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Spectrophotometric Determination of Amiodarone and Ondansetron with Precipitation Reagents

T. SIVA RAO, P.S.N.H. RAMACHANDRA RAO, U.VIPLAVA PRASAD and C.S.P. SASTRY*

Department of Organic Chemistry, Foods, Drugs and Water
Andhra University, Visakhapatnam-530 003, India

Amiodarone (AD) and ondansetron (SOT) form insoluble molecular complexes with iodine (I_2, method A), ammonium molybdate (AM, method B) or phosphomolybdic acid (PMA, method C) under acid conditions. In addition, colour reactions have been combined to estimate the precipitants (in turn AD or OST). They are based on the colour formation with either unreacted precipitant in the filtrate (I_2) or released precipitant from the precipitate (AM or PMA) with chromogenic reagents such as *p*-N-methyl amino phenol sulphate-sulphanilic acid (PMAP-SAc, λ_{max} 520 nm for I_2, method A), potassium thiocyanate (SCN^-, λ_{max} 465 nm for AM, method B) or cobalt nitrate-ethylene diamine tetraacetic acid disodium salt complex (CoII-EDTA, λ_{max} 840 nm for PMA, method C). The methods obey Beer's law and the precision and accuracy of the methods were checked by UV reference methods; standard deviations were typically ≤ 0.584. Recoveries were > 99.33%. Regression analysis of Beer's plots showed good concentration ranges (2–12, 0.8–4.8), (4–24, 5–30) and (20–100, 8–48) $\mu g\ mL^{-1}$ for AD or OST in methods A, B and C respectively. The applicability of the methods was examined by analysing tablets of AD or OST.

Key words: Spectrophotometric, determination, amiodarone, ondansetron

INTRODUCTION

Amiodarone hydrochloride (AD) is a class III anti-arrhythmic drug and is chemically known as 2-butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone. Ondansetron (OST) is an antinauseant and antiemetic and is chemically known as 1,2,3,9-tetrahydro-9-methyl-3-(2-methyl-1H-imidazol-1-yl) 4H-carbazol-4-one, monohydrochloride. Amiodarone is official in IP[1], while ondansetron is official in USP[2]. A number of methods such as HPLC (OST)[3–6], mass spectroscopy (OST)[7,8] UV (AD)[9] and visible spectrophotometric (SOT)[10–12] are reported in the literature. No visible spectrophotometric method

**Address for correspondence*: Prof. C.S.P. Sastry, D.No. 9–36–4, Opp. N.C.C. Office, Andhra Bank Road, Pithapuram Colony, Visakhapatnam-530 003, India.

for the determination of AD has been reported. The aim of the present work is to provide simple and sensitive visible spectrophotometric methods for the estimation of AD or OST which are basic in bulk and formulations. The efforts in this accord resulted in developing the present methods.

AD or OST undergo quantitative precipitation in the form of molecular complexes with iodine (I_2, method A), ammonium molybdate (AM, method B) or phosphomolybdic acid (PMA, method C) when used in excess. In addition to precipitation reactions, colour reactions have also been combined to estimate AD or OST. They are based on the colour formation with either unreacted precipitant in the filtrate (I_2) or released precipitant from the molecular complex (AM or PMA) with chromogenic reagents such as PMAP-SAc (for I_2)[13], SCN^- (for AM)[14], Co(II)-EDTA complex (for PMA).

EXPERIMENTAL

A Milton Roy Spectronic 1201 and a Systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for the spectral and absorbance measurements in the UV and visible regions respectively.

All the chemicals and reagents used were of analytical grade and the solutions were prepared freshly. Aqueous solution of I_2 (E. Merck, 3.5×10^{-3} M), in 5.35×10^{-2} M of KI, PMAP (Loba, 5.81×10^{-2} M), SAc (Sd. Fine, 2.31×10^{-2} M), HCl (E. Merck, 1 M) for method A; AM (E. Merck, 1.62×10^{-2} M), KCNS (Ranbaxy, 1.029 M), conc. HCl (used as it is) for method B; PMA (Rea Chem. 4%), cobalt nitrate (BDH, 1.03×10^{-1} M), EDTA disodium salt (Sd. Fine, 1.07×10^{-1} M) for method C; 0.01 M HCl for methods B and C were prepared in triply distilled water.

Standard drug solutions: A 1 mg mL^{-1} solution was prepared by dissolving 100 mg of drug (AD or OST) in 100 mL of 0.01 M HCl and this stock solution was diluted stepwise with 0.01 M HCl to obtain the working standard solutions of concentrations for AD (100 μg mL^{-1}, method A; 200 μg mL^{-1}, method B; 1000 μg mL^{-1}, method C) and OST (40 μg mL^{-1}, method A; 250 μg mL^{-1}, method B; 400 μg mL^{-1}, method C).

Sample solutions: An accurately weighed amount of tablet powder equivalent to 100 mg of drug (AD or OST) was dissolved in few mL of 0.01 M HCl and filtered. The volume of filtrate was made up to 100 mL with 0.01 M HCl to get 1 mg mL^{-1} solution. This solution was diluted stepwise with 0.01 M HCl to get working sample solutions and analysed as under procedures described for bulk samples.

Analysis of bulk samples

Method A: Aliquots of the standard drug solution (0.5–3.0 mL, 100 μg mL^{-1} for AD or 0.5–3.0 mL, 40 μg mL^{-1} for OST) were transferred into a series of centrifuge tubes. Then 2 mL of 1 M HCl and 2 mL of iodine were added successively. The volume was made up to 7 mL with distilled water and kept

aside for 15 min and centrifuged for 5 min. The precipitate was collected through filtration and subsequently washed with 2 mL of distilled water. The filtrate and washings were collected in a 25 mL graduated test tube. Then 3.0 mL of PMAP and 2 mL of SAc solutions were added successively and the volume was made up to the mark with distilled water. The absorbance was measured after 25 min at 520 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance and in turn the drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of drug present was calculated from its calibration graph.

Method B: Aliquots of standard drug solution (0.5–3.0 mL, 200 μg mL^{-1} for AD or 0.5–3.0 mL, 250 μg mL^{-1} for OST) were delivered into a series of centrifuge tubes and the volume in each tube was adjusted to 3.0 mL with 0.01 M HCl. 1 mL of ammonium molybdate was added and centrifuged for 5 min. The precipitate was collected through filtration followed by washing with 50% alcohol until it was free from the reagent. The precipitate in each tube was dissolved in 5 mL of acetone and transferred into a 25 mL graduated test tube. Then 5 mL of concentrated HCl and 3 mL of potassium thiocyanate were successively added. The test tubes were kept aside for 20 min at laboratory temperature. The solution in each tube was made up to the mark with distilled water. The absorbance was measured at 465 nm against a similar reagent blank. The amount of the drug was calculated from Beer's law plot.

Method C: Aliquots of standard drug solution (0.5–3.0 mL, 1000 μg mL^{-1} for AD or 0.5–3.0 mL, 400 μg mL^{-1} for OST) were delivered into a series of centrifuge tubes and the volume in each tube was adjusted to 3.0 mL with 0.01 M HCl. Two mL of phosphomolybdic acid was added and centrifuged for 5 min. The precipitate was collected through filtration followed by washing with distilled water until it is free from the reagent. The precipitate in each tube was dissolved in 5 mL of acetone and transferred into a 25 mL graduated test tube. 1 mL of cobalt nitrate and 1 mL of EDTA solutions were successively added and the tubes were heated for 12 min at 60°C. The test tubes were cooled and the solution in each tube was made up to the mark with distilled water. The absorbance was measured at 840 nm against a similar reagent blank. The amount of the drug was calculated from the calibration graph.

RESULTS AND DISCUSSION

The optimum conditions for the maximum colour development of each method A, B or C were established by varying the parameters one at a time, keeping the others fixed in both the steps (precipitation and colour formation) and observing the effect produced on the absorbances of the coloured species. The conditions so obtained were incorporated in the recommended procedures. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity for the method are given in Table-1. The precision of the method was found by measuring absorbance of six replicate samples containing known amounts of drugs and the results obtained are incorporated in Table-1.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS FOR AD AND OST

	Method A		Method B		Method C	
	AD	OST	AD	OST	AD	OST
λ_{max} (nm)	520	520	465	465	840	840
Beer's law limits ($\mu g\ mL^{-1}$, C)	2.0–12.0	0.8–4.8	4.0–24.0	5.0–30.0	20.0–100.0	8.0–48.0
Molar absorptivity ($1\ mole^{-1}\ cm^{-1}$)	2.73×10^4	5.58×10^4	1.81×10^4	8.42×10^3	3.64×10^3	4.31×10^3
Sandell's sensitivity ($\mu g\ cm^2$/0.001 absorbance unit)	2.5×10^{-2}	6.56×10^{-3}	3.77×10^{-2}	4.35×10^{-2}	1.87×10^{-1}	8.49×10^{-2}
Regression equation (Y)*						
Slope (b)	4.0×10^{-2}	1.53×10^{-1}	2.65×10^{-2}	2.3×10^{-2}	5.31×10^{-3}	1.17×10^{-2}
Intercept (a)	2.66×10^{-4}	1.33×10^{-4}	8.66×10^{-4}	2.66×10^{-4}	2.0×10^{-4}	8.0×10^{-4}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
Relative standard deviation (%)†	0.584	0.444	0.413	0.406	0.581	0.545
% range of error (confidence limits)†						
0.05 level	0.613	0.466	0.434	0.426	0.610	0.523
0.01 level	0.960	0.730	0.680	0.666	0.957	0.898
% error in bulk samples†	0.200	0.205	–0.070	0.087	–0.094	–0.265

*Y = a + bC, where C is concentration.
†six replicate samples.

Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and are presented in Table-1. The accuracy of the methods was ascertained by comparing the results[15] by proposed and reference methods (UV) statistically by the t- and F-tests (Table-2). The results of the recovery experiments by the proposed methods are listed in Table-2.

The comparison shows that there is no significant difference between the results of proposed methods and those of the reference one, while the similarity of the results is an obvious evidence that during the application of method the additives and excipients that are usually present in tablets do not interfere with the assay of proposed methods.

Coloured species formation: All the three proposed methods involve two steps. The first step is the quantitative precipitation of drug (AD or OST) with I_2, AM or PMA. The second step varies in each method. In method A, the unreacted I_2 in the filtrate oxidises PMAP to *in situ* and highly reactive *p*-N-methylbenzoquinone monoimine which in turn involves in coloured charge-transfer formation with SAc. In method B, the molybdate released from the precipitate with acetone when treated with KCNS in strong acid medium to get orange yellow coloured complex $[Mo(NCS)_6^{3-}]$. In method C, the PMA released with acetone undergoes reduction by Co(II)-EDTA to yield molybdenum blue.

TABLE-2
ASSAY OF AD AND OST IN PHARMACEUTICAL FORMULATIONS

Samples*	Labelled amount (mg/tab)	Amount found by proposed methods†			Reference method	% recovery by proposed methods‡		
		Method A	Method B	Method C	AD[9], OST[Δ]	Method A	Method B	Method C
I	100	99.77 ± 0.21	99.83 ± 0.16	99.83 ± 0.16	99.79 ± 0.24	99.77 ± 0.240	99.83 ± 0.160	99.83 ± 0.16
	(AD)	F = 1.21, t = 0.36	F = 2.25, t = 0.58	F = 2.25, t = 0.58				
	4	3.99 ± 0.0056	3.98 ± 0.015	3.99 ± 0.015	3.99 ± 0.01	99.85 ± 0.141	99.61 ± 0.292	99.68 ± 0.36
	(OST)	F = 3.23, t = 1.48	F = 2.25, t = 1.61	F = 1.96, t = 1.03				
II	100	99.5 ± 0.66	99.62 ± 0.50	99.62 ± 0.5	99.66 ± 0.356	99.50 ± 0.660	99.62 ± 0.50	99.62 ± 0.50
	(AD)	F = 3.56, t = 0.875	F = 1.98, t = 0.46	F = 1.97, t = 1.7				
	4	3.92 ± 0.31	3.99 ± 0.017	3.99 ± 0.0167	3.99 ± 0.011	99.81 ± 0.310	99.8 ± 0.330	99.76 ± 0.40
	(CST)	F = 1.33, t = 0.99	F = 2.38, t = 1.0	F = 2.33, t = 1.03				
III	200	199.55 ± 0.422	199.66 ± 0.32	199.66 ± 0.32	199.62 ± 0.33	99.77 ± 0.210	99.83 ± 0.160	99.83 ± 0.16
	(AD)	F = 1.64, t = 0.6	F = 1.03, t = 0.48	F = 1.06, t = 0.48				
	8	7.983 ± 0.014	7.97 ± 0.03	7.975 ± 0.29	7.97 ± 0.02	99.83 ± 0.240	99.69 ± 0.290	99.69 ± 0.36
	(CST)	F = 2.04, t = 1.84	F = 2.25, t = 0.5	F = 1.15, t = 1.14				
IV	200	199.58 ± 0.59	199.15 ± 0.96	199.15 ± 0.96	199.34 ± 0.715	99.44 ± 0.636	99.57 ± 0.480	99.84 ± 0.226
	(AD)	F = 1.46, t = 1.44	F = 1.35, t = 1.45	F = 1.0, t = 1.7				
	8	7.961 ± 0.027	7.954 ± 0.025	7.946 ± 0.034	7.95 ± 0.0.032	99.51 ± 0.420	99.40 ± 0.260	99.33 ± 0.42
	(CST)	F = 1.18, t = 0.76	F = 1.63, t = 0.3	F = 1.12, t = 0.76				

*Different batches of tablets from different pharmaceutical companies.

†Average ± standard deviation of six determinations, the t- and F-test values refer to comparison of the proposed method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.57

‡Recovery of 10 mg added to the preanalysed pharmaceutical formulations (average of three determinations)

[Δ]Developed in the laboratory using methanolic solution of OST whose λ_{max} is 266 nm.

Conclusions

The proposed methods are applicable for the assay of drug (AD or OST) and have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and the increasing order of λ_{max} among the proposed methods (A–C) for AD or OST are:

method A > method B > method C

and method B < method A < method C

or method A > method B > method C

and method B < method A < method C

respectively. As the formation of coloured species differs from one another in the proposed methods depending upon the nature of precipitant and the chromogenic reagent, the appropriate methods can be used for the assay of AD or OST in bulk form and tablets depending on the situation.

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