Journal of Chromatography, 469 (1989) 209–222
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 327

MASS SPECTRAL INVESTIGATIONS ON TRICHOTHECENE MYCOTOXINS

VII. LIQUID CHROMATOGRAPHIC–THERMOSPRAY MASS SPECTROMETRIC ANALYSIS OF MACROCYCLIC TRICHOTHECENES

THAIYA KRISHNAMURTHY*
Research Directorate, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD 21010-5423 (U.S.A.)
DEBRA J. BECK and ROBERT K. ISENSEE
Oneida Research Services, One Halsey Road, Whitesboro, NY 13492 (U.S.A.)
and
BRUCE B. JARVIS
Department of Chemistry, University of Maryland, College Park, MD (U.S.A.)
(First received May 2nd, 1988; revised manuscript received January 6th, 1989)

SUMMARY

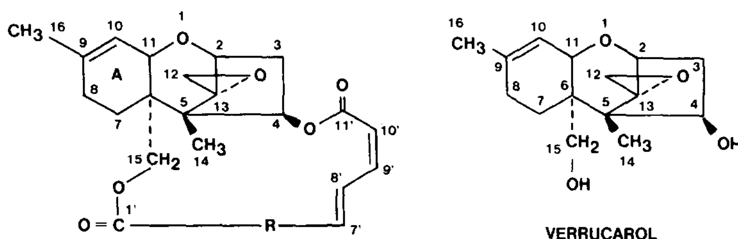
Thermally labile, polar toxic roridins and biologically active, isomeric baccharinoids were separated on a reversed-phase high-performance liquid chromatography column and effectively ionized under thermospray ionization conditions. The mass spectra indicated the formation of corresponding molecular ion–ammonium adducts in great abundance. Experiments designed for monitoring specific ions of these analytes at predesignated intervals were utilized for the accurate analysis of these macrocyclic trichothecenes in real, crude samples. A synthetically modified macrocyclic trichothecene, 8-ketoverrucarin A, was used as the internal standard for the detection and quantification of these compounds. Minimum detectable limits, during this first reported method for the unambiguous analysis of these structurally related macrocyclic trichothecenes, were determined to be 2–5 ng.

INTRODUCTION

Trichothecenes are toxic fungal metabolites, which have been associated with human health hazards, loss of farm products, live stock and other animals for decades[1–5]. They were also alleged to have been used as chemical warfare agents[6–9]. Recently, the growth of trichothecene producing fungi has been also detected in the U.S. urban environments[10]. Thus, the development of rapid, specific, sensitive, and accurate methods for the analysis of these toxins in environmental, agricultural, and biological samples has become vital. The large number of trichothecenes present and their polar and labile properties pose a great challenge in developing one common methodology to analyze all the derivatives of interest[11,12].

0021-9673/89/$03.50 © 1989 Elsevier Science Publishers B.V.

Recently, several mass spectrometric (MS) and direct tandem MS methods have been developed for the analysis of simple trichothecenes[13–23]. We have previously reported several such methods having excellent speed, selectivity, and sensitivity with minimal sample clean-up[17–23]. Some of these procedures were the first ever methods reported for the analysis of macrocyclic trichothecenes (Fig. 1), the most toxic variety[1,2,24–26]. However, none of them were direct and could be used for the unambiguous detection of the isomeric macrocyclics. Development of one single MS



COMPOUND	MOL. WT.	R	OTHER
RORIDIN A	532	$-CHOH\,CH\,CH_3CH_2\,CH_2OCH\,CHOH\,CH_3$ (2′, 3′, 12′, 4′, 5′, 6′/13′, 14′)	—
RORIDIN D	530	$-CH-CH\,CH_3\,CH_2\,CH_2OCH\,CHOH\,CH_3$ (2′–O–3′ epoxide; 12′, 4′, 5′, 6′/13′, 14′)	—
RORIDIN E	514	$-CH=CHCH_3\,CH_2\,CH_2\,OCH\,CHOH\,CH_3$ (2′, 3′, 12′, 4′, 5′, 6′/13′, 14′)	—
RORIDIN H	512	$-CH=CH\,CH_3\,CH_2\,CH$ (–O–6′CH–, –O–CHCH₃) (2′, 3′, 12′, 4′, 5′, 6′, 13′, 14′)	—
BACCHARINOID-1 AND 2	548	$-CH_2\,CH\,CH_3CHO\,HCH_2O\,CH\,CHOH\,CH_3$ (2′, 3′, 12′, 4′, 5′, 6′/13′, 14′)	8β-OH
BACCHARINOID-3 AND 7	548	$-CHO\,HCH\,CH_3\,CH_2\,CH_2O\,CH\,CHOH\,CH_3$ (2′, 3′, 12′, 4′, 5′, 6′/13′, 14′)	8β-OH
BACCHARINOID-4	562	$-CH-CHCH_3\,CHOH\,CH_2O\,CHCHOH\,CH_3$ (2′–O–3′ epoxide; 12′, 4′, 5′, 6′/*13′, 14′)	8β-OH
BACCHARINOID-5	562	$-CH-CHCH_3\,CHOH\,CH_2O\,CHCHOH\,CH_3$ (2′–O–3′ epoxide; 12′, 4′, 5′, 6′/*13′, 14′)	9,10-EPOXY
8-KETOVERRUCARIN A (KVA)	516	$-CHOH\,CH\,CH_3\,CH_2\,CH_2\,O\,C(=O)-$ (2′, 3′, 12′, 4′, 5′, 6′)	8-KETO

* CENTER OF EPIMERIZATION

Fig. 1. Structures of roridins and baccharinoids.

methodology for the accurate and unambiguous detection of all known types of trichothecenes including the isomers present in complex, aqueous matrices is highly desirable.

Hence, we have recently investigated the thermospray ionization[27] of some of the most toxic (roridins) and few known benevolent (baccharinoids) and isomeric macrocyclic trichothecenes and developed a simple liquid chromatography–mass spectrometry (LC–MS) procedure for their analysis. It was applied to the analysis of these compounds in crude, aqueous extracts from Brazilian *Baccharis megapotamica* and *B. coridifolia* plants. A semi-synthetic macrocyclic trichothecene, 8-ketoverrucarin A (KVA)[28], which was used earlier as an internal standard for the analysis of macrocyclic trichothecenes by direct, chemical ionization tandem MS methods[20–22], was found to be an adequate internal standard under these conditions as well. The unambiguous identification of the isomeric baccharinoids, which was not possible by earlier methods, and their quantification were possible during this newly developed procedure.

EXPERIMENTAL

A Finnigan-MAT TSQ tandem mass spectrometer, Waters Assoc. standard high-performance liquid chromatograph with dual pumps, automatic gradient controller (680), Waters 600 multisolvent delivery system, Rheodyne injector (1125), Waters universal U6K injector and Vestec thermospray LC–MS interface were used throughout this investigation.

All methanolic standard solutions were stored in reactivials fitted with mininert valves (Supelco) at 2°C. Glass distilled HPLC grade solvents, purchased from Burdick & Jackson Labs., were used as received. The reversed-phase C_8 LC column (25 cm × 4.6 mm, 5 μm) manufactured by Waters Assoc. was used for all measurements.

Standard or sample solutions (20 μl or less) were injected onto the LC column and separated using 0.1 *M* aqueous ammonium acetate–methanol (50:50) at a flow-rate of 1.2 ml/min. The effluent from the column was introduced into the mass spectrometer via the thermospray LC–MS interface. The probe tip and the source block temperatures were maintained at 205°C and 265°C, respectively. The aerosol temperature was kept at 255°C. The source pressure was kept at 3.3 Torr. The ionized molecules were analyzed by scanning quadrupole 3 from 100 to 600 a.m.u. in 0.5 s. Alternatively, specific programs were used for monitoring only selected ions at predesignated intervals using quadrupole 3. Each sample analysis was preceded by a blank run regardless of the mode of analysis.

Extraction of baccharis plants

A 5-g sample of dried plant material was crushed in small pieces and soaked overnight in 40 ml of 90% aqueous methanol. The solvent was decanted and the residue was treated again with 40 ml of 90% aqueous methanol and left to extract overnight. The combined extracts were filtered and washed twice with 50 ml of hexane. The methanol solution was concentrated on a rotary evaporator and the resulting solution was extracted three times with 20 ml of methylene chloride. The combined methylene chloride solution was dried over anhydrous sodium sulfate and decanted; and the solvent was removed on a rotary evaporator. The residue was dissolved in

a minimum amount of methylene chloride and loaded on a silica gel column, packed in a pasteur pipette and washed with methylene chloride (5 ml), methanol–methylene chloride (10:90) (5 ml) and methanol–methylene chloride (25:75) (5 ml). The combined effluent was concentrated under vacuum, dissolved in methanol (1 ml) and stored in a vial (2 ml) at 2°C prior to analysis.

RESULTS AND DISCUSSION

The optimum conditions for the thermospray ionization of the individual macrocyclic trichothecenes were determined by introducing them (500 ng) directly into the mass spectrometer via a blank column and the thermospray interface and a methanol–0.1 *M* ammonium acetate buffer (50:50). This solvent system, a midpoint of the possible solvent mixture to be used during the LC separation, was chosen for the measurements. Hence, the experimentally determined optimum ionization conditions could be modified slightly, if required, during the LC–MS analysis of the mixtures. The individual temperatures of the source block, probe tip, and aerosol jet were varied step-wise, keeping two out of three constant, and acquiring the positive ion MS data. The recorded total ion counts and that of the ammonium adducts provided the required information. In like manner, the optimum conditions for the ionization of the mixture containing all of the analytes were determined.

The analytes were ionized most efficiently when the probe tip and the source block temperatures were kept at 205°C and 265°C, respectively. Optimum aerosol temperature was determined to be 255°C. The ionization efficiency of the analytes were comparable, when the above temperatures were maintained which required occasional manual adjustment of the temperature, regardless of the variation in the composition of the eluting solvent (buffer–methanol) during the HPLC separation of the components. Optimum thermospray temperatures were checked everyday prior to measurements. Only slight modifications if any was required throughout this investigation. The source pressure was maintained at 3.3 Torr. Similarly, the optimum flow-rates (1.2 ml/min) of the solvents and minimum concentration of ammonium acetate (20%) required for maximizing the ionization were determined.

Optimum conditions for the LC separation of the roridins and baccharinoids were determined after a series of controlled experiments. The eluted compounds were ionized and their full-scan mass spectra (*m/z* 100–600) were acquired. The corresponding mass chromatograms of the ammonium adducts were traced to determine the resolution of compounds. The mass spectra of all resolved components were obtained. Conditions where a base line separation was achieved with well defined mass spectra of the components were sought after during these experiments. Isocratic conditions involving solvent mixtures ranging from 20 to 50% methanol were tried. Alternatively, we used gradient programming (curve 6) from the initial concentration (20–60% methanol) maintained for 2 min and increased to final concentration (60–80% methanol) in 4 min and maintained until all components were eluted. Most of the components except baccharinoids-2 and -3 were well resolved in a shorter duration under isocratic elution using 0.1 *M* ammonium acetate–methanol (35:65) or when the initial concentration of 50% methanol was maintained for 2 min and increased to 70% methanol in 4 min using the gradient curve 6 (Fig. 2).

Alternatively, solvent mixtures of different ratios were introduced, as specified

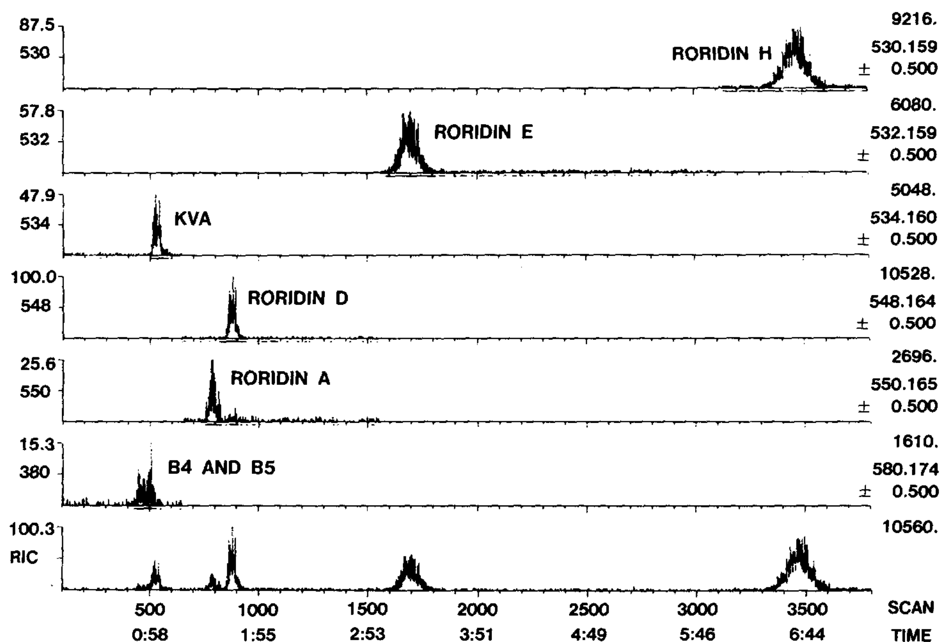


Fig. 2. Mass chromatograms of roridins and baccharinoids standards. Initial concentration of methanol–0.1 *M* ammonium acetate (50:50) maintained for 2 min and increased to 70% methanol in 4 min and maintained during rest of the period.

below, into the column at predesignated intervals using the Waters 600 multisolvent delivery system. The initial concentration ratio of buffer–methanol (59:41) was maintained for 1 min and changed to 56:44 and maintained for 19 min. Then the ratio was rapidly changed to 20:80 and maintained until the end of the run. All components including baccharinoids were resolved and unambiguously detected under these following conditions (Fig. 3) and hence used throughout the investigation.

A mixture containing 1 μg of all the roridin and baccharinoid standards was analyzed under the above experimenally determined optimum conditions. The list of observed positive ions in the mass spectra and their relative abundances are listed in Table I. Most of the analytes formed fewer ions under these softer thermospray ionization conditions and invariably the molecular ion–ammonium adduct ions were the most abundant ones.

Two Brazilian *Baccharis* plant extracts, labelled as "meg10" and "cor3", were analyzed under the same conditions. They were obtained from *B. megapotamica* and *B. coridifolia* plants, respectively. Roridins A, D, and E were detected in "cor3" sample. The presence of roridins A (traces), D, E and baccharinoids 1–5, and 7 was observed in "meg10" sample. The observed relative retention times of the compounds in standard and sample solutions are listed in Table II. The standard spectrum of roridin A along with the sample spectrum (cor3) are shown in Fig. 4. The spectra of baccharinoid 5 in the standard and "meg10" sample are indicated in Fig. 5. The characteristic ions with their relative abundances, in comparison with abundances observed for the standards, are noted in the sample spectra. The relative retention times observed for compounds in samples were comparable to those of standards. These are sufficient for the

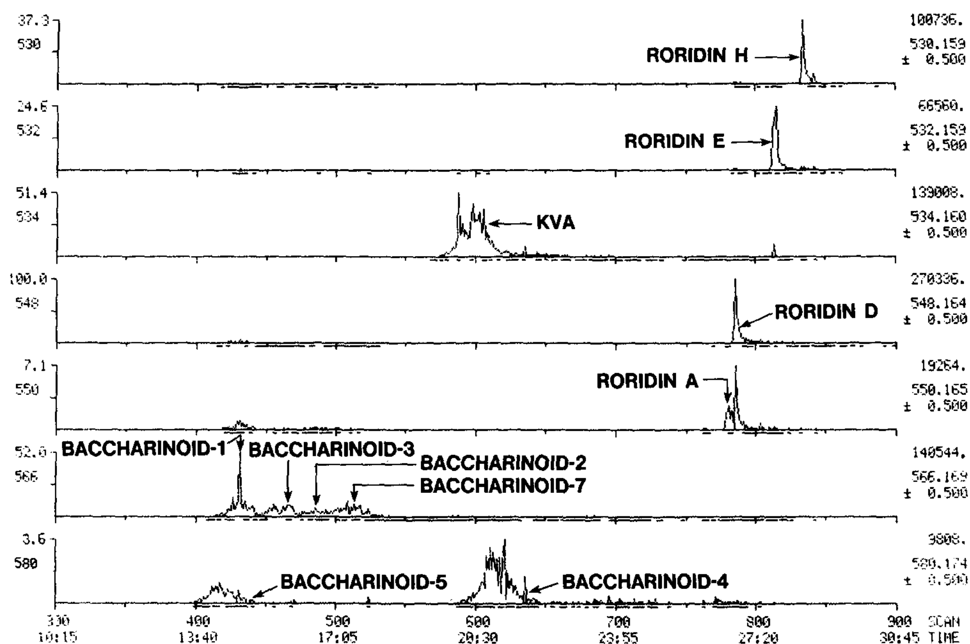


Fig. 3. Reconstructed ion chromatogram of resolved baccharinoids and roridins. Initial concentration of methanol–0.1 M ammonium acetate (59:41) was maintained for 1 min and changed to 56% methanol and kept for 19 min and then changed to 20% methanol and maintained until the end of the run.

TABLE I

MASS SPECTRA OF AMMONIUM ADDUCTS (THERMOSPRAY IONIZATION) OF MACROCYCLIC TRICHOTHECENES

Only 10 most abundant ions are tabulated.

Trichothecenes	*m/z (relative abundances)*				
Roridin D	548 (100)	504 (68)	394 (2)	318 (1)	266 (16)
	249 (22)	231 (3)	182 (3)	148 (2)	106 (6)
Roridin E	532 (100)	489 (12)	488 (56)	378 (2)	249 (3)
	150 (3)	124 (5)	120 (3)	109 (1)	
Roridin H	530 (100)	513 (5)	266 (5)	249 (4)	209 (1)
	124 (2)	106 (1)			
Baccharinoid-1	566 (85)	548 (4)	530 (8)	504 (3)	282 (94)
	265 (100)	257 (43)	247 (12)	229 (4)	110 (11)
Baccharinoid-2	566 (100)	548 (6)	530 (5)	522 (7)	504 (9)
	282 (36)	265 (73)	247 (12)	229 (6)	150 (9)
Baccharinoid-3	566 (100)	549 (37)	531 (2)	419 (9)	401 (15)
	349 (24)	265 (28)	247 (66)	229 (25)	155 (13)
Baccharinoid-4	580 (20)	534 (100)	298 (2)	282 (4)	265 (12)
	263 (10)	245 (24)	227 (2)	150 (12)	148 (32)
Baccharinoid-7	566 (100)	548 (5)	522 (29)	505 (3)	282 (4)
	265 (5)	247 (3)	229 (1)	217 (1)	110 (3)
KVA	534 (100)	392 (2)	298 (3)	280 (6)	263 (6)
	245 (7)	228 (2)	148 (28)		

TABLE II

RELATIVE RETENTION DATA

Retention time for the internal standard, KVA, is 19:59 minutes.

Compound	*Standard*	*Sample*	
		Cor3	*Meg10*
Roridin A	1.30	1.30	–
Roridin D	1.31	1.31	1.30
Roridin E	1.36	1.36	1.35
Roridin H	1.40	–	–
Baccharinoid-1	0.72	–	0.73
Baccharinoid-2	0.82	–	0.82
Baccharinoid-3	0.77	–	0.78
Baccharinoid-4	1.03	–	1.03
Baccharinoid-5	0.69	–	0.70
Baccharinoid-7	0.86	–	0.86
KVA	1.00	1.00	1.00

identification of these trichothecenes and possibly for their analysis in samples. They are in agreement with earlier results observed during the isolation of these compounds from several kilogram quantities of the plant materials and their characterization by spectrometric methods[29].

However, the high noise levels observed in the sample spectra cannot be ignored. Either more rigorous sample clean-up procedures prior to analysis or careful analysis of sample and standard spectra with respect to the relative abundances of the characteristic ions should be performed for their unambiguous identification and quantification in unknown samples.

Alternate methods for the detection and confirmation of these compounds with increased sensitivities were developed. Two procedures (Table III) were devised by programming the data system to monitor the one or more selected ions (Table I) for each analyte at predesignated intervals. These intervals were based on the retention times of the compounds. These procedures are more efficient than detecting the specific ions of all compounds during the entire analysis. The added advantage of such procedures is that the detected analytes could be confirmed by selectively monitoring only the characteristic ions and not the background noise during the LC–MS sample runs. Such approaches were successful during the accurate analysis of several simple trichothecenes by gas chromatography (GC)–MS methods[17–19].

Analysis of a mixture containing 5 ng of each of the standards was performed by procedure L_1, described in Table III. Each analyte was detected with good resolution and signal to noise ratio of 1/5 or better. The mass chromatogram observed while analyzing the *B. megapotamica* sample under the same condition is shown in Fig. 6. A simple experiment for the confirmation of baccharinoid-4 by monitoring the six most characteristic ions was devised (Table IV). The less abundant ions were monitored with longer dwell times for higher sensitivity. Ions specific to the internal standards were also monitored at appropriate intervals during the same run (Table IV). The compounds were eluted isocratically using acetonitrile–0.1 *M* aqueous

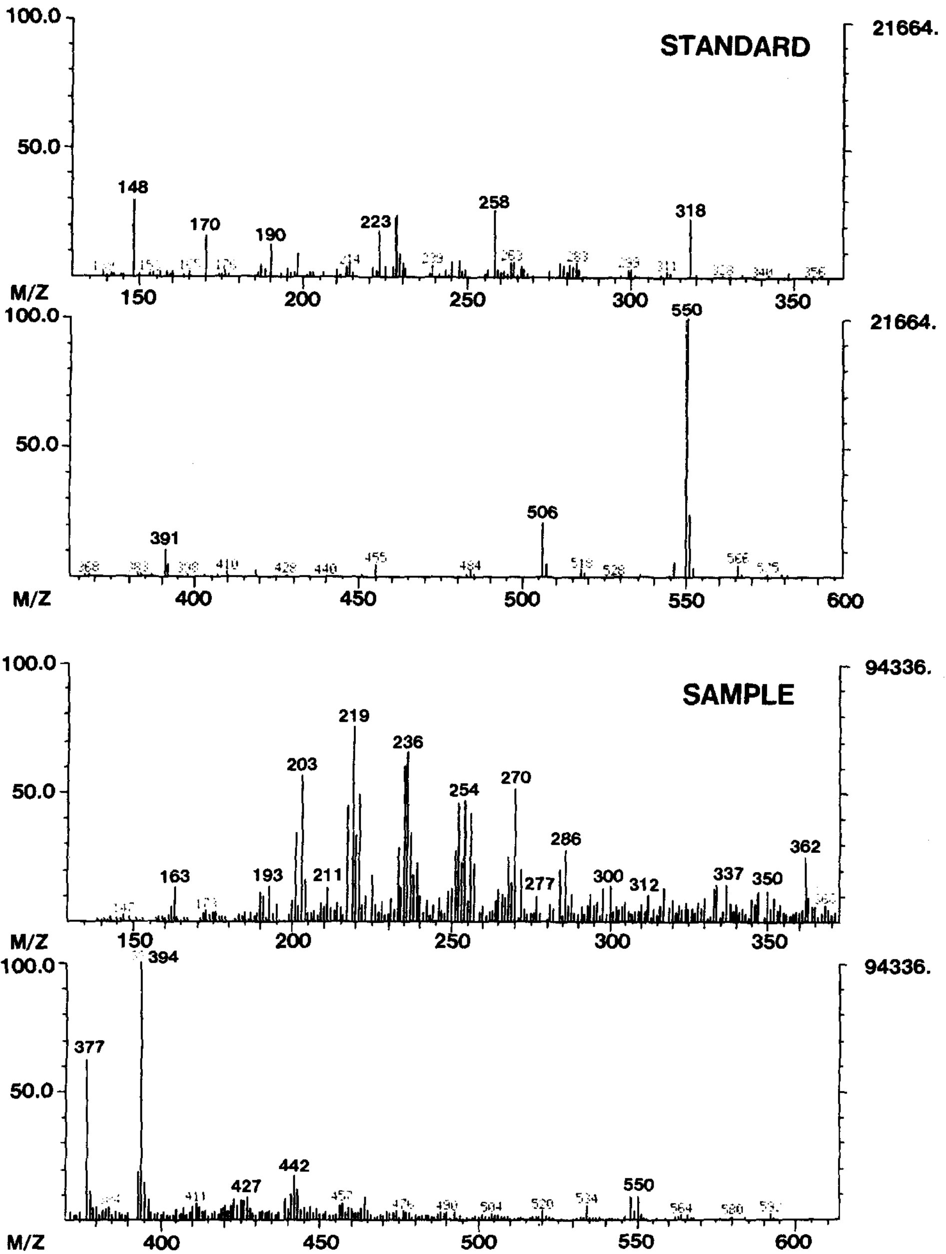


Fig. 4. Mass spectra of roridin A in standard and *B. coridifolia* ("cor3") sample.

ammonium acetate (60:40), in order to reduce the analysis time. Results observed during the standard and sample ("meg10") analyses are shown in Fig. 7. The relative abundance values observed for the standard and the sample are quite adequate for the unambiguous detection of baccharinoid-5 in the sample. Analysis of crude samples by

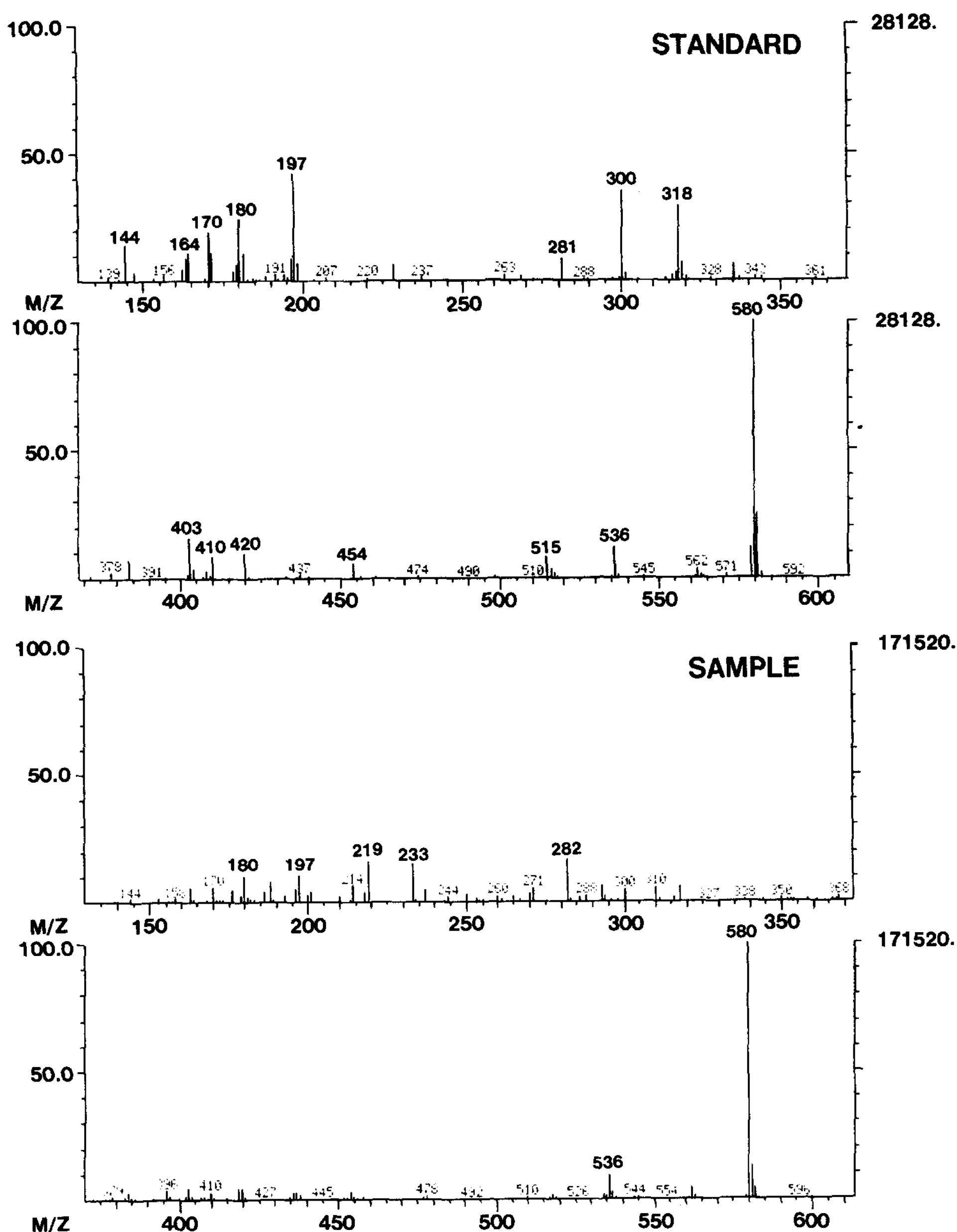


Fig. 5. Mass spectra of baccharinoid-5 in standard and *B. megapotamica* ("meg10") sample.

such selected ion monitoring procedures (L_1 in Tables III and IV) for the detection and confirmation of analytes is highly effective and applicable to the analysis of unknowns.

The procedure such as L_3 (Table III) could be used for the simultaneous detection and confirmation of analytes, during a single analysis, in an unknown sample

TABLE III

SELECTED ION MONITORING AT SPECIFIC INTERVALS

Compounds	*Monitored ions (m/z)*		*Monitoring time (min)*		
	L_1	L_3	*Start*	*Run*	*End*
KVA	534, 566, 580	245, 263, 534	0	24.00	24.00
Baccharinoids		247, 566, 580			
Roridin A	548, 550	249, 504, 506	24.00	3.00	27.00
Roridin D		548, 550			
Roridin E	530, 532	249, 488, 513	27.00	5.00	32.00
Roridin H		530, 532			

of limited quantity. A standard mixture containing 20 ng of all the roridins and baccharinoids was analyzed with good sensitivity.

The minimum detectable limits under the conditions expressed in experiments L_1 for roridins (and KVA) and baccarinoids were 2 and 5 ng, respectively. Under L_3 experimental conditions, 20 ng of each of the analytes could be detected. The signal-to-noise ratio observed under both conditions was at least 10/1. Full-scan (*m/z* 100–600) mass spectra of the roridins and baccharinoids could be obtained from 100 ng of the standards.

A procedure such as L_1 (Table III) can be utilized for the quantification of the detected analytes as well. Mixtures containing each of the analytes (5–100 ng) and KVA (10 ng) were analyzed under these conditions. The relative amounts of the analytes with respect to the internal standard were plotted against their corresponding

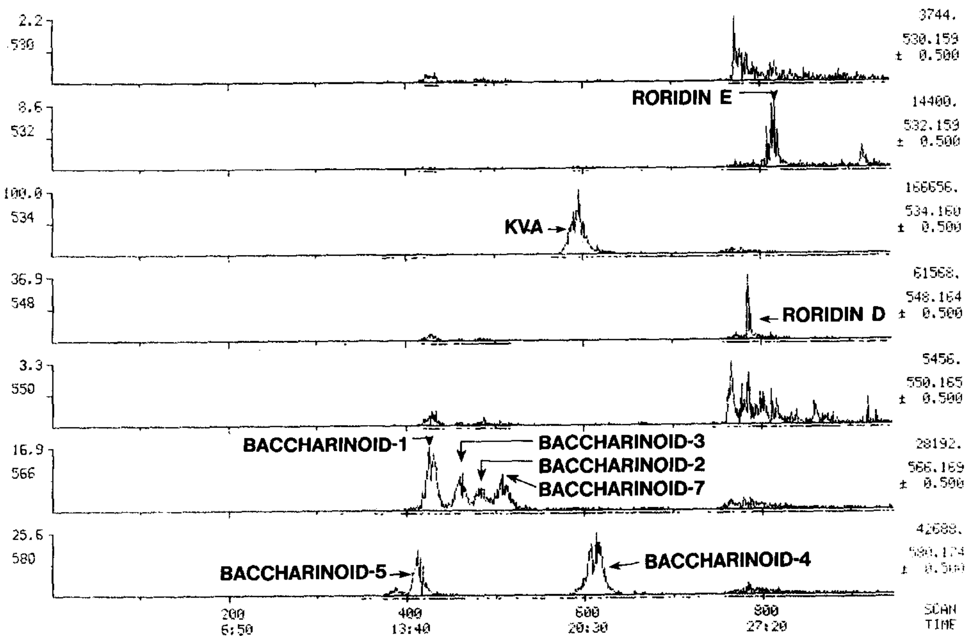


Fig. 6. Selected reaction monitoring of *B. megapotamica* sample.

TABLE IV

CONFIRMATION OF ANALYTES IN SAMPLE

Compound	Ions (m/z)[a]					
Baccharinoid-4	580 (0.03)	536 (0.06)	282 (0.06)	265 (0.06)	263 (0.12)	245 (0.06)
KVA	534 (0.03)	517 (0.12)	280 (0.12)	263 (0.12)	245 (0.06)	148 (0.06)
Roridin A	550 (0.03)	533 (0.12)	506 (0.12)	284 (0.03)	266 (0.03)	249 (0.03)

[a] Monitoring time (s) for each ion is mentioned in the parenthesis.

TABLE V

CALIBRATION DATA

Internal standard (8-ketoverrucarin A), 25 ng.

Compound	Response factor	Linear regression constants		
		Correlation coefficient	Slope	Intercept
Roridin A	0.37 ± 0.03	0.999	0.449	−0.082
Roridin D	5.93 ± 1.83	0.961	7.884	−1.032
Roridin E	1.30 ± 0.53	0.931	1.256	0.523
Roridin H	1.03 ± 0.15	0.978	0.851	0.280
Baccharinoid-1	1.42 ± 0.33	0.999	1.084	1.564
Baccharinoid-2	0.35 ± 0.08	0.993	0.265	0.140
Baccharinoid-3	0.68 ± 0.17	0.993	0.895	0.413
Baccharinoid-4	0.22 ± 0.05	0.996	0.289	0.136
Baccharinoid-5	0.26 ± 0.06	0.990	0.305	−0.069
Baccharinoid-7	0.55 ± 0.02	0.999	0.525	0.089

TABLE VI

SAMPLE ANALYSIS DATA*

KVA, 25 ng; spiked amounts, 25–50 ng.

Compound	Amount detected[a]		Recovery Meg10 (%)
	Cor3	Meg10	
Roridin A	21.75	–	22.5
Roridin D	14.38	9.68	59 ± 15
Roridin E	4.35	5.4	79 ± 13
Roridin H	–	–	60 ± 23
Baccharinoid-1	–	0.11	71 ± 18
Baccharinoid-2	–	3.38	73 ± 9
Baccharinoid-3	–	1.20	113 ± 11
Baccharinoid-4	–	195.9	53 ± 4
Baccharinoid-5	–	105.9	60 ± 8
Baccharinoid-7	–	1.27	69 ± 11

[a] Amount (ng) of analyte detected in the analyzed volume.

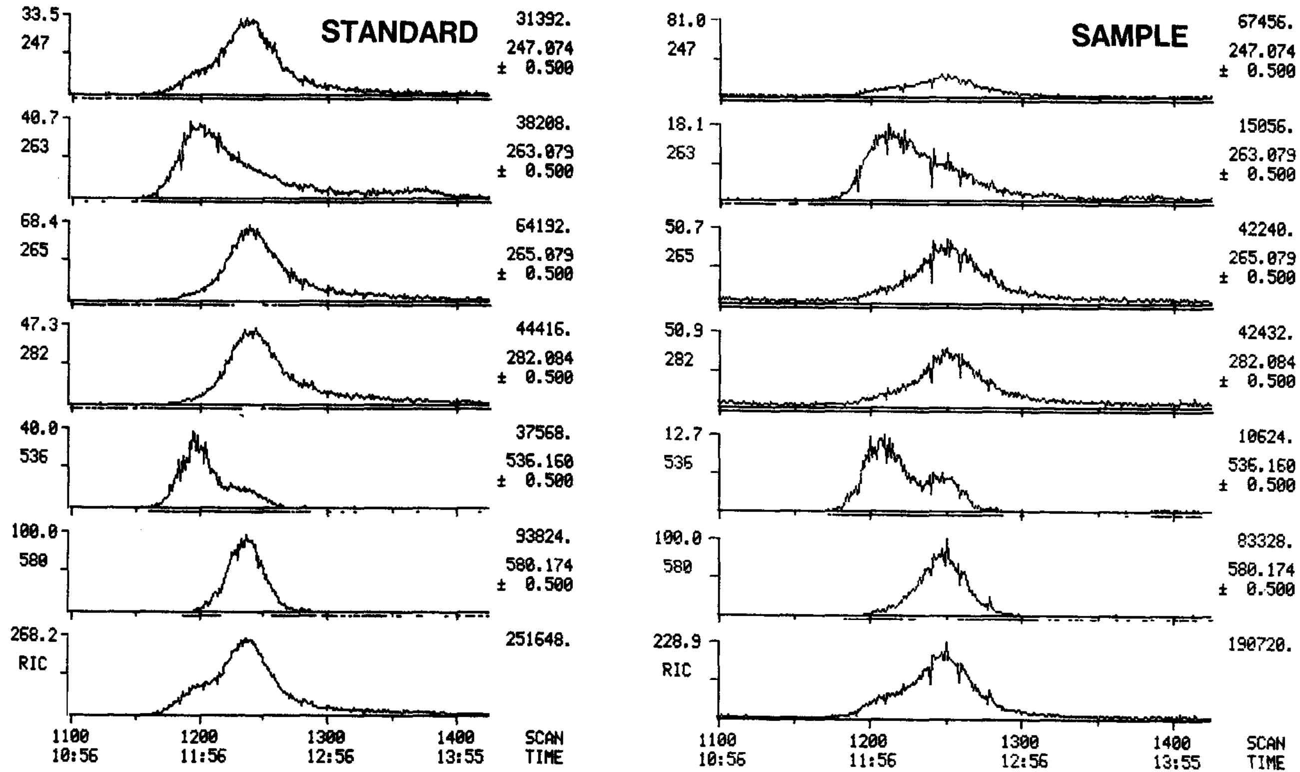


Fig. 7. Confirmation of baccharinoid-4 in *B. megapotamica* by selected ion monitoring.

relative ion abundances. A linear correlation between these two measured factors was observed for all analytes (Table V). Samples "cor3" and "meg10" and spiked solutions were also analyzed under the same conditions. The observed quantification data of the samples are listed in Table VI. It was clearly demonstrated that 5-ng quantities of these macrocyclic trichothecenes could be accurately analyzed in real samples via carefully designed experiments for monitoring selected ions at specific intervals.

In summary, the thermospray ionization of toxic roridins and biologically active baccharinoids, followed by MS analysis appears to be adequate for analyzing a number (10) of macrocyclic trichothecenes (including some isomeric compounds). KVA appears to be a suitable internal standard for the LC–MS analysis of these compounds. Selective ion monitoring procedures for the specific and sensitive analysis of roridins and baccharinoids are preferred to obtaining full-scan mass spectra. The high noise levels observed during the analysis of crude samples are thereby avoided.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mrs. Marguerite E. Brooks, and Dr. Richard Smardzewski of U.S. Army Chemical Research, Development, and Engineering Center, Aberdeen Proving Ground, MD, U.S.A. for their interest and valuable suggestions rendered throughout this investigation.

REFERENCES

1 Y. Ueno, in Y. Ueno (Editor), *Developments in Food Science. 4-Trichothecenes, Chemical, Biological and Toxicological Aspects*, Elsevier, Amsterdam, 1983, pp. 1–6.
2 H. Kurata and Y. Ueno (Editors), *Toxigenic Fungi—Their Toxins and Health hazards*, Elsevier, Amsterdam, 1984.
3 J. V. Rodrick, C. W. Hesseltine and M. A. Mehlman, *Mycotoxin in Human and Animal health*, Pathotox Publishers, Park Forest, IL, 1st ed., 1977, pp. 189–340.
4 J. Palti, *Toxigenic Fusaria, Their Distribution and Significance Causes of Disease in Animals and Men*, Paul Parey, Berlin, 1978.
5 D. Eaker and T. Wadstrom (Editors), *Natural Toxins*, Plenum, New York, 1980.
6 A. M. Haig, *Chemical Warfare in Southeast Asia and Afganistan*, U.S. Department of State Special Report Number 98, Washington, DC, March 1982.
7 G. P. Shultz, *Chemical Warfare in Southeast Asia and Afganistan: An Update*, U.S. Department of State Special Report Number 104, Washington, DC, November 1982.
8 R. T. Rosen and J. D. Rosen, *Biomed. Mass Spectrom.*, 9 (1982) 443.
9 C. J. Mirocha, R. A. Pawlosky, K. Chatterjee, S. Watson, W. Hayes, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 1485.
10 W. A. Croft, B. B. Jarvis and C. S. Yatawara, *Atmos. Environ.*, 20 (1986) 549.
11 K. Ishi, in Y. Ueno (Editor), *Developments in Food Science. 4-Trichothecenes, Chemical, Biological and Toxicological Aspects*, Elsevier, Amsterdam, 1983, pp. 7–19.
12 B. B. Jarvis in Y. Ueno (Editor), *Development in Food Science. 4-Trichothecenes, Chemical, Biological and Toxicological Aspects*, Elsevier, Amsterdam, 1983, 20–38.
13 P. M. Scott, P. Lau, S. R. Kanhue, *J. Assoc. Off. Anal. Chem.*, 64 (1981) 1364.
14 J. M. Rothberg, J. L. McDonald and J. C. Swims, *ACS Symp. Ser.*, 234 (1983) 272.
15 R. D. Voyksner, W. M. Hagler, Jr., K. Tyczkowska and C. A. Haney, *J. High Resolut. Chromatogr. Chromatogr. Comm.*, 8 (1985) 119.
16 R. D. Plattner, G. A. Bennett, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 1470.
17 T. Krishnamurthy, M. B. Wasserman and E. W. Sarver, *Biomed. Mass Spectrom.*, 13 (1986) 503.
18 T. Krishnamurthy, E. W. Sarver, B. B. Jarvis and S. L. Greene, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 132.
19 T. Krishnamurthy and E. W. Sarver, *J. Chromatogr.*, 355 (1986) 253.

20 T. Krishnamurthy and E. W. Sarver, *Biomed. Environ. Mass Spectrom.*, 15 (1988) 13.
21 T. Krishnamurthy and E. W. Sarver, *Biomed. Environ. Mass Spectrom.*, 13 (1987) 185.
22 T. Krishnamurthy and E. W. Sarver, *Anal. Chem.*, 59 (1987) 1272.
23 T. Krishnamurthy, E. W. Sarver and B. B. Jarvis, in J. F. J. Todd (Editor), *Adv. Mass Spectrometry*, Wiley, New York, NY, 1986, pp. 595–596.
24 B. B. Jarvis, N. B. Pena, M. M. Rao, N. S. Comezoglu, T. F. Comezoglu and N. B. Mandava, in A. C. Thompson (Editor), *The Chemistry of Allelopathy*, *Am. Chem. Soc. Symp. Ser.*, 268, 1984, p. 149.
25 G. G. Habermehl and L. Busam, *Liebigs. Ann. Chem.*, (1984) 1746.
26 B. B. Jarvis and E. P. Mazzola, *Acc. Chem. Res.*, 15 (1983) 388.
27 M. L. Vestal, *Int. J. Mass Spectrom. Ion Phys.*, 15 (1982) 388.
28 B. B. Jarvis, G. P. Stahly, G. Pavanasasivam, E. P. Mazzola, *J. Med. Chem.*, 23 (1980) 1054.
29 B. B. Jarvis, Department of Chemistry, University of Maryland, College Park, MD, 1984, unpublished results.