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Research Article

Development and validation of HPTLC method for simultaneous estimation and stability indicating study of metformin HCl and linagliptin in pharmaceutical formulation

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ABSTRACT

A simple, precise, rapid, selective, and economic high-performance thin layer chromatographic method has been established for simultaneous estimation of Metformin Hydrochloride and Linagliptin in formulation. The chromatographic separation was performed on precoated silica gel 60 GF_{254} plates with acetone-methanol-toluene-formic acid 4:3:2:1 (v/v/v/v) as mobile phase. The plates were developed to a distance of 8 cm at ambient temperature. The developed plates were scanned and quantified at their single wave length of 259 nm. Experimental conditions such as band size, chamber saturation time, migration of solvent front, slit width, etc. was critically studied and the optimum conditions were selected. The drugs were satisfactorily resolved with R_f 0.61 and 0.82 for metformin hydrochloride and linagliptin respectively. The method was validated for linearity, accuracy, precision, and specificity. The calibration plot was linear between 400-2000 (ng/spot) and 20-100 (ng/spot) for metformin hydrochloride and linagliptin respectively. The limits of detection and quantification for metformin hydrochloride and linagliptin 20 (ng/spot) and 10 (ng/spot) respectively.

Keywords: *Metformin, Linagliptin, HPTLC, Stability indicating assay*

INTRODUCTION

Metformin (MET) is chemically **N,N-Dimethyl imidodicarbonimidicdiamide** (fig 1). The mechanism of Metformin action in the treatment of diabetes involves the inhibition of hepatic gluconeogenesis and the stimulation of glucose uptake in muscle. These effects are achieved by AMPK-mediated transcriptional regulation of genes involved in gluconeogenesis in the liver and those encoding glucose transporters in the muscle, such as peroxisome proliferator-activated receptor-g coactivator 1a (PGC-1a) and glucose transporter type 4 (GLUT4), respectively. Consequently, metformin enhances insulin sensitivity and lowers fasting blood glucose and insulin in diabetes.

Linagliptin (LINA) is chemically known as 1H-Purine-2,6-dione, 8-((3R)-3 aminopiperidin-1-yl)-7-(2-butyn-1-yl)-3,7-dihydro-3-methyl-1-((4-methylquinazolin-2-yl) methyl) (fig 2). Linagliptin is an oral drug that reduces blood sugar (glucose) levels in patients with type 2 diabetes. From the extensive literature review it was found that, many HPLC methods were reported for the estimation of metformin with various drug combinations [1-6]. A Spectrophotometric method [7] and many RP-HPLC methods and stability indicating study has been reported for the simultaneous estimation of Metformin Hydrochloride and Linagliptin [8-10]. A Validated HPTLC Method for Simultaneous Estimation of Metformin Hydrochloride, Atorvastatin and Glimepiride in Bulk Drug and Formulation was reported [11]. But no information related to stability- indicating HPTLC method for this drug combination (MET & LINA) has ever been mentioned in literature. Based on the above facts, HPTLC method was developed and validated for the simultaneous estimation and stability indicating study for the combination of Metformin Hydrochloride and Linagliptin.

MATERIALS AND METHODS

Materials: Linagliptin and Metformin drug samples (pure) are procured as gift sample from Eli Lilly, Delhi. and tablet dosage form is procured from Boheringer Eli Lilly pharma.

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Instrumentation: The samples were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60 F-254, (20×10) cm with 250 µm thickness; E.Merck, Germany using a Camag Linomat IV (Switzerland). The mobile phase consisted of acetone-methanol-toluene-formic acid (4:3:2:1). The plates were prewashed by methanol and activated at 120°C for 5 min prior to chromatography. Samples were applied as bands 6mm long at 5 mm intervals under a stream of nitrogen. The slit dimensions were 6 × 0.90 mm and sensitivity was kept at auto mode. A constant application or spraying rate of 10 s μl^{-1} and scanning speed 20 mm/sec was employed. Linear ascending chromatogram development to distance of 8 cm was performed in 20×10 cm twin trough TLC developing chamber (Camag) at room temperature and previously saturated for 30 min with mobile phase. Subsequent to the development, TLC plates were dried at 100°C. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 259 nm. The source of radiation utilized was deuterium lamp.

Calibration curves: A stock solution of metformin hydrochloride and linagliptin 4000 ng/spot and 20 ng/spot was prepared in methanol respectively. Different volumes of stock solution were spotted on the TLC plate to obtain concentrations 4000 to 20000 ng/spot and 20 to 100 ng/spot for metformin hydrochloride and linagliptin respectively. The data of peak area versus drug concentration was treated by linear least square regression analysis and was selected as working range for the assay and recovery.

Method Validation

a) Accuracy: Accuracy of the method was determined by recovery experiments. The reference standards of the respective drug were added to the sample solution 4000 (ng/spot) of MET and 20 (ng/spot) of LINA at the level of 50%, 100% and 150%. These were further diluted by procedure as followed in the estimation of formulation. The concentrations of the drugs present in the resulting sample solution were determined by using assay method.

b) Linearity and range: From the standard stock solutions, a suitably mixed standard solution was prepared. The solutions were examined by the assay procedure. The calibration curve was plotted using peak area vs concentration of the standard solution. From the calibration curve, the slope and intercept were calculated.

c) Precision: Precision of the method was determined by:
Intra-day precision
Inter-day precision
Repeatability

a) Intra-day Precision: Intra-day precision was found out by carrying out the analysis of the standard drug solutions at concentration of 4000-12000 (ng/spot) of MET and 20-60 (ng/spot) of LINA for three times on the same day. The Percentage RSD was calculated.

b) Inter-day precision: Inter-day precision was found out by carrying out the analysis of the drug solution at a concentration of 4000-12000 (ng/spot) of MET and 20-60 (ng/spot) of LINA for three different days and the percentage RSD was calculated.

c) Repeatability: Repeatability of measurement of the peak area was determined by spotting 8000 (ng/spot) MET and 40 (ng/spot) LINA of drug solution on a pre-coated TLC plate. The separated spots were scanned five times without changing the position of the plate and the percentage RSD was calculated.

d) Limit of Detection (LOD) and Limit of Quantification (LOQ): The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a standard which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were experimentally verified by the known concentration of a standard solution of Metformin Hydrochloride and Linagliptin until the average response approximately 3 or 10 times the standard deviation of the responses for five replicate determinations.

e) Specificity: The peak purity of the Metformin Hydrochloride and Linagliptin was assessed by comparing the spectra at three different levels, viz. peak start, and peak apex and peak end positions of the spot.

f) Robustness of the method: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small deliberate changes in the developed method. In the present study change in the mobile phase composition, development distance, detection wavelength and slit dimension were slightly changed and the effects on the results were examined.

g) Ruggedness: It expresses the precision within laboratory variations like different days, different analyst, and different equipments. Ruggedness of the method was assessed by spiking the standard concentrations of MET 8000 (ng/spot) and LINA 40 (ng/spot), five times in two different days with different analyst.

Stress Degradation study of metformin hydrochloride and linagliptin: A stock solution containing 500 mg metformin and 2.5 mg of linagliptin in 10 ml methanol was prepared. This solution was diluted with methanol to get the final concentration of 12000 (ng/spot) for MET and 30 (ng/spot) for LINA. The formulation was used for forced degradation to provide an indication of specificity of the proposed method. All the degradation studies (acid and base, hydrogen peroxide, and photolytic degradation) were performed as per ICH guidelines [12-14] and the average peak areas of both MET and LINA after injection of five replicates were recorded.

RESULTS AND DISCUSSION

HPTLC method development and validation: The TLC procedure was optimized with a view to develop a stability indicating assay method. Both the pure and degraded products were spotted on the HPTLC plate and run in different solvent systems. The mobile phase containing acetone-methanol-toluene-formic acid (4:3:2:1) gave good resolution, sharp, and symmetrical peak with R_f value of 0.61 and 0.82 for metformin hydrochloride and linagliptin respectively. It was observed that prewashing of HPTLC plates with methanol and pre-saturation of TLC chamber with mobile phase for 5 min to ensure good reproducibility and peak shape of both metformin hydrochloride and linagliptin.

Validation: Using the optimized chromatographic conditions, the HPTLC method developed was validated in terms of linearity, LOD, LOQ, precision, accuracy and specificity.

Analysis of Formulation: The percentage of drug in formulation, mean and relative standard deviation were calculated. The result of analysis showed that the amount of drug present in the formulation is in good correlation with the label claim of the formulation (Table 1).

Linearity: Metformin Hydrochloride and Linagliptin were found to be linear in the range of 4000 to 20000 (ng/spot) and 20 to 100 (ng/spot) respectively (Table 2, Fig 5-10). The correlation coefficient of Metformin Hydrochloride and Linagliptin were found to be 0.9907 & 0.999 respectively (Fig 3 and 4**)**.

Accuracy (Recovery studies): The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulations at 50%, 100% and 150% levels. The recovery studies were carried out 6 times of each level and the percentage recovery and percentage relative standard deviation were calculated and given in Table 3**.** The percentage recovery of Metformin Hydrochloride and Linagliptin were found to be in the range of 99.93-100 % and 99-100.9 % respectively. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.

Precision: The precision of the method was determined by studying reproducibility and repeatability. The area of drug peaks and percentage relative standard deviation of intraday and inter day were calculated and presented in Table 4. The results revealed that the developed method was found to be reproducible in nature.
Acceptance criteria: The results complied with an acceptance criteria since the percentage relative standard deviation of peak areas of MET and LINA were found to be within the limit ie, NMT 2%.

Repeatability
The results complied with an acceptance criteria, since the percentage relative standard deviation was found to be within limit ie, NMT 2% (Table 5).

Ruggedness: The sample was analyzed by a different chemist and same instruments on a different day had been performed (Table 7). The method is rugged since the percentage relative standard deviation was found to be within the limit ie, NMT 2%.

Robustness: The Robustness studies were performed for the standard solutions and presented in Table 8. This method was found to be robust because the % recovery was within the limit of ± 2%.

Stress Degradation Studies: Complete degradation pathway of the drugs was established using stress degradation technique employing HPTLC method as shown in Table 9.
Base hydrolysis: Base degradation with 0.1M NaOH, 1M NaOH and 2M NaOH for 3 h at 80˚C resulted in complete degradation of LINA and 59.03 to 80.27% hydrolysis of MET with a additional peak for degradation product were observed. (Fig 11, 12 & 13)

Acid Hydrolysis: Severe hydrolytic degradation was observed in acidic (0.1M HCl, 1M HCl and 2M HCl) condition at 80°C for 3 h. Complete degradation of LINA and 42.54 to 42.63% degradation of MET were observed. (Fig 14, 15 & 16).

Oxidation: Oxidative degradation was performed for formulation with 3% hydrogen peroxide at 80ºC for 3 hrs. The complete degradation of LINA and 53.36% degradation of MET were observed under oxidative condition (Fig. 17).

Photolysis: Photolysis was performed for the formulation under direct sun light for 48 h. The complete degradation of LINA and 23.43% degradation of MET were observed. (Fig 18)

CONCLUSION

The developed method is found to be simple, rapid, sensitive, specific, accurate, and reproducible and this method can be used for routine estimation and also for stress degradation study of metformin hydrochloride and linagliptin in pharmaceutical formulation.

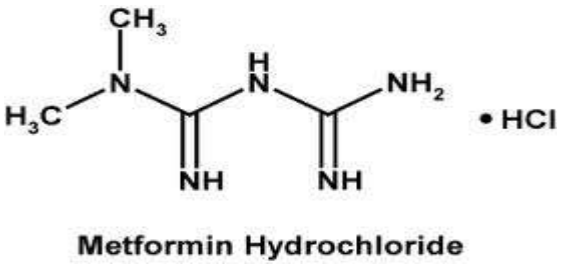

Fig: 1 Metformin hydrochloride

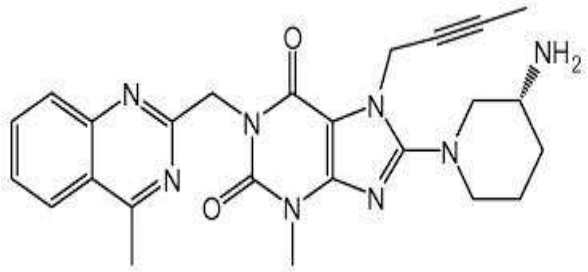

Fig: 2 Linagliptin

Table 1. Assay of Metformin HCl and Linagliptin tablet Dosage Form

Formulation	Labelled amount (mg)		Amount Found (mg)		Percentage assay (%)		%R.S.D*	
	MET	LINA	MET	LINA	MET	LINA	MET	LINA
	500	2.5	499.69	2.51	99.74	100.2	0.56	0.51

*** mean of five observations**

Table 2. Linearity range of Metformin HCl and Linagliptin

Concentration µg/ml (ng/spot)	MET		Concentration (ng/spot)	LINA	
	R_f value	Peak area*		R_f value	Peak area*
4000	0.56	7125	20	0.76	2546
8000	0.56	9693	40	0.77	4048
12000	0.58	12463	60	0.78	5545
16000	0.57	15019	80	0.78	7019
20000	0.56	17962	100	0.77	8539

*** mean of five observations**

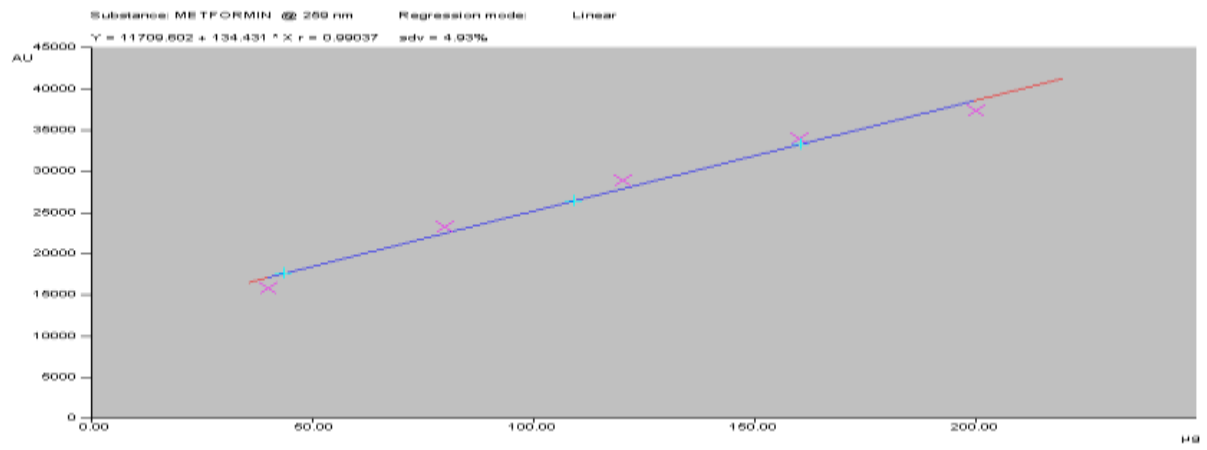

Fig 3. Calibration curve for Metformin Hydrochloride

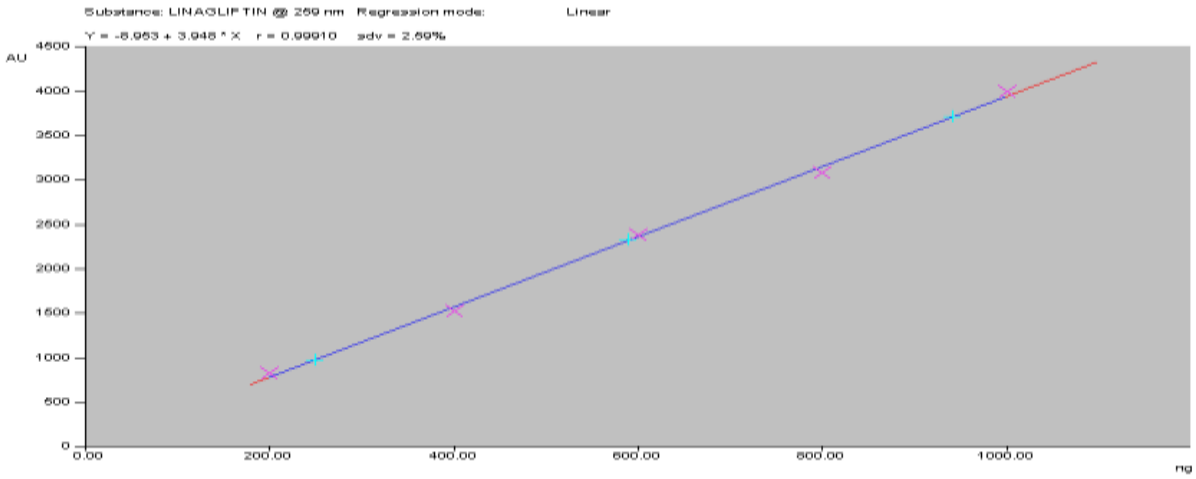

Fig 4. Calibration curve for Linagliptin

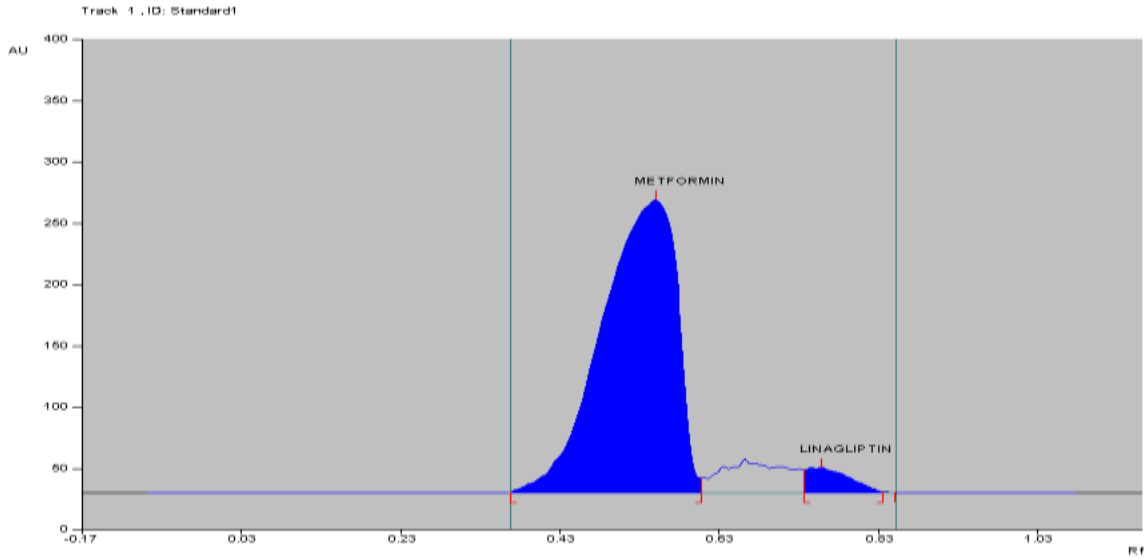

Fig 5. Chromatogram of standard (4000 ng/spot of MET and 20 ng/spot LINA)

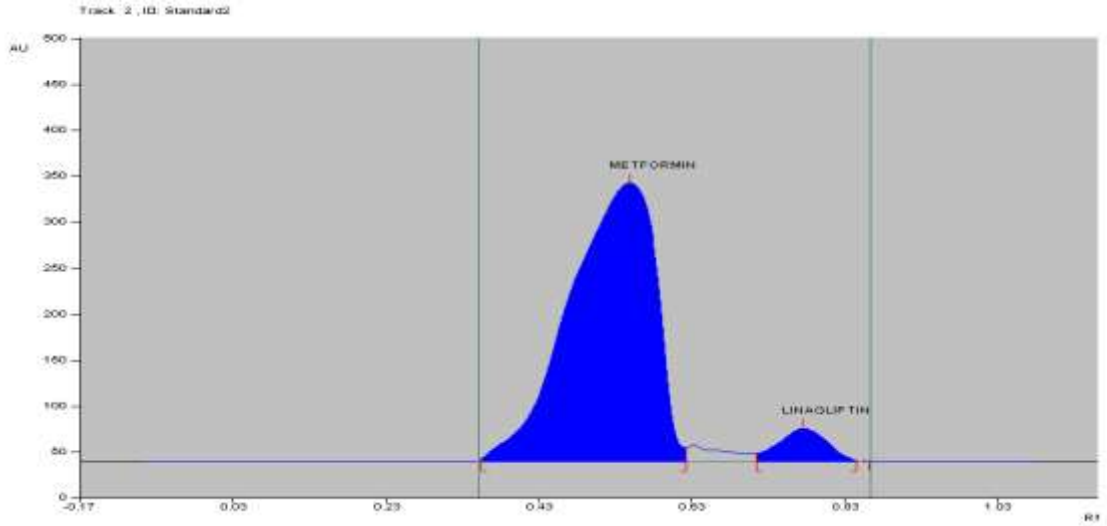

Fig 6. Chromatogram of standard (8000 ng/spot of MET and 40 ng/spot LINA)

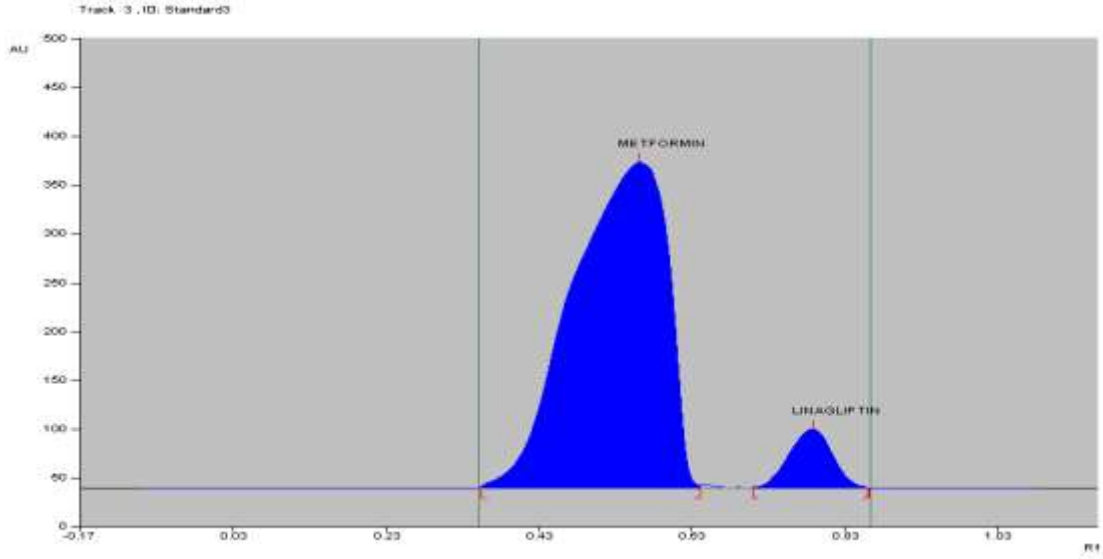

Fig 7. Chromatogram of standard (12000 ng/spot of MET and 60 ng/spot LINA)

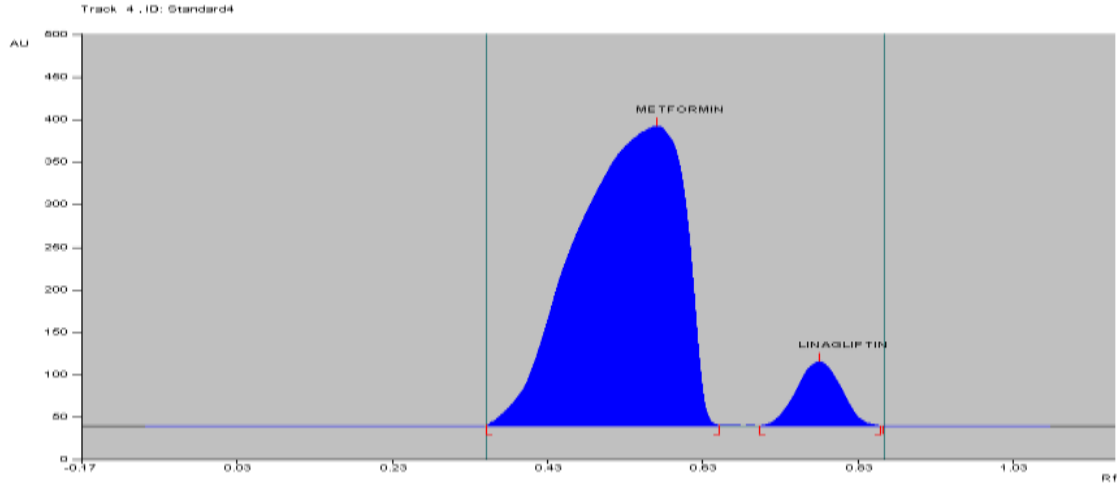

Fig 8. Chromatogram of standard (16000 ng/spot of MET and 80 ng/spot LINA)

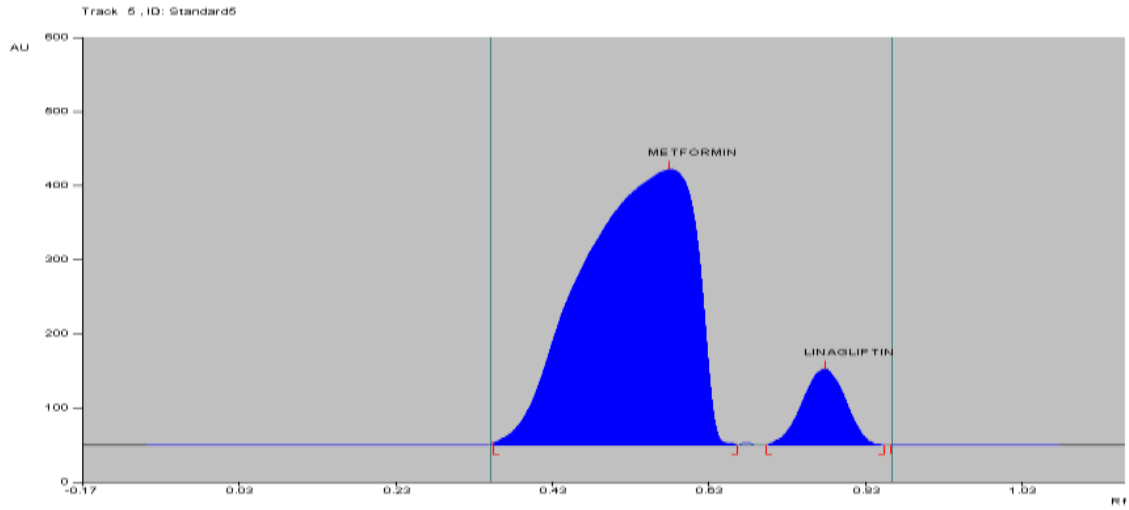

Fig 9. Chromatogram of standard (20000 ng/spot of MET and 100 ng/spot LINA)

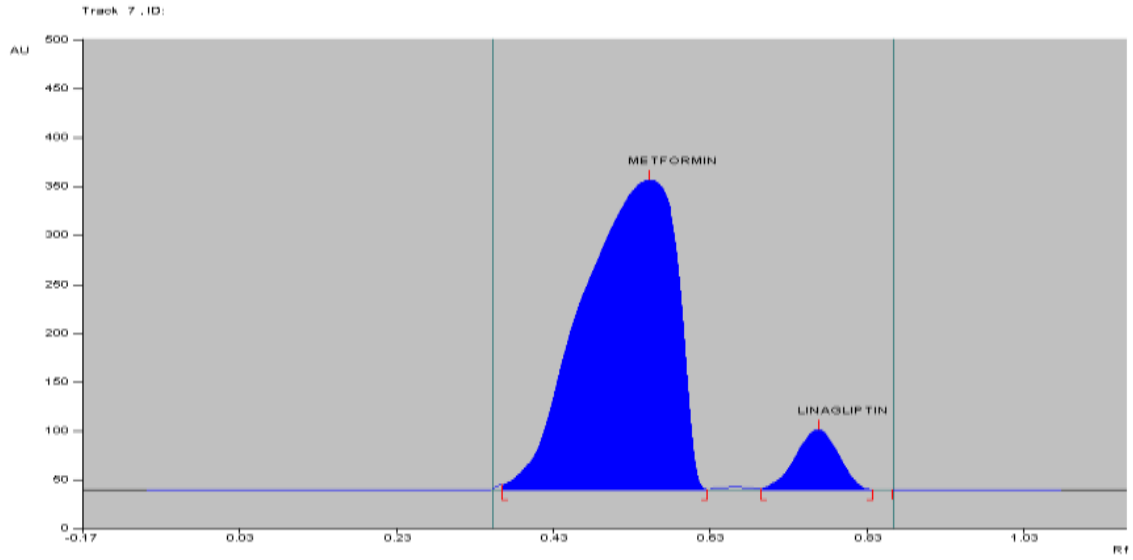

Fig 10. Chromatogram of sample (12000 ng/spot of MET and 60 ng/spot LINA)

Table 3. Accuracy (Recovery studies)

Drug	Label Claim mg/tab	Spike Level (%)	Amount of drug added (µg /ml) (ng/spot)	Amount of drug recovered (µg/ml) (ng/spot)	Percentage Recovery	%RSD*
MET	500	50	2000	1998.92	99.95	0.98
		100	4000	3997.1	99.93	1.02
		150	6000	6000.1	100	0.75
LINA	2.5	50	10	10.09	100.9	0.76
		100	20	19.8	99	0.81
		150	30	29.7	99.01	0.92

***mean of five observations**

Table 4. Intra-day and inter-day precision of the developed method

Concentration (ng/spot)	Intraday			Interday		
	Peak area*	SD	%RSD*	Peak area*	SD	%RSD*
MET						
4000	15839	64.83	0.94	15734	66.46	1.13
8000	23513	61.15	0.72	23821	59.61	0.75
12000	29009	75.49	0.57	28982	73.48	0.69
LINA						
20	821	33.38	1.04	852	20.88	0.93
40	1550	36.26	0.98	1652	53.03	1.51
60	2412	75.39	0.92	2541	83.69	1.41

***mean of five observations**

Table 5 . Repeatability

Conc MET (ng/spot)	Peak Area*	% RSD*	Conc. LINA (ng/spot)	Peak Area*	% RSD*
8000	23479	0.73	40	1652	0.78

***** mean of five observations**

Table 6. LOD and LOQ

Parameter	MET (ng/spot)	LINA (ng/spot)
LOD	20	10
LOQ	100	20

*** mean of five observations**

Table 7. Ruggedness

Drug	Concentration (ng/spot)	Mean Peak area*	% R.S.D*
Day I, Analyst I			
MET	8000	23513	0.85
LINA	40	1550	1.04
Day II, Analyst II			
MET	8000	24528	0.89
LINA	40	1682	0.96

***mean of five values.**

Table 8. Robustness studies

Parameter	Modification	MET Recovery (%)	LINA Recovery (%)
Mobile Phase Ratio	4:4:2:1	98.96	99.54
	5:3:2:1	99.32	100.2
Development Distance	9 mm	99.42	98.97
Detection Wavelength(nm)	257 nm	99.81	99.42
Slit Dimension	5.00 x .30m micro	99.65	99.53

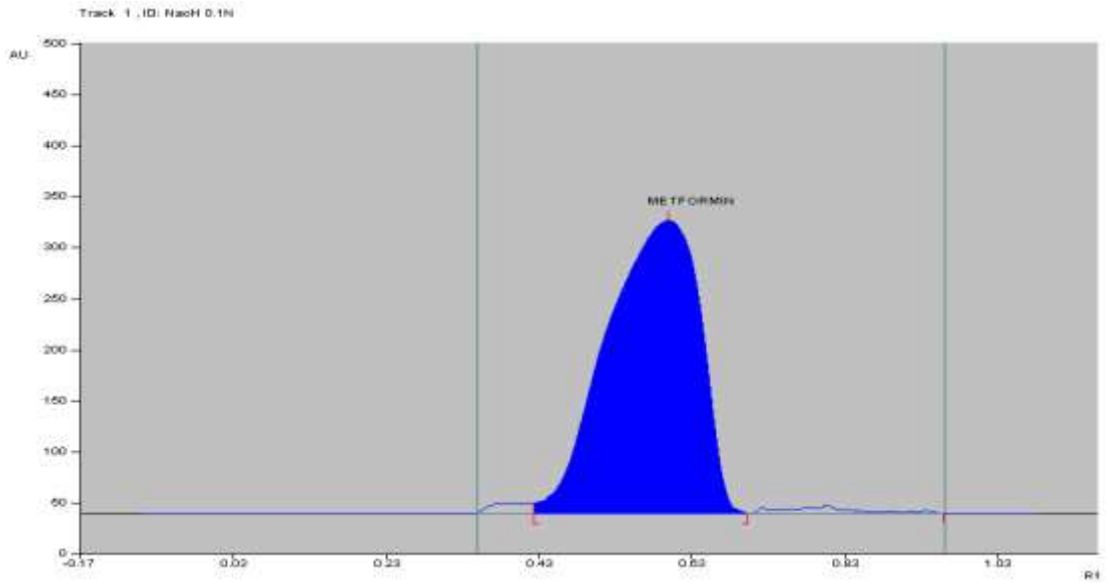

Fig 11. Densitogram of MET and LINA subjected to alkali degradation in 0.1N NaOH

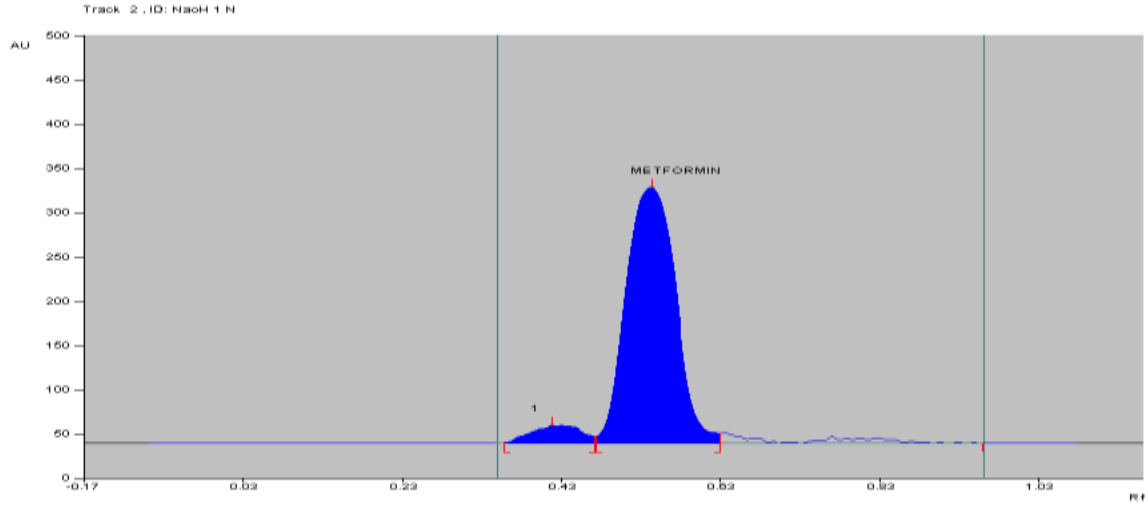

Fig 12. Densitogram of MET and LINA subjected to alkali degradation in 1N NaOH

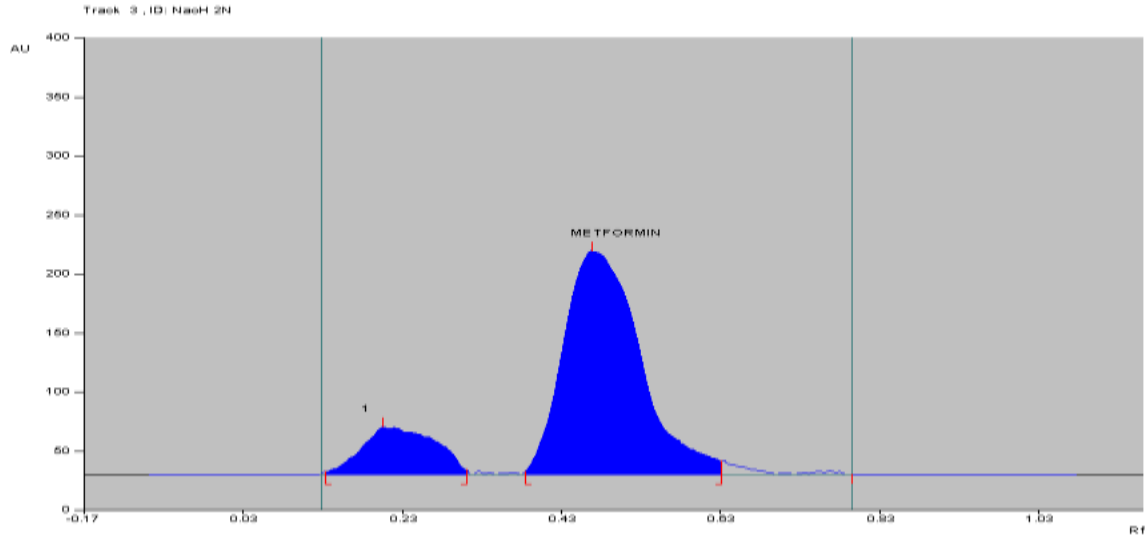

Fig 13. Densitogram of MET and LINA subjected to alkali degradation in 2N NaOH

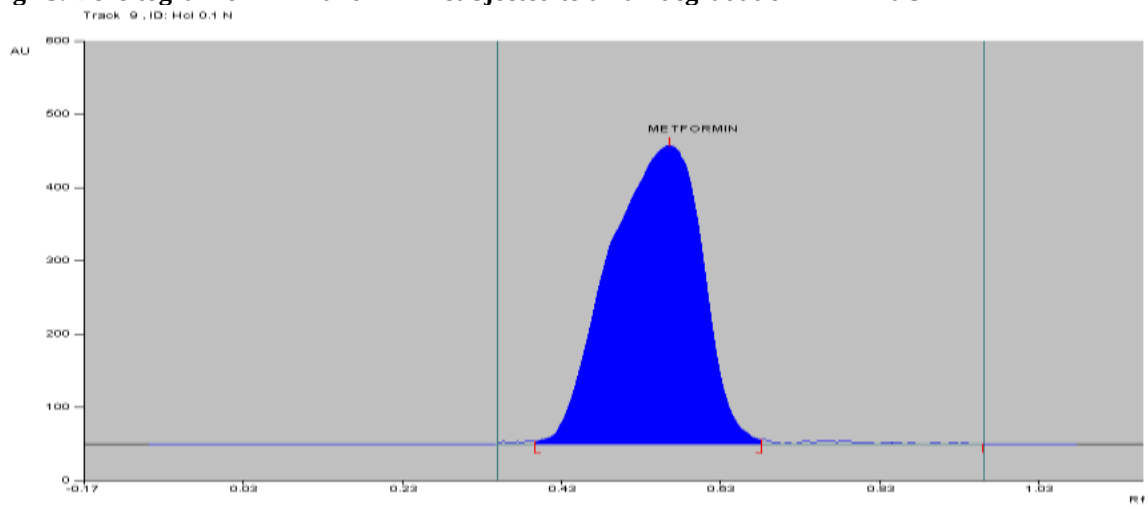

Fig 14. Densitogram of MET and LINA subjected to acid degradation in 0.1N HCl

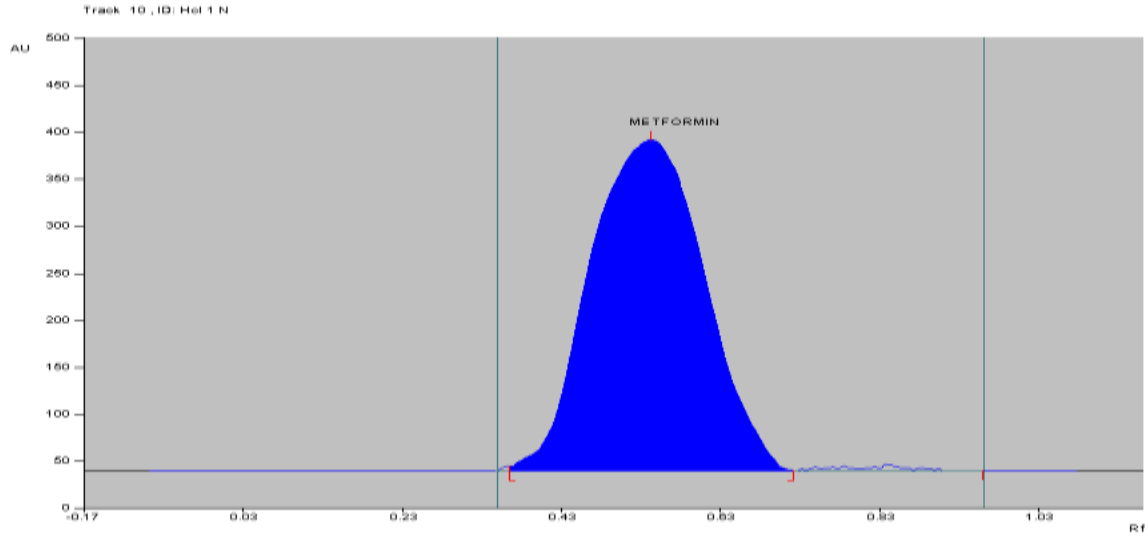

Fig 15. Densitogram of MET and LINA subjected to acid degradation in 1N HCl

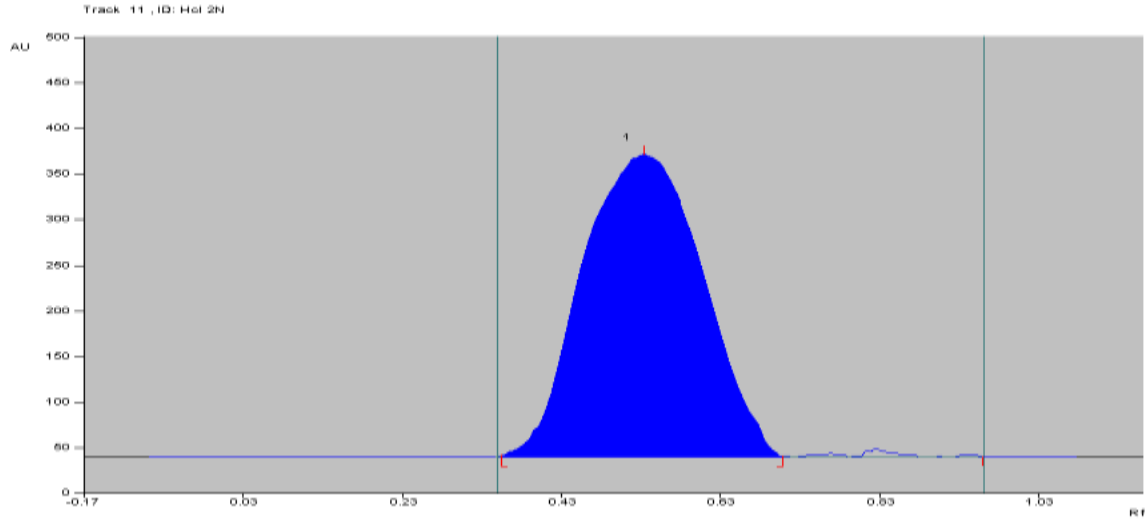

Fig 16. Densitogram of MET and LINA subjected to acid degradation in 2N HCl

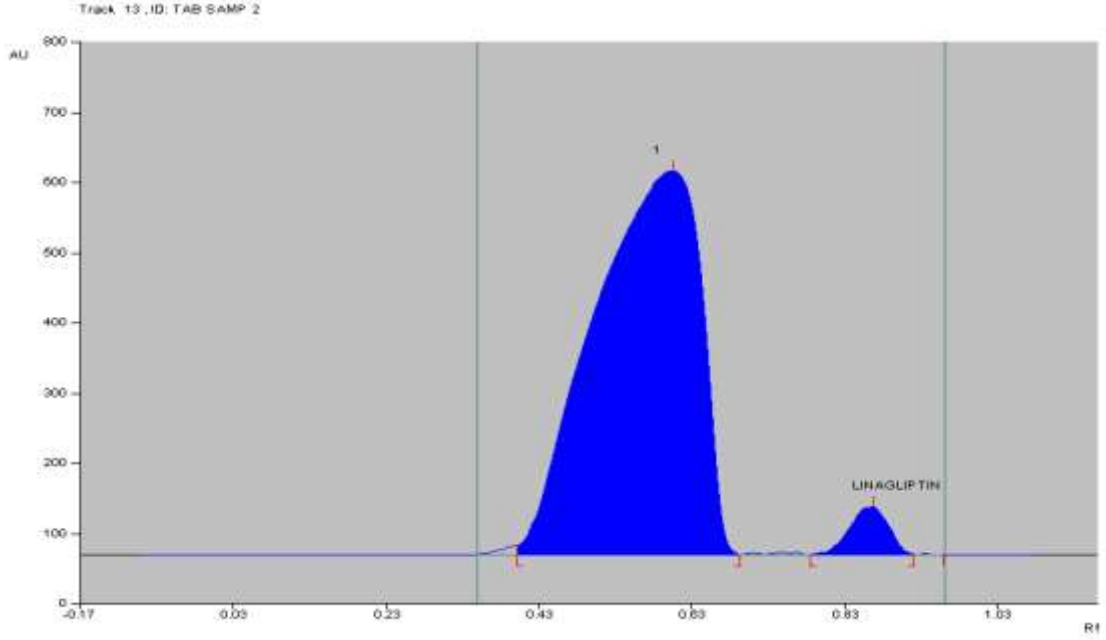

Fig 17. Densitogram of MET and LINA subjected to Oxidation by H_2O_2

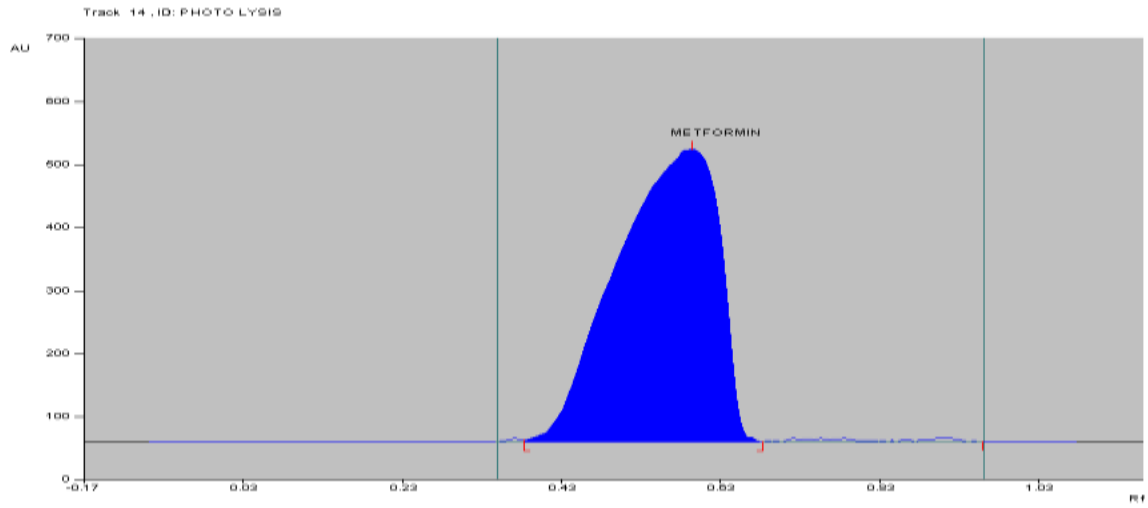

Fig 18. Densitogram of MET and LINA subjected to Photolysis

Table 9. Results of forced degradation studies on MET and LINA

Stress condition/Duration/State	Degradation (%)	
	MET	LINA
Basic/ 0.1N NaOH / 3 h/solution / 80ºC	59.03	100
Basic / 1N NaOH / 3 h/solution / 80ºC	77.37	100
Basic / 2N NaOH / 3 h/solution / 80ºC	80.27	100
Acidic/0.1N HCl/ 3 h/solution/80ºC	42.54	100
Acidic/ 1N HCl/ 3 h/solution/80ºC	42.59	100
Acidic/ 2N HCl/ 3 h/solution/80ºC	42.63	100
Oxidation / 3% H_2O_2/ 3 h /solution / 80ºC	53.36	100
Photolysis / direct sun light	23.43	100

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