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# Method Development and Validation of Sucralfate and Oxetacaine in Bulk Drug and Formulation by Rp-Hplc

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**ABSTRACT**

A simple and reliable RP-HPLC chromatographic method was developed for the quantitative determination of Sucralfate and Oxetacaine. Chromatography was carried out by reversed-phase technique on a $C_{18}$ column with a mobile phase composed of acetonitrile and phosphate buffer (pH 8.9) in the proportion of 30:70 v/v, at a flow rate of 1mL/min with a $\lambda_{max}$ of 282 nm. This method was found to be specific and accurate with the mean recovery of 99.60% and 100.32% for Sucralfate and Oxetacaine respectively. The linearity of the proposed method was investigated in the range of 500-1500 µg/mL and 10-30 µg/mL for sucralfate and Oxetacaine respectively with a run time of 20min (Retention time of Sucralfate 3.5min and for Oxetacaine 5.4min). Assay content of Sucralfate and Oxetacaine was determined and the mean assay was found to be 99.93% and 100.2%.The method was also evaluated for robustness, LOD, LOQ and the results obtained were satisfactory. Overall, the proposed method is highly sensitive, precise and accurate and can be used for the reliable quantitation of Sucralfate and Oxetacaine.

**Keywords:** Sucralfate, Oxetacaine, IR, RP-HPLC, ICH guidelines, Validation.

## INTRODUCTION

Sucralfate is a Hexdeca-µ-hydroxytetracosahydroxy [$\mu_8$-[1, 3, 4, 6-tetra-O-sulfo-β-Dfructofuranosyl-α-D-glucopyranoside tetrakis (hydrogen sulfato) 8-)]] hexadecaaluminium. This anti-ulcer drug is a sucrose sulfate-aluminium complex that binds to the mucosa, thus creating a physical barrier that impairs diffusion of hydrochloric acid in the gastrointestinal tract and prevents degradation of mucus by acid(Jensen SL and Funch Jensen P, 1992). Oxetacaine is a 2, 2'-(2-hydroxyethylimino) bis [N-(1, 1-dimethyl-2-phenylethyl)-N-methyl acetamide], which is a local anesthetic drug (Seifter J *et al*., 1962). 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. This combination is used in the treatment of Peptic ulcers. Sucralfate and Oxetacaine were determined by high performance liquid chromatography with UV detection at 282nm. Linearity for detector response was observed in the concentration range of 500-1500µg/mL for sucralfate and 10-30µg/mL for oxetacaine. The aim of this study was to develop a simple, sensitive precise liquid chromatographic method with PDA detection for the determination of sucralfate and oxetacaine.

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## MATERIALS AND METHODS

### Chemicals and reagents

Dipotassium hydrogen phosphate, Methanol, Acetonitrile were obtained from MERCK Chemicals, Mumbai. Milli Q Water, Distilled Water were procured from In House Production (Lara drugs Private Ltd).

### High performance liquid chromatography

Waters HPLC with Empower2 software, 2695 Photodiode Array Detector with data processing capacity was used. A Hypersil BDS column $C_{18}$, 250 X 4.6mm, 5µ was used. The $p^H$ measurement was performed by using Labotronics. As a degasser, Enerteck ultrasonicator was used. Typical operating conditions include Flow rate 1mL/min; Injection Volume 10 µL; Wavelength 282nm; Column Temperature $30^0$C; Run time of 20min.

### Preparation of solutions

### Preparation of solution A

Dissolve 17.418g of Dipotassium hydrogen phosphate in 1000mL of water (pH 8.9). Filter through 0.45µ or finer porosity membrane filter.

### Preparation of solution B

Take Acetonitrile in 1000mL beaker and degas it to remove air bubbles.

**Preparation of mobile phase**

Prepare a degassed mixture of solution A & solution B in the ratio of 70:30 v/v.

**Preparation of diluent**

Prepare a degassed mixture of water & Methanol in the ratio of 70:30 v/v.

**Assay Calculation**

**FORMULA:**

$$\frac{AT}{AS} X \frac{WS}{DS} X \frac{DT}{WT} X \frac{Avg.Wt}{LC} X \text{ Purity of STD}$$

$$\% \text{ Assay of Sucralfate} = \frac{382685}{383857} X \frac{500}{100} X \frac{5}{25} X \frac{100}{5000} X \frac{25}{5} X \frac{5000}{500} X 99.2$$

$$= 99.93\%$$

$$\% \text{ Assay of Oxetacaine} = \frac{774593}{773580} X \frac{10}{100} X \frac{5}{25} X \frac{100}{5000} X \frac{25}{5} X \frac{5000}{500} X 99.8$$

$$= 100.2\%$$

**Validation of the developed HPLC method**

**System suitability**

**Standard or System Suitability Solution for Sucralfate**

Weigh and transfer accurately 500mg of Sucralfate working standard into a 100mL clean, dry volumetric flask, add some amount of diluent and sonicate to dissolve. Make upto the volume with diluent. Dilute 5mL of this solution to 25mL with diluent.

**Standard or System Suitability Solution for Oxetacaine**

Weigh and transfer accurately 10mg of Oxetacaine working standard into a 100mL clean, dry volumetric flask, add some amount of diluent and sonicate to dissolve. Make upto volume with diluent. Dilute 5mL of this solution to 25mL with the diluent.

**Evaluation of system suitability**

Inject 10µl of system suitability solution before and after the analysis into the chromatograph and record the chromatograms. The column efficiency as determined from the Sucralfate peak is NLT 2500 USP plate count and USP tailing for the same peak is NMT 2.0. %RSD for peak areas of five injections of standard solution is NMT 2.0.

**Precision**

The precision of the method was demonstrated through two parameters which are injection reproducibility (system precision) and the method precision.

**System precision (Injection reproducibility)**

For injection reproducibility, consider five injections of system suitability solution were made and the relative standard deviation for the replicate injections was calculated.

**Method Precision**

For method precision, 6 individual preparations were made from the same batch and the individual peaks areas were measured. 5g of the suspension is taken in a 100mL volumetric flask. Add some amount of diluent. Sonicate and degas it. Then it is made upto the mark with the diluent. 5mL of the above solution is transferred to a 25mL volumetric flask and made upto the mark with the diluent. Six samples were prepared with a target concentration of about 1000µg/mL from sucralfate and 20µg/mL from oxetacaine.

**Accuracy**

The accuracy of an analytical method is the closeness of the results obtained by that method to the true value for the sample. It is expressed as recovery (%).The experiment was performed in triplicate for 100%, six times for 50%, and 150%.

**6. Limit of Detection**

LOD of Sucralfate = $3.3\sigma/S$

= 3.3×25.0066/385.4

= 0.2141 µg/mL

LOD of Oxetacaine = $3.3\sigma/S$

= 3.3×538.2/7349

= 0.2416 µg/mL

**7. Limit of Quantification**

LOQ of Sucralfate = $10\sigma/S$

= 0.643 µg/mL

LOQ of Oxetacaine = $10\sigma/S$

= 0.7248 µg/mL

## Figure.1 Standard chromatogram of Sucralfate and Oxetacaine

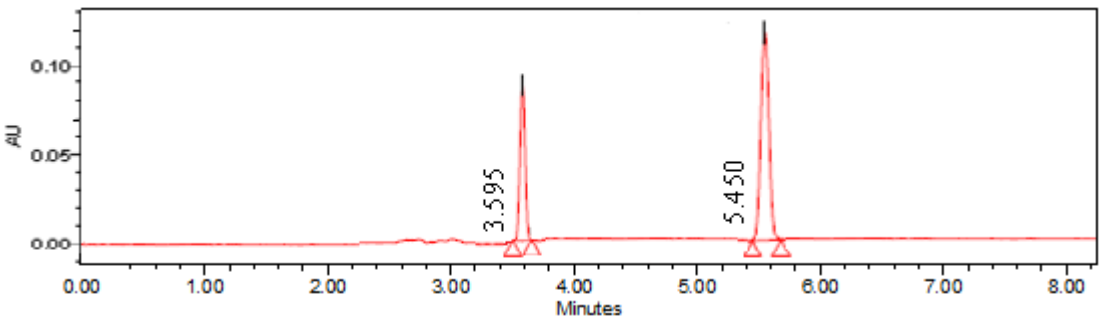
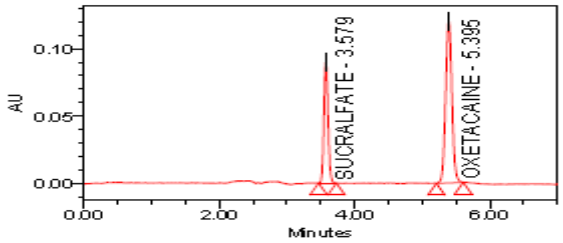
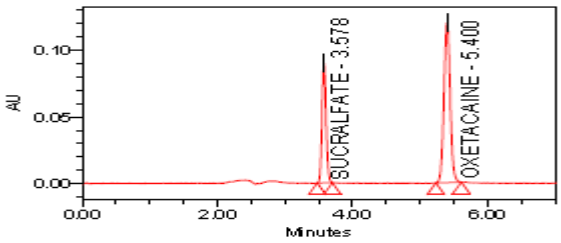


**Figure.2 System suitability**

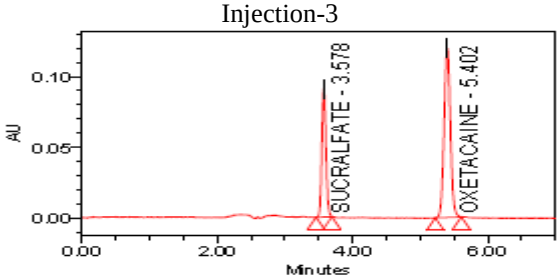
Injection-1



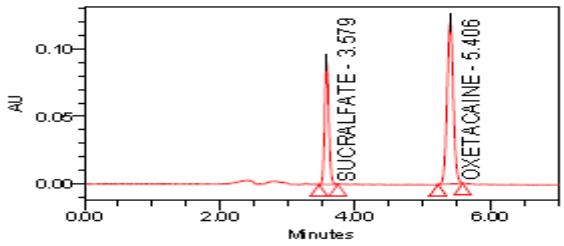

Injection-2




Injection-3




Injection-4




Injection-5

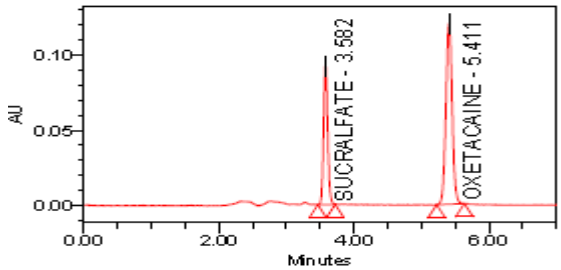
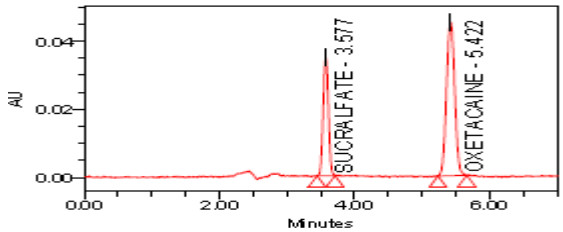
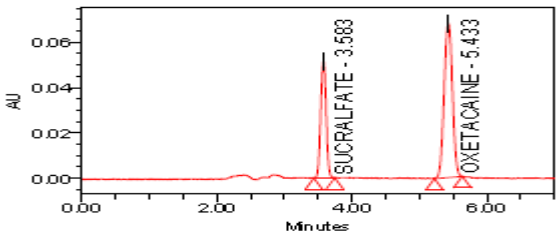



**Figure.3 Linearity of sucralfate and oxetacaine**

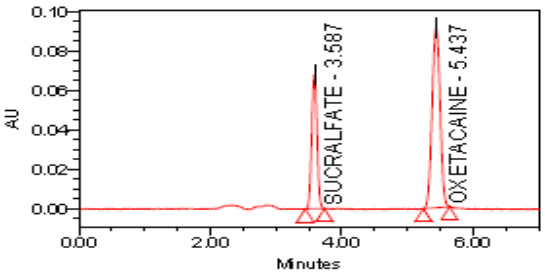
50% Linearity of sucralfate and oxetacaine



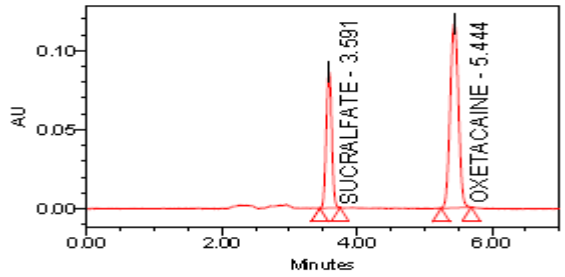

75% Linearity of sucralfate and oxetacaine




100% Linearity of sucralfate and oxetacaine




125% Linearity of sucralfate and oxetacaine




150% Linearity of sucralfate and oxetacaine

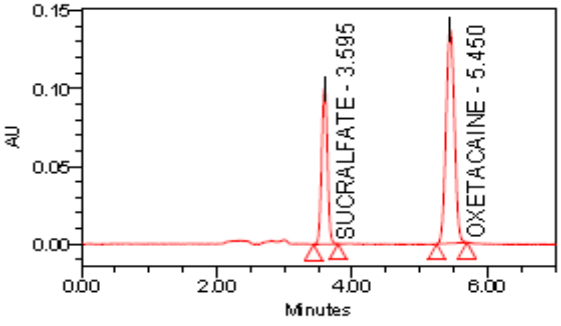


**Figure.4 Calibration graph for sucralfate**

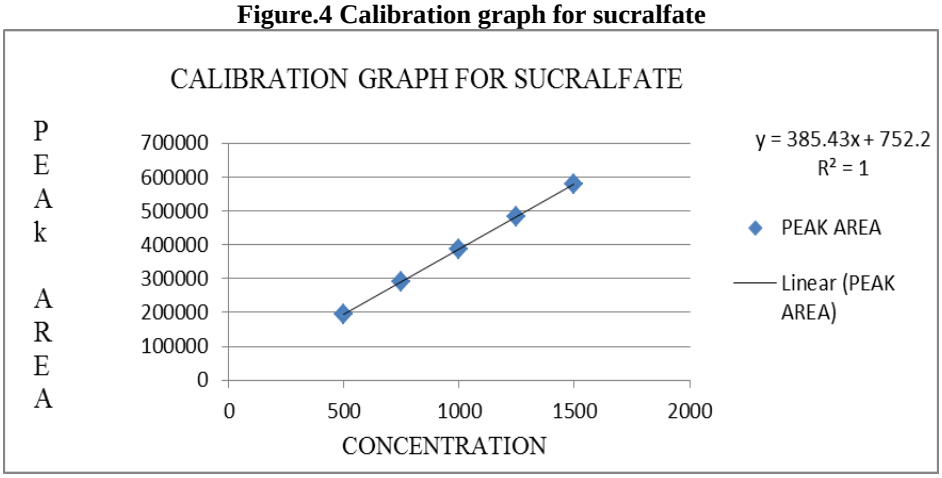



**Figure.5 Calibration graph for oxetacaine**

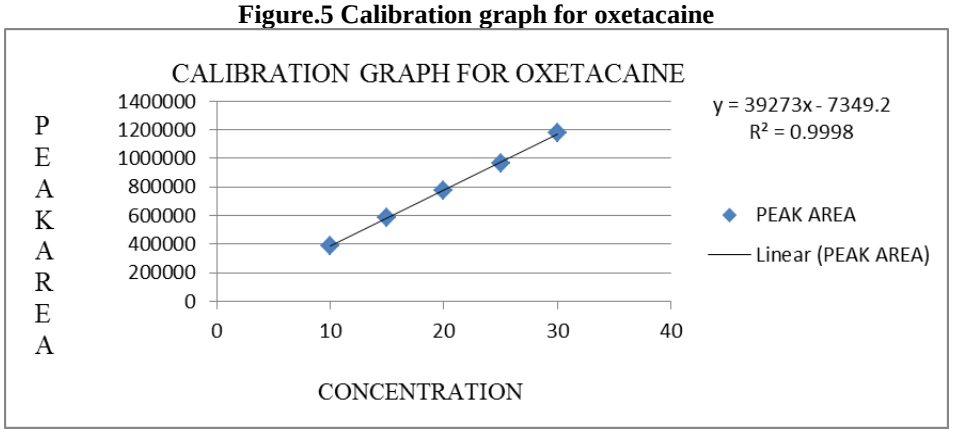



**Table.1 System suitability for sucralfate**

| S. No | Inj | Name | RT | Area | Tailing | Plate count |
|---|---|---|---|---|---|---|
| 1 | 1 | SUCRALFATE | 3.579 | 380907 | 1.128 | 16691 |
| 2 | 2 | SUCRALFATE | 3.578 | 378636 | 1.122 | 16965 |
| 3 | 3 | SUCRALFATE | 3.578 | 380652 | 1.118 | 16518 |
| 4 | 4 | SUCRALFATE | 3.579 | 385972 | 1.126 | 16437 |
| 5 | 5 | SUCRALFATE | 3.582 | 393120 | 1.129 | 16587 |
| Mean | | | | 383857 | | |
| Std. Dev | | | | 5842 | | |
| %RSD | | | | 1.5 | | |

**Table.2 System suitability for oxetacaine**

| S. No | Inj | Name | RT | Area | Tailing | Plate count |
|---|---|---|---|---|---|---|
| 1 | 1 | OXETACAINE | 5.395 | 775432 | 1.050 | 16288 |
| 2 | 2 | OXETACAINE | 5.400 | 771889 | 1.052 | 16360 |
| 3 | 3 | OXETACAINE | 5.402 | 773217 | 1.047 | 16381 |
| 4 | 4 | OXETACAINE | 5.406 | 770853 | 1.033 | 16380 |
| 5 | 5 | OXETACAINE | 5.411 | 776512 | 1.040 | 16168 |
| Mean | | | | 773580 | | |
| Std.Dev | | | | 2369 | | |
| %RSD | | | | 0.3 | | |

**Table.3 Linearity of Sucralfate**

| S. No | Level Solution for Linearity | Concentration of Sucralfate (µg /mL) | Area |
|---|---|---|---|
| 1 | 50% | 500 | 193551 |
| 2 | 75% | 750 | 289706 |
| 3 | 100% | 1000 | 386004 |
| 4 | 125% | 1250 | 482884 |
| 5 | 150% | 1500 | 578744 |

**Table.4 Linearity of Oxetacaine**

| S. No | Level Solution for Linearity | Concentration of Oxetacaine (µg /mL) | Area |
|---|---|---|---|
| 1 | 50% | 10 | 387960 |
| 2 | 75% | 15 | 582188 |
| 3 | 100% | 20 | 775383 |
| 4 | 125% | 25 | 968339 |
| 5 | 150% | 30 | 1176718 |

**Table.5 Injection reproducibility of sucralfate**

| S. No | Inj | Name | RT | Area | Tailing | Plate count |
|---|---|---|---|---|---|---|
| 1 | 1 | SUCRALFATE | 3.579 | 380907 | 1.128 | 16691 |
| 2 | 2 | SUCRALFATE | 3.578 | 378636 | 1.122 | 16965 |
| 3 | 3 | SUCRALFATE | 3.578 | 380652 | 1.118 | 16518 |
| 4 | 4 | SUCRALFATE | 3.579 | 385972 | 1.126 | 16437 |
| 5 | 5 | SUCRALFATE | 3.582 | 393120 | 1.129 | 16587 |
| Mean | | | | 383857 | | |
| Std. Dev | | | | 5942 | | |
| %RSD | | | | 1.3 | | |

**Table.6 Injection reproducibility of oxetacaine**

| S. No | Inj | Name | RT | Area | Tailing | Plate count |
|---|---|---|---|---|---|---|
| 1 | 1 | OXETACAINE | 5.395 | 775432 | 1.050 | 16288 |
| 2 | 2 | OXETACAINE | 5.400 | 771889 | 1.052 | 16360 |
| 3 | 3 | OXETACAINE | 5.402 | 773217 | 1.047 | 16381 |
| 4 | 4 | OXETACAINE | 5.406 | 770853 | 1.033 | 16380 |
| 5 | 5 | OXETACAINE | 5.411 | 776512 | 1.040 | 16168 |
| Mean | | | | 773580 | | |
| Std.Dev | | | | 2369 | | |
| %RSD | | | | 0.3 | | |

**Table.7 Method precision for sucralfate**

| S.No | Solution ID | Peak area |
|---|---|---|
| 1 | Preparation-1 | 383960 |
| 2 | Preparation-2 | 379687 |
| 3 | Preparation-3 | 383258 |
| 4 | Preparation-4 | 381622 |
| 5 | Preparation-5 | 383925 |
| 6 | Preparation-6 | 383659 |
| SD | | 1706.353 |
| %RSD | | 0.445 |

**Table.8 Method precision for oxetacaine**

| S.No | Solution ID | Peak area |
|---|---|---|
| 1 | Preparation-1 | 775987 |
| 2 | Preparation-2 | 773417 |
| 3 | Preparation-3 | 769664 |
| 4 | Preparation-4 | 771317 |
| 5 | Preparation-5 | 779802 |
| 6 | Preparation-6 | 777372 |
| SD | | 3826.485 |
| %RSD | | 0.493 |

**Table.9 Method precision and assay of sucralfate**

| Solution ID | Percentage assay of sucralfate |
|---|---|
| Preparation-1 | 99 |
| Preparation-2 | 98 |
| Preparation-3 | 99 |
| Preparation-4 | 99 |
| Preparation-5 | 99 |

| | |
|---|---|
| Preparation-6 | 99 |
| Mean | 99 |
| Standard deviation | 0.44 |
| %RSD | 0.45 |

**Table.10 Method precision and assay of oxetacaine**

| Solution ID | Percentage assay of oxetacaine |
|---|---|
| Preparation-1 | 100 |
| Preparation-2 | 100 |
| Preparation-3 | 99 |
| Preparation-4 | 100 |
| Preparation-5 | 101 |
| Preparation-6 | 100 |
| Mean | 100 |
| Standard deviation | 0.49 |

**Table.11 Accuracy for sucralfate**

| S.No | Spiked Level | µg/mL added | µg/mL found | Area | %Recovery | %Mean | %RSD |
|---|---|---|---|---|---|---|---|
| 1. | 50% | 495.000 | 486.79 | 188366 | 98.01 | 98 | 0.4 |
| | | 495.000 | 485.08 | 187702 | 98 | | |
| | | 495.000 | 490.73 | 189888 | 99 | | |
| | | 495.000 | 485.29 | 187785 | 98.37 | | |
| | | 495.000 | 484.99 | 187668 | 98 | | |
| | | 495.000 | 486.14 | 188112 | 98.05 | | |
| 2. | 100% | 990.000 | 988.95 | 382678 | 100 | 100 | 0.3 |
| | | 990.000 | 983.66 | 380631 | 99 | | |
| | | 990.000 | 988.61 | 382545 | 100 | | |
| 3. | 150% | 1485.000 | 1501.60 | 581047 | 101 | 101 | 0.3 |
| | | 1485.000 | 1492.57 | 577554 | 101 | | |
| | | 1485.000 | 1504.47 | 582157 | 101 | | |
| | | 1485.000 | 1499.39 | 580192 | 101 | | |
| | | 1485.000 | 1495.49 | 578683 | 101 | | |
| | | 1485.000 | 1497.81 | 579580 | 101 | | |
| | | | | | | Mean %RSD | 0.3 |

**Table.12 Accuracy for oxetacaine**

| S.No | Spiked level | µg/mL added | µg/mL found | Area | %Recovery | %Mean | %RSD |
|---|---|---|---|---|---|---|---|
| 1. | 50% | 9.900 | 9.65 | 374025 | 97 | 98 | 1.3 |
| | | 9.900 | 9.62 | 372888 | 97 | | |
| | | 9.900 | 9.87 | 382657 | 100 | | |
| | | 9.900 | 9.89 | 383242 | 100 | | |
| | | 9.900 | 9.58 | 371296 | 97 | | |
| | | 9.900 | 9.74 | 377401 | 98 | | |
| 2. | 100% | 19.800 | 20.09 | 778557 | 101 | 101 | 0.8 |
| | | 19.800 | 19.80 | 767363 | 100 | | |
| | | 19.800 | 20.01 | 775644 | 101 | | |
| 3. | 150% | 29.7 | 30.38 | 1177258 | 102 | 102 | 0.1 |
| | | 29.7 | 30.37 | 1177040 | 102 | | |
| | | 29.7 | 30.44 | 1179637 | 102 | | |
| | | 29.7 | 30.48 | 1181389 | 103 | | |
| | | 29.7 | 30.41 | 1178593 | 102 | | |
| | | 29.7 | 30.41 | 1178675 | 102 | | |
| | | | | | | Mean %RSD | 0.73 |

**Robustness**

**Table.13 Robustness parameters of Sucralfate (± 0.2mL/min)**

| S. No | Parameter | Condition | System suitability results for sucralfate | | | |
|---|---|---|---|---|---|---|
| | | | RT | Area | USP tailing | Theoretical plates |
| 1 | Flow rate by ± 0.2mL/min | 1.2 mL/min | 2.988 | 382678 | 1.102 | 7183 |
| | | 0.8 mL/min | 4.465 | 380631 | 1.053 | 9147 |

**Table.14 Robustness parameters of Sucralfate (± 5°C)**

| S. No | Parameter | Condition | System suitability results for sucralfate | | | |
|---|---|---|---|---|---|---|
| | | | RT | Area | USP tailing | Theoretical plates |
| 1 | Column Oven temperature by ± 5°C | 35°C | 3.595 | 389171 | 1.058 | 8024 |
| | | 25°C | 3.581 | 390722 | 1.026 | 8924 |

**Table.15 Robustness parameters of oxetacaine (± 0.2mL/min)**

| S. No. | Parameter | Condition | System suitability results for Oxetacaine | | | |
|---|---|---|---|---|---|---|
| | | | RT | Area | USP tailing | Theoretical plates |
| 1 | Flow rate by ± 0.2mL/min. | 1.2 mL/min | 4.536 | 778557 | 1.117 | 7247 |
| | | 0.8 mL/min | 6.788 | 767363 | 1.063 | 9368 |

**Table.16 Robustness parameters of oxetacaine (± 5°C)**

| S. No. | Parameter | Condition | System suitability results for Oxetacaine | | | |
|---|---|---|---|---|---|---|
| | | | RT | Area | USP tailing | Theoretical plates |
| 1 | Column Oven temperature by ± 5°C | 35 °C | 5.450 | 785501 | 1.117 | 7247 |
| | | 25 °C | 5.414 | 786834 | 1.063 | 9368 |

**Summary and conclusion**

A simple and reliable RP-HPLC chromatographic method was developed for the quantitative determination of Sucralfate and Oxetacaine. Chromatography was carried out by reverse-phase technique on a $C_{18}$ column with a mobile phase composed of acetonitrile and phosphate buffer (pH 8.9) in the proportion of 30:70 v/v, at a flow rate of 1mL/min with a $\lambda_{max}$ of 282 nm. This method was found to be specific and accurate with the mean recovery of 99.60% and 100.32% for Sucralfate and Oxetacaine respectively. The linearity of the proposed method was investigated in the range of 500-1500 µg/mL and 10-30 µg/mL for sucralfate and Oxetacaine respectively with a run time of 20min (Retention time of Sucralfate 3.5min and for Oxetacaine 5.4min). Assay content of Sucralfate and Oxetacaine was determined and the mean assay was found to be 99.93% and 100.2%.The method was also evaluated for robustness, LOD, LOQ and the results obtained were satisfactory.

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