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Development and Validation of RP-HPLC Method for Simultaneous Determination of Saroglitazar and DPP4 Inhibitors

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Abstract – A modern, highly efficient system of liquid chromatography (RP-HPLC) reversed phase has been developed and validated with a saroglitazar, linagliptin, and teneligliptin photodiode-array (PDA) detector. Phenomenex Luna C-18 column (250 x 4.6 mm, 5 µ) with a combination of the mobile process, phosphate buffer pH 7.0 and acetonitrile with a gradient flow rate of 1.0 mL/min were collected for separation. The detection was conducted for all analytes at 243 nm. The linagliptin, teneligliptin and saroglitazar retention periods were 7.124 minutes, 7.565 minutes and 8.569 minutes respectively. The calibration curve demonstrated strong linearity over the 0.1-0.5 µg/mL range. Data collected following system validation revealed that the process built was precise, reliable, linear, accurate and repeatable.

Keywords: RP-HPLC, Saroglitazar, Linagliptin, Teneligliptin

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INTRODUCTION

Form 2 diabetes mellitus (T2DM) is more than 70% the main cause of cardiovascular disease (CVD) and CVD related morbidity. Dyslipidemia attributable to T2DM is one of CVD's main comorbidities [1,2]. Currently, several anti-diabetic medications are used to avoid symptoms in diabetics. Single treatment counselling, though is not adequate to inhibit metabolic syndrome triggered by diabetes. Thus an adequate medication combination treatment must be found that has substantial success in reducing diabetic complications.

Peroxisome-activated receptor α/µ(PPARα/µ) dual agonist, saroglitazar is used in diabetic dyslipidemia & hypertriglyceridemia[3,4]. [3,3]. Saroglitazar is biologically a benzenepropanoic acid, alpha-ethoxy-4-[2-[2-methyl-5-[4-(methyl)phenyl]-1H-pyrolean-1-yl]ethoxy]– magnesium salt, shown in figure 1,[4] Saroglitazar, due to its advantages in significant triglyceride and low level lipoprotein [4,6], is the first Glitazar-class medicament approved for the treatment of diabetic dyslipidemia. Clinical studies have documented a positive impact of the T2DM patients' saroglitazaron lipid and glycemic parameters without any side effects such as infibrates and pioglitazone medications. In addition, there was no chance of associated hypoglycaemia [7,8,9]. In Phase III clinical trials, the Saroglitazar Prospective Randomized Effectiveness and Protection [PRESS] V study and the PRESS VI analysis, the safety, efficacy and tolerability of saroglitazzar action have been established via the unique receptors [10,11] of PPARα. Saroglitazar's dual activity allows it a promising molecule for controlling the cardiovascular danger of T2DM[12,13].

DPP-4 inhibitors offer a novel clinical strategy to managing type 2 diabetes, which acts by inducing glucose-dependent insulin release and reducing glucagon levels. In specific, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are obstructed by inhibition of the glycemic controls. (14) Chemically, linagliptin is 8-[(3R)-3-aminopiperidine-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazoline-2-yl) ethyl]-3,7-dihydro-1H-purine-2,6-dione] (Figure 1) and is dipeptidylpeptidase-4 inhi-a. The special structure, distinguished by five consecutives Rings (Figure 1) and peptidomimetic [17], is Teneligliptin, {(2S,4S)-4-[4-(3-methyl-1-phenyl-1 H-pyrazol-5-yl)piperazine-1-yl]Pyrrolidine-2-yl}(1,3-thiazolidine-3-yl).

High-performance liquid chromatography (HPLC), for the calculation of analyte in the difficult

biological matrices, is considered a valuable separation technique. A effective testing of medicines in biological fluids by the HPLC is needed to establish an analytical method for the removal of matrix interferences. No HPLC method for the simultaneous determination of saroglitazar and DPP4 inhibitors is currently known. In this research, a modern, accurate, fast, robust, reproducible and economic method for the simultaneous determination of Saroglitazar and DPP4 inhibitors using RP-HPLC was established and validated.

MATERIALS AND METHODS:

Materials:

As a gift sample, saroglitazar (Cadila Healthcare Ltd., Vadodara, Gujarat, Inde), Linagliptin (Glenmark Generics Ltd., Bharuch, Gujarat, India) and Teneliptin (Glenmark Generics Ltd., Bharuch, Gujarat, India) were obtained.

Chromatographic System:

The method was developed using the auto sampler, online degaasser and 2998 photodiode array (PDA) detector device of Waters ez695 partnership with HPLC.

Method development and Optimization of Chromatographic Conditions:

Phenomenex Luna C-18 (250 to 4.6 mm, 5 µm) with a gradient flow pump was used for chromatographic separation. The wavelength was calculated by PDAscanning at 200 nm to 400 nm and for further study 243 nm was chosen for anoptimum reaction. Originally, the process formulation was carried out by the combination of water: acetonitrile with various ratios, but none of the ratios were able to provide a right picture resolution. The mobile step pH of the RP-HPLC is avital variable in retention control as the sample comprises ionic or ionizable compounds. A range of research was conducted utilising phosphate buffers with altered pH along with various percentages of acetonitrile and in the pH 7.0 of the mobile phase A and acetonitrile phase B greater isolation and peak symmetry than others. The mobile phase proportion was optimised with a variety of phosphate buffers with pH 7.0 and acetonitrile. Finally, the phosphate buffer (pH 7.0) and acetonitrile (different ratio) were found to be useful for evaluating inhibitors of saroglitazar and DPP4 (Table 1). The amount of injection was 10 µL. The phase period was 12 minutes and the ambient temperature was held throughout the study.

Preparation of solutions:

A stock solution of saroglitazar, linagliptin and teneligliptin was prepared by specific weighing of 1 mg API of saroglitazar, linagliptin and teneligliptin and by 10 ml of volumetric flask in the mobile process as diluents. At room temperature (22°C ± 1°C) the inventory solution was preserved and held in the dark before study. The stock solution was used for the calibration analysis for progressive dilutions.

RESULTS:

The RP-HPLC system developed was validated in compliance with ICH-Q2B requirements as regards parameters such as linearity, exact, detection limit (LOD), quantification limits (LOQ), recovery, precision and robustness.[18]

Linearity & Range:

The predictability of an analysis methodology is its capacity to yield effects that are equivalent to the sample concentration of an analyte in a specified range, directly or by a well-defined statistical conversion. By evaluating the various concentrations of a regular saroglitazar solution, linagliptin and teneligliptin linearity were assessed. The solution of the triplicate and curved API injected by saroglitazar, linagliptin and teneligliptin was obtained by contrasting the image with the concentration of a substance. The spectrum of linearity was 0.1-0.5 µg/mL. A linear method for saroglitazar, linagliptin and teneligliptin with R2>0.999 was discovered. The system developed was reliable with R2>0.99 and relative standard deviation (RSD)<2 percent.

Precision:

The precision of the calculation was evaluate by measuring 3 replicates of each low quality control (LQC) concentration at different periods on the same day (intra-day) and on different days (inter-day) and its standard deviation(SD) and RSD percent at different intervals and the findings were presented in Table 2.

Accuracy:

The consistency of a research system represents the closeness to the true meaning of the test outcomes produced by a process. Accuracy may be calculated through applying the analytical technique to an established pureness assay (for the medication substance) or by remediation tests with a defined normal increase in placebo (for the pharmaceutical). The accuracy has been determined in the current research by recuperation studies (50%, 100% and 150%) by taking various quantities. The estimated recuperation percentage values are displayed in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The detection maximum is the volume pumped that exceeds a peak height of at least two to three times the Baselinenoise frequency (Signal/Noise = 2 – 3). LOD is the lowest quantity of a sample that can be detected and not quantified considerably. The LOQ determines the lower study in a study that can be quantitatively calculated with sufficient precision and accuracy (signal/noise = 10-20). In this analysis, LOD and LOQ were calculated by steadily injecting a low concentration of the regular analyte solution. The LOD value for saroglitazar, tenneligliptin and linagliptin was observed to be 0.037µg/mL, 0.014µg/mL and 0.096µg/mL. A combined LOQ value of 0.188 µg/mL, 0.074 µg/mL and 0.482 µg/mL was identified (Table 4).

Specificity:

Except for talc's, magnesium stearate, povidone, microcrystalline cellulose (MCC), colloidal siliqua and croscarmellose sodium, excipients widely used in tablet formulation were mixed. The regular solution with a known saroglitazar concentration was spiked into the mixture and analysed using the method established. The spiked solution chromatogram was equivalent to the normal solution chromatogram for measurement. Following reference, the analytical approach developed was found to be specific, since no intervention was detected during the preservation of all three analytes.

Robustness:

The robustness is an analytical tool which tests the method's ability to remain unchanged by a slight yet deliberate variance, i.e. pH, flow rate, the column temperature and injection capacity. It also shows the efficiency of the operation by regular use. The robustness of the system developed was seen in Table 5 at the variant flow rate and mobile step composition.

Ruggedness:

Ruggedness is a test of effects replicability in multiple environments, such as climate, labs, analysts, equipment, operators and materials. The ruggedness of the approach built was tested by analysing the outcomes of intraday and inter-day analyses carried out by two separate analysts. The RSD percentage was less than 2 percent suggested that the method built was robust enough.

System suitability:

Table 6 was determined on the suitability of the device and chromatographic parameters, for example, astail factor, asymmetry factor and number of theoretical plates.

DISCUSSION:

The current work has improved chromatographic conditions for preclinical pharmacokinetic tests. The findings of the present research indicate that the approach being implemented is simple, fast, economical, sensitive, reliable and precise. It can also effectively be used for routine review in quality management. The findings indicated that the procedure proposed was sufficient for simultaneous determination of inhibitors saroglitazar and DPP4. In conjunction with acetonitrile organic solvent, various mobile phases were used. Among them is the composition of mobile phase mixture, which resulted in a phosphate buffer (pH 7.0): a 25:75 (percent v/v) ratio, which provided a sufficient resolution of saroglitazar peak, linagliptin, and teneligliptin. The flow rate in the established system of 1 mL/min and 12 minutes suggests that the method developed is economically approved. The procedure was tested in compliance with the ICH guideline[18] in order to make it technically appropriate. The calibration curve was illustrated against the concentration of the standard solution by drawing standard peak regions. Low percent intra-day and inter-day RSD values have shown that the approach established is reliable. Recovery experiments were carried out by analysing analytical findings for the recovered samples at three stages (50%, 100% and 150%) to maintain accuracy and reproducibility. The findings of the precision evaluation were also in line with the ICH approval criterion. Repeatability of the highest zones, tail factor and number of theoretical plates (N) examined in the Device Test (SST) suggested that the procedure for simultaneous saroglitazar, linagliptine, and teneligliptin determination was sufficient.

CONCLUSION:

For the identification of saroglitazar, linagliptine and teneligliptin, a modern, accurate and efficient RP-HPLC method was created. The key advantage of the approach developed is cost-effectiveness, quick examination, sufficient consistency and precision. The reproducibility of this approach is distinguished by low RSD values intra-day and inter-day examination and excellent recoveries. High quality data and critically tested methods represent the highly responsive, precise and rigorous methods developed. Considering all the parameters of the validated process, it seems quite useful in raw material and dose research at different stage in industry for saroglitazar, linagliptin and teneligliptin.

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REFERENCES:

1. Joshi S, Anjana R, Deepa, M, Pradeepa R, Bhansali A, Dhandania V, Joshi P, Unnikrishnan R, Nirmal E, Subashini R. Prevalence of Dyslipidemiain Urban and Rural India: The ICMR–INDIAB Study. PLoS ONE 2014;9: pp. e96808.

2. Misra A, Devi R, Khanna N, Sharma R, Pandey R. High Prevalence of Obesity, Dyslipidemiaand Diabetes in Poor Urban Slum Population in Northern India. Diabetes Research and Clinical Practice 2000; 50: pp. 351.

3. Ramakrishnan S. From 'Make in India' To 'Made in India': The Saroglitazar Story. Indian Heart Journal 2015;67: pp. 8-10.

4. Agrawal R. The First Approved Agent in the Glitazar's Class: Saroglitazar. Current Drug Targets 2014;15: pp. 151-155.

5. Majumder A. Diabetic Dyslipidemia - Role of Saroglitazar. Medicinal Chemistry 2014;4: pp. 684-687.

6. Joshi S. Saroglitazarfor the Treatment of DyslipidemiaIn Diabetic Patients. Expert Opinion on Pharmacotherapy 2015;16: pp. 597-606.

7. Shetty S, Kumar S, Mathur R, Sharma K, Jaiswal A. Observational Study to Evaluate the Safety and Efficacy of Saroglitazarin Indian Diabetic Dyslipidemia Patients. Indian Heart Journal 2015;67: pp. 23-26.

8. Chatterjee S, Majumder A, Ray S. Observational Study of Effects of Saroglitazaron Glycaemic and Lipid Parameters on Indian Patients with Type 2 Diabetes. Sci. Rep. 2015;5: pp. 7706.

9. Sharma A, Amarnath S, Kushwah DS, Ramaswamy S. Saroglitazar, A Novel Cardiometabolic Agent for Diabetic Dyslipidemia - A Review. Journal of Young Pharmacists 2015;7: pp. 2-6.

10. Pai, V.; Paneerselvam, A.; Mukhopadhyay, S.; Bhansali, A.; Kamath, D.; Shankar, V.; Gambhire, D.; Jani, R.; Joshi, S.; Patel, P. A Multicenter, Prospective, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Saroglitazar 2 And 4 Mg Compared to Pioglitazone 45 Mg in Diabetic Dyslipidemia (PRESS V). Journal of Diabetes Science and Technology 2014, 8, pp. 132-141.

11. Jani R, Pai V, Jha P, Jariwala G, Mukhopadhyay S, Bhansali A, Joshi S. A Multicenter, Prospective, Randomized, Double-Blind Study to Evaluate the Safety and Efficacy of Saroglitazar 2 and 4 mg Compared with Placebo in Type 2 Diabetes Mellitus Patients having Hypertriglyceridemia not Controlled with Atorvastatin Therapy (PRESS VI). Diabetes Technology & Therapeutics 2014; 16: pp. 63-71.

12. Jain, M.; Giri, S.; Trivedi, C.; Bhoi, B.; Rath, A.; Vanage, G.; Vyas, P.; Ranvir, R.; Patel, P. Saroglitazar, A Novel PPAR α/ γ Agonist with Predominant PPAR α Activity, shows Lipid-Lowering and Insulin-Sensitizing Effects in Preclinical Models. Pharmacol Res Perspect 2015;3(3):e00136.

13. Jani R, Kansagra K, Jain M, Patel H. Pharmacokinetics, Safety and Tolerability of Saroglitazar (ZYH1), A Predominantly PPARα Agonist with Moderate PPARγAgonist Activity in Healthy Human Subjects. Clinical Drug Investigation 2013; 33: pp. 809-816.

14. Nirogi R, Kandikere V, Mudigonda K, Komarneni P, Aleti R, Boggavarapu R. Sensitive liquid chromatography tandem mass spectrometry method for the quantification of sitagliptin, a DPP-4 inhibitor, in human plasma using liquid-liquid extraction; Biomedical Chromatography, (2008);22: pp. 214–222.

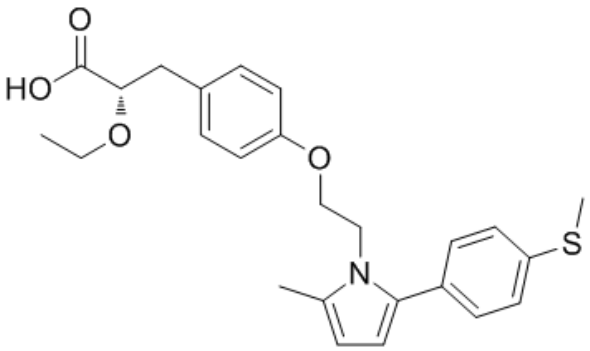
15. Kirby M, Yu D, Conor S. Inhibitor selectivity in the clinical application of dipeptidyl peptidase-4 inhibition; Clinical Science. (2010);118: pp. 31–41.

16. Sekaran B, Rani P. Development and validation of spectrophotometric method for the determination of DPP4 inhibitor, sitagliptin, in its pharmaceutical dosage forms; International Journal of Pharmacy and Pharmaceutical Sciences. 2010;2: pp. 138–142.

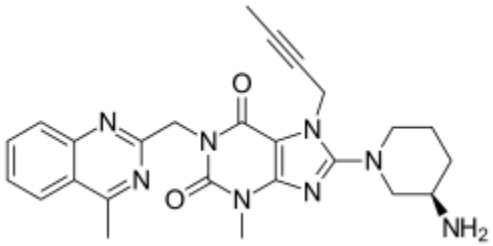
17. Yoshida T, Akahoshi F, Sakashita H, et al. Discovery and preclinical profile of teneligliptin(3-[(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl]pyrrolidin-2-ylcarbonyl]thiazolidine): a highly potent, selective, long-lasting and orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2

diabetes. Bioorg Med Chem. 2012;20(19): pp. 5705–5719.

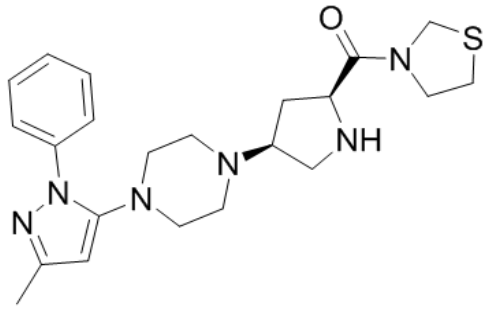
18. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf (accessed Sep 17, 2015).



Saroglitazar



Linagliptin



Teneligliptin

Figure-1: Chemical structure of saroglitazar, linagliptin and teneligliptin

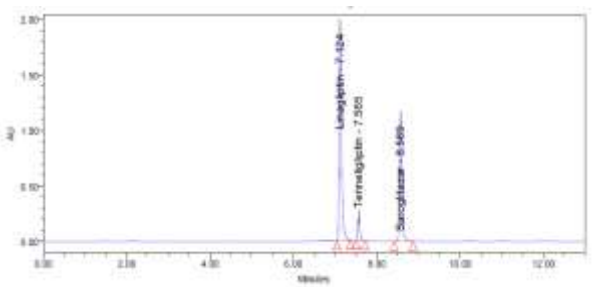
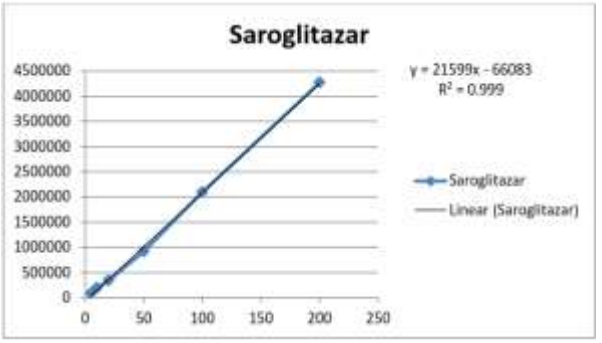
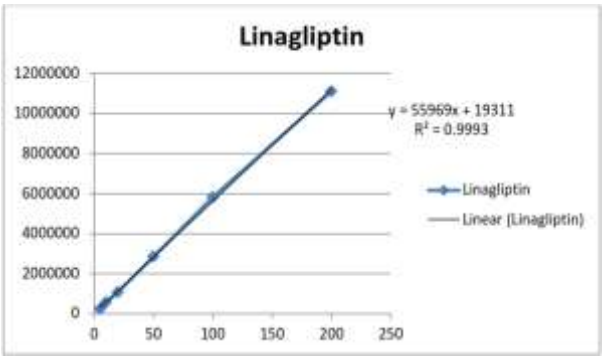


Figure-2: Representative chromatogram of standard saroglitazar, linagliptin and teneligliptin





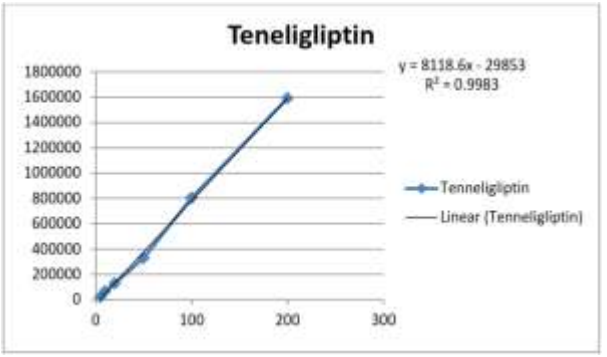


Figure-3: Linearity of saroglitazar, linagliptin and teneligliptinin the range 5 to 200µg/ml.

Table-1: Gradient program for the mobile phase during analytical method development.

Time (min.)	Phosphate buffer pH 7.0 Mobile Phase A	Acetonitrile Mobile Phase B
0.0	70%	30%
3.0	70%	30%
4.5	45%	55%
9.0	25%	75%
10.0	70%	30%
12.0	70%	30%

Table-2: Intraday and Interday of precision study results

Drug	Concentration (µg/mL)	Intra-day Concentration		Inter-day Concentration	
		Mean* (µg/mL)	RSD (%)	Mean* (µg/mL)	RSD (%)
Saroglitazar	0.2	0.198	0.77	0.188	1.33
	0.3	0.294	0.94	0.280	1.68
	0.4	0.395	1.04	0.391	1.41
Linagliptin	0.2	0.199	0.67	0.192	1.12
	0.3	0.296	0.98	0.288	1.34
	0.4	0.396	1.02	0.391	1.38
Teneligliptin	0.2	0.198	0.81	0.189	1.30
	0.3	0.294	0.94	0.285	1.64
	0.4	0.394	1.08	0.390	1.51

*Mean of three observations

Table-3: Results of recovery studies

Drug	Level	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery
Saroglitazar	50%	0.2	0.20	100.00
	100%	0.3	0.298	99.33
	150%	0.4	0.397	99.17
Linagliptin	50%	0.2	0.20	100.00
	100%	0.3	0.299	99.66
	150%	0.4	0.398	99.33
Teneligliptin	50%	0.2	0.20	100.00
	100%	0.3	0.298	99.33
	150%	0.4	0.397	99.17

Table-4: LOD and LOQ of developed analytical method

Drug	LOD (µg/mL)	LOQ (µg/mL)
Saroglitazar	0.037	0.188
Teneligliptin	0.014	0.074
Linagliptin	0.096	0.482

Table-5:Robustness of developed analytical method

Parameters	Saroglitazar		Linagliptin		Teneligliptin	
Change in Flow rate (mL/min)	Retention Time * (min)	RSD (%)	Retention Time * (min)	RSD (%)	Retention Time * (min)	RSD (%)
1.0 (Original)	8.57	0.237	7.13	0.166	7.50	0.142
0.9	8.60	0.256	7.21	0.110	7.65	0.167
1.1	8.46	0.273	7.09	0.149	7.42	0.183

*Mean of three observations

Table-6:Results of system suitability

Factors	Saroglitazar	RSD (%)*	Linagliptin	RSD (%)*	Teneligliptin	RSD (%)*
Retention time	8.57min	0.237	7.13 min	0.166	7.50 min	0.142
Tailing Factor	1.54	-	1.59	-	1.29	-
Theoretical plates	96477	-	86564	-	91363	-

*Mean of three observations

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