



## Analytical Methods for Determination of Sitagliptin: An Updated Review

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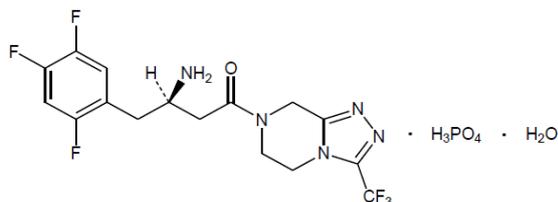
### ABSTRACT

Sitagliptin is an oral anti-hyperglycemic agent of the dipeptidyl peptidase-4 (DPP-4) inhibitor class used in the treatment of type-2 diabetes. It stimulates insulin release and reduces glucagon level by inhibition of inactivation of the incretin in a glucose-dependant manner. In 2013 it was the second best-selling drug in the U.S. This review explores the reported analytical methods so far in the literature for the estimation of sitagliptin in bulk drug, pharmaceutical formulations and in biological matrix. This assessment encompasses various analytical methods such as spectrometry, high performance liquid chromatography (HPLC), liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS-MS), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis (CE), ultra performance liquid chromatography (UPLC), high performance thin layer chromatography (HPTLC) and gas chromatography-mass spectrometry (GC-MS) for the estimation of sitagliptin in single and/or in combination.

**Keywords:** Sitagliptin, Analytical methods, Type-2 diabetes.

### INTRODUCTION

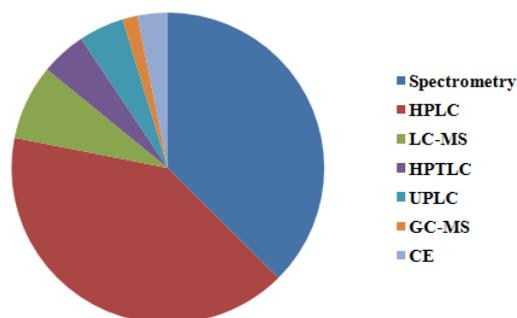
Sitagliptin (SITA) chemically (3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5h-[1,2,4] triazolo [3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one (Figure 1), is an oral anti-diabetic agent that blocks DPP-4 activity, used in the treatment of type 2 diabetes.<sup>1,2</sup> DPP-4 enzyme breaks down the incretin hormones including glucagon like peptide-1 (GLP-1) and glucose dependent insulintropic polypeptide (GIP). GLP-1 and GIP are 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.<sup>3,4</sup> The absolute bioavailability of SITA is approximately 87%. The co-administration of high fat meal with SITA has no effect on the pharmacokinetics. It may be administered with or without food.<sup>1</sup> Approximately 80% of the SITA excreted unchanged in urine. The fecal route accounts for 13% of elimination.<sup>5</sup> The benefit of this medicine is its lower side effects like less hypoglycemic and less weight gain. It is used alone or in combination with metformin or simvastatin to treat diabetes.



**Figure 1:** Structure of Sitagliptin

SITA is a white to off white, crystalline, non-hygroscopic powder and has a molecular formula of

$C_{16}H_{15}F_6N_5O \cdot H_3PO_4 \cdot H_2O$ . The molecular weight is 523.32. It is soluble in water and N, N-Dimethyl formamide; slightly soluble in methanol; very slightly soluble in ethanol, acetone and acetonitrile; and insoluble in isopropanol and isopropyl acetate.<sup>1</sup> In this review the various analytical methods published in the literature hitherto for the determination of sitagliptin in bulk drug, pharmaceutical formulations and in biological matrix are compiled. Techniques like spectrometry, high performance liquid chromatography (HPLC), liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS-MS), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis (CE), ultra performance liquid chromatography (UPLC), high performance thin layer chromatography (HPTLC) and gas chromatography-mass spectrometry (GC-MS) have been used for analysis of SITA. Overview of these methods for determination of SITA is shown in figure 2.



**Figure 2:** overview of Analytical methods for determination of Sitagliptin in Biological and Pharmaceutical samples.

## Sample Preparation

### Solubility

According to biopharmaceutical classification system (BCS), the SITA falls in BCS class-I, meaning high solubility and high permeability.<sup>6</sup> The pH of a saturated water solution of SITA is 4.4. The partition coefficient is 1.8 and pKa is 7.7.<sup>7</sup> The solubility of the drug was tested in solvents routinely used for analytical methodology.

### Sample preparation strategies

About 80% of the total analysis time is spent on sample preparation in most of the methods. The quality of sample preparation is a key factor for success of analysis.<sup>8</sup> Figure 3 shows various diluents used in the analysis of SITA. In most of the spectrometric methods distilled water, in some cases methanol is used as diluent. The sample preparation techniques for the extraction of SITA from biological matrices (plasma, urine) include protein precipitation with acetonitrile (ACN) and methanol, solid phase extraction (SPE) using methanol-phosphate buffer (PB), methanol-water and liquid-liquid extraction (LLE) using tert butyl methyl ether (TBME) and ethyl acetate.

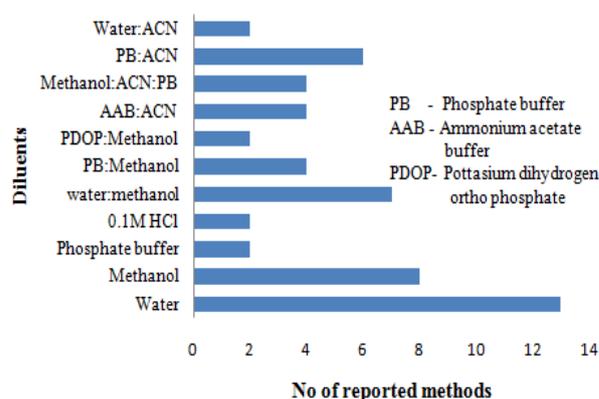
## Analytical Methods

### Spectrometry

In the literature about 25 methods were reported for the estimation of SITA using spectrometry, of which 11 methods are for determination of SITA alone, while the others are for quantifying SITA in combination with other drug substances. The summary of reported spectrometric methods indicating the basic principle,  $\lambda_{max}$ , solvent and limit of detection (LOD) is shown in table 1.<sup>2, 9-32</sup>

**Table 1:** Summary of spectrometric methods for the analysis of SITA either alone or in combination with other drugs like Gliclazide (GLZ), Metformin (MET), Simvastatin (SIMV).

Compounds	Methods	$\lambda_{max}$ (nm)	Solvent/Procedure	LOD ( $\mu\text{g}/\text{mL}$ )	Ref.
SITA	First order derivative	275	Water	2.38	2
SITA	Method A Method B	267 275	Methanol & Water	0.09636 0.4125	9
SITA	-	267	Methanol	6.03	10
SITA	Method A (Abs. ratio) Method B (AUC)	267 261- 270	Water	11.85 14.05	11
SITA	Extractive Visible Method A-BTB Visible Method B- BCG	412 419	Methanol	-	12
SITA	-	267	Water	0.2269	13
SITA	Method for dissolution study	267	0.01M HCl/ USP apparatus 1(basket)	-	14
SITA	Zero order, First order, Second order derivative	267, 213, 276	Methanol	6.03, 4.14, 3.43	15
SITA	-	267	0.1N HCl	0.139	16
SITA	Visible method	430	Water/ Primary amino group of SITA with acetyl acetone & HCHO gives yellow color.	1.947	17



**Figure 3:** Various diluents used for the analysis of Sitaagliptin

## HPLC

### Biological samples

Anil Dubala et al. developed a method in human plasma for estimation of SITA for application in pharmacokinetic studies. The mobile phase used in this method is a mixture of 0.5%v/v triethylamine and ACN (77:23 v/v). They performed the validation studies and the LOD and LOQ was found to be 1ng/mL and 10 ng/mL.<sup>33</sup> Arun M.Kashid et al. reported a method in human plasma for estimation of SITA from dosage form during pharmacokinetic study. ACN: Methanol: Buffer (2:3:5 v/v) is used as mobile phase. The LLOQ was found to be 25  $\mu\text{g}/\text{mL}$ .<sup>34</sup>

### Pharmaceutical samples

Analytical methods for the determination of SITA in bulk drug and pharmaceutical dosage forms using HPLC are shown in table 2.<sup>35-50</sup>

SITA	Visible method	338	Water/ SITA with OPA, NAC & borate buffer gives chromogen.	1.1	18
SITA, GLZ	Multi-wavelength method	267, 226	Methanol & Water	0.2269,0.31	19
SITA, MET	Multi-wavelength method	267, 231	Water	0.954, 1.2	20
SITA, MET	Vierodt's method Second order derivative	267, 232 249, 278	Water	0.825,0.086 0.106,0.630	21
SITA, SIMV	Vierodt's method	267, 238	Methanol: Water (90:10)	-	22
SITA. MET	Vierodt's method	266, 232	Water	0.01693, 0.2638	23
SITA, MET	Absorption maxima method Area under curve method	266, 232. 244-279, 222-240.	Water	-	24
SITA, SIMV	Q-Absorbance ratio method	267, 239	Methanol: Water (40:60)	0.0012, 0.091	25
SITA, GLZ	Q-Absorbance ratio method	267, 226	Methanol & Water	-	26
SITA, SIMV	Q-Absorbance ratio method	267, 237	Methanol: Water (60:40)	14.05,0.78	27
SITA, SIMV	-	-	Buffer: Methanol (25:75)	0.964, 3.6	28
SITA, MET	Vierodt's method	273, 237	Methanol: Water (40:60)	0.3784, 0.0196	29
SITA	Spectrofluorimetric method	267, 297	Water	0.07878	30
SITA, MET	Spectrofluorimetric, Zero, First.	263, 475 267, 275&246	Methanol	0.05, 2.88, 14.92,0.24	31
SITA	Spectrofluorimetric method	270, 300	Deionized water	0.00531	32

**Table 2:** Reported HPLC methods for determination of SITA either alone or in combination with other drugs in pharmaceutical dosage forms.

Study aim	Column	Mobile phase	Detection	$\lambda_{max}$ (nm)	Flow rate mL/min	LOD $\mu\text{g/mL}$	Ref.
Content analysis & Dissolution study	-	Buffer:ACN (60:40) v/v	UV	257	1	-	35
In bulk and Tablet dosage form	ZorbaxEclipse XDB C18(150 x 4.6mm x 5 $\mu$ )	0.01M KH <sub>2</sub> PO <sub>4</sub> : Methanol (50:50)v/v	PDA	267	0.7	0.6	36
Chiral separation of SITA enantiomer	Chiralpak AD-H (250 x 4.6mm x 5 $\mu$ )	Heptane: Ethanol: Diethylamine (35:65:0.1) v/v	UV	265	1.0	0.15	37
Simultaneous estimation with MET	Hypersil BDS C18 (150 x 4.6mm x 5 $\mu$ )	PDOP: Methanol (50:50) v/v	PDA	260	1	-	38
Simultaneous with MET	Xterra Symmetry C8 (100 x 4.6mm x 5 $\mu$ )	Methanol: ACN: PB(20:35:45) v/v	UV	254	1.0	0.24, 0.42	39
Simultaneous with MET	Hypersil BDS C18 (250 x 4.0mm x 5 $\mu$ )	PB:ACN (60:40) v/v	UV	260	1.0	-	40
Simultaneous with SIMV	Inertsil ODS-3 C18 (75mm x 4.6mm x 5 $\mu$ )	0.05M AAB: ACN (60:40)v/v	UV	253	1.0	-	41
Simultaneous with MET in bulk & dosage form	Inertsil ODS (250 x 4.6mm x 5 $\mu$ )	Ammonium dihydrogen PB: ACN (74:26) v/v	UV	246	1.0	0.06, 0.13	42
Simultaneous with MET & Atorvastatin	Hypersil GOLD (150 x 4.6mm x 5 $\mu$ )	Buffer: Methanol (30:70)v/v	UV	254	1	0.82, 0.4, 0.09	43

Simultaneous with MET in bulk & tablet dosage form	Hypersil BDS C18 (100 x 4.6mm x 5 $\mu$ )	PDOP: Methanol (50:50)v/v	UV	215	1.0	0.07, 0.08	44
Simultaneous with SIMV in bulk & tablet dosage form	Nucleosil C18 (150 x 4.6mm x 5 $\mu$ )	PB:ACN (30:70) v/v	UV	254	1.0	1.305, 0.257	45
Simultaneous with MET in tablet dosage form	Symmetry C18 (250 x 4.6mm x 5 $\mu$ )	Methanol:PB (60:40) v/v	UV	258	1.0	3.0, 2.9	46
Simultaneous with MET	Phenomenex C18 (250 x 4.6mm x 5 $\mu$ )	0.02M KH <sub>2</sub> PO <sub>4</sub> :ACN (55:45)v/v	UV	252	1.0	1.24, 2.64	47
Simultaneous with SIMV in bulk & tablet dosage form	Hypersil C18 (150 x 4.6mm x 5 $\mu$ )	Buffer:ACN (30:70) v/v	UV	254	1.0	2.95, 3.02	48
Simultaneous with SIMV in bulk & tablet dosage form	Hi-Q Sil C18 (250 x 4.6mm x 5 $\mu$ )	ACN: Methanol: 10 mM PB (65:25:10) v/v	PDA	250	1.2	51.50, 6.80	49
Simultaneous with SIMV	Agilent C8 (250 x 4.6mm x 5 $\mu$ )	Methanol: Water (25:75) v/v	PDA	266	1.0	-	50

### Stability indicating method

In the literature eight stability indicating methods were reported. Table 3 shows the summary of the methods.<sup>51-58</sup>

**Table 3:** Summary of stability indicating HPLC methods for determination of SITA either alone or in combination with other drugs.

Study aim	Stress condition	Detection	Type of study	Ref.
SITA in bulk drug & tablets	Acid, alkali, oxidation, thermal, light	DAD- 260nm	Separation in presence of degradation product.	51
SITA in tablets	Acid, alkali, oxidation, thermal, light	PDA-264nm	Isolation, characterization of degradation products.	52
SITA simultaneous with MET	Alkali	UV-220nm	Separation of SITA & MET in presence of SITA alkaline degradation product.	53
SITA simultaneous with MET in tablet	-	UV-245	Separation of SITA & MET in presence of added impurities.	54
SITA simultaneous with SIMV in tablet	Acid, alkali, oxidation, thermal, light	UV-252	Separation of SITA & SIMV in presence of degradation products.	55
SITA simultaneous with SIMV in tablet	Acid, alkali, oxidation, thermal, light	PDA-236	Separation of SITA & SIMV in presence of degradation products.	56
SITA simultaneous with MET in bulk drug & tablet	Acid, alkali, oxidation, thermal, light	PDA-254	Separation of SITA & MET in presence of degradation product.	57
SITA simultaneous with SIMV in tablet	Acid, alkali, oxidation, thermal, light	PDA-253	Separation of SITA & SIMV in presence of degradation products.	58

### LC-MS

Sitadevi et al. developed a method for comparison of conventional and supported liquid extraction methods for the determination of SITA and Simvastatin (SIMV) in rat plasma by LC-ESI-MS/MS. Three extraction methods were compared for their efficiency to analyze SITA and SIMV including LLE, SPE and supported liquid extraction (SLE). Venlafaxine hydrochloride was used as internal standard (IS). Chromatographic separation was performed on an Oyster ODS3 (4.6 mm x 50 mm, 3  $\mu$ m). Comparison of recoveries of analytes revealed that SLE was the best

extraction method. The detection was facilitated with ion-trap mass spectrometer by multiple reactions monitoring (MRM) in a positive ion mode with ESI. The transitions monitored were m/z 441.1 $\rightarrow$ 325.2 for SIMV, 408.2 $\rightarrow$ 235.1 for SITA and 278.1 $\rightarrow$ 260.1 for the IS. The authors demonstrated that the efficient SLE rendered the method useful in high-throughput analysis. The method was applied to pharmacokinetic study in rats.<sup>59</sup>

Rapid LC-ESI-MS-MS method was discussed by Samanthula Gananadhamu et al. for the simultaneous determination of SITA and Pioglitazone (PIO) in rat plasma



and its application to pharmacokinetic study. The method was based on HPLC separation on the reverse phase phenomenex synergy C18 column (36 mm length, 4.6 mm internal diameter and 4  $\mu$ m particle size) at a temperature of 40°C using a binary gradient mobile phase consisting of methanol and 2M ammonium acetate buffer pH adjusted to 4.5 with acetic acid, at a flow rate of 1 mL/min. Tolbutamide was used as an internal standard (IS). Detection of analytes was achieved with LC/MS/MS system in MRM mode. The method was validated over concentration range of 10.98-2091.77 ng/mL for SITA and 8.25 – 1571.63 ng/mL for PIO. Within batch and between batch recovery for SITA was found within 96.9 – 100.3 and for PIO 100.0 – 104.3. The authors successfully applied this method to monitor the pharmacokinetics profile of both SITA and PIO on simultaneous oral administration to rats. This method can be applicable for pharmacokinetic drug-drug interaction studies.<sup>60</sup>

A simple and sensitive LC/MS/MS method was developed by Bhonde et al. for simultaneous determination of Metformin (MET) and SITA in human plasma using Metformin-d6 HCl and Sitagliptin-d4 HCl as IS respectively. After ACN- induced protein precipitation of plasma samples, MET, SITA and IS were chromatographed on reverse phase C18 (50mm x 4.6mm i.d., 5 $\mu$ m) analytical column. Quantitation was performed on a triple quadrupole mass spectrometer employing ESI technique and operating in MRM and positive ion mode. The run time was 2.0 min and calibration curves were linear over the concentration range of 25-3000 ng/mL for MET and 5-800 ng/mL for SITA. The recoveries obtained for MET and its IS was  $\geq$ 39% and SITA and its IS was  $\geq$ 64%. Inter-batch and intra-batch coefficient of variation across five validation runs (LLOQ, LOC, MQC1, MQC and HQC) was less than 7.5% for both MET and SITA. The authors reported that the proposed method is suitable for measuring accurate plasma concentration in bioequivalence study and therapeutic drug monitoring, following combined administration.<sup>61</sup>

For analysis of the anti-diabetic drugs MET and SITA and the renal clearance marker creatinine in the same human dried blood spot (DBS) extract two liquid chromatography methods employing HPLC/UV and LC-ESI-MS/MS have been developed and validated. An accurate volume of 40 $\mu$ L blood was spotted on a sampling paper which was extracted using 90% ACN with 10% formic acid. The validated ranges were 0.2-5 $\mu$ g/mL for MET, 1.5-15 $\mu$ g/mL for creatinine and 3-500ng/mL for SITA. Since drug analysis in DBS determines whole blood concentrations as opposed to the typically used plasma levels the partition ratios between human plasma and blood cells, c(P)/c(BC), were elucidated in-vitro to gain insight into the significance of blood cells as compartment of distribution for both compounds. The c(P)/c(BC) was found to be 4.65 $\pm$ 0.73 for MET and 5.58 $\pm$ 0.98 for SITA. The analytical methods were successfully applied to authentic capillary blood samples from two diabetic patients regularly taking a combination of MET and SITA. Both samples revealed

analyte trough concentrations well above the lower limit of quantification of the respective compounds. This study offered a methodological basis for the DBS analysis of MET and SITA in relation to the patient's creatinine concentration.<sup>62</sup>

Burugula et al. developed a simple, rapid and sensitive LC-MS/MS assay method for simultaneous quantification of SITA and SIMV in human plasma. Carbamazepine was used as an IS. The analytes and IS were extracted from the human plasma by LLE technique. The reconstituted samples were chromatographed on an Alltima HP C18 column using an isocratic solvent mixture [acetonitrile-5 mM ammonium acetate (pH 4.5), 85:15 (v/v)] at a flow rate of 1.0 mL/min. The calibration curves obtained were linear ( $r^2 \geq 0.99$ ) over the concentration range of 0.10-501 and 0.05-105 ng/mL for SITA and SIMV, respectively. The results of the intra-day and inter-day precision and accuracy studies were well within the acceptable limits. Both the analytes were found to be stable in a battery of stability studies. A run time of 3.0 min for each sample made it possible to analyze more than 300 plasma samples per day. The developed assay was successfully applied to a pharmacokinetic study in human volunteers.<sup>63</sup>

#### HPTLC

Rathod Sonali et al. reported a HPTLC method for simultaneous estimation of SITA and SIMV. A precoated silica gel 60 F254 (0.2 mm thickness) on aluminium sheets was employed to carry out the separation. Chloroform: methanol in the ratio of 8:2 v/v was used as mobile phase. The developing chamber was run up to 8 cm. The R<sub>f</sub> values were found to be 0.13 and 0.75 for SITA and SIMV. The plate was scanned and quantified at 217 nm. The linear detector response was observed between 2000 ng/spot to 7000 ng/spot and 250 ng/spot to 750 ng/spot for SITA and SIMV. The LOD and LOQ were found to be 660, 2000 ng/spot and 50, 150 ng/spot, respectively for SITA and SIMV. The Average recovery was found to be 92.80 % and 98.01 % for SITA and SIMV. The proposed method was specific and accurate.<sup>64</sup>

A simple, sensitive and accurate HPTLC method was developed by Chirag Patel et al. for simultaneous determination of MET and SITA in marketed formulation. The mobile phase used was 1% w/v ammonium acetate in methanol. The detection of spots was carried out densitometrically using a UV detector at 257 nm in absorbance mode. The R<sub>f</sub> value for MET and SITA was found to be 0.43 $\pm$ 0.009 and 0.60 $\pm$ 0.013. The calibration curve was found to be linear between 600 to 2000 and 1000 to 7000 ng/spot for MET and SITA respectively. The limits of detection and quantitation were found to be 76.257 and 231.083 ng/spot, respectively for MET and 65.080 and 197.212 ng/spot, respectively for SITA. The authors demonstrated that the proposed HPTLC method was highly reproducible and reliable.<sup>65</sup>



Malathi et al. proposed a rapid and accurate HPTLC method for the simultaneous estimation of SITA and SIMV in pharmaceutical formulation. They have used Merck TLC aluminum sheets of silica gel G60 F254 with the thickness of 200  $\mu\text{m}$  to carry out the separation. Ethyl acetate: toluene: methanol (6:2:2 v/v/v), was used as mobile phase. Analysis of the compounds was carried out by densitometry in the absorbance mode at 254 nm. The mobile phase gave the well defined peak at the Rf value of 0.6 +0.02 and 0.3+0.02 for SITA and SIMV respectively. The linear regression analysis data for the calibration plots showed good linear relationship with the regression coefficient ( $r^2$ ) of 0.9999 and 0.9994 for the SITA and SIMV respectively, in the concentration range of 2-6  $\mu\text{g}/\text{spot}$ , 0.2-0.6  $\mu\text{g}/\text{spot}$  respectively. The LOD and LOQ were 5, 20 ng/spot, 50,100 ng/spot respectively for SITA and SIMV. The authors recommended this method for routine analysis of SITA and SIMV in combined formulations.<sup>66</sup>

### UPLC

A novel approach was used by Chellu Malleswara rao et al. to develop accurate and precise stability indicating UPLC method for the simultaneous determination of SITA and MET in pharmaceutical dosage forms. The chromatographic separation was achieved on Aquity UPLC BEH C8 100 x 2.1 mm, 1.7  $\mu\text{m}$ , column using a buffer consisting of 10 mM potassium dihydrogen phosphate and 2 mM hexane-1-sulfonic acid sodium salt (pH adjusted to 5.50 with diluted phosphoric acid) and ACN as organic solvent in a gradient program. The flow rate was 0.2 mL/min and the detection wavelength was 210 nm. The LOD for SITA and MET was 0.2 and 0.06  $\mu\text{g}/\text{mL}$ , respectively. The LOQ for SITA and MET was 0.7 and 0.2  $\mu\text{g}/\text{mL}$ , respectively.<sup>67</sup>

A rapid and consistent UPLC method for simultaneous estimation of Sitagliptin and Simvastatin in combined dosage forms was reported. UPLC separation was achieved on a Symmetry C18 (2.1 x 100mm, 1.7 $\mu\text{m}$ , Make: BEH) or equivalent under an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (30%) whose pH was adjusted to 4.0 by using TEA & ACN (70%). The flow rate was monitored at 0.4 mL per min. The wavelength was selected for the detection was 213 nm. The run time was 3min. The retention time found for the drugs SITA and SIMV were 0.509 min. & 1.623 min. respectively. The linearity was established in the range of 500 to 900 ppm for the drug SITA & 200 to 360ppm for the drug SIMV. The LOD for the drugs SITA & SIMV were found to be 0.18  $\mu\text{g}/\text{mL}$  & 0.17 $\mu\text{g}/\text{mL}$  respectively. The LOQ for the drugs SITA & SIMV were found to be 0.61 $\mu\text{g}/\text{mL}$  & 0.56 $\mu\text{g}/\text{mL}$  respectively. The proposed method was adequate sensitive, reproducible, and specific for the determination of SITA and SIMV in tablet dosage form.<sup>68</sup>

Kavitha et al. developed a reverse phase UPLC method for the simultaneous estimation of SITA and SIMV. The method was carried out on waters Aquity BEH C18 100 x

2.1 mm, 1.7  $\mu\text{m}$ , UPLC column and a mobile phase consisting of water: ACN (30:70) adjusted to pH 4.0 with orthophosphoric acid. The flow rate was 0.35 mL/min and the effluent was monitored at 236 nm. The authors recommended that the method can be used for routine quality control analysis due to its simplicity and accuracy.<sup>69</sup>

### GC-MS

A simple and rapid GC-MS method for the determination of SITA in human urine was developed. SITA was derivatized by N-methyl-trimethylsilyltrifluoroacetamide prior to GC-MS analysis and converted to its N-TMS amine derivative. It was extracted from urine by using carbonate buffer (pH 9.0) and ether. LOQ was found to be 50 ng/mL. The calibration curve was linear in the range of 50–600 ng/mL with a coefficient of determination ( $r^2$ ) above 0.997. The intra-day and inter-day precisions were less than 8.76%, and the intra-day and inter-day accuracies were found between 0.83 and 4.53%. The method was successfully applied to urine samples obtained from diabetic patients.<sup>70</sup>

### Capillary Electrophoresis (CE)

Tamàs Sohajda et al. developed an aqueous capillary electrophoretic method developed for chiral analysis of the SITA. The acid–base profiling of the analyte was carried out using both capillary electrophoresis and nuclear magnetic resonance pH titrations. The apparent complex stability and chiral separation properties were investigated with 30 different cyclodextrins under acidic conditions. The effect of concentration and pH of the background electrolyte (BGE), temperature of the capillary, and the type and concentration of the chiral selector on the enantiomer resolution were thoroughly investigated. The effects of dual cyclodextrin systems on separation were also extensively studied. Complete separation of racemic SITA with good resolution (RS52.24) was achieved within a short time (15 min) with optimized parameters (101C, pH54.4, 40mM phosphate buffer) of a sulfobutylether-b-cyclodextrin (averaged degree of substitution  $\sim 4$ ) and native b-cyclodextrin dual system. The averaged stoichiometry of the inclusion complex was determined using the Job plot method with both  $^1\text{H}$  and  $^{19}\text{F}$  NMR experiments and resulted in a 1:1 complex. The structure of the inclusion complex was elucidated using 2-D ROESY NMR experiments.<sup>71</sup>

A novel and simple capillary zone electrophoresis CZE method was reported by Mohamed Salim et al. for the simultaneous determination of SITA and MET in pharmaceutical preparations. Separation was carried out in fused silica capillary (50.0 cm total length and 43.0 cm effective length, 49  $\mu\text{m}$  i.d.) by applying a potential of 15 KV (positive polarity) and a running buffer containing 60 mM phosphate buffer at pH 4.0 with UV detection at 203 nm. The samples were injected hydrodynamically for 3 s at 0.5 psi and the temperature of the capillary cartridge was kept at 25 °C. Phenformin was used as IS. The



method showed good linearity in the ranges of 10–100 µg/mL and 50–500 µg/mL with limits of detection of 0.49, 2.11 µg/mL and limits of quantification of 1.48, 6.39 µg/mL for SITA and MET, respectively. The proposed method was successfully applied for the analysis both drugs in their synthetic mixtures and co-formulated tablets without interfering peaks due to the excipients present in the pharmaceutical tablets. The method was further extended to the in-vitro determination of the two drugs in spiked human plasma. The estimated amounts of SITA/MET were almost identical with the certified values, and their percentage relative standard deviation values (% R.S.D.) were found to be 1.50% (n = 3).<sup>72</sup> The authors compared the results with a reference method<sup>31</sup> reported in the literature and no significant difference was found statistically.

## CONCLUSION

This review aimed at focusing various analytical methods reported for the assay of SITA. A broad range of techniques are available for the estimation of SITA in biological samples and pharmaceutical dosage forms. The analysis of published data revealed that spectrometric methods are the simple and economical methods for estimation of SITA in pharmaceutical formulations. For analysis of SITA, HPLC-UV provides accurate results and low cost compared to advanced detection techniques. HPLC with PDA detection was extensively used for the development of stability indicating assay methods for separation and quantification of SITA in presence of degradation products. This survey also highlights the hyphenated techniques that incorporate the efficient separation of metabolites of SITA using HPLC with sensitive MS detection has become an indispensable tool for quantification of SITA in biological fluids and pharmacokinetic studies. When compared with chromatographic methods the application of capillary electrophoresis is considered as an alternative technique for separation of SITA. This review includes the complete detail of analytical methods available on SITA which will be supportive for further research on the drug.

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