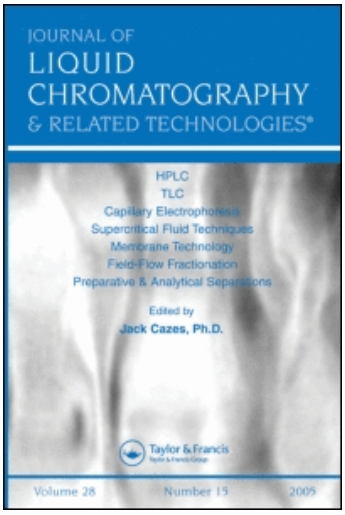

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chromatography of Trichothecene Mycotoxins

P. J. Martin[a]; H. M. Stahr[a]; W. Hyde[a]; M. Domoto[a]
[a] Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa

To cite this Article Martin, P. J. , Stahr, H. M. , Hyde, W. and Domoto, M.(1986) 'Chromatography of Trichothecene Mycotoxins', Journal of Liquid Chromatography & Related Technologies, 9: 7, 1591 — 1602
To link to this Article: DOI: 10.1080/01483918608076705
URL: http://dx.doi.org/10.1080/01483918608076705

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(7), 1591-1602 (1986)

CHROMATOGRAPHY OF TRICHOTHECENE MYCOTOXINS

P. J. Martin, H. M. Stahr,
W. Hyde, and M. Domoto
Veterinary Diagnostic Laboratory
Iowa State University
Ames, Iowa 50011

ABSTRACT

Trichothecene mycotoxins occur in agricultural commodities and can cause problems from feed refusal to death in animals. This paper describes chromatographic methods for selective analysis for trichothecene mycotoxins. These methods include gas chromatography (GC), thin layer chromatography (TLC), and high pressure liquid chromatography (HPLC). The trichothecene analysis methods by GC and TLC are shown to have a greater sensitivity than in HPLC for the underivatized mycotoxins.

INTRODUCTION

The trichothecene mycotoxins are toxic secondary metabolites of the Fusarium mold species. These mycotoxins occur on agricultural commodities, foods,

 0148-3919/86/0907-1591$3.50/0

and feeds. They are acutely irritating and can cause a variety of symptoms from feed refusal to death. Bamburg and Strong[1] have described the occurrence, analysis, and effects of trichothecene mycotoxins on animals.

The Veterinary Diagnostic Laboratory at Iowa State University has a need for analyzing hundreds of samples for trichothecene mycotoxins. This type of analysis aids the agricultural community.

There are various chromatographic methods for analysis of trichothecenes. Ikediobi[2] published a trichothecene method which utilized GC for derivatized trichothecenes. Stahr[3] presented analytical methods for analyzing underivatized and derivatized trichothecenes. Eppley[4] reviewed analysis methodology for trichothecenes. Stacks[5] reviewed TLC methods for trichothecenes. Schmidt et al[6] and Palmisano et al[7] have published HPLC methods for trichothecenes analysis. Some chromatographic methods of analysis for trichothecenes are described in this paper. These methods are useful in diagnostic toxicology analysis work.

MATERIALS AND METHODS

Equipment and Reagents:

Gas chromatographs used were the Bendix 2500 and the Packard 427, a flame ionization detector was used

on the former and an HNU photoionization detector (Delta Instruments, Fairview, IL) with a 10.2 ev UV light source for the latter.

Linear Instruments recorders were used and gas chromatographic supplies were obtained from Applied Science Laboratories (State College, PA). Ten microliter Hamilton syringes (Las Vegas, NV) were used.

A Varian Cary 219 UV-visible spectrophotometer was used with Varian Pascal software on an Apple II Plus computer.

A Waters Associates (Milford, MA) model 45 pump, 6000A pump and a 660 gradient programmer were used with a 25 cm Waters C-18 μBondapak reverse phase column.

Whatman (Clifton, NJ) KC18 reverse phase thin layer plates, with 200 micron thickness, were used.

The solvents used were nanograde Mallinckrodt or HPLC grade from Fisher Scientific. Anisealdehyde reagent, acetic anhydride, and pyridine were all from Eastman White Label obtained from Fisher Scientific. Chromotropic acid was ACS reagent grade from Fisher Scientific.

T-2 toxin and Vomitoxin were obtained from Mycolabs (Chesterfield, MO). T-2 tetraol, HT-2 toxin and Diacetoxyscirpenol (DAS) were obtained from the Sigma Corporation (St. Louis, MO). Roridan A, Verrucarins A and J were gifts from B. Jarvis (University of Maryland).

Gas Chromatographic Conditions:

In the Packard 427 a 6 ft. glass column (ID 3 mm OD 6 mm) of 3% OV101 on Gas Chrom Q 100-120 mesh was used in conjunction with a photoionization detector. The injector temperature was at 250°C, column temperature was at 225°C, and detector temperature was at 270°C. In the Bendix 2500 a 6 ft. glass column (ID 3 mm OD 6 mm) of 4% SE30 6% QF1 on Gas Chrom Q 100-120 mesh was used in conjunction with a flame ionization detector. The injector temperature was at 280°C and the column temperature was at 250°C.

High Pressure Chromatography Conditions:

A Waters Associates 660 solvent programmer in conjunction with the 6000 A and M 45 pumps were used with a 25 cm Waters C-18 ODS μBondapak column. A concave gradient #7 from 45% to 60% MeOH (MeOH/H_2O mobile phase) for 30 min. then 60% MeOH isocratic till all compounds eluted. The flow rate was 1 ml/min to achieve the separation. The Micromeritics Chromonitor UV detector (Norcross, GA) was used at 208 nm for detection. A mixture of trichothecene standards were prepared by adding 100 microliters of each 1μg/μl standard into a vial and evaporating the solvent on a steam bath under N_2 to dryness. The standards were then resolvated in MeOH before chromatography was performed.

Ultraviolet Detection:

A Varian Cary 219 UV-visible spectrophotometer was used to produce UV spectra of the trichothecenes. Varian Pascal software was used on an Apple II Plus computer.

Thin Layer Chromatography:

Reverse phase TLC separations were achieved with a mobile phase for 65:35:1 (ethanol:water:acetic acid) + 0.5% NaCl.

Visualization of the compounds was achieved with anisealdehyde and chromotropic spray reagents. The anisealdehyde spray reagent[8] was prepared by mixing 0.5 ml p-anisealdehyde in 85:10:5 (methanol:concentrated glacial acetic acid:concentrated sulfuric acid). Chromotropic acid spray reagent[9] was made by mixing 1 part 10% chromotropic acid aqueous solution with 5 parts H_2SO_4:H_2O (5:3). This spray is good for 3-4 weeks. Plates are heated with a heat gun to produce distinctive colors formed between reagent spray and trichothecene compounds. (Note aniseladehyde spray reagent should be refrigerated between uses). Also if the spray turns the TLC plate pink, new spray reagent should be prepared. In using the chromotropic reagent spray too heavy a spray will result in water droplets on the TLC causing gray spots to appear after heating.

Preparation of Trichothecene Standards:

Trichothecene compounds were weighed and then dissolved in methanol to a concentration of 1 µg/µl.

Preparation of Acetate Derivatives:

Trichothecene compounds were added to one dram vials and solvent evaporated to dryness on a steam bath under N_2. To this vial 100 microliters of reagent grade pyridine and 100 microliters of reagent grade acetic anhydride were added and vortexed for a few seconds. The vials were allowed to sit at room temperature overnight. The next day this solution in the vials was again evaporated to dryness on a steam bath under N_2. The appropriate solvent (usually methanol) was then added to acetate derivatized samples and the appropriate procedure was followed.

RESULTS

The GC retention times of the trichothecene compounds on the 3% and 10% liquid phase columns are shown in Table 1. A microgram of most compounds gives a half-scale deflection on a 1 mv linear recorder. By using varied temperatures or different GC packing phases qualification and quantification of the trichothecene mycotoxins can be accomplished. The two column phases illustrated in Table 1 work well in our laboratory. We have been using these phases for detection of trichothecenes in diagnostic samples.

TABLE 1

GC Retention Ratios for Trichothecenes or their Acetate Derivatives (Ac)

Trichothecenes	Retention Ratios (Relative to Vomitoxin Ac)	
	3% OV101	4% SE30 6% QF1
1. Verrucarol Ac	0.51	0.52
2. DAS	0.74	0.79
3. DAS Ac	0.84	0.79
4. Vomitoxin Ac	1.00	1.00
5. T-2 tetraol Ac	1.42	1.36
6. T-2	2.58	2.26
7. HT-2	2.65	2.63
8. HT-2 Ac	2.69	2.26
9. T-2 Ac	2.72	2.26

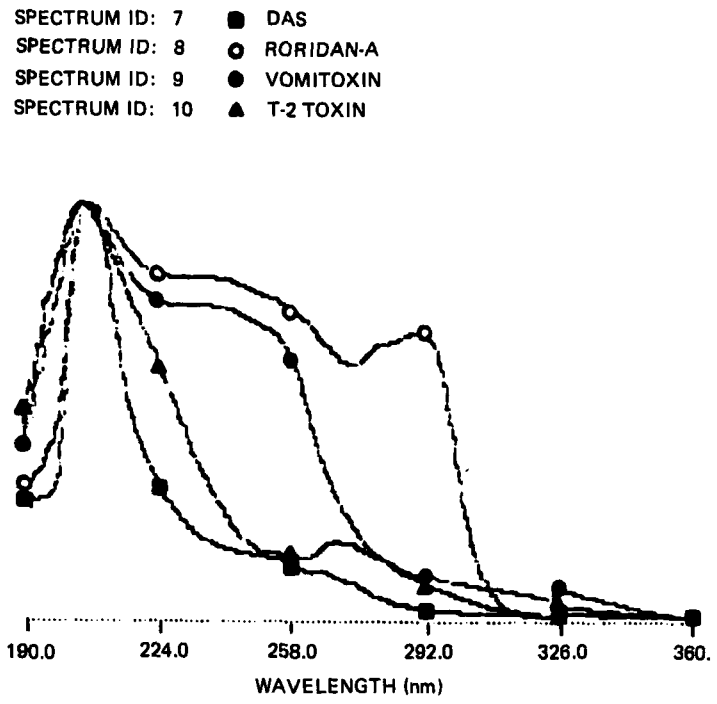

Figure 1. UV Spectra of Four Trichothecene Mycotoxins

The ultraviolet spectra of four trichothecenes are shown in Figure 1. Utilization of the proper wavelength allows selective analysis of trichothecene compounds. Also selection of a wavelength common to many trichothecenes can aid in HPLC detection.

HPLC separation of the trichothecene mycotoxins illustrating relative retentions to DAS are shown in Table 2. The sensitivity of this method is about five times less than that obtained by TLC or GC. The advantage of HPLC separation of mycotoxins is that the

TABLE 2

HPLC of Trichothecenes

	Trichothecene	Retention Ratios (Relative to DAS)
1.	T_2 tetraol	0.20
2.	Vomitoxin	0.26
3.	Verrucarol	0.36
4.	DAS	1.00
5.	HT-2 toxin	1.62
6.	T-2 toxin	2.05
7.	Verrucarin A	2.16
8.	Roridan A	2.27
9.	Roridan D	2.47
10.	Verrucarin J	3.52

Table 2 Trichothecenes were separated on a 25 cm Whatman C-18 ODS μBondapak column by a concave gradient #7 from 45-60% MeOH for 30 min. then isocratic at 60% MeOH till all compounds were eluted, flow rate was 1 ml/min.

less volatile compounds such as the Verrucarins and Roridans can be analyzed. Perhaps a fluorescent tag or electrochemical detection would prove more sensitive for this method of chromatography.

The Rf values of trichothecene mycotoxins on reverse phase TLC are shown in Table 3, along with the colors produced by the two visualization spray reagents. Detection of 0.5-1.0 microgram can be observed with either the anisealdehyde or chromotropic acid spray reagent.

Mass spectral confirmation is possible at the microgram level from nearly any matrix with suitable

TABLE 3

Rf values for Trichothecenes and their Acetate Derivatives (Ac) on Reverse phase TLC

Trichothecene		Color		Rf Value
		Anisealdehyde	Chromotropic Acid	
1.	Verrucarol	purple	yellow	0.74
2.	Verrucal Ac	purple	yellow	0.54
3.	DAS	pink	yellow	0.66
4.	DAS Ac	pink	yellow	0.54
5.	Vomitoxin	yellow	purple	0.82
6.	Vomition Ac	yellow	purple	0.61
7.	T-2 toxin	pink-brown	purple	0.53
8.	T-2 toxin Ac	pink-brown	purple	0.42
9.	T-2 tetraol	pink	brown	0.87
10.	T-2 tetraol Ac	pink	brown	0.62
11.	HT-2 toxin	brown	purple	0.59
12.	HT-2 toxin Ac	brown	purple	0.41
13.	Verrucarin J	purple	yellow	0.72
14.	Verrucarin J Ac	purple	yellow	0.69
15.	Verrucarin A	purple	yellow	0.53
16.	Verucarin A Ac	purple	yellow	0.38
17.	Roridan A	blue	yellow	0.53
18.	Roridan A Ac	blue	yellow	0.26

cleanup. Stahr et al[10] has published spectra of parent and acetate derivatives of the commonly available tricothecenes. Also R. Cole and R. Cox[11] have published mass spectra of many of the trichothecene mycotoxins.

CONCLUSION

Selective analysis of trichothecene mycotoxins can be accomplished by various chromatographic techniques including GC, TLC, and HPLC. The most sensitive methods being GC and TLC.

A method of extraction analysis was published by Stahr, et al[12]. Cleanup of samples can be done by silica gel or reverse phase column chromatography[13].

All of these various chromatographic methods provide a trichothecene analysis useful for nearly any laboratory preference and/or budget.

REFERENCES

1) Bamburg, J.R. and Strong, F.M. Algal and Fungal Toxins, Vol. 7, Chapter 7, Edited by A. Ciegler, S. Kadis, A. Ayl, Academic Press, NY (1971).

2) Ikediobi, C.O., Bamburg, J.R., Strong, F.M. Anal. Biochem. 43:327 (1971).

3) Stahr, H.M. Editor. Analytical Toxicology Methods Manual, ISU Press, Ames, IA 1975.

4) Eppley, R.M. Trichothecenes and Their Analysis. J. of Am. Oil Chemists Soc. 56:824-29 (1979).

5) Stacks, M.E. and Eppley, R.M. "The Analysis of Trichothecene Mycotoxins by TLC," Chapter in Advances of Thin Layer Chromatography, Edited by J.C. Touchstone, John Weleny and Sons, New York, NY (1982).

6) Schmidt, R., Ziegenhagen, E., and Dose, K. J. Chrom. 212:370-73 (1983).

7) Palinisano, F., Viconti, A., Bottalico, A., Levario, P. and Zambori, P.G. Analyst. 106:992-98 (1981).

8) Stoloff, L. Analytical Methods for Mycotoxins, Clinc. Toxicol. 5(4):465-94 (1972)

9) Baxter, J.A., Terhume, S.J. and Qureshi, S.A. J. Chrom. 261:130-33 (1983)

10) Stahr, H.M., Lerdel, D., Pfeiffer, R. and Hyde, W. Applied Spectroscopy 37(4):396-400 (1983)

11) Cole, R.J. and Cox, R.H. Handbook of Toxic Fungal Metabolites, Academic Press, NY (1981).

12) Stahr, H.M. Editor. Analytical Toxicology Methods Manual, ISU Press, Ames, Iowa (1975)

13) Stahr, H.M., Lerdel, D., Pfeiffer, R. and Hyde, W. Applied Spectroscopy 37:396-400 (1983).