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New Analytical Method Development and Validation of Some Oral Hypoglycemic Drugs

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ABSTRACT

A validated method for the determination of Sitagliptin and Pioglitazone has been developed by using reverse phase high performance liquid chromatography and UV spectrophotometry in pharmaceutical dosage forms. Spectrophotometric determination was carried out at an absorption maximum of 267 nm for sitagliptin and 225nm for pioglitazone using methanol. The linearity over the concentration range of 20-60 µg/ml for sitagliptin and 6-14 µg/ml for pioglitazone with correlation coefficient 0.999 were obtained for the both drugs. Chromatographic separation was carried out using mobile phase as Acetonitrile and 10mM Potassium di Hydrogen Phosphate buffer (pH adjusted to 3.0±0.1 with Ortho phosphoric acid) in the ratio of 30:70 (V/V) for 8min and 28:72(v/v) for 8-15 min on Agilent chromatographic system equipped with C18 column (250 X 4.6 mm, 5µm) in an gradient mode at a flow rate of 1.0 ml/min with detection at 270 nm using a UV detector. The developed methods were found to be precise and accurate for the estimation of Sitagliptin and pioglitazone in pharmaceutical dosage forms.

Keywords: Sitagliptin, pioglitazone, RP-HPLC, Spectrophotometry.

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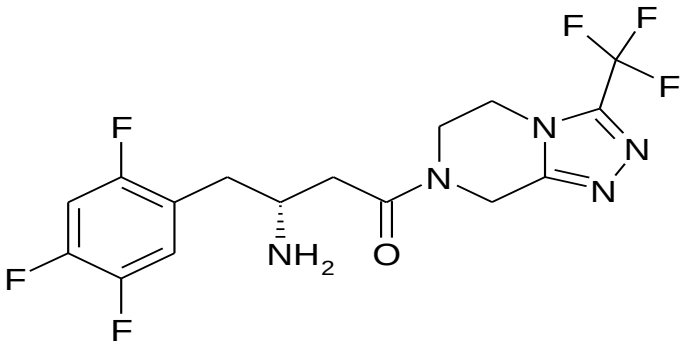
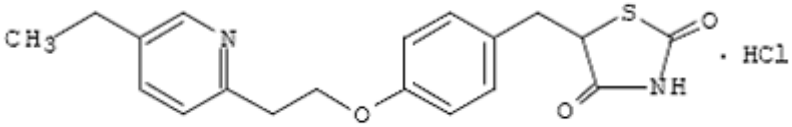
INTRODUCTION



SITAGLIPTIN



PIOGLITAZONE HYDROCHLORIDE

Sitagliptin [1] is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor [2,3]. Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the pancreas. This drives blood glucose levels towards normal. As the blood glucose level approaches normal, the amounts of insulin released and glucagon suppressed diminishes, thus tending to prevent an "overshoot" and subsequent low blood sugar (hypoglycemia) which is seen with some other oral hypoglycemic agents. Pioglitazone [4] is a class thiazolidinedione with hypoglycemic (antihyperglycemic, antidiabetic) action. Pioglitazone hydrochloride is an oral antidiabetic agent that acts primarily by decreasing insulin resistance. Pioglitazone is used in the management of type 2 diabetes mellitus (also known as non-insulin-dependent diabetes mellitus [NIDDM] or adult-onset diabetes). Pharmacological studies indicate that it improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis. Pioglitazone improves glycemic control while reducing circulating insulin levels.

Significance of combination

The anti-diabetic actions of the DPP-4 inhibitors are based on the two incretin hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP). Both GLP-1 and GIP stimulate insulin secretion after meals. Moreover, GLP-1 exerts additional actions that target postprandial hyperglycemia: inhibition of postprandial glucagon secretion, delay of gastric emptying, and possible induction of early satiety [5,6]. However, it was not practical to directly use GLP-1 and GIP as pharmacological agents for treatment of diabetes because of their

very short half-lives as result of their rapid breakdown by the enzyme DPP-4. For example, the half-life of GLP-1 is approximately 2 minutes after intravenous administration [7]. To overcome this problem, inhibitors of the enzyme DPP-4 were used as alternatives to GLP-1 and GIP. Thus, DPP-4 inhibitors, also called incretin enhancers, prolong the effects of native GLP-1 and GIP and increase their serum levels by approximately 2-fold. The best-studied DPP-4 inhibitors are sitagliptin and vildagliptin. The two drugs stimulate insulin secretion and inhibit glucagon release after meals. They do not delay gastric emptying or induce early satiety presumably because the rise in GLP-1 circulating levels is not sufficiently high to exert such effects [8]. Available evidence does not support consistent effects of DPP-4 inhibitors on insulin sensitivity [9-17]. On the other hand, improving insulin sensitivity is the main mechanism whereby thiazolidinediones (TZDs) lower plasma glucose. Therefore, due to the marked differences in mechanisms of actions between DPP-4 inhibitors and TZDs, it was plausible to evaluate the therapeutic potential of the combination between the 2 drug classes. The DPP-4 inhibitor sitagliptin as part of combination therapy with the TZD pioglitazone for treatment of type 2 diabetes.

Literature review reveals that NO analytical methods have been evoked for the estimation of sitagliptin and pioglitazone by spectrophotometric and HPLC methods were reported. The present study the authors were developed a sensitive, accurate and reliable method for the estimation of sitagliptin and pioglitazone in bulk and pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation

The method involved in estimation of the sitagliptin and pioglitazone uses HPLC Agilent (1120) Module with UV Detector Chromatograph and Agilent, C_{18} 250× 4.6 mm. 5µ as column, and Shimadzu UV-1800 spectrophotometer was used for the estimation by UV connected to a computer loaded with Shimadzu UV Probe 2.10 software. In the present work the method development and validation of the sitagliptin and pioglitazone is carried out by using the RP-HPLC chromatographic method and UV spectrophotometric method.

Chemicals and Reagents

HPLC grade acetonitrile, water and grade Phosphate buffer (10m M), Orthophosphoric acid, HPLC grade Methanol, ethanol.

Chromatographic conditions

HPLC chromatographic separation was carried out in a gradient mode utilizing Agilent C18 column with dimensions (5µ, 250mm x 4.6mm) as stationary phase with injection volume

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of 20µl. The mobile phase composed of Acetonitrile and 10mM Potassium dihydrogen Phosphate buffer (pH adjusted to 3.0±0.1 with Ortho phosphoric acid) in the ratio of 30:70 for 8 min, 28:72 for 8-15 min at a flow rate of 1.0 ml/min with UV-detection at 270 nm Rt was found at 5.7min and 7.4 min shown in Fig.2.

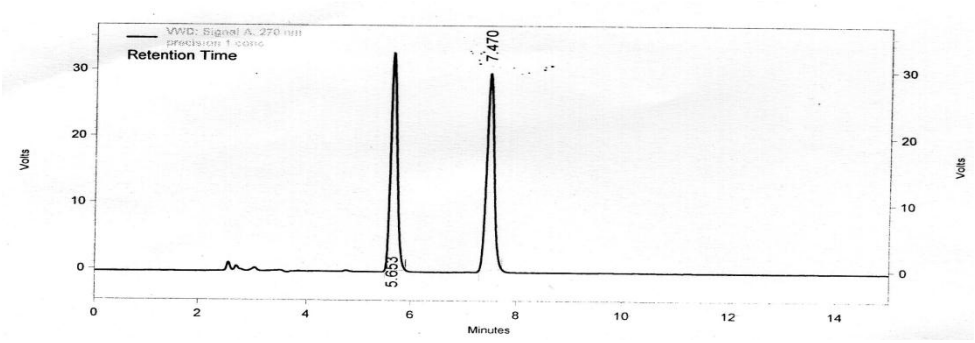

Fig.2. Chromatogram showing Retention Time (R_t) at 5.7min and 7.5 min for Sitagliptin and pioglitazone.

Spectrophotometric conditions

In this method sitagliptin and pioglitazone were dissolved in methanol which showed the absorption maximum at 267 nm and 225 nm respectively.

Preparation of standard solutions:

Spectrophotometry

About 25mg of sitagliptin and pioglitazone were accurately weighed and transferred into a 25 ml volumetric flask and diluted to volume with methanol to get the stock solution (1mg /ml). From this stock solution, 5ml was pipette into 50ml volumetric flask and volume was made up to mark with water to give a solution containing 100µg/ml.

HPLC

Accurately weighed 25 mg of Capecitabine and transferred into 25 mL volumetric flask and dissolved in methanol and the volume was made up to the mark with the solvent. From the above 5 mL solution were pipette out into two 50 mL seperate volumetric flask for sitagliptin and pioglitazone and volume was made up to the mark with the same solvent. This gave the concentration of 100 µg mL^{-1} of Capecitabine, from this five dilutions were prepared. five dilutions in between 50-90 µg $mL^{-1}$of sitagliptin and 6-14 µg mL^{-1} of pioglitazone with methanol was used in spectrophotometric estimation, but in case of HPLC the final working concentration make up by using mobile phase as a solvent.

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Sample preparation

Spectrophotometry and HPLC:

Twenty tablets were weighed and finely powdered. The tablets of sitagliptin and pioglitazone were accurately weighed and powdered and transferred to volumetric flask of 100ml capacity containing 25ml of the methanol and sonicated for 5 min. The flask was shaken and volume was made upto the mark with methanol. The above solution was filtered through Whatmann filter paper (No.41). From this solution, solution of 100µg/ml was prepared and used for the estimation. But in case of HPLC, the final working concentration prepared by using mobile phase as a solvent. From this, suitable dilutions were made to obtain the concentrations.

Procedure

Spectrophotometry

Five aliquots of sitagliptin and pioglitazone solution were transferred to respective 10 ml volumetric flasks in such amounts as to obtain final concentrations of 20-60 µg/ml and 6-14 µg/ml for sitagliptin and pioglitazone respectively. Volume was made with methanol and each flask content was measured at 267nm and 225nm wave length.

HPLC

Various standard concentrations of sitagliptin and pioglitazone ranging from 60-90 µg/ml and 6-14 µg/ml were prepared in mobile phase. The contents of the mobile phase were filtered before use through 0.45 µm membrane filter, degassed with a sonicator (EQUITRON-230VAC, 50Hz) for 15 min. And pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug, the mobile phase was pumped for about 30 min. To saturate the column there by to get the base line corrected, then 20 µl of each of the drug solution was injected for five times. Quantitative determinations were made by comparison of the peak area from a standard injection. The amount of sitagliptin and pioglitazone present in the sample were calculated through the calibration curve.

RESULTS

Linearity

Calibration curve for spectrophotometric method was constructed by plotting absorbance Vs concentration of solution. For chromatographic method it was constructed by plotting peak area against concentration of solution. Figs. 3,4,5 and 6 show spectrophotometric and HPLC linearity curves of sitagliptin and pioglitazone. Linearity ranges and correlation coefficients obtained from these methods are presented in Table 1.

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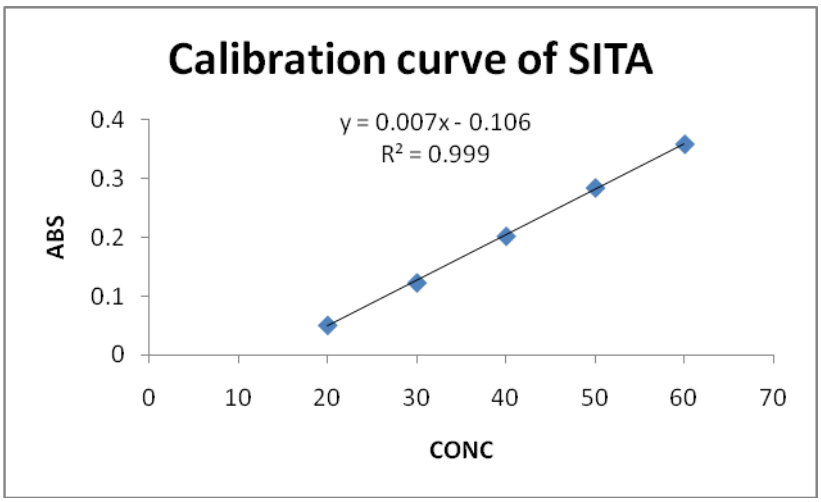

Fig 3: Linearity Curve of sitagliptin (UV)

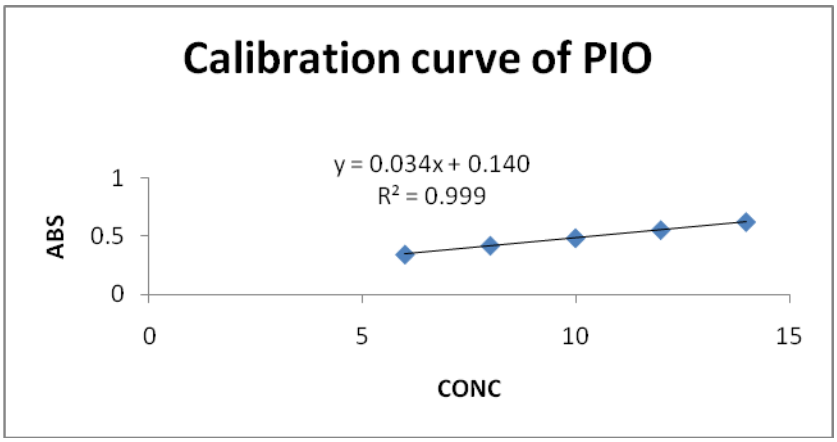

Fig 4: Linearity Curve of sitagliptin (UV)

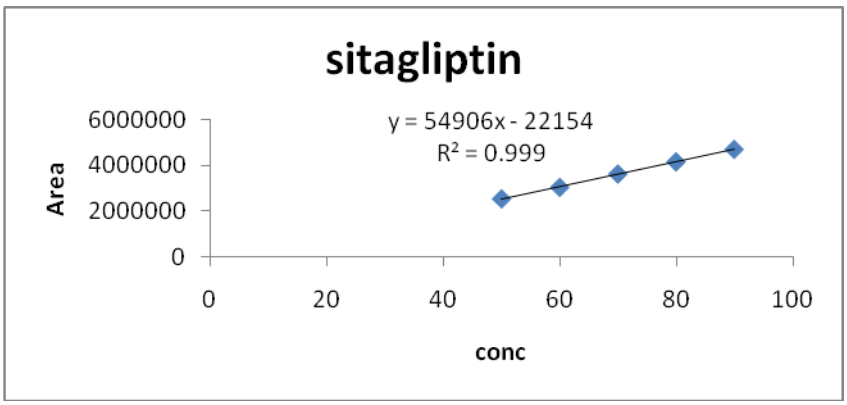

Fig 5: Linearity Curve of sitagliptin (HPLC)

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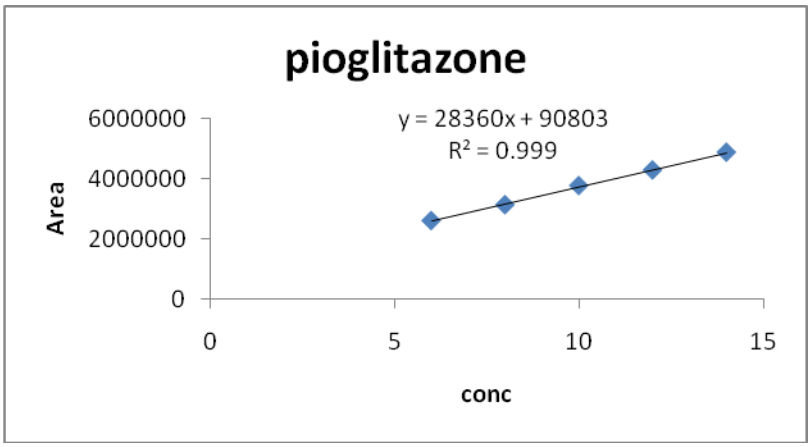

Fig 6: Linearity Curve of sitagliptin (HPLC)

System suitability parameters

The system suitability tests were carried out on freshly prepared standard stock solution of sitagliptin and pioglitazone under the optimized chromatographic conditions. The parameters that were studied to evaluate the suitability of the system were: a) Number of theoretical plates b) tailing factor c) retention time d) calibration range e) LOD and LOQ. These values are presented in Table 2.

Precision for HPLC

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

It was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for the title ingredient. The repeatability (within-day in triplicates) and intermediate precision (for 3 days) were carried out at six concentration levels for compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The value of %RSD for sitagliptin and pioglitazone was found to be 0.9 and 1.5 respectively for intra-day studies. The %RSD of sitagliptin and pioglitazone for inter-day studies were 0.5 and 1.2 respectively. This shows that the developed method is precise. The precision was expressed in % RSD is given in Table 3 and Table 4.

Precision for UV

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

Sample to sample precision was evaluated using three samples of three different concentrations, which were prepared and analyzed on same day. Day to day variability was

assessed using three concentrations analyzed on two different days. These results show the reproducibility of the assay. Thus, it was concluded that there was no significant difference of samples which were tested on intra – day and inter – day basis. The value of %RSD for Capecitabine was found to be 0.4 and 0.1 for both drugs for intra-day studies. The values for inter-day studies were 0.3 and 0.1. This shows that values are not more than 2%, indicates that the developed method is precise. The precision expressed as % RSD is given in Table 5.

Assay and recovery study

To determine the accuracy of the proposed methods, recovery experiments were carried out by standard addition method. The values of recovery experiments and assay of commercial formulations are presented in Table 6.

DISCUSSION

The linearity was obeyed in the range of 20-60 µg/ml and 6-14 µg/ml for sitagliptin and pioglitazone respectively for spectrophotometric method and 60-90µg/ml for sitagliptin and 6-14 µg/ml for pioglitzone for HPLC method. Quantitative estimation of formulations showed the %Recovery for Spectrophotometry is 99 to 101% For Chromatographic method the %Recovery was 99 to 100 for HPLC.

Linearity data for SITA and PIO

Sl.no	Conc(µg/ml) of Sitagliptin	Absorbance at 267nm	Conc(µg/ml) of Pioglitazone	Absorbance at 225nm
1	20	0.051	6	0.343
2	30	0.123	8	0.418
3	40	0.202	10	0.482
4	50	0.284	12	0.551
5	60	0.358	14	0.619

Intra day precision (repeatability)

Sl. No.	Conc. (µg ml)		Absorbance*		% RSD	
	SITA	PIO	SITA	PIO	SITA	PIO
1	20	6	0.1118±0.00083	0.2882±0.000836	0.7483	0.2903
2	30	8	0.1348±0.00083	0.3928±0.0008367	0.6206	0.2129
3	40	10	0.2078±0.0004472	0.4862±0.0004472	0.2158	0.0919
4	50	12	0.2138±0.0008366	0.5474±0.0005477	0.3913	0.1000
5	60	16	0.2676±0.200054	0.642±0.000707	0.2046	0.1101
Average % RSD					0.4361	0.1610

*= an average of, 2 experiments each of three replicates five concentration levels within-day

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Inter day precision (intermediate precision)

Sl. No.	Conc. (µg ml)		*Absorbance		% RSD	
	SITA	PIO	SITA	PIO	SITA	PIO
1	20	6	0.1122±0.000836	0.2874±0.000547	0.7456	0.1905
2	30	8	0.1344±0.000547	0.3926±0.000547	0.4075	0.1395
3	40	10	0.2066±0.000547	0.486±0.0007071	0.2651	0.1454
4	50	12	0.2126±0.000547	0.5476±0.000547	0.2576	0.1000
5	60	14	0.267±0.0007071	0.642±0.00	0.2648	0
Average % RSD					0.3881	0.1150

* an average of, 2 experiments each of three replicates five concentration levels within-day

Analysis of commercial formulation

Level of standard addition in %	Amount of labeled formulated drug(mg)		Amount of API drug added(mg)		*Recovery of sample (mg)		%recovery of sample		Mean Recovery	
	SITA	PIO	SITA	PIO	SITA	PIO	SITA	PIO	SITA	PIO
80%	80	40	64	32	79.8	40.1	99.75	100.25	100.5%	100.16%
100%	80	40	80	40	80.2	40.2	100.25	100.5		
120%	80	40	96	48	81.2	39.9	101.5	99.75		

Characteristic parameters of calibration equation for the proposed HPLC method

Parameters	HPLC	
	SITA	PIO
Calibration range (µg /ml)	50-90	6-14
Slope (b)	54906	28360
Intercept (a)	22154	90803
Correlation coefficient	0.999	0.999
Theoretical plates per meter	12808	14317
ASymmetry factor	1.312	1.113
Resolution	7.814	

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Precision (Repeatability) study results of prepared binary mixture

Precision	Conc. (µg/ml)	Area± SD	%RSD	Peak Symmetry±SD	%RSD
SITA	50	1890059±17235	0.911	1.41±0.019	1.22
	60	2719430±35760	1.314	1.47±0.018	1.14
	70	3556239±66543	1.88	1.48±0.03	1.94
	80	4092515±42392	1.03	1.42±0.028	1.73
	90	4877967±47564	0.96	1.40±0.026	1.63
PIO	6	2595871±17328	0.667	1.33±0.025	1.90
	8	3128325±23296	0.744	1.33±0.026	1.96
	10	3736150±41833	1.119	1.32±0.020	1.53
	12	4199187±28129	0.669	1.23±0.020	1.63
	14	4454594±47924	1.075	1.28±0.022	1.72

an average of, 2 experiments each of three replicates five concentration levels within-day

Precision (Intermediate Precision) study results of prepared binary mixture

Precision	Conc. (µg/ml)	Area± SD	%RSD	Peak Symmetry±SD	%RSD
SITA	50	2570409±18905	0.735	1.605±0.029	1.866
	60	3124279±56729	1.815	1.589±0.024	1.537
	70	3679701±37517	1.01	1.587±0.031	1.958
	80	4123224±40477	0.98	1.66±0.027	1.644
	90	4505247±38103	0.845	1.601±0.026	1.672
PIO	6	1825031±10678	0.58	1.356±0.020	1.53
	8	2711830±44926	1.65	1.324±0.021	1.60
	10	3501920±50038	1.42	1.323±0.019	1.37
	12	3917618±21243	0.54	1.320±0.020	1.51
	14	4980339±75379	1.51	1.30±0.021	1.62

a n average of, 2 experiments each of three replicates of five concentration levels between-days (3-days)

Results of recovery of SITA AND PIO

Level of standard addition in %	Amount of labeled formulated drug(mg)		Amount of API drug added(mg)		*Recovery of sample (mg)		%recovery of sample		Mean Recovery	
	SITA	PIO	SITA	PIO	SITA	PIO	SITA	PIO	SITA	PIO
80%	80	40	64	32	80.018	39.740	100.3	99.35	99.88%	99.85%
100%	80	40	80	40	79.960	40.011	99.2	100.02		
120%	80	40	96	48	80.003	40.112	100.07	100.28		

*Average of three determinations

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CONCLUSION

The developed method is a new method to determine sitagliptin and pioglitazone in pharmaceutical dosage forms that contain it as unique active principle with quite satisfactory results for the specific purposes of its design. Its advantages of methods are its simplicity, fastness and low-cost. The developed methods are simple, accurate and reproducible, so these methods are suitable to determine sitagliptin and pioglitazone in formulations.

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