IJBPAS, December, 2023, 12(12): 5352-5367

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SUCROFERRIC OXYHYDROXIDE

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Received 19th Oct. 2022; Revised 16th Nov. 2022; Accepted 23rd March 2023; Available online 1st Dec. 2023

https://doi.org/10.31032/IJBPAS/2023/12.12.7391

ABSTRACT

A simple, accurate and precise Stability indicating RP-HPLC method was developed and validated for estimation of Sucroferric oxyhydroxide in presence of its degradation products in API. A simple and easy UV spectrophotometric method used for developing the simple calibration curve of drug. The method based on the measurement of absorbance at 254 nm using distilled water as a solvent. The RP-HPLC method has shown adequate separation for Sucroferric oxyhydroxide from its degradation product using the kromosil C18, 150 µm X 4.6 mm, 5µm column with a mobile phase consisting of methanol: water in the ratio of (10:90 v/v) at flow rate of 1ml/min, using 25°C column oven temperature with UV detection at 254nm. The retention time for Sucroferric oxyhydroxide was 2 ± 0.4 min. The linearity was found to be in the concentration range of 20-100 µg/ml and correlation coefficient was 0.999. The limit of detection was 2.598 µg/ml and limit of quantification was 7.873 µg/ml. The % recoveries at 80%, 100% and 120% were found to be within the limit of 98-102%. Sucroferric oxyhydroxide were exposed to acidic, basic, oxidative, thermal and photolytic stress conditions for forced degradation studies according to ICH guideline. The forced degradation behaviour showed that Sucroferric oxyhydroxide was more prone to degradation.

In specific acidic and thermal conditions shows more prone to degradation than the other stress conditions. Degradation was found to be lesser extent in oxidative degradation.

Developed stability indicating method was validated as per ICH guideline for linearity, specificity, accuracy, precision and robustness for estimation of Sucroferric oxyhydroxide was found to be satisfactory.

Keywords: Sucroferric oxyhydroxide, Chronic Kidney Disease, High Performance Liquid Chromatography, Validation, Force degradation study

INTRODUCTION

Sucroferric oxyhydroxide is a novel non-calcium-based phosphate binder therapy which used in chronic kidney disease patient undergoes on dialysis for specific hyperphosphatemia.

Currently in covid19 plague coronavirus increasing world widely some studies and research found that the when covid 19 affect in all body organ which also including kidney also and they discuss that due to affecting of covid19 after recovery they might have acute kidney disease sign if proper treatment not occur, they might affect with chronic kidney disease. Chronic Kidney Diseases [CKD] is the most death causing disease. In 2020, a rapidly progressive of CKD, unexplained by diabetes and hypertension, had increase histrionically in occurrence over a few periods in several countries. In 2016 worldwide 753 million people: 417 million females & 336 million males globally affected by CKD. in 2015, around 1.2 million deaths & from 1990 around 409000 deaths are caused by chronic kidney disease **[1]**. Initially CKD is type of kidney disease which involves the gradual loss of kidney function more than 3 months **[2]**. Characteristically, chronic kidney disease [CKD] is defined by a reduction in glomerular filtration rate [GFR] and featuring structural or functional abnormalities of the renal tract. it considered by the increased urinary albumin excretion, or a combination of the two **[3]**. The kidney stages were identified by the kidney disease outcomes Quality Initiative [K/DOQI] according to those five stages of CKD are elaborate on basis of level of kidney function, defined in terms of the GFR **[4]**. stages of chronic kidney disease is categorized into five stages on the basis of its renal function and that are clarify in Table1, the higher the CKD stage, the more severe the renal insufficiency **[5]**. A Glomerular filtration rate (GFR) greater than or equal to 60 ml/min/ 1.73 m^2 is measured normal without chronic kidney disease if there is no kidney injury existing **[6]**.

Table 1: stages of chronic kidney disease

Stages of Chronic Kidney Disease (CKD)		
Stage of CKD	**Description**	**Glomerular Filtration Rate**
Stage 1	**Kidney damage with normal or increased GFR**	**>90 ml/min + proteinuria**
Stage 2	**Kidney damage with mildly decreased GFR**	**60-89 ml/min + proteinuria**
Stage 3	**Moderate reduction in GFR**	**30-59 ml/min**
Stage 4	**Severe reduction in GFR**	**13-29 ml/min**
Stage 5	**Kidney failure**	**<15 ml/min**

As CKD develops more progressive in last two stage and it practically effect on entirely body systems is unpleasantly affected by kidney damage **[2]**. In chronic kidney disease (CKD), stage 4 has unconventional kidney damage with a severe decline in the glomerular filtration rate (GFR) to 13-29 ml/min. As kidney function decline, waste products like uremic toxins accumulate in the blood which causing a severe condition. So, during that stage it necessary to need dialysis or a kidney transplant **[1]**. In that condition, kidney replacement therapy is usually required, in the form of either dialysis or a kidney transplant **[1]**.

Chronic kidney disease (CKD) is an advanced decline of mineral homeostasis due to deteriorating kidney function and that leads to irregular blood serum and tissue levels of phosphorus **[8]**. International clinical practice guidelines suggest lowering serum phosphorus levels towards the normal range in CKD patients on dialysis **[8]**. Due to hyperphosphatemia, the kidney failure or damage is a virtually universal problem and that convoyed by hypocalcaemia and decrease in blood serum levels of vitamin d. In case of lacking of treatment they lead to severe secondary hyperparathyroidism, brown tumours and generalized osteopenia they lead painful fractures. It has been basis healing therapy to dietary restrictions on phosphate **[9]**. hyperphosphatemia is simply defined as insufficient filtration of phosphate form the blood by poorly functioning kidney. It means that certain amount of phosphate remaining in the blood and form abnormally elevated levels **[10]**. The normal range of phosphorous is 4.5-5.3 mg/dl if above the 6.4 mg/dl it associated with higher risk of all-cause of mortality and if appears the lack of phosphorus in serum level it also produces the numbers of complication **[11]**. However, since dietary phosphate restriction is problem for most CKD cases which are on dialysis and frequently insufficient to continue controlled serum phosphorus levels then oral phosphate binder therapy is an essential component of hyperphosphatemia management **[12]**. Numbers of Different research study relives

that significant reduction in mortality when phosphate binders use to control serum phosphate level in CKD **[9, 13, 14]**. The phosphate binder which uses in therapy include the calcium, magnesium, and aluminium-based phosphate binder they all have properties of phosphate-binding capacity **[15]**. According to UK treatment guideline the major support therapy is calcium-based phosphate binder therapy which continue from many years, that therapy suggested as a first-line therapy some of drug therapy are as such in below table **[16]**. In some case calcium-based binders led increase the risk of hypercalcaemia and calcification. So, that non-calcium-based phosphate binders are need to developed in recent year **[17]**.

Sucroferric oxyhydroxide is the oral, non-calcium-based phosphate binder which use in treatment of HYPERPHOSPHATEMIA in CHRONIC KIDENEY DISEASE patient undergoing dialysis specific haemodialysis or peritoneal dialysis in form of chewable tablet. Sucroferric oxyhydroxide [SOH] structure is explained in as followed **(Figure 1)**. SOH is containing mainly polynuclear iron (III)-oxyhydroxide, sucrose and starch in that carbohydrate shell is stabilised the iron (III)-oxyhydroxide core to preserve the phosphate absorption capacity. The carbohydrate shell composed of sucrose and starch **[18]**.

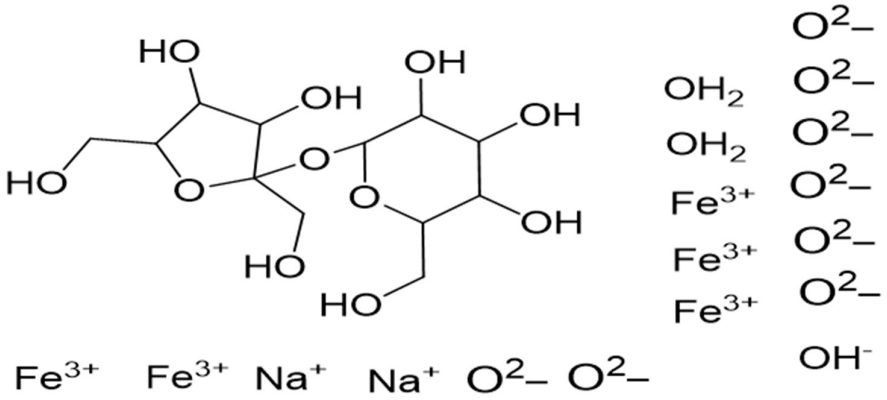

Figure 1: Structure of sucroferric oxyhydroxide

MATERIALS AND METHODS:

Chemical and reagents:

Sucroferric oxyhydroxide was obtained as a gift sample from pure chem Pvt Ltd, Akhleshwar, Gujarat, India.

Methanol and water used were of HPLC grade.

Instrumentation:

A shimadzu HPLC system with lab solution software with a UV-visible detector (SPD 20-A) are used for the analysis.

Chromatographic condition:

An HPLC system (shimadzu) which is operated using lab solution software, with a kromosil C18 with particle size 5µm, Length 150 µm, Diameter 4.6 mm column was used for the analysis. Low pressure gradient pump with a 1 ml/min flow rate was preferred for a specific drug.

Preparation of mobile phase:

Gradient mobile phase A (methanol) and gradient mobile phase B(water) was used as mobile phase.

Diluents:

The SOH was dissolved in water HPLC grade as a dilution.

Preparation of stock solution:

Accurately weighed 10mg Sucroferric oxyhydroxide was dissolved in a water and make up to the 10 ml water to get the solution of 1000µg/ml concentration.

The chromatogram of standard Sucroferric oxyhydroxide solution was shown in below figure and the average retention time was found to be 2 min.

Method Validation:

The proposed method was validated according to the ICH guidelines for system suitability. Linearity, precision. recovery, limit of detection (LOD), limit of quantitation (LOQ), robustness, specificity.

System suitability parameter:

It is an essential part of chromatographic techniques. It is performed to ensure that the analytical system is working properly and can give the accurate and precise result. Standard solution is used.

It is performed by injecting six replicate injections of standard solution of Sucroferric oxyhydroxide at 100% level (1000µg/ml concentration). And was evaluated for peak area, retention time, tailing factor and theoretical plates of standard chromatogram. The result was represented in Table.

Linearity:

linearity is the ability of method to produce test result that are directly proportional to the concentration of the analyte present in the sample. It was found to be in the range of 5-30 µg/ml.

the standard stock solution was taken in a series of standard solution were auto injector (SIL-10 AD vp) injected sample according to 5, 10, 15, 20, 25 and 30 µl into HPLC system and the peak area of chromatogram was noted for Sucroferric oxyhydroxide. the calibration graph was plotted and the drug was found to be liner with the correlation coefficient (r^2) of 0.9989 linearity data was represented in table. And the curve was shown in figure.

Precision:

It is the degree of agreement among individual test results when the applied rpeatedly to multiple samples. It was determined by studying repeatbility, intra-day and inter-day precision of method.

Repeatbility was evalued at six replicated solution of one concentration and the areas of six were mewsured and % RSD was calculated.

The intra-day and inter-day precision of the assay method was evaluted by three different concentration levels (10µl,15 µl and 20 µl) of the standard solution were injected and chromatogran were recored. The peak area was calculated for each concentration.the experiment was repeated 3 times in a day and hours and average % RSD was calculated.

Accuracy :

The % recovery was performed by standard addition method. In this method, fixed amount of SOH solution are spikied in the formulation in the increasing amount of its standatd solution were addes at 80%, 100% and 120% levels of pure drug solution. Standrrad addition and recovery eperments were conducted to determine the accuracy of the method for the quanitation of sucroferric oxyhydroxide in the drug product.

Limit of detction(LOQ) and limit of quantification (LOQ)

the limit of detection (LOD) and limit of quantification were calculated by following formula:

limit of detection = 3.3* σ/s

limit of quantification = 10* σ/s

where σ= the standard deviation of y-intercept

s =the slope of the calibration curve

Robustness:

The robustness of the assay method was calculated by introducing small or delibreted chages in the optimized HPLC condition which included flow rate(0.9 ml/min and 1.1 ml/min), mobile phase ratio in that percentage of methanol in the mobile phase (92:7 v/v and 88:12 v/v) wavelength(252 nm and 256 nm). Roubustness of the method was studies using three replicates at a concentration levels of 10µg/ml od SOH.

Specificity:

Specificity is the ability of the method to measure an analyte in ppresence of its degradation product, excipient or impuritrs. Sucroferric oxyhydroxide was subjected to various stress condition like hydrolytic, oxidative,thermal and photolytic. Degraded samples were injected inti HPLC syatem and developed chromatograms were observed for resolution of degraded products.

Forced degradation study

A stock solution of SOH was prepared by dissolving 100 mg of SOH in 10ml of

HPLC grade water for get the 10,000 µg/ml concentration. This stock solution was used for degradation study.

Hydrolytic conditions

Acid degradation

1ml of stock solution of SOH was taken in 10 ml of volumetric flask, 1 ml of 0.1N HCl was added and the solution was keeping a side in room temperature for 1 hr. than after it was neutralized with 0.1N NaOH. Then solution was make up to the mark in 10 ml volumetric flask to get the 1000 µg/ml. the solution was sonicated for 5 min and filtered through 0.45 µ nylon 6,6 membrane syringe filter.

Base degradation

1ml of stock solution of SOH was taken in 10 ml of volumetric flask, 1 ml of 1N NaOH was added and the solution was keeping a side in room temperature for 2 hr. than after it was neutralized with 1N HCl. Then solution was make up to the mark in 10 ml volumetric flask to get the 1000 µg/ml. the solution was sonicated for 5 min and filtered through 0.45 µ nylon 6,6 membrane syringe filter.

Oxidative degradation

Hydrogen peroxide-induced degradation 2 ml of stock solution of SOH was taken in 10 ml of volumetric flask, 1 ml of 6% hydrogen peroxide (H_2O_2) was added. The solution was kept at room temperature for 3 hr. the solution was make up to volume with water. the solution was filtered through 0.45µ nylon 6,6 membrane syringe filter.

Thermal degradation

Pipette out 1 ml solution of standard stock solution in 10ml volumetric flask and place it in water bath at the 60°C for 30 min under wet heat condition and then cooled to room temperature. Volume was make up to with water to get a 1000µg/ml concentration.

Photolytic degradation

Sucroferric oxyhydroxide was evenly spread in a Petridis and kept in a UV chamber for 24 hrs. After 24 hrs accurately weighed 10 mg Sucroferric oxyhydroxide powder was transferred into a 10 ml volumetric flask, dissolved it and sonicated for 5 minutes. After sonication of sample solution was diluted up to with water to get concentration of 1000 µg/ml.

Force degradation	Stress condition	Time	Temperature
No degradation	No stress	----	Room temperature
Hydrolytic			
Acid degradation	0.1 M HCl	1 hr	Room temperature
Base degradation	1 M NAOH	2 hr	Room temperature
Oxidative degradation	6 % H2O2	3 hr	Room temperature
Photolytic degradation	Uv light	6 hr	Room temperature
Thermal degradation	Water bath	30 min	60°C

RESULTS AND DISCUSSION

Method development and optimization

A detection wavelength 254 nm was selected the range UV spectral information due to its high sensitivity for all degradation products and negligible difference in response factors. SOH having pKa value for Strongest Acidic its 11.84 and for Strongest basic its -3. The optimized low pressure gradient mobile phase A (methanol) and gradient mobile phase B (water) 10:90 v/v on C-18 column are selected as per availability. That Kromosil C18 Particle size is 5 µm, Length is 150 µm, Diameter is 4.6 mm at room temperature provided the highest number of peak and better tailing and theoretical plate. Thus, further experiments were caried out using that C-18 column.

Method validation

System suitability method

The developed method has produced results in that specification such that theoretical plate more than 2000 with tailing factor less than 2 and the % RSD of peak area is less than 2% which ensure that the developed method is suitable. The developed method result is summarized in **Table 2**.

Linearity and range

In the proposed validation method, the retention time of SOH was 2.1 min. the calibration curve for SOH was created by plotting area against their corresponding concentrations, linearity was found over the range 5-30µg/ml with a coefficient of relation (r^2) results of linearity are shown in figure and **Table 3**.

Table 2: System suitability parameters of SOH

System suitability parameter	Observed value
Retention time	2.104
Number of theoretical plates	3765
Tailing factor	1.452
Peak Area	1221007

Table 3: calibration curve of SOH

Concentration (µg/ml)	Mean	Standard Deviation	% RSD
5	2053695	18185.95767	0.88552378
10	4657891.333	27978.76254	0.60067444
15	6757574.333	42789.04217	0.63320121
20	9222186.333	52132.0243	0.56528921
25	11573292.67	169001.3234	1.46027002
30	13581611	178913.1545	1.31731909

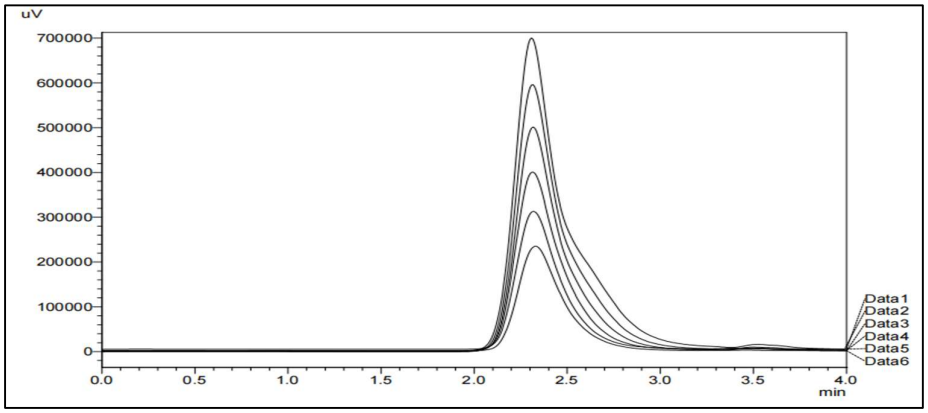

Figure 2: calibration curve of SOH

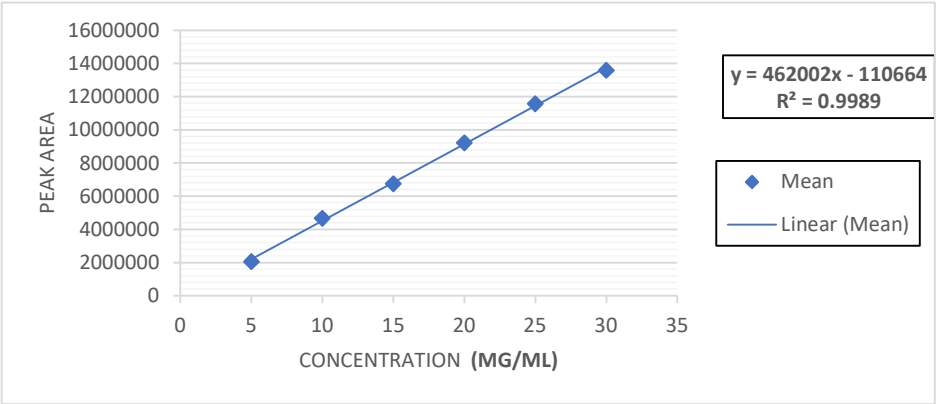

Figure 3: linearity graph

Table 4: calibration data of SOH

Parameters (unit)	Sucroferric oxyhydroxide
Regression equation	462002x-110664
Linearity range(µg/ml)	5 -30
R2	0.9989
slope	462002
Intercept	110664

Precision

the within-day (intraday) precision and for that proposed method were at three concentration levels of SOH using three replicate determinations for each concentration within one day. Same as that but between-day(inter-day) precision and for that tested by analysing the same three concentration three replicate determinations repeated on three days. Were the % RSD were calculated were suitable. The percentage of relative standard deviation (%RSD) was less than 2 % providing the high repeatability and intermediated precision of developed method for the estimation of SOH in bulk form.

Sr no	Concentration (µg/ml)	Peak Area	Standard deviation	%RSD (Not more than 2)
1	10	4301916		
2	10	4299004	33865.8312	
3	10	4279281		
4	10	4210291		0.793400507
5	10	4264601		
6	10	4255552		
Average		4268440.833		

Table 5 :Intra-day precision data for Sucroferric oxyhydroxide

Sr no	Concentration (µg/ml)	Time Interval	Peak Area	SD	% RSD
1	10	1 hr	4011342	43504.08448	1.074171919
2	10	2hr	4041577		
3	10	3 hr	4097115		
	Average Peak Area		4050011		
1	15	1 hr	7289312	43765.52052	0.601758775
2	15	2hr	7306149		
3	15	3 hr	7223342		
	Average Peak Area		7272934		
1	20	1 hr	9210089	29327.79011	0.317315125
2	20	2hr	9267232		
3	20	3 hr	9250122		
	Average Peak Area		9242481		

Table 6:Inter-day precision data for Sucroferric oxyhydroxide

Sr no	Concentration (µg/ml)	Time Interval	Peak Area	SD	% RSD
1	10	1 day	4218401	44801.59134	1.049333014
2	10	2 day	4301916		
3	10	3 day	4288274		
	Average Peak Area		4269530		
1	15	1 day	7141654	67173.37599	0.942909593
2	15	2 day	7180673		
3	15	3 day	7049831		
	Average Peak Area		7124052		
1	20	1 day	9141749	87159.89511	0.955427735
2	20	2 day	9198601		
3	20	3 day	9027464		
	Average Peak Area		9122604		

Accuracy (%Recovery)

To pre-analysed sample solution and in that a known amount of standard stock solution of SOH (8.2, 10.3- and 12.4-ml of 1000 µg/ml) were added i.e., 80%, 100% and 120%. The solutions were reanalyzed by proposed method. Calculate the mean % recovery from peak areas obtained.

% Spiking amount	Amount of sample solution (µg/ml)	Spiking concentration of API (µg/ml)	Calculated total amount of recovery	% Recovery	% Recovery (mean)	% Recovery (SD)	% Recovery (%RSD)
	10.3	8.2	18.66	100.18			
80%	10.3	8.2	18.79	100.9	100.2066	0.6803	0.6789
	10.3	8.2	18.54	99.54			
	10.3	10.3	20.74	100.24			
100%	10.3	10.3	20.54	98.08	99.1833	1.08075	1.0896
	10.3	10.3	20.3	99.23			
	10.3	12.4	23.06	101.31			
120%	10.3	12.4	22.41	98.45	99.6166	1.5009	1.5067
	10.3	12.4	22.74	99.09			
		Average			96.688	1.0873	1.0917

Robustness

The robustness was examined by evalution the influce of small variation in difrrent condition such as flow rat, wavelength and mobile phase ratio. The averge value of % RSD for determination of SOH less than 2% revealed robustness of the method. results of robustness studies are summarized in table.

Variation	Mean	SD	%RSD	System suitability parameter		
				R_t	Tailing factor	Theoretical plate
Mobile phase methanol: water (v/v)						
12:88	**3857596**	**44842.95565**	**1.162458579**	**1.765**	**1.345**	**2431**
10:90	**4065516**	**39725.44056**	**0.977131576**	**1.987**	**1.453**	**2796**
8:92	**4263683**	**26473.37208**	**0.620903757**	**2.152**	**1.524**	**2386**
Wavelength (nm)						
252	**4101873**	**77910.15377**	**1.899379961**	**2.122**	**1.765**	**2341**
254	**4358227**	**35214.51615**	**0.808000964**	**2.310**	**1.392**	**2498**
256	**4553095**	**24244.87745**	**0.532492199**	**2.412**	**1.285**	**2811**

Forced degradation study

The results from the stress testing studies indicates that the method is highly specific for SOH. Degradation products were completely distinguishable from the parent compound. The drug undergoes significant degradation under acid, alkene conditions. Acid degradation was faster than alkaline degradation. the SOH was observed relatively more stable in oxidative condition than the other condition such as the thermal and photolytic degradation. Three degraded products are obtained in thermal degradation. In oxidative degradation H_2O_2 shows its additional peak.

The chromatogram of Acid, Base, Oxidation, Photolytic and thermal degradation conditions the sample of Sucroferric oxyhydroxide showed additional peak, other than Sucroferric oxyhydroxide peak at different retention time are shown in **Figure A-E**.

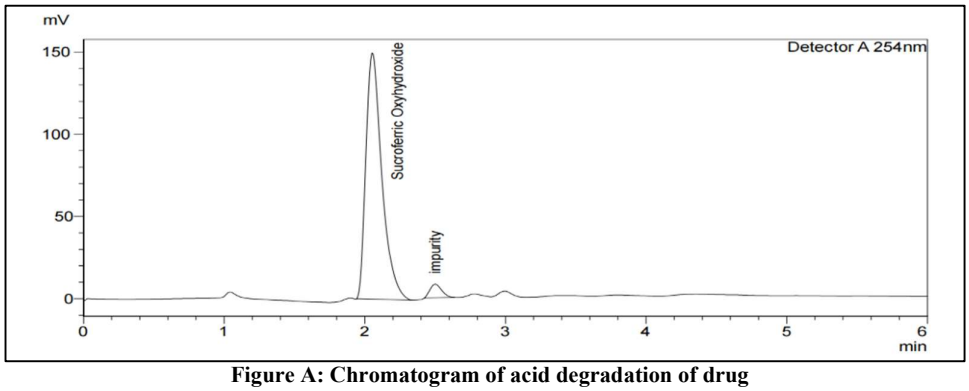

Figure A: Chromatogram of acid degradation of drug

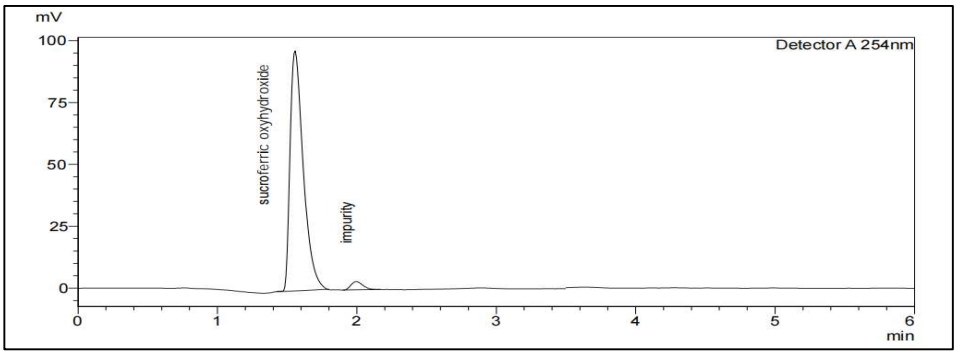

Figure B: Chromatogram of base degradation of drug

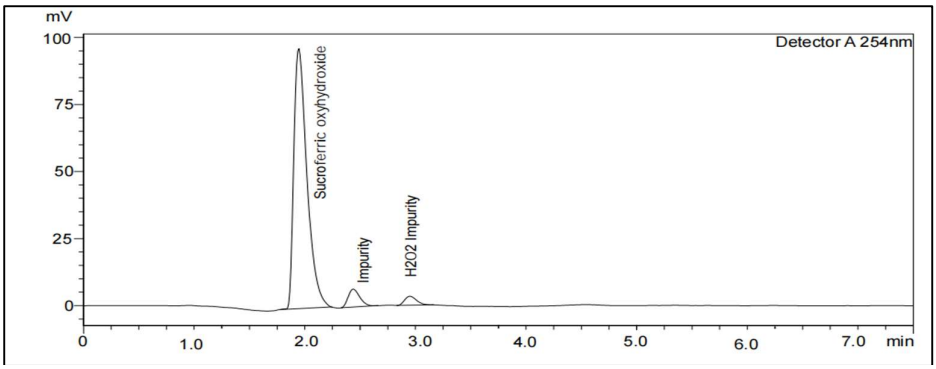

Figure C: Chromatogram of oxidative degradation of drug

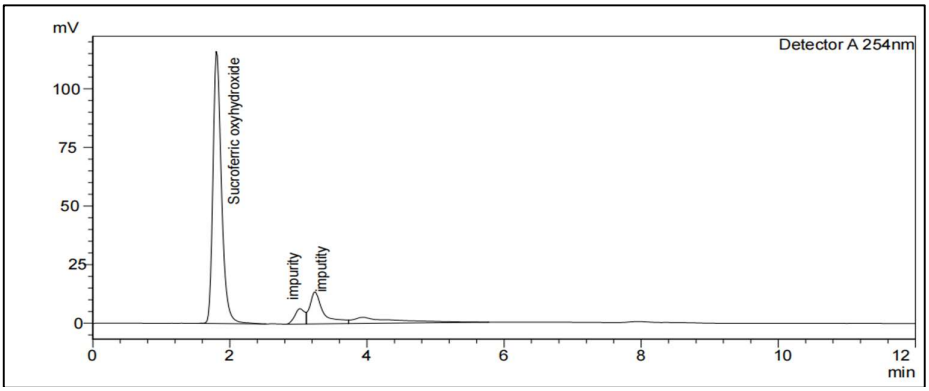

Figure D: Chromatogram of photolytic degradation of drug

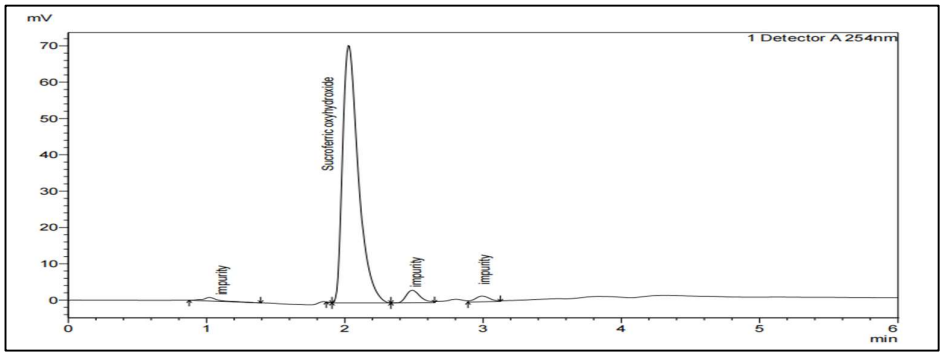

Figure E: Chromatogram of thermal degradation of drug

CONCLUSION:

Table: Summary of Stress Degradation Study of Sucroferric oxyhydroxide

Sample exposure	Time and temperature	R_f value of drug	R_f value of degraded product	% Degradation
Acidic, 1ml of 0.1 M acid (HCl) for	1 hr at room temperature	2.054	2.500	13.87%
Basic, 1ml of 1M base (NAOH) for	2 hr at room temperature	2.006	2.479	16.22 %
Hydrogen peroxide 6%, 2ml (H_2O_2)	3 hr at room temperature	1.840	2.250	8.69%
Sunlight	6 hr at room temperature	1.853	2.962	14.84%
Thermal water bath	30 min at 60°C	1.973	1.008 1.806 2.444 2.953	20.51%

A sensitive, selective, precise stability indicating analytical method developed and validated as per the international council of harmonization [ICH] for determination of Sucroferric oxyhydroxide. Here, in table the summary validation parameters for estimation of Sucroferric oxyhydroxide was found that the parameters were within the acceptance criteria as per guidelines. so, it can conclude that the method is simple, economic, easy, precise, rapid, accurate, specific and robust. for that developed method stability-indicating HPLC method the drug was exposed in acidic, basic, oxidative, photolytic, and thermal condition in that the drug Sucroferric oxyhydroxide found that the proper separation of degraded product and drug peak and % degradation are shown in table 7.2. and it conclude that the drug shows degradation in greater extent in acidic and thermal condition than the other condition. Degradation was found to be lesser extent in oxidative degradation.

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