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Research Article

New spectrophotometric techniques for the estimation of Perphenazine in bulk drug form

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ABSTRACT

Perphenazine is an atypical anti-psychotic drug. It indicated for the treatment of a agitated depression in low doses together with an anti-depressant. Perphenazine has sedation and anxiolytic properties and also useful for the treat psychosis like schizophrenia and manic phases of bi-polar dis-orders. The simple and accurate and precise absorption ratio method has been developed for the simultaneous estimation of Perphenazine in the pure drug form. The absorption maxima was found to be 310nm in the Method A (0.1N HCL Buffer) and shows linearity over the concentration range of 0.002-0.02 µg/mL with regression equation $y=0.5372x-0.0099$ ($r^2 = 0.9990$). In Method B (Sodium acetate buffer, pH 4.5) the drug obeys Beer Lambert's law (λ_{max} 310nm) in the concentration range of 0.002-0.02 µg/mL with regression equation $y=0.4257x - 0.0084$ ($r^2= 0.9992$). In Method C (Phosphate buffer, pH 6.8) the drug obeys Beer Lambert's law (λ_{max} 310nm) in the concentration range of 0.002-0.02 µg/mL with regression equation $y=0.482x - 0.0074$ ($r^2= 0.9991$). In Method D (phosphate buffer, pH 7.2) the drug obeys Beer Lambert's law (λ_{max} 310nm) in the concentration range of 0.002-0.02 µg/mL with regression equation $y=0.3686x - 0.0055$ ($r^2= 0.9992$). In Method E (0.1N NaoH Buffer) and shows linearity over the concentration range of 0.002-0.02 µg/mL with regression equation $y=0.4864x-0.0081$ ($r^2 = 0.999$). In Method F (Methanol) the drug obeys Beer Lambert's law (λ_{max} 300nm) in the concentration range of 0.002-0.02 µg/mL with regression equation $y=0.6323x - 0.003$ ($r^2= 0.999$). In Method G (Ethanol) the drug obeys Beer Lambert's law (λ_{max} 300nm) in the concentration range of 0.002-0.02 µg/mL with regression equation $y=0.3686x - 0.0055$ ($r^2= 0.9991$). First derivative spectrophotometric methods (A1, B1, C1, D1, E1, F1 and G1) were developed in 0.1NHCl and Sodium acetate pH 4.5 and phosphate buffer, in pH 6.8 and phosphate buffer, pH 7.2, 0.1N NaoH Buffer, which Perphenazine obeys Beer Lambert's law (λ_{max} 310nm) in the concentration range of 0.002-0.02 µg/mL and 0.002-0.02 µg/mL and 0.002-0.02 µg/mL and 0.002-0.02 µg/mL with regression equations $y=0.0357x - 0.0006$ and $y=0.0201x+0.0004$ and $y=0.0196x-0.0002$ and $y=0.0162x+0.0002$ and $y=0.0239x - 0.0002$ and Perphenazine obeys Beer Lambert's law (λ_{max} 300nm) for methanol and ethanol in the concentration range of 0.002-0.02 µg/mL and 0.002-0.02 µg/mL with regression equations $y=0.0423x-0.0003$ and $y=0.0371x+0.0003$ respectively. The proposed spectrophotometric methods were validated as per the ICH guidelines and can be applied for the determination of Perphenazine in pharmaceutical formulations.

Keywords: Perphenazine, Derivative spectroscopy, Spectrophotometry, Validation.

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1. INTRODUCTION

Perphenazine, chemically known as 2[4-(3-(2-chlorophenothiazin-10-yl) propyl) piperazin-1-yl] ethanol indicates for the treatment of an agitated depression¹ and also for the treat psychosis- like schizophrenia and manic phases of bi-polar dis-order. Perphenazine is a phenothiazine derivative and a dopamine antagonist with anti-emetic and anti-psychotic dopamine 2(D2) receptors in the mesolimbic and medullary chemoreceptor trigger zone (CTZ) thereby preventing the excess of dopamine in the brain. This leads to reduction in psychotic symptoms, such as hallucinations and delusions².

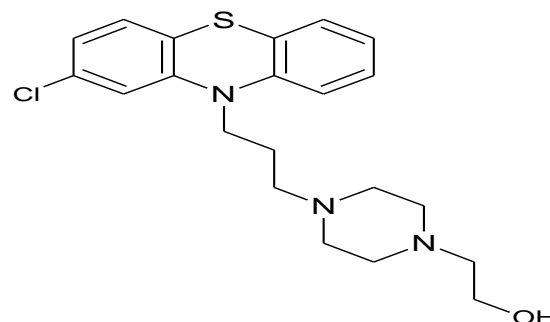


Figure 1: Structure of Perphenazine

Perphenazine appears to exert its anti-emetic activity by blocking the dopamine and histamine-1 receptors in CTZ thereby relieving nausea and vomiting in the brain. In addition, Perphenazine binds to alpha-adnergic receptors. Literature survey revealed that Perphenazine was determined by UV-Visible spectroscopy³. In the present study, the authors have proposed seven simple validated spectrophotometric methods³ for the determination of Perphenazine in the pure drug form.

MATERIALS AND METHODS

2.1 Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) associated with a computer loaded with spectra manager programming UV Probe was utilized with a spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm way length matched quartz cells. For scanning, the wavelength range chosen was 400nm to 200nm with medium scanning speed. All weights were taken on the electronic balance (Shimadzu).

2.2 Chemicals and reagents

HPLC grade Methanol (Merck), glacial acetic acid (Merck), sodium acetate trihydrate (Merck), Hydrochloric acid (Rankem), potassium Di-hydrogen phosphate (Merck), sodium hydroxide (Merck), Ethanol (Merck) was used. Perphenazine, obtained as a gift sample from, CEMO (India), was used.

2.3 Preparation of 0.1N Hcl

8.5mL of 35% Conc. Hydrochloric acid was added to the 1000mL volumetric flask containing 900mL de-ionized water and mixed well. The volume was then made up to 1000mL with water.

2.4 Preparation of Sodium Acetate Buffer (pH 4.5)

Sodium acetate trihydrate (2.99 gm) were weighed accurately in a 1000ml volumetric flask containing 1000ml deionized water and mixed well, then 1.7mL glacial acetic acid was added to it and the pH adjusted to 4.50 with glacial acetic acid.

2.5 Preparation of phosphate buffer pH 6.8:

6.8grams of potassium Di-hydrogen phosphate and 0.9grams of sodium hydroxide were weighed accurately and was added in a 1000ml volumetric flask containing 900ml deionized water and mixed well. The volume was made up to 1000ml with water.

2.6 Preparation of phosphate buffer pH 7.2:

6.8grams of potassium Di-hydrogen phosphate and 1.4grams of sodium hydroxide were weighed accurately and was added in a 1000ml volumetric flask containing 900ml deionized water and mixed well. The volume was made up to 1000ml with water.

2.7 Preparation of 0.1N NaoH Buffer:

4grams of sodium hydroxide was weighed accurately and was added in a 1000ml volumetric flask containing 900ml deionized water and mixed well. The volume was made up to 1000ml with water.

2.8 Preparation of stock solution:

The standard solution of Perphenazine was prepared by dissolving accurately about 25 mg of the Perphenazine with methanol in a 25 ml volumetric flask.

The stock solution was further diluted with 0.1N HCl and pH

4.5 sodium acetate buffer and phosphate buffer pH 6.8 and phosphate buffer 7.2 and 0.1N NaoH buffer and Methanol buffer and Ethanol buffer for method A (0.002-0.02 μ g/mL) and method B (0.002-0.02 μ g/mL) method C (0.002-0.02 μ g/mL) and method D (0.002-0.02 μ g/mL) and method E (0.002-0.02 μ g/mL) method F (0.002-0.02 μ g/mL) and method G (0.002-0.02 μ g/mL) respectively as per the requirement.

2.9 Procedure for preparation of Calibration curve:

The drug solutions were scanned (200-400 nm) against the reagent blank (0.1N HCl for method A and sodium acetate buffer pH 4.5 for method B and phosphate buffer pH 6.8 for method C and phosphate buffer pH 7.2 for method D and 0.1N NaoH buffer for method E and Methanol buffer for method F and Ethanol buffer for method G) the absorption spectra were recorded. The absorption maximum (λ_{max}) was observed at 310 nm for methods A, B, C, D and E and absorption maximum (λ_{max}) was observed at 300 nm for methods F and G. The absorption spectra so obtained were converted in to first derivative spectra by the inbuilt software of the instrument and the resulting spectrum shows both maxima and minima and therefore the magnitude of the amplitude was recorded against concentration for method A, B, C, D, E, F and G. Calibration curves were constructed by taking the concentration of the drug solutions on the x-axis and

The corresponding absorbance values on the y-axis.

2.10 Validation Procedure:

The proposed method was optimised using methanol as stock solvent. Hcl(Method A) and various buffers (method B, C, D, E, F and G) as diluted solvents. The present method was validated for the various parameters as per ICH guidelines [3].

2.11 Precision and Accuracy

The precision and accuracy studies were performed as per the ICH guidelines. The absorbance of six replicates (0.01 μ g /mL) and the % RSD was calculated [3].

Accuracy was evaluated as per the ICH guidelines by the percent recovery studies by the addition of 50%, 100%, and 150% of Perphenazine solution was taken and the % RSD was calculated.

2.12 Limit of Detection and Limit of Quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal to- noise ratio and the use of standard deviation of the response and the slope of the calibration curve. The LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/S$ and $10 \sigma/S$ criterions, respectively, where σ is the standard deviation of the S-intercepts of the regression lines and σ is the slope of the calibration curve.

3. RESULTS

New spectrophotometric methods were developed for the determination of perphenazine in pharmaceutical preparations. Perphenazine has shown absorption maxima (λ_{max}) at 310 nm in 0.1N Hcl (Method A) and Sodium acetate buffer pH 4.5 (Method B) and phosphate buffer pH 6.8 (method C) and phosphate buffer pH 7.2 (method D) and 0.1N NaoH Buffer (Method E) and Perphenazine has shown absorption maxima (λ_{max}) at 300 nm in Methanol buffer (Method F) and Ethanol buffer (Method G) the corresponding absorption spectra were shown in Figures.

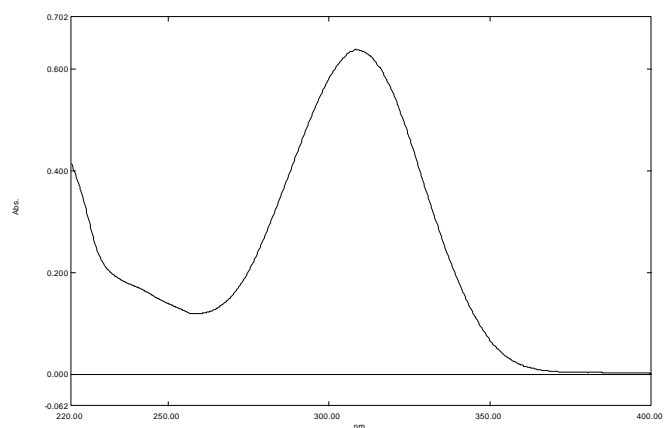


Figure 2: Absorption spectrum of Perphenazine (1.0µg/mL) in 0.1N HCl Buffer

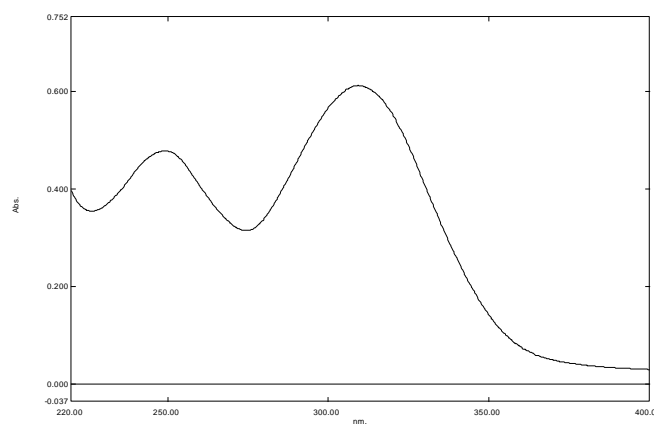


Figure 6: Absorption spectrum of Perphenazine (1.2µg/mL) in 0.1N NaOH Buffer

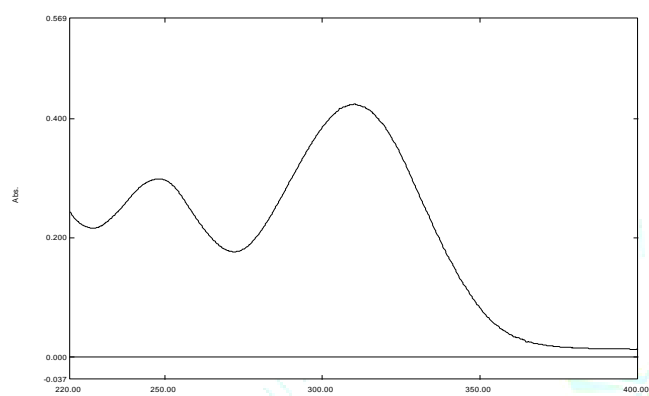


Figure 3: Absorption spectrum of Perphenazine (1.0µg/mL) in Acetate Buffer pH4.5

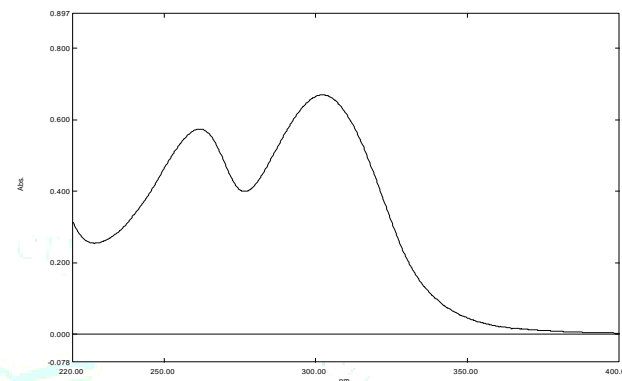


Figure 7: Absorption spectrum of Perphenazine (1.0µg/mL) in Methanol buffer

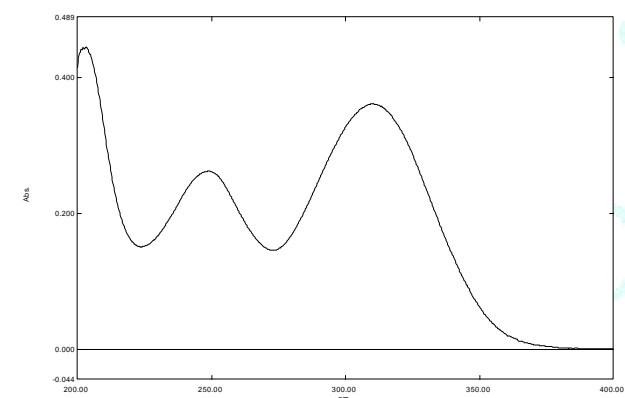


Figure 4: Absorption spectrum of Perphenazine (1.0µg/mL) in Phosphate Buffer pH6.8

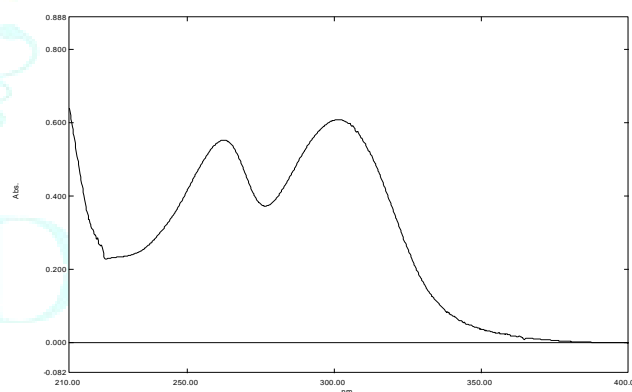


Figure 8: Absorption spectrum of Perphenazine (1.0µg/mL) in Ethanol Buffer

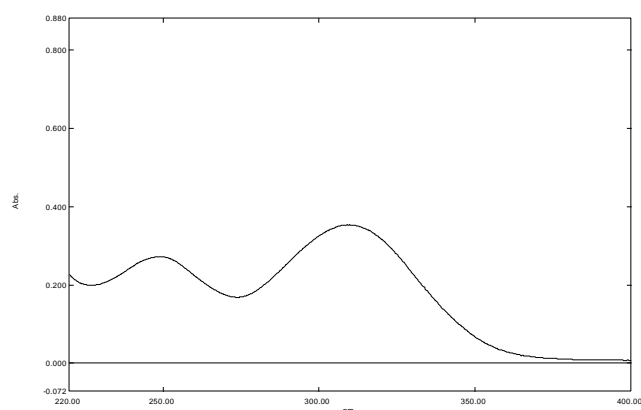


Figure 5: Absorption spectrum of Perphenazine (1.0µg/mL) in Phosphate Buffer pH7.2

In method A1 for 0.1N HCl buffer, Perphenazine has shown zero crossing points at 202.10, 257.78, 307.78, 398.20, with maxima at 288.62 nm and minima at 328.15 nm in Figure 9 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve. Similarly, in method B1 for Acetate buffer pH4.5, has shown zero crossing point at 206.38, 228.98, 248.17, 272.31, 301.19, 373.35 and with maxima at 239.21 nm and minima at 328.43 nm in Figure 10 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve and therefore the amplitude has been taken against the concentration for the construction of the calibration curve. In method C1 for phosphate buffer pH 6.8, Perphenazine has shown zero crossing points at 202.05, 225.75, 248.50, 272.16, 310.48, 380.45, with maxima at 237.91 nm and minima at 333.62 nm in Figure 11 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve.

Similarly, in method D1 for phosphate buffer pH7.2, has shown zero crossing point at 201.77, 204.40, 204.88, 224.17, 248.26, 272.67, 310.02, 397.27 and with maxima at 237.20 nm and minima at 327.61 nm in Figure 12 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve and therefore the amplitude has been taken against the concentration for the construction of the calibration curve. In method E1 for 0.1N NaoH buffer , Perphenazine has shown zero crossing points at 210.78, 225.16, 247.14, 311.01, 397.89 and with maxima at 288.21 nm and minima at 327.89 nm in Figure 13 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve. Similarly, in method F1 for Methanol buffer has shown zero crossing point at 205.98, 227.24, 261.48, 276.94, 301.92, 393.67 and with maxima at 248.60 nm and minima at 320.60 nm in Figure 14 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve and therefore the amplitude has been taken against the concentration for the construction of the calibration curve. Similarly, in method G1 for Ethanol buffer has shown zero crossing point at 204.49, 223.39, 262.43, 276.67, 301.14, 395.02 and with maxima at 250.16 nm and minima at 320.78 nm in Figure 15 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve and therefore the amplitude has been taken against the concentration for the construction of the calibration curve.

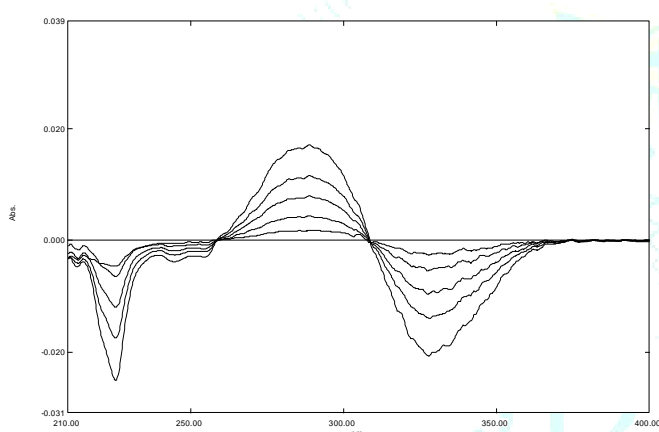


Figure 9: Overlay first derivative spectra (D_1) of Perphenazine in 0.1N HCl

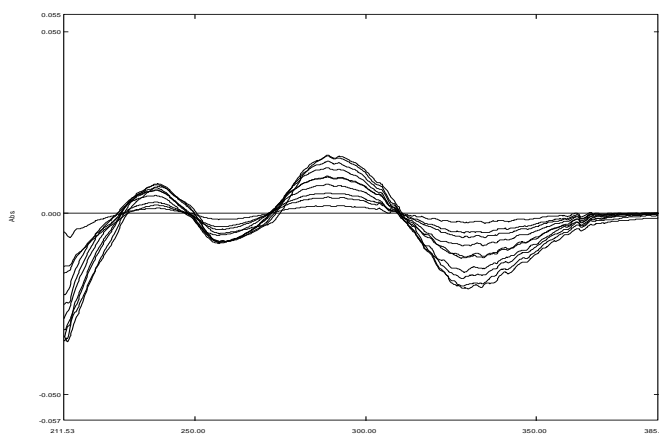


Figure 10: Overlay first derivative spectra (D_1) of Perphenazine in acetate buffer pH 4.5

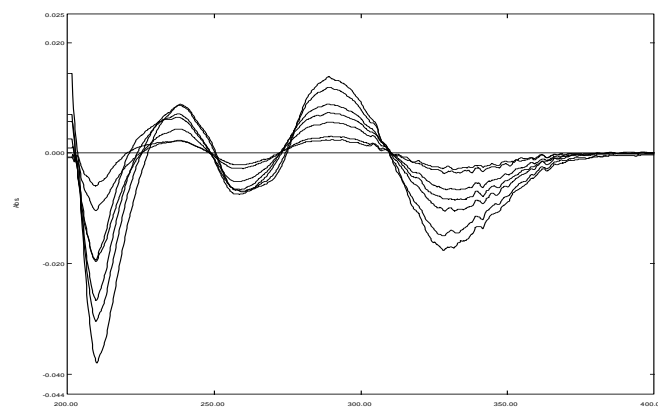


Figure 11: Overlay first derivative spectra (D_1) of Perphenazine in phosphate buffer pH 6.8

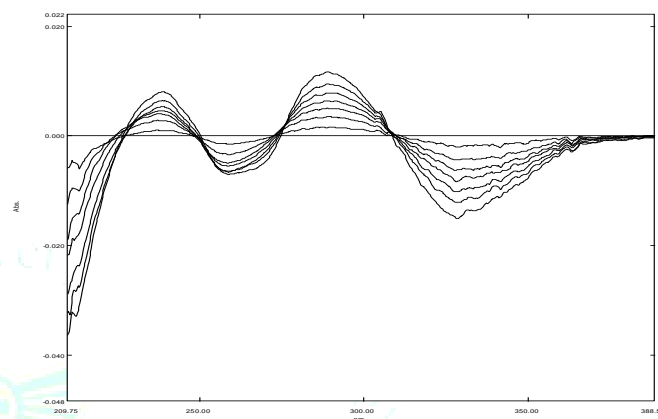


Figure 12: Overlay first derivative spectra (D_1) of Perphenazine in phosphate buffer pH 7.2

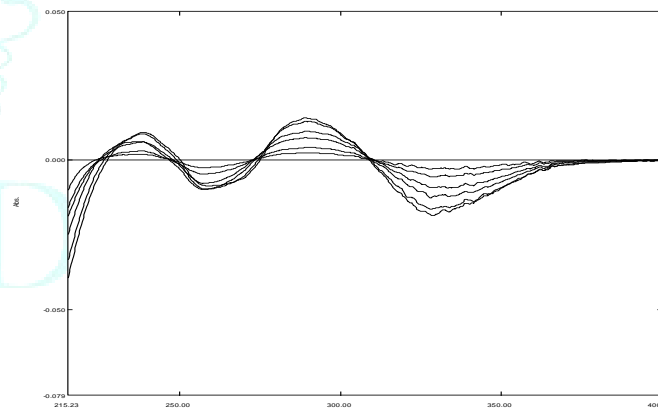


Figure 13: Overlay first derivative spectra (D_1) of Perphenazine in 0.1N NaoH

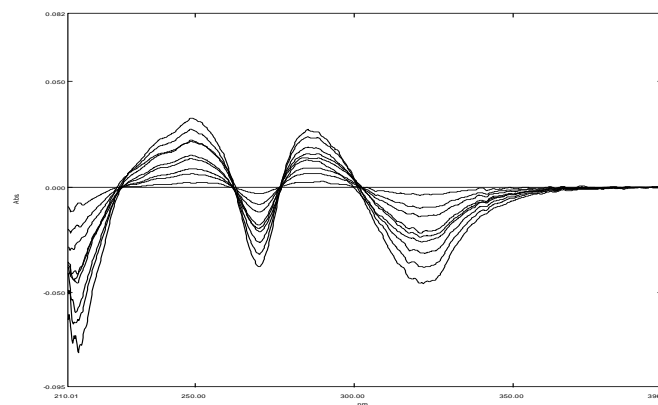


Figure 14: Overlay first derivative spectra (D_1) of Perphenazine in Methanol buffer

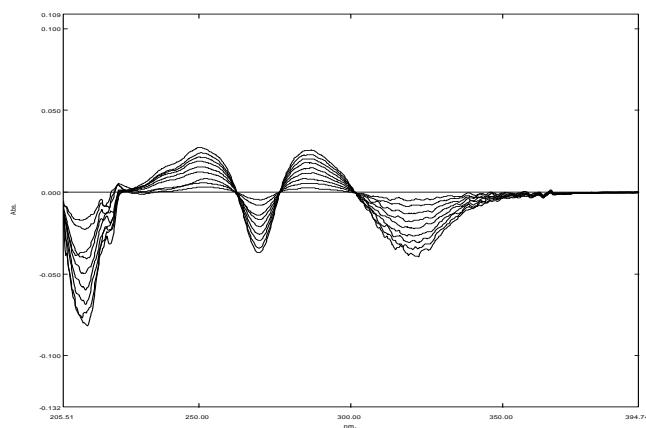
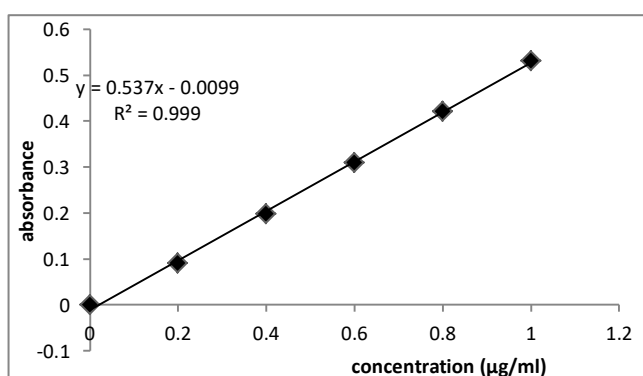
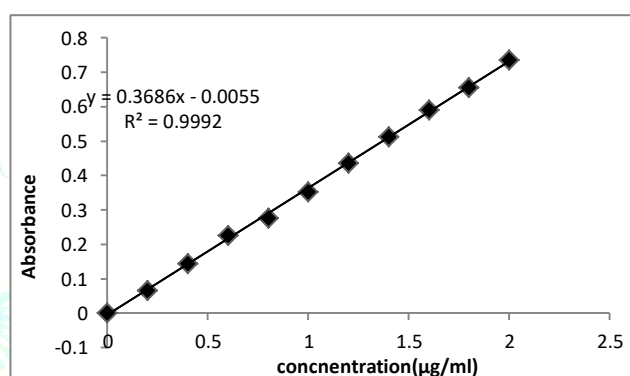


Figure 15: Overlay first derivative spectra (D₁) of Perphenazine in Ethanol buffer

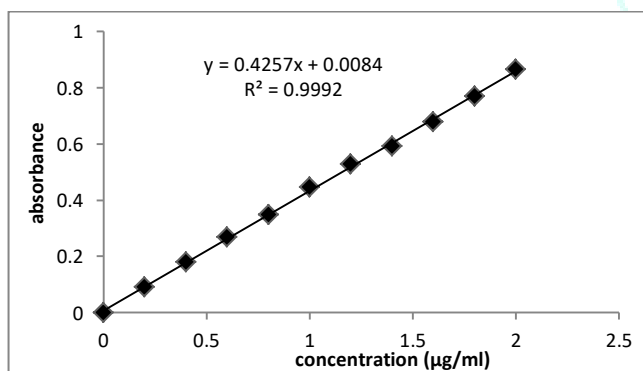
Beer's law was obeyed in the concentration range of 0.002-0.02 $\mu\text{g/mL}$ for method A and A1 and 0.002-0.02 $\mu\text{g/mL}$ for Method B and B1 and 0.002-0.02 $\mu\text{g/mL}$ for Method C and C1 and 0.002-0.02 $\mu\text{g/mL}$ for Method D and D1 and 0.002-0.02 $\mu\text{g/mL}$ for Method E and E1 and 0.002-0.02 $\mu\text{g/mL}$ for Method F and F1 and 0.002-0.02 $\mu\text{g/mL}$ for Method G and G1. The linear regression equations were found to be $y = 0.5372x - 0.0099$, $y = 0.4257x - 0.0084$, $y = 0.482x - 0.0074$, $y = 0.3686x - 0.0055$, $y = 0.6323x + 0.003$, $y = 0.5955x + 0.0128$, $y = 0.0357x - 0.0006$, $y = 0.0201x + 0.0004$, $y = 0.0196x - 0.0002$, and $y = 0.0162x - 0.0002$, $y = 0.239x + 0.0002$, $y = 0.0423x - 0.0003$, $y = 0.0371x + 0.0003$ for method A, B, C, D, E, F, G and H and also for A1, B1, C1, D1, E1, F1 and G1 respectively (Figure 10) with correlation coefficient 0.999, 0.9992, 0.9991, 0.9992, 0.999, 0.999 and 0.9991 and also for 1st derivatives 0.9995, 0.9993, 0.9993, 0.9993, 0.9996, 0.9994, and 0.9992 respectively (Table 1).



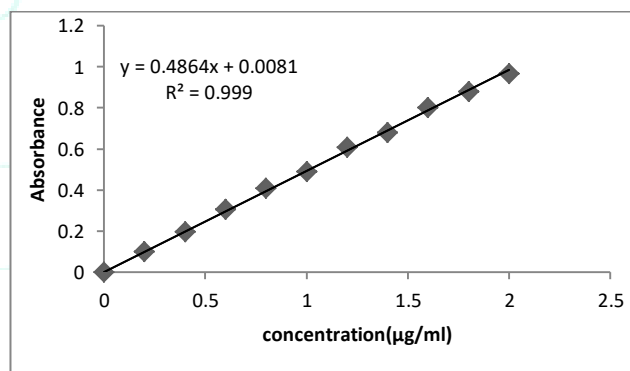
(A)



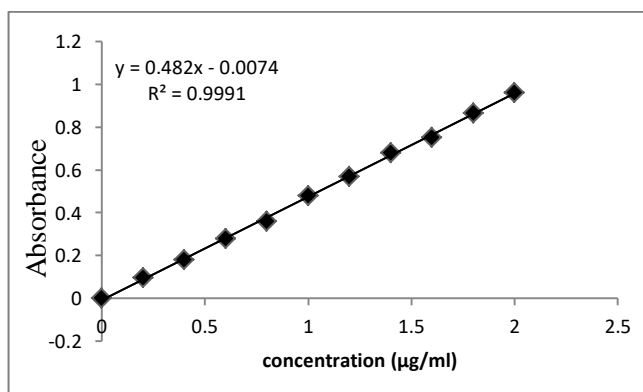
(D)



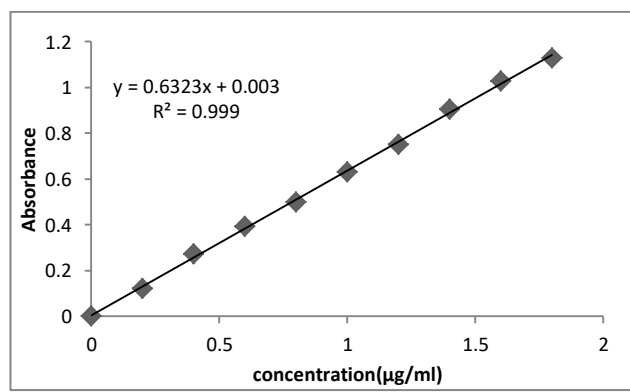
(B)



(E)

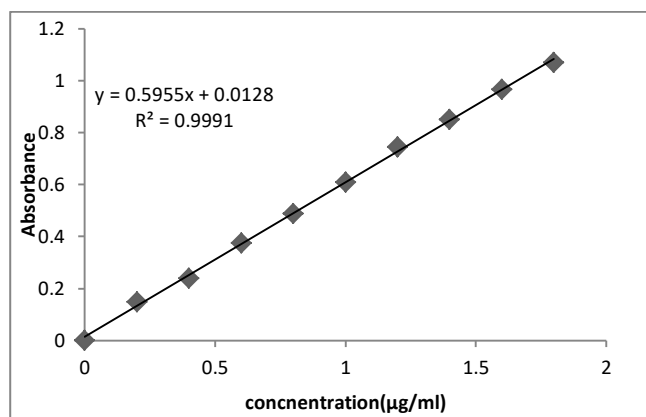


(C)

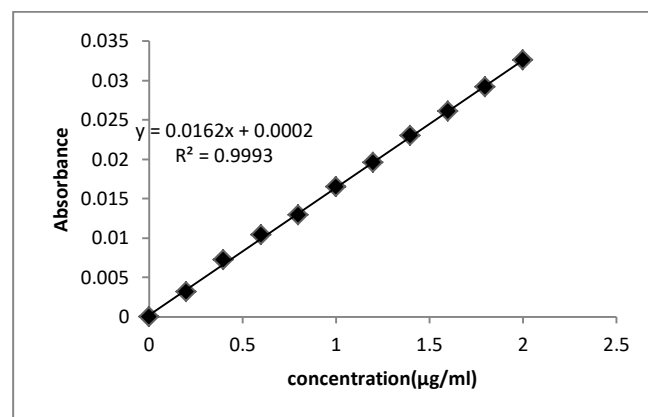


(F)

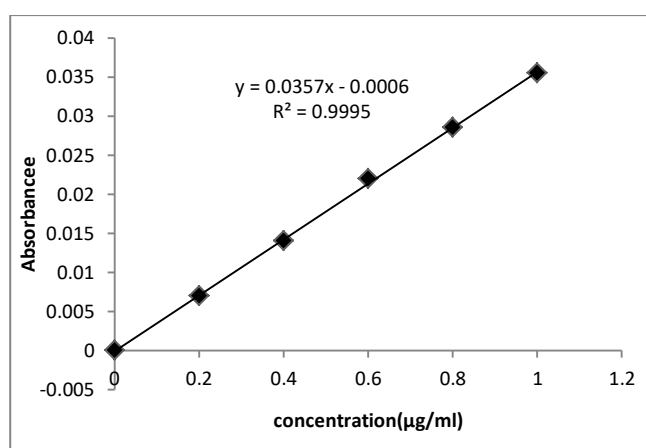
Figure 16: Calibration curves of Perphenazine in method A, B, C, D, E & F



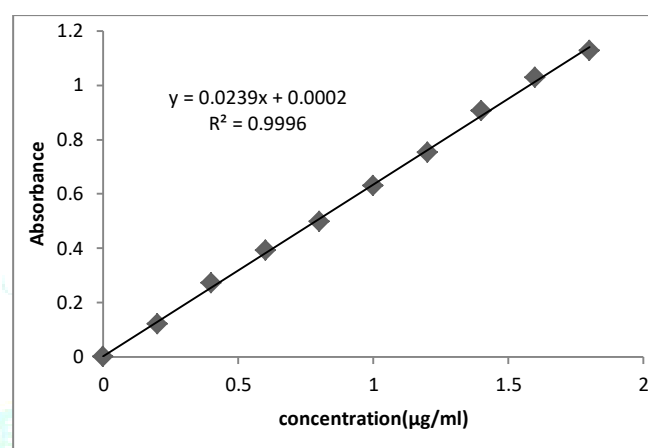
(G)



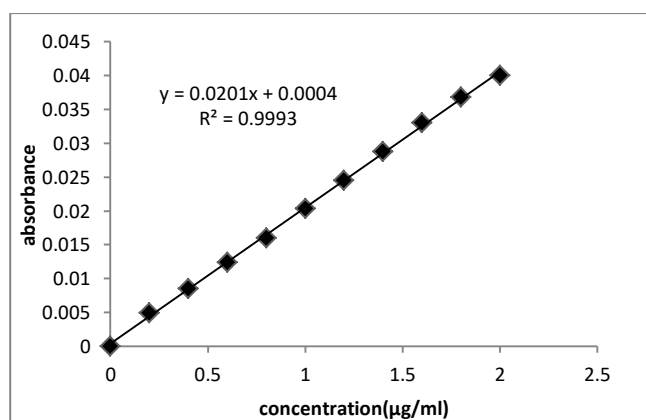
(D1)



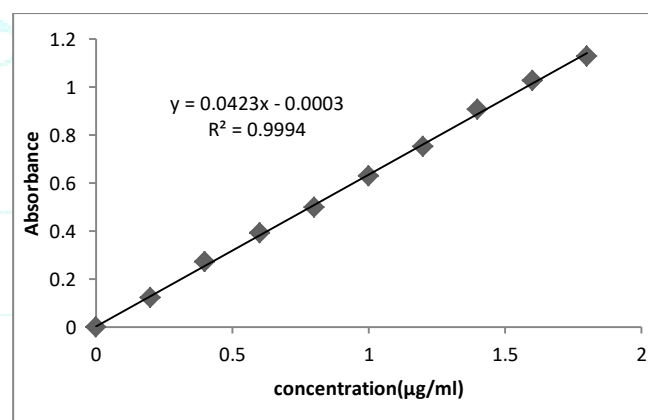
(A1)



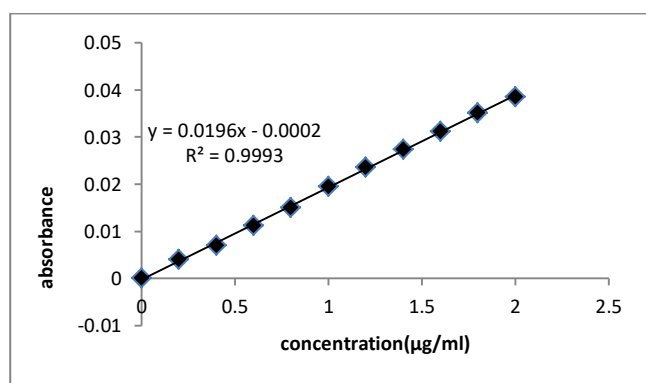
(E1)



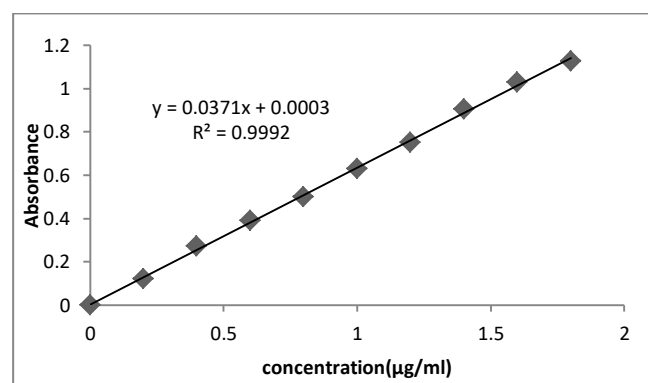
(B1)



(F1)



(C1)



(G1)

Figure 16: Calibration curves of Perphenazine in method G and also for A1, B1, C1, D1, E1, F1 and G1

Table1: Optical characteristics of Perphenazine

Parameters	Method							
	A	B	C	D	A1	B1	C1	D1
Beer-Lambert's limits ($\mu\text{g}/\text{mL}$)	0.002-0.02	0.002-0.02	0.002-0.02	0.002-0.02	0.002-0.02	0.002-0.02	0.002-0.02	0.002-0.02
λ_{max} /Amplitude range (nm)	310	310	310	310	289.00-327.81	288.81-329.81	289.00-328.61	288.98-327.62
Regression equation	$0.5372x + 0.0099$	$0.4257x - 0.0084$	$0.482x - 0.0074$	$0.3686x - 0.0055$	$0.0357x - 0.0006$	$0.0201x + 0.0004$	$0.0196x - 0.0002$	$0.0162x + 0.0002$
Slope	0.5372	0.4257	0.482	0.3686	0.0357	0.0201	0.0196	0.0162
Intercept	0.0099	0.0084	0.0074	0.0055	0.0006	0.0004	0.0002	0.0002
Correlation coefficient	0.999	0.9992	0.9991	0.9992	0.9995	0.9993	0.9993	0.9993
Sandells Sensitivity	$1.56 \times 10^{-5} \mu\text{gcm}^{-2}$	$2.33 \times 10^{-5} \mu\text{gcm}^{-2}$	$2.02 \times 10^{-5} \mu\text{gcm}^{-2}$	$2.39 \times 10^{-5} \mu\text{gcm}^{-2}$	$2.65 \times 10^{-4} \mu\text{gcm}^{-2}$	$5.45 \times 10^{-4} \mu\text{gcm}^{-2}$	$5.33 \times 10^{-4} \mu\text{gcm}^{-2}$	$6.13 \times 10^{-4} \mu\text{gcm}^{-2}$

Table 2: Optical characteristics of Perphenazine

Parameters	METHOD					
	E	F	G	E1	F1	G1
Beer-Lambert's limits ($\mu\text{g}/\text{mL}$)	0.002-0.02	0.002-0.02	0.002-0.02	0.002-0.02	0.002-0.02	0.002-0.02
λ_{max} /Amplitude range (nm)	310	300	300	335.16-341.15	250.03-322.19	250.18-320.80
Regression equation	$0.4864x + 0.0081$	$0.6323x - 0.003$	$0.5955x + 0.0128$	$0.0239x + 0.0002$	$0.0423x - 0.0003$	$0.0371x + 0.0003$
Slope	0.4864	0.6323	0.5955	0.0239	0.0423	0.0371
Intercept	0.0081	0.003	0.0128	0.0002	0.0003	0.0003
Correlation coefficient	0.999	0.999	0.9991	0.9996	0.9994	0.9992
Sandells Sensitivity	$2.68 \times 10^{-5} \mu\text{gcm}^{-2}$	$1.94 \times 10^{-5} \mu\text{gcm}^{-2}$	$1.64 \times 10^{-5} \mu\text{gcm}^{-2}$	$2.58 \times 10^{-4} \mu\text{gcm}^{-2}$	$2.57 \times 10^{-4} \mu\text{gcm}^{-2}$	$2.64 \times 10^{-4} \mu\text{gcm}^{-2}$

The % RSD values in precision studies were found to be 0.12, 0.28, 0.34, 0.45, 0.11, 0.28 and 0.35 for method A, B, C, D, E, F and G respectively (RSD <2%) and 0.24, 0.57, 0.69, 0.91, 0.11, 0.28 and 0.35 for method A1, B1, C1, D1, E1, F1 and G1 respectively (RSD <2%) indicating that the method is more precise. The % Recovery values (Tables 3 to 6) were found to be 99.20%, 99.33%, 99.44%, 99.54%, 99.30 %, 99.44% and 99.54% with RSD 0.13, 0.39, 0.45, 0.56, 0.14, 0.49 and 0.55 for method A, B, C, D, E, F and G respectively (RSD <2%) and the % Recovery values (Tables 3 to 6) were found to be 99.42%, 99.22%, 99.57%, 99.60%, 99.66 %, 99.22% and 99.64% with RSD 0.35, 0.68, 0.71, 0.93, 0.35, 0.48 and 0.69 for method A1, B1, C1, D1, E1, F1 and G1 respectively (RSD <2%) indicating that the proposed methods can be applied for the determination of pharmaceutical formulations and the method is more accurate.

LOQ is defined as the lowest amount of analyte which can be detected. LOD was defined as the lowest amount of analyte which can be quantitatively determined. LOD and LOQ of the drug were calculated as per ICH guidelines. The Limit of Detection and Limit of Quantification was found to be 0.33 $\mu\text{g}/\text{mL}$ and 0.0165 $\mu\text{g}/\text{mL}$ (Method A and A1) and 0.165 $\mu\text{g}/\text{mL}$ and 0.00825 $\mu\text{g}/\text{mL}$ (Method B and B1) and 0.66 $\mu\text{g}/\text{mL}$ and 0.0274 $\mu\text{g}/\text{mL}$ (Method C and C1) and 0.495 $\mu\text{g}/\text{mL}$ and 0.033 $\mu\text{g}/\text{mL}$ (Method D and D1) and 0.99 $\mu\text{g}/\text{mL}$ and 0.01625 $\mu\text{g}/\text{mL}$ (Method E and E1) and 0.33 $\mu\text{g}/\text{mL}$ and 0.00825 $\mu\text{g}/\text{mL}$ (Method F and F1) and 0.66 $\mu\text{g}/\text{mL}$ and 0.00825 $\mu\text{g}/\text{mL}$ (Method G and G1) respectively.

Table 3: Assay of API

Level	*Amount obtained (mg)							
	Method							
	A	B	C	D	A1	B1	C1	D1
50	0.4949	0.4954	0.4965	0.4974	0.4967	0.4964	0.4976	0.4953
100	0.9959	0.9920	0.9971	0.9982	0.9942	0.9950	0.9963	0.9974
150	0.9975	0.9940	0.9989	0.9996	0.9961	0.9989	0.9971	0.9985

*Each value is average of three determinations

Table 4: Assay of API

Level	*Amount obtained (mg)					
	Method					
	E	F	G	E1	F1	G1
50	0.4939	0.4944	0.4958	0.4966	0.4954	0.4964
100	0.9959	0.9920	0.9970	0.9979	0.9961	0.9979
150	0.9989	0.9940	0.9980	0.9995	0.9981	0.9985

*Each value is average of three determinations

Table 5: Assay of API

Level	% Recovery*							
	Method							
	A	B	C	D	A1	B1	C1	D1
50	99.20	99.33	99.44	99.54	99.42	99.22	99.57	99.60
100	99.51	99.22	99.61	99.74	99.41	99.50	99.71	99.81
150	99.71	99.30	99.72	99.81	99.69	99.60	99.89	99.90

Table 6: Assay of API

Level	% Recovery*					
	Method					
	E	F	G	E1	F1	G1
50	99.30	99.44	99.54	99.66	99.22	99.64
100	99.59	99.33	99.71	99.89	99.51	0.9967
150	99.89	99.40	99.60	99.95	99.61	99.85

*Each value is average of three determinations

4. DISCUSSION

The objective of the analytical procedure is to govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below: Linearity, Accuracy, Precision, LOD, and LOQ. The calibration plot for linear regression data was indicative of a good linear relationship between concentration and peak area concentration over a wide range. The results have shown best recoveries (%) of the spiked drug at each added concentration, indicating that the method was accurate. The precision of Perphenazine was evaluated and the percentage relative standard deviation (%RSD) was found to be which proves that the method was precise. And the Limit of Detection and Limit of Quantification was found to be 0.33µg/ml and 0.0165µg/ml (Method A and A1) and 0.165µg/mL and 0.00825µg/mL (Method B and B1) and 0.66µg/ml and 0.0274µg/ml (Method C and C1) and 0.495µg/ml and 0.033µg/ml (Method D and D1) and 0.01625µg/ml (Method E and E1) and 0.33µg/mL and 0.00825µg/mL (Method F and F1) and 0.66µg/ML and 0.00825 µg/ML (Method G and G1) respectively. Hence the proposed method was sensitive.

CONCLUSION

The present methods can be employed for the determination of Perphenazine in API.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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