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Feasible discussion about aflatoxin B_1 determination in TartaryBuckwheat products by HPLC

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ABSTRACT

Feasibility of determining aflatoxin B_1 in TartaryBuckwheat products by HPLC was studied by analyzing the Standard curve, sensitivity, accuracy, precision, the system error and sensitivity. The results showed that using HPLC to determine the aflatoxin B_1 in TartaryBuckwheat products is feasible, the detection range was 0.20-200.00μg /L, determination coefficient R^2 was 0.9968, the relative error was 7.09%, 5.28%, 4.55%, 0.23% respectively, the average recovery of standard addition in buckwheat core flour was 84.58%, the variation coefficient of buckwheat noodles, buckwheat soup and buckwheat tea was 9.36%, 5.25%, 8.22% respectively, and the detection sensitivity reached 0.20 μg /L.

KEYWORDS

HPLC; Aflatoxin B_1; Tartary Buckwheat products; Methodology.

INTRODUCTION

Systematic study about the feasibility of determining the quantity of aflatoxin B_1 in Tartary Buckwheat products by HPLC was done in this paper. Through analyzing the standard curve, sensitivity, accuracy, precision and effects on different Tartary Buckwheat products of HPLC, the evaluation index can be found, quality control parameters can be determined and the feasibility of determining aflatoxin B_1 in Tartary Buckwheat products by HPLC can be proved.

MATERIALS AND METHODS

Reagents and Materials

Trifluoroacetic acid (TFA), analytical reagent; n-hexane, extracted acetonitrile, analytical reagent; mobile phase acetonitrile, chromatographic pure; aflatoxin B_1 standard product, National Bureau of Standard and Metrology, China; Tartary Buckwheat powder, Tartary Buckwheat noodles, Tartary Buckwheat soup, Tartary Buckwheat tea, XichangHangfei Tartary Buckwheat Technology Development Co., Ltd.

Apparatus and Equipment

High Performance Liquid Chromatography (HPLC) System (with fluorescence detector, a reversed-phase C18 column), Agilent 1100; HZAK-FA210 electronic balance, Huazhi Scientific Instrument Co., LTD. China Fuzhou; transfer liquid gun, Black&Decker Technology Co.,Ltd. Germany; benchtop high speed refrigerated centrifuge, Xingke Scientific Instrument CO. Ltd. China Hunan; fume hood, drying box, Yarong Biochemical Instrument Factory, China Shanghai.Electric oscillator, multifunctional purifying column.

Test Methods

(1) Drawing the Standard Curve of Aflatoxin B_1 by HPLC

To determinate the range of text methods, it is necessary to plot the standard curve of aflatoxin B_1 by HPLC. Aflatoxin B_1 solutions with the concentration of0.00μg /L, 25.00μg /L, 50.00μg /L, 100.00μg /L, 200.00μg /L respectively are added to the High Performance Liquid Chromatography one by one. The steps strictly follow the HPLC detect instructions. The peak areas can be derived. The curves are drawn with solution concentrations as abscissa and peak areas as ordinate.

(2) Precision Determination of Aflatoxin B_1 by HPLC

Here, relative error is used to determinate the precision of this method[1]. In order to derive the precision of aflatoxin B_1 by HPLC, aflatoxin solution with the concentration of 0.00μg /L, 25.00μg /L, 50.00μg /L, 100.00μg /L, 200.00μg /L respectively are taken 20μL of three times. Relative error can be worked out in this way with its value less than 10%.

$$\text{Relative Error} = \frac{Mesured Value - Real Value}{Real Value} \times 100\%$$

(3) Error Determination of Aflatoxin B_1 by HPLC

Error is derived by the standard addition recovery test [2]. 50μL Aflatoxin solution with the concentration of 0.00μg /L, 25.00μg /L, 50.00μg /L, 100.00μg /L, 200.00μg /L respectively are put into 5g Tartary Buckwheat powder to make the sample with different pollution degrees[3]. After extraction, purification and derivative, the mass of aflatoxin should be detected with HPLC. The recovery rate can be no less than 70% [4].

$$P = \frac{C_2 - C_1}{C_3} \times 100\%$$

Here, P is the standard addition recovery rate; C_1 is the sample concentration; C_2 is the addition sample concentration; C_3 is the amount of standard addition.

(4) Accuracy Determination of Aflatoxin B_1 by HPLC

The coefficient of variation is used to measure the accuracy[5]. To study the accuracy of aflatoxin B_1 by HPLC, aflatoxin B_1 included is measured 5 times, 5 grams of Tartary Buckwheat powder, Tartary Buckwheat tea and Tartary Buckwheat noodles respectively each time. The coefficient of variation is calculated as the formula below. The less the coefficient of variation is, the accuracy is better. While the coefficient of variation is less than 10%, the accuracy of the test is considered to be good.

$$SD = \sqrt{\frac{\sum \left(X_i - \overline{X}\right)^2}{n-1}} \quad CV = \frac{SD}{\overline{X}}$$

Here, SD is standard deviation; X_i is the measured amount of aflatoxin B_1 in each group; X is the average amount of aflatoxin B_1 in this sample; n is the number of sample groups; CV is the coefficient of variation.

(5) Sensibility Determination of Aflatoxin B_1 by HPLC

The sensibility is measured with signal noise ratio[6]. To study the sensibility of aflatoxin B_1 by HPLC, the noise peak height N of pure solvent must be measured firstly and followed by measuring the peak height in chromatogram of solution with different concentration: 0.10μg /L, 0.15μg /L, 0.20μg /L, 0.25μg /L of aflatoxin B_1 sample. When the ratio of signal and noise S/N equals 3, this concentration should be the minimal text concentration.

Quantity Determination of Aflatoxin B1 by HPLC

(1) Disposition and Preparation of Sample

Put fully crushed Tartary Buckwheat 5g in centrifuge tube; add to 20mL acetonitrile-water (84+16) extracting solution. Put the solution on the electric oscillator to oscillate for 30 minutes with 1000r/min, and then centrifuge in vacuum for 15 minutes. Take the clear liquid in the higher level.

(2) Purification of Sample

Put 8mL clear liquid in glass tube of multifunctional purifying column. Put the filler tube in the glass tube and push it slowly. The purifying liquid can be collected in the collect pool of multifunctional purifying column.

(3) Derivative of Sample

Transport 2μL purifying liquid from collect pool to small bottle with plug. Avoid light. Dry the bottle at 85ºC±1ºC in the drying machine. Add 200μLn-hexane and 100μLTrifluoroacetic acid to the liquid and mix them avoid air for 30 seconds. Put the bottle in drying box for 15 minutes at 40ºC±1ºC. Then cool and dry at room temperature. Dissolve in water-acetonitrile (85+15), mix homogeneously for 30 seconds. After that, centrifuge in vacuum for 15 minutes with 1000r/min. Take the upper level clear liquid in the High Performance Liquid Chromatographysample bottle for the following measurement.

(4) Preparation of Standard Solution

Transport exactly2.00μg /mL aflatoxin B_1 solution to prepare the standard solution with concentration of 25.00μg /L. Take 200μL of the standard solution and dry in the drying box under 85ºC. Derivative process, see 1.4.3.

(5) Measurements and Conditions

Chromatographic Column: 4.6*150mm, 5μm, C18
Column Temperature: 30ºC
Mobile Phase: acetonitrile, chromatographic pure 17%, water 83%
Fluid Velocity: 0.50mL/min
Enter Sample Amount: 20μL
Fluorescence Detector: excitation wavelength: 360nm; emission wavelength: 440nm [7].

(6) Determination

After the Appearance of the peak, draw the peak area of standard sample as a function of concentration. Comparing the sample chromatography with standard one derives the peak. Make use of standard curve and peak area to calculate of aflatoxin B_1 amount. The concentration of aflatoxin B_1 in the sample can be calculated according to the following formula:

$$C = \frac{A \times V}{m \times f}$$

Here, C is the aflatoxin B_1 concentration in the sample with unit of μL/L; A is thecorrespondingconcentration in standard curve with standard addition methodwith unit of μL/L; V is the extracting solution volume with unit of mL; M is the mass of sample with unit of g; F is concentration multiple after derivatives compared with before [8].

RESULTS AND ANALYSIS

Standard Curve of Aflatoxin B_1 by Using HPLC

Aflatoxin B_1 solutions with the concentration of 0.00μg /L, 25.00μg /L, 50.00μg /L, 100.00μg /L, 200.00μg /L is derived and then measure under the measurement conditions as excitationwavelength of 360nm,emission wavelength of 440nm and column Temperature of 30ºC with enter sample amount 20μL. Measure the peak area AFB_1 (shown in Figure 1).

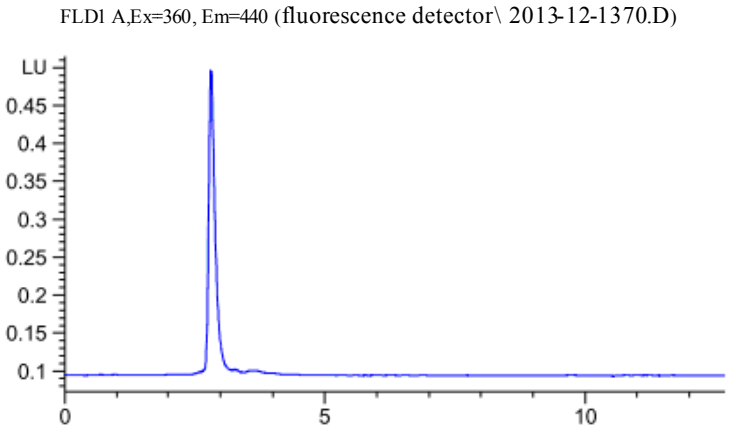

Figure 1: Chromatogram of AFB_1

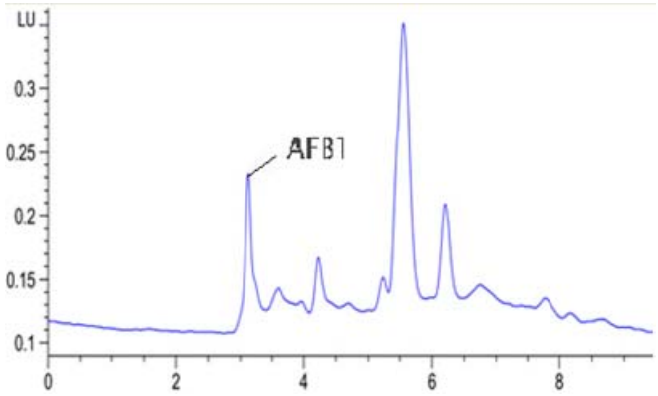

Figure 2: Chromatogram of AFB_1

Here, the concentration of aflatoxin B_1 is set as abscissa and peak area as ordinate. The regression equation is $Y=0.0741X+0.033, R^2=0.9968$. The standard curve of aflatoxin B_1 is shown in Figure 2. From this figure, it can conclude that peak area is proportional with amount of aflatoxin B_1. With a larger peak area, the amount of aflatoxin B_1 is more and the determination coefficient is 0.9968, almost 1. It is said that in the range of 0.20-200.00μg /L, the linear dependence of standard curve derived by HPLC is acceptable, shown as Figure 3.

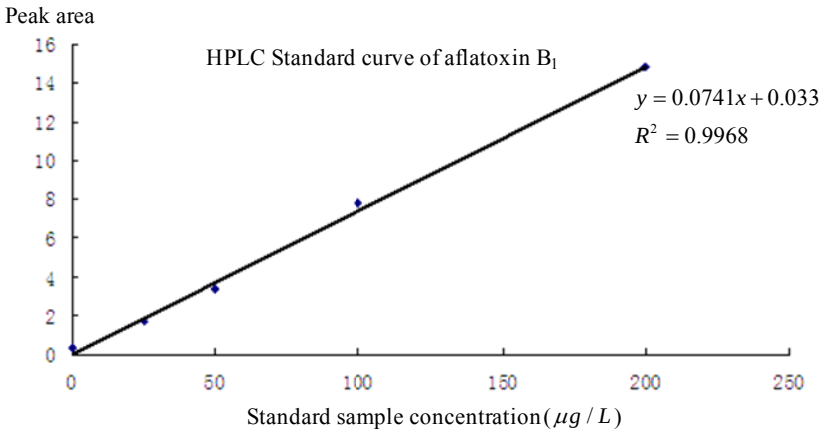

Figure 3: Standard curve of aflatoxin B_1 derived by HPLC

Precision of Aflatoxin B_1 by Using HPLC

In order to derive the precision of aflatoxin B_1 by HPLC, aflatoxin standard solution with the concentration of 0.00μg /L, 25.00μg /L, 50.00μg /L, 100.00μg /L, 200.00μg /L respectively are taken 20μL of three times (shown in Table 1).

Table 1: Precision of Aflatoxin B_1 by Using HPLC

No.	AFB_1 Concentration (μg/L)	Peak Area 1	Peak Area 2	Peak Area 3	Average Peak Area	Calculated Concentration (μg/L)	Relative Error (%)
1	0.00	0.33	0.39	0.36	0.36		
2	25.00	1.28	2.24	1.73	1.75	23.23	7.09
3	50.00	4.21	3.78	2.63	3.54	47.36	5.28
4	100.00	7.77	6.91	8.66	7.78	104.55	4.55
5	200.00	15.09	14.32	15.05	14.82	199.55	0.23

From the table above, it is shown that the relative error (7.09%, 5.28%, 4.55% and 0.23% respectively) is always inferior 10%, the National Standard. So the precision of HPLC is excellent.

Errors of Aflatoxin B_1 by HPLC

Error is derived by the standard addition recovery test. 50μL Aflatoxin solution with the concentration of 0.00μg /L, 25.00μg /L, 50.00μg /L, 100.00μg /L, 200.00μg /L respectively are put into 5g Tartary Buckwheat powder to make the sample with different pollution degrees. After extraction, purification and derivative, the mass of aflatoxin should be detected with HPLC(shown in Table 2).

Table 2: Measure the Standard Addition Recovery Rate of Aflatoxin B_1 in Tartary Buckwheat Powder

No.	Sample AFB_1 Concentration (μg/L)	Addition AFB_1 Concentration (μg/L)	Measured Concertraion (μg/L)	Recovery Rate (%)
1	42.68	12.50	52.71	80.20
2	42.68	25.00	64.21	86.12
3	42.68	50.00	85.38	85.40
4	42.68	100.00	124.38	81.70
5	42.68	200.00	221.68	89.50
Average Recovery Rate				84.58

From Table 2, when the concentration range is between 12.50 and 200.00μg/L, the recovery rate of Tartary Buckwheat powder is between 80.20% and 89.50%. This fits the technology requirements. The average recovery rate is 84.58%, higher than the minimal recovery rate 70%.

Accuracy of Aflatoxin B_1 by Using HPLC

To study the accuracy of aflatoxin B_1 by HPLC, take 5 samples of 5g Tartary Buckwheat noodles, Tartary Buckwheat soup and Tartary Buckwheat tea. After handling, measure the concentration of aflatoxin B_1andcalculate the coefficient of variation (shown as Table 3).

Table 3: Accuracy of Aflatoxin B_1 by using HPLC

Tartary Buckwheat Product	PeakArea1	Peak Area2	Peak Area3	Peak Area4	Peak Area5	Average Peak Area	Calculated Concentration (μg/L)	Coefficient of Variation (%)
Noodle	2.18	2.43	2.00	1.89	2.12	2.12	11.29	9.36
Soup	3.28	2.96	3.03	3.32	3.04	3.13	16.69	5.25
Tea	1.91	1.88	2.01	1.76	2.18	1.95	10.34	8.22

In Table 3, the coefficient of variation of aflatoxin B_1 in Tartary Buckwheat noodle, Tartary Buckwheat soup and Tartary Buckwheat tea are 9.36%, 5.25%, and 8.22% respectively. In International Standard GB/T17480-2008, the coefficient of variation is ruled to be less than 10%. All the data above fit this rule. So the accuracy of HPLC is good.

Sensibility of Aflatoxin B_1 by Using HPLC

Firstly, let the 0.00μg /Lsolutions enter to detect the noise N near the peak. N is 0.0015mAU. And then let the 0.10μg/L, 0.15μg/L, 0.20μg/L, 0.25μg/L of aflatoxin B_1 solutions enter to detect signal S. Then calculate the ratio S/N (shown in Table 4).

Table 4: Sensibility of Aflatoxin B_1 by Using HPLC

Concentration / Peak Height	0.10μg /L	0.15μg /L	0.20μg /L	0.25μg /L
Peak Height S	0.0025 mAU	0.0035 mAU	0.0045 mAU	0.0055 mAU
S/N	1.6	2.3	3	3.6

It is shown as Table 4 that when the concentration of aflatoxin B_1 is 0.2μg /L, the ratio of S/N is 3. So the threshold concentration is 0.2μg /L.

DISCUSSION AND CONCLUSION

This paper built the quality control parameter for HPLC and analyzed the functionality and reliability for HPLC. The result showed all the information as following. Determination coefficient of standard curve, that drawn by HPLC, R^2 is 0.9968, almost 1. It fits the test requirement. When the aflatoxin B_1 solution concentration in the range of 0.20μg /L and 200.00μg /L,the recovery rate of Tartary Buckwheat powder is between 80.20% and 89.50%. This fits the technology requirements.The average recovery rate is 84.58%, higher than the minimal recovery rate 70%.The coefficient of variation of aflatoxin B_1 in Tartary Buckwheat noodle, Tartary Buckwheat soup and Tartary Buckwheat tea are 9.36%, 5.25%, and 8.22% respectively. In International Standard GB/T17480-2008, the coefficient of variation is ruled to be less than 10%. And the threshold concentration is 0.2μg /L.

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