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Original Article

DEVELOPMENT AND VALIDATION OF ZERO AND FIRST ORDER SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF OPIPRAMOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: Two simple, precise and accurate zero and first order spectrophotometric methods were developed and validated for the quantification of opipramol in bulk and tablet dosage form.

Methods: The quantitative analysis of the drug was carried out using the zero order and first order derivative values were measured at 254 nm and 266 nm respectively. The estimation of the drug was carried out by regression equations with the standard solution.

Results: Calibration graph was found to be linear r^2 = 0.996 for zero order and r^2 = 0.998 for first order derivative over the concentration range of 2-10 µg/ml. Precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 100.77 %), specific and robust. No obstruction was observed from general pharmaceutical adjutants.

Conclusion: The developed derivative methods can be utilized in its routine analysis opipramol in quality control division.

Keywords: Opipramol, Zero order spectra, First order, UV-spectrophotometric, Analytical method validation.

INTRODUCTION

Opipramol 2-HCl (4-[3-(5H-dibenz [b, f]-azepine-5-yl)-propyl]-1-piperazine-ethanol dihydrochloride) (fig. 1) is an atypical anxiolytic and antidepressive drug. It is a psychotropic drug normally used for treatment of anxious-depressive states, somatoform disorders and general anxiety disorders [1-4]. Literature survey indicated that few analytical methods have been reported for analysis of opipramol. They include some HPLC methods associated with potentiometric detection in human plasma [5], electroanalytical [6] detectors. There is no any spectropotometric method for the analysis of opipramol in pharmaceutical formulation. Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Under computer-controlled instrumentation, derivative spectrophotometry is acting a very important role in the single or multicomponent analysis of drugs by UV molecular absorption spectrophotometric method.

These developed methods have their relative merits but the majorities of them uses relatively expensive instrument [5] and involve extraction [6]and are time consuming. Hence in this present study, we developed two simple, rapid and inexpensive derivative methods for the analysis of opipramol in pure and pharmaceutical dosage form. The proposed derivative methods are validated using standard ICH guidelines [7, 8].

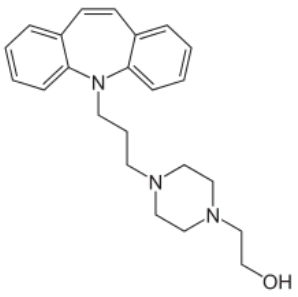

Fig. 1: Chemical structure of Opipramol

MATERIALS AND METHODS

The spectrophotometric measurements were carried out using a Shimadzu UV/Vis spectrophotometer with a pair of 10 mm matched quartz cells, Shimadzu digital balance for weighing and sonicator were used for the study. A tablet used was "Opiprol 100" of Sun Pharma Laboratories, Mumbai, and each film coated tablet contains 100mg of Opipramol dihydrochloride as per the label claim.

Reagents

Opipramol was obtained as a gift sample from SRL fine chemicals, Bangaluru. Distilled water was used as solvent throughout the experimentation. A pharmaceutical preparation was purchased from the local pharmacy.

Standard solutions

Analytical method development

A standard stock solution of Opipramol HCl was prepared by dissolving accurately weighed 10 mg of Opipramol HCl in 100 ml of distilled water (100 µg/ml). Appropriate dilutions were prepared to obtain 10 µg/ml solutions was scanned on a UV spectrophotometer in the wavelength range of 200-400 nm in 1.0 cm cell against solvent blank and the spectra was recorded. Spectrum was taken, and λmax was determined. Optical parameters of the developed methods are tabulated in table 1.

Assay procedure

A total of 20 tablets of opipramol were opened and the contents were weighed and mixed. Accurately weighed and powdered. An aliquot of powder equivalent to the weight of 1 tablet was accurately weighed and transferred to the volumetric flask and was dissolved in 100 ml of water and made up to volume with same solvent. The solutions were filtered through a 0.45 µm nylon filter and sonicated for about 15 min and then volume made up with water. This solution was filtered to eliminate any insoluble matter. The filtrate was collected in a clean flask. Appropriate dilutions were made with water and measured solution (100 µg/ml) for both zero order at 254 nm and first order derivative at 266 nm spectrophotometric methods.

Development of the methods

Method A: Zero order spectroscopic method

The solutions were scanned in the range of 400- 200 nm, and the peak was observed and gives maximum absorbance at 254 nm. So, the wavelength selected for the analysis of the drug was 254 nm. The drug followed the Beer's- Lamberts law in the range of 2-10 µg/ml. The overlay spectra and linearity graph of opipramol were shown in fig. 2 and 3 respectively.

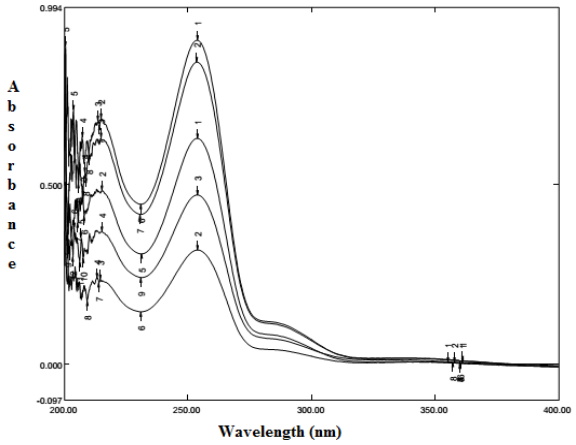

Fig. 2: Overlay spectra of opipramol for zero order method

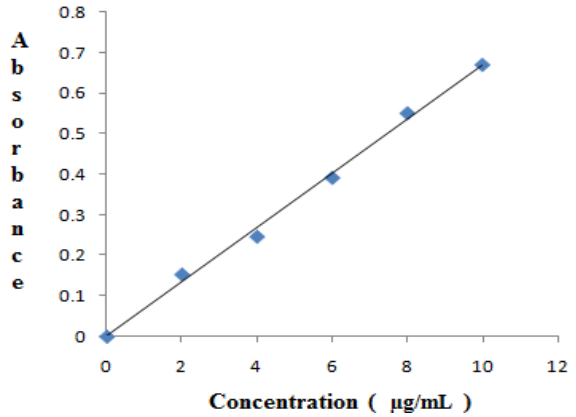

Fig. 3: Linearity graph of opipramol for zero order method

Method B: First order derivative spectroscopic method

The standard drug solution was diluted so as obtain the final concentration in the range of 2-10 µg/ml and scanned in the first order derivative spectra. The first order derivative spectra showed at 266 nm. The amplitude of absorbance was measured at 266 nm was plotted against concentration to give a calibration curve, and the regression equation was calculated. The amplitude was linear in the concentration range of 2-10 µg/ml. The overlay spectra and linearity graph of opipramol were shown in fig. 4 and 5 respectively.

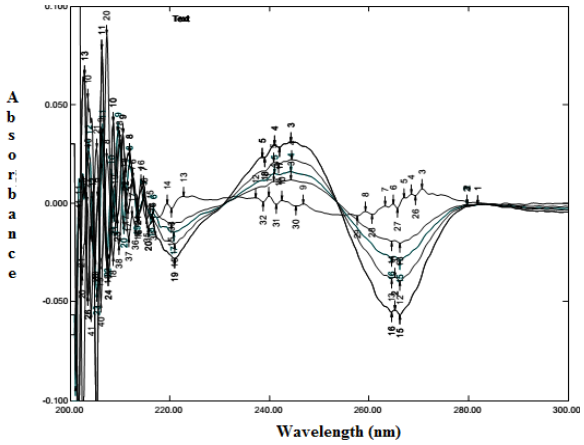

Fig. 4: Overlay spectra of opipramol for first order method

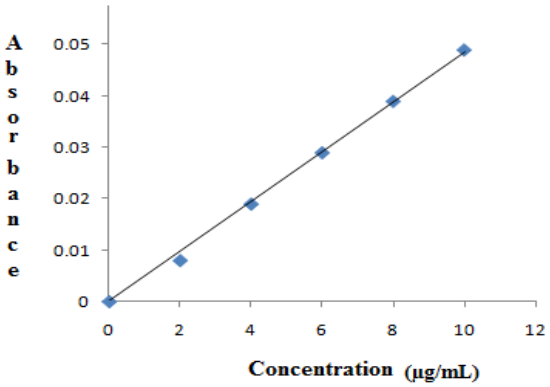

Fig. 5: Linearity graph of opipramol for First order method

Analytical method validation

Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the preanalysed tablet powder and the proposed method was followed. Standard quantity equivalent to 80%, 100% and 120% is to be added to sample. The result is shown that best recoveries (98.44-102.18%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate. Recovery data of the method is shown in table 2. From the amount of drug estimated, percentage recovery was calculated. The method was found to be accurate with the average accuracy percentage of 100.0 ± 1.25 and 103 ± 0.75 for zero order and first order method.

Table 1: Optical parameters of the proposed derivative methods

Parameters determined	Obtained values	
	Zero derivative	I-Derivative
λ_{max}	254 nm	266 nm
Linearity(µg/ml)	2 - 10	2 - 10
Slope	0.066	0.004
Intercept	0.01	0.00
Regression coefficient	0.996	0.997
LOD (µg/ml)	0.2181	0.1363
LOQ (µg/ml)	0.7272	0.4545
Molar absorptivity (L $mol^{-1}cm^{-1}$)	2.1809×10^3	7.2699×10^2
Sandell Sensitivity (mg cm^{-2} per 0.001 absorbance unit)	0.0013 A. U.	0.05 A. U.

Table 2: Recovery studies of the method

% Recovery level	% recovery	Mean % recovery	SD	% RSD
80	101.53	101.71	0.1512	0.1486
	101.90			
	101.70			
100	102.75	102.18	0.663174	0.649025
	101.25			
	102.54			
120	98.18	98.44	0.389102	0.395268
	98.99			
	98.15			

Table 3: Precision data of the proposed methods

Sample no.	% assay	
Set	Intra day	Inter day
1.	0.379	0.319
2.	0.365	0.313
3.	0.378	0.311
4.	0.378	0.321
5.	0.372	0.314
6.	0.374	0.312
Mean	0.3743	0.315
SD	0.0048	0.0036
%RSD	1.2965	1.1736

Precision

It was ascertained by replicate analysis of the homogeneous sample of tablet powder and % RSD of the estimations is shown in table 3 for the given brand of the sample by the proposed method.

Inter day and Intraday precision

An accurately weighed quantity of Tablet powder equivalent to about 10 mg of opipramol maleate was transferred to 100 mL volumetric flask, shaken for 15 min with distilled water solution and diluted, up to the mark with distilled water. The contents were filtered through Whatman filter paper no. 41. Aliquot portions were further diluted with distilled water to get concentration of 10 µg/ml of opipramol (on label claim basis). The absorbances of the final solutions were read after 0 hr, 3hr and 6 hr in 1.0 cm cell at a selected wavelength. Similarly the absorbance of the same solution was read on 1st, 3rd and 5th day. The amount of opipramol was estimated by comparison of standard and by taking the regression equation at 254 nm for zero order and 243 nm for the second order.

Linearity and range

Six points calibration curve were obtained in concentration range from 2-10 µg/ml for opipramol. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.066 x+0.001 with correlation coefficient 0.996 for zero order derivative method and y = 0.004 x + 0.00 with correlation coefficient 0.998 for first order method. The concentrations of drug against absorbance are shown in the table 4.

Table 4: Concentration Vs absorbance for linearity study

Concentration (µg/ml)	Absorbance (Zero Order)	Absorbance (First Order)
2	0.153	0.008
4	0.246	0.019
6	0.391	0.029
8	0.55	0.039
10	0.669	0.049

Limit of detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated based on the standard deviation of the analytical response (absorbance at 530 nm) and the slope of the calibration curve using the equations LOD = 3.3 σ/S and LOD = 10 σ/S, where σ is the SD of the response and S is the slope of the calibration curve. It was found that LOD and LOQ for zero order method were 0.2181 µg/ml and 0.7272 µg/ml respectively. The LOD and LOQ for first order method were 0.1363 µg/ml and 0.4545 µg/ml respectively.

Ruggedness

It was carried out by analysing the same by three different analysts and estimation of drug by the proposed method.

Robustness

The evaluation of robustness should be measured during the development phase and depends on the type of procedure, conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Table 5: Evaluation data of system suitability study

Sample no.	Absorbance
1.	0.153
2.	0.150
3.	0.149
4.	0.144
5.	0.155
6.	0.148
Average	0.1516
SD	0.0028
%RSD	1.8519

System suitability

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation was taken and %RSD of standard reading were taken for same. Acceptance criteria for system suitability, %RSD of standard reading not more than 2.0%, were full fill during all validation parameter (table 5).

RESULTS AND DISCUSSIONS

The zero and first order derivative spectra of standard solutions of opipramol was studied in water. The absorption spectra showed that the clear sharp defined peaks were observed in both the zero and first order spectra. All the validation parameters showed values within standard validation limits. The percent recovery was considered to be 98.44-101.71% indicating reproducibility and accuracy of the method. Precision study was carried to find out intra-day and inter-day variations. The results of precision studies are reported in table 2 and values of percentages of standard deviations are 1.2965 for zero order and 1.1736 for first order method indicates a high degree of precision. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) value was found to be 0.2181µg/ml and 0.7272µg/ml for zero order method and 0.1363 µg/ml and 0.4545 µg/ml for the first order method respectively. The developed method was found to be simple, precise and economical and can be adopted for routine quality control of drug.

CONCLUSION

The present analytical method for the quantification of opipramol was validated as per ICH Q2(R1) guideline and it meets to specific acceptance criteria. The analysis of the drug does not involve any extraction procedure and the method was limit of detection developed in nano gram concentration proves that it very sensitive and accurate. The excipients usually present in pharmaceutical dosage forms did not interferes in the developed methods. It is concluded that the analytical method was specific, precise, linear, accurate, robust and having stability indicating characteristics. The present analytical method can be used for its intended purpose. Results also prove that the developed methods can be successfully applied for a regular analysis and quantitative control of drug.

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CONFLICT OF INTERESTS

Declared None.

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