HAND-PORTABLE GAS CHROMATOGRAPHY - ION MOBILITY SPECTROMETER FOR THE DETERMINATION OF THE FRESHNESS OF FISH

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ABSTRACT

A hand-held, portable gas chromatography-ion mobility spectrometer (GC-IMS) device was used to detect the presence of volatile amine compounds in the headspace of decomposing fish. The Food and Drug Administration (FDA) largely relies on olfactory discrimination with respect to fresh and spoiled, frozen and unfrozen fish. The fish are delivered at ship docks on pallets, and each pallet of fish can range from $30-40K in value. Fresh fish were placed in a teflon bag and the direct headspace was interrogated. In the first three days, only low molecular weight volatile amines were detected. On the fourth day, a number of spectral signatures were observed which indicated the presence of 1,5-diaminopentane, cadaverine. Analyses typically took from 0.5-1 minute.

INTRODUCTION

Recently, a hand-held device has been introduced which embodies two separate analytical stages for separation in the detection and identification of chemical species [1,2]. The first stage is a gas chromatograph (GC) and the second stage is an ion mobility spectrometer (IMS). This hand-held GC-IMS was shown to provide for the rapid (seconds to minutes) separation of a number of compounds belonging to different chemical classes including phosphoryl species, chlorophenols, ketones and aromatic amines. The concept was to take two analytical techniques, that have been mated in the laboratory since the early 1980's, and produce a GC-IMS system that could have potential as a field screening or environmental compound monitor. Thus, this system would be able to produce two separate, yet concurrent (or hyphenated), dimensions of information which has greater information content with respect to a one-dimensional or parallel instrumental arrangement.

GC is a widely used analytical technique and its fundamentals as well as its combinations with other instruments can be found in many places in the literature. IMS has shown a resurgence in popularity in the late 1980s and early 1990s and was the topic of a number of recent reviews [3,4] and a book on its fundamentals, theory and applications [5].

One of the charges of the U.S. Food and Drug Administration is to administer inspections of commercial catches of fish when they are delivered at dockyards and ship ports as well as in storage areas. A pallet of fish can cost over $30,000, and as such, inspections are performed on fresh and frozen fish in order to ensure that no spoilage of the seafood has taken place. Inspections routinely take the form of

organoleptic characterization, by inspectors who are trained to use their sense of smell for these purposes. This procedure can de-sensitize or overwhelm nasal discrimination in a very short time. A hand-held analytical instrument that could discriminate between fresh and spoiled fish would be a welcome tool for the FDA inspection personnel.

There are important characteristics that a hand-held instrument should have when considering that a person should be relatively unhindered and unencumbered when performing investigations of seafood quality in a dockyard scenario. Some of these include lightweight, portability (considered to be one-hand portability), ease of use, information production of the unit and speed of response.

A desirable characteristic of analytical instrument interrogation is where there is a minimal to negligible handling/processing of the sample. This is especially true for outdoor or away-from-the-laboratory situations. The literature shows that various classes of compounds have been investigated from fresh and decomposing fish for their degree of freshness and include saturated and unsaturated low and high molecular weight free fatty acids, aldehydes, ketones, mono- and polyamines, selected amino acids, alcohols and C_2-C_4 esters. The esters and carbonyl containing compounds appear to have utility as indicators of the freshness of fish in the long term (greater than 2 months) under frozen conditions [6]. However, in the short term, the only reliable chemical compound indicators have been the amine class of compounds that produce an odor [6, 7-9]. Virtually all investigations in the determination of the freshness of fish have utilized extensive processing and handling methods, culminating in the analyses of extracts and/or the chemical derivatization of extracts [10-13]. The few experiments that dealt with headspace of vapors entailed sparging homogenates [6,9] or steam distillation [14] of a sample of fish and directing the vapors onto the head of a GC column. The latter studies reported on non-amine compounds and the former provided no information on the identification of the volatile compounds.

The present study addressed the possibility of determining volatile biochemical markers with a hand-held, two-dimensional GC-IMS system from freshly caught fish. Volatile compounds were sought in order to alleviate for sample handling and processing procedures. Direct headspace was used in sampling for volatiles from the fish, and the mono- and di- amine classes of compounds were targeted.

EXPERIMENTAL

Gas Chromatography -Ion Mobility Spectrometry

A modified Environmental Vapor Monitor, EVM, (Graseby Ionics, Ltd., Watford, Herts, UK) was used (Figure 1). The EVM is comprised of a capillary gas chromatograph integrated with a hand-held ion mobility spectrometer, IMS [1,2]. The IMS operates with an internal sample gate repetition rate of 33 Hz. The gating pulse was 180 microseconds in duration and provided the trigger for the data collection. The modifications include the introduction of temperature and pressure sensors inside the IMS cell and the construction of a modular, easy-to-replace and disposable GC module with temperature programming. The modifications in the GC-IMS system were designed to allow a more complete separation, improved detection and identification of chemical compounds, increased ease of maintenance of the system, and a more robust hand-held detector. Typical experimental conditions used for the GC-IMS are shown in Table 1. Sample introduction to the GC column was accomplished by using an Automated Vapor Sampling unit (AVS) [15]. Conventional power supplies were used to provide power to the GC-IMS unit. The sample pulse was user controlled, with a range of 0.2 seconds to 20 seconds duration.

The IMS data was collected using an AT-MIO-16X multifunction I/O board (National Instruments Corporation, Austin, TX). The data collection algorithms for the GC-IMS were written using Labwindows

Software Version 2.2 (National Instruments, Austin, TX) in both C and QuickBasic programming languages. The programs were compiled using Microsoft C Version 5.1 and Microsoft QuickBasic as appropriate. The compiled versions of the software were then run under Microsoft DOS Version 6.0. Labwindows versions of the executable code were all created using the Labwindows Run Time System.

Gas Chromatography - Mass Spectrometry

A Finnigan-MAT Magnum GC-ion trap MS system was used to assist in confirmation of GC-IMS data. GC parameters were: 10 m DB-1, 0.25 mm ID, 0.25 *um* film thickness, 35-280°C or 50-280°C at 25°C/min column temperature. The GC value was splitless for the first one-half minute and then switched to split mode. The injector and transfer line were both set at 280°C.

Fish Samples

Fish (bluegill, perch) were caught from local streams (by DBS) and were placed in a teflon bag equipped with a septum sampling port. The headspace of the bag containing one or two fish was sampled periodically either directly by the GC-IMS or by injection of 1, 10 or 20 *ul* volumes into the GC-MS with a gas-tight syringe.

Standards

Chemical amine standards were purchased from either Aldrich (Milwaukee, WI) or Alfa (Ward Hill, MA).

RESULTS AND DISCUSSION

The decay of fish occurs in the form of different biological processes and as such produces different metabolic products. Two key processes are muscle and fat (rancidity) decay. Muscle decay produces monoamine and polyamine by-products and results from proteolysis caused by the decarboxylase enzymes in microorganisms. Fat decay (phospholipid oxidation) produces volatiles such as aldehydes, ketones, fatty acids and alcohols [10, 11, 16].

Monoamines

Currently, the presence of the amine class of decomposition analyte is the most reliable indicator of fish spoilage, because they correlate most closely with organoleptic (nasal) judgment of fish freshness. Very low molecular weight monoamines are known to be produced by muscle amino acid decay. In the various GC-IMS experiments, whether there was one or two fish in a teflon bag, headspace sampling of the volatiles at various intervals from 1-3 days produced spectra typical of that as shown in Figure 2. In addition to the water reactant ion peak (RIP), a mobility peak at shorter times was observed. This phenomenon is typical of low molecular weight amine compounds. Table 2 shows that compounds such as ammonia, dimethylamine and trimethylamine all have drift times in the 5.23-5.35 msec range, which is faster than the water RIP of 5.56 msec. They also have very short GC retention times on the order of 0.7 sec. Therefore, any or all of these compounds could comprise the 5.23-5.35 msec peak.

GC-MS analyses of the fish headspace confirmed this hypothesis. A representative total ion current (TIC) GC-MS profile of a headspace injection of fish volatiles is shown in Figure 3a. Essentially a broad air void volume and low molecular weight fish volatiles were found in the first minute and small amounts of column degradation silane products were found later in the run at temperatures greater than

170°C. The TIC of an equivalent volume of laboratory air injected into the GC-MS (Figure 3b) is very similar in appearance to that of the fish headspace (Figure 3a). Upon close inspection for low molecular weight amine compounds, Figure 4 shows relative intensities of m/z 40, 44 and 45. m/z 40 is that of the background atmospheric argon gas, and m/z 44, 45 are characteristic of dimethylamine. Note that m/z 40 is the base peak in the air blank mass spectrum while it is relatively lower in intensity with respect to m/z 44 and 45 in the fish samples. In other words, m/z 44 and 45 increase significantly with respect to m/z 40 in a fish headspace sample. Figure 5 shows a TIC of dimethylamine and note that it elutes in the same time frame as the air peak. Figure 6 shows a series of mass spectra. Figures 6a and 6b show pure dimethylamine and the fish headspace, respectively, from scan numbers 21-30 of the TIC in Figure 3a. Figures 6c-e are library compound mass spectra. N-methyl methanamine (dimethylamine) appears to give a good match (compare Figure 6a-b) while the low operating temperatures of the GC preclude decomposition of the silane phase coating to produce methysilane.

The possibility of the presence of trimethylamine was investigated. Figure 7 shows that the pure compound also elutes in the air background window of time and Figure 8a-d shows a series of mass spectra including pure trimethylamine and its library mass spectrum. Characteristic masses are m/z 58 and 59 in a roughly 2:1 intensity ratio, respectively. Figure 9 shows reconstructed ion chromatograms of m/z 58 and 59, with the approximate 2:1 ratio in the features between 20-40 sec. This ratio was a typical occurrence in the 21-30 mass scan number interval as shown in Figure 3a. However, the relative intensities of the peaks indicative of trimethylamine (Figure 9) are approximately 10-100 times lower than that of dimethylamine (Figure 4).

An enlargement of Figure 6b is shown in Figure 10 to indicate the relative intensities of m/z 44, 45, and 58, 59. If trimethylamine was present, it was only in very low quantities. It is possible that in the teflon bag-enclosed headspace, trimethylamine decomposed to dimethylamine.

Polyamines

The monoamine analysis was indicative of the sampled fish headspace in the 1-3 day timeframe. Polyamines also indicates fish spoilage and their appearance follows microbial muscle degradation and the deleterious organoleptic odor evaluations. Putrescine $NH_2(CH_2)_4NH_2$, cadaverine $NH_2(CH_2)_5NH_2$, spermidine $NH_2(CH_2)_4NH(CH_2)_3NH_2$, and spermine $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ are included among these compounds. Standard analyses with the GC-IMS are found in Table 2.

Table 3 presents a retention time and drift time summary on the fourth day of the fish volatile characterization study; a complete gas chromatogram - ion mobility spectrum is shown in Figure 11. The first IMS peak consists of the low molecular weight amines (5.20 msec drift time) as observed in the first three days of the study. A second set of ion mobility peaks elutes at a retention time of 5-7 sec with K_0 of 1.73 and 1.40 cm/V*sec. Table 2 shows that a standard cadaverine sample has a component that elutes at 7.14 sec with a K_0 of 1.73 cm/V*sec and Figure 12 shows a GC-IMS record of pure cadaverine (summarized in Table 2). There is a close match of the second eluting peak in Table 3 (peak marked "a" in Figure 11) to that of the peak marked "a" in Figure 12. The K_0 1.40 CM/V*sec peak from the second eluting compound in Table 3 was not observed in the cadaverine standard. The third and fourth eluting peaks in Table 3 were unidentified in that they did not match any of the suspected amine compounds in Table 3. Unfortunately, confirmation assistance of the fish headspace vapor by GC-MS did not take place because of instrumental failure. Later, another experiment with one fish in a teflon bag failed to show the information shown in Figure 11 in a one week time frame analysis, either by GC-IMS or GC-MS.

Despite the relatively high boiling points of the polyamines (up to 180°C) and the relatively low 126°C temperature limit of the column in the GC-IMS device, these compounds can elute from the GC and be observed by the IMS due to the short column length and relatively high flow rates.

CONCLUSIONS

The hand-held GC-IMS system was capable of detecting spoilage/decomposition amine compounds of fish stored at room temperatures in a teflon bag by sampling the headspace vapors over a period of one week. Volatile amines are the most indicative substances of fish spoilage. GC-MS confirmation of dimethylamine and trimethylamine was used to implicate their presence in GC-IMS experiments of fish stored at room temperature. Ion mobilities of these compounds fell in a drift time window which was faster than that of the RIP and were consistently present after the first day of fish decomposition. One GC-IMS experiment indicated the presence of cadaverine, among other unidentified compounds, that produced ion mobilities slower than that of the RIP and was observed at the fourth day of fish decomposition. This correlates with the observation that trimethylamine and volatile amine compounds appear to a relatively greater extent after the second day of fish storage under room temperature conditions [7].

Further experimentation needs to be done. For example, better characterization of the potential for hand-held GC-IMS utility in the determination of fish decomposition products would be to sample the headspace of containers with more than 2 fish, e.g. - 10-15 fish. This would be closer to actual conditions where pallets of fresh fish are routinely screened. This would necessarily increase the amount of analyte available in the vapor phase for instrumental monitoring.

ACKNOWLEDGEMENT

The authors acknowledge the assistance of Dwight Miller, USFDA National Center for Toxicological Research, Jefferson, AR, who brought the application to our attention and provided some standard amine samples.

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TABLE 1
EXPERIMENTAL OPERATING CONDITIONS OF THE GC-IMS

Disposable GC Module:		
GC Column:	Liquid Phase:	DB-1 (0.25 micrometer)
	Temperature (°C):	45°C/min programmable
	Carrier Gas:	Clean dry air
	Flow Rate:	2.1 ml/min
	Length:	1 m
	Sample injection:	0.2 sec
	AVS unit:	90°C
Ion Mobility Spectrometer:		
Ionization Source:		^{63}Ni
Gating Pulse Repetition Rate:		30 Hz
Cell Temperature:		30°C
Cell Pressure:		640 torr
Drift Gas:		Clean dry air
Drift Gas Flow:		400 ml/min
Positive Mode		

TABLE 2

GC retention times and ion mobility values of various amines, Drift Time of the RIP was 5.56 msec

K_0 relative to 2,4-Lutidine at K_0 = 1.95 $cm^2/V*sec$

Chemical Name	MW	bp°C	R.T. (sec)	t_{Drift}	K_0
Ammonia NH_3	17	--	.7-.8	5.23	2.20
Dimethylamine	45	7	.7-.8	5.35	2.15
Trimethylamine	59	3	0.71	5.27	2.19
Diethylamine	73	55	1.1	5.78	1.99
Diisopropylamine	101	84	1.3	6.07	1.90
Butylamine	73	78	1.37	6.26	1.84
1,4 Diaminobutane; putrescine	88	160	1.32	6.36	1.81
1,3 Diaminopropane	74	140	2.8	6.65	1.73
1,5 Diaminopentane; cadaverine	102	180	1.54	6.35	1.81
			3.46	5.93	1.94
				7.05	1.63
			7.14	6.68	1.73
			13.1	6.13	1.88
				7.68	1.50
2,4 Lutidine	107	159	7.2	5.91	1.95
				7.57	1.52

TABLE 3
GC retention times and ion mobility values of a head space sample of two, 4-day old fish
Drift time of the RIP was 5.55 msec

K_0 relative to 2,4-Lutidine at K_0 = 1.95 $cm^2/V*sec$

Fish component	R.T. (sec)	t_{Drift}	K_0
#1	0.71	5.20	2.22
#2	5-7	6.65	1.73
		8.23	1.40
#3	7-8.5	5.88	1.96
		7.58	1.52
#4	21.5-22.5	6.80	1.69
		7.31	1.58

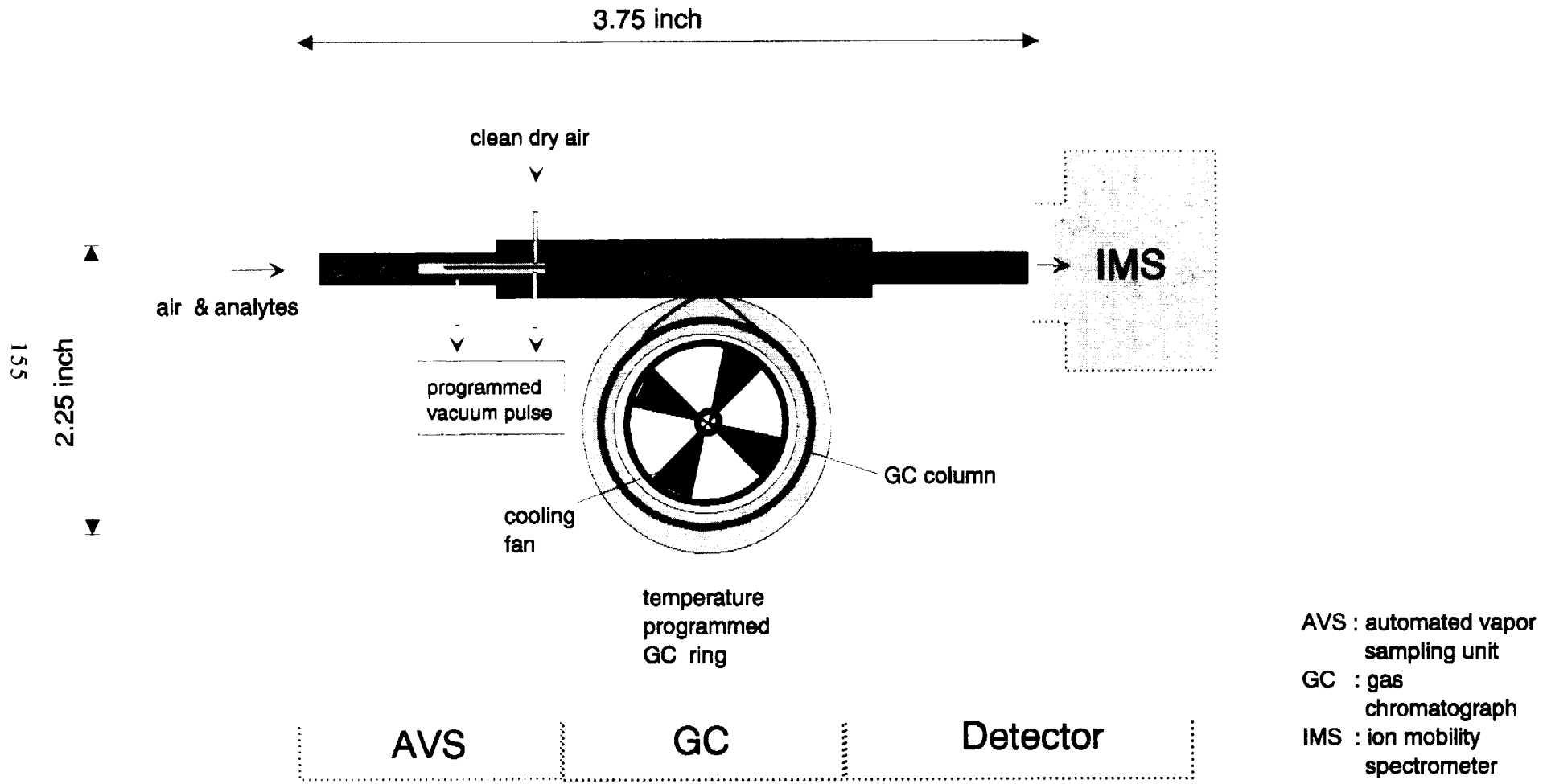

Figure 1. Schematic of the improved GC-IMS device.

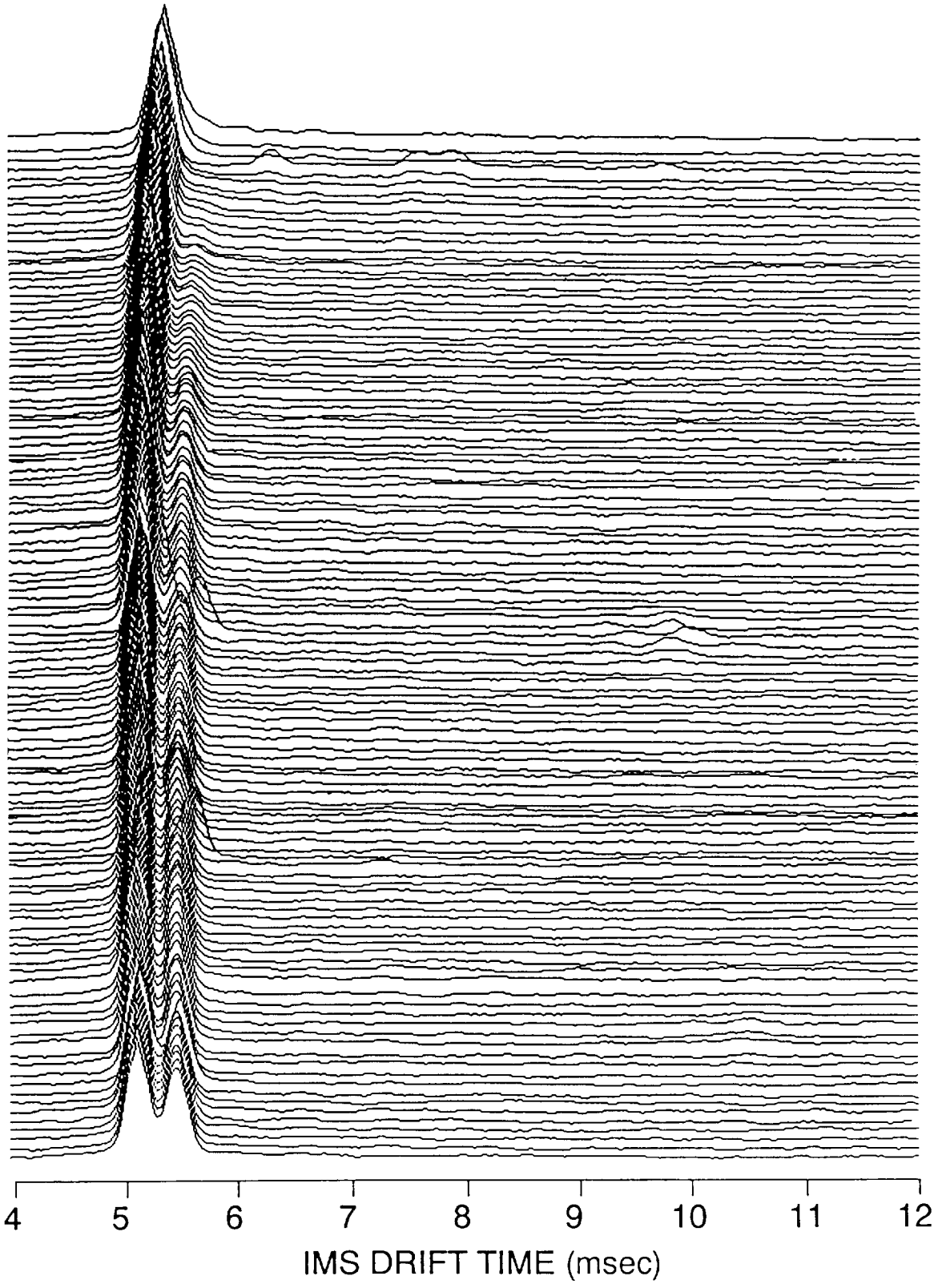

Figure 2. GC/IMS chromatogram of the headspace of a 3-day old fish sample in a teflon bag. Note the faster volatile amine drift time (5.14 msec) with respect to the water RIP (5.47 msec).

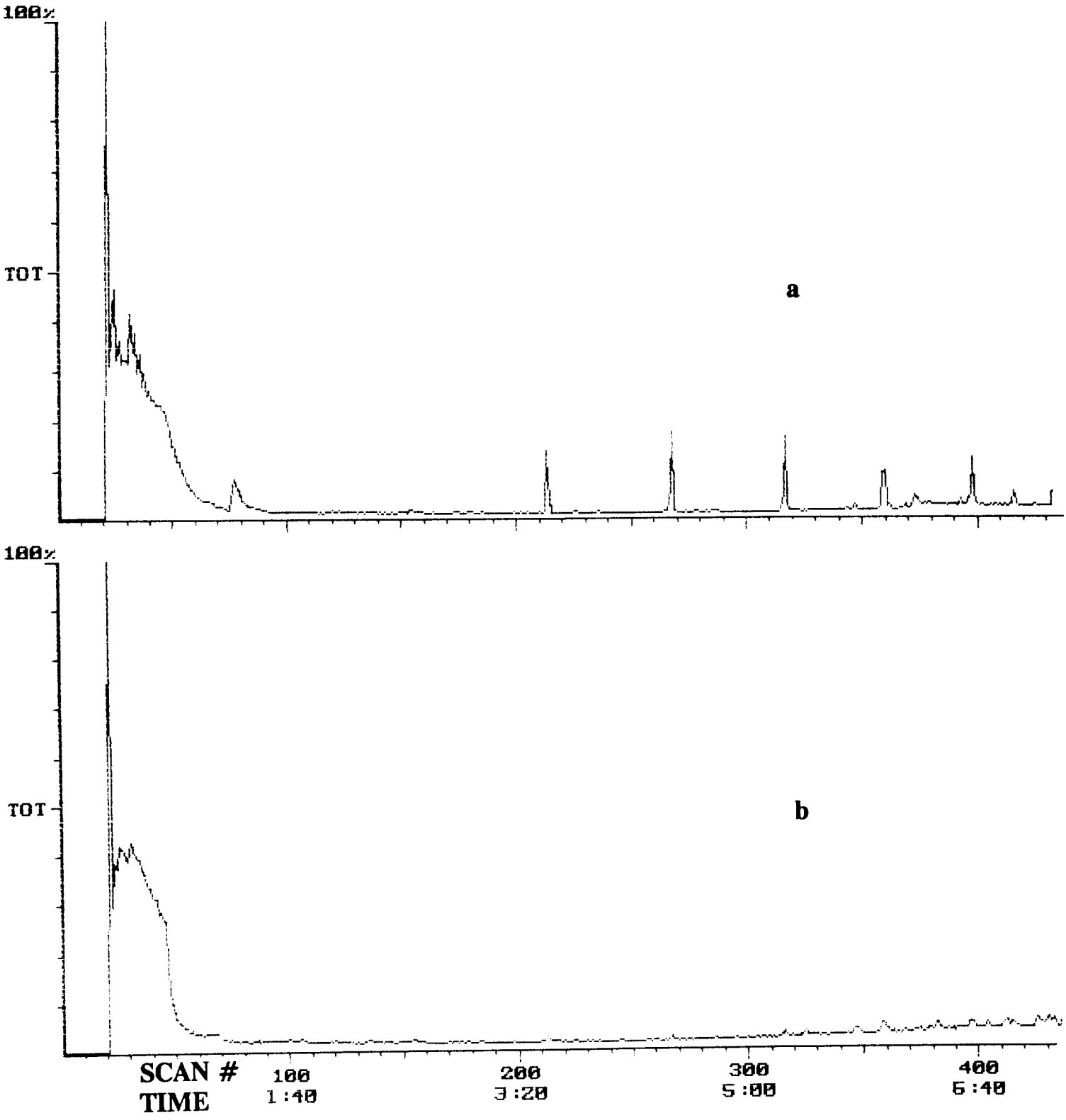

Figure 3. GC-MS TICs of (a) fish headspace and (b) air blank.

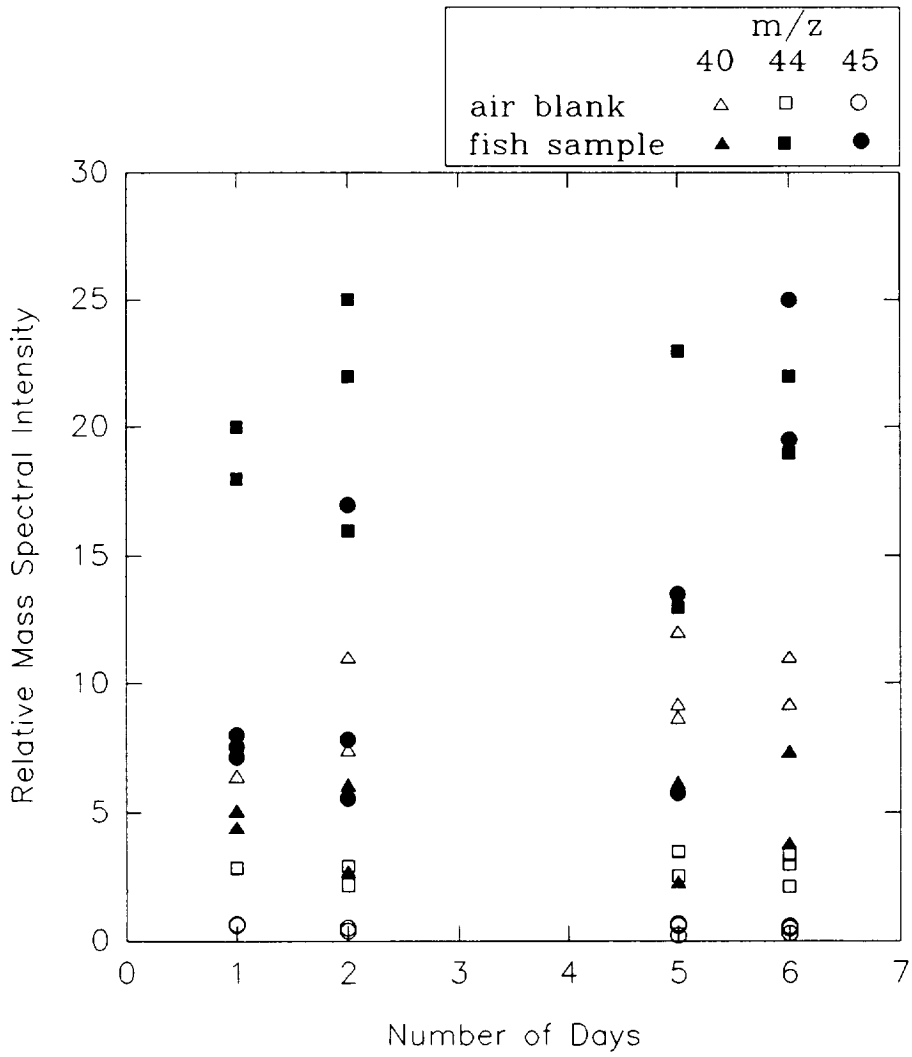

Figure 4. Graph of the maximum value of the reconstructed ion currents of m/z 40, 44 and 45 in air blank and fish headspace GC-MS analyses.

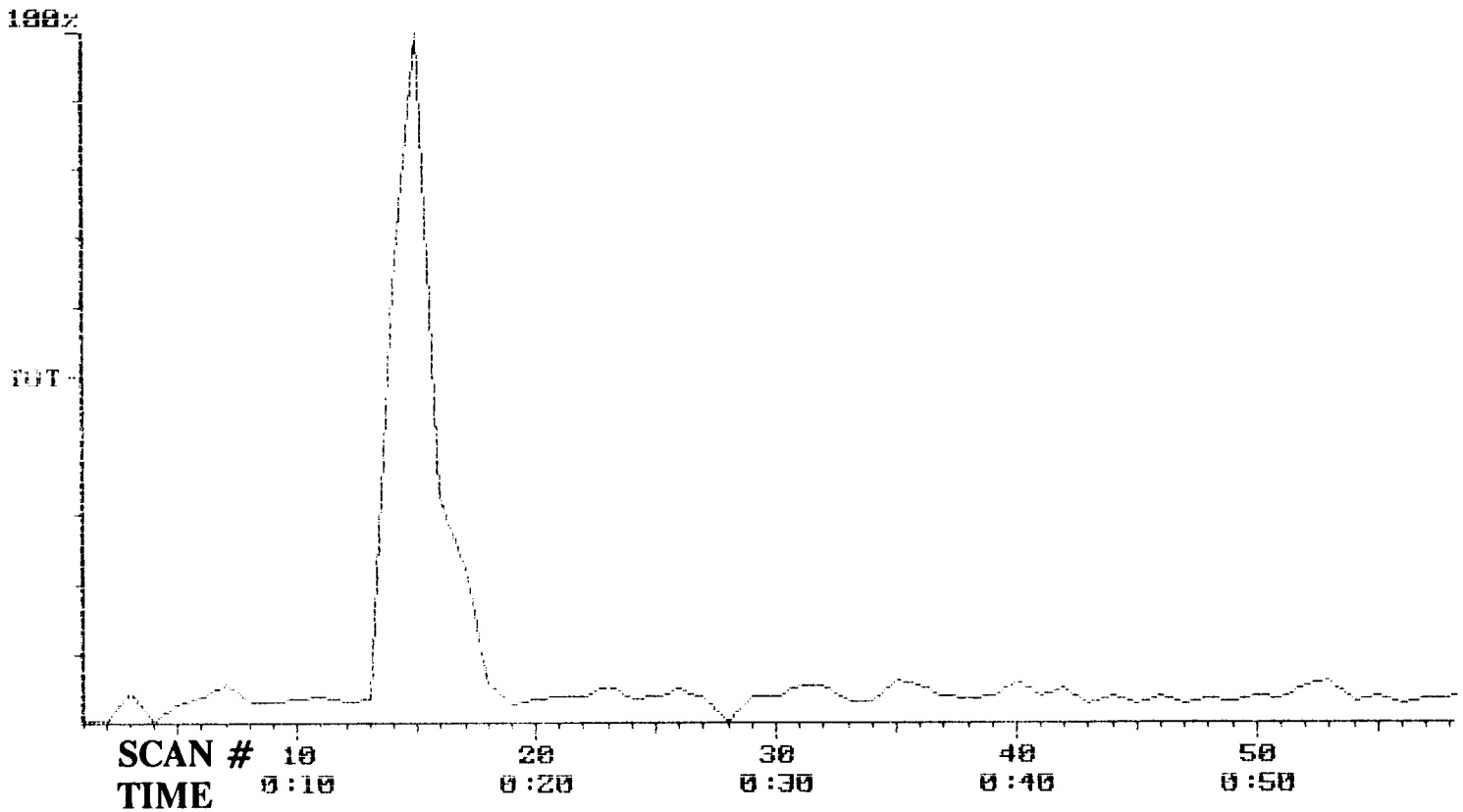

Figure 5. GC-MS TIC of pure dimethylamine.

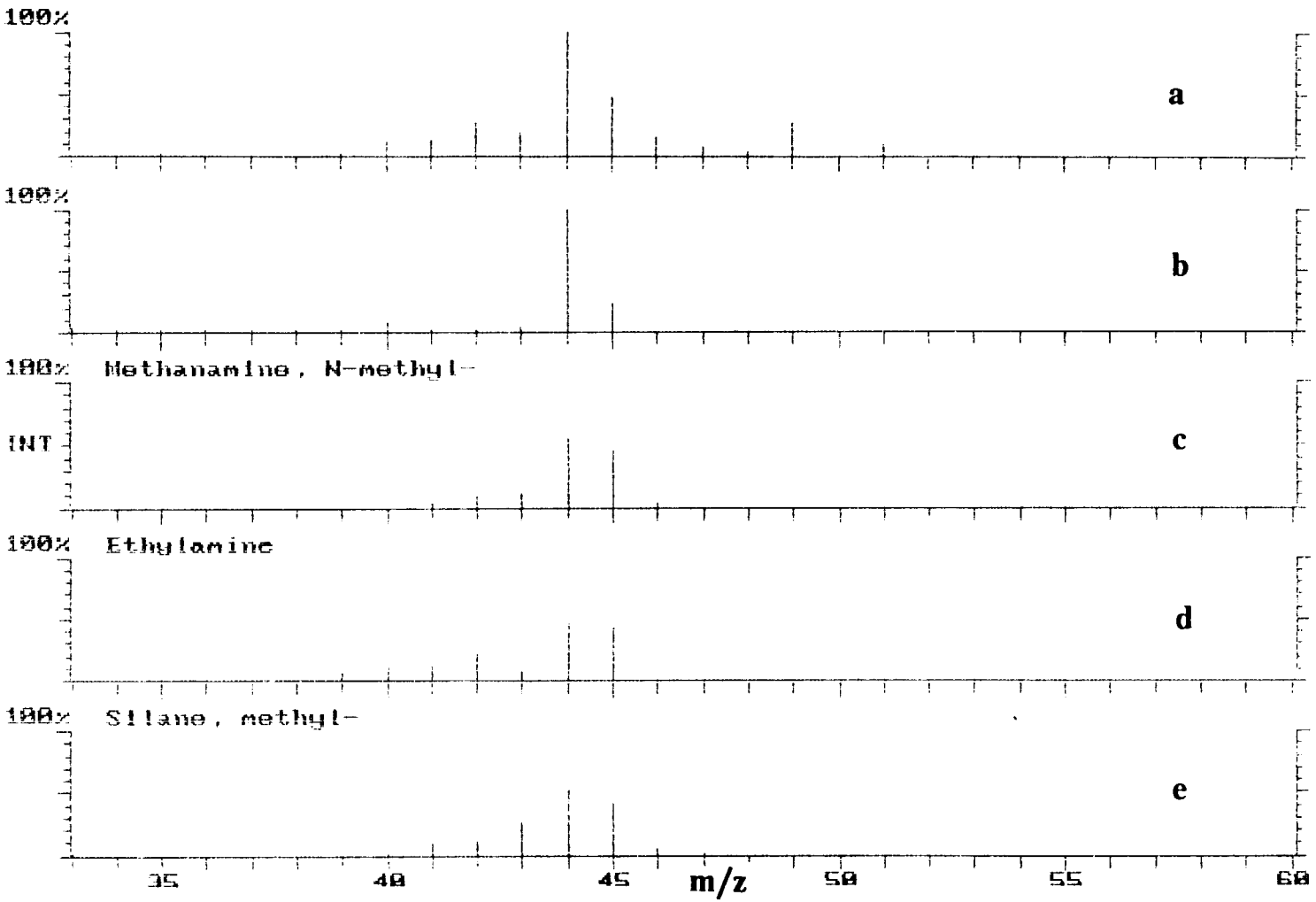

Figure 6. Mass spectra of (a) pure dimethylamine, (b) fish headspace and library mass spectra of (c) N-methylmethanamine, (d) ethylamine and (e) methylsilane.

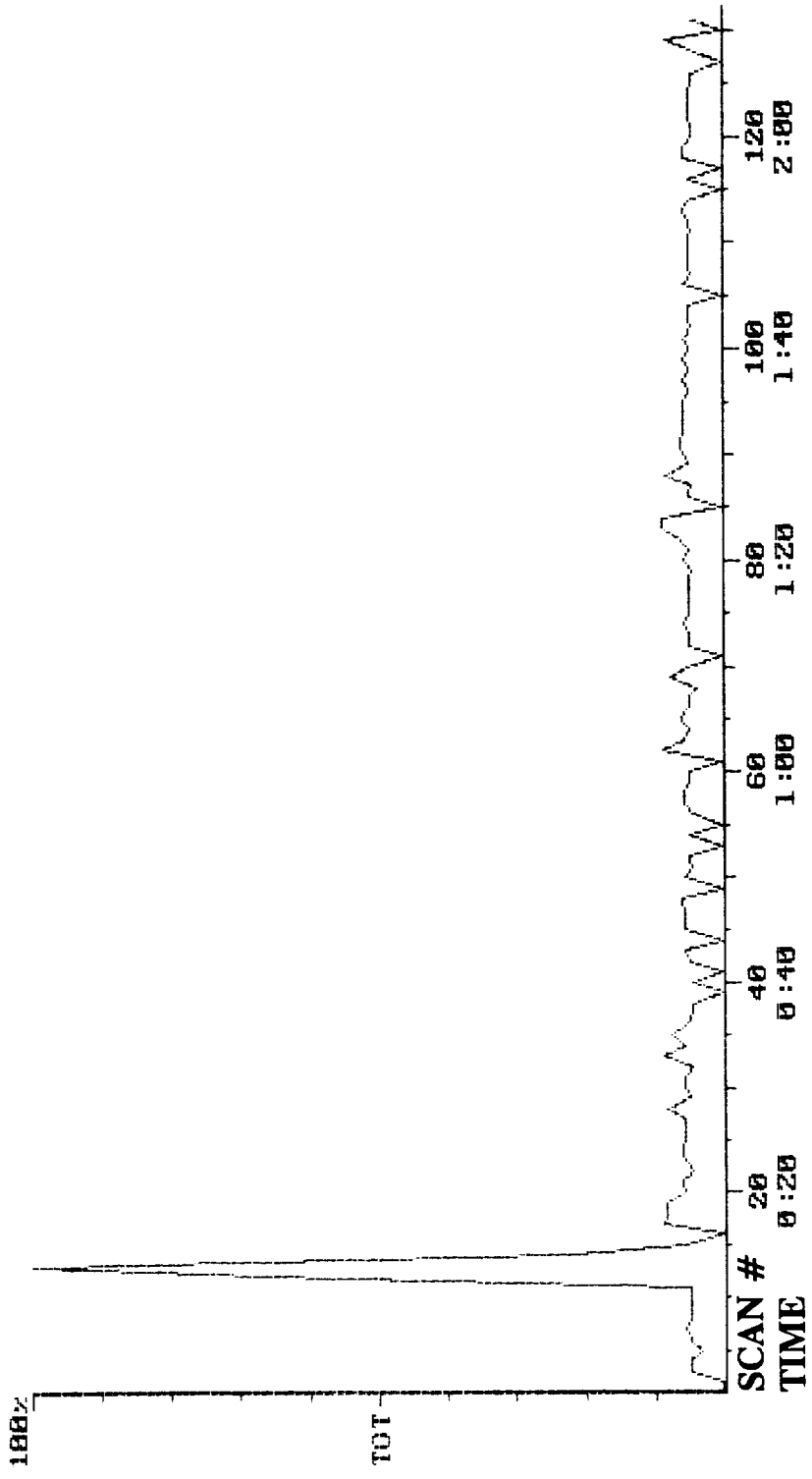

Figure 7. GC-MS TIC of pure trimethylamine.

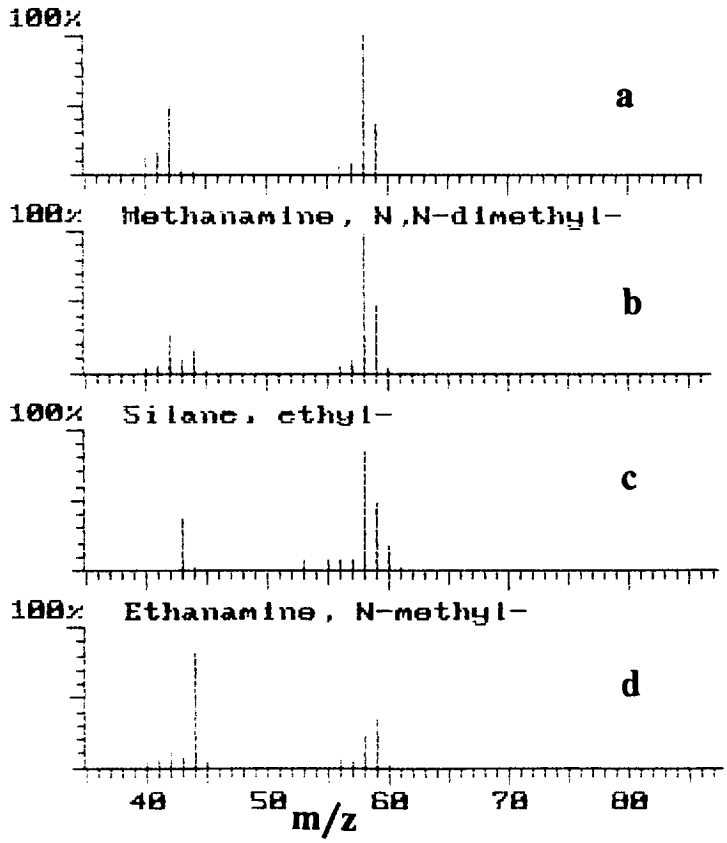

Figure 8. Mass spectra of (a) pure trimethylamine standard, and library mass spectra of (b) trimethylamine, (c) ethylsilane and (d) N-methylethanamine.

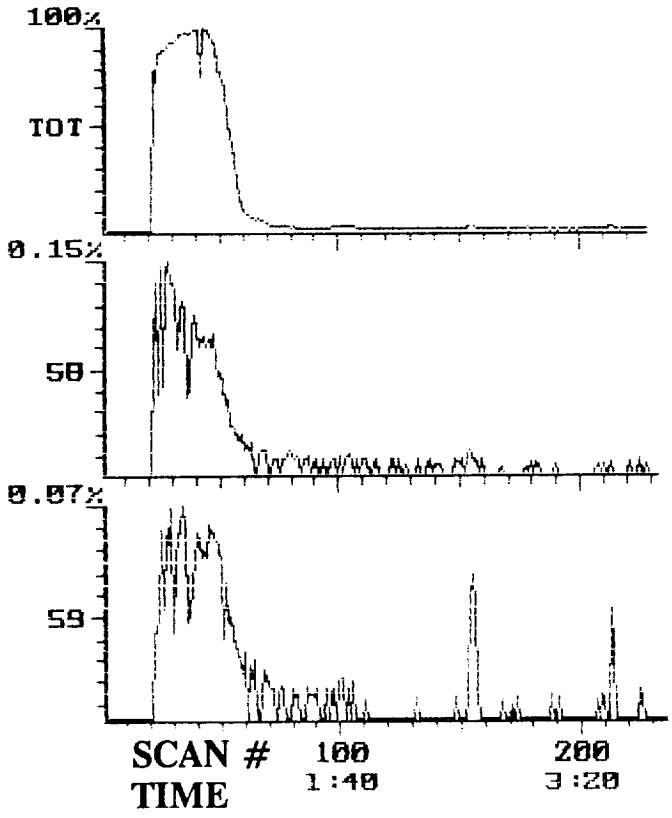

Figure 9. TIC and reconstructed ion chromatograms of m/z 58 and 59 in a fish headspace analysis.

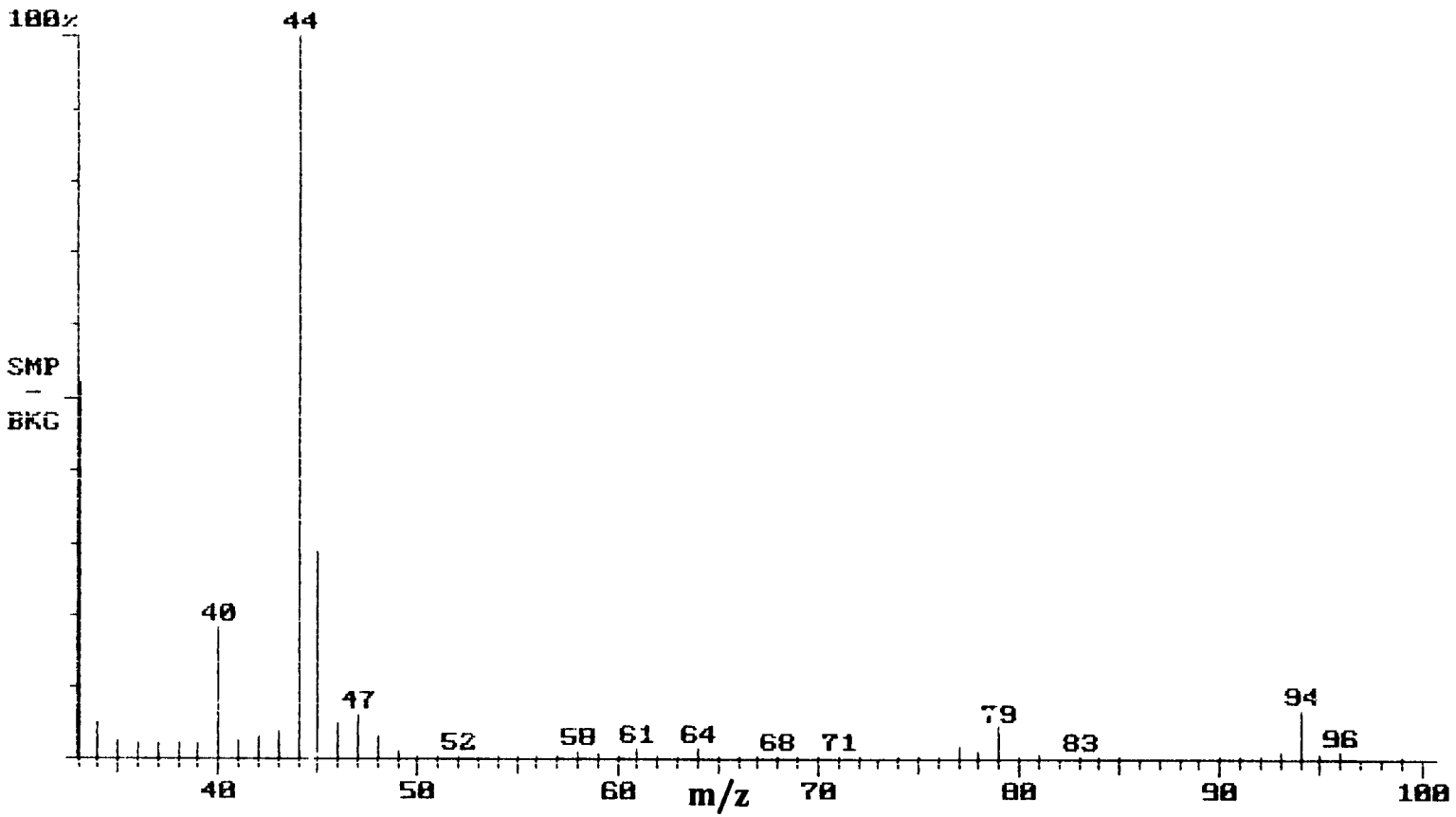

Figure 10. Mass spectrum of the 21-30 scan number interval in Figure 3a.

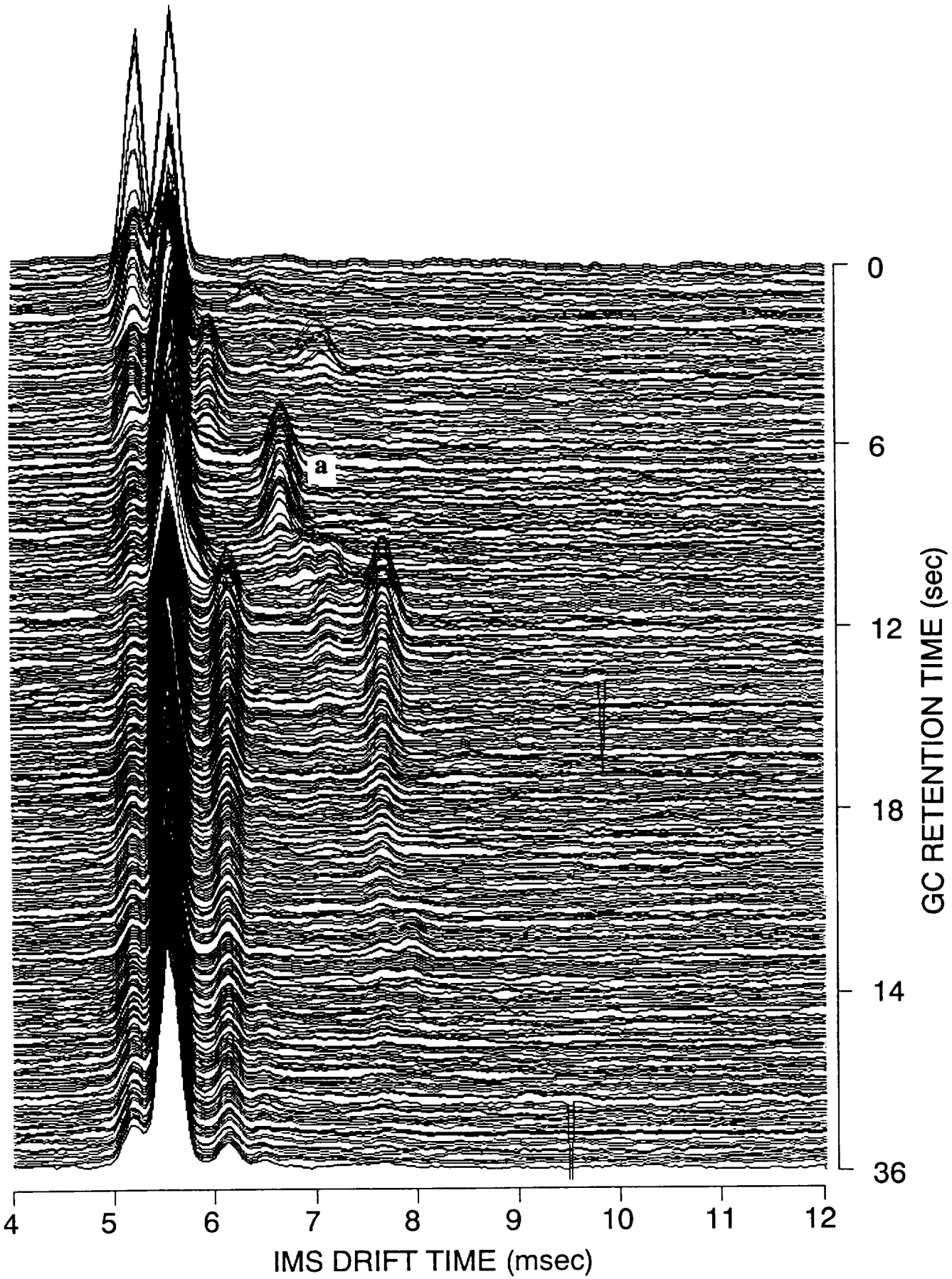

Figure 11. GC-IMS chromatogram of fish headspace on the fourth day of the study.

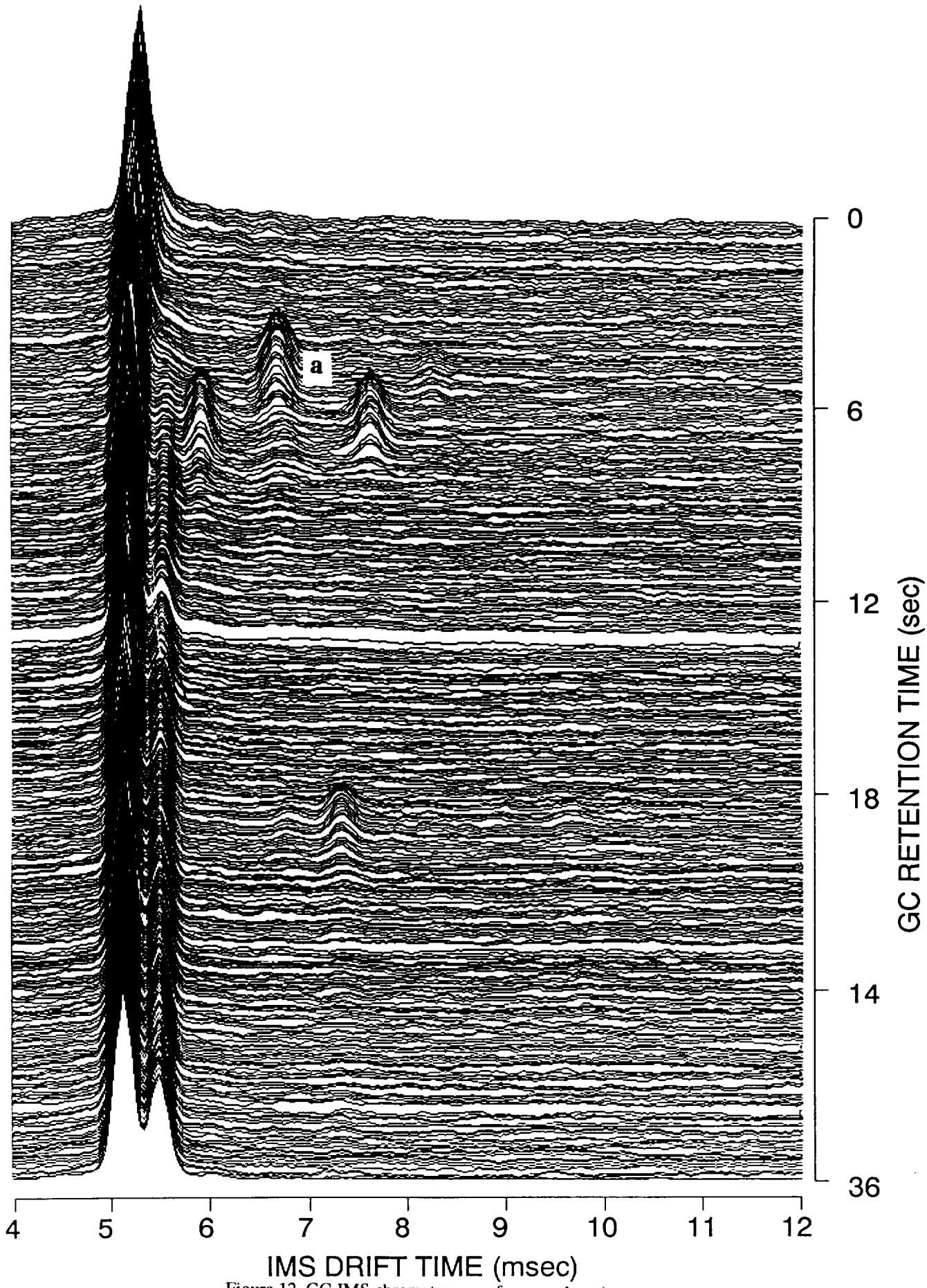

Figure 12. GC-IMS chromatogram of pure cadaverine.