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Research/Review

A novel stability indicating methoddevelopment and validation of tafamidis by RP-HPLC technique

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Abstract

This study presents a novel stability-indicating method for the quantification and validation of Tafamidis using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). Tafamidis, primarily used to treat cardiomyopathy, demands stringent quality control due to its complex structure and sensitivity to environmental conditions. Our approach employs an optimized chromatographic process utilizing a YMC Triart C18 column with isocratic elution, and an initial mobile phase composed of 20mM Ammonium Acetate and Acetonitrile in a 60:40 ratio. The method showcases robust performance with a retention time of 3.3 minutes and detection at 206 nm. Validation of the method adhered to International Conference on Harmonization (ICH) guidelines, confirming the system's suitability through rigorous testing of parameters like linearity, precision, accuracy, and robustness. The method demonstrated excellent linearity within the 5-25 µg/ml range with a correlation coefficient (R^2) of 0.998. Precision tests, both inter-day and intra-day, indicated consistent results with low relative standard deviation (RSD), underscoring the method's reliability. Forced degradation studies further validated the method's efficacy in providing stability data for Tafamidis under varied conditions, revealing significant resilience to oxidative, acidic, alkaline, and thermal stresses. This validated method is both effective and efficient for routine quality control and stability testing of Tafamidis in pharmaceutical formulations, ensuring compliance with pharmacopeial standards and therapeutic efficacy.

Keywords: Tafamidis, RP-HPLC, stability-indicating method, forced degradation, chromatographic validation.

INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry concerned with the resolution, separation, identification, determination, and purification of a specific sample of a medication or pharmaceutical, as well as the detection and quantification of any impurities present. The quality/standard of a pharmaceutical product is monitored and maintained throughout the process of manufacture and alsoin the finished product by a series of tests from quality control. Quality control processes might include chemical, physicochemical, or instrumental procedures, as well as microbiological or biological treatments.

Precursors, or the raw materials from which the purity and quality of amedicament are derived, are also examined in pharmaceutical analysis. The quality ofthe drug and its formulations can only be determined once the validity of the drug hasbeen established. Physical phenomena that originate from chemical processes arestudied using physico-chemical techniques. In Physico-Chemical analysis, the most essential methods are optical (Refractometry, Polarimetry, Emission, and Fluorescence procedures), photometric (Photocolorimetry and Spectrophotometry including UV-Visible, IR Spectroscopy, and Nephloturbidimetry), and chromatographic (Column, Paper, Thin Layer, Gas Liquid, and High PerformanceLiquid Chromatography). They're getting increasingly popular: Nuclear Magnetic Resonance (NMR), and Para Magnetic Resonance (PMR). The combination of MassSpectroscopy (MS) and Gas Chromatography (GC) is a highly effective technology. Gravimetric and volumetric activities can be performed using acid-base, precipitation, and other chemical processes based on complex formation. The pharmacologicalassessment of drugs has also used non-aqueous media titrations and complexometry.

Drugs are entering the market at a faster rate than ever before. It is possible that some drugs are entirely novel substances, or that they are only partially restructured versions of previous ones. When a medicine enters the market, there is often a period of time before it is included in pharmacopoeias. These include reports of novel toxicity, patient resistance to medicine; and the introduction of newer and better medications by competitors. The pharmacopoeias may not have standards or analytical techniques for these medications under certain circumstances. Because of this, it is vital to create new analytical techniques for these medications.

HPLC and Method Development[2-6]

The term ‘Chromatography refers to processes that aim to separate different species of amixture based on their distribution characteristics between a stationary and a mobile phase.

Some of the advantages are:

- Speed (analysis can be accomplished in 20 minutes or less),
- Increased sensitivity (various detectors can be employed),
- Improved resolution (wide variety of stationary phases),
- Columns that can be reused (expensive columns but can be used for manyanalysis),
- Ideally suited for low volatility substances,
- “Simple sample recovery, handling and maintenance”,
- “Instrumentation is prone to automation and quantitation (less time and lesslabour)”
- “Precise and reproducible”
- “Calculations are performed by integrator itself”
- “Suitable for preparative liquid chromatography on a much larger scale”. Chromatographic methods are most simply classified according to their stationary andmobile phases, as seen below.

Classification of chromatographic methods

Table 1: Classification of Chromatographic Methods

Stationary phase	Mobile phase	Method
Solid	Liquid	Adsorption, thin-layer, ionexchange, High performance liquid chromatography
Liquid	Liquid Gas	Partition, column, thin-layer chromatography (TLC) HPLC, paper chromatography. Gas-Liquid chromatography.

High performance liquid chromatography

Chromatography is used to identify, quantify, and purify the various components of a mixture by separating them from each other in the chromatographic procedure. Many different stationary phases, a pump that drives the mobile phase along, and a detector that offers a specific retention time for each analyte are all components of HPLC.For example, if the detector is equipped with UV/Vis spectroscopic data for the analyte,it can provide additional information about the sample. The degree of interactions between the stationary phase and analyte, the ratio/composition of solvents used, and theflow velocity of the mobile phase all influence analyte retention time.

In HPLC, the increased pressure required to push the mobile phase and analyte through the tightly packed column is supplied by a pump rather than gravity. The smaller particlesizes lead to an increase in density. In comparison to conventional column chromatography, this method allows for a more complete separation on shorter columns.High-pressure sample introduction pumps and injectors, eluent storage, stationary phasedelivery columns, detectors, and recorders are the main parts of the system. The method'sflexibility and ability to analyse multi-component mixtures have been substantially boosted by the introduction of very efficient micro particle bound phases.

Adsorption, partition, ion exchange and size-exclusion.

- As in ion-exchange chromatography, an anionic or cationic stationary phase attracts molecules with opposite charges to its surface.
- Size-exclusion The utilisation of a solid stationary phase with carefully regulated porediameters is the foundation of chromatography. Solutes are classified based on their molecular size, with larger molecules being able to pass through pores and elute first.

Analytical method development

Method development is done

- "For new products"
- "For existing products"

When there are no formal methods for a new product, methods are invented. Existing (non-Pharmacopoeial) products are being redesigned in order to save money and time while improving precision and toughness. Trials are carried out, and the procedure is refined and validated. Comparative laboratory data, including both advantages and disadvantages, is made available when a new method is recommended to replace an existing methodology.

Method development Analyte standard characterization

a) Analyte properties include toxicity, purity, hygroscopicity and solubility as well as solubility and stability.
b) In order to obtain the standard analyte (purity 100 percent). In order to ensure adequate storage, all the essential preparations are being performed (refrigerator, desiccators, and freezer).
c) The number of components in the sample matrix is documented, data is gathered, and the availability of standards is determined for each one during the examinationof numerous components.
d) Only methods compatible with sample stability (MS, GC, HPLC, etc.) are considered.

Method requirements

The method's objectives and requirements determine how to determine analytical figures of merit. The detection limits, selectivity, linearity, range, accuracy, and precision necessary for each type of detection are described.

Analyte standard characterization

a) The analyte and its structure are thoroughly investigated, including physical and chemical characteristics, toxicity, purity, hygroscopicity, solubility, and stability.
b) This is accomplished by obtaining the standard analyte (purity 100 percent). The required preparations for proper storage are being made (refrigerator, desiccators, and freezer).
c) A sample matrix's multiple components can be broken down and examined to determine how many different standards are available for each one.
d) Only methods that are compatible with sample stability (MS, GC, HPLC, etc.) are considered.

Literature search and prior methodology

Analyte synthesis, physical and chemical characteristics as well as the ability to be dissolved in water and the appropriate analytical procedures are gathered from scientific literature.' In addition to books and journals, we also examine chemical manufacturer's compendia and publications from regulatory agencies including USP/NF, the AOAC, and ASTM. An automatic computerized literature search is made possible via the Chemical Abstracts Service (CAS).

Choosing a method

a) The approach is adapted based on information gathered from scientific publications. As needed, changes are made to the procedures. In order to reproduce, alter, enhance, or validate current procedures for in-house analytes and samples, extra apparatus may be required.
b) The analyte's structure and chemical characteristics can be compared to similar compounds in order to

investigate and work out the analyte's properties. Every analyte can typically be analysed using a technique developed for another chemical.

Instrumental setup and initial studies

a) The instrumentation has been installed. Instrument installation, operation, and performance qualification are all verified using laboratory standard operating procedures (SOPs).
b) New consumables (e.g., solvents, filters, and gases) are always used; for example, method development is never started on a previously used HPLC column.

MATERIALS AND INSTRUMENTS

Pure drug samples

The drug samples of Tafamidis was received as gift sample from Intas Pharmaceuticals Pvt Ltd respectively.

Chemicals and Solvents

Table 2: Chemicals and Solvents

S.no	Chemicals/Solvents	Manufacturer
1	HPLC Grade Acetonitrile	Lab Fine Chem Industries Ltd., Mumbai, India
2	HPLC Grade Methanol	Lab Fine Chem Industries Ltd., Mumbai, India
3	Ammonium Acetate	Gaurav Scientific and Chemicals, Mumbai, India
4	Hydrochloric acid	Lab Fine Chem Industries Ltd., Mumbai, India
5	Sodium Hydroxide	Lab Fine Chem Industries Ltd., Mumbai, India
6	Hydrogen per oxide	V.L. Products, Mumbai, India

Instruments Used

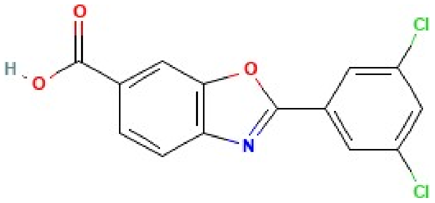
Table 3: Details of Instruments

S.no	Instruments	Model
1	UV Spectrophotometer	Shimadzu UV1800S
2	Ultra performance Liquid Chromatography	Agilent UHPLC-MS, E6125B
3	Liquid Chromatography Mass Spectrometry	Agilent 1290 Infinity II

Drug profile

Tafamidis

Structure: Tafamidis



Drug Profile of Tafamidis

IUPAC name : 2-(3,5-Dichlorophenyl)-1,3-benzoxazole-6-carboxylicacid
Molecular formula : C 14H 7CI2 NO3
Molecular weight : 308.1 g/mol
Category : Tafamidis is indicated to treat cardiomyopathy
Class : Sodium-glucose co-transporter2(SGLT2) inhibitors
Solubility : "soluble in organic solvents such as ethanol and DMSO" slightly soluble water
:
Brand name : VYNDAMAX

Hplc system

Instrument Specifications

Instrument	-	High Performance Liquid Chromatography
Injector	-	Rheodyne (20µl loop)
Software	-	LC solutions
Detector	-	PDA

Initial chromatographic conditions

Selection of chromatographic method for separation

Reverse phase chromatographic techniques was selected since the drug is polar in nature.

Selection of wavelength

Selectivity of HPLC method with PDA detector is dependent on suitable wavelength selection. A precise wavelength must be chosen for the medications to have a distinct and distinctresponse. 206 nm was chosen as the study wavelength from the Tafamidis drug's UV spectra.

Selection of mobile phase

The HPLC method's PDA detector selectivity is based on the wavelength of the PDA detector. For the drug to have a specific and definite response, a precise wavelength must be chosen. A wavelength of 206 nm was selected from the UV spectra of the Tafamidis drug becauseit was the most relevant. Different mobile phases tried and their observations are given in the Table 4.

Table 4: Selection of mobile phase trials

Mobile Phase condition	Observation
Water : Acetonitrile (75:25)	Tailing, Broad peaks
5mM K_2HPo4 : Acetonitrile pH5 (70:30)	Fronting, Tailing, Splited Peaks
5mM K_2HPo4 : Acetonitrile pH4.5 (70:30)	Tailing, Splited Peaks
5mM K_2HPo4 Acetonitrile pH4 1ml/min (70:30)	Tailing, Broad Peaks
Water : methanol (70:30)	Good Symmetric Peaks

Table 5: Optimized Chromatographic conditions

Parameter	Condition
Column	YMC Triart C_{18} column (100 x 2 mm, 1.9)
Flow rate	1ml/minute
Detector Wavelength	206nm
Injection volume	20µl
Column Temperature	60^0C
Auto sample Temperature	2^0C
Elution mode	Isocratic
Run time	**10** minutes
Diluent	Ammonium acetate(20mM):Acetonitrile in the ratio of 60:40

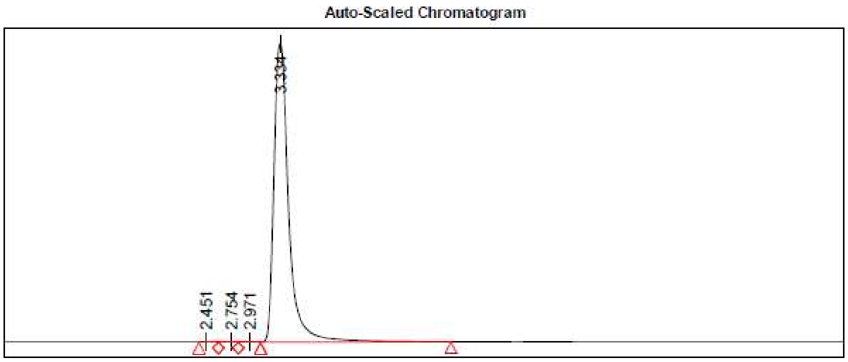


Fig 1: Optimized Chromatogram of Tafamidis

Validation of rp-hplc method

System suitability studies

System suitability parameters like Retention time, number of theoretical plates (N), Tailing factor, resolution (Rs) etc., were studied, and results are given in table 6.

Table 6: System suitability studies of Tafamidis

Area

Drug	Theoretical Plates (N)	Retention time(Rt)	Tailing facto
Tafamidis	3711	3.3	1.5

Linearity and range

The Calibration curve was used to establish linearity using least squares regression. Tafamidis calibration curves (5-25 µg/ml) were linear. Peak areas were plotted against concentrations, and the resulting curves were subjected to linear regression analysis. Tafamidis's slope and intercept have a 0.999 correlation coefficient.

Table 7: Linearity of Tafamidis

Concentration (µg/ml)	Mean peak area
5	9304252
10	19414872
15	27025768
20	34551193
25	42207683
Linear regression equation (y=mx+c)	Y=17.29x+12.82
Slope(m)	17.295
Intercept(c)	12.828
Correlation coefficient (R^2)	0.998

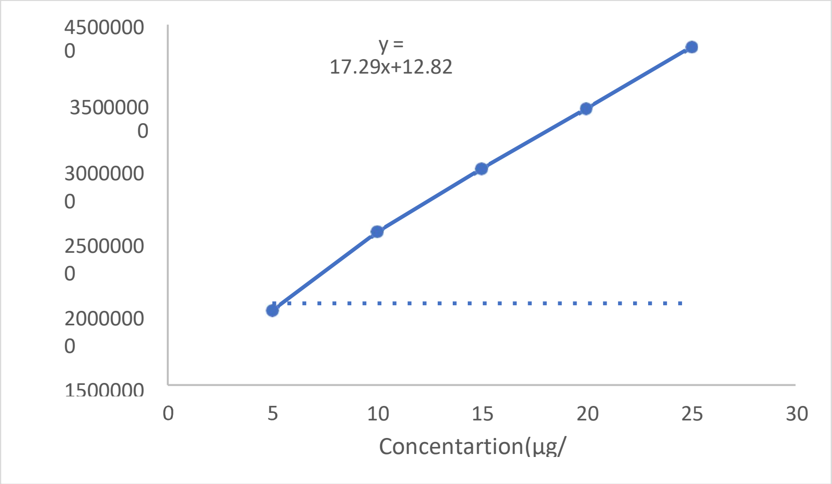


Fig 2: Calibration curve of Tafamidis at 286nm

Precision

Precision of method was demonstrated by

a) Intraday precision
b) Inter day precision

Intraday precision

Analysis of standard drug solutions in the linearity range at five different concentrations on thesame day yielded intraday precision by calculating percent RSD, table.no.5.2.5.

Table 8: Intraday Precision of Tafamidis

Drug	Conc (µg/ml)	Peak area	Time intervals of samples in precision					Average	%RSD
Tafamidis	10	176.75	176.75	176.77	176.80	176.84	176.87	176.806	0.029
	20	370.081	370.01	370.01	353.03	370.03	370.04	370.024	0.037
	30	534.878	534.87	534.87	534.88	534.88	534.89	534.878	1.568
	40	720.048	720.04	720.04	720.05	720.05	720.06	720.048	1.180
	50	872.461	872.46	872.46	872.48	872.48	872.44	872.466	1.259

Inter day precision

Inter-day precision was achieved by analysing reference drug solutions at five concentrations in the linearity range for five days over a one-week period and calculating percent RSD, table.no. 5.2.6.

Table 9: Intraday Precision of Tafamidis

Drug	Conc (µg/ml)	Peak area	Time intervals of samples in precision					Average	%RSD
Tafamidis	10	176.752	176.74	176.75	176.77	176.78	176.76	176.766	0.0119
	20	370.081	370.03	370.07	370.05	370.09	370.08	370.064	0.0617
	30	534.87	534.87	534.88	534.86	534.84	534.85	534.854	0.027
	40	720.04	720.04	720.05	720.06	720.07	720.08	720.06	0.023
	50	871.461	871.44	871.45	871.47	871.48	871.49	871.46	0.023

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated mathematically. The LOD and LOQ of Tafamidis were found tobe 0.0039µg/ml and 0.0119µg/ml respectively. It can be shown in table.no.652.7.

Table 10: LOD and LOQ of Tafamidis

Drug	Limit of Detection	Limit of Quantification
Tafamidis	0.0040	0.0120

Robustness

It is possible to assess an analytical procedure's robustness by observing how well it holds up undertypical conditions, even when tiny but deliberate changes are made to the technique parameters. Changes in the composition of the buffer in the mobile phase and flow rate were used to test the method's robustness. Each condition's RSD percentage was computed. Although several procedureparameters were deliberately varied, the findings derived from these changes were remarkably consistent. and results are shown in table 11.

Ruggedness

The ruggedness of the procedure was determined by comparing the intra-day and inter-day Tafamidis assay findings done by two analysts. The percent RSD values for assays done by two analyzers in the same laboratory did not surpass 2, showing the method's ruggedness.

Table 11: Robustness studies of Tafamidis

Parameters	Modifications	Robustness	
		Plate count	tailing factor
		Tafamidis	Tafamidis
Flow Rate	0.8	3513	1.41
	Optimized	3712	1.51
	1.2	3705	1.42
Mobile PhaseComposition	10% less	3712	1.42
	Optimized	3712	1.52
	10% more	3013	1.47
Wavelength	+5nm	3413	1.46
	Optimized	3712	1.51
	-5nm	3313	1.46

Column Temperature	$+5^{o}$C	3213	1.43
	Optimized	3712	1.51
	-5^{0}C	3413	1.47

Accuracy

The accuracy of the drug's recovery was determined by conducting recovery experiments. The sample formulation was reanalyzed using the suggested approach after it was mixed with a known quantity of standard pharmaceuticals. Both a 50 and a 100 percent sample was used in this study table 12 displays the recovery results.

Table 12: Accuracy studies of Tafamidis

Drug	Levels (%)	Amount taken (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% recovered
CANA	80	50	40	89.86	99.88%
	100	50	50	100.37	100.36%
	120	50	60	120.19	100.15%

Analysis of formulation

For formulation analysis, fixed chromatographic conditions were used.

Preparation of standard solutions

Dissolving 1 milligram of Tafamidis in 10 ml of mobile phase yielded stock solutions. Transferred aliquots from the standard stock solution of Canaglifozin were made into 10 ml volumetric flanks and a solution was created as needed to meet the Linearity ranges.

Preparation of sample solutions

Measurement and powdering of individual Invokana pills yielded an average weight ofTafamidis. A 10-ml volumetric flask was required for the transfer of 100 mg of Tafamidis. This can only be removed by using the mobile phase. It was necessary to use a 20-minute ultrasonication procedure to ensure that the medications were completely dissolving in the volumetric flask. The HPLC was used to obtain aconcentration within the linearity range by injecting the formulation solution in appropriate aliquots.

Recording of chromatograms

Standard drug solutions were injected into the chromatographic conditions that had been constructed, and chromatograms were recorded. 3.801 minutes was found to be the Tafamidis retention time. Next, the formulation was tested in the form of an injection, in order to construct calibration curves, the peak areas of standard pharmaceuticals were plotted against the matching standard solutions' concentrations.
Table .no.652.10 shows the amount of Tafamidis in the sample chromatograms relativeto the peak regions of the chromatograms, as shown in fig.no.5.2,8

Table 13: Analysis of marketed formulation

Drugs	Labeled amount	Amount found	%label claim	%RSD
Tafamidis	100	99.2	99.2%	0.5

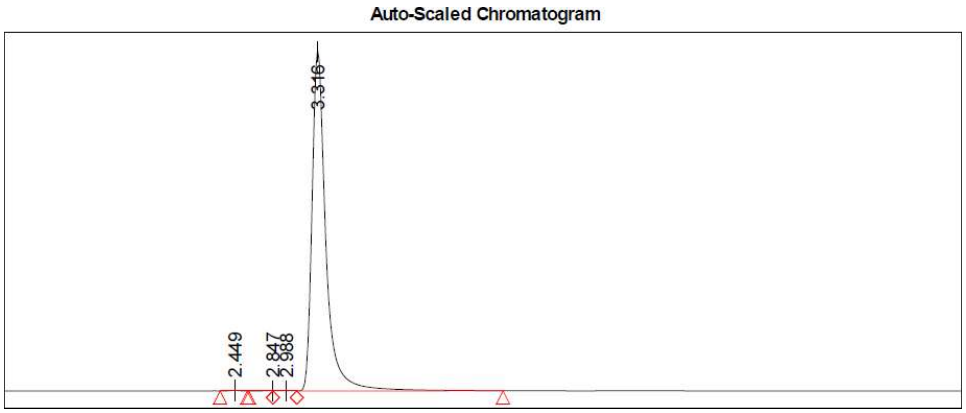


Fig 3: Formulation Chromatogram of TafamidisForced Degradation Study for

Tafamidis

Tafamidis drug individual samples was forcibly degraded under the stress conditions mentioned in the below table. the details of the degradation were recorded. Themother sample and forcibly degraded samples of Tafamidis was analyzed for Description,Related substances by HPLC as per the final method.

Degradation Procedure

The stability of the developed method was established by performing forceddegradation studies of the drug in the presence of acid, alkali, H_2O_2, temperature, UV light, and HPLC grade water.

Acid degradation

Over a wide range of pH, hydrolysis is a frequent degradation chemical process. Hydrolysis is a chemical process in which a chemical component is brokendown by the addition of water to the reaction. Ionizable groups in molecules can beused to catalyse hydrolysis in acidic conditions. Drug substances are exposed to an acidic environment in order to produce primary degradants in a desired range of concentrations. The stability of the drug material specifies the type and concentrationof acid used. For acid degradation to break down a, use hydrochloric acid (0.1 to 1 M). Co-solvents can be used to dissolve substances for stress testing that are insoluble in water. In order to choose a co-solvent, it is necessary to know the drug'sstructure. Normal stress testing trials begin at ambient temperature and progress to increased temperatures ranging from 50–70ºC if no degradation is seen. Stress testing should not last for longer than seven days at the most. To prevent further breakdown, an acid or buffer is used to neutralise the degraded sample.

Table 14: Acid Degradation of Tafamidis

S.no	Optimized and Degradation types	Retention time	Peak Area
1	Tafamidis Peak	4.257	8.356
2	Impurity-A	0.455	74.554
3	Impurity-B	0.740	14.306
4	Impurity-C	1.403	10.417
5	Impurity-D	1.767	103.703
6	Impurity-E	2.524	93.171
7	Impurity-F	2.879	8.228

Alkali degradation

Over a wide range of pH, hydrolysis is a frequent degradation chemical process. Hydrolysis is a chemical process in which a chemical component is brokendown by the addition of water to the reaction. Under acidic and basic conditions, ionizable functional groups in the molecule are used as catalysts for hydrolysis. Stress testing with acid or base includes exposing a pharmacological material to acidic or basic conditions in order to develop primary degradants that are in the desired concentration range. The stability of the drug substance dictates the type and concentration of base used. For base hydrolysis, reagents such as sodium hydroxide or potassium hydroxide (0.1–1M) are recommended. Co-solvents can beemployed to dissolve the stress testing chemicals in NaOH if they are insoluble in water. In order to choose a co-solvent, it is necessary to know the drug's structure. Normal stress testing trials begin at ambient temperature and progress to increasedtemperatures ranging from 50–

701C if no degradation is seen. Stress testing shouldnot last for longer than seven days at the most. To prevent further degradation, thesample is neutralized with a base, or buffer.

Table 15: Alkaline Degradation of Tafamidis

S.no	Optimized and Degradation types	Retention time	Peak Area
1	Tafamidis Peak	4.305	2.854
2	Impurity-A	0.344	40.237
3	Impurity-B	0.475	1.244
4	Impurity-C	1.813	15.256

Table 16: %Alkaline Degradation of Tafamidis

S.no	Impurities	Peak Area	% Degradation	% Asssay ofActive Substance
1	Impurity-A	40.237	0.3249	32.49
2	Impurity-B	1.244		
3	Impurity-C	15.256		

Oxidative degradation

Other oxidizing agents, such as metal ions, oxygen, and radical initiators (e.g., azobisisobutyroni-trile, AIBN), can also be utilised in forced degradation investigations. Itis important to know the drug material before selecting an oxidising agent, its concentration, and the conditions for use. Hydrogen peroxide exposure at neutral pH and room temperature for seven days or up to a maximum of 20% degradation is stated to be able to produce meaningful degradation products. Electrons are transferred between anionsand cations as a result of the oxidative decomposition of drugs. Electron transfer oxidation of amines, sulphides, and phenols produces N-oxides, hydroxylamine, sulfones, and sulfoxides. Oxidation of labile hydrogen-containing functional groups such as those in thebenzylic carbon, the allylic, and the tertiary carbon positions with regard to the Hetro atomcan result in hydro peroxides, hydrogen or ketone formation. As shown in fig no.6.2.11 and results in table.16,17.

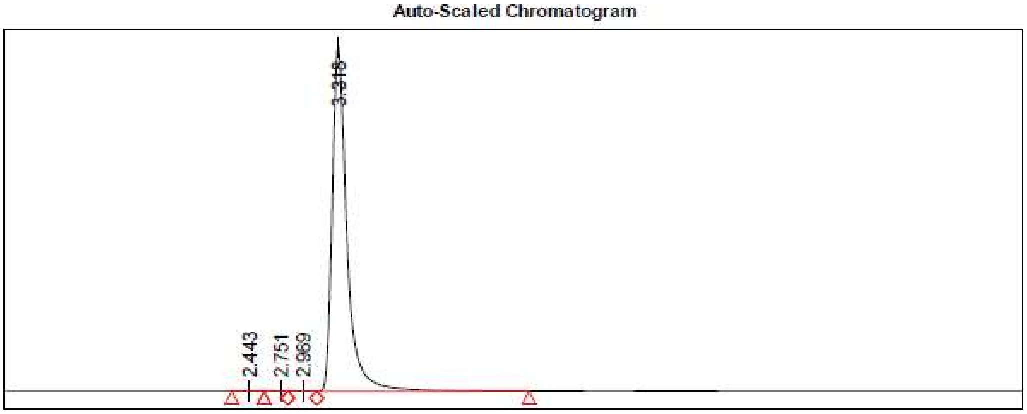


Fig 4: Oxidative Degradation of Tafamidis

Table 17: Oxidative Degradation of Tafamidis

S.no	Optimized and Degradation types	Retention time	Peak Area
1	Tafamidis Peak	4.259	12.411
2	Impurity-A	0.471	1.709
3	Impurity-B	1.539	74.776

Table 18: % Oxidative Degradation of Tafamidis

S.no	Impurities	Peak Area	% Degradation	% Asssay of Active Substance
1	Impurity-A	1.709	0.4352	43.52

2	Impurity-B	74.776

Thermal degradation

Thermal degradation (e.g., dry heat and wet heat) should be performed under more challenging settings than those prescribed by ICH Q1A accelerated testing. Solid-state drug substances and drug products should be treated with dry and wet heat, whereas liquid drug productsshould be treated with dry heat. In research, higher temperatures for a shorter period of time may be used. As illustrated in table 18.

Table 19: Degradation of Tafamidis

	%Impurity							
Stress condition	**Imp -A**	**Imp-B**	**Imp-C**	**Imp- D**	**Imp-E**	**Imp-F**	**% Degradation**	**% Asssayof Active Substance**
Acid hydrolysis	74.553	14.304	10.417	103.702	93.171	8.228	0.2883	28.83
Alkaline Hydrolysis	40.237	1.244	15.256	ND	ND	ND	0.3248	32.49
Oxidative Hydrolysis	1.709	74.776	ND	ND	ND	ND	0.4351	43.52
Thermal Hydrolysis	76.795	14.593	10.601	104.180	93.281	8.807	0.2929	29.29

SUMMARY AND CONCLUSION

Acetonitrile and Ammonium Acetate were used as the initial mobile phase systemfor the determination of both medicines in this study. For this investigation, 206 nm waschosen as the wavelength. Initially, several concentrations of Ammonium acetate were tested. Ammonium acetate at 20 mM was found to be the optimal concentration for the experiment. Ammonium acetate (20 mM) and acetonitrile were the only ones that produceclear peaks. The mobile phase ratio was then calculated by altering the 20mM and acetonitrile proportions. Ammonium acetate (60:40 v/v) and Acetonitrile (60:40 v/v) wereused to measure the substance. Tafamidis was shown to have retention times of 3.3 minutes. ICH principles were followed in the validation of the new approach. The standard peak regions vs. concentration of standard solutions were used to plot calibration graphs. In this study, Tafamidis had a slope of 17.311, an intercept of 12.494, and a correlation coefficient of 0.998. From 5 to 25 µg/ml, Tafamidis was shown to be linear. Tafamidis'sLOD and LOQ were reported to be 0.0039 and 0.0119 micrograms per litre, respectively.The developed method's precision was tested under both intraday and interday precisionconditions. The approach is precise if the percent RSD values are low. In order to ensurethe method's reproducibility and reliability, recovery experiments were conducted at threelevels: 80 percent, 100 percent, and 120 percent, with known concentrations of standard medicines, and analysis was performed in accordance with the formulation protocol. Anaccurate approach was discovered with recovery values falling well within acceptable ranges. We were able to confirm that the approach we devised was dependable. Retention time (Rt), Tailing factor and Resolution were all examined to determine the system's appropriateness. Studies on Tafamidis's stability were conducted using the previously validated liquid chromatographic technique. Stability studies of Tafamidis was conducted through Forced degradation studies such as Acid, Alkali, Neutral, Oxidative, Photolytic and Thermal Degradation. It was found that in oxidative condition the drug was degradaed nearly about43.51% and in acidic, alkaline and thermal conditions the drug was degraded about 28.82%, 32.48% and 29.28% respectively.

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