International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2024; 12(4): 54-59

Received: 05-06-2024
Accepted: 10-07-2024

Venkata Rao Basa
Department of Chemistry, Government College, Rajahmundry, Andhra Pradesh, India

Srinivasa Rao Tirunagari
Department of Chemistry, Government College, Rajahmundry, Andhra Pradesh, India

Corresponding Author:
Venkata Rao Basa
Department of Chemistry, Government College, Rajahmundry, Andhra Pradesh, India

A visible spectrophotometric assay of Di[RS]-3-[4-(2-methoxy ethyl)phenoxy-1-(isopropyl amino) propan-2-ol] tartrate (metoprolol tartrate) by using cobalt thiocyanate

Venkata Rao Basa and Srinivasa Rao Tirunagari

DOI: https://doi.org/10.22271/chemi.2024.v12.i4a.12419

Abstract
Background: In the present research study, a new, simple, sensitive and accurate spectrophotometric method has been developed for the assay of metoprolol tartrate (MPT), which is based on the complexation of drug with cobalt thiocyanate. Metoprolol tartrate (MPT) is a selective β1 receptor blocker drug mostly administered in cardiovascular diseases. Due to the highly probable over dosage, a rapid and easy to perform method for the quantitative determination of MPT in biological matrices would be useful in its therapeutic monitoring as well as in intoxication cases.
Experimental: The study presents a validated method for the U.V.-VIS quantitative assay of MPT in bulk and tablet formulations at λ_{max} of 625 n.m. with molar absorptivity of 1.705×10^3 L. mole^{-1} cm^{-1}. The parameters targeted for the validation were linearity, sensitivity, accuracy and precision. The presence of secondary amine group in MPT molecule is the basis for the proposed visible spectrophotometric method. Cobalt thiocyanate (CTC) has been proved to be a valuable chromogenic reagent for the detection and determination of amino compounds.
Results: The colored species formed is the coordination complex of the drug and the central metal atom of CTC, which is extractable into nitrobenzene from aqueous solution. Formation of blue colored complex when MPT is treated with CTC due to the presence of secondary amine group in it is the basis in the present investigation.
Conclusion: The developed method is more selective, sensitive, reproducible, rapid, cheap and simple than in most of the analogous methods. For these reasons, it can be used in routine analysis and can be applied for the determination of MPT in pharmaceutical formulations and biological samples.

Keywords: Cardiovascular, method validation, β-blockers, spectrophotometry, cobalt thiocyanate

Introduction
Metoprolol tartrate (MPT) is a cardio selective β1 adrenergic receptor antagonist mainly used in hypertension, angina pectoris, cardiac arrhythmia, congestive heart failure, myocardial infarction, supraventricular tachycardia, ventricular tachycardia and prevention of migraine headaches [1, 2, 3, 4]. Chemically MPT is Di[RS]-3-[4-(2-methoxy ethyl)phenoxy-1-(isopropyl amino)propan-2-ol] tartrate (Figure 1). It is a white crystalline powder with molecular formula $(C_{15}H_{25}NO_3)_2\ C_4H_6O_6$ and molecular mass 684.82. It is freely soluble in water with elimination half-life of 3-7hrs [5, 6].

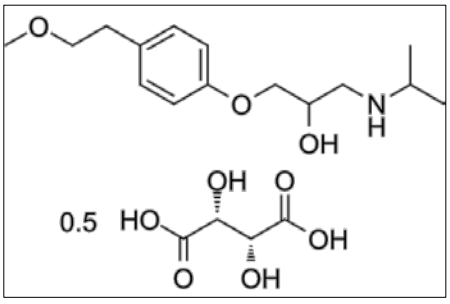

Fig 1: Chemical Structure of MPT

Mode of action of MPT is by reducing agonistic effect of catecholamine's 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 catecholamine's is reduced [7]. The drug is quite sensitive, even small amount of drug doses giving significant blockade of β1adreno receptors [8, 9] However, the β-Blockers are also misused as doping agents in sports and therefore these drugs have been added to the list of forbidden drugs by the International Olympic Committee [10]. Therefore the development of an analytical method for the determination of MPT is of great significance.

The drug is officially listed in Martindale. The Extra Pharmacopoeia [11]. The recommended assay of the drug is listed in the British Pharmacopoeia, which describes a potentiometric titration method [12] The various analytical methods such as thin layer chromatography, [13, 14] high performance liquid chromatography, [15-17] gas chromatography, [18] capillary electrophoresis, [19] infrared spectroscopy, [20] and electrochemical methods [21] have been described for its determination. The above-mentioned techniques are good and sensitive but require a laborious clean up procedure prior to analysis of the drug. Spectrophotometry in the visible region is attractive because of its speed, no pretreatment steps and simplicity.

In the present study, a successful attempt has been made to estimate the drug by U.V. spectrophotometric analysis which is simple, rapid, accurate, reproducible and economical method which has been described for the determination of MPT in bulk as well as in tablet formulations. The validation was performed in respect of the ICH Q2 R1 regulations [22-36] for analytical procedures.

Materials and Methods

Instrumentation

All the spectral and absorbance measurements were made in a Shimadzu UV-2600 model UV-Visible spectrophotometer with 1cm matched quartz cuvettes. pH measurements were made on an Elico LI-120 digital pH meter.

Chemicals and Reagents

Pure drug samples of MPT were obtained from pharma companies. Commercial tablet formulations containing MPT were procured from local pharmacies. All chemicals and reagents used were of analytical grade. Double distilled water was used for preparing solutions.

Preparation of Standard Stock Solutions

A stock solution (1 mg/ml) of MPT was prepared by dissolving 100 mg of it in 100 ml of distilled water. A portion of this stock solution was diluted with distilled water to obtain working standard MPT solution of 500 µg/ml concentration for the proposed method.

Study of Spectra and Selection of Wavelengths

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in spectrophotometric method, specified amount of MPT was taken in final solution and the colors were developed by following the mentioned procedures. Absorption maximum is observed in the wavelength region of 400-800 nm. The absorption spectra were scanned on a spectrophotometer in a wavelength region of 400-800 nm against a corresponding reagent blank. The results were graphically represented in Figure 2.

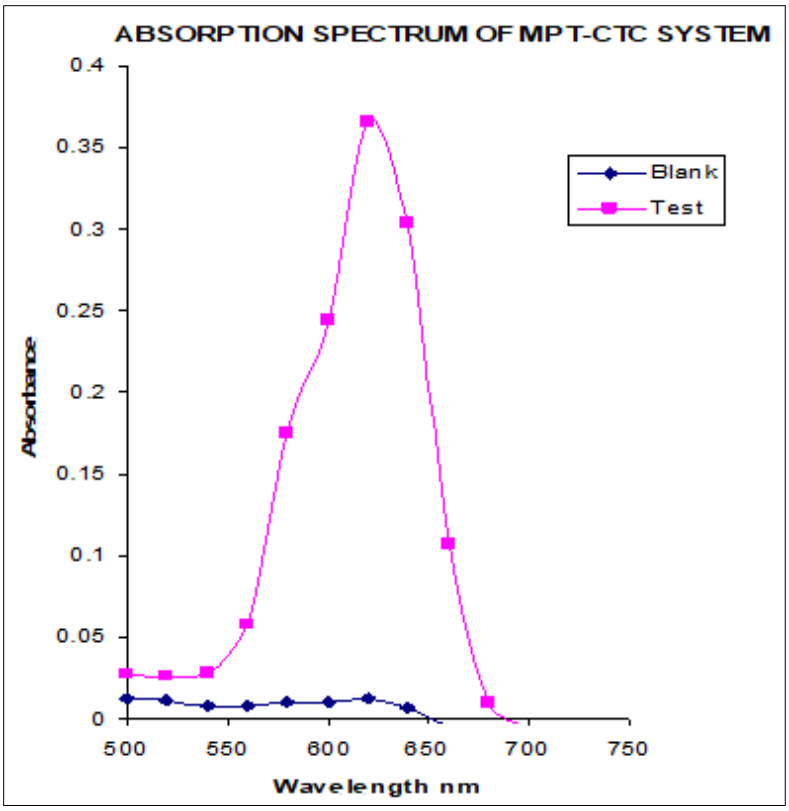

Fig 2: Absorption spectrum of MPT-CTC System

The absorption curves of colored species show characteristic absorption maxima, whereas the blank in the method has low or no absorption in this region.

Preparation of Sample Solution

CTC solution (2.5×10^{-1} M): It is prepared by dissolving 7.25 gm of cobaltous nitrate (BDH) and 3.89 gm of ammonium thiocyanate (BDH) in 100 ml of distilled water.

Buffer solution (pH 2.0): Prepared by mixing 306 ml of trisodium citrate (0.1 M) with 694 ml of 0.1 M HCl and adjusted the pH to 2.0. Nitrobenzene (Qualigens) used directly.

Proposed Procedure for the Determination of MPT: Cobalt thiocyanate (CTC) (formed by combination of ammonium thiocyanate and cobalt nitrate) has been proved to be a valuable chromogenic reagent for the detection and determination of amino compounds. The colored species formed is the co-ordination complex of the drug (electron donor) and the central metal atom (cobalt) of cobalt thiocyanate, which is extractable in to nitrobenzene from aqueous solution. Blue colored complex is formed when MPT is treated with CTC due to the presence of secondary amine group in it. This is the basis in the present investigation. The probable sequence of reactions is presented in the Figure 3, as below.

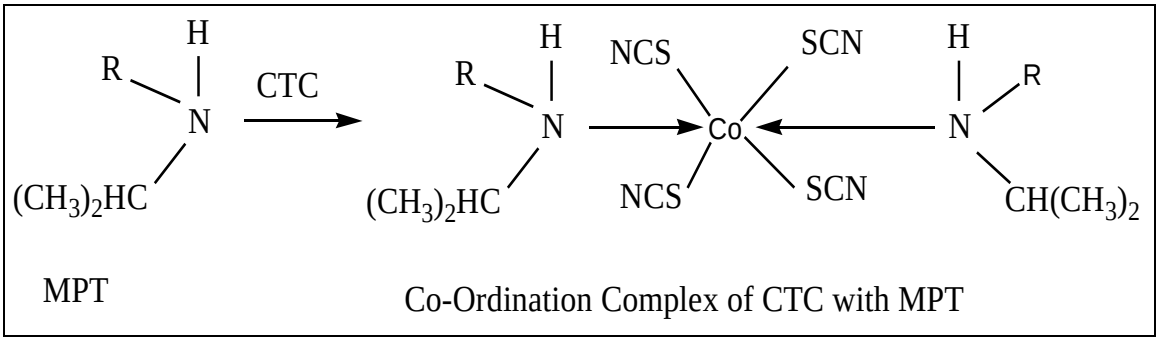

Fig 3: Sequence of reaction.

Recommended Procedure for Bulk Samples: In to a series of 125 ml separating funnels, containing aliquots of standard MPT solution (1-4 ml, 500 µg/ml), 2.0 ml of buffer (pH 2.0) and 5.0 ml CTC ($2.5x10^{-1}$ m) solutions were added. The total volume of the aqueous phase in each separating funnel was adjusted to 15.0 ml with distilled water. To each separating funnel, 10.0 ml of nitrobenzene was added and the contents were shaken for 2 minutes. The phases were allowed to separate and the absorbance of the separated nitrobenzene layer was measured at 625 nm against a similar reagent blank. The amount of the drug was computed from the calibration graph.

Procedure for Pharmaceutical Formulations: Preparation of MPT stock solution for pharmaceutical formulations: An accurately weighed portion of the tablet /capsule powder equivalent to 100 mg of MPT was extracted with 3x10 ml portions of chloroform and filtered after the deletion of spores. The combined filtrate was evaporated to dryness and some portion of the residue was dissolved in distilled water to get a concentration of 1 mg/ml. This solution was further diluted stepwise with distilled water as under the preparation of standard drug solutions and reference method. Another portion of the residue was dissolved in DMF to get a concentration of 1 mg/ml. This solution was further diluted with DMF as under the preparation of standard drug solution. Then the procedures given under bulk samples were followed for the assay of MPT in formulations.

Analysis of Formulations: To find out the suitability of proposed method for the assay of pharmaceutical formulations, different samples containing MPT were analyzed by the proposed method and reference method. The results obtained from each of the proposed methods and the reference method were compared statistically by the T and F-tests and were found not to differ significantly. The results were recorded in Table 1.

Recovery studies: Recovery studies were conducted by analyzing each pharmaceutical preparation in the first instance for the active ingredient by the proposed method. 10 mg of MPT was added to each one of the previously analyzed pharmaceutical preparations and the total amount of the MPT was once again determined by the proposed method after bringing the concentration within Beer's law limits. The results are recorded in the Table1.

Interference studies: The effect of wide range of excipients and other additives usually present in formulations in the determination of MPT under optimum conditions were investigated. It was found that none of the excipients present in pharmaceutical preparations, interfere in the estimation of MPT, by the recommended procedure. However preliminary treatment is necessary prior to its estimation which has been described under procedures for pharmaceutical formulations.

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The proposed UV spectrophotometric method was completely validated according to the procedure described in ICH guidelines and United States Pharmacopoeia for validation of analytical methods. The performance parameters evaluated for the method were linearity, precision, accuracy.

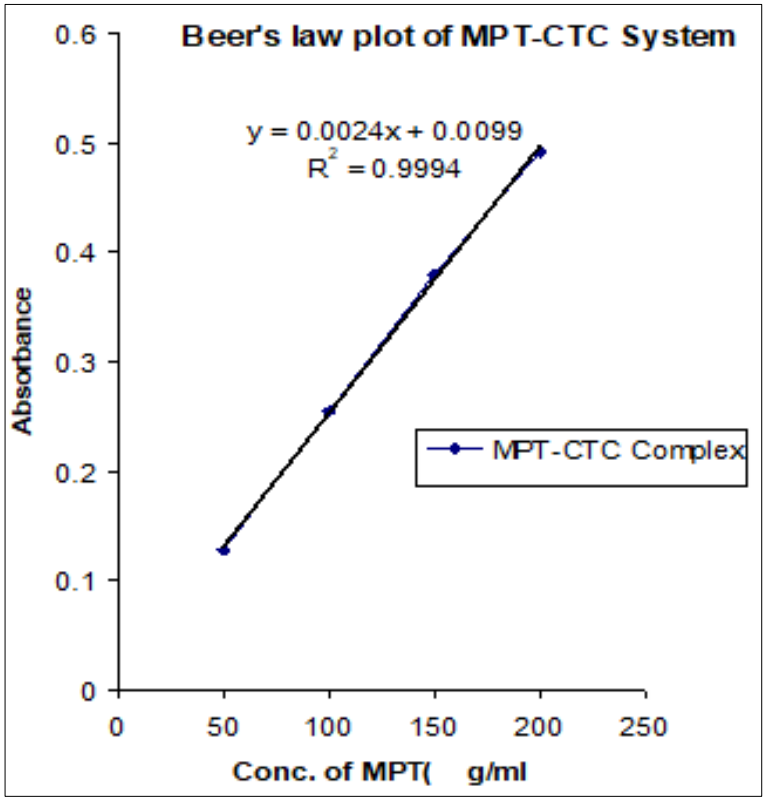

Fig 4: Beer's law plot of MPT-CCT System

Linearity and sensitivity of the method: Knowledge of the sensitivity of the color is important and the following terms are commonly employed for expressing sensitivity.

According to the Bouguer-Lambert-Beer's law

$$A = \log \frac{\text{Intensity of incident radiation}}{\text{Intensity of transmited light}} = \varepsilon ct$$

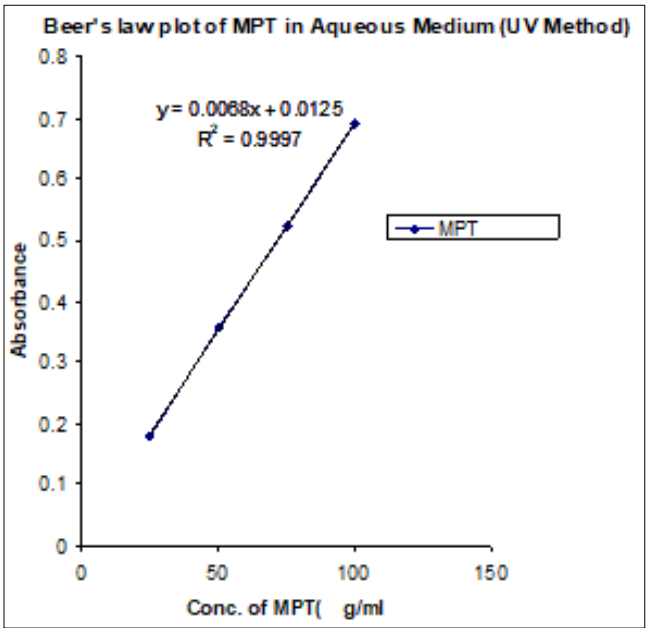

Fig 5: Beer's law plot of MPT in Aqueous Medium

The absorbance (A) is proportional to the concentration (C) of absorbing species, if absorptivity (ϵ) and thickness of the medium (t) are constant. When C is in moles/lit, the constant is called molar absorptivity; Beer's law limits and the ϵmax values are expressed as µg/ml and L mol^{-1} cm^{-1} respectively. Sandell's sensitivity refers to the number of µg of the drug to be determined, converted to the colored product, which is in a column solution of cross section $1Cm^2$ shows an absorbance of 0.001(expressed as $\mu g/Cm^2$)

Ringbom's plot: The relative concentration error depends inversely upon the product absorbance and transmittance. The relative error increases at the extremes of the transmittance scale. The slope of plot 'C' versus 'T' i.e., Ringbom plot gives relative error coefficient (i.e. plot of log C vs T).

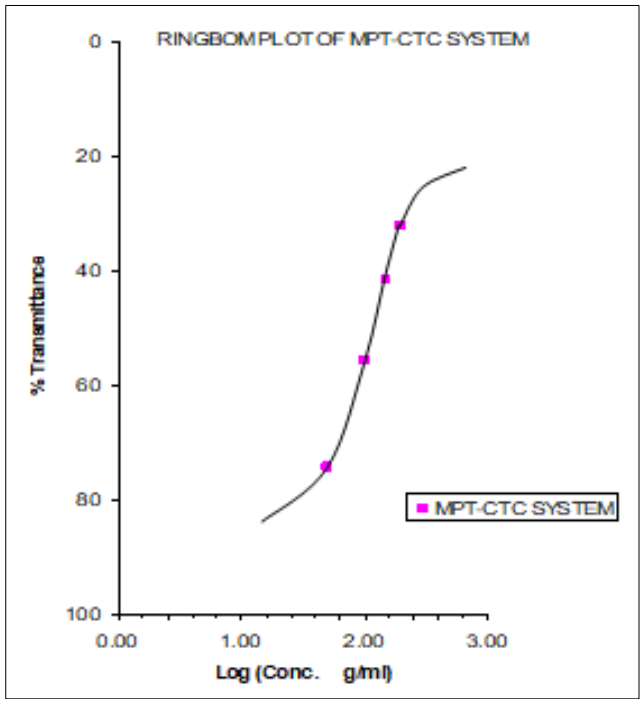

Fig 6: Ringbom's plot of MPT-CCT System

The main limitations of Ringbom's plot is that it provides no information concerning the concentration range of good precision unless it is combined with ΔT vs T relations. The above expression is valid whether the Beer's law is followed or not.

Precision and Accuracy

The purpose of carrying out a determination is to obtain a valid estimate of true values. Precision and accuracy together determine the error of an individual determination. They are among the most important criteria for judging analytical procedures by their results.

Precision

Precision refers to the reproducibility of measurement within a set, that is to scatter or dispersion of a set about its central value. The term 'Set' is defined as referring to a number (n) of independent replicate measurements of some property.

Table 1: Optical characteristics, precision and accuracy of the Proposed method

Data	Value
$l_{max}^{(nm)}$	625
Beer's law limits (mg/ml)	50-200
Molar absorptivity ($L.mole^{-1}.cm^{-1}$)	$1.705x10^3$
Sandell's sensitivity (mg/cm^2/0.001 absorbance unit)	0.04016
Standard error of estimate (S_e)	$7.38x10^{-3}$
Standard deviation of slope (S_b)	$6.6x10^{-5}$
Standard deviation of intercept (S_a)	$9.03x10^{-3}$
Slope (b)	0.0024
Intercept (a)	0.0099
Correlation coefficient (r)	0.9994
RSD	0.5455
0.05 level	0.5726
0.01 level	0.8979
Error in bulk samples	0.55

The standard deviation 'S' is given by,

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

Standard deviation has the same units as the property being measured.

% Relative standard deviation=100 X (s/x).

Precision

The precision of each proposed method was ascertained by measuring the substance of six samples each containing $2/3^{rd}$ the amount of the upper Beer's law limits. The percent of relative standard deviation and percent range of error were calculated and summarized in Table 1.

Accuracy

To determine the accuracy of the proposed method different amounts of bulk samples of the MPT were taken and analyzed by the proposed method and theresults (percent error) are given in Table 1.

Results and Discussion

The proposed method has been planned to serve as a key to visible spectrophotometric methods which have been developed (reported and proposed) and successfully applied to the analysis of sample (bulk and pharmaceutical formulations) containing MPT. The various functional groups present in the MPT molecule permits the visible spectrophotometric methods for its determinations, through a wide range of more or less specific reactions and it was felt that it would be worth presenting them from a critical stand point taking into account simplicity, sensitivity, selectivity (specificity), precision and accuracy.

The optimum conditions incorporated in the proposed procedure for the determination of MPT were established through control experiments based on the maximum color development and stability of the colored species formed in different reactions.

Validity of the analytical methods proposed for the determination of MPT was established from the precision (calculating percent relative standard deviation, percent range of error at confidence limits with D=0.05 and 0.01 levels from six determinations) and accuracy (percent error in bulk samples, comparisons of results, obtained by the proposed method with the reference method in the case of pharmaceutical formulations and recovery experiments) studies. The sensitivity of the method was ascertained through molar extinction coefficient, Sandell's sensitivity, optimum photometric range and Beer's law limits. The regression analysis using the method of least squares was made for slope (b), intercept (a), and correlation coefficient (r) obtained from different concentrations. The data obtained in the determination of MPT with different reagents are summarized in Tables 1. For comparison of results obtained by proposed method, a UV reference method in water was developed for the quantification of MPT at an appropriate l_{max}.

The selectivity (or specificity) of the proposed method was ascertained through interference studies with other active and inactive ingredients usually present in pharmaceutical preparations. The results obtained from the proposed method and the UV reference method were compared statistically by the 't' and 'F' tests and were found that the proposed method not to differ significantly in precision and accuracy from the UV reference method (Table1). The sensitivity data (ε_{max} and Beer's law limits) of the proposed method in the determination of MPT is furnished in Table-1.

Conclusion

The present study was undertaken with an objective of developing simple, sensitive and reliable analytical method like UV-Visible spectrophotometry for estimation of MPT with CTC in tablet dosage form. The method has sufficiently good accuracy, precision and permitted as a cost effective as other methods. The results of validation tests were found to be satisfactory. The analytical method is simple, sensitive, rapid and specific. For these reasons, it can be used in routine analysis and can be applied for the determinations of MPT in pharmaceutical formulations and biological samples in the laboratories of research, hospitals and pharmaceutical industries.

Acknowledgements

The authors extend their sincere thanks to Dr. Ramachandra RK, the Principal, and also Board of Research Studies, Government College Autonomous, Rajahmundry for providing seed money to carry out the research work vide proceedings No: 004/GCRJY/Acad. Cell/CREATE Fund/ Research Funding/2023. Also thankful to all the faculty members of the chemistry department, Government College (A), Rajahmundry.

Conflicts of interests if any: The authors have no conflicts of interest regarding this investigation.

References

1. Nahar S, Islam SMA, Khandaker SI, Nasreen W, Hoque O, Dewan I. Formulation and evaluation of Metoprolol Tartrate loaded niosomes using 2^3 factorial design. J Pharm Res Int. 2018;22(6):1-17. DOI: 10.9734/JPRI/2018/42068.
2. Ouerfelli N, Vrinceanu N, Mliki E, *et al*. Modeling of the irradiation effect on some physicochemical properties of Metoprolol Tartrate for safe medical uses. Sci Rep. 2020;10:67. https://doi.org/10.1038/s41598-019-56805-0.
3. Kiefer O, Fischer B, Breitkreutz J. Fundamental investigations into Metoprolol Tartrate deposition on orodispersible films by inkjet printing for individualised drug dosing. Pharmaceutics. 2021;13:247. https://doi.org/10.3390/pharmaceutics13020247.
4. Jaiswal J, Anantawar SP, Narkhede MR, Gore SV, Mehta K. Formulation and evaluation of thermo reversible in situ gel of Metoprolol Succinate. Int. J Pharm Pharm. Sci. 2012;4(3):96-102.
5. The Merck Index. 14th Ed. New Jersey, USA: Merck Res. Lab. Division of Merck and Co. Inc. Whitehouse Station; c2009. p. 6151.
6. Gaikwad V. Formulation and evaluation of in-situ gel of Metoprolol Tartrate for nasal delivery. J Pharm Res. 2010;3(4):788-793.
7. Dhole SM, Chaple DR, Harde MT. Int J Anal Bioanal Chem. 2013;3(3):82-85.
8. Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and postmortem material. 3rd Ed. London: Pharmaceutical Press; c2004. p. 1275-1276.
9. British Pharmacopoeia. London: The British Pharmacopoeia Commission. 2010;2:14-19.
10. Drug Bank Metoprolol (DB00264). Available from: http://www.drugbank.ca/drugs/DB00264, Accessed 19 November 2013.
11. International Conference on Harmonization (ICH). Q2A: Text on; validation of analytical procedures. Federal Register. 1995;60(40):11260-11262.
12. International Conference on Harmonization (ICH). Q2B: Validation of analytical procedures: Methodology, availability. Federal Register. 1997;62(96):27463-27467.
13. Martindale W. Martindale: The complete drug reference. 36th ed. London: Pharmaceutical Press; c2009.
14. Royal Pharmaceutical Society of Great Britain. Martindale: The extra pharmacopoeia. 33rd Ed. London: Pharmaceutical Press; c2002. p. 932.
15. British Pharmacopoeia. London: H.M. Stationery Office. 1998;1:889.
16. Bhusan R, Arora M. Biomed Chromatogr. 2003;17:226.
17. Lucic B, Radulovic D, Vujic Z, Agbaba D. J Planar Chromatogr Mod TLC. 2005;18:294.
18. Park YJ, Lee DW, Lee WY. Anal Chim Acta; c2002, 47-51.
19. Rao KVK, Rao MEB, Nagoji KEV, Rao SS. Indian J Pharm Sci. 2003;65:204.
20. Li C, Shi J, Shan W. Yuowu Fenxi Zazhi. 2004;24:205.
21. Ternes TA, Hirsch R, Mueller R, Haberer K. Fresenius J Anal Chem. 1998;362:329.
22. Sadecka J, Polonsky J. J Chromatogr A. 1996;735:403.
23. Blanco M, Coello J, Iturriaga H, Maspoch S, Pou N. Analyst. 2001;126:11-29.
24. Hassan SSM, Abou-Sekkina MM, El-Ries MA, Wassel AA. J Pharm Biomed Anal. 2003;32:175.
25. International Conference on Harmonization (ICH). ICH guidelines Q2(R1): Validation of analytical procedures: Methodology. Geneva: International Conference on Harmonization; c2005.
26. Ringbom A. Z Anal Chem. 1938;115:332.
27. Ayres GH. Anal Chem. 1949;21:652.
28. Guide for use of terms in reporting data. Anal Chem. 1982;54:157.
29. Massart DL, Vandeginste BGM, Deming SN, Michotte Y, Kaufman L. Chemometrics: A textbook. Amsterdam: Elsevier; c1988.
30. Zarapkar SS, Rele RV, Doshi VJ. Indian Drugs. 1987;24:560.
31. Bhatkar RG, Chodankar SK. Indian J Pharm. Sci. 1980;42:145.
32. Bhatkar RG, Madkaiker DC. Indian J Pharm. Sci. 1981;43:20.
33. Indian Pharmacopoeia. New Delhi: The Controller of Publication, Govt. of India. 2010;2:1681.
34. Rahman N, Haque SM, Azmi SNH. J Chin Chem. Soc. 2007;54:1511-1520.
35. Hinge MA, Mahida RJ, Sojitra P. Int J Pharm Sci. Rev Res. 2015;31(1):217-222.
36. Cesme M, Tarinc D, Golcu A. Pharmaceuticals. 2011;4:964-975.