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Research Article

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Spectrophptometric Analysis of Clathromycin with Tpooo, Ars and WFB

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Abstract

One simple and sensitive procedure (simple spectrophotometric method) for the assay of drug clarithromycin in pure form and formulations. This method involves the formation of ion-association complex between CAM and the TPOOO, ARS and WFB. 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} 480nm,420nm and580nm of respective 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: Clarithromycin, Spectrophotometer and TPOOO, ARS,WFB

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 lactones containing 12 to 20 carbon atoms in the primary ring bind to the 50s 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 [1-4]. Existing analytical methods are reveal that little attention paid in developing the spectrophotometric methods for its determination.

In the visible spectrophotometry (colourometry), both the oxidant and analyte being used are present at very low concentrations and the reaction rate is 1/1000 to 1/100000th of the rate, at the concentration commonly used. This magnifying of the time scale confers some selectivity to oxidants and makes it possible to oxidize certain compounds specifically in the presence of other more stable compounds. Useful differences in reaction rate, however will exist only between compounds in different structural classes (basic moiety, functional groups present or both differ), but not between the compounds in the same classes. Selectivity can also be attained by using different oxidants and by varying the experimental conditions, but the suitability of an oxidant depends upon the associating ingredients. The mentioned oxidants are selective as they react only with certain functional groups under controlled experimental conditions.

Existing analytical methods are revealing that little attention paid in developing the visible spectrophotometric methods for its determination. The present paper describes the determination of the drug namely clarithromycin by reaction with the reagent TPOOO, alizarin red S (ARS) and WFB by exploiting its structural features of tertiary amine.

Experimental

Instrumentation

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.

Standard Solution of Clarithromycin Method M1

One mg ml^{-1} stock solution of CAM in aqueous medium was prepared by dissolving 100 mg of CAM in 5 ml of 0.1M HCl followed by dilution to 100 ml with distilled water.

Pharmaceutical Formulations

Sample Stock Solution

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 method.

Recommended Procedures

Method1 TPOOO for CAM

Into a series of 100ml separating funnels containing aliquots of drug (CAM: 0.5-3.0 ml, 50 $\mu g.ml^{-1}$;) solutions, 6.0ml of 0.1M HCl and 2.0ml of (5.709×10^{-3}M) TPOOO solutions were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. To each separating funnel, 10.0ml of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of separated chloroform layer was measured at 480 nm. Against a similarly prepared reagent blank. The amount of drug was calculated from the calibrated curve (Figure 1).

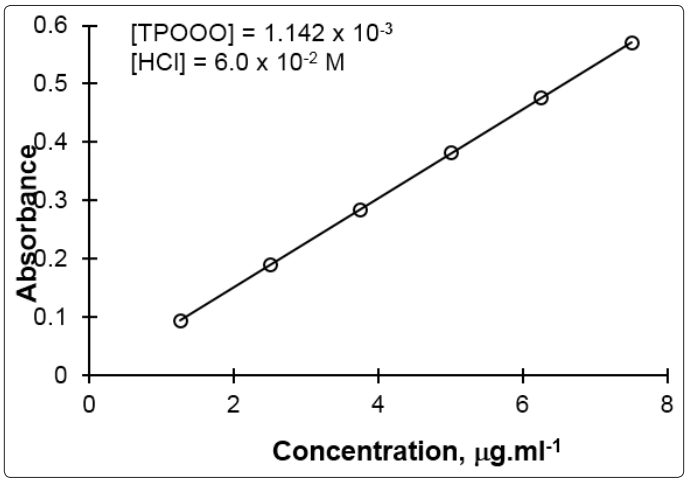


Figure 1: Method M_2 (Alizarin Red S) for CAM

Into series 100 ml separating funnels containing aliquots of drug (CAM: 0.5-3.0ml, 50 $\mu g.ml^{-1}$;) solutions, 2.0 ml of 0.1M HCl and 2.0 ml of (5.68×10^{-3}M) alizarin red S solutions were added. The aqueous layer was brought 15.0ml with distilled water. Then 10ml of chloroform was added to each separating funnel and shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 420 nm. Against a reagent blank and the amount of the drug was computed from its calibrated graph (Figure 2)

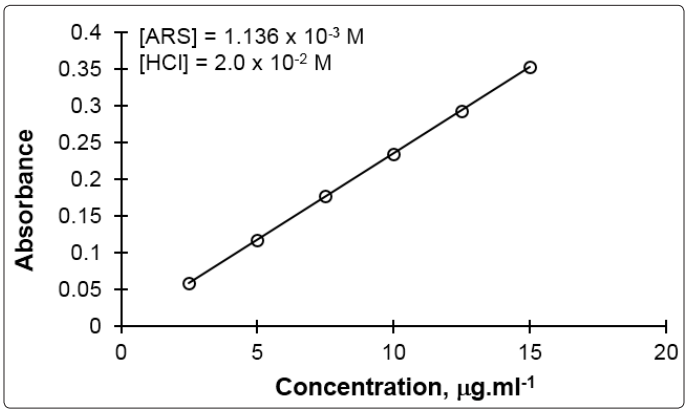


Figure 2: Method M_3 (WFB) for CAM

Into a series of 100ml separating funnels containing aliquots of standard drug (CAM: 0.5-3.0 ml, 50 $\mu g.ml^{-1}$;) solutions 6.0 ml of buffer solution (pH 1.5) and 2.0 ml of (3.26×10^{-3}M) WFBBL solutions were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. To each separating funnel 10.0ml of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of separated chloroform layer was measured at 580nm against a reagent blank prepared under similar conditions. The amount of the drug was deduced from the calibration graph (Figure 3).

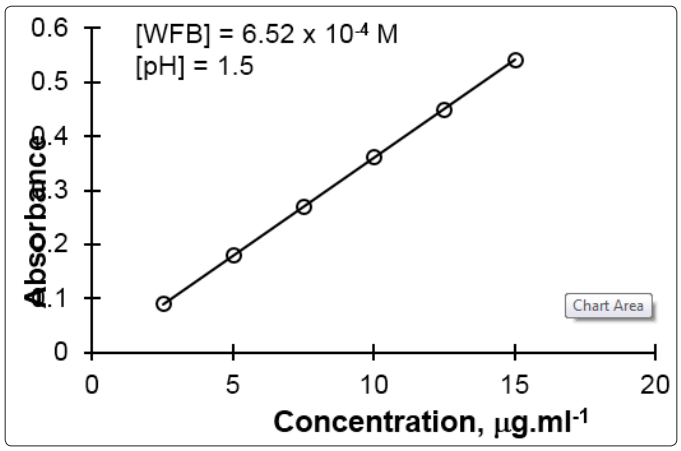
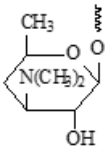


Figure 3

Results and Discussion

This method involves the formation of Ion-association complex between CAM and the dyes TPOOO, ARS (Alizarine Red S) and WFB. 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 480nm, 420nm and 580nm 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 Methods M1-M3.

Preliminary investigations were carried out using three acidic dyes (TPOOO, ARS and WFBBL) by extraction spectrophotometric technique for the assay of the drug clarithromycin [5-7]. The chemical features of dyes used in ion association complex formation are verified. The sensitivity and selectivity of the ion-association complex formation method depends upon the structural features of drug and dye. The involved basic moiety portions in the drug is



(Tertiary amine, CAM), the ion-association complex formation products of preferred methods (based on λ_{max} and ε_{max} values).

Spectral Characteristics of the Proposed Methods

I. Ion association complex formation

(1) Method M_1 for CAM, M_2 for CAM, and M_3 for CAM.

It was found that each dye is extractable from the aqueous phase into organic phase only in the presence of drugs (CAM) under experimental conditions. The drug- dye complexes were separately prepared for each dye in solution as under recommended procedures given in as above and then extracted into chloroform. After separation of chloroform and aqueous layers, the chloroform layer was collected in each case and scanned in the wavelength region 400-700nm against a reagent blank and the results are shown graphically in Figs. 1,2and 3. The λ_{max} values were found to be 480nm for M_1, 420nm for M_2 and 580nm for M_3. The λ_{max} value of each dye in aqueous phase was almost the same as the complex in organic phase.

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 (Table 1 & 2).

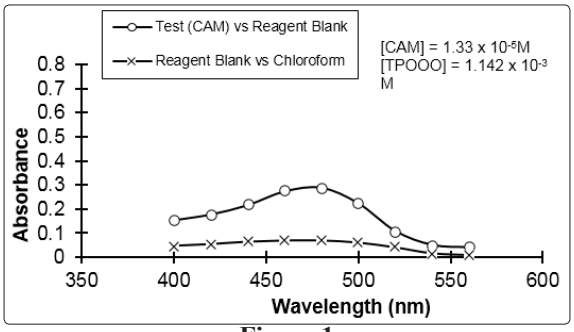


Figure 1

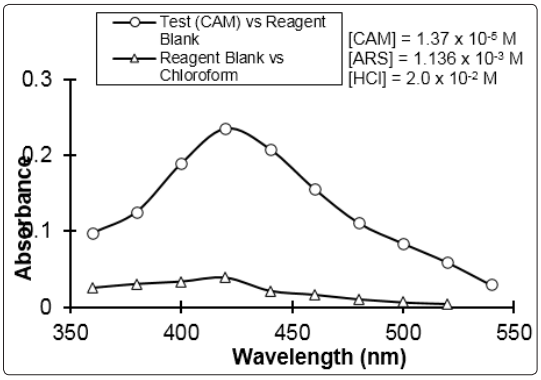


Figure 2

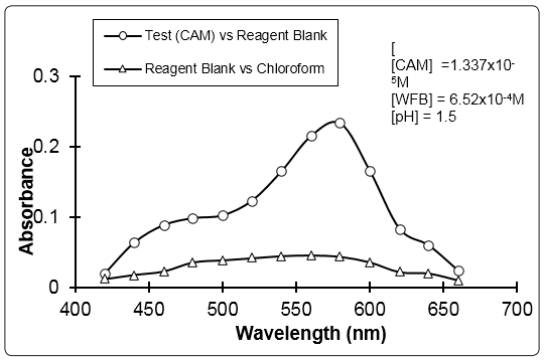


Figure 3

Table 1: Optical and Regression Characteristics, PRECISION and Accuracy oF the proposed methods For CAM

Parameter	M1 TPOOO	M2 ARS	M3 WFB
λ_{max} (nm)	480	420	580
Beer's Law Limits (µg. $m1^{-1}$)	1.75-50	2.0-15.0	2.5-15
Detection limit (µg.$m1^{-1}$)	$8.769x10^{-2}$	$7.216x10^{-2}$	$3.902x10^{-2}$
Molar absorptivity (mole-$1cm^{-1}$)	$2.16x10^{4}$	$1.757x10^{4}$	$2.10891x10^{4}$
Sandell's sensitivity (µg.cm^{-2} / 0.01 absorbance unit)	$3.44x10^{-2}$	$4.25x10^{-2}$	$2.762x10^{-2}$
Optimum photometric rang (µg.$m1^{-1}$)			
Regression equation (y=a+bc)			
Slope (b)	0.02882	$2.358x10^{-2}$	$3.617x10^{-2}$
Standard deviation on slope (S_b)	$8.653x10^{-5}$	$5.826x10^{-5}$	$4.833x10^{-3}$
Intercept (a)	$4.666x10^{-4}$	$-4.0x10^{-4}$	$6.666x10^{-4}$
Standard deviation in intercepts (S_a)	$8.4246x10^{-4}$	$5.672x10^{-4}$	$4.705x10^{-5}$
Standard error of estimation (S_e)	$9.05x10^{-4}$	$6.094x10^{-4}$	$6.346x10^{-4}$
Correlation coefficient (r)	0.9998	0.9999	0.9999
Relative standard deviation (%)*	0.1776	0.3191	0.5066
% rang of error (confidence limits)*			
0.05 level	0.1864	0.335	0.5316
0.01 level	0.2923	0.5255	0.8341
% Error in bulk samples**	-0.1034	0.2553	0.2592

*Average of six determinations considered
**Average of three determinations

Table 2: Assay of CAM in Pharmaceutical Formulations

Formulations*	Labelled amount (mg)	Amount found by proposed methods **			Reference	% Recovery by proposed methods***				
		M_1 TPOOO	M_2 ARS	M_3 WFB	Method	M_1 TPOOO	M_2 ARS	M_3 WFB	M_3 Picric acid	M_4 CTC
Tablets	125	123.99 ±0.99	123.99 ±0.87	124.00 ±1.030	124.04 ±0.96	99.19 ±0.72	99.19 ±0.69	99.20 ±0.83	98.78 ± 0.92	99.04 ±0.74
		F = 1.13	F = 1.21	F = 1.16						
		t = 0.07	t = 0.03	t = 0.03						
Tablets	250	248.65 ±2.33	248.46 ±1.59	247.38 ±1.61	248.7 ±2.22	99.46 ±0.91	99.38 ±0.63	98.95 ±0.64	99.48 ± 0.77	99.09 ±0.90
		F = 1.10	F = 1.92	F = 1.89						
		t = 0.02	t = 0.15	t = 0.61						
Tablets	250	247.98 ±1.80	248.23 ±2.20	247.7±2.43	248.4 ±2.00	99.19 ±0.72	99.29 ±0.88	99.10 ±0.97	99.26± 0.63	99.29 ±0.63
		F = 1.23	F = 1.21	F = 1.48						
		t = 0.67	t = 1.40	t = 0.27						
Tablets	500	492.69 ±2.87	498.11 ±3.26	494.76 ±3.22	498.7 ±2.22	98.53 ±0.57	99.62 ±0.65	98.95 ±0.64	99.23 ± 0.36	98.89 ±0.63
		F = 1.67	F = 2.15	F = 2.11						
		t = 2.15	t = 0.99	t = 1.28						

*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).

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