THE USE OF STABLE ISOTOPE INTERNAL STANDARDS FOR ENVIRONMENTAL ANALYSIS

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Abstract

Low level environmental analysis (ppb levels) is complicated by many factors. The use of isotopically labeled internal standards can simplify the situation considerably. The use of a deuterated internal standard for the analysis of DDAC (didecyldimethyl ammonium chloride) in rainwater runoff samples will illustrate the advantages of this technique.

Introduction

Kop-Coat's Analytical Need

The environmental and toxicological effects of Kop-Coat's Sapstain Control Product, NP-1, have been well documented (1). It is now the most widely used anti-sapstain treatment for freshly cut lumber. Although it is a replacement for far more toxic treatment chemicals (chlorophenols) there is still environmental concern attributable to the toxicity of its components (DDAC and IPBC) to certain aquatic organisms. We were approached to assist in the development of highly sensitive and specific analytical methods to allay some of these concerns. The sample matrix is complex, rainwater runoff. A method was needed to measure levels of quaternary amine in this matrix at the sub-ppm level.

The Role of a Responsible Chemical Manufacturer

The formulator and/or user of a biocidal formulation must rely upon the chemical manufacturer for detailed chemical knowledge of the chemical components. Particularly important in this environmentally sensitive decade is the ability to detect and quantify the components in environmental samples. Typically, the levels are in the low ppm or even ppb range. Of utmost importance is specificity; false positives can lead to undue concern and cause
lengthy regulatory delays.

A company supplying chemicals into heavily regulated areas, such as the biocide area, may fairly be judged upon its ability to supply this type of technical support. The example, given later in this paper, illustrates our commitment to respond to the needs of the wood preservation industry.

The Need for a Reference Method

The British Columbia Ministry of the Environment had already commissioned BC Research to develop sensitive methodology for the detection of DDAC (didecydimethylammonium chloride). This is the major component (>95%) of Bardac 2280, supplied by Lonza, Inc.

Scientists at BC Research had already done an excellent job of developing sensitive and specific chromatographic methods when we entered the picture. Their concerns at that point were for the stability of the quat in collected water samples and for maximizing the total recovery of the DDAC. We felt that we could contribute synthetic and analytical resources to help resolve some of these concerns. The studies described in this paper were completed within one month of the initial request by Kop-Coat.

The surfactant nature of DDAC and the pyrolysis of the quaternary ammonium center during gas chromatography are factors that complicate this analysis. The use of a stable isotope labeled standard minimizes problems in both areas.

Specificity in Organic Analysis

The most popular wet chemical tests for quaternary chemicals are not specific and are certainly not appropriate for environmental analysis. Colorimetric and spectroscopic methods also lack the necessary specificity. In order to achieve the desired sensitivity and specificity, a chromatographic method coupled with specific detection is required.

Quaternary ammonium compounds have been analyzed for years using gas chromatography (2). The pyrolysis of the compound that occurs in the heated injection port results from a reaction at the quaternary center that is similar to a Hoffmann degradation. Chromatography then separates the resultant degradation products (3). The amine peaks are of greatest analytical utility. When temperatures and injection conditions are tightly controlled, the decomposition is very reproducible and accurate quantitation is possible.

Most reported work employs the conventional flame ionization detector. Additional specificity may be obtained by use of a nitrogen selective detector, one approach taken by BC Research. The ultimate specificity, however, is obtained by use of mass spectrometric detection, either MS or MS-MS. The MS detector also allows the use of labeled standards to minimize the effects of losses during recovery on quantitation.

Stable Isotopes Used for Organic Analysis

Advantages

Most people when they hear of "labeled" or "isotopically labeled" compounds think immediately of radioactive materials. The "stable" isotopes are not radioactive. The analyst is interested in using the less abundant, higher mass isotopes that differ only in mass from their naturally occurring, most abundant cousins. There are great advantages to the analytical use of these "stable isotopes".

The first table shows the isotopes of hydrogen, carbon and nitrogen. The 17O and 18O isomers have not been included but are also of use.

Safety. Certainly, the elimination of the need for the special precautions required for handling radioactive materials simplifies things considerably. Normal laboratory procedures are employed and there are no disposal problems. Also, the radioactive isotope licensing requirements are eliminated.

Cost. Labeled materials are not "cheap", but costs have fallen considerably in recent years and the availability of various labeled reagent chemicals has improved. The normal chemical supply houses, Aldrich for example, include in their catalogs a wide variety of deuterated and 13C
labeled reagents. For comparison, 5 grams of methyl iodide-d6 (99.5+% D) lists in the 1990-91 Aldrich catalog for $32.15, while 5 grams of 13C labeled methyl iodide (99% 13C) runs $185.95.

Chemical Identity. Obviously, the synthetic procedures employed are similar to those normally used. One difference, however, due to the cost of the reagents, is that reactions are usually run to minimize the use of labeled material and are necessarily of small scale.

The synthesized materials are chemically identical to their unlabeled analogs. They behave identically in adsorption, extraction and most physical separations. In multiply deuterated molecules of low molecular weight, one must be careful of effects introduced by significant increase of the molecular weight, for example, retention times of gas chromatographic peaks may be slightly shifted. (See Fig. 1)

There are frequently differences in the rates of chemical reactions involving deuterated centers, due to isotope effects. The analytical chemist may see this in the mass spectral fragmentation of isotopically labeled molecules. Ion intensities of labeled molecules may differ from their unlabeled counterparts, hence the need for standard calibration curves, as opposed to straight peak intensity ratioing.

All of these factors should be taken into consideration in planning out the experimental strategy. The analyst has a number of options, both in choice of isotope and in the site of incorporation.

Synthetic Considerations

Choice of Nuclide. Cost is certainly a factor, albeit not as significant as in earlier years. 13C labeling is approximately 5 times the cost of deuterium labelling and 15N labeling is 10 times as costly. Of far greater significance is the suitability of the label to the experiment.

For mass spectral detection, it is important that the mass fragments of the labeled molecule do not overlap with mass fragments of the unlabeled molecule. A labeled standard that has only a single mass unit of molecular weight increase will in all cases overlap with the M+1 peaks of the unlabeled material. The M+1 peak intensity will be:

$$\text{peak intensity} = (\text{number of carbon atoms}) \times 1.1\%$$

The effect of this upon the mass spectra of 13C labeled (single 13C atom) and deuterated (3 labeled atoms) material is shown in Figure 2. This can further complicate things.

Deuterium labeling on the other hand is not without it's problems. If the deuterium label is near a reactive center or involved in a rearrangement (either chemical or mass spectral) it can easily be lost.

These are certainly not difficult problems to overcome, but the best option is to spend time up front to consider the alternatives. One has the choice of labels, but one also has the choice of where in the molecule to place them.

Site of Incorporation. Usually this is determined by ease of synthesis and availability of suitable labeled reagents. If label stability is an issue, incorporation far from active centers is preferred.

Mode of Synthesis

The focus of this paper is necessarily narrow; it is quaternary amine detection. The two most common (also the cheapest) reagents for synthesis of 13C labeled compounds are methyl iodide-13C and potassium cyanide-13C. Methyl iodide-d6 is also a common reagent for deuterium introduction.

Labeling of a dimethyl quat is straightforward and can be carried out in high yield. The corresponding dialkylamine is reacted with methyl iodide (2D or 13C). The resultant quat iodide can then be exchanged to yield the quat chloride.

Alternatively one can use decyl chloride, labeled in either the C-1 or C-10 position, to react with dimethylamine to generate labeled material. This is a quite expensive route but gives a stable long chain labeled material.

Analytical Considerations

The goal in producing isotopically labeled materials is to achieve accurate quantitation in complex matrices. All elements must come together for optimal sensitivity - detection, separation and quantitation.

Detection. The detection method must be highly sensitive and allow discrimination of labeled and non-labeled material. Mass spectroscopy provides this necessary combination of sensitivity and selectivity. Sub-nanogram (10^{-9} g) amounts of material can be detected by modern mass spectrometers. With selective ion detection, picogram (10-12 g) sensitivity is possible.

There are also ionization options available to the mass spectroscopist. Use of chemical ionization techniques can limit fragmentation in molecules that dissociate readily. In this study we were able to use the standard electron impact mode of ionization with good results.

The ultimate weapon, if required, is MS-MS. This technique is frequently referred to as tandem mass spectroscopy. A beam of ions of a particular molecular weight (characteristic of the substance of interest) is channeled from a first mass spectrometer into a collision chamber filled with inert gas. Collision with the inert gas produces further characteristic fragmentation. The fragments produced are then separated and quantified by a second stage of mass spectroscopy. Suitable choice of ions for both stages can result in absolute specificity. The detector can be operated at extremely high gain, as only the ions of the compound of interest ever reach the detector.

In normal applications, the selectivity of high resolution chromatography coupled with mass spectral single ion monitoring is sufficient.

Separation. The ideal method of separation for use with mass spectroscopy is gas chromatography. Most modern mass spectrometers are interfaced to high-resolution, capillary column gas chromatographs. The capillary column can either deliver its output directly into the source or into some sort of pre-separator, either a jet-separator or a membrane separator. In all cases, efficiency is very high.

Great strides have been made in recent years in the use of liquid chromatography with mass spec detection. Various types of interfaces have been used, with thermospray (and chemical ionization) emerging as perhaps the most popular. This is a temperamental system and in use primarily in specialist laboratories.

Quantitation. One cannot ignore the contribution of a good data handling system to accurate analysis. One must be particularly careful in handling capillary column GC-MS

spectral data. The digitizer must sample the peaks with sufficient speed to allow reproducible, reliable integration of the narrow peaks.

In doing the final quantitation, one should analyze a full range of standards, as well as checking the accuracy via multiple spiking experiments in order to verify the accuracy, linearity and precision of the technique.

Analysis for DDAC from Kop-Coat NP-1 in Rainwater Runoff

All of the forgoing discussion has been focused at explaining the general aspects of stable isotope standard use. Now we will deal with a real case.

We should first review the analytical problem. When lumber is treated with Kop-Coat NP-1 the DDAC component adsorbs tightly to the cellulose where it prevents growth of the fungi that result in sap staining of the wood. There is concern that the rainwater runoff might carry the quat into bodies of water where it might cause damage to marine life. This is unlikely for a number of reasons, but no analytical data exists on level of quat in the runoff. The situation is outlined in Figure 5.

Quaternary amines like DDAC are very surface active; they adsorb readily to glassware, soil, sediment, etc (4,5). Ideally the internal standard should be added as soon as practical into the system. This is one of the appeals of stable isotopes, they can be added in the field. In this preliminary study, however, the internal standard was not added until the samples arrived in our lab in New Jersey.

Before detailing the quantitation itself, a brief review of the internal standard preparation is appropriate.

Synthesis

The synthetic scheme for DDAC-d6 is shown in Figure 6.

Reaction. The reaction to generate the quat iodide was carried out in isopropanol with no real purification, other than evaporation of the solvent, required. The resultant iodide was then treated with HCl in IPA in the presence of AmberliteR IRA-400 ion exchange resin to yield the quat chloride. Overall yield of the reaction was >95%.

Characterization. In addition to the standard "wet chemical" characterization of the quat product, the material was chromatographically identical to unlabeled DDAC. Shown in Figure 7 are the comparative CMR spectra of labeled and unlabeled DDAC. When deuterium is substituted on the methyl carbons, the usually sharp singlet peak seen in the broadband decoupled 13C spectrum (50 ppm downfield from TMS), is transformed into a low intensity septet. This validates both the high level of 2H incorporation and its position in the molecule.

Extraction

The surfactancy of quats makes their extraction a matter of concern. This concern is what makes this method of internal standardization so appealing. In addition, the thorough work done by BC Research minimized the amount of effort required in our lab.

Surfactancy of Quats. Figure 8 shows some of the sample quat adsorption problems.

Method of sample extraction. The extraction protocol followed was quite conventional, differing only in the addition of tetramethylammonium chloride to the evaporator flask to minimize glass wall adsorption of DDAC. The process is outlined in Figure 9. The final sample is taken up in a mixture (1:1) of dichloromethane and toluene.

Separation

Again, at the risk of sounding repetitious, we are greatly indebted to the researchers at BC Research for sharing the results of their method development effort with us. We have used their GC methods with little modification, except for MS detection.

Gas Chromatography. Gas chromatography was performed on a Perkin-Elmer Model 8420 Gas Chromatograph fitted with a 30 m x 0.015 mm SPB-5 capillary column. Helium flow was maintained at a flow of 1.5 ml/min. The chromatograph was programmed with an initial temperature of 80 oC for a hold time of 0.5 min followed by a rapid temperature rise of 30 deg/min to a final column temperature of 250 oC for a hold time of 8 additional minutes. The column flowed directly into the helium makeup system of a Perkin-Elmer (Finigan) Ion Trap. A typical total ion chromatogram (TIC) is shown later in this paper in Figure 11.

Liquid Chromatography. While we have not used HPLC as part of this effort, it is included here not only for completeness, but because it may well find use as an additional preparative cleanup step for particularly dirty samples. Again, because of the chemical identity of the internal standard, even this can be done as part of the sample cleanup.

Also, if one has access to LC-MS, it would be possible to avoid the pyrolysis that occurs during GC separation.

Detection

We used a simple benchtop system, a Finigan Ion Trap, as our detector system. The ion trap, if utilized correctly, gives results consistent with normal EI mass spectroscopy. One must, however, be very careful to watch the amount of sample being introduced into the system. If the sample concentration in the ionizing chamber gets too high, one begins to see atypical features in the spectra - protonated molecular ions and ion-molecule interaction peaks.

Selectivity. We have discussed selectivity previously. In Figure 10 a series of reconstructed ion chromatograms are overlayed to show the selectivity possible with the ion trap detector. Compare these to the full total ion chromatogram shown in Figure 11.

Sensitivity. This is an older ion trap system and does not have the sensitivity of a research grade mass spectrometer. It's single ion monitoring mode does not give the same enhancement in sensitivity that one expects in a quadrupole or magnetic sector instrument. The reconstructed ion chromatograms are adequate and certainly selective enough, but do not approach the sensitivity levels one could achieve with a full mass spectrometer system. Nevertheless, the example given here will illustrate the advantages of this approach.

The total ion chromatogram for an authentic rainwater sample is shown in Figure 11. The didecylmethyl amine peak is clearly seen in the chromatogram and represents approximately 300 ppb of total material, 100 ppb of native material and 200 ppb of added internal standard.

Quantitation

The pyrolysis of the DDAC sample in the injector of the GC system produces decyldimethylamine and didecylmethylamine. These are separated on the GC column and passed on to the ion trap. The primary fragments of each compound are shown in Figure 12. The standard differs by 3 mass units for the monomethyl amine and by 6 for the dimethyl amine. The actual mass spectra for the two decomposition products are seen in Figures 13 and 14.

In order to quantify DDAC in a sample, the analyst prepares a calibration curve using a series of known concentrations. The ratio of unlabled to labeled (standard) material is plotted as a function of added DDAC. These calibration curves are shown in Figure 15 and 16. They show excellent linearity. We have also verified the accuracy of the technique by comparing spiked samples against the standard curves. Reproducibility is excellent, given the level.

Conclusion

Limits of Detection

We limited the water sample extracted to 250 ml in our first efforts. We could easily increase this volume to achieve additional sensitivity. Given the signal:noise ratio seen in these trials we can estimate the limit of detection as approximately 20 ppb. At the 100 ppb level the 95% confidence limit appears to be about 15% relative.

Applicability

The method is applicable to water samples of all types. Water with large amounts of sediment have not been examined yet and would likely cause some difficulties in extraction and filtration.

Other Applications

The method is quite general in nature and can likely be extended into a variety of applications. Two of immediate interest come to mind.

Quat in Treated Wood. One could presumably use the excellent sensitivity of the technique to look at the degree of penetration of quat into the wood surface. One should not ignore the possibility of using a 13C label and 13C NMR spectroscopy, as well as mass spectroscopy, to look for quat decomposition in biological systems. One might also be able to use deuterium labels with 2H NMR spectroscopy (possibly even NMR tomography).

Quat in Biological Samples. With the possibility of low ppb analysis of quat one can imagine a variety of biological experiments (in the environment). The absence of radioactivity definitely simplifies things.

Work to be Done

As a primary manufacturer of quats, we obviously have a great interest in being able to quantify them in a variety of sample matrices. We feel that is also of great importance to be able to do so in an absolutely specific manner. We hope that our findings will be of use to the scientific community in general and to our customers in specific.

Acknowledgements

Lonza, Inc. - Paul Metz, Barb Reilly, Bob Sloan, Leigh Walker, Kop-Coat, Inc. - Alan Ross, Carol Hope, We also acknowledge the cooperation of the British Columbia Ministry of the
Environment and thank BC Research for sharing their preliminary research results with us.

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3. Goetz, N., Good, D., Kaba, G., Lasserre, P., Characterization and Identification of Quaternary Ammonium Compounds Using Pyrolysis Gas-Chromatography, Cosmet. Sci. Technol., Vol. 4, 1985.

4. Daly, D., Soil/Sediment Adsorption-Desorption of 14C-didecyldimethylammonium chloride (DDAC). Final Report #37009 to Lonza, Inc., Fair Lawn, NJ, ABC Laboratories, Inc., Dec. 29, 1989.

5. Dykes, J. and Fennessey, M., Hydrolysis of Didecyldimethylammonium chloride (DDAC) as a Function of pH at 25oC. Final Report #37004 to Lonza, Inc., Fair Lawn, NJ, ABC Laboratories, Inc., Apr. 21, 1989.

Isotopes of Common Organic Elements

Symbol	Relative Abundance	Alternate Name/Symbol	Detection Method
^{1}H	99.985%		NMR or MS
^{2}H	0.015%	Deuterium D	NMR or MS
^{3}H	(Radioactive)	Tritium T	
^{12}C	99.89%		NMR or MS
^{13}C	1.11%		NMR or MS
^{14}C	(Radioactive)		
^{14}N	99.63%		NMR or MS
^{15}N	0.37%		NMR or MS

TABLE 1

Shift of Retention Time with Deuteration

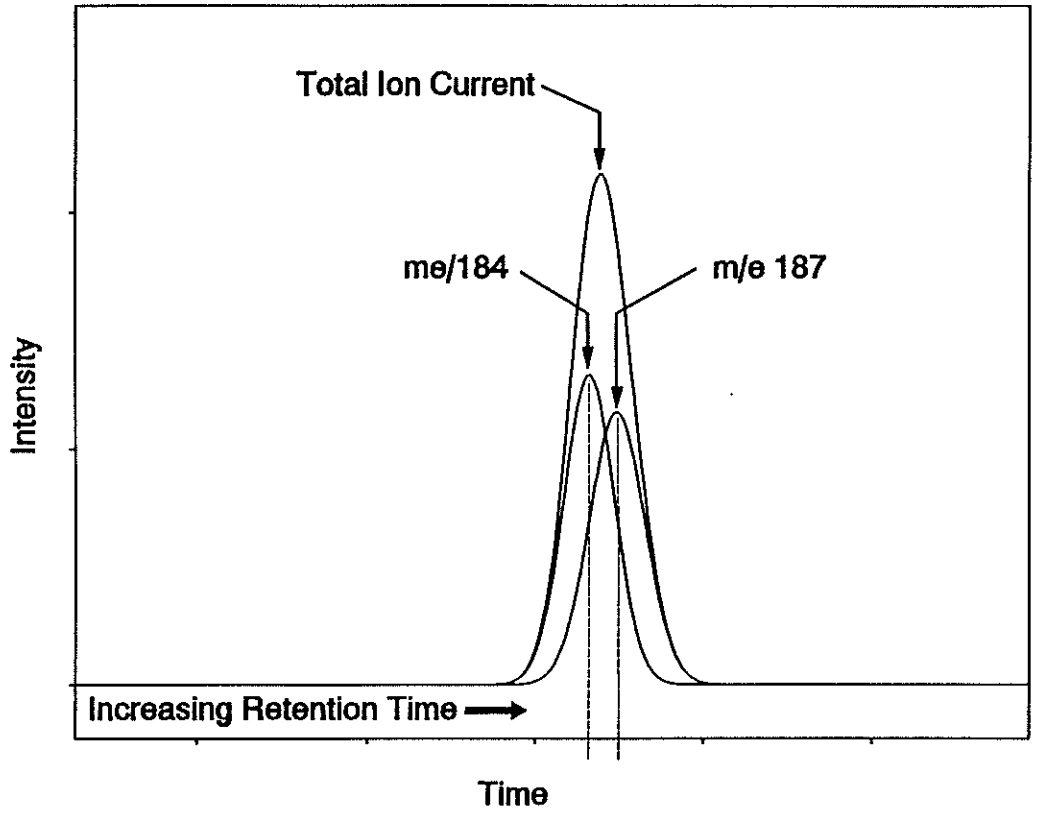


FIGURE 1

Comparison of Mass Spectra (Different Labels)

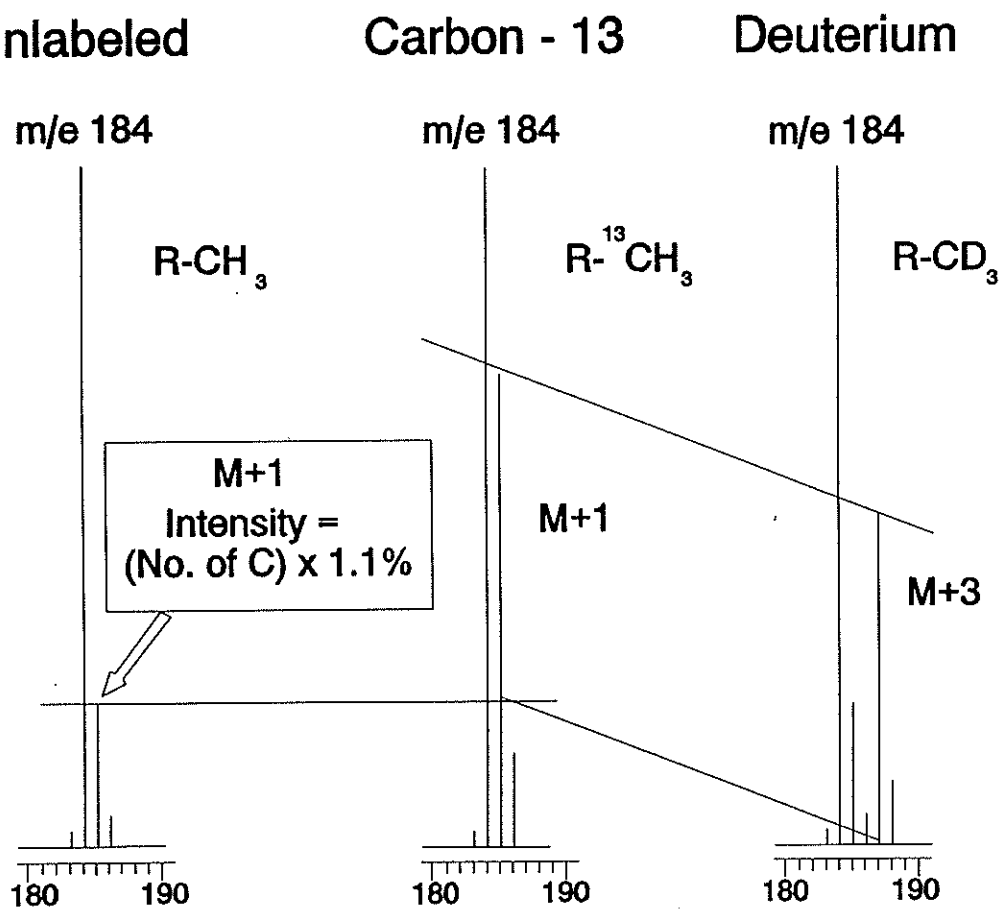
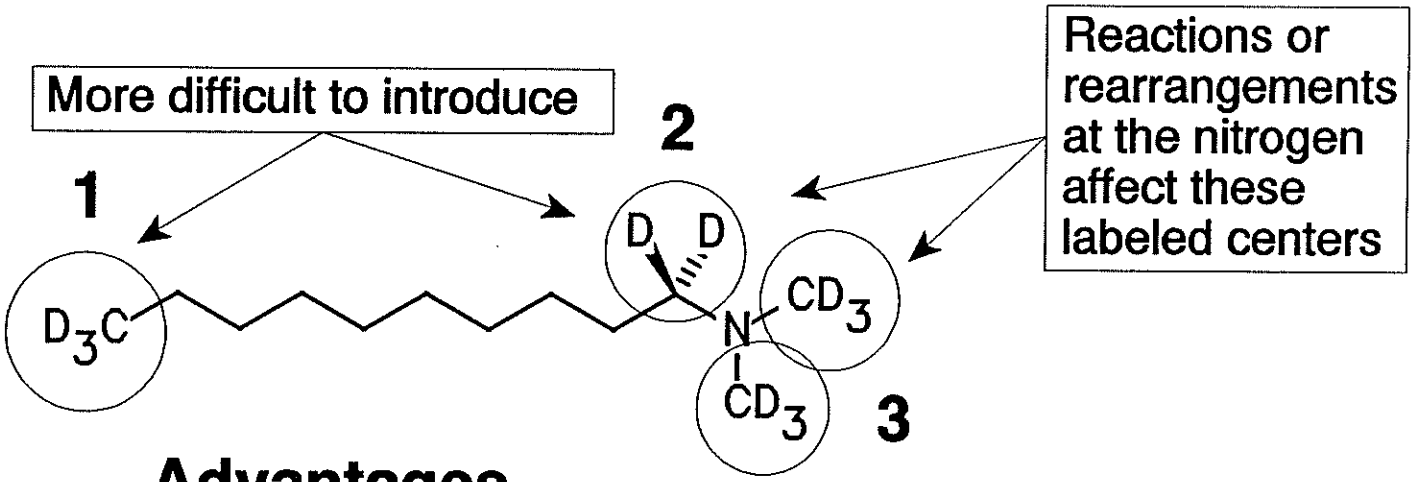


FIGURE 2

Deuterium Labeling



Advantages

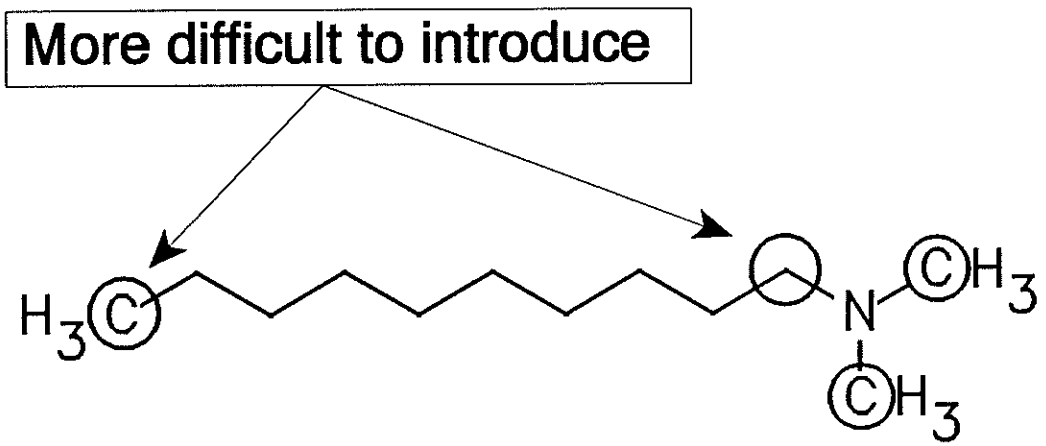
Inexpensive and readily available reagents
Higher mass increase for MS detection

Disadvantages

Isotope effects on chemical reactions and MS fragmentation
Rearrangement or loss of labels is possible

FIGURE 3

^{13}C Labeling



Advantages

Label is very chemically stable

Little isotope effect on reactions or MS fragmentation

Disadvantages

Costs more than deuterium labeling

Only increases mass by 1 unit

FIGURE 4

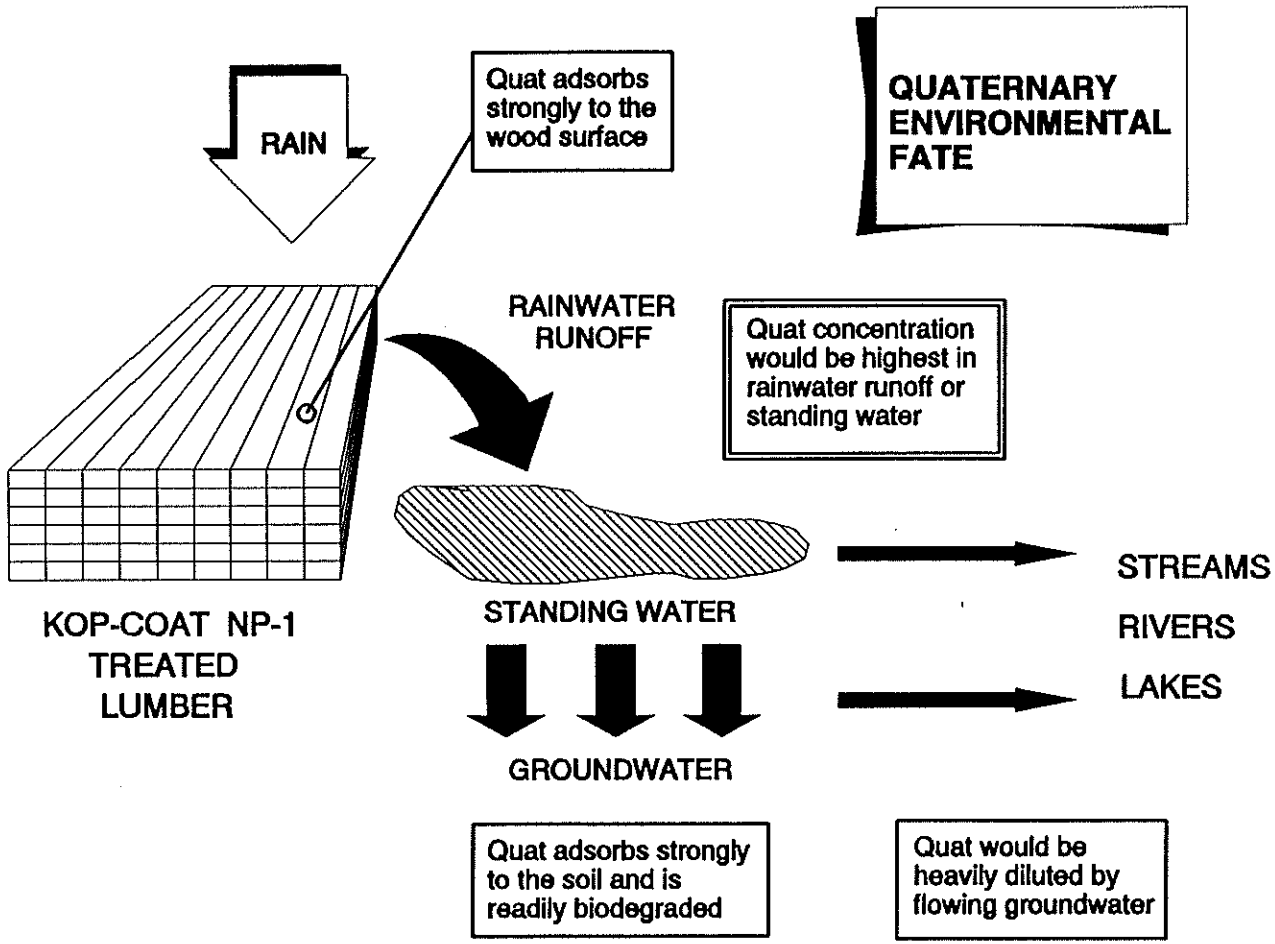

QUATERNARY ENVIRONMENTAL FATE
RAIN
Quat adsorbs strongly to the wood surface
RAINWATER RUNOFF
Quat concentration would be highest in rainwater runoff or standing water
KOP-COAT NP-1 TREATED LUMBER
STANDING WATER
STREAMS
RIVERS
LAKES
GROUNDWATER
Quat adsorbs strongly to the soil and is readily biodegraded
Quat would be heavily diluted by flowing groundwater

FIGURE 5

Synthesis of Deuterated DDAC

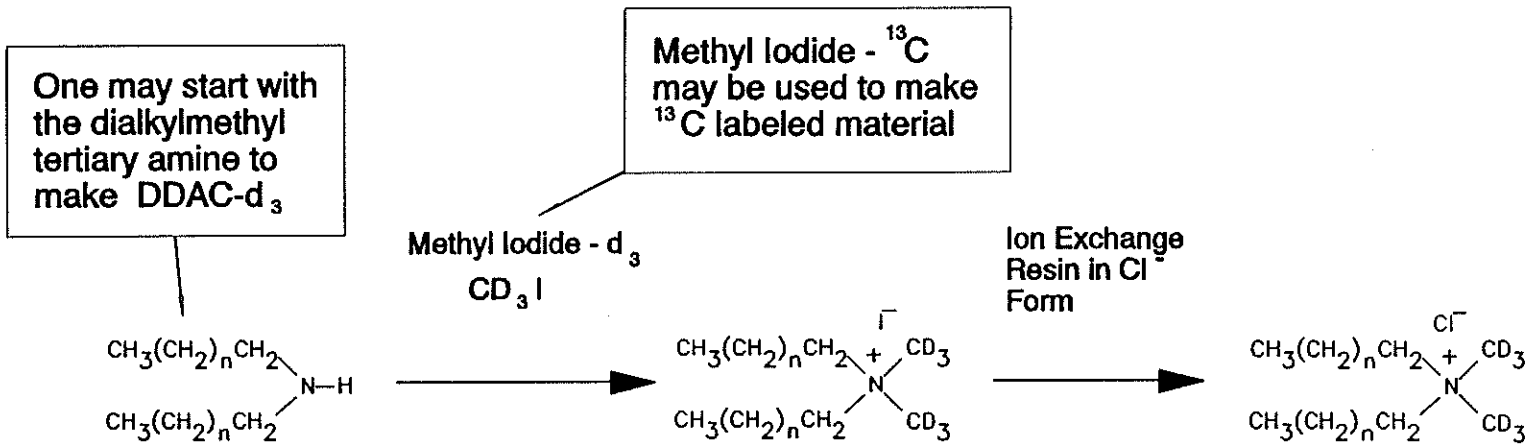


FIGURE 6

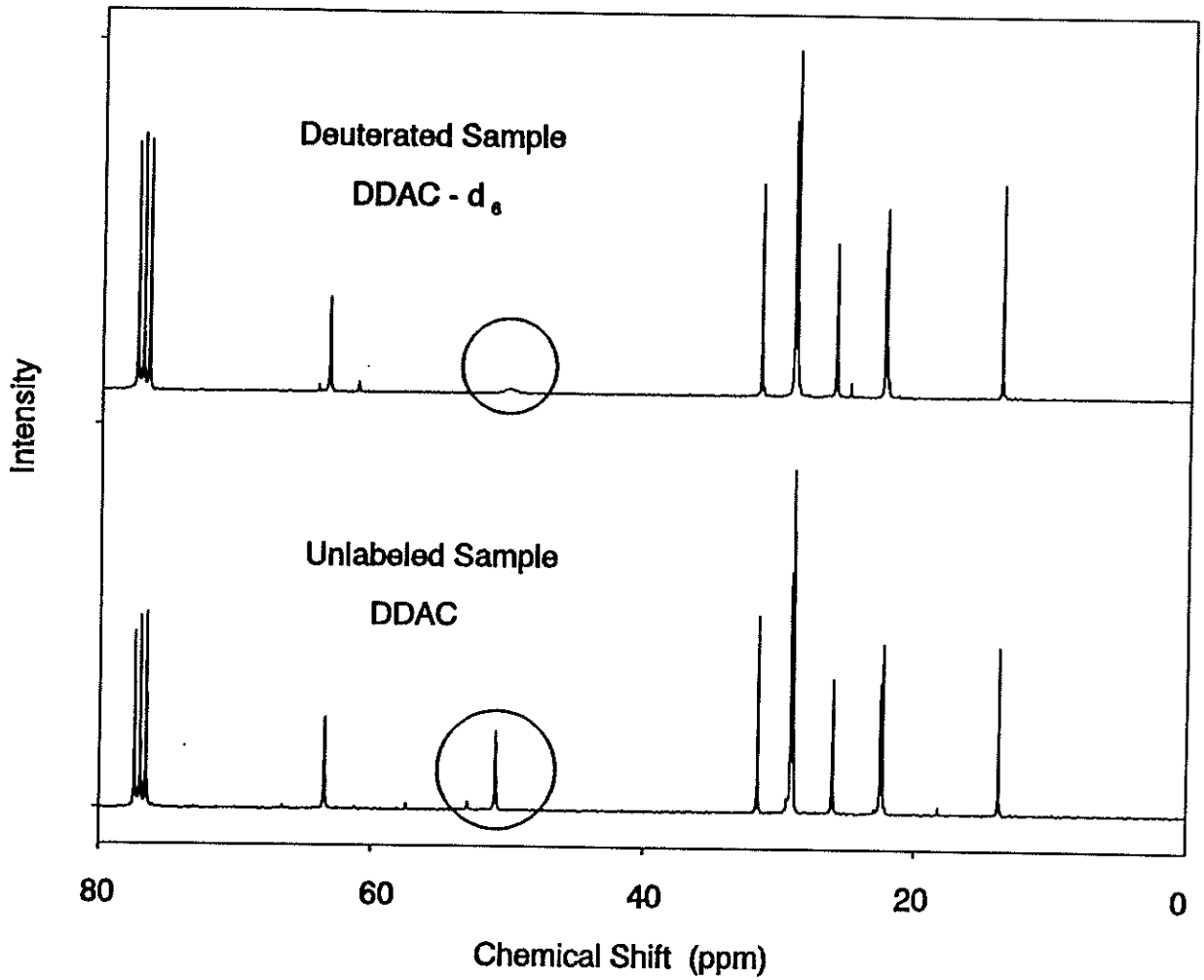

Deuterated Sample
DDAC - d6
Unlabeled Sample
DDAC
Intensity
80
60
40
20
0
Chemical Shift (ppm)

FIGURE 7

Sources of Quaternary Loss During Sampling

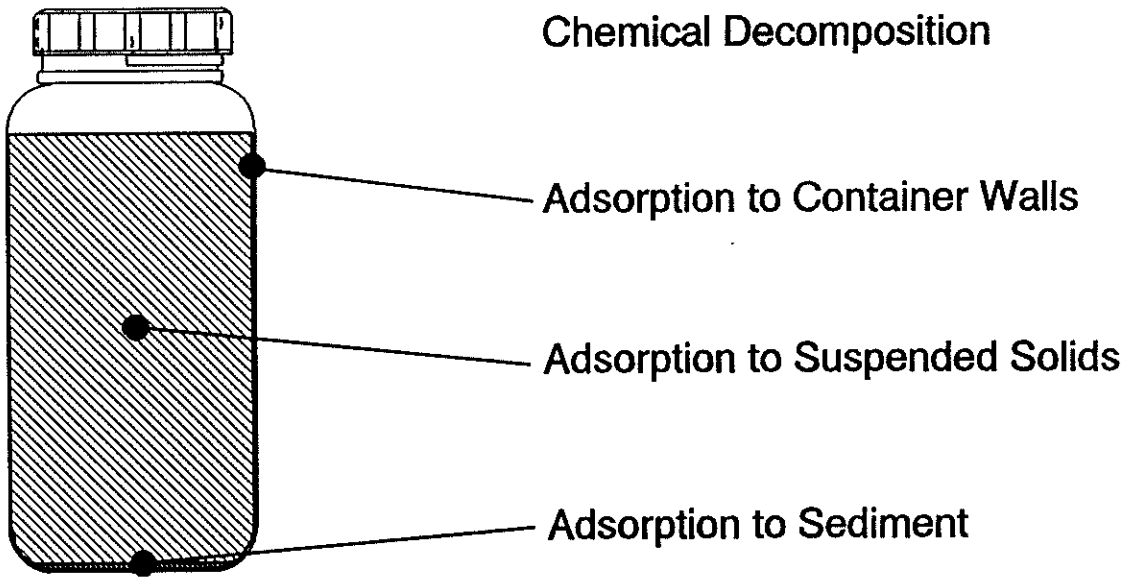


FIGURE 8

Selected Ion Chromatograms of DDAC plus DDAC - d_6

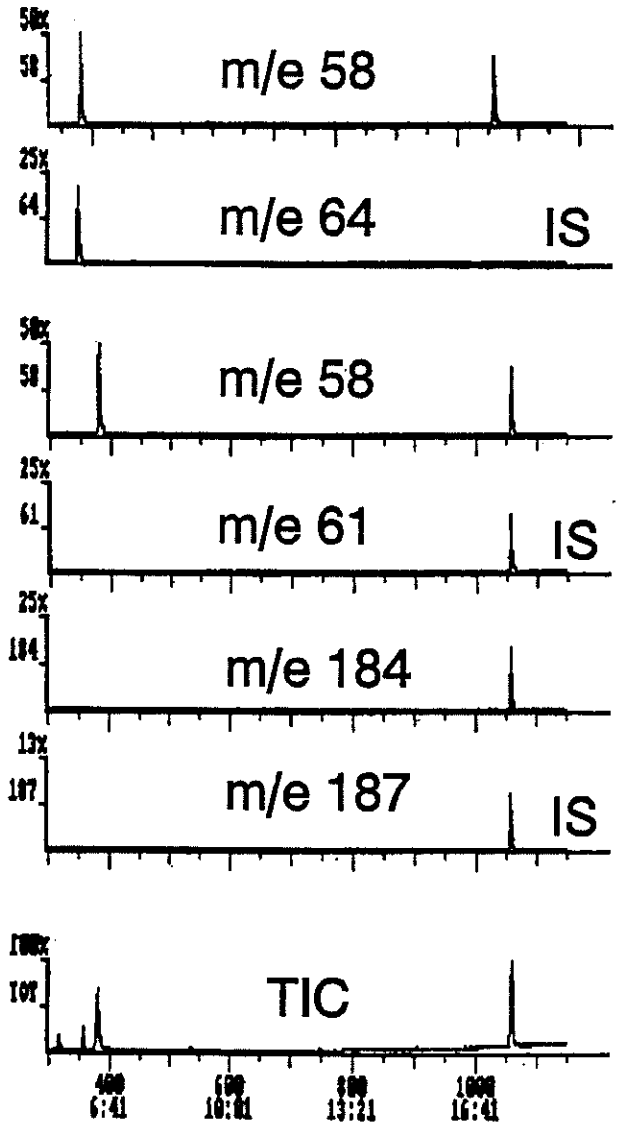


FIGURE 10

Total Ion Chromatogram of Authentic Sample

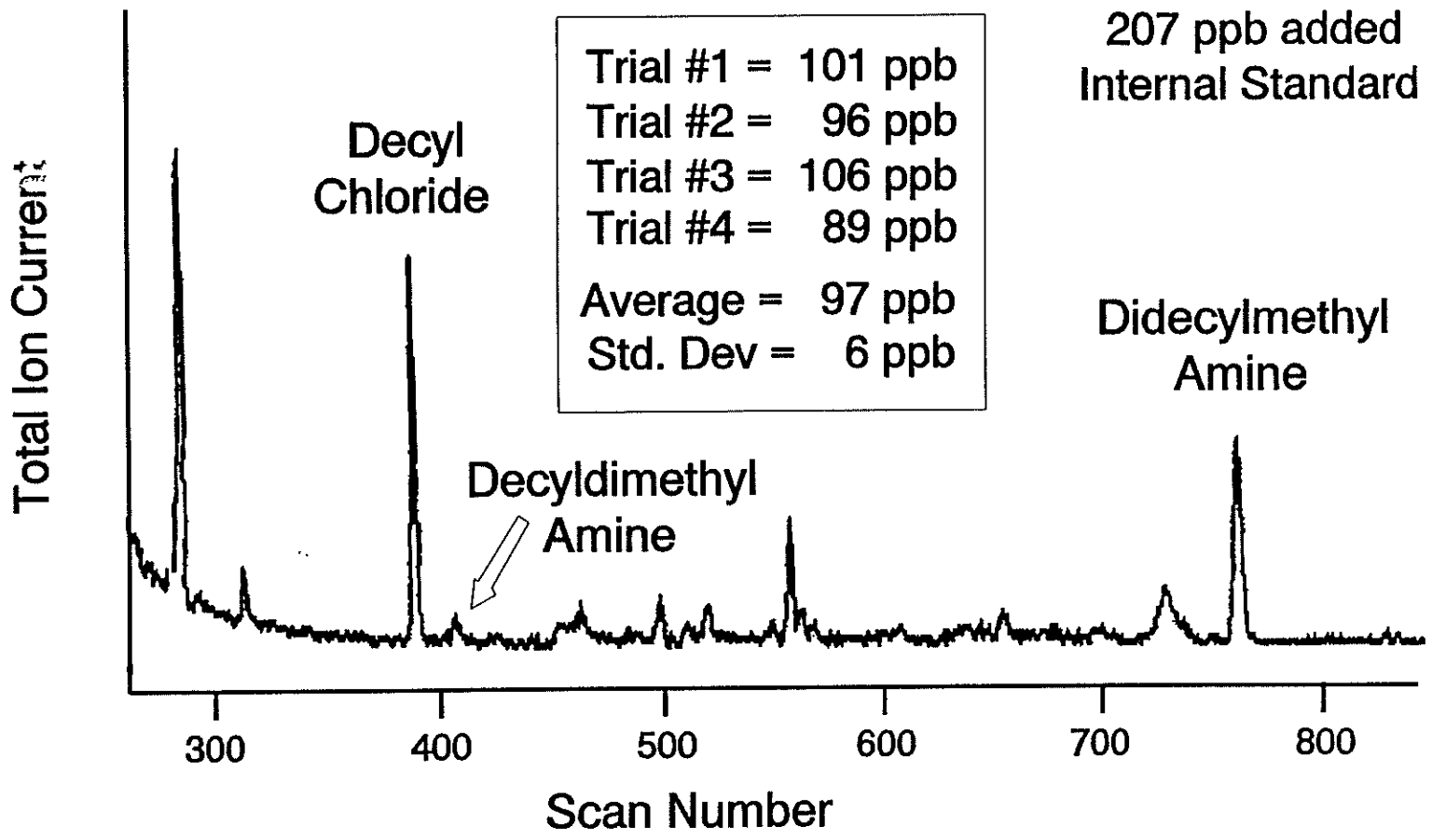


FIGURE 11

GC Pyrolysis and MS Fragmentation of DDAC - d_6

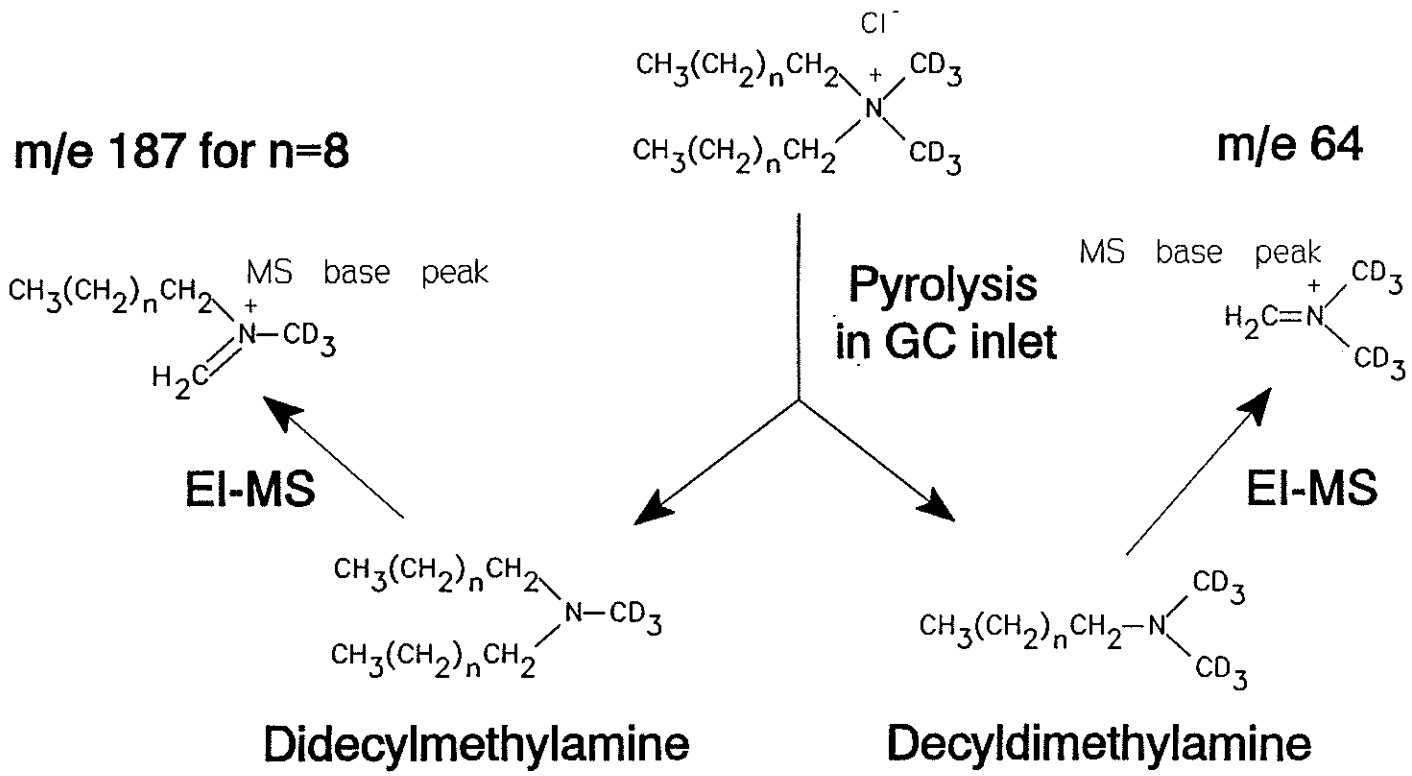


FIGURE 12

EI Mass Spectrum of Decyldimethyl Amine GC Peak

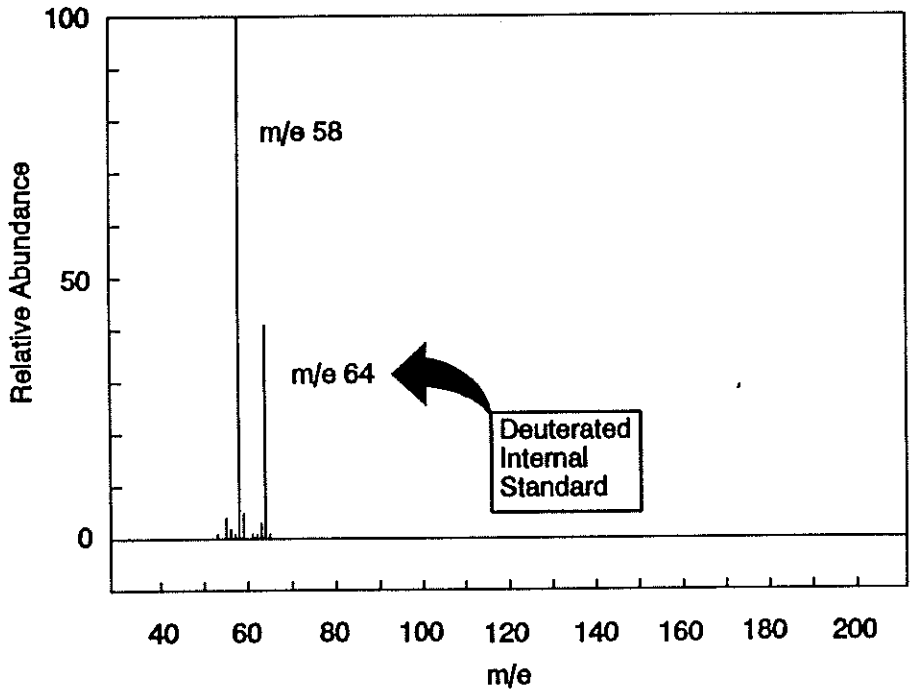


FIGURE 13

EI Mass Spectrum of Didecylmethyl Amine GC Peak

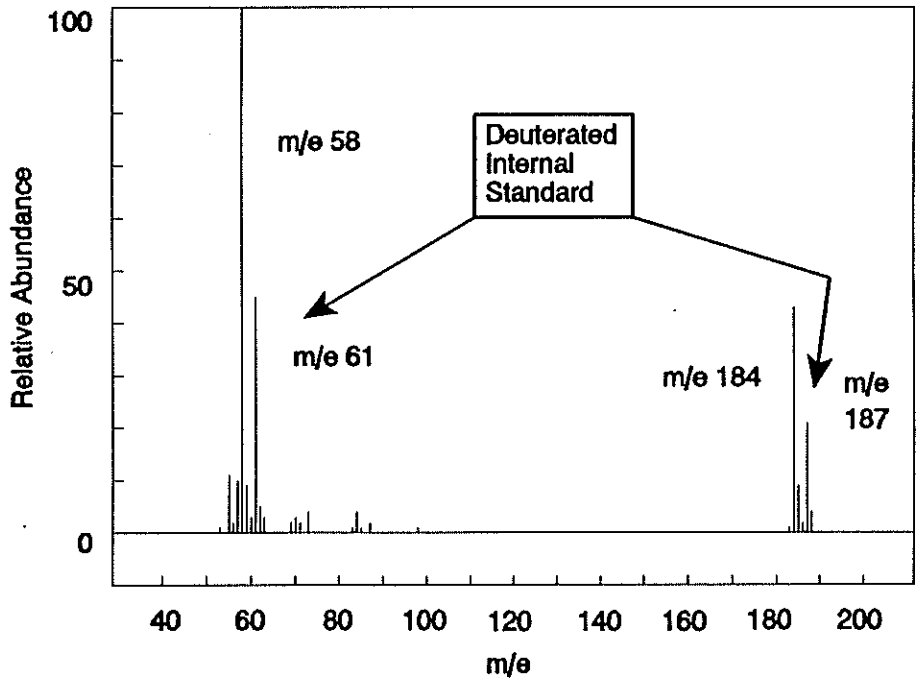


FIGURE 14

GC-ITMS of DDAC Pyrolysis Product

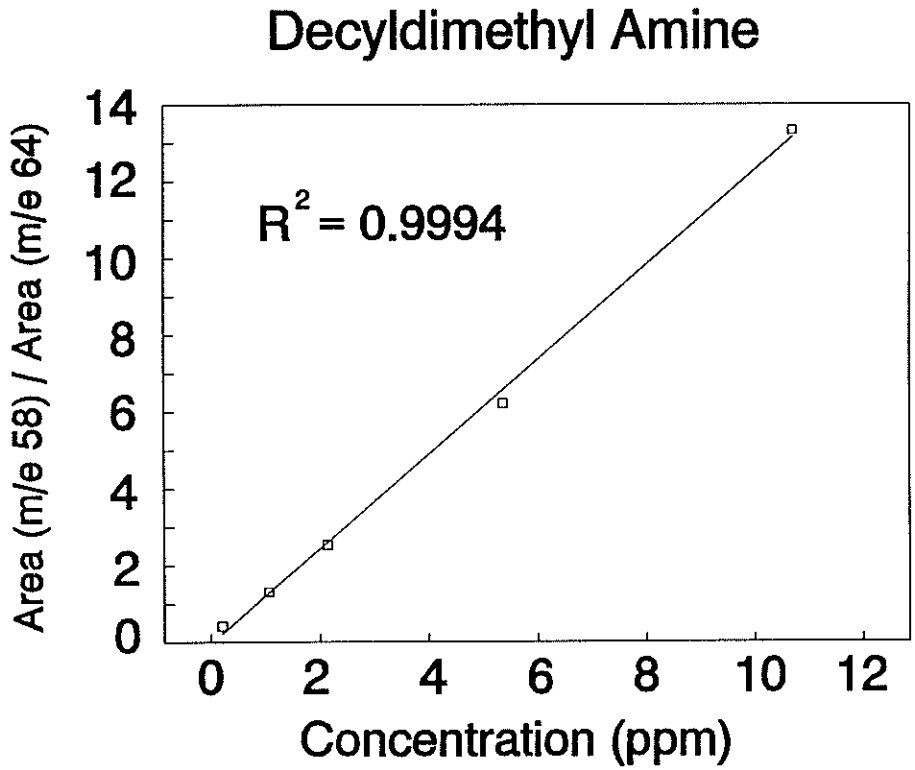


FIGURE 15

GC-ITMS of DDAC Pyrolysis Product

Didecylmethyl Amine

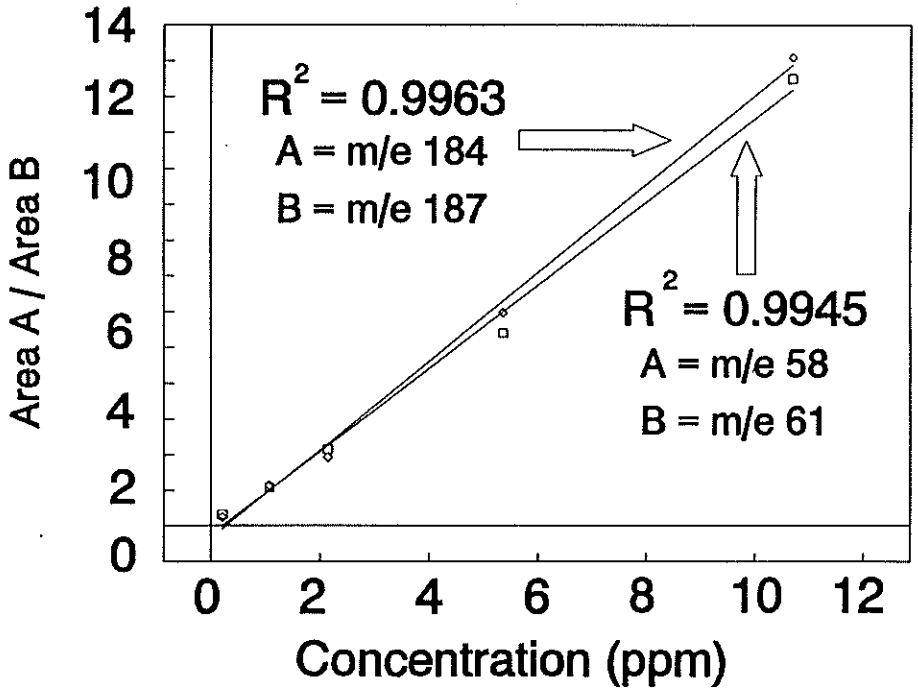


FIGURE 16