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RP-HPLC Method for The Estimation of Amifostine in Pharmaceutical Dosage Forms

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ABSTRACT

A simple and precise RP-HPLC method was developed and validated for the determination of Amifostine in pharmaceutical dosage forms. Chromatography was carried out using Hypersil C_{18} 150 x 4.6 mm, 5, ACN: Methanol (70:30) as the mobile phase at a flow rate 1.2 ml/min. The analyte was monitored using UV detector at 246nm.The Retention time of the drug was 3.34 min for Amifostine. The proposed method was found to have linearity in the concentration range of 25–150 µg/ml with correlation coefficient of r2=0.9999.The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for Amifostine were in the range 100.6-101.9%. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining Amifostine in bulk and dosage forms.

INTRODUCTION

Amifostine chemical name is 2-(3-aminopropylamino) ethylsulfanyl phosphoric acid. It is a cytoprotective adjuvant used in cancer chemotherapy involving DNA-binding chemotherapeutic agents. Also commonly known as WR-1065 in its active form. It is marketed by Med Immune under the trade name Ethyol[1,4]. Amifostine is used therapeutically to reduce the incidence of neutropenia-related fever and infection induced by DNA-binding chemotherapeutic agents including alkylating agents (e.g. cyclophosphamide) and platinum-containing agents (e.g. cisplatin). It is also used to decrease the cumulative nephrotoxicity associated with platinum-containing agents. Amifostine (Fig. 1) is also indicated to reduce the incidence of xerostomia in patients undergoing radiotherapy for head and neck cancer.

Only very few HPLC methods have been reported in the literature for the estimation of Ethyol present in biological fluids[1-3]. There are no reported methods for the determination of Ethyol by RP-HPLC in pharmaceutical dosage forms. Hence the author has made an attempt to develop a HPLC method for the determination of Ethyol in pharmaceutical formulations (10ml vial).

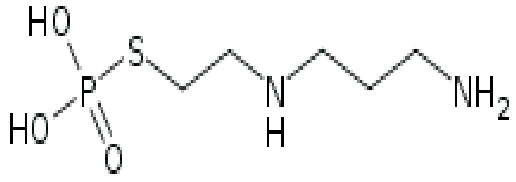

Fig. 1: Amifostine

MATERIALS AND METHODS

Instrumentation

Agilent 1200 series separation module with an auto injector and U.V-Visible detector equipped with Chemstation software.

Chemicals and reagents

Amifostine was obtained from local market. Water (HPLCgrade), acetonitrile (HPLC grade) and metanol (HPLC grade).

Chromatographic conditions

Mobile phase consists of acetonitrile and methanol in the ratio (70:30), filter through 0.45µ nylon membrane filter and degas. The mobile phase was pumped from the solvent reservoir to the column at a flow rate 1.2 ml/min. The column was maintained at 50°C and the volume of each injection was 20µL. Prior to injection of the solutions, column was equilibrated for at least 20min with mobile phase flowing through the system. The eluents were monitored at 246nm.

Standard Preparation

Weigh and transfer accurately about 50.0 mg of Amifostine working standard into a 100 ml clean dry volumetric flask, add about 70 ml of diluent, sonicate for 5 minutes, and dilute to volume with diluents.

Sample Preparation

10 ml vial(consisting of 500mg) of Amifostine were taken into a 100 ml volumetric flask, dissolved in working mobile phase and then the solution was made up to the mark with mobile

Table No.1: System suitability, precision and accuracy of the proposed methods for Amifostine

Parameter	Results
Retention Time (t) (Min)	3.337
Theoretical Plates (n)	11831
Plates per Meter (N)	118306
Linearity range (µg/ml)	7.5-17.5
Peak asymmetry	1.11
Regression equation	
Slope (b)	35.88
Intercept (a)	-4.845
Standard deviation of intercept (S_a)	3.526
Correlation Coefficient (r)	0.9999
(%)Relative standard deviation	1.724
Percentage range of errors*	
0.05 level	0.0214
0.01 level	0.03367

phase,from the above solution take 1ml and dissolved in 10ml volumetric flask containg dilutent and filtered through 0.45 µ membrane filter.

RESULTS AND DISCUSSION

Several systematic trials were performed to optimize the Chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of Amifostine in pharmaceutical dosage vial forms. The present method contains mobile phase ACN: Methanol (70:30) which was found to be the most suitable as the peak obtained with good peak shape and symmetry. Hence this method was finalized for the estimation of Amifostine.

Linearity

A Series of dilutions are prepared using Amifostine working standard (500µg/ml) at concentration levels from 25% to 150% of target concentration.

Accuracy

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Amifostine into each volumetric flask for each spike level to get the concentration of Amifostine equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Amifostine was calculated.

Table No.2: Results from analysis of Amifostine in vail

Label claim. mg	500
Amount found, mg	495.5mg
Amount found, %, n = 6	98.9
RSD, %, n = 6	0.56

Precision

The precision of the method was ascertained from the peak area of Amifostine obtained by determination of six replicates of fixed amount of Amifostine. The percent relative standard deviation and percent range of errors were calculated and were presented.

Robustness

Robustness of the proposed methods was evaluated by making small changes in flow rate, organic modifier concentration and temperature. The results were found to be not affected by these small alterations.

CONCLUSION

From the obtained results it can be concluded that the proposed method is quite precise and accurate. The absence of additional peaks in the Chromatogram indicated that there is no interference of the common excipients used in the tablets. The proposed HPLC Method is sensitive and reproducible for the analysis of Amifostine in VIAL forms. The method was duly validated by using required statistical parameters.

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