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STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF GLIMEPIRIDE AND SITAGLIPTIN PHOSPHATE MONOHYDRATE IN SYNTHETIC MIXTURE

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ABSTRACT: A new, precise, rapid and accurate RP-HPLC method was developed for the simultaneous estimation of Glimepiride and Sitagliptin phosphate monohydrate in synthetic mixture. RP-HPLC method was designed using an HPLC system equipped PDA detector. After optimization of good chromatographic separation was achieved with a mixture of Acetonitrile: Methanol: Phosphate buffer (pH- 4.5 with orthophosphoric acid) in the volume ratio of 35:30:35 %v/v/v as the mobile phase with, C18 column (250 nm x 4.6 mm, 5 µm) as stationary phase at flow rate of 1 mL/min and detection wavelength of 240 nm. The retention time of Dexamethasone and Metoclopramide was found to be 5.640 min and 3.504 min respectively. The linearity of this method was found in the concentration range of 50 to 300 µg/ml for Glimepiride and Sitagliptin phosphate monohydrate. The correlation coefficient r^2 value was found to be 0.994 for Glimepiride and 0.9984 for Sitagliptin phosphate monohydrate. The method was extensively validated according to ICH guidelines for Linearity, Range, Precision, specificity and Robustness.

KEYWORDS: RP-HPLC, Glimepiride, Sitagliptin phosphate monohydrate, Simultaneous estimation, Method validation

INTRODUCTION

The precise and reliable estimation of pharmaceutical compounds in synthetic mixtures is essential for quality control and assurance in drug manufacturing. Glimepiride, a sulfonylurea, is widely used in the treatment of type 2 diabetes mellitus due to its ability to stimulate insulin secretion from pancreatic beta cells, thereby reducing blood glucose levels [1]. Sitagliptin Phosphate Monohydrate, a dipeptidyl peptidase-4 (DPP-4) inhibitor, enhances the body's own ability to lower elevated blood sugar by increasing and prolonging the action of incretin hormones [2]. The combination of Glimepiride and Sitagliptin Phosphate Monohydrate offers a synergistic approach to managing blood glucose levels in diabetic patients [3].

The simultaneous estimation of these compounds in a synthetic mixture poses significant analytical challenges due to their differing chemical properties and potential interactions. High-Performance Liquid Chromatography (HPLC) is a preferred analytical technique for such analyses due to its high resolution, accuracy, and ability to handle complex matrices [4]. Stability-indicating HPLC methods are particularly important as they can separate and quantify the drug components from their degradation products, ensuring the stability and efficacy of the pharmaceutical formulation [5].

Developing a stability-indicating HPLC method involves a systematic approach to method development, including the selection of appropriate chromatographic conditions, such as the mobile phase, column type, and detection wavelength, to achieve optimal separation [6]. Method validation, following International Council for Harmonisation (ICH) guidelines, ensures that the method is reliable and reproducible, meeting the criteria for accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), and robustness [7].

This study aims to develop and validate a stability-indicating HPLC method for the simultaneous estimation of Glimepiride and Sitagliptin Phosphate Monohydrate in a synthetic mixture. The successful validation of this method will provide a robust tool for the pharmaceutical industry, ensuring the quality and stability of combined Glimepiride and Sitagliptin Phosphate Monohydrate formulations, ultimately benefiting patients with type 2 diabetes mellitus.[8-10]

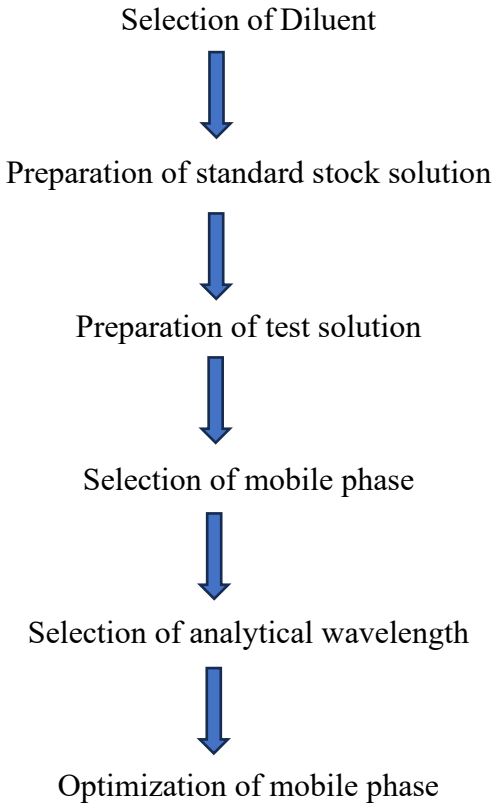
METHODS AND MATERIALS

Instrument - several methods for determination of glimepiride and sitagliptin phosphate monohydrate in biological matrixes have been developed, including UV- spectrophotometric, HPTLC and HPLC.

Reagent and standard: These were prepared by weighing 10 mg of Sitagliptin Phosphate Monohydrate (SIT) and Glimepiride (GLI) accurately and by transferring it to a 10 ml volumetric flask. A three-fourth quantity of diluent was poured into the flask and sonicated for 5 minutes to eliminate the dissolved gases present if any and the remaining volume was brought up to the required level using diluent. 1 ml of the stock solution was pipetted out and transferred into a 10 ml volumetric flask after diluting it to the right concentration with diluent yielded a suitable dilution of 100 µg/ml of SIT and GLI the working standard solution. [11]

METHOD FOR VALIDATION:

Various conditions used during the development of analytical methods should be optimizing for developing sensitive, accurate and reproducible method. Parameters which were optimized were as follows-



VALIDATION OF RP-HPLC METHOD: As per ICH guidelitiines the developed method was validated for validating the analytical procedures. The validation parameters include system suitability

test, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, robustness and specificity were also performed. [12-14]

1. **System suitability studies** - The appropriateness of the system was determined by examining six replicates of SIT and GLI in mixture concentrations of 200µg/ml and 2µg/ml. For peak area and retention time, the acceptance criteria are less than 2% R.S.D., theoretical plates higher than 2000, tailing factor less than 2.0, and capacity factor greater than 3.0. The results of the system suitability analysis met the required standards.
2. **Forced degradation studies** - The International Conference on Harmonization (ICH) guideline entitled stability testing new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. These studies are performed at various stress conditions to describe the stability of the pure drug substance and are helpful in determining a suitable storage condition. These studies include base, peroxide, acid, neutral hydrolysis, photo, and thermal degradation. The aim of this work was to perform the stress degradation studies on the SIT and GLI using the proposed method.
3. **Specificity** - To verify the interferences in the optimized chromatogram, specificity was performed. By correlating the chromatograms of the placebo, blank and standard solution at working concentration, specificity was determined. The method has not shown any interfering peaks.
4. **Linearity and range** - Pipette out 1, 2, 3, 4, and 5ml of SIT working standard solution (1000µg/ml) and 1, 2, 3, 4, and 5ml of GLI stock solution (10µg/ml) into a series of 10ml volumetric flasks and adjust the volume to mark with methanol to give solution strength 100, 200, 300, 400 and 500 µg/ml for SIT and 1, 2, 3, 4, and 5 µg/ml for GLI. For SIT and GLI, the linearity response was determined by evaluating 5 separate levels of calibration curve in the range (n=6). The correlation coefficient is determined by plotting the calibration curve of peak area vs. concentration.
5. **Precision** –
   - Repeatability : The method and system precision were demonstrated by preparing the standard solution at a known concentration and six repeatable injections were given to ensurethe repeatability of the proposed method. The repeatability was determined byanalyzing solutions containing 300µg/ml and 2µg/ml for SIT and GLI, respectively, and analyzing the identical solutions and calculating the percent R.S.D.
   - Intra day precision : The new method's intra-day precision was evaluated by analyzing solutions comprising concentrations of 100, 300, 500µg/ml for SIT and 1, 3, 5µg/ml for GLI and three replicates (n=3) each on the same day.
   - Inter day precision : The new method's inter-day precision was tested by analyzing sample solutions comprising concentrations of 100, 300, 500µg/ml for SIT and 1, 3, 5µg/ml for GLI on three different days.
   - Accuracy - Recovery studies were performed to evaluate the method's accuracy by spiking various concentrations of pure drug in the analyte sample solutions of three different concentrations of standard having 50%, 100%, and 150% of pure drug. The percentage recovery and percentage RSD were measured to assess the accuracy. The acceptance limits of % recovery should be in the range 98%– 102%.
   - Robustness - Small and purposeful variations in instrumental settings such as mobi1le phase composition, flow rate, and wavelength of detection were used to test the method's robustness

by injecting triple injections of standard solutions of 300µg/ml and 3µg/ml of SIT and GLI, respectively. The effect was investigated by measuring the peak area of solutions and calculating the percent RSD.

- LOD (limitation of detection) and LOQ (limit of qualification) - The Limit of detection (LOD) and Limit of Quantification (LOQ) of the developed method was calculated from the five-calibration curve.

6. **Analysis of synthetic mixture** - The synthetic mixture of SIT and GLI was made in a ratio of 100:1 mg each weigh andother excipient as per above tablet weight which is equivalent for 20 tablets. The synthetic mixture was accurately weighed as 0.170 g and transferred into a 10- mL volumetric flask containing a few mL of methanol, and it was sonicated for 15 min. The solution was filtered using Whatman filter paper No. 42, and the filtrate was collected in a 10 ml volumetric flask, where the volume was adjusted up to the mark with methanol to obtain conc. 1000 µg/ml of SIT and 10 µg/ml of GLI. Pipette 3 ml ofthe aforesaid solution into a new 10 ml volumetric flask and adjust the volume with methanol to the mark to get conc. 300 µg/ml of SIT and 3 µg/ml of GLI. Three times the process was carried out. Each solution was analyzed and a chromatogram was produced using the recommended chromatographic conditions. SIT and GLI concentrations were determined using a regression equation. [15]

RESULT AND DISCUSSION:

Selection of analytical wavelength - The RP-HPLC method uses with PDA detector for detection of Sitagliptin PhosphateMonohydrate (SIT) and Glimepiride (GLI). The spectrum has shown 230 nm as a suitable wavelength for the present RP-HPLC method. Both drugs have shown good absorption at this wavelength.

Table 1: HPLC Trials for optimization of mobile phase

Trials	Mobile phase	Drug	Inference
1.	Acetonitrile: Methanol: Water (50:10:40 v/v/v)	SIT andGLI	One Drug eluted only
2.	Acetonitrile: Water: Methanol (20:40:40 v/v/v)	SIT andGLI	One Drug eluted only and splitting observed
3.	Acetonitrile: Methanol: Water (30:30:40 v/v/v)	SIT andGLI	One Drug eluted only and splitting observed
4.	Acetonitrile: Methanol: Buffer (pH 3.5 adjust with ortho phosphoric acid) (30:40:30 v/v/v)	SIT andGLI	Peak shape not proper, and Tailing observed
5.	Acetonitrile: Methanol: Buffer (pH 4.5 adjust with ortho phosphoric acid) in the ratio of 30:35:35 v/v/v	SIT andGLI	GLI eluted at Rt = 3.481 and SIT at 8.639 with proper shape, theoretical plate greater than 2000 and resolution is greater than 2.

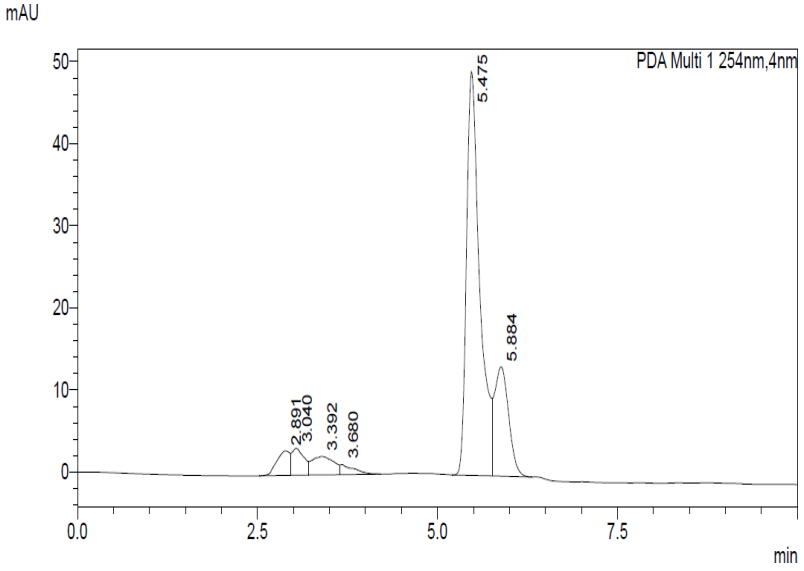


Figure 1: Trail 1: Chromatogram of mixture using Acetonitrile: Methanol: Water (50:10:40)

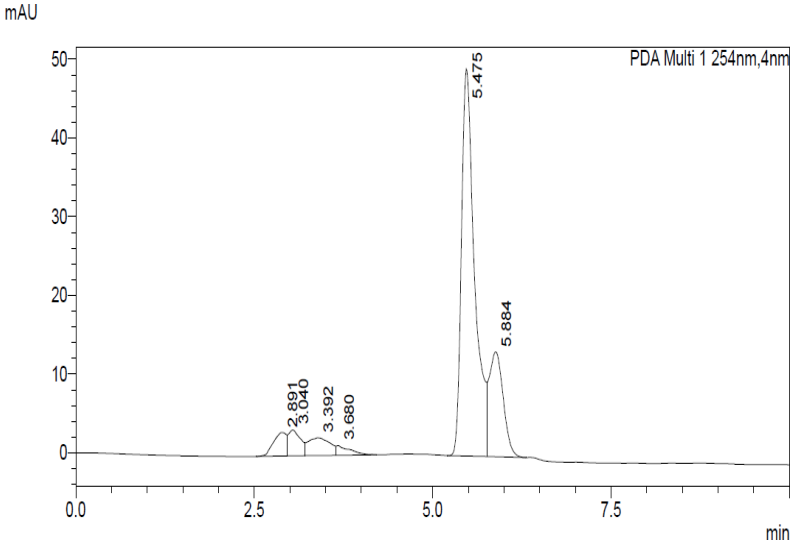


Figure 2: Trail 2: Chromatogram of mixture using Acetonitrile: Water: Methanol (20: 40:40 v/v/v)

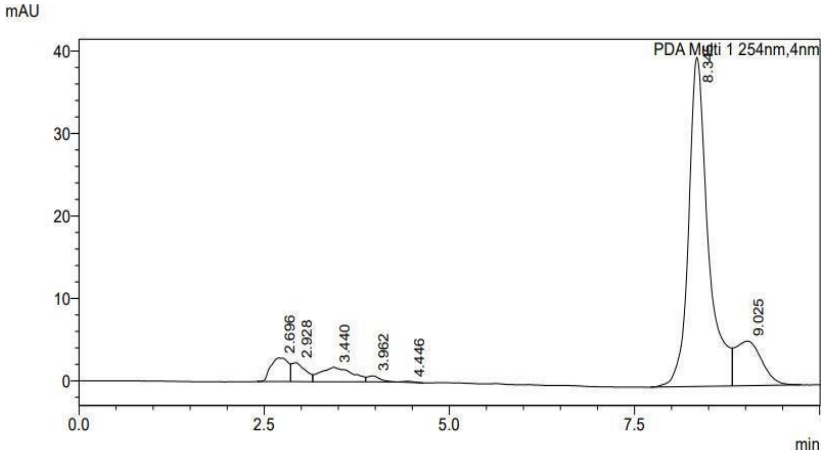


Figure 3: Trail 3: Chromatogram of mixture using Acetonitrile: Methanol: Water (30:30:40 v/v/v)

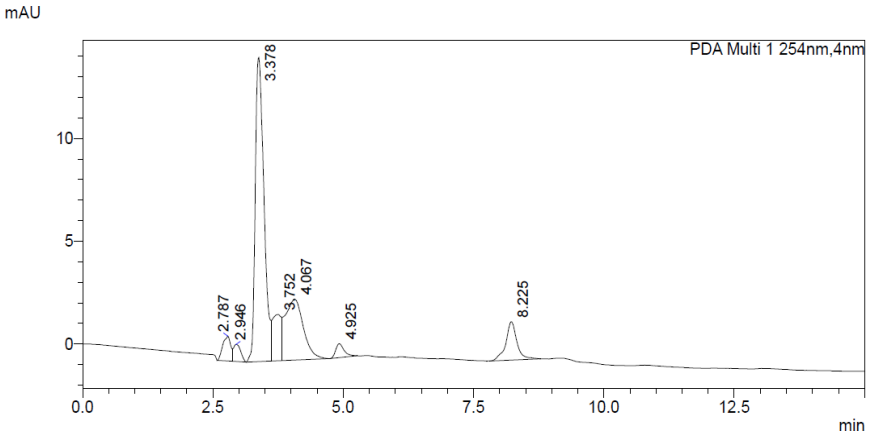


Figure 4: Trial 4: Chromatogram using Acetonitrile: Methanol: Buffer (pH 3.5 adjust with ortho phosphoric acid) (30:40:30 v/v/v)

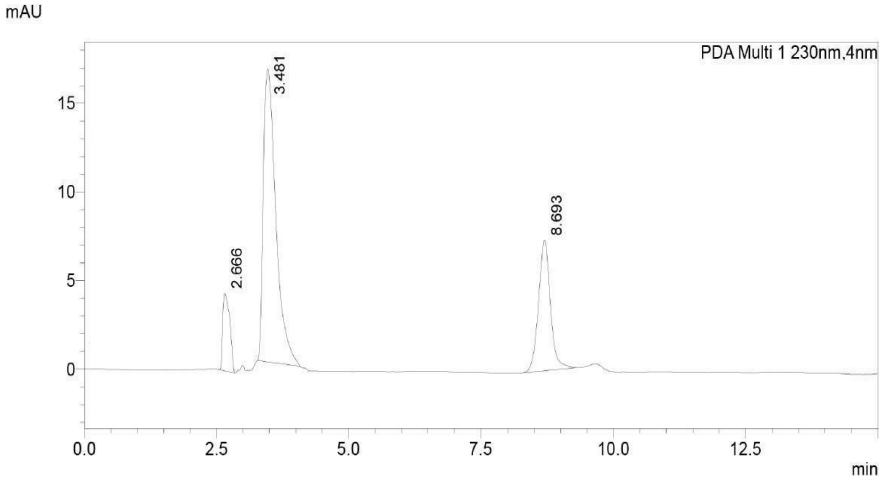


Figure 5: Chromatogram using optimized mobile phase of 300ppm Sitagliptin Phosphate Monohydrate (SIT) and 3ppm Glimepiride (GLI)

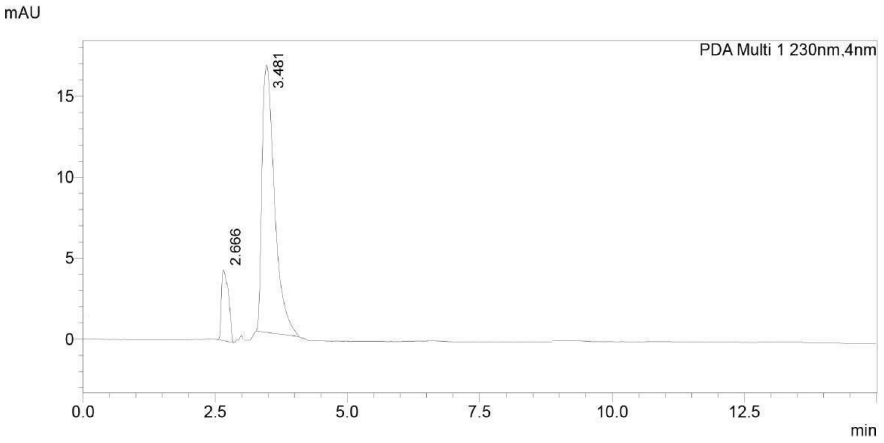


Figure 6: Chromatogram of 3PPM Glimepiride (GLI)

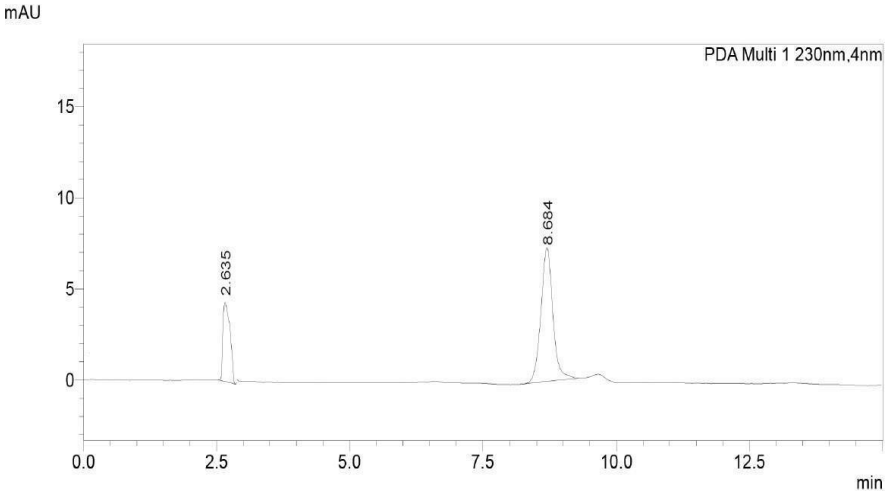


Figure 7: Chromatogram of 300PPM Sitagliptin Phosphate Monohydrate (SIT)

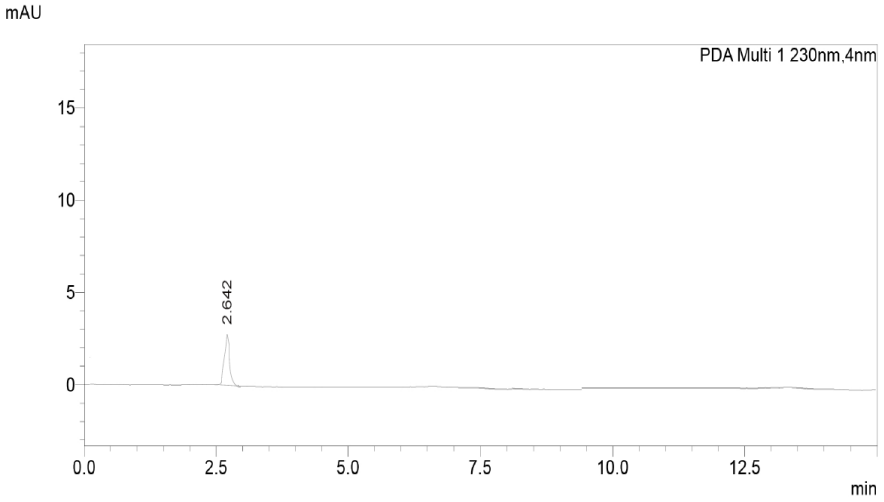


Figure 8: Blank Chromatogram

Optimize chromatographic condition - The final optimized mobile phase is shown in table after optimization of the mobile phase.

Table 2: Optimized mobile phase

Sr.no	**Parameter**	**Condition**
1	Mobile phase	Acetonitrile: Methanol: Buffer (pH 4.5 adjust with ortho phosphoric acid) in the ratio of 30:35:35 v/v/v
2	Flow Rate	1 ml/min
3	Run Time	15 min

4	Volume of Injection	10 µl
5	Detection of Wavelength	PDA detector at 230 nm
6	Column	Shimpack ODS C18 (250 mm x 4.6 mm, 5µm)
7	Column Temperature	40 ° C

METHOD VALIDATION

1. **System suitability**: Analyzing GLI and SIT at mixture concentrations of 3µg/ml and 300µg/ml was used to determine system applicability. For peak area and retention time, the acceptance criteria are less than 2% R.S.D., theoretical plates higher than 2000, tailing factor lessthan 2.0, and capacity factor greater than 3.0. The results of the system suitability analysis met the required standards.

Table 3: System suitability analysis

Drugs	Parameters	Mean ± S.D. (n=6)	% R.S.D.
GLI	Retention Time	3.478 ± 0.012	0.34
	Tailing Factor	1.35 ± 0.021	1.55
SIT	Retention Time	8.489 ± 0.102	1.20
	Resolution	12.19 ± 0.174	1.42
	Tailing Factor	1.073 ± 0.013	1.21

2. **Forced degradation studies** - Forced degradations were performed to show the stability indicating properties of the analytical method, particularly when there is no information available about the potential degradation product
3. **Acid Hydrolysis** - The acid hydrolysis was done by pipetted out 3 ml of solution along with 3 ml of1 N HCl into 10 ml volumetric flask. This was kept at 60°C for 3 hours and then neutralized with 1 N NaOH, followed by filtration with 0.45 µm syringe filter and placement in vials. The acidic condition applied on the active drug substances for above condition induced the hydrolysis of GLI causing assay loss of about 10.51% degradative peak observed at 4.746, 4.979 and SIT causing assay loss of about 9.07 % and degradative peak observed at 8.167 shown in chromatogram.

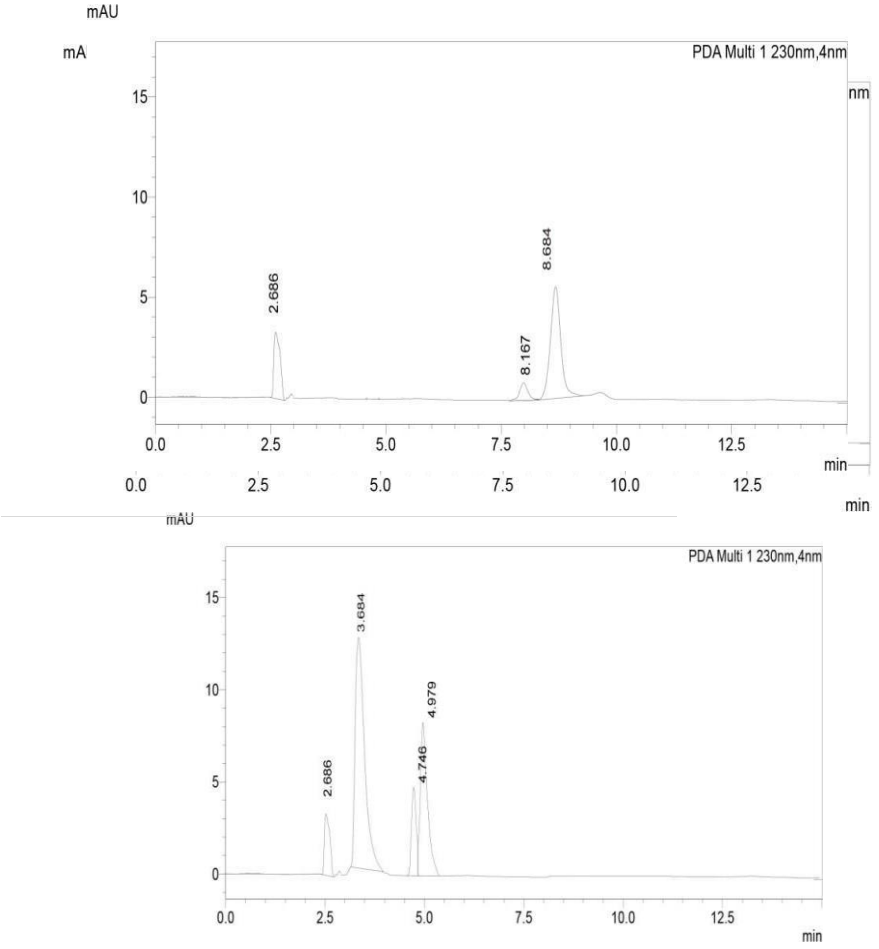


Figure 9: (a) Acid Hydrolysis chromatogram of GLI and SIT mixtures (b) Acid Hydrolysis chromatogram of SIT (c) Acid Hydrolysis chromatogram of GLI

4. **Base Hydrolysis** - The base hydrolysis was carried out by pipetted out 3 ml of solution along with3 ml of 1 N NaOH into 10 ml volumetric flask. This was kept at 60°C for 3 hours and then neutralized with 1 N HCl, followed by filtration with 0.45 µm syringe filter and placed in vials. The alkaline condition applied on the active drug substances for above condition induced the hydrolysis of GLI causing assay loss of about 8.43 % and degradative peak observed at 4.955 and SIT causing assay loss of about 12.46% and degradative peak observed at 8.124 drugs shown in chromatogram.

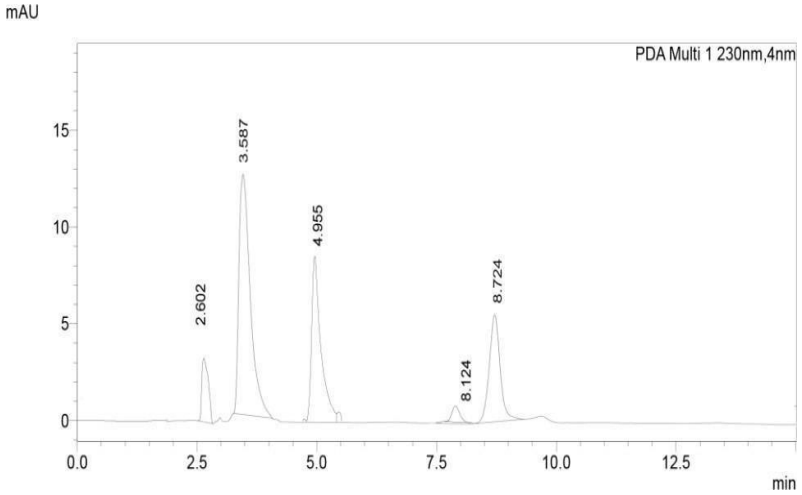


Figure 10: (a) Base Hydrolysis chromatogram of GLI and SIT mixtures (b) Base Hydrolysis chromatogram of SIT (c) Base Hydrolysischromatogram of GLI

5. **<u>Oxidative degradation</u>** - The oxidative degradation was carried out by pipetted out 3 ml of solution along with 3 ml of 10% w/v of hydrogen peroxide into a 10 ml volumetric flask. This was then kept at room temperature for 30 min, followed by filtrationwith 0.45 µm syringe filter and placed in vials. The oxidative condition appliedon the active drug substances for above condition induced the oxidation of GLI causing assay loss of about 4.40 % and degradative peak observed at 4.881 andSIT causing assay loss of about 3.57 % degradative peak observed at 8.071.

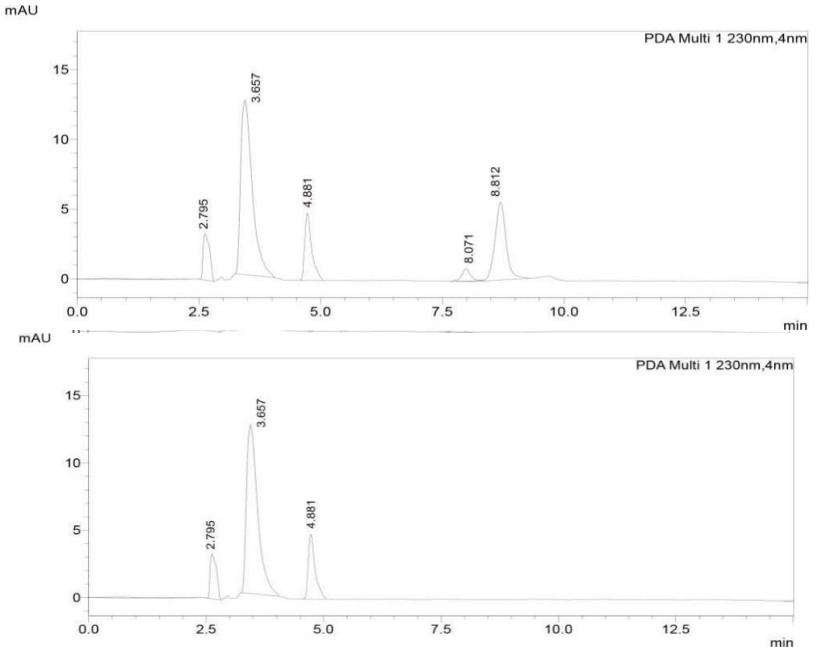


Figure 11: (a) Oxidative degradation chromatogram of GLI and SIT mixtures (b) Oxidative degradation chromatogram of SIT (c) Oxidative degradation chromatogram of GLI

6. **Thermal degradation** - Thermal degradation was carried out by placing solid samples in a Petri dish and keeping these in a hot air oven at 110°C for 3 hrs, followed by filtration with 0.45 µm syringe filter and placed in vials. Thermal degradation was carriedout by placing solidsamples in a Petri dish and keeping these in a hot air oven at110°C for 3 hrs, followedby filtration with 0.45 µm syringe filter and placed in vials. The thermal condition applied on the active drug substances for above condition induced the degradation of GLI causing assay loss of about 7.22 %and SIT causing assay loss of about 9.09 % and no major degradative peak observed for both drugs.

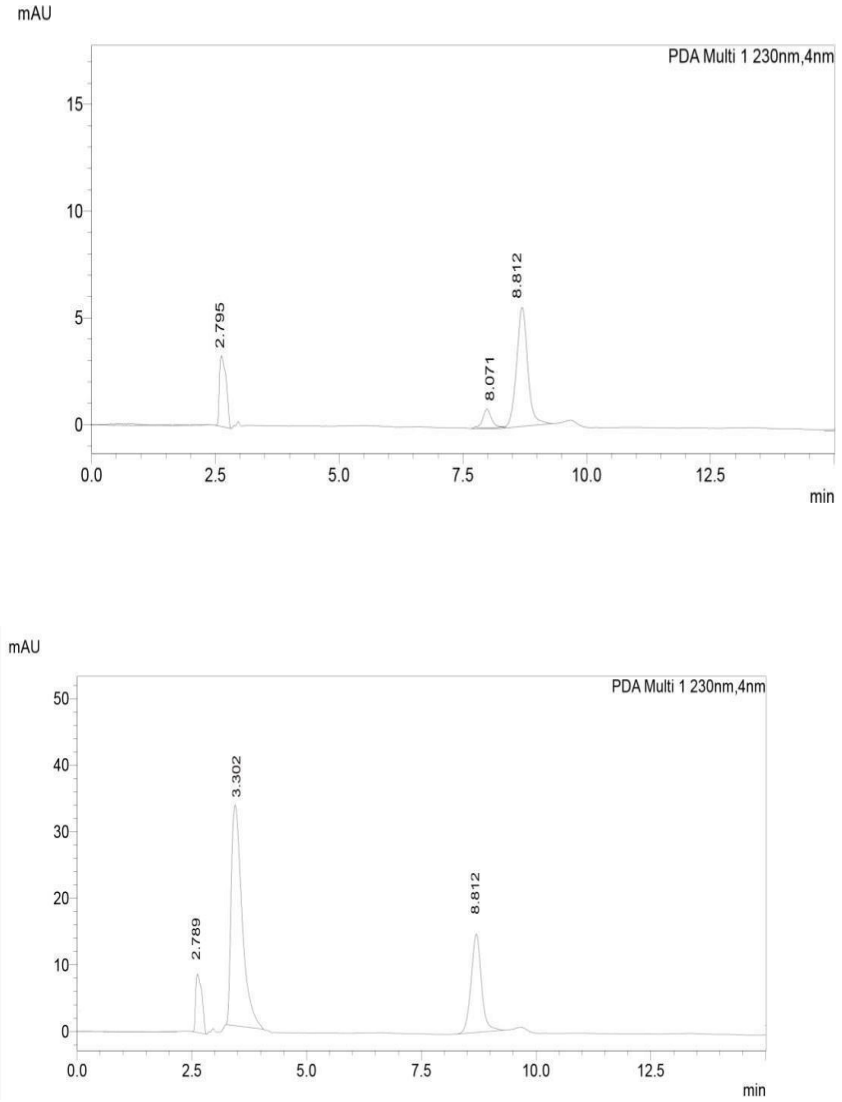


Figure 12: Thermal degradation chromatogram of GLI and SIT mixtures

7. **Photo degradation** - The photolytic degradation was carried out by taking solid samples spread out as a thin layer on a Petri plate. It subsequently exposed to UV light in a chamber for 48 hrs. The stressed sample was filtered through 0.45 µm syringe filter before its analysis. The thermal condition applied on the active drugsubstances for above condition induced the hydrolysis of GLI causing assay loss of about 6.50 % and SIT causing assay loss of about 8.68 % and no degradative peak observed for both drugs.

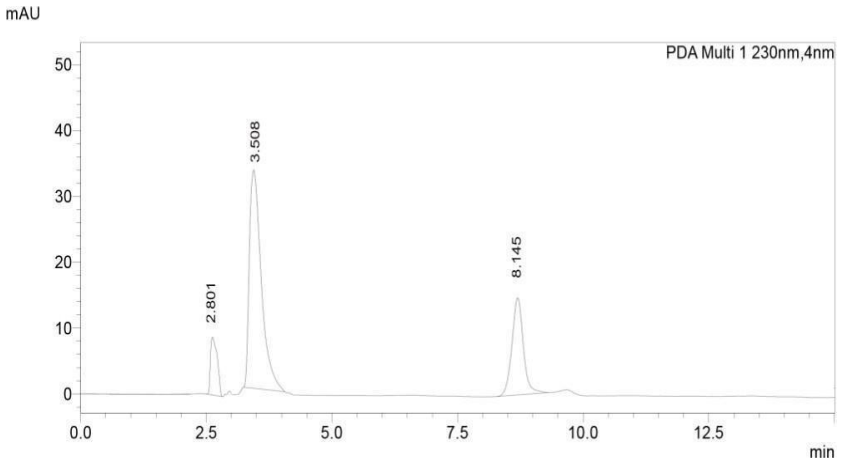


Figure 13: Photo degradation chromatogram of GLI and SIT mixtures

Table 4: Forced degradation data of the method

Stress Condition	**Amount of GLI degraded (%)**	**Amount of GLI recovered (%)**	**Amount of SIT degraded (%)**	**Amount of SIT recovered (%)**
Acidic	10.51	89.49	9.07	90.93
Alkali	8.43	91.57	12.46	87.54
Oxidative	11.12	88.88	8.24	91.76
Thermal	7.22	92.78	9.09	90.91
Photo stability	6.50	93.50	8.68	91.32

8. **Specificity** - The method's specificity was determined by evaluating reference pharmaceuticals as well as GLI and SIT samples. The presence of an excipient in the synthetic combination does not affect the outcome. As a result of the findings, it appears that the proposed method is unique.

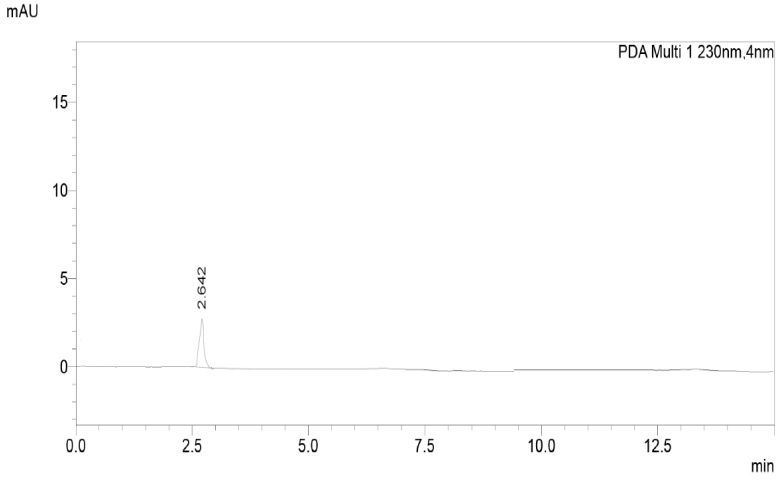


Figure 14: Blank Chromatogram

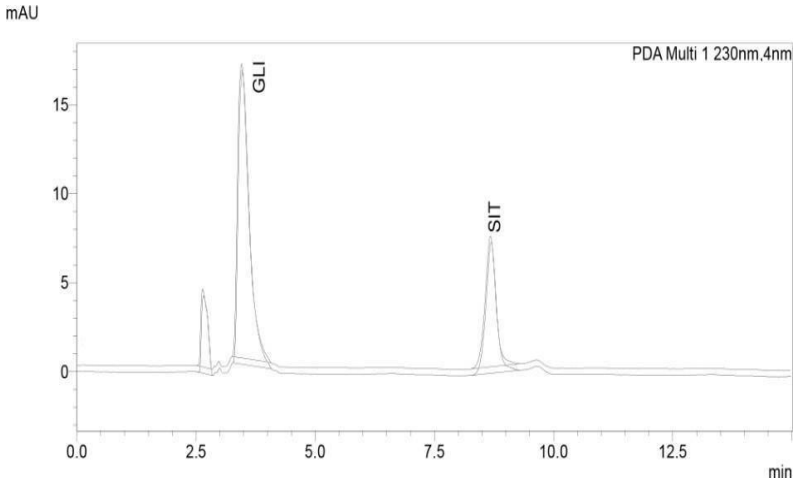


Figure 15: Chromatogram of sample GLI (3µg/ml) and SIT (300µg/ml) on optimized mobile phase

9. **Linearity and range** - Plotting the mean peak area of GLI and SIT against concentration over the ranges of 1-5µg/ml and 100-500 µg/ml for GLI and SIT, respectively, yielded a representative calibration curve (Figure 14 and 15). In the above conc. range, responses were found to be linear, with correlation values of 0.9945 for GLI and 0.9995 for SIT. The linearity results for GLI and SIT are reported in Tables 6 and 7, respectively. Figure 13 shows an overlay chromatogram of 1-5µg/ml and 100-500 µg/ml for GLI and SIT. The calibration range was designed so that the combination ratio was maintained throughout the simultaneous estimation of both bulk and synthetic mixture medicines. Table 7 shows the calibration curve's outcome as well as a regression analysis of the calibration curve.

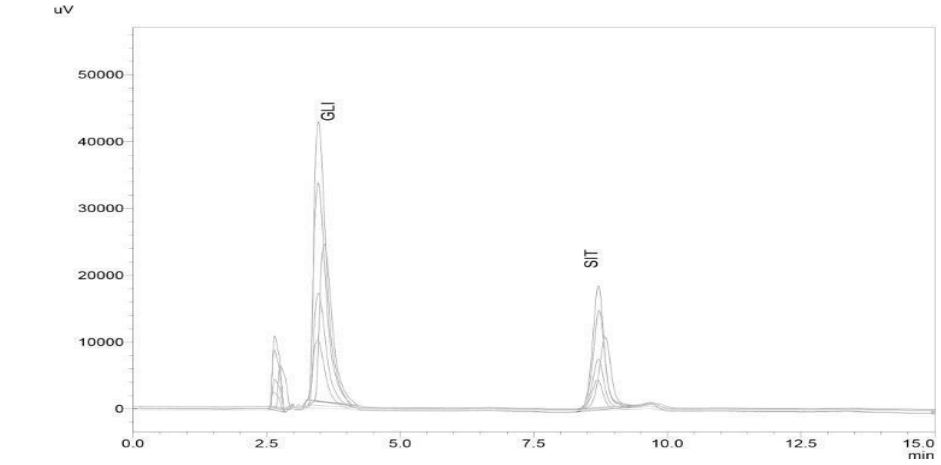


Figure 16: Overlay Chromatogram of 1-5µg/ml and 100-500 µg/ml for GLI and SIT

Table 5: Linearity data of Glimepiride (GLI)

Concentration (µg/ml)	Area Mean ± SD (n=6)	% RSD
1	108515 ± 1330.73	1.23
2	195058 ± 3421.18	1.75
3	276827 ± 4346.18	1.57
4	385549 ± 2158.40	0.56
5	501277 ± 3731.09	0.74

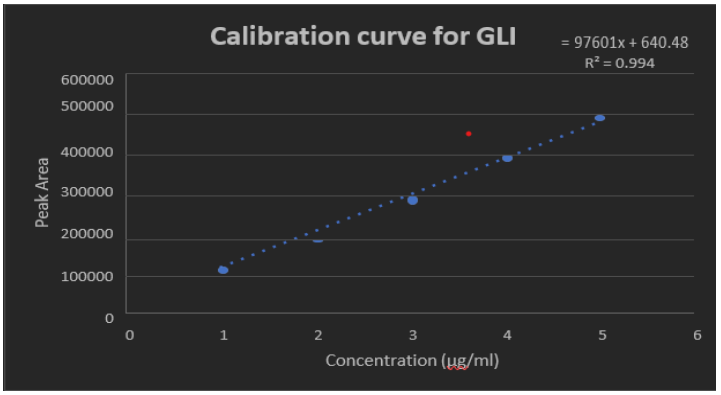


Figure 17: Calibration curve for GLI

Table : 6 Linearity data of SIT

Concentration (µg/ml)	Area Mean ± SD (n=6)	% RSD
100	37572 ± 429.33	1.14
200	71349 ± 1146.73	1.61
300	116309 ± 2060.01	1.77
400	153186 ± 2967.77	1.94
500	189719 ± 3047.57	1.61

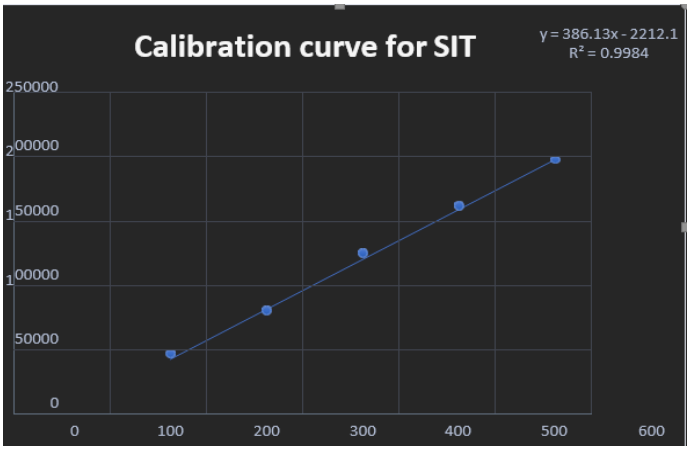


Figure 18: Calibration curve for SIT

Table 7: Regression Analysis of calibration curve

Parameter	GLI	SIT
Concentration range (µg/ml)	1-5 µg/ml	100-500 µg/ml
Regression equation	y = 97601x + 640.48	y = 386.13x - 2212.1
Regression coefficient (r^2)	0.9941	0.998 4
Average of slope	9760 1	40526
Standard deviation of Intercept	3129.41	2119.70
Limit of detection (µg/ml)	0.10	0.17
Limit of quantification (µg/ml)	0.32	0.52

10. Precision:

- Repeatability - GLI and SIT solutions containing 3µg/ml and 300µg/ml, respectively, and the samesolution were examined seven times GLI had a %RSD of 1.60 % andSIT had a %RSD of 0.54%. The fact that the %RSD value was less than 2.0 suggested that theapproach was precise.

Drug	Concentration (µg/ml)	Mean peak area ± S.D. (n=7)	% R.S.D.
GLI	3	277637 ± 4450.39	1.60
SIT	300	116768 ± 2016.75	1.72

Sr. No.	GLI 3 µg/ml	SIT 300 µg/ml
1	273356.08	114373.29
2	277034.10	115787.98
3	278097.90	114254.00
4	280601.00	115741.00
5	281647.00	116321.00
6	280353.05	116856.18
Average	278514.85	115555.57
SD	3050.27	1044.77
% RSD	1.09	0.90

- Intraday precision - GLI and SIT solutions containing 1, 3 and 5µg/ml and 100, 300 and 500µg/ml were examined three times on the same day using a devised HPLC method, and %RSD was calculated. GLI had a %RSD of 0.32-1.64%, while SIT had a % RSD of 0.43- 1.50%. The fact that the %RSD value was less than 2.0 suggested that the approach was precise. (Figure 10)

Table 9: Intraday precision data for estimation of GLI and SIT (n=3)

Conc. (µg/ml)		Mean peak area ± SD	% RSD	Mean peak area ± SD	% RSD
GLI	SIT	GLI		SIT	
1	100	108788.33 ± 1403.76	1.29	37518.33 ± 161.66	0.43
3	300	275395.33 ± 4510.65	1.64	116429.51 ± 1500.77	1.29
5	500	502830.00 ± 1601.90	0.32	189614.33 ± 2849.09	1.50

- Inter-day precision - GLI and SIT solutions containing 1, 3 and 5µg/ml and 100, 300 and 500µg/ml were examined three times on the same day using a devised HPLC method, and %RSD was calculated. GLI had a %RSD of 0.52-1.78%, while SIT had a % RSD of 1.12- 1.75%.

Table 10: Inter-day precision data for estimation of GLI and SIT (n=3)

Conc. (µg/ml)		Mean peakarea ± SD	% RSD	Mean peakarea ± SD	% RSD
GLI	SIT	GLI		SIT	
1	100	108774.33 ± 1935.46	1.78	37474.33 ± 615.97	1.64
3	300	278258.33 ± 4552.98	1.64	116949.48 ± 2050.82	1.75
5	500	501425.66 ± 2620.07	0.52	190869.96 ± 2140.20	1.12

- Accuracy - Recovery studies from synthetic mixtures at three levels of standard addition (50%, 100%, and 150%) validated the method's accuracy. The data in Tables 12 and 13 show that the developed procedure is reliable. GLI and SITwere reported to have %recovery rates of 98.21 – 101.65% and 98.40 – 101.72%, respectively.

Table 11: Accuracy data of GLI (n=3)

	Conc. of GLI from Synthetic mixture (µg/ml)	Amount of Std .GLI added (µg/ml)	Total amount of GLI (µg/ml)	Mean peakarea ± SD	Total amount of GLI Recovered (µg/ml) Mean ± SD	% Recovery
0	2	0	2	193531.33 ± 3345.24	1.98 ± 0.03	98.82
50%	2	1	3	28205.21 ± 4305.58	2.93 ± 0.04	98.21
100%	2	2	4	384481.33 ± 1482.97	3.93 ± 0.02	98.32
150 %	2	3	5	496701.86 ± 7375.54	5.08 ± 0.08	101.65

Table 12: Accuracy data of SIT (n=3)

Level	Conc.of SIT from Synthetic mixture (µg/ml)	Amount of Std. SITadded (µg/ml)	Total amount of SIT (µg/ml)	Mean peakarea ± SD	Total amount of SIT Recovered (µg/ml) Mean ± SD	% Recovery
0	200	0	200	73775.80 ± 3879.26	196.79± 10.04	98.40
50%	200	100	300	115618.50 ± 1416.22	305.16 ± 3.67	101.72
100%	200	200	400	152874.33 ± 3717.98	401.64 ± .63	100.41
150 %	200	300	500	188022.33 ± 3625.37	492.67 ± 9.39	98.53

- Robustness - To test robustness, a slight purposeful adjustment in HPLC conditions was performed. For both GLI (3µg/ml) and SIT (300µg/ml), two changes were measured using this approach. The effects of ratio of mobile phase ratio, flow rates as well as changes in detection wavelengths were studied. The % RSD value of robustness HPLC method were found less than 2% indicating that developed method was robust

Table 13: Robustness data of GLI and SIT (n=3)

Parameters	Change in condition	GLI		SIT	
		Peak Area	%RSD	Peak Area	%RSD
Optimized Chromatographycondition		275541.33 ± 4382.38	1.59	117977.03 ± 1359.34	1.15
Detection wavelengt h(230nm)	227 nm	272513.31 ± 5161.42	1.89	116913.83 ± 1614.85	1.38
	233 nm	273984.08 ± 4770.42	1.74	117306.75 ± 1128.63	0.96
Flow rate Changed (1 ml/min)	0.9	275953.87 ± 4316.21	1.56	118154.08 ± 1373.48	1.16
	1.1	273521.47 ± 4442.92	1.62	117111.08 ± 1255.43	1.07
Mobile	Acetonitrile: Methanol: Buffer (pH 4.5 adjust	276729.23 ± 4009.53	1.45	118488.30 ± 1486.29	1.25

Proportion Changed Acetonitrile: Methanol: Buffer (pH 4.5 adjust with ortho Phosphoric acid) in theratio of 30:35:35 v/v/v	with ortho phosphoricacid) (35:35:30 v/v/v)				
	Acetonitrile: Methanol: Buffer (pH 4.5 adjust withortho phosphoric acid) (25:35:40 v/v/v)	276789.71 ± 2284.16	0.83	120089.08 ± 400.75	0.33

- Limit of Detection and Quantitation - Limit of Detection and limit of Quantitation of GLI and SIT shown inTable 7.15indicating sensitivity of method.

Table 14: LOD and LOQ data of GLI and SIT (n=6)

	GLI (µg/ml)	**SIT (µg/ml)**
LOD	0.10	0.17
LOQ	0.32	0.52

Table 15: Summary of validation parameters

PARAMETERS	**HPLC method**	
	GLI	**SIT**
Concentration range(µg/ml)	1-5 µg/ml	100-500 µg/ml
Regression equation	y = 97601x + 640.48	y = 386.13x - 2212.1
Correlation Coefficient(r^2)	0.9941	0.9984
Accuracy(%Recovery) (n=3)	98.21 – 101.65%	98.40 – 101.72%
Repeatability (%RSD) (n=7)	1.60	1.72
Intra-day Precision (%RSD) (n=3)	0.32-1.64	0.43-1.50
Inter-day precision (%RSD) (n=3)	0.52-1.78	1.12-1.75

Assay (%Recovery) (n=3)	99.40 ± 1.78	97.03 ± 1.17
Specificity	Specific	
Robustness	Robus t	
LOQ(µg/ml)	0.10	0.17
LOD(µg/ml)	0.32	0.52

For two compounds, all of the parameters satisfied the ICH method validation criteria and were deemed to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The linearity, accuracy, and precision of the results revealed to be with in limits, with lower detection and quantification limits.

CONCLUSION:

The literature review reveals that the no stability indicating RP-HPLC method for estimation of Sitagliptin Phosphate Monohydrate (SIT) and Glimepiride (GLI) in synthetic mixture.

A novel attempt in a field of research has been made to develop and validate stability indicating assay method and forced degradation performed by HPLC. To develop stability indicating RP-HPLC method for the Sitagliptin Phosphate Monohydrate (SIT) and Glimepiride (GLI).

In RP- HPLC method, good resolution was achieved using mobile phase Acetonitrile: Methanol: Buffer (pH 4.5 adjust with ortho phosphoric acid) in the ratio of 30:35:35 v/v/v at a flow rate of 1 mL/min and at detection using PDA detector at 230nm using a C18 column (4.6×250 mm, 5 mm) column. The retention time of Glimepiride (GLI) and Sitagliptin Phosphate Monohydrate (SIT) is 3.481 and 8.693 min, respectively. The proposed method was accurate and precise. So, it can be used for routine analysis of Glimepiride (GLI) and Sitagliptin Phosphate Monohydrate (SIT).

The suitability, performance and applicability of developed method has been validated as per ICH guideline by applying various validation parameters like specificity, linearity accuracy, precision, robustness, LOD and LOQ.

A simple, sensitive, accurate, rapid and economical RP-HPLC method was developed and validated for the determination of Glimepiride (GLI) and Sitagliptin Phosphate Monohydrate (SIT).

Based on the results of the HPLC method used to analyses GLI and SIT in bulk drug as well as their synthetic mixture, it can be concluded that the method has linearity in the range of 1-5µg/ml for GLI and 100-500µg/ml for SIT. The regression coefficients (R2) for GLI and SIT were greater than 0.99. The limits of detection for GLI and SIT were found to be 0.10µg/ml and 0.17µg/ml, respectively, and the limits of quantification for GLI and SIT were 0.32µg/ml and 0.52µg/ml. The accuracy of GLI and SIT were % recovery rates of 98.21 –101.65% and 98.40 – 101.72%, respectively. For precision, repeatability, intra- day, and inter-day studies, the %R.S.D. was determined to be less than 2%. Th % RSD value of robustness HPLC method were found less than 2% indicating that developed method was robust.

Glimepiride (GLI) and Sitagliptin Phosphate Monohydrate (SIT) is undergoing significant degradation in acid, base, photo, thermal and oxidation. Hence, a method of the analysis of Glimepiride (GLI) and Sitagliptin Phosphate Monohydrate (SIT) shows that the degradation product doesn't interfere with the analytical determination.

Hence, the proposed analytical method is also useful for the determination of Glimepiride (GLI) and Sitagliptin Phosphate Monohydrate (SIT) stability in a sample of the Synthetic mixture as

well as for further quality control analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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