"MICROWAVE ASSISTED ACCLERATED STABILITY STUDY OF METOPROLOL TARTRATE IN SOLID STATE"

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

Pharmaceutical Analysis

BY

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Under the guidance of

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May 2020

CERTIFICATE

This is to certify that the dissertation work entitled "Microwave assisted accelerated stability study of Metoprolol tartrate in solid state" submitted by Shivani P. Shah (18MPH308) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Analysis" is a Bonafede research work carried out by the candidate at the Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University under my guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

Guide

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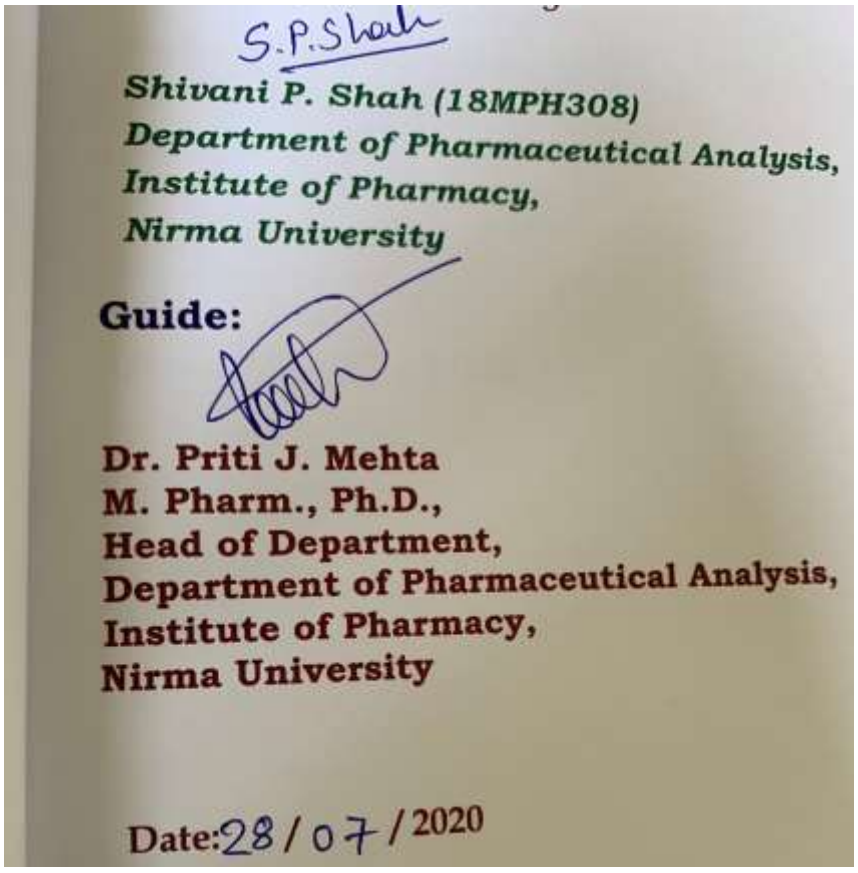
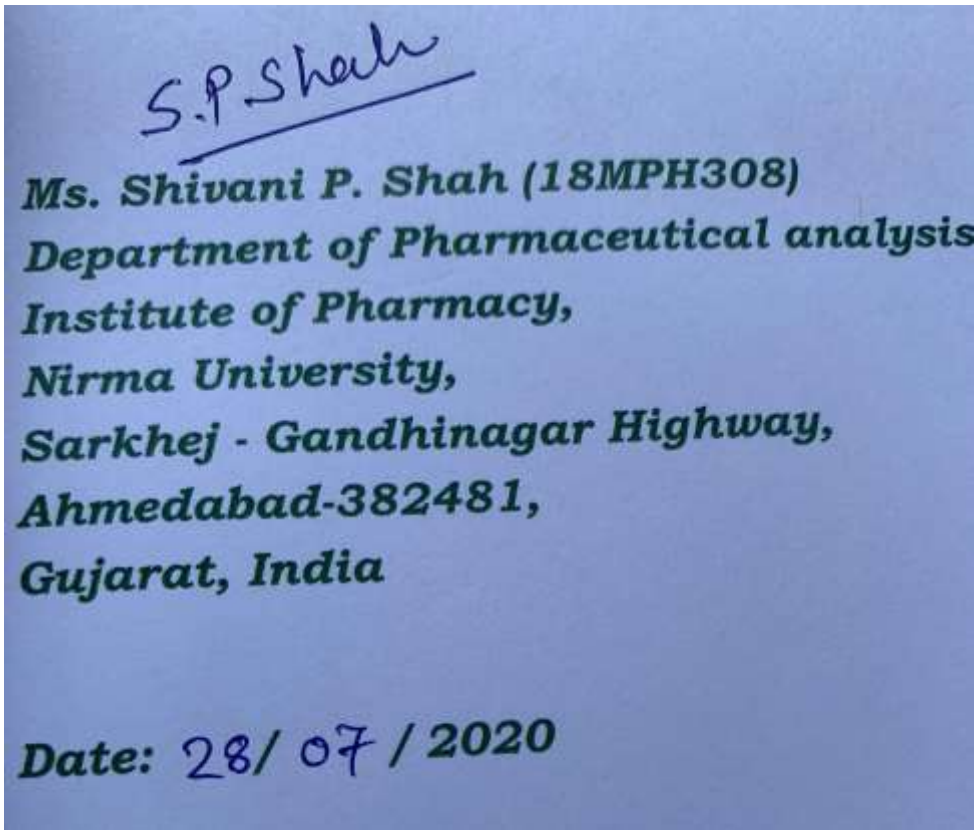
CERTIFICATE OF ORIGINALITY OF WORK

This is to undertake that the dissertation work entitled "Microwave assisted accelerated stability study of Metoprolol tartrate in solid state" Submitted by Shivani P. Shah (18MPH308) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Analysis" is a Bonafede research work carried out by me at the "Department of Pharmaceutical Analysis", Institute of Pharmacy, Nirma University under the guidance of "Dr. Priti J. Mehta". I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, this work is original and not reported anywhere as per best of my Knowledge.



DECLARATION

I hereby declare that the dissertation entitled "Microwave assisted accelerated stability study of Metoprolol tartrate in solid state", is based on the original work carried out by me under the guidance of Dr. Priti J. Mehta, Head of the department under the Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.



Dedicated to My Family

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"No research is ever quite complete. It is the glory of a good bit of work that it opens the way for something still better, and this repeatedly leads to its own eclipse"

First I thanks to my family with my heart for giving their love and encouragement, my family is my backbone and tower of strength. They are providing me all facilities in any situation. My support system is **My Family**, without them I am nothing.

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Shivani P. Shah

Contents

List of Abbreviations

Symbol	Full Form
API	Active pharmaceutical ingredient
AR	Analytical Reagent
ACN	Acetonitrile
MeOH	Methanol
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
H2O2	Hydrogen peroxide
HPLC	High Performance Liquid chromatography
UV	Ultra Violet
ASS	Accelerated Stability Study
°C	Degree Celsius
gm	gram
mg	Milligram
µg	Microgram
mL	Millilitre
µm	Micrometre
cm	Centimetre
nm	Nanometre
%	Percentage
hr	Hour
min	Minute
sec	Second
DP	Degradation Product
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceutical for human use
FDA	Food and Drug Administration

RSD	Relative Standard Deviation
Rt	Retention Time
RRT	Relative Retention time
ND	No Degradation
Temp, T	Temperature
W	Watt
GHz	Gigahertz

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ABSTRACT

Stability studies are essential to confirm drug stability and quality till the time of ingestion by patients. Actual stability studies are long and therefore accelerated stability study are executed at high temperature and moisture condition to enhance the speed of reaction. Conferring towards the 'International Conference on Harmonization (ICH)' guidelines, accelerated stability studies are completed next to '40ºC ± 75%' (RH) for around 6 months. Microwave-assisted heating has been used in chemistry for many years to enhance the speed of reaction which there by reduces the time for synthesis. The practice of using a microwave to perform accelerated stability studies instead of the conventional method might diminish the time for calculating the constancy of the treatment ingredient. However, there remains a lack of any substantial evidence about the use of microwave-assisted stability studies in the literature. Hence, the goal of work being done was to assess the possibility of the microwave assisted heating method in the estimation of drug stability. In this project, an effort was made to work out accelerated stability study of Metoprolol tartrate using microwave heating and conventional method. Degradation from both the methods were analyzed to compare the results. The Metoprolol tartrate sample were kept in the stability chamber at 40ºC ± 75 % RH and were collected at different time intervals. Correspondingly, samples were also kept in microwave at different conditions having power range from 200W - 600W; temperature range from 80 ˚C - 100 ˚C and water level from 50µl to80 µl. The degradation behavior was analyzed by the aid of HPLC (High-performance liquid chromatography).The chromatograms obtained from microwave-assisted method were compared with the conventional method.

The study confirmed a substantial correlation between conventional and microwave-assisted stability. The microwave-assisted stability study under conditions with (80µL water; 600W; 100T; 30min) showed no degradation product in mixture with PVP K30 to that compared the conventional method.

Therefore, to conclude microwave-assisted heating is an efficient way to perform the accelerated stability study of APIs.

Chapter-1

Introduction

1. Introduction

1.1 Drug substance/drug product stability and regulatory significance

Stability testing in addition with various aspects permits to assess the stability of various drug products. Several aspects such as active pharmaceutical ingredients (API) stability, the process of manufacturing used, the interaction between API and excipients, type of dosage form, packaging, handling care, shipping, exposure to atmosphere during storage, and susceptibility of API to oxidation, reduction, hydrolysis, photolysis, and racemization .Stability testing enables to determine retesting period of a drug, expiry date of substance, and storage conditions of Active Pharmaceutical Ingredients or drug substances. (Huynh-Ba, 2008; Yoshioka et al., 2000; Baertschi, 2010)

Different regulatory bodies recommends creating a stability profile of pharmaceutical products during drug development process. On a major aspects, the International Conference on Harmonization (ICH) guidelines are being followed in practice. This guidelines commends validating the stability method using forced degradation samples which may contain all degradation impurities. Various ICH guidelines are given in **Table 1.1.** (Singh et al., 2013)

Table 1.1 : ICH guidelines on stability testing

Guideline	References
Q1A	*"Stability Testing of New Drug Substances and Products (Second Revision)"*
Q1B	*"Stability testing: Photostability Testing of New Drug Substance sand Products"*
Q1C	*"Stability testing of New Dosage Forms"*
Q1D	*"Bracketing and Matrixing Designs for stability testing of Drug Substances and Products"*
Q1E	*"Evaluation of stability data"*
Q1F	*"Stability data package for Registration Applications in Climatic Zones III and IV"*
Q5C	*"Stability Testing of Biotechnological/Biological Products"*

1.2 Drug Excipient incompatibility

Choice of excipients is very important during the design of drug product. Excipients and their concentrations in a drug formulary are carefully chosen grounded on their functional behavior and similarly on the compatible nature amongst the excipients and Active pharmaceutical ingredient. (Narang et al. 2009).Unsuitability can be situated well-defined as an adverse drug interface with other mechanisms of a preparation which might result in variations to its physical, biochemical and healing be longings of the dose form (Narang et al. 2009).It is always essential to evaluate the risk condition, whether this interface would diminish the bioavailability of a medicine substance. Unintentional physical and chemical interface of an excipient with drug can outcome in the complexation or also can effect binding properties of the drug. Drug and excipient binding properties in the dosage form can disturb in-vitro drug proclamation in the dissolution medium. Huang et al. reported a strong ionic interface among croscarmellose sodium and metformin (Huang et al. 2006). This lead to frame-up of metformin in the matrix of croscarmellose sodium, important to reduced diagnostic properties. It stood overcomes by the use of arginine, which is a tougher binder, representative the interaction was reversible and ionic.

1.3 Accelerated Stability Testing

Accelerated stability instructions examine the stability of the drug product or drug substance at unsafe temperatures to estimate constancy below long-term storage situations at lower temperatures. This method offers significant advantages in scientific decision-making during the development of drug materials and drug products. (Huynh-Ba, 2008). Accelerated stability is used across the pharmaceutical industry and had direct exposure across the global regulatory agencies. The notion is suggested as an enhancement on practical working practices for the assignment of retest periods for intermediate and starting materials and as essential knowledge of the stability of a product. In this method samples are stored under various conditions of humidity and temperature determined by experimental design. Alterations in the characteristic of interest (impurity level, an assay of drug substances, efficiency of dissolution) are monitored by periodic sampling over the course of the study, and the output is modeled to provide e a long-term stability prediction.

(Huynh-Ba, 2008; Yoshioka and others, 2000). According to ICH guidance document accelerated testing is defined as "*studies designed to increase the rate of chemical degradation or physical change of an active drug substance or drug product by using exaggerated storage conditions as part of the formal, definitive, storage programme*." During these studies, it uses as the accelerated condition sample storage duration of 6 months below 40ºC ± 75% Relative Humidity (RH). *"A drug product that is stable at 37-40°C for three months and 75% or higher relative humidity may be given a tentative expiry period of two years from the date of manufacture, according to the FDA"*. (Huynh-Ba, 2008; Yoshioka and others, 2000).

Many faster stability testing copies are originated on the Arrhenius equation i.e.

'$K = Ae^{-Ea/RT}$'

Where,

K = Degree of rate constant

A = Frequency factor (Frequency of collision among rectants)

R = Gas Constant (8.314 J mol^{-1})

T = Temperature (K)

The Arrhenius equation describes the relationship between the rate of degradation with storage temperature to predict stability. It is said that 24-month shelf-life is grounded on long-term twelve-month stability or 6-month data at accelerated stress. (Baertschi et al., 2011).

1.4 Microwave

1.4.1 Heating Principle

Microwave chemistry was initially used primarily to perform several process such as ashing, fat analysis, digestion and protein hydrolysis. The electromagnetic radiation region, located between infrared radiation and radio waves, is used to heat the sample. In general very local and engineering warm devices intended for heating system are regulated by a frequency of 2.455 GHz, corresponding to a wavelength of 122 nm, which reflects to an energy of

approximately 10-5 eV of the microwave photon that cannot break any chemical bonds. (Gaba et al., 2011).

Two mechanism of heating are mainly involved in microwave irradiation.

1) Dipolar Polarization

The ideal material for the dipolar polarization method is polar molecules. It must have a dipole moment (like water molecule) in order for a material to produce heat when exposed with the microwave. A dipole is sensitive to the electric field is applied and will attempt to align itself by rotation with the field. (Lidström et al., 2001)

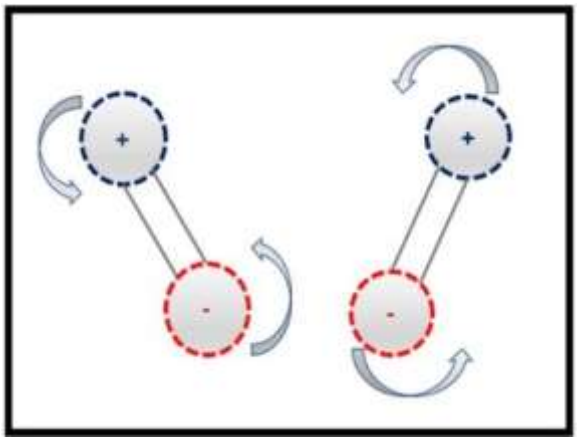

Figure1.1 Dipolar polarization in molecules

2) Conduction Mechanism

Solution which contains ions or even a single isolated ion with hydrogen bond, in the sample will interchange through the solution below the consequence of an electric field, which will result in the outflow of energy due to an increased collision rate, which will eventually convert the kinetic energy into heat. (Kommanaboyina, 1999)

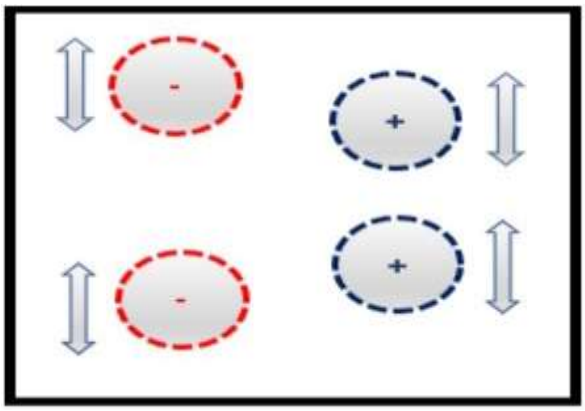

Figure 1.2 Conduction mechanism in molecule

1.4.2 Advantages of microwave heating

The microwave is an efficient source of energy and process being convenient due to non-contact heat source. (Taylor et al 2005; Wagner, 2006)

1) Efficient source of heat
2) Reproducibility
3) Selective heating
4) Eco – friendly
5) Higher reaction rate and percentage yield
6) Uniform heating

1.4.3 Microwave Apparatus

In the previous, near all the responses by warms were done using local ovens, which usually do not let temperature change throughout the degradation procedure and are non to be used for technical tests. The outcomes stayed majorly dangerous due to the fast heating system of organic solvents below locked container circumstances. In a local heat oven, the degradation power is usually maintained by series of the magnetron, and it is virtually not accessible to screen the response temperature in a dependable means. (Wagner, 2006)

Figure 1.3. demonstrate schematic diagram of the microwave apparatus. It consists of magnetron which produces microwave that radiates from the antenna into the waveguide. The wall of reflective material that directs microwave into the oven cavity. The stirrer and turntable on which samples are rotated ensure that field distribution is as homogenous as possible. The wall of the cavity is made of reflective material which inhibits microwave leak and increases the cavity's productivity. The vessel is placed into microwave path and bottom part is heated. The upper part of the vessel is out of microwave field which remains cool, providing the conduction mechanism. (Kappe, 2003; Kuhnert, 2002)

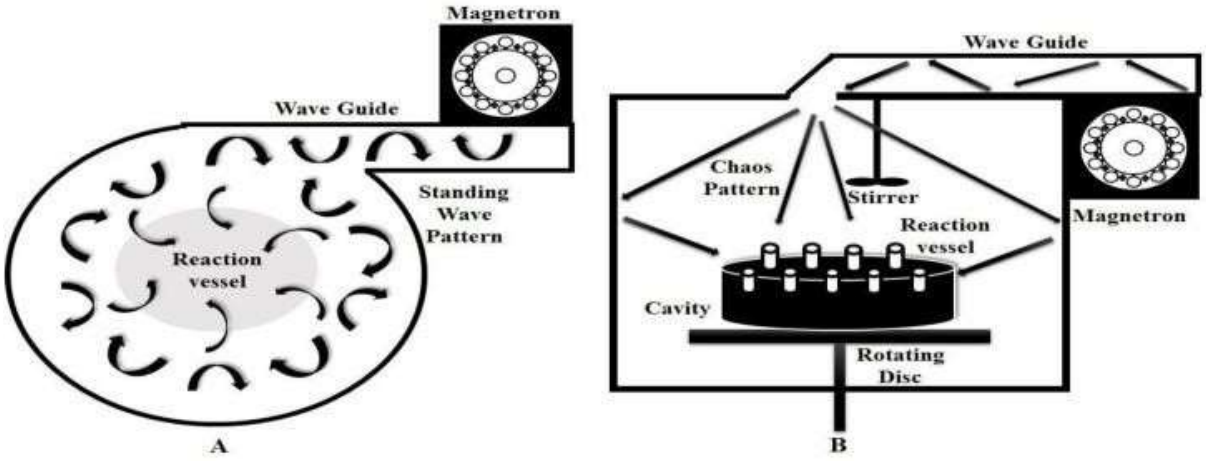

Figure 1.3 (A) Schematic Diagram of Mono – mode of Microwave

(B) Schematic Diagram of multi – mode microwave

Presently, two dissimilar viewpoints by esteem to microwave reactor design are developing: multimode and mono mode (single mode) reactors. The mono mode apparatus is having the capacity to generate a standing wave pattern. As only one container is exposed at a time, better control of the reaction parameter. The desired temperature is achieved faster and after completion, the response mix is fast chilled due to the built-in cooling system. The single-mode apparatus has become more user-friendly and commonly applied for small scale discovery. While in multimode apparatus, a standup trend form is deliberately avoided. Here, the goal is to create chaos inside the apparatus. Better the chaos, advanced spreading of microwave energy resulting in effective heating inside the apparatus. Fundamentally, here are now four gadget producers that yield microwave reactors for research laboratory gage organic fusion.

1.5 Drug profile for Metoprolol Tartrate

Metoprolol Tartrate is a Cardio selective beta-1 adrenergic receptor antagonist. It is mainly used for the dealing of hypertension, angina pectoris and myocardial infarction.

- **Mechanism of action:**

Metoprolol tartrate is a Beta – 1 Cardio selective adrenergic receptor blocker. This preferential result is not comprehensive, however, and at higher plasma

concentrations. It also helps in inhibiting Beta - 2 Adrenoreceptors, which are present in the bronchial and vascular musculature. Pharmacology examines have confirmed the beta-blocking activity of Metoprolol tartrate.

(1) Reducing heart rate and cardiac output
(2) Reducing systolic blood pressure
(3) Inhibiting isoproterenol induced tachycardia
(4) Reducing reflex tachycardia.

- **General name:** Metoprolol Tartrate
- **Chemical structure:**

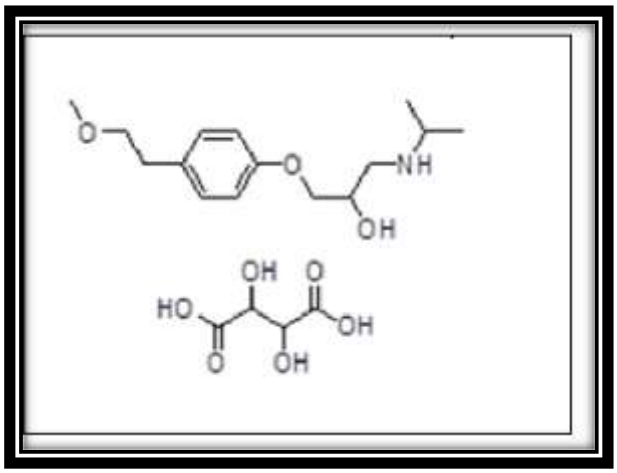

Figure 1.4 Chemical Structure of Metoprolol Tartrate

- **IUPAC Name:** (2R, 3R) – 2,3 – di hydroxy butanedioic acid; bis (1-[4-(2-methoxyethyl) phenoxy] – 3 – [(propan – 2 – yl)amino]propan – 2 – ol)
- **Molecular Formula:** $C_{34}H_{56}N_2O_{12}$
- **Molecular Weight:** 684.84 g/mol
- **Melting Point:** 120°C
- **Description:** White powder
- **pKa:** 14.09(Strongest Acidic) , 9.67(Strongest Basic)
- **Solubility:** Soluble in water, methanol, chloroform, ethanol and Dimethyl sulfoxide
- **Storage:** Protected from light
- **Drug Category:** Beta 1 blocker

- **Clinical Pharmacology:** Metoprolol Tartrate is a Cardio selective competitive beta-1 adrenergic receptor blocker with antihypertensive efficacy and lacking of sympathomimetic activity. It antagonizes beta-1 adrenergic receptors in the myocardium, which reduces the rate of myocardial contraction, and a diminished cardiac output. This drug will also help to reduce the secretion of renin with reduction in levels of angiotensin II thereby decreasing sympathetic activation, vasoconstriction and aldosterone secretion.
- **Dose and Administration:** The usual initial antihypertensive oral dose of Metoprolol tartrate is prescribed100 mg, and the maximum dose is 450 mg either single or divided.

Chapter-2

Literature review

2. Literature review

Several stability indicating High Pressure Liquid Chromatographic methods of Metoprolol Tartrate are summarized in the following section.

Table 2.1 summary of reported stability-indicating HPLC method for the estimation of Metoprolol Tartrate

Sr. No	Drug	Chromatographic Conditions				Reference
		Column	Mobile Phase	Flow Rate	Wave length	
1	Metoprolol Tartrate tablets	C_{18} with 150mm width, I.D. 4.6mm, 5.0 um particle size	0.06M ammonium acetate and acetic acid pH – 3.7	1mL/min	280 nm	(R. B. Reddy, More, Gupta, Jha, & Magar, 2016)
2	Metoprolol Tartrate tablets	Agilent 1260, thermostatic 25cm C_{18} column	10mM Phosphate buffer in combination with acetonitrile (50:50) pH – 3.0	1mL/min	235 nm	(Mabrouk, Hammad, El-malla, &Elshenawy, 2019)
3	Metoprolol Tartrate salt	PFP ACE® C_{18} with 150mm width, 4.6mm I.D , 5um size	Acetonitrile: Water (90:10) formic acid 0.1%(v/v)	0.7mL/min	222 nm	(Domingues & Silvestre, 2016)
4	Metoprolol Tartrate API	C_{18} with 250mm width, I.D. 4.6mm ,5.0 um particle size	10mM Acetate buffer:Acetonitrile (60:40)	0.8mL/min	222 nm	Y. Gao, Zhang, Li, Tian, &Gao, 2020)
5	Metoprolol Tartrate salt	Cogent Bidentate C_{18} with 50mm width, 2.1mm I.D, 4um size	Acetonitrile – Water (90:10) acidified with formic acid	0.8mL/min	222 nm	(Koba, Golovko, Kode,

			0.1%(v/v)			&Grabic, 2016)
6	Metoprolol Tartrate API	Agilent ZORBAX XDB C_{18} with 150mm width, 4.6mm I.D , 5um size	Acetonitrile: Water: Trifluroacetic acid (15:66:19)	0.8mL/ min	Excitation – 216 Emisssion - 312nm	(Xu et al., 2013)
7	Metoprolol Tartrate tablets (50mg)	C_{18} with 250mm width, I.D. 4.6mm ,5.0 um particle size	Acetonitrile – Water (90:10) acidified with formic acid 0.1%(v/v)	0.8mL/ min	222 nm	(Yang et al., 2010)
8	Metoprolol Succinate tablets (50mg) and Telmisartan tablets (40mg)	HiQ Sil C_{18} with 250mm width, 4.6mm I.D and 5um particle size	Methanol: 10mM Pottasium phosphate buffer: 10mM hexane sulphonic acid (80:10:10)	1mL/min	223 nm	(Taylor, n.d.)
9	Metoprolol Succinate tablets (50mg)	Acquity UPLC HSS T3 with 100mm width, 2.1mm I.D and 1.8um particle size	Sodium Hydrogen Phosphate : Acetonitrile (95:5)	0.5mL/ min	232 nm	(Shitole et al., 2014)
10	Metoprolol API	Supelcosil™ LC-18 with 150 mm width, 4.6mm I.D and 3um size	Acetonitrile – Water (90:10) acidified with formic acid 0.1%(v/v)	1.0mL/ min	223 nm	(Apci-ms, Johnson, & Lewis, 2006)
11	Metoprolol Succinate pure form	C_{18} with 250mm width, I.D. 4.6mm, 5.0 um particle size	20mM Ammonium formatebuffer : methanol (80:20) pH – 3	1mL/min	232 nm	(Borkar, Raju, Srinivas, & Patel, 2012)

12	Metoprolol API	C_{18} ODS – 3(5um) with 150mm width and 4.6mm I.D.	25mM Na_2HPO_4 :1-butanol:TEA (93:6:1) pH – 3	2mL/min	Excitation -230 Emmision – 311	(Soltani, n.d.)
13	Racemic Metoprolol solution	Chiralpak® with 250mm width, 4.6mm I.D and 10um size	Hexane:Ethanol:isopropanol:diethylamine (88:10:1:1)	1.2mL/min	232 nm	(Antunes et al., 2013)
14	Metoprolol Tartrate API	Atlantis C_{18} with 50mm width, 4.6mm I.D and 3mm size	Acetonitrile:Water with 0.2% formic acid (75:25) pH-3	1.0mL/min	235 nm	(R. Reddy, Ramesh, & Seshagirirao, 2013)
15	Metoprolol Tartrate API	Chiralcel with 250mm width, 4.6mm I.D and 8um size	Diethylamine:1-propanol:hexane (25:25:50)	0.25 – 1.75 ml/min	Excitation – 272nm Emmision – 306 nm	(P. Chemistry, 1991)
16	Metoprolol Tartrate Tablet	Ultimate XB C_{18} with 150mm width, 2.1mm I.D and 5um size	Methanol:Water with 0.1% formic acid (65:35)	0.2mL/min	235 nm	(Li et al., 2012)
17	Racemic Metoprolol Tartrate	Chiral – AGP with 100mm width, 4mm I.D and 5um particle size	Phosphate buffer:Acetonitrile (95:5)	0.5ml/min	Excitation – 228nm Emission – 272nm	(B. Chemistry & Chemistry, 1990)
18	Metoprolol Tartrate API	Agilent HC – C_{18} with 250mm width, 4.6mm I.D and 5mm particle size	Methanol:Water containing 0.1% formic acid (39:61)	1.0mL/min	223 nm	(Ma et al., 2016)
19	Metoprolol	Venusil MP – C_{18} with	Methanol:Ammoniu	0.8mL/	235nm	(F. Gao et

	Tartrate API	100mm width, 4.6mm I.D and 5um particle size	macetate (50:50)	min		al., 2010)

Table 2.2 Reported analytical methods for microwave-assisted heating in forced degradation study of pharmaceuticals

Drugs and its Formulations	**Conventional Forced Degradation**				**Microwave – Assisted Degradation**				**Reference**
	Stress Conditions		**% Degradation**		**Stress Conditions**		**% Degradation**		
	Strength Of	Temp	Time		Strength of	Temp	Time		
Imatinib Mesylate	Acid	NR	NR	NR	2M HCL	15 s/ Cycle	15 min	5 DP	(Bende et al., 2007)
	Base	NR	NR	NR	2 M NaOH	2.45 GHz	15 min	5 DP	
	Neutral	NR	NR	NR	Neutral	300 W	15 min	5 DP	
	6% H_2O_2	25 ºC	12 hr	NR/3DP		80%			
	Thermal	90 ºC	12 hr	NR/1DP					
Tolterodine Tartarate	1N HCl	80 ºC	12 hr	7DP/0.5 %	1 N HCl	15 s/cycle	5 min	9DP/0.2%	(Madhavi et al., 2008)
	1N NaOH	80 ºC	12 hr	7DP/34.7%	1 N NaOH	2.45 GHz	15 min	5DP/32.3%	
	Neutral	80 ºC	12 hr	5DP/0.2 %	Neutral	300 W	15 min	4DP/0.6%	
	6% H_2O_2	RT	48 hr	2DP/0.6 %	6% H_2O_2		15 min	5DP/0.3%	
	Thermal	60 ºC	10 Days	ND					
Rebamipide in bulk	0.5N NaOH	Reflux	1 hr	2DP/8 %	0.55 N NaOH	525 W	6.7 min	2DP/8.74%	(Sonawane et

and Tablet form	Acid	NR	NR	ND	Acid	NR	NR	ND	al., 2011)
	Neutral	NR	NR	ND	Neutral	NR	NR	ND	
	30% H_2O_2	RT	24 hr	ND					
	Thermal	80 ºC		ND					
Indomethacin	0.1 M HCl	RT	72 hr	24%	0.1 M HCl	60 ºC	1 min	27%	(Prekodravac et al., 2011)
	0.1 M H_2SO_4	100 ºC	30 min	24%					
	0.1 M NaOH	100 ºC	120 min	7%					
Losartan Potassium and Ramipril Tablets	Acid	NR	NR	NR	2 M HCl	15 s/cycle	5 min	ND	(Kollipara et al., 2012)
	Base	NR	NR	NR	2 M NaOH	2.45 GHz		1DP of LS	
	Neutral	NR	NR	NR		300W		2DP of RS	
	3% H_2O_2	25 ºC	12 hr	3DP	Neutral			ND	
	Thermal	90 ºC	12 hr	NR					
Levamisole	0.1 M NaOH	100 ºC	150 min	17%/2DP	0.1 M NaOH	100 ºC	150 min	73.6%/2DP	Da Silvaet al., 2013)
		100 ºC	120 min	41.38%		100 ºC	30 min	19.3%	
		130 ºC	15 min	41.38%		100 ºC	60 min	24%/2DP	
Cephalosporin oral dosage form	Acid	NR	NR	NR	Cefixime				
	Base	NR	NR	NR	0.1N HCl	210 W	2 min	5.7%	Sonawane et al., 2013)
	Neutral	NR	NR	NR	0.3N HCl	245 W	2 min	16.4%	

		0.3N HCl	280 W	2 min	20.6%	
		0.01 N NaOH	210 W	20 sec	7.3%	
		0.03 N NaOH	245 W	20 sec	16.4 %	
		0.03 N NaOH	280 W	30 sec	21.0 %	
		Cefadroxil				
		0.1N HCl	210 W	2 min	10%	
		0.3N HCl	245 W	2 min	14.5%	
		0.3N HCl	280 W	2 min	21.5%	
		0.01 N NaOH	210 W	20 sec	15.0%	
		0.03 N NaOH	245 W	20 sec	16.4%	
		0.03 N NaOH	280 W	30 sec	24.5%	
		Cefpodoxime				
		0.1N HCl	210 W	2 min	43.1%	
		0.3N HCl	245 W	2 min	60.6%	
		0.3N HCl	280 W	2 min	98.3%	
		0.01 N NaOH	210 W	20 sec	56.9%	
		0.03 N NaOH	245 W	20 sec	87.0%	

					0.03 N NaOH	280 W	30 sec	99.4%	
Venlafaxin in bulk and Capsule Formulation	0.1 N HCl	Reflux	1 hr	21.2%	0.1 N HCl	490 W	10 min	18.15 %	(Prajapati et al., 2015)
	0.5 N NaOH	Reflux	2 hr	24.52 %	0.5 N NaOH	560 W	7 min	19.25 %	
	Wet heat	Reflux	6 hr	ND					
	30% H_2O_2	RT		ND					
	Dry heat	80 ºC	48 hr	ND					
Atenolol		40 ºC	>67 2 hr	2.3 – 15% Amide Product		120 ºC	NA	2.3-15% Amide Product	(Sonawane et al., 2016)
		80 ºC	15 hr			180 ºC	0.05 hr		

Chapter-3

Aim and Objective

3. Aim and Objective

3.1 Aim

A main task in the pharmaceutical engineering is a betterment in explore and development productivity. The stability studies are carried out usually for the confirmation of the product's quality. But these studies are taking considerable time and thus accelerated stability study are approved out at increasing temperature and moisture conditions to increase the rate of reaction. However, the accelerated stability studies carried at 40 ºC ± 75% RH required 6 months to predict the drug degradation behavior.

Since several years, microwave heating principle is known to increase the rate of reaction in synthetic chemistry. Instead of conventional ovens or water baths, microwave reaction can act as a very useful heating source leading to a reduction in the time of reaction during stability studies. The literature regarding the use of microwave degradation in forced degradation study is infrequent. Furthermore, there is no literature reported on the use of microwave heating for accelerated stability studies.

Hence, it was hypothesized that the use of microwave heating to perform accelerated stability studies replacing the conventional method can eliminate the time period in evaluating drug product stability.

So, the purpose of the current project was to perform accelerated stability studies of metoprolol tartrate using conventional and microwave -assisted methods and to compare the resulting degradation profiles to evaluate the probability of the use of the microwave for stability studies.

3.2 Objective

- To perform accelerated stability examination of Metoprolol tartrate by microwave-assisted heating technique.
- To perform accelerated stability examination of Metoprolol tartrate by the conventional method using a stability chamber.
- To compare microwave-assisted accelerated stability data of Metoprolol tartrate with conventional accelerated stability data. .

Chapter-4

Experimental work

4. Experimental work

4.1 Chemicals and materials

HPLC grade ammonium acetate, Milli Q water, acetonitrile, ortho- phosphoric acid, glacial acetic acid, and 0.45 µ PVDF filter were procured from Merck (Mumbai, India).

The (API) of Metoprolol tartrate was a sample gifted from the torrent research center, Ahmedabad.

The PVP K30 was purchased from Sisco research Laboratories (SRL).

4.2 Instruments and equipment

All the necessary equipment and instruments used during the project were calibrated from time to time according to in house SOP of Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University.

Below is the list of instruments used during the project:

- ***Melting point apparatus***: T0603160; EIE Instruments Pvt. Ltd., Ahmadabad, India Analytical balance: CX220, Citizen, USA.
- ***Ultrasonicator*:** D-compact, EIE instrument Pvt. Ltd., Ahmedabad, India. Digital pH meter: PH-MV-TEMP. Meter, PH -206, LTLUTRON, Taiwan.
- ***UV-visible spectrophotometer*:** UV1800 UV-Visible NIR-specrophotometer, Jasco with Software: Spectra Manager
- ***Fourier Transform Infrared spectrometer (FT-IR)*:** Jasco 6100 Japan with Spectramanger software
- ***Water bath*:** EIE 406, EIE, instrument Pvt. Ltd., Ahmadabad India
- ***Hot air oven*:** EIE 108, EIE Instruments Pvt. Ltd., Ahmadabad India
- ***Stability Chamber*:** Emcure Pharmaceutical Pvt. Ltd.
- ***High-Performance Liquid Chromatography (HPLC)*:**
  Jasco(Japan), LC-2000 Plus series fortified by a PDA detector. Data collection and analysis were performed using Jasco Borwin Version 1.50 software.
- ***Microwave synthesizer*:** Anton Paar Synthos 3000

4.3 Identification of drug

The following techniques were used for the identification of Metoprolol tartrate:

1) Melting point

2) UV-Spectroscopy

3) FT-IR Spectroscopy

4.3.1 Melting point

The melting point of Metoprolol tartrate was performed through an open capillary method where liquid paraffine was used as a heating medium.

Drug	Reported melting point (°C)	Observed melting point(°C)
Metoprolol tartrate	120˚C	119-121˚C

Table 4.1 Reported and observed melting point for Metoprolol Tartrate

4.3.2 UV – spectroscopy

The UV spectrum of Metoprolol tartrate (25 µg/mL) in distilled water was recorded by using UV-Visible spectrophotometer. The scanning range of the sample was 200-400 nm where distilled water was used as blank.

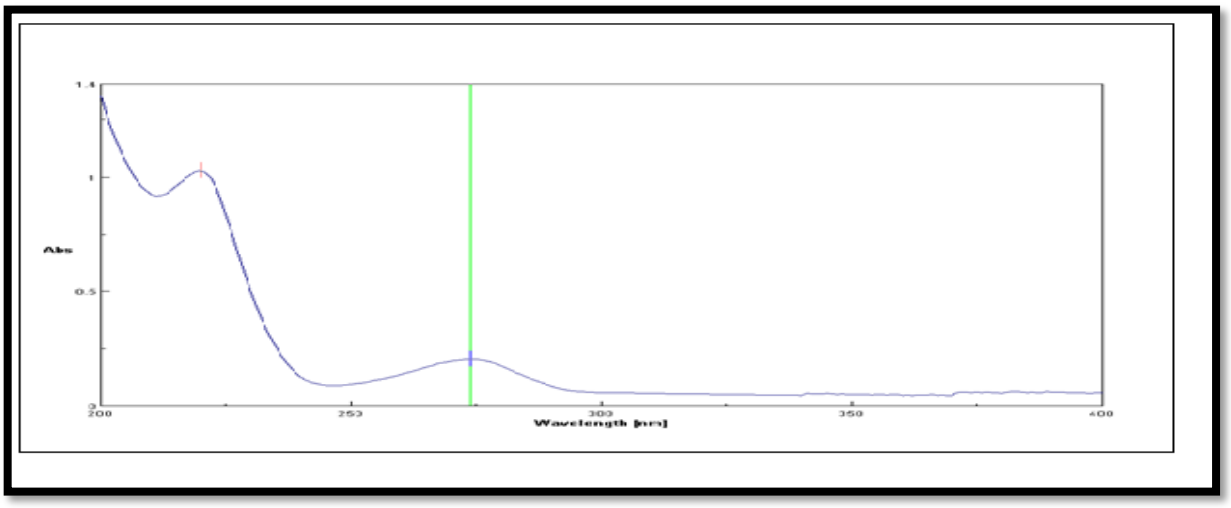

Figure 4.1 UV-Spectrum of Metoprolol Tartrate (25 µg/mL in distilled water)

Drug	Reported λ max	Observed λ max
Metoprolol tartrate	274nm 221nm	274nm 220nm

Table 4.2 Comparison of reported and observed λ_{max} of Metoprolol Tartrate

4.3.3 FT-IR Spectroscopy

The FT-IR spectrum of Metoprolol tartrate was noted in the array of 400-4000 cm^{-1}. Obtained spectrum was compared with the reported spectrum of Metoprolol tartrate. Theoretical values of wave numbers responsible for functional groups are compared with observed values of wave numbers.

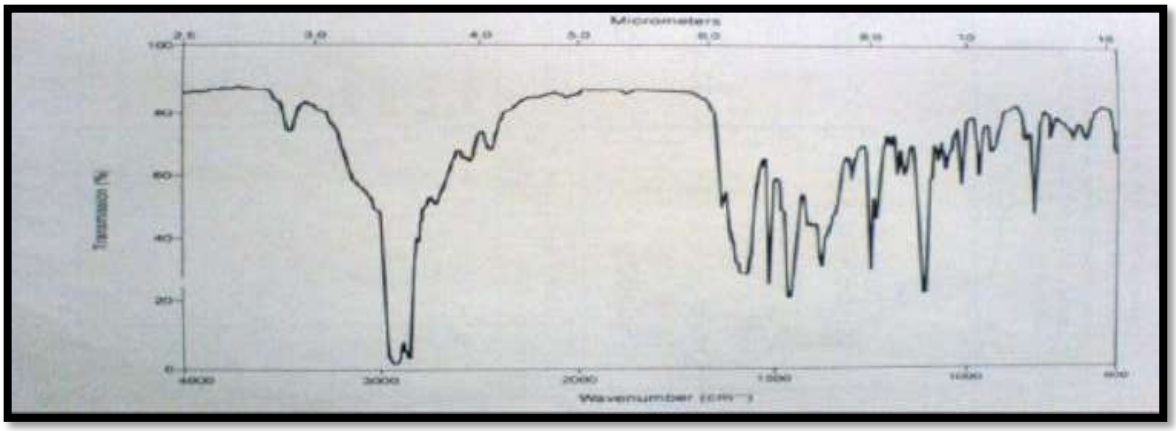

Figure 4.2 Reported FT-IR spectrum of Metoprolol Tartrate

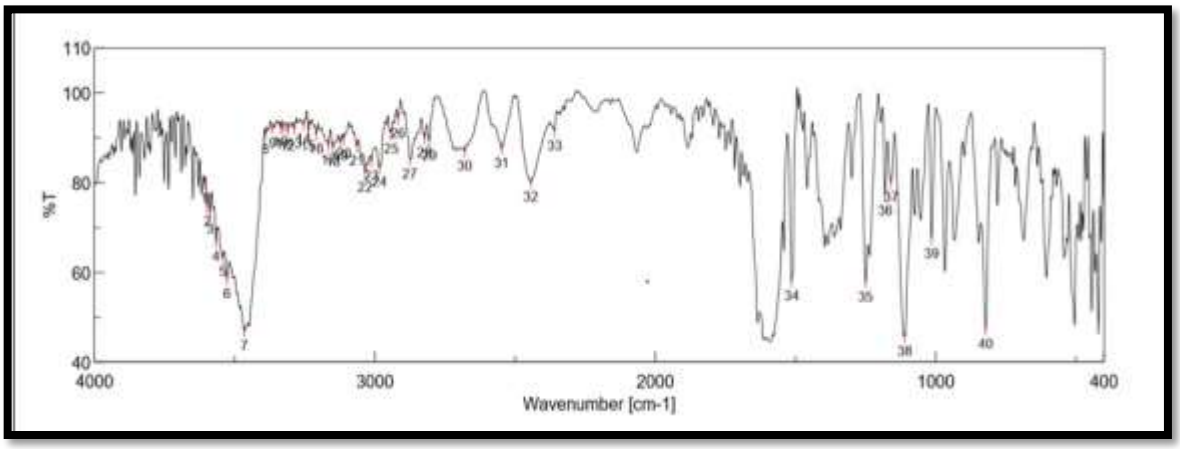

Figure 4.3 Observed FT-IR spectrum of Metoprolol Tartrate

Reported wavenumber (cm-1)	Observed wavenumber (cm-1)	Assignment
3600-2300	2985.27	Aliphatic and Aromatic CH
1580	1580.24	Aromatic ring
1015	1012.67	Aromatic ether
1180	1177.53	Isopropyl group
1100	1102.34	Aliphatic ether

Hence from the results, it was identified that the obtained gift sample was Metoprolol tartrate.

4.4 Preliminary trials on hot air oven

4.4.1 Hot air oven conditions

The samples for trials of hot air oven conditions include,

1. Metoprolol Tartrate API
2. A mixture of Metoprolol Tartrate and PVP K30 (1:1)

Each sample was transferred individually into a Petri plate. Further, 25% w/v of HPLC grade water was added and mixed well. All the models were kept in a hot air oven at 100 °C. The samples were analyzed at a defined time point after 24, 48 and 72 hrs. They were analyzed by UV – visible spectrophotometry.

4.4.2 Sample preparations

After collection from hot air oven, each sample was prepared by the following method for sample analysis: Accurately weighed 10 mg of drug and drug mixtures corresponding to 10 mg of the standard drug were added to 10 mL flasks separately. About 5 mL of distilled water was added and sonicated for 15 minutes. Then capacity was made equal to 10 mL with

diluent to get a solution of 1000 µg/mL. A solution comprising 100 µg/mL was ready by further concentrating 1mL of the above solution with 10 mL of diluent. Aliquot 2.5 mL was moved to 10 ml flask and the final concentration was made 25 µg/mL. Each sample was analyzed by UV – visible spectrophotometry

4.5 Accelerated stability studies

Accelerated stability study remained carried out through a conventional method by means of stability chamber and microwave-assisted technique using microwave synthesizer.

The samples for accelerated stability study include:

1. Metoprolol Tartrate API
2. A mixture of Metoprolol Tartrate and PVP K30 (1:1)

4.5.1 Preparation of samples for Accelerated stability study by a conventional method

For accelerated stability study, all four samples were kept in a different glass bottle. Each bottle was kept in the stability compartment for 6 months at '40ºC ± 75 % RH' as per ICH Q1A guideline. The samples were collected at definite time interval which being0 to 6 months and analyzed by HPLC.

4.5.2 Preparation of samples for Accelerated stability study by microwave-assisted method

For microwave accelerated stability study, all four samples were taken in different microwave vials and about 50µl & 80µl water was added and mixed well. Each vial was placed into microwave synthesizer at different power, temperature and time.

Sr. No	Time (min)	Water (µl)	Power (Watt)	Temperature (°C)
1	5	20	400	80
2				90
3				100
4	5	20	600	80
5				90

6				100

Table 4.4 Trial 1 for optimization of temperature

Sr No	Time (min.)	Water (µl)	Power (Watt)	Temperature (°C)
1	30	30	400	80
2				90
3				100
4	30	50	600	80
5				90
6				100
7	30	50	200	80
8				90
9				100
10	30	80	400	80
11				90
12				100
13	30	80	600	80
14				90
15				100

Table 4.5 Trial 2 for optimization of time

4.5.3 Sample preparation for HPLC

After the collection of samples from the stability chamber or microwave synthesizer, each sample was prepared by the following method for sample analysis.

A.) Sample preparation of MET stock solution

Accurately weight 20 mg of MET and then transferred to the 10 mL volumetric flask. About 5 mL of diluent (2:8 ACN: water) were added and subsequently sonicated for 15min. Finally, water was added up to the mark (2000 µg/mL).

B.) Sample preparation of MET with PVP K30 stock solution

Accurately weighed 20 mg of MET and excipient mixtures equivalent to 20 mg of the standard drug were transfered into 10 mL volumetric flask. Then 5 mL diluent was additional and sunsequently sonicated for 15 min. Water was added up to the mark (2000 µg/mL).

4.6 Chromatographic conditions of samples

Throughout the HPLC analysis chromatographic conditions were:

- **Column:** Waters C18, 250nm ×4.6 nm , 5 µm
- **Wavelength:** 280nm
- **Flow Rate:** 1.0ml/min
- **Injected Volume:** 50µl
- **ColumnTemperature:**40°C
- **Run time:** 30 minutes
- **Mobile Phase used:** Dissolve 3.90 g of ammonium acetate in 800 ml of water, then add 2 ml of TEA, 10 ml of glacial acetic acid,3 ml of ortho-phosphorric acid and 156 ml of acetoniitrile and mix.
- **Diluent:** Water: ACN (80:20 %v/v)

4.7 System suitability study

System suitability test of all selected method was done to analyze the resolution and reproducibility of the adopted stability-indicating method, using six replicate injection of working standard solution (µg/mL) Metoprolol tartrate. The limitations measured were peak area, retention time of drug products, asymmetry, and theoretical plates. The system suitability results were discussed in the following section.

Chapter-5

Results and Discussion

5. Result and Discussion

The present work includes Metoprolol tartrate, Metoprolol tartrate API with excipient PVP K30. The preliminary trials were performed in a hot air oven. Further, the study was performed on conventional and microwave-assisted heating method. The results of the study are presented below.

5.1 Preliminary trials on hot air oven

Firstly, the samples from the preliminary trials were placed in a hot air oven. The chemical modification was observed. The physical variation was not seen and chemical variation was measured using the UV- visible spectrophotometer through a reduction in the absorbance.

5.1.1 Physical changes

The samples were withdrawn at an interval of 24 hours up to 72 hours. After 72 hours, no color change was observed in the MET API.

Figure 5.1 Colour change in a mixture of Metoprolol tartrate

5.1.2 UV-Visible analysis

After the collection of the samples at an time period of 24 hours up to 72 hours the absorbance of the samples at every 24 hours decreased. The final concentration of the sample solution was 25 µg/mL.

5.1.2.1 Metoprolol Tartrate

The preliminary trials in hot air oven on Metoprolol tartrate API at 100 °C showd that with an increase in time, a decrease in the absorbance.

Time (Hours)	Absorbance at 221nm	% Of Degradation
Standard	0.762	-
24	0.702	7.84%
48	0.669	12.20%
72	0.619	18.76%

Table 5.1 Degradation of Metoprolol tartrate APIafterhotairovenat100°Cafter24,48and 72hours

5.1.2.2 Metoprolol Tartrate and PVP K30

Time (Hours)	Absorbance at221nm	% of Degradation
Standard	0.699	0%
24	0.347	50.35%
48	0.262	62.51%
72	0220	68.52%

Table 5.2 Degradation of Metoprolol tartrate API with PVP K30 afterhotairovenat100°Cafter24,48and 72hours

5.1.3 Conclusion

The hot air oven trials were carried out on Metoprolol tartrate API and Metoprolol tartrate API with PVP K30 at 100ºC for 72 hours. The samples were withdrawn at 24, 48 and 72 hours and absorbance were measured using UV at 221 nm. From 24 hours to 72 hours, a decrease in the absorbance was observed that signifies substantial degradation. Hence, the samples were further selected for the accelerated stability study.

5.2 Stability study of Metoprolol Tartrate

The optimized HPLC method was used to examines the degradation performance of Metoprolol tartrate below various conditions. The following Figures represent HPLC chromatograms of Metoprolol tartrate standard (figure 5.2) at 2000 µg/mL.

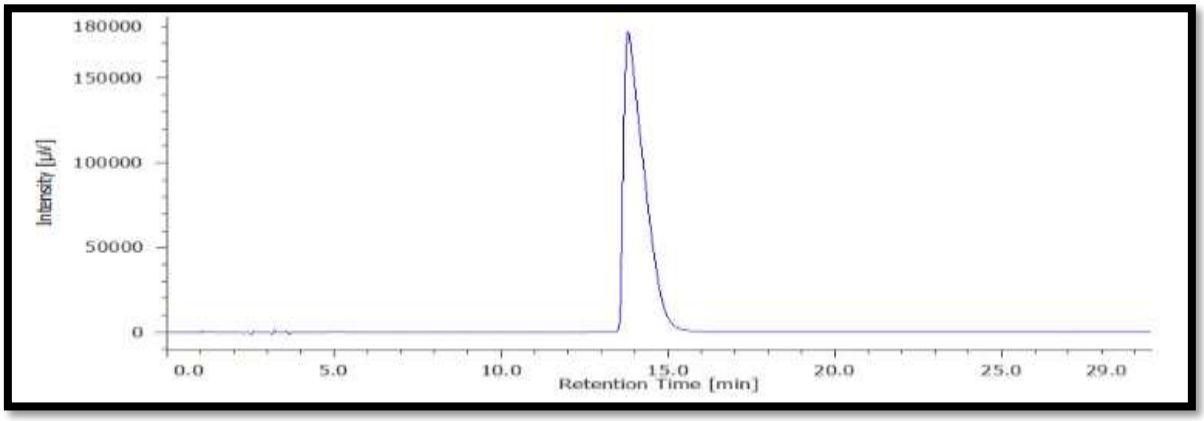

Figure 5.2 HPLC chromatogram of standard Metoprolol tartrate (2000ug/mL)

5.2.1 Linearity of the Metoprolol Tartrate

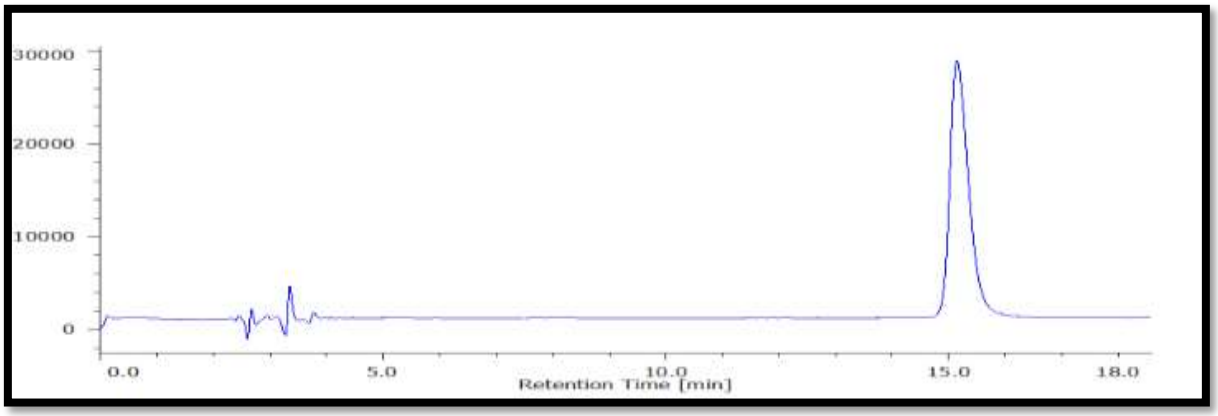

Figure 5.3 HPLC chromatogram of Blank solution (Water : ACN, 80:20)

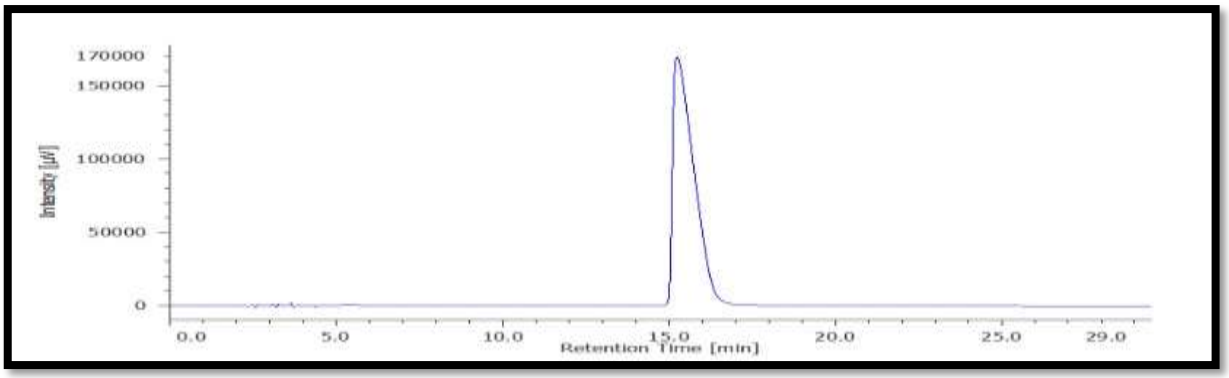

Figure 5.4 HPLC chromatogram of Metoprolol Tartrate (1600 µg/mL)

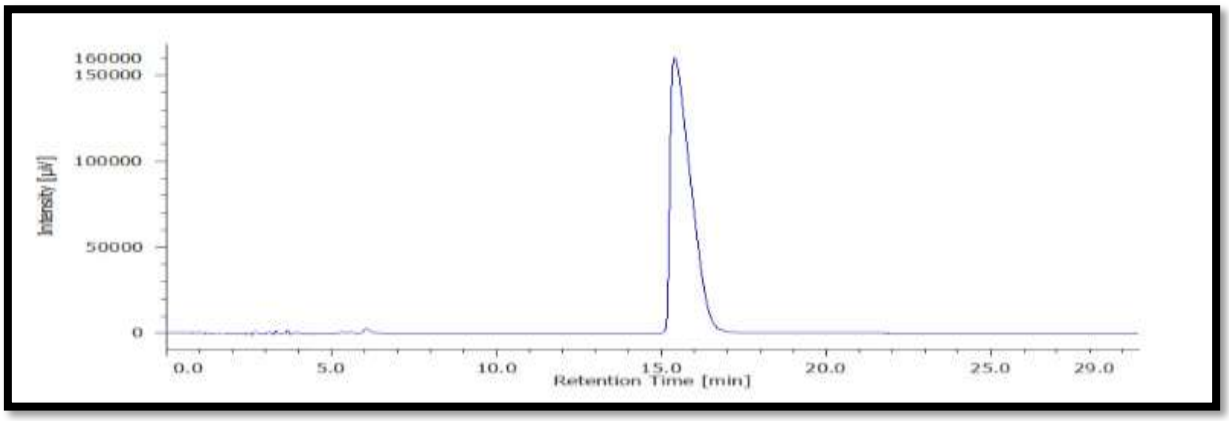

Figure 5.5 HPLC chromatogram of Metoprolol Tartrate (1800 µg/mL)

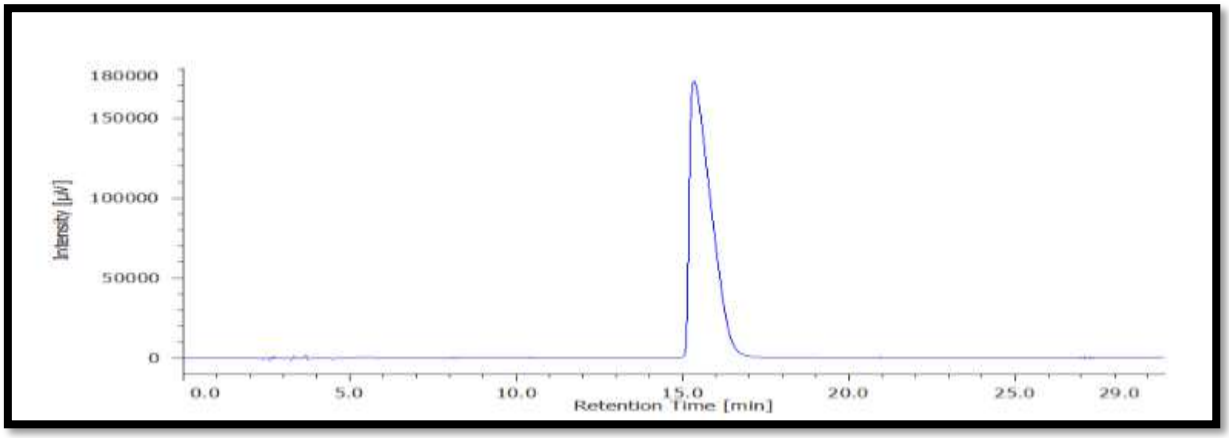

Figure 5.6 HPLC chromatogram of Metoprolol Tartrate (2000 µg/mL)

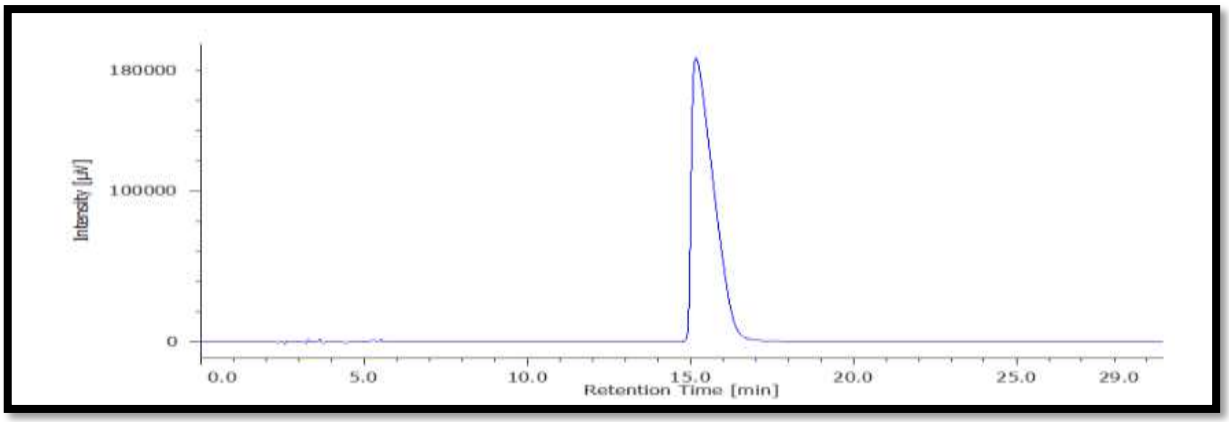

Figure 5.7 HPLC chromatogram of Metoprolol Tartrate (2200 µg/mL)

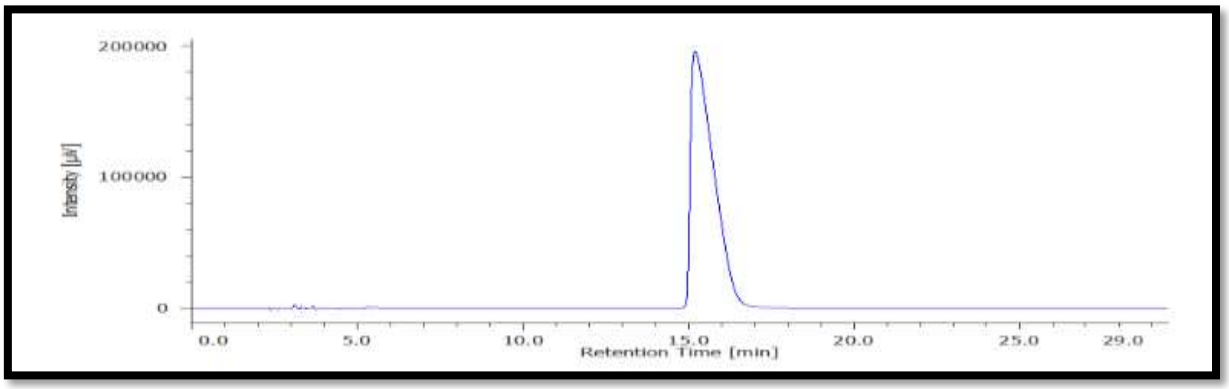

Figure 5.8 HPLC chromatogram of Metoprolol Tartrate (2400 µg/mL)

5.3 Accelerated stability study

All samples were placed in the stability chamber and there accelerated stability study data were generated by preserving the samples for 6 months in a stability compartment at '40ºC ± 75 %'. The samples were composed each calendar month for six months. The important part of this research work is the use of microwave irradiation on the accelerated stability testing of the selected samples and to observe influence on the timescale without affecting stability profile. Degradation product generated by microwave heating and from accelerated stability study were compared by the relative retention time of peak through HPLC chromatogram. Every month samples were collected from the stability chamber and HPLC chromatogram was taken.

5.3.1 Accelerated stability study of Metoprolol tartrate API

In the sample of Metoprolol tartrate API, there was no color change observed after 6 months

Figure 5.9 Observed color change after 6 months for Metoprolol Tartrate

Analysis of HPLC chromatogram of Metoprolol tartrate after 6 months. On 1,2,3 and 6month HPLC chromatogram was taken. The impurities generated in Metoprolol tartrate is less than 0.1% so the impurities were below the acceptance criteria. Hence the drug was stable after accelerated stability study.

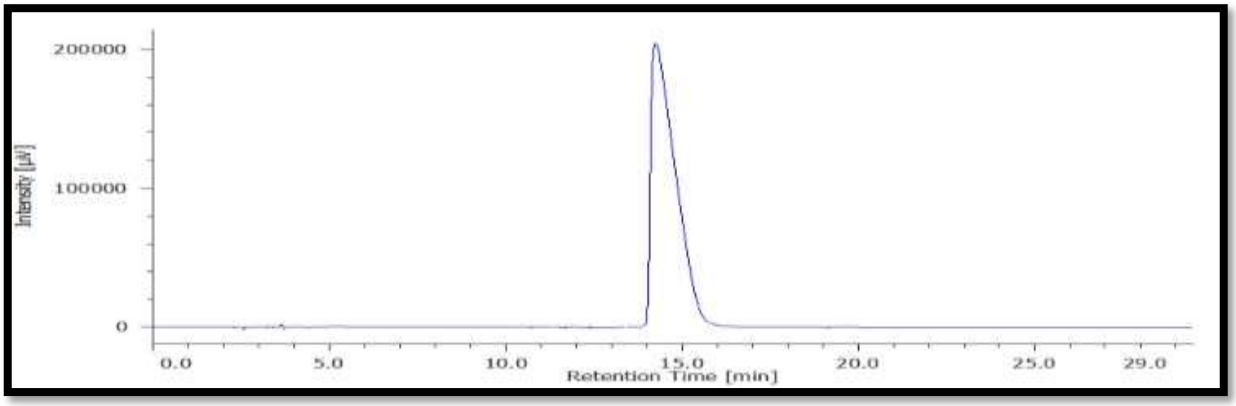

Figure 5.10 Accelerated stability study chromatogram of Metoprolol tartrate(1 month)

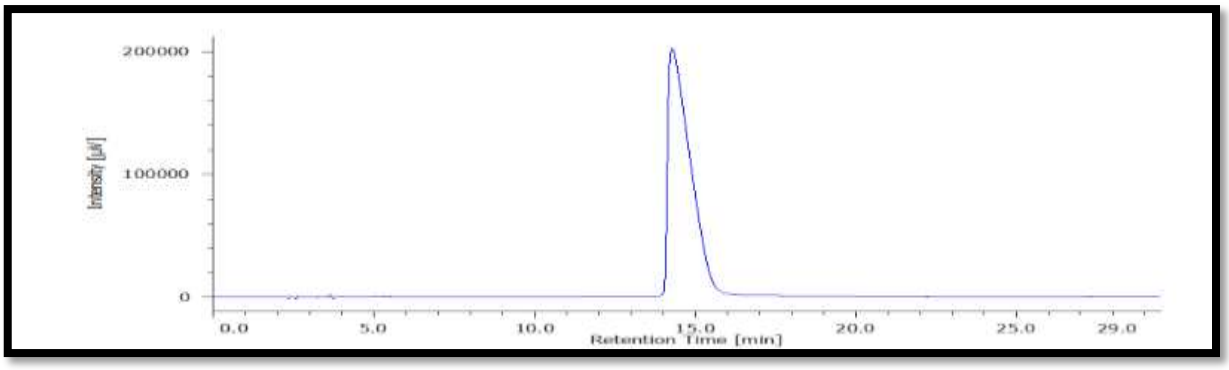

Figure 5.11 Accelerated stability study chromatogram of Metoprolol tartrate (2 month)

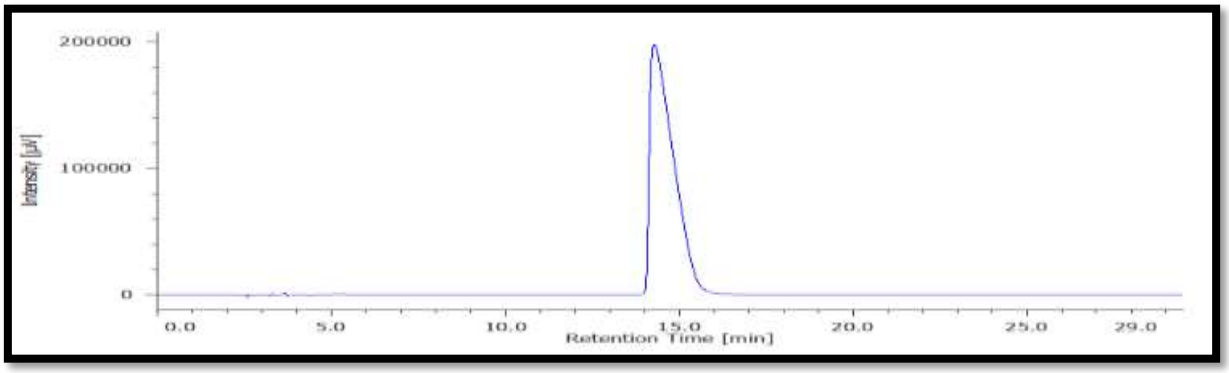

Figure 5.12 Accelerated stability study chromatogram of Metoprolol tartrate (3 month)

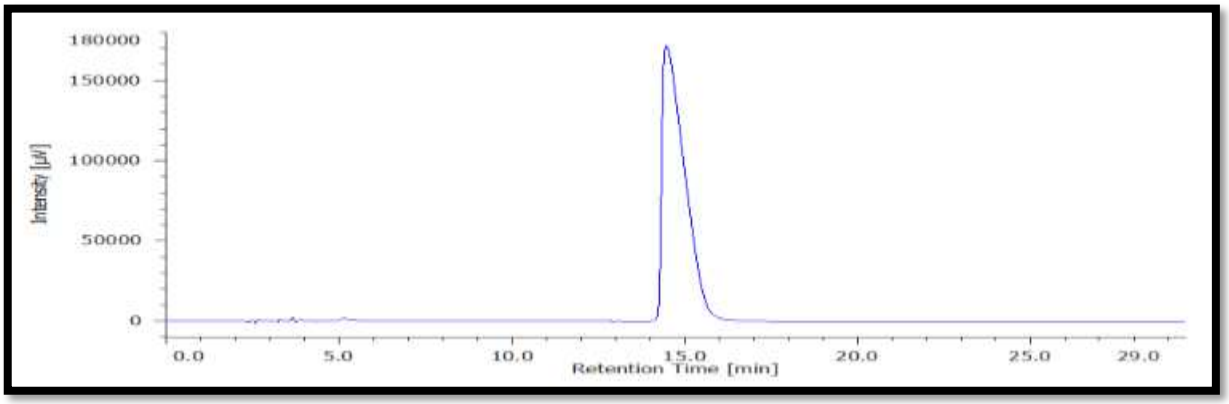

Figure 5.13 Accelerated stability study chromatogram of Metoprolol tartrate (6 month)

5.3.2 Accelerated stability study of Metoprolol tartrate API with PVP K30

The sample mixture of Metoprolol tartrate API, and PVP K30 darkened every month. The light yellow color was observed after 6 months. Figure 5.14 shows the color change observed in the sample at the end of every month. The HPLC chromatogram of the mixture with Metoprolol tartrate API, and excipient after 6 months were carried out. On every month HPLC chromatogram was taken.

Figure 5.14 Observed color change after 6 months for Metoprolol Tartrate and PVPK30

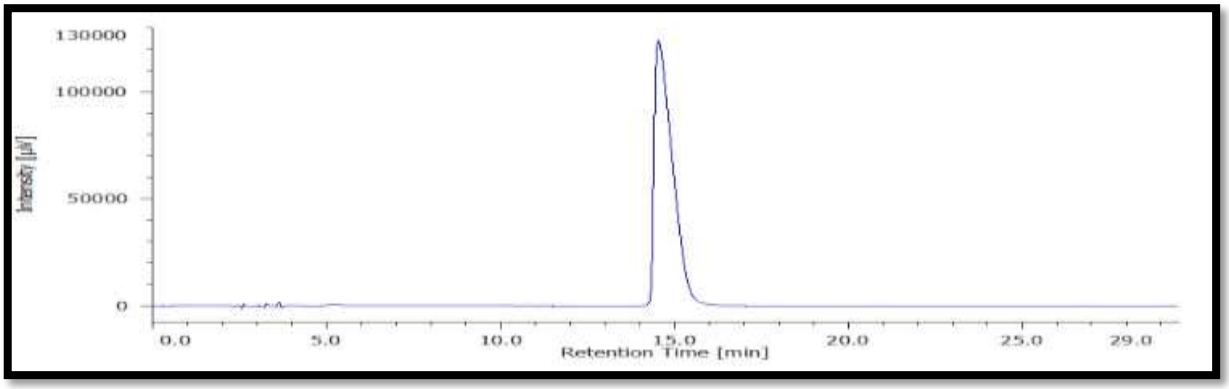

Figure 5.15 Accelerated stability study chromatogram of Metoprolol tartrate with PVP K30 (1 month)

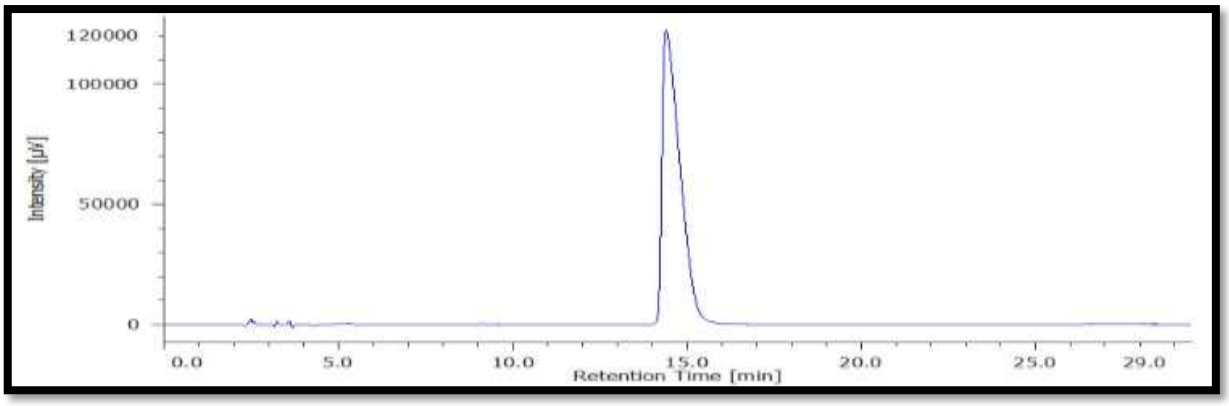

Figure 5.16 Accelerated stability study chromatogram of Metoprolol tartrate with PVP K30 (2 month)

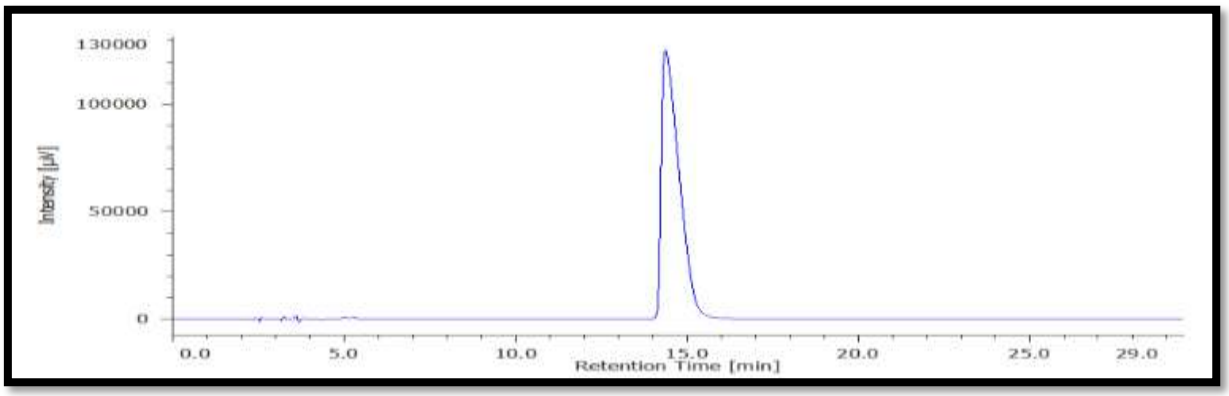

Figure 5.17 Accelerated stability study chromatogram of Metoprolol tartrate with PVP K30 (3 month)

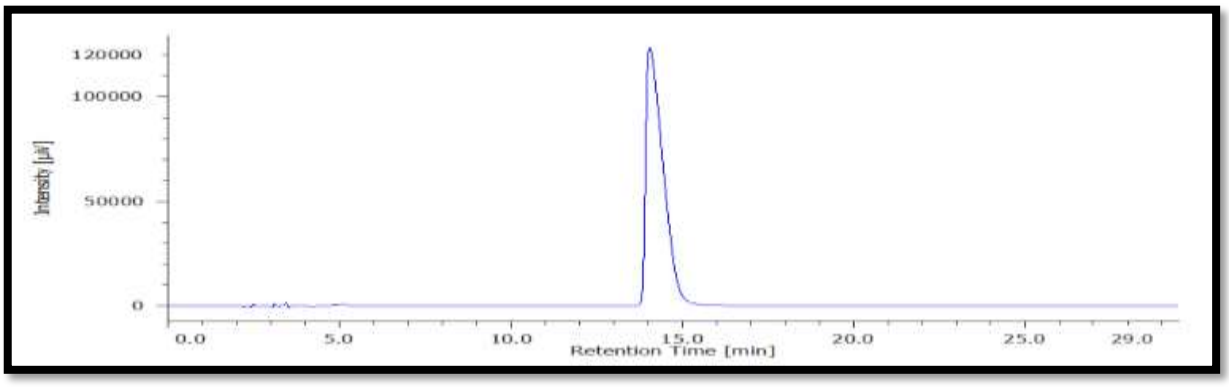

Figure 5.18 Accelerated stability study chromatogram of Metoprolol tartrate with PVP K30 (6 month)

Table 5.3 3 Accelerated stability study results of Metoprolol tartrate + PVP K30 till 6 month

Sample	Total %of Degradation	No. Of DP above 0.1%	DP RRT(%Degradation)
1 month	0.12%	-	0.83(0.12%)
2 month	0.14%	-	0.85 (0.14%)
3 month	0.11%	-	0.81(0.11%)
6 month	0.12%	-	0.83(0.12%)

5.4 Microwave-assisted accelerated stability study

5.4.1 Trial 1

For microwave -assisted stability study, two samples (Metoprolol tartrate API and Metoprolol tartrate API with PVP K30) were kept in the microwave to check the degradation. The samples were placed at different power (400W and 600W) for 15 minutes at a different temperature (80 ˚C, 90 ˚C and 100 ˚C).

A.) Metoprolol Tartrate Samples

a) Metoprolol Tartrate at 400W &600W in 15 minutes different temperature (80ºC,90ºC, and 100ºC)

Data of HPLC chromatographic analysis of Metoprolol Tartrate (Figure 5.19 TO Figure 5.24) show no degradation was observed.

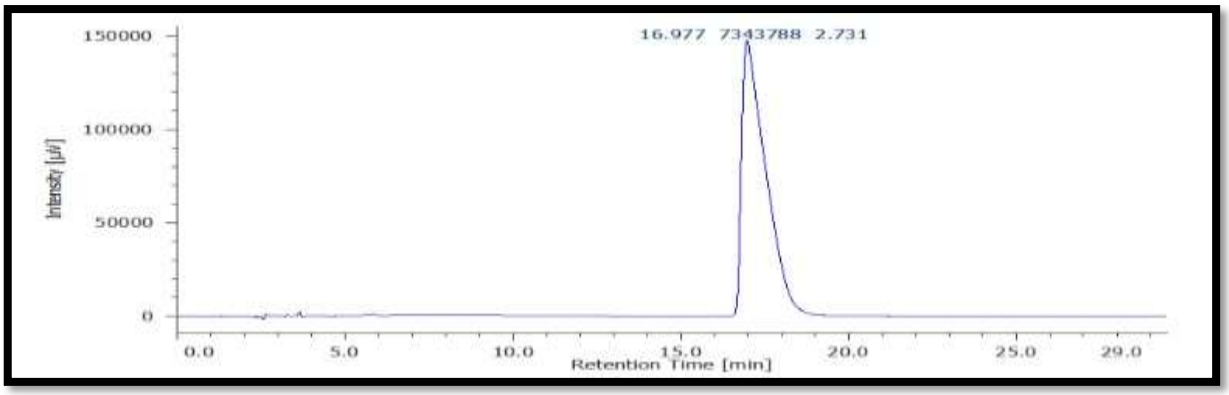

Figure 5.19 Chromatogram of Metoprolol Tartrate at 400W for 15 minutes at temperature (80ºC)

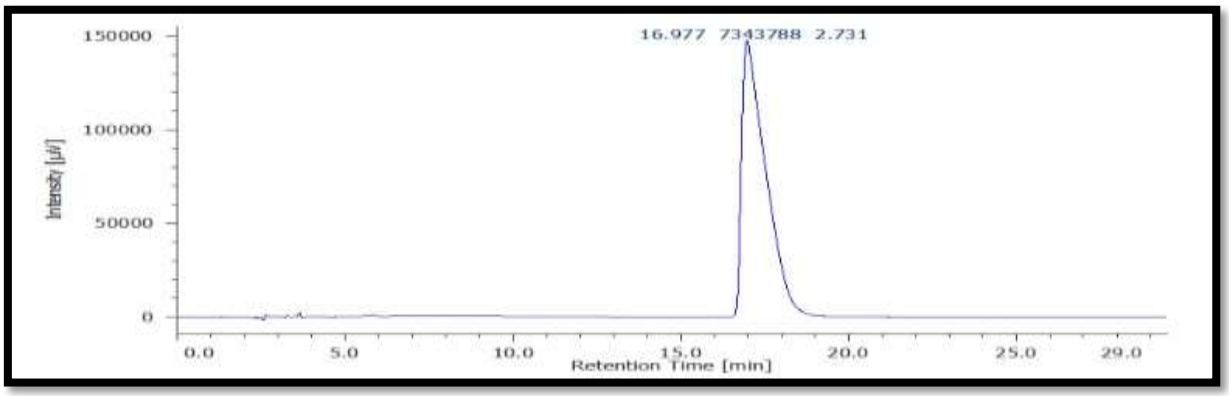

Figure 5.20 Chromatogram of Metoprolol Tartrate at 400W for 15 minutes at temperature (90ºC)

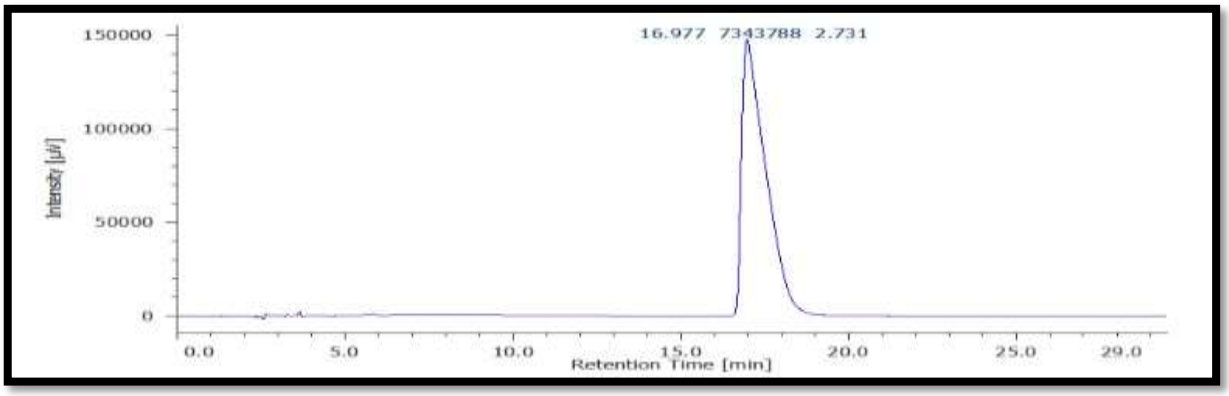

Figure 5.21 Chromatogram of Metoprolol Tartrate at 400W for 15 minutes at temperature (100ºC)

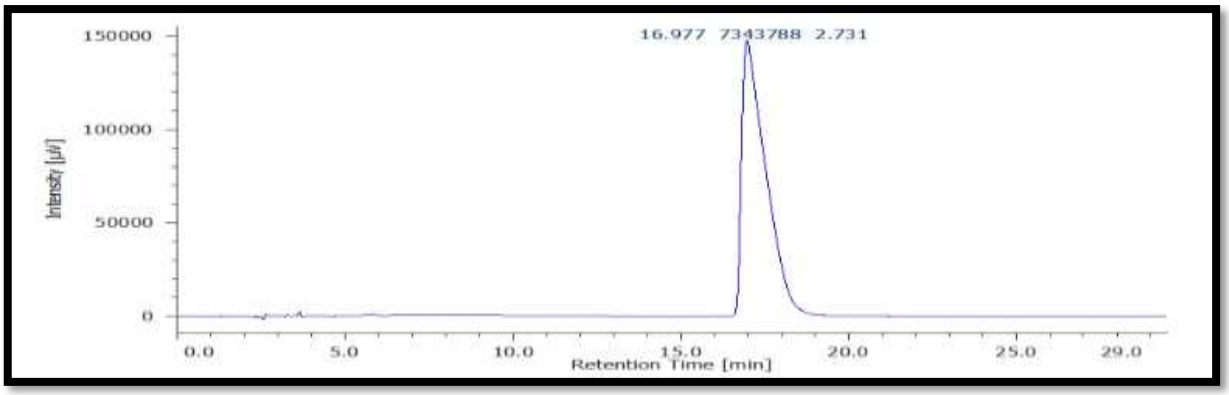

Figure 5.22 Chromatogram of Metoprolol Tartrate at 600W for 15 minutes at temperature (80ºC)

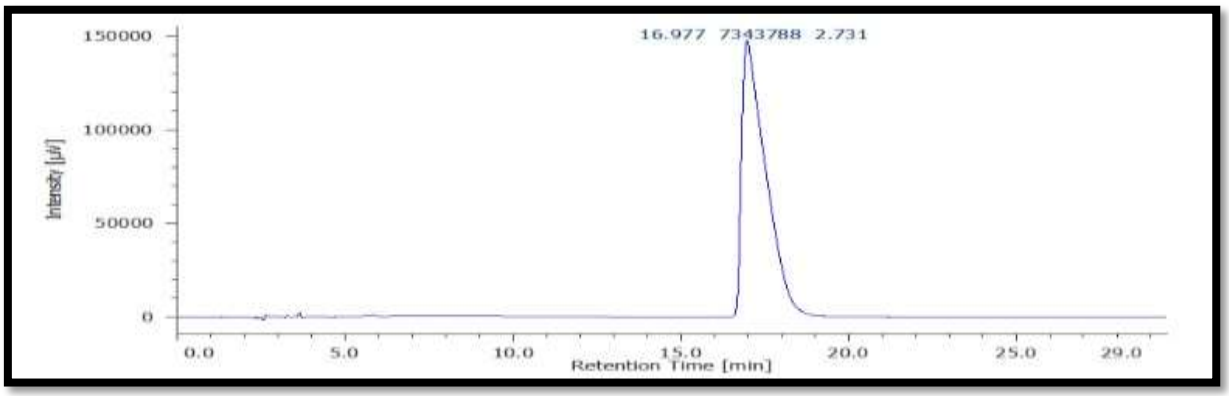

Figure 5.23 Chromatogram of Metoprolol Tartrate at 600W for 15 minutes at temperature (90ºC)

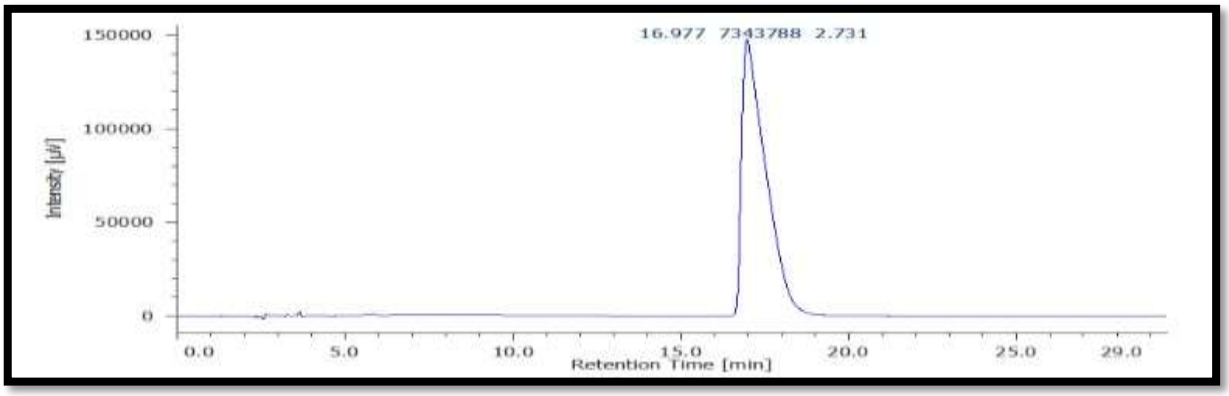

Figure 5.24 Chromatogram of Metoprolol Tartrate at 600W for 15 minutes at temperature (100ºC)

B.) Metoprolol Tartrate with PVP K30

a) Metoprolol Tartrate with PVP K30 at 400W & 600W in 15 minutes at different temperature (80ºC,90ºC, and 100ºC)

Data of HPLC chromatographic analysis of Metoprolol Tartrate (Figure 5.25 to Figure 5.30) show no degradation was observed.

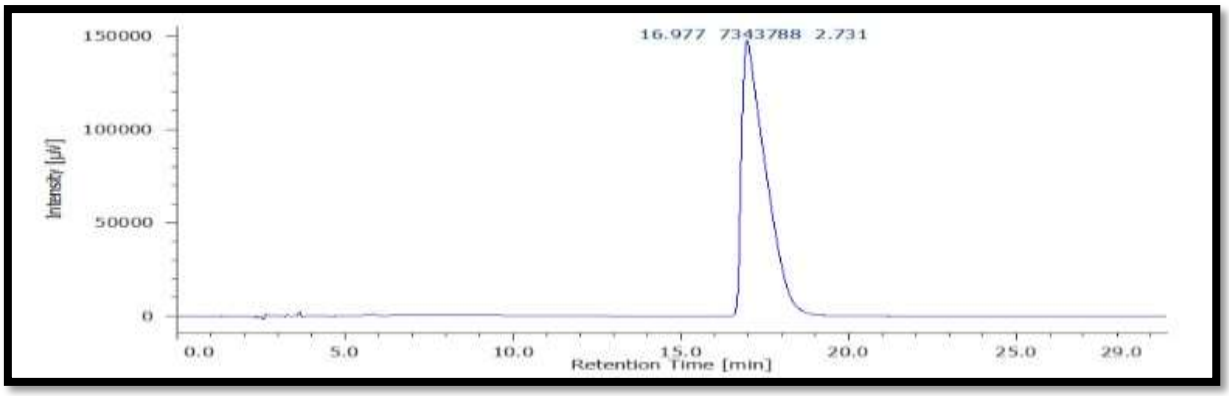

Figure 5.25 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 15 minutes at temperature (80ºC)

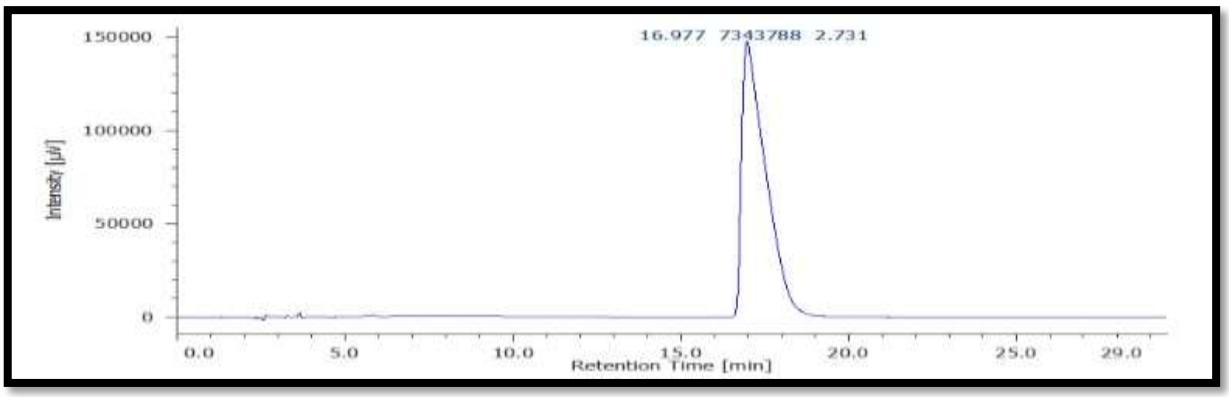

Figure 5.26 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 15 minutes at temperature (90ºC)

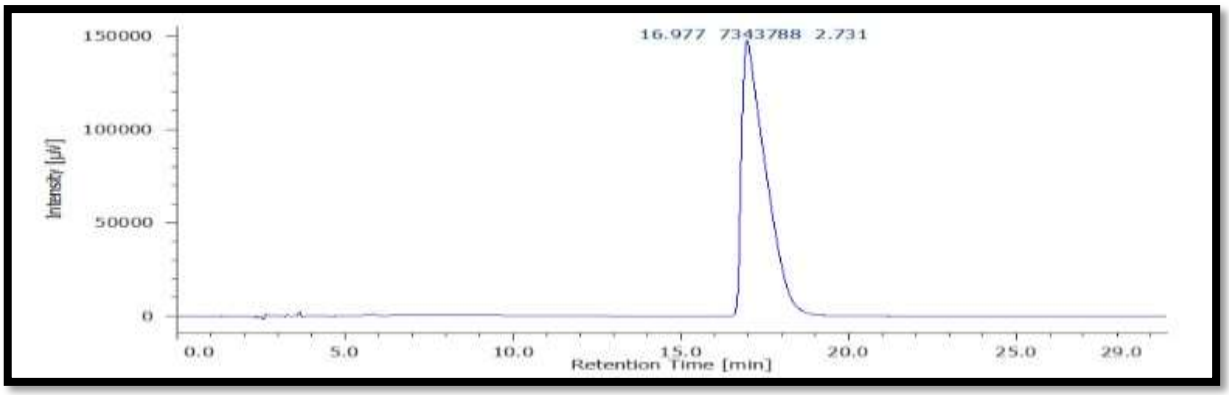

Figure 5.27 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 15 minutes at temperature (100ºC)

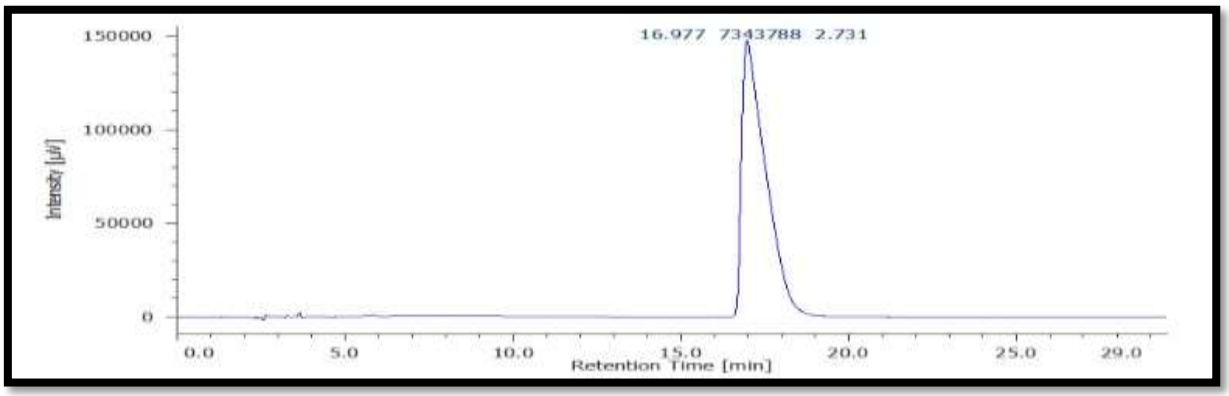

Figure 5.28 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 15 minutes at temperature (80ºC)

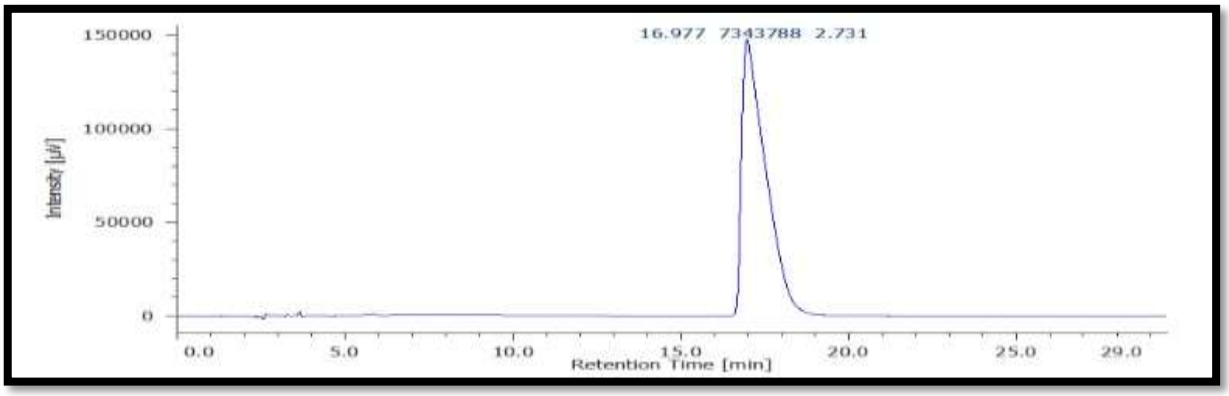

Figure 5.29 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 15 minutes at temperature (90ºC)

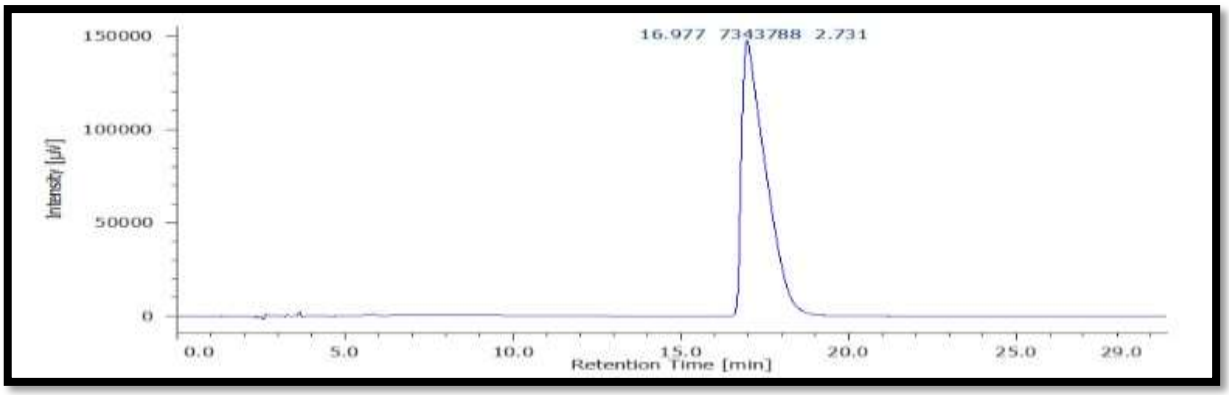

Figure 5.30 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 15 minutes at temperature (100ºC)

5.4.1.1 Conclusion

There was no degradation detected at different power like 400W, 600W for 15 minutes at a different temperature like 80˚C, 90˚C, and 100˚C. So for further study, the time was increased from 15 minutes to 30 minutes.

5.4.2 Trial 2

The four samples (Metoprolol tartrate API and Metoprolol tartrate API with PVP K30) were taken. Then, 50µL and 80µL water were added in each sample. Finally, the samples were charged in the microwave at different power (200W, 400W, and 600W) at different temperature (80°C, 90°C, and 100°C) for 30 minutes. After exposure to radiation, the total % of degradation was calculated only when the single impurities have degradation more than 0.1 %.

5.4.2.1 Metoprolol Tartrate API Samples

HPLC chromatogram of the sample containing Metoprolol Tartrate in the presence of 50µL and 80µL at 200W, 400W and 600W for 30 min at different temperature.

A) Metoprolol Tartrate API at 200W

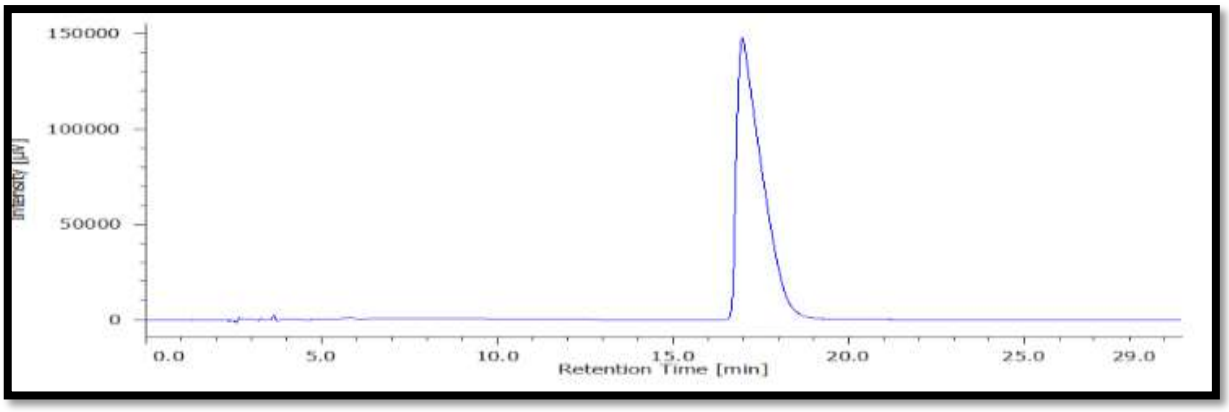

Figure 5.31 Chromatogram of Metoprolol Tartrate at 200W for 30 minutes at 80ºC upon addition of 50 µL of water

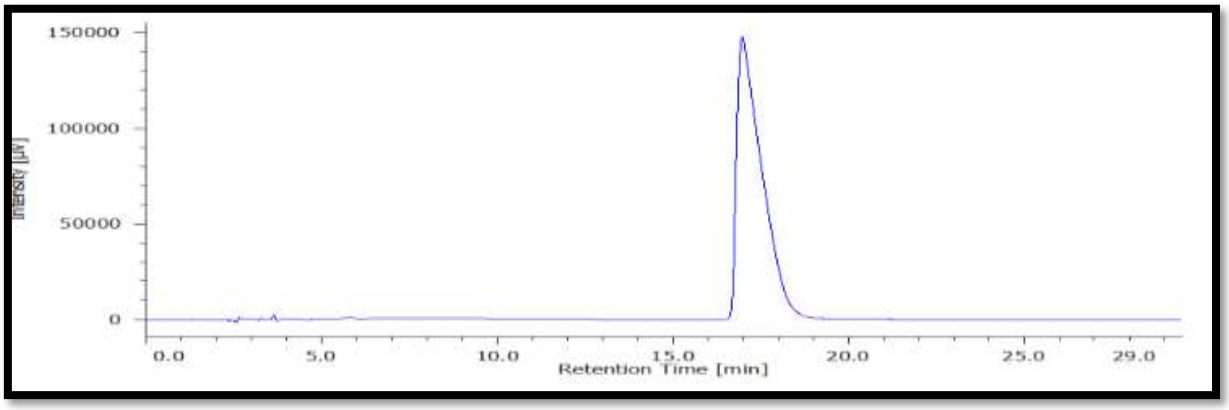

Figure 5.32 Chromatogram of Metoprolol Tartrate at 200W for 30 minutes at 80ºC upon addition of 80 µL of water

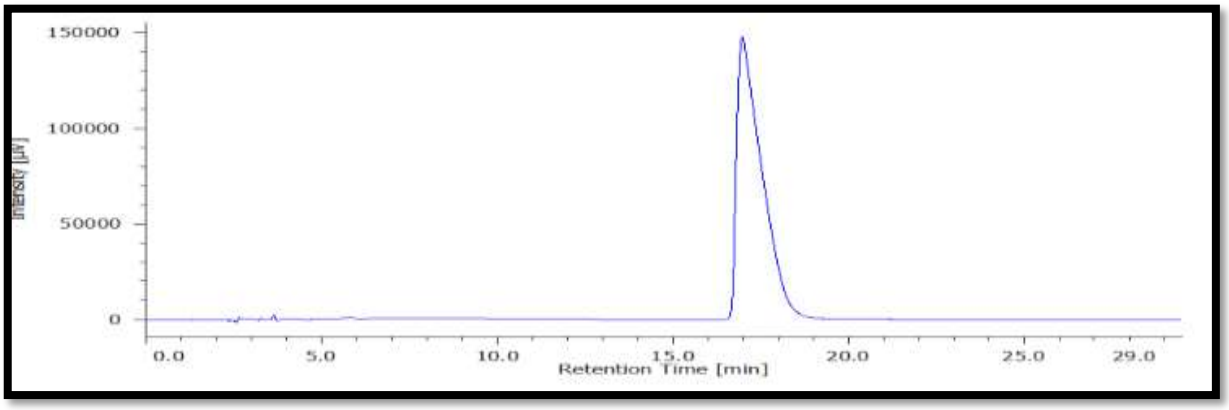

Figure 5.33 Chromatogram of Metoprolol Tartrate at 200W for 30 minutes at 90ºC upon addition of 50 µL of water

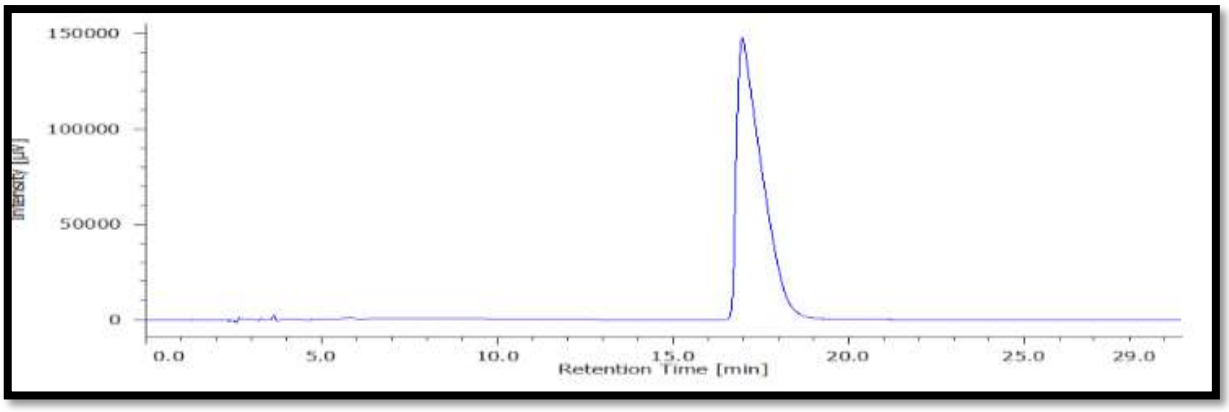

Figure 5.34 Chromatogram of Metoprolol Tartrate at 200W for 30 minutes at 90ºC upon addition of 80 µL of water

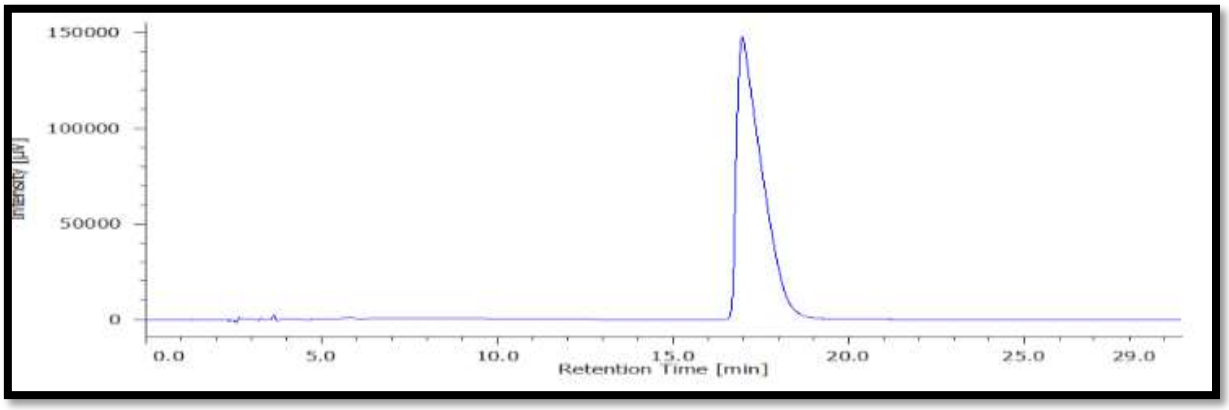

Figure 5.35 Chromatogram of Metoprolol Tartrate at 200W for 30 minutes at 100ºC upon addition of 50 µL of water

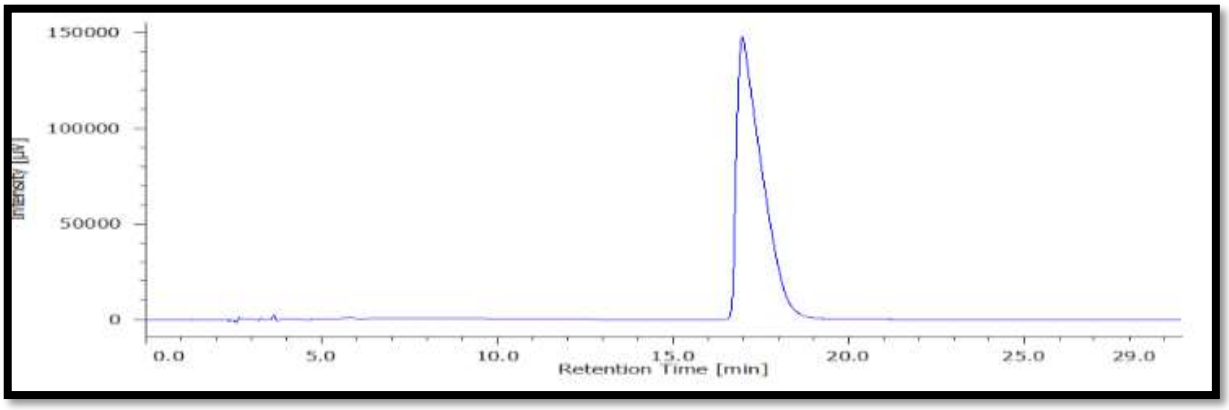

Figure 5.36 Chromatogram of Metoprolol Tartrate at 200W for 30 minutes at 100ºC upon addition of 80 µL of water

Table 5.4 Degradation summary of Metoprolol Tartrate at 200W,80ºC,90ºC,100ºC for 30 min with different volumes of water

Water	50µLwater			80µLwater		
Temp. (°C)	Total %Degradation	RRT				
80	-	-	0	-	-	0
90	0.10%	1	0	0	1	0
100	0.10%	1	0	0	1	0

B) Metoprolol Tartrate API at 400W

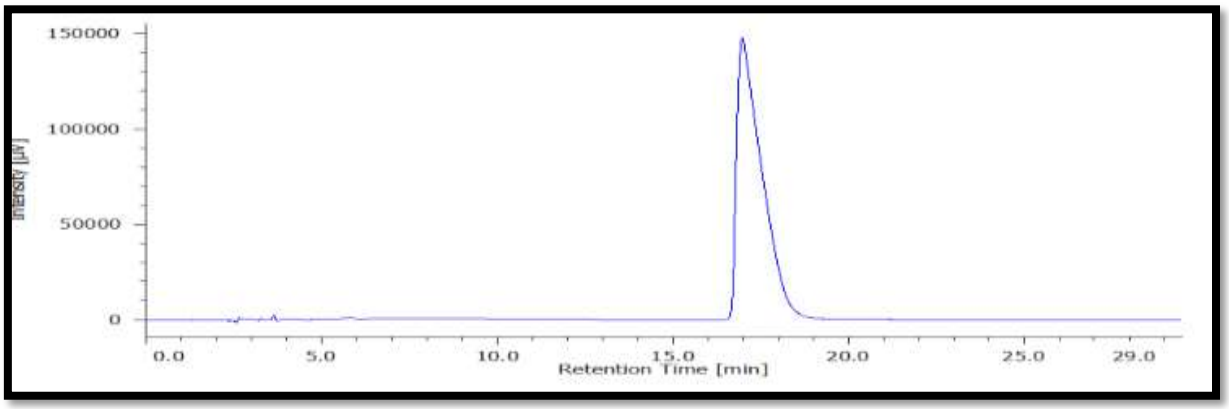

Figure 5.37 Chromatogram of Metoprolol Tartrate at 400W for 30 minutes at 80ºC upon addition of 50 µL of water

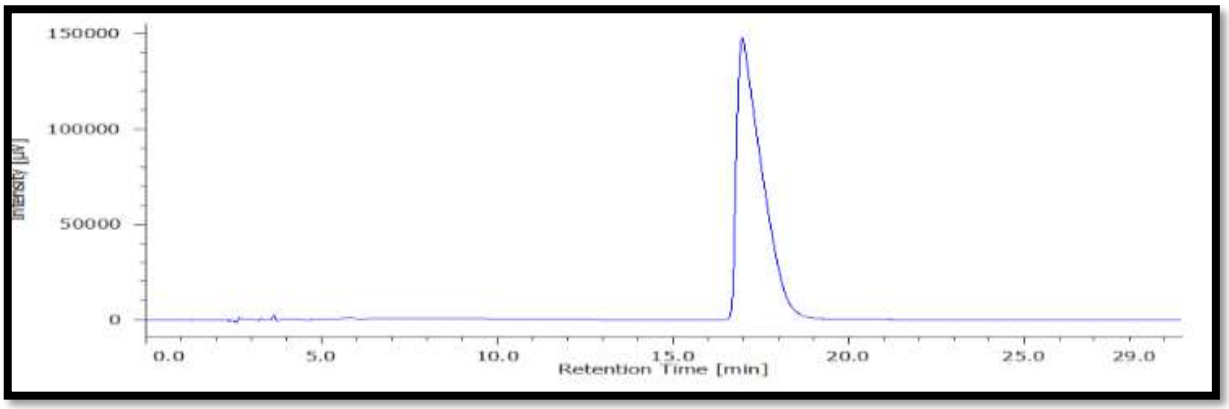

Figure 5.38 Chromatogram of Metoprolol Tartrate at 400W for 30 minutes at 80ºC upon addition of 80 µL of water

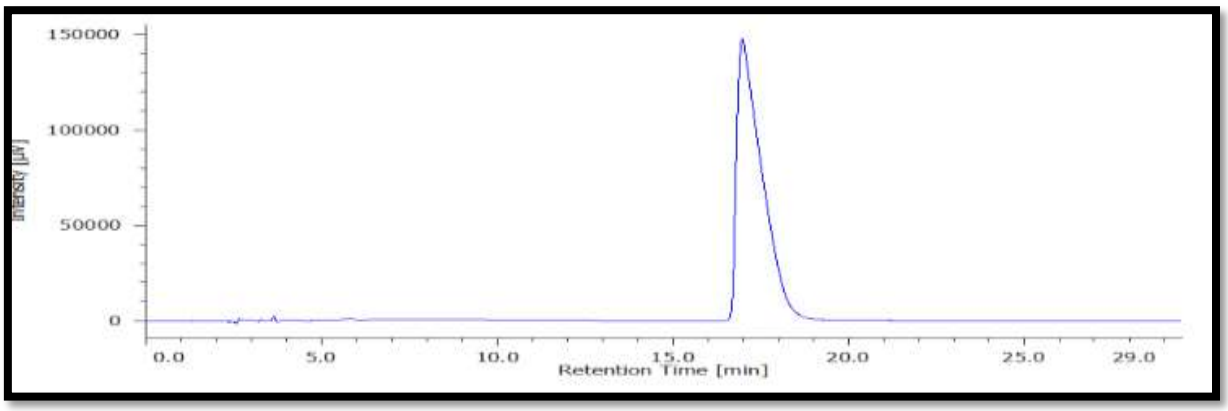

Figure 5.39 Chromatogram of Metoprolol Tartrate at 400W for 30 minutes at 90ºC upon addition of 50 µL of water

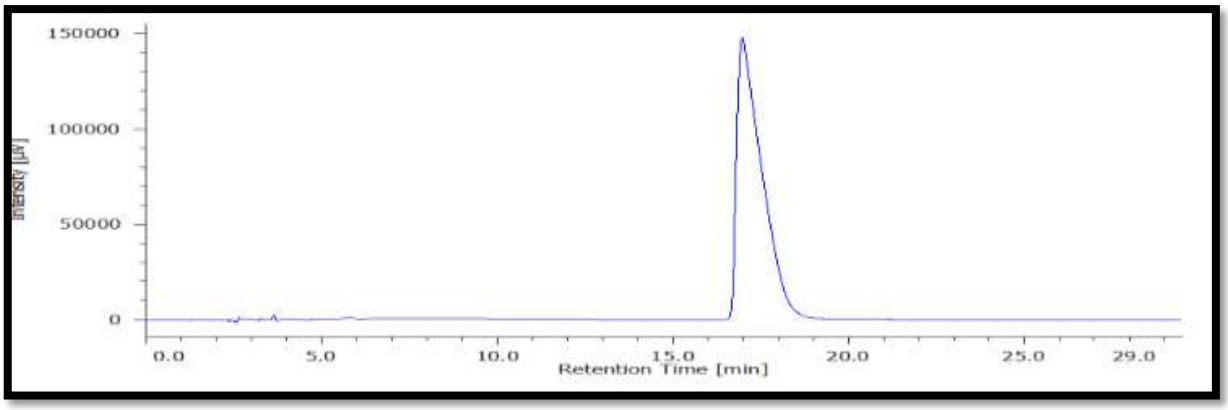

Figure 5.40 Chromatogram of Metoprolol Tartrate at 400W for 30 minutes at 90ºC upon addition of 80 µL of water

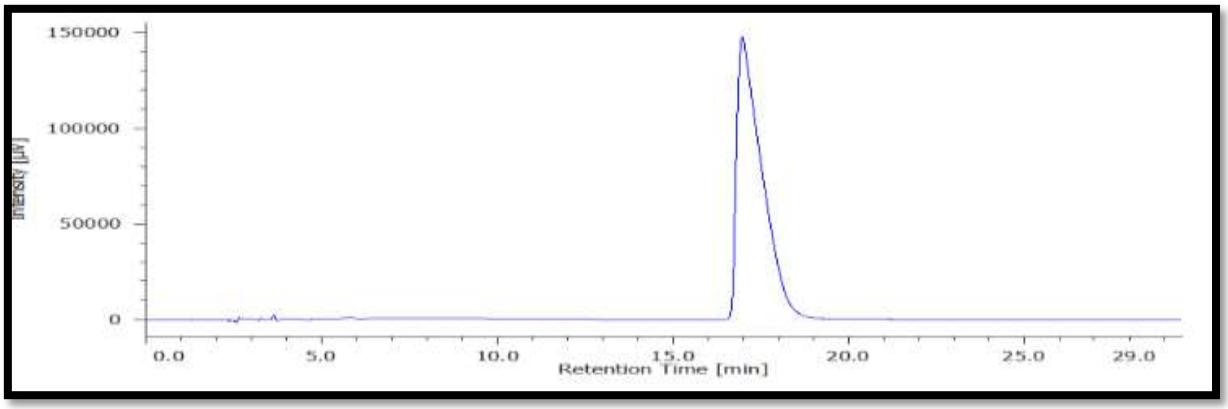

Figure 5.41 Chromatogram of Metoprolol Tartrate at 400W for 30 minutes at 100ºC upon addition of 50 µL of water

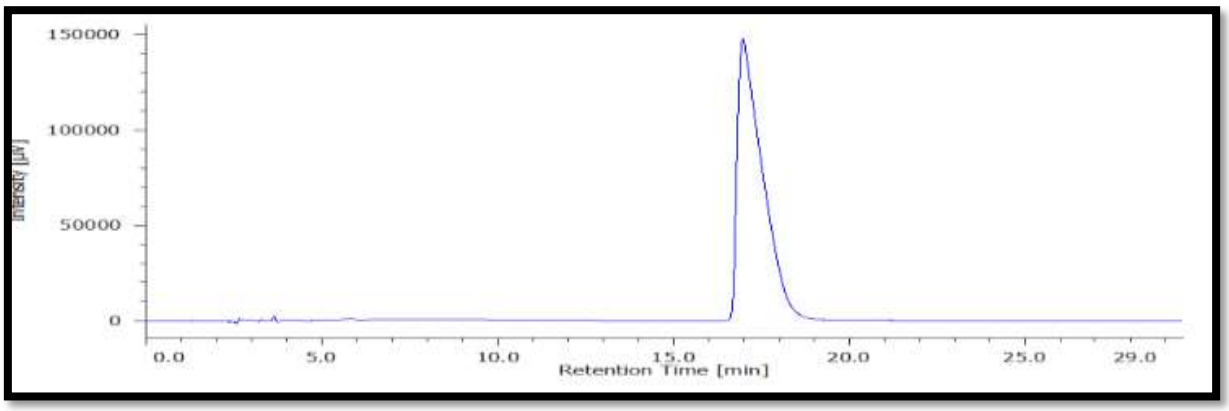

Figure 5.42 Chromatogram of Metoprolol Tartrate at 400W for 30 minutes at 100ºC upon addition of 80 µL of water

Table 5.5 Degradation summary of Metoprolol Tartrate at 400W,80ºC,90ºC,100ºC for 30 min with different volumes of water

Water	**50µLwater**			**80µLwater**		
Temp. (°C)	**Total %Degradation**	**RRT**				
80	**-**	**-**	**0**	**-**	**-**	**0**
90	**0.10%**	**1**	**0**	**0**	**1**	**0**
100	**0.10%**	**1**	**0**	**0**	**1**	**0**

C) Metoprolol Tartrate API at 600W

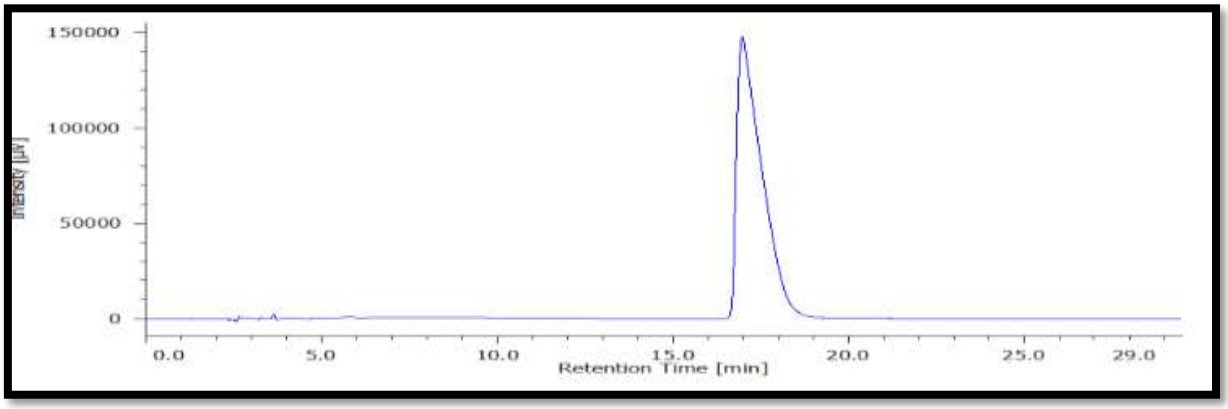

Figure 5.43 Chromatogram of Metoprolol Tartrate at 600W for 30 minutes at 80ºC upon addition of 50 µL of water

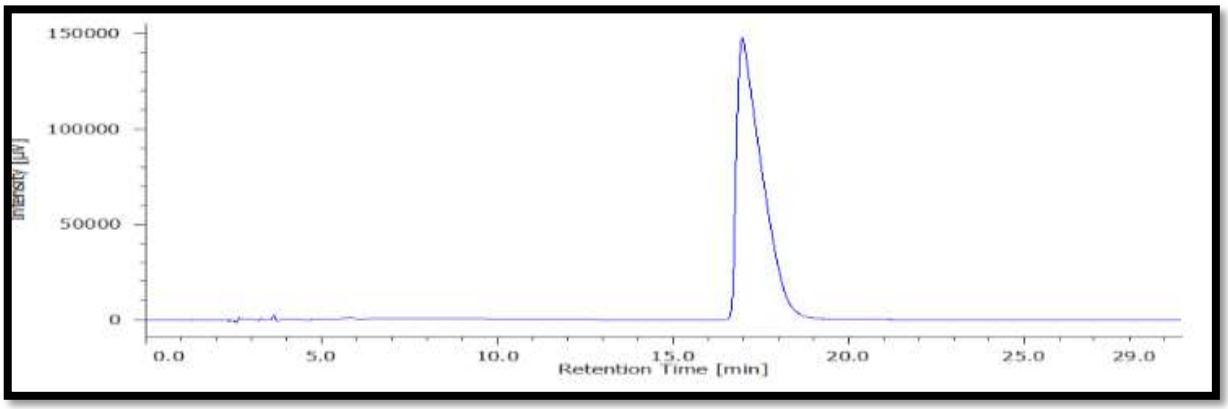

Figure 5.44 Chromatogram of Metoprolol Tartrate at 600W for 30 minutes at 80ºC upon addition of 80 µL of water

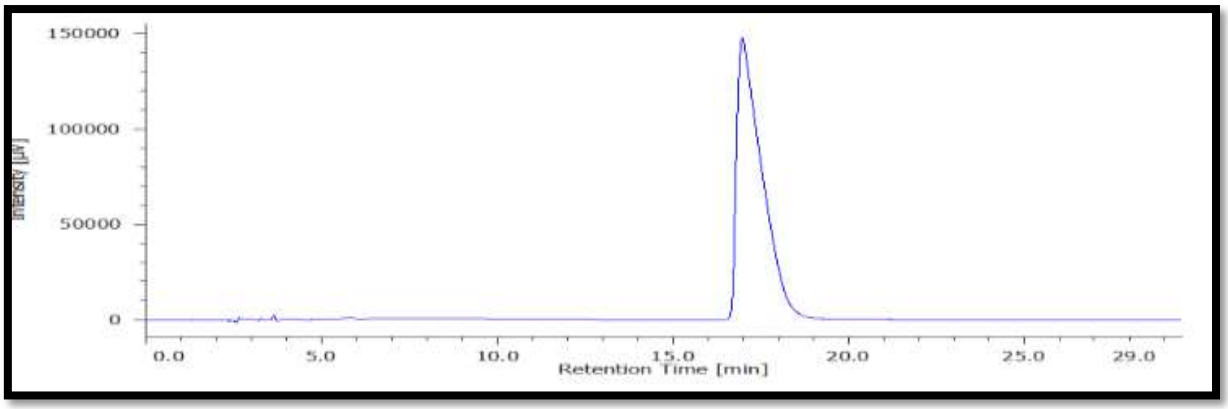

Figure 5.45 Chromatogram of Metoprolol Tartrate at 600W for 30 minutes at 90ºC upon addition of 50 µL of water

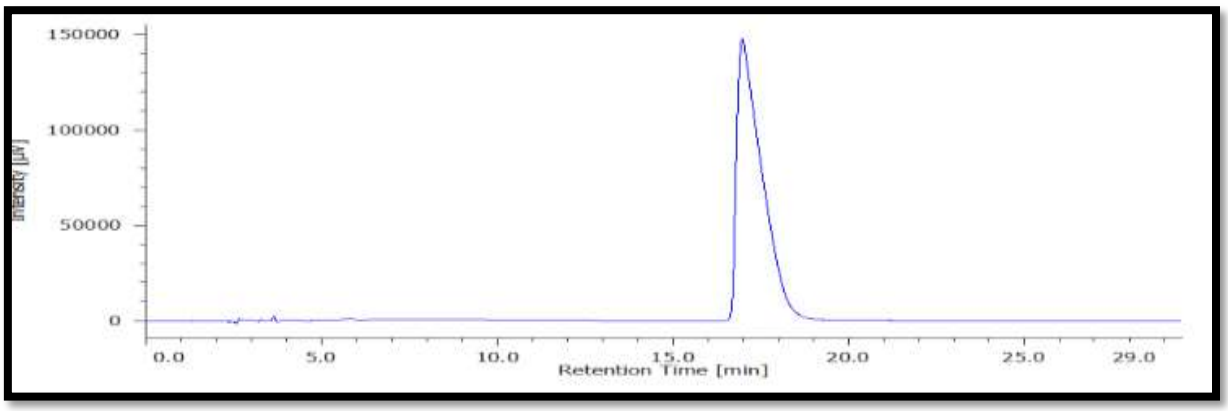

Figure 5.46 Chromatogram of Metoprolol Tartrate at 600W for 30 minutes at 90ºC upon addition of 80 µL of water

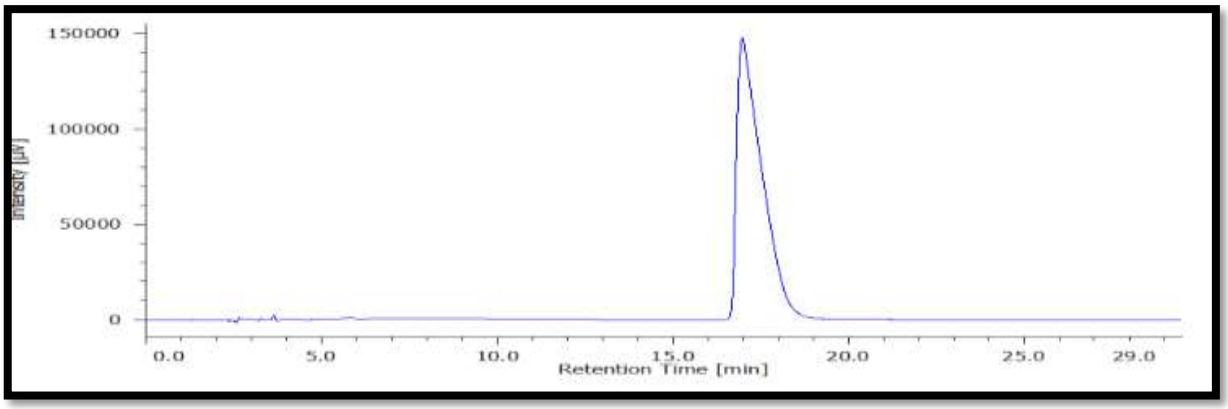

Figure 5.47 Chromatogram of Metoprolol Tartrate at 600W for 30 minutes at 100ºC upon addition of 50 µL of water

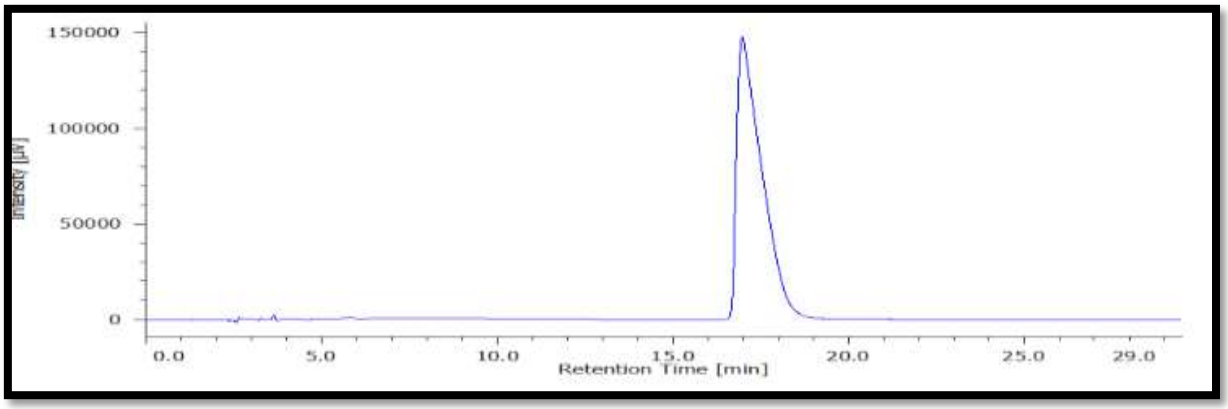

Figure 5.48 Chromatogram of Metoprolol Tartrate at 600W for 30 minutes at 100ºC upon addition of 80 µL of water

Table 5.6 Degradation summary of Metoprolol Tartrate at 600W,80ºC,90ºC,100ºC for 30 min with different volumes of water

Water	50µLwater			80µLwater		
Temp. (°C)	Total %Degradation	RRT				
80	-	-	0	-	-	1
90	0.10%	1	0	0	1	0
100	0.10%	1	0	0	1	0

5.4.2.2 Metoprolol Tartrate API with PVP K30

HPLC chromatogram of the sample containing Metoprolol Tartrate with PVP K30 in the presence of 50µL and 80µL at 400W and 600W for 30 min at different temperature .

A) Metoprolol Tartrate API samples with PVP K30 at 400W

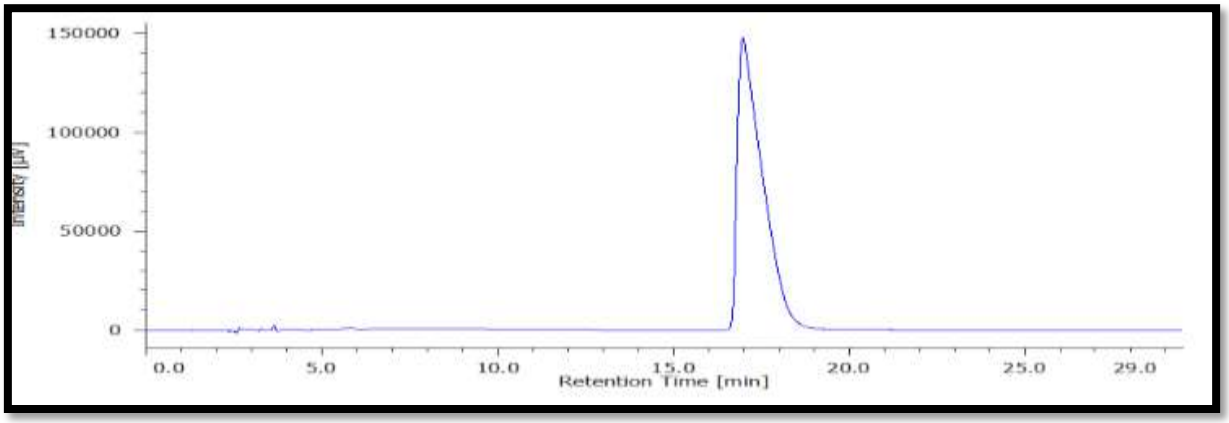

Figure 5.49 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 30 minutes at 80ºC upon addition of 50 µL of water

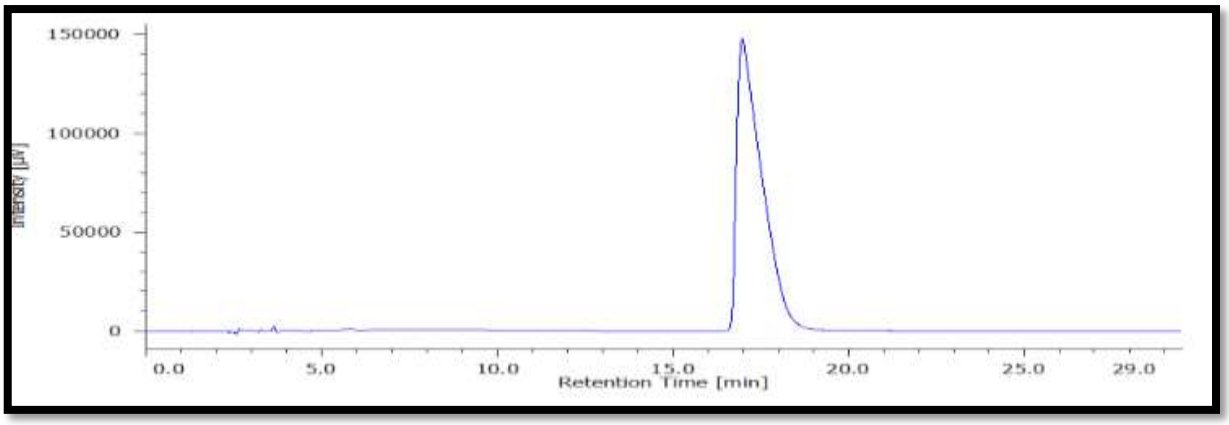

Figure 5.50 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 30 minutes at 80°C upon addition of 80 µL of water

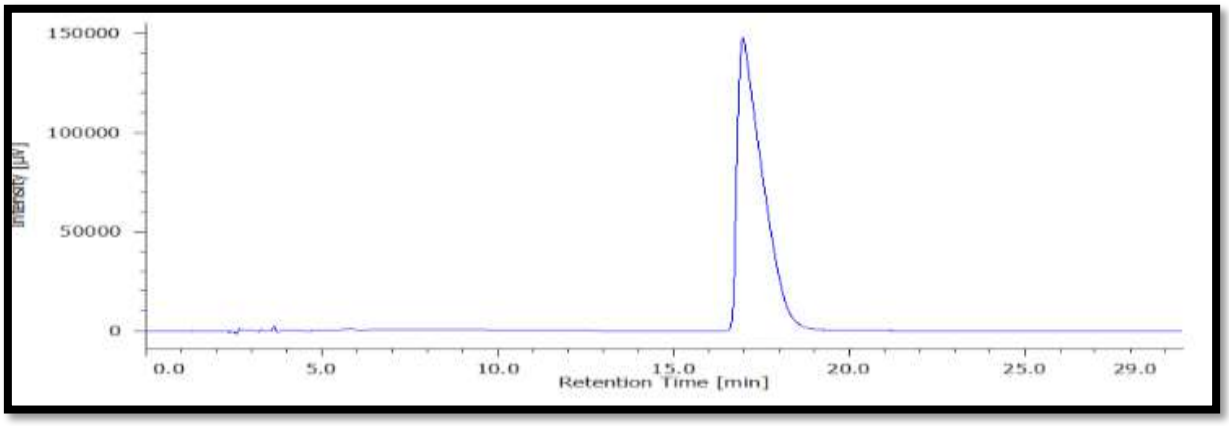

Figure 5.51 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 30 minutes at 90°C upon addition of 50 µL of water

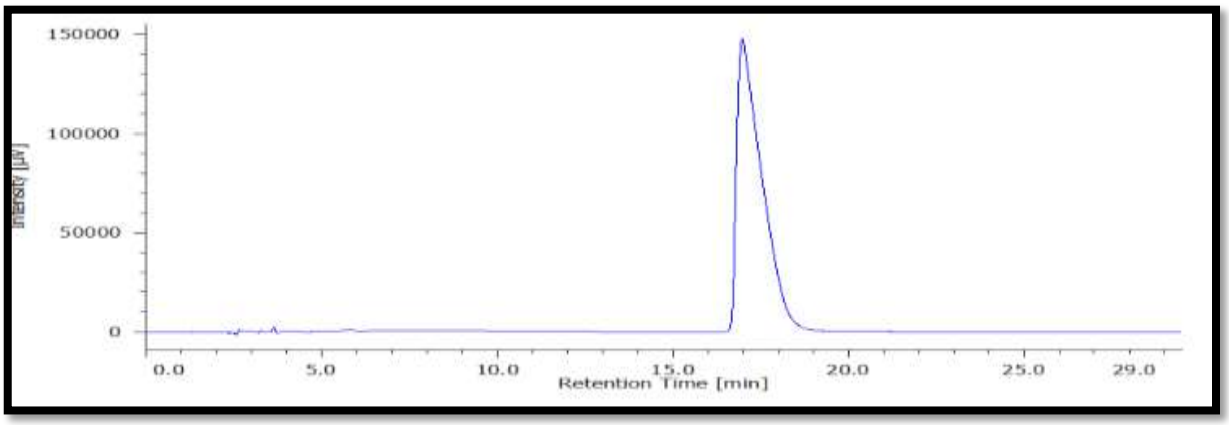

Figure 5.52 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 30 minutes at 90ºC upon addition of 80 µL of water

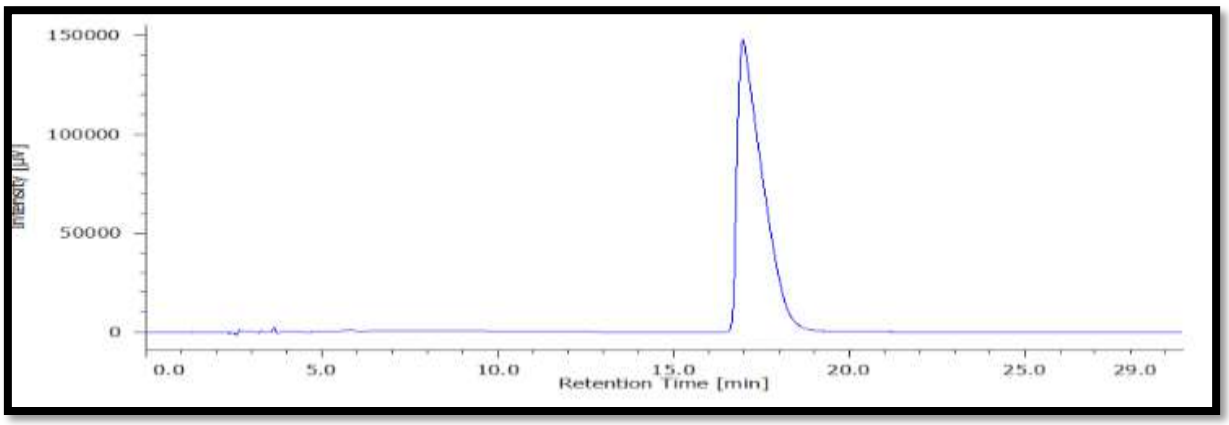

Figure 5.53 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 30 minutes at 100ºC upon addition of 50 µL of water

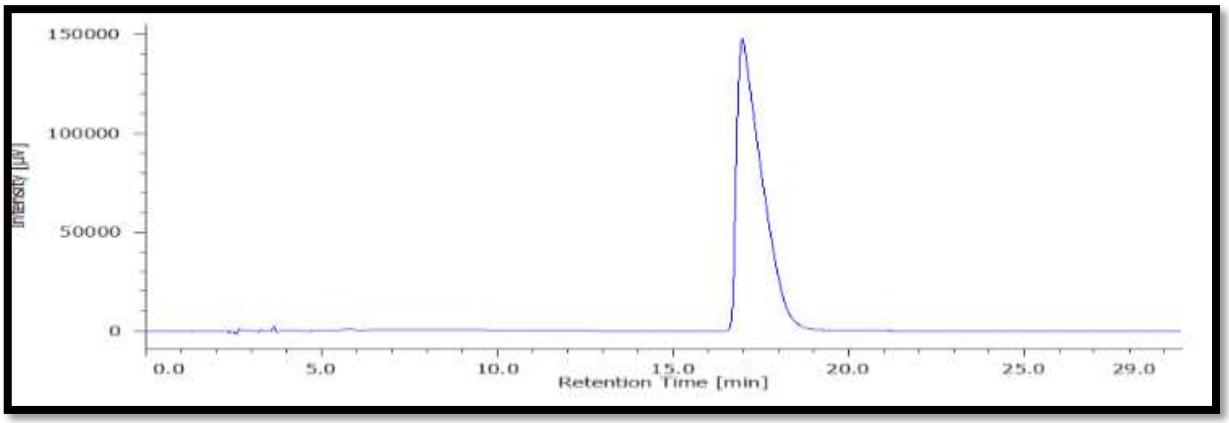

Figure 5.54 Chromatogram of Metoprolol Tartrate with PVP K30 at 400W for 30 minutes at 100ºC upon addition of 80 µL of water

Water	**50µLwater**			**80µLwater**		
Temp. (°C)	**Total %Degradation**					
80						
90						

100						

Table 5.7 Degradation summary of Metoprolol Tartrate +PVP K30 at 400W,80ºC,90ºC,100ºC for 30 min with different volumes of water

B) Metoprolol Tartrate API samples with PVP K30 at 600W

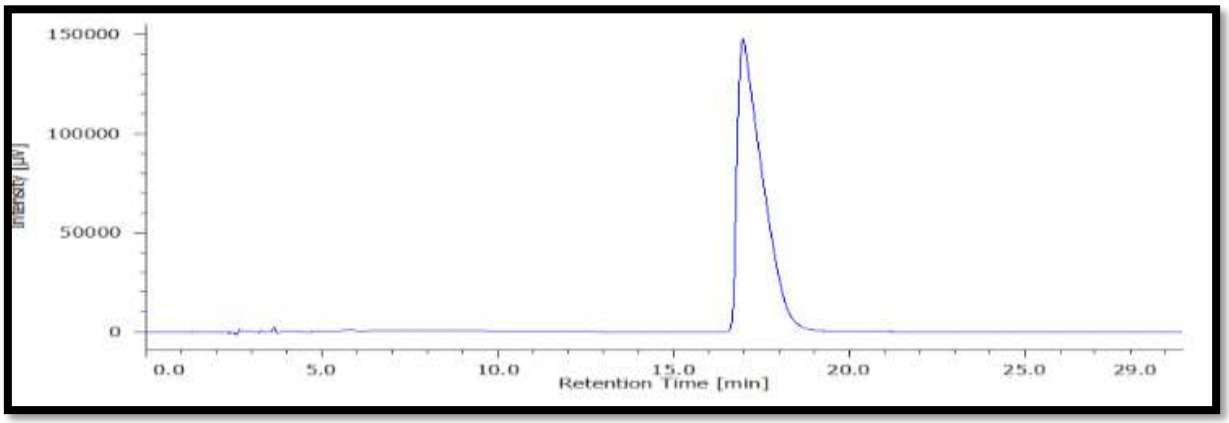

Figure 5.55 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 30 minutes at 80ºC upon addition of 50 µL of water

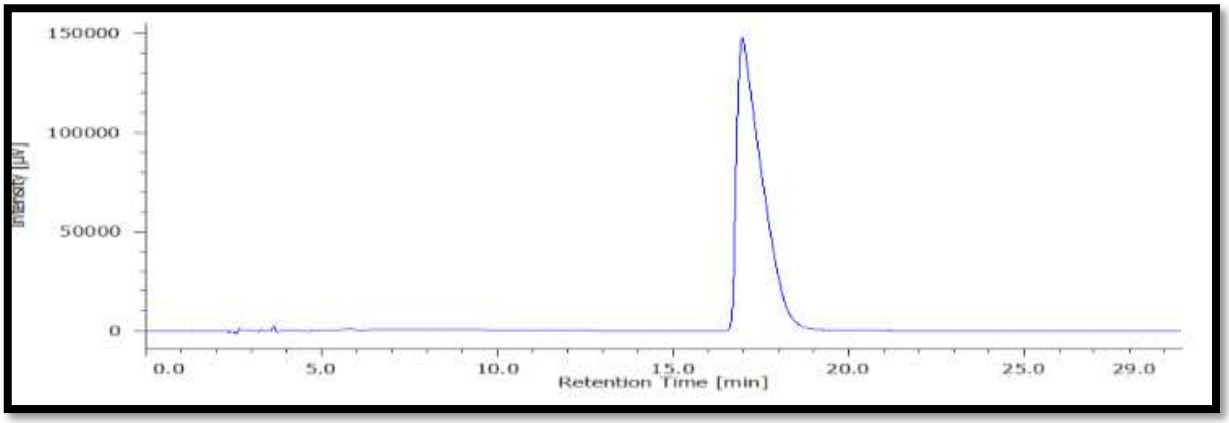

Figure 5.56 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 30 minutes at 80ºC upon addition of 80 µL of water

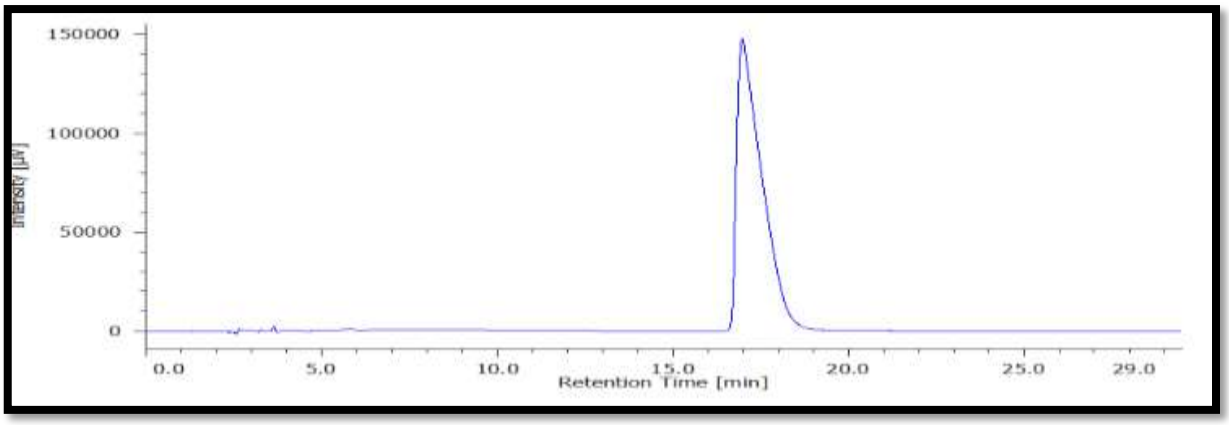

Figure 5.57 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 30 minutes at 90°C upon addition of 50 µL of water

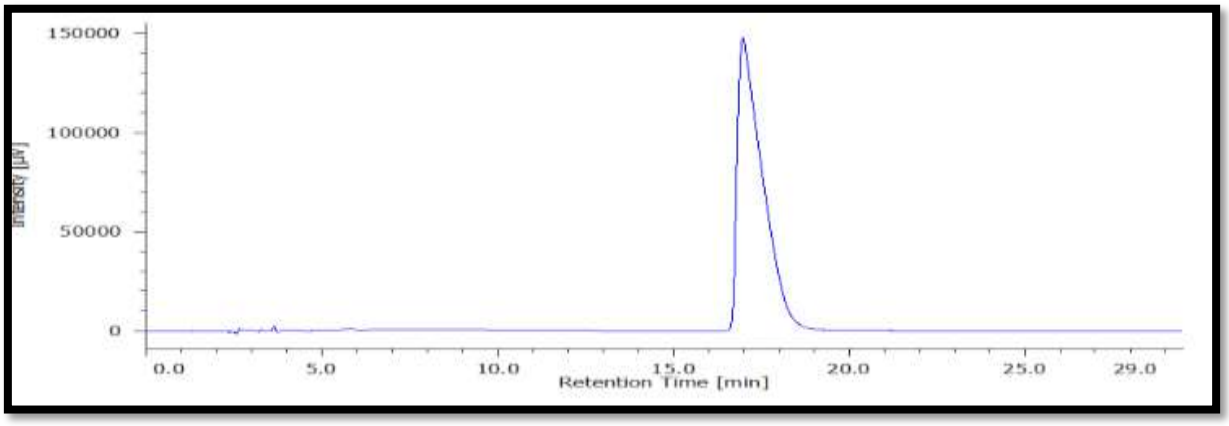

Figure 5.58 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 30 minutes at 90°C upon addition of 80 µL of water

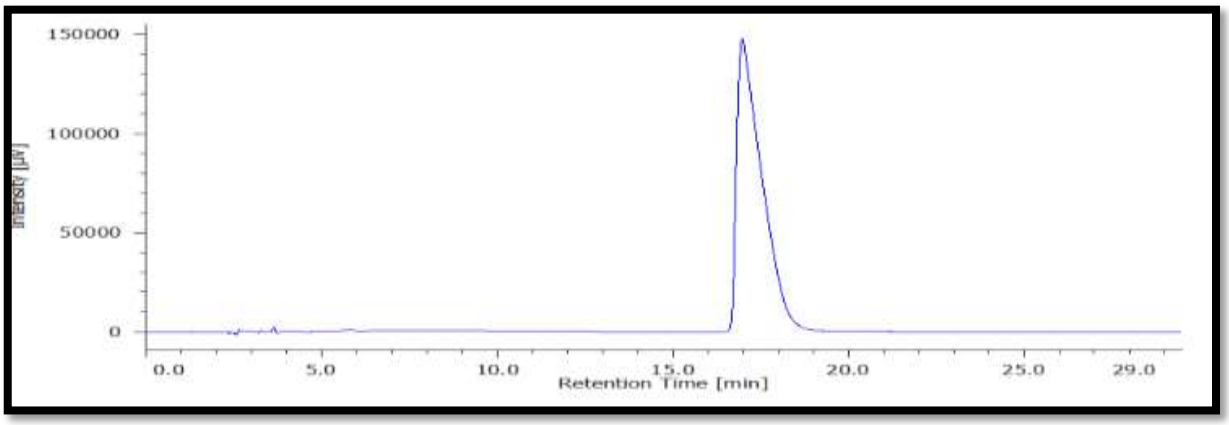

Figure 5.59 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 30 minutes at 100ºC upon addition of 50 µL of water

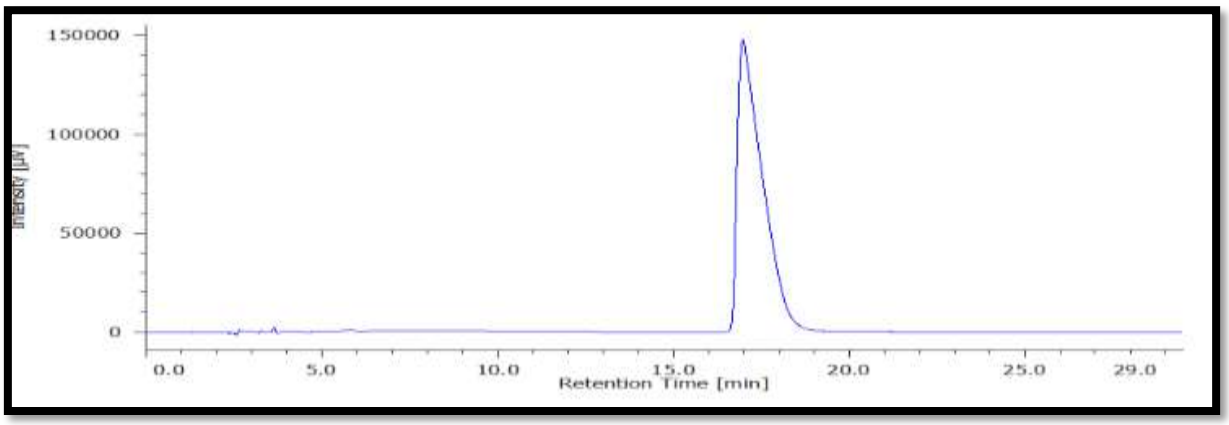

Figure 5.60 Chromatogram of Metoprolol Tartrate with PVP K30 at 600W for 30 minutes at 100ºC upon addition of 80 µL of water

Water	50µLwater			80µLwater		
Temp. (°C)	Total %Degradation	RRT				
80						
90						
100						

Table 5.8 Degradation summary of Metoprolol Tartrate +PVP K30 at 600W,80ºC,90ºC,100ºC for 30 min with different volumes of water

Chapter-6

Summary and Conclusion

SUMMARY

The feasibility of microwave-assisted heating based accelerated stability testing of Metoprolol Tartrate API mixture with PVP K30 in the presence of magnesium stearate, were evaluated. The accelerated stability examinations of Metoprolol Tartrate was accepted available by means of microwave-assisted heating and conventional method. The degradation profile generated by microwave-assisted heating was comparable with the conventional method through relative retention time (RRT) comparison. Additionally, the results confirm that microwave-assisted heating produces no degradation for API+PVP K30 compared with conventional accelerated stability testing.

The study also demonstrated a substantial correlation in the pattern of degradation products between conventional and microwave-assisted stability. The microwave-assisted stability study under 50µL water content; 600W; 100T; 30 min conditions showed no degradation product in mixture with PVP K30 to that of the conventional method.

The present work successfully demonstrated that the use of microwave-assisted heating is a feasible option to enhance the rate of reaction reducing the time required for stability studies. The results obtained after 6 months using the conventional stability method were predicted using microwave-assisted heating technique within 30 minutes. Hence, the microwave-assisted heating technique can serve as an efficient alternative to predict drug product stability. The use of microwave-assisted heating technique saves the time required to estimate stability and quality of drug product enhancing the productivity of the pharmaceutical industries.

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Remaining Work:

- Forced degradation study of the Metoprolol tartrate is pending.
- Also forced degradation study of Atenolol were planes.

Chapter-7
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