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Impurity profile study of pioglitazone and glimepiride combination drug product by liquid chromatography

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ABSTRACT

A reverse phase high- performance liquid chromatographic (RP-HPLC) method in gradient mode has been developed and evaluated for its ability to simultaneously establish the level of known impurities as well as the unknown impurities in pioglitazone and glimepiride tablets. The best separation was achieved on Eclipse XDB-C8, 5µm, 4.6 × 150mm column. Use of 0.1N sodium dihydrogen phosphate dihydrate buffer pH 3.0, acetonitrile and methanol, as mobile phase at flow rate of 1.2 mL min^{-1} enabled acceptable resolution of pioglitazone and glimepiride from possible impurities.UV detection was performed at 230nm. The developed method was validated in term of selectivity, linearity, accuracy using spiked levels of impurities, precision (repeatability and reproducibility), limit of detection, limit of quantification and ruggedness. Overall, the proposed method was found to be highly sensitive, suitable and accurate for quantitative determination of known and unknown impurities in dosage form without any interference from the excipients.

KEYWORDS

Pioglitazone hydrochloride;
Glimepiride;
Related impurities;
Analytical chemistry;
Chromatography;
Formulation;
Relative retention time;
Relative response factor;
Validation;
HPLC.

1. INTRODUCTION

Many patients with type 2 diabetes require treatment with more than one antihyperglycaemic drug to achieve optimal glycaemic control. Pioglitazone, 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl] thiazolidine-2,4-dione,belonging to the thiazolidine diones is one of the novel oral antihyperglycaemic drug that improve glycaemic control primarily by decreasing insulin resistance by sensitizing the skeletal muscle, liver and adipose tissue to the actions of insulin. Glimepiride, 1-[[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl]sulphonyl]-3-trans-(4-methyl cyclohexyl)urea is an third generation sulphonyl urea used to reduce blood glucose levels by stimulating insulin secretions from the beta cells of pancreas and also known to increase peripheral insulin sensitivity thereby decreasing insulin resistance.Pioglitazone as mono therapy and in combination with sulfonyureas not only reduce glycosylated hemoglobin levels, but also effect changes in blood lipid concentrations and have the potential to ameliorate cardiovascular disease risk[1], hence necessitates the development of combination dosage forms.

Further, an safety of a drug is dependent not only on the toxicological properties of the active substance itself, but also on its pharmaceutical impurities, which consist of reaction by-products, generated during synthesis of drug substances and degradation products formed during the formulation manufacturing process and/or storage of drug substances or formulated products. Impurity profiling is increasingly viewed as a valu-

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able and essential part of quality requirement. Establishment of monitoring methods for impurities and degradation products during pharmaceutical development is necessary because of their potential toxicity[2,3]. HPLC is an extensively used technique in the pharmaceutical industry due to the availability of fully automated systems, excellent quantitative precision, accuracy, broad linear dynamic range and availability of a wide variety of column stationary phases. The aim of this study was to develop LC method for simultaneous determination of known, unknown and degradation impurities in pioglitazone and glimepiride combination pharmaceutical drug product.

Glimepiride is officially recognized in the European and United states Pharmacopeia in the pure form and not in the dosage form[4], whereas Pioglitazone is not official in any. Analytical method has been reported for determination of related impurities as well as degradants in glimepiride active[5,6]. Literature search also revealed methods for the determination of pioglitazone and its metabolites as well as impurities on HPLC[7,8]. Determination of pioglitazone and glimepiride individually and in combination with other drugs by HPLC has also been reported[9-12]. However, to our knowledge, there are no published reports on quantitative analysis of potential impurities in Pioglitazone and Glimepiride combination dosage form. The Pharmacopeial impurities of glimepiride examined were glimepiride sulfonamide (GS) denoted as Glimepiride Related compound B , glime piride urethane (GU) denoted as Glimepiride Related compound C and glimepiride -3- Isomer (GS) denoted as Glimepiride Related compound D along with the pioglitazone impurity, (5-[4-{2-(5-ethyl-2-pyridinyl) ethoxy} benzyl]-2, 4-thiazolidinedione (Figure 1).This paper describes accurate quantification of these impurities in pharmaceutical dosage form along with the method validation as per the ICH norms. The developed and validated method is specific, precise, accurate and stable with improved sensitivity

2. MATERIALS AND METHODS

2.1 Chemical and reagents

USP reference standard of glimepiride(G), glimepiride Ortho Isomer (GI),glimepiride Urethane (GU) and glimepiride Sulphonamide (GS), working standard of pioglitazone (P) and its related impurity (5-[4-{2-(5-ethyl-2-pyridinyl) ethoxy} benzyl]-2, 4-thiazolidinedione (PI) were supplied by Ipca laboratories Ltd.Mumbai. Pioglitazone and Glimepiride tablets R and D sample manufactured Ipca Laboratories Ltd were used for investigations.One tablet contains 30mg of P active and 2 mg of G active along with other ingredients. The average mass of tablet was 270mg. HPLC grade of Acetonitrile, Methanol and AR grade of Sodium dihydrogen phosphate dihydrate, ortho phosphoric acid AR grade (88%) were procured from Merck. Milli-Q water was used. GF/C filter paper was obtained from Whatmann. All dilutions were prepared in standard volumetric flasks.

2.2. Instrumentation and chromatographic conditions

Chromatography was performed using HPLC of

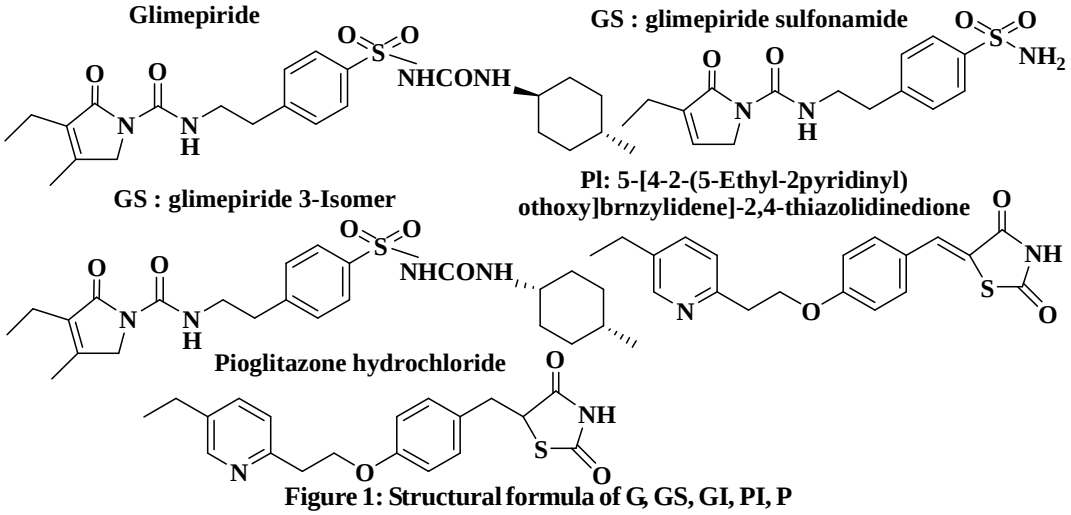

Figure 1: Structural formula of G, GS, GI, PI, P

Waters 2695 Alliance separation module system, Waters 2996 with PDA detector and column oven. Chromatograms and data were recorded by means of Empower software version 2.10. Separation was achieved on Eclipse XDB-C8,(150mm × 4.6mm dimensions) having particle size 5µm, with flow rate as 1.2mL min^{-1} and column oven temperature as 50^0C. The mobile phase consists of 0.1N Sodium dihydrogen phosphate dihydrate buffer, pH adjusted to 3.0 with ortho phosphoric acid, methanol and acetonitrile.The isocratic gradient was 50% buffer and 50% methanol for initial 15min, then decrease to 5% of methanol with increase in acetonitrile to 45% for further 10mins.The injection volume was 20µl and the detection wavelength was 230nm. A typical HPLC chromatogram obtained for simultaneous determination of PI, GS, GU, GI along with P and G is shown in figure 2.

2.3. Diluent

Prepare a solution of 80% acetonitrile in water.

2.4. Standard preparation

Prepare a standard solution containing P and G of concentration 1.5g mL^{-1} and 0.2µg mL^{-1} respectively in diluent.

2.5. Sample preparation

Five tablets were weighed and transferred in 100mL volumetric flask containing about 70mL of diluent. The flask was sonicated for about 15 minutes with intermittent shaking allowing the tablets to disintegrate, cooled to room temperature and diluted to the mark with the diluent. Filtered the solution through GF/C.

3. RESULTS AND DISCUSSION

The optimization of the proposed method was studied to examine conditions that would affect the results. For this purpose, the influence of column type, mobile phase composition, buffer type, buffer pH, column oven temperature and flow rate was systematically investigated[13]. Pharmacopoeial methods were utilized for the development. Also references listed in this paper were taken as a base. In case of reverse phase –HPLC, various columns are available of which C8 column was preferred over the other columns to achieve the best separation. Agilent's Zorbax Eclipse XDB-C8 having less carbon loading and pore size gave a better separation especially between GS and P as compared to other C8 columns. The next goal was to improve chromatographic performance with respect to peak shape and resolution. The combined effect of pH and mobile phase compositions on reverse phase liquid chromatographic behavior of P,G and its impurities were also studied in the range of conditions providing acceptable retention, resolution, tailing factor and number of theoretical plates.pH between 2.6 to 3.2 had no effect on the results.Optimum pH was 3.0, because higher pH resulted in low sensitivity. The preliminary experiments carried out with only sodium dihydrogen phosphate dihydrate buffer and acetonitrile / methanol in different ratio were not successful in the separation of the impurities. Hence, gradient program combined with column oven temperature was introduced and optimized to achieve resolution between GS and GU as well as between P and PI in initial 15mins with methanol and then the separation between G and GI was achieved by introducing acetonitrile along with methanol.Wavelength was selected by scanning both the drugs and the known impurities over the wide range of wavelength 200nm to 400nm. All the components show reasonably good response at 230nm (Figures 2 and 3).

Hence, after studying different column make and composition of mobile phase of buffer, methanol and acetonitrile, the above method has been finalized to optimize the retention time of P, G along with its related impurities.

4. Method validation

Validation of method establishes that its performance characteristics are adequate for the intended purpose. The study was performed as per the ICH guidelines for impurities[14,15], taking into consideration the specified limits for GS as 0.4%, GU as 0.1%, GI as 0.2% as per the USP monograph, for PI as 0.1% and

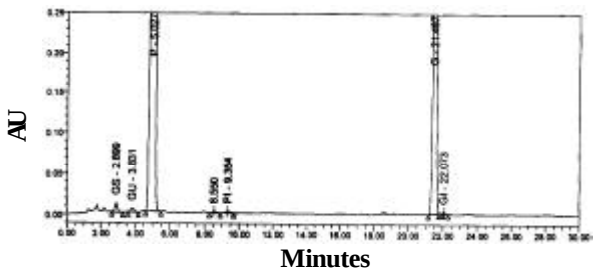

Figure 2: Chromatogram showing the separation between known impurities and the actives

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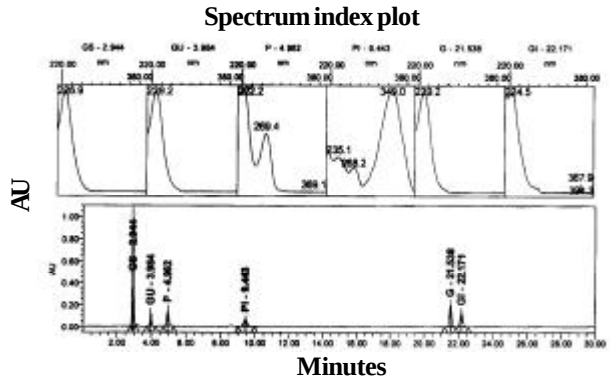
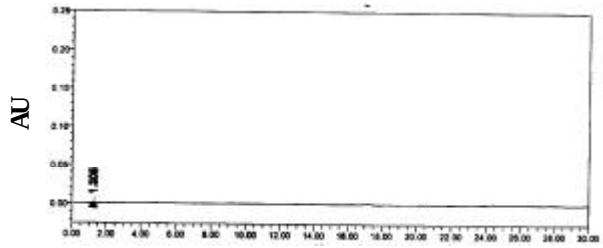


Figure 3: Spectrum Index plot of G, GS, GU, GI, PI, P with HPLC chromatogram



aFigure 4: Chromatogram of diluent

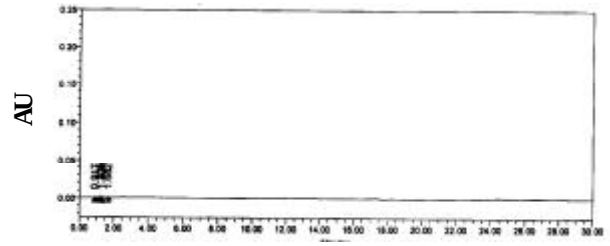


Figure 5: Chromatogram of placebo

for single maximum unknown impurity as 0.1%.

4.1. System suitability

System suitability was performed by injecting Relative retention time solution and determining resolution between closely eluting peaks of GS and GU, GU and P and between G and GI. Also the RSD of peak responses of P and G in standard solution in six replicates, along with the tailing factor and theoretical plates was calculated (TABLE 1).

4.2. Specificity

The specificity of the method was studied by injecting the placebo (containing all the ingredients of the formulation except the analytes) of the tablets as per the procedure applied to sample solution. Individual impurities, actives and the mixture were analyzed. No peak was detected at the retention time of P, G and their related impurities hence proving the specificity of the method (figures 4 and 5).

TABLE 1: Results of system suitability

Parameter	Value
Resolution between GS and GU	2.78
Resolution between GU and P	3.14
Resolution between G and GI	2.24
% RSD of P	0.24
% RSD of G	0.47
Tailing Factor / Theoretical plates of P	0.80/6241
Tailing Factor / Theoretical plates of G	0.90/106925

TABLE 2 : Relative retention time and response factor

Component	Retention time	Relative retention time	Response factor
P	5.03	1.0	1.00
G	21.49	1.0	1.00
GS	2.89	0.13	2.71
GU	3.83	0.18	0.42
GI	22.07	1.03	0.68
PI	9.35	1.86 (with respect to P)	0.74 (with respect to P)

Further forced degradation of P and G drug substances and drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions.

For thermal stress, samples of drug substances and drug product were placed in a controlled temperature oven at 55^0C for 7 days. For photolytic stress drug substances and drug product were exposed to light of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/ square meter[9].

During the initial forced degradation experiments, it was observed that acidic hydrolysis with 0.5N hydrochloric acid was a fast reaction for G. Thus, hydrolytic degradation for drug substances and drug product was carried out using 0.1N hydrochloric acid and 0.1N sodium hydroxide solution at 100^0C for 1 hr.For oxidative stress, actives and drug product were treated with 10% of hydrogen peroxide solution and kept on standing for 30mins. Forced degradation study showed the major formation of GS both in G alone as well in drug product concluding GS as the main degradant (Figures 6 and 7). The peak purity of both the drugs and their related impurities in the degraded samples was established from the purity plot, peak angle and peak thresh-

old indicating no other co-eluting peak was found proving its spectral homogeneity and the stability indicating nature of the method.

4.3. Response factor

Response factor for GI, GS, GU and PI was determined by injecting solution containing mixture of all known impurities and actives at same concentration. The results are listed in TABLE 2.

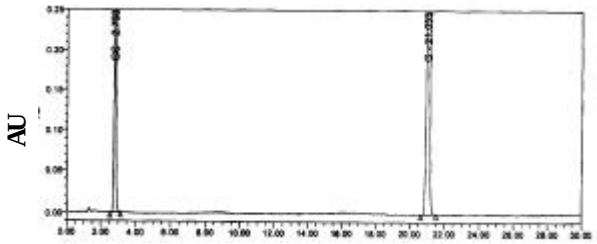


Figure 6: Chromatogram of forced degradation of Glimipiride active showing the formation of GS

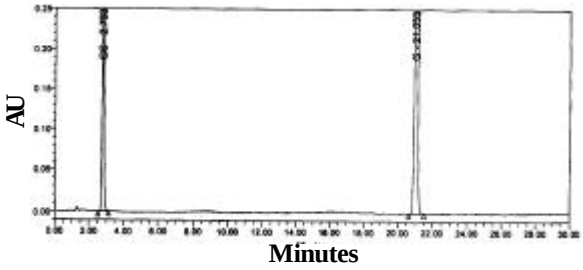


Figure 7: Chromatogram of forced degradation of P and G tablet showing the formation of GS

4.4. Linearity

Linearity was evaluated by analyzing different concentration levels from 10 -200% of the specified limit for related impurities and 0.1% limit for both the actives. The regression data obtained are listed in TABLE 3.

4.5. LOD and LOQ

The limit of detection and limit of quantitation of the known impurities and actives were established from the standard deviation of the response and the slope of the corresponding calibration curve (LOD =3s/n; LOQ = 10s/n) (TABLE 3).

4.6. Accuracy

The accuracy of the method was checked by recovery study using standard addition method, at three different concentration levels i.e. multilevel recovery study.The pre-analyzed samples were spiked with the GI, GU, GS and PI at the specified limit at 80,100 and 120% level (Figure 7). The mean recoveries of the impurities were found to be in the range of 99 -101% (TABLE 4) indicating that the method enables highly accurate estimation of the impurities from the drug product.

TABLE 3 : Linearity, LOD and LOQ results

Analyte	Linearity range	Slope	Intercept	Correlation coefficient	LOD(in μgmL^{-1})	LOQ(in μgmL^{-1})
P	0.90ppm- 2.10ppm	48286.04	79.79	0.9991	0.12	0.41
G	0.12ppm - 0.28ppm	79837.53	77.52	0.9994	0.02	0.05
GS	0.48ppm - 1.12ppm	145393.3	11394.5	0.9898	0.07	0.22
GU	0.12ppm - 0.28ppm	113402.9	81.266	0.9994	0.01	0.04
GI	0.12ppm - 0.28ppm	40828.54	99.56	0.9992	0.04	0.11
PI	0.90ppm - 2.10ppm	37951.87	-555.68	0.9989	0.04	0.13

TABLE 4 : Summary of the results of amount added vs. amount recovered

Level	preparation	GS			GU			GI			PI		
		Active added in μgmL^{-1}	Active recovered in μgmL^{-1}	% Recovery	Active added in μgmL^{-1}	Active recovered in μgmL^{-1}	% Recovery	Active Added in μgmL^{-1}	Active recovered in μgmL^{-1}	% Recovery	Active added in μgmL^{-1}	Active recovered in μgmL^{-1}	% Recovery
80	1	0.637	0.639	99.7	0.156	0.157	99.4	0.319	0.320	100.3	1.236	1.218	98.5
	2	0.635	0.623	98.1	0.158	0.159	100.6	0.324	0.322	99.4	1.149	1.158	100.8
	3	0.639	0.640	100.2	0.166	0.163	98.2	0.326	0.323	99.1	1.190	1.195	100.4
100	1	0.794	0.797	100.4	0.196	0.198	101.0	0.398	0.399	100.3	1.479	1.490	100.7
	2	0.798	0.799	100.1	0.202	0.201	99.5	0.390	0.395	100.3	1.470	1.485	101.0
	3	0.818	0.809	98.9	0.192	0.194	101.0	0.396	0.398	100.5	1.542	1.521	98.6
120	1	0.946	0.953	100.7	0.247	0.243	98.3	0.489	0.485	99.2	1.774	1.787	100.7
	2	0.939	0.949	101.1	0.246	0.243	98.8	0.484	0.482	99.6	1.753	1.776	101.3

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TABLE 5: Results of precision and ruggedness

	GS	GU	GI	PI	Unknown impurity
RSD (Precision)	2.61	2.67	NIL	4.69	3.26
RSD(Ruggedness)	2.56	2.85	NIL	4.58	3.52

4.7. Precision

Precision study was assessed by injection repeatability and sample repeatability. Injection repeatability was confirmed by performing replicate injection of the standard solution and calculating the % RSD of the peak area responses for both the content. The data show good precision of the system with the RSD = 2.0% (TABLE 1). The sample repeatability was studied by analyzing the same sample for six times and calculating the % impurities and RSD.Refer TABLE 5.

4.8. Solution stability

The stability of the analytical solutions of the method was studied by analyzing the standard and sample solution immediately as well as till 24 hrs with two intermediate time point. The stability was assessed by comparing the area response for standard preparation and % impurity in case of sample preparation. The sample results were found within ± 0.05% of the initial value indicates that sample solution can be considered stable under the condition investigated.

4.9. Ruggedness

The ruggedness study was carried out by analyzing same sample six times by different analyst, on different day using different instrument (TABLE 5).

5. CONCLUSION

The proposed method for the simultaneous detection and quantitation of GI, GU, GS, PI and unknown impurities in P and G tablets is highly sensitive, accurate and precise. This procedure can be easily adopted for the routine quality control analysis of tablet dosage form without any interference from the excipients or each other. Method was validated for its performance parameters such as Specificity (placebo interference), Linearity and range, Recovery, LOD, LOQ Precision and Ruggedness. The specificity of the method proves that the method is stability indicating. It was concluded that the developed method offers several advantages such as single chromatographic condition for the determination of impurities of two drugs, simple mobile phase and sample preparation steps, improved sensitivity makes it specific and reliable for its intended use.

5. ACKNOWLEDGMENTS

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