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Titrimetric and Spectrophotometric Determination of Metaprolol tartrate in Pharmaceuticals Using N-Bromosuccinimide

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Abstract: One titrimetric and two spectrophotometric methods are presented for the assay of metaprolol tartrate (MPT) in bulk drug and in tablets. The methods employ N-bromosuccinimide (NBS) as the oxidimetric reagent and two dyes, methyl orange and indigo carmine as spectrophotometric reagents. In titrimetry, an acidified solution of MPT is treated with a known excess amount of NBS and after a definite time, the unreacted oxidant is determined by iodometric back titration. Spectrophotometry involves adding a measured excess of NBS to MPT in acid medium followed by determination of residual NBS by reacting with a fixed amount of either methyl orange and measuring the absorbance at 520 nm (Method A) or indigo carmine and measuring the absorbance at 610 nm (Method B). In all the methods, the amount of NBS reacted corresponds to the amount of MPT. Reaction conditions have been optimized. Titrimetry allows the determination of 1 - 12 mg of MPT and the calculations are based on a 1: 4 (MPT: NBS) reaction stoichiometry. In spectrophotometry, the measured absorbance is found to increase linearly with the concentration of MPT serving as basis for quantitation. The systems obey Beer's law for 0.5 - 4.0 μg mL^{-1} and 1.25 - 10.0 μg mL^{-1} for method A and method B, respectively. The apparent absorptivities are calculated to 1.07 x 10^5 be and 4.22 x 10^4 L mol cm^{-1} for method A and method B, respectively. The methods developed were applied to the assay of MPT in commercial tablet formulations, and the results were compared statistically with those of a reference method. The accuracy and reliability of the methods were further ascertained by performing recovery tests via standard-addition method.

Keywords: Metaprolol tartrate, assay, titrimetry, spectrophotometry, N - bromosuccinimide, tablet formulations.

Introduction

Metaprolol tartrate (MPT) which is chemically known as (±)-(isopropylamino)-3-[4-(2-methoxyethyl) phenoxy]-2-propanol tartrate (Fig. 1) is a cardiovascular beta adrenergic blocker and has been in clinical use for over 30 years. This is one of the most widely prescribed drugs in the world to-day for the treatment of various cardiovascular disorders such as angina pectoris, cardiac arrhythmia and hypertension[1]. In the last 35 years, nearly 200 articles have been published concerning with the detection and determination of MPT and its metabolites using various techniques, a great majority of them chromatographic, but, most of them are limited for the analysis of biological samples. The drug is official in US Pharmacopoeia[2] which describes non-aqueous titrimetry and high performance liquid chromatography as assay procedures for bulk drug and tablets, respectively.

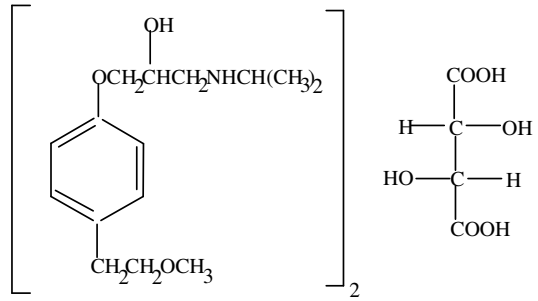

Figure 1. Structure of Metoprolol tartrate

For the determination of the drug in dosage forms, various analytical techniques including uv-spectrophotometry[3-6] diffuse reflectance and NIR spectrometry[7], fluorimetry[8,9], densiometry[10], AAS[11,12], liquid chromatography[13], high performance liquid chromatography[14-19], high performance thin layer chromatography[20], gas chromatography-mass spectroscopy[21], non-suppressed ion-chromatography[22], ion-selective electrode based potentiometry[23] and voltammetry[24] have been reported. But, such techniques are time-consuming because of extensive sample pretreatment, require expensive instrumentation and beyond the reach of small laboratories, particularly in under developed and developing countries. The assay procedures, from a pharmaceutical analysis view point, should be simple, rapid and cost-effective, without, of course, compromising on the requirements of accuracy and precision, and sensitivity. Considering from this angle, titrimetry and visible spectrophotometry may serve as useful alternatives to many of the aforesaid sophisticated techniques because of their cost-effectiveness, ease of operation, sensitivity, remarkable accuracy and precision and wide applicability.

It is revealed from the literature survey that the only titrimetric method[25] available for MPT using metavanadate as the oxidimetric reagent requires high H_2SO_4 concnetration and is applicable over a narrow range (1-5mg). Visible spectrophotometric methods based on diverse reaction chemistries have been proposed for assay in pharmaceuticals. Nitration of MPT in H_2SO_4 medium to yield a yellow product has been used as basis for the assay of the drug by Sanghavi and Vyas[26]. The greenish-yellow chromogen resulting from the reaction of MPT with iron(III) chloride in HCl medium was used by Patel et al[27] for the estimation of the drug in pharmaceutical preparations. MPT was caused to react with formaldehyde and chloranil in the presence of Ag_2O to form a blue colour for quantification by Shingbal and Sardesai[28]. One of the same authors[29] has used 1-fluoro-2,4-dinitro benzene (FDNB) as a chromogenic reagent for the estimation of the drug. Based on the formation of charge-

transfer complex with 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) in methanolic-aqueous medium, an assay method has been devised by Amin et al[30].Other Π-acceptors employed for assay based on a similar reaction scheme include tetracyanoethylene (TCNE) or chloranilic acid (CAA)[12]. Alpdogan and Sungur[11] have recently proposed a sensitive method based on the formation of Cu(II)dithiocarbamate complex by derivatisation of the secondary amine group of MPT with CS_2 and $CuCl_2$ in the presence of ammonia, and the complex was extracted into chloroform for measurement. The method has very recently been modified[12] in which the copper(II)chelate formed in the presence of CS_2 at pH 7.5 was extracted into isobutylmethylketone and measured, thereby offering enhanced sensitivity. Ion-pair complex formation followed by extraction into organic solvents before absorbance measurements is another approach found in the literature for the assay of MPT. Benzyl orange[31] and bromothymol blue[32] have been used as chromogenic reagents for the purpose. But, most of the above methods suffer from one or other deficiency such as heating or extraction step, critical dependence on acid/pH condition, use of non-aqueous medium/expensive chemicals, poor sensitivity and/or narrow range of linear response, as indicated in Table 1.

We have previously demonstrated the applicability of NBS as a useful reagent for the assay of certain bioactive substances[33,35]. The present work extends the utility of NBS as an oxidimetric reagent fro the assay of MPT in pharmaceutical formulations.

Experimental

Apparatus

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and standards

All chemicals used were of analytical purity grade and all solutions were prepared in distilled water.

N-Bromosuccinimide (NBS)

0.01 M NBS solution was prepared by dissolving about 1.8 g of chemical (SRL Research Chemicals, Mumbai, India) in water with the aid of heat, and diluted to one litre with water and standardized[36]. The solution was stored in an amber coloured bottle and used for titrimetry. It was diluted appropriately to get 90 and 340 μg mL^{-1} NBS for use in spectrophotometric method A and method B respectively. The NBS solution was stored in a refrigerator when not in use.

Sodium thiosulphate (0.01 M)

Prepared by dissolving 2.48 g of chemical (Sisco Chem Industries, Bombay India) in 1 litre of water and standardized using pure potassium dichromate[37].Hydrochloric acid 1M, Acetic acid 5 M, Methyl Orange 50 μg mL^{-1}, Indigocarmine 200 μg mL^{-1} and Starch Indicator (1%) were prepared usual manner.

Standard drug solution (2 mg mL^{-1})

Pharmaceutical grade MPT was received from Astra-Zeneca, Bangalore, India, which was reported to be 99.7 % pure as gift and was used as received. A stock standard solution containing 2 mg mL^{-1} MPT was prepared by dissolving 500mg of pure drug in water and diluting the solution to the mark in a 250 mL calibrated flask and used in titrimetric work.

The stock solution (2000 μg mL^{-1}) was diluted appropriately with water to yield 10 and 50 μg mL^{-1} MPT for use in spectrophotometric method A and method B, respectively.

Table 1. Comparison of the existing spectrophotometric methods with the proposed methods for MPT

S No	Reagent/s used*	$\lambda_{max,}$ nm	Linear range, μg mL^{-1}	Remarks	Ref
1	KNO_3	440	Upto120	Uses high H_2SO_4 concen tration; less sensitive	26
2	Fe (III) Cl_3	380	40 - 200	Less sensitive	27
3	$HCHO^-$ - chloranil- Ag_2O	680	25 - 75	Involves incubation at 75°C for 10 min; less sensitive; narrow linear range	28
4	FDNB	380		Involves incubation at 75°C for 40 min; requires partially non-aqueous medium	29
5	NBD-Cl	470	0.4 – 60.0	Requires partially non-aqueous medium	30
6	a) TCNE	415	10 -170 ($3.49x10^3$)	Less sensitive; requires partially non-aqueous medium	12
	b) CAA	510	20 -230 ($1.73x10^3$)		
7	CS_2-$CuCl_2$-dithiocarbamate	440	11 - 55	Involves liquid-liquid extraction; less sensitive	11
8	CS_2-$CuCl_2$	435.4	Upto 60 ($1.08x10^4$)	Involves liquid-liquid extraction; less sensitive	12
9	Benzyl orange	401	3.4- 34.2	Requires strict pH control; involves liquid-liquid extraction; less sensitive	31
10	Bromothymol blue	410	2 - 14	Requires strict pH control; involves liquid-liquid extraction	32
11	a) NBS-methyl orange	520	0.5-4.0 ($1.07x10^5$)	Free from heating or extraction step, non-rigid optimum conditions; long linear range of response, highly sensitive	Present methods
	b) NBS-indigo carmine	610	1.25-10.0 ($4.22x10^4$)		

Solution from dosage forms

Preparations containing MPT were purchased from local commercial sources and subjected to analysis. A quantity of finely ground tablet powder equivalent to 200 mg of MPT was accurately weighed into a 100 mL volumetric flask and shaken with 60 mL of water for 20 min; the volume was made upto the mark with water and mixed. Then, filtered using a Whatmann No. 42 filter paper. The first 10 mL portion of filtrate was discarded and a convenient aliquot of the subsequent portion was used for assay by titrimetric procedure.

The tablet extract (2000 μg mL^{-1} in MPT) was appropriately diluted to get working concentrations for assay by spectrophotometric methods.

Procedures

Titrimetry

A 10 mL aliquot of standard drug solution containing 1-12 mg of MPT was accurately measured and transferred into a 100 mL titration flask and acidified with 5 mL of 5 M acetic acid. Ten mL of NBS (0.01 M) was pipetted into the flask, the content mixed and kept aside for 5 min. Then, 5 mL of 10 % potassium iodide solution were added, and the liberated iodine was titrated against sodium thiosulphate (0.01M) using starch indicator. A blank titration was performed under identical conditions. The amount of drug in the measured aliquot was calculated from:

$$\text{Amount (mg)} = \frac{(B-S)\, M_w R}{4}$$

where B = volume of thiosulphate solution used in blank titration, mL
S = volume of thiosulphate solution used in sample titration
Mw = relative molecular mass of MPT
R = molarity of thiosulphate solution

Spectrophotometric method A

In each of a series of 10 mL calibrated flasks were placed 0.5, 1.0, 2.0 ….. 4.0 mL of standard 10 μg mL^{-1} MPT solution and the total volume was adjusted to 4 mL with water. To each flask was added 1.5 mL of 1 M hydrochloric acid followed by 1 mL of NBS solution (90 μg mL^{-1}). The flasks were stoppered and let stand for 20 min with occasional shaking. Finally, 1 mL of 50 μg mL^{-1} methyl orange solution was added to each flask, volume diluted to the mark with water, mixed well and absorbance measured at 520 nm against a water blank after 5 min.

Spectrophotometric method B

Different aliquots (0.25, 0.5, 1.0, ….. 2.0 mL) of standard (50 μg mL^{-1}) MPT solution were accurately measured into a series of 10 mL calibrated flasks by means of a microburette and the total volume was adjusted to 2 mL by adding water. One mL of 1M hydrochloric acid was added to each flask followed by 1 mL of NBS solution (340 μg mL^{-1}). The flasks were stoppered and let stand for 20 min with occasional shaking. Lastly, 1 mL of 200 μg mL^{-1} indigo carmine dye solution was added to each flask, the volume was diluted to the mark with water, mixed well and absorbance of each solution was measured at 610 nm against a water blank after 5 min.

In either spectrophotometric method, a calibration curve was prepared by plotting absorbance versus concentration of drug or regression equation was derived using the calibration curve data. The concentration of the unknown was read from the calibration curve or computed from the regression equation.

Results and Discussion

All the three methods described here are based on the oxidation reaction involving MPT and NBS in acid medium. The methods are indirect and are based on the determination of residual NBS after having allowed the oxidation reaction to go to completion under the specified experimental conditions. The amount of NBS reacted corresponds to the drug content in all the methods.

Titrimetry

Direct titration of MPT with NBS in acid medium was not successful. However, a back titrimetric assay was found to be possible when the reactants were allowed to stand for some time in acid medium. Reproducible and stoichiometric results were obtained when acetic acid medium was employed. The results were found to be unaffected when 0.2 - 2.0 M acid concentration was maintained, and thus a 1.0 M acetic acid concentration was employed in the investigation. The reaction was found to be quantitative with a stoichiometry of 1:4 (MPT: NBS) for the range investigated (1-12 mg) for a contact time of 5-15 min. Beyond 15 min and upto 30 min a small quantity of NBS was consumed but without yielding any significant reaction stoichiometry. The relationship between the titration end point and the amount of drug was evaluated by calculating the correlation coefficient, which was found to be -0.9924 indicating a fixed stoichiometric reaction between MPT and NBS under the stated experimental conditions. Based on the reaction stoichiometry.

Spectrophotometric methods

The ability of NBS to oxidize MPT and bleach the colors of methyl orange and indigo carmine dyes has been used for the indirect spectrophotometric assay of the drug. In both methods, the drug is reacted with a known excess of NBS in acid medium, and the unreacted oxidant is determined by reacting with a fixed amount of either methyl orange or indigo carmine and measuring the absorbance at either 520 nm or 610 nm. In either method, the absorbance increased linearly with increasing concentration of drug.

MPT, when added in increasing amounts to a fixed amount of NBS, consumes the latter and there will be a concomitant fall in its concentration. When a fixed amount of either dye is added to decreasing amounts of NBS, a concomitant increase in the concentration of dye results. This is observed as a proportional increase in the absorbance at the respective wavelengths of maximum absorption with increasing concentration of MPT as indicated by the correlation coefficients of 0.9996 and 0.988 for method A and method B, respectively.

Preliminary experiments were performed to determine the maximum concentrations of the dyes spectrophotometrically, and these were found to be 5 and 20 μg mL^{-1} for methyl orange and indigo carmine, respectively. A NBS concentration of 9 μg mL^{-1} was found to destroy the red colour due to 5 μg mL^{-1} methyl orange whereas 34 μg mL^{-1} NBS was required in the case of blue colour due to 20 μg mL^{-1} indigo carmine. Hence, different amounts of MPT were reacted with 1 ml of 90 μg ml^{-1} NBS in method A and 1 ml of 340 μg mL^{-1} NBS in method B before determining the residual NBS as described under the respective procedures.

Hydrochloric acid was the ideal medium for the oxidation of MPT by NBS as well as the latter's determination employing either dye. The reaction between MPT and NBS was unaffected when 1.0 – 2.5 mL of 1 M hydrochloric acid in a total volume of about 7 mL was used. Hence, 1.5 mL of 1 M hydrochloric acid in method A and 1 mL in method B were used for both steps in the assay procedures. For a quantitative reaction between MPT and NBS, a contact time of 20 min was found necessary in both procedures and constant absorbance readings were obtained when contact times were extended upto 20 and 30 min in method A and method B, respectively. A standing time of 5 min was necessary for the bleaching of the dye colour by the residual NBS. The measured colour was found to be stable for several hours in the presence of the reaction product/s in both methods.

Analytical parameters of spectrophotometric methods

A linear relation was found to exist between absorbance at λ_{max} and concentration ranges given in Table 2 for both methods. The graphs are described by the equation

$$Y = a + bX$$

(where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration of MPT in μg mL^{-1}) obtained by the method of least squares. The apparent molar absorptivity and Sandell sensitivity values together with the limits of detection and quantification are compiled in Table 2 and are indicative of the high sensitivity of both methods.

Table 2. Analytical and regression parameters of spectrophotometric methods

Parameter	Method A	Method B
λ_{max}, nm	520	610
Beer's law limits, μg mL^{-1}	0.5 - 4.0	1.25 - 10.0
Molar absorptivity, L mol^{-1} cm-1	1.07×10^5	4.22×10^4
Sandell sensitivity, $ngcm^{-2}$	6.36	16.21
Limit of detection, μg mL^{-1}	0.041	0.105
Limit of quantification, μg mL^{-1}	0.124	0.318
Regression equation, Y*		
Intercept (a)	0.011	0.011
Slope (b)	0.151	0.058
Sa	±0.016	±0.005
Sb	±0.005	±0.0006
Correlation coefficient, (r)	0.9996	0.9880

*Y = a+bx, where y is the absorbance and X concentration in μg mL^{-1}
Sa. Standard deviation of intercept
Sb. Standard deviation of slope.

Method validation

Accuracy and precision

The accuracy and precision of the methods were evaluated by performing seven replicate analysis on pure drug solution at three amount/concentration levels (within the working ranges). The relative error (%), an indicator of accuracy was within 3.0 and within day precision, also called the repeatability, expressed as relative standard deviation (RSD) (%) was less than 2.5 indicating high accuracy and repeatability of the methods. The results of the study are given in Table 3. The reproducibility of the methods also known as the day-to-day precision was evaluated by performing replicate analyses on pure drug solution at three levels over a period of five days, preparing all solutions afresh. The day-to-day RSD values were less than 4 % reflecting the usefulness of the methods in routine analysis.

Table 3. Evaluation of accuracy and precision

Method*	MPT taken	MPT found**	Range	RE %	SD	RSD %	ROE***
Titrimetry	3.0	2.98	0.17	0.67	0.076	2.55	±2.55
	6.0	5.97	0.26	0.50	0.095	1.59	±1.59
	9.0	8.99	0.17	0.11	0.069	0.77	±0.77
Spectrophotometric method A	1.0	1.03	0.03	3.0	0.011	1.06	±1.06
	2.0	2.02	0.06	2.0	0.024	1.15	±1.149
	3.0	3.02	0.07	0.67	0.027	0.86	±0.859
Spectrophotometric method B	2.5	2.44	0.06	2.4	0.023	0.96	±0.959
	5.0	4.97	0.14	0.6	0.048	0.97	±0.969
	7.50	7.65	0.12	2.0	0.045	0.52	±0.519

*In titrimetry, MPT taken/found, range and SD are in mg while in spectrophotometric methods, the quantities are in µg mL^{-1}

Re relative error; SD. Standard deviation; RSD. Relative standard deviation; ROE. Range of error

** Mean value of seven determinations; ***At the 95% confidence level for 6 degrees of freedom.

Application

Commercial tablets were successfully analysed by the proposed methods. Co- formulated substances did not interfere. For the purpose of comparison the same batch pharmaceutical preparations were simultaneously analysed by the reference UV method [4] which consisted of the measurement of the absorbance of the tablet extract in 0.1 M hydrochloric acid at 224 nm. The results are presented in Table 4. As shown in the table, the results of analysis obtained by the proposed methods are in accordance with those obtained by the reference method. The performance of the methods was further judged by applying Student's t-test for accuracy and F-test for precision. At the 95 % confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F = 6.39) suggesting that the proposed methods are as accurate and precise as the reference method.

Table 4. Results of determination of Ranitidine in formulations and statistical comparison with the reference method

Brand name[#] and dosage form	Label claim, mg/ tablet	% found* ± SD			
		Reference method	Titrimetric method	Method A	Method B
BETALOC[a]	50	102.3±0.96	99.68±1.22	100.3±1.30	101.5±1.05
			t=3.58	t=2.63	t=1.19
			F=1.61	F=1.83	F=0.40
METAPRO[b]	25	96.54±1.24	95.75±1.02	94.99±1.20	95.01±0.67
			t=1.04	t=1.89	t=2.39
			F=1.47	F=1.67	F=3.42
METOLAR[c]	100	101.3±0.62	100.2±1.42	99.65±1.32	98.74±1.62
			t=1.61	t=2.53	t=5.19
			F=5.24	F=4.53	F=1.88

*Mean Value of five determinations

#Marketed by: a).AstraZeneca Pharm Ltd.India; b) Microvascular Pharm Ltd.India.c)Cipla Pharm Ltd. India.

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. The results summarized in Table 5 reveal good accuracies and non-interference from excipients and diluents such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate. This is also evident from the results of analysis presented in Table 4.

Table 5. Results of Recovery experiments by Standard Addition method

Formulation studied	Titrimetry				Spectrophotometric Method A				Spectrophotometric Method B			
	Amount of drug in formulation mg	Amount of pure drug added, mg	Total found mg	Pure drug Recovered* %	Amount of drug in formulation µg	Amount of pure drug added µg	Total found µg	Pure drug recovered* %	Amount of drug in formulation µg	Amount pure drug added, µg	Total found µg	Pure drug Recovered* %
METOLAR 100 mg tablets	3.01	1.5	4.57	104.0	4.98	10	15.10	101.2	19.75	10	29.65	99.00
	3.01	3.0	6.07	102.03	4.98	15	19.97	99.93	19.75	20	40.17	102.1
	3.01	4.5	7.48	99.33	4.98	20	25.42	102.2	19.75	40	61.11	103.4

*Mean value of three determinations

Conclusions

The results demonstrate that micro level determination of metaprolol tartrate is possible by titrimetry which can be performed with ease, rapidly and by using inexpensive chemicals. The method has the advantage of being applicable over a long range compared the narrow range offered by the only existing titrimetric method. Unlike most currently available spectrophotometric methods, the present methods are free from unwelcome steps such as heating or extraction and also from critical pH or acid/alkaline conditions. A significant advantage of the spectrophotometric methods is their remarkable sensitivity which is higher than that of the existing methods and is comparable to the sensitivity offered by some sophisticated techniques such as voltammetry, HPLC, HPTLC, densitometry and fluorimetry. An additional advantage is that the absorbance measurement is made at longer wavelengths (520 or 610 nm) where the interference from tablet excipients is expected to be less compared to shorter wavelengths (about 400 nm) used in most available methods. These advantages coupled with a fairly good accuracy and precision lend the methods optly suitable for routine quality control.

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