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Collection, Validation and Generation of Asphalt Roofing Fumes for Reproductive/Developmental Toxicity Study

Prepared for the AMERICAN PETROLEUM INSTITUTE HPV TESTING GROUP

CONSORTIUM REGISTRATION # 1100997

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Table of Contents

Summary

Personal exposure samples from an built up roofing (BUR) operation using Type III asphalt were analyzed and compared to the corresponding fume condensate collected from above a storage tank using the same asphalt. Acceptance criteria was designed to match parameters that could be analyzed both on the workplace samples and the asphalt fume condensate collected from the tank. These parameters include boiling point distributions, fluorescence analysis, qualitative comparisons between individual PAHs and qualitative characterization of selected extracted ions from gas chromatography/ mass spectroscopy (GC/MS) analyses. As a result, 2400 grams of roofing asphalt fume condensate, representative of roofing worker exposures of the toxicologically significant components for this reproductive/developmental toxicity study have been collected. The protocol used in this study was adopted from a similar study for the collection of asphalt roofing fumes sponsored by the Asphalt Roofing Environmental Council (AREC). Additionally, a Scientific Advisory Committee (SAC) to the AREC was created to assist with the study design and protocol development of its proposed dermal carcinogenicity assay. Moreover, the same asphalt batch that was used for one of the AREC asphalt fume condensate test materials (identified as TR-A) was used for this study.

1 Introduction

This research program was conducted for the American Petroleum Institute (API) Petroleum High Production Volume (HPV) Testing Group for its inhalation reproductive/developmental toxicity-screening test, which was identified as a data gap to complete the hazard profile for streams in this category. This fume condensate is intended for use in the OECD 421 protocol in rats and a satellite micronucleus assay (OECD 474) to clarify current cytogenetic data. No developmental or reproductive toxicity studies have been conducted on an asphalt fume condensate, so it is intended to provide critical toxicology information relevant to the roofing asphalt industry and its workers.

2 Objectives

Two basic goals were addressed in preparation for the final goal of a reproductive developmental toxicity study using Type III roofing asphalt. First was the collection of industrial hygiene (IH) exposure samples during hot asphalt built up roofing application.

Secondly, a large volume of condensed storage tank asphalt fumes that chemically mimic select physical and chemical characteristics of the corresponding IH exposures were collected from the roofing asphalt. The same batch of source asphalt was used for both the IH portion and the large volume storage tank collection scheme of this study.

3 Validation Criteria for Acceptance

Criteria for acceptance are outlined in Table 1. This was determined after many discussions with the Scientific Advisory Committee.

Table 1. Acceptance Criteria

Analytical Test	Acceptance Criteria	
Simulated Distillation	% Distilled	Criteria
	10	$\pm$ 20°C
	50	$\pm$ 15°C
	90	$\pm$ 10°C
UV Fluorescence	$\pm$ 15% RPD*	
PAH Profile	Qualitative	
Selected Ion GC/MS Fingerprinting	Qualitative	

*Relative Percent Difference = [(difference x 2)/sum]

4 Industrial Hygiene Sampling

4.1 Overview

While valid fumes from the industrial hygiene measurements of worker exposure are of utmost importance in this study, the industrial hygiene sampling was designed to identify and estimate relative proportions of materials in the fume believed to be most important for evaluating the toxicological endpoint of interest.

At the Heritage facility in Indianapolis, Indiana, the roofing bitumen was received as a bulk hot liquid. A portion of the bitumen was poured into 100-pound cartons and solidified upon cooling. These cartons were then transported to the job site in Atlanta, Georgia. The remaining asphalt was retained for large volume fume collection from the tank headspace. Arrangements were made with Tip Top Roofers, Inc. (the contractor) to use this study asphalt (BURA Type III) while workers were monitored. For this study site, the crew consisted of twelve workers; four who were monitored due to their specific job duties; one kettleman, two moppers, and one felt layer/cutter. To minimize a typical field confounder, all crewmembers were asked not to smoke on the job, and all workers complied during sampling.

Each of the four workers were equipped with two separate sampling assemblies; one a MSA Escort LC personal sampling pumps calibrated to 2 L/min, connected to a 2.0 micron PTFE membrane filter laminated to PTFE (SKC Cat. No. 225-17-07 or equivalent) hydrophobic filter, with cellulose support pad in a 37-mm opaque cassette filter holder (NIOSH Method 5042) (1), backed up with a XAD-2 sorbent tube (SKC 226-30-04). This sampler contained a pre-filter to allow quantitative exposure comparisons to historical data. The other excluded a pre-filter to avoid complications when separating the filter portion from the XAD-2 sorbent tube. Complications have included contaminants introduced by the filter portion or connector to the

XAD-2 and loss of some of the components during the benzene soluble matter (BSM) extraction process. XAD-2 sorbent tubes were eluted with methylene chloride to a final volume of ten milliliters as per other Heritage studies (2, 3).

Similar to the worker collection systems, two background areas (free of asphalt fumes) were also monitored to assure that exposures were not confounded by other environmental factors. Background sample 1 (XAD-2 + filter) was located on the south side of the 6^{th} floor and background 2 (XAD-2 only) was located on the north side of the 5^{th} floor of the building.

In addition to worker and background samplers, mannequins were also utilized to hold XAD-2 sorbent tubes (without pre-filters) to obtain more data points at higher concentrations. Thirteen samplers were attached to the rooftop mannequin at various heights that represented the potential breathing zone of a worker while mopping. Specifically, the open XAD-2 ends were placed between 23 and 35 inches above the rooftop. For the kettle mannequin, three samplers were positioned near the kettle with the XAD-2 tubes positioned at approximately the height of the kettleman's breathing zone.

Each mannequin was designed and positioned in a strategic location to represent employee asphalt fume exposures. Three samplers were attached to a mannequin positioned near the kettleman that remained near the kettle throughout the duration of the sampling period. The second mannequin carried 13 samplers and was frequently maneuvered around the rooftop following the moppers to simulate worker exposure. Figure 1 is a photograph taken during the IH monitoring process on the rooftop. With an attempt to minimize interruption of the normal workflow, the mannequin was moved around by the industrial hygienist to capture emissions in the area of the workers. Table 2 provides details of all samples collected during the roofing project.

4.2 Roofing Conditions

In Atlanta, Georgia, the project occurred atop a nine-story, 3400 square foot rooftop. The 250-gallon kettle used was placed near the center of the rooftop during sampling. Heritage personnel verified that this kettle was empty with photographs prior to the asphalt being loaded, to limit possible contamination from residual products. For the purposes of this study, "industrial" clean was defined as less than one percent contamination from residual products. A sample of this asphalt residue was scraped from the kettle and tested for coal tar contamination using the Heritage full scan fluorescence method (4). This data is provided in Appendix A and demonstrates that the kettle was free of any coal tar contamination prior to use in this study.

On the afternoon prior to the asphalt roofing application the 100-pound asphalt study cartons were transported to the rooftop via crane. On the day of application the kettle was heated in the early morning hours, with the first mop bucket of asphalt being applied at 10:15 am. Pumps on the workers and mannequins were turned on when the roofers first started working with the hot asphalt. Figure 2 demonstrates the worker mopping application technique, where a thin layer of hot asphalt is spread with a mop prior to mounting the board. This picture also shows the position of the rooftop mannequin.

Circumstances about this job that may have influenced the concentration of the asphalt fume exposures include the small size of the rooftop and kettle used, the blockage of the wind by a one-story wall and a safety wall around the roof perimeter (see Figure 1). All workers appeared to be simultaneously exposed to kettle fumes and the mop-bucket fumes, since the area was so small.

Figure 1: A Mobile Mannequin is Positioned Near the Workers during a Hot Asphalt Built up Roofing Application

Figure 2. Mopping Application Techniques:

Table 2: Description of Samples Collected Including ID, Sampling Time, and Volume of Air

Description	Sample ID	Sampling Time (minutes)	Volume (Liters of Air)
Kettleman	XAD-146	360	714
Kettleman	XAD-147, AR-94	360	734
Mopper 1	XAD-148	360	716
Mopper 1	XAD-149, AR-95	360	703
Mopper 2	XAD-150	360	741
Mopper 2	XAD-151, AR-96	360	718
Felt Layer/Cutter	XAD-152	360	735
Felt Layer/Cutter	XAD-153, AR-97	360	714
Background 1	XAD-141	360	736
Background 2	XAD-142, AR-93	360	718
Rooftop Mannequin Sampler 1	XAD-154	360	727
Rooftop Mannequin Sampler 2	XAD-155	360	734
Rooftop Mannequin Sampler 3	XAD-156	360	738
Rooftop Mannequin Sampler 4	XAD-157	360	715
Rooftop Mannequin Sampler 5	XAD-158	360	748
Rooftop Mannequin Sampler 6	XAD-159	360	735
Rooftop Mannequin Sampler 7	XAD-160	360	714
Rooftop Mannequin Sampler 8	XAD-161	360	715
Rooftop Mannequin Sampler 9	XAD-162	360	729
Rooftop Mannequin Sampler 10	XAD-163	360	732
Rooftop Mannequin Sampler 11	XAD-164	360	740
Rooftop Mannequin Sampler 12	XAD-165	360	735
Rooftop Mannequin Sampler 13	RM-13	360	704
Kettleman Mannequin Sampler 1	XAD-143	360	709
Kettleman Mannequin Sampler 2	XAD-144	360	726
Kettleman Mannequin Sampler 3	XAD-145	360	724

4.3 Meteorological Information

Strong winds and other weather conditions can sometimes hinder collection of asphalt fumes. In general, the conditions during the industrial hygiene-sampling day were relatively calm, consisting of < 2 mph sustained winds, >55 ^{o}F ambient air temperature and no rain after the samplers were started. Weather conditions collected at interval times throughout the day are presented in Table 3.

Table 3. Summary of Meteorological Information

Time	Temperature (^oF)	Relative Humidity (%)	Wind Speed (mph)	Other
9:20 AM	55	58	0 to 1.4	cloudy, light drizzle
11:30 AM	56	56	0 to 3.1	cloudy
12:15 PM	62	54	0.4 to 0.8	cloudy
2:15 PM	64	52	0 to 1.7	partial sunshine

4.4 Hot Asphalt Temperatures

While sampling in the field, liquid asphalt temperatures were monitored at the kettle and mop bucket at various times throughout the workday. A total of twelve different temperature readings were collected using a handheld Cole Palmer thermocouple thermometer. Table 4 provides where the temperature was taken (mop bucket or kettle), the time, and the temperature reading.

Table 4. Roofing Asphalt Temperatures at the Job Site

	Reading 1	**Reading 2**	**Reading 3**	**Reading 4**
Time	10:34 AM	10:36 AM	11:25 AM	11:25 AM
Temperature (^oF)	478	525	505	398
Location	Mop Bucket	Kettle	Kettle	Mop Bucket
	Reading 5	**Reading 6**	**Reading 7**	**Reading 8**
Time	12:01 PM	12:01 PM	1:25 PM	1:27 PM
Temperature (^oF)	517	486	482	545
Location	Kettle	Spreader	Mop bucket	Kettle
	Reading 9	**Reading 10**	**Reading 11**	**Reading 12**
Time	1:50 PM	1:50 PM	2:15 PM	2:30 PM
Temperature (^oF)	434	526	404	503
Location	Mop bucket	Kettle	Mop bucket	Kettle

4.5 Analytical Descriptions

Demonstrating that the fume condensate samples to be used for toxicity studies mimic select physical and chemical characteristics of the fumes from actual roofing operations was a critical component of this study. To accomplish this task, a series of analytical techniques were used, outlined with their respective methodologies in Table 5.

Table 5. Summary of Analytical Methodologies.

Test	**Method**
Particle Size Monitoring	Using TSI® 3320 Particle Size Analyzer
TPM/BSM (worker only)	NIOSH 5042 (1)
TOM (field only)	SW-846-8015B Mod. (5)
Simulated Distillation	ASTM D-2887 (6)
Fluorescence	Heritage Method (4)
PAH Analysis	GC/MS SW-846 8270C (7)
Fingerprinting	Selected Ion GC/MS (8)

Real time particle size was monitored using a TSI Aerosol Particle Size Analyzer® Model 3320 during the collection process. This technique allowed instantaneous characterization of a snapshot (20 seconds) of particulate exposure, which helped determine if parameters were set up correctly and being maintained during the large volume, storage tank fume collection process. Field use of this instrument allowed comparison of particle size distributions and exposure concentrations between the various workers and their respective mannequin samples.

Gas chromatography with flame ionization detection (GC/FID) was used to analyze two parameters. Total organic matter (TOM) for field samples provided information regarding the amount of fume collected (modified SW846-8015B (5). Simulated distillations were performed using ASTM Method D-2887 (6) to determine the boiling point range of all asphalt fume extracts.

Fluorescence analysis was performed using a Perkin Elmer Luminescence Spectrometer LS50B following the asphalt fume fluorescence (AFF) test protocol outlined in a separate publication (4). This is a screening test designed to optimize response to any carcinogenic compounds within asphalt fumes, if present. Specifically it optically isolates the 4-6 ring polycyclic aromatic compounds (PACs) believed to be responsible for any carcinogenic activity.

GC/MS was utilized for analysis of the traditional EPA sixteen polycyclic aromatic hydrocarbon (PAH) compounds following a modified SW-846 8270 method (7). GC/MS total ion chromatograms were also obtained to determine the presence or absence of similar compound classes in both IH and tank samples. Specifically, samples were validated by comparing n-alkanes, naphthalene, and some of their alkylated homologous series of isomers. Fingerprints from these extracted ion chromatograms were very similar between the field and tank fumes. Further characterizations were provided for additional assurance of compositional similarity by obtaining the extracted ions listed in Table 6. This table contains the extracted ions chosen to characterize the roofing fume.

Table 6. List of Extracted Ions (m/z) for Validation and Characterization

Compounds	**Extracted Ions (m/z)**	**Use**
n-alkanes	85	Validation
naphthalene	128	Validation
alkylated naphthalenes	142,156,170	Validation
monocycloalkanes	69	Characterization
benzothiophene	134	Characterization
alkylated benzothiophenes	148,162,176	Characterization
dibenzothiophene	184	Characterization
alkylated dibenzothiophenes	198,212	Characterization
benzofuran	118	Characterization
alkylated benzofurans	132, 146	Characterization
dibenzofuran	168	Characterization
alkylated dibenzofurans	182, 196	Characterization

All fume samples were individually analyzed by GC/FID to obtain a simulated distillation before combining to verify the validity of each sample. To demonstrate that these mannequin samples were consistent with worker exposure, an equal amount of sample from each of the valid mannequin samples (specific to each site) was combined for analysis as specified in the protocol used for the Asphalt Roofing Environmental Council (AREC) collection, validation and generation of asphalt roofing fumes intended for other research studies. To simulate typical ratios of rooftop workers to kettleman a ratio of 4.3 to 1 (based on 13 rooftop mannequin samples and 3 kettle mannequin samples) was used during preparation of the "Official IH" sample. By using this combined extract, it is apparent that the intent of this study was to collect typical asphalt fume exposures rather than looking for worst-case conditions. This composite mannequin extract was compared to a similarly prepared composite worker sample. These composite extracts were characterized using the techniques described above. The characterization ions are also outlined in Table 6. Oxygenated heterocyclic PACs were included in this list due to the oxidation that generally occurs in roofing products.

Finally, a Modified Ames assay was conducted on the final fume testing material. Mutagenicity is the potential for a chemical to increase the frequency of mutations by directly or indirectly modifying the genome or its expression. The slope of the line relating a material's dose to reverterant is the mutagenicity index (MI) (9). This test allowed comparison to the AREC TR-A asphalt fume condensate.

4.6 Results

4.6.1 <u>Gravimetric Data (TPM and BSM)</u>

Following NIOSH analytical method 5042 (1), gravimetric data as Total Particulate Matter (TPM) and Benzene Soluble Matter (BSM) are outlined in Table 7, showing the individual worker exposures as well as an average result. Possibly due to the job conditions as discussed at the end of section 4.2, these concentrations are all above the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV®) of 0.5 mg/m^3 as benzene extractable inhalable particulate. Industrial hygiene reports were distributed to the contractor with individual reports for each worker.

Table 7. Industrial Hygiene Gravimetric Data (TPM and BSM)

Description	**Volume of Air (Liters)**	**TPM (mg/m^3)**	**BSM (mg/m^3)**	**TWA TPM (mg/m^3)**	**TWA BSM (mg/m^3)**
Kettleman	734	1.71	1.5	1.29	1.12
Roof Worker 1	703	1.04	0.84	0.78	0.63
Roof Worker 2	718	1.21	0.95	0.91	0.72
Roof Worker 3	714	0.9	0.69	0.68	0.52
Average	**717**	***1.22***	***1.00***	***0.91***	***0.75***
Background Average	718	0.06	-0.02	0.05	-0.01

TWA=Time weighted average TPM=Total Particulate Matter BSM=Benzene Soluble Matter

4.6.2 Simulated Distillations

Table 8 outlines the boiling point distributions as provided by GC/FID simulated distillation data described in the ASTM D-2887 (6) method. The 10, 50 and 90 percent-distilled data are listed below in degrees centigrade to aid comparison. Averaging the kettle worker and mannequin exposures, results show a 10% distilled at 219°C, a 50% at 343°C and a 90% at 432°C for the kettle area. The average rooftop area and worker results show a slightly lighter boiling point distribution: 10% distilled at 211°C, 50% at 333°C and 90% at 430°C. GC and GC/MS chromatograms are shown in the storage tank results section for comparison between the IH samples and the tank fume condensate.

Table 8. Industrial Hygiene Simulated Distillation Data (^oC)

	Kettleman			Kettle Area Mannequin		
Asphalt	10%	50%	90%	10%	50%	90%
Roofing	215	340	431	222	345	433
	Roof Worker			Roof Area Mannequin		
Asphalt	10%	50%	90%	10%	50%	90%
Roofing	215	326	426	207	339	434

4.6.3 Total Organic Matter

Total organic matter (TOM) includes organics captured by the XAD-2 sampler plus the BSM. Average results for the worker and mannequin samples are listed in Table 9 below.

Table 9. Average Industrial Hygiene TOM Results (mg/m^3)

Description	mg/m^3
Rooftop Workers	1.19
Rooftop Mannequins	0.76
Kettle Worker	2.28
Kettle Mannequins	2.19

4.6.4 Fluorescence

Fluorescence results obtained from a Perkin Elmer Luminescence Spectrometer (LS50B) are listed in Table 10 for the official industrial hygiene sample, the kettle mannequin, the roof mannequin, the kettle worker, the roof workers and the official HPV tank sample. Differences between the mannequin and the worker data are within the acceptance criteria of 15% relative difference. Moreover, the difference between the official IH sample and the official tank sample was 1.8% RPD.

Table 10. Fluorescence results for Industrial Hygiene Samples (EU/g)

Source	**EU/g**	Reference is **Bold**	**%RPD***
Official HPV Tank Official IH	**163** **166**	Official Tank vs. **IH**	**1.8**
Kettle Mannequin Kettle Workers	**120** **113**	Kettle Mann. vs. **Kettle Worker**	**6**
Roof Mannequin Roof Workers	**150** **162**	Roof Mann. Vs. **Roof Worker**	**-7.7**

**Difference times 2 divided by the sum times 100*

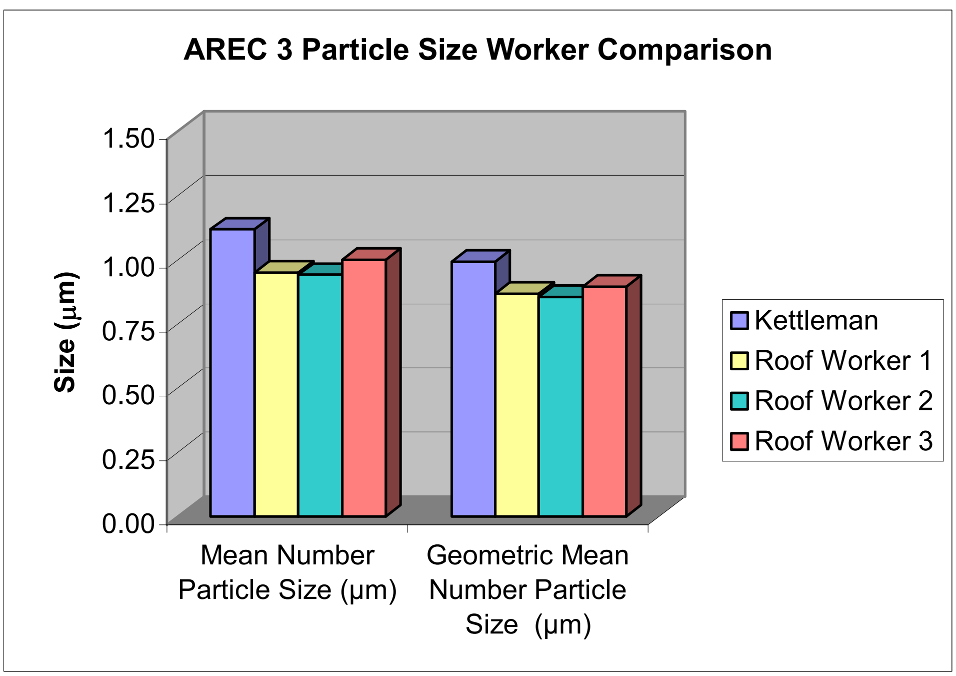
4.6.5 <u>Particle Size Data</u>

Aerodynamic particle size data were collected twice during this exposure assessment project, once in the morning and once in the afternoon. During these two sample collection times a total of three 20-second snapshots were obtained and geometric means calculated. Size distributions from 0.5 to 20 μm were counted and light-scattering intensity for particles from 0.3 to 20 μm detected. Instrument set-up conditions included a 20 second sampling time in summed mode (continuous) with a baud rate of 9600 and an inlet pressure of ~980 mbar. With a total flow rate of 5 liters per minute (lpm), the sheath flow rate was 4-lpm and the aerosol flow rate was 1-lpm. Special carbon impregnated conductive silicone tubing (8 ohms/cm), 7.62 m in length and 11.2 mm internal diameter, was used for all particle size samplings. The particle size analyzer provided data that include the mean number particle size data (microns) and the geometric mean number particle size (microns). Table 11 displays the results for the morning and afternoon sampler by location. Figure 3 graphically displays the mean number particle size and geometric mean number particle size for each worker. Results show consistency of particle size for all roofing workers with a relative standard deviation of <15%.

Table 11. Particle Size Summary Data from the Industrial Hygiene Sampling

Description		Mean Number Particle Size (µm)	Geometric Mean Number Particle Size (µm)
Background	AM	0.83	0.75
	PM	0.8	0.73
Kettleman	AM	1.17	1.02
	PM	1.07	0.96
Mopper 1	AM	0.85	0.8
	PM	1.06	0.94
Mopper 2	AM	0.88	0.81
	PM	1	0.9
Felt Layer/Cutter	AM	1.18	1.04
	PM	0.85	0.77
Rooftop Mannequin	AM	1.07	0.98
	PM	0.97	0.89
Kettle Mannequin	AM	1.2	1.04
	PM	1.15	1.02
Average		***1.04***	***0.93***
Std. Deviation		*0.13*	*0.10*
Relative Std. Deviation		*12.4*	*10.4*

Figure 3. Comparison of Worker Particle Size Data Collected



5 Storage Tank Fume Condensate

5.1 Overview

Large volume fume condensates above asphalt storage tanks, containing ~5,500 gallons (22 metric tons), were collected over a period of several weeks and compared to IH field samples. A total of 2400 grams was collected for the reproductive/developmental toxicity assays and retention of an archived sample of the fume.

5.2 Collection Description

Heritage used a fume collection system set-up modeled after that of the Fraunhofer Institute of Toxicology and Experimental Medicine (10). Figure 4 shows a set of photos showing the tanker with cartons, the heated hose used to transport the fumes from the tank headspace to condensation and collection assembly contained in a shed as pictured in Figure 5. This portion of the study occurred at Asphalt Materials, Inc. on 86th St. in Indianapolis, IN. Figure 6 (10) is a schematic of the Fraunhofer setup, which is near two asphalt tanks used for sampling. The fumes of the heated asphalt in the tank were directed through a ½ inch diameter heated tube and a cooling spiral into a Peltier condenser, with the cooler set at a temperature of 5^{o}C. The asphalt condensate was sampled in a 10-liter polyethylene receiver bottle at a vacuum pressure of 800 mbar. A second polyethylene bottle served as a water trap to protect the chemical resistant vacuum pump, which operated at flow rates between 30 and 40 liters/minute.

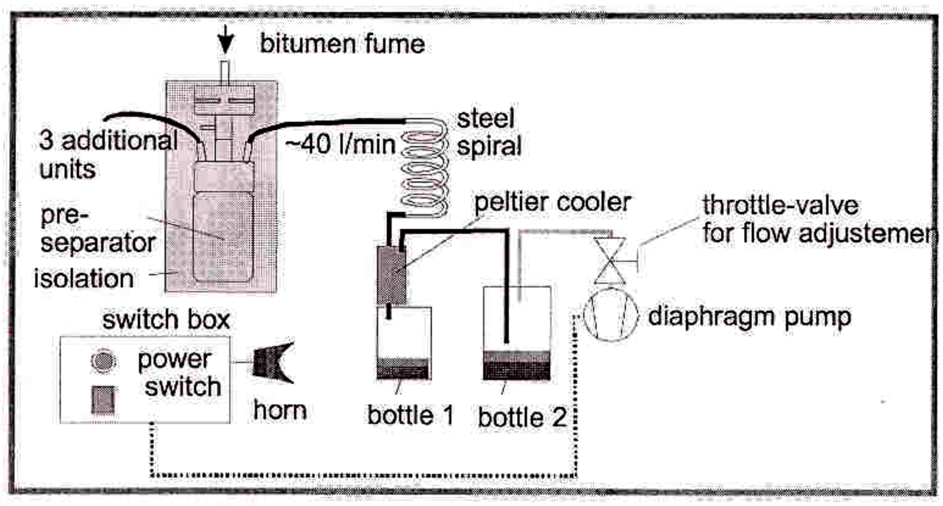
5.3 Asphalt Description

Type III roofing grade asphalt (CAS 64742-93-4) was used in this study and shipped to the HRG facility in Indianapolis, Indiana for generation of the asphalt fume condensate test material. Type III roofing asphalt was selected based on the fact it is the most prominent used grade in the United States. The Type III asphalt used in this was stored in a 10,000-gallon (40 m^3) horizontal tank dedicated for this fume collection study. Upon receiving the asphalt at the collection facility, more than three hundred 100-pound cartons of asphalt were poured for the industrial hygiene portion of the roofing project. Information was provided from the asphalt producer regarding the crude slate and general refining conditions employed at the time of manufacture, including theoretical cut point.

Figure 4. Photograph of Tanker, Cartons and Heated Hose used to Collect Roofing Asphalt Fume Condensate

Figure 5. Photograph of Shed Housing the Collection Components of the System

Figure 6. Schematic of Fraunhofer Set-up



5.3.1 <u>Softening Point Data</u>

To assure that the grade of asphalt remained the same during large volume collection, a softening point using method ASTM D36-95 (11) was performed at the beginning and end of the tank sample collection period. Results confirm that at the end of the collection period the Type III grade had been retained. To qualify as Type III grade asphalt, the softening point value must range between 185°F and 205°F. Table 12 provides the before and after sampling softening point values.

Table 12. Softening Point Values Pre and Post Fume Collection

	Softening Point Temperature (°F)
Before Collection of Large Volume	197
After Collection of Large Volume	202

5.4 Extraction Procedures

Asphalt fumes collected in the Fraunhofer condensation process were placed into a two-liter separatory funnel allowing the oil and water phase to separate. After draining the water phase into a second separatory funnel, the oil layer was passed through sodium sulfate to remove any residual water. Oil retained in the sodium sulfate was rinsed with methylene chloride into the water phase, which was extracted in triplicate, using 100 mL for the first extraction and 50 mL for the next 2 extractions, for 2 minutes each time. Methylene chloride extracts were also passed through sodium sulfate. These combined extracts were rotary-evaporated at 40°C under reduced pressure. The residue from this process was combined with the original oil to obtain the final fume condensate test material.

5.5 Other Analytical Tests

The storage tank fume condensate was characterized using the analytical methodologies previously described in the Industrial Hygiene Analytical Descriptions Section 3.4. Only the IH field samples were analyzed for TPM, BSM, and TOM. Simulated distillation, fluorescence, PAH analysis and GC/MS fingerprinting were performed on the storage tank condensates as well as the IH field samples.

Additionally, a modified Ames test (MI) was performed on the HPV final tank sample composite in accordance with the procedures described in ASTM Standard Method E 1687-04 (9). Mutagenicity is the potential for a chemical to increase the frequency of mutations by directly or indirectly modifying the genome or its expression. The slope of the line, relating dose to reverterant, is the mutagenicity index (MI).

Other material properties such as densities and kinematic viscosities were performed on the storage tank condensates and are listed in the result section.

5.6 Results

5.6.1 Physical Properties of Fume Condensate

5.6.1.1 Density

As a measure of the mass per unit volume, the density of the HPV roofing asphalt fume condensate is 0.8745 g/mL.

5.6.1.2 Kinematic Viscosity

As a coefficient that describes the diffusion of momentum, Kinematic viscosity for the fume condensate is 8.3616 centi stokes at 100^{o}F.

5.6.2 Chemical and Biological Testing

5.6.2.1 Simulated Distillation

Simulated distillation data are listed for the tank fume condensate as well as the “Official IH” sample in Table 13 along with the differences in ^{o}C between these data at the % distilled points specified in the acceptance criteria.

Chromatograms from the GC/FID, which plot time on the x axis versus intensity on the y axis are shown below in Figure 7 visually demonstrating the agreement in simulated distillation between the official industrial hygiene sample and the HPV tank sample. TR-A, the fume condensate collected from the same asphalt batch for the AREC study, is listed for comparison.

Table 13. Tank vs. Official IH Simulated Distillation Data

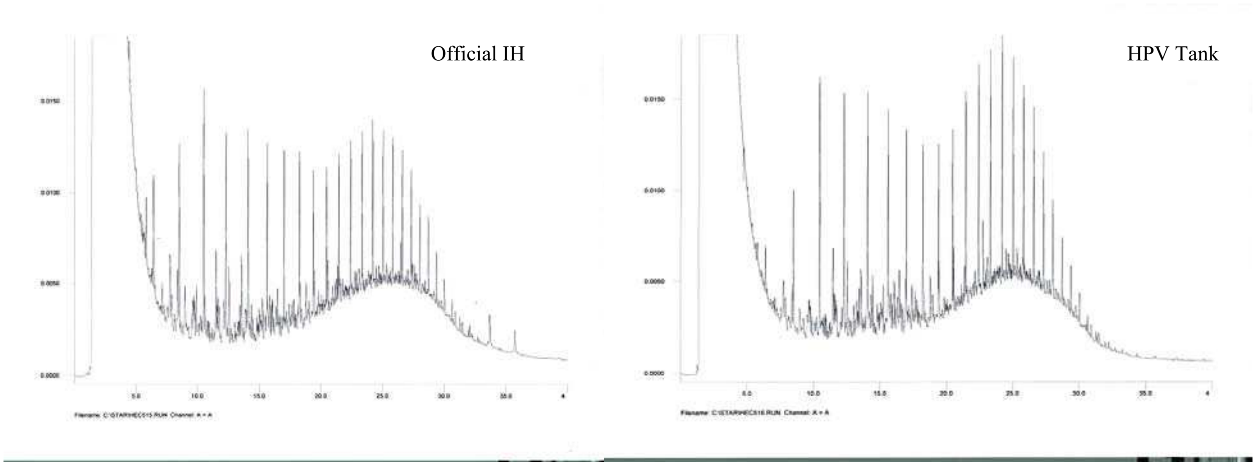
	Simulated Distillation ^oC			**^oC Differences**		
	10%	50%	90%	^oC	^oC	^oC
IH_o	215	350	436			
HPV Tank	218	345	427	*3*	*5*	*9*
TR-A	208	342	432	*11*	*3*	*-8*
Acceptance Criteria Requirement				≤20	≤15	≤10

IH_o = Official IH Sample

5.6.2.2 Fluorescence

The fluorescence response for the HPV tank sample is 166 EU/g versus 163 EU/g for the official IH sample and differs by only 1.8%RPD. As required in the acceptance criteria, this fluorescence data combined with the simulated distillation data quantitatively validate that the test material matches the asphalt fumes to which workers are exposed. The qualitative tests via GC/MS also confirm these similarities.

Figure 7.



5.6.2.3 GC/MS PNA data

GC/MS PNA data are listed in Table 14. Only three compounds are detected. The tank fume condensate could have been analyzed at lower concentrations resulting in lower detection limits, but since the goal was to compare it to the field, it was analyzed at the same concentration as found on the worker samples. The 16 PNAs on EPA's list that are not listed in this table were below the detection limit (BDL) of 130 mg/kg.

Table 14. GC/MS PNA Data (mg/kg) on HPV Roofing Asphalt Fumes

	HPV Tank	IH	Roof Worker	Roof Mannequin	Kettle Worker	Kettle Mannequin
Acenaphthene	BDL	BDL	BDL	BDL	BDL	BDL
Anthracene	BDL	BDL	BDL	BDL	BDL	BDL
Fluoranthene	BDL	BDL	BDL	BDL	BDL	BDL
Fluorene	**300**	**350**	**230**	**230**	**130**	**150**
Naphthalene	**180**	**520**	**480**	**540**	**240**	**280**
Phenanthrene	**230**	**520**	**550**	**620**	**300**	**380**
Pyrene	BDL	BDL	BDL	BDL	BDL	BDL

BDL= below detection limit of 130 mg/kg.

5.6.2.4 GC/MS Fingerprints

GC/MS Fingerprints are shown in Figures 8 through 12 for the extracted ions used in the validation of the fume. These include extracted ions (m/z) for the n-alkanes (85), naphthalene (128) and alkylated naphthalenes (142, 156 & 170). All of the remaining extracted ion chromatograms used for characterization are presented in Appendix B. The official IH composite sample chromatogram is always presented at the top and the HPV tank fume condensate is always presented at the bottom in an inverted manner within these comparative chromatograms. Results show excellent agreement between what the workers are exposed to and the composition of the HPV test material.

Figure 8. HPV Roofing Extracted Ion 85

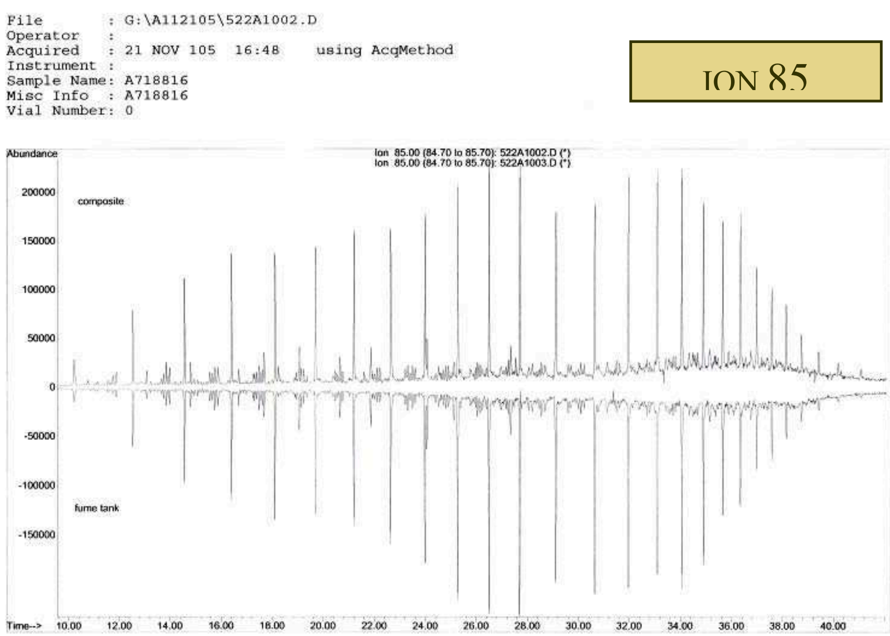


Figure 9. HPV Roofing Extracted Ion 128

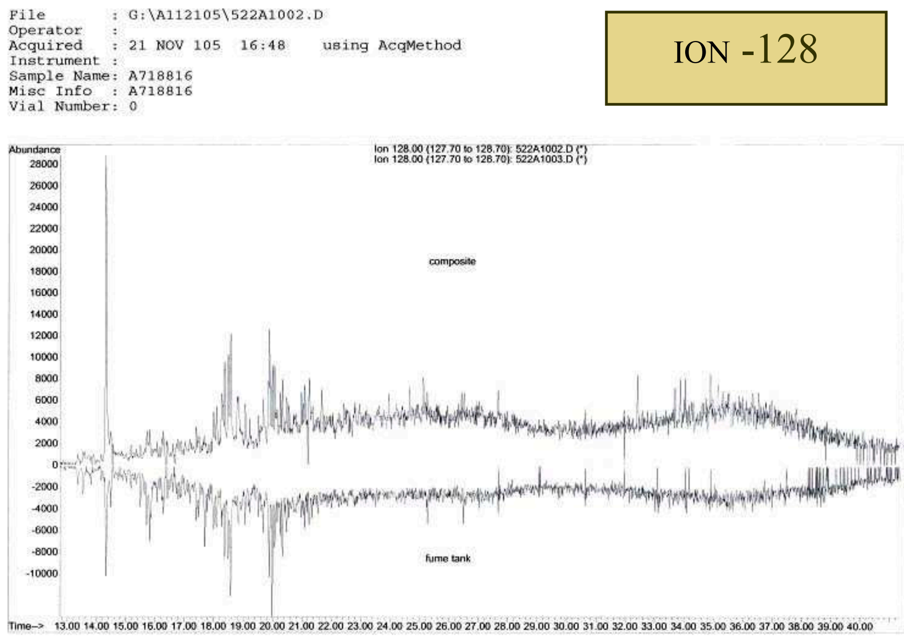


Figure 10. HPV Roofing Extracted Ion 142

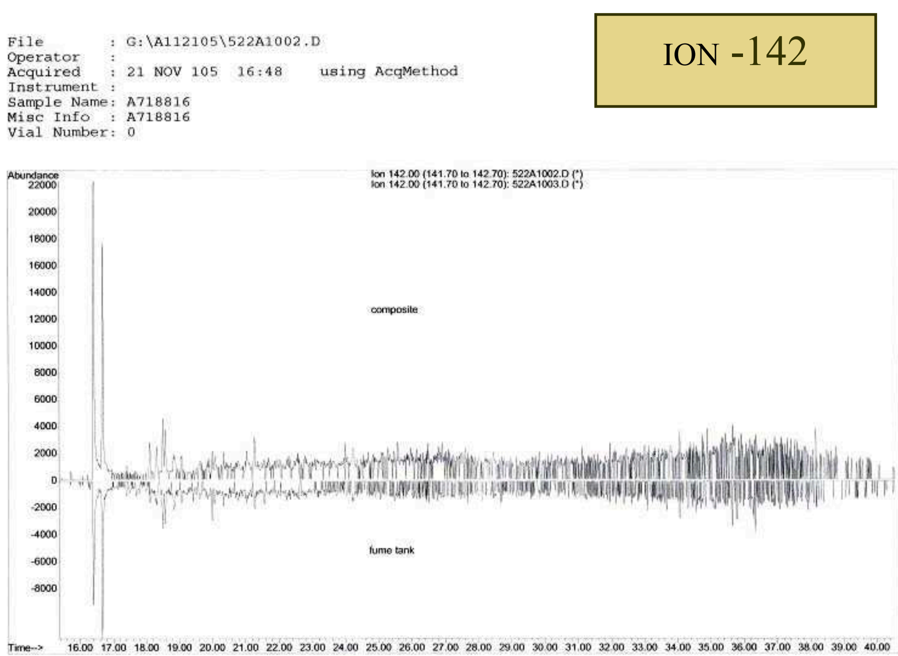


Figure 11. HPV Roofing Extracted Ion 156

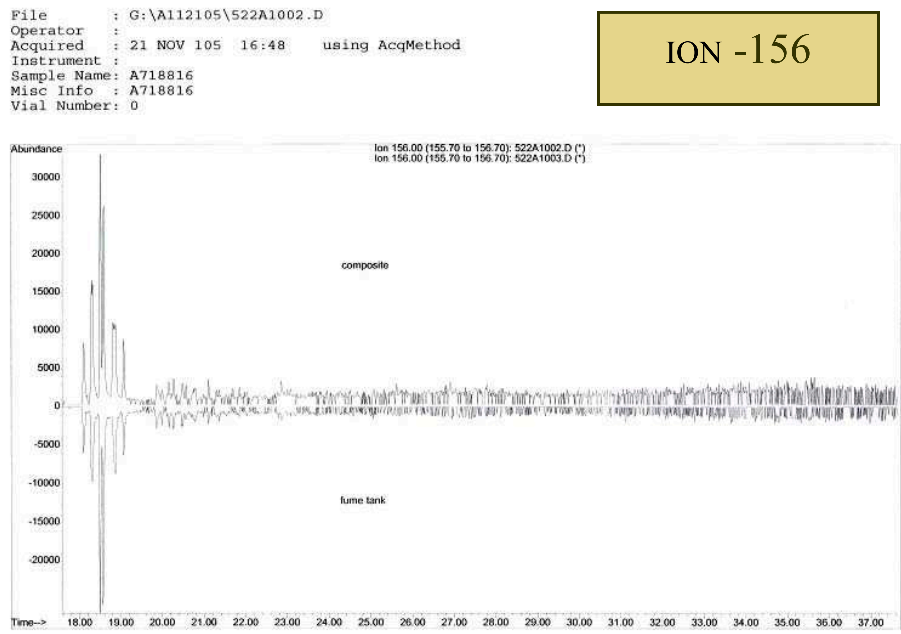
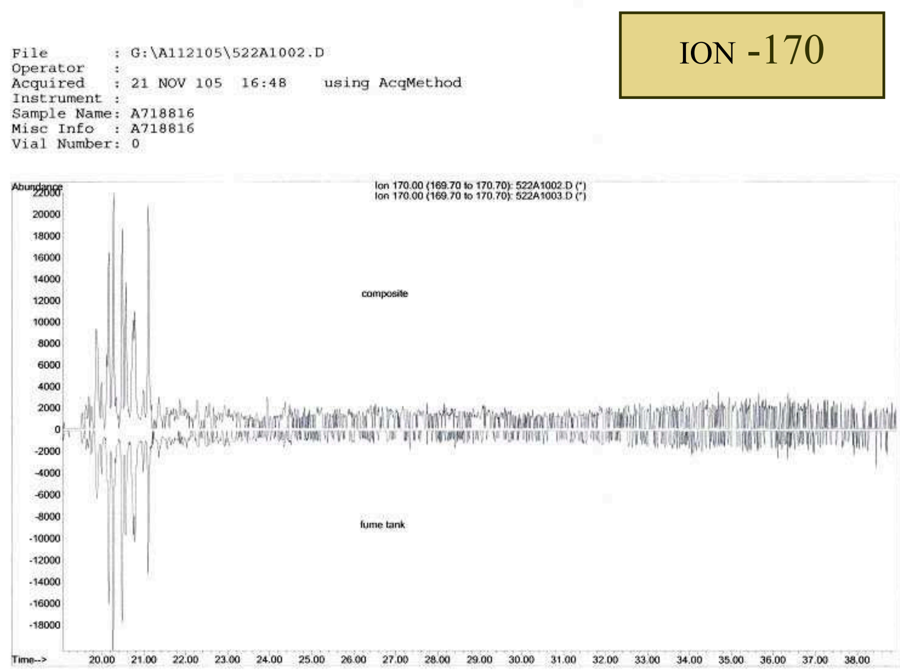


Figure 12. HPV Roofing Extracted Ion 170



5.6.2.5 Mutagenic Index (MI)

The Mutagenic Index (MI) for the HPV fume condensate is 1.2. This result is exactly the same as the original tank (TR-A) roofing fume condensate sample from the research sponsored by AREC.

6 Discussion and Conclusions

Figure 13 show the boiling point distributions between the "Official" IH sample and the HPV tank fume condensate. Excellent agreement in boiling point distribution between worker exposure and the tank fume condensate is demonstrated in these curves. This fume condensate also matches that of the AREC study (see Table 13).

Figure 14 shows the comparison of the fluorescence results, where all criteria are met for the worker samples, official IH sample and the HPV roofing tank fume condensate within 15 % RPD. This fluorescent measure for the HPV fume condensate also matches that of the AREC fume condensate (TR-A), with a fluorescence response of 166 EU/g versus 157 EU/g (5.6% RPD).

Although the PAH data is minimal due to detection limit restrictions for the IH samples, the three compounds that were detected are in the same order of magnitude for both samples. More importantly, the extracted ions for both the validation and characterization show excellent agreement between the IH sample and the HPV tank asphalt roofing fume condensate.

In summary, results of the industrial hygiene workplace samples compare to the large-volume fume condensate for the HPV roofing Type III asphalt based on all acceptance criteria established by the SAC. This test material meets the predetermined criteria of acceptance for the reproductive toxicity study. Since the IH portion was identical for both the AREC and this API study, it is not surprising that the HPV test material shows such excellent agreement with that of the TR-A test material in its physical, chemical and biological properties.

Figure 13. Simulated Distillation Comparisons Between IH and Tank Sample

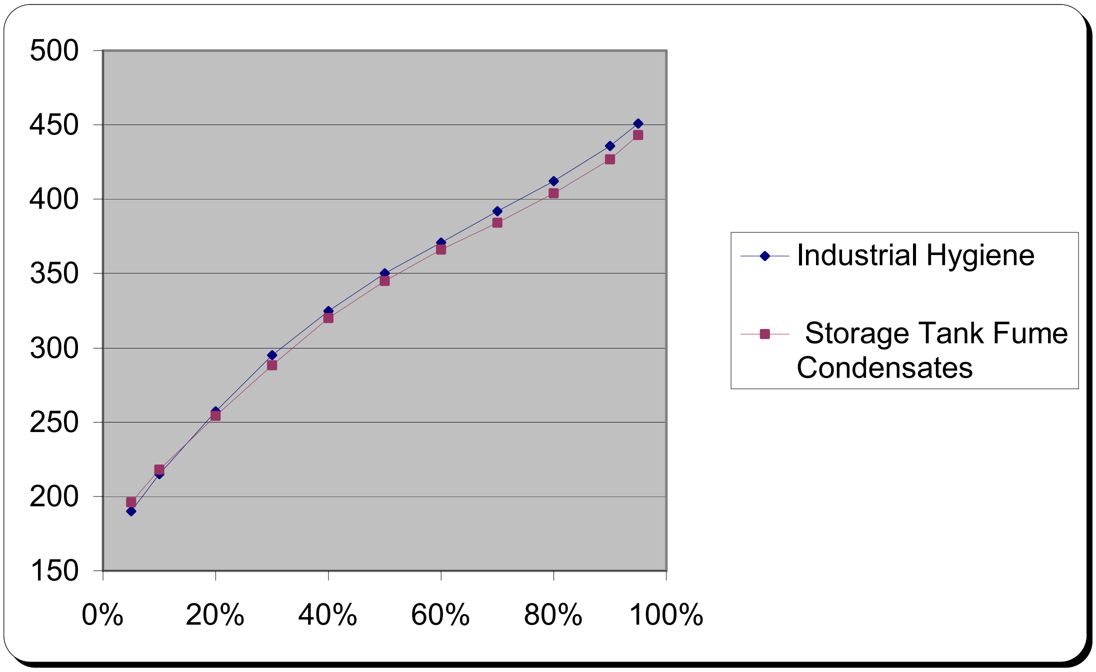
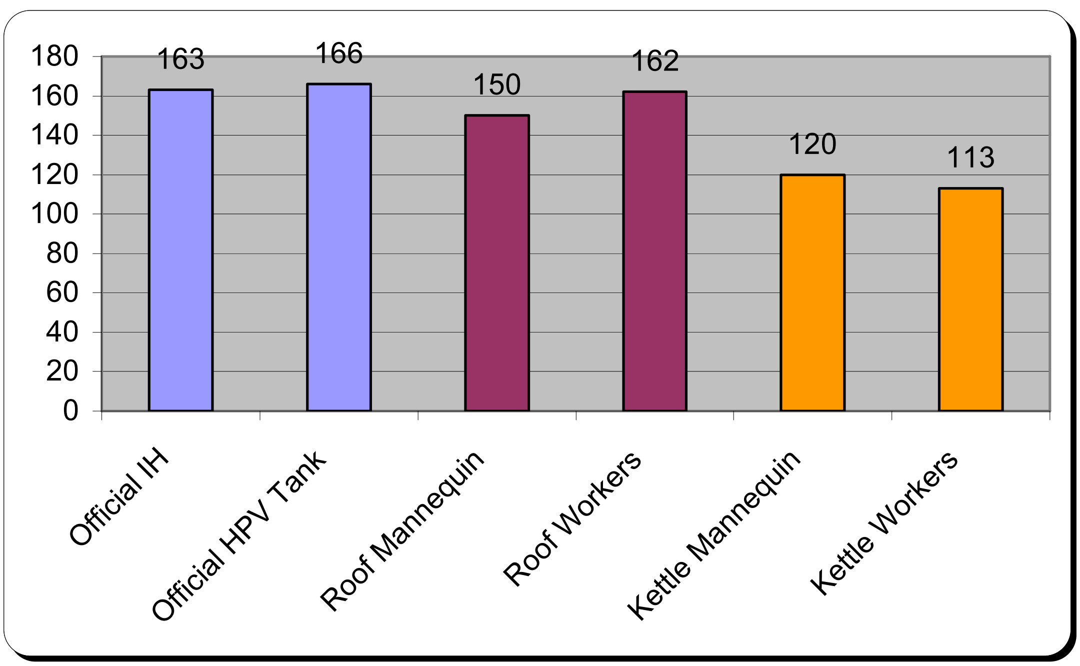


Figure 14. Fluorescence (EU/g) Comparison



7 Test Material Handling Procedures

Stored in a refrigeration unit below $^{-}18^{o}C$, the bulk fume is currently contained in a four-liter amber glass bottle with Teflon® lined lid. Upon instructions from the study sponsor, this test material will be thawed, slightly warmed, thoroughly mixed and carefully transferred to smaller glass bottles to minimize the need to interrupt the sample integrity with multiple openings and transfers. Borosilicate glass amber bottles with Teflon®-lined caps will be used for aliquots in amounts specified by the laboratory conducting the reproductive toxicity studies. Each bottle will be custody sealed and inventoried. Information on all labels will include the name "API-HPV Roofing" and the month and year. Finally, any remaining fume condensate will be designated for repository storage and will contain the word "Repository" and indicate bottle 1 of 4, 2 of 4, etc.

It is important to note that this fume condensate contains straight chain aliphatic compounds that are solid at room temperature and may require slight warming. It is recommended that each vial be checked for homogeneity prior to use in the animal studies.

A chain of custody will accompany any sample exchange. On this form, the receiving laboratory will document that the custody seals are present and intact, the containers are not broken, the COC agrees with the sample labels, and that the containers meet the protocol guidelines.

8 References

(1). **National Institute for Occupational Safety and Health (NIOSH)**: *Benzene solubles and total particulate (asphalt fume) (Analytical Method 5042*). In P.M. Eller, editor, NIOSH Manual of Analytical Methods, 4th ed., Cincinnati, Ohio: NIOSH, 1998.

(2). **Kriech, A.J., L.V. Osborn, D.C. Trumbore, J.T. Kurek, H.L. Wissel and K.D. Rosinski.** *Evaluation of Worker Exposure to Asphalt Roofing Fumes: Influence of Work Practices and Materials*. Journal of Occupational and Environmental Hygiene, 2004, 1, p. 88-98.

(3). **Kriech, A.J., J.T. Kurek, H.L. Wissel, L.V. Osborn and G.R. Blackburn.** *Evaluation of Worker Exposure to Asphalt Paving Fumes Using Traditional and Nontraditional Techniques*. American Industrial Hygiene Association Journal, 63, 5, 2002.

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(5). **U.S. Environmental Protection Agency (EPA):** *Method SW846-8015B, Nonhalogenated organics using GC/FID*. In U.S. EPA Test Methods for Evaluating Solid Waste, Physical/Chemical Method SW846, 3rd rev. ed. Washington, D.C.: EPA, 1996.

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(7). **U.S. Environmental Protection Agency (EPA):** *Method SW846-8270C, Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)*. In U.S. EPA Test Methods for Evaluating Solid Waste, Physical/Chemical Method SW846, 3rd rev. ed. Washington, D.C.: EPA, 1996.

(8). **Olsen, Larry**. Personal communication. 13 July 2004.

(9). **American Society for Testing and Materials (ASTM)**: *Standard Test Method for Determining Carcinogenic Potential of Virgin Base Oils in Metalworking Fluids* (Designation: E 1687-04). West Conshohocken, PA: ASTM, 2004.

(10). **Heinrich, Prof. Dr. Uwe**. *Collection, Validation and Generation of Bitumen Fumes for Inhalation Studies on Rats*. Fraunhofer Institute of Toxicology and Experimental Medicine Draft Report, 2003.

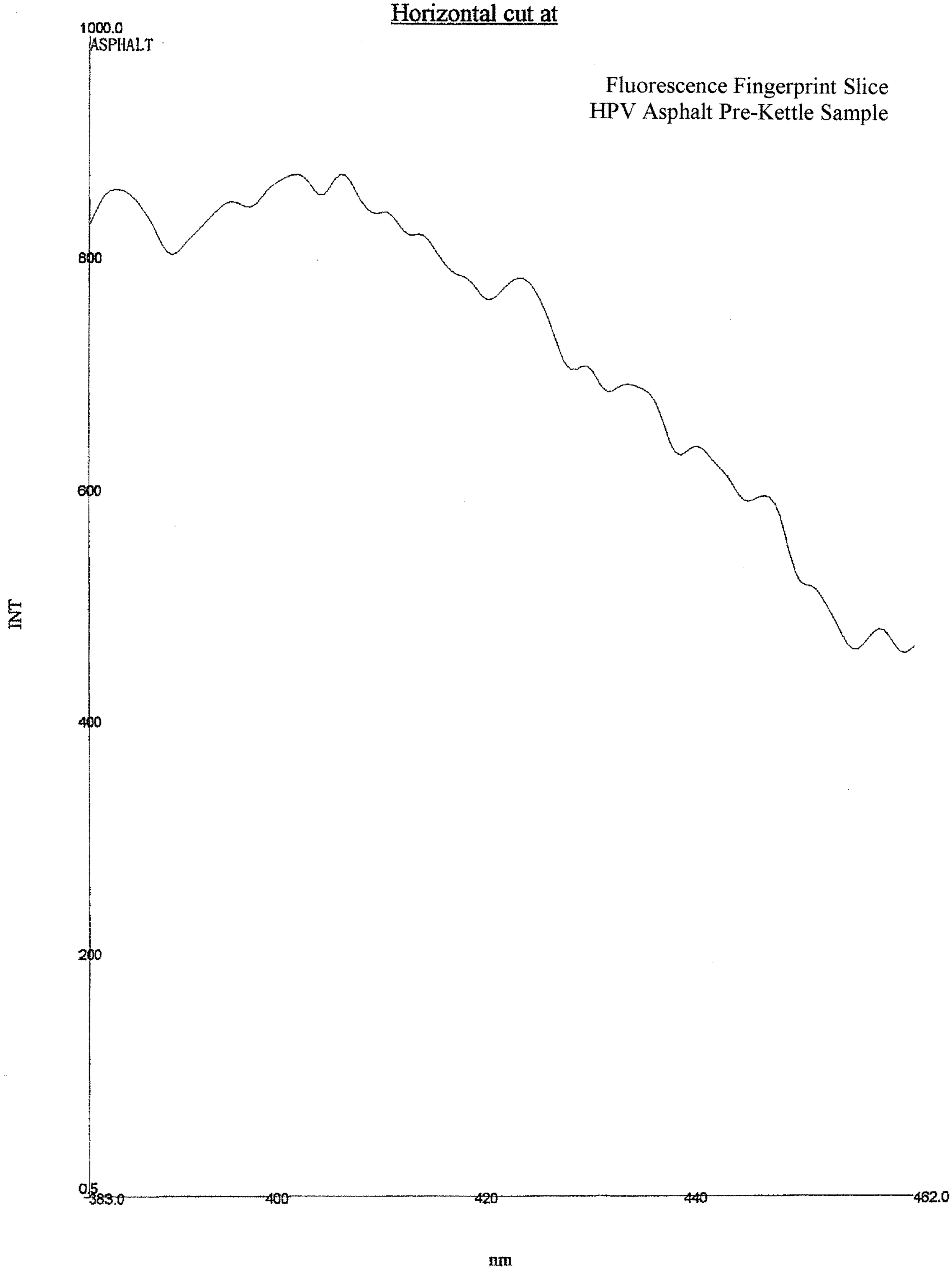
(11). **American Society for Testing and Materials (ASTM)**: *Test Method for Softening Point of Bitumen (Ring & Ball Apparatus)* (Designation: D 36-95 (2000)). West Conshohocken, PA: ASTM, 1995.

Appendix A

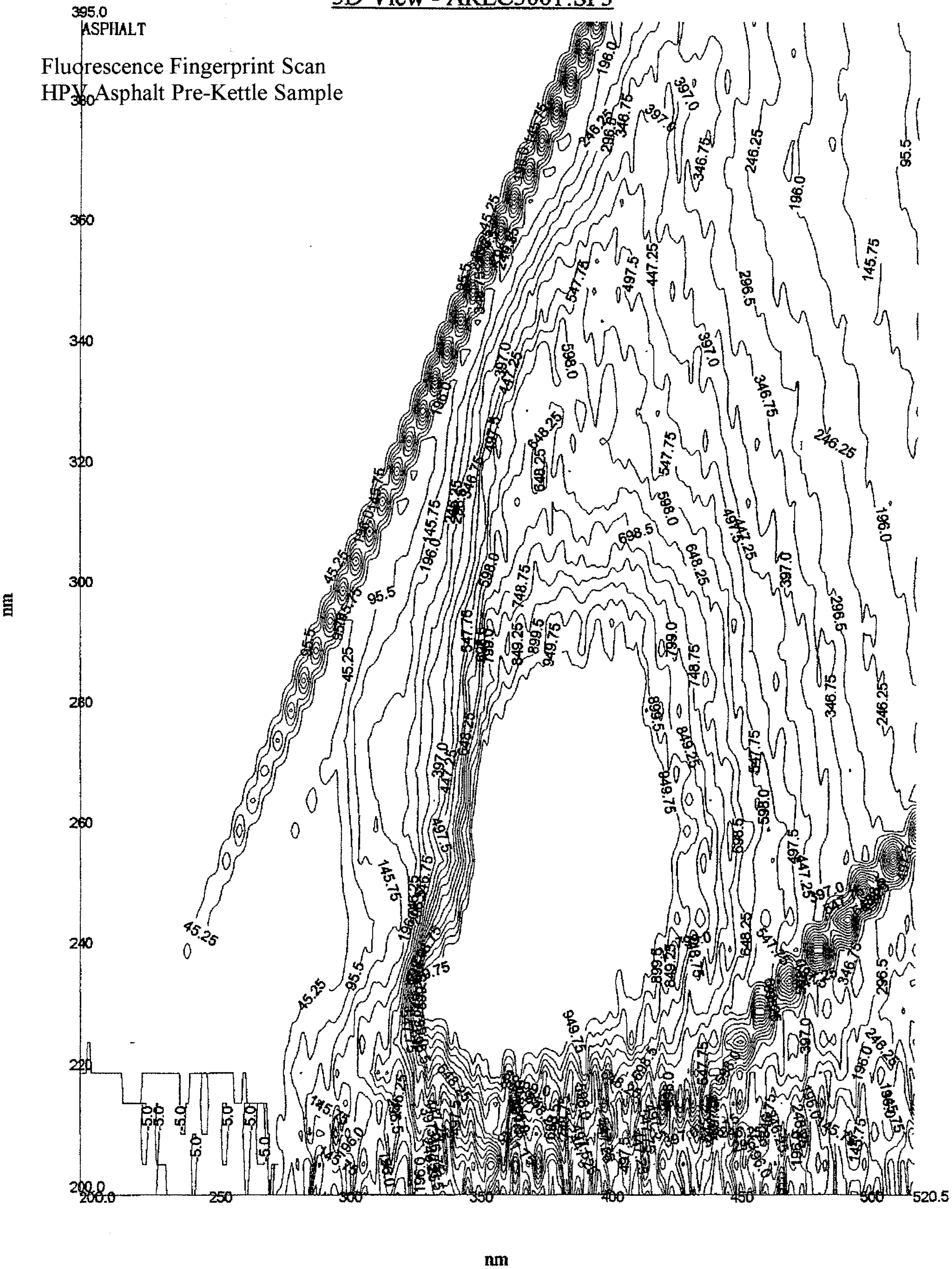
Fluorescence 3-D Scans

to verify the absence of coal tar in the kettle residue prior to use during this study

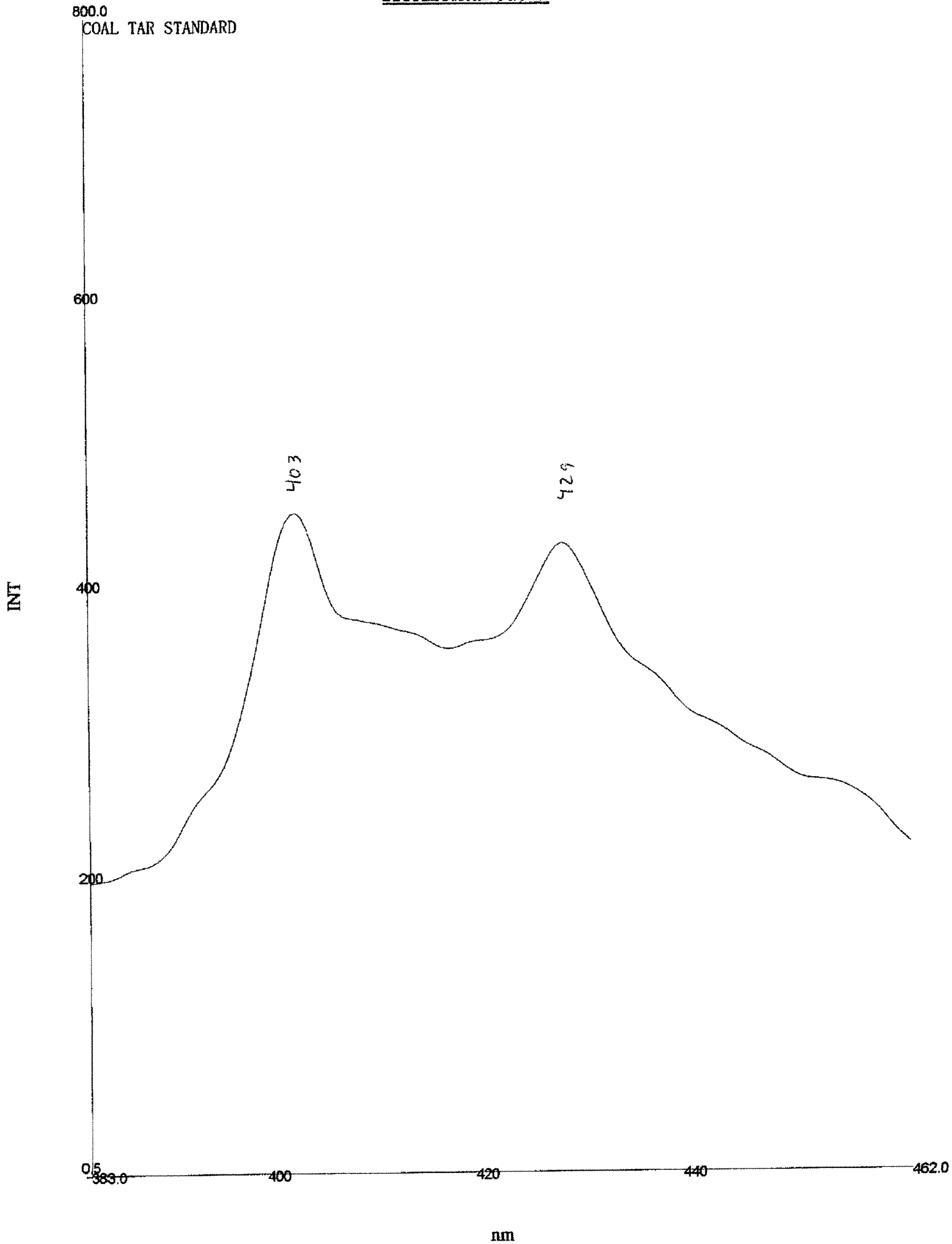
Horizontal cut at



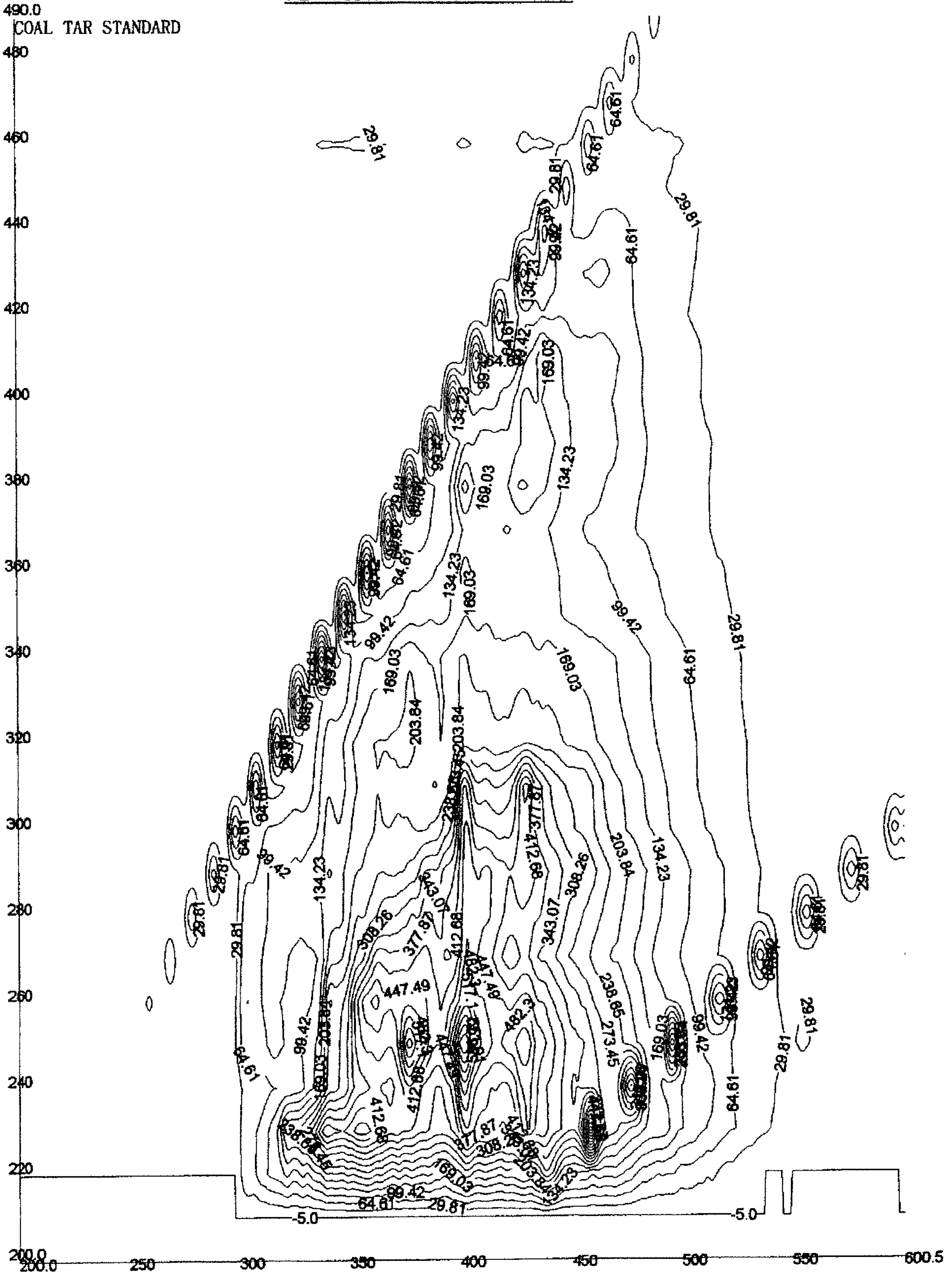
3D View - AREC3001.SP3



Horizontal cut at



3D View - 829CT001.SP3



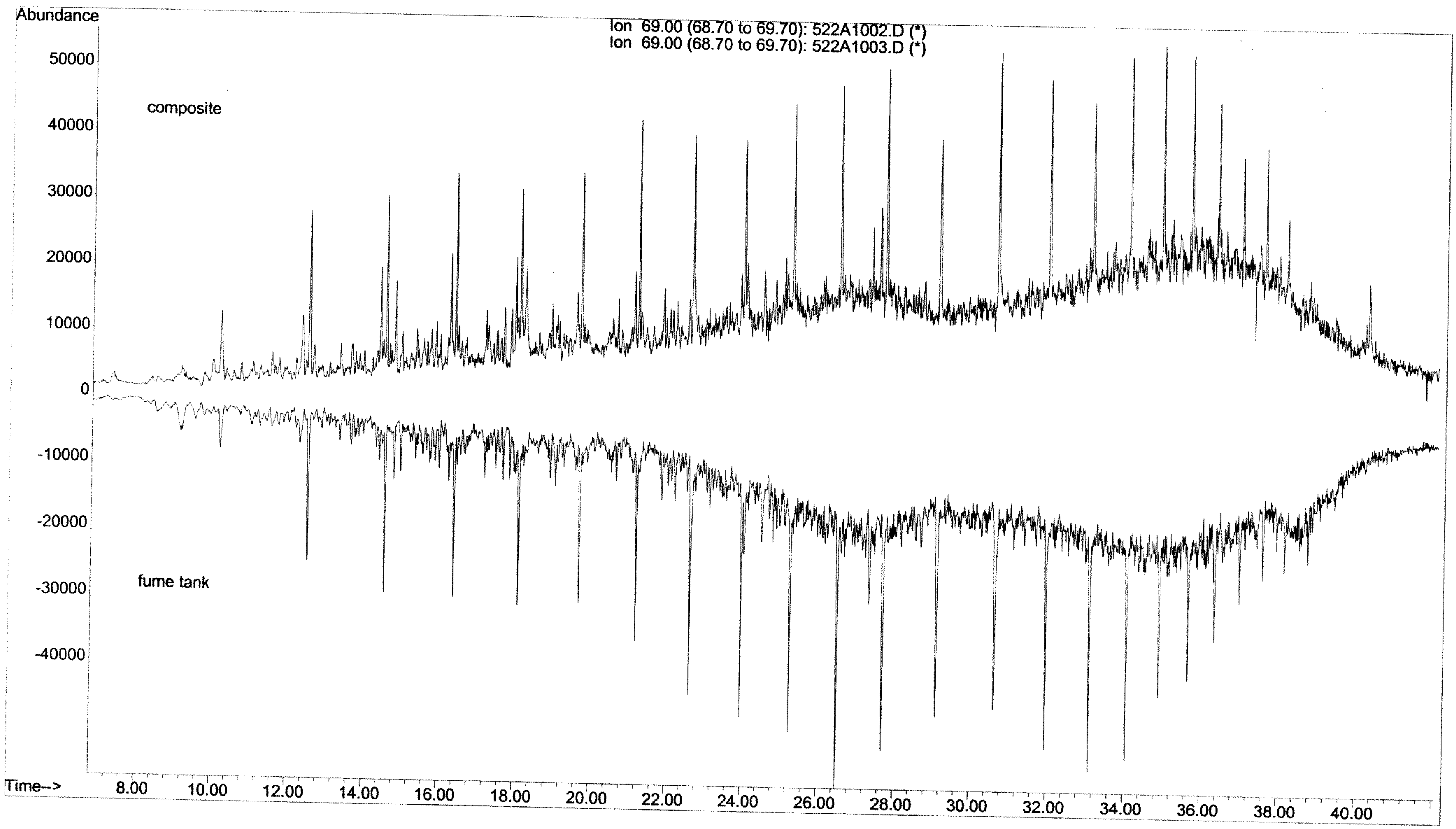
Appendix B

GC/MS Scans

Extracted Ions (m/z) For Characterization

File : G:\A112105\522A1002.D
Operator :
Acquired : 21 NOV 105 16:48 using AcqMethod
Instrument :
Sample Name: A718816
Misc Info : A718816
Vial Number: 0

ION 69



File : G:\A112105\522A1002.D
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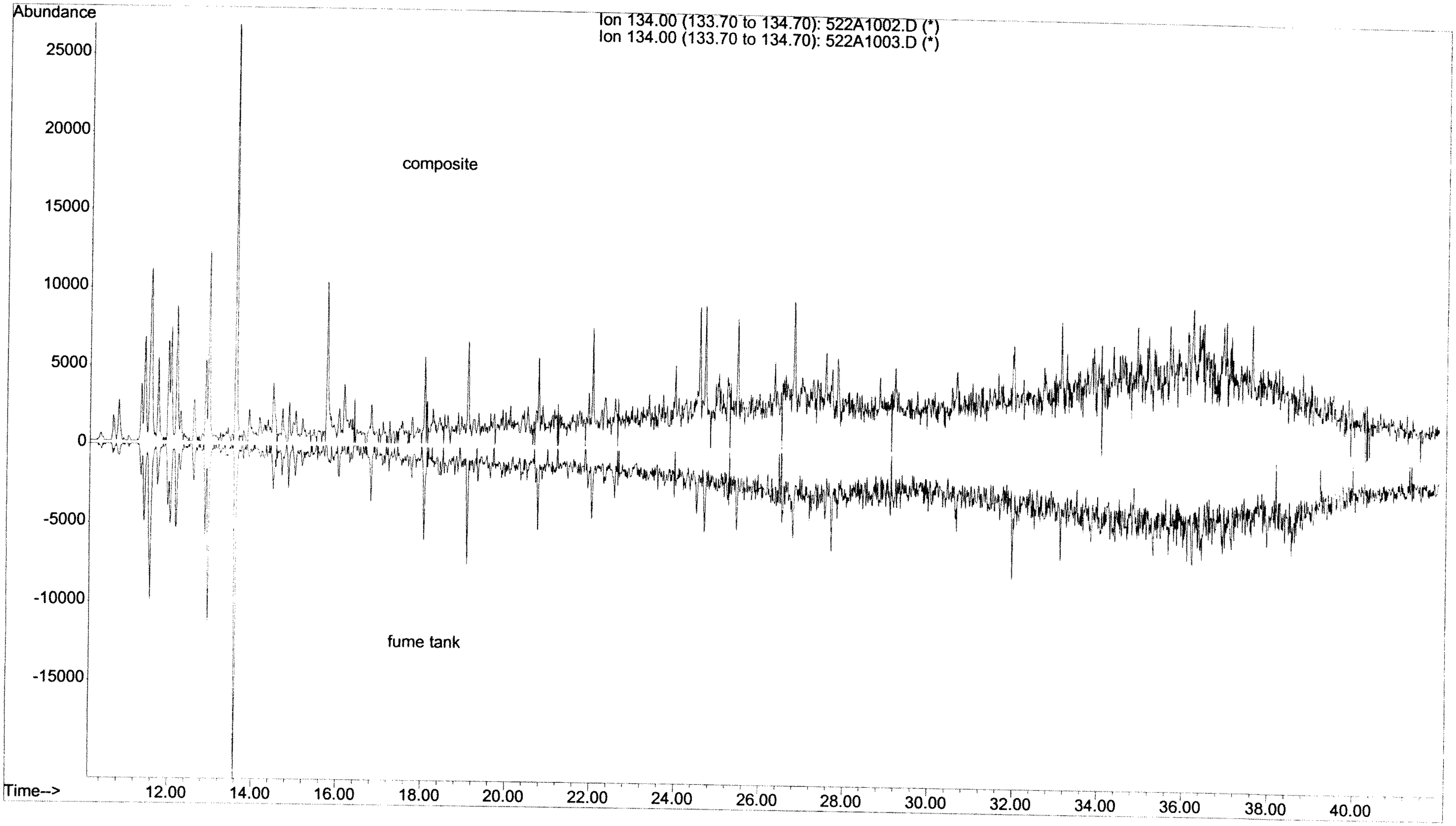
Misc Info : A718816
Vial Number: 0

ION 134



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Instrument :
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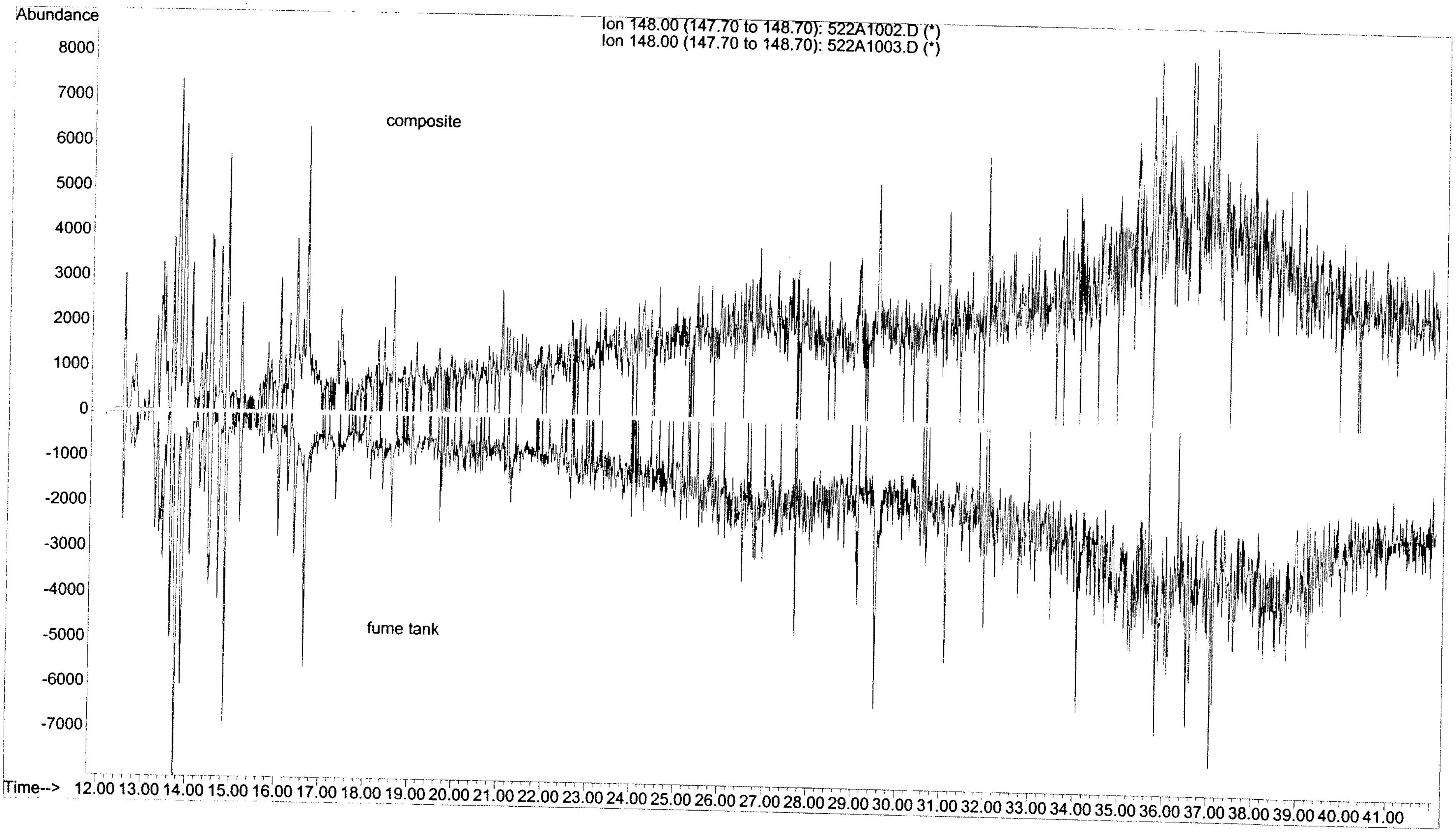
Vial Number: 0

ION 148



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Instrument   :
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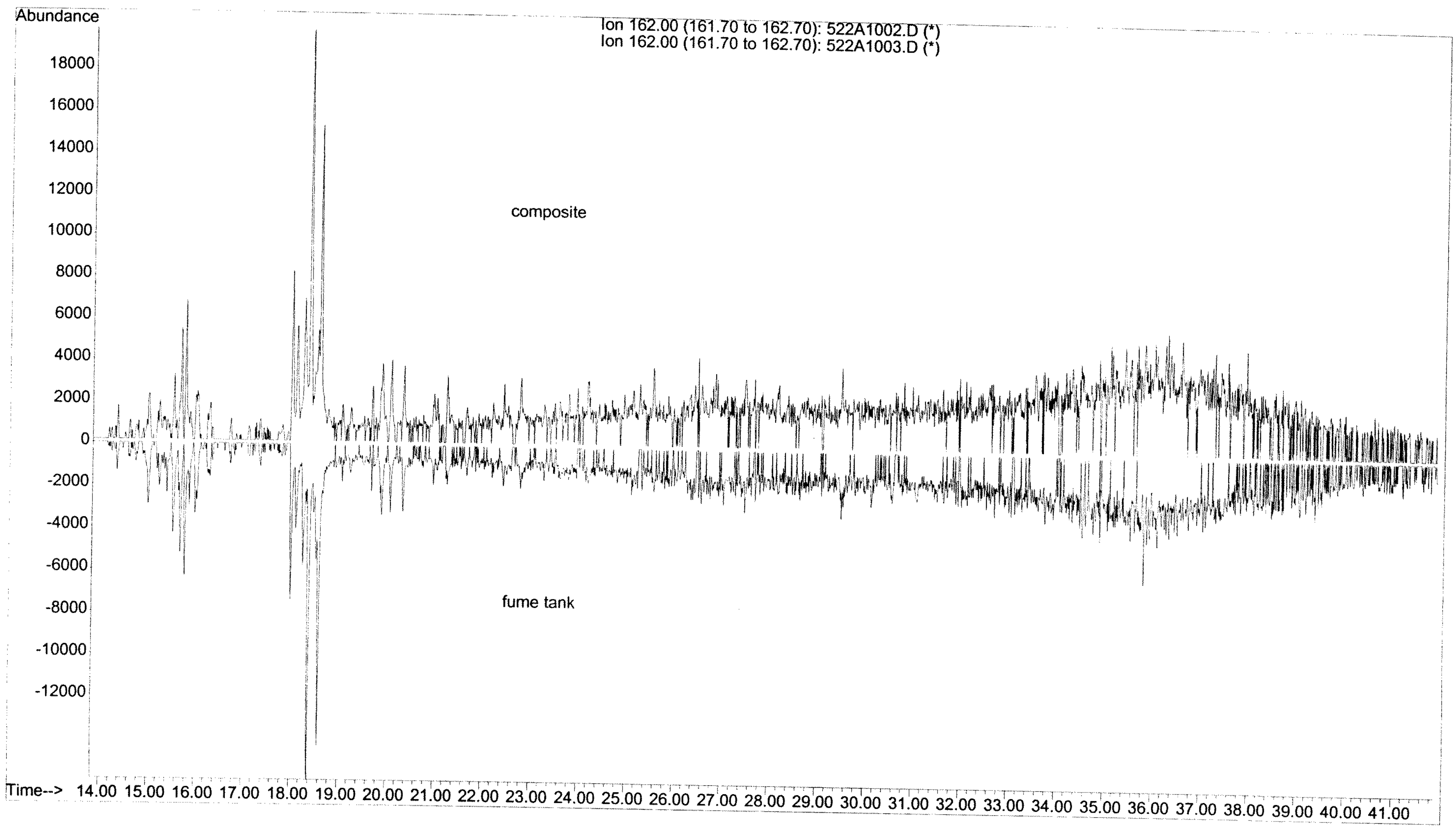
Misc Info    : A718816
Vial Number: 0
```

ION 162



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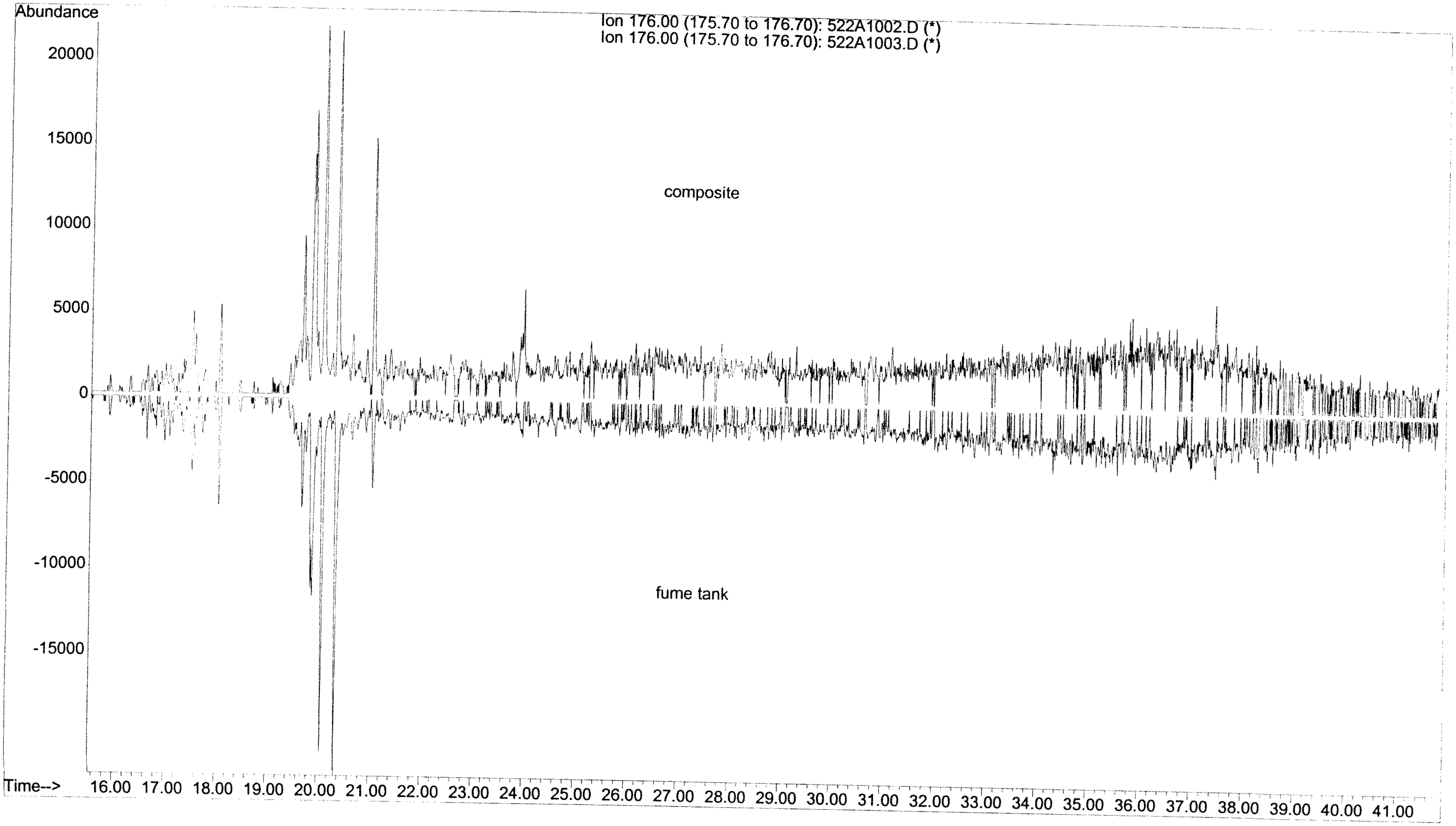
Misc Info : A718816
Vial Number: 0

ION 176



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Instrument    :
Sample Name: A718816
Misc Info     : A718816
Vial Number: 0
```
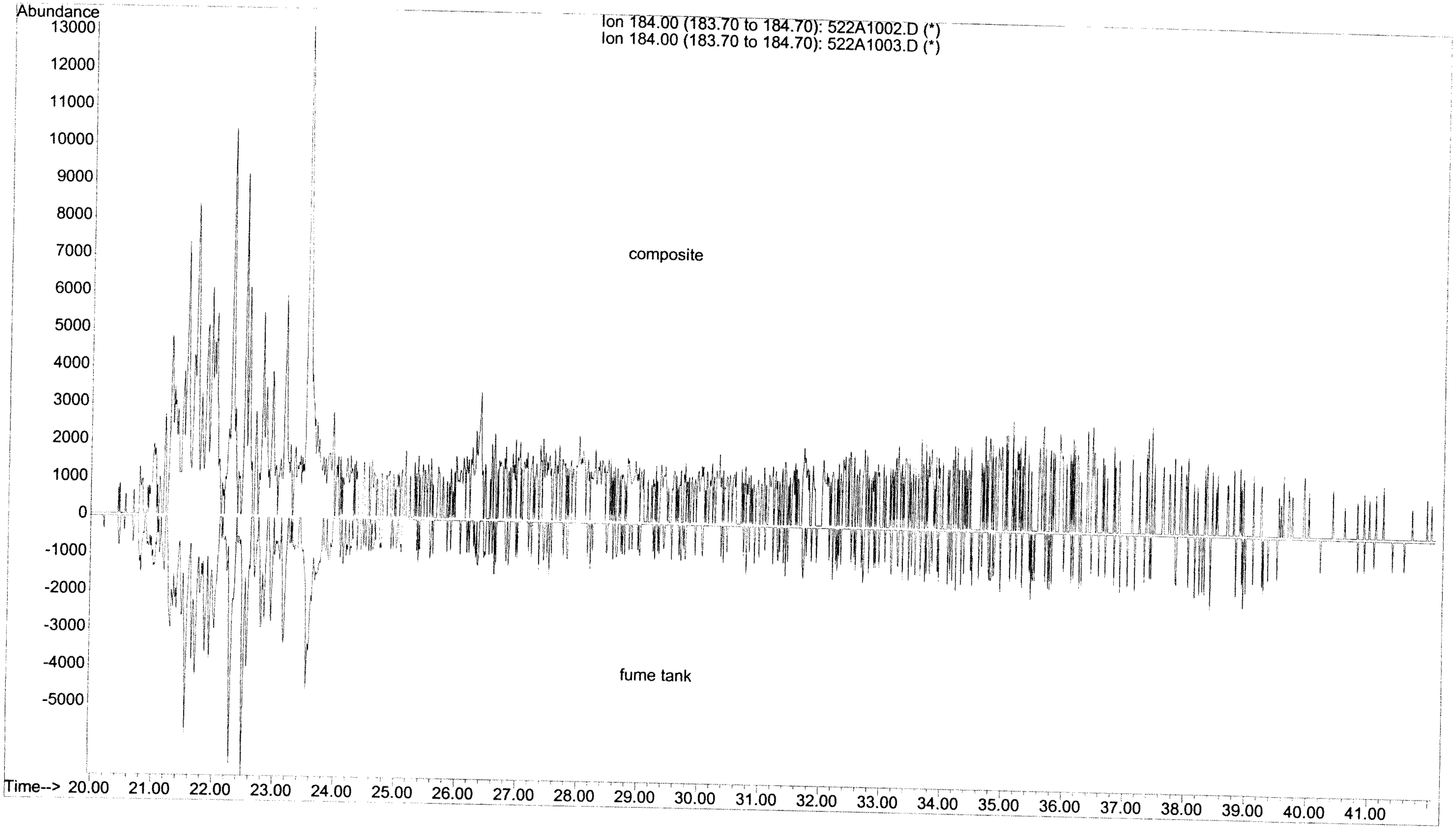

ION 184



File : G:\A112105\522A1002.D
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Instrument :
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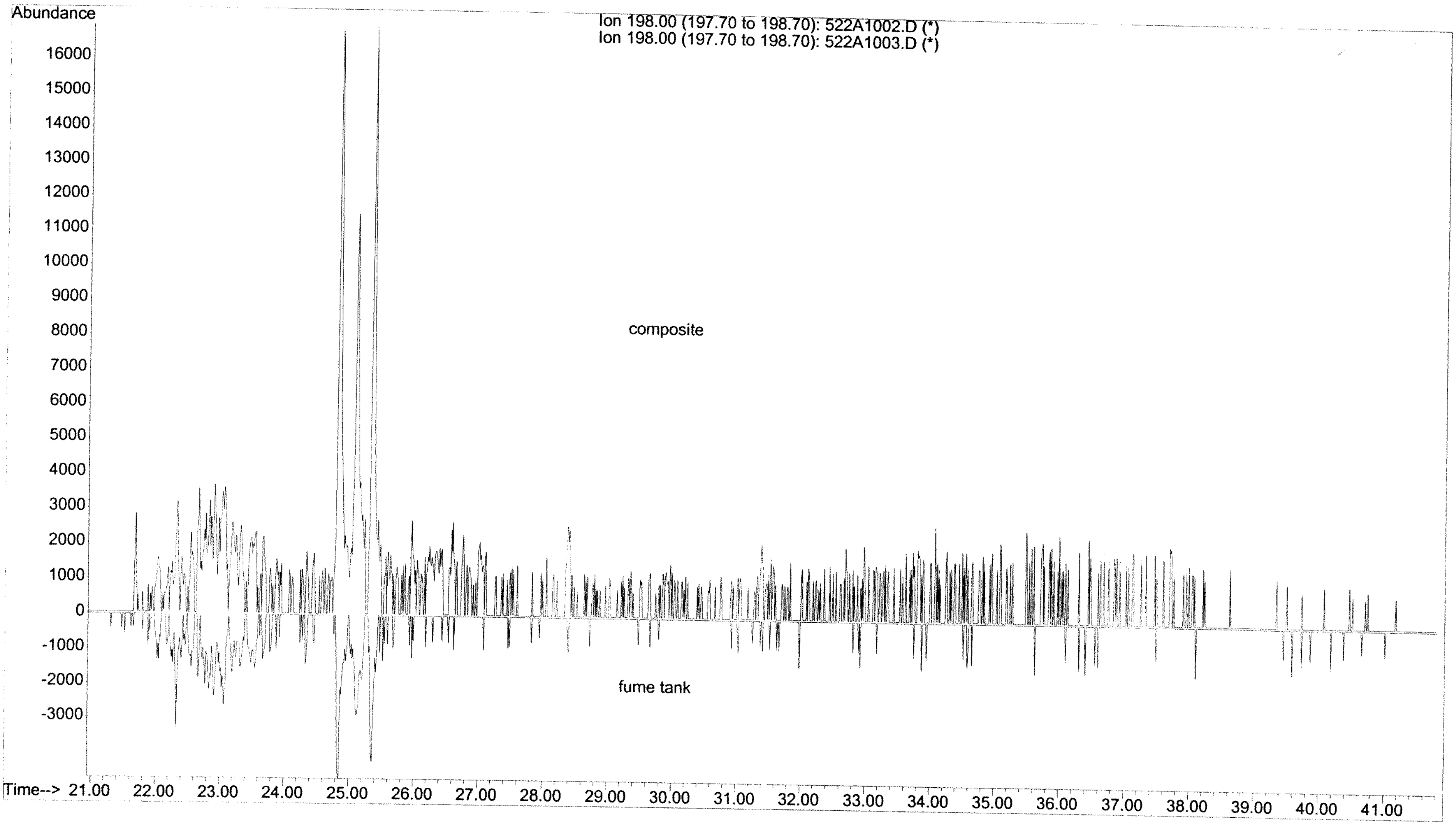
Misc Info : A718816
Vial Number: 0

ION 198



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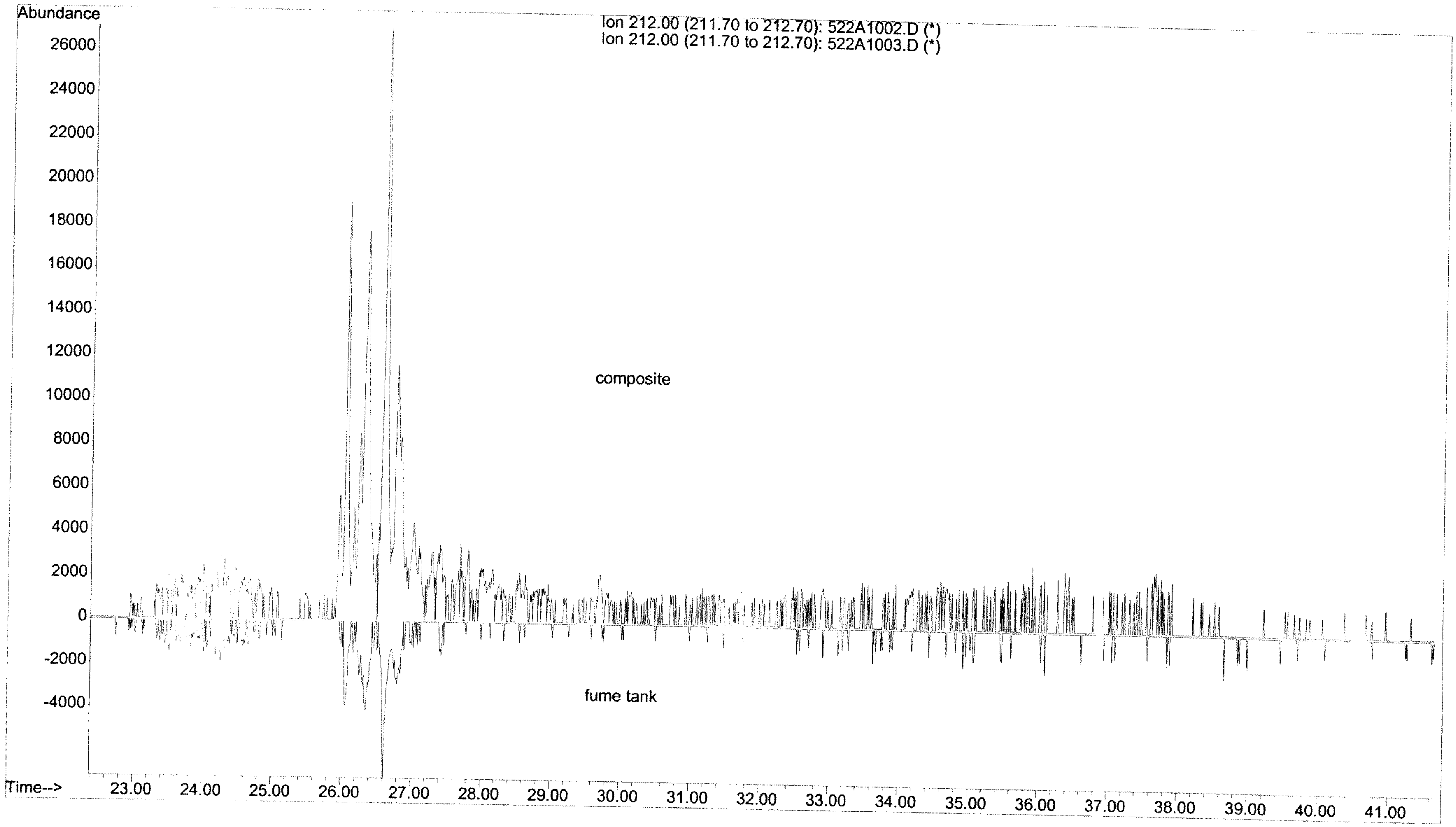
Vial Number: 0

ION 212



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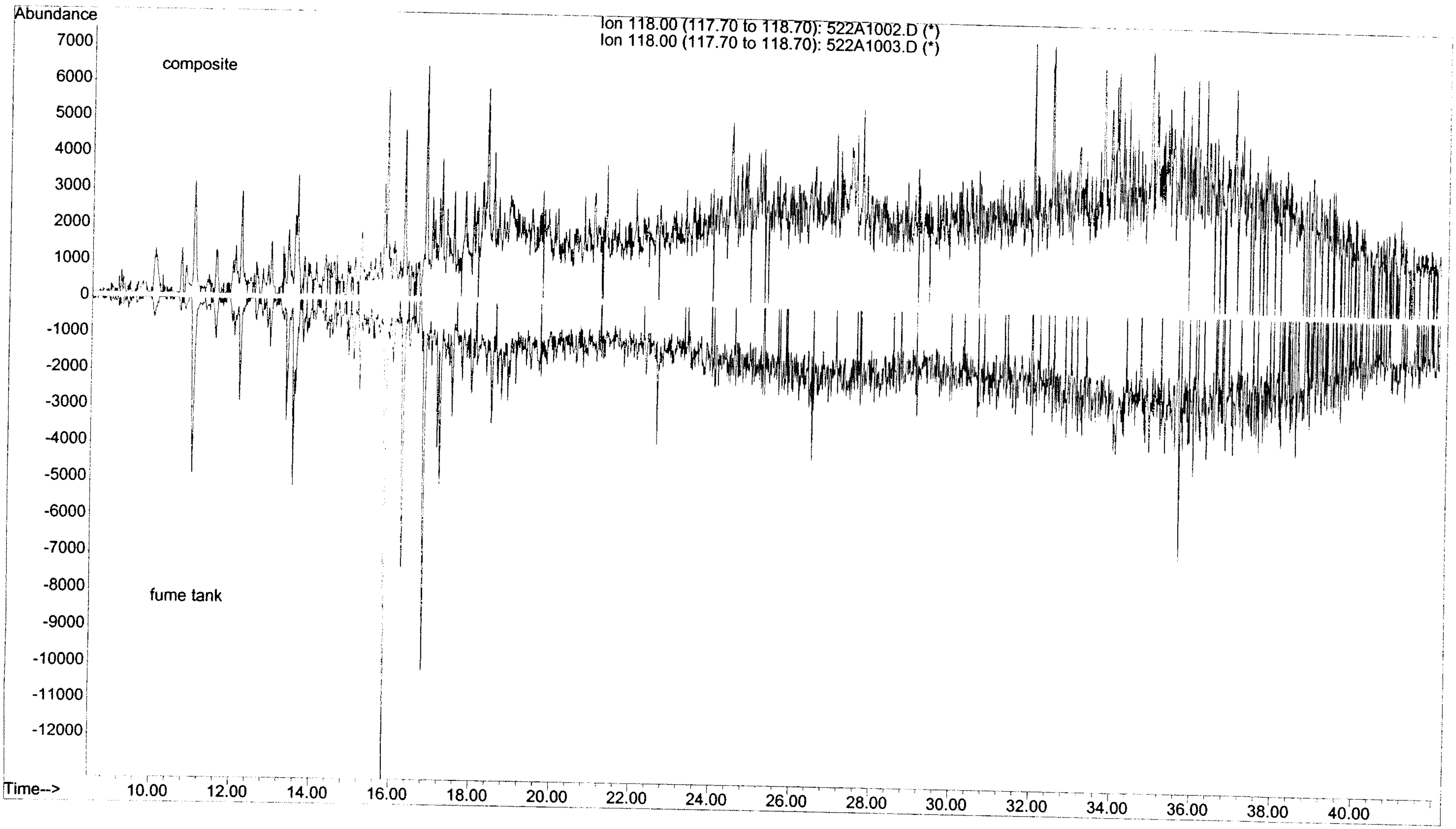
Misc Info : A718816
Vial Number: 0

ION 118



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Instrument :
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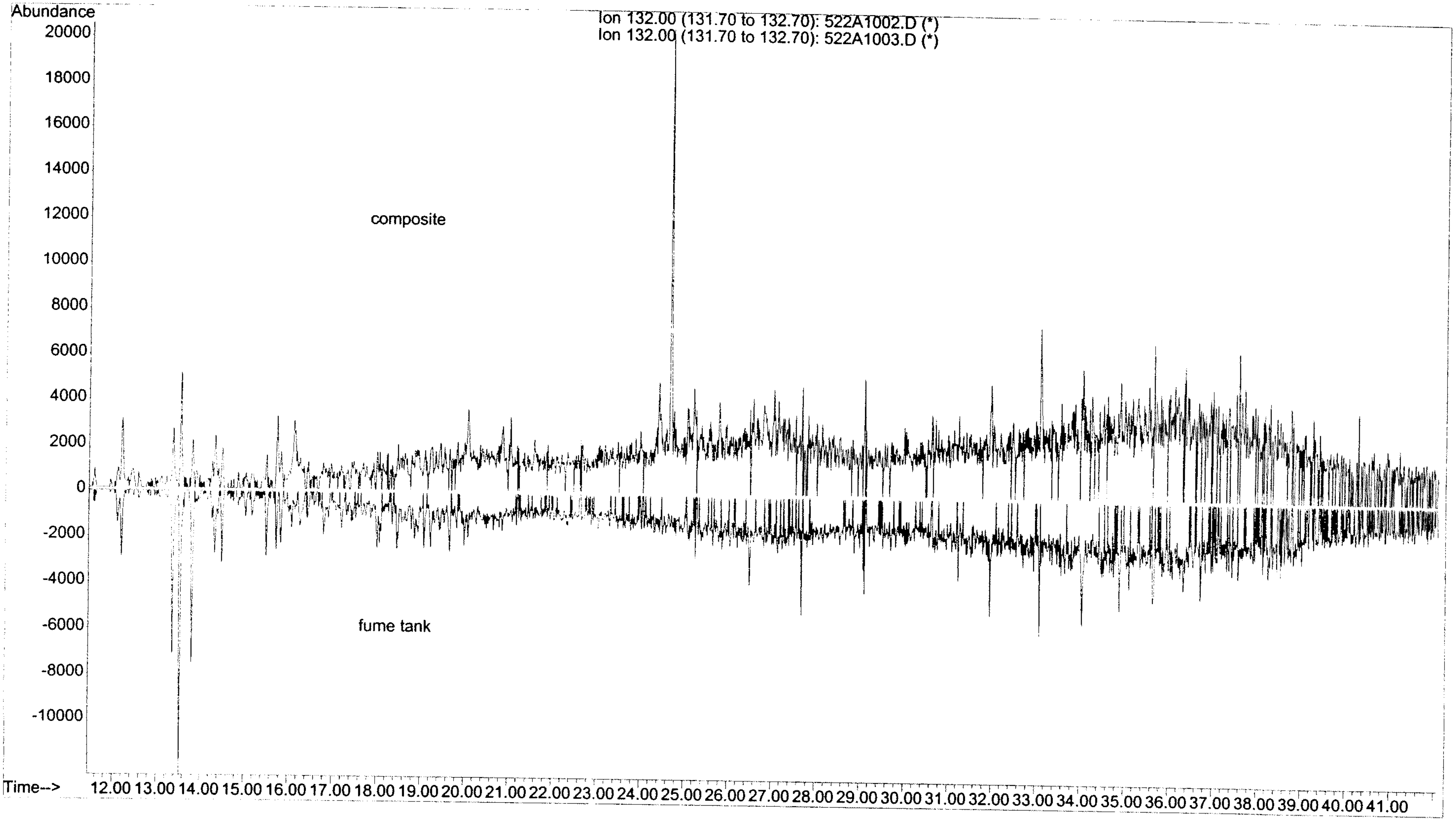
Vial Number: 0

ION 132



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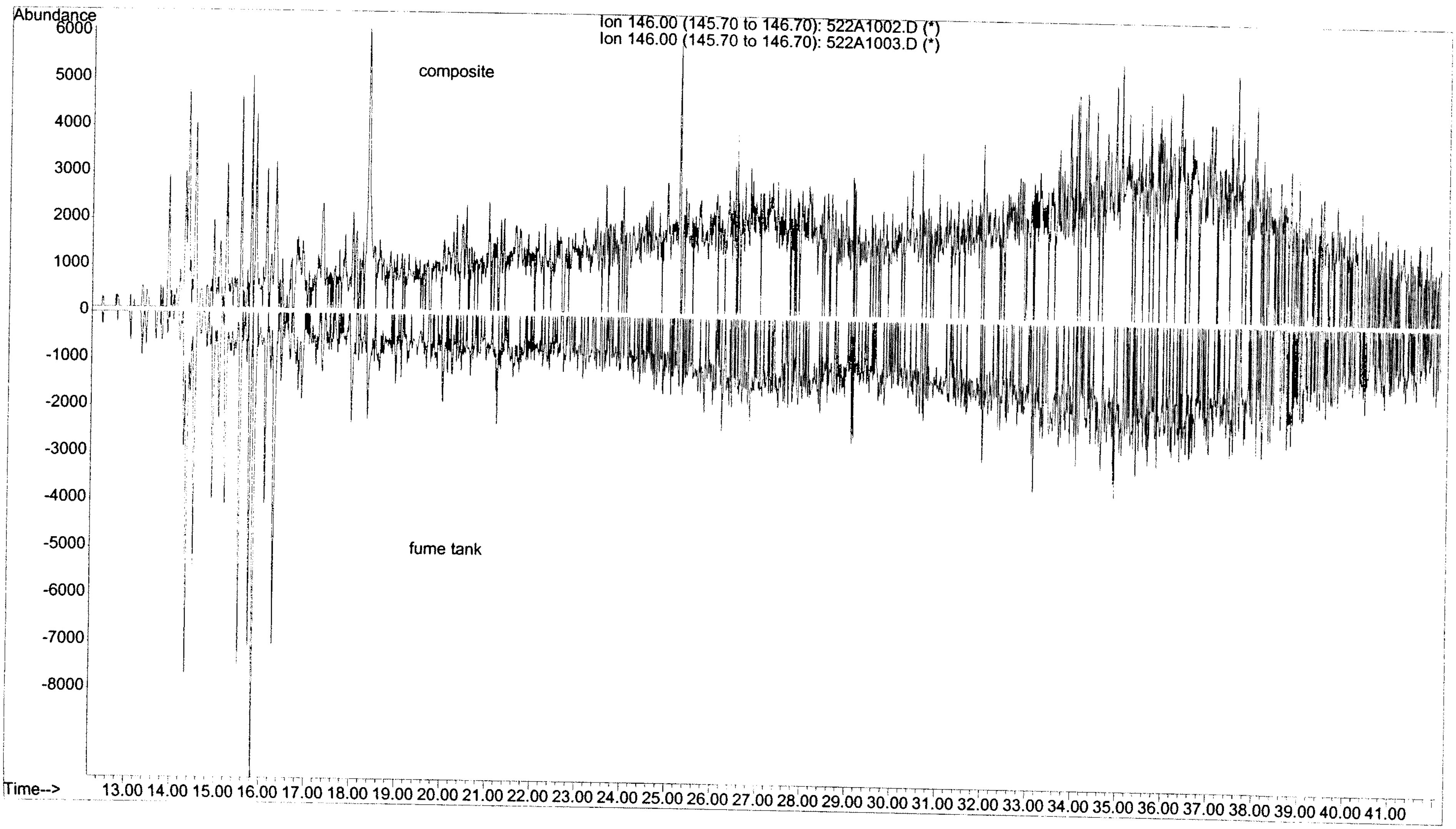
Misc Info   : A718816
Vial Number: 0

ION 146



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Instrument   :
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Misc Info    : A718816
Vial Number: 0
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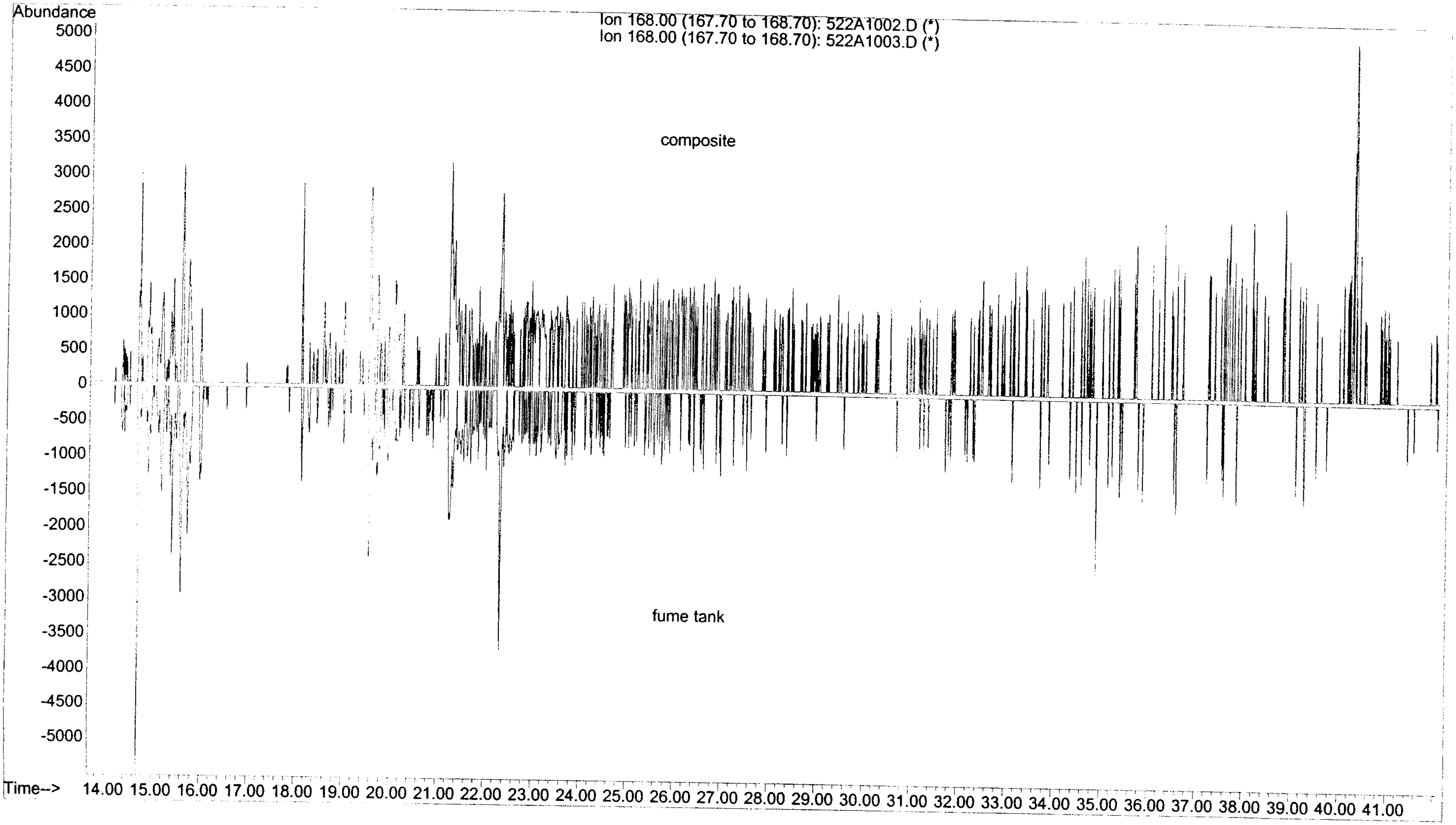
ION 168



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Instrument :
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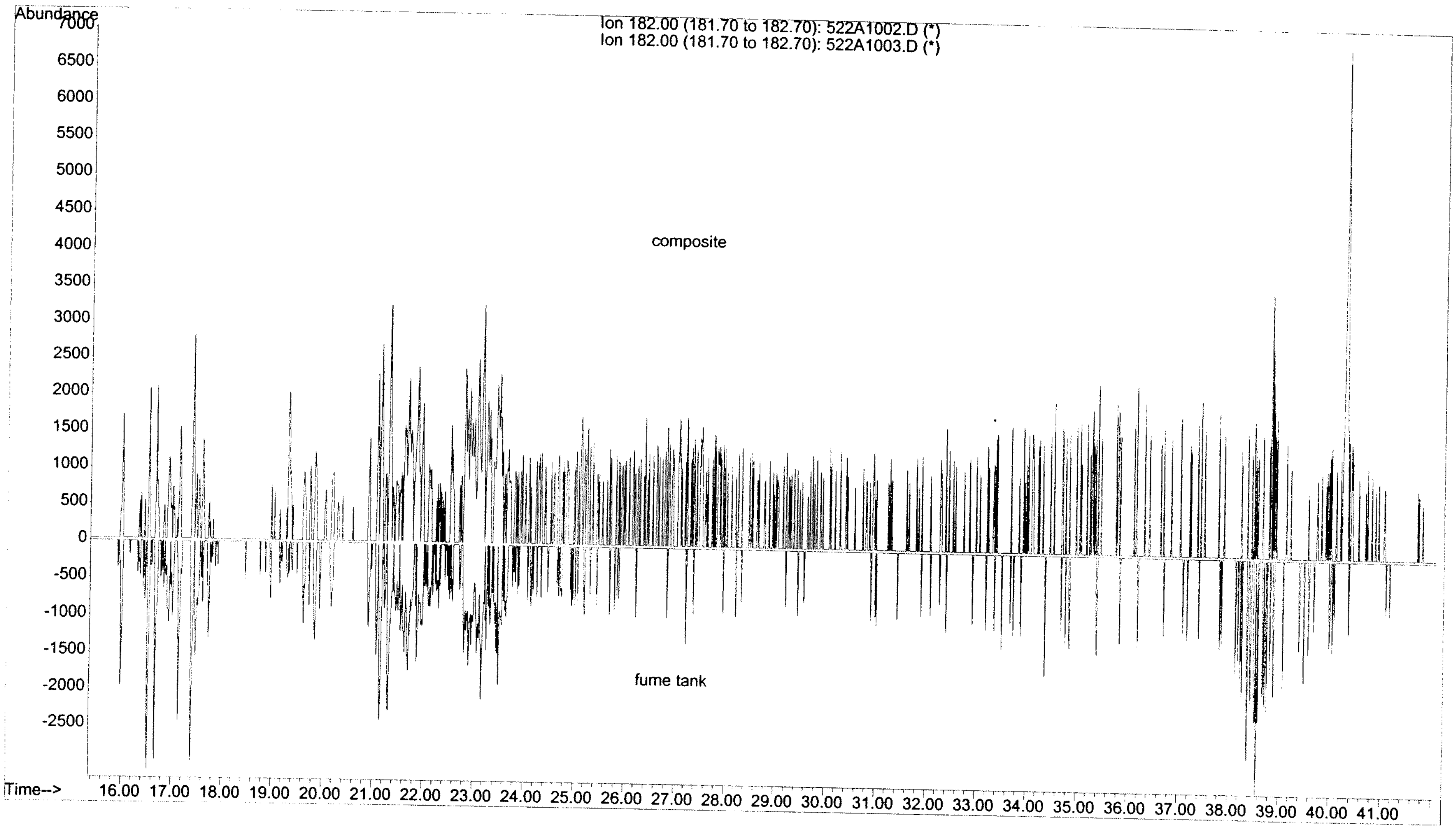
Misc Info : A718816
Vial Number: 0

ION 182



```
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Operator      :
Acquired      : 21 NOV 105   16:48     using AcqMethod
Instrument    :
Sample Name: A718816
Misc Info     : A718816
Vial Number: 0
```

ION 196