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Research Article

Development and Validation of LC-MS/MS method for Simultaneous Determination of Rosiglitazone and Glimepiride in Rat Plasma and its Application to Pharmacokinetic Studies

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Abstract

Rosiglitazone (ROS) and Glimepiride (GLM) are widely used to treat Type 2 diabetes (T2DM). In order to investigate drug-drug interactions between ROS and GLM, a method was developed and validated for simultaneous determination of ROS and GLM in rat plasma employing solid-phase extraction for the separation of analytes and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for quantitation. The compounds were eluted isocratically on a Zorbax SB-Phenyl column, ionized using an atmospheric pressure electrospray ionization source, and analyzed in positive ion mode with multiple reaction monitoring. The ion transitions monitored were 358.13 → 135.06 for ROS, m/z 491.5 → 352.6 for GLM, and m/z 271.3 →172.1 for the internal standard (IS: Tolbutamide). The chromatographic run time was 5 min per injection, with retention times of 1.92, 2.73, and 3.48 min for GLM, IS, and ROS, respectively. The calibration curves of ROS and GLM were over the range of 0.1-6.0 ($r^2 > 0.999$) and 0.05-3.0 µg/ml ($r^2 > 0.9987$) in the combined matrix of rat plasma and a mobile phase (60:40 (v/v) mixture of acetonitrile and 10 mM ammonium acetate buffer with 0.02% TFA (pH 3.00 ± 0.05). The results of the intra- and inter-day precision and accuracy studies were well within the acceptable limits. In addition, all the analytes were found to be stable in a group of stability studies. Thus, the method is precise and sensitive enough for its intended purpose. The developed assay method was successfully applied to a pharmacokinetic study in human male volunteers.

Keywords: Rosiglitazone; Glimepiride; LC-MS/MS; Pharmacokinetics; Method Validation; Chromatography

Introduction

Increased serum glucose either because of impaired insulin secretion or resistance to normal serum levels is the key characteristic of T2DM. Multiple drug therapy is generally preferred in most cases when patients are not recommended for insulin therapy and the cases where monotherapy gets failed. Multidrug therapy involves administering a combination of oral antidiabetic drugs, having different mechanisms of action to provide greater control in maintaining the elevated serum glucose level. A delay in insulin therapy does not adversely affect the serum glucose levels during multidrug therapy, making the treatment more acceptable to the patients over subcutaneous injections [1,2]. Among many

currently existing combinations of multidrug therapy, a diversified combination of ROS and GLM is one of the successful combinations for treating T2DM. ROS, 5-((4-(2-(methyl-2-pyridinylamino) ethoxy) phenyl) methyl)-2, 4-thiazolidinedione (Figure 1) is a thiazolidinedione anti diabetic agent used in the treatment of T2DM. It exerts its effect through the peroxisome proliferator-activated receptor-gamma, facilitating the expression of genes responsible for glucose and lipid metabolism [3]. GLM, 3-ethyl-4-methyl-N-(4-[N-((1r,4r)-4 methyl cyclohexyl carbamoyl) sulfamoyl] phenethyl)-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide (Figure 1) it is a medium-to-long acting sulfonylurea anti-diabetic drug. It is the first of the third-generation sulfonylurea and is very potent [4]. Based on a literature review, it has been found only a few analytical methods for determining ROS in pharmaceutical preparations and biological fluids, including Spectrophotometric approaches [5,6], Voltametric [7], and HPLC [8-10] procedures. In the case of GLM, several methods have been published concerning its analysis in pharmaceutical formulations and biological fluids Viz; spectrophotometric [11], polarographic [12] and HPLC [13-15]. To the best of our knowledge, no published reports have dealt with the quantitation of ROS and GLM using the LC-MS/MS method in a kind of plasma source. In order to evaluate potential drug-drug interactions between ROS and GLM, a sensitive and selective LC-MS/MS method was needed for simultaneous determination of ROS and GLM in plasma. Therefore, this paper describes a method employing extraction of ROS and GLM using solid-phase extraction from rat plasma followed by LC-MS/MS quantitation of ROS and GLM in rat plasma. Because analytical methods must be validated before any pharmaceutical industry can use them, the proposed LC-MS/MS method was validated in accordance with International Conference on Harmonization (ICH) guidelines [16] by assessing its validation parameters.

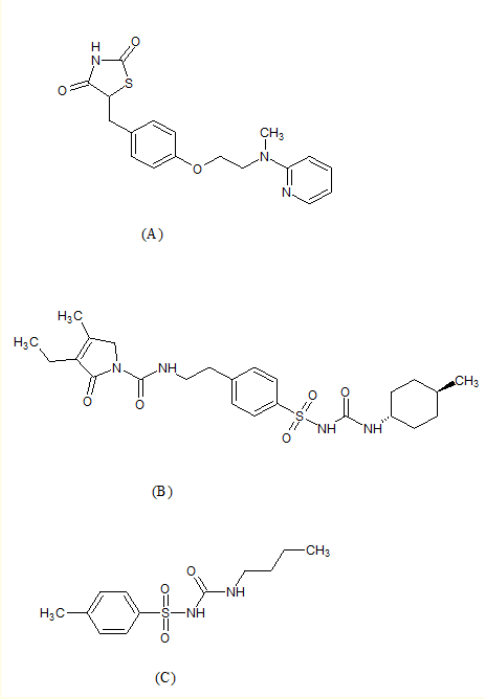

Figure 1: Structural representation of ROS (A), GLM (B) and IS (C).

Materials and Methods

Materials and reagents

The reference samples of ROS (98.84%), GLM (99.19%), and tolbutamide (98.61%) were procured from Neucon Pharma Pvt. Ltd., (Goa, India). The chemical structures of these molecules are presented in figure 1. Water used for the present LC-MS/MS analysis was prepared from a Milli-Q water purification system procured from Millipore (Bangalore, India). Acetonitrile and methanol were HPLC grade and purchased from JT Baker (Phillipsburg, NJ, USA). Analytical grade ammonium acetate and formic acid were purchased from Merck Ltd. (Mumbai, India). The control rat plasma sample was procured from Deccan's Pathological Labs (Hyderabad, India).

Instrumentation and chromatographic conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of an Agilent Zorbax SB-Phenyl, column (150 x 2.1 mm; 5µm, Wilmington, DE, USA), a binary LC-20 AD prominence pump, an autosampler (SIL-HTc), and a solvent degasser (DGU-20 A_3) were used for the study. Aliquots of the processed samples (10 µL) were injected into the column, kept at room temperature. The isocratic mobile phase, 60:40 (v/v) mixture of acetonitrile and water with 10 mM ammonium acetate and 0.02% TFA (pH 3.00 ± 0.05) at a flow rate of 0.3 ml/min with a run time of 5 mints, was delivered into the electrospray ionization chamber of the mass spectrometer. System carry-over was determined by injecting the highest calibration standard, followed by a blank. No carry-over was observed. Quan-

titation was achieved with MS-MS detection, using an MDS Sciex API- 4000 mass spectrometer (Foster City, CA, USA) equipped with a Turboionspray™ interface at 550°C was operated in positive ion mode (ESI^+) for both the analytes and the internal standard. The ion spray voltage was set at 4800 V. The source parameters viz. the nebulizer gas, curtain gas, auxiliary gas, and collision gas were set at 35, 18, 37, and 7 psi, respectively. The compound-specific parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP), and collision cell exit potentials (CXP) were set at 100, 33, 10, and 7V for ROS, 40, 28, 10, and 6V for GLM and 66, 18.5, 15, 8.5V for IS (tolbutamide). Detection of the ions was carried out in the multiple reaction monitoring (MRM) mode by monitoring the transition pairs of 358.13 → 135.0 for ROS, m/z 491.5 → 352.6 for GLM, and m/z 271.3 →172.1 for the IS Quadrupoles Q1 and Q3 were set on unit resolution. The analysis data obtained were processed by Analyst software™ (version 1.4.2).

Preparation of stock and working solutions of analytes and IS

Standard and quality control (QC) samples were prepared from different primary stock solutions of ROS and GLM by separate weighing. Primary stock solutions of ROS, GLM, and IS at 1 mg/mL were prepared in acetonitrile, and these stocks were stored at -20°C; they were found to be stable for 15 days. The stock solutions were suitably diluted with a mixture of acetonitrile and water (60:40, v/v; diluent) to prepare working standard solutions to plot the calibration curve (CC). Another set of working solutions of ROS and GLM were made in diluent (from primary stock) at appropriate dilutions to prepare QC samples. A working IS solution (5µg/mL) was also prepared in a diluent. Finally, working standard solutions of ROS and GLM were prepared in combination.

Preparation of calibration and QC samples

Calibration curve (CC) standards of ROS and GLM were prepared by spiking 430µL of blank rat plasma with 30 (0.12 µM) and 20µL (0.08 µM) of ROS and GLM working standard solutions respectively along with the addition of 20µL of IS, which directly giveaway the final concentrations of 0.1, 0.2, 0.5, 1, 2, 3, 4, and 6 µg/mL of ROS and 0.05, 0.1, 0.2, 0.4, 0.8, 1.5, 2, and 3 µg/mL of GLM. The CC samples were analyzed along with the QC (QC) samples for each batch of plasma samples. The QC samples were prepared at five different concentration levels in blank plasma, namely lowest limit of quantification (LLOQ), lowest quality control (LQC), middle quality control-1 (MQC-1), middle quality control-2 (MQC-2), and highest quality control (HQC) according to ascending concentration order. The prepared concentration levels of LLOQ is ROS/GLM: 0.1/0.05 µg/ml, LQC is ROS/GLM: 0.3/0.15 µg/ml, MQC-1 is (ROS/GLM: 1.80/0.90 µg/ml) MQC-2 is ROS/GLM: 3.5/1.8 µg/ml and HQC (ROS/GLM: 5.00/2.50 µg/ml. All the prepared plasma samples were stored at -70°C.

Sample preparation

All frozen rat plasma samples, calibration standards, and QC samples were thawed and allowed to equilibrate at room temperature prior to analysis. The samples were vortexed to mix for 10 s before spiking. 250 µL aliquot of rat plasma sample was mixed with 10 µL of the internal standard working solution (5 µg/mL of tolbutamide). To this, 500 µL of 2% formic acid was added, followed by vortex mixing for 10 s. The sample mixture was loaded onto Oasis HLB cartridges (1 cm^3, 30 mg/ 1mL) pre-conditioned with 1.0 mL of methanol followed by 1.0 mL water. The extraction cartridge was washed twice with 1.0 mL of 10 mM ammonium acetate solution followed by 1.0 mL of water. ROS, GLM, and IS were eluted with 1.0 mL of the mobile phase. An aliquot of 10 µL of the extract was injected into the LC-MS/MS system.

Method validation

The method was validated for the fundamental validation parameters following the guidelines of USFDA and the International Conference on Harmonization (ICH). The parameters selectivity, linearity, sensitivity, precision, accuracy, matrix effect, recovery, and stability were determined.

Selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank plasma obtained from six different sources, including one lipemic and one hemolyzed plasma. Sensitivity was determined by analyzing six replicates of plasma samples spiked with the lowest level of the CC concentrations. The determination value of precision within 20% was thought to be acceptable.

Linearity

For checking, the linearity standard calibration curves containing at least eight points (non-zero standards) were plotted (0.1-6.0 µg/mL for ROS & 0.05-3 µg/mL GLM).

Accuracy and precision

The precision and accuracy were determined by analyzing six replicates at LLOQ and four different QC levels on six different runs. Six replicates of LLOQ (ROS/GLM: 0.1/0.05 µg/ml), LQC (ROS/GLM: 0.3/0.15 µg/ml), MQC-1 (ROS/GLM: 1.80/0.90 µg/ml), MQC-2 (ROS/GLM: 3.5/1.8 µg/ml) HQC (ROS/GLM: 5.00/2.50 µg/ml) were estimated during the intra-day and inter-day assay precision and accuracy. The precision was expressed by the relative standard deviation (RSD, %) and the accuracy by relative errors (RE, %), respectively. The extraction recovery of ROS, GLM, and IS were evaluated at LQC, MQC-1, MQC-2, and HQC levels in six replicates by comparing the peak areas of the analytes and IS from regular extracted QC samples to the mean area of the analytes and IS from blank extracts spiked after extraction. The matrix effect of ROS and GLM were tested in six different sources of rat plasma at four QC concentration levels (LQC, MQC-1, MQC-2, and HQC), and it was evaluated by comparing the peak areas of analytes and IS from blank extracts spiked after extraction to the mean area of the analytes and IS from neat solutions at equivalent concentration. The stability of the analytes was evaluated by analyzing low, middle and high concentration QC samples in six replicates that were exposed to different storing and handling conditions. Analyte stability was tested using QC samples for multiple freeze/thaw (F/T) cycles on the bench at room temperature (short-term stability) or at -80°C in the freezer (long-term storage). Autosampler stability and working solution stability were also determined. These results were compared with the nominal values expressed in RSD (%) and RE (%).

Pharmacokinetic study

Male Wister rats (250-270 g) were obtained from the animal facility, Gitam University, Visakhapatnam, Andhra Pradesh, and the experimental protocol for animal studies was approved by the Institutional Animal Ethical Committee (IAEC approval file no. IAEC/GIP-1287/SG-F/APPOVED/8/2019-2020). Animals were maintained on 12 h light, 12 h dark cycle under controlled temperature (25 ± 2°C) and humidity (60 ± 5% RH) conditions in polypropylene cages filled with sterile paddy husk. Before drug administration, the rats fasted overnight with free access to water. The dose selected for the present study for ROS was 0.5 mg/kg bw and for GLM 1 mg/kg bw. In combination, specified doses of ROS & GLM in 0.5% sodium carboxymethylcellulose as a suspension in distilled water administrated orally. Animals were provided with a standard diet 3 h post-dose. The rats were anesthetized using diethyl ether, and blood samples (~ 0.40 mL) were collected from the retro-orbital plexus into a microcentrifuge tube (containing 10 µL of saturated EDTA) at 0.0 (pre-dose) 0.5, 1, 2, 3, 4, 8, 12 and 24 h after dosing, and plasma was harvested by centrifuging at 2500 x g for 5 min. The plasma samples were transferred into new tubes and stored frozen at -70°C until analysis. Plasma (150 µL) samples were spiked with IS and processed as described in above section. The plasma concentrations of ROS and GLM at different time points were expressed as mean ± SEM, and the mean concentration-time curves were plotted. Plasma concentration-time data of ROS and GLM were analyzed by non-compartmental methods using Phoenix Winnonlin 6.2.1 software (Pharsight Corporation, Mountain View, CA, USA).

Statistical analysis

Data were reported as the mean ± SD. The main pharmacokinetic parameters of ROS & GLM were calculated using Phoenix Winnonlin 6.2.1 software. In addition, the PK parameters between the combination group and the single drug group were compared in paired t-test by Statistic Package for Social Science (SPSS) 20.0 software. A value of $P<0.05$ was considered statistically significant, and $P < 0.01$ was very significant.

Results and Discussion

Sample pre-treatment

Sample pre-treatment is to remove the interference of endogenous plasma constituents with a high relative extract recovery of the analyte. Several sample pre-treatment methods were investigated and compared. Liquid-liquid extraction with various organic solvents such as diethyl ether, n-butanol, ethyl acetate, and MTBE gave the low extraction recovery and strong interferences from endogenous substances in plasma. The solid-phase extraction is based on a polymeric matrix with a high hydrophilic-lipophilic balance, as ROS and GLM are lipophilic molecules that serve the better choice for extraction of these drugs from the biological matrix. In preliminary studies, after loading the plasma samples to the SPE cartridge, the optimization of the washing solvent was carried out using different concentrations of ammonium acetate buffer of varying pH to remove plasma interferences and avoid drug loss. Out of several trials, the cleanest extraction procedure with the highest recoveries was achieved by washing the biological matrix with two

times of 10mM ammonium acetate buffer followed by water. In addition, different solvents for compound elution from the HLB Oasis sorbent cartridge were tested. Finally, the mobile phase, (60:40 (v/v) mixture of acetonitrile and water with 10 mM ammonium acetate and 0.02% TFA (pH 3.00 ± 0.05)) was selected to elute ROS and GLM from the column, resulting in good recovery with a clear baseline and more negligible plasma endogenous interferences on the chromatogram.

Chromatography

The main objective of this work was to develop a novel, simple and sensitive method for the simultaneous determination of ROS and GLM in rat plasma using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The development of the HPLC method followed systematic changes of the chromatographic factors. The process involved selecting appropriate conditions and their optimization, which includes the type of column packing, column dimensions, mobile phase composition, flow rate, oven temperature, and sample amount. The stationary and mobile phases play an important role in theoretical plate, peak shape, symmetry, and resolution. Therefore, to obtain a symmetrical peak with better resolution and peak purity, various chromatographic conditions were investigated and optimized for the simultaneous determination of ROS and GLM. In order to obtain a suitable stationary phase, a lot of commercially available columns were assessed in respect of the peak shape, retention time, sensitivity, carry-over, and baseline noise for analytes. Out of all the stationary phases tested, the Agilent Zorbax SB-Phenyl, column (150 x 2.1 mm; 5µm) was optimal, which exhibited good peak shape and retention time. In order to achieve the successful elution of two analytes, the composition of mobile phase and variable buffer systems were also investigated. Consequently, an isocratic mobile phase, 60:40 (v/v) mixture of acetonitrile and water with 10 mM ammonium acetate and 0.02% TFA (pH 3.00 ± 0.05) at a flow rate of 0.3 ml/min was selected as optimal mobile phase. The retention time for ROS, GLM, and IS were 3.48, 1.92, and 2.73, respectively.

Optimization of mass spectrometric conditions

The mass spectrometry parameters were optimized by directly infusing the 1.0 µg/mL standard solution of ROS & GLM separately into the mass spectrometry. Considering the signal response, the positive mode was chosen to quantify ROS and GLM. Under the favorable ESI conditions, ROS and GLM produced predominantly protonated molecules $[M+H]^+$ at m/z 358 for ROS and 491.5 for GLM in Q1 full scan mass spectra. The corresponding product ion mass spectra were showed in figure 2. We were chosen transition of 358.13 → 135.06 for ROS, m/z 491.5 → 352.6 for GLM, and m/z 271.3 →172.1 for the IS quantification analysis in multiple reaction monitoring (MRM) chromatogram.

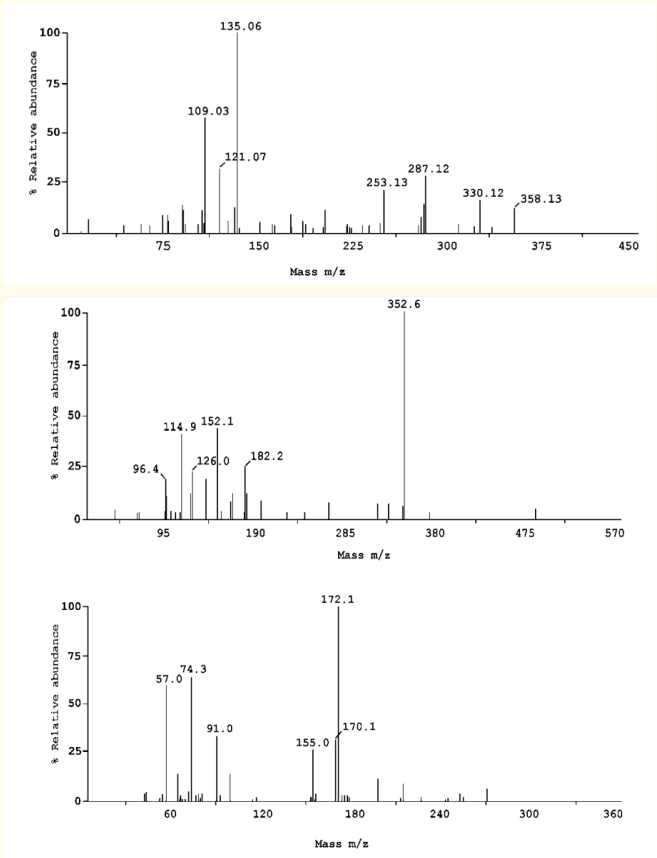

Figure 2: Product ion mass spectra of (A) ROS, (B) GLM and (C) IS.

Method validation

Selectivity

The representative chromatograms of blank plasma samples (Fig 3A, 3D, and 3G) and extracted blank plasma samples at LLOQ (Fig 3B, 3E, and 3H) and extracted plasma sample from a male Wistar rat at 1 h after oral administration (Fig 3C, 3F, and 3I) of ROS,

GLM and IS at their respective retention times were shown in figure 3. ROS and GLM were successfully resolved at 3.48 ± 0.01 min, 1.92 ± 0.02 min, from IS which was eluted at 2.73 ± 0.02 min, which reflect that ROS, GLM, and IS peaks were well separated, and no significant interferences from the endogenous plasma components were observed at the analytes and IS retention times.

Calibration curve and LLOQ

The calibration curves of the two analytes were linear over the concentration range of 0.10-6.00 µg/ml for ROS and 0.05-3.00 µg/ml GLM. The typical equations were y = 0.003x - 0.065 (R^2 = 0.999) for ROS and y = 0.003x - 0.044 (R^2 = 0.998) for GLM. The LLOQ values for ROS and GLM were 0.1 and 0.05 µg/mL respectively, with precision of 8.912 (% RSD) and accuracy of -5.831 (% RE) for ROS and 12.358 (% RSD) and -3.512 (% RE) for GLM (Table 1). Figure 3B and 3E depicts representative chromatograms of 0.1 and 0.05 µg/mL for ROS and GLM respectively.

Precision and accuracy

The results for intra-day and inter-day precision and accuracy in plasma QC samples are summarized in table 1. The intra-day and inter-day precision deviation values were within 15% of the relative standard deviation (RSD) at low, middle-1, middle-2, and high-QC levels, whereas within 20% at LLOQ QCs. The intra-day and inter-day accuracy (RE%) were in the range of -7.77 to 0.55 for ROS and -6.59 to 1.03 for GLM at overall levels of QC samples, including LLOQ. Thus, the results revealed good precision and accuracy of the developed LC-MS/MS method.

Analyte	Measurement Level	Nominal Conc. In µg/mL	Intra day (n = 6)			Inter day (n = 18)		
			Measured conc. (µg/mL) (Mean ± SD)	RSD (%)	RE (%)	Measured conc. (µg/mL) (Mean ± SD)	RSD (%)	RE (%)
ROS	LLOQ	0.1	0.091 ± 0.023	8.912	-5.831	0.095 ± 0.021	11.482	-4.61
	LQC	0.3	0.286 ± 0.071	11.354	-6.012	0.293 ± 0.069	13.293	-7.77
	MQC-1	1.8	1.693 ± 0.395	9.587	-0.906	1.701 ± 0.405	10.568	-1.54
	MQC-2	3.5	3.182 ± 0.674	10.542	-0.637	3.203 ± 0.689	9.524	0.55
	HQC	5	4.913 ± 0.921	8.635	-0.932	4.859 ± 0.835	12.285	-0.75
GLM	LLOQ	0.05	0.043 ± 0.011	12.358	-3.512	0.041 ± 0.018	8.427	-2.36
	LQC	0.15	0.142 ± 0.032	10.582	-2.634	0.139 ± 0.029	9.534	-6.57
	MQC-1	0.9	0.816 ± 0.205	9.523	-1.112	0.827 ± 0.264	11.269	1.03
	MQC-2	1.8	1.704 ± 0.418	13.254	-6.596	1.695 ± 0.425	14.856	-5.22
	HQC	2.5	2.345 ± 0.562	12.876	-0.936	2.316 ± 0.519	12.248	-1.09

Table 1: LLOQ, Accuracy, and precision of ROS and GLM in rat plasma by LC-MS/MS method.

Recovery and matrix effect

The extraction recoveries for ROS and GLM at LQC, MQC-1, MQC-2, and HQC levels are listed in table 2. Extraction recoveries for ROS were 87.23 ± 7.59, 89.41 ± 9.73, 85.78 ± 8.21 and 92.19 ± 12.34 and for GLM, 85.23 ± 9.85, 90.48 ± 11.49, 88.76 ± 8.47and 86.97 ± 9.66 at LQC, MQC-1, MQC-2 and HQC levels respectively. The mean extraction recovery of IS was 94.31 ± 5.03% (n = 18). The results showed that the developed method had good extraction efficiency, and the recovery of both the analytes in rat plasma was consistent, precise, and reproducible. IS-normalized matrix effects of ROS were 1.02, 1.00, 1.01, 1.00 and the RSD were 2.60%, 1.30%, 3.01% and 1.09%, at LQC, MQC-1, MQC-2 and HQC levels respectively. IS-normalized matrix effects of ROS were 1.02, 1.00, 1.01, and 1.03 with the RSD of 4.60, 5.33, 4.01, and 6.09 at LQC, MQC-1, MQC-2, and HQC levels, respectively. IS-normalized matrix effects of GLM were 1.01, 0.99, 0.98, and 1.02 with RSD of 3.02, 5.98, 7.75, and 5.84 at LQC, MQC-1, MQC-2, and HQC levels, respectively. The above results are all within the acceptance limit, and it was illustrated that the rat plasma matrix had no interference in the analysis of ROS and GLM.

Analyte	Measurement Level	Nominal Conc. In µg/mL	Extraction Recovery		Matrix effect	
			Recoveries in % (Mean ± SD)	RSD (%)	IS-Normalized matrix factor	RSD (%)
ROS	LQC	0.3	87.23 ± 7.59	8.346	1.026	4.602
	MQC-1	1.8	89.41 ± 9.73	6.542	1.005	5.306
	MQC-2	3.5	85.78 ± 8.21	9.373	1.018	4.014
	HQC	5	92.19 ± 12.34	12.324	1.032	6.092
GLM	LQC	0.15	85.23 ± 9.85	14.086	1.013	3.023
	MQC-1	0.9	90.48 ± 11.49	11.017	0.996	5.986
	MQC-2	1.8	88.76 ± 8.47	9.601	0.981	7.754
	HQC	2.5	86.97 ± 9.66	8.792	1.027	5.847

Table 2: The recoveries and matrix effects of ROS and GLM in rat plasma by LC-MS/MS method.

Figure 3: Typical MRM chromatograms representing blank plasma analysis at the retention times of (3A) ROS, (3D) GLM, and (3G) IS and blank plasma extracted samples at LLOQ of (3B) ROS, (3E) GLM, and (3H) IS and extracted plasma samples collected after 1 h of post-administration of dosage regimen of (3C) ROS (3F) GLM and (3I) IS.

Stability

QC samples at four concentrations were analyzed in six replicates for studying the possible conditions to which the samples might be exposed during storage and handling. It was found that ROS & GLM were stable in rat plasma after being stored at room temperature for 24 h, after repeated three freeze-thaw cycles, and after being stored at -70°C for 30 days. In addition, the treated samples were found to be stable in the autosampler for a period of 24 h, and the results were found to be within the assay variability limits during the entire process. All results of the stability tests are summarized in table 3.

Pharmacokinetic study

In order to verify the sensitivity and selectivity of this method in a real-time situation, the present method was used to examine the pharmacokinetic status of ROS and GLM in Wistar rats (n=6). The validated method was used to evaluate the plasma concentrations of ROS & GLM in male Wistar rats. We compared the pharmacokinetic parameters (Table 4) of ROS alone with those of ROS plus GLM, administered orally to the rats. The mean areas under the concentration-time curve ($AUC_{0\text{-}36\ h}$) for ROS were 9.75 mg h/L and 9.86 mg h/L in the absence and presence of GLM, respectively, and mean areas under the concentration-time curve ($AUC_{0\text{-}48\ h}$) for GLM were 5.69 mg h/L and 6.12 mg h/L in the presence and absence of ROS respectively. We observed that the co-administration of these drugs had affected the elimination half life's ($t_{1/2}$) of both

Analyte	Measurement Level	Nominal Conc. In µg/mL	Working solutions		Autosampler for 24 hr's		Freeze-Thaw Cycles		Room temp. For 24 hr's		-80°C for 1 month	
			RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)
ROS	LQC	0.3	6.127	-4.56	12.551	-0.16	13.194	0.69	11.435	-2.89	12.613	-5.49
	MQC-1	1.8	9.581	-1.71	10.382	-1.76	11.042	0.03	9.023	-3.29	11.427	-1.65
	MQC-2	3.5	7.342	-0.57	9.178	-6.72	10.324	0.60	7.537	-1.44	9.402	-0.55
	HQC	5	10.263	-0.47	8.095	-0.45	13.152	-0.53	10.164	-0.76	14.323	-0.47
GLM	LQC	0.15	14.186	-4.48	12.663	-4.77	8.055	-6.44	8.782	-9.78	12.997	-5.15
	MQC-1	0.9	11.604	-1.37	10.957	-0.56	11.112	-2.30	10.573	-2.26	10.504	-2.47
	MQC-2	1.8	13.367	-0.76	7.229	-1.12	10.371	-0.62	9.397	0.19	9.533	-1.10
	HQC	2.5	9.205	-0.64	9.028	-1.56	8.182	-0.87	12.133	-0.76	13.472	-0.41

Table 3: Stability of ROS and GLM under various storage conditions.

the analytes. Elimination half-life ($t_{1/2}$) of ROS decreased from 3.46 to 3.41 when co-administered with GLM, as well as $t_{1/2}$ of GLM was greatly reduced from 7.61 to 5.98 when co-administered with ROS. The concentration-time curve ($AUC_{(0-t)}$) and $AUC_{(0-\infty)}$ and $t_{1/2}$ of GLM, when administered alone, was significantly different from it when co-administered with ROS, indicating that co-administration of ROS increased the amount of GLM in the body and reduced the elimination half-life of ROS. Besides, the present study also revealed that the co-administration of GLM with ROS also increases the clinical favourability of ROS (Figure 4A and 4B).

Parameter	ROS alone administered group	GLM alone administered group	ROS and GLM combination group	
	ROS	GLM	ROS	GLM
C_{max} (µg/mL)	3.09 ± 0.81	1.47 ± 0.33	3.21 ± 0.92	1.63 ± 0.76
t_{max} (h)	1.13 ± 0.29	1.53 ± 0.61	1.07 ± 0.18	1.21 ± 0.61
$t_{1/2}$ (h)	3.46 ± 0.78	7.61 ± 1.03	3.41 ± 0.65	5.98 ± 1.47
AUC_{0-t} (µg h/L)	9758 ± 775.24	5698 ± 312.18	9863 ± 575.29	6124 ± 311.85
$AUC_{0-\infty}$ (µg h/L)	110239 ± 946.19	7136 ± 459.25	113254 ± 834.83	8249 ± 582.84

Table 4: Key pharmacokinetic parameters of ROS and GLM.

Figure 4: Comparison of mean plasma concentration-time profile of ROS (Figure 4A) and GLM (Figure 4B) in single analyte administered group and ROS and GLM combined groups following oral administration to healthy rats.

Conclusion

We have developed and validated an LC-MS/MS method that can concurrently quantitate Rosiglitazone, and Glimepiride in rat plasma. The method was fast, selective, and sensitive; the drug extraction process was straightforward; the run time was short; the LLOQ for both Rosiglitazone, and Glimepiride was 0.1 and 0.05 µg/mL, respectively. This study is the first validated industrially suitable LC-MS/MS method, to our knowledge, for the simultaneous quantification Rosiglitazone, and Glimepiride in rat plasma. Thus, this method can be used for therapeutic monitoring of Rosiglitazone and Glimepiride and evaluating the possible impact on the therapeutic efficacy of both the drugs when used in combination for the treatment of type 2 diabetes.

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