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Trace-level quantification of NDMA in levosulpuride active pharmaceutical ingredient and tablet formulation Using UFLC-MS/MS

Hemanth Vikram P.R [a,b], Gunjan Kumar [b], Rajashree Deka [c], Umme Hani [d], Nazima Haider [e], Sirajunisa Talath [f], Adil Farooq Wali [g], Dilipkumar Reddy Kandula [h], Narasimha M. Beeraka [i,j,k,l], Sinchana B Gopalaiah [m], Devi Sri Chiriki [a], Namitha Bannimath [n], Pramod Kumar [o], Bannimath Gurupadayya [a,*]

[a] Department of Pharmaceutical Chemistry, JSS College of Pharmacy Mysuru, JSS Academy of Higher Education and Research (JSSAHER), Mysuru, 570015, India
[b] Xenone Healthcare Pvt. Ltd., New Delhi, 110076, India
[c] Department of Zoology, Bhattadev University, Bajali, Pathsala, 781325, Assam, India
[d] Department of Pharmaceutics, College of Pharmacy, King Khalid University, Abha 62529, Saudi Arabia
[e] Department of Pathology, College of Medicine, King Khalid University, Abha 62529, Saudi Arabia
[f] Department of Pharmaceutical Chemistry, RAK College of Pharmacy, RAK Medical and Health Sciences University, Ras Al Khaimah 11172, United Arab Emirates
[g] Department of Pharmaceutical Chemistry, RAK College of Pharmacy, RAK Medical and Health Sciences University, Ras Al Khaimah, United Arab Emirates
[h] Department of Pharmacy, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, 333010, Rajasthan, India
[i] Department of Human Anatomy and Histology, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, 119991, Russia
[j] Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Ananthapuramu, 515721, India
[k] Herman B. Wells Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, 46202, USA
[l] Department of Studies in Molecular Biology, University of Mysore, Mysore, Karnataka 570006, India
[m] Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, India
[n] Department of Pharmacology, University of Galway, University Road, Galway, H91 TK33, Ireland
[o] Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER)-Guwahati, Changsari, 781101, India

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ABSTRACT

Nitrosamine impurities identified in several pharmaceuticals during recent times has raised concerns leading to product recalls worldwide and necessitating sensitive liquid and gas chromatographic methods for trace level detection of nitrosamine impurities. This study developed and validated a ultra-fast liquid chromatography-tandem mass spectrometry (UFLC-MS/MS) method for the quantification of NDMA in Levosulpuride drug substance and tablet formulation. Current method utilizes a triple quadrupole analyzer, atmospheric pressure chemical ionization (APCI) ionization source and multiple reaction monitoring (MRM) scan mode for the analysis. Chromatographic separation was achieved on a Gemini NX-C18 column (150 × 4.6 mm, 3 μm) maintained at 40 °C. The mobile phase consisted of a binary gradient of solvent A (0.1 % formic acid in water) and solvent B (methanol), with a total run time of 18 minutes. Current method achieved excellent linearity, recovery, precision, and sensitivity. Greenness of the developed method was evaluated using the GAPI, AGREE, and AES metrics. Current method is sensitive and selective for NDMA in levosulpuride drug substance and tablet formulations and can be employed for routine quality control analysis in pharmaceutical industry.

1. Introduction

Levosulpiride (LVS) is a levo-enantiomer of sulpiride and a benzamide derivative used in treatment of various diseases and disorders. LVS is used as a prokinetic agent, in treating gastrointestinal disorders like irritable bowel syndrome, gastroesophageal reflux disease, and diabetic gastroparesis [1]. LVS exhibits antipsychotic properties by acting as an antagonist on central and peripheral dopamine D2 receptors [2]. LVS is also used in treating schizophrenia and anxiety disorders [3], ulcers [3], vertigo [3] and premature ejaculation [4]. As per Biopharmaceutics Classification System (BCS) LVS is categorised as a Class IV drug, presenting challenges in terms of solubility and permeability [5]. Chemical

* Corresponding author.
E-mail address: bmgurupadayya@jssuni.edu.in (B. Gurupadayya).

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structure of LVS is presented in Fig. 1**a.**

N—Nitrosamines are considered as possible carcinogens to human [6] and are referred to as "cohort of concern" compounds [7]. Contamination of pharmaceuticals with nitrosamine impurities above the acceptance limits has become a significant concern in recent times and lead to withdrawal of multiple pharmaceutical products from the markets [8,9]. Toxicity of nitrosamine impurities is attributed to their ability to undergo cytochrome P450 (CYP) mediated metabolic activation and subsequently forming DNA-reactive species [10].

N-nitrosodimethylamine (NDMA) is a nitrosamine with the formula $C_2H_6N_2O$, having the potential to cause cancer and has attained the attention of the scientific community due to its identification as an impurity above the permissible levels in some medications. NDMA is recognized as a probable carcinogen by the International Agency for Research on Cancer (IARC) [11]. USFDA set acceptable limits for NDMA as 96 ng/day [12]. N—Nitrosodimethylamine (NDMA) is detected in valsartan [13], ranitidine [14], nizatidine [15], and metformin (Glucophage) extended release products [16]. Chemical structure of LVS is presented in Fig. 1**b.**

The recent surge in nitrosamine incidents and market withdrawals of pharmaceuticals underscores an urgent need for sensitive analytical methods capable of detecting and quantifying these impurities at trace levels. USP has established a virtual platform "Nitrosamines Exchange" to facilitate knowledge exchange on various aspects and updates on ongoing nitrosamine crisis. Nitrosamine exchange features a segment 'Analytical Methods Hub' that discusses and updates the novel analytical methods for analysis of nitrosamine impurities provide updates on novel analytical methods for testing nitrosamines. Despite significant efforts, by regulatory bodies globally, a substantial number of drug products remain unexamined for nitrosamine contamination due to lack of specific analytical methods. To address this critical gap, there is a need for developing sensitive gas and liquid chromatography coupled with mass spectrometry.

Greenness assessment of analytical methods is gaining popularity in recent times. Greenness assessment involves evaluating several parameters like solvent consumption, energy usage, waste generation, and the toxicity of reagents. It emphasizes the reduction of environmental impact. Metrics like the National Environmental Methods Index (NEMI), Analytical Greenness (AGREE), Analytical Eco-scale, Green Analytical Procedure Index (GAPI), and are commonly used to assess greenness profile of analytical methods [17–19]. By abiding green chemistry principles analysts aim to minimize the generation of hazardous waste, conserve resources, and improve laboratory safety. Ultimately, greenness assessment fosters developing sustainable and environmentally responsible analytical methods.

To address the critical gap of sensitive analytical methods for NDMA detection in pharmaceuticals, our study presents a novel and sensitive and validated UFLC-MS/MS method that could quantify NDMA levels in levosulpiride API and tablet formulations. The method is suitable for routine quality control analysis to employ in pharmaceutical industries. Additionally, greenness assessment of the developed method is performed by greenness metrics such as AGREE, GAPI and analytical eco-scale (AeS).

2. Materials and methods

2.1. Chemicals and reagents

Levosulpuride API, having a purity of (99.82 %) was procured from Sigma-Aldrich, Mumbai, India. NDMA nitrosamine impurities standard was obtained from Vivan Life Sciences Pvt. Limited, Thane, India. Drug product of Levosulpuride had been procured from the Indian market. LC-MS/MS grade methanol (99.9 %) was procured from J.T Baker (100 Matsonford Road, Radnor, PA 19,087, USA). Rankem analytical grade formic acid is used for buffer preparation. Millipore HPLC grade water used obtained from the Millipore Direct-Q® water purification system.

2.2. Preparation of solutions

2.2.1. Preparation of Mobile phase a

Add 1 mL of formic acid in 1000 mL of water (0.1 %), degased and sonicated for 5 min.

2.2.2. Preparation of Mobile phase b

Methanol

2.2.3. Preparation of standard solutions

Add 10 mg of LVS standard in 100 mL of methanol to prepare a stock solution of 100μg/mL. From the prepared stock solutions required concentrations of solutions are further prepared. Prepared solution is sonicated for 10 min, filtered with syringe filter and then transferred into the vials.

2.2.4. Preparation of sample solutions

Added 250 mg equivalent of test sample in 15 mL centrifuge tube, 5 mL of methanol is further added to it, resultant solution is vortexed, centrifuged for 10 min with 3000 rpm, and filtrered through a 0.22 μm PTFE syringe filter and then transferred into the vials for analysis.

2.3. Instrumentation

For weighing the standard analyte and sample drug SARTORIUS (QUINTIX125D- IN), Mumbai 400,076, India, weighing balance is used. Dilutions were prepared using micropipette (0.5–10 μL) LAB-QUEST (RF647966) by BOROSIL, Pune, India.

Chromatographic separation and quantification was done using ultra-fast liquid chromatography coupled with mass spectrometry system, SHIMADZU LC-20AD Pump with a maximum pressure of 35 MPa

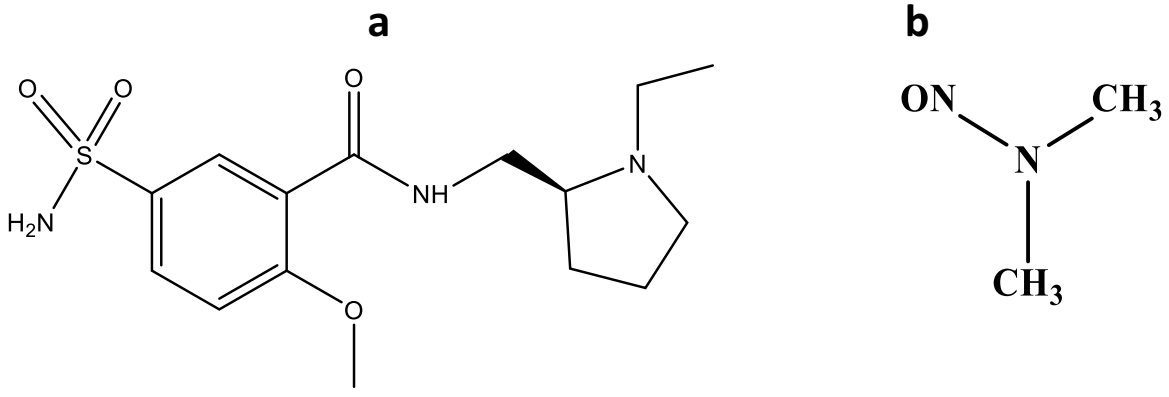

Fig. 1. Chemical Structures of Levosulpiride (LVS) and NDMA.

equipped with SIL-20AC HT Auto-sampler, with a 100 µl loop volume and CTO-10A VP column oven. Mass Identification was done using the SCIEX API 4000 system.

LAB-QUEST micropipette by BOROSIL (0.5–10 µL) is used for dilutions. PCI India sonicated was used to degas and sonicate the samples. Eutech pH meter was used.

2.4. Mass spectrometry and chromatographic conditions

Mass parameters were tuned for the analysis of NDMA in the levosulpuride. SCIEX API 4000 triple quadrupole instrument coupled with Atmospheric pressure chemical ionization (APCI) ionisation source and multiple reaction monitoring (MRM) scan mode were employed. The quantifier ion pair selected for NDMA were 75 *m/z* (precursor ion) and 58 *m/z* (product ion). Collision gas and curtain gas were set as 30 psig, ion source gas1 was 40 psig, ion spray voltage was 5000 V, temperature was set to 350.0 °C, interface heater was kept on during the run, dwell time of 200 msec was employed, Delustering potential was 54.0 Vs, entrance potential was 9.0 Vs, collision energy was 16.0 eV, and collision cell exit potential of 9.0 V were set and tuned for optimum response. Chromatographic separation was achieved by using Gemini NX-C18 column (150 × 4.6 mm, 3 µm) column in combination with a binary gradient time program of 0.1 % formic acid in water and methanol. The flow was set at 0.6 mL/min and at 40 °C column temperature, while auto sampler temperature was set as 15 °C and an injection volume of 20 µL. Run time was 18 min with equilibration time of 60 min for the analysis.

3. Results

3.1. LC-MS/MS method optimization

During method optimization, appropriate quantifier ion pair is chosen. The mass parameters for NDMA were tuned to obtain good sensitivity. The curtain gas flow rate was optimized to reduce the contamination of the ion source while it stabilized ion transmission. Ion source gas1, controlled temperature and flow rate were optimized for efficient desolvation and proper ionization of analyte. Declustering potential and collision energy were optimized achieve maximum response for the analyte with least interference from other fragment ions. Finally, the LC gradient was optimized to elute the NDMA with good sensitivity, accurate detection and quantitation and without any interference from matrix. MRM chromatograms for NDMA standard is displayed in Fig. 2, while a sample chromatogram of the levosulpuride tablet (control) is shown in Fig. 3

3.2. Method validation

3.2.1. System suitability or system precision

Standard Solutions at LOQ level were injected as per the developed method into the UFLC-MS/MS system. System suitability/System precision parameters like % Relative Standard Deviation (%RSD) for peak areas and retention times for six replicates of LOQ level (0.39 ng/mL)

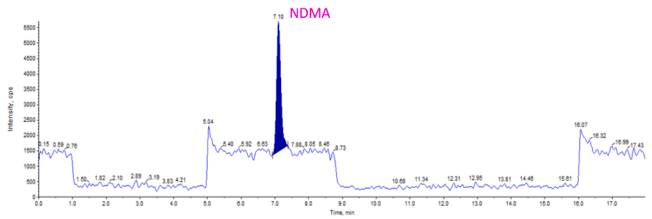

Fig. 2. Multiple Reaction Monitoring (MRM) chromatogram of NDMA. The figure provides visual representation of elution, separation and detection of NDMA impurity, facilitating the assessment of their presence and quantitative analysis in levosulpuride samples.

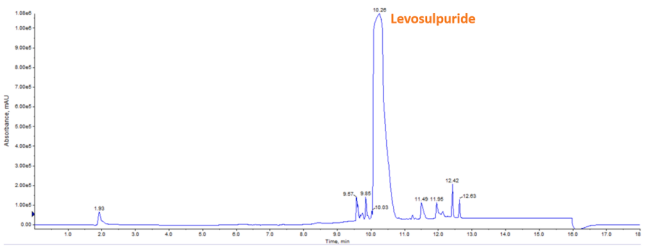

Fig. 3. Sample chromatogram demonstrating elution of levosulpuride tablet formulation (Control). This chromatogram illustrates the elution profile of a levosulpuride tablet control sample, depicting the retention time and peak characteristics associated with levosulpuride. By comparing the chromatographic profile of the control sample to that of standards or test samples, analysts can verify the identity and specificity of NDMA and levosulpuride within pharmaceutical formulations.

were evaluated and found well within the acceptable limits (%RSD should not be greater than 20.0 %). System suitability results are summarized in **Table S1**.

3.2.2. Specificity

Specificity of the developed method is evaluated by injecting Blank, Standard solution, control sample solution and spiked sample solutions into UFLC-MS/MS to Specificity results depicts no interference from matrix components at the *m/z* values of NDMA. Hence it is concluded that the method is specific.

3.2.3. Linearity

A series of solutions of NDMA were prepared and a 10 point calibration curve is plotted in the concentration ranging from 0.195 ng/mL to 133 ng/mL and injected into the UFLC-MS/MS system to assess the linearity for the developed method. The results are summarized in the **Table S2** in **supplementary file s1**. Correlation coefficients for NDMA were well within acceptable limits (NLT 0.999) indicating linear relation in responses.

3.2.4. Limit of detection (LOD) and Limit of quantification (LOQ)

LOD & LOQ is established based on visualization method. Injected LOQ solution in six replicates into LC-MS/MS system for LOQ precision. LOD and LOQ chromatograms are presented in Fig. 4.

3.2.5. Accuracy

The accuracy of the developed method was evaluated through recovery experiments. Known amounts of NDMA nitrosamine impurity

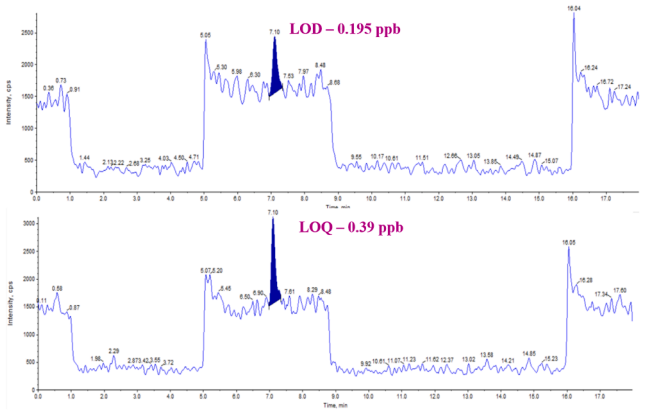

Fig. 4. Representative chromatograms of Limit of Detection (LOD), Limit of Quantification (LOQ) for NDMA impurity. These chromatograms serve as visual indicators of the method's sensitivity and ability to detect and quantify trace levels of NDMA in levosulpuride formulations.

was spiked into samples, and the resulting concentrations and % recovery was calculated and compared with that of previously added values. Accuracy of the method evaluate the method's ability to correctly quantify nitrosamines within the complex sample matrix. Analysed three test preparations of six replicates for the levels of 0.195 ppb, 0.39 ppb and 1.17 ppb. The individual recoveries of all the samples at each preparation and average recovery percentages were within the acceptable range of 70.0 % to 130.0 % proving the reliability and accuracy of the newly developed method. The results are summarized in the Table 1.

3.2.6. Method and intermediate precision

The method and intermediate precision was evaluated by analysing six replicates of spiked samples and the method precision results obtained on different days were compared. The individual and cumulative % RSD values for peak areas, % recovery values for twelve determinations (six from method precision and six from intermediate precision) were found to be well within the acceptance criteria. Intermediate precision results are summarized in the below Table 2.

3.3. Assessment of greenness of the developed method

The greenness profile of the developed method is comprehensively evaluated using multiple tools, including the Green Analytical Procedure Index (GAPI), the Analytical GREEnness metric (AGREE), and the Analytical Eco-Scale (AES). GAPI provides a visual assessment of the method's environmental impact through a series of coloured fields representing different aspects of the analytical procedure. AGREE offers a more detailed numerical evaluation based on 12 principles of green chemistry, quantifying the greenness of the method. AES, on the other hand, assigns penalty points for factors that negatively impact the environment, such as the use of hazardous reagents or high energy consumption, thus providing a semi-quantitative measure of the method's environmental footprint.

3.3.1. Green analytical procedure index (GAPI) assessment

In GAPI method, the greenness profile of the analytical method is assessed across eleven categories. GAPI results for the developed method are depicted in the form of pentagrams in Fig. 5**a**. Green and yellow sectors indicate favourable environment approach, whereas red sectors indicate deviation from the greenness. Usage of minimal solvent per analysis, sample preparation step, and lack of sample preservation, storage, and transportation steps contributed in favour of greenness of the method. Although methanol is classified as a hazardous substance, it is relatively less harmful compared to solvents such as acetonitrile. Its overall impact on the greenness of the method is deemed manageable. Consequently, the GAPI assessment revealed that the developed UFLC-MS/MS method exhibits a favorable greenness profile.

Table 1
Accuracy results at three test preparations.

Accuracy	Peak area	Spiked NDMA Concentration in solution (ng/mL)	Recovered NDMA Concentration in solution (ng/mL)	% Recovery
Std 0.195 ng/mL	8155	0.2	0.2	104.09
	5278		0.12	59.9
	8462		0.21	108.82
	6094		0.14	72.44
	7846		0.19	99.34
	6861		0.16	84.22
Std 0.39 ng/mL	17,606	0.39	0.49	124.64
	13,847		0.37	95.77
	16,864		0.46	118.94
	16,879		0.46	119.06
	17,156		0.47	121.18
	15,370		0.42	107.46
Std 1.17 ng/mL	36,345	1.17	1.05	89.52
	45,523		1.32	113.02
	35,264		1.02	86.76
	44,588		1.29	110.63
	43,691		1.27	108.33
	39,841		1.15	98.47
Control Sample_API 39mg/mL	0	0	No Peak	N/A
	0		No Peak	N/A
	0		No Peak	N/A

Table 2
Intermediate Precision Results for NDMA impurity in levosulpuride samples. This table presents the intermediate precision results for the quantification of eight nitrosamine impurities in Valsartan drug substances and tablet formulations.

Name of the Solution	% recovery
Method precision Sample −1	93.21
Method precision Sample −2	102.99
Method precision Sample −3	115.55
Method precision Sample −4	106.04
Method precision Sample-5	73.88
Method precision Sample-6	91.25
Intermediate Precision sample-1	95.24
Intermediate Precision sample-2	108.23
Intermediate Precision sample-3	118.25
Intermediate Precision sample-4	108.32
Intermediate Precision sample-5	82.58
Intermediate Precision sample-6	99.24
Average	**99.57**
%RSD	**13.15**

3.3.2. AGREE assessment

In the AGREE method, the greenness profile of an analytical method is assessed by considering the 12 principles of Green Analytical Chemistry (GAC). Each principle is evaluated, and scores are assigned to reflect the method's adherence to these principles. The overall AGREE score ranges from 0 to 1, with a score closer to 1 indicating a greener method. AGREE metrics are visually represented in Fig. 5**b**.

An AGREE score of 0.6 suggests that the method is moderately green. This indicates a good balance between analytical performance and environmental impact. The method performed satisfactorily in areas such as recycling and waste management, achieving a score of 0.6. This implies that the method employs efficient waste reduction strategies and recycles materials wherever possible. However, there is still room for improvement, particularly in the use of alternative, greener solvents. This could further enhance the method's environmental sustainability by reducing the reliance on potentially hazardous solvents and minimizing the overall ecological footprint.

3.3.3. Analytical Eco-Scale (AES) assessment

In the AES method, Waste management, occupational hazards, solvent energy, and instrumental energy were all taken into account. Current analytical method involved the usage of methanol as the mobile phase solvent, contributing to the overall energy consumption resulted in 6 penalty points. UFLC-MS/MS instrumental energy consumption, resulted in 2 penalty points (> 1.5 kWh per sample). The use of methanol and the hermitization process eliminates any possible occupational hazards. The run time employed in the method was 18 min that resulted in 3 penalty points (0–20 mL). Overall AES score obtained is 89 with a total of 11 penalty points (PP) indicating greenness of the method. Penalty points obtained are the result of the usage of methanol, and energy consumed by UFLC-MS/MS instrument which is >1.5 kWh per sample. The aforementioned factors had a significant influence on the overall greenness profile.

4. Discussion

The increasing nitrosamines crisis, marked by the emergence of diverse nitrosamine compounds and an increasing number of susceptible pharmaceutical products, describes the urgent need for the development

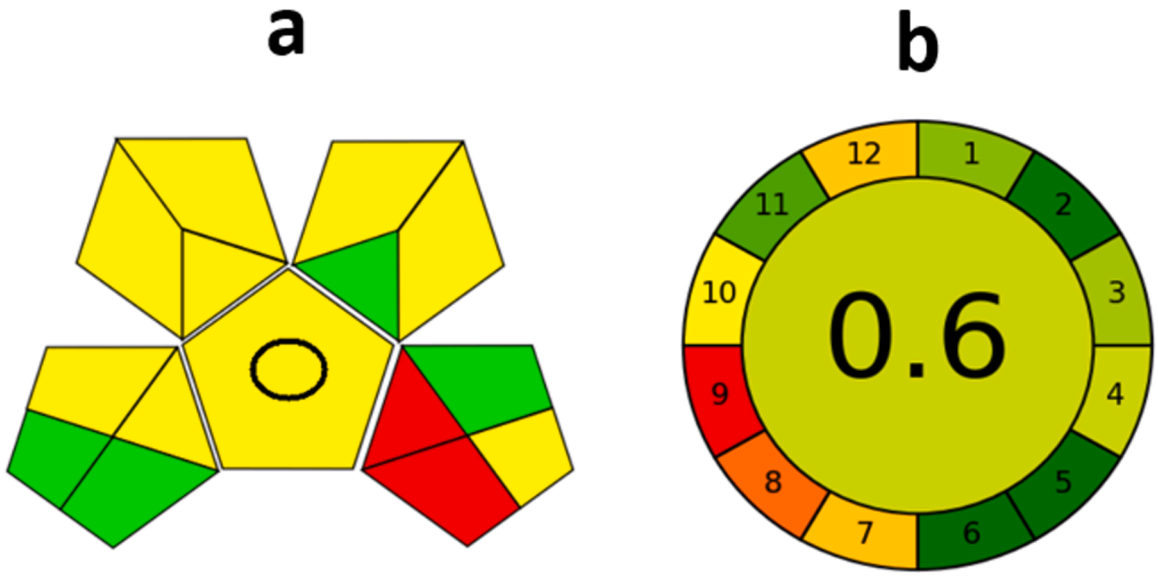

Fig. 5. **(a)** GAPI assessment and **(b)** AGREE metrics. Pictograms visually represent the greenness evaluation results for the developed analytical method. The GAPI pictogram illustrates the method's environmental impact through a color-coded system, indicating aspects such as solvent usage, energy consumption, and waste generation. The AGREE metric provides a detailed visual representation based on the 12 principles of Green Analytical Chemistry, quantifying the method's adherence to these principles and highlighting areas of strength and potential improvement.

of sensitive analytical methods. Traditional detection techniques for NDMA have primarily relied on gas chromatography (GC), methodologies originally designed for the analysis of large volumes of wastewater and drinking water, typically ranging from 20 mL to 1 L [20–23]. However, these volumes are impractical for bioanalytical applications in clinical studies, where sample sizes are often limited to approximately 1 to 2 mL. Additionally, conventional GC methods often require elevated temperatures, which can typically convert ranitidine into NDMA or even cause the degradation of NDMA, introducing potential artifacts into the analysis. Previous studies were performed to develop a low sample volume, low-temperature LC-MS/MS method capable of detecting and quantifying NDMA in human urine or plasma [24–28]. We have optimized a novel Ultra-Fast Liquid Chromatography-Tandem Mass Spectrometry (UFLC-MS/MS) method specifically designed to quantify genotoxic impurity NDMA in both the active pharmaceutical ingredient (API) and tablet formulations of levosulpuride. The method was validated according to the guidelines outlined in ICH Q2 (R1) and the United States Pharmacopeia (USP) analytical testing guidelines for nitrosamines [29].

One of the major challenges encountered during method development was overcoming matrix effects, which could compromise the accuracy and reliability of the analysis. The chromatographic results demonstrated no interference from excipients, highlighting the method's high sensitivity and specificity. A binary gradient time program mode was employed to achieve effective separation of the impurity. Linearity was established across a concentration range of 0.195 ng/mL to 133 ng/mL for all targeted impurities. Recovery rates ranging from 70 % to 130 % indicated the method's accuracy and its capacity to selectively quantify the eight impurities in drug formulations. The LOD and quantification LOQ for NDMA were determined to be 0.195 ppb and 0.39 ppb, respectively. Intermediate precision, assessed through relative standard deviation (%RSD) values for peak areas, recovery percentages, and cumulative %RSD for twelve determinations, confirmed the method's repeatability and reproducibility.

In alignment with the principles of green analytical chemistry, methanol was selected as a safer alternative to more hazardous solvents like acetonitrile. Greenness assessment tools, including the Green Analytical Procedure Index (GAPI), Analytical Eco-Scale (AES), and AGREE metrics, were employed to evaluate the environmental impact of the method. The GAPI assessment revealed favorable eco-friendly attributes, with predominantly green and yellow sectors in the pentagrams, while the red sectors indicated areas for potential improvement. The AGREE score of 0.6 reflected a moderate level of method greenness, and the AES score of 89, with 11 penalty points, fell within the desirable range for green methods. Collectively, these metrics confirmed the method's environmentally friendly performance. To further enhance the sustainability of the method, we recommend the substitution of methanol with even more eco-friendly solvents and the implementation of waste minimization strategies. The greenness of this method was also compared with other analytical methods reported in the literature, further demonstrating its environmentally responsible approach.

NDMA and NDEA, classified as Group 2A carcinogens by the IARC, were identified in several pharmaceutical products, including metformin (Glucophage), ranitidine, and various sartans [25]. These discoveries led to widespread recalls across the United States and Europe, highlighting the urgent need for more robust and sensitive detection methods. To address these challenges, future research should focus on the development and integration of sustainable solvent systems, which align with the principles of green chemistry, reducing the environmental impact of analytical processes. Moreover, enhancing automation within these methods could significantly decrease the potential for human error and increase throughput, making the processes more reliable and efficient.

The versatility of the developed method can be expanded to encompass a wider array of analytes and matrices, thus broadening its applicability beyond pharmaceuticals to include environmental monitoring, food safety, and other critical fields. By adapting the method to diverse contexts, it can serve as a valuable tool in detecting contaminants across various industries, ensuring public safety and compliance with regulatory standards. Continuous innovation in analytical technologies, combined with a commitment to green chemistry, will be essential for maintaining the relevance and environmental compliance of these methods. Future advancements should also focus on minimizing waste, improving sensitivity and specificity, and ensuring that the methods can be applied to new and emerging contaminants. This forward-looking approach will ensure that the analytical methodologies remain at the forefront of scientific research and regulatory practices, providing robust tools for safeguarding human health and the

environment.

5. Conclusions

A highly sensitive and selective UFLC-MS/MS method has been successfully developed, optimized, and rigorously validated, demonstrating exceptional precision, and accuracyin the quantitative analysis of the target analytes. The method's validation parameters, including linearity, LOD, LOQ, and recovery rates, all meet the stringent criteria established by regulatory bodies such as the ICH and USP, underscoring the method's reliability and suitability for routine analytical applications. In addition to its analytical performance, the method's environmental sustainability has been significantly ascertained using multiple greenness assessment tools, including GAPI, AES, and AGREE metrics. These assessments confirm that the method adheres to green chemistry principles, minimizing the use of hazardous substances and implementing effective waste management practices and this report concluded the method as not only scientifically robust but also environmentally responsible. However, the continuous evolution of green chemistry provides opportunities for further enhancing the method's sustainability. One significant approach for improvement is the substitution of current solvents with even greener alternatives, which could further reduce the environmental footprint and align the method with the latest advances in eco-friendly analytical practices. As analytical chemistry continues to progress, integrating these greener technologies will be crucial in maintaining the method's relevance and ensuring its compliance with evolving environmental standards.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Abbreviations

UFLC-MS/MS: Ultra-fast liquid chromatography-tandem mass spectrometry.
NDMA: N-Nitrosodimethylamine.
NDEA: N-nitrosodiethylamine.
NDPA: N-Nitrosodi-n-propylamine.
NEIPA: N-Nitrosoethylisopropylamine.
NDIPA: N-nitrosodiisopropylamine.
NMBA: N-nitroso-N-methyl-4-aminobutanoic acid.
NDBA: N-nitrosodibutylamine.
NMPA: N-nitrosomethylphenylamine.
APCI: Atmospheric pressure chemical ionization.
CEM: Channel electron multiplier.
MRM: Multiple reaction monitoring.
ICH M7: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use mutagenic impurities.
GC–MS/MS: Gas Chromatography Tandem Mass Spectrometry.
IARC: International Agency for Research on Cancer.
RSD: Relative standard deviation.
API: Active pharmaceutical ingredient.
PTFE: Polytetrafluoroethylene.
CAD: collision-activated dissociation.
LOD: Limit of detection.
LOQ: Limit of quantification.
Ppb: Parts per billion.

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CRediT authorship contribution statement

Hemanth Vikram P.R: Writing – original draft, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gunjan Kumar:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization. **Rajashree Deka:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Umme Hani:** Project administration, Funding acquisition, Conceptualization. **Nazima Haider:** Project administration, Funding acquisition, Data curation, Conceptualization. **Sirajunisa Talath:** Project administration, Investigation, Funding acquisition, Conceptualization. **Adil Farooq Wali:** Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Dilipkumar Reddy Kandula:** Writing – review & editing, Visualization, Software, Conceptualization. **Narasimha M. Beeraka:** Writing – review & editing, Supervision, Conceptualization. **Sinchana B Gopalaiah:** Validation, Software, Conceptualization. **Devi Sri Chiriki:** Visualization, Validation, Conceptualization. **Namitha Bannimath:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pramod Kumar:** Writing – review & editing, Visualization, Conceptualization. **Bannimath Gurupadayya:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Supplementary materials

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Data availability

The authors do not have permission to share data.

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