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MASS SPECTRAL INVESTIGATIONS ON TRICHOTHECENE MYCOTOXINS

III. SYNTHESIS, CHARACTERIZATION AND APPLICATIONS OF PENTAFLUOROPROPIONYL AND TRIFLUOROACETYL ESTERS OF SIMPLE TRICHOTHECENES

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SUMMARY

The pentafluoropropionyl (PFP) and trifluoroacetyl (TFA) esters of several naturally occurring and synthetically modified simple trichothecenes were synthesized in nanogram amounts and characterized. Optimum conditions for the gas chromatographic (GC) separation of these derivatives and their analysis by negative ion chemical ionization (NICI) mass spectrometric technique were determined. These perfluoroacyl derivatives under the NICI conditions undergo limited but characteristic fragmentations similar to the fragmentations of heptafluorobutyryl esters of trichothecenes under the same conditions. Characteristic ions for the specific detection and accurate quantification of these PFP and TFA derivatives were chosen. Preliminary results indicated that the PFP derivatives are better suited for the analysis of simple trichothecenes by GC–NICI-MS technique. Ultra trace (0.5–2.0 pg) amounts of these PFP derivatives were detected by the developed procedure.

INTRODUCTION

Routine monitoring of agricultural products for the presence of fusarium fungal metabolites, specifically simple trichothecenes, has recently become mandatory in order to prevent any serious loss of crops and farm animals[1–6]. Consumption of food contaminated with trichothecenes has been linked to various human illnesses as well[2–4,7]. Recently the alleged use of these trichothecenes to induce human health hazards and possibly death has also been brought to the attention of the general public[8–11]. Hence the detection and analysis of trichothecenes in environmental and agricultural samples is very essential in the prevention and control of losses and disasters caused by these toxins introduced either by natural or induced exposures.

Several analytical, including gas chromatography–mass spectrometry (GC–MS), methods have been reported recently for the analysis of these simple trichoth-

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ecenes[5,6,8,9,12]. The procedures in general involve the conversion of the polar trichothecenes into their volatile, non-polar derivatives followed by the separation and analysis by GC–MS methods. Synthesis of heptafluorobutyryl (HFB) esters of trichothecenes and monitoring the negative ions formed by these derivatives under chemical ionization conditions has led to an ultra sensitive, accurate and applicable method of analysis for the simultaneous detection of several, simple trichothecene molecules[12]. Two synthetically modified trichothecenes, deoxyverrucarol[13] and 16-hydroxyverrucarol[14,15], have been found to be adequate internal standards for the quantification of simple trichothecenes by this MS procedure[12]. The latter method despite its sensitivity and applicability for the analysis of simple and macrocyclic trichothecenes in real samples is not suited for the analysis of some of the simple but important trichothecenes. The limitation was whenever the molecule contained more than three derivatizable hydroxyl groups and the molecular weights of their HFB derivatives (T-2 tetraol, nivalenol, etc.) exceeded the mass ranges of the commonly available mass spectrometers. This led us to investigate the synthesis and application of other perfluoroacylated esters of trichothecenes for the detection and quantification of at least these toxins preferably all simple trichothecenes in samples.

Pentafluoropropionyl (PFP) and trifluoroacetyl (TFA) esters of several simple trichothecenes (Table I) were synthesized and their negative ion chemical ionization (NICI) spectra were recorded. The mode of fragmentation of these derivatives were found to be similar to that of HFB esters[12]. However, the PFP derivatives were found to be better suited for the desired application.

EXPERIMENTAL

The origin of trichothecene standards, preparation and storage of standard solutions, synthesis and analysis of HFB derivatives of trichothecenes by GC–MS technique etc. are described elsewhere in detail[12].

Synthesis of trifluoroacetyl esters

Methanolic standard solution containing mixtures of trichothecenes (250 ng) was stransferred into a vial (2 ml) fitted with PTFE lined screw cap. The solvent was evaporated under nitrogen and the residue was dissolved in 10% acetonitrile–toluene (0.5 ml) and treated with trifluoroacetylimidazole (0.5 ml). The reaction mixture was heated at 70°C for 30 min, cooled and treated with 5% aqueous sodium carbonate solution (0.5 ml). The aqueous layer was removed using a disposable pipette and the organic layer containing the derivatives was further washed with 5% aqueous sodiumbicarbonate (0.5 ml) and twice with water (0.4 ml). An aliquot (1 μl) of the organic layer containing the TFA derivatives of trichothecenes (500 pg/μl) was analyzed by GC–MS.

Synthesis of pentafluoropropionyl esters

The residue containing the simple trichothecenes (250 ng) in a vial (2 ml) was dissolved in 10% acetonitrile–toluene (0.5 ml) and treated with 0.05 *M* triethylamine (0.1 ml) followed by pentafluoropropionic anhydride (0.1 ml). The reaction mixture was heated at 60°C for 15 min and cooled. The product was washed twice with 5% aqueous ammonia solution (0.5 ml) and water (0.4 ml), removing the aqueous solution using a disposable pipette.

GC–MS analysis of peracylated esters

A volume of 1 μl of the esters was injected into the DB-5 fused-silica capillary column via grobe injector in the splitless mode. The GC column was heated from 120°C to 300°C at a rate of 10°/min for TFA esters; and from 100°C at a rate of 10°/min up to 11 min and then at a rate of 25°/min to 300°C for PFP derivatives. The resolved esters were introduced into the chemical ionization source (100°C), maintaining the source pressure from 0.5–1.0 torr with CI reagent gas, methane. The negative ions were monitored either by total or selected ion monitoring mode.

RESULTS AND DISCUSSION

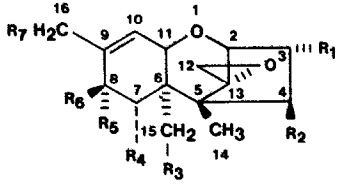
We had earlier reported[12] an ultra sensitive and accurate GC–MS method of analysis for the simultaneous detection of fourteen simple trichothecenes and related molecules, and their quantification using structurally similar, synthetically modified trichothecenes, deoxyverrucarol (DOVE) and 16-hydroxyverrucarol (HOVER) as internal standards. The developed procedure consisted of the conversion of polar trichothecenes with limited volatility into their highly volatile, and stable HFB esters for the successful GC separation, followed by analysis under NICI mode using methane as the CI reagent gas. The seven fluorine atoms substituted during the derivatization of each hydroxyl group contributed immensely in the production of negative ions by capturing the thermal electrons formed under CI conditions. The method was found to be extremely applicable in analyzing the real-life samples with excellent sensitivity and accuracy[12]. However, the trichothecenes with more than three hydroxyl groups could not be analyzed by this procedure since the molecular weights of their HFB esters exceeded the mass ranges of most of the commercial mass spectrometers.

The alternate derivatives considered to be investigated, in order to overcome the mass range problem, would preferably be PFP esters. The five fluorine atoms replacing each of the hydroxyl groups should be sufficient for the desired sensitivity of detection of trichothecenes by GC–NICI-MS technique, since the sensitivity of any molecule under this condition is directly proportional to the number of electron attracting groups present. However, the TFA derivatives were also investigated under NICI conditions even though a lesser sensitivity was expected during the analysis.

The mixture of trichothecenes as shown in Table I along with the intended internal standards, DOVE and HOVER, in 10% acetonitrile–toluene were converted into their PFP derivatives using the commercially available pentafluoropropionyl anhydride. The synthesized PFP derivatives were immediately subjected to a GC separation on a DB-5 fused-silica capillary column and analyzed under CI (methane) conditions. The negative ions formed were detected either by total or selected ion monitoring modes. The two simple trichothecenes, nivalenol and T-2 tetraol, which could not be analyzed earlier due to the higher molecular weights of their HFB esters were also detected in the mixture along with neosolaniol. The HFB ester of the latter was formed in poor yield and found to be unstable. In addition the M^- ions essential for the unambiguous detection of any molecules were detected with less than 15% relative abundance in comparison with the most abundant, non-specific heptafluorobutyrate ion (*m/z* 213). Similar results were also observed during the synthesis and GC–MS analysis of the PFP derivative of neosaloniol. However, the reconstructed

TABLE I

SIMPLE TRICHOTHECENES AND RELATED MOLECULES



	Molecular weight	R_1	R_2	R_3	R_4	R_5	R_6	R_7
T-2	466	OH	CH_3COO	CH_3COO	H	$(CH_3)_2CHCH_2COO$	H	H
HT-2	424	OH	OH	CH_3COO	H	$(CH_3)_2CHCH_2COO$	H	H
T-2 Tetraol (TET)	298	OH	OH	OH	H	OH	H	H
Monoacetoxyscirpenol (MAS)	324	OH	OH	CH_3COO	H	H	H	H
Diacetoxyscirpenol (DAS)	366	OH	CH_3COO	CH_3COO	H	H	H	H
Deoxynivalenol (DON)	296	OH	H	OH	OH	=O		H
Fusarinon-X (FUSX)	354	OH	CH_3COO	OH	OH	=O		H
Nivalenol (NIV)	312	OH	OH	OH	OH	=O		H
Deoxyverrucarol (DOVE)	250	H	H	OH	H	H	H	H
Verrucarol (VER)	266	H	OH	OH	H	H	H	H
Scirpentriol (SCIR)	282	OH	OH	OH	H	H	H	H
8α-Hydroxyverrucarol (8αHOVER)	282	H	OH	OH	H	OH	H	H
8β-Hydroxyverrucarol (8βHOVER)	282	H	OH	OH	H	H	OH	H
16-Hydroxyverrucarol (HOVER)	282	H	OH	OH	H	H	H	OH

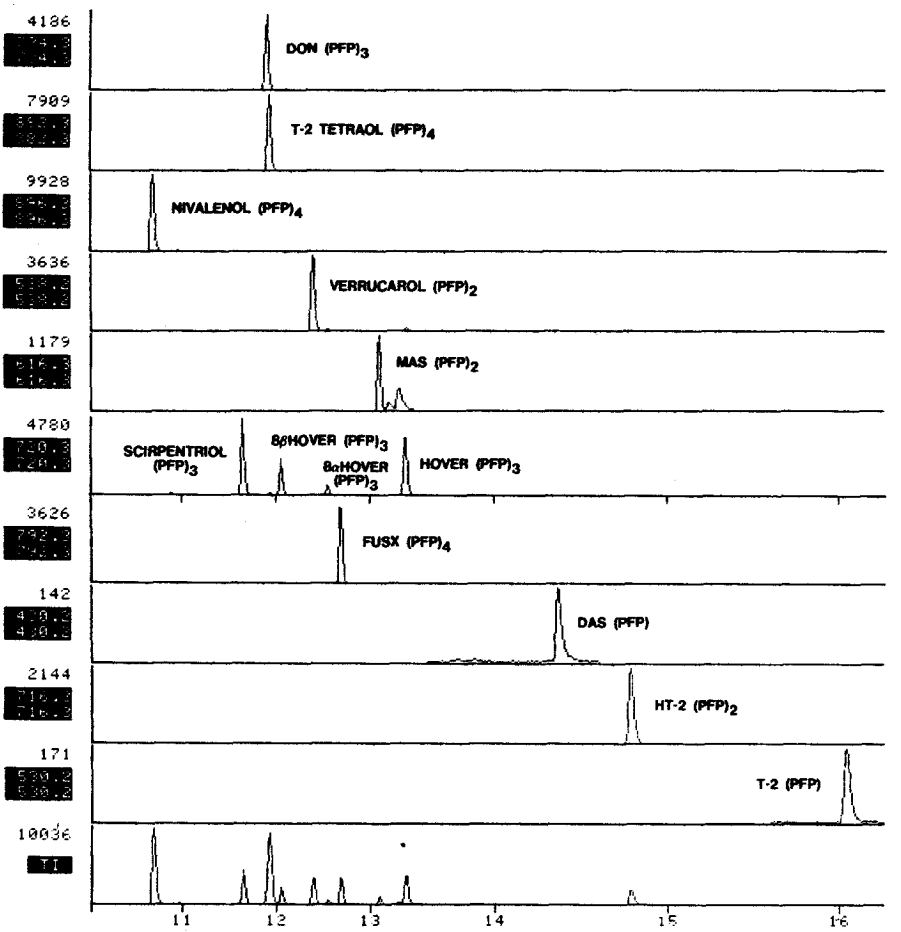


Fig. 1. Reconstructed mass chromatogram of pentafluoropropionyl derivatives of simple trichothecenes.

ion chromatogram as shown in Fig. 1 indicated the good resolution of the PFP derivatives during the chromatographic separation. The full-scan NICI spectra of these well separated esters were recorded.

Careful evaluation of the NICI spectra of the PFP derivatives was conducted and the results of the evaluation are listed in Table II. In most instances the most abundant ions are either the molecular ions M^- or the ions $(M-20_n)^-$ formed by the removal of one HF molecule from the molecular ions. The exceptions are HOVER $(PFP)_3$ with the base peak (m/z 163) due to the pentafluoropropionic ($CF_3CF_2CO_2^-$ or PFP) ion; the T-2 and DAS derivatives showing $(M-82)^-$ ions as the most abundant ions. The latter ions are proposed to be formed by the removal of two molecules of HF and one molecule of $CH_2=C=O$. Such a transformation was earlier noted in the NICI spectra of HFB esters of T-2 and DAS as well. Fusarinon-X with an acetate group at the C-4 atom also showed the ion formed by the removal of $CH_2=C=O$ from the M^- of its PFP ester. The derivatives with acetate group at the C-15 atom (T-2, HT-2, MAS and DAS) form ions due to the removal of neutral

TABLE II

RELATIVE ABUNDANCES (RA) OF NEGATIVE (CI) IONS OF PENTAFLUOROPROPIONYL ESTERS

HF, 20; $CH_2=CO$, 42; CH_3CHO, 44; $CH_3CO_2^-$, 59; CH_3CO_2H, 60; $(CH_3)_2CHCH_2CO_2^-$, 101; CF_3CF_2CO-, 147; CF_3CF_2CHO, 148; $CF_3CF_2CO_2^-$, 163; $CF_3CF_2CO_2H$, 164.

Derivative	*Retention time (min)*	*163*	*M^-*	*$M-(20)_n-(148)_m$*			*$M-(20)_n-(163)_m$*			*$M-(20)_n-(164)_m$*			*Other (m/z, RA)*
				n	*m*	*RA*	*n*	*m*	*RA*	*n*	*m*	*RA*	
T-2 (PFP)	16.1	5.7	20.4		–		1	0	19.4		–		(M−20−20−42), 100;
							2	0	9.7				(M−H−164), 12.4;
							3	0	2.2				(M−H−60−164), 6.5;
							0	1	6.5				(M−101−59−59), 15.6;
													(M−101−59−59−163), 1.6
HT-2 $(PFP)_2$	14.8	3.1	100	0	1	10	1	1	2.0	1	1	1.0	(M−H−60), 1.0
										1	2	1.0	
T-2 Cetraol (PFP)	11.9	48.3	100		–		1	0	61.6	0	1	2.7	(M−20−163−164), 2.0
							0	1	12.2				
							1	1	13.1				
							2	1	1.3				
							2	2	1.8				
							3	2	32.9				
MAS $(PFP)_2$	13.1	56.4	100		–		1	0	8.3	0	2	1.0	(M−59), 1.0
							1	1	1.2				
DAS (PFP)	14.3	25.7	3.1		–		1	0	30.7	1	1	3.8	(M−44), 10.7; (M−1−20−44), 10.7
							2	0	20.7	3	1	74.9	(M−20−20−42), 100
							1	1	14.1				
DON $(PFP)_3$	11.9	8.5	100	0	1	6.2	0	1	7.8	0	1	5.8	
							1	1	4.9				
							0	2	48.9				

FUSX $(PFP)_3$	12.7	10.2	100		–		1	0	1.0	3	2	5.2	(M – H – 42), 1.5
							3	3	1.0				(M – H – 20 – 20), 1.3; (M – 147), 1.2;
							0	1	1.3				(M – 147 – 147), 7.8; (M – 147 – 148), 47.2
NIV $(PFP)_4$	10.7	9.8	100	1	0	3.2		–			–		–
DOVE (PFP)	11.9	–	2.5	1	20	100		–			–		–
VER $(PFP)_2$	12.4	7.1	28.6		–		1	0	100		–		–
SCIR $(PFP)_3$	11.7	12.3	100		–		1	0	84.3		–		(M – H – 164 – 164), 10.0;
							2	0	1.0				(M – H – 20 – 164 – 164), 1.4
							1	1	4.3				(M – 163 – 163 – 164), 1.0
							2	1	1.0				
8αHOVER $(PFP)_3$	12.5	68.6	100		–		1	0	48.2	0	1	7.1	–
							2	0	1.0	1	1	15.1	
							0	1	22.9	0	2	14.3	
							1	1	33.5	1	2	11.2	
										2	2	6.5	
8βHOVER $(PFP)_3$	12.0	50.2	100		–		1	0	86.4		–		(M – H – 163 – 163), 1.4;
							2	0	1.2				(M – H – 164 – 164), 13.5
							0	1	10.8				
							1	1	12.1				
							2	1	1.4				
							3	1	3.2				
HOVER $(PFP)_3$	13.3	100	50.4	1	1	1.2	1	0	24.9	0	1	1.3	(M – 59 – 163 – 163), 24.5
							2	0	8.4				
							0	1	3.0				
							1	1	16.8				
							2	1	8.1				

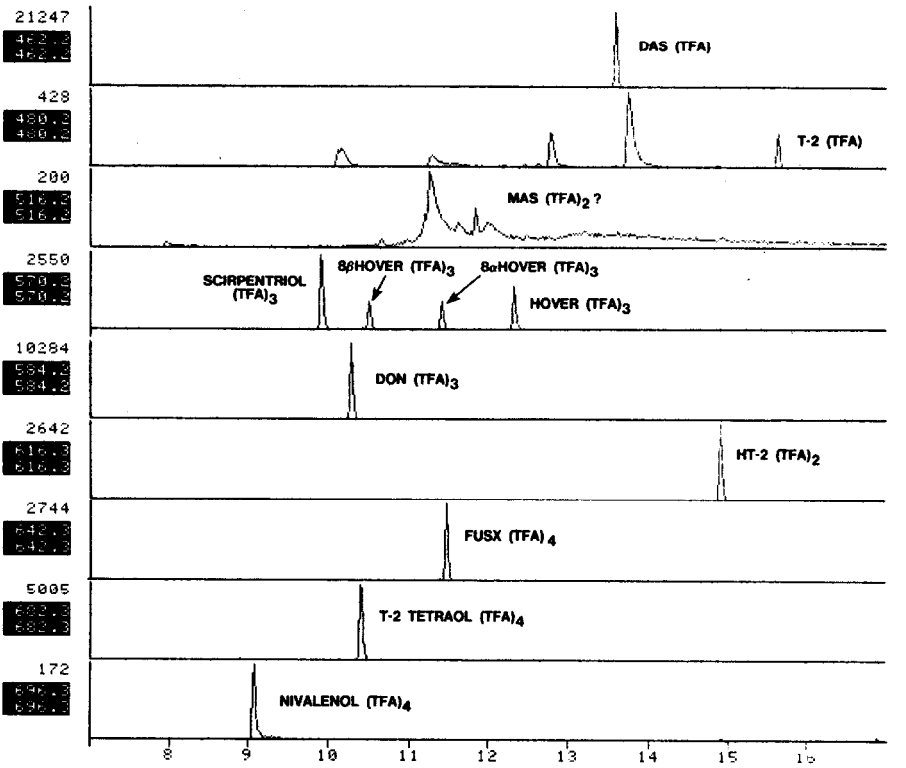


Fig. 2. Reconstructed mass chromatogram of trifluoroacetyl derivatives of sample trichothecenes.

acetic acid molecule or acetate ion from their corresponding M^- ions. The other transformations leading to the formation of the negatively charged fragments are due to the removal of one or more of neutral HF (*m/z* 20) and PFPH (*m/z* 164) molecules and/or the PFP (*m/z* 163) ions. The NICI spectra of the PFP esters of the structural isomers 8α- and 8β-hydroxyverrucarol were found to be different. The former formed ions with the removal of both PFP ions and PFPH and the latter mostly with the removal of PFP ions. The mode of fragmentations of PFP esters of trichothecenes in general seems to be similar to that of the corresponding HFB derivatives[12]. This was highly useful in chosing 5–6 most characteristic higher mass ions for the confirmation of the presence of these trichothecenes in samples by selected ion monitoring (SIM) mode of analysis.

The reconstructed mass chromatograms of the TFA derivatives of simple trichothecenes are shown in Fig. 2. The abundant fragments from the NICI spectra of the TFA esters of trichothecenes are listed in Table III. The types of fragments observed are similar to that of the other perfluoroacyl esters. The most abundant ions with the exception of some hydroxyverrucarols and MAS are either the M^- or $(M-82)^-$. The fragments were formed by the removal of one or more $CF_3CO_2^-$, CF_3CO_2H, CH_3CO_2H, CH_3CO^-, $CH_2=C=O$ etc. groups from the molecular ions. The TFA esters of MAS and Nivalenol were formed in poor yields, and hence the measured relative abundances may not be accurate due to the high background val-

TABLE III

THE RELATIVE ABUNDANCES (RA) OF NEGATIVE (CI) IONS OF TRIFLUOROACETYL DERIVATIVES

CF_3CHO-, 98; $CF_3CO_2^-$, 113; CF_3CO_2H, 114. For other groups see Table II.

Derivative	*Retention time (min)*	*113*	M^-	$M-(20)_n-(98)_m$			$M-(20)_n-(113)_m$			$M-(20)_n-(114)_m$			*Other (m/z, RA)*
				n	*m*	*RA*	*n*	*m*	*RA*	*n*	*m*	*RA*	
T-2 (TFA)	12.63	5.4	100	0	1	4.1		–			–		(M−98), 4.1 [M−(20)$_2$−42], 100; (M − 42), 17.0 (M−43), 73.8; (M−44), 71.6 (M−102), 1.5; (M−1−60), 9.5 (M−44−101), 15.5 (M−H−98−98), 10.7 [M−44−(113)$_2$], 10.1
HT-2 (TFA)$_2$	11.92	30.7	100		–			–			–		(M−102), 1.1; (M−101−98), 1.4 (M−102−98), 2.1 [M−(20)$_4$−98−101], 1.0
T-2 tetraol (TFA)$_4$	7.5	8.8	100		–		2	2	21.8		–		(M−98−114), 2.2
							3	3	21.4				[M−113−(114)$_3$], 1.2
MAS (TFA)$_2$	9.00	100	7.7		–			–			–		(M−60), 1.6; (M−69), 1.0 (M−20−60), 1.0
DAS (TFA)	10.62	–	100		–			–			–		–
FUSX	8.53	35.0	100		–			–			–		(M−2H−20−20−98), 61.2
NIV (TFA)$_4$	6.33	2.0	100	2	0	2.3	0	1	2.3	0	1	3.2	–
				0	1	8.3	0	2	1.8	0	2	2.2	
SCIR (TFA)$_3$	7.07	79.7	100		–		2	1	4.7	2	1	26.2	[M−H−(114)$_2$], 11.8 [M−H−20−(114)$_2$], 11.8
8βHOVER (TFA)$_3$	7.62	20.5	100		–		0	1	40.3	0	1	11.4	[M−H−(113)$_3$], 5.9 (M−113−114), 1.1
8αHOVER (TFA)$_3$	8.47	100	21.3		–		1	0	1.0		–		–
							0	1	6.2		–		–
HOVER (TFA)$_3$	9.35	100	18.9		–		1	0	1.2		–		–

TABLE IV

MOST ABUNDANT NEGATIVE (CI) IONS OF PERFLUOROACYLATED DERIVATIVES

Compound	*Derivative*		
	TFA	*PFP*	*HFB*
T-2	480	530	580
HT-2	616	716	816
T-2 Tetraol	682	882	–
MAS	113	616	716
DAS	462	430	480
DON	113 (M^-, 90%)	734	884
FUSX	642	792	942
NIV	696	896	–
DOVE	326	376	426
VER	–	538	638
SCIR	570	720	870
8αHOVER	113 (M^-, 20%)	720	870
8βHOVER	113 (M^-, 10%)	720	870
HOVER	–	720	870

ues. Despite repeated attempts the synthesis of TFA derivative of verrucarol was not successful. In general the ion intensities due to the derivatives were lower in comparison with the PFP esters and the noise level during the total ion monitoring was noted to be higher. The most abundant ions for each of these esters are listed in Table IV.

After the careful evaluation of NICI spectra of PFP and TFA derivatives, the former was chosen for further study; since they formed higher mass ions than the corresponding trifluoroacetyl esters, required for the specific detection of the molecules and the most abundant ions in all cases were either M^- or $(M-20)^-$ or

TABLE V

SIM CONDITIONS FOR THE PFP DERIVATIVES OF SIMPLE TRICHOTHECENES

Group	*Time (min)*		*Trichothecene*	*Ions (m/z)*	*Dwell time (ms)*
	Start	*End*			
1	10.0	12.3	DOVE	376.2	50
			SCIR and 8βHOVER	720.3	50
			DON	734.3	50
			T-2 Tetraol	882.3	50
			NIV	896.3	50
2	12.3	13.7	VER	538.2	50
			MAS	616.3	50
			8αHOVER and HOVER	720.3	50
			FUSX	792.3	50
3	13.7	14.7	DAS	430.2	200
4	14.7	15.9	HT-2	716.3	200
5	15.9	16.0	T-2	530.2	200

TABLE VI

DETECTION LIMITS OF TRICHOTHECENE DERIVATIVES

Trichothecene	*SIM ion (m/z)*		*Detection limit (pg)**	
	PFP	*HFB*	*PFP*	*HFB*
DOVE	376	426	–	0.50
T-2	530	580	2.00	2.00
HT-2	716	816	0.50	0.50
T-2 tetraol	882	–	0.50	–
MAS	616	716	0.50	0.50
DAS	430	480	0.50	2.0
DON	734	884	0.50	0.10
FUSX	792	942	0.50	0.20
NIV	896	–	0.50	–
VER	538	638	0.50	0.25
SCIR	720	870	0.50	0.20
8αHOVER	720	870	0.50	0.50
8βHOVER	720	870	0.50	0.40
HOVER	720	870	0.50	0.20

* PFP, under conditions specified in Table V; HFB, ref. 12.

$(M-82)^-$ ions. In the case of the TFA esters they were due to non-specific $CF_3CO_2^-$ ions. The yields of formation of TFA derivatives of T-2, MAS, verrucarol and HOVER were very poor in comparison with their corresponding PFP esters. In addition the number of electron-capturing fluorine atoms, required for higher sensitivity of detection, in PFP derivatives are more than the TFA esters.

Several derivative solutions containing the mixtures of trichothecenes (0.1–10 pg/μl) were prepared and minimum detectable limits for each compounds were determined by analyzing under NICI conditions and monitoring a single most characteristic ion for individual derivatives. The SIM experimental conditions used for these measurements, the same intended for the routine monitoring of samples for the presence of simple trichothecenes, are indicated in Table V. More dwell times were allotted for the less intense ions in order to improve their sensitivity during the analysis. The measured values are shown in Table VI along with that of HFB esters. They are comparable with that of the HFB derivatives and were good. The PFP derivatives were stored at −2°C for 24 h after their synthesis and analyzed by the usual method. Their stability for the specified period was good. Further investigations with respect to the synthesis of TFA derivatives with improved yields, stability of PFP derivatives on prolonged storage and evaluation and comparison of all three of these perfluoroacylated derivatives for the application of simple trichothecenes analysis are underway. However, it is reasonable to conclude at this point that the PFP derivatives are suitable alternative to HFB derivatives of simple trichothecenes, at least for the ones with more than three derivatizable groups, for their analysis and quantification by GC–NICI-MS method.

CONCLUSION

Investigation on the synthesis and NICI mass spectral data of the perfluoropropionyl and trifluoroacetyl esters of trichothecenes indicated the former to be an adequate derivative for the analysis of simple trichothecenes, especially the ones with four or more hydroxyl groups.

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