Original research article

ISSN 2321-0125

Journal of Pharmaceutical and BioSciences
JPBS
An International Journal

www.jpbs-online.com

Stability- Indicating HPTLC densitometric method for determination of Metformin hydrochloride in tablet formulation

Jain Pritam*, Girase Devendra, Bedse Anjali, Surana Sanjay

R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist. Dhule 425405 MS, India.

Received on 20 May 2013, Accepted on 03 June 2013, Available online from 10 June 2013

Abstract

A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of metformin hydrochloride both as a bulk drug and in formulation was developed and validated. The method employed HPTLC aluminium plates precoated with silica gel 60F254 as the stationary phase. The solvent system consisted of water: methanol: tri-ethylamine (1:3.5:0.2 v/v). The system was found to give compact spot for metformin hydrochloride (RF value of 0.48±0.02). Densitometric analysis of metformin hydrochloride was carried out in the absorbance mode at 247 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r2 = 0.9965 ± 0.0013 with respect to peak area in the concentration range 100–600 ng per spot. The mean value ± S.D. of slope and intercept were 9.047 ± 0.11 and 553.0 ± 39.06 with respect to peak area. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 9.40 and 28.51 ng per spot, respectively. Metformin hydrochloride was subjected to hydrolysis (in acid, alkali, and neutral solutions), oxidation and photo- degradation. The drug undergoes degradation under acid, alkali, neutral, H2O2, and photolytic conditions. This indicates that the drug is susceptible to hydrolysis, oxidation and photo degradation. Statistical analysis proves that the method is selective, precise and accurate for the estimation of said drug. The proposed developed HPTLC method can be applied for identification and quantitative determination of metformin hydrochloride in bulk drug and dosage forms.

Keywords: Metformin hydrochloride, HPTLC, Stability, Tablet.

INTRODUCTION

Metformin hydrochloride (Fig. 1) chemically is, N,N-Dimethyl imidodicarbonimide diamine hydrochloride biguanide class of antidiabetic drug used in treatment of type-2 diabetic patients.

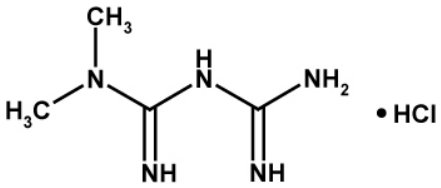

Figure 1: Chemical structure of metformin hydrochloride

Metformin Hydrochloride lowers blood glucose by a mechanism that is complex and incompletely understood. It increases the uptake and utilization of glucose in skeletal muscle (thereby reducing insulin resistance) and reduces hepatic glucose production (gluconeogenesis). It prevents hyperglycemia. It is a white in color and has a crystalline nature; freely soluble in water, slightly soluble in ethanol, insoluble in acetone and chloroform. The melting point is 2320 C; and it is administered in the form of tablets. It is an official drug mentioned in Merck Index, and United States Pharmacopoeia [1, 2].

Ion pair liquid chromatography technique has been reported for the quantification of metformin hydrochloride in human plasma [3]. A LC/MS/MS method is reported for quantification of metformin hydrochloride in biological samples [4] and several high performance liquid chromatography methods has been reported for its analysis in human plasma [5-12].

*Corresponding author *E - mail:* pritash79@yahoo.com

The objective of this study was to develop a new analytical method to establish the inherent stability of metformin hydrochloride by use of stress studies under a variety of ICH recommended test conditions [13, 14]. The new method was easy to use, specific and separation of all metformin hydrochloride degradants b the method, demonstrated the stability indicating nature of proposed HPTLC method.

MATERIALS AND METHOD

Metformin hydrochloride was a gift sample from Cipla Pharma, India. All chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

HPTLC INSTRUMENTATION: The samples were spotted in the form of bands of width 6 mm with a Camag microliter syringe on precoated silica gel aluminium Plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat 5 (Switzerland). A constant application rate of 200nl/s was employed and space between two bands was 8 mm. The slit dimension was kept 6 mm × 0.45 mm micro, 20 mm/s scanning speed was employed. The mobile phase consisted of water: methanol: triethylamine (1:3.5:0.2, v/v). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature. The length of chromatogram run was approximately 75 mm. Subsequent to the development; TLC plate was dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner 3 in the absorbance mode at 247 nm. The source of radiation utilized was tungsten lamp.

CALIBRATION CURVE OF METFORMIN HYDROCHLORIDE: A stock solution of metformin hydrochloride (1000 µg/mL) was prepared in methanol. From the stock solution 1.0mL was taken in 10mL volumetric flask and the vol. was adjusted with methanol to give 100µg/mL. From this 1, 2, 3, 4, 5 and 6 µl of the solution were spotted on TLC plate to obtain concentrations of 100, 200, 300, 400, 500 and 600 ng per spot of Metformin hydrochloride, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

Table 1: Linear regression data for the calibration curves[a]

Linearity range (ng per spot)	100–600
r2 ± S.D.	0.9965±0.0013
Slope ± S.D.	9.047±0.72
Confidence limit of slope[b]	8.86-9.15
Intercept ± S.D.	553.0±8.26
Confidence limit of interceptb	518.62-587.29

[a] n = 3, [b] 95% confidence limit.

METHOD VALIDATION

PRECISION: Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (200 ng per spot of Metformin hydrochloride). The intra- and inter-day precision for the determination of metformin hydrochloride was carried out at three different concentration levels of 200, 300 and 400 ng per spot, Table 2.

Table 2: Intra- and Inter-day precision of HPTLC method[a]

ng/ spot)	Intra-day precision				Inter-day precision			
	Mean area	**S.D. %**	**R.S.D.**	**S.E.[b]**	**Mean area**	**S.D.**	**%R.S.D.**	**S.E.[b]**
200	2344.8	28.53	1.21	16.49	2334.36	12.91	0.55	7.49
300	3311.15	35.47	1.22	20.50	307.21	34.29	1.03	19.82
400	4204.28	45.74	1.08	26.44	4273.43	21.53	0.50	15.31

[a] *n*=6., [b] Standard error.

ROBUSTNESS OF THE METHOD: By introducing small changes in the mobile phase composition, the effects on the chromatographic results were examined. Mobile phases having different composition of water: methanol: triethylamine (1.5:3:5:0.2 and 2.0:3.5:0.2, v/v) were used and chromatographic runs were carried out. The amount variation of mobile phase composition was in the range of ±5%. The plates were prewashed by methanol and activated at 60±50C for 2, 5 and 7 min prior to chromatography. Time from spotting, chromatographic run and scanning varied from 0, 20, 40 and 60 min. Robustness of the method was done at three different concentration levels: 300, 500 and 700 ng per spot. Robustness of the method was done at three different concentration levels: 200, 300 and 400 ng per spot. Robustness parameters were mention in Table 3.

Table 3: Robustness of the method[a]

Parameter	S.D. of peak area	%R.S.D.
Mobile phase composition	2. 82	1.41
Amount of mobile phase	2.82	1.44
Plate pretreatment	2.21	1.10
Time from spotting to chromatography	0.44	0.38
Time from chromatography to scanning	0.38	0.33

[a] *n*=6

LIMIT OF DETECTION AND LIMIT OF QUANTITATION: In order to determine detection and quantitation limit, metformin hydrochloride concentrations in the lower levels of the linear range of the calibration curve were used. Metformin hydrochloride solutions of 200, 220, 240, 260, 280 and300 ng/spot were applied in triplicate. The amounts of metformin hydrochloride versus average response (peak area) were plotted in a linear regression and the regression equation was determined. The standard deviations (S.D.) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by (3.3×A.S.D.)/b and quantification limit was calculated by (10×A.S.D.)/b, where "b" corresponds to the slope obtained in the linearity study of method.

SPECIFICITY: The specificity of the method was ascertained by analyzing standard drug and sample. The spot for metformin hydrochloride in sample was confirmed by comparing the RF values and spectra of the spot with that of standard Metformin hydrochloride. The peak purity of Metformin hydrochloride was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot, (Fig. 2-3).

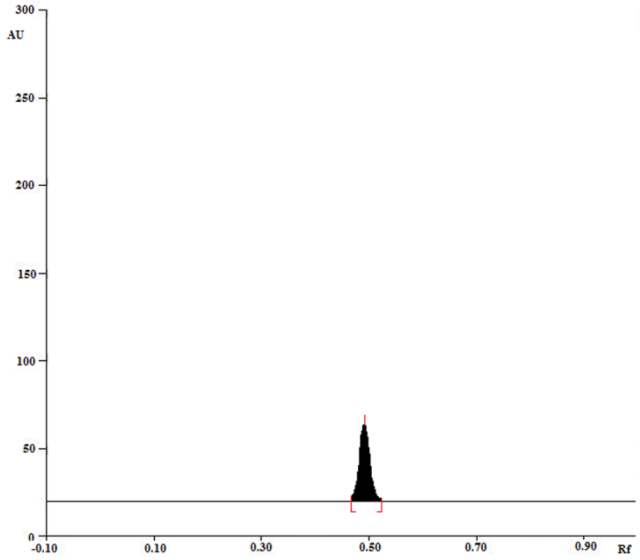

Figure 2: A typical HPTLC chromatogram of metformin hydrochloride (RF = 0.48) in water: methanol: triethylamine (1:3.5:0.2, v/v) at 247 nm

RECOVERY STUDIES: Recovery studies were carried out by applying the method to drug samples, in which known amounts of metformin hydrochloride corresponding to 80, 100 and 120% were spiked. For each stated level, six determinations were performed, data of recovery studies listed in Table 4.

ANALYSIS OF METFORMIN HYDROCHLORIDE IN FORMULATION: A commercially available tablet was analyzed by the proposed method. Marketed formulation of tablet contained 500 mg of metformin hydrochloride (Glyciphage, Batch No. PT12146, Mfg. Nov. 2012 and Exp. Oct. 2015).

*Corresponding author *E - mail:*

Table 4: Recovery studies[a]

Excess drug added to analyte (%)	Amount recovered (mg)	Recovery (%)	%R.S.D.	S.E.
0	197.98	98.99	1.26	0.37
80	341.08	98.66	0.79	0.43
100	394.71	98.67	0.71	0.40
120	441.64	100.37	0.39	0.22

[a] *n*=6.

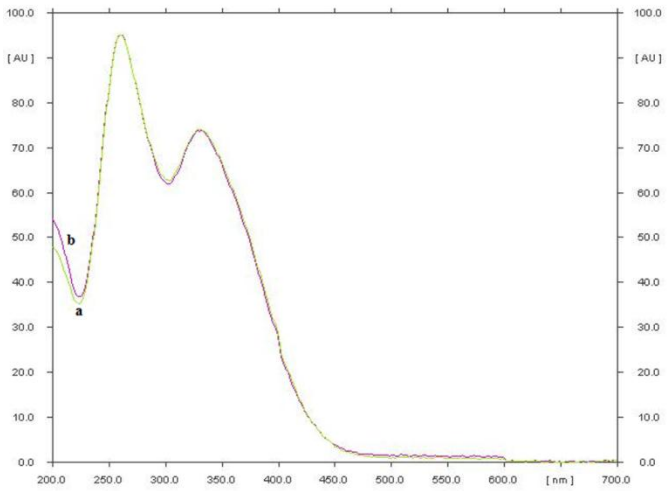

Figure 3: A typical overlain spectra of metformin hydrochloride in bulk (a) and in pharmaceutical formulation (b).

The sample preparation was done by adding tablet powder weight equivalent to 10 mg of pure metformine hydrochloride and dissolve in 100 ml of methanol to obtain concentration of 1000 µg/mL. After filtration through 0.41 µm filter (millifilter, Milford, MA), a concentration of 200ng/spot followed by development and scanning. The analysis was carried out in triplicates. The possibility of excipient interferences in the analysis was evaluated.

FORCED DEGRADATION OF METFORMIN HYDROCHLORIDE

ACID AND BASE INDUCED DEGRADATION: The 10 mg of metformin hydrochloride was separately dissolved in 10.0 mL of methanolic solution of 0.1M HCl and 0.1 M NaOH. The solutions were kept for 8 hrs at room temperature in the dark in order to exclude the possible degradative effect of light. Then, 1.0 mL of above solution was neutralized, and diluted up to 10.0 mL with methanol. The resultant solution was applied on TLC plate in triplicate (4.0 µl each, i.e. 400 ng per spot). The chromatographic analysis was carried out as shown in (Fig.4-5).

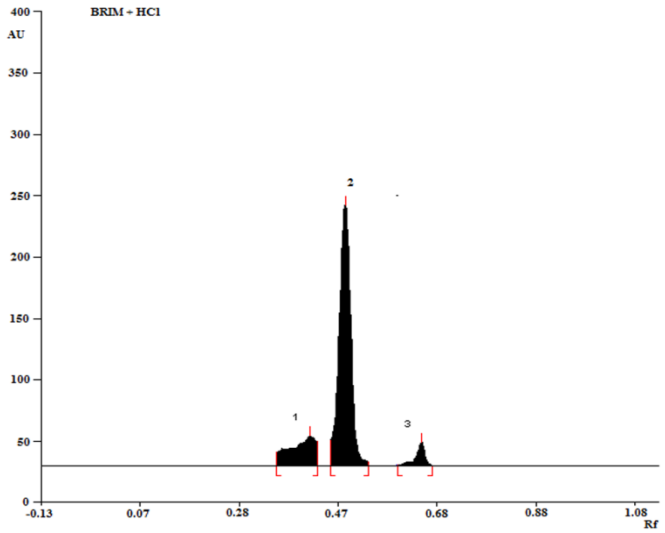

Figure 4: HPTLC Chromatogram of acid (0.1 M HCl, 8h, R.T.) treated metformin hydrochloride; peak1 (impurity) (RF: 0.37), peak 2 (metformin hydrochloride) (RF: 0.48), peak3 (impurity) (RF: 0.55).

HYDROGEN PEROXIDE INDUCED DEGRADATION: About 10 mg of metformin hydrochloride was separately dissolved in 10.0 mL of methanolic solution of hydrogen peroxide (3.0%, v/v). The solution was kept for 8 hrs at room temperature in the dark in order to exclude the possible degradative effect of light. Then, 1.0 mL of the resultant solution was taken and diluted up to 10.0 mL with methanol. The resultant solution was applied on TLC plate in triplicate (4.0 µl each, i.e. 400 ng per spot). The chromatographic analysis was carried out similarly, as shown in (Fig.6).

PHOTO DEGRADATION: The photochemical stability of the drug was also studied by exposing the stock solution (1000 µg/mL) to direct sunlight for 8 hrs.

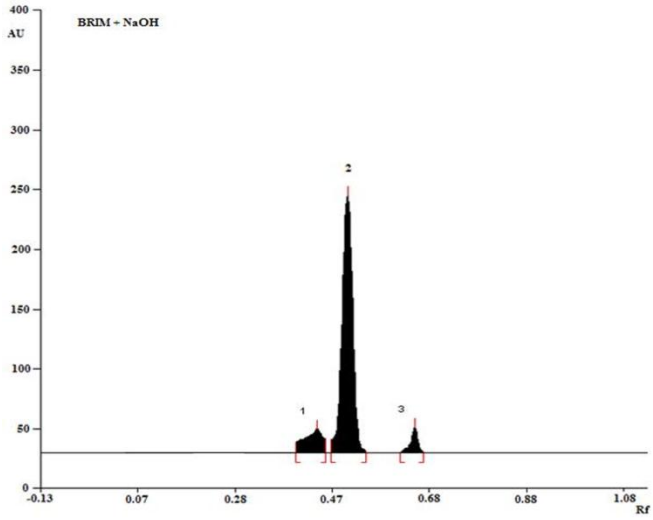

Figure 5: HPTLC Chromatogram of base (0.1 M NaOH, 8h, R.T.) treated metformin hydrochloride; peak1 (impurity) (RF: 0.39), peak2 metformin hydrochloride(RF: 0.48), peak 3 (impurity) (RF: 0.61).

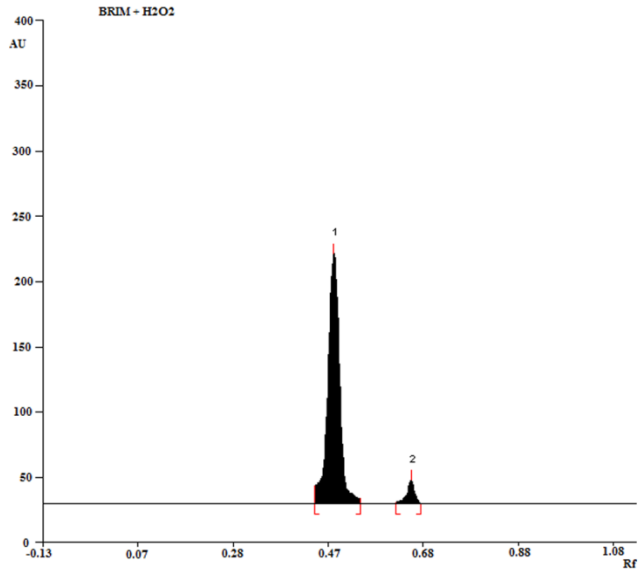

Figure 6: HPTLC Chromatogram of 3% hydrogen peroxide (8h, R.T.) treated metformin hydrochloride; peak1 (metformin hydrochloride) (RF: 0.48), peak2 (impurity) (RF: 0.64).

After suitable dilution, 4 µl of the solution (400 ng per spot) was applied on the TLC plate in triplicate and analysis was carried out and shown in (Fig.8).

NEUTRAL HYDROLYSIS: About 10 mg of metformin hydrochloride was separately dissolved in 10.0 mL of methanolic solution.

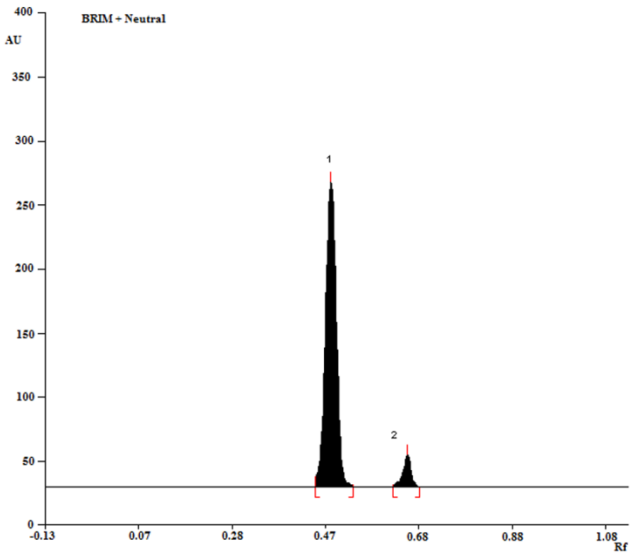

Figure 7: HPTLC Chromatogram of neutral solution (8h, R.T.) treated metformin hydrochloride; peak1 (metformin hydrochloride) (RF: 0.48), peak2 (impurity) (RF: 0.62).

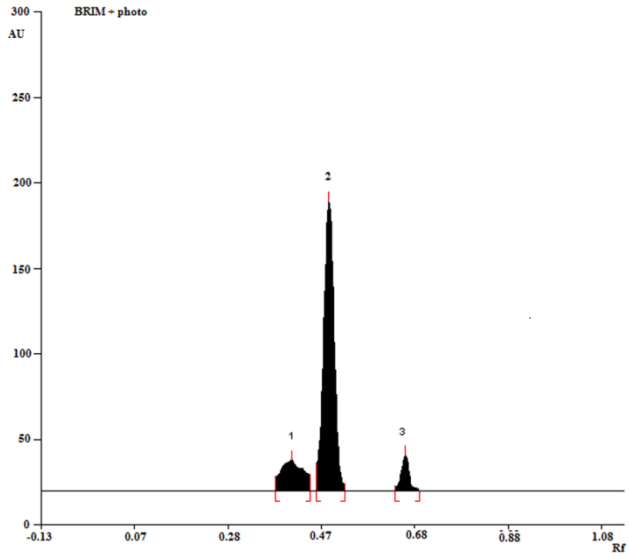

Figure 8: HPTLC Chromatogram of photo degradation (8h, R.T.) treated metformin hydrochloride; peak1 (impurity) (RF: 0.38), peak 2 (metformin hydrochloride) (RF: 0.48), peak3 (impurity) (RF: 0.62).

The solution was kept for 8 hrs at room temperature in the dark in order to exclude the possible degradative effect of light. Then, 1.0 mL of above solution was taken and diluted up to 10.0 mL with methanol. The resultant solution was applied on TLC plate in triplicate (4.0 µl

each, i.e. 400 ng per spot) and chromatographic run was carried out and shown in (Fig.7).

RESULTS AND DISCUSSION

DEVELOPMENT OF OPTIMUM MOBILE PHASE: The TLC procedure was optimized in view to develop a stability-indicating assay method. Both the pure drug and degraded products were spotted on TLC plates and run in different systems. Initially, water: methanol (2:5 v/v) in a varying proportion was tried. The mobile phase water: methanol (1: 3.5 v/v) gave good resolution with a RF value of 0.48 but typical peak nature was missing. To resolve this, 0.2 mL of triethyl amine was added to the solvent system. Finally, the mobile phase consisting of water: methanol: triethylamine (1:3.5:0.2 v/v) gave a sharp and well defined peak at RF value of 0.48 (Fig. 1). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature.

CALIBRATION CURVES: The linear regression data for the calibration curves (n=5) as shown in Table 1 exhibited a good linear relationship over the concentration range 100–600 ng per spot with respect to peak area. No significant difference was observed in the slopes of standard curves (ANOVA, P > 0.05)

VALIDATION OF THE METHOD

PRECISION: The repeatability of sample application and measurement of peak area were expressed in terms of %R.S.D. and results are depicted in Table 2. The intra- and inter-day variation of metformin hydrochloride at three different concentration levels of 200, 300 and 400 ng per spot was found to be <2%.

ROBUSTNESS OF THE METHOD: The standard deviation of peak areas was calculated for each parameter and % R.S.D. was found to be less than 2%. The values of % R.S.D. are shown in Table 3, and it thus indicates the robustness of the method.

LOD AND LOQ: The S/N 3:1 and 10:1 was considered as LOD and LOQ. The LOD and LOQ were found to be 9.40 and 28.51.

SPECIFICITY: The peak purity of metformin hydrochloride was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e., r2 (S, M) = 0.9997 and r2 (M, E) = 0.9998. Good correlation (r2 = 0.9989) was also obtained between standard and sample spectra of metformin hydrochloride, (fig 3).

RECOVERY STUDIES: The proposed method when used for extraction and subsequent estimation of metformin hydrochloride from pharmaceutical dosage forms after spiking with 80, 100 and 120% of additional drug afforded recovery of 98–101% as listed in Table 4. The data of summary of validation parameters are listed in Table 5.

Table 5: Summary of validation parameters

Parameter	Data
Linearity range (ng per spot)	100–600
Correlation coefficient	0.9965±0.0013
Limit of detection (ng per spot)	9.40
Limit of quantitation (ng per spot)	28.51
Recovery (*n* = 6)	99.15±0.76
Inter-day (*n* = 6)	0.69
Precision (% R.S.D.) Repeatability of application (*n* = 6)	1.57
Intra-day (*n* = 6)	1.14
Robustness	Robust
Specificity	Specific

ANALYSIS OF PREPARED FORMULATION: A single spot of RF 0.48 was observed in chromatogram of the metformin hydrochloride samples extracted from tablet formulation. There was no interference from the excipients commonly present in the formulation. The metformin hydrochloride content was found to be 99.09% with a %R.S.D. of 0.84. It may therefore be inferred that degradation of metformin hydrochloride had not occurred in the formulation that was analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of metformin hydrochloride in pharmaceutical dosage forms.

STABILITY-INDICATING PROPERTY: The chromatogram of samples degraded with hydrogen peroxide and heat

showed well separated spots of pure metformin hydrochloride as well as some additional peaks at different RF values. The spots of degraded product were well resolved from the drug spot as shown in Figs. 4–8. The conditions of degradation, number of degradation products with their RF values, content of metformin hydrochloride determined, and percentage recovery were calculated and listed in Table 6.

Table 6: Forced degradation of metformin hydrochloride

Sample exposure condition 8 hrs, RT	Number of degradation product (R_F value)	Metformin hydrochloride Remained (ng/400 ng) (±S.D, *n*=3)	S.E	Recovery (%)
0.1M HCl	2(0.37, 0.55)	359.93(±41.13)	23.41	87.20
0.1 M NaOH	2(0.39, 0.61)	329.26(±34.33)	19.84	82.82
3% H_2O_2	1 (0.64)	352.21(±46.22)	26.71	87.67
Photo	2(0.38, 0.62)	291.11(±34.73)	20.07	73.96
Neutral	1(0.62)	384.12(±67.45)	38.98	94.43

CONCLUSION

The proposed HPTLC method provides simple, accurate, reproducible and stability indicating assay for the quantitative determination of metformin hydrochloride in pharmaceutical formulations, eliminating interference from the excipients and in the presence of its acidic, alkaline, oxidative and photolytic degradation products. The method was validated according to the ICH guidelines. Statistical tests indicated that the proposed method is suitable for the routine analysis in pharmaceutical formulation in quality control laboratories with less time required for analysis. This study separates the drug from its degradation products, and, hence, it is a typical example of stability indicating assay.

REFERENCES

1. Budavari S., 1996. The Merck Index', 14th Ed., Merck and Co. Inc, USA, 1025.
2. United States Pharmacopoeia 31, 2008, Asian edition NF 26, The Official compaounds of standards, 2, 2, 2211-12.
3. Vasudevan M., Ravi J., Ravisankar S., Suresh B., 2001. Ion-pair liquid chromatography technique for the estimation of metformin in its multicomponent dosage forms. Journal of Pharmaceutical and Biomedical Analysis, 25, 77-84.
4. Heinig K., Bucheli F., 2004. Fast liquid chromatographic-tandem mass spectrometric (LC-MS-MS) determination of Metformin in plasma samples. Journal of Pharmaceutical and Biomedical Analysis, 34, 1005-1011.
5. Amini H., Ahmadiani A., Gazerani P., 2005. Determination of metformin in human plasma by high-performance liquid chromatography. Journal of Chromatography B. 824, 319-322.
6. Cheng C., Chou C., 2001. Determination of metformin in human plasma by high-performance liquid chromatography with spectrophotometric detection. Journal of Chromatography B. 762, 51-58.
7. Marlice A., Marques S., Soares A., Olivia A., Barroso W., Douglas M., Eduardo W., 2007. Simple and rapid method determination for metformin in human plasma using high performance liquid chromatography tandem mass spectrometry: Application to pharmacokinetics studies. Journal of Chromatography B. 852, 308-316.
8. Vesterqvist O., Nabbie F., Swanson B., 1998. Determination of metformin in plasma by high-performance liquid chromatography after ultra-filtration. Journal of Chromatography B. 716, 299-304.
9. Zhanga M., Mooreb G. A., Leverc M., Gardinera S. J., Kirkpatrick M. J., Begg E. J., 2001 Rapid and simple high performance liquid chromatographic assay for the determination of metformin in human plasma and breast. Journal of Chromatography B. 766, 175-179.
10. Zhao X. H., Song B., Zhong D. F., Zhang S. Q., Chen X. Y., 2007. Simultaneous determination of metformin and glipizide in human plasma by

liquid chromatography-tandem mass spectrometry. Biomedical Chromatography. 42, 1087-1091.
11. Zarghi A., Foroutan S. M., Shafaati A., Khoddam A., 2003. Rapid determination of metformin in human plasma using ion-pair HPLC. Journal of Pharmaceutical and Biomedical Analysis. 31, 197-200.
12. Bandarkara F. S., Khattaba I. S., 2010. Simultaneous estimation of Glibenclamide, Gliclazide, and Metformin hydrochloride from bulk and commercial products using a validating Ultra fast liquid chromatography technique. Journal of Liquid Chromatography and Related Technologies. 33, 1814-1830.
13. Bakshi M., Singh S., 2002. Development of validated stability-indicating assay methods-critical review. Journal of Pharmaceutical and Biomedical Analysis. 28, 1011-1040.
14. ICH-Guidelines Q1(A) (R2), Stability testing of new drug substances and products. IFPMA Geneva, February (2003).