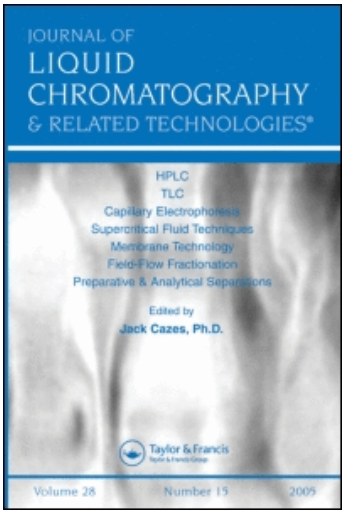

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Thin Layer Chromatographic Analysis of Mycotoxins: A Review of Recent Literature

Louise S. Lee[a]; Dorothy B. Skau[a]
[a] U.S. Department of Agriculture, Southern Regional Research Center[(1)], New Orleans, Louisiana

To cite this Article Lee, Louise S. and Skau, Dorothy B.(1981) 'Thin Layer Chromatographic Analysis of Mycotoxins: A Review of Recent Literature', Journal of Liquid Chromatography & Related Technologies, 4: 11, 43 — 62
To link to this Article: DOI: 10.1080/01483918108069351
URL: http://dx.doi.org/10.1080/01483918108069351

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(Suppl. 1), 43-62 (1981)

THIN LAYER CHROMATOGRAPHIC ANALYSIS OF MYCOTOXINS: A REVIEW OF RECENT LITERATURE

Louise S. Lee and Dorothy B. Skau

Southern Regional Research Center(1)
U.S. Department of Agriculture
New Orleans, Louisiana 70179

ABSTRACT

A review of the recent literature pertaining to thin layer chromatographic (TLC) separation of 17 mycotoxins is presented. It includes limits of detection, preferred visualization techniques, and solvent systems. Where applicable, TLC detection is compared with high pressure liquid chromatographic (HPLC) detection.

INTRODUCTION

Toxic secondary metabolites, termed mycotoxins, are relatively small molecular weight compounds. They often have chemical characteristics conducive to chromatographic separation coupled with an outstanding physical property enabling detection of extremely small quantities. Fluorescence of the aflatoxins is an example. Because of these physical and chemical properties, thin layer chromatography (TLC) has been

used for separation and quantitation of mycotoxins. This detection technique is being challenged by high pressure liquid chromatography (HPLC), a more sophisticated, precise, expensive chromatographic technique. However, because of the expense and sophistication of HPLC, TLC remains the most convenient means of detecting and quantitating mycotoxins.

Whether detection is by HPLC or TLC, in all cases the sample to be examined must be extracted in a system that ideally removes all of the toxin without also extracting excessive amounts of other materials. The mixture should be further purified without destruction of the toxin. These preliminary column or partition chromatographic steps are a critical part of the analysis; they are dictated by the substance being analyzed as well as by the particular mycotoxin. This review covers mainly the TLC step in separation and detection in mycotoxin analysis rather than complete methodology. It covers TLC in single-assay procedures, multiple-toxin separations and, where applicable, a comparison between TLC and HPLC detections. Toxins listed in Table 1 are found as contaminants of food and feed. Those listed in Table 2 are toxins seldom found as natural contaminants, but are produced by molds infecting food and feed. The question arises with this group - would they be found as natural contaminants if methods of detection were more sensitive? Most methods use the comparison of intensity of an

Table 1

Toxins Occurring Naturally in Food or Feed

Toxin	Producing Fungus	Food or Feed Source
Aflatoxins	Aspergillus flavus	Peanuts, cottonseed, corn, copra, rice
B_1, B_2, G_1, G_2, M_1, M_2, parasiticol	Aspergillus parasiticus	
Ochratoxins A+B	Penicillium viridicatum	Grains
Zearalenone (F-2)	Fusarium graminearum	Corn
Penicillic acid	Penicillium cyclopium	Corn
Patulin	Penicillium patulum	Apples
Citrinin	Penicillium citrinum	Barley, wheat, rye, oats

aliquot of the sample with an aliquot of the standard. Comparison of the sample and standard spot is always made on the same plate because the adsorbent layer affects the spot intensity. Availability of pure standards is then necessary.

Aflatoxins

The major closely related toxins, B_1, B_2, G_1, G_2, M_1, M_2, and parasiticol are considered together. There are numerous TLC techniques in the separation of these highly toxic and sometimes potent carcinogens. Schuller et al. (2) reviewed the sampling plans and collaboratively studied methods for their

analysis. Methods judged suitable for use by agencies in regulatory control are termed "Refereed." These are published in Chapter 26, AOAC (3). The official method for given commodities is prescribed. TLC procedures for each commodity are described in detail. Jones (4) gives an excellent survey of qualitative and quantitative TLC methods for aflatoxins, and Scott (5) a similar one for other mycotoxins. Silica gel is the preferred coating for most aflatoxin analyses. Durackova et al. (6) describe the quantitation of aflatoxin along with 36 other mycotoxins. They prepared plates from silica gel G (Merck, Darmstadt, G.H.R.) 0.25 mm thick (7). Before use, the plates were cleaned by developing them in the solvent system that was subsequently used for their development with the samples. They developed plates in eight solvent systems, each to a height of 10 cm and compared Rfs of the aflatoxins to those obtained on precoated silufol sheets (Kavalier, Votice, Czechoslovakia) developed in the same solvents. Chloroform:acetone (9:1) and chloroform:methanol (4:1) were the solvent systems best separating aflatoxins B_1, B_2, G_1, G_2, and M_1. In addition to fluorescence in UV, they used p-anisaldehyde as a spray reagent. B_1 appears yellow in UV, B_2 green, G_1 pink, G_2 green-blue, and M_1 orange. Stubblefield (8) found isopropyl alcohol:acetone:chloroform (5:10:85) and Adsorbisil 1 plates (Applied Science Laboratories, Inc., State

College, Pa.) best for separating aflatoxin B_1, B_2, G_1, G_2, M_1, and M_2 and parasiticol. They used the method recommended by Pohland (9) to make the water adducts of various toxins. These adducts increase fluorescence intensity and allow greater sensitivity in toxin detection. Such derivative formation increases fluorescence, and hence sensitivity. Levels of 0.01 to 0.02 µ/kg of aflatoxins can be detected in purified extracts of eggs by the adduct formation (Trucksess, 10). Heathcote and Hibbert (11) found that freeze-drying of extracts before TLC much improved the subsequent separation of aflatoxins. They found that SilicAR TLC-7G (Mallinckrodt) was the best of the five adsorbents tested for preparative TLC. Any plates containing acid could cause the formation of catalyzed hydration derivatives. Such plates are not recommended for aflatoxin analyses. When standards alone are spotted on Adsorbisil-1 plates, 2 ng can be visualized by fluorescence (12).

Ochratoxins

Ochratoxin A (13), and ochratoxin B (14) have been encountered as natural contaminants in foodstuffs. Methods of analysis of foodstuffs for ochratoxins have been well reviewed by Nesheim (15). Several chemical methods have been developed, with limits of detection as low as 2 µg/kg. (16,17). Nesheim (16) recommends SilicAR TLC-7G, Adsorbisil-1 (Applied Science

Laboratories, Inc.), or silica-gel G-HR (Brinkmann Instruments, Inc.) as TLC adsorbents and quantitates by fluorescence. Trenk (17) recommends exposure to ammonia as an alternative quantitation technique. Scott (18) used Adsorbisil-5 silica-gel plates developed in toluene:ethyl acetate:formic acid (6:3:1) and benzene:methanol:acetic acid (24:2:1) as developing solvents. Fifty µg was spotted, but authors did not state that this was the lowest level detectable.

Citrinin and Zearalenone

These mycotoxins are possible contaminants of corn. Stoloff (19) describes a multimycotoxin method, later modified by Scott (20), that can be used for citrinin. Marti (21) dipped inactivated silica gel 60 (F-254) in 10% oxalic acid. Twenty ng was the smallest amount of citrinin detectable by its yellow-green fluorescence in UV. Stubblefield (22) detected 10 ng on TLC plates prepared with Na_2EDTA developed in acetic acid:benzene (5:95). An excellent review of assay procedures for zearalenone is given by Shotwell (23). Scott (20) and Durackova et al. (6) included this mycotoxin in studies discussed earlier. Recently Malaiyandi (24) published on the use of a bis-diazotized benzidine spray that forms a brick-red derivative with zearalenone on TLC plates. Eppley (25) used aluminum chloride to enhance zearalenone fluorescence. In a study on maize in Africa, Martin (26) used this spray to detect

zearalenone on plates developed in toluene:ethyl acetate: 90% formic acid (60:30:10). In his 1978 report, Scott (27) used Fast Violet B salt as a spray reagent and could detect 5 ng of zearalenone on a TLC plate as compared with 2 ng zearalenone detection claimed by Malaiyandi (24).

Patulin

This mycotoxin is a natural contaminant of apple juice. Scott (28) detected patulin by TLC with phenylhydrazine hydrochloride as a spray reagent (detection limit 100-300 µg/L of juice). In 1973 Scott (29) improved sensitivity to enable detection of 20-25 µg/L or 10 ng patulin/spot. They developed silica gel plates with toluene:ethyl acetate: 90% formic acid (5:4:1) and sprayed the dried plate with 0.5% 3-methyl-2-benzothiazolinone hydrazone (MBTH) hydrochloric acid solution. Patulin appears as a yellow-brown fluorescent spot. Young (30) detected patulin by TLC of its aniline imine. Quantitation was effected by measurement of fluorescence.

Penicillic Acid

Although this mycotoxin is found as a natural contaminant of food, relatively few TLC methods for its detection have been reported. Wilson (31) used Adsorbisil-1 precoated plates (Applied Science Laboratories, Inc.) developed in glacial acetic acid:benzene (1:9). They visualized penicillic acid by

first spraying with 3-methyl-2-benzothiazolinone hydrazone (MBTH) hydrochloride solution, then heating 10 min. at 130°C. The penicillic acid derivative appears as a yellow fluorescent spot under longwave UV. Fifty nanograms penicillic acid/spot can be detected. As an alternative, for quantitation Ciegler (32) proposed exposure to ammonia fumes - a reaction that forms a fluorescent derivative. Scott (28) utilized a phenylhydrazine HCL spray reagent.

Toxins from Table 2

Methods for the detection of these toxins are fewer because interest is not as great. Interest is not as great because they have not been found in food. It follows that development of methods of sufficient sensitivity may reveal such toxins in foods and feeds (33).

Table 2

Potential Feed Toxins

Toxin	Producing Fungus	Potential Feed Source
Vomitoxin	Fusarium graminearum	Corn
Xanthomegnin	Penicillium viridicatum	Cereals
Viomellein	Penicillium viridicatum	Cereals
Secalonic acid	Pencillium oxalicum	Corn
Sterigmatocystin	Aspergillus versicolor	Coffee, cereals

Sterigmatocystin

This mycotoxin is a potent carcinogen (34) and is produced by Aspergillus versicolor, a mold found on certain foods. Durackova et al. (6) included sterigmatocystin in a study in which they developed cleaned TLC plates (silica gel G) in chloroform:methyl isobutyl ketone (4:1) and visualized by p-anisaldehyde and $FeCl_3$ spray. Shannon (35) developed plates in benzene:ethanol:acetic acid (90:5:5) and visualized with aluminum chloride. They recommend a confirmatory test by spraying with trifluoroacetic acid. Athnasios (36) also recommends aluminum chloride spray reagent and developed plates in benzene:acetic acid (10:10). Reiss (37) used a gray scale to semiquantitate sterigmatocystin.

Vomitoxin

This toxin is a trichothecene produced by Fusarium graminearum that causes vomiting by swine ingesting extremely mold-damaged corn. An excellent analytical method developed by Vesonder (38) used TLC as an alternate to GLC for quantitation. They used silica gel 60 plates with the fluorescor 254 developed in $CHCl_3$:CH_3OH (80ml:2ml + 1 drop of water). Vomitoxin quenches fluorescence and appears as a dark spot in short wave UV. On spraying the plates with p-anisaldehyde and heating at 110°C, vomitoxin appears as a yellow spot in visible

light. Another method proposed by Romer and Boling (39) utilized a similar TLC system and reports sensitivity of 0.1 to 3 µg per gram of sample.

Secalonic Acid

Secalonic acid has been isolated from Penicillium oxalicum, a common fungus in cereals. Steyn (40) identified secalonic acid as the major toxin produced by five strains of this species. Ciegler (41) quantitated by TLC on Brinkmann precoated plates (Silica Gel 60, F-254, 0.25 mm thick) using benzene:ethyl acetate:formic acid (100:40:10) for developer. As in vomitoxin, quenching of fluorescence is used for quantitation; 0.5 µg of standard could be detected.

Xanthomegnin and Viomellein

These pigments are two secondary metabolites of Penicillium viridicatum and P. oxalicum, molds that have been isolated from corn. TLC methods for their detection (42) utilize benzene:methanol:acetic acid (18:1:1) or toluene:ethyl acetate:formic acid (6:3:1) for developing on Bakerflex silica gel plates. A serial dilution procedure was used to detect the minimal amount of pigments by visual observation. After standing for 6 hours, xanthomegnin turns from yellow to orange and viomellein turns from yellowish green to yellowish brown.

Exposure to ammonia fumes turns the pigments from yellow to purple. Detection limits were 0.1 µg for xanthomegnin and 0.3 µg for viomellein. The lack of sensitivity of detection methods no doubt accounts for the lack of detection of these toxins in foods and feeds. Formation of derivatives may help in increasing sensitivity.

Other Toxins

Scott, in an excellent review (5) states that preparative TLC has been used to isolate tremortin A (penitrem) from grain extracts. Rubritoxin B, alternariol, and trichothecenes such as T-2 toxins can also be detected using TLC. Rubratoxin B forms a fluorescent spot after heating the TLC plate at 200°C, alternariol and its monomethyl ether are fluorescent under shortwave UV; and the trichothecenes are visualized with 20% H_2SO_4 at 100°C or p-anisaldehyde at 130°C. T-2 toxin then gives a blue fluorescence under UV.

Multimycotoxin Methods

Several screening methods have been proposed for the simultaneous detection of a number of mycotoxins (43, 44, 45). Stoloff (19) describes a TLC method for aflatoxins, ochratoxins, zearalenone, sterigmatocystin, and patulin in a number of agricultural products. They used silica gel plates with internal fluorescors developed in benzene:methanol:acetic

acid (18:1:1) or hexane:acetone:acetic acid (18:2:1) and viewed developed plates under both short and longwave UV. The limits of detection ranged from 20 µg (aflatoxin) to 450 µg of patulin per kilogram of commodity. Josefsson (44) reports similar detection limits: 5 µg aflatoxin, 10 µg ochratoxin A, 50 µg patulin, 10 µg sterigmatocystin and 35 µg zearalenone per kilogram of commodity by using gel filtration as a cleanup procedure prior to TLC. The method is primarily intended for the simultaneous detection of several mycotoxins, each of which may then be quantitated separately by TLC. These separate TLC methods are similar to those described in this paper for the individual toxins. Roberts and Patterson (45) describe a membrane cleanup procedure to determine 12 mycotoxins. Sensitivity was not as good as that of the Josefsson method (44). As stated in the introduction, the sensitivity of the method is generally governed by the commodity being examined as well as the mycotoxin. Cottonseed, for example, contains many pigment glands and materials that interfere with TLC analysis of mycotoxins. These interfering substances should be removed without destroying the mycotoxin in question.

HPLC vs TLC for Mycotoxin Detection

Since the advent of HPLC this analytical technique has been applied to the quantitation of several mycotoxins. A paper by

Seitz (46) showed that the four major naturally occurring aflatoxins could be separated on a microparticulate silica column. UV detection was used, but an increase in sensitivity for the two G toxins can be achieved by the use of fluorescence detection (47, 48). Takahashi (49) used reverse phase partition for separation. Zimmerli (50) and Panalaks and Scott (51) have shown a considerable improvement in sensitivity for aflatoxins by the use of a fluorimeter flow cell packed with silica gel. Detection limits of 0.02 µg/L of wine are claimed by Zimmerli (50). Pons and Franz (52) used this cell in their method for detecting aflatoxin in cottonseed products.

Several other naturally occurring toxins have been investigated by HPLC. These include patulin in apple juice (53-56),ochratoxin, penicillic acid (57, 58) and zearalenone (59-60). Holder (61) used GC with HPLC in his chromatographic analysis of zearalenone. Ito et al. (62) and Stack et al. (63) used HPLC for the detection of sterigmatocystin and xanthomegnin (64).

Because HPLC is a relatively new detection technique, comparisons between detection by TLC and HPLC are limited. Results from studies of two mycotoxins--zearalenone and aflatoxins show little difference between the two detection systems. Pons et al. (65) collaboratively studied a cottonseed method in which TLC and HPLC were interchangeable for

quantitation of aflatoxins, and Scott (27) showed a similar correlation for zearalenone.

HPLC is the preferred method for patulin (53). As more sensitive detectors are developed for HPLC quantitation, it may be that this detection technique will be preferred for all mycotoxins. But TLC is now the most widely used, economic, and certainly the easiest means for quantitating mycotoxins.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Alex Ciegler for advice in organization.

REFERENCES

(1) One of the facilities of the Southern Region Science and Education Administration, U.S. Department of Agriculture.

(2) Schuller, P. L., Horwitz, W., and Stoloff, L., A review of sampling plans and collaboratively studied methods of analysis for aflatoxins. J. Assoc. Off. Anal. Chem. 59, 1315 (1976).

(3) Association of Official Analytical Chemists. Natural Poisons: mycotoxins. Official Methods of Analysis, Ed. 13. Washington, 1980, Method 26.001-26.132.

(4) Jones, B. D., Methods of aflatoxin analysis. Trop. Prod. Inst. Rep. G-70 (1972).

(5) Scott, P. M., Thin layer chromatographic methods for mycotoxins other than aflatoxins. FDA By-lines 6, 305 (May 1977).

(6) Durackova, A., Betina, V., and Nemec, P., Systematic analysis of mycotoxins by thin-layer chromatography. J. Chromatogr. 116, 141 (1976).

(7) Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

(8) Stubblefield, R. D., Shotwell, O. L., Shannon, G. M., Weisleder, D., and Rohwedder, W. K., Parasiticol: a new metabolite from Aspergillus parasiticus, J. Agric. Food Chem. 18, 391 (1970).

(9) Pohland, A. E., Yin, L., and Dantzman, J. G., Rapid chemical confirmatory method for aflatoxin B_1. I. Development of the method, J. Assoc. Off. Anal. Chem. 53, 101 (1970).

(10) Trucksess, M. W., Stoloff, L., Pons, W. A., Jr., Cucullu, A. F., Lee, L. S., and Franz, A. O., Jr., Thin layer chromatographic determination of aflatoxins B_1 in eggs, J. Assoc. Off. Anal. Chem. 60, 795 (1977).

(11) Heathcote, J. G. and Hibbert, J. R., Improvements in the thin-layer chromatography of natural products, J. Chromatogr. 108, 131 (1975).

(12) Lee, L. S., Personal Observation.

(13) Van der Merwe, K. J., Steyn, P. S., Fourie, L., Scott, De B., and Theron, J. J., Ochratoxin A, a toxic metabolite produced by Aspergillus ochraceus Wilh, Nature (London) 205, 1112 (1965).

(14) Steyn, P. S. and Holzapfel, C. W., The synthesis of ochratoxins A and B., metabolites of Aspergillus ochraceus Wilh, Tetrahedron 23, 4449 (1967).

(15) Nesheim, S. The ochratoxins and other related compounds, Adv. Chem. Ser. No. 149, 276 (1976).

(16) Nesheim, S., Hardin, N. F., Francis, O. J., Jr., and Langham, W. S., Analysis of ochratoxins A and B and their esters in barley using partition and thin layer chromatography. I. Development of the method, J. Assoc. Off. Anal. Chem. 56, 817 (1973).

(17) Trenk, H. L. and Chu, F. S., Improved detection of ochratoxin A on thin layer plates, J. Assoc. Off. Anal. Chem. 54, 1307 (1971).

(18) Scott, P. M., Lawrence, J. W., and van Walbeek, W. Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts. Appl. Microbiol. 20, 839 (1970).

(19) Stoloff, L., Nesheim, S., Yin, L., Rodricks, J. V., Stack, M., and Campbell, A. D., A multimycotoxin detection method for aflatoxins, ochratoxins, zearalenone, sterigmatocystin, and patulin, J. Assoc. Off. Anal. Chem. 54, 91 (1971).

(20) Scott, P. M., van Walbeek, W., Kennedy, B., and Anyeti, D., Mycotoxins (ochratoxins A, citrinin, and sterigmatocystin) and toxigenic fungi in grains and other agricultural products, J. Agric. Food Chem. 20, 1103 (1972).

(21) Marti, L. R., Wilson, D. M., and Evans, B. D., Determination of citrinin in corn and barley, J. Assoc. Off. Anal. Chem. 61, 1353 (1978).

(22) Stubblefield, R. D., Thin layer chromatographic determination of citrinin, J. Assoc. Off. Anal. Chem. 62, 201 (1979).

(23) Shotwell, O. L., Assay methods for zearalenone and its natural occurrence, In Mycotoxins in Human and Animal Health; proceeding of a conference, eds., J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman. Pathotox, Park Forest South, Ill., 1977, p. 403.

(24) Malaiyandi, M., Barrette, J. P., and Wavrock, P. L., Bis-diazotized benzidine as a spray reagent for detecting zearalenone on thin layer chromatoplates, J. Assoc. Off. Anal. Chem. 59, 959 (1976).

(25) Eppley, R. M., Stoloff, L., Trucksess, M. W., and Chung, C. W., Survey of corn for Fusarium toxins, J. Assoc. Off. Anal. Chem. 57, 632 (1974).

(26) Martin, P. M. D. and Keen, P., The occurrence of zearalenone in raw and fermented products from Swaziland and Lesotho, Sabouraudia, 16, 15 (1978).

(27) Scott, P. M., Panalaks, T., Kanhere, S., and Miles, W. F., Determination of zearalenone in cornflakes and other corn-based foods by thin layer chromatography, high pressure liquid chromatography, and gas-liquid chromatography/high

resolution mass spectrometry, J. Assoc. Off. Anal. Chem. 61, 593 (1978).

(28) Scott, P. M. and Somers, E., Stability of patulin and penicillic acid in fruit juices and flour, J. Agric. Food Chem. 16, 483 (1968).

(29) Scott, P. M. and Kennedy, B. P. C., Improved method for the thin layer chromatographic determination of patulin in apple juice, J. Assoc. Off. Anal. Chem. 56, 813 (1973).

(30) Young, J. C., Fluorescence detection and determination of patulin by thin-layer chromatography of its aniline imine, J. Environ. Sci. Health, B14, 15 (1979).

(31) Wilson, D. M., Tabor, W. H., and Trucksess, M. W., Screening method for the detection of aflatoxin, ochratoxin, zearalenone, penicillic acid, and citrinin, J. Assoc. Off. Anal. Chem. 59, 125 (1976).

(32) Ciegler, A. and Kurtzman, C. P., Fluorodensitometric assay of penicillic acid, J. Chromatogr. 51, 511 (1970).

(33) Steyn, P. S., Mycotoxins, excluding aflatoxin, zearalenone and the trichothecenes, In Mycotoxins in Human and Animal Health; proceeding of a conference, eds., J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman. Pathotox, Park Forest South, Ill., 1977, p. 419.

(34) Purchase, I. F. H. and van der Watt, J. J., Carcinogenicity of sterigmatocystin, Food Cosmet. Toxicol. 8, 289 (1970).

(35) Shannon, G. M. and Shotwell, O. L., Thin layer chromatographic determination of sterigmatocystin in cereal grains and soybeans, J. Assoc. Off. Anal. Chem. 59, 963 (1976).

(36) Athnasios, A. K. and Kuhn, G. O., Improved thin layer chromatographic method for the isolation and estimation of sterigmatocystin in grains, J. Assoc. Off. Anal. Chem. 60, 104 (1977).

(37) Reiss, J., Semiquantitative determination of the mycotoxin sterigmatocystin on thin-layer chromatograms with a gray scale, Fresenius' Z. Anal. Chem. 275, 30 (1975).

(38) Vesonder, R. F., Ciegler, A., Rogers, R. F., Burbridge,

K. A., Bothast, R. J., and Jensen, A. H., Survey of the 1977 crop year preharvest corn for vomitoxin, Appl. Environ. Microbiol. 36, 885 (1978).

(39) Romer, T. R. and Boling, T. M., A screening method for vomitoxin in corn, Presented at the 93rd Annual Meeting, Assoc. Off. Anal. Chem., Washington, D.C., 1979.

(40) Steyn, P. S., The isolation, structure and absolute configuration of secalonic acid D, the toxic metabolite of Penicillium oxalicum, Tetrahedron 26, 51 (1970).

(50) Zimmerli, B. J., Verbesserung der Nachweisgrenze von Aflatoxinen in der Hochdruck-Flüssigkeits-Chromatographie durch Verwendung eines-kieselgefullten Fluoreszenzdetektors, J. Chromatogr. 131, 458 (1977).

(51) Panalaks, T. and Scott, P. M., Sensitive silica gel-packed flowcell for fluorometric detection of aflatoxins by high pressure liquid chromatography, J. Assoc. Off. Anal. Chem. 60, 583 (1977).

(52) Pons, W. A., Jr. and Franz, A. O., Jr., High performance liquid chromatography of aflatoxins in cotton-seed products, J. Assoc. Off. Anal. Chem. 60, 89 (1977).

(53) Ware, G. M., High pressure liquid chromatographic method for the determination of patulin in apple butter, J. Assoc. Off. Anal. Chem. 58, 754 (1975).

(54) Brackett, R. E. and Marth, E. H., Use of high-performance-liquid-chromatography to determine patulin in apple juice, In Liquid Chromatographic Analysis of Food and Beverages, ed. George Charalambous, Academic Press, N.Y., 1979, Vol. 2, p. 499.

(55) Leuenberger, U., Gauch, R., and Baumgartner, E., Neue Bestimmungsmethode des Mykotoxins Patulin mit Hilfe der Dünnschicht- und Hochdruckflüssigchromatographie, J. Chromatogr. 161, 303 (1978).

(56) Buchanan, J. R. and Sommer, N. F., High-pressure liquid chromatography in the identification and analysis of mycotoxins in foodstuffs, Toxicon 1976 Suppl. 1(Toxins: Animals, Plant Microb.) 703 (pub. 1978).

(57) Hunt, D. C., Bourdon, A. T., Wild, P. J., and Crosby, N. T., Use of high performance liquid chromatography

combined with fluorescence detection for the identification and estimation of aflatoxins and ochratoxin in food, J. Sci. Food Agric. 29, 234 (1978).

(41) Ciegler, A., Hayes, A. W., and Vesonder, R. F., Production and biological activity of secalonic acid D, Appl. Environ. Microbiol. 39, 285 (1980).

(42) Robbers, J. E., Hong, S., Tuite, J., and Carlton, W. W., Production of xanthomegnin and viomellein by species of Aspergillus correlated with mycotoxicosis produced in mice, Appl. Environ. Microbiol. 36, 819 (1978).

(43) Wilson, D. M., Tabor, W. H., and Trucksess, M. W., Screening method for the detection of aflatoxin, ochratoxin, zearalenone, penicillic acid, and citrinin, J. Assoc. Off. Anal. Chem. 59, 125 (1976).

(44) Josefsson, B. G. E. and Moller, T. E., Screening method for the detection of aflatoxins, ochratoxin, patulin, sterigmatocystin, and zearalenone in cereals, J. Assoc. Off. Anal. Chem. 60, 1369 (1977).

(45) Roberts, B. A. and Patterson, D. S. P., Detection of twelve mycotoxins in mixed animal feedstuffs, using a novel membrane cleanup procedure, J. Assoc. Off. Anal. Chem. 58, 1178 (1975).

(46) Seitz, L. M., Comparison of methods for aflatoxin analysis by high-pressure liquid chromatography, J. Chromatogr. 104, 81 (1975).

(47) Garner, R. C., Aflatoxin separation by high-pressure liquid chromatography, J. Chromatogr. 103, 186 (1975).

(48) Pons, W. A., Jr., Resolution of aflatoxins B_1, B_2, G_1, and G_2 by high-pressure liquid chromatography, J. Assoc. Off. Anal. Chem. 59, 101 (1976).

(49) Takahashi, D. M., Reversed-phase high-performance liquid chromatographic analytical system for aflatoxins in wines with fluorescence detection, J. Chromatogr. 131, 147 (1977).

(58) Hunt, D. C., Bourdon, A. T., and Crosby, N. T., Use of high performance liquid chromatography for the identification and estimation of zearalenone, patulin and penicillic acid in food, J. Sci. Food Agric. 29, 234 (1978).

(59) Ware, G. M. and Thorpe, C. W., Determination of zearalenone in corn by high pressure liquid chromatography and fluorescence detection, J. Assoc. Off. Anal. Chem. 61, 1058 (1978).

(60) Schweighardt, H., Boehm, J., and Leibetseder, J., Zearalenonbestimmung in Getreide mittels Hochdruck-Flussigheitschromatographie (HPLC), Z. Tierphysiol., Tierernaehr. Futtermittelkd. 41, 39 (1978).

(61) Holder, C. L., Nony, C. R., and Bowman, M. C., Trace analysis of zearalenone and/or zearalanol in animal chow by high pressure liquid chromatography and gas-liquid chromatography, J. Assoc. Off. Anal. Chem. 60, 272 (1977).

(62) Ito, K., Yamane, A., Hamasaki, T., and Hatsuda, Y., An assay of sterigmatocystin and related metabolites of Aspergillus versicolor by high speed liquid chromatography, Agric. Biol. Chem. 40, 2099 (1976).

(63) Stack, M. E., Nesheim, S., Brown, N. L., and Pohland, A. E., Determination of sterigmatocystin in corn and oats by gel permeation and high-pressure liquid chromatography, J. Assoc. Off. Anal. Chem. 59, 966 (1976).

(64) Stack, M. E., Brown, N. L., and Eppley, R. M., High pressure liquid chromatographic determination of xanthomegnin in corn, J. Assoc. Off. Anal. Chem. 61, 590 (1978).

(65) Pons, W. A., Jr., Lee, L. S., and Stoloff, L., A revised method for aflatoxins in cottonseed products, including a comparison of thin layer and high performance liquid chromatography determinative steps: Collaborative study, J. Assoc. Off. Anal. Chem. 63, 899 (1980).