Spectrophotometric determination of Clinidipine, Efavirenz, Prazosin drugs by using Folin-Ciocalteu Reagent

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Abstract : For the determination of three drugs namely Cilnidipine (CDP), Efavirenz (EVZ), Prazosin (PRZ), a simple, fast, selective and accurate spectrophotometric method has been outlined. This method is based on the development of blue coloured chromogen due to reduction of tungstate and/ or molybdate in Folin-Ciocalteu reagent by Drug in alkaline medium. The coloured species has an absorption maximum at 730 nm, 745 nm and 770 nm and the Beer's law obeys over the concentration range of 12-72 μg/mL, 10-70 μg/mL, 3-21 μg/mL respectively for the drugs CDP, EVZ, PRZ. This method has been validated according to ICH guidelines and applied to the analysis of pharmaceuticals.

Keywords : Spectrophotometry, Folin-Ciocalteu reagent, drugs, pharmaceutical formulations.

1. Introduction

1.Cilnidipine (CDP) is chemically known as 3-(E)-3-Phenyl-2-propenyl 5-2-methoxyethyl 2,6-dimethyl-4-(m nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. Cilnidipine is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals that supply blood vessels. It has a long-lasting, slow onset vasodilating effect.

Literature survey reveals that various analytical methods reported for the Determination of Cilnidipine in human plasma and pharmaceutical preparations either as a single drug or in conjunction with other medications. Those are Spectrophotometry [1-3], HPLC [4-6], HPTLC [7-9], Spectrofluorimetric [10].

2. Efavirenz (EVZ) is chemically (4S)-6-chloro-4-(cyclopropylethynyl)- 1,4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one. It is a non-nucleoside reverse transcriptase inhibitors (NNRTIs) class of HIV medicines. NNRTIs bind and block a reverse transcriptase HIV enzyme. NNRTIs prevent HIV from multiplying by blocking reverse transcriptase, and can reduce the amount of HIV in the body.

Due to its physiological importance, it has been quantified using several methods mentioned in the recent reference, such as Spectrophotometry [11-15], HPLC [16-20], HPTLC [21].

3. Prazosin (PRZ) is chemically [4-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-1-piperazinyl](2-furyl) methanone. Prazosin belongs to the α1-adrenoblocker group and is used to treat arterial hypertension and prosthetic hypertrophy in clinical practice. The drug is used to treat post-traumatic stress disorder successfully in children and servants working in difficult conditions.

The literature survey found that different methods for analysing and estimating of Prazosin are reported, those are Spectrophotometric [22-24], HPLC [25-28], HPTLC [29], Voltammetry [30].

Chromatographic methods are the most widely used ones. Visible spectrophotometry is perhaps the most generally used method of assaying the drugs mentioned. The various methods listed above suffer from one or more of the drawbacks such as extreme experimental conditions, use of organic solvent, longer standing time, low sensitivity and narrow linear range. Folin-Ciocalteu (FC) reagent has been widely used to evaluate a wide range of pharmaceutically relevant organic compounds. The main aim of the present work was to study the utility of FC reagent in drug assay i.e., CDP, EVZ and PRZ. The method had sufficiently good accuracy and provided a simple, time-saving assay of the drugs listed. The Significance of the method is the proposed method is used in drug formulations and also in quality control laboratories. Moreover, the suggested approach was used for the determination of drugs in commercial pharmaceutical dosage forms, which were statistically correlated with reference methods by t-test and F-test and found not to differ significantly at confidence levels of 95 per cent. The method is distinguished by its simplicity with precision and accuracy.

2. Experimental

2.1. Instrumentation

All absorption spectra were recorded on SHIMADZU 2600 UV-Visible double beam spectrophotometers using quartz cells of 10mm path length. A Dhona 200 single pan electrical balance is used for weighing the samples.

2.2. Materials and Reagents

All chemicals and reagents used were of analytical or pharmaceutical grade. Double distilled water was used throughout the investigation. Folin-Ciocalteu reagent (Merck, mumbai), Sodium hydroxide (Hetero Drugs Pvt Ltd, Hyd) were of analytical reagent grade and used without further purification. The pharmaceutical grade pure drug samples were kindly provided by GVK biosciences Pvt Ltd, Hetero Drugs Pvt Ltd, Dr Reddy's Laboratories, Hyderabad.

A stock standard solution of each drug containing 100 μg/mL were prepared by dissolving accurately weighed 10 mg of the respective drug transferred in to a 100 mL volumetric flask and made up to mark with distilled water. The solutions were further diluted quantitatively according to their linearity range. The pharmaceutical preparations were purchased from a local market and analysed.

2.3. Method development

Different aliquots of the drug solution (1 to 7 ml) were transferred into a series of 10 mL calibrated flasks. To each flask 1 mL 10% NaOH was added, followed by 2 mL 2N Folin-Ciocalteu reagent. The contents were mixed and they were set aside for 20 minutes under occasional shaking. The volume was made up to the mark with water and the absorbance of each solution was measured at 730-770nm range for respective drugs against a reagent blank.

2.4. Construction of calibration curves

The calibration curve was plotted by taking concentration of the drugs in X-axis and absorbance in Y-axis. The calibration curves were constructed by taking absorbance data in six replicate experiments. The absorbance to concentration called relative response is calculated. Those points falling between 95% to 105% of the average relative response are only considered for construction of calibration.

2.5. Accuracy and Precision studies

Accuracy of the methods developed are evaluated from the recovery studies on pure drug sample. At least four known concentration of drug solutions have been brought into Beer's law limit and recovery studies have been carried out. Excellent recovery showed the validity of the calibration curves for each drug. Precision of the procedure is illustrated by repeating experiment (n=6) and %RSD is found out. %RSD being less than 1 in each case speaks the high precision of the method.

2.6. Procedure for analysis of pharmaceuticals

Cilnidipine

A quantity of finely ground tablet [CILACAR, 10 mg] powder equivalent to 20 mg of Cilnidipine was correctly weighed and taken into a 100 mL calibrated flask, the flask was shaken roughly 3 minutes after adding 60 mL of water and finally the volume was made up to the mark with distilled water. For 20 minutes the content was kept aside, and filtered using Whatman No. 42 filter paper. The filtrate's first 10mL portion was discarded, and a sufficient aliquot of the corresponding portion was diluted sufficiently to achieve a working concentration and the assessment was carried out in accordance with the procedure mentioned above.

Efavirenz

About two tablets (EFAVIR, 600mg) were weighed and pulverized into fine powder.quantity equivalent to 20mg of the Efavirenz was dissolved in methanol. The contents were filtered through Whatman filter paper No. 42. The methanol was evaporated and the residue was dissolved in 100mL of distilled water. The content was kept aside for 15 minutes. This solution was further diluted to get a working concentration for the estimation of the drug.

Prazosin

Twenty tablets (PRAZOPRESS, 5mg) were weighed and pulverized into a fine powder. An accurately weighed quantity containing 10mg of Prazosin drug was transferred into a 100mL volumetric flask, 40mL water added, shaken well for 5 minutes and made up to mark with distilled water. The solution kept aside for 20 min and then filtered through Whatman filter paper No. 42. The solutions were further diluted according to their linearity range and analysed by the above described procedure.

The drug solutions extracted from tablet formulations were subjected to redox reaction with Folin-Ciocalteu reagent and subsequent determination was done. The concentration of tablet solutions falling under Beer's law limit was chosen for drug assay in the tablet. An outstanding tally between the concentration of drugs taken and found indicated the applicability of methods for formulations.

3. Results and Discussions

The Folin-Ciocalteu reagent is used to evaluate several pharmaceutical-interest substances. The structural features of above-mentioned drugs permitted Folin-Ciocalteu reagent to be used for its analysis. The proposed technique is based on the development of a blue coloured chromogen, following the reduction of phosphor-molybdo tungstic mixed acid of the F-C reagent by phenolic drugs, in the presence of sodium hydroxide, which could be estimated at 730-770nm range for respective drugs. The mixed acids in the F-C reagent are the final chromogen and involve the following chemical species:

$3H_2O.P_2O_5 .13 WO_3 .5MoO_3 .10H_2O$ and

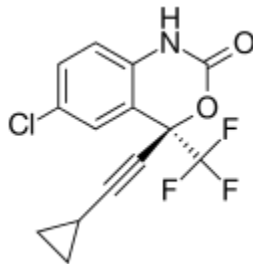$3H_2O.P_2O_5 .14 WO_3 .4 MoO_3 .10H_2O$.
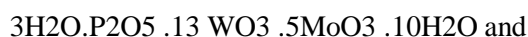
Drugs are likely to reduce tungstate and/or molybdate oxygen atoms in the F-C reagent by producing one or more possible reduced species which have characteristic intense blue colour. The method is based on the red-ox reaction of drugs in sodium hydroxide medium with Folin-Ciocalteu reagent and the resulting blue coloured chromogen is evaluated at 730-770nm range for respective drugs.
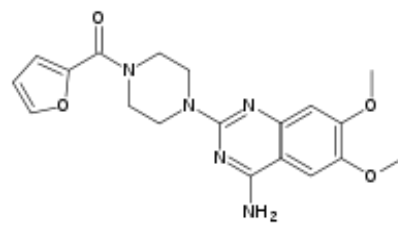
Structures of Drugs
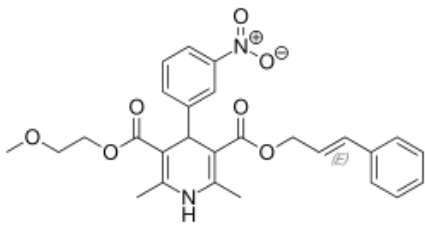
Fig-1: Cilnidipine   **Fig-2: Efavirenz**   **Fig-3: Prazosin**

Calibration Curves

Fig-4: Calibration curve of Cilnidipine

$y = 0.0101x - 0.0632$
$R^2 = 0.9992$
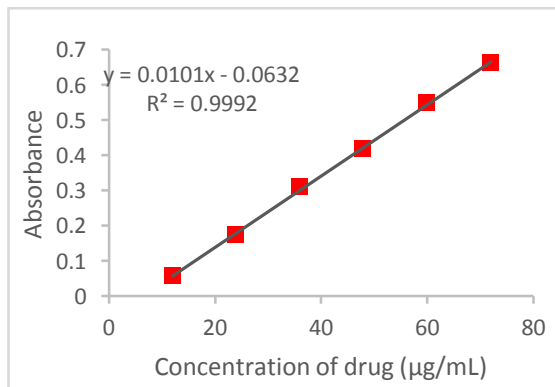
Fig-5: Calibration curve of Efavirenz

$y = 0.0099x + 0.0302$
$R^2 = 0.9989$
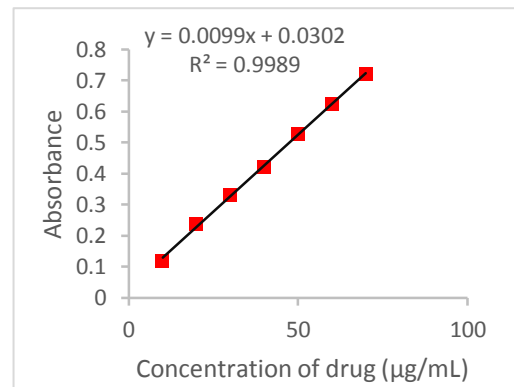
Fig-6: Calibration curve of Prazosin

$y = 0.0505x - 0.0739$
$R^2 = 0.9979$
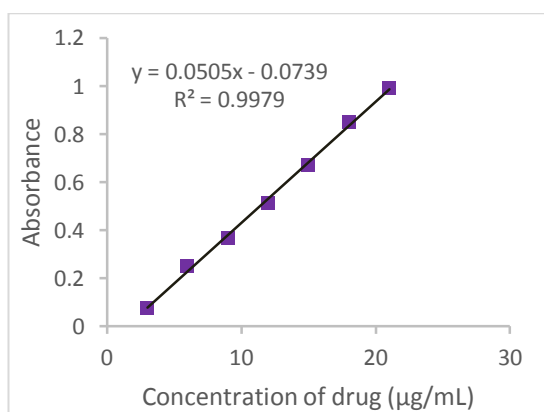
Table-1: Analytical Parameters for determination of drugs By Red-Ox reaction with Folin-Ciocalteu reagent.

Parameters	CDP	EFZ	PRZ
λm*ax (nm)*	730	745	770
Beer's law Limits(μg/mL)	12-72	10-70	3-21
Regression equation, Y	=0.0101x-0.0632	=0.0099x+0.0302	=0.0505x-0.0739
Intercept (a)	0.0632	0.0302	0.0739
Slope (b)	0.0101	0.0099	0.0505
SD of Intercept (Sa)	0.003	0.012	0.016
SD of Slope (Sb)	0.0049	0.009	0.008
Limit of Detection μg ML^{-1}	0.98	4	1.04
Limit of Quantification μg mL^{-1}	2.97	12.12	3.16
Correlation coefficient (r)	0.9995	0.9994	0.9989
Sandell sensitivity (μg cm^{-2})	0.099	0.1	0.019
Molar absorptivity L mol^{-1} cm^{-1}	2.35×10^{3}	3.74×10^{3}	9.7×10^{3}

Table-2: Determination of accuracy and precision of the methods on pure drug samples.

Drug	Taken (μg/mL)	Found (μg/mL)	Error (%)	Recovery (%)	RSD (%)	Proposed method mean±SD
CDP	24	24.01	0.04	100.04	0.569	99.72±0.568
	36	35.96	0.12	98.88		
	48	48.04	0.08	100.08		
	60	59.94	0.10	99.90		
EFZ	20	19.99	0.05	99.95	0.071	99.95±0.071
	30	30.02	0.06	100.06		
	40	39.96	0.1	99.90		
	50	49.96	0.08	99.92		
PRZ	9	9.00	0.0	100.0	0.179	99.93±0.179
	12	12.02	0.16	100.16		
	15	14.97	0.2	99.80		
	18	17.96	0.22	99.78		

Table-3: Results of assay of tablets by proposed method and statistical evaluation and recovery experiments by standard addition method.

Drug	Taken (µg/mL)	Found (µg/mL)	Error (%)	Recovery (%)	RSD (%)	Proposed method mean$\pm$ SD	Reference method mean$\pm$SD	t-Test	F-Test
CDP	26 34 46 58	25.97 33.98 46.04 57.94	0.11 0.06 0.08 0.10	99.89 99.94 100.08 99.90	0.087	99.95$\pm$0.087	99.97$\pm$0.183	1.0	0.22
EFZ	15 24 32 45	15.02 24.01 31.96 45.01	0.13 0.04 0.125 0.02	100.13 100.04 99.875 100.02	0.104	100.01$\pm$0.105	100.04$\pm$0.197	0.92	0.29
PRZ	8 12 16 20	08.01 11.99 15.98 20.01	0.125 0.08 0.125 0.05	100.125 99.92 99.875 100.05	0.115	99.99$\pm$0.115	100.06$\pm$0.104	0.2	1.3

5. Conclusion

A simple, selective, rapid and sensitive method has been proposed for the assay of drugs and in pharmaceutical formulations. The method is focused on the well-characterized and proven red-ox reaction and uses very simple, cheaper chemicals and easily accessible instruments. Other features like short performance time, ease of handling and the non-use of organic solvents also suggest this procedure as a routine laboratory method. The method is successfully applied to quantifying drugs in tablets and injecting them without intervention from common excipients. The current method is ideal for evaluation of CDP, EVZ and PRZ in bulk drugs and pharmaceuticals; therefore, this method can be used in laboratories for quality control.

6. References

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