

Accepted Manuscript

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PII: S1570-0232(10)00364-8
DOI: doi:10.1016/j.jchromb.2010.05.043
Reference: CHROMB 16920

To appear in: *Journal of Chromatography B*

Received date: 18-1-2010
Revised date: 27-5-2010
Accepted date: 27-5-2010

Please cite this article as: A. Medina, F.M. Valle-Algarra, M. Jiménez, N. Magan, Different sample treatment approaches for the analysis of T-2 and HT-2 toxins from oats-based media, *Journal of Chromatography B* (2008), doi:10.1016/j.jchromb.2010.05.043

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Different sample treatment approaches for the analysis of T-2 and HT-2 toxins from oats-based media

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Abstract

A LC-DAD method is proposed for the determination of the T-2 and HT-2 toxins in cultures of *Fusarium langsethiae* in oat-based and other in vitro media. Test media consisted of freshly prepared milled oats to which T-2 and HT-2 toxin stock solutions were added. Different mixtures of extraction solvent (acetonitrile:water and methanol:water), extraction times (30', 60' or 90') and drying methods were investigated. Results showed that extraction with methanol:water (80:20 v/v) for 90 minutes, drying with N₂ and subsequent analysis by LC-DAD was the fastest and most user friendly method for detecting HT-2 and T-2 toxins production by *F. langsethiae* strains grown on oat-based media at levels of 0.459 and 0.508 mg of toxin/Kg of agar, respectively. The proposed method was used to investigate toxin production of 6 *F. langsethiae* strains from northern Europe and provided clear chromatograms with no interfering peaks in media with and without glycerol as water activity modifier.

Keywords: Analysis, Type A trichothecenes, diode array, cereals

1. Introduction

The toxins T-2 and HT-2 occur in various cereal crops, especially oats, where they are produced by *Fusarium langsethiae*. T-2 toxin is rapidly metabolized to HT-2 toxin which is also the main metabolite *in vivo* (1). T-2 toxin, the most toxic trichothecene, is a potent inhibitor of DNA, RNA and protein synthesis, and shows immunosuppressive and cytotoxic effects both *in vivo* and *in vitro* (2-3). Recent data collected to evaluate the risk of dietary exposure to *Fusarium* toxins by the populations of EU member states showed that T-2 and HT-2 toxins are quite common contaminants in cereals in the EU (4).

F. langsethiae, has been isolated from infected oats, wheat and barley in central and northern Europe (5-6). This species has been implicated in the production of high levels of HT-2 and T-2 mycotoxins in cereals in Norway (7) and in oats in the UK (8). However, there is little knowledge of the ecology of this pathogen and it is necessary to develop reliable and fast methods that could allow chemical and ecological characterisation. Thus, studies on oat-based nutritional media are important when screening the ability of strains to produce these toxins.

Today, one of the commonest and quick methods for the characterisation of toxin/no toxin production is the agar plug method. This method was introduced by Frisvad and Filtenborg in 1983 and up to now has been used in numerous studies to identify the relative ability of strains to produce specific mycotoxins and the amounts produced (9-11).

Generally, detection of trichothecenes has been performed by ELISA, thin-layer chromatography (TLC), liquid chromatography (LC) or gas chromatography (GC). Regarding type A trichothecenes, due to the lack of any ultra-violet (UV) absorbing or fluorescent chromophore, the detection via LC-UV or LC-RID (refractive index detection) is difficult.

Usually, separation is achieved on a RP-C₁₈ column with methanol-water (14:86) as the mobile phase (12-13). Regarding UV detection, in the presence of methanol, interfering absorption at low wavelengths, and sometimes low pH, reduces the UV absorption. Thus, UV detection of type A trichothecenes was only applicable to cultures with high toxin concentrations or used to discriminate producing/non-producing strains just for screening purposes (14-15). Several studies have described determination of HT-2 and T-2 by LC coupled with fluorescence detectors (FLD) after derivatization (16-17). The trichothecenes have been derivatized with 1-naphthoyl chloride (1-NC); 2-naphthoylchloride (2-NC); 1-anthroylnitrile (1-AN) and, pyrene-1-carbonyl cyanide (PCC). 2-NC and PCC performed better than 1-AN and 1-NC in terms of sensitivity and selectivity (18).

GC has been the most widely used method, but it requires derivatisation in order to obtain good results (7, 19-20), although methods without derivatisation have been published (21). Today LC-MS² is the technique of choice for the determination of type-A trichothecenes in foodstuffs (22-25). However, the high cost makes the use of this technique difficult for routine strain characterization.

The aim of this work was to find a fast and easy-to-use method for the analysis of T-2 and HT-2 toxins in cultures grown *in vitro* on media. In our case the medium consisted of 2% (w/v) ground oats. Two different extraction solvents, 3 different extraction times, and two different extract drying procedures were tested using liquid chromatography to optimize the procedure. The proposed method was tested by quantifying the levels of the mycotoxins in *in vitro* cultures of different *Fusarium langsethiae* strains on an oat-based medium modified to different water availabilities using glycerol.

2. Material and methods

2.1. Reagents and standards

Trichothecene standards, including T-2 and HT-2 were supplied by Sigma (Sigma-Aldrich, UK). Acetonitrile and methanol were purchased from Fisher Scientific (Fisher Scientific UK Ltd., UK). All solvents were HPLC grade. Pure water was obtained from a Milli-R/Q watersystem (Millipore, Billerica, MA, USA).

2.2. Chromatographic analysis

HPLC equipment consisted of an Agilent 1100 Series HPLC system equipped with a UV diode-array detector set at 200 nm (Agilent Technologies, Palo Alto, CA, USA). The column was a Phenomenex® Gemini C₁₈, 150 mm × 4.6 mm, 3 µm (Phenomenex, Macclesfield, UK) preceded by a Phenomenex® Gemini C₁₈ 3 mm, 3 µm guard cartridge. Signals were processed by Agilent ChemStation software Ver. B Rev: 03.01 [317]. Analysis was performed in the gradient mode. Water was solvent A and acetonitrile solvent B. Gradient conditions were initiated by holding the mobile phase composition 3 minutes with 30% B, after that it was changed linearly to 55% B during 18 min. The composition was then changed to 99% B in 1 minute and maintained for 5 min as a cleaning step in order to improve the results. After cleaning, the eluent composition was returned to the initial 30% B. The flow rate of the mobile phase was 1 mL min⁻¹ and injection volume was 50 µL.

2.3. Preparation of standard solutions

T-2 standard was dissolved in acetonitrile at a concentration of 2.0 mg mL⁻¹ and stored at -20 °C in a sealed vial until use. HT-2 standard solution in acetonitrile was purchased at a concentration of 100.2 µg mL⁻¹. Working standards (50, 20, 10, 5, 2.5, 1, 0.5 and 0.2 µg mL⁻¹) were prepared by appropriate dilution of known volumes of the stock solutions with acetonitrile and used to obtain calibration curves for LC-DAD analysis.

2.4. Medium preparation and fungal culture preparation

Milled oats were prepared by homogenisation for 5 mins. Five different sets of a mixture of 2% (w/v) oat flour in water were prepared and 2%(w/v) agar was added. The culture medium was prepared and after autoclaving, the required volume of T-2 and HT-2 toxin stock solution were added (at around 45°C) to get the desired concentration (0.1, 0.25, 0.5, 1 and 5mgKg⁻¹). The medium was vigorously shaken and poured into Petri dishes. Controls consisted of non-spiked culture medium.

For experiments with six *F. langsethiae* strains (Ref. 2004/57 and 2004/59 from UK; 44P and 88E from Norway; 05010 and 05014 from Finland), the medium was modified with glycerol to the required water activity levels (a_w ; 0.99, 0.95). The six strains of *F. langsethiae* were inoculated using agar discs (4 mm diameter) at three points equidistant apart onto freshly prepared 2% oat medium and incubated at 25 °C for 12 days.

2.5. Sample preparation

Using a cork borer, five-six discs of agar (4 mm diameter) weighing around 0.75 grams were removed from the spiked plates and placed in previously weighed 2 mL volume safe-lock Eppendorf tubes. A total of 3 replicates per treatment were collected, weighed again and immediately frozen at -20°C freezer.

2.6. Extraction procedures

Samples were thawed and extracted by mixing the agar plugs with one ml of a mixture of water and organic solvents. Afterwards the tubes were shaken at 150 rev min⁻¹ at 25°C in the dark in an orbital shaker. They were then centrifuged at 1150 g for 15 min and 750 µL of supernatant were transferred to a 2 mL chromatography silanized amber vial. Extracts were completely dry in a stream of nitrogen. Dry extract was redissolved in 300 µl of acetonitrile:water (50:50 v/v) and injected in the chromatograph.

a) Comparison of extraction solvents

Extraction was made using two different extraction solvents, acetonitrile:water (84:16 v/v) or methanol:water (80:20 v/v) for 45 minutes. Then samples were dried overnight in an air forced 50°C dryer, and analysed using LC-DAD.

b) Comparison of extraction times

Once the best extraction solvent was identified, different extraction times were tested (30, 60 or 90 minutes).

c) Comparison of drying methods

The best solvent and best time were used and finally samples were dried using two different methods. One set was dried placing the tubes overnight in a forced air dryer at 50°C and the other set was dried under a gentle stream of N₂ at 50°C in an aluminium heated block.

2.8. Statistical analysis

Statistical analysis was performed using the package STATISTICA 8 (StatSoft® Inc., 2007. Tulsa, OK, USA).

3. Results

3.1. Comparison of extraction solvents

Ten samples (5 per treatment) of each concentration were defrosted and toxin extraction was made using the two different mixtures: acetonitrile:water (84:16 v/v) or methanol:water (80:20 v/v) for 30 minutes and then analysed using the LC-DAD procedure. There were no statistically significant differences ($p > 0.05$) between treatments. Finally, a methanol:water (80:20 v/v) mixture was selected for further experiment due to the lower consumption of methanol and also its lower price in comparison to acetonitrile.

3.2. Comparison of extraction times

Fifteen samples of each concentration, five for each different time treatment were used. The mean recoveries and relative standard deviations (RSD) obtained extracting toxins after different times are shown in Table 1. In general an increase in the mean recovery was observed when the extraction time was increased. HT-2 and T-2 recoveries for the lower concentration (0.1 mgKg^{-1} of agar) were predominantly higher than 100% and RSD values greater than 10%, showing that this value was near the limit of detection. With all the other concentrations the best results for both toxins were observed when the extraction step lasted 90 minutes. In both cases recoveries were higher than 95% and RSD values (excluding both toxins at the lowest concentration) were smaller than 7%.

3.3. Comparison of drying methods

Six samples of 0.5, 1 and $5 \mu\text{g}$ of both toxins g^{-1} of agar were defrosted. Samples were extracted using the best options described before and 3 samples of each concentration treatment dried using forced air at 50°C overnight. The remaining samples were dried under a gentle stream of N_2 at 50°C in an aluminium heated block.

Concerning HT-2 toxin, drying with both methods gave very similar results despite a slight reduction of 5.3 % of the toxin being observed when samples were dried overnight in a dryer.

Regarding T-2 toxin, larger differences were found. In this case values of toxin after drying using forced air at 50°C overnight were 12.3 % lower than those obtained using N_2 drying. Based on these results, drying with a gentle stream of N_2 at 50°C in an aluminium heated block was selected as the best method.

3.4. Method performance

Determination of T-2 and HT-2 toxins was carried out using a LC-DAD and extracting the chromatogram signal obtained at $\lambda=200.4$ nm. Figure 1A shows a chromatogram obtained after injection of a mixture of toxin standards containing 0.05 μ g of HT-2 and 0.05 μ g of T-2. Figure 1B shows the chromatogram obtained after 90 min extraction of an oats-based medium spiked with T-2 and HT-2 (0.25mg Kg⁻¹ of agar) using methanol:water (80:20 v/v), corresponding to 0.015 μ g of each toxin per injection.

HT-2 and T-2 peaks were observed at 11.78 and 17.66min, respectively. Run time was 20 minutes and after each injection a cleaning step was performed in order to improve results and avoid 'ghost' peaks in the next run. Total time used for each sample was around 35minutes. Direct injection without clean-up provided clean chromatograms and flat baselines allowing very good integration of the T-2 and HT-2 toxin peaks.

The limit of detection (LOD) was calculated according to the following equation (Long and Winefordner, 1983)(26):

$$LOD = \bar{X}_n + 3 \cdot S_{n-1}$$

Where, $\bar{X}$ is the concentration mean and S_n the standard deviation. The limit of quantification (LOQ) was calculated according to the next equation:

$$LOQ = \bar{X}_n + 10 \cdot S_{n-1}$$

LC-DAD limits of detection (LOD) and limits of quantification (LOQ) were 0.1612 and 0.2294 mg Kg⁻¹ agar for HT-2 and 0.1648 and 0.2541 mg Kg⁻¹ agar for T-2, respectively.

According to the results obtained, extraction of oats-based medium with methanol:water (80:20 v/v) for 90 minutes, drying with a gentle stream of N₂ at 50°C and subsequent analysis by LC-DAD was the optimum condition for to analysis HT-2 and T-2 in culture media.

The mean recovery for HT-2 and T-2 on oat-based media spiked at a level from 0.25 to 5 mgKg⁻¹ of agar was 101.28 ± 3.11% and 99.0 ± 1.53% respectively.

The regression coefficients (*r*) for T-2 and HT-2 were all > 0.998. The results of the study reflected that the use of the proposed method gave clean chromatograms and high recoveries in the range between 0.25 and 5 mg of T-2 and HT-2 Kg⁻¹ of agar.

Precision was calculated in terms of intra-day repeatability (*n*=5) and inter-day reproducibility (3 different days) on samples containing 1 mgKg⁻¹ of agar of both T-2 and HT-2 toxins. The RSD for intra-day repeatability ranged from 4.5 to 7.8%. The inter-day reproducibility was < 10% in all cases.

3.6. Application to *F. langsethiae* cultures in oats-based culture medium

The method proposed was applied to investigate the T-2 and HT-2 production ability of 6 *F. langsethiae* strains isolated from oats from different countries of northern Europe. Oat-based media were modified to 0.99 and 0.95 a_w using glycerol. A sample chromatogram obtained in these analyses is shown in Figure 2. No interfering peaks were observed in glycerol-modified water availability media. This shows that HT-2 and T-2 toxin were easily analysed using the proposed method. Results for all samples are shown in Table 2.

4. Discussion

This study considered different aspects of the analysis procedures such as extraction efficiency, sample processing and trichothecene type A detection. It has shown that extraction with methanol:water (80:20 v/v) for 90 minutes, drying with N₂ and subsequent analysis by LC-DAD is an appropriate method for detecting HT-2 and T-2 toxin production in cultures of *F. langsethiae* grown on oat-based media.

There are a myriad of different extraction mixtures which have previously been used for HT-2 and T-2 toxin. Among them, acetonitrile or methanol mixtures with water have been the most common (16, 20, 27). In the present study both gave good results. No significant differences were found after statistically analyzing mean recoveries using both options and the final selection was made based on the lower amount of organic solvent used in the methanol mixture, and the higher cost of acetonitrile.

Although many studies have described problems with DAD for type Atrichothecenes (12, 20, 28) our results show that, under the conditions used in this study, detection of HT-2 and T-2 is possible in *Fusarium langsethiae* cultures containing only 0.23 and 0.25 mg of toxin kg⁻¹ agar, respectively. Culture media containing only 2% of freshly milled oats were used. Under these conditions the fungi are able to grow and produce both toxins but the concentration of the cereal is low enough to allow analysis by the proposed method. Since this approach was first investigated more than 20 years ago, the improvements showed here would depend on improvements in the chromatographic equipment (DAD equipment and stationary phase) and in the better quality of organic solvents available today, because of their enhanced purity, and lower absorption at reduced wavelengths.

The proposed method does not require any derivatisation process, and the extract is injected directly into the system. In this way separation of HT-2 and T-2 using a C₁₈ analytical column, widely used in mycotoxin analysis, can be done in around 35 min per sample, including the column washing.

Potential application to real *F. langsethiae* samples was demonstrated by the results obtained in the analysis of both toxins in 12-day cultures of 6 different strains. The method provided clear chromatograms with no interfering peaks in all media including experiments carried out using glycerol to modify the media a_w.

5. Conclusions

The present method provides a rapid system for detecting production of HT-2 and T-2 in cultures of *F. langsethiae* grown on oat-based media. For physiological and ecological studies it has several advantages over other conventional methods which use large amounts of natural substrates or culture media, and subsequently, are quite costly in terms of solvents and amount of time required. The simplicity of the method, despite the cereal composition of the medium, makes it very useful for screening approaches to examine intra-strain variation and effect of fungicides on control of HT-2 and T-2 toxins. A further advantage is that the extracts are very clean allowing the analysis by LC-DAD, without any clean-up or time-consuming derivatisation steps.

6. Acknowledgements

We are grateful to Dr. Marika Jestoi, Prof. Simon Edwards and Dr. Mona Torp for the supply of strains. A. Medina wants to acknowledge funding from the Spanish Ministry of Science and Innovation (Programa Nacional de Movilidad de Recursos Humanos del Plan Nacional de I+D+I 2008-2011). F.M. Valle-Algarra and M. Jimenez wish to thank financial support from FEDER and Spanish Government “Ministerio de Ciencia e Innovación” (Project AGL2007-66416-C05-01/ALI, and two research grants) and the Generalitat Valenciana (Project ACOMP2009/371).

7. References

- (1) Eriksen, G.S.; Alexander, J. Fusarium toxins in cereals – a risk assessment. Nordic Council of Ministers; Tema Nord 1998. Eriksen, G.S.; Alexander, J. Eds . Copenhagen, 502 (1998) p. 7.
- (2) R.A. Canady, R.D. Coker, S.K. Egan, R. Krska, M. Olsen, S. Resnik, J. Schlatter, T-2 and HT-2 toxins. In Safety Evaluation of Certain Mycotoxins in Food, FAO Food and Nutrition Paper 74, WHO Food Additives Series 47. WHO (Ed.), Geneve, Switzerland, (2001) p. 557.
- (3) A.Visconti, Bull. Inst. Compr. Agr. Sci. Kinki Univ. 9 (2001) 39.
- (4) R.C.; Schothorst, H.P. van Egmond, Toxicolog. Lett. 153 (2004) 133.
- (5) M. Torp, A. Adler, Int. J. Food Microbiol. 95 (2004) 241.
- (6) M. Torp, H.I. Nirenberg, Int. J. Food Microbiol. 95 (2004) 247.
- (7) W. Langseth, T. J.Rundberget, Mycopathologia 147 (1999) 157.
- (8) Edwards, S.G. Investigation of Fusariummycotoxins in UK barley and oat production. In HGCA Project Report No. 415. London: HGCA. 2007.
- (9) J.C. Frisvad, J. Smedsgaard, R.A. Samson, T.O. Larsen, U. J. Thrane, Agric. Food Chem., 55 (2007) 9727.
- (10) H. Hestbjerg, K.F. Nielsen, U. Thrane, S. Elmholt, J. Agric. Food Chem. 50 (2002) 7593.
- (11) D. Mitchell, R. Parra, D. Aldred, N.Magan, J. Appl. Microbiol. 97 (2004) 439.
- (12) J.C. Frisvad, U. J. Thrane, Chromatogr., 404 (1987) 195.
- (13) R. Schmidt, E. Ziegenhagen K. J. Dose, Chromatogr. 212 (1981) 370.
- (14) P. Kuronen, Archiv. Environ. Contam. Toxicol. 18 (1988) 336.
- (15) U. Thrane, A. Adler, P.-E. Clasen, F. Galvano, W. Langseth, H. Lew, A. Logrieco, K.F. Nielsen, A. Ritieni, Int. J. Food Microbiol. 95 (2004) 257.

- (16) A. Trebstein, w. Seefelder, U. Lauber, H.U. Hump, J. Agric. Food Chem. 56 (2008) 4968.
- (17) A. Visconti, V.M.T. Lattanzio, M. Pascale, M. Haidukowski, J. Chromatogr. A 1075 (2005) 151.
- (18) V.M.T. Lattanzio, M. Pascale, A. Visconti, Trends Anal. Chem. 28 (2009) 758.
- (19) R.Krska, S. Baumgartner, R. Josephs, Fresenius' J. Anal. Chem. 371 (2001) 285.
- (20) P. Majerus, J. Hain, M. Scheer, Mycotoxin Research 24 (2008) 24.
- (21) Y. Onji, Y. Aoki, N. Tani, K. Umebayashi, Y. Kitada, Y. Dohi, J. Chromatogr A 815 (1998) 59.
- (22) F. Berthiller, R. Schuhmacher, G. Buttinger, R. Krska, J. Chromatogr. A 1062 (2005), 209.
- (23) S. Biselli, C. Hummert, Food Addit. Contam. 22 (2005) 752.
- (24) M. Schollenberger, U. Lauber, H.T. Jara, S. Suchy, W. Drochner, H.-M. Muller, J. Chromatogr. A 815 (1998) 123.
- (25) K. Schwadorf, H.-M. Muller, Chromatographia 32 (1991) 137.
- (26) G.L. Long, J.D. Winefordner, 1983. Anal. Chem. 55, 713A.
- (27) J. Gilbert, A. Boeneke, P. J. Wegstafte, Food Addit. Contam. 9 (1992) 71 .
- (28) D.R. Lauren, R. J. Greenhalgh, Assoc. Off. Anal. Chem. 70 (1987) 479.

7. Figure captions

Figure 1. Chromatogram obtained after injection of (A) mixture of toxin standards containing 0.025 μg of HT-2 and 0.0125 μg of T-2 and (B) obtained after 90 min extraction of an oats-based medium spiked with T-2 and HT-2 (0.5 mg Kg^{-1} of agar) using methanol:water (80:20 v/v).

Figure 2. Chromatogram of an oats-based medium (a_w , 0.99) inoculated with strain 2004/57 for 12 days, after 90 min methanol:water (80:20 v/v) extraction, containing 0.64 and 10.92 mg kg^{-1} of HT-2 and T-2 respectively.

Figure 2

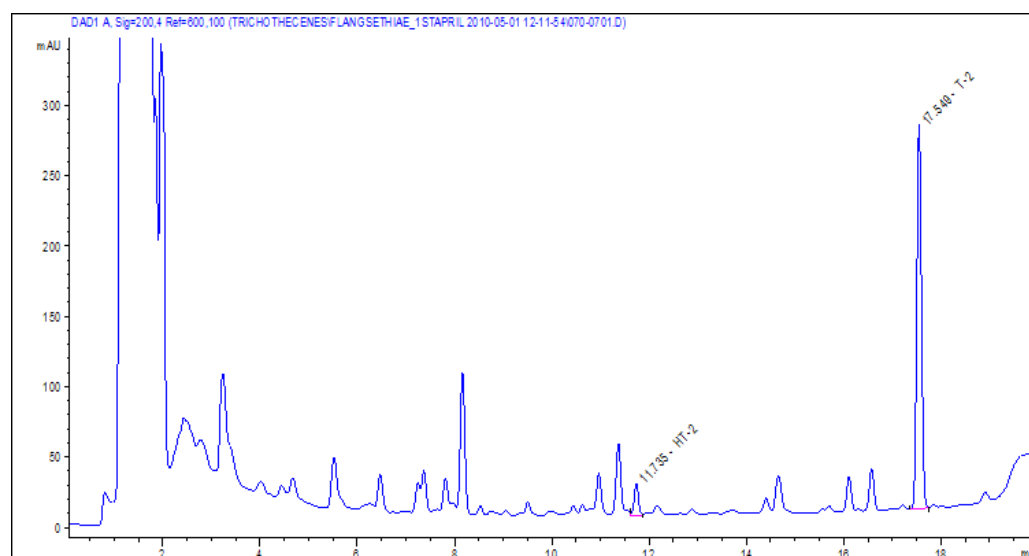


Figure 1

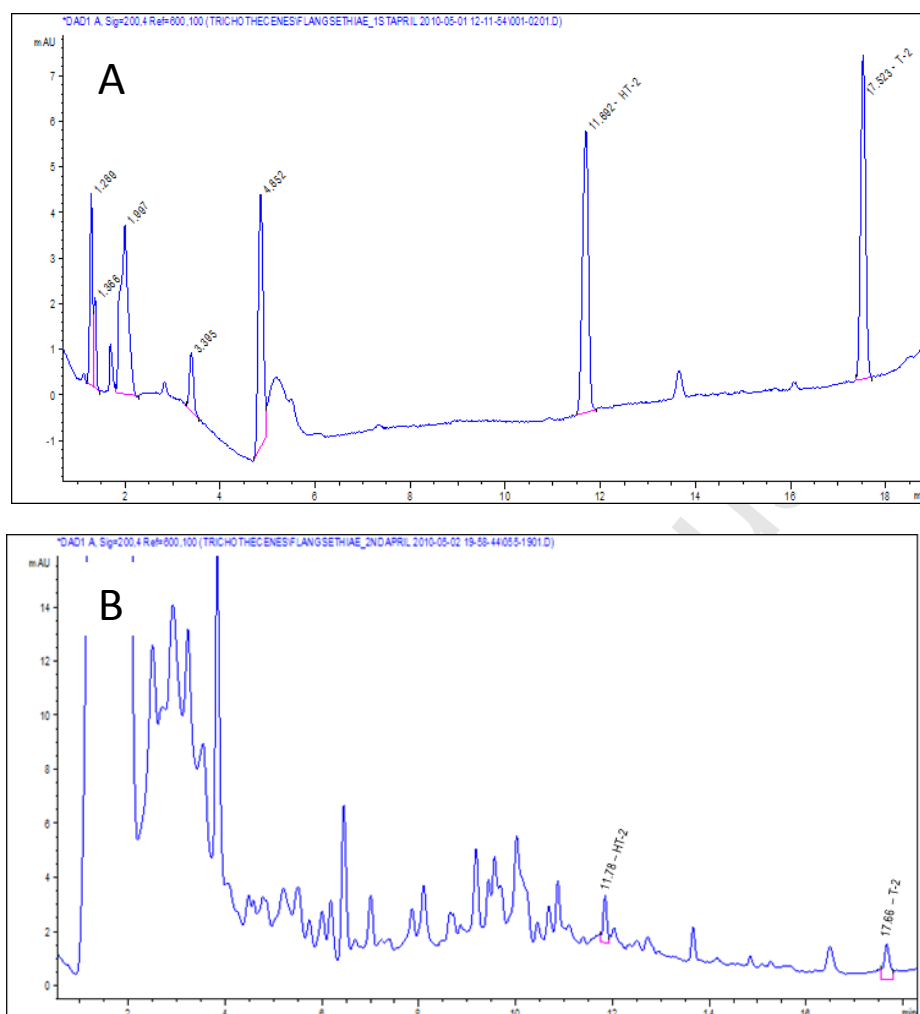


Table 1: HT-2 and T-2 mean recoveries (%) and relative standard deviations (RSD) from oat-based medium obtained testing different extraction times. Analysis was performed using LC-DAD.

Concentrations (mg/K g of agar)	30'		60'		90'	
	Mean recovery %	RSD %	Mean recovery	RSD %	Mean recovery	RSD %
HT-2						
0.1	141.01	15.46	131.78	14.09	128.42	11.47
0.25	89.54	6.99	92.95	6.99	105.63	5.45
0.5	80.68	7.32	86.54	5.47	98.45	5.85
1	80.22	5.72	83.41	7.33	101.12	6.42
5	78.65	5.11	84.12	6.76	99.78	5.01
T-2						
0.1	130.68	12.63	115.39	8.96	119.08	10.39
0.25	91.62	6.65	92.85	6.21	101.27	6.28
0.5	90.99	8.07	93.96	5.35	98.55	5.66
1	95.72	9.01	96.34	7.02	98.21	4.45
5	98.92	8.54	97.19	5.53	97.97	3.83

Table 2. HT-2 and T-2 levels obtained after extraction from 6 different *F. langsethiae* strains grown on an oat-based medium, adjusted to water activities of 0.99 and 0.95 using glycerol for 12 days.

Strains	0.99		0.95	
	HT-2 (mg Kg ⁻¹ agar)	T-2 (mg Kg ⁻¹ agar)	HT-2 (mg Kg ⁻¹ agar)	T-2 (mg Kg ⁻¹ agar)
2004/57	0.64 ± 0.23 ^a	10.92 ± 1.11	TR	2.18 ± 0.98
2004/59	0.87 ± 0.12	10.91 ± 2.45	TR	2.01 ± 0.18
44P	TR	2.67 ± 0.67	0.23 ± 0.07	1.14 ± 0.21
88E	0.23 ± 0.08	5.12 ± 0.99	ND	TR
05010	1.26 ± 0.31	12.12 ± 2.12	TR	0.66 ± 0.14
05014	0.84 ± 0.16	6.73 ± 1.56	ND	1.19 ± 0.32

^a: Standard Deviation

TR: Below LOQ

ND: Not detected



DSPACE

<https://dspace.org/>

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2010-08-01T00:00:00Z

Elsevier Science B.V., Amsterdam.

<http://dx.doi.org/10.1016/j.jchromb.2010.05.043>

Angel Medina, Francisco M. Valle-Algarra, Misericordia Jimenez, Naresh Magan; Different sample treatment approaches for the analysis of T-2 and HT-2 toxins from oats-based media, Journal of Chromatography B, Volume 878, Issue 23, 1 August 2010, Pages 2145-2149