[16] Its determination has been documented using a variety of analytical techniques, including thin

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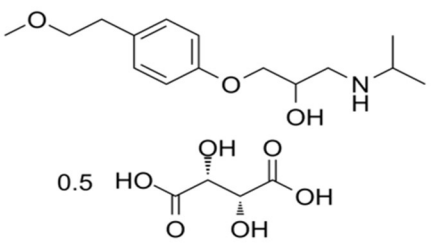
layer chromatography[17,18], high-performance liquid chromatography[19], gas chromatography[20], and electrochemical approaches.[21]

Table-1: Official Status of Metoprolol

Drug	IP[22]	EP[23]	USP[24]	Merck[25] Index	Remington's[26] pharmaceutical Sciences	PDR[27]
Metoprolol Tartrate	486-487	1192-1193	1015	802-803	902	634

Table-2: Structural Features of Metoprolol as Tartrate Salt

Chemical name	Structure	Analytically useful functional groups
Di[R,S]-3-[4-(2-methoxyethyl)phenoxy-1-(isopropylamino)propan-2-ol] tartrate		Methoxyl, secondary hydroxyl, secondary amine, imino ol.

The aforementioned are sensitive and effective methods, however, they need a tedious cleanup process before the medication can be analyzed. The speed, simplicity, and lack of pretreatment processes make spectrophotometry appealing in the visible region. Surveys on the existing literature revealed that there are only a few reports available on simple visible spectrophotometric methods (Ninhydrin[28], NBS[29], Bromanil[30], and related moieties[31-43]) for the determination of MPT. Due to the chemical characteristics of MPT drug molecules, there is plenty of room for improvement in the sensitivity, specificity, precision, and accuracy of visible spectrophotometry (Table-2). Table-3 shows the unique qualities and medicinal significance of metoprolol as tartrate salt.

Table-3: Characteristics and Therapeutic Importance of 'Metoprolol' as Tartrate Salt

S. No.	Characteristics		Therapeutic importance
1.	Molecular formula	$(C_{15}H_{25}NO_3)_2{\cdot}C_4H_6O_6$	A selective β_1-antagonist with slight β_2- antagonist activity. Its β_2-blocking activity is sufficient that in therapeutic dosage it can suppress adrenergic-induced hyperglycemia and also frequently cause bronchospasm. It only slightly increases airway resistance in normal persons and moderately in persons with obstructive airway disease. Presently this drug is approved only for treating angina pectoris, hypertension, and after myocardial infarction.
2.	Molecular weight	684.82	
3.	Color	White	
4.	odor	NA	
5.	Taste	Bitter	
6.	State of Existence	crystalline powder	
7.	Melting point range	121^0-124^0C	
8.	Therapeutic category	β-adrenoceptor antagonist or β-adrenergic blocking agent	
9.	Solubility of MPT	Soluble in water, 95 % ethanol, chloroform, and dichloromethane; whereas, soluble slightly in acetone, and ether insoluble.	

This method, which is based on MPT's reactivity with reagent TPOOO, generates color species with a fair amount of stability and makes it possible to use visible spectrophotometry to determine MPT and its pharmaceutical formulations.

This work successfully attempted to estimate the drug's properties using UV spectrophotometric analysis. This straightforward, quick, accurate, repeatable, and cost-effective approach has been reportedly used to assess MPT in tablet and bulk formulations under the ICH Q2 R1 regulations[44-46].

EXPERIMENTAL

Equipment

A spectrophotometer of Shimadzu, model UV-2600, was employed to measure the spectral parameters using 1 cm matched quartz cuvettes. An Elico pH meter, bearing model number LI-120, was used to monitor the pH of the constituents.

Chemicals and Reagents

Pharma firms provided pure medication samples of MPT. We purchased MPT-containing commercial tablet formulations from pharmacies nearby. Table 4 lists the pharmaceutical formulations that were considered. Analytical grade reagents and chemicals were all utilized. Water that had been double-distilled was used to prepare solutions.

Table-4: Pharmaceutical Preparations Available in the Local Market

Generic Name	Company	Formulations	Proprietary Name	Strength of the formulation
Metoprolol	Dr. Reddy's	Tablet	BETAONE-XL	100mg.
	Novartis India	Tablet	LOPRESOR	50mg
	Micro Cardicare	Tablet	METAPRO	50mg
	Micro Cardicare	Tablet	METAPRO-XL	50mg
	Orchid	Tablet	METO	25mg 50mg 100mg
	Astra Zeneca	Tablet	BETALOC	25mg 50mg 100mg
	Astra Zeneca	Capsule	BETALOC	25mg
	Cipla	Tablet	METOLAR	25mg 50mg 100mg

Standard Stock Solutions Preparatory Measures

The process is to dissolve 100 milligrams of MPT powder in 100 milliliters of double distilled (dd) water to create a stock solution (1mg/ml). To create an MPT standard solution with a conc. of 500 µg/ml for the suggested procedure, the solution was diluted using dd water.

Examining the Spectra and Choosing Wavelengths

A predetermined quantity of MPT was added to the prepared solution, and the colors were created by following the indicated processes to determine the optimal wavelength at maximum absorption (λ_{max}) of that colored species formed in the spectrophotometry approach. λ_{max}, the absorption maxima is noted in the 400–800 nm wavelength range. A spectrophotometer was used to scan the absorption spectra in the 400–800 nm wavelength range in comparison to a matching reagent blank. Figure-1 provides a graphical representation of the results. Colored species' absorption curves have distinctive absorption maxima, whereas the method's blank exhibits little to no absorption in this area.

Sample Solution Preparation

Solution of TPOOO (Fluka chemical; 0.2%, 5.70×10^{-3}M)

By dissoluting 200 mg of TPOOO in hundred ml of dd water, the required solution of TPOOO can be obtained.

Solution of Hydrochloric Acid, HCl

(E-Merck, 0.1M): Made by standardizing 8.6 milliliters of concentrated HCl in 1000 milliliters of distilled water.

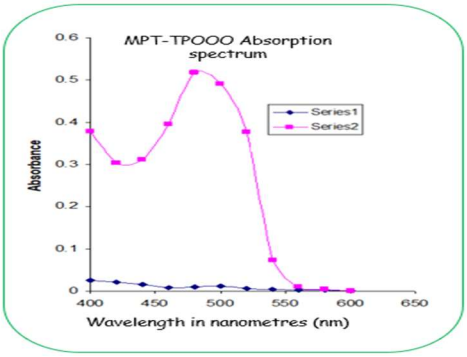


Fig.-1: MPT-TPOOO Complex Absorption Spectrum

Chloroform

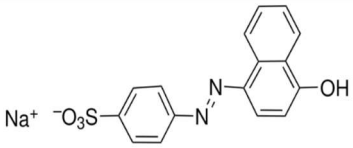
Analytical reagent (AR) Grade Chloroform was used.

Detection Method

The Following is the Suggested Process for Determining MPT

Ion Association Complexes Formation: A unique type of molecular combination called an ion-association complex, or adduct, is produced when two components that are extractable into organic solvents from an aqueous phase at an appropriate pH are combined. One part is a chromogen that is insoluble in organic solvents due to its charge (it can be either cationic or anionic). The second one is colorless and has the opposite charge from chromogen. Table-5 displays the chemical characteristics of the dye utilized in the production of ion-association complexes.

Table-5: Dye Chemical Features

Name of the Dye	Chemical category	Structure	Chemical Name
Tropaeolin OOO (TPOOO)	Azo (Mono Azo)		4-(4-Hydroxy-1-naphthyl azo) benzenesulfonic acid sodium salt (C.I. 15510)

Several compounds with basic moieties (2^0 or 3^0 aliphatic amino groups) have been estimated using ion-association complex extraction, which uses a chlorinated solvent as the extractant and an acid dye as the reagent. The parameters of the experiment (concentration of components, aqueous phase pH) may have an impact on the species' structure. When acidified, the color can change or become more intense, or it can be extracted again into the buffer. Extraction of the complexed species into the organo-phase is frequently hindered due to the presence of substituents that are hydrophilic in nature like -OH and -COOH. Using the right organic solvent as an extractant can improve the reaction's selectivity; however, this is dependent on factors like the dye and amine's polarities. TPOOO is utilized in the current study's MPT test. Together with TPOOO, the drug MPT produces an ion-association complex that may be extracted into chloroform from the aqueous phase. As shown in Fig.-2, the positively charged protonated nitrogen of MPT is predicted to attract the component of opposite charge of the dye and behave as unity which is held together by electrostatic attractions.

Analytical Discussion

Suggested Process for Mass Samples

A set of six separating funnels, with a volume of 125 ml each, were taken and filled with a standard MPT solution (0.5 - 2.5 ml, 100 µg/ml concentration). To these funnels, 2.0 milliliters of TPOOO and 6.0 milliliters of 0.1M solution of hydrochloric acid were added respectively.

After adding dd water, the aqueous phase volume in the glass equipment was brought to 15 ml. 10 milliliters of chloroform was added to these separating funnels, and the mixture was then agitated for two minutes and left to separate. After gathering the organic layer, the absorbance calibrated at 485 nm was promptly in comparison to the blank reagent. For one hour, the colored species was allowed to remain steady. Then Beer's law trace was employed to determine the quantity of MPT in the sample solution.

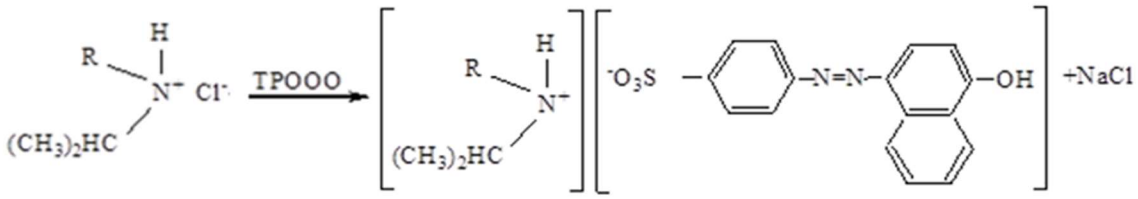


Fig.-2: Scheme Showing MPT and TPOOO Reaction Sequence

Procedure to Prepare Pharmaceutical Formulations

Making MPT stock solutions for use in drug formulations: After the spores were removed, a precisely weighed piece of caplet or capsule in powder form corresponding to 100 milligrams of MPT was extracted using three separate 10 ml volumes of chloroform and filtered. After the combined filtrate was completely evaporated, part of this precipitate was then dissolved in dd water to get a 1 mg/ml concentration. The creation of standard drug solutions and reference techniques required a step-by-step dilution of this solution using dd water. To get the required concentration of 1 mg/ml, another part of the residue shall be dissolved in DMF. As part of the preparation process for a standard medication solution, this solution was diluted further with DMF. The procedure described under bulk samples was then used to assay the MPT in formulations.

Formulations Analysis

Several samples containing MPT were examined using the suggested technique and the reference method to determine if the proposed approach was appropriate for the assay of pharmaceutical formulations. Then T-tests and F-tests were used to compare data of each of the suggested ways with the reference method statistically, and it was discovered that there was no significant difference. The outcomes are noted in Table-6.

Recovery Studies

The suggested approach was used to analyze each pharmaceutical preparation for the active component in the first place. After putting the concentration within the bounds of Beer's law, ten milligrams of MPT were added to each one of the preparations that had been previously examined. The total quantity of MPT was then once more calculated using the suggested approach. Figures-3 and 4 depict Beer's law plots of the MPT-TPOOO System and MPT in an aqueous medium, respectively. Table-6 lists the results.

Interference Studies

A variety of excipients and other additives that are typically included in formulations were examined for their potential impact on the optimal circumstances for determining MPT. In the study, it was discovered that none of the excipients used in these pharma preparations affected this suggested procedure's ability to estimate MPT. However, before its calculation, preparatory treatment is required, as explained under the methods for pharma formulations.

Method Validation

The practice of establishing recorded proof provides a high degree of assurance that a given action will consistently generate the anticipated outcome or a product that fulfills its set specifications and quality standards. The process outlined in the literature, like United States Pharmacopoeia and ICH recommendations for the validation of analytical techniques, was followed for the proposed UV spectrophotometric method. The method's linearity, accuracy, and precision were assessed as performance metrics.

The Method's Linearity and Sensitivity

Understanding a color's sensitivity is crucial, and the adjectives listed below are frequently used to convey sensitivity. The Bouguer-Lambert-Beer law states,

$$\text{Absorbance, } A = log \frac{Incident\ radiation\ intensity}{Transmitted\ ligh\ \ intensity} = \epsilon ct$$

If absorptivity (€) and medium thickness (t) are constant, then absorbance, A is directly proportional (∞) to the concentration (C) of the medium of absorption. The constant is known as molar absorptivity when it is represented in moles/lit; the values of λ_{max} and Beer's law limitations are given as L mol^{-1} cm^{-1} and µg/ml, correspondingly. A number of micrograms of the pharmaceutical preparation to be determined, which in turn is transformed into the colored product, which is present in a cross-sectional column solution $1Cm^2$, is known as Sandell's sensitivity. The absorbance of 1 Cm^2 is 0.001 (represented as µg/Cm^2).

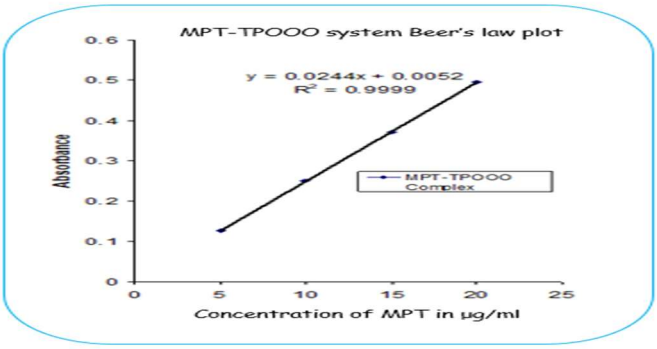


Fig.-3: Graph of Beer Law for MPT-TPOOO System

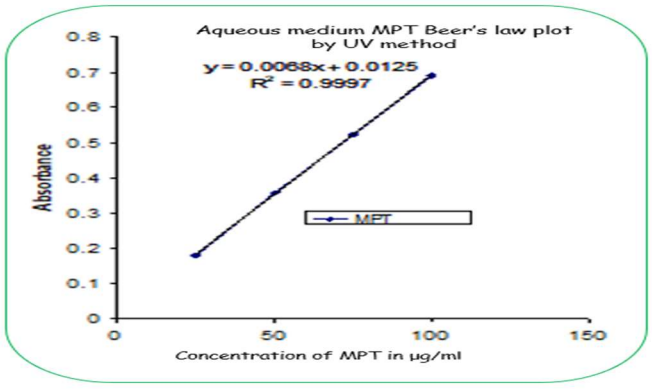


Fig.-4: Beer's Law Graph of MPT Taken in Aqua Medium

Ringbom Trace

The product absorbance and transmittance have an inverse relationship with the relative concentration error. At the transmittance scale's extremities, the relative inaccuracy rises. As per the findings that are presented in Figure-5, the relative error coefficient (i.e., a trace of log C vs. T) is given by the slope of the trace "C" vs "T," or the Ringbom trace.

The primary limitation of the Ringbom trace is the absence of the combination of ΔT vs.T relations, it offers no information about the concentration range with any degree of precision. Whether Beer's rule is observed or not, the aforementioned statement remains true.

Precision and Accuracy

Getting a reliable estimate of the real values is the goal of making a determination. The combination of accuracy and precision yields the error of the single determination. They are among the most crucial standards by which to evaluate analytical techniques and their outcomes.

Precision: Measurement repeatability within a set, or the dispersion of a set around its center value, is referred to as precision. According to its definition, a "set" is any number (n) of independent duplicate measurements of a particular feature.

RESULTS AND DISCUSSION

The proposed approach serves as a reference for visible spectrophotometric techniques that have been developed, published, and effectively proposed for analysis of MPT-containing samples (both bulk as well as formulations). The visible spectrophotometric methods for its measurements are made possible by numerous functional groups present in the MPT molecule, which undergo a broad range of specialized

combinations. Presenting them from a critical perspective, including simplicity, sensitivity, selectivity (specificity), precision, and accuracy, were considered valuable.

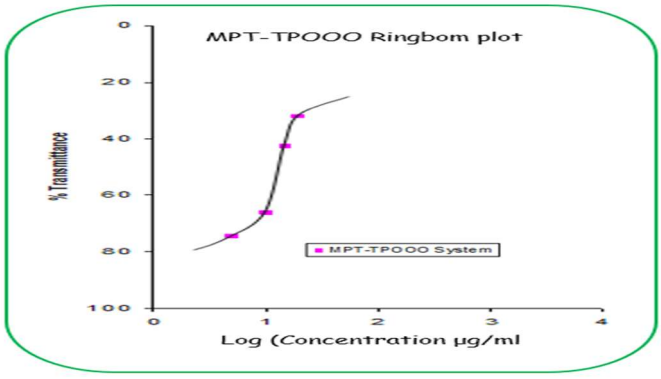


Fig.-5: Ringbom Trace of MPT-TPOOO System

Control experiments were carried out to figure out the optimal parameters that comprise the suggested approach for quantifying MPT. The results were based on the most significant color development and stability of the colored species created in different reactions. The accuracy (calculating percent error in bulk samples, comparing the results obtained by the proposed method with the reference method in the case of pharmaceutical formulations and recovery experiments) and precision (calculating percent relative standard deviation, percent range of error at confidence limits with D = 0.05 and 0.01 levels from six species) studies established the validity of the analytical methods proposed for the determination of MPT. By analyzing the contents of six samples, each containing two-thirds the quantity of upper Beer law limits, the accuracy of each suggested approach was determined. Table-6 provides an overview of the percent range of error and percent of relative standard deviation that were computed. Accuracy: A variety of bulk MPT samples were collected and subjected to the suggested method's analysis. The findings (represented as a percentage of inaccuracy) are shown in Table-6.

Table-6: Ocular Properties, Accuracy, and Precision of the Suggested Approach

Data presented	Corresponding Value
λ_{max} (in nanometers)	485
Beer law limits (µg/ml)	5-20
Molar absorptivity (L/mole.cm)	$1.746x10^4$
Sandell sensitivity (µg/cm^2/0.001 absorbance unit)	0.003992
Standard error of estimate (S_e)	$1.18x10^{-3}$
The standard deviation of slope (S_b)	$1.06x10^{-4}$
The standard deviation of intercept (S_a) Regression equation (y = a + bx)	$1.4x10^{-3}$
Slope (b)	0.0244
Intercept (a)	0.0052
Correlation coefficient (r)	0.9999
Relative Standard Deviation, RSD Range of error (confidence limits)	0.6591
At 0.05 level	0.6919
At 0.01 level	1.085
The error associated with bulk samples	0.72

'S' which is the standard deviation, is represented as,

$$S = \sqrt{\frac{1}{(n-1)}\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}$$

The property being measured and the 'S', standard deviation has similar units, as; Percentage relative standard deviation = 100 × (s/x). Sandell sensitivity, molar extinction coefficient, optimal photometric

range, and Beer's law restrictions were used to determine the method's sensitivity. Regression analysis was performed for the slope (b), and the intercept (a), the correlation coefficient (r) derived from varying conc. Using the least squares approach. Tables-6 describes the information gathered throughout the MPT determination process using various reagents. A UV reference technique for the measurement of MPT at an acceptable λ_{max} in water was devised to compare the findings produced by the suggested approach. Through the testing of interference with additional active and inactive chemicals often found in pharmaceutical compositions, the selectivity (or specificity) of the present technique is determined. When the findings from the UV reference technique and the suggested approach were statistically evaluated using the "t" and "F" tests, it was discovered that there was no discernible difference between the two methods' precision and accuracy (Table-6). Table-6 provides the sensitivity data (λ_{max} and Beer law limitations) for the suggested approach for calculating the presented approach for MPT.

CONCLUSION

The objective of the current research work was to create an analytical technique using UV-visible spectrophotometry that would be easy to use, sensitive, and trustworthy for estimating MPT while using TPOOO in tablet form. The approach is allowed to be as cost-effective as other methods and has adequate precision and accuracy. Validation test findings were determined to be good. The analytical process is straightforward, quick, precise, and sensitive. For such reasons, this procedure may be employed for routine analysis and utilized to determine MPT in biological samples and pharmaceutical formulations in research laboratories, hospitals, and the pharmaceutical industry.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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