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Spectrophotometric determination of metoprolol in pharmaceutical formulation by charge transfer complexation

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Abstract

Two simple and selective spectrophotometric methods are described for the determination of metoprolol tartar as base form metoprolol (MTP) in bulk drug, and in tablets and capsules. The methods are based on the molecular charge transfer complexation of metoprolol base MTP with Bromothymol blue (BTB) or 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The yellow and orange colored radical anions formed on dissociation, are quantitated at 413 nm (BPB method) or 457 nm (DDQ method). The assay conditions were optimized. Beer's law is obeyed in the concentration ranges 2.0-40.0 $\mu\text{g mL}^{-1}$ in BPB method and 5.0-25.0 $\mu\text{g mL}^{-1}$ in DDQ method, with respective molar absorptivity values of 5.78×10^3 and $4.05 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$. The reaction stoichiometry in both methods was evaluated by Job's method of continuous variations and was found to be 1:1 MPT-BPB, MPT-DDQ. The developed methods were successfully applied to the determination of MTP in pure form and commercial tablets with good accuracy and precision. Statistical comparison of the results was performed using Student's t-test and F-ratio at 95% confidence level and the results showed no significant difference between the reference and proposed methods with regard to accuracy and precision. Further, the accuracy and reliability of the methods were confirmed by recovery studies via standard addition technique.

Keywords: Spectrophotometry; Charge-transfer complexes; Metoprolol; Pharmaceutical.

1. Introduction

Metoprolol (MTP) is cardio selective β_1 adrenergic receptor antagonist mainly used in hypertension, angina pectoris, cardiac arrhythmia, congestive heart failure and myocardial infarction [1, 2, 3]. Chemically Metoprolol is (RS)-1-(Isopropylamino)-3-[4-(2-methoxyethyl) phenoxy] propan-2-ol (Figure 1) with molecular mass 267.364 g mol^{-1} . Freely soluble in water and elimination half life of 3-7 hrs [4, 5]. Mode of action of metoprolol is by reducing agonistic effect of catecholamines on the heart (which is released during physical and mental stress). This means that the usual increase in heart rate, cardiac output, cardiac contractility and blood pressure, produced by the acute increase in catecholamines is reduced [6]. The drug is quite sensitive, even small amount of drug doses giving significant blockade of β_1 adrenoreceptors [7, 8].

Several methods have been reported for determination of metoprolol including gas chromatography-mass spectrometry (GC-MS) [9-11], high-performance liquid chromatography (HPLC) [12-16], LC-MS [17-19], LC-MS-MS [20] and spectrophotometry [21-25].

This study describes simple, direct, sensitive, accurate and precise spectrophotometric methods for the determination of metoprolol (MTP) reaction with sigma and pi-acceptors in their common dosage forms and irrespective of the presence of contaminants and additives.

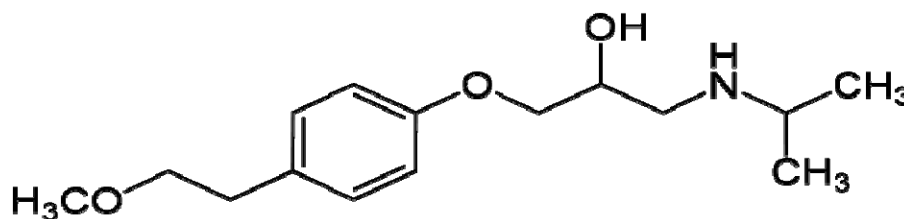


Fig 1: Structure of metoprolol (MTP).

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2. Material and Methods

2.1 Apparatus

A GENESYS 10S UV-Vis double beam spectrophotometer (Thermo Spectronic, USA) with a fixed slit width (1.8nm) connected to an IBM computer loaded with Thermo Spectronic VISION Lite version 4 software and 1-cm quartz cell were used for the registration and treatment of absorption spectra.

2.2 Materials and Reagents

All Chemicals used were of analytical reagent grad unless otherwise is mentioned, Metoprolol tartrate standard powder (purity 99.8%) were kindly supplied by SPIMACO ADDWAEIH, Al-Qassim, Saudi Arabia.

Bromothymol blue (BTB) (Sigma –Aldrich Co), 0.1% (w/v) solution was prepared by dissolving 0.01 g of the dye in double distilled deionized water was diluted to a final volume of 10 ml with distilled water. Working solutions were freshly prepared by subsequent dilutions.

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Sigma – Aldrich Co); 0.1 % (w/v) solution was prepared by dissolving 0.01 g of the DDQ in 5 ml of acetonitrile and then the solution was diluted to a final volume 10 ml with acetonitrile. Working solutions were freshly prepared by subsequent dilutions. This solution is prepared daily using red- glass volumetric flask because it is a light sensitive reagent.

Phthalate buffer of pH ranging from 2.2-4.0 was prepared by dissolving 20.4 g potassium hydrogen phthalate in 1.0 L distilled water and pH of solution was maintained by adding 0.1M HCl.

2.3 Preparation of standard stock solution:

The stock standard solution of MPT was prepared in methanol to a concentration of 100 $\mu\text{g}\cdot\text{mL}^{-1}$ and kept stored at $-20\text{ }^{\circ}\text{C}$ in dark glass flasks. Working standard solutions were prepared from the stock standard solutions.

2.4 Pharmaceutical formulations:

Twenty tablets were weighed accurately and ground into a fine powder. The powder equivalent to 100 mg of MPT was dissolved in methanol and shaken well for proper mixing. This solution was allowed to stand for 30 min and then sonicated for complete solubilization of drugs. Then the contents were filtered to separate the insoluble excipients and volume was completed with same solvent to get the final concentration of 1000 $\mu\text{g}\cdot\text{mL}^{-1}$. The procedure was continued as described under the procedure for pure MTP.

2.5 Procedure

• Method for BTB

MTP solution in the concentration range of 2-40 $\mu\text{g}\cdot\text{mL}^{-1}$ was transferred in to 10 mL volumetric flasks, to this 3.0 mL buffer of pH 3.4 and 2.5 mL BTB solution was added and the volume was completed with distilled water. The mixture was then transferred into 50 mL separating funnel and extracted with 20 mL chloroform in two portions. The absorbance of extracted organic layer was measured against reagent blank treated similarly. The standard calibration graph was prepared by plotting absorbance vs. concentration of MTP.

• Method for DDQ

Aliquots of stock standard solution of MTP in the concentration range of 5-25 $\mu\text{g}\cdot\text{mL}^{-1}$ was transferred into two separate series of 10 mL volumetric flasks; to each flask 1 mL of DDQ. These solutions were allowed to react at room

temperature for 15 min. The volume was made up to the mark with acetonitrile and the absorbance was measured against reagent blank treated similarly.

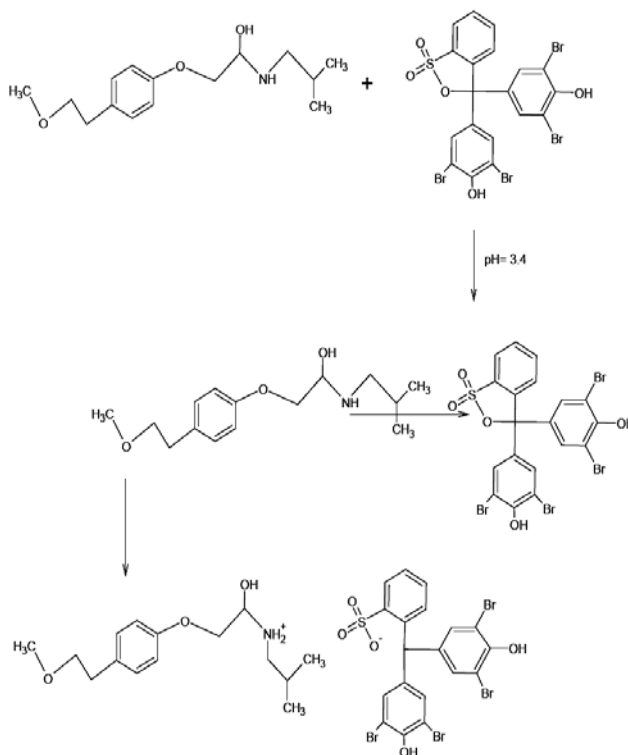
2.6 Synthesis of solid CT Complexes

Solid charge transfer complexes were synthesized by refluxing the MTP with each of the complexing agent separately for 2 hrs in the ratio of 1:1 as found through the stoichiometric study by applying Job's method. The resulting product in each case was filtered off and washed with minimum amount of methanol. The excess solvent was evaporated to dryness.

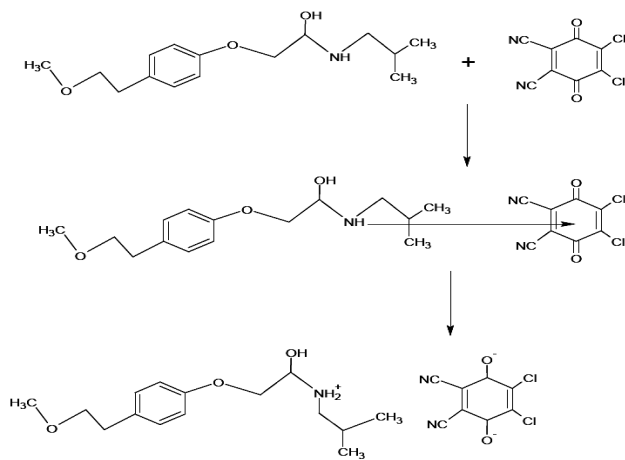
3. Results and Discussion

MTP, being a nitrogenous drug is present in the positively charged protonated form. It is reported to form charge transfer complexes with many of compounds. In the present study, efforts have been made to develop the complexes of MTP with BTB and DDQ. It was observed that MTP interact with all of these agents in the stoichiometric ratio of 1:1 as determined by applying Job's method of continuous variation [26] leading to the formation of yellow and orange charge transfer complexes. In all resulting product, MTP is in positively charged protonated form, whereas BTB forms sulfonephthalein group and DDQ forms radical anion Scheme 1 and 2. The newly formed complexes show intense absorption bands at 413, and 457nm respectively. The electronic absorption spectra of these complexes are given in Figure 2 and 3.

Different parameters influencing the intensity of absorption bands were investigated to establish the optimum experimental conditions for the assay. It was observed that the complete complexation occurs instantaneously for DDQ and absorbance remains constant for 24 hrs. The optimum reaction time for MTP-BTB complex was determined by investigating the absorbance of complex at every minute while shaking the reaction mixture. It is apparent from the Figure 4 that complete interaction occurs after proper shaking of mixture for 3 min and it also found to be stable for 24 hr.



Scheme 1: MTP-BBT charge transfer complex



Scheme 2: MTP-DDQ charge transfer complex

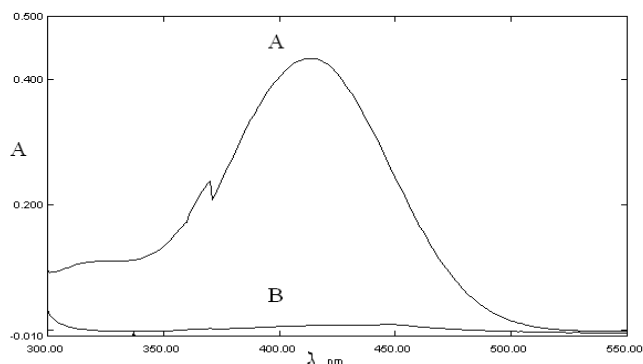
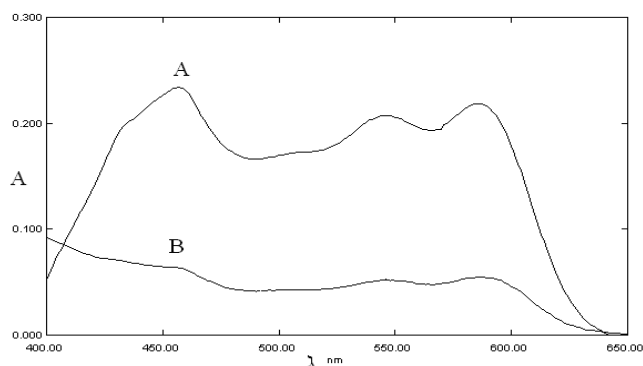
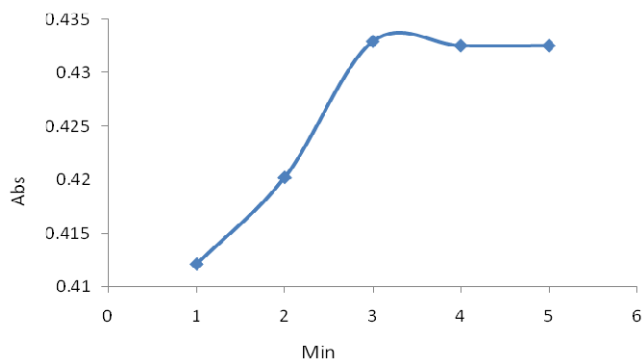
Fig 2: Absorption spectra of A: 20 µg.mL⁻¹ MTP–BTB charge transfer complex against reagent blank, B: reagent blank against chloroform, under optimum conditions.Fig 3: Absorption spectra of A: 15 µg.mL⁻¹ MTP–DDQ charge transfer complex, against reagent blank, B: reagent blank against acetonitrile, under optimum conditions.

Fig 4: Effect of shaking time on MTP-BTB complex

The effect of reagent concentration was examined by adding different volumes of 6.2×10^{-3} M BTB and 2.7×10^{-3} M DDQ in to a fixed amount of MTP. The 2.5 mL BTB and 1 mL DDQ were found to be appropriate for complete complexation. For BTB method, buffer of suitable pH was selected by testing them in the range of 2.0-4.0 and the maximum absorbance was obtained at pH 3.4 Figure 5. For proper extraction of complex, solvents like benzene, carbon tetra chloride, dichloromethane and chloroform were tested. Double extraction with chloroform was supposed to be adequate for maximum recovery.

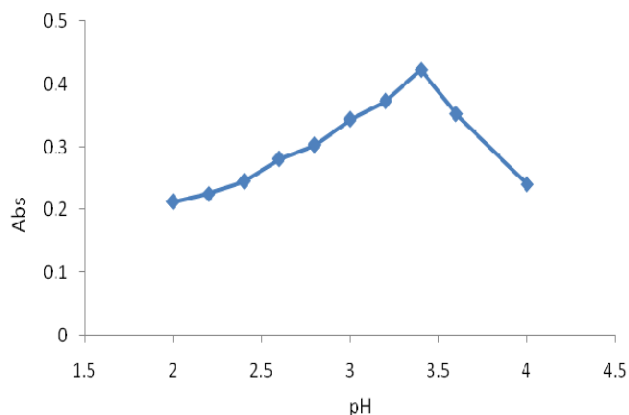


Fig 4: Effect of pH on MTP-BTB complex

3.2 Method validation

• Analytical data

At the established experimental conditions, standard calibration curves for MTP with BTB and DDQ were constructed by plotting absorbance verses concentration. The linear regression curves were obtained in the Beer's law range of 2.0-40 and 5.0-25 µg.mL⁻¹ with correlation coefficient 0.9999 and 0.9991 in each case respectively. Regression characteristics including slope, intercept, correlation coefficient and also the molar absorptivity values for each proposed method are given in Table 1. The detection limit (LOD) and quantification limit (LOQ) were calculated by using the following equations [27]:

$$LOD = \frac{3.3 \times \sigma}{S} \quad \& \quad LOQ = \frac{10 \times \sigma}{S}$$

where, σ is the standard deviation of seven replicate determinations under the same conditions as for the sample in the absence of the analyte and S is the slope of the calibration graph. The LOD values were calculated to be 0.363 and 0.746 µg.mL⁻¹ respectively (Table 1).

Table 1: Optimum conditions and analytical parameters

Parameters	MTP-BTB complexes	MTP-DDQ complexes
λ_{max} (nm)	413	457
Linearity range µg.mL ⁻¹	2.0 - 40	5.0 - 25
Molar absorptivity	5787.6	4051.3
Slope	0.020	0.014
Intercept	0.037	0.012
Correlation coefficient	0.9999	0.9991
LOD µg.mL ⁻¹	0.363	0.746
LOQ µg.mL ⁻¹	1.10	2.286

• Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, pure drug MTP solution at three different concentration levels (within the working range) were prepared and analyzed in seven replicates during the same day (intra-day precision) and on five consecutive days (inter-day

precision) and the results are presented in Table 2. The percentage relative error (RE%) was ≤ 1.04 which indicates that the accuracy of the methods is satisfactory. Percentage relative standard deviation (RSD%) for intra-day was $\leq 0.2.10$ and for inter-day it was ≤ 1.25 indicating repeatability and usefulness of the proposed methods in the routine analysis.

Table 2: Evaluation of intra-day and inter-day precision and accuracy.

Method	MTP taken $\mu\text{g.mL}^{-1}$	Intra-day (n=7)			Inter-day (n=5)		
		Found ^a $\mu\text{g.mL}^{-1}$	%RSD ^b	% RE ^c	Found $\mu\text{g.mL}^{-1}$	%RSD	% RE
BTB method	5	4.95	1.35	1.04	4.96	1.23	0.70
	10	9.93	1.41	0.68	10.03	1.25	0.30
	30	30.21	1.83	0.68	30.15	1.14	0.49
DDQ method	5	4.96	1.83	0.76	4.98	1.02	0.38
	10	9.96	1.78	0.43	9.97	0.77	0.29
	20	20.18	2.10	0.91	20.09	0.85	0.92

^aMean value of five determinations; ^bRelative standard deviation (%); ^cRelative error (%).

The assay results were in good agreement with the label claim. Also, the effect of commonly found excipients was determined by scanning the blank solution of MTP and the placebo solutions. The percent recovery values given in Table 3 indicate that excipients of tablet did not found to interfere during the assay.

Table 3: Percent recovery for 20 $\mu\text{g.mL}^{-1}$ of MTP in the presence of 250 $\mu\text{g.mL}^{-1}$ of Excipients.

Excipients	Recovery% *	
	BTB method	DDQ method
Lactose	98.89	99.14
Sucrose	101.15	100.98
Talc	100.40	99.29
Starch	99.44	101.51
Magnesium stearate	101.58	99.31
Sodium Citrate	99.01	101.25
Sodium chloride	101.67	98.99

*Average of three determinations.

• Robustness and ruggedness

To evaluate the robustness of the methods, two important experimental variables volume of reagent and reaction time, were slightly altered and the effect of this change on the absorbance of the Charge transfer complexes was studied. The results of this study are presented in Table 4 and indicated that the proposed methods are robust. Method ruggedness was evaluated by performing the analysis following the recommended procedures by three different analysts and on three different spectrophotometers by the same analyst. From the %RSD values presented in Table 4, one can conclude that the proposed methods are rugged.

Table 4: Robustness and ruggedness.

Method	MTP taken $\mu\text{g.mL}^{-1}$	Method robustness		Method ruggedness	
		Parameters altered		Inter-analysts RSD%, (n=4)	Inter-cuvettes RSD%, (n=4)
		Reagent volume, ml ^a RSD% (n=3)	Reaction time ^b RSD%, (n=3)		
BTB Method	5	0.96	1.18	1.42	1.05
	10	1.06	0.92	1.04	1.23
	30	0.81	1.29	1.16	1.09
DDQ Method	5	1.22	0.79	1.56	1.27
	10	1.18	1.27	1.33	1.34
	20	1.35	1.28	1.46	1.10

^a In both methods, the volume of reagent was 0.8, 1.0 and 1.2 mL. ^b The reaction time was 4, 5 and 6 min.

3.3 Application to analysis of formulations:

The proposed methods were applied to the determination of MTP in tablets and capsules (Table 5). The results obtained were statistically compared with those of the official method ^[5] by applying the Students t-test for accuracy and F-test for precision. The official method describes a potentiometric titration of ethanolic solution of MTP with sodium hydroxide. As can be seen from the Table 5, the calculated t-test and F-value at 95 % confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, for four degrees of freedom. The results indicated that there is no difference between the proposed methods and the official method with respect to accuracy and precision.

Table 5: Results of analysis of tablets by the proposed methods.

Tablet brand name	Labeled amount mg/tablet	Method	Amount found* (in mg)	%Recovery \pm SD*	T-test**	F-test***
Lopresor	50	BPB method	51.05	102.11 \pm 0.98	1.05	2.34
		DDQ Method	50.50	101.00 \pm 1.03	1.03	2.59
		Official method	50.82	101.56 \pm 0.64	-	-
Metoprol	50	BPB method	50.81	101.63 \pm 1.25	1.64	2.78
		DDQ method	50.64	101.28 \pm 1.08	1.22	2.07
		Official method	50.28	100.56 \pm 0.75	-	-
Metoprol	100	BPB method	100.99	100.99 \pm 1.59	0.94	2.25
		DDQ method	99.07	99.07 \pm 1.30	1.49	1.50
		Official method	100.19	100.19 \pm 1.06	-	-

*Mean value of five determinations.

**Tabulated t-value at the 95% confidence level is 2.78.

***Tabulated F-value at the 95% confidence level is 6.39.

3.4 Recovery studies

Accuracy of the proposed methods was further confirmed by standard-addition procedure. Pre-analyzed tablet powder was spiked with pure MTP at three different concentration levels

(50, 100, and 150 % of the quantity present in the formulation) and the total was found by the proposed methods. The result of the recovery study is shown in Table 6.

Table 6: Results of recovery study by standard addition method.

Tablets studied	BPB method				DDQ method			
	MTP in tablets $\mu\text{g.mL}^{-1}$	Pure MTP added $\mu\text{g.mL}^{-1}$	Total found $\mu\text{g.mL}^{-1}$	%Recovery* $\pm\text{SD}$	MTP in tablets $\mu\text{g.mL}^{-1}$	Pure MTP added $\mu\text{g.mL}^{-1}$	Total found $\mu\text{g.mL}^{-1}$	%Recovery* $\pm\text{SD}$
Lopresor	20	10	33.66	102.2 \pm 0.83	10	5	15.29	101.9 \pm 1.52
	20	20	39.70	99.25 \pm 0.54	10	10	20.16	100.8 \pm 0.97
	20	30	50.55	101.1 \pm 1.21	10	15	25.55	102.2 \pm 0.96
Metoprol	20	10	30.90	103.0 \pm 0.83	10	5	14.88	99.22 \pm 0.87
	20	20	40.80	102.0 \pm 1.64	10	10	20.48	102.4 \pm 0.56
	20	30	50.80	101.6 \pm 0.74	10	15	25.45	101.8 \pm 0.48
Metoprol	20	10	29.40	98.00 \pm 2.17	10	5	14.76	98.39 \pm 0.87
	20	20	39.63	99.08 \pm 0.82	10	10	20.38	101.9 \pm 0.56
	20	30	50.70	101.4 \pm 27	10	15	25.23	100.9 \pm 0.28

*Mean value of three determinations.

4. Conclusion

Formation of n- π charge-transfer complex between n-electron donor MTP and π -acceptor BPB or DDQ was used to develop two rapid, accurate and precise methods for Metoprolol tartrate MTP in its dosage forms. Possibly, these are the simplest methods ever for MTP since they involve simple mixing of the drug and absorbance measurement. Both methods are absolutely free from any critical experimental variables. This is reflected in high accuracy and precision of results (intra-day %RE and %RSD being <1%). The methods follow a single step reaction and use a single reagent unlike the reported methods. The methods are characterized by wide linear dynamic ranges, and are far more sensitive. The principal advantage of the proposed methods is their suitability for the drug alone and in its dosage forms without fear of interference caused by the excipients expected to be present in tablets.

5. Reference

- Jaiswal J, Anantawar SP, Narkhede MR, Gore SV, Mehta K. Formulation and evaluation of thermo reversible in situ gel of metoprolol succinate. *International journal of pharmacy and pharmaceutical sciences* 2012; 4(3):96-102.
- The Merck Index, Merck Res. Lab. Division of Merck and Co. Inc Whitehouse station. 14th Ed, New Jersey, USA 2009, 6151.
- Gaikwad V. Formulation and evaluation of In-Situ gel of metoprolol tartarate for nasal delivery. *Journal of Pharmacy Research* 2010; 3(4):788-793.
- Moffat AC, Osselton MD, Widdop B. Clarke's Analysis of Drugs and Poisons in pharmaceuticals, body fluids and postmortem material. 3rd Ed, Pharmaceutical Press, London, 2004, 1275-1276.
- British Pharmacopoeia, The British Pharmacopoeia Commission, Vol II, London, 2010, 1419.
- [http://www.Drug Bank Metoprolol \(DB00264\). Htm](http://www.Drug Bank Metoprolol (DB00264). Htm) 19, November, 2013
- Q2A: Text on; Validation of Analytical Procedures. In International Conference on Harmonization. Federal Register 1995; 60(40):11260-11262.
- Q2B: Validation of Analytical Procedures: Methodology, Availability. In International Conference on Harmonization. Federal Register 1997; 62(96):27463-27467.
- Kim KH, Lee JH, Ko MY, Shin KS, Kang JS, Mar WC *et al.* Determination of metoprolol enantiomers in human urine by GC-MS using (-)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride as a chiral derivatizing agent. *Chromatographia* 2002; 55:81-85.
- Angier MK, Lewis RJ, Chaturvedi AK, Canfield DV. Gas chromatographic-mass spectrometric differentiation of atenolol, metoprolol, propranolol, and an interfering metabolite product of metoprolol. *J Anal Toxicol* 2005; 29:517-521.
- Hartonen K, Riekkola ML. Detection of β - blockers in urine by solid-phase extraction supercritical fluid extraction and gas chromatography-mass spectrometry. *J Chromatogr B* 1996; 676:45-52.
- Albers S, Elshoff JP, Volker C, Richter A, Laer S. HPLC quantification of metoprolol with solid-phase extraction for the drug monitoring of pediatric patients. *Biomed Chromatogr* 2005; 19:202-207.
- Braza AJ, Modamio P, Lastra CF, Marino EL. Development, validation and analytical error function of two chromatographic methods with fluorometric detection for the determination of bisoprolol and metoprolol in human plasma. *Biomed Chromatogr* 2002; 16:517-522.
- Chiu FCK, Damani LA, Li RC, Tomlinson B. Efficient high-performance liquid chromatographic assay for the simultaneous determination of metoprolol and two main metabolites in human urine by solid-phase extraction and fluorescence detection. *J Chromatogr B* 1997; 696:69-74.
- Boralli VB, Coelho EB, Cerqueira PM, Lanchote VL. Stereoselective analysis of metoprolol and its metabolites in rat plasma with application to oxidative metabolism. *J Chromatogr B* 2005; 823:195-202.
- Schuster D, Modi MW, Lakla D, Gengo FM. Reversed-phase high-performance liquid chromatographic assay to quantitate diastereomeric derivatives of metoprolol enantiomers in plasma. *J Chromatogr B* 1988; 433:318-325.
- Johnson RD, Lewis RJ. Quantitation of atenolol, metoprolol, and propranolol in postmortem human fluid and tissue specimens via LC/APCI-MS. *Forensic Sci Int* 2006; 156:106-117.
- Jensen BP, Sharp CF, Gardiner SJ, Begg EJ. Development and validation of a stereoselective liquid chromatography-tandem mass spectrometry assay for quantification of S- and R-metoprolol in human plasma. *J Chromatogr B* 2008; 865:48-54.

19. Dupuis C, Gaulier JM, Pelissier-Alicot AL, Marquet P, Lachatre G. Determination of three β -blockers in biofluids and solid tissues by liquid chromatography-electrospray-mass spectrometry. *J Anal Toxicol* 2004; 28:674-679.
20. Gowda KV, Mandal U, Selvan PS, Solomon WDS, Ghosh A, Sarkar AK *et al.* Liquid chromatography tandem mass spectrometry method for simultaneous determination of metoprolol tartrate and ramipril in human plasma. *J Chromatogr B* 2007; 858:13-21.
21. Alpdoğan G, Sungur S. AAS and spectrophotometric methods for the determination metoprolol tartrate in tablets. *Spectrochim Acta Part A* 1999; 55:2705-2709.
22. Yilmaz B. Determination of metoprolol in pharmaceutical preparations by zero-, first-, second- and third- order derivative spectrophotometric method. *Int J of Pharma and Bio Sci* 2010; 12(1):1-15.
23. Jadhav AS, Tarkase KN, Deshpande AP. Quantitative determination of metoprolol succinate in bulk and tablet Dosage form through comparative study of UV and derivative Spectroscopy. *Der Pharmacia Lettre* 2012; 4(3):763-767.
24. Pagar SA, Shinkar DM, Saudagar RB. Development and validation of spectrophotometric method for determination of metoprolol succinate. *Int J Pharm Bio Sci* 2013; 3(4):224-228.
25. Mustafa C, Derya T, Aysegul G. Spectrophotometric determination of metoprolol tartrate in pharmaceutical dosage forms on complex formation with Cu(II), *Pharmaceuticals* 2011; 4:964-975
26. Tang PH, Miles MV, Glauser TA, DeGrauw T. Automated microanalysis of gabapentin in human serum by high-performance liquid chromatography with fluorometric detection. *J Chromatogr B Biomed Sci Appl* 1999; 727:125-129.
27. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology, dated 06 November 1996, incorporated in November London, 2005.