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NEWER DEVELOPMENTS IN MYCOTOXIN METHODOLOGY

A. E. Pohland, C. W. Thorpe and S. Nesheim

Food and Drug Administration, Division of Chemistry and Physics (HFF-144)

200 "C" Street, S. W., Washington, D. C. 20204

Abstract- The development of methods with high specificity and low limits of determination, as well as screening methods that are convenient for detection and rough quantitation of mycotoxins, has proceeded at an accelerating rate over the past few years. Of particular interest has been the widespread application of high performance liquid chromatographic and immunoassay techniques in mycotoxin methodology. An overview of the latest developments in mycotoxin methodology will be presented with emphasis on the need for attention to sampling and sample preparation, on the basic principles supporting the analytical methodology, on the need for techniques to confirm analyte identification, and on the requirement for high quality reference materials.

INTRODUCTION

It is a real pleasure for me to speak to you this afternoon on a subject which I believe to be basic to all other areas of mycotoxin research; certainly it is the key to assessing the extent and eventually controlling the risk to human health associated with the contamination of foods and feeds by mycotoxins. Any effort made to assess and control exposure to mycotoxins will necessarily rely heavily on the availability of good analytical methodology. It is for this reason that the U.S. Food and Drug Administration, and other organizations as well, have invested a great deal of effort in developing accurate, sensitive and rugged methods for detecting mycotoxins, and particularly aflatoxin, in foods, feeds and biological tissues.

The amount of effort applied to the development of mycotoxin methodology can perhaps best be appreciated by considering the following statistics, which although admittedly imcomplete, can give an estimate of where the effort has been applied and what trends, if any, may be apparent (See Tables I and II). The FDA Mycotoxin Literature file covers the period during which mycotoxin methods were being developed. During that period of time 1339 papers describing a "method" for mycotoxins were entered into the data base; some of these were modifications, extensions or adaptations of other methods. A large number (38%) of these methods were for aflatoxin, and the great majority of all methods were based on classical thin layer chromatography (TLC).

TABLE 1. Mycotoxin Methods in FDA Data Base

Mycotoxin	Analytical Methods			
	Total	%	1977-9	%
Aflatoxin	510	38.1	96	28.5
Citrinin	22	1.6	9	2.7
Ochratoxin	95	7.1	22	6.5
Patulin	85	6.3	30	8.9
Penicillic Acid	41	3.1	12	3.6
Sterigmatocystin	95	7.1	25	7.4
Trichothecenes	150	11.2	35	10.4
Zearalenone	88	6.6	34	10.1
Other	253	18.9	74	21.9
Total	1339	100	337	100

TABLE 2. Mycotoxin Methods in FDA Data Base

Analytical Type	Total	Aflatoxin
TLC	400	
TLC-Densitometry	27	27
HPLC	61	34
Mass Spectrometry	17	
Fluorometry	17	
IR Spectroscopy	8	
UV Spectroscopy	5	
Immunoassay	7	
Polarography	5	
Screening Procedures	18	
Bioassays	Many	

It is apparent on inspection of this data that there has been no slackening of interest in mycotoxin methodology; although there has been a clearly discernible shift in interest away from aflatoxin toward other mycotoxins. There has been a decided trend toward the application of high performance liquid chromatography (HPLC) to the detection and quantitation of mycotoxins; of the 61 such methods developed for aflatoxin, 34 appeared during the 1977-9 period.

In speaking to you this afternoon it may be useful if, at the outset, I clearly indicate those subjects of mycotoxin methodology which I do not intend to address; i.e. I do not intend to outline in detail and review all analytical methods which have been developed for mycotoxins. Nor do I intend to discuss the "most widely used" methods, nor those used or recommended by the U.S. FDA. Rather, I would like to describe some novel approaches to the analysis for some of the more common mycotoxins, some techniques which seem to have high potential for application to mycotoxin methodology, and some new approaches to the confirmation of identity of mycotoxins. Finally I would like to review some of the methods developed recently by my research group in the Bureau of Foods, FDA.

SAMPLING AND SAMPLE PREPARATION

My discussion will concentrate primarily on the detection, quantitation and confirmation stages of the analysis. This should in no way be construed to indicate a lack of importance or applied significance in the sampling and sample preparation stages of an analysis. It must be appreciated that if the analytical sample is not representative of the lot from which it is derived, then the analytical result, no matter how accurate in relation to the sample analyzed, is meaningless. Sampling and subsampling errors can be so large that the coefficient of variation for the analytical method itself becomes insignificant. (Note that a careful, statistically designed sampling plan is essential to control as far as is possible this sampling/subsampling error and to estimate the magnitude of the error). For example, it has been estimated that in the analysis of peanuts for aflatoxin using a 48 lb. sample, 98% of the total error is due to the sampling/subsampling errors, and only 2% is due to analysis error (1). Then if one considers only the analysis error, we find that the biggest factor contributing to this error lies in the preparation of the sample for quantitation (extraction/clean-up). In a recent collaborative study (2) of a method for aflatoxin in cottonseed products the collaborators compared results obtained using both TLC and HPLC (UV and packed cell) in the determinative step. The results showed that the repeatability (within laboratory error) was essentially the same whether the collaborators used TLC or HPLC in the determinative step. The greater precision of HPLC over TLC had only a minor effect on the overall precision of the method.

Of course any analytical procedure for the quantitative estimate of the level of a particular mycotoxin is necessarily dependent upon the integrity of the analytical standard used. The concentration of such standards must be known and checked frequently. This point is often ignored, and although detailed instructions for checking both the purity and concentration of reference standards have been published as part of the "official methods," one frequently finds a lack of awareness on the part of the analyst of the importance of such a check. The result can only be a large increase in the analytical error. For example, in 1978 the Smalley Committee of the American Oil Chemists Society distributed, as part of the Aflatoxin Check Sample Series, a standard solution of aflatoxin B_1, B_2, G_1 and G_2 as one of

samples to be analyzed by measurement against the analyst's reference standard. The major source of error, therefore, in the analysis of this sample should theoretically have been the error associated with the calibration of the standard (c.v. ± 5%) and that resulting from the visual estimation of fluorescence intensity (c.v. ± 20%). Rather than the expected c.v. of ca. ± 25%, the between labooratory c.v. on this solution of the four aflatoxins was found to be ca. ± 90% (3).

SAMPLE CLEAN-UP

The most important, the most difficult and the most time consuming step in any analysis of a natural substrate is that involving clean-up of the extract containing the substance to be analyzed. A great deal of effort has been applied to this step which usually involves liquid-liquid partition, preparatory TLC or open bed column chromatography. Each of these techniques have undesireable characteristics: for example, the first often leads to intractable emulsions; the second is labor intensive and exposes the compound of interest to possible undesirable changes; and the third requires the use of large volumes of solvent. To circumvent these problems Dr. J. D. Lowry of DuPont Instruments, Inc. has developed an automated sample processor which uses centrifugal force to elute a small column packed with a high performance, small particle size packing (<25 u). The technique had been developed originally by Neal and Florini (4) to desalt a small volume of protein solution, and by McGhee (5) to separate formaldehyde from polynuclear hydrocarbons.

In this procedure a 4.0 x 0.7 cm i.d. column with a 12 ml reservoir is prepared by pipetting 5 ml of a slurry of the column packing material (e.g. 75 g TLC silica gel/225 ml solvent) onto the column. The column is placed into a 16 x 125 mm culture tube, placed into a centrifuge, and centrifuged at 1000 RPM for 2 minutes. The result is a uniformly and reproducibly packed column. To use this column, the crude sample extract is pipetted onto the top of the column; the column is then eluted using centrifugal force with the most polar solvent system possible to remove extraneous materials but not the compound of interest. Finally the analyte is eluted with the appropriate solvent.

Dr. Lowry has applied this technique in the development of a new microprocessor-controlled, sample preparation system recently marketed by DuPont (6) which can handle up to 12 samples simultaneously. This system uses an extraction cartridge consisting of an upper cap, clean-up column, effluent cup and recovery cup. The sample extracts are applied to the clean-up columns in these cartridges, which are loaded into the "dual fluid path" rotor of the centrifuge, while the rotor is turning in a clockwise direction. This direction of rotation aligns the exit tip of the column with the effluent cup. After elution of the column with a suitable clean-up solvent incapable of eluting the analyte, the direction of rotation is reversed to align the exit of the column with the recovery cup. The appropriate elution solvent is then applied to the column to elute the analyte which is then collected in the recovery cup. The solvent may then be evaporated if desired by a stream of dry air directed into the recovery cups. The result is the automatic clean-up of up to 12 extracts using a micro-processor controlled instrument to yield clean, dry extracts for further analysis by GLC, HPLC, etc. Efforts are currently underway to apply this technique to mycotoxin methodology.

Let us consider now the detection-quantitation phases of the analytical method. The major analytical techniques applied in the past to the detection of mycotoxins are thin layer chromatography (TLC), gas-liquid chromatography (GLC), and high performance liquid chromatography (HPLC). In addition to these basic techniques, there have been a plethora of less extensively used procedures, including a large number of bioassay procedures, procedures based upon IR and UV spectrophotometry, immunoassay procedures and colorimetric procedures. Advances in each of these areas are detailed below.

THIN LAYER CHROMATOGRAPHY

An excellent review of the factors effecting the TLC analysis for aflatoxins has recently been published (7). Included are excellent discussions of such important considerations in performing a TLC analysis as type of adsorbent, binder, plate backing materials, proper preparation and care of TLC plates, the proper handling of standards, spotting techniques, developing solvents, and the like. These considerations apply whenever a TLC analysis is contemplated, for any material, and I heartily recommend this paper to you.

In recent years several important advances have occurred in the application of TLC analytical techniques to mycotoxin analysis. One of these has been the switch to two-dimensional TLC techniques in which the sample is developed in one direction using a given solvent, followed by development in a second direction, perpendicular to the first, using a second solvent. The technique is particularly applicable to "dirty" extracts, which because of large amounts of extraneous materials, make identification and quantitation by one-

dimensional chromatography difficult. In effect one is using the TLC in one direction as a clean-up step, and the TLC in the second direction as the actual detection-quantitation step. A two-stage development also allows reaction of the separated spot with a specific reagent before the second development; i.e. derivative formation for confirmation.

Recently a new technique was developed at FDA (8) for accomplishing TLC clean-up when the interferences are slower-migrating than the compounds of interest. The technique is simple and involves the use of a deep solvent-trough. In using this technique one first develops a 20 x 20 cm. plate using a standard trough until the solvent front reaches R_f of ca. 0.4. The plate is removed and the solvent evaporated. The target compound is located, a score is drawn across the plate about 2 cm. below the target compound, which may be derivatized for identification. The plate is then placed in the deep trough and solvent is added until the solvent level is ca. 0.5 cm. above the score line. The plate is then further developed in the normal fashion with a more polar solvent than the original one. The slow moving, interfering compounds cannot develop past the score line; those materials that work off the plate are diluted sufficiently by the solvent not to effect the second stage development. The result is, effectively, a double TLC development using a single direction. The two dimension development unfortunately introduces a degree of imprecision in the quantitation, the extent of which is currently being explored.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

In recent years high performance liquid chromatography has become popular for many reasons including: (a) the high precision and excellent sensitivity of the modern analytical HPLC instruments; (b) the ease of adaptation to a wide variety of compound types; (c) the ability to analyze easily degraded (by heat, light, air) compounds; (d) ease of adaptation to confirmatory techniques; and (e) high potential for automation. These advantages may be obtained without the purchase of expensive equipment. For example, one can easily construct a complete HPLC system for aflatoxin analysis for as little as $5365.00 (1978 list prices - (See Table 3). Such a system has been in use in our laboratories for some time and I recommend it heartily to you.

TABLE 3. Construction of a High Pressure Liquid Chromatograph

Pump - Milton Roy (5000 psi)	$600.00
Valve - Valco	350.00
Syringe - Hamilton	40.00
Column - (SS., 30 cm x 4.6 mm id)	275.00
Amplifier Noise Filter	300.00
Fluorichrom Analyzer (Varian)	2200.00
Recorder (Hewlett-Packard)	1600.00
	$5365.00

The original HPLC procedures involved the use of an ultraviolet (UV) detector set at 350-365 nm; as a result such methods were relatively insensitive (detection limits of >10 ng/g) because of the frequent presence of UV absorbing materials in the sample extract. With the advent of sensitive fluorescence detectors and good reverse phase columns, detection limits improved dramatically. This resulted in one instance from the finding that aflatoxins B_{2a} and G_{2a} in solution were much more fluorescent than B_1 and G_1 (9). A method was subsequently developed based upon these considerations which has a limit of detection for aflatoxin B_1 in corn as low as 0.75 ng/g and an absolute detection limit on pure standard material as low as 10 pg (10). Recently a procedure capable of detecting M_1 at the 0.5 ppb level has been developed for aflatoxin M_1 in milk based upon the formation of the "M_1-hemiacetal" derivative and fluorescence detection (11).

The next major advance resulted from the observation, known for many years, that the aflatoxins fluoresce more strongly when adsorbed to silica gel than in solution (12). A flow-through cell was therefore incorporated into the fluorescence detector of a liquid chromatograph (13-14). The result was an extremely sensitive detector in which the fluorescence intensity of the aflatoxin adsorbed bands was close to that observed on a TLC plate. A procedure was subsequently published using this idea for detection and quantitation of aflatoxin in corn with a lower limit of detection of lng/g (12); the major advantage of this technique of course is that it obviates the need for preparation of the hemiacetal derivative. When this technique was incorporated into a rapid procedure for the detection of aflatoxin in cottonseed products it was found in a collaborative study that there was no

appreciable difference in analysis repeatability when HPLC or TLC was used in the determinative step, although the between laboratory error component was less when HPLC was used (15). A commercial fluorescence detector containing a LiChrosorb Si-60 packed flow cell is available (16) which gives excellent resolution of the four aflatoxins. The silica gel column is quite sturdy and can be used for many hundreds of analyses provided the extracts are properly cleaned-up. Our analysts have found they can prepare their own flow cells. They are easily dry packed with silica gel of 30-35 um size. It is important, in order to prevent overloading of these columns, that the HPLC column packing material have a smaller surface area than that of the packed flow cell. New standard curves must be prepared for each new packing material or flow cell, since the intensity ratios change with packing material.

One of the major advantages of HPLC is the potential for the application of detectors specific to chemical structural types, e.g. UV detectors for UV-absorbing compounds, fluorescence detectors for compounds which fluoresce strongly, polarographic detectors for anthraquinones, etc. (Table 4) Recently a detector was described (17) which uses a 325 nm He-Cd ion laser excitation source. The laser beam is focused on a 4 ul droplet of solvent eluting from the analytical column. The droplet serves as a windowless cell. The aflatoxin fluorescence is then detected. Using this technique as little of 750 fg aflatoxin B_{2a} may be detected; the lower limit of detection in corn was found to be ca. 2 ng/g. The technique has also been applied to the detection of zearalenone in corn (27).

TABLE 4. HPLC Methods for Mycotoxins

Analyte	Substrate	Detection Limit	Basis	Reference
Aflatoxin	Corn	2 ng/g	Laser-Fl.	17
Ergot Alkaloids	Plant extracts		UV	18
Ochratoxin	Grains, Coffee	50 ng/g	Fl.	19
Patulin	Apple Juice	1 ug/l	UV	20
Penicillic Acid	Grains, Beans Swiss Cheese	20 ng/g	UV	21
Roquefortine	Cheese	16 ng/g	UV Electro-chemical	22
Sterigmatocystin	Corn, Oats	25 ng/g	UV	23
Satratoxins G & H	Grains	200 ng/g	UV	24
Xanthomegnin	Corn	750 ng/g	UV	25
Zearalenone	Corn	10 ng/g	Fl.	26
		5 ng/g	Laser-Fl.	27

An important feature of any analytical method involves confirmation of identity of the entity measured. This is a matter of major importance in regulatory analysis. The HPLC technique is easily adapted to confirmation procedures, first because of its non-destructive nature, and second because two different detectors may be placed in series with no loss in sensitivity for either one. In many of the published HPLC procedures provisions are made for collection of the compound of interest as it elutes from the detector; the identity of the compound is then confirmed by mass spectrometry. Although no confirmatory techniques of this nature based upon infrared (IR) spectrophotometry have been developed, there is no reason to believe, with the advent of FT-IR, that such a confirmation might not be equally as valid. The HPLC methods for aflatoxin, ochratoxin, patulin, penicillic acid, roquefortine, sterigmatocystin, xanthomegnin and zearalenone listed in Table 4 include a mass spectrometric confirmation step. In the case of roquefortine, UV and electrochemical detectors were placed in series to provide two independent detection systems, the one complementing the other. In the case of zearalenone confirmation of identity was attained by measuring the fluorescence intensity using four different excitation wavelengths. In this confirmation procedure the fluorescence response is compared to that obtained from standard zearalenone under identical conditions (fluorescence ratioing).

MISCELLANEOUS PROCEDURES

The emphasis placed on TLC and HPLC procedures does not mean that other analytical techniques are being neglected (Table 5). For some mycotoxins and for some types of samples

other techniques are preferred. For example, many of the trichothecenes are very difficult to detect and quantitate using either TLC (they are non-fluorescent) or HPLC (they absorb poorly). These compounds at the present time are most amenable to analysis by GC coupled with a mass spectrometer for confirmation of identity (28). Many other mycotoxins have been detected and quantitated by GLC including aflatoxin, patulin, and penicillic acid. There is little advantage to these methods over the simpler and generally more sensitive TLC and HPLC methods. However, Salhab, et al. (31) has reported a GC-MS procedure for the analysis of wheat, rice, barley and corn for sterigmatocystin using gel permeation for extract clean-up; levels of sterigmatocystin as low as 1 ng/g were estimated using single ion monitoring. GLC capillary columns have been applied to the detection of zearalenone in corn (32); the method was sensitive to ca. 100 ppb. The major advantage of glass capillary GLC is the high resolution obtained on these columns; the application of the technique to mycotoxin analysis is relatively new, although capillary columns have been used for some time for flavor component analysis.

TABLE 5. Selected Mycotoxin Methods - GLC

Analyte	Substrate	Analysis Type	Detection Limit	Reference
T-2, DAS	Grain, Corn	GC-FID -EC	20 ng/g	29
T-2, HT-2	Milk, Corn	GC-MS	300 ng/g	30
Deoxy-nivalenol	Corn, Wheat	GC-FID -EC	50 ng/g	19
Sterigmatocystin	Grains	GC-MS	1 ng/g	31
Zearalenone	Corn	GC-FID	100 ng/g	32

TABLE 6. Selected Mycotoxin Methods - Immunoassay

Analyte	Substrate	Analysis Type	Detection Limit	Reference
Aflatoxin	Urine	RIA	750 pg/ml	33
	Peanut butter	RIA	1 ng/g	33
Aflatoxin	Urine	RIA	10 pg/ml	34
	Blood			34
Aflatoxin	Biological fluids	EIA	10 pg/ml	35
Aflatoxin		RIA		36
Ochratoxin	Biological fluids	RIA	20 ng/g	37

There has been a great deal of interest in the development of simple, rugged, specific immunoassay procedures for detection of various mycotoxins in biological fluids and tissues where only limited amounts of samples are available, where extremely low concentrations are expected, and where large numbers of analyses (many samples) are expected. Several assay techniques have been developed (See Table 6); however, none of the procedures have been found to be generally applicable for a variety of reasons including: the lack of antibody specificity, the requirements for expensive radio counting instrumentation, and the lack of confirmation of identity capability at the extremely low levels of detectability. A great deal of effort is currently being applied, therefore to the development of monoclonal antibodies, and enzyme and spin immunoassay techniques, which would overcome some of these undesirable characteristics in the current methods.

SCREENING PROCEDURES

In the analysis of large numbers of food and feed samples, most of which are mycotoxin free, it is desirable to have available simple, economical, rugged and practical screening procedures which can be used by inexperienced field personnel. The first such method was developed by Holaday in 1968 (38) for detection of aflatoxin in peanuts. It involved a small (75 mm x 4 mm id.) glass dip column filled with silica gel. The column was dipped into the

aflatoxin containing extract, the aflatoxin eluted up the column by capillary action of the solvent, and finally, the aflatoxin was detected by fluorescence on the column under longwave UV light. Many modifications of this original idea have since been made (39). The most widely used procedure today is the modified Velasco minicolumn (40, 41) which consists of a glass tube (15 cm x 6 mm id.) with a glass wool plug in the bottom, covered with a layer of calcium sulfate, a layer of florisil, a layer of silica gel, a layer of alumina, and finally a layer of calcium sulfate. The aflatoxin containing extract is placed on the top of this column and eluted with chloroform/acetone (9+1). The calcium sulfate acts as a drying agent and the alumina removes most colored impurities; the aflatoxin elutes to the top of the Florisil layer to which it becomes tightly bound. The column is then examined under longwave UV light. A rough estimate of the amount of total aflatoxin present can be made by comparing the fluorescent intensity of the band with that of a reference column prepared with a known amount of aflatoxins. Such analyses will generally pick up aflatoxins in most commodities at levels as low as 5 ppb; a positive result should trigger a second analysis by conventional quantitative methods. It is now possible to purchase inexpensive prepacked minicolumns (42).

Although the minicolumn technique has been applied principally to the detection of aflatoxin, including aflatoxin M_1, (43), minicolumn methods are now available for the detection of ochratoxin A in barley down to ca. 12 ppb (44) and zearalenone in corn (45) at the 40 ppb level.

Most multimycotoxin detection procedures based upon TLC may be considered to be screening procedures in that such procedures are rarely quantitative, are not normally amenable to confirmation of identity and are rapid in terms of total information gained. For example, Gimeno (46) recently published a TLC procedure for detecting aflatoxins, ochratoxins, sterigmatocystin, zearalenone, citrinin, T-2 toxin, diacetoxyscirpenol, penicillic acid, patulin and penitrem A in mixed feeds. Carrying the idea to its extreme, Durackova et al. (47) published a TLC procedure for the systematic analysis of 37 mycotoxins. The procedure represents a relatively inexpensive technique using a combination of different solvent systems and a series of spray reagents for characterization of the mycotoxins. These procedures, however, are not a substitute for an intelligent conjecture based on commodity, mold flora, and circumstance.

With the finding of a high incidence and level of aflatoxin in corn, a great deal of effort was applied to the development of a fast, inexpensive way of detecting contamination in the field and at the commodity purchasing station. Such a screening procedure was developed (48) based upon the detection of a bright green-yellow (BGY) fluorescence under longwave UV light associated with corn contaminated by aflatoxin. It has been found that the molds which produce aflatoxin also produce kojic acid in copious amounts; this kojic acid, in the presence of the peroxidase enzymes normally found in living plant tissues, is converted into the BGY-fluorescing compound. Consequently the presence of this BGY-fluorescence may be taken as presumptive evidence for the co-occurrence of aflatoxin. However, one must realize that not all commodities susceptible to aflatoxin contamination have the necessary enzymes to form the kojic acid reaction product, the observation is inapplicable if the commodity has been treated in such a way as inactivate the enzymes. Also, the fluorescent reaction product is water soluble and therefore may be leached from the substrate. To date the technique has been applied successfully only to corn although laboratory studies have demonstrated BGY-fluorescence in other grains inoculated with A. flavus (49).

Davis and Diener (50) recently developed a new approach to the screening of corn for aflatoxin eliminating most of the organic solvents normally used. The technique has been designated as the fluorimetric iodine rapid screening (FL-IRS) method. In this procedure the corn (10g) is extracted with methanol-water (8+2), the extract cleaned up using a zinc-acetate precipitation, and the filtered extract treated with an aqueous iodine solution. The fluorescence of the resulting solution is then measured using a simple photofluorometer. The method reportedly requires 7-8 minutes and ca. 5 cents worth of reagent per sample and gives fewer false answers than the BGY screen.

Of course there are many other types of screening procedures including a myriad of bioassay procedures, which generally lack specificity, and an interesting spectrophotometric procedure for detection of ochratoxin A in barley (51) based upon the use of carboxypeptidase A to cleave ochratoxin A to ochratoxin α and phenylalanine. In the latter procedure detection is based upon the loss of fluorescence intensity due to conversion of ochratoxin A (380 nm max.) to ochratoxin α (340 nm max.). All such procedures should trigger a second, more conventional analysis.

METHODS DEVELOPED AT FDA

I should like to conclude this summary of newer methods of mycotoxin analysis with a summary of the methods developed at FDA in the last several years (see Tables 7 and 8). Details of each method may be found in the Tables. Notice that each method includes a

TABLE 7. MYCOTOXIN METHODS DEVELOPED AT BF/FDA (1978-9)

-AFLATOXIN-

Mycotoxin	Commodity Analyzed	Sample Size	Extraction Purification	Chromatography	Quantitation	Detection Limit (Recovery)
Aflatoxin B_1 (52)	Eggs	100g	Acetone-H_2O $Pb(OAc)_2$ Pet Ether $CHCl_3$	Silica Gel $CHCl_3$-Acetone(9+1)	2D TLC Anh. Et_2O Acetone-$CHCl_3$(1_9) Densitometry	0.1 ng/g 75%
Aflatoxin B_1 M_1 (53)	Liver Kidney Muscle	100g	NaCl-Citric Acid Acetone $Pb(OAc)_2$, $(NH_4)_2SO_4$ Pet Ether $CHCl_3$	Silica Gel $CHCl_3$-MeOH(97+3)	2D TLC $CHCl_3$-Acetone-iPrOH (85-10-5) Et_2O-MeOH-H_2O (96-3-1)	0.1 ng/g B_1-100-14% $M_1$123-10%
Aflatoxin B_1 (54)	Ginger Root Oleoresin	50g(Root) 0.7(Oleo)	CH_2Cl_2 Root MeOH-NaCl(Oleo) Hexane CCl_4 CH_2Cl_2	Silica Gel CH_2Cl_2 Benzene-HOAc(9+1) Et_2O $CHCl_3$-Acetone(8+2)	TLC Acetone-$CHCl_3$(1+9)	5 ng/g 83%
Aflatoxin B_1 M_1 (28)	Urine	30ml	$CHCl_3$	Silica Gel Acetone-$CHCl_3$(2+8)	RIA HPLC-Fluorescence	20 pg/ml B_1-79% 200 pg/ml

TABLE 8. MYCOTOXIN METHODS DEVELOPED IN THE BF/FDA (1978-9)

Mycotoxin	Commodity Analyzed	Sample Size	Extraction-Purification	Chromatography aq.	Quantitation	Detection Limit (Recovery)
Ochratoxin A&B (19)	Corn Oats Wheat Barley Green Coffee	25g	$CHCl_3$-H_3PO_4 (10+1)	Celite-aq.$NaHCO_3$ Benzene-HOAc (98+2)	HPLC - Fluorescence TLC - Silica Gel Benzene-MeOH-HOAc (95+5+5)	50 ng/g 85%
Penicillic Acid (21)	Corn Oats Barley Pinto Beans Swiss Cheese	50g	Ethyl Acetate 3% $NaHCO_3$ 1N HCl Ethyl Acetate	Silica Gel Hexane-Ethyl Acetate-gl. HOAc (750+250+2)	HPLC - UV (270 nm) TFA Derivative TLC - Screening-Adsorbosil-1 p-Anisaldehyde	20 ng/g 70-80%
Roquefortine Isofumigaclavine A (22)	Blue Cheese Blue Cheese Dressing	25g	Ethyl Acetate aq. HCl 3% $NaHCO_3$ Ethyl Acetate	-	TLC - Screening (Silica Gel) $CHCl_3$-MeOH-NH_4OH(90-10-1) MeOH-H_2SO_4 HPLC UV (312 nm) Electrochemical	15 ng/g 70-80%
Trichothecenes Deoxynivalenol (19)	Corn Wheat	100g	Acetone-H_2O Ethyl Acetate	Silica Gel CH_2Cl_2-MeOH(95+5) CH_2Cl_2-Acetone(80+20)	GC - FID or EC Silyl Ethers	50 ng/g 70%
T-2 Toxin Diacetoxyscirpenol (29)	Corn Oats Wheat Barley	60g	aq. Ethyl Acetate MeOH-HOAc-$(NH_4)_2SO_4$ CH_2Cl_2 Hexane	Silica Gel CH_2Cl_2-Acetone(95+5) CH_2Cl_2-Acetone(80+20)	GLC - FID or EC Silyl Ethers	20 ng/g 102% (T-2) 84% (DAS)
Satratoxins G&H (24)	Corn Oats Wheat Barley Rice	50g	MeOH-H_2O(55+45) Hexane $CHCl_3$	Silica Gel Hexane Ethyl Ether MeOH-$CHCl_3$(3+97)	HPLC - Silica Gel Cell UV (254 nm)	200 ng/g (?) 65% (G) Wheat 71% (H)
Xanthomegnin (25)	Corn	50g	$CHCl_3$-H_3PO_4	-	HPLC - UV (405 nm) $CHCl_3$-MeOH-HOAc (98+1+1)	750 ng/g 41%
Zearalenone (26)	Corn	50g	aq. $CHCl_3$ aq. NaOH Benzene-Citric Acid	-	HPLC - Fluorescence $CHCl_3$-MeOH	10 ng/g 89%

confirmation of identity. For the immediate future our efforts will be directed principally toward the development of sensitive techniques for detection of the trichothecene type mycotoxins, toward the development of detailed procedures for the mass spectrometric confirmation of identity of aflatoxin and to the development of newer methods for detection, quantitation and confirmation of mycotoxins by HPLC. I hope that I have given to you, in this report, an indication of the great amount of interest, effort and success in the area of mycotoxin methodology which has occurred in the last few years, and the excitement, pride and feeling of accomplishment which is generated by work in this important area of research.

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