Author’s response to reviews

Title: Determination of Parecoxib and Valdecoxib in Rat Plasma by UPLC-MS/MS and its Application to Pharmacokinetics Studies

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Author’s response to reviews:

Dear Editor and reviewers,

We received the letter with pleasure on the required changes to our submitted manuscript (Submission-ID: PHAT-D-19-00201R1) and appreciated very much for offering us an opportunity to improve our work. Following the revision, we tried answering questions point by point. We made some changes and also highlighted them using the red font in our manuscript accordingly. Here we submit the revised manuscript for your reference and hope it may have an opportunity to appear in this prestigious journal.

Thanks again for your kindest attention. We look forward to hearing from you soon,

Sincerely yours,
Yijie Chen and Ledan Wang

Part 1: Reviewer Comments for Manuscript.

Reviewer 1

Due to growing use of parecoxib (PCX) alone and in combination with other drugs, the present study aimed to develop and validate a sample and convenient UPLC-MS/MS method to simultaneously quantify PCX and valdecoxib (VCX) in plasma samples. The authors argue that conventional high-performance liquid chromatographic methods used for the determination of PCX and VCX were have lower sensitivity and that there is a need for a more sensitive, specific, and straightforward UPLC-MS/MS method to determine PCX and VCX concentrations in plasma samples. These authors also
argue that current methods of sample preparation aimed at detecting PCX and its metabolite VCX in samples have disadvantages which render these methods both complex and time-consuming. These authors aim to improve the sample preparation process and sensitivity by combining by UPLC-MS/MS with one-step protein precipitation with acetonitrile. This effectiveness method was evaluated by analyzing rat plasma samples collected after the sublingual vein administration of 5 mg/kg PCX in rats. The authors conclude that their one step method improves upon the method of Jin et al. who used methanol to precipitate plasma protein with two-step centrifugations.

Q1: Unfortunately this reviewer does not view this technical methods paper as adding sufficiently to the basic pharmacology and toxicology literature to warrant publication in BMC PT. Recommend transfer to BMC research notes.

A1: Thanks for the reviewer’s comments on the current manuscript. This study aims to develop and validate a rapid, selective, and reproducible UPLC-MS/MS method for the simultaneous determination of PCX and VCX in rat plasma. As we know, the establishment of quantitative methodology is critical for pharmacokinetic research. Also, we retrieved some articles regarding pharmacokinetic study has already been published in BMC Pharmacology and Toxicology, that’s why we submitted our work to this journal that may provide as supplement for the library of pharmacokinetic studies on PCX and its metabolite VCX.


Reviewer 2

Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors.

Q1: Improve the language from native English speaker.

A1: Thanks for the reviewer’s comments on the current manuscript. A native English speaker has edited the revised manuscript, and we hope it would be more suitable for publication in this prestigious journal.

Q2: Provided the statistical analysis.
Thanks for the reviewer’s suggestion. For now, we added the statistical analysis by using DAS (Drug and Statistics) software (Version 3.0, Wenzhou Medical University, China). Furthermore, pharmacokinetics parameters of the PCX and VCX after sublingual vein administration of 5 mg/kg PCX in rats (n = 6) were displayed in Table 4.

Q3: Rewrite the introduction section.

We appreciate the reviewer to point out this problem. Following by reviewer’s concern, the introduction section has been rewritten in the revised manuscript. Please see on page 3.

Reviewer 3

I have nothing to comment. It is well organized work.

We appreciate the reviewer’s positive comments.

Reviewer 4

The manuscript by Chen et al. shows the Establishment of Quantitative Methodology in Parecoxib and Its Main Metabolite Valdecoxib Analysis and the Further Application of Its Pharmacokinetics in Rat.

Q1: The title of the article should be revised, and it's long.

A1: Thanks for the reviewer’s suggestion. Now that we already revised the title, please see on page 1.

Q2: At the end of the introduction the purpose is not well mentioned.

A2: We appreciate the reviewer to point out this problem. As the same part mentioned from the other reviewer, the introduction section has been rewritten in the revised manuscript. Please see on page 3.

Q3: The code of ethics is not mentioned in the Pharmacokinetic study section.

A3: Thanks for the reviewer’s concern about the code of ethics. We had written the ethical approval number in 2.6 section. Please see page 6, and we highlighted them using the red font in our manuscript as well.

Q4: How were the doses and times selected? Are they based on the literature or on epidemiologic/exposure studies?

A4: Thanks for the reviewer’s concern on the experimental design aspects. The recommended dose of PCX in clinic use is 40 mg intravenously or intramuscularly, following by 20 mg or 40 mg if the pain is not suppressed between 6 and 12 hours, but a total daily dose is no more than 80 mg. In our study, rats were given PCX via sublingual vein at a dose of 5 mg/kg, which was equivalent to 56 mg in the person of 70 kg body weight that just located in the range of 40-80 mg window. Actually, we performed the preliminary animal assays for optimizing the dose before our research. As a result, we found that the tmax of PCX and VCX were 0.1 and 0.8 h, respectively. We then chose three points close to Cmax, and
nine points picked around the eliminate the phase. Overall, the entire sampling period lasted five t1/2. Therefore, blood samples (0.3 mL) were collected from the tail vein into heparinized 1.5 mL polythene tubes regarding given time points as 0 (before administration), 0.083, 0.167, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after dosing. Also, some publications support our research design as below.


Q5: Write the names of the compounds in full in the tables.

A5: Thanks for the reviewer’s suggestion. For now, we had written the names of the compounds in full in the tables. Please check it in tables.

Q6: Significant in the tables are not mentioned.

A6: Thanks for the reviewer’s concern about the significant in the tables. This study aims to develop and validate a UPLC-MS/MS method for the simultaneous determination of parecoxib (PCX) and valdecoxib (VCX) levels in rat plasma. Since the whole study just want to determine the pharmacokinetics of PCX and VCX in rats, while it doesn’t implicate in inter-group comparisons. Based on this fact, it is unable to do statistical analysis in our study. To further confirmation, we listed a few of studies that did not add significant statistical analysis for their data as well.


Q7: The discussion of the article is not well written.

A7: We appreciate the reviewer to point out this problem. We have gone through the entire discussion part and rewrite some sentences, and we hope it would be more suitable for publication in this prestigious journal.

Q8: The English language needs to be revised.

A8: Thanks for the reviewer’s comments on the current manuscript. As the same requirements from the other reviewer, we have asked a native English speaker to help improve the copy editing.