



**CONFIDENTIAL REPORT FOR:**  
**GABRIELE LANG**



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Sample Description	Avomeen ID
T61 Euthanasia Product	032612-LA569

## Analysis of Euthanasia Product

Thank you for contacting Avomeen Analytical Services for the analysis of the T61 Euthanasia Product. Following are the results, methodology, and data associated with our analysis of the sample.

### Executive Summary

Table 1: Analysis Results

Compound	Approximate Concentration (mg/mL)
Dimethylformamide (DMF)	662.5
Epinephrine (Adrenaline)	not detected
Mebozonium Iodide	2.2 <sup>1</sup>
Tetracaine HCl	2.6 <sup>1</sup>
Emutramide	69.8 <sup>1</sup>

<sup>1</sup>Estimated Concentration based on Toluene.

Quantitative analysis by GC-FID of the sample (032612-LA569) determined the concentration of dimethylformamide (DMF) was 662.5 mg/mL. Quantitative analysis of the sample by HPLC, determined that Epinephrine (Adrenaline) was not detected in the sample, with a detection limit of 0.5 mg/mL. Active ingredients were identified by GC-MS, and semi-quantitatively determined (based on the response of Toluene), were 2.2 mg/mL of Mebozonium Iodide, 2.6 mg/mL of Tetracaine HCl, and 69.8 mg/mL of Embutramide.

### Analytical Testing:

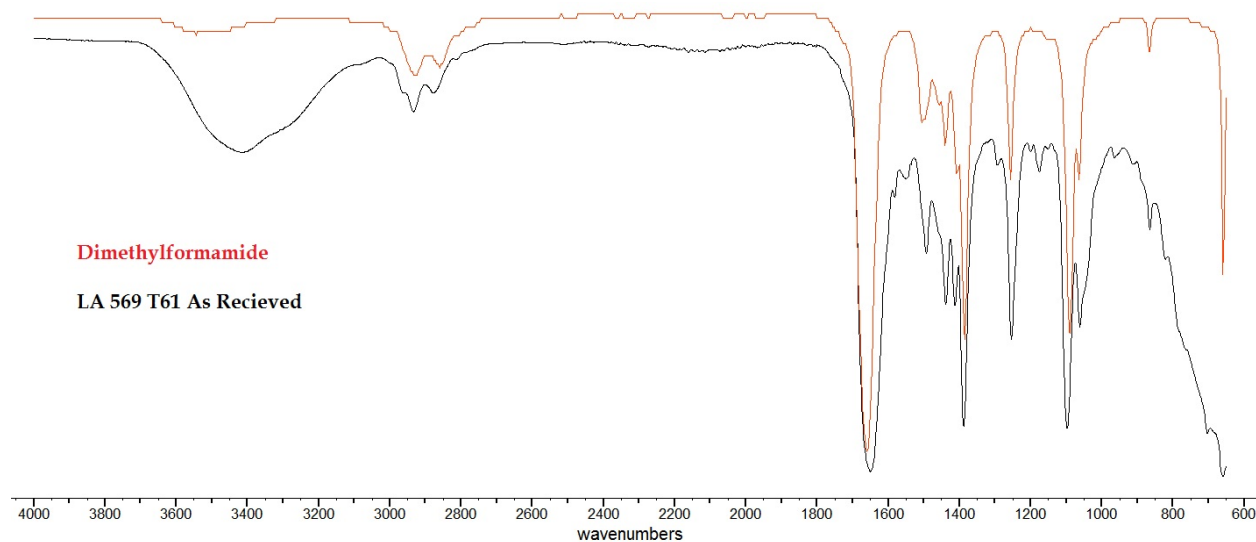
1. FT-IR analysis of the “as received” sample resembled a match to the reference FT-IR spectrum of DMF (Figure 1).
2. The sample was diluted 1:10 with isopropyl alcohol (IPA) and analyzed directly by GC-MS. The presence of DMF, Mebozonium Iodide, Tetracaine HCl, and Embutramide were confirmed based on the resulting mass spectra (Figure 1).
3. Standards of 1%, 5%, and 10% DMF in IPA were made and analyzed by GC-FID. The sample solution (sample diluted 1:10 with IPA) was analyzed and an internal standard of Toluene was maintained throughout the analysis. The concentration of DMF was calculated using linear regression, and the concentration of Mebozonium Iodide, Tetracaine HCl, and Embutramide were semi-quantitatively determined based on the response of Toluene. All results were corrected for the dilution factor.
4. A standard of 53.5 ppm Epinephrine in IPA and the sample solution were made and analyzed by HPLC. The sample results determined that Epinephrine was not present in the sample in the sample. The detection limit was adjusted for the dilution factor.

## Discussion of Results and Methodology:

Fourier Transform-Infrared Spectroscopy (FT-IR), Gas Chromatography Mass Spectrometry (GC-MS) and Gas Chromatography Flame Ionization Detection (GC-FID) were used to identify, quantitate, and semi quantitate the major and minor components of the sample. The following were the key steps in the analyses.

### Fourier Transform-Infrared Spectroscopy (FT-IR) Analysis

FT-IR analysis was conducted on the “as received” sample. The spectrum was obtained and compared to a library of over 236,000 spectral matches. FT-IR spectrum is shown in Figure 1 below:



**Figure 1.** FT-IR Spectrum of Sample “As Received” (Black) vs. Nearest Library Spectral Match (Red)

## Gas Chromatography Mass Spectrometry (GC-MS)

The GC-MS analysis was performed on the sample solution. Compounds were identified using the mass spectrum for each respective peak. GC-MS chromatograms and mass spectra are shown in Figures 2-6 below:

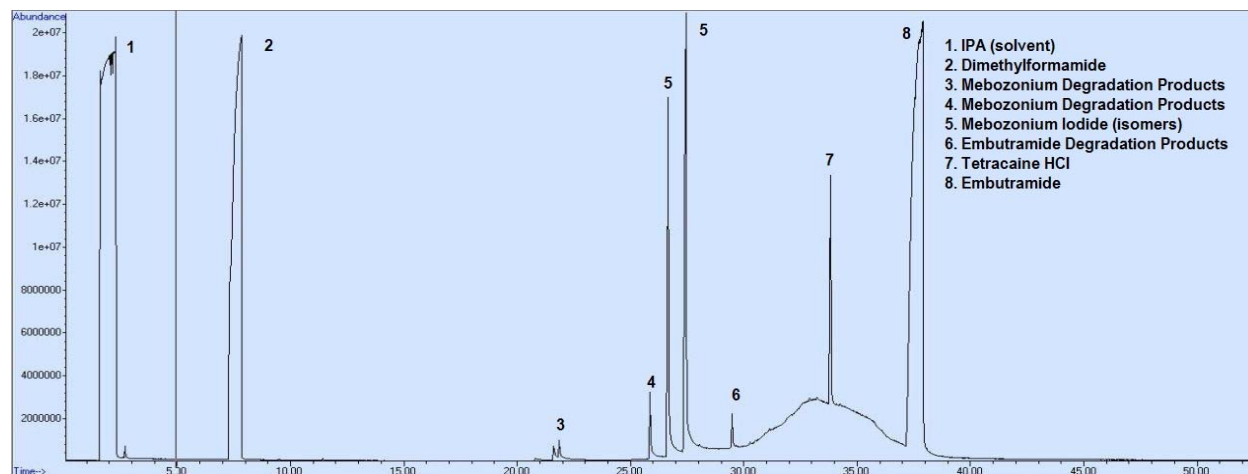


Figure 2. TIC of 1:10 Dilution of LA 569

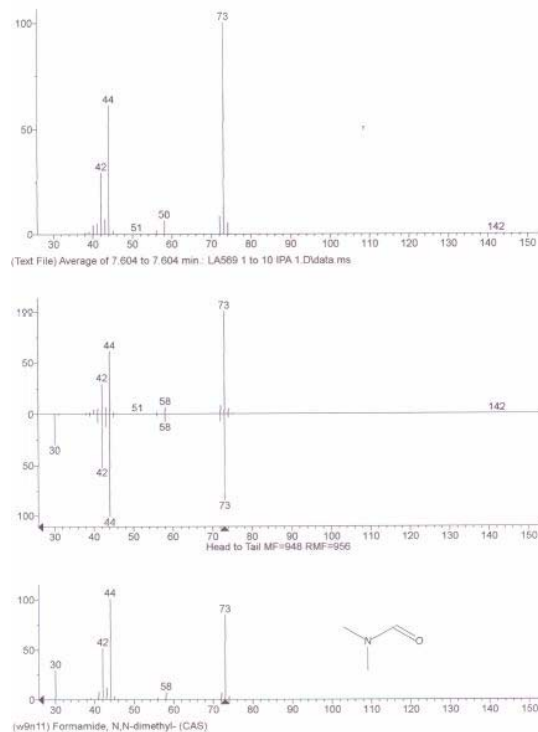
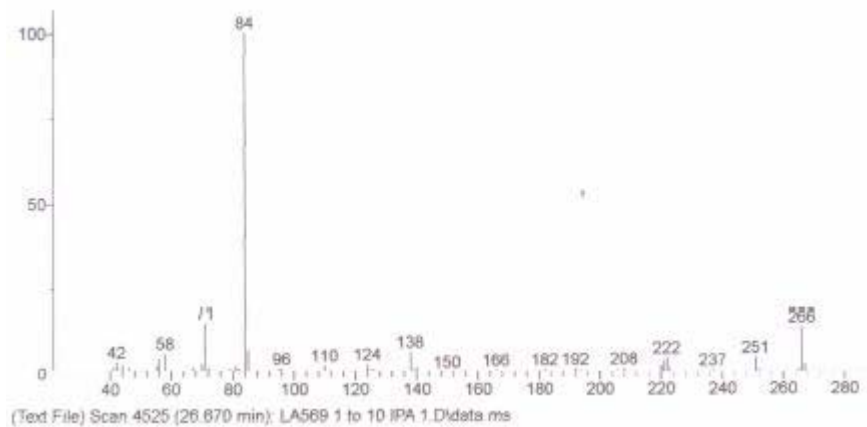
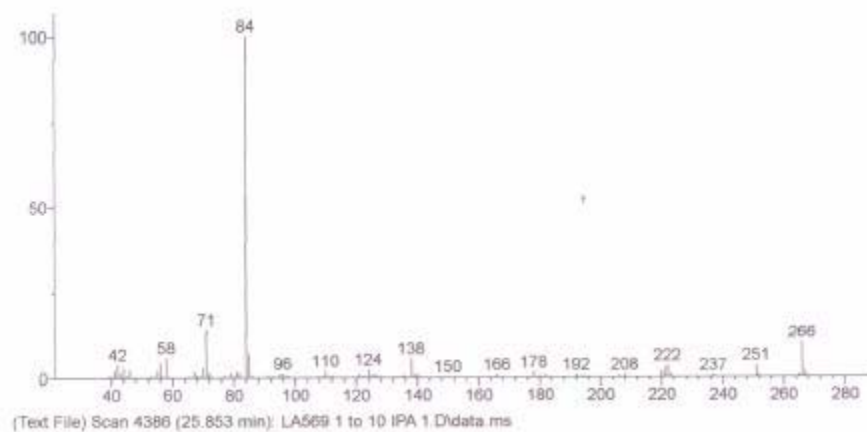


Figure 3. Mass Spectrum of DMF (Peak 2-Figure 2)



**Figure 4.** Mass Spectral Identification of Mebozonium Iodide (Peak 3 Figure 2)

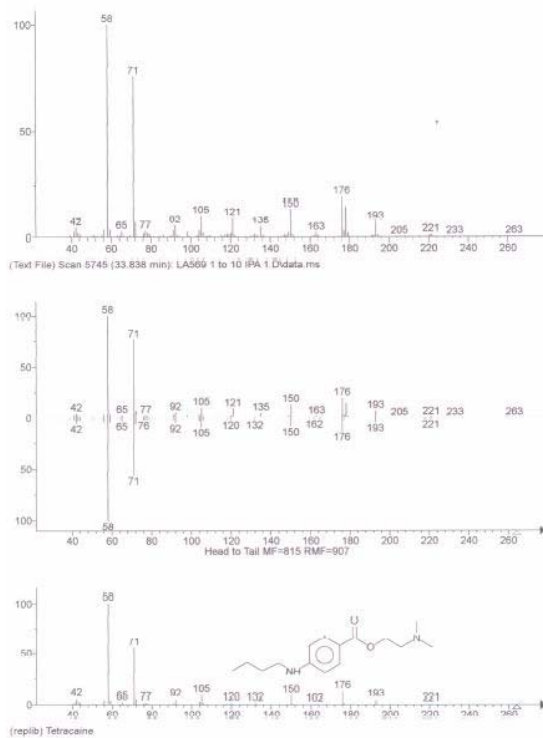


Figure 5. Mass Spectrum of Tetracaine HCl (Peak 4 Figure 2)

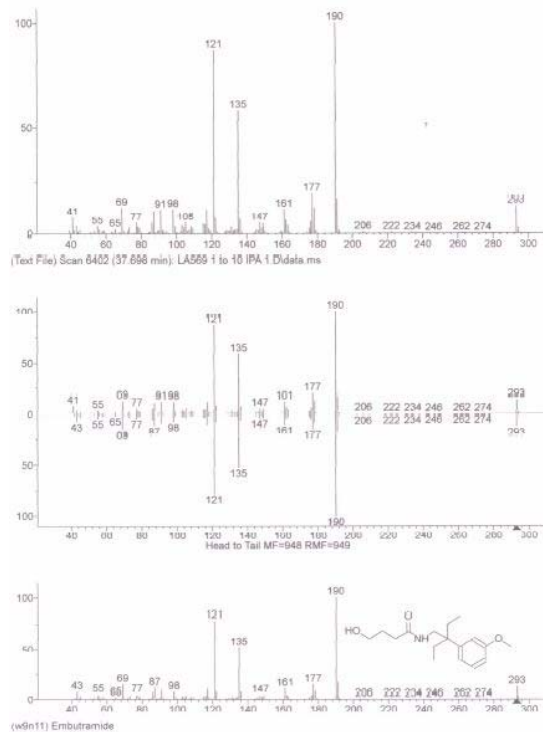
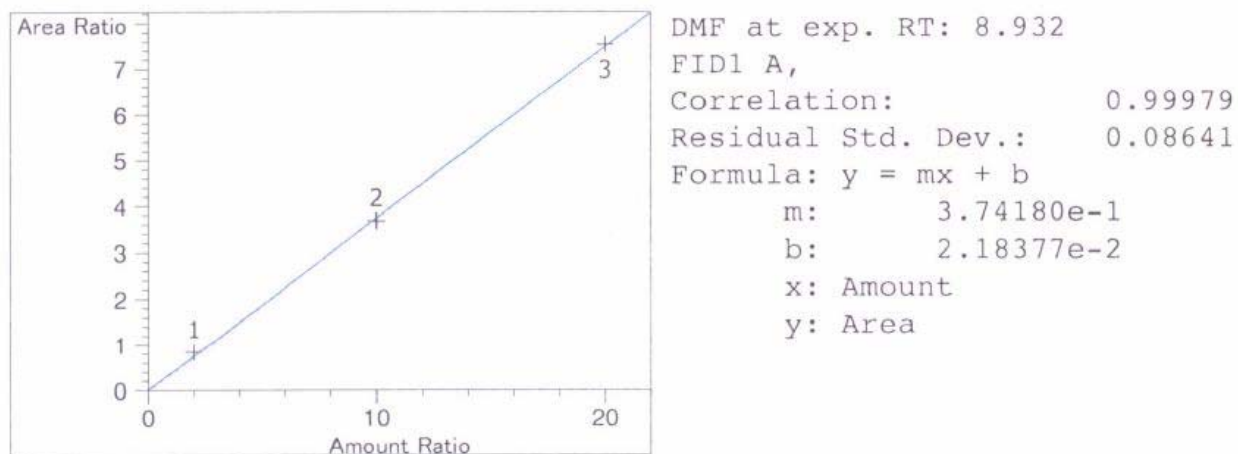


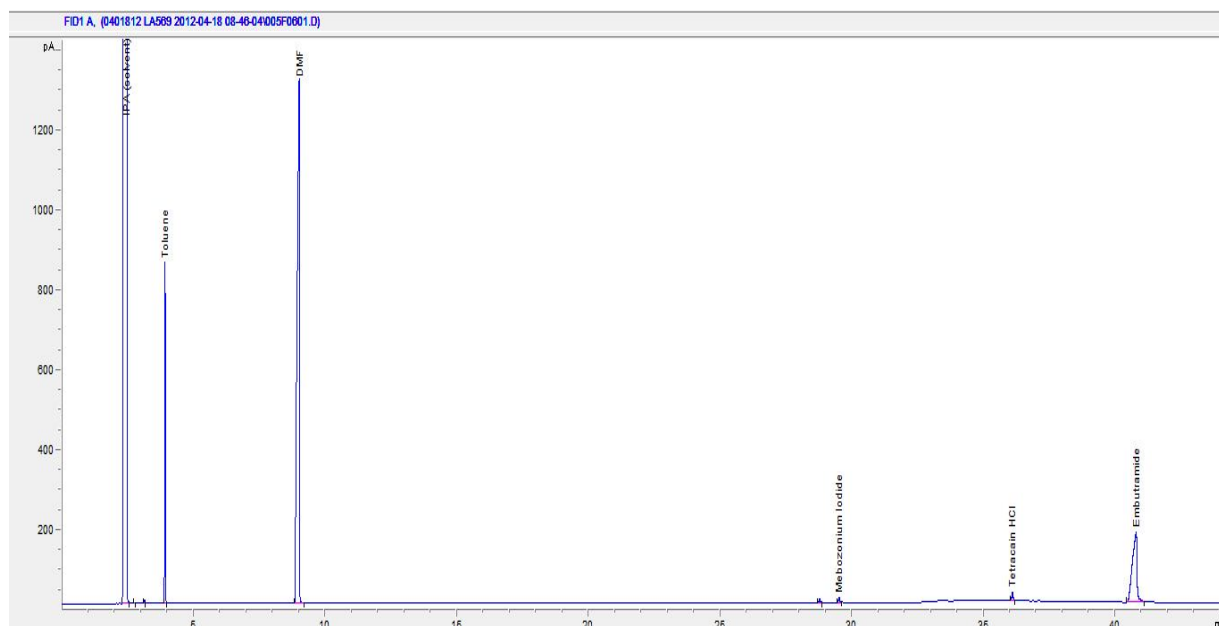
Figure 6. Mass Spectrum of Embutramide (Peak 5 Figure 2)

### GC-FID Quantitative Analysis

Standards were analyzed at 1-10% DMF by GC-FID and the concentration in the sample solution was determined using linear regression. Toluene was used as an internal stand and to semi-quantitate Mebozonium Iodide, Tetracaine HCl, and Embutramide. Linear regression analysis (Figure 7) and a representative sample chromatogram (Figure 8) are shown below:



**Figure 7.** Linear Regression Analysis for DMF Standards

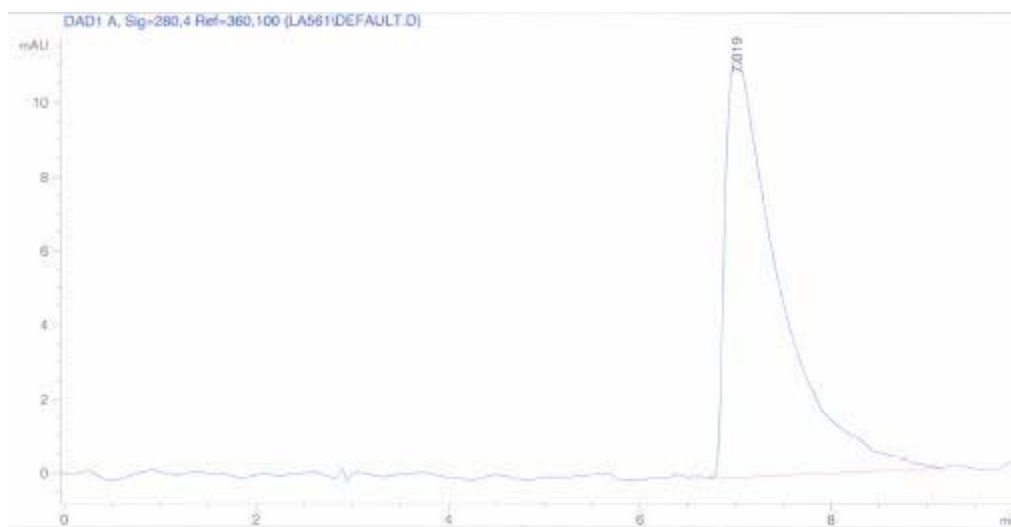


**Figure 8.** Chromatogram of Sample Diluted 1:10 with IPA

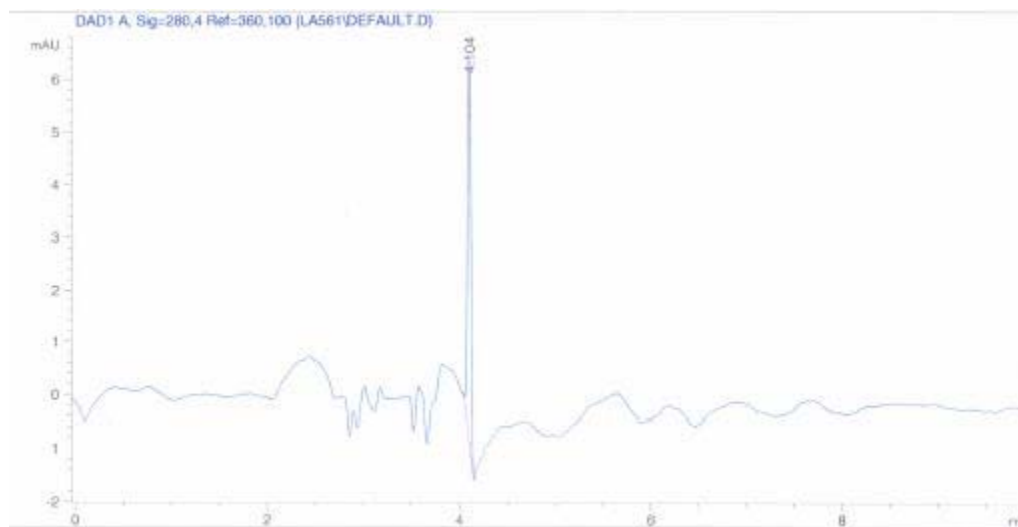


### HPLC Analysis for Epinephrine

Epinephrine standard was prepared at 53.5 ppm and analyzed at 280 nm by HPLC (Figure 9). The retention time was 7.0 minutes and no peak was detected for the sample solution (Figure 10)



**Figure 9.** HPLC-UV Chromatogram of Epinephrine at 280 nm



**Figure 10.** HPLC-UV Chromatogram of Sample Solution at 280 nm

## Description of Instrumentation Used:

**Fourier Transform Infrared Spectroscopy (FT-IR):** Avomeen utilizes FT-IR technology to obtain molecular fingerprints of unknown compounds, using transmittance measurements captured after firing an infrared laser beam at the sample compounds. Unknown fingerprints are then searched against a database of over 230,000 known FT-IR spectra in order to develop a conclusive identification. Avomeen's state-of-the-art PerkinElmer Spectrum 65 FT-IR is equipped with an Attenuated Total Reflectance (ATR) accessory, which allows our scientists to use diamond cell technology to test solid, liquid, or gas state samples in their natural state at much lower sample sizes.

**Gas Chromatography/Mass Spectroscopy (GC-MS):** GC-MS testing allows for the analysis of samples along multiple dimensions of chemical properties, providing specific identification of the different compounds separated during the GC analysis. The gas chromatograph separates a complex mixture into its individual components and delivers each one to the mass spectrometer. This analysis generates a chromatogram consisting of different peaks, one for each component of a mixture. The area of each peak is used to measure quantity. GC-MS analysis can be used both for qualitative and quantitative determinations of chemical composition.

**Gas Chromatograph (GC-FID):** GC analysis is commonly used to separate and analyze vaporized volatile compounds. This system uses an inert gas to carry the sample through a separatory column, and then detects the retention time of different compounds in the column. Avomeen's scientists often use gas chromatography to help in the identification of an unknown compound, or mixture of compounds. Avomeen's Gas Chromatography capabilities include autosampling, flame ionization detection, and the use of a range of polar and non-polar columns.

**Reverse Phase High Performance Liquid Chromatography (HPLC):** Avomeen uses a Agilent 100 HPLC with a variable wavelength detector and quaternary pump. This instrument allows one to analyze samples that absorb light in the UV-region between 190-400 nm. The system is optimized for reverse phase chromatography, which allows the analyst to utilize a highly polar mobile phase solution that carries the compound of interest through the chromatography column. The column contains a non-polar stationary phase that interacts with the compound of interest as it is pumped through the column. The compound eventually is released from the column and travels to the detector where a signal arises based on the compound's characteristic absorbance and retention time. The analog signal is converted to a usable chromatogram where the information about the compound can be analyzed with high accuracy and precision.

## Wrap Up:

Thank you for consulting with Avomeen Analytical Services. If you have any questions regarding this analysis, or if we can be of any further assistance, please call us at (800) 930-5450. Following the receipt of this final report, a final invoice will be sent to you. We will safely and securely dispose of all samples and confidential information in our possession in 30 days, unless otherwise instructed by your company.

It has been a pleasure working with you and we look forward to serving you again.

Sincerely,

**Avomeen Analytical Services**

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