

**Leaf Processing in Streams and the Determination
of Fungal Biomass via a Chemical Index**

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by Emily Stricker
Lycoming College
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Abstract

Leaf processing of two plant species, sugar maple (*Acer saccharum*) and river birch (*Betula nigra*), was studied in two, north central Pennsylvania streams of different orders during the summer and fall. Processing rates, or k values, organic content, and macroinvertebrates were monitored at 7, 21, 28, and 35-day intervals in the summer and at eight intervals from 8 to 48 days in the fall. Ergosterol was extracted from incubated leaves using procedures by Newell (1988) and measured with HPLC. The effect of incubation time, plant species, season, and stream pH on leaf processing was assessed. Organic contents of both plant species decreased over incubation time due to nutrient leaching and microbial degradation. Processing rates for *Acer saccharum* and *Betula nigra* were significantly lower in the third-order stream than the second-order stream because of a significantly lower pH and colder water temperatures ($P=0.786$, $P=0.159$). *Acer saccharum* decomposed significantly faster in the summer than *Betula nigra* in both Mill Creek and Big Bear Creek ($P=0.787$, $P=0.689$, $\alpha=0.05$). Summer fungal biomass levels were significantly higher in the second-order stream due to the lower pH of the third-order stream ($P=0.066$, $\alpha=0.05$). The highest fungal biomass concentration found was $2.28 \mu\text{g}/\text{mg}$ for the 7-day, *Acer saccharum* incubation. A significant difference was found between the summer and fall fungal biomasses of *Betula nigra* ($P=0.500$, $\alpha=0.05$). However, *Acer saccharum* had no significant difference in its summer and fall fungal biomass, possibly due to its fast decomposition rate ($P=0.024$, $\alpha=0.05$). Total invertebrates in the summer increased as fungal biomass decreased. In conclusion, this study showed increased fungal biomass in the fall and increased processing rates in the summer. Future studies should try other methods of incubation and extraction, along with a larger sample size because uncontrollable weather conditions cause sample loss.

Introduction

Allocthonous material such as leaf litter is a primary energy source for woodland stream ecosystems. Fungi, particularly aquatic hyphomycetes, are the main microorganisms involved in initial leaf breakdown in these streams. The fungi soften leaf tissue with pectin-degrading enzymes. This softening increases the cell-sloughing rate, which leads to increased availability of structural polysaccharides like cellulose. Fungi are absorptive heterotrophs, therefore, they also feed on the leaves, which aids in leaf particulation. Then, the macroinvertebrates feed upon the detritus, or decaying leaf matter, because the fungi make the detritus more nutritious. Shredders are the primary macroinvertebrate involved in this processing of leaves by insects (Suberkropp 1994). Figure 1 summarizes the food web of a stream and emphasizes the importance of fungi in leaf processing.

The aquatic hyphomycetes's membrane contains a sterol called ergosterol, similar to a human's cholesterol (Newell 2000). Therefore, ergosterol's presence in leaf litter indicates fungal life and functions as a valuable fungal index molecule because previous research shows that ergosterol is not found in vascular plants (Gessner and Chauvet 1994). In addition, the 5,7 double bonds of ergosterol allow sensitive detection of an ergosterol extraction's ultraviolet absorption because it peaks at 282 nanometers (Newell 1988).

Researchers have found that a stream's fungal activity is controlled by internal characteristics of leaf tissue such as tannin and lignin and environmental conditions like the water's temperature and nutrient concentration. Evidence shows that leaf-inhabiting fungi obtain their inorganic nutrition, like phosphorus, from the stream water (Suberkropp 1995). Sridhar and Barlocher (2000) also state that external sources of phosphorus and

nitrogen promote fungal growth and metabolism that increases leaf decay; nitrogen possibly makes leaves more appetizing for invertebrates.

Environmental factors, like pH, temperature, and season, assist in determining leaf processing in a stream. For example, energy and material availability to a stream's macroconsumers decreases when a stream becomes acidic. In addition, leaves from different tree species have been shown to decompose at different rates. Varying leaf species can be assigned to decomposition groups through a determination of leaf processing (k). Processing rates, k values of >0.01 are usually considered in Group I, or the fast decomposers, according to Peterson and Cummins (1974). Group II, the medium decomposers, have k values of $0.005-0.010$ and Group III, the slow decomposers, have k values <0.005 . Consistent with Solada et al. (2000) processing rates, or k values, are significantly lower in streams with a low pH. Studies in a Tennessee woodland stream have shown that ergosterol levels are the lowest in the summer and peak in the fall to early winter (Suberkropp 1997). Normally, fungal biomass is positively correlated with temperature, but in many cases, temperature's effect can be overridden by the accessibility of leaves as an organic substrate. Furthermore, one West Virginia stream study showed that streams with a lower pH have lower fungal biomass levels along with increased invertebrate density (Engstrom et al. 2000).

The main objective of this study was to assess fungi's role in leaf decomposition in two Pennsylvania mountain streams using an ergosterol assay. A comparison of summer and fall leaf processing rates in two different watersheds was made for two species of trees, *Acer saccharum* (sugar maple) and *Betula nigra* (river birch). The macroinvertebrate component of the food web was also determined.

Methods and Materials

Study Sites Description

This study was conducted in two, north central Pennsylvania streams of two different orders. Both streams are in Lycoming County. The Mill Creek site is a second-order stream below Warrensville, PA adjacent to Dr. Zimmerman's property. Big Bear Creek site is a third-order stream flowing through the Dunwoody Sportsmens' Club near Barbours, PA. However, the Big Bear Creek site was not used in the fall because of the construction of Rosgen structures in September 2000 that were being used for a separate study to improve trout habitat. Using the EPA habitat assessment by Plafkin et al. (1989), as presented in Figure 2, the Big Bear Creek site runs through a denser forested area and has a well-developed riparian zone indicated by a high habitat assessment score of 187, as opposed to the Mill Creek site with a score of 141, as shown in Table 1. The Mill Creek site is the downstream end of about 7 miles of stream flowing through a mixed agricultural and residential area.

Physical and Chemical Water Analysis

Physical measurements of each stream site were taken at both the beginning and end of all incubation periods. Dissolved oxygen (DO) and temperature ($^{\circ}\text{C}$) were determined using a hand-held YSI model 55 DO meter. Velocity (m/s) was assessed using a Swoffer Model 2100 flow meter. Depth (cm) was measured using a meter stick and width (m) was determined using a meter tape. Chemical analyses of pH, alkalinity (ppm CaCO_3), nitrate (ppm NO_3^-), nitrite (ppm NO_2^-), orthophosphorus (ppm PO_4^{3-}), and conductivity (μS) were done in the laboratory following Standard Methods procedures (American Public Health Association 1995) within 24 hours of sample collection on water samples taken from each site. Between collection and water analysis, samples were

placed in ice during transport and later refrigerated in the lab. Water samples were collected at three intervals during the summer and four intervals during the fall. The titration method using 0.2 N H₂SO₄ was used for alkalinity and pH was assessed on a Corning pH Meter 440. Nitrate and nitrite was measured on a HACH DR/4000 Spectrophotometer and orthophosphorus was measured on a HACH DR/2000 Spectrophotometer. A Hanna Instrument Conductivity/TDS meter model HI 9635 was used to determine conductivity. Standards were run on all instruments.

Leaf Litter Organic Content and Processing Rates

Leaf litter decomposition was tested using two species of leaves: *Acer saccharum* (sugar maple) and *Betula nigra* (river birch). Sugar maple and river birch leaves were picked preabscission in early June and early September. *Acer saccharum* leaves were picked from a tree at Lycoming College, Williamsport, PA, while the *Betula nigra* leaves were picked from a tree at the Mill Creek site. Leaves were kept in a cold room at 5°C until they could be incubated. Each leaf pack consisted of five leaves that were placed on a numbered brick and fastened with 3 rubber bands, as shown in Figure 3. The surface area (cm²) of individual leaves was taken using the LI-COR Model LI-3000A portable area meter. For the summer period, 48 leaf packs were incubated in Big Bear Creek and Mill Creek (4 collection dates x 2 leaf species x 3 replicates x 2 sites). Leaves were placed in the streams on June 14, 2000 and incubated for periods of 7, 21, 28, and 35 days. Also, over 70 leaf packs of sugar maple and river birch were incubated beginning on July 11, 2000 in Big Bear Creek and Mill Creek for long-term incubation until September. At each collection date, three leaf packs were removed from the stream and placed in Ziploc bags to be transported back to the laboratory. Once at the laboratory, the leaves were rinsed with deionized water and invertebrates were collected off of the leaves and preserved in

70% ethanol. For the fall period, twenty-four leaf packs (6 collection dates x 2 leaf species x 2 replicates) were incubated beginning on September 19, 2000 in Mill Creek for 8, 17, 24, 31, 36, 41, 45, and 48 days. Two leaf packs were recovered at each collection date and transported back to the laboratory in Ziploc bags, where invertebrates were collected and preserved in 70% ethanol.

After taking the post-incubation surface area, several leaves were placed in the drying oven at 80°C for approximately 24 hours. Individual leaves were ground using a mortar and pestle. The ground leaves were placed in clean crucibles that were labeled accordingly and pre-weighed on an analytical balance. The leaves were ignited in a muffle oven at 550°C for one hour. The percent organic matter content was calculated as the weight loss due to muffling. Processing rates for each species were determined using the equation $W_t = W_0 e^{-kt}$, where W_t is the post-incubation surface area, W_0 is the pre-incubation surface area, and t is the time in days. Therefore, $k = -\{\ln(W_t/W_0)\} / t$ (Peterson and Cummins 1974).

Invertebrate Analysis

Collected macroinvertebrates were sorted by species and incubation period length and identified to a functional feeding group as set by Cummins and Wilzbach (1985). Functional feeding groups include shredders, collectors (gathering and filtering), scrapers, and predators. Figure 4 shows the general food web of a stream and the importance of each functional feeding group in breaking down organic material. Shredders depend on large organic matter like leaves, wood, and needles, and other plant material derived from the riparian zone. Collectors use small particles of organic matter by either gathering from deposits on the stream bed or filtering from the flowing water. Scrapers remove attached algae from rocks or logs in the current. Predators have specific body parts for capturing

prey. Total numbers of invertebrates were also tallied for each species and incubation date.

Fungal Biomass Determination

Another study was conducted to determine fungal biomass concentrations in leaf detritus by ergosterol quantification. In the summer, Big Bear and Mill Creek were yet again the two study sites. The leaf packs consisted of five leaves per pack of *Acer saccharum*, sugar maple, and *Betula nigra*, river birch, leaves that had been collected preabscission, stored, and incubated, as were the leaves in the leaf decomposition study. Forty-eight leaf packs were again incubated between the two sites for 7, 21, 28, and 35 days. At each collection date, the leaf packs were transported back to the laboratory in Ziploc bags where the leaves were rinsed with deionized water and the invertebrates were collected from the leaves and preserved. In the fall, Mill Creek was again the only site able to be studied. Twenty-four leaf packs of five leaves/pack (12 *Acer saccharum* and 12 *Betula nigra*) were incubated there, as in the leaf decomposition study. Incubation periods were again 8, 17, 24, 31, 36, and 48 days. At each collection date, the leaf packs were placed in Ziploc bags to be taken back to the laboratory where leaves were rinsed and the invertebrates were collected from the leaves and preserved. Leaves were placed in a freezer until ergosterol extraction could be performed.

A stock ergosterol standard solution was prepared by dissolving 0.2001 g of 95% ergosterol (Aldrich) in 200 mL of HPLC-grade methanol. One mL of the stock ergosterol standard solution was diluted to 50 mL to reach a final working ergosterol standard solution of 19.01 μg ergosterol/mL methanol (see Appendix I). High-Pressure Liquid Chromatography (HPLC) was used to detect ergosterol peaks. The HPLC system used consisted of a Waters 510 pump, a Whatman Partisil 5 OD5-3 25 cm x 4.6 mm column set

to monitor 282 nm (the wavelength of maximum absorption by ergosterol), 100 μL sample loop, and a Waters 991 photodiode array detector with Millennium software. Varying volumes of the standard solution ranging from 5 μL to 80 μL were injected into the HPLC system to establish a standard curve by plotting peak areas against known ergosterol amounts, as shown in Figure 5.

Extraction was done using procedures, with some modifications, as stated by Newell et al. (1988). For each sample, 10 discs were cut from the leaves using a 13 mm cork borer and placed in 25 mL of HPLC-grade methanol in a round-bottom flask. The flask was lowered into an 80°C water bath and refluxed for 30 minutes. Five mL of 4% KOH was added and the solution was refluxed for an additional 30 minutes. When the solution cooled to room temperature, it was filtered by water aspiration through a 60 mL Buchner funnel (glass frit, coarse, 40-60 μm) to remove any debris and transferred to a 65-mL screw cap vial. Five mL of 20% (w/v) salt water were placed in the vial to promote layer separation. Three consecutive portions of pentane (10 mL, 5 mL, and 5 mL) were added. After each addition, the vial was repeatedly inverted, pressure was released, and the top pentane layer containing the ergosterol was removed and combined in a separate vial. The pentane layer was filtered through a 0.45 μm nylon membrane with a glass microfiber prefilter (Whatman Autovial, Cat # AV125UNAO) and the bottom layer of methanol was removed. Uncovered vials were placed in a hood overnight with air circulation to evaporate the pentane. After evaporation, sample residues were redissolved in 1 mL of HPLC-grade methanol and sonicated until all residues was dissolved. The sample was then filtered through a 13 mm 0.45 μm nylon membrane. The HPLC-grade methanol used as the solvent was degassed in the solvent bottle by simultaneous application of vacuum and sonication (Cole-Parmer sonnicator bath 8845-30). Prior to

sample injection, a baseline was established on the HPLC for twenty minutes to assure that no impurities were in the system. A sample was injected into the system and run with HPLC-grade methanol through a Whatman Partisil 5 0D5-3 25 cm x 4.6 mm column at a flow rate of 1.5 mL/minute and a detection wavelength of 282 nm. Experimental peak areas (see HPLC printout example in Figure 6, see Appendix II for all experimental HPLC printouts) were manually reintegrated (see Figure 7) to eliminate underlying base area caused by carryover from preceding peaks. The peak areas were compared to the standard curve to obtain experimental ergosterol amounts. These amounts were then corrected for the volume injected and the dissolution volume. Finally, the experimental ergosterol concentration was converted to grams of fungal biomass using a conversion factor of 182 g fungal biomass/g ergosterol (see Gessner and Chauvet 1992). Finally, the grams of fungal biomass/sample were divided by the original leaf disc mass that underwent a reflux extraction. Lastly, g fungal biomass/g detritus was converted to μg fungal biomass/mg detritus.

Before running sample extractions, extractions were run on fresh leaves, ones that were picked off the same trees that were used for leaves to be incubated in the stream. These extractions were done to verify that there was no ergosterol present in vascular plants, only in aquatic hyphomycetes that colonize the incubated plant material.

Furthermore, two duplicate recovery studies for the reflux extraction procedure were performed. Ergosterol (20 μg) was reflux extracted, following the same procedures as described above for the leaves, and run on the HPLC. Also, a 20 μg sample ergosterol sample was run directly on the HPLC (no prior reflux). The peak area of the known ergosterol was compared to the ergosterol peak from the extracted ergosterol to obtain the amount of ergosterol recovered. The resulting amount was shown as a percent recovered

from the original 20 μg . This determined the efficiency of the extraction procedure employed.

Statistical Analysis

Data analysis was done using a two-sample hypothesis test at a α -level of 0.05.

Analysis was performed on SPSS 10.0 Windows computer program (SPSS 2000).

Results

Physical and Chemical Water Analysis

The results of summer (Mill Creek and Big Bear Creek) and the fall (Mill Creek) chemical water analysis are presented in Tables 2-3 and the physical water analysis is presented in Tables 4-5. In general, alkalinity, nitrates, nitrites, and orthophosphorus were higher in Mill Creek than in Big Bear Creek. The summer pH in Big Bear Creek was significantly lower than the summer pH in Mill Creek ($P=0.079$). Big Bear Creek exhibited the lowest pH of 5.3 in the summer study. Mill Creek had a significant pH increase from the summer to fall study and showed the highest pH of 8.09 in September ($P=0.144$). Alkalinity was higher in Mill Creek in the fall than in the summer; conductivity was also higher in Mill Creek in the fall study. Mill Creek's temperature was higher than Big Bear Creek's temperature in the summer study with a high of 15.4°C. Mill Creek's temperature was lower in the fall than in the summer, with a low temperature of 6.1°C.

Percent Organic Content

Percent organic content results are shown in Figures 8-10. Data gaps are due to weather conditions not leaving enough leaves to sample for that period. In the summer study of Mill Creek, *Acer saccharum*, sugar maple, percent organic content decreased slightly over incubation time. Its organic content decreased by 16% from 28 to 35

incubation days. Overall, *Acer saccharum* had a higher organic content than *Betula nigra*, river birch. In the summer study of Big Bear Creek, sugar maple and river birch organic content generally decreased. Fall organic content (Mill Creek) for sugar maple decreased slightly as incubation time increased. River birch organic content also generally decreased over incubation time. In general, river birch organic contents were higher in the fall than in the summer in Mill Creek. However, sugar maple organic contents were higher in the summer than the fall in Mill Creek.

Leaf Processing Rates

Processing rates (k) are shown in Tables 6. Both sites' summer values for *Acer saccharum*, sugar maple, and *Betula nigra*, river birch leaves are $k > 0.01$, so they are considered fast decomposers by Peterson and Cummins (1974). Summer leaf processing values were significantly lower in Big Bear Creek than in Mill Creek for both sugar maple and river birch leaves ($P=0.786$, $P=0.159$). In both Mill Creek and Big Bear Creek, sugar maple had significantly higher summer k values of 0.110 and 0.027, respectively, than the river birch leaves ($P=0.787$, $P=0.689$). However, the fall k values of *Acer* and *Betula* leaves at Mill Creek exhibited no significant difference ($P=0.014$). Finally, summer k values at Mill Creek were significantly higher than the fall k values for both sugar maple and river birch leaves ($P=0.751$, $P=0.060$).

Invertebrate Analysis

Results of the invertebrate analysis are shown in Figures 11-14. For both leaf species for the summer study in Mill Creek, the total number of invertebrates increased between days 7 and 21 and decreased for the rest of the incubation. The *Acer saccharum*, sugar maple, and *Betula nigra*, river birch, leaves contained mainly filtering collectors. For the summer study in Big Bear Creek, total invertebrates for both leaf

species increased between days 7 and 28, but decreased from days 28 and 35. The prevalent functional feeding group in Big Bear Creek on *Acer* leaves was the gathering collectors for the 7-, 28-, and 35-day samples. The predominant group on *Betula* leaves at this site was the filtering collectors for the 7- and 28-day samples and the gathering collectors for the 21- and 35- day samples. In the fall study of Mill Creek, total invertebrates for both leaf species increased slowly to the 41-day mark and decreased until 48 days. The sugar maple leaves held predominantly gathering collectors for the 8-, 24-, and 31-day samples, while the 17-, 41-, and 48-day samples had a prevalence of filtering collectors. Fall-incubated river birch leaves contained predominantly gathering collectors for the 8-, 17-, and 24-day sample. The 31-day sample has mainly scrapers, while the 41- and 48-day samples had mainly shredders. Any data gaps are due to high water conditions destroying or washing leaf packs downstream.

Fungal Biomass

The results of the recovery study revealed the extraction procedure yielded 16.8 μg ergosterol and 17.7 μg ergosterol from 20 μg ergosterol. This corresponds to 84% and 89% efficiency of the reflux extraction procedure.

Fungal biomass concentrations are shown in Figures 15-18. Any data gaps are because the incubated leaves for that date were gone from the brick or the brick had been washed downstream. Ergosterol eluted in the HPLC between 5.3 and 5.8 minutes. Summer fungal biomass levels of *Acer saccharum*, sugar maple, leaves were significantly higher in Mill Creek than in Big Bear Creek ($P=0.066$). The highest concentration was 2.28 $\mu\text{g}/\text{mg}$ detritus, found at the 7-day Mill Creek incubation. Though Mill Creek had generally significantly higher river birch fungal biomass concentrations, the highest value of 2.24 $\mu\text{g}/\text{mg}$ detritus was exhibited in Big Bear Creek at the 7-day incubation ($P=0.153$).

The *Betula nigra*, river birch, biomass levels at Big Bear Creek declined from the 7-day mark, as time increased, as did the sugar maple biomass. In the fall, fungal biomass (Mill Creek) concentrations were higher for river birch leaves compared to sugar maple leaves. The highest amount found was 8.43 ug/mg detritus at the 41-day incubation. Fall *Betula* fungal biomass concentrations generally increased over incubation time, though they peaked at 41 days and then decreased. Fall *Acer* biomass levels, on the other hand, generally remained at the same low level throughout the incubation time. The highest amount that was found was 1.12 ug/mg detritus at the 45-day mark. When comparing fall and summer fungal biomass concentrations, *Betula* exhibited significantly higher fungal biomass levels in the fall than the summer ($P=0.500$). On the other hand, *Acer* showed no significant difference in fungal biomass levels between the fall and the summer incubation in Mill Creek ($P=0.024$).

All of the leaf packs that had been incubating in the streams for 3 months were either entirely gone because the brick had been carried downstream or the leaf pack was gone. Two river birch leaf packs, though, at Mill Creek were intact enough to analyze the fungal biomass content. These results are shown in Figure 18. Sample 1 had a fungal biomass content of 1.94 ug/mg detritus, while Sample 2 had a fungal biomass level of 0.89 ug/mg detritus, roughly a 2 to 1 ratio of fungal biomass to fungal biomass. These results are comparable to the fungal biomass amounts in the 8- and 17-day river birch samples in the fall study.

The fresh leaf extractions that were performed showed no presence of ergosterol after being run on the HPLC.

Discussion

The governing energy source of woodland stream ecosystems is allocthonous input such as leaf litter (Peterson and Cummins 1974). As leaves enter a stream, they are colonized by fungi, mainly a group called aquatic hyphomycetes. The membranes of these particular fungi contain a sterol called ergosterol. Ergosterol has been proven not to be a component of vascular plants, so its presence can be used to quantify the amount of fungal biomass on leaf litter (Gessner and Chauvet 1994).

Summer and fall organic contents generally decreased over incubation time for both *Acer saccharum* and *Betula nigra* at both sites. This trend was expected because increased nutrient leaching and microbial degradation over incubation time would cause organic content to decrease.

Summer leaf processing values (k) were significantly lower in Big Bear Creek than in Mill Creek for both *Acer saccharum* and *Betula nigra* ($P=0.786$, $P=0.159$). This finding may also be due to the significantly lower pH in Big Bear Creek because Solada et al. (2000) found that k values are significantly lower in acidic streams because the acidification reduces the nutrient base for aquatic consumers ($P=0.079$). Summer k values for *Acer saccharum* and *Betula nigra* in Mill Creek were significantly higher than Mill Creek fall k values ($P=0.751$, $P=0.060$). Maloney and Lamberti's (1995) research on leaf decomposition of various leaf species, including sugar maple, shows that summer leaf processing may be higher than fall processing because of the invertebrate abundance in the summer. The higher summer k values may also be attributed to by the higher summer water temperatures, which increase leaf decay. Furthermore, for both Mill Creek and Big Bear Creek in the summer, sugar maple had a significantly higher k value (0.110, 0.0271) than river birch leaves ($P=0.787$, $P=0.689$). This data is confirmed by Peterson and

Cummins (1974) research that places sugar maple in the fast decomposers category, $k > 0.01$. Additionally, sugar maple leaves may be processed faster because of the increased surface area of sugar maple leaves that provides more surface area for microbial colonization (Maloney and Lamberti 1995).

Summer fungal biomass concentrations for *Acer saccharum* and *Betula nigra* were significantly higher in Mill Creek than in Big Bear Creek, possibly due to the significantly more acidic conditions of Big Bear Creek ($P=0.066$, $P=0.153$). Invertebrate densities, though, were generally higher in Big Bear Creek than in Mill Creek. These results coincide with Engstrom et al.'s (2000) findings that acidic streams have lower fungal biomass along with increased invertebrate densities because acidic conditions decrease energy and other material accessibility to stream macroconsumers. River birch fungal biomass levels in Mill Creek were found to be higher in the fall than in the summer, which coincides with Suberkropp's (1997) and Aimer's (1985) research that found ergosterol levels to peak in the fall to early winter and to be lowest in the summer. However, sugar maple fungal biomass levels were not consistent with the literature because this study showed no significant difference between the summer and fall fungal biomass values for sugar maple leaves ($P=0.024$). This finding could be due to the significantly faster processing of sugar maple leaves, which means less leaf material remains as a substrate for microbial colonization. Compared to Suberkropp's (1997) research that determined fungal biomass concentrations of leaf litter in a Tennessee woodland stream, this study's ergosterol levels are relatively low. This finding could be due to the method of leaf pack incubation or the type of ergosterol extraction procedure.

Summer total invertebrates at both sites showed an increasing trend as fungal biomass decreased. This is an expected trend because fungi condition incubated leaves

making them more nutritious for invertebrates (Suberkropp 1997). Therefore, invertebrates will colonize the leaves more when there are more microbes present. As a result, fungal biomass concentrations will eventually decrease with increased invertebrate colonization.

There are drawbacks to the leaf pack construction technique employed in this study. First, the use of rubber bands in fastening leaves to the brick does not allow determination of how much loss is due to just physical breaks and not decomposition. Fishing line may be used in future studies because it has less surface area than rubber bands. Moreover, many leaf packs were lost due to high water events tearing the leaf packs off of the bricks or carrying the bricks completely downstream. Another leaf pack construction technique that should be evaluated is placing leaves in nylon mesh bags. Boulton and Boon (1991) state that this method provides similar environmental conditions inside the bag as is outside the bag. Also, major quantities of detritus material will not escape through the bag. However, mesh bags may exclude large shredders and change water currents around the bag, which may alter potential microbial colonization. Overall, in further studies, both techniques could be employed for comparison of invertebrate, decomposition, and microbial effects.

Additionally, a further study could be performed to identify exact species of aquatic hyphomycetes on the leaf litter that had been incubated in these two sites. One study researched which species of aquatic hyphomycetes dominate varying temperature period (Suberkropp 1984). Leaf species preference may also be taken into consideration as a factor for dominating fungi. Suberkropp (1984) outlines three incubation methods that can be used to assess background aquatic hyphomycetes that would potentially colonize leaf packs.

The pattern of fungal biomass' presence was significantly correlated with season, temperature, and pH. Furthermore, leaf-processing values were consistent with other studies in that k values were higher in the summer than in the fall. Future studies on this subject should consider a larger sample size and the other research methods previously mentioned.

Overall, fungal biomass amounts were higher in the fall than in the summer and were higher in the less acidic stream. Leaf processing, or the k values, was faster in the warmer stream and in the summer. Also, leaf processing was more rapid for the sugar maple leaves than the river birch leaves.

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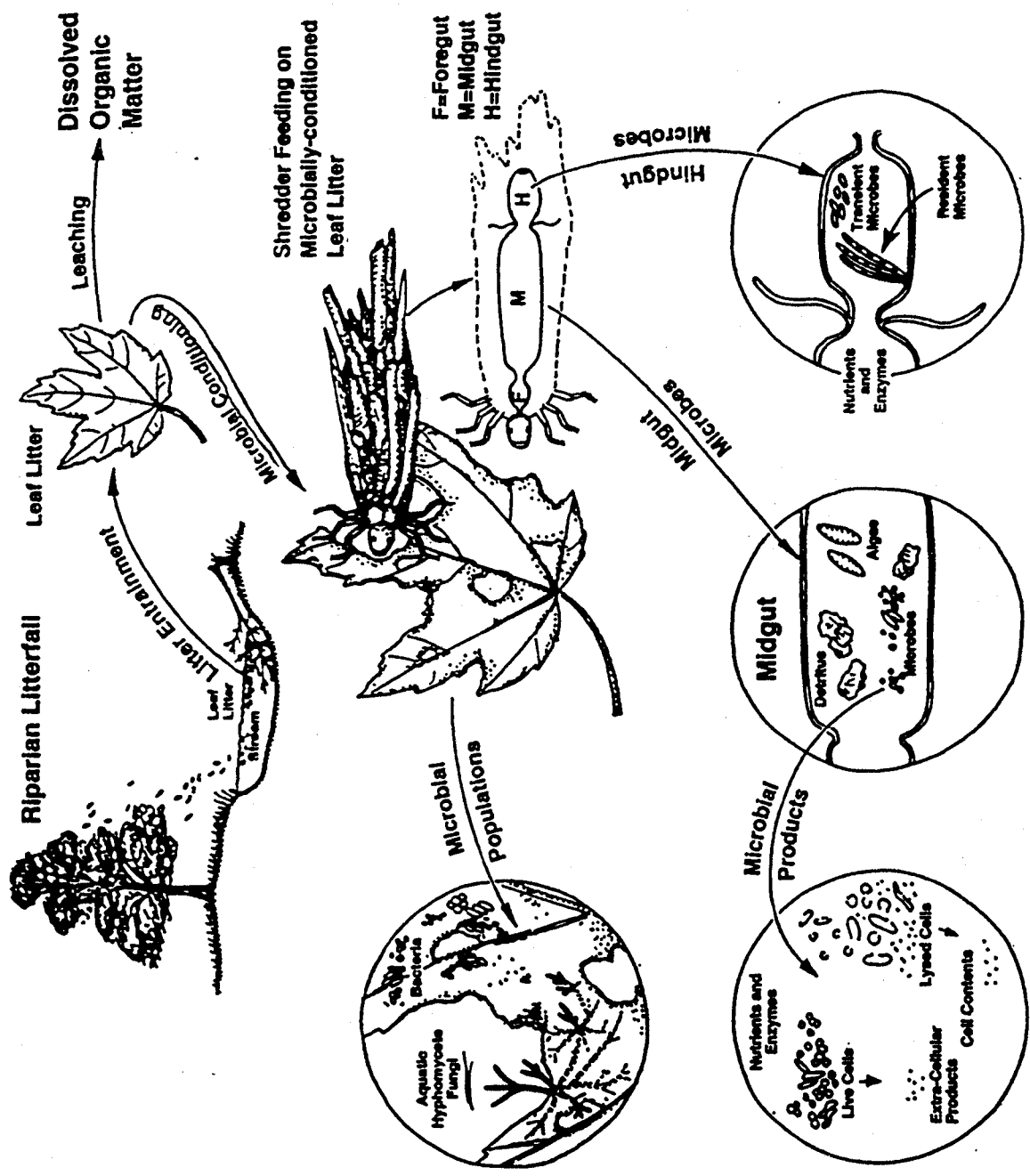


Figure 1: A stream ecosystem model showing the importance of fungi in leaf conditioning modified from Cummins 1975

HABITAT ASSESSMENT FIELD DATA SHEET

RIFPLE/RUN PREVALENCE

Habitat Paramotor	Category			
	Optimal	Suboptimal	Marginal	Poor
<p>1. Instream Cover (Fish)</p> <p>Greater than 50% mix of boulder, cobble, submerged logs, undercut banks, or other stable habitat.</p>	<p>30-50% mix of boulder, cobble, or other stable habitat; adequate habitat.</p>	<p>10-30% mix of boulder, cobble, or other stable habitat; habitat availability less than desirable.</p>	<p>Less than 10% mix of boulder, cobble, or other stable habitat; lack of habitat is obvious.</p>	
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<p>2. Epifaunal Substrate</p> <p>Well-developed riffle and run; riffle is as wide as stream and length extends two times the width of stream; abundance of cobble.</p>	<p>Riffle is as wide as stream but length is less than two times width; abundance of cobble; boulders and gravel common.</p>	<p>Run area may be lacking; riffle not as wide as stream and its length is less than 2 times the stream width; gravel or large boulders and bedrock prevalent; some cobble present.</p>	<p>Riffles or run virtually nonexistent; large boulders and bedrock prevalent; cobble lacking.</p>	
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<p>3. Embeddedness</p> <p>Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment.</p>	<p>Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.</p>	<p>Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.</p>	<p>Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.</p>	
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<p>4. Velocity/Depth Regimes</p> <p>All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow).</p>	<p>Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).</p>	<p>Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).</p>	<p>Dominated by 1 velocity/depth regime (usually slow-deep).</p>	
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<p>5. Channel Alteration</p> <p>No channelization or dredging present.</p>	<p>Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.</p>	<p>New embankments present on both banks; and 40 to 80% of stream reach channelized and disrupted.</p>	<p>Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted.</p>	
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<p>6. Sediment Deposition</p> <p>Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.</p>	<p>Some new increase in bar formation, mostly from coarse gravel; 5-30% of the bottom affected; slight deposition in pools.</p>	<p>Moderate deposition of new gravel, coarse sand on old and new bars; 30-50% of the bottom affected; sediment deposits at obstruction, constriction, and bends; moderate deposition of pools prevalent.</p>	<p>Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.</p>	
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Figure 2: The EPA's habitat assessment form developed by Plafkin et al. (1989)

Figure 2 continued

RIFFLERUN PREVALEN

Habitat Parameter	Category			
	Optimal	Suboptimal	Marginal	Poor
7. Frequency of Riffles	Occurrence of riffles relatively frequent; distance between riffles divided by the width of the stream equals 5 to 7; variety of habitat.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream equals 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is between ratio > 25.
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Channel Flow Status	Water reaches base of both lower banks and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
9. Condition of Banks	Banks stable; no evidence of erosion or bank failure.	Moderately stable; infrequent, small areas of erosion mostly healed over.	Moderately unstable; up to 60% of banks in reach have areas of erosion.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; on side slopes 60-100% of bank has erosional scars.
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
10. Bank Vegetative Protection	More than 90% of the streambank surfaces covered by vegetation.	70-90% of the streambank surfaces covered by vegetation.	50-70% of the streambank surfaces covered by vegetation.	Less than 50% of the streambank surfaces covered by vegetation.
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
11. Grazing or Other Disruptive Pressure	Vegetative disruption, through grazing or mowing, minimal or not evident; almost all plants allowed to grow naturally.	Disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	Disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Disruption of streambank vegetation is very high; vegetation has been removed to 2 inches or less in average stubble height.
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
12. Riparian Vegetative Zone Width	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.
SCORE _____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Total Score _____

	Mill Creek	Big Bear Creek
<i>Instream Cover</i>	13	20
<i>Epifaunal Substrate</i>	12	17
<i>Embeddedness</i>	16	18
<i>Velocity/Depth Regimes</i>	14	20
<i>Channel Alteration</i>	12	14
<i>Sediment Deposition</i>	10	17
<i>Frequency of Riffles</i>	13	18
<i>Channel Flow Status</i>	12	13
<i>Condition of Banks</i>	10	10
<i>Bank Vegetative Protection</i>	10	11
<i>Grazing or Other Disruptive Protection</i>	13	15
<i>Riparian Vegetative Zone Width</i>	6	14
Total	141	187

Table 1: Habitat Assessment Scores for Mill Creek and Big Bear Creek



Figure 3: Leaf packs on bricks before incubation

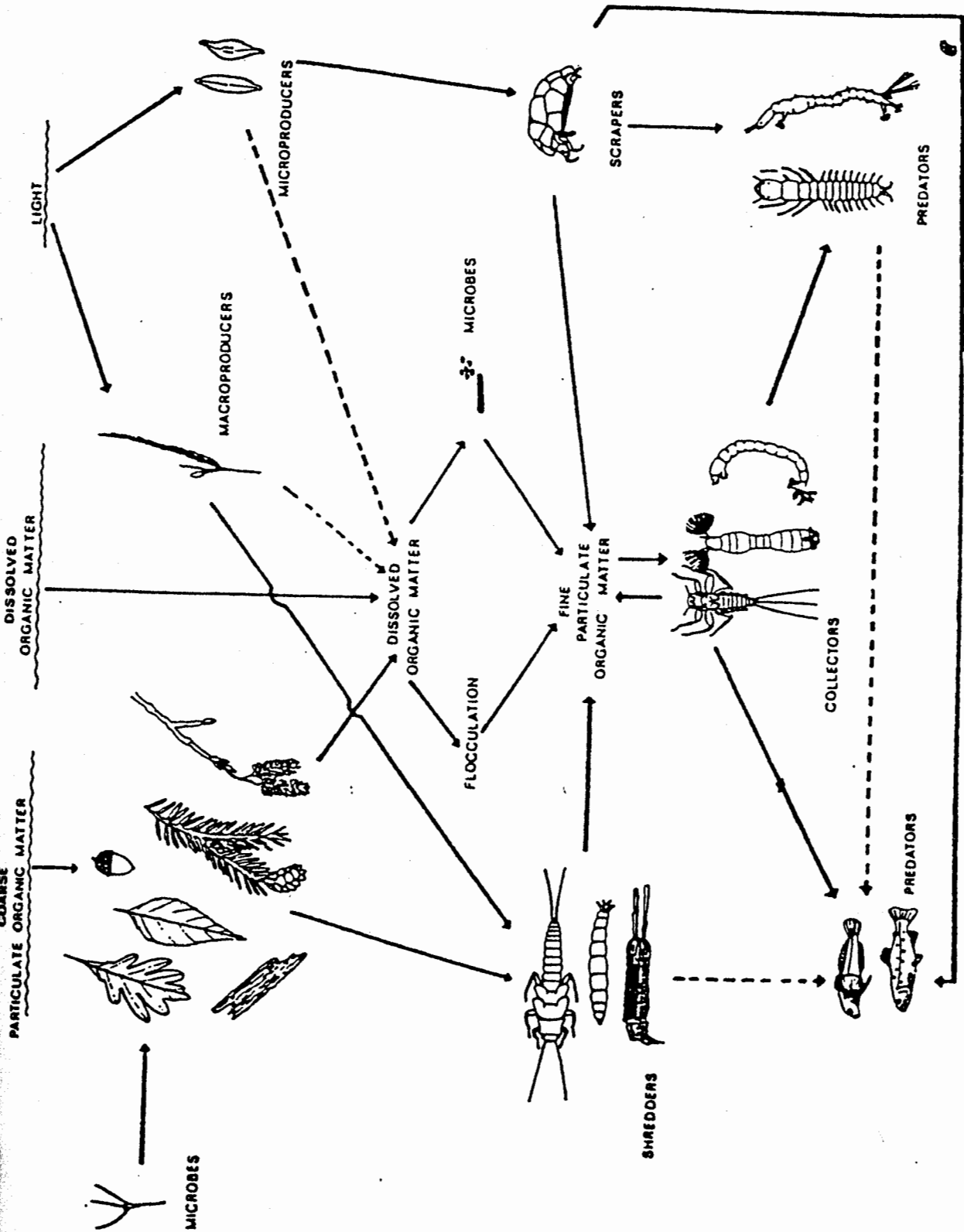


Figure 4: A model of macroinvertebrate functional feeding group's interactions in a stream ecosystem modified from Cummins 1975

Ergosterol Standard Curve

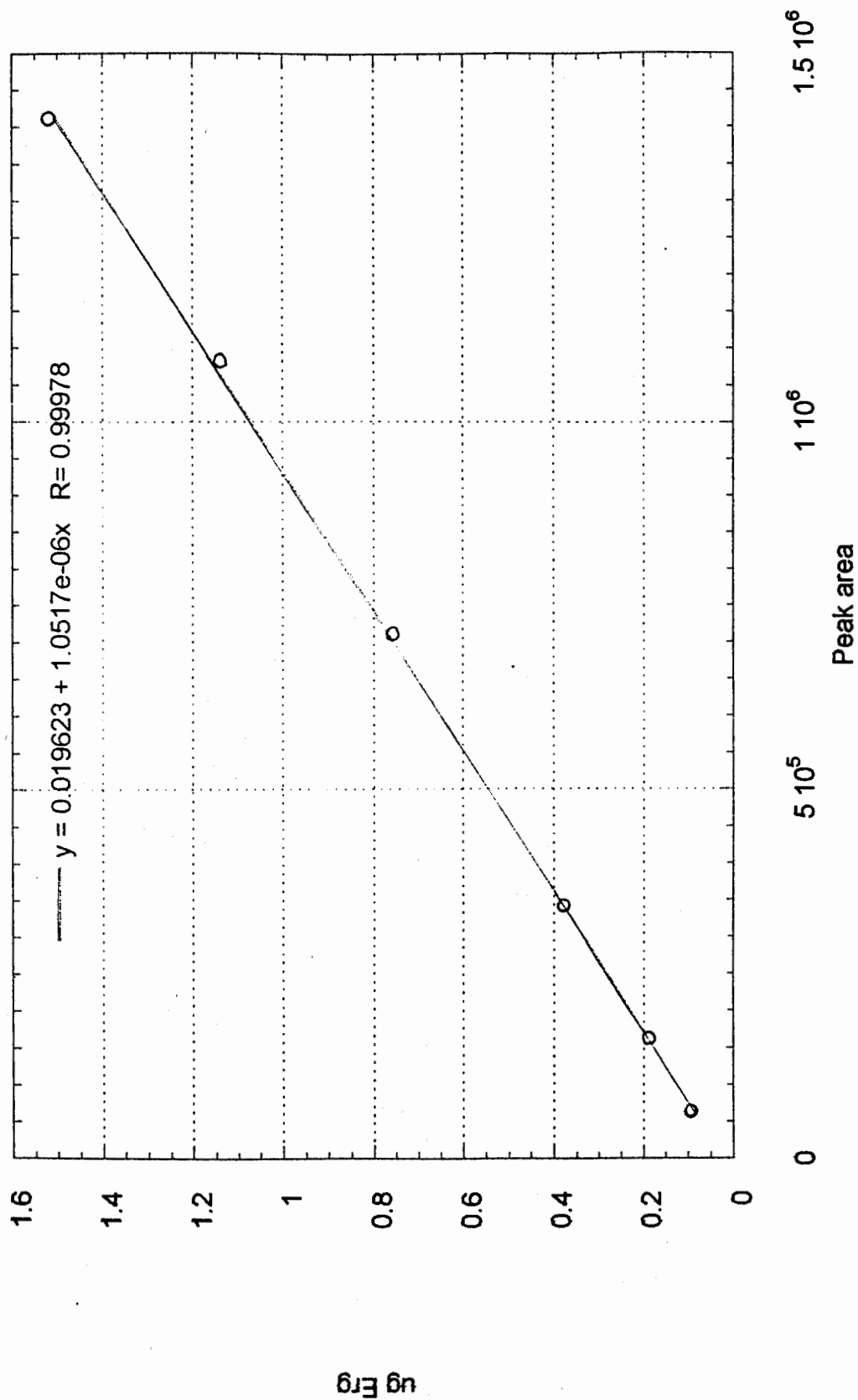
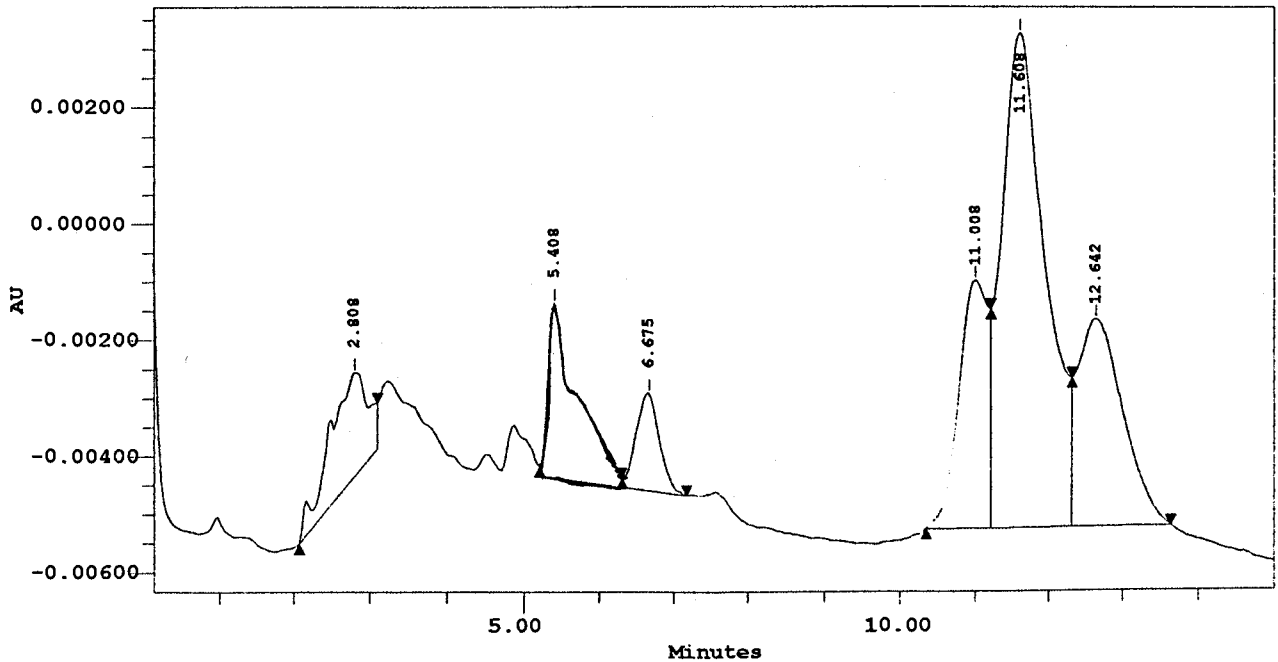


Figure 5: Standard curve from known ergosterol concentrations versus ergosterol peak areas from HPLC

Millennium Results Report	August 1, 1984	Page: 1 of 1
Report Method: MetCarb_RM	Version: 2.15	
For Sample: 60 ul of #64	Vial: 5	Injection: 1
Channel: 991M	Proc Chan: PDA_282.0nm	Processed: 08/01/84 09:55:46 PM
Channel Descr: PDA 282.0 nm		

Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: 60 ul of #64
Vial: 5	Sample Origin:
Sample Type: Unknown	Solvent: meoh
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 08/01/84 09:40:22 PM	Volume: 60.00
Sample Weight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.808	64098	1792	BV	2.075	3.108	8.00
2		5.408	80011	3017	VV	5.208	6.308	9.98
3		6.675	36103	1681	VB	6.308	7.175	4.50
4		11.008	113611	4283	BV	10.342	11.208	14.17
5		11.608	354408	8508	VV	11.208	12.308	44.21
6		12.642	153351	3581	VB	12.308	13.642	19.13

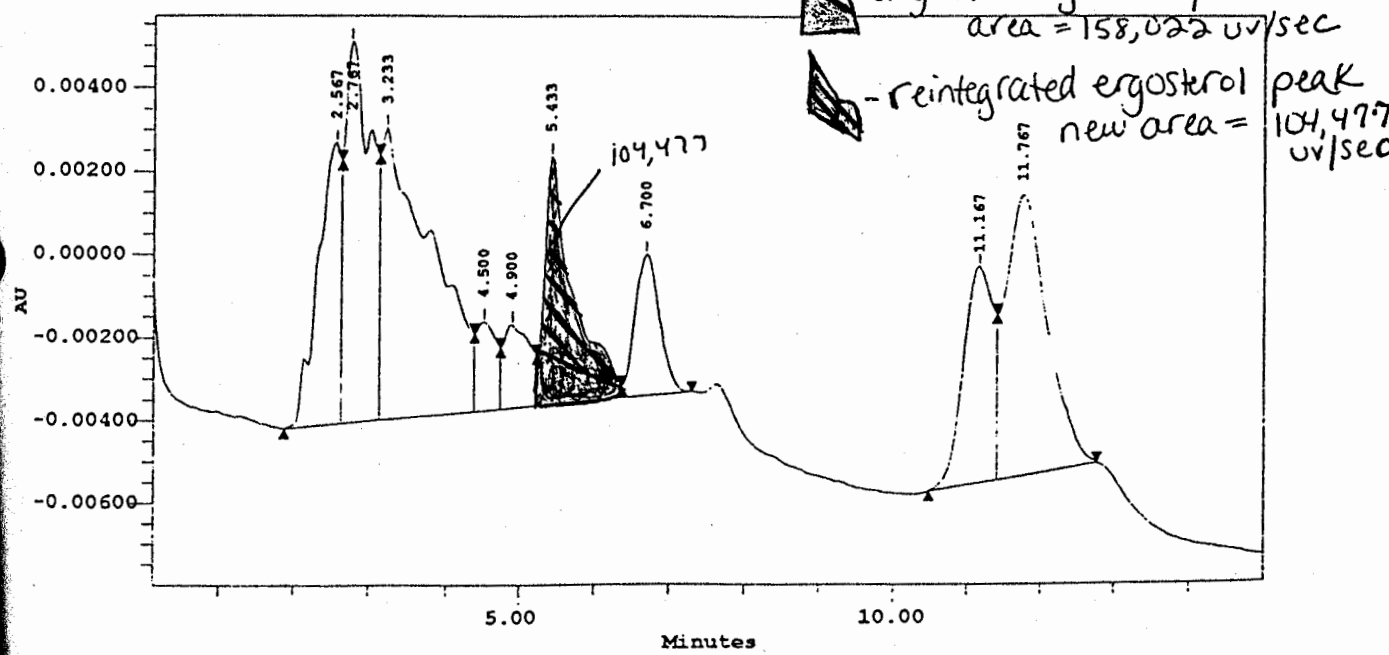
Figure 6: A sample HPLC printout (Dunwoody, sugar maple, 35-day sample) with ergosterol peaking at 5.408 seconds (area=80011 uv/sec)

D-m-21-7-13-00

Millennium Results Report July 12, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #54 Vial: 2 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/12/84 09:13:51 PM
 Channel Descr: PDA 282.0 nm

1ml Lycoming College, Department of Chemistry Z1 Dunwoody S. Ripe Jul 7

Project Name: Ergosterol Sample Name: 60 ul of #54
 Vial: 2 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 Flow Rate: 1.500
 Channel: 991M Level:
 Date Acquired: 07/12/84 08:53:09 PM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.567	139542	6749	BV	1.867	2.633	9.81
2		2.767	222590	9136	VV	2.633	3.133	15.65
3		3.233	317458	6993	VV	3.133	4.367	22.32
4		4.500	41949	2142	VV	4.367	4.733	2.95
5		4.900	50722	2005	VV	4.733	5.233	3.57
6		5.433	(158022)	5966	VV	5.233	6.367	11.11
7		6.700	76461	3393	VB	6.367	7.300	5.38
8		11.167	143429	5213	BV	10.467	11.400	10.08
9		11.767	272305	6762	VB	11.400	12.767	19.14

Figure 7: An example of an HPLC printout including the reintegration of the ergosterol peak

54

	pH	alkalinity(ppm CaCO ₃)	Nitrate (ppm NO ₃ ⁻)	Nitrite(ppm NO ₂ ⁻)	Orthophosphorus (ppm PO ₄ ³⁻)
6/14/00	6.45	21	7.7	0.0105	0.1
7/5/00	6.92	20	1.5	0.0102	0.01
7/14/00	6.91	21.5	1.3	0.003	n/a
9/27/00	8.09	13	1.3	0.0122	0.16
10/13/00	7.32	36	1.5	0.006	0.01
10/30/00	7.54	28	1.5	0.0051	0.01
11/6/00	7.18	20	1.3	0.0067	0.02

Table 2: Summer and Fall Chemical Water Analysis for Mill Creek

	pH	alkalinity(ppm CaCO ₃)	Nitrate (ppm NO ₃ ⁻)	Nitrite(ppm NO ₂ ⁻)	Orthophosphorus (ppm PO ₄ ³⁻)
6/19/00	5.3	1.5	0.7	0.0043	0.02
7/10/00	6.39	5	0.5	0.0013	0.06
8/1/00	6.43	5	0.6	0.0049	0.096

Table 3: Summer Chemical Water Analysis for Big Bear Creek

	Velocity (m/s)	Depth (cm)	Width (m)	DO (ppm)	Temp (°C)	Conductivity (µs)
6/23/00	n/a	n/a	n/a	11	10.9	n/a
7/7/00	0.75	22	n/a	10.19	13	86.8
7/14/00	0.35	n/a	8.8	10.02	n/a	79.9
7/21/00	0.62	16	8.45	6.61	12.7	93.4
9/5/00	0.76	n/a	8.59	7.6	13.2	n/a
9/12/00	0.31	9.5	8.33	8.16	19.2	n/a

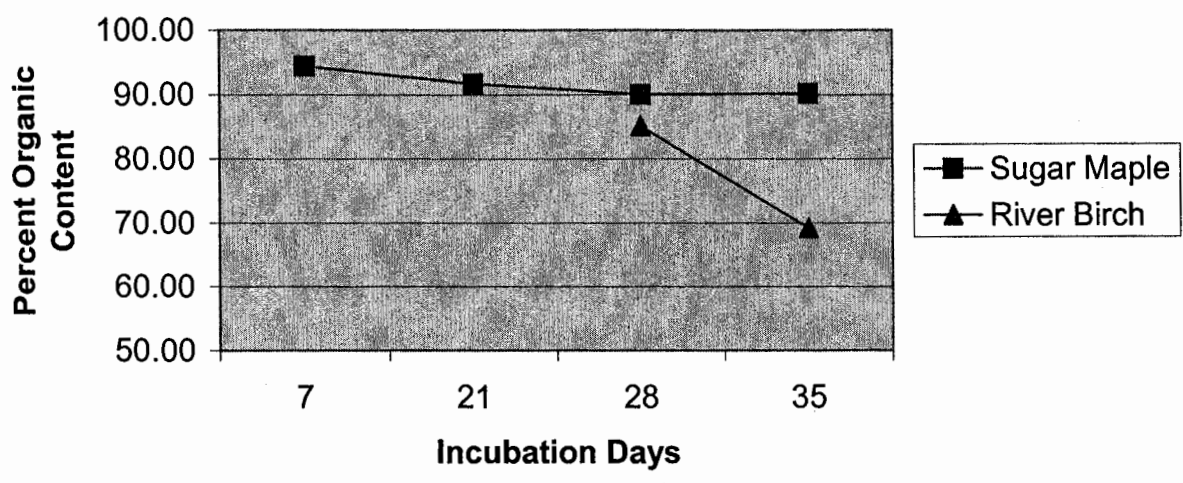
Table 4: Summer Physical Water Analysis for Big Bear Creek

	Velocity (m/s)	Depth (cm)	Width (m)	DO (ppm)	Temp (°C)	Conductivity (µs)
6/21/00	n/a	n/a	n/a	9.56	15.3	86.8
7/5/00	0.38	18	8.8	10.9	11	79.9
7/12/00	0.35	15	8.8	10.19	13.6	n/a
7/19/00	0.26	15	8.44	10.02	15.4	93.4
9/27/00	0.15	n/a	n/a	76.3	14.3	122.5
10/6/00	0.37	15	n/a	62.3	13.7	n/a
10/20/00	0.32	16	8	5.15	9	n/a
10/25/00	0.19	12	n/a	6.8	10.3	n/a
10/30/00	0.2	11	8	7.41	7.3	242
11/3/00	0.14	10.5	n/a	8.78	7.5	n/a
11/6/00	0.24	17	n/a	10.66	6.1	525

Table 5: Summer and Fall Physical Water Analysis for Mill Creek

8a

Summer Percent Organic Content of Leaves in Mill Creek



8b

Summer Percent Organic Content of Leaves in Big Bear Creek

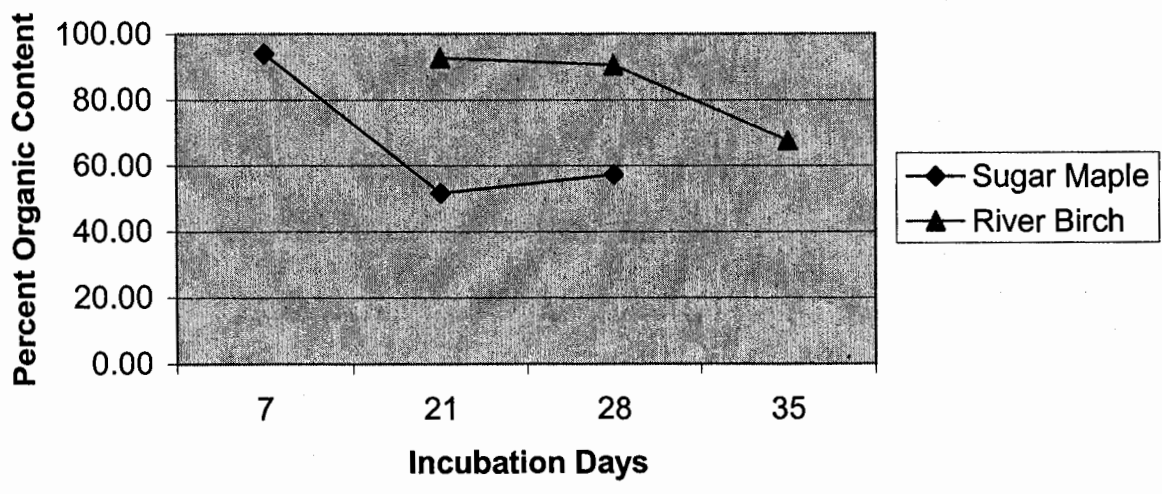


Figure 8a-b: Percent Organic Content of Sugar Maple and River Birch Leaves in Mill Creek and Big Bear Creek during the Summer Study

Fall Percentage Organic Content of Leaves in Mill Creek

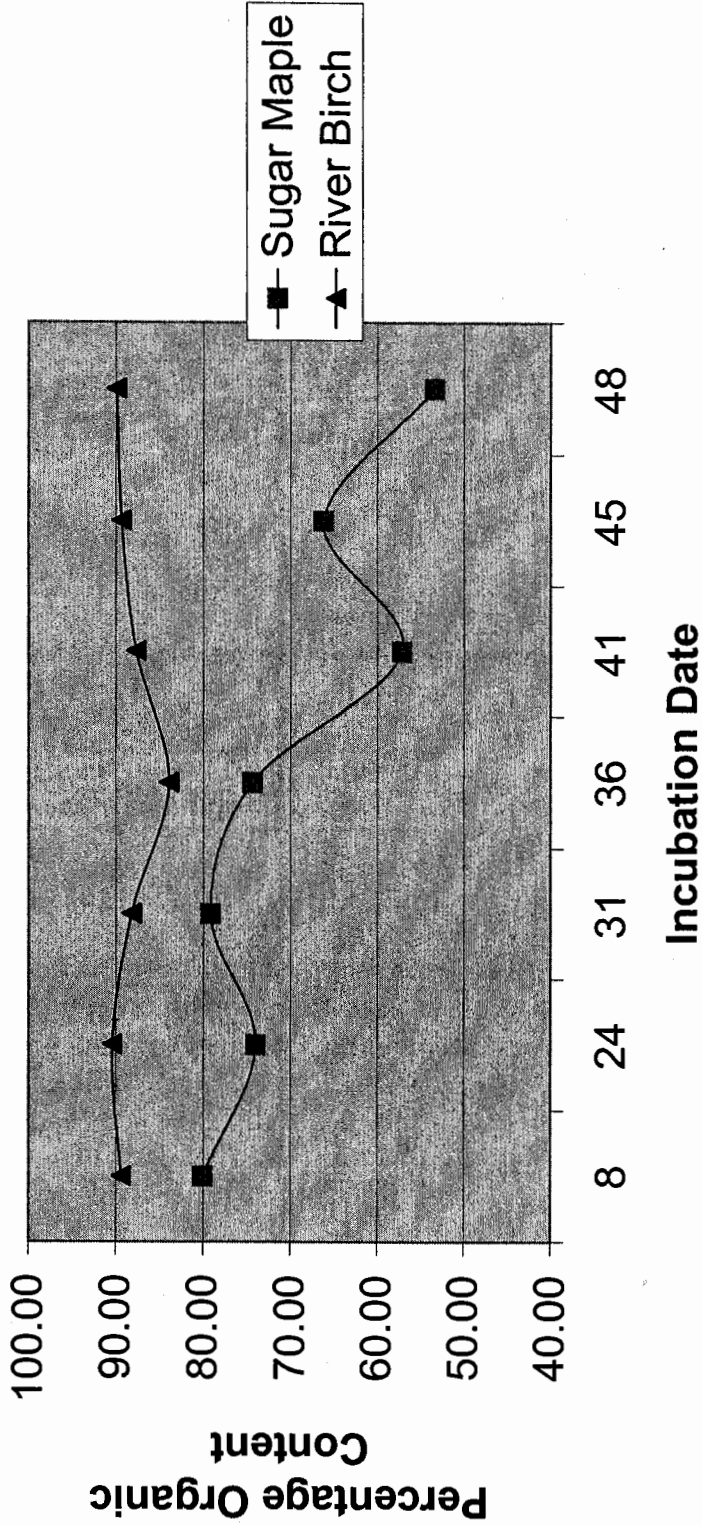


Figure 9: Percent Organic Content of Sugar Maple and River Birch Leaves in Mill Creek during the Fall Study

Table 6a: Summer Mill Creek mean k values

River Birch	0.0285+/.0188
Sugar Maple	0.110

Table 6b: Summer Big Bear Creek mean k values

River Birch	.0173+/-0.0107
Sugar Maple	0.0271+/-0.0186

Table 6c: Fall Mill Creek mean mean k values with standard deviations

River Birch	0.0008 +/- 0.0006
Sugar Maple	0.0015 +/- 0.001

Tables 6a-c : Fall and Summer k values calculated as the natural log of the post-incubation surface area divided by the pre-incubation surface area, all divided by the incubation time in days

Figure 10a

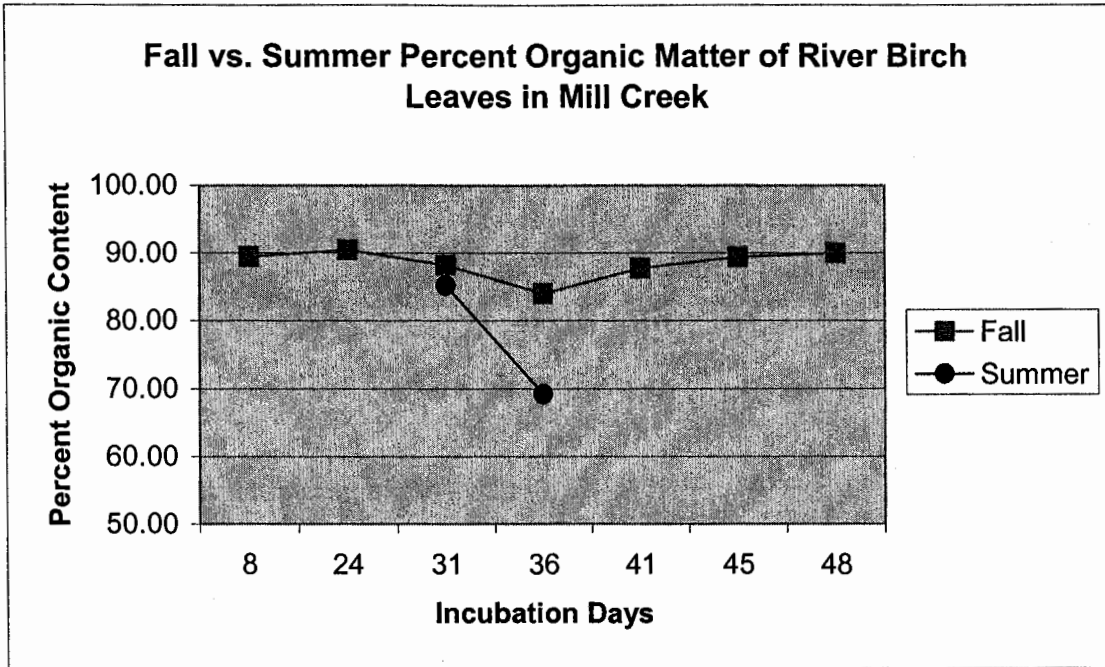


Figure 10b

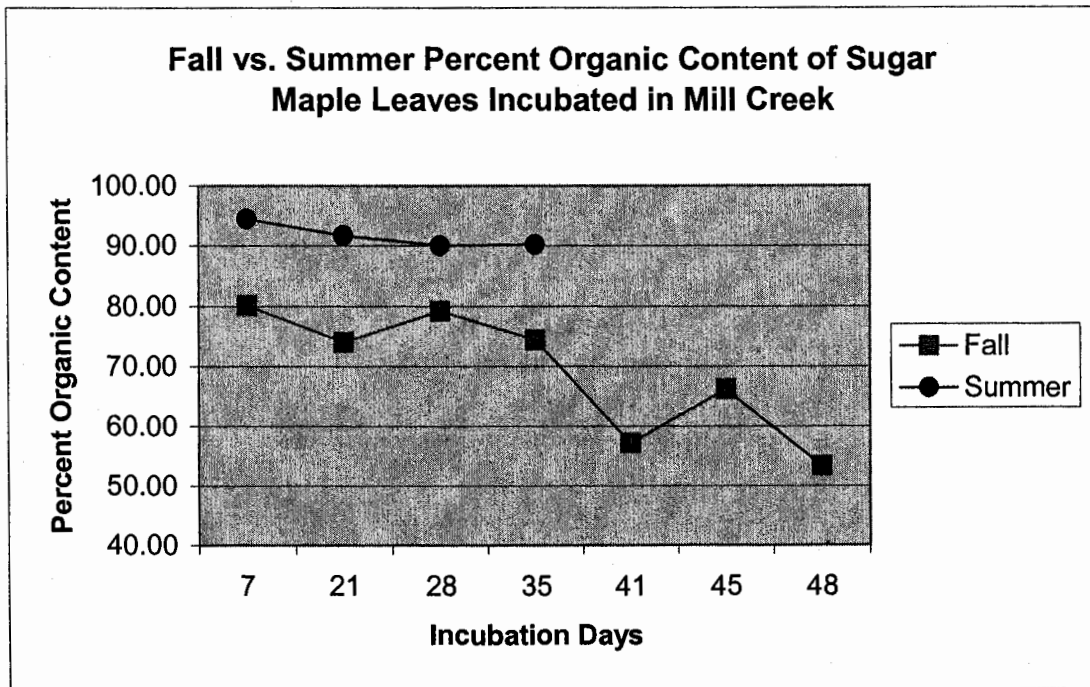


Figure 10a-b : Percent Organic Content of Fall vs. Summer Incubated Sugar Maple and River Birch Leaves in Mill Creek

Figure 11a

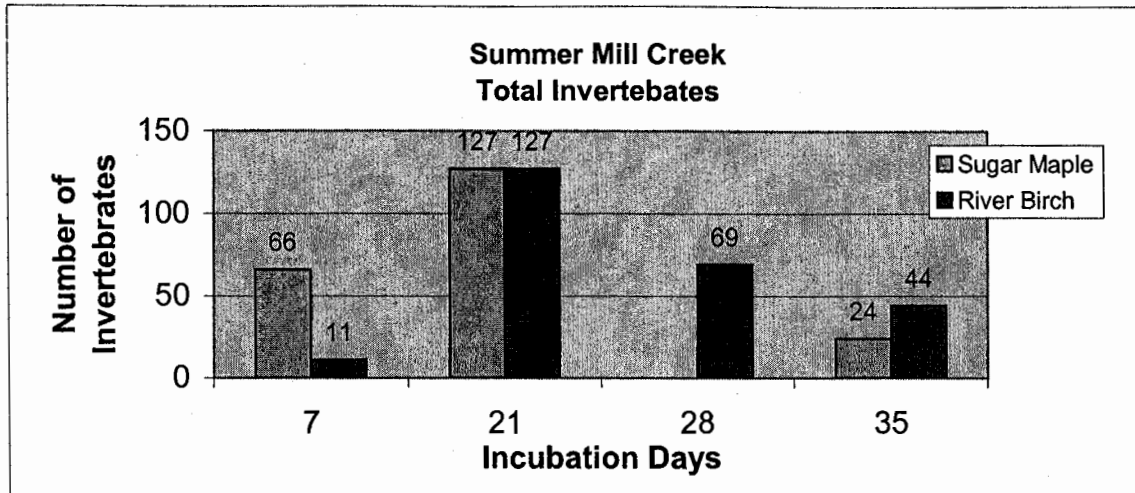


Figure 11b

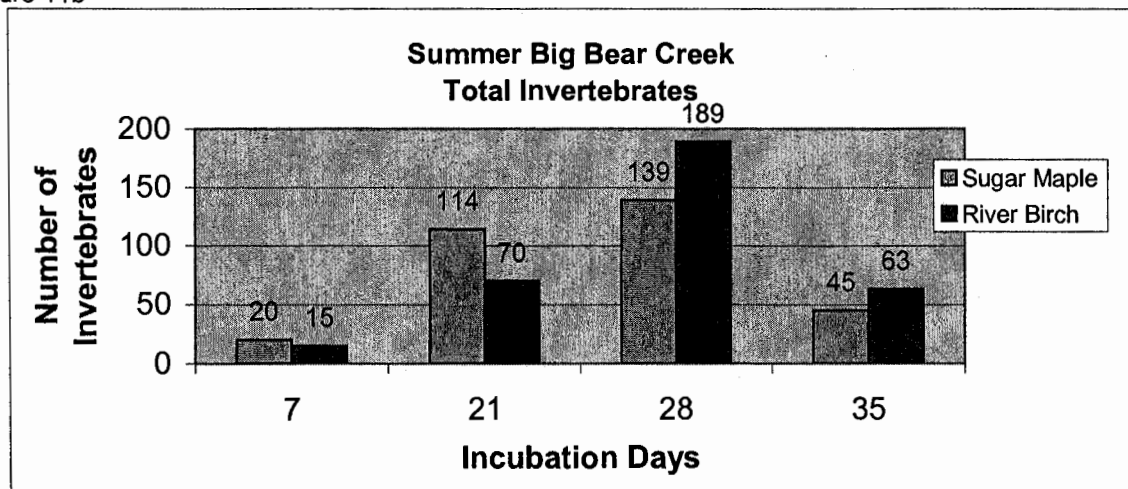


Figure 11c

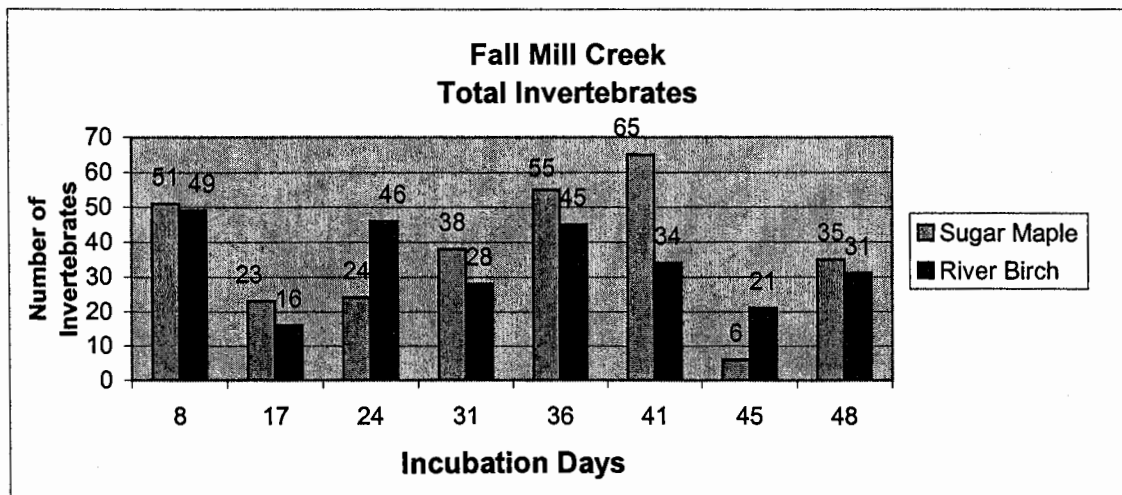


Figure 11 a-c: Total Number of Invertebrates on all Leaf Packs Collected for Each Incubation Date

Figures 12a-d: Invertebrates Sorted into Functional Feeding Groups for the Summer Study of Mill Creek

Figure 12a

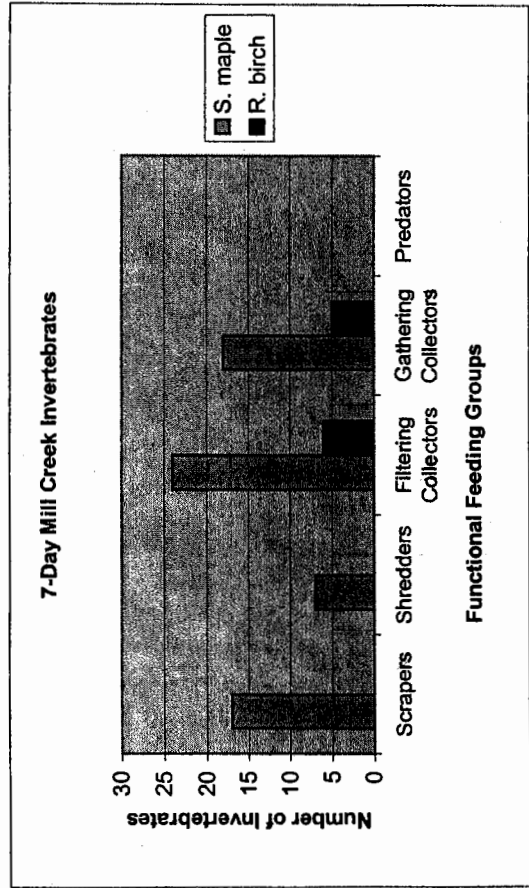


Figure 12b

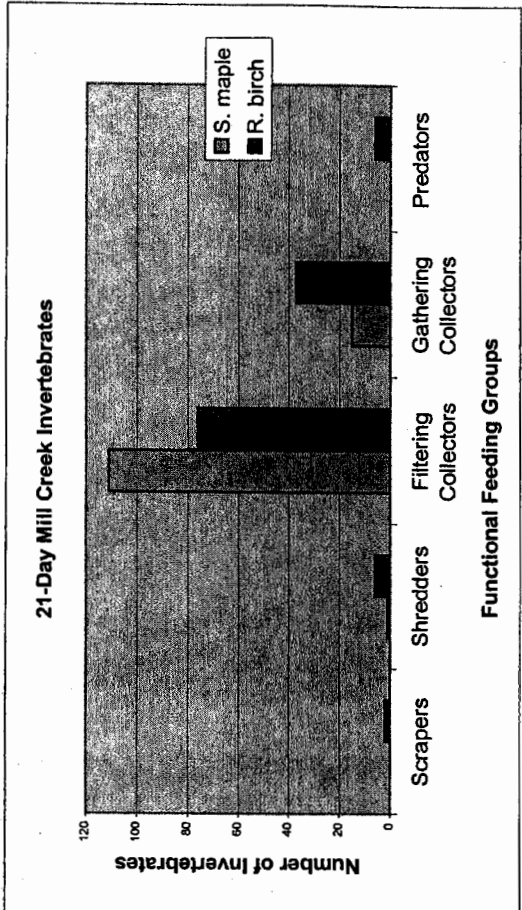


Figure 12c

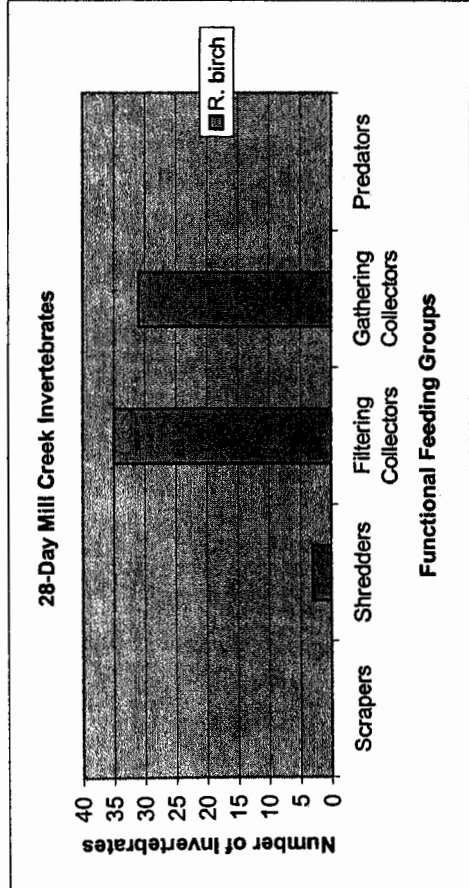


Figure 12d

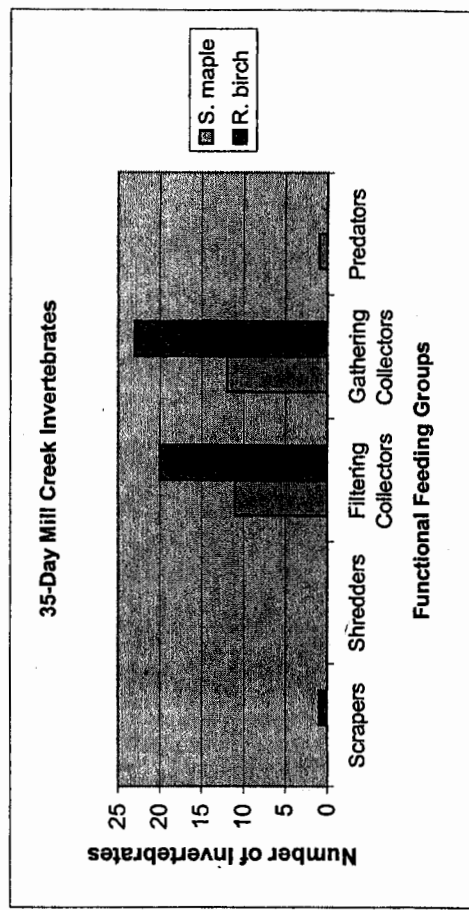


Figure 13 a-d: Invertebrates Sorted into Functional Feeding Groups for the Summer Study of Big Bear Creek

Figure 13a

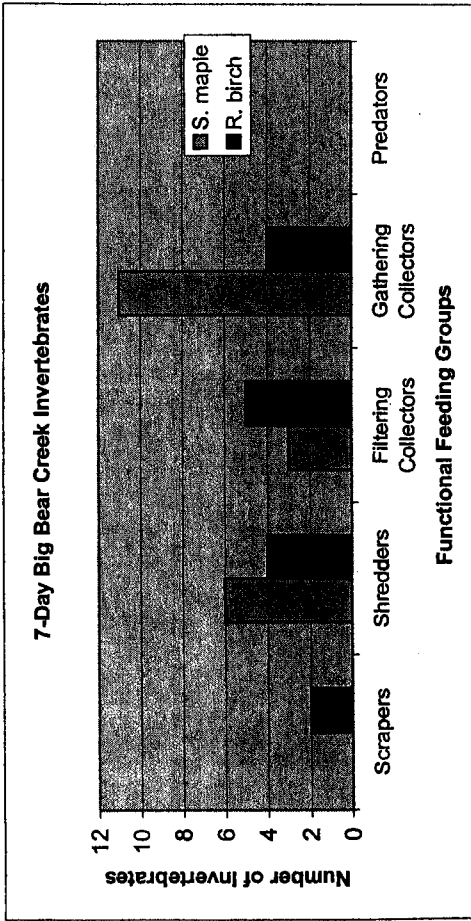


Figure 13b

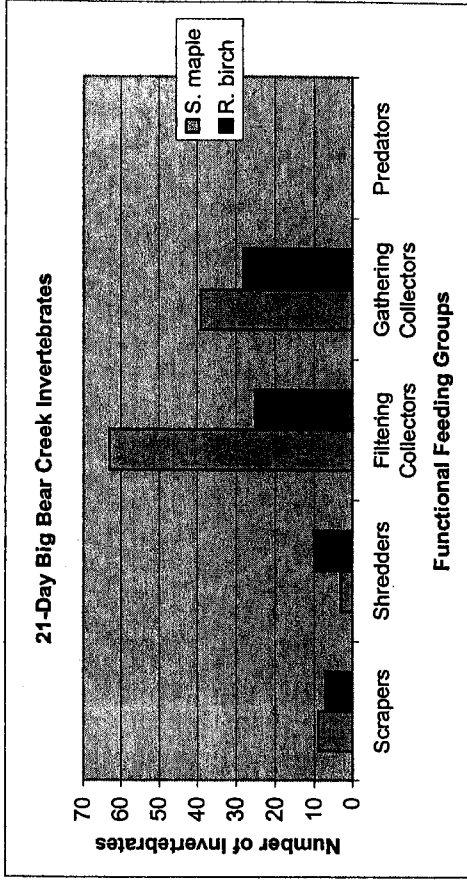


Figure 13c

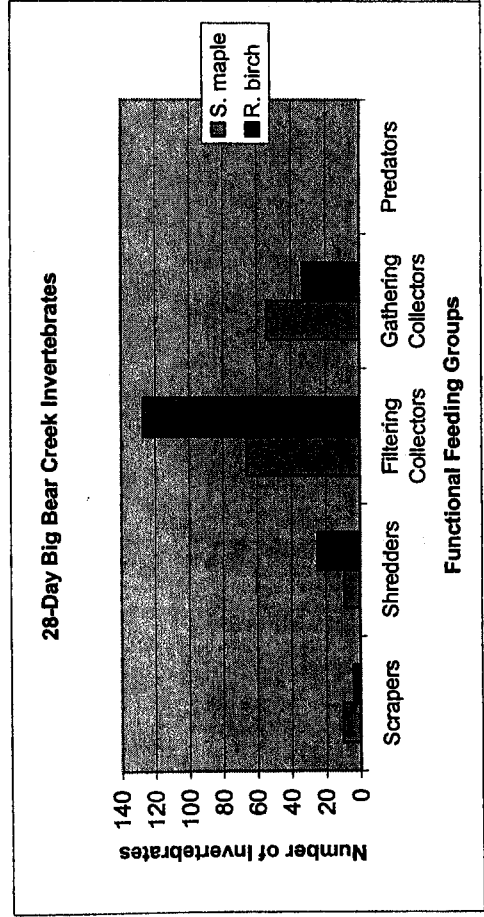
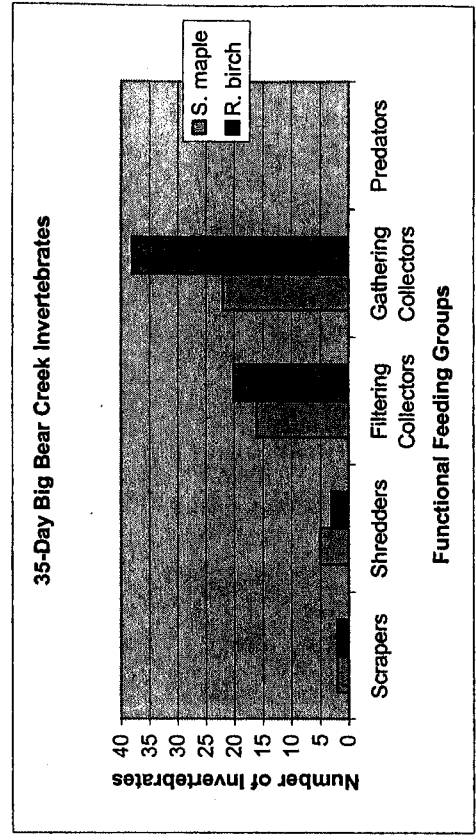


Figure 13d



Figures 14 a-f: Invertebrates Sorted into Functional Feeding Groups for the Fall Study of Mill Creek

Figure 14a

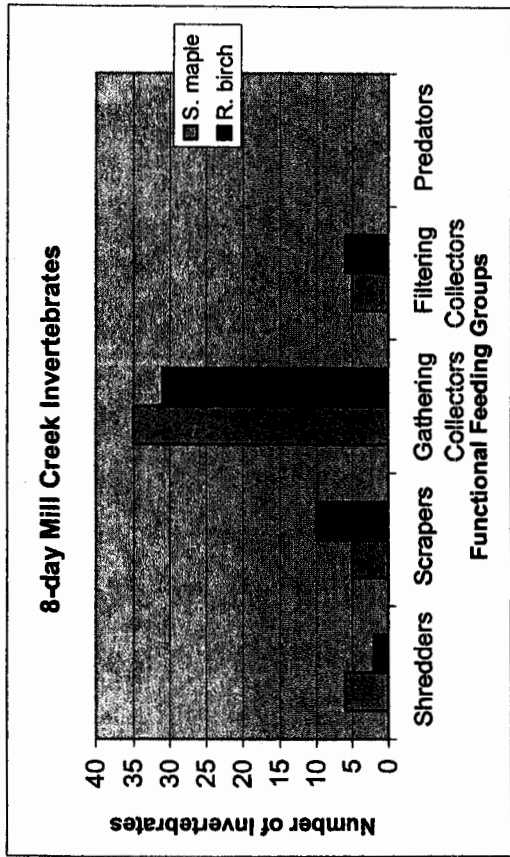


Figure 14b

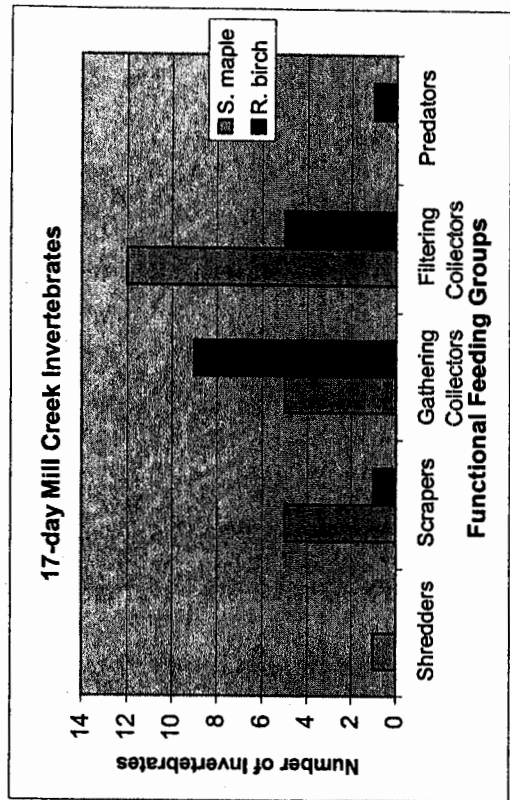


Figure 14c

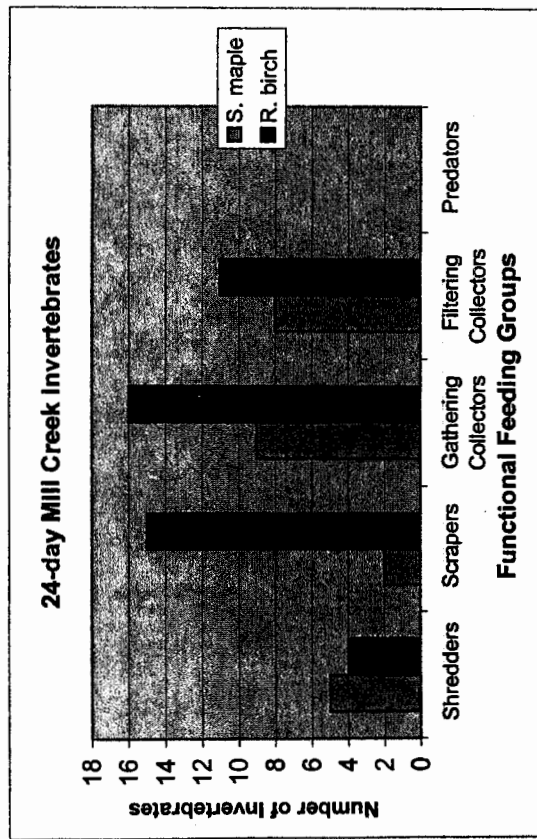


Figure 14d

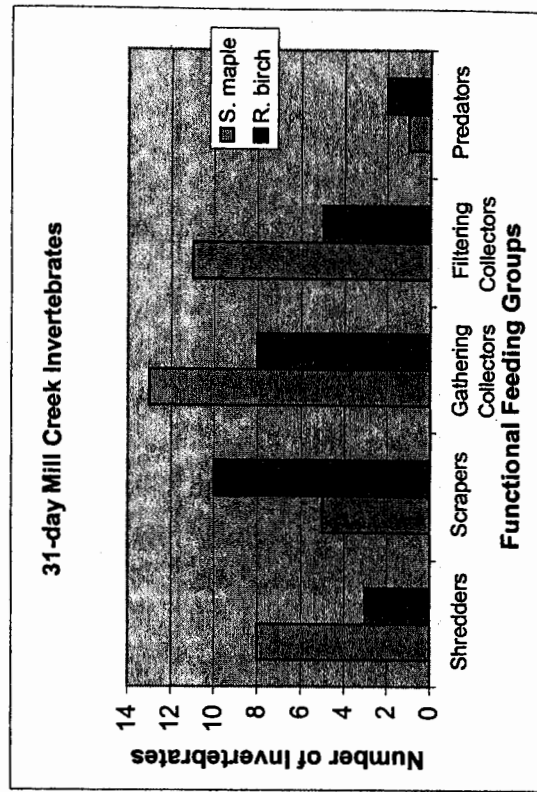


Figure 14f

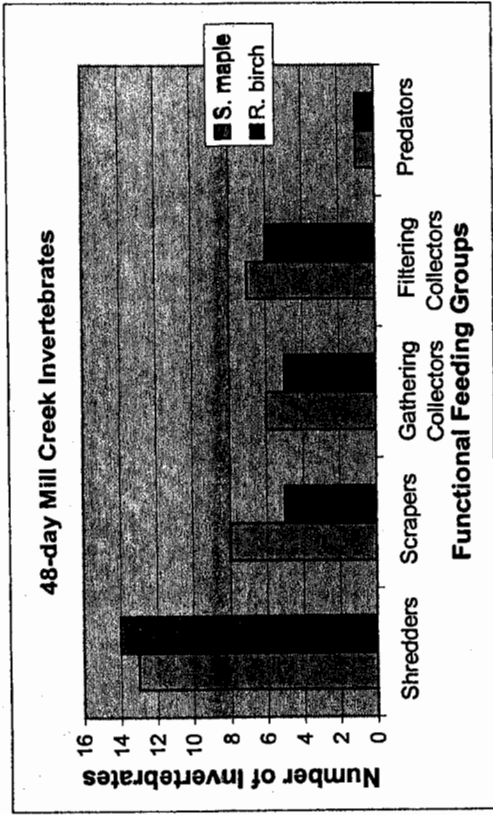


Figure 14e

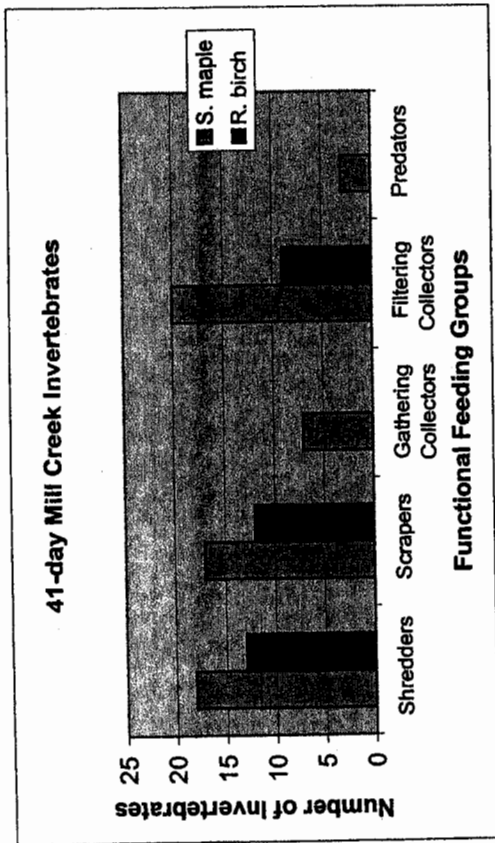


Figure 15a

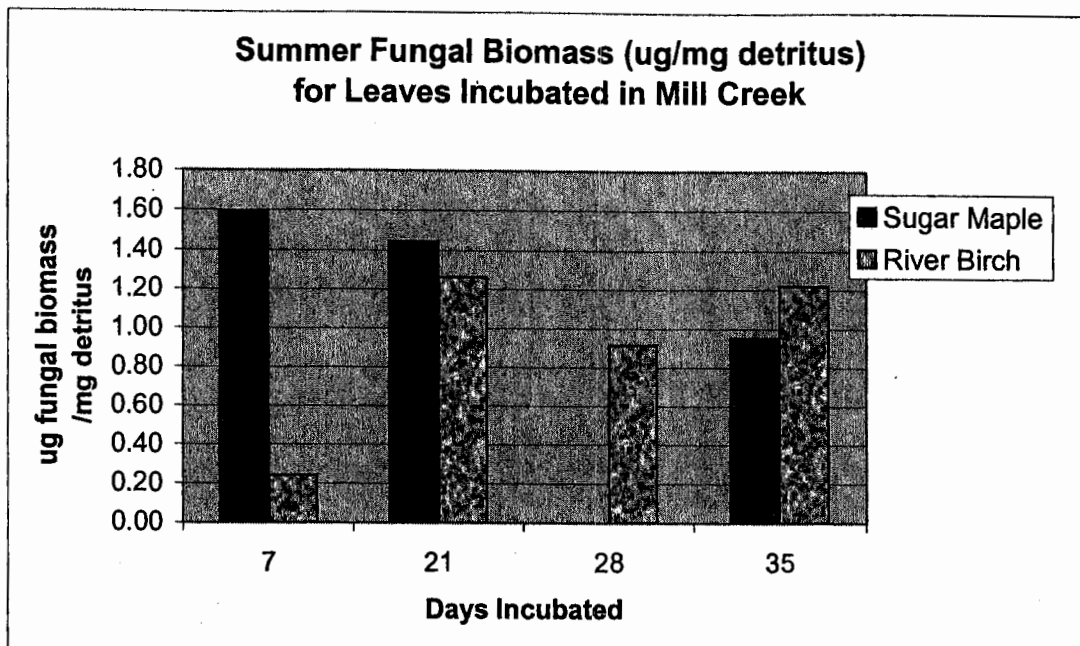
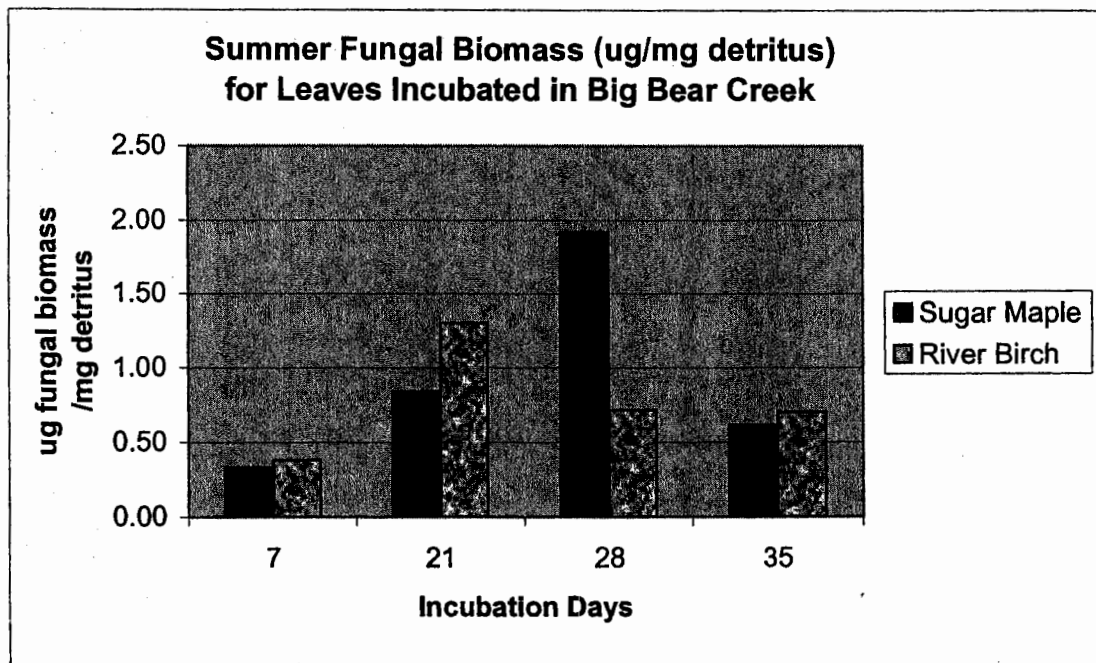


Figure 15b



Figures 15a-b: Fungal Biomass Concentrations for Big Bear Creek and Mill Creek for the Summer Study. Each incubation period's values are an average of that period's samples.

Fall Fungal Biomass (ug/mg detritus) of Leaves Incubated in Mill Creek

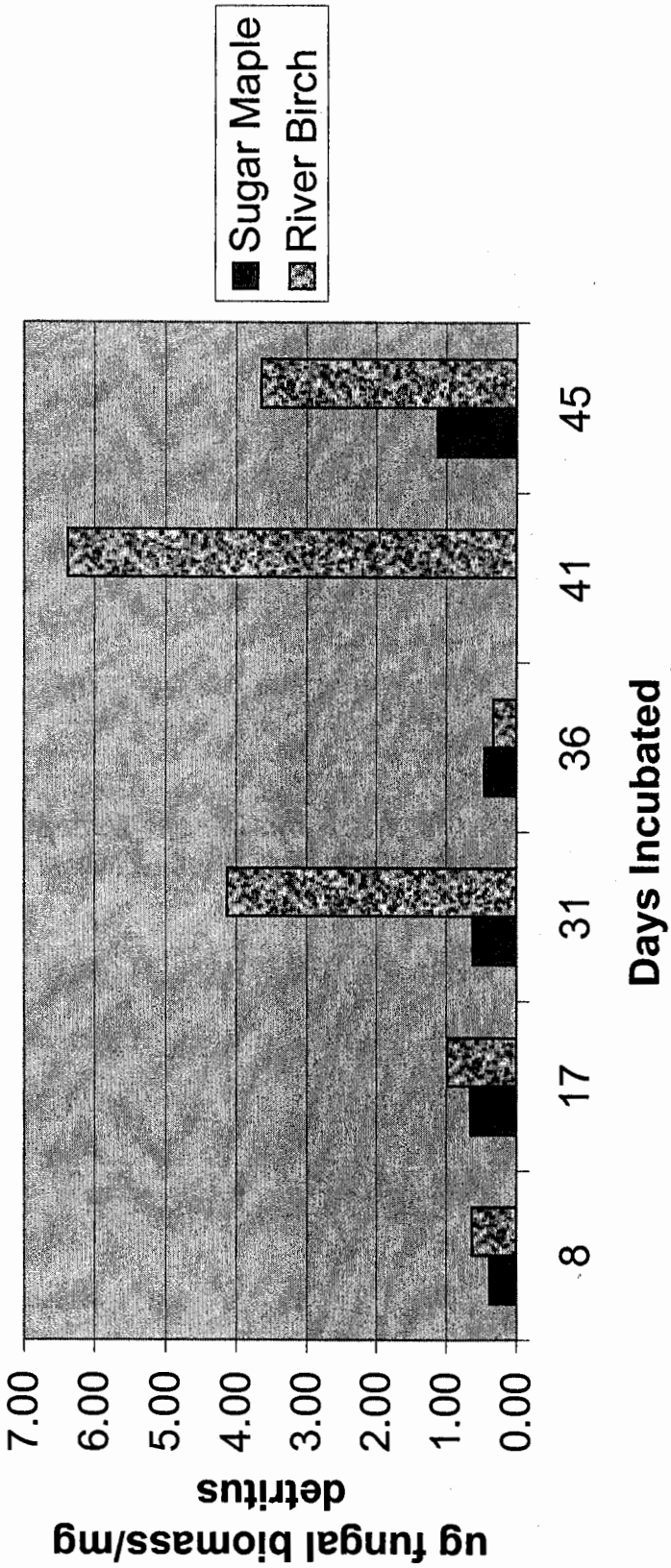


Figure 16 : Fungal Biomass(ug/mg detritus) found in Sugar Maple and River Birch Leaves Incubated in Mill Creek in the Fall Study. The 41-day, sugar maple sample was lost.

Figure 17a

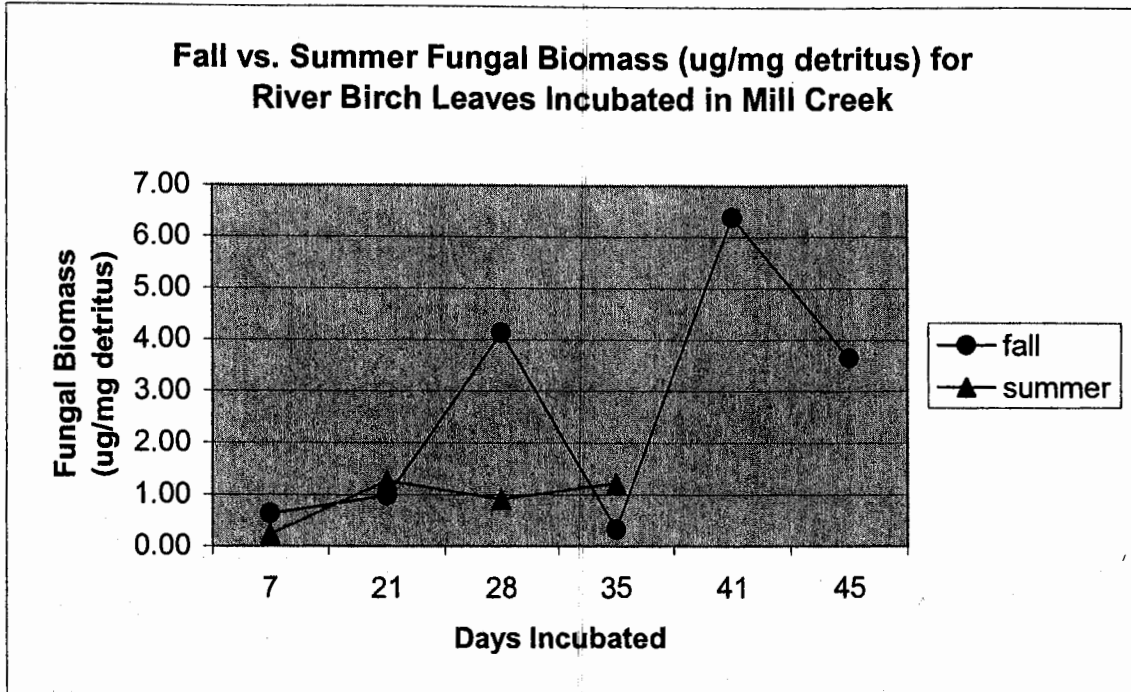
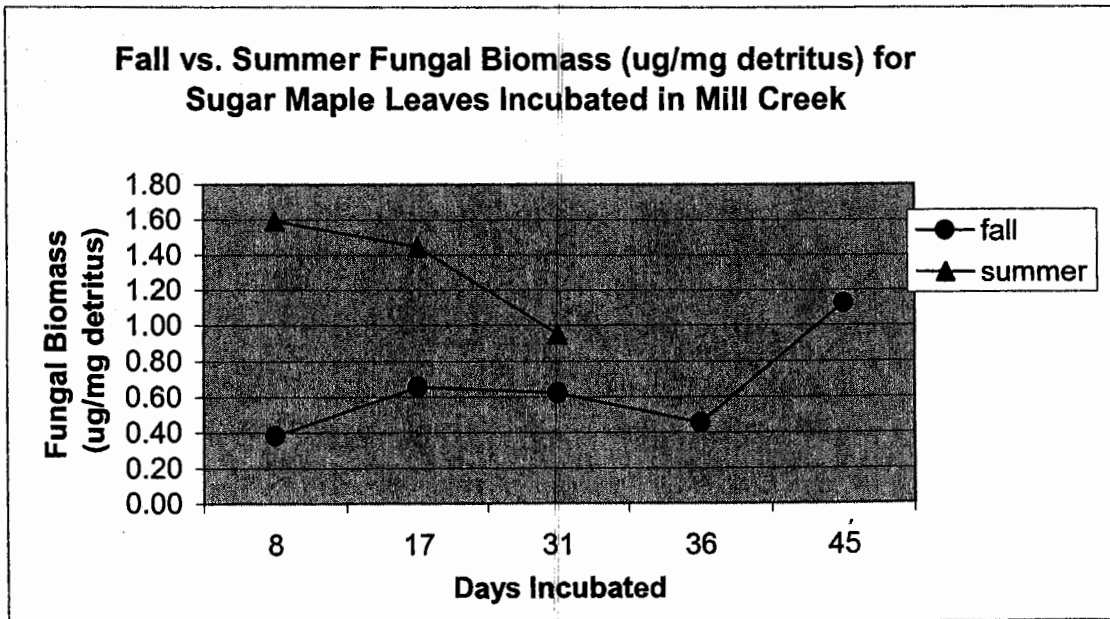


Figure 17b



Figures 17 a-b: Fungal Biomass Concentrations for Fall and Summer Incubated River Birch Leaves in Mill Creek

Fungal Biomass (ug/mg detritus) of Two River Birch Samples Incubated in Mill Creek for ~3 months

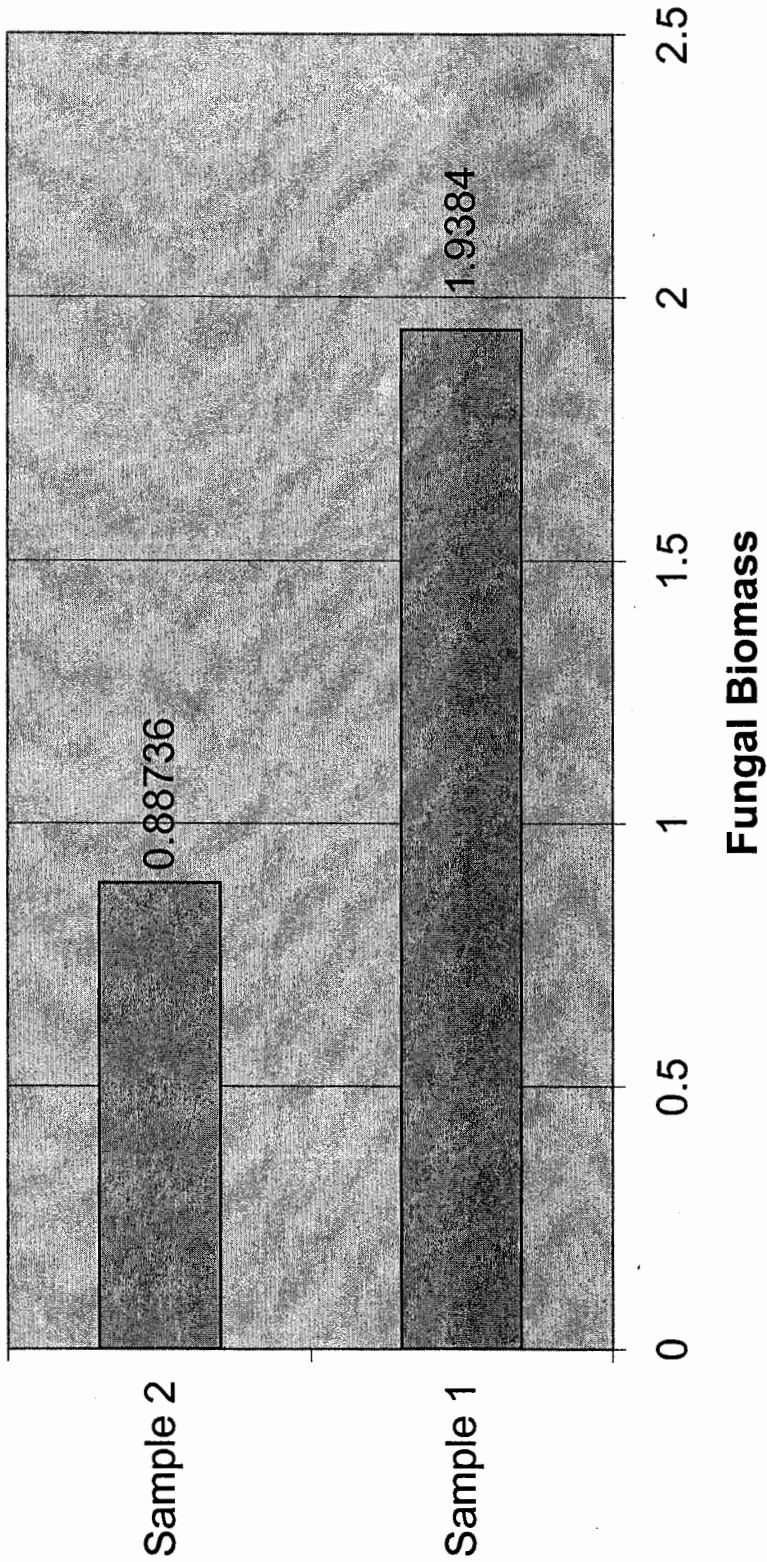


Figure 18: Fungal Biomass Concentrations of 2 River Birch Samples Incubated in Mill Creek for Approximately 3 months (July-October)

Appendix I

Sample Calculations

1) Preparation of Stock Ergosterol Standard

0.2001 g of 95% ergosterol (Aldrich) in 200 mL of HPLC-grade methanol

$$\frac{0.2001 \text{ erg} \times 0.95 \times (\mu/10^{-6})}{200 \text{ mL}} = 950.5 \mu\text{g ergosterol/ mL methanol}$$

2) Preparation of Working Ergosterol Standard

Take 1.00 mL of stock ergosterol standard and dilute with HPLC-grade methanol to 50 mL

$$C_1V_1=C_2V_2$$

$$\frac{(1.00\text{mL})(950.5 \mu\text{g ergosterol/mL Methanol})}{(50.0 \text{ mL})} = 19.01 \mu\text{g ergosterol/ mL methanol}$$

3) Inject varying known volumes into HPLC to establish a working standard curve

Injection volume (μL)	Injection volume (mL) x Standard Volume ($\mu\text{g/mL}$)	Final concentration of standard injections (μg)
5	$0.005 \times 19.01 =$	0.095
10	$0.010 \times 19.01 =$	0.190
20	$0.020 \times 19.01 =$	0.380
40	$0.040 \times 19.01 =$	0.760
60	$0.060 \times 19.01 =$	1.14
80	$0.080 \times 19.01 =$	1.52

4) Entered μg ergosterol values from 3) on Kaleidograph and plotted a graph which gave the equation of line

$$\mu\text{g ergosterol} = 1.0517 \times 10^{-6} * (\text{experimental peak area}) + 0.019624$$

Example: Sample Z-R-41(2)

$$1.0517 \times 10^{-6} * (1.4852 \times 10^6) + 0.019624 = 1.5817 \mu\text{g ergosterol}$$

5) Experimental peak areas were manually reintegrated to eliminate underlying base area caused by carryover from preceding peaks as shown in Figure __

$$\mu\text{g ergosterol} = 1.0517 \times 10^{-6} * (\text{reintegrated peak area}) + 0.019624$$

Example: Sample Z-R-41(2)

$$1.0517 \times 10^{-6} * (1.1605 \times 10^6) + 0.019624 = 1.2401 \mu\text{g ergosterol}$$

6) Determining total μg ergosterol accounting for the injected and dissolution (by HPLC-grade methanol) volumes

$$\frac{\mu\text{g ergosterol}}{\text{mL injected}} \times \text{dissolution vol (mL)} = \mu\text{g ergosterol / refluxed sample (10 discs)}$$

Example: Sample Z-R-41(2)

$$\frac{1.2401 \mu\text{g ergosterol}}{0.060 \text{ mL injected}} \times 1.00 \text{ mL (dissolution vol)} = 20.668 \mu\text{g ergosterol / refluxed sample (10 discs)}$$

7) Weight % ergosterol

$$\frac{\text{Total } \mu\text{g ergosterol/10 discs}}{\text{Leaf weight/10 discs}} = \text{Total } \mu\text{g ergosterol/mg leaf detritus}$$

Example: Sample Z-R-41(2)

$$\frac{20.668 \mu\text{g ergosterol/10 discs}}{446 \text{ mg/10 discs}} = 0.04634 \mu\text{g ergosterol/mg leaf detritus}$$

8) Fungal Biomass Determination

$$\text{Conversion factor} = \frac{182 \text{ g fungal biomass}}{\text{g ergosterol}}$$

Example: Sample Z-R-41(2)

$$\frac{182 \text{ g fungal biomass}}{\text{g ergosterol}} \times \frac{0.04634 \mu\text{g ergosterol}}{\text{mg leaf detritus}} = 8.43388 \mu\text{g ergosterol/mg leaf detritus}$$

Appendix II

Experimental HPLC Printouts

#92

D-m-7

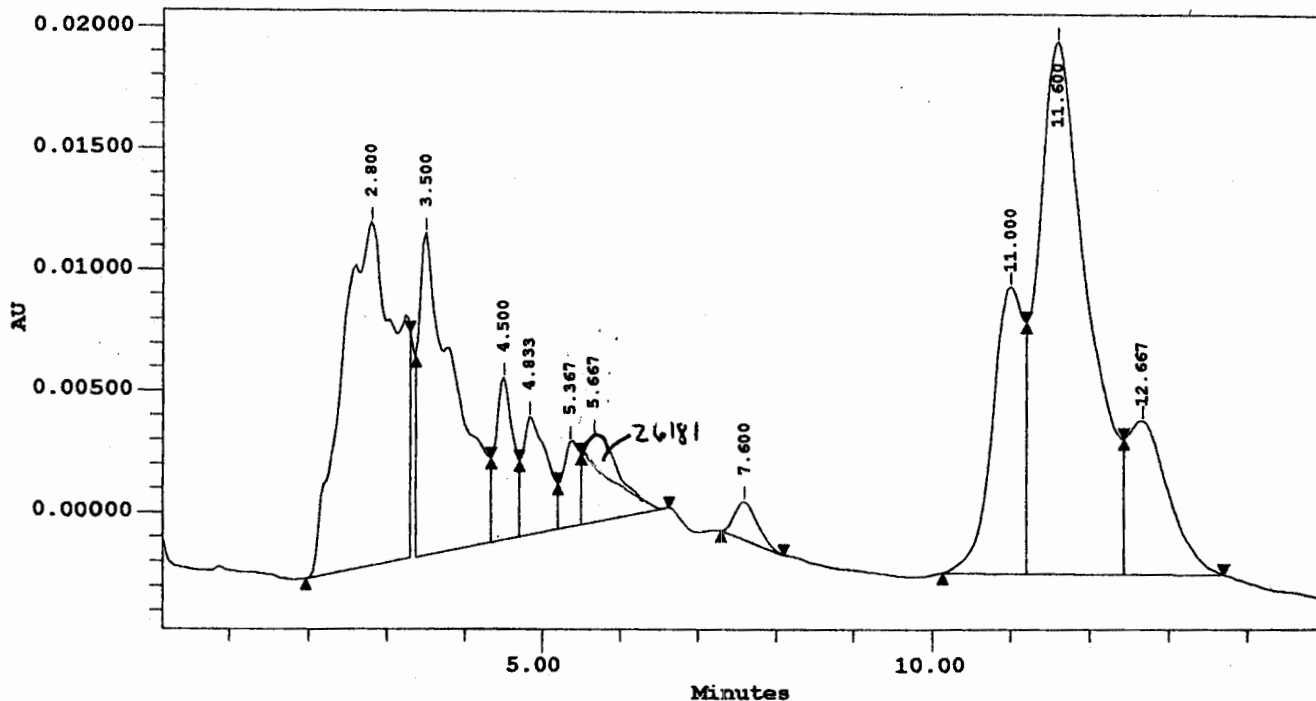
6/27/00

Millennium Results Report	June 26, 1984	Page: 1 of 1
Report Method: MetCarb_RM	Version: 2.15	
For Sample: 60 ul of #92	Vial: 5	Injection: 1 Channel: 991M
Proc Chan: PDA_282.0nm	Processed: 06/26/84 09:29:50 PM	
Channel Descr: PDA 282.0 nm		

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 Lycoming College, Department of Chemistry 7

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 S. Meigs
 June 23rd

Project Name:	Ergosterol	Sample Name:	60 ul of #92
Vial:	5	Sample Origin:	
Sample Type:	Unknown	Solvent:	meoh
Injection:	1	FlowRate:	1.500
Channel:	991M	Level:	
Date Acquired:	06/26/84 09:14:19 PM	Volume:	60.00
Sample Weight:	1.00000	Run Time:	15.0 min
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



Peak Results

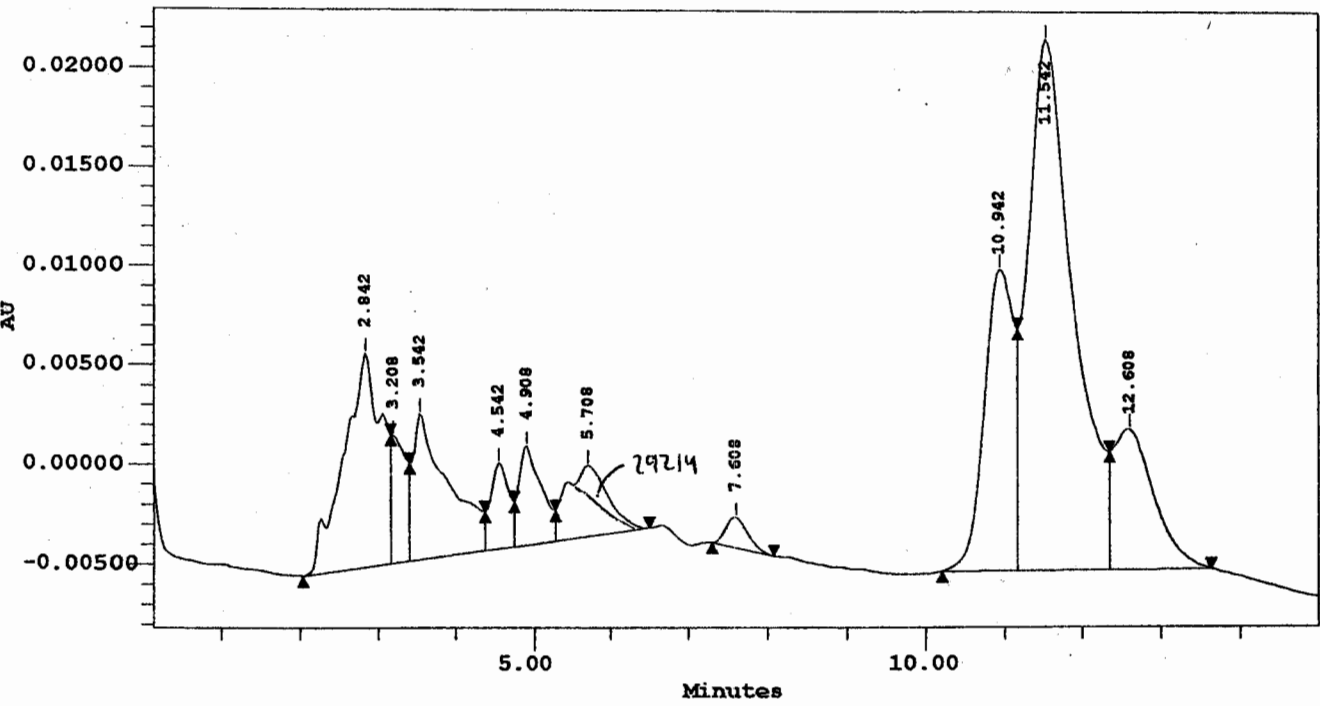
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.800	678116	14131	BV	1.967	3.300	22.23
2		3.500	426251	13274	VV	3.367	4.333	13.97
3		4.500	107112	6654	VV	4.333	4.700	3.51
4		4.833	106139	4863	VV	4.700	5.200	3.48
5		5.367	(51698)	3525	VV	5.200	5.500	1.69
6		5.667	(113643)	3599	VB	5.500	6.633	3.73
7		7.600	31878	1569	BB	7.300	8.100	1.04
8		11.000	321209	11870	BV	10.133	11.200	10.53
9		11.600	982660	22025	VV	11.200	12.433	32.21
10		12.667	232011	6367	VB	12.433	13.700	7.61

0-M-76-28-00

illennium Results Report June 28, 1984 Page: 1 of 1
 eport Method: MetCarb_RM Version: 2.15
 or Sample: 60 ul of #95 Vial: 2 Injection: 1 Channel: 991M
 roc Chan: PDA_282.0nm Processed: 06/28/84 02:14:47 AM
 hannel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry 7 Overwood Sample Jun 23

Project Name: Ergosterol Sample Name: 60 ul of #95
 Vial: 2 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 06/28/84 01:59:23 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

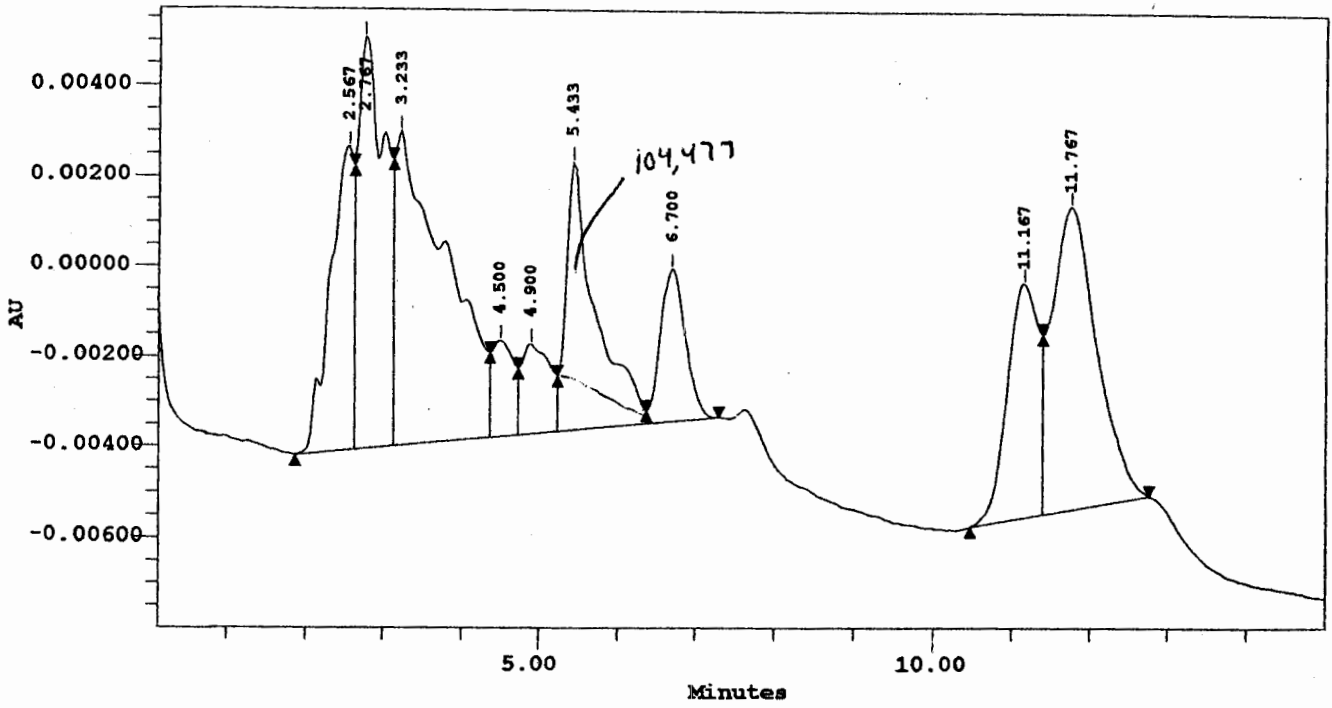
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.842	365750	10748	BV	2.042	3.175	13.21
2		3.208	81497	6426	VV	3.175	3.408	2.94
3		3.542	232552	7349	VV	3.408	4.375	8.40
4		4.542	68910	4315	VV	4.375	4.742	2.49
5		4.908	102242	4983	VV	4.742	5.275	3.69
6		5.708	(135226)	3581	VB	5.275	6.508	4.88
7		7.608	28931	1527	BB	7.308	8.075	1.05
8		10.942	399294	15244	BV	10.208	11.175	14.42
9		11.542	1094436	26834	VV	11.175	12.342	39.53
10		12.608	259452	7108	VB	12.342	13.642	9.37

D-m-21 7-13-00

Millennium Results Report July 12, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #54 Vial: 2 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/12/84 09:13:51 PM
 Channel Descr: PDA 282.0 nm

1ml Lycoming College, Department of Chemistry Z1 Dunnedy S. Maple
Jul 7

Project Name: Ergosterol	Sample Name: 60 ul of #54
Vial: 2	Sample Origin:
Sample Type: Unknown	Solvent: MeOH
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 07/12/84 08:53:09 PM	Volume: 60.00
SampleWeight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

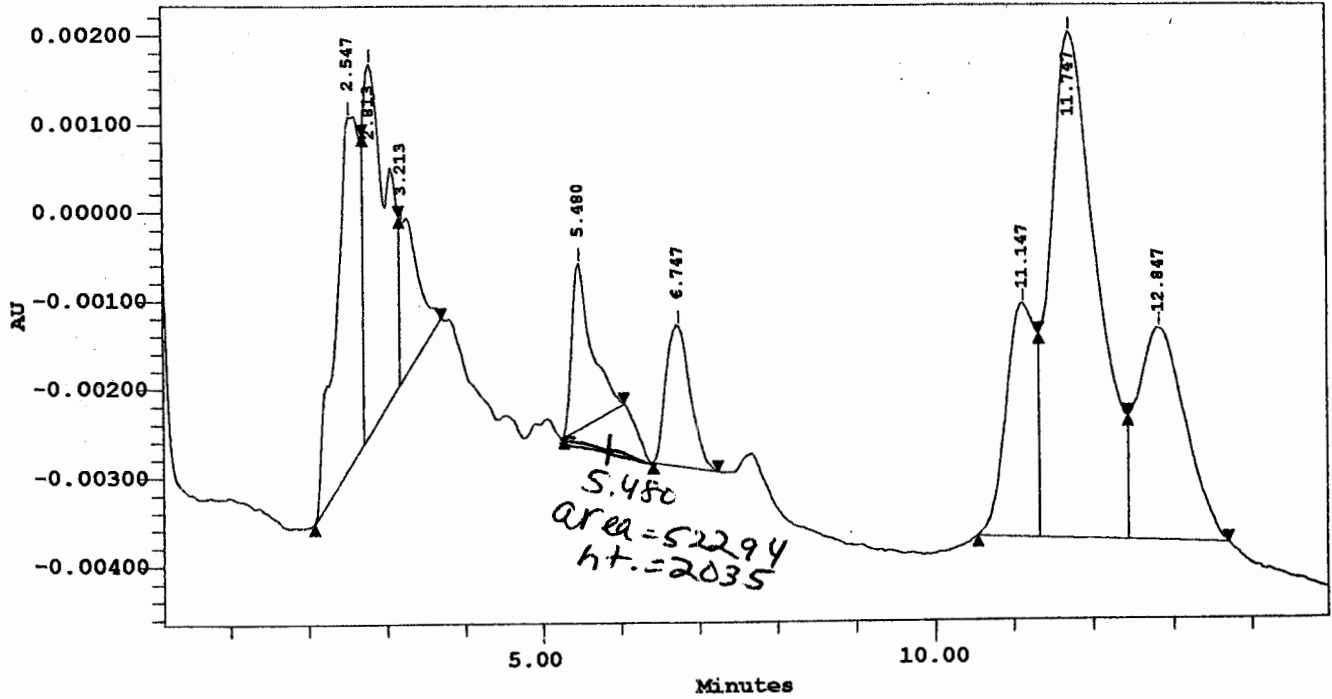
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.567	139542	6749	BV	1.867	2.633	9.81
2		2.767	222590	9136	VV	2.633	3.133	15.65
3		3.233	317458	6993	VV	3.133	4.367	22.32
4		4.500	41949	2142	VV	4.367	4.733	2.95
5		4.900	50722	2005	VV	4.733	5.233	3.57
6		5.433	(158022)	5966	VV	5.233	6.367	11.11
7		6.700	76461	3393	VB	6.367	7.300	5.38
8		11.167	143429	5213	BV	10.467	11.400	10.08
9		11.767	272305	6762	VB	11.400	12.767	19.14

D-M-21

Millennium Results Report July 12, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #114 Vial: 3 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/12/84 10:43:57 PM
 Channel Descr: PDA 282.0 nm

(1 ml) Lycoming College, Department of Chemistry 21 Dunn
S. Mope
Jul 7

Project Name:	Ergosterol	Sample Name:	60 ul of #114
Vial:	3	Sample Origin:	
Sample Type:	Unknown	Solvent:	MeOH
Injection:	1	FlowRate:	1.500
Channel:	991M	Level:	
Date Acquired:	07/12/84 10:28:32 PM	Volume:	60.00
Sample Weight:	1.00000	Run Time:	15.0 min
Acq Meth Set:	Ergosterol_MS		
Processing Method:	Ergosterol_PM		



Peak Results

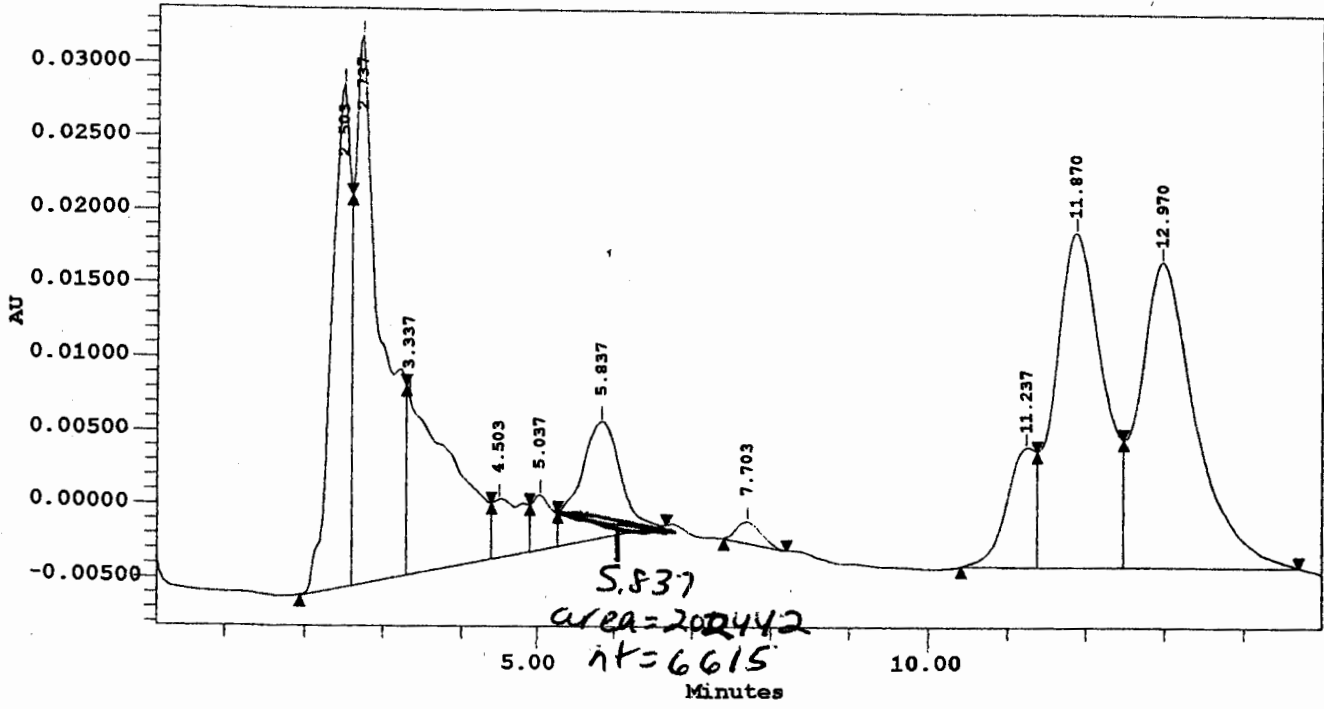
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.547	85609	3949	BV	2.080	2.713	12.76
2		2.813	85490	4154	VV	2.713	3.180	12.74
3		3.213	29532	1802	VB	3.180	3.713	4.40
4		5.480	(32809)	1884	BB	5.280	6.047	4.89
5		6.747	34324	1599	BB	6.413	7.247	5.12
6		11.147	69801	2660	BV	10.547	11.347	10.41
7		11.747	231849	5757	VV	11.347	12.447	34.56
8		12.847	101415	2420	VB	12.447	13.713	15.12

D-M-28

Millennium Results Report July 25, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 100 ul of #117 Vial: 6 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/24/84 03:11:44 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry 28
 Dunwoody
 s. maple
 July 14

Project Name: Ergosterol Sample Name: 100 ul of #117
 Vial: 6 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 Flow Rate: 1.500
 Channel: 991M Level:
 Date Acquired: 07/24/84 02:56:18 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

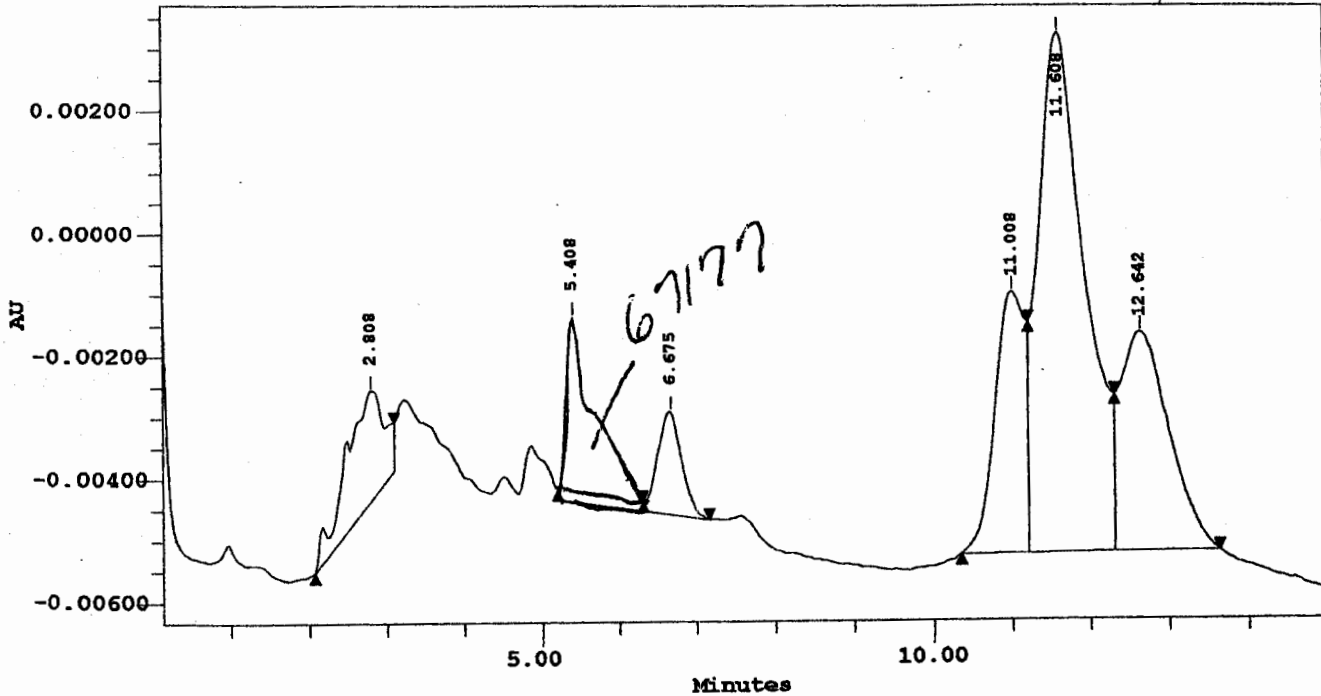
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.503	558647	34289	BV	1.937	2.603	11.91
2		2.737	896663	37277	VV	2.603	3.303	19.11
3		3.337	500620	12226	VV	3.303	4.403	10.67
4		4.503	106147	3950	VV	4.403	4.903	2.26
5		5.037	68144	3698	VV	4.903	5.270	1.45
6		5.837	(295452)	7948	VB	5.270	6.670	6.30
7		7.703	31335	1439	BB	7.403	8.203	0.67
8		11.237	203774	8162	BV	10.403	11.370	4.34
9		11.870	983966	22947	VV	11.370	12.470	20.98
10		12.970	1046341	20938	VB	12.470	14.703	22.30

D-M-35

Millennium Results Report August 1, 1984 Page: 1 of 1
Report Method: MetCarb_RM Version: 2.15
For Sample: 60 ul of #64 Vial: 5 Injection: 1 Channel: 991M
Proc Chan: PDA_282.0nm Processed: 08/01/84 09:55:46 PM
Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol Sample Name: 60 ul of #64
Vial: 5 Sample Origin:
Sample Type: Unknown Solvent: meoh
Injection: 1 FlowRate: 1.500
Channel: 991M Level:
Date Acquired: 08/01/84 09:40:22 PM Volume: 60.00
SampleWeight: 1.00000 Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS
Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.808	64098	1792	BV	2.075	3.108	8.00
2		5.408	80011	3017	VV	5.208	6.308	9.98
3		6.675	36103	1681	VB	6.308	7.175	4.50
4		11.008	113611	4283	BV	10.342	11.208	14.17
5		11.608	354408	8508	VV	11.208	12.308	44.21
6		12.642	153351	3581	VB	12.308	13.642	19.13

#82

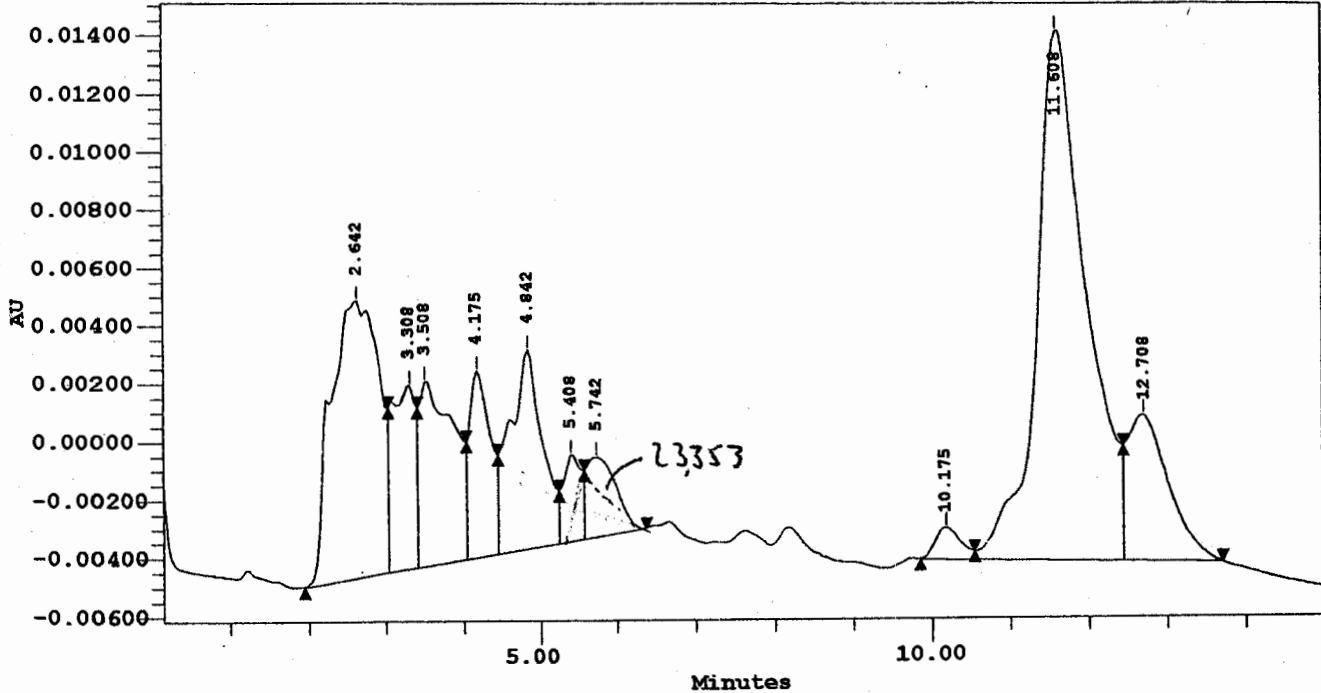
0-R-7 6/27/00

Millennium Results Report June 26, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #82 Vial: 7 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 06/26/84 10:03:11 PM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry 7

Unknown
s. sample
Jun 23

Project Name: Ergosterol Sample Name: 60 ul of #82
 Vial: 7 Sample Origin:
 Sample Type: Unknown Solvent: mech
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 06/26/84 09:47:48 PM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.642	409394	9536	BV	1.942	3.042	18.66
2		3.308	129280	6303	VV	3.042	3.408	5.89
3		3.508	198367	6358	VV	3.408	4.042	9.04
4		4.175	118908	6408	VV	4.042	4.442	5.42
5		4.842	203921	6837	VV	4.442	5.242	9.30
6		5.408	(49484)	2986	VV	5.242	5.575	2.26
7		5.742	(73300)	2754	VB	5.575	6.375	3.34
8		10.175	24586	1099	BV	9.842	10.542	1.12
9		11.608	801139	18328	VV	10.542	12.442	36.52
10		12.708	185333	5046	VB	12.442	13.708	8.45

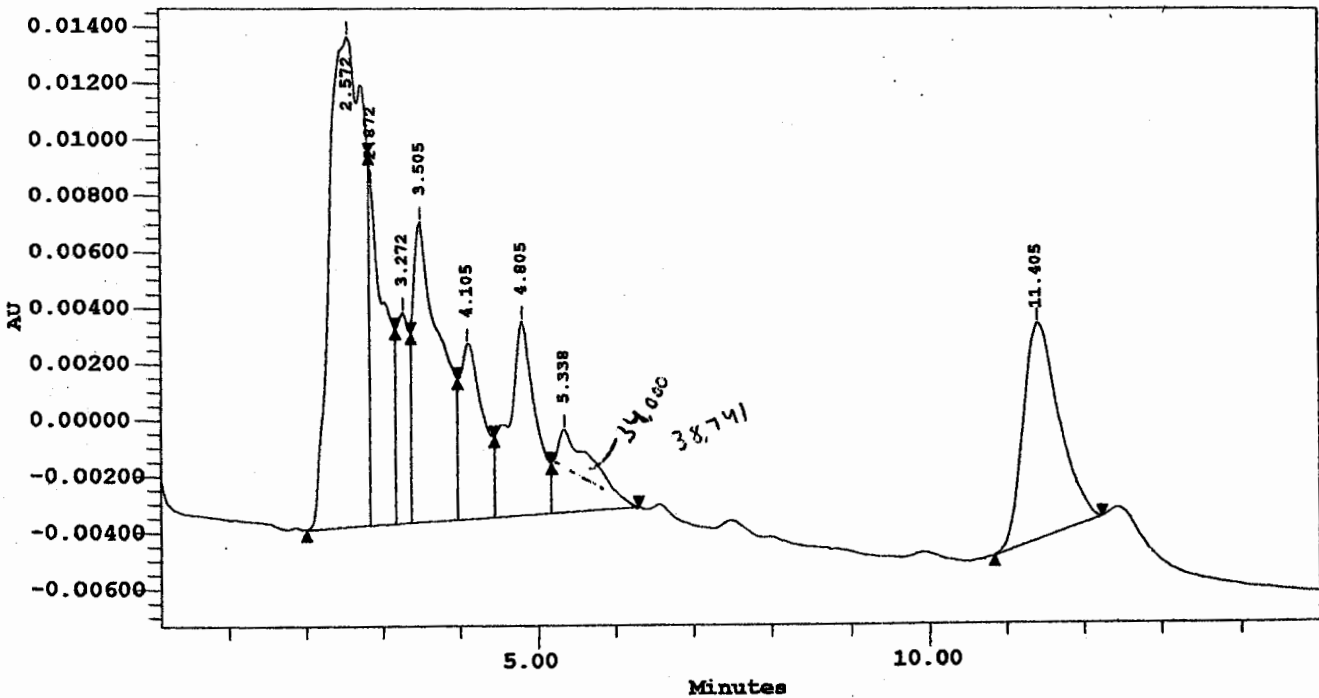
D-R-7

6-26-00

Millennium Results Report June 26, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul 72 Vial: 2 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 06/26/84 12:42:18 AM
 Channel Descr: PDA 282.0 nm

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 June 23

Project Name: Ergosterol Sample Name: 60 ul 72
 Vial: 2 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 06/26/84 12:26:50 AM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Extractions performed 6-23

Peak Results

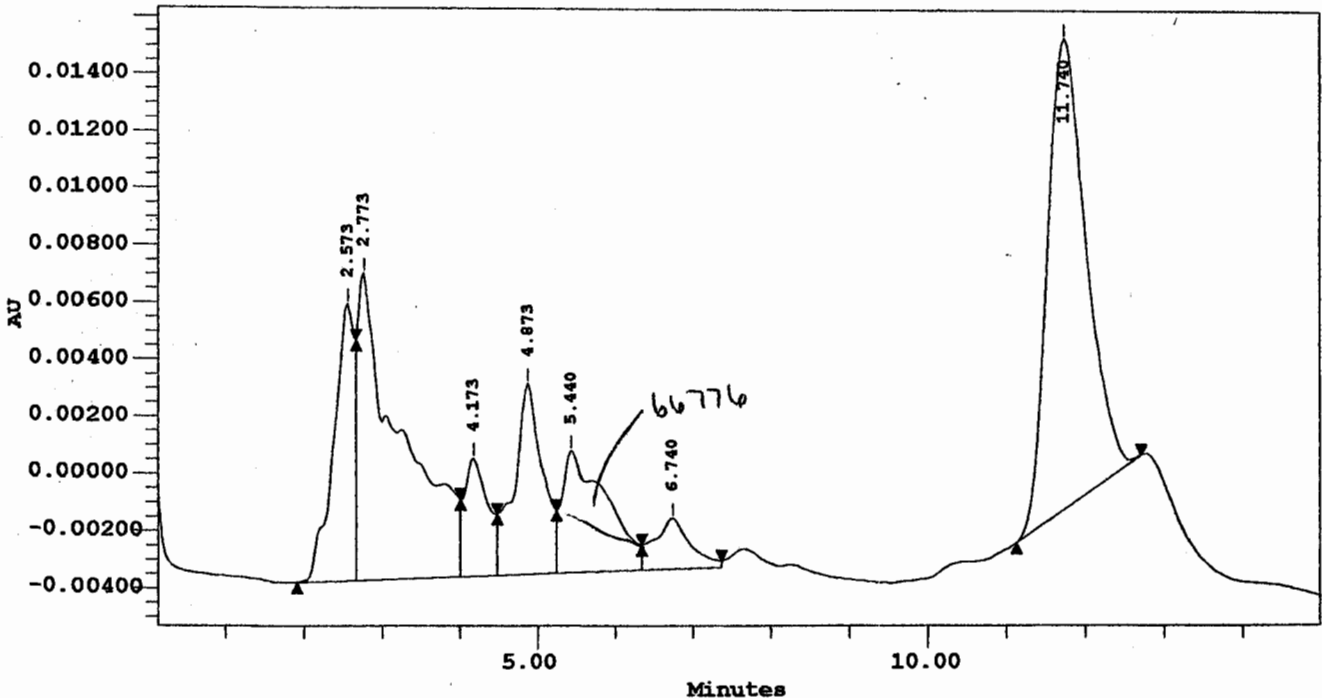
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.572	536289	17472	BV	2.005	2.838	31.22
2		2.872	177094	12127	VV	2.838	3.172	10.31
3		3.272	85787	7510	VV	3.172	3.372	4.99
4		3.505	267927	10707	VV	3.372	3.972	15.60
5		4.105	130263	6258	VV	3.972	4.438	7.58
6		4.805	171027	6915	VV	4.438	5.172	9.96
7		5.338	99426	2970	VB	5.172	6.305	5.79
8		11.405	249700	7726	BB	10.838	12.238	14.54

D-R-21

Millennium Results Report July 12, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #65 Vial: 2 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/12/84 10:27:36 PM
 Channel Descr: PDA 282.0 nm

(1) Lycoming College, Department of Chemistry 21 Dumway
Lab
Jul 7

Project Name: Ergosterol	Sample Name: 60 ul of #65
Vial: 2	Sample Origin:
Sample Type: Unknown	Solvent: MeOH
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 07/12/84 10:12:11 PM	Volume: 60.00
Sample Weight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

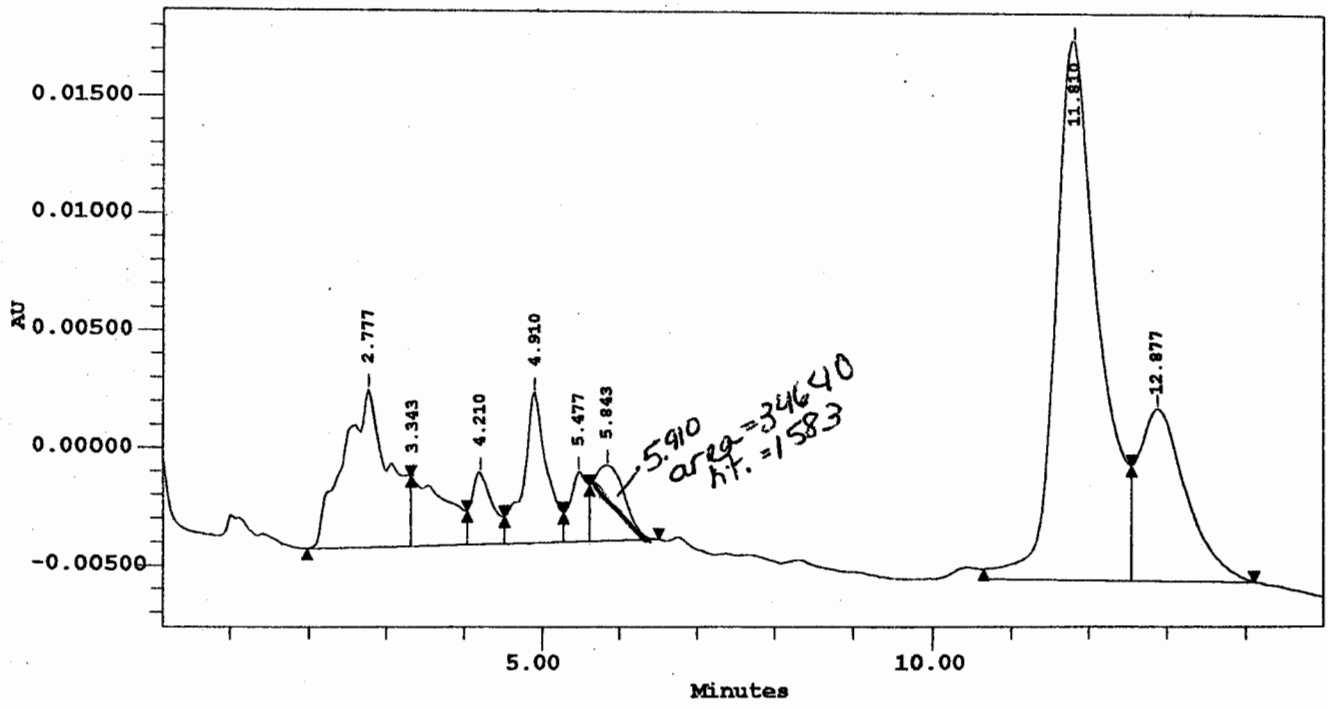
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.573	175708	9711	BV	1.907	2.673	10.75
2		2.773	415068	10756	VV	2.673	4.007	25.40
3		4.173	85810	4120	VV	4.007	4.473	5.25
4		4.873	172035	6689	VV	4.473	5.240	10.53
5		5.440	168245	4266	VV	5.240	6.340	10.30
6		6.740	57406	1778	VV	6.340	7.373	3.51
7		11.740	559854	16580	BB	11.140	12.707	34.26

DR-21

Millennium Results Report July 12, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #66 Vial: 1 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/12/84 10:00:50 PM
 Channel Descr: PDA 282.0 nm

1 ml Lycoming College, Department of Chemistry 21 Dunnedy
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Jul 7

Project Name: Ergosterol	Sample Name: 60 ul of #66
Vial: 1	Sample Origin:
Sample Type: Unknown	Solvent: MeOH
Injection: 1	Flow Rate: 1.500
Channel: 991M	Level:
Date Acquired: 07/12/84 09:16:27 PM	Volume: 60.00
Sample Weight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

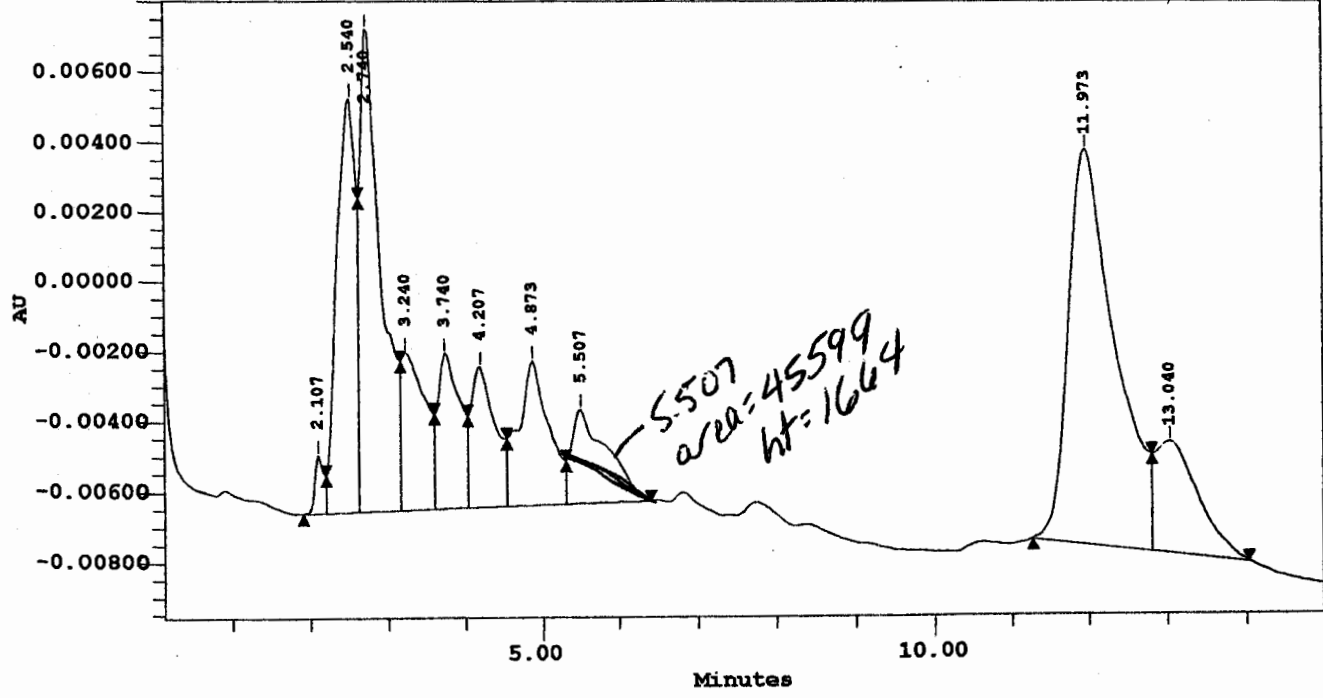
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.777	270541	6721	BV	1.977	3.310	14.25
2		3.343	92313	2852	VV	3.310	4.043	4.86
3		4.210	55706	3045	VV	4.043	4.510	2.93
4		4.910	135904	6408	VV	4.510	5.277	7.16
5		5.477	(44673)	2966	VV	5.277	5.610	2.35
6		5.843	(90945)	3212	VB	5.610	6.510	4.79
7		11.810	894104	23101	VV	10.643	12.543	47.09
8		12.877	314423	7372	VB	12.543	14.110	16.56

D-R-28

Millennium Results Report July 23, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of # 77 Vial: 3 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/23/84 10:10:02 PM
 Channel Descr: PDA 282.0 nm

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 Dunaway
 R. Birch

Project Name: Ergosterol Sample Name: 60 ul of # 77
 Vial: 3 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 07/23/84 09:54:33 PM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.107	12737	1656	BV	1.907	2.207	0.83
2		2.540	200929	11808	VV	2.207	2.640	13.02
3		2.740	261619	13773	VV	2.640	3.173	16.96
4		3.240	95908	4489	VV	3.173	3.607	6.22
5		3.740	88642	4415	VV	3.607	4.040	5.75
6		4.207	86892	4000	VV	4.040	4.540	5.63
7		4.873	113792	4108	VV	4.540	5.307	7.38
8		5.507	(85972)	2672	VB	5.307	6.407	5.57
9		11.973	474681	11339	BV	11.273	12.807	30.77
10		13.040	121482	3195	VB	12.807	14.040	7.87

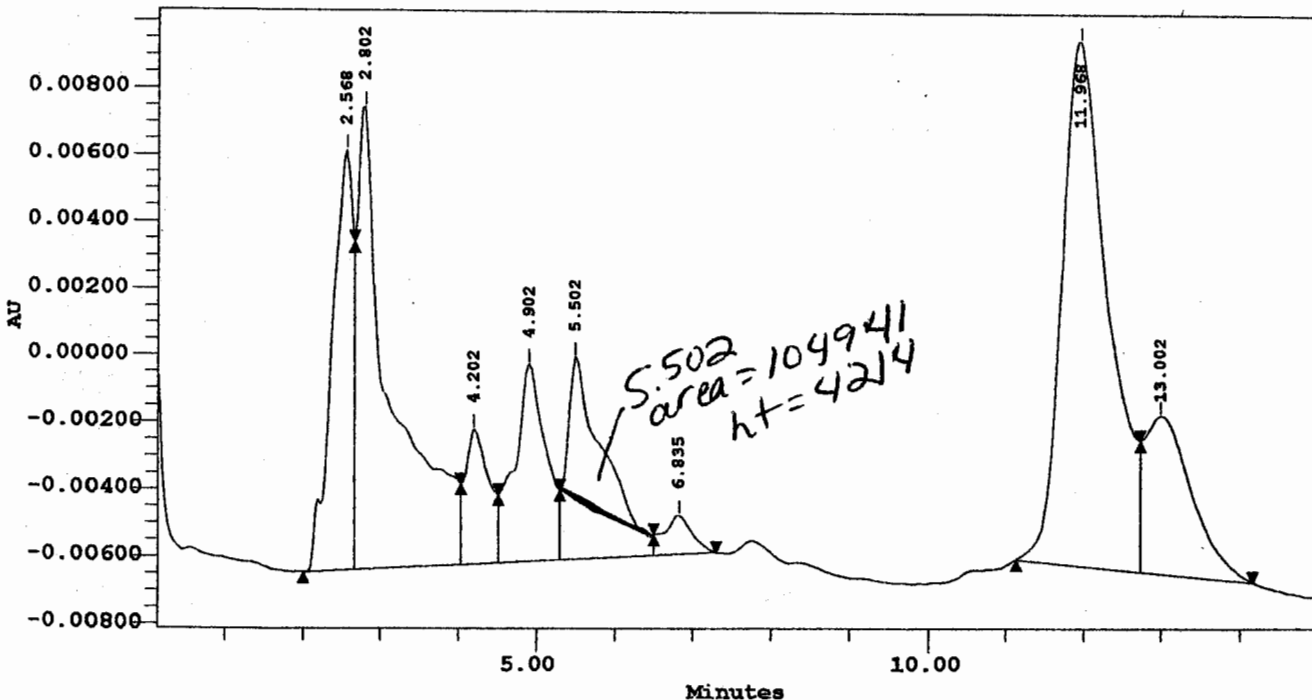
D-R-28

Millennium Results Report July 24, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #62 Vial: 3 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/24/84 02:20:26 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry 28

Dunwoody
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Project Name: Ergosterol Sample Name: 60 ul of #62
 Vial: 3 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 07/24/84 02:04:59 AM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

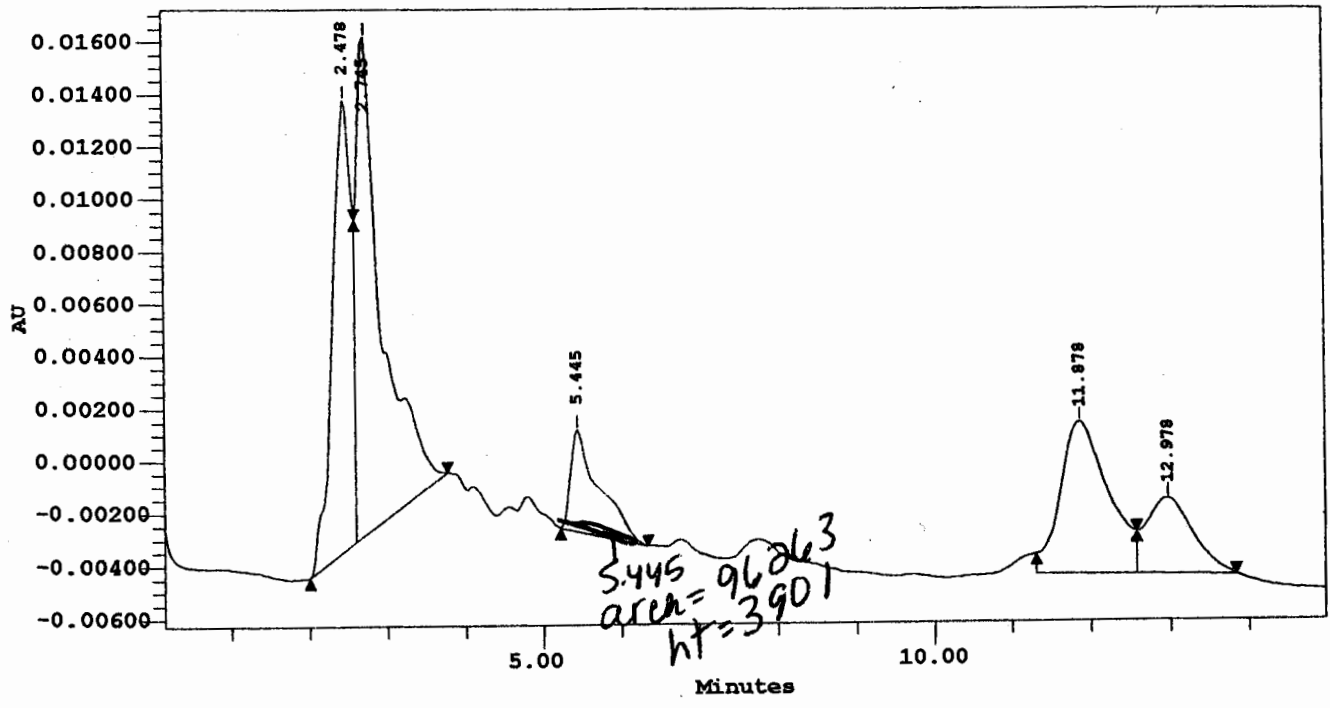
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.568	226058	12556	BV	2.002	2.668	11.37
2		2.802	431620	13834	VV	2.668	4.035	21.71
3		4.202	82619	4001	VV	4.035	4.502	4.16
4		4.902	166812	5883	VV	4.502	5.302	8.39
5		5.502	104941	6027	VV	5.302	6.502	10.05
6		6.835	28495	1134	VB	6.502	7.302	1.43
7		11.968	652772	15765	BV	11.135	12.735	32.83
8		13.002	200048	4730	VB	12.735	14.168	10.06

DR-28

Millennium Results Report July 24, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of 165 Vial: 4 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/24/84 02:37:08 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry 28
 Jimmy
 n. birch
 Jul 12

Project Name: Ergosterol Sample Name: 60 ul of 165
 Vial: 4 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 07/24/84 02:21:41 AM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



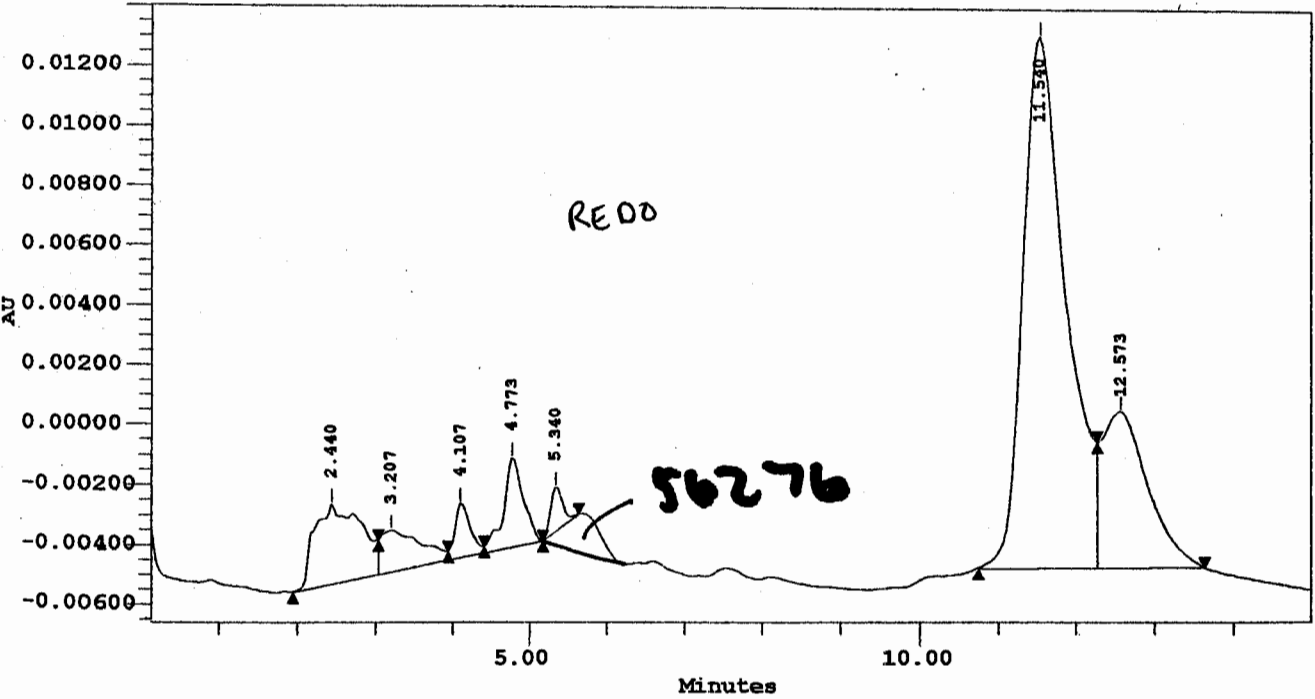
Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.478	296490	17150	BV	2.012	2.612	25.20
2		2.745	422333	18941	VB	2.612	3.778	35.89
3		5.445	97226	3909	BB	5.212	6.345	8.26
4		11.878	242658	5824	VV	11.312	12.578	20.62
5		12.978	117922	2883	VB	12.578	13.845	10.02

Millennium Results Report August 1, 1984 Page: 1 of 1
Report Method: MetCarb_RM Version: 2.15
For Sample: 60 ul of #75 Vial: 7 Injection: 1 Channel: 991M
Proc Chan: PDA_282.0nm Processed: 08/01/84 10:28:31 PM
Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol Sample Name: 60 ul of #75
Vial: 7 Sample Origin:
Sample Type: Unknown Solvent: meoh
Injection: 1 FlowRate: 1.500
Channel: 991M Level:
Date Acquired: 08/01/84 10:13:07 PM Volume: 60.00
SampleWeight: 1.00000 Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS
Processing Method: Ergosterol_PM



Peak Results

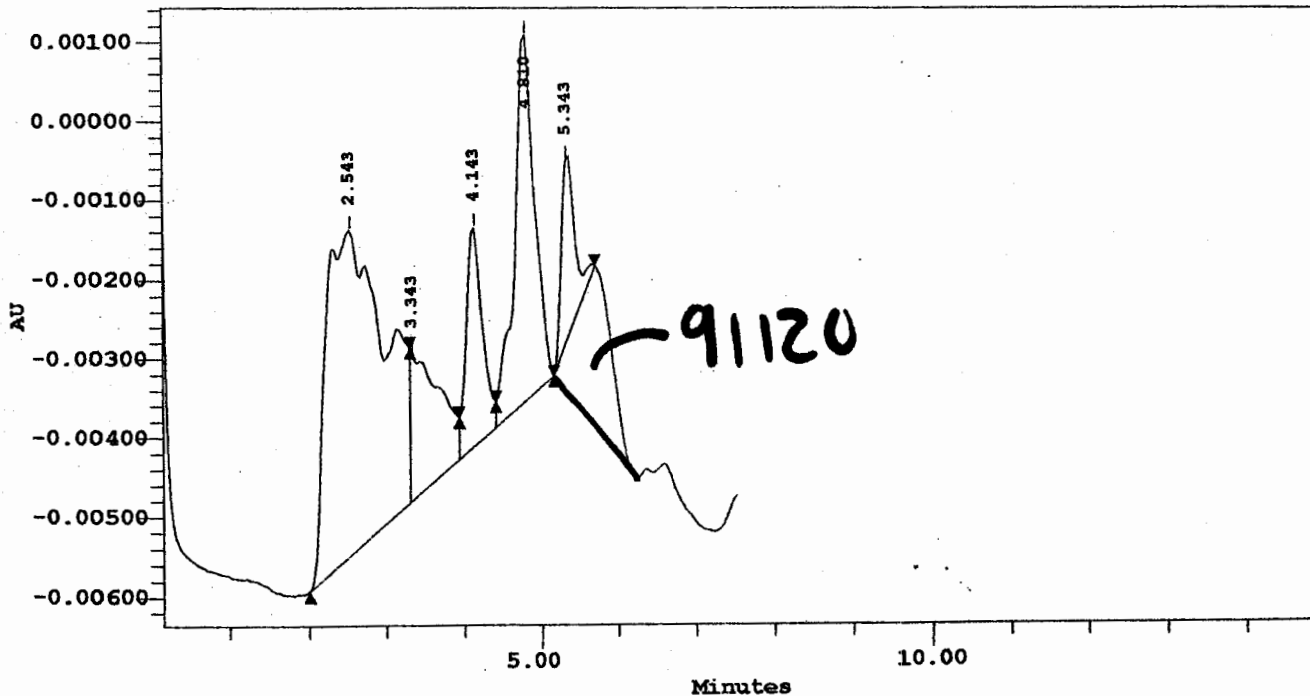
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.440	108881	2677	BV	1.940	3.040	9.46
2		3.207	49325	1397	VV	3.040	3.940	4.29
3		4.107	25002	1821	VV	3.940	4.407	2.17
4		4.773	53407	2970	VB	4.407	5.173	4.64
5		5.340	15367	1462	BB	5.173	5.640	1.34
6		11.540	684282	17867	BV	10.740	12.273	59.48
7		12.573	214093	5240	VB	12.273	13.640	18.61

DR-35

Millennium Results Report August 2, 1984 Page: 1 of 1
Report Method: MetCarb_RM Version: 2.15
For Sample: 100 ul of #75 Vial: 4 Injection: 2 Channel: 991M
Proc Chan: PDA_282.0nm Processed: 08/02/84 01:41:03 AM
Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol Sample Name: 100 ul of #75
Vial: 4 Sample Origin:
Sample Type: Unknown Solvent: MeOH
Injection: 2 FlowRate: 1.500
Channel: 991M Level:
Date Acquired: 08/02/84 01:33:09 AM Volume: 60.00
SampleWeight: 1.00000 Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS
Processing Method: Ergosterol_PM



Peak Results

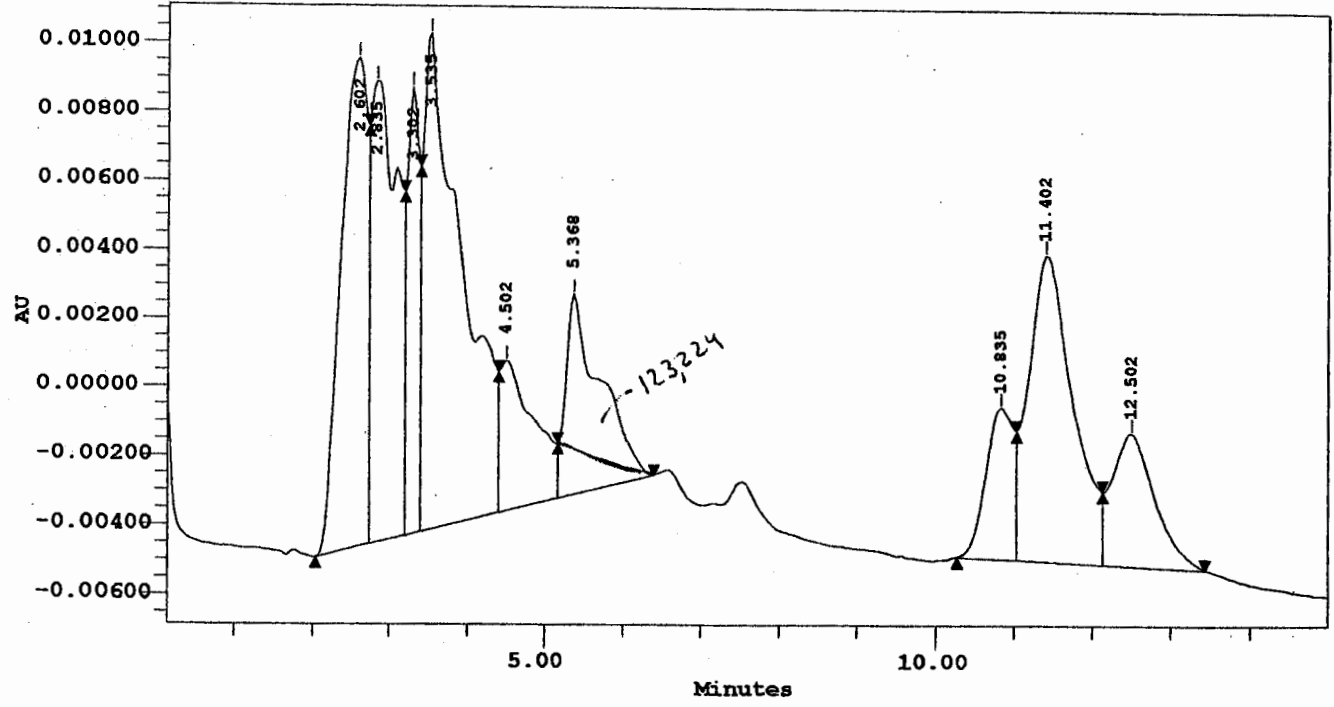
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.543	212513	4093	BV	2.010	3.310	51.66
2		3.343	46898	1844	VV	3.310	3.943	11.40
3		4.143	40023	2753	VV	3.943	4.410	9.73
4		4.810	85062	4621	VB	4.410	5.177	20.68
5		5.343	26847	2281	BB	5.177	5.710	6.53

ZM-7 626-00

Millennium Results Report June 26, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul 6 Vial: 3 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 06/26/84 01:09:48 AM
 Channel Descr: PDA 282.0 nm

Zml Lycoming College, Department of Chemistry June 21
 2mm Sample

Project Name: Ergosterol Sample Name: 60 ul 6
 Vial: 3 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 06/26/84 12:54:23 AM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.602	337054	14184	BV	2.035	2.735	15.14
2		2.835	323813	13394	VV	2.735	3.202	14.54
3		3.302	139501	12957	VV	3.202	3.402	6.26
4		3.535	499546	14396	VV	3.402	4.402	22.43
5		4.502	133451	4363	VV	4.402	5.168	5.99
6		5.368	(181395)	5854	VB	5.168	6.402	8.15
7		10.835	108679	4423	BV	10.268	11.035	4.88
8		11.402	356656	8962	VV	11.035	12.135	16.02
9		12.502	146860	3877	VB	12.135	13.435	6.59

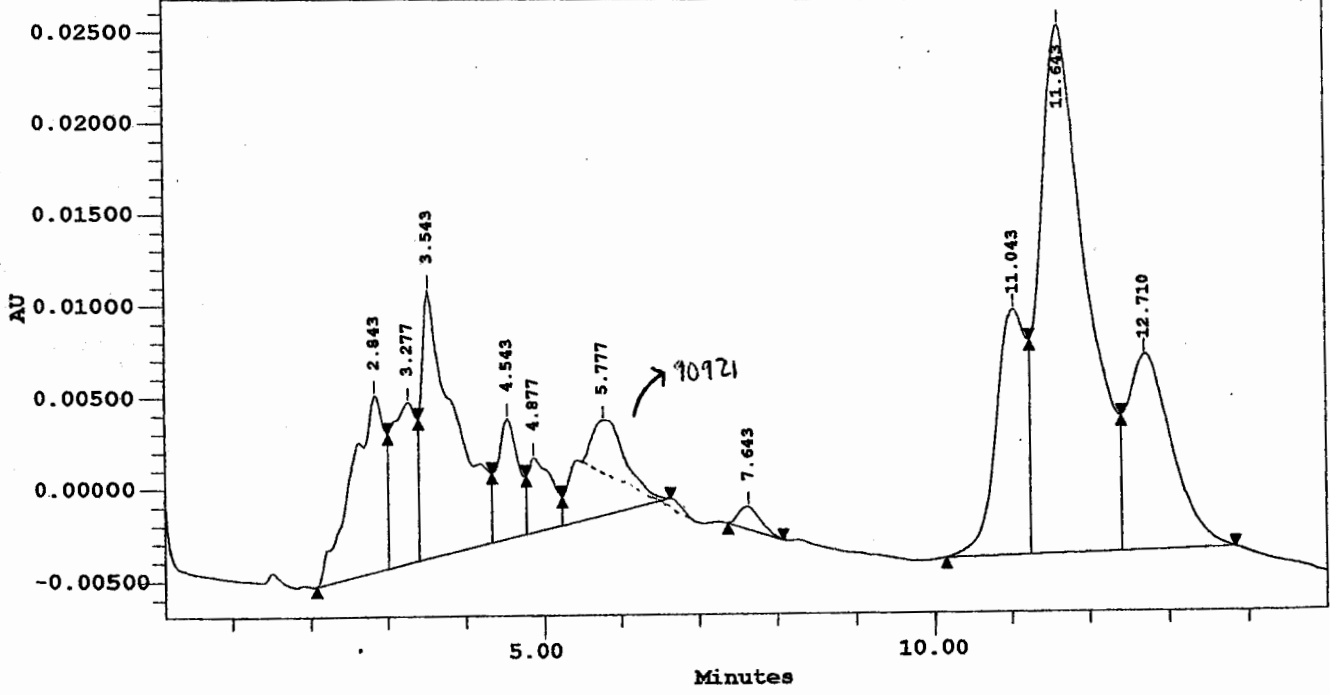
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2-11-7 6/27/00

Millennium Results Report June 26, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of # 1 Vial: 6 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 06/26/84 09:46:31 PM
 Channel Descr: PDA 282.0 nm

(ML) Lycoming College, Department of Chemistry 7 21-Jun
 Zimm's
 S. Maple

Project Name: Ergosterol Sample Name: 60 ul of # 1
 Vial: 6 Sample Origin:
 Sample Type: Unknown Solvent: meoh
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 06/26/84 09:31:04 PM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

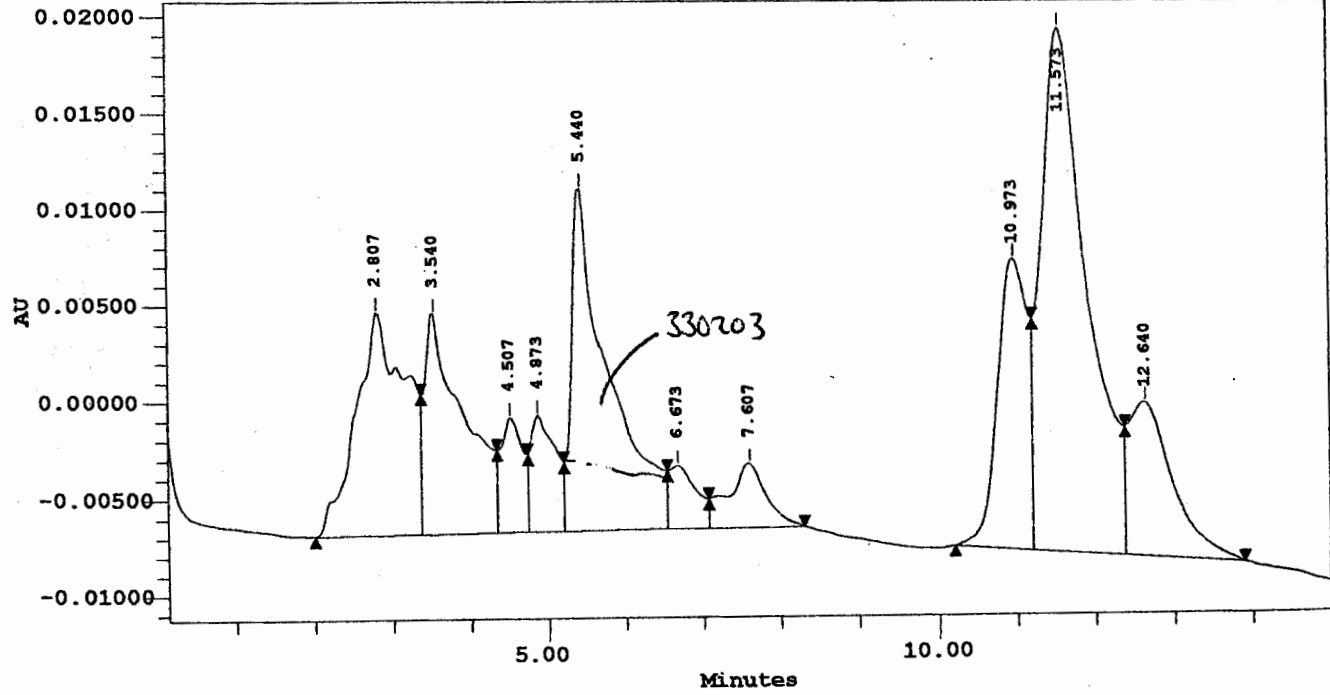
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.843	284790	9616	BV	2.077	3.010	8.44
2		3.277	195099	8782	VV	3.010	3.410	5.78
3		3.543	436552	14655	VV	3.410	4.343	12.94
4		4.543	123538	6577	VV	4.343	4.777	3.66
5		4.877	86721	4091	VV	4.777	5.243	2.57
6		5.777	219923	5170	VB	5.243	6.643	6.52
7		7.643	23261	1232	BB	7.377	8.077	0.69
8		11.043	363078	13481	BV	10.143	11.243	10.76
9		11.643	1219524	29035	VV	11.243	12.410	36.14
10		12.710	422247	10763	VB	12.410	13.843	12.51

2-m-7 6-28-00

Millennium Results Report June 28, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #17 Vial: 2 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 06/28/84 01:32:05 AM
 Channel Descr: PDA 282.0 nm

Imb Lycoming College, Department of Chemistry **7** *2mm sample Jun 21*

Project Name: Ergosterol Sample Name: 60 ul of #17
 Vial: 2 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 06/28/84 01:16:38 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.807	502759	11484	BV	2.007	3.373	13.33
2		3.540	396726	11365	VV	3.373	4.340	10.52
3		4.507	116818	5877	VV	4.340	4.740	3.10
4		4.873	134507	5984	VV	4.740	5.207	3.57
5		5.440	(590681)	17746	VV	5.207	6.540	15.67
6		6.673	79313	3237	VV	6.540	7.073	2.10
7		7.607	112681	3274	VB	7.073	8.307	2.99
8		10.973	403205	15115	BV	10.207	11.207	10.69
9		11.573	1119559	27313	VV	11.207	12.373	29.69
10		12.640	314140	8003	VB	12.373	13.907	8.33

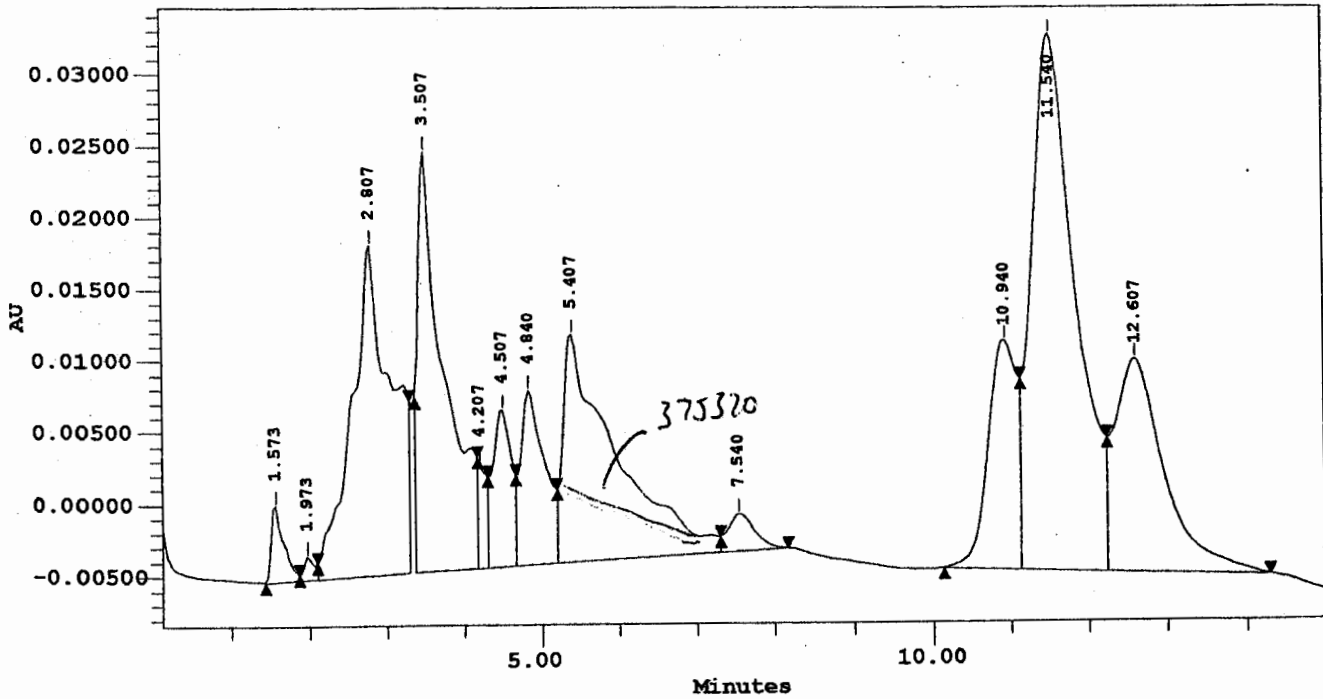
Z-M-7 6-28-00

Millennium Results Report June 28, 1984 Page: 1 of 2
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #15 Vial: 1 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 06/28/84 01:58:14 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Zimmer's
 Sample
 June 21

Project Name: Ergosterol Sample Name: 60 ul of #15
 Vial: 1 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 06/28/84 01:42:43 AM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

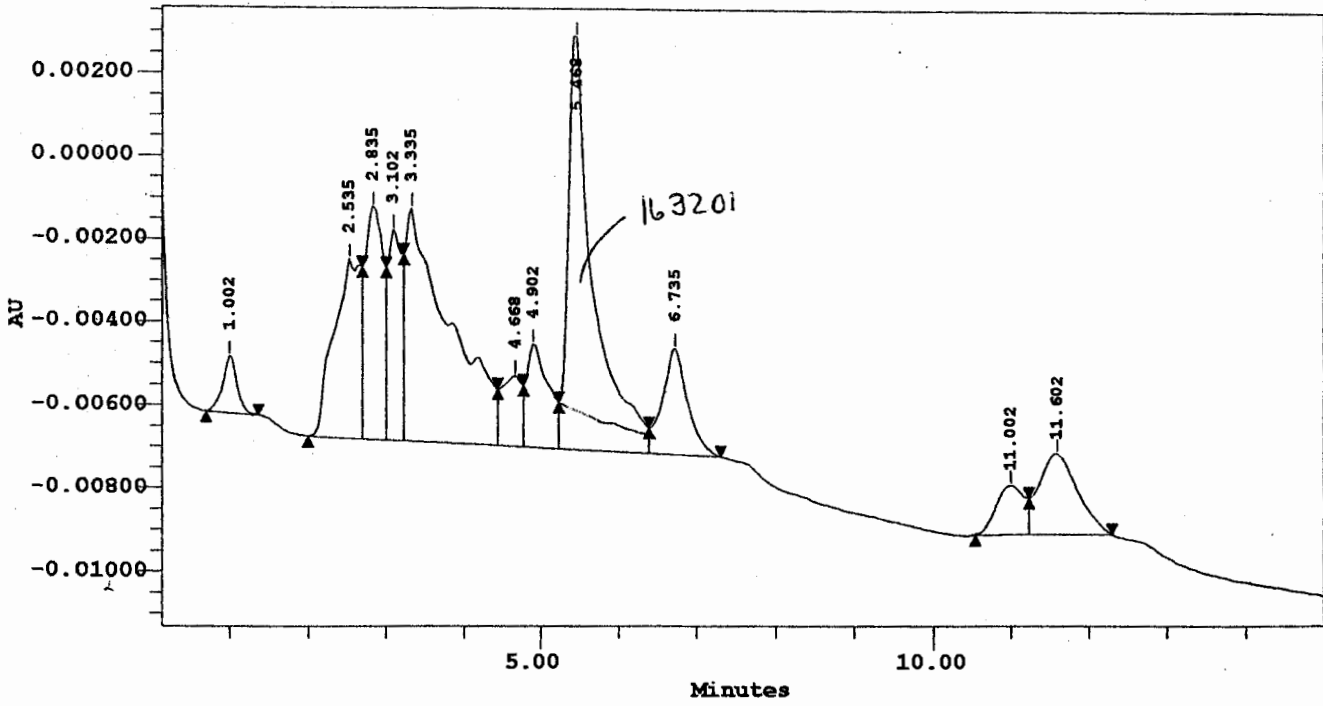
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		1.573	57524	5241	BV	1.440	1.873	1.06
2		1.973	14730	1629	VV	1.873	2.107	0.27
3		2.807	819262	23000	VV	2.107	3.307	15.09
4		3.507	703433	29312	VV	3.373	4.173	12.96
5		4.207	54996	7239	VV	4.173	4.307	1.01
6		4.507	185448	10897	VV	4.307	4.673	3.42
7		4.840	261718	12120	VV	4.673	5.207	4.82
8		5.407	(746850)	15841	VV	5.207	7.307	13.76
9		7.540	61057	2576	VB	7.307	8.173	1.12
10		10.940	412412	15972	BV	10.140	11.140	7.60
11		11.540	1463791	37547	VV	11.140	12.240	26.97

Z-M-21

Millennium Results Report July 7, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of # 3(8) Vial: 2 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/07/84 12:58:46 AM
 Channel Descr: PDA 282.0 nm

ZML
Lycoming College, Department of Chemistry Z1
Zimms
S. maple
July 5

Project Name: Ergosterol	Sample Name: 60 ul of # 3(8)/12
Vial: 2	Sample Origin:
Sample Type: Unknown	Solvent: MeOH
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 07/07/84 12:43:18 AM	Volume: 60.00
Sample Weight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

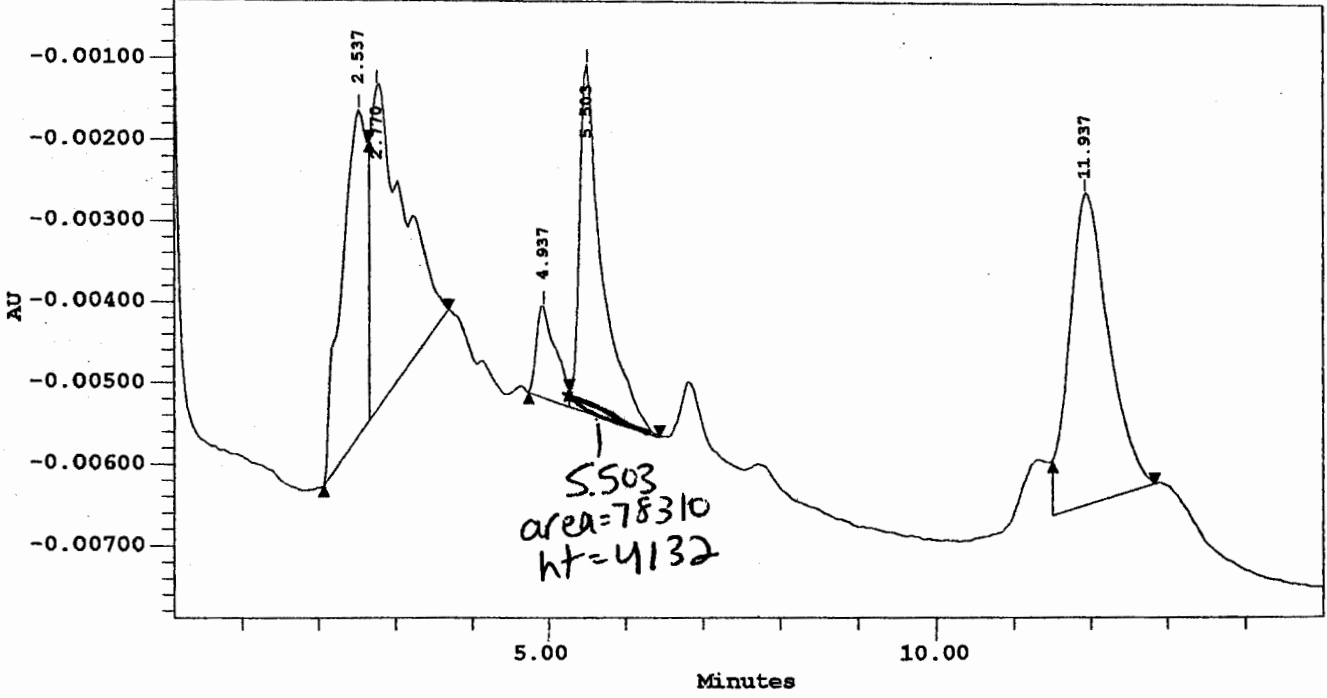
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		1.002	16743	1354	BB	0.702	1.368	1.76
2		2.535	99919	4318	BV	2.002	2.702	10.51
3		2.835	88474	5601	VV	2.702	3.002	9.31
4		3.102	64943	5067	VV	3.002	3.235	6.83
5		3.335	220967	5606	VV	3.235	4.435	23.25
6		4.668	30753	1705	VV	4.435	4.768	3.24
7		4.902	50005	2477	VV	4.768	5.235	5.26
8		5.468	(224946)	9967	VV	5.235	6.402	23.67
9		6.735	56811	2563	VB	6.402	7.302	5.98
10		11.002	29637	1185	BV	10.535	11.235	3.12
11		11.602	67192	1946	VB	11.235	12.302	7.07

2-M-35

Millennium Results Report July 23, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #4 Vial: 5 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/23/84 10:45:12 PM
 Channel Descr: PDA 282.0 nm

1 mL Lycoming College, Department of Chemistry 7-19
 Zimm's
 J. Maple

Project Name: Ergosterol Sample Name: 60 ul of #4
 Vial: 5 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 07/23/84 10:29:41 PM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

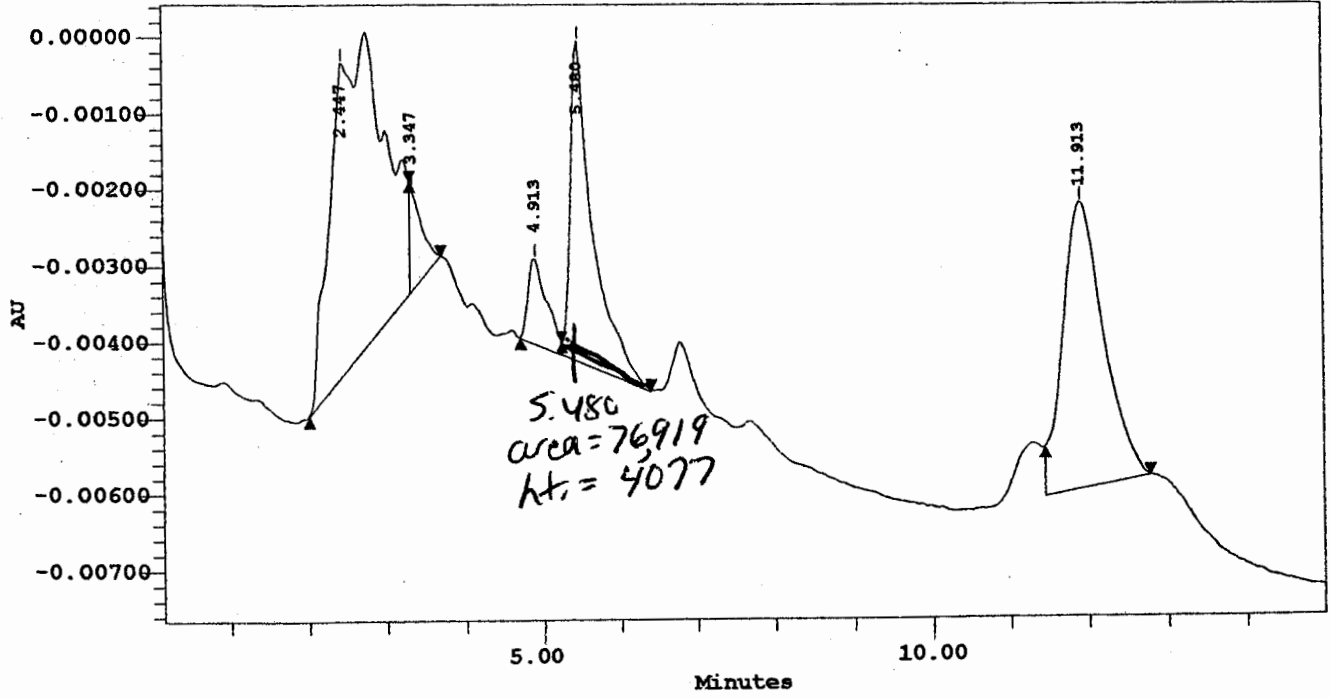
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.537	92579	4012	BV	2.070	2.670	20.26
2		2.770	118334	4003	VB	2.670	3.703	25.90
3		4.937	19907	1137	BV	4.737	5.270	4.36
4		5.503	(86684)	4307	VV	5.270	6.437	18.97
5		11.937	139403	3875	VB	11.503	12.837	30.51

2m-35

Millennium Results Report July 24, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #4 Vial: 2 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/24/84 02:03:00 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: 60 ul of #4
Vial: 2	Sample Origin:
Sample Type: Unknown	Solvent: MeOH
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 07/24/84 01:47:33 AM	Volume: 60.00
SampleWeight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

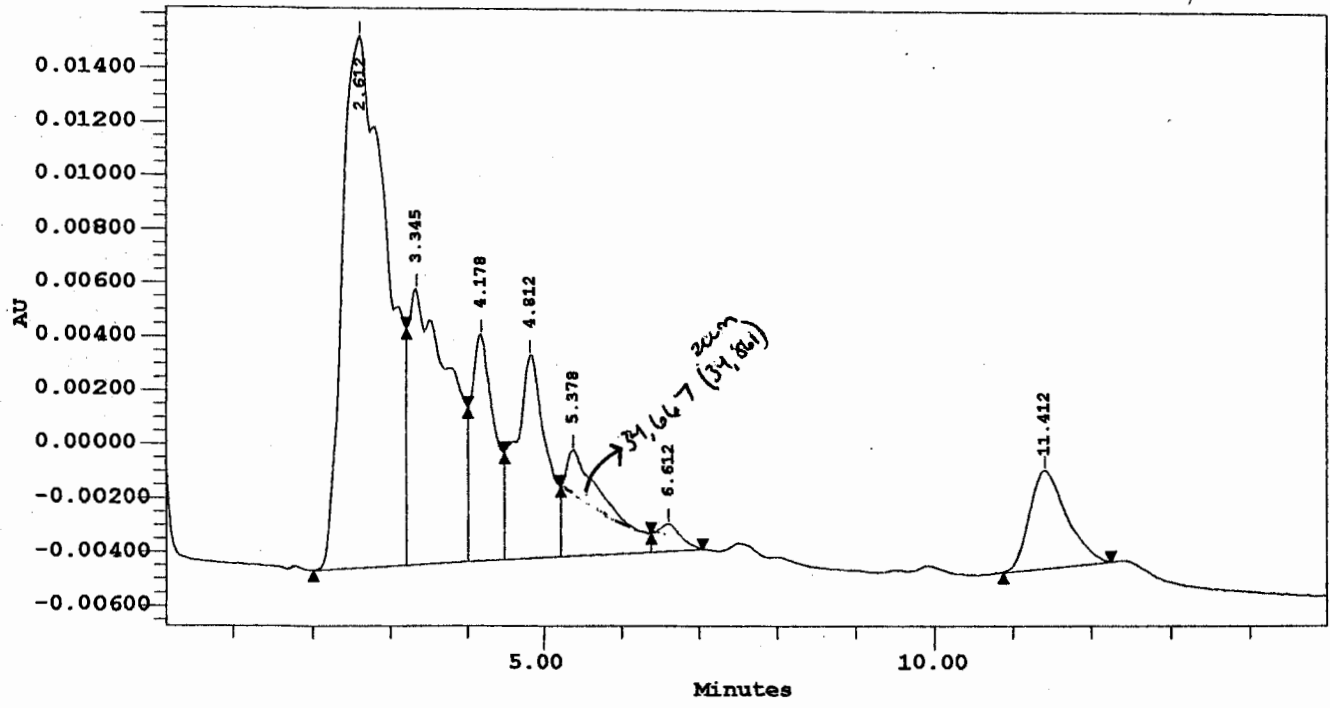
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.447	201062	4103	BV	2.013	3.313	44.11
2		3.347	14696	1314	VB	3.313	3.713	3.22
3		4.913	19061	1124	BV	4.713	5.247	4.18
4		5.480	(84413)	4225	VV	5.247	6.413	18.52
5		11.913	136620	3797	VB	11.447	12.780	29.97

227 6/26/84

Millennium Results Report June 26, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul 150 Vial: 4 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 06/26/84 01:26:20 AM
 Channel Descr: PDA 282.0 nm

2mL Lycoming College, Department of Chemistry 7? Zimm's r. birch

Project Name: Ergosterol Sample Name: 60 ul 150
 Vial: 4 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 Flow Rate: 1.500
 Channel: 991M Level:
 Date Acquired: 06/26/84 01:10:55 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.612	787842	19785	BV	2.012	3.212	42.57
2		3.345	385133	10252	VV	3.212	4.012	20.81
3		4.178	173924	8414	VV	4.012	4.478	9.40
4		4.812	213347	7570	VV	4.478	5.212	11.53
5		5.378	(146157)	3941	VV	5.212	6.378	7.90
6		6.612	22723	1008	VB	6.378	7.045	1.23
7		11.412	121553	3676	BB	10.878	12.245	6.57

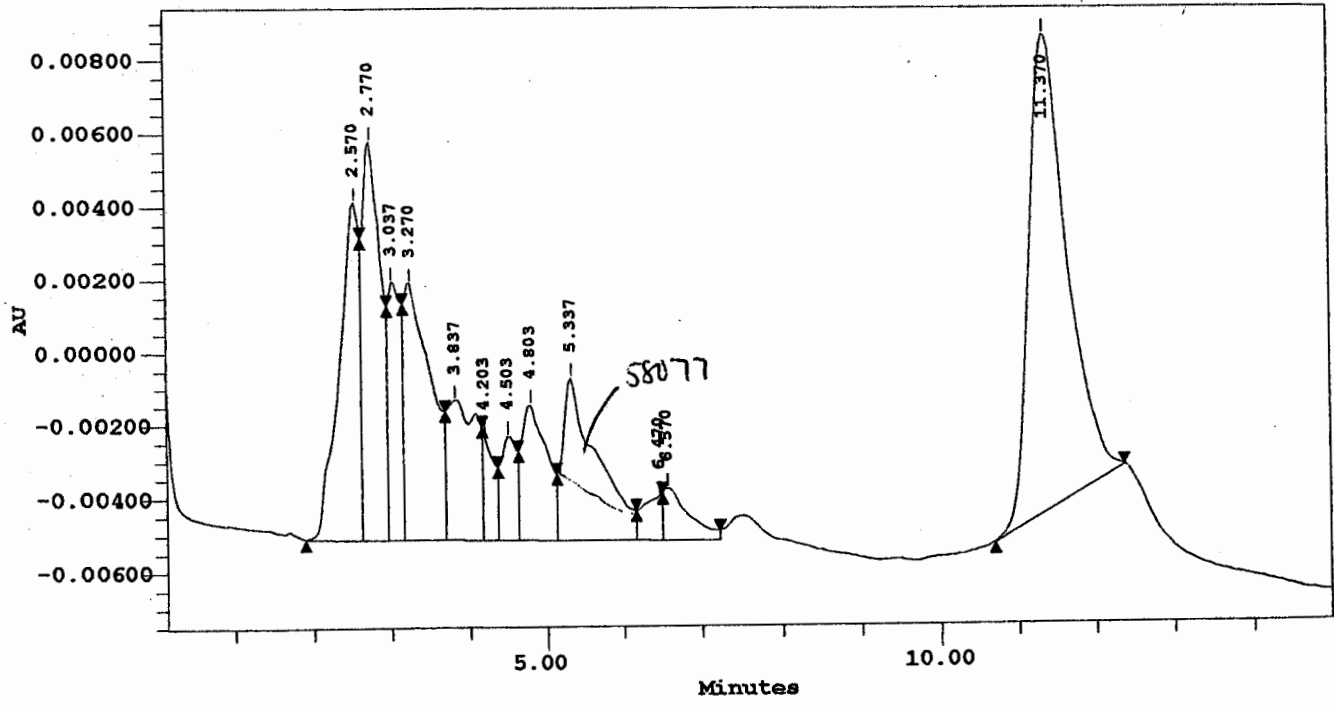
260
280

C-R-21

Millennium Results Report July 7, 1984 Page: 1 of 2
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #142(1) Vial: 4 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/07/84 01:32:28 AM
 Channel Descr: PDA 282.0 nm

1ml
Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: 60 ul of #142(1) again
Vial: 4	Sample Origin:
Sample Type: Unknown	Solvent: MeOH
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 07/07/84 01:17:05 AM	Volume: 60.00
SampleWeight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

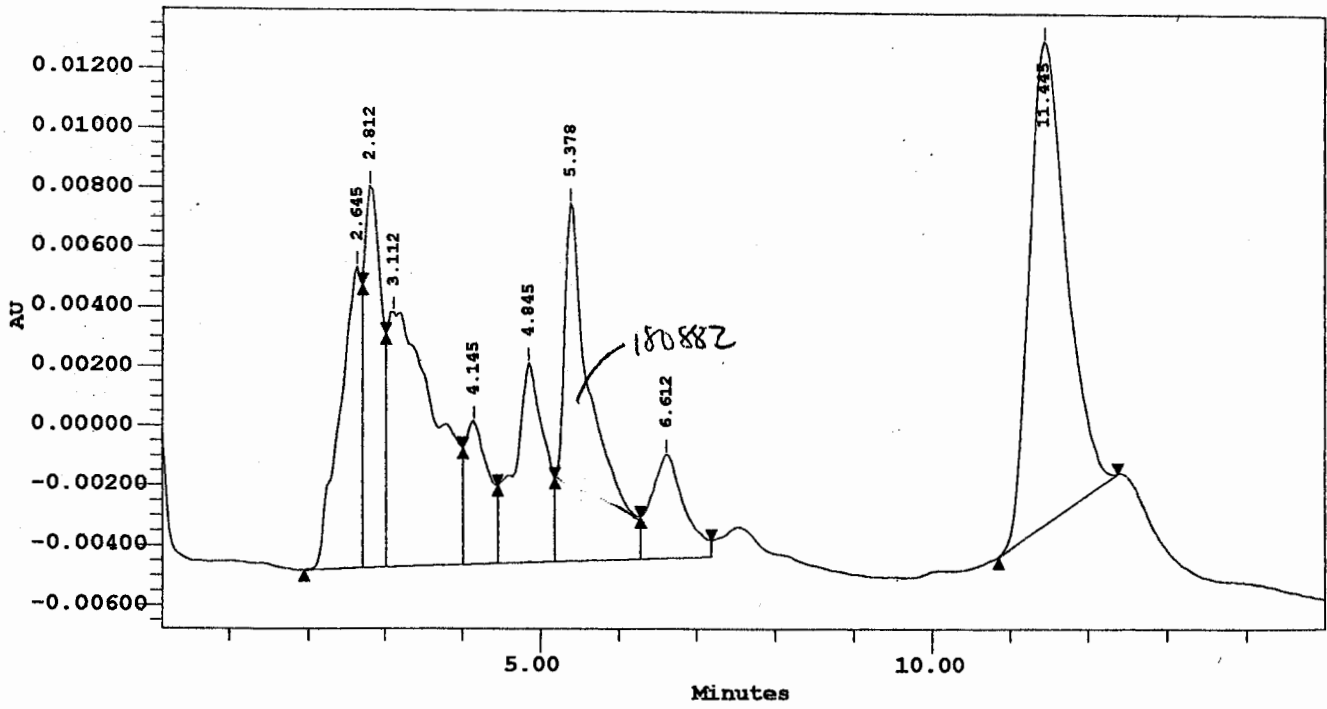
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.570	163675	9246	BV	1.903	2.637	11.09
2		2.770	179174	10900	VV	2.637	2.970	12.14
3		3.037	80686	7058	VV	2.970	3.170	5.47
4		3.270	169067	7015	VV	3.170	3.703	11.46
5		3.837	97353	3811	VV	3.703	4.170	6.60
6		4.203	28819	2818	VV	4.170	4.370	1.95
7		4.503	39621	2805	VV	4.370	4.637	2.69
8		4.803	83997	3670	VV	4.637	5.137	5.69
9		5.337	(137452)	4420	VV	5.137	6.170	9.32
10		6.470	19993	1161	VV	6.170	6.503	1.35
11		6.570	31301	1383	VV	6.503	7.237	2.12

2-R-21

Millennium Results Report July 7, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #142(2) Vial: 5 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/07/84 01:52:09 AM
 Channel Descr: PDA 282.0 nm

(1ml) Lycoming College, Department of Chemistry July 5
 Zimm
 r. birch (s.m.)

Project Name: Ergosterol Sample Name: 60 ul of #142(2)
 Vial: 5 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 07/07/84 01:36:46 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.645	191795	10122	BV	1.945	2.712	9.60
2		2.812	193976	12802	VV	2.712	3.012	9.71
3		3.112	376156	8553	VV	3.012	4.012	18.84
4		4.145	98064	4796	VV	4.012	4.445	4.91
5		4.845	180900	6691	VV	4.445	5.178	9.06
6		5.378	318319	12038	VV	5.178	6.278	15.94
7		6.612	102490	3472	VV	6.278	7.178	5.13
8		11.445	535129	16313	BB	10.845	12.378	26.80

Report Method: MetCarb_RM

Version: 2.15

For Sample: 60 ul of #142(1

Vial: 4

Injection: 1

Channel: 991M

Proc Chan: PDA_282.0nm

Processed: 07/07/84 01:32:28 AM

Channel Descr: PDA 282.0 nm

Peak Results

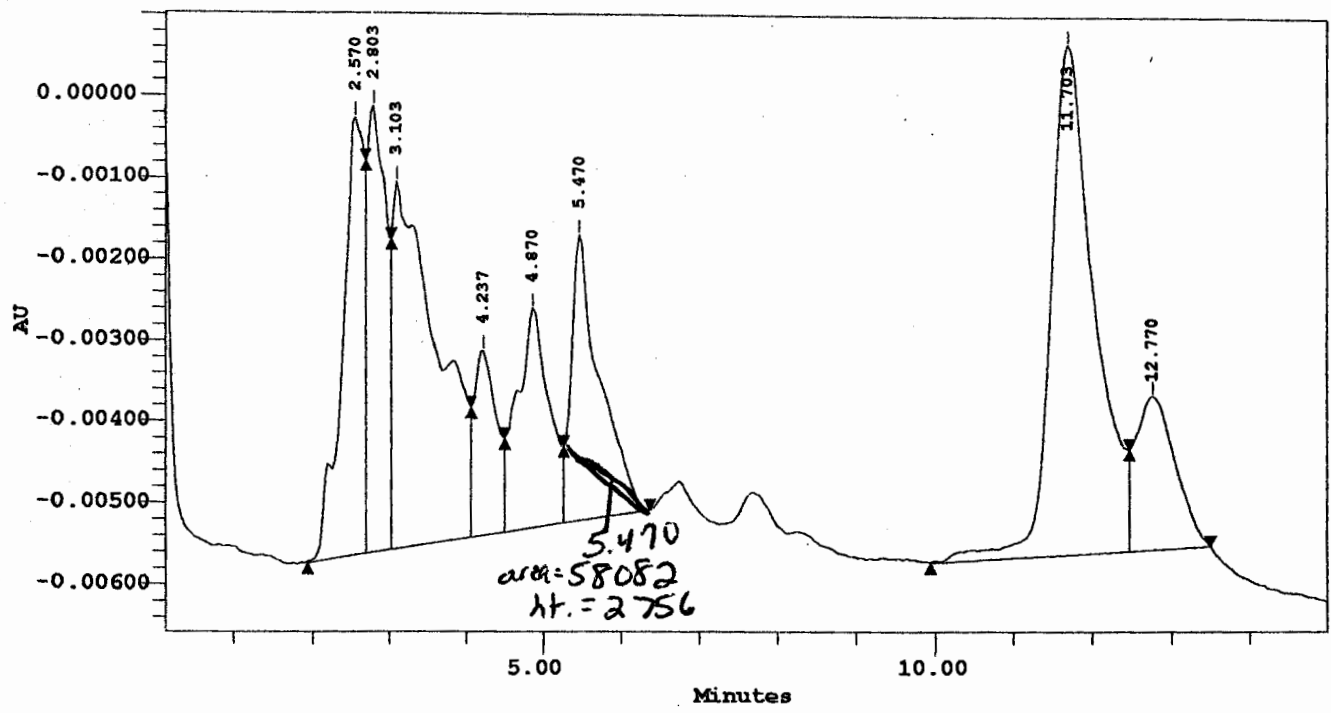
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
12		11.370	444387	13112	BB	10.703	12.370	30.12

2-A-21 7/14/84

Millennium Results Report
 Report Method: MetCarb_RM
 For Sample: 60 ul of #157
 Proc Chan: PDA_282.0nm
 Channel Descr: PDA 282.0 nm
 July 14, 1984
 Version: 2.15
 Vial: 4
 Injection: 1
 Processed: 07/14/84 03:09:41 AM
 Page: 1 of 1
 Channel: 991M

1ml
 Lycoming College, Department of Chemistry
 Zimm, r. birch
 7-12

Project Name: Ergosterol
 Vial: 4
 Sample Type: Unknown
 Injection: 1
 Channel: 991M
 Date Acquired: 07/14/84 02:54:11 AM
 Sample Weight: 1.00000
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM
 Sample Name: 60 ul of #157
 Sample Origin:
 Solvent: MeOH
 Flow Rate: 1.500
 Level:
 Volume: 60.00
 Run Time: 15.0 min



Peak Results

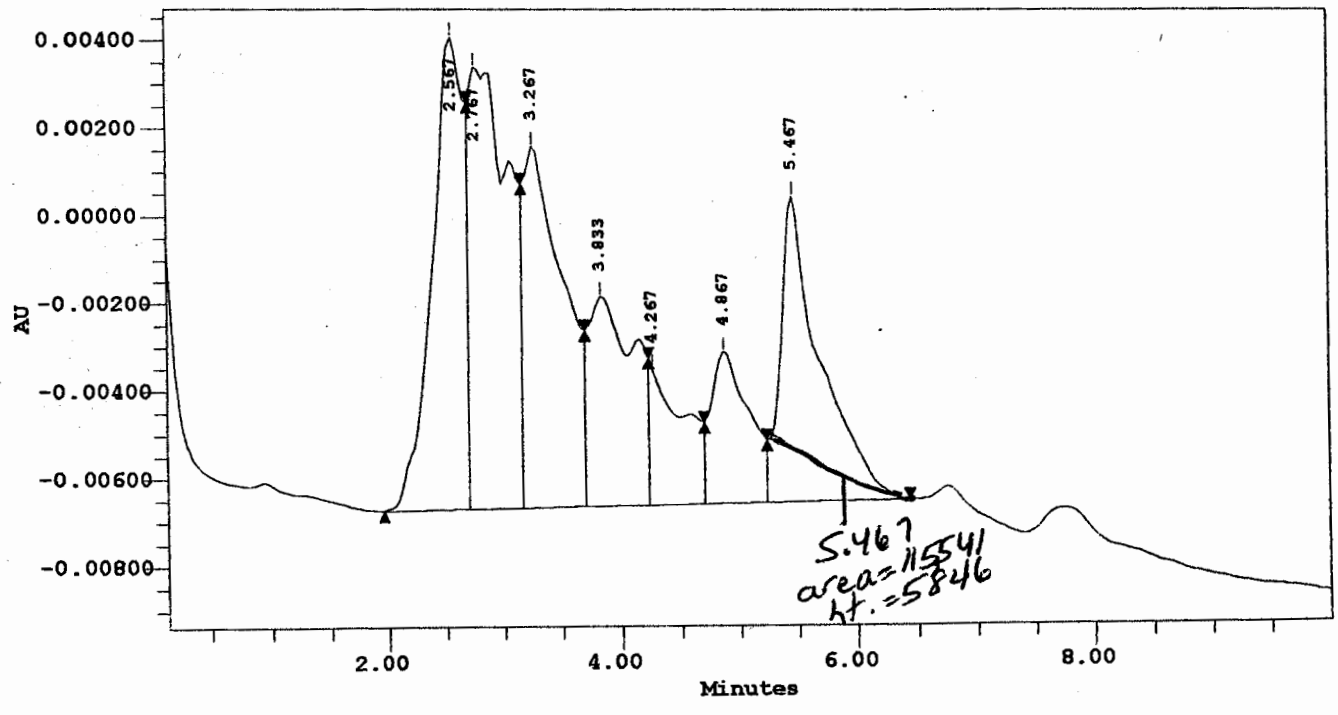
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.570	105471	5387	BV	1.937	2.703	11.65
2		2.803	95410	5492	VV	2.703	3.037	10.54
3		3.103	181895	4521	VV	3.037	4.070	20.09
4		4.237	45804	2278	VV	4.070	4.503	5.06
5		4.870	78465	2710	VV	4.503	5.270	8.67
6		5.470	(89047)	3530	VB	5.270	6.370	9.84
7		11.703	242680	6312	BV	9.937	12.470	26.81
8		12.770	66564	1910	VB	12.470	13.503	7.35

2-R-21 7/14/00

Millennium Results Report July 14, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: 60 ul of #156 Vial: 2 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 07/14/84 01:23:45 AM
 Channel Descr: PDA 282.0 nm

1ml r. birch
2mm
7-12
 Lycoming College, Department of Chemistry

Project Name: Ergosterol Sample Name: 60 ul of #156
 Vial: 2 Sample Origin:
 Sample Type: Unknown Solvent: meoh
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 07/14/84 01:13:18 AM Volume: 60.00
 SampleWeight: 1.00000 Run Time: 10.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.567	204303	10776	BV	1.967	2.700	18.86
2		2.767	246338	10078	VV	2.700	3.167	22.74
3		3.267	197772	8237	VV	3.167	3.700	18.26
4		3.833	128198	4771	VV	3.700	4.233	11.83
5		4.267	63530	3080	VV	4.233	4.700	5.86
6		4.867	77773	3456	VV	4.700	5.233	7.18
7		5.467	(165409)	6989	VB	5.233	6.433	15.27

151

2-R-21

Millennium Results Report
 Report Method: MetCarb_RM
 For Sample: 60 ul #149
 Proc Chan: PDA_282.0nm
 Channel Descr: PDA 282.0 nm

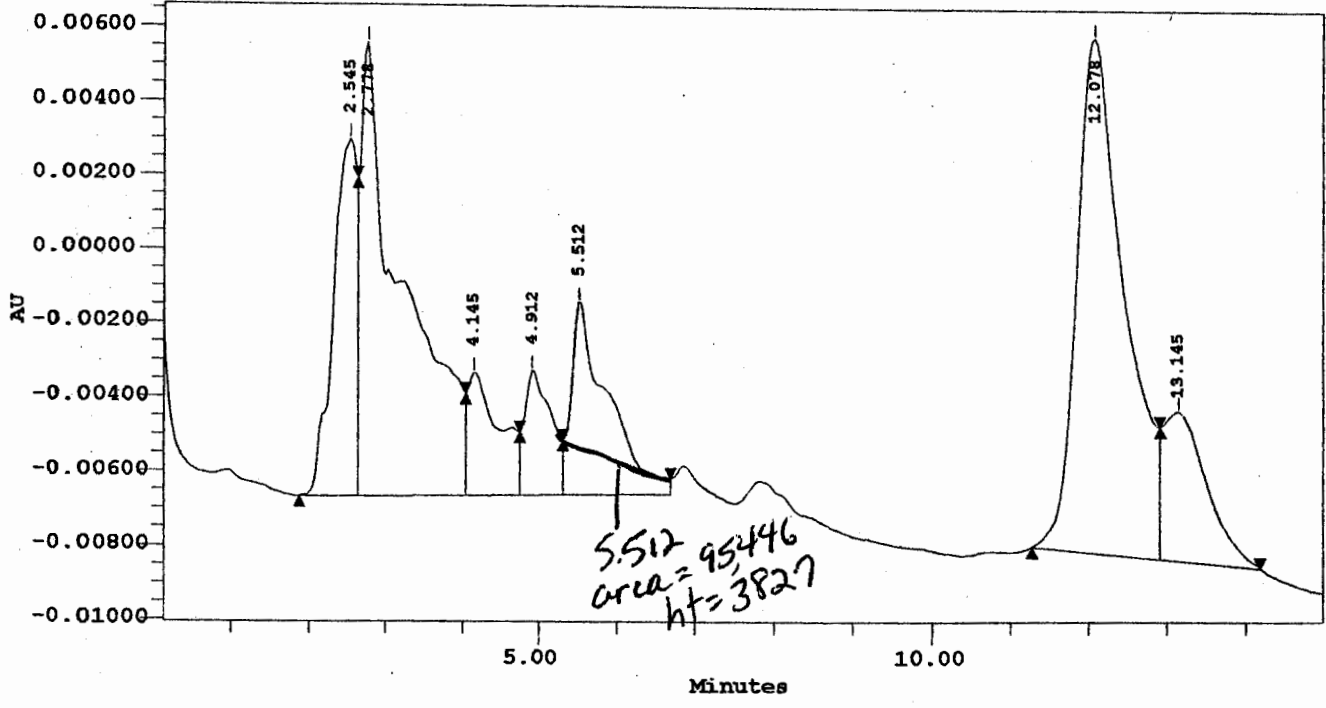
July 23, 1984
 Version: 2.15
 Vial: 2
 Injection: 1
 Processed: 07/23/84 09:53:22 PM

Page: 1 of 1
 Channel: 991M

1ml
 Lycoming College, Department of Chemistry 21
 Zimm's r.b.
 7-5-88
 (for s.c. high)

Project Name: Ergosterol
 Vial: 2
 Sample Type: Unknown
 Injection: 1
 Channel: 991M
 Date Acquired: 07/23/84 09:37:55 PM
 Sample Weight: 1.00000
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM

Sample Name: 60 ul #149
 Sample Origin:
 Solvent: MeOH
 Flow Rate: 1.500
 Level:
 Volume: 60.00
 Run Time: 15.0 min



Peak Results

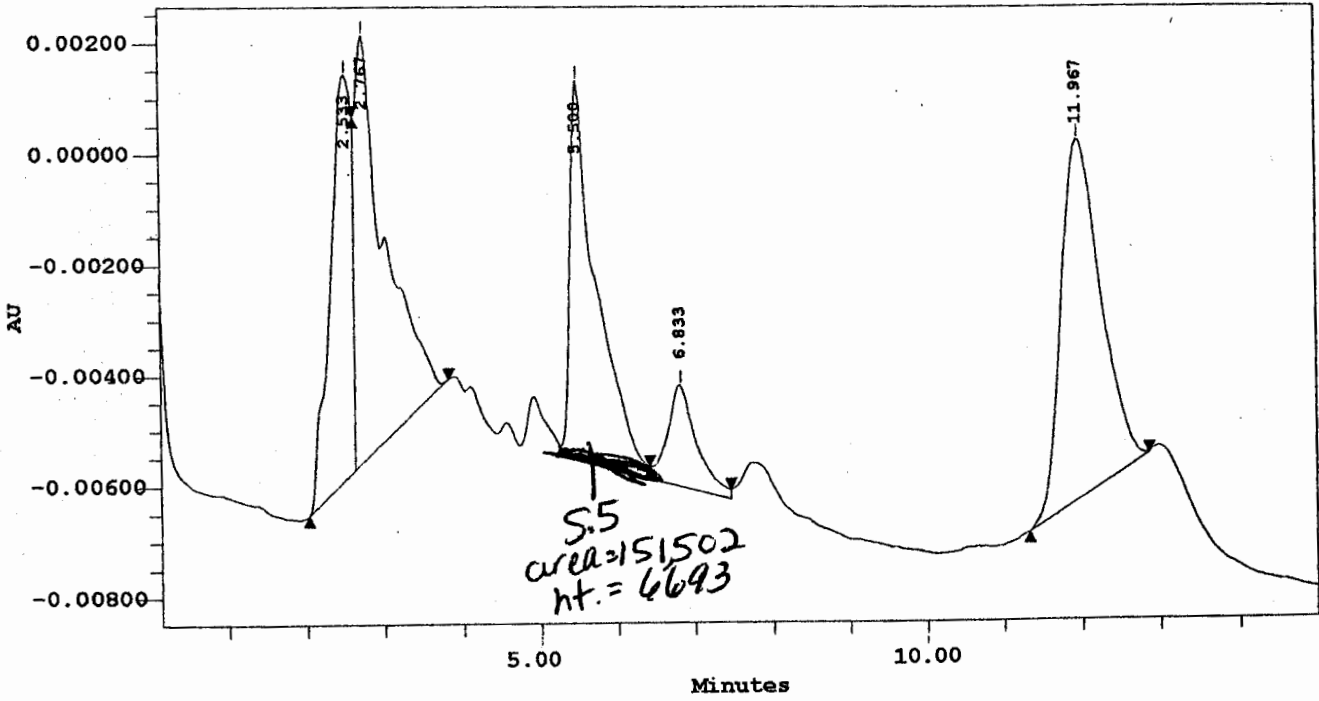
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.545	199940	9647	BV	1.878	2.645	11.19
2		2.778	479086	12248	VV	2.645	4.045	26.81
3		4.145	96026	3300	VV	4.045	4.745	5.37
4		4.912	82908	3374	VV	4.745	5.312	4.64
5		5.512	(171712)	5216	VV	5.312	6.678	9.61
6		12.078	599865	14016	BV	11.278	12.912	33.57
7		13.145	157161	4078	VB	12.912	14.178	8.80

ZR 35

Millennium Results Report July 23, 1984 Page: 1 of 1
Report Method: MetCarb_RM Version: 2.15
For Sample: 60 ul of # 45 Vial: 4 Injection: 1 Channel: 991M
Proc Chan: PDA_282.0nm Processed: 07/23/84 10:27:45 PM
Channel Descr: PDA 282.0 nm

1 ml Lycoming College, Department of Chemistry 35
7-19
Zimm
r. birch

Project Name: Ergosterol Sample Name: 60 ul of #145
Vial: 4 Sample Origin:
Sample Type: Unknown Solvent: MeOH
Injection: 1 Flow Rate: 1.500
Channel: 991M Level:
Date Acquired: 07/23/84 10:12:20 PM Volume: 60.00
Sample Weight: 1.00000 Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS
Processing Method: Ergosterol_PM



Peak Results

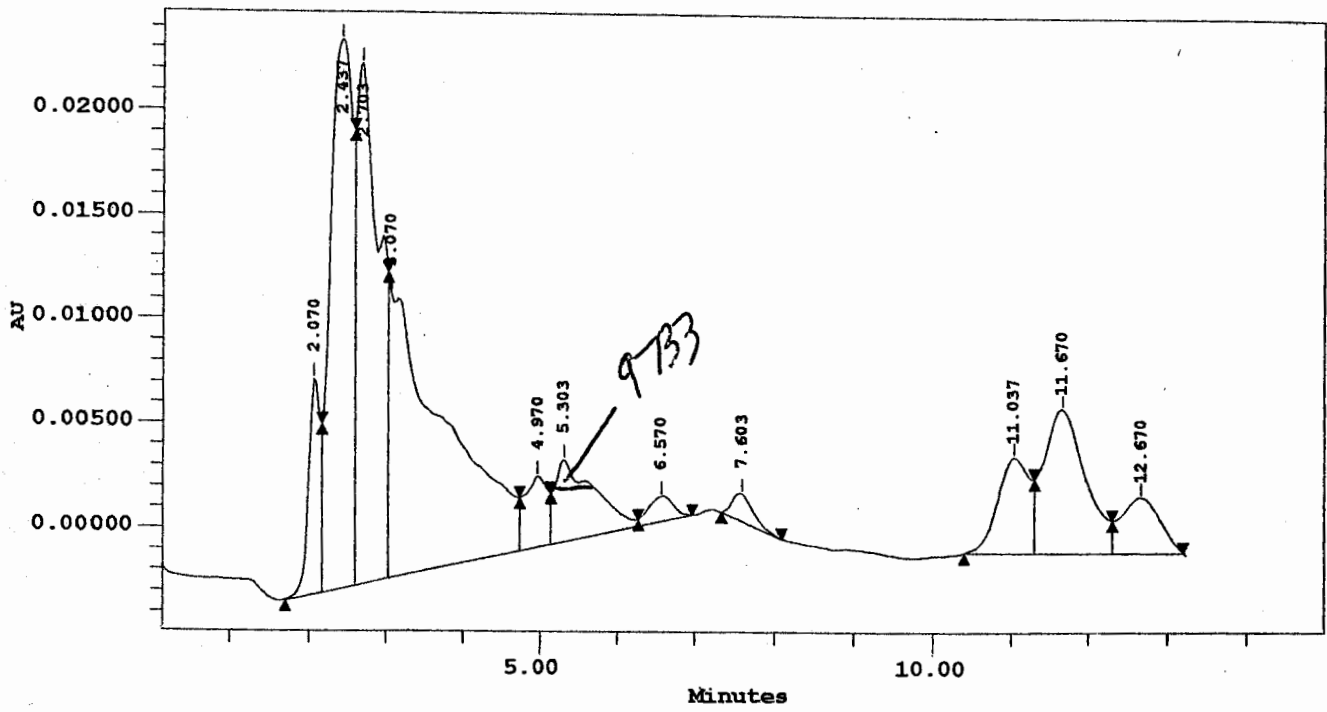
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.533	140234	7286	BV	2.033	2.633	17.70
2		2.767	209201	7675	VB	2.633	3.833	26.41
3		5.500	(165573)	6880	VV	5.267	6.433	20.90
4		6.833	47214	1840	VV	6.433	7.467	5.96
5		11.967	229969	6546	BB	11.333	12.867	29.03

CM-8 10/3/80

Millennium Results Report October 11, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: z-m-8 Vial: 8 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 10/11/84 03:10:37 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: z-m-8
Vial: 8	Sample Origin:
Sample Type: Unknown	Solvent: meoh
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 10/11/84 02:56:51 AM	Volume: 100.00
Sample Weight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.070	102233	10340	BV	1.703	2.170	3.96
2		2.437	536718	26299	VV	2.170	2.603	20.81
3		2.703	509760	24964	VV	2.603	3.037	19.77
4		3.070	687698	13649	VV	3.037	4.737	26.67
5		4.970	69350	3396	VV	4.737	5.137	2.69
6		5.303	137389	3938	VV	5.137	6.270	5.33
7		6.570	26738	1190	VB	6.270	6.970	1.04
8		7.603	23868	1330	BB	7.337	8.103	0.93
9		11.037	130884	4642	BV	10.403	11.303	5.08
10		11.670	261685	6963	VV	11.303	12.303	10.15
11		12.670	92293	2704	VB	12.303	13.203	3.58

z-m-8 10/3/06

Millennium Results Report
 Report Method: MetCarb_RM
 For Sample: z-m-8
 roc Chan: PDA_282.0nm
 Channel Descr: PDA 282.0 nm

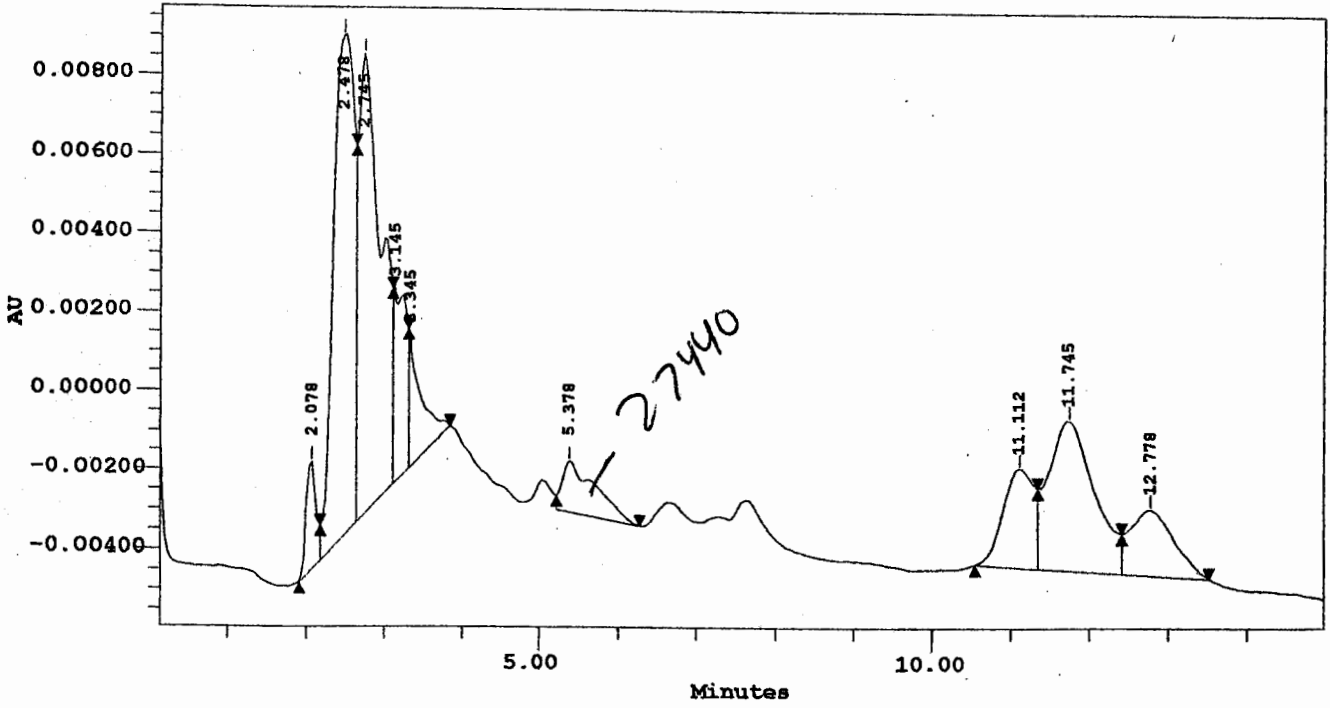
October 11, 1984
 Vial: 7
 Injection: 1
 Processed: 10/11/84 02:38:49 AM

Page: 1 of 1
 Version: 2.15
 Channel: 991M

Lycoming College, Department of Chemistry

Project Name: Ergosterol
 Vial: 7
 Sample Type: Unknown
 Injection: 1
 Channel: 991M
 Date Acquired: 10/11/84 02:23:19 AM
 Sample Weight: 1.00000
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM

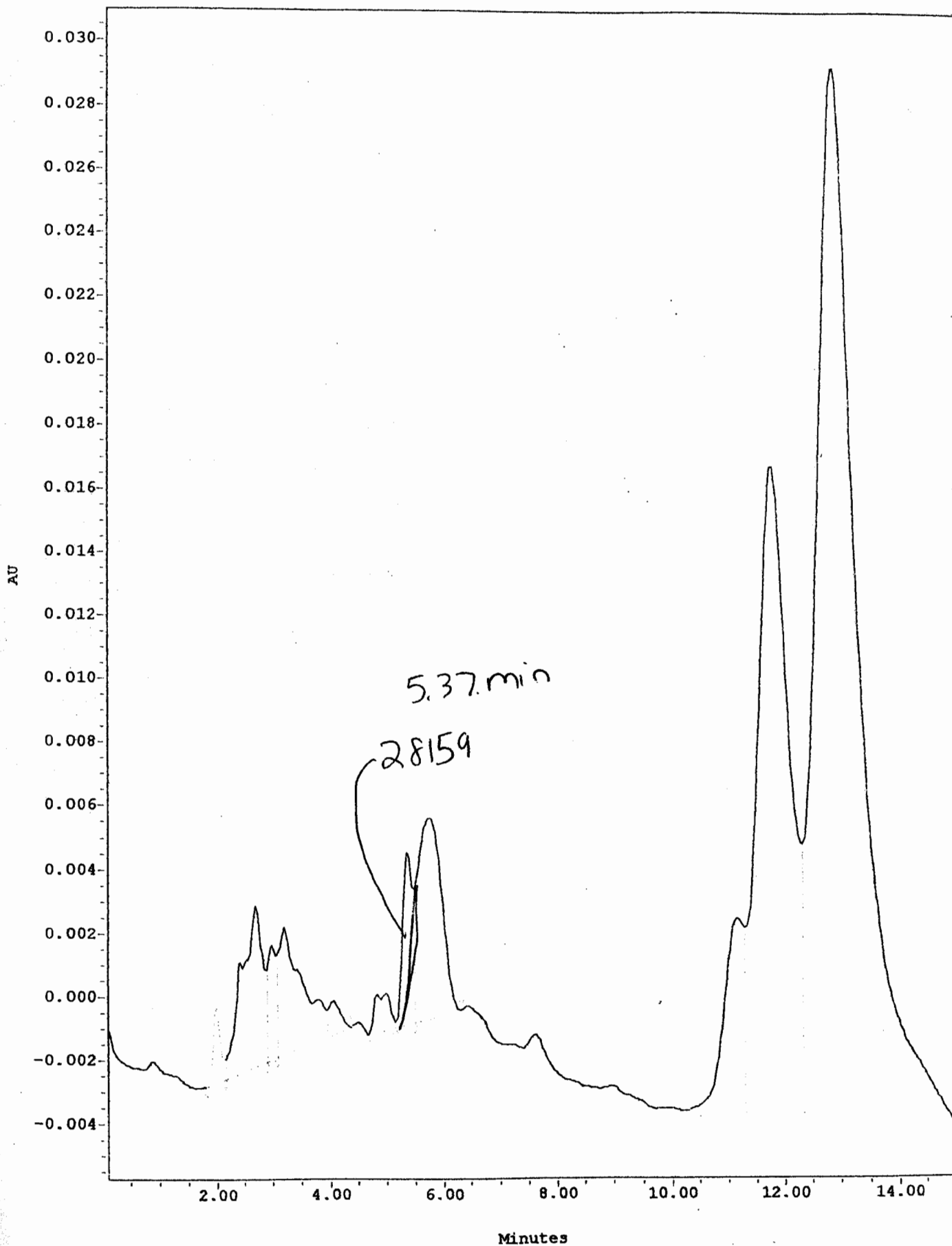
Sample Name: z-m-8
 Sample Origin:
 Solvent: meoh
 Flow Rate: 1.500
 Level:
 Volume: 60.00
 Run Time: 15.0 min



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.078	22353	2683	BV	1.912	2.178	2.47
2		2.478	235329	12691	VV	2.178	2.645	25.96
3		2.745	231977	11690	VV	2.645	3.112	25.59
4		3.145	52948	4547	VV	3.112	3.312	5.84
5		3.345	36833	2954	VB	3.312	3.845	4.06
6		5.378	38562	1281	VB	5.212	6.278	4.25
7		11.112	67552	2507	BV	10.545	11.345	7.45
8		11.745	156501	3826	VV	11.345	12.412	17.27
9		12.778	64340	1669	VB	12.412	13.512	7.10

z-m-17



Z-m-17

Result Table

#	Retention Time (min)	Area (uV*sec)	Height (uV)
1	2.003	21395	2334
2	2.703	132702	5032
3	3.003	39564	3554
4	3.203	111823	3987
5	4.070	14613	1041
6	4.837	22778	1172
7	5.370	66706	5398
8	5.770	184013	6299
9	11.170	142616	6019
10	11.803	808534	20365
11	12.870	1688280	33013

Z-M-31 11/13/84

Millennium Results Report

November 13, 1984

Page: 1 of 1

Report Method: MetCarb_RM

Version: 2.15

For Sample: z-m-31

Vial: 6

Injection: 1

Channel: 991M

roc Chan: PDA_282.0nm

Processed: 11/13/84 01:47:47 AM

Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol

Sample Name: z-m-31

Vial: 6

SampleOrigin:

Sample Type: Unknown

Solvent: MeOH

Injection: 1

FlowRate: 1.500

Channel: 991M

Level:

Date Acquired: 11/13/84 01:32:15 AM

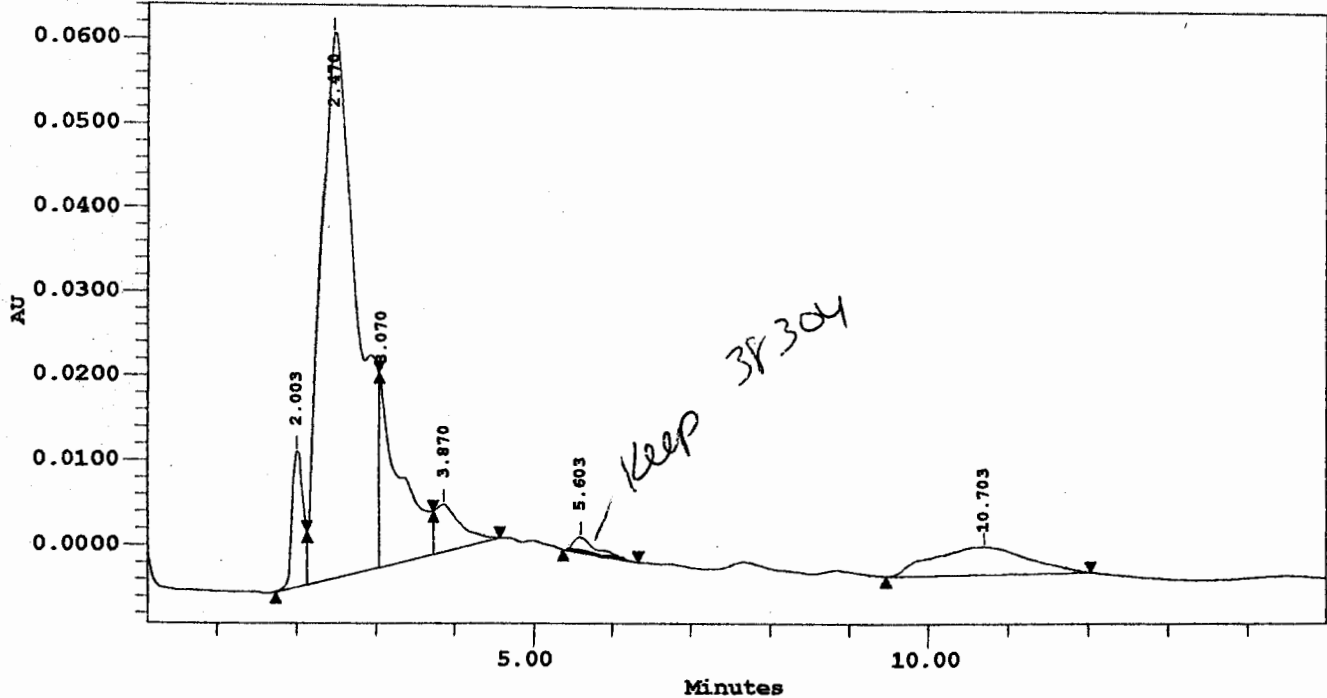
Volume: 60.00

SampleWeight: 1.00000

Run Time: 15.0 min

Acq Meth Set: Ergosterol_MS

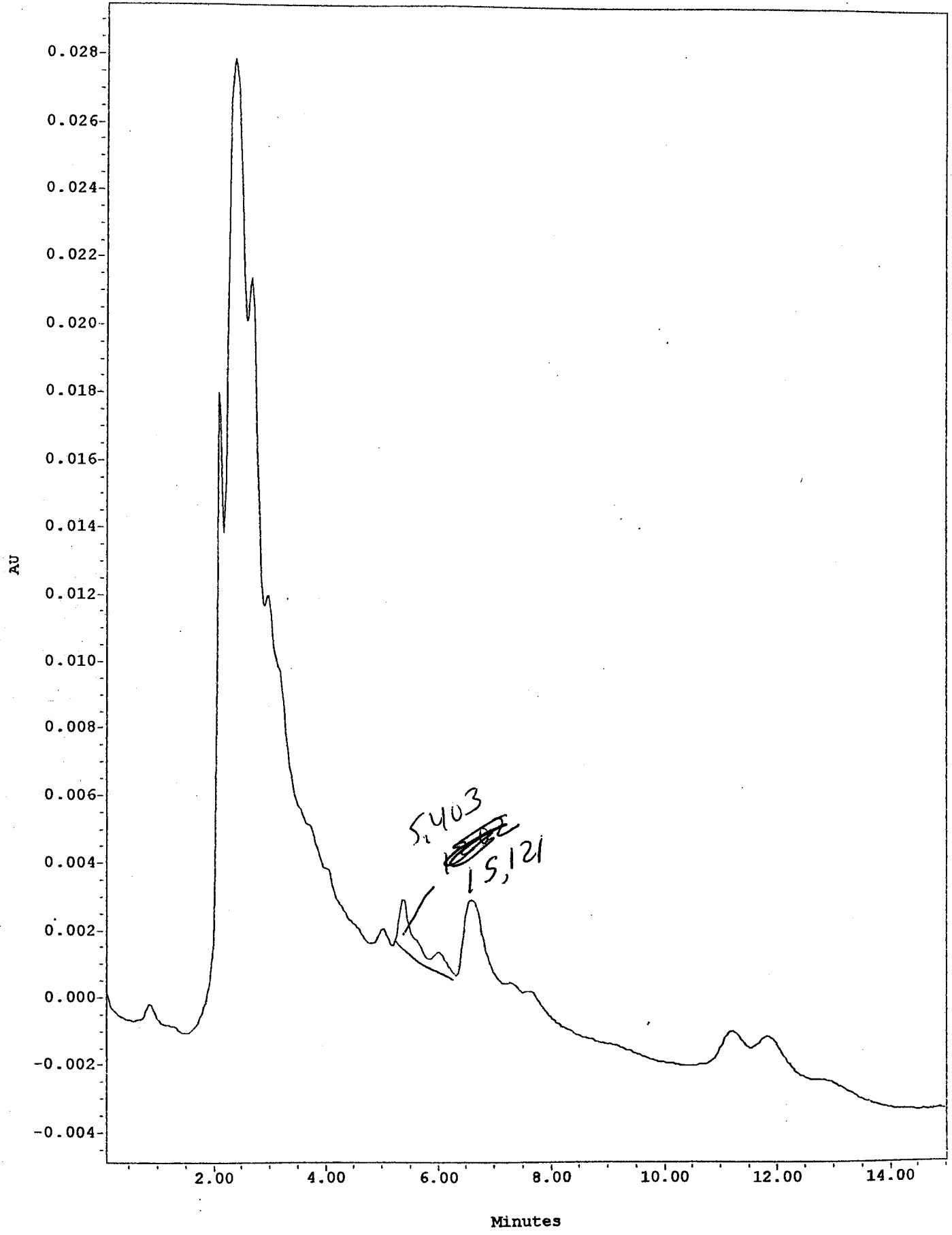
Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.003	156245	16011	BV	1.737	2.137	5.14
2		2.470	2023102	64682	VV	2.137	3.037	66.54
3		3.070	418116	20881	VV	3.037	3.737	13.75
4		3.870	125688	5592	VB	3.737	4.570	4.13
5		5.603	38304	1888	BB	5.370	6.337	1.26
6		10.703	279184	3341	BB	9.470	12.037	9.18

z-m-36(1)



SampleName: z-m-36(1) Vial: 2 Inj: 1 Ch: PDA_282.0nm Type: Unknown

Z-m-366i

Result Table

#	Retention Time (min)	Area (uV*sec)	Height (uV)
	2.103	200775	18770
2	2.403	760453	28498
3	2.803	709614	16768
4	5.037	21766	1333
5	5.370	36565	2013
6	6.603	54248	2332

- 5.403, (15121)^{area}

zm-36(2) 11/15/84

Millennium Results Report
 Report Method: MetCarb_RM
 For Sample: z-m-36(2)
 roc Chan: PDA_282.0nm
 Channel Descr: PDA 282.0 nm

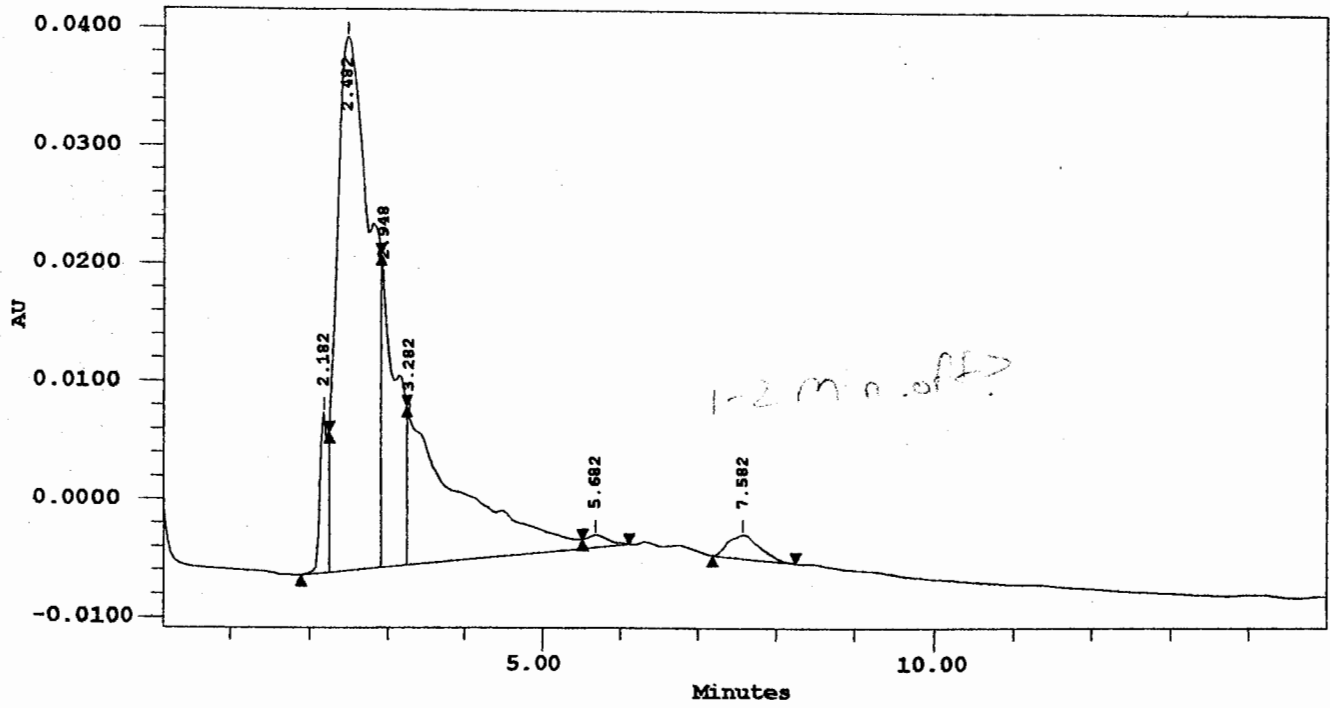
November 15, 1984
 Version: 2.15
 Vial: 2
 Injection: 1
 Processed: 11/15/84 12:41:41 AM

Page: 1 of 1
 Channel: 991M

Lycoming College, Department of Chemistry

Project Name: Ergosterol
 Vial: 2
 Sample Type: Unknown
 Injection: 1
 Channel: 991M
 Date Acquired: 11/15/84 12:26:14 AM
 Sample Weight: 1.00000
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM

Sample Name: z-m-36(2)
 Sample Origin:
 Solvent: meoh
 Flow Rate: 1.500
 Level:
 Volume: 60.00
 Run Time: 15.0 min



Peak Results

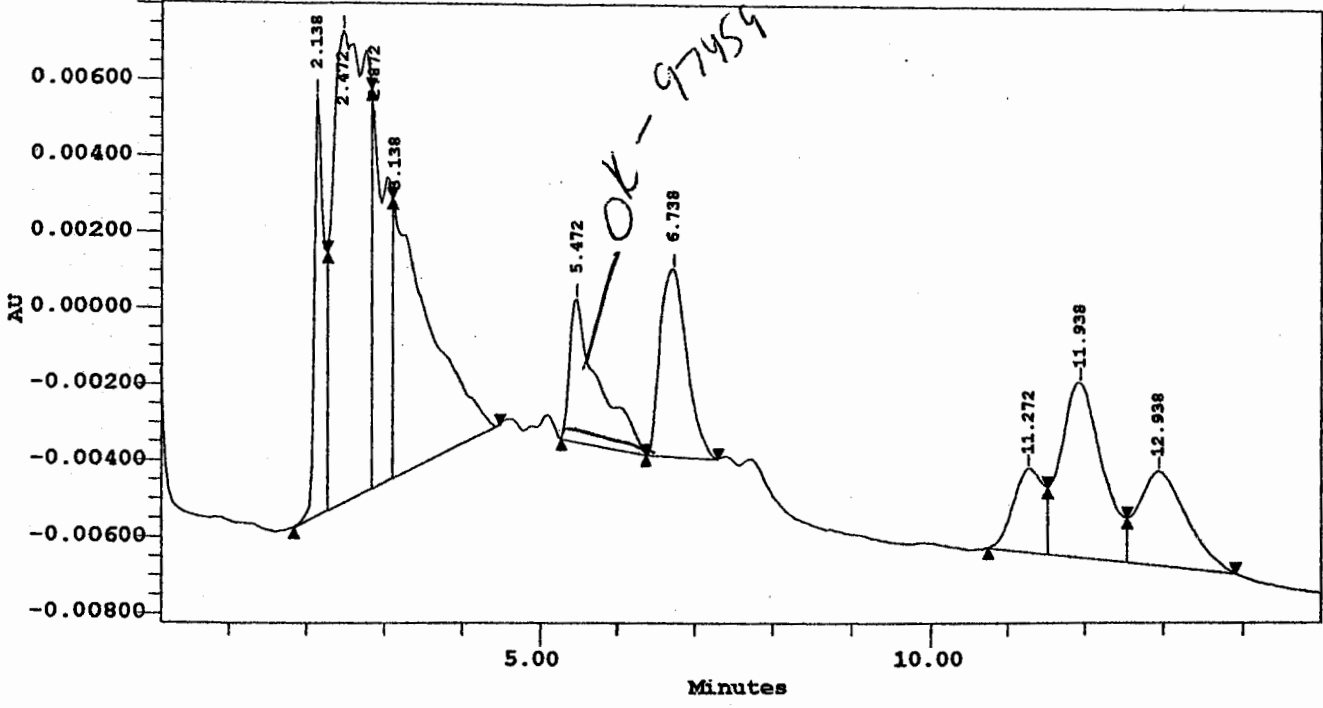
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.182	92305	13598	BV	1.882	2.248	3.70
2		2.482	1334905	45281	VV	2.248	2.915	53.49
3		2.948	355667	23948	VV	2.915	3.248	14.25
4		3.282	633923	12501	VV	3.248	5.515	25.40
5		5.682	21494	1079	VB	5.515	6.115	0.86
6		7.582	57201	2050	BB	7.182	8.248	2.29

2M-45 11/10/84

Millennium Results Report November 10, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: z-m-45 Vial: 4 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 11/10/84 01:13:18 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: z-m-45
Vial: 4	Sample Origin:
Sample Type: Unknown	Solvent: MeOH
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 11/10/84 12:57:50 AM	Volume: 60.00
SampleWeight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

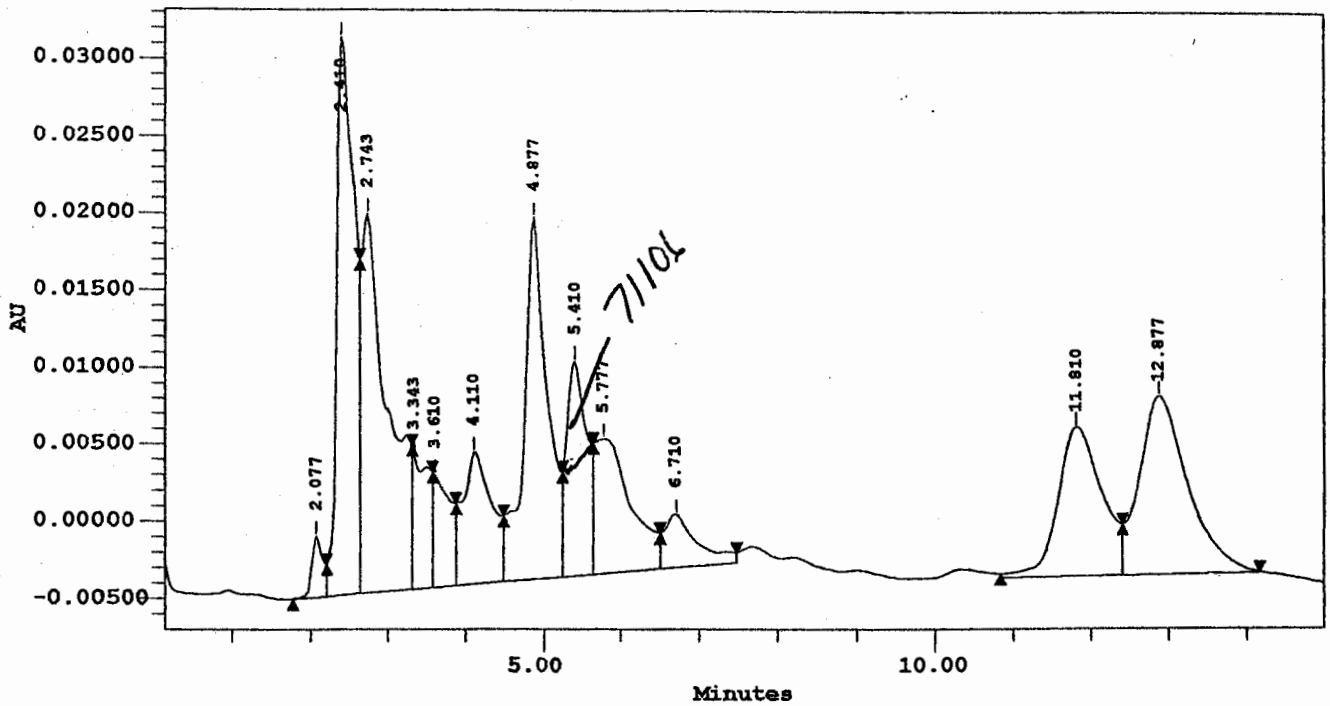
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.138	96447	11071	BV	1.838	2.272	6.93
2		2.472	374055	12397	VV	2.272	2.838	26.87
3		2.872	131067	9524	VV	2.838	3.105	9.42
4		3.138	236063	6873	VB	3.105	4.472	16.96
5		5.472	97459	3768	BB	5.272	6.372	7.00
6		6.738	116817	4975	BB	6.372	7.305	8.39
7		11.272	58217	2212	BV	10.738	11.505	4.18
8		11.938	176653	4627	VV	11.505	12.538	12.69
9		12.938	105125	2497	VB	12.538	13.905	7.55

Z-R-8 10/3/00

Millennium Results Report
Report Method: MetCarb_RM
For Sample: z-r-8
roc Chan: PDA_282.0nm
Channel Descr: PDA 282.0 nm
October 11, 1984
Version: 2.15
Vial: 6
Injection: 1
Processed: 10/11/84 02:20:21 AM
Page: 1 of 2
Channel: 991M

Lycoming College, Department of Chemistry

Project Name: Ergosterol
Vial: 6
Sample Type: Unknown
Injection: 1
Channel: 991M
Date Acquired: 10/11/84 02:04:54 AM
SampleWeight: 1.00000
Acq Meth Set: Ergosterol_MS
Processing Method: Ergosterol_PM
Sample Name: z-r-8
SampleOrigin:
Solvent: meoh
FlowRate: 1.500
Level:
Volume: 60.00
Run Time: 15.0 min



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.077	37560	3965	BV	1.777	2.210	1.02
2		2.410	568275	36154	VV	2.210	2.643	15.46
3		2.743	591080	24626	VV	2.643	3.310	16.08
4		3.343	126758	8606	VV	3.310	3.577	3.45
5		3.610	112091	7289	VV	3.577	3.877	3.05
6		4.110	218743	8529	VV	3.877	4.477	5.95
7		4.877	502292	23504	VV	4.477	5.243	13.66
8		5.410	252703	13961	VV	5.243	5.643	6.87
9		5.777	288097	8711	VV	5.643	6.510	7.84
10		6.710	99502	3428	VV	6.510	7.477	2.71
11		11.810	378859	9739	VV	10.843	12.410	10.31

Millennium Results Report	October 11, 1984	Page: 2 of 2
Report Method: MetCarb_RM	Version: 2.15	
For Sample: z-r-8	Vial: 6	Injection: 1
roc Chan: PDA_282.0nm	Processed: 10/11/84 02:20:21 AM	Channel: 991M
Channel Descr: PDA 282.0 nm		

Peak Results

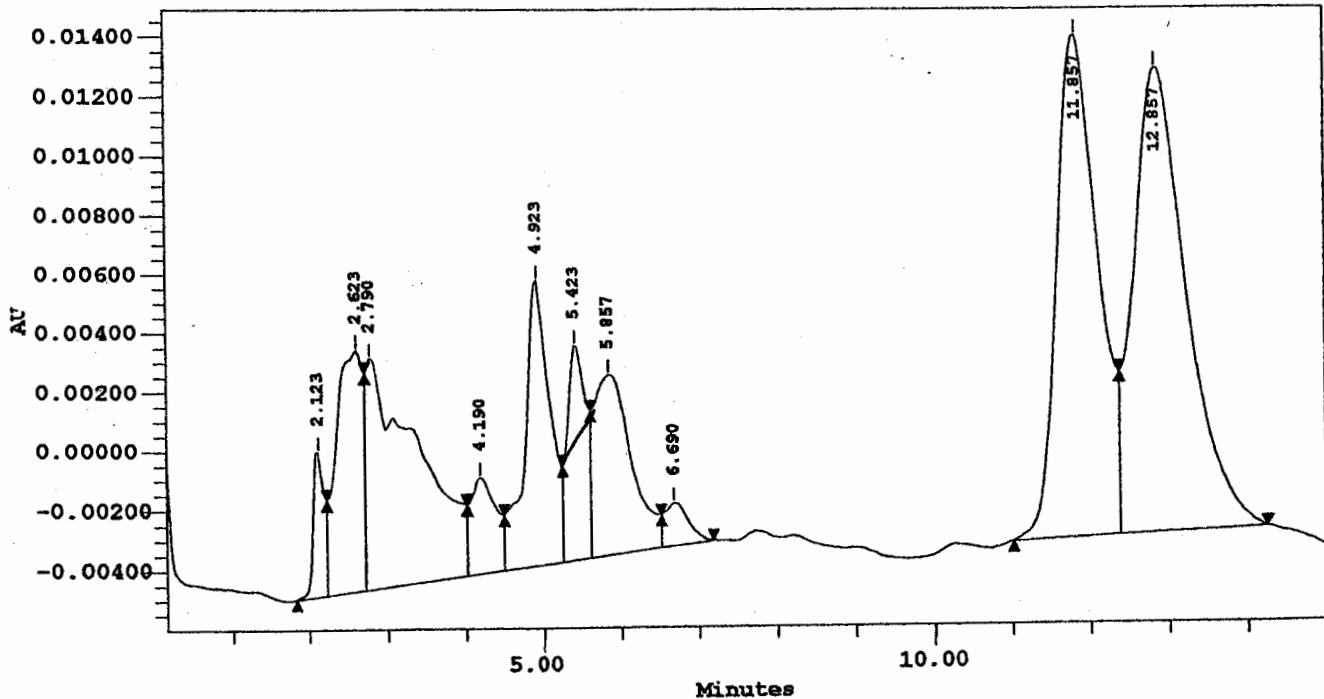
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
12		12.877	499990	11647	VB	12.410	14.177	13.60

Z-R-17 11/8/00

Millennium Results Report November 8, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: replayZ-R-17 Vial: 7 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 11/08/84 04:32:21 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: replayZ-R-17
Vial: 7	Sample Origin:
Sample Type: Unknown	Solvent: meoh
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 11/08/84 04:16:54 AM	Volume: 60.00
Sample Weight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

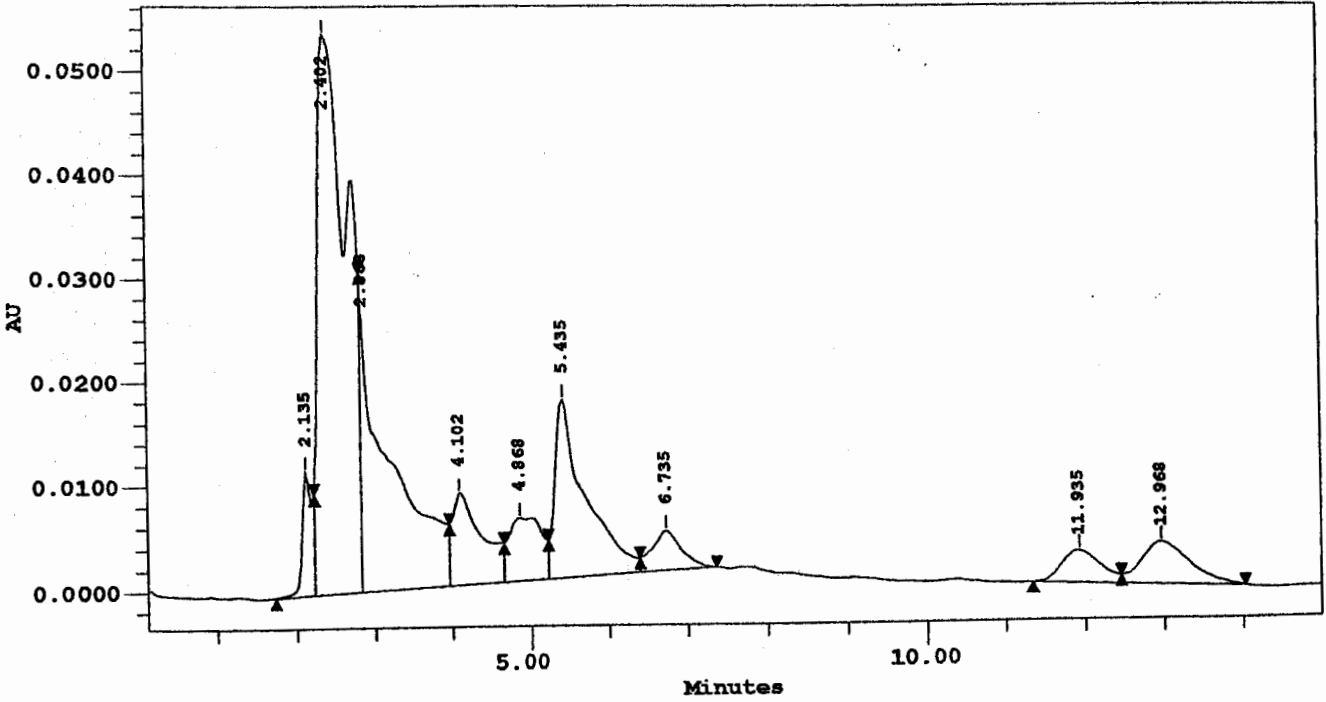
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.123	46234	4843	BV	1.823	2.223	1.78
2		2.623	192856	8096	VV	2.223	2.723	7.42
3		2.790	359296	7750	VV	2.723	4.023	13.82
4		4.190	71489	3208	VV	4.023	4.490	2.75
5		4.923	223351	9629	VV	4.490	5.257	8.59
6		5.423	123163	7223	VV	5.257	5.623	4.74
7		5.857	201832	6075	VV	5.623	6.523	7.76
8		6.690	30132	1438	VB	6.523	7.190	1.16
9		11.857	623927	17068	BV	11.023	12.390	24.00
10		12.857	727073	15811	VB	12.390	14.257	27.97

Z-R-31 11/10/84

Millennium Results Report	November 10, 1984	Page: 1 of 1
Report Method: MetCarb_RM	Version: 2.15	
For Sample: z-r-31	Vial: 6	Injection: 1 Channel: 991M
Proc Chan: PDA_282.0nm	Processed: 11/10/84 01:48:37 AM	
Channel Descr: PDA 282.0 nm		

Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: z-r-31
Vial: 6	Sample Origin:
Sample Type: Unknown	Solvent: MeOH
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 11/10/84 01:33:09 AM	Volume: 60.00
SampleWeight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

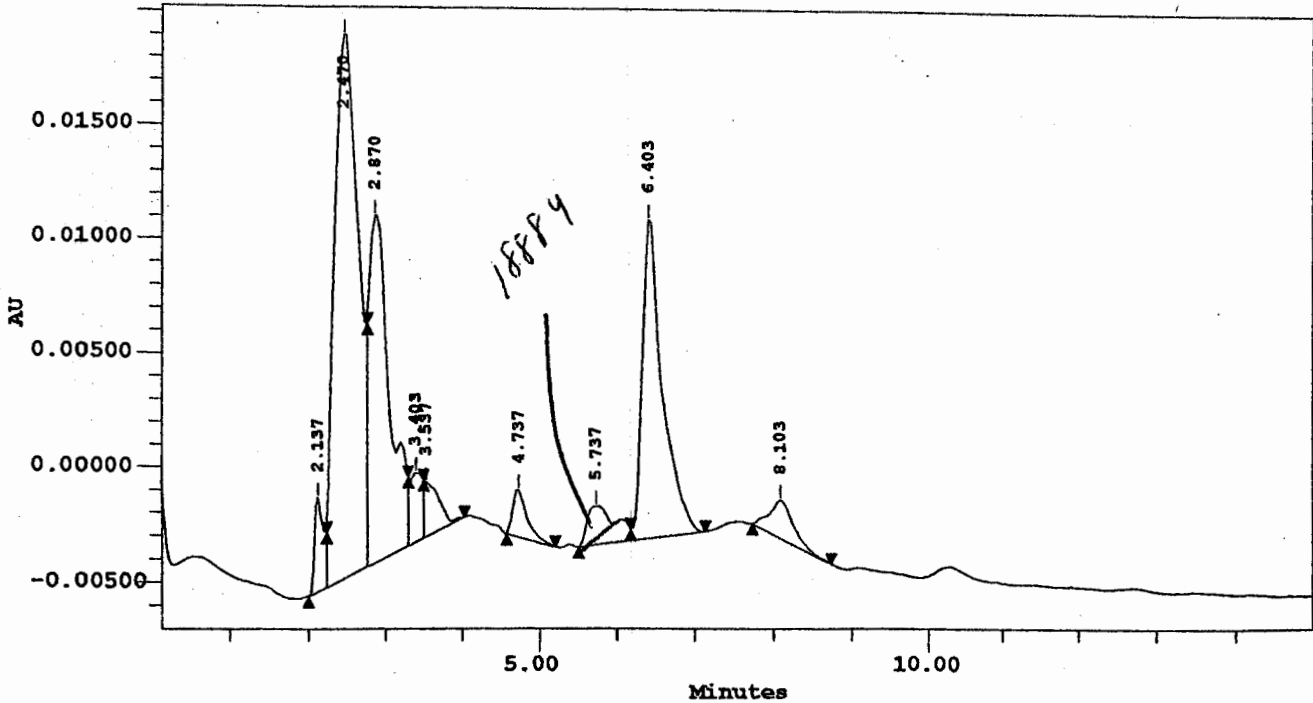
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.135	100937	11876	BV	1.735	2.235	2.88
2		2.402	1414856	53811	VV	2.235	2.835	40.38
3		2.868	739868	24867	VV	2.835	3.968	21.12
4		4.102	234867	8757	VV	3.968	4.668	6.70
5		4.868	174193	5998	VV	4.668	5.235	4.97
6		5.435	463211	17134	VV	5.235	6.402	13.22
7		6.735	99131	3769	VB	6.402	7.368	2.83
8		11.935	106878	3130	BV	11.335	12.468	3.05
9		12.968	169927	4071	VB	12.468	14.035	4.85

Z-R-36(1) 11/14/84

Millennium Results Report
Report Method: MetCarb_RM
For Sample: Z-R-36(1)
roc Chan: PDA_282.0nm
Channel Descr: PDA 282.0 nm
November 14, 1984
Version: 2.15
Vial: 6
Injection: 1
Processed: 11/14/84 12:13:53 AM
Page: 1 of 1
Channel: 991M

Lycoming College, Department of Chemistry

Project Name: Ergosterol
Vial: 6
Sample Type: Unknown
Injection: 1
Channel: 991M
Date Acquired: 11/13/84 11:58:26 PM
SampleWeight: 1.00000
Acq Meth Set: Ergosterol_MS
Processing Method: Ergosterol_PM
Sample Name: Z-R-36(1)
SampleOrigin:
Solvent: meoh
FlowRate: 1.500
Level:
Volume: 60.00
Run Time: 15.0 min



Peak Results

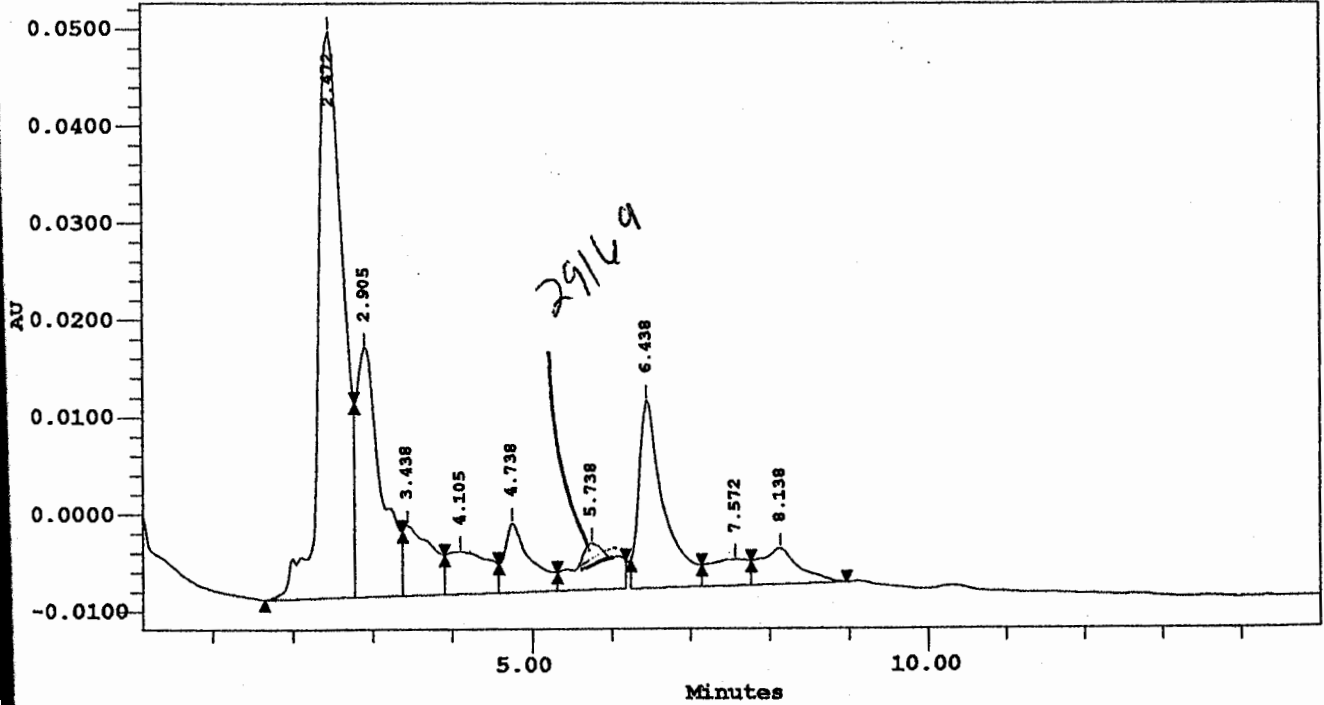
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.137	31198	4047	BV	2.003	2.237	2.50
2		2.470	513396	23794	VV	2.237	2.770	41.11
3		2.870	279857	15177	VV	2.770	3.303	22.41
4		3.403	34110	3021	VV	3.303	3.503	2.73
5		3.537	31587	2388	VB	3.503	4.037	2.53
6		4.737	26249	2086	BB	4.570	5.203	2.10
7		5.737	41253	1665	BV	5.503	6.170	3.30
8		6.403	255380	14021	VB	6.170	7.137	20.45
9		8.103	35902	1692	BB	7.737	8.737	2.87

Z-R-36(2) 11/14/84

Millennium Results Report November 14, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: Z-R-36(2) Vial: 7 Injection: 1 Channel: 991M
 roc Chan: PDA_282.0nm Processed: 11/14/84 12:30:50 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol Sample Name: Z-R-36(2)
 Vial: 7 Sample Origin:
 Sample Type: Unknown Solvent: meoh
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 11/14/84 12:15:28 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

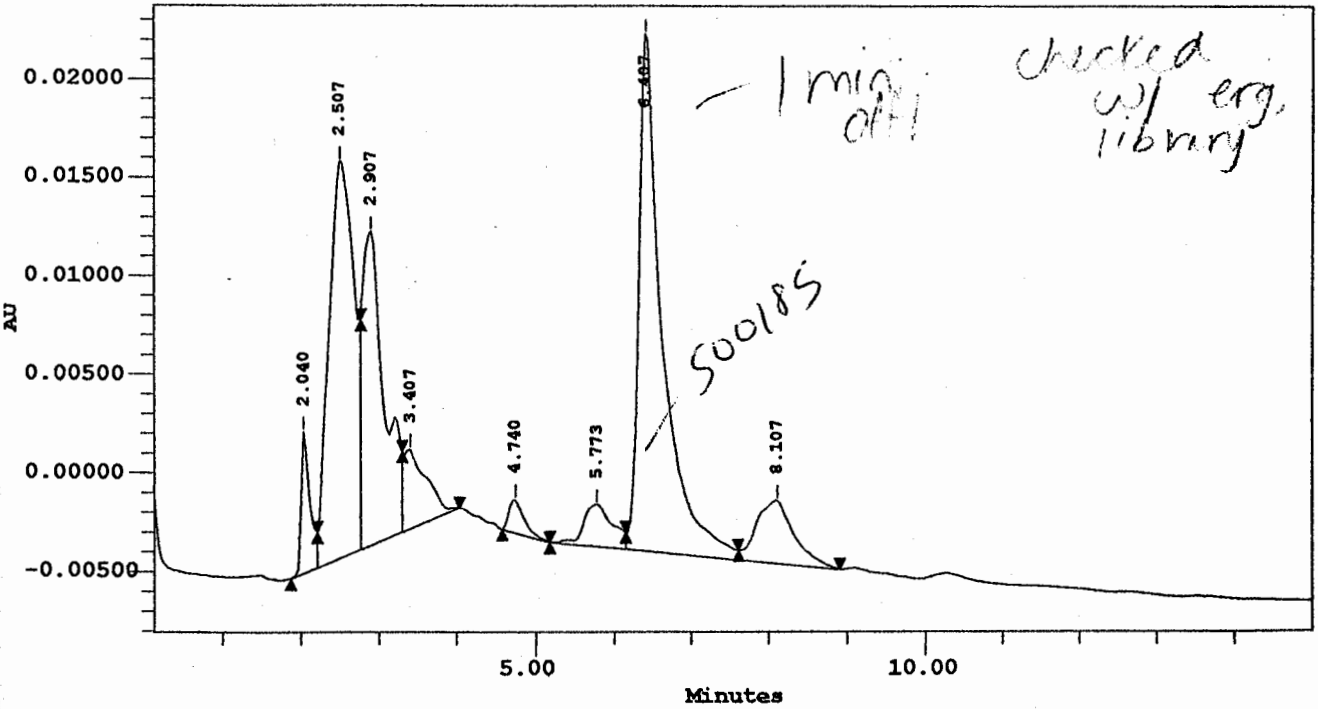
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.472	1243609	58268	BV	1.638	2.772	40.11
2		2.905	547950	25603	VV	2.772	3.372	17.67
3		3.438	180881	7172	VV	3.372	3.905	5.83
4		4.105	155253	4330	VV	3.905	4.572	5.01
5		4.738	165153	7057	VV	4.572	5.305	5.33
6		5.738	166928	4750	VV	5.305	6.172	5.38
7		6.438	422485	19310	VV	6.238	7.138	13.62
8		7.572	93964	2645	VV	7.138	7.772	3.03
9		8.138	124596	3672	VB	7.772	8.972	4.02

ZR-41(1) 11/14/84

Millennium Results Report	November 13, 1984	Page: 1 of 1
Report Method: MetCarb_RM	Version: 2.15	
For Sample: Z-R-41(1)	Vial: 5	Injection: 1
Channel: PDA_282.0nm		Channel: 991M
Channel Descr: PDA 282.0 nm		Processed: 11/13/84 11:57:13 PM

Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: Z-R-41(1)
Vial: 5	Sample Origin:
Sample Type: Unknown	Solvent: meoh
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 11/13/84 11:41:44 PM	Volume: 60.00
Sample Weight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.040	52246	7178	BV	1.873	2.207	3.19
2		2.507	453962	20115	VV	2.207	2.773	27.69
3		2.907	308444	15908	VV	2.773	3.307	18.81
4		3.407	82297	4025	VB	3.307	4.040	5.02
5		4.740	21966	1679	BB	4.573	5.173	1.34
6		5.773	54180	2125	BV	5.173	6.140	3.30
7		6.407	561376	26279	VV	6.140	7.607	34.24
8		8.107	105020	3196	VB	7.607	8.907	6.41

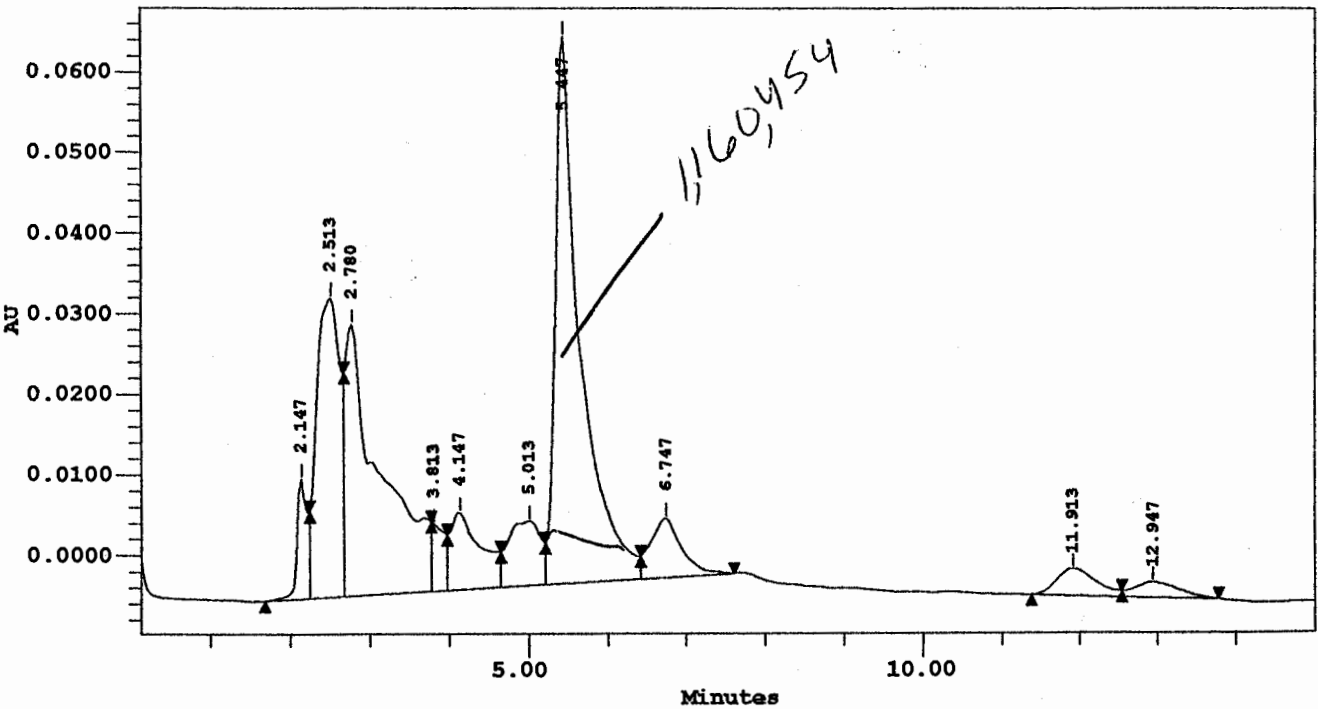
z-r-41(2)

11/10/84

Millennium Results Report November 10, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: z-r2-41 Vial: 5 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 11/10/84 01:31:00 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol Sample Name: z-r2-41
 Vial: 5 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 Flow Rate: 1.500
 Channel: 991M Level:
 Date Acquired: 11/10/84 01:15:30 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

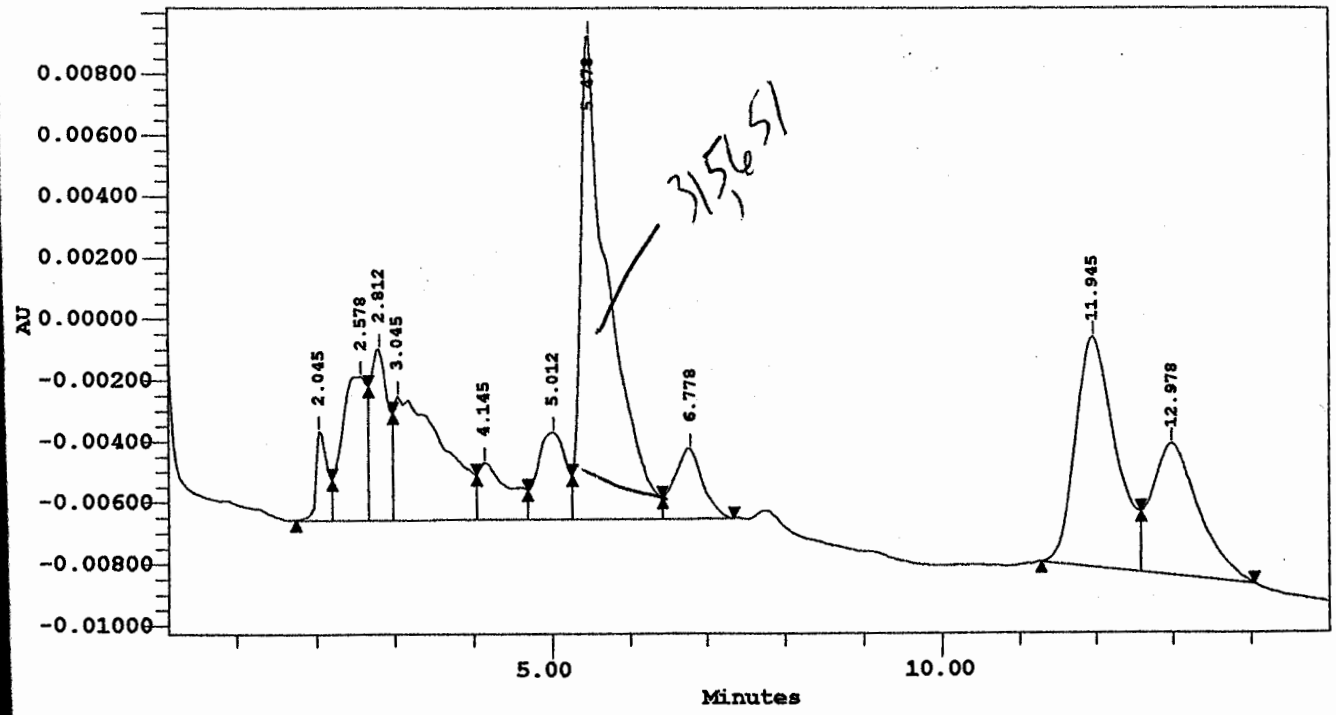
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.147	130668	14867	BV	1.680	2.247	2.97
2		2.513	758228	37122	VV	2.247	2.680	17.23
3		2.780	1054946	33626	VV	2.680	3.780	23.98
4		3.813	92268	8283	VV	3.780	3.980	2.10
5		4.147	255981	9521	VV	3.980	4.647	5.82
6		5.013	227730	7983	VV	4.647	5.213	5.18
7		5.447	1485242	67872	VV	5.213	6.413	33.76
8		6.747	203810	7304	VB	6.413	7.613	4.63
9		11.913	116916	3319	BV	11.380	12.547	2.66
10		12.947	73602	1883	VB	12.547	13.780	1.67

Z-R-45 11/10/84

Millennium Results Report November 10, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: z-r-45 Vial: 3 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 11/10/84 12:55:32 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol Sample Name: z-r-45
 Vial: 3 Sample Origin:
 Sample Type: Unknown Solvent: MeOH
 Injection: 1 Flow Rate: 1.500
 Channel: 991M Level:
 Date Acquired: 11/10/84 12:40:02 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.045	29092	2905	BV	1.745	2.212	2.03
2		2.578	101724	4671	VV	2.212	2.678	7.10
3		2.812	83760	5579	VV	2.678	2.978	5.85
4		3.045	180072	4028	VV	2.978	4.045	12.57
5		4.145	49546	1862	VV	4.045	4.678	3.46
6		5.012	70621	2830	VV	4.678	5.245	4.93
7		5.478	401955	15774	VV	5.245	6.412	28.06
8		6.778	58844	2270	VB	6.412	7.345	4.11
9		11.945	275026	7530	BV	11.278	12.578	19.20
10		12.978	181716	4299	VB	12.578	14.045	12.69

Summer \approx 2mo. \approx 60 days

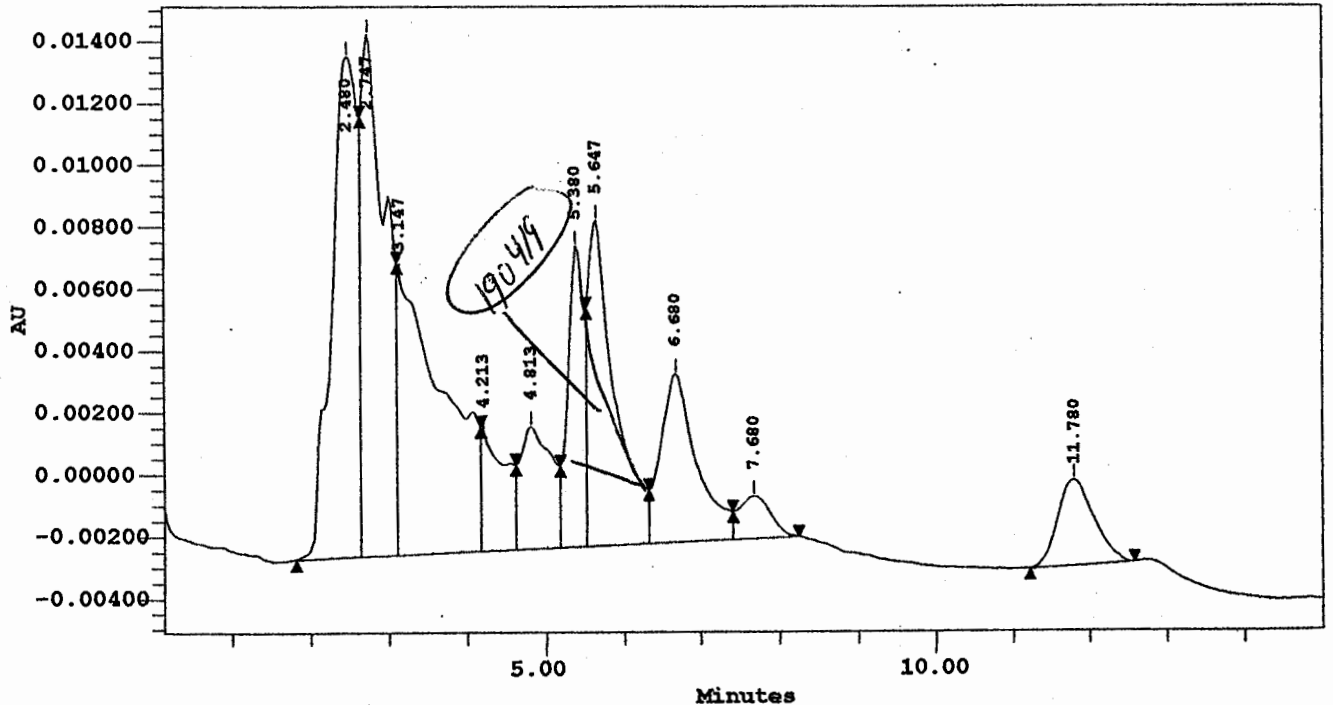
10/3/00

Z-R

Millennium Results Report	October 11, 1984	Page: 1 of 1
Report Method: MetCarb_RM	Version: 2.15	
For Sample: z-r-1	Vial: 4	Injection: 1 Channel: 991M
Proc Chan: PDA_282.0nm	Processed: 10/11/84 01:44:21 AM	
Channel Descr: PDA 282.0 nm		

Lycoming College, Department of Chemistry

Project Name: Ergosterol	Sample Name: z-r-1
Vial: 4	Sample Origin:
Sample Type: Unknown	Solvent: meoh
Injection: 1	FlowRate: 1.500
Channel: 991M	Level:
Date Acquired: 10/11/84 01:28:53 AM	Volume: 60.00
Sample Weight: 1.00000	Run Time: 15.0 min
Acq Meth Set: Ergosterol_MS	
Processing Method: Ergosterol_PM	



Peak Results

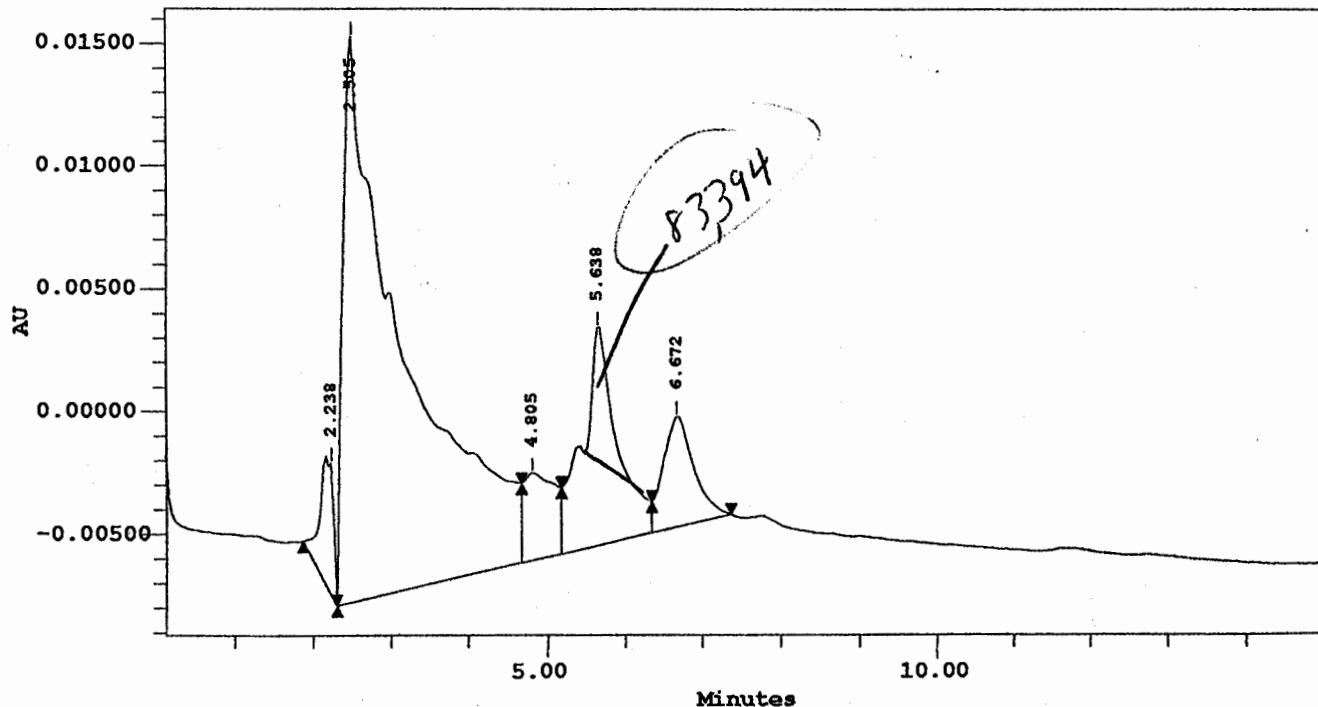
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.480	370767	16151	BV	1.813	2.647	18.50
2		2.747	365016	16832	VV	2.647	3.113	18.21
3		3.147	381496	8925	VV	3.113	4.180	19.04
4		4.213	79446	3764	VV	4.180	4.613	3.96
5		4.813	110147	3938	VV	4.613	5.180	5.50
6		5.380	136105	9699	VV	5.180	5.513	6.79
7		5.647	250890	10505	VV	5.513	6.313	12.52
8		6.680	180662	5424	VV	6.313	7.413	9.01
9		7.680	37791	1372	VB	7.413	8.247	1.89
10		11.780	91829	2778	BB	11.213	12.580	4.58

10/3/00 Z-2

Millennium Results Report October 11, 1984 Page: 1 of 1
 Report Method: MetCarb_RM Version: 2.15
 For Sample: z-r-2 Vial: 5 Injection: 1 Channel: 991M
 Proc Chan: PDA_282.0nm Processed: 10/11/84 02:01:40 AM
 Channel Descr: PDA 282.0 nm

Lycoming College, Department of Chemistry

Project Name: Ergosterol Sample Name: z-r-2
 Vial: 5 Sample Origin:
 Sample Type: Unknown Solvent: meoh
 Injection: 1 FlowRate: 1.500
 Channel: 991M Level:
 Date Acquired: 10/11/84 01:46:12 AM Volume: 60.00
 Sample Weight: 1.00000 Run Time: 15.0 min
 Acq Meth Set: Ergosterol_MS
 Processing Method: Ergosterol_PM



Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Int Type	Start Time (min)	End Time (min)	% Area
1		2.238	63653	5399	BB	1.872	2.305	3.51
2		2.505	1247905	23014	BV	2.305	4.672	68.82
3		4.805	95743	3560	VV	4.672	5.172	5.28
4		5.638	279396	8902	VV	5.172	6.338	15.41
5		6.672	126708	4506	VB	6.338	7.372	6.99

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