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Multivariate Calibration Technique for the Spectrophotometric quantification of Zaleplon in bulk drug and pharmaceutical formulations

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Abstract

The present study focuses on the development and validation of a rapid, sensitive and precise multivariate calibration technique employing UV spectrophotometric method. Multivariate calibration method utilizes the linear regression equations by correlating the relation between concentration and absorbance at five different wavelengths. The results were treated statistically. The developed method was validated as per the ICH guidelines and was found to be accurate, precise and reproducible. The method is accurate, precise and linear within the range 8 - 12 µg/mL. This statistical approach gives optimum results by eliminating the fluctuations arising from the instrumental or experimental conditions.

Key words: Zaleplon, Sedative – Hypnotic, UV spectrophotometer, Multivariate calibration

INTRODUCTION

Zaleplon is a pyrazolopyrimidine derivative that acts as an effective hypnotic, selectively binds to the α1 benzodiazepine receptors [1]. Zaleplon [Fig. 1] is chemically N-[3-(3-Cyanopyrazolo [1,-5-a] pyrimidin-7-yl) phenyl]-N-ethylacetamide. The molecular formula is $C_{17}H_{15}N_5O$ and has a molecular weight of 305.33 g/mol [2]. The drug is not official in any pharmacopoeias. The literature survey reveals the reported methods for the determination of Zaleplon includes spectrophotometry [3], spectroflurimetry [4], voltammetry [5], capillary electrophoresis [6], HPTLC [3], HPLC [3, 7-11], LC-MS [12-16], and a GC-MS [17] either in pharmaceutical formulations or in biological fluids.

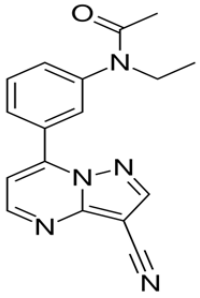

Figure 1- Chemical structure of Zaleplon

The proposed and developed method is based upon the direct determination of Zaleplon with a high degree of precision and accuracy. The method is easy, inexpensive and can be applied to bulk drug and pharmaceutical formulations. The present manuscript describes the applicability of UV spectral multivariate calibration technique having simple mathematical content for the estimation of Zaleplon in pharmaceutical dosage from.

Multivariate calibration represents the transition of common single species analysis from one dependant variable to 'm' dependant variables. e.g. wavelengths or sensors, which can be simultaneously included in the calibration model[18].

Under optimized conditions the applied statistical method provides considerable resolving power, sensitivity, rapidity and low cost for the quantitative analysis, quality control and routine analysis of the investing compounds.

If the absorbance of an analyte(X) is measured at five wavelengths set (λ = 226, 228, 230, 232 and 234 nm), the following equation can be written for each selected wavelength.

$$A_{\lambda 226} = a \text{ X } C_x + k_1 \quad \ldots\ldots (1)$$

$$A_{\lambda 228} = b \text{ X } C_x + k_2 \quad \ldots\ldots (2)$$

$$A_{\lambda 230} = c \text{ X } C_x + k_3 \quad \ldots\ldots (3)$$

$$A_{\lambda 232} = d \text{ X } C_x + k_4 \quad \ldots\ldots (4)$$

$$A_{\lambda 234} = e \text{ X } C_x + k_5 \quad \ldots\ldots (5)$$

where A_λ represents the absorbance of the analyte; a, b, c, d, e are the slopes of the linear regression functions of the analyte; k_1, k_2, k_3, k_4, k_5 are the intercepts of the linear regression functions at the five selected wavelengths and C_x represents the concentration of the analyte. The above five equation systems (1-5) can be summarized as

$$A_T = a \text{ x } C_x + b \text{ x } C_x + c \text{ x } C_x + d \text{ x } C_x + e \text{ x } C_x + K_T \quad \ldots\ldots (6)$$

which can be further simplified to

$$A_T = C_x (a+b+c+d+e) + K_T \quad \ldots\ldots (7),$$

where A_T and K_T represents the sum of the absorbance obtained and the sum of intercepts of regression equations at five wavelength set respectively. The concentration of the analyte X in a solution can be calculated by using the equation

$$C_x = \frac{A_T - K_T}{(a+b+c+d+e)}$$

EXPERIMENTAL

Chemicals and solvents

- Distilled water
- Methanol
- Analytical grade Zaleplon was obtained as gift sample from Orchid Pharma, Chennai. The marketed capsule formulation used was Zaplon (label claim - 10 mg), Torrent Pharmaceuticals, India, procured from the local market.

Solubility

- Very freely soluble in methanol, acetone, acetic acid, ethyl acetate, acetonitrile
- Sparingly soluble.

Instrumentation

- Perkin-Elmer UV-Visible double beam spectrophotometer
- Sonicator
- Electric balance

Solubility studies

Zaleplon was found to be freely soluble in methanol and sparingly soluble in water. Hence methanol: water (8:2 v/v) was used as the solubilizing agent throughout the study.

Standard preparation

100 mg of Zaleplon was weighed out accurately and transferred to a 100 mL volumetric flask; about 50 mL of the solubilizing agent was added followed by sonication for 15 mins. The resulting solution was filtered and suitable dilutions were made from the filtrate to make a concentration of 1 mg/mL. The above stock solution was serially diluted to yield the concentration ranging between 8–12 µg/mL.

Determination of absorption maxima (λmax)

The λmax was determined on a 10 µg /mL solution prepared by appropriate dilution of the standard stock solution and the λmax was found to be 230 nm [Fig.2]. To improve the correlation coefficient and minimize the instrumental fluctuations, absorbance of the solutions were measured covering the range of λmax (230 nm) i.e., 226, 228, 230, 232, 234 nm.

Preparation of sample solution

Twenty capsules of Zaleplon were weighed and the contents were transferred in to a mortar and mixed well. A weight equivalent to 10 mg of Zaleplon was weighed and solubilized in 10 mL of solubilizing agent to get 1 m λmax g/mL solution, filtered and diluted in the working concentration range of 8-12 µg/mL. The absorbance versus concentration gave a linear plot.

Method validation [19]

The method was validated as per ICH Q2B guidelines for linearity, sensitivity, precision and accuracy.

Linearity

Stock solution of Zaleplon was diluted with the solvent to get a concentration in the range of 8-12 µg/mL. Now in order to improve the correlation and minimize the instrumental fluctuations, absorbance of the solutions were measured over a range surrounding 230 nm i.e., 226, 228, 230, 232, 234 nm. The overlay spectra showing linearity at the λmax, absorbance of the different concentrations recorded at five different wavelength were shown in Figure 3, Table 1.

Precision

Intraday and inter day precision was performed by scanning the absorbance of 8, 10 and 12 µg/mL solution at all the five wavelength. The aliquots were scanned 3 times a day and for three days at the same time for intraday and interday precision [Table 2, Table 3].

Accuracy

The accuracy of the developed method was determined by standard addition method at 80%. 100% and 120%. From the prepared stock solutions of standard and sample, 0.5 mL of standard solution was pipetted into 3 volumetric flasks and 0.3, 0.5, 0.7 mL of the sample solution was added to the above volumetric flasks and the volume was made up to 10 mL with solvent. The aliquots were scanned using UV spectrometer and the % recovery was calculated.

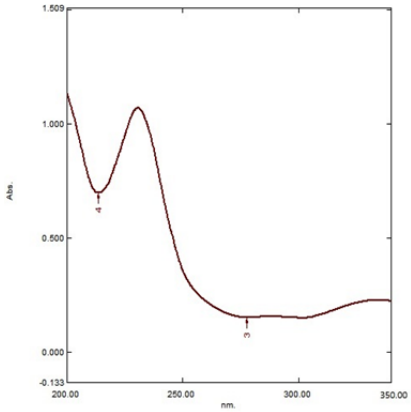

Figure 2 - UV spectrum of Zaleplon

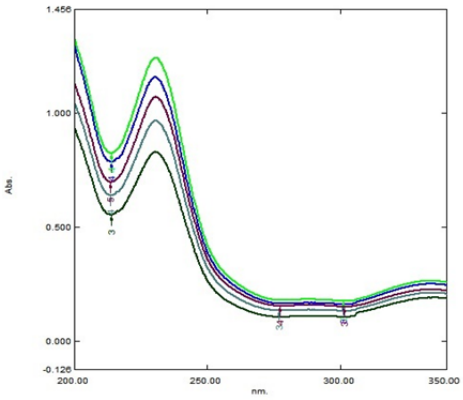

Figure 3 - UV spectrum showing linearity of Zaleplon at 230 nm.

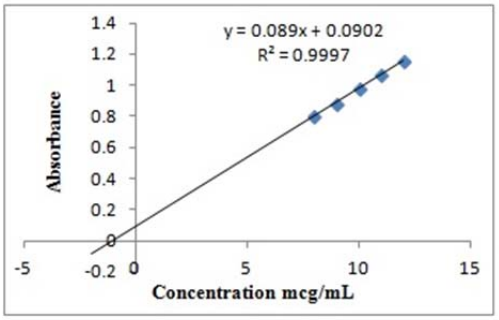

Figure 4 - Calibration graph at 226 nm

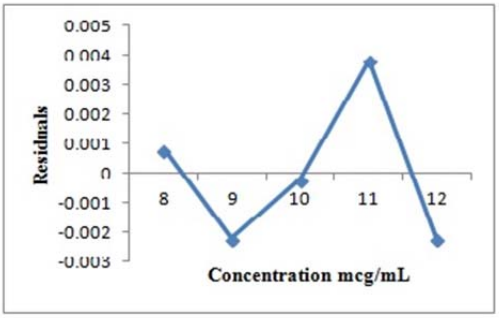

Figure 9 - Residual plot at 226 nm

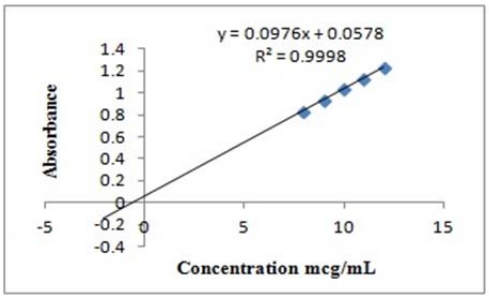

Figure 5 - Calibration graph at 228 nm

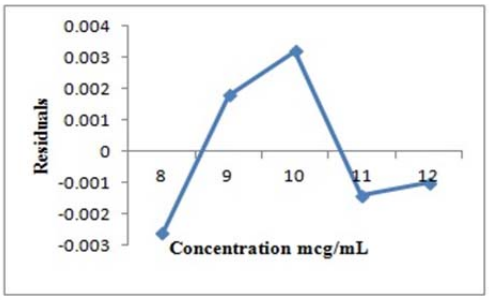

Figure 10 - Residual plot at 228 nm

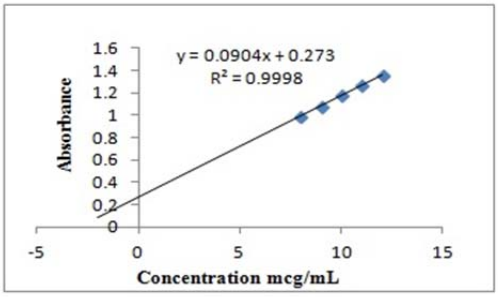

Figure 6 - Calibration graph at 230 nm

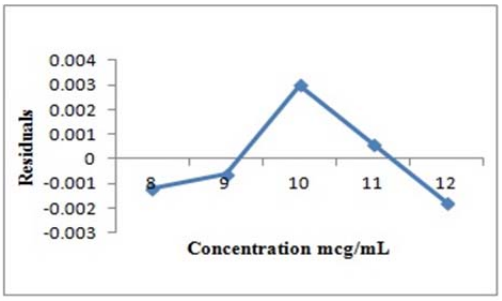

Figure 11 - Residual plot at 230 nm

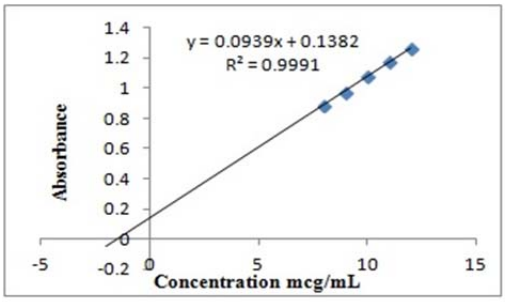

Figure 7 - Calibration graph at 232 nm

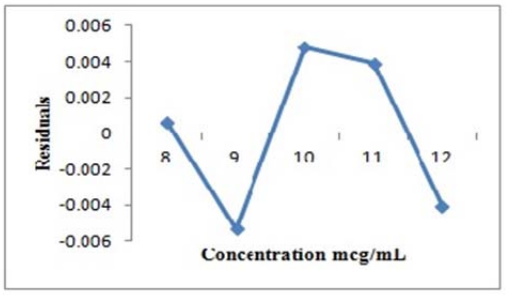

Figure 12 - Residual plot at 232 nm

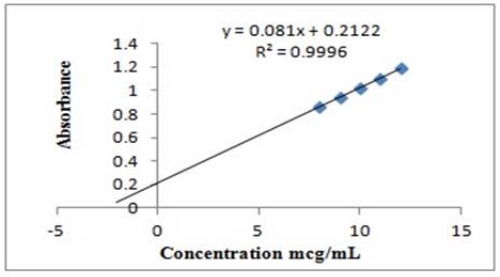

Figure 8 - Calibration graph at 234 nm

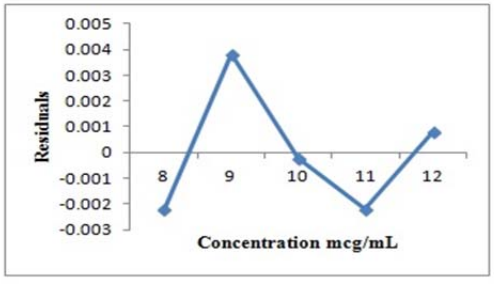

Figure 13 - Residual plot at 234 nm

Table 1 - Multivariate UV calibration obtained at five wavelengths

Conc (µg/ml)	Absorbance				
	226 nm	228 nm	230 nm	232 nm	234 nm
8	0.803	0.836	0.995	0.890	0.858
9	0.889	0.938	1.086	0.978	0.945
10	0.980	1.037	1.180	1.082	1.022
11	1.073	1.130	1.268	1.175	1.101
12	1.156	1.228	1.356	1.261	1.185

Table 2 – Results of Intraday Precision

Con (µg/mL)	No. of Repetitions	Absorbance				
		226 nm	228 nm	230 nm	232 nm	234 nm
8	1	0.803	0.836	0.995	0.89	0.858
	2	0.799	0.828	0.99	0.895	0.852
	3	0.812	0.842	1.013	0.902	0.863
10	1	0.981	1.037	1.18	1.082	1.022
	2	1.011	1.049	1.189	1.099	1.029
	3	0.979	1.031	1.171	1.073	1.012
12	1	1.156	1.228	1.356	1.261	1.185
	2	1.149	1.219	1.359	1.259	1.192
	3	1.163	1.235	1.367	1.251	1.179

Table 3 – Results of Interday Precision

Con (µg/mL)	Day	Absorbance				
		226 nm	228 nm	230 nm	232 nm	234 nm
8	1	0.801	0.839	0.991	0.897	0.853
	2	0.791	0.831	0.985	0.889	0.847
	3	0.815	0.845	1.008	0.911	0.869
10	1	0.991	1.031	1.186	1.086	1.032
	2	1.005	1.047	1.181	1.091	1.018
	3	0.982	1.023	1.198	1.077	1.027
12	1	1.126	1.237	1.364	1.264	1.189
	2	1.143	1.223	1.357	1.251	1.197
	3	1.167	1.217	1.349	1.249	1.209

Table 4 - Linearity data showing statistical parameters at all five wavelengths

Wavelength (nm)	Regression equation	Slope	Intercept	% Intercept	R^2
226	Y = 0.0890x + 0.0902	0.0890	0.0902	9.204	0.9997
228	Y = 0.0976x + 0.0578	0.0976	0.0578	5.573	0.9998
230	Y = 0.0904x + 0.2730	0.0904	0.2730	23.135	0.9998
232	Y = 0.0939x + 0.1382	0.0939	0.1382	12.772	0.9991
234	Y = 0.0810x + 0.2122	0.0810	0.2122	20.763	0.9996

Table 5 – Statistical data for Intraday Precision

Con (µg/mL)	Description	226 nm	228 nm	230 nm	232 nm	234 nm
8	Mean	0.805	0.835	0.999	0.896	0.858
	SD	0.007	0.007	0.012	0.006	0.006
	% RSD	0.827	0.841	1.210	0.673	0.642
10	Mean	0.990	1.039	1.18	1.085	1.021
	SD	0.018	0.009	0.009	0.013	0.009
	% RSD	1.810	0.882	0.763	1.217	0.837
12	Mean	1.156	1.227	1.361	1.257	1.185
	SD	0.007	0.008	0.006	0.005	0.007
	% RSD	0.606	0.654	0.481	0.421	0.549

Table 6 - Statistical data for Interday Precision

Con (µg/mL)	Description	226 nm	228 nm	230 nm	232 nm	234 nm
8	Mean	0.802	0.838	0.995	0.899	0.856
	SD	0.012	0.007	0.012	0.011	0.011
	% RSD	1.503	0.835	1.199	1.239	1.328
10	Mean	0.993	1.034	1.188	1.085	1.026
	SD	0.012	0.012	0.009	0.007	0.007
	% RSD	1.168	1.184	0.735	0.654	0.692
12	Mean	1.155	1.226	1.357	1.255	1.187
	SD	0.012	0.01	0.008	0.008	0.011
	% RSD	1.040	0.837	0.553	0.649	0.893

Table 7 - Recovery studies

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery
226	5	3	7.9823	99.78
		5	9.9921	99.92
		7	11.9682	99.74
228	5	3	7.8995	98.74
		5	10.0257	100.26
		7	11.9915	99.93
230	5	3	8.0054	100.07
		5	9.9839	99.84
		7	11.9976	99.98
232	5	3	7.9836	99.80
		5	9.9591	99.59
		7	11.9893	99.91
234	5	3	7.9728	99.66
		5	9.9913	99.91
		7	11.9568	99.64

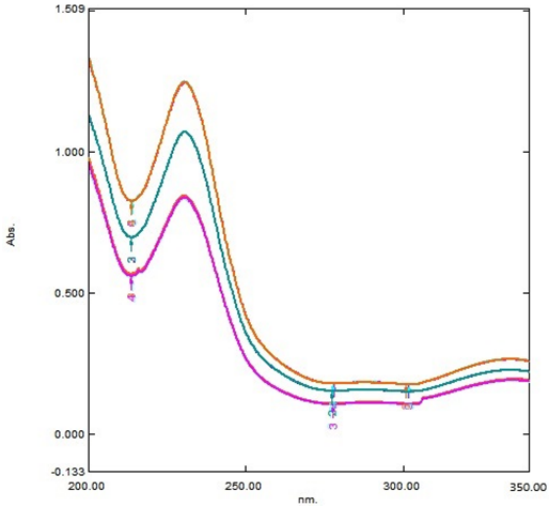

Figure 14 - Overlay UV Spectrum showing Intraday precision

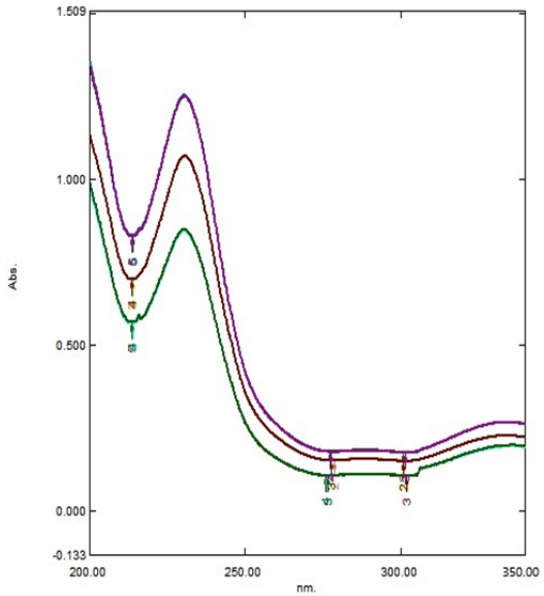

Figure 15 - Overlay UV Spectrum showing Interday Precision

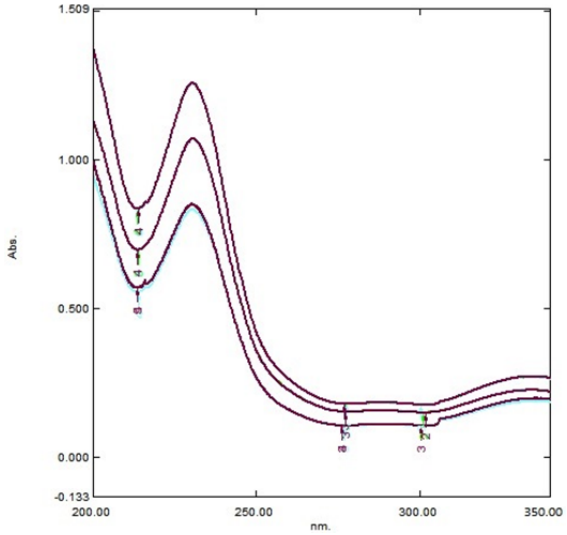

Figure 16 - UV Spectrum showing accuracy of Zaleplon

RESULTS AND DISCUSSION

The λmax of Zaleplon was found to be 230 nm using methanol: distilled water (8:2 v/v) as the solvent. All the calibration curves were linear over the concentration range 8-12 µg/mL. The linear regression analysis data for the calibration plots showed good linear relationship with R^2=0.9998. The linear regression equation was found to be Y = 0.0904x + 0.2730. The percentage RSD for precision was found to be 0.763 and 0.735. The percentage recovery was found to be within the prescribed limit. Hence all the parameters were found to be within the acceptance criteria as per the ICH guidelines.

Linearity

The linearity for different concentration 8-12 µg/mL were recorded at 226, 228, 230, 232 and 234 nm, which was shown in Fig.3 and their calibrations graphs and residual plots are shown in Figures 4 to 8 and Figures 9 to 13 respectively.

Precision

The low value of SD at all wavelength indicates that the method was precise and the %RSD for intraday and interday precision were found to be in the range of 0.421 -

1.810 and 0.649 - 1.503 and is well within the acceptance criteria of less than 2% at all the wavelength. The low value of % RSD indicates that the proposed method was precise and accurate [Figure 14 & 15, Table 5, 6].

Recovery

The percentage recovery of the drug from the synthetic mixture was found to be in the range of 98.74 - 100.26 % w/w, which was within the acceptance limit of 97 - 103 % w/w as per the ICH guideline as shown in Table 7 and Figure 16.

CONCLUSION

The suggested UV spectrophotometric method employing multivariate calibration technique was novel, uncomplicated, accurate, precise, profitable and sensitive for the quantification of Zaleplon in its pharmaceutical formulations. Hence, this method is very useful with very simple mathematical contents, is more reliable than the other spectrophotometric methods and strongly recommends the developed method for a routine analysis of Zaleplon in pharmaceutical formulations.

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