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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND TENELIGLIPTIN HYDROBROMIDE IN COMBINED DOSAGE FORM

Ankita Malani, Lata Kothapalli, Rachana Bhimanwar *
Department of Pharmaceutical Quality Assurance, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Researches, Pimpri, Pune, India
*Corresponding Author Email: r.09joshi@gmail.com

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ABSTRACT

The stability indicating High performance thin layer chromatographic method was developed using Camag HPTLC system. The separation was carried out using Silica G60 F_{254}precoated TLC plates used as stationary phase. The mobile phase used was Toluene: Methanol: GAA: TEA (5:4:0.5:0.5). Densitometric analysis was carried out in the absorbance mode at 257 nm. A well resolved peaks were obtained for MET and TENE at Rf 0.57 and 0.79 respectively. The calibration curves show linearity in the concentration range of 300-800ng and 7.5–20 µg band^{-} for MET and TENE with correlation coefficients of 0.993 and 0.992 respectively. Degradation study of both drug were carried out in acidic, alkaline, Thermal, oxidation, photolytic conditions. Degraded products peaks were also well resolved. The developed method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limit of detection, limit of quantification. The developed HPTLC method was simple, accurate and precise and used for the quantitative analysis of drug in the presence of their degradation products.

Keywords: Metformin hydrochloride, Teneligliptin hydrobromide, Stability Indicating High Performance Thin Liquid Chromatography

INTRODUCTION

Teneligliptin hydrobromide hydrate (TENE) is chemically described as {(2S,4S)-4-[4-(3-methyl-1phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl} (1,3-thiazolidin-3-yl) methanone hemipenta hydrobromide hydrate is a dipeptidyl peptidase inhibitor (Figure 1). TENE slows the inactivation of incretin hormones, thereby increasing bloodstream concentrations and reducing fasting and postprandial glucose concentrations in a glucose-dependant manner in patients with type 2 diabetes mellitus. The inhibition of DPP-4 increases the amount of active plasma incretins which helps with glycemic control. Metformin hydrochloride (MET) is 1,1-dimethylbiguanide hydrochloride, a biguanide antidiabetic (Figure 2). It is given orally in the treatment of type 2 diabetes mellitus and is the drug of choice in overweight patients. They do not stimulate insulin release but require that some insulin be present in order to exert their antidiabetic effect. Possible mechanism of action includes the delay in the absorption of glucose from the GIT and increase in insulin sensitivity and glucose uptake in to cells and inhibition of hepatic gluconeogenesis. For effective control of blood sugar in diabetic patients more than one medication is required. TENE shows effective control of blood sugar when combined with MET. The objective of the present study was to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Metformin Hydrochloride and Teneligliptin and validate the method with forced degradation studies according to ICH guidelines[1].

Literature survey reveals various HPLC and UV methods for the determination of MET and TENE in bulk and dosage forms, some also reveals stability studies using HPLC[2-5]. Many methods for estimation of TENE in dosage form were also reported[6-9]. Literature survey also reveals the methods for estimation of MET with other drugs combination[10-12]. Till date there is no HPTLC method reported for this combination. HPTLC is use for routine analysis because of its advantages like many samples can be run at a time using small volume of mobile phase unlike HPLC and nearby lowers the cost and time of the analysis. Mobile phase having higher p^H can also be used for the analysis. HPTLC is also used for Simultaneous estimation of multicomponents in a formulation.[13, 14]

In the development of a pharmaceutical formulation it is important to determine the intrinsic stability of the drug to predict possible reactions and degradation products. The intrinsic stability of the substance should be evaluated in terms of temperature, humidity, oxidation, UV light exposure, and hydrolysis. Stability can be observed in the drug when subjected to the official conditions of the stability studies.

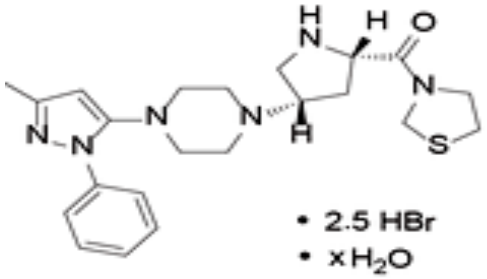

Figure 1: Structure of Teneligliptin hydrobromide

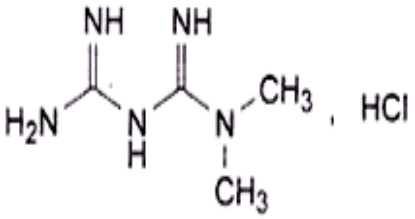

Figure 2: Structure of Metformin hydrochloride

MATERIALS AND METHODS

Pure samples for MET and TENE were procured from Spectrum Research Private Limited, Hyderabad and USV Limited, Baddi, Himachal Pradesh, India respectively. Toluene, methanol, glacial acetic acid and triethyl amine of AR grade were used for the analysis of drugs and were purchased from Merck. The tablet sample, Tenglyn [M] 500 manufactured by Cadila Healthcare Limited, Goa was purchased from the local market.

Chromatographic conditions

The chromatographic separation was performed on 10×10cm aluminum plates pre-coated with a layer of silica gel $60F_{254}$. The TLC plates were prewashed with methanol and dried in an oven at 100°C for 10min. Samples were spotted on a TLC plate by a Linomat V semi-automatic spotter using following parameters: bandwidth, 6mm; track distance, 11.6mm; and application volume 0.5µl. The TLC plate was developed in twin trough chamber using toluene: methanol: GAA: TEA (5:4:0.5:0.5, v/v/v) as mobile phase; chamber saturation time, 10min; migration distance, 90mm. The TLC plate was scanned by camag TLC Scanner and analyzed using Win CATS software at detection wavelength of 257nm. Deuterium lamp was used as a source of radiation in scanner.

Methods of preparation of solutions

Preparation of standard solution and calibration curves

Standard stock solution of MET and TENE were prepared in methanol to get the final concentration of 25mg/ml and 1mg/ml for MET and TENE respectively. The spots were applied on TLC plate and peaks were obtained.

Analysis of MET and TENE in marketed formulation

Weigh 20 tablets (Tenglyn M 500) of MET and TENE and powdered. Appropriately weighed tablet powder equivalent to 500 mg of MET and 20 mg of TENE was transferred to 25 ml volumetric flask, sonicate for 30 min using methanol and made up the volume upto the mark with methanol. The sample over the plate in such a way that the final concentration of sample solution was found to be 12.50µg/band and 500 ng/band of MET and TENE respectively**. (Figure 7)**

Preparation of sample solutions for the stability study of MET and TENE

The stability study i.e. forced degradation study of MET and TENE were carried out under the different conditions like treatment with acid, alkali and oxidizing reagent (H_2O_2), thermal degradation, exposure to UV light and stability chamber.

Acid hydrolysis - Accurately weigh the quantity of MET and TENE and transferred to 10 ml volumetric flask. Add 3 ml of 1 N HCl to it and heated at 80^0C for 2 hrs on water bath. Cool the sample and make up the volume up to the mark with methanol and analyze the sample using HPTLC method.

Alkaline hydrolysis - Accurately weigh the quantity of MET and TENE and transferred to 10 ml volumetric flask. Add 3 ml of 0.5 N NaOH to it and heated at 60^0C for 2 hrs on water bath. Cool the sample and make up the volume up to the mark with methanol and analyzed the sample using HPTLC method.

Oxidative degradation - Accurately weigh the quantity of MET and TENE and transferred to 10 ml volumetric flask. Add 3 ml of 3% $H_2O_2$to it and heated at 80^0C for 2 hrs on water bath. Cool the sample and make up the volume up to the mark with methanol and analyzed the sample using HPTLC method.

Thermal degradation - Accurately weigh the quantity of MET and TENE and kept in hot air oven at 80^o **C** for 60 min. After 60 min take out the samples and transferred to 10 ml volumetric flask and make up the volume with methanol and analyzed the sample using HPTLC.

Photolytic degradation - Accurately weigh the quantity of MET and TENE and kept in UV chamber at 254 nm for 24 hrs. Take out the samples and transferred to 10 ml volumetric flask and make up the volume with methanol and analyzed the sample using HPTLC.

Stability study -Accurately weigh the quantity of MET and TENE and kept in stability chamber at 40^o C and 75 % RH for 28 days. Take out the samples and transferred to 10 ml volumetric flask and make up the volume with methanol and analyzed the sample using HPTLC.

Method validation

Linearity

Calibration curve was plotted of mean peak area of 3 determinants against the concentration of MET and TENE in the range of 7.5µg – 20.0µg/ band and 300-800ng / band of MET and TENE respectively (Figure 4A and 4B).

Precision

The precision of the method is closeness of results between a series of measurements obtained from the homogeneous samples under the same condition. It was studied in terms of Interday and Intraday variations.

Intraday precision

It was assessed by analyzing the working solution (12.5µg for MET and 500ng for TENE) within the range of calibration curve, after short interval of time within a day (n= 3) and calculate the % RSD and it should not be more than 2.

Inter day precision

It was assessed by analyzing the working solution (12.5µg for MET and 500ng for TENE) within the range of calibration curve on 3 consecutive days (n= 3) and calculate the % RSD and it should not be more than 2.

Accuracy

The accuracy of the analytes was calculated in terms of percentage recovery. The recovery study was carried out in triplicates at 3 different levels i.e. 80, 100 and 120% by spiking the standard drugs to the sample.

Limit of Detection (LOD) – LOD is the lowest amount of analyte that can be detected but not necessarily quantified and it was calculated using following formula[1]

$$LOD = 3.3 \times N/S$$

Limit of Quantitation (LOQ) – LOQ is the lowest amount of analytes that can be quantified and calculated using following formula[1]

$$LOQ = 10 \times N/S$$

Where N is the standard deviation and S is the slope of the calibration curve.

Table 1: Optimized chromatographic condition

Stationary phase	Aluminium plate precoated with silica gel 60 f 254
Mobile phase	Toluene : Methanol : GAA : TEA (5 : : 4 : 0.5 : 0.5)
Saturation time	10 min
Sample application volume	0.5 µl
Screening wavelength	257 nm
Scanning mode	Absorbance / Reflectance
Plate size	10 × 10
Development chamber	Twin trough chamber with stain- less steel lid

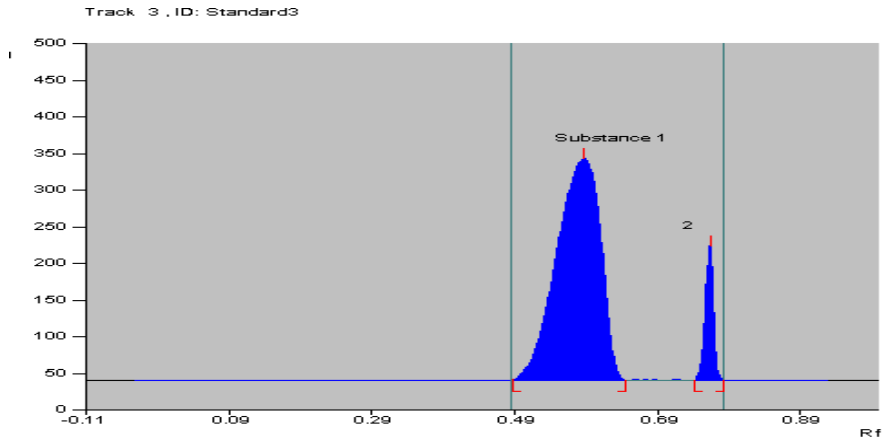

Figure 3: Typical densitogram of MET and TENE

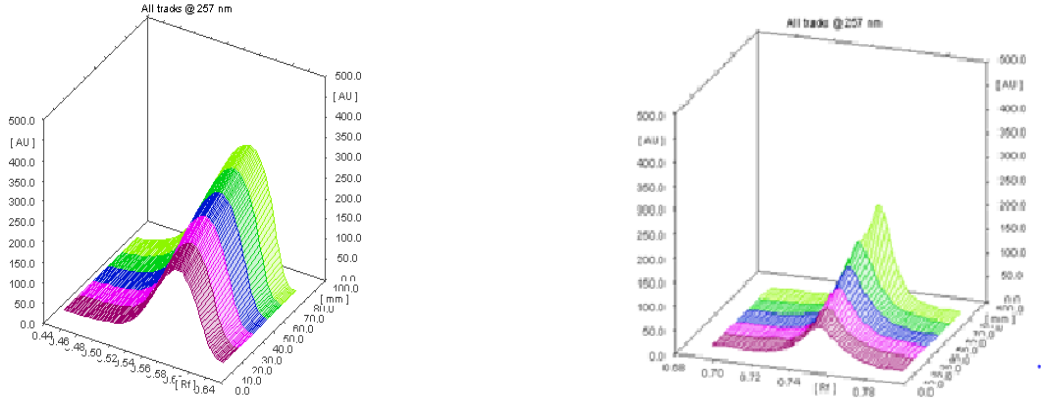

Figure 4: 3D Densitogram of (A) MET [7.5µg – 20.0µg/ band] (B) TENE [300-800 ng / band]

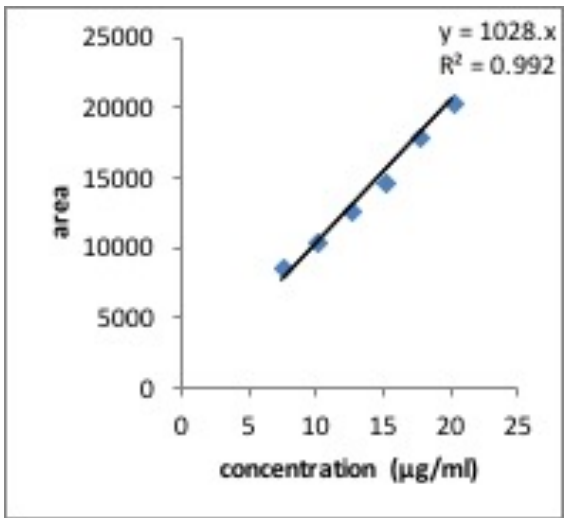

Figure 5: Calibration curve for MET

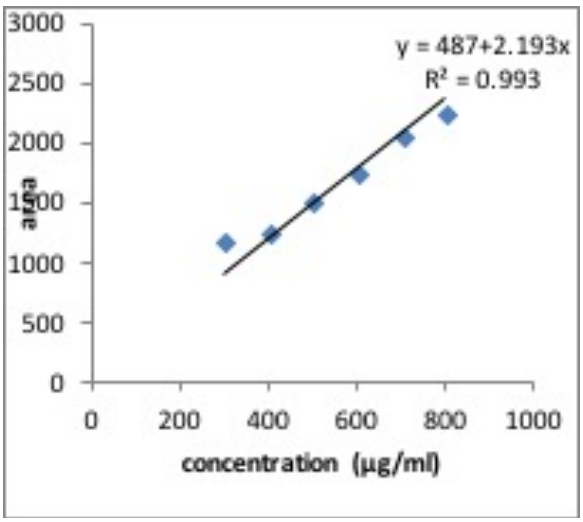

Figure 6: Calibration curve for TENE

Table 2: Determination of accuracy for MET and TENE

Drugs	Recovery level (%)	Initial amount (mg)	Amount added (mg)	% Recovery	% RSD
MET	80	500	400	101.64	0.7647
	100	500	500	99.10	0.3917
	120	500	600	101.21	0.2211
TENE	80	20	16	99.80	0.3025
	100	20	20	98.50	0.5175
	120	20	24	99.93	0.5163

Table 3: Summary of validation parameters

Validation parameters		Results	
		MET	TENE
Linearity		0.992	0.993
Precision	Intra day precision	0.085	0.74
	Inter day precision	0.73	0.83
Accuracy	80 % level	101.64	99.80
	100% level	99.10	98.50
	120% level	101.21	99.93
LOD		0.0244	0.0267
LOQ		0.07399	0.0811

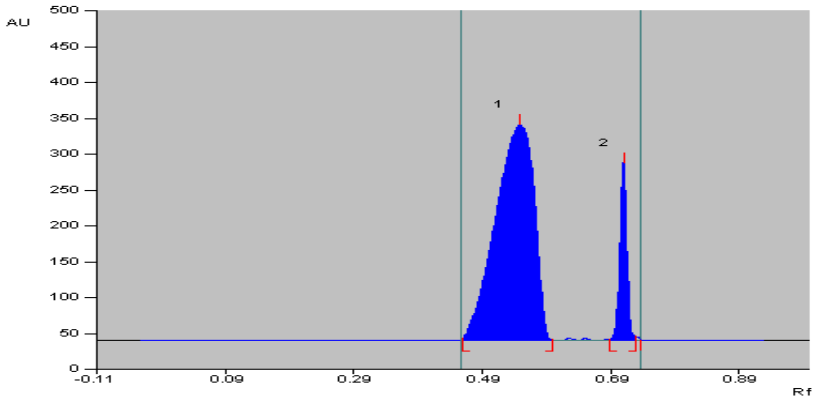

Fig .7: Typical densitogram of marketed formulation

Table 4: Assay of marketed formulation

Formulation	Tenglyn [M] 500	
	MET	TENE
Actual concentration (mg)	500	20
Concentration obtained (mg)	493.05	19.59
% Label claim	98.61	97.98
% RSD	0.9356	0.2628
Limit (%)	95 - 105	95 - 105

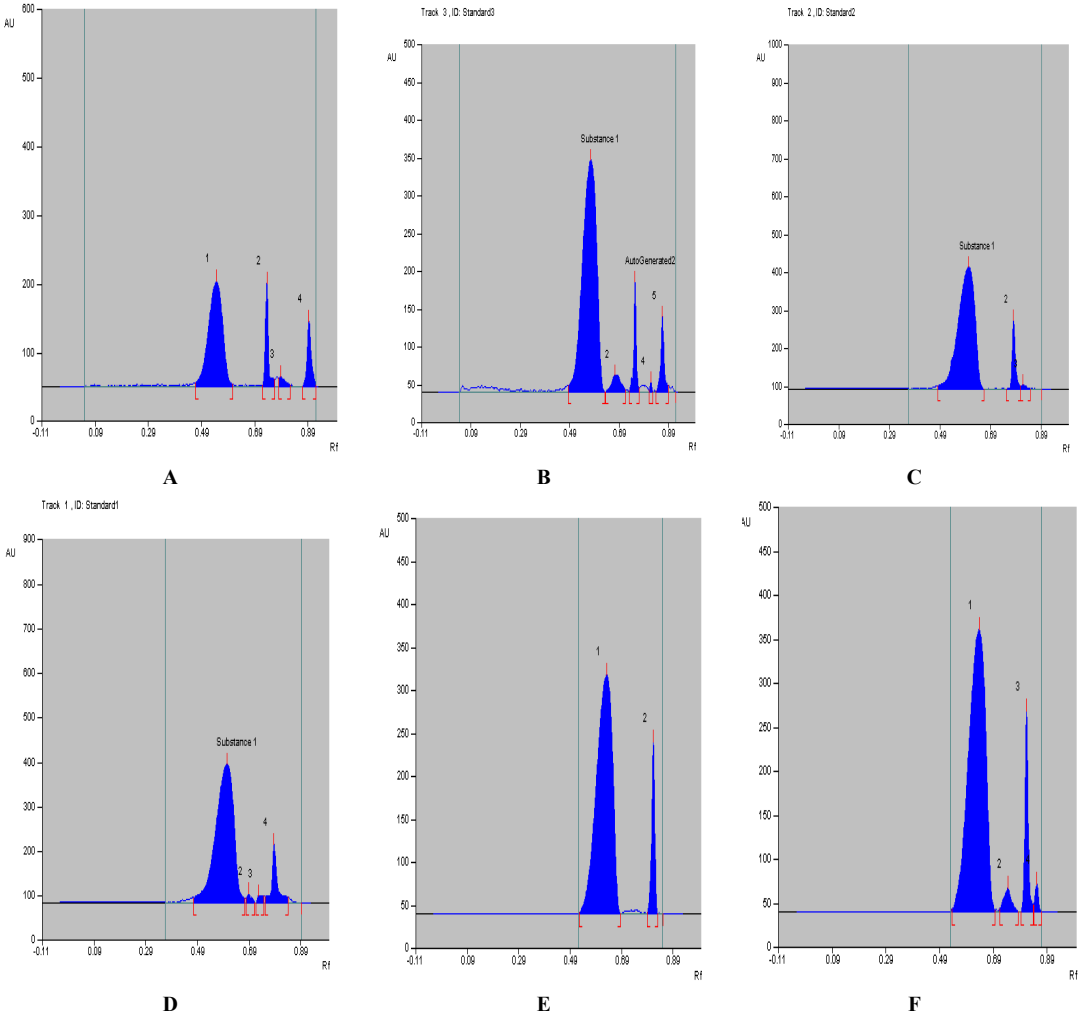

Figure 8: Densitogram of MET and TENE (A) Acid treated (B) Alkali treated (C) Thermal degradation (D) Oxidative degradation (E) Photolytic degradation (F) Stability study

Table 5: Summary of Forced degradation study

Degradation parameters / Condition	% drug estimated		% drug degradation		Rf of degraded products
	MET	TENE	MET	TENE	
Acid hydrolysis (1 N HCl at 60^0 C for 2 hrs)	80.28	73.73	19.72	26.27	0.87, 0.89
Alkaline hydrolysis (0.5 N NaOH at 60^0 C for 2 hrs)	83.53	92.03	16.46	7.97	0.69, 0.87, 0.89
Oxidative degradation (3% H_2O_2) at 60^0 C for 2 hrs)	87.12	72.06	12.88	27.94	0.63, 0.68
Thermal degradation (80^0 C for 1 hr)	90.99	91.23	9.01	8.77	0.74
Photolytic degradation (at 254 nm for 24 hrs)	-	-	-	-	-
Stability chamber (40^0 C / 75 % RH for 28 days)	91.84	54.6	8.16	45.4	0.84, 0.89

RESULT AND DISCUSSION

A simple HPTLC method was developed for the simultaneous estimation of MET and TENE in the presence of their degradation products. Stability studies were performed for both the drugs. Aqueous alkaline degradation was found to affect the stability of the drugs.

HPTLC method

Optimization of chromatographic conditions

Standard solution of MET and TENE was spotted on the TLC plates and run the plates in different solvent system. The mobile phase toluene: methanol: GAA: TEA (5:4:0.5: 0.5) found to give good resolution with Rf of 0.57 and 0.79 for MET and TENE respectively (Figure 3)

Method Validation

Linearity – The calibration curve for MET showed linearity in the range of 7.5µg – 20.0µg/ band with coefficient of correlation of 0.993 (figure 5) and for TENE it was in the range of 300-800 ng / band for TENE with coefficient of correlation of 0.991 (figure 6). The results are represented in 3D Densitogram (Figure 4A and 4B).

Precision

The precision was expressed in terms of Intraday and Inter day precision. The % RSD of intra – day precision for MET and TENE was found to be 0.085 & 0.74 respectively while inter day precision was found to be 0.73 & 0.82 for MET and TENE respectively. The % RSD of precision was found to be less than 2% and hence the developed method is precise.

Accuracy

Accuracy was calculated in terms of % recovery. The recovery study was performed by standard addition technique at 3 different levels i.e. 80% – 120% by the proposed method. The percent recovery for MET found in the range of 99.10 – 101.64 % and for TENE it was 98.50 – 99.93 % which shows that developed method is accurate. (Table 2)

Limit of detection and quantitation

The LOD and LOQ were calculated using the standard deviation of response (N) and from the slope of the calibration curve (S) using following formula. The LOD and LOQ for MET was found to be 0.0244 and 0.07399 µg / band and for TENE was found to be 0.0267 and 0.0811 ng / band respectively. (Table 3)

Assay of marketed formulation

The developed method was used to assay the marketed formulation containing MET and TENE in combination. The percent amount of MET and TENE in marketed formulation was found to be 98.61 and 97.98 % respectively (Table 4). The densitogram of marketed formulation do not show any additional peak except for the MET and TENE which shows that the excipients and other additives do not interfere in the assay of MET and TENE (Figure 7)

Analysis of forced degradation samples

MET and TENE were subjected to various conditions for the degradation like acidic, alkaline, oxidative degradation, thermal, photolytic and stability chamber. The developed method was used to measure the analyte response in the presence of its degradation products.

The summary of degradation is given in Table 5. From the densitogram of degradation under different conditions, it was observed that MET and TENE degrades under acidic, alkaline, oxidative, and photolytic stress conditions (Figure8: A-F).

CONCLUSION

Stability indicating HPTLC method was developed and validated using aluminium plate precoated with silica gel as stationary phase and toluene: methanol: GAA: TEA (5 : 4 : 0.5 : 0.5) as mobile phase . The developed method quantifies the MET and TENE in the presence of their degradant products formed in different stress conditions like acidic, alkaline, oxidative, Photolytic and excipients which indicates that the developed method was found to be selective for the estimation of TENE in dosage forms. The developed method was simple precise and accurate and can be applied for the assay of pharmaceutical in dosage form.

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