AD-0015-GM

Access to your success
SHIMADZU
Asia Pacific

Application News
GCMS

December 2005

Analysis of Trichothecenes by Gas Chromatography-Chemical Ionization Mass Spectrometry

Novalina Lingga, Ph.D., Cynthia M. Lahey
Customer Support Centre, Shimadzu (Asia Pacific), Pte. Ltd., Singapore

Trichothecenes is one group of toxins produced by fungi (mycotoxins). Trichothecenes have been classified into four groups, A, B, C and D. Langseth and Rundberget[1] reviewed some instrumental methods for the determination of trichothecenes in foodstuffs.

Among the instrumental methods used is gas chromatography with mass spectrometric detection (GC/MS). Here we report the analysis of eight trichothecenes by GC/MS based on the method described by Schollenberger et al.[2] The literature described the use of GC/MS with chemical ionization.

In GC/MS analysis, the accuracy of quantitation and identification of a compound in a complex matrix will be improved if the compound's mass spectrum has ions that are relatively characteristic to the compound. The higher the m/z values the more characteristic the ions are. The most characteristic ion of a compound would be the ion whose m/z value corresponds to the molecular weight of the compound. Meanwhile, the sensitivity of detection for the compound will be improved if such characteristic ions have relatively high abundances.

Chemical ionization mass spectrometry is very suitable for obtaining ions with high m/z of a compound, due to the "soft" nature of this ionization technique, that is, the energy involved in the ionization process is relatively less harsh than that involved in the more commonly used electron impact ionization mass spectrometry.

The eight trichothecenes analyzed belong to type A and type B trichothecenes, and are shown in Table 1 and Figure 1 below.

Type A

Type B

Figure 1. Structures of Type A and Type B trichothecenes.

Table 1. Trichothecenes analyzed in this study.

	Substituent at position					
Name	R_1	R_2	R_3	R_4	R_5	MW
HT-2 toxin (HT-2)	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$	424
T-2 toxin (T-2)	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$	466
Diacetoxyscirpenol (DAS)	OH	OAc	OAc	H	H	366
Deoxynivalenol (DON)	OH	H	OH	OH	-	294
3-Acetyl-deoxynivalenol (3-ADON)	OAc	H	OH	OH	-	338
15-Acetyl-deoxynivalenol (15-ADON)	OH	H	OAc	H	-	338
Nivalenol (NIV)	OH	OH	OH	OH	-	312
Fusarenon-X (FUS-X)	OH	OAc	OH	OH	-	354

---

[1] W. Langseth, T. Rundberget, Instrumental methods for determination of nonmacrocyclic trichothecenes in cereals, foodstuffs and cultures, *Journal of Chromatography A*, **815** (1998), 103-121

[2] M. Schollenberger et al, Determination of eight trichothecenes by gas chromatographymass spectrometry after sample clean-up by two-stage solid-phase extraction, *Journal of Chromatography A*, **815** (1998), 123-132

Experimental

Trichothecenes standards and trifluoroacetic anhydride (TFAA) were purchased from Sigma-Aldrich (Singapore). The trichothecenes standards were derivatized by trifluoroacetylation according to the method described by Schollenberger et al.,[2] and outlined in Figure 2.

The analysis was performed by using Shimadzu GCMS-QP2010nc in the Chemical Ionization (CI) mode. The analytical conditions are shown in Figure 3.

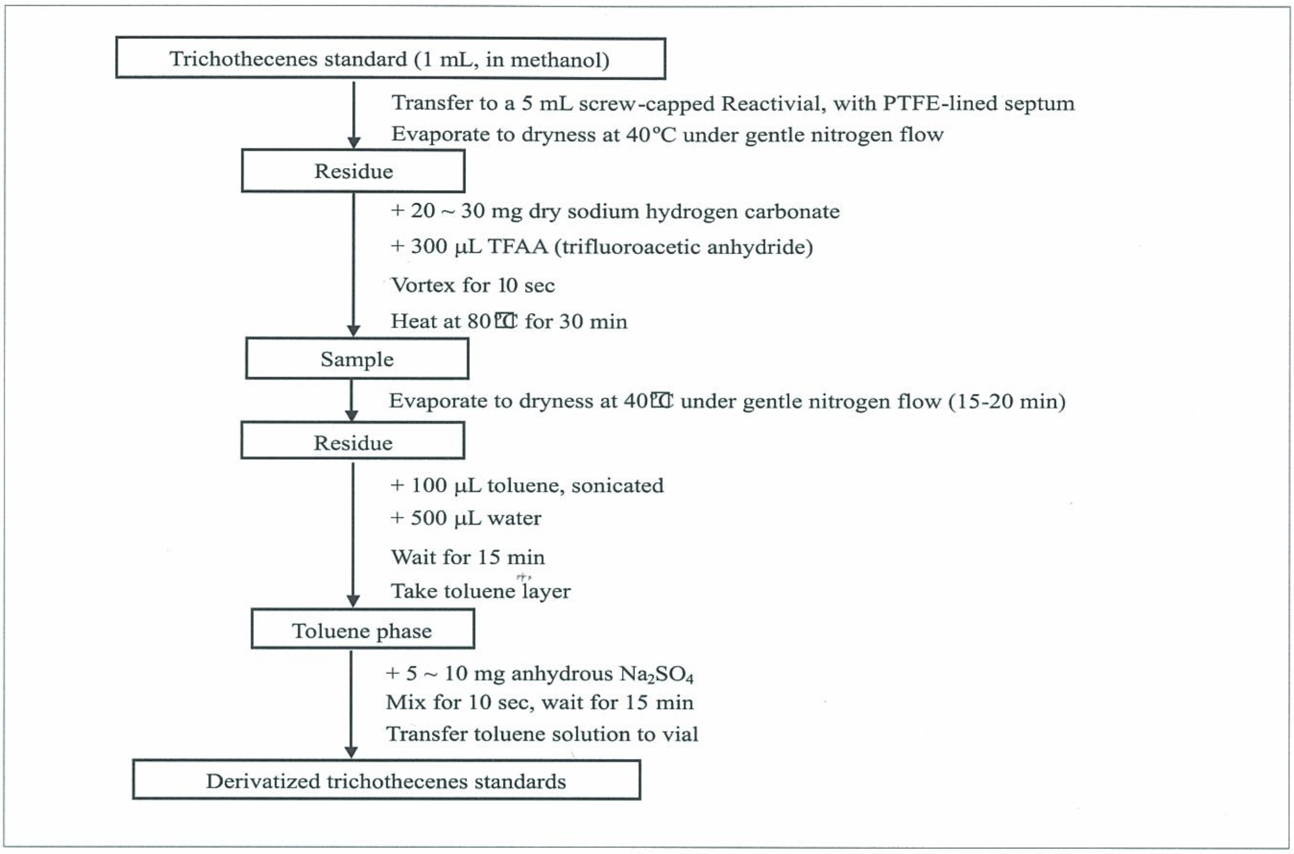


Figure 2. Derivatization steps of trichothecenes.

ANALYTICAL CONDITION
Instrument: Shimadzu GCMS-QP2010nc + AOC-20i auto-injector

GC Condition	
Injector temp.:	260 ^{0}C
Injection mode:	Pulsed Splitless
Sampling time:	1.5 min
High Pressure injection:	250 kPa (2min)
Flow control mode:	Linear velocity
Linear velocity:	40 cm/s
Column flow:	1.17 ml/min
Column:	Rtx-5SilMS 30m, 0.25mm ID, 0.25um df
Column temp. Program:	90^{0}C (2min) - 23^{0}C/min - 275^{0}C(2min) - 30^{0}C/min - 290^{0}C (15min)

MS Condition	
Ionization mode:	Chemical Ionization
Reagent gas:	Isobutane, 99.9%, 0.5bar
Ion source:	200^{0}C
Interface temp:	290^{0}C
Solvent cut time:	3min
Acquisition mode:	SCAN & SIM
SCAN m/z range:	280-900 u
SIM m/z:	Refer to NOTE in Table 2

Figure 3. GCMS analysis conditions.

[2] M. Schollenberger et al, Determination of eight trichothecenes by gas chromatographymass spectrometry after sample clean-up by two-stage solid-phase extraction, *Journal of Chromatography A*, **815** (1998), 123-132

Results

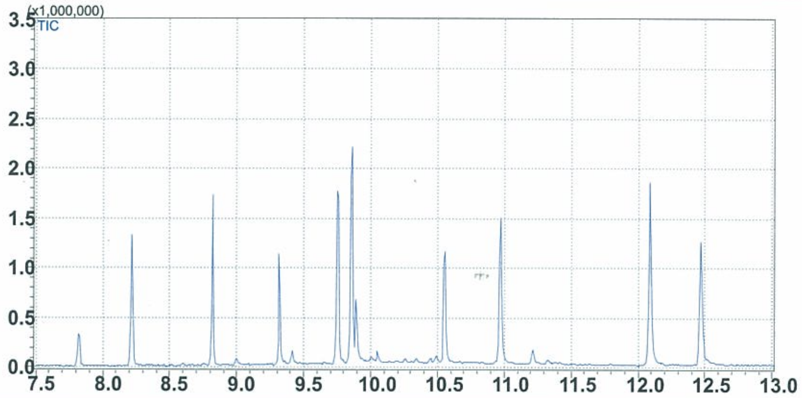
Trichothecenes Chromatogram & Mass Spectra

The total ion chromatogram of the 10 ppm trichothecenes mixture (after derivatization) obtained in Full scan mode was shown in Figure 3. The mass spectra of each compounds are shown in Figure 5. Identification of the compounds was based on the comparison of mass spectra and elution order obtained with those reported in literature.[2]

The results obtained by GCMS-QP2010 showed that for Type B trichothecenes analyzed, the molecular ion was the most abundant ion in the mass spectrum (see Table 2). Whereas for Type A trichothecenes analyzed, the molecular ion was the second most abundant ion in the mass spectrum.

Trichothecenes Calibration Curves

Figure 6 shows the SIM data and calibration curves for the trichothecenes derivatives.



NIV RT = 8.217 min
DON RT = 8.818 min
FUS-X RT = 9.318 min
15-ADON RT = 9.754 min
3-ADON RT = 9.856 min
DAS RT = 10.555 min
HT-2 RT = 10.973 min
T-2 RT = 12.467 min

Figure 4. Total Ion Chromatogram (TIC) of 10 ppm trichothecenes standard solution (derivatized, analyzed in SCAN mode).

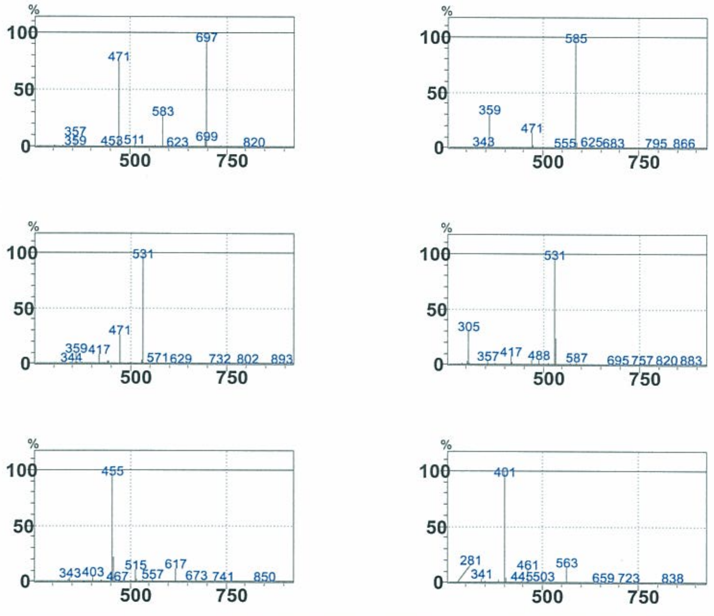


Figure 5. Chemical ionization mass spectra of the trichothecene derivatives.

Table 2. Mass spectral analysis results of trifluoroacetyl derivatives of eight trichothecenes (CI mode, isobutane as reagent gas).

	Observation				*Literature[2]*		
Name	*Molecular ion $(M+H)^+$ (u)*	*Main fragment (u)*	*Secondary fragment (u)*	*Tertiary fragment (u)*	*Molecular ion $(M+H)^+$ (u)*	*Main fragment (u)*	*Secondary fragment (u)*
NIV	*697*	*697*	*471*	*583*	*697**	*583*	*469*
DON	*585*	*585*	*359*	*471*	*585*	*585*	*479*
FUS-X	*643*	*643*	*417*	*529*	*643*	*529*	*469*
15-ADON	*531*	*531*	*471*	*417*	*531*	*471*	*417*
3-ADON	*531*	*531*	*305*	*417*	*531*	*531*	*471*
DAS	*463*	*403*	*463*	*445*	*463*	*403*	*289*
HT-2	*617*	*455*	*617*	*515*	*617*	*455*	*617*
T-2	*563*	*401*	*563*	*461*	*563*	*401*	*563*
NOTE: main fragment and secondary fragment ions were used for the SIM analysis					**out of mass range of instrument; not reported*		

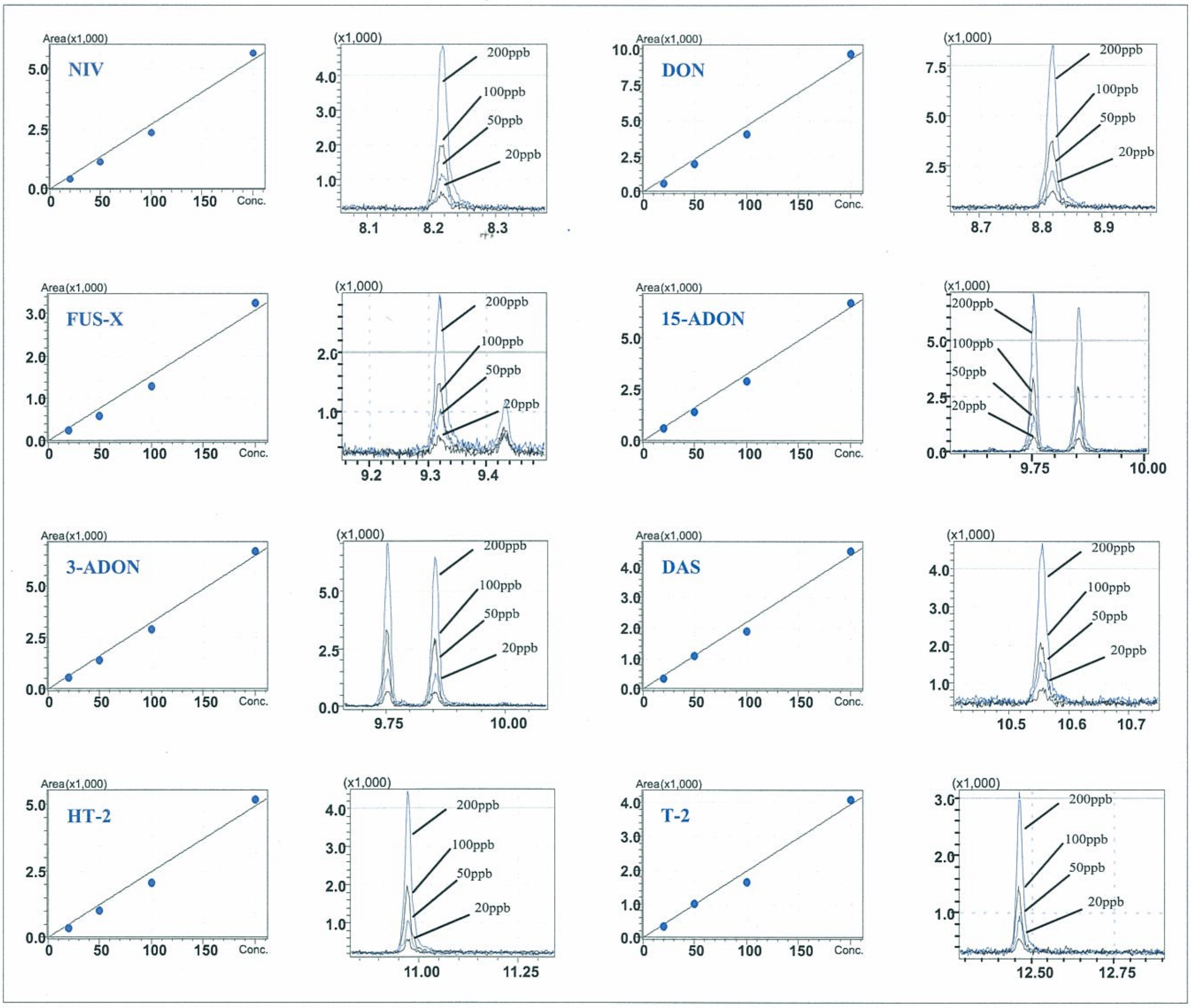


Figure 6. Calibration curves and SIM data for trichothecenes derivatives.

SHIMADZU (Asia Pacific) Pte. Ltd.
Customer Support Centre
16 Science Park Drive #01-01, The Pasteur
Singapore Science Park I, Singapore 118227
Tel: +(65) 6778-6280
Fax: +(65) 6778-2050

[IPDL] Printed in Singapore.

Related Solutions

- Price Inquiry
- Product Inquiry
- Technical Service / Support Inquiry
- Other Inquiry