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ORIGINAL RESEARCH

Simultaneous quantitation of rosuvastatin and gemfibrozil in human plasma by high-performance liquid chromatography and its application to a pharmacokinetic study†

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ABSTRACT: A simple, sensitive and specific high-performance liquid chromatography method is described for simultaneous determination of rosuvastatin (RST) and gemfibrozil (GFZ) in human plasma using celecoxib as an internal standard (IS). The assay procedure involved extraction of RST, GFZ and IS from plasma into acetonitrile. Following separation and evaporation of the organic layer the residue was reconstituted in the mobile phase and injected onto an X-Terra C_{18} column (4.6 × 150 mm, 5.0 μm). The chromatographic run time was less than 20 min using flow gradient (0.0–1.60 mL/min) with a mobile phase consisting of 0.01 M ammonium acetate:acetonitrile:methanol (50:40:10, v/v/v) and UV detection at 275 nm. Nominal retention times of RST, GFZ and IS were 6.7, 13.9 and 16.4 min, respectively. Absolute recovery of both analytes and IS was greater than 90%. The lower limit of quantification (LLOQ) of RST and GFZ was 0.03 and 0.30 μg/mL, respectively. Linearity was excellent (r^2 = 0.999) in the 0.03–10 μg/mL and 0.3–100 μg/mL ranges for RST and GFZ, respectively. The inter- and intra-day precisions in the measurement of RST quality control (QC) samples 0.03, 0.09, 2.50 and 8.00 μg/mL were in the range 2.37–9.78% relative standard deviation (RSD) and 0.92–10.08% RSD, respectively. Similarly, the inter- and intra-day precisions in the measurement of GFZ quality control (QC) samples 0.30, 0.90, 25.0 and 80.0 μg/mL were in the ranges 2.79–6.27 and 0.96–9.69% RSD, respectively. Accuracies in the measurement of QC samples for RST and GFZ were in the range 85.43–107.23 and 84.98–102.35% respectively, of the nominal values. RST and GFZ were stable in the array of stability studies viz., bench-top, auto-sampler and freeze–thaw cycles. Stability of RST and GFZ was established for 1 month at –80°C. The application of the assay in an oral pharmacokinetic study in rats co-administered with RST and GFZ is described. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: rosuvastatin; gemfibrozil; human plasma; method validation; HPLC; pharmacokinetics; rats

INTRODUCTION

Rosuvastatin (RST, Crestor®, Fig. 1) produces marked dose-dependent decreases in LDL cholesterol in patients with primary hypercholoesterolaemia (Olsson *et al.*, 2001) and was found to be more effective than atorvastatin, simvastatin and pravastatin in reducing LDL cholesterol in hypercholesterolaemic patients, while exerting beneficial effects on triglyceride and HDL cholesterol levels and other lipid measures associated with atherogenic risk (Olsson *et al.*, 2001; Paoletti *et al.*, 2001; Davidson *et al.*, 2002).

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Abbreviations used: EDTA, ethylene diamine tetra acetic acid disodium salt; GFZ, gemfibrozil; RST, rosuvastatin.

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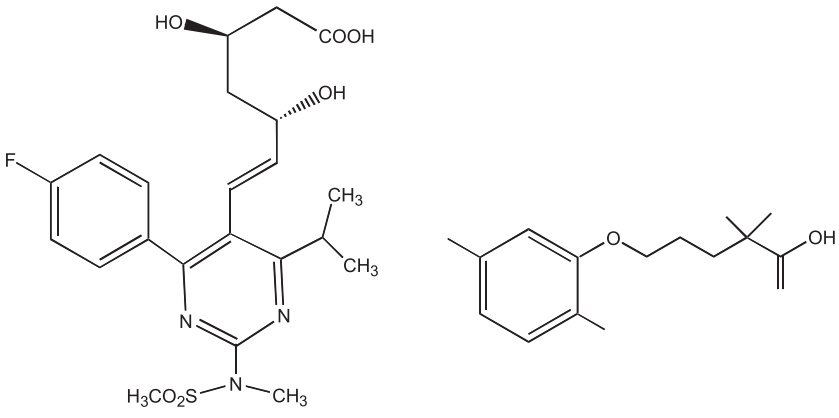
Gemfibrozil (GFZ, CAS no. 25812-30-0; Fig. 1) is a lipid-regulating agent which is generically classified as a fibric acid derivative, but exhibits different pharmacological effects from other such drugs. Published data indicate that in patients with all types of dyslipidaemia (except type I) gemfibrozil 800–1200 mg/day is particularly effective in reducing total plasma triglyceride concentrations. It lowers the incidence of coronary heart disease in humans (Sallustio and Fairchild, 1995). GFZ is well absorbed in the intestinal tract with a plasma half-life of ~1.5 h (Smith and Reynard, 1992). It is extensively metabolized and excreted in urine as a glucuronide together with four oxidized metabolites (Nakagawa *et al*., 1991).

RST and GFZ act by unique and different mechanism of action in controlling elevated lipid levels in the body. Generally it is believed that co-administration of statins and gemfibrozil is associated with an increased risk for myopathy, which may be due in part to a pharmacokinetic interaction. Schneck *et al.* (2004)



ROSUVASTATIN GEMFIBROZIL

Figure 1. Structural representation of RST and GFZ.

studied the effect of GFZ on RST pharmacokinetics and found that GFZ increases the AUC and C_{max} of RST 2-fold, so care is warranted when GFZ is co-administered with RST. Several bioanalytical methods (HPLC and LC-MS/MS) exist for quantitation of GFZ (Hengy and Kolle, 1985; Forland *et al.*, 1987; Knauf *et al.*, 1990; Nakagawa *et al.*, 1991; Sallustio and Fairchild, 1995; Benkone Markus 1996; Dix *et al.*, 1999; Hermening *et al.*, 2000; Gonzalez-Penas *et al.*, 2001; Kim *et al.*, 2003; Borges *et al.*, 2005). To date there have only been two methods reported for the estimation of RST in human plasma, which utilizes high-performance liquid chromatography coupled with mass spectrometry (Hull *et al.*, 2002; Trivedi *et al.*, 2005). Of late, we have reported the method validation for RST in rat plasma using HPLC (Kumar *et al.*, 2006). To the best of our knowledge currently there has been no bioanalytical method reported in the literature describing the full details of a complete methodology and validation for simultaneously assaying RST and GFZ using HPLC. We believe simultaneous quantitation of RST and GFZ on HPLC offers the ease and convenience of routine clinical monitoring for an important combination treatment used to mange lipid levels in dyslipidemic patients. In this paper, we present a simple sensitive and reproducible HPLC assay with commercially available IS for the simultaneous determination of RST and GFZ in human plasma and its application to a pharmacokinetic study in rats.

EXPERIMENTAL

Chemicals and reagents. RST, GFZ and celecoxib (internal standard, IS) were synthesized by the Medicinal Chemistry Group, Discovery Research, Dr Reddy's Laboratories Ltd (DRL), Hyderabad, and were characterized using chromatographic and spectral techniques by Analytical Research Group, Discovery Research, DRL, Hyderabad. Purity was found to be more than 99% for all the compounds. HPLC-grade acetonitrile, methanol and analytical-grade ammonium acetate, ethylene diamine tetra acetic acid disodium salt (EDTA) and glacial acetic acid were purchased from Qualigens (Glaxo Mumbai, India). All aqueous solutions including the buffer for the HPLC mobile phase were prepared with Milli Q (Millipore, Milford, MA, USA)-grade water. The control human plasma was purchased from Cauvery Diagnostics and Blood Bank (Secunderabad, India).

Instrumentation and chromatographic conditions. The HPLC system was a Dinoex (Dionex GmbH, Waldbronn, Germany) LC system equipped with degasser (G1322A), isopump (P680A LPG), auto-injector with sample cooler (ASI-100T) and photodiode array detector (UVD340U). The data were acquired and processed with Chromeleon® software (Version 6.60 SP1a). An X-Terra C_{18} column (4.6 × 150 mm, 5.0 µm, Waters, UK), which was kept at ambient temperature, was used for the analysis. An isocratic mobile phase system consisting of 0.01 M ammonium acetate:acetonitrile:methanol (50:40:10, v/v/v) was run as per the flow-gradient program (0.60 mL/min, 0.0–7.5 min; 1.60 mL/min, 8.50–18.0 min; 0.6 mL/min, 18.5–20.0 min) through the column to elute the analytes. The eluate was monitored by the UV detector set at 275 nm.

Preparation of stock and standard solutions. Primary stock solutions of RST and GFZ for preparation of standard and quality control (QC) samples were prepared from separate weighing. The primary stock solutions of the analytes and IS were prepared in methanol (1.0 mg/mL) and stored at –20°C, and were found to be stable for one month. Appropriate dilutions were made in methanol for RST and GFZ to produce two-in-one working stock solutions of 100, 50, 20, 10, 5, 2, 0.6, 0.3 and 1000, 500, 200, 100, 50, 20, 6, 3 µg/mL, respectively. On the day of analysis these stocks were used to prepare a calibration curve (CC). Another set of working stock solutions of RST and GFZ was made in methanol (from primary stock) at 80.0, 25.0, 0.90, 0.30 and 800, 250,

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9.00, 3.00 μg/mL, respectively, for preparation of QC samples. Working stock solutions were stored at approximately 5°C for a week (data not shown). A working IS solution (100 μg/mL) was prepared in methanol. Calibration samples were prepared by spiking 450 μL of control human plasma with the appropriate amount of the analytes (50 μL containing both RST and GFZ) and IS (10 μL) on the day of analysis. Samples for the determination of recovery, precision and accuracy were prepared by spiking control human plasma in bulk at appropriate concentrations [0.03 μg/mL (LLOQ), 0.09 μg/mL (QC low), 2.50 μg/mL (QC medium) and 8.00 μg/mL (QC high) for RST and 0.30 μg/mL (LLOQ), 0.90 μg/mL (QC low), 25.0 μg/mL (QC medium) and 80.0 μg/mL (QC high) for GFZ] and 500 μL volumes were aliquoted into different tubes and, depending on the nature of the experiment, samples were stored at –80°C until analysis.

Sample preparation procedure. To 500 μL of plasma sample, 10 μL of IS solution (equivalent to 1 μg) was added and mixed for 15 s on a cyclomixer (Remi Instruments, Mumbai, India). After the addition of 3 mL of acetonitrile, the mixture was vortexed for 3 min, followed by centrifugation for 5 min at 3200 rpm on a tabletop centrifuge (Remi Instruments). The organic layer (2.7 mL) was separated and evaporated to dryness at 40°C using a gentle stream of nitrogen (Zymark® Turbovap®, Kopkinton, MA, USA). The residue was reconstituted in 150 μL of the mobile phase and 100 μL were injected onto HPLC system.

Specificity and selectivity. The lack of chromatographic interference from endogenous plasma components was investigated using pooled blank samples.

Calibration curve. Calibration curves were acquired by plotting the peak area ratio of analyte (RST or GFZ): IS against the nominal concentration of calibration standards. The concentrations used were 0.03, 0.06, 0.20, 0.50, 1.00, 2.00, 5.00 and 10.0 μg/mL for RST and 0.30, 0.60, 2.00, 5.00, 10.0, 20.0, 50.0 and 100 μg/mL for GFZ. The results were fitted to linear regression analysis using $1/X^2$ as weighting factor. The calibration curve had to have a correlation coefficient (r^2) of 0.999 or better. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value except at LLOQ, which was set at 20% (Shah *et al.*, 1992).

Precision and accuracy. The intra-assay precision and accuracy were estimated by analyzing four replicates containing RST and GFZ at four different QC levels, i.e. 0.03, 0.09, 2.50, 8.00 and 0.30, 0.90, 25.0, 80.0 μg/mL, respectively. The inter-assay precision was determined by analysing the four levels QC samples on three different runs. The criteria for acceptability of the data included accuracy within ±15% deviation (DEV) from the nominal values and a precision of within ±15% relative standard deviation (RSD), except for LLOQ, where it should not exceed ±20% of CV (*United States Pharmacopoeia*, 1995; Shah *et al.*, 1992).

Recovery. The recovery of RST, GFZ and IS through the liquid–liquid extraction procedure were determined by comparing the responses of the analytes extracted from replicate QC samples ($n = 4$) with the response of analytes from non-extracted standard solutions at equivalent concentrations. Recoveries of RST and GFZ were determined at low, middle and high concentrations (viz. 0.09, 2.50, 8.00 and 0.90, 25.0, 80.0 μg/mL, respectively) and at the lower limit of quantification (LLOQ; 0.03 and 0.30 μg/mL, for RST and GFZ, respectively). The recovery of the IS was determined at a single concentration of 10 μg/mL.

Stability experiments. The stability of RST, GFZ and IS in the injection solvent was determined periodically by injecting replicate preparations of processed samples up to 20 h after the initial injection. The peak-areas of the RST, GFZ and IS obtained at initial cycle were used as the reference to determine the relative stability of the analytes at subsequent points. Stability of RST and GFZ in the biomatrix during 8 h (bench-top) was determined at ambient temperature (25 ± 3°C) at four concentrations in quadruplicates. Freezer stability of RST and GFZ in human plasma was assessed by analyzing the QC samples stored at –80°C for at least 1 month. The stability of RST and GFZ in human plasma following repeated freeze–thaw cycles was assessed using QC samples spiked with RST and GFZ. The samples were stored at –80°C between freeze–thaw cycles. The stability of RST and GFZ was assessed after three freeze–thaw cycles. The samples were processed using the same procedure as described in the sample preparation section. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (i.e. ±15% DEV) and precision (i.e. 15% RSD), except for LLOQ, where it should not exceed ±20% of CV.

Application of method. The pharmacokinetic study was carried out in male Wistar rats. The animals were fasted overnight (~14 h) and had free access to water throughout the experimental period. RST and GFZ were administered by oral gavage at a dose of 50 and 100 mg/kg, respectively, as a suspension in 0.25% sodium carboxymethylcellulose. Animals were provided with standard diet 3 h post-dosing. The rats were anaesthetized in ether and blood samples (~1 mL, sparse sampling protocol) were collected from retro-orbital plexus into microfuge tube (containing 30 μL of saturated EDTA) at 0.5, 1.5, 3, 5, 8, 10 and 24 h post-dosing. Plasma was harvested by centrifuging the blood using Biofuge (Hereaus, Germany) at 12,800 rpm for 5 min. Plasma (500 μL) samples were spiked with IS and processed as described above.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated by employing a non-compartmental analysis (Gibaldi and Perrier, 1982). The peak plasma concentration (C_{max}) and the corresponding time (T_{max}) were directly obtained from the raw data. The area under the plasma concentration vs time curve up to the last quantifiable time point, $AUC_{(0-t)}$ was obtained by the linear and log–linear trapezoidal summation. The $AUC_{(0-t)}$ extrapolated to infinity (i.e. $AUC_{(0-\infty)}$) by adding the quotient of C_{last}/K_{el}, where C_{last} represents the last measurable time concentration and K_{el} represents the apparent terminal rate constant. K_{el} was calculated by the linear regression of the log-transformed concentrations of the drug in the terminal phase. The half-life

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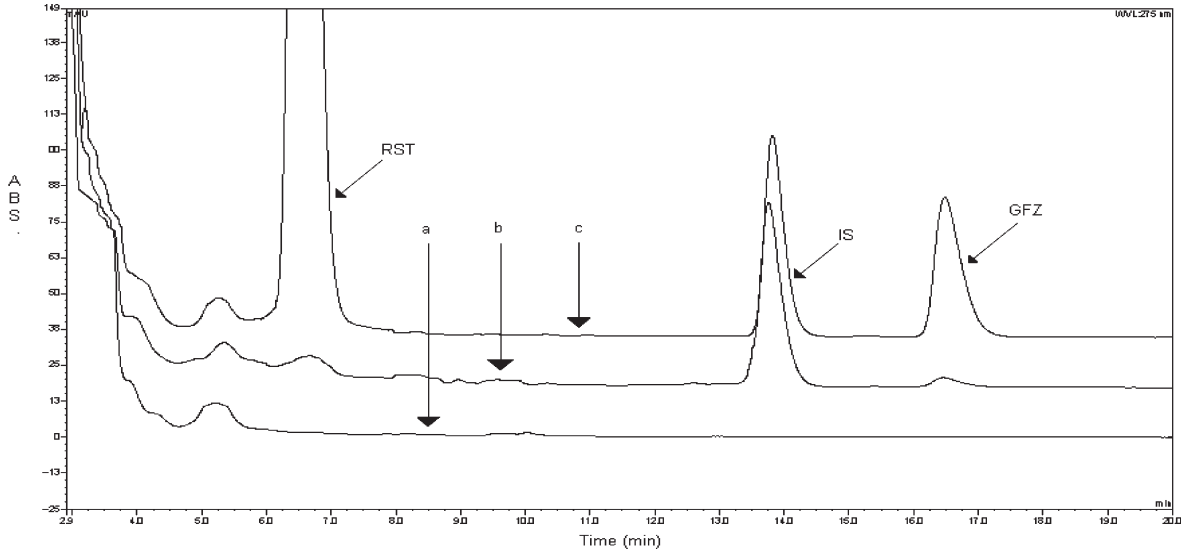

Figure 2. HPLC chromatograms of a 100 µL injection of (a) human blank plasma (b) human plasma spiked with RST and GFZ at the concentration of 0.03 and 0.3 µg/mL, respectively with IS (1.0 µg/mL) and (c) a 0.5 h *in vivo* plasma sample obtained from rat dosed with RST and GFZ each at 50 and 100 mg/kg, p.o, respectively.

($t_{1/2}$) of the terminal elimination phase was obtained using the relationship $t_{1/2} = 0.693/K_{el}$.

RESULTS AND DISCUSSION

Optimization of the experimental conditions

Preliminary experiments were carried out to optimize the experimental parameters affecting the chromatographic separation of RST and GFZ along with IS in the pre-selected LC-column selected and their detection by UV. RST and GFZ have maximum absorbance at 240 and 275 nm, respectively. In order to detect RST and GFZ simultaneously with good sensitivity, 275 nm was selected as UV_{max}. Feasibility of different mixture of solvents such as acetonitrile and methanol using different buffers such as phosphate, formic acid and ammonium acetate, along with variable pH range (3–5) and different flow-rates (in the range of 0.5–1.0 mL/min) were tested for complete chromatographic resolution of RST, GFZ and IS. It was found that with the optimized flow-gradient (0.60 mL/min, 0.0–7.5 min; 1.60 mL/min, 8.50–18.0 min; 0.6 mL/min, 18.5–20.0 min) the resolution of RST, GFZ and IS was satisfactory and the analytical run time was 20 min. Following optimization of the analytical conditions were optimized we have checked the versatility, suitability and robustness of the method with several C_{18} columns from various manufacturers, viz. Kromasil C_{18} (250 × 4.6 mm, 5 µm, Hichrom, Berkshire, UK), Symmetry shield RP_{18} (250 × 4.6 mm, 5 µm, Waters Corporation, Milford, Ireland), X-Terra C_{18} column (150 × 4.6 mm, 5 µm, Waters Corporation, Milford, Ireland) and C_{18} Inertsil® ODS 3V column (4.6 × 250 mm, 5 µm, GL Sciences Inc., Tokyo, Japan) by running four replicates of RST and GFZ along with IS under identical HPLC conditions. On the basis of the specific evaluation, we concluded that the X-Terra C_{18} column was best suited with regard to chromatographic resolution, selectivity and sensitivity.

Specificity and chromatography

In the chosen completely optimized chromatographic conditions, specificity was indicated by the absence of any endogenous interference at retention times of peaks of interest as evaluated by chromatograms of blank human plasma and plasma spiked with RST, GFZ and IS. When single analytes were injected at the highest concentration in the chromatographic system, at the retention times of all analytes no interference was observed (data not shown). RST, GFZ and IS were well separated with retention time of 6.7, 13.9 and 16.4 min, respectively. Figure 2 shows a typical overlaid chromatogram for the control human plasma (free of analyte and IS), human plasma spiked with RST and GFZ at their respective LLOQ and IS at 1.00 µg/mL concentration and 0.5 h *in vivo* sample collected from rats following oral administration of RST and GFZ.

Calibration curve

Peak-area ratios of each analyte spiked in human plasma to the IS were measured and acted as a surrogate for quantitation. A representative calibration graph of peak-area ratio (each analyte to IS) vs each analyte concentration in the range of 0.03–10.0 µg/mL

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Table 1. Intra- and inter-day precision of determination of RST in human plasma

Theoretical concentration (μg/mL)	Run	Measured concentration (μg/mL) Mean	SD	RSD	Accuracy (%)
Intra-day variation (four replicates at each concentration)					
0.03	1	0.03	0.00	2.58	85.43
	2	0.03	0.00	5.52	101.87
	3	0.03	0.00	6.72	97.61
	4	0.03	0.00	5.59	107.23
0.09	1	0.09	0.01	7.85	98.36
	2	0.08	0.01	7.38	93.02
	3	0.08	0.01	10.08	94.39
	4	0.09	0.01	6.57	94.76
2.50	1	2.42	0.03	1.13	96.61
	2	2.47	0.05	1.97	98.75
	3	2.42	0.02	0.92	97.00
	4	2.38	0.09	3.66	95.22
8.00	1	8.07	0.09	1.08	100.93
	2	8.12	0.29	3.55	101.48
	3	8.34	0.13	1.51	104.28
	4	8.29	0.31	3.78	103.57
Inter-day variation (16 replicates at each concentration)					
0.03		0.03	0.00	9.78	98.03
0.09		0.09	0.01	7.53	95.13
2.50		2.42	0.06	2.37	96.89
8.00		8.21	0.23	2.84	102.57

RSD, relative standard deviation (SD × 100/mean).

for RST and 0.30–100 μg/mL for GFZ was found to be linear. The average regression ($n = 3$) was 0.999 for all the analytes. The standard curve had a reliable reproducibility for each analyte across the calibration range. The lowest concentration with the RSD <20% was taken as LLOQ (Shah *et al*., 1992) and was found to be 0.03 and 0.30 μg/mL for RST and GFZ, respectively.

Accuracy and precision

As displayed in Tables 1 and 2, the assay had adequate accuracy and precision for intra- and inter-day, simultaneous determination of RST and GFZ from plasma samples.

Extraction recovery

The results of the comparison of neat standards vs plasma-extracted standards were estimated at LLOQ, low, medium and high QC concentration for both RST and GFZ and found that the recovery was >90% for both the analytes. The absolute recovery of IS was ~100%.

Stability

Autosampler and bench top stability. Over a period of 20 h injection time in the auto-sampler at 5°C and over the bench-top for 8 h period, the predicted concentrations for RST at 0.03, 0.09, 2.50 and 8.00 μg/mL and GFZ at 0.30, 0.90, 25.0 and 80.0 μg/mL samples deviated within the nominal concentrations. The results were found to be within the assay variability limits (Tables 3 and 4).

Freeze–thaw stability. Tables 3 and 4 show the results of the analyses of the QC samples following repeated three freeze–thaw cycles. RST and GFZ were shown to be stable in the frozen plasma at −80°C for at least three freeze–thaw cycles.

Freezer stability. Both RST and GFZ were found to be stable when stored at −80°C for at least 30 days. Both accuracy and precision of QC samples in this evaluation were within the assay variability of ±15% (Tables 3 and 4).

Application of the method

Although the complete validation was done in human plasma, we selected rodents for *in vivo* study to show the applicability of the newly developed bioanalytical method. The rat plasma samples generated following concurrent administration of RST and GFZ were analyzed by the newly developed validated method along with QC samples. Recovery of RST, GFZ and IS from rat plasma was >90%. Additionally all QC

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Table 2. Intra- and inter-day precision of determination of GFZ in human plasma

Theoretical concentration (μg/mL)	Run	Measured concentration (μg/mL) Mean	SD	RSD	Accuracy (%)
Intra-day variation (four replicates at each concentration)					
0.30	1	0.27	0.01	3.32	89.80
	2	0.28	0.01	2.85	93.10
	3	0.25	0.01	5.30	84.98
	4	0.27	0.03	9.69	90.09
0.90	1	0.90	0.02	1.95	100.21
	2	0.92	0.03	2.75	102.15
	3	0.92	0.03	2.84	101.88
	4	0.87	0.08	9.48	97.14
25.0	1	24.37	0.36	1.47	97.47
	2	24.20	0.89	3.66	96.81
	3	24.45	0.23	0.96	97.82
	4	24.10	1.10	4.58	96.42
80.0	1	79.84	1.24	1.56	99.80
	2	79.79	3.53	4.42	99.74
	3	81.09	0.94	1.15	101.37
	4	81.88	2.95	3.60	102.35
Inter-day variation (16 replicates at each concentration)					
0.30		0.27	0.02	6.27	89.49
0.90		0.90	0.05	5.00	100.34
25.0		24.28	0.68	2.79	97.13
80.0		80.65	2.35	2.92	100.81

RSD, relative standard deviation (SD × 100/mean).

Table 3. Stability data of RST quality controls in human plasma

QC (spiked) concentration (μg/mL)	Stability	Mean ± SD[a] (μg/mL), $n = 4$	Accuracy (%)[b]	Precision (%CV)
0.03	0 h (for all)	0.026 ± 0.001		
	3 F/T	0.031 ± 0.003	101.69	9.13
	8 h (B.T.)	0.032 ± 0.003	106.24	8.13
	20 h (in-injector)	0.030 ± 0.004	99.33	15.01
	30 days at −80°C	0.031 ± 0.003	104.06	9.65
0.09	0 h (for all)	0.089 ± 0.007		
	3 F/T	0.087 ± 0.005	96.75	6.03
	8 h (B.T.)	0.089 ± 0.002	98.74	2.58
	20 h (in-injector)	0.090 ± 0.006	99.93	6.95
	30 days at −80°C	0.084 ± 0.006	93.35	7.57
2.50	0 h (for all)	2.42 ± 0.03		
	3 F/T	2.34 ± 0.03	93.62	1.11
	8 h (B.T.)	2.31 ± 0.04	92.29	2.50
	20 h (in-injector)	2.37 ± 0.05	94.99	2.07
	30 days at −80°C	2.52 ± 0.05	100.72	2.07
8.00	0 h (for all)	8.07 ± 0.09		
	3 F/T	8.17 ± 0.16	102.14	1.99
	8 h (B.T.)	7.93 ± 0.18	99.12	2.23
	20 h (in-injector)	8.20 ± 0.14	102.50	1.76
	30 days at −80°C	8.87 ± 0.17	110.91	1.88

QC, quality control; %CV, coefficient of variation; F/T, freeze–thaw; B.T.: bench-top.
[a] Back-calculated plasma concentrations.
[b] (Mean assayed concentration/mean assayed concentration at 0 h) × 100.

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Table 4. Stability data of GFZ quality controls in human plasma

QC (spiked) concentration (μg/mL)	Stability	Mean ± SD[a] (μg/mL), $n = 4$	Accuracy (%)[b]	Precision (%CV)
0.30	0 h (for all)	0.27 ± 0.01		
	3 F/T	0.28 ± 0.02	94.21	7.13
	8 h (B.T.)	0.29 ± 0.03	96.76	9.66
	20 h (in-injector)	0.31 ± 0.02	102.63	5.84
	30 days at –80°C	0.30 ± 0.04	98.77	12.61
0.90	0 h (for all)	0.90 ± 0.02		
	3 F/T	0.92 ± 0.01	101.99	1.17
	8 h (B.T.)	0.93 ± 0.03	103.09	3.68
	20 h (in-injector)	0.94 ± 0.006	104.72	3.15
	30 days at –80°C	0.93 ± 0.08	103.57	8.14
25.0	0 h (for all)	24.37 ± 0.36		
	3 F/T	23.89 ± 0.22	95.55	0.92
	8 h (B.T.)	23.46 ± 0.42	93.82	1.36
	20 h (in-injector)	24.40 ± 1.00	97.60	4.11
	30 days at –80°C	25.62 ± 0.89	102.48	3.48
80.0	0 h (for all)	79.84 ± 1.24		
	3 F/T	79.69 ± 1.46	99.61	1.83
	8 h (B.T.)	77.51 ± 1.80	96.88	2.32
	20 h (in-injector)	78.33 ± 1.25	97.92	1.59
	30 days at –80°C	87.467 ± 1.30	109.32	1.49

QC, quality control; %CV, coefficient of variation; F/T, freeze–thaw; B.T., bench-top.
[a] Back-calculated plasma concentrations.
[b] (Mean assayed concentration/mean assayed concentration at 0 h) × 100.

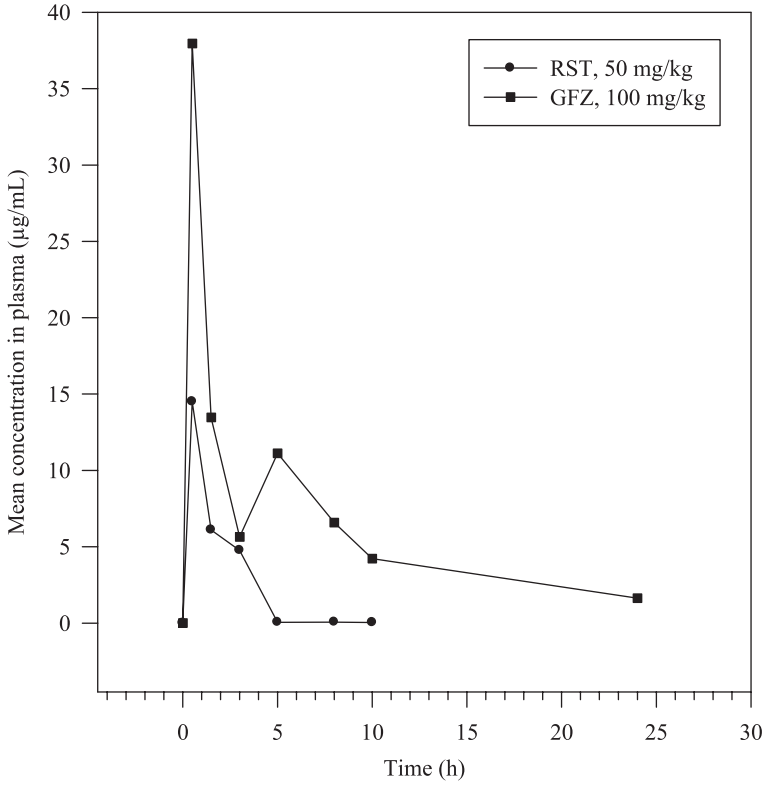

Figure 3. Plasma concentration vs time profiles of RST and GFZ after single dose oral administration of 50 and 100 mg/kg, respectively in male Wistar rats.

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samples prepared in rodent plasma met the criteria as per the guidelines using standard curve samples prepare in human plasma (data not shown). After a single oral administration of 50 and 100 mg/kg of RST and GFZ, respectively, to male Wistar rats, the plasma concentrations of RST and GFZ were determined by the described method. The mean plasma concentration vs time profiles for RST and GFZ is depicted in Fig. 3. Inspection of Fig. 3 revealed that the newly developed analytical method had the required sensitivity to characterize the absorption, distribution and elimination phases of both RST and GFZ following oral dosing. The pharmacokinetic parameters were calculated using a non-compartmental analysis. The maximum concentration in plasma (C_{max}) was achieved at 14.50 and 37.95 μg/mL for RST and GFZ, respectively. The half-life ($t_{1/2}$) was found to be 6.19 and 8.65 h for RST and GFZ, respectively. The $AUC_{(0-\infty)}$ for RST and GFZ was found to be 27.53 and 164.83 μg·h/mL, respectively.

CONCLUSIONS

A HPLC-UV method utilizing flow-gradient elution with single wavelength has been developed for simultaneous analysis of RST and GFZ in human plasma. The validated method is specific, accurate, precise and reproducible. We have used the method successfully to determine the pharmacokinetic profile of RST and GFZ in rats and demonstrated that this assay is effective and inexpensive analytical tool.

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