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Visible Spectrophotometric Method for Determination of Lisinopril dihydrate and Enalaprilmaleate in Bulk and Pharmaceutical Formulations by DDQ/DMF/Dioxane

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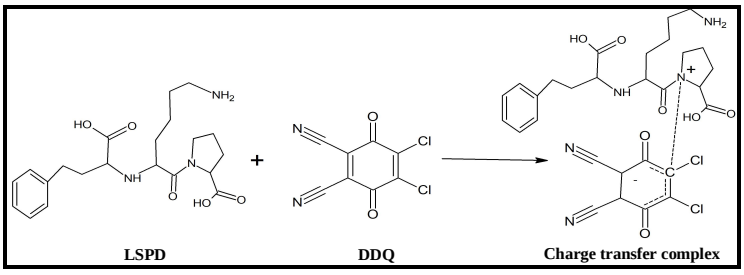
ABSTRACT

A simple and sensitive Spectrophotometric method for the determination of Lisinopril dihydrate and Enalapril maleate in bulk and in pharmaceutical formulations has been developed and validated. This method is based on the formation of colored product as a charge transfer complex when the drug reacts with DDQ and Dioxane. The optimum conditions of the reactions for the proposed method were studied and optimized. Results of the assay were statistically validated and recorded. The proposed method was applied successfully for the determination of lisinopril dihydrate and enalapril maleate in commercial dosage forms and no significant interference was observed from the excipients commonly used as pharmaceutical aids with the assay procedure. System suitability, specificity, linearity, accuracy and precision were performed.

Graphical Abstract



Keywords: Lisinopril dihydrate, Enalapril maleate, DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone), DMF(n, n-dimethyl formamide), Dioxane, Visible spectrophotometer.

INTRODUCTION

Lisinopril dihydrate (LSPD) is a potent [1], competitive inhibitor of angiotensin-converting enzyme (ACE), the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the rennin-angiotensin aldosterone system

(RAAS). [2-4] Lisinopril may be used to treat hypertension and symptomatic congestive heart failure, to improve survival in certain individuals following myocardial infarction and to prevent progression of renal disease in hypertensive patients with diabetes mellitus and micro albuminuria or overt nephropathy [5-6].

Enalapril maleate (EPM) is a potent angiotensin converting (ACE) enzyme inhibitor [7-9]. It is a pro-drug without direct biological activity which is rapidly absorbed after oral administration and de-esterified *in vivo* to its active metabolite Enalapril at diketopiperazine derivative (DKP) and has little pharmacologic activity until hydrolyzed in the liver to Enalapril [10-17]. This agent is able to reduce cardiovascular mortality and morbidity in patients with heart failure.

LSPD and EPM were some published methods for estimation of assay and impurity profile by HPLC, UV/Visible spectroscopy techniques [18-22]. The aim of present work is to validate the method for assay of LSPD and EPM in formulation to show specificity, linearity, precision, accuracy and stability in analytical solution by visible spectrophotometer. Method validation has performed as per the ICH and regulatory guidelines and review articles were revealed for method development and validation.

MATERIALS AND METHODS

Instrument and Chemicals: A Systronics-119 UV-Visible spectrophotometer with pc connection was used for spectral and absorbance measurements. Sartorius BT 224s analytical balance was used for this research experiments. The reference samples of LSPD and EPM were supplied as a gift sample from Hetero labs limited, Hyderabad. The commercially available LSPD and EPM solid dosage forms were procured from the local market. All the chemicals used were of analytical grade and the solutions were prepared with double distilled water.

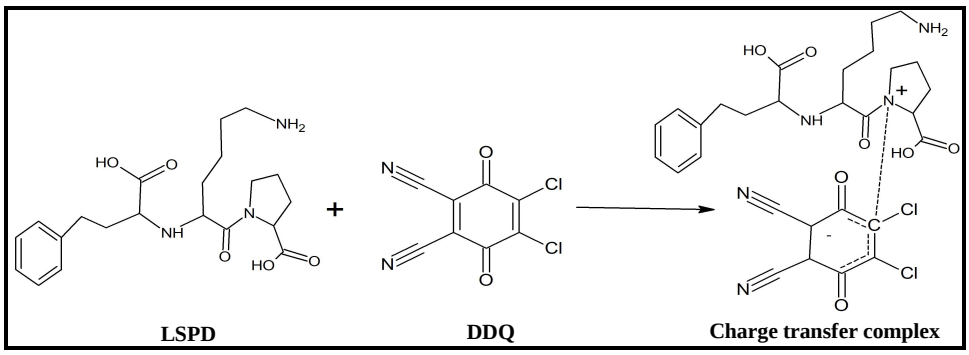
Preparation of standard drug solution

LSPD: The stock solution (1 mg mL^{-1}) of LSPD was prepared by dissolving 100 mg of it in 100mL of 0.05M hydrochloric acid. A portion of this stock solution was diluted sequentially with the same solvent to obtain the working standard drug solution of concentrations of 100 µg mL^{-1}.

EPM: The stock solution (1 mg mL^{-1}) of EPM was prepared by dissolving 100 mg of it in 100 mL of 0.05M hydrochloric acid. A portion of this stock solution was diluted sequentially with the same solvent to obtain the working standard drug solution of concentrations of 100 µg mL^{-1}.

Preparation of reagents

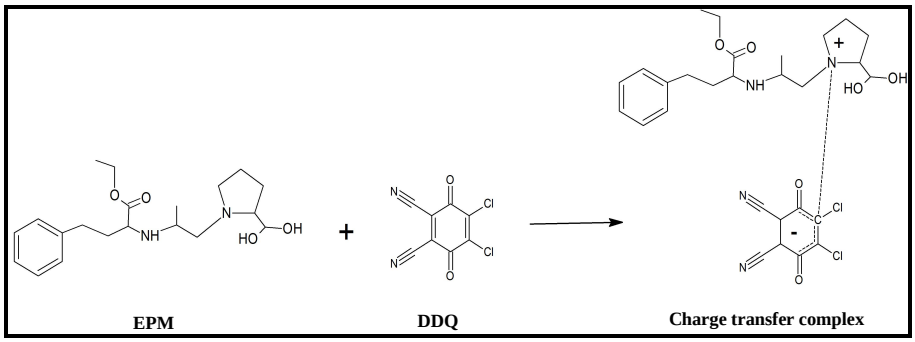
DDQ solution (0.1%): Prepared by dissolving 100 mg of DDQ, initially in 10 mL of DMF followed by dilution to 100 ml with dioxane. **Dioxane (Qualigens**): AR grade dioxane was used as it is.



Scheme I

Scheme-I: DDQ forms charge-transfer complex with a drug containing basic nitrogen. In the present investigation, a blue colored charge transfer complex is formed between DDQ (e acceptor) with LSPD. In this investigation LSPD (e donor) gives intermediate Sigma complex with DDQ. This has been utilized in the visible spectroscopic assay of donors. The probable mechanism was shown below.

Scheme- II: DDQ forms charge-transfer complex with EPM containing basic nitrogen. In the present investigation, a blue colored charge transfer complex is formed between DDQ (e acceptor) with EPM. In this investigation EPM (e donor) gives intermediate Sigma complex with DDQ. This has been utilized in the visible spectroscopic assay of donors. The probable mechanism was shown below.



Scheme II

General procedure for LSPD/EPM: Different aliquots of working standard solution (0.2 to 1.2 mL) of LSPD/EPM were transferred into a series of 10 mL volumetric flask, so as to get concentration range 0.02 – 0.12 mg mL^{-1} and the volume in each tube was adjusted to 3.0 mL with dioxane. Then 2.0 ml of DMF and 2.5 mL of DDQ solutions were added and the total volume in each tube was adjusted to 10 mL with dioxane. The absorbance of the blue colored species was measure at 476 nm for LSPD and 480 nm for EPM against a reagent blank during the stability period (60 min). The amount of the LSPD/EPM present was calculated from the Beer's plot.

RESULTS AND DISCUSSION

LSPD and EPM contain active tertiary and secondary amine groups. An attempt has been made to indicate the nature of colored species formed in the proposed method for the determination of LSPD and EPM based on analogy.

Optimization of the conditions on absorption spectrum of the reaction product: The condition under which the reaction of LSPD and EPM with DDQ and dioxane fulfills the essential requirements was investigated. All conditions studied were optimized at room temperature (32±2°C).To find a suitable medium for the reaction; different acidic media have been used. The best results were obtained when 0.05M hydrochloric acid for LSPD and EPM. From the absorption spectra it was evident that the solutions were found optimum. Larger volumes had no significant effect on the absorbance of the colored species.

Several experiments were carried out to study the influence of DDQ concentration on the color development by keeping the concentrations of drug and dioxane constant and changing reagent concentration (0.5–3.0 mL). It was apparent that 2 mL of DDQ for LSPD and EPM gave maximum color. Several experiments were carried out to study the influence of DMF concentration on the color development by keeping the concentrations of drugs, DDQ constant and changing DMF concentration. It was apparent that 2.5 mL of reagent gave maximum color. The color reaction was not instantaneous. Maximum color was developed within 5 min of mixing the reactants and was stable for 2 h thereafter.

Absorption spectrum and calibration graph: Absorption spectrum of the colored complex was scanned at 400-600 nm against a reagent blank. The reaction product showed absorption maximum at 476 nm for LSPD and 474 nm for EPM. Calibration graph was obtained according to the above general procedure and graphs were shown in the figures 1-6. The linearity replicates for six different concentrations of LSPD and EPM were checked by a linear least - squares treatment. All the spectral characteristics and the measured or calculated factors and parameters were summarized in table1.

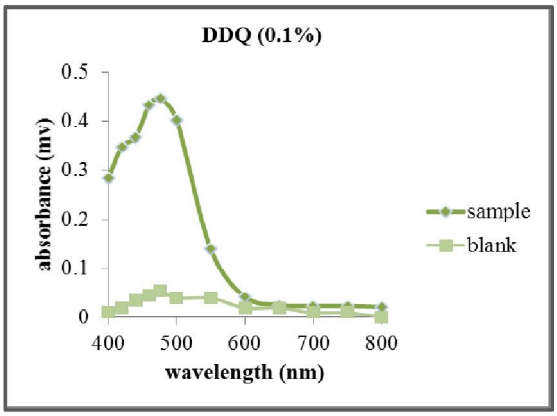


Figure1. Absorption spectrum of LSPD with DDQ

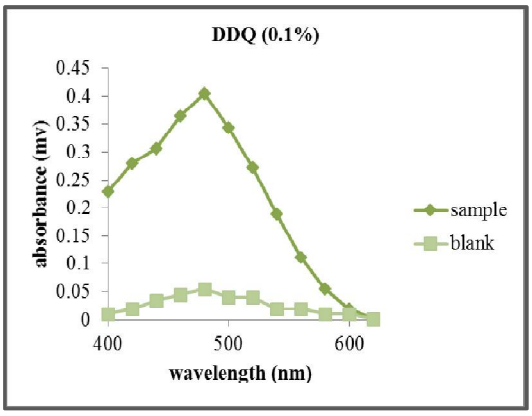


Figure 2. Absorption spectrum of EPM with DDQ

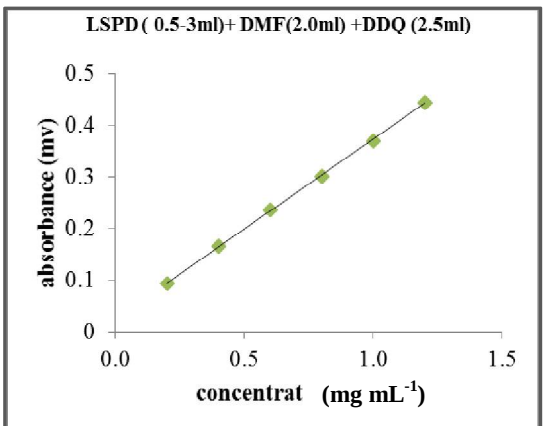


Figure 3. Beer's law plot of LSPD with DDQ

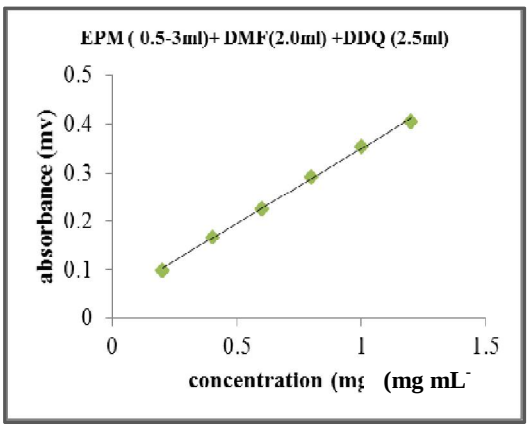


Figure 4. Beer's law plot of EPM with DDQ

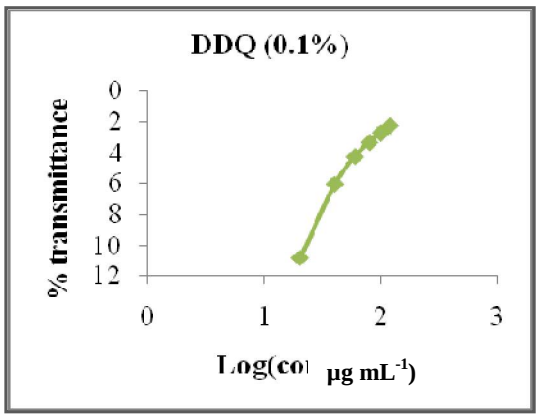


Figure 5. Ringbom plot of LSPD with DDQ

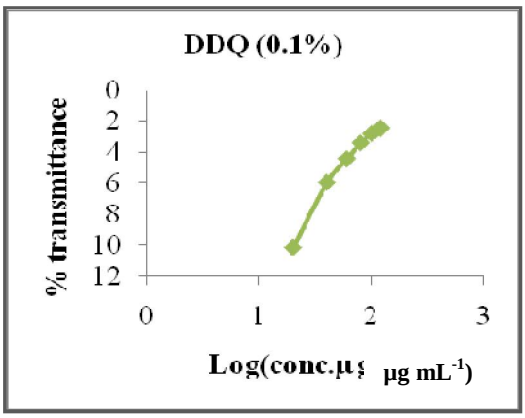


Figure 6. Ringbom plot of EPM with DDQ

Table 1. Optical and regression characteristics of the proposed methods

Parameter	LSPD	EPM
λ max (nm)	476	480
Beer's law limit (µg mL^{-1})	20-120	20-120
molar absorptivity, L $mol.cm^{-1}$	$1.6373x10^3$	$1.662x10^3$
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.2697	0.2964
Slope(b)	0.3493	0.3087
Intercept(a)	0.0237	0.0411
Correlation coefficient ®	0.9998	0.9991
r^2	0.9996	0.9982
SD(Standard Deviation)	0.1307	0.1156
Standard error on estimation(s_e)	0.0597	0.0528
Standard deviation on slope (s_b)	0.0713	0.0631
Standard deviation on Intercept (s_a)	0.0555	0.0491
LOD(Limit of Detection)	0.5248	0.5251
LOQ(Limit of Quantification)	1.5902	1.5913
% RSD(Relative Standard Deviation)	0.1111	0.2395
Precision: 0.01level	0.0713	0.0985
Precision: 0.05 level	0.0496	0.0686

System suitability: A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and %RSD of standard reading were taken for same. Acceptance criteria for system suitability, %RSD of standard reading not more than 2.0%, were fulfilled during all validation parameters.

Specificity: Results of tablet solutions showed that there is no interference of the excipients when compared with the working standard solution. Thus, the method was said to be specific.

Accuracy: For the accuracy of proposed methods, recovery studies were performed by standard method at three different levels (50%, 75 % and 125% of final concentration). A known amount of standard pure drug was analyzed by proposed methods. Results of recovery studies were found to be satisfactory.

Precision: The repeatability of the proposed method was ascertained by three replicates of fixed concentration (1.2 mg/ml) within the Beer's range and finding out the absorbance by the proposed method. The method precision was carried out by intraday and inter-day measurement. From this absorbance % RSD was calculated. The calculated % RSD observed is well below 0.1111% for LSPD and 0.2395% for EPM indicates that the methods are precise.

LSPD and EPM were estimated successfully by the developed extractive spectrophotometric methods, a pure compound and as a pharmaceutical formulation. The proposed method was suitable and valid for application in laboratories lacking of liquid chromatographic or other sophisticated instruments. These methods were simple, rapid, accurate, and does not involve any critical reaction conditions, or tedious sample preparation. It is unaffected by slight variations in experimental conditions such as pH, dye concentration, shaking time and temperature. Hence, these proposed methods can be used for the routine analysis of the cited drugs in their available dosage form.

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified, under the stated conditions of the test. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of test. The LOD and LOQ can be expressed as

$$LOD=3S_a/b,$$

$$LOQ=10S_a/b,$$

Where S_a is the standard deviation of the response and b is the slope of the calibration curve.

APPLICATION

Proposed method in the present study based on the reactivity of Lisinopril dehydrate and Enalapril maleate was used to produce color species with reasonable stability paving possibility for the determination of the drugs in bulk and pharmaceutical formulations by visible spectrophotometry. The results are tabulated in the table 2.

Table 2. Assay and recovery studies of proposed methods for drugs in pharmaceutical formulations

LSPD				EPM			
Formulation Tablet	**Labeled amount**	**Avg± Std.dev**	**% recovery**	**Formulation Tablet**	**Labeled amount**	**Avg±Std.dev**	**% recovery**
Acinopril Cipril	10 mg	9.896±0.068885 T = 0.27 F =1.00	99.84%	Oren Enapril	20 mg	19.87±0.100429 T =0.37 F =0.38	99.95%
Acebitor Lisnopril	5 mg	4.851± 0.09815 T = 0.94 F =1.06	99.67%	Hytrol Enapril	10 mg	9.867± 0.0970 T = 0.95 F =0.62	99.65%

CONCLUSION

Lisinopril and Enalapril were estimated effectively by the development extractive spectrophotometric methods, a pure compound and as a pharmaceutical formulation. The proposed methods were suitable and valid for application in laboratories lacking of liquid chromatographic or other sophisticated instruments. These methods were also simple, rapid and accurate does not involve any critical reaction conditions or tedious sample preparation. It is unaffected by slight variations in experimental conditions such as pH units, dye concentration, shaking time and temperature. Hence, these methods were suggested and can be used for the routine analysis of cited drugs in their available dosage forms.

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