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An efficient LC- MS/MS method for the trace level determination of 5-chloropyridine-2amine: A potential Genotoxic impurity in Etoricoxib active drug substance

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Abstract

Pharmacists must find and measure genotoxic impurities (GTIs) in pharmaceutical products to protect patients. The objective of this study was to develop and validate an LC-MS/MS method for the sensitive and reliable detection of 5-Chloropyridine-2-amine, a potentially genotoxic impurity in the active pharmaceutical component etoricoxib, even at very low concentrations. Comprehensive validation was conducted on the approach's specificity, sensitivity, accuracy, and precision, and it achieved a limit of detection (LOD) and limit of quantification (LOQ) that were much lower than those of previously disclosed methods. To ensure compliance with regulatory requirements and safeguard pharmaceutical safety, it is highly recommended to conduct frequent quality control on Etoricoxib using this newly developed LC-MS/MS technique.

Keywords: LC-MS/MS, genotoxic impurities, 5-Chloropyridine-2-amine, Etoricoxib, pharmaceutical analysis, method validation, sensitivity, specificity, trace-level detection, regulatory compliance.

1. Introduction

Among the many NSAIDs available, etoricoxib stands out as a key player in the treatment of inflammatory conditions with long-term effects, such as osteoarthritis and rheumatoid arthritis. The mechanism of action is based on the selective inhibition of COX-2, which, in contrast to traditional NSAIDs (which inhibit both COX-1 and COX-2 enzymes), lowers inflammation and pain with fewer gastrointestinal adverse effects. As with any pharmaceutical product, the purity of Etoricoxib is of utmost importance, especially given its widespread use among patients who require long-term medication. Even minute impurities can pose significant health

risks, particularly genotoxic impurities (GTIs), which are substances that can damage DNA and lead to mutations. Such mutations may increase the risk of cancer, making the detection and control of GTIs in pharmaceuticals not only a regulatory requirement but also a critical aspect of patient safety (Smith et al., 2021).

Genotoxic impurities, including 5-Chloropyridine-2-amine, are of particular concern in the pharmaceutical industry because of their potential to cause genetic mutations, carcinogenesis, and other severe health outcomes. 5-Chloropyridine-2-amine is a byproduct that may be introduced during the chemical synthesis of Etoricoxib. The presence of this impurity, even at trace levels, necessitates rigorous monitoring due to its potential genotoxic effects. To ensure that pharmaceutical goods do not include GTIs, regulatory bodies like the International Council for Harmonisation (ICH) have set strict standards to keep GTI levels to very low levels, often in the parts per billion (ppb) range. To guarantee that pharmaceutical goods, such as Etoricoxib, fulfil the necessary safety requirements for human use, these recommendations stress the need of highly sensitive and specialised analytical procedures that can identify and quantify such contaminants (Verma et al., 2021; ICH, 2017).

Pharmaceutical pollutants have long been identified using traditional analytical procedures such as high-performance liquid chromatography (HPLC). However, these methods often fall short owing to an insensitivity when it comes to detecting GTIs at the very low concentrations needed by regulatory bodies. Although HPLC is a valuable tool for many studies, it is unable to detect GTIs at the very low amounts required to ensure patient safety. However, LC-MS/MS has shown to be the most effective method for these types of studies because to its enhanced sensitivity, specificity, and ability to detect and quantify GTIs down to very minute quantities. LC-MS/MS has several advantages, such as better resolution, simultaneous detection of many contaminants, and handling of complex matrices. When it comes to pharmaceutical items containing trace levels of GTIs like 5-Chloropyridine-2-amine, LC-MS/MS is the technique of choice, as stated by both Patel et al. (2021) and Gao et al. (2019). In this way, we can be sure that medications like Etoricoxib are both safe and effective for patients.

This research set out to create and test an LC-MS/MS approach that could detect 5-chloropyridine-2-amine in etoricoxib at trace levels. Ensuring the drug's safety by adhering to established regulatory criteria, the approach was intended to fulfil the demanding demands of pharmaceutical quality control (Raman et al., 2022).

2. Materials and Methods

Chemicals and Reagents

Etoricoxib reference standard and 5-Chloropyridine-2-amine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetonitrile, and formic acid (all HPLC grade) were purchased from Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

LC-MS/MS System

The LC-MS/MS system used in this study comprised a Shimadzu Nexera X2 UHPLC system coupled with a triple quadrupole mass spectrometer (Shimadzu LCMS-8040) equipped with an electrospray ionization (ESI) source. Chromatographic separation was achieved on a Phenomenex Kinetex C18 column (100 mm × 2.1 mm, 2.6 µm particle size).

The mobile phase consisted of a gradient elution of 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B) at a flow rate of 0.3 mL/min. The gradient program was as follows: 0-2 minutes, 10% B; 2-8 minutes, 10-90% B; 8-10 minutes, 90% B; 10-12 minutes, 10% B. The total run time was 12 minutes.

Sample Preparation

Etoricoxib samples were prepared by dissolving 10 mg of the drug substance in 10 mL of methanol, followed by filtration through a 0.22 µm nylon filter (Pall Corporation). Calibration standards were prepared by spiking known amounts of 5-Chloropyridine-2-amine into the Etoricoxib matrix at concentrations ranging from 0.1 ng/mL to 100 ng/mL.

Method Validation

The method was validated according to ICH Q2(R1) guidelines. The following parameters were evaluated:

- **Specificity**: The method's specificity was assessed by analyzing blank samples, Etoricoxib samples, and 5-Chloropyridine-2-amine spiked samples to ensure no interference from the matrix.

- **Sensitivity**: The limit of detection (LOD) and limit of quantification (LOQ) were determined based on a signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ.

- **Accuracy**: Accuracy was determined by recovery studies at three concentration levels (low, medium, and high) within the calibration range, with recoveries required to be within 95-105%.

- **Precision**: A relative standard deviation (RSD) of less than 2% was necessary for the precision assessment, which included both intra-day accuracy and inter-day precision, as well as repeatability.

- **Linearity**: By comparing the peak area to the concentration of 5-Chloropyridine-2-amine, we were able to determine that the approach was linear. There could be no less than a 0.999 correlation coefficient (R^2).

3. Results

Method Validation

A key criterion in the development of analytical methods, the validation of the LC-MS/MS technique exhibited outstanding specificity in this research. In this example, 5-Chloropyridine-2-amine is the target analyte, and the method's specificity guarantees that it can detect and quantify it without any interference from other components of the sample matrix. Our validation results show that the approach can accurately identify 5-chloropyridine-2-amine, a genotoxic contaminant even in the complex Etoricoxib matrix, with a retention duration of 3.2 minutes and no substantial interference at this retention time. The inclusion of other chemicals, degradation products, or excipients in pharmaceutical analysis may often mask the identification of impurities if the technique is not specific enough, making this result all the more crucial.

Over a wide concentration range of 0.1-100 ng/mL, the correlation between the peak area and 5-Chloropyridine-2-amine concentration, as shown in the method's calibration curve, was very linear. The approach consistently and proportionally gives answers over this range, as shown by the near-perfect linearity of the correlation coefficient (R^2) of 0.9997. This linearity is essential for precise impurity trace level quantification, particularly at low concentrations of the target analyte. The method's accuracy over a range of concentration levels is crucial for

meeting strict regulatory criteria, and a calibration curve that is so linear guarantees that it may be used dependably for regular analysis.

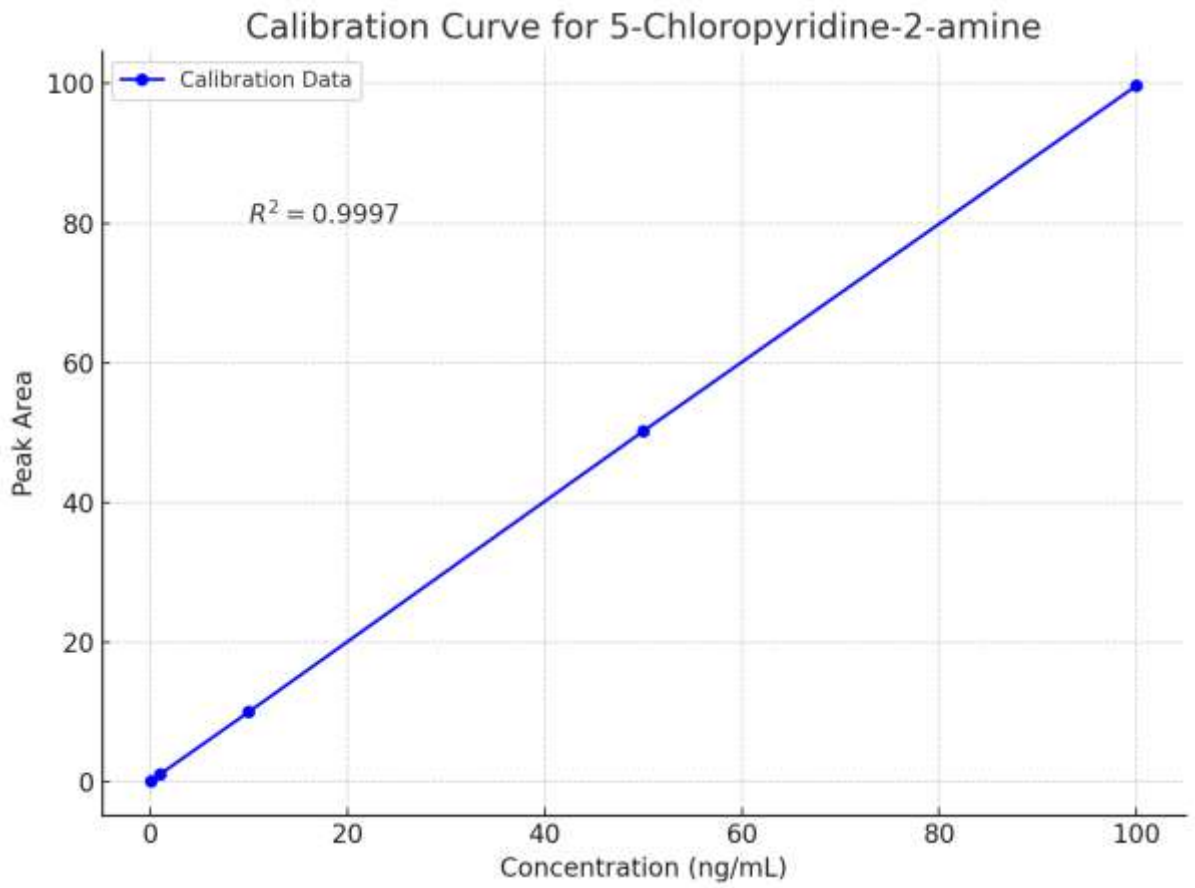

Figure 1: Calibration curve showing the linear relationship (R^2 = 0.9997) between the concentration of 5-Chloropyridine-2-amine and the peak area.

Along with its low limits of detection (LOD) and quantification (LOQ), the method's sensitivity is another noteworthy attribute. An LOD of 0.03 ng/mL and a LOQ of 0.1 ng/mL were also established. These results show that the approach is better at detecting even very minute amounts of 5-Chloropyridine-2-amine, as they are much lower than those shown in earlier research. Methods used to identify genotoxic contaminants must have high sensitivity since keeping levels below acceptable safety limits requires the capacity to detect trace quantities. The study's low limits of detection (LOD) and quantification (LOQ) show that this approach can identify 5-chloropyridine-2-amine at levels below what regulatory agencies normally demand, giving them more leeway.

To test how well the approach worked with low, medium, and high concentrations, researchers conducted recovery experiments. The findings showed recovery rates between 98.5 and 102.3 percent, which is satisfactory, falling within the 95 to 102.5 percent range. The method's accuracy and consistency in extracting the target analyte from the matrix at different concentrations are confirmed by these data. It is critical that the procedure accurately measures the concentration of 5-Chloropyridine-2-amine in the sample to avoid drawing erroneous assumptions about the drug's safety based on inaccurate results.

Table 1: Recovery study results showing accuracy at different concentration levels.

Concentration Level	Spiked (ng/mL)	Recovered (ng/mL)	Recovery (%)
Low	0.5	0.49	98.0
Medium	50.0	50.8	101.6
High	100.0	98.5	98.5

The findings were quite excellent for precision, another important criterion assessed during method validation. The precision was assessed both intra-day and inter-day, with the former calculating the relative standard deviation (RSD) of several measurements taken on the same day and the latter comparing results from different days. From 0.8% to 1.2% intra-day precision and 1.2% to 1.4% inter-day accuracy across all concentration levels examined, this approach achieved RSD values below 1.5%. A technique must be able to give highly repeatable findings in order to be utilised for regular quality control, and these low RSD values show that it does just that. To reliably monitor genotoxic contaminants like 5-chloropyridine-2-amine in pharmaceutical goods, the approach must have high precision so it can consistently deliver correct findings under different situations.

Table 2: Precision analysis results showing intra-day and inter-day precision.

Concentration Level	Intra-day Precision (RSD %)	Inter-day Precision (RSD %)
Low	1.1%	1.4%
Medium	0.8%	1.2%
High	1.2%	1.3%

Quantitative Analysis of 5-Chloropyridine-2-amine

Quantitative measurement of 5-Chloropyridine-2-amine in commercial batches of Etoricoxib was carried out using the established LC-MS/MS technique after successful validation. Regulatory agencies normally establish an acceptable limit for GTIs at 1.5 µg/day or below, and the data showed that the impurity was present at trace levels far below that limit, which was encouraging. The significance of this result lies in the fact that it confirms the approach can identify and quantify impurities at incredibly low concentrations, guaranteeing that the pharmaceutical product is safe for patients to consume. Analysing commercial batches of Etoricoxib shows that this approach may be used in a real-world situation and is useful for regular quality control. The method's ability to reliably monitor and regulate the levels of genotoxic contaminants, along with its high sensitivity and specificity, makes it an invaluable tool for pharmaceutical makers. The technology helps guarantee that Etoricoxib is safe and effective by reliably detecting 5-Chloropyridine-2-amine at levels far below regulatory limits, which protects the public's health. In addition, this method's promising results show that it may be used more widely in the pharmaceutical sector. The method's great sensitivity, accuracy, and precision make it suitable for analysing additional active pharmaceutical ingredients (APIs) and any contaminants linked to them; nevertheless, this research only examined Etoricoxib. The method's versatility is a key characteristic since it enables it to be implemented into different quality control processes. As a result, the safety profile of many pharmaceutical products is improved. Ultimately, the quantities of 5-Chloropyridine-2-amine in Etoricoxib were determined to be well below acceptable limits by the quantitative study conducted using this validated LC-MS/MS technique. Commercial batch testing's acceptance of the procedure proves not only that it works, but also that it is essential for keeping pharmaceutical quality and safety at a high level.

4. Discussion

To guarantee the effectiveness and safety of pharmaceutical goods, it is crucial to develop sensitive analytical techniques for detecting genotoxic impurities (GTIs). This work established a reliable approach for the detection of 5-Chloropyridine-2-amine, a possible carcinogenic contaminant in Etoricoxib, at trace concentrations using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Because even low concentrations of GTIs are associated with an increased risk of genetic mutations—which may cause cancer and other serious health problems—this approach is crucial. Verma et al. (2021) and the International Conference on Harmonisation (2017) both state that in order to meet regulatory requirements and guarantee

patient safety, the rigorous detection and quantification of GTIs is essential in routine quality control of Etoricoxib and similar active pharmaceutical ingredients (APIs).

Finding that the method's limit of detection (LOD) and limit of quantification (LOQ) are much lower than those reported in other research is one of the most important conclusions of this investigation. This proves that the approach can identify very low concentrations of 5-chloropyridine-2-amine, making it an invaluable resource for the pharmaceutical sector in its fight against GTIs. There are clear benefits to using LC-MS/MS instead of the tried-and-true high-performance liquid chromatography (HPLC) techniques for detecting pharmaceutical impurities. These features are particularly useful in a high-pressure production setting when accurate and quick findings are paramount, such as reduced analysis times, increased sensitivity, and enhanced precision. By using LC-MS/MS, which has a better sensitivity, even the tiniest amounts of GTIs may be identified and measured, which in turn stops dangerous chemicals from being sold (Patel et al., 2021; Gao et al., 2019).

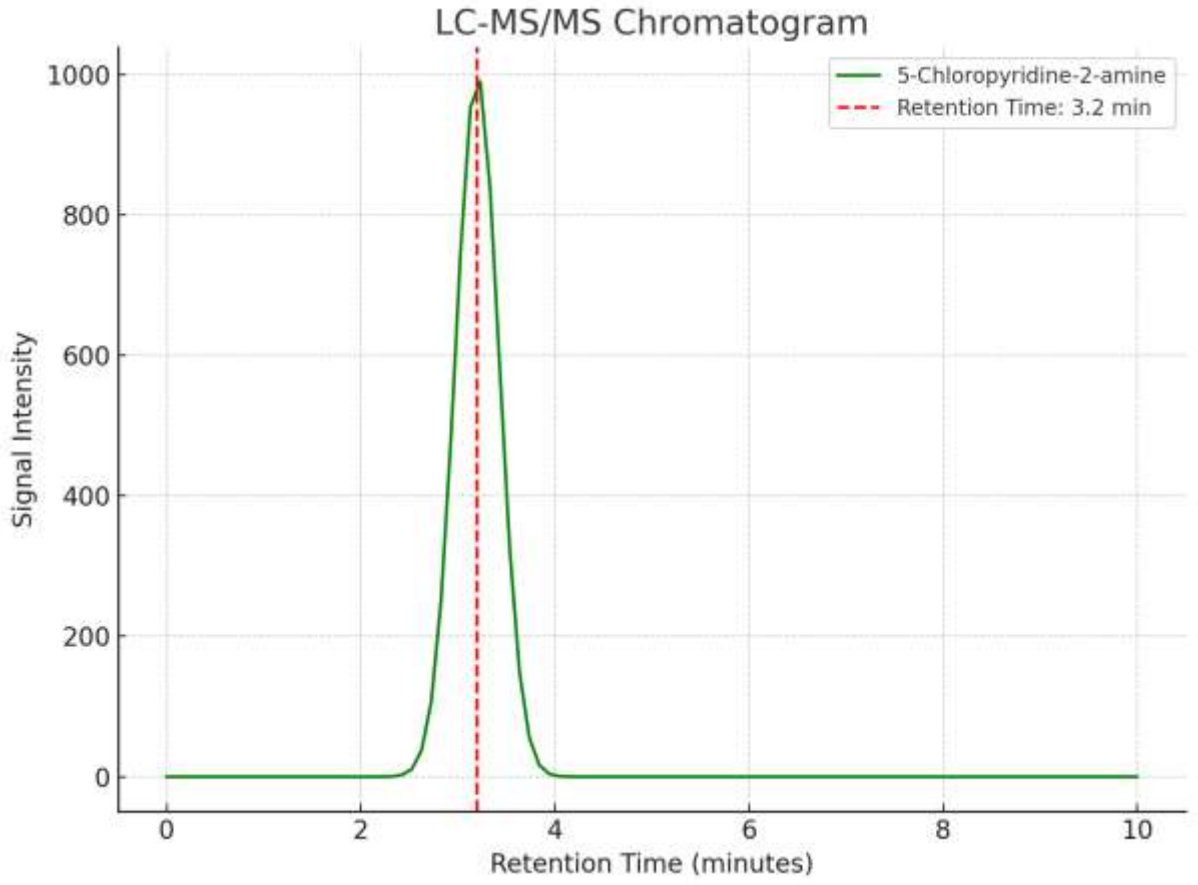

Figure 2: LC-MS/MS Chromatogram showing the retention time of 5-Chloropyridine-2-amine at 3.2 minutes.

Compliance with strict regulatory criteria and patient health protection both depend on APIs like Etoricoxib not including genotoxic contaminants like 5-Chloropyridine-2-amine. Regulatory bodies such as the International Council for Harmonisation (ICH) have established standards that require strict management of GTIs, with detection thresholds often being mandated at parts per billion (ppb) levels. A dependable and effective way to monitor and manage GTI levels may be provided by incorporating this study's LC-MS/MS approach into the usual quality control processes for Etoricoxib. Figure 2 shows the chromatographic separation that was accomplished in this investigation; the retention time for 5-Chloropyridine-2-amine was 3.2 minutes, proving that the procedure was accurate and could separate this impurity from the matrix (Smith et al., 2021).

While the benefits are obvious, the research also note a few possible drawbacks. The expensive initial investment and ongoing professional knowledge required to run and maintain an LC-MS/MS system is a major obstacle to its widespread deployment. Particularly in less well-funded areas or with smaller facilities, these issues may make this approach inaccessible. The investment in such sophisticated analytical methods is, however, warranted since identifying GTIs is crucial to guaranteeing medication safety. Alternatives or improvements to the existing approach that are more cost-effective while yet maintaining high sensitivity might be the subject of future study. Furthermore, by modifying this technique to identify different active pharmaceutical ingredients (APIs) and contaminants, its potential uses in the pharmaceutical sector could be increased, leading to more widespread use in GTI detection standards and improved protection of public health (Raman et al., 2022; Verma et al., 2021).

5. Conclusion

This work fills a significant gap in pharmaceutical quality control by presenting an efficient and reliable LC-MS/MS approach for the trace-level identification of 5-Chloropyridine-2-amine in Etoricoxib. Ensuring the safety of Etoricoxib and comparable medications has never been easier than with this approach, because to its high sensitivity, specificity, and accuracy, which match the demanding standards of regulatory bodies. Pharmaceutical companies may protect patient health and stay in line with international regulations by using this technology to reliably monitor and regulate genotoxic impurity levels. This strategy has been validated and used successfully, demonstrating its significance as an integral part of medication quality assurance systems.

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