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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 × 4.6 mm, 5 µm column. Mobile phase A consists of buffer (ammonium acetate 50 mM) pH 5.0: Acetonitrile: Water, in ratio 10:10:80 (v/v), whereas mobile phase B consists of acetonitrile and water in ratio 80:20 (v/v). Gradient program was set as follows: time in min/% of mobile phase A 0/100, 10/80, 15/80, 30/20, 35/100, and 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 approach, offering a systematic optimization strategy employing Box-Behnken design. The developed method was aligned and validated according to the ICH guidelines.

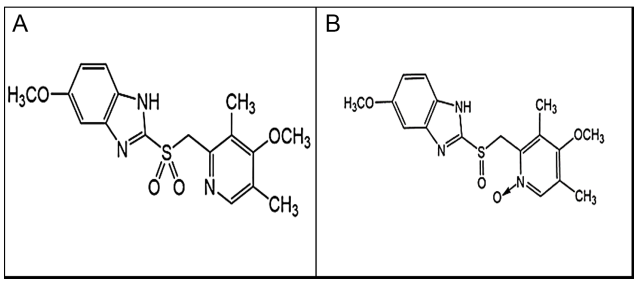
Keywords: esomeprazole, levosulpiride, degradation products, validation, quality by design

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 conditions [3]. Furthermore, light exposure induces photodegradation, 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 in Figure 1.

Figure 1
Omeprazole-related compounds 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, either

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independently or in combination with other pharmaceutical drugs [7–12]. 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 are 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 optimizes 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

Water HPLC with Empower version 3.0 software and Shimadzu system with lab solution software were used. Minitab software version 17.0 and Design Expert software version 11.0 were used. Acetonitrile and methanol were used for 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 was performed by use of gradient program. 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 program was set as follows: time in min/% of mobile phase A 0/100, 10/80, 15/80, 30/20, 35/100, and 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 disodium 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 min 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 min 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 min 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 (OFAT)

The 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

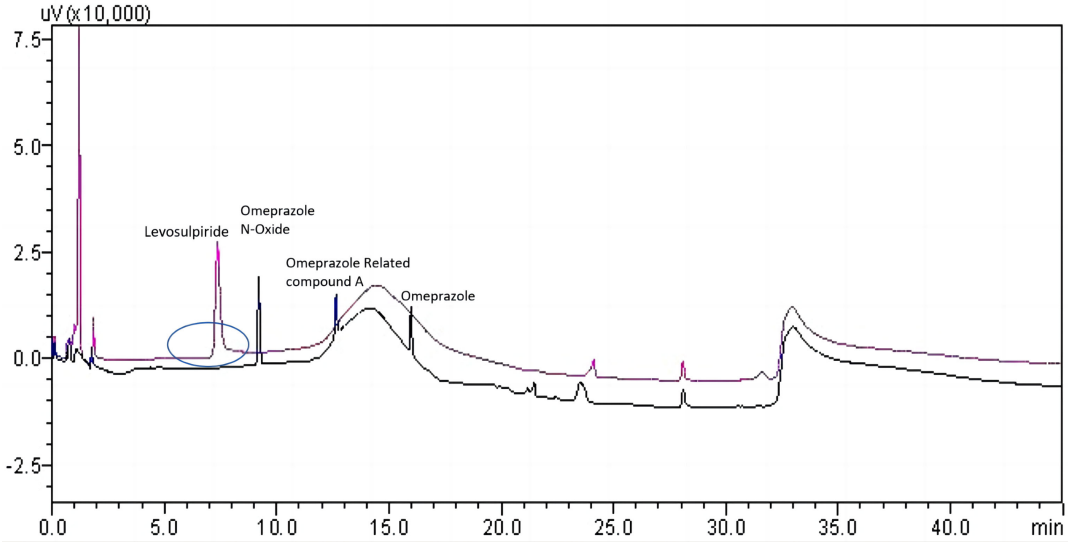
The developed method was validated as per ICH guidelines with respect to parameters like specificity, precision, accuracy, linearity, robustness, ruggedness, solution stability, limit of detection (LOD), and limit of quantification (LOQ) [18–21].

3. Result and Discussion

3.1. Method development by 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

Figure 2
Overlay of omeprazole imp mixture and levosulpiride standard



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 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%; and 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 × 4.6 mm, 3 µm 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.

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

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).

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

To separate placebo peak 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 were changed. Various columns were tried including YMC Triart C18 250 × 4.6 mm, 5 µm, Agilent XDB

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

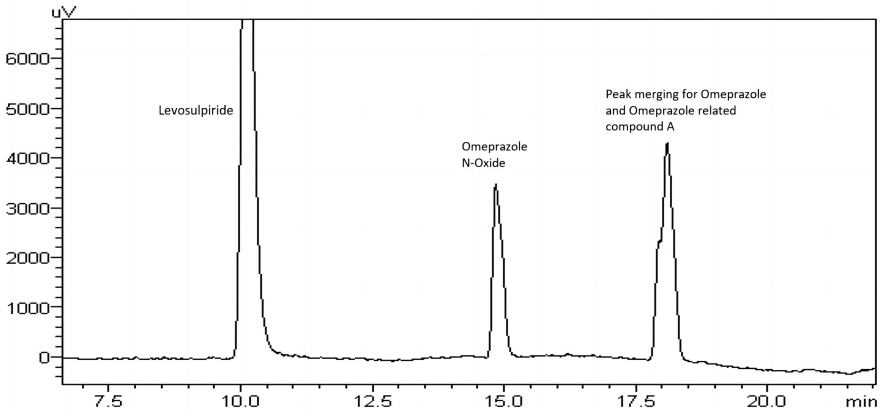


Figure 4
Effect of buffer pH 7.2 on separation of omeprazole and omeprazole-related compound A peaks. (a) Effect of buffer pH 7.2 on separation of omeprazole and omeprazole-related compound A peaks. (b) Effect of buffer pH 6.5 on separation of omeprazole and omeprazole-related compound A peaks

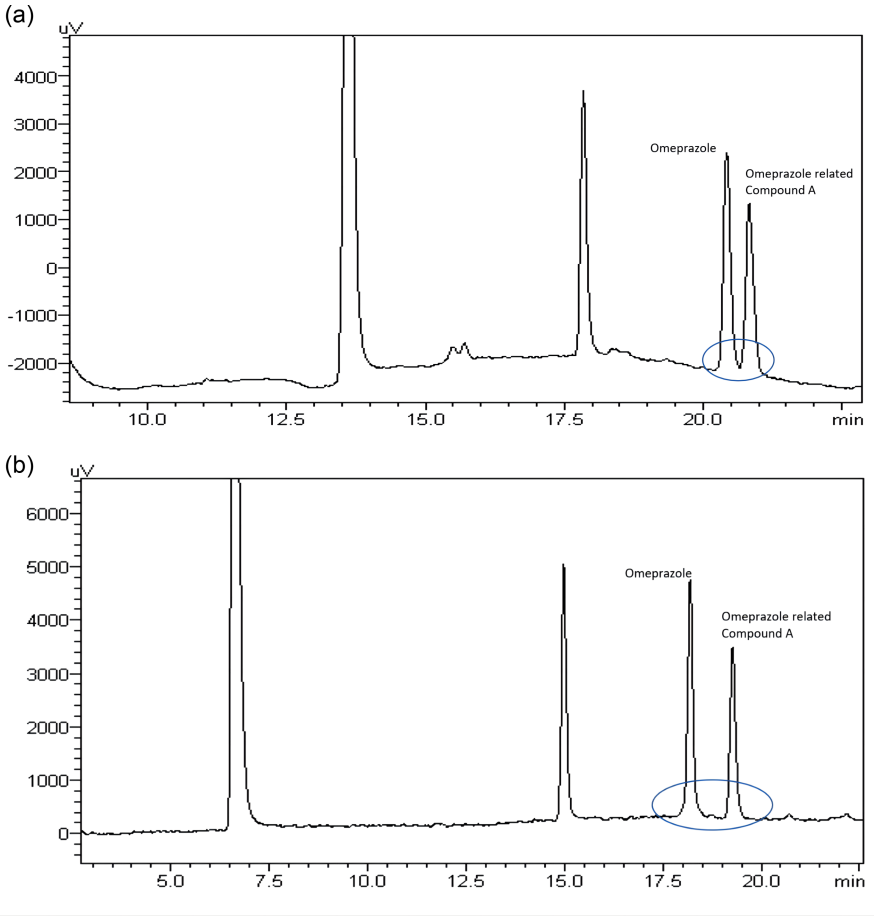


Figure 5
Overlay of placebo chromatogram with sample chromatogram

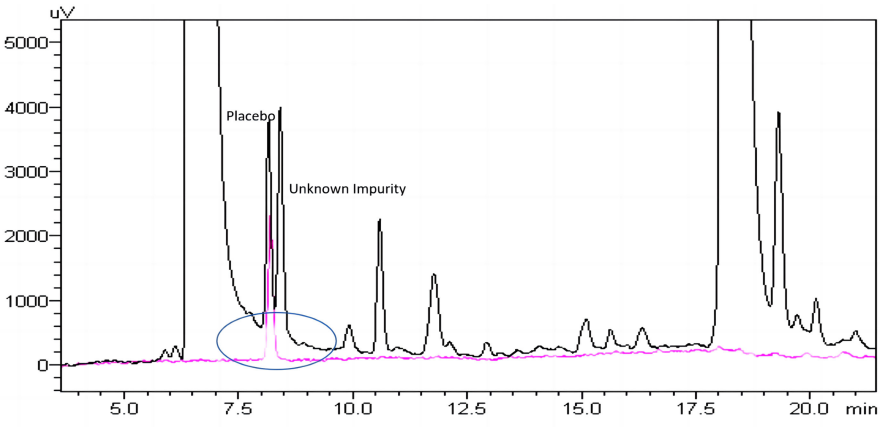
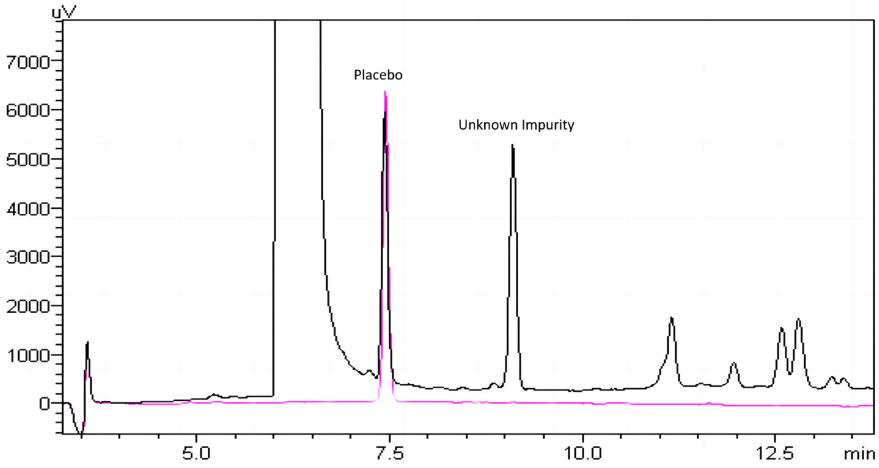


Figure 6
Separation of placebo and unknown impurity



C18 250 × 4.6 mm, 5 µm, Inertsil ODS 250 × 4.6 mm, 5 µm, Bakerbond C18 250 × 4.6 mm, 5 µm. The latter column, i.e., Bakerbond C18 250 × 4.6 mm, 5 µm, showed separation of placebo peak in Figure 6.

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 optimization. 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.

3.2. Optimization through QbD approach

The method optimization 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 are detailed in Table 2 [22–25].

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 as follows:

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

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

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

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

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

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.

The ANOVA outcomes, as delineated in Table 4, unequivocally validate the statistical significance of the predictive efficacy of the model concerning the resolution 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 substance method. Moreover, the graphical depiction of these influential factors and responses via contour plot in Figure 8 provides a succinct and visually intuitive representation of their respective impacts within the analytical framework.

3.3. Optimized method parameters

With the help of Minitab software, optimized conditions 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).

The final method parameters entailed the utilization of an octadecylsilane Bakerbond column of dimensions 250 × 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 50 mM 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 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 min, maintaining this composition until 15 min. Then, a gradual transition to 20% mobile phase A was executed in 30 min. At 35 min, the mobile phase composition was reverted to 100% mobile phase A and held until the completion of the run at 45 min.

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

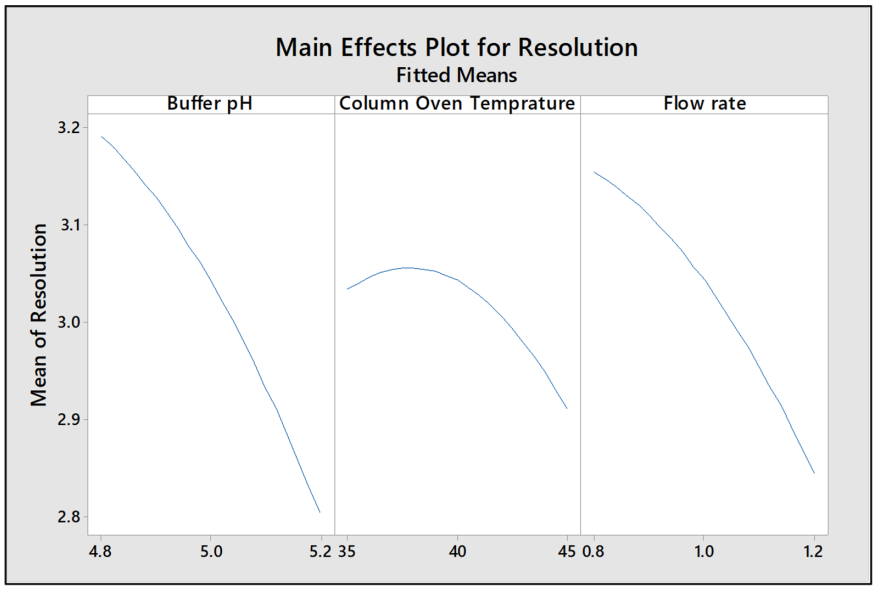
3.4.2. Linearity

Prepared a series of linearity standard solutions of levosulpiride, omeprazole N-Oxide impurity, omeprazole, and omeprazole-related

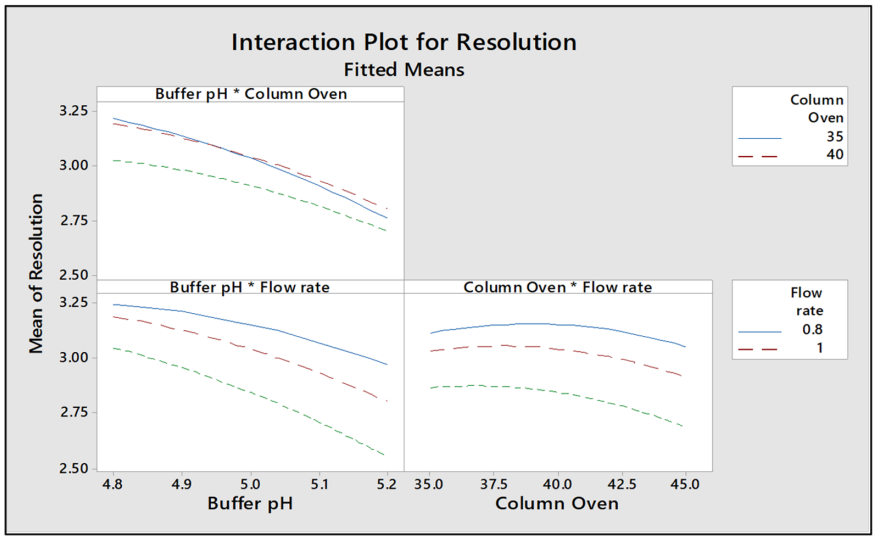
Figure 7

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

(a)



(b)



(c)

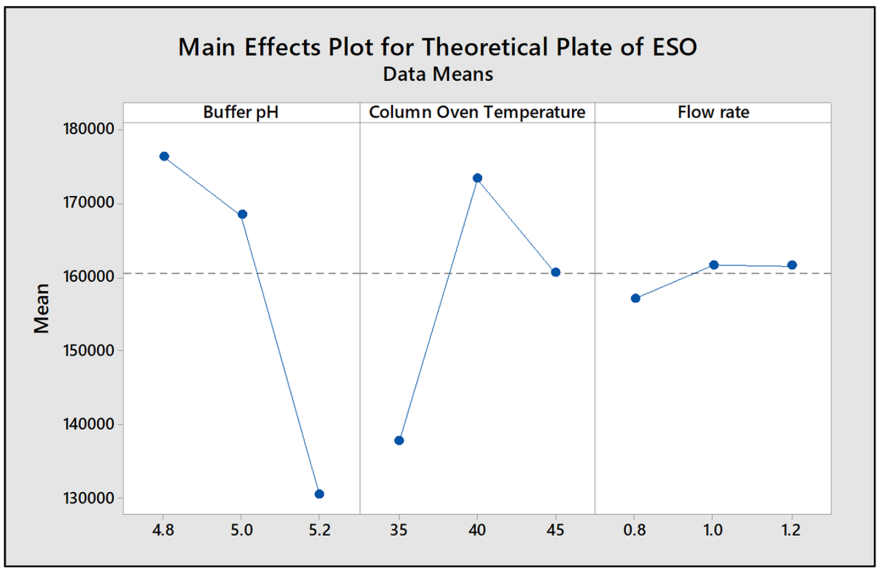
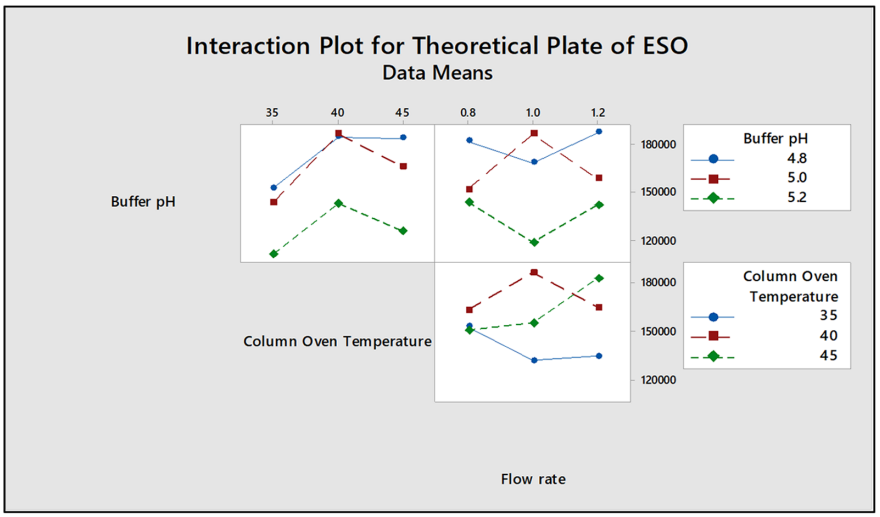
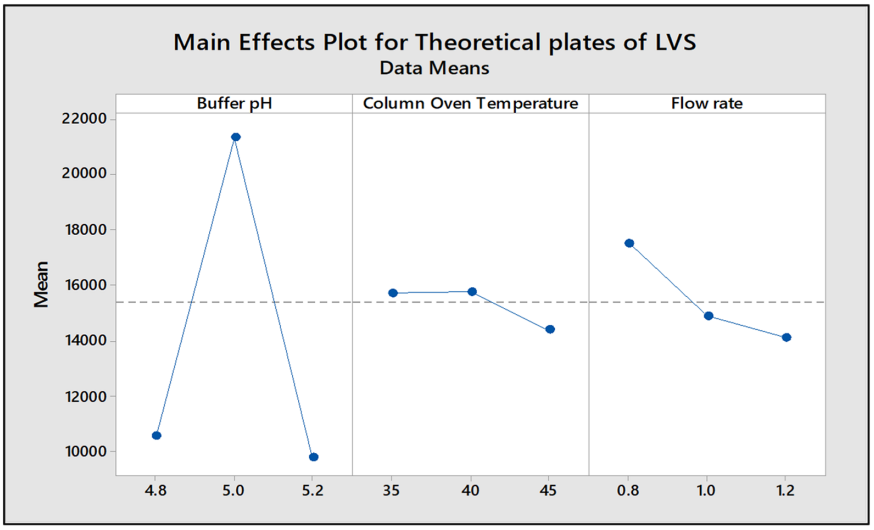


Figure 7
(Continued)

(d)



(e)



(f)

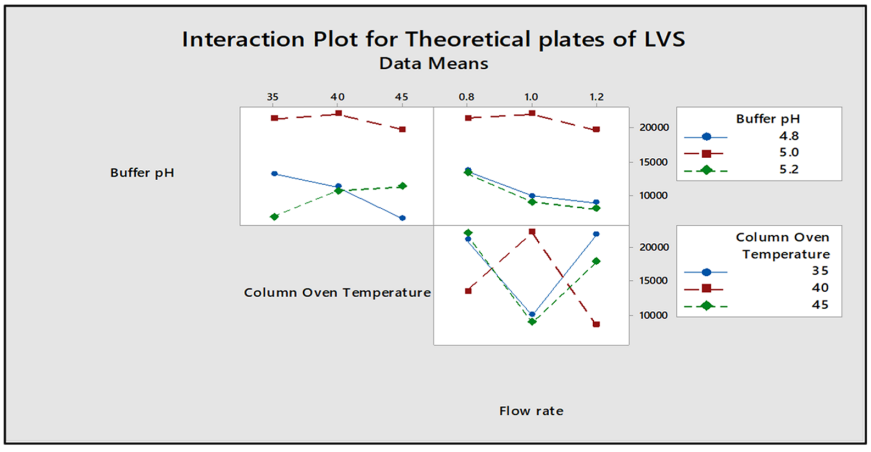


Table 3
Estimated regression coefficients for resolution between omeprazole and omeprazole-related compound A (*Y*1), theoretical plate of esomeprazole (*Y*2) and theoretical plate of levosulpiride (*Y*3) (quadratic) *X*1: buffer pH, *X*2: column oven temperature and *X*3: flow rate

Factors	Coef			SE Coef			T			P		
	*Y*1	*Y*2	*Y*3	*Y*1	*Y*2	*Y*3	*Y*1	*Y*2	*Y*3	*Y*1	*Y*2	*Y*3
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

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
*Y*1	9	0.57	0.06	6.58	0.000	92.22	78.21	0.00	0.027
*Y*2	9	9311189102	1034576567	116.52	0.000	99.53	98.67	92.57	0.036
*Y*3	9	535807876	59534208	42.32	0.000	98.70	96.37	83.22	0.313

Note: 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

(a)

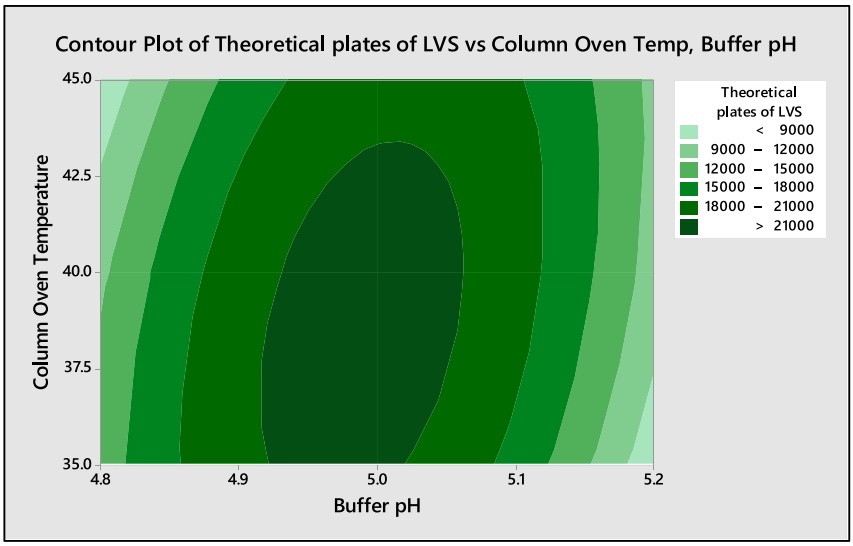
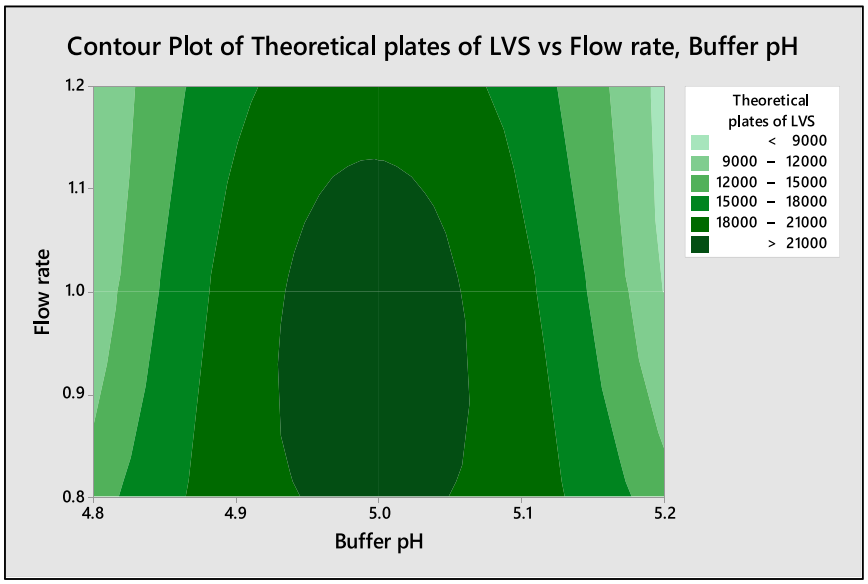
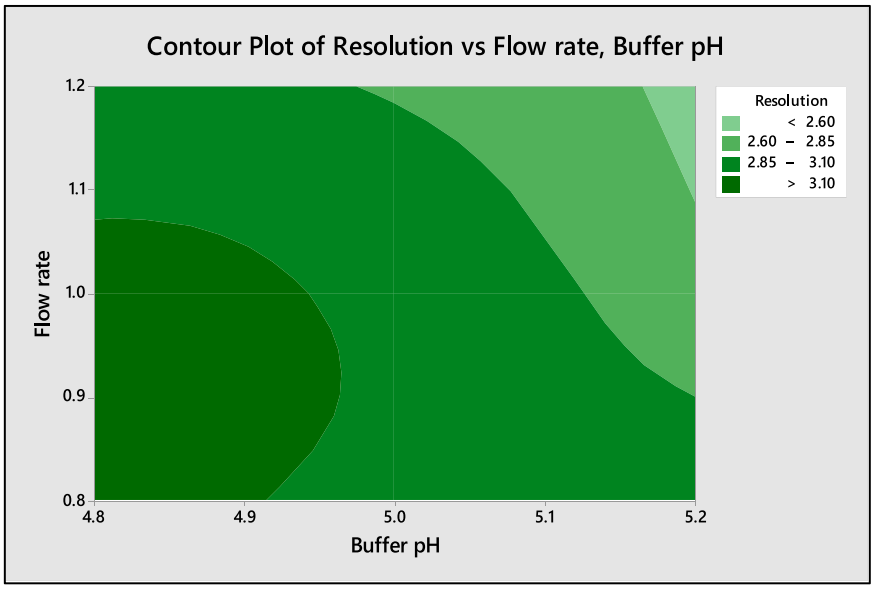


Figure 8
(Continued)

(b)



(c)



(d)

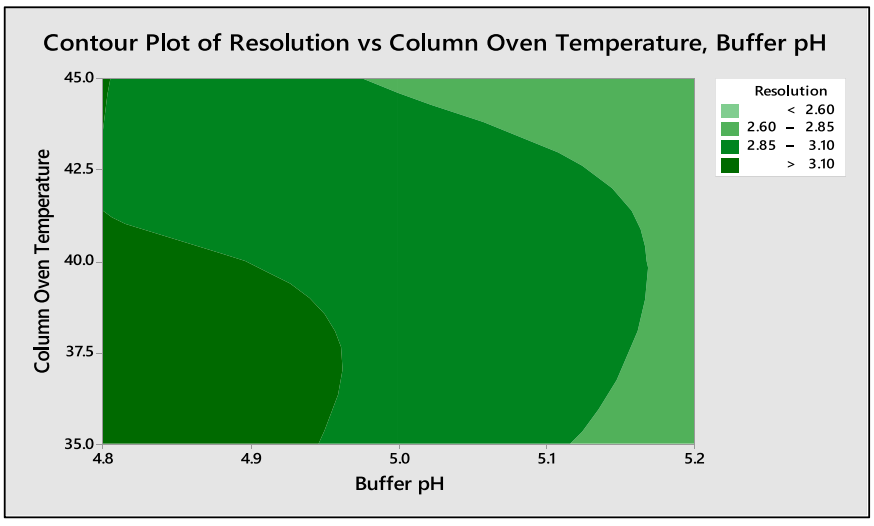
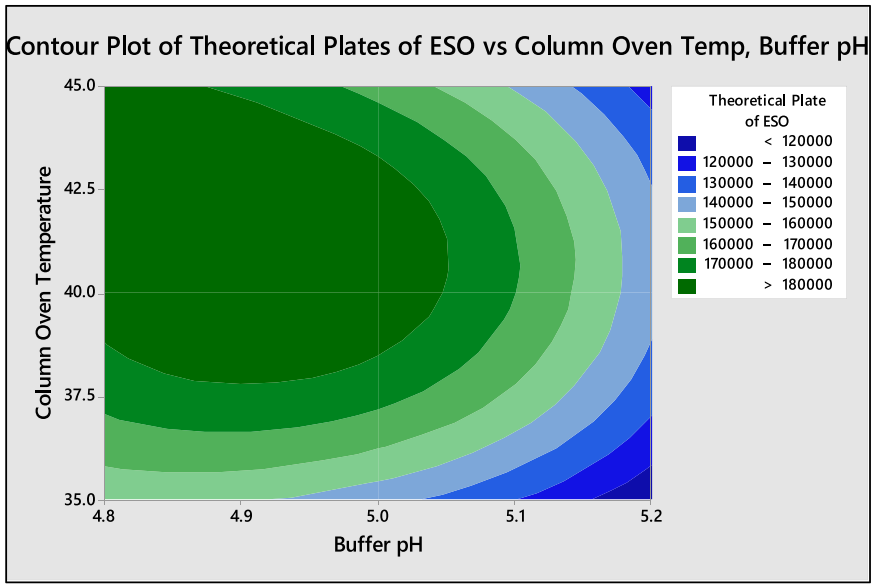
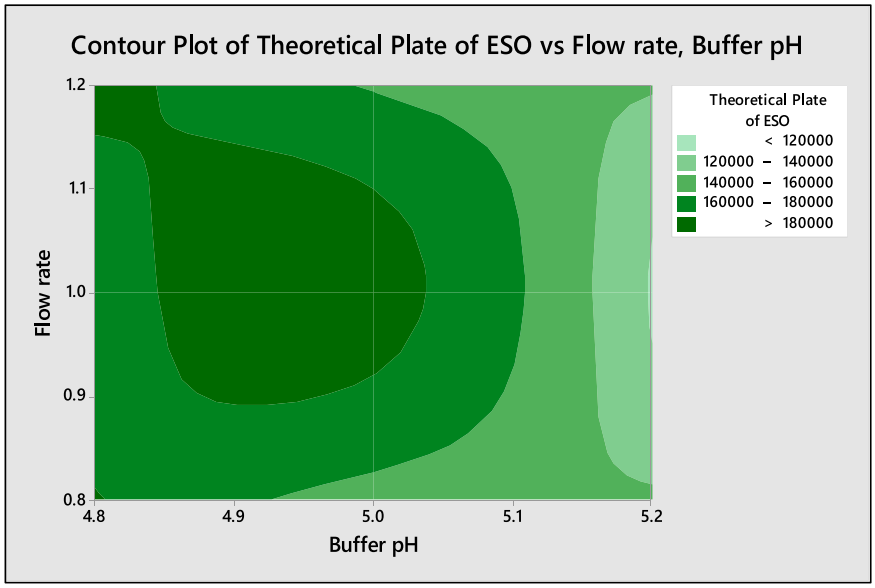


Figure 8
(Continued)

(e)



(f)



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 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.

3.4.4. Precision

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

Figure 9
Optimized conditions for target

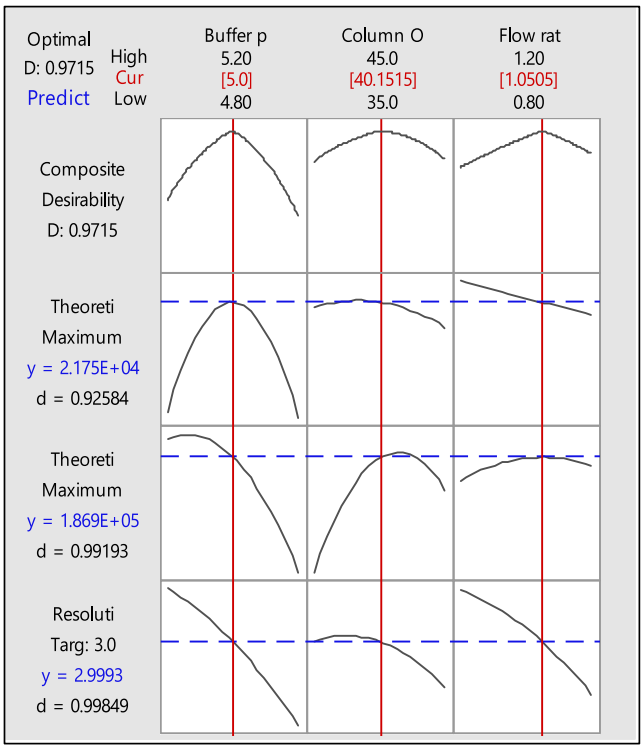


Figure 10
Blank

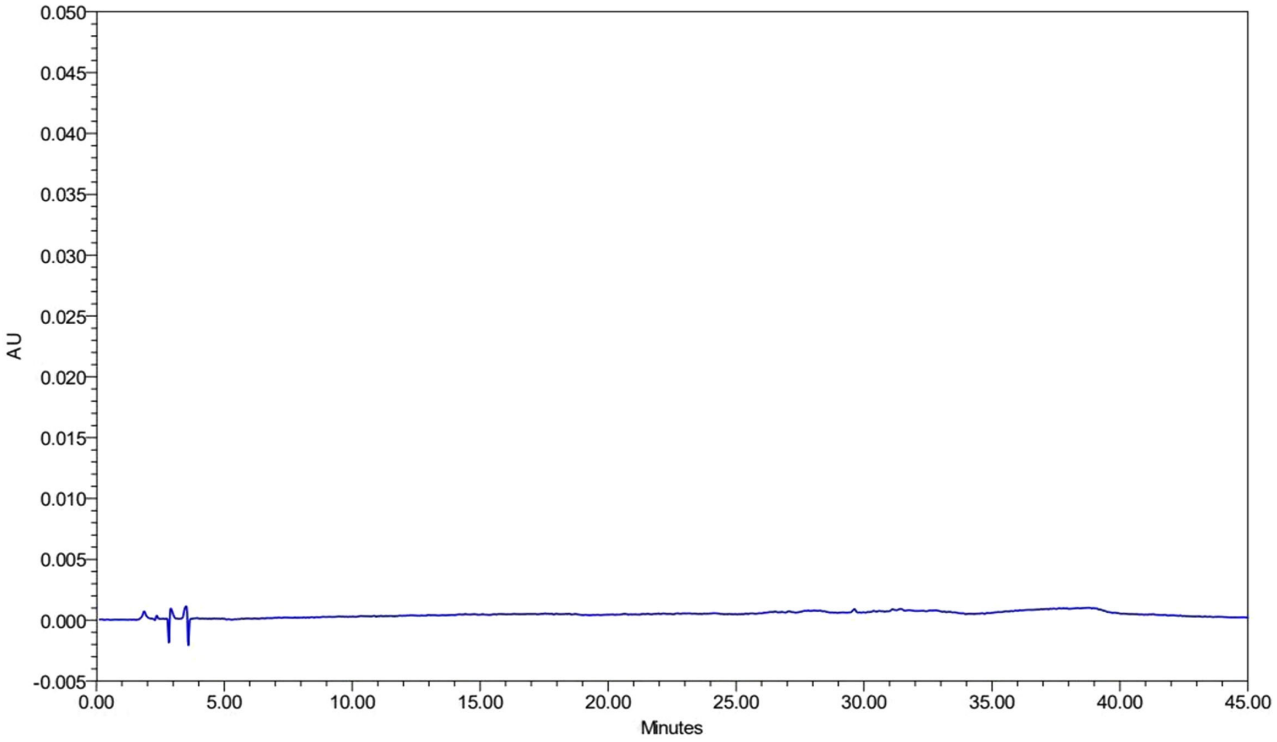


Figure 11
Placebo

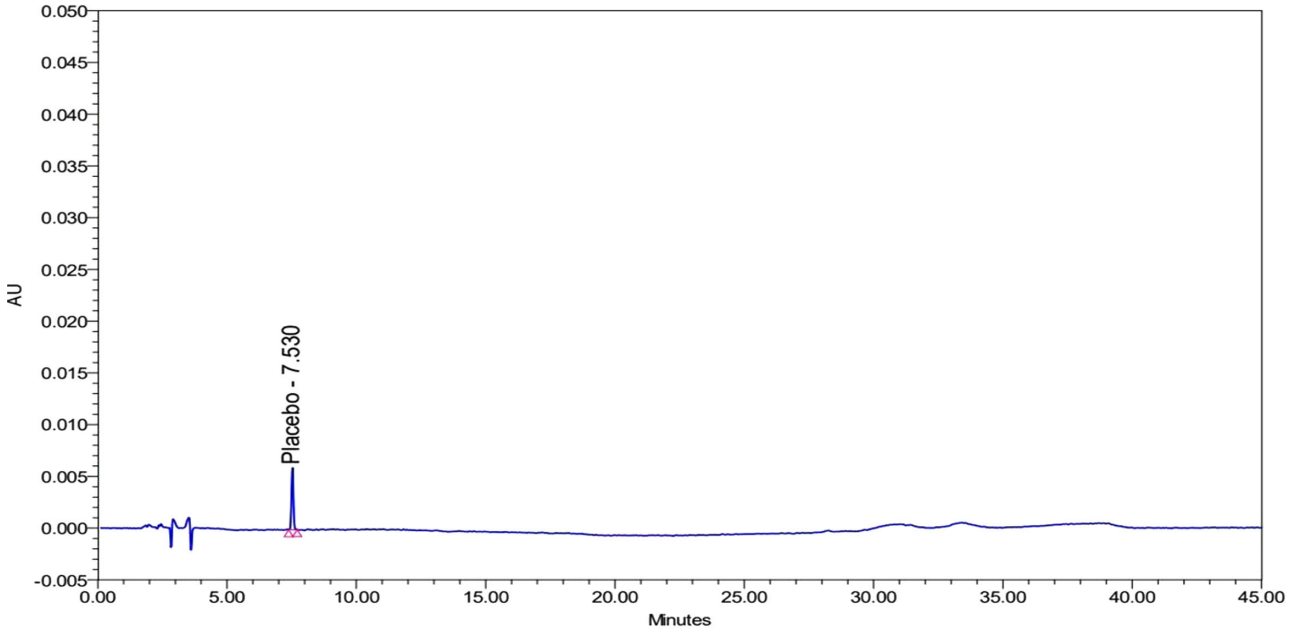


Figure 12
Selectivity standard solution

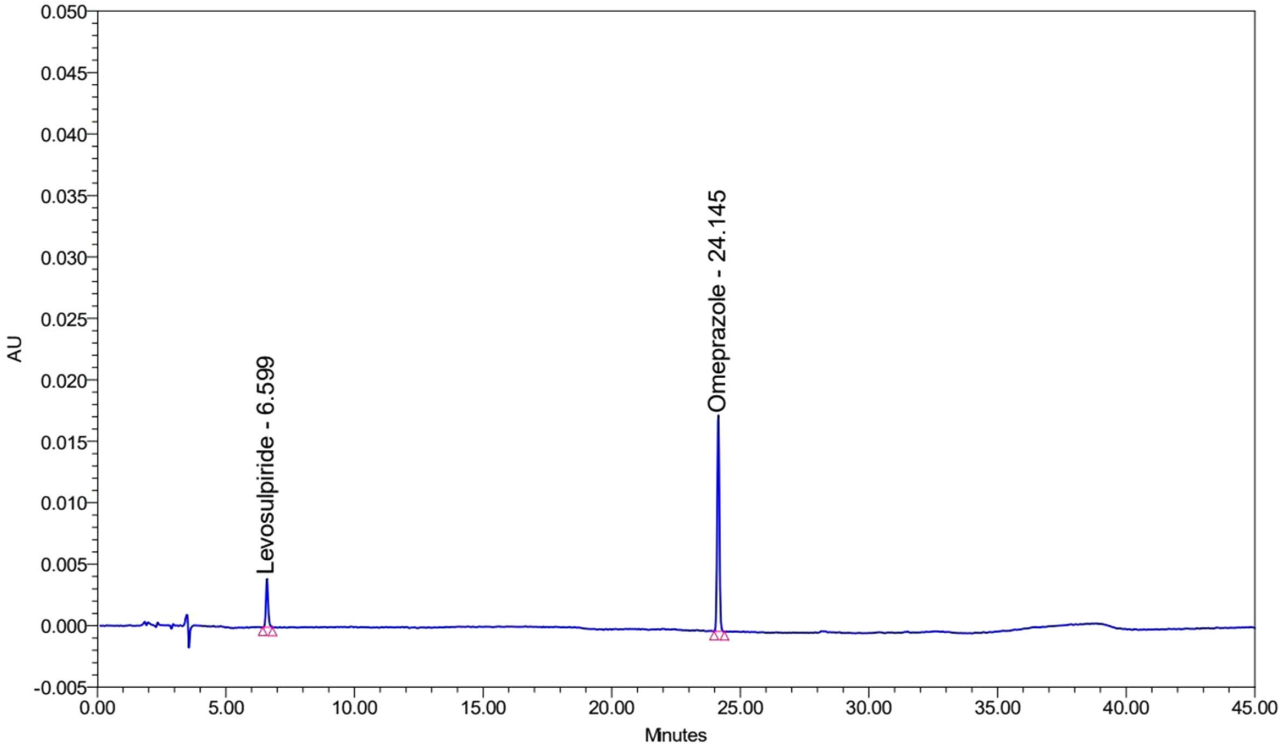


Figure 13
Selectivity levosulpiride ID solution

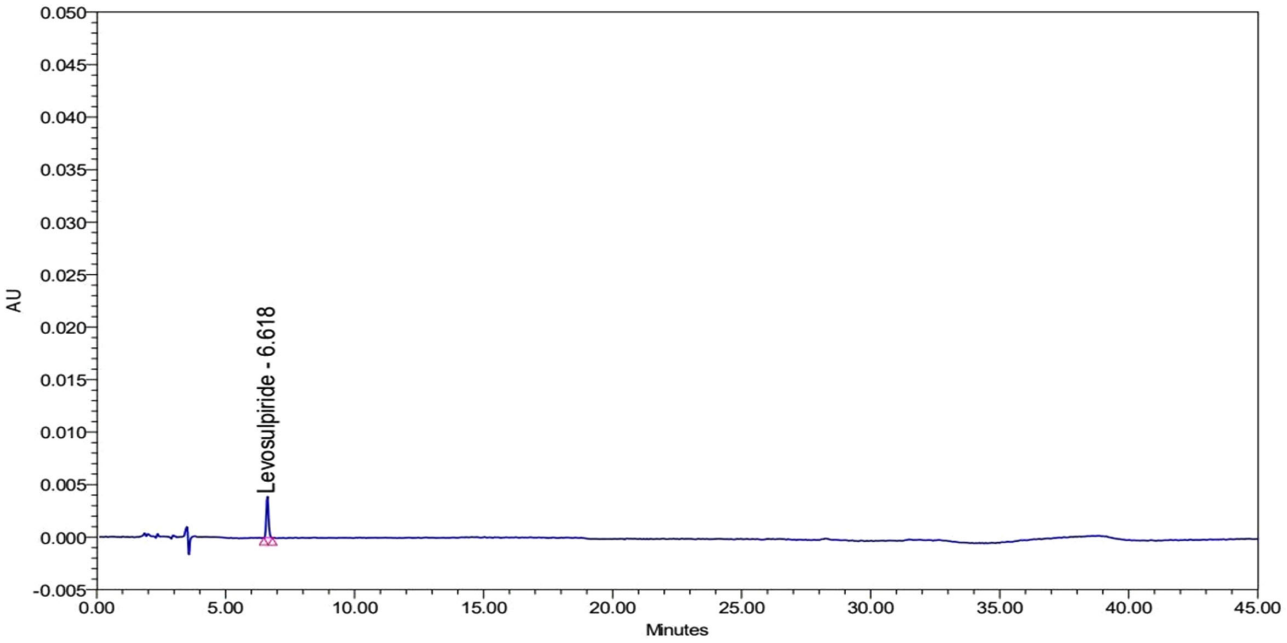


Figure 14
Selectivity omeprazole ID solution

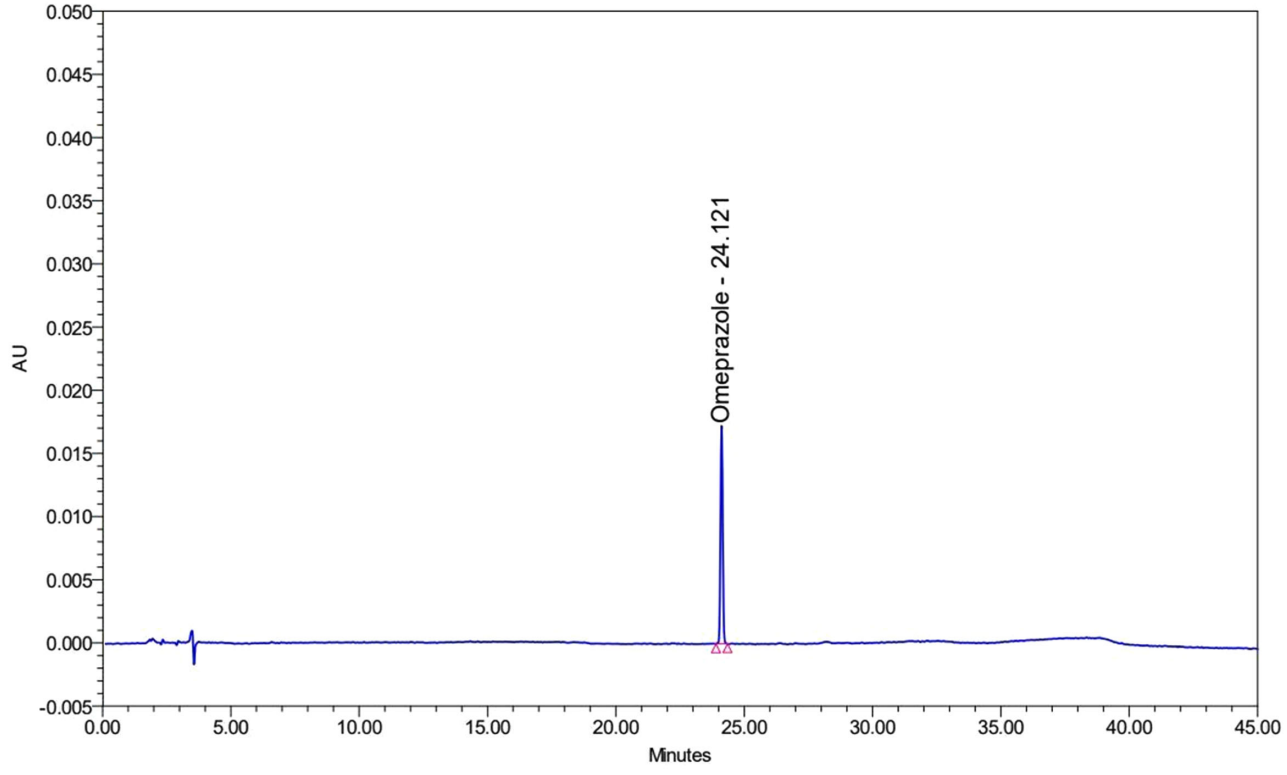


Figure 15
Selectivity omeprazole N-Oxide ID solution

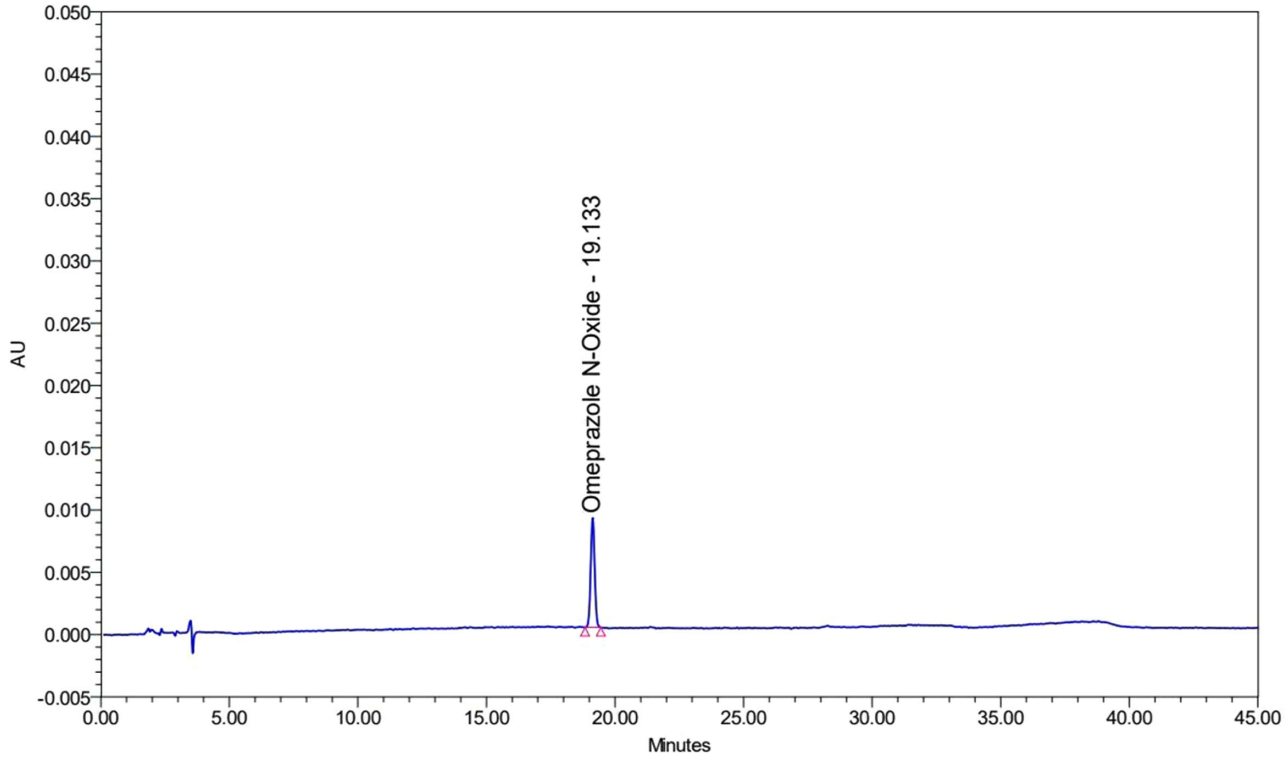


Figure 16
Selectivity omeprazole-related compound A solution

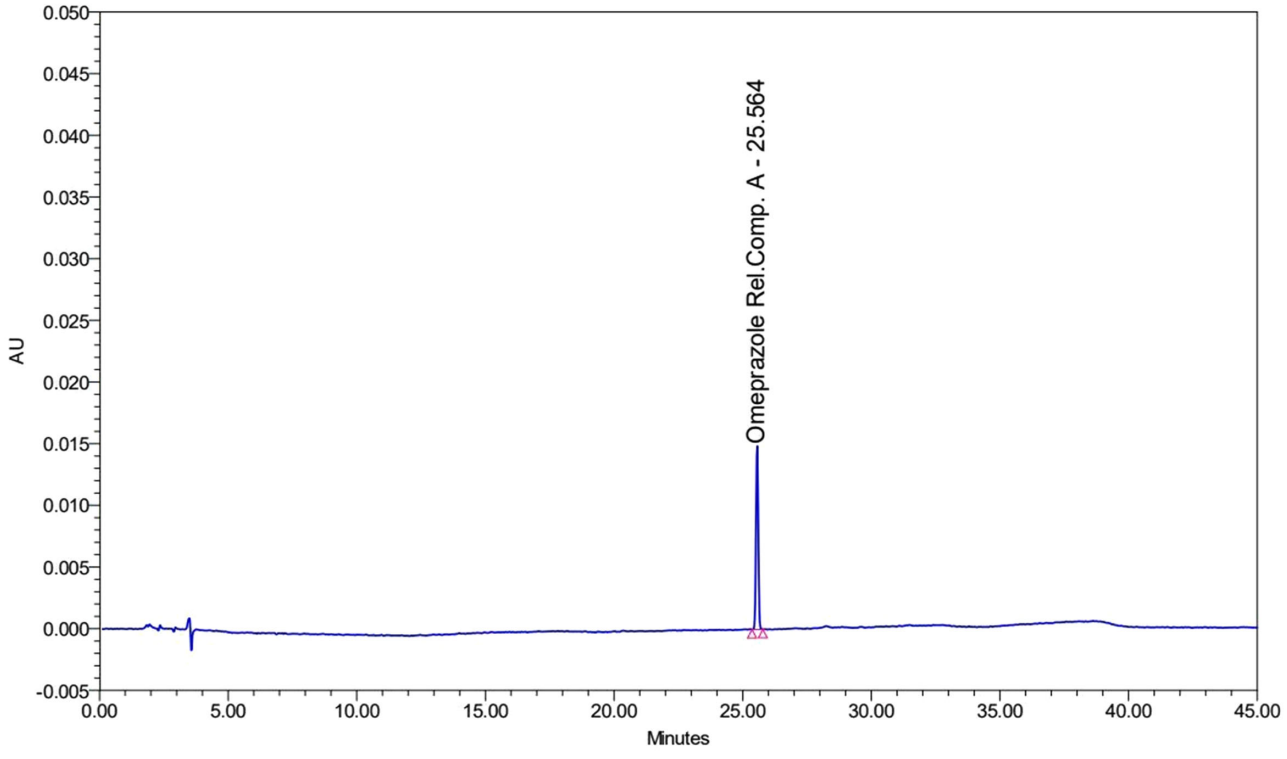


Figure 17
Selectivity test solution

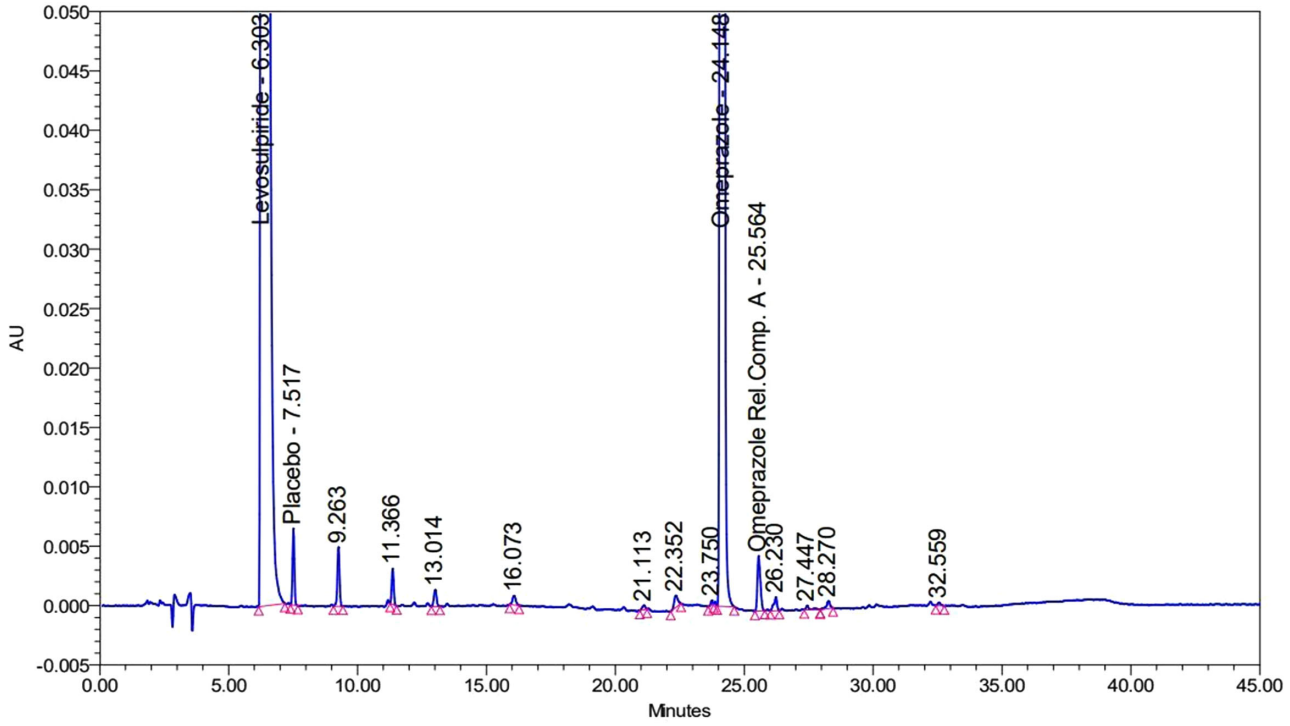


Figure 18
Selectivity impurity spike test solution

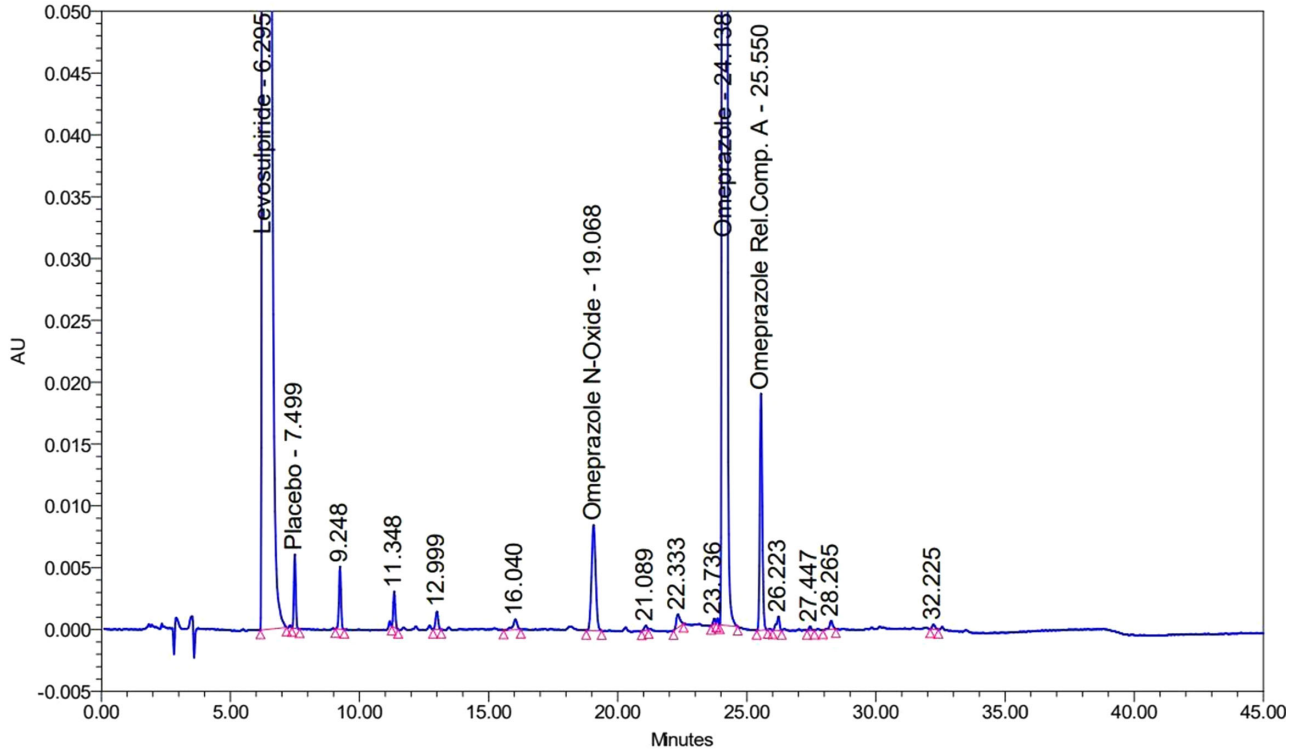


Table 5
Peak purity of impurity solutions and test solution

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

Note: #: Diluted solution

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

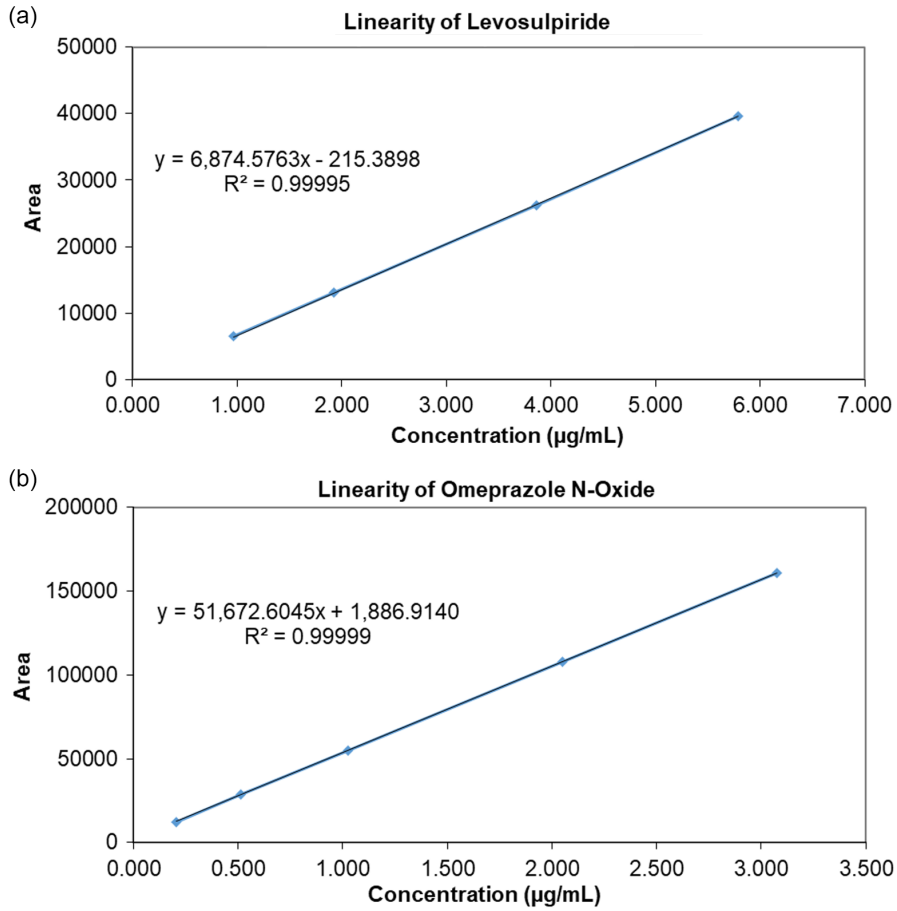


Figure 19
(Continued)

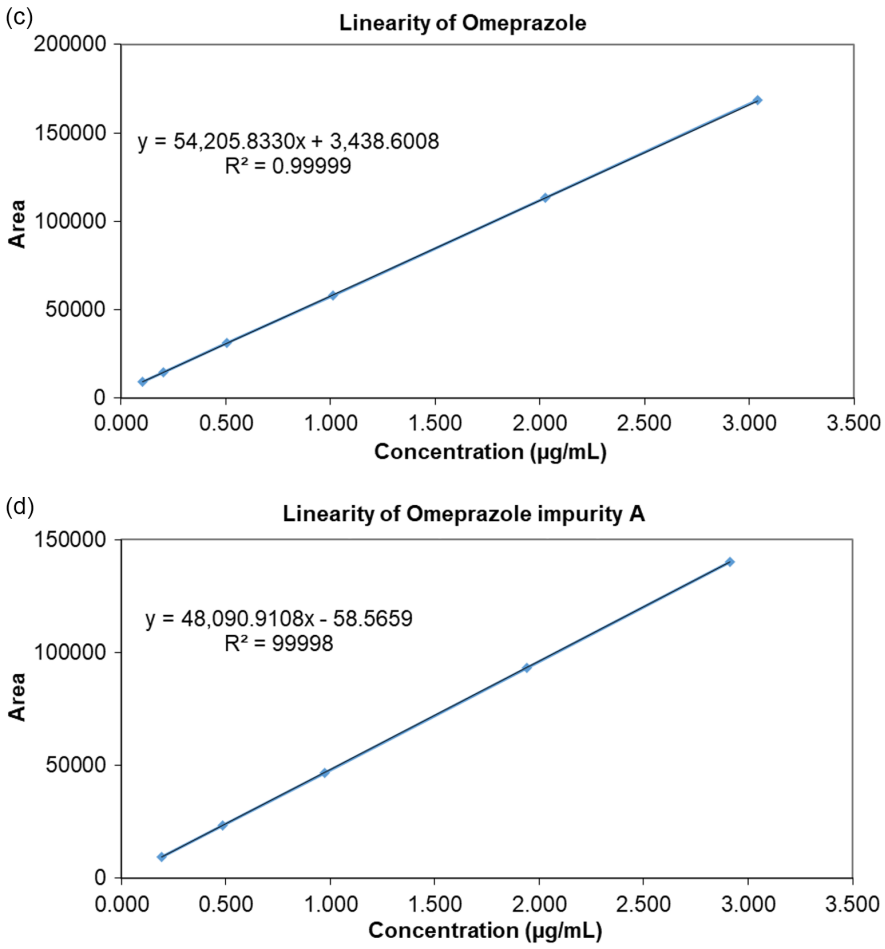


Table 6
Recovery for omeprazole-related compound A and omeprazole N-Oxide impurity at LOQ, 50%, 100%, and 150%, respectively

% 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)

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				
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%/0.101 ppm	2.5%/0.051 ppm	1.13
Levosulpiride	25%/0.965 ppm	10%/0.386 ppm	1.04
Omeprazole-related compound A	10%/0.194 ppm	5%/0.097 ppm	1.38
Omeprazole N-Oxide Impurity	10%/0.205 ppm	5%/0.102 ppm	1.19

3.4.5. Force degradation study (Table 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 min)	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 min.)	BDL	0.199	0.347	0.986
4	Oxidation degradation (10 mL, 1.0% H2O2, kept sample solution in water bath at 70 °C for 60 min.)	BDL	1.846	5.128	10.182
5	Heat degradation (solid State) (Exposed the sample at 70 °C for 24 h in oven.)	BDL	0.209	0.139	0.698
6	Photolytic degradation (kept in a photostability 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 h.)	BDL	0.207	0.146	0.762
8	Hydrolysis degradation (10 mL Water, kept sample solution in water bath at 70 °C for 60 min)	BDL	0.204	0.138	0.725

3.4.6. Solution stability (Table 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 H	BDL	0.197	0.131	0.727
24 H	BDL	0.193	0.132	0.743
36 H	BDL	0.191	0.134	0.773
48 H	BDL	0.188	0.135	0.747
60 H	BDL	0.185	0.153	0.805
72 H	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, and flow rate. However, change in analyst, change in HPLC system, and change in HPLC column were employed to study the ruggedness of method. The % RSD was found <2.0 with all the changes which established the method's robustness and ruggedness.

Optimized 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 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 are available 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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