Dear Editor,

Please find enclosed our revised manuscript entitled **“Speciation of chromium through aqueous two-phase extraction ofcomplexes of Cr(III) with pyridylazoresorcinol and Cr(VI) with diphenylcarbazyde”**, authored by **Tamara N. Simonova, Valentina A. Dubrovina, Andriy B. Vishnikin**

We included also a detailed and itemized response to the Referees’ comments.

We would like to thank the Referees for their thorough work and greatly appreciate their valuable suggestions. We fully agree with all of the comments and have corrected the manuscript accordingly.

Sincerely yours,

Andriy Vishnikin

# Detailed replies to the Referees’ comments

Reviewers' comments:
Reviewer #1:

**Comment:** The manuscript presents a speciation method for chromium species in aqueous samples by two-phase extraction systems using pyridylazoresorcinol chelating reagent for Cr(III) and diphenylcarbazide for Cr(VI). Two detectors were examined, a UV-Vis spectrophotometer (535/545 nm) and a Graphite Furnace AAS. The study was well conducted and critical factors affecting the extractions were investigated and optimized. The work can be accepted for publication after detailed responding to some important comments listed below. **Nevertheless, I have to mention that the analytical novelty is poor, since it makes use of rather known agents for chromium.**

**Response**: We want to attract your attention to the fact that we did not intended to find the new analytical forms for the separation and determination of chromium forms. The manuscript is devoted to the one of the first attempts to combine the green extraction method – aqueous two-phase extraction (ATPE) - with speciation analysis of metal forms. Consequently, it was reasonable to use as analytical reagents most reliable, widespread and approved reagents. In addition, the selectivity of the method for the determination of chromium(III) with pyridylazoresorcinol was significantly improved by using the method proposed in the present investigation.

**Comment:** 1. The authors must elaborate more on the text because the two described extraction procedures are not rapid and by no means simple. In addition very large quantities of salts are required for each determination and although there are commercially available in reasonable prices (consider that
analytical grade salts are required and more than 30 g are consumed for each
determination!!

**Response**: In accordance with criticism of reviewer, such characteristics of the proposed extraction procedure as “simple” or “rapid” are removed from abstract, conclusions and the text of the manuscript. It was additionally checked and shown that up to ten-fold reduction in volumes or quantities of reagents used is possible in the proposed procedures for the determination of chromium forms that is especially useful in connection with ET AAS detection.

**Comment:** 2. The authors should provide also spectra for the UV-Vis determination, in order to reveal the absence of interferences, since the two absorbance lines are close (535-545 nm) and the spectrum of each chelate is broad.

**Response**: Really, the spectra of both colored products formed in the reactions between chromium forms and analytical reagents used are close each to other. But as it is shown in the manuscript, chromium(III) does not react with diphenylcarbazide owing to the kinetic inertness. Therefore, even 150-fold excess of Cr(III) does not interfere to the determination of Cr(VI). In one`s turn, 450-fold excess of Cr(VI) can be separated from Cr(III) by using the proposed procedure. Hence, both chromium forms do not interfere to the determination one another in the ratios far from typical for natural waters. The spectra of both analytical forms are well known and we believe that there is no need to provide them.

**Comment:** 3. Please check the legend and text for Fig.1, to which complex refer to Cr(VI) or to Cr(III)?

**Response**: The legend to the Fig. 1 is correct. As well as product of the reaction between Cr(VI) and diphenylcarbazide is complex between Cr(III) and diphenylcarbazone exactly extraction yield of this compound was interconnected with extraction efficiency for Cr(VI).

**Comment:** 4. I noticed also in Table IV a measurement in Waste Water sample which is 0.32 ug/L although the authors already stated in the Abstract that the
“calibration graphs obtained … were linear over the concentration ranges
…. Corresponding concentration ranges determined by using ET AAS method
were from 1 to 10 μg L-1 for both chromium species.” Taking into account
the detection limit, I assume that the quantitation limit is something
between 0.7 to 1 μg L-1.So how this sample was measured??

**Response**: We are agree with this comment. Actually we were not able to determine such low Cr(VI) concentration and have used preliminary 10-fold evaporation of the sample. The appropriate changes are made in Table IV.

**Comment:** Correct some useless phrases like “heat-resistant beaker”, etc.

**Response**: We have improved the text of the manuscript.

**Comment:** In line 356 if possible check again with more references the information for hexavalent chromium “In some countries, the maximum permitted concentration is 20 mg L-1 [12]”.

**Response**: Really, there was a mistake. We have corrected the phrase to «California’s drinking water standard established a maximum contaminant level of 10 μg L-1 for Cr(VI) in drinking water.36 » and added corresponding reference.

Reviewer #2:

**Comment:** This paper is written clear and correctly, **but described methods of
chromium preconcentration and speciation in natural water samples have
already been applied and published, and in that way, so there is no novelty
in this work.**

**Response**: We have already responded to this comment in response to the first reviewer.

**Comment:** Introduction section should be supported by later references in order to make visible advantages and novelty of this work.

**Response**: The significance and novelty of the conducted investigation was emphasized by providing 8 additional references (№ 23-29, 34). The references are devoted to the using of salting-out effect in the present-day applications of ATPS systems, including salt-assisted liquid-liquid microextraction (SALLE) and salting-out homogeneous liquid-liquid extraction (SHLLE). Appropriate discussion has been included into the text of the manuscript in introduction section.

**Comment:** Experiment is well designed and described but there are corrections needed to be done mostly for paragraph Effect of foreign ions and application: The effect of some anions that are present in the environmental water samples, such as Cl-, NO3-, CO32-, SO42-, PO43- , should be taken in consideration.

**Response**: Following sentence was added to the section “Effect of foreign ions and application”.

The determination of Cr(III) and Cr(VI) is possible in the presence of high excess of anions: 5∙105-fold for NO3-, 2∙105-fold for SO42- and Cl-, 9∙104-fold for CO32-, and 4∙104-fold for PO43-.

**Comment:** Please complete Table IV with all results, SP and ET AAS.

**Response**: Table IV was completed with necessary dates.

**Comment:** The method validation should be improved by using some certified material.

**Response**: We are sorry, but the samples of certified reference materials for waters investigated in this work were unavailable.

**Comment:** According to all this, Conclusion section could be more specific and
offering something new in this sphere.

**Response**: Description of the novelty in Conclusion section was improved and supplemened.

**Comment:** In Reference paragraph there is mistake in Ref. 3., is not correctly written author name: correct is Z. Stasicka instead J. Stasicka.

**Response**: Corrected.