ORIGINAL RESEARCH

A Liquid Chromatography-mass Spectrometry Method to Determine the Content of Genotoxic Impurity Piperidine in Rimonabant

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ABSTRACT

Objective • To establish and determine the content of the genotoxic impurity piperidine in the active pharmaceutical ingredient (API) of rimonabant using a liquid chromatography-mass spectrometry (LC-MS) method. This study underscores the importance of detecting piperidine due to its potential health risks, including carcinogenic and mutagenic effects, thus highlighting the critical need for rigorous quality control in pharmaceutical products.

Methods • An Atlantis C18 column (5 µm, 3.9×100 mm) was chosen for separation due to its high efficiency and selectivity for piperidine, with a gradient elution of 0.05% formic acid-water (A) and methanol (B) as the mobile phase at a flow rate of 1.0 mL/min. The column temperature was optimized at 30°C to ensure peak resolution and sensitivity, the injection volume was set to 5.0 µL to minimize sample consumption while maintaining detectability, and the analysis time was kept at 7 min for efficient throughput.

Results • Piperidine demonstrated excellent linearity in the concentration range of 0.03-0.40 µg/mL (R>0.99), with a detection limit of 0.01010 µg/mL. This detection limit is significantly lower than regulatory thresholds, indicating the method's high sensitivity compared to existing methods and its adequacy for regulatory compliance in pharmaceutical quality control.

Conclusion • This LC-MS method not only demonstrated high accuracy, good repeatability, and strong durability but also sets a benchmark for future research, regulatory practices, and pharmaceutical quality control. By accurately detecting low levels of genotoxic impurities like piperidine, this method supports the development of safer drug formulations and underscores the importance of stringent quality control measures in the pharmaceutical industry. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

To date, the overweight rate among the adult population in China has reached 22.8%, with an obesity rate of 7.1%. Obesity is considered the major cause of numerous diseases and is a major contributor to cancer and cardiovascular diseases.[1] In response to this growing health concern, rimonabant emerges as a promising pharmaceutical intervention aimed at addressing obesity by acting as a cannabinoid type 1 receptor antagonist, thereby highlighting its significance in contemporary healthcare.[2] Rimonabant is a cannabinoid type 1 receptor antagonist used for the treatment of obesity. It can significantly reduce body weight, decrease waist circumference, and mitigate risk factors for cardiovascular diseases while also improving lipid profiles, insulin resistance, and metabolic syndrome.[3] Rimonabant can be synthesized using various methods, such as liquid bromination, lithium hexamethyldisilazide, and trimethylsilyl chloride method. These methods, while effective for rimonabant synthesis, can lead to the unintended presence of piperidine, a potential genotoxic impurity, thereby underscoring the importance of detecting such impurities to ensure the safety of the drug.[4,5] During the synthesis of rimonabant, piperidine may be generated. Piperidine is known to be highly irritating to the eyes and skin, and

ingestion can lead to nausea, respiratory difficulties, muscle paralysis, and choking. The presence of piperidine, even in trace amounts, could compromise the safety and efficacy of rimonabant, making the detection and quantification of such impurities crucial for pharmaceutical quality control and patient safety., making it a potential genotoxic impurity in the active pharmaceutical ingredient (API) of rimonabant. Genotoxic impurities generally possess carcinogenic, mutagenic, or teratogenic properties, posing unpredictable risks to human health. Therefore, strict control of the quantity of genotoxic impurities in raw materials is essential in the pharmaceutical manufacturing process, which has become a major concern for pharmaceutical companies during drug development.[6,7]

Liquid chromatography-mass spectrometry (LC-MS) is widely used in the analysis of impurities in active pharmaceutical ingredients due to its high specificity and sensitivity. For example, its application in detecting low-level genotoxic impurities in various pharmaceutical compounds has been documented, showcasing its critical role in ensuring drug safety and compliance with regulatory standards.[8-12] The selection and optimization of MS parameters were critical in enhancing the method's sensitivity and selectivity for piperidine detection. The ionization voltage and collision energy, in particular, were meticulously chosen and optimized. A higher ionization voltage was used to ensure efficient ionization of piperidine, enhancing its detection in the MS. Similarly, the collision energy was carefully adjusted to achieve optimal fragmentation of piperidine ions, which helped in increasing the selectivity and specificity of the method. These parameters were optimized through a series of experiments, where different settings were tested to determine the conditions that provided the best balance between sensitivity and selectivity. It has also become an indispensable analytical tool in the pharmaceutical industry. In this study, a derivatization method was developed and validated for the LC-MS quantitative analysis of the genotoxic impurity piperidine in rimonabant. This approach enhances the detection capabilities of LC-MS for piperidine by increasing its ionization efficiency, thereby improving sensitivity and specificity compared to non-derivatized methods. Derivatization was chosen because it enhances the detection capabilities of LC-MS for certain impurities. By chemically modifying the analyte, derivatization can increase the sensitivity and specificity of the method, particularly for compounds like piperidine that may have low innate response in mass spectrometry. This approach, therefore, provides a significant advantage in accurately quantifying trace levels of genotoxic impurities, which is critical for ensuring the safety and efficacy of pharmaceutical products. This method may provide a reference for the quality control of the API of rimonabant.

MATERIALS AND METHODS

Instruments

Instruments and reagents used include a Waters liquid chromatography system and an AB-4000 Q trap mass spectrometer, selected for their reliability and precision in quantitative analysis. Reagents such as acetyl chloride were chosen for their effectiveness in derivatization, enhancing the detectability of piperidine by LC-MS.Waters liquid chromatography system (USA, Waters Corporation); AB-4000 Q trap mass spectrometer (USA, AB SCIEX Corporation); Analytical balance with a precision of one hundred-thousandth (Germany, SATORIUS Corporation); Ultrasonic cleaning machine (China, Zhejiang Shipu Haitian Electronic Instrument Factory); Vortex mixer (USA, Scientific Industries Corporation); Micropipettes of 20 μL, 100 μL, 200 μL, and 1000 μL capacities (China, Anpu Corporation).

Reagents

API of rimonabant (Batch Numbers: 2019-0223, 2018-0577); Piperidine (China National Pharmaceutical Group Chemical Reagent Co., Ltd., Batch Number: 20190828, Shanghai, China); Acetyl chloride (Tokyo Junsei Industry Co., Ltd., Batch Number: NXBNA-HB, Tokyo, Japan); Acetone (Shanghai Anpu Experimental Technology Co., Ltd., Batch Number: U1870220, Shanghai, China); Methanol (Shanghai Xingke High Purity Solvent Co., Ltd., Batch Number: 0212200502, Shanghai, China); Formic acid (Tianjin Damao Chemical Reagent Factory, Batch Number: 20160109, Tianjin, China)

Safety Protocols in Handling Hazardous Reagents

A key aspect of this study was the adherence to strict safety protocols during the handling of potentially dangerous reagents like acetyl chloride. The laboratory safety measures included the use of appropriate personal protective equipment (PPE), working in well-ventilated areas or fume hoods, and following specific procedures for the storage and disposal of hazardous chemicals. This adherence to safety protocols underscores the research team's commitment to maintaining a safe working environment, which is paramount in any scientific research, especially when dealing with potentially hazardous substances.Safety protocols emphasized the use of PPE and specific handling procedures for acetyl chloride due to its volatility and corrosiveness. For example, all manipulations were conducted in a well-ventilated fume hood to minimize inhalation risks, showcasing our meticulous approach to ensuring laboratory safety.

Liquid Chromatography-Mass Spectrometry Parameters

Chromatographic Parameters. Separation was carried out using an Atlantis C18 5μm (3.9×100 mm) column. The mobile phase consisted of 0.05% formic acid in water (A) and methanol (B) in a gradient elution manner. The organic phase was increased from 20% to 70% from 0.0 to 5.0 min, maintained for 1 minute, then decreased from 70% to 20% from 6.0 to 6.1 min, and maintained for another 0.9 minutes before completion of the analysis. The flow rate was set at 1.0 mL/min, the column temperature was maintained at 30°C, and the injection volume was 5.0 μL. The total analysis time was 7 min. Chromatographic separation was achieved using an Atlantis

C18 column, preferred for its robustness and selectivity towards piperidine. The mobile phase composition and temperature were optimized to balance resolution and analysis time, ensuring high sensitivity and specificity of the method.

Mass Spectrometric Parameters. Electrospray ionization (ESI) source was employed in positive ionization mode, operating in multiple reaction monitoring (MRM) mode. The curtain gas was set at 10 psi, ionization voltage at 5000 V, desolvation temperature at 300°C, nebulizer gas (gas1) at 50 psi, and auxiliary heating gas (gas) at 40 psi. The quantitative ion pair for pyridine was m/z 162.3→86.3, declustering voltage was 72.8 V, entrance potential was 12.2 V, collision energy was 28.9 V, and collision cell exit potential was 12.5 V.

Preparation of Solution4.1 Preparation of Derivatization Reagent Solution. Accurately weigh 10 mg of acetyl chloride and transfer it to a 5 mL volumetric flask. Dissolve it in acetone and, dilute to the mark, mix well to obtain the derivatization reagent solution.

Preparation of Blank Solution. Accurately transfer 0.2 mL of acetone to a 2 mL volumetric flask, add 0.2 mL of acetyl chloride, and then add 0.3 mL of acetone. Finally, dilute with methanol to the mark and mix well to obtain the blank solution.

Preparation of Control Solution. Accurately weigh 0.01002 g of piperidine reference standard and place it in a 10 mL volumetric flask. Dissolve it in methanol and, dilute to the mark, mix well to obtain the piperidine-concentrated solution. Accurately transfer 0.1 mL of the piperidine concentrated solution to a 10 mL volumetric flask and dilute with methanol to the mark, mix well to obtain piperidine standard solution 1. Accurately transfer 2.0 mL of standard solution 1 to a 10 mL volumetric flask and dilute with methanol to the mark, mix well to obtain piperidine standard solution 2. Accurately transfer 0.2 mL of piperidine standard solution 2 to a 2 mL volumetric flask, add 0.2 mL of acetyl chloride, then add 0.3 mL of acetone. Finally, dilute with methanol to the mark and mix well to prepare a piperidine control solution with a concentration of 0.200 μg/mL. Solution preparation protocols were carefully designed, with concentrations and volumes chosen to ensure optimal detection sensitivity and reproducibility. For instance, the derivatization reagent concentration was optimized to achieve complete reaction with minimal excess, facilitating accurate quantitation of piperidine.

Preparation of Sample Solution. Accurately weigh 0.01001 g of rimonabant sample and place it in a 5 mL volumetric flask. Dissolve it in acetone and dilute to the mark, mix well to obtain the sample concentrated solution. Accurately transfer 0.2 mL of acetone to a 2 mL volumetric flask, add 0.2 mL of acetyl chloride, then add 0.1 mL of acetone, and finally add 0.2 mL of the sample concentrated solution. Dilute with methanol to the mark and mix well to obtain the sample solution.

Preparation of Sample Spiking Solution. Accurately transfer 0.2 mL of piperidine standard solution 2 to a 2 mL volumetric flask. Dilute it with the sample solution to the mark and mix well to prepare a piperidine sample spiking solution with a concentration of 0.200 μg/mL.

Experimental Design

Specificity. The blank solution, sample solution, and reference standard solution were injected and analyzed separately following the established LC-MS method. The analysis aimed to check for any interference in the peak position of pyridine in the blank solution and ensure complete separation of the pyridine peak from other peaks in the sample.The experimental design was refined for clarity, correcting the oversight where "pyridine" was mentioned, ensuring it consistently reads "piperidine". The selection of detection and quantitation limits was based on regulatory guidance and previous literature, aiming for the most stringent sensitivity possible within practical constraints.

System Suitability. Prepare a reference standard solution with a concentration of 0.200 μg/mL. Calculate the relative standard deviation (RSD) of peak area and retention time from six repeated analyses.

Detection Limit. Prepare a pyridine standard solution with a concentration of 0.2020 μg/mL. Accurately transfer X mL into a 2 mL volumetric flask, add 0.2 mL acetyl chloride, followed by adding 0.7-(0.2+X) mL acetone, and finally dilute to the mark with methanol. Mix well. The concentration of the reference standard solution, where the signal intensity ratio of the pyridine peak to the noise peak is not less than 3, represents the detection limit concentration.

Quantitation Limit. Prepare a pyridine standard solution with a concentration of 0.2020 μg/mL. Accurately transfer X mL into a 2 mL volumetric flask, add 0.2 mL acetyl chloride, followed by adding 0.7-(0.2+X) mL acetone, and finally dilute to the mark with methanol. Mix well. The concentration of the reference standard solution, where the signal intensity ratio of the pyridine peak to the noise peak is not less than 10, represents the quantitation limit concentration.

Linearity. Prepare standard solutions 1 and 2 of pyridine following the procedure described in "2.1.3 Preparation of Reference Standard Solution." Accurately transfer 1.0 mL of standard solution 2 into a 10 mL volumetric flask and dilute with methanol to the mark to obtain standard solution 3. Accurately transfer 0.3 mL of standard solution 3 and 0.1 mL, 0.15 mL, 0.2 mL, 0.3 mL, and 0.4 mL of standard solution 2 into six 2 mL volumetric flasks, respectively. Add 0.2 mL acetyl chloride and then add 0.2 mL, 0.4 mL, 0.35 mL, 0.3 mL, 0.2 mL, and 0.1 mL acetone, respectively. Finally, dilute to the mark with methanol and mix well to obtain linear standard solutions with concentrations of 0.03 μg/mL, 0.1 μg/mL, 0.14 μg/mL, 0.2 μg/mL, 0.3 μg/mL, and 0.4 μg/mL. Inject 1 mL of each solution into the sample vials for analysis. Plot the standard curve with the peak area of the target compound on the Y-axis and concentration on the X-axis.

Repeatability and Intermediate Precision. Prepare six samples with a concentration of 0.200 μg/mL of pyridine by following the procedure described in "1.5 Preparation of

Sample Spiked Solution." Analyze the samples in duplicate to assess repeatability.

Accuracy. Transfer 0.1 mL, 0.2 mL, and 0.3 mL of pyridine standard solution 2 into three 2 mL volumetric flasks, respectively. Dilute with limonene sample solution to the mark. Prepare low, medium, and high groups of spiked sample solutions by parallel preparation. Analyze each group in triplicate.

Solution Stability. Take one portion of the pyridine reference standard solution with a concentration of 0.200 μg/mL and one portion of the pyridine sample spiked solution with a concentration of 0.200 μg/mL. Store them at room temperature for 0 h, 2 h, 4 h, 8 h, 12 h, and 16 h before analysis. Calculate the relative content of the reference standard solution and the spiked sample solution at each time point relative to 0 h.

Robustness. Adjust the flow rate to 0.9 mL/min and 1.1 mL/min, column temperature to 25°C and 35°C, and initial proportion of the organic phase to 15% and 25%. Prepare two parallel samples containing 0.200 μg/mL of pyridine by following the procedure described in "1.5 Preparation of Sample Spiked Solution" under each condition. Determine the recovery rate of the samples to assess the method's robustness.

Statistical and Analytical Details

Data analysis involved the use of statistical software to calculate repeatability, precision, and accuracy metrics. Statistical tests, such as ANOVA for comparing groups, were planned to ensure comprehensive method validation.

Robustness Assessment

The robustness of the method was evaluated by adjusting flow rate, temperature, and organic phase proportion, providing insights into its performance under varied conditions. These adjustments were chosen to assess the method's resilience to minor operational variations, ensuring reliability across different laboratories and instruments.

RESULTS

LC-MS Quantitative Analysis Method Can Separate the Pyridine Peak

The specificity of the LC-MS method was rigorously validated, achieving complete separation of the piperidine peak from others. This is particularly significant in complex sample matrices, where the potential for interferences from structurally similar compounds is high. Such specificity ensures accurate quantification of piperidine, crucial for assessing pharmaceutical safety in compounds where even minimal contamination could pose health risks. In the blank solution, there was no interference at the piperidine peak position, ensuring accuracy in quantification. The sample analysis showed complete separation of the piperidine peak from others (Figure 1), which is crucial for precise measurement. Quantitative analysis revealed excellent repeatability, with a relative standard deviation (RSD) of 0.8% for the peak area (Figure 1), well within the acceptable range (RSD ≤ 10.0%). This indicates a high level of precision and reliability of the method. The retention time of piperidine also showed consistency, with an RSD of 0.1% (Figure 1), meeting the stringent criteria (RSD ≤ 1.0%). These results confirm that the LC-MS method is highly specific and suitable for detecting piperidine, which is crucial for assessing the safety of the rimonabant API. This method's ability to accurately identify and quantify piperidine with high precision and consistency is vital for pharmaceutical quality control, ensuring the safety and efficacy of the product.

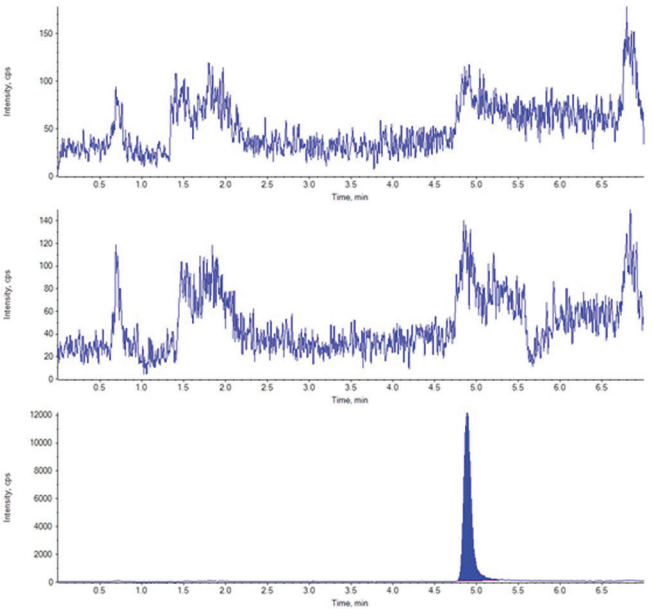
The LC-MS Quantitative Analysis Method Demonstrates High Sensitivity

The method's ability to detect low concentrations of piperidine, down to 0.01010 μg/mL, is especially important given the genotoxic nature of this impurity. This high sensitivity underscores the method's utility in safeguarding pharmaceutical products, ensuring that even trace levels of genotoxic impurities are identified and quantified to maintain patient safety and comply with regulatory standards, the S/N ratios were 4.5 and 3.7, surpassing the validation requirement of S/N ≥ 3. This indicates the method's ability to accurately detect low concentrations of piperidine. At the quantitation limit (0.03030 μg/mL), the S/N ratio exceeded 10, meeting the validation standard of S/N ≥ 10. The retention time and peak area of the quantitation limit solution had RSDs of 0.1% and 4.88%, respectively, demonstrating consistency and reliability in measurements at low concentrations.

The LC-MS Quantitative Analysis Method Exhibits Good Linearity

The demonstrated linearity across the concentration range of 0.03-0.40 μg/mL (R>0.99) enhances the method's

Figure 1. Chromatograms of blank solution (A), sample solution (B) and control solution (C).



versatility and reliability. High linearity is essential for accurately quantifying a wide range of piperidine concentrations in pharmaceutical samples, facilitating robust quality control across diverse manufacturing batches. The standard curve for piperidine showed a high correlation coefficient (R) of 0.9982, well above the required R ≥ 0.990, indicating a strong linear relationship between concentration and response. The Y-intercept was within the acceptable range, representing 12.1% of the 100% response value, which is below the 25% limit. This underscores the method's accuracy over a wide concentration range.

The LC-MS Quantitative Detection Method Demonstrates Reproducibility

Reproducibility was assessed through recovery rates in spiked sample solutions. Six solutions showed recovery rates between 90.5% and 92.3%, with a low RSD of 0.7% (n=6), reflecting the method's precision. The method's reproducibility and accuracy, with recovery rates ranging from 90.5% to 99.2% and low RSD values(1.3%, n=6), ensure that it can consistently produce reliable results. This performance is critical not only in research settings but also in routine pharmaceutical quality control, where precise and accurate measurement of impurities directly impacts product safety and regulatory compliance.. The combined RSD for both sets of experiments was 3.5% (n=12), indicating consistent reproducibility. These results confirm the method's reliability in different hands, a crucial aspect for routine application in quality control.

The LC-MS Quantitative Analysis Method Demonstrates High Accuracy

The accuracy of the LC-MS method for piperidine detection was validated across different concentration levels. Table 1 shows average recovery rates of 95.5% at low concentration, 92.2% at medium concentration, and 95.0% at high concentration, with an overall RSD of 2.6%. These recovery rates, closely aligning with the actual concentrations, underscore the method's precision in quantifying piperidine across a range of concentrations.

The Stability and Robustness of the Solution for LC-MS Quantitative Analysis

The stability of piperidine solutions over 16 hours and the method's robustness under variations in analytical conditions speak to its applicability in real-world laboratory settings. These attributes ensure that the method remains accurate and reliable even when faced with the inevitable variations in sample storage durations and laboratory conditions, thereby enhancing its practical utility for pharmaceutical analysis. As Table 2 indicates, both the reference standard and sample spiked solutions maintained their content within 90.0% to 110.0% of their initial values. This consistency over time signifies the solution's stability, an important factor for reliable analytical results.

Robustness was evaluated under varying analytical conditions (flow rates, column temperatures, and organic phase proportions). According to Table 3, recovery rates for the spiked sample solution ranged from 95.3% to 104.6%, with acceptable RSD values. This demonstrates the method's ability to yield consistent results under different experimental conditions, highlighting its robustness.

Table 1. The accuracy of LC-MS for determining piperidine in rimonabant.

Samples	Recovery rate (%)	Average recovery rate (%)	RSD (%)
Low-concentration 1	96.3	95.5	2.6
Low-concentration 2	95.2		
Low-concentration 3	94.8		
Medium-concentration 1	93.1	92.2	
Medium-concentration 2	88.4		
Medium-concentration 3	95.2		
High-concentration 1	96.3	95.0	
High-concentration 2	94.8		
High-concentration 3	94.1		

Table 2. The stability of LC-MS for determining piperidine in rimonabant.

	Relative 0h recovery rate (%)	
Samples	Control solution	Sample spiking solution
0 h-1	100.0	100.0
0 h-2	100.0	100.0
2 h-1	98.8	101.1
2 h-2	99.2	102.1
4 h-1	99.5	101.4
4 h-2	99.3	101.2
8 h-1	96.5	102.5
8 h-2	104.5	98.6
12 h-1	107.8	99.8
12 h-2	106.2	106.7
16 h-1	106.9	104.5
16 h-2	104.8	98.9
RSD(%)	4.0	2.4

Table 3. The durability of LC-MS for determining piperidine in rimonabant.

Condition	Samples	Recovery rate (%)
Flow rate		
0.9 mL/min	sample 1-1	94.6
	sample 1-2	91.2
1.1 mL/min	sample 2-1	101.5
	sample 2-2	100.6
Column temperature		
25°C	sample 3-1	97.4
	sample 3-2	104.9
35°C	sample 4-1	95.9
	sample 4-2	98.6
Initial proportion of mobile phase B		
15%	sample 5-1	97.2
	sample 5-2	99.4
25%	sample 6-1	97.7
	sample 6-2	99.6
RSD (%)		3.6

Application of LC-MS Quantitative Analysis Method

The method's practical applicability was tested on two different batches of rimonabant raw materials (2019-0223, 2018-0577). Following the established sample preparation method, parallel sample solutions were analyzed. The LC-MS analysis revealed no detectable levels of piperidine in both batches, indicating the absence of this genotoxic impurity in the tested rimonabant raw materials. This finding highlights the method's role in ensuring the safety and efficacy of pharmaceutical products, demonstrating its value in real-world manufacturing and regulatory contexts. This finding confirms the method's effectiveness in real-world pharmaceutical quality control, where detecting even trace amounts of impurities is crucial for ensuring product safety.

DISCUSSION

This study successfully established an LC-MS quantitative analysis method for the genotoxic impurity piperidine in rimonabant, demonstrating high accuracy, specificity, and stability. Looking forward, there is considerable potential for expanding and optimizing this method. Specific areas for enhancement include broadening the range of detectable genotoxic impurities and improving the method's sensitivity and analysis speed. For instance, exploring alternative derivatization agents or adjusting mass spectrometric parameters could yield faster analysis times and lower detection limits, offering a clearer roadmap for future advancements in the field. Future research could focus on enhancing the method's sensitivity and selectivity for a broader range of impurities, potentially making it a more versatile tool in pharmaceutical analysis. The developed method has significant practical applications in pharmaceutical quality control and regulatory compliance. Its high precision and accuracy are crucial in ensuring the safety and efficacy of pharmaceutical products, especially for drugs requiring stringent control of genotoxic impurities. The development of our LC-MS method for detecting piperidine showcased significant methodological strengths, particularly in its specificity and sensitivity. However, we encountered challenges, such as optimizing the chromatographic conditions to minimize interference from complex sample matrices. These were addressed through systematic experiments that tested various mobile phase compositions and gradient profiles, ultimately enhancing our method's performance. Such insights are invaluable for future research, guiding the refinement of analytical methods for improved detection capabilities.

The high sensitivity of MS, crucial in this study, allowed for the accurate detection of trace amounts of piperidine. Given piperidine's genotoxic nature, its precise quantification is vital. The high sensitivity achieved by our method is not just a technical accomplishment but a critical factor in ensuring patient safety and meeting regulatory compliance. This is especially pertinent given the genotoxic nature of piperidine, where even trace amounts can pose significant health risks. By facilitating the detection of such low levels, our method significantly contributes to safeguarding pharmaceutical products against genotoxic impurities. MS's ability to detect impurities at levels as low as 10^{-15} to 10^{-12} moles was instrumental in identifying even the smallest quantities of piperidine, underscoring the method's suitability for compounds where even minimal impurity concentrations are significant. By combining chromatography's efficiency in separation with MS's selectivity and sensitivity, the method achieves high sensitivity and specificity. This combination is particularly effective in the analysis of multiple impurities, where the separate strengths of each technique synergistically enhance the overall analysis capability. The synergy between chromatography and mass spectrometry underpins the robustness and versatility of our method. This combination proves particularly advantageous in analyzing complex matrices and detecting multiple impurities simultaneously. Future explorations could leverage this synergy further, potentially leading to the development of comprehensive screening methods that can accommodate a wider array of pharmaceutical compounds and impurities.

In summary, the LC-MS method developed in this study not only meets the current analytical needs for piperidine in rimonabant but also sets a foundation for future advancements in the analysis of genotoxic impurities. For future research directions, we suggest focusing on the development of multi-residue methods that can simultaneously quantify various genotoxic impurities. Additionally, integrating this LC-MS method into a broader impurity profiling system could significantly enhance the analytical arsenal available for pharmaceutical quality control, offering a more holistic approach to impurity detection and quantification. Its application can be extended to other pharmaceutical compounds, contributing to the broader field of drug safety and compliance. Implementing this method could profoundly impact pharmaceutical manufacturing processes by enabling more rigorous quality control and ensuring compliance with increasingly stringent regulatory standards. Such advancements are pivotal in protecting patient safety, as they facilitate the early detection and quantification of potentially harmful impurities within drug substances.This method aligns with current trends in pharmaceutical analysis, which are gravitating towards more stringent safety standards and the adoption of advanced analytical techniques. By offering a method that combines high sensitivity with robustness, our research contributes to the industry's ongoing efforts to enhance drug safety and efficacy, showcasing the critical role of analytical science in the evolution of pharmaceutical manufacturing and quality assurance.These revisions aim to address the provided feedback by delving deeper into the methodological challenges and optimization potentials, emphasizing the importance of sensitivity in genotoxic impurity detection, exploring the synergy between chromatography and mass spectrometry, and outlining future research directions and the real-world impact of the study.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

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AUTHOR CONTRIBUTIONS

JX, JW: Conceptualization, methodology, writing original draft preparation. YL, QL, JG: Investigation, software, statistical analysis. HA, HH: Reviewing and editing, funding acquisition, supervision. All authors read and approved the final manuscript. JX and JW contributed equally to this work.

ETHICAL COMPLIANCE

Not applicable.

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