Preprints.org

Article

Not peer-reviewed version

Study on Time-Resolution Fluorescence Immunochromatography of Aflatoxin B1 in Yellow Rice Wine

Mengjie Zhu , Dun Wang * , Du Wang , Xue Wang , Qi Zhang * , Jing Dong , Peng Feng

Posted Date: 23 January 2024

doi: 10.20944/preprints202401.1688.v1

Keywords: Yellow rice wine; Aflatoxin B1; Time-resolution fluorescence immunochromatography

Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Study on Time-Resolution Fluorescence Immunochromatography of Aflatoxin B_1 in Yellow Rice Wine

Mengjie Zhu [1,2], Dun Wang [1,2,*], Du Wang [1,2], Xue Wang [1], Qi Zhang [2,*], Dong Jing [1] and Peng Feng [1]

[1] Xiangyang Academy of Agricultural Sciences, Xiangyang 441057, China; zmj2280880980@163.com (M.Z.); wang416929@gmail.com(D.W.); maysxuer@126.com (X.W.); xynkydongjing@163.com (J.D.); 2295851430@qq.com

[2] Key Laboratory of Detection for Biotoxins and Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture and Rural Affairs, Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China

* Correspondence: wangdunzju@163.com (D.W.); zhangqi01@caas.cn (Q.Z.)

Abstract: (1) Background: There is a risk of Aflatoxin B_1(AFB_1) pollution in yellow rice wine[1], threatening human life and health, the risk is a major focus of government attention and social concern. The current detection technology cannot meet the needs of large-scale testing due to the disadvantages of high false positive, cumbersome operating procedures or high cost. Therefore, it is urgent to study and establish a high-sensitivity and high-fast detection method for AFB_1 to provide technical support for yellow rice wine quality supervision and risk assessment.; (2) Methods: In this study, three-factor and three-level orthogonal experiments were carried out for oscillation time, reaction temperature and tomography time to determine the best reaction conditions for pretreatment. AFB_1 detection was carried out by aflatoxin time-resolved fluorescence immunochromatography technology，Through the ratio of the T signal value of the detection line to the C signal value of the quality control line and the natural logarithmic value of the standard solution concentration[2], a rapid detection technology of time-resolution fluorescence immunochromatography is established. The technology was methodically assessed, the actual yellow rice wine samples were tested, and the results were compared with the HPLC method; (3) Results: The results show that the optimal reaction conditions for pretreatment are: the sample oscillation time is 20min, the sample reaction temperature is 37° C, and the sample chromatography time is 6min. In the actual sample detection of yellow rice wine, The time-resolved fluorescence immunochromatography detection technology established in this study detects AFB_1. The detection limit is 0.3 $\mu g \cdot kg^{-1}$, The linear range is 0.8-12.0$\mu g \cdot kg^{-1}$, The linear equation of the corresponding standard curve y=-0.1913x+0.5206 (R^2=0.9948), The standard deviation is 0.029. The results of the in-batch accuracy and precision of this method show that the addition and recovery rate is 77.9% - 105.7%, The coefficient of variation is in 4.5% - 10.3%; Inter-batch accuracy and precision test results show that the addition recovery rate is75.9% - 85.8%, the coefficient of variation is in 8.1% - 13.9%, explain that the detection technology has good accuracy and precision. Compared with HPLC, the relative error is less than 10%, It shows that aflatoxin time-resolution fluorescence immunochromatography technology is in good agreement with the test results of national standard methods; (4) Conclusions:he rapid detection technology of time-resolution fluorescence immunochromatography of AFB_1 in yellow rice wine is easy to operate with fast detection speed, high sensitivity, good repeatability and stability, and has broad application prospects.

Keywords: yellow rice wine; aflatoxin B_1; time-resolution fluorescence immunochromatography

© 2024 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

1. Introduction

Yellow rice wine is a traditional liquor with unique health care functions in China. Compared with wines such as beer and wine, research on the metabolic mechanism and control technology of harmful substances in yellow rice wine started late, foreign mature research results and available experience are lacking[3]. As a secondary metabolite of related microorganisms, fungal toxin has always been a safety hazard in the food industry, AFB_1 is a highly toxic and highly carcinogenic substance, which is more toxic than potassium cyanide and arsenic. It is very easy to contaminate food crops, meat and dairy products. The melting point of aflatoxin is higher (265～269°C), the crystallization state is relatively stable under high temperature conditions[4-6]. The traditional yellow rice wine brewing glycochemical agent is a naturally fermented raw wheat curd, but yellow rice wine factories in various places often make their own rice, the climate, environment and room conditions vary greatly from place to place. The composition of microorganisms in the curd is very different, and aflatoxin-producing aspergillus aflatox is very likely to breed[3], and most of the yellow rice wine brewing process is carried out openly without sterilization., There are a large number of microorganisms in all kinds of raw materials and various tools. The brewing process environment and the air have the opportunity to invade beneficial and harmful microorganisms[7]. There are many researches showed that the AFB1 in the production of distilled liquor(Daqu, fermented grains, lost rice, yellow water, base wine, finished wine)is far below $5\mu g \cdot kg^{-1}$. Especially AFB_1 is almost non-existent in base wines and finished wines[8-10]. Unlike liquor, the mash and yellow rice wine are nutritious medium in the brewing process of yellow rice wine, although the low pH, alcohol and mash has a certain inhibitory effect on microorganisms, there have not a distillation process, some non-cultured bacteria may also reproduce and increase possibility of aflatoxinB_1 pollution[11].26 samples of bottled yellow rice wine were determined by Ji Xiaofeng[1], although The content of AFB_1 does not exceed the limit standard of starting fermented food (5μg/kg), the detection rate is as high as 100%, and is very stable under normal conditions, especially once formed in the wine body, it is extremely stable and difficult to decompose. From this point of view, with the rapid growth of the yellow rice wine consumption market and improvement of consumers' nutritional health and safety awareness, the quality and safety issues of AFB_1 in yellow rice wine are also becoming more and more important to consumers, study on the establishment of AFB_1 with high sensitivity and high-fast detection methods are very urgent and necessary.

The existing detection methods of AFB_1 in yellow rice wine are mainly instrumental analysis and enzyme-linked immuno assay (ELISA) method. Enzyme-linked immunosorbent technology is the most commonly used in enzyme-linked immuno assay, but because it is prone to false positive results, it can only be used for preliminary screening. Instrument analysis methods such as HPLC-MS/MS and GC-MS improve the accuracy and sensitivity of analysis, but due to the high cost of equipment and the need for professional and technical personnel, the high detection cost is not conducive to carry out the detection of large-scale samples[12-13]. Immune analysis technology has the characteristics of strong specificity, high sensitivity, large analysis capacity, fast analysis and low cost, but traditional radioimmunology is limited due to the environment pollution and background fluorescence interference, The Time-resolution fluorescence immune analysis Technique(TRFIA) can mark trivalent rare earth ions which can produce fluorescence, such as Eu (III) 、Tb(III), Sm(III), Use its long fluorescence decay time features of waiting for the natural fluorescence decay in the sample before measuring the fluorescence of rare earth ions can eliminate the interference of natural fluorescence. It has the advantages of high sensitivity, good anti-m matrix interference and strong stability[14-17].

Therefore, this paper is based on time-resolution fluorescence immuno assay technology, Set up rapid detection technology of AFB_1 in yellow rice wine with high resolution, high sensitivity, high security, thus filling the gap of rapid detection technology of AFB_1 in yellow rice wine.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

2. Results and Discussions

2.1. Detection limit and linear range of the method

The results show that（Figures 1 and 2） when the contention of AFB_1 is less than 0.8μg·kg^{-1}, the concentration is not linearly correlated with the detection card. The detection limit of time-resolution fluorescence immunochromatography in yellow rice wine of AFB1 is 0.3μg·kg^{-1}, the linear range is 0.8-12.0μg·kg^{-1}. The linear equation of the corresponding standard curve is y=-0.1913x+0.5206（R^2=0.9948), the standard deviation is 0.018.

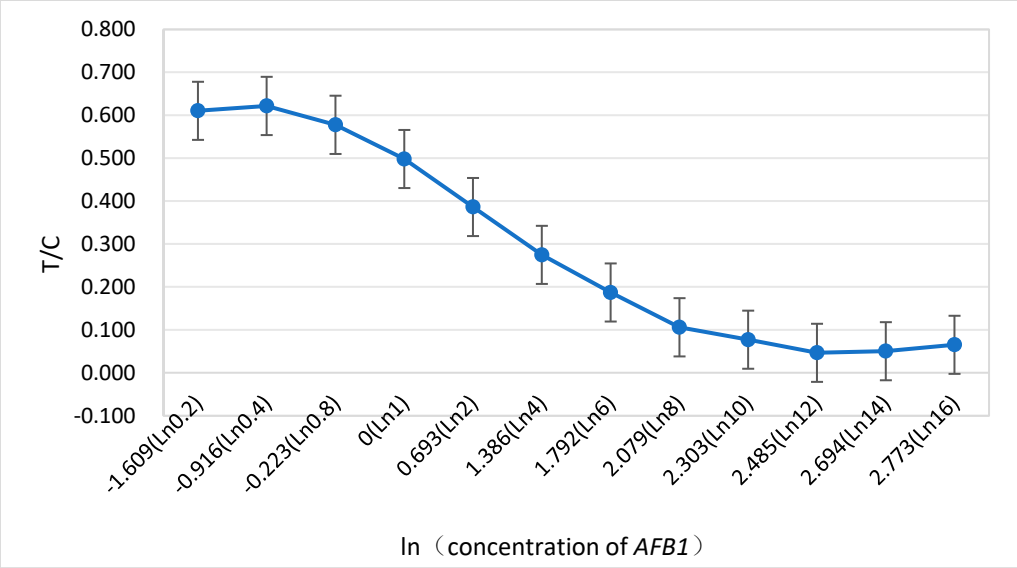


Figure 1. AFB_1 dose-response curve in yellow wine.

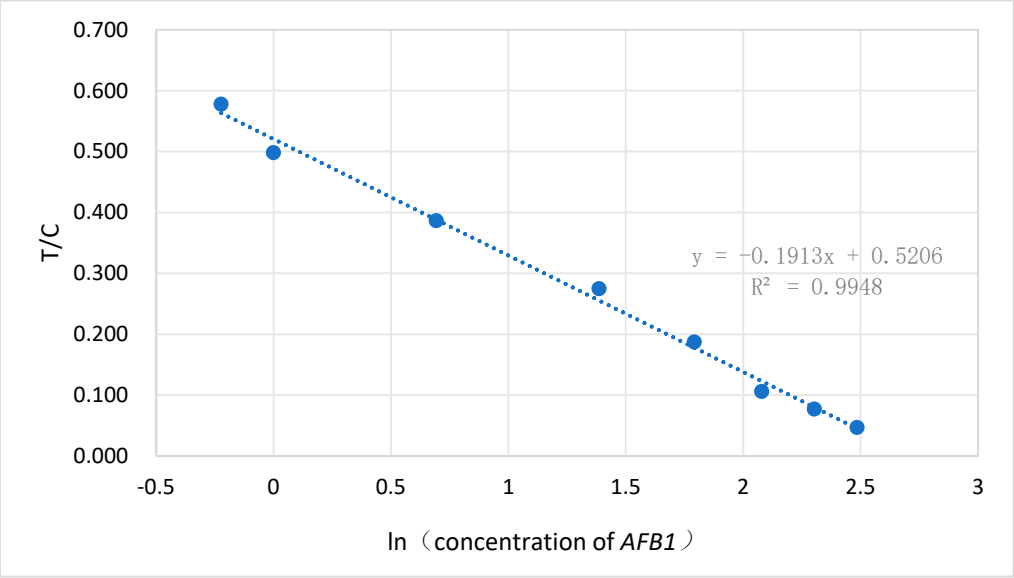


Figure 2. AFB_1 standard curve in yellow wine.

2.2. Preprocessing optimal reaction conditions

Through the recovery rate experiment (Table 1) and R extreme difference analysis (Table 2) display RB>RC>RA, indicating the influencing factors order is B > C > A, althrough intuitive analysis (Figure 3) determine that the optim combination is A2B3C2. Variance analysis (Table 3) Results show

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

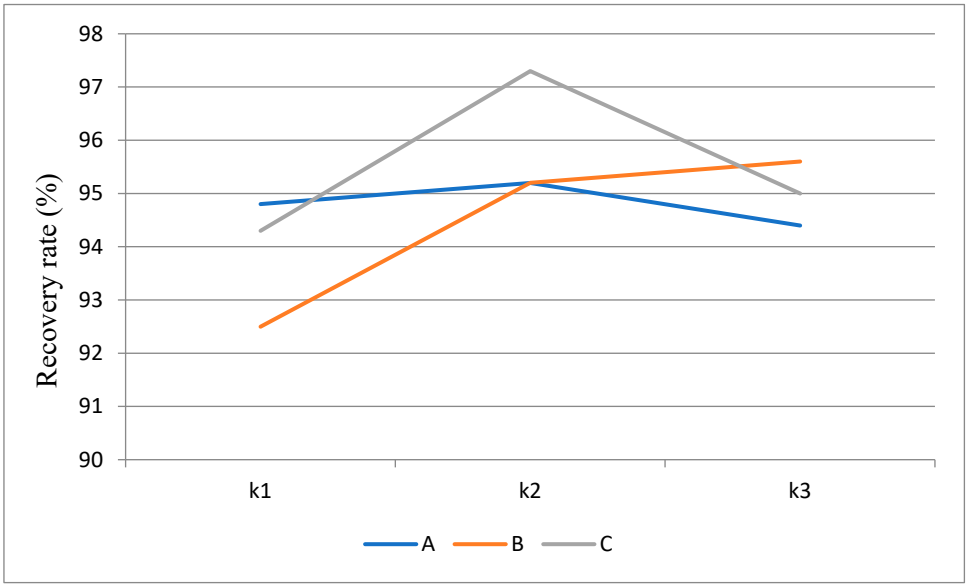
: B is significant, but A/C is not significant, FB>FC>FA, so B>C>A, consistent with the R extreme analysis results.

Table 1. Orthogonal test sheet for recovery rate experiment.

Level	A	B	C		
Level Factor FactorFG	Oscillation time（min）	Reaction temperature(℃)	Chromatography time（min）	Empty column	Recovery rate (%)
1	1	1	1	1	92.1
2	1	2	2	2	95.6
3	1	3	3	3	96.7
4	2	1	2	3	92.9
5	2	2	3	1	95.7
6	2	3	1	2	96.9
7	3	1	3	2	92.5
8	3	2	1	3	94.2
9	3	3	2	1	98.4

Table 2. Extreme difference analysis.

Extreme difference extreme	A	B	C	Empty column
Factor factorFactor				
K1	284.4	277.5	283.2	284.4
K2	285.5	285.5	286.9	285.5
K3	285.1	292	284.9	285.1
K1	94.8	92.5	94.4	94.8
K2	95.2	95.2	95.6	95.2
K3	95.0	97.3	95.0	95.0
R extreme	0.4	4.8	1.2	0.9



Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

Figure 3. Intuitive analysis.

Table 3. Variance analysis.

Source of variance	Square sum	Degree of freedom	Mean square	F value	F(2,2) A=0.05	Salience
A	0.21	2	0.11	0.22		Not significant
B	35.17	2	17.59	36.64	19	Significant
C	2.29	2	1.15	2.39		Not significant
Error	0.96	2	0.48			
Sum total	38.63	8				

2.3. Accuracy and precision of the method

2.3.1. In-batch accuracy and precision of the method

Table 4 shows that the addition and recovery rate of the same batch of time-resolution fluorescent immunochromatography test strips is 77.9% - 105.7%. With the increase of the marked concentration and the increase of the recovery rate, the coefficient of variation of the measurement results becomes smaller, mainly because the difference between the signal is small, and the interference of the signal has a greater impact on the results. The coefficient of variation is in4.47% - 10.26%, It shows that the detection technology has good accuracy and precision.

Table 4. Results of in-batch recycling experimental.

Spiked level ($\mu g \cdot kg^{-1}$)	Average finding ($\mu g \cdot kg^{-1}$)	Standard deviation ($\mu g \cdot kg^{-1}$)	Recovery (%)	CV (%)
1	0.779	0.0799	77.9%	10.26
5	4.987	0.0663	97.7%	6.80
10	10.569	0.0501	105.7%	4.47

2.3.2. Inter-batch accuracy and precision of the method

For yellow rice wine samples, the addition and recovery rate of time-resolution fluorescence immunochromatography test strips between different batches is 75.9% - 89.8%, coefficient of variation8.10% - 13.86%(Table 5), indicating that the batch of the detection technology has good accuracy and precision.

Table 5. Results of inter-batch recycling experimental.

Spiked level ($\mu g \cdot kg^{-1}$)	Average finding ($\mu g \cdot kg^{-1}$)	Standard deviation ($\mu g \cdot kg^{-1}$)	Recovery (%)	CV (%)
1	0.759	0.1052	75.9%	13.86
5	4.210	0.0682	84.2%	8.10
10	8.980	0.0426	89.8%	10.10

2.4. Comparison of results on AFB_1 detection in actual sample test results between time-resolution fluorescence immunochromatographys and HPLC

By the Table 6, It can be seen that The relative error of the detection results between time-resolution fluorescence immunochromatography technology established in this study and national food safety standards [18] (GB5009.22—2016) is less than 10%, explain the test results of time-resolution fluorescence immunochromatography technology are consistent with the the standard method and meet the requirements of rapid detection of aflatoxin in agricultural products.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

Table 6. Comparison of result between TRFIA and HPLC.

Sample Number	Finding by TRFIA (μg·kg^{-1})	Finding by HPLC (μg·kg^{-1})	Relative error (%)
Sample 1	0.949	1.051	-9.70
Sample 2	N.D.	N.D.	
Sample 3	4.932	4.994	-1.24
Sample 4	1.754	1.839	-4.63
Sample 5	6.710	6.910	-2.89
Sample 6	N.D.	N.D.	
Sample 7	1.200	1.330	-9.78

ND：No detected.

Materials and methods

3.1. Establish a standard curve

3.1.1. Preparation of sample diluent

Weigh 1.0 g Sucrose,0.5 g Bovine serum albumin and 2.5g Twain20 (Comes from China National Pharmaceutical Group Chemical Reagent Beijing Co., Ltd.), mix well and use ultra-pure water (Comes fromUPH-III-10Preparation of Upu ultra-pure water manufacturing system) fixed capacity to 100.0 mL[19].

3.1.2. Selection of blank matrix solution

Choose yellow rice wine samples with few impurities and bright color（alll purchased in large local supermarkets）. Adopt the third method in national of food safety standards (GB5009.22—2016) --high performance liquid chromatography- Post-column derivative method for testing（HX-G Photochemical post-column derivative instrument, Wuhan Hengxin Reagent Technology Co., Ltd.; Shimazu LC-20ALiquid chromatograph , Total aflatoxin immune affinity columnComes fromJiangsu Su Weiwei Biological Research Co., Ltd.), choose the sample without AFB_1 as a blank matrix solution.

3.1.3. Standard working solution preparation

Buy AFB_1 standard products （2 mg·kg^{-1}, Beijing Tanmo Quality Inspection Technology Co., Ltd.), use methanol（United StatesFisher）fixed capacity to 10 mL, the quality concentration of the formulated product is 200μg·kg^{-1}, to save the standard reserve solution at -20°C. Then dilute the reserve liquid gradient with a blank matrix solution to receive a series of standard working solutions of 0.2、0.4、0.8、1.0、2.0、4.0、6.0、8.0、10.0、12.0、14.0 and 16.0μg·kg^{-1}, test from low to high concentrations, according to the detection line T Signal value and quality control line C, the ratio of signal value (T/C) and the natural logarithm of the standard solution concentration (lnc), draw standard curves.

3.2. Certain sample pretreatment optimal reaction conditions

3.2.1. Sample pretreatment and AFB_1 content determine

Use an electronic balance（CP4102Mould， Aohaus Instruments (Changzhou) Co., Ltd.）to weigh 5.0 g yellow rice wine into centrifugal tube, mix evenly in the oscillator（KB5010Type, Haimen Qilin Bell Instrument Manufacturing Co., Ltd.）, High-speed centrifugation（5810RHigh-speed low-temperature centrifuge, Eppendorf）for 5 minutes（6000r/min）, add 3.0mLsample diluentto 1.0mL

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

cleansing liquid, The sample diluent is mixed evenly with an vortex meter and filtered by an organic filter membrane to obtain the liquid to be tested for later use. Open the sample cup and add 150μLsample, the sample to be tested is fully dissolved, mixed, and warmed in the heater, Remove the test strip from the test cartridge, insert it down into the sample cup according to the arrow, and chromatography. After the hardware self-test of the instrument according to the operation steps of the aflatoxin time-resolution fluorescence meter, take it out after tomography and dry the lower liquid. Use a time-resolution fluorescence reader to read signal values and determine AFB_1 content within 2 minutes(Aflatoxin time-resolution fluorescence meter, incubator, sample bottle and other accessories all by Jointly developed by the Institute of Oil Crops of the Chinese Academy of Agricultural Sciences and Shanghai U You Biotechnology Co., Ltd).

3.2.2. Pretreatment for the optimal reaction conditions

Add AFB_1 standard working solution to the blank matrix sample of yellow rice wine, ensure the final concentration of addition is 5μg·kg $^{-1}$, carry out three factors and three levels of orthogonal experiments according to 3.2.1. The oscillation time (A) is respectively 10min、20min、30min, reaction temperature (B) respectively is 28°C, 32°C, 37°C, chromatography time (C) is respectively 4min、6min、8min. The best reaction conditions for sample pretreatment are determined through extreme difference and variance analysis of recovery rate and variance analysis.

Table 7. Experimental factors of recovery rate.

	A	B	C
Level Level Level Factor Factor	**Oscillation time (min)**	**Reaction temperature (°C)**	**Chromatography time (min)**
1	10	28	4
2	20	32	6
3	30	37	8

3.3. AFB_1 detection limit and linear range

The detection is limited to the minimum amount (or minimum concentration) that the object to be tested can be recognized from the background detection of the blank matrix sample. According to national standards GB/T 27404—2008(Riterionon Quality Control of Laboratories-Chemical Testing of Food[20], calculate the detection limit with the formula in (1). The linear range is obtained according to the recovery test of blank matrix samples.

$$CL=3Sb/b \quad (1)$$

Formula (1): CL- Methodological detection limit; Sb- Standard deviation of blank value; b -Method standard curve slope

3.4. AFB_1 detection of in-batch precision

A blank matrix sample was added with a recovery test to obtain the in-batch precision of this method. Add AFB_1 standard working solution into the blank matrix sample of yellow rice wine to make the final concentration of AFB_1 is 1、5、10μg·kg^{-1}, using the same batch of aflatoxin time-resolution fluorescence immunochromatography test strips to test six times, calculate the average addition recovery rate and variation coefficient in the in-batch（CV）.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

3.5. AFB_1 detection of inter-batch precision

A blank matrix sample was added with a recovery test to obtain the inter-batch precision of this method. Add AFB_1 standard working solution into the blank matrix sample of yellow rice wine to make the final concentration of AFB_1 is 1 、5、10μg·kg^{-1}, using different batches of aflatoxin time-resolution fluorescence immunochromatography test strips to test six times, calculate the average addition recovery rate and variation coefficient between batches（CV）.

3.6. Comparison of test results from actual samples between Time-resolution fluorescence immunochromatography and HPLC

Choose seven actual samples of yellow rice wine randomly, aflatoxin time-resolution fluorescence immunochromatography test strip method and the national food safety standard respectively (GB5009.22—2016) is compared and tested to compare the consistency of the two methods.

4. Conclusion

AFB_1 in yellow rice wine is a very important safety hazard, and it is very necessary to test it. In the existingdetection method, HPLC-MS/MS and GC-MS operate complexly and are not easy to popularize. Although enzyme-linked immunization methods are fast and convenient, they are prone to false positives. This article is studied about Time-resolution fluorescence immunochromatography rapid detection technology of AFB_1 in yellow rice wine for the first time. Compared with national standard method, this method has the advantages of simple operation, fast detection speed, high sensitivity, good repeatability and stability, low dosage of organic reagents, and high safety for the environment and inspectors, filling the blanks of quick and accurate detection of AFB_1 in yellow rice wine， and has broad application prospects.

Author Contributions: Conceptualization, M.Z.; methodology, D.W. (Du Wang); formal analysis, X.W.; investigation, M.Z., D.W. (Dun Wang), D.W. (Du Wang); X.W, J.D., P.F.; resources, D.W.; data J.D., P.F.; writing—original draft preparation, M.Z.; writing—review and editing, D.W. (Dun Wang)., Q.Z.; project administration, D.W.(Dun Wang), X.W.; funding acquisition, D.W. (Dun Wang)., Q.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the open project of the Key Laboratory of Biotoxin Detection of the Ministry of Agriculture and Rural Affairs (SWDSJC2018001) and the Youth Fund of Xiangyang Academy of Agricultural Sciences (YFXYAAS-2022).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request from the authors.

Acknowledgments: Thanks to the open project of the Key Laboratory of Biotoxin Detection of the Ministry of Agriculture and Rural Affairs (SWDSJC2018001) and the Youth Fund of Xiangyang Academy of Agricultural Sciences (YFXYAAS-2022) for funding this project.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. JI, X.F.; CHEN, X.Y.; WEI, W. Determination of aflatoxin B_1 in bottled Chinese rice wine by enzyme-linked immunosorbent assay (ELISA). *Acta Agriculturae Zhejiangensis*.**2015**,27(11): 2006 -2010.
2. WANG, D.; ZHANG, W.; LI, P.W.; ZHANG, Qi.; ZHANG, Z.W.; DING, X.X.; JIANG, J. Rapid and quantitative detection of aflatoxin B_1 in plant grain and oilseeds products using colloid golden immuno - chromatographic method. *Chinese Journal of Oil Crop Sciences*.**2014**,36(4): 529 - 532.
3. REN, Q.; XU, J.L.; LIU, J.H. Safety risk and its control of yellow rice wine. *Journal of Food Science and Technology*.**2016**,34(3): 12 - 19.
4. ZHUANG, Z.H.; ZHENG, C.Q.; WANG, S.H. Optimization of Aspergillus flavus Culture Conditions and Extraction of Aflatoxin B1. *Chin J Appl Environ Biol*.**2010**,16 (5): 724-729.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 January 2024 doi:10.20944/preprints202401.1688.v1

5. WILSON, C.L., DROBY, S. Microbial food contamination. *New York: CRC Press*.**2001**.
6. GUO.X.; WEN.F.; ZHENG.N.; et al. Development of an ultrasensitive aptsensor for the detection of aflatoxin B_1. *Biosens Bioelectron*. **2014**, 56 (1): 340-344.
7. MAO, Q.Z.; LU, W.G.; CHEN, B.L.; YU, G.S.; WU, B.Y. Study and analysis on safety of rice wine. *LIQUOR MAKING*. **2006**,(03)60- 65.
8. YANG, X.D.; LUO, H.B.; YE, G.B.; LI, D.Y.; WANG, C.H.; WANG, Y. Verification of the Safety of Aflatoxin B_1 in Nong-flavor Liquor Production by Enzyme-linked Immunosorbent Assay(ELISA). *LIQUOR —MAKING SCIENCE & TECHNOLOGY*.**2013**,(4)92-94.
9. Han, X.W. Analysis Methods Development for Three Mycotoxins and Application in liquor production. *Tianjin University of Science and Technology*.2014.
10. MAGATOMI, Y.; INOUE, T.; UYAMA, A.; et al. The fate of mycotoxins during the distillation process of barley shochu, a distilled alcoholic beverage. *bioscience, biotechnology, and biochemistry*.**2012**,76(1):202-204.
11. XU, Z.; TAN, S.M.; JIAO, Y.C.; ZHANG, Y.F. Determination of Aflatoxin B_1 in Dry Capsicum by Enzyme-linked Immunosorbent Assay (ELISA). *Food Science*.**2009**,30(10): 245 - 247.
12. LIU, J.; XIONG, N.; LIU, L.; YU, D. Verification of rapid detection of Aflatoxin B_1 in grain by immunochromatographic test parer method. *Journal of Henan University of Technology*.**2011**,32(5): 51 -57.
13. LI, P.W.; MA, L.; YANG, J.E.; ZHANG, W.; YANG, C.H.; YANG, M.A. Reiew on analytical methods for aflatox in B_1 in grains and oilseeds products. *Chinese journal of oil crop sciences*.**2005**,27 (2): 77 - 81.
14. Cusati. T.; Granucci. G.; Persico. M. Photodynamics and time-resolved fluorescence of azobenzene in solution: a mixed quantum-classical simulation. *Journal of the American Chemical Society*.**2011**, 133(13): 5109-5123.
15. Mizukami. S.; Tonai. K.; Kaneko. M.; Kikuchi. K. Lanthanide-based protease activity sensors for time-resolved fluorescence measurements. *Journal of the American Chemical Society*.**2008**, 130(44): 14376-14377.
16. Bunzli. J.C.G.; Piguet. C. Taking advantage of luminescent lanthanide ions. *Chemical Society Reviews*. **2005**, 34(12): 1048-1077.
17. Shi, J.; Huang, B.; Zhang, L.; Mi, X.l.; et al. Use of ultrasensitive time-resolved fluoroimmunoassay for rapid detection of clenbuterol hydrochloride in urine. *Journal of Hygiene Rrsearch*.**2006**,35(6): 798 - 801.
18. Determination of AFB and AFG groups in food. *National Food Safety Standards*. GB 5009.22-**2016**:11-17.
19. ZHANG, Z.W.; LI, P.W.; ZHANG, Q.; DING, X.X. Study on Time-Resolved Fluorescence Immunochromatography for Afaltoxin Determination in Agricultural Product. *Scientia Agricultura Sinica*.**2014**,47(18):3668-3674.
20. Riterion on Quality Control of Laboratories-Chemical Testing of Food. *Chinese National Standards*. GB/T 27404-**2008**:29-27.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.