Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 722 - 727

Research Article **Pharmacy**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ASSAY OF AMIFOSTINE FOR INJECTION BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

BASHEER AHMAD.S*, HARITHA PAVANI.K, SURESH BABU.U, HAIMA.R AND SHIVAJI.K

Nimra College of Pharmacy, Nimra Nagar, Ibrahimpatnam, Vijayawad-521456

ABSTRACT

A simple, economic, selective, precise, and accurateReverse phase High Performance Liquid Chromatography method for analysis of Amifostine for injection, was developed and validated according to ICH guidelines. The quantification of the drug was carried out using Lunar C_8, 250 mm X 4.6 mm, 5 μm columnor its equivalent in isocratic mode, with mobile phase compressing of Buffer: Methanol (72:28)the flow rate was 1.0ml/min and the detection was carried at 220 nm. The retention time for Amifostine for injection was found to be 4.5. The percent assay was found to be 97.3%. Proposed method was validated for precision, accuracy, linearity range, specificity and robustness.

KEY WORDS: Amifostine for injection, RP-HPLC, Validation.

BASHEER AHMAD.S
Nimra College of Pharmacy, Nimra Nagar, Ibrahimpatnam, Vijayawad-521456

Corresponding author

INTRODUCTION

Amifostine is chemically 2-(3-aminopropylamino) ethylsulfanylphosphonic acid. It is a cytoprotectiveadjuvant used in cancer chemotherapy involving DNA-binding chemotherapeutic agents, and 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-relatedfever and infection induced by DNA-binding chemotherapeuticagents including alkylating agents (e.g. cyclophosphamide) andplatinum-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 radiotherapyfor head and neck cancer.Other possible effects include accelerated DNA repair, induction of cellular hypoxia, inhibition of apoptosis, alteration of gene expression and modification of enzyme activity. Only few HPLC methods have been reported in the literature review for the estimation of Ethyol present in biological fluids [1-3]. There are no reported methods for the determinationof Ethyol by RP-HPLC [5] in pharmaceutical dosage forms. Hencethe author has made an attempt to develop a HPLC method for thedetermination of Ethyol in pharmaceutical formulations (10mlvial).

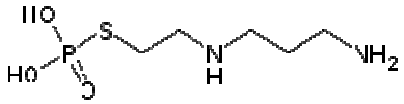

Figure 1
Amifostine Structure

MATERIALS AND METHODS

Chemicals and Reagents

Amifostine anhydrous was procured from NATCO Parenteral Ltd. (Hill colony, Nagarjuna sagar, A.P, India). Commercial Pharmaceutical preparations from NATCO Pharma, which were claimed to contain 500 mg of Amifostine was used in analysis.Methanol (HPLC grade), Phosphoric acid (HPLC grade) and Sodium 1hexane sulfonate (AR Grade) were of reagent grade.

Instrumentation

A HPLC (Waters 2690 series) with UV/VIS Detector/PDA detector and Lunar C_8, 250 mm X 4.6 mm, 5 μm columnwas used with auto sampler injector was used. The HPLC system was equipped with Empower software for data processing.

Chromatographic Condition

The mobile phase containing Buffer: Methanol (72:28) was found to resolve Amifostine. Phosphoric acid was used for pH adjustment of Sodium 1hexane sulfonatebuffer. The mobile phase was filtered on a 0.45 nylon membrane filter and then ultrasonicated for 30 min. The flow rate was set to1.0ml/min. The drug shows good absorbance at 220 nm, which was selected as wavelength for further analysis.

Buffer Preparation

Accurately weighed and transfer 0.94gm of sodium 1-hexane sulfonate into 1000ml of water and adjust $p^H$with phosphoric acid to 3.0. Filter the solution through 0.45μm nylon filter paper.

Preparation of Mobile Phase
Preparedly filtered and degassed mixture of buffer and Methanol in the ratio of 72:28 v/v

Diluent solution
Water (HPLC grade).
Preparation of Standard solution
Weighed and transferred accurately about 36 mg of Amifostine working standard into a 100ml cleandry volumetric flask add 10 ml of diluent, sonicated for 5 minutes and make up to the mark with diluent.

Preparation of Sample solution
The amount powdered lyophilized active ingredient supposed to be present in 5 vials was accurately weighed and transferred into 100ml volumetric flask and made up to the mark with diluent and sonicated for about 30 minutes. Transfer 3.0ml of above solution into a 25ml volumetric flask and made up to the volume with diluent.

Preparation of Placebo
The amount of powdered inactiveingredient supposed to be present in 5 vials was accurately weighed and transferred in to 100 ml volumetric flask, 100ml of diluent was added and sonicated for about 30 minutes.

METHOD VALIDATION
1) System Suitability/System Precision
System Suitability was performed by injecting six replicate injections of standard solutions of Amifostine at 100% and expressed as %RSD of peak area.

2) Linearity of Amifostine
The linearity of the HPLC method was demonstrated for Amifostine solutions ranging from 80% to 120% of standard concentrations.

3) Specificity
To demonstrate that diluents and placebo are not interfering with analytic peak. Solutions of placebo, blank, Standard and Sample were preparedas per test procedure and injected into the HPLC system.

4) Precision
Precision was measured in terms of repeatability of application and measurement. Repeatability of sample application was carried out using six replicates of the same sample concentration.

5) Accuracy (%Recovery)
%Recovery studies were carried out at three different levels of 80%, 100% and 120% of standard solution (i.e.Amifostine API spiked to the placebo) in triplicate in each level.

6) Robustness
The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, column temperature and buffer composition which may differ but the responses were still within the specified limits.

7) Ruggedness
The variability of the results obtained with the analysis of Amifostine sample solution six times by two different analysts, two different reagents, two different columns, two different instruments on two different days to assess the method ruggedness.

RESULTS AND DISCUSSION

Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for amifostine. The Mobile phase methanol : buffer (phosphate buffer, 28:72) was found to be satisfactory and gave symmetric and well resolved peak for amifostine.

Table.1
System suitability data

Parameter	Amifostine
Tailing Factor	1.14
Theoretical Plates	5110
%RSD of Peak area	0.29

The correlation coefficient (r^2) was found to be 0.9999 and shows good linearity. The data of the calibration curve was given in Table.2.

Table.2
Linearity data for amifostine

Level	Peak Area
80%	763566
90%	856303
100%	951760
110%	1045528
120%	1141900
Slope	9467.95
Intercept	5376.4
Correlation Coefficient	0.9999

There is no interference of blank and placebo at the retention time of Amifostine which indicate good elution of compound & chromatograms were shown in Fig.3, 4 and 5.

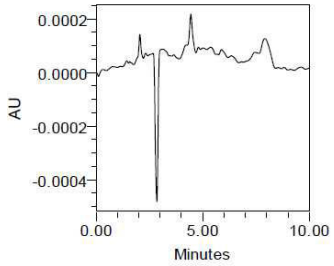

Figure 3
Blankchromatogram

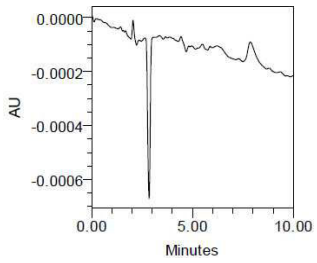

Figure 4
Placebochromatogram

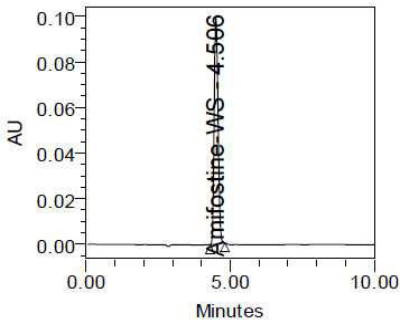

Figure 5
Samplechromatogram

Precision was determined & the results are represented in the form of %RSD which was found to be below 2% & shows that the test method was highly precise and results given in Table-4.

Table.4
Repeatability data

Parameter	Amifostine
Mean	945417
SD	1091.15
%RSD	0.115

The % mean recovery for amifostinewas found to be in range of about 100.31% to100.32%.The results were summarized in Table.5.

Table.5
Accuracy data

Drug	%Level	Mean Peak Area	Mean % Recovery	% RSD
Amifostine	80%	762409	100.31	0.11866
	100%	940997	99.94	0.94882
	120%	1138020	100.32	0.176417

As part of the robustness, deliberate changes in the flow, column temp& buffer composition was made to impact on the method. RT was significantly changed but within the acceptance limit and results given in Table.6.

Table.6
Robustness data

Parameter	RT	Peak Area of Amifostine	%RSD Peak Area
Actual	4.51	928598	0.29
High Flow Rate	4.1	863027.7	0.01
Low Flow Rate	4.96	1055147	0.04
Low Temperature	4.4	963528.8	0.03
High PH	4.6	978096.0	0.03
Low PH	4.4	953901.2	0.02
Column Variation	4.5	932151.0	0.03

The % bias for ruggedness was within the limit i.e. less than 2.0 and results shown in Table.7.

Table.7
Ruggedness data

Parameter	Amifostine		Bias (%)
	Day-1	Day-2	
Mean assay	101.6%	100.2%	+1.4%
%RSD	0.05	0.08	-

CONCLUSION

It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, specific, robust, rugged and reproducible for the simultaneous analysis of Amifostine with less tailing and is also economical.

AKNOWLEDGEMENT

I gratefully acknowledge Mr. Balakrishna Rao, General Manager, NATCO Pharma Pvt.Ltd. Nagarjuna Sagar, for referring me to the industrial project and training at NATCO Pharma Parentrals Ltd., which was the most valuable opportunity in my career starting in Pharma field.

REFERENCES

1. Feng Bai, M. N. Kirstein, S. K. Hanna and C. F. Stewart,New liquid chromatographic assay with electrochemical detection for the measurement of amifostine and WR1065.J.Chromatogr., B: Anal. Technol. Biomed. Life Sci., 2002, 772(2),257-265.
2. T. K. Mandal and I. Womack, HPLC analysis ofamifostine.Pharm.Pharmacol.Commun., 1999; 5(9): 541-543.
3. Bachy C, Fazenbaker C, Kifle G, et al: Tissue levels ofWR-1065, theactive metabolite of Amifostine (Ethyol), areequivalent following intravenousor subcutaneous administration in cynomolgus monkeys. Oncology, 2004; 67:187-193.
4. Indian Pharmacopoeia; Vol 3, 2007. p.1586.
5. Anupama. B*, Kavitha. B, Jagathi. V, Varaprassad. K, RP-HPLC Method for the estimation of Amifostine in pharmaceutical dosage forms, Asian J. Pharm. Health. Sciences, Apr-Jun 2011, Vol-1, Issue-2, pg.no-89-90.