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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF DESVENLAFAXINE AND CLONAZEPAM IN TABLET DOSAGE FORM

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Abstract: A simple, rapid, accurate and precise Spectrophotometric method for Simultaneous Estimation of Desvenlafaxine and Clonazepam in tablet dosage form have been developed. The method was second order derivative technique which was based on Zero Crossing Point (ZCP) values. The wavelength 293 nm was selected for the determination of Desvenlafaxine (ZCP of Clonazepam) and wavelength 340nm was selected for determination of Clonazepam (ZCP of Desvenlafaxine). Beer-Lambert's law obeyed in linearity range of 200-700 µg/mL and 2-7 µg/mL for Desvenlafaxine and Clonazepam respectively. The regression coefficient for Desvenlafaxine and clonazepam was 0.9964 and 0.9983 respectively. Method is validated according to ICH guideline and carried out for analysis of Desvenlafaxine and Clonazepam in pure and in marketed tablet dosage form.

Keywords: Desvenlafaxine, Clonazepam, Second-Derivative, UV spectrophotometeric, Validation

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INTRODUCTION

Desvenlafaxine (DES) chemically 4-[2-dimethylamino-1- (1-hydroxycyclohexyl) ethyl] phenol. It is Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) that is approved by the USFDA for the treatment of adult major depressive disorder (MDD). The clinical effect of Desvenlafaxine is thought to occur via potentiation of serotonin and norepinephrine in the central nervous system[1-3]. The structure of Desvenlafaxine is shown in figure 1.

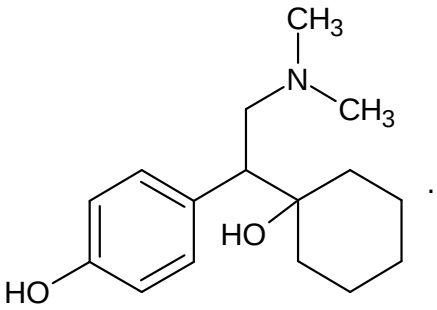

Figure 1: Structure of Desvenlafaxine

Clonazepam (CLO) chemically 5-(2-chlorophenyl)-7-nitro-1,3-dihydro-2*H*- 1,4- benzo- diazepin-2-one. It is Anticonvulsants Benzodiazepines, GABA Modulators that is Used as an anticonvulsant in the treatment of the Lennox-Gastaut syndrome (petit mal variant), akinetic and myoclonic seizures. It is work by acting on GABA receptor. Allosteric interactions between central benzodiazepine receptors and gamma-aminobutyric acid (GABA) receptors potentiate the effects of GABA. As GABA is an inhibitory neurotransmitter, this results in increased inhibition of the ascending reticular activating system. Benzodiazepines, in this way, block the cortical and limbic arousal that occurs following stimulation of the reticular pathways[4, 5]. The structure of Clonazepam is shown in figure 2.

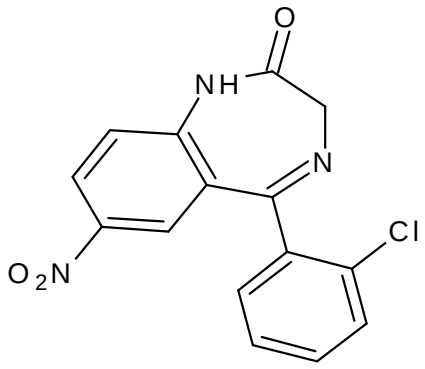

Figure. 2: Structure of Clonazepam

MATERIALS AND METHODS

Instruments

A double beam UV-Visible Spectrophotometer (UV-2600 shimazdu) with a paired of matched quartz cell of 1cm of width was used for measuring absorbance. Acculab ALC-210.4 Digital balance was used for weighing and ultrasonicator of Enertech Fast Clean used for sonicating the drug and sample solutions.

Materials

Desvenlafaxine was gifted from Zydus Cadila, Ahmedabad and Clonazepam was kindly gifted from Apostte Remedies, Vadodara. Tablets of ZyVen OD Plus were purchased from local market; each tablet was labelled to contain 50 mg of DES and 0.5 mg of CLO. All chemical use for development were analytical grade.

Selection of Derivative Condition:

Using memory channels, the second-order derivative spectra were overlapped. The zero crossing point (ZCP) values of DES at which the CLO showed some derivative response, were recorded. The wavelength 293 nm was selected for the determination of DES (where the derivative response for CLO was zero). Similarly, 340 nm was selected for the determination of CLO (where the derivative response for DES was zero). Characteristics wavelengths (ZCPs) for DES and CLO were confirmed by varying the concentration of both drugs.

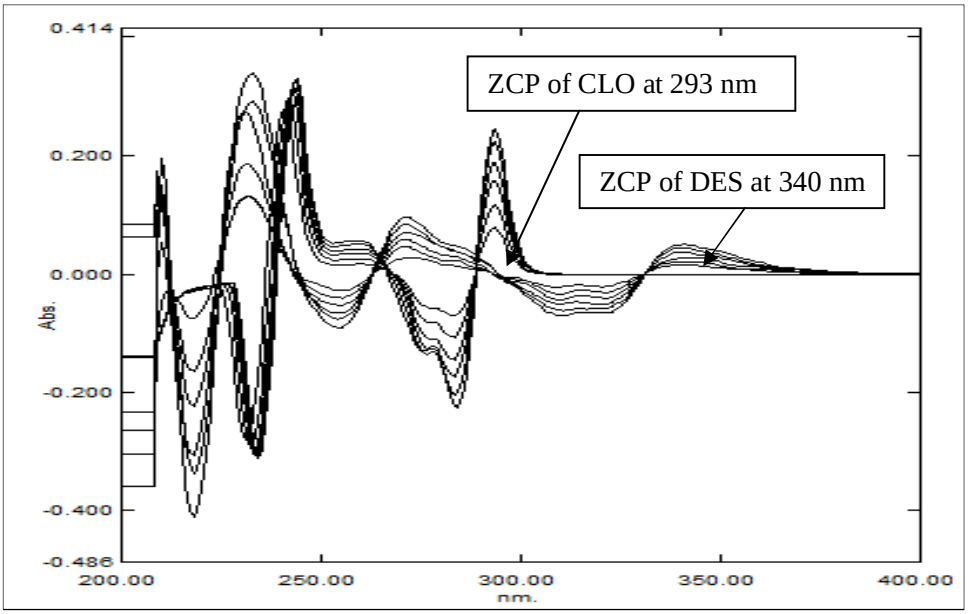

Figure 3: Overlay second derivative spectra of DES and CLO Standard

Preparation of Standard Stock Solution of DES (1000 µg/mL)

A standard stock solution containing 1000 µg/mL of DES was prepared by dissolving accurately weighed 50 mg quantity of DES in methanol and diluting to volume with methanol in a 50 mL volumetric flask.

Preparation of Standard Stock Solution of CLO (100 µg/mL)

A standard stock solution containing 100 µg/mL of CLO was prepared by dissolving accurately weighed 50 mg quantity of CLO in methanol and diluting to volume with methanol in a 50 mL volumetric flask. Take 1 mL and diluted up to 10 mL with methanol.

Preparation of Sample Solution

Twenty ZyVen OD Plus tablets were accurately weighed, calculate average weight and made fine powdered. An amount of tablet powder equivalent to 50 mg DES and 0.5 mg CLO was weighed and transferred into a 50 mL volumetric flask and 30 mL of methanol was added into it. The contents of the flask were sonicated for 15 min to dissolve the active ingredients completely. The solution was then diluted to 50 mL with methanol and solution was filtered through whatman filter paper .Take 3 mL aliquot from the filtered solution was transferred into a 10 mL volumetric flask to get concentration 300 µg/mL DES and 3 µg/mL CLO was then analysed for assay determination.

Preparation of Standard Calibration Solutions

Standard calibration solutions having concentrations of 200, 300, 400, 500, 600 and 700 µg/mL of DES & 2, 3, 4, 5, 6 and 7 µg/mL of CLO were prepared in 10 mL volumetric flasks by taking appropriate aliquots from the standard stock solution of DES and diluting them to volume with methanol.

VALIDATION OF THE DEVELOPED METHOD[6]

Linearity

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. Standard calibration curves were constructed daily, for three consecutive days, using six standard calibration solutions in a concentration range of 200-700 µg/mL of DES and 2-7 µg/mL of CLO.

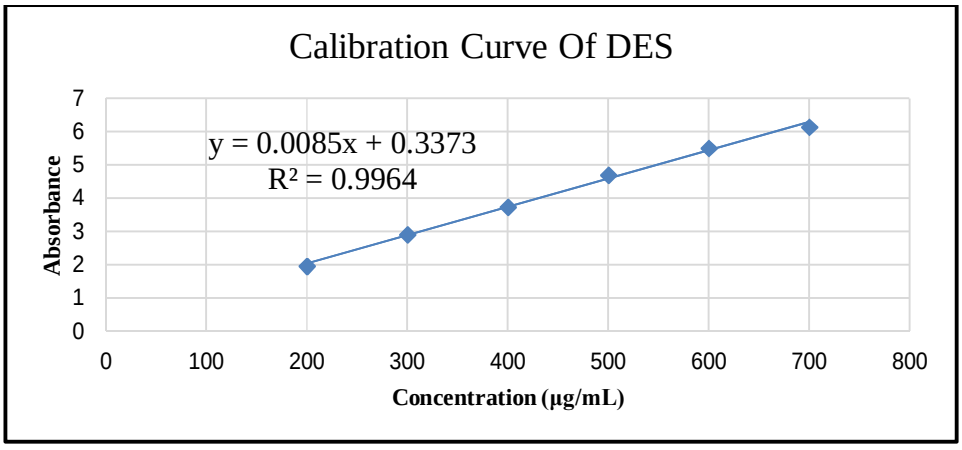

Figure 4: Calibration curve of DES at 293.0 nm

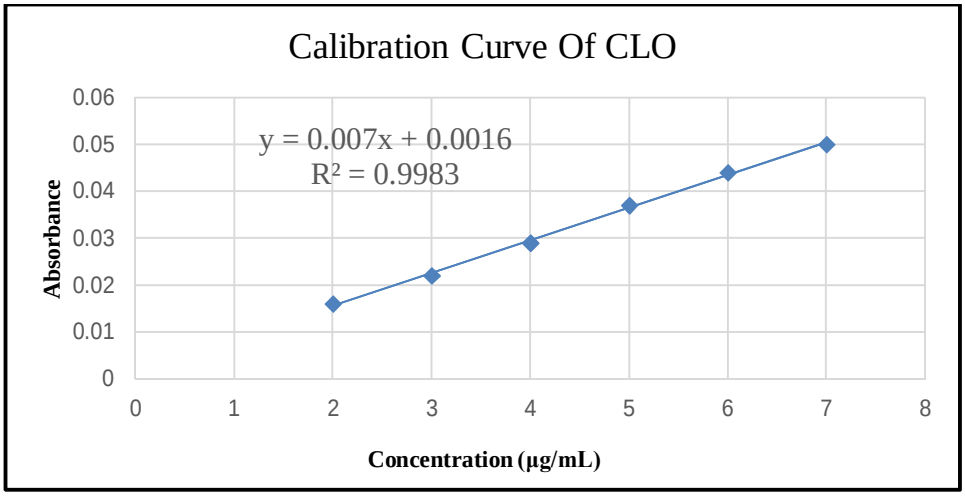

Figure 5: Calibration curve of CLO at 340.0 nm

Table 1: Linearity data for DES at 293 nm and CLO at 340 nm

Sr No.	Conc. of DES (µg/ml)	Abs of DES at 293 nm*± SD	% RSD	Conc. of CLO (µg/ml)	Abs of CLO at 340 nm* ± SD	% RSD
1	200	1.953 ± 0.018	0.97	2	0.016 ± 0.00027	1.71
2	300	2.904 ± 0.022	0.76	3	0.022 ± 0.00031	1.45
3	400	3.736 ± 0.030	0.81	4	0.029 ± 0.00053	1.83
4	500	4.693 ± 0.032	0.69	5	0.037 ± 0.00062	1.68
5	600	5.507 ± 0.040	0.73	6	0.044 ± 0.00067	1.54
6	700	6.139 ± 0.051	0.84	7	0.050 ± 0.00064	1.29

***=**Average of three determination

Table 2: Result of Regression study

Parameters	DES	CLO
Linearity Range	200-700 µg/mL	2-7 µg/mL
Regression line equation	y=0.0085+0.3373	y=0.007+0.0016
Slope	0.0085	0.007
Y-intercept	0.3373	0.0016
Correlation coefficient	0.9964	0.9983

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of DES and CLO by the standard addition method. Known amount of standard solutions of DES (240, 300 and 360 µg/mL) and (2.4, 3 and 3.6 µg/mL) were added to a pre quantified sample solution of DES (300 µg/mL) and CLO (3 µg/mL). The derivative response of DES and CLO were recorded. The percentage recovery was calculated by measuring the absorbance and fitting these values into the regression equation of respective calibration curves. Each response was average of three determinations.

Table 3: Recovery data for DES

Amount of sample (µg/mL)	Amount of Standard added (µg/mL)	Amount recovered Mean* ± S.D (µg/mL)	%Recovery	%RSD
300	240	554.99 ± 1.68	100.73	0.31
300	300	607.26 ± 2.55	101.21	0.42
300	360	658.87 ± 1.51	99.83	0.23

***=**Average of three determination

Table 4: Recovery data for CLO

Amount of sample (µg/mL)	Amount of Standard added (µg/mL)	Amount recovered Mean* ± S.D (µg/mL)	%Recovery	%RSD
3	2.4	5.34 ± 0.03	98.94	0.57
3	3	6.05 ± 0.07	100.95	1.27
3	3.6	6.48 ± 0.06	98.26	1.03

***=**Average of three determination

Precision

Repeatability (Intra-day Precision):

Repeatability (Intra-day precision) was determined by analysing of DES and CLO standard solutions in the range 300, 400, 500 µg/mL and 3, 4, 5 µg/mL for DES and CLO respectively in triplicate. Calculate % RSD for DES and CLO.

Table 5: Results of Repeatability

Concentration (µg/mL)		Amount Found* ± S.D		%RSD	
DES	CLO	DES	CLO	DES	CLO
300	3	2.904 ± 0.027	0.023 ± 0.00031	0.95	1.37
400	4	3.734 ± 0.011	0.028 ± 0.00040	0.32	1.43
500	5	4.694 ± 0.032	0.034 ± 0.00041	0.69	1.23

***=**Average of three determination

Inter-day Precision:

Inter-day precision was determined by analysing of DES and CLO standard solutions in the range 300, 400 and 500 µg/mL and 3, 4 and 5 µg/mL for DES and CLO respectively on the different days. Calculate % RSD for DES and CLO.

Table 6: Results of Inter-day precision

Concentration (µg/mL)		Amount Found* ± S.D		% RSD	
DES	CLO	DES	CLO	DES	CLO
300	3	2.905 ± 0.018	0.025 ± 0.00046	0.62	1.85
400	4	3.736 ± 0.021	0.027 ± 0.00040	0.57	1.50
500	5	4.697 ± 0.039	0.035 ± 0.00046	0.84	1.34

***=**Average of three determination

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of y-intercept of calibration curve (N) and slope (S) of the calibration curve.

$$LOD = \frac{\sigma}{s} * 3.3$$

$$LOQ = \frac{\sigma}{s} * 10$$

Where σ is the SD of the response

S is the slope of the calibration curve.

Table 7: LOD and LOQ limit for DES and CLO

DES (µg/mL)		CLO (µg/mL)	
LOD	LOQ	LOD	LOQ
6.98	21.17	0.12	0.38

Specificity

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre-weight quantity of drug and then absorbance was measured and calculation done to determine quantity of drugs.

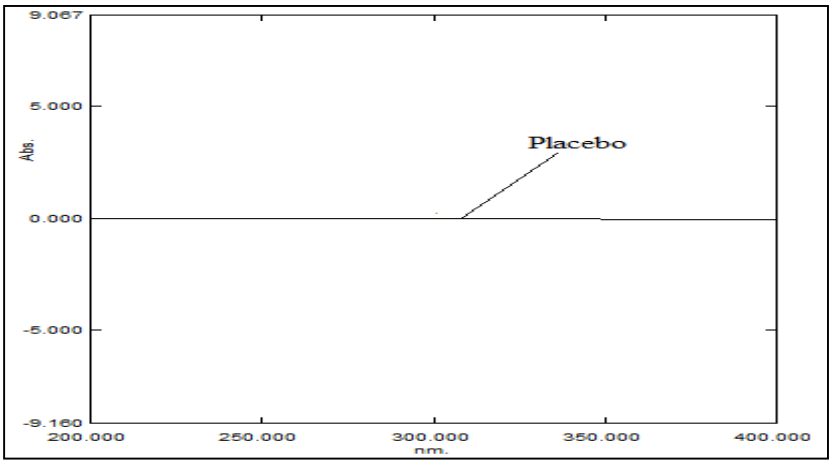

Figure 6: UV spectra of Placebo

Table 8: Analysis marketed formulation

Drug	Amount of drug (mg)		%Assay*	% RSD
	Labelled	Estimated		
DES	50	50.390	100.78 % w/w	0.51
CLO	0.5	0.495	99.04 % w/w	0.74

***=**Average of six Determination

RESULT AND DISCUSSION:

The DES and CLO shows solubility in Methanol and having the ZCP 293 nm and 340 nm for DES and CLO respectively. The linearity range for DES and CLO is 200-700 µg/mL and 2-7 µg/mL respectively at selected ZCPs (Table No. 1). The correlation of coefficient for DES at 293 nm and for CLO at 340 nm is 0.9964 and 0.9983 respectively, it shows good result (Table No. 2). DES and CLO have limit of detection (LOD) 6.98 µg/mL and 0.12 µg/mL and limit of quantitation (LOQ) 21.17 µg/mL and 0.38 µg/mL, respectively (Table no. 7). Both drugs shows good regression values at their respective ZCPs and result of recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by proposed method (Table No. 4). The % assay of DES and CLO from tablet dosage form is 100.78 % and 99.74 % with % RSD <1 (Table No. 8). Precision is determined by studying Repeatability and Inter-day. Repeatability and inter-day precision indicates the precision under same operating conditions over a short time period interval at same day and different day interval respectively. All precision study shows the % RSD <2%, which indicates good repeatability, intra-day precision

and good reproducibility of developed method (Table No. 5 and 6). Specificity of the method is indicated as there is no interference of placebo was found in the in to the standard mixture (Figure No. 6).

CONCLUSION

The proposed method found to be simple, economic, accurate and precise for the routine determination of Desvenlafaxine and Clonazepam in Tablet Dosage Form. Accuracy studies were carried out to check the validity and reproducibility of the developed method which was indicates good results for method. The method was validated as per ICH Q2(R1) in terms of linearity, accuracy, precision and specificity. So the method can be successfully used for simultaneous estimation of Desvenlafaxine and Clonazepam.

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