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Method Development and Validtion of Melphalan Injection by RP-HPLC Method

Gummi Vishwanth*, P. Sunitha, Naga Jyothi. C

Dept. of Pharmaceutical Analysis and Quality Assurance, Teegala Krishna Reddy College of Pharmacy, Hyderabad, India

A B S T R A C T

A simple, rapid, sensitive, accurate and precise RP-HPLC method has been developed and validated for the estimation of melphalan in injection dosage form. The mobile phase used was a combination of Methanol, Acetonitrile, TEA in the ratio of 55:10:35 respectively. Retention time was found to be 3.99mins. The developed method was validated as per ICH Guidelines. The % purity of Melphalan was found to be 99.11% and Linearity was observed in the range of 60-140 µg /ml with correlation co-efficient of 0.998%. Validation results serve as an authentic proof that the developed method is Reliable. Thus, this method can be preferred for the routine analysis of melphalan in injection dosage forms.

Keywords: Melphalan, RP-HPLC, TEA

A R T I C L E I N F O

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***Corresponding Author**
Gummi Vishwanth
Dept. of Pharmaceutical Analysis and Quality Assurance, Teegala Krishna Reddy College of Pharmacy, Hyderabad, India
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1. Introduction

The main aim was to develop a new RP-HPLC method for the estimation of melphalan in injection dosage form. The method should be an accurate and more over economical. In the present work the main goal was to reduce the retention time of the drug.

2. Materials and Methods

Sample-Melphalan

Reagents- Methanol, Acetonitrile and Tri Ethyl amine are used which wer HPLC grade

Apparatus- HPLC used was shimadzu and uv-detector was Nicolet evolution 100

Experimental work

Chromatographic condition-This method was developed by using**x terra** c_{18} column. Mobile phase was sonicated for 10min. Run time was set to 10 mintites. Injection volume is 20µl. Now the analyte is injected into HPLC and retention time is obtained.

Method Validation

Linearity

A stock solution of 1000µg/ml was prepared by using the mobile phase as diluent. From the above stock solution, different concentrations were prepared .i.e., 60, 80, 100, 120,140µg/ml respectively. Prepared dilutions were injected serially. Calibration curve was plotted by taking the peak area and concentration of the prepared dilutions.

Accuracy

The solutions of accuracy 80%, 100% and 120%were injected into chromatographic system.The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated

Precision

Prepare stock sample solution of 1000µg/ml preparations and injected 6 times in to the column.

Assay

%assay =AT/AS * WS/DS * DT/WT * P

Where

At = average test peak area

As = average standard area

Ws = weight of standard

Ds= dilution of standard

P= standard purity

Specificity

The specificity was performed by injecting blank.

Robustness

In robustness flow rate is changed and results were evaluated. Flow rate was maintained at 0.8min/ml, 1.0min/ml, and 1.2min/ml

LOD

LOD's are calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) LOD calculated according to the formula**.**

Lod=3.3σ/s

Where

σ=standard deviation of the response

s= slope of the calibration curve

LOQ

Loq= 10σ/s

3. Results and Discussion

Method Development

Various trials were performed by using Acetonitrile, Methanol, Tri ethyl amine. Trails were continued until the method is optimized.

Optimized chromatogram

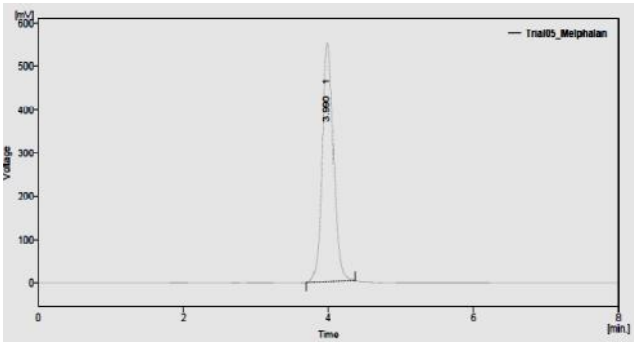

Figure 1: Chromatogram Showing Sample Injection

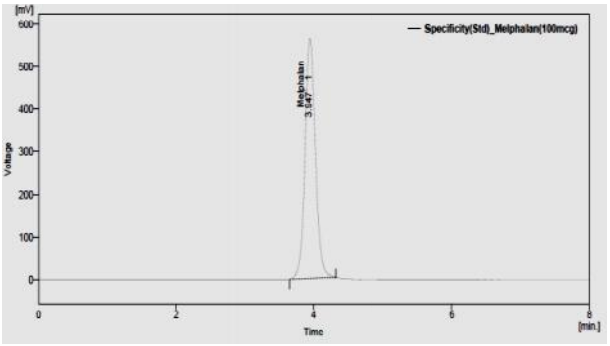

Figure 2: Chromatogram Showing Standard Injection

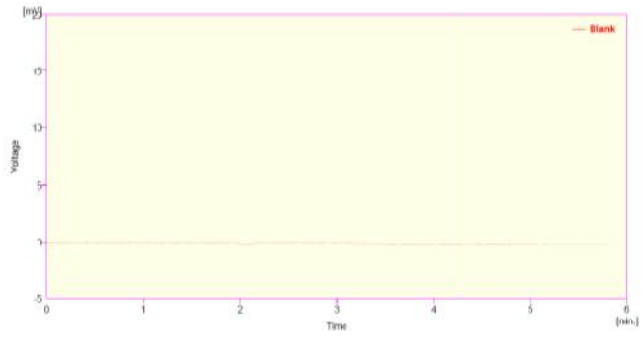

Figure 3: Chromatogram Showing Blank

Table 1: Optimized conditions

Para meter	Condition
Mobile phase	Acetonitrile: Methanol: Tri ethyl amine (55:10:35)
Flow rate	1ml/min
Column temperature	Room temperature

Validation

Linearity

The linearity study was performed for the concentration of 60 to 140 µg/ml.The area of each level was used for calculate. Correlation Coefficient was found to be 0.999. Low values of standard deviation, standard error, etc serve as a proof to show that the calibration plot did not deviate from linearity.

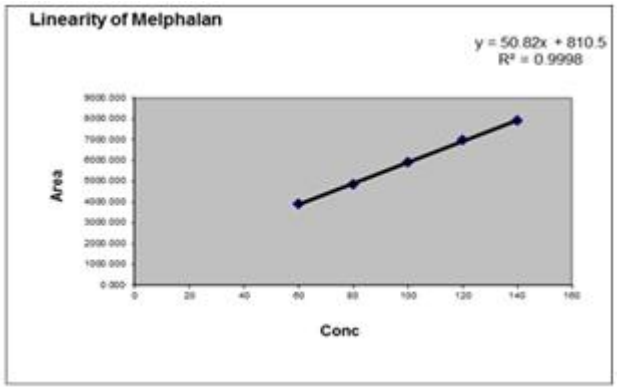

Figure 4: Linearity Graph of Melphalan

Accuracy

Accuracy was determined at 3 different levels i.e at 80%, 100%, 120%. And percentage recovery and percentage RSD was calculated.

Table 2: Accuracy Results

Concentration	% Recovery
80	99.57
100	99.27
120	100.52

Precision:

Determined RT and %RSD was calculated to prove that method is validated

Table 3

s.no	RT
1.	4.020
2.	3.980
3.	3.967
4.	3.470
5.	3.937
6.	3.937
Avg	3.9647
St. Dev	0.0321
%RSD	0.81

Limit of Detection

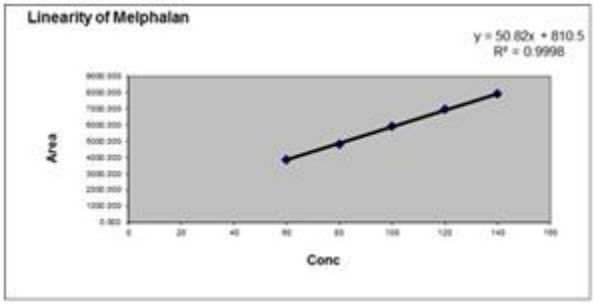

Figure 5

LOD=3.3σ/s

The LOD for this method was found to be 2.05 µg/ml & area 104.39

Limit of detection

LOQ= 10σ/s

The LOQ for this method was found to be 6.22 µg/ml & area 316.32 for Melphalan.

Robustness

The method is said to be robust if there is no effect at different conditions

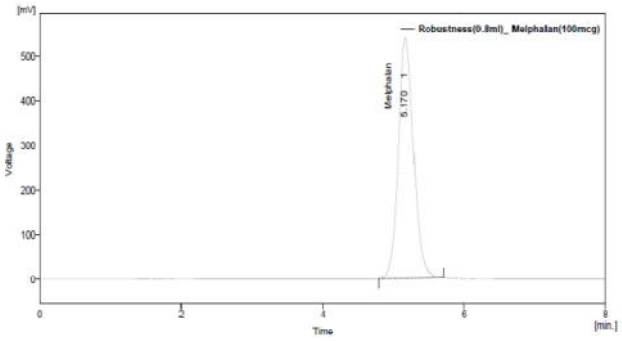

Figure 5: Chromatogram of Robustness (0.8 ml/min)

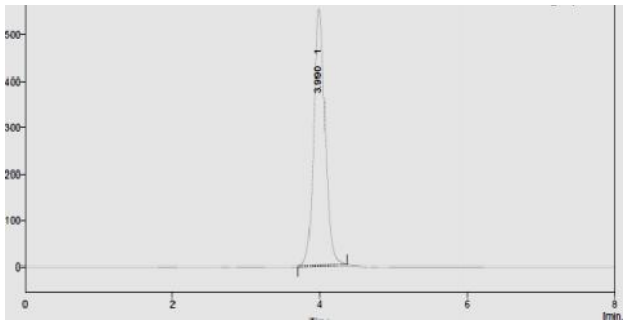

Figure 6: Chromatogram of Robustness (1ml/min)

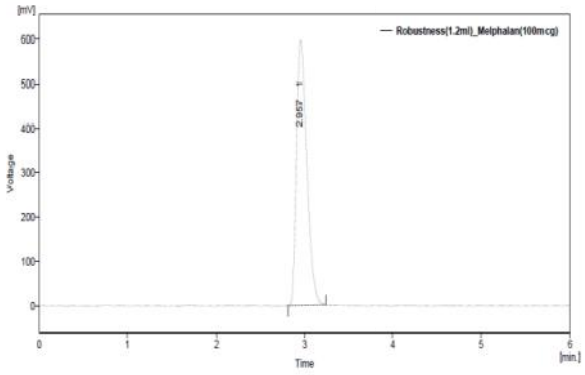

Figure 7: Chromatogram of Melphalan for (1.2 ml/min)

Table 4

Parameter (flow rate)	RT	Tailing factor
0.8ml/min	5.170	1.280
1ml/min	4.020	1.263
1.2ml/min	2.907	1.231

4. Conclusion

The method was validated for system suitability, precision, accuracy, linearity, ruggedness. Therefore it was concluded that the proposed method can be used for routine analysis of melphalan in its injection dosage form.

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