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Simultaneous Determination of Antidiabetic and Antihypertensive Drugs in Pharmaceutical Formulations by RP-LC

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Abstract : The objective of the study was the development and validation of an isocratic RP-LC method for the simultaneous estimation of rosiglitazone, glimepiride and amlodipine in a combined dosage form. The separation was achieved with a C_{18} 5micron {250 mm x 4.6 mm i.d.}column, using a mobile phase comprising of a mixture of methanol, water and ortho phosphoric acid (75: 25: 0.2, v/v), the pH of which was adjusted to 4.5 with the help of liquid ammonia. The flow rate was kept at 1 mL min^{-1}, with UV detection at 230 nm. The retention time for rosiglitazone, amlodipine and glimepiride was found to be 2.62, 3.9 and 7.387 minutes, respectively. The LOD was found to be 16.23, 19.88 and 15.81 ng mL^{-1}; while LOQ was found to be 54.16, 66.28 and 52.69 ng mL^{-1} for rosiglitazone, amlodipine and glimepiride, respectively. The developed method was rapid, isocratic, specific, sensitive, accurate and precise and has been successfully applied to the analysis of pharmaceutical dosage forms.
Keywords : Rosiglitazone Maleate**,** Amlodipine Besylate**,** Glimepiride, High Performance Liquid Chromatography.

Introduction

Diabetes rates are skyrocketing worldwide and have nearly doubled in the past three decades due to increase in obesity and sugary diets. An estimated 422 million adults were living with diabetes in 2014, up from 108 million in 1980[1]. Diabetes kills 1.5 million people every year worldwide: this number is expected to double by 2030[2].The burden of diabetic vasculopathy on the global population is enormous and ever growing. Besides the well-known microvascular complications in type 2 diabetes (T2DM), there is a growing epidemic of macrovascular complications. People with T2DM have a higher risk of death from cardiovascular (CV) diseases than persons without diabetes. This calls for an early detection and intervention in patients with T2DM as well as impaired glucose tolerance (IGT), not only to delay progression of IGT to T2DM but also to treat early macrovascular diseases in both groups [3]. The patients with both hypertension and diabetes have a particularly high risk of developing coronary artery disease. These patients take both antidiabetic and antihypertensive drugs. Literature survey revealed that attempts are going on to develop a single medicine which contains both antidiabetic and antihypertensive drugs, to reduce the number of pills taken by the patients having diabetes mellitus associated with hypertension [4-8]. The development of such a medicine requires the data providing drug-drug interaction, stability and pharmacokinetic parameters. For these studies, a quantitative

analytical method is required. Therefore, it is rationale to integrate antidiabetic and antihypertensive drugs and to develop a reliable assay method for the estimation of both types of drugs in combination.

On the basis of market survey and available combinations, two antidiabetics {Rosiglitazone maleate (RGZ)} and Glimepiride (GLM)} and one antihypertensive drug {Amlodipine besylate (AML)} were selected for integration and to develop an assay method to simultaneously estimate the three drugs. The structures of these three drugs are shown in Figure1.Rosiglitazone maleate (5-[[4-[2-(methyl-2-pyridinylamino) ethoxy] phenyl] methyl]-2, 4-thiazolidinedione) is one of the available member of the thiazolidinedione family that acts primarily by reducing insulin resistance [9]. Glimepiride, 1-[[p-(2-(3-ethty-4-methyl-2-oxo-2, 5-dihydro-1-H-pyrrole-1-carboxamido) ethyl] phenyl] sulfonyl)-3-(trans-4-methylcyclohexyl) urea is a third generation sulphonyl urea used as modern oral hypoglycemic agent that can be given as a single daily dose. It acts by stimulating insulin release from pancreatic β-cells and possibly also by extra-pancreatic mechanisms [10]. Amlodipine besylate (2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-Methoxycarbonyl-6-methyl-1, 4-dihydropyridine) is a dihydropyridine derivative which acts by blocking calcium channels, thus producing a negative inotropic effect.

There are various methods reported for estimation of RGZ in tablets [11, 12], in human plasma [13-15] and in urine [16] and for GLM in tablets [17] and in human plasma [18]. The methods are also available for the simultaneous estimation of RGZ and GLM in combination with other antidiabetics [19-24]. There are various methods available for the estimation of AML in human serum [25] and also with combination of other cardiovascular drugs [26, 27]. However, there is no single method reported for the simultaneous estimation of RGZ, AML and GLM in a pharmaceutical dosage form. Thus, an LC-UV method was developed and validated as per ICH guidelines.

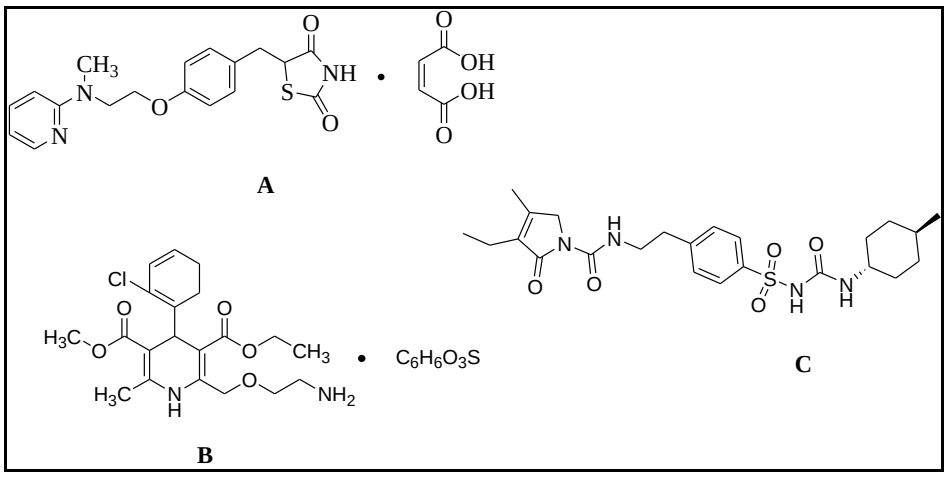


Figure 1.Chemical structure of analytes. A: RGZ, B: AML and C: GLM.

Materials and Methods

Instrumentation and chromatographic conditions

The LC system (SHIMADZU, Japan) consisted of a solvent delivery module (LC-10AT VP), Rheodyne manual injector 7725i, fitted with a 20 µL injection loop and a UV detector (SPD-10 A VP). The chromatographic separation was performed using Lichrocart C_{18} 5micron {250 mm x 4.6 mm i.d.} column. The mobile phase was prepared by mixing methanol: water: ortho phosphoric acid in the ratio of 75: 25: 0.2, v/v; and the final pH was adjusted to 4.5 with liquid ammonia. Mobile phase was degassed by ultrasonication (Toshcon, India).The flow rate was kept at 1 mL min^{-1}. The detection wavelength was set to 230 nm. All the determinations were carried out at an ambient temperature. Operation, data acquisition and analysis were performed using Spinchrom 1.7 software.

Reagents and chemicals

All the solvents and reagents used for the analysis were of HPLC grade. While the solvents used for thin layer chromatography were of analytical reagent grade (AR), HPLC grade water was obtained from Millipore DirectQ3, BBDNITM, Lucknow.

Reference standards of RGZ and GLM were procured from Zydus Research Center (Ahmadabad, Gujarat, India), and AML was supplied by Cadila Pharmaceuticals (Ahmadabad, Gujarat, India). Methanol was of LC grade (Rankem, Delhi), ortho-phosphoric acid (Merck, Mumbai) and liquid ammonia (S. D. fine Chem., Mumbai) were of analytical reagent grade. Deionised water was prepared using a Millipore Direct Q3 ultra-pure water system. For the tablet analysis, Rosicon-G® (containing 2 mg RGZ and 1 mg GLM; Glenmark Pharmaceuticals, India) and Amlodac® (containing 2.5 mg AML; Zydus Medica, India) were purchased from the local market of Lucknow, India.

Preparation of standards solution (SSS-1)

5 mg of compound (RGZ/AML/GLM) was weighed accurately and transferred into a clean and dry 10 mL volumetric flask. The drug was dissolved in 2 mL of methanol and mixed with the help of a vortex mixer for 30 sec, and finally remaining 8mL of methanol was added. SSS-1 thus prepared was stored in a refrigerator. 2 mL of SSS-1 was transferred to a 10 mL volumetric flask and 8 mL of mobile phase was added to obtain standard stock solution of 100 µg/mL. These solutions were kept at 4 ^{0}C.

Preparation of working solution

Further dilutions were prepared in mobile phase to obtain working standards in a concentration range of 0.25-16.00 µg/mL. The quality control samples were prepared in mobile phase to obtain a concentration range of 3-12 µg/mL for RGZ and AML, and 1.5-6 µg/mL for GLM.

Preparation of solutions of mixture of drugs

2 mL each of SSSR-1, SSSA-1 and SSSG-1 was transferred to a 10 mL volumetric flask and 4 mL of mobile phase was added. Thus, the solution containing 100 µg/mL of each drug was obtained.

Preparation of quality control sample

Three quality control (QC) standards of mixture of drugs were made. The three QC standards represent three levels of concentrations viz. high, medium and low. Concentrations of 12µg/mL, 6µg/mL and 3µg/mL represent the high, medium and low levels, respectively for RGZ and AML. Since the tablets taken for the analysis contained half concentration of GLM (1 mg) as compared to RGZ (2 mg), therefore high, medium and low levels of QC standards contained 6µg/mL, 3µg/mL and 1.5µg/mL, respectively. Here, the concentration shown represents the concentration of RGZ and AML; while the same solution contains GLM in half concentration.

Sample Preparation

Five tablets were weighed and finely powdered in a mortar. A quantity of the powder equivalent to one tablet was accurately weighed and transferred to a 10 mL volumetric flask and dissolved in 10 mL of methanol. The flask was sonicated for 15 min. An aliquot was centrifuged at 5,000 rpm for 10 min. Clear supernatant was transferred to a 10 mL volumetric flask. Further, the working test standards were prepared accordingly by serial dilution method to obtain a concentration range of 3-12 µg/mL for RGZ and AML. Since the quantity of GLM in the tablet was half the quantity of RGZ, all the test samples contained half the concentration of the GLM as compared to RGZ.

Method validation

The developed method was validated as per ICH guidelines using various parameters like accuracy, precision, LOD, LOQ, linearity and solution stability.

Results

Method Development

Selection and optimization of chromatographic conditions

The chromatographic conditions were developed by following a series of experiments in an effort to elute RGZ, AML and GLM at a retention time that is suitable for analysis with adequate resolution.

Table1: Selected and optimized chromatographic conditions.

Parameters	Optimized Conditions
HPLC	LC-10AT VP solvent delivery module, S. No. C20974113117 NJ, Shimadzu Corp., Japan.
Injector	Rheodyne Model 7725i, fitted with 20µl loop
Syringe	Hamilton Bonadaz AG microliter syringe (volume 25µl), Switzerland
Detector	UV-VIS detector- Shimadzu, model SPD- 10A VP, S. No. C20994171179, USA.
Software	Spinchrom 1.7 software
Column	Lichrocart C_{18} 5micron {250 mm x 4.6 mm i.d.}
Mobile phase	Methanol: water: ortho phosphoric acid (75: 25: 0.2)
pH	4.5 ± 0.01 adjusted with liquid Ammonia
Flow rate	1.0 mL/min
Wavelength	230 nm
Temperature	Ambient

Determination of solubility of drugs

The solubility profile of all the three drugs selected for analysis RGZ, AML and GLM) was determined in various solvents such as acetonitrile, methanol, isopropanol, absolute ethanol, water and also in the mobile phase which was selected after method development phase. The solubility of RGZ, AML and GLM was determined in different solvents like acetonitrile, methanol, isopropanol, absolute ethanol, water and mobile phase. All the three drugs were soluble in the mobile phase {methanol: water: ortho-phosphoric acid, (75: 25: 0.2); pH 4.5}. RGZ and AML were soluble even at a higher concentration of 3 mg/mL; while 10 mg of GLM was soluble at 15 mL of mobile phase, i.e. solubility of GLM in mobile phase was found to be 666.67 µg/mL.

Selection of mobile phase

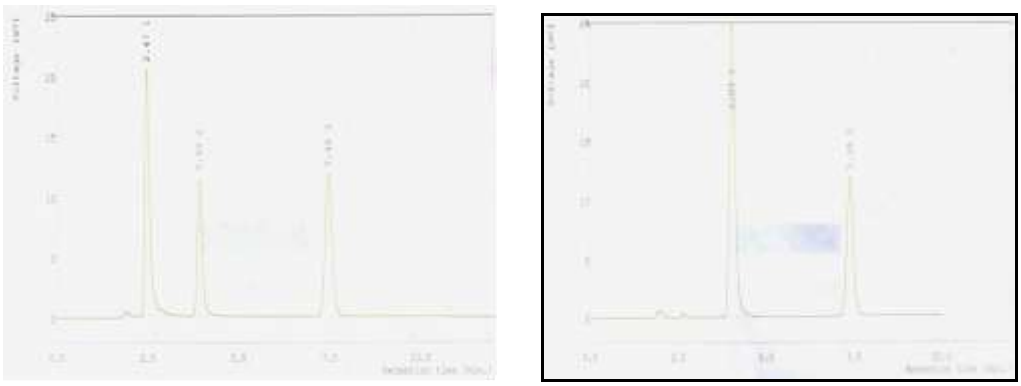
Initially, all the drugs were dissolved in methanol due to greater solubility of selected drugs into methanol. Then, for mobile phase optimisation, the drugs were injected individually in order to determine the retention time of the drugs under analysis. The mobile phase that gave the best peak shape and separation was selected as optimised mobile phase. Mixture of methanol: water: ortho phosphoric acid (75: 25: 0.2) was found satisfactory over other mobile phases tried; as the drugs had satisfactory retention time along with good peak shape.

Selection of pH

After mobile phase optimisation, effect of pH was seen on the resolution of peaks, of all the three drugs, from each other. For the optimisation of pH, injection of individual as well as mixture of RGZ, AML and GLM was used and data were analysed. Further, the pH of mobile phase was adjusted to 4.5, 5.0 and 5.5, and the injection of drugs, individually as well as in mixture, was analysed and the response was observed in context of retention time, resolution and peak shape.

At pH 5.5, RGZ and AML had the same retention time. Therefore when the mixture of both drugs was given, they combined together and gave a single peak. Both the drugs were resolved at pH 5.0 and 4.5; while no significant difference was found in the retention time of GLM at pH 4.5, 5.0 and 5.5. pH 4.5 was selected as the best pH condition because the resolution between RGZ and AML was high as compared to pH 5.0. At pH 4.0,

the RGZ was poorly retained and had short retention time and thus appeared at the retention time of void volume.



(a) **(b)**

Figure 2: Chromatogram of mixture of drugs (a) at pH 4.5 (b) at pH 5.5.

Selection of peak modifier

A peak modifier is added in mobile phase to sharpen the peak. The recommended maximum limit of a modifier is 1%. Different peak modifiers (acidic and basic) were used to select the best analytical condition (Triethylamine, tetrahydrofuran, glacial acetic acid and ortho-phosphoric acid were used as peak modifiers). Triethylamine and tetrahydrofuran were not found satisfactory as the peak shapes obtained were broad and tailing was more. Further, using acidic modifiers increases the peak height and the peak shape also becomes better, with lesser tailing. Ortho-phosphoric acid was selected over glacial acetic acid because glacial acetic acid produced some negative peaks. After the selection of peak modifier, the concentration of the modifier was determined and the drugs were individually, and in mixture, were analysed using 0.2 and 0.5 % of peak modifier.

No significant change was observed in the peak shape and peak area of RGZ, AML and GLM. However, the retention time of GLM was affected significantly and was increased as the concentration of modifier was increased. Therefore, 0.2% modifier was selected over 0.5%.

Selection of wavelength

UV scans of all the three drugs were recorded on a Sytsronics Double Beam UV-VIS Spectrophotometer-2201. The overlain spectra (Figure 3) of the three drugs showed that the drugs had best absorbance at wavelength of 230 nm. On the basis of overlain spectra of the drugs, the wavelength of 230 nm was selected for the optimisation of mobile phase, pH and peak modifiers.

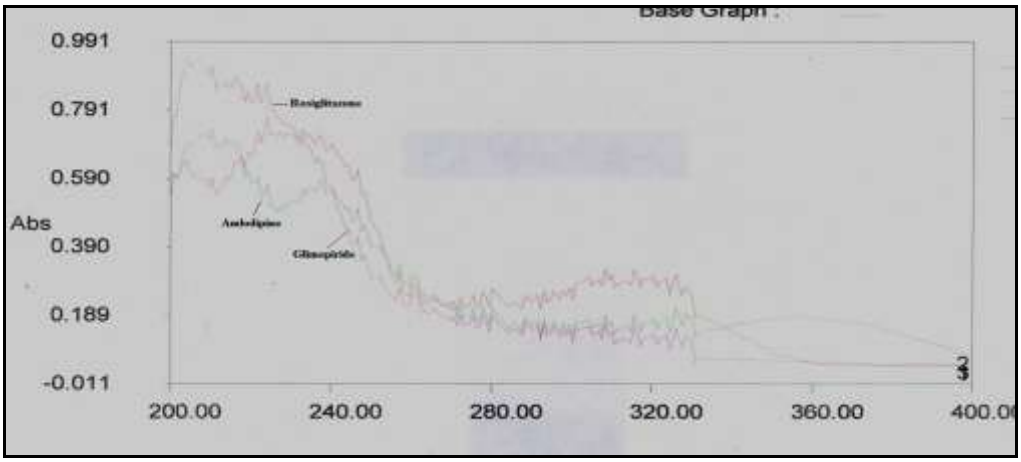


Figure 3: Overlain UV spectra of RGZ, AML and GLM in mobile phase.

Method Validation

The proposed method was validated with respect to stability, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision according to the ICH Guidelines [23].

Calibration and linearity

A standard curve of seven points was plotted. Standard solutions were prepared in the concentration range of 0.25-16 µg/mL for all the three drugs. Quality control samples were prepared at the concentration of 3 µg/mL LQC (low quality control), 6 µg/mL MQC (medium quality control) and 12 µg/mL HQC (high quality control) for RGZ and AML, and 1.5 µg/ mL LQC, 3 µg/mL MQC and 6 µg/mL HQC for GLM.

Specificity

To demonstrate the specificity of the method, blank sample (mobile phase) and commonly used tablet excipients (lactose, magnesium stearate, dextrose, carboxymethyl cellulose and talc) in mobile phase were analyzed. Representative chromatograms were generated and compared with the chromatogram of the mixture of drugs to ascertain that peaks of other components, if any were well resolved from the parent analyte.

A calibration curve of the three drugs was constructed and linearity was assessed by least square regression analysis. The correlation coefficient (r^2) was determined which should be 0.999 or better. The acceptance criteria of standard concentration were ±15% deviation from the nominal value except LLOQ, which was set as ±20%.

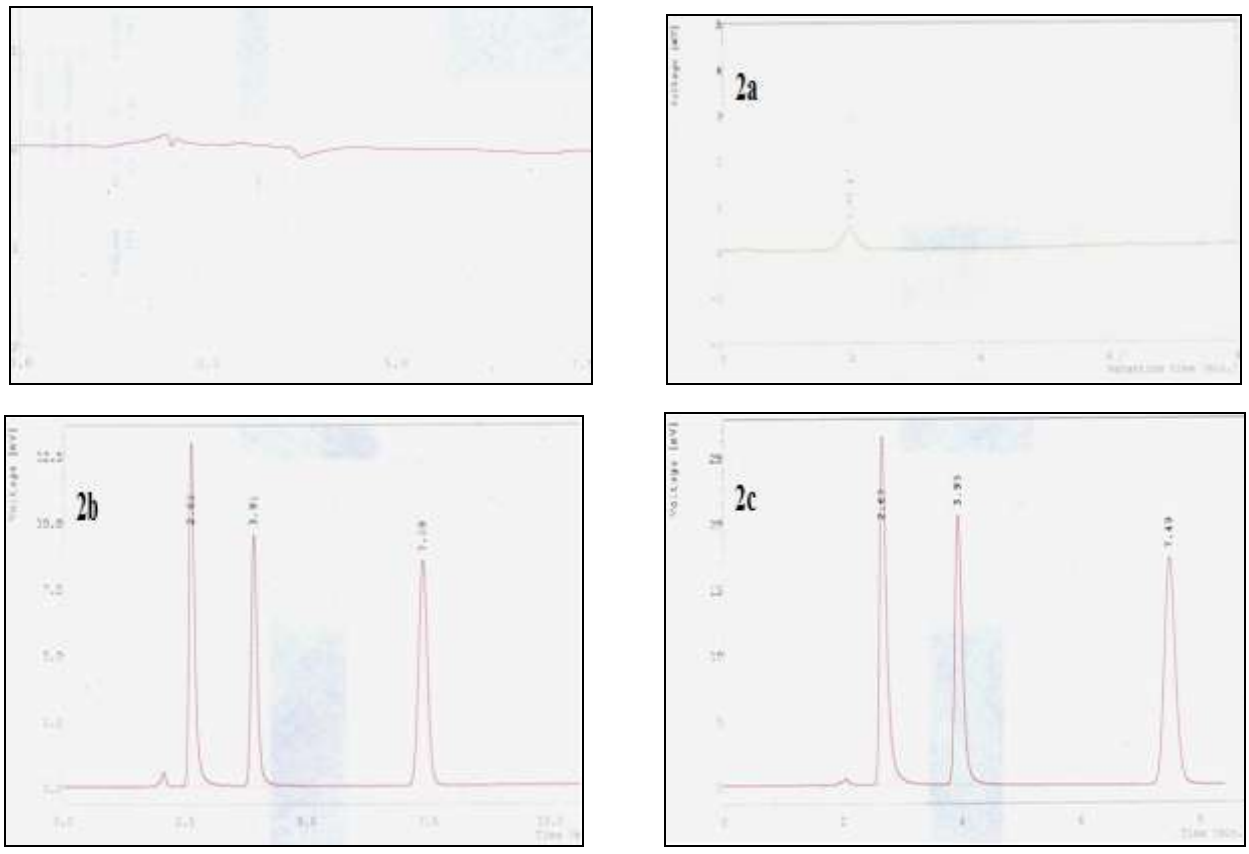


Figure 4: Chromatogram of blank sample (mobile phase). 2a.Representative chromatogram of tablet excipients (placebo); 2b.Representative chromatogram of standard solutions; 2c.Representative chromatogram of commercial pharmaceutical preparations containing RGZ, AML and GLM.

Interaction Studies

The interaction study was done by comparing the peak area of individual drug with the peak area of the same drug in mixture at the same concentration. A difference of ±20% in peak area of individual drug versus

peak area of drug in mixture is often acceptable. The data of analysis for interaction study revealed that the proposed method did not have any interaction with the drug solutions. Also, the drugs were stable in the mixture of other two drugs as the difference in peak area was found to be in an acceptable range of ±20%.

Precision and accuracy

Precision and accuracy were determined by the analysis of three concentrations chosen from the high, medium and low range of the standard curves for all the three drugs (1, 4, and 16 µg mL^{-1}). Triplicates of each samples (n=9) were analyzed on day 1 to determine intra-day precision and accuracy. Inter-day precision and accuracy were determined by triplicate samples of these concentrations on day 1 to 14. Mean, standard deviation and relative standard deviation were calculated from these concentration values and used in the estimation of intra- and inter-day precision.

Table2. Intra-day and inter-day accuracy and precision data of RGZ, AML and GLM.

Drug	Spiked Conc. (µg/mL)	Intra-day			Inter-day		
		Found[a] (µg/mL)	Precision %CV	Accuracy[b] %Bias	Found[a] (µg/mL)	Precision %CV	Accuracy %Bias
RGZ	1	1.018 ± 0.021	2.012	+1.796	0.982 ± 0.042	4.227	-1.781
	4	3.957 ± 0.089	2.266	-1.085	4.117 ± 0.148	3.603	+2.932
	16	16.03 ± 0.243	1.517	+0.196	15.997 ± 0.23	1.442	-0.02
AML	1	1.014 ± 0.037	3.677	+1.43	0.993 ± 0.01	1.039	-0.66
	4	4.007 ± 0.093	2.316	+0.17	3.971 ± 0.05	1.265	-0.732
	16	16.041 ± 0.19	0.159	+0.258	16.024 ± 0.12	0.746	+0.148
GLM	1	1.018 ± 0.028	2.758	+1.77	0.99 ± 0.014	1.438	-1.03
	4	3.991 ± 0.14	3.518	-0.213	4.016 ± 0.111	2.774	+0.41
	16	16.00 ± 0.335	2.092	+0.008	15.949 ± 0.02	0.098	-0.321

a-Mean± standard deviation , b-Bias % =[(found- spiked)/spiked]x10

Accuracy (bias) is expressed as the percent difference between calculated mean concentrations relative to the nominal concentration. The precision (%CV) of ≤5% and accuracy (%bias) of ≤ ±15% are acceptable. The RSD values of intra- and inter-day studies varied from 0.9884 to 4.56%, which showed that the precision of the method was satisfactory (Table 2).System precision was determined from nine replicate injections of the mixed standard solutions. The data show good precision of the system with a RSD of ≤5% (Table 3). Method precision was determined from the results from seven independent determinations at 100% of the sample concentrations of RGZ, AML and GLM.

Table3. System precision data for RGZ, AML and GLM.

Factors	Rosiglitazone	Amlodipine	Glimepiride
Regression equation Y=mx+c;slpoe;intercept	y = 20.249x +1.383 20.249; 1.383	y = 18.0307x + 0.794 18.0307; 0.794	y = 12.744x + 0.3675 12.744; 0.3675
Correlation coefficient(r^2)	1	0.9999	1
Retention time (min)	x* = 2.62 ± 0.0047 %CV = 0.178	x = 3.906 ± 0.0069 %CV = 0.179	x = 7.383 ± 0.017 %CV = 0.226
Capacity factor	x = 1.6222 ± 0.0067 %CV = 0.411	x = 2.9056 ± 0.0073 %CV = 0.25	x = 6.3844 ± 0.0167 %CV = 0.261
Peak asymmetry	x = 1.785 ± 0.0781 %CV = 4.373	x = 1.366 ± 0.026 %CV = 1.904	x = 1.1014 ± 0.0434 %CV = 3.944
Resolution	-	x = 5.752 ± 0.0699 %CV = 1.2155	x = 11.886 ± 0.1112 %CV = 0.936
LOD (ng/ml)	16.23	19.88	15.81
LOQ(ng/ml)	54.16	66.28	52.69

*** = Mean ± standard deviation, CV = coefficient of variance**

Y=mx+c ; m:slope, c: y axis intercept

Stability studies

Stability of the standard solutions of RGZ, AML and GLM was evaluated under different storage conditions. The long-term stability was assessed after storage of stock solutions at 4 ^{0}C for 14 days. The stock solutions of individual drugs as well as mixed standard solutions were analysed on 3rd, 7th and 14th day and the response were compared with the response obtained from the analysis of stock solutions on 1st day. The results showed that the retention time and peak area were almost unchanged (RSD % < 5) and that no significant degradation was observed within the given period, indicating that the solutions were stable for at least two weeks.

Specificity

Specificity, described as the ability of a method to discriminate the analyte from all potential interfering substances, was evaluated by preparing the analytical placebo and it was confirmed that there were no peaks obtained at the same retention time of the drugs. Also, the extraneous peak if obtained was well resolved from the peak of the analyte. A solution of an analytical placebo (containing tablet excipients namely lactose, magnesium stearate, dextrose, carboxymethyl cellulose and talc, except the analytes) was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of the inactive ingredients (placebo) (Figure 4.2a), standard solutions (Figure 4.2b) and the commercial pharmaceutical preparations including RGZ, AML and GLM (Figure4.2c) were analyzed by the proposed method. The representative chromatograms show no other peaks at the retention time of the analytes, which confirmed the specificity of the method.

Linearity

The linearity was determined by plotting the graph between peak area and concentration of the drug. The peak area responses for seven concentrations were determined. The concentrations used for analysis were in the range of 0.25-16 µg/mL for RGZ, AML and GLM. The linearity curves were defined by the following equations: $y = 20.249x + 1.3836$, $r^2 = 1$ for RGZ, $y = 18.0307x + 0.7946$, $r^2 = 0.9999$ for AML and $y = 12.744x + 0.3675$, $r^2 = 1$ for GLM; where y is the peak area and x is the concentration expressed in µg/ mL.

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

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the residual standard deviation of a regression line and slope was adopted. To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analytes in the range of 0.25-16 µg/mL for all the three drugs. The LOD was found to be 16.23, 19.88 and 15.81ng/mL; while LOQ was found to be 54.16, 66.28 and 52.69ng/mL for RGZ, AML and GLM, respectively.

Analysis of pharmaceutical preparations

The developed and validated method was applied to the simultaneous determination of RGZ, AML and GLM in pharmaceutical preparations. Satisfactory results were obtained for each compound and were found to be in agreement with the label claim (Table 3). The results indicated that the amount of each drug in the tablets was within the requirements of 90 to 110% of the label claim.

Table 3: Estimation of the three drugs in mixture of tablet

Drug	Quantity claimed (mg/tab)	Quantity found mg/tab	% Quantity found (± SD)
RGZ	2.0	1.98	99.0 (± 0.022)
AML	2.5	2.51	100.4 (± 0.047)
GLM	1.0	1.01	1.0.0 (± 0.026)

SD = Standard deviation

Discussion

The objective of this work was development and validation of a reliable, simple, specific, and accurate HPLC method for the simultaneous estimation of RGZ, AML and GLM in pharmaceutical dosage forms. Different mobile phases were experimented with to optimize the best mobile phase that shall give a good peak shape, better resolution and shorter retention time. Changing the mobile phase composition did not provide good resolution between AML and RGZ. Therefore, in order to resolve the RGZ and AML; alteration of pH was done. Finally, separation was achieved with a mobile phase consisting of methanol: water: ortho phosphoric acid (75: 25: 0.2, v/v/v), at pH 4.5. The flow rate was kept at 1 mL/min and the peaks were integrated by UV detector at 230 nm. The retention time of RGZ, ALM and GLM was found to be 2.62, 3.91 and 7.383 min. respectively. The specific study revealed the absence of any other compound in the area of interest. Also, there was no extraneous peak present or eluted at the retention time of RGZ, AML and GLM when the tablet excipients and blank samples were analysed. The linearity results showed that an excellent correlation existed between response factor and concentration of drugs within the concentration range, showing that the drug did not have any interaction in the mixture. The sample solutions were stable over the period of analysis (7 days). The value of capacity factor for RGZ, AML and GLM indicated that the peaks were well resolved with respect to each other. RSD values less than 1.0% expressed as %CV, indicated good injection repeatability. The results of analysis of marketed formulations of RGZ, AML and GLM showed that the method was selective for the routine analysis of RGZ, AML and GLM in the industry. The value of analysis of tablets obtained by the proposed method were between 99.0-101.0%, which showed that the estimation of dosage forms were accurate and they were within the acceptance level of 95-105%.

Conclusion

From the results, it can be concluded that the method has been successfully applied for the analysis of marketed tablets and can be used for the routine analysis of formulations containing any one of the selected drugs or their combinations, without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Since the method was successfully applied for the estimation of selected drugs in bulk as well; therefore this method can also be adopted for the study of pharmaceutical release patterns of the drugs while designing new dosage forms. The proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations are analysed by different chromatographic conditions. The simplicity, selectivity, rapidity, reproducibility and economy of the proposed method completely fulfil the objective of the research.

The developed method exhibits excellent chromatographic performance (e.g. good peak shapes, good resolution and short analysis time). It was concluded that the developed method offers several advantages such as rapid and simple mobile phase and sample preparation step. This makes the method suitable for routine analysis in quality control laboratories.

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