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A sensitive green analytical LC-MS/MS method for the quantification of trace levels of nine nitrosamine impurities in Zaltoprofen bulk drug

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ABSTRACT

Zaltoprofen, a selective cyclooxygenase-2 (COX-2) inhibitor and a member of the nonsteroidal anti-inflammatory drugs, has been associated with the formation of nitrosamine impurities under specific conditions involving secondary or tertiary amines and nitrite salts, particularly in acidic environments. This research presents a detailed, exacting, and structured analytical approach for quantifying nine nitrosamine impurities (N-Nitroso dimethyl amine, N-Nitroso-N-methyl-4-aminobutyric acid, N-Nitroso diethyl amine, N-Nitroso morpholine, N-Nitrosoethylisopropylamine, N-Nitroso diisopropylamine, N-Nitrosomethylphenylamine, N-Nitroso dibutyl amine, and 1-Methyl-4-Nitrosopiperazine) utilizing the Acquity UPLC HSS T3 (100 x 3.0 mm, 1.8µ) column on the Waters UHPLC-MS/MS Acquity H-Class platform. The method established a gradient program utilizing 0.1% formic acid and methanol as mobile phases A and B, respectively, at a flow rate of 0.5 ml/minutes, with ionization performed using atmospheric pressure chemical ionization in positive mode. Quantification was performed utilizing multiple reaction monitoring mode, achieving sensitivity at the parts per million (ppm) level. The validation of the proposed analytical method adhered to the guidelines established by the International Council for Harmonisation Q2, incorporating parameters such as system precision, specificity, linearity (R^2 > 0.990 from limit of quantification (LOQ) to 200%), accuracy, method precision, intermediate precision, limit of detection (0.0014 ppm), and LOQ (0.0041 ppm) for the nine nitrosamine impurities. The method further assesses environmental sustainability through the Analytical GREEnness and Analytical Eco-Scale evaluations, which validated the method's commendable eco-friendliness. This thorough approach highlights the essential requirement for the oversight of nitrosamine impurities in active pharmaceutical ingredients to uphold safety and conformity with regulatory standards.

1. INTRODUCTION

Zaltoprofen is classified as a nonsteroidal anti-inflammatory drug (NSAID) that has been thoroughly examined for its analgesic, antipyretic, and anti-inflammatory pharmacological properties. Specifically, it is recognized for its selective inhibition of the cyclooxygenase-2 (COX-2) enzyme and its ability to suppress pain responses triggered by bradykinin without obstructing the bradykinin receptors. Consequently, it is applicable for the treatment of various pain and inflammatory disorders, which include dental pain, musculoskeletal pain, postoperative pain, and osteoarthritis. Zaltoprofen functions as a selective COX-2 inhibitor by obstructing the COX-2 enzyme, which is responsible for the synthesis of prostaglandins associated with inflammation and pain. Additionally, it inhibits the activity of bradykinin, a peptide known to induce pain and inflammation. This pharmaceutical agent is categorized within the BCS class II (characterized by low solubility and high permeability), presenting challenges in bioavailability and necessitating innovative formulation strategies to enhance its solubility and dissolution rate. The subsequent sections will provide a comprehensive overview of the chemical structure

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and solubility characteristics, along with the physicochemical properties of Zaltoprofen, followed by methodologies aimed at improving the pharmaceutical efficacy of this drug. The chemical designation of Zaltoprofen is 2-(10,11-Dihydro-10-oxodibenzo[b,f]thiepin-2-yl) propionic acid [1–3].

Nitrosamine impurities represent a distinct category of compounds defined by the incorporation of a nitroso group (-N=O) bonded to an amine, and they are recognized as potential genotoxic impurities classified under the Cohort of Concern in accordance with ICH M7 guidelines. It is imperative that these impurities are quantified at trace levels to mitigate the carcinogenic risks associated with human consumption. Throughout the pharmaceutical manufacturing process, nitrosamines may arise in the presence of secondary or tertiary amines coupled with nitrosating agents. The utilization of specific reagents, catalysts, or solvents that either contain or generate nitrosating agents is accountable for such occurrences. Additionally, contamination can be exacerbated by raw materials and excipients utilized in drug formulation. In these components, the presence of nitrosating agents or amines can facilitate the synthesis of nitrosamines. Pharmaceuticals, such as Zaltoprofen, have notably attracted heightened scrutiny regarding nitrosamine impurities due to the potential carcinogenic consequences associated with these contaminants [4–7].

Nitrosoamine impurities [N-Nitroso dimethyl amine (NDMA), N-Nitroso-nmethyl-4-aminobutyric acid (NMBA), N-Nitroso diethyl amine (NDEA), N-Nitroso morpholine (NMOR), N-Nitrosoethylisopropylamine (NEIPA), N-Nitroso disopropylamino (NDIPA), N-Nitrosomethylphenylamine (NMPA), N-Nitroso dibutyl amine (NDBA), and 1-Methyl-4-Nitrosopiperazine (MENP)] may be generated during the manufacturing processes of drug substances and products through the reactions of secondary or tertiary amines with nitrosating agents under specific conditions. In light of the emergence of these impurities, regulatory authorities have formulated a comprehensive policy aimed at their detection and management. The identification of nitrosamines in Zaltoprofen, a non-steroidal anti-inflammatory medication, necessitates an assessment of the drug's production process to identify possible contamination sources and ensure adherence to safety regulations. The concentrations of all nine nitrosamines in Zaltoprofen must be regulated in accordance with established regulatory standards of green analytical chemistry (GAC), which emphasizes the formulation of environmentally sustainable, safe, and efficient analytical methodologies, which is an expanding area of study. The principles of GAC, particularly in the context of drug quantification utilizing UHPLC-MS/MS, are established to mitigate environmental impact while maintaining robust analytical performance. This methodology is increasingly vital within the pharmaceutical sector, where there is a demand for sensitive and specific analytical techniques. The tenets of GAC advocate for the reduction or complete removal of hazardous organic solvents from analytical procedures. This objective can be achieved by substituting these solvents with more environmentally benign alternatives such as ethanol or water, significantly lowering toxic waste and enhancing safety conditions for laboratory personnel. Analytical GREEnness (AGREE) and the Analytical Eco-Scale are also employed in the evaluation of the environmental friendliness of analytical methods. These frameworks incorporate tools that analyze various factors such as solvent consumption, energy utilization, and waste production, ultimately providing a comprehensive score that encompasses these elements and identifies areas for improvement [8–10].

Nevertheless, a comprehensive review of the literature concerning analytical techniques for the quantification of Zaltoprofen and its associated impurities in bulk pharmaceuticals and/or formulations across various matrices utilizing UPLC, HPLC, or LC-MS/MS has been documented. There is a lack of scholarly work focused on the quantification of nitrosamine impurities in Zaltoprofen employing LC-MS/MS methodologies [11–17].

This scholarly article aims to clarify the UHPLC-MS/MS methodology for the detection of nine potential genotoxic nitrosamine contaminants, which include NDMA, NDEA, NMOR, NEIPA, NNMBA, NDIPA, NMPA, MENP, and NDBA in Zaltoprofen, as illustrated in Figure 1. This analytical technique underwent validation through parameters such as system suitability/system precision, specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, and method precision, which were evaluated in accordance with ICH Q2 guidelines, alongside an assessment of the method's environmental impact using green analytical tools including the AGREE and the Analytical Eco-Scale [18].

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All solvents and reagents that were acquired are of LC-MS grade and possess an exceptional purity level exceeding 99.8%. Methanol was procured from Honeywell, located in Charlotte, NC, USA. Formic acid was supplied by Fluka. Samples of Zaltaprofen alongside six nitrosamine impurities were obtained from Hetero Lab, situated in Hyderabad, India. Water was acquired utilizing the Milli-Q Water purification system from Millipore Technologie Ltd.

2.2. Analytical instruments, columns, and software

A Waters UHPLC–MS/MS Acquity H–Class system, which includes Binary pumps, an auto injector, a sample cooler, and a column heater, was integrated with a Waters Xevo–TQ–XS Mass Spectrometer for the analysis. The data obtained from mass spectrometry were processed using the MassLynx software. An Acquity UPLC HSS T3 (100 x 3.0) mm 1.8µ Part No. 186004680 column was utilized for the analytical procedures. An Analytical balance and a Micro Balance manufactured by Sartorius were utilized for the precise weighing of the standard and sample. The filtration of the mobile phase was conducted using a microfiltration unit equipped with Millipore 0.22 µm PVDF filters. Both the sample and standard underwent sonication with the Power Sonic 410 Ultra Sonicator. Pipetting was performed with the micropipette provided by Eppendorf.

2.3. Optimized LC-MS conditions

For the UHPLC mobile phase, mobile phase A was prepared with 0.1% formic acid dissolved in Milli-Q water,

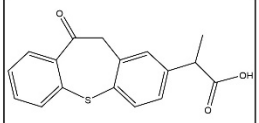
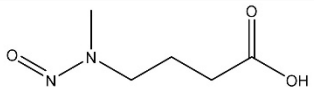
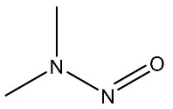
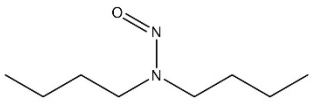
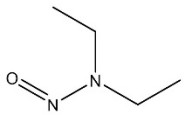
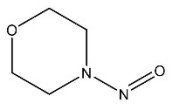
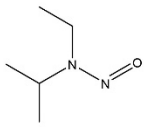
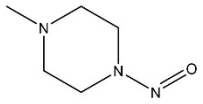
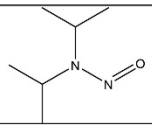
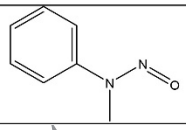
S. No.	Structure	Name, Chemical formula, Exact mass	S. No.	Structure	Name, Chemical formula, Exact mass
1		Zaltoprofen 2-(10,11-Dihydro-10-oxodibenzo[b,f]thiepin-2-yl) propionic acid Chemical Formula: $C_{17}H_{14}O_3S$ Exact Mass: 298.07	6		N-nitroso-N-methyl-4-aminobutanoic acid. Chemical Formula: $C_5H_{10}N_2O_3$ Exact Mass: 146.07
2		N-nitrosodimethylamine Chemical Formula: $C_2H_6N_2O$ Exact Mass: 74.05	7		N-nitrosodibutylamine Chemical Formula: $C_8H_{18}N_2O$ Exact Mass: 158.14
3		N-nitrosodiethylamine. Chemical Formula: $C_4H_{10}N_2O$ Exact Mass: 102.08	8		N-nitrosomorpholine Chemical Formula: $C_4H_8N_2O_2$ Exact Mass: 116.06
4		N-nitrosoethylisopropylamine Chemical Formula: $C_5H_{12}N_2O$ Exact Mass: 116.09	9		1-Methyl-4-Nitrosopiperazine Chemical Formula: $C_5H_{11}N_3O$ Exact Mass: 129.09
5		N-nitrosodiisopropylamine Chemical Formula: $C_6H_{14}N_2O$ Exact Mass: 130.11	10		N-nitrosomethylphenylamine. Chemical Formula: $C_7H_8N_2O$ Exact Mass: 136.06

Figure 1. Represents the chemical structures of Zaltoprofen, NDMA, NDEA, NEIPA, NDIPA, NMBA, NDBA, NMOR, NMPA, and MENP.

whereas mobile phase B was formulated with 0.1% formic acid in Methanol, following a gradient program with an overall runtime of 30 minutes. The specific program for mobile phase B included the following time (minutes)/%B parameters: (0.0/5, 3.0/5, 8.0/30, 12.0/60, 11.0/90, 14/95, 21/95, 22.0/5, 30.0/5). The column utilized for this analysis was Part No. 186004680, identified as the Acquity UPLC HSS T3 (100 x 3.0) mm 1.8µ column. A flow rate of 0.5 ml/minutes was maintained, with the HPLC column and sample temperature regulated at 50°C and 10°C, respectively, while the injection volume was set at 20.0 µl. The diluent employed during this procedure consisted of Milli-Q Water and Methanol in an 80:20 v/v ratio. The mass spectrometry (MS) experimental protocols were conducted on a Waters UHPLC-MS/MS system, which was integrated with a quadrupole-time of flight (QTOF LC/MS, Waters, USA) utilizing an atmospheric pressure chemical ionization (APCI) source in positive scan mode. The multiple reaction monitoring (MRM) approach for data acquisition was facilitated through the Mass Lynx software. The operational conditions for the mass source were as follows: probe temperature at 250°C, source temperature at 120°C, cone gas flow rate at 150 l/hour, desolvation gas flow at 850 l/hour, nebulizer gas flow at 4.00 bar, Corona at 4.0 kV, dwell time (s) at 0.003, cone voltage (V) at 30.00, collision energy (eV) at 8.00, and delay (s) set to auto. The initial (start) time was established at 0.00 min, with a duration (end) time of 30.0 minutes.

2.4. Analytical solutions

2.4.1. Preparation of NDMA and NMBA standard solution

A reference stock solution for NDMA and NMBA nitrosamine impurities was formulated in methanol, achieving a concentration of 1,000 µg/ml. The aforementioned solution was then diluted by adding 1.0 ml into a 50 ml volumetric flask, with the volume completed to the calibrated mark using a diluent, followed by thorough mixing to yield a concentration of 20 µg/ml. From the stock solution, 1.2 ml was transferred to a 50 ml volumetric flask, brought to the mark with a diluent, and adequately mixed to produce a solution of 0.48 µg/ml. As the final step, 1.0 ml of this solution was placed into a 10 ml volumetric flask, solubilized, diluted to the mark with a diluent, and mixed thoroughly to achieve a concentration of 0.048 µg/ml.

2.4.2. Preparation of NDEA, NMOR, NEIPA, NDIPA, NMPA, MENP, and NDBA standard solution

A stock solution containing nitrosamine impurities, including NDEA, NMOR, NEIPA, NDIPA, NMPA, MENP, and NDBA, was prepared in methanol to achieve a final concentration of 1,000 µg/ml. From the resultant solution, 0.5 ml was meticulously transferred into a 50 ml volumetric flask and diluted to the calibration mark with an appropriate diluent, ensuring thorough mixing to establish a concentration of 10 µg/ml. Subsequently, 0.66 ml of the previously prepared stock solution was transferred into a 50 ml volumetric flask, with the volume adjusted to the calibration mark using a suitable diluent, ensuring complete mixing to attain a concentration of 0.132 µg/ml. Finally, 1.0 ml of this solution was added to a 10 ml volumetric flask, where it was dissolved and diluted with diluent to the designated mark, mixing thoroughly to achieve a concentration of 0.0132 µg/ml.

2.4.3. Preparation of the sample as such a solution

Weigh approximately 600.00 mg of the samples and transfer them into a clean and dry 10.00 ml polypropylene tube;

subsequently, add around 2 ml of diluent and thoroughly mix using a vortex for several minutes. Adjust the volume to the 5.00 ml mark with diluent and ensure thorough mixing. Filter the resultant solution through a 0.45 µm PVDF filter.

2.5. Analytical method validation

2.5.1. System suitability/system precision

System suitability, commonly known as system precision, has been confirmed by conducting six replicate injections of a standard solution. The relative SD (%RSD) related to the ion counts for the nine nitrosoamine impurity peaks obtained from these six replicate injections should not surpass 20.0% Table 1.

2.5.2. Specificity

Specificity has been assessed via the utilization of a diluent (blank), a reference solution, the analyte, and a sample fortified with impurities at the predetermined threshold. It is imperative to confirm that there is no interference from the blank throughout the retention period of the nine nitrosamine impurity peaks.

2.5.3. Limit of quantification (LOQ) and limit of detection (LOD)

The LOQ was established at the 30% level, which aligns with the designated standard concentration, whereas the LOD was determined at the 10% level. The precision at the LOQ has been assessed through the repeated analysis of standard solutions at the LOQ concentration (n = 6). The % RSD for the peak areas of nine nitrosamine impurities resulting from six replicate injections of the LOQ solution should not surpass 25.0.

2.5.4. Linearity

The validation of the linearity concept was conducted by incorporating the impurities NDEA, NMOR, NEIPA, NDIPA, NMPA, NDBA, and MENP at the concentration levels of LOQ, 50%, 100%, 150%, and 200%, along with specific concentrations of 0.00396 µg/ml, 0.00660 µg/ml, 0.01320 µg/ml, 0.01980 µg/ml, and 0.02640 µg/ml, respectively, in accordance with established parameters. The assessment of impurities NDMA and NMBA was performed at LOQ, 50%, 100%, 150%, and 200% with defined concentration values of 0.0144 µg/ml, 0.0240 µg/ml, 0.0480 µg/ml, 0.0720 µg/ml, and 0.0960 µg/ml. It is imperative to document both the slope and the intercept, and the coefficient of determination (r^2) for the impurity should not fall below 0.990.

2.5.5. Method precision

The precision of the analysis has been verified through the assessment of the performance of six spiked sample preparations at the specified threshold (100% level) incorporating nine nitrosamine impurities. The % RSD determined for the content ppm of the nine nitrosamine impurities across the six distinct spiked sample preparations must not exceed 25.0.

2.5.6. Accuracy (Recovery)

The accuracy has been validated through the introduction of the spiked sample containing nine nitrosamine impurities at levels of 100%, 200%, and the LOQ in accordance with the specifications. The recovery of the content in ppm for the nine nitrosamine impurities in each formulation at all levels should not deviate more than ± 30%.

2.6. Green analytical principles

GAC encompasses the advancement of environmentally friendly, safe, and sustainable methodologies. Among the three primary instruments, these include the AGREE and the Analytical Eco-Scale. The AGREE tool evaluates methodologies based on the 12 principles of GAC and assigns a comprehensive score that highlights areas necessitating further focus, lauded for its straightforward and automated functionality. The Analytical Eco-Scale operates on a system of five penalty points, where a score of zero is optimal, indicating that a higher score correlates with a more environmentally friendly method. While some tools may be more user-friendly than others, certain instruments provide more comprehensive insights; for example, NEMI is more straightforward but offers less detail compared to AGREE. Ultimately, integrating these tools into method development facilitates a holistic approach to minimizing environmental impact while maintaining analytical efficacy.

3. RESULTS AND DISCUSSION

3.1. Analytical method validation

The analytical validation of the UHPLC MS/MS technique was performed in accordance with the established guidelines (ICH Q2), encompassing system suitability/system precision, specificity, accuracy, method precision, linearity, LOQ, and LOD.

3.1.1. System suitability/system precision

Six replicate injections of standard solutions containing nine nitrosamine impurities were conducted in accordance with ICH Q2 guidelines to assess the procedure's effectiveness. The percentage (% RSD) of the areas corresponding to the nine nitrosamine impurity peaks from the six replicate injections does not exceed 20.0. The acceptance criteria were successfully achieved for the parameters pertaining to system precision and system suitability for the standard preparation. Consequently, the system is deemed appropriate for analytical applications, as illustrated in Table 3.

3.1.2. Specificity

The specificity of the optimized methodology was assessed through the injection of a blank (diluent), a standard solution containing nine nitrosamine impurities, the sample solution, and a nitrosamine-spiked sample solution. The specificity of the method was definitively established as no interference was detected in the blank at the retention time corresponding to the nine nitrosamine impurities. Figure 2 presents a representative ion chromatogram for the blank, while Figure 3 depicts the representative chromatogram for the standard solution. Furthermore, Figure 4 illustrates the representative chromatograms for the sample solution, and Figure 5 provides the representative chromatograms for the LOQ

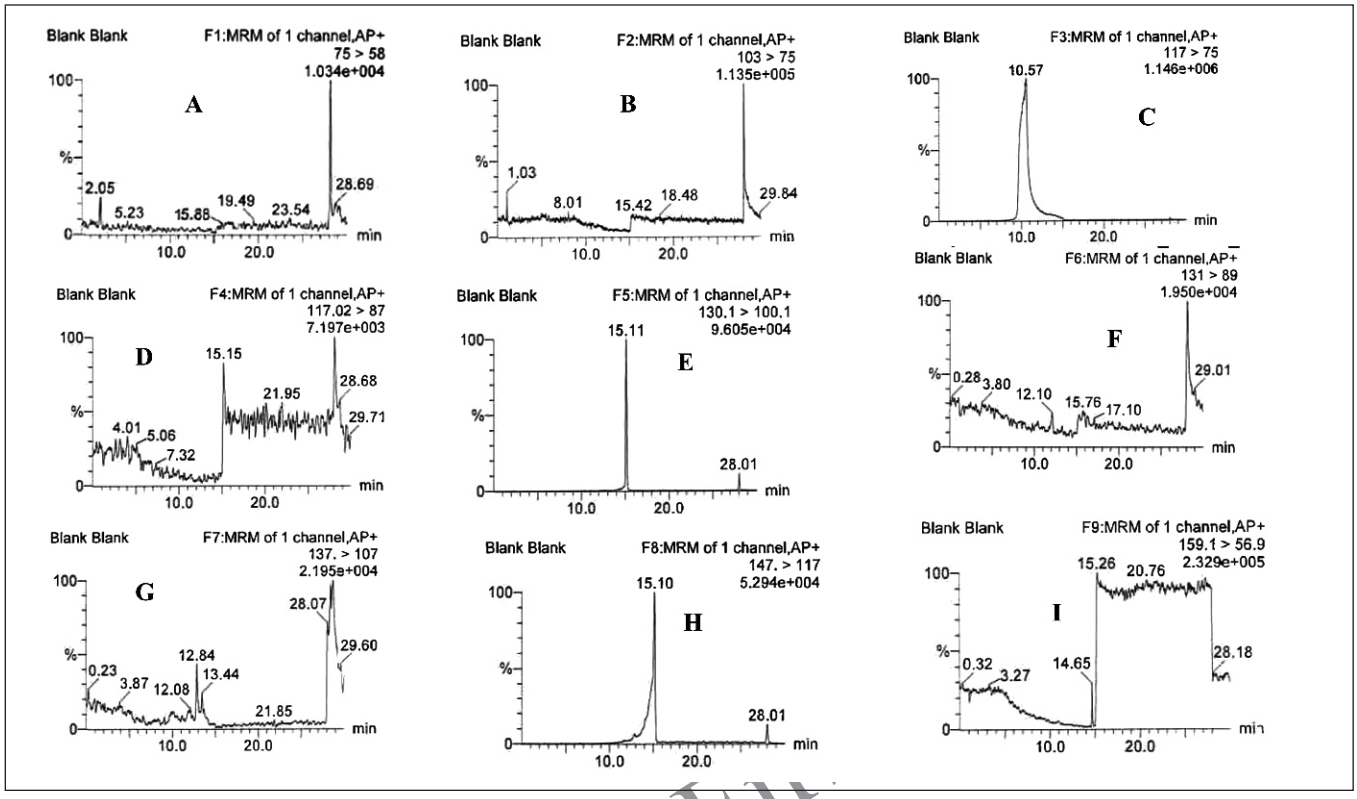


Figure 2. Typical selected Ion chromatograms of blank solutions A. NDMA, B. NDEA, C. NEIPA, D.NMOR, E. MENP, F. NDIPA, G.NMPA, H. NMBA, I. NDBA.

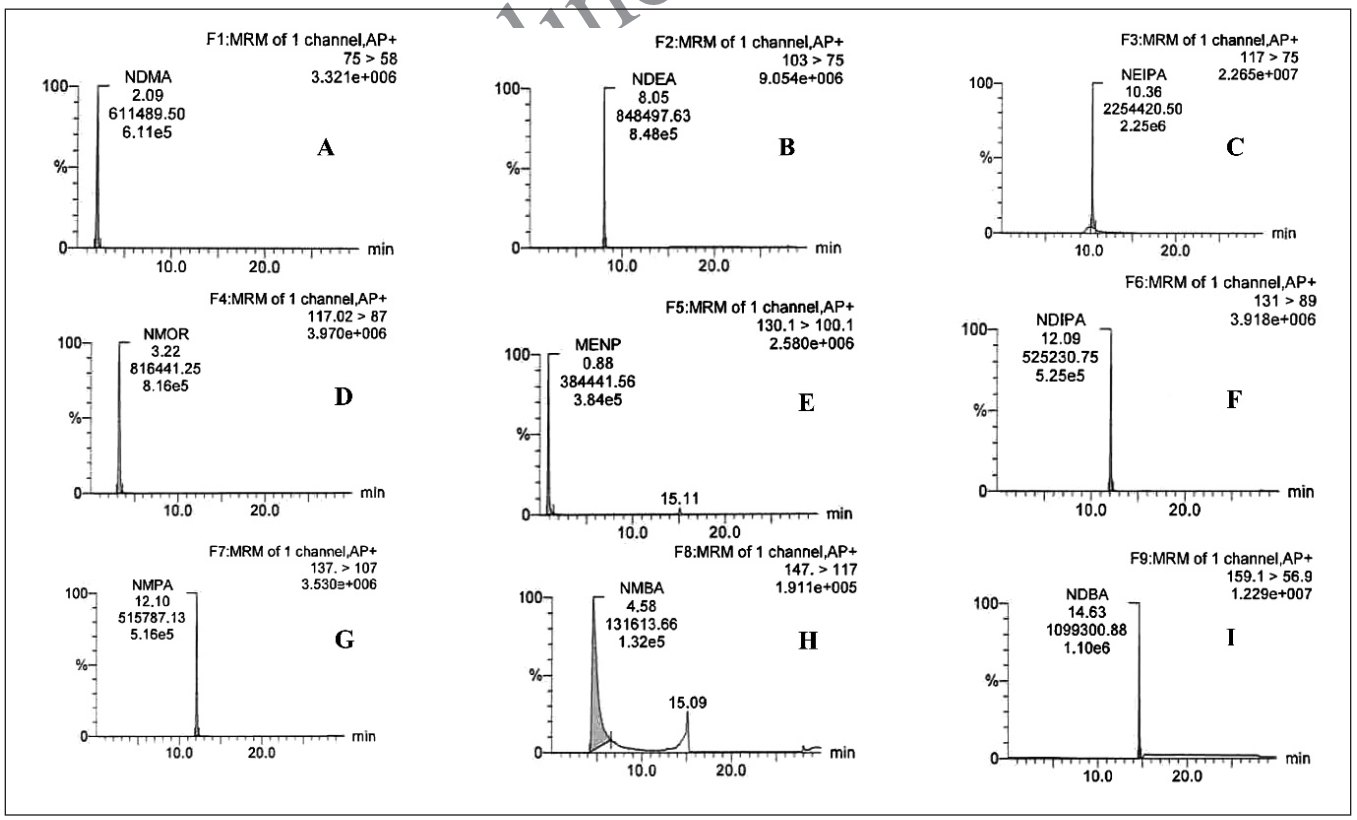


Figure 3. Typical selected ion chromatograms of standard solutions A. NDMA, B. NDEA, C. NEIPA, D.NMOR, E. MENP, F. NDIPA, G.NMPA, H. NMBA, I. NDBA.

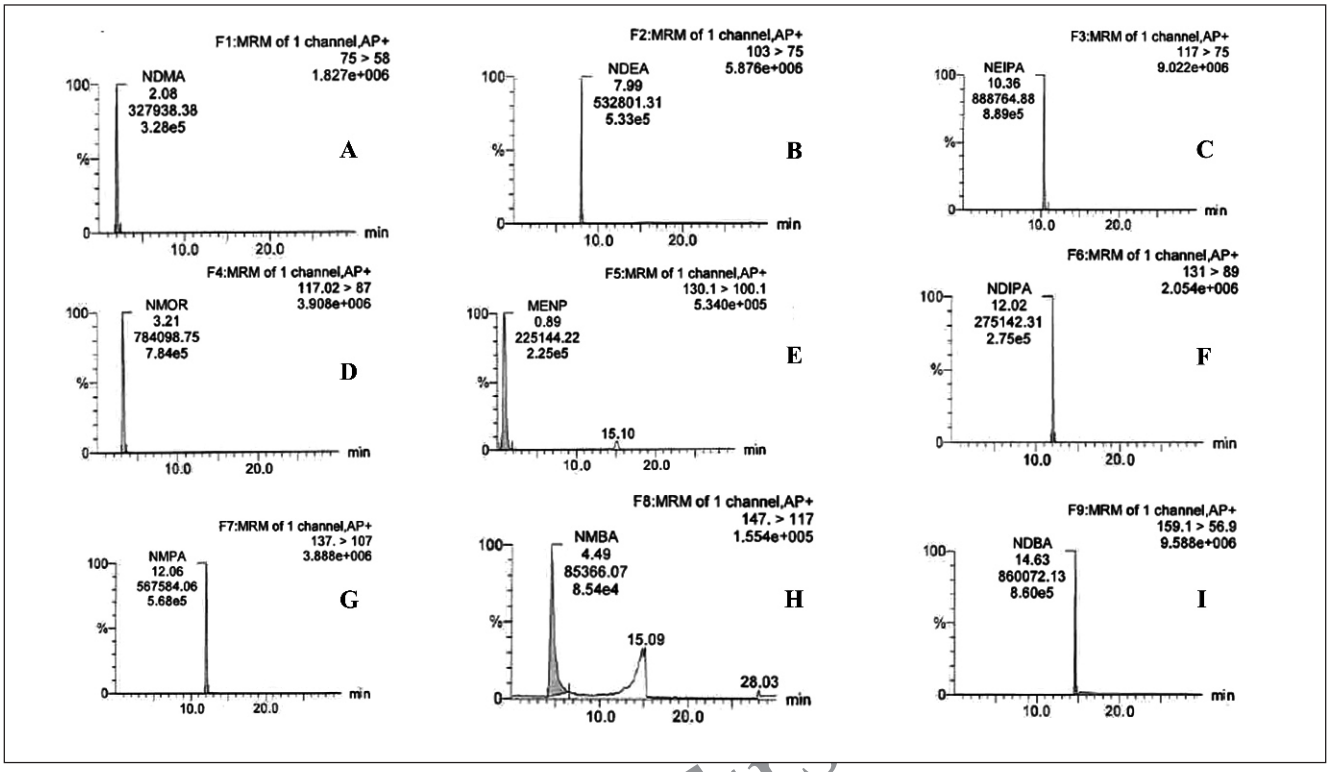


Figure 4. Typical selected ion chromatograms of sample solutions A. NDMA, B. NDEA, C. NEIPA, D. NMOR, E. MENP, F. NDIPA, G. NMPA, H. NMBA, I. NDBA.

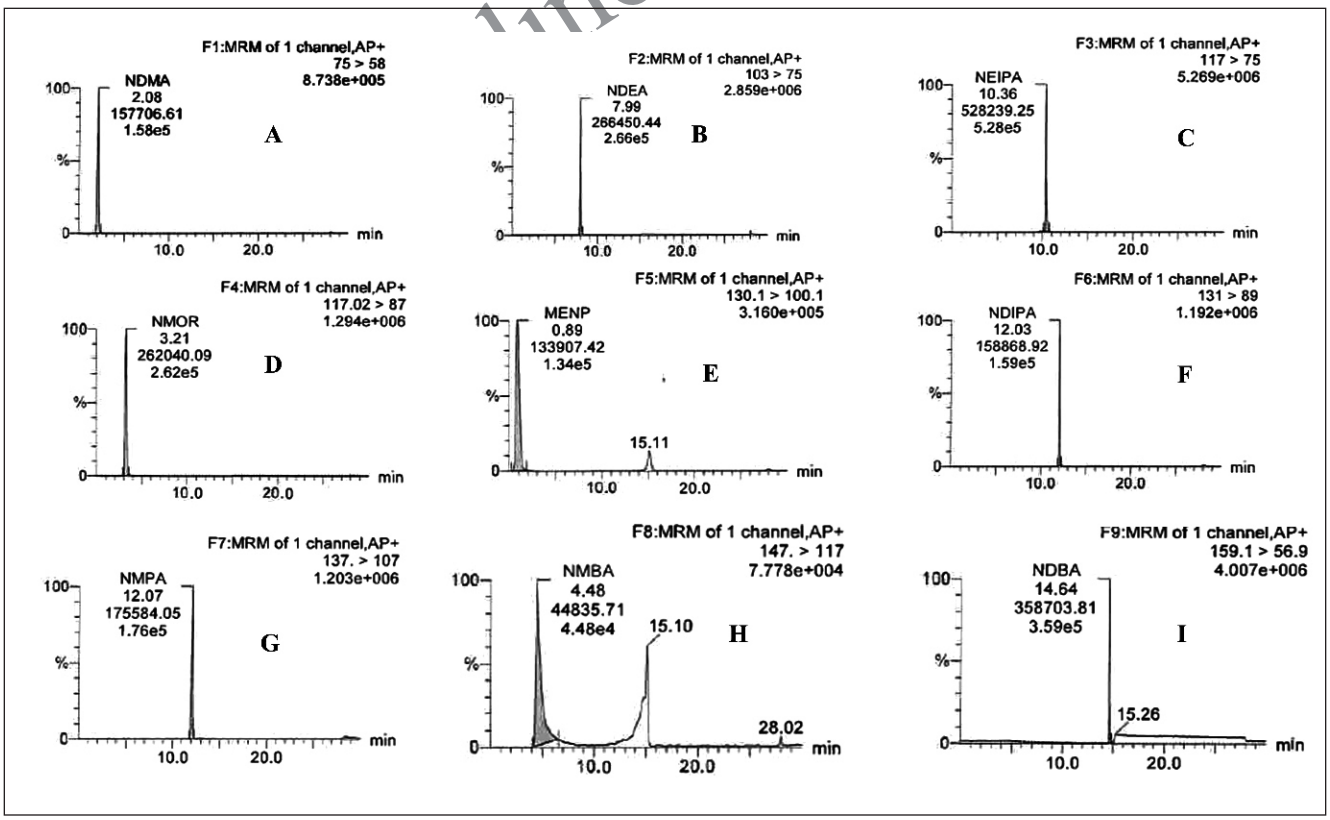


Figure 5. Typical selected ion chromatograms of LOQ solutions A. NDMA, B. NDEA, C. NEIPA, D.NMOR, E. MENP, F. NDIPA, G. NMPA, H. NMBA, I. NDBA.

Table 1. Represents the MS parameter for each nitrosamine compound.

S. No	Compound name	MS Parameters
1	NDMA	Parent ion (Da): 75.00 Daughter ion (Da): 58.00
2	NDEA	Parent ion (Da): 103.00 Daughter ion (Da): 75.00
3	NEIPA	Parent ion (Da): 117.00 Daughter ion (Da): 75.00
4	NMOR	Parent ion (Da): 117.02 Daughter ion (Da): 87.00
5	MENP	Parent ion (Da): 130.10 Daughter ion (Da): 100.10
6	NDIPA	Parent ion (Da): 131.00 Daughter ion (Da): 89.00
7	NMPA	Parent ion (Da): 137.00 Daughter ion (Da): 107.00
8	NMBA	Parent ion (Da): 147.00 Daughter ion (Da): 117.00
9	NDBA	Parent ion (Da): 159.10 Daughter ion (Da): 56.90

Table 2. Represents the retention time of Nitrosamine impurities in Standard and Spiked Samples.

Name of the impurity	Standard RT	Spike sample RT
NDMA	2.09	2.08
NDEA	8.05	8.00
NEIPA	10.43	10.37
NMOR	3.24	3.21
MENP	0.88	0.88
NDIPA	12.09	12.04
NMPA	12.13	12.08
NDBA	14.69	14.65
NMBA	4.58	4.50

solution. Table 2 details the retention times of the nitrosamine impurities identified in both the standard and spiked samples. Table 3 summarizes the results obtained.

3.1.3. Linearity

The linearity of the optimized analytical method was assessed through the injection of standard solutions of nine nitrosamines at levels of LOQ, 50%, 100%, 150%, and 200%. The ion counts were subsequently plotted against the concentrations of nitrosamines to generate a calibration curve. The validation of the method's linear characteristics was confirmed through the resulting calibration curve, which exhibited a correlation coefficient exceeding 0.990. The acceptance criteria for the results are specified in Table 3, and the obtained results conform to the established criteria.

3.2. Method precision

The precision of the employed methodology was assessed through the examination of six spiked sample solutions, each containing nine nitrosamines at the concentration delineated in the Zaltoprofen sample solution (100% standard concentration). It was determined that the %RSD values remained below 5.4 for the nine nitrosamine impurities. Additionally, Table 3 illustrates that the outcomes conformed to the established acceptance criteria for results.

3.3. Intermediate precision

Intermediated precisions are assessed through the preparation of six distinct sample solutions infused with varying nitrosamine impurities at the specification level corresponding to the Zaltoprofen sample solution, followed by analysis in accordance with the aforementioned testing procedure (conducted on a different day by a different analyst). The computed %RSD for the content in ppm from nine nitrosamine impurities across six spiked sample preparations does not exceed 25.0, as detailed in Table 3. The %RSD calculated for the content in ppm of nine nitrosamine impurities (derived from method precision and intermediate precision) is not greater than 30.0 for the cumulative total of 12 preparations, with the results presented in Table 3.

3.3.1. Accuracy

As a result, the accuracy of the methodology was meticulously assessed through the standard addition method for nine nitrosamine contaminants, utilizing the refined analytical technique. This evaluation was conducted in triplicate at the limits of quantification (LOQ), as well as at 100% and 200% of the specification level. The data pertaining to recovery percentages were subsequently organized and systematically presented in Table 3. The percentage recovery for the content of nine nitrosamine impurities across each preparation at all specified levels falls within the range of 70% to 130%. The accuracy findings satisfied the established acceptance criteria.

3.3.2. LOD and LOQ

LOD represents the minimal concentration of nine nitrosamine impurities at which they can be identified; LOQ denotes the quantitative concentration of nine nitrosamines at which they can be measured. Standard solutions at reduced concentrations were evaluated for signal-to-noise ratio (S/N). The S/N for LOD exceeds 3.0, while LOQ surpasses 10.0. The areas of the nine nitrosamine impurity peaks obtained from six replicate injections of the LOQ solution do not surpass the %RSD threshold of 25.0. The LOQ precision results were deemed satisfactory according to the specified criteria in Table 3.

3.4. Green analytical assessment using green metrics

The proposed methodology employed methanol and formic acid as solvents within the mobile phase, while utilizing methanol and water as diluents. A column with dimensions of 100 mm x 3.0 mm and a particle size of 1.8 µm was utilized, enabling the analysis of nine nitrosamine impurities present

Table 3. Represents the method validation results of nitrosamine impurities for the proposed analytical method.

Validation Parameter	NDMA	NDEA	NEIPA	NMOR	MENP	NDIPA	NMPA	NMBA	NDBA
System Suitability									
%RSD	7.2	3.3	2.1	1.9	1.4	1.7	3.1	2.4	4.1
Specificity									
Interference observed	No	No	No	No	No	No	No	No	No
Method Precision									
ppm (n=6)	0.41411	0.10814	0.11289	0.11261	0.10552	0.10905	0.11065	0.41695	0.11237
%RSD	5.4	2.7	2.0	2.1	1.9	2.1	2.4	3.7	3.9
I.P.									
ppm (n=6)	0.42047	0.11739	0.11082	0.11345	0.11170	0.11011	0.10267	0.43710	0.11341
%RSD	5.9	2.9	2.2	2.2	1.6	2.2	1.1	3.8	2.3
LOD									
Conc. (ppm)	0.0049	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0050	0.0014
S/N ratio	765.572	3.5.705	243.365	446.103	2955.893	716.863	143.839	189.145	402.540
LOQ									
Conc. (ppm)	0.0147	0.0042	0.0041	0.0041	0.0041	0.0041	0.0038	0.015	0.0041
S/N ratio	2232.208	819.630	367.458	1826.878	3070.946	2435.993	213.642	94.270	517.384
LOQ Precision									
%RSD	6.7	1.9	2.8	2.0	2.3	1.9	2.7	7.2	2.9
Linearity									
r^2	0.9959	0.9995	0.9998	0.9998	0.9995	0.9999	0.9996	0.9998	0.9991
Accuracy (%)									
LOQ (*n* = 3)	99.6	100.9	118.4	101.0	100.5	99.3	94.6	97.2	104.4
100% (*n* = 3)	98.6	101.8	116.0	100.4	100.8.	98.8	94.3	106.9	100.9
200% (*n* = 3)	99.9	99.2	111.6	99.4	97.9	97.2	92.7	109.6	102.2

in Zaltoprofen drug substances to be completed within a total runtime of 30 minutes, requiring less than 6 ml of methanol per sample for evaluation.

A thorough assessment of environmental impact was conducted utilizing green metric tools, including the AGREE and the Analytical Eco-Scale. The Analytical Eco-Scale assessment tool, as illustrated in Table 4, evaluates the chemicals or reagents utilized, the energy consumption of the instrumentation, the waste generated, and the management practices associated with the proposed methodology. The environmental sustainability of the technique is evaluated through the application of penalty points, wherein an optimal green analysis achieves an eco-scale value of 100, superior green analysis scores exceed 75 on the eco-scale, a reasonable green analysis is classified as above 50, and any method receiving a score below 50 is deemed an unsatisfactory green method. The calculation of penalties for each chemical utilized is established through the equation (amount of penalty points x hazard penalty points). The determination of hazard penalty points involves multiplying the total number of pictograms present in the material safety data sheet of the chemical by the score assigned to the signal word (safe = 1, danger = 2). The amount of penalty points is assigned according to the guideline that (less than 10 ml = 1, 10–100 ml = 2, more than 100 ml = 3). For methanol, the penalty points are calculated as 3 pictograms x 2 (danger) x 2 (amount 10–100 ml) = 12 penalty points. The results indicate that the proposed method yielded a total penalty of 26 points, resulting in an acceptable greenness score of 72 points.

The AGREE tool illustrated in Figure 6 is employed to indicate the environmental sustainability of the method, achieving a score of 0.60, which surpasses the established threshold of 0.50. The assessment of the GAP integrated within the proposed methodology is comprehensively outlined in Table 5. The highest energy techniques (LCMS/MS) are highlighted in red, while the operator safety section 12 and the line measurement section 3 are marked in orange. Additionally, the off-line analysis and sample preparation section 1, along with the toxic reagents utilized in section 10, are indicated in yellow, denoting their non-green status in Figure 6. In terms of sample preparation, the inclusion of the toxic solvent “methanol” contributes to the method type being categorized with a red color; consequently, all sections in Figure 6 predominantly appear yellow, reflecting a slight reduction in greenness.

Table 4. Represents the analytical greenness using the Analytical Eco-Scale.

Proposed analytical method for assessing the Analytical Eco-Scale		
S.No	**Name**	**Penalty points**
	Chemicals or reagents	
1	Formic acid	6
2	Methanol	12
	Instruments	
1	Energy required for each sample in LC-MS/MS.	2
2	Occupational Hazard	0
	Waste	
1	Total Amount of waste produced (>10 ml)	5
2	Waste treatment	3
	Penalty points (total)	28
	Total score (100-penalty points)	72
	Greenness evaluation	Acceptable

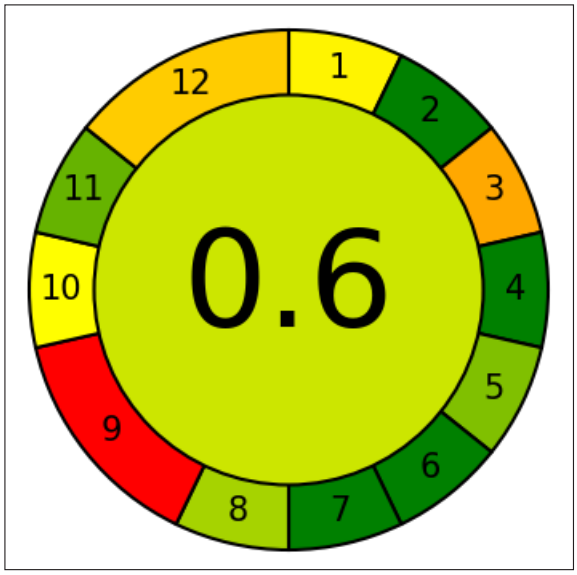


Figure 6. Represents AGREE pictograms for the proposed method.

Table 5. Represents the green analytical assessment using the AGREE tool.

S.No	GAC principles	Sample procedure
1	Direct analytical methods for sample preparation.	Off-Line Analysis
2	Minimal sample size.	1 g
3	On-site measurements	At-line
4	Combining analytical procedures and operations to conserve energy and lowers reagent consumption.	4 distinct steps.
5	Selection of automated and compact techniques.	Semi-Automatic and miniaturized
6	Derivatization processed.	No Derivatization
7	Reduce excessive generation of analytical waste	1 g
8	Analyze number of analytes in single run and samples per hour.	9 nitrosamine impurities quantified in single run; 2 samples analyzed per hour
	The use of energy should be minimized	
9	Technique that requires the highest energy.	UHPLC-MS/MS
	Calculate the total energy consumption of a single analysis in kWh.	1.5
10	Reagents derived from renewable sources.	Some reagents are toxic
11	Toxic substances ought to be removed.	No toxic reagents or solvents used
12	Enhance the safety of the operator.	The threats that are not avoided are a. Toxic to aquatic life b. Bio accumulative c. Highly flammable d. Explosive

4. CONCLUSION

The validation of the optimized experimental results demonstrates that the UHPLC-MS/MS technique is proficient in precisely quantifying nine nitrosamine contaminants (NDMA, NDEA, NEIPA, NDIPA, NDBA, NMBA, NMPA, MENP, and NMOR) in Zaltoprofen through the utilization of suitable stationary phases, chromatographic conditions, and mass spectrometric parameters. The validation of the proposed analytical method was conducted in accordance with the criteria outlined in ICH Q2 (R1) guidelines. The outcomes of this methodology exhibit specificity, linearity, accuracy, and precision, yielding satisfactory LOD and LOQ values. Furthermore, this method was assessed for its environmental impact utilizing the same GAP evaluation tools: Analytical Eco-Scale and AGREE. The evaluation indicates that the proposed method is environmentally friendly and adheres to green principles (with an Analytical Eco-Scale score of 72). The precise determination of nine impurities in bulk Zaltoprofen is

crucial for quality control (QC) and research and development laboratories to ensure accurate identification and quantification.

5. LIST OF ABBREVIATIONS

AGREE: Analytical GREEnness; Conc: Concentration; GAC: Green analytical chemistry; GAP: green analytical principles; LC-MS/MS: Liquid chromatography tandem mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; MENP: 1-Methyl-4-Nitrosopiperazine; NDBA: N-Nitroso dibutylamine; NDEA: N-Nitroso diethylamine; NDIPA: N-Nitroso diisopropylamine; NDMA: N-Nitroso dimethylamine; NEIPA: N-Nitroso ethylisopropylamine; NFPA: National Fire Protection Association; NMBA: N-Nitroso N-methyl-4-aminobutyric acid; NMOR: N-Nitrosomorpholine; NMPA: N-Nitrosomethylphenylamine; ppm: parts per million; RSD: relative standard deviation; RT: retention time; S/N: signal-to-noise ratio.

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All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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We affirm that the article has been studied, accepted, and approved by all listed authors.

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The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Hirate K, Uchida A, Ogawa Y, Arai T, Yoda K. Zaltoprofen, a non-steroidal anti-inflammatory drug, inhibits bradykinin-induced pain responses without blocking bradykinin receptors. Neurosci Res. 2006;54:288–94. doi: https://doi.org/10.1016/j.neures.2005.12.016.
2. Higuchi T, Takeuchi A, Munesue S, Yamamoto N, Hayashi K, Harashima A, *et al*. A nonsteroidal anti-inflammatory drug, zaltoprofen, inhibits the growth of extraskeletal chondrosarcoma cells by inducing PPARγ, p21, p27, and p53, Cell Cycle. 2023; 22:939–50. doi: https://doi.org/10.1080/15384101.2023.2166195.
3. Muruganantham S, Krishnaswami V, Kandasamy R, Alagarsamy S. Potentiating the solubility of BCS class II drug zaltoprofen using nanodispersion technology. J Dispers Sci Technol. 2024;45:632–41. doi: https://doi.org/10.1080/01932691.2023.2173224.
4. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Control of Nitrosamine Impurities in Human Drugs Guidance for Industry. 2024. Available from: https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs
5. M7(R2) Addendum: Application of the principles of the ICH M7 guideline to calculation of compound-specific Acceptable Intakes Guidance for Industry. 2023. Available from: https://www.regulations.gov/docket/FDA-2022-D-0055
6. Nitrosamine impurities in drugs: what health care professionals need to know.
7. Recommended acceptable intake limits for nitrosamine drug substance-related impurities (NDSRIs). Guidance for Industry, 2023.
8. Bhukya VN, Beda DP. Implementation of green analytical principles to develop and validate the HPLC method for the separation and identification of degradation products of Panobinostat, and its characterization by using LC-QTOF-MS/MS and its in-silico toxicity prediction using ADMET software. Green Anal Chem. 2024;8:100090. doi: https://doi.org/10.1016/j.greeac.2023.100090.
9. Divya Bhargavi P, Lolla S, Sugunan S, Shiva Gubbiyappa K, Ali Khan A, Alanazi AM, *et al*. The simultaneous quantification of Sitagliptin and Irbesartan in rat plasma using the validated LC-MS/MS method is applied to a pharmacokinetic study. J Chromatogr B. 2023;1221:123677. doi: https://doi.org/10.1016/j.jchromb.2023.123677.
10. Bhukya VN, Beda DP, Gubbiyappa KS, Pathuri R. Combined green analytical principles and quality by design for ultraperformance liquid chromatography analytical method development, the characterization and in-silico toxicity prediction of Ixazomib degradation products using mass spectrometry. Green Anal Chem. 2024;8:100105. doi: https://doi.org/10.1016/j.greeac.2024.100105.
11. Boovizhikannan T, Nagaraja S, Petapalli B, Shankar PM, Sampath K. Reported estimation techniques for quantification of zaltoprofen: a review. World J Bio Pharm Health Sci. 2022;12:30–9. doi: https://doi.org/10.30574/wjbphs.2022.12.2.0188.
12. Deshpande MM, Kasture VS, Mohan M, Chavan MJ. Development and validation of RP-HPLC method and forced degradation of powerful bradykinin inhibitor zaltoprofen. CPA. 2018;14:604–10. doi: https://doi.org/10.2174/1573412913666171005161737.
13. Bhosale VM, Sonvane SM, Birajdar MJ, Satpute KL, Shetkar BM, Gadgul AB, *et al*. RP-HPLC method development and validation for of paracetamol & zaltoprofen in pharmaceutical dosage form. IJBPAS. 2024;13:9634. doi: https://doi.org/10.31032/IJBPAS/2024/13.11.9634.

14. Jang J-H, Jeong SH, Cho H-Y, Lee Y-B. Comparison of UPLC-MS/MS and HPLC-UV methods for the determination of zaltoprofen in human plasma. J Pharm Investig. 2019;49:613–24. doi: https://doi.org/10.1007/s40005-018-00416-w.
15. Yang HK, Kim SY, Kim JS, Sah H, Lee HJ. Application of column-switching HPLC method in evaluating pharmacokinetic parameters of zaltoprofen and its salt. Biomed Chromatogr. 2009;23:537–42. doi: https://doi.org/10.1002/bmc.1151.
16. Chu VM, Kim KT, Kim SH, Lee W, Lee KC, Nguyen VL, *et al*. Chiral pharmacokinetics of zaltoprofen in rats by HPLC with solid-phase extraction. J Pharm Biomed Anal. 2012;70:567–73. doi: https://doi.org/10.1016/j.jpba.2012.05.019.
17. Patil JD, Dhankani AR, Pawar SP. Spectrophotometric and chromatographic review on NSAIDs include: zaltoprofen, loxoprofen, ketoprofen and flurbiprofen. AJPA. 2023;11:261–6. doi: https://doi.org/10.52711/2231-5675.2023.00043.
18. ICH. Harmonised Tripartite guideline validation of analytical procedures: text and methodology Q2(R1), 2005.

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