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OPEN PEER REVIEW

Simultaneous Screening of Nitrofuran Metabolites in Honey Using Biochip Array Technology: Validation Study According to the Decision 2002/657/EC of the European Union

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

0 comments

No public comments in this round.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

August 22, 2022

Dr. Shahhosseini
Editor In Chief
Journal of Iranian Journal of Pharmaceutical Research

RE: IJPR-129432

Dear Dr. Shahhosseini

Thank you very much for reviewing our manuscript. We also greatly appreciate the associate editor and reviewers for their useful comments. Please find in the following our answer to the comments of associate editor and reviewers.

We hope that you find our responses satisfactory and that the manuscript is now acceptable for publication.

,Sincerely
Samira Eslamizad, MSc, Ph.D

Assistant Professor
Food Safety Research Center

Shahid Beheshti University of Medical Sciences

I.R. Iran

Reviewer and associate Editor 1:

Question 1- The Title of the Manuscript should be shortened.

Author's response: Thank you very much for your valuable comments. It was corrected.

Question 2. It is suggested Key words become different from works of Title. Also, the Keywords must be selected from MeSH-NCBI.

Author's response: It was corrected.

Question 3. What is the reason for choosing nitrofuran metabolites? Is it not mentioned in the introduction?

Author's response: Nitrofurans are a class of broad-spectrum antibiotics that were widely used in food-producing animals. Due to concerns about their potency as carcinogens and, they were banned outright from use (EC 1442/95, 1995). However, occurrences of nitrofuran contaminants are the most frequent source of alerts in RASFF (the EU Rapid Alert System for Food and Feed Annual Reports, 2005 and 2006), although the number of transgressions is declining.

It was mentioned in the introduction in paragraph 4.

Question 4. What is the basis for choosing this number of samples for evaluation?

Why were Germany and Netherlands samples chosen along with Iranian samples?

Iranian samples belong to which region?

Author's response: choosing 20 blank and spiked samples are based on European guideline (reference 9) and described in the Experimental section, Validation procedure part.

We provide many samples as blank and real samples from the Netherlands (Wageningen University). Most Netherlands honey samples were used for the validation procedure because they were blank according to their and our tests (we checked them with the Randox evidence investigator). Regarding German samples, during sampling, we found only 2 German samples from the north of Tehran.

Iranian samples were collected from Tehran (north, south, West, east, and center of Tehran).

Question 5. Why are the number of samples not equal? It is not possible to draw a correct conclusion.

Author's response: The goal of this study was the validation of biochip array technology for Simultaneous screening of nitrofuran metabolites in honey based on the European Decision No. CE/2002/657 (C.D 657) and based on the European guideline for the validation of screening methods for veterinary drug residues, after that showing the applicability of this kit, a number of real samples were screened. This is the primary monitoring of nitrofuran metabolites in honey and for deciding about nitrofuran metabolites in honey in Iran should be analysed more samples from different provinces.

Question 6. All parts of the Method must be appropriately referenced.

Author's response: The sample preparation and screening procedures were carried out according to the instructions provided by the company. It was added.

Question 7. In the Methods section, write the CAS number of the Chemicals and Reagents.

Author's response: This is a detail data of the chemicals and solvents that we used but I didn't see the CAS number in any articles.

NO.	Name	Code	Lot NO.
1	4-NP-AOZ	33347-50 mg	BCBS5220
2	4-NP-AMOZ	33349-50 mg	SZBF160XV
3	4-NP-ADH	33655-100 mg	BCBS3643V
4	4-NP- SEC	33656-100 mg	BCBV0139

Name	Cat. Num.	Co.
Methanol (for liquid)	1.06007.2500	Merck
n-Hexane (for liquid)	1.04391.2500	Merck
Hydrochloric Acid 32%	1.00319.2500	Merck
Ethyl Acetate (for analysis)	1.09623.2500	Merck
Sodium hydroxide (pellets for analysis)	1.06498.1000	Merck
4-Nitrobenzaldehyde for synthesis	8.06766.0025	Merck
di-potassium hydrogen phosphate (for analysis)	1.05104.1000	Merck

Question 8. All data are descriptive. There is no visual data in this study.

Author's response: Part of validation data is visual (fig 1) but other information is such that cannot be visualized.

Question 9. The Discussions section needs more explanation.

Author's response: It was corrected.

Question 10. All tables must be reported in the same format.

Author's response: It was corrected.

Question 11. What are your suggestions for future studies?

Author's response: According to the results of the former and the real samples, monitoring antibacterial residues of different types of honey in Tehran and other provinces of IR Iran requires more extensive screening studies.

Associate Editor 2:

Question 1. Please organized abstract in 5 sections, 1- Background, 2- Objectives, 3- Patients and Methods or (Materials and methods), 4- Results and 5-Conclusions.

Author's response: Thank you very much for your invaluable comments. It was organized.

Question 2. Please check your Keywords with Mesh data base. It seems that the key words need some revisions

Author's response: It was corrected.

Question 3. Please write the type of study, sample size, sampling strategy and date and country of study in abstract

Author's response: It was corrected and heighted in abstract.

Question 4. It is better that in abstract result mentioned numerical result .for example mean of group1, group 2, .., group4 with p value=? also country of study should be mentioned in abstract

Author's response: This method is a screening method and the signal in RLU was used for analysis, Samples with RLUs above the cut-off were flagged as presumed negative. Samples

with an RLU below the cut-off level were flagged as positive for screening, so we could not report mean and p value.

Question 5. By what mechanism was the honey purchased? Are you sure that the honeys are really from the same countries?

Author's response: We provide many samples as blank and real samples from the Netherlands (Wageningen University). Most Netherlands honey samples were used for the validation procedure because they were blank according to their and our tests (we checked them with the Randox evidence investigator). Regarding German samples, during sampling, we found only 2 German samples from the north of Tehran.

Iranian samples were collected from Tehran randomly (north, south, West, east, and center of Tehran)

The goal of this study was the validation of biochip array technology for Simultaneous screening of nitrofuran metabolites in honey based on European Decision No. CE/2002/657 (C.D 657) and based on the European guideline for the validation of screening methods for veterinary drug residues, after that showing the applicability of this kit, a number of real samples were screened.

Question 6. Why is the sample size so unbalanced?

Author's response: The goal of this study was the validation of biochip array technology for Simultaneous screening of nitrofuran metabolites in honey based on European Decision No. CE/2002/657 (C.D 657) and based on the European guideline for the validation of screening methods for veterinary drug residues, after that showing the applicability of this kit, a number of real samples were screened.

Question 7. What is your type of study? Experimental study, Cross sectional, cohort, case-control, clinical trial, systematic review or … please mentioned it in method section.

Author's response: This is an Experimental study; it was added in the method section.

Question 8. Did have any you inclusion and exclusion criteria? Please speak about it and then tell about how many samples include and exclude according to these criteria

Author's response: In the validation procedure, only blank samples were used for this part any contaminated samples were excluded. 20 blank samples were included.

Question 9. What was your sample size formula? What is your expected power? Please mention in main text

Author's response: We don't have a sample size formula because the goal of this study was the validation of biochip array technology for Simultaneous screening of nitrofuran metabolites in honey based on European Decision No. CE/2002/657 (C.D 657) and based on the European guideline for the validation of screening methods for veterinary drug residues, after that showing the applicability of this kit, a number of real samples were screened. This is the primary monitoring of nitrofuran metabolites in honey and for deciding on nitrofuran metabolites in honey in Iran should be analysed more samples.

Question 10. Discuss more about sampling strategy .it is a little vague.

Author's response: As stated in response to question 9, the goal of this study was validation of this novel technique (not precise monitoring), the sample was collected from Tehran randomly (north, south, West, east, and center of Tehran).

Question 11. Please mention the city, country and date of study in method section

Author's response: It was mentioned.

Question 12. How many observers did you have? if you had more than one observer, you must mention agreement index like kappa coefficient (write in method section)

Author's response: During the validation process and routine analyses, positive and negative quality controls (QCs) were used. During validation and analyses of the 35 real honey samples, spiked samples and control in the kit were used as positive QCs and blank samples used as negative QCs. When the QCs during analyses of real samples in each run were not valid, the samples were reanalyzed.

It was added in the text.

Question 13. What statistical software did you use? And also what statistical methods did you use? Please mention them in method section

Author's response: It was mentioned. The Excel software was used for the analysis of the data.

Question 14. Did you check normal assumption? Please mention it. It seems that some variables have not normal distribution and you must have used nonparametric method

Author's response: We did not use statistical methods to analysis the data.

Question 15. There are some spelling and grammatical errors in the text. Please correct them

Author's response: It was corrected.

Question 16. Your sample size is so low. Why did not you use exact method like boot strap and Jackknifing method for reporting pvalue and CI

Author's response: The sample size is low because the samples were used only for showing the applicability of this kit, the goal of this study was the validation of biochip array technology for Simultaneous screening of nitrofuran metabolites in honey.

We could not report p value and CI because the signal in RLU was used for analysis; Samples with RLUs above the cut-off were flagged as presumed negative. Samples with an RLU below the cut-off level were flagged as positive for screening.

Question 17. Most of article references are out of date (2019 or upper). Please use more up to date references and also arrange references according to journal format

Author's response: It was added.

Question 18. Please mention the weak and strong points of your study

Author's response: strong point- this study represents the first validation of an antibacterial array III assay for honey in compliance with the European Instruction regarding Iran's veterinary drug screening methods. This method can be used in laboratories of food and drug organization, laboratories of deputy food and drug administration, universities of medical sciences, and laboratories of entry points in the country.

Weak points- monitoring antibacterial residues of different types of honey in other provinces of IR Iran require

Question 19. The main text is too long. Please decrease main text.

Author's response: According to other reviewers, we could not decrease the text.

Question 20. Did you have any missing value in your study? If it was, please write what was your strategy for solving this problem. It is critical points for control non response bias. Please mention it

Author's response: Some well of biochips made an error.

For controlling the response we had two types of positive control (spiked samples and positive control of the kit) and negative control (blank samples).

Question 21. The novelty of your work must be discussed, it seems it is so weak The Code and date of ethical approval should be mentioned in main text. Also ethical considerations should be mentioned with more detail

Author's response: In our country, we don't have a rapid screening test for screening nitrofuran metabolites simultaneously in honey, so the valid, simple, and rapid screening method for screening of nitrofuran metabolites in honey seemed necessary.

The Code and date of ethical approval were mentioned in the main text.

2 Revision 1

1 comment · 5 Sep 2022

Associate Editor

MINOR REVISION 5 Sep 2022

Comments:
1. Some reviewer comments have not been addressed in the manuscript text, such as the request to add information about the test compound to the Introduction section.
Some reviewer comments have not yet been addressed in the text of the revised article, such as the request to add a description of the test compound to the Introduction section.

2. Contrary to the author's opinion, all the results are presented as tables and numbers, and there are no visual (chromatography) data.
Contrary to the author's opinion, all the results are tabular, and there are no visual (chromatography) data.

3. Do not use Track changes to make revisions requested by the reviewers in the main text of the manuscript. Use yellow highlighting to mark the revisions in the manuscript text.
Do not use Track changes to make revisions requested by the reviewers in the main text of the manuscript. Use yellow highlighting to mark the revisions in the manuscript text.

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

September 6, 2022

Reviewer and associate Editor 1:

Question 1. Do not use Track changes to make corrections.

Author's response: Thank you very much for your valuable comments. It was corrected.

Question 2. Some Reviewer's comments in the text have not been corrected.

Author's response: It was corrected.

Question 3. In the text, Mark the correct comment with yellow tape.

Author's response: It was corrected.

Question 4. All data are descriptive. There is no visual data in this study

Author's response: Figure 1 was added to the text. Line number is 176.

3 Revision 2

0 comments

No public comments in this round.

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

October 04, 2022

Reviewer and associate Editor 1:

Question 1. The purpose of comment 3 is to use analysis peaks as visual data.

Author's response: Thank you very much for your valuable comments. The biochip based method (Evidence Investigator Biochip Array Technology), employing chemiluminescent immunoassay principles. The signals are recorded by a charge-coupled device (CCD) camera and are presented in RLU and part per billion (ppb). Each analytes concentration is then calculated from a calibration curve. As mentioned before part of validation data is visual (fig 1) but other information is such that cannot be visualized.

Question 2. Please show your chromatography peak(s) to prove your data and include your chromatography peak(s) in your paper a figure.

Author's response: We did not use chromatography method, this analysis method is biochip based method that is employing chemiluminescent immunoassay principles.

4 Revision 3

0 comments

No public comments in this round.