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# Stability Indicating RP-HPLC Method for Determination and Validation of Repaglinide in Pharmaceutical Dosage Form

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**Abstract:** A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of Repaglinide in pure and tablet forms. The drug was found almost stable to neutral and photolytic condition. Resolution of drug and the degradation products formed under different stress conditions were successfully achieved on a Luna $C_{18}$ (5µm×25cm×4.6mm) column utilizing mixture of Acetonitrile and potassium dihydrogen phosphate buffer (pH 4.5 adjusted with orthophosphoric acid) in the ratio of 60:40 (*v/v*) as mobile phase at a flow rate of 1.0 ml $min^{-1}$ and detection was performed at 278 nm. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, forced degradation, solution stability and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed; the % RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.
**Keywords:** Repaglinide, RP-HPLC, Degradation studies.

## Introduction

Stability indicating methods have become an important aspect of any analytical method validation and a part of US FDA requirements [1]. Repaglinide is a meglitinide antidiabetic used in the management of type 2 diabetes mellitus, chemically *S*(+)2-ethoxy-4(2((3- methyl-1-(2-(1-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid [2-3]. It is official in USP [4] which describes liquid chromatographic method for its quantitation. Literature survey reveals that one HPLC method in human plasma [5], two HPLC [6-7], one RPTLC [8] and one spectrophotometric method [9] in pharmaceutical dosage form. In recent times, there is an increase tendency towards the development of stability-indicating assay, using the approach of stress testing as mentioned in the ICH guidelines (Q1A). It also recommends carrying out of stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure.

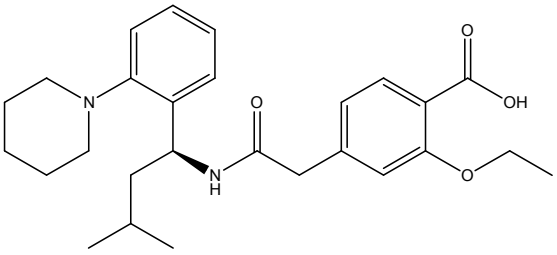


**Fig: 1 Chemical Structure of Repaglinide**

The stress testing encompasses the influence of temperature, humidity, light, oxidizing agent as well as susceptibility over a wide range of pH values [10, 11]. The purpose of this work was to develop and validate simple, specific, sensitive, accurate, precise, rapid and cost effective HPLC method for the estimation of Repaglinide in bulk and its formulations. Therefore, we have made an attempt to develop a new, simple, accurate stability indicating RP-HPLC method for the determination of Repaglinide in pure and tablet forms.

## Experimental

All analytical works were performed on Shimadzu HPLC LC 2010 CHT series equipped with quaternary constant flow pump, auto injector, SPDM 10 A VP Shimadzu Photo Diode array detector, LC Solution Version 1.22 SP1 software, Phenomenex Gemini C18 column (4.6 mm × 25 cm, 5 µm particle size) as stationary phase, a calibrated electronic single pan balance Sartorius CP 225 D, pH Meter of LABINDIA, Enertech Fast Clean Ultrasonic cleaner were also used during the analysis. Single component tablet formulations of repaglinide (2 mg) (formulation A Eurepa, manufactured by Torrent Pharma. Ltd., Ahmedabad) were purchased from local market. All chemicals and reagents used were of AR/HPLC grade, Chloroform, ammonia (SD'S) and methanol (A.R., Ranbaxy Ltd., New Delhi) were used for mobile phase preparation and as solvent.

**Instrumentation:**

Precision mentol heater (Biotech, Mumbai) with temperature regulator equippted with a reflux condencer were used for degradation study in acid, alkali and neutral conditions. Dry air oven was used to study the effect of dry heat. Photolytic study was carried out by exposing the drug to direct sunlight for 8 hrs.The HPLC system equippted with an LC-10 AT *vp* solvent-delivery system with universal loop injector (Rheodyne 7725 i) of injection capacity of 20 µL. Detector consists of photodiode array detector SPD-10 AVP UV-Visible detector. Separation was carried out on a Phenomenex Luna $C_{18}$ (5µm×25cm×4.6mm i.d) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC workstation. The work was carried out in an air-conditioned room maintained at temperature $25\pm2^0$C. Chromatograms were recorded using CLASS-VP software (Shimadzu, Kyoto, Japan).

**Degradation studies:**

Drug at a concentration of 2 mg $ml^{-1}$ was used in all degradation studies. The pH of the potassium dihydrogen phosphate buffer was checked before and after reaction and no change was observed. Conditions employed for stability studies were as follows.

**Hydrolytic studies:**

For acid hydrolysis studies, 2 mg $ml^{-1}$ solution of the drug was prepared by diluting required amount of drug in 0.1N HCl and the solution was refluxed for 8h and then for 12h. Same concentration of drug was subsequently prepared in 1 N HCl and refluxed for 13h. Studies in alkali conditions were done at a drug concentration of 2 mg $ml^{-1}$ in 0.1 N NaOH and the solution was refluxed for 8h. For neutral condition 2mg $ml^{-1}$ solution of the drug was prepared in water and refluxed initially for 10h and subsequently for 14h.

**Oxidative studies:**

For oxidative degradation study, initially 2mg $ml^{-1}$ strength of drug was prepared in 10% $H_2O_2$.The drug was kept under the conditions of room temperature for a period 12h and then for 24h. Subsequently the drug was exposed to 10% $H_2O_2$ at room temperature for a period of 24h.

**Photolytic studies:**

Photolytic study was done by exposing the dry drug to direct sunlight for 10 days [12]. The photochemical stability of the drug was studied by exposing the stock solution (2 mg.mL-$^1$) as well as solid drug to direct sunlight for 10 days on a wooden plank and kept on terrace.

**Thermal (dry heat) studies:**

Susceptibility of the drug to dry heat was studied by exposing the solid drug to $60^0$ C for 15 days in a hot air oven. Sampling was carried out every day to study its degradation behaviour. For all the stability study, the formation of degradable product was conformed by comparing the chromatogram of the degradable mixture with the blank solvent stored under normal condition and the drug solution kept under normal condition.

**Preparation of samples for HPLC analysis:**

For hydrolysis study during 1.0 N HCl, 0.1 N NaOH and oxidative study during 10% $H_2O_2$ the samples were diluted 10 times with water where as the samples were diluted 100 times with water during higher acidic, higher alkali and 20 % $H_2O_2$ conditions. The solid samples for neutral, thermal and photolytic degradation study were suitably diluted with water.

**Separation studies on stressed samples:**

In all HPLC runs, the mobile phase was filtered through 0.2µm nylon membrane under vaccum and degassed before use. The injection volume was 20µl and the mobile phase flow rate was 1.0 ml $min^{-1}$, the

analytical wavelength selected was 278 nm. HPLC studies were carried out on all reaction solution individually. Initially analysis were performed $C_{18}$ column and mobile phase composed of acetonitrile: potassium dihydrogen phosphate buffer (pH 4.5 adjusted with orthophosphoric acid). As the satisfactory resolution of the drug and the degradation products was not achieved, hence to get good resolution the method was further optimized by increasing the ratio of acetonitrile and it was found good resolution in the ratio of 60:40 (*v/v*) of acetonitrile: potassium dihydrogen phosphate buffer.

## Validation of the Method:

Validation of the optimized HPLC method was done with respect to following parameters as per ICH norms [13].

**Linearity and range:**

A stock solution of the drug (2mg $ml^{-1}$) was prepared in water. From this stock solution five concentrations of the drug were prepared within the concentration range of 200-1000 µg $ml^{-1}$. The solutions were injected in triplicate into the HPLC column, keeping all the conditions constant.

**Precision:**

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentration of (200,400,700 µg $ml^{-1}$) drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating the studies on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision).

**Accuracy:**

Accuracy of the method was tested by fortifying a mixture of decomposed reaction solutions with three concentration of the drug and determining the percentage of recovery of added drug.

**Specificity and selectivity:**

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. Where as selectivity was established through determination of purity for each degradation product peak using PDA detector.

## Results and Discussion

**Degradation behaviour of Repaglinide**

HPLC studies of sample obtained on stress testing of Repaglinide under different conditions using acetonitrile: potassium dihydrogen phosphate buffer (60:40) as the mobile solvent system suggested the following degradation behaviour.

***Acidic condition:***

It was observed that the drug gets slowly degraded in strongly acidic conditions over a period of time. On reflux in 0.1 N HCl ( 8hrs) and further for 12h there is no degradation. The degradation of the drug resulted in the rise of one extra peak at 4.261 in 1.0 N HCl (12h) (fig .2c). This indicates that the drug is hydrolysed under acid conditions, to a chromatographic compound.

***Degradation in alkali:***

In alkali, the drug was found to decompose almost 55-70% and then 75-90% after refluxing for 8 h and then continuing 12 h in 0.1 N NaOH respectively. As shown in chromatogram (Fig .2b), degradation of the drug resulted in the rise of one extra peak at 5.162 min.

**Table I Precision studies**

| Actual concentration (µg $ml^{-1}$) | Measured concentration ± S.D. (µg $ml^{-1}$).; %COV | | |
|---|---|---|---|
| | Repeatability (n=6) | Intradayprecision (n=3) | Inter day precision (n=3) |
| 200 | 200.214±0.121; 0.216 | 199.892±0.143; 0.023 | 201.325 ±0.431; 0.143 |
| 400 | 400.138 ±0.432; 0.221 | 399.290±0.218; 0.067 | 400.268±0.284; 0.365 |
| 700 | 700.982±0.241; 0.365 | 700.432±0.782; 0.265 | 699.905±1.204; 0.045 |

**Table II: Recovery studies (n=3)**

| Actual concentration (µg $ml^{-1}$) | Calculated concentration (µg $ml^{-1}$) ±S.D.; % COV | Recovery (%) |
|---|---|---|
| 200 | 200.651 ± 0.652; 0.027 | 100.075 |
| 400 | 400.325 ± 0.314; 0.031 | 100.15 |
| 700 | 700.243 ± 0.265; 0.038 | 100.03 |

**Table III. Summary of degradation study results.**

| Stress condition | Time (hours) | Assay of active substance (%) |
|---|---|---|
| Acidic hydrolysis (0.1 N HCl) | 12 h | 71.3 |
| Basic hydrolysis(0. 1 N NaOH.) | 8 h | 14.9 |
| Aqueous hydrolysis(at 60˚C) | 12 h | 63.1 |
| Oxidation(10% $H_2O_2$.) | 24 h | 59.3 |
| Photolytic studies | 10 days | 99.7 |
| Thermal studies (60˚C) | 10 days | 93.8 |

***Oxidation studies:***

The drug was found to be stable in 10 % $H_2O_2$ for 6h at room temperature. However about 5-10 % drug degradation was observed on exposure to 10% $H_2O_2$ for 24h. One small degradation product peaks at 2.431 min was seen and there was significant rise in the height of the peak with time (fig .2d). This signifies that the drug was degraded in oxidative conditions to chromatographic compounds.

***Photolytic studies:***

Photolytic study was carried out in dry form. Here the drug was directly exposed to the sunlight for 10 days on a hot sunny day. Repaglinide was found to be degraded in very negligible amount with a reduction in peak height (fig .3). Only (~2%) of the drug was found to be degraded in to non-chromatographic product. It shows Repaglinide was almost stable in the photolytic studies.

***Thermal stress study:***

Repaglinide was found to be degraded in negligible amount as two new peaks of degradation products were appear at 1.382 and 2.973 min. Only (8-14%) of the drug was found to be degraded hence Repaglinide was almost stable after exposing the drug to 60 $^0$ C for 10 days.

**Development of stability-indicating method**

It was observed that satisfactory resolution of Repaglinide and its degradation products formed under various conditions was achieved when the analyses was performed by using a mixture of acetonitrile and potassium dihydrogen phosphate buffer ( pH 4.5 adjusted with orthophsophoric acid) in the ratio of 60:40 (*v/v*) as mobile phase at a flow rate of 1.0 ml $min^{-1}$. UV detection was performed at 278nm.

## Validation of the developed stability-indicating HPLC method

The developed method was validated by using following criteria.

***Linearity:***

The response curve of the drug was linear in the concentration range of 200-1000 µg $ml^{-1}$. The mean values of slope, intercept and correlation coefficient were 0.9999 ($r^2$) respectively.

*Precision:*

The results of repeatability and intermediate precision study are given in Table 1. The developed method was found to be precise as the % COV values for repeatability and intermediate precision studies were < 2.

***Accuracy:***

The accuracy of the developed HPLC methods was checked by performing recovery study. The result of the recovery study was given in Table 2. Good recoveries (100.25-101.15%) of the drug and low % COV (< 2) were obtained at each added concentration, indicating that the method was accurate.

***Selectivity:***

The method was also selective to degradation products as all the peaks were pure. The peaks were well separated also.

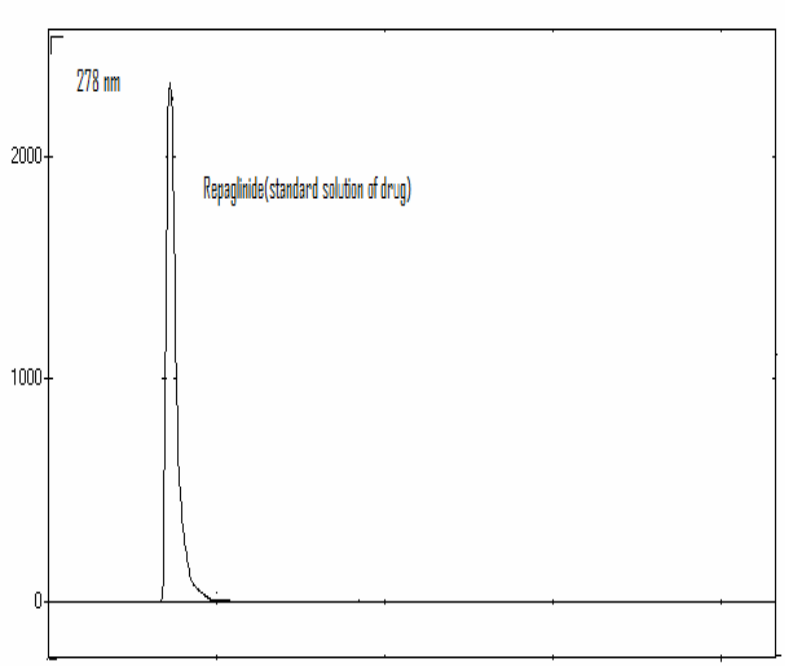


**Fig. 2(a). Representative HPLC chromatograms of Repaglinide, standard solution of drug**

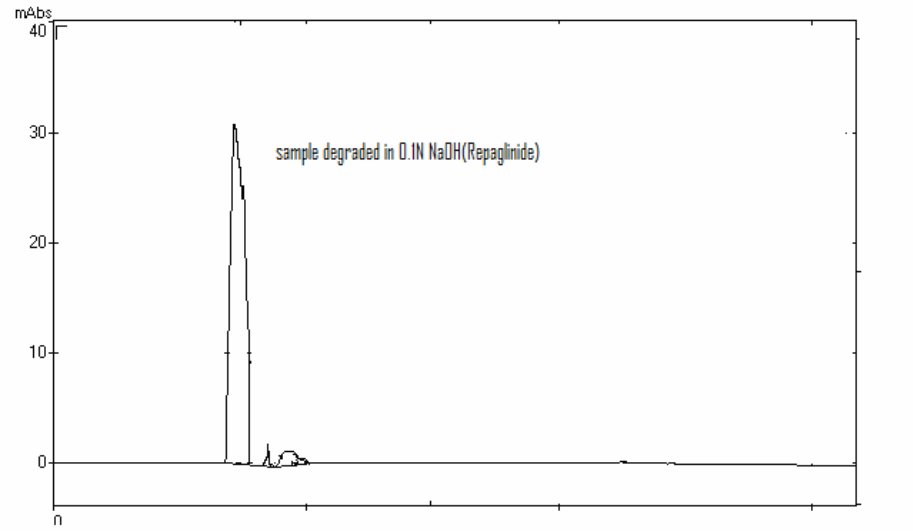


**Fig. 2 (b). Representative HPLC chromatograms of Repaglinide sample degraded in 0.1N NaOH**

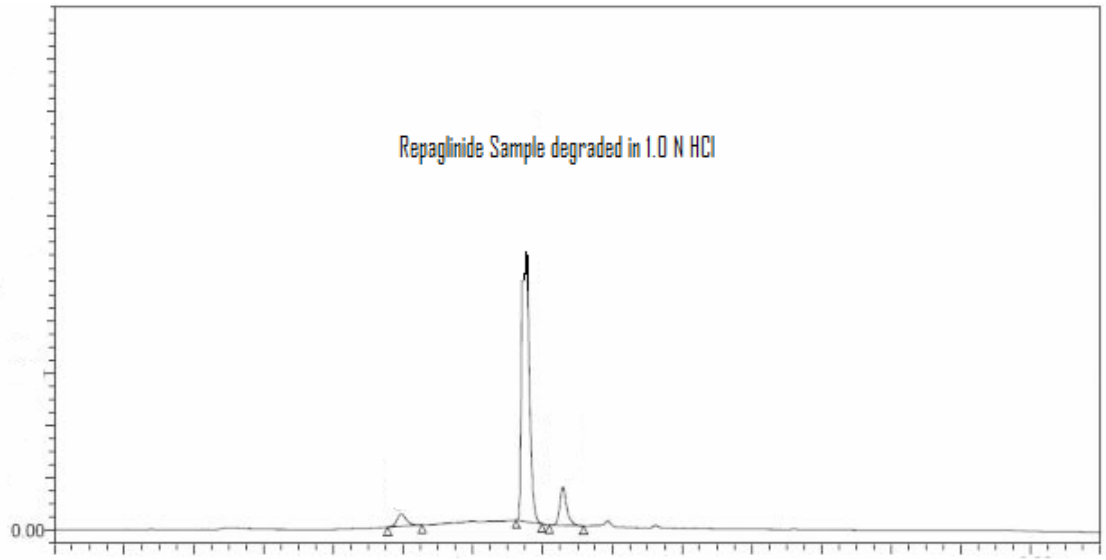


**Fig. 2(c). Representative HPLC chromatograms of Repaglinide Sample degraded in 1.0 N HCl**

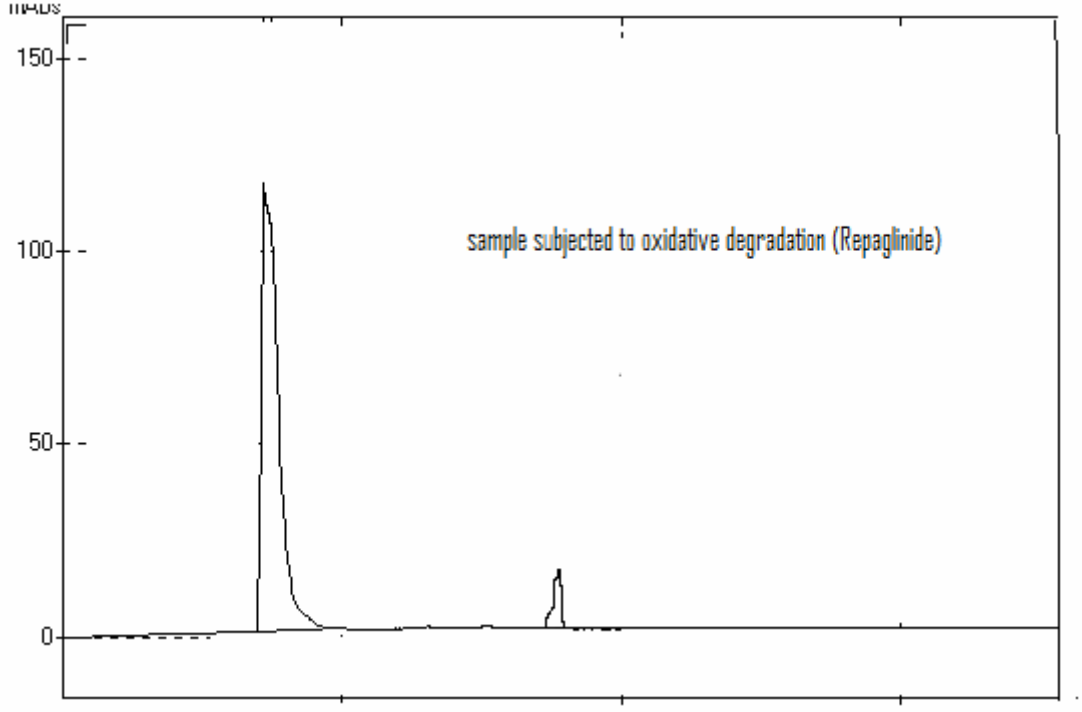


**Fig. 2(d). Representative HPLC chromatograms of Repaglinide Sample subjected to oxidative degradation.**

## Conclusions

In the present study, a stability-indicating assay method for Repaglinide was established by following the ICH recommended stress conditions. The drug was found to be degraded extensively in alkaline medium, where as mild degradation of drug occurred in higher acidic, higher oxidative stress and in the thermal stressed conditions. But the drug was stable in photolytic and neutral stress conditions. A new, reversed-phase HPLC method was developed, which is simple, accurate, precise, selective and specific and can be helpful for the manufacturers to analyses the drug in stability samples.

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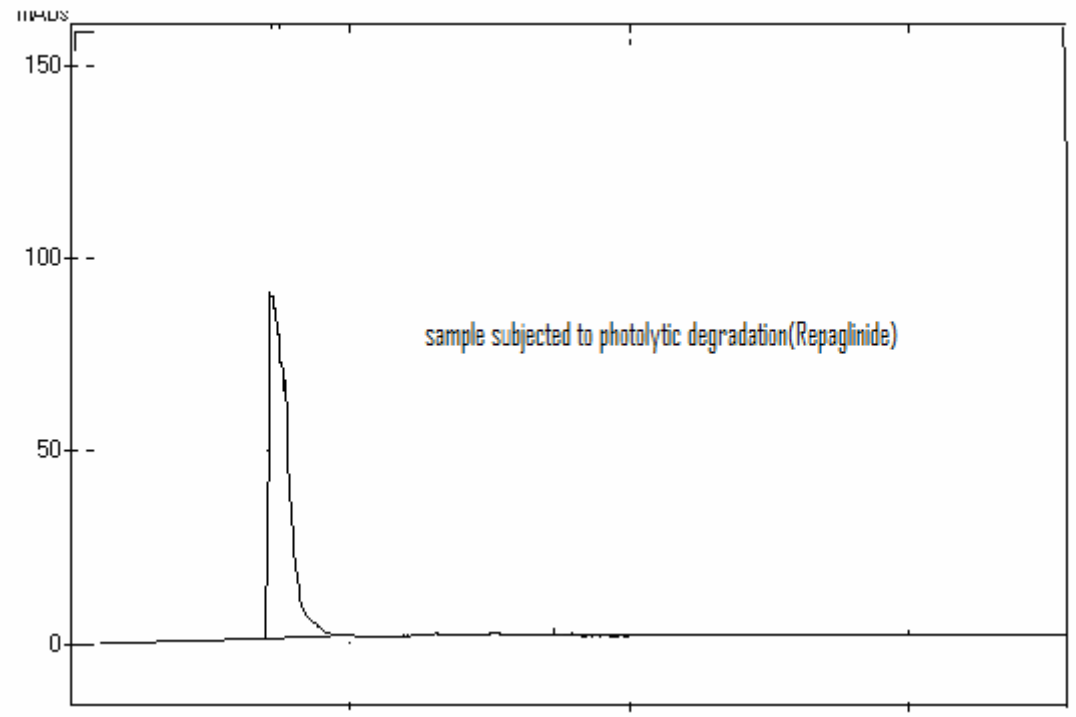


**Fig. 3. Representative HPLC chromatograms of Repaglinide: sample subjected to photolytic degradation in dry form**

## References:

[1]M.D.Rockville, United States Pharmacopoeia; 25th (eds) United States Pharmacopoeial Convention Inc, 2002,2150 ;

[2] S.Budavari, The Merck Index. 13th (eds) Whitehouse Station (NJ, USA) Merck and Co Inc; 2001. pp. 790;

[3] J.E.F. Reynolds, Martindale, 33 rd ed. (London), The Complete Drug Reference
Pharmaceutical Press, 2002 pp. 334;

[4] M .D. Rockville, United State Pharmacopoeia, (Asian eds) USP Convention Inc,
2003. pp. 623;

[5]A.B.Ruzilawati, M.S.Wahab, A.Imran, Z.Ismail, S.H.Gan. J. Pharm. Biomed. Anal, 2007, 43 (5), 1831-1835;

[6]M.Gandhimathi, T.K.Ravi, S.K.Renu. Anal. Sciences, 2003, 19 (12), 1675-1677;

[7] R.H. Khan, S. Talegaonkar, R.M. Singh, S.C. Mathur, R.Shiv,G.N.Singh. Indian.Drugs, 2007,44(6), 428-433.

[8] A .Gumieniczek A, Berecka, H.Hopkała. Journal of Planar Chromatography, 2005, 18 (2), 155-159;

[9] A.Goyal, I. Singhvi. Indian. J. Pharm Sci, 2006,68,656-657;

[10] ICH, Stability testing of new drug substances and products, in: Proceeding of the International Conference on Harmonisation, IFPMA, Geneva, 2003.

[11] M.Bakshi ,S.Singh. J. Pharm. Biomed. Anal, 2002,28, 1011;

[12] S.Singh, M. Bakshi. Pharm. Tech. On-line,2000, 24, 1;

[13] D.D.Hong, M. Shah, J. T. Carstensen,C. T. Rhodes (Eds.), Drug Stability Priniciple and Practices, Marcel Dekker, Inc. New York, 358 (2005)

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