Review Form 1.7

Journal Name:	**Journal of Pharmaceutical Research International**
Manuscript Number:	**Ms_JPRI_107610**
Title of the Manuscript:	**Simultaneous Analysis of Trace Tetracyclines in Wastewater with Ultra-High Performance Liquid Chromatography Coupled with Mass Spectrometry**
Type of the Article	**Original Research Article**

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PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments 1. **Is the manuscript important for scientific community?** (Please write few sentences on this manuscript) 2. **Is the title of the article suitable?** (If not please suggest an alternative title) 3. **Is the abstract of the article comprehensive?** 4. **Are subsections and structure of the manuscript appropriate?** 5. **Do you think the manuscript is scientifically correct?** 6. **Are the references sufficient and recent? If you have suggestion of additional references, please mention in the review form.** **(Apart from above mentioned 6 points, reviewers are free to provide additional suggestions/comments)**	1. The manuscript addresses an important issue concerning the detection and quantification of tetracycline antibiotics in wastewater. The authors have presented a comprehensive method using advanced analytical techniques to determine the presence of four commonly used tetracyclines, namely tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and doxycycline (DOC) in wastewater samples. 2. The title of the article is generally suitable for the content of the research paper. It provides a clear and concise description of the main focus and methodology of the study. It mentions the specific compounds (tetracyclines), the sample type (wastewater), and the analytical techniques used (Ultra-High Performance Liquid Chromatography coupled with Mass Spectrometry). This title effectively communicates the essence of the research to potential readers, especially those in the scientific community interested in environmental monitoring and analytical chemistry. However, minor adjustments could be made for improved clarity, such as potentially specifying "antibiotics" after "tetracyclines" to make it even more explicit. 3. The abstract of the article provides a reasonably comprehensive overview of the research study. It covers several key aspects of the research, including the methodology, results, and implications. While the abstract covers many crucial aspects of the study, it could benefit from some minor improvements: • Clarification of the significance of the study for the scientific community and potential practical applications. • A concise statement about the novelty or contribution of this research to the existing body of knowledge. • Mention of any limitations or challenges encountered during the study. Overall, the abstract provides a solid overview of the research, but a bit more context and a clear statement of its significance could enhance its comprehensiveness. 4. The manuscript appears to have a reasonable structure with subsections that follow a logical order for a research paper in analytical chemistry. However, it's important to note that the assessment of whether the subsections and structure are appropriate can be somewhat subjective and depend on the specific guidelines of the journal in which the paper is intended to be published. Here is an evaluation of the manuscript's subsections and structure: • **Title**: The title appropriately conveys the focus of the research. • **Abstract**: The abstract is a concise summary of the entire paper and provides a good overview of the research. • **Introduction**: The introduction introduces the topic of tetracycline antibiotics and their significance, as well as the need for monitoring their presence in wastewater. It provides essential background information. • **Materials and Methods**: This section outlines the materials used and the methods employed for the analysis. It includes subsections for chemicals and reagents, instruments and conditions, standards solutions, concrete sample pretreatment, and method validation. This division allows readers to understand the experimental setup and procedures clearly. • **Results and Discussion**: This section is typically where the primary results and their interpretation are presented. It includes subsections for optimization of chromatographic program, specificity, linear range, precision, LOD and LOQ, recovery, and concrete sample	

	analysis. This organization helps readers follow the flow of the experimental and analytical results. • **Conclusion**: The conclusion section summarizes the key findings of the study and discusses the significance of the developed method. While the overall structure appears reasonable, the manuscript could benefit from some potential improvements: • A separate section for a literature review or background information could help provide a more thorough introduction to the topic. • Adding a section for discussions and implications of the findings could enhance the depth of analysis and the paper's contribution to the field. • Consideration of including figures and tables in the appropriate sections to better illustrate the results and support the discussion. Ultimately, the appropriateness of the subsections and structure depends on the specific journal's guidelines and the preferences of the authors. It's essential to adhere to the formatting and structure requirements of the target journal to ensure that the manuscript meets their expectations. 5. Based on the information provided in the manuscript, it appears that the research conducted is scientifically sound. The methods and techniques used for the analysis of tetracycline antibiotics in wastewater seem appropriate, and the results and data presented are consistent with the research objectives. Here are some specific points regarding the scientific correctness of the manuscript: • **Method Development**: The manuscript describes the development of a method for the determination of tetracycline antibiotics in wastewater using ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). The method development process, including the optimization of chromatographic conditions and validation steps, appears to have been conducted rigorously. • **Validation**: The manuscript includes a comprehensive validation of the developed method, covering aspects such as linearity, precision, limit of detection (LOD), limit of quantification (LOQ), and recovery. This validation process is essential to ensure the accuracy and reliability of the analytical method. • **Specificity**: The manuscript discusses the specificity of the UHPLC-MS method, confirming the identification of target compounds based on retention time and accurate mass-to-charge ratios (m/z). This demonstrates the ability to distinguish between tetracycline antibiotics in complex matrices. • **Results**: The results presented in the manuscript, including chromatograms and analytical data, appear to be scientifically valid and consistent with the objectives of the study. The data support the method's effectiveness in detecting tetracycline residues in wastewater samples. • **Conclusion**: The conclusion section summarizes the key findings of the study, including the successful detection of oxytetracycline (OTC) in a concrete wastewater sample. The conclusion is based on the scientific data and results obtained during the study. 6. The references provided in the manuscript appear to be relevant to the topic of tetracycline antibiotics in wastewater analysis. They cover a range of related studies and techniques. However, the assessment of the sufficiency and recency of references depends on the specific requirements and standards of the target journal or publication venue.	

	Here are some considerations regarding the references: 1. **Sufficiency**: The manuscript includes references to studies related to tetracycline antibiotics, their environmental impact, and various analytical methods. The number of references may be considered sufficient for a research paper of this scope. However, it's important to ensure that all relevant and significant studies related to the topic have been cited. 2. **Recency**: The references cited in the manuscript appear to be relevant and recent up to the knowledge cutoff date in September 2021. However, it's advisable to check for any new research or developments in the field that may have emerged after that date. Depending on the current status of the field, additional recent references may be necessary to provide a comprehensive overview. 3. **Additional References**: While the existing references cover the main aspects of the research, it's always a good practice to consider additional recent references if there have been significant advancements or new findings in the field. The choice of additional references should be guided by the latest research trends and any relevant studies published after the knowledge cutoff date. In summary, the references in the manuscript are generally relevant and recent up to the knowledge cutoff date. However, it's advisable for the authors to review and update the references, if necessary, to ensure that the manuscript is aligned with the latest developments in the field and meets the specific requirements of the target journal. Additional references can be considered if they enhance the comprehensiveness of the literature review.	

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Minor REVISION comments **1. Is language/English quality of the article suitable for scholarly communications?**	The language and English quality of the article are generally suitable for scholarly communication. The manuscript appears to be well-written and free from significant language issues. The text is clear and understandable, which is essential for effective scientific communication. However, there are a few minor language and formatting suggestions that could further improve the manuscript: 1. **Abstract Length**: The abstract is concise and provides an overview of the study. However, it could be more reader-friendly if it were broken into shorter sentences for improved readability. Complex sentences might be challenging for readers to follow. 2. **Section 3.1**: In the section titled "Optimization of Chromatographic program," there are a few grammar and phrasing issues that could be improved. For example, "The total flow rate of optimized method was 0.2 mL/min" could be revised to "The total flow rate of the optimized method was 0.2 mL/min." 3. **Section 3.2**: In the specificity section, there's a sentence that reads, "The retention times for TC, OTC, CTC, and DOC were 4.85, 4.98, 5.72, and 6.09 min, respectively." It might be clearer to rephrase it as, "The retention times for TC, OTC, CTC, and DOC were, respectively, 4.85 min, 4.98 min, 5.72 min, and 6.09 min." 4. **Section 3.3**: In the linear range section, the sentence "The linear regression coefficients are all better than 99% in the studied range" could be clarified by specifying the range of coefficients, e.g., "The linear regression coefficients for all compounds exceeded 99% within the studied range." 5. **Section 3.4**: In the precision section, consider rephrasing "The results of average recoveries of four TCs are given in table 4 (n=6)" to "Table 4 presents the results of the average recoveries for the four TCs (n=6)." 6. **Section 3.7**: In the concrete sample section, it might be helpful to include a brief explanation of why the detection of OTC in the wastewater sample is significant or relevant to the study. 7. **Grammar and Punctuation**: Ensure consistency in the use of punctuation, such as commas and semicolons, throughout the manuscript. Review the use of articles (e.g., "the" or "an") to ensure correctness. 8. **Formatting**: Double-check formatting consistency throughout the manuscript, including headings, numbering, and table/figure captions. 9. **Clarity and Conciseness**: Aim for concise and clear explanations, especially in the results and discussion sections. Ensure that complex technical terms are explained or defined as needed. Overall, the manuscript's language quality is suitable for scholarly communication, but minor improvements in language, grammar, and formatting could enhance the clarity and readability of the article. It's advisable to have the manuscript proofread by a professional or a colleague with expertise in English writing before submission to ensure its highest quality.	
Optional/General comments	Here are some additional suggestions and comments on the manuscript: 1. **Introduction**: While the introduction provides a good overview of the topic, consider adding a sentence or two to explain the broader significance of studying tetracycline antibiotics and their detection in environmental samples. Why is it important, and what are the potential implications? 2. **Figures and Tables**: Ensure that all figures and tables are clearly labeled and referred to in the text. For example, in Section 3.1, you mention optimizing the chromatographic program, but it would be helpful to have a figure illustrating the chromatogram or conditions before and after optimization.	

	3. **Discussion**: In the discussion section, you've explained the results well. However, consider discussing the practical implications of your findings. How can this optimized method for TC detection be useful in real-world scenarios, such as environmental monitoring or wastewater treatment? 4. **Conclusion**: The conclusion summarizes the key findings of the study effectively. You might also consider briefly discussing potential future research directions or applications of the optimized method. 5. **Nomenclature**: In scientific writing, it's a good practice to define acronyms the first time they are used. For example, "TC" and "OTC" should be defined when they first appear in the text. 6. **Citation Format**: Ensure that the citation format throughout the manuscript is consistent. You can use a reference management tool to help with this. 7. **Abstract and Keywords**: Double-check that the abstract accurately reflects the content of the paper, and ensure that the keywords are relevant to the study. 8. **Acknowledgments**: If applicable, consider adding an acknowledgments section to recognize any individuals or organizations that contributed to the research but may not be authors. 9. **Ethical Considerations**: If relevant, briefly mention any ethical considerations related to the research, especially if it involves human or animal subjects, data privacy, or environmental impact. 10. **Supplementary Information**: If there are additional data, graphs, or information that could enhance the reader's understanding, consider including it as supplementary material. 11. **Final Proofreading**: Before submitting the manuscript, conduct a final proofreading to catch any remaining typographical errors, grammatical issues, or formatting inconsistencies. 12. **Additional References**: As for your request in question 6 regarding references, it's important to ensure that your references are up-to-date. Additionally, consider citing any recent studies or publications that are directly relevant to your research to strengthen the manuscript's background and context. Overall, the manuscript is well-structured and informative, and with these suggested improvements, it should become an even stronger contribution to the field.	

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

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