Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Research Article

HPTLC – Stability Indicating Densitometric Method for Determination of Metformin Hydrochloride in Tablet Formulation.

Pankaj Chaudhari*, Pankaj Patil, Sachin Mahajan, Prakash Patil

K.V.P.S., Institute of Pharmaceutical Education and Research, Boradi, Shirpur Dist: Dhule-425405, Maharashtra, India.

Received 26 January 2019; received in revised form 12 February 2019; accepted 22 February 2019
***Corresponding author E-mail address:** daal15@rediffmail.com*

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 aluminum plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of water:methanol:triethylamine (1:3.5:0.2 v/v). The system was found to give compact spot for metformin hydrochloride (R_F 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 r^2 = 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 28.51ng 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, H_2O_2, and photolytic conditions. This indicates that the drug is susceptible to hydrolysis, oxidation and photo degradation. 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 Formulation

1. INTRODUCTION:

Metformin hydrochloride chemically N, N-Dimethyl imido dicarbonimide diamine hydrochloride biguanide class of antidiabetic drug used in treatment of type-2 diabetic patient. Metformin Hydrochloride is lowers blood glucose by mechanisms that is complex & incompletely understood. They increase glucose uptake & utilization in skeletal muscle (there by reducing insulin resistance) and reduce hepatic glucose production (gluconeogenesis). It prevents hyperglycemia. It is a white to crystalline powder; freely soluble in water, slightly soluble in ethanol, insoluble in acetone and chloroform; melting point 2320 C; administered in the form of tablets. It is official in Merck Index– An encyclopedia of chemicals, drugs and biological and United States of pharmacopoeia [1, 2].

Ion pair liquid chromatography technique has been reported for the quantification of metformin hydrochloride in human plasma [3]. One LC/MS/MS method is also reported for quantification of metformin hydrochloride in biological samples [4] whereas seven High performance liquid chromatography methods have been reported in human plasma [5-11]. Ultra fast liquid chromatography method has been developed on combination of three antidiabetic drugs [12].

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

2. MATERIALS AND METHODS

2.1. Materials

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.

2.2. HPTLC instrumentation

The samples were spotted in the form of bands of width 6mm with a Camag microliter syringe on precoated silica gel aluminium Plate 60F-254 (20 cm×10 cm with 0.2mm 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 6mm×0.45mm 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.

2.3. 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 this1, 2, 3, 4, 5and 6µl of the solution were spotted on TLC plate to

obtain concentration of 100, 200, 300, 400, 500 and 600ng per spot of metformin hydrochloride, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

2.4. Method Validation

2.4.1. 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.

2.4.2. Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of water: methanol:triethylamine(1.5:3:5:0.2and 2.0:3.5:0.2, v/v) were tried and chromatograms were run. The amount of mobile phase was varied 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. The amount of mobile phase was varied in the range of ±5%.Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and60 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.

2.4.3. Limit of detection and limit of quantification

In order to determine detection and quantification limit, metformin hydrochloride concentrations in the lower part of the linear range of the calibration curve were used. Metformin hydrochloride solutions of 200, 220, 240, 260, 280 and 300 ng/spot were applied in triplicate. The amount of metformin hydrochloride by spot versus average response (peak area) was graphed and the equation for this 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.

2.4.4. 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. 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.

2.4.5. Recovery studies

Recovery studies were carried out by applying the method to drug sample to which known amount of metformin hydrochloride corresponding to 80, 100 and 120% had been added. At each of the amount, six determinations were performed.

2.5. Analysis of Metformin Hydrochloride in Formulation

To determine the concentration of Metformin hydrochloride in tablets (labeled claim: 100 mg per tablet), the contents of 20 tablets were weighed, their mean weight determined and they were finely powdered. The powder equivalent to 10 mg of Metformin hydrochloride was weighed.

The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min and the volume was made up to 10 ml. The resulting solution was filtered using 0.45 µm filter (Millfilter, Milford, MA). The above solution (200 ng per spot) was applied on TLC plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate.

2.6. Forced Degradation of Metformin Hydrochloride

2.6.1. 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 h" at room temperature in the dark in order to exclude the possible degradative effect of light. The 1.0ml of above solution was taken and neutralized, then diluted up to 10.0 ml with methanol. The resultant solution was applied on TLC plate in triplicate (4.0µl each, i.e. 400ng per spot). The chromatograms were run as described in section2.2.

2.6.2. Hydrogen Peroxide induced degradation

The 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 "8h" at room temperature in the dark in order to exclude the possible degradative effect of light. The 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). The chromatograms were run as described in section 2.2.

2.6.3. Photo degradation

The photochemical stability of the drug was also studied by exposing the stock solution (1000µg/ml) to direct sunlight for "8 h" on a wooden plank and kept on terrace. After suitable dilution 4µl of the solution (400 ng per spot) was applied on the TLC plate in triplicate. The chromatograms were run as described in section 2.2.

2.6.4. Neutral hydrolysis

The 10 mg of metformin hydrochloride was separately dissolved in 10.0 ml of methanolic solution. The solution was kept for "8 h" at room temperature in the dark in order to exclude the possible degradative effect of light. The 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). The chromatograms were run as described in section 2.2.

3. RESULTS AND DISCUSSION

3.1. 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 the above mobile phase 0.2 ml triethyl amine was added. 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 0.48shoen in Fig. 1. Well-defined spots were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature.

3.2. Calibration curves

The linear regression data for the calibration curves (n=5)as shown in Table 1 showed 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, $P > 0.05$)

3.3. Validation of the Method

3.3.1. 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 of200, 300 and 400 ng per spot was found to be <2%.

3.3.2. 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 low values of %R.S.D. values as indicated are shown in Table 3 indicated robustness of the method.

3.3.3. 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

3.3.4. 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.

3.3.5. 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.

3.4. 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.

3.5. 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. 3(a)–3(e).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.

4. CONCLUSION

The proposed HPTLC method provide simple, accurate, reproducible and stability indicating assay for the quantitative determination of metformin hydrochloride in pharmaceutical formulation, without 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 reduce the duration analysis and appear to be equally suitable for the routine analysis in pharmaceutical formulation in quality control laboratories. This study separates the drug from its degradation products, and, hence, it is a typical example of stability indicating assay.

5. REFERENCES

1. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biological, Edited by M.J. O'Neil, Royal Society of Chemistry, Cambridge, 15, (2013).
2. United States Pharmacopoeia 31, Asian edition NF 26, The Official compounds of standards, 2, (2006).
3. M. Vasudevan, J. Ravi, S. Ravisankar, B. Suresh. (2001). Ion-pair liquid chromatography technique for the estimation of metformin in its multicomponent dosage forms. *Journal of Pharmaceutical and Biomedical Analysis*. 25, 1, 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, 1-2, 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*. 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 ultrafiltration. *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., ZhongD. 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, 10, 1087-1091.

11. Zarghi A., Foroutan S. M., Shafaati A., Khoddam A.(2003). Rapid determination ofmetformin in human plasma using ion-pair HPLC. *Journal of Pharmaceutical and Biomedical Analysis.* 3, 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 & Related Technologies*. 33, 1814-1830.

13. Bakshi M., Singh S. (2002). Development of validated stability-indicating assay methods critical review. *Journal of Liquid Chromatography & Related Technologies.* 28, 1011-1040.

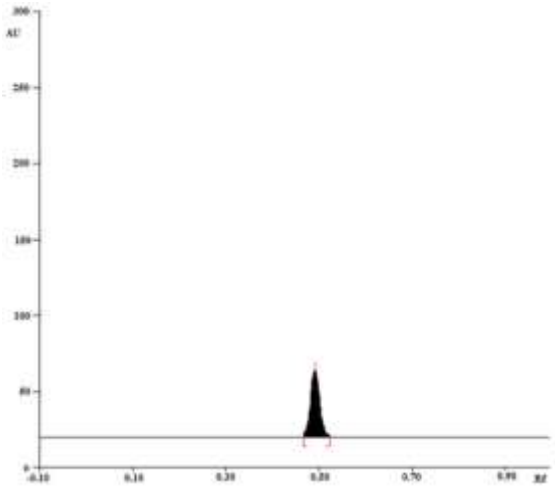

Figure 1. A typical HPTLC chromatogram of metformin hydrochloride (R_F = 0.48) in water: methanol: triethylamine (1:3.5:0.2, v/v) at 247 nm

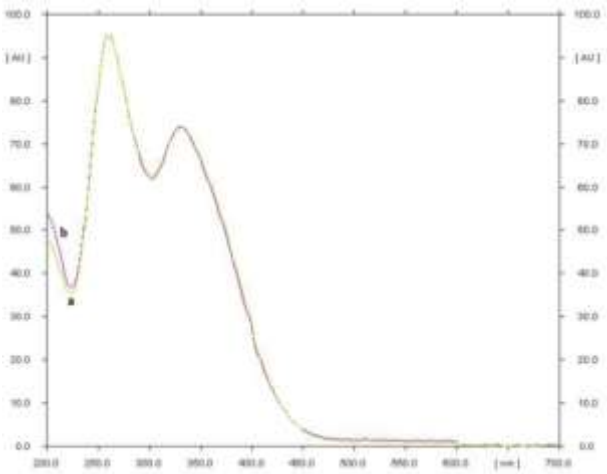

Figure 2. Typical overlain spectra of metformin hydrochloride in bulk (a) and in pharmaceutical formulation (b).

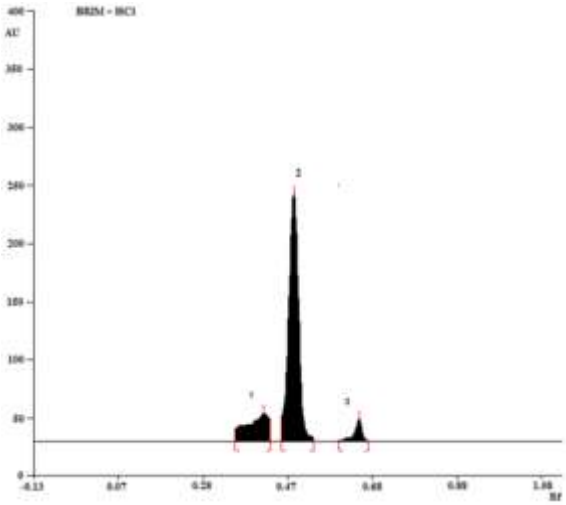

Figure 3a. HPTLC Chromatogram of acid (0.1 M HCl, 8h, R.T.) treated metformin hydrochloride; peak1 (impurity) (R_F: 0.37), peak 2 (metformin hydrochloride) (R_F: 0.48), peak 3 (impurity) (R_F: 0.55).

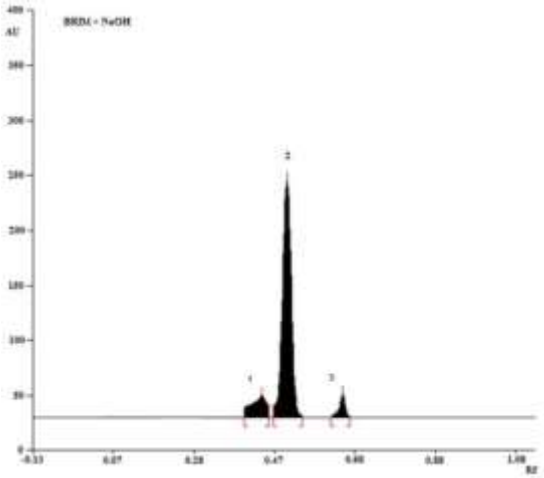

Figure 3b. HPTLC Chromatogram of base (0.1 M NaOH, 8h, R.T.) treated metformin hydrochloride; peak1 (impurity) (R_F: 0.39), peak2 metformin hydrochloride (R_F: 0.48), peak 3 (impurity) (R_F: 0.61).

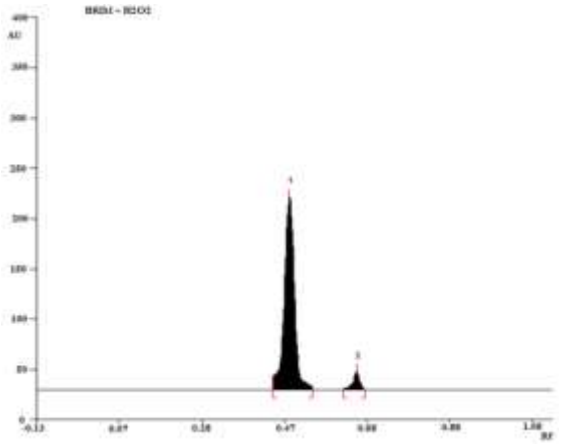

Figure 3c. HPTLC Chromatogram of 3% hydrogen peroxide (8h, R.T.) treated metformin hydrochloride; peak1 (metformin hydrochloride) (R_F: 0.48), peak2 (impurity) (R_F: 0.64).

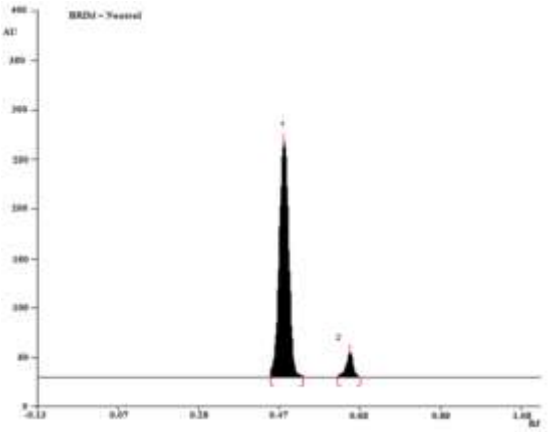

Figure 3d. HPTLC Chromatogram of neutral solution (8h, R.T.) treated metformin hydrochloride; peak1 (metformin hydrochloride) (R_F: 0.48), peak2 (impurity) (R_F: 0.62).

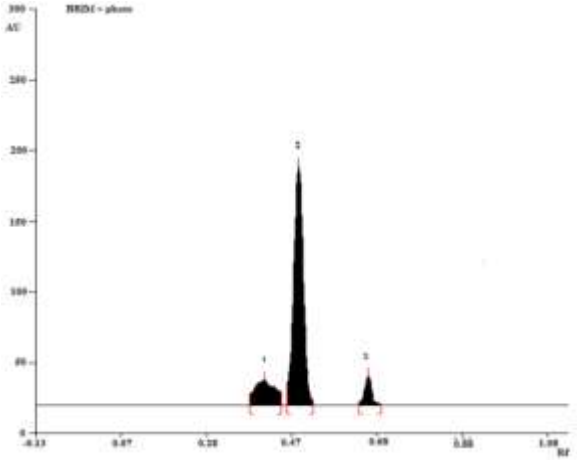

Figure 3e. HPTLC Chromatogram of photo degradation (8h, R.T.) treated metformin hydrochloride; peak1 (impurity) (R_F: 0.38), peak 2 (metformin hydrochloride) (R_F: 0.48), peak3 (impurity) (R_F: 0.62).

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

Parameter	Data
Linearity range (ng per spot)	100–600
r^2 ± 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 intercept[b]	518.62-587.29

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

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

Initial Amt.	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

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

Table 4. Recovery Study

Excess drug added to the analyte (%)	Amount recovered	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.

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
Precision (% R.S.D.)	
Repeatability of application (n = 6)	1.57
Inter-day (n = 6)	0.69
Intra-day (n = 6)	1.14
Robustness	Robust
Specificity	Specific

Table 6. Forced degradation of metformin hydrochloride

Serial No.	**Sample exposure Condition "8 h", RT**	**Number of degradation product (R_F value)**	**Metformin hydrochloride Remained (ng/400 ng) (±S.D, n=3)**	**S.E**	**Recovery (%)**
1	0.1M HCl	2(0.37, 0.55)	59.93(±41.13)	23.41	87.20
2	0.1MNaOH	2(0.39, 0.61)	329.26(±34.33)	19.84	82.82
3	3% H_2O_2	1 (0.64)	352.21(±46.22)	26.71	87.67
4	Photo	2(0.38, 0.62)	291.11(±34.73)	20.07	73.96
5	Neutral	1(0.62)	384.12(±67.45)	38.98	94.43