RJC
http://www.rasayanjournal.com

RASĀYAN *J. Chem.*
Vol.4, No.4 (2011), 863-867
ISSN: 0974-1496
CODEN: RJCABP

A VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF MELPHALAN IN TABLET DOSAGE FORMS

K.Kiran Kumar[1*] and R. Venkata Nadh[2]

[1]Department of Chemistry, Nalanda P.G. College, Vijayawada-5200010, India.
[2]School of Biotechnology, Vignan University, Vadlamudi, Guntur-522231, India.
*E-mail: kiran_79@ymail.com

ABSTRACT

A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the determination of melphalan in tablet. The analyte was resolved by using a mobile phase (Acetonitrile, water and 1% ortho phosphoric acid in the ratio 70:27:3 v/v/v) at a flow rate 1 ml/min on an isocratic HPLC system (PEAK) consisting UV-Visible detector, ODS C-18, RP column (4.6 mm i.d x250 mm) at a wavelength of 275 nm. The linear dynamic range for melphalan was 2.0 µg/mL – 14.0µg/mL. The limit of detection [LOD] and Limit of quantification [LOQ] for melphalan was 0.5µg/mL and 1.5µg/mL respectively.

Keywords: Melphalan, HPLC, linearity, validation.

© 2011 RASĀYAN. *All rights reserved.*

INTRODUCTION

Melphalan[1]{*4-[Bis (2-chloro ethyl) amino]-L-phenyl alanine*} is an antineoplastic agent commonly prescribed for the treatment of multiple myeloma (cancer) [2-3]. Literature survey reveals that a very few HPLC methods[4-7] and LC-MS[8] have been reported for the estimation of melphalan. The present work describes a simple RP-HPLC method using C18 column for determination of melphalan in tablet combined dosage form. The method was validated as per ICH guidelines[9,10]. The chemical structure of the melphalan is as follows:

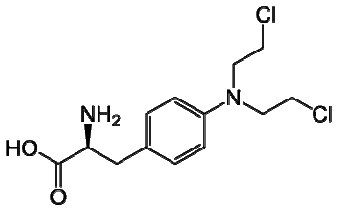


Fig.-1: Chemical Structure of Melphalan

EXPERIMENTAL

Chemicals and materials

The pharmaceutical grade pure sample of melphalan (99.28%) was procured from CELON Laboratories limited, Andhra Pradesh, India. Acetonitrile solvent of analytical grade was obtained from E Merck Ltd, Mumbai, India. Orthophosphoric acid AR grade was procured from Qualigens Fine Chemicals, Mumbai, India. The HPLC grade water was obtained from a Milli-QRO water purification system.

Instrumentation

The development and validation of the method was performed on a isocratic HPLC system (PEAK) consisting of Isocratic liquid pump, LC 8200 variable wavelength UV-Visible detector. The analytical column used to achieve chromatographic separation was a stainless steel ODS C-18 RP column (4.6mm i.d. x 250mm) purchased from Waters Corporation (Bedford, MA, USA) protected by a guard column of the same material.

Table-1: Optimized chromatographic conditions

Chromatograph	PEAK HPLC
Elution	Isocratic
Mobile phase	Acetonitrile: water : 1 % ortho phosphoric acid (70:27:3 v/v/v)
API Concentration	10 µg/ml
Column	ODS C-18 RP (4.6 mm i.d x 250 mm)
Flow rate	1 min/ ml
Detection	UV at 275 nm
Injection volume	20 micro liters
Temperature	Ambient
Retention time	4.57 minutes
Run time	10 minutes
Area	768173.3 mAU
Concentration	37.5 ug
pH	4.8
Theoretical plates	3978
Pressure	20-25 Mpa
Tailing factor	1.17

Table -2: Calibration of the RP HPLC for the estimation of Melphalan

Concentration in $\mu g.mL^{-1}$	Area (mAU)
2	168687.1
4	335787.0
6	502576.6
8	643699.3
10	782350.3
12	914253.3
14	1120622.0
Regression equation	Y = a X + b
Slope (a)	76651.98
Intercept (b)	25066.37
Correlation coefficient	0.9985

Table-3: Precision data

Day	Precession Area Mean	R.S.D.
Day-1	944509	0.289
Day-2	946797.7	0.128

All the values are the averages of five determinations

Table-4: Recovery studies of the proposed HPLC method

Labelled amount µg/ml	Amount added µg/ml	Total amount µg/ml	Amount found µg/ml	% of Recovery	Mean
4	2	6	5.775	96.25%	98.65%
4	4	8	7.9132	98.91%	
4	6	10	10.088	100.8%	

All the values are the averages of three determinations

Standard stock solution

An accurately weighted sample of 10 mg of melphalan was dissolved in methanol to give standard stock solution of 100µg/ml. A series of working standard solutions (2.0µg/mL - 14µg/mL were obtained by diluting the stock solutions with mobile phase (acetonitrile, water and 1% ortho phosphoric acid in the

ratio of 70:27:3 v/v/v). All the volumetric flasks containing melphalan were wrapped with aluminium foil and stored in the dark.

Table-5: Results of analysis of tablet containing Melphalan & recovery studies

Pharmaceutical formulation	Amount of Melphalan (mg)		% of recovery
	Labelled	Found	
Alkeran	5.0	4.97	99.4 %

All the values are the averages of three determinations

Preparation of tablets containing the drug

Ten tablets of melphalan were ground to fine powder. Accurately weighed powder sample equivalent to 10 mg of melphalan was dissolved in methanol in a 100mL volumetric flask. The flask was placed in an ultrasonic bath at room temperature for 10min. After sonication, the solution was allowed to stand for 5.0 min. 1.0mL was transferred into a 100 ml volumetric flask and diluted to the mark with mobile phase. A sample of 20 µL of this solution was directly injected. The average content of the tablets was determined either from the calibration graph or using the corresponding regression equation.

Chromatographic conditions

The mobile phase was filtered by passing through a 0.45µm membrane filter (Millipore, Bedford, MA, USA). Chromatographic analysis was carried out at ambient temperature. The compounds were separated isocratically with a mobile phase consisting of acetonitrile, water and 1% ortho phosphoric acid in the ratio of 70:27:3 (v/v/v). The flow rate was 1.0mL/min. The effluent was monitored spectrophotometrically at a wavelength of 275nm. The optimized chromatographic conditions for the determination of melphalan are represented in Table.1.

RESULTS AND DISCUSSION

Method development

Several tests were performed in order to get satisfactory separation-resolution of melphalan in different mobile phases with various ratios by using C18 column. The ideal mobile phase used is acetonitrile, water and 1% ortho phosphoric acid in the ratio of 70:27:3 (v/v/v) to obtain satisfactory and good resolution. The retention of melphalan on analytical column was evaluated at a flow rate of 1.0mL.min^{-1}. The injection volume was 20µL. The typical chromatogram of sample solution of melphalan is shown in Fig.2. The retention time was same for standard and sample of melphalan.

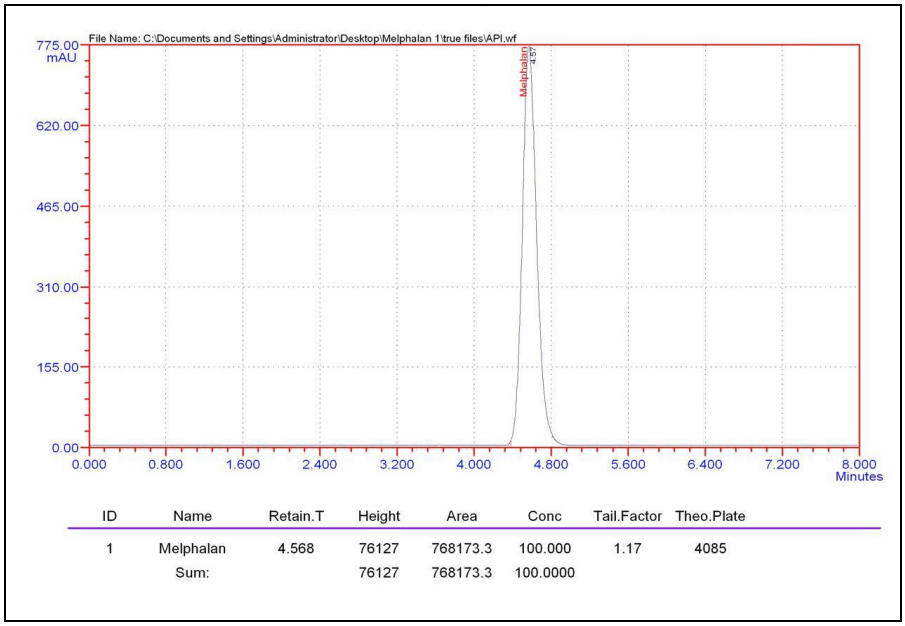
Method validation

Linearity

The linearity for HPLC method was determined at eight concentration levels of melphalan ranging from 2.0 –14.0µg.mL^{-1}. The calibration curve was constructed by plotting response factor against concentration of melphalan (Fig.3). The slope and intercept value for calibration curve were y = 766518X + 25066.37 (R^2= 0.9985) for melphalan, where Y represents the peak area of analyte and X represents analyte concentration. The results were satisfactory and a significant correlation exists between response factor and concentration of drug within the concentration range indicated on Y-axis (Table.2).

Sensitivity

The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) for melphalan were found to be 0.5µg.mL^{-1} and 1.5µg.mL^{-1} respectively.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Melphalan	4.568	76127	768173.3	100.000	1.17	4085
	Sum:		76127	768173.3	100.0000		

Fig.-2: HPLC Chromatogram of Melphalan

Precision

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in Table.3. From the data obtained, the developed RP-HPLC method was found to be precise.

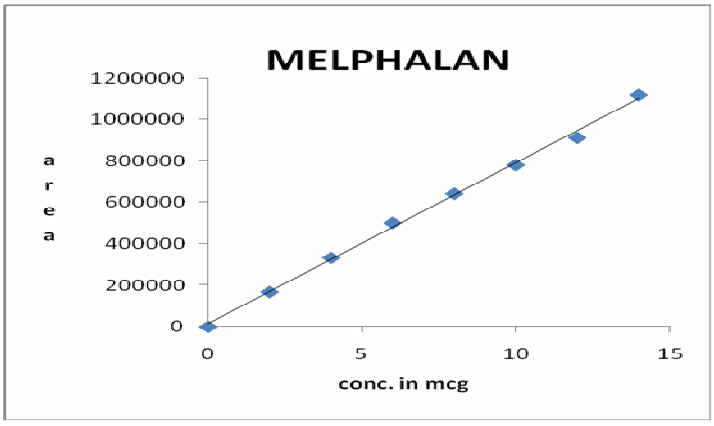


Fig.-3: Calibration curve of Melphalan

Recovery studies

Recovery study carried out for the drug was performed by spiking the known standard drug in powdered formulations. The assay procedure was repeated for six times for each standard addition to the sample. The concentration of drug was calculated from mean peak area. The percentages of drug found in formulation, mean, standard deviation in formulation were calculated. The results of the recovery analysis were found to be 96.28 ± 1.120 to 100.08 ± 0.164 and reported in Table.4. The analysis of results (Table.5) shows that the amount of drug was in good agreement with the label claim of the formulation.

Ruggedness and Robustness

Ruggedness test was determined between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase, pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

CONCLUSION

The HPLC method developed in this study has the sensitivity, selectivity, reproducibility, and stability which make it versatile. The method can also be readily adapted to routine quality control analysis.

REFERENCES

1. Indian pharmacopeia, (**1),** 1006 (1996).
2. S.C. Sweetman, Martindale: The Complete Drug Reference, Pharmaceutical Press; 34th edition, 566 (2005).
3. T. Facon, J.Y. Mary, and C. Hulin, *Lancet,* **370 (9594),** 1209 (2007).
4. K. Brightman, G. Finlay, I. Jarvis, T. Knowlton, and C. Manktelow, *J. Pharm. Biomed. Anal.*, **20(3),** 439 (1999).
5. C.E. Nath, L.Zeng, A.Eslick, J.Trotman and J.W. Earl, *Journal Acta Chromatogrphica,* **20(3),** 383 (2008).
6. Z.Y. Wu, M.J. Thomspon, M. S. Roberts, R. S. Addison, G.R. Cannell, A.J. Grabs, B.M. Smithers, *J.Chromatogr.-B: Biomed. Appl.,* **673(2),** 267 (1995).
7. S.Y.Chang, D.S. Alberts, L.R.Melnick, P.D.Walson, S.E.Salmon, *Journal of Pharmaceutical Sciences*, **67(5),** 679 (1978).
8. A. Mirkou, B. Vignal, S. Cohen, M. Guillaumont, O. Glehen and J. Guitton, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, **877(27),** 3089 (2009).
9. ICH Q2A, Text on validation of analytical procedures, International Conference on Harmonization, Geneva (1994).
10. ICH Q2B, Validation of analytical procedures: Methodology, International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use, Geneva (1996).

[RJC-776/2010]

International Journal of
Chemical, Environmental and Pharmaceutical Research
www.ijcepr.com
ISSN: 2229-3892(Print); ISSN: 2229-5283(Online)
[Abstracted in : Chemical Abstracts Service , American Chemical Society, USA and CAB(I) , UK]

ijCEPr widely covers all fields of **Chemical, Environmental** and **Pharmaceutical Research.**
Manuscript Categories*: Full-length paper, Review Articles, Short/Rapid Communications.*
Manuscripts should be addressed to:
E-mail: ijcepr@gmail.com