New UV Spectrophotometric method development and validation for the estimation of Sitagliptin and Glimepiride in pharmaceutical dosage forms and bulk drug

Dillan Kumar[1*], Kumari Khushboo[1], Ashu[2], Sreenivas Rao T[1], Chandanam Sreedhar[1]

[1]Department of Pharmaceutical Analysis,
Karnataka College of Pharmacy, Bangalore-64

[2]Kharvel Subharti College of Pharmacy, Meerut.

***Abstract*: New UV Spectrophotometric method has been developed for the simultaneous estimation of Sitagliptin phosphate and Glimepiride in pharmaceutical dosage forms and bulk drugs. The proposed method were based on the application of simultaneous equation. Absorbance maxima for Sitagliptin and Glimepiride were found to be 267nm and 225 nm respectively. The Calibration curves were linear with correlation coefficient of 0.9959 over the concentration range of 20-70µg/ml for Sitagliptin phosphate and 5-30µg/ml for Glimepiride and with correlation coefficient of 0.9969 The mean percent recovery was found to be 99.7and 100.1 for Sitagliptin phosphate and Glimepiride. The results of analysis were validated statistically. The proposed simple UV methods were rapid, accurate, precise and economical and can be used in the quality control of pharmaceutical formulations and routine laboratory analysis.**

***Keywords*: Sitagliptin, Glimepiride, UV Spectrophotometry, Linearity**

1.Introduction

Type 2 diabetes is a progressive condition in which the body becomes resistant to the normal effects of insulin and/or gradually loses the capacity to produce enough insulin in the pancreas[1]. For the treatment of type-2 diabetes various classes of anti-diabetic drugs are used. Anti-diabetic drugs are pharmacological agents that have been approved for hypoglycaemic treatment in type-2 diabetes mellitus. The various classes of anti-diabetic drugs used in the treatment of type-2 diabetes are, biguanides, sulfonylureas, meglitanides, thizolidinones and DPP4 inhibitors [2]. Glimepiride lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors and chemically it is 4-ethyl-3-methyl-*N*-[2-[4-[(4-methylcyclohexyl) carbamoylsulfamoyl]phenyl]ethyl]-5-oxo-2*H*-pyrrole-1-carboxamide. Sitagliptin is an orally-active inhibitor of the DPP4 enzyme. Sitagliptin is described chemically as (3*R*)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5*H*- [1,2,4] triazolo[4,3-a] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-onehexane-3-carbonitrile [4]. In combination therapy of Glimepiride and DPP4 inhibitors of type-2 diabetes, Glimepiride has been shown to have a durable glucose-lowering effect and a potential for preserving beta-cell function. DPP4 inhibitors are characterized by sustained efficacy and have been shown to be safe with respect to CV risk. These agents may also have potential in preserving beta-cell function, making a rational combination with Glimepiride while potentially attenuating some of the side effects of the latter, particularly if lower doses of Glimepiride are used [5]. Literature survey revealed that UV Spectroscopic methods were reported for individual drugs[6-9] but no method has been reported for the above combination. The present work describes a simple, accurate, and precise method for simultaneous determination of these two drugs by verdoit method. The method was validated as per the current ICH guidelines [10–12].

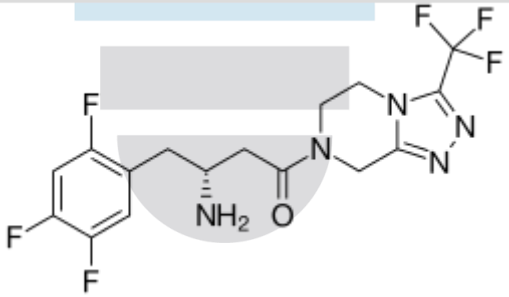

Fig. 1 Structure of Sitagliptin

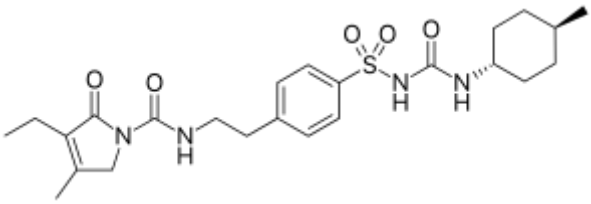

Fig. 2 Structure of Glimepiride

2. Materials and Methods

2.1Apparatus and software in spectrophotometric estimation of SITA and GMP

Shimadzu UV-1800 double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.33 software was used for all the spectrophotometric measurements. The spectral bandwidth was 1nm and the scanning speed was very fast. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm.

2.2 Reagents and materials used in spectrophotometric estimation of SITA and GMP

Sitagliptin(99.5% purity) and Glimepiride (99.8% purity) was received as gift samples from Micro Labs, Bangalore, Karnataka. A.R grade Methanol (Merck Index), Pharmaceutical formulation tablets (label claim 80 mg SITA and 40 mg GMP) was used in UV analysis.

2.3 Preparation of stock solutions:

100mg of standard SITA and GMP were weighed and transferred to a 100ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000µg/ml. From this stock solution, pipette out 10ml, placed in to 100 ml volumetric flask and volume was made up to mark with methanol to give a solution containing 100µg/ml.

2.3 Determination of Maximum Absorbance (max)

Solutions of 10 µg/mL of both drugs were prepared from working stock solution and scanned in the range of 200 nm to 400 nm against methanol as blank. Individual spectrums of SITA and GMP were used to determine absorption maxima.

2.4 Selection of analytical concentration ranges:

From the standard stock solution of SITA and GMP, appropriate aliquots were pipette out in to 10ml volumetric flask and dilutions were made with methanol to obtain working standard solutions of concentrations from 20-70µg/ml and 5-30 µg/ml. Absorbance for these solutions were measured at 267nm and 210nm respectively.

3. Method validation

3.1 Linearity and range

The proposed zero order method shows good linearity in the concentration range of 2-12 µg/ml for SITA and 2-12 µg/ml for GMP. The correlation co-efficient was found to be 0.9959 for SITA and The correlation co-efficient was found to be 0.9966.conc and its results were shown in table 2.

3.2. Application of the Proposed Method for Estimation in Standard Laboratory Mixture

The absorptivity coefficient of both drugs was determined and the individual concentration of SITA and GMP was determined using the following equations.

$$C_X = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2}$$

$$C_Y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2}$$

3.3 Precision

The binary mixtures of SITA and GMP at five levels within their linearity range were prepared and experiment was repeated five times (repeatability) and two different days (intermediate precision)

Intraday and inter day precision for determination of SITA and GMP by proposed zero order spectroscopy were evaluated in terms of % RSD. The average % RSD of repeatability for determination of SITA and GMP were found to be 0.4614 , 0.6388 and 0.501 ,0.5406 in zero order and average % RSD of intermediate precision for determination of SITA and GMP was found to be 0.4606, 0.7311 and 0.275, 0.5135 in zero order. Results were shown in table 3, 4, 5 and 6.

3.4 Accuracy

Twenty tablets each containing 2.5 mg of SITA and 40 mg of GMP were weighed and powdered for further study. The powder equivalent to 10 mg of SITA and 40 mg of GMP were accurately weighed and transferred to 100 ml volumetric flask containing 50 ml of the methanol and sonicated for 10 min. make up the volume with Methanol. The above solution was carefully filtered through Whatmann filter paper (No. 41). From this solution, required dilutions for UV method were prepared within the linearity range using methanol as solvent,

The study was performed by increasing standard addition of known amount of studied drugs to an unknown concentration (constant volume) of the commercial pharmaceutical formulation. A constant volume of the unknown solution is added to each of four volumetric flasks. Then a series of increasing volumes of working standard solutions are added. The resulting mixtures were analyzed and recoveries were determined. The results obtained are compared with expected results. The excellent mean recoveries and standard deviation (Table 7) suggested good accuracy of the proposed method and no interference from formulations excipients.

3.5 LOD and LOQ

Calibration curve was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated. The values of LOD and LOQ are given in (Table 1).

Table 1 LOD and LOQ of SITA and GMP

	SITA	GMP
LOD (µg/ml)	0.2606	0.2064
LOQ (µg/mL)	0.7899	0.618

4. Results of analysis of commercial tablet formulation

Applicability of the proposed methods was tested by analyzing the commercially available tablet formulation containing the binary mixture of SITA and GMP. The values of % recovery from formulation as shown in the (Table 4.1.5) are found to be very close to each other as well as to the label value of commercial pharmaceutical formulation, which shows that the method is applicable for simultaneous determination of SITA and GMP from their binary mixture formulation.

5. Results and Discussion

The solutions Sitagliptin phosphate and Glimepiride hydrochloride were analysed in pharmaceutical dosage forms and bulk drugs. And Absorbance maxima for Sitagliptin and Glimepiride found to be 267 nm and 225 nm(fig. 3 and fig 4)

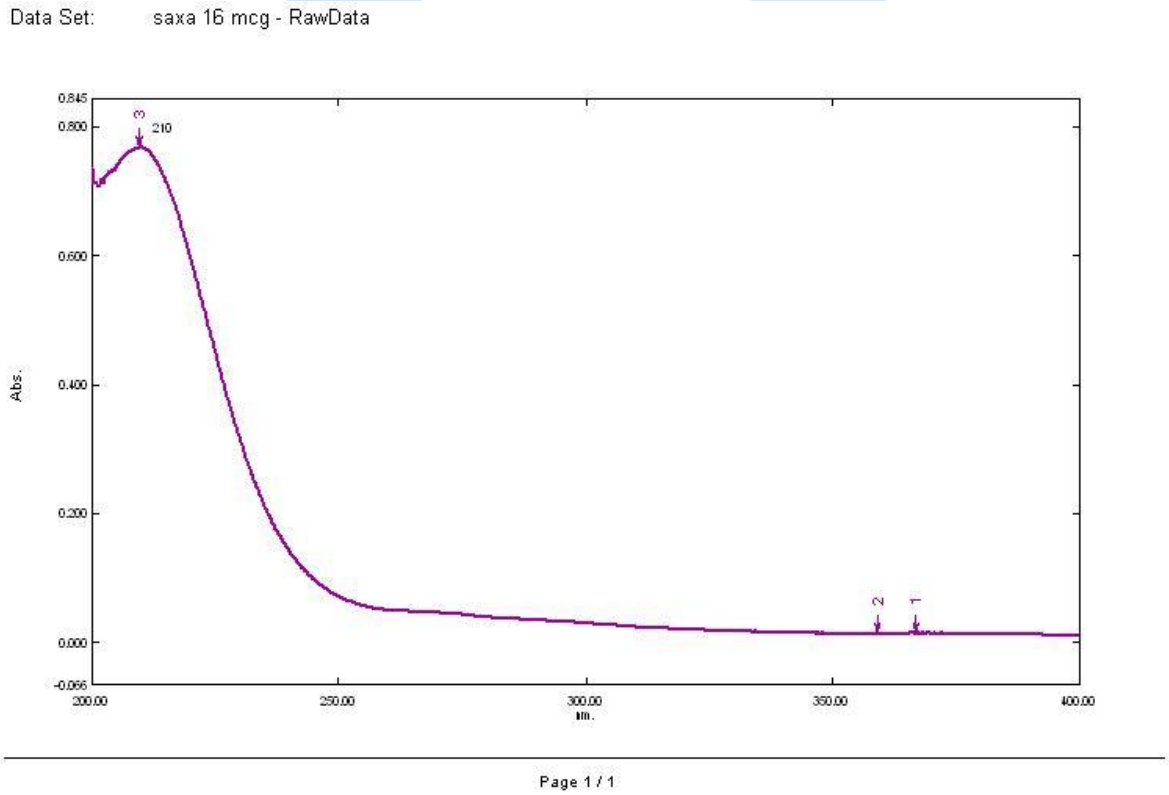

Fig 3 Sitagliptin absorption maxima

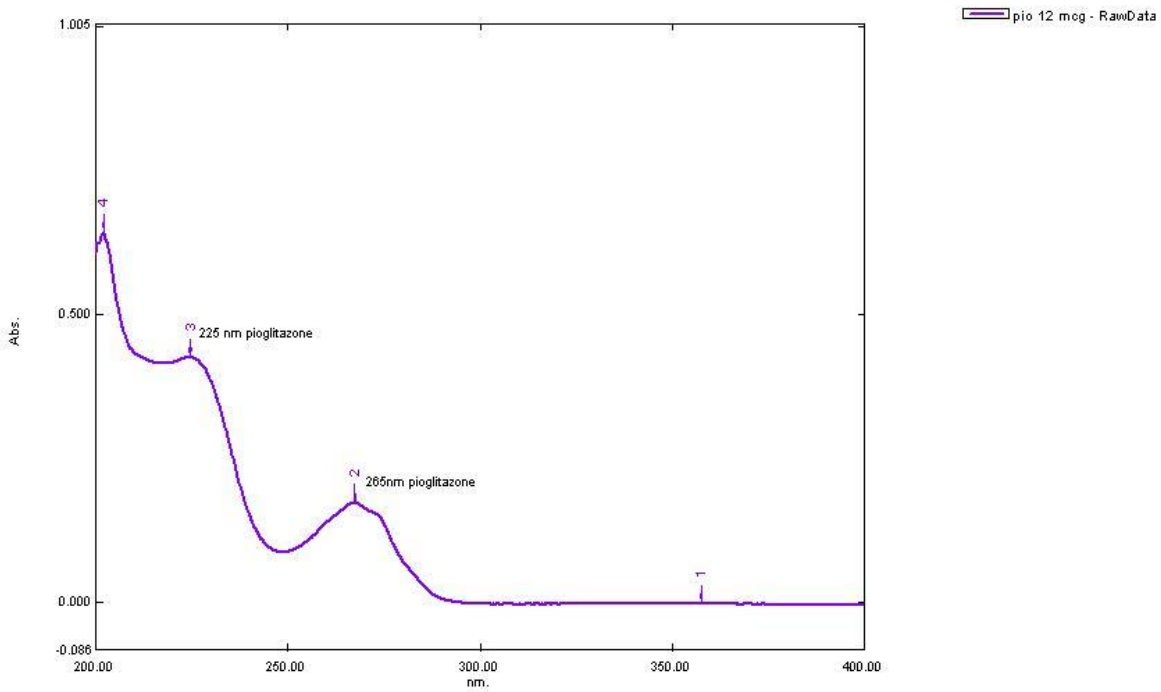

Fig.4 Glimepiride absorption maxima

The Calibration curves were linear with correlation coefficient of.9959 over the concentration range of 20-70µg/ml for Sitagliptin phosphate and 5-30µg/ml for Glimepiride and with correlation coefficient of 0.9969 The mean percent recovery was found to be 99.7and 100.1 for Sitagliptin phosphate and Glimepiride. The results of analysis were validated statistically. The proposed simple UV methods were rapid, accurate, precise and economical and can be used in the quality control of pharmaceutical formulations and routine laboratory analysis.

Table 2 Calibration Concentrations of SITA and GMP

Sl.no	Conc(µg/ml) of Sitagliptin	*Absorbance at 210nm	Conc(µg/ml) of Glimepiride	*Absorbance at 225nm
1	20	0.153	5	0.285
2	30	0.289	10	0.352
3	40	0.443	15	0.418
4	50	0.569	20	0.492
5	60	0.769	25	0.572
6	70	0.914	30	0.624

Table 3 Intraday precision (repeatability)morning

Sl. No.	Conc. (µg ml)		Absorbance		% RSD	
	SITA	GMP	SITA	GMP	SITA	GMP
1	40	20	0.371	0.409	0.4614	0.6388
2	40	20	0.373	0.414		
3	40	20	0.376	0.416		
4	40	20	0.372	0.415		
5	40	20	0.374	0.411		
6	40	20	0.373	0.414		
Mean			0.373167	0.413167		
Std. dev			0.001722	0.002639		

Table 4 Intraday precision (repeatability)afternoon

Sl. No.	Conc. (µg ml)		Absorbance		% RSD	
	SITA	GMP	SITA	GMP	SITA	GMP
1	40	20	0.372	0.414	0.501	0.5406
2	40	20	0.376	0.412		
3	40	20	0.373	0.414		
4	40	20	0.374	0.416		
5	40	20	0.375	0.411		
6	40	20	0.371	0.41		
Mean			0.3735	0.412833		
Std.dev			0.001871	0.002229		

Table 5 Inter day precision (intermediate precision) day-1

Sl. No.	Conc. (µg ml)		Absorbance		% RSD	
	SITA	GMP	SITA	GMP	SITA	GMP
1	40	20	0.374	0.408	0.4606	0.7311
2	40	20	0.373	0.41		
3	40	20	0.371	0.415		
4	40	20	0.375	0.416		
5	40	20	0.376	0.413		
6	40	20	0.374	0.412		
Mean			0.374	0.412333		
Std			0.001414	0.003011		

Table 6 Inter day precision (intermediate precision) day-2

Sl. No.	Conc. (µg ml)		Absorbance		% RSD	
	SITA	GMP	SITA	GMP	SITA	GMP
1	40	20	0.376	0.414	0.275	0.5135
2	40	20	0.373	0.412		
3	40	20	0.372	0.409		
4	40	20	0.371	0.415		
5	40	20	0.374	0.411		
6	40	20	0.373	0.41		
Mean			0.372667	0.411833		
Std			0.001033	0.002115		

Table 7 Recovery Studies

Level of	Amount of		Amount of		*Recovery		%recovery		Mean Recovery	
	SITA	GMP	SITA	GMP	SITA	GMP	SITA	GMP	SITA	GMP
80%	10	40	8	32	9.89	40.12	98.9	100.3	99.7	100.11
100%	10	40	10	40	10.1	40.1	101	100.25		
120%	10	40	12	48	9.92	39.92	99.2	99.8		

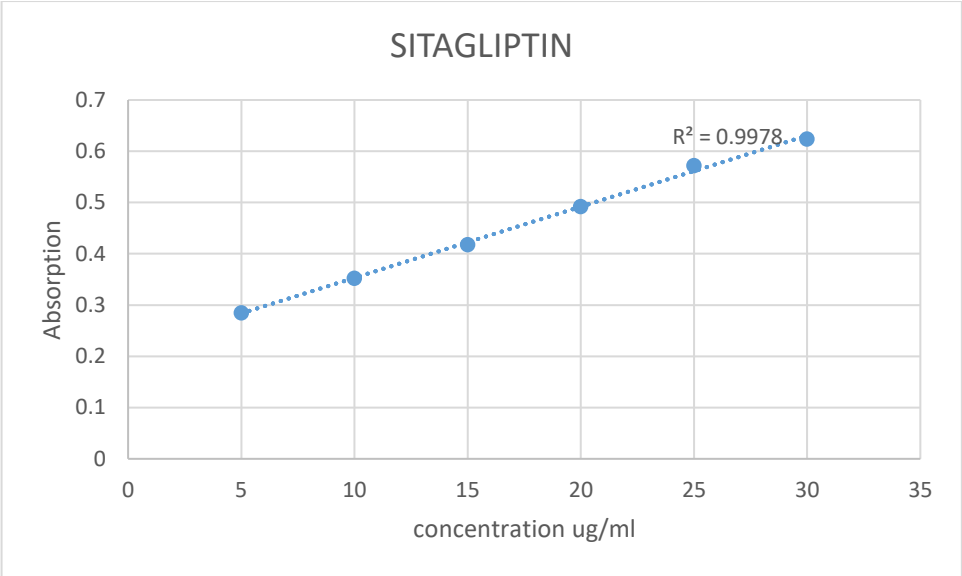

Fig. 5 Calibration curve of sitagliptin at 267 nm

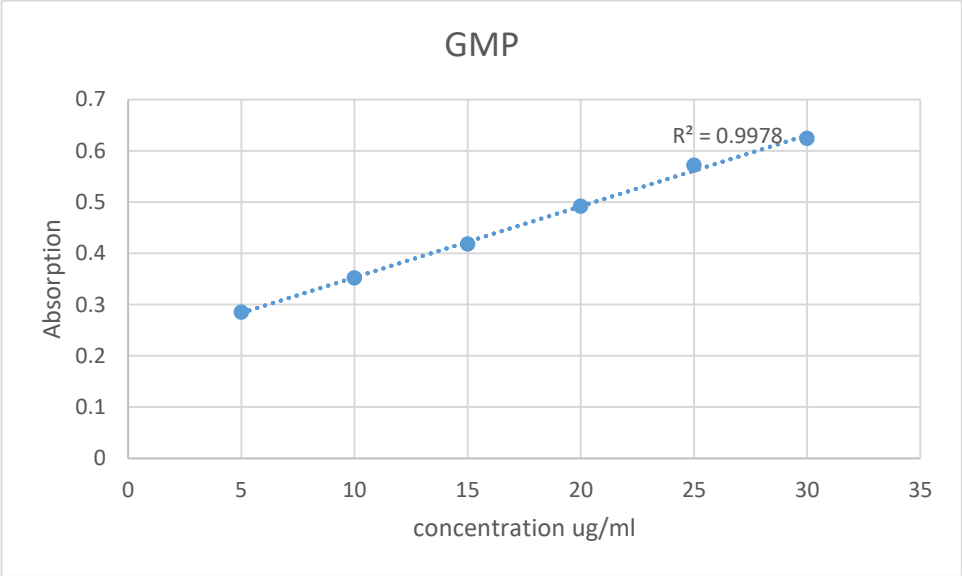

Fig. 6 Calibration curve of Glimepiride at 225nm

6 Conclusion

The UV spectrophotometric –simultaneous equation method was developed and validated for the simultaneous analysis of SITA and GMP. The results together established that the method is simple, accurate, precise, reproducible, rapid, and sensitive. The method could be applied successfully and economically for the simultaneous estimation of SITA and GMP in laboratory samples for efficient data generation and for combination formulations of these two drugs in the future.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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