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Application of DDQ and p-Chloranilic Acid for the Spectrophotometric Estimation of Milrinone in Pharmaceutical Formulations

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Abstract: Two simple, rapid and sensitive spectrophotometric methods have been proposed for the determination of milrinone in pharmaceutical formulations. The first method is based on the charge transfer complexation reaction of milrinone with 2, 3-Dichloro-5, 6-Dicyanobenzoquinone, DDQ while the second method is based on charge transfer reaction of milrinone with p-Chloranilic Acid (pCA). Under the optimized experimental conditions Beer's law is obeyed in the concentration range of 2-40 μg mL^{-1} for method A and 5-100, 2-40 μg mL^{-1} for method B. The recovery ranged from 100.06 -100.11 for method A and from 99.34 -99.97 for method B. The coefficient of correlation was found to be 0.9998 for A and 0.9999 for B and the detection limit for method A and method B was found to be 0.765 and 3.35, respectively. Both the methods have been applied to determination of milrinone in the pharmaceutical formulation. Results of the analysis are validated statistically.

Key words: Milrinone, spectrophotometry, charge transfer, DDQ, pCA

INTRODUCTION

Milrinone, chemically known as 1, 6-dihydro-2-methyl-6-oxo-[3,4'-bipyridine]-5-carbonitrile) is a cardiovascular drug which possesses inotropic and potent vasodialating properties. Milrinone , has little chronotropic activity different in structure and mode of action from either the digitalis glycosides or catecholamines. The drug is a specific inhibitor of phosphodiesterase III and exerts its pharmacological effects by increasing cyclic AMO (cAMP) levels in the heart and vascular smooth muscle, which causes an increase in contractility of heart muscle and reduction of peripheral resistance (Lehtonen *et al*., 2004; Ooi and Colucci, 2001). This inhibitory action is consistent with cAMP mediated increases in intracellular ionized calcium and contractile force in cardiac muscle. Another literature suggest that its experimental evidence indicates that milrinone, is not a beta-adrenergic agonist nor does it inhibit sodium-potassium adenosine triphosphate activity as do the digitalis glycosides. Milrinone is commonly used following open heart surgery to prevent the occurrence of low cardiac output syndrome. Unlike other medication used for this purpose such as catecholamines, milrinone affords an advantage in that it does not commonly causes increase heart rate or the risk of dysrhythmias, both of which could pose a risk of increase ventricular after load (Bailey *et al*., 2004).

The therapeutic importance of milrinone was behind the development of few methods for its determination. The method adopted for the analysis of milrinone, include High

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Performance Liquid Chromatography (HPLC) (Nguyen *et al*., 2009; Brocks *et al*., 2005; Edelson *et al*., 1983; Oddie *et al*., 1986; Wilson *et al*., 1986; Akkerman *et al*., 1999).

The DDQ (2, 3-dichloro-5, 6-dicyanobenzoquinone), which is a stronger oxidant than 1, 4-benzoquinone, is used as reagent for oxidative couplings and cyclization reactions and dehydrogenation of hydroaromatic compounds and was proved to be useful for a wide range of reactions (Mohammed *et al*., 2006).

p-chloranilic acid, PCA is also used as π-acceptor in charge transfer reactions (El-Zeany *et al*., 2003; Rahman and Azmi, 2000). The literature shows the analysis of roxatidine by spectrophotometry using charge transfer agents, DDQ and PCA, allowing the charge transfer reactions (Rahman and Kashif, 2005). Thus, similar technique is employed for the analysis of milrinone as no such method is available for its analysis by spectrophotometry. Since, spectrophotometry is one of the cheapest technique for the analysis, its method could be useful in quality control and research laboratories.

The present communication describes two sensitive, fast, simple and economical methods for the determination of milrinone in dosage forms. Both the methods are based on the charge transfer complexation reaction of milrinone with DDQ and p-chloranillic acid in methanolic and acetonitrile medium, respectively.

MATERIALS AND METHODS

Experimental

The experiments were started in the month of January 2009 at Venus Medicine Research Centre, Baddi, India. The spectral runs were performed on Shimadzu 1701 spectrophotometer (Shimadzu, Kyoto, Japan) with 1 cm matched quartz cuvette.

Chem draw Ultra 7.0 version (Chembridge soft) was used to draw the chemical structure while Origin 6.0 (Originlab) was used to plot the effect of the drugs and reagents. Both the reagents are reported to be used for the analysis of pharmaceuticals (El-Ragehy *et al*., 1997):

- Milrinone was procured from Hubei Pharmaceutical Company, China
- Pharmaceutical preparation of milrinone such as Primacor IV was procured from the local market
- DDQ (Merck India Limited) was prepared as 0.05% (w/v) solution in methanol
- Chloranilic acid (Merck India Limited) was prepared as 0.18% (w/v) solution in Acetonitrile

Standard Drug Solution

For Method A(DDQ method)-Take 20 mg of milrinone in 100 mL standard flask dissolve the content in minimum amount of dimethyl sulfoxide and then dilute upto the mark with methanol, thus having a final concentration of 0.2 mg mL^{-1}.

For Method B (pCA method)-Take 50 mg of milrinone in 100 mL standard flask dissolve the content in minimum amount of dimethyl sulfoxide and then dilute upto the mark with methanol, thus having a final concentration of 0.5 mg mL^{-1}.

PROCEDURE FOR THE DETERMINATION OF MILRINONE

Method A (DDQ Method)

Aliquots of milrinone standard (0.2 mg mL^{-1}) equivalent to 2-40 μg of drug were transferred into a series of 5 mL volumetric flask. To each flask 1.0 mL of 0.05% DDQ was

added and brought up to the volume with methanol. The color developed immediately at room temperature (25±1°C) and absorbance was measured after 2 min at 356 nm against the reagent blank prepared similarly omitting the drug.

Method B (pCA Method)

Into a series of 5 mL volumetric flasks, volumes of milrinone standard solution (0.5 mg mL^{-1}) equivalent to 5-100 μg of drug were transferred. To each flask 1 mL of 0.18% pCA was added and brought upto the volume with acetonitrile. The colored product formed immediately at room temperature (25±1°C) and absorbance was measured after 2 min of mixing at 519 nm against the reagent blank prepared similarly without adding the drug.

Analysis of Milrinone in Pharmaceutical Preparation

For Method A

Twenty milliliter of milrinone injection (Primacor IV) having a label claim of 1 mg mL^{-1} were diluted to 50 mL volume with methanol. It was further diluted according to the need and then analyzed following the proposed procedure.

For Method B

Twenty five milliliter of milrinone injection (Primacor IV) having a label claim of 1 mg mL^{-1} were diluted to 50 mL volume with methanol. It was further diluted according to the need and then analyzed following the proposed procedure.

RESULTS AND DISCUSSION

The results of the experiment performed were found to be satisfactory. Experiment were performed at 356 and 529 nm for DDQ and PCA method, respectively. Linear regression plots were obtained from the experimental data for both the methods, $A = 6.08534\times10^{-5}+0.01836C$, $r = 0.99988$ for method DDQ and $A = 4.61197\times10^{-4}+0.00869\times C$, $r = 0.99996$ for method PCA. The limit of detection for DDQ method was found to be 0.765 and 3.35 μg mL^{-1} for the PCA method. Accuracy was determined by applying the described method, mean recoveries (mean, RSD) from the drug is found to be 100.11, 0.29 and 100.06, 0.17 for method DDQ and 99.34, 1.48 and 99.97, 0.74 for method PCA indicating good accuracy of the method for determination of the drug.

These results indicate the efficiency of the developed method for this particular drug. Earlier (Darwish, 2005; Hesham, 2008, 2009) shows the use of DDQ in charge transfer reactions but no such studies were done using DDQ and PCA with milrinone thus these results serves as the additional data for the analysis of milrinone, one can fulfill its purpose as this method is cheap and effective. The aim of the study was to develop a cheap and effective method for the analysis of milrinone which was successfully achieved as shown by the results obtained.

Determination of Analytical Performance Parameters

Methods A and B have been validated for specificity, linearity, limit of detection, precision, accuracy and recovery.

Linearity

The linearity was evaluated by considering concentration levels of 2, 4, 8, 20, 24, 28, 32 and 40 μg mL^{-1} for method A (DDQ method) (Fig. 1) and by considering

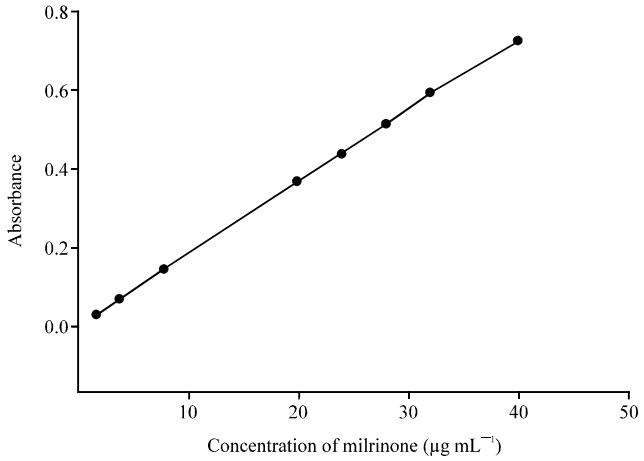


Fig. 1: Effect of drug on the color development in DDQ method

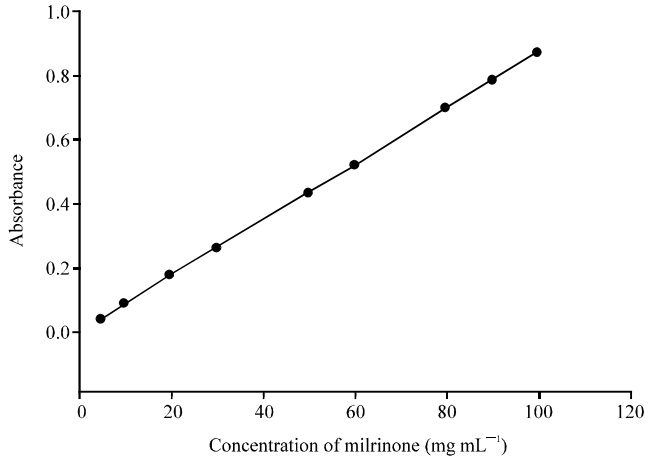


Fig. 2: Effect of drug in the color development in pCA method

concentration level of 5, 10, 20, 30, 50, 60, 80, 90 and 100 µg mL^{-1} method B (Fig. 2). The determination was completed 5 times at each concentration level.

Accuracy and Precision

Intra day and inter day precisions were performed at three concentration levels: 4, 20, 32 g mL^{-1} for method A and 10, 50, 90 g mL^{-1} for method B. The determination was repeated 5 times at each concentration level using DDQ and p-chloranilic acid obtained from Merck India Limited Mumbai.

Recovery Studies

Method A (DDQ Method)

Recovery experiments were carried out by standard addition method. For this 0.2 mL (or 0.6 mL) of reference milrinone (0.2 mg mL^{-1}) was transferred into 5 mL volumetric flask followed by 0.3 mL of sample solution (0.2 mg mL^{-1}). The volume was completed with methanol and the nominal value was determined by the proposed procedure.

Method B (pCA Method)

In this 0.1 mL (or 0.6 mL) of reference milrinone (0.05 mg mL^{-1}) was transferred into 5 mL volumetric flask followed by 0.6 mL of sample solution (0.05 mg mL^{-1}). The volume was completed with acetonitrile and the nominal value was determined by the proposed procedure.

Selectivity and Specificity

Selectivity of the proposed method was estimated by determining the content of milrinone in a synthetic mixture of following composition milrinone (100 mg), dextrose (40.7 mg) lactose (60 mg) and mannitol (60 mg). The solutions were prepared as described in analysis of milrinone in pharmaceutical preparation. The content of milrinone was determined in the presence of excipients the recovery was found to be satisfactory.

Limits of Detection (LOD) and Quantitation (LOQ)

The values of the LOD and LOQ were calculated using the expression:

$$LOD = 3.3 \times S_0/b$$

and

$$LOQ = 10 \times S_0/b$$

where, S_0 and b are standard deviation and slope of the calibration line, respectively.

Stability of Analytical Solution

The stability of the test solution of milrinone dissolved in minimum amount of dimethylsulfoxide and diluted with methanol was studied by recording the UV absorption spectra of the drug for thee days. The drug solution having a λ_{max} at 202 nm, showed no change in the absorption spectra of the test and sample solution of drugs for at least three days, when the solutions were stored at room temperature in dark.

Robustness

The proposed method conditions for the determination of milrinone in pure form and in drug formulation are very robust. Each operational parameter was tested and challenged for the robustness of the method. The operational parameter investigated is:

- Volume of 0.05% DDQ (±0.4 mL) for method A
- Volumes of 0.18% pCA (±0.5 mL) for method B

Under these condition sample solutions from the sole dosage form, 20 and 50 μg mL^{-1} of active milrinone was assayed by performing five independent analyses by the proposed methods. The results of mean recovery, SD and relative standard deviation indicate good sensitivity and appreciable recovery. Thus, the conditions of the proposed method are very robust.

Optimization of Variables

Different parameters affecting the color development were extensively studied to determine the optimum condition for assay procedures. The optimum values of the variables were maintained throughout the determination process.

Method A (DDQ Method)

Effect of Reaction Time

Reaction time was evaluated by monitoring the color development at room temperature. It was observed that the reaction got stabilized within two minutes and color developed remained stable at room temperature for about 1.5 h.

Effect of DDQ Concentration

The study the effect of the volume of the reagent on the absorbance of the charge transfer complex, varying volume of 0.05% DDQ was mixed with 1 mL of 0.0 5% drug in a 5 mL standard flask and diluted to volume with methanol. The results (Fig. 3) showed that the highest absorbance was obtained with 0.8 mL, which remained unaffected by further addition of DDQ. Hence, 1 mL of the reagent was used for the determination process.

Method B (pCA Method)

Effect of Time on the Reaction

The interaction of pCA with milrinone resulted in the formation of a colored product which is stabilized within 2 min. The colored product developed by the reaction of milrinone with pCA remained stable for 2 h.

Effect of pCA Concentration

To establish the optimum experimental condition, milrinone 100 μg was allowed to react with different volumes (0.1-1 mL) of 0.18% pCA w, the results (Fig. 4) shows that the highest absorbance was obtained with 0.6 mL which remained unaffected upto 1.1 mL thus 1.0 mL of pCA was selected for further studies.

Analytical Data

Under the optimized experimental conditions, the absorbance measured in methods A and B were proportional to the milrinone concentration over the range shown in Table 1. The results of regression analysis of the calibrated date for both the methods are shown in Table 1.

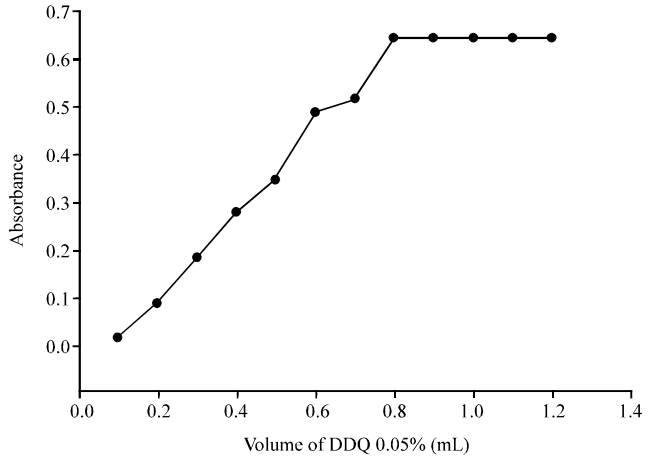


Fig. 3: Effect of DDQ on color development

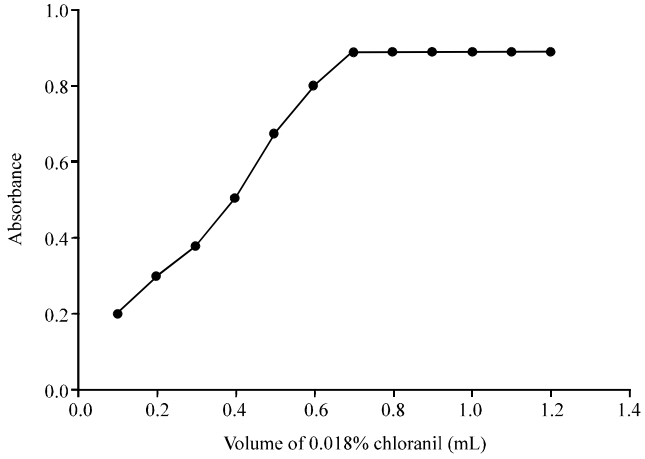


Fig. 4: Effect of pCA on the color development

Table 1: Optical and regressional characteristics of the proposed method

	Methods	
Parameters	DDQ	PCA
λ_{max} (nm)	356	529
Beer's Law limit (μg mL^{-1})	2-40	5-100
Linear regression equation	$A = 6.08534\times10^{-5}+0.01836\times C$	$A = 4.61197\times10^{-4}+0.00869\times C$
tSa	0.00274	0.00168
tSb	1.16087×10^{-4}	2.81386×10^{-5}
Correlation coefficient	0.99988	0.99996
Variance (So2)	1.81476×10^{-5}	7.9524×10^{-6}
Detection limit	0.765	3.35

±tSa: Confidence limit for intercept, ±tSb: Confidence limit for slope

Accuracy and precision of method A were evaluated by determining the content of milrinone at three concentration levels 4, 20 and 32, while the same for method B was evaluated also at three concentration level of 10, 50 and 90 μg mL^{-1}. The accuracy of the proposed method was expressed as the mean percent relative error. As shown in Table 2, in all case the error was less than 0.31%. Interday and Intra day precisions of methods A and B were expressed as percentage of Relative Standard Deviation (% RSD). It is apparent from Table 2 that in all cases the RSD% was lower than 1.76. The accuracy of the proposed method was also tested in recovery experiments applying standard addition method. The recovery results are shown in Table 3. Recovery ranged from 100.06-100.11 for method A and from 99.34-99.97 for method B. It appears from the table that the presence of excipients did not interfere with the determination process.

The methanolic solution of milrinone absorbs maximally at 202 nm whereas the absorption spectra of DDQ shows that it has a λ_{max} of 454 nm. Addition of DDQ to the drug solution resulted in the formation of a new characteristic band peaking at 356 nm (Fig. 5). The DDQ is π a acid known to react with variety of Lewis base forming charge transfer complexes which are rapidly reduced to radical anion. Absorption spectrum of p-chloranillic acid in methanol shows a band at 300 nm, while the drug as mentioned above is peaking at 202 nm. Addition of pCA to the drug solution caused an immediate shift of absorption band to 528 (Fig. 6). It is well known that pCA is a λ-acceptor and forms a charge transfer complexes with n-donor compounds. The literature survey (Rahman and Haque, 2008) reveals that pCA exits

Table 2: Interday and intra day assays (Determination of milrinone in drug formulation)

Drug formulation	Concentration (μg mL^{-1}) Taken	Found	Recovery %±SD	RSD[a]	SAE[b]	CI[c]
DDQ method (Inter day assay)						
Primacor IV	4.0	3.973	99.32±0.066	1.68	0.31	0.082
	20.0	19.978	99.89±0.372	0.37	0.033	0.090
	32.0	31.990	99.96±0.198	0.62	0.089	0.240
DDQ method (Intra day assay)						
Primacor IV	4.0	4.016	100.41±0.071	1.76	0.031	0.088
	20.0	20.094	100.20±0.054	0.27	0.024	0.067
	32.0	31.978	99.93±0.105	0.33	0.045	0.130
pCA method (Inter day assay)						
Primacor IV	10.0	9.850	98.54±0.24	0.24	0.01	0.030
	50.0	49.870	99.75±0.44	0.88	0.20	0.540
	90.0	89.970	99.97±0.67	0.74	0.30	0.830
pCA method (Intra day assay)						
Primacor IV	10.0	9.940	99.37±0.05	0.53	0.02	0.060
	50.0	50.280	100.56±0.56	1.12	0.25	0.700
	90.0	90.100	100.11±0.70	0.78	0.31	0.870

[a]RSD: Relative standard deviations (n = 5), [b]SAE: Standard analytical error, [c]CL: Confidence limit at 95% confidence level

Table 3: Standard addition method (Evaluation of the proposed method for the recovery of milrinone)

Drug formulation	Concentration (μg mL^{-1}) Taken	Added	Found±SD	Recovery %±SD	RSD[a]	SAE[b]	CI[c]
DDQ method							
Primacor IV	4.0	12.0	16.02±0.046	100.11	0.29	0.021	0.058
	24.0	12.0	36.02±0.062	100.06	0.17	0.028	0.077
pCA method							
Primacor IV	10.0	30.0	39.74±0.590	99.34	1.48	0.260	0.730
	60.0	30.0	89.97±0.670	99.97	0.74	0.300	0.830

[a]RSD: Relative standard deviations (n = 5), [b]SAE: Standard analytical error, [c]CL: Confidence limit at 95% confidence level

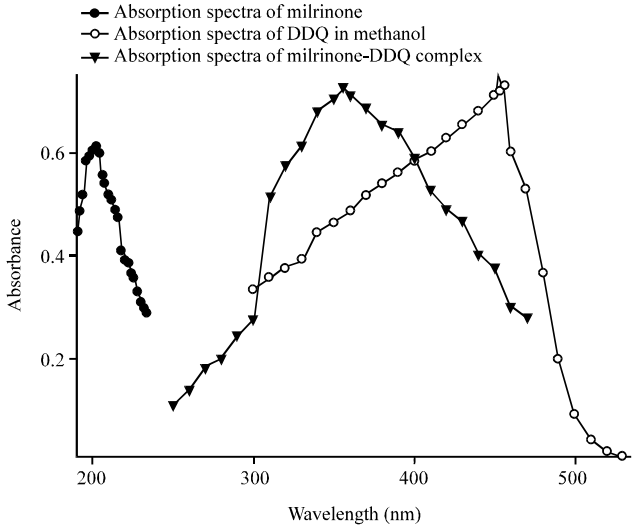


Fig. 5: Absorption spectra of milrinone, DDQ in methanol and milrinone-DDQ Complex

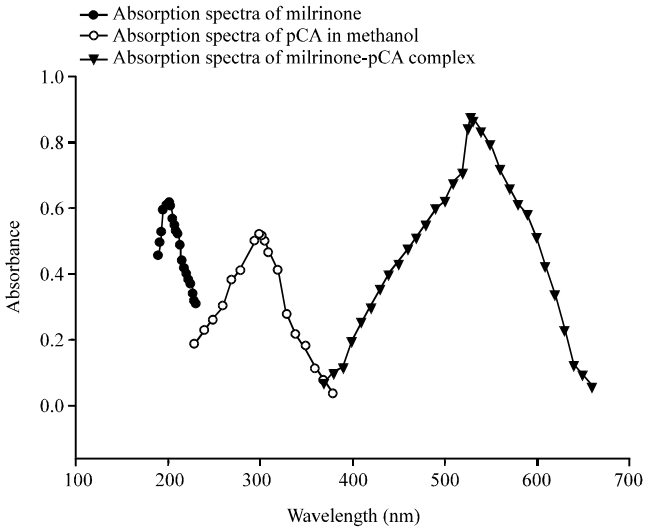
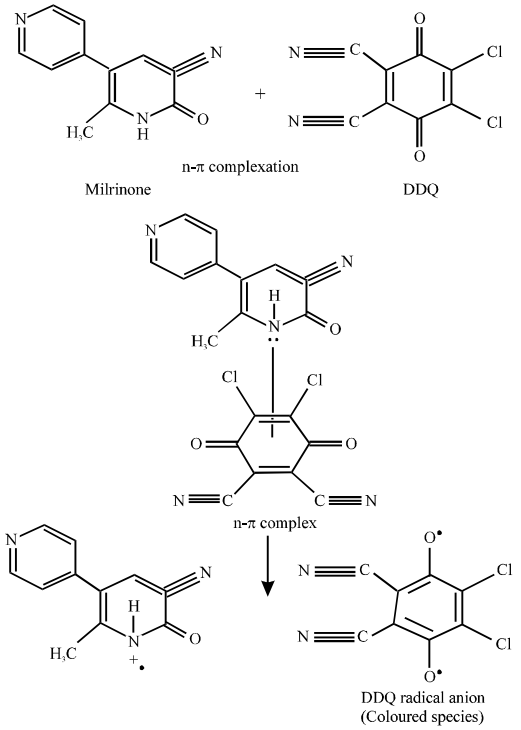
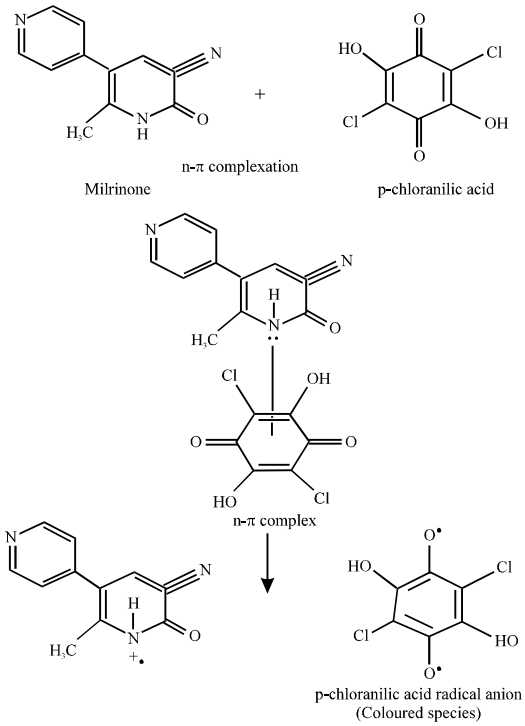


Fig. 6: Absorption spectra of milrinone, pCA in acetonitrile and milrinone-pCA complex



Scheme 1: Charge transfer complexation reaction sequence of milrinone-DDQ complex

in three forms. In this study, the interaction of milrinone with π acceptor pCA at room temperature was found to yield colored charge transfer complex. In polar solvents, complete electron transfer from milrinone (D), as an electron donor, to the acceptor moiety (A) takes



Scheme 2: Charge transfer complexation reaction sequence of milrinone-pCA complex

place resulting in the formation of intense colored radical anions. The reaction sequence can be shown in Scheme 1 for DDQ method and Scheme 2 for pCA method, respectively.

The literature reveals that pCA exists in three forms: (1) H_2A at very low pH which is yellow orange in color and (2) colorless A^{-2} which is stable at high pH. The inter conversion of these species can be represented as:

$$H_2A \rightleftharpoons H^+ + HA^-$$

$$HA^- \rightleftharpoons H^+ + A^-$$

CONCLUSION

The proposed methods are simple and reliable. They are based on the charge transfer complexation reaction and further spectrophotometric determination of the products at 356 and 529 nm for method A and B, respectively. Moreover, the proposed method can be performed at room temperature without the need of heating or any pretreatment of the drug sample. The proposed method are specific can be applied to the determination of drug in dosage forms in the presence of adjuvant and excipients.

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