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UV Spectrophotometric Analysis of Drugs Terbinafine Hydrochloride and Clarithromycin

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Abstract

One simple and sensitive procedure (UV spectophotometric method) for the assay of two drugs namely terbinafine hydrochloride and clarithromycin in pure form and formulations. This method involves the formation of ion-association complex between TRB or CAM and the picric acid. In order to establish the optimum conditions necessary for rapid and quantitative formation of coloured product with maximum stability and sensitivity, the author performed experiments by measuring the absorbance at λ_{max} 350 nm of a series of solutions, varying one and fixing the other parameters in each case such as type, volume and concentration of acid, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, shaking time and temperature. The variable parameters were optimized. The results were statistically validated.

Keywords: Terbinafine hydrochloride; Clarithromycin; Spectrophotometer; Picric acid

Introduction

Terbinafine hydrochloride (TRB) [1-naphthalene methenamine N-[(2E) 6,6 dimethyl-2-heptene-4ynyl]-N-methyl] is a synthetic allylamine anti-fungal compound exists in the market in the form of tablets and cream. It is official in USP, Merck Index, and Martindale Extra Pharmacopoeia [1-3]. Clarithromycin or 6-0 methyl erythromycin or [2R, 3S, 4S, 5R, 6R, 8R, 10R, 11R, 12S, 13R)-3-(2,6-Dideoxy-3C, 3-0-dimethyl- α -L-ribo-hexopyranosyloxy)]-11,12-dihydroxy-6- methoxy, 2,4,6,8,10,12-hexamethyl-9-oxo- 5-(3,4,6-trideoxy-3-dimethylamino β -D-xylo- hexopyranosyloxy) pentadecan-13-olide. is a macrolide antibacterial hydroxylated macro cyclic lactone containing 12 to 20 carbon atoms in the primary ring bind to the 50 s sub units of bacterial ribosomes indicated to treat infections caused by bacteria. It is official in, USP, Merck index, Martindale's extra pharmacopoeia, Remington, PDR [1,3-5]. Existing analytical methods are reveal that little attention paid in developing the UV spectrophotometric methods for its determination. The present paper describes the determination of two drugs namely terbinafine hydrochloride and clarithromycin by reaction with the reagent picric acid.

Picric acid is a chemical known as symmetrical trinitrophenol and is acidic in nature and forms an adduct with aromatic hydrocarbons such as naphthalene and anthracene. It also forms picrate of the amine extractable into chloroform which is in a yellow colour but the absorption maxima are located in the UV region. Picric acid has also been used for the determination of reducing sugars as they undergo reduction to orange color picramic acid in alkaline medium. Picric acid is also proposed for the estimation of cardio glycosides [6-9].

This method involves the formation of Ion-association complex between TRB or CAM and the picric acid. In order to establish the optimum conditions necessary for rapid and quantitative formation of coloured product with maximum stability and sensitivity, the author performed experiments by measuring the absorbance at 350 nm of a series of solutions, varying one and fixing the other parameters in each case such as type, volume and concentration of acid, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, shaking time and temperature. Picric acid has also been used for the determination of reducing sugars as they undergo reduction to orange color picramic acid in alkaline medium. Picric acid is also proposed for the estimation of cardio glycosides.

Experimental

All spectral and absorbance measurements were made on a Systronics 106 model visible spectrophotometer with 1 cm matched glass cells or Milton Roy spectronic 1201 UV-visible spectrophotometer with 1 cm matched quartz cells.

All pH measurements were made on a Systronics 335 model digital pH meter or an Elico LI 120 digital pH meter.

Reagents and solutions

Picric acid solution: (Loba: 0.1%, 4.36×10^{-2} M): 100 mg of picric acid was dissolved in 100 ml of distilled water.

Buffer pH-9.8: Prepared dissolving 300 g of monosodium phosphate dihydrate and 9 g of NaOH in 750 ml of water.

Tebinafine hydrochloride drug solution: All the reagents were of analytical grade and all the aqueous solutions were prepared in double distilled water. Freshly prepared solutions were always used. One mg ml^{-1} stock solution of TRB HCl was prepared by dissolving 100 mg of TRB initially in 5 ml of 0.1 N HCl followed by dilution to 100 ml with double distilled water. For pharmaceutical formulations of the drug a quantity of tablet powder or cream equivalent to 100 mg of TRB HCl was treated with 4 × 20 ml portions of chloroform and the chloroform

extract was transferred to 100 ml volumetric flask and made upto 100 ml with chloroform to obtain 1 $mg.ml^{-1}$ stock solution. From this solution required concentrations are prepared for further experiments.

Clarithromycin drug solution: One mg ml^{-1} stock solution of CAM in aqueous medium was prepared by dissolving 100 mg of CAM in 5 ml of 0.1 M HCl followed by dilution to 100 ml with double distilled water. Tablet powdered equivalent to 100 mg of CAM was dissolved and diluted to 100 ml with chloroform and the insoluble portion was removed by filtration to get 1 $mg.ml^{-1}$. For pharmaceutical formulations of the drug twenty-five ml of above stock solution was taken and the chloroform portion was evaporated to dryness and the residue was initially dissolved in 2 ml of 0.1 N HCl followed by dilution to 25 ml with distilled water.

Recommended procedure

Method (Picric acid) for TRB or CAM: Into a series of 50 ml separating funnels containing aliquots of drug (TRB:0.5-3.0 ml, 50 $\mu g.ml^{-1}$; CAM:0.5-3.0 ml, 100 $\mu g.ml^{-1}$) solutions, 2 ml of pH 9.8 buffer and 1 ml of 0.1% picric acid solutions were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 10 ml with distilled water. To each separating funnel 10 ml of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate, and the absorbance of the separated chloroform layer was measured at 350 nm against a reagent blank prepared under similar conditions. The amount of drug was deduced from the calibration graph (Figures 1 and 2).

Pharmaceutical formulations for TRB HCl and CAM: A quantity of tablet powder or cream equivalent to 100 mg of TRB HCl was treated with 4 × 20 ml portions of chloroform and the chloroform extract was transferred to 100 ml volumetric flask and made up to 100 ml with chloroform to obtain 1 $mg.ml^{-1}$ stock solution.

Fifty milliliters of the above stock solution (1 $mg.ml^{-1}$) was taken and chloroform portion was evaporated to dryness and the residue was transferred to a 50 ml volumetric flask by dissolving it in 5 ml of 0.1 N HCl and diluted to the mark with distilled water.

Twenty-five ml of above stock solution was transferred in to a 25 ml separating funnel and washed with 5 ml 0.05 N NaOH to get the free base formed Twenty-five ml portion of this was evaporated to dryness and the residue was dissolved in 25 ml methanol to get 1 $mg.ml^{-1}$ stock solution.

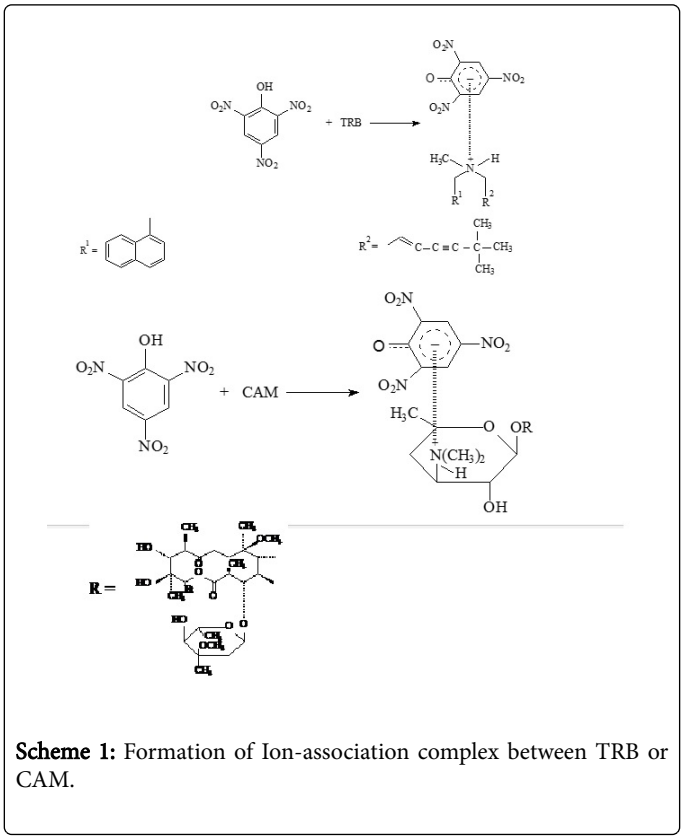
Ten ml of the above stock solution was taken, and chloroform portion was evaporated to dryness and the residue was initially dissolved in 2 ml of with glacial acetic acid and diluted to 10 ml with distilled water.

Tablet powdered equivalent to 100 mg of CAM was dissolved and diluted to 100 ml with chloroform and the insoluble portion was removed by filtration to get 1 $mg.ml^{-1}$.

Twenty-five ml of above stock solution was taken, and the chloroform portion was evaporated to dryness and the residue was initially dissolved in 2 ml of 0.1 N HCl followed by dilution to 25 ml with distilled water.

Results and Discussion

This method involves the formation of Ion-association complex between TRB or CAM and the picric acid. In order to establish the optimum conditions necessary for rapid and quantitative formation of coloured product with maximum stability and sensitivity, the author performed experiments by measuring the absorbance at 350 nm of a series of solutions, varying one and fixing the other parameters in each case such as type, volume and concentration of acid, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, shaking time and temperature. The method involves the coloured species formation is as shown in Scheme 1 as shown below.



Scheme 1: Formation of Ion-association complex between TRB or CAM.

Fixation of optimum conditions for the proposed methods and optical characteristics as described below: The picric acid complex was prepared in solution as under recommended procedures given in given above and then extracted in to chloroform. After separation of chloroform and aqueous layers, the chloroform layer was collected in each case and scanned in the wavelength region 200-400 nm against a reagent blank and the results are shown graphically in Figure 1. The λ_{max} value was found to be 350 nm in UV region. The λ_{max} value of picric acid in aqueous phase was almost the same by the complex in the organic phase.

- In order to test whether each one of the drug with specified reagent adheres to Beer's law, the absorbance at appropriate wavelengths of a set of solutions containing varying amounts of drug and specified amounts of reagents and treated as described in the corresponding recommended procedure were noted against appropriate reagent blank. (The Beer's law plots of the systems are presented graphically in Figures 2 and 3. The Beer's law limits of each drug with appropriate reagents were calculated in $\mu g.ml^{-1}$ and the results are incorporated in tables. Detection limits, molar absorptivity, Shandell's sensitivity and optimum photometric range for each drug with mentioned reagents were calculated and also recorded.

The regression analysis using the method of least squares was made for the slope (b), standard deviation on slope (S_b), intercept (a),

standard deviation on intercept (S_a), standard error of estimation (S_e) and correlation coefficient (r) obtained from different concentrations of each drug and the results are also summarized in Tables 1 and 2.

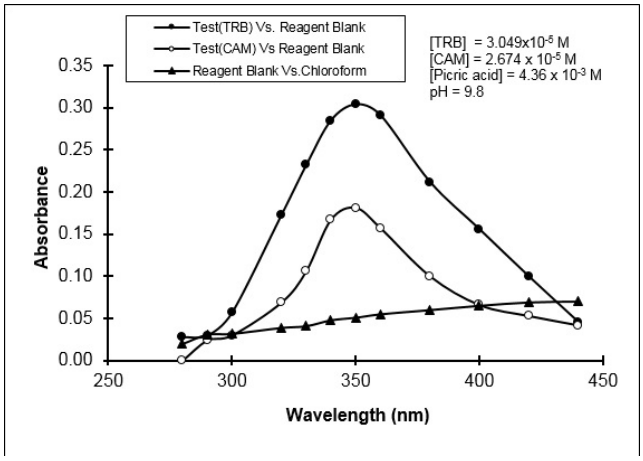


Figure 1: Absorption spectrum of TRB and CAM-picric acid.

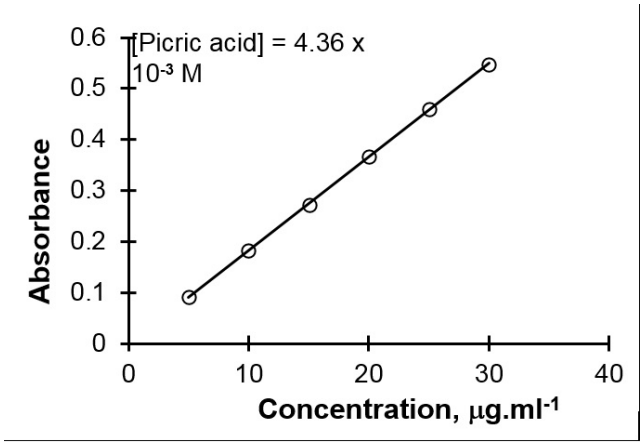


Figure 2: Beer's law plot of TRB-picric acid M1.

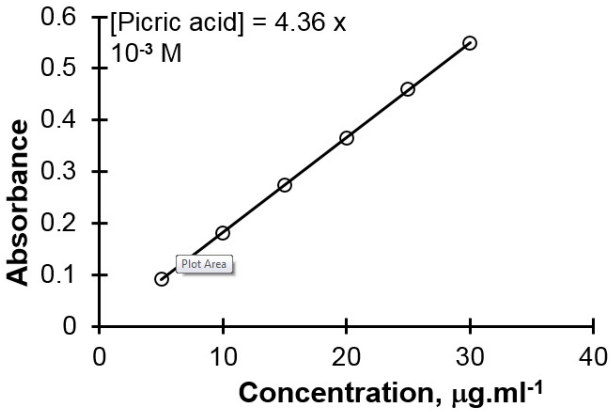


Figure 3: Beer's law plot of CAM-picric acid M2.

Parameter	MTRB	MCAM
	Picric Acid	Picric Acid
λmax (νμ)	350	350
Beer's Law Limits (mg.ml^{-1})	2.5-15	5-30
Detection limit (mg.ml^{-1})	1.299 × 10^{-1}	0.2737
Molar absorptivity (mole^{-1}cm^{-1})	9.968 × 10^{3}	1.353 × 10^{4}
Shandell's sensitivity (mg.cm^{-2} / 0.01 absorbance unit)	3.289 × 10^{-2}	5.52 × 10^{-2}
Regression equation (y=a+bc)		
Slope (b)	3.013 × 10^{-2}	1.829 × 10^{-2}

Standard deviation on slope (Sb)	1.34×10^{-4}	8.571×10^{-5}
Intercept (a)	4.666×10^{-4}	-1.2666×10^{-3}
Standard deviation in intercepts (Sa)	1.305×10^{-4}	1.668×10^{-3}
Standard error of estimation (Se)	1.403×10^{-3}	1.7928×10^{-3}
Correlation coefficient (r)	0.9999	0.9999
Relative standard deviation (%)*	0.3843	0.4034
% rang of error (confidence limits)*		
0.05 level	0.4034	0.4234
0.01 level	0.6330	0.6641
%Error in bulk samples**	0.2302	0.2739

Table 1: Optical and regression characteristics, precision and accuracy of the proposed methods for TRB and CAM. *Average of six determinations considered, **Average of three determinations.

Formulations*	Labeled Amount mg)	Amount found by proposed methods **		Reference Method for TRB	Reference method for CAM	% Recovery by proposed methods***	
		Picric acid for CAM	Picric acid for TRB	Picric acid for CAM		Picric acid for TRB	
Tablets	125	123.47	123.89	124.36 ± 0.72	124.04 ± 0.96	98.78 ± 0.92	99.11 ± 0.764
		± 1.15	± 0.766				
		F=1.43	F=1.74				
		t=1.01	t=1.32				
Tablets	250	248.7	248.72	249.52 ± 0.82	248.7 ± 2.22	99.48 ± 0.77	99.48 ± 0.458
		± 1.94	± 1.45				
		F=1.30	F=1.94				
		t=0.005	t=1.155				
Cream	TRB 10	248.17	9.98	9.99 ± 0.016	248.4 ± 2.00	99.26 ± 0.63	99.89 ± 0.189
		± 1.58	± 0.019				
Tablet	CAM 250	F=1.54	F=1.34				
		t=0.27	t=1.0				
Cream	TRB 250	496.18	247.5	247.86 ± 3.09	498.7 ± 2.22	99.23 ± 0.36	99 ± 1.454
		± 1.84	± 3.63				
Tablet	CAM 500	F=1.44	F=1.37				
		t=1.09	t=1.174				

Table 2: Assay of CAM and TRB in Pharmaceutical Formulations. * Formulations from four different pharmaceutical companies. ** Average +standard deviation on six determinations, the t- and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F=5.05, t=2.57. *** Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations).

Conclusion

An important step in the validation process of spectrophotometric method is the fixation of appropriate system suitability parameters to ensure successful analysis. They defined as a range of acceptance values for a series of key parameters such as precision, accuracy, linearity of detector response and recovery as deemed appropriate for the scope of analysis.

The validity of the proposed methods for the determination of a fore mentioned drugs were established from the precision (calculating percent relative standard deviation, percent range of error at confidence limits with P=0.05 and 0.01 level from six determinations) and accuracy (percent error in pure samples, comparison of results obtained with proposed and reported methods in the case of pharmaceutical preparations and recovery experiments) studies. The sensitivity of each method was ascertained through molar extinction coefficient, Shandell's sensitivity, optimum photometric range beer's law limits. The regression analysis using the method of least squares was made for the slope (b), standard deviation (S_b), intercept (a), standard deviation on intercept (S_a), standard error of estimation (S_e) and correlation coefficient (r) obtained from different concentrations. The data obtained in the determination of each drug with different reagents are summarized in this section. The selectivity (or specificity) of each proposed method was ascertained through interference studies with other active and inactive ingredients usually present in pharmaceutical preparations.

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