THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN -2319-2119

RESEARCH ARTICLE

ANUSHA DEVI.V et al, The *Experiment, OCTOBER.2012, Vol. 3(3), 193-198*

RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF GLATIRAMER ACETATE FOR INJECTION IN PHARMACEUTICAL DOSAGE FORMS

ABSTRACT

A simple RP-HPLC method for the determination of Glatiramer acetate in pharmaceutical dosage forms. Numerous HPLC conditions were tested for determination of Glatiramer acetate. The best result was achieved by using Develosil 300, C-4 (250×4.6mm) 5µm column and a mobile phase consisting of Acetonitrile: Trifluoroacetic acid at flow rate of 1.0 ml/min with ultraviolet detection at 220nm. The retention time of the drug was 9.039 min. The method produced liner responses in the concentration range of 8 to 12 ppm of Glatiramer acetate. The method was found to be applicable for determination of the drug in injection.

KEYWORDS: Glatiramer acetate(GA) Estimation, RP-HPLC, Validation, Acetonitrile, Trifluoro acetic acid.

INTRODUCTION

Glatiramer acetate, the active ingredient of Copaxone, is the acetate salt of synthetic polypeptides, containing four naturally occurring amino acids resembling myelin basic protein (MBP): L-glutamic acid, L-alanine, L-tyrosine and L-lysine with an average molar fraction 0.141, 0.427, 0.095 and 0.338, respectively[1].GA has been shown to be effective in preventing and suppressing experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis. It was tested in several clinical studies and approved for the immunomodulatory treatment of relapsing-type MS in 1996. Glatiramer acetate demonstrates a strong promiscuous binding to major histocompatibility complex molecules and inhibits the T cell response to several myelin antigens. In addition, it was shown to act as a T cell receptor antagonist for the 82–100 MBP epitope. Glatiramer acetate treatment causes in vivo changes of the frequency, cytokine secretion pattern and effector function of GA-specific T cells. It was shown to induce GA-specific regulatory CD4+ and CD8+ T cells and a TH1–TH2 shift with consecutively increased secretion of anti-inflammatory cytokines. GA-specific TH2 cells are able to migrate across the blood–brain barrier and cause in situ bystander suppression of auto-aggressive TH1 T cells. In addition glatiramer acetate was demonstrated to influence antigen presenting cells (APC) such as monocytes and dendritic cells. Literature survey reveals that there were some papers on analysis of Glatiramer acetate by using UV-spectrophotometry[2], visible spectrophotometry[2], HPLC[2], HPLC-MS,GC-MS and various pharmacological methods for determination of Glatiramer acetate in biological fluids and tissues[3,4,5,6,7].

Now the authors report a simple, reliable and reproducible RP-HPLC method which was duly validated by statistical parameters precision, accuracy and recovery. The method has been satisfactorily applied to the determination of Glatiramer acetate in pharmaceutical preparation

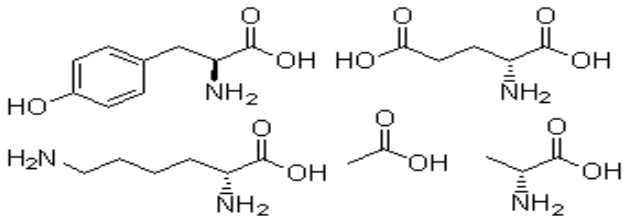

Figure- 1. Chemical structure of Glatiramer acetate

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Chemicals and solvents:

HPLC grade Acetonitrile, HPLC grade Trifluoroacetic acid was used for mobile phase preparation. Pure sample of Glatiramer acetate was a gift sample from a local pharmaceutical industry. Commercial samples of injections containing the drug Glatiramer acetate were purchased from the local pharmacy.

Chromatographic Conditions:

A High pressure liquid chromatography (Waters HPLC**)** with LC-P 100 pump, variable wavelength programmable UV-Visible detector 2489, system controller (Waters) and Develosil C- 4 column was used. The HPLC system was equipped with the soft ware EM power (Cyber lab). A freshly prepared mixture of acetonitrile and trifluoroacetic acid was used as the mobile phase. Diluent water was used as diluents. Acetonitraile and Trifluoroacetic acid were filtered through a 0.45µm membrane filters and sonicated before use. The flow rate of the mobile phase was maintained at 1ml/min. The detection was carried out by UV detector at 220 nm.

Method Development Conditions:

S.no	Mobile phase Composition	Type of Column	Flow Rate	Column Temperature	Glatiramer acetate Retention time	Tailing Factor
1	Acetonitrile:Trifluor-oacetic acid	kromosil	1ml	ambient	9.081	0.83
2	Acetonitrile:Trifluoroace tic acid	Develosil	0.8ml	ambient	10.236	0.84
3	Acetonitraile:Trifluoroac etic acid	Develosil	0.9ml	ambient	9.069	0.83
4	Acetonitraile:Trifluoroac etic acid	Develosil	1.1	ambient	9.204	0.83
5	Acetonitraile:Trifluoroac etic acid	Develosil	1.0ml	40^{o}c	9.039	0.90

Table 1

Estimation of Glatiramer acetate:

About 104.0 mg of Glatiramer acetate was weighed accurately and transferred into a 100 ml volumetric flask and dissolved in 50 ml diluent. The solution was sonicated for 20 min and then the volume was made up with a further quantity of the diluent to get a 1mg/ml solution. Subsequent dilutions of this solution ranging from 8 to 12 ppm were made in 100 ml volumetric flasks with diluents. 50µl of the solution was injected each time into the column, at a flow rate of 1ml/min. Each of the dilutions was injected 5 times into the column and the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug were noted. The regression equation of the drug concentrations was computed. This equation was later used to estimate the amount of Glatiramer acetate in pharmaceutical dosage forms.

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Estimation of the Drug in injection Dosage Forms:

Accurately weigh and transfer about 5ml of the sample equivivalent to 100mg of drug into 100ml of volumetric flask, dissolve and dilute to volume with purified water. Inject equal volumes 50 µl of blank, standard preparation and sample preparation into chromatograph. Filter the solution through membrane filter having the pore size 0.45µ. 50µl of the solution was then injected into the column. The mean peak area of the drug of five such determinations was calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample column.

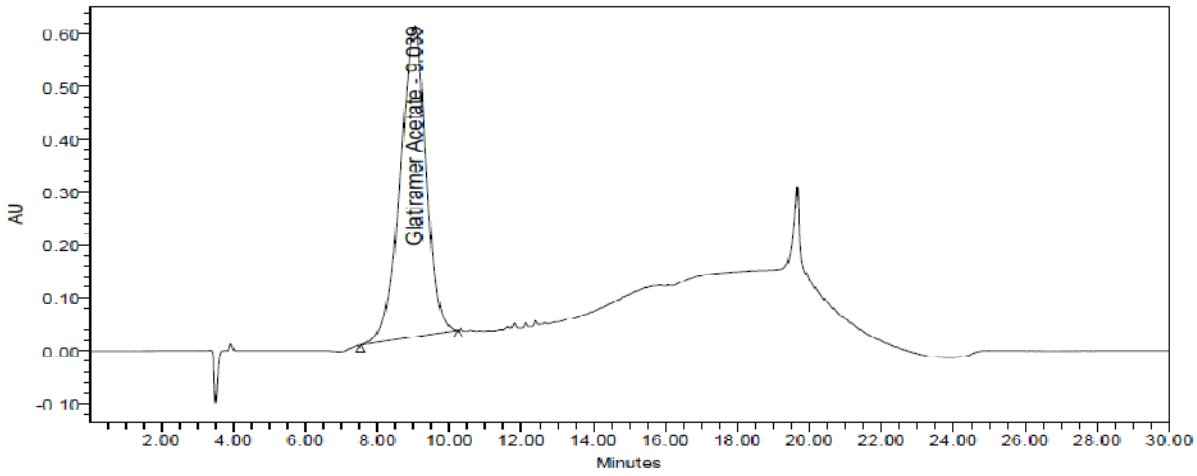

Figure - 2. A model chromatogram of Glatiramer acetate

	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Glatiramer Acetate	9.039	29128034	100.00	0.90	750

CONCLUSION

A method was developed for the determination of Glatiramer acetate for injection which is simple, quick, reliable, inexpensive and simple. The results indicate that the described method can be used for quantitative analysis of the compound.

ACKNOWLEDGEMENTS

This work was supported by NIMRA COLLEGE OF PHARMACY, Ibrahimpatnam, for their continuous support and encouragement and for providing the necessary facilities.

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Calibration of the proposed method:

Linearity range (ppm)	8-12 (ppm)
$r^2 \pm$ S.D.	0.9999

Table 2

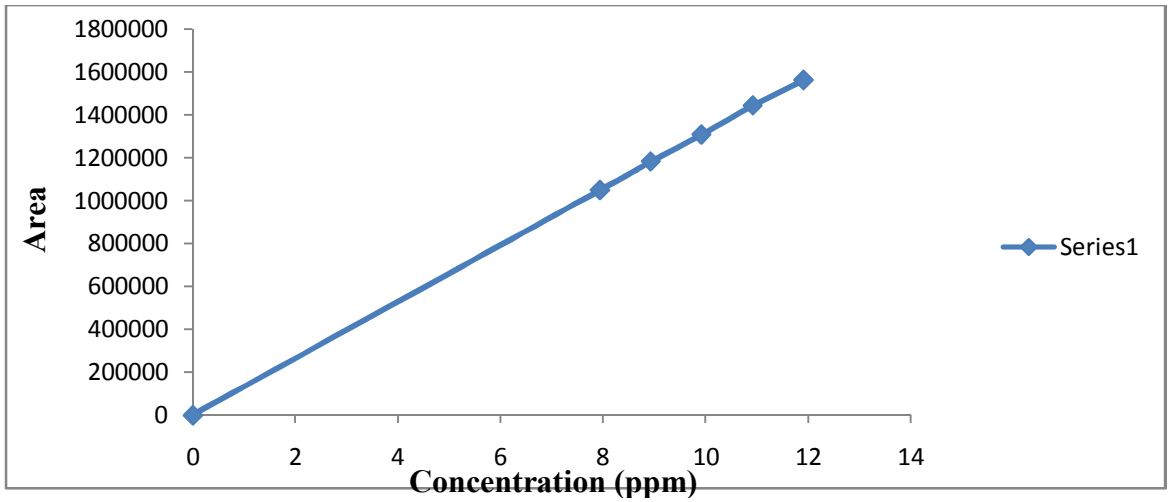

Figure - 3. Linearity of Glatiramer acetate

Repeatability and reproducibility Precision of the Proposed Method

Repeatability	Mean *	%RSD
	9.02	0.16
Reproducibility	9.01	0.20

Table 3

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Assay of Glatiramer acetate in injection Dosage Forms

Brand	Labelled amount of drug(mg)	Amount found by proposed method (mg/ml)	Amount found by proposed method (%)
COPAXONE	20mg	20.18	100.9

Table 4

% Recovery Results for Glatiramer acetate

S.NO	AREA (80%)	AREA (100%)	AREA (120%)
Preparation1	23197372	28898445	34686486
Preparation2	23177390	28879940	34672728
Preparation3	23150757	28884594	34671599
Average	23175173	28887660	34676938
Obtained assay(mg/ml)	0.81	1.01	1.21
Spiked assay(mg/ml)	0.81	1.01	1.21
% recovery	100.2	99.74	99.77

Table 5

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8.

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