ELSEVIER

Journal of Chromatography A, 695 (1995) 65–73

JOURNAL OF CHROMATOGRAPHY A

Analysis of irritants by capillary column gas chromatography–tandem mass spectrometry

Paul A. D'Agostino*, Lionel R. Provost

Defence Research Establishment Suffield, Box 4000, Medicine Hat, Alberta, T1A 8K6, Canada

First received 6 October 1994; revised manuscript received 29 November 1994; accepted 29 November 1994

Abstract

Daughter spectra were obtained for the molecular and principal electron impact fragmentation ions of four irritants, 1-methoxycycloheptatriene, 2-chloroacetophenone, *o*-chlorobenzylidenemalononitrile and dibenz[*b*,*f*]-1,4-oxazepin, during capillary column GC–MS/MS analysis. The use of standardized collisional activated dissociation cell conditions resulted in the acquisition of daughter spectra suitable for identification and data-base generation purposes. Daughter operation detection limits of 100 pg ($S/N > 10:1$), for the highest molecular mass irritant, dibenz[*b*,*f*]-1,4-oxazepin, were obtained. This level of sensitivity was approximately the same as that routinely obtained for chemical warfare agents during capillary column GC–MS analysis under electron impact ionization conditions. The specificity of GC–MS/MS was demonstrated by spiking a complex diesel exhaust extract, with 1-methoxycycloheptatriene.

1. Introduction

Chemical warfare agents can be classified into two general categories, those that exert a lethal effect and those that act in an incapacitating manner. Lethal chemical warfare agents include nerve agents such as sarin, soman and tabun, while incapacitating agents include irritants (tear gases or riot control agents). Acute exposure to irritants causes a number of incapacitating effects including burning or irritation of the skin and eyes, coughing, nausea and vomiting. The incapacitating nature of these chemicals has led to the development of dispersal devices for their use in riot control situations, during military training exercises and to a lesser extent as chemical weapons on the battlefield [1]. The most commonly employed irritants are *o*-chlorobenzylidenemalononitrile, often referred to as tear gas, and 2-chloroacetophenone. Dibenz[*b*,*f*]-1,4-oxazepin has been used less frequently and 1-methoxycycloheptatriene was evaluated as a possible military training agent.

The text of the 1994 "Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and their Destruction" states in Article 1 that: "Each state party undertakes not to use riot control agents as a method of warfare". United Nations peacekeeping forces could encounter use of irritants during active duty in regions of the world where there is a threat of chemical warfare agent use. Intelligence gathering, through the collection of contaminated samples, and subsequent analysis of the samples would enable identification of the suspect chemical and con-

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)01181-8

firm use of a controlled chemical for warfare purposes. The results of such analyses would likely contribute to the development of appropriate strategic and political positions.

Gas chromatographic (GC) methods, including methods based on GC retention indices [2–7], have been used for the detection of irritants in suspect samples. These methods and others involving the use of chromatographic techniques for the detection of irritants and other chemical warfare agents have recently been reviewed [8]. In cases where positive identification is required, most laboratories analyse suspect extracts by GC–MS, as this technique offers the greatest sensitivity and certainty for chemical warfare agent identification. Electron impact (EI) ionization has been used frequently for irritant identification and the EI data for the common irritants, 2-chloroacetophenone, *o*-chlorobenzylidenemalononitrile and dibenz[*b*,*f*]-1,4-oxazepin [9–14], and related benzylidenemalononitriles [12,15] have been reported.

However under an allegations of use scenario, collected samples would typically be environmental samples such as soil, water and air, or man-made materials such as paint, concrete, and munitions or munition fragments from the scene of an attack. In many cases the samples taken may have been exposed to rain, heat, sunlight or wind, for days or weeks and much of the original chemical warfare agent may have evaporated or undergone degradation (e.g., hydrolysis of *o*-chlorobenzylidenemalononitrile). This "weathering" process makes identification of the chemical warfare agent that much more difficult. The levels of contamination could be extremely low in these cases and require sophisticated methods for the detection and identification of these compounds in the presence of naturally occurring chemical interferences. Similar difficult analytical situations could also be envisioned during the analysis of forensic samples for irritants.

GC–MS, while generally accepted as the technique of choice for the confirmation of irritants, has limitations in the presence of high levels of chemical interferences. Tandem mass spectrometry offers considerable advantages over traditional mass spectrometry under this scenario, as it is a more specific and sensitive technique for the identification and confirmation of chemical warfare agents and related compounds [16–21] in complex environmental samples [17–19]. Daughter spectra of lethal chemical warfare agents have been published in these papers, but they do not include tandem mass spectrometric data for common irritants.

GC–MS/MS characterization of irritants was investigated, since this technique may enable selective identification of these compounds in complex samples. This report summarizes the daughter spectra obtained for the irritants, 1-methoxycycloheptatriene, 2-chloroacetophenone, *o*-chlorobenzylidenemalononitrile and dibenz[*b*,*f*]-1,4-oxazepin, during GC–MS/MS analysis of these compounds and illustrates the utility of GC–MS/MS for the specific identification of 1-methoxycycloheptatriene in the presence of numerous interfering hydrocarbons collected in a diesel exhaust environment.

2. Experimental

2.1. Samples

Four irritants, 1-methoxycycloheptatriene, 2-chloroacetophenone, *o*-chlorobenzylidenemalononitrile and dibenz[*b*,*f*]-1,4-oxazepin were provided by the DRES Organic Chemistry Laboratory. Standard solutions containing the irritants were prepared at the 10 and 0.2 ng/μl level in dichloromethane for use during capillary column GC–MS/MS analyses.

Air from a diesel exhaust environment was sampled through a R51A charcoal chemical cartridge (American Optical Corp., Southbridge, MA, USA) for 2 h at a rate of 20 l/min. The charcoal (50 g) was Soxhlet extracted overnight with dichloromethane (250 ml) and concentrated by nitrogen blowdown to 10 ml. 1-Methoxycycloheptatriene was spiked into a portion of the extract at the 0.5 ng/μl level to evaluate the detection of an irritant in a complex environmental matrix, similar to what would be expected during battlefield sampling in the presence of diesel powered vehicles.

2.2. Instrumental

Capillary column GC–MS/MS analyses were performed with either a VG AUTOSPEC-Q or VG-70SQ hybrid tandem mass spectrometer, each of which was equipped with a Hewlett Packard Model 5890 gas chromatograph. Either a 15 m × 0.32 mm ID DB-1701 or DB-5 J&W capillary column (0.25 μm film thickness) was used for GC–MS/MS analyses with the following temperature program: 40°C (2 min hold), 10°C/min to 280°C (5 min hold). GC injections were cool on-column using an injector of our own design [2] with the AUTOSPEC-Q instrument and were splitless with the VG-70SQ instrument.

EI-MS operating conditions were similar for both mass spectrometers: source pressure, $3 \cdot 10^{-6}$ Torr (1 Torr = $1.3 \cdot 10^{2}$ Pa); source temperature, 200°C; electron energy, 70 eV; electron emission, 100 or 200 μA and accelerating voltage, 8 kV. CAD (collisional activated dissociation) cell conditions [CAD cell argon pressure of $8–9 \cdot 10^{-7}$ Torr and an energy of 25 eV (laboratory scale)], based on the best compromise between sensitivity and spectral content were used for all daughter analyses [20,21]. This argon pressure reduced the intensity of the perfluorokerosene ion at *m/z* 219 to 50% of its intensity with residual air in the CAD cell. A typical daughter spectra for *m/z* 219 at 30 eV (with no detectable signal below 12 eV) under this CAD cell condition follows:

Argon (50% reduction): *m/z* 219:*m/z* 131:
m/z 69 = 1:0.25:0.15

Daughter spectra were obtained under these CAD cell conditions for the molecular and principal EI fragmentation ions for each of the four irritants during capillary column GC–MS/MS analysis with the AUTOSPEC-Q instrument. The quadrupole was operated at unit resolution and scanned from 250 to 50 u at 0.7 s/scan and, the sector resolution was set at 1000 (10% valley definition). Daughter spectra for all four irritants were obtained during each chromatographic analysis (DB-1701 column) by monitoring for 1-methoxycycloheptatriene from 1:00 to 6:00 min, 2-chloroacetophenone from 6:00 to 12:00 min, *o*-chlorobenzylidenemalononitrile from 12:00 to 14:00 min and dibenz[*b*,*f*]-1,4-oxazepin from 14:00 and 17:00 min.

Similar GC–MS/MS conditions were employed with the VG-70SQ during multiple reaction ion monitoring analyses of the diesel exhaust extract and 1-methoxycycloheptatriene spiked diesel exhaust extract. Both the *m/z* 122 to 107 and *m/z* 122 to 92 processes were alternately monitored for 80 ms (20 ms delay) to provide data from three characteristic 1-methoxycycloheptatriene ions, during chromatographic analyses.

A sector resolution of 10 000 (10% valley definition) was employed during high resolution GC–MS analyses with the AUTOSPEC-Q instrument.

3. Results and discussion

Fig. 1 illustrates typical capillary column GC–MS/MS chromatograms obtained during the

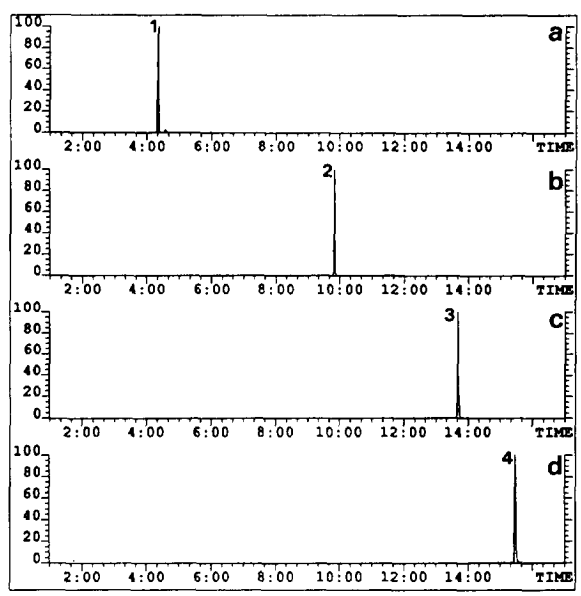

Fig. 1. Capillary column GC–MS/MS chromatograms for daughters of (a) *m/z* 122 [$M^{+\cdot}$ for 1-methoxycycloheptatriene, (1)], (b) *m/z* 154 [$M^{+\cdot}$ for 2-chloroacetophenone, (2)], (c) *m/z* 188 [$M^{+\cdot}$ for *o*-chlorobenzylidenemalononitrile, (3)] and (d) *m/z* 195 [$M^{+\cdot}$ for dibenz[*b*,*f*]-1,4-oxazepin, (4)]. [Time scale in minutes, DB-1701 column]

analysis of a 10-ng standard containing the four irritants, 1-methoxycycloheptatriene, 2-chloroacetophenone, *o*-chlorobenzylidenemalononitrile and dibenz[*b*,*f*]-1,4-oxazepin with the DB-1701 column. Daughter spectra for the molecular ions obtained during this analysis are illustrated in Figs. 2a, 3a, 4a and 5a. The reproducibility of the daughter spectra obtained were found to be similar to that obtained for phosphate esters, with the standard deviation of the daughter ion relative intensities being approximately 30% [21].

Typical sensitivity of daughter detection was evaluated by monitoring the molecular ion (*m*/*z* 195, EI relative intensity: 100%) of the highest molecular mass irritant, dibenz[*b*,*f*]-1,4-oxazepin (Fig. 6). An interpretable daughter spectrum (inset in Fig. 6) was obtained for 100 pg of dibenz[*b*,*f*]-1,4-oxazepin ($S/N > 10{:}1$). This spectrum contains two characteristic ions, and while this may be satisfactory for the detection of this compound for some applications, it should be noted that confirmation under MS/MS conditions requires the acquisition of a minimum of three characteristic ions. To achieve this level of certainty, acquisition of an additional daughter

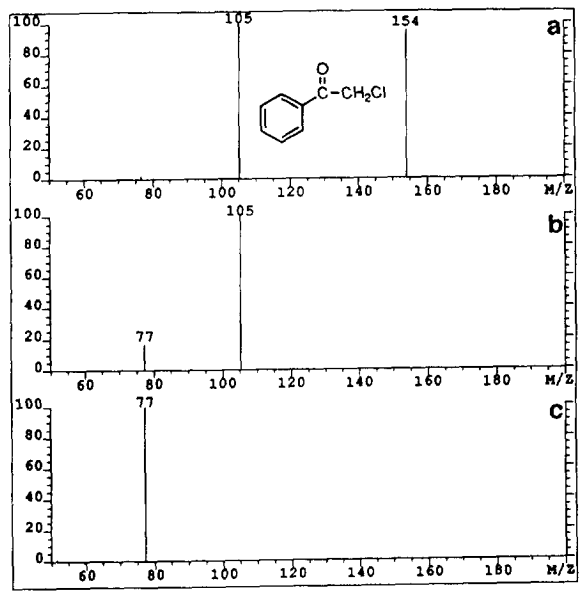

Fig. 3. Daughter spectra of (a) *m*/*z* 154, (b) *m*/*z* 105 and (c) *m*/*z* 77 for 2-chloroacetophenone.

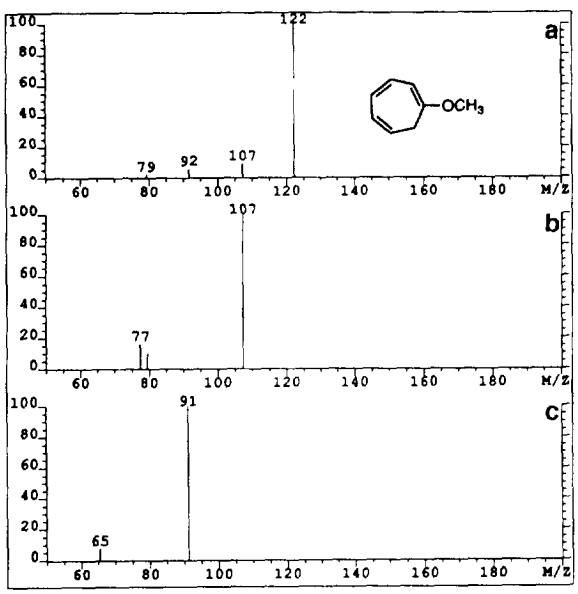

Fig. 2. Daughter spectra of (a) *m*/*z* 122, (b) *m*/*z* 107 and (c) *m*/*z* 91 for 1-methoxycycloheptatriene.

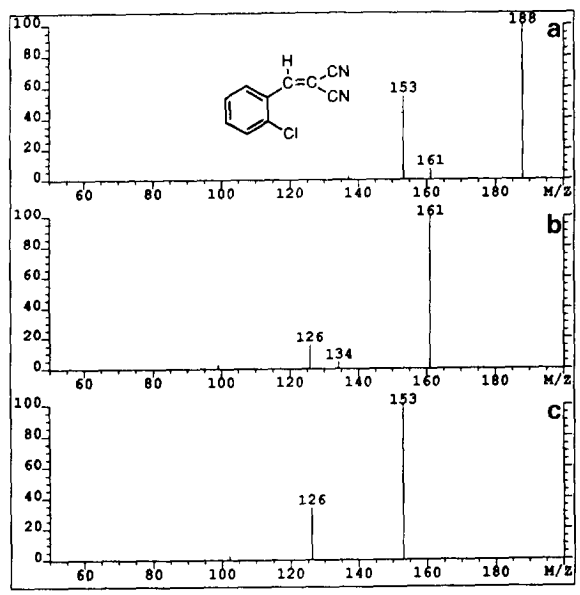

Fig. 4. Daughter spectra of (a) *m*/*z* 188, (b) *m*/*z* 161 and (c) *m*/*z* 153 for o-chlorobenzylidenemalononitrile.

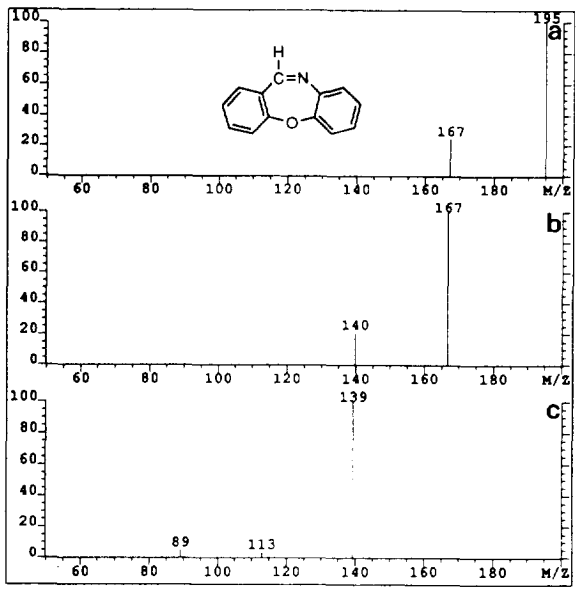

Fig. 5. Daughter spectra of (a) m/z 195, (b) m/z 167 and (c) m/z 139 for dibenz[b,f]-1,4-oxazepin.

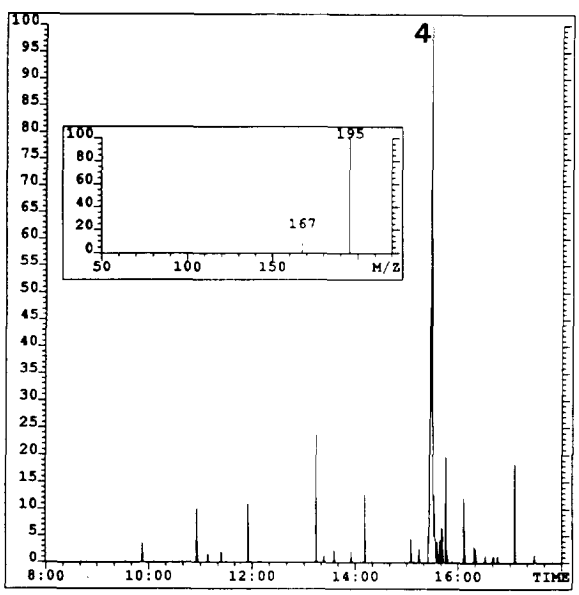

Fig. 6. Capillary column GC–MS/MS chromatogram for daughters of (a) m/z 195 (M^+ for 100 pg of dibenz[b,f]-1,4-oxazepin, [4]). Daughter spectrum obtained for 100 pg of dibenz[b,f]-1,4-oxazepin inset on Figure. [Time scale in minutes, DB-1701 column]

spectrum for another parent ion (e.g., m/z 167 or m/z 139) would be required.

Daughter sensitivity depends directly on the relative abundance of the parent ion, as these compounds have similar EI detection limits. As a result, sensitivities would be similar for most of the irritant ions monitored during daughter study, with the exception of the molecular ion of 2-chloroacetophenone due to its very low EI relative intensity (1.8%).

In general, an interpretable full scanning EI mass spectrum may be acquired for 100 pg to 500 pg of chemical warfare agent, depending on the compound, during capillary column GC–MS analysis. Similar sensitivity has been observed during daughter study and the following has been noted. If an interpretable full scanning EI mass spectrum can be acquired, then the analyst can expect to acquire an interpretable daughter spectra for the compound at the same level, provided the parent ion has an EI relative abundance above 25%.

Table 1 lists the EI ions monitored during the acquisition of daughter spectra, their relative intensity and the elemental composition of each ion, as confirmed during high-resolution GC–MS analysis. Figs. 2 to 5 illustrate typical daughter spectra for the molecular and principal EI fragmentation ions of 1-methoxycycloheptatriene, 2-chloroacetophenone, *o*-chlorobenzylidenemalononitrile and dibenz[b,f]-1,4-oxazepin, respectively.

1-Methoxycycloheptatriene exhibits a molecular ion at m/z 122 and two higher mass fragmentation ions at m/z 107 and m/z 91 during EI-MS operation. The molecular ion at m/z 122 fragments in the CAD cell to form daughter ions at m/z 107, m/z 92, m/z 79 and m/z 77, due to $[M-CH_3]^+$ and $[M-CH_2O]^+$, $[M-CH_3-CO]^+$ and $[M-CH_3-CH_2O]^+$, respectively (Fig. 2a). The EI fragmentation ion at m/z 107, $[M-CH_3]^+$, produced daughters at m/z 79 and m/z 77 due to neutral loss CO and CH_2O, respectively (Fig. 2b). Only one daughter at m/z 65, due to neutral loss of C_2H_2 was observed in the daughter spectrum of m/z 91 (Fig. 2c).

The EI mass spectrum of 2-chloroacetophenone contains a weak molecular ion (refer to

Table 1
Ions monitored during GC–MS/MS study

Irritant[a]	EI mass measured[b]	EI mass calculated	Error (mmu)	% R.I.[c]	Composition
a) CH	122.0722	122.0732	−1.0	100	$C_8H_{10}O$
	107.0501	107.0497	+0.4	55	C_7H_7O
	91.0536	91.0548	−1.2	70	C_7H_7
b) CN	154.0198	154.0185	+1.3	1.8	C_8H_7OCl
	105.0324	105.0340	−1.6	100	C_7H_5O
	77.0343	77.0391	−4.8	54	C_6H_5
c) CS	188.0132	188.0141	−0.9	54	$C_{10}H_5N_2Cl$
	161.0033	161.0032	+0.1	17	C_9H_4NCl
	153.0457	153.0453	+0.4	100	$C_{10}H_5N_2$
d) CR	195.0709	195.0684	+2.5	100	$C_{13}H_9NO$
	167.0730	167.0735	−0.5	52	$C_{12}H_9N$
	139.0540	139.0548	−0.8	25	$C_{11}H_7$

[a] CH = 1-methoxycycloheptatriene; CN = 2-chloroacetophenone; CS = *o*-chlorobenzylidenemalononitrile; CR = dibenz[*b*,*f*]-1,4-oxazepin
[b] Obtained during GC–MS analysis at 10 000 resolution (10% valley definition).
[c] %Relative intensity.

Table 1) and two higher mass EI fragmentation ions at *m/z* 105 and *m/z* 77. Daughters at *m/z* 105 and *m/z* 77, due to $[M - CH_2Cl]^+$ and $[M - CH_2Cl - CO]^+$, respectively, were observed during GC–MS/MS analysis of the molecular ion at *m/z* 154 (Fig. 3a). The EI fragmentation ion at *m/z* 105 produced a single daughter ion at *m/z* 77 due to neutral loss of CO (Fig. 3b) and this ion did not produce any significant daughter ions (Fig. 3c).

o-Chlorobenzylidenemalononitrile, the most commonly used irritant, contains a molecular ion and two higher mass EI fragmentation ions at *m/z* 161 and *m/z* 153. Significant daughter ions due to loss of HCN and Cl, at *m/z* 161 and *m/z* 153, respectively, were observed in the daughter spectrum of the *o*-chlorobenzylidenemalononitrile molecular ion at *m/z* 188 (Fig. 4a). Daughter ions at *m/z* 134 and *m/z* 126, due to loss of HCN and Cl, respectively, were detected in the daughter spectrum of *m/z* 161, $[M - HCN]^{+\cdot}$ (Fig. 4b). An ion at *m/z* 126, due to loss of HCN, was the principal daughter ion in the spectrum acquired for $[M - Cl]^+$ at *m/z* 153 (Fig. 4c). Several minor daughter ions at *m/z* 137, *m/z* 126 and *m/z* 102, detected in the acquired daughter data for *o*-chlorobenzylidenemalononitrile, were likely due to C_7H_4NCl, C_9H_4N and C_4H_5NCl, respectively.

The molecular ion of dibenz[*b*,*f*]-1,4-oxazepin, at *m/z* 195, and two higher mass EI fragmentation ions (*m/z* 167 and *m/z* 139) were investigated during GC–MS/MS operation. An ion at *m/z* 167, due to loss of CO, was the principal daughter in the spectrum acquired for *m/z* 195 (Fig. 5a). The daughter spectrum of *m/z* 167, due to $[M - CO]^{+\cdot}$, exhibited a significant daughter ion at *m/z* 140 due to neutral loss of HCN (Fig. 5b). Two daughters at *m/z* 113 and *m/z* 89, likely due $[C_9H_5]^+$ and $[C_7H_5]^+$ were observed in the daughter spectrum of $[C_{11}H_7]^+$ at *m/z* 139 (Fig. 5c).

Fig. 7 illustrates the capillary column GC–MS chromatogram of the dichloromethane extract of the collected diesel exhaust sample. This complex extract, containing numerous hydrocarbons (refer to Table 2), was selected to evaluate the selectivity of GC–MS/MS, as it is typical of what one might collect during battlefield sampling in the presence diesel powered vehicles. 1-Methox-

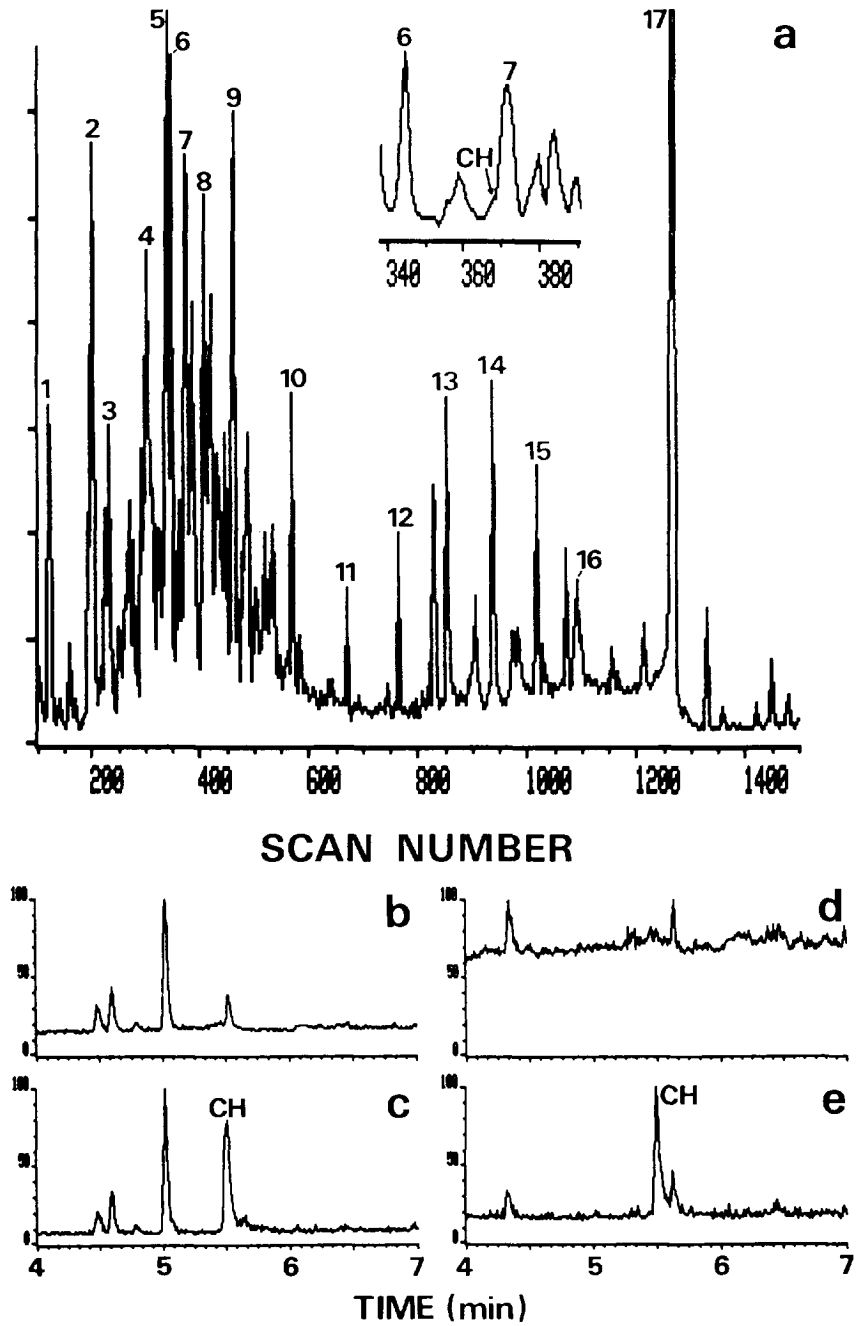

Fig. 7. (a) Capillary column GC–MS total ion-current chromatogram (300 to 40 u) of the diesel exhaust extract (Numbers of sample components are listed in Table 2). The expanded insert illustrates the expected retention time for 1-methoxycycloheptatriene (Each scan number represents about 0.6 s, DB-5 column). Reaction ion monitoring chromatograms for m/z 122 to 107 obtained during analysis of the (b) diesel exhaust extract and (c) the same diesel exhaust extract spiked at the 500 pg level with 1-methoxycycloheptatriene. Reaction ion monitoring chromatograms for m/z 122 to 92 obtained during analysis of the (d) diesel exhaust extract and (e) the same diesel exhaust extract spiked at the 500 pg level with 1-methoxycycloheptatriene.

Table 2
Major diesel exhaust extract components

Peak No.[a]	Compound
1	Methyl benzene
2	C_2-Substituted benzene
3	nC_9-Alkane
4,5,7	C_3-Substituted benzene
6	nC_{10}-Alkane
8	C_4-Substituted benzene
9	nC_{11}-Alkane
10	nC_{12}-Alkane
11	nC_{13}-Alkane
12	nC_{14}-Alkane
13	nC_{15}-Alkane
14	nC_{16}-Alkane
15	nC_{17}-Alkane
16	nC_{18}-Alkane
17	Sulfur (S_8)

[a] Refer to Fig. 7.

ycycloheptatriene, at the apex of the hydrocarbon envelope, was selected to illustrate the specificity of GC–MS/MS as it was completely masked by chemical interferences and could not be detected at the 5 ng spiked level during GC–MS analysis under EI conditions.

Multiple reaction ion monitoring of m/z 122 to 107 and m/z 122 to 92 processes resulted in the detection of 500 pg of 1-methoxycycloheptatriene with a $S/N > 10{:}1$ in the presence of numerous interfering hydrocarbons in the diesel exhaust extract (Fig. 7). A minor interference was observed for the m/z 122 to 107 process. This contribution was likely due to loss of CH_3 from a C_9H_{14} ion in the diesel exhaust extract. No interferences were detected for the m/z 122 to 92 process.

4. Conclusions

Daughter spectra were obtained for the molecular and principal electron impact fragmentation ions of four irritants, 1-methoxycycloheptatriene, 2-chloroacetophenone, *o*-chlorobenzylidenemalononitrile and dibenz[*b*,*f*]-1,4-oxazepin, during capillary column GC–MS/MS analysis. The use of standardized collisional activated dissociation cell conditions resulted in the acquisition of daughter spectra suitable for identification or data-base generation purposes. Daughter operation detection limits of 100 pg ($S/N > 10{:}1$), for the highest molecular mass irritant, dibenz[*b*,*f*]-1,4-oxazepin, were obtained. This level of sensitivity was approximately the same as that routinely obtained for other chemical warfare agents during capillary column GC–MS analysis under electron impact ionization conditions. The specificity of GC–MS/MS was demonstrated by detection of 1-methoxycycloheptatriene in the presence of numerous chemical interferences in the diesel exhaust extract.

Tandem mass spectrometry may be used for the acquisition of complementary daughter data for the identification of irritants and other chemical warfare agents. Use of MS/MS data as well as traditional MS data would increase the level of confidence in the identification of irritants in samples suspected to contain these compounds.

Acknowledgement

The authors acknowledge the assistance of Dr. P.W. Brooks and Energy, Mines and Resources (Calgary, Alberta, Canada) during acquisition of the multiple reaction ion monitoring data.

References

[1] S.M. Somani (Editor), *Chemical Warfare Agents*, Academic Press, New York, 1992.
[2] P.A. D'Agostino and L.R. Provost, *J. Chromatogr.*, 331 (1985) 47–54.
[3] B.R. Gandhe, R.C. Malhotra and P.K. Gutch, *J. Chromatogr.*, 479 (1989) 165–169.
[4] J.R. Hancock and G.R. Peters, *J. Chromatogr.*, 538 (1991) 249–257.
[5] A. Kaipainen, O. Kostiainen and M.-L. Riekkola, *J. Microcol. Sep.*, 4 (1992) 245–251.
[6] M. Kokko, *J. Chromatogr.*, 630 (1993) 231–249.
[7] J.F.K. Huber, E. Kenndler, G. Reich, W. Hack and J. Wolf, *Anal. Chem.*, 65 (1993) 2903–2906.
[8] Z. Witkiewicz, M. Mazurek and J. Szulc, *J. Chromatogr.*, 503 (1990) 293–357.
[9] H.W. Avdovich, A. By, J.-C. Ethier and G.A. Neville, *Can. Soc. Forens. Sci. J.*, 14 (1981) 172–178.

[10] J. Nowicki, *J. Forensic Sci.*, 27 (1992) 704–709.
[11] R.M. Martz, D.J. Reutter and J.D. Lasswell, *J. Forensic Sci.*, 28 (1983) 200–207.
[12] E.R.J. Wils and A.G. Hulst, *J. Chromatogr.*, 330 (1985) 379–382.
[13] E.R.J. Wils and A.G. Hulst, *Fresenius Z. Anal. Chem.*, 320 (1985) 357–360.
[14] K.E. Ferslew, R.H. Orcutt and A.N. Hagardorn, *J. Forensic Sci.*, 31 (1986) 658–665.
[15] S.S.M. Hassan, J.M. Abdalla and N.E. Nashed, *Mikrochimica Acta*, II (1984) 27–38.
[16] A. Hesso and R. Kostiainen, *Proceedings of the 2nd International Symposium on Protection Against Chemical Warfare Agents, Stockholm, June 15–19, 1986*, National Defence Research Institute, Umea, 1986, pp. 257–260.
[17] P.A. D'Agostino, L.R. Provost, J.F. Anacleto and P.W. Brooks, *J. Chromatogr.*, 504 (1990) 259–268.
[18] P.A. D'Agostino, L.R. Provost and P.W. Brooks, *J. Chromatogr.*, 541 (1991) 121–130.
[19] P.A. D'Agostino and C.J. Porter, *Rapid Commun. Mass Spectrom.*, 6 (1992) 717–718.
[20] P.A. D'Agostino and L.R. Provost, presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, May 30–June 4, 1993.*
[21] P.A. D'Agostino and L.R. Provost, *J. Chromatogr.*, 670 (1994) 127–134.