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CAPILLARY COLUMN GAS CHROMATOGRAPHIC - TANDEM MASS SPECTROMETRIC CHARACTERIZATION OF IRRITANTS (U)

BY

P.A. D'AGOSTINO AND
L.R. PROVOST

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Capillary Column Gas Chromatographic - Tandem Mass Spectrometric Characterization of Irritants

by

Paul A. D'Agostino and Lionel R. Provost

January 1995

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 reproducible 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 weight 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 of standards under electron impact ionization conditions.

INTRODUCTION

Chemical warfare agents can be classified into two general categories, those that exert a lethal effect and those that act as incapacitating agents. Lethal chemical warfare agents include nerve agents such as sarin, soman, tabun and VX, 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 most commonly used irritants are 2-chloroacetophenone and o-chlorobenzylidenemalononitrile, with dibenz[b,f]-1,4-oxazepin having been used less frequently. A fourth irritant, 1-methoxycycloheptatriene, was evaluated at Defence Research Establishment Suffield for military training purposes. o-Chlorobenzylidenemalononitrile and other irritants have been used as chemical agents on the battlefield, but use of these agents has usually been restricted to riot control or military training exercises (1).

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". The Canadian Forces (CF) could encounter use of irritants in a warfare role during active duty in regions of the world where there is a significant threat of chemical/biological warfare agent use. Intelligence gathering, through the collection of contaminated samples, and subsequent off-site analysis of the samples would lead to the identification of the chemical used. The Canadian chemical/biological identification capability resides at Defence Research Establishment Suffield, where analytical specialists confirm and identify chemical warfare agents in suspect samples for the CF. The results of such analyses would contribute to the development of strategic and political positions regarding future Canadian military operations and facilitate the dissemination of technical advice to in-theatre field commanders and medical personnel confronted with the threat of chemical warfare agent use.

Gas chromatographic (GC) methods, including methods based on GC retention indices (2-7), have been used extensively for the detection of irritants in suspect samples. These methods and others involving the use of GC and other 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 civil and defence laboratories analyse suspect extracts by GC-MS, as this spectrometric technique offers the greatest sensitivity and certainty for chemical warfare agents identification. Reproducible electron impact (EI) mass spectrometric data have been reported for common irritants, including 1-methoxycycloheptatriene, 2-chloroacetophenone, o-chlorobenzylidenemalononitrile and dibenz[b,f]-1,4-oxazepin (9-15), and related benzylidenemalononitriles (12,16).

However, samples suspected to contain chemical warfare agents or their degradation products are typically 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. 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.

Mass spectrometry, and in particular 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 (17-23) in complex environmental samples (18-20). These publications include the daughter spectra of lethal chemical warfare agents, but do not include tandem mass

spectrometric data for any irritants.

GC-MS/MS characterization of irritants was investigated, since this technique may enable selective identification of these compounds in samples collected by the CF in an intelligence gathering role. This report summarizes the first 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 under standardized collisional activated dissociation (CAD) cell conditions (21,22).

EXPERIMENTAL

Standards

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 all capillary column GC-MS/MS analyses.

Instrumental

Capillary column GC-MS/MS analyses were performed with a VG AUTOSPEC-Q (EBEQQ geometry) hybrid tandem mass spectrometer equipped with a Hewlett Packard model 5890 gas chromatograph. A 15 m x 0.32 mm ID DB-1701 J&W capillary column (0.25 μm film thickness) was used for all GC-MS/MS analyses with the following temperature program: 40°C (2 min hold) 10°C/min to 280°C (5 min hold). All GC injections were cool on-column using an injector of our own design (2).

The EI-MS operating conditions were as follows: source pressure, 3 x 10^{-6} Torr; source temperature, 200°C; electron energy, 70 eV; electron emission, 100 or 200 μA and accelerating voltage, 8 kV. Standardized CAD cell conditions [CAD cell argon pressure of 8-9 x 10^{-7} Torr and an energy of 25 eV (laboratory scale)], based on the best compromise between sensitivity and spectral content (21,22), were used for all daughter analyses. This argon pressure reduced the intensity of the PFK 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. The quadrupole was operated at unit resolution and scanned from 250 to 50 u at 0.7 sec/scan and, the sector resolution was set at 1000 (10% valley definition). Daughter spectra for the four irritants were obtained during chromatographic analyses by monitoring for 1-methoxycycloheptatriene from 1:00 to 6:00 minutes, 2-chloroacetophenone from 6:00 to 12 minutes, o-chlorobenzylidenemalononitrile from 12:00 to 14:00 minutes and dibenz[b,f]-1,4-oxazepin from 14:00 and 17:00 minutes.

During high resolution GC-MS analyses a sector resolution of 10,000 was employed (10% valley definition).

RESULTS AND DISCUSSION

Figure 1 illustrates typical capillary column GC-MS/MS chromatograms obtained during the analysis of a 10 ng standard containing the four irritants, 1-methoxycycloheptatriene, 2-

chloroacetophenone, o-chlorobenzylidenemalononitrile and dibenz[b,f]-1,4-oxazepin. Daughter spectra of the molecular ions obtained during this analysis are illustrated in Figures 2a, 3a, 4a and 5a. The reproducibility of the daughter spectra obtained were evaluated and found to be similar to that obtained for lethal chemical warfare agents (23), with the standard deviation of the daughter ion relative intensities being less than 30%. While this is probably a factor of two or more than that routinely observed during EI operation, it is quite similar to that obtained during chemical ionization study. The differences in relative intensity would result in somewhat poorer fit values than are routinely demonstrated during EI data-base searching, but would be sufficiently reproducible for data-base generation and subsequent searching.

The sensitivity of daughter detection was evaluated by monitoring the daughters of the molecular ion (m/z 195) of the highest molecular weight irritant, dibenz[b,f]-1,4-oxazepin (Figure 6). A good quality, interpretable daughter spectrum (inset in Figure 6) was obtained for 100 pg of dibenz[b,f]-1,4-oxazepin (S/N > 10:1). This detection limit is typical of full scanning EI detection limits which often vary between 100 to 500 pg, depending on the compound. Lower levels of irritants may be confirmed by multiple reaction ion monitoring (RIM; minimum of two transitions) in a mode analogous to selected ion monitoring (SIM).

Table I 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. Figures 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 $[M\text{-}CH_3]^+$ and $[M\text{-}CH_2O]^+$, $[M\text{-}CH_3\text{-}CO]^+$ and $[M\text{-}CH_3\text{-}CH_2O]^+$, respectively (Figure 2a).

The EI fragmentation ion at m/z 107, $[M\text{-}CH_3]^+$, produced daughters at m/z 79 and m/z 77 due to neutral loss CO and CH_2O, respectively (Figure 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 (Figure 2c).

The EI mass spectrum of 2-chloroacetophenone contains a weak molecular ion (refer to Table I) 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 $[M\text{-}CH_2Cl]^+$ and $[M\text{-}CH_2Cl\text{-}CO]^+$ respectively, were observed during GC-MS/MS analysis of the molecular ion at m/z 154 (Figure 3a). The EI fragmentation ion at m/z 105 produced a single daughter ion at m/z 77 due to neutral loss of CO (Figure 3b) and this ion did not produce any significant daughter ions (Figure 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 (Figure 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, $[M\text{-}HCN]^{+\cdot}$ (Figure 4b). An ion at m/z 126, due to loss of HCN, was the principal daughter ion in the spectrum acquired for $[M\text{-}Cl]^+$ at m/z 153 (Figure 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 MS/MS operation. The principal daughter, at m/z 167, in the daughter spectrum of m/z 195 was due to loss of CO from the molecular ion (Figure 5a). The daughter spectrum of m/z 167, due to $[M\text{-}CO]^{+\cdot}$, exhibited a significant daughter ion at m/z 140 due to neutral loss of HCN (Figure 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 (Figure 5c).

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 reproducible 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 weight 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 of standards under electron impact ionization conditions.

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

Table I: Ions monitored during GC-MS/MS study

Irritant	EI mass measured[a]	EI mass calculated	Error (mmu)	% R.I.[b]	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	+1.4	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	$C_{11}H_7$

CH: 1-methoxycycloheptatriene
CS: o-chlorobenzylidenemalononitrile
CN: 2-chloroacetophenone
CR: dibenz[b,f]-1,4-oxazepin

[a] Obtained during GC-MS analysis at 10,000 resolution (10% valley definition).
[b] Percent Relative Intensity.

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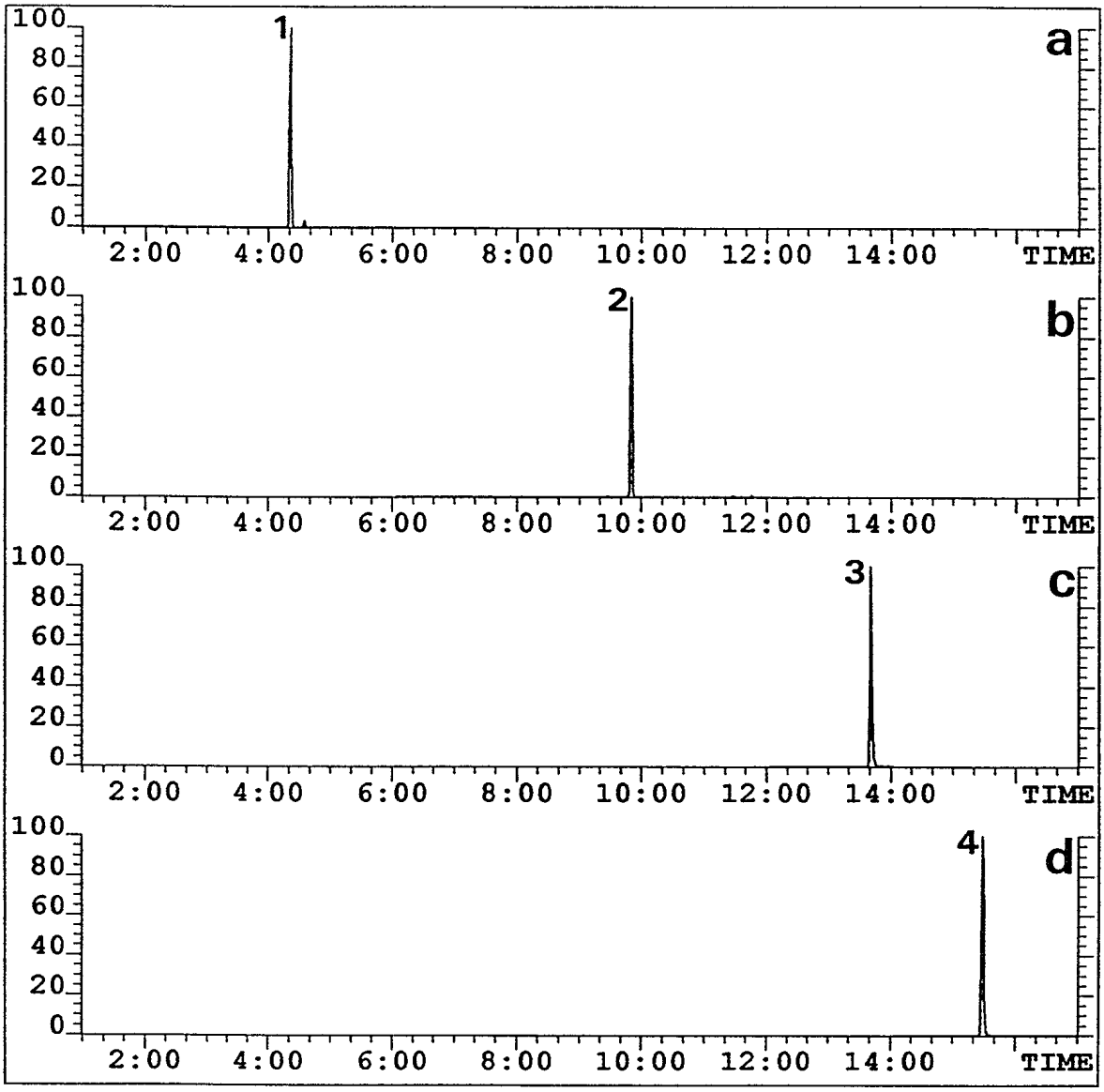

Figure 1: Capillary column GC-MS/MS chromatograms for daughters of a) m/z 122 ($M^{+\cdot}$ for 1-methoxycycloheptatriene, [1]), b) m/z 154 ($M^{+\cdot}$ for 2-chloroacetophenone, [2]), c) m/z 188 ($M^{+\cdot}$ for o-chlorobenzylidenemalononitrile, [3]) and d) m/z 195 ($M^{+\cdot}$ for dibenz[b,f]-1,4-oxazepin, [4]). [Time scale in minutes; Sector resolution of 1000]

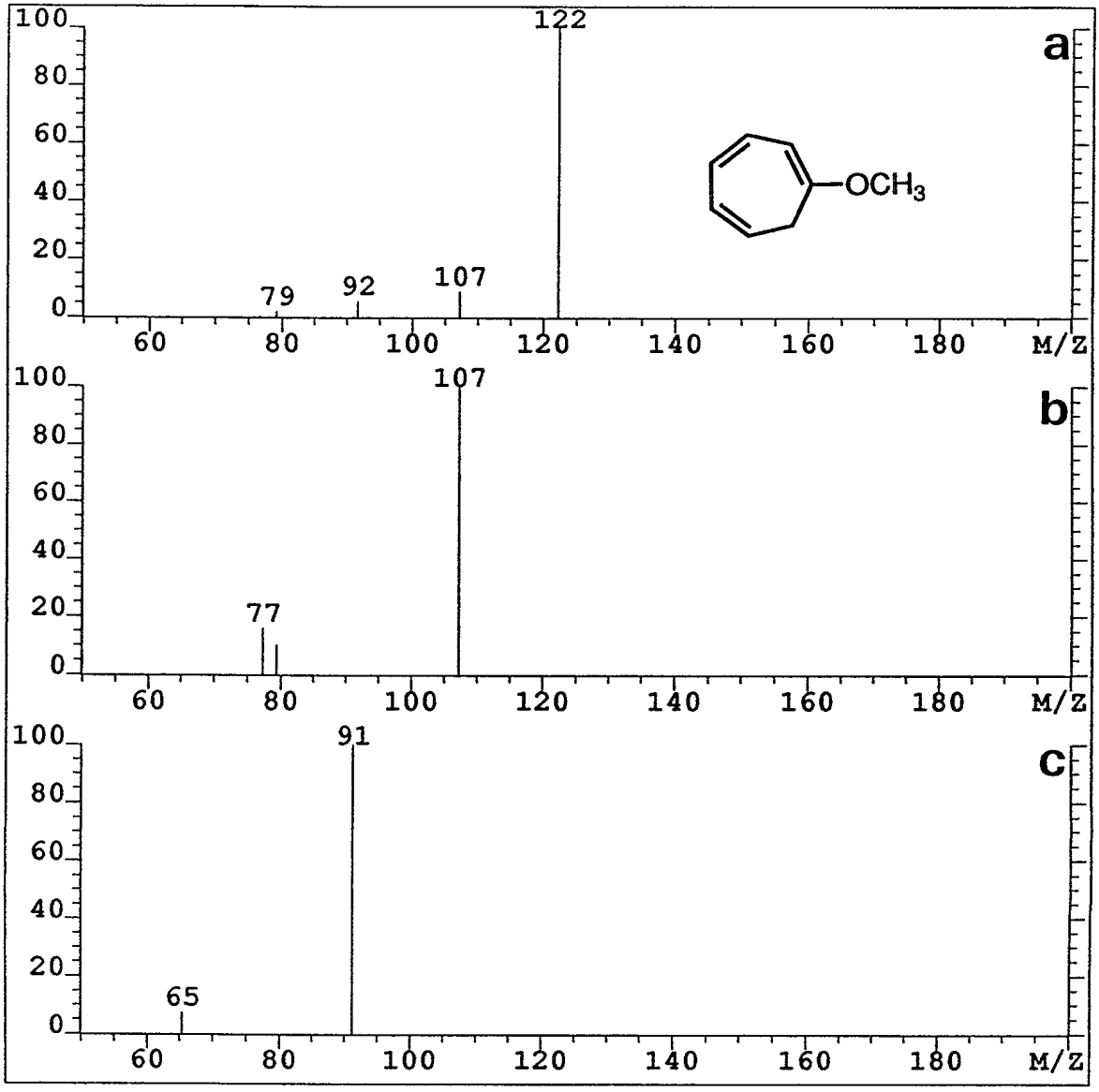

Figure 2: Daughter spectra of a) m/z 122, b) m/z 107 and c) m/z 91 for 1-methoxycycloheptatriene.

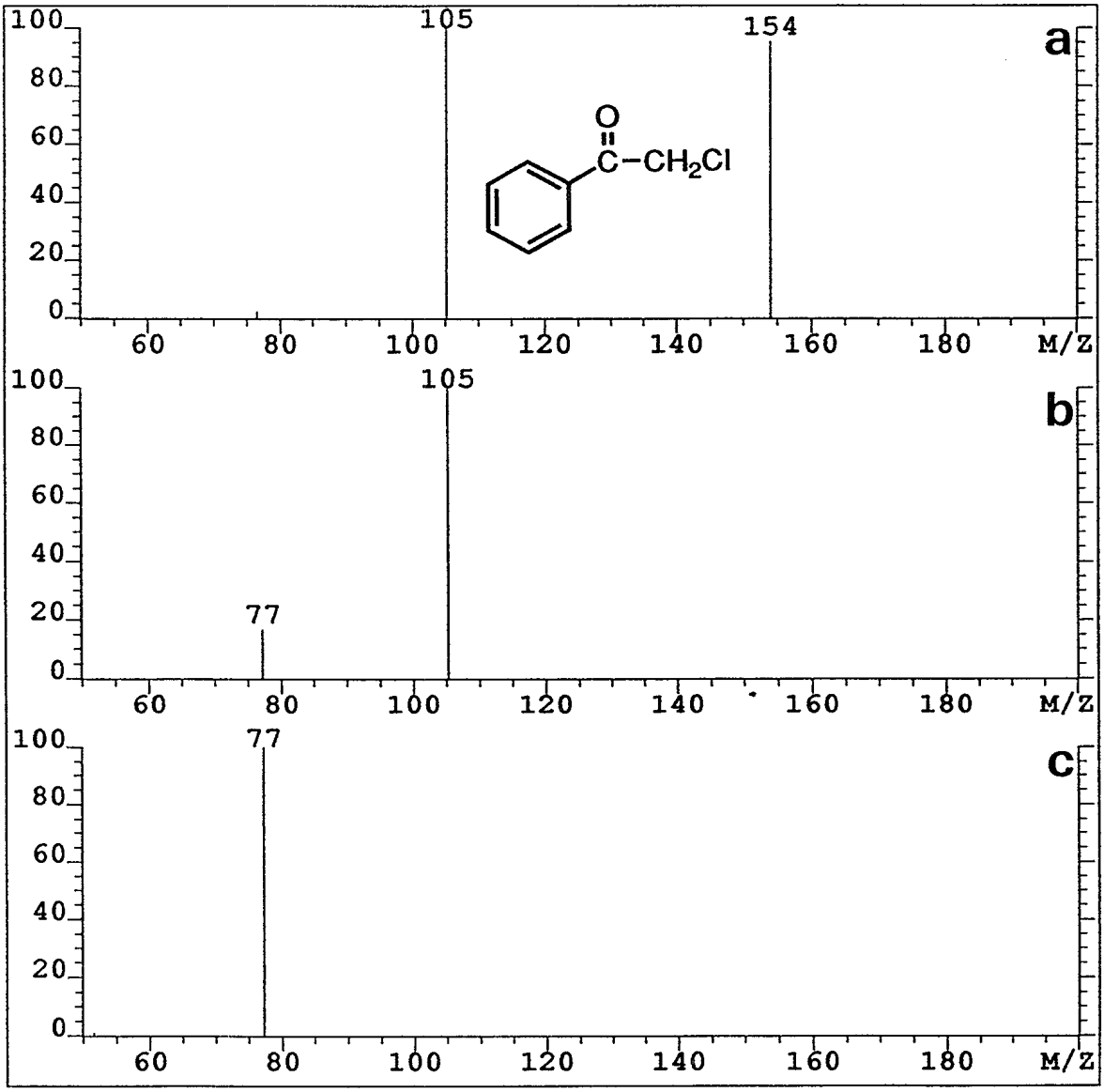

Figure 3: Daughter spectra of a) m/z 154, b) m/z 105 and c) m/z 77 for 2-chloroacetophenone.

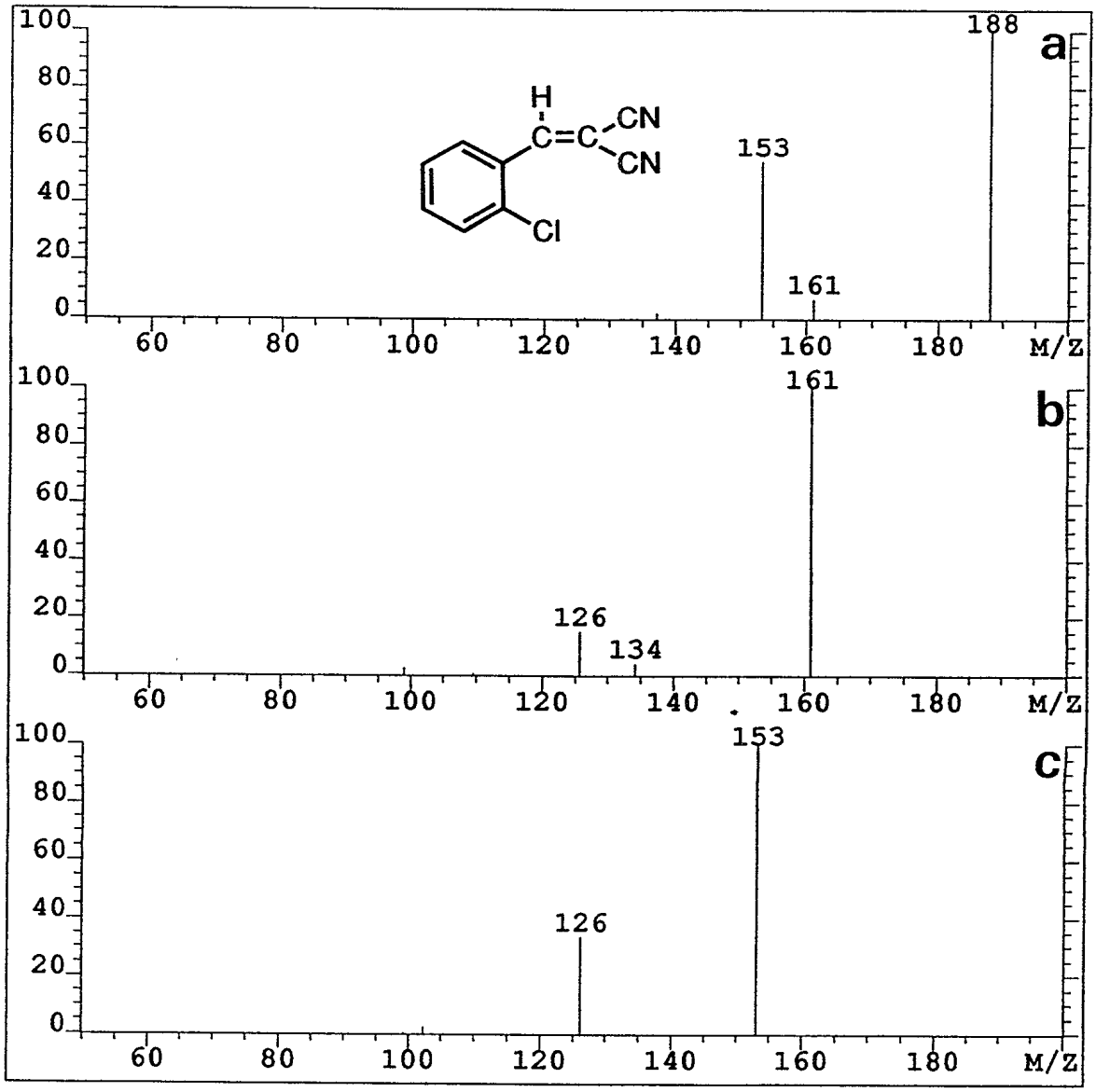

Figure 4: Daughter spectra of a) m/z 188, b) m/z 161 and c) m/z 153 for o-chlorobenzylidenemalononitrile.

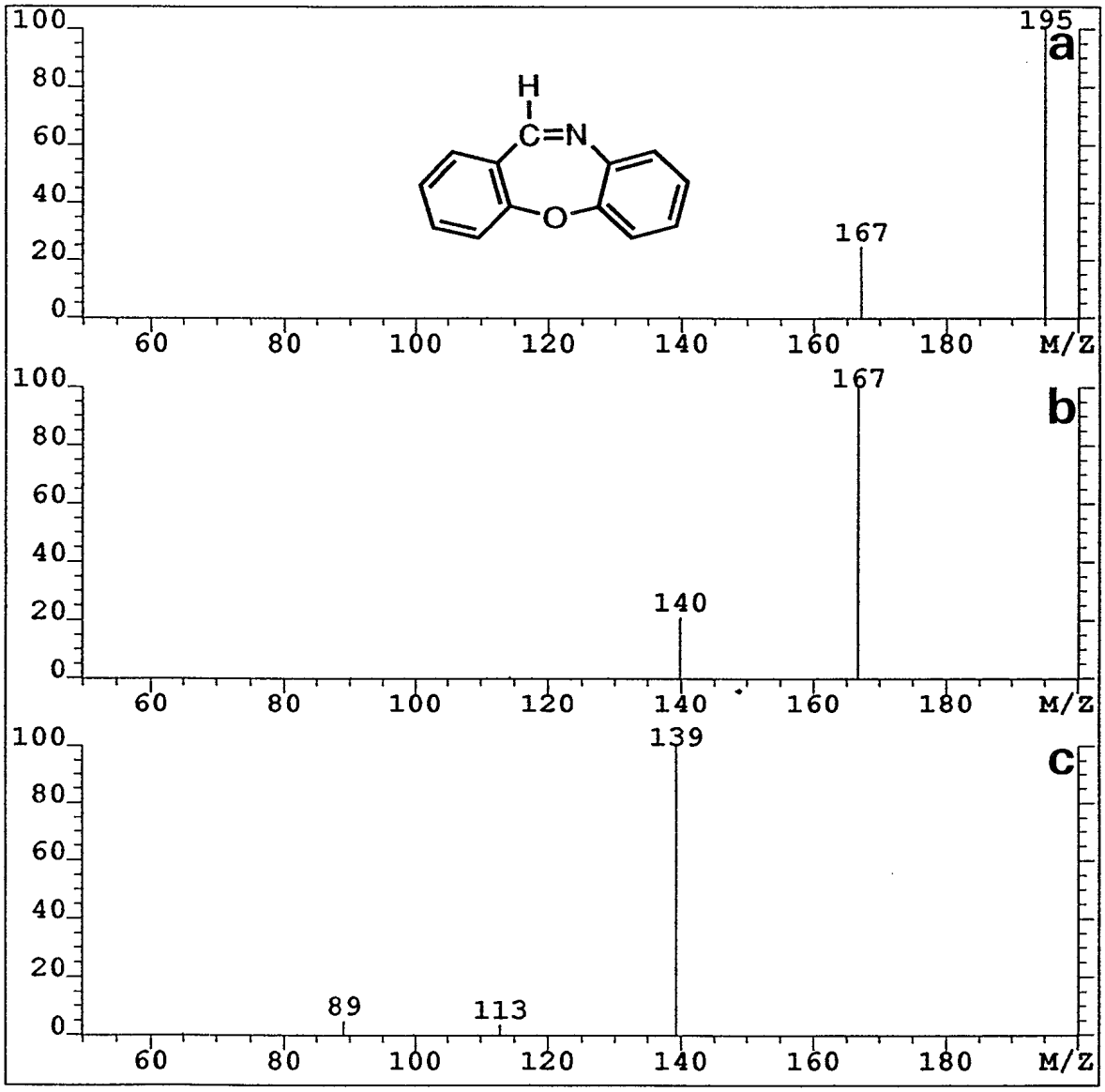

Figure 5: Daughter spectra of a) m/z 195, b) m/z 167 and c) m/z 139 for dibenz[b,f]-1,4-oxazepin.

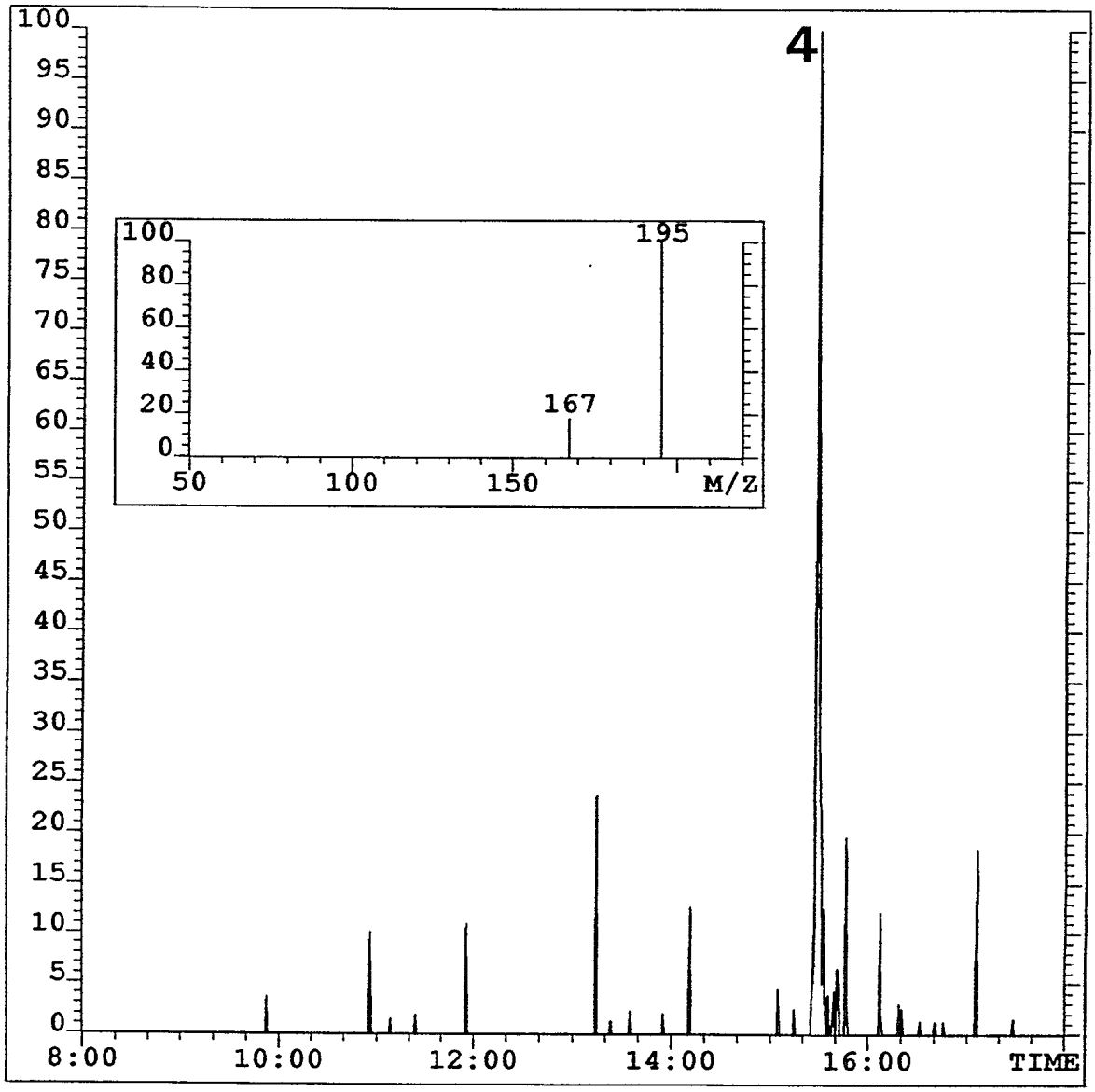

Figure 6: Capillary column GC-MS/MS chromatogram for daughters of a) m/z 195 ($M^{+\cdot}$ for 100 pg of dibenz[b,f]-1,4-oxazepin, [4]). Daughter spectrum obtained for 100 pg of dibenz[b,f]-1,4-oxazepin inset on Figure. [Time scale in minutes; Sector resolution of 1000]

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