Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(5): 426-433
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

A Validated Stability Indicating HPLC method for the Determination of Impurities in Pioglitazone Hydrochloride

N. Rashmitha[a], Hiriyanna S.G[a], CH. Sreenivasa Rao[a], K. Chandra Sekhar Reddy[a], M. Hari Kiran[a], Hemant Kumar Sharma[a]*, K. Mukkanti[b]

[a] *Department of Analytical Research, APL Research Centre (A Division of Aurobindo Pharma Ltd.), Bachupally, Quthubullapur, Hyderabad, India*
[b] *Head of the Department Centre for Pharmaceutical Science, Institute of Science and Technology, J. N. T. University, Hyderabad, India*

ABSTRACT

The present paper describes the development of a reversed-phase high performance liquid chromatographic (RP-HPLC) method for Pioglitazone hydrochloride in the presence of its impurities and degradation products, generated from forced degradation studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of Pioglitazone hydrochloride was observed under base and oxidative stress conditions. The drug was found to be stable in other stress conditions studied. Successful separation of the drug from the process related impurities and degradation products formed under stress conditions were achieved on an Inertsil ODS-3V (150 x 4.6 mm), 5 µm column. The gradient LC method employs solution A and solution B as mobile phase. The solution A contains phosphate buffer pH 3.1 and acetonitrile as Solution B. The developed RP-LC method was validated with respect to specificity, linearity, accuracy, precision and high sensitivity with detection limits and quantification limits ranging from 0.033 µg/ml to 0.049 µg/ml and 0.100 µg/ml to 0.150 µg/ml respectively. To the best of our knowledge, a rapid LC method, which separates all the impurities, disclosed in this investigation was not published elsewhere.

Key words: Pioglitazone hydrochloride, RP-HPLC, Impurities, Degradation products and Validation.

INTRODUCTION

Pioglitazone is a thiazoldineone oral antidiabetic drug. It is used in the management of Type-2 diabetes mellitus [1]. It is chemically designated as (±)-5-[4-(2-(5-Ethyl-2-Pyridinyl)ethoxy)benzyl]-2,4-thiazolidinedione.hydrochloride. The literature survey reveals that

chromatographic methods were reported for determination of Pioglitazone and its metabolites, in human plasma [2-4], human serum [5-6], urine [7] and in pharmaceutical formulations [8-10].

Organic impurities can arise during the manufacturing process and storage of the drug substances. The criteria for their acceptance up to certain limits are based on pharmaceutical studies or known safety data [11]. Two unknown impurities were detected consistently in HPLC along with four known impurities i.e. Impurity-B, Impurity -C, Impurity-D and Impurity-E were reported in US Pharmacopeial Forum [12]. However, there was no report of these new impurities i.e., Impurity-A and Impurity-F in the literature. As per regulatory guidelines, the pharmaceutical studies using a sample of the isolated impurities can be considered for safety assessment. Therefore, it is essential to isolate and characterize unidentified impurities present in the drug sample. During the development of an analytical procedure, the LC method was developed for the determination of In-house synthesized Pioglitazone hydrochloride and to separate impurities arising during its synthesis. In the present study, we describe a reverse phase liquid chromatography method for the separation and quantification of process related and degradation impurities of Pioglitazone hydrochloride. The accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness of the method were determined in accordance with ICH guidelines [13]. This research paper reports for the first time a rapid, efficient, simple and validated LC method for the separation of process related impurities and degradation products.

MATERIALS AND METHODS

Chemicals

Reference standard of Pioglitazone hydrochloride and six impurities namely, Impurity-A, Impurity-B, Impurity-C, Impurity-D, Impurity-E and Impurity-F (Figure 1) were synthesized and characterized by using LC-MS, NMR and IR in Aurobindo Pharma Ltd., Research Centre, Hyderabad, India. The commercial samples of Pioglitazone hydrochloride are also manufactured by Aurobindo Pharma Ltd. All reagents used were of analytical reagent grade unless stated otherwise. Milli Q water, HPLC-grade acetonitrile and HPLC-grade orthophosphoric acid (OPA) were purchased from Merck (Darmstadt, Germany).

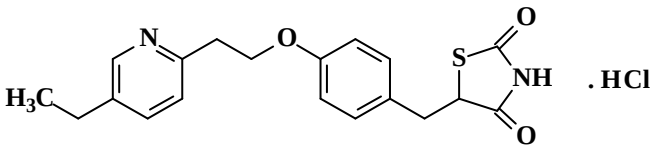
The LC system was equipped with quaternary gradient pumps with autosampler and auto injector (Alliance 2695, Waters, Milliford, MA, USA) connected to a photodiode-array detector (PDA; Waters model 2996); all were controlled by Empower software (Waters).

Preparation of Standard Solution

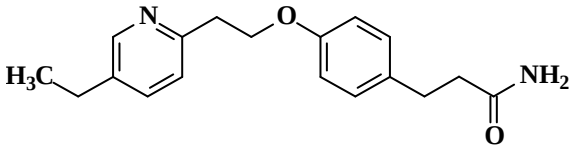
Accurately weighed 60 mg of Pioglitazone hydrochloride was transferred to a 100 ml volumetric flask, added about 50 ml of diluent and sonicated to dissolve, diluted upto the mark with diluent and mixed. Further, 5 ml of the resulting solution was diluted to 100 ml with diluent and further 5 ml of the solution was diluted to 100 ml with diluent to prepare a final standard concentration of 0.0015 mg/ml.

Preparation of Sample Solution

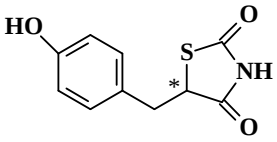
Accurately weighed 50 mg of Pioglitazone hydrochloride was transferred to 50 ml of volumetric flask, added about 25 ml of diluent sonicated to dissolve, diluted upto the mark with diluent and mixed.



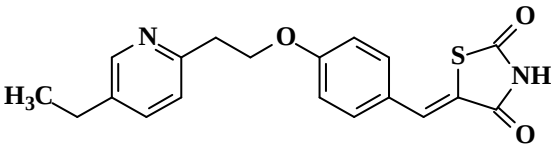
PIOGLITAZONE HYDROCHLORIDE



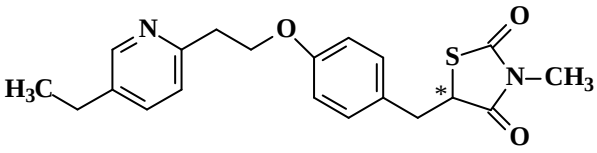
IMPURITY-A



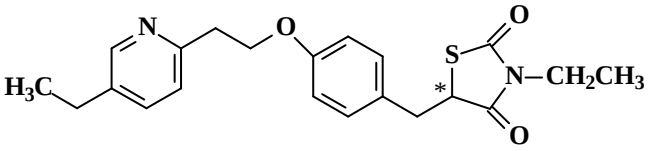
IMPURITY-B



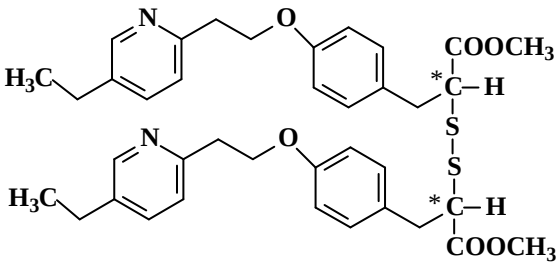
IMPURITY-C



IMPURITY-D



IMPURITY-E



IMPURITY-F

** asymmetric centre*

Figure 1. Chemical structures of Pioglitazone Hydrochloride and its impurities.

Chromatographic Conditions

The chromatographic separation was achieved on an Inertsil ODS-3V (150 x 4.6 mm), 5 µm particle size. The gradient LC method employs solution A and B as mobile phase. The solution A contains phosphate buffer pH 3.1 and acetonitrile as Solution B. The flow rate of the mobile phase was 1.5 ml/min. The column temperature was maintained at 30°C and the detection was

monitored at a wavelength of 225 nm. The injection volume was 20 µl. Standard and test solutions were prepared in mixture of 0.1% OPA solution : acetonitrile (50:50, v/v) was used as diluent.

Validation of the method

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [11]. The specificity of the developed LC method for Pioglitazone hydrochloride was carried out in the presence of its impurities namely, Impurity-A, Impurity-B, Impurity-C, Impurity-D, Impurity-E and Impurity-F. Stress studies were performed for Pioglitazone hydrochloride drug substance to provide an indication of the stability indicating property and specificity of the proposed method. ICH guidelines degradation was attempted to stress condition of heat (105°C, 120 h), acid (5M HCl, 85°C, 120 min), base (1M NaOH, 85°C, 90 min), oxidation (30% H_2O_2 , 85°C, 240 min) and photolytic degradation (10 K Lux, 120 h) to evaluate the ability of the proposed method to separate Pioglitazone hydrochloride from its degradation product. For heat study, period was 120 h, for acid 120 min, for base 90 min, for oxidation 240 min, for photolytic degradation 120 h. Peak purity test was carried out for the Pioglitazone peak by using a PDA detector. The peak purity factor is within the threshold limit obtained in all stressed samples demonstrates the analyte peak homogeneity.

Precision

The precision of the method was assessed by performing six individual preparations of Pioglitazone hydrochloride (1.00 mg/ml) spiked with 0.20% of Impurity-C and 0.15% of Impurity-A, Impurity-B, Impurity-D, Impurity-E and Impurity-F with respect to the Pioglitazone analyte concentration. The % RSD of the area for each impurity (Impurities-A, -B, -C, -D, -E and -F) was calculated.

The intermediate precision of the method was also evaluated using different analyst and different instrument in the same laboratory.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined by measuring the magnitude of analytical background. The LOD and LOQ were determined from slopes of linear regression curves. The LOD and LOQ for Impurity-A, Impurity-B, Impurity-C, Impurity-D, Impurity-E and Impurity-F were determined by injecting a series of dilute solutions with known concentrations [13].

Linearity

The Linearity of peak areas verses different concentrations was evaluated for Pioglitazone hydrochloride and all the related substances using 10 concentration levels ranging from LOQ to 150% of the specification level. The above tests were carried out for three consecutive days in the same concentration range for related substance method.

Accuracy

The accuracy of the method for all the related substances was determined by analyzing Pioglitazone hydrochloride sample solutions spiked with all the related substances at three different concentration levels of 50, 100 and 150% of each in triplicate at the specified limit.

Robustness

To determine the roubustness of the developed method, experimental conditions were deliberately altered and the resolution between Pioglitazone hydrochloride Impurity-A, Impurity-

B, Impurity-C, Impurity-D, Impurity-E and Impurity-F was recorded. The parameters selected were mobile phase compositon (±2% of gradient composition), pH of the mobile phase (±0.2 units), flow rate (± 10%), wavelength (± 5 nm) and column temperature (± 5°C). The effect of the percent organic strength on the resolution was studied by varying acetonitrile by -5 to +5% while other mobile phase components were held constant.

Solution stability and mobile phase stability

To determine the stability of sample solution, the sample solutions of Pioglitazone hydrochloride spiked with related substances at specified level were prepared and analyzed immediately after preparation and after different time intervals up to 15 h, while maintaining the sample cooler temperature at about 25°C. The results from these studies indicated that the sample solution was stable at room temperature for atleast 15 h.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The main objective of the chromatographic method was to seperate Pioglitazone hydrochloride from Impurity-A, Impurity-B, Impurity-C, Impurity-D, Impurity-E and Impurity-F. Impurities were coeluted using different stationary phases such as C8, phenyl and cyano as well as different mobile phases. The chromatographic separation was achieved on an Inertsil ODS-3V (150 x 4.6 mm), 5 µm particles. The gradient LC method employs solution A and B as mobile phase. The solution A contains phosphate buffer pH 3.1 and acetonitrile as Solution B. The flow rate of the mobile phase was 1.5 ml/min and the peak shape of the Pioglitazone hydrochloride was found to be symmetrical. In the optimized chromatographic conditions of Pioglitazone hydrochloride, Impurity-A, Impurity-B, Impurity-C, Impurity-D, Impurity-E and Impurity-F were separated with a resolution greater than 3, typical relative retention times were approximately 0.31, 0.58, 1.62, 1.85, 2.61, 5.21 with respect to Pioglitazone eluted at 7.415 (Figure 2). The system suitability results are given in Table 1 and the developed LC method was found to be specific for Pioglitazone and all of its six impurities namely Impurity-A , Impurity-B, Impurity-C, Impurity-D , Impurity-E and Impurity- F (Figure 2).

Table 1. System suitability

Parameter	Impurity-A	Impurity-B	Pioglitazone	Impurity-C	Impurity-D	Impurity-E	Impurity-F
RT	2.280	4.337	7.415	12.006	13.730	19.360	38.610
RRT	0.31	0.58	1.00	1.62	1.85	2.61	5.21
R	-	6.66	10.12	11.09	3.76	11.11	29.88
N	6454	5883	6226	11750	14033	20750	43620
Asymmetry factor	1.63	1.01	1.02	1.05	1.03	1.06	1.03

RT = Retention time; RRT = Relative retention time, R = Resolution, N = Theoretical plates

Validation of the Method

Forced Degradation

No considerable degradation observed in Pioglitazone hydrochloride bulk samples under stress conditions such as thermal, photolytic and acid hydrolysis (Figure 2). The degradation of drug substance was observed during base hydrolysis and oxidative stress condition. Pioglitazone

hydrochloride was degraded to Impurity-A under Base conditions (1M NaOH/85°C/90 min) and it was confirmed by co-injection with a qualified Impurity-A standard. Mild degradation was observed under oxidative environment (treated with 30% H2O2/85°C/240 min) leads to the formation of some unknown degradation peaks. Peak purity test results obtained by using a PDA detector confirmed that the Pioglitazone hydrochloride peak is homogenous and pure in all the analyzed stress samples. The summary of the forced degradation studies was given in Table 3.

Table 3. Forced degradation studies data

Stress Condition	Degradation Condition	Peak Area	Degradation (%)	Peak Purity	
				Purity Angle	Purity Threshold
-	Undegraded Sample	10891666	-	0.024	0.258
Acid	5M HCl / 85°C / 120 min.	11043667	Nil	0.020	0.256
Base	1M NaOH / 85°C / 90min	10021304	14	0.015	0.267
Peroxide	30% H_2O_2 / 85°C / 240 min	10703868	1.6	0.019	0.256
Thermal	105°C / 120 Hours	10935181	Nil	0.016	0.256
Photolytic	10K Lux / 120 Hours	10945655	Nil	0.016	0.256

Precision

In the study of the precision of the Pioglitazone hydrochloride related substance method, RSD of peak area of impurities Impurity-A to F was 1.3%. In the intermediate precision study, %RSD for the area of all the six impurities was well within 0.8%, conforming good precision of the method.

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

The values of LOD and LOQ for Pioglitazone were 0.033 µg/ml, 0.101 µg/ml and they were for related substances, in the ranges; 0.033-0.150 µg/ml respectively. The calculated LOD and LOQ concentrations were verified for precision. RSD was in the range of 12.2-14.8 for LOD and 2.2-4.3 % for LOQ respectively. The results were depicted in Table 2.

Table 2: Linearity, LOD, LOQ, Precision and Accuracy data

Parameter	Impurity-A	Impurity-B	Pioglitazone	Impurity-C	Impurity-D	Impurity-E	Impurity-F
r	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
Slope	26250	45376	40076	39974	38873	43454	43665
Intercept	63	-549	265	149	68	-941	-309
RF	1.18	0.88	1.00	1.00	1.03	0.92	0.92
Limit of Detection							
Con (µg/ml)	0.049	0.033	0.033	0.034	0.033	0.033	0.034
%RSD	12.3	11.9	14.8	11.3	12.4	12.7	12.2
Limit of Quantification							
Con (µg/ml)	0.150	0.101	0.101	0.103	0.100	0.100	0.102
%RSD	2.7	4.3	2.9	3.6	2.7	2.2	4.0
Precision %RSD(n=6)	0.8	0.6	1.2	0.9	0.7	1.3	1.3
Accuracy %Recovery(n=3)	98.4-101.1	101.3-103.4	98.3-100.0	98.5-101.2	98.7-101.3	98.3-101.3	99.2-101.3

r = Correlation coefficient; RF = Response Factor

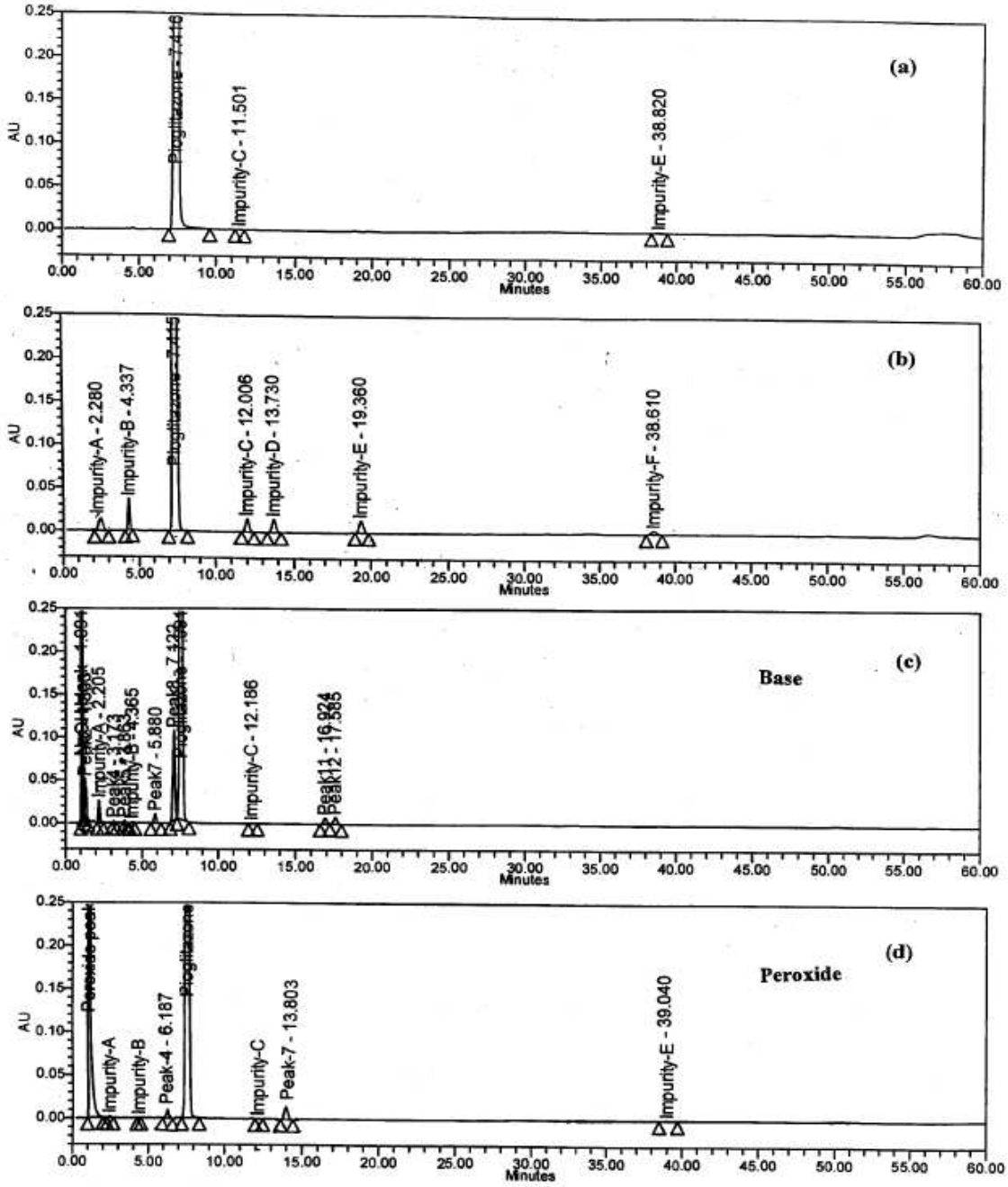


Figure 2. Chromatograms of Pioglitazone HCl, a sample, b spiked with 0.15% of all impurities, c base degradation and d peroxide degradation.

Linearity

Linear calibration plot for the related substance method was obtained over the calibration ranges tested, i.e., LOQ 10 % to 150% for impurities, Impurity-A, Impurity -B, Impurity-C, Impurity-D, Impurity-E and Impurity-F. The correlation co-efficient obtained was greater than 0.9999. Linearity was checked for related substance method over the same concentration range for three consecutive days. The above result shows that an excellent correlation existed between the peak area and the concentration of all six impurities.

Accuracy

The accuracy of all these related substances was found to be in between the predefined acceptance criterion of 98.3-103.4 and the data was given Table 2.

Robustness

When the chromatographic conditions flowrate, column temperature amount of organic solvent in the mobile phase, pH was deliberately varied and resolution between the critical pair, i.e. Impurity-C and Impurity-D, was greater than 3, illustrating the robustness of the method.

Solution Stability

There were no significant changes in the amounts of the impurities during solution stability experiment performed using the related substances method. The results from the studies indicated, the sample solution was stable at room temperature for at least 15 h.

CONCLUSION

A new, accurate and selective gradient RP-HPLC method is proposed for the determination of Pioglitazone related substances in Pioglitazone drug substance and validated as per the ICH guidelines. The method is found to be simple, selective, precise, accurate and robust. Therefore, this method can be used for routine testing as well as stability analysis of Pioglitazone drug substance. All statistical results (Percentage, Mean, RSD, Percentage difference and recovery %) were within the acceptance criteria.

Acknowledgements

The authors wish to thank the management of Aurobindo Pharma Limited Research Centre for their support, encouragement and permitting this work to communicate for publication. Authors also wish to thank the Chemical Research and Analytical Research Departments for their cooperation.

REFERENCES

[1] C.Sean Sweetmann, Martindale-*The complete drug reference*, **2007**, 35th Edition p.414.
[2] Z.John-Lin, W. Ji, D. Desai-Karieger, L. Shum, *J. Pharm. Biomed. Anal*., **2003,** 33, 101-108.
[3] E. Souri, H. Jalalizadeh, S. Saremi, *J. Chromatogr. Sci*.**, 2008**, 46 (9) 809-812.
[4] P. Sripalakit, P. Neamhom, A. Saraphanchotiwittaya, *J.Chromatogr. B*, **2006,** 843 (2) 164-169.
[5] W Z. Zhong , M G. Williams, *J. Pharm. Biomed. Anal*., **1996**, 14, 465-473.
[6] Y.J Xue, C.K.Turner, B. J.Meeker, J. Pursley, M.Arnold, S.Unger, *J. Chromatogr. B*, **2003**, 795 (2), 215-226.
[7] K.Yamashita, H. Murakani, T. Okuda, M. Motohashi, *J. Chromatogr A*, **1996,** 677, 141-146.
[8] R.S. Mehta, D.M. Patel, K.K. Bhatt, M.B. Shankar, *Ind. J. Pharm Sci*., **2005**, 67 (4), 487-489.
[9] A. Jedlicka, J. Kimes , T. Grafnetterova, *Pharmazie*. **2004**, 59 (3), 178-182.
[10] T. Radhakrishna, D. Sreenivas Rao, G. Om Reddy, *J. Pharm. Biomed. Anal*., **2002**, 29 (4), 593-607.
[11] International Conferences on Hormonization Q3A (R2): Draft Revised Guidance on impurities in New Drug Substances, **2006**.
[12] US Pharmacopeial Forum, **2010,** 36 (1), 126-129.
[13] International Conferences on Hormonization Q2 (R1) Validation of analytical Procedures: text and methodology, **2005**.