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Monitoring of The Validated Method for Aflatoxin Analysis with Internal and External Quality Control Analysis

Valide Adilmiş Aflatoksin Analiz Metodunun İç ve Dış Kalite Kontrol Analizleri ile İzlenmesi

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Abstract

In this research work, quality control analyses were conducted in order to monitor and evaluate the validated method for the determination of aflatoxins B1, B2, G1 and G2 in food by using immunoaffinity column and liquid chromatography with postcolumn derivation and fluorescence detection. Quality control analyses used in this study are, duplicate sampling, blank analysis, CRM, RM or QCTM material analyses, spike samples analysis, proficiency testing programs, calibration control and the use of quality control samples & control charts. In addition to these analyses, the OQ-PV test, the homogeneity of sample/sampling procedures, which can be considered as part of quality control analyses have also been used.

Private and official food control laboratories should develop inner quality assurance programs for their validated methods and constantly monitor whether these programs have been functioning as specified. At the same time, as provided by the article 5.9 of TS EN ISO/IEC17025 all laboratories must also develop quality control systems in order to monitor the validation of methods and testing. At the same time, they should participate in proficiency tests from time to time.

Keywords: Aflatoxins Validation, Quality Control Analysis; Duplicate Sample; Blank Analysis; CRM, RM or QCTM Material Analysis; Spike Samples Analysis; Proficiency Testing Programs; Calibration Control.

Özet

Bu çalışma da; B1, B2, G1 ve G2 aflatoksinlerinin floresans dedektörlü likit kromatografisi ile kolon sonrası türevlendirme yöntemiyle valide edilmiş analiz metodunun izlenmesi amacıyla farklı kalite kontrol analizleri uygulamaları yapıldı. Bu çalışmada yapılan kalite kontrol analizleri; paralel analiz, blank analiz, QCTM materyalleri analizleri, geri alma analizi, yeterlik test programları, kalibrasyon kontrolü, kalite kontrol numuneleri ve kontrol kartlardır. Bu analizlere ilave olarak, OQ-PV testi, kalite kontrol analizlerinin bir parçası olarak düşünülebilen örnek/örnekleme prosedürlerinin homojenliği de yapıldı. Özel ve resmi gıda kontrol laboratuvarları, onaylanmış yöntemleri için iç kalite güvencesi programları geliştirmeli ve bu yöntemlerin belirtilen şekilde işleyip işlemediğini sürekli izlemelidir. Aynı zamanda, TS EN ISO/IEC17025'in 5.9. maddesinde öngörüldüğü gibi, tüm laboratuarlar, yöntemlerin ve deneylerin geçerliliğini izlemek için kalite kontrol sistemleri geliştirmelidirler. Aynı zamanda, zaman zaman yeterlik testlerine katılmalıdırlar.

Anahtar Kelimeler: Aflatoksin Validasyonu, Kalite Kontrol Analizi, Blank Analizi, CRM, RM veya QCTM Malzeme Analizi, Spike Örnekleri Analizi, Yeterlilik Test Programları, Kalibrasyon Kontrolü.

Abbreviations

CRM:Certified reference material, RM: Reference material, QCTM: Quality control test material, OQ-PV: Operational Qualification-Performance Verification, QC: Quality control

1. Introduction

A new method that will be used by laboratories must be validated or confirmed. Some of the methods used also need revalidation or at least verification. When important changes are made in a method, an entire validation is necessary (Taverniers at al. 2004). When a methodology is changed or adapted to novel circumstances (e.g., various sample matrix, etc.), depending on the nature of the modification and the new situation of the method, for example, when a method for the analysis of the aflatoxin is put in practice for the hazelnut; a revalidation and verification must be necessary. No action is required where a modification is only a small one (Anonymous 2002). A monitoring and evaluation program is not only the collection and laboratory analyses of samples, but it also consists of things more than these. Quality control operations, techniques and activities cover quality requirements. In other words, quality control provides the general precautionary measures a laboratory makes use of to guarantee the quality assurance of its activities (Anonymous 2005). To succeed in this, the proceedings should be monitored, and the performance problems must be resolved. After a method has been validated or verified and implemented, there is a continuous need in any quality assurance system to monitor that the method is still performing within its specifications (Thompson and Wood 1993). Quality control stipulates that methodology of analysis is feasible for the goals, which means the last accomplishment of the analytical effects with the default requirements. The aims of quality control is checking the accuracy of the validated method that is practiced together with the data obtained in the laboratory on a daily basis. In this context, both random and systematic errors that cause deviation errors and inconsistencies should be monitored. To be able to watch these mistakes, they must stay permanent attention. In the laboratory, usually these conditions can be obtained in an analytical study.

Quality Control involves any trial which is fulfilled to show that the conclusions are dependable. The quality control must be practiced in order to check one of every twenty samples analyzed. This process of monitoring involves the on-going quality controls of the method, analysis of binary, CRM analyses, recovery work, external proficiency testing programs, analyses of the samples that are analyzed again, OQ-PV testing devices, controls and calibrators, creation of the control card, homogeneity of sample/sampling and testing of the components of the system (this is sometimes referred to as system suitability testing).

2. Materials and Methods

2.1. Chemicals and Reagents

All reagents used were analytical or HPLC grade. Supelco aflatoxin standard solution consisting 1,0 µg/mL AFB1, 0,3 µg/mL AFB2, 1,0 µg/mL AFG1, 0,3 µg/mL AFG2 were obtained from Sigma-Aldrich (St. Louis, MO, USA, catalog number: 46304-U). Immunoaffinity columns were obtained Romer Labs (Romer Labs Diagnostic, Tulln, Austria). Calibrated equipments were used for analysis.

2.2. Samples and Sample Analysis

Samples of hazelnut, hazelnut paste, peanut, walnut, pistachio, corn samples for the aflatoxin analysis were taken from companies in Trabzon, and sampling was executed in accordance with the Sample Preparation Methodology in Turkish Food Codex sampling for official control of mycotoxin limits in the food, sample preparation and analysis method criteria communication (Anonymous 2011). Analyses were performed according to Single-Laboratory Validation for the Determination of Aflatoxin B1, B2, G1, and G2 in Foods Based upon the Immunoaffinity Column and Liquid Chromatography with Postcolumn Derivation and Fluorescence Detection (Baltacı et al. 2013).

2.3. Parallel Analysis

The parallel samples (one per 20 samples or batch (min. frequency 5%)) were analyzed simultaneously in laboratory. For acceptance of the calculated concentrations for any compound. The 2,8xSDr equation was used for the repeatability limit of 95% confidence interval.

2.4. Blank Analysis

Blank samples were run high concentration (Total AFs of 8,0 □g/kg and 15,0 □g/kg) after every 20 samples. No attempt at the retention time of aflatoxin was detected in all the blind sample analyses.

2.5. QCTM Material Analysis

FAPAS test material (T0495) samples were analyzed once a month. The results obtained were printed on the control cards and evaluated both for the recovery and satisfactory range.

2.6. Fortified Sample Analysis

The hazelnut, hazelnut paste, peanut, walnut, pistachio, corn samples were analyzed in a period of one month Samples spiked with aflatoxins at three different concentrations were prepared to perform the recovery study. The spiked samples (six replicates) were analyzed with the method (Baltacı et al. 2012).

2.7. Proficiency Testing Program

The technical competence of the laboratory was tested by being participated the FAPAS (an organization supports laboratories for analysis and demand their costumer, accreditation and manage) Proficiency Test 04203 in October 2012. FAPAS test material (hazelnut) was analyzed in triplicate. The Z-score calculated by the FAPAS was used to evaluate the results.

2.8. Homogeneity of Sample/Sampling

In this study, 10 kg of the sample "A" was weighed and grounded in laboratory grinder for 2 min at 3600 rpm. The sample "B" was spiked with standard solutions of 1s of 10,0 µg mL-1 AFB1, 5,0 µg mL-1 AFB2, 10,0 µg mL-1 AFG1, 5,0 µg mL-1 AFG2 at the levels of 0,6 mL, 1 mL, 2 mL, and 4 mL, respectively, prior to extraction. These samples were left for 30 min and then grounded for 2 min at 3600 rpm. 100 samples were prepared from both A and B group hazelnuts (each 100 g), and 10 samples were taken randomly and analyzed twice.

2.9. Statistical Analysis

Microsoft Excel Statistical Computer Software was used for data processing (Microsoft Office Excel 2007, Microsoft Corp., Redmond, WA USA). Very high and different results between triple measurements obtained were removed according to the Cochran test and Grubbs test. The linear regression model was performed using the least squares approach.

3. Results and Discussion

3.1. Work Forms and Quality Control

Laboratory notebooks, work paper or form sheets are used by the analyst for two purposes: the former, to record data; and the latter, to edit data on a regular basis. In general, there aren't standard forms for QC in testing laboratories. Most laboratories can usually develop their own forms to record analysis results. The forms are made in a shape that makes it simple to save the data, overview it, and retrieve it when necessary. Our forms include the following items: Date and time of the validated method, head of the unit, duplicate samples, blank analyses, CRM, RM or QCTM material analyses, spike samples analysis, proficiency testing programs, calibration control, use of quality control samples & control charts, homogeneity of sample/sampling. Each of the analyses was determined for each period. For example, participation in proficiency tests is once a year.

3.2. Parallel Analysis of The Samples

Couple sample analysis is performed to determine the consistency of the repeatability of the method used. Couples (divided or parallel samples) that were taken from the same source as parallel samples were processed and analyzed. The analyte is concentrated and tested, thus being parallel between the diversity and acceptance. In general, each parallel analysis is performed for every 20 samples analyzed. For instance, provided 1-20 samples are compiled per sampling, 1 field parallel analysis is necessitated. Provided that 20-40 samples are compiled per sampling fact, 2 field parallel analysis are needed. The samples are parallel-analyzed simultaneously in laboratory and reanalyzed for acceptance of the calculated concentrations for any compound. The 2,8xSDr equation was used for the repeatability limit of 95% confidence interval. Standard deviations of repeatability (SDr) was obtained from the validation report for each aflatoxin. SDr were 0,12, 0,14, 0,19 and 0,18 for AFB1, AFB2, AFG1 and AFG2, respectively. About 360 samples and 18 duplicate samples were analyzed in a period of one month (Table 1). The results of all parallel analyses were found to be suitable.

3.3. Blank Analysis

A blank detection is the working of the samples without the analyte added, or an analysis without a sample. The refinement of testing solution, lab distilled water, and the cleanup of glassware and appliances were observed with blank-monitoring. The analysis of reagent blanks were performed after any sample of high level concentration that may be reason of the contamination to after than sample. Blank samples were run sample of high concentration (Total AFs of 8,0 µg/kg and 15,0 µg/kg) and after every 20 samples. No attempt at the retention time of aflatoxin was detected in all the blind sample analyses (Figure 1). Total 360 samples and 18 blank samples were analyzed in a period of one month.

Table 1. 360 Samples and 18 Duplicates in a Period of One Month.

n	Aflatoxin B_1			Aflatoxin B_2			Aflatoxin G_1			Aflatoxin G_2		
	X_1	X_2	$\lvert X_1-X_2\rvert$	X_1	X_2	$\lvert X_1-X_2\rvert$	X_1	X_2	$\lvert X_1-X_2\rvert$	X_1	X_2	$\lvert X_1-X_2\rvert$
1	3,45	3,65	0,20	0,76	0,71	0,05	1,26	1,35	0,09	0,01	0,01	0,00
2	4,89	4,75	0,14	0,58	0,56	0,03	2,31	2,42	0,11	0,45	0,42	0,03
3	2,85	2,97	0,12	0,45	0,42	0,03	1,92	1,85	0,07	0,53	0,52	0,05
4	3,21	3,20	0,01	0,56	0,55	0,01	2,31	2,15	0,16	0,42	0,41	0,01
5	1,69	1,78	0,09	0,45	0,45	0,00	0,96	0,91	0,05	0,01	0,01	0,00
6	2,03	2,09	0,06	0,36	0,38	0,02	1,23	1,29	0,06	0,52	0,50	0,02
7	3,05	3,09	0,04	0,43	0,41	0,03	1,95	1,84	0,11	0,31	0,32	0,01
8	2,78	2,85	0,07	0,29	0,30	0,01	1,03	1,15	0,12	0,45	0,42	0,03
9	2,55	2,55	0,00	0,27	0,27	0,00	1,56	1,49	0,07	0,29	0,32	0,03
10	7,96	7,85	0,11	0,86	0,89	0,03	3,25	3,02	0,23	0,85	0,80	0,05
11	3,21	3,26	0,05	0,52	0,58	0,06	1,56	1,42	0,14	0,63	0,59	0,04
12	0,98	0,85	0,13	0,01	0,01	0,00	0,01	0,01	0,00	0,25	0,25	0,00
13	1,21	1,23	0,02	0,45	0,43	0,02	0,56	0,52	0,04	0,01	0,01	0,00
14	1,32	1,35	0,03	0,32	0,33	0,01	0,01	0,01	0,00	0,01	0,01	0,00
15	2,31	2,45	0,14	0,29	0,30	0,01	1,05	1,03	0,02	0,23	0,24	0,01
16	3,20	3,10	0,20	0,68	0,64	0,04	0,75	0,85	0,10	0,45	0,46	0,01
17	1,02	1,04	0,02	0,33	0,34	0,01	0,32	0,42	0,10	0,33	0,32	0,01
18	1,75	1,85	0,10	0,45	0,44	0,01	0,56	0,52	0,04	0,10	0,10	0,00
	r_{AFB1}=0,34		acceptable	r_{AFB2}=0,39		acceptable	r_{AFG1}=0,53		acceptable	r_{AFG2}=0,50		acceptable

Toxin contamination was not detected in any of the 18 blank samples. For the method the LOQ values were 0,07, 0,02, 0,07, and 0,03 µg/kg for AFB1, AFB2, AFG1, and AFG2, respectively.

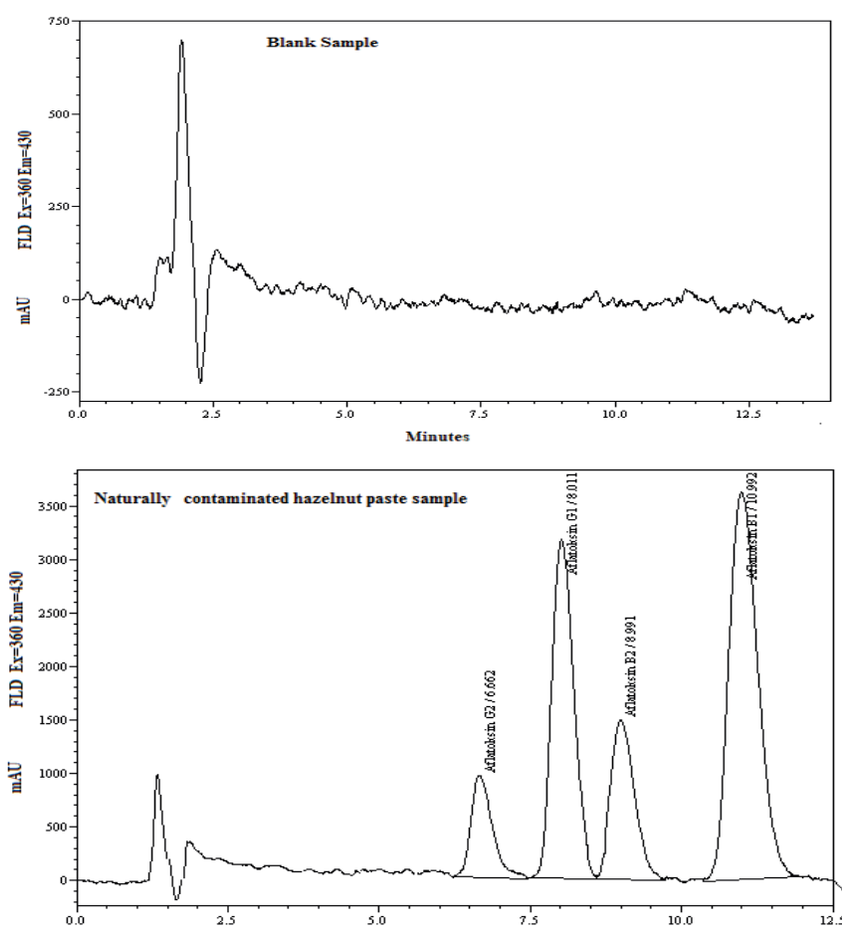

Figure 1: Blank Sample and Naturally Contamined Sample

Table 2. Mean Results of Analysis 12 Months Quality Control Test Material (FAPAS Test Material T0495)

Analyte	Unit	Assigned Value(X)	Satisfactory Range	No. of labs producing X	Our results (mean of 12 months)	Recovery %
Aflatoxin B_1	µg/kg	2,36	1,32 – 3,40	63	2,29	97,0
Aflatoxin B_2	µg/kg	0,93	0,52 – 1,34	61	0,83	89,2
Aflatoxin G_1	µg/kg	1,75	0,98 – 2,52	60	1,59	90,9
Aflatoxin G_2	µg/kg	0,43	0,24 – 0,62	58	0,41	95,3
Aflatoxins (total)	µg/kg	5,39	3,02 – 7,76	62	5,12	95,0

3.4. CRM, RM or QCTM Material Analysis

Certified reference materials that include one or more property values of whose confidence intervals are given for each value of uncertainty (Anonymous 1992) were used for quality control analyses. Reference materials can play an important role in quality guarantee, and help to the operation of formation traceability of units used to report laboratory results. The use of adequate reference materials is important in accreditation for ISO/IEC 17025. QCTM have a few properties of real food matrix that have been obtained from the outputs of the laboratories joined in the proficiency testing. The remaining samples will be utilized by test laboratories in the form of quality control test materials. The values given in the certificate have been obtained from the analysis results of the various analyses methods used in many laboratories. The acceptable working range illustrates the range between which outcomes would have been given an acceptable z-score in the proficiency test. The satisfactory z-results of the proficiency test obtained are thought to be fit for the purpose, and reflect the anticipated inter laboratory reproducibility. CRMs, RMs and QCTM are being widely used in food analyses (Anonymous 2000). Also, FAPAS test material (T0495) was used as the QCTM material in the method. The reclamation is predicted by dividing the determined average value to the certified value. The work of quality control test materials was analyzed once a month. The results obtained were suitable both for the recovery and satisfactory range (Table 2).

3.5. Fortified Sample Analysis

Fortified sample analysis of the method was conducted to test the varying concentrations of the analyte. A certain quantity of analyte was spiked to the sample, and the recovery was calculated. If interferences are present in the sample, results may be obtained which are significantly higher or lower than the actual concentration. To detect the efficiency of a method, fortified experiments can be executed. In practice, control samples are most generally used for fortifying. Analysis of samples is made at least 10 times, and the percentage recovery is calculated. For in-house validation, the repeatability is detected, whereas for the quality control, the inside-laboratory reproducibility is detected and the data recorded on control charts. The concentration degree of the fortification connected with the aim: For routine control work, the level will largely correspond to those of the test samples, and a concentration midway the operating rank is a convenient choice. After the 20 samples, the spike samples were analyzed. The results are given in Table 3

3.6. Proficiency Testing Program

The valuing of analytical performance of a laboratory is fulfilled by participating in inter-laboratory comparisons. The statistical parameters of both precision and accuracy can be tested with these tools which standard deviations, repeatability and reproducibility obtained from the gathered knowledge. In addition, these organizations can be a beneficial resource of QCTM samples which can be offered and used internally by the joining laboratories. The common procedure is that the subsamples of a larger sample are dispatched to joining laboratories at regular intervals. Many times, the subsamples of certain larger samples are dispatched repeatedly without the participants knowing this (Anonymous 1993, Anonymous 1997, Anonymous 1999a and Anonymous 1999b). The technical competence of the Laboratory's was checked by participating in a proficiency test of The Food and Environment Research Agency (FAPAS) Proficiency Test 04203 in October 2012. The FAPAS test material (hazelnut slurry) was examined and the result was sent in. The Z-scores made by the FAPAS were 0,3, 0,0, 0,0, 0,0 and 0,2 for AFB1, AFB2, AFG1, AFG2 and total AFs, and in turn (L.N:11). These values of representing the technical competence of laboratories are below 2 of Z scores (absolute) reference value.

Table 3. The Recovery Values of Spiked Samples at Three Different Concentrations and Different Samples

Spike samples analysis (Recovery %)							
Analyte	**Added Concentration µg/kg**	**Hazelnut**	**Hazelnut Paste**	**Peanut**	**Walnut**	**Pistachio**	**Corn**
Aflatoxin B_1	2,50	101,8	91,3	93,8	94,3	94,8	92,8
	5,00	92,3	92,4	93,3	93,3	93,3	92,3
	7,50	93,4	95,4	101,1	94,2	95,4	96,4
Aflatoxin B_2	0,75	91,5	94,5	91,5	95,5	95,5	100,8
	1,50	95,3	94,3	94,3	102,4	94,3	92,7
	2,25	97,6	95,4	95,6	93,6	96,6	95,6
Aflatoxin G_1	2,50	94,3	91,5	93,5	95,7	93,5	94,5
	5,00	93,5	93,9	102,5	98,5	92,2	95,5
	7,50	96,8	94,8	94,8	94,8	95,8	96,8
Aflatoxin G_2	0,75	83,0	86,1	85,6	89,5	85,6	84,6
	1,50	88,8	87,5	87,8	89,6	82,8	85,0
	2,25	89,3	89,4	89,3	86,9	84,3	83,7
Aflatoxins (total)	8,00	96,9	93,4	93,2	101,5	97,1	92,5
	13,00	93,5	92,3	95,2	98,0	96,7	93,6
	19,50	95,8	94,5	94,4	93,6	98,1	94.5

3.7. Use of Quality Control Samples & Control Graphs

Control graphics was used to detect the statistical control of the experiment. Actually, a control graph is a operate graph (characterized earlier) that contains statistically formed upper and lower check limits (Schafer et al. 2011 yazılacak). The aim of a control graph is to define any undesirable change in the process. This alternation will be indicated by unusual dots on the graph. A broad study by Dr. Shewhart showed that by installing upper and lower limits at three times the standard deviation of the processing (extra and negative, in order of), 99,73% of the general causation variation would decrease within these limits. A procedure is said, thus, to be in statistical control. When the operation measurements vary randomly within the check limits; namely, the variation present in the operation is stable and predictable over time. 10 kg of natural contaminated hazelnut sample was weighed and grinded for 2 min at 3600 rpm. 100 samples were prepared from natural contaminated hazelnut and 12 samples were taken randomly and analyzed twice. Upper check limit (UCL) and lower check limit (LCL) of the process were determined in Figure 2. The Quality Control Samples were analyzed about two times in one month. For each toxins were given figs 2-6 (Total AFs ✦, AFB1 ●, AFB2 ★, AFG1 ⬤, AFG2 ▲)

3.8. Calibration Control

The performance of a chromatographic system may change in the course of time. Prior to calibration, simple standard sample prepared by fortified clean samples in a certain concentration may be used for the calibration control. The peak areas and retention times of peaks in liquid chromatography isolation might be a helpful diagnosis tool to detect the troubles with an isolation. Little alterations (for instance ± 0,1 min) are normal for methods. Alteration in the retention times or peak areas ensure a signal of trouble on the column temperature, mobile phase constitution or column deterioration. The heaping up of sample pollutions by stages influences the functioning of the column with a loss of separation capability and an increase of mobile phase back pressure over time. Control chart for retention times of analytes can be a useful tool to follow the column disruption and to decide when the column needs to be washed or replaced. In addition to the plate number, some constituents can be computed and observed. In our study, the calibration mix standard sample (AFB1 2,00 µg/kg, AFB2 0,60 µg/kg, AFG1 2,00 µg/kg, AFG2 0,60 and total AFs 5,20 µg/kg) was employed. With this standard sample, peak shape, retention times, gradient performance, sensibility (peak area/height) and analytes concentrations were especially evaluated. Calibration was monitored on a daily basis, and the data on the charts were recorded as analyte concentrations. Calibration control studies for each toxins were given figure 2-6 (Total AFs ✖, AFB1 ★, AFB2 ●, AFG1 ✦, AFG2 ▲)

3.9. Homogeneity of Sample/Sampling

The homogeneity of the test material is required for all laboratory studies. The results of experiments can be affected tremendously if materials are not well-homogenized. The homogeneity of the tested materials has to be confirmed. The international homogenized protocol defines a test for adequate homogeneity. Therefore, particular care should be taken in handling and preparation of samples before the analysis.

The IUPAC revised statistical models were used in this study (Michael et al. 2006). According to the harmonized protocol, the Cochran test procedure is proposed for duplicate results. The largest D2max/ΣD2i ratio of each of the naturally-contaminated and non-contaminated hazelnut samples were calculated where Di shows the differences of each pair of duplicates and Dmax showing the largest proportion difference. The calculated ratio was compared with the critical values for Cochran test statistics for duplicates. Outlying pairs were detected at 95% level of confidence. Analytical variance (san2) and sampling variance (ssam2) were estimated by ANOVA. Allowable sampling variance (σall) was calculated as σall=(0,3x σp) where σp is the target standard deviation. Then the critical value for (c) the test is calculated and if the calculated ssam2 value is less than the critical value (c), the test for homogeneity has been passed (Table 4).

4. Conclusions

Today, it is a necessity for all official and private analysis laboratories conducting food analyses to perform validation studies for their methods. Besides, these institutions should develop quality assurance programs for their validated methods and constantly monitor whether these programs have been functioning as specified. At the same time, as provided by the article 5.9 of TS EN ISO/IEC 17025: General Requirements for the Competence of Testing and Calibration Laboratories, all laboratories must also develop quality control systems in order to monitor the validation of methods for calibration and testing (Anonymous 2012). The review of data quality involves several levels of evaluation. In general, the unit chief will be responsible for reviewing and validating. Unit chief should do a complete audit the data generated. The emphasis should be on the data acceptability reliability to the data quality indicators and the accuracy of the final data summaries. All analytical problems encountered during the sample analysis should be properly addressed, documented and resolved. Additional verification by the Unit of Quality Control should be accomplished through routine audits of the data collection and flow procedures and by monitoring the results of Quality Control check samples. The unit chief should carry out minimum monitoring requirements for each validated method. It must be done by selecting the appropriate one or more from the following monitoring conditions.

- The least; one calibration control point, plus blank point and check standard or as method appropriates and continuing calibration check using an AOAC, ISO, vs NIST reference, if available.
- Working of quality control check sample can do included in each analysis or per 20 samples or once in a month whenever possible
- Working of blank samples can do one reagent blank per matrix and per concentration level for every sample batch analyzed (i.e., one for 20 samples or one month).
- Working of samples spike, and sample duplicates per matrix for every sample batch analyzed (i.e., one per 20 samples or one month).
- Review of sample documents for completeness by the analyst(s) at each step of the analysis.
- Review of instrument logs, performance test results, and analyst performance.
- Random calculation checks.
- Review of data with emphasis on reasonability, spectral interference, significant figures, and analysis detection limits.
- As often as possible you should participate in proficiency tests. Or participate in inter laboratory benchmarking studies

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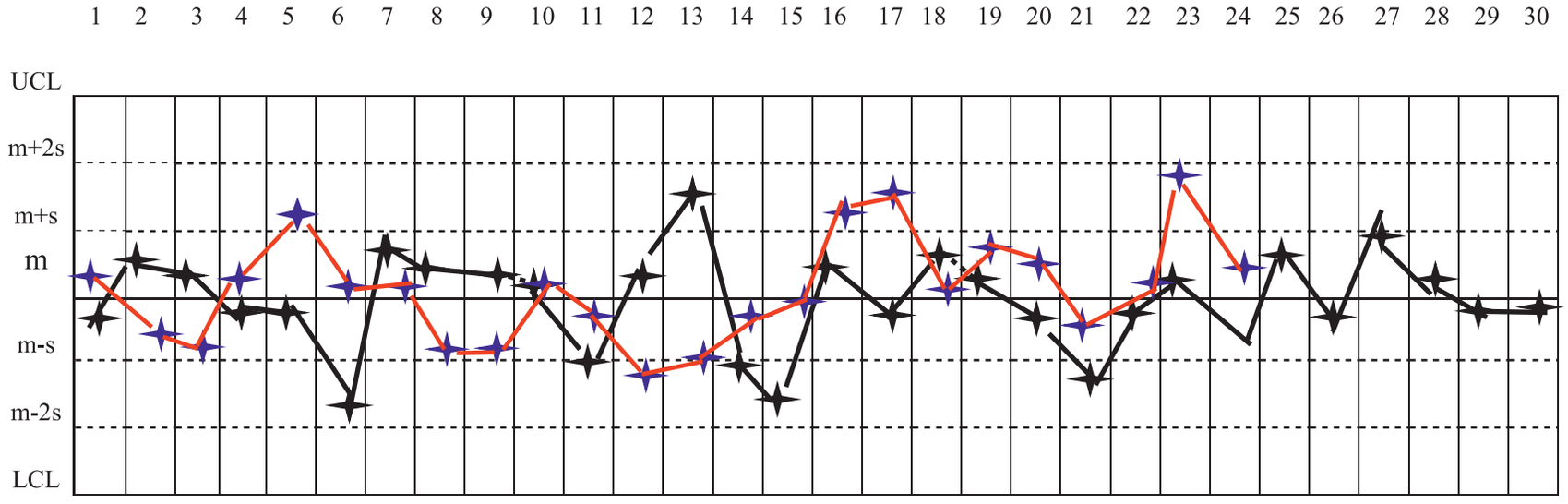
Table 4. Homogeneity Data for Hazelnut Material

	AFB_1 µg/kg		$AF\ B_2$ µg/kg		$AF\ G_1$ µg/kg		$AF\ G_2$ µg/kg		Total AF µg/kg	
Mean, n	1.36	20	0.64	20	2.95	20	0.52	20	5.45	20
origin of target sd (σ_p)	Horwizt	<120 ppb	Horwizt	<120 ppb	Horwizt	<120 ppb	Horwizt	<120 ppb	Horwizt	<120 ppb
abs target sd (σ_p)& RSD_R%	0.299	20	0.141	20	0.649	20	0.114	20	1.199	20
S_{an}^2	0.010		0.0004		0.0002		0.0021		0.007	
S_{am}^2	0.007		0.0001		0.039		0.0021		0.051	
σ_{all}^2	0.08		0.0002		0.038		0.0012		0.129	
critical	0.023		0.004		0.072		0.0043		0.250	
S_{am}^2< *critical ?*	*0.007 < 0.023*		*0.0001 < 0.004*		*0.039 < 0.072*		*0.052 < 0.061*		*0.051 < 0.250*	
	Accept		***Accept***		***Accept***		***Accept***		***Accept***	

Figure 2. Control Chart of Daily Calibration Control (Bolor Black) for 5.20 µg/kg and Quality Control Sample (color blue) of Total AFs



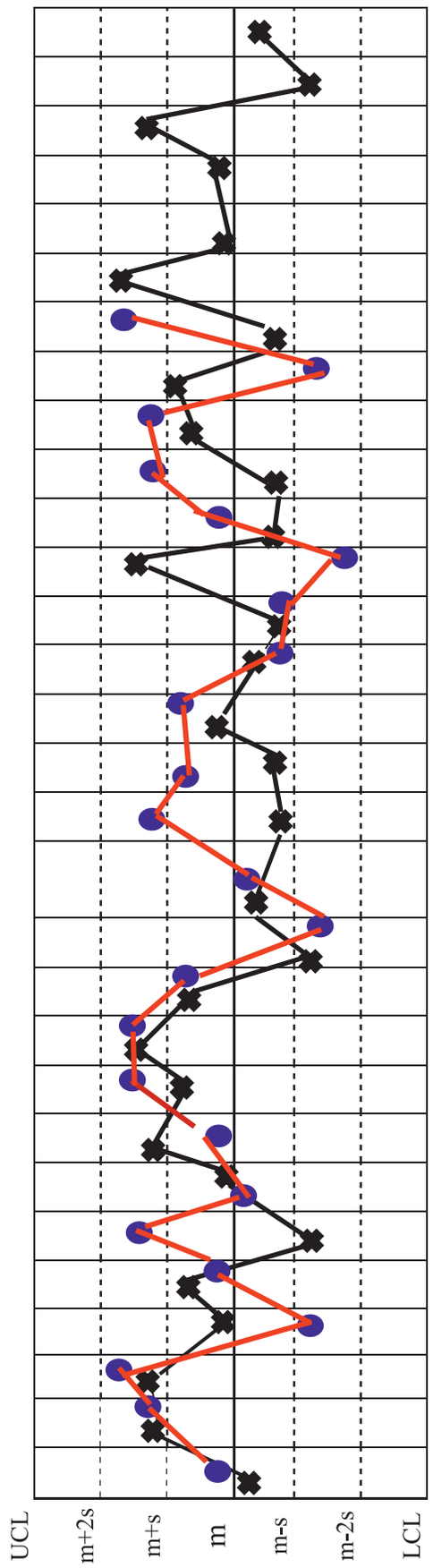


Figure 3. Control Chart of Daily Calibration (Color Black) Control for 2.00 µg/kg and Quality Control Sample (Color Blue) of AFB_1

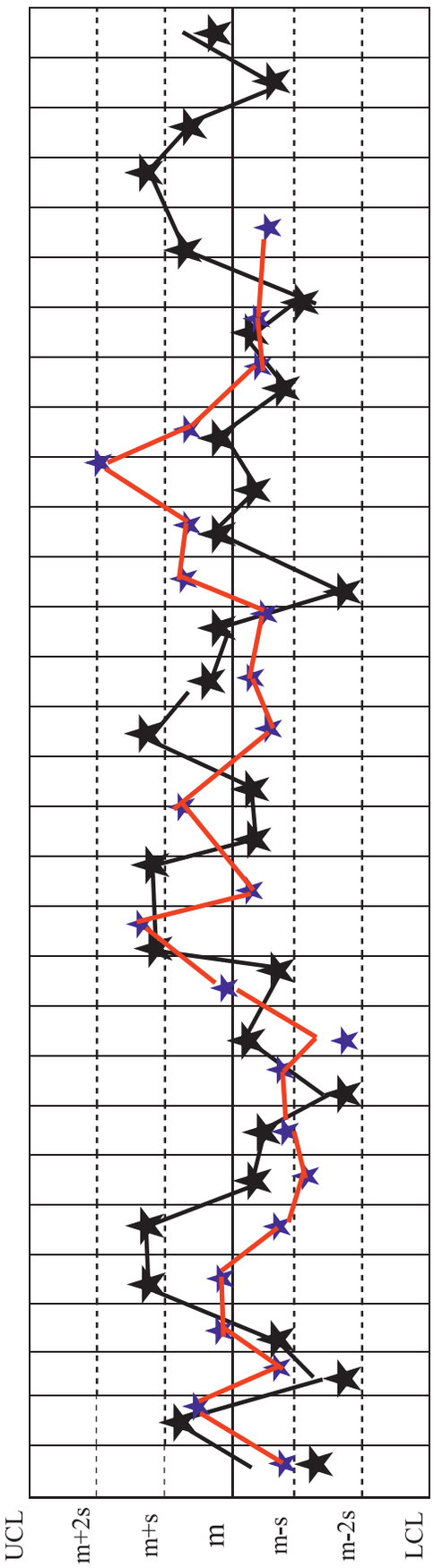


Figure 4. Control Chart of Daily Calibration (Color Black) Control for 0.60 µg/kg and Quality Control Sample (Color Blue) of AFB_2

Figure 5. Control Chart of Daily Calibration (Color Black) Control for 2.00 µg/kg and Quality Control Sample (Color Blue) of AFG_1

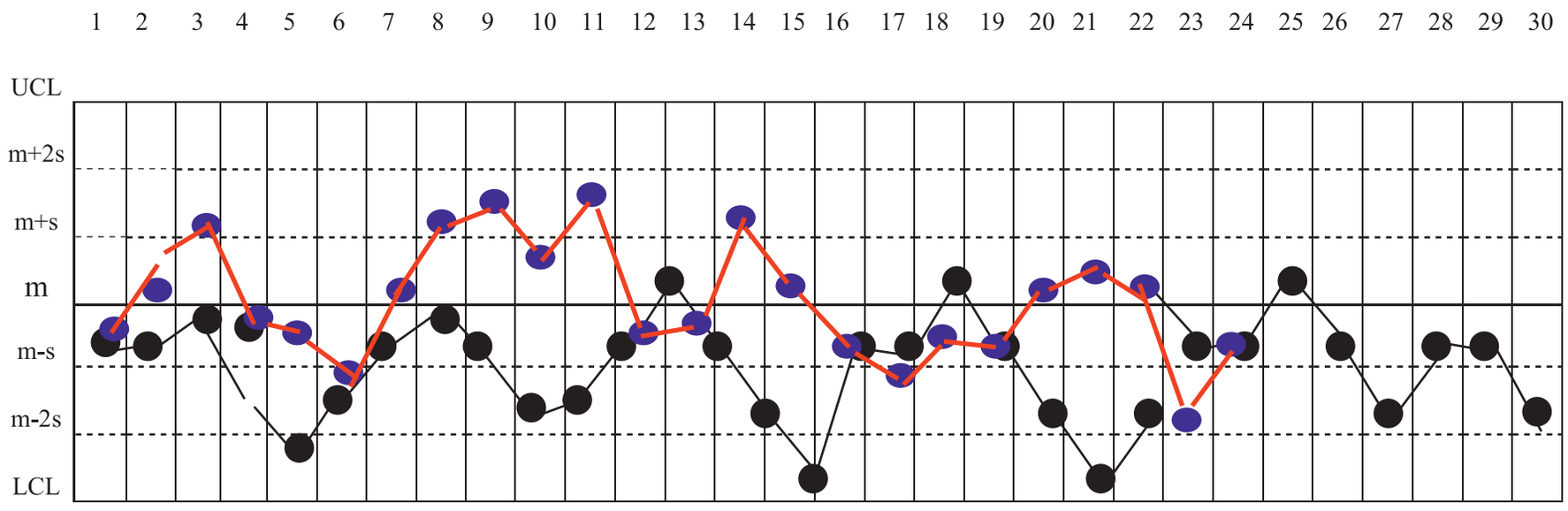


Figure 6. Control Chart of Daily Calibration (Color Black) Control for 0.60 µg/kg and Quality Control Sample (Color Blue) of AFG_2