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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF SUCRALFATE AND OXETACAINE IN BULK AND MARKETED FORMULATION BY RP-HPLC

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Keywords:

Sucralfate, oxetacaine, RP-HPLC, PDA detector

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ABSTRACT: The objective of present study is to develop and validate a method for the estimation of sucralfate and oxetacaine in suspension dosage form. An isocratic reversed phase high performance liquid chromatographic method was developed to estimate sucralfate and oxetacaine using thermohypersil BDS (4.6 x 150mm, 5µ). The mobile phase was prepared by mixing ammonium acetate (pH- 4.4) and methanol in the ratio of (60:40 v/v) which is run isocratically at the flow rate of 0.8ml/min. the wavelength at which detector (PDA) was set is 263nm. The retention time of oxetacaine and sucralfate is 2.08 and 3.4 minutes. Linearity was observed in the concentration range of 20-60µg/ml for oxetacaine and 1000-3000µg/ml for sucralfate. The percent recovery of sucralfate and oxetacaine was found to be 100. The limit of detection of oxetacaine and sucralfate is 0.178 µg/ml and 2.93 µg/ml and the limit of quantification of oxetacaine and sucralfate is 0.541 µg/ml and 8.879 µg/ml. The formulation was subjected to acid, base, peroxide, sunlight and heat and stressed samples were analysed by proposed method. The results confirmed that the proposed method is specific, rapid, reproducible and suitable for the routine determination of sucralfate and oxetacaine.

INTRODUCTION: Sucralfate has traditionally been classified as a topical site-protective or cytoprotective agent of ulcer-healing drugs with a high affinity for the gastric mucosa.[1] It is chemically known as hexadeca-µ-hydroxytetra cosahydroxy [μ_8- [1,3,4,6- tetra-O-sulfo-β-Dfructofuranosyl-α-D-glucopyranoside tetrakis (hydrogen sulfato) 8-)]] hexadecaaluminium.[2] This antiulcer drug is a sucrose sulfate-aluminium complex that [3] binds to duodenal and gastric ulcers and to gastric erosions produced by ethanol and anti-inflammatory drugs.[2]

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After oral administration, sucralfate dissociates in the acid environment of the stomach into its primary components—aluminum hydroxide and sucrose octasulfate. The latter undergoes polymerization to form a viscous paste-like complex with a strong negative charge that binds electrostatically to positively charged proteins in the base of ulcers or erosions up to 6 hours.

This insoluble complex forms a barrier that protects the ulcer from further damage by preventing back diffusion of hydrogen ions, inactivating pepsin, and absorbing gastric-damaging bile acids refluxed from the duodenum. The cytoprotective effects of sucralfate are further augmented by its ability to stimulate formation of local mediators, such as prostaglandins (PGs) and growth factors, that protect the gastric mucosa. PG release enhances mucosal blood flow and increases secretion of

mucus and bicarbonate, thereby accelerating ulcer healing. [6]

Oxethazaine, or 2,2'-(2-hydroxyethylimino) bis[N-(l,l-dimethyl-2-phenylethyl)- N-methylacetamide], a glycine amide resembling lidocaine, is a potent, safe local anaesthetic agent. Following topical application, it provides prolonged anaesthesia of mucous membranes. Its salient feature includes its ability to remain un-ionised even at a pH of 1, unlike most of the other local anaesthetics. [4] It interacts with a receptor situated within the voltage sensitive sodium channel and raises the threshold of channel opening thereby sodium ions permeability fails to increase in response to an impulse or stimulus. [3]

Literature survey revealed that one analytical method has been reported for simultaneous estimation of sucralfate and oxetacaine.

In the present investigation an attempt has been made to develop reverse phase- high performance liquid chromatographic method using simple mobile phase which was sensitive and rapid for estimation of sucralfate & oxetacaine in suspension dosage form and subsequent validation of the developed method as per ICH guidelines.

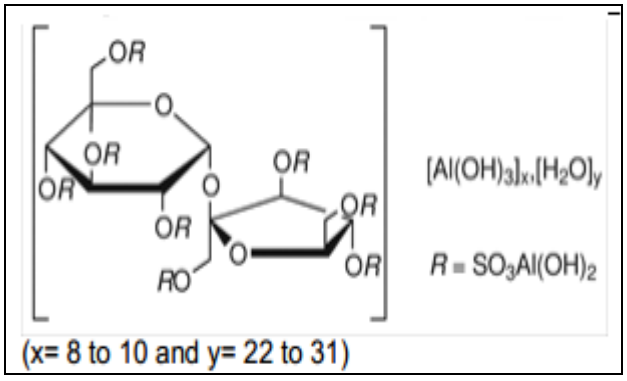

FIG. 1: STRUCTURE OF SUCRALFATE

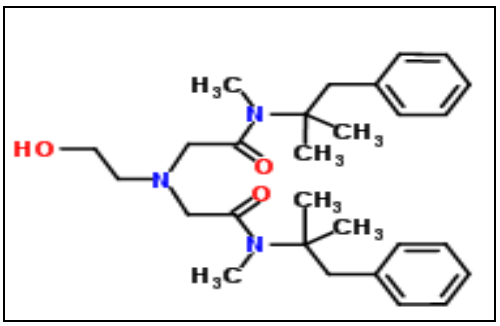

FIG. 2: STRUCTURE OF OXETACAINE

Chemicals & Reagents:

Sucralfate and oxetacaine were procured from Lara drugs pvt ltd (Telengana, India) sodium hydroxide, ammonium acetate, (Merck) used were of analytical grade. Methanol and water (Rankem) were of HPLC grade.

Chromatographic conditions:

The HPLC separation and quantification were achieved on thermohypersil BDS (4.6 x 150mm, 5µ). The mobile phase was prepared by mixing ammonium acetate (pH- 4.4) and methanol in the ratio of (60:40)v/v that run isocratically at the flow rate of 0.8ml/min. The temperature maintained in sample compartment is 25°C. The injection volume is 10µl. the wavelength at which detector was set is 263nm.

Preparation of 0.1M Ammonium acetate Buffer (pH 4.4):

To prepare 0.1M ammonium acetate solution dissolve 7.7gm of ammonium acetate ($C_2H_3O_2NH_4$) in 200ml of HPLC grade water, add 1ml of glacial acetic acid and dilute to 1000 ml with HPLC grade water. The prepared buffer was filtered through nylon membrane filter having a pore size of 0.45 µm and degassed with the help of sonicator.

Preparation of standard solution:

Standard stock solution of sucralfate & oxetacaine is prepared by adding 500mg of sucralfate & 10mg of oxetacaine to 50ml volumetric flask containing few ml of 0.1M NaOH (diluent). After dissolving the solids make up the volume up to mark with 0.1 M NaOH yielding a solution containing 10,000µg/ml of sucralfate & 200µg/ml of oxetacaine. This is labeled as stock 1 solution. From stock 1 solution 5ml was transferred to 25ml volumetric flask, the volume was made up to the mark with HPLC grade water.

Preparation of sample solution:

To 50 ml volumetric flask add 5ml of suspension. To this add 45 ml of 0.1 M sodium hydroxide (NaOH) solution which is used as diluent. Sonicate the solution for 30 min to dissolve the drug completely, then filter the solution through nylon membrane filter having a pore size of 0.45µm, then filter it through vacuum filter until a clear solution is obtained. Label it as stock- I solution. From stock I pipette out 5ml of clear solution to 25 ml

volumetric flask add 20 ml of HPLC grade water, shake the solution and fill it in vial.

Determination of λ_{max} of sucralfate and oxetacaine:

The aliquots of standard stock solution of sucralfate and oxetacaine was diluted appropriately with water to obtain a concentration of 2,000 µg/ml of sucralfate and 40 µg/ml of oxetacaine. The solutions were scanned in the range of 200-400 nm using a PDA detector. The maximum absorption of drug was found to be at 263nm.

Evaluation of System Suitability:

The 10 µl of standard solution was injected in six duplicate, the chromatogram were recorded. System suitability parameter like column efficiency, plate count and tailing factor were also recorded. The column efficiency was found to be more than 2000 plate count, tailing for the same peak is not more than 2.0 and % RSD of six injection of the standard solution is not more than 2.0%.

Formula to calculate assay %:

$$\text{Assay} = \frac{\text{sample area}}{\text{stadard area}} X \frac{\text{conc of std}}{\text{conc of sample}} X \frac{\text{Purity of working std}}{100} X \frac{\text{Avg weight}}{\text{label claim}} X 100$$

Assay result of oxetacaine:

$$\text{Assay} = \frac{3204994.33}{3204893} \times \frac{40}{23160} \times \frac{99.1}{100} X \frac{5790}{10} \times 100 = 99.1\ \%$$

$$\text{Assay} = \frac{6934250.33}{6931892} \times \frac{2000}{23160} \times \frac{100}{100} X \frac{5790}{500} \times 100 = 100\ \%$$

Validation:

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.[5] The linearity was determined at five levels over the range of 50%-150% of standard concentration in a diluents. Accurate aliquots of standard stock solution were taken in five different 25 ml volumetric flask. The volume was made up to the mark with diluents to obtain a final concentration of 20, 30, 40, 50, 60µg/ml of oxetacaine and 1000, 1500, 2000, 2500, 3000µg/ml of sucralfate. The calibration curves were obtained by plotting the peak area versus concentration. The calibration curves are shown in **Figure 4** and **5**.

Precision:

Precision was measured in terms of repeatability. The study was carried out by injecting six replicates of standard concentration of 2000 µg/ml of sucralfate & 40 µg/ml of oxetacaine. The measurement of the peak areas were expressed in terms of % RSD and were found to 0.12 and 0.04 for oxetacaine & sucralfate respectively. (Standard Value is % RSD should not be more than 2).

Accuracy:

It is expressed as recovery. Accuracy is determined at three different levels 50%, 100%, 150%. The recovery experiment was carried out by standard addition method. In which the amount of placebo was kept constant and the amount of pure drug was varied that is 255mg, 510mg, 765mg for 50%, 100%, 150%. The solution was prepared in triplicates and the accuracy was indicated by % recovery.

Limit of Detection (LOD):

Limit of detection was calculated from the values of standard deviation and slope of calibration curve. LOD is calculated from the formula; LOD = 3.3 S.D/Slope. The limit of detection of oxetacaine and sucralfate is 0.178 µg/ml and 2.930 µg/ml respectively as mentioned in **Table 6**

Limit of Quantification (LOQ):

Limit of detection was calculated from the values of standard deviation and slope of calibration curve. LOQ is calculated from the formula; LOQ = 10 S.D/Slope and was found to be 0.541 µg/ml and 8.879 µg/ml of oxetacaine and sucralfate respectively as mentioned in **Table 6**

Robustness:

In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The studied parameters were: flow rate (± 0.2ml/min) and temperature (±5°C). The flow rate in increased and decreased by 0.2ml/min and temperature is varied by 5°C. The optimized flow rate is 0.8ml/min and temperature of sample compartment is 25°C. The

results as shown in **Table 4** and **5** indicated that the proposed method was robust and was not affected by variations.

Forced Degradation:

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, & photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity,

a) Hydrolytic Degradation In Acidic Condition:

Sample preparation procedure: Transfer 5ml (5790mg) of sample in to50ml volumetric flask and add 10mlof 0.1M HCL and sonicate for 30 minutes at 60^{o}C and add 10ml of 0.1M NaOH make up the volume with diluent (0.1M NaOH). From above solution transfer 5ml in to 25ml volumetric flask make up the volume with HPLC grade water.

b) Hydrolytic Degradation In Alkaline Condition:

Sample preparation procedure:

Transfer 5ml (5790mg) of sample in to 50ml volumetric flask and add 10mlof 0.1M NaOH and sonicate for 30 minutes 60^{o}C and add 10ml of 0.1M HCL make up the volume with diluent. From above solution transfer 5ml in to 25ml volumetric flask make up the volume with HPLC grade water.

c) Oxidative Degradation:

Sample preparation procedure:

Transfer 5ml (5790mg) of sample in to 50 ml volumetric flask and add 10 ml of peroxide and sonicate for 30 minutes at 60^{o}C and make up the volume with diluent (0.1M NaOH). From above solution transfer 5ml in to 25ml volumetric flask make up the volume with HPLC grade water.

d) Thermal Degradation:

Sample preparation procedure: Before weighing, sample is exposed at 105^{0}C in an oven for 1 hour.

Transfer 5ml (5790mg) of sample which was exposed to heat in to 50ml volumetric flask and add 15ml of diluent (0.1M NaOH) and sonicate for 30 minutes. Make up the volume with diluent. From above solution transfer 5ml in 25ml volumetric flask and make up the volume with HPLC grade water.

e) Photolytic Degradation:

Procedure:

Before weighing, the sample was exposed in sunlight for 12 hours.

Transfer 5ml (5790mg) of sample in to 50ml volumetric flask. Add 15ml of diluent (0.1M NaOH) and sonicate for 30 minutes. Make up the volume with diluent. From above solution, transfer 5ml in 25ml volumetric flask dilute it with HPLC grade water.

RESULTS & DISCUSSIONS: The present study was aimed at developing a simple, precise, accurate and economical HPLC method for the analysis of sucralfate and oxetacaine in bulk and pharmaceutical dosage form. The HPLC separation and quantification were achieved on thermohypersil BDS (4.6 x 150mm, 5µ). The mobile phase was prepared by mixing ammonium acetate (pH- 4.4) and methanol in the ratio of (60:40) v/v that run isocratically at the flow rate of 0.8ml/min. The temperature maintained in sample compartment is 25^{o}C. The injection volume is 10µl. the wavelength at which detector was set is 263nm. The optimized chromatogram is shown in **Figure 3**.

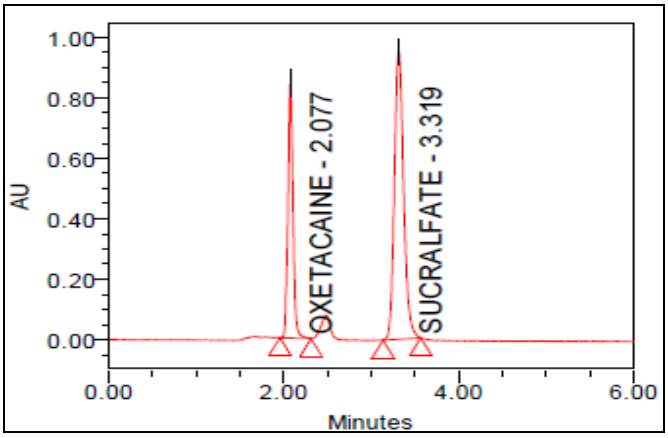

FIG 3: OPTIMIZED CHROMATOGRAM OF OXETACAINE & SUCRALFATE

The developed method was validated by evaluating system suitability, recovery, linearity, precision, robustness, specificity, LOD, LOQ. System suitability was evaluated by injecting 10 µl of standard solution six times, the chromatogram were recorded. System suitability parameter like column efficiency, plate count and tailing factor were also recorded. The column efficiency was found to be

more than 2000 plate count, tailing for the same peak is not more than 2.0 and % RSD of six injection of the standard solution is not more than 2.0%. A good linear relationship was observed for sucralfate & oxetacaine. The calibration curves were obtained by plotting the peak area Vs concentration. The calibration curves are shown in **Figure 4** and **5** and results are tabulated in 1.

TABLE 1: LINEARITY DATA OF OXETACAINE AND SUCRALFATE

OXETACAINE			SUCRALFATE		
Concentration level %	**Area**	**µg/ml**	**Concentration Level %**	**Area**	**µg/ml**
50	1608973	20	50	3460024	1000
75	2408937	30	75	5198685	1500
100	3207976	40	100	6933236	2000
125	4007812	50	125	8664580	2500
150	4905976	60	150	10300114	3000

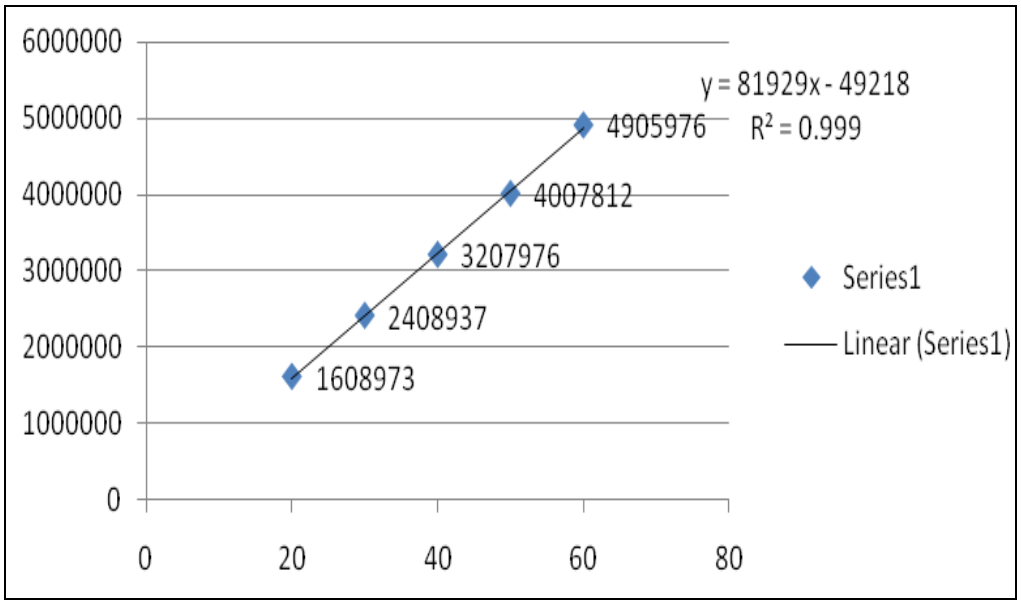

FIG. 4: CALIBRATION CURVE OF OXETACAINE

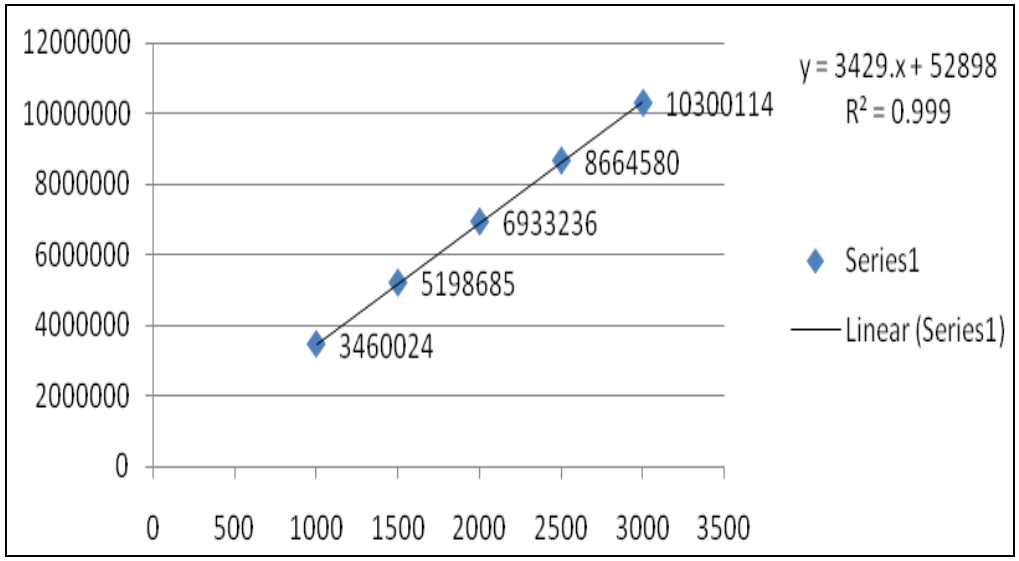

FIG. 5: CALIBRATION CURVE OF SUCRALFATE

Accuracy is determined at three different levels 50%, 100%, 150%. The recovery experiment was carried out by standard addition method. The percent recovery for sucralfate ranges from 99%-100% & for oxetacaine is 100% which is presented in the **Table 2** and **3**.

TABLE 2: RECOVERY STUDIES OF OXETACAINE

Level	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	1604837	19.800	19.88	100	100
100%	3204788.66	39.600	39.60	100	
150%	4806107.16	59.400	59.46	100	

TABLE 3: RECOVERY STUDIES OF SUCRALFATE

Level	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	3465079.66	1000.000	995.94	100	
100%	6936105	2000.000	1994.50	100	100
150%	10347313.33	3000.000	2974.87	99	

The precision of each method was ascertained separately from the peak area obtained by actual determination of six replicates of a fixed amount of drug i.e. (2000 µg/ml of sucralfate and 40µg/ml of oxetacaine). The RSD of oxetacaine & sucralfate is found to be 0.12 and 0.04 respectively. Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (± 0.2ml/min) & temperature (± 5^{o}C). The results of robustness are given in **Table 4 & 5**.

TABLE 4: ROBUSTNESS OF OXETACAINE

S.no	Parameter	Optimized	Used	Retention time	Plate count	Peak area	Tailing
1.	Flow rate	0.8ml/min	0.6ml/min	2.815	5864	4606668	1.068
			1.0ml/min	1.713	3825	2616180	0.969
2.	Temperature	25^{o}C	20^{o}C	2.131	3982	3362993	0.990
			30^{o}C	2.118	3997	3326635	0.983

TABLE 5: ROBUSTNESS OF SUCRALFATE

S.no	Parameter	Optimized	Used	Retention time	Plate count	Peak area	Tailing
1.	Flow rate	0.8 ml/min	0.6ml/min	4.739	5713	10552616	1.159
			1.0ml/min	2.899	3746	6036419	1.094
2.	Temperature	25^{o}C	20^{o}C	3.583	4526	7801604	1.113
			30^{o}C	3.573	4702	7720317	1.109

The limit of detection of oxetacaine and sucralfate is 0.178 µg/ml and 2.930 µg/ml. and limit of quantification of oxetacaine and sucralfate is 0.541 µg/ml and 8.879 µg/ml respectively. Results of LOD & LOQ is tabulated in **Table 6**.

TABLE 6: LOD &LOQ OF OXETACAINE AND SUCRALFATE

Parameter	Oxetacaine	Sucralfate
LOD	0.178 µg/ml	2.930 µg/ml
LOQ	0.541 µg/ml	8.879 µg/ml

Sucralfate and oxetacaine was subjected to various stress conditions like hydrolytic degradation under

acidic and alkaline condition, thermal degradation, oxidative degradation, photolytic degradation.

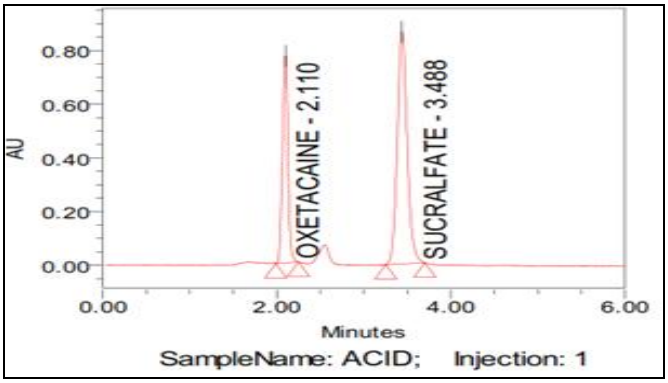

FIG. 6: CHROMATOGRAM OF ACID DEGRADATION

The results of forced degradation is shown in the **Table 7** and the chromatograms of forced degradation of oxetacaine and sucralfate is shown in **Figure 6** and **7**.

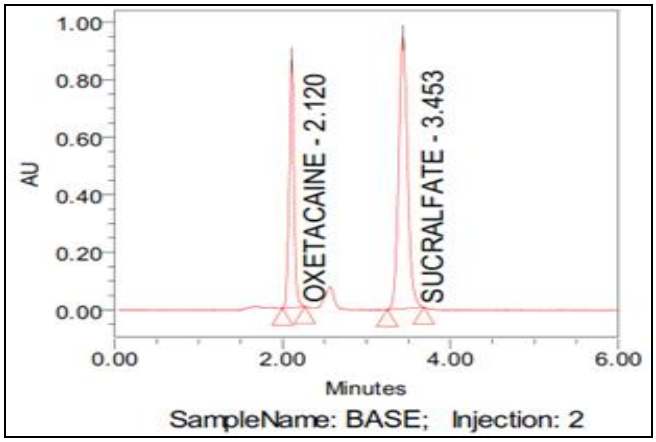

FIG. 7: CHROMATOGRAM OF ALKALI DEGRADATION

CONCLUSION: This HPLC method is accurate, precise, reproducible, specific and stability indicating. The method has found to be better than previously reported methods, because of its wide range of linearity, use of an economical and readily available mobile phase, the retention time is very low. The separation of two components was done with 5 minutes. The result assay of sucralfate and oxetacaine obtained by the proposed method was found to quite concurrent and reproducible indicating that the method is precise and rugged. The recoveries of the drug were about 100% indicating accuracy of the method and non-interference of the excipients states that the method is specific. The developed method is stability indicating and can be used or routine analysis of sucralfate and oxetacaine free of interference from their degradation products in suspension formulation. All these factors make this method suitable for quantification of sucralfate and oxetacaine in bulk and pharmaceutical dosage form.

TABLE 7: DEGRADATION RESULTS OF SUCRALFATE AND OXETACAINE

Parameter	Area of oxetacaine	Area of sucralfate	% Assay of oxetacaine	%Assay of sucralfate	% Degradation of oxetacaine	% Degradation of sucralfate
Acid	3204908	5906934	99.097	84.958	0.002	14.776
Base	3213537	5914514	99.364	85.067	0.265	14.667
Peroxide	3205498	5968494	99.115	85.844	- 0.016	13.890
Sunlight	3193305	6648185	98.738	95.619	0.361	4.115
Heat	3206818	6018463	99.156	95.192	- 0.057	4.542

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