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| Description: vin_front_page_logo.gif | **Institute of Nuclear Sciences *VINČA***  **Department of Physical Chemistry 050**  Account no. 205-113593-70 Mike Alasa 12-14  tel (011) 6453 967 PO BOX 522  fax (011) 8066 434 11001 Belgrade, Serbia  VAT SR101877940 www.vinca.rs/050/ |

Subject: Response to Reviewers - Submission of the revised manuscript to Journal of the Serbian Chemical Society

Dear Editor,

I am submitting the revised manuscript version: Validation and uncertainity estimation of analitycal method for determination of phenolic compounds in concrete.

Thank you for giving us the opportunity to revise our manuscript. We have dealt with the referees’ comments carefully and made all required corrections, which are listed below in our reply to the referees. We hope that these corrections will contribute to the general improvement of the quality and relevance of the manuscript, and that this will be acceptable.

Respectfully,

Branislava G. Savić

Vinca Institute of Nuclear Sciences,

Department of Physical Chemistry

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Dear Branislava Goran Savić,

Thank you for the interest to publish your paper in the J. Serb. Chem. Soc..

Your manuscript entitled: "Validation and uncertainity estimation of analitycal method for determination of phenolic compounds in concrete" has been evaluated by referees, the reports of which are enclosed.

Please consider the suggested changes and upload (by logging in with your registered username and password to the JSCS OnLine article processing service http://www.shd-pub.org.rs) within 60 days (detailed instructions are available at <http://www.shd-pub.org.rs/index.php/JSCS/about/submissions#authorGuidelines>, heading MANUSCRIPT REVISION):

1. The corrected manuscript (as .doc file in "REVIEW" Section – Editor Decision - Upload author version option);

2. Your comments on the changes you made in the text as .doc or .pdf file in "add a supplementary file" option under "SUMMARY" Section of your submission (in the Title field please write "Response to Reviewers")

3. corrected Figure(s), Schemes..., if any (also as supplementary file(s) - Title: Corrected Fig.xxx, Type: Illistration(s)....). Please note that all files supplied in revision have to be presented to reviewers - hence, please mark "Present file to reviewers" during the upload of supplementary files.

The filling-in of the metadata is obligatory (please correct it in "SUMMARY" Section, option EDIT METADATA, if necessary)

Upon completion, send the Notify Editor email to the Sub-editor ("REVIEW" Section - Editor Decision option)

Prof. Slavica Ražić

Department of Analytical Chemistry, Faculty of Pharmacy, University of

Belgrade

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JSCS : : Analytical Chemistry Sub Editor

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**Reviewer A**. comment: Please indicate the page numbers for suggested corrections. Please, be as specific as possible if major correction by the author(s) is recommended! While this Manuscript undoubtedly has scientific merit, it is of the paramount importance that the following issues be addressed before it is considered for publication. Some of the point by point remarks are given below also as an incentive to the authors to rewrite and improve their Manuscript. I must therefore recommend that this Manuscript be rewritten and resubmitted. In my opinion, this manuscript should: be published after major revision and additional review. If manuscript is suitable for publishing, referees recommendation: Short communication.

Thank you for careful review of our work. We hope that these corrections will contribute to the general improvement of the quality and relevance of the manuscript, and that this will be acceptable for you. Please, find our reply to your comments below.

Reviewer A.

***1. Comment*/P.2 Line 37 - 43:**

No all phenolic compounds are toxic! Please define which of these compounds are toxic and why only nine of them were analyzed in this study.

Author reply: Thank you for your suggestion. Yes, not all phenols are toxic. We reformulated our statement in the manuscript (Line 37). However, we concentrated our study on toxic phenolic compounds. Also, we examined only nine compounds because the extraction process did not prove to be the best for nitrophenols present in the standard mixture Phenol-Mix 15.

***2. Comment*/P.3 Line 86:**

Phenol-Mix 15 contains 13 phenols?!! Where are the missing two phenols?

Author reply: The calibration standard "Phenol-Mix 15" contains 13 phenols. We attach the certificate to the *supplementary file* (name: Certificate Phenol-Mix 15).

***3. Comment*/P.3 Line 98:**

How much concentrated hydrochloric acid was added? It is important to realize if the extracting solvent during sonication is non-aqueous!

Author reply: 1 ml concentrated hydrochloric acid was added.

***4. Comment/P*.4 Line 102:**

Why the volume of 1 mL of acetic anhydride was added in this step?

Author reply: The acetic anhydride is added in high excess to provide a high yield of the reaction, preferably is 100%. This step of esterification has a direct effect on yield (recovery). Also, the acetic anhydride as a reagent greatly moves the balance of the reaction to the right and prevents transesterification with the possibly present impurities that could affect the course of the reaction.

***5. Comment*/P.4 Line 108: 109:**

In this sample preparation procedure an internal standard is missing!

Author reply: Thank you for your observation. The analysis was performed without use of an internal standard. In procedure with internal standard, sample preparation took long time, while the aim of the current study was to develop a rapid, accurate, precise, and low-cost analysis for the determination of phenol compound in concrete by GC-MS without using an internal standard. In order to avoid the use of an appropriate internal standard, a quality control chart was constructed and the internal quality sample was analyzed after five different runs External calibration curves were used as quantification techniques. In general, the use of an internal standard in the analysis is commonly used to correct random errors from the injection repeatability and systematic errors from the instrumental drift and the procedural errors. However, as defined in the review by Hewavitharana (2009)\*, the internal standard can become a foe “if the analyst is unaware of the linear range of the internal standard and uses a concentration that is outside the linear range, a severe loss of accuracy will occur”. Furthermore, the appropriate use of the internal standard suggests that all samples must be spiked with the appropriate content of the internal standard during the first step of the preparation step, meaning the sample weighing. In this step, usually few microliters are spiked in some grams, and for this reason the accuracy of the method depends on the homogenization of the sample mixture. The whole procedure is time- and cost-consuming and for this reason, in the current work, no internal standard was used with regard to special precautions.

\**A. K. Hewavitharana, Critical Reviews in Analytical Chemistry,* ***34:4*** *(2009) 272-275. (*[*https://doi.org/10.1080/10408340903001201*](https://doi.org/10.1080/10408340903001201)*)*

***6. Comment*/P.4 Line 118 - 119:**

Please explain how this column temerature program is selected since it containes extremely high temperature ramps!

Author reply: The high temperature ramps is in accordance with physical and chemical properties of phenolic compounds.

*\* J. M. Stellman, Phenols & Phenolic Compounds: Physical & Chemical Properties, Encyclopaedia of Occupational Health and Safety, International Labor Organization, Geneva, 2011*

***7. Comment*/P.7 Line 187:**

The peaks in Fig.2. is not correctly labelled.

Author reply: Thank you for your suggestion. It has been corrected.

***8. Comment*/P.7 Line 199:**

The linear range was not estimated! How the range (from 0.01 to 7.5 mg kg-1) is estimated?

Author reply: A linear range from 0.05 to 1.0 mg L-1 was estimed which corresponds to 0.01 to 7.5 mg kg-1 of the solid sample.

***9. Comment*/P.8 Line 206:**

The terms "Accuracy" is confused with the term "Trueness"!

Author reply: Thank you for your observation. Measurement ‘accuracy’ expresses the closeness of a single result to a reference value. Accuracy is, therefore, normally studied as two components: ‘trueness’ and ‘precision’ (\*Eurachem). Dueto absence of the certified material containing the analyte in con-crete’s matrix. We make a practical assessment of the trueness (This assessment is normally expressed quantitatively in terms of ‘bias’). Trueness was determined by recovery experiments using spiked samples.

It has been corrected in manuscript.

*\*Eurachem - The Fitness for Purpose of Analytical Methods,* [*https://www.eurachem.org/images/stories/Guides/pdf/MV\_guide\_2nd\_ed\_EN.pdf*](https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf)

***10. Comment*/P.10 Line 262:**

No details about uncertainity calculation is given. Please include details, so it could be reprocessed. The major problem in this manuscrript is the uncertainity values in table III ! For instance, the estimated expanded uncertainity of 0.95 is less then the individual uncertainity comming only from the recovery component (Table II) !

Author reply: Thank you for your suggestion. Yes, there are no enough details for calculation to be reprocessed - the parameters which are required for the uncertainty calculation are not given for all nine phenolic compounds (because we thought that in that case there would be too many tables). Instead of that, the paper contained the required parameters only for one phenol compound (Table II) as an example.

After revision, the required parameters for uncertainty calculation for the eight other phenolic compounds (together with the results) are now involved in *supplementary file* (please find attached Supplementary material) in Tables I-XVI.

Also, we had omitted to present the chosen number of histories in Monte Carlo simulation. Several sentences about the choice of the number of histories in Monte Carlo simulation have been added in line 262 (The last parameter we need to define is the number of histories M in Monte Carlo calculations. According to the ''Evaluation of measurement data – Supplement 1 the ''Guide to the expression of uncertainty in measurement'' – Propagation of distribution using a Monte Carlo method'' 22 this number can be chosen a priory in the following way:

 where p is a selected coverage probability. Therefore, when the coverage probability is 95% (as in our case) M should be at least higher than 2·105. Since a larger number of histories M causes better convergence of result we chose M to be 5·106).

So, now all uncertainty calculations can be reprocessed.

Considering the uncertainty values in Table III, there was probably a misunderstanding. We have repeated our calculations by using directions from GUM guide, and also checked the results with Box-Muller’s method, and we didn’t find any contradiction.

**Reviewer B.** comment:

Please indicate the page numbers for suggested corrections. Please, be as specific as possible if major correction by the author(s) is recommended!: Pp. 1, 5, 7, 8, 10. In my opinion, this manuscript should: be published after major revision and additional review. If manuscript is suitable for publishing, referees recommendation: Original scientific paper.

Thank you for the careful review of our work. We have accepted most of your remarks. We performed a reorganization of the manuscript in order to improve transparency of the results, provide better demonstration of the idea and contribute to the better quality of the manuscript. Please find a list of replies to the specific comments below.

Reviewer B.

***1. Comment*/P.1 Line 27:**

The keywords are not adequate to the content of the paper. I propose to replace ‘building material’ by ‘concrete’; to add ‘phenolic compounds’, ‘method validation’, and ‘measurement uncertainty’.

Author reply: The instructions for preparing the manuscript write: "Do not use words appearing in the manuscript title", so we decided on these key words.

***2. Comment*/P.5 Line 137 and further:**

Variables should be shown using Italic font.

Author reply: Thank you. It has been corrected in manuscript.

***3. Comment*/P.7 Lines 196-199:**

In Table 1 correlation is characterized by R (is it not correlation coefficient r in line 199?). Since all R > 0.99, why r < 0.99?

Author reply: Thank you, it was a typos mistake and is corrected. It has been corrected in manuscript.

***4. Comment*/P.8 Line 207:**

Accuracy is not recovery. For characterization of measurement accuracy (VIM, clause 2.13) the ‘true’ value is necessary – a theoretical concept. Trueness (VIM, clause 2.14) is characterized using the practically available reference quantity value. See ISO 1725 “Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions”. When recovery is only the source of the bias (a deviation of a test result from the reference value), this hould be commented/discussed/explained or mentioned as minimum. In particular, there are a number of concrete certified reference materials, and it is not clear why they were not used for the trueness evaluation. Recovery of spikes is a poor way for trueness characterization, since the behavior of the analytes (phenolic compounds in a concrete sample) in the extraction may be different than of the same compounds added to the same sample for spiking. Moreover, there are a number of other bias sources in the measurement process, e.g. calibration with a perfect linearity doesn’t mean the bias absence, etc.

Author reply: Thank you for your observation. Measurement ‘accuracy’ expresses the closeness of a single result to a reference value. Accuracy is, therefore, normally studied as two components: ‘trueness’ and ‘precision’. /citat eurochem. Dueto absence of the certified material containing the analyte in con-crete’s matrix. We make a practical assessment of the trueness (This assessment is normally expressed quantitatively in terms of ‘bias’). Trueness was determined by recovery experiments using spiked samples.

It has been corrected in manuscript, "trueness" instead of "accuracy".

***5. Comment*/P.10 Lines 267-268:**

When the expanded uncertainty by GUM in Table 3 is understandable (about 27% of the measured analyte concentration), the Monte Carlo estimate shows that the uncertainty may be larger than the measured value. In such a case it is impossible to know at all, is the phenolic compound in the concrete or absent. That is when pdfs shown in Fig. 4 are very close, very similar. Probably the authors should check again their calculations and describe them in more details. Therefore, conclusions are also under question. Note, the authors are not the first who compare the law of propagation of uncertainty and Monte Carlo method. There are a number of publication on the topic. See, for example, a paper by M. Sega, F. Pennecchi, S. Rinaldi, F. Rolle “Uncertainty evaluation for the quantification of law masses of benzo[a]pyrene: comparison between the law of propagation of uncertainty and Monte Carlo method”, Analytica Chimica Acta 920 (2016) 10-17. Thus, a minimal review is desirable before the own contribution.

Author reply: When we talk about GUM method, the expanded uncertainty for a level of confidence of 95% is about 27% of the measured analyte concentration. On the other hand, according to the ''Evaluation of measurement data – Supplement 1 the ''Guide to the expression of uncertainty in measurement'' – Propagation of distribution using a Monte Carlo method''22 the following parameters should be presented: the estimation of the output quantity, the chosen coverage probability, the endpoints which represent the limits of the shortest-length of the chosen coverage probability, and the estimation of the PDF.

Therefore, a value from Table III 4.94 mg kg-1 is not expanded uncertainty, but the high endpoint of the shortest-length 95% confidence interval. Since the low endpoint is 2.39 mg kg-1 it means that it is possible to estimate whether the phenolic compound is present in the concrete or not.

Since we understand that data from the table can easily be misunderstood, we have modified some explanations in paper at line 264 (The results for eight other compounds are presented in Tables II, IV, VI, VIII, X, XII, XIV, XVI in Supplementary material. In these tables, the following parameters obtained by Monte Carlo simulation-low endpoint for 95% and high endpoint for 95%, represent the low limit and the high limit of the shortest-length 95% confidence interval, respectively. It could be seen that for all compounds, Monte Carlo approach predicts a larger spread of data around a central value. For example, from Table III we can notice that one half of the shortest interval is 1.29 mg kg-1 compared to the expanded uncertainty of 0.95 mg kg-1.) and at line 272 (i.e. the resulting PDFs from Monte Carlo simulations are skewed to the right and their coverage intervals are larger than coverage intervals obtained by GUM approach).

At the end, we have included a short review about previous publications at the line 76 (The Monte Carlo approach was introduced in the Supplement 1 to the GUM - "Propagation of distributions using a Monte Carlo method" by the Joint Committee for Guides in Metrology in 2008 22. Since then, the newly initiated method has found wide applications. Further, some authors showed advantages and/or necessity of Monte Carlo method for evaluating measurement uncertainty by comparison of Monte Carlo and GUM approaches in different areas: chemistry23,24 pharmacy25, geodesy26, spectrometry27, biomedical science28 etc.).

23. M. Sega, F. Pennecchi, S. Rinaldi, F. Rolle, Analytica Chimica Acta 920 (2016) 10 (https://doi.org/10.1016/j.aca.2016.03.032)

24. D. Theodorou, L. Meligotsidou, S. Karavoltsos, A. Burnetas, M. Dassenakis, M. Scoullos, Talanta 83 (2011) 1568 (https://doi.org/10.1016/j.talanta.2010.11.059)

25. A. M. Saviano, F. R. Lourenco, Measurement 46 (2013) 3924 (https://doi.org/10.1016/j.measurement.2013.08.005)

26. W. Niemeier, D. Tengen, J. Applied Geodesy 11 (2017) 67 (https://doi.org/10.1515/jag-2016-0017)

27. O. Sima, M. C. Lepy, Applied Radiation and Isotopes 109 (2016) 493 (https://doi.org/10.1016/j.apradiso.2015.11.097)

28. A. Chen, C. Chen, Measurement 87 (2016) 27 (https://doi.org/10.1016/j.measurement.2016.03.007)

References have been added in the manuscript.