FOOD CHEMICAL CONTAMINANTS

Immunoaffinity Column Cleanup with Liquid Chromatography for Determination of Aflatoxin M_1 in Liquid Milk: Collaborative Study

SYLVIANE DRAGACCI and FREDERIC GROSSO
Agence Française de Sécurité Sanitaire des Aliments, Unite Toxines Microbiennes, 10, Rue Pierre Curie, 94704, Maisons-Alfort, Paris, France
JOHN GILBERT
Ministry of Agriculture, Fisheries and Food, Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK

Collaborators: M. Agnedal; L. Hyndrick; G. Jamet; K. Jorgensen; J. Miller; L. Oliveira Palavras; A. Pittet; V. Rousi; P. Sharron; E.A. Sizoo; M. Spott; E. Strassmeier

A collaborative study was conducted to evaluate the effectiveness of an immunoaffinity column cleanup liquid chromatographic method for determination of aflatoxin M_1 in milk at proposed European regulatory limits. The test portion of liquid milk was centrifuged, filtered, and applied to an immunoaffinity column. The column was washed with water, and aflatoxin was eluted with pure acetonitrile. Aflatoxin M_1 was separated by reversed-phase liquid chromatography (LC) with fluorescence detection. Frozen liquid milk samples both naturally contaminated with aflatoxin M_1 and blank samples for spiking, were sent to 12 collaborators in 12 different European countries. Test portions of samples were spiked at 0.05 ng aflatoxin M_1 per mL. After removal of 2 noncompliant sets of results, the mean recovery of aflatoxin M_1 was 74%. Based on results for spiked samples (blind pairs at 1 level) and naturally contaminated samples (blind pairs at 3 levels) the relative standard deviation for repeatability (RSD_r) ranged from 8 to 18%. The relative standard deviation for reproducibility (RSD_R) ranged from 21 to 31%. The method showed acceptable within- and between-laboratory precision data for liquid milk, as evidenced by HORRAT values at the low level of aflatoxin M_1 contamination.

Methodology for determination of aflatoxin M_1 in milk improved markedly with the application of immunoaffinity column technology to provide a combined extraction and cleanup stage to the analysis (1). Previously, methods involved either liquid–liquid extraction (2, 3) or solid phase extraction (4) followed by silica gel column or other cleanup (3, 5) with thin layer chromatography or liquid chromatographic (LC) determination. These critically compared methods (6) have formed the basis of full collaborative studies and are still extant as AOAC INTERNATIONAL Official Methods for aflatoxin M_1 in liquid and powdered milk (7). Although an immunoaffinity LC method for determining aflatoxin M_1 in milk powder was collaboratively tested under the auspices of the International Dairy Federation (IDF; 8), the study lacked samples to establish method recovery and was not submitted to AOAC INTERNATIONAL for proposed adoption.

European Commission Regulations (9) for aflatoxin M_1, implemented in January 1999, set a limit of 0.05 ng/mL in liquid milk. The existing AOAC method (10) has not been tested at <0.08 ng/mL, and the IDF method (8) has only been tested for powdered milk at a 10-fold lower limit when expressed on an equivalent weight basis. As part of a project funded by the European Commission Standards Measurement and Testing (SMT) Programme on method validation, a full collaborative study was undertaken at the low European limit required by the new regulations. This validated method will ultimately be submitted for consideration for adoption as a European Standard (CEN), and will be aimed at fulfilling AOAC INTERNATIONAL requirements for a collaborative study.

Because contamination levels involved in the present study were very low, particular care was taken in the preparation, homogeneity testing, packaging, and storage of liquid milk test samples. All laboratories were provided with a common standard of aflatoxin M_1, the concentration of which was confirmed by 3 independent laboratories at the outset of the trial. To ensure that all collaborative trial participants rigorously followed the protocol, a precollaborative trial workshop was held in January 1998. The workshop did not involve any hands-on

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analytical work, but did provide opportunities to discuss and raise any potential difficulties before the start of the trial.

Collaborative Study

Test Materials

Preparation of milk samples.—Naturally contaminated milk was prepared by feeding cows with aflatoxin B_1-contaminated peanut meal. Two cows were fed with 2 kg contaminated peanut meal (containing about 2 mg/kg aflatoxin B_1) added to their daily ration for 3 days. The morning milkings on the fourth day (about 11 L milk) were collected and gently homogenized to keep the fat well dispersed. Analysis of the aflatoxin M_1 content of this milk indicated a level of contamination of 2.2 ng/mL. The milk was stored at –30°C until use.

Control milk (30 L) containing <0.005 ng/mL aflatoxin M_1, determined by the method described in this study, was obtained from a local farm. The milk was stabilized with penicillin at about 0.06 μg/mL, and divided into 12 L for use as the control sample for the study and 18 L for use in blending to generate the naturally contaminated samples.

Preparation of test materials to be sent to laboratories.—Different volumes (68.5, 137, and 274 mL) of the highly contaminated milk (containing 2.2 ng/mL aflatoxin M_1) were diluted with 6 L blank milk in each case. After thorough mixing, the milk was subdivided into 125 mL Nalgene plastic bottles (56 samples in each case). All samples were frozen at –30°C.

Homogeneity testing of milk samples.—Every sixth sample of contaminated milk and every twelfth sample of blank milk were removed from each batch, providing 10 samples of milk in each instance for homogeneity testing. Each sample was analyzed by the method described here in duplicate for aflatoxin M_1 content.

Organization of the Collaborative Study

The 12 collaborators from 12 different European countries represented a cross-section of government, food control, university, and food industry affiliations. Before the trial, each collaborator received a practice sample of blank milk and a calibrant solution for spiking. Collaborators met at a precollaborative trial workshop where any problems experienced with analyzing the practice sample were discussed, and details of the organization of the trial were outlined by the coordinators.

For the collaborative trial, each participant received the following: (*1*) a set of 8 randomly coded samples of liquid milk; (*2*) a pair of blank milk samples for spiking; (*3*) one labeled ampule of aflatoxin M_1 calibrant solution provided by the European Commission, SMT Programme, with an independently established aflatoxin M_1 content of 10 μg/mL; (*4*) 2 ampules of aflatoxin M_1 calibrant solution labeled A and B, with aflatoxin M_1 content unknown to participants; (*5*) 10 immunoaffinity columns containing anti-aflatoxin M_1 antibodies, which were supplied from the same batch; (*6*) a copy of the method of analysis; and (*7*) instructions for undertaking the collaborative study.

Frozen milk samples, together with ice-packs, were sent to the laboratories by express delivery. Each participant was required to prepare one extract from each milk sample and analyze by LC. Participants were also provided with a spiking protocol and 2 bottles of milk blank materials assumed to contain <0.005 aflatoxin M_1 ng/mL. Participants were asked to spike blank materials by opening aflatoxin M_1 ampule A, transfer 50 μL of the calibration solution into a vial, evaporate it to dryness under a gentle stream of nitrogen, and add 1 mL 10% acetonitrile solution. After labeling this solution "vial 1," participants were to agitate it vigorously with a vortex-like stirrer. Then, participants were to transfer 50 μL from vial 1 to 950 μL 10% acetonitrile solution, label this solution as "vial 2," and shake it vigorously with a vortex-like stirrer. Participants were to measure 70 mL of blank milk and transfer 1 mL milk from the 70 mL volume of blank material to a 2 or 2.5 mL tube, and add 140 μL from "vial 2." After shaking vigorously with a Vortex-like stirrer for about 30 s, participants were to dilute this spiked solution in the remaining volume (original volume less 1 mL) of blank material, and shake it again vigorously for a further 30 s. Participants were to analyze this spiked material by following exactly the procedure given in the method protocol, taking a test portion of 50 mL. The whole spiking sequence was repeated with the second bottle of blank material and the aflatoxin M_1 ampule B. This spiking protocol led to a spiking level of 0.050 ng/mL.

AOAC Official Method 2000.08
Aflatoxin M_1 in Liquid Milk
Immunoaffinity Column by Liquid Chromatography
First Action 2000

(Applicable to determination of aflatoxin M_1 in raw liquid milk at > 0.02 ng/mL).

Caution: This method requires the use of solutions of aflatoxin M_1. Aflatoxins are carcinogenic to humans. *See* introductory statement to this chapter (11). Aflatoxins are subject to light degradation. Protect analytical work from the daylight and keep aflatoxin standard solutions protected from light by using amber vials or aluminum foil. The use of non acid-washed glassware (e.g., vials, tubes, flasks) for aflatoxin aqueous solutions may cause a loss of aflatoxin. Special attention should be taken with new glassware. Thus, before use, soak glassware in dilute acid (e.g., sulfuric acid, 110 mL/L) for several hours; then, rinse extensively with distilled water to remove all traces of acid (check with pH paper).

See Table **2000.08A** for the results of the interlaboratory study supporting acceptance of the method.

A. Principle

The test portion is extracted and cleaned up by passing through an immunoaffinity column containing specific antibodies bound onto a solid support. Antibodies selectively bind

with any aflatoxin M_1 (antigen) contained in the extract, to give an antibody–antigen complex. Other components of matrix are washed off the column with water. Aflatoxin M_1 from the column is eluted with acetonitrile. After the eluate is concentrated, the amount of aflatoxin M_1 is determined by LC with fluorometric detection.

B. Performance Standards for Immunoaffinity Columns

The immunoaffinity column shall contain antibodies against aflatoxin M_1 with a capacity of not less than 100 ng aflatoxin M_1 (which corresponds to 2 ng/mL when 50 mL test portion is applied). Recovery of not less than 80% must be obtained for aflatoxin M_1 when a calibrant solution containing 4 ng toxin is applied (which corresponds to 80 ng/L for a loaded volume of 50 mL).

Any immunoaffinity column meeting the above specifications can be used. Check the performance of the columns regularly, at least once for every batch of columns.

C. Apparatus

(**a**) *Disposable syringe barrels*.—To be used as reservoirs (10 and 50 mL capacity).

(**b**) *Vacuum system*.—For use with immunoassay columns.

(**c**) *Centrifuge*.—To produce a radial acceleration of at least $2000 \times g$.

(**d**) *Volumetric pipets*.

(**e**) *Microsyringes*.—100, 250, and 500 μL (Hamilton or equivalent).

(**f**) *Glass beakers*.

(**g**) *Volumetric flasks*.—50 mL.

(**h**) *Water bath*.—37 ± 2°C.

(**i**) *Filter paper*.—Whatman No. 4, or equivalent.

(**j**) *Conical glass tubes*.—5 and 10 mL, stoppered.

(**k**) *Spectrophotometer*.—Wavelength 200–400 nm, with quartz face cells of optical length 1 cm.

(**l**) *Liquid chromatography equipment*.—With pump delivering a steady flow rate of 0.8 mL/min; loop injection system of 50–200 μL capacity; fluorescent detection with 365 nm excitation and 435 nm emission; and recorder, integrator, or computer-based processing system.

(**m**) *Reversed-phase LC analytical column*.—The following columns have been used satisfactorily: Octadecylsilane (ODS, ODS-1, ODS-2, ODS Hypersil, Nucleosil C18 [Machery-Nagel], Chromospher C18, Nova-Pak C18 [Waters Corp.], LiChrosorb RP18 [Merck KGaA, Darmstadt, Germany], Microsphere C18); dimensions (mm): 100×2.3, 4.6, 5; 125×4; 200×2.1, 3, 4; 250×4.6; with and without guard columns.

(**n**) *Mobile phases*.—Water–acetonitrile (75 + 25) or (67 + 33); water–acetonitrile–methanol (65 + 25 + 10); or water–isopropanol–acetonitrile (80 + 12 + 8). Degas before use.

D. Reagents

(**a**) *Chloroform*.—Stabilized with 0.5–1.0% ethanol.

(**b**) *Nitrogen*.

(**c**) *Aflatoxin M_1 standard solutions*.—(*1*) *Stock standard solution*.—1 μg/mL. Suspend a lyophilized film of reference standard aflatoxin M_1 in chloroform to obtain the required concentration. Determine the concentration of aflatoxin M_1 by measuring its absorbance at the maximum (ca 365 nm) in a calibrated spectrophotometer against chloroform as a blank between 200–400 nm. Check purity by noting an undistorted shape of the recorded peak. Calculate the mass concentration (C, μg/mL) from the equation:

$$C = \frac{1000AM}{\varepsilon}$$

where A is the measured absorbance at the maximum wavelength, M is the molecular mass of aflatoxin M_1 (328 g/mol), and ε is the absorption coefficient of aflatoxin M_1 in chloroform (1995 m^2/mol; 12, 13).

Store this stock solution in a tightly stoppered amber vial below 4°C. Solution is stable ca 1 year.

(*2*) *Working standard solution*.—1 μg/mL. Transfer by means of a syringe 50 μL of the standard stock solution, (**c**)(*1*), into an amber vial and evaporate to dryness under a steady stream of N. Dissolve the residue vigorously in 500 μL acetonitrile using a Vortex mixer. Store this solution in a

Table 2000.08A. Interlaboratory study results for aflatoxin M_1 in liquid milk immunoaffinity column LC method

Sample ID	No. of labs, a(b)[a]	$\bar{x}$ Average (ng/mL)	r	s_r	RSD_r, %	R	s_R	RSD_R, %	HORRAT value	Rec., %
Spiked	10 (2)	0.037[b]	0.019	0.007	18	0.032	0.011	31	0.42	74
a	12 (0)	<0.005	—	—	—	—	—	—	—	—
b	12 (0)	0.023	0.011	0.004	17	0.017	0.006	27	0.33	93
c	12 (0)	0.046	0.016	0.006	12	0.029	0.010	23	0.31	94
d	12 (0)	0.103	0.022	0.008	8	0.062	0.022	21	0.33	107

[a] a = Number of labs retained after eliminating outliers; (b) = number of labs removed as outliers.

[b] Spike level = 0.05 ng/mL.

Note: Statistical analysis was not carried out on the blank milk (a).

tightly stoppered amber vial below 4°C. Solution is stable ca 1 month.

(*3*) *Calibrant standard solutions*.—Prepare on day of use. Bring working standard solution, (**c**)(*2*), to ambient temperature. Prepare a series of standard solutions in the mobile phase, **C**(**n**), of concentrations that depend upon the volume of the injection loop in order to inject, e.g., 0.05–1.0 ng aflatoxin M_1.

E. Preparation of Test Solution

Warm milk before analysis to ca 37°C in a water bath, and then gently stir with magnetic stirrer to disperse the fat layer. Centrifuge liquid milk at $2000 \times g$ to separate the fat and discard thin upper fat layer. Filter through one or more paper filters, collecting at least 50 mL. Let immunoaffinity columns reach room temperature. Attach syringe barrel to top of immunoaffinity cartridge. Transfer 50 mL (V_s) of prepared test portion with volumetric flask volumetric pipet into syringe barrel and let it pass through immunoaffinity column at slow steady flow rate of ca 2–3 mL/min. Gravity or vacuum system can be used to control flow rate.

Remove syringe barrel and replace with a clean one. Wash column with 20 mL water at steady flow rate. After washing completely, blow column to dryness with N stream. Put another dry clean barrel on the cartridge. Slowly elute aflatoxin M_1 from column with 4 mL pure acetonitrile. Allow acetonitrile to be in contact with column at least 60 s. Keep steady slow flow rate. Collect eluate in conical tube. Evaporate eluate to dryness using gentle stream of N. Dilute to volume V_f of mobile phase, i.e., 200 μL (for 50 μL injections) to 1000 μL (for 250 μL injections).

F. LC Determination with Fluorescence Detection

Pump mobile phase at steady flow rate through LC column. Depending on the kind of column, the acetonitrile–water ratio and flow rate of the mobile phase may be adjusted to ensure optimal separation of aflatoxin M_1 from other extract components. As a guideline for conventional columns (with a length of 250 mm and id of 4.6 mm), a flow rate of ca 0.8 mL/min gives optimal results. Check optimal conditions with aflatoxin M_1 calibrant solution and spiked milk before analyzing test materials.

Check linearity of injection calibrant solutions and stability of chromatographic system. Repeatedly inject a fixed amount of aflatoxin M_1 calibrant solution until stable peak areas or heights are obtained. Peak areas or heights corresponding to consecutive injections must be within ± 5%. Retention times of aflatoxin M_1 can vary as a function of temperature and must be monitored by injecting a fixed amount of aflatoxin M_1 calibrant solution at regular intervals.

(*1*) *Calibration curve of aflatoxin M_1*.—Inject in sequence suitable volumes V_i, depending on the injection loop, aflatoxin M_1 standard solutions containing from 0.05 to 1 ng. Prepare a calibration graph by plotting the peak area or peak height against the mass of injected aflatoxin M_1.

(*2*) *Analysis of purified extracts and injection scheme*.—Inject suitable volume V_i (equivalent to at least 12.5 mL milk) of eluate into LC apparatus through injection loop. Using the same conditions as for calibrant solutions, inject calibrants and test extracts according to stipulated injection scheme. Inject an aflatoxin M_1 calibrant with every 10 injections. Determine aflatoxin M_1 peak area or height corresponding to the analyte, and calculate aflatoxin M_1 amount W_a in test material from the calibration graph, in ng. If aflatoxin M_1 peak area or height corresponding to test material is greater than the highest calibrant solution, dilute the eluate quantitatively with mobile phase and re-inject the diluted extract into the LC apparatus.

G. Calculation

Calculate aflatoxin M_1 mass concentration of the test sample, using the following equation:

$$W_m = W_a \times (V_f / V_i) \times (1/ V_s)$$

where W_m = the numerical value of aflatoxin M_1 in the test sample in ng/mL (or μg/L); W_a = the numerical value of the amount of aflatoxin M_1 corresponding to area or height of the aflatoxin M_1 peak of the sample extract (ng); V_f = the numerical value of the final volume of redissolved eluate (μL); V_i = the numerical value of the volume of injected eluate (μL); V_s = the numerical value of volume of prepared test portion passing through the column (mL).

Express the results to 3 significant figures.

Ref.: *J. AOAC Int*. **84**, 438–440(2001)

Results and Discussion

Homogeneity of Test Materials

The replicate analysis of every sixth sample of milk from each batch indicated that at all 3 levels, the contaminated milk samples were homogeneous. No trend was observed for either sampling or analysis order for all samples, thus, confirming overall that the samples were homogeneous.

Precollaborative Trial Workshop

Only minor points to clarify details of the method were requested at the workshop. This resulted in a closer definition of the centrifugation conditions (>2000 g but <4000 g) and optimization of the acetonitrile–water ratio for the LC mobile phase.

Collaborative Trial

Participants 3 and 9 reported receiving milk samples that were curdled upon arrival or became curdled after storage. Participants 3 and 9 were sent a second set of samples.

The method protocol allowed a choice of LC column for the analysis, and information was collected on the instrumentation used. A diversity of LC columns (type, dimension, manufacturer) were used by participants. Many participants chose to use short columns (100×4 or 5) to reduce the amount of solvents used. Most participants used the recommended LC mobile phase (water–acetonitrile, 75 + 25) but 2 participants selected a ternary mobile phase (water–acetonitrile–methanol, 65 + 25 + 10) for an ODS-1 column and (water–isopropanol–acetonitrile, 80 + 12 + 8) for an ODS

Hypersil column but without indication of evident advantage. The flow rate for delivery of mobile phase in the LC apparatus varied according to the length of the LC columns.

The cleanup step was carried out manually or with the help of a vacuum system such as the VacElut™ system. No participant chose to use an automated system such as the ASPEC™. However, only one participant used a manual injection system (participant 3). The injection volumes ranged from 10 to 500 μL. No particular analytical effects were observed in relation to this wide discrepancy in the equipment of laboratories, which may be taken as tangible proof of the ruggedness of the method.

Comments from Collaborative Trial Participants

Some comments were made on the reporting sheets from participants. Laboratory 4 observed the possibility that an earlier-eluting peak corresponded to the occurrence of aflatoxin M_2 in the naturally contaminated milk. Irrespective of the identity of this earlier-eluting peak, it did not interfere with the aflatoxin M_1 peak and, therefore, did not hinder its accurate measurement. Laboratory 6 found the recommended acid washing and water rinsing of vials to be problematic. Laboratory 8 reported a different aflatoxin M_1 concentration in calibrant solution (25 μg/mL instead of 10 μg/mL). Laboratory 9 found it better to centrifuge the liquid milk at low temperature, and as with Laboratory 4, detected the presence of aflatoxin M_2 in all positive samples. Laboratory 11 observed that it would be easier to work with a test portion of 40 mL rather than the recommended 50 mL for extracting samples. Finally, except for Laboratory 6 which found that the method protocol was not clear enough with respect to the calculation equation, all other participants had no particular remarks concerning the understanding of the method protocol.

Spiking Experiment for Determining Recovery Yield of the Method

For determining the method recovery, laboratories were asked to undertake the spiking experiment. Laboratory raw data are reported in Table 1. Results from Laboratories 2 and 8 were removed as noncompliant as they had not adequately followed the spiking protocol. The running of Cochran and Grubbs tests did not identify any outliers. For the spiked samples, a repeatability RSD_r = 18%, and a reproducibility RSD_R = 31% were obtained for a mean overall recovery of 74%. Thus, notwithstanding the evident problems with recovery in the case of 3 participants (41, 45, and 51%), the performance characteristics for the spiked samples are still acceptable as confirmed by the HORRAT value of 0.42.

Although outside the scope of the statistical evaluation of collaborative trial data according to the International Harmonized Protocol (14), it was thought worthwhile to examine the influence of recovery on method performance. The data were, thus, reanalyzed after removing individual laboratory results where individual recovery was below an arbitrarily chosen 70%, i.e., removing 5 data sets from Laboratories 2, 3, 5, 6, and 8. This approach to data handling was previously used in consideration of laboratory intercomparison data for BCR, M&T (Measurement and Testing), and SMT certification exercises of reference materials, with 70% chosen as a minimum acceptable recovery. Removal of these data sets increased the mean percentage recovery to 87% and generated significantly better RSD_r and RSD_R values of 14% in both instances, with a HORRAT value of 0.19. The poor score for the 5 laboratories exhibiting a recovery <70 % was clearly related to mishandling in the spiking experiment or in the filtration step, as the milk used in this trial was raw milk, and not an indication of the recovery performance of the method itself. Indeed, labora-

Table 1. Collaborative trial results of determination of aflatoxin M_1 in liquid milk by LC

	Aflatoxin M_1 concentration ng/mL									
Lab ID	0.05	0.05	a[a]	a	b[a]	b	c[a]	c	d[a]	d
1	0.047	0.046	<0.004	<0.004	0.026	0.026	0.052	0.054	0.116	0.116
2	0.028[b]	0.028[b]	<0.0005	<0.0005	0.020	0.017	0.034	0.033	0.084	0.09
3	0.027	0.018	0.008	<0.002	0.021	0.024	0.035	0.045	0.068	0.1
4	0.043	0.045	<0.004	<0.004	0.029	0.030	0.055	0.057	0.127	0.127
5	0.024	0.027	<0.005	<0.005	0.024	0.019	0.044	0.036	0.092	0.1
6	0.030	0.011	<0.005	<0.005	0.020	0.008	0.041	0.025	0.093	0.09
7	0.029	0.042	<0.005	<0.005	0.029	0.028	0.060	0.056	0.134	0.114
8	0.018[b]	0.016[b]	<0.0015	<0.0015	0.025	0.025	0.050	0.046	0.110	0.106
9	0.046	0.044	<0.004	<0.004	0.027	0.028	0.054	0.049	0.118	0.114
10	0.042	0.042	<0.010	<0.010	0.024	0.016	0.048	0.049	0.113	0.114
11	0.035	0.052	<0.005	<0.005	0.009	0.020	0.025	0.041	0.051	0.06
12	0.049	0.044	<0.002	<0.002	0.027	0.030	0.051	0.058	0.120	0.121

[a] a, b, c, d = blind duplicate pairs of naturally contaminated samples.

[b] Noncompliant data (failure to correctly follow spiking procedure).

tories producing poor recoveries declared they had trouble in the filtration step (Laboratories 2, 3, 5, and 6) and Laboratories 5 and 6 had trouble filtering their spiked solutions. Laboratory 6 noted a slight flocculation of milk when dissolving the spiking solution. Laboratory 2 had diluted the calibrant solution in a too large volume of milk (30 mL) instead of the recommended volume (1 mL). Laboratory 8 did not evaporate the chloroform of the standard test portion for the spiking experiment which led to a lack of proper dissolution of aflatoxin M_1 calibrant solution in milk. It is also noteworthy that the LC injection volume for Laboratory 6 was quite small (10 μL) and for Laboratory 8 quite large (500 μL). This could possibly lead to a less accurate estimation in the measurement of the aflatoxin M_1 peak.

Precision Characteristics of Method

Raw data obtained from the interlaboratory study are given in full in (Table 1) and were not corrected for recovery. For the blank milks (sample 'a'), all data with one exception (Laboratory 3 for one sample) were <0.005 μg/L which is unanimously considered as confirmation of the limit of quantification of the method at the signal-to-noise ratio of 5:1. This demonstrated that in no instances were any problems of interferences or co-extractives evident in the analysis of the milk extracts. The statistical evaluation was performed on uncorrected data according to the IUPAC/AOAC International Harmonized Protocol (14). The mean levels, precision parameters, and HORRAT values are given in Table **2000.08A**. No straggling nor outlying data were found. The precision parameters are acceptable when considering the very low studied level of aflatoxin M_1 detection (i.e., below the μg/L level). The RSD_R is <31%. The acceptability of the precision values is confirmed by the very low HORRAT values (0.31–0.42) produced in this trial. There was no evidence of a significant overall improvement in precision data through selecting laboratories on the basis of recoveries above 70%, and subsequent recovery correction of the data.

Comparing results from this interlaboratory exercise to those already published on the validation of an LC fluorescence detection method for aflatoxin M_1 in liquid milk, the interlaboratory precision and HORRAT values are very similar for the same range of aflatoxin M_1 levels (i.e., roughly between 0.03 and 0.60 μg/L). In the present trial, RSD_R values of 21–27% are of the same order of magnitude as those of Tuinstra et al. (11–19%; 8), and better than those given in AOAC Method **986.16** (37–62%; 10), and the RSD_R of 28% obtained by Dragacci and Fremy (15) in proficiency testing where all participants used a very similar protocol.

Recommendation

It is recommended that the immunoaffinity column cleanup method by reversed-phase LC analysis with fluorescence detection be adopted Official First Action for determination of aflatoxin M_1 in liquid milk at >0.02 ng/mL.

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J. Miller, Unilever Research, Sharnbrook, UK

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P. Sharron, State Laboratory, Dublin, Ireland

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E.A. Sizoo, RIVM, Bilthoven, The Netherlands

E. Strassmeier, Federal Institute for Food Analysis and Research, Wien, Austria

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