Simple, Sensitive, Specific and Validated Calorimetric Method Development for the Quantitative Estimation of Melphalan Hydrochloride in Bulk and Pharmaceutical Dosage forms

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ABSTRACT
In the present work, simple, sensitive, specific, and validated colorimetric method has been developed for the quantitative estimation of Melphalan in bulk and pharmaceutical dosage form. The method was based on the reaction of Melphalan with ferric chloride and potassium ferricyanide to form a green colored species having absorption maxima at 768nm. The linearity was found in concentration range of 0.5-4.0µg/ml. The correlation coefficient was found 0.999. The regression equation was found as Y= 0.24426X +0.25861. The method was validated for linearity, accuracy, precision and ruggedness. The LOD and LOQ for estimation of Melphalan were found as 0.0157, 0.0518respectively. Recovery of Melphalan was found to be 98.20%. Proposed method was successfully applied for the quantitative estimation of Melphalan in marketed formulations.

Key words: Melphalan Hydrochloride, linearity, precision, ruggedness.

INTRODUCTION

Spectroscopy deals with the transitions induced in a chemical species by its interaction with the photons of electromagnetic radiation or the measurement and interpretation of Electro Magnetic Radiation (EMR) absorbed or emitted when the molecule or atoms or ions of a sample move from one energy state to another energy state. In a modern industrialized society the analytical chemist has a very important role to play. Thus most manufacturing industries rely upon both qualitative and quantitative chemical analysis to ensure that the raw material used meet certain specification, and also to check the quality of the final product. The examination of raw materials is carried out to ensure that there is

no unusual substance present which might be deleterious to the manufacturing process or appear as a harmful impurity in the final product Further, since the value of the raw materialmay be governed by the amount of the required

Ingredient which it contains, aquantitative analysis is performed to establish the proportion of the essentialcomponent: this procedure is often referred to as assaying.

Melphalan hydrochlorideis a chemotherapy drug belonging to the class of nitrogen mustardagents. An alkylating agent adds an alkyl group (C_nH_{2n+1}) to DNA.The IUPAC name is *4-bis (2-chloroethyl) amino-L-phenylalanine*, it has a molecular weight of 305.20

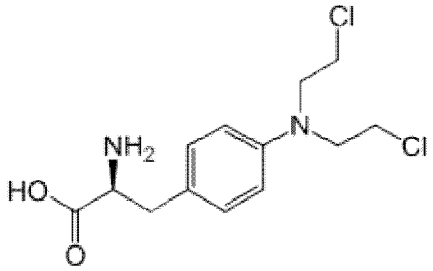

Figure1: Structure of Melphalan

Melphalan is an alkylating agent of the bischloroethylamine type. As a result, its cytotoxicity appears to be related to the extent of its interstr and cross-linking with DNA, Probably by binding at the N7 position of guanine. Like other bi functional alkylating agents, it is active against both resting and rapidly dividing tumor cells.

Melphalan is used to treat multiple myeloma (a type of cancer of the bone marrow). Melphalan is also used to treat a certain type of ovarian cancer.Up to now there are many methods developed like spectrofluorimetric and Derivative absorption spectrophotometric, LC-MS spectroscopic method developed etc... but No methods develop on colorimetric for Melphalan. So here aim is to develop new colorimetric method on Melphalan. Andvalidation will be done on Melphalan. The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, and Limit of quantitation, Linearity and range, Ruggedness,

MATERIALS AND METHODS

Working standard sample Melphalan was obtained from well reputed research laboratory. Formulation sample was purchased from local pharmacy. Spectrophotometric coloring reagents i.e Potassium ferry cyanide and Ferric Chloride were purchased from Merk chemicals pvt limited, Mumbai, India

Instrumentation:

Tec cop UV -230 double beam UV –visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software .standard cuvettes of 10mm path length were used for analysis. Sonication [1.5L] ultra sonicator was used to sonication the standard formulation sample. Standard and sample were weighed by using Denver electronic analytical balance [SI-234]

EXPERIMENTAL

Preparation of standard stock solution of Melphalan:

Standard stock solution prepared by accurately weighing 100 mg of Melphalan in 100 ml calibrated volumetric flask and made up the volume with ethanol up to 100 ml.

Preparation of working standard solution of Melphalan:

Working standard was prepared by transferring 10ml of the standard stock solution into 100 ml calibrated volumetric flask and made up the volume with ethanol to get concentration of 100µg/ml.

PREPARATION OF REAGENTS:

Potassium Ferricyanide: 100mg of Ferri cyanide was weighed accurately and was dissolved in 100ml hot distilled water

Ferric Chloride: 100mg of anhydrous Ferric Chloride was weighed accurately and was dissolved in 100ml distilled water

Preparation of Sample Solution:

Ten tablets (Alphalan 10mg) were weighed and powdered. The amount of tablet powder equivalent to 10mg of Melphalan was weighed accurately and transferred into few ml of water and kept for 15min in sonicator and volume was made up to mark with water in 10ml Volumetric flask. 100µg/ml stock solution was prepared by taking 1 ml of the above solution into 10 ml volumetric flask and made up to the mark with distilled water. 1 ml of the above stock solution was taken and diluted to 10 ml to get a concentration of 10µg/ml. The solution was then filtered through Whatmann filter paper. The absorbance was measured against blank. The drug content of the preparation was calculated using standard calibration curve.

Parameter fixation

Determination of absorption maxima

An absorption maxima (or) max are the wavelength at which maximum absorption takes place. It is important to know the absorption maxima of the substance under study, since it helps to avoid any interfering impurities

PROCEDURE:

100µg/ml of stock was taken in 10ml volumetric flasks, 1.0ml of Ferric chloride solution and 1ml of Ferricyanide solutions were added and waited for 10 Minutes to differentiate the blank and sample. Then the absorbance's of the formed color was scanned in the visible region i.e800nm-400nm against a reagent blank and identified the wavelength maxima for the developed color.

PROCEDURE: In 10 ml volumetric flask, 1.0ml of Ferric chloride solution and 1ml of Ferricyanide solutions were added and waited for 10 Minutes to differentiate the blank and sample as the blank appeared as Light Green and the sample appeared as intense Blue

Optical characters

a) Determination of concentration range

For spectrophotometric analysis determination of the concentration range which obeys the Beer- Lambert's law is necessary for accuracy and reproducibility.

b) Preparation of standard curve

Standard curve was prepared by using pure Melphalan in the concentration range of 0.5 -4.0µg/ml by this method and selecting absorbance maximum at 768 nm.

c) Procedure:

Eight 10mlvolumetric flasks were taken. Then 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0ml working standard solution of Melphalan Hydrochloride was added. 1.5ml of Ferric chloride solution and 1ml of Ferricyanide solutions were added and waited for 10 more minutes. Then the absorbance's of the formed color was scanned in the visible region and it was measured at 768nm against a reagent blank.

The result is recorded in table no. 8 and graph is given in figure no. 9 .The six such linearity was taken for regression co-efficient and eight such linearity was taken for standard deviation separately.

Method validation

1. LINEARITY:

Aliquot of the drug was (0.5-4 ml of 10µg/ml) taken in a series of 10ml volumetric flasks, 1.5ml of Ferric chloride solution and 1ml of Ferricyanide solutions were added and waited for 10 more minutes. Then the absorbance's of the formed color was scanned in the visible region and it was measured at 768nm against a reagent blank.

The absorbances of all the formed colored solutions were measured and calibration curve was constructed with concentration against absorbance. Good linearity was observed within the concentrations under the study. Linearity range was found to be 0.5-4µg/ml and linear equation was found to be y = 0.244286x + 0.25861. Results were shown in Table 1. Linearity graph was shown in Figure 1

2. Recovery (%Accuracy)

The accuracy of the methods was determined by calculating % recovery of Melphalan by standard addition method. Known volumes of standard solutions of Melphalan were taken for recovery

Studies. It is mentioned in table 3.

3. Method precision (% Repeatability)

The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 0.5 µg/ml without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD). Reported in table 4.

4. Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed methods were performed by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Melphalan (0.5,1.0,1.5,2.0,2.5,3.0,3.5,4.0µg/ml).

The results were reported in terms of relative standard deviation (RSD). Reported in Table 4.

5. Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline. Reported in Table5.

LOD = 3.3 X σ/S and LOQ = 10 X σ/S

Where, σ = the standard deviation of the response,S = slope of the calibration curve.

6. Statistical evaluation

The precision of each proposed method was ascertained by analyzing the same concentration in freshly prepared sample solution of Melphalan six times of each three samples combination of drug and extracted solutions of drug. The set absorbance values obtained were then used to calculate the drug content per

tablet and this was used to obtain standard deviation (s), standard Error (S.E), and precision (P) value. Reported in Table 6.

RESULT AND DISCUSSION

The result obtained in this method was based on reduction of the Fe3+ in $Fecl_3$ to Fe2+ by standard drug in the presence of K_3Fe(CN_6). Subsequently, the formed Fe2+ reacts withK_3Fe (CN_6) to form soluble Prussian blue (K_3FeIII [FeII (CN) 6]) whose absorbance was measured at 768nm against the corresponding reagent blank.

CONCLUSION

In this proposed method, a new visible spectrophotometric method was developed for the estimation of Melphalan in pharmaceutical formulation

The determination procedures were characterized by Low detection limit, Simple, reproducible and economic. The statistical data and the proposed method were in good agreement with those of the known methods. The method was free from experimental variables such as heating or solvent extraction steps. The proposed method relies on the use of simple and cheap chemicals and techniques and can be used for rapid routine determination and quality control of Melphalan in pure form, bulk sample, and pharmaceutical preparations.

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. Melphalan is official in Indian Pharmacopoeia. A very few analytical methods appeared in the literature for the determination of Melphalan includes LC, HPLC, HPTLC, and UV-Visible spectrophotometric methods. In view of the

Above fact, some simple analytical method was planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, colorimetric method for the quantitative estimation of Melphalan in bulk drug and pharmaceutical formulations has been developed. The results are expressed in Tables and figures.

Table 1: Standard curve for Melphalan

Concentration	Absorbance
0.5	0.400
1	0.500
1.5	0.628
2	0.729
2.5	0.846
3.0	1.001
3.5	1.105
4.0	1.257
INTERCEPT	0.25861
SLOPE	0.244286

Table 2: Formulation results of Melphalan

S.NO	Brand name	Available form	Label claim	Concentration	Amount found	% Assay
1	Alphalan	Tablet	10mg	0.5µg/ml	0.491	98.20

Table 3: Accuracy of Melphalan

% of Recovery	Accuracy				
	Target Conc., (µg/ml)	Spiked conc., (µg/ml)	Final Conc., (µg/ml)	Amount found	% of Assay
50%	1.0	0.5	1.5	0.625	99.522
	1.0	0.5	1.5	0.628	100.00
	1.0	0.5	1.5	0.632	100.64
100%	1.0	1.0	2.0	0.729	100.00
	1.0	1.0	2.0	0.723	99.176
	1.0	1.0	2.0	0.719	98.628
150%	1.0	1.5	2.5	0.843	99.645
	1.0	1.5	2.5	0.846	100.00
	1.0	1.5	2.5	0.846	100.827

Table 4: Method Precision (% Repeatability) of Melphalan

S.NO	Concentration in µg/ml	Precision	
		Intraday	Interday
1	0.5	0.400	0.398
2	0.5	0.401	0.403
3	0.5	0.401	0.405
4	0.5	0.402	0.407
5	0.5	0.403	0.402
6	0.5	0.400	0.406
	MEAN	**0.401166667**	**0.4035**
	S.D	**0.001169045**	**0.003271085**
	%RSD	**0.291**	**0.810**

Table 5: LOD and LOQ of Melphalan

Drug	LOD (µg/ml)	LOQ (µg/ml)
MELPHALAN	**0.0157**	**0.0518**

Table 6: Statistical data of Melphalan

PARAMETER	RESULT
λmax (nm)	768
Beer's law limits (µg/ml)	0.5-4.0
correlation coefficient (R2)	0.997
Regression equation (y = a+bc) a) Slope (b) b) Intercept (a)	 0.244286 0.25861
%RSD	**Intraday:**0.291 **Interday :** 0.810

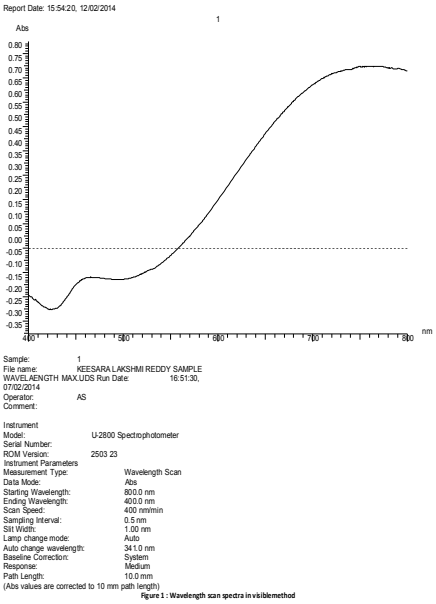

Figure 1 : Wavelength scan spectra in visiblemethod

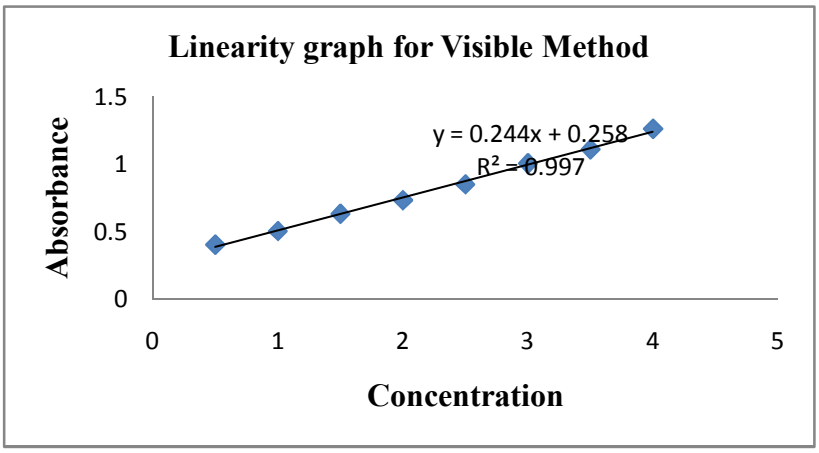

Figure 2: Linearity graph for the developed method

REFERENCES

1. ONS Clinical Practice Committee. Cancer Chemotherapy Guidelines and Recommendations for Practice. Pittsburgh, PA: Oncology Nursing Society; 1999:32-41.
2. Recommendations for the safe handling of parenteral antineoplastic drugs. Washington, DC: Division of Safety, Clinical Center Pharmacy Department and Cancer Nursing Services, National Institutes of Health; 1992. US Dept of Health and Human Services. Public Health Service publication NIH 92-2621.
3. AMA Council on Scientific Affairs. Guidelines for handling parenteral antineoplastics. JAMA. 1985; 253:1590-1591.
4. National Study Commission on Cytotoxic Exposure. Recommendations for handling Cytotoxic agents. 1987. Available from Louis P. Jeffrey, Chairman, National Study Commission on Cytotoxic Exposure. Massachusetts College of Pharmacy and Allied Health Sciences, 179 Longwood Avenue, Boston, MA 02115
5. Clinical Oncological Society of Australia. Guidelines and recommendations for safe handling of antineoplastic agents. Med J Australia. 1983; 1:426-428.
6. Jones RB, Frank R, Mass T. Safe handling of chemotherapeutic agents: a report from the Mount Sinai Medical Centre. CA-A Cancer J for Clin. 1983; 33:258-263.
7. Society of Hospital Pharmacists. ASHP technical assistance bulletin on handling cytotoxic and hazardous drugs. am J Hosp Pharm. 1990; 47:1033-1049?
8. Controlling Occupational Exposure to Hazardous Drugs. (OSHA Work-Practice Guidelines.) Am J Health-Syst Pharm. 1996; 53:1669-1685
9. Facon T, Mary JY, Hulin C, *et al.* (October 2007). "Melphalan and prednisone plus thalidomide versus melphalan and prednisone alone or reduced-intensity autologous stem cell transplantation in elderly patients with multiple myeloma (IFM 99-06): a randomised trial". Lancet370 (9594): 1209–18.
10. Gobin YP, Dunkel IJ, Marr BP *et al.* (Jun 2011). "Intra-arterial chemotherapy for the management of retinoblastoma: four-year experience". *Arch Ophthalmol*129 (6): 732–7.