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# Assessment and Computational Estimation of Omeprazole and Levosulpiride Impurities in Fixed Dose Combination by AQbD Approach

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**Abstract:** This study focuses on novel high-performance liquid chromatography (HPLC) method to simultaneously identify and quantify degradation products of esomeprazole and levosulpiride in capsule dosage form, enhancing analytical precision and reliability. The gradient was thoroughly optimized for proper separation of all the peaks in the chromatogram. Chromatographic separation was obtained on Octadecylsilane 250 x 4.6 mm, 5 µm column. Mobile phase A consisting of buffer (Ammonium acetate 50 mM) pH 5.0: Acetonitrile: Water, in ratio 10:10:80 (v/v), whereas mobile phase B consisting of Acetonitrile and Water in ratio 80:20 (v/v). Gradient programme was set as follow: time in min/% of mobile phase A 0/100, 10/80, 15/80, 30/20, 35/100, 45/100. The flow rate was 1.0 mL/min$^{-1}$ with UV detection at 302 nm. The method development incorporated an analytical quality by design (AQbD) approach, offering a systematic optimization strategy employing Box-Behnken design. The developed method was aligned and validated according to the ICH guidelines.

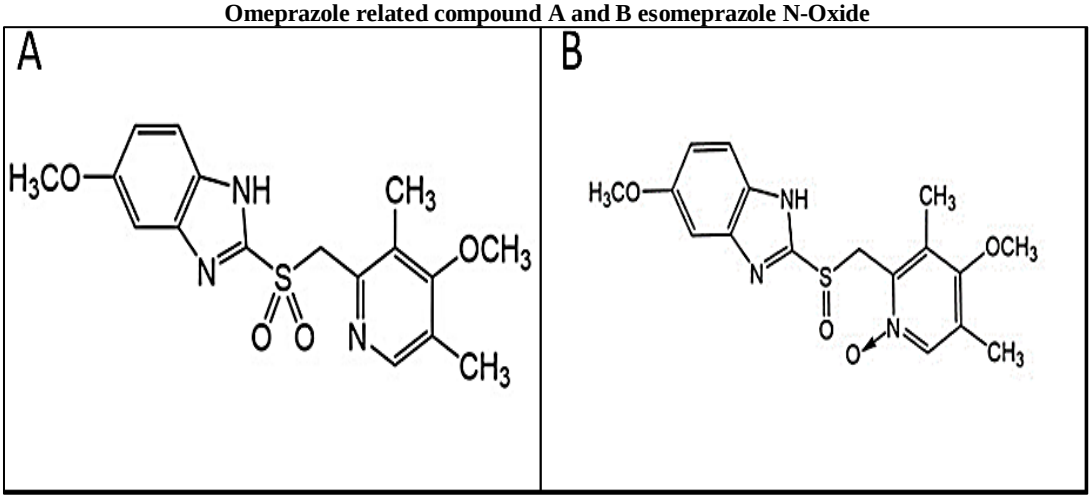
**Keywords:** esomeprazole (ESO), levosulpiride (LVS), degradation products, validation, quality by design (QbD)

## 1. Introduction

Esomeprazole, the S enantiomer of Omeprazole, is a potent proton pump inhibitor utilized in the treatment of gastric acid-related disorders. Its chemical structure comprises a benzimidazole core with a sulfinyl moiety [1].

Esomeprazole exhibits susceptibility to degradation via various pathways, including hydrolysis, oxidation, and photolysis. Hydrolysis, modulated by pH, represents a prominent degradation route [2], while oxidative degradation can occur in the presence of oxygen, particularly under harsh condition [3]. Furthermore, light exposure induces photo degradation, necessitating stringent light protection measures during storage and handling. Levosulpiride, the L-isomer of sulpiride, is characterized by a benzamide structure with a sulfamoyl group [4-5]. It is commonly employed in the management of psychiatric disorders. Degrading studies conducted on levosulpiride have elucidated its stability profile under various environmental stressors. Specifically, the compound demonstrated resilience to sunlight exposure, wet hydrolysis, and dry heat stress conditions. However, it exhibited susceptibility to base hydrolysis, acid hydrolysis, and oxidation induced by 3% Hydrogen peroxide [6]. Esomeprazole exhibits robust stability under basic conditions but demonstrates pronounced instability in acidic environments. It is prone to form acid degradation product thus it is necessary to examine the degradation of esomeprazole and levosulpiride in combination. Within the parameters outlined by the United States Pharmacopeia and the British Pharmacopeia, two impurities have been identified: 4-Methoxy-2-[[(RS)-(5-methoxy-1H-benzimidazol-2-yl) sulfinyl] methyl]-3, 5-dimethylpyridine 1-oxide, commonly known as Omeprazole N-Oxide, and Omeprazole related compound A.

**Figure 1**
**Omeprazole related compound A and B esomeprazole N-Oxide**




A comprehensive literature survey highlights various analytical techniques employed for the quantification of esomeprazole and levosulpiride, encompassing UV spectroscopy, [7-12] either independently or in combination with other pharmaceutical drugs. However, impurity profiling of combined formulation for esomeprazole and levosulpiride is not reported.

Thus, it is necessary to have comprehensive understanding of its chemical properties and degradation pathways for ensuring its stability and efficacy in pharmaceutical formulation. Despite one of the popularly indicated antacid combination, its simultaneous impurity determination methodologies not well studied. This highlighted the need of current study [13-17].

Currently, pharmaceutical analysts face significant challenges during the separation of impurities. Our main goal was to create a methodology for the simultaneous estimation of esomeprazole and levosulpiride, along with their related compounds in capsules. This method aims to utilize a singular sample processing and chromatographic procedure, minimizing mobile phase utilization and analytical time. The methodology includes performance criteria to accurately quantify both esomeprazole and levosulpiride, as well as their impurities within the specified framework.

This study systematically optimises an analytical method using Box-Behnken design. Independent variables, including column oven temperature, flow rate, and mobile phase pH, were examined alongside dependent factors like resolution, theoretical plate number, and tailing factor. Fractional factorial design efficiently identified key factors, while central composite design explored response surfaces. Statistical analysis ensured robustness, providing insights into variable interactions and yielding a validated methodology for precise chromatographic analysis.

## 2. Materials and Method

### 2.1. Experimental condition

Waters HPLC with empower version 3.0 software and Shimadzu system with lab solution software was used. Minitab software version 17.0 and Design expert software version 11.0 was used. Acetonitrile and Methanol were used of HPLC grade. Levosulpiride and Omeprazole working standards were provided by Zuventus Healthcare Limited. Omeprazole N-Oxide and Omeprazole Related Compound A were supplied by Zuventus Healthcare Limited. All chemicals of AR grade were used. Chromatographic separation of impurities for esomeprazole and levosulpiride were performed by use of gradient programme. Mobile phase A prepared from (80:10:10) Acetonitrile: Water: Buffer (Ammonium acetate 50 mM, pH 5.0 adjusted with Ortho phosphoric acid) and Acetonitrile: Water (80:20) as mobile phase B. Flow rate was 1.0 mL/min$^{-1}$. Gradient programme was set as follow: time in min/% of mobile phase A 0/100, 10/80, 15/80, 30/20, 35/100, 45/100. UV detection was performed at 302 nm and temperature 40°C for column oven was found to be the best for analysis.

### 2.2. Preparation of buffer solution for diluent

Dissolve 5.24 g of Tribasic Sodium Phosphate Dodecahydrate and 7.81 g of Di Sodium Phosphate anhydrous in 1000 mL of water. Adjust the pH 11.0 with Orthophosphoric acid if necessary.

### 2.3. Preparation of diluent

Mix buffer solution for diluent and water in the proportion of 20: 80 & degas.

### 2.4. Reference solution preparation

Accurately weighed about 20.0 mg Omeprazole standard and 37.5 mg of levosulpiride Standard and transferred into 100 mL of clean & dry volumetric flask. Added 70 mL of Methanol and sonicated for about 10 minutes to dissolve. Volume made up to the mark with diluent & mixed well.

Transferred 1.0 mL of solution into a 100 mL of clean & dry volumetric flask. Volume made up to the mark with diluent & mixed well.

## 2.5. Test solution preparation

Twenty capsules were crushed, and a powder equivalent to 80 mg of esomeprazole was transferred to a 200 mL volumetric flask. After adding 20 mL of Methanol, 40 mL of buffer was included, and the mixture was sonicated for 15 minutes with intermittent shaking. The volume was then brought up to the mark with water, thoroughly mixed, and filtered through a 0.45 µm PVDF filter, discarding the initial few milliliters of the filtrate.

## 2.6. Placebo solution preparation

Weighing 550 mg of placebo powder, it was transferred to a 200 mL volumetric flask. Next, 20 mL of Methanol was added, followed by 40 mL of buffer. The mixture was sonicated for 15 minutes with intermittent shaking. The volume was then adjusted to the mark with water, mixed thoroughly, and filtered through a 0.45 µm PVDF filter, discarding the initial few milliliters of the filtrate.

## 2.7. One factor at a time approach

The one factor at a time (OFAT) approach is a method used in experimental design and process optimization. It involves changing one variable or factor at a time while keeping all other variables constant to determine its effect on the outcome. Mobile phase B (Water: Acetonitrile) composition, Flow rate, Column Temperature and Buffer pH were identified as critical factors and its effects were studied.

## 2.8. QbD approach

The quality by design (QbD) approach in analytical development applies the principles of QbD to the development and validation of analytical methods. The goal is to ensure that analytical methods are robust, reliable, and capable of consistently delivering accurate and precise results, which are critical for ensuring product quality in pharmaceutical industries. Resolution between Omeprazole and Omeprazole Related Compound A, Theoretical Plate of esomeprazole and Theoretical Plate of levosulpiride were identified as analytical target profile (ATP) and Buffer pH, Column Oven Temperature and Flow rate were identified as critical method parameters (CMA). Their correlation was studied using Box-Behnken design.

## 2.9. Method validation

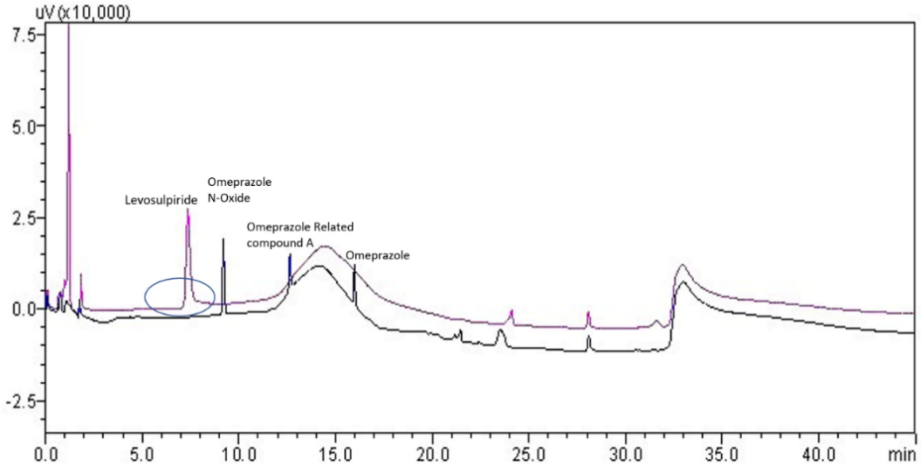
The developed method was validated as per ICH guidelines with respect to parameters like specificity, precision, accuracy, linearity, robustness, ruggedness, solution stability, limit of detection (LOD) and limit of quantification (LOQ). [18-21]

# 3.Result and Discussion

## 3.1. Method development by one-factor-at-a-time (OFAT) approach

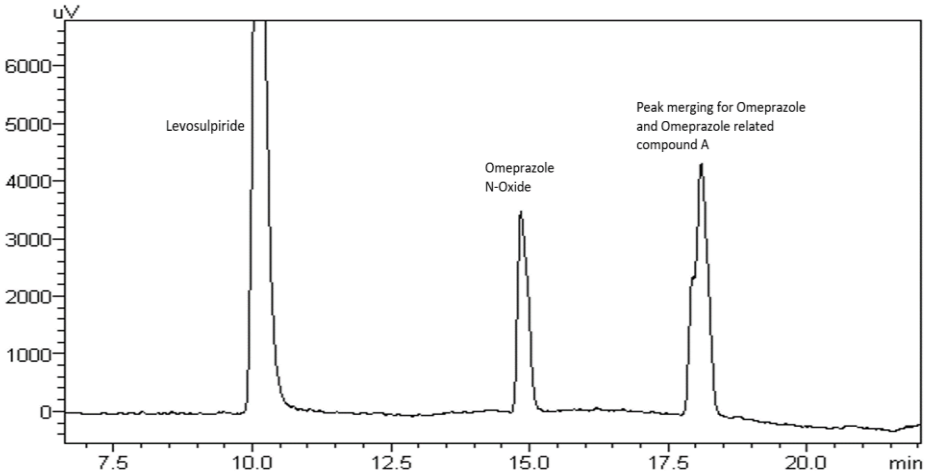
The experimental trials commenced with the utilization of USP reference substances method for esomeprazole capsule dosage form. This entailed employing a mixture of 5.2 mM Sodium phosphate buffer and 31.5 mM Disodium phosphate buffer at a pH of 7.6 within the gradient program. Mobile Phase A consisted of a mixture of Buffer, Acetonitrile, and Water in a ratio of 10:10:80 (v/v), while Mobile Phase B comprised a mixture of buffer, Acetonitrile, and Water in a ratio of 1:80:19 (v/v). The gradient program detailed alterations in the percentage composition of Mobile Phase A as follows: 0 min, 100%; 10 min, 80%; 30 min, 0%; 31 min, 100%; 45 min, 100%. The flow rate was maintained at 1.0 mL/min, and chromatographic separation was conducted using an Inert sustain C18 column (100 mm x 4.6 mm, 3µm particle size). UV detection was performed at 302 nm, with the column oven temperature set at 25 °C. However, an issue arose with the chromatographic profile, as evidenced by improper peak shape and observed peak tailing, particularly in the case of levosulpiride, as depicted in Figure 2.

**Figure 2**
**Overlay of omeprazole imp mixture and levosulpiride standard**



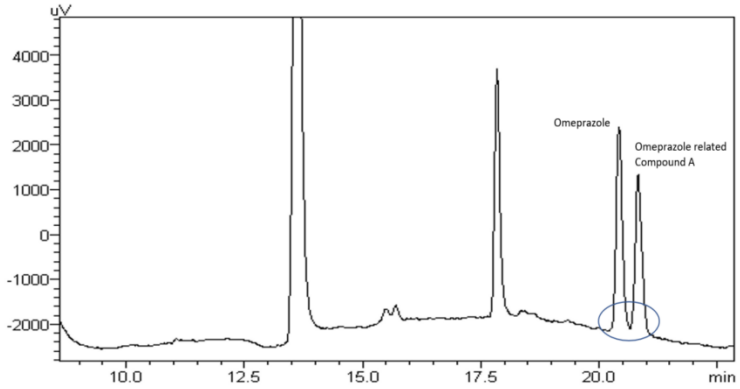

To tackle this issue, we attempted an alternative with the YMC Triart column (150 x 4.6 mm, 5-µm). However, we observed the merging of peaks for Omeprazole and Omeprazole Related Compound A. (Figure 3).

**Figure 3**
**Merging of omeprazole and omeprazole related compound A peaks**

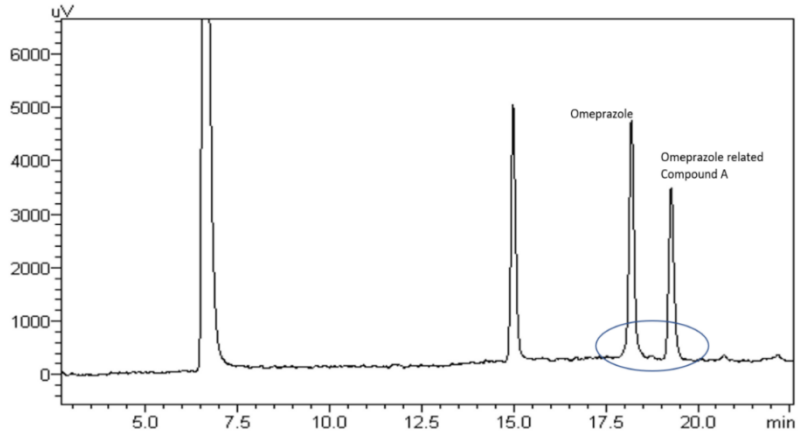



To separate these peaks, several trials were conducted, including adjustments to the gradient, variations in mobile phase composition, changes in column oven temperature, and alterations in buffer pH. Among these approaches, changing the buffer pH yielded the most promising results (Figure 4).

**Figure 4**
**(a) Effect of buffer pH 7.2 on separation of omeprazole and omeprazole related compound A peaks**

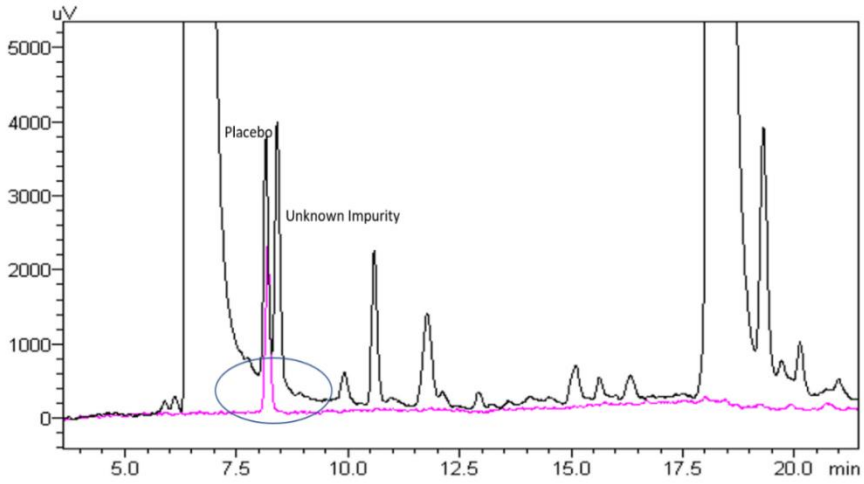



**(b) Effect of buffer pH 6.5 on separation of omeprazole and omeprazole related compound A peaks**



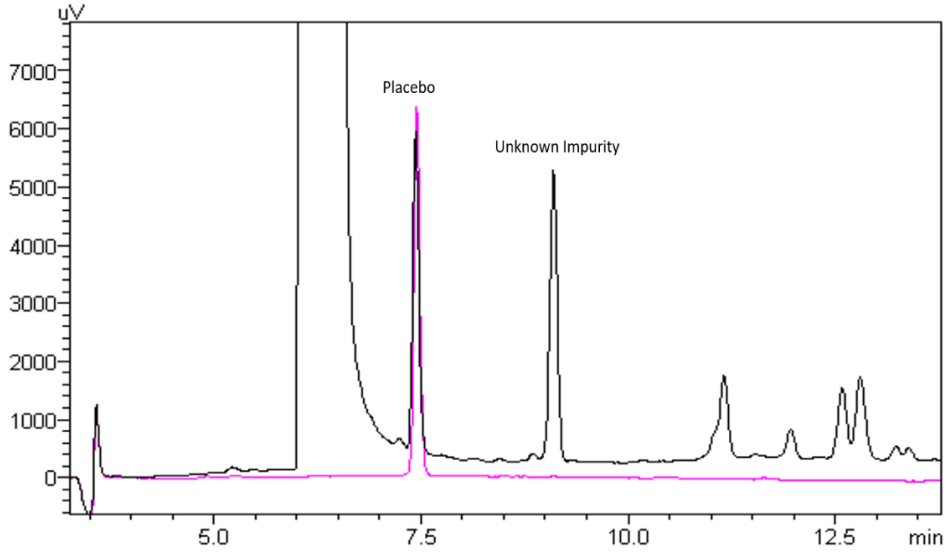

But with this trial it was observed that placebo peak is merging with unknown peak in sample (Figure 5).

**Figure 5**
**Overlay of Placebo chromatogram with sample chromatogram**



To separate placebo from unknown impurity, peak various trials were taken such as change in gradient, Methanol was introduced in mobile phase B but has shown no effect, buffer was excluded from mobile phase B to further simplify method and has shown no effect on separation. Continued with Water: Acetonitrile (20:80) (v/v), column oven temperature was changed. The higher column temperature showed improvement in theoretical plates. Considering this column oven temperature was set at 40 °C. As separation was not observed with these trials column dimension and make was changed. Various columns were tried including YMC triart C18 250 x 4.6 mm, 5 µm, Agilent XDB C18 250 x 4.6 mm, 5 µm, Inertsil ODS 250 x 4.6 mm, 5 µm, Bakerbond C18 250 x 4.6 mm, 5 µm. The later column i.e. Bakerbond C18 250 x 4.6 mm, 5 µm showed separation of placebo peak.

**Figure 6**
**Peak merging of omeprazole and omeprazole related compound A was observed**




To achieve effective separation of Omeprazole and its related compound A, a series of trials were conducted, as detailed in Table 1. Notably, trials conducted within the pH range of 5.5 to 4.5 exhibited optimal resolution, with a discernible enhancement in resolution as the pH of the buffer decreased. Specifically, trials within the pH range of 5.5 to 4.5 demonstrated resolutions exceeding 2.5, a requisite threshold for satisfactory peak separation. However, minimal variation in resolution was observed upon further reduction of pH from 5.0 to 4.5. Consequently, Buffer pH, Column oven temperature and Flow rate were chosen for further optimisation. Upon achieving a satisfactory outcome at a pH of 5.0, the buffer solution was subsequently replaced with a 50 mM solution of Ammonium acetate. It is noted that Acetate buffer exhibits superior buffering capacity within the pH range of 4.0 to 5.5 when compared with Phosphate buffer.

**Table 1**
**Chromatographic trials for separation of omeprazole and omeprazole related compound A**

| Parameter | ELT1 | ELT2 | ELT3 | ELT4 | ELT5 | ELT6 | ELT7 | ELT8 | ELT9 | ELT10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Mobile phase B (Water: Acetonitrile)** | 30:70 | 10:90 | 20:80 | 20:80 | 20:80 | 20:80 | 20:80 | 20:80 | 20:80 | 20:80 |
| **Flow rate** | 1 | 1 | 1.2 | 0.8 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| **Column Temperature** | 40 | 40 | 40 | 40 | 45 | 35 | 40 | 40 | 40 | 40 |
| **Buffer pH** | 6.5 | 6.5 | 6.5 | 6.5 | 6.5 | 6.5 | 6.0 | 5.5 | 5.0 | 4.5 |
| **Results** | | | | | | | | | | |
| **Resolution between Omeprazole and Omeprazole related compound A** | 1.21 | 1.14 | 1.07 | 1.76 | 1.17 | 1.25 | 1.92 | 2.61 | 2.98 | 3.05 |

## 3.2. Optimisation through QbD approach

The method Optimisation study was performed using design of experiment. Minitab application was used. To investigate the influence of Buffer pH (X1), Column Oven Temperature (X2), and Flow rate (X3) on the Resolution between Omeprazole and Omeprazole Related Compound A (Y1), Theoretical Plate of esomeprazole (Y2) and Theoretical Plate of levosulpiride (Y3) a Box-Behnken design employing three factors at three levels was utilized. Experimental runs, detailed in Table 2. [22-25].

**Table 2**
**Box-Behnken design**

| Buffer pH | Column Oven Temperature | Flow rate | Resolution | Theoretical Plate of esomeprazole | Theoretical plates of levosulpiride |
|---|---|---|---|---|---|
| 5.2 | 40 | 1.2 | 2.54 | 141932 | 7972 |
| 5.0 | 40 | 1.0 | 3.06 | 186258 | 22424 |
| 5.0 | 45 | 0.8 | 2.96 | 150468 | 21963 |
| 5.2 | 35 | 1.0 | 2.68 | 111076 | 6725 |
| 5.0 | 35 | 0.8 | 3.11 | 152704 | 21128 |
| 5.0 | 35 | 1.2 | 2.96 | 134535 | 21788 |
| 5.0 | 45 | 1.2 | 2.69 | 182278 | 17773 |
| 5.0 | 40 | 1.0 | 3.05 | 185469 | 22968 |
| 4.8 | 40 | 0.8 | 3.26 | 181698 | 13646 |
| 5.0 | 40 | 1.0 | 3.02 | 186935 | 21241 |
| 4.8 | 45 | 1.0 | 3.11 | 183698 | 6555 |
| 5.2 | 45 | 1.0 | 2.71 | 125698 | 11246 |
| 4.8 | 40 | 1.2 | 2.96 | 187469 | 8938 |
| 4.8 | 35 | 1.0 | 3.21 | 152704 | 13237 |
| 5.2 | 40 | 0.8 | 3.06 | 143596 | 13312 |

A polynomial model for Y was constructed via regression analysis as follows: $Y = b0 + b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X1^2 + b22X2^2 + b33X3^2$. Here, Y represents the response, while X1, X2, and X3 denote Buffer pH, Column Oven Temperature, and Flow rate, respectively. The interaction effect of factors is denoted by X1X2X3, and quadratic effects by $X1^2$, $X2^2$, and $X3^2$. Coefficients (b0, b1, b2, b3, etc.) represent the model parameters. Significance of factors on the response was assessed through p-values of b1–b2-b3, determined via ANOVA. Factors with p-values < 0.05 were deemed significant. Equations are:

Y1 = -26.4 + 10.5 X1 + 0.081 X2 + 9.45 X3 - 1.14 X1*X1 - 0.00282 X2*X2 - 1.07 X3*X3 + 0.0325 X1*X2 - 1.38 X1*X3 - 0.0300 X2*X3 (1)

Y2 = -12384195 + 4376793 X1 + 92815 X2 + 14763 X3 - 428115 X1*X1 - 1032.1 X2*X2 - 135558 X3*X3 - 4093 X1*X2 - 46469 X1*X3 + 12495 X2*X3 (2)

Y3 = -6581660 + 2698251 X1 - 8006 X2+ 60307 X3 - 280828 X1*X1 - 61.5 X2*X2 - 272 X3*X3 + 2801 X1*X2 - 3950 X1*X3 - 1213 X2*X3 (3)

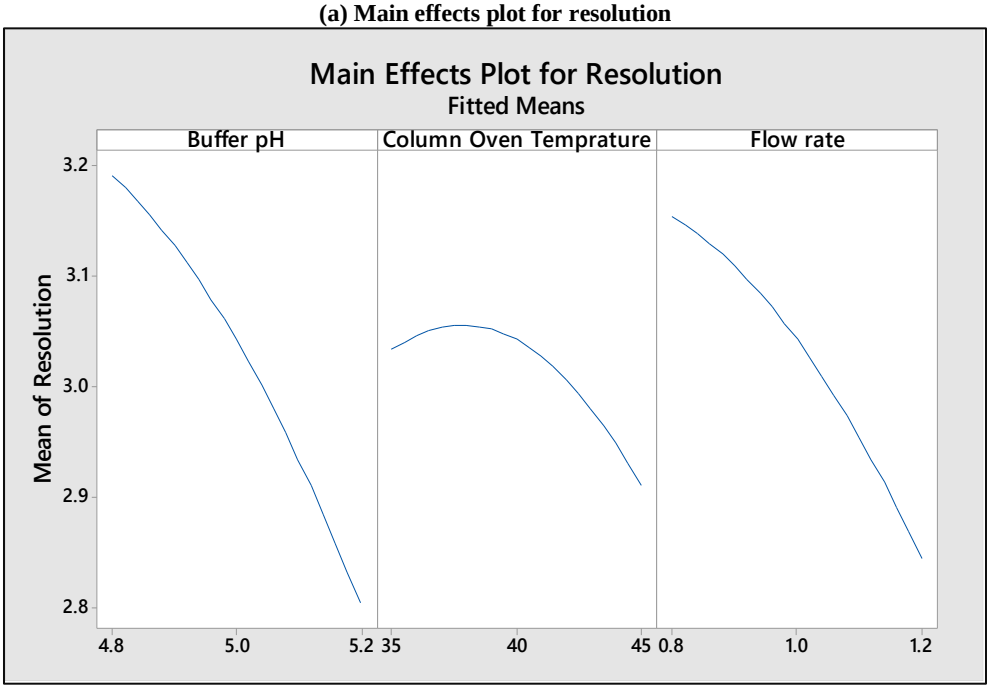
Analysis revealed significant impacts of all factors on Resolution, Theoretical Plate of esomeprazole, and Theoretical Plate of levosulpiride. Notably, all factors exhibited a negative effect on Resolution, consistent with observations from main effect plots (Figure 7). Additionally, interaction effects between Buffer pH and Flow rate were observed on Resolution, with Buffer pH negatively affecting Theoretical Plate of esomeprazole (Y2), and Flow rate negatively impacting Theoretical Plate of levosulpiride (Y3). Moreover, the interaction of all factors significantly influenced Theoretical Plate of esomeprazole, while Theoretical Plate of levosulpiride was unaffected by the interaction between Buffer pH and Flow rate. Refer Table 3.

**Table 3**
**Estimated regression coefficients for resolution between omeprazole and omeprazole related compound A (Y1), theoretical plate of esomeprazole (Y2) and theoretical plate of levosulpiride (Y3) (Quadratic) X1: buffer pH , X2: column oven temperature and X3: flow rate**

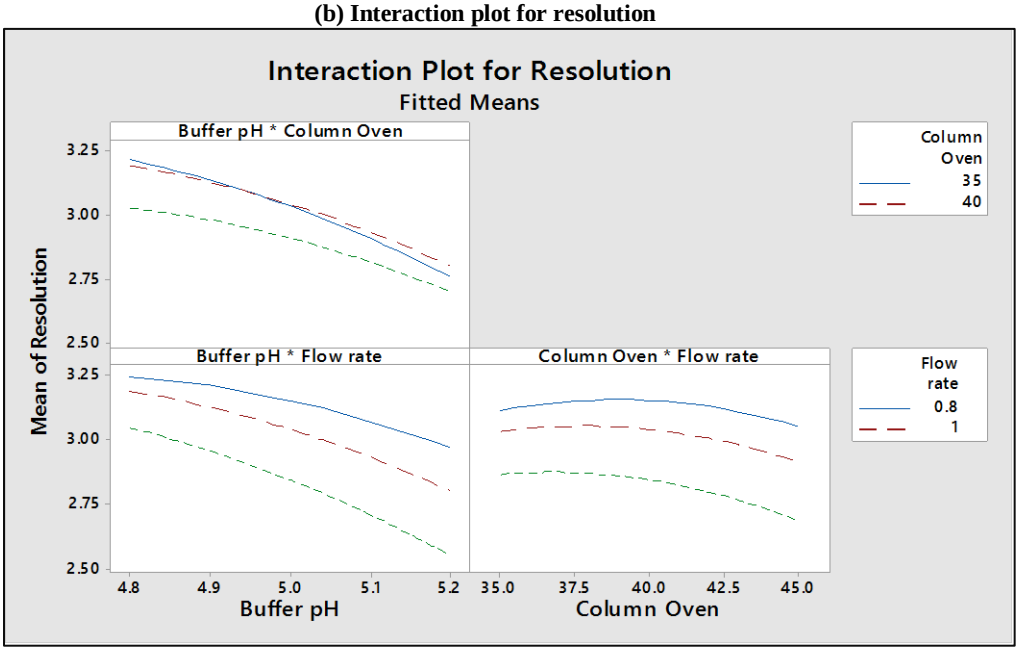
| Factors | Coef | | | SE Coef | | | T | | | P | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Y1 | Y2 | Y3 | Y1 | Y2 | Y3 | Y1 | Y2 | Y3 | Y1 | Y2 | Y3 |
| Constant | 3.0433 | 186221 | 22211 | 0.0567 | 1720 | 685 | 53.69 | 108.24 | 32.44 | 0.000 | 0.000 | 0.000 |
| X1 | -0.1937 | -22908 | -390 | 0.0347 | 1054 | 419 | -5.58 | -21.74 | -0.93 | 0.003 | 0.000 | 0.395 |
| X2 | -0.0613 | 11390 | -668 | 0.0347 | 1054 | 419 | -1.76 | 10.81 | -1.59 | 0.138 | 0.000 | 0.172 |
| X3 | -0.1550 | 2218 | -1697 | 0.0347 | 1054 | 419 | -4.47 | 2.11 | -4.05 | 0.007 | 0.089 | 0.010 |
| X1*X1 | -0.0454 | -17125 | -11233 | 0.0511 | 1551 | 617 | -0.89 | -11.04 | -18.20 | 0.415 | 0.000 | 0.000 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| X2*X2 | -0.0704 | -25802 | -1537 | 0.0511 | 1551 | 617 | -1.38 | -16.64 | -2.49 | 0.227 | 0.000 | 0.055 |
| X3*X3 | -0.0429 | -5422 | -11 | 0.0511 | 1551 | 617 | -0.84 | -3.50 | -0.02 | 0.439 | 0.017 | 0.987 |
| X1*X2 | 0.0325 | -4093 | 2801 | 0.0491 | 1490 | 593 | 0.66 | -2.75 | 4.72 | 0.537 | 0.040 | 0.005 |
| X1*X3 | -0.0550 | -1859 | -158 | 0.0491 | 1490 | 593 | -1.12 | -1.25 | -0.27 | 0.313 | 0.267 | 0.801 |
| X2*X3 | -0.0300 | 12495 | -1213 | 0.0491 | 1490 | 593 | -0.61 | 8.39 | -2.04 | 0.568 | 0.000 | 0.096 |

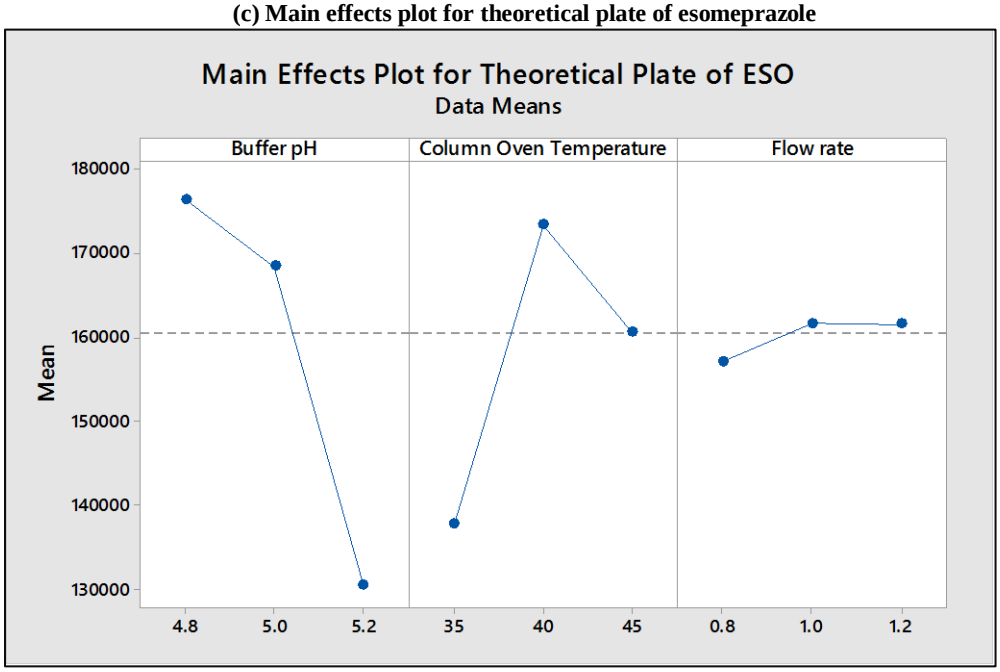
**Figure 7**
**Main effect plots and interaction plots**
**(a) Main effects plot for resolution**



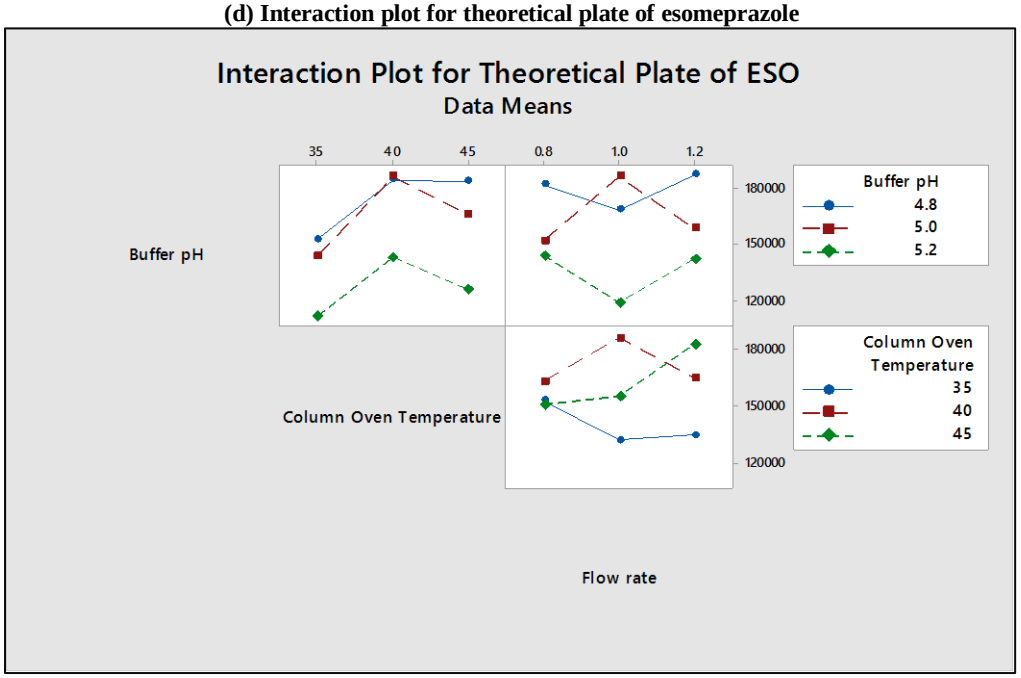

**(b) Interaction plot for resolution**



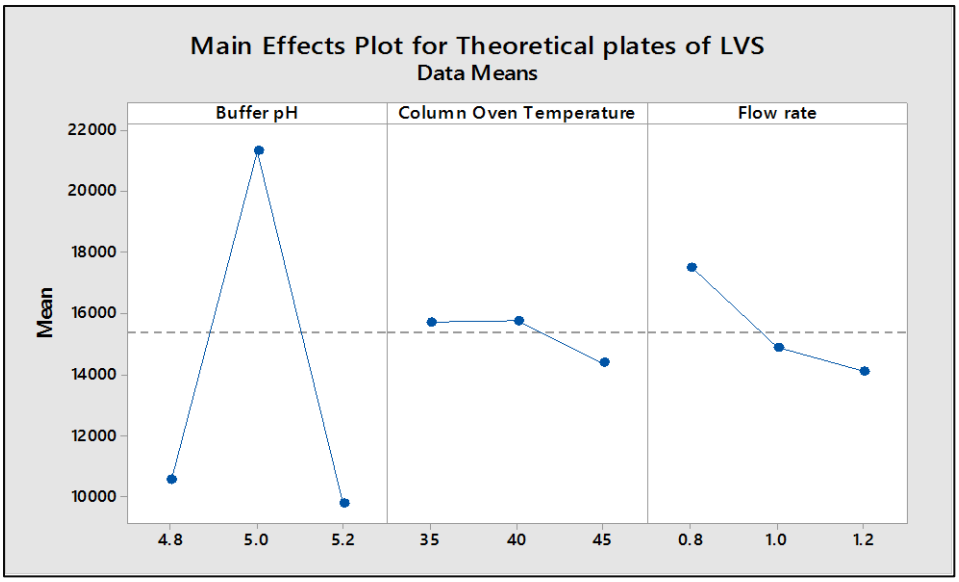
**(c) Main effects plot for theoretical plate of esomeprazole**



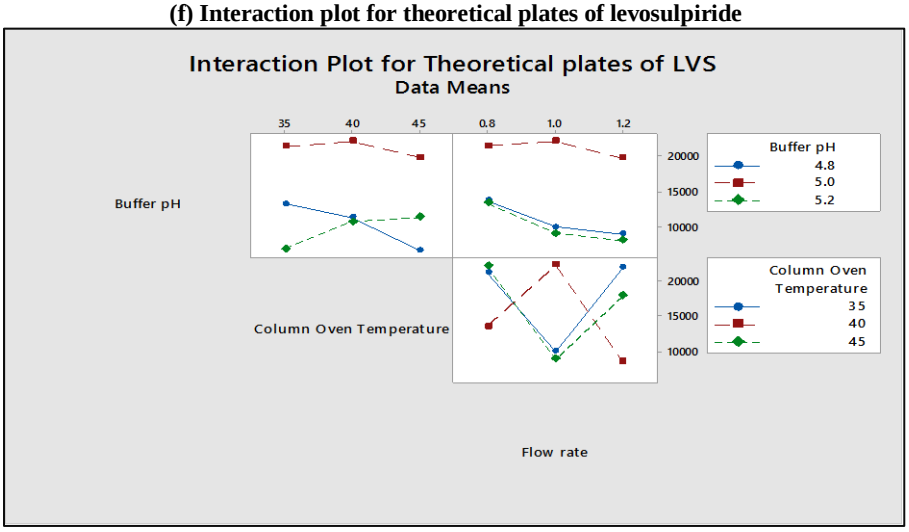

**(d) Interaction plot for theoretical plate of esomeprazole**




**(e) Main effects plot for theoretical plate of levosulpiride**




**(f) Interaction plot for theoretical plates of levosulpiride**




The ANOVA outcomes, as delineated in Table 4, unequivocally validate the statistical significance of the predictive efficacy of the model concerning the resolution of between Omeprazole and Omeprazole Related Compound A, as well as the theoretical plate metrics of esomeprazole and levosulpiride. These results underscore the pivotal role played by the selected independent and response factors within the model, each demonstrating statistical significance in relation to the Related Substances Method. Moreover, the graphical depiction of these influential factors and responses via Counter plots in Figure 8 provides a succinct and visually intuitive representation of their respective impacts within the analytical framework.

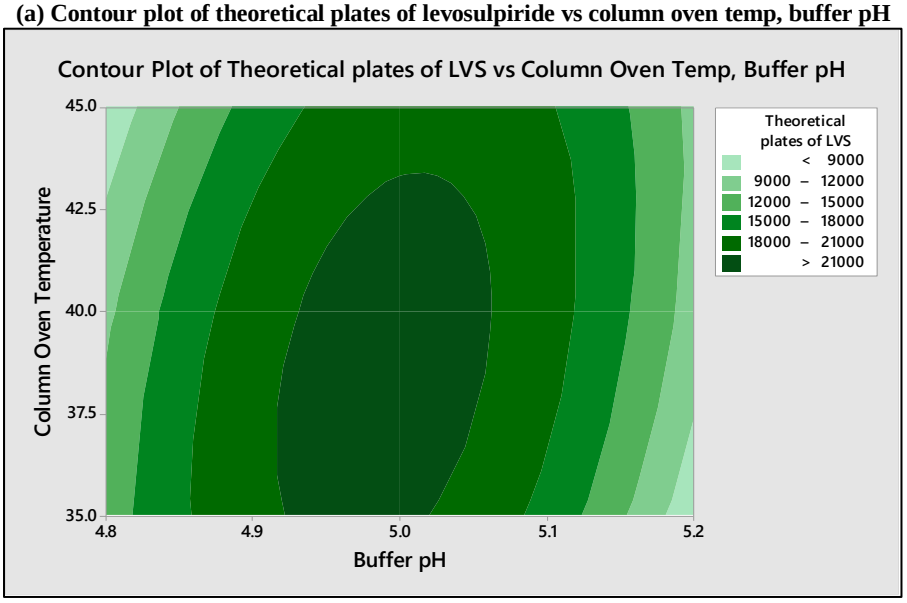
**Table 4**
**ANOVA parameter for evaluation of full model**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Factors** | **DF** | **SS** | **MS** | **F-Ratio** | **Prob>F** | **$R^2$** | **$R^2$ (adj)** | **$R^2$ (Pred)** | **Lack of Fit** |
| Y1 | 9 | 0.57 | 0.06 | 6.58 | 0.000 | 92.22 | 78.21 | 0.00 | 0.027 |

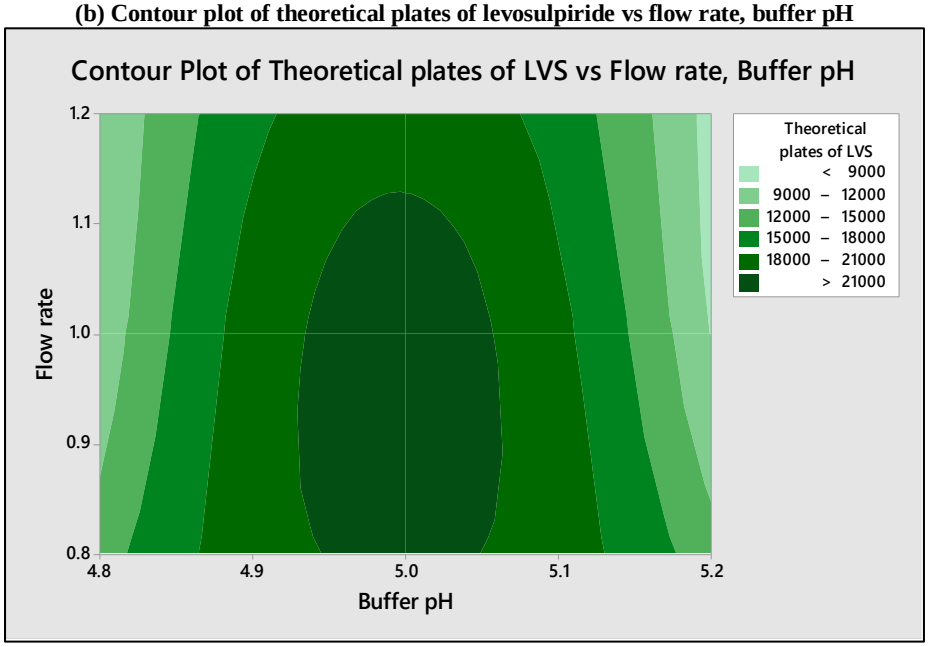
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Y2 | 9 | 9311189102 | 1034576567 | 116.52 | 0.000 | 99.53 | 98.67 | 92.57 | 0.036 |
| Y3 | 9 | 535807876 | 59534208 | 42.32 | 0.000 | 98.70 | 96.37 | 83.22 | 0.313 |
| DF: - Degree of freedom, SS: - sum of square, MS: - Mean square | | | | | | | | | |

**Figure 8**

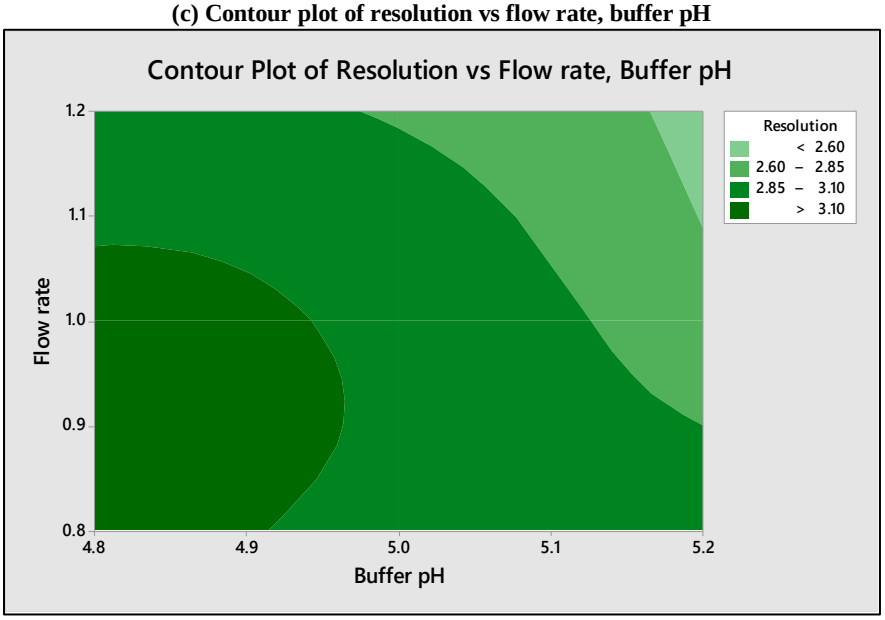
**(a) Contour plot of theoretical plates of levosulpiride vs column oven temp, buffer pH**



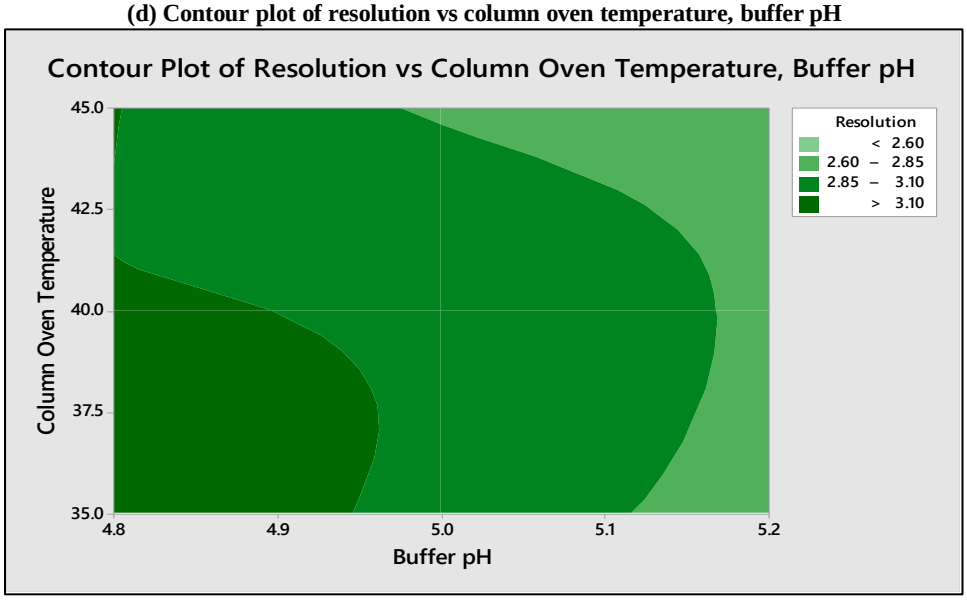

**(b) Contour plot of theoretical plates of levosulpiride vs flow rate, buffer pH**



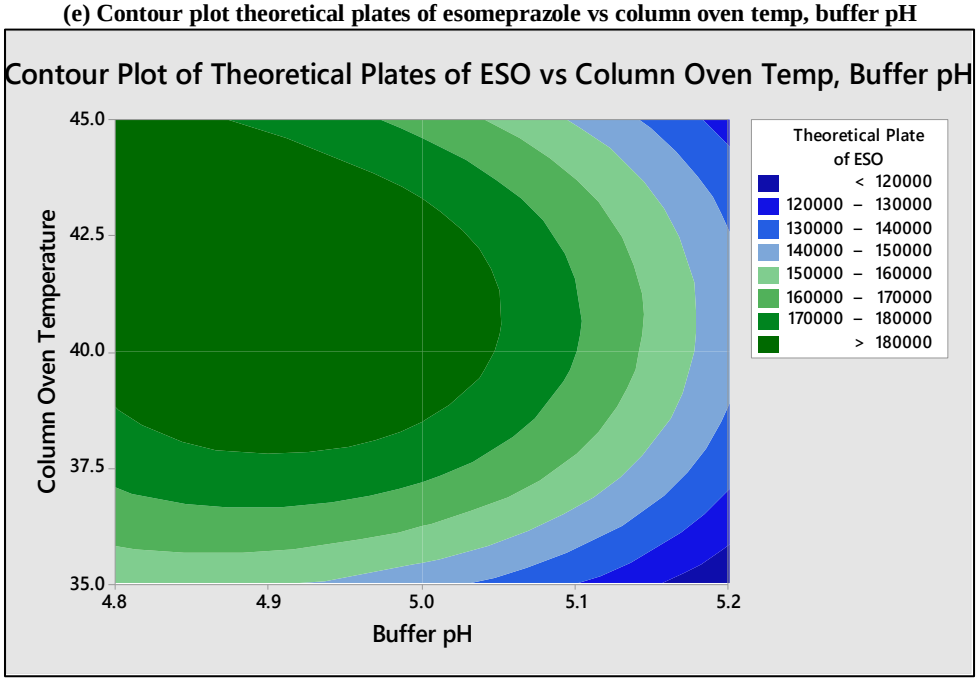
**(c) Contour plot of resolution vs flow rate, buffer pH**



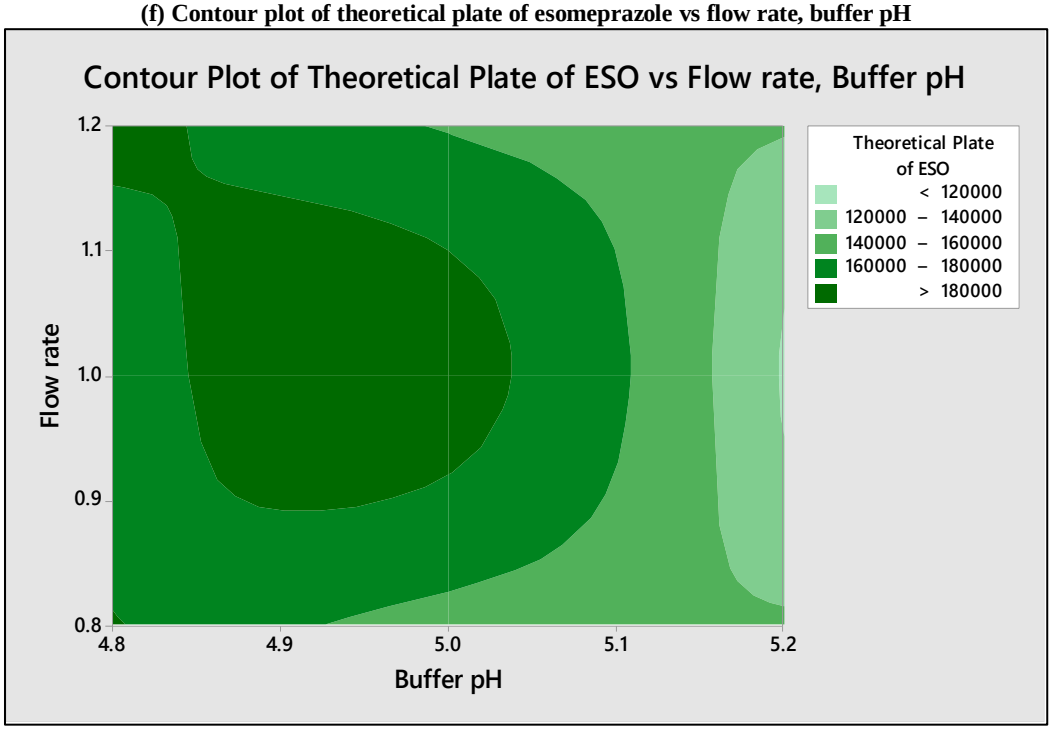

**(d) Contour plot of resolution vs column oven temperature, buffer pH**



**(e) Contour plot theoretical plates of esomeprazole vs column oven temp, buffer pH**




**(f) Contour plot of theoretical plate of esomeprazole vs flow rate, buffer pH**

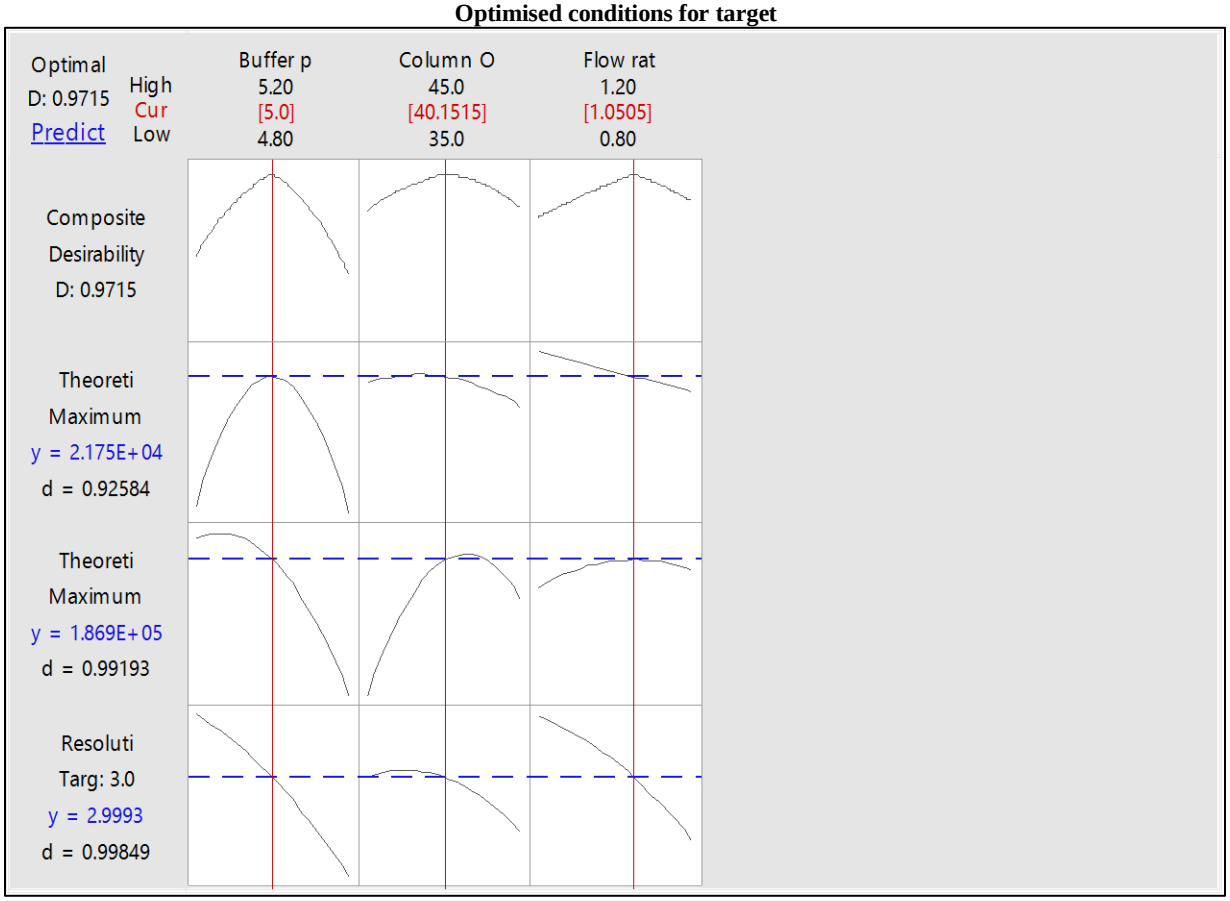



## 3.3. Optimised method parameters

With help of Minitab software optimised conditions was predicted the obtained model was evaluated by performing validation. Optimum condition for parameter suggested is as follows:

X: Buffer pH is 5.0, X2: Column Oven Temperature is 40.15 round of to 40.0 and X3: Flow rate is 1.05 round of to 1.00 (Figure 9).

**Figure 9**
**Optimised conditions for target**




The final method parameters entailed the utilization of an Octadecylsilane Baker Bond column of dimensions 250 x 4.6 mm and 5 µm particle size. The chromatographic method employed a binary mobile phase system. Mobile phase A was composed of a buffer solution containing 50mM solution of Ammonium acetate, adjusted to a pH of 5, followed by a mixture of Acetonitrile and Water in a volumetric ratio of 10:10:80 (Buffer: Acetonitrile: Water, v/v/v). On the other hand, mobile phase B comprised a mixture of Acetonitrile and Water in a volumetric ratio of 80:20 (v/v).

A gradient elution program was meticulously designed to optimize separation. Initially, the composition was 100% mobile phase A. Subsequently, a linear decrease to 80% mobile phase A was implemented over 10 minutes, maintaining this composition until 15 minutes. Then, a gradual transition to 20% mobile phase A was executed by 30 minutes. At 35 minutes, the mobile phase composition was reverted to 100% mobile phase A and held until the completion of the run at 45 minutes.

The flow rate throughout the chromatographic analysis was maintained at 1.0 mL/min, with UV detection set at 302 nm to facilitate precise detection and quantification of analytes.

## 3.4. Method validation

### *3.4.1. Selectivity and specificity*

Prepared the blank solution, placebo solution, standard solution and test solution as per the optimised method. Representative chromatograms obtained from blank solution and blank spiked with LOQ standard of each analyte and impurity solution are presented in Figures 10 to 18. There were no significant interferences observed at the retention time of each analyte and impurity in blank and placebo solution (Table 5).

**Figure 10**
**Blank**

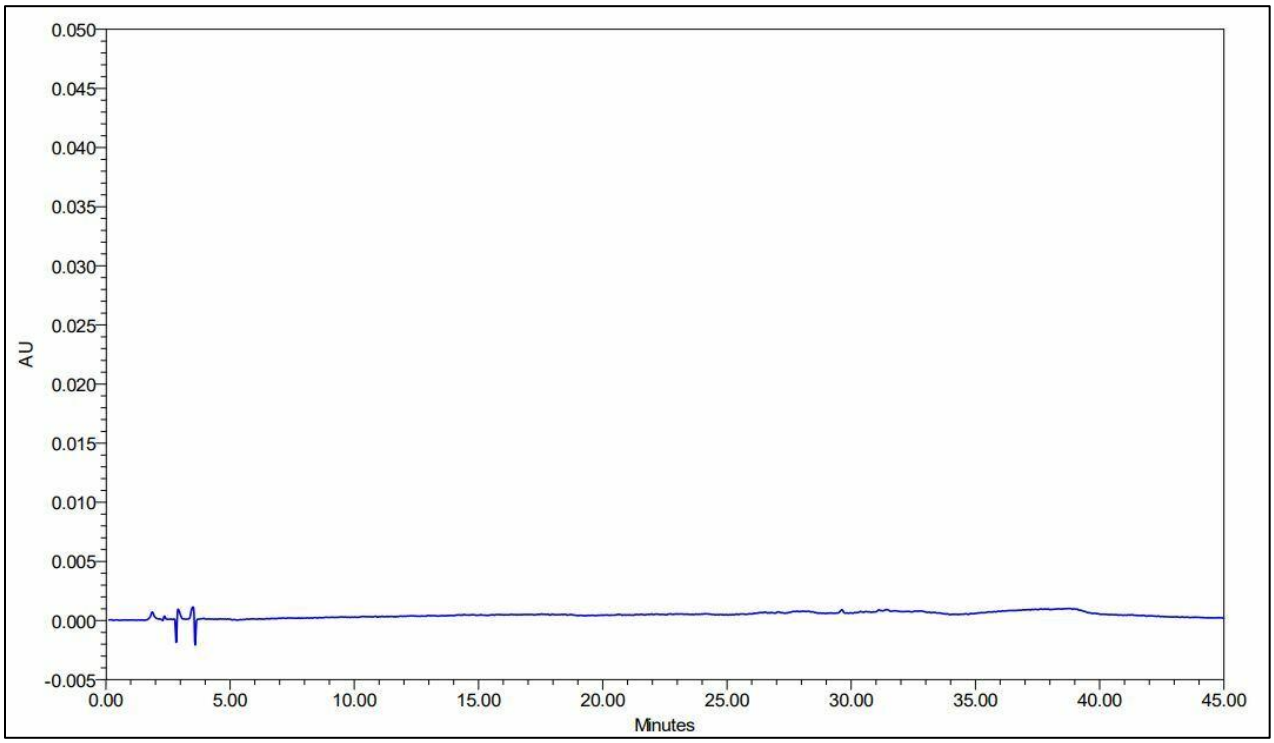



**Figure 11**
**Placebo**

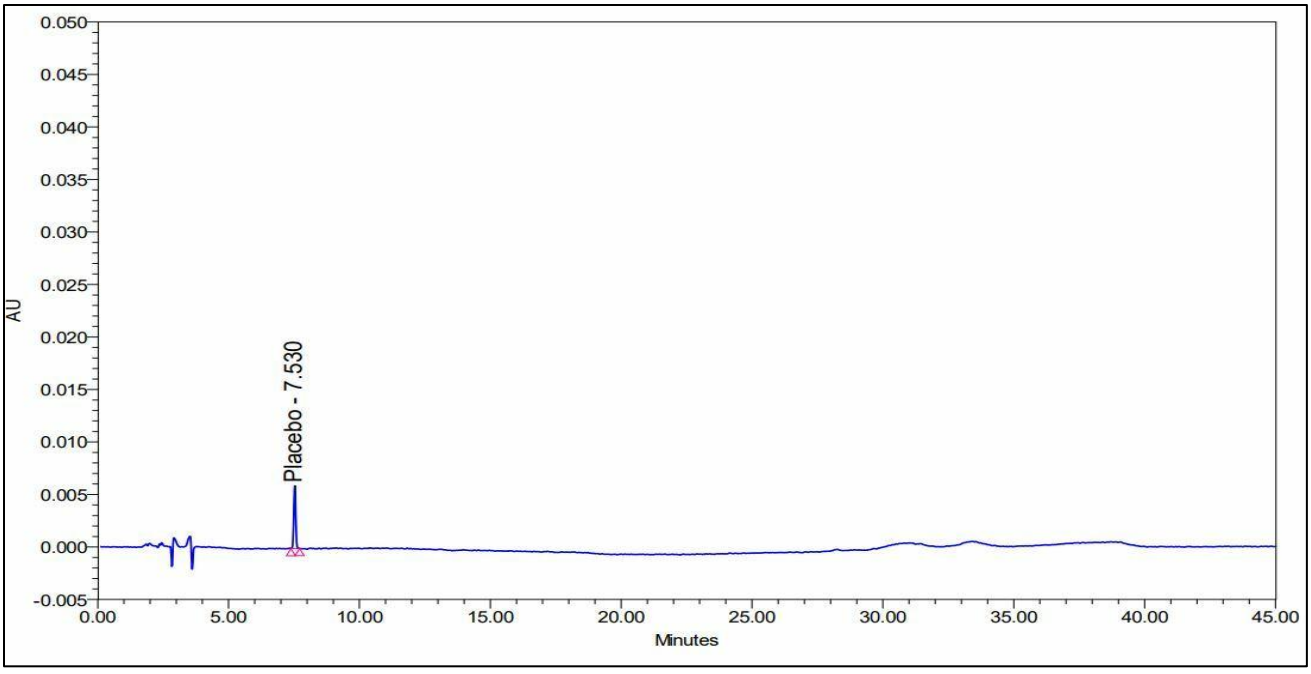


**Figure 12**
**Selectivity standard solution**

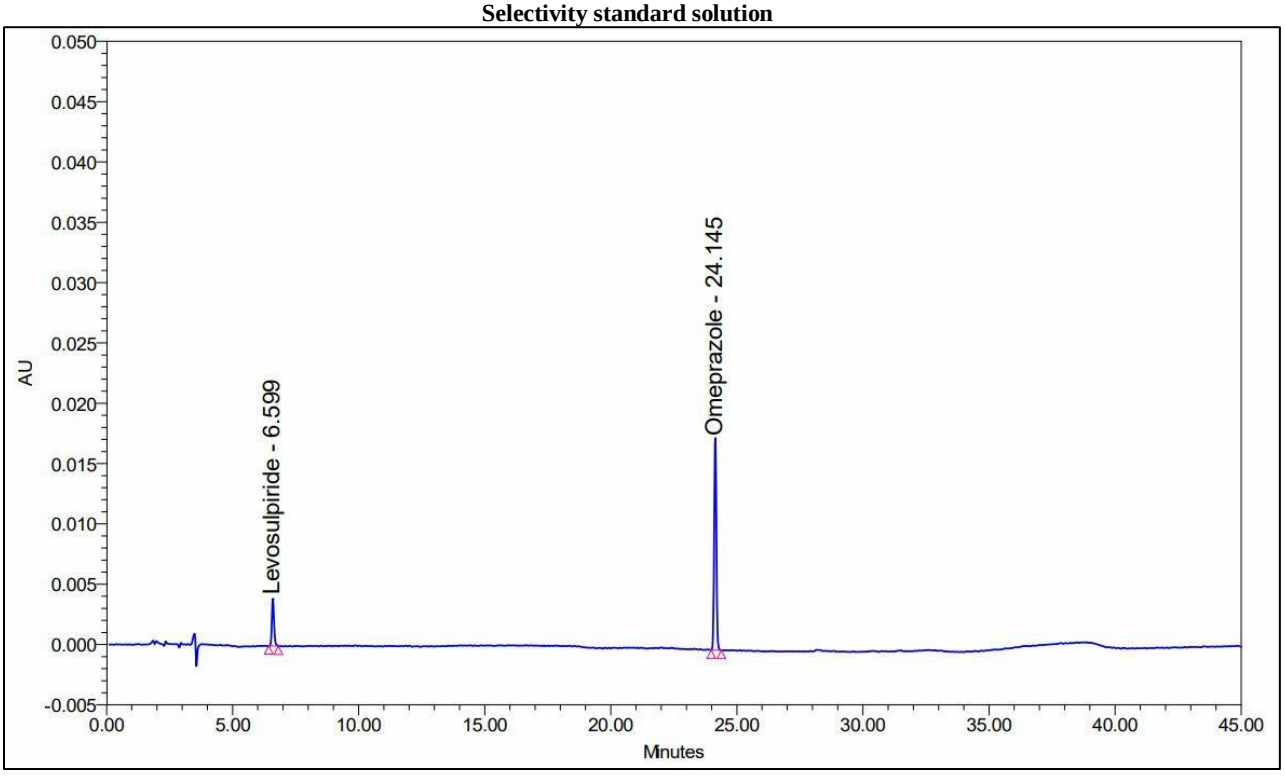



**Figure 13**
**Selectivity levosulpiride ID solution**

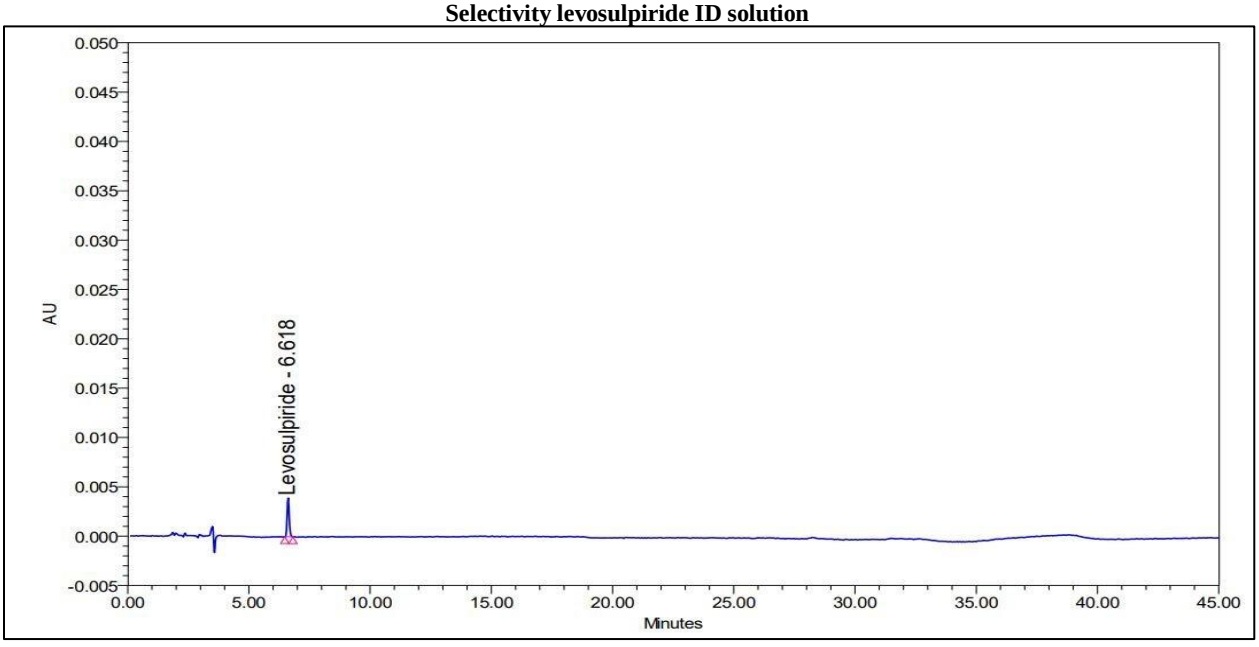


**Figure 14**
**Selectivity omeprazole ID solution**

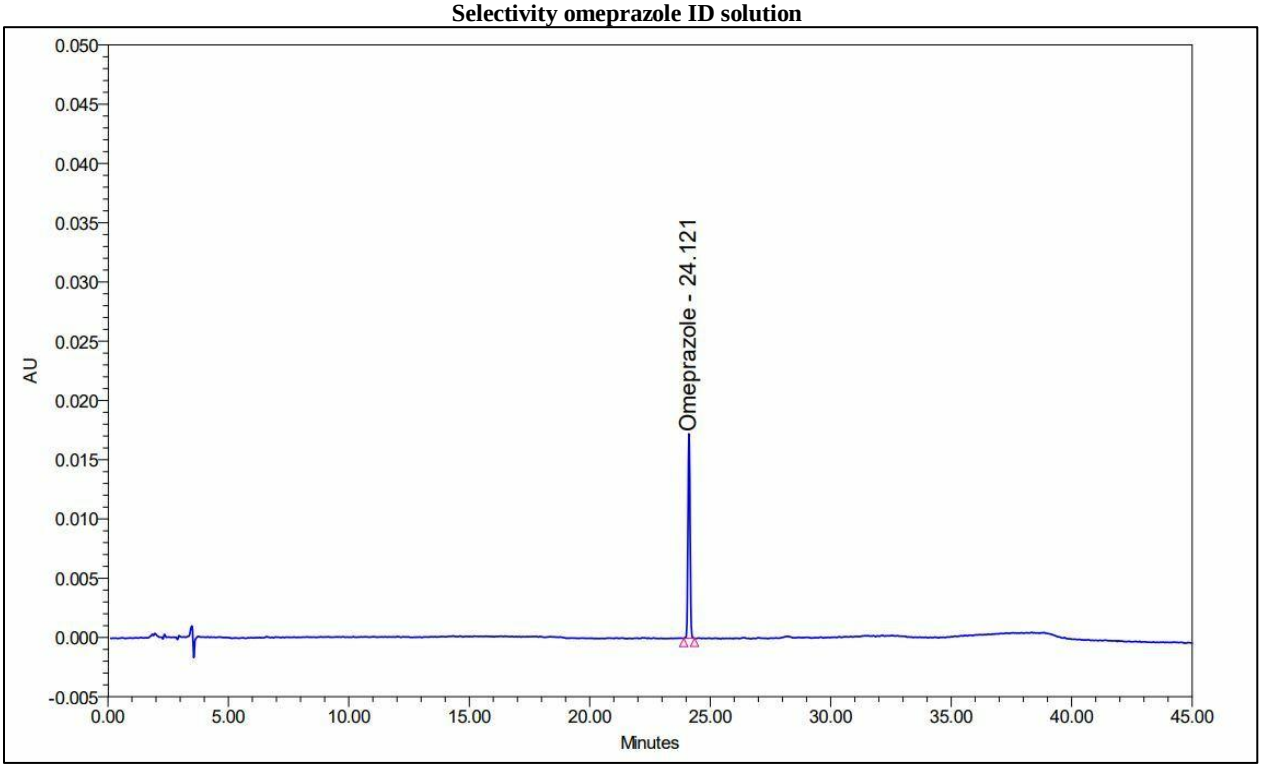



**Figure 15**
**Selectivity omeprazole N-Oxide ID solution**

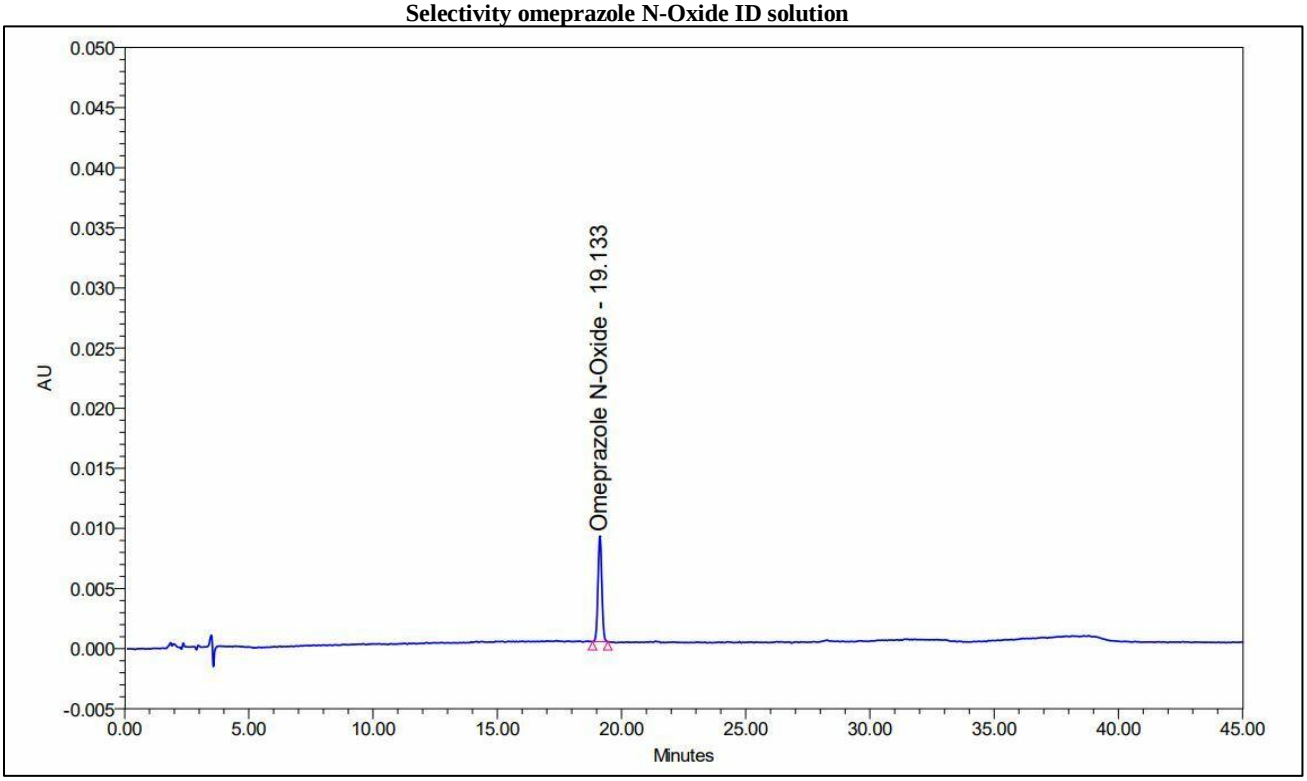


**Figure 16**
**Selectivity omeprazole related compound A solution**

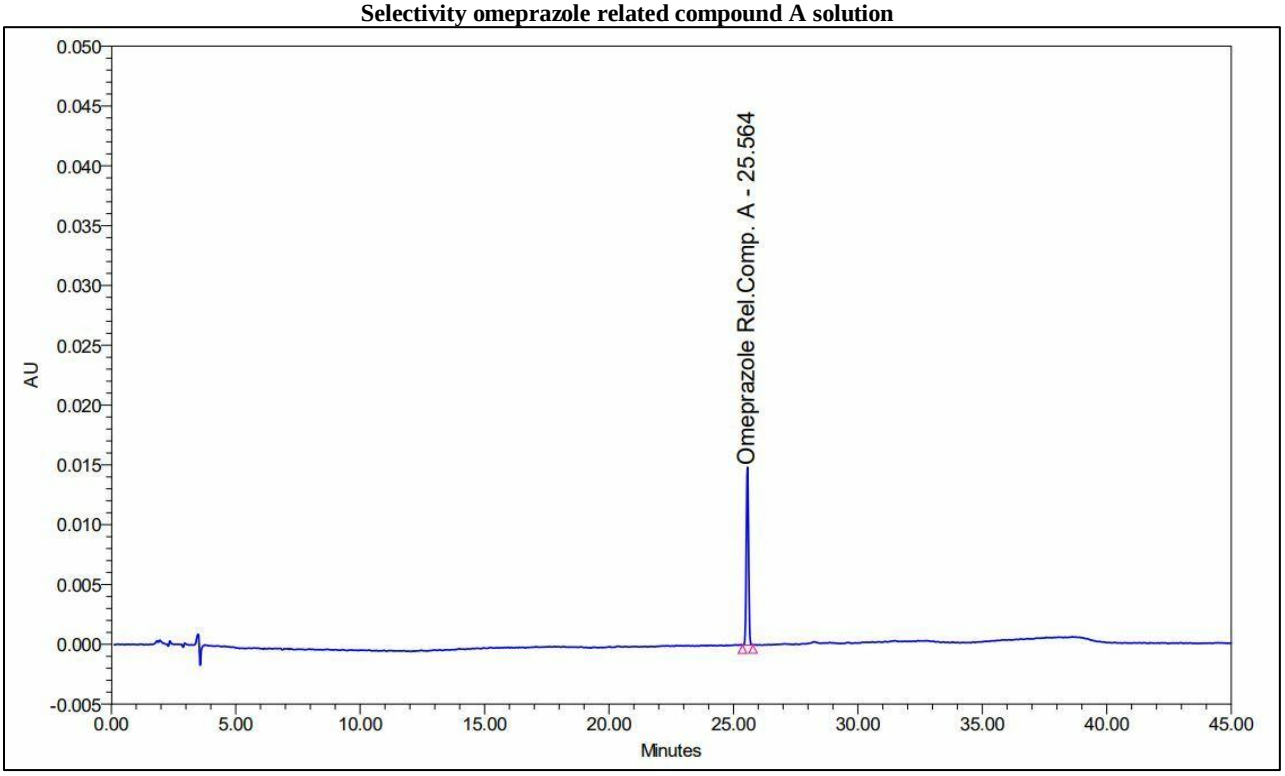



**Figure 17**
**Selectivity test solution**

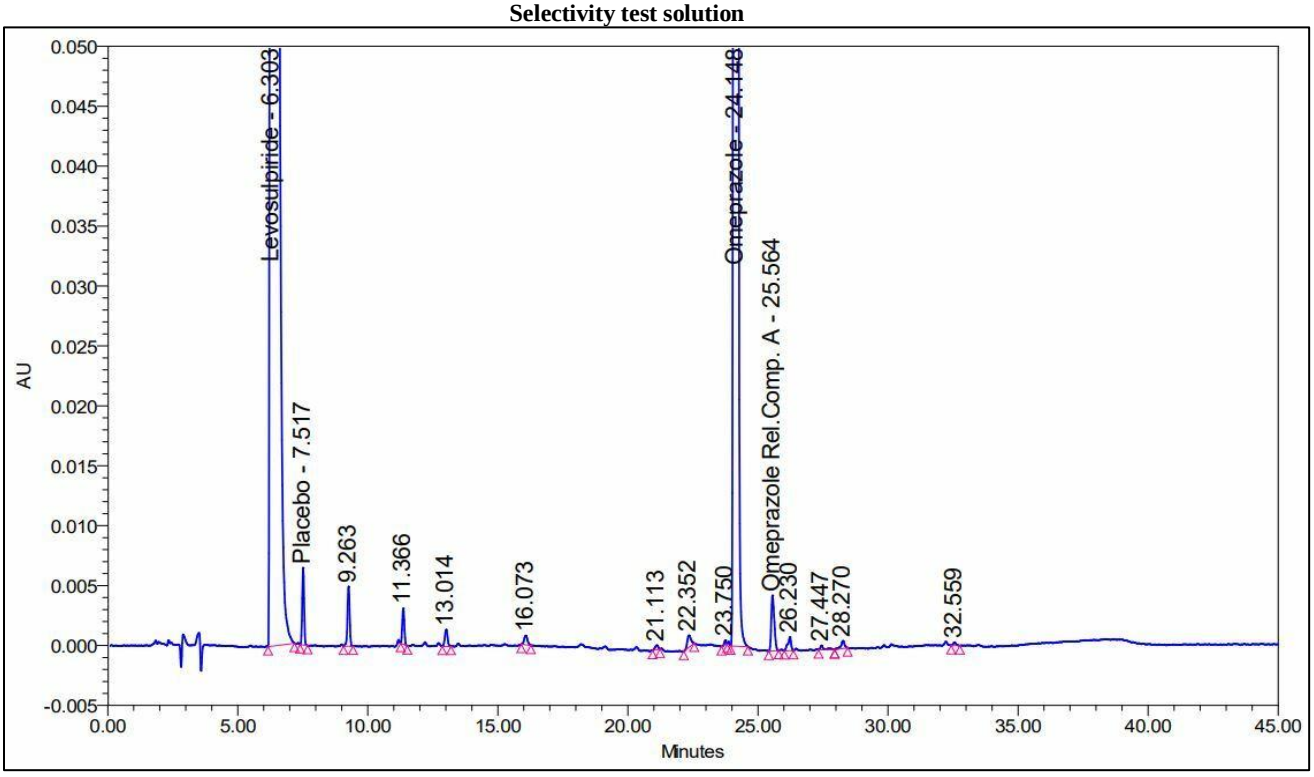


**Figure 18**
**Selectivity impurity spike test solution**

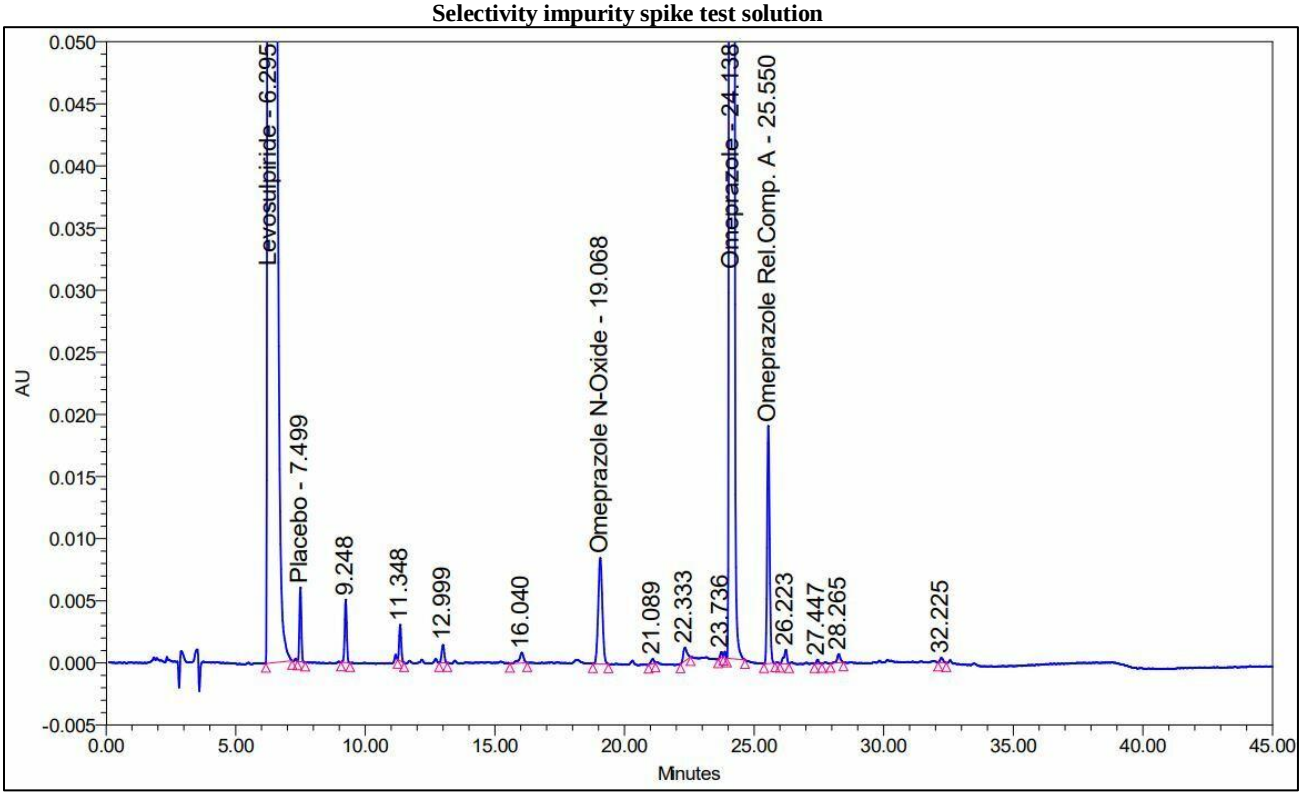



**Table 5**
**Peak purity of impurity solutions and test solution**

| Sample details | Retention time | Peak purity Threshold | Peak purity Angle | Peak purity |
|---|---|---|---|---|
| Test solution | | | | |
| Levosulpiride | 6.30 | 1.007 | 0.144 | Pass |
| #: Omeprazole | 24.15 | 1.003 | 0.139 | Pass |
| Impurity Spike test solution | | | | |
| Omeprazole N-Oxide impurity | 19.07 | 1.353 | 0.471 | Pass |
| Omeprazole Related compound A impurity | 25.55 | 1.241 | 0.809 | Pass |
| Identification solution | | | | |
| Levosulpiride | 6.62 | 1.613 | 0.614 | Pass |
| Omeprazole N-Oxide impurity | 19.13 | 1.375 | 0.504 | Pass |
| Omeprazole | 24.12 | 1.282 | 0.339 | Pass |
| Omeprazole Related compound A impurity | 25.56 | 1.321 | 0.327 | Pass |

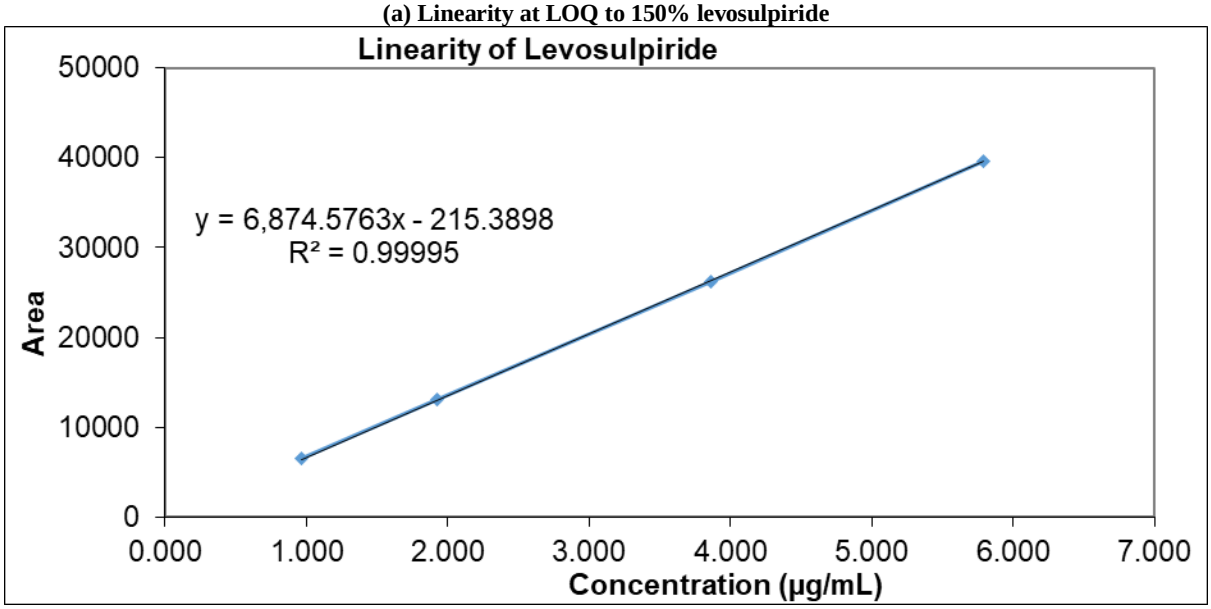
#: Diluted solution

### *3.4.2. Linearity*

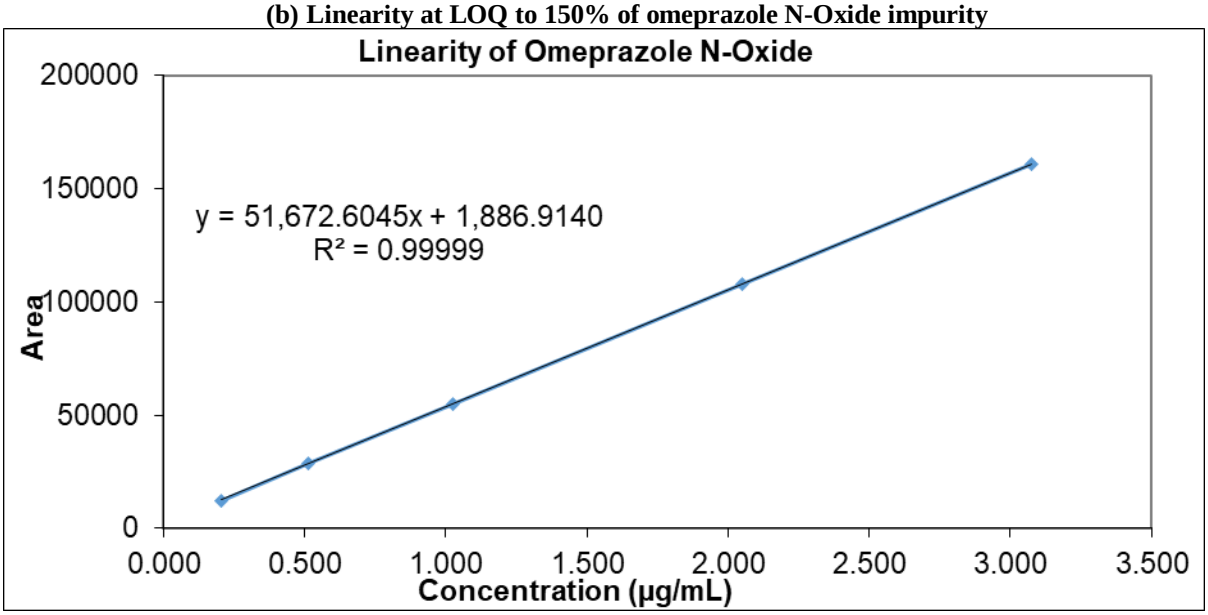
Prepared a series of linearity standard solutions of levosulpiride, Omeprazole N-Oxide impurity, Omeprazole and Omeprazole related compound A impurity over a range starting from the 0.3125 % to 150% of specification limit concentration. The linearity graph at LOQ to 150 % of standard concentration for levosulpiride (0.965 ppm to 5.790 ppm), Omeprazole N-Oxide impurity (0.205 ppm to 3.074 ppm), Omeprazole (0.101 ppm to 3.040 ppm) and Omeprazole related compound A impurity (0.194 ppm to 2.915 ppm) are presented in Figure 19. The method was found linear in the range of LOQ % to 150 % of specified concentration of the corresponding compound.Figure 19A) Linearity at LOQ to 150% levosulpiride, B) Linearity at LOQ to 150% of Omeprazole N-Oxide impurity, C) Linearity at LOQ to 150% of Omeprazole, D) Linearity at LOQ to 150% of Omeprazole related compound A impurity.

**Figure 19**

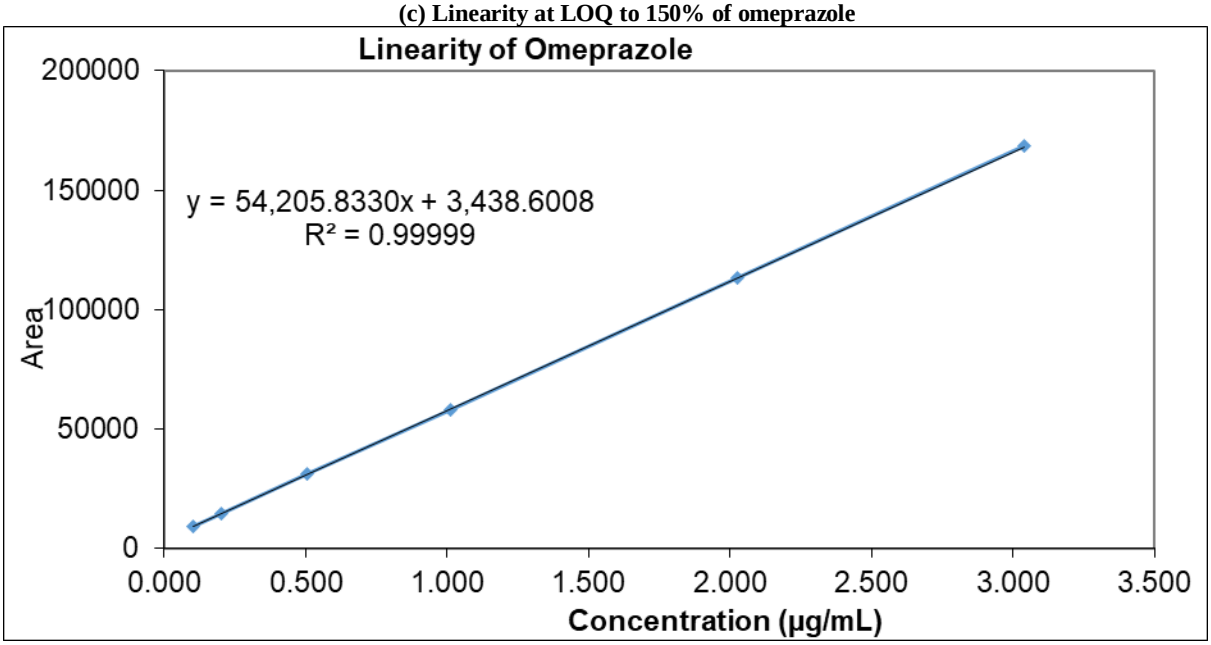
**(a) Linearity at LOQ to 150% levosulpiride**



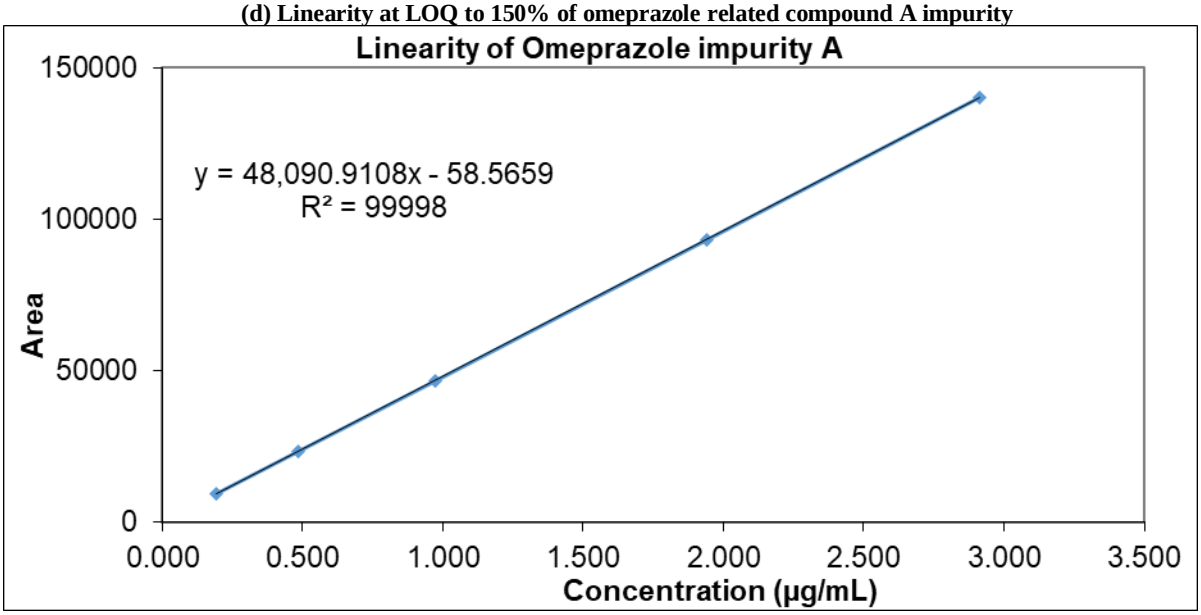

**(b) Linearity at LOQ to 150% of omeprazole N-Oxide impurity**



**(c) Linearity at LOQ to 150% of omeprazole**




**(d) Linearity at LOQ to 150% of omeprazole related compound A impurity**




### *3.4.3. Accuracy*

All the impurities were spiked at LOQ, 50%, 100% and 150% levels of specification concentration of esomeprazole and levosulpiride. Results are tabulated in Table 6.

**Table 6**
**Recovery for omeprazole related compound A and omeprazole N-Oxide impurity at LOQ, 50%, 100% and 150%, respectively**

| % Level | Omeprazole Related compound A (% RSD) | Omeprazole N-Oxide impurity (%RSD) |
|---|---|---|
| LOQ | 101.64 (1.56) | 104.69 (3.40) |
| 50 | 100.79 (0.35) | 100.40 (0.46) |

| | | |
|---|---|---|
| 100 | 98.84 (0.08) | 99.13 (0.28) |
| 150 | 100.49 (0.47) | 100.69 (0.49) |

### *3.4.4. Precision*

Prepared blank solution, placebo solution, standard solution and six preparations of test solution as per optimised method. The % RSD of specified impurity, any unspecified impurity & total impurities of six test solutions of each of was calculated. The results are tabulated in Table 7 & 8.

**Table 7**
**Results of precision**

| **Method Precision** | | | | |
|---|---|---|---|---|
| Parameter | % Omeprazole N-Oxide impurity | % Omeprazole Rel. comp. A | % Any unspecified impurity | % Total impurities |
| Mean | BDL | 0.201 | 0.130 | 0.690 |
| SD | NA | 0.009 | 0.001 | 0.012 |
| % RSD | NA | 4.38 | 1.13 | 1.74 |
| **Intermediate Precision** | | | | |
| Parameter | % Omeprazole N-Oxide impurity. | % Omeprazole Rel. comp. A | % Any unspecified impurity | % Total impurities |
| Mean | BDL | 0.205 | 0.142 | 0.652 |
| SD | NA | 0.003 | 0.003 | 0.036 |
| % RSD | NA | 1.55 | 1.93 | 5.57 |

**Table 8**
**Results of LOQ precision**

| Compound | LOD (% / ppm) | LOQ (% / ppm) | LOQ Precision (% RSD) |
|---|---|---|---|
| Omeprazole | 5% / 0.101ppm | 2.5% / 0.051ppm | 1.13 |
| Levosulpiride | 25% / 0.965ppm | 10% / 0.386ppm | 1.04 |
| Omeprazole Related compound A | 10% / 0.194ppm | 5% / 0.097ppm | 1.38 |
| Omeprazole N-Oxide Impurity | 10% / 0.205ppm | 5% / 0.102ppm | 1.19 |

### *3.4.5. Force degradation Study (Table no. 9)*

**Table 9**
**Results of forced degradation study**

| Sr. No. | Stress condition | % Omeprazole N-Oxide imp. | % Omeprazole Rel. comp. A | % Any unspecified impurity | % Total impurities |
|---|---|---|---|---|---|
| 1 | As such sample | BDL | 0.192 | 0.134 | 0.625 |
| 2 | Acid degradation (10 mL 0.1 M HCl, kept sample solution in water bath at 70°C for 60 minutes) | BDL | 0.188 | 3.159 | 8.599 |
| 3 | Alkali degradation (10 mL 0.1 M NaOH, kept sample solution in water bath at 70°C for 60 minutes.) | BDL | 0.199 | 0.347 | 0.986 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | Oxidation degradation (10mL, 1.0% H2O2, kept sample solution in water bath at 70°C for 60 minutes.) | BDL | 1.846 | 5.128 | 10.182 |
| 5 | Heat degradation (solid State) (Exposed the sample at 70°C for 24 hours in oven.) | BDL | 0.209 | 0.139 | 0.698 |
| 6 | Photolytic degradation (kept in a photo stability chamber for exposure of about 1.2 million-lux hours and near UV at 200-Watt hrs/m2.) | BDL | 0.208 | 0.140 | 0.775 |
| 7 | Humidity degradation (Exposed the sample at 40°C/75% RH for 24 hours.) | BDL | 0.207 | 0.146 | 0.762 |
| 8 | Hydrolysis degradation (10 mL Water, kept sample solution in water bath at 70°C for 60 minutes) | BDL | 0.204 | 0.138 | 0.725 |

### *3.4.6. Solution stability (Table no.10)*

**Table 10**
**Results of analytical solution stability**

| Test No. | Omeprazole N-Oxide impurity | Omeprazole Rel. comp. A | % Any unspecified impurity | % Total impurities |
|---|---|---|---|---|
| Initial | BDL | 0.208 | 0.131 | 0.686 |
| 12 Hours | BDL | 0.197 | 0.131 | 0.727 |
| 24 Hours | BDL | 0.193 | 0.132 | 0.743 |
| 36 Hours | BDL | 0.191 | 0.134 | 0.773 |
| 48 Hours | BDL | 0.188 | 0.135 | 0.747 |
| 60 Hours | BDL | 0.185 | 0.153 | 0.805 |
| 72 Hours | BDL | 0.181 | 0.184 | 0.812 |
| Mean | BDL | 0.192 | 0.143 | 0.756 |
| SD | NA | 0.009 | 0.020 | 0.044 |
| % RSD | NA | 4.61 | 13.81 | 5.87 |

### *3.4.7. Robustness*

Robustness was performed by slightly and deliberately changing various method parameters such as Buffer pH, Column oven temperature, Flow rate. Whereas change in analyst, change in HPLC system, change in HPLC column was employed to study the ruggedness of method. The % RSD was found <2.0 with all the changes which established the method robustness and ruggedness.

Optimised method obtained from QbD was successfully validated as per ICH guidelines and further can be used for testing of product at QC.

## 4. Conclusion

A simple, Accurate, precise method was developed and validated for the simultaneous estimation of degradation products of esomeprazole and levosulpiride in capsule dosage form. The method development supported with a quality by design (QbD) approach that offered a systematic optimization strategy for critical method parameters. The ANOVA results clearly establish the statistical significance of the model's predictive effectiveness. Chromatographic conditions demonstrated as optimum using Minitab software for Box-Behnken design were validated. . Results derived from validation were found within acceptance criteria stated in current ICH guidelines (Q2R2).

The technique's ability to distinguish esomeprazole from its contaminants, as demonstrated in the specificity evaluation, is crucial for guaranteeing precise and dependable outcomes in pharmaceutical quality assurance. The formulation of a response function and compliance with established acceptance criteria in the linearity profile further underscore the technique's precision and reliability. Additionally, the method's stability, accuracy, and tolerance ranges, as described in the comprehensive summary, bolster its repeatability and reproducibility, making it exceptionally suitable for routine evaluation. The adherence to specified accuracy benchmarks accentuates its precision and relevance for quantitative analysis. Furthermore, the investigation into induced degradation not only affirms the method's specificity but also yields significant insights into the behavior of levosulpiride

and esomeprazole under extreme conditions, thereby enriching our understanding of their stability-indicating characteristics.

In conclusion, the meticulous design and validation of this method for levosulpiride, esomeprazole, and their contaminants establish it as an essential tool for drug investigation and quality assurance. Its precision, stability-indicating characteristics, and ability to manage various impurity profiles and concentrations highlight its reliability and relevance across a spectrum of analytical contexts. This technique empowers researchers and analysts in the drug industry to ensure the safety and efficacy of treatments containing levosulpiride and esomeprazole, ultimately improving patient outcomes globally.

## Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

## Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

## Data Availability Statement

Data available on request from the corresponding author upon reasonable request.

## Author Contribution Statement

**Vikram Gharge:** Conceptualization, Supervision, Project administration; **Satish Jadhao:** Conceptualization, Methodology, Supervision, Project administration; **Balasaheb Jadhav:** Methodology; **Pranav Bang:** Software, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization; **Gurudatt Hendge:** Validation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization; **Nayan Jadhav:** Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization; **Rutuja Mulik:** Formal analysis, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization; **Shubham Bhange:** Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization; **Laxman Ingole:** Formal analysis, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization.

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