Asian Journal of Chemistry; Vol. 26, No. 2 (2014), 621-624

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16407

Ion Pair Colorimetric Estimation of Dronedrone HCl in Solid Dosages Using Methyl Orange

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Received: 21 September 2013; *Accepted*: 7 November 2013; *Published online*: 15 January 2014; AJC-14600

New ion pair colorimetric method has been developed for the estimation of dronedrone in bulk and in tablet formulation using methyl orange dye as an ion paring complexant using pH 4 buffer. The developed method has been validated for its sensitivity and specificity. The intensity of ion pair complex of dronedrone and methyl orange found linear over the range of 2-20 µg mL^{-1} with a correlation coefficient of 0.9958. The inter and intraday precision, accuracy, stability, LOD, LOQ, placebo interference, robustness and ruggedness were evaluated during validation. The % RSD found for the recovery of the method resulted with low value of about 0.72-0.99; and with good precision. From the results obtained the developed method was found suitable and comparable in terms of estimating dronedrone in formulation with that of existing methods.

Keywords: Dronedrone, Methyl orange, Ion association, Spectrophotometric.

INTRODUCTION

Dronedarone (DO) is an amiodarone group of anti-arrhythmic agent chemically known as N-[2-butyl-3-[4-(3-dibutyl-aminopropoxy)benzoyl]methane sulfonamide, hydrochloride]. Dronedrone is noniodinated benzofuran derivative with a sulfonamide group on the benzofuran ring (Fig. 1). The mechanism of action of dronedarone is close to amiodarone and its derivative. Both agents belong to all 4 Vaughan-Williams classes. These derivatives were used mainly for the treatment of cardiac arrhythmias and also for the treatment of atrial fibrillation and atrial flutter[1-7].

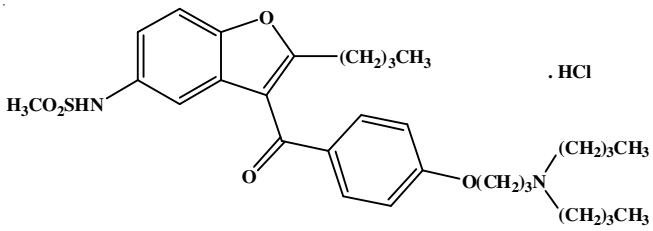

Fig.1. Structure of dronedarone HCl

The methods UV spectroscopic[8], HPLC[9], stability indicating HPLC[10-14], HPTLC[15] for the estimation of dronedrone in bulk and formulations and LC-MSMS method for its estimation in biological matrics were reported[16]. Until now none of colorimetric methods have been reported for its estimation in bulk and pharmaceuticals. Colorimetric methods are the choice of method when sophisticated instruments such as HPLC and LC-MSMS are not available. Further they are more selective than UV methods. Even though the HPLC and LC-MSMS methods are more sensitive to estimate the drug, they need very stringent control over separation, expensive solvents and time consuming while comparing to colorimetric methods. Colorimetric methods are affordable and attractive tool to estimate the drugs, in case of small scale laboratories and for academic labs where the sophisticated instruments are not available. Among the other colorimetric procedure, extractive colorimetric procedure was recently adopted by many pharmaceutical scientist due to their minimal solvent and reagents requirement. Hence in this present study, we reported a simple, selective, sensitive and reproducible ion pair colorimetric method for the estimation of dronedrone in bulk and pharmaceutical dosage forms and to validate the method by following ICH and USP[17,18].

EXPERIMENTAL

All chemicals used here were of analytical reagent grade procured from Daejung Chemicals & Metals. De ionized water was used to prepare all solutions throughout the study. methyl orange of concentration 0.05 % w/v was prepared using ethanol-water (1:9). Standard dronedrone was procured from Sigma Aldrich and tablets with 400 mg of dronedrone were purchased from a retail pharmacy.

All spectral analysis was carried using a Shimadzu UV mini-1240 UV-visible spectrophotometer (Japan) with 1 cm quartz cells and with Shimadzu UV Probe (version 2.1) system software. Neomet-Iste pH meter was used for pH measurements.

Standard solution of the drug: The standard stock solution of dronedrone was prepared by dissolving accurately weighed quantity of the drug in little methanol and further diluted to produce 1 mg mL^{-1}. Suitable serial dilutions were preferred to make working standards.

Sample preparation: To prepare samples with different concentration various quantity of 100 µg mL^{-1} working standard solution was transferred into a series of 100 mL separating funnels. To each of this funnel a definite quantity of (0.5 mL) of 0.05 % w/v methyl orange was added and shaken well to produce colored ion pair. Then the ion pair was extracted using 10 mL of chloroform by shaking the solution for few minutes and set aside for separation.

Assay of formulations: From the available marketed formulation twenty tablets were taken and weighed, average weight of tablet was calculated and made into fine powder. From that a quantity of powder to make definite dilution was accurately weighed into a 50 mL volumetric flask. Then little amount methanol and water was added to dissolve the drug in the powder. Finally the volume was made up with water, shaken well and the insoluble diluents contained in the powder were filtered through a whatman filter paper No. 40 and used for final dilution.

RESULTS AND DISCUSSION

The full scan absorption spectrum of the yellow dronedrone-methyl orange (DO-MO) ion-pair was obtained by scanning the chromogen after chloroform extraction from 400-800 nm. The results of the over lay spectra is presented in Fig. 2. A maximum absorbance (λ_{max}) was noted at 422 nm and the same is used for further studies of estimation.

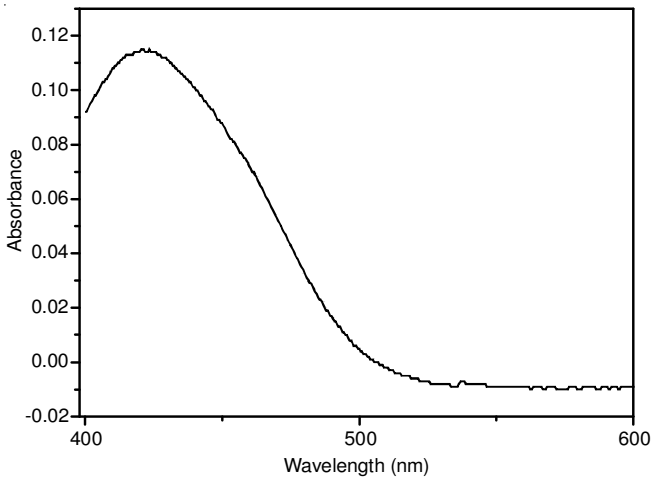

Fig. 2. Full scan absorption spectra of DO-MO ion-pair complex

Validation of the method: The ICH[17] and USP[18] guidelines were followed for method validation after an initial method optimization. From the trials it was noted that formation of ion pair needs about 0.5 mL of 0.05 % wt/V methyl orange and 4 mL of pH 4 phosphate buffer. Further from various trials it was verified that chloroform was the best solvent for extraction while compared with other tested organic solvents.

Linearity and range: Beer's law linearity and molar absorptivity were determined and the results are given in Fig. 3 and Table-1. A calibration curve of absorbance *vs.* concentrations was plotted (µg mL^{-1}) to know the Beer's law limit. The results of regression equation is given as follows:

$$A = 0.0158x - 0.0101 \quad (r = 0.9958)$$

where A, the absorbance at 422 nm, x, concentration of dronedrone in µg mL^{-1} and r, correlation coefficient. The molar absorptivity (ε) was found to be 1.3871×10^4 L mol cm^{-1}. The Sandell's sensitivity was also determined and presented in the same Table-1. Job's continuous variation method was used to study the drug-dye stoichiometric ratio and was determined that the dronedrone, methyl orange forms a 1:4 complex[19].

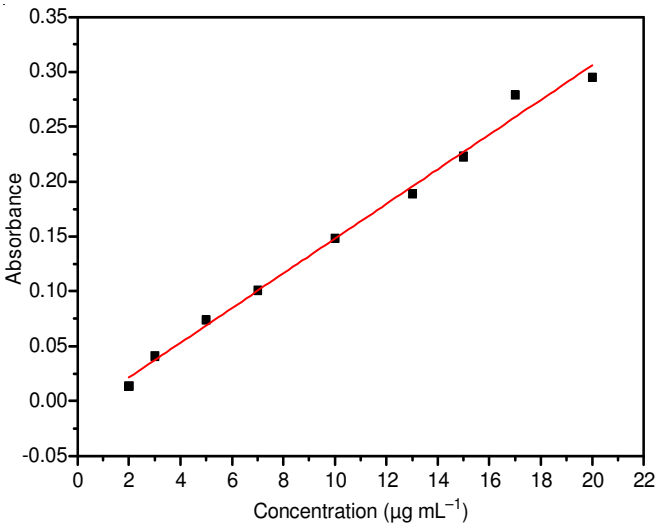

Fig. 3. Beer's law standard plot

TABLE-1
OPTICAL PROPERTY OF THE CHROMOGEN

Parameters	Values
Beer's law limit	2-20 µg mL^{-1}
Molar absorptivity (ε) (lit mol cm^{-1})	1.3871×10^4
Sandell's sensitivity (µg cm^{-2}/0.001 abs unit)	0.06757
Regression equation	A = ax + b
Slope (a)	0.0158
Intercept (b)	-0.010
Standard error on slope	0.0065
Standard error on intercept	5.4688×10^{-4}
Correlation coefficiency (r)	0.9958

LOD and LOQ: To determine the method sensitivity towards the concentration limit of detection (LOD) and limit of quantitation (LOQ) were established using the formula: LOD or LOQ = K σa/b, where K = 3 for LOD and 10 for LOQ, 'σ' is the standard deviation of intercept (a) and slope (b). The LOD and LOQ were 0.009 and 0.08 µg mL^{-1}, respectively.

Application of the proposed method to formulation: The method was successfully applied to the analysis of the bulk drug. From the results mean recovery value was found to

be 99.88 ± 0.57 %. This proves the suitability of the method to determine dronedrone in bulk. To check the applicability of the proposed method to pharmaceutical formulation the assay of marketed tablets dosage was carried by proposed method and reported UV method[8]. About 99.98 and 99.48 % assay results were obtained with proposed and reported methods, respectively to that of label claim. Student *t*- and *F*-test were used for the statistical comparison of proposed and reported methods. From that there were no significant differences detected between the calculated and theoretical values at 95 % confidence level which prove comparability method with that of reference method (Table-2).

Precision of the method (repeatability): To determine the intra-day assay precision six fold replicate analysis of sample on the same day was carried out. For the inter-day precision determination, analysis of the same sample examined for 5 successive days. The percentage relative standard deviation (% RSD) values were found to be 0.765 and 0.891 for inter, intra-day precision respectively, these evidencing the repeatability (precision) of the method (Table-3).

Method recovery (accuracy): This was attained by standard spiking procedure, *i.e*. a known quantity of standard drug was spiked to the pre-analyzed sample and the recovery estimation was carried out by the proposed method. The results of recovery studies are given in Table-3. About 0.72-0.99 % mean % RSD found at three spiking levels. Further, these results were within the acceptance limit for accuracy (< 2 % RSD).

Study on methyl orange concentration and quantity: The effect of the methyl orange was studied by measuring the absorbance of solutions containing dronedrone (12 μg mL^{-1}) and 0.5 mL of methyl orange solution at various concentration (0.025-0.15 % wt/v). The results are described in Fig. 4(a) evidences about 0.05 % wt/V of methyl orange gave a good maximum absorbance hence, it was chosen as suitable concentration for complexation. Regarding the quantity methyl orange needed, the volume of the dye added was varied form 0.2-1.2 mL by maintaining the dye concentration at 0.05 % wt/v with 10 μg mL^{-1} drug's concentration Fig. 4(b). From the results obtained it was recognized that 0.05 mL of 0.05 % wt/V methyl orange is sufficient to make good ion pair with maximum color intensity. There were any marked effect on intensity was noted above 0.05 mL methyl orange on intensity of color.

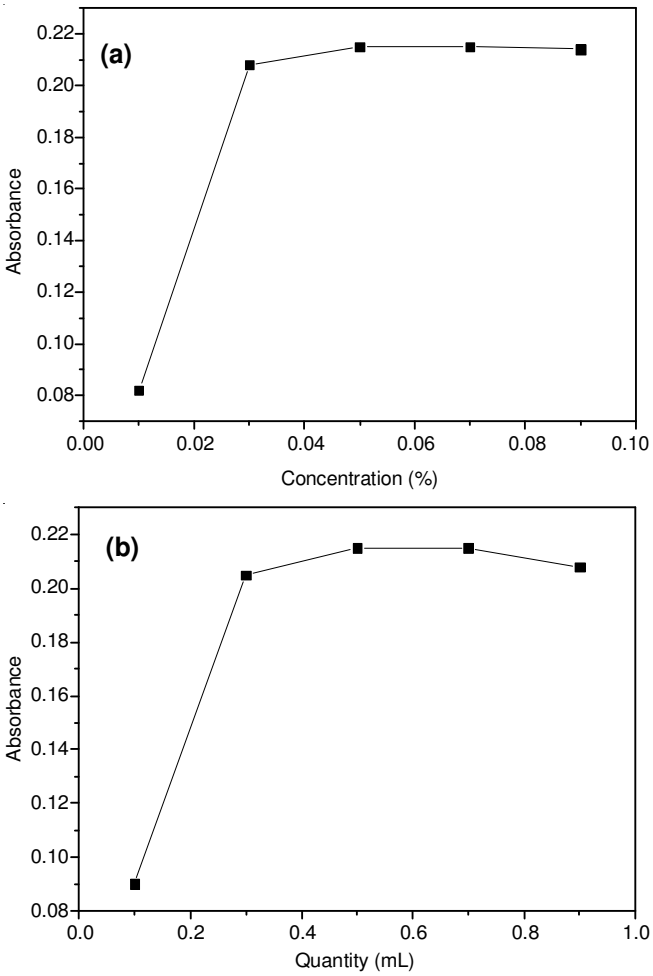

Fig. 4. (a) Effect of methyl orange concentration (b) Effect of methyl orange quantity

Study of interference and placebo study: Studies on interference by common excipients that might be added during formulations were carried by mixing known amount of dronedrone (100 mg) with about 10 mg of the common excipients such as lactose, starch and magnesium stearate then dronedrone's recoveries were calculated. Form the results about 98.95 ± 0.748, 99.65 ± 0.38 and 99.50 ± 0.455 were obtained

TABLE 2
RESULTS OF ASSAY

Sample	Label claim (mg/tab)	% Amount[a]		% RSD[a]		Confidence	
		Proposed	Reported	Proposed	Reported	*t*-test[b]	*F-test*[b]
I	400	99.98	99.48	0.79	0.98	1.4	2.32

[a] Mean of six determinations, [b] The tabulated values of *t* and *F* at 95 % confidence limit are 2.67 and 6.02 respectively

TABLE-3
RESULTS OF PRECISION AND ACCURACY

Conc. (μg mL^{-1})	Precision				Recovery		
	Inter-day		Intra-day		% Spike level	% Recovered[b]	% RSD[b]
	Amt found (μg mL^{-1})[a]	% RSD[a]	Amt found (μg mL^{-1})[a]	% RSD[a]			
10	10.95	0.765	10.87	0.891	50	99.895	0.99
					100	99.567	0.85
					150	100.121	0.72

[a] Mean of six determinations, [b] Mean of five determinations

TABLE-4.
RESULTS OF ROBUSTNESS AND RUGGEDNESS

Wavelength (nm)	Robustness				Ruggedness		
	% RSD*	Conc. methyl orange (% w/v)	% RSD*	Analyst	% RSD*	System	% RSD*
420	0.452	0.03	0.865	I	0.345	I	0.782
422	0.321	0.07	0.958	II	0.421	II	0.565

*Mean of five replicated determination.

for lactose, starch and magnesium stearate, respectiviely. Form that it is clear that the excipients do not show any interference in the estimation of the drug. Similarly only placebo mixture was prepared and the procedure was followed and there was no color observed in the extract revealed the selectivity of the present method for the analyte of interest.

Bench top stability of ion pair: To study the stability of chromogen, specified quantity of stock was mixed with above optimized quantity of methyl orange and extracted. Then the absorbance of the chromogen noted from the time of extraction (considered as 0 min) to various time interval and the results are plotted against time (Fig. 5). The plot shows that the chromogen was stable more than 2 h.

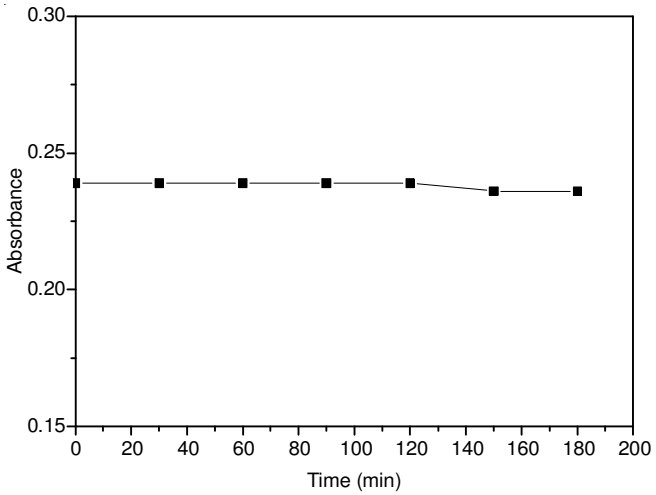

Fig. 5. Stability of DO-MO ion-pair complex

Robustness and ruggedness: To evaluate robustness (study of effect of deliberate change) effect of slight changes in wavelength of estimation and dye's concentration on the estimation of DO in tablet were considered. % RSD for the results were within the suggested limits for robustness (< 2 %) (Table-4). Likewise ruggedness was established by studying the effect using two different spectrophotometer Shimadzu UV mini-1240 (system I) and SCINCO, Neosys-2000 DRS-UV with liquid sample port (system II) and two different analysts (I and II)for estimating the drug in tablet. The results obtained were within the recommended % RSD limit (< 2 %) (Table-4).

Conclusion

The ion-pair extractive colorimetric estimation of dronedrone HCl in bulk and in formulation proposed here is more sensitive, specific (selective), rapid and cost effective. The highest % recovery obtained in the proposed method proved the accuracy of the present method. The selectivity of the method towards the drug analyzed was proved from the placebo interference as it resulted with low % RSD. Hence, the proposed method is good alternative choice to other method because of the use of simple and easily available reagent. From that, we conclude the developed method is suitable for regular determination of dronedrone in its solid formulations and can be consider as alternative choice to other sophisticated and non selective methods like HPLC and UV in terms of its simplicity and specificity respectively.

ACKNOWLEDGEMENTS

The authors acknowledged the financial support by grants from Korea CCS R & D centre, funded by Ministry of Education, Science and Technology of the Korean Government.

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