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Development and Validation of New Analytical Methods for the Determination of Endoxan in Pharmaceutical Formulations by UV-Visible Spectrophotometry

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Abstract: ***In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were undertaken by varying one parameter at a time and controlling all other parameters to get maximum color development, minimum blank color, reproducibility and the reasonable period of stability of final colored species formed. Method 1: A standard solution of endoxan of concentration of 0.1mg/ml was prepared in the water and then the absorbance studies were conducted for the solutions by using the uv-visible spectrophotometry. Solvent: Distilled water, Reagents: 1%1, 10Phenanthroline, 2%Fecl$_3$, Wavelength: 420nm, Concentration range: 2-4µg/ml, in this method, the spectra recorded was very nice and absorbance values gave good linearity. Here the range is extended up to 10µg/ml. Method 2: A standard solution of Endoxan of concentration of 0.1mg/ml was prepared in water and then the absorbance studies were conducted for the solution by using uv-visible spectrophotometry. Solvent: Distilled water, Reagents: FcRe agent, Wavelength: 410nm, Concentration range: 2-10µg/ml. In this method the spectra recorded was very nice and absorbance values gave good linearity. Here the range is extended up to 10µg/ml. Method 3: A standard solution of endoxan of concentration of 0.1mg/ml was prepared in water and then the absorbance studies were conducted for the solutions by using uv-visible spectrophotometry. Solvent: Distilled water, Reagents: Acetaldehyde solution, 3%NaoH, Wave length: 410nm, Concentration range: 2-10µg/ml. In this method the spectra recorded was very nice and the absorbance values gave good linearity, here the range is extended up to 10µg/ml. The absorption curves of colored species formed in each method shows characteristic absorption maximum; whereas the blank in each method has low or no absorption in this region .In developing these methods, a systematic study of the effects of various relevant parameters validate this method according to ICH Q2 (B) to get a suitable method which meets the regulatory requirements. In the methods concerned were undertaken by varying one parameter at a time and controlling all other parameters to get maximum color development, minimum blank color, reproducibility and the reasonable period of stability of final colored species formed.***

Keywords: Endoxan, UV, visible, validation, analytical

1. Introduction

Cyclophosphamide is used to treat various types of cancer. It is a chemotherapy drug that works by slowing or stopping cell growth .Cyclophosphamide also works by decreasing your immune system's response to various diseases. In the methods concerned were undertaken by varying one parameter at a time and controlling all other parameters to get maximum color development, minimum blank color, reproducibility and the reasonable period of stability of final colored species formed A survey of literature on the selected drug endoxan revealed that there are very few visible spectrophotometric methods for their estimation at the time of commencement of this investigation. The analytically important functional groups of selected drug do not seem to be fully exploited for designing visible spectrophotometric methods for their determinations .The developed method is a simple and sensitive UV-VISIBLE method using chromogenic reagents like Folin-Ciocalteu'sphenolreagent, 1,10-Phenanthroline monohydrate reagent, Acetaldehyde Solution.

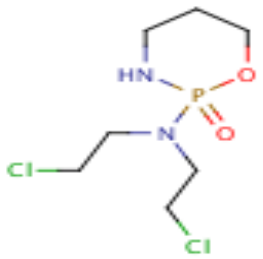

Figure 1: Structure of cyclophosphamide

2. Experimental

2.1 Materials

Folin-ciocalteu's phenol reagent, Merck specialties private Ltd. Shiv sagar estate A, Dr.annie Beasent Road, Worli, Mumbai,1,10 Phenanthroline mono hydrate, Finer Chemicals Ltd, Ahmedabad, India. Acetaldehyde solution, Loba chemicals Ltd. 107, Wodehouse road, Mumbai, India. Ferric chloride(III)anhydrous Purified**,** Merck specialties private Ltd. Shiv sagar estate A, Dr. Annie Beasent Road ,Worli, Mumbai**,** India. SodiumHydroxidePellets Thermo Fisher Scientific India, Wing, Delhi, Hiranandani Business park, Powai, Mumbai, India, Methanol ,Merck specialties private Ltd. Shiv sagar

estate A. Cyclophosphamide standard was supplied by Aziant Drug Research Solutions, Hyderabad, India.

2.2 Equipments

An Elico single beam UV-visible spectrophotometer Aquamate plus with 1 cm matched quartz cells was used for all spectral and absorbance measurements. A Systronics digital pH meter was used for all pH measurements. Digital pH meter Model DI-45P Hyderabad, Balance: Electronic balance type BL-220h, Schimadzu corporation, Japan.

2.3 Standard Preparation

A standard solution of endoxan of concentration of 0.1mg/ml was prepared in the water and then the absorbance studies were conducted for the solutions by using the uv-visible spectrophotmetry.

2.4 Standard Solution and Calibration Graph

Method 1: A standard solution of endoxan of concentration of 0.1mg/ml was prepared in the water and then the absorbance studies were conducted for the solutions by using the uv-visible spectrophotometry.
Solvent: Distilled water,
Reagents: 1%1, 10Phenanthroline, 2%$Fecl_3$,
Wavelength: 420nm,
Concentration range: 2-4µg/ml, in this method, the spectra recorded was very nice and absorbance values gave good linearity. Here the range is extended up to 10µg/ml.

Method 2: A standard solution of Endoxan of concentration of 0.1mg/ml was prepared in water and then the absorbance studies were conducted for the solution by using uv-visible spectrophotometry.
Solvent: Distilled water,
Reagents: FcReagent,
Wavelength: 410nm,
Concentration range: 2-10µg/ml. In this method the spectra recorded was very nice and absorbance values gave good linearity. Here the range is extended up to 10µg/ml.

Method 3: A standard solution of endoxan of concentration of 0.1mg/ml was prepared in water and then the absorbance studies were conducted for the solutions by using uv-visible spectrophotometry.
Solvent: Distilled water
Reagents: Acetaldehyde solution, 3%NaoH, Wave length: 410nm,
Concentration range: 2-10µg/ml. In this method the spectra recorded was very nice and the absorbance values gave good linearity, here the range is extended up to 10µg/ml.

3. Method Validation

The UV-VISIBLE method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Assay method precision was determined using six-independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on different days. The accuracy of the assay method was evaluated by adding the cyclophosphamide drug substance on placebo in the range of about50 to 150% level. . Linearity test solutions were prepared the spectra recorded was very nice and the absorbance values gave good linearity. To determine the robustness of the method, the final experimental conditions were altered and the results were examined. The λ max was varied by (±) 0.1 nm.

4. Results and Discussion

Optimization of the spectrophotometric conditions Parameters Fixation: In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were undertaken by varying one parameter at a time and controlling all other parameters to get maximum color development, minimum blank color, reproducibility and the reasonable period of stability of final colored species formed. In order to ascertain the optimum wave length of maximum absorption, the spectra were scanned in the wave length region of 400- 800nm against a corresponding reagent blank. The reagent blank absorption spectrum of the method was recorded against the solvent employed in the method. The absorption curves of colored species formed in each method shows characteristic absorption maximum; whereas the blank in each method has low or no absorption in this region. Reagents preparation 1,10-Phenanthroline(1%w/v),Weigh about 0.1 gm of 1,10-phenanthrolineand dissolve it in 10ml of methanol.$Fecl_3$ solution(2%w/v) ,In a 100ml volumetric flask weigh about 2gm of $Fecl_3$ and dissolve it in a minimum quantity of distilled water and make it up the volume with distilled water. NaoH solution(3%w/v) , In a 100ml volumetric flask add 3gm of NaoH to this add small quantity of water and dissolve NaoH and make it up to the volume with distilled water.

Method 1: A standard solution of endoxan of concentration of 0.1mg/ml was prepared in the water and then the absorbance studies were conducted for the solutions by using the uv-visible spectrophotometry.
Solvent: Distilled water,
Reagents: 1%1, 10Phenanthroline, 2%$Fecl_3$,
Wavelength: 420nm,
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Method 2: A standard solution of Endoxan of concentration of 0.1mg/ml was prepared in water and then the absorbance studies were conducted for the solution by using uv-visible spectrophotometry.
Solvent: Distilled water,
Reagents: FcReagent, Wavelength: 410nm,
Concentrating range: 2-10µg/ml. In this method the spectra recorded was very nice and absorbance values gave good linearity. Here the range is extended up to 10µg/ml.

Method 3: A standard solution of endoxan of concentration of 0.1mg/ml was prepared in water and then the absorbance studies were conducted for the solutions by using uv-visible spectrophotometry.
Solvent: Distilled water
Reagents: Acetaldehyde solution, 3%NaoH, Wave length: 410nm,
Concentration range: 2-10µg/ml. In this method the spectra recorded was very nice and the absorbance values gave good linearity, here the range is extended up to 10µg/ml. (Figure 1-1)

5. Validation of Method

5.1 Specificity

The specificity of the uv-visible method is where complete separation of endoxan was noticed in presence of impurities. In addition there was no any interference of impurities' at the absorbance of endoxan in the spectrum. There was no any interference at the absorbance of endoxan in the spectra of placebo solution. This shows that the absorbance peak of analyses was pure and excipients in the formulation did not interfere the analyte.

5.2 Accuracy

Accuracy of the method was calculated by recovery studies at six levels for 50% and 150% level and three levels for 75%, 100%, and 125%. (Table 1).The mean percentage recovery obtained for endoxan was found to be in between 99.90 and 101.0% respectively (Table 1)

5.3 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analyses of the same working solution. The relative standard deviation [R.S.D.)] obtained for endoxan was 0.2. The intra- and inter-day variability or precision data are summarized in (Table3). The intra-day precision of the developed colorimetry method was determined by preparing the samples of the same batch. A standard solution of Endoxan of concentration of 0.1mg/ml was prepared in water and then the absorbance studies were conducted for the solution by using uv-visible spectrophotometry. Blank solution and six replicates of repeatability solutions were seen for absorbances. The %R.S.D, % assay of the assay results was used to evaluate the method precision. The inter-day precision was also determined by the same procedure. The results indicated the good precision of the developed method. (Table 2)

5.4 Linearity

Linearity was determined for endoxan in the range of Concentration range: 2-4µg/ml for method-1, 2-10µg/ml for method-2, 2-10µg/ml for method-3. The correlation coefficient ('r') value for the drug was >0.998. (Table 3)

5.5 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the method, the final experimental conditions were altered and the results were examined. The λ max was varied by (±) 0.1 nm. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

6. Conclusion

A simple, specific, linear, precise, accurate and uv-visible method has been developed and validated for quantitative determination of endoxan in pharmaceutical formulations. Statistical analysis proves that method is repeatable and selective for the analysis of endoxan in pharmaceutical formulations .The developed method can be applicable for pharmaceutical dosage forms and in process testing.

Table 1: Results of the recovery analysis endoxan

S. No.	*% added*	*Amount added (mg)*	*Amount recovered (mg)*	*% Recovery*	*% Mean Recovery*	*% RSD*
1.	50	93.71	93.60	99.9	100.2	0.4
2.		93.91	93.63	99.7		
3.		93.79	94.32	100.6		
4.		93.72	94.18	100.5		
5.		93.65	94.04	100.4		
6.		93.62	94.00	100.4		
1.	75	148.01	147.96	100.0	99.9	0.2
2.		148.19	148.20	100.0		
3.		148.16	147.74	99.7		
1.	100	198.68	199.09	100.2	100.2	0.1
2.		198.73	199.42	100.3		
3.		198.23	198.37	100.1		
1.	125	243.69	245.84	100.9	101.0	0.2
2.		243.75	246.56	101.2		
3.		243.57	245.87	100.9		
1.	150	299.84	302.39	100.9	100.9	0.3
2.		299.48	301.45	100.7		
3.		299.51	302.65	101.1		
4.		299.80	302.24	100.8		
5.		299.85	301.51	100.6		
6.		299.84	303.82	101.3		

Table 2: Intra -and inter- day assay precision data (n=12)

Sample No.	*Method Precision*	*Intermediate Precision*	*Over all % RSD (n=12)*
1	98.3	101.8	1.0
2	100.2	101.3	
3	100.1	98.6	
4	99.9	100.2	
5	99.9	100.3	
6	100.5	101.1	
Mean	99.8	100.6	
% RSD	0.8	1.1	

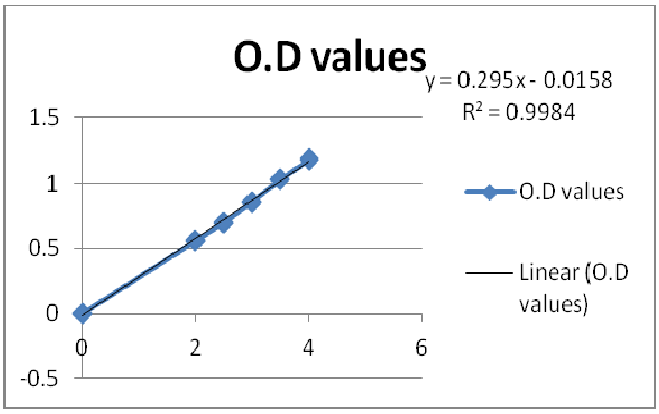

Figure 1: Beer`s Law plot of Endoxan with 1, 10-Phenanthroline

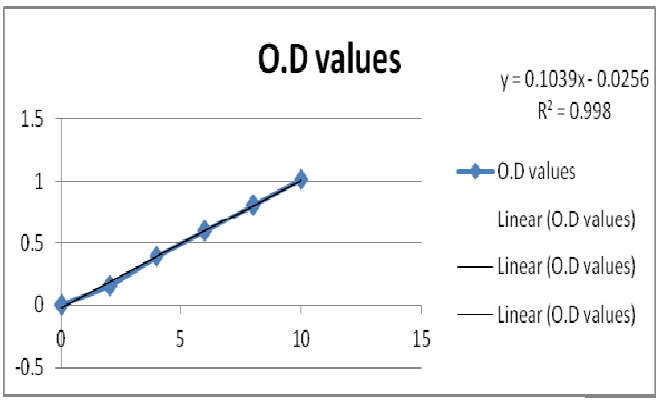

Figure 2: Beer`s Law plot of Endoxan with FC reagent

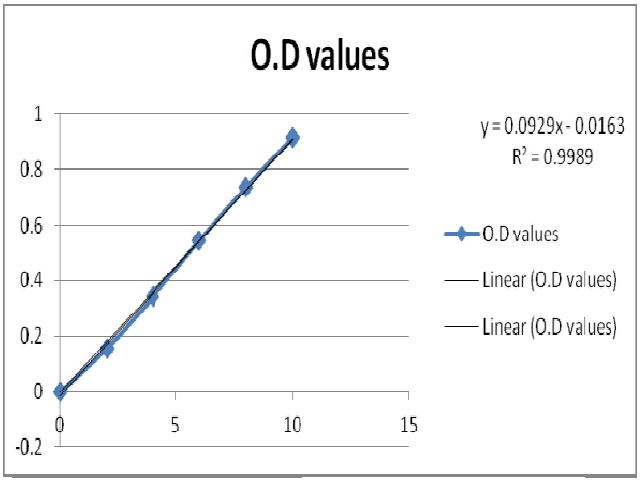

Figure 3: Beer`s Law plot of Endoxan with Acetaldehyde reagent

References

[1] Hobrat H.Willard. Instrumental Method of analysis; 1st edition, 2005, PP 580-, 622.
[2] H.Kaur, Instrumental method of Chemical Analysis; 4th edition, 2006, Pragati Prakashan, PP 798-813.
[3] Keceley.D and Hceines. P.J, Analytical chemistry; 2nd edition, 2002, PP 1-9
[4] Seamus P.J.Higson, Analytical Chemistry; 4th edition, 2005, Longman group U.K Ltd, PP 230-232.
[5] Loyal R. Snyder, Joseph J.Kirkland and Joseph L.Glajch, Practical HPLC method development; 2nd edition, 2005, PP 42--705.
[6] Validation of analytical methods and procedures, http:// www.lab compliance.com/info/links/methods/guidelines.html/
[7] Analytical method validation, http:// www. Asi-rtp.com/method – validation-process.html/
[8] Validation of analytical procedures: Text and Methodology Q_2 (B):ICH Harmonized Tripartite Guidline.2007 Nov. 2
[9] International Conference on Harmonization (ICH), Q2B, Validation of Analytical procedures Methodology. 1997,62,US FDA Federal Register
[10] http://www.drugbank.ca/drugs/DB000207.
[11] http://www. Life-extension-drugs.com