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SIMULTANEOUS ESTIMATION OF PIOGLITAZONE,GLIMEPIRIDE AND GLIMEPIRIDE IMPURITIES IN COMBINATION OF DRUG PRODUCT BY HPLC METHOD

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ABSTRACT

Pharmaceutical analysis, in general, refers to the techniques required to ascertain the identification, strength, quality, and purity of such goods. However, it would be appropriate to expand the scope of this definition to include the study of intermediates and raw materials used in the production of medications for potential reasons. Both the pharmaceutical industry and the chemical industry that creates pharmaceutical raw materials must do this kind of analytical chemistry. Thousands of different organic compounds are used as raw materials in the synthesis of modern pharmaceuticals as well as as intermediates throughout research, development, and synthesis. As a result, in addition to having particular expertise in the evaluation of pharmaceutical products, the pharmaceutical analyst needs to have a solid understanding of fundamental organic analysis.

KEY WORDS: Simultaneous, Impurities, Combination, Drug Product, HPLC Method.

INTRODUCTION

The purity of a pharmaceutical medication product is the best indicator of its quality. When chromatographic methods were not yet developed, the early years of drug analysis relied on non-specific titrimetric and photometric methods to determine the active ingredient content. These methods were backed up by the determination of physical constants and a few limit tests for known impurities, primarily based on colour reactions. The drawbacks of this strategy are widely acknowledged. Numerous instances exist when even highly impure drug components satisfy the standards established in the initial printings of various pharmacopoeias.

A condition known as type 2 diabetes is characterised by elevated blood glucose levels. The most typical type of diabetes is this one. People who are impacted by this condition must live with it for the rest of their life. For the treatment of type 2 diabetes, glimepiride, pioglitazone hydrochloride, and metformin hydrochloride

extended release are combined. It is believed that glimepiride's main mechanism for decreasing blood sugar depends on encouraging the release of insulin from active pancreatic beta cells. A powerful and extremely specific agonist for peroxisome proliferators-activated receptor gamma is pomglitazone hydrochloride. By enhancing peripheral glucose uptake and utilisation, metformin hydrochloride improves insulin sensitivity by reducing hepatic glucose synthesis, reducing intestinal glucose absorption, and decreasing intestinal glucose absorption. As a result, this combination aids in improving glycemic control when treating type 2 diabetes. The prevention of related macrovascular and microvascular problems is also likely impacted by it.

Estimation for glimepiride was in USP. The primary approach for determining the purity of both pharmaceutical formulations and raw materials is known as the HPLC method. Liquid chromatography and derivative spectroscopy are two techniques for the detection of glimepiride in pharmaceutical dosage forms that are described, worked with compounds and degradation pathway techniques related to glimepiride.

PURPOSE OF THE WORK

For the simultaneous assessment of pioglitazone, glimepiride, and glimepiride impurities in the combination medication product, no stability-indicating HPLC approach has yet been published. Pioglitazone and glimepiride are extremely unstable drugs. Although this combination medicine product has been marketed by a number of pharmaceutical companies, there is no analytical approach available to identify it using standard quality control and stability sample analysis processes. The unstable compounds in glimepiride and pioglitazone require the development of a stability-indicating assay technique. Additionally, glimepiride significant degradations of impurities B and C were injected and estimated in the combination tablets to demonstrate the method's selectivity. A single HPLC method for the simultaneous quantification of pioglitazone, glimepiride, glimepiride impurity B, and impurity C from the combination drug product is being developed as part of the current investigation.

RESEARCH METHODOLOGY

Pioglitazone and glimepiride pharmaceutical grade standards were provided by M/S Pharma Lab and are chemically described as 5-(4-[2- (5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione and 3-ethyl-4-methyl-N-(4-[N-((1r,4r)-4-methyl cyclohexylcarbamoyl) s (Baddi, India). Chemically known as 3-Ethyl-4-methyl-2-oxo-N-[2-(4-sulphamoylphenyl) ethyl] Glimepiride Impurity B -2,3-dihydro-1H-pyrrole-1-carboxamide) and impurity C, which is chemically known as methyl [[4-[2-[[(3-Ethyl-4-methyl-2-oxo-1H-

pyrrol-1-yl) carbonyl]amino]ethyl]phenyl]- sulphonyl] carbamate, were bought from LGC Standards (Mumbai, India).

The chemical structures bought combination tablets from the market that had 15 mg of pioglitazone, 2 mg of glimepiride, and 500 mg of metformin hydrochloride (PRICHEK GMP®-produced by Indoco Rem). Acetonitrile for HPLC, potassium dihydrogen phosphate for analytical reagents, and orthophosphoric acid were purchased from Rankem (India). The Milli-Q plus water purification system produced Millipore water (Bedford, MA, USA).

INSTRUMENTATION

For the development and validation, a Waters HPLC system made up of a 2695 binary pump plus auto sampler, a 2996 photo diode array, and a 2487 UV detector (Waters Corporation, Milford, USA) was employed.

SOLUTION FOR SYSTEM SUITABILITY

By dissolving the required amounts in methanol, stock solutions of glimepiride impurity B, impurity C, and glimepiride (1000 g/mL) were created. From the aforementioned stock solutions, system suitability solutions containing 0.2 g/mL of impurity B and impurity C and 0.5 g/mL of glimepiride were created using an 8:2 acetonitrile to water diluent mixture.

PREPARATION OF STANDARD SOLUTION

The right quantity of pioglitazone and glimepiride standard were dissolved in diluent to create a standard solution that included 750 g/mL of pioglitazone and 100 g/mL of glimepiride.

SAMPLE SOLUTION PREPARATION

The mortar and pestle instrument was used to weigh and pulverise twenty pills. In a 100 mL volumetric flask, powder tablets containing 10 mg of glimepiride (or 75 mg of pioglitazone) were added. To completely disperse the material, 60 mL of diluent was added, kept on a rotating shaker for 10 minutes, then sonicated for 10 minutes (during sonicating, the bath temperature was maintained at 25°C), and then diluted to 100 mL with diluent. Pioglitazone and glimepiride had concentrations of 750 g/mL and 100 g/mL, respectively. Centrifuging the resultant solution for five minutes at 10,000 rpm. The measurement of pioglitazone, glimepiride, and glimepiride impurities was done using the supernatant solution.

RESULT AND DISCUSSION

CHROMATOGRAPHIC METHOD IMPROVEMENT

To create a method that could indicate stability, the HPLC method was refined. The sample matrices and degradation products from the stability-indicating method shouldn't interfere with the proper measurement of the active components. Due to the degradability of pioglitazone and glimepiride, the gradient method was chosen over the isocratic method in order to obtain a complete degradation product and provide acceptable resolution between closely eluting molecules. The original trials used glimepiride and pioglitazone in their pure medication forms that had been tainted by glimepiride impurities B and C. Methanol and acetonitrile-containing solvent systems and various buffer pH (2–7) ranges were examined. The initial attempt utilised the C18 reverse phase column chemistry. With a flow rate of 0.8 mL/min, the gradient programme including solutions A (phosphate buffer at pH 3.2) and B (acetonitrile) successfully separated the two solutions. All of the forced degradation samples were injected under the ideal circumstances to demonstrate the method's stability-indicating properties. Because of the influence from degradation chemicals, the peak purity of glimepiride and pioglitazone was unsuccessful. A little change to the gradient, column temperature, and flow rate was made to address this issue, however these trials did not produce the expected outcomes. Therefore, various column chemistry was tested. At first, a known chemical was combined into the C8 column. One degradation peak was seen when the phenyl column was used to analyse the glimepiride peak. The pioglitazone peak purity remained unchanged, however the glimepiride peak purity was satisfactory. The cyano column was then employed for development. More than 2.0 resolutions separated the pioglitazone peak from the primary base degradation peak. According to our knowledge, this is the first approach in which the known compound received a very good resolution despite a number of documented degradation peaks. At 230 nm, pioglitazone, glimepiride, glimepiride impurity B, and impurity C were all detected with a sufficient response. In the case of a stressed sample, a chromatogram was extracted with the full 200–400 nm wavelength range to check for a new impurity at various wavelengths, however only the 230 nm wavelength detected peaks revealed any further peaks. Using 100 g/mL of glimepiride sample preparation and a 25 L injection volume, the needed LOQ value of glimepiride impurities B and C was determined. A fresh sample preparation was made and employed because it was noticed that the impurity B forms quickly throughout the development process. Temperatures in the sonicator bath were kept below 25 °C while the sample solution was being made. In comparison to the current USP monograph glimepiride tablet method, the critical near eluting impurities of glimepiride impurities B and C were discovered at a higher resolution.

Table: 1- Optimized chromatographic method

Mobile phase-A	20 m mol/L potassium dihydrogen phosphate, pH adjusted to 3.2 using dilute ortho phosphoric acid		
Mobile phase-B	Acetonitrile		
Diluent	Mixture of acetonitrile and water (8:2, v/v)		
Column	Zorbax cyano, 250 mm x 4.6 mm, 5 micron		
Column oven temperature	25°C		
Detection wavelength	230 nm		
Injection volume	25 µL		
Flow rate	0.8 mL/min		
Gradient programme	Time (min)	Mobile phase-A (%)	Mobile phase-B (%)
	0.01	80	20
	13	80	20
	50	50	50
	55	20	80
	60	20	80
	63	80	20
	70	80	20

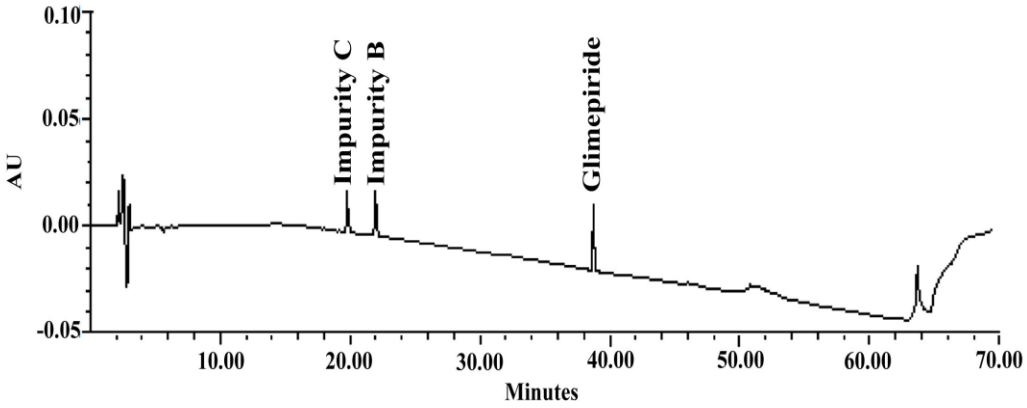
METHOD VALIDATION

According to ICH and FDA criteria, the developed chromatographic technique underwent validation for system applicability, selectivity, specificity, linearity, precision, accuracy, LOD, LOQ, and robustness.

SYSTEM SUITABILITY

Table 2.2 displays the observed analyte retention time (RT) and relative retention time (RRT). The system appropriateness parameter was established at the resolution between the closely eluting pair of glimepiride impurities B and C (> 6.0). Additionally, the peak area of pioglitazone and glimepiride's RSD percentages were computed. Figure 2.5 displays the chromatogram for system appropriateness.

Figure :1- System suitability chromatogram (Containing glimepiride,glimepiride impurity B and



glimepiride impurity C)

SPECIFICITY AND SELECTIVITY

Through the use of forced degradation studies, the specificity of the developed approach was evaluated. The new HPLC method's specificity was assessed in the presence of various sample matrices and its degradation products. In order to demonstrate the specificity and stability-indicating property of the proposed approach, forced degradation investigations were carried out on a tablet sample.

The sample solutions were exposed to oxidation (using 3% H2O2 for 2 hours), UV radiation, and acid and base hydrolysis (using 0.1 N HCl and 0.1 N NaOH, respectively) (254 nm for 48 hours). Minor degradation of the medicine was seen when it was subjected to acid and a peroxide state, but considerable degradation was seen when it was exposed to a base environment. All acid, base, and peroxide stressed samples showed an increase in impurity B, whereas impurity C was only present in the peroxide condition. No signs of deterioration or photolysis were found in the medicines. Peak purity was discovered to be within acceptable bounds in every stressed sample (the purity angle is below the purity threshold), demonstrating the method's specificity. Table 3 displays the results.

All individual chemicals, including pioglitazone, glimepiride, metformin, glimepiride impurity B, and glimepiride impurity C, were injected in the improved process to demonstrate the method's selectivity. By

infusing the sample diluents, blank interference was evaluated. The chemicals under discussion caused no interference. The chromatograms of specificity are displayed.

Table :2- System suitability results

Parameter	Pioglitazone	Glimepiride	Impurity B	Impurity C
% RSD	1.1	1.3	4.1	3.2
Retention time	31.93	38.73	21.99	19.82
Relative retention time	-	1.00	0.57	0.51
USP resolution	-	-	6.50	-
USP tailing factor	1.01	0.99	1.22	1.13
USP theoretical Plates	15011	18123	8012	7532

Table :3- Forced degradation results

Condition	Time	% Assay of Glimepiride	% Assay of Pioglitazone
Unstressed sample	-	99.2	99.0
Acid hydrolysis (0.1 N HCl)	2 hours	96.0	97.2
Base hydrolysis (0.1 N NaOH)	2 hours	91.9	82.3
Oxidation (3 % H_2O_2)	2 hours	95.3	96.3
Light (254 nm)	48 hours	100.2	99.2

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

We measured the level of analytical background and calculated LOD and LOQ. Serial dilutions of the glimepiride impurity B and impurity C solutions were used to calculate the LOD and LOQ. Next, the signal-to-noise ratio was calculated. LOD and LOQ were defined as signal-to-noise ratios of 3 and 10, respectively. For a 25 L injection volume, 0.005% (i.e., 0.005 g/mL) and 0.02% (i.e., 0.02 g/mL) of the LOD and LOQ solutions of glimepiride impurity B and glimepiride impurity C, respectively, were attained by injecting six preparations of the solutions. For glimepiride impurities B and C, the accuracy at the LOQ concentration (six different preparations) was less than 5.0%.

Table.4 LOQ level precision for impurities

Peak Area		
Injection	**Impurity B**	**Impurity C**
1	16950	15591
2	16985	15659
3	17001	16000
4	17500	15350
5	16680	15455
6	17100	15377

Mean	17036	15572
SD	267.34	241.71
% RSD	1.57	1.55

LINEARITY

By measuring five concentration levels at three preparations ranging from 50% to 150% of the analyte concentration, or 750 g/mL for pioglitazone and 100 g/mL for glimepiride, the linearity of the test method was assessed. For both chemicals, the obtained correlation was determined to be more than 0.9999. Six concentration levels from LOQ to 200% (LOQ, 25%, 50%, 100%, 150%, and 200% for impurity B and impurity C) were created by dilution of the impurity stock solution to the necessary concentrations. The obtained correlation coefficient was higher than 0.9999.

Table- 5: Linearity data for drug substances and impurities

Compound	Range (µg/mL)	Regression Parameters (n = 3)	
		Equation of regression line	R^2 value
Pioglitazone	375-1125	Y = 332706x - 479991	0.9999
Glimepiride	50-150	Y = 123014x + 160007	0.9999
Impurity B	0.02-0.4	Y = 867436x - 115.18	0.9999
Impurity C	0.02-0.04	Y = 780417x - 524.47	0.9999

PRECISION

For pioglitazone and glimepiride, respectively, the assay value for the percent RSD of six sample preparations was 1.1. For pioglitazone and glimepiride, the average assay was determined to be 98.2% and 100.2%, respectively. Different columns, methods, and analysts evaluated the assay method's intermediate precision. Both pioglitazone and glimepiride's% RSDs were within 2.0 on various days. Assay value was discovered to range between 98% and 102%, demonstrating the method's robustness. By injecting six separate preparations (in a single injection) of 100 g/mL glimepiride spiked with 0.2% of the aforementioned impurities, the accuracy of impurity B and impurity C was confirmed. Impurity B and impurity C had percent RSDs of 3.2 and 2.9, respectively. The percentage RSDs for contaminants were considerably below the limit of (5.0) in the intermediate precision.

Table :6- Summary of method precision

Injection	Pioglitazone(%)	Glimepiride(%)	Impurity B (%)	Impurity C (%)
1	98.5	99.0	0.26	0.21
2	97.5	99.2	0.27	0.22
3	97.3	100.1	0.27	0.21
4	98.0	101.0	0.27	0.21
5	97.5	101.1	0.25	0.22
6	100.5	100.5	0.27	0.22
Mean	98.22	100.15	0.27	0.21
SD	1.20	0.89	0.01	0.01
% RSD	1.22	0.89	3.16	2.87

Table :7 - Summary of intermediate precision

Injection	Pioglitazone(%)	Glimepiride(%)	Impurity B (%)	Impurity C (%)
1	98.9	97.1	0.27	0.20
2	99.3	98.3	0.26	0.21
3	99.1	99.2	0.26	0.20
4	98.4	99.5	0.26	0.19
5	98.0	99.8	0.27	0.20
6	100.1	99.1	0.26	0.20
Mean	98.97	98.83	0.26	0.20
SD	0.73	0.99	0.01	0.01
% RSD	0.74	1.00	1.96	3.16

ACCURACY

For pioglitazone, glimepiride, and glimepiride impurities, the recovery of three sample preparations at five concentration levels—50%, 75%, 100%, 125%, and 150% of working concentration levels—was calculated.

The recovery of pioglitazone and glimepiride, which ranged from 98% to 102%, was achieved within the acceptable range. The recovery rates for impurity B and impurity C were, respectively, 96.1% to 101.3% and 98.1% to 102.1%. Table 2.8 presents the recovery findings.

Table :8 - Accuracy results for developed HPLC method

Compound	Level (%)	Amount added (µg/mL)	Recovery(%)	% RSD(n = 3)
Pioglitazone	50	375	98.3	1.1
	75	563	98.5	1.3
	100	750	100.1	0.9
	125	938	100.3	1.2
	150	1125	99.2	0.8
Glimepride	50	50	98.1	0.9
	75	75	99.3	1.1
	100	100	99.1	1.2
	125	125	98.7	0.8
	150	150	100.2	0.5
Impurity B	50	0.10	100.3	1.1
	75	0.15	101.2	1.4
	100	0.20	96.1	3.1
	125	0.25	100.1	0.8
	150	0.30	101.3	0.9
Impurity C	50	0.10	99.1	1.3
	75	0.15	98.1	1.2
	100	0.20	98.7	2.0
	125	0.25	102.1	1.9

	150	0.30	101.5	0.9

ROBUSTNESS

To assess the method's robustness, chromatographic settings were drastically changed. We looked at the recovery for the primary components in the sample solution as well as the system appropriateness characteristics. The flow rate (0.1 mL/min), pH (0.2), and organic content (5%) in the mobile phase were the three parameters that were changed. The outcomes of the intentional modifications were perfectly inside the bounds. In all of the adjustments, a resolution better than 5.0 was attained between impurity B and impurity C. The robustness of the procedure was confirmed by the assay value of pioglitatone and glimepiride, which was obtained between 98% and 102%.

Table 9 Robustness result for flow rate variation

Compound	**0.7 mL/min**	**0.8 mL/min**	**0.9 mL/min**
Resolution between impurity B and impurity C	6.8	6.6	6.5
Pioglitazone (%)	98.3	99.1	98.9
Gimepiride (%)	99.1	99.5	98.6
Impurity B (%)	0.06	0.07	0.07

Table 10 Robustness result for buffer pH variation

Compound	**pH 3.0**	**pH 3.2**	**pH 3.4**
Resolution between impurity B and impurity C	6.6	6.6	6.7
Pioglitazone (%)	98.8	99.1	99.2
Gimepiride (%)	99.5	99.5	98.2
Impurity B (%)	0.07	0.07	0.07

Table 11 Robustness result for organic concentration variation

Compound	Acetonitrile (95 %)	Acetonitrile (100 %)	Acetonitrile (105 %)
Resolution between impurity B and impurity C	7.1	6.6	6.1
Pioglitazone (%)	98.2	99.1	99.7
Gimepiride (%)	99.1	99.5	98.4
Impurity B (%)	0.06	0.07	0.07

CONCLUSION

Commercial preparations (PRICHEK GMP®-manufactured by Indoco Rem-Tablets comprising 15 mg of pioglitazone, 2 mg of glimepiride, and 500 mg of metformin hydrochloride) were analysed to assess the applicability of the presented approach. The contents of pioglitazone, glimepiride, glimepiride impurity B, and glimepiride impurity C were calculated after six preparations of the commercial samples. Pioglitazone, glimepiride, and glimepiride impurity B all had average test values of 98.2%, 100.1%, and 0.07%, respectively. The commercial sample that was analysed did not contain glimepiride impurity C.

For the purpose of simultaneously estimating pioglitazone, glimepiride, glimepiride impurity B, and impurity C from the combination medicine product, a single reversed phase stability-indicating RP-HPLC method has been developed. All of the technique validation parameters were successfully checked, and the data were deemed to be adequate. Both quality control departments and commercial sample purity checks can easily employ the established approach for routine chemical analysis.

Pharmaceutical analysis is primarily utilised in the production of pharmaceutical chemicals. The pharmaceutical analyst is a key player in any research on the synthesis of novel chemicals. The analytical work that needs to be done includes everything from the standardised elemental analysis of organic compounds to the highly specialised chemical or instrumental functional group determination in complicated and medicinally significant molecules. The analyst's next responsibility is to provide techniques for process control for the analysis of the intermediates when a novel medicine has been created on a lab scale. Both the pilot plant stage and the full-scale production of the medicine must continue this process. The control division, which is organizationally separate from both production and research, is present in most drug manufacturing companies. This team is in

charge of certifying to the firm management that each manufactured lot of medications satisfies the relevant quality criteria prior to being made available for distribution.

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