Research Article

UV Visible Spectrophotometric Method Development and Validation for the Estimation of Ifosfamide in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

The main objective of this research work was to develop and validate simple, economic and rapid UV spectrophotometric method for Ifosfamide in bulk drug and pharmaceutical dosage form. The proposed method utilized a Thermoelectron Corporation UV-double beam spectrophotometer with matched pair of 10mm quartz cells was used throughout the experimental work. UV Vision pro software was used to acquire data and all determinations were made at a wavelength of 249 nm. Methanol was used as a solvent. Experiments were designed for determining linearity, limit of detection and quantitation, accuracy, precision and specificity of this analytical method as per the International Organization for Standardization guidelines. The proposed method was found to be linear in the concentration ranges from 2-10µg/ml with the linear correlation coefficient of R2= 0.999 and the mean recoveries were 99.63 to 101.17 %.The stated method can be used as a method with high degree of linearity, accuracy and precision for assay of Ifosfamide in routine pharmaceutical analysis. A simple, sensitive and a cost effective UV-visible spectroscopic method was developed and validated for the estimation of ifosfamide in bulk drug and pharmaceutical dosage form.

Keywords: Validation, linearity, specificity, UV/Vis spectrophotometer.

INTRODUCTION

Ifosfamide is a chemically 3-(2-chloroethyl)-2-((2-chloroethyl)amino)tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide. Ifosfamide is a chemotherapeutical agent and it is chemically associated to the nitrogen mustards and it is a synthetic analog of cyclophosphamide. It is active as an alkylating agent and an immunosuppressive agent. It is a chemotherapy medication used to treat a number of types of cancer. This includes testicular cancer, soft tissue sarcoma, osteosarcoma, bladder cancer, small cell lung cancer, cervical cancer, and ovarian cancer. It is administered by injection into a vein.[1,2]

Figure 1: Structure of Ifosfamide

After the development of an analytical procedure, it is must important to assure that the procedure will consistently produce the intended a precise result with high degree of accuracy. The method ought to provide a specific result which will not be affected by external matters. This creates a demand to validate the analytical procedures.

Method validation is thought to be one of the foremost most accustomed areas in analytical chemistry. The global Organization for Standardization defines validation because the confirmation by examination and provision of objective proof that the particular needs for a specified intended use are fulfilled. This diction primarily implies that a closed examination has been carried out and provides proof that the utility of the analytical method with a high degree of accuracy consequently produces results that are fit for purpose. The use of valid strategies is exigent for an analytical laboratory to parade its qualification and proficiency.

UV/Vis spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis, which is simple, rapid, specific, precise, accurate and applicable to small quantities of compounds. The main principle of UV/Vis spectrophotometry is that the measuring of the amount of UV or visible light absorbed by a substance in solution. Beer- Lambert law that governs the quantitative spectrophotometric analysis, that states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules as it passes through a medium of homogeneous thickness.

Therefore, the main objective of this research work was to develop and validate simple, economic and rapid UV spectrophotometric method for Ifosfamide in bulk form and pharmaceutical formulation .[3-9]

MATERIALS AND METHODS

UV/Vis spectra were recorded on a Thermoelectron Corporation UV-double beam spectrophotometer with matched pair of 10mm quartz cells. All weighing's were done on an electronic balance (Metler Toledo). Bath sonicator was used to aid dissolution. Glassware used in each procedure were bleached entirely with detergent and rinsed thoroughly with double-distilled water and dried in

a hot air oven. Pure drug samples of ifosfamide were obtained from celon laboratories pvt ltd. All other chemicals and reagents used were of analytical grade. Methanol, Sodium hydroxide (NaOH), Hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were purchased from rankem laboratories. Locally available brand of Ifosfamide have been purchased from a local pharmacy to conduct the assay with the developed method.

Preparation of standard and sample solution

Standard Solution

About 10 mg Ifosfamide standard weighed and transferred into 10 ml volumetric flask dissolved in methanol and the volume was made up to mark with methanol. From the above solution 1 ml was pipetted into 10 ml volumetric flask dissolved in methanol and the volume was made up to mark with methanol (100µg/ml).

Sample Solution

Weighed a quantity of powder equivalent to 100mg of Ifosfamide powder and transferred into 100 ml volumetric flask dissolved in methanol and the volume was made up to mark with methanol. The above solution was filtered and 10ml of filtrate transferred into 100 ml volumetric flask and the volume was made up to mark with methanol (100µg/ml).

RESULTS

Absorption maxima method

The solutions were scanned in the range of 400-200 nm against 0.1N NaOH as reference, and the peaks were observed in the spectra at 249nm. The wavelength selected for analysis of drug was 249nm. The drug obeys the lamberts law in the range of 2-10µg/ml. By using linearity plot the quantification was carried out.

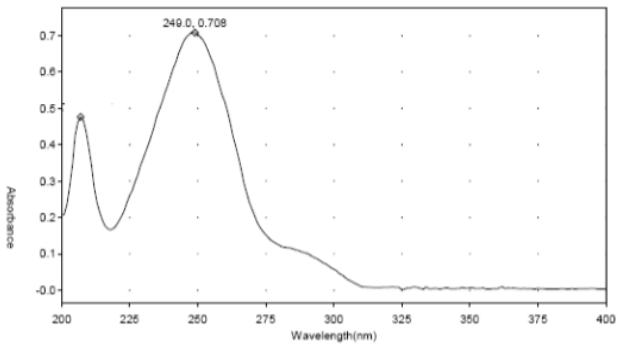

Figure 2: UV Spectrum of Ifosfamide

Assay of Formulation

The Injection powder equivalent to 100 mg of Ifosfamide was accurately weighed, transferred to a 100 mL of volumetric flask containing 10 ml methanol and diluted up to mark with water. The solution was filtered with Whatmann filter paper No. 41. and absorbance was measured. This procedure was repeated six times.

Table 1: Assay of ifosfamide

Marketed formulation	Label claim	Amount taken (equivalent wt)	%amount found +/- SD
IFOMID	1gm	100mg	99.06+/- 0.008

Validation of Analytical Method

The analytical methods were validated according to ICH validation parameters.

Linearity

Fresh aliquots were prepared from standard stock solution ranging from 2-10 µg/ml and the absorbance values of each concentration was recorded at 249nm for this method using NaOH as blank. The drug shows linearity between 2-10µg/ml for this method.

Table 2: Linearity of Ifosfamide

S.NO	Conc(µg/ml)	Absorbance
1	2	0.175
2	4	0.356
3	6	0.586
4	8	0.772
5	10	1.029

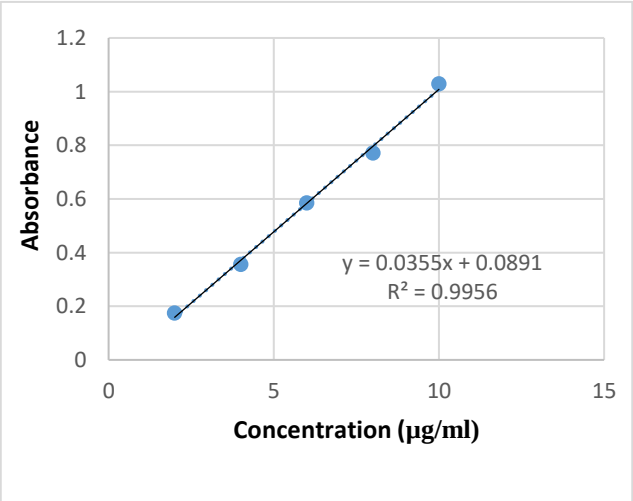

Figure 3: Calibration curve of ifosfamide at 249nm.

Precision

In intraday study concentration of drug were calculated on the same day after 6 hour. In inter day study the concentration of drug contents were calculated on two different days. In both intra and inter-day precision study for the methods % RSD were calculated.

Table 3: Results of inter day and intra day precision

S.NO	Intraday precision (249nm)	Interday Precision (249nm)
1	0.988	0.975
2	0.986	0.977
3	0.982	0.982
4	0.989	0.971
5	0.984	0.984
6	0.987	0.981
AVG	0.986	0.978
SD	0.003	0.005
%RSD	0.264	0.499

Accuracy

Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges 40%, 60%, 100% each one in triplicate. From the recovery studies it was clear that the method is very accurate for quantitative estimation of tablet as the statistical results were within the acceptance range.

Table 4: Accuracy studies of Ifosfamide

%level	Absorbance	Conc Recovered	% Recovered	Mean recovered
40%	0.352	3.96	98.88	
40%	0.357	4.01	100.28	99.63 %
40%	0.355	3.99	99.72	
60%	0.578	5.92	98.63	
60%	0.581	5.95	99.15	99.60 %
60%	0.592	6.06	101.02	
100%	1.034	10.05	100.49	
100%	1.041	10.12	101.17	101.17 %
100%	1.048	10.18	101.85	

Robustness

Robustness of the method was determined by carrying out the analysis at th4ree different wavelengths (±10nm). The respective absorbance was noted and the result was indicated by % RSD.

Table 5: Results of Robustness

S.NO	239nm	249nm	259nm
1	0.981	0.995	0.989
2	0.978	0.991	0.992
3	0.989	0.994	0.985
4	0.985	0.995	0.981
5	0.982	0.990	0.987
6	0.987	0.992	0.984
Avg	0.984	0.993	0.986
SD	0.004	0.002	0.004
%RSD	0.415	0.215	0.394

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification of ifosfamide by proposed methods were determined using calibration graphs. LOQ and LOD were calculated as; LOD = 3.3 X S.D/S LOQ = 10 X S.D/S Where S is the slope of the calibration curve and SD is the standard deviation of response of least concentration of calibration curve in three replicates.

Table 6: Results for LOD and LOQ

Active ingredient	LOD (µg/ml)	LOQ (µg/ml)
Ifosfamide	0.27	0.84

Table 7: Summary Of Validation

S.NO	Parameter	Result
1	Linearity Indicated by Correlation Coefficient	0.9956
2	Precision Indicated By % RSD -Intraday Day -Inter Precision	0.264 0.499
3	Accuracy Indicated By % Recovery	99.63-101.17
4	Robustness Indicated By %RSD	0.394
5	LOD	0.27µg/ml
6	LOQ	0.84µg/ml
7	Assay	99.06%

DISCUSSION

This study provides a simple, accurate, precise and most economical method for the analysis of ifosfamide in bulk and pharmaceutical dosage form using UV spectrometry. The wavelength corresponding to maximum absorbance in methanol was found to be at 249nm.The method obeyed the beers law in the concentration range of 2-10µg/ml with correlation coefficient(R^2 =0.9956). The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (%) of the drug were obtained at each added concentration, which indicates that the method was accurate. The method was also found to be robust as indicated by the %RSD values which are less than 2%. The LOD and LOQ was found to be 0.27µg/ml and 0.84µg/ml. The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (99.06%). The validation parameters are summarized in table 7.

CONCLUSION

The proposed method is found to be simple, sensitive, and cost effective. The proposed UV/Vis spectrophotometric method was validated successfully as per the ICH Q2B guideline. It is concluded that the analytical method was

specific, precise, linear, accurate, robust. The present analytical method can be used for its intended purpose.

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