Dear Editor and Reviewers,

First of all, the authors are grateful and appreciative for the Editor’s and Referees’ effort and feedback to improve the quality of paper. We answer the positive comments about our concerned problems by reviewers and we are encouraged by their kind and insightful suggestions. In the revised version, we thank for the reviewers’ constructive comments and we acknowledge their valuable suggestions. There may be still some points which may require further clarifications, so we are ready to make our best. Any feedback regarding with the manuscript will be grateful. The manuscript has been resubmitted to your journal and we look forward to take your positive response.

 Our manuscript (8607-48417-2-SM) titled *“Determination of tramadol in pharmaceutical forms and urine samples using a boron-doped diamond electrode”* is based on the development of a new electrochemical methodology for the determination of tramadol. The modified items have been highlighted in the Revised Version of the manuscript by using highlighted colored text. We have addressed all the issues indicated in the review reports and believed that the revised version of the manuscript. Our reply is arranged in Q's and A's point by point. They are shown as follows.

1. **Reply to Reviewer #A comments**

**Q1.** Experimental, lines119-120: Procedure and experimental parameters for CV measurements are missing and should be added.

**A1.**These parameters are already given in the "Cyclic voltammetric behavior of TRH on the BDD electrode" section. Therefore, there is no need to repeat this data in this section.

**Q2.**Page 6, line 166: Authors can use the slope of the linear dependence log I = f(log v) to verify the adsorption nature of the oxidation process.

**A2**. The related equation has been added to the text.

**Q3.** Page 6, lines 181-183: The slope of Ep = f(pH) of 0.215V/pH is discussable regarding to number of protons involved. To clarify this, the effect of pH on CV, rather than SWV, should be analyzed.

**A3.** The slope of Ep = f(pH) is given in the relevant section as follows:

The slope was found to be 0.0215 V per pH unit which indicated that the numbers of electron and proton taking part in the electrode reaction are unequal. The previous paper has been cited.

On the other side, as you have mentioned, the pH optimization in electroanalytical methods is mostly investigated by the cyclic voltammetry technique. However, there are many studies such as https://doi.org/10.1007/s12161-019-01486-8,http://jes.ecsdl.org/content/166/12/B933, http://dx.doi.org/10.1098/rsos.170324, etc. in pH optimizations that are examined by using pulse methods such as square wave and differential pulse. In our study, we prefer the square wave method to produce a faster and more sensitive response at lower analyte concentration. Moreover, the analysis will also be more economical because less analytes are consumed.

**Q4.** Page 7, line 202: Some data should be included in the paragraph “Effect of accumulated time and accumulated potential” to support the choice of optimum Eacc and tacc values.

**A4.** The related information has been added to the text.

**Q5.** Page 8, Fig. 4 and lines 248-250: Authors wrote that above the SDS concentration of 8x10-4 mol L-1, a very small change in signal was remarked, but it can not be seen in the Fig.4. Therefore, please correct the Figure 4- Inset.

**A5.** Thank you for your attention and valuable contributions. Figure 4 and inset have been rearranged.

**Q6.** Page 12, lines 364-368: Voltammograms of important compounds AA, DOP and especially UA, should be presented in the figure as the proof of the statement (lines 366-368), and an explanation of the additional peak at +1,00V in Fig.6.

**A6.** We would like to thank you for the suggestion of our article for the improvement of the quality. In line with your suggestions, the relevant figure is added as proof of our statement between lines 366 and 368. The explanation has been done in the text.

**Q7.** Page 13, Fig. 6: The complete voltammogram of the urine sample should be added dashed line in the potential ranged +1.45V - +1.65V is not informative enough.

**A7**. Thank you for your valuable comments and suggestions. The corresponding voltammogram of the urine sample (from +0.40V to 1.65V) was added as figure 7B.

**Q8.** Literature, pages 14-15: Additional references concerning the oxidation mechanism of tramadol should be included.

**A8.** In accordance with your suggestion, the relevant references on the oxidation mechanism of tramadol(M. Soleimani, M. G. Afshar, A. Shafaat, G. A. Crespo, Electroanalysis 25 (2013) 1159 and E. Mynttinen, N. Wester, T. Lilius, E. Kalso, J. Koskinen, T. Laurila, *Electrochim. Acta* **295** (2019) 347) added to the references. Also, it was highlighted in color in the bibliography (22nd and 23nd  references).

**Q9.** Please add concentrations in molL-1 everywhere in the text where only mg mL-1 is given.

**A9.** Since tramadol concentration in the drug sample was given in mg mL-1, we chose this concentration for comparison. In some places, the SI unit is given in parenthesis for comparison with other studies.

**Q10.** Some technical errors are highlighted in the uploaded PDF file of the manuscript.

**A10.** The technical errors highlighted in the uploaded PDF file of the article have been corrected and these corrected items are shown in the Revised Version of the article using highlighted colored text.

**Q11.** This study was based on tramadol oxidation peak present at highly
positive potential (+1.58V) in pH range 2-8 at BDDE. According to literature
tramadol is not electroactive at pHs less then 4; above that value a pH
dependent oxidation peak is observable at Ep =1.0 V at graphite electrodes.
[E.M.P.J. Garrido et al. J. Pharm. Biomed. Anal. 32 (2003) 975-981,
references 21, 23, and references within]. This new findings, and
differences with respect to the literature data, should be clearly pointed
out and compared in the Introduction part. Also the origin of the peak at
+1.58V should be explained in Results and discussion.

**A11.** We think the information (tramadol is not electroactive at pHs less then 4) is not true. Probably, the researcher have been used carbon based electrodes which don’t have large potential window. As you known, BDD electrodes have a large potential window and enable to work in even the most aggressive environments etc. The previous studies (A.M. Santos, F.C. Vicentini, L.C.S. Figueiredo-Filho, P.B. Deroco, O. Fatibello-Filho, Flow injection simultaneous determination of acetaminophen and tramadol in pharmaceutical and biological samples using multiple pulse amperometric detection with a boron-doped diamond electrode, Diam. Relat. Mater., 60 (2015), pp. 1-8) has been done at pH 2.0 and Elsi Mynttinen at al. (Simultaneous electrochemical detection of tramadol and O-desmethyltramadol with Nafion-coated tetrahedral amorphous carbon electrode, Electrochimica Acta 295 (2019) 347-353) have been shown TRH oxidation peak at pH 3.

**Q12.**  In this work the urine samples were used without any pretreatment.
Usually, some pretreatment like protein precipitation by centrifugation
followed by filtration through membrane is used. The complete voltammogram
of the urine sample should be included in the text as a proof that no
pre-treatment is needed, together with some explanation and comments.

**A12.** Thank you for your valuable comments and suggestions. The corresponding voltammogram of the urine sample (from +0.40V to 1.65V) was added next to figure 7. The paragraph has been added to the text. “The related voltammograms of the urine sample by standard addition method are depicted in Fig. 7A. It can be concluded that an appeared oxidation peak at about +1.55 V is due to the TRH oxidation since its peak current increased after each TRH standard addition. In the absence of TRH, there were no detectable oxidation peaks in the working potential range where the analytical peak observed (Fig. 7B). On the other hand, an unknown oxidation peak at about +1.00 V was observed in blank urine samples, which could be due to the oxidation of uric acid (UA) 28,29.”

1. **Reply to Reviewer #B comments**

**Q1.** English should be improved on some places (for example lines 78-81) The authors describe determination of tramadol in pharmaceutical forms and urine samples by square wave adsorptive stripping voltammetry using a boron-doped diamond electrode. The manuscript contains complete applicability examination of the electrode and method through electrochemical studies by cyclic voltammetry and SW-AdSV, effect of supporting electrolyte, pH of the solution, effect of the adsorption time and potential, effect of anionic surfactant for improving response, range of applicability (LOD, LOQ), interfering compounds and applicability in real samples. The results are presented and discussed properly and compared with literature data available. Thus I recommend acceptance of this manuscript

with minor revision. The authors should improve English, for example the sentence: To the best of our knowledge, it was not found any study on the electrochemical behaviors and quantitative analysis of TRH using unmodified BDD electrodes except for the flow injection analysis method with multi-pulse amperometric detection using cathodically pretreated BDD

electrode should be “To the best of our knowledge, any study on the electrochemical behavior and quantitative analysis of TRH using unmodified BDD electrodes has not been found so far except for the flow injection analysis method with multi-pulse amperometric detection using cathodically pretreated BDD electrode.

**A1.** The relevant sentence has been rearranged in the text.

To the best of our knowledge, any study on the electrochemical behavior and quantitative analysis of TRH using unmodified BDD electrodes has not been found so far except for the two papers which perform simultaneous electroanalytical determination of acetaminophen and TRH using a BDD electrode19,20.

**Q2.** support electrolyte should be supporting electrolyte, etc..

**A2.** The entire text was reviewed and the places that refer to "support electrolyte" were replaced by "supporting electrolyte".

1. **Reply to Reviewer #C comments**

**Q1.** Please specify concentration expressions in mol L-1 instead of mol L-1 (for instance on p1 line 21, 8×10-4)

**A1.** The article was thoroughly reviewed. Spelling errors corrected.

**Q2.** Please place a gap space on page 2, line 21.

**A2.** Throughout the whole text, missing and extra spaces have been checked according to journal rules and the necessary corrections have been made in the direction of your suggestions.

**Q3.** p3, line 72: This paper should be cited:

S. Pysarevska, L. Dubenska, S. Plotycya, Ľ. Švorc: A state-of-the-art approach for facile and reliable determination of benzocaine in pharmaceuticals and biological samples based on the use of miniaturized boron-doped diamond electrochemical sensor; Sens. Actuators B 270, 9-17, 2018.

**A3.** The relevant article proposed by the referee C was cited. The related article is also marked as color in the references section.

**Q4.** Did the authors check the stability of the stock solution of analyte? e.g. by spectrophotometry

**A4.** No, we prepared the stock solution of TRH day by day.

**Q5.** Were these experiments undertaken in approval with respective law in Turkey? e.g. with the informed consent obtained from the volunteer prior to the voltammetric experiments.

**A5.** Yes. According to respective laws, the volunteer consent form was approved by the volunteer before the voltammetric experiments.

**Q6.** Page 5,line 150, gap should be removed.

**A6.** All spelling errors and gaps in the text were examined and corrected.

**Q7.** Page 6, line 166: Herein, for total confirmation of adsorption-controlled electrode process on the working electrode, the logarithmic analysis "log Ip vs. log v" should be carried out.

**A7.** The related equation has been added to the text.

**Q8.** How did it look like in pH higher than 8? e.g. at pH 9-12. Can the authors explain it?

**A8.** The peak current intensity at pH 8 decreased due to the formation of a second oxidation peak at about 1.70V. This decrease increased further in pH 9. Therefore, pHs after pH8 were not given in Fig 3.

**Q9.** Page 6, line 187: The term "supporting electrolyte" should be used instead of "support electrolyte".

**A9.** The entire text was reviewed and the places that refer to "support electrolyte" were replaced by "supporting electrolyte".

**Q10.** In figure 3: Use TRH instead of tramadol.

**A10.** Except for the first mention in the text, the abbreviation TRH was used instead of tramadol in all other parts of the text.

**Q11.** Did the authors perform also DPV measurements or only SWV?

**A11.** We performed the experiments only with the SWV method with the assumption that the speed and sensitivity of the analyzes were better. No study was performed to compare pulse techniques. Therefore, we did not use DPV technique in our study.

**Q12.**Page8, line 226: Use “evaluate of” instead of “evaluated”.

**A12.** The necessary correction has been made.

**Q13.** Page 8, line 251: Use 2.5 instead of 2.53.

**A13.** The proposed change has been made.

**Q14.** Page9,figure4: Use TRH instead of tramadol

**A14.** Except for the first mention in the text, the abbreviation TRH was used instead of tramadol in all other parts of the text.

**Q15.** Page 8, line 256: Use CSDS instead of CSDS

**A15.** The necessary correction has been made.

Q16. In p9 inset figure5 graph, specify peak current in Ip

**A16.** All figures were reviewed. Peak currents in the inset graph were corrected as *Ip*.

**Q17.** In figure 5, use TRH instead of tramadol

**A17.** Except for the first mention in the text, the abbreviation TRH was used instead of tramadol in all other parts of the text.

**Q18.** On p10 line 303, type “suggests” instead of suggest

**A18.** The necessary correction has been made.

**Q19.** Line 369 and line 370 use “did not effect” and “show” instead of “not affected” and “shown” respectively.

**A19.** The entire text was examined grammatically and corrections were made carefully.

**Q20.** On p13 line 393, Is the oxidation peak of uric acid a speculation or the authors really proved it?

**A.20.** The paragraph has been added to the text. “The related voltammograms of the urine sample by standard addition method are depicted in Fig. 7A. It can be concluded that an appeared oxidation peak at about +1.55 V is due to the TRH oxidation since its peak current increased after each TRH standard addition. In the absence of TRH, there were no detectable oxidation peaks in the working potential range where the analytical peak observed (Fig. 7B). On the other hand, an unknown oxidation peak at about +1.00 V was observed in blank urine samples, which could be due to the oxidation of uric acid (UA) 31,32.”

**Q21.** In figure 6, use TRH instead of tramadol.

**A21.** Except for the first mention in the text, the abbreviation TRH was used instead of tramadol in all other parts of the text.

**Q22.** Line 409, the text should be rearranged in the following:

“BDD electrode was used for the first time to develop a simple…”

**A22.** The text has been rearranged in line with your suggestion.

**Q23**. Line 412, use “showed” instead of “shown”.

**A23.** The necessary correction has been made.

**Q24.** Page 13, line 416: "detection" should be removed from the sentence and the sentence should be rewritten as follows:

“This method was validated on model and spiked samples.”

**A24.** The necessary correction has been made.