A validated specific stability-indicating RP-HPLC method for pioglitazone hydrochloride

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ABSTRACT

A simple and rapid specific stability-indicating high-performance liquid chromatographic (HPLC) method developed for a leading anti diabetic drug substance namely Pioglitazone hydrochloride. The developed method is very much compatible with LC-MS and shows the superior resolution between the Pioglitazone, its process related impurities and as well the degradation products generated from the forced degradation studies includes hydrolysis, oxidative, photolytic and thermal degradation. The method was developed using Zorbax bonus RP column with water/acetonitrile/trifluoroacetic acid as a mobile phase using a simple linear gradient. The detection was carried out at 225 nm. The method was validated with respect to linearity, precision, accuracy, specificity and robustness. The limit of detection and the limit of quantification for the Pioglitazone and its process related impurities were established. The developed method was found to be suitable to check the quality of bulk samples of Pioglitazone at the time of batch release and also during its storage.
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INTRODUCTION

Pioglitazone hydrochloride[(±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-] thiazolidine dione monohydrochloride belongs is an antidiabetic agent that acts primarily by decreasing insulin resistance. It is used in the management of type 2 diabetes mellitus known as non-insulin-dependent diabetes mellitus [NIDDM] or adult-onset diabetes. It has a different pharmacological action than the sulfonylureas, metformin or the α-glucosidase inhibitors. Pharmacological studies indicate that ACTOS improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis. Pioglitazone improves glycemic control while reducing circulating insulin levels[1].

Sofar few articles were published on analytical method of Pioglitazone hydrochloride and its metabolites for its biological studies[2,3,4,5,6,7]. Some papers were published on the analytical methods for the bulk drug and formulation of pioglitazone hydrochloride and its combination with Glimepiride and Metformin[8,9,10,11]. Two different techniques on the related substance method of pioglitazone hydrochloride have been published in the year 2002[12]. One of the published paper describes the stability-indicating HPLC method for the formulated Pioglitazone hydrochloride[13]. The primary

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objective of the current research work is to develop a LC-MS compatible, rapid stability- indicating method for the Pioglitazone hydrochloride bulk drug which is superior in system suitability parameters. The current article deals only with development and stability- indicating capability of method and its validation. The developed method gives superior LOD and LOQ values for the impurities and better tailing factor when compared to the earlier reported methods[2,3,4,5,6,7,8,9,10,11,12,13]. Forced degradation was performed by hydrolysis in acid, alkaline, oxidation in peroxide, stressed in photolytic and temperature to prove the stability- indicating capability of the developed method. The same method was validated as per ICH requirements.

EXPERIMENTAL

Chemicals and reagents

Pioglitazone hydrochloride and its related impurities were received from Process Research Department of Custom Pharmaceutical Services of Dr. Reddy's Laboratories Limited, Hyderabad, India. Sodium hydroxide, hydrochloric acid (Rankem, New Delhi, India) and Hydrogen peroxide (S.D. Fine Chem., Mumbai, India) were used. HPLC-grade Acetonitrile (Rankem, New Delhi, India), Across make of trifluoro acetic acid were used. High pure water was prepared by using Millipore Milli Q plus purification system

Equipment

The method development and forced degradation studies were done using Agilent 1100 series HPLC system with diode array detector. The data were collected and the peak purity of the pioglitazone peak was checked using chemstation as software. The photolytic degradation was carried out using Binder KBS240 photolytic chamber. The peak homogeneity of degraded samples were studied in Agilent LCMS 6410 instrument with triple quadrapole. The chromatographic separations were achieved on Zorbax Bonus RP18 (Agilent, Milford, USA) column (150mm×4.6mm i.d., with a particle size of 3.5μ).

Sample preparation

For the related substance method, pioglitazone hydrochloride was prepared 0.25mg/ml in water : aceto- nitrile in the ration of 60:40(v/v) as a diluent. For the assay determination the concentration was fixed as 50 μg/ml by diluting the related substance solution.

Chromatographic conditions

Linear gradient elution was employed in Zorabax Bonus RP18 column with water :trifluoroacetic acid in the ratio of 100:0.05(v/v) as a mobile phase-A and acetonitrile :trifluoroacetic acid in the ratio of 100:0.05(v/v) as a mobile phase - B. The gradient program: time/% of MP-B is 0/10, 12/65,16/65, 17/10 with post run 5 min.

The flow rate of the mobile phase was 1.0ml/min. The column was maintained at $30^{0}C$ and the wavelength was monitored at a wavelength of 225nm. The injection volume was 10μl.

Generation of stress samples for establishment of stability-indicating assay

Stock solutions of pioglitazone were prepared in different stressed solutions and further diluted to nominal concentration each time before injecting them in HPLC[14,15,16,17,18]. The degradation was attempted at 0.5 N Hydrochloric acid, 0.5 N Sodium hydroxide and 5%w/v hydrogen peroxide for the 2.5mg/ml solutions. The stressed solutions were injected after further dilution to get 0.25mg/ml. The stress conditions were as follows:

(i) Stress study under hydrolytic condition : **Acidic hydrolysis:** Stressed solution in 0.5N HCl was studied for 10 days at ambient temperature. **Alkaline hydrolysis:** Stressed solution in 0.5N NaOH was studied for 48 h at ambient temperature.
(ii) Stress study under oxidative condition :Stressed solution in 5.0% w/v H_2O_2 was studied for 10 days at ambient temperature.
(iii) Stress study under light: Bulk drug was exposed to UV and fluorescent light as ICH-recommended conditions[19] for 11 days.
(iv) Thermal stress: Bulk drug was subjected to dry heat at $60^{0}C$ for 10 days.

Separation studies

The initial analyses of different stressed samples for pioglitazone hydrochloride was performed on HPLC system using reported C-18 column with linear gradient with the mobile phase described in the chromatographic conditions.

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Optimization studies

The system suitability parameters were improved dramatically using Zorbax Bonus RP column. The separations of Pioglitazone from their degradation products were optimised by changing the gradient program as it described in the chromatographic conditions. Using the optimized condition, the specificity of the pioglitazone peak was checked in DAD for the study period. The peak homogeneity was also studied by LCMS by extracting the mass number under the Pioglitazone peak.

Validation of the developed method

1. System suitability

The pioglitazone hydrochloride was spiked with the Imp-F and Imp-G and the USP resolution of each peak was checked. Also the capacity factor, theoretical plates on tangent method and the USP tailing of each peak was recorded

2. Precision

The precision of the related substance method was checked by injecting six individual preparations of (0.25mg/ml) pioglitazone hydrochloride spiked with 0.15% of the potential impurities Imp-D, Imp-E, Imp-F and Imp-G. The%RSD of area% of each impurity was calculated. The precision of the assay in the developed method was evaluated by carrying out six independent assays of test sample at 50µg/ml of Pioglitazone hydrochloride against qualified reference standard and calculated the% RSD of assay. The intermediate precision of the method was also evaluated using different analyst and a different instrument in the same laboratory.

3. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for Imp-D, Imp-E, Imp-F and Imp-G were determined at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration[8]. Precision study was also carried at the LOQ level by injecting six individual preparations of Imp-D, Imp-E, Imp-F and Imp-G and calculating the% R.S.D. of the area.

4. Linearity

The linearity of the method was assessed by least squares linear regression analysis in the concentration verses the response of compounds. Test solutions for related substance method were prepared by diluting the impurity stock solution to the six different concentration levels from LOQ to 200% (i.e. 0.30% with respect to pioglitazone hydrochloride) of the specification. Test solutions for assay were prepared at five concentration levels from 25µg/ml to 75µg/ml of pioglitazone hydrochloride. The calibration curve was drawn by plotting the concentration of the impurities versus corresponding peak area. The slope and *Y*-intercept of the calibration curve was calculated.

5. Accuracy

The bulk sample provided by process research department of Custom Pharmaceutical Services does not show the presence of any of the process related impurities. Standard addition and recovery experiments were conducted to determine accuracy of the related substance method for the quantification of potential impurities in bulk drug samples. The study was carried out in triplicate at 0.075, 0.15 and 0.225% of the analyte concentration (0.25mg/ml). The percentages of recoveries for impurities were calculated from the slope and *Y*-intercept of the calibration curve obtained in linearity section of related substances. The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e. 25, 50 and 75 µg/ml in bulk drug sample. The percentages of recoveries were calculated from the slope and *Y*-intercept of the calibration curve obtained in linearity section of assay.

6. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between pioglitazone and the degradation impurity which is eluting closely was recorded. To study the effect of flow rate on the resolution, it was changed by 0.1 units i.e from 0.9 to 1.1ml/min while the other chromatographic conditions were held constant. The effect of change in the gradient of mobile phase-B was checked by changing the same to ± 5% (i.e MP-B ends with 65±4%). The effect of column temperature on resolution was studied at 25 and 35°C instead of 30°C while the other chromatographic conditions were held constant.

7. Solution stability and mobile phase stability

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The solution stability in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 48h. The same sample solutions were assayed for every 6 h interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions for 6 h interval up to 48h. Mobile phase prepared was kept constant during the study period. The% R.S.D. of assay of Pioglitazone hydrochloride was calculated for the study period during mobile phase and solution stability experiments. The solution stability of the related substance method was established by analysing the sample solution spiked with the impurities in tightly capped volumetric flask at room temperature for 2 days. Content of Imp-D, Imp-E, Imp-F and Imp-G were determined for every 6 h interval up to the study period. Mobile phase stability was also carried out for 2 days by injecting the freshly prepared sample solutions for 6 h interval. Contents of Imp-D, Imp-E, Imp-F and Imp-G were checked in the test solutions. The prepared mobile phase was kept constant during the study period.

RESULTS AND DISCUSSION

Development of optimized stability-indicating methods

The system suitability parameters in Zorbax XDB C18 column[20] were good when compared to the other reported methods with C-18 columns [2,3,4,5,6,7,8,9,10,11,12,13]. But by changing the column to Zorbax Bonus RP which is having triple end capping, the peak width, USP tailing factor and theoretical plat counts were till improved significantly. Trifluoroacetic acid used here as not only a volatile buffer, but it acts as very good additive or modifier. During the initial runs the linear gradient ends with more% of mobile phase-B as a usual practice to elute the non polar impurities with in short runtime. But it led to the resolution of close eluting alkaline degradation impurity at around 2.0. Considering the long term QC runs to get base to base separation of close eluting impurity, the gradient program was optimized and the resolution was achieved >3.5.

The results of forced degradation studies were shown that there was no degradation in the acid hydrolysis, thermal and photolytic stressed conditions as per ICH. The peak purity of stressed sample in all above conditions were checked using DAD after 10 days. The peak homogeneity in LCMS was checked and it was proved that no additional mass number were there under the peak of Pioglitazone other than pioglitazone mass. The base hydrolysis shows significant degradation and number of unknown peaks formed during the stress period. Major degradant formed in the RRT of 1.05 in the range of 10% a/a. Apart from that 4 more significant impurities formed in the level of 1-3% a/a. The peak purity in DAD and homogeneity in LCMS were confirmed that there were no impurities under the peak of Pioglitazone. Mild degradation observed in oxidative stress study and a small impurity at RRT 1.44 was raised in the level of 0.25% a/a. (TABLE 1). The peak purity in DAD and homogeneity in LCMS were confirmed that there were no impurities under the peak of Pioglitazone in the oxidative forced degradation.

Validation of the developed stability-indicating methods

TABLE 1 : Summary of forced degradation results

Stress condition	Duration	Purity of pioglitazone after degradation	Area% of major degradant	Remarks
Acid hydrolysis	10 days	99.9	-	No degradation products formed
Base hydrolysis	48 h	79.9	9.7	Major degradant formed at the tailing of Pioglitazone peak at the RRT of 1.05
Oxidation	10days	99.5	0.25	Mild degradation observed
Thermal (60 °C)	10 days	99.9	-	No degradation products formed
Photolytic as per ICH	11 days	99.9	-	No degradation products formed

TABLE 2: System suitability results

Compound (n = 3)	Capacity factor (k^{1})	Resolution (Rs)	USP tailing factor (T)	No. of theoretical plates (N) tangent method
Pioglitazone	4.3	-	1.1	89155
Imp-F	4.9	7.9	0.9	46689
Imp-G	5.6	7.3	1.1	100065

TABLE 3 : Results of LOD and LOQ concentrations

Compound	LOD concentration In ng/ml	LOQ concentration In ng/ml
Imp-D	43	128
Imp-E	35	93
Pioglitazone	22	58
Imp-F	23	61
Imp-G	31	85

TABLE 4 : Results of accuracy for related substances

Compound	Level (%)	Spiked quantity (ng/ml) (n=3)	Recovered quantity (ng/ml)	Recovery in%
	0.075	175.3	169.5	96.7
Imp-D	0.150	350.6	333.8	95.2
	0.225	525.9	523.3	99.5
	0.075	173.5	177.5	102.3
Imp-E	0.150	347.0	335.1	96.6
	0.225	520.5	513.2	98.6
	0.075	168.7	170.7	101.2
Imp-F	0.150	337.4	349.3	103.5
	0.225	506.1	527.9	104.3
	0.075	172.65	169.0	97.9
Imp-G	0.150	345.3	334.3	96.8
	0.225	517.95	498.8	96.3

TABLE 5 : Results of accuracy for assay

Compound	Level (%)	Spiked quantity (µg/ml) (n=3)	Recovered quantity (µg/ml)	Recovery in%
	50	24.3	24.1	99.2
Pioglitazone	100	48.6	48.5	99.8
	150	72.9	73.8	101.3

The method was validated for parameters such as linearity, precision, accuracy, specificity and robustness. The results of system suitability test performed for each parameter was quit well. The averages of the result was tabulated. (TABLE 2)

1. Precision

The% RSD of area% of impurities in related substance method precision study were with in 3.2% and the% RSD of assay results obtained in assay method precision study was with in 1.0%. The% RSD of area%

of impurities was below 4.5% in the intermediate precision representing good precision of the developed method. The% RSD of assay results obtained in intermediate precision study was also below 0.9%.

2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for impurities were ranging from 22 to 128ng/ml for 10µl injection volume(TABLE 3). The method precision for the impurities at LOQ level was below 8.7% RSD.

3. Linearity

Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.3% for each impurities with correlation coefficient greater than 0.999. Linearity was checked for related substance method over the same concentration range for three consecutive days. The Y-Intercept value of each impurity is less than 3.2% of responses at 0.15% level is indicating that the plot is going almost through the origin which will minimise the error in recovery calculation. The results shows that an excellent correlation existed between the peak area and concentration of impurities.

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 25 µg/ml to 75µg/ml for pioglitazone hydrochloride; the corresponding linear regression equation was $y = 7.6633x + 583.06$ with correlation coefficient greater than 0.999. The results shows that an excellent correlation existed between the peak area and concentration of Pioglitazone hydrochloride.

4. Accuracy

The percentage recovery of impurities in bulk drugs samples were ranged from 95.2 to 104.3 (TABLE 4). The percentage recovery of pioglitazone hydrochloride in bulk drug samples were ranged from 99.2 to 101.3 (TABLE 5).

5. Robustness

In all the deliberate varied chromatographic conditions (flow rate, gradient program, column temperature) the resolution between pioglitazone and the closely eluting impurity during the forced degradation in NaOH
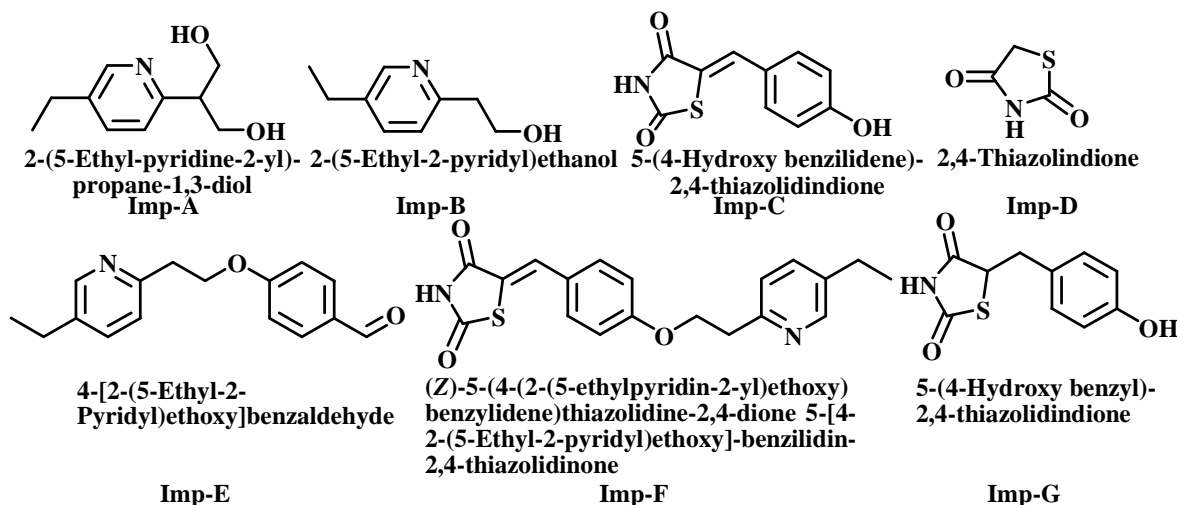
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2-(5-Ethyl-pyridine-2-yl)-
propane-1,3-diol
Imp-A

2-(5-Ethyl-2-pyridyl)ethanol
Imp-B

5-(4-Hydroxy benzilidene)-
2,4-thiazolidindione
Imp-C

2,4-Thiazolindione
Imp-D

4-[2-(5-Ethyl-2-
Pyridyl)ethoxy]benzaldehyde
Imp-E

(Z)-5-(4-(2-(5-ethylpyridin-2-yl)ethoxy)
benzylidene)thiazolidine-2,4-dione 5-[4-
2-(5-Ethyl-2-pyridyl)ethoxy]-benzilidin-
2,4-thiazolidinone
Imp-F

5-(4-Hydroxy benzyl)-
2,4-thiazolidindione
Imp-G

Figure 1: Chemical structures of impurities of pioglitazone hydrochloride
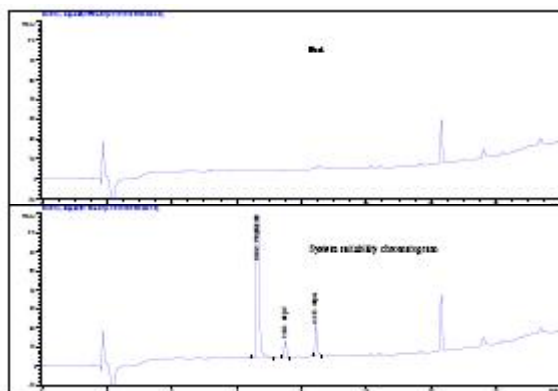
Figure 2: Chromatogram of blank and system suitability
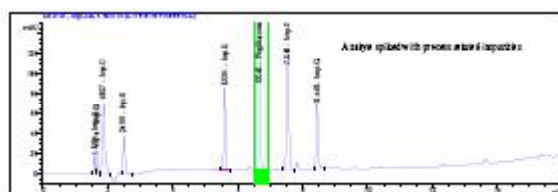
Figure 3 : Chromatogram of analyte spiked with process related impurities
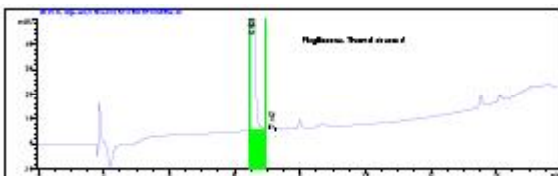
Figure 4 : Chromatogram of stressed sample in temperature
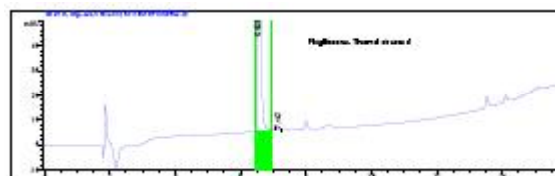
Figure 5: : Chromatogram of stressed sample in photolytic condition

Abundance vs. Acquisition Time (min)
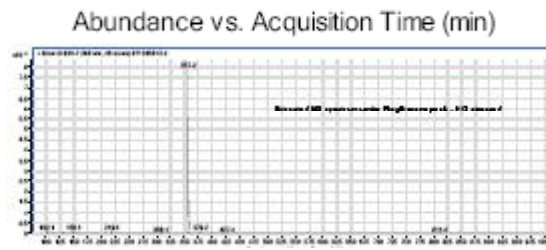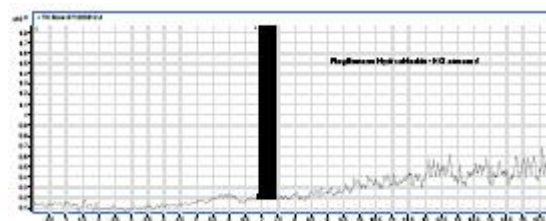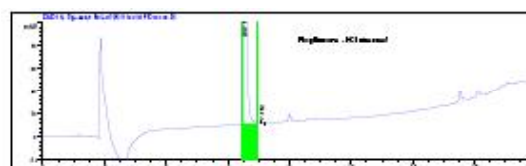
Abundance vs. Mass-to-Charge (m/z)

Figure 6: Chromatogram of stressed sample by acid hydrolysis and peak homogeneity by LCMS

was greater than 3.2, illustrating the robustness of the method.

6. Solution stability and mobile phase stability

The solution stability and mobile phase stability experiments data confirms that Pioglitazone hydrochloride sample solutions and mobile phase used during

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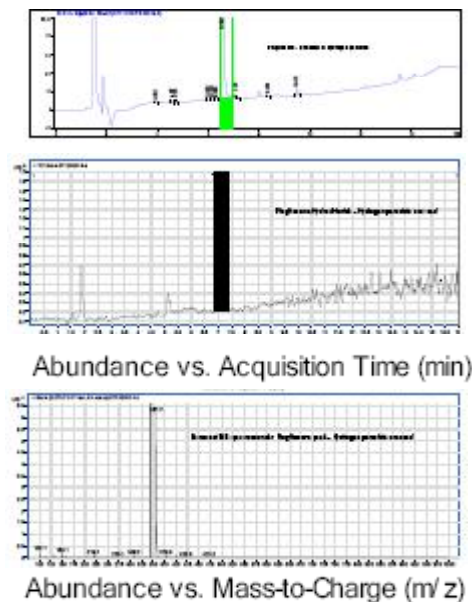
Figure 7: Chromatogram of stressed sample by oxidation condition and peak homogeneity by LCMS
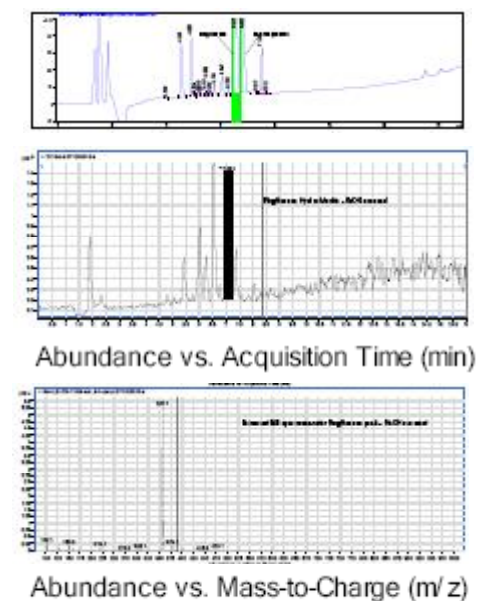
Figure 8: Chromatogram of stressed sample by base hydrolysis and peak homogeneity by LCMS

assay and related substance determination were stable for at least 48h.

CONCLUSION

Validated LCMS compatible and rapid stability-indicating HPLC methods was developed for Pioglitazone hydrochloride after subjecting the drug to ICH-recommended stress conditions. The method was validated for linearity, precision and accuracy in the studied concentration range quantitatively. The methods were found to be 'specific' to the drug as the peaks of the degradation products as well as the process related impurities did not interfere with the drug peaks. The above method can be used in the quality control laboratory during the testing and release of Pioglitazone hydrochloride bulk samples. The above validated method can be conveniently used for assessing the storage stability of bulk samples.

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REFERENCES

[1]   T.Sohda, Y.Momose, K.Meguro, Y.Kawamatsu, Y.Sugiyama, H.Ikeda; Osaka, Japan Arzneimittel-Forschung, **40(1)**, 37-42 **(1990)**.

[2]   N.W.Leung, Gary, K.K.Leung, David, Wan, S.M.Terence, Wong, H.F.Colton; Journal of Chromatography, A, **1156(1-2)**, 271-279 **(2007)**.

[3]   P.Venkatesh, T.Harisudhan, Choudhury, Hira; Mullangi, Ramesh, R.Srinivas, Nuggehally; Biomedical Chromatography, **20(10)**, 1043-1048 **(2006)**.

[4]   Sripalakit, Pattana, Neamhom, Penporn, Saraphanchotiwitthaya, Aurasorn; Journal of Chromatography, **B843(2)**, 164-169 **(2006)**.

[5]   Berecka, Anna, Gumieniczek, Anna, Hopkala, Hanna; Journal of Planar Chromatography Modern TLC, **18(101)**, 61-66 **(2005)**.

[6]   Ho, N.M.Emmie, Yiu, C.H.Kenneth, Wan, S.M. Terence, Stewart, D.Brian, Watkins, L.Keith; Journal of Chromatography, **B811(1)**, 65-73 **(2004)**.

[7]   A.Jedlicka, J.Klimes, T.Grafnetterova; Pharmazie, **59(3)**, 178-182 **(2004)**.

[8]   N.Jyothi, D.P.Sujan, Kumar, P.Ravi Rao, J.V.L.N. Seshagiri Rao, V.Jayathirtha; International Journal of Chemical Sciences , **4(2)**, 279-284 **(2006)**.

[9]   Chandna, Shveta, A.V.Kasture, P.G.Yeole; Indian Journal of Pharmaceutical Sciences, **67(5)**, 627-

Full Paper

629 **(2005)**.

[10] R.T.Sane, S.N.Menon, Inamdar, Shafi, Mote, Mandar, Gundi, Gunesh; Chromatographia, **59(7/8)**, 451-453 **(2004)**.

[11] B.L.Kolte, B.B.Raut, A.A.Deo, B.A.Bagool, D.B. Shinde; Journal of Chromatographic Science, **42(1)**, 27-31 **(2004)**.

[12] T.Radhakrishna, D.Sreenivas Rao, G.Om Reddy; Journal of Pharmaceutical and Biomedical Analysis, **29(4)**, 593-607 **(2002)**.

[13] D.B.Wanjari, N.J.Gaikwad; Indian Journal of Pharmaceutical Sciences, **67(2)**, 256-258 **(2005)**.

[14] ICH, Stability Testing of New Drug Substances and Products (Q1AR), International Conference on Harmonization, IFPMA, Geneva, **(2000)**.

[15] M.Bakshi, S.Singh; J.Pharm.Biomed.Anal., **28**, 1011-1040 **(2002)**.

[16] M.Bakshi, B.Singh, A.Singh, S.Singh; J.Pharm. Biomed.Anal., **26**, 891-897 **(2001)**.

[17] 'Pharmaceutical stress testing-Predicting Stress Degradation', Drug and the Pharmaceutical Science, 153

[18] ICH: Guidance for Industry Q2(R)Validation of Analytical Procedure; Text and Methodology, Fed.Regist., **62(96)**, 27464-27467 **(2006)**.

[19] ICH, Photo stability Testing of New Drug Substances and Products - Q1B

[20] 'HPLC Methods for Recently Approved Pharmaceuticals',George Lunn, Published by John Wiley & Sons, Inc, 511 **(2005)**.