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Determination of some Cardiovascular Drugs through Condensation with p-Dimethylaminocinnamaldehyde

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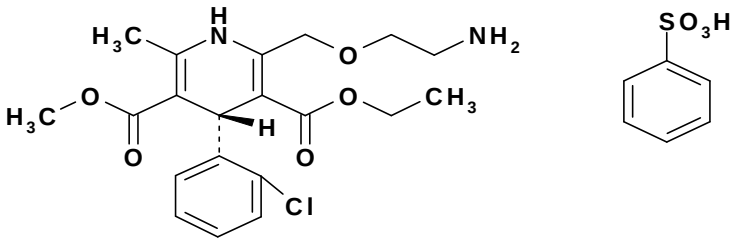
Summary: This work presents simple, sensitive and validated spectrophotometric method for the determination of some cardiovascular drugs namely Amlodipine besylate (AM), dobutamine HCl (DO), and carvedilol (CA) in their drug substances and drug products. The method depends on condensation of the primary or secondary amino group with p-dimethylaminocinnamaldehyde (p-DAC) in methanol to form colored products measured at 472, 484, and 488 nm for AM, DO, and CA respectively. Under the optimum reaction conditions, good linearity were obtained in the concentration range of 1.0-20.0, 0.5-8.0, and 1.0-12.0 µg ml^{-1} for the three mentioned drugs respectively. The method was validated according to ICH guidelines and the results were statistically compared with the official methods. The limit of detection (LOD) and limit of quantification (LOQ) were calculated. Apparent molar absorbtivity, sensitivity index, stability constant (K_f), and free energy change (ΔG) of the reaction products were also calculated.

Introduction

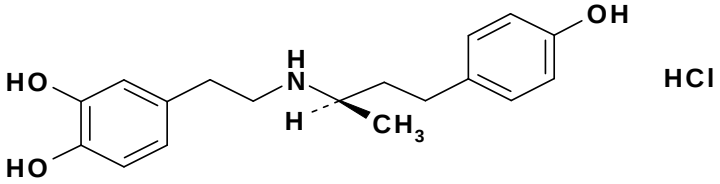
Amlodipine besylate (AM); (**scheme 1A**) 3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzenesulphonate is a second generation 1, 4-dihydropyridines derivative of the prototypical molecule nifedipine. It is used in the treatment of chronic stable angina and in the management of mild to moderate essential hypertension and heart failure. It is marked as the benzene sulfonic acid salt (besylate)[1, 2]. AM has been determined in its drug substance and drug product by potentiometric titration method[3]. UV spectrophotometry[4-6], electrochemical method[7], Thin layer chromatography[8], HPLC[9-10] and Capillary zone electrophoresis[11].



Scheme 1A. Structure of Amlodipine besylate

Dobutamine HCl (DO) ;(**scheme 1B)** (RS)-4-[2-[[3-(4-hydroxyphenyl)-1-methylpropyl] - amino] ethyl] benzene-1, 2-diol hydrochloride is a compound that structurally can be viewed as an analogue of dopamine. In vivo, racemic DO increases the inotropic activity of the heart to a much greater extent than it increases chronotropic activity. This pharmacological profile has led to its use in treating congestive heart failure. It is given by intravenous infusion since it is not effective orally. It has a plasma half-life of about 2 minutes[1, 2].

Reviewing the literature showed that the drug can be determined electrochemically[12], spectrophotometricaly[13, 14], HPLC[15], capillary electrophoresis[16], and flow injection chemiluminescences[17].

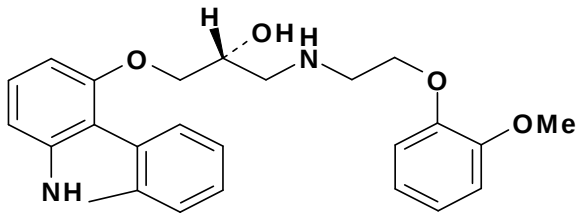


Scheme 1B: **Structure of Dobutamine HCl**

Carvedilol (CA) ;(**scheme 1C)** (2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol is a third-generation β receptor antagonist that has a unique pharmacological profile. It blocks β_1, β_2, and α_1 receptors similarly to labetalol, but also has antioxidant effect. CA produces vasodilatation. It is thought that the additional properties contribute to the beneficial effects seen in treating congestive heart failure[1, 2].

CA is the subject of monographs in British pharmacopoeia[1] and European pharmacopoeia[18] whereby a non aqueous titrimetric method is recommended for its determination. Several methods have been published for its determination either in bulk powder or in pharmaceutical preparation and biological fluids.

These methods include: non-aqueous titration[19], spectrophotometry[19-20], fluorimetry [21- 22], Chemiluminescence method[23], electrochemical method[24], gas chromatography[25], HPLC[26, 27], TLC[28-29] and Capillary electrophoresis[30].



Scheme 1C: Structure of Carvedilol

p-DAC was used as a reagent for spectrophotometric determination of some drugs having primary or secondary amino function and sometimes active methylene group via its aldehydic group[31-32]. The present study is devoted to investigate the reaction of AM, DO and CA with p-DAC reagent, and employment the reactions in the development of new simple spectrophotometric method for determination of AM, DO, and CA in its drug products.

The suggested method has the advantage of being simple, sensitive and suitable for routine analysis in quality control laboratories.

Experimental

Materials and reagents

All chemicals used were of analytical grade and solvents were of spectroscopic grade.

Amlodipine besylate was kindly supplied by PFIZER Co. Egypt, The purity of the sample was found to be 99.50±0.48% according to the official HPLC method[1]. Alkapress tablets 5 mg B.N. 072, manufactured by ALKAN PHARMA Co., Egypt, was purchased from local market.

Dobutamine HCl was kindly supplied by PHARCO Co., Egypt. The purity of the sample was found to be 99.30±0.59% according to the official HPLC methods[1]. Dobutamine HCl injection 250 mg/20 ml B.N.120 manufactured by Abbot Co., was purchased from local market. Carvedilol was kindly supplied by Eva Co. Egypt and assayed for purity according to the official titrimetric

methods[1,18] to be found 99.80±0.19%. Dilator tablets B.N. 19503, each tablet was labeled to contain 25 mg of carvedilol manufactured by Chemipharm Pharmaceutical Industries S.A.E, Egypt. Methanol (LAB-SCAN) Dublin, Ireland.

P-Dimethylaminocinnamaldehyde (p-DAC) (Merck) solution, 0.5% w/v dissolved in methanol.

Apparatus

SHIMADZU Dual-beam (Japan) UV-Visible spectrophotometer, model UV-1601 PC, connected to an IBM compatible computer and HP 600 ink Jet printer. The bundled software, UV-PC personal spectroscopy software version 3.7 (SHIMADZU) was used to process absorption. The spectral band width was 2 nm and scanning speed 2800 nm min^{-1}.

Preparation of solutions

Stock solutions (0.1 $mgml^{-1}$) for each drug

Accurately weighed 10 mg of each of AM, DO, and CA were transferred into three separate 100 ml volumetric flasks. About 50 ml methanol was added to dissolve and then the volumes were completed to the mark with methanol.

Working standard solutions (50 μgml^{-1}) for each drug

Aliquots equivalent to 0.5 mg of each AM, DO, and CA were transferred from their stock solutions into three series of 10 ml volumetric flasks. Five ml methanol were added and mixed well and the volume was completed to the mark with methanol.

General Recommended Procedures

Scanning of the absorption spectra

Aliquots equivalent to (180, 80, and 120 µg) from AM, DO, and CA working standard solutions respectively were transferred into test tubes, p-DAC solution (0.5% w/v), 1 ml for DO and 2 ml for AM and CA were added. The mixtures were heated on a boiling water bath for (10 min for DO and 15 min for AM and

CA), solutions were carefully observed, 1 ml of methanol was added if the volume of solution decreased, then cooled. The solutions were then transferred to volumetric flask 10 ml and completed to volume with methanol. The absorption spectra of the resulting solutions were recorded in the range of 250-600 nm against blank solutions which were prepared similarly without the drugs.

Application on drug products

Ampoules (Dobutamine HCl ampoules 250 mg/20 ml)

The contents of 3 ampoules were mixed well in 250 ml dried beaker. Aliquot equivalent to 5 mg of DO was transferred quantitatively into 50 ml volumetric flask and completed to the mark with methanol and preceded as directed under general recommended procedure.

Tablets **(**Alkapress tablets 5 mg), (Dilatrol tablets 25 mg)

The contents of 10 tablets were finely pulverized and mixed thoroughly. An accurate weight of the mixed powder equivalent to one tablet of AM and CA were transferred into two separated 100 ml conical flasks, 50 ml methanol was added, mechanically shacked for 30 min. filtered and transferred quantitatively into two separate 100 ml volumetric flask. The solutions were completed to the mark with methanol, and preceded as described under general recommended procedure

Results and discussion

Absorption Spectra

In this work p-DAC was used to evaluate AM, DO, and CA, where the first drug contains both primary and secondary amino group, while second and third drugs contain secondary amino function. The spectra of the colored reaction products for the selected cardiovascular drugs

show characteristic λ_{max} (472, 484, and, 488 nm) for AM, DO, and CA, respectively as shown in **Fig. 1.**

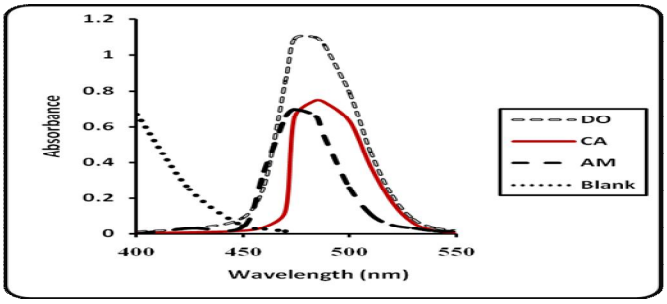
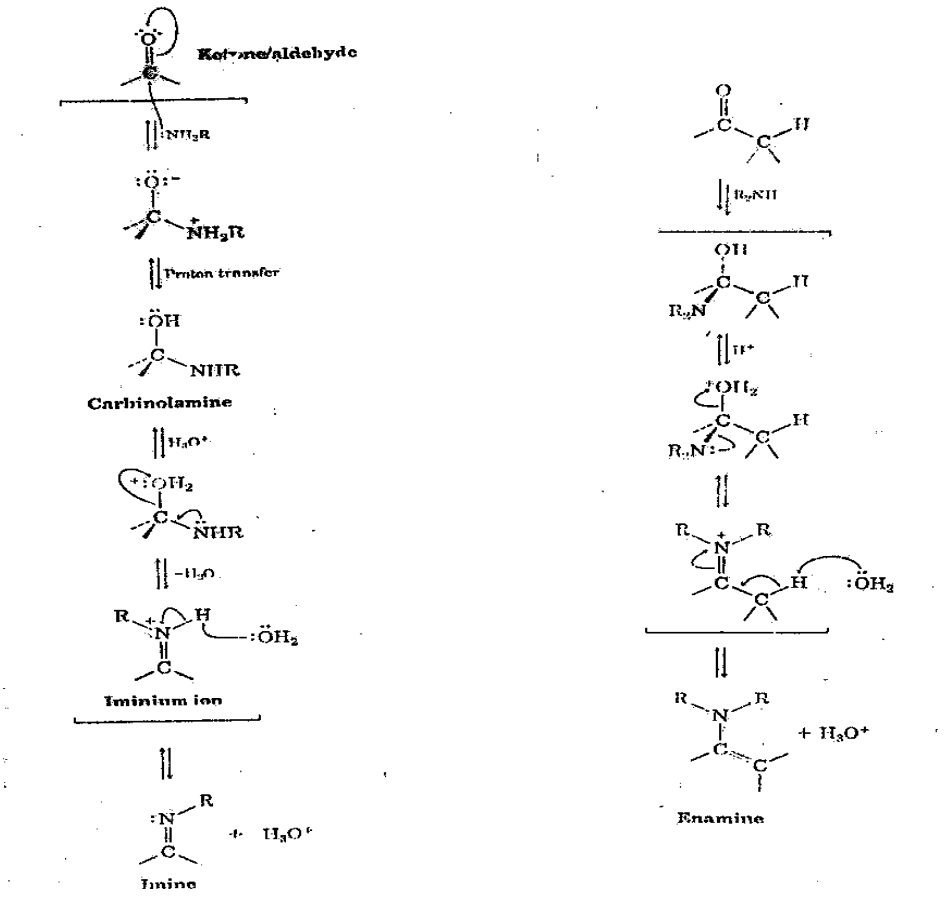


Fig. 1: Absorption spectra of DO (8 µgml^{-1}), AM (12 µgml^{-1}), and CA (12 µgml^{-1}) –p-DAC (0.5%w/v) reaction products.

In general aldehydes are known to react with primary and secondary amines. Primary amines undergo nucleophilic addition to aldehydes to form imines, while secondary amines add similarly to give enamines [33]; the reaction pathway is proposed to be proceeded as shown in scheme 2.



Scheme 2: The suggested structure of the formed reaction product between primary, secondary amines and p-DAC.

The general reaction of primary and secondary amines with p-DAC in weak acid medium was reported to stop at the iminium ion step due to the formation of resonating structure [34].

Although the addition of acid is omitted in the present work, the reaction occurred with reproducible results that may be due to the weak acidic properties of the drugs (pH of the reactants 5.5). however, a study of the effect of different acids on the reaction between p-DAC and the studied drugs revealed that the use of 1 ml of 0.1 M (hydrochloric, sulfuric or glacial acetic acids) gave no maxima above 400 nm (below 400 nm the reagent interferes).

Optimization of Reaction Variables

The spectrophotometric properties of the colored products, as well as the different experiments parameters affecting the development of the reaction product and its stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. The factors include the concentration of reagent, the temperature, the heating time and diluting solvents.

Effect of reagent concentration and volume

The influence of the concentration of p-DAC was studied using different volumes of 0.5% w/v of the reagent solution. It was found that the absorbance increased with the increase in the reagent volume. The highest absorbance was attained at volume ranges of 0.25-3 ml. For high precise values, further experiments were carried out using 1 ml of 0.5% w/v for DO, and 2 ml of 0.5% w/v for AM and CA as shown in **Fig.2**

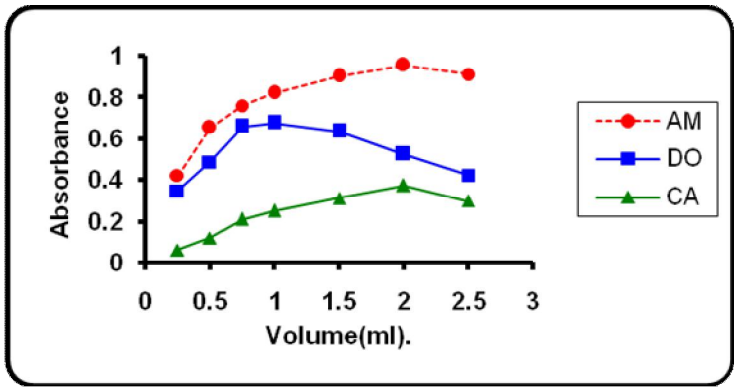


Fig. 2: Effect of volume of (0.5% w/v) p-DAC on absorbance of AM (16 µgml^{-1}), DO (6 µgml^{-1}), and CA (6 µgml^{-1})-p-DAC (0.5% w/v) reaction products.

Effect of temperature and time

The effect of temperature on the reaction was studied by carrying out the reaction at different temperatures (25-95 ^{o}C). It was found that reaction of AM, DO, and CA with p-DAC were applied after heating the solutions in a boiling water bath 10 minutes was adequate for DO and 15 minutes for both AM and CA as shown in **Fig. 3**

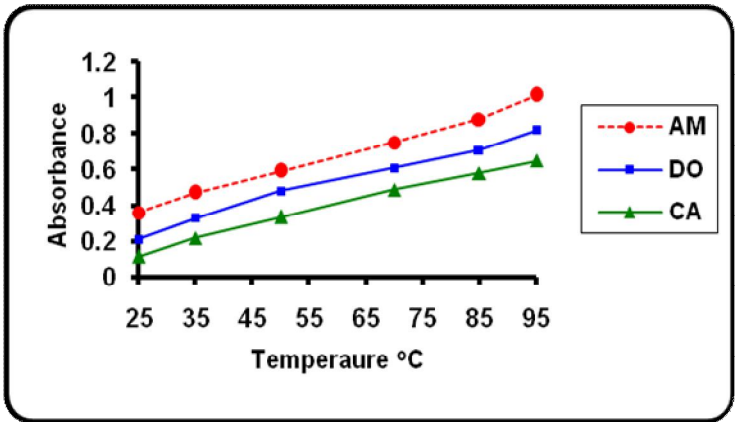


Fig. 3: Effect of temperature on the absorbance of AM (18 µgml^{-1}), DO (6 µgml^{-1}) and CA (10 µgml^{-1})-p-DAC (0.5 % w/v) reaction product.

Effect of diluting solvents

Upon diluting the reaction solutions with water, turbidity was obtained indicating the incomplete solubility of AM, DO and CA –p-DAC derivatives in water. Therefore, water could not be used for dilution. In order to select the most appropriate organic solvent for diluting the reaction solutions, different solvents

were tested: methanol, ethanol, acetone, chloroform, and isopropyl alcohol. The highest readings were obtained when methanol was used for dilution.

Stability of the chromophore

After dilution the reaction solutions, it was found that the absorbance of the chromophore (AM-p-DAC), (DO-p-DAC), and (CA-p-DAC) remained stable for at least 2 hours.

Under the optimum experimental conditions the calibration curves were plotted representing the relationship between the absorbance at 474, 484 and 488nm and the corresponding concentration of the three cited drugs (AM, DO and CA). Linear correlation coefficients were obtained within the concentration range 1-20 μgml^{-1} for AM, 0.5-8.0 μgml^{-1} for DO and 1-12 μgml^{-1} for CA.

Stoichiometry of the reaction

Under the optimum conditions, the stoichiometries of the reaction of AM, DO, and CA with p-DAC were determined adopting the Job's method of continuous variation [35]. The results revealed that AM and DO reacted with p-DAC in a ratio of 3:2 under the optimum condition attained while CA reacted with p-DAC in a ratio 1:1 as shown in **Fig. 4**

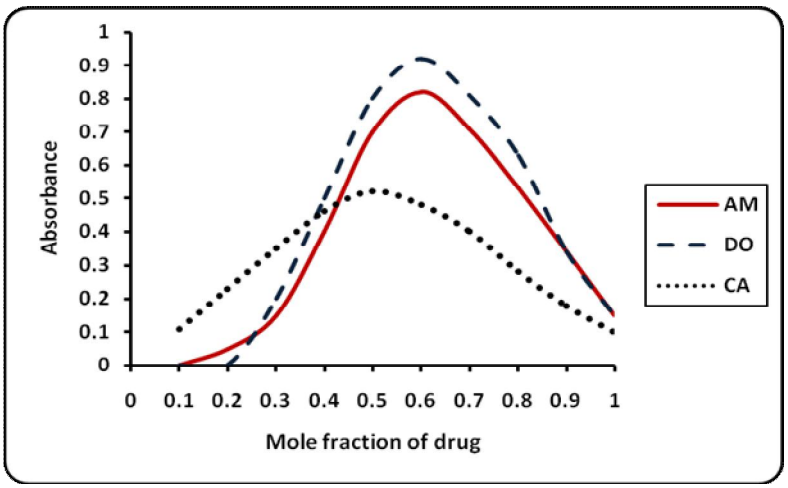


Fig. 4: Determination of Stoichiometry of the reaction of amlodipine besylate, dobutamine HCl and carvedilol – p-DAC by continuous variation method using (2 x 10^{-4} M) solutions.

Determination of the stability constant

The stability constant (K_f) of the reaction products are calculated according to the follow equation:

$$K_f = (A / A_m) / [1 - A / A_m]^{n-1} C^n n^n$$ (36)

Where: A = maximum fluorescence intensity of the continuous variation curve **Figure 4**,

A_m = fluorescence intensity corresponding to intersection of two tangents of the continuous variation curve, n = number of molecules of the reagent in the reaction product, C = molar concentration of the drug and K_f = formation constant of the complex.

The stability constant of the reaction products of DO, AM and CA with p-DAC were 7.2×10^4, 2.8×10^4, and 4.4×10^3, respectively.

The Gibbs free energy change of the reaction (ΔG)[37] was also calculated adopting the following equation: $\Delta G = -2.303 R T \log K_f$

Where: ΔG = Gibbs free energy change of the reaction (k.J. mol^{-1})

R = Universal gas constant (8.314 joules)

T = Absolute temperature ($273+25^oC$)

K_f = Formation constant of reaction

The free energy changes (ΔG) of the reaction DO, AM and CA with p-DAC was found to be -5.05×10^4, -4.25×10^4 and -2.07×10^4 k.J.$mole^{-1}$ respectively, The higher K_f and ΔG values obtained indicate very stable reaction products.

Validation of the Method

Linearity

In the proposed method, linear plots with good correlation coefficients were obtained in the concentration ranges of 1-20, 0.5-8.0, and 1-12 µgml^{-1} for AM, DO, and CA, respectively as shown in **Table 1**.

Limit of detection and limit of quantification

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2B[38], **Table 1**. The

limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected, and results are also abridged in **Table 1**

$$\mathbf{LOQ = 10\ S_a / b \quad and \quad LOD = 3.3\ S_a / b}$$

Where S_a = stander deviation of intercept of the calibration curve and b = slope of the calibration curve.

Accuracy

The accuracy of the proposed method was evaluated by the recovery studied for added concentrations of AM, DO, and CA of 2.0-18.0, 1.0-7.0 and 2.0-10.0 μgml^{-1} respectively.

The recovery values were 99.63%±0.204, 99.57%±0.325, and 99.78%±0.157 for AM, DO and CA, respectively **Table 1**, indicating the accuracy of the proposed method.

The results of the proposed method were statistically compared with those obtained by the official methods. Statistical analysis of the results, using Student's "*t* " test and variance ratio "*F*" test revealed no significant difference between the performance of the proposed and reference methods **Table 2**. The validity of the method was proved by statistical evaluation of the regression lines, using the standard error of intercept and standard error of slope. The results are abridged in **Table 1**.

Precision

The repeatability for cited drugs were evaluated by assaying a freshly prepared solutions in triplicate in the concentration ranges 1.0-17.0, 0.5-6.0 and 1.0-11.0 μgml^{-1} for AM, DO, and CA, respectively, and a relative standard deviation *(%RSDs*) were found to be ±0.212%, ±0.280%, and ±0.238% for three drugs. The intermediate precision was calculated by assaying freshly prepared solutions in triplicate for three days. The relative standard deviation *(%RSDs*) were found to be ±0.337%, ±0.204% and ±0.319% for AM, DO and CA respectively, as shown in **Table 1**.

Robustness and Ruggedness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, such as change in the volume of p-DAC (0.5% w/v), (1±0.5 ml for DO), (2±0.5 ml for AM and CA), also the change temperature (95±5 ^{o}C). The minor changes did not affect the absorbance. Ruggedness was also tested by applying the method to the assay of AM, DO and CA using the same operational conditions but using two different instruments at different laboratories and different elapsed time. No significant difference was obtained from lab-to-lab and day-to-day in this study.

Method Validation for Drug Products

It is evident from the above-mentioned results that the proposed method gave satisfactory results with AM, DO and CA in its drug substances. Thus its drug products were subjected to the analysis of their AM, DO and CA contents by the proposed and the reported UV method for AM [39], official HPLC method for DO [1] and reported HPLC method for CA [40]. The label claim percentages were 99.84%±0.151, 99.63%±0.0.813, and 100.0%±0.0.726 for AM, DO and CA, respectively **Table 3**. These results were compared with those obtained from the reported and official methods by statistical analysis with respect to the accuracy (by *t*- test) and precision (by *F*- test). No significant differences were found between the calculated and theoretical values of *t*-test and *F*-test at 95% confidence level proving similar accuracy and precision in the determination of AM, DO and CA by UV method.

Conclusions

The present paper described the evaluation of p-DAC as analytical reagent in the development of simple, sensitive, and accurate spectrophotometric method, for the determination of AM, DO, and CA in drug substances and drug products. The described method is superior reported spectrophotometric method in term of the simplicity and sensitivity. The proposed method has comparable analytical

performances and devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Therefore, this method can be recommended for the routine analysis of AM, DO, and CA in quality control laboratory.

Table 1: Results of assay validation by applying by the proposed spectrophotometric method using p-DAC for the determination of amlodipine besylate, dobutamine HCl, and carvedilol in drug substances.

Parameters	Amlodipine besylate	Dobutamine HCl	Carvedilol
Linearity range ($\mu g ml^{-1}$)	1.0-20.0	0.5-8.0	1.0-12.0
Apparent Molar absorbtivity (L mol^{-1}- cm^{-1}).	$3.3x\ 10^4$	$4.6x\ 10^4$	$2.5x\ 10^4$
Sensitivity Index ($\mu g\ cm^{-2}$)	0.017	0.007	0.016
Regression equation*	A = 0.0587C + 0.001	A = 0.1351C + 0.004	A = 0.062C+ 0.0029
Slope	0.0587	0.1360	0.0627
S E of slope	0.0002	0.0011	0.0004
Confidence limit of slopeb	0.0582- 0.0592	0.1331- 0.1390	0.0616- 0.0638
Intercept	0.0012	0.0042	0.0029
S E of intercept	0.0028	0.0048	0.0031
Confidence limit of interceptb	-0.0053- 0.0076	-0.0092- 0.0175	-0.0109- 0.0051
Correlation coefficient (r)	0.9998	0.9996	0.9997
SE of (r)	0.0049	0.0071	0.0043
Accuracy (Mean**±RSD %)	99.63±0.204	99.57±0.325	99.78±0.157
Precision			
Repeatability	99.62±0.212	99.68±0.280	99.49±0.238
Intermediate precision	99.56±0.337	99.78±0.204	99.74±0.319
LOD ($\mu g ml^{-1}$)	0.281	0.120	0.224
LOQ ($\mu g ml^{-1}$)	0.850	0.370	0.680

*A = Absorbance C = Concentration $\mu g ml^{-1}$

** mean of six different experiments. (b) 95% confidence limit

Table 2: Statistical comparison between the results obtained by the suggested spectrophotometric method using p-DAC and official HPLC method for AM, official titrimetric method for DO and CA for determination in drug substances**.**

Parameters	Amlodipine besylate		Dobutamine HCl		Carvedilol	
	Using p-DAC	official HPLC method (1)	Using p-DAC	official titrimetric method (1)	Using p-DAC	official titrimetric method (1)
Accuracy Mean*±RDS %	99.63± 0.204%	99.28± 0.323%	99.57± 0.325%	99.29± 0.316%	99.78± 0.157%	99.61± 0.197%
SD	0.203	0.321	0.324	0.314	0.157	0.197
Variance(V)	0.041	0.103	0.105	0.098	0.246	0.038
SE	0.083	0.131	0.132	0.128	0.064	0.080
t- test (2.228)a	1.750		1.550		1.700	
F-test (5.1)b	2.500		1.071		4.200	

* Average of six different experiments.

a) Tabulated t- value for 6 degrees of freedom at 95% confidence limit.

b) Tabulated F- value for 6 degrees of freedom at 95% confidence limit.

Table 3 : Application of the proposed method using p-DAC for the determination of amlodipine besylate, dobutamine HCl and carvedilol in its drug products.

Parameters	Amlodipine besylate Alkapress tablets 5 mg B.N. 072		Dobutamine HCl Dobutamine HCl Ampoules 250 mg/25ml B.N. 120.		Carvedilol Dilator tablets 25 mg B.N. 19503	
	Using p-DAC	Reported UV method [39]	Using p-DAC	official HPLC method [1]	Using p-DAC	Reported HPLC method [40]
Accuracy Mean*±RDS%	99.57± 0.483%	99.53± 0.49%	99.77± 0.216%	99.65± 0.182%	99.61± 0.272%	99.54± 0.253%
SD	0.481	0.488	0.216	0.181	0.271	0.252
Variance (V)	0.231	0.238	0.046	0.033	0.073	0.064
SE	0.196	0.199	0.086	0.074	0.111	0.103
t- test (2.228)a	0.144		0.902		0.461	
F-test (5.1)b	1.030		1.39		1.14	

* Average of six different experiments.

a) Tabulated t- value for 6 degrees of freedom at 95% confidence limit.

b) Tabulated F- value for 6 degrees of freedom at 95% confidence limit.

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