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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC ASSAY METHOD FOR ESTIMATION OF SITAGLIPTIN PHOSPHATE

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ABSTRACT: An Accurate, sensitive, precise and stability indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the estimation of SITA-P. The method was developed using TLC aluminium plates precoated with silica gel 60F254 as the stationary phase using ethyl acetate: methanol: formic acid (8.5:1:0.5 v/v/v) as mobile phase. Densitometric analysis of SITA-P was carried out in the absorbance mode at 265 nm. The retention factor for SITA-P was found to be 0.50 ± 0.04. Linearity was found to be 500 - 2500 ng/band for SITA-P. The method was found to be accurate, precise, and robust according to acceptance criteria. The limit of detection (LOD) and (LOQ) was found to be 124.36 ng/band and 376.87 ng/band for SITA-P respectively. The drug was subjected to stress condition. The method was validated for different parameters as per the International Conference for Harmonization guidelines (ICH). This HPTLC method can be used for the determination for the stability indicating assay methods for bulk drug and its formulations.

INTRODUCTION: SITA-P previously identified as MK-0431 and marketed as the phosphate salt under the trade name Januvia) is an oral anti-hyperglycemic (antidiabetic drug) of the dipeptidyl peptidase - 4 (DPP - 4) inhibitor class. It was developed, and is marketed, by Merck and Co. This enzyme-inhibiting drug is used either alone or in combination with other oral antihyperglycemic agents (such as metformin or a thiazolidinedione) for treatment of diabetes mellitus type 2 [12]. The drug works to competitively inhibit a protein / enzyme, dipeptidyl peptidase 4 (DPP-4), that results in an increased amount of active incretins (GLP-1 and GIP), reduced amount of release of glucagon (diminishes its release) and increased release of insulin [13]. SITA - P has been shown to lower haemoglobin (a blood pigment that carries oxygen) HbA1c level by about 0.7% points versus placebo. It is slightly less active than Metformin when used as a monotherapy. It does not cause weight gain and has less hypoglycemia compared to sulfonylureas. SITA-P is mentioned as a second line drug (in combination with other drugs) after the blend of diet/exercise and metformin fails.

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Various functional groups in structure of the drug makes it susceptible to degradation due to chemical reactivities of these groups under hydrolytic, oxidative, and photolytic environments, which will ultimately produce varied impurities or degradation products.

Hence, the present study is undertaken to conduct ICH prescribed systematic forced degradation study on SITA-P to identify its potential degradation products and to develop and validate a SIAM by using RP-HPLC and HPTLC for accurate determination of SITA-P in the presence of its impurities and degradation products. The aim is to provide new and prove the present data on impurities and determination methods. Chemical structure of SITA-P as shown in **Fig. 1.**

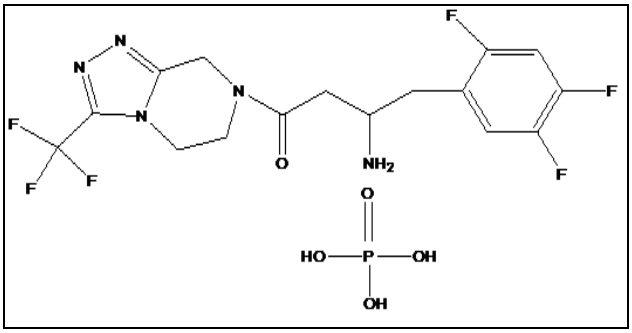

FIG. 1: SITA-P

MATERIALS AND METHODS:

Material: Pharmaceutical grade Sitagliptin phosphate (SITA-P) working standard was kindly supplied by Mylan Pvt. Ltd., (Nashik, India). The pharmaceutical dosage form used in this study was Januvia tablets (MSD Pharmaceuticals Ltd., Italy) labelled to contain 50 mg of SITA-P was procured from the local market. Toluene (AR grade), Ethyl acetate and Formic acid (AR grade), Methanol (HPLC grade) was purchased from Merck specialties Pvt. Ltd., (Mumbai, India).

Equipment: Camag HPTLC system consisting Linomat 5 applicator, camag TLC scanner 3 and Win CATS software V-1.4.2 was used for chromatographic separation. Spotting of samples was done by using Hamilton microliter syringe.

Methods:

Preparation of Standard Stock Solution: Working standard stock solution was prepared by dissolving 50 mg of drug in 10 ml methanol to get concentration of 5 mg/ml from which 1 ml was further diluted to 10 ml to get standard stock solution of 500 ng/µl.

Validation of Analytical Method:

Linearity: Standard stock solution of SITA-P (500 ng μl^{-1}) was applied by on the TLC plates in range of 1, 2, 3, 4 and 5 µl using semiautomatic spotter under nitrogen stream. TLC plates were dried, developed and densitometrically analyzed as described earlier. The linear regression data for calibration curves (n = 6) showed good linear relationship over a concentration range of 500 - 2500 ng/band **Fig. 2**.

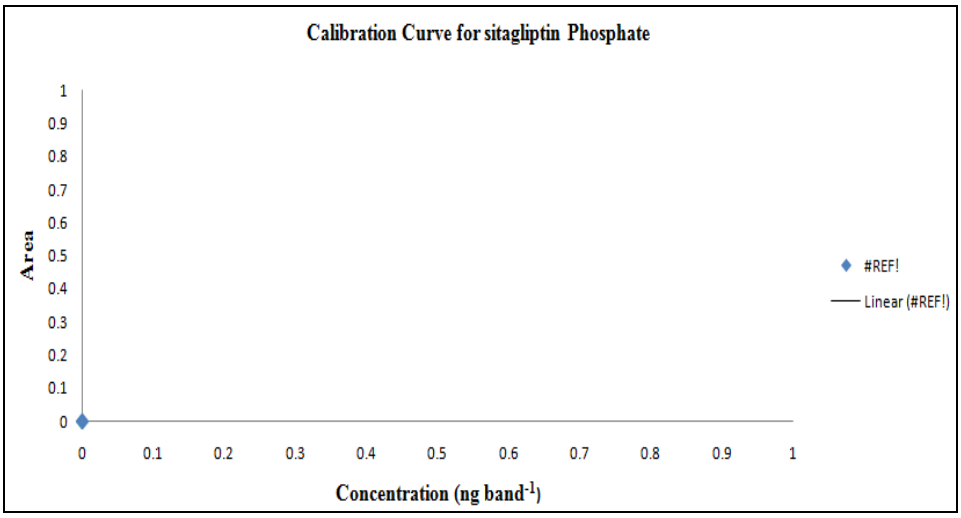

FIG. 2: CALIBRATION CURVE FOR SITA-P

Precision: The precision of the method was demonstrated by intra-day and inter-day variation studies. Intra-day precision was determined by analyzing the standard solutions of SITA-P (1000, 1500, 2000 ng/band) in linearity range at three different time intervals on same day.

Inter-day precision was determined by analyzing the standard solutions of SITA-P (1000, 1500, 2000 ng/band) in linearity range at three consecutive days. The percentage RSD was calculated. The result obtained for SITA-Pare shown in **Table 1** and **2.**

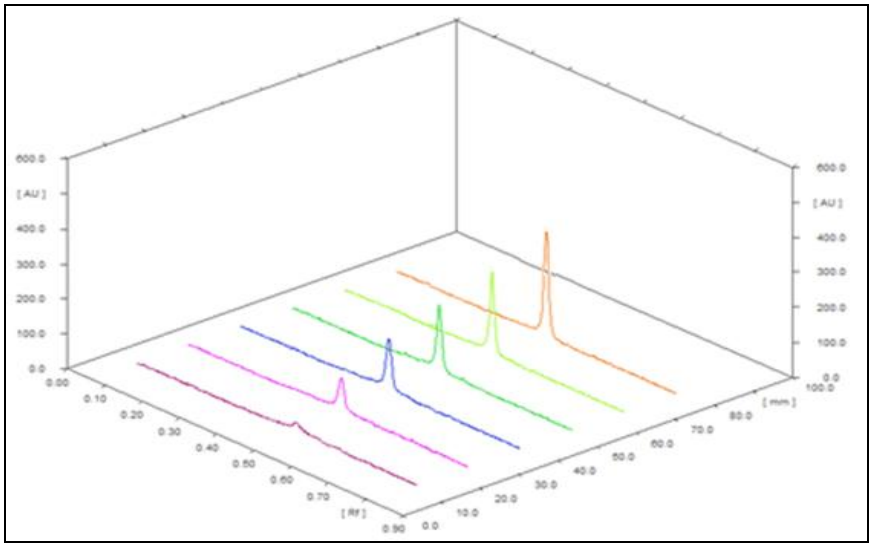

FIG 3: 3D SPECTRA OF LINEARITY OF SITA-P (500 - 2500 ng/band)

TABLE 1: INTRA-DAY PRECISION STUDIES FOR SITA - P

Concentration (ng/band)	Average Area	Amount recovered (ng/band)	S. D.	% RSD
1000	2500.09	1000.44	15.70	1.57
1500	3270.71	1503.46	17.10	1.13
2000	4027.65	1997.54	14.01	0.70

TABLE 2: INTER-DAY PRECISION STUDIES FOR SITA - P

Concentration (ng/band)	Average Area	Amount recovered (ng/band)	S. D.	% RSD
1000	2514.33	1009.74	18.33	1.81
1500	3277.65	1507.99	15.83	1.04
2000	4021.75	1993.70	14.85	0.74

Accuracy: Accuracy was determined at three different level 80%, 100% and 120% of the target concentration 1000 ng/band of SITA-P in triplicate. The results obtained for SITA-P are shown in **Table 3**.

TABLE 3: RECOVERY STUDIES FOR SITA-P

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	S. D.	% R.S.D.*
SITA-P	1000	800	1796.49	99.80	13.95	0.77
	1000	1000	2001.70	100.08	9.38	0.46
	1000	1200	2203.96	100.17	15.49	0.69

Limit of Detection and Limit of Quantification: LOD and LOQ of the drug were derived by calculating the signal-to-noise (*i.e.* 3.3 for LOD and 10 for LOQ) ratio using the following equations as per ICH guideline.

$$LOD = 3.3 \times \sigma/S \quad LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response. S = slope of the calibration curve.

Robustness: The effect of small, deliberate variation of the analytical conditions on the peak areas of the drugs was examined. Change in chamber saturation time and change in volume of mobile phase were investigated and % RSD was calculated.

Stress Degradation Studies:

Densitogram of SITA-P: Three micro-litres of standard solution of SITA-P (1500 ng μl^{-1}) was applied on pre-washed and activated plate under nitrogen stream using semiautomatic spotter. They were developed at constant temperature in a Camag twin-trough chamber previously saturated for 15 min with mixture of ethyl acetate: methanol: formic acid (8.5: 1: 0.5, v/v/v) as mobile phase. The plates were removed from the chamber and dried in air. Densitometric measurements were performed at 265 nm with Camag TLC Scanner 3 using win CAT software version 1.4.2. using deuterium lamp as source of radiation. The retention factor of SITA-P was found to be: 0.50 ± 0.04 **Fig. 4.**

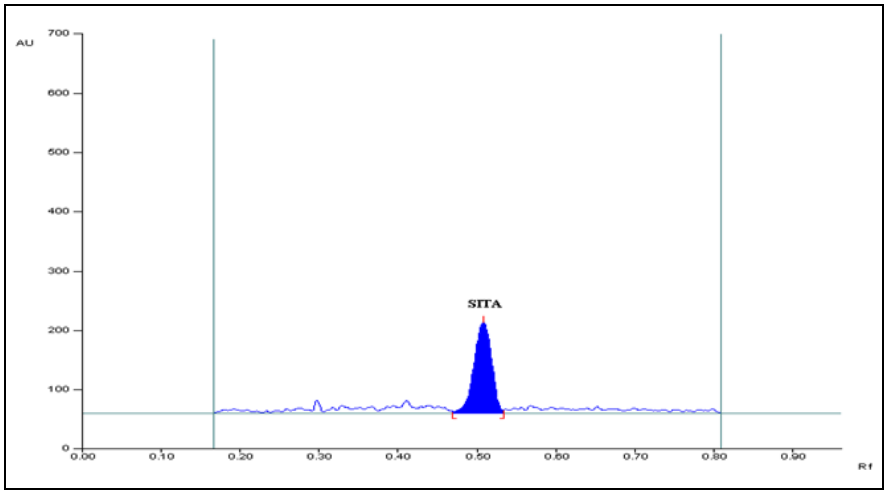

FIG. 4: DENSITOGRAM OF WORKING STANDARD SOLUTIONS OF SITA - P (1500 ng/band)

Stress Degradation in Acidic Condition: Initially degradation study perform by keeping drug in 0.1N, 0.5N, 1N, 1.5N hydrochloric acid solution and kept at RT for 3 hours along these solutions degradation was found at 1N hydrochloric acid. 1 mL working standard solution of SITA-P (5000 ng μL^{-1}) was mixed with 1 mL of 1 N HCl and 8 mL of methanol. Solution was kept at room temperature for 1 h. The 3 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions **Fig. 5.**

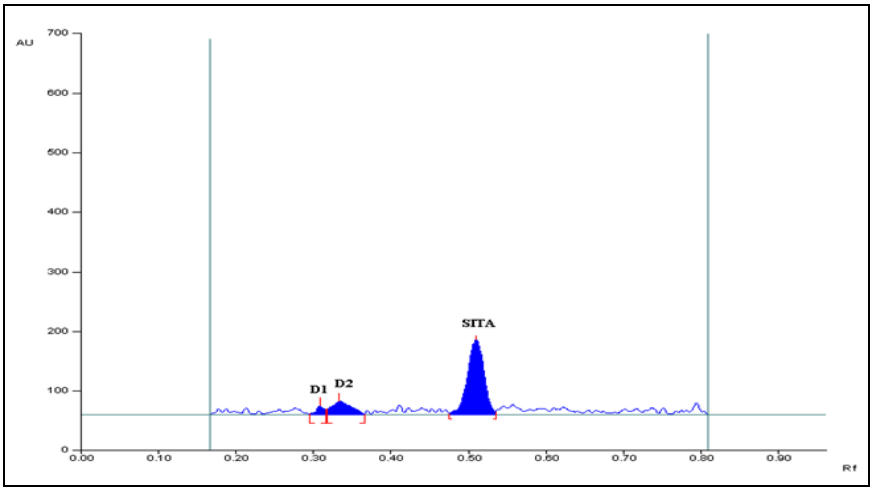

FIG. 5: DENSITOGRAM OF SITA - P AND ITS DEGRADATION PRODUCTS IN 1N HCl AT ROOM TEMPERATURE FOR 1 hr

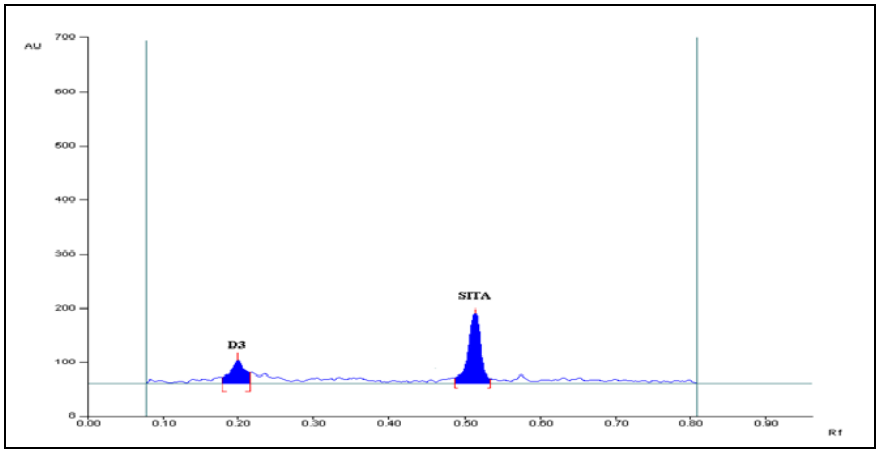

FIG. 6: DENSITOGRAM OF SITA - P AND ITS DEGRADATION PRODUCTS IN 1N NaOH AT ROOM TEMPERATURE FOR 1 hr

Stress Degradation in Basic Condition: Initially degradation study perform by keeping drug in 0.1N, 1N, 1.5N, 2N, 2.5N, 3N sodium hydroxide solution kept at RT for 3 hours along these solutions degradation was found in 1N sodium hydroxide. Therefore 1 mL working standard solution of SITA-P (5000 ng μL^{-1}), 1 ml of 1 N NaOH and 8 mL of methanol was added. Solution was kept at room temperature for 2 h. 3 µL volume of resulting solution was applied on TLC plate and developed under optimized chromatographic conditions **Fig. 6.**

Stress Degradation in Photolytic Condition: The photochemical stability of the drug was studied by exposing the drug sample to UV light up to 200 watt hour square $meter^{-1}$. Sample was weighed, dissolved in methanol to get concentration of 500 ng μL^{-1}. 3 µL of the resulting solution was applied to HPTLC **Fig.7.**

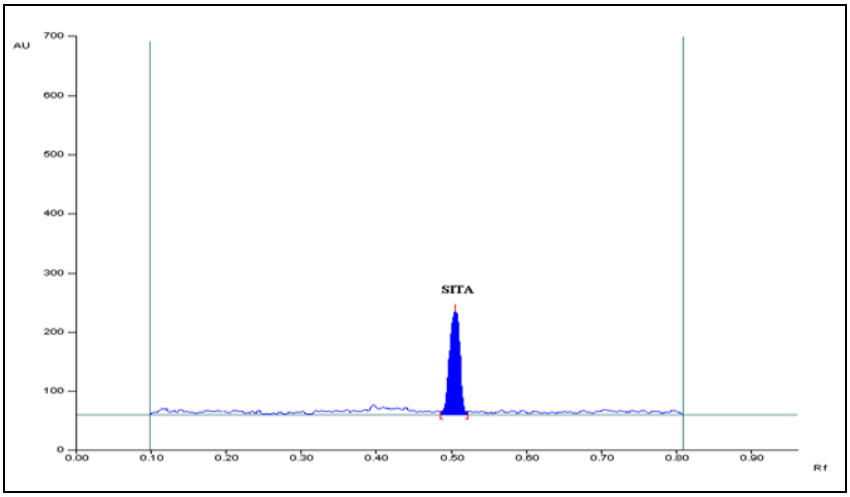

FIG. 7: DENSITOGRAM OF SITA – P AND ITS DEGRADATION PRODUCTS IN UV LIGHT UP TO 200 watt hr SQUARE METER

Stress Degradation in Oxidative Condition: 1 mL working standard solution of SITA-P (5000 ng μL^{-1}) was mixed with 1 mL of 6 % solution of H_2O_2 and the volume was made with solvent. Solution was kept at room temperature for 30 min. The 6 µL of resulting solution was applied on TLC plate and developed under optimized chromatographic condition **Fig. 8.**

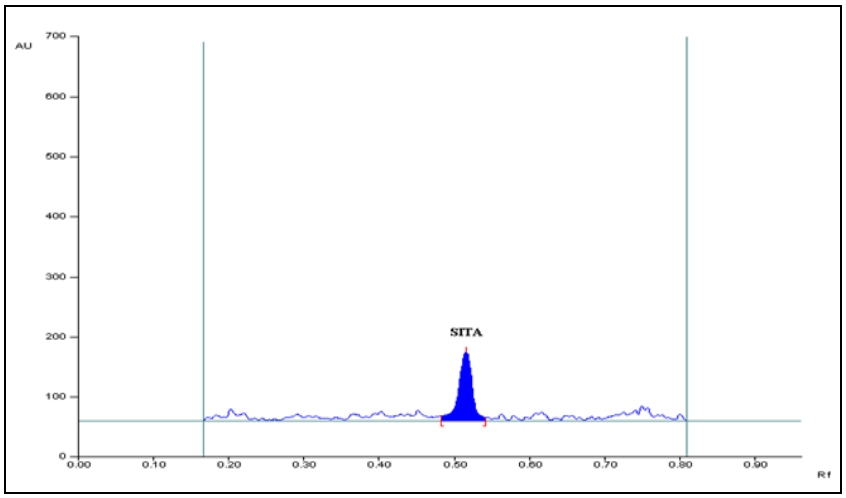

FIG. 8: DENSITOGRAM OF SITA - P AND ITS DEGRADATION PRODUCTS IN 6% HYDROGEN PEROXIDE AT ROOM TEMPERATURE FOR 30 min

Stress Degradation in Thermal Condition: Dry heat studies were performed by keeping drug sample in oven at 80 °C for a period of 24 hours. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 500 ng μL^{-1}. 3 µL of the resulting solution was applied to HPTLC **Fig. 9.**

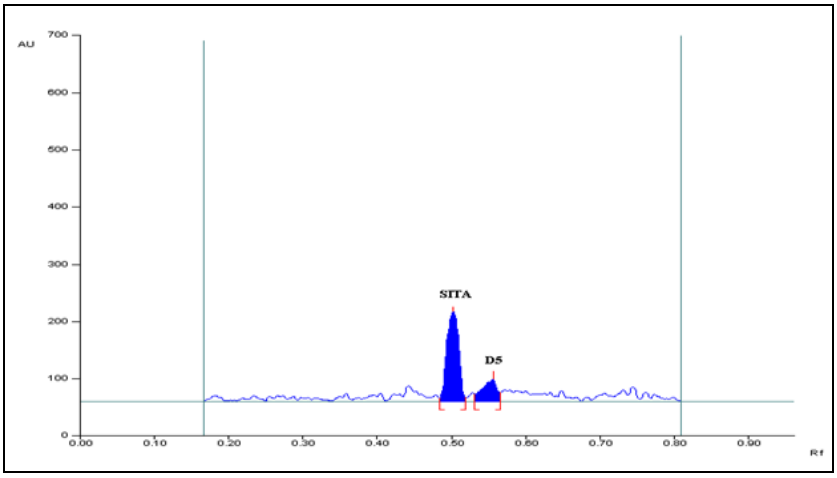

FIG. 9: DENSITOGRAM OF SITA - P AND ITS DEGRADATION PRODUCTS IN DRY HEAT 80 °C FOR 24 HOURS

RESULTS AND DISCUSSION: HPTLC method was validated as per ICH guidelines. The developed method was found to be linear within the range of 500 - 2500 ng/band with R^2 = 0.994 for SITA-P. The accuracy of method was determined at 80%, 100%, 120% level. The % recoveries were found to be 98% - 102% for SITA-P. The LOD for SITA-P was found to be 124.36 ng/band and LOQ was found to be 376.87 ng/band. The developed method was found to be precise as the % RSD values for intra-day and inter-day were found to be less than 2%. The method was also found to be robustness indicated by the % RSD values which are less than 2%. The summary of validation parameters of proposed HPTLC method is shown in **Table 4.** The stress degradation studies were carried out for the drug in acid, base, photolytic, oxidation and thermal conditions. Summary of the results of stress degradation studies of SITA - P shown in **Table 5**.

TABLE 4: SUMMARY OF VALIDATION PARAMETERS

Sr. no.	Validation Parameters	SITA-P
1.	Linearity Equation	Y=1.532x+967.4 R^2 =0.994
2.	Accuracy (% mean recovery)	99.80-100.17%
3.	LOD	124.36ng/band
4.	LOQ	376.87ng/band
5.	Precision (% RSD) Intra-day; Inter-day	1.13%; 1.19%
6.	Roubustness (% RSD)	0.66%, 0.94%

TABLE 5: SUMMARY OF STRESS DEGRADATION STUDIES FOR SITA-P

Sr. no.	Stress degradation parameter	Peak Area	% degradation	R_f of degraded of Product
1.	1500 ng/band	3268	0.00%	0.50
2.	Acidic /1 N HCl / Kept at room temperature for1h	2784	20.06%	0.35, 0.30
3.	Alkaline / 1 N NaOH / Kept at room temperature for 2h	2867	17.45%	0.20
4.	Oxidative / 6% H_2O_2 / Kept at room temperature for 30 min	2895	16.12%	…..
5.	Dry heat / 80 ºC / 24 h	2941	14.12%	0.54
6.	Photo degradation	2983	12.29%	…..

CONCLUSION: The proposed methods are precise, specific, accurate, robust and stability-indicating ones. SITA-P was found to be unstable in acidic, alkaline and oxidative condition and stable in photo and thermal conditions. Keep this medication in the container it came in, tightly closed. Store it at room temperature and away from excess heat and moisture. SITA - P can be determined in bulk and pharmaceutical formulation and percentage degradation.

ICH guidelines were followed throughout the study for method validation and stress testing.

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CONFLICT OF INTEREST: None.

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