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**SUPERCRITICAL FLUID APPLICATION
FOR THE ANALYSIS OF LEWISITE (L) AND
3-QUINUCLIDINYL BENZILATE (BZ)**

**Michael W. Ellzy
Foy E. Ferguson
Paul C. Bossle**

RESEARCH DIRECTORATE

February 1990

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PREFACE

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SUPERCRITICAL FLUID APPLICATION FOR THE ANALYSIS OF LEWISITE (L) AND 3-QUINUCLIDINYL BENZILATE (BZ)

1. INTRODUCTION

The analysis of lewisite [dichloro (2-chlorovinyl) arsine] (L) and 3-quinuclidinyl benzilate (BZ) has been investigated by several researchers.^{1,2} What is new, and until now has not been investigated, is the use of supercritical carbon dioxide (CO₂) to extract and chromatograph these two compounds.

Currently, these two compounds cannot be chromatographed by gas chromatography (GC) even with the advent of new fused silica column technology.^{3,4} Our method provides a means of extracting these compounds from complex matrices (i.e., soils) and chromatographing them by a supercritical fluid chromatograph (SFC).

2. EXPERIMENTATION

With a SUPREX Corporation (Pittsburgh, PA) SFC/200A fluid chromatograph equipped with a flame ionization detector (FID), a 1- μ L injection loop, an extractor unit able to hold a gram of soil, and a KRATOS Division (Newark, New Jersey) Spectroflow 757 UV detector, BZ was chromatographed using the instrument parameters outlined in Figure 1. Lewisite was chromatographed using the pressure program outlined in Figure 2. The fused silica restrictor attached to the end or exit line of the absorbance detector provided a CO₂ flow measured at the restrictor of 30 mL/min. All separations were performed on a Keystone Scientific (State College, PA) 1-mm by 10-cm packed C18 column. Data was collected on a Hewlett Packard (Rockville, MD) 3390 integrator. Standards were prepared between 1 μ g/mL and 1000 μ g/mL using ultraviolet (UV) grade methanol or hexane obtained from Fisher Scientific (Springfield, NJ). Ethyl ethylcarbonate (EEC) and ethyl n-ethylcarbanilate (ENEC) were obtained from Aldrich Chemical Company, Incorporated (Milwaukee, WI). The BZ and L were obtained from the U.S. Army Chemical Research, Development and Engineering Center (CRDEC) Chemical Transfer Facility. The JHP CO₂ and bone dry CO₂ were purchased from the Matheson Gas Products Company (East Rutherford, NJ).

3. RESULTS

Initial SFC results obtained with the UV detector resulted in a minimum detection level of 10 μ g/mL when monitoring at the 225-nm wavelength using a 1- μ L injection loop. The minimum detection limit was established as 250 μ g/mL when FID was employed. The identity of the peak (Figure 1, bottom) for BZ was established using mass spectrographic analysis of the standard solutions running a calibration curve and analyzing the BZ with the use of two internal standards. Figure 3 shows a 3-component mixture of EEC, ENEC, and BZ. The lower portion of Figure 3 shows a chromatogram of the 2-component mixture and an attenuated 3-component mixture of the top chromatogram. Methyl benzilate was chromatographed and coeluted with the BZ component under our chromatographic conditions.

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DETECTOR 410 DEGREES C

PRESSURE PROGRAM

ISOTHERMAL OVEN TEMPERATURE 70 DEGREES C

STAGE	PRESSURE ATM	TIME MIN	RATE ATM/MIN	TOTAL MIN
1	50			
2	50	8.00	0.0	8.00
3	150	15.00	2.5	23.00
4	200	10.00	5.0	43.00
5	200	2.00	0.0	45.00

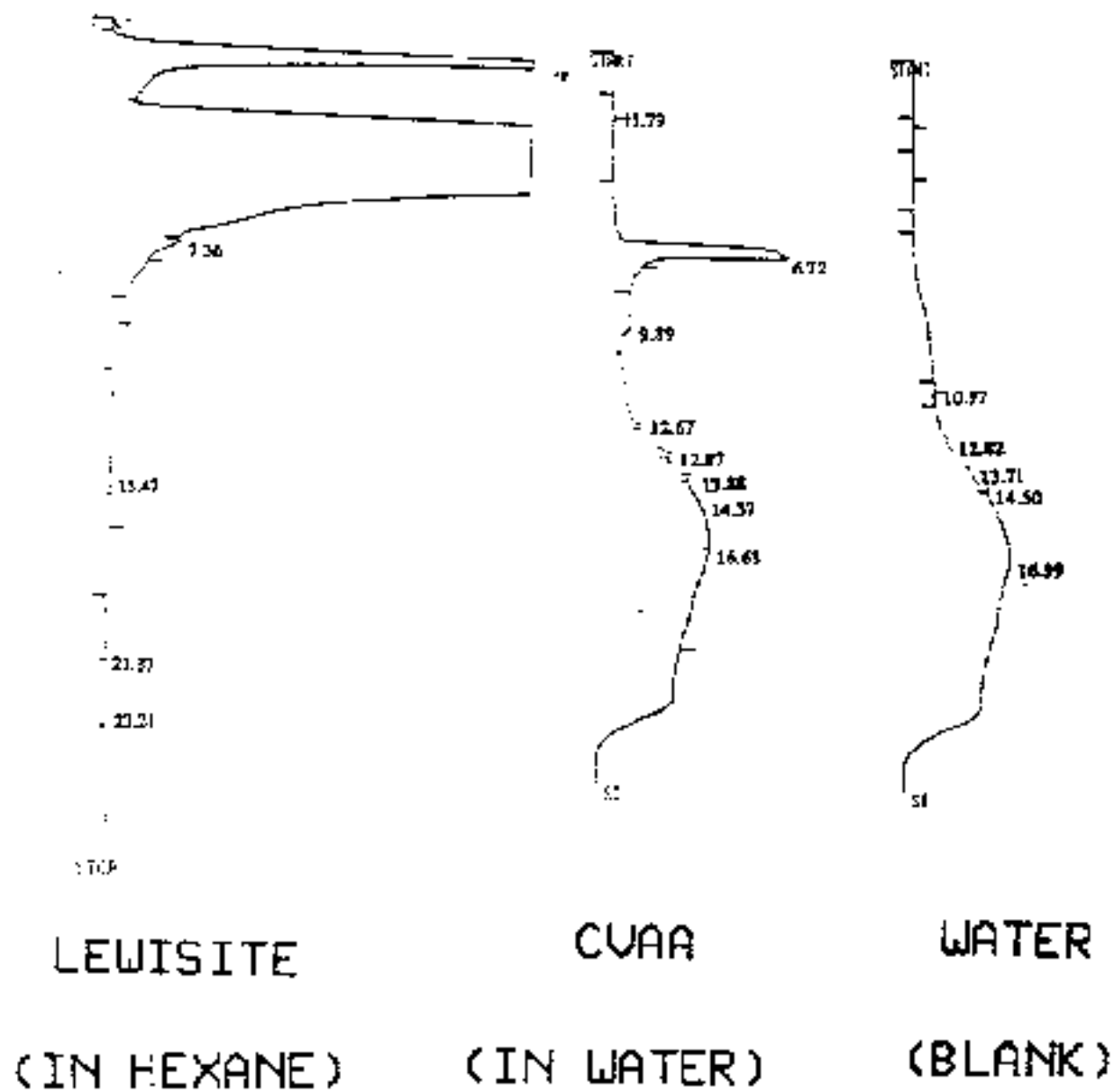
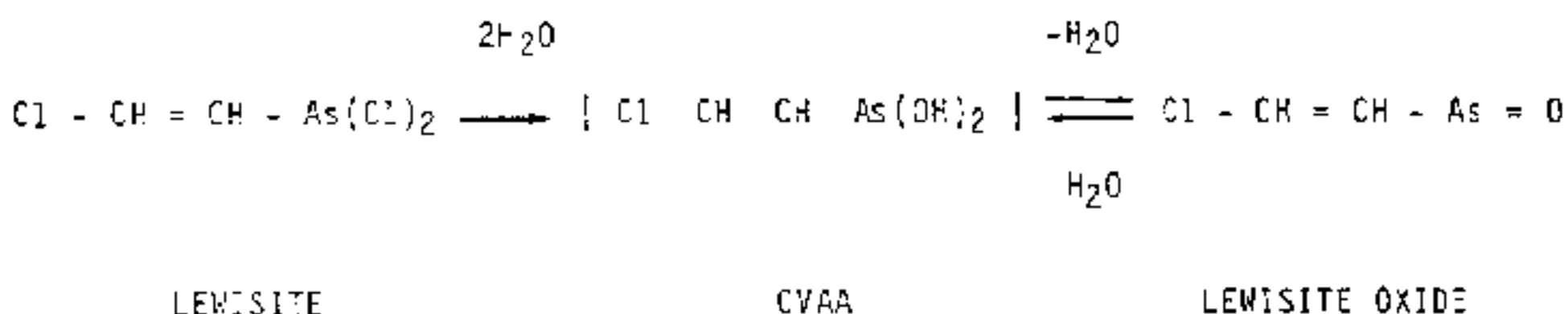


Figure 2. Pressure Program for Lewisite

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Typical chromatography resulting from L analysis is presented in the lower portion of Figure 2. The L peak in hexane is detected first using the FI and UV detectors. Identification of the L was accomplished by UV detector at 225 nm. It was necessary to disconnect the UV due to the pressure limitation (350 atm) of that detector. Lewisite oxide requires a pressure of 400 atm before it begins to elute from the column.

Lewisite is hydrolyzed rapidly and quantitatively in moisture to stable, water-soluble, and highly toxic, 2-chlorovinylarsenious acid (CVAA)¹ as shown below. The CVAA is the hydrated form of 2-chlorovinylarsenious oxide (lewisite oxide) and can exist only in aqueous solution. During our attempts to chromatograph L, the 0.01% moisture content of the CO₂ resulted in the formation of L oxide. To prevent this formation we switched to bone dry CO₂.



In our identification technique, we converted the L to chlorovinylarsenious acid and made water injections directly into the SFC (Figure 2, bottom). The hydrolyzed L was also analyzed using the high-performance liquid chromatography (HPLC) method developed in August 1988.⁴ This process allowed us to compare overall concentration responses for the L and CVAA. If we hydrolyzed L (CVAA), the chromatogram would look similar to that of chromatograph labeled CVAA in water (Figure 2).

In addition, the overall molar response for L would not equate to the molar response obtained when analyzing for a standard that has been converted to CVAA.

We have established a minimum detection limit of 0.3 mg/mL using an FID. The UV detection for L has been established as 0.03 mg/mL. The CVAA or its solid analog, L oxide, is more likely to be encountered in the natural environment than L itself. Consequently, the amount of pre-existing L that was present in an aqueous matrix can be determined by analyzing for CVAA. The CVAA and L oxide are also potent blistering agents;² therefore it is necessary to include the two as part of any detection protocol for L. Lewisite treated with water results in conversion of both L and L oxide to CVAA. The molar response of CVAA allows one to determine the amount of L present in the standard solution. From this, we have established a minimum detection limit of 0.3 mg/mL using an FID. The UV detection for L has been established as 0.03 mg/mL.

A 1-g soil sample was extracted before spiking with BZ, EEC, and ENEC to identify any components in the soil. Out of five spike trials per concentration recovery, 3 components did not deviate more than $\pm 0.05\%$ of the expected concentration value. This value showed good agreement based on the

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SUPERCRITICAL FLUID APPLICATION FOR THE ANALYSIS
OF LEWISITE (L) AND 3-QUINUCLIDINYL BENZILATE (BZ)

M.W. ELLZY, F.E. FERGUSON, AND P.C. BOSSLE

Abstract:

The main thrust of the research was the study of supercritical fluids as applied to chromatographing of L and BZ. The most important results obtained were: (1) Parts-per-billion/parts-per-million detection limits for BZ and L using flame ionization and ultraviolet detectors; (2) Compound separation on reverse phase 1 mm packed columns or delta bonded 1 mm packed columns. Most importantly the extraction of BZ from soil using this approach appears to be feasible and easily accomplished.

Introduction:

The analysis of lewisite [dichloro (2-chlorovinyl) arsine] (L) and 3-quinuclidinyl benzilate (BZ) has been investigated by several researchers¹⁻². What is new and until now has not been investigated is the use of supercritical carbon dioxide to extract and chromatograph these two compounds.

Currently, these two compounds cannot be chromatographed by gas chromatography even with the advent of new fused silica column technology³⁻⁴. Our method provides a means of extracting these compounds from complex matrices (i.e., soils) and chromatographing them by a supercritical fluid chromatograph (SFC).

Experimental:

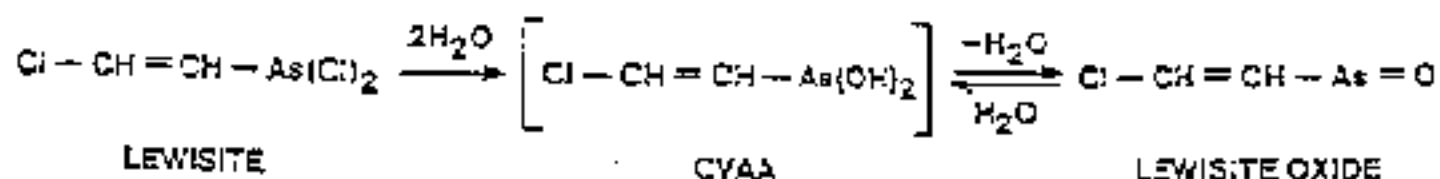
Using a SUPREX SFC/200A fluid chromatograph equipped with a flame ionization detector (FID), 1 ul injection loop, extractor unit able to hold a gram of soil, and a KRATOS Division Spectroflow 757 UV detector, BZ was chromatographed using the instrument parameters outlined in figure 1. Lewisite was chromatographed using the pressure program outlined in figure 2. The fused silica restrictor attached to the end or exit line of the absorbance detector provided a CO₂ flow measured at the restrictor of 30 ml/min. All separations were performed on a Keystone Scientific 1 mm x 10cm packed C18 column. Data was collected on a Hewlett Packard 3390 integrator. Standards were prepared between 1 ug/ml and 1000 ug/ml using UV grade methanol or hexane obtained from Fischer Scientific. Ethyl ethylcarbanate (EEC) and ethyl n-ethylcarbanilate (ENEC) were obtained from Aldrich. BZ and L was obtained from CRDEC Chemical Transfer Facility. UHP CO₂ and bone dry CO₂ was purchased from Matheson Company.

Results:

Initial SFC results obtained with the UV detector resulted in a minimum detection level of 10 ug/ml, when monitoring at the 225 nanometer wavelength using a 1 ul injection loop. Minimum detection limit was established as 250 ug/ml. The identity of the peak (figure 1, bottom) for BZ was established using mass spectrographic analysis of the standard solutions running a calibration curve and analyzing the BZ with the use of two internal standards. Figure 3 shows a 3 component mixture of EEC ENEC and BZ. The lower portion of figure 3 shows a chromatogram of the 2 component mixture and an attenuated 3 component mixture of the top chromatogram. methyl benzoate was chromatographed and found to coelute with the BZ component under our chromatographic conditions.

Typical chromatography resulting from Lewisite analysis is presented in the lower portion of figure 2. The Lewisite peak in hexane is detected first using the FI and UV detectors. Identification of the Lewisite was accomplished by UV detector at 225 nanometers. It was necessary to disconnect the UV due to the pressure limitation (30 atm) of that detector. Lewisite oxide requires a pressure of 400 atm. before it begins to elute from the column.

Lewisite is hydrolyzed rapidly and quantitatively in moisture to stable, water-soluble, and highly toxic, 2-chlorovinylarsenious acid¹ as shown below. CVAA is the hydrated form of 2-chlorovinylarsenious oxide (Lewisite oxide) and can exist only in aqueous solution. During our attempts to chromatograph Lewisite the one hundredth of a percent moisture content of the CO₂ resulted in the formation of Lewisite oxide. To prevent this formation we switched to bone dry CO₂.



As our identification technique we converted the Lewisite to chlorovinylarsenious acid and made water injections directly into the SFC (figure 2, bottom). This hydrolyzed Lewisite was also analyzed using HPLC method developed in August 1985⁴. This process allowed use to compare overall concentration responses for both the Lewisite and CVAA. If we hydrolyzed Lewisite (CVAA) the chromatogram would look similar to that of chromatograph label CVAA in water (figure 2). Furthermore the overall molar response for Lewisite would not equate to the molar response obtained when analyzing for a standard which has been converted to CVAA.

We have established a minimum detection limit of 0.3 mg/ml using an FID. UV detection for Lewisite has been established as 0.03 mg/ml. CVAA or its solid analog, Lewisite oxide, are more likely to be encountered in the natural environment than Lewisite itself. Consequently, the amount of pre-existing Lewisite that was present in an aqueous matrix can be determined by analyzing for CVAA. CVAA and Lewisite oxide are also potent blistering agents² and should of necessity be part of any detection protocol for Lewisite. Lewisite treated with water results in conversion of both Lewisite and Lewisite oxide to CVAA. The molar response of CVAA allows one to determine the amount of Lewisite present in the standard solution. From this we have established a minimum detection limit of 0.3 mg/ml using an FID. UV detection for Lewisite has been established as .03 mg/ml.

A one gram soil sample was extracted before spiking with BZ, EEC, and ENEC to identify any components in the soil. Using 5 trials per concentration recovery of all 3 components did not deviate more than + or - 0.05%. This value based on the expected when the spike concentration was injected directly in the SFC unit. This data was generated using the FID and a concentration range of 3 mg/ml to 0.5 mg/ml. Standard deviations were computed using area count deviations.

Conclusions:

Lewisite and BZ have been chromatographed using Supercritical Fluid Carbon Dioxide. Bone dry carbon dioxide was required to chromatograph Lewisite. The hydrolysis rate of Lewisite is so rapid that the hexane solvent used to prepare standard solutions had to be dried for 30 days before linear dilution could be accomplished. Following the parameter provided in figures 2 and 3 BZ and Lewisite have respective retention times of 17.08 and 0.9 with actual retention deviation being effected by manual injection. All separations were accomplished using a 1 mm x 10 cm packed C18 column.

Minimum detections have been established as 0.025 mg/ml and 0.3 mg/ml using FI detection for BZ and Lewisite respectively. While it was possible to extract Lewisite from soil the soil had to be moisture free and extraction efficiency was at best 75%. BZ on the other hand was in most cases quantitatively recovered from soil with the worst recovery of 87% based on amount used to spike. This study indicates the feasibility of reproducibly recovering BZ from soil and chromatographing the extracted compound. Chromatographing Lewisite can be successful using this method, although due to the rapid elution of Lewisite we believe a slower flow rate should be employed to increase separation. One must remember at 50 atm and 70 degrees C Lewisite is chromatographed in the sub-supercritical CO₂ range. Decreasing the flowrate by decreasing restrictor inner diameter will result in frequent plugging if SFC system to include standards are not extremely water free. Finally, though it is not advisable to inject

water into the SFC unit, CVAA in water does chromatograph using the pressure program outlined in figure 2.

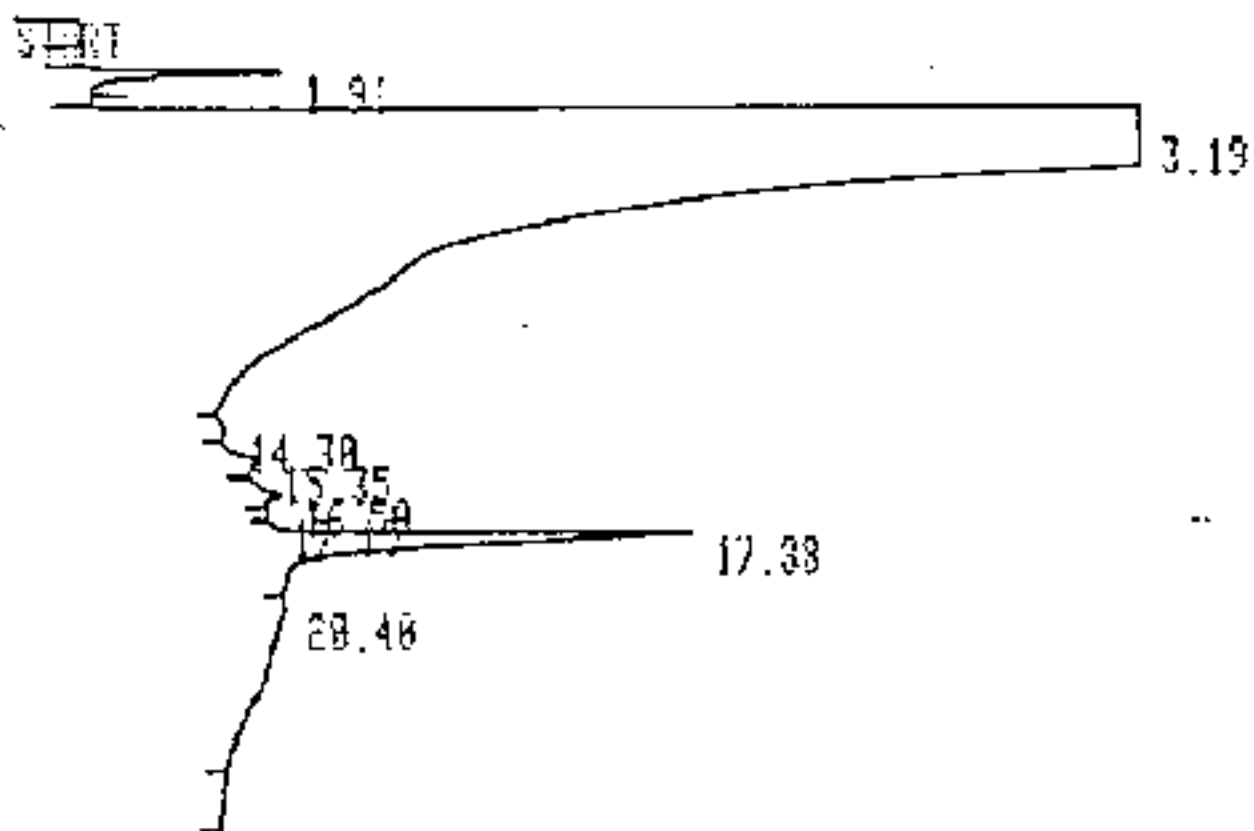
References:

1. Boudier, N. M., "Lewisite (M-1), 1940 Summary of Data", EATR 214, Copy No. 1, 21 August 1940.
2. Deiner, A., Shaw, F., Vigus, E., Keller, J., "Automated 3-Quinuclidinyl Benzilate (BZ) Analysis and Feasibility Studies to Develop an Alarm, CRDC-TR-84075, March 1985.
3. RESTEK Corporation, HALFMIL Capillary Columns, Second Edition.
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5 COMPONENT MIXTURE
 DETECTOR 410 DEGREES C
 PRESSURE PROGRAM

ISOTHERMAL OVEN TEMPERATURE 70 DEGREES C

STAGE	PRESSURE ATM	TIME MIN	RATE ATM/MIN	TOTAL MIN
1	100			
2	100	8.00	0.0	8.00
3	150	5.00	10.0	13.00
4	200	10.00	5.0	23.00
5	200	2.00	0.0	25.00
6	300	5.00	10.0	30.00



BZ in Methanol [5mg/ml]

FIGURE 1

DETECTOR 410 DEGREES C

PRESSURE PROGRAM

ISOTHERMAL OVEN TEMPERATURE 70 DEGREES C

<u>STAGE</u>	<u>PRESSURE ATM</u>	<u>TIME MIN</u>	<u>RATE ATM/MIN</u>	<u>TOTAL MIN</u>
1	50			
2	50	8.00	0.0	8.00
3	150	15.00	2.5	23.00
4	200	10.00	5.0	43.00
5	200	2.00	0.0	45.00

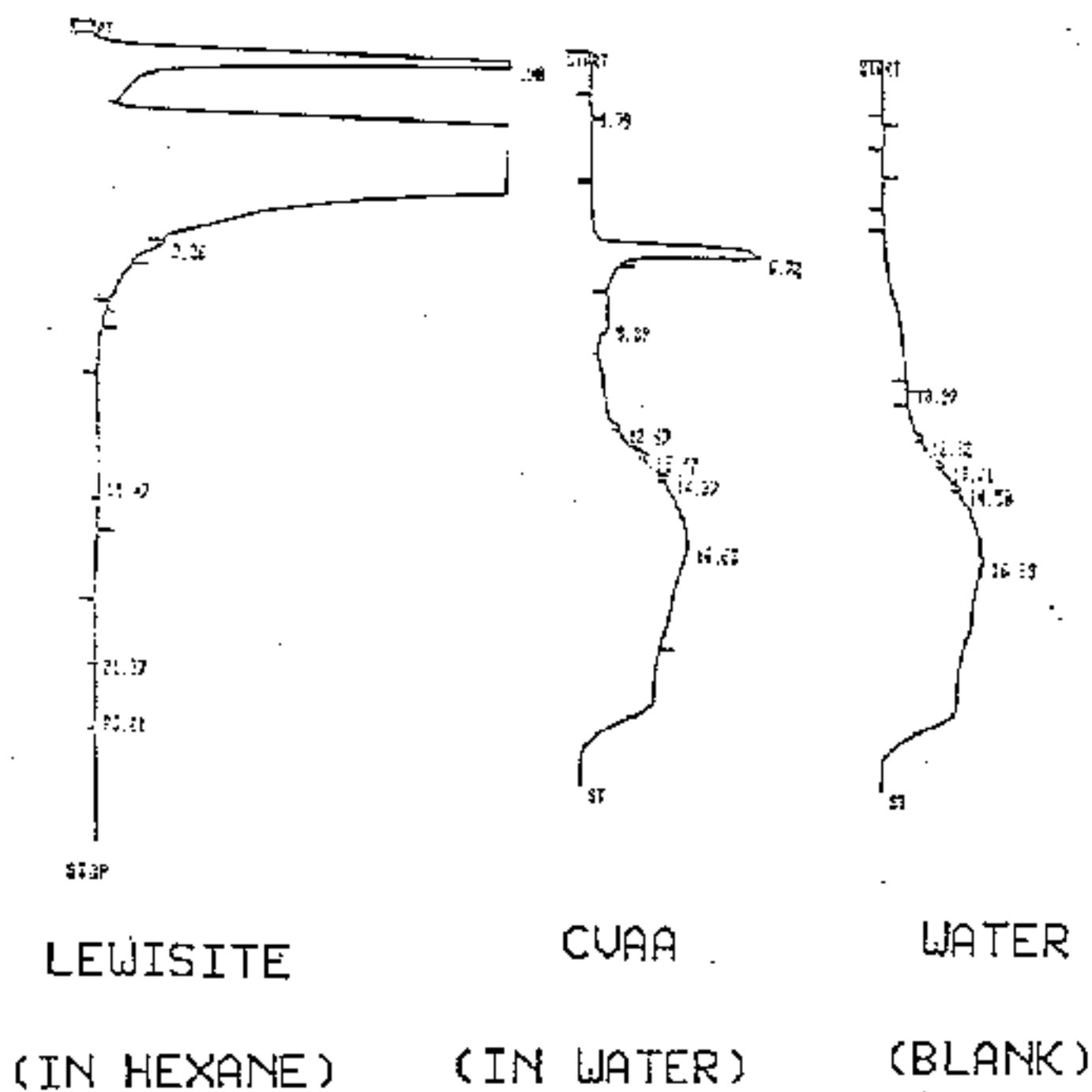
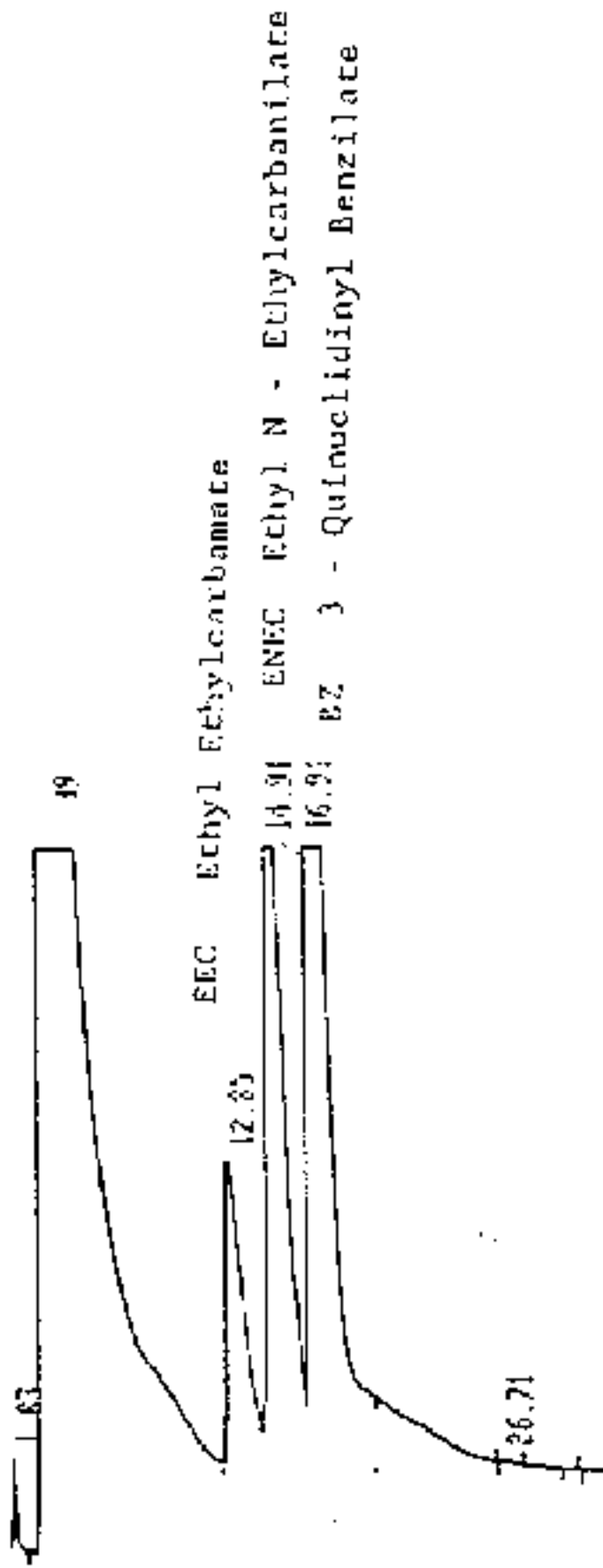
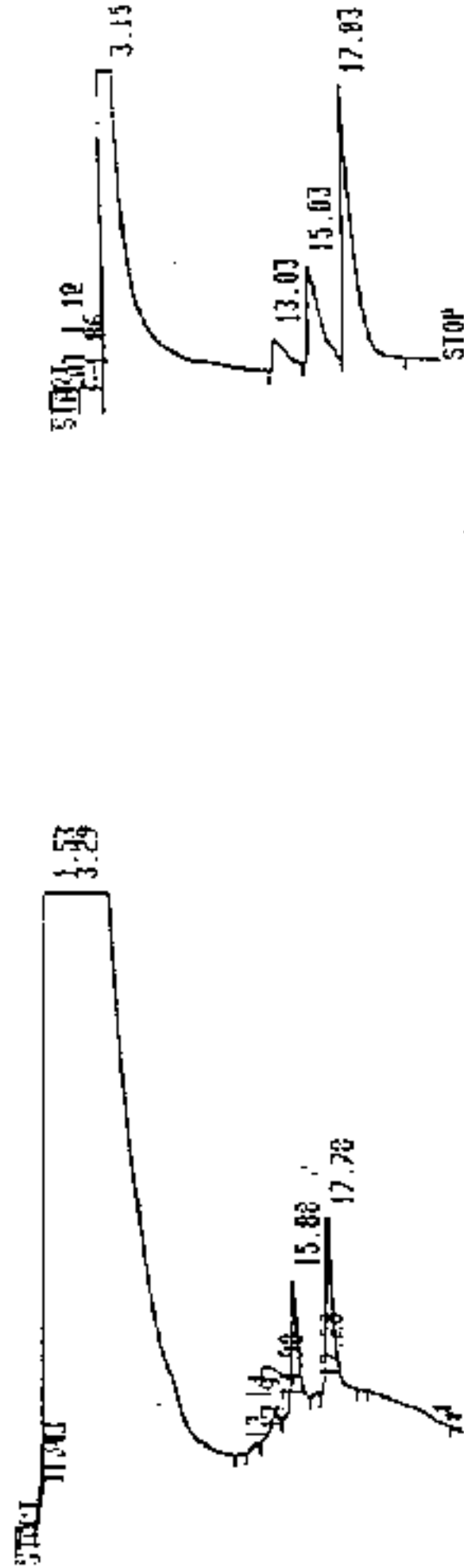


Figure 2



3 Component Mixture Attenuation 8



Mixture of EEC (2.72mg/ml) and ENEC (3.26mg/ml)

3 Component Mixture Attenuation 12
Concentration 10,20 and 30mg/ml respectively