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Original Research Article

Development of Vanadometric System for Spectrophotometric Determination of Timolol in Pure and Dosage Forms

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Abstract

Purpose: *To develop a simple vanadometric spectrophotometric method for the assay of timolol.*
Methods: *The oxidation reactions were performed at optimum conditions of 3 mL 20 % v/v H_2SO_4, 6.5 % w/v ammonium metavanadate, 40 - 60 ºC and 5 min for full colour (blue) development. The proposed method was validated in accordance with International Council on Harmonization (ICH) guidelines Q2 (R1) and applied to assay commercial timolol.*
Results: *Oxidation of timolol occurred at 504 nm wavelength. The developed method recovered 99.25 - 102.00 % of timolol in pre-analyzed formulations and 99.85 - 102.00 % of the manufacturers' claim in commercial samples with RSD < 2 %. Linearity of 2 - 20 ppm (R^2 = 0.9995),as well as accuracy, 98 - 101 %; precision, 0.98 % (intraday) and 1.25 % (interday); robustness, 0.95 %; LOD, 0.256 ppm; LOQ, 0.425 ppm; and robustness, 0.95 - 1.10 %, were obtained.*
Conclusion: *The developed method is simple, sensitive, low-cost, accurate, reproducible, robust and rugged, and compares well with some complex methods for assay of timolol maleate in pure and dosage forms.*

Keywords: *Ammonium metavanadate, Spectrophotometry, Vanadometric, Timolol*

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INTRODUCTION

Transition element-based compounds have great potentials in quality control (QC) of pharmaceuticals due to the presence of lone electron pair(s), and the consequent ability to form complexes with many drugs. Ammonium metavanadate (AMV) is a yellow crystalline solid that has been used variously as a catalyst for alpha-hydroxyphosphonate [1], insulin mimic [2], oxidizer and sensor for N-acetyltransferase activity in urine analysis post-isoniazid administration [3] and is known to exhibit pH-dependent variable oxidative states of +5 to +2 due to 3d34s2 configuration of central vanadium with colour variations in solution of reductants [4]. These unique properties of AMV have been utilized in the analysis of many pharmaceutical dosage forms and inorganic cations.

Timolol maleate (Scheme 1) is a first generation non-selective beta-adrenergic receptor antagonist used in the treatment of myocardial infarction, migraine, chronic open-angle glaucoma, heart attack, angina pectoris and ocular hypertension [5]. It is chemically designated as (S)-1-[(1, 1-dimethylethyl) amino]-3-[[4-(4-morpholinyl)-1, 2, 5-thiadiazol-3-yl] oxy]-

2-propanol (Z)-2-butenedioate (1:1) salt. Timolol, in combination with a diuretic, has remained the first line drug of choice in a stepped-care approach to anti-hypertensive drug therapy and has remained among the top prescribed agents for management of cardiac disorders among the blacks.

The therapeutic importance of timolol necessitated development of spectrophotometric [6-10], HPLC [11], HPTLC [12], chemiluminescence [13], voltammetric [14], electrophoretic [15], TLC densitometric [16], complexometric by charge transfer [17], RP-HPLC [18-21] and LC [22] methods for its determination in single dosage and/or combined forms by analysts. Most of the reported methods require complex instrumentation; delicate sample preparation methods, costly reagents, expertise and a lot of time.

Literature survey indicated no report(s) of developed vanadometric assay method for timolol in single or combined forms. Against this backdrop, we developed and validated an oxidometric spectrophotometric assay method for timolol, applied the developed validated method for assay of the drug in a single dosage form and compared the validation parameters with the earlier developed methods of assay. Attempts were also made to propose the possible route and mechanisms of oxidation; the principle of which the developed method is dependent.

EXPERIMENTAL

Materials

Whatman 0.2 µm membrane filter, 25 mm diameter size (Cole-Palmer, Nigeria), U-2900 double beam UV-visible spectrophotometer, 190 - 1100 nm, 1.5 nm spectral bandpass (Hitachi High-Tech Co, Japan). All the reagents are of analytical grade. All the timolol formulations (0.25 and 0.5 %) used were procured from reputable drug outlets and were still within their expiry date.

Methods

Optimization of experimental variables

The effect of AMV concentration was determined by adding aliquot 1.0 mL of 0.5 - 10 % w/v AMV to equal volume (1 mL) of 2.5 ppm timolol in different test tubes. The nature and concentration of acidic medium (H_2SO_4, HCl, HNO_3, H_3PO_4 and $HClO_3$) were studied by adding various volumes (0.5 - 6 mL) of concentrated acids and the optimum concentration of oxidant to 2.5 ppm timolol solution. Optimum temperature was determined by carrying out the reaction in a thermostatically controlled water bath at different temperatures 25 - 100 ºC at the optimum conditions so far determined, while the reaction time was studied for different periods of time (0-60 mins). The stability of the developed colour was studied up to 4 h, while the order of addition of AMV, acid, and temperature to the drug solution were determined appropriately. For each variable, the rate of blue colour development and the absorbance values recorded at 504 nm were used as parameter for selecting optimum condition for the analysis.

General timolol assay method

The standard timolol solution (1 mL) was transferred into 10 mL calibrated flask and 1 mL of the 6.5 % w/v AMV was added. The optimum volume (3 mL) of 20 % v/v of H_2SO_4 was added (Table 1). The contents of the flasks were thoroughly mixed and the reactions were allowed to proceed for different periods of time (Table 1) at different temperature (40 - 60 °C), and then cooled to room temperature. After completion of the reactions, the solutions were completed to volume with distilled water. The absorbance of the resulting solutions was measured at the λ_{max} of 504 nm against reagent blanks treated similarly.

Method validation

The proposed method was validated in accordance with the ICH guidelines Q2 (R1) for linearity, precision, accuracy, limits of detection and quantization, interference, ruggedness and robustness [9]. The linearity was determined by preparing six replicates of standard solution of timolol in the range of 2-20 ppm and the construction of standard calibration curves using the optimum conditions. Regression analysis was carried out using least square method. Precision was determined by replicate analysis of nine different solutions of timolol at 10 ppm and estimating the relative standard deviation, RSD. Robustness was determined by carrying out the reaction at temperature of 40 ºC instead of 50 ºC, and then 5 % w/v AMV instead of 6.5 % w/v to ascertain the influences of AMV concentration and temperature variations (or any other parameter) on the performance of the proposed method while keeping other optimum conditions unchanged. The percentage recovery and the RSD were determined. Ruggedness was performed by carrying out the analysis on different days and using two different spectrophotometers in different laboratories. The percentage recovery and % RSD were

determined for day-to-day and laboratory-laboratory assay. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula 3S/slope and 10S/slope respectively, where S is the standard deviation of the intercept of the Beer's calibration.

Recovery studies

Recovery studies were carried out by adding a 10 ppm solution of pre-analyzed timolol eye-drop to standard solution of pure timolol at 80, 100 and 120 % levels [6]. To 1 mL of pre-analyzed eye-drop solution in three different test tubes, 0.8, 1.0 and 1.2 mL of 10 ppm standard solution were added to each of the eye-drop solution respectively and then 1 mL AMV + 3 mL sulphuric acid. The solutions were diluted to 10 mL volume and assayed.

Analysis of timolol formulations by proposed method

A quantity of eye-drop equivalent to 1 mg of timolol was accurately measured and transferred into a 10 mL volumetric flask, appropriately diluted with 1 mL 6.5 % w/v AMV and 3 mL 20 % v/v of sulphuric acid, made up to mark with distilled water and then warmed to the required temperature and allowed some time for colour development to finally produce a sample solution for UV/VIS spectrophotometric analysis as already described. The amount of drug present in the sample solution was determined using the regression equation of calibration curve of standard [8].

RESULTS

The preliminary findings (Table 1) showed that the absorption intensity of timolol increased progressively with increasing concentration of AMV and H_2SO_4, reaction time and temperature until the maximum absorbance was obtained. However, further increase in the concentration of AMV or acid, temperature or time had no effect on the absorbance.

The parameters selected as optimum conditions (Table 1) were considered as the parameter (concentration, time and temperature) at which maximum absorbance was obtained or full colour development observed in the near plateau region of the parameter-absorbance curves.

Table 1: Optimization of experimental variables

Parameter	Value
AMV concentration	6.5 % w/v
Concentration and nature of acid	20 %v/v, H_2SO_4 3 mL
Temperature	40-60 ± 0.4-1.6 ºC
Reaction Time	5.0 ± 0.6 mins
Complex stability	3.4 ± 0.9 hours
Order of addition	Drug = AMV > H_2SO_4 > Time = Temperature

**Values are mean ± SD*

Table 2: Parameters of the proposed method validation

Parameter	Value	Parameter	Value
λ_{max}	504 nm	Precision	
R^2	0.9995	Intra-day (% RSD)	0.98 %
Slope	0.665	Inter-day (Ruggedness)	1.250-1.251 %
Intercept	0.0015	Accuracy (Recovery)	98-101 %
Beer's limit (Linearity)	2-20 µg/mL	Robustness (% RSD)	0.95-1.10 %
LOD (µg/mL)	0.256-0.921	LOQ (µg/mL)	0.425-1.611

Table 3: Recovery of timolol from pre-analyzed eye-drops

Formulation	Amount of timolol spiked (%)	Amount recovered (%)	% RSD
Eye-drop A	80	99.25 ± 1.03	1.28
	100	100.60 ± 0.94	0.96
	120	101.90 ± 0.29	0.11
Eye-drop B	80	102.00 ± 0.78	.082
	100	99.80 ± 1.00	1.01
	120	99.50 ± 1.01	1.05
Eye-drop C	80	100.05 ± 0.47	0.66
	100	100.70 ± 0.39	0.13
	120	99.70 ± 1.29	1.90

Table 4: Analysis of commercial formulations of timolol

Formulation	Label claim (mg/mL)	Amount estimated (mg/mL)	Estimated (%)	% RSD	SD
Eye-drop A	2.5	2.541	101.65	1.56	1.86
Eye-drop B	2.5	2.496	99.85	0.87	0.98
Eye-drop C	5.0	5.100	102.00	1.85	1.90

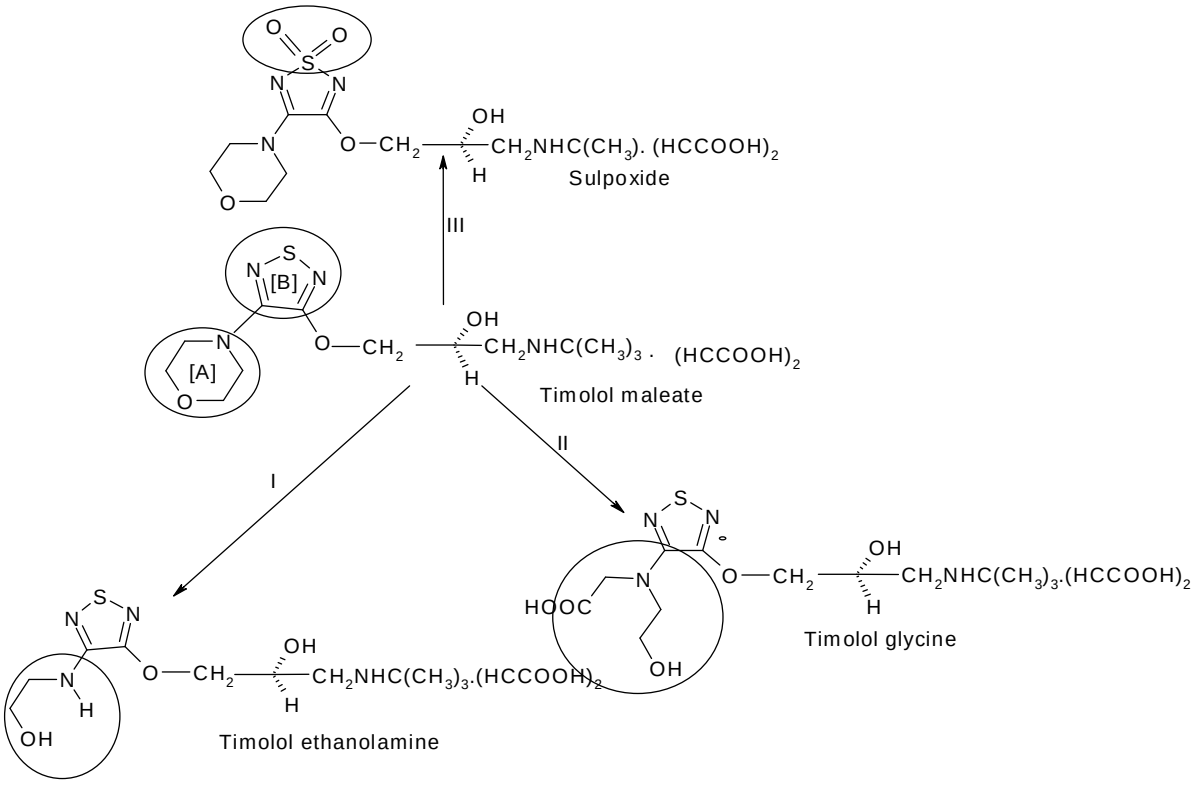
DISCUSSION

The stability of the complex was observed beyond 3.4 h which is an indication that this assay could be done for a longer time than usual, thereby providing flexibility of the method in cases of large scale assay in QC. The order of addition was performed to determine effect sequence of inclusion of the parameters during timolol assay. The result showed that the acidic medium should be created first once the drug comes in contact with the oxidant before adjusting the time or the temperature or both. Other acids did not provide intense colour development compared to H_2SO_4.

The result of method validation showed that Beer's law was obeyed at 2 - 20 ppm concentration with good correlation coefficient (0.9995). The RSD was less than 2 % (0.98 %) signifying good reproducibility of the proposed method for routine timolol assay. The percentage recovery of timolol was 98 - 101 % while the % RSD was 1.1 %. Variations of optimum parameters did not affect the recovery and RSD significantly showing the reliability and flexibility of the proposed method of timolol analysis. The % RSD obtained for lab-to-lab and day-to-day assay were 1.25 and 1.251 respectively showing the reproducibility of the proposed method.

Timolol is a morpholine ring-containing (A) β-adrenoceptor antagonist. Cleavage of the morpholine ring constitutes one of the major oxidation pathways, aside S-oxidation of thiadiazole ring (B) as shown in Scheme 1.

The morpholine cleavage involves two pathways- (I) initial oxidative attack on one of the carbon atoms adjacent to the nitrogen atom of the morpholine ring forming hydroxyl-ether intermediates and the timolol ethanolamine and (II) cleavage of the morpholine ring of timolol occurs prior to oxidative attack at one of the carbon atoms adjacent to the oxygen atom;



Scheme 1: Proposed oxidation pathways of timolol maleate

further oxidation would form timolol glycine [23]. The other route (III) involves S-oxidation (sulpoxidation). Theoretically, oxidation of timolol via routes I and II represents *in vivo* metabolic pathways and the mechanisms have not been established unequivocally [24]. Postulations also revealed that the oxidation pathways cannot be harnessed *ex vivo* or *in vitro* quantitatively to warrant its usefulness in the present studies as the metabolism (by oxidation) of several drugs (including metoprolol and timolol) is linked to the debrisoquine oxidation phenotype [24]. Several drugs which possess structures (ring B) similar to timolol have been found to be oxidized quantitatively to its sulphoxide in acidic medium [25] suggesting the possibility of timolol oxidation via such mechanism.

The validation parameters of timolol maleate in this study compare very well with reported parameters of other developed methods, suggesting the suitability of this method in the routine assay of timolol. The UV detection ʎ differs due to the differences in the methods developed and solvents applied. Theoretically, the sensitivities of some of the earliest developed methods are higher than the present method; however, most of them are too expensive and cumbersome to be used in the routine assay of timolol especially in developing economies where their procurement, expertise and maintenance are difficult to come by.

Table 5: Comparison of validated parameters of timolol assay

Method	Validated assay parameters	Ref.
Chemometric (CLS, PCR, PLS)	ʎ $_{max}$ 295 nm, % mean recovery 99.85, % S.E 1.0, R^2 0.9727-0.9993, ʎ $_{max}$ 234-377 nm, IC_{95} 93.67-105.75 %.	[6]†
Vierordt's and bivariate calibration algorithm spectrophotometry	ʎ $_{max}$ 257 nm, linearity 5-85 ppm, R^2 0.9999, LOD 1.40-1.47 ppm, accuracy 99.9-100.3 %, precision (SD) 0.702-0.996, %RSD 0.700-0.967, % error 0.221-0.306, repeatability 99.7-100.5, specificity 100.2-100.6 %,	[7]†
Charge transfer	ʎ $_{max}$ 543 nm, linearity 6.7-24.8 ppm, molar absorbance 0.6 l/mol.cm, R^2 0.99702, LOD 0.129692 ppm, LOQ 0.431873 ppm, precision % RSD (interday) 0.349-0.505 % intraday 0.39-0.78 % , recovery (serum) 99-99.332%, (urine) 98.667-99.334%, Sandell's sensitivity0.001667 µg.cm-2/00.001 A.U	[17]†
Simultaneous equation method, First Derivative Method and Ratio First Derivative UV spectrophotometry	ʎ $_{max}$ 255-294; 302.3; 290 nm, linearity 2-50; ppm, R^2 0.996-0.998, LOD 0.24-0.5; 0.14; 0.091 ppm, LOQ 0.1515-0.727; 0.424, 0.276 ppm, recovery 98.94-100.8; 99.73-101.1; 99.26-100.36 %, repeatability RSD 0.369-0.734; 1.02; 0.696 %, RSD precision (interday) 0.329-0.940; 0.603-1.376; 0.388-0.870 % (intraday) 0.165-0.722; 0.276- 0.361; 0.298-0.379 %	[8]†
TLC-densitometry	ʎ $_{max}$ 294 nm, linearity 1000-6000 ng/spot, recovery 99.87 %, RSD precision (intraday) 0.74-1.42 % (interday) 1.15-1.46 %, recovery 99.75-99.96 %	[16]
RP-HPLC	ʎ $_{max}$ 285 nm, linearity 5-30 ppm, recovery 99.75-100.06 %, LOD 1.24 ppm, LOQ 3.77 ppm, robustness RSD 0.053-0.260 %, regression y=47.28x-15.57, R^2 0.999, precision RSD (interday) 0.04 (intraday) 0.16, commercial drug estimate 100.42 %.	[18]†
UV spectrophotometry	ʎ $_{max}$ 295 nm, linearity 2-10 ppm, regression equation y=0.0258x-0.00076, R^2 0.9999, LOQ 1.0 ppm.	[9]
RP-HPLC	ʎ $_{max}$ 295 nm, linearity 10-20 ppm, precision RSD (intraday) 1.24-1.68 % (interday) 1.31-1.86 %, recovery 97.3-99.4 %. LOQ 1.4 ppm, R^2 0.989, regression equation y=35.56x-10.49, LOD 0.4 ppm.	[12]
RP-HPLC	ʎ $_{max}$ 210 nm, linearity 250-750 ppm, LOD 0.3 ppm, LOQ 0.8 ppm, regression equation y=17537.67x+59803.88, R^2 0.9994, precision RSD 0.09 %, recovery 99.9-100.1 %.	[20]
RP-HPLC	ʎ $_{max}$ 295 nm, linearity 10-30 ppm, regression equation y=16109x-48294, R^2 0.999, LOD 0.755 ppm, LOQ 2.289 ppm, precision RSD (intraday) 0.788 (interday) 0.366, recovery 99.31 %.	[19]
Derivative and derivative ratio spectrophotometry	ʎ $_{max}$ 295 nm, linearity 3-20 ppm, regression equation y=0.0394x-0.002, R^2 0.99992, LOD 0.32 ppm, LOQ 0.97, precision RSD<2 %.	[10]†
This study (vanadometric spectrophotometry)	Tables 2-4	†

Classical least square (CLS), principal components regression (PCR), partial least square (PLS), †developed methods successfully applied to commercial timolol dosage forms

CONCLUSION

The developed method is simple, rapid, sensitive, reproducible, accurate and economical for assay of timolol compared to some chromatographic methods earlier developed. The probable mechanism of oxidation is by S-oxidation with little or no contribution from the morpholine ring oxidation. The method should be suitable in quality control and assurance for rapid and routine assay of timolol in pure and dosage formulations.

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