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UV Spectrophotometric Methods for the Determination of Perphenazine in Dosage Form

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ABSTRACT:

A Simple, Sensitive and rapid UV Spectrophotometric methods have been developed for the determination of Perphenazine(PER). The absorption spectrum of PER standard solution (10ug/mL) in methanol was recorded and the wavelength of maximum absorbance was found to be 254.80nm. First and second derivative spectra were also recorded for the same solution. From the first derivative spectrum, it is found that a valley at 261.40nm showed the maximum amplitude and therefore validation of the method was carried out by measuring the amplitudes at this wavelength. The second derivative spectrum has the maximum amplitude in a negative valley at 256.80nm, hence the second derivative was validated by measuring amplitudes at 256.80 nm. Standard deviation and percent of relative standard deviation were calculated and found within limits. The mean percent of recovery were evaluated at 0%, 80%, 100%, 120% concentration levels and found to be within range. The developed methods were found to be linear within the range of concentrations 2-10ug/mL, and slope, intercept and correlation coefficient were calculated and found to be satisfactory. The precision (Repeatability, Intraday, Interday) was evaluated and the result found to be satisfactory. The developed methods were found to be precise, accurate, robust and stable, therefore readily adapted for routine quality control of PER.

KEY WORDS: Perphenazine, UV spectrophotometry, zero-order Derivative, first-order Derivative, second-order Derivative.

INTRODUCTION

Perphenazine (PER) is chemically, 2-[4-[3-(2-chlorophenothiazin- 10- yl) propyl] piperazine- 1-yl]ethanol. It is an antipsychotic phenothiazine derivative with actions and uses similar to those of chlorpromazine. This compound belongs to the category of phenothiazines. These polycyclic aromatic compounds contain a phenothiazine ring that is a linear tricyclic system with two benzene rings joined by a parathiazine ring. It acts by binding to the dopamine D1 and dopamine D2 receptors and inhibits their activity. The mechanism of the anti-emetic effect is due predominantly to blockage of the dopamine D2 neurotransmitter receptors in the chemoreceptor trigger zone and vomiting center. Perphenazine also binds the alpha-adrenergic receptor. This receptor's action is mediated by association with G proteins that activate a phosphatidyl inositol-calcium second messenger system. [1, 2]

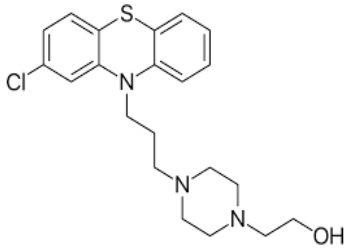


Figure: 1 Perphenazine[1]

Derivative UV-spectrophotometry is an analytical technique commonly used for qualitative and quantitative concerning spectra which are of unresolved bands, concerning qualitative and quantitative analysis, it uses first or higher derivatives of absorbance following wavelength. [3] Plotting absorbance versus wavelength gives a graph, showing peak with maxima and minima that are supposed to pass through zero on the ordinate [4]

Zero-order derivative spectrum

Zero-order derivative is the initial step for producing its next derivatives i.e., the zeroth-order spectrum can give nth order derivative. In derivative spectroscopy, the D^0 spectrum i.e. zeroth order is a representative feature of the normal absorption spectrum [4]. The zero-order derivative spectra helps in obtaining the 1st 2nd n 3rd order derivative. An increase in the order of derivatives increases the sensitivity of determination [5]. If a spectrum is expressed as absorbance (A) as a function of wavelength (λ), the derivative spectra are given as,

$$A=f(\lambda),$$

First-order derivative spectrum

It is a plot of change of absorbance with wavelength against wavelength[6] i.e. rate of change of the absorbance with wavelength,

$$dA/d\lambda=f'(\lambda)$$

Even if in the derivatized form it is more complex than zero-order spectrum. In First order spectra, the absorbance band passes from zero as λ max. The absorbance band of first-order derivative shows certain positive and negative bands with maxima and minima[7]. To obtain first-derivative spectra can be obtained by scanning the spectrum with a minimum and constant difference between two wavelengths[8].

Second-order derivative spectrum

This type of spectra is obtained by derivatizing the absorbance spectrum twice. It is a plot of curvature of the absorption spectrum against wavelength[9].

$$d2A/d\lambda2=f'(\lambda)$$

The second derivative has a direct relation with concentration i.e. directly proportional. $d^2A/d\lambda^2$ must be large, large the ratio greater is the sensitivity[10].

MATERIAL AND METHOD

Instrumentation

UV-Visible spectrophotometer: An UV-Visible spectrophotometer Shimadzu (UV-1800) with 1cm matched quartz cells was used for the spectral and absorbance measurements.

Digital balance: A REPTECH-RA123digitalWeighing balance was used for weighing purposes.

Preparation of standard and sample solutions

Preparation of PER standard stock solution (1000 µg/mL)

10mg of Perphenazine was weighed and transferred to 10 mL volumetric flask and volume were made up to the mark with methanol to get 1000 µg/mL.

Preparation of PER working stock solution (100 µg/mL)

Aliquot of 1 mL from above standard stock solution was pipette out into 10 ml of volumetric flask and volume was made up to the mark with methanol to give a solution containing 100µg/mL.

Preparation of PER sample solution

A series of solutions were prepared by transferring 0.2 mL-1 mL from above-working stock solution into 10 mL volumetric flask and volume was made up to mark with methanol.

Method development

The zero-order, first and second derivative spectra were of a solution of 10 µg/mL of PER were recorded by scanning the absorbance values in the range of wavelength 200-400nm. From the absorption spectra (fig 2) it was found that the wavelength of maximum absorbance was 254.80nm. The first derivative spectrum D^1(fig3) crossed the zero point at a wavelength of 250nm and producing positive valley at a wavelength of 247.60nmand negative valley at 261.40nm on either side of the zero-crossing point of the spectrum. The valley at 261.40 nm showed a maximum amplitude than the first valley. The second derivative spectrum D^2 (fig4) crossed the x-axis at 256.80. The maximum amplitude was observed at a negative valley; therefore the method was validated by measuring amplitudes at 256.80nm.

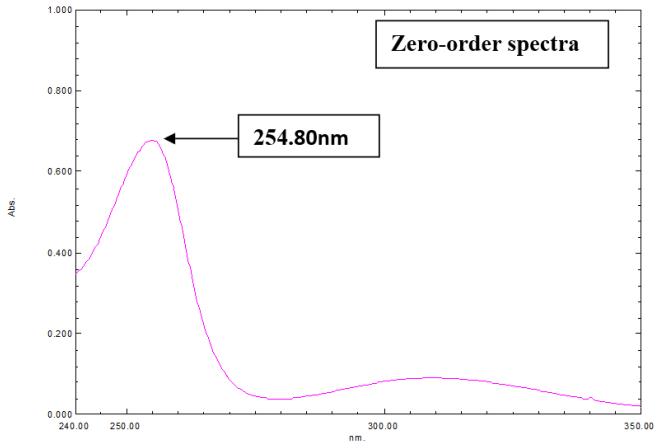


Figure: 2 Zero Order derivative spectrum of PER (10 µg/mL)

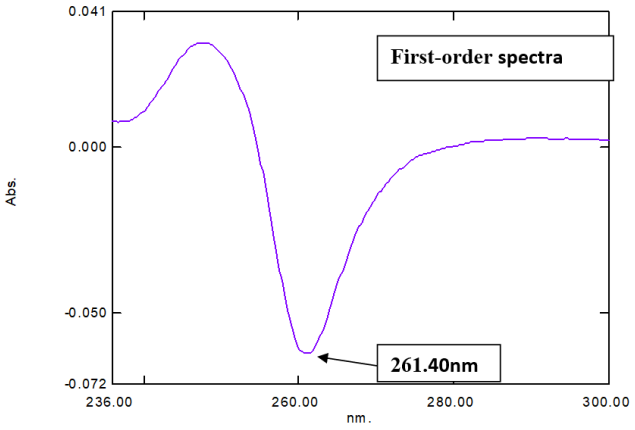


Figure: 3 First Order derivative spectrum of PER (10 µg/mL)

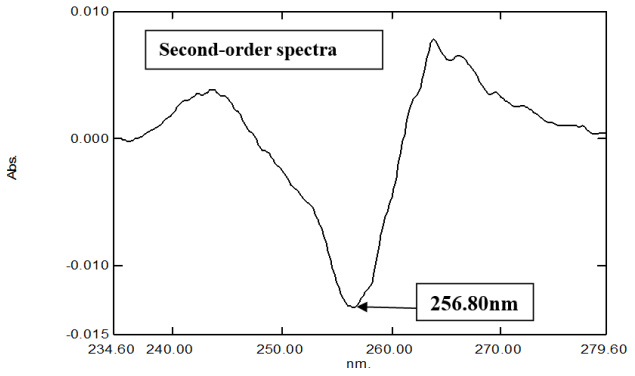


Figure: 4 Second Order Derivative Spectrum of PER (10 µg/mL)

Method Validation[11-13]

Validation:

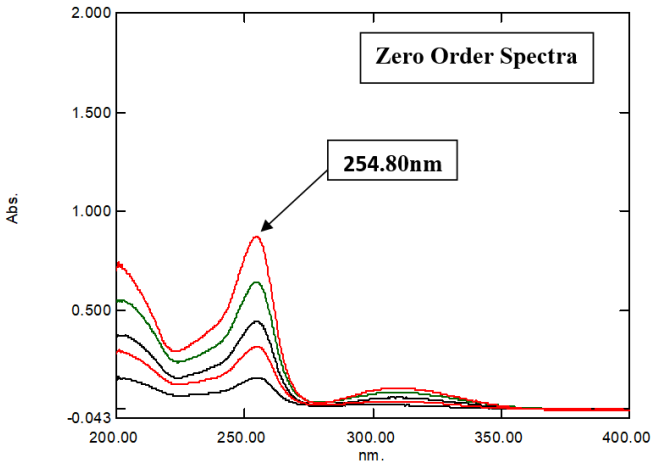
Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce the desired product meeting its predetermined specifications and quality attributes.

Validation characteristics/parameter:

a. Linearity
b. Precision
   1. Repeatability
   2. Intermediate precision
   3. Reproducibility
c. Accuracy
d. Range
e. Limit of Detection (LOD)
f. Limit of Quantitation (LOQ)

Linearity (n-=5):

The linearity response was determined by analyzing 5 independent levels of the calibration curve in the range of 2 – 10 µg/mL for PER. The zero, first and second-order derivative spectra for each of the concentrations were recorded over a wavelength range 200-400nm against methanol.



. Figure: 5 Linearity overlay of Zero Order Absorption spectra of PER(2-10 µg/mL)

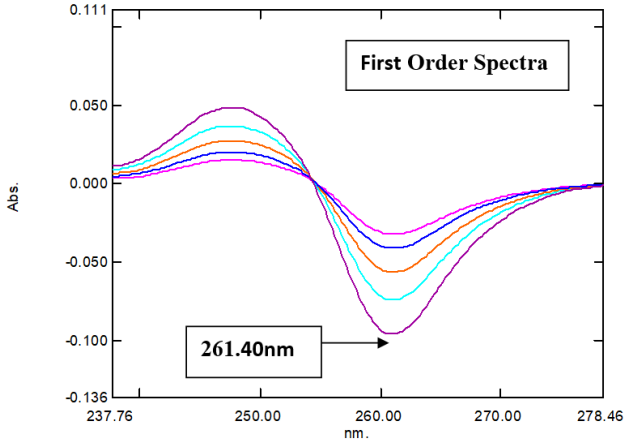


Figure: 6 Linearity overlay of First Order Derivative spectra of PER (2-10 µg/mL)

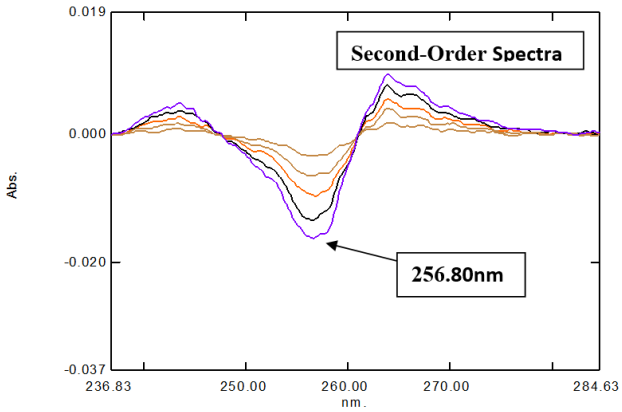


Figure: 7 Linearity overlay of Second Order Derivative spectra of PER(2-10 µg/mL)

Calibration curve: Calibration curve for PER consisted of different concentration of standard PER solution ranging from 2-10 µg/mL. the solution was prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1.0 of the working stock solution of PER (100 µg/mL) into series of 10 mL volumetric flasks and the volume was adjusted to mark with methanol to produce 2, 4, 6, 8 and 10 µg/mL respectively. In zero order (D^0), a linear straight line was drawn by taking absorbance values on the y-axis and concentration on the x-axis (fig 8). In the case of derivative method, maximum D^1 and D^2amplitudes were plotted against the concentration of drug (fig 9-fig 10).linear least square regression analysis was applied in three cases and slope-intercept and correlation parameters were calculated and were presented in table-1.

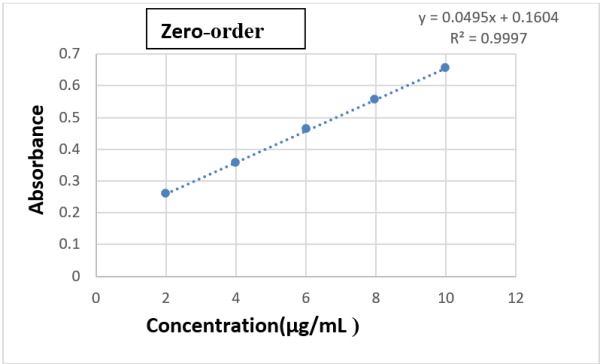


Figure 8 Zero Order Linearity plot of absorbance against the concentration of PER.

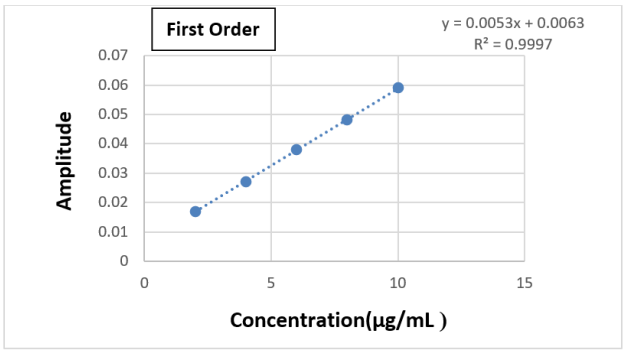


Figure: 9 Linearity plot of the first derivative amplitude against the concentration of PER

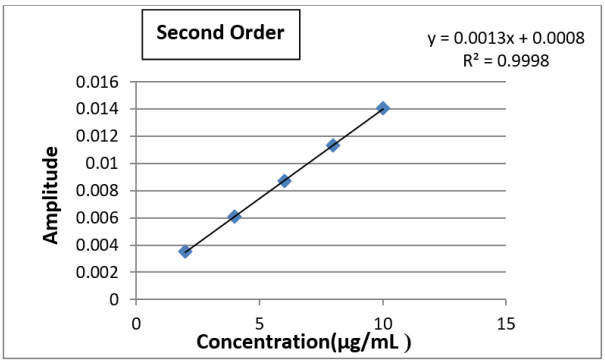


Figure: 10 Linearity plot of Second derivative amplitude against the concentration of PER

Table no: 1 Linearity data of PER for Zero Order, First Order, and Second-Order Derivative

Sr. No	Conc. (µg/ml)	Mean ABS. D^0	Mean Amp. D^1	Mean Amp. D^2
1	2	0.2629	0.0172	0.0035
2	4	0.3568	0.0278	0.0062
3	6	0.4615	0.0376	0.0086
4	8	0.5559	0.0482	0.0113
5	10	0.6514	0.0582	0.0140
	SLOPE	0.0495	0.0053	0.0013
	INTERCEPT	0.1604	0.0063	0.0008
	SD	0.0061	0.0005	0.00009
	%RSD	1.2606	1.6061	1.1780

Precision:

A. Repeatability (n=6)

0.6 mL of working stock solution of Perphenazine (100 µg/mL) was transferred to a 10 mL volumetric flask. The

volume was adjusted up to mark with methanol to get 6 µg/mL solution of Perphenazine. The solution was analyzed 6 times on the same day spectrophotometry for D^0, D^1, D^2 and Standard deviation and % RSD was calculated.

Table no 2: Repeatability data for PER.

Sr. No	CONC.	ZERO ORDER	FIRST ORDER	SECOND ORDER
Avarage		0.4671	0.038	0.0113
SD	6 µg/mL	0	0	0
%RSD		0.01	0.1664	0.0791

B. Intraday precision (n=3)

Aliquots of 0.4, 0.6 and 0.8 ml of working stock solution of Perphenazine (100 µg/mL) were transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 4, 6 and 8 µg/mL solution of Perphenazine. The solution was analyzed three times on the same day and Standard deviation and % RSD was calculated.

Table no 3: Intraday precision for PER.

Sr. No	ZERO ORDER			FIRST ORDER			SECOND ORDER		
	4ppm	6ppm	8ppm	4ppm	6ppm	8ppm	4ppm	6ppm	8ppm
Avg.	0.3591	0.4649	0.5508	0.028	0.0378	0.0484	0.0061	0.0086	0.0114
SD	0.0055	0.0069	0.0083	0.0002	0.0003	0.0003	0	0	0.0001
RSD	0.0155	0.015	0.0151	0.8966	0.8067	0.725	0.8108	0.5597	0.8849

Table no 4: Interday precision for PER

Sr.	ZERO ORDER			FIRST ORDER			SECOND ORDER		
No	4ppm	6ppm	8ppm	4ppm	6ppm	8ppm	4ppm	6ppm	8ppm
Avg.	0.3564	0.4547	0.5475	0.0284	0.0385	0.049	0.0068	0.0088	0.0114
SD	0.0124	0.0109	0.0146	0.0003	0.0003	0.0003	0.0001	0.0001	0.0001
RSD	0.0348	0.0241	0.0266	1.0732	0.9365	0.7358	1.8459	1.7292	1.336

C. Interday precision (n=3)

Aliquots of 0.4, 0.6 and 0.8 mL of working stock solution of Perphenazine (100 µg/mL) were respectively transferred to the same above series of 10 mL volumetric flask. The volume was adjusted up to mark with methanol to get 4, 6 and 8 µg/mL solution of Perphenazine. The solution was analyzed three times on the different day using spectrophotometry and % RSD was calculated

Accuracy (n=3)

A. Preparation of Dosage form

4 mg equivalent of PER was taken into 10 ml of volumetric flask. The volume was adjusted up to mark with methanol to get 400 µg/of PER. From the above solution, 1.0 mL was pipette out and transferred to10 ml of volumetric flask and volume was made up to the mark with methanol to get PER(40µg/mL) solution.

Tablet solution X: PER (40 µg/mL)

Solution Y: PER (40µg/mL)

B. Preparation of sample solution for PER

Table no 5: Steps for accurate measurement for PER

Sr No.	Step 1	Step 2	Step 3	Total AMI Conc. (µg/ml)
1	Take 1 mL of solution X	-	Make up the volume to 10mL with methanol	10
2	Take 1 mL of solution X	Add 0.8 mL of solution Y	Make up the volume to 10mL with methanol	18
3	Take 1 mL of solution X	Add 1.0 mL of solution Y	Make up the volume to 10mL with methanol	20

4	Take 1 mL of solution X	Add 1.2 mL of solution Y	Make up the volume to 10mL with methanol	22

Each solution was scanned from 200-400 nm against methanol as a blank. The absorbance of the solution was measured at selected wavelengths for PER. The amount of PER was calculated at each level (80 %, 100 %, and 120 %) and % recoveries were computed.

Table no 6: Accuracy of the developed method

Concentration	Amount added	Zero-order	First-order	Second-order
		% Recovery	% Recovery	% Recovery
0%	4	98.93	99.01	98.75
80%	7.2	98.96	98.58	99.25
100%	8	99.86	100.90	99.9
120%	8.8	99.97	100.70	100.43
Mean % Recovery		99.43	99.80	99.58

LOD and LOQ

- The LOD (Limit of Detection) was estimated from the information obtained from the set of 5 calibration curves that were used to determine the linearity of the method. The LOD was calculated by using the formula; LOD = 3.3 x S.D. / Slope and LOQ = 10 x S.D. / Slope.

Table no 7: LOD AND LOQ

Parameter	Zero Order (µg/mL)	First Order (µg/mL)	Second Order (µg/mL)
LOD	0.2870	0.1765	0.1928
LOQ	0.8699	0.5348	0.5844

RESULT AND DISCUSSION

The absorption spectrum, first and second derivative spectra of a solution of 10 µg/mL of PER were presented in Fig.2-Fig.4.The wavelength of maximum absorbance of Zero Order was found to be 254.80nm. The first derivative spectrum (Fig.3) shows the maximum amplitude (valley) at 261.40 nm, therefore, validation of the method was carried out by measuring the amplitudes at this wavelength. In the second derivative spectrum, the maximum amplitude was observed in the negative valley at 256.80nm hence the method was validated by measuring amplitudes at 256.80nm. The developed method was found to be linear within the range of concentrations of 2-10 µg/mL. Slope, intercept and correlation coefficient for the developed method were found to be 0.0495, 0.1604 and 0.9997; 0.0053,0.0063 and 0.9997; 0.0013, 0.0008 and 0.9998for zero-order, first-order and second-order derivative methods respectively. Standard deviation and Percent of relative standard deviation (%RSD) values for zero-order, first and second derivative methods were found to be 0.0000& 0.0100, 0.0000& 0.1664and0.0000& 0.0791 respectively. The mean percent of recovery and percent of relative standard deviation were evaluated at 0%, 80%, 100%, and 120% concentration levels. The mean percent of recovery and percent of relative standard deviation were found to be 99.43%, 99.80%, 99.58%. The LOD and LOQ for PER of zero-order, first-order and second-order derivative was found to be 0.2870 & 0.8699; 0.1765 &0.5348; 0.1928 & 0.5844 respectively.

CONCLUSION

The developed UV spectrophotometric methods were effective for the quantitative determination of PER. The developed methods were found to be simple, sensitive, selective, reproducible, and stable. The developed methods could be readily adapted to routine quality control of PER by laboratories.

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