Author’s response to reviews

Title: Nutrition assessment of Vitamin A and Vitamin D in northeast Chinese population based on SPE/UPLC/PDA

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Author’s response to reviews:

Dear Editor:

Thank you very much for the review of our manuscript (Manuscript Number BMC Nutrition - NUTN-D-17-00210 “Nutrition assessment of Vitamin A and Vitamin D in northeast Chinese population based-on SPE/UPLC/PDA”). We have read the comments of the editor and three reviewers carefully. It is undisputable that all these comments are very valuable and precise for us to revise our manuscript.

We have revised this manuscript point-to-point as detailed below:

I. Response to the issues raised by Reviewer 1

Reviewer 1: Wang et al. aimed to establish a reliable method for simultaneous determination of serum 25OHD3 and retinol, and assess the nutritional status of VA and VD among northern
Chinese population. In the current study, they established a new method, SPE/UPLC/PDA, which could simultaneously detect the serum levels of 25OHD3 and retinol. And using this new method, they tested serum VA and VD levels among elementary school students, middle school students and adults in Harbin. They found VD deficiency was common with the prevalence > 60% in all three age groups and the high prevalence of VA deficiency was only observed in the elementary school students. According to the authors, this method could spontaneously measure serum VA and VD levels with good precisions, and could process 100 samples within 12 hours.

Thanks very much for your comments.

Several concerns need to be addressed while it is considered for publication:

1. The biggest concern I have is how to approve the new method is reliable. I would suggest to randomly select participants from each group and used traditional method, e.g., HPLC/MS to measure the serum concentration of VA and VD and further compare them with the VA/VD concentrations tested by the new SPE/UPLC/PDA method.

Thank you very much for your valuable suggestion. LC-MS/MS has already been introduced as a simple, rapid, and robust reference method for the determination of steroid hormones, such as 25OHD3. Therefore, LC-MS/MS was used as the reference method to validate our established method. We have established a method of measuring the serum concentration of VA and VD using UPLS/Xevo™ TQ MSMS. We randomly selected 30 participants and used UPLS/Xevo™ TQ MSMS to measure the serum concentration of VA and VD and compared the results with the VA/VD concentrations from the new SPE/UPLC/PDA method. The method and comparison of results have been added in the revisions.

2. The new method developed in this study is the combination of SPE, UPLC and PDA. There might be not such combined method before, but I am wondering if the authors could provide evidence that these methods have been used before to test VA and VD or something else similar and showed reliable and accurate performance.

Thank you very much for your valuable suggestion. The combinations of HPLC/PDA and SPE/HPLC/MS have been used in others articles, these articles were cited in our revised revision.

13). Alvarez JC, De MP. Rapid and sensitive high-performance liquid chromatographic method for simultaneous determination of retinol, α-tocopherol, 25-hydroxyvitamin D_3 and 25-


3. Is there any published data on the prevalence of serum VA and VD in Harbin city? If so, is the probabilities of deficiency comparable to what was showed in this study?

Thanks very much for such a professional, accurate suggestion.

We have added the published data it in our revised article1, 2.


4. One typo on page 7 line 161, ".... 307 elementary school students were included in current students" should be edited to "the current study", right?

Thanks very much for such a professional, accurate suggestion. We have corrected it in our revised article.

5. In the abstract, all abbreviations, e.g., VA,VD, SPE, if were used for the first time, should be given the full terms.

Thanks very much for such a professional, accurate suggestion. We have corrected it in our revised article.
Reviewer 2: This study does not bear any true novelty in the field, as its rationale and methodology is flawed by the fundamental absence of covariates and a correlation/regression statistics with a further commonly used method or a gold standard to assay retinol and calcidiol in the recruited subjects.

Thank you very much for your valuable suggestion. LC-MS/MS has already been introduced as a simple, rapid, and robust reference method for the determination of steroid hormones, such as 25OHD3. Therefore, LC-MS/MS was used as the reference method to validate our established method. We have established a method of measuring the serum concentration of VA and VD using UPLS/Xevo™ TQ MSMS. We randomly selected 30 participants and used UPLS/Xevo™ TQ MSMS to measure the serum concentration of VA and VD and compared the results with the VA/VD concentrations from the new SPE/UPLC/PDA method. The method and comparison of results have been added in the revisions.

No ROC analysis was attempted, to assess of false positives...

Thank you for your advice. The main aim of our article was to describe the epidemiology of vitamin A and D status. Therefore, ROC analysis was not performed.

Furthermore, no dietary and metabolic information on the enrolled individuals have been addressed. The paper is completely unsuitable for publication in this form.

Thank you for your comment. Our purpose was to establish a rapid detection method and evaluate different vitamin levels in the Harbin region using this approach. During the design of the study, we did not record the dietary or metabolic information, unfortunately.

Therefore, we did not consider other parameters, such as diet and other metabolic markers and did not added in the revisions.

Minor:

Please, check and revise English language and typos in the text

Thank you very much for your valuable suggestion.

Reviewer 3: The present paper is well written and is interesting. I have some question that i would consider i the way the aim is described

Thanks very much for your comments.
1.- the present paper has two different aims. First is to describe a new technique to measure vitamin A and D that can be used instead of gold standard techniques. And the second hand we have the aim to describe the epidemiology of Vitamin A and D status.

If we have to answer the first research question that is validate the method i consider that it has a good intra assay validation however the inter assay validation seems not very clear to me. It say that they measure in serum samples creating standards adding Vitamin D and Vitamin A in different concentration. In my opinion if you want to validate a test you must have to compare to a gold standar measurement. In the way is written i agree that you reach an internal validation of the test but not sure if you can reach an external validation of the test.

Thank you very much for your valuable suggestion. LC-MS/MS has already been introduced as a simple, rapid, and robust reference method for the determination of steroid hormones, such as 25OHD3. Therefore, LC-MS/MS was used as the reference method to validate our established method. We have established a method of measuring the serum concentration of VA and VD using UPLS/Xevo™ TQ MSMS. We randomly selected 30 participants and used UPLS/Xevo™ TQ MSMS to measure the serum concentration of VA and VD and compared the results with the VA/VD concentrations from the new SPE/UPLC/PDA method. The method and comparison of results have been added in the revisions.

I think the second research question is answered properly.

Thanks very much for your comments.

I can not say if the conclusion of the abstract are correct because the study design is not performed to recommend or not a supplementation with vitamin A or D.

Thank you very much for your valuable suggestion.

Our purpose was to establish a rapid detection method and measure vitamin levels in the Harbin region with this established tool.
Therefore, we recommended in the conclusion that vitamin A supplementation should be considered for elementary school students and that vitamin D supplementation is highly recommended for all age groups in Harbin.

We have read all the suggestions of the two reviewers carefully and revised our manuscript point-to-point. Thanks very much for the three reviewers’ precious advices. Undoubtedly, these comments gave us much help on improving the quality and accuracy of our manuscript.

According to the reviewers’ comments, this manuscript has been edited by Edanz.

If it still needs to be revised, please don’t hesitate to tell us. We will be very grateful and appreciate to revise it until it is qualified for publication on your journal.

I’m looking forward to hearing from your good news.

Sincerely yours,

Wang Maoqing