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U.S. ARMY CHEMICAL AND BIOLOGICAL DEFENSE COMMAND
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ABERDEEN PROVING GROUND, MARYLAND 21010-5423

REPLY TO
ATTENTION OF

August 11, 1998

Freedom of Information and Privacy Act Office

Mr. John Greenewald, Jr.
[redacted]

Dear Mr. Greenewald:

In response to your June 2, 1998, Freedom of Information Act (FOIA) request, I have enclosed a copy of "Evaluation of Analytical Methods of the Determination of BZ", as you requested.

Fees incurred while processing this request have been waived.

Sincerely,

Cheryl S. Fields

Cheryl S. Fields
Freedom of Information and Privacy Act Officer

Enclosure

Battelle Columbus Laboratories

Report EA 4-1

EVALUATION OF ANLYTICAL METHODS FOR THE DETERMINATION OF BZ

B.A. Petersen
and Others

Contract DAAK40-73-C-0142

December 1977

REPORT EA 4-1

10 FEB 1978

EVALUATION OF ANALYTICAL METHODS FOR THE DETERMINATION OF BZ

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The objectives of this research program were to survey current analytical techniques and develop two methods for the analysis of BZ (an incapacitating agent): a highly sensitive and specific method capable of detecting low levels, and a simpler, less sensitive method which could be used (preferably by less experienced personnel) to screen BZ at higher levels.

A gas chromatography-mass spectrometry (GC-MS) method was developed which is capable of definitively identifying sub-nanogram quantities of BZ

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in environmental media. GC techniques which can easily detect nanogram quantities of BZ in environmental samples were also developed. It appears that the latter GC techniques will be of value when the medium to be analyzed is simple, e.g., washings from metal parts, effluents, and stack samples. However, additional work needs to be performed with actual samples to confirm this.

FOREWORD

This research was supported by the Chemical Systems Laboratories, Aberdeen Proving Ground, Maryland, and was monitored by the U. S. Army Missile Research and Development Command, Redstone Arsenal, Alabama, under Contract No. DAAK-73-C-0142 sponsored by the Defense Advanced Research Projects Agency.

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EVALUATION OF ANALYTICAL METHODS FOR THE DETERMINATION OF BZ

by

B. A. Petersen, R. M. Riggin, K. H. Shafer,
R. E. Wyant, and A. P. Graffeo

INTRODUCTION

The Chemical Systems Laboratory (CSL) has been investigating techniques for the detection/analysis and neutralization of BZ (an incapacitating agent) for the Program Manager, Chemical Demilitarization and Installation Restoration. Regardless of the disposal process used for the demilitarization process, analytical methods for the detection of low levels of BZ must be developed in order to comply with laws regulating occupational safety and health and environmental pollution. CSL requested Battelle's Columbus Laboratories to conduct a study to evaluate analytical techniques for BZ detection to assist them in this program.

We have evaluated a number of analytical methods that might eventually be used to monitor the presence of BZ in the environment. For the sake of completeness, we included spectroscopic, chromatographic, and mass spectrometric techniques. The major thrust was in gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques, but the feasibility of the others was also examined. These included colorimetric and fluorometric analysis. It was also possible, within the time constraints of the study, to conduct investigations of brine extracts and recoveries from glass-fiber filters.

OBJECTIVES

The objectives of this research program were to survey current analytical techniques and develop two methods for the analysis of BZ, a highly-sensitive and specific method capable of detecting low levels, and

a simpler, less-sensitive method which could be used (preferably by less experienced personnel) to screen BZ at higher levels. In this way, when demilitarizing BZ, the fast screening method could be used to quickly measure high levels of BZ from stack samples, brine, effluents, and washings from metal parts. When very low levels of BZ need to be analyzed (i.e., for environmental assessment), the highly-sensitive procedure could be used. Both of these objectives have been accomplished.

- A GC-MS method has been developed which is capable of definitively identifying sub-nanogram quantities of BZ in environmental media. This technique has been evaluated on extracts from brine samples.
- GC techniques have been developed which can easily detect nanogram quantities of BZ in environmental samples. These techniques will be valuable when the medium to be analyzed is simple such as washings from metal parts, and possibly stack samples and effluents. The validity of this statement needs to be checked on real samples. However, when the sample matrix becomes too complex (i.e., extracts of brine), cleanup techniques will have to be developed for this technique or the GC-MS procedure used for analysis.

DISCUSSION

Ultimately, the analysis of BZ will require three separate steps: (1) sample collection, (2) sample preparation, and (3) sample analysis. The sample analysis procedure must be developed first since the collection and preparation of the sample will ultimately depend on the method selected. The goal of the present study was to evaluate current analytical methods for the analysis step, recognizing that the complexity of environmental samples oftentimes dictates the ultimate analysis method and possible cleanup techniques to be used.

The principal effort of the study was directed to the evaluation of GC and GC-MS techniques, however colorimetric and fluorimetric techniques were also investigated. All of the techniques were judged on the basis of three criteria: (1) sensitivity, (2) selectivity, and (3) simplicity.

A discussion of three GC techniques [flame ionization detection (FID), alkali flame detection (AFD), and electron capture detection (ECD)] is presented first. The GC techniques chosen as the simpler, less-sensitive method of choice are discussed next under Quantitative Methods. The chemical ionization (CI) and electron impact (EI) mass spectrometric properties of BZ are then discussed followed by the development of a highly-sensitive and specific quantitative method using GC/CI-MS. Procedures and results are given of the colorimetric and fluorimetric analyses next, and finally, an evaluation of simulated "real" systems is presented.

TECHNICAL PROGRAM

Gas Chromatographic Methods Development

Three GC techniques are described in this section: FID, AFD, and ECD. Laboratory studies indicate that both GC-AFD and GC-ECD can be used to detect nanogram and greater quantities of BZ in environmental samples. These analysis methods appear to be valuable when the medium to be analyzed is simple; e.g., washings from metal parts, effluents, and stack samples; however, studies of actual samples need to be undertaken to confirm this. When the sample matrix is too complex (e.g., extracts of brine), cleanup techniques must be developed prior to the analysis step.

Using GC techniques, results can be quickly obtained, and they are simple enough to be used by less-experienced personnel.

Flame Ionization Detection (FID)

This mode of detection is most commonly used because of the relative simplicity and general applicability. A detection limit of 5-10 ng was obtained for BZ using FID. However, because of the lack of specificity of FID, its successful use in the analysis of BZ in complex mixtures is doubtful.

Detection Limit - 5 ng

Linear Range - 5 ng - 20 µg

Precision - 2 percent at 100 ng level

Interferences - Quinuclidinol, benzilic acid, and benzophenone all elute much earlier than BZ, thus, BZ can be monitored in large excesses of these components by using temperature programming, however, FID is a universal carbon detector and probably will not be specific enough for BZ detection in complex mixtures.

Alkali Flame Detection (AFD)

This detection system was chosen for evaluation because of its high selectivity and sensitivity for nitrogen containing compounds, such as BZ. Evaluation was done using a Hewlett-Packard Model 5740A equipped with dual AFD/FID detectors. Since temperature programming affects the response of the AFD, all GC separations were done isothermally. Figure 1 shows the response for .5 nanograms of BZ using the AFD. The detection limit for BZ using the AFD was ∿.1 nanograms injected. The AFD selectivity was found to be quite good since such expected hydrolysis/pyrolysis products as benzophenone, benzilic acid, and benzohydrol were not detected at the 1 microgram level. However, quinuclidinol (QN) did give a response equal to BZ and this may cause interference problems when present at high levels. Figure 2 shows the response due to QN and BZ in a 20:1 ratio. Tailing of the QN is substantial and, unfortunately, temperature programming, which would aid the QN-BZ separation, cannot be used with AFD (when detecting low levels of material). Despite this drawback, AFD is highly-sensitive to BZ (see below), and, therefore, can be used effectively to monitor nanogram levels of BZ with little difficulty.

Due to variations in the AFD response, it was necessary to use an internal standard in order to quantitate BZ. The internal standard chosen was n-allyl piperidyl benzilate, which is structurally very similar

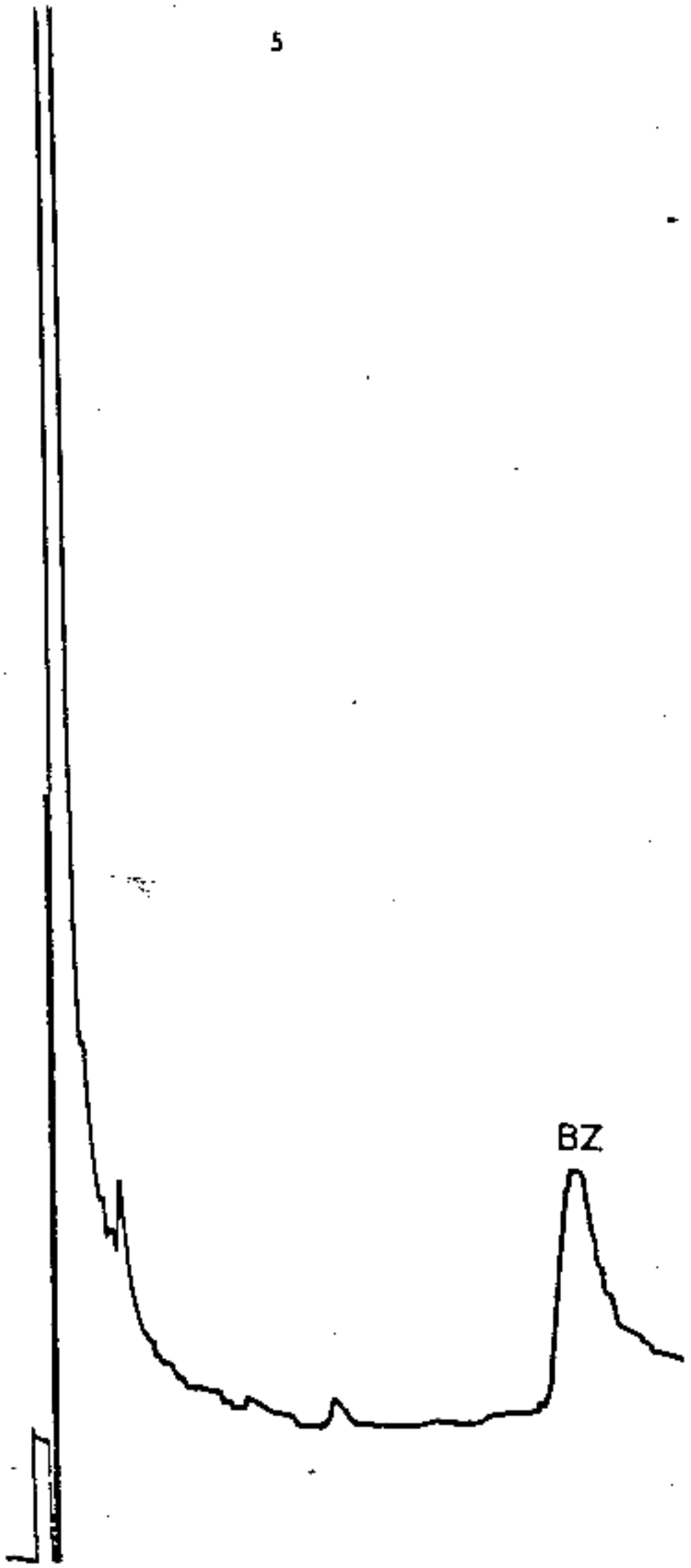

FIGURE 1. RESPONSE FOR BZ ON AFD

Conditions - 3 percent OV-17 on 100/120
Gas Chrom Q 6' x 2 mm
30 ml/min He
Isothermal 240°
Attn x 2.
BZ Retention time 16.2 min
Amt BZ inj - .5 ng

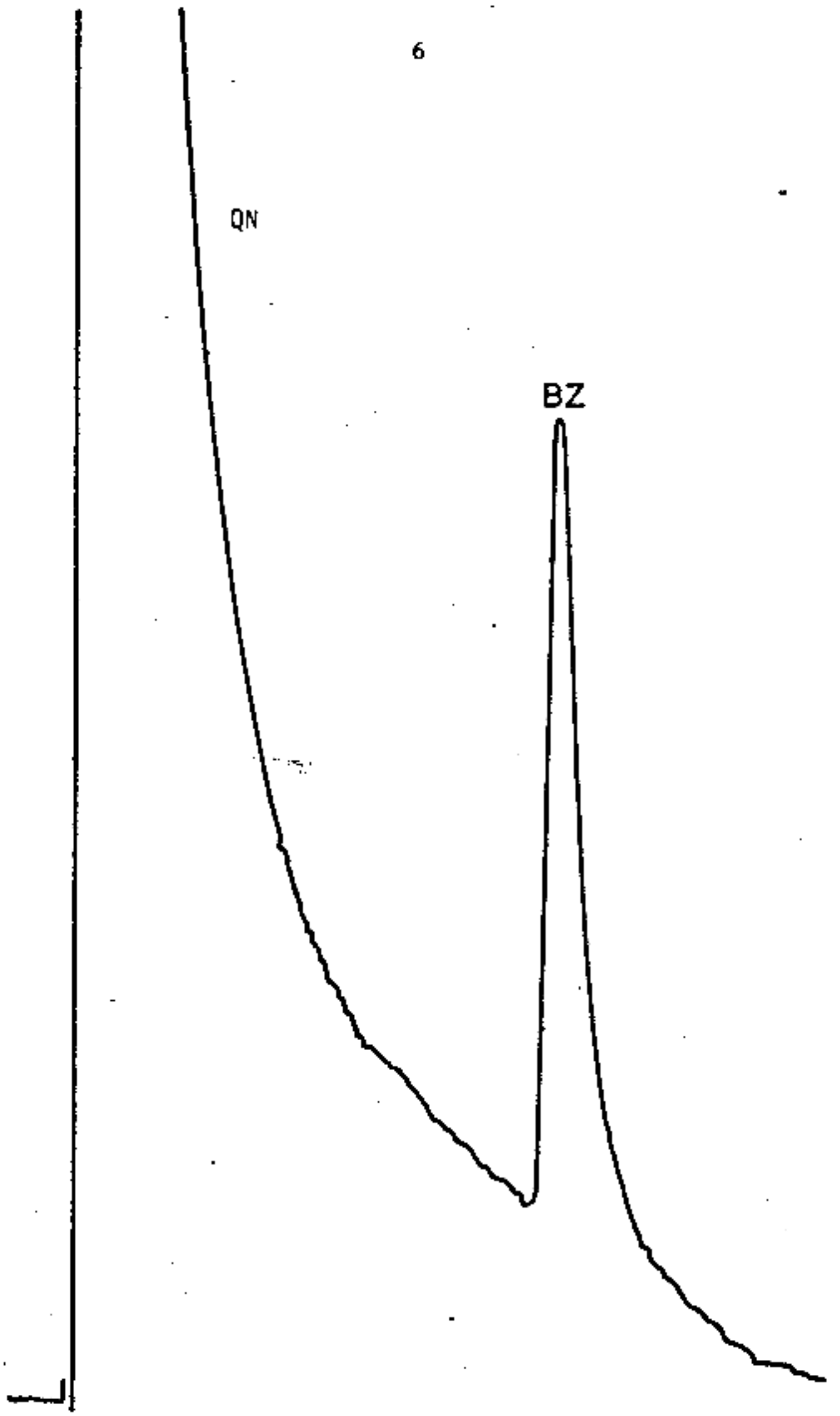

FIGURE 2. RESPONSE FOR 3-QUINUCLIDINOL AND BZ ON AFD

Conditions - see Figure 1
Quantity BZ - 10 ng, QN - 200 ng

to BZ. Using the internal standard (its separation is shown in Figure 3), the AFD precision was found to be 5-10 percent. The response was approximately linear from 1-25 ppm (.2-5 nanograms) as shown in Figure 4.

Detection Limit - .1 ng

Linear Range - .1 ng - 10 ng

Precision - 5.4 percent at 20 ng level

~10 - 15 percent at .5 ng level

Interferences - Quinuclidinol (QN) responds equally well as BZ but elutes more quickly. Unfortunately, temperature programming cannot be used very effectively with AFD, so QN tailing will limit the detection of BZ when present in large excess. However, the high sensitivity of AFD for BZ will easily allow its detection at nanogram levels or greater.

Electron Capture Detection (ECD)

The electron capture detector is a relatively specific and sensitive detector which is very useful for analyzing compounds which contain electron withdrawing groups (e.g., halogen, nitro, etc.). BZ is somewhat responsive to the EC detector and, thus, the properties of this detector towards BZ were evaluated.

Figure 5 shows the response for 50 nanograms of BZ using a Hewlett-Packard 5730 equipped with a ^{63}Ni electron capture detector. The detection limit for BZ was found to be 2-5 ng. Benzilic acid gives a response but benzophenone and QN do not. The benzilic acid has been shown to decarboxylate to benzophenone in the GC so its response is rather puzzling (perhaps it is due to a product arising from an alternate pyrolytic path).

In order to enhance the responsiveness of BZ using the ECD, a fluorinated derivative was prepared. Two hundred µg of BZ dissolved in

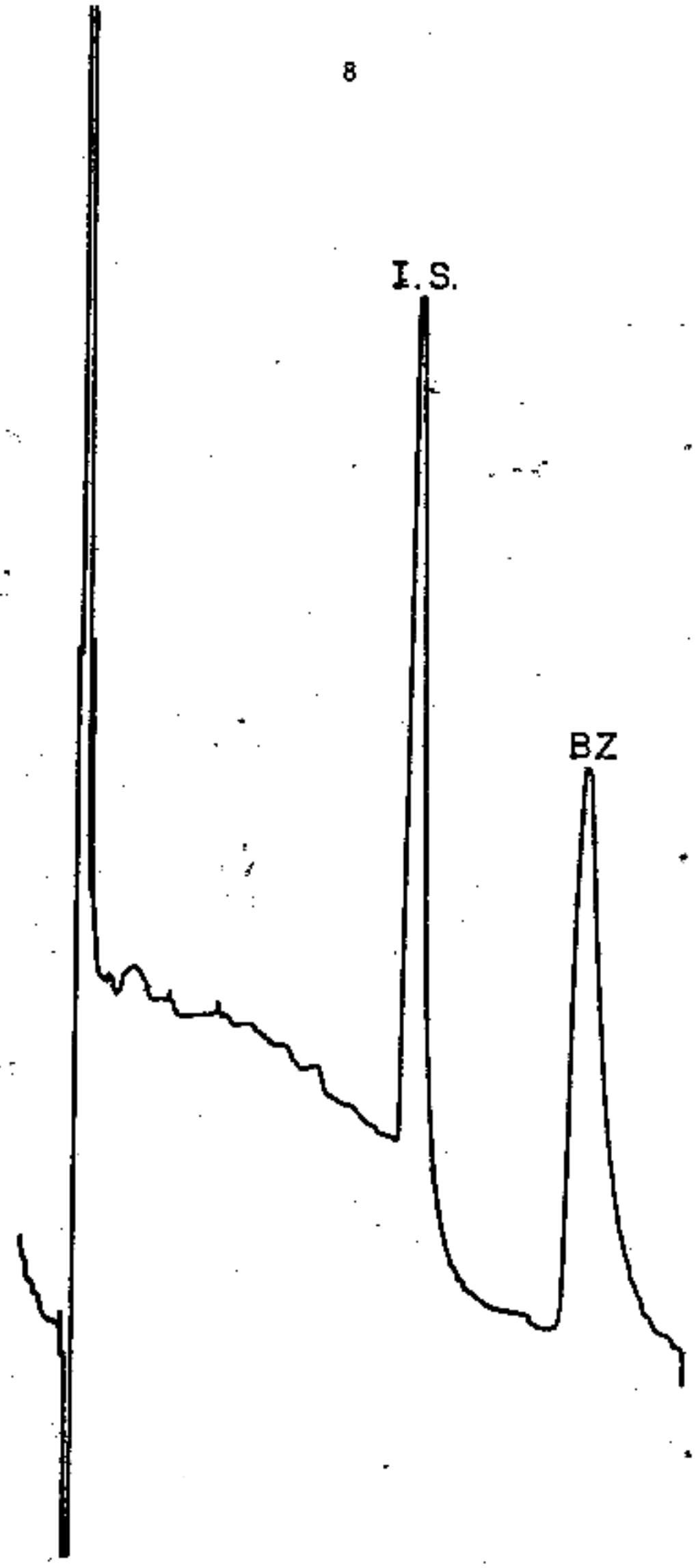

FIGURE 3. RESPONSE FOR BZ PLUS INTERNAL STANDARD USING AFD

(N-allyl-3-piperidyl benzilate)
Conditions - see Figure 1
Quantity - BZ - 5 ng, I.S. - 5 ng
Retention times - BZ - 16.2 min
I.S. - 11.7 min

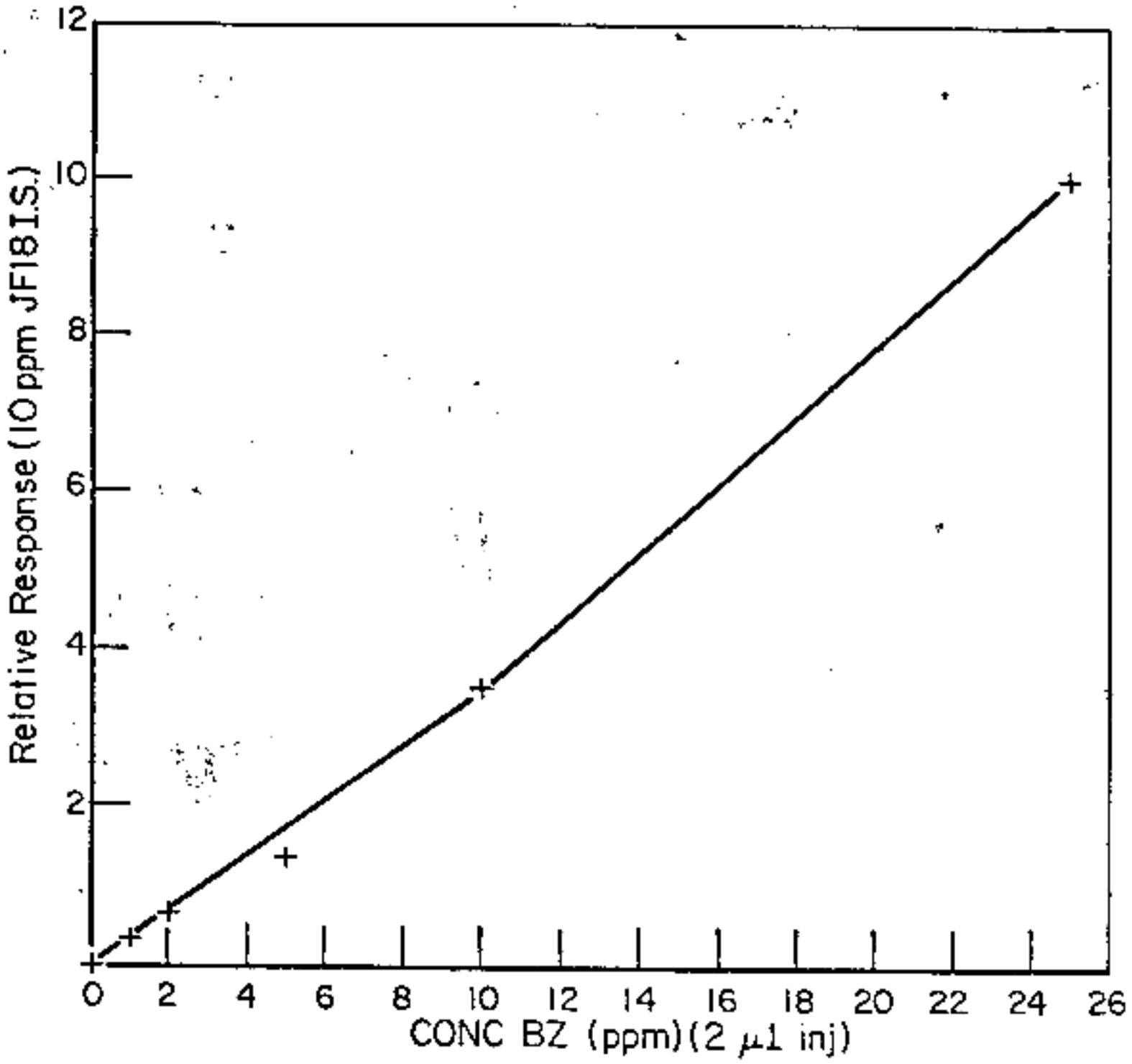

FIGURE 4. LINEARITY CURVE FOR BZ USING AFD

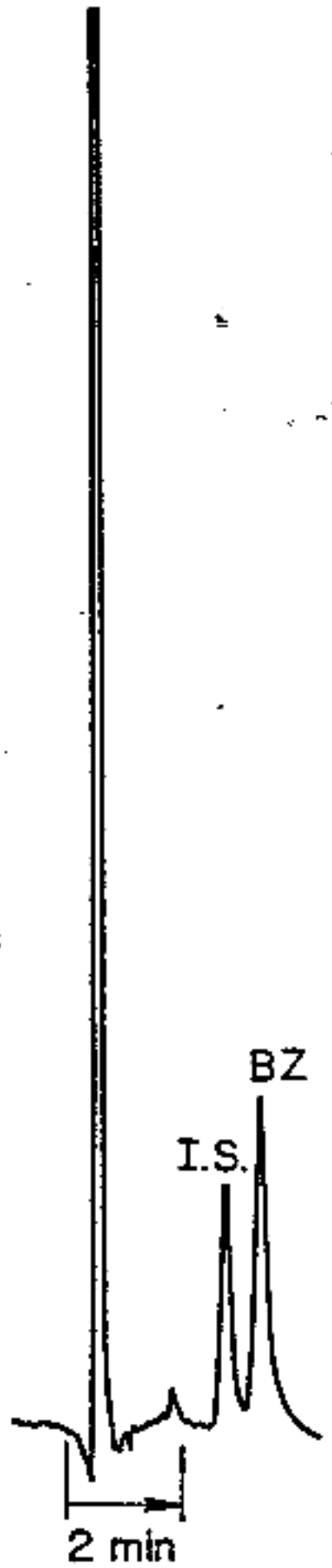

FIGURE 5. RESPONSE FOR BZ AND I.S. ON GC/ECD

Conditions - 3 percent OV-1 6' x 2 mm I.D., 30 ml/min He
Amounts - BZ - 50 ng, I.S. - 50 ng
Attn: x32
Isothermal - 250°

500 µl of benzene was placed in a 12 ml centrifuge tube. Ten µl of pentafluoroproprionic anhydride and 20 µl of 5 percent triethylamine in benzene was added and the mixture heated at 50 C for 15 min. The benzene was extracted with 2 ml of water and then 2 ml of 5 percent ammonia. The benzene layer was placed in a septum capped vial for analysis.

The separation of the BZ-PFPA derivative from BZ is shown in Figure 6. The BZ-PFPA derivative was found to give a response fifty times that of the underivatized BZ. The results of this experiment indicate that this particular derivatization procedure is effective in providing enhanced sensitivity for BZ. Therefore, ECD is a highly-selective and quite sensitive technique which can be used effectively for BZ analysis.

Free BZ

Detection Limit - 2 - 5 ng
Linear Range - 10 ng - 250 ng (at least)
Precision - 3 percent at 50 ng level
Interferences - Benzilic acid responds but benzophenone and 3-quinuclidinol do not. Since temperature programming can be used with the ECD (with some loss in sensitivity), separation of benzilic acid from BZ is no problem even when the benzilic acid is in large excess.

PFPA Derivatized BZ

Detection Limit - .1 ng
Other parameters not investigated.

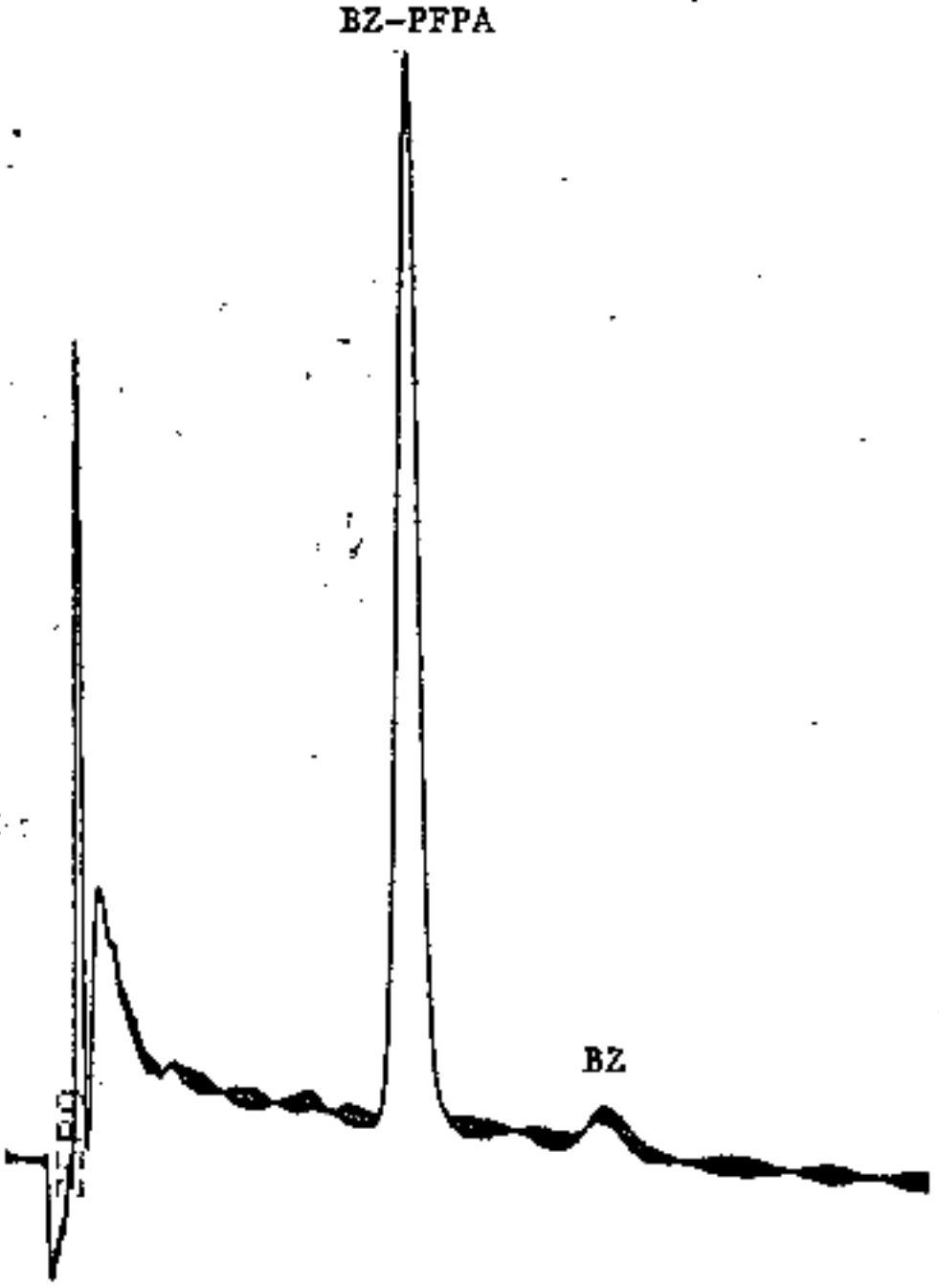

FIGURE 6. SEPARATION OF BZ-PFPA AND BZ USING THE ECD
Conditions:
Column - 5 percent OV-101 on Gas Chrom Q
80-100 mesh, 4 ft x 2 mm I.D.
Flow - 20 ml/min 10 percent CH_4/Ar
Col. Temp - 280 C Attn - X1024
Inj. Temp - 280 C Amt. Injected - 2 ng each
Det. Temp - 300 C

Quantitative Analysis of BZ
by Gas Chromatography

As mentioned earlier, quantification of BZ is strongly dependent on the matrix in which it is contained. Therefore, our evaluation of GC techniques for analysis is only one step in the method development process. There remains two important experiments to be done, the evaluation of possible interferences in the analysis method from a wide variety of real samples, and the development of cleanup procedures which can effectively remove these interferences without jeopardizing the quantification of BZ.

Based on our laboratory studies, both GC-AFD and GC-ECD can be effectively used to monitor low levels of BZ. The advantages of GC-AFD are two: (1) a lower limit of detection than GC-ECD, and (2) selectivity for nitrogen compounds only. Two main disadvantages are: (1) the unability of temperature program, and (2) the unstability of AFD as compared to ECD detection. The advantages of GC-ECD are: (1) selectivity to electron capturing compounds only, (2) the stability and ruggedness of ECD detection, and (3) the familiarity of GC-ECD in most analytical laboratories.

A key element in choosing one of these two methods will be their selectivity to the real samples analyzed. GC-AFD was used to look at an extract of brine sample and significant interferences from nitrogen containing compounds were present (see Evaluation of Simulated "Real Systems section). Due to the time limitations of the study, we were unable to run the extract by GC-ECD.

Although both of these methods can be effectively used for quantifying BZ, a few additional experiments along with the needs of the Chemical Systems Laboratory will ultimately decide the choice.

GC-MS Methods Development

Gas chromatographic-mass spectrometric (GC-MS) analysis has been performed on BZ and its possible degradation products; benzilic acid, benzophenone, benzhydrol and 3-quinuclidinol. The objective of this study was to:

- examine the GC-MS characteristics of BZ and its degradation products,
- develop a sensitive and specific analysis for BZ, using GC/CIMS, and
- determine the lower limit of detection of BZ.

As a result of these studies, a highly-sensitive and specific analysis method has been developed using GC/CIMS with selected ion monitoring. Using this method, definitive identification of BZ can be accomplished on every sample at the ppb and lower levels. Also, little interferences are expected from even complex environmental matrices (see the Evaluation of Simulated "Real" Systems section). Although experienced personnel are needed for the required analysis, this method unquestionably represents the best method available for BZ analysis of complex samples.

Electron Impact Studies

The electron impact (EI) mass spectra of BZ, shown in Figure 7, exhibited an easily recognizable molecular ion peak (m/e 337, $[M^{+\cdot}]$) of very low relative intensity. Fragment ions are formed by retention of the charge with either the benzilic or quinuclidinyl moieties. The presence of the benzilate function can be determined by the characteristic series of ions at mass-to-charge ratios (m/e) 183, 165, 105, 77. Preliminary screening for the benzilate system can be best accomplished by selectively monitoring the m/e 183 ion in this mode, in view of its high relative intensity. Unfortunately, this ion appears in the EI mass spectra of various degradation products of BZ, such as benzophenone. Cleavage of the

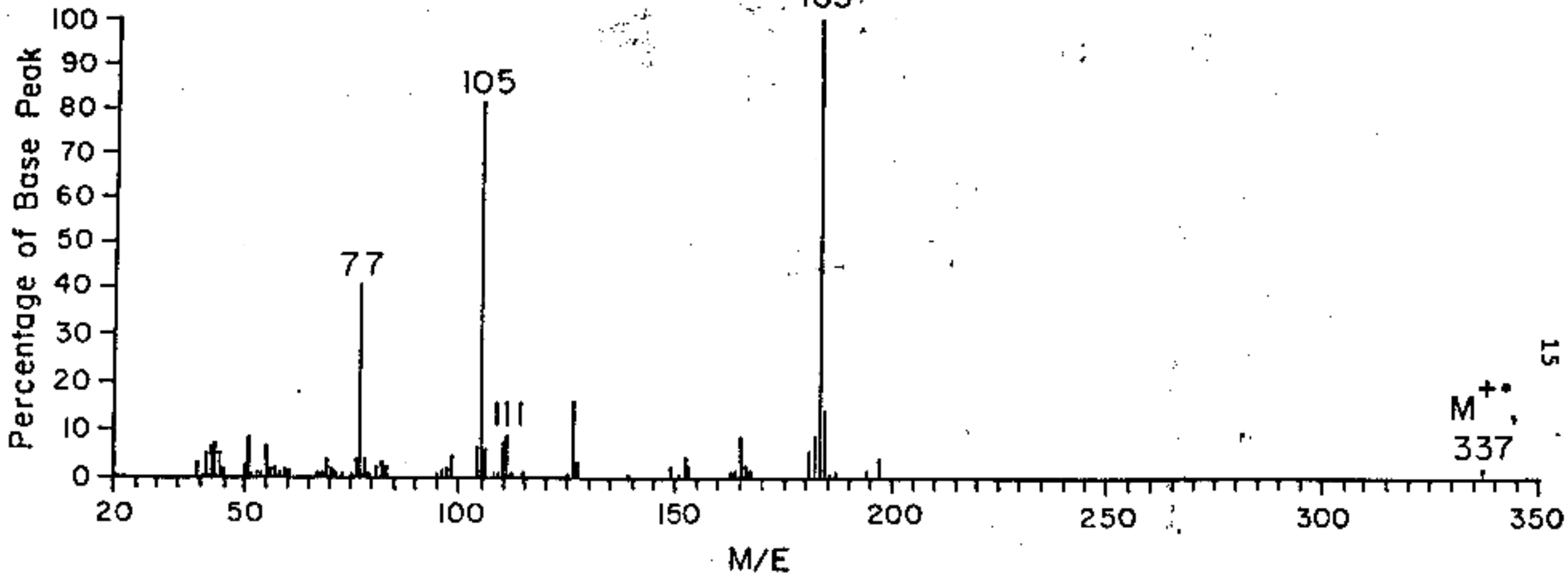

FIGURE 7. ELECTRON IMPACT (70 eV) MASS SPECTRUM OF BZ

quinuclidinyl ester oxygen bond and retention of the charge with the quinuclidinyl function results in the formation of a relatively abundant ion appearing at m/e 111. GC-MS using electron impact ionization is not suitable for trace analysis of BZ due to the low molecular ion intensity. In addition, monitoring the prominent m/e 183 ion peak is not suitable for BZ analysis since this ion is present in pyrolysis/hydrolysis products of BZ.

Chemical Ionization Studies

Chemical ionization (CI) mass spectrometry is a technique in which a sample is introduced into the ion source with an excess of reagent gas (i.e., 10^3:1) at a pressure of ~1 torr. High energy electron bombardment of the mixture results in initial ionization of the reagent gas molecules followed by a series of ion-molecule reactions to produce a variety of reagent gas ions. Ionization of the sample molecules is usually accomplished by proton transfer upon reaction with the reagent gas ions. As the method of ion formation in CI differs from that of other mass spectrometric techniques, the resultant spectra are unique to CI and provide additional information concerning the compounds in question. CI mass spectra of many organic compounds are almost exclusively dominated by formation of a molecular adduct ion $(M+H)^+$.

Chemical ionization mass spectra of BZ have been obtained using methane, isobutane, and ammonia as the reagent gas. We have found that a methane/ammonia (CH_4/NH_3) reagent gas mixture produces a CI mass spectrum of BZ with the highest molecular adduct ion intensity [$(M+H)^+$, m/e 338]. The CI mass spectra of BZ using this reagent gas mixture is shown in Figure 8. In this spectrum, the molecular ion species is the predominant ion peak representing about 25 percent of the total ionization of the sample. The electron impact mass spectrum of BZ (Figure 7) yielded a molecular ion peak much less than 1 percent of total ionization. The specificity and sensitivity of GC-MS using chemical ionization can be used to identify and quantify BZ

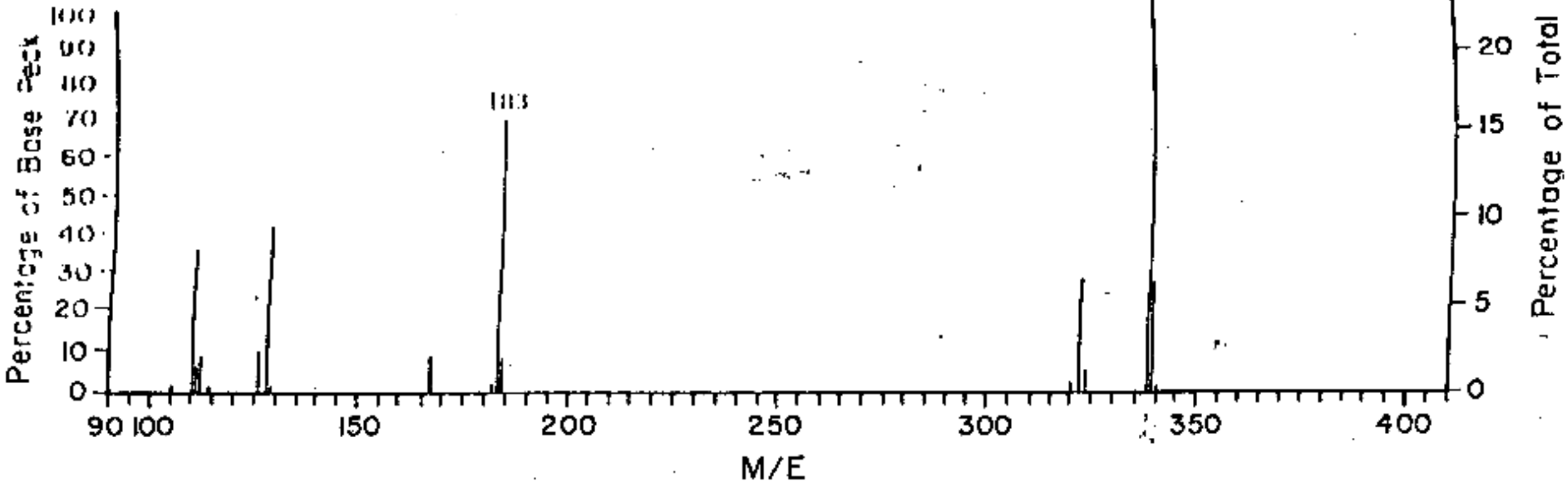

FIGURE 8. CH_4/NH_3 CHEMICAL IONIZATION MASS SPECTRUM OF BZ

by monitoring the m/e 338 ion. This technique is known as selective ion monitoring (SIM) and should allow trace analysis of BZ without interferences from pyrolysis/hydrolysis products as these compounds give ion peaks at m/e values below 200.

The GC/CI-MS properties of the possible degradation products were examined in order to determine if the presence of these compounds would interfer with the analysis of BZ. A mixture containing 3-quinuclidinol, benzhydrol, benzophenone, and BZ (100 ppm each) was injected into the GC-MS. As demonstrated in Figure 9, BZ is well separated from the other compounds in the mixture. The CI mass spectra of the three possible degradation products are almost dominated exclusively by a single ion. Quinuclidinol (MW 127) and benzophenone (MW 182) yield molecular adduct ion peaks at m/e 128 and 183, respectively, and their spectra are shown in Figures 10 and 11. Benzhydrol (MW 184) does not give a molecular adduct ion peak at m/e 185, but an ion peak corresponding to the dehydrated adduct ion $(M+H - H_2O)^+$ to give the ion at m/e 167 (Figure 12). Although benzhydrol and benzophenone coelute under the conditions used in Figure 3, the compounds can be separated using their ion current profiles. Benzilic acid (MW 228) is not expected to elute from the GC column as the free acid, and decomposes to benzophenone in the GC injector. Since BZ can be separated from these possible impurities, and its molecular adduct ion appears at a high m/e value (m/e 338), interference in either detection or quantification is not expected.

Quantitative Analysis of BZ by GC/CIMS

Quantitative analysis of BZ can best be performed by introducing an internal standard to the sample before any pretreatment or cleanup. For high accuracy and precision, the internal standard for BZ should possess similar:

(1) chemical and physical properties,

(2) gas chromatographic retention, and

(3) mass spectrometric properties.

A stable isotope derivative of BZ, such as a deuterium labeled analog would fulfill all of these requirements. Furthermore, a labeled

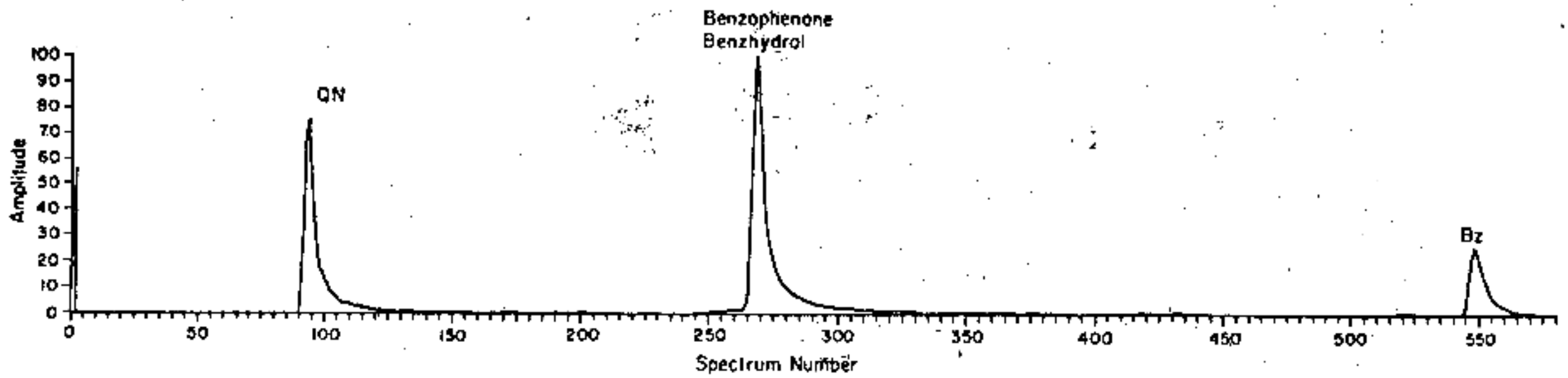

FIGURE 9. GC/CIMS SEPARATION OF QUINUCLIDINOL, BENZHYDROL, BENZILIC ACID, BENZOPHENONE, AND BZ

Column - 6' x 2mm I.D. Glass 3% OV-1 on Supelcoport 80/100 mesh

Carrier - CH_4 12 ml/min
Temp - 100°-280° @ 10°/min

Ionization voltage - 135 eV
NH_3 pressure - 300 microns
CH_4 pressure - 700 microns

Source temp - 200°
Transfer temp - 280°

Scan range - 100-400 AMU
Integration time - 5 mSEC/AMU

Emission current - 1 mA

Electron multiplier voltage - 2200
Electron multiplier gain - 1×10^4

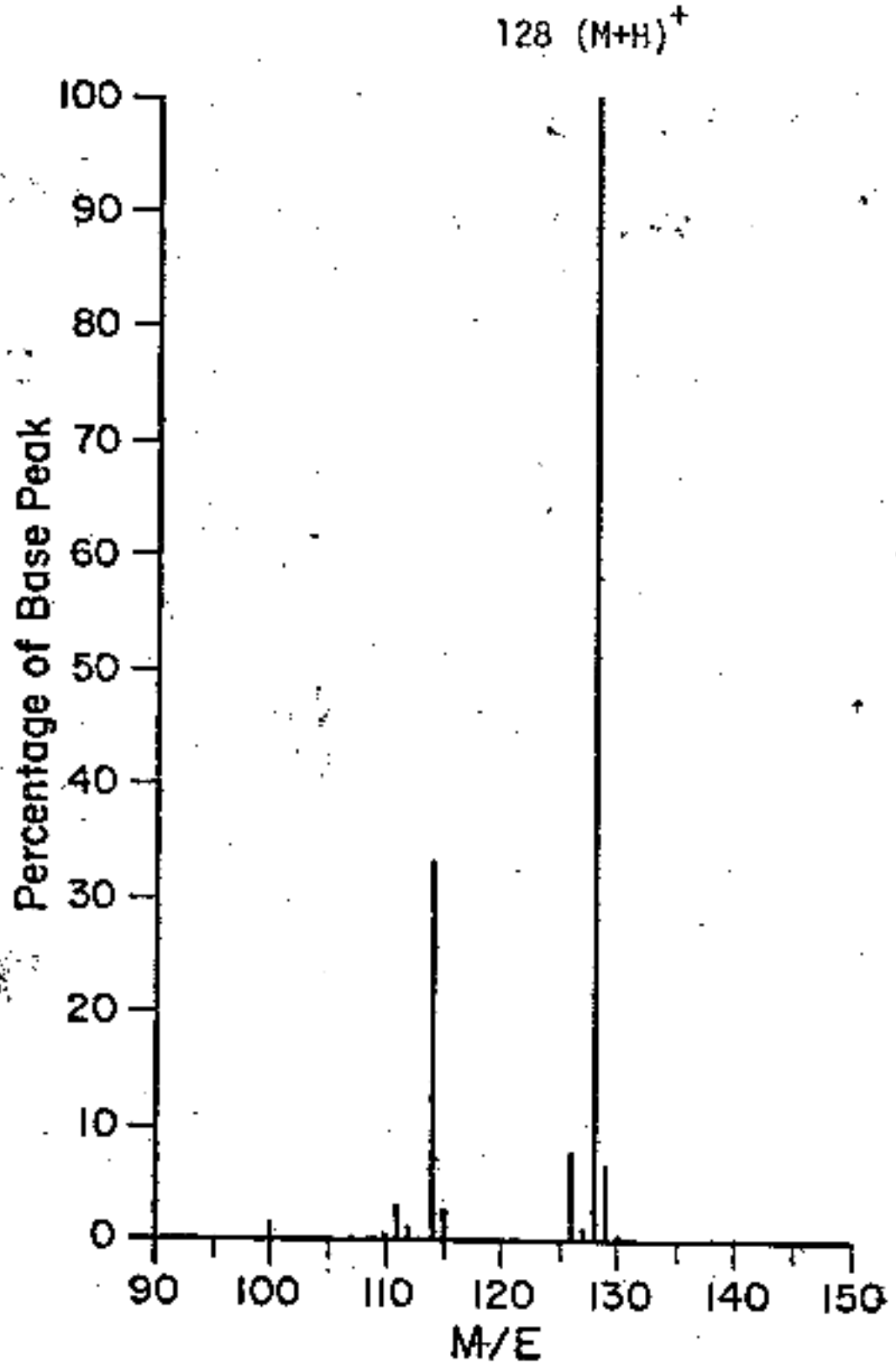

FIGURE 10. CH_4/NH_3 CHEMICAL IONIZATION MASS SPECTRUM OF QUINUCLIDINOL (MW 127)

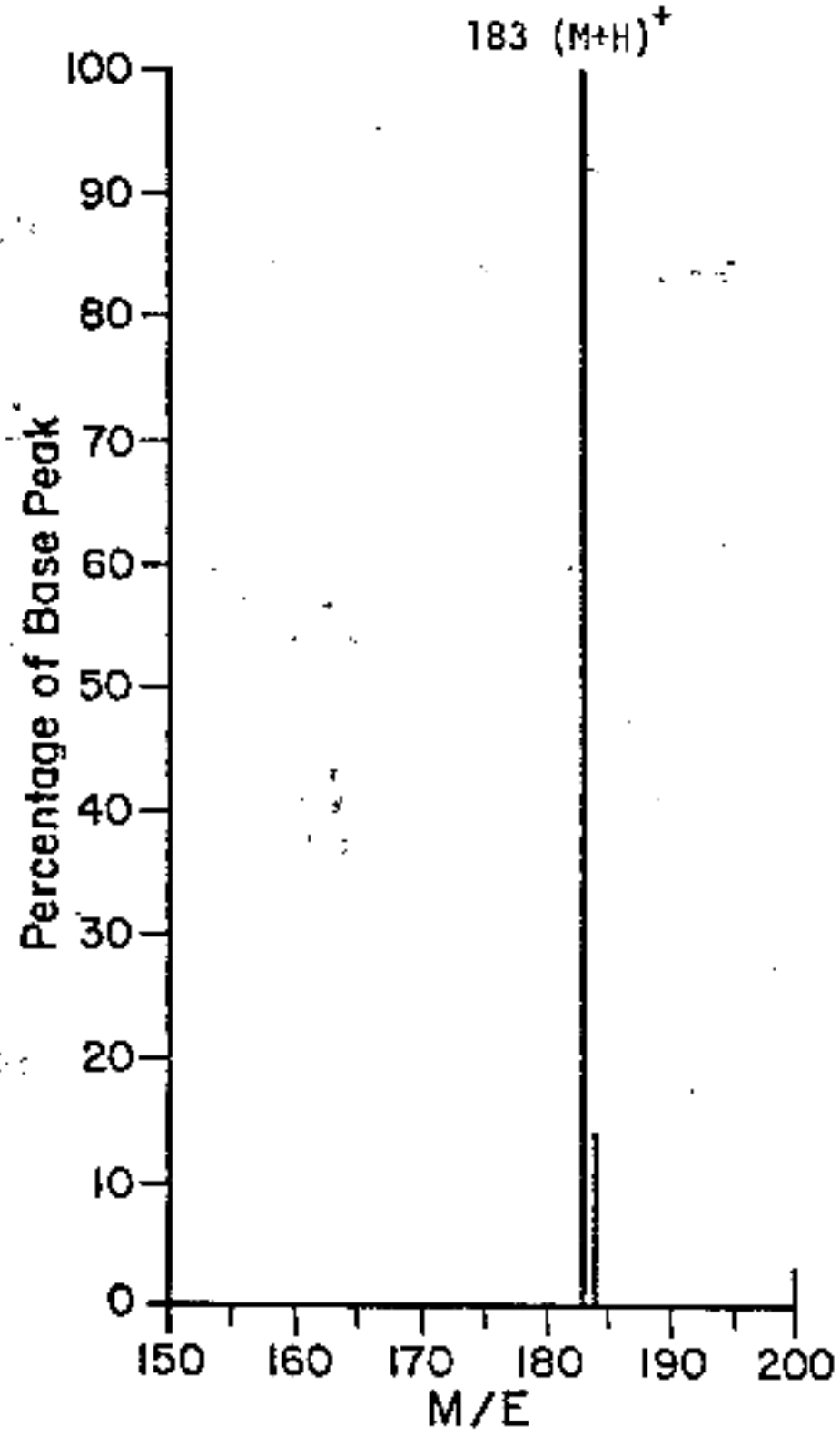

FIGURE 11. CH_4/NH_3 CHEMICAL IONIZATION MASS SPECTRUM OF BENZOPHENONE (MW 182).

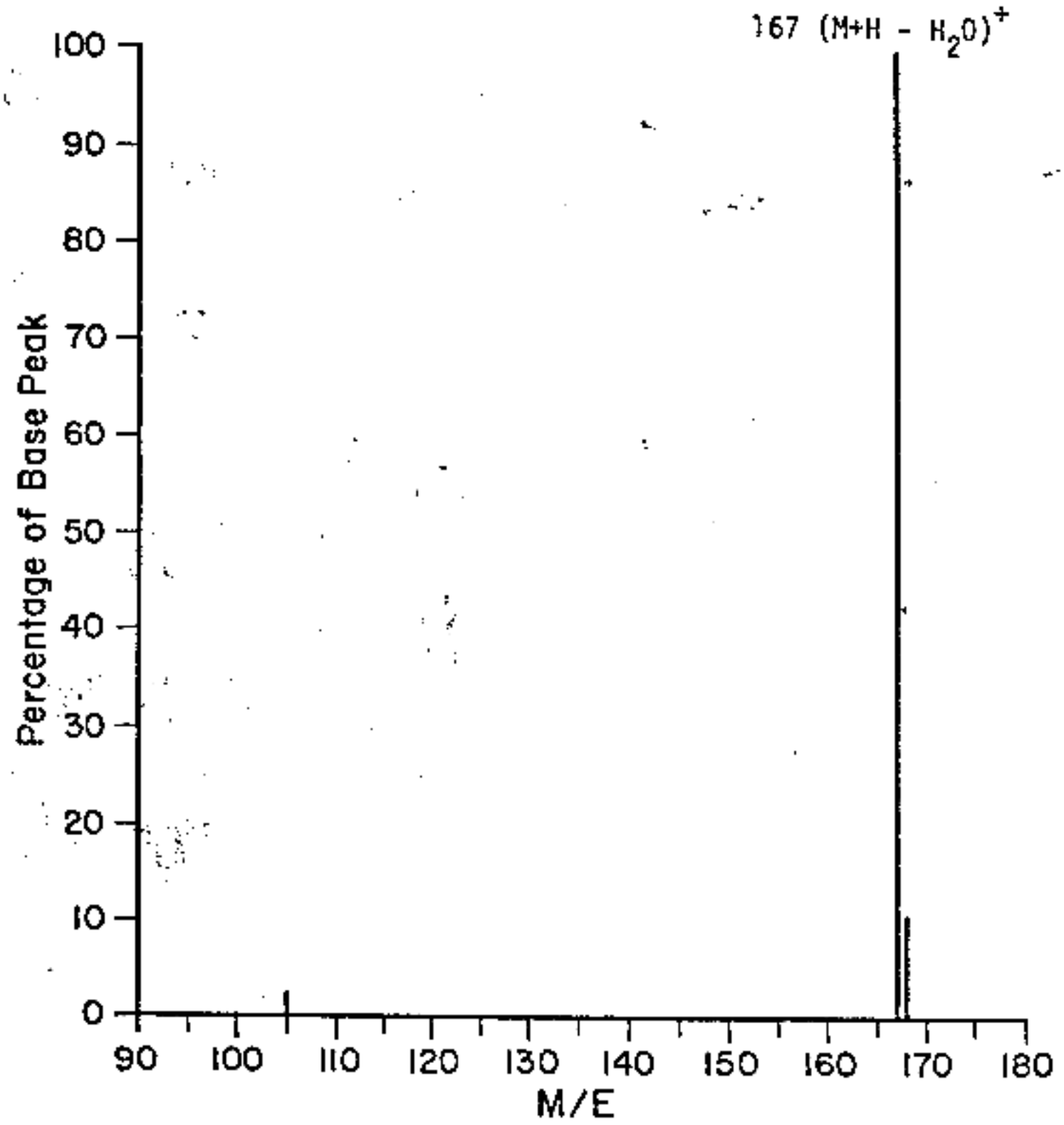

FIGURE 12. CH_4/NH_3 CHEMICAL IONIZATION MASS SPECTRUM OF BENZHYDROL (MW 184)

internal standard would coelute with BZ and act as a carrier. This carrier effect would also minimize loss of BZ due to adsorption. Unfortunately, we have experienced difficulty in obtaining commercially available deuterium labeled compounds for the purpose of synthesizing labeled BZ, and as a result, we have had to use another type of internal standard.

The chromatographic and mass spectrometric properties of BZ are very similar to a related class of compounds; piperidyl benzilates. Shown in Figure 13 is a GC/CI-MS chromatogram of a mixture containing 100 ng each of BZ and two piperidyl benzilates, namely N-ethyl-3-piperidyl diphenyl acetate (JB-305), and N-allyl-3-piperidyl benzilate (JF-18). In order to determine if JF-18 can be used as an internal standard, a series of 1 ml benzene solutions containing 1, 5, 10, 20, 50, and 100 ng of BZ and 20 ng of JF-18 were prepared. These solutions were evaporated to approximately 100 µl under a stream of N_2 at 50 C, 5 µl of each injected into the GC-MS, and the molecular ion adduct peaks of JF-18 (m/e 352) and BZ (m/e 338) were simultaneously monitored. For this analysis, these samples were injected into the GC-MS at 250 C and the temperature increased to 280 C at 10 C/min. The analysis time for BZ using this method is 3 min. A standard curve for these data was obtained and is shown in Figure 14. Although the linear dynamic range for this method was only 10^2, there should not be any difficulty in extending the range to 10^3 or 10^4, if needed. The correlation coefficient for these data was 0.95 indicating that JF-18 can be used as an internal standard. However, higher precision and accuracy can be obtained using a deuterium labeled analog of BZ as an internal standard. Using this method, the minimum detectable quantity of BZ was 1 part per billion (ppb) at a signal-to-noise ratio of ~10:1, and this selective ion monitoring analysis is shown in Figure 15.

For the highly-sensitive, rapid, and specific analysis of BZ in environmental samples, GC-MS is the best choice of the analytical methods.

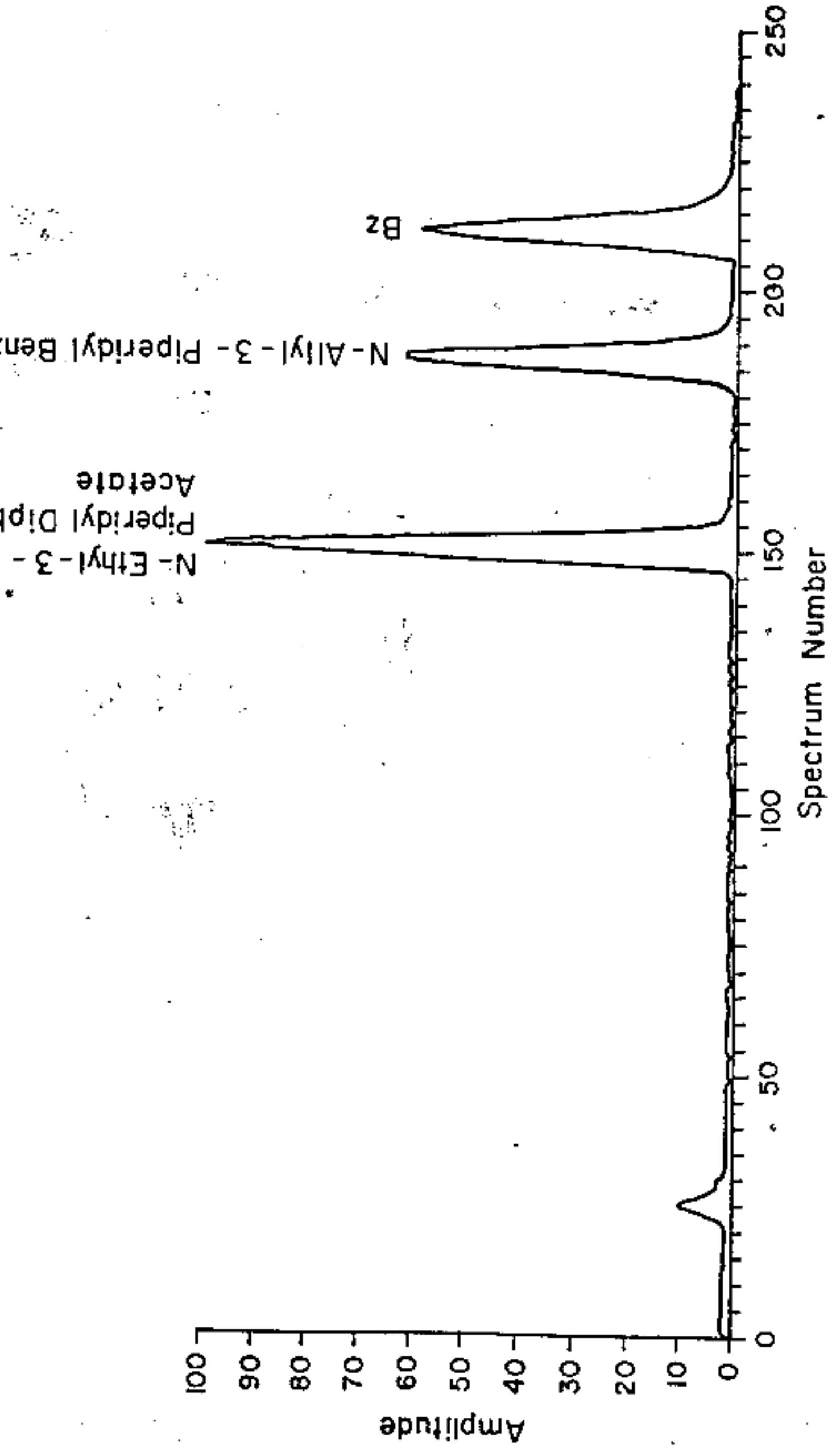

FIGURE 13. CHEMICAL IONIZATION GC-MS ANALYSIS OF PIPERIDYL GLYCOLATES AND BZ
Column - 6' x 2 mm I.D. 2 percent OV-17 on gas chrom Q 80-100 mesh
Temp - 250 C - 280 C at 10 C/min
Other conditions identical to Figure 9

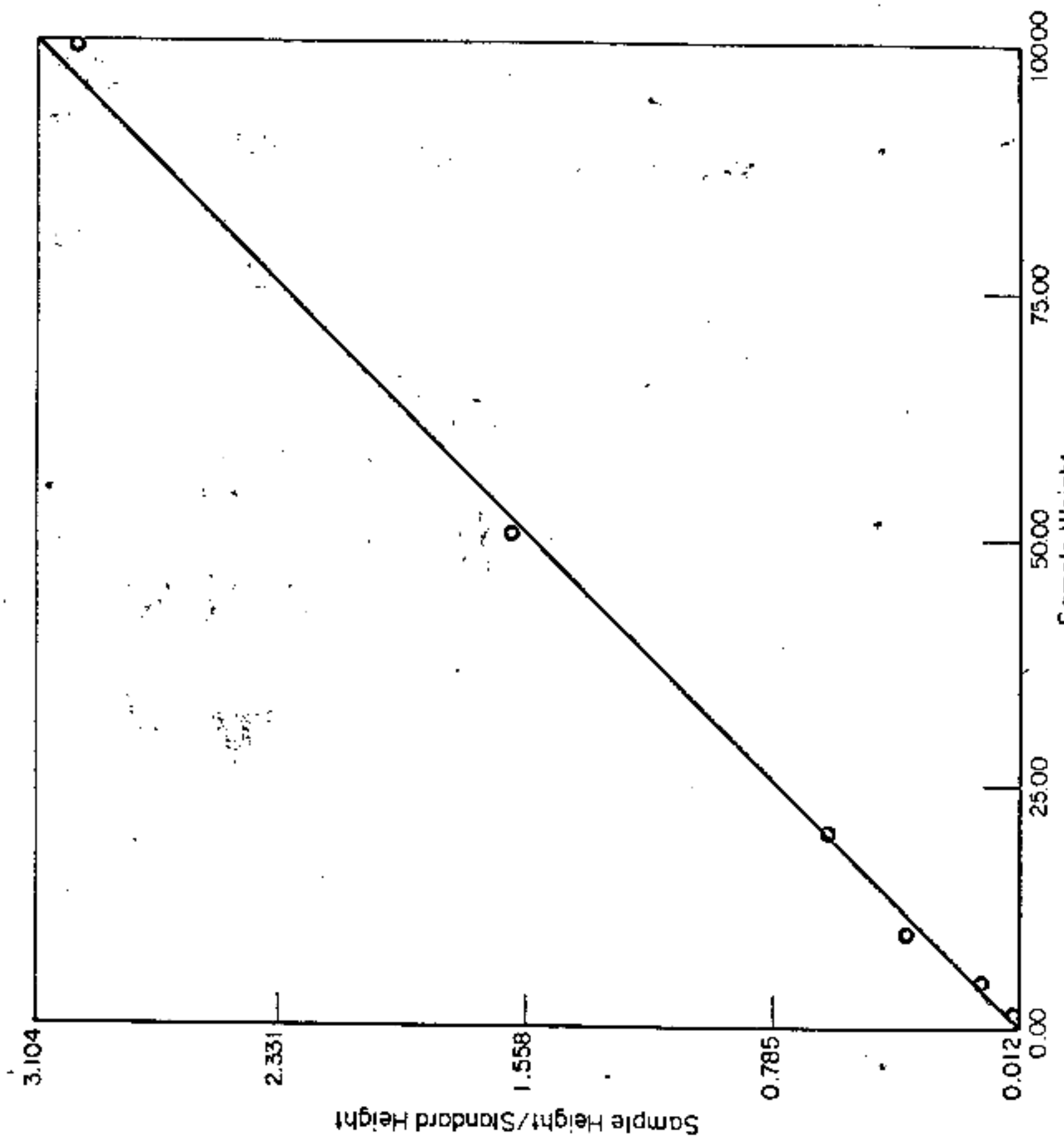

FIGURE 14. CALIBRATION CURVE FOR 1-100 NG BZ USING N-ALLYL-3-PIPERIDYL BENZILATE AS INTERNAL STANDARD

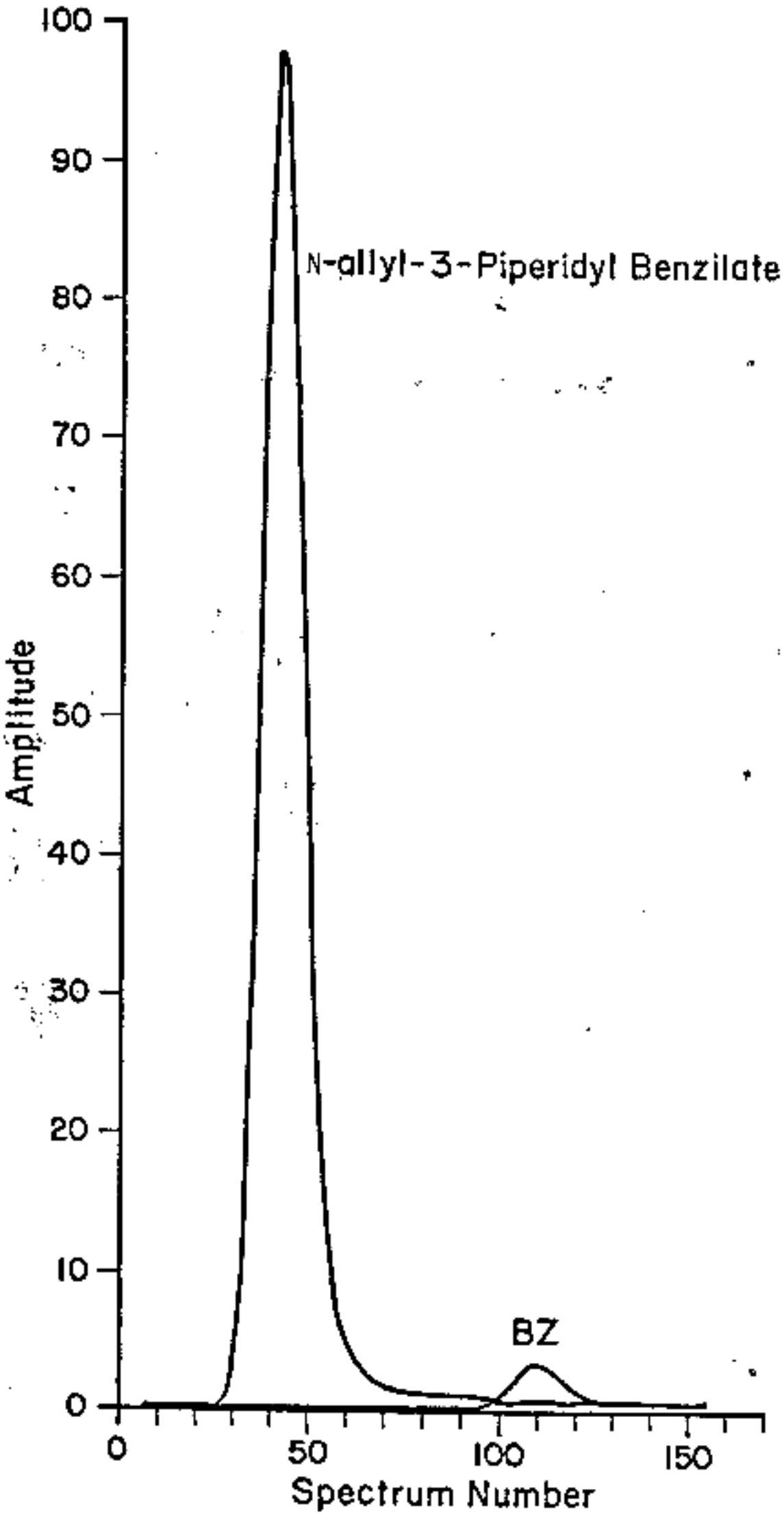

FIGURE 15. ANALYSIS OF 1 PPB OF BZ BY GC/CIMS
By selectively monitoring m/e 352 (QF-18) and 338 (BZ)
Temperature 250 - 280 C at 10 c/min
GC-MS conditions same as Figure 9

Ancillary Methods Development

At the request of the Project Officer, we investigated additional methods for the analysis of BZ: spectroscopic analysis after complexation with Tropaeolin oo; spectroscopic analysis after complexation with iodine; and fluorimetric analysis after complexation with indandione. The results of these investigations indicate that although in certain cases (spectroscopic analysis) the sensitivity is sufficient for the analysis of BZ, the selectivity of these methods were poor, and interferences would most likely cause erratic results in quantification. In the case of BZ-indandione, we showed evidence of the instability of the complex under TLC elution conditions.

Tropaeolin OO Complexation

It is reported in the literature that the BZ-Tropaeolin OO ion pair complex is completely extracted from water at a pH near 4 via certain halogenated solvents and can be used to detect BZ at a level of 0.01 ug/ml. by reading the absorbance of the extract at 420 mu. We have determined that BZ can probably be measured at the level quoted but that detection at levels lower than this probably can not be easily achieved. Confirmation of the reported sensitivity is based on a single experiment run in our laboratory.

To confirm the reported sensitivity data, a standard curve was prepared according to a published procedure and the data obtained plotted to determine whether the system involved obeyed the Beer-Lambert law. The procedure used was as follows: to 250 ml of distilled water in a 500 ml separatory funnel (teflon stopcock) was added 5 ml of pH 4.5 0.1 M citrate-phosphate buffer and after mixing well, the buffered solution was spiked with an accurately measured volume of a standard 50 ppm (1 µg/µl) solution of BZ in acetone. After mixing well, 5 ml of a solution of Tropaeolin OO in 1,1,2,2-tetrachloroethane (washed with dilute sodium hydroxide, distilled and stored over silica gel) was added and the mixture shaken well and separated. It should be pointed out that the concentration of Tropaeolin OO should be 0.375 g/l but this level could not be achieved even though

several batches of the dye were examined. After separation of the layers, the absorbance (optical density) of the 1,1,2,2-tetrachloroethane layer was measured at 420 mu using a Beckman Model DU with a Guilford attachment. s-Tetrachloroethane was used as the reference. The data obtained from this experiment are collected in Table 1.

These data are plotted in Figure 16. It can be seen that the Beer-Lambert Law is valid between the concentrations of 0.01 µg/ml (2.5 g total BZ added) and 0.08 µg/ml (20 µg total BZ added). No higher levels of BZ were examined. From this plot it does appear that levels of BZ as low as 0.01 µg/ml can be detected via this procedure.

Since in the hydrolysis of BZ the hydrolysis products are likely to be present in much larger quantities than the residual unhydrolyzed BZ, it is very important that any BZ detection selected be effective in the presence of these massive amounts of BZ hydrolysis products. It is reported that the Tropaeolin OO procedure is effective even in the presence of 10^4-fold excess of 3-quinuclidinol. This did not prove to be the case in our experiments. To determine the effect of large amounts of 3-quinuclidinol on this detection system, the 245 ml water-5 ml citrate phosphate buffer solution was spiked with 66 mg and 164 mg of 3-quinuclidinol and the extraction carried out with Tropaeolin OO solution as described above in the preparation of the standard curve. These quantities of 3-quinuclidinol are approximately 0.3×10^4 and 0.8×10^4-fold excess of the highest level of BZ examined. The absorbance of the Tropaeolin OO complex of these extracts were 0.063 and 0.073, respectively. Thus it can be readily seen from Table I and Figure 16, that massive amounts of 3-quinuclidinol are likely to interfere with the measurement of small amounts of BZ via this procedure. The effect of smaller amounts of 3-quinuclidinol on the procedure were not investigated.

Iodine Complexation

When BZ in aqueous acid is mixed with a chloroform solution of iodine, a complex is formed which is extracted into the chloroform layer. The visible iodine absorption is diminished and new peaks are formed in the ultraviolet region at 288 mv and 273 mv, the latter being the largest peak. These represent a 1:1 complex. The extraction of this iodine

TABLE 1. TROPAEOLIN OO - BZ STANDARD CURVE DATA

Distilled Water, ml	Buffer, ml	BZ Standard Added, µl	BZ Added, µg	Tropaeolin OO Added, ml	Absorbance at 420 µg
245	5	0	0	5	.039
245	5	50	2.5	5	.045
245	5	100	5.0	5	.052
245	5	200	10	5	.066
245	5	300	15	5	.081
245	5	500	20	5	.101

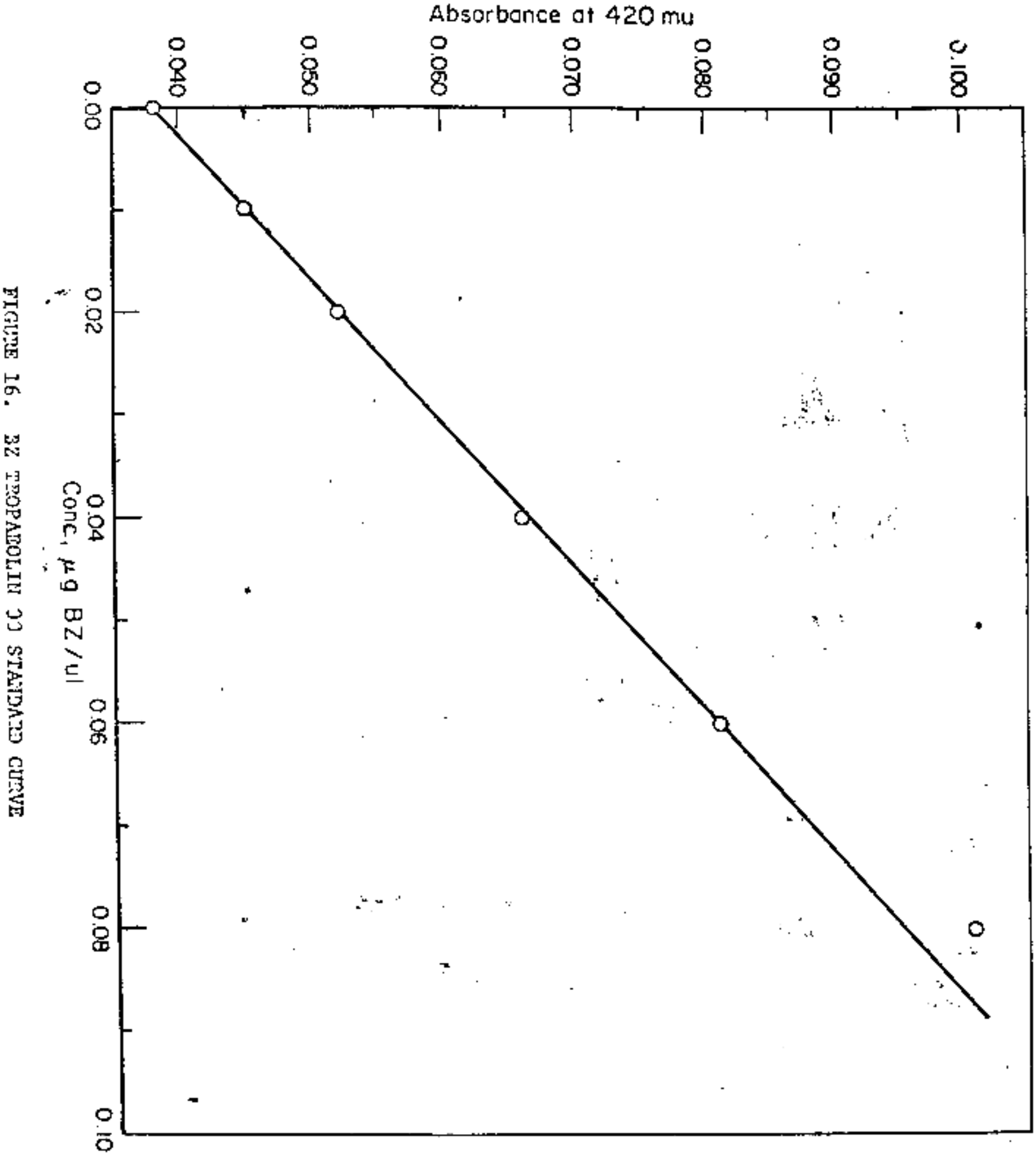

FIGURE 16. BZ TRIOPANOLIN STANDARD CURVE

complex has been applied to the automated analysis of field samples for BZ, the sensitivity found to be in the order of 1 µg/ml. It has been determined that this method does have the sensitivity quoted above and that BZ can readily be detected at the 1 µg/ml level. Actually, under the conditions of our experiment, this method appears to have greater sensitivity than reported.

To confirm the sensitivity data quoted, a standard curve was prepared according to directions obtained from personnel at Edgewood Arsenal. The procedure used was as follows: a 30 ml separatory funnel (teflon stopcock) was charged with 5 ml 0.1N sulfuric acid and then spiked with accurately measured quantities of a 50 ppm (1 µg/ml) solution of BZ in acetone. To this was then added 5 ml of a tris buffer (prepared by mixing 250 ml 0.2M tris-hydroxymethylaminomethane, 287.5 ml 0.1N hydrochloric acid, diluting to 1 liter and adding 4.0 g sodium hydroxide) followed by 5 ml of .001M iodine in chloroform. After mixing well, the chloroform layer was separated and the absorbance (optical density) measured at 273 mu using a Beckman DU equipped with a Guilford attachment. The reference was the extract from the experiment in which BZ was not added. The data obtained from these experiments are collected in Table 2.

The standard curve for the BZ-iodine complex is presented in Figure 17. It can be seen that Beer-Lambert law is valid for the levels of BZ examined. No higher levels of BZ were examined. From this plot, it definitely appears that levels of BZ in the order of 1 µg/ml and probably even lower can be readily detected.

As mentioned above under Tropaeolin OO discussion, it is very important that any BZ analytical method selected be uneffected by the high levels of 3-quinuclidinol that are likely to occur during the BZ hydrolysis. Accordingly 3-quinuclidinol in the amounts of 10, 50, and 150 mg ($.7 \times 10^4$, 3.4×10^4, 10.7×10^4-fold over the highest level of BZ used) were added to the 0.1N sulfuric acid and the iodine complexation and extraction carried out as described above in the standard curve preparation. Although there was no consistency in the 273 mu absorbance readings of the extracts, all were in the 1.5 - 3.0+ range. Thus it definitely appears that

TABLE 2. IODINE - BZ STANDARD CURVE DATA

0.1 N H_2SO_4, ml	BZ Standard Added, µl	BZ Added, µg	Buffer added, ml	I_2 - $CHCl_3$ added, ml	Absorbance at 273 mu
5	5	0.25	5	5	.195
5	10	0.50	5	5	.336
5	15	0.75	5	5	.524
5	22	1.10	5	5	.828
5	25	1.25	5	5	.908
5	28	1.40	5	5	.954

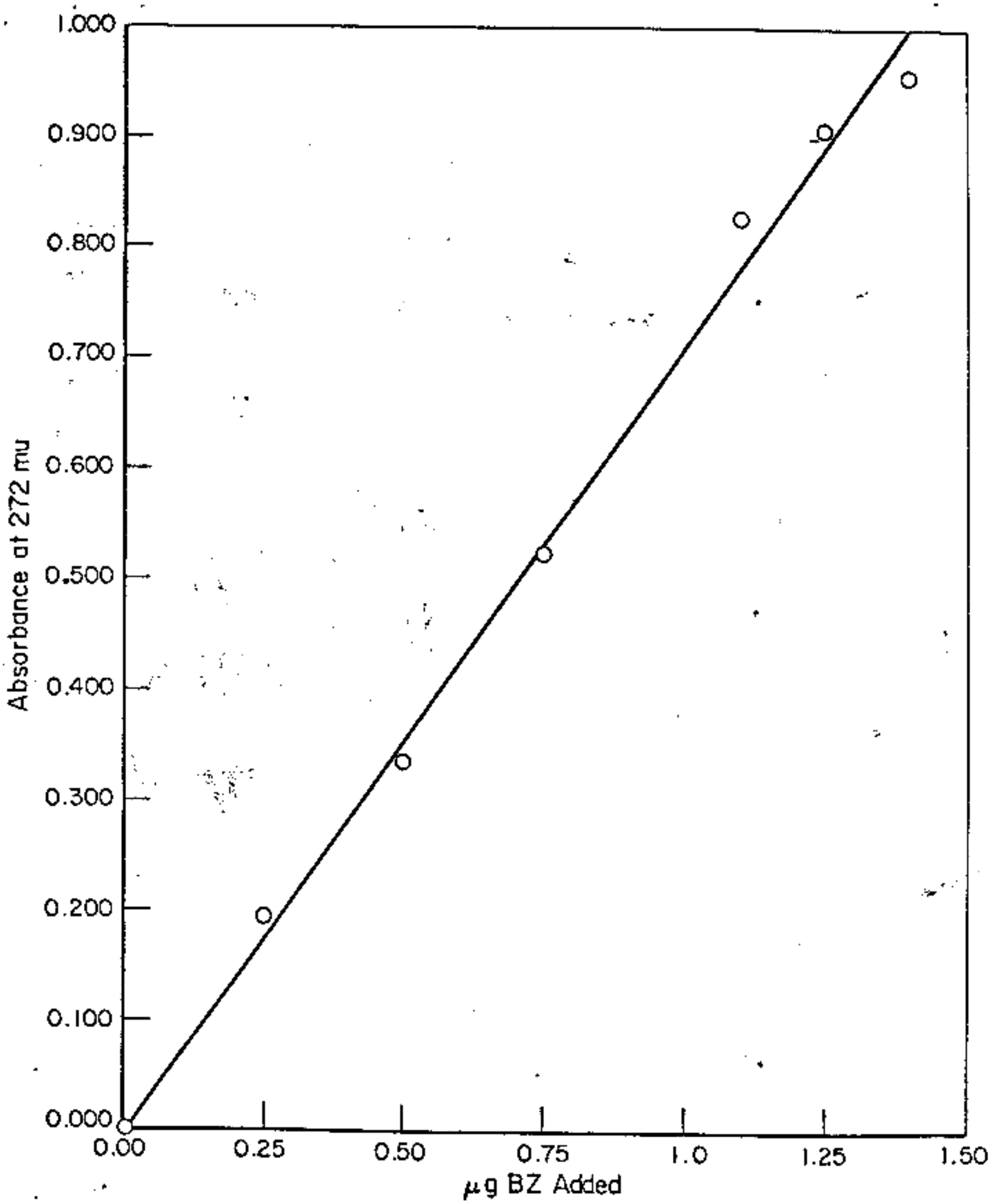

FIGURE 17. BZ-IODINE STANDARD CURVE

massive amounts of 3-quinuclidinol are likely to interfere with the measurement of low levels of BZ via the iodine complexation method.

Although colorimetric methods do allow one to rapidly measure levels of BZ, the presence of interferences leading to high results seriously impair the ability of this technique in environmental analysis.

Fluorometric Analysis with Indandione

The complex formation of 3-quinuclidinyl benzilate (BZ) with modified indandione was investigated with respect to its fluorescence on thin-layer chromatography (TLC) plates. It was hoped that if a stable BZ-indandione complex could be formed and chromatographed, then HPLC using fluorescence detection might be used as a simple and sensitive analysis method. The degree of binding between BZ and the fluorescent reagent, indandione, was examined briefly, and shown to be unstable under TLC elution conditions.

First, a number of solvents were evaluated for the elution of BZ by TLC. Silanized silica and silica gel-60 were used with methanol-chloroform, -tetrahydrofuran, -water, and -5 percent acetic acid mixtures as eluting solvents. The results are shown in Table 3. Iodine vapor provided a means of visualizing the developed BZ spots of 8 µg quantities. As expected, the silica gel stationary phase exhibits a stronger affinity for BZ (shorter R_f values) than does the silanized stationary phase. BZ has the most retention using the $MeOH/H_2O$ solvent mixture and the least retention using the $MeOH/CHCl_3$ solvent system.

Next, the amount of fluorescence produced by the indandione-BZ complex was examined. A TLC plate of silanized silica gel-60 was spotted with 15 to 150 µg quantities of BZ in 15 µg increments. A 0.125 g/100 ml solution of indandione in chloroform was sprayed onto the spotted TLC plate which was then illuminated with long and short wavelength UV radiation. A stronger fluorescence was observed at the longer wavelength portion of the UV source setting. An increase in fluorescence starting with the 30 µg spot and proceeding to the 150 µg spot was observed. However, no noticeable fluorescence was seen for the 15 µg spot and the 30 µg spot exhibited slight fluorescence.

TABLE 3. THIN LAYER CHROMATOGRAPHIC RETENTION DATA FOR BZ

Number	Stationary Phase	R_f	Solvent Mixture
1	S	0.143	90% MeOH/H_2O
2	R	0.190	90% MeOH/H_2O
3	S	0.259	90% MeOH/$CHCl_3$
4	S	0.309	50% MeOH/THF
5	S	0.400	50% MeOH/$CHCl_3$
6	S	0.456	10% MeOH/$CHCl_3$
7	R	0.570	10% MEOH/THF
8	R	0.599	90% MeOH/$CHCl_3$
9	S	0.632	90% MeOH/5% Acetic Acid
10	R	0.644	50% MeOH/THF
11	R	0.747	90% MeOH/5% Acetic Acid
12	R	0.839	50% MeOH/$CHCl_3$
13	R	0.892	10% MeOH/$CHCL_3$

R = Silanized

S = Silica

The stability of a complex between BZ and the fluorescent compound indandione was then studied. Silica gel-60 was used with an eluent of methanol-tetrahydrofuran (1:1). This solvent system was chosen because an intermediate R_f value is obtained for BZ compared to other solvent mixtures tested. A silanized TLC plate was spotted with 60 µg of BZ in three locations, afterwhich 12.5 µg of indandione was added to two of the BZ spots (BZ-I, see Figure 18). Two spots of 12.5 µg each of indandione were also spotted. Figure 18 shows that the BZ indandione complex disacociates upon elution with THF/H_2O.

Low microgram quantities of BZ appear to be detectable by observing the fluorescence of the BZ-indandione complex on the TLC plates. The complex once formed is very weakly bound and is evidently easily separated into indandione and BZ by simple elution of the complex with methanol/THF on silica gel-60. HPLC separation of the BZ-indandione complex is unlikely and indications are that the sensitivity needed to monitor nanogram quantities of BZ are not met with this procedure.

Evaluation of Simulated "Real" Systems

Brine Analysis

A brine containing a hydrolyzed BZ mix was received from Pine Bluff Arsenal and was used for the assessment of types of interferences (and their levels) likely to be encountered during analysis. The brine (100 ml) was adjusted to pH 8.5 and extracted with 3 x 50 ml of diethyl ether. The diethyl ether was washed with 2 x 25 ml of distilled water, dried with Na_2SO_4, and concentrated to 1 ml for analysis. This procedure was repeated with 1 µg (10 ppb) of BZ (dissolved in benzene) added to the brine after pH adjustment. Chloroform and methylene chloride were evaluated as extracting solvents, but were abandoned due to the precipitation of large amounts of solid material during the concentration step.

The brine extract was injected onto a GC/CIMS system and spectra were collected during a temperature program run. The resulting reconstructed

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FIGURE 18.

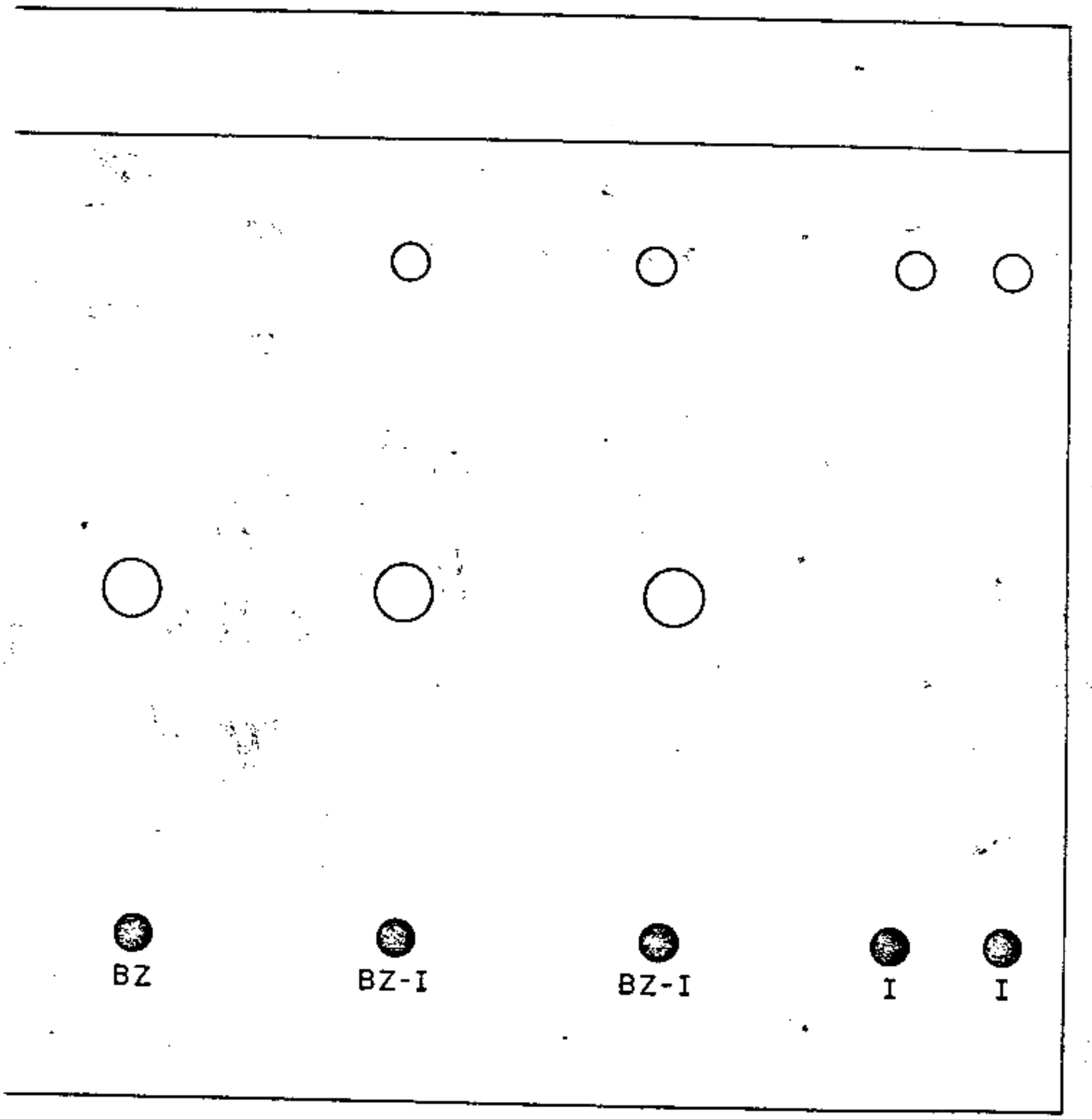

FIGURE 18. TLC OF BZ – INDANDIONE COMPLEX

gas chromatogram is shown in Figure 19. Only QN (Figure 20) and benzophenone (Figure 21) were detected. As discussed earlier, benzilic acid decomposes to benzophenone on column so that the detection of benzophenone implies the presence of benzilic acid in the extract.

GC/CIMS/SIM was used to specifically detect BZ in the spiked and unspiked extracts. No BZ was found in the unspiked extract, but the BZ was readily detected in the spiked extract as shown in Figure 22. By comparison with external standards, the BZ recovery was found to be >80 percent. The extract was also injected onto a GC/AFD system, and a large amount of material was detected (as shown in Figure 23). The BZ was not detected using AFD due to the level of interferences. The amount of BZ necessary to give a detectable response (relative to the other components) was estimated to be .5 ppm.

This experiment strongly points out the selectivity of GC-MS relative to other techniques and gives some insight as to the actual detection limit of AFD in brine samples. It is possible that an alternative extraction procedure, or a column chromatographic cleanup step would remove most of these interferences, allowing AFD or ECD to be used at lower levels. We, unfortunately, were unable to chromatograph the brine extract using ECD detection, due to time limitations.

Air Analysis

In order to analyze low levels of BZ in air, it is desirable to sample as large a volume of air as possible. The most convenient method for sampling large volumes of air is to use a glass fiber filter. However, loss of sample from the filter during collection is a possible problem in using this approach.

In order to determine the degree of loss of BZ from a glass fiber filter, the following experiment was performed. One µl of a 1000 ppm solution of BZ (1 µg) was deposited on each of 2 glass fiber filters and allowed to air dry. One filter was extracted immediately with 10 ml of benzene. Five ml of the benzene was removed and concentrated to .2 ml. This concentrate was then analyzed for BZ by GC/AFD. The second filter

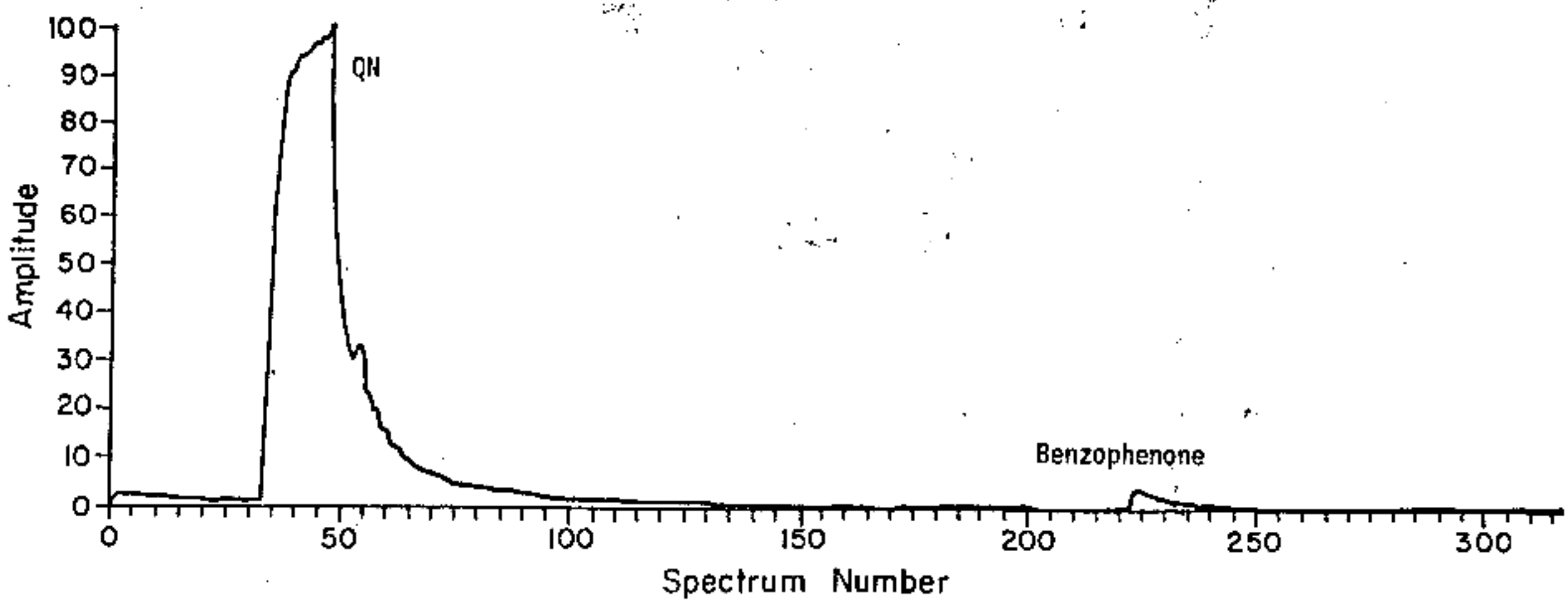

FIGURE 19. GC/CIMS RECONSTRUCTED GAS CHROMATOGRAM OF BRINE EXTRACT USING CH_4/NH_3 REAGENT GAS

Conditions - same as Figure 9

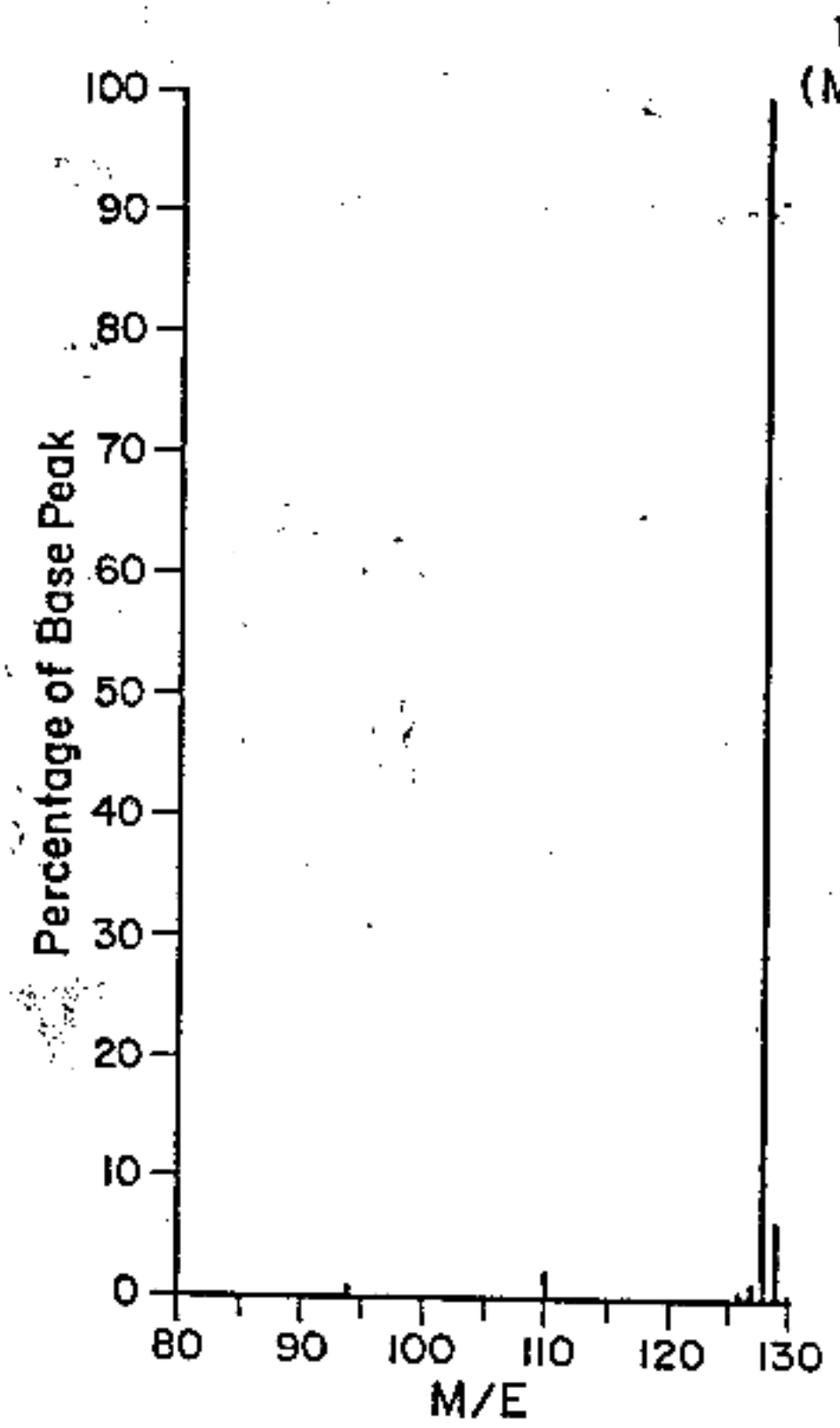

FIGURE 20. CH_4/NH_3 CHEMICAL IONIZATION MASS SPECTRUM OF QUINUCLIDINOL (MW 127) IN BRINE EXTRACT

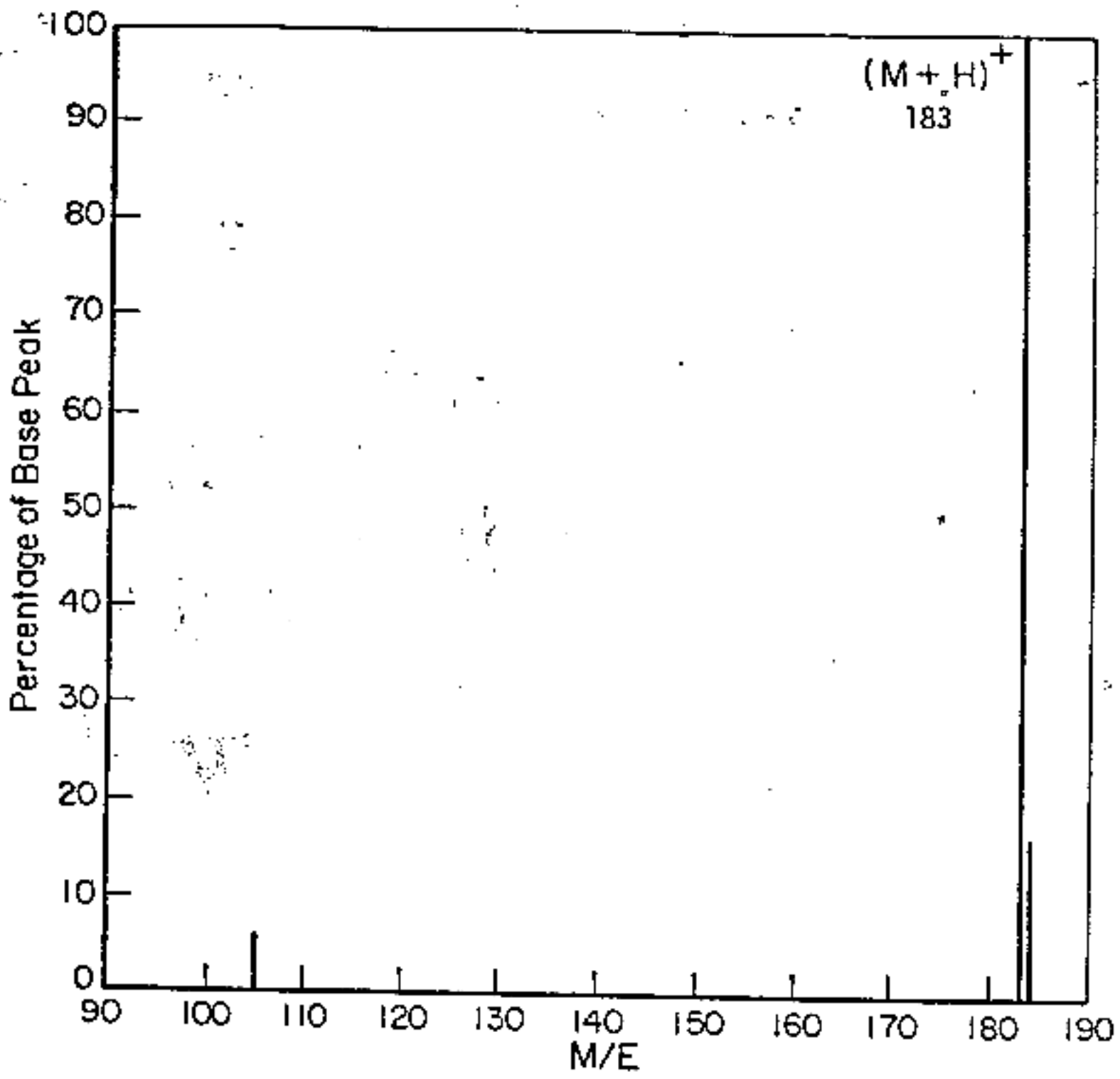

FIGURE 21. CH_4/NH_3 CHEMICAL IONIZATION MASS SPECTRUM OF BENZOPHENONE (MW 182) IN BRINE EXTRACT

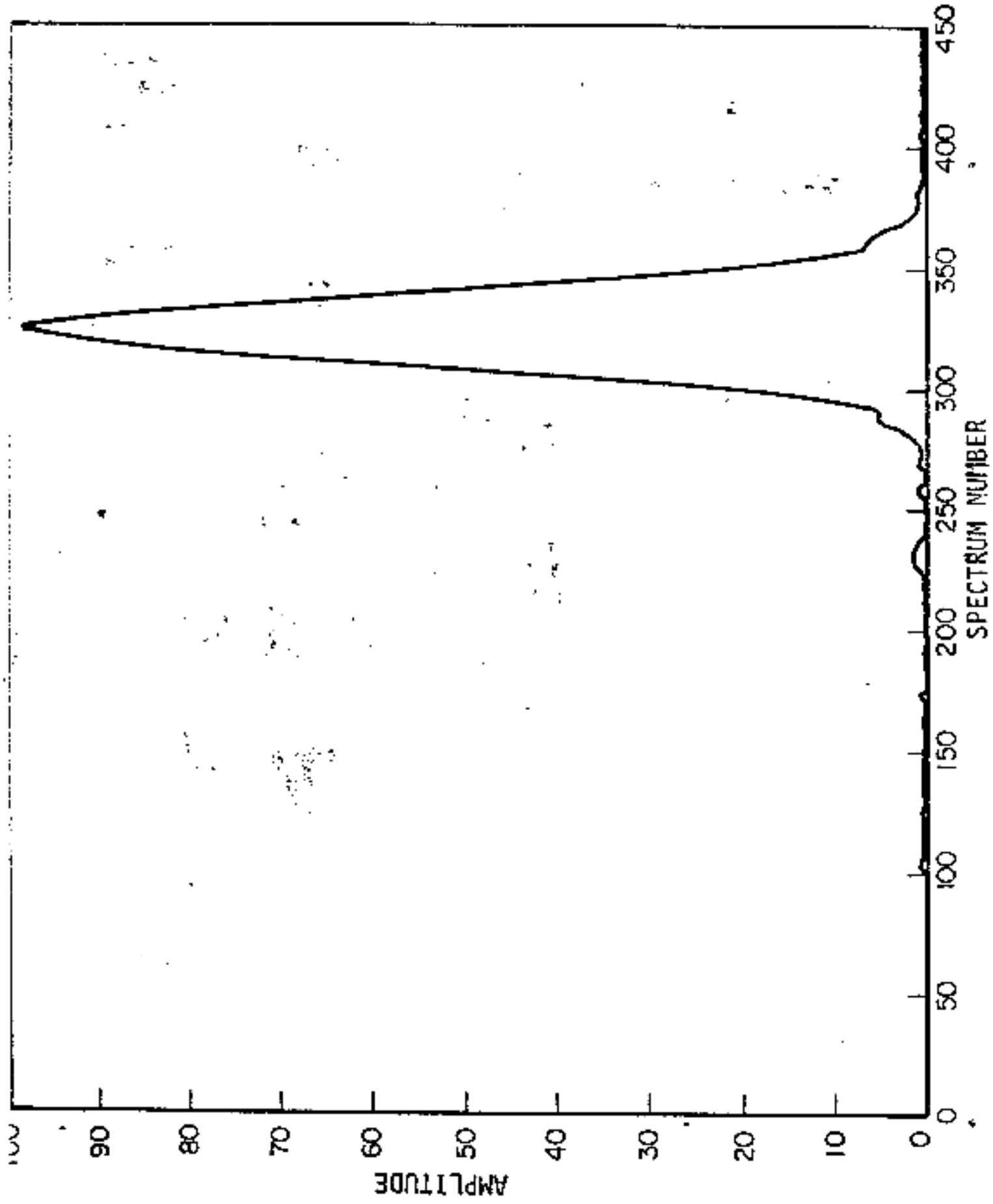

FIGURE 22. GC/CIMS CHROMATOGRAM BZ BRINE EXTRACT

Ion monitored - 338.4
Integration time - 15 msec
Scans/point - 15
Temp program - 250°-280° at 10°/min
Analysis time - 3 min
Other conditions - see Figure 9

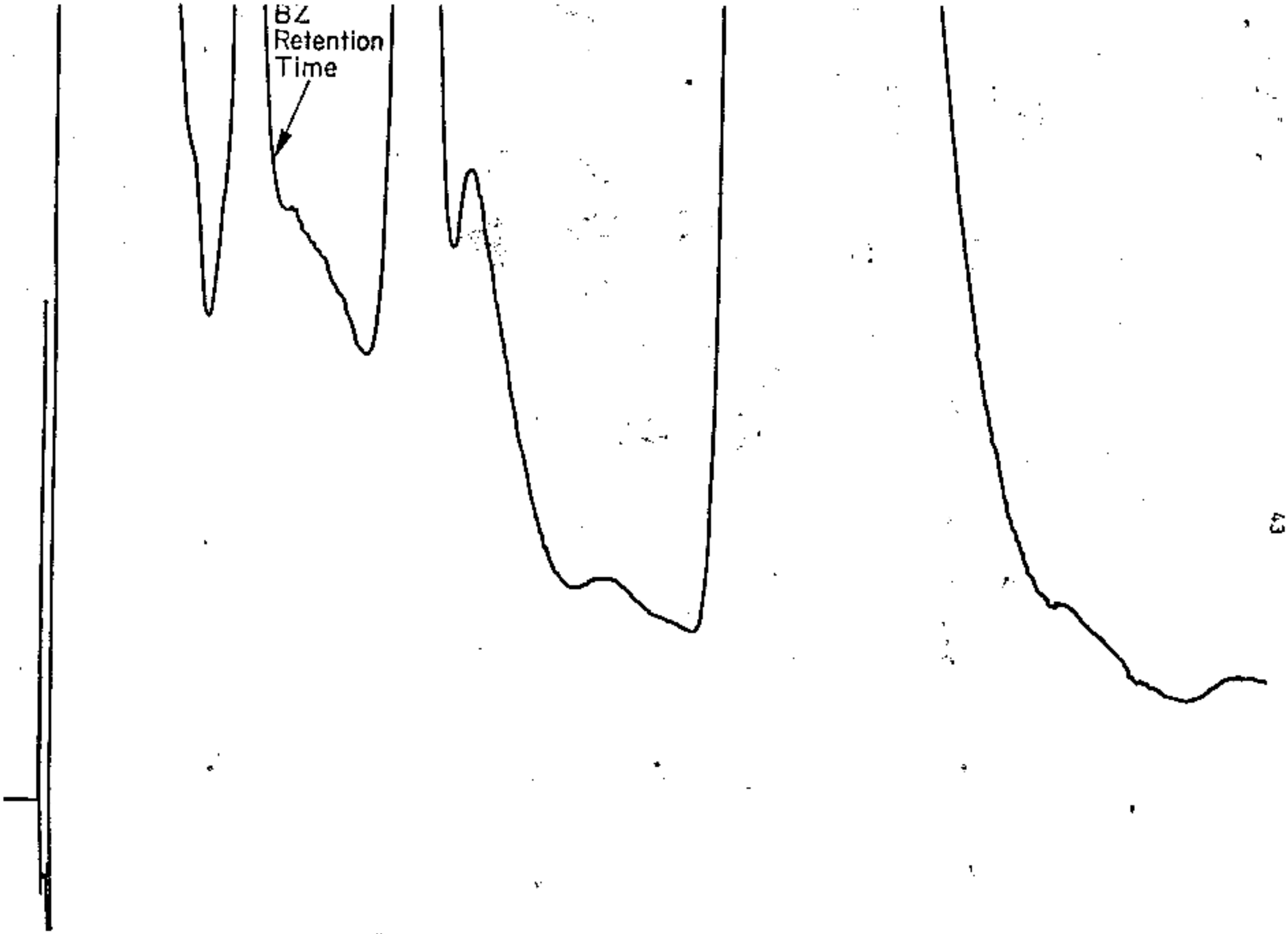

FIGURE 23. CHROMATOGRAM OF SPIKED BRINE EXTRACT USING AFD

Conditions - see Figure 1
Attenuation - X8

was placed in a filter holder and .5 m^3 of air was drawn through it at the rate of .04 m^3/min. This filter was then extracted as described above. Recovery of BZ from the first filter was 85 ± 10 percent, whereas recovery from the second filter (after sampling .5 m^3 of air) was <10 percent. Thus, very substantial losses of BZ can occur using this method of sampling.

This experiment is very preliminary and does not exclude the possibility that BZ adsorbed to particulate matter in air would be retained on the filter. However, at this point it seems that an adsorbant trapping method for the analysis of BZ in air would be necessary in order to get quantitative recovery of BZ.

CONCLUSIONS AND RECOMMENDATIONS

The findings of the study provide a good base for planning further investigations into the BZ neutralization/disposal problem. It is obvious from this work that a number of analytical techniques are capable of analyzing BZ at low levels and at least one technique (GC/CIMS) can analyze for BZ at the ppb level in hydrolyzed brine samples with excellent specificity.

It would not be wise at this point to designate particular analytical methods as being "best", since little is presently known concering the actual complexity of the samples to be assayed. However, one can make certain judgements as to the circumstances under which certain techniques are likely to be most useful and what studies need to be undertaken to validate their utility. In general, consideration should not be given to the spectroscopic and/or fluorimetric methods for trace analysis of BZ due to their lack of specificity and sensitivity. However, in certain cases, rapid screening for BZ at relatively high levels might be best accomplished using these methods.

For the trace analysis of BZ, analytical methods based on gas chromatographic separation are most likely to be successful. ECD, AFD, and GC/CIMS detection modes each show promise for BZ detection and should be further evaluated using real samples. The GC/CIMS was shown to be

extremely sensitive and selective and should be given preference when ultimate sensitivity and specificty are required. However, GC-MS is a very sophisticated technique requiring highly-trained personnel and should not be employed when less sophisticated techniques are adequate. AFD is also highly-sensitive towards BZ but the specificity is not nearly as good, thus, requiring more extensive sample preparation to remove interferences. ECD is also highly sensitive towards BZ, especially when the PFPA derivative is prepared. The specificity of ECD is reasonably good, and may not require quite as extensive sample purification as for AFD. Consequently, both AFD and ECD should be considered for the routine analysis of low levels of BZ.

Our recommendations for future work in this are summarized below:

- Each of the procedures studied should be evaluated using "real" samples such as pyrolysis/oxidation effluents, ambient air, and hydrolyzed brine mixtures.
- Other derivatization procedures should be evaluated in order to further enhance ECD detection of BZ.
- Sample cleanup procedures (e.g. liquid chromatography, solvent extraction, etc.) should be evaluated for real samples using AFD and ECD detection modes.