FABAD J. Pharm. Sci., 51, 3, 1191-1212, 2026
Doi: 10.55262/fabadeczacilik.1929489
RESEARCH ARTICLE

A Quality-by-Design Enabled Green and White Analytical UPLC Method for the Comprehensive Quantification of Nitroso Impurities in Tegoprazan Bulk Drug and Formulations

Krishna Vamsi KANDIMALLA[*°], Ajay MANUKONDA[**], Sambasiva Rao TUMMALA[***], Tangeti Venkata SWAMY[****], Govindarao YEDLAPALLI[*****]

A Quality-by-Design Enabled Green and White Analytical UPLC Method for the Comprehensive Quantification of Nitroso Impurities in Tegoprazan Bulk Drug and Formulations

SUMMARY

A rapid, reliable, environmentally sustainable, and Quality-by-Design (QbD)-assisted UPLC method was proposed for the simultaneous quantification of Tegoprazan and its nitroso impurities in bulk drug and pharmaceutical formulations. Design of Experiments (DoE) was employed to investigate the influence of organic solvent composition, mobile-phase pH, and flow rate on key chromatographic responses, such as resolution and peak symmetry. The quadratic models generated for resolution between Nitroso impurities 2 and 3 and Nitroso impurities 4 and 5 demonstrated excellent statistical performance, with R^2 values of 0.9644 and 0.9641, and adjusted R^2 values of 0.8718 and 0.8708, respectively. The model corresponding F-values of 10.41 and 10.33 and p-values < 0.01 confirm the high significance of the models and strong predictive capability. The model corresponding to the tailing factor of Nitroso impurity 5 also exhibits a strong fit (R^2 = 0.9583, adjusted R^2 = 0.8499) and statistical significance (F-value = 8.84, p = 0.013). The method validation confirms excellent linearity ($r^2 \geq 0.999$), precision (%RSD < 1%), accuracy (98.01–100.97%), and high sensitivity (LOQ 0.105–0.125 µg/mL). The sustainability assessment using AGREE, GAPI, and RGB tools produced a green score of 0.83, an E-factor of 1.8×10^4, and a brilliance score of 83.8%, with an overall classification of "white analytical method". In conclusion, this study delivers the first QbD-enabled, statistically significant, green, and white analytical method for nitroso impurities of Tegoprazan with a robust, eco-friendly, and regulatory-compliant platform for routine quality control.

Keywords: *Tegoprazan, nitroso impurities, quality by design approach, method greenness, method whiteness*

Tegoprazan Ham Maddesi ve Formülasyonlarındaki Nitroso Safsızlıklarının Kapsamlı Kantitasyonu için Tasarım Odaklı Kaliteyi Sağlayan Yeşil ve Beyaz Analitik UPLC Yöntemi

ÖZ

Toplu ilaç ve farmasötik formülasyonlarda Tegoprazan ve nitroso safsızlıklarının eş zamanlı kantifikasyonu için hızlı, güvenilir, çevre dostu ve Kalite Odaklı Tasarım (QbD) destekli bir UPLC yöntemi önerilmiştir. Organik çözücü bileşiminin, mobil faz pH'sının ve akış hızının çözünürlük ve pik simetrisi gibi temel kromatografik yanıtlara etkisini araştırmak için Deney Tasarımı (DoE) kullanılmıştır. Nitroso safsızlıkları 2 ve 3 ile Nitroso safsızlıkları 4 ve 5 arasındaki çözünürlük için oluşturulan ikinci dereceden modeller, sırasıyla 0,9644 ve 0,9641 R^2 değerleri ve 0,8718 ve 0,8708 düzeltilmiş R^2 değerleri ile mükemmel istatistiksel performans sergilemektedir. Modelin karşılık gelen F değerleri 10,41 ve 10,33 ve p değerleri < 0,01, modellerin yüksek önemini ve güçlü tahmin yeteneğini doğrulamaktadır. Nitroso safsızlığı 5'in kuyruk faktörüne karşılık gelen model de güçlü bir uyum (R^2 = 0,9583, düzeltilmiş R^2 = 0,8499) ve istatistiksel anlamlılık (F değeri = 8,84, p = 0,013) göstermektedir. Yöntem doğrulaması, mükemmel doğrusallığı ($r^2 \geq 0,999$), hassasiyeti (%RSD < 1%), doğruluğu (%98,01–100,97) ve yüksek duyarlılığı (LOQ 0,105 – 0,125 µg/mL) doğrulamaktadır. AGREE, GAPI ve RGB araçları kullanılarak yapılan sürdürülebilirlik değerlendirmesi, 0.83'lük bir yeşil puan, $1,8 \times 10^4$'lük bir E faktörü ve %83,8'lik bir parlaklık puanı ile "beyaz analitik yöntem" genel sınıflandırmasını ortaya koymaktadır. Sonuç olarak, bu çalışma, rutin kalite kontrolü için sağlam, çevre dostu ve mevzuata uygun bir platform ile Tegoprazan'ın nitroso safsızlıkları için ilk QbD destekli, istatistiksel olarak anlamlı, yeşil ve beyaz analitik yöntemi sunmaktadır.

Anahtar Kelimeler: *Tegoprazan, nitroso safsızlıkları, tasarımla kalite yaklaşımı, yöntem yeşilliği, yöntem beyazlığı*

Recieved: 13.04.2026
Revised: 25.07.2026
Accepted: 08.08.2026

[*] ORCID: 0009-0004-4526-6045, Northeastern University, 360 Huntington Ave, Boston, Massachusetts - 02115, United States
[**] ORCID: 0009-0000-9896-4796, Northeastern University, 360 Huntington Ave, Boston, Massachusetts - 02115, United States
[***] ORCID: 0000-0001-6686-7318, Stira Pharma Private Limited, Regulatory Affairs, Manjeera Trinity Corporate, Hyderabad - 500072, Telangana, India
[****] ORCID: 0000-0001-8969-6806, Tagore Government Arts and Science College (Affiliated to Pondicherry University), Puducherry - 605008, Andhra Pradesh, India
[*****] ORCID: 0000-0003-2088-6438, Malineni Perumallu Pharmacy College, Pulladigunta, Guntur - 522017, Andhra Pradesh, India

[°] Corresponding Author; Vamsi KANDIMALLA
E-mail: vamsikandimalla07@gmail.com

INTRODUCTION

Tegoprazan (Figure 1A.) is a novel, fast-acting potassium-competitive acid blocker used to treat acid-related gastrointestinal disorders, such as gastroesophageal reflux disease, erosive oesophagitis, and peptic ulcer disease (Kim Dong et al., 2019). Tegoprazan reversibly inhibits the H^+/K^+-ATPase proton pump in gastric parietal cells through competitive blockade of the K^+-binding site. This inhibition results in rapid and sustained suppression of gastric acid secretion with minimal dependence on activation by acidic pH (Lee et al., 2019). This pharmacological advantage offers quick relief of symptoms, improves nighttime acid control, and reduces therapeutic response variability (Takahashi & Yukinori, 2019). The side effects reported during the use of tegoprazan include headache, diarrhea, constipation, abdominal discomfort, dizziness, and transient elevations in liver enzymes (Wang et al., 2025).

Nitroso impurities are unwanted chemical contaminants that contain–N=O (nitroso) groups in their structure. These compounds can be generated during drug synthesis or storage of the active pharmaceutical ingredient or formulation (Cioc, Ciarán, Monika, & Robert, 2023). Nitroso impurities associated with tegoprazan arise due to nitrosation reactions of functional groups present in the drug substance or residual amine-containing reagents used during synthesis. These impurities are of significant concern because of their potential genotoxic and carcinogenic risks, even at trace concentrations (Hemanth et al., 2024). Global regulatory agencies recommend stringent allowable intake limits for nitroso impurities (Kruhlak et al., 2024). Therefore, their detection and control are essential during API synthesis, formulation development, and long-term product stability evaluation.

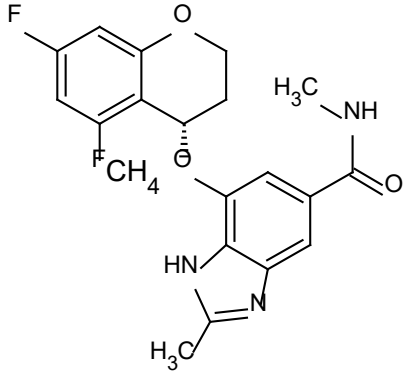
Analytical approaches reported for the quantification of Tegoprazan suggest the presence of various analytical approaches. The reported LC–MS/MS method quantified Tegoprazan and its major metabolite M1 in dog plasma (Joohwan, Sungjun, Hyun-Jung, Shin-Young, & Soo-Jin, 2019), but this method is not suitable for the resolution of Tegoprazan impurities. The stability-indicating method using LC-HRMS, LC-MS^n, and GC-QTOF successfully reported multiple degradation products of Tegoprazan, but this method was designed for structural elucidation rather than validated quantitative analysis of nitroso impurities at regulatory limits (Kaijing, Xiao, H., Hongyi, Z., & Chen, 2023). The RP-HPLC-based related-substance method was reported for the quantification of impurities A to D of Tegoprazan. However, this method was not designed or validated for the determination of nitroso impurities (Lei et al., 2025). The reported chiral HPLC method was applicable for the enantiomeric purity assessment of Tegoprazan, but the method does not offer applicability for nitroso impurity monitoring due to limited selectivity and absence of trace-level validation (Lei, Shutao, Shujuan, Lihua, & Yingde, 2024). The UPLC-MS/MS procedures reported for human and rat plasma support clinical pharmacokinetic and bioequivalence studies but remain unsuitable for impurity profiling (Mengjie, Yize, Yu, Manjie, & Qinghua, 2025). These reported methods are insufficient for the quantification of nitroso impurities of Tegoprazan.

Conventional impurity profiling analytical methods predominantly depend on HPLC techniques with acetonitrile-based mobile phases. These methods produce adequate resolution and sensitivity, but they suffer from long run time, higher solvent consumption, toxic solvent use, and poor environmental sustainability. In light of the increase in regulatory emphasis on green analytical chemistry (GAC), there is a growing need to design methods that minimize environmental impact without compromising analytical performance (Varma, Pavani, Subhashini, Kandula, & Babu, 2024). The implementation of GAC principles in analytical methods can eliminate the involvement of hazardous substances in the analytical workflow by promoting the usage of safe solvents with minimum waste generation. The greenness concept evaluates the

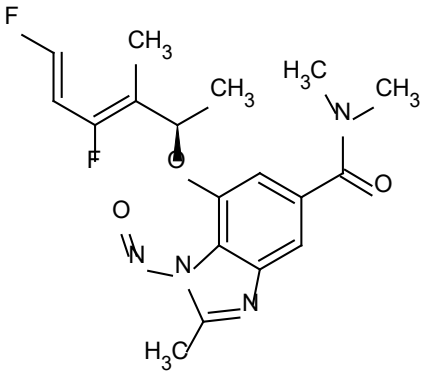
environmental impact of analytical methods and does not fully capture other key aspects, such as cost-efficiency, operator safety, and overall method performance. The concept of White Analytical Chemistry (WAC) emerged to address these limitations. The WAC integrates the "green" (environmental) and "red" (analytical efficiency and quality) aspects of the method and provides a more holistic evaluation of method sustainability (Veerendra, Kumar, Mankumare, & Kumar, 2024).

Further, the application of the Quality by Design (QbD) paradigm in analytical development transformed the traditional trial-and-error approach into a systematic, science-based, and risk-managed process (Sravanthi & Kumaraswamy, 2025). QbD focuses on the identification of Critical Quality Attributes (CQAs) and Critical Method Parameters (CMPs) that influence method performance. This approach optimizes the method using multivariate statistical tools such as the Design of Experiments (DoE). This systematic framework ensures robust method performance across a defined Design Space with enhanced reliability, reproducibility, and regulatory compliance (Nassef, Ahmed, El-Atawy, Alanazi, & Mohamed, 2024).

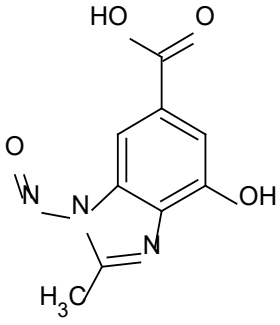
Therefore, the present study aimed to develop a Quality-by-Design (QbD)-assisted, green, and white analytical UPLC method for the simultaneous quantification of tegoprazan and its nitroso impurities. To improve the environmental sustainability of the analytical procedure, ethanol was deliberately selected as the organic modifier in place of conventional solvents such as acetonitrile and methanol. Ethanol is recognized as a greener solvent because of its lower toxicity, renewable origin, reduced environmental impact, and improved operator safety while maintaining satisfactory chromatographic performance. Thus, the proposed method was designed to achieve efficient chromatographic separation with minimal solvent consumption and a reduced ecological footprint. Furthermore, the greenness and overall sustainability of the developed method were evaluated using AGREE, GAPI, and the White Analytical Chemistry (RGB12) assessment tools. Nitroso impurities 1–5 of tegoprazan were included in this study, and their chemical structures are presented in Figure 1.



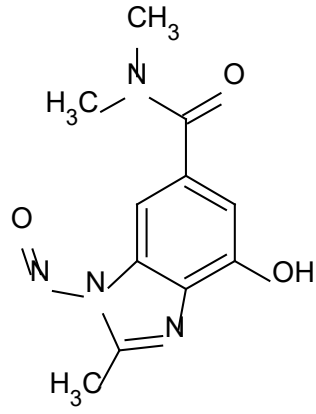
A) Tegoprazan



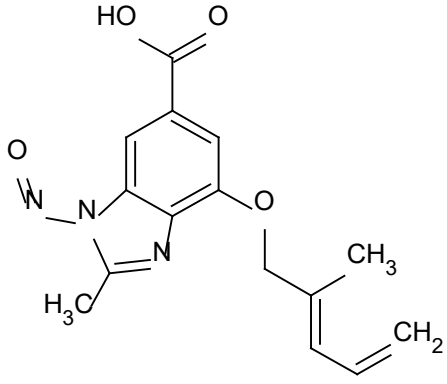
B) Nitroso impurity 1



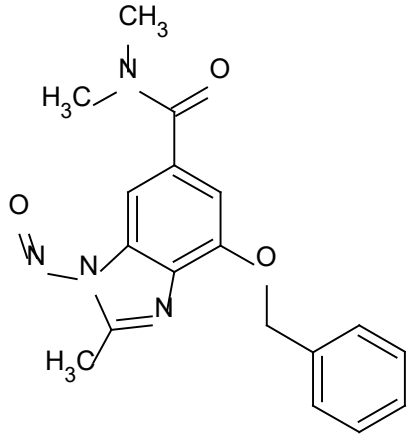
C) Nitroso impurity 2



D) Nitroso impurity 3



E) Nitroso impurity 4



F) Nitroso impurity 5

Figure 1. Molecular structure of Tegoprazan and its nitroso impurities

MATERIALS AND METHODS

Chemicals and reagents

The tegoprazan active pharmaceutical ingredient with a purity of 98.25% and nitroso impurities was generously provided by Dr. Reddy's Laboratories Ltd., Hyderabad, Telangana, India. The marketed tegoprazan tablet formulation (PCAB® 50 mg) was procured from a local pharmacy and used directly for analysis. UPLC-grade ethanol (LiChropur™) and formic acid were obtained from Merck Chemicals, Mumbai, India. Ultrapure water was produced using a Millipore Direct-Q 3UV purification system (Merck Millipore, Darmstadt, Germany) and used throughout the study. All chemicals, solvents, and reagents employed in the study were of analytical or chromatographic grade and were filtered through 0.22 µm membrane filters before use.

Instrumentation and analytical conditions

The ACQUITY™ (Waters Corporation, Milford, MA, USA) UPLC system was used for the analysis of tegoprazan and its nitroso impurities. This system includes a Binary Solvent Manager for mobile-phase delivery, a flow-through needle sample manager for precise sample injection, a column module to accommodate the column, and a photodiode array (PDA) detector. Empower™ 3 (Waters, USA) software was utilized for instrument control, chromatographic data acquisition, and peak processing during the analysis. All experiments were conducted under controlled laboratory conditions at 25 ± 2 °C.

Chromatographic separation was achieved on an ACQUITY BEH C18 column (100 mm × 2.1 mm, 1.7 µm). The mobile phase consisted of 10 mM ammonium acetate at pH 3.5 (adjusted with formic acid) and ethanol in a 45:55 (v/v) ratio at an isocratic delivery of 0.3 mL/min. The 2 µL portion of the sample was injected, and the analytes were monitored at a wavelength of 262 nm.

Preparation of standard and calibration solutions

Stock solutions of tegoprazan and each of its nitroso impurities were prepared separately at a concentration of 1000 µg/mL. In the preparation, 10 mg of each reference standard was accurately weighed and dissolved in 10 mL of diluent (equal volumes of ethanol and water). Then, a 50 µg/mL solution was prepared from these stock solutions by appropriate dilution and used as a 100% reference level solution for quantification. The linear standard solutions of tegoprazan were freshly prepared within a 12.5 to 100 µg/mL (representing 25% to 200% of the target concentration) concentration range. The nitroso impurity working solutions were diluted similarly manner to obtain levels equivalent to 1% of the tegoprazan test concentration. Equal volumes of the respective tegoprazan and impurity solutions were mixed to prepare combined calibration samples. Each calibration level was injected in triplicate (n = 3) under optimized UPLC conditions.

Test solution preparation

Twenty PCAB® tablets containing 50 mg of tegoprazan were accurately weighed and crushed into a fine powder using a clean mortar and pestle. A portion of the powder equivalent to one tablet was transferred to a 50 mL volumetric flask. It was mixed with approximately 50 mL of the diluent and sonicated for 15 min to ensure complete extraction of the drug. The solution was then diluted to volume with the same diluent and filtered through a 0.22 µm membrane filter. An aliquot of this filtrate was further diluted to obtain the test solution corresponding to the 100% level (50 µg/mL). The spiked sample was also prepared by adding each nitroso impurity at 1% of the test concentration to the 100% solution. The unspiked and spiked samples were analyzed under the optimized UPLC conditions to evaluate the resolution, recovery, and ability of the method to accurately quantify nitroso impurities.

Method development

The method development process began with the assessment of the solubility and UV absorption behavior of tegoprazan and its nitroso impurities. The UV spectrum was recorded between 200 and 400 nm to identify the most suitable wavelength that showed strong absorbance for all analytes. Different column lengths of the Acquity BEH C18 (100 and 50 mm) were evaluated at temperatures between 25 and 35 °C to obtain good separation, peak shape, and efficiency. The mobile phase composition was then optimized using ethanol as the organic solvent to support green analytical chemistry. Preliminary experiments were performed to optimize the chromatographic conditions through the evaluation of different aqueous modifiers, buffer pH, organic solvent composition, column temperature, and flow rate. Based on these screening experiments, organic solvent composition (% ethanol), mobile-phase pH, and flow rate were identified as the CMPs that had the greatest influence on chromatographic performance. These three factors were therefore selected for systematic optimization using the Design of Experiments (DoE) approach.

QbD approach in method optimization

The CCD approach in Design-Expert® 11 was utilized for the optimization of the method. The clear separation and reliable quantification of tegoprazan and its nitroso impurities was the primary goal in this QbD-guided method development. This study aimed to obtain baseline resolution (Rs > 2) and short run time with less solvent usage to support green analytical practices. Key performance characteristics, such as retention time, peak separation, and peak symmetry, were defined as CMAs. The preliminary optimi-

zation studies identified organic solvent composition (% ethanol), mobile-phase pH, and flow rate as CMPs influencing chromatographic performance. These three variables were selected for optimization using a Design of Experiments. A CCD within DoE approach was employed to evaluate these variables systematically. The effects of organic solvent composition, mobile-phase pH, and flow rate were systematically investigated to establish the design space and identify the optimum chromatographic conditions.

Method validation

The suitability of the proposed method for routine quality assessment of tegoprazan and its nitroso impurities was confirmed by performing validation studies as per ICH Q2(R2) guidelines (Prasad, Kasimala, & Anna, 2021). The linearity range of the proposed method was assessed over 25-200% of the target concentration for tegoprazan with 1% of its nitroso impurities. Calibration plots were prepared by relating the peak area to the analyte concentration. A corresponding correlation coefficient (r^2) of at least 0.999 was accepted as evidence of good linearity. The system precision, repeatability (intra-day), and intermediate precision (inter-day) were evaluated using the 100% level solution. The %RSD values for the peak areas and retention times were within 2.0%, were considered to indicating satisfactory reproducibility of the proposed method. The accuracy was assessed through recovery studies at 50%, 100%, and 150% levels by spiking the tegoprazan tablet matrix with known amounts of nitroso impurities. The recovery results fall between 98.0% and 102.0%, which is considered an acceptable recovery of the proposed method. The sensitivity of the proposed method for the quantification of nitroso impurities was proved by evaluating the limit of detection (LOD) and quantification (LOQ) using the signal-to-noise ratio (S/N) approach. The method robustness was assessed by introducing small, deliberate variations in the proposed analytical conditions such as flow rate (±0.05 mL/min), detection wavelength (±3 nm), and mobile phase ratio (±5%). The chromatographic results were verified for minimal changes in the peak response and system suitability values. Stability studies were performed on 100% solutions stored at room temperature (25 ± 2 °C) and under refrigeration (2-8 °C) for 24 h to confirm the stability of tegoprazan and its nitroso impurities.

Analytical sustainability assessment

The sustainability of the developed UPLC method for the determination of tegoprazan and its nitroso impurities was comprehensively evaluated using AGREE, GAPI tools, and the RGB 12 algorithm (White Analytical Chemistry model). These tools are widely recognized to assess the compliance of analytical methods with the principles of Green Analytical Chemistry (GAC). The AGREE metric provides the numerical greenness score based on the twelve principles of GAC and generates a value between 0.00 (poor compliance) and 1.00 (excellent compliance). This metric offers an integrated, objective interpretation of the proposed method in alignment with modern sustainability requirements. The Green Analytical Procedure Index (GAPI) was utilized to examine each stage of the analytical workflow, such as sample preparation, solvent and reagent selection, instrument energy demand, and waste generation. The resultant color-coded pentagonal GAPI pictogram highlights the relative environmental burden of each step and helps identify areas of reduced ecological impact.

The overall methodological sustainability of the proposed method was further assessed using the RGB 12 algorithm. This tool combines three essential dimensions: analytical performance (red), environmental friendliness (green), and operational/economic practicality (blue). Parameters such as method simplicity, analysis time, solvent safety, energy consumption, waste output, cost, and robustness were scored and integrated into a single "whiteness" value. The whiteness score reflects the holistic balance of the proposed method. The results of this study confirm the analytical efficiency, environmental responsibility, and routine applicability of the proposed method.

RESULTS AND DISCUSSION

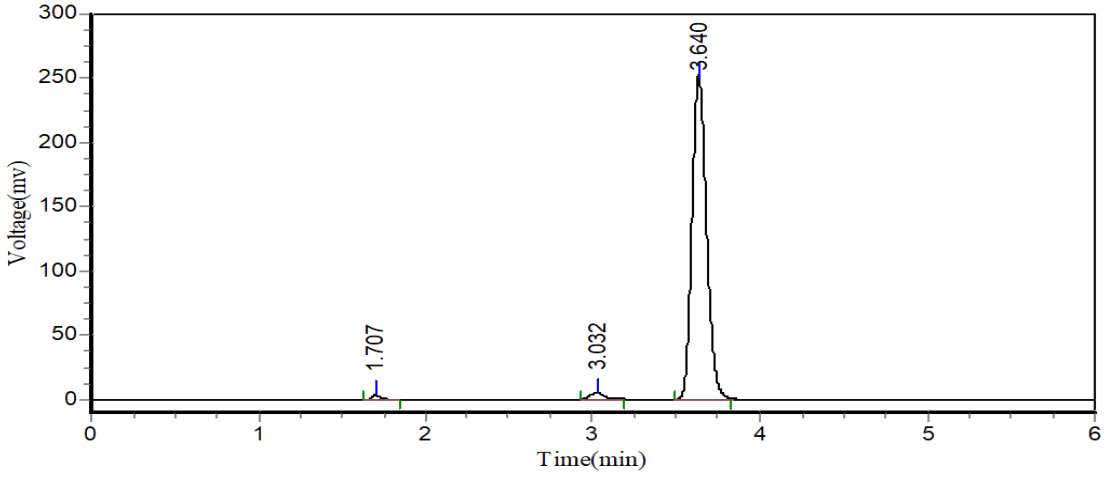
The main goal of this research was to develop a reliable, fast, and environmentally friendly UPLC method for the simultaneous assessment of tegoprazan and its associated nitroso impurities in both the active pharmaceutical ingredient and finished dosage forms. As no fully sustainable or green analytical procedure is currently available to resolve and quantify the nitroso impurities of tegoprazan, this study was designed to fill the gap in the literature. The chromatographic conditions were carefully optimized to obtain the best possible separation, symmetric peak shapes, and high detection sensitivity required for accurate impurity profiling. The chromatographic conditions were systematically optimized through a series of experimental trials to achieve baseline separation of tegoprazan and its five nitroso impurities with acceptable peak symmetry and system suitability.

The chromatographic conditions for the simultaneous resolution of tegoprazan and its nitroso impurities were systematically optimized through the evaluation of different mobile phase compositions, buffer systems, column dimensions, and flow rates. In the first trial, a mobile phase comprising of 0.1% aqueous formic acid and ethanol (50:50, v/v) was employed on an ACQUITY BEH C18 (150 mm) column at 0.3 mL/min with detection at 262 nm. Under these conditions, no resolution of the analytes was achieved, and this indicated that the method was unsuitable for analysis (Figure 2A.). In the second trial, mobile phase composition was changed to 0.1% aqueous formic acid and ethanol (70:30, v/v) using an ACQUITY BEH C18 (100 mm) column with the maintenance of the same flow rate and detection wavelength. The chromatogram under this conditions displayed improper resolution accompanied by baseline disturbances, and hence this method was unsuitable for the resolution of analytes (Figure 2B.).

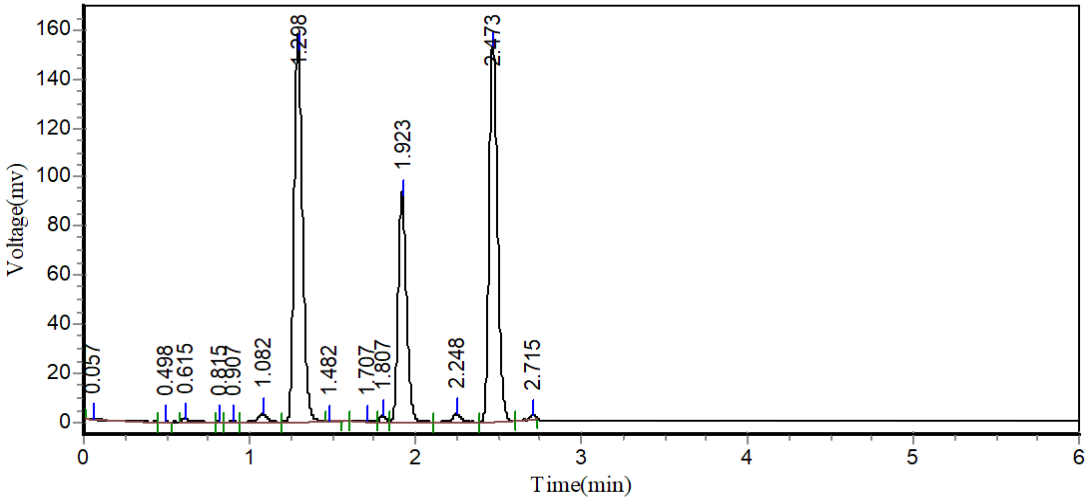
The effect of organic phase on the resolution was further investigated with the increase of ethanol content. In this condition, 0.1% aqueous formic acid and ethanol (30:70, v/v) were utilized. However, no significant improvement in chromatographic performance was observed in this condition compared with the previous trial. This suggests that formic acid was not an appropriate buffer additive to achieve satisfactory separation of tegoprazan and its nitroso impurities (Figure 2C.). Subsequently, the mobile phase was modified with the replacement of formic acid with 5 mM ammonium acetate buffer at pH 3.8 and ethanol (20:80, v/v). The chromatographic run produced a stable baseline with poor separation. This result indicates that the condition needs further optimization (Figure 2D.).

In the fifth trial, the buffer concentration was increased to 10 mM ammonium acetate at pH 4.2 with ethanol in a ratio of 25:75 (v/v). Under these conditions, tegoprazan peak and its nitroso impurities were detected. Nevertheless, the peak symmetry and overall system suitability parameters were not within acceptable limits, and hence this condition was rejected (Figure 2E.). Finally, the mobile phase composition was optimized to 10 mM ammonium acetate buffer (pH 3.8) and ethanol in 60:40 (v/v), with a 0.35 mL/min flow rate on an ACQUITY BEH C18 (100 mm) column. This condition produces a significant improvement in chromatographic resolution, peak symmetry, and overall system suitability with a stable baseline. Hence, this condition was considered satisfactory and was selected for subsequent QbD studies.

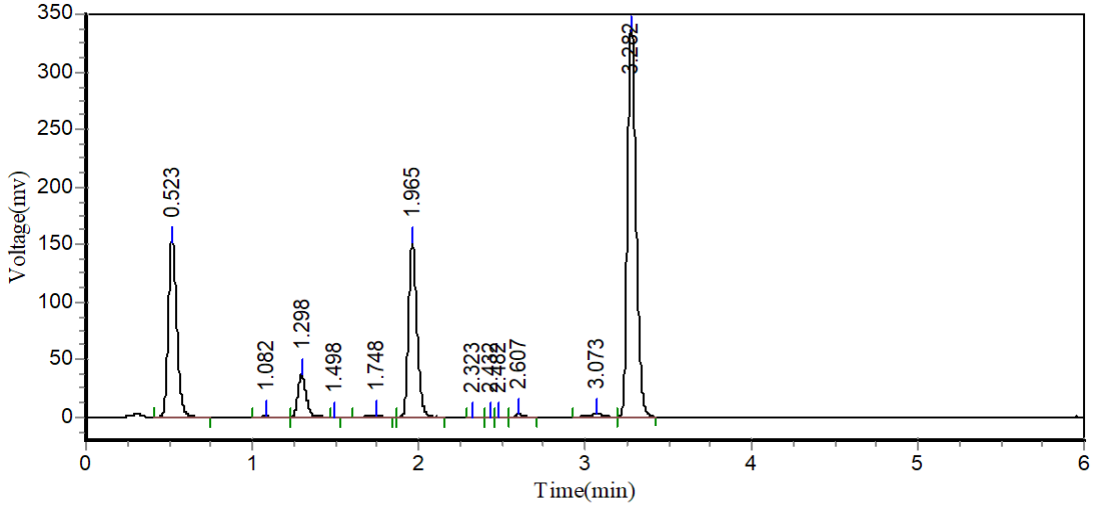
A


Voltage(mv)
Time(min)
1.707
3.032
3.640

B


Voltage(mv)
Time(min)
0.057
0.498
0.615
0.815
0.907
1.082
1.298
1.482
1.707
1.807
1.923
2.248
2.473
2.715

C


Voltage(mv)
Time(min)
0.523
1.082
1.298
1.498
1.748
1.965
2.323
2.607
3.073
3.282

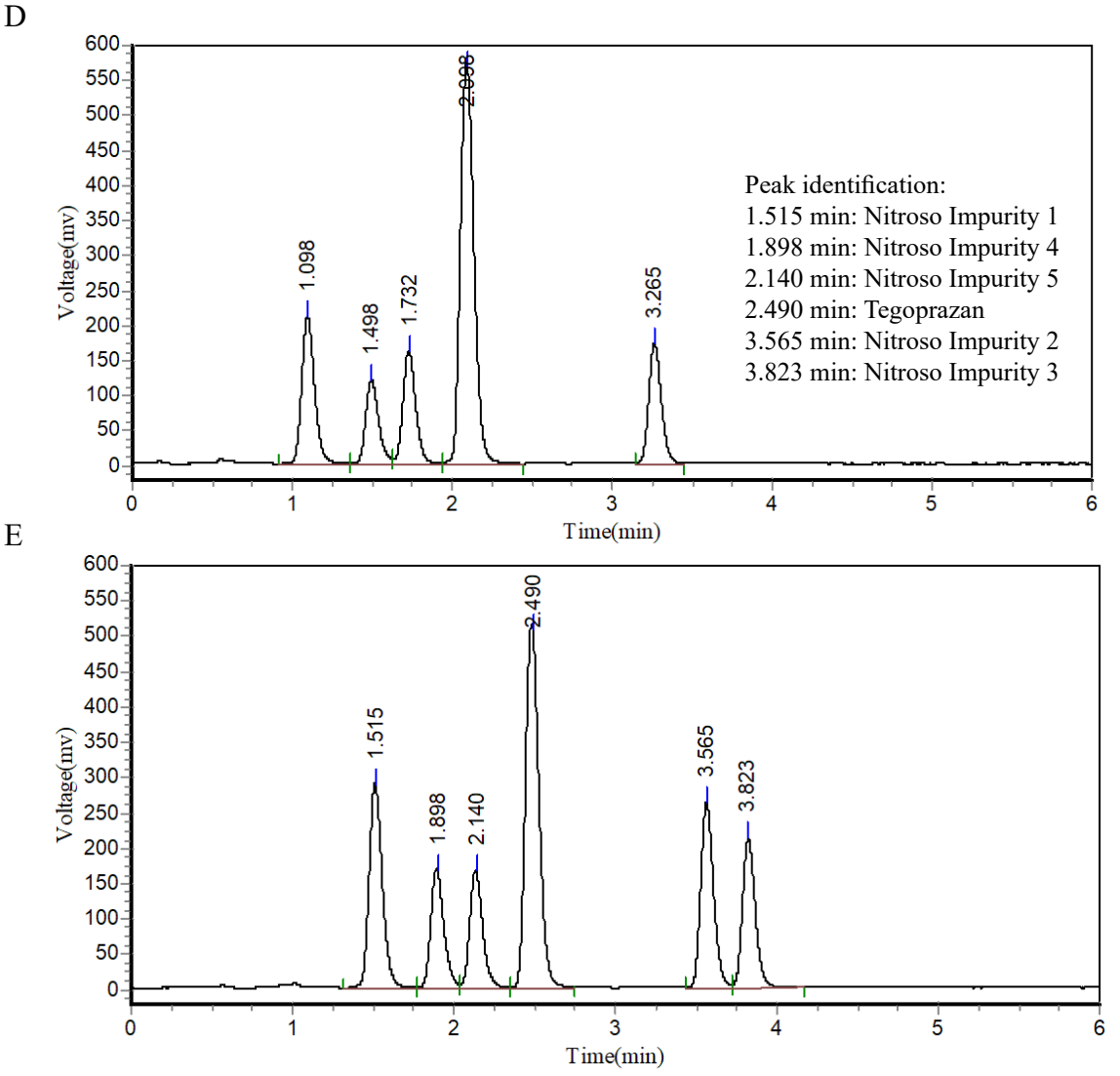


Figure 2. Chromatograms observed during the method optimization steps for the UPLC analysis of tegoprazan and its nitroso impurities

A) Trial 1: 0.1% aqueous formic acid–ethanol (50:50, v/v); (B) Trial 2: 0.1% aqueous formic acid–ethanol (70:30, v/v); (C) Trial 3: 0.1% aqueous formic acid–ethanol (30:70, v/v); (D) Trial 4: 5 mM ammonium acetate (pH 3.8)–ethanol (20:80, v/v); and (E) Trial 5: 10 mM ammonium acetate (pH 4.2)–ethanol (25:75, v/v). The chromatograms are presented at an enlarged scale to facilitate clear visualization of peak separation, peak symmetry, and baseline characteristics during method development

QbD-aided method optimization

The QbD approach was used for the systematic optimization of a robust and reliable analytical method for the quantification of tegoprazan and its nitroso impurities. DoE-guided method optimization facilitated the evaluation of the influence of the selected CMPs such as organic solvent composition (% ethanol), mobile-phase pH, and flow rate, on the chromatographic responses. A CCD experimental design was used to identify the most influential factors that affect analyte separation. The results from 20 experimental runs (Table 1.) reveal that the organic solvent composition displays the most significant effect on the resolution between close compounds such as nitroso impurities 2 and 3, and 4 and 5. The full factor ANOVA table for these responses was presented in supplementary tables S1, S2 and S3, respectively, for R1, R2, and R3.

Table 1. Experimental design and chromatographic responses observed during the QbD-based method optimization for the analysis of tegoprazan and its nitroso impurities

Run	Factor 1: Organic Solvent (%)	Factor 2: pH	Factor 3: Flow Rate (mL/min)	Response 1: Resolution (Nitroso impurity 2 and 3)	Response 2: Resolution (Nitroso impurity 4 and 5)	Response 3: Tailing Factor (Nitroso impurity 5)
1	60	3.7	0.35	2.68	2.47	0.98
2	55	3.5	0.30	2.64	2.43	1.00
3	60	3.3	0.35	2.73	2.50	0.97
4	50	3.3	0.35	2.66	2.46	0.99
5	60	3.3	0.25	2.78	2.55	1.02
6	55	3.5	0.30	2.74	2.52	1.04
7	50	3.7	0.25	2.72	2.51	1.05
8	55	3.5	0.30	2.81	2.58	1.01
9	55	3.5	0.30	3.10	2.78	0.96
10	50	3.3	0.25	2.62	2.43	0.98
11	60	3.7	0.25	3.14	2.80	0.94
12	50	3.7	0.35	2.71	2.52	0.96
13	55	3.5	0.22	2.68	2.47	0.98
14	55	3.5	0.30	2.64	2.43	1.00
15	55	3.5	0.30	2.73	2.50	0.97
16	55	3.5	0.38	2.66	2.46	0.99
17	55	3.8	0.30	2.78	2.55	1.02
18	63	3.5	0.30	2.74	2.52	1.04
19	55	3.2	0.30	2.72	2.51	1.05
20	47	3.5	0.30	2.81	2.58	1.01

The resolution between nitroso impurities 2 and 3 was selected as Response 1 in the DoE model to evaluate the effect of critical chromatographic parameters on separation efficiency. The impact of CMPs on the resolution between nitroso impurities 2 and 3 was assessed statistically using a quadratic model generated through RSM. The model exhibits a high coefficient of $R^2 = 0.9644$, indicating that approximately 96.44% of the variability in resolution could be explained by the selected model. The adjusted R^2 value was 0.8718. These values were in good agreement, proving the strong predictive power and minimal overfitting of the model. The model F-value of 10.41 and the associated p-value of < 0.0088 demonstrate that the model was highly significant.

The experimental design matrix (Table 1.) shows that the resolution between nitroso impurities 2 and 3 was in the range of 2.6 to 3.2. The maximum separation was reported at a moderate organic composition of 47- 63% at a 0.3 mL/min flow rate.

The experimental design demonstrated that the resolution between nitroso impurities 2 and 3 was varied across the investigated organic solvent range (47–63%). As illustrated in the response surface and contour plots (Figure 3.), the chromatographic resolution was observed to be improved as the organic solvent composition approached the optimized region around 55%. This improvement reflects the enhancement of analyte partitioning between the mobile and stationary phases. At the upper end of the investi-

gated design space (63% ethanol), a slight reduction in resolution was observed compared with the optimum conditions. Since the experimental design was restricted to 47–63% ethanol, no conclusions were drawn regarding chromatographic behaviour beyond this investigated range. Similarly, the flow rate exhibits a strong inverse relationship with the resolution. The lower flow rates (0.25 mL/min) improve the analyte interaction with the stationary phase but cause peak broadening. The higher flow rate (0.35 mL/min) can reduce retention and compromise separation efficiency. The optimized flow rate of 0.35 mL/min provides the balance between analysis time and peak sharpness and yields a resolution value of 3.1 for nitroso impurities 2 and 3. The pH displayed a minimal effect in the study. The response surface and contour plots clearly show a slightly curved 3D surface, confirming the nonlinear relationship between the organic phase percentage and flow rate on resolution.

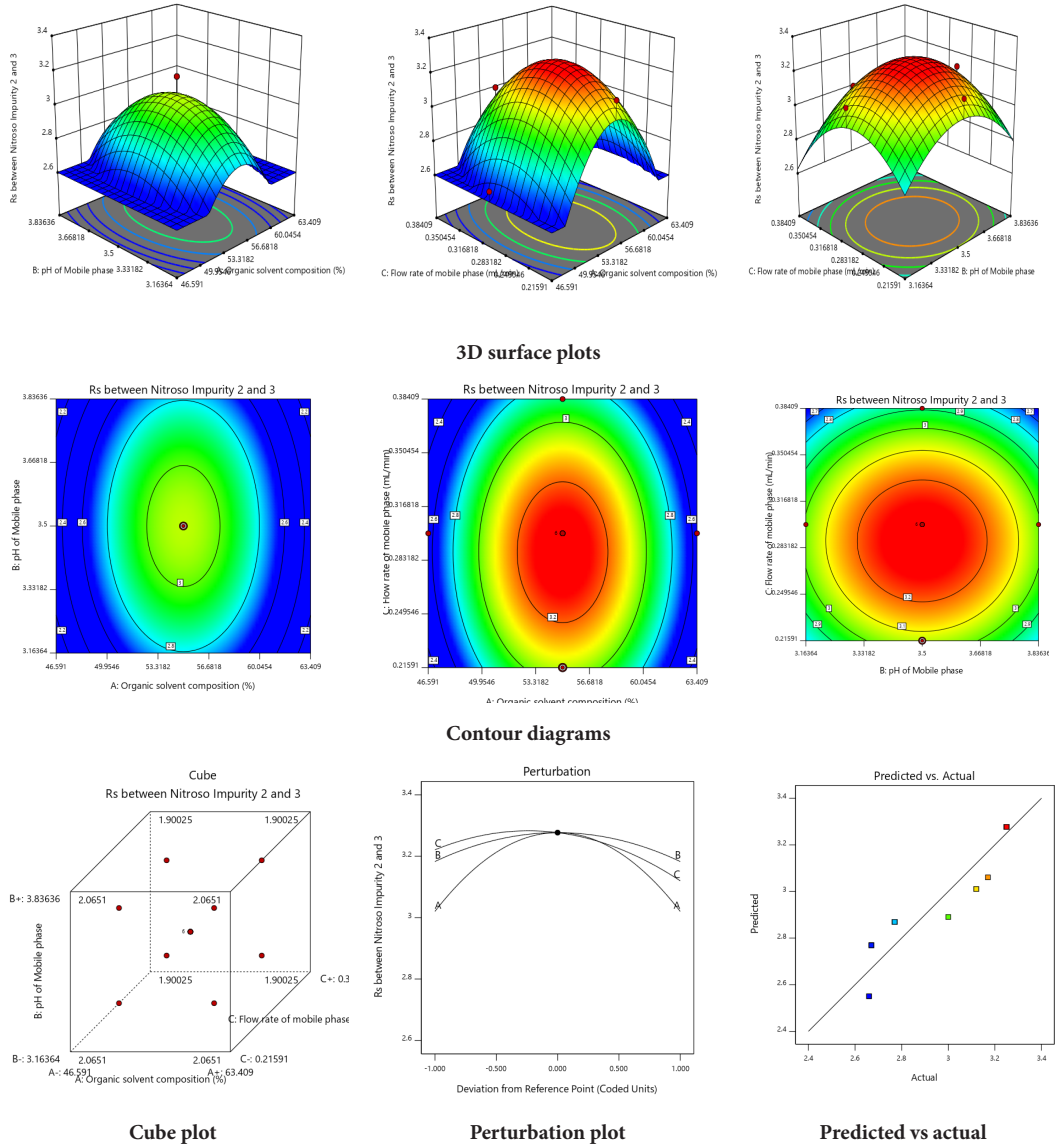
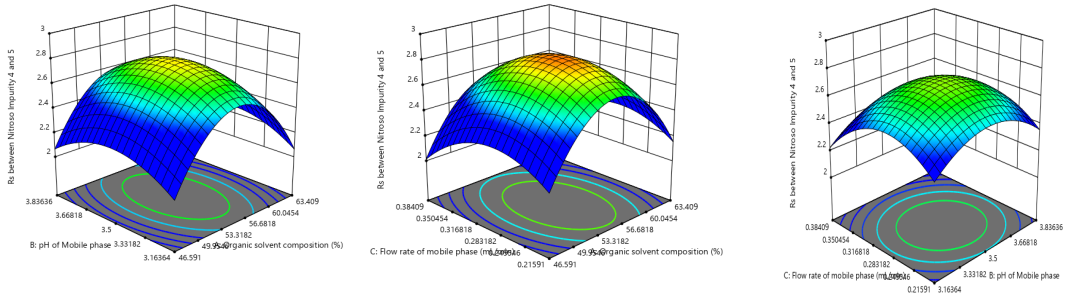


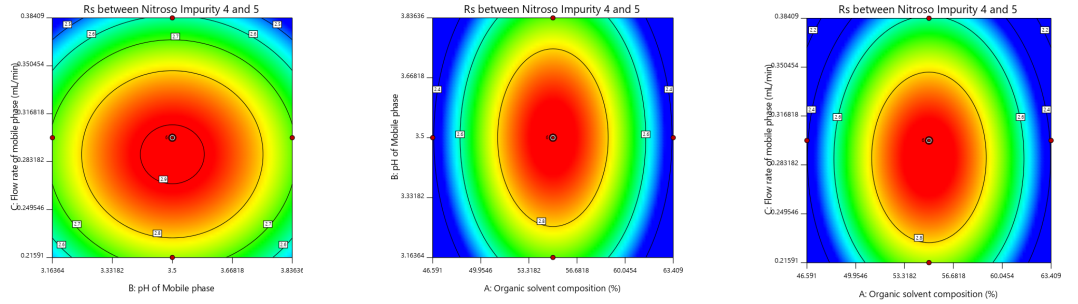
Figure 3. QbD-DoE model graphs observed during the assessment of the impact of organic solvent composition and flow rate on the resolution between nitroso impurities 2 and 3

The resolution between Nitroso Impurities 4 and 5 was selected as Response 2 to confirm that baseline separation of this critical adjacent peak pair was consistently maintained throughout the experimental design space. This analysis evaluates the robustness of the chromatographic method under deliberate variations in the CMPs. Although the resolution between Nitroso Impurities 4 and 5 was noticed to be exceed the predefined system suitability criterion (Rs > 2.0) under all experimental conditions, this response was intentionally kept in the AQbD optimization because these impurities represent one of the critical adjacent peak pairs in the developed chromatographic method. The objective of the AQbD approach was not merely to achieve the minimum acceptable resolution but to establish a robust Method Operable Design Region (MODR) in which adequate chromatographic performance is consistently maintained despite deliberate variations in the CMPs. Hence, monitoring the resolution between Nitroso Impurities 4 and 5 provides additional assurance that baseline separation remains unaffected throughout the experimental design space. This proves the robustness and reliability of the optimized analytical method.

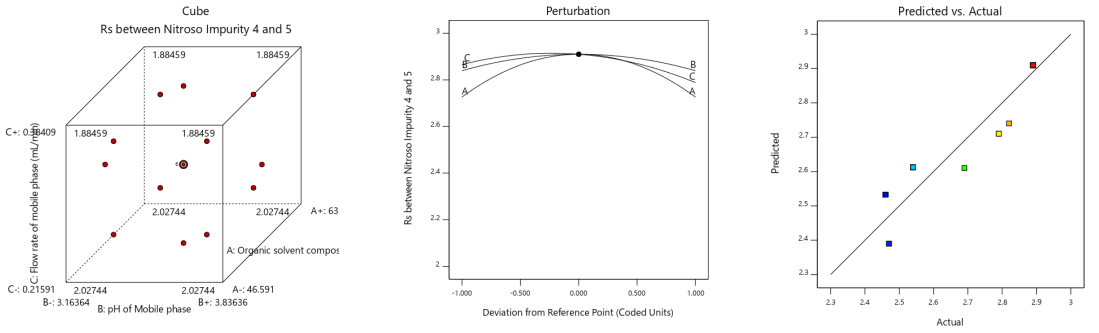
The statistical analysis demonstrated an excellent model fit with an R^2 of 0.9641. The high R^2 value indicates that approximately 96.41% of the variation in resolution could be explained by the selected variables. The adjusted R^2 value was 0.8708. These results confirm the high predictive accuracy and minimal model bias. The model F-value (10.33) and the associated p-value (< 0.0001) indicate that the model was highly significant and that the studied factors had a pronounced influence on the separation of nitroso impurities 4 and 5. The experimental results (Table 1.) show that the resolution between nitroso impurities 4 and 5 was in the range of 2.46 to 2.89. The 3D response surface and contour plots (Figure 4.) reveal a clear nonlinear relationship between the two critical factors: organic solvent percentage and flow rate. The model graphs observed in the DoE evaluation of the CMPs on the resolution between impurities C and D are presented in Figure 4.



3D surface plots



Contour diagrams



Cube plot **Perturbation plot** **Predicted vs actual**

Figure 4. QbD–DoE response surface illustrating the combined influence of organic solvent composition, pH, and flow rate of mobile phase on the resolution between Nitroso Impurity 4 and 5

The tailing factor is considered a critical chromatographic parameter that reflects the peak symmetry and column performance. This factor directly influences the quantitative accuracy and robustness of the method. Hence, in the QbD-based optimization, the tailing factor of Nitroso Impurity 5 was evaluated as Response 3. This response evaluated the combined effects of organic solvent composition, mobile-phase pH, and flow rate on the peak symmetry of Nitroso Impurity 5. The results reveal a well-behaved quadratic model with strong predictive capacity. The R^2 value was observed to be 0.9583 with an adjusted R^2 of 0.8499, indicating that the majority of the variation in peak symmetry was explained by the studied factors. The significant model F-value (8.84) and corresponding p-value of 0.013 confirm that the combined effect of organic solvent composition, mobile-phase pH, and flow rate has a meaningful influence on the tailing behaviour. The 3D surface plots and contour maps prove that the selected chromatographic factors influence the peak symmetry of nitroso impurity 5. The optimization objective for this response was to obtain a tailing factor as close as possible to the ideal value of 1.0. The tailing factor of 0.92 was achieved in optimized chromatographic conditions. Although the observed value was noticed to be slightly lower than the ideal value, this result falls within the generally accepted chromatographic limits and indicates satisfactory peak symmetry suitable for reliable quantitative analysis. Deviations from this central point produce a gradual increase in tailing, confirming that the method exhibits a well-defined design space. The high flow rates (0.35 mL/min) display distortion in peak shape, whereas very low flow (0.25 mL/min) also causes mild deterioration in symmetry. These results are in alignment with the perturbation plot, where the flow rate displays the steepest curvature. The organic solvent composition similarly demonstrates a parabolic effect with maximum peak symmetry at an organic solvent composition of 55 solvent. Organic solvents at 50% and 60% resulted in visibly greater tailing. The cube plot illustrates these trends consistently across extreme factor levels. The predicted vs. actual plot confirms the high model reliability because all the data points were observed to be close to the regression line with minimal deviation. Collectively, these findings validate that precise control of organic content, pH, and flow rate is essential for maintaining the optimal symmetry of Nitroso Impurity 5. This result reinforces the robustness of the proposed optimized UPLC conditions for routine quality assessment of tegoprazan and its nitroso impurities. Representative model graphs are presented in Figure 5.

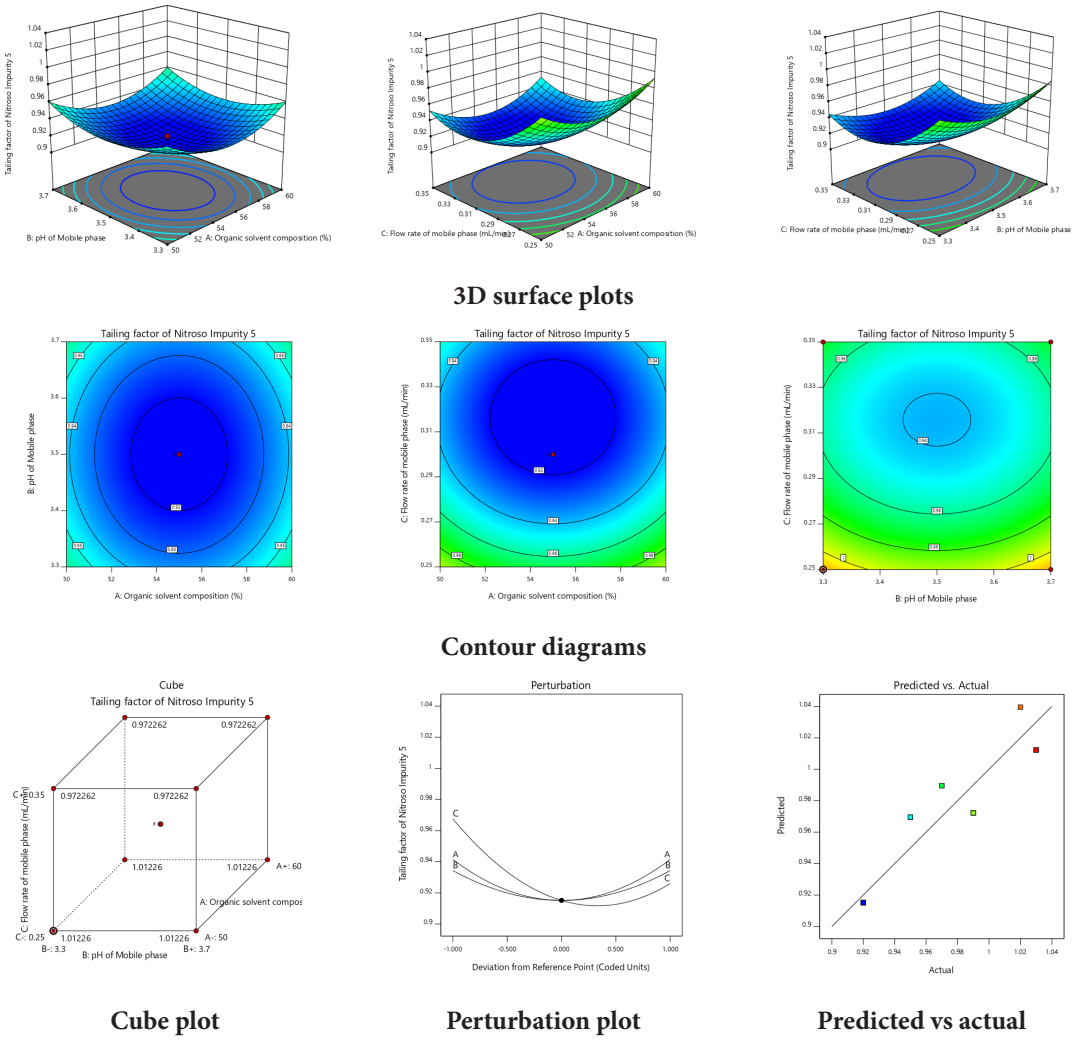


Figure 5. QbD–DoE response surface illustrating the combined influence of organic solvent composition, pH, and flow rate of mobile phase on the tailing factor of Nitroso Impurity 5

Numerical optimization using desirability function

Numerical optimization was performed using the desirability function approach in Design-Expert® software to identify the optimum chromatographic conditions that simultaneously satisfy all predefined analytical objectives. The optimization criteria were established to maximize the resolution between nitroso impurities 2 and 3 and between 4 and 5, along with the tailing factor of nitroso impurity 5 as close as possible to the ideal value of 1.0. The investigated factors were constrained within their respective experimental ranges. Based on these optimization criteria, the software predicted the optimum chromatographic conditions as 55% ethanol, mobile-phase pH 3.5, and a flow rate of 0.30 mL/min with an overall desirability value of 1.000. This indicates that all analytical objectives were simultaneously satisfied. The predicted optimum conditions were subsequently verified experimentally and were found to provide satisfactory chromatographic performance. The desirable graphs observed in the DOE study were presented in Figure S1. The numerical optimization criteria used for desirability function analysis was presented in Table 2.

Table 2. Numerical optimization criteria used for desirability function analysis

Parameter	Goal	Lower Limit	Upper Limit	Weight	Importance
Organic solvent (%)	In Range	47	63	1	3
Mobile-phase pH	In Range	3.2	3.8	1	3
Flow rate (mL/min)	In Range	0.22	0.38	1	3
Resolution (NI2–NI3)	Maximize	2.60	3.30	1	5
Resolution (NI4–NI5)	Maximize	2.40	2.90	1	3
Tailing factor (NI5)	**Target = 1.0**	0.90	1.10	1	3

Numerical optimization based on the desirability function identified 55% ethanol, pH 3.5, and a flow rate of 0.30 mL/min as the optimum chromatographic conditions that yield an overall desirability of 1.000 and simultaneously satisfy all predefined optimization criteria. Under these optimized conditions, the method produced resolution values of 3.25 for Nitroso Impurities 2 and 3 and 2.89 for Nitroso Impurities 4 and 5, with acceptable tailing factors and theoretical plates. The system suitability chromatogram (Figure 6.) further demonstrates the clear and well-resolved elution of all components, with retention times of 0.74 min (Nitroso Impurity 1), 1.16 min (Nitroso Impurity 4), 1.45 min (Nitroso Impurity 5), 2.20 min (Tegoprazan), 3.19 min (Nitroso Impurity 2), and 3.54 min (Nitroso Impurity 3). The DoE findings and system suitability results confirmed that the optimized UPLC method was robust, precise, and capable of reliably separating tegoprazan and its nitroso impurities for routine quality control applications.

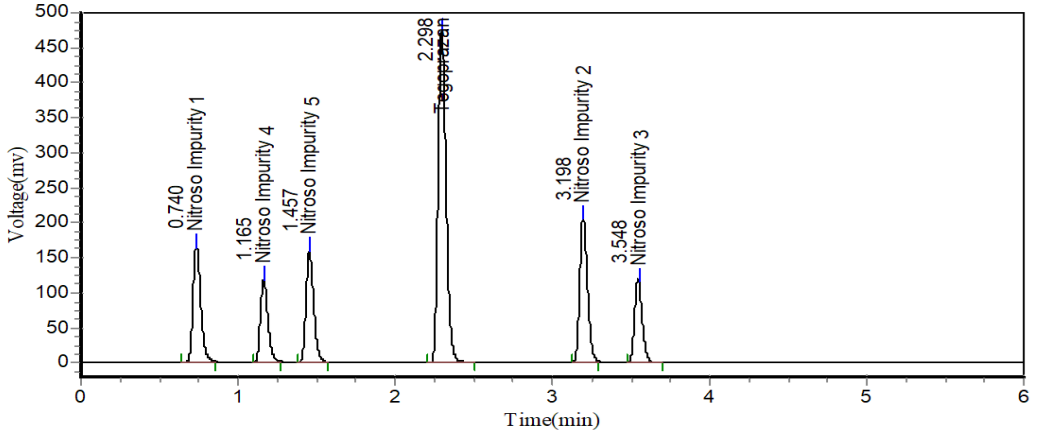


Figure 6. System suitability chromatogram showing baseline separation of Tegoprazan and Nitroso Impurities 1–5 obtained using the optimized UPLC conditions (55% ethanol, pH 3.5, 0.30 mL/min)

Method validation

The proposed method was validated in accordance with the guidelines of ICH Q2(R2) to prove the applicability of the method for the routine analysis of tegoprazan and its nitroso impurities. The selectivity of the proposed UPLC method was evaluated by analyzing blank, standard, individual nitroso impurity solutions, and a spiked sample containing tegoprazan along with all nitroso impurities under the optimized chromatographic conditions. The chromatogram displays no interfering peaks at the retention times of tegoprazan or its nitroso impurities. This chromatographic response demonstrates that the excipients, diluent, and other matrix components did not interfere with the analyte responses. Baseline separation of tegoprazan and all nitroso impurities was achieved with acceptable peak symmetry and resolution, proving the ability of the method to selectively identify

and quantify each analyte in the presence of the other components. The method demonstrates excellent linearity for over 12.5–100 μg/mL for tegoprazan and 0.125–1.0 μg/mL for its nitroso impurities. Since all nitroso impurities are potential mutagenic impurities that are monitored at comparable specification levels, a common calibration range was selected to encompass the expected working concentration for routine quality control analysis. This concentration range adequately covers the LOQ and extends to concentrations above the specification level. The correlation coefficient (r^2) of the linearity range was 0.9996 for tegoprazan and higher than 0.999 for all nitroso impurities. This result establishes a strong linear relationship between the concentration and detector response over the studied concentration range. Intra-day (repeatability), inter-day (intermediate precision), and ruggedness studies were performed to assess the precision of the method. The % RSD in intra-day precision was observed to be in the range of 0.21–0.56%, and the inter-day precision was found to be 0.51–0.93%. Ruggedness of the developed method was evaluated by a second analyst under identical chromatographic conditions using independently prepared sample solutions at the test concentration. The % RSD in ruggedness assessment was noticed to be 0.49–0.88%. The %RSD values in all these studies were within the acceptance limit of 2% for tegoprazan and its nitroso impurities, confirming the reproducibility and robustness of the method. Recovery studies at 50%, 100%, and 150% spiked levels were conducted to assess the method accuracy. The mean recovery values were within the ranges of 98.01% and 100.97% for tegoprazan and its nitroso impurities, respectively. These results meet the acceptance criteria of 98–102% and prove the accuracy and reliability of the method for impurity quantification.

The method also shows high sensitivity for the determination of nitroso impurities. The LOD and LOQ were determined experimentally using the signal-to-noise (S/N) approach in accordance with ICH Q2(R2). The baseline noise was measured from a blank chromatogram with the selection of a region adjacent to the analyte peak free from any interfering signals, and the S/N ratio was calculated using the chromatographic software. The LOD and LOQ corresponded to concentrations producing S/N ratios of approximately 3:1 and 10:1, respectively. The experimentally determined LOD values observed in the range of 0.032 to 0.038 μg/mL, whereas the LOQ values ranged from 0.105 to 0.125 μg/mL for the nitroso impurities. The calibration curve was intentionally established from the validated LOQ concentration to ensure reliable and accurate quantification throughout the analytical range. Consequently, the lowest calibration standard (0.125 μg/mL) corresponds to the validated LOQ for those impurities with an LOQ of 0.125 μg/mL, rather than by coincidence. These low LOD and LOQ values confirmed the capability of the method to detect and quantify trace levels of nitroso impurities with high precision. A summary of the validation results is presented in Table 3.

Table 3. Validation results of the developed UPLC method for the quantification of tegoprazan and its nitroso impurities in accordance with ICH Q2(R2) guidelines

S No	Test	Parameter	Results observed					
			Tegoprazan	NI 1	NI 2	NI 3	NI 4	NI 5
1	Linearity	Range (µg/mL)	12.5-100	0.125-1.0	0.125-1.0	0.125-1.0	0.125-1.0	0.125-1.0
2		Slope	19923	190433	222864	134260	146722	185367
3		Intercept	7524.1	785.91	3121.6	6208.1	6784.2	13776
4		Correlation coefficient	0.9996	0.9995	0.9999	0.9991	0.9994	0.9992
5	Repeatability (% RSD)[$]	Intra-day precision	0.38	0.40	0.21	0.29	0.56	0.47
6	Intermediate Precision (% RSD)[$]	Inter-day precision	0.69	0.85	0.72	0.51	0.68	0.93
7		Ruggedness	0.51	0.54	0.74	0.88	0.76	0.49
8	Recovery/ Accuracy (% recovery)[$$]	50 % spiked level	98.19±0.17	98.58±0.32	99.01±0.13	99.69±0.13	98.76±0.09	99.36±0.17
9		100 % spiked level	100.89±0.25	99.01±0.19	98.19±0.11	99.85±0.21	98.91±0.12	98.01±0.09
10		150 % spiked level	100.97±0.19	98.46±0.35	99.28±0.36	99.65±0.19	99.85±0.15	98.56±0.36
11	Sensitivity (µg/mL)	LOD	-	0.032	0.038	0.038	0.032	0.032
12		LOQ	-	0.105	0.125	0.125	0.105	0.105

NI = Nitroso impurity; % RSD values for n = 3 ($); average±SD for n=6

The robustness assessment results summarized in Table 4 demonstrate that the proposed method remains stable and reliable under small, deliberate variations in critical method parameters. Adjustments in the organic solvent composition (±3%) produced negligible changes in the peak area (<0.6%) and maintained an adequate resolution for both impurity pairs. The resolution was observed to be in the range of 3.19 to 3.29 for Nitroso impurities 2 and 3, and 2.81 to 2.93 for Nitroso impurities 4 and 5. A similar trend was observed with pH modification of ±0.2 units. In this case, the peak area deviation was less than 0.5%, and the resolution values were within the acceptance criterion of > 2.0. This indicates that the ionization-dependent selectivity of the analytes does not significantly impact this pH change. The variations in the detection wavelength (±5 nm) also displayed minimal influence on both the peak area and chromatographic separation. The consistency of all responses across these stress conditions highlights the ruggedness of the method and validates that none of the tested parameters exert a critical effect on quantification or resolution.

Table 4. Influence of minor changes in organic solvent composition, mobile-phase pH, and detection wavelength on the chromatographic performance of tegoprazan and its nitroso impurities under optimized UPLC conditions

S No	Parameter	Change	% change in peak area of Tegoprazan	Resolution between Nitroso impurity 2 and 3	Resolution between Nitroso impurity 4 and 5
1	Organic solvent	+3% change (58% ethanol)	0.52	3.19	2.81
2		-3% change (52% ethanol)	0.19	3.29	2.93
3	pH	+0.2 units (3.7)	0.36	3.21	2.76
4		-0.2 units (3.3)	0.47	3.33	2.77
5	Wavelength	+5 nm (267 nm)	0.35	3.36	2.85
6		-5 nm (257 nm)	0.31	3.37	2.79

Application to pharmaceutical formulation

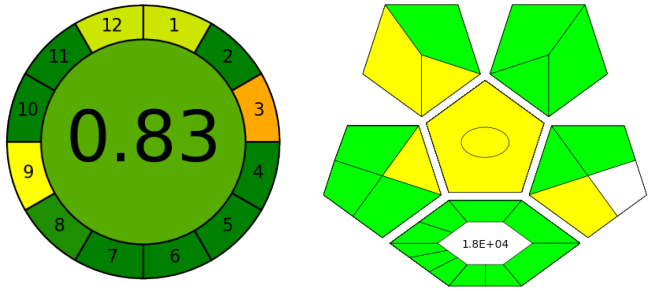
The applicability of the developed UPLC method was evaluated for the analysis of a marketed tegoprazan pharmaceutical formulation. The chromatogram of the formulation sample shows no detectable peaks corresponding to the nitroso impurities, indicating the absence of interference from formulation excipients and confirming the selectivity of the method. The assay of tegoprazan in the formulation was calculated to be 98.75%. The assay demonstrates the accuracy of the proposed method for quantitative estimation of the active pharmaceutical ingredient. The formulation sample was spiked with known concentrations of all nitroso impurities at the precision level and was tested for further assessed to the suitability of the method for impurity analysis. All impurity peaks were well resolved from the tegoprazan and excipient peaks, and recoveries more than 98% were obtained for each impurity. These results confirm that the developed method was suitable for the simultaneous determination of tegoprazan and its nitroso impurities in pharmaceutical formulations and can be reliably employed for routine quality control applications.

Greenness assessment

The greenness of the developed UPLC method was comprehensively evaluated using the AGREE metric and GAPI. These tools collectively assess compliance with the 12 principles of GAC. The AGREE assessment produced an overall score of 0.83 indicating excellent environmental sustainability (Figure 7.). The high score was primarily due to the direct analytical approach with minimal sample preparation, which reduces sample handling, the absence of derivatization and thereby minimizing reagent consumption and analytical steps. The method utilizes ethanol as an organic modifier instead of conventional solvents such as acetonitrile or methanol, significantly reducing toxicity while maintaining satisfactory chromatographic performance. The use of a low mobile-phase flow rate (0.30 mL/min) and a short chromatographic run time (6 min) low solvent consumption, energy requirement, and analytical waste. The method also generated only a small volume of waste per analysis and did not employ highly hazardous or persistent chemicals, and thereby improving operator safety and reducing environmental impact. Moderate AGREE scores were assigned to principles related to reagent hazards because the method required ammonium acetate buffer and ethanol, both of which require routine laboratory handling precautions despite being less hazardous than many conventional chromatographic solvents. Overall, none of the 12 AGREE principles were rated in the red zone, demonstrating excellent compliance with GAC principles.

The GAPI pictogram further confirmed the environmental sustainability of the developed method. The green sectors correspond to direct sample prepa-

ration, minimal reagent consumption, minimal waste generation, and low occupational and environmental hazards. The yellow sectors indicate moderate environmental impact associated with the use of buffered mobile phases and laboratory instrumentation, which are unavoidable in chromatographic analysis but were minimized through the selection of ethanol and a low-flow UPLC method. No red zones associated with highly toxic reagents, excessive waste generation, or hazardous sample preparation were observed. Furthermore, the calculated E-factor of 1.8×10^4 was substantially lower than those typically reported for conventional liquid chromatographic methods (10^6–10^9), confirming a significant reduction in waste generation. Collectively, the AGREE and GAPI assessments demonstrate that the proposed method successfully integrates high analytical performance with the principles of Green Analytical Chemistry, making it suitable for environmentally sustainable routine quality control analysis.



A B

Figure 7. Integrated greenness assessment of the developed UPLC method for Tegoprazan and its Nitroso impurities using AGREE (A) and GAPI (B) tools

Assessment of method whiteness

The overall sustainability of the method for the analysis of tegoprazan and its nitroso impurities was further assessed using the RGB model. This model produces a green and white score for the proposed method by integrating analytical performance (Redness), environmental safety (Greenness), and practical efficiency (Blueness). As shown in Figure 8, the method produced a redness score of 78.2%, which reflects strong analytical performance based on excellent precision, accuracy, and linearity across all analytes. The Greenness score was observed to be 87.8%, and the score highlights environmentally favorable characteristics. The observed blueness score (85.8%) demonstrates high practical feasibility. The combined brilliance value of 83.8% was observed, and the value classifies the method as "white." This indicates a well-balanced integration of analytical reliability, ecological compatibility, and operational efficiency. This holistic sustainability evaluation confirms that the proposed method was not only robust and sensitive for routine quantification of tegoprazan and its nitroso impurities, but was also environmentally responsible and practically efficient.

		w=3			w=3			w=3			
REDNESS (analytical performance)	W=3	Precision (RSD)			Accuracy (RE)			Linearity			
	LAV=33.3	2.0%			2.0%			0.999			
	LSV=66.6	0.5%			0.5%			1.0000			
CS: 78.2%	Result	0.38%			0.41%			0.9996			
	Score (0-100)	88	88	88	81	81	81	67	67	67	
		w=3			w=3			w=3			
GREENNESS (safety and eco-friendliness)	W=3	Reagent consumption			Waste amount			Other occupational hazards			
	LAV=33.3	1200 mL			800 mL			4 hazards			
	LSV=66.6	200 mL			400 mL			2 hazards			
CS: 87.8%	Result	150 mL			280 mL			0 hazards			
	Score (0-100)	89	89	89	95	95	95	80	80	80	
		w=3			w=3			w=3			
BLUENESS (productivity / practical effectiveness)	W=3	Cost of analysis			Time of analysis			Sample consumption			
	LAV=33.3	250			600 min / 20 runs			acceptable			
	LSV=66.6	125			300 min / 20 runs			satisfactory			
CS: 85.8%	Result	88			120 min / 20 runs			satisfactory			
	Score (0-100)	95	95	95	100	100	100	66.6	66.6	66.6	
FINAL COLOR:		REDNESS		GREENNESS		BLUENESS		BRILLIANCE (MB):		83.8%	
WHITE		≥33.3%	≥66.6%	≥33.3%	≥66.6%	≥33.3%	≥66.6%				
		yes	yes	yes	yes	yes	yes				
Short annotation: 83.8white		Long annotation: 83.8white(78.2/3red-87.8/3green-85.8/3blue)									

Figure 8. RGB sustainability assessment results observed for the method developed for the analysis of tegoprazan and its nitroso impurities

CONCLUSION

This study successfully establishes a QbD-driven, environmentally sustainable method for the simultaneous determination of tegoprazan and its nitroso impurities by addressing the lack of green, high-resolution analytical procedures reported in the literature. DoE-based optimization revealed the dominant influence of the organic solvent composition and mobile phase pH of on chromatographic selectivity. The optimized method employs ethanol-based mobile phases and a low flow rate, which demonstrates robust separation of all nitroso impurities with excellent system suitability and strong predictive agreement between experimental and model-predicted responses. Method validation confirmed the high precision, accuracy, linearity, sensitivity, and ruggedness that support its suitability for routine quality assessment. The Greenness evaluation through AGREE and GAPI tools verifies the low ecological footprint of the method, while the RGB assessment classifies the method as "white," which reflects the superior balance between analytical quality, environmental compatibility, and operational efficiency. Overall, the proposed method provides a reliable, sustainable, and regulatory-aligned analytical platform for routine monitoring of tegoprazan and its nitroso impurities in pharmaceutical environments with significant analytical performance and environmental responsibility.

Future studies need to focus on extending the method to high-resolution mass spectrometric detection for structural confirmation of low-level nitrosamines. Further studies are needed to explore greener mobile-phase modifiers, column technologies with reduced environmental impact, and solvent-free or microfluidic chromatographic platforms to enhance method sustainability.

ACKNOWLEDGEMENTS

The authors extend their sincere thanks to Dream Step Research Solutions for their support in carrying out this research work.

AUTHOR CONTRIBUTION STATEMENT

KKV: project administration, writing – original draft, writing – review & editing. KKV, MA, TSR, TVS, and YG: Conceptualization, methodology, investigation, literature review, data curation, formal analysis, and visualization. KKV, MA, TSR, TVS, and YG authors reviewed the manuscript and approved it for publication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Cioc, R. C., Ciarán, J., Monika, M., & Robert, N. (2023). Formation of N-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies. *Organic Process Research & Development, 27,* 1736-1750. doi:10.1021/acs.oprd.3c00153.

Hemanth, P. R., Vikram, T., Pramod, K., Gunjan, K., Narasimha Beeraka, M., & Rajashree, D. (2024). Nitrosamines crisis in pharmaceuticals – Insights on toxicological implications, root causes and risk assessment: A systematic review. *Journal of Pharmaceutical Analysis, 14,* 100919. doi:10. 1016/j.jpha.2023.12.009.

Joohwan, K., Sungjun, K., Hyun-Jung, N., Shin-Young, R., & Soo-Jin, K. (2019). Simultaneous quantification of tegoprazan and its major metabolite M1 in dog plasma using liquid chromatography-tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis, 171,* 99-103. doi: 10.1016/j.jpba.2019.03.045.

Kaijing, G., Xiao, H., Hongyi, Z., & Chen, M. (2023). Characterisation of degradation products of tegoprazan by LC-MS and GC-MS. *Journal of Pharmaceutical and Biomedical Analysis, 228,* 115323. doi:10.1016/j.jpba.2023.115323.

Kim Dong, K., Keun-Ho, L., Sung-jun, K., Soo-Jin, K., Song Jin, L., & Chi Hye, P. (2019). Effects of Tegoprazan, a Novel Potassium-Competitive Acid Blocker, on Rat Models of Gastric Acid–Related Disease. *The Journal of Pharmacology and Experimental Therapeutics, 369,* 318-327. doi:10.1124/jpet.118.254904.

Kruhlak, N. L., Marianne, S., Roland, F., Stefan, G., Bodo, H., Irene, H., & Stephen, H. (2024). Determining recommended acceptable intake limits for N-nitrosamine impurities in pharmaceuticals: Development and application of the Carcinogenic Potency Categorization Approach (CPCA). *Regulatory Toxicology and Pharmacology, 150,* 105640. doi:10.1016/j.yrtph.2024.105640.

Lee, K. J., Son, B. K., Kim, G. H., Hye-Kyung, J., Hwoon-Yong, J., & Il-Kwun, C. (2019). Randomised phase 3 trial: tegoprazan, a novel potassium-competitive acid blocker, vs. esomeprazole in patients with erosive oesophagitis. *Alimentary Pharmacology & Therapeutics, 49,* 864-872. doi:10.1111/apt.151853.

Lei, Z., Shutao, D., Shujuan, X., Lihua, W., & Yingde, S. (2024). Determination of Enantiomeric Impurity in Tegoprazan Drug Substance by HPLC. *Separation Science Plus, 7,* e202400125.doi:10.1002/sscp.202400125.

Lei, Z., Shujuan, X., Qingshan, B., Lihua, W., Yingde, S., & Shufeng, M. (2025). Determination of Four Related Substances in Tegoprazan by HPLC. *Separation Science Plus, 8,* e70123. doi:10.1002/sscp.70123.

Mengjie, X., Yize, C., Yu, W., Manjie, L., & Qinghua, W. (2025). A Novel UPLC–MS/MS Method for Determining Tegoprazan in Rat Plasma: An Application in a Rat Pharmacokinetic Study. *Biomedical Chromatography, 39,* e70264. doi:10.1002/bmc.70264.

Nassef, H. M., Ahmed, H. A., El-Atawy, M. A., Alanazi, T. Y. A., & Mohamed, M. A. (2024). Green assessment of RP-UPLC method for estimating Triamcinolone Acetonide and its degraded products compared to Box-Behnken and Six Sigma designs. *Green Chemistry Letters and Reviews, 17*. doi:10.1080/17518253.2023.2301315.

Prasad, S. S., Kasimala, B. B., & Anna, V. R. (2021). QBD-Based Analytical Method Development and Validation for The Separation and Quantification of Agomelatine and Its Impurities in Solid Oral Dosage Forms Using HPLC. *RASĀYAN Journal of Chemistry, 14,* 2183-2190. doi:10.31788/RJC.2021.1446426.

Sravanthi, G., & Kumaraswamy, G. (2025). Development of a quality by design-based ultra-performance liquid chromatography method for the simultaneous estimation of casirivimab and imdevimab with greenness metrics. *Green Analytical Chemistry, 13,* 100248. doi:10.1016/j.greeac.2025.100248.

Takahashi, N., & Yukinori, T. (2019). Tegoprazan, a Novel Potassium-Competitive Acid Blocker to Control Gastric Acid Secretion and Motility. *Pharmacology and Experimental Therapeutics, 364,* 275-286. doi:10.1124/jpet.117.244202.

Varma, R. B., Pavani, P., Subhashini, E., Kandula, R., & Babu, B. K. (2024). Green Analytical Approach for HPLC Method Development for Quantification of Sorafenib and Its Pharmacopeia Impurities: LC–MS/MS Characterization and Toxicity Prediction of Stress Degradation Products. *Separation Science Plus, 7,* e202400106. doi:10.1002/sscp.202400106.

Veerendra, Y. V. S., Kumar, P. B., Mankumare, S. D., & Kumar, V. C. (2024). Evaluation of analytical greenness metric for an eco-friendly method developed through the integration of green chemistry and quality-by-design for the simultaneous determination of Nebivolol hydrochloride, Telmisartan, Valsartan, and Amlodipine besylate. *Heliyon, 10,* e35376. doi:10.1016/j.heliyon.2024.e35376.

Wang, W., Yaseen, H. S., Li, X., Muhammad Zia, A., Yaowu, C., & Guorong, L. (2025). Tegoprazan: a novel, highly selective, and potent potassium-competitive acid blocker (P-CAB). *European Journal of Clinical Pharmacology, 81,* 1103-1117. doi:10.1007/s00228-025-03850-6.