Research Article

A Novel, Stability Indicating RP-HPLC Method for Determination of Tafamidis in Capsule Dosage Form

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ABSTRACT

A novel, stability-indicating, rapid, simple, accurate, precise, and reproducible RP-HPLC method has been advanced and validated for the estimation of Tafamidis in capsule dosage form. The chromatographic analysis of the drug was performed isocratically and the separation was obtained using Ascentis® Express 90 A° C18 (25 cm X 4.6 mm, 5 µm) column. The mobile phase consisting of 25 mM potassium dihydrogen phosphate buffer (pH 6.5) and acetonitrile in the ratio of 60:40% v/v was used and the column temperature was maintained at 25°C with a flow rate of 1mL/min. The diluent used was methanol, and 10 µL of the solution was injected, and the analyte was monitored at 206 nm. The method validation was carried out as per ICH Q2 (R1) guidelines. The retention time of Tafamidis was found to be about 4.9 min. The drug established linearity in the concentration range of 3-18 µg/mL with correlation coefficient 0.9992. The mean percent recovery of Tafamidis was found to be 100.5 %. The LOD and LOQ values of Tafamidis were found to be 0.12 µg/mL and 0.35 µg/mL respectively. Forced degradation studies were also carried out by exposing the drug to various stress conditions such as acidic, alkaline, hydrolytic, oxidative, thermal, and photolytic degradation to establish the specificity of the method.

Keywords: Tafamidis, RP-HPLC, Stability indicating, Method development, Validation.

INTRODUCTION

Tafamidis delay disease progression in adults with certain forms of transthyretin amloidosis. It falls under the category of central nervous system drug and is a member of the class of benzoxazoles used for the amelioration of transthyretin-related hereditary amyloidosis[1]. Tafamidis (CAS 594839-88-0) is a member of the class of 1,3-benzoxazoles, a monocarboxylic acid and a dichlorobenzene. It can be used to treat familial amyloid polyneuropathy and wild type transthyretin amyloidosis. It works by stabilising the quaternary structure of the protein transthyretin[2,3]. The IUPAC name of Tafamidis is 2-(3,5-dichlorophenyl)-1,3-benzoxazole-6-carboxylicacid. The structure of tafamidis is shown in Fig.1. The molecular formula and molecular weight of Tafamidis are $C_{14}H_7Cl_2NO_3$ and 308.1g/mol respectively[4]. For the treatment of transthyretin mediated amyloidosis in adults, Tafamidis was approved by U.S. Food and Drug Administration on May 3, 2019. It was granted a European Medicine Agency market authorisation on November 16, 2011[5, 6].

The literature survey revealed that the monograph of Tafamidis is not official in any pharmacopoeia and only few instrumental methods are reported. A LC-MS method is reported for the assay of Tafamidis in rat plasma, and another article for the determination of Tafamidis in amyloidosis patients with Glu89Gln mutation by HPLC-UV Detection is reported[7,8]. However no stability indicating HPLC method was found during the literature survey for quantitative analysis of the Tafamidis in pharmaceutical dosage form. Hence, it was thought worthwhile to develop a novel, simple, rapid, precise, accurate, and stability indicating RP-HPLC method to estimate Tafamidis in the pharmaceutical capsule dosage form.
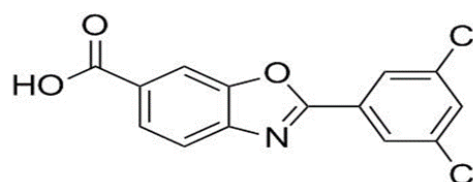

Figure 1: Chemical structure of Tafamidis

MATERIALS AND METHODS

Chemical and reagents

The Tafamidis reference standard with a defined potency of 99.7 % and Vyndaqel (61mg) capsules manufactured by Pfizer pharmaceuticals were obtained from Central Drugs Testing Laboratory, Mumbai. Acetonitrile, potassium dihydrogen phosphate, methanol (HPLC grade) from Finar Ltd. were used. The ultra-purified HPLC grade distilled water was obtained from the Milli-Q® system (Millipore, Milford, MA, USA) water purification unit. A high flow

nylon membrane filter (0.45μm) was purchased from Axiva Sichem Pvt. Ltd.

Instrumentation

Lab India UV3000+ UV/Vis Spectrophotometer having UV win 5 software version was used for all spectrophotometric measurements. The chromatographic separation and forced degradation studies were performed using Waters Alliance 2695 separation module equipped with a database version 7.30 using photo diode array detector. Sartorius analytical balance was used for all the weighing.

Selection of wavelength

Tafamidis standard 20 mg was weighed accurately and transferred to a 20 mL volumetric flask and the volume was made up to the mark with methanol (1000μg/mL) and further dilutions were made to get a concentration of 12 μg/mL. The above solution was then scanned in the range of 400.0 nm to 200.0 nm.Tafamidis showed maximum absorbance at 206.0 nm. Hence, 206 nm was selected for the analysis of Tafamidis.

Preparation of the buffer for the mobile phase

25 mM potassium dihydrogen phosphate buffer (pH 6.5) was prepared by weighing 3.4022 gm of potassium dihydrogen phosphate, transferring to a 1000 mL mobile phase bottle with water to dissolve, and sonicating for a few minutes using an ultra sonicator. Further, pH was adjusted to 6.5 with triethylamine and vacuum filtered through a 0.45 μm high flow nylon membrane filter.

Selection of the diluent

Considering the chemical nature of Tafamidis, methaol was selected as the diluent for standard and sample preparations.

Preparation of mobile phase

Potassium dihydrogen phosphate buffer (pH 6.5) and acetonitrile in the ratio of 60:40% v/v were used as mobile phase for the present study and sonicated, degassed before use.

Preparation of standard solution

The Tafamidis standard solution with a concentration of 12 μg/mL was obtained using methanol as diluent.

Analysis of marketed formulation

Average weight was determined using 20 capsules of Vynadaqel (61mg) and an amount of sample equivalent to 61mg of Tafamidis was accurately weighed and transferred into a 50 mL volumetric flask, dissolved in 25 mL of methanol. The mixture was subjected to sonication for 10 min, and volume was made using methanol. Further dilutions were made to get a concentration of 12.2 μg/mL.

10μL of standard and sample solutions were injected in triplicates into the HPLC system for performing the assay on the above capsules. % Assay, mean, SD and % RSD were calculated and reported.

Method optimization

Tafamidis is a weak acid. Based on the chemical nature of the molecule, different mobile phase systems and HPLC columns were tried to obtain a proper separation of the molecule.

The intial trials were carried out on Spherisorb waters-C18 column with a mobile phase of 0.1% trifluroacetic acid : acetonitrile (50:50% v/v) with an injection volume of 10 μL and a flow rate of 1mL/ min. However, poor peak shape was observed even after varying the ratio of mobile phase components.

Further trials were carried out on the Synchronis C18 column using potassium dihydrogen phosphate(pH 6.5): acetonitrile (60: 40% v/v) as mobile phase but the peak shape obtained did not meet the system suitablility parameters. Further trials were carried using an Ascentis® Express 90 A° C18 HPLC Column (25 cm×4.6mm×5μm) with mobile phase consisting of potassium dihydrogen phosphate (pH 5.5) : acetonitrile (60:40 % v/v).The chromatogram obtained showed good peak shape, but the tailing factor was below the specified limit. Then the mobile phase pH was increased to 6.5 and the remaining conditions were kept the same. Under these conditions, the peak was eluted with good peak shape and acceptable system suitability parameters.

Method validation

The developed RP-HPLC method was validated for system suitability testing, specificity, linearity, precision, accuracy and recovery, LOD, LOQ, and robustness as per ICH guidelines[9].

System suitability studies:

System suitability studies were carried out to check the system performance. This was performed by injecting six replicates of the standard solution of Tafamidis (12 μg/mL) and a single injection of blank preparation into the HPLC. The chromatograms were recorded and the mean, SD and % RSD of area and retention time were calculated. The tailing factor and theoretical plates were also evaluated.

Specificity:

For specificity, forced degradation studies were performed at different conditions such as acidic, alkaline, oxidative, hydrolytic, thermal, and photolytic conditions.

Linearity:

The linearity studies on Tafamidis were carried out in the concentration range of 3-18 μg/mL and the correlation coefficient was found to be 0.9992. The regression equation obtained was found to be y=993.73+524.82. The linearity graph was plotted by taking the concentration of the drug on the X-axis and the corresponding peak area on the Y-axis as shown in Fig. 2.

Precision:

System precision: This parameter was performed by injecting six replicate injections of a standard solution (12 µg/mL) . The mean, SD, and %RSD of peak areas of six replicate injections was calculated and reported.

Method precision (Assay repeatability): Six replicates of a sample solution of Tafamidis (12.2 µg/mL) were injected into the HPLC system. % Assay, mean, SD, and %RSD were calculated and reported.

Intermediate precision:

This parameter was performed on two different days, and six replicates of standard solution (12 µg/mL) and three replicates of sample solution (12.2 µg/mL) were injected into the HPLC system. Its % Assay, average, SD, and %RSD were calculated. The results of all precision parameters are summarized in Table 1.

Accuracy and recovery (Standard addition method):

Accuracy was performed by the standard addition method, using pre-analysed samples at three different levels of 80%, 100%, 120%. At each level, three determinations were performed. % Recovery and % RSD were calculated and reported. The results of accuracy and recovery are described in Table 2.

LOD and LOQ:

Limit of detection (LOD) and limit of quantitation (LOQ) of Tafamidis were determined from the calibration curve method using the following formula:

$$LOD = 3.3 \times \propto/s, \quad LOQ = 10 \times \propto/s$$

Where $\propto$ represent the standard deviation of the response of the regression line and s represent slope obtained from the calibration curve. After calculating, solutions of the desired concentration for LOD and LOQ were prepared and injected.

Robustness:

Robustness was performed by varying the flow rate (± 0.02 mL/min), column temperature (±2 °C), wavelength (±2 nm), and the ratio of mobile phase (±5 %v/v). Six replicates of sample preparations of 12.2 µg/mL were prepared and injected along with six replicate injections of standard solution of 12 µg/mL under different chromatographic conditions. Its % Assay, mean, SD, and %RSD were calculated and the results are described in Table 3.

Forced degradation studies

The chemical stability of pharmaceutical molecules is a significant matter of concern as it affects the safety and efficacy of the drug product. Forced degradation data helps to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors. According to ICH guidelines, stress testing helps in the identification of degradation products, which helps in the determination of the intrinsic stability of the molecule and establishing degradation pathways, as well as validating the stability indicating procedures used[10]. Forced degradation of Tafamidis was carried out under acidic, alkaline, oxidative, hydrolytic, thermal, and photolytic conditions.The peak purity of Tafamidis was evaluated by photo diode array detector in each condition. Stress conditions and results are given in Table 4.

RESULTS AND DISCUSSION

System suitability studies

All the system suitablility parameters were found to be within the acceptance limits.

Method validation

The proposed method for determination of Tafamidis in capsule dosage form is specific and was established by forced degradation studies. The method was found to be linear in the concentration range of 3-18 µg/mL with a correlation coefficient of 0.9992.

In system precision, the % RSD for peak areas of Tafamidis standard solution was found to be 0.25, and the mean assay percentage results of Tafamidis sample solutions were found to be within the specified limits. The % RSD was found to be 0.15. The chromatogram for the standard and sample solution (12.2 µg/mL) is shown in Fig.3 and 4. Hence, the developed method was found to be precise. The % RSD values of intermediate precision and assay were found to be within the acceptance limits.

The mean percent recovery and accuracy of Tafamidis sample solutions was found to be 100.5%, which was within the limit of 98-102%. The LOD and LOQ values were found to be 0.12 µg/mL and 0.35 µg/mL. The lower values of LOD and LOQ indicate that the method developed is accurate, precise, and sensitive as it can detect and quantify the analyt at very low concentrations.

The % RSD of different parameters in robustness was less than 2.0 and reproducible results were obtained, which proves the method to be robust. High percent recovery values and low SD and % RSD values confirm that the developed method is suitable for routine analysis of Tafamidis in its pharmaceutical capsule dosage form.
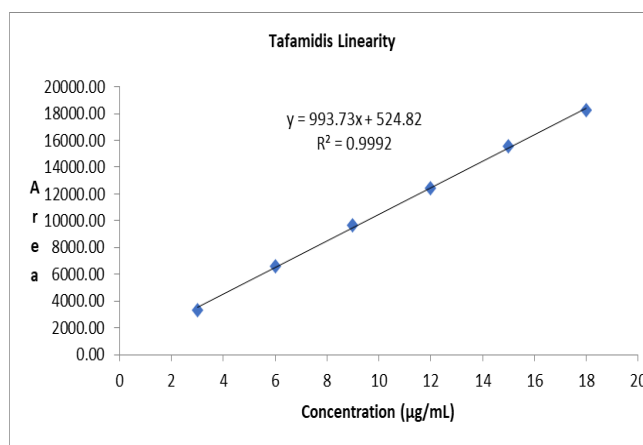

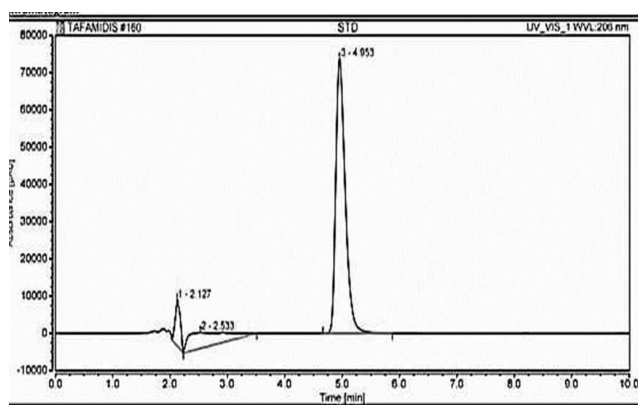**Figure 2:** Linearity graph of Tafamidis

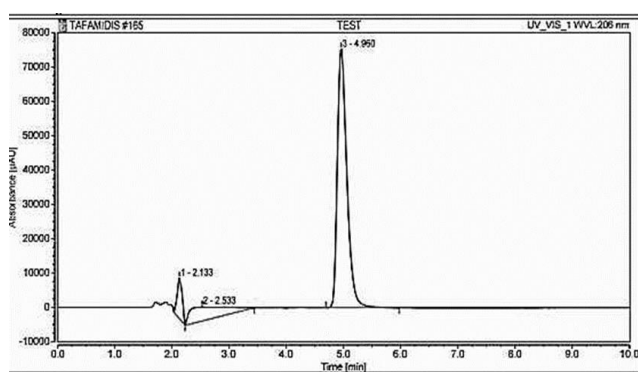**Figure 3:** Chromatogram of standard solution of Tafamidis

Figure 4: Chromatogram of sample solution of Tafamidis

Table 1 Precision

Parameters	% RSD	Limit of % RSD
System precision	0.25	NMT 2.0 %
Method precision	0.15	
Intermediate precision (Day 1)	0.60	
Intermediate precision (Day 2)	0.48	

Forced degradation studies

Chromatograms of acidic and alkaline degradation showed extra peaks indicating mild degradation, i.e. 2.96 % degradation in acidic conditions and 11.56 % in basic conditions. Tafamidis was found to be stable to water hydrolytic, oxidative, thermal and photolytic stress conditions and hence no degradation was observed. Degradation products formed were well separated under developed conditions. The peak purity of the main peak was found to be 100.00 %, as the peaks of degradation products were successfully separated from the main peak of Tafamidis without any interference. The acceptance limit of peak purity is not less than 99 %. This confirms the stability indicating nature of the proposed method.

Table 2: Accuracy data of Tafamidis

% level	Amount present in pre-analyzed sample (µg/mL)	Standard spiked (µg/mL)	Theoretical amount (µg/mL)	% amount recovered	% recovery	Mean % recovery
80	305	240	10.9	80.02	100.03	
100	305	300	12.1	100.70	100.70	100.5
120	305	360	13.3	121.14	100.95	

Table 3: Robustness studies of Tafamidis

Parameter	Change in parameter (±)	% Assay estimation	Average	SD	% RSD of assay	Limit of % RSD
Flow rate (±0.02 mL/min)	0.98	100.53	101.62	0.94	0.93	NMT 2%
	1	102.16				
	1.02	102.17				
Column temperature (±2°C)	23	100.91	101.14	0.58	0.57	
	25	101.80				
	27	100.71				
Wavelength (±2 nm)	204	101.95	100.24	0.62	0.61	
	206	100.98				
	208	100.80				
Mobile phase ratio (±5% v/v)	65-35	100.56	100.99	0.47	0.46	
	60-40	100.91				
	55-45	101.49				

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Table 4: Data of forced degradation studies of Tafamidis.

Sr No.	Degradation	Condition	Duration	Retention time of degradation products (min)	% Residual drug	Observed peak purity (%)
1	Acid degradation	1mL of 2N HCl at 60°C	1h	3.48	97.05	100.0
2	Alkali degradation	1 mL of 0.1N NaOH at 60°C	1h	4.52	88.44	100.0
3	Oxidative degradation	1mL of 3% H_2O_2 at 60°C	1h	-	100.0	100.0
4	Water hydrolysis	1mL of water at 60°C	1h	-	100.0	100.0
5	Thermal degradation	Hot water bath at 100°C	1h	-	100.0	100.0
6	Photolytic degradation	UV lamp (254 nm)	24 h	-	100.0	100.0

NLT= Not less than

CONCLUSION

The developed RP-HPLC method was found to be novel, simple, rapid, accurate, precise, sensitive, and stability-indicating. The method was validated for parameters like specificity, linearity, precision, accuracy and recovery, LOD, LOQ, and robustness as per ICH Q2 (R1) guidelines. This method development is constructive, since no stability-indicating RP-HPLC method was reported for estimation of Tafamidis in pharmaceutical capsule dosage form before. The results of all validation parameters were found within the specified limits. The stress study demonstrates that Tafamidis undergoes mild degradation in acidic and alkaline conditions and it was found to be stable in oxidative, thermal, water hydrolytic and photolytic stress conditions. The degradation products were separated during forced degradation studies from the active pharmaceutical ingredient. Hence, the proposed RP-HPLC method can be used for routine analysis of Tafamidis in pharmaceutical capsule dosage form.

ACKNOWLEDGMENT

DECLARATION OF INTEREST

Authors declare that there is no conflict of interest, financially or otherwise.

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