Reviewer's report

Title: Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry with multivariate statistical analysis for exploring potential chemical markers to distinguish between raw and processed Rheum palmatum

Version: 5
Date: 10 June 2014
Reviewer: Ammar Saleem

Reviewer's report:

Response to Reviewer 1

Major Compulsory Revisions:

3. Are the data sound?

Fig 2. this overlayed chromatogram is not very informative. Fig 2s is not extracted ion chromatograms but mass spectra with no assignment of the signals. Please replace fig 2 with extracted ion chromatograms of the compounds under study or assign signals of 2s.

Fig 3. Revise with improved font size.

-Revise with improved font size.

-If the authors applied pareto scaling to generate the pac plot it must be mentioned in the methods section and in the figure legend.

-Legends 1-5 most probably represent different preparations, please assign these numbers in the Sample Preparation section.

Fig 4B (trend plots are not readable).
Please adjust the font by applying correct parameters available in the report section of the MarkerLynx software.

Fig 5. Please adjust the font by applying correct parameters available in the report section of the MarkerLynx software.

Table 1. Molecular formula column can not have entries 'unidentified'. Please use elemental composition portion of the MarkerLynx software to generate molecular formula and then attempt to search them in Chemspider

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?

The conclusions are drawn on the basis of relative intensity of the signals. Since the ion intensities were not optimized for the markers by using combined flow analysis the applied QTOF conditions are very general and could be misleading. Absolute quantity of each analyte that represent the changes from raw to
processed material is warranted.

5. Are the discussion and conclusions well balanced and adequately supported by the data?

Chromatographic conditions of UPLC. During the optimization the PCA analysis is warranted to validate statistical approach. In many cases optimization of the ionization is preferable besides the separation. Authors do not report any MS optimization and hence the method be cannot be claimed to be novel (as mentioned in Conclusion section).

Table 2. Please include tentative identities of the discriminant signals

Table 3 and section, Preliminary study on processing mechanism.

Adding references does not help improve this table. The data is not your work. If the toxicology tests on raw and processed material were performed the experimental section should report the procedure.

Minor and Discretionary revisions:

6. Are limitations of the work clearly stated?

No

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?

Not clearly

8. Do the title and abstract accurately convey what has been found?

No

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests