THE DEVELOPMENT OF A METHOD

FOR THE

ANALYSIS OF MAHEWU

BY

Richard G de Goede

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Supervisors: Dr J.H. Adamson (Technikon Natal)

Dr D.N. Neethling (Technikon Natal)

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AUTHOR: Richard Goodwin de Goede

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I hereby declare that this dissertation represents my own work both in conception and execution.

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R.G. de Goede, DURBAN, 1994-01-30

Approved for final submission

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Dr J.H. Adamson, Supervisor

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ABSTRACT

A comparison was made of various methods for the analysis of the odoriferous components of Mahewu, a fermented mealie meal porridge. The most satisfactory procedure was found to be that of dynamic headspace sampling. This technique, used in conjunction with gas chromatography and mass spectrometry, allowed the positive identification of several components.

SUMMARY

Mahewu (also known as amahewu and magou) is a non-alcoholic beverage drunk by African people. It is made by fermenting a runny mealie meal gruel(porridge). The resulting ferment is sour to the taste because a product of the fermentation is lactic acid.

A comparison was made of various methods for the analysis of the odoriferous components of Mahewu. The most satisfactory procedure was found to be that of dynamic headspace sampling. This technique, used in conjunction with gas chromatography and mass spectrometry, allowed the positive identification of several components.

The dynamic headspace sampling was performed to concentrate the odoriferous components on a Tenax column, using a Tekmar Headspace Concentrator for small samples. Samples were also taken from the manufacturer's tanks, but the water vapour in this headspace made this procedure unsatisfactory. This sample was then transferred as a plug to a GC column where it was separated in the GC. The dynamic headspace concentration process had to be optimised in order to produce a satisfactory response in the detector. The GC conditions had to be optimised to produce satisfactory separation of the components, hence resolved peaks on the chromatograms. A mass spectrometer was used as the detector. The mass spectra data were stored on a computer data disc and the spectral data for each peak compared with the mass spectra of compounds in the library in the computer. This library search yield three possibilities for each reasonable peak. These were studied, together with properties of the

OPSOMMING

Mahewu (ook bekend as amahewu en magou) is 'n nie-alkoholiese drank wat deur swartmense gedrink word. Dit word gemaak deur 'n loperige mieliemeelpap te laat fermenteer. Die gevolglike ferment smaak suur want een van die produkte van fermentasie is melksuur.

'n Vergelyking van verskillende metodes vir die ontleding van die reukgewende komponente van Mahewu is gemaak. Monsterneming van die dinamiese kopruimte is as die mees bevredigende prosedure bevind. Tesame met gaschromatografie (GC) en massaspektrometrie (MS) maak hierdie tegniek die positiewe identifisering van verskeie komponente moontlik.

Monsters van die dinamiese kopruimte is geneem om die reukgewende komponente met behulp van 'n Tekmar-kopruimtekonsentrator vir klein monsters op 'n Tenax-kolom te konsentreer. Monsters is ook in die vervaardiger se tenks geneem, maar as gevolg van die waterdamp in die kopruimte was hierdie prosedure onbevredigend. Die monster van die Tenax-kolom is toe in gekonsentreerde vorm na 'n GC-kolom oorgedra waar dit in die GC afgeskei is. Die konsentrasieproses van die dinamiese kopruimte moes geoptimaliseer word om 'n bevredigende reaksie in die detektor te verkry. Die gaschromatografiese toestande moes ook geoptimaliseer word om 'n bevredigende skeiding van die komponente te verkry, vandaar die geskeide pieke op die chromatogramme. 'n Massaspektrometer is as die detektor gebruik. Die massaspektradata is op 'n rekenaardataskyf vasgelê en die spektrale data vir elke piek is met die massaspektra van verbindings in die rekenaar se biblioteek vergelyk. Hierdie proses het drie moontlikhede vir elke redelike piek opgelewer.

substances. In this way the number of possible substances was decreased. Because of their availability, commercial samples of six of these were obtained and used to positively identify three of the components. The positive identification could not be performed using the MS because it ceased to function so a flame ionisation detector was used instead, the components being positively identified by spiking a mahewu sample with the standards in turn. The components that were positively identified were ethyl acetate, ethyl propionate and propyl benzene.

An attempt was made to quantify these components in a mahewu sample but dynamic headspace sampling does not lend itself to consistent quantitation.

Several solvent extraction techniques were investigated to check whether the odoriferous components in the headspace could be isolated from the gruel and thus concentrated in the solvent. The solvents tried, which ranged in polarity, included dichloromethane, methyl ethyl ketone, hexane, ether and sunflower oil that is used for cooking. Portions of these extracts were admitted to the GC/MS for separation and identification, but none of the solvents nor methods proved satisfactory.

Die moontlike stowwe, tesame met hul eienskappe, is bestudeer. Die getal moontlike stowwe is sodoende verminder. Kommersiële monsters van ses van hierdie stowwe is vanweë hul bekombaarheid gekies en gebruik om drie van die komponente positief te identifiseer. Die massaspektograaf het onklaar geraak en kon dus nie gebruik word om die positiewe identifikasie te doen nie. 'n Vlamionisasiedetektor is in plaas daarvan gebruik en die komponente is positief geïdentifiseer deur die standaarde om die beurt by 'n mahewu-monster te voeg. Die positief geïdentifiseerde komponente was etielasetaat, etielpropionaat en propielbensien.

Daar is gepoog om hierdie komponente in 'n mahewu-monster te kwantifiseer, maar monsterneming van dinamiese kopruimte leen hom nie tot konsekwente kwantifisering nie.

Verskeie extraksietegnieke met oplosmiddels is ondersoek om vas te stel of the reukgewende komponente in die kopruimte van die dun pap afgeskei kon word en sodoende in die oplosmiddel gekonsentreer kon word. Oplossers van wisselende polariteit is probeer, insluitende dichlorometaan, metieletielketoon, heksaan, eter en sonneblomolie (vir kookdoeleindes). Gedeeltes van hierdie ekstrakte is in die GC/MS vir afskeiding en identifisering ingevoer, maar geeneen van die oplossers of metodes het bevredigend geblyk te wees nie.

ACKNOWLEDGEMENTS

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I thank those members of staff of the Department of Chemical Sciences who helped in many ways.

I would like to thank the staff of Clover Dairies who supplied samples and allowed me to sample their tanks.

I am grateful to Mr B Parel of the Department of Chemistry at the University of Natal for his help in printing many of the chromatograms.

Finally, I acknowledge the financial support of the FRD and of Technikon Natal, whose support enabled this work to be performed.

ABBREVIATIONS

GC	Gas Chromatograph(y)
GC/MS	Gas Chromatograph(y)/Mass Spectrometer(y)
Tekmar	Tekmar Headspace Concentrator
FID	Flame ionization detector
°C	degrees Celsius
mm	millimetres
cm	centimetres
cm^3	centimetres cubed
dm^3	decimetres cubed
al	alcohol (ethanol)
eth	diethylether
ac	2-propanone (acetone)
bz	benzene
chl	trichloromethane (chloroform)
DCM	dichloromethane (or methylene chloride)
MEK	2-butanone (or methyl ethyl ketone)
ether	diethyl ether
rpm	revolutions per minute
µl	microlitre

CHAPTER 1

INTRODUCTION

1.1 MAHEWU

Mahewu (also known as amahewu and magou) is a non-alcoholic beverage drunk by African people. It is prepared by firstly making a fairly runny mealie meal porridge, then adding some bacteria-containing substance which ferments the mixture producing lactic acid. The lactic acid imparts a sour taste to the porridge. The traditional lactobacillus-containing additive is wheat flour. Mahewu is also produced on a large scale in industry, the lactobacillus having been developed in order to reduce the souring time from about 36 hours using the traditional method to a matter of 3 hours. This also has the effect of ensuring that quality is more uniform from batch to batch as well as eliminating undesirable products such as ethanoic acid and butanoic acid. [1].

1.2 REASON FOR THE INVESTIGATION

The project originated when one of the manufacturers of mahewu, Clover Dairies, encountered a problem on their plant in Congella. Every now and again they would make a batch which the human tester at the end of the line would reject. Testing was entirely subjective using the nose. Seeing that each batch was 2000 litres, such rejection was costing the manufacturer a large

amount of money. It was not known what caused the off-odour which led to rejection of the batch. It was decided to attempt to isolate and identify the offending gaseous substance so that the microbiologist could attempt to decide what was producing this substance. A strategy for dealing with the contamination could then be developed. However, before the basic profiles of uncontaminated mahewu could be ascertained, the manufacturer moved to a new site where they had installed a new mahewu plant in Queensburgh. The off-odour was no longer a problem so the need to identify the offending substance disappeared.

The main thrust of the project changed to analyzing the headspace of mahewu, without trying to identify off-odour components. If basic profiles of the headspace could be established under given analysis conditions then, if an off odour occurred in a batch, this would facilitate the identification of the peaks due to these undesired components.

1.3 SUMMARY OF WORK ALREADY DONE IN THE FIELD OF ANALYSIS OF THE OLFACTORY COMPONENTS OF MAHEWU

In the field of the analysis of the olfactory components of mahewu there is no work documented in the literature covered by Chemical Abstracts. However, the manufacturers that had the problem did supply a report of a headspace analysis done by PA Torline of the National Food Research Institute, CSIR[2]. Details of the procedure were not supplied, only a list of possible

components as determined by GC/MS analysis.

Because lactic acid, CH_3 - CH(OH) - COOH, is a product of the fermentation it was expected that propanoate esters were likely to be present in mahewu. The lower molecular mass esters are sweet-smelling and volatile.

In dealing with the olfactory components one of the main problems is that some substances produce a very strong response in the olfactory receptors even in extremely low concentrations, e.g. hydrogen sulphide, whereas other substances produce an extremely small or no response even in fairly high concentrations. Therefore in the headspace of mahewu there are likely to be components in large concentrations that do not contribute to its smell. Also there are likely to be components in extremely small concentrations that contribute greatly to its smell[3].

A great deal of work has been performed on the analysis of headspaces above many similar beverages. "Several methods have been used for concentration. Cryogenic techniques (e.g. cold traps, freezing of sample in empty capillaries, direct introduction in cooled open tubular columns), adsorption on solid adsorbents (charcoal, silicagel, alumina, porous polymers) and conventionally coated GC supports have been applied."[4]

Because of the availability of a headspace concentrator and a GC/MS it was decided to follow this path in the analysis of the headspace.

CHAPTER 2

HEADSPACE ANALYSIS

2.1 INTRODUCTION

Some preliminary investigations were required to establish flow rates in the sampler and in the GC capillary column.

Because of the low concentrations of the odoriferous components of the headspace a technique for concentrating them was necessary. Dynamic headspace sampling is the continuous removal of the headspace vapour above a liquid by means of an inert gas flow with subsequent trapping of the sample components by adsorption or cold trapping[5]. Trapping was done by adsorption by a Tenax column. The GC/MS was connected to the dynamic headspace sampler in a way such that the carrier gas supply line went through the sampling instrument, but bypassing the sample and adsorbent, then lead through a heated line to the injection port of the GC. When the sample on the adsorbent is desorbed a valve enables the carrier gas to be diverted over the flash-heated adsorbent, thence to the GC.

2.2 EQUIPMENT

Gas Chromatograph: Varian 6000

Headspace Concentrator: Tekmar Model 4000 Dynamic Headspace Concentrator

Tekmar Model 4100 Heated Sampler Module

GC/MS: Finnigan 1020

When the Tekmar is connected to the Finnigan GC/MS the set of equipment is known by the Environmental Protection Agency as the 'Organics in Water Analyzer'.

2.3 INSTRUMENT CONDITIONS

Tekmar Headspace Concentrator:

Purge time	10 min.(unless otherwise specified)
Purge ready temperature	30 °C
Desorbtion temperature	150 °C
Purge flow rate	40 cm3/min.(unless otherwise specified)
Desorbtion time	0.2 min.
Baking temperature	225 °C
Baking time	10 min. (unless otherwise specified)

GC:

Carrier gas, nitrogen	4 cm^3/min.
Make-up gas, nitrogen	30 cm^3/min.
Detector	FID
Air flow rate	300 cm^3/min.
Hydrogen flow rate	30 cm^3/min.
Injector temperature	120 °C
Detector temperature	250 °C

GC/MS:

Zone temperature	280 °C
Separator oven temperature	240 °C
Manifold temperature	80 °C
Current injection mode	Capillary
Splitless time	0
Turn filament off at	0
Current filament mode	A
Scanning	30 - 600
Scan rate	0.95 s
Threshold	2
Min. peak area	20

Temperature program (unless otherwise specified):

Initial temperature	40 °C
Final temperature	170 °C
Initial time	4 min.
Ramp rate	5 °C/min.
Final time	10 min.

GC columns

The following commercial open tubular capillary columns were used:

BP-1: 25 m. A non-polar column containing 100% methyl silicone.

BP-5: 30 m. A non-polar column containing 5% phenyl methyl silicone.

BP-20: 25 m. A polar column containing Carbowax 20M.

PEG-20M: 25 m. A polar column (equivalent to the BP-20) containing polyethylene glycol 20M.

2.4 PRELIMINARY INVESTIGATIONS

The optimum gas flow rate in the capillary column was ascertained by performing a Van Deemter plot.[6] From this a carrier gas flow rate of 4 cm^3/min was found to be satisfactory, realising that with capillary columns the Van Deemter plot is much flatter than for a packed column. Therefore it is not necessary to use a flow rate that is very close to the optimum as determined by the Van Deemter plot.

2.5 PRELIMINARY RUNS FOR DETERMINATION OF CONDITIONS

In these runs the Tekmar was connected to the GC. The column used was the BP-1. At this stage the sample heater was in operation. The sparge tube was positioned 5 mm above the liquid level of the sample. It was not bubbled through the sample because when it was bubbled through too much foam was produced. A 5 min preheat

and purge flow rate of 40 cm^3/min. with an attenuation setting of 32 x 10^{-12} were used on the following and the corresponding chromatograms obtained as shown:

Blank sample tube at 80 °C, 5 min. purge (CHROMATOGRAM 1)

The same tube repurged at 80 °C, 5 min. purge (CHROMATOGRAM 2)

5 cm^3 mahewu at 80 °C, 5 min. purge (CHROMATOGRAM 3)

5 cm^3 mahewu at 80 °C, 10 min. purge (CHROMATOGRAM 4)

5 cm^3 mahewu + 3 g sodium chloride at 80 °C, 10 min. purge (CHROMATOGRAM 5)

Because of the differences in the chromatograms obtained for the purges of the blank tubes it was decided that all sample tubes would be heated in an oven at 120 °C for at least one hour, before being used.

The sodium chloride was added to saturate the mahewu sample to see if this would have a marked effect on the decrease of the solubilities of the headspace components, thus increasing the concentration of the components in the headspace. The peaks were only a little larger so it was decided that sodium chloride would not be added to future samples.

2.6 EFFECT OF TEMPERATURE OF MAHEWU SAMPLE ON THE HEADSPACE.

Using the temperature program:

Initial temperature	50 °C
Final temperature	250 °C
Initial time	10 min.
Ramp rate	10 °C/min.
Final time	2 min.

chromatograms were obtained for the sample at room temperature and at 90 °C (CHROMATOGRAMS 6 & 7).

The chromatograms show marked differences in the number of peaks and in the heights of common peaks. Because the mahewu is usually consumed at ambient temperature all further headspace work that was carried out on it in the laboratory was performed at room temperature.

2.7 EFFECT OF PURGE TIME ON CHROMATOGRAMS

Considering CHROMATOGRAMS 3 & 4 it was decided to purge all further mahewu samples at 40 cm^3/min. for 10 minutes when using the Tekmar, employing a similar temperature programme to ensure that all components are eluted before the next injection.

ALL SUBSEQUENT QUALITATIVE ANALYSES OF THE HEADSPACE WERE PERFORMED USING THE TEKMAR CONNECTED TO THE GC/MS.

From time to time the source of the MS was cleaned, taking all the necessary precautions as prescribed in the manufacturers' manual.

A number of runs were done on various mahewu samples (Files DEGOEDE02 through to DEGOEDE37). The analysis of the headspace components of these is used in a later chapter.

2.8 LONG PURGES

It was decided that it would be desirable to sample the headspace of the producer's tanks. This necessitated the construction of a portable sampling apparatus. Tenax was conditioned by placing it in the oven at 120 °C for 3 hours. The ends of 90 mm glass tubes with inside diameters of 5 mm were fire glazed and each packed with 0.15 g of Tenax. The Tenax was held in each tube by plugs of glass wool.

For each long purge performed in the laboratory nitrogen from a cylinder was passed through 600 cm^3 of mahewu sample in a 1 dm^3 glass bottle fitted with stainless steel inlet and outlet tubes, thence through the tube containing Tenax. The nitrogen thus bubbled through the sample for 48 hours. The Tenax with the adsorbed components was transferred to a clean sample tube and fitted to the Tekmar. Because the heater unit was not working the sample tube containing the Tenax was heated using a spirit burner while being purged with nitrogen at a flow rate of 12 cm^3/min. for 4 min. Desorption was for 0.5 min. at 135 °C.

2.9 TANK PURGING

Having developed a method for long purges in the laboratory a starter tank at the suppliers' plant was sampled by drawing the headspace directly from the tank using a portable pump which pumped at a set rate of 1 dm^3/min. A special stainless steel plate had to be made to fit the sampling port on the tank and to facilitate the tubing to the Tenax tube thence to the pump. The headspace above the tank was thus sampled for 1 hour. Back in the laboratory the Tenax was removed with difficulty from the tube. The process was difficult because the Tenax was wet from the water vapour in the headspace above the hot mahewu in the tank. The same conditions were used to produce CHROMATOGRAM 12. The chromatogram showed evidence of column-bleed caused by water vapour.

A purge of the starter tank was repeated with a drying tube in line before the Tenax tube. The drying tube consisted of a U-tube, of inside diameter 2 cm, containing anhydrous sodium sulphate with glass wool keeping the drying agent in place. This did not prove satisfactory because the sodium sulphate and glass wool removed from the tube had a definite smell, indicating that odoriferous components were not reaching the Tenax in their entirety.

The line heater controller was not operational so this was done manually.

The temperature program used for this run was:

initial temperature	60 °C for 4 min.
ramp rate	5 °C/min.
final temperature	200 °C for 10 min.

and CHROMATOGRAM 8 obtained.

To check if any artifacts remained on the original Tenax, the sample tube was heated in an oil bath at 150 °C during the purge cycle. For this run a final temperature of 230 °C was employed and CHROMATOGRAM 9 obtained.

The same mahewu was purged again after 37 days, this time purging with nitrogen at 12 cm^3/min. for 36 hours. The adsorbed material was delivered to the GC/MS as before except that the sample tube on the Tekmar was heated in an oil bath at 150 °C, resulting in CHROMATOGRAM 10.

With a fresh mahewu sample and the same procedure as for CHROMATOGRAM 10, CHROMATOGRAM 11 was obtained.

One problem associated with the GC/MS was that if the mains voltage dipped during a run the computer automatically reset the instrument. This occurred during the chromatographic run of a 36 hour purge of a home-fermented sample of mahewu, resulting in the loss of all the data.

At this stage no more work was possible on the GC/MS because it has not worked satisfactorily since then. The electrometer was faulty, then when it was fixed it was not possible to tune the MS satisfactorily. At one stage the disc drive damaged someone else's data disc, necessitating a shutdown of some time until repaired. The printer required for the output of chromatograms ceased to function so this had to be done on an identical system at another institution.

CHAPTER 3

SOLVENT EXTRACTION

3.1 INTRODUCTION

Because the headspace components originate from the mother liquor it was decided to attempt to extract these compounds from mahewu using solvent extraction techniques, to separate them and identify them. In this way it may be possible to identify off-odour compounds without working on the headspace.[7]

The solvents were chosen because of their range of polarities. Their properties are shown in the following table.[8]

TABLE 3.1

SOLVENT	DENSITY at $20^{o}C/g\ cm^{-3}$	BOILING POINT $/^{o}C$
DCM	1,325	40
MEK	0,806	79
Hexane	0,659	69
Ether	0,714	35

The concentration of the extracts in the solvents may be

increased by rotary evaporation or by passing a stream of inert gas over the surface to encourage evaporation of the solvent. The inherent dangers involve attrition of very volatile solutes and possibly including impurities from the gas stream.[9]

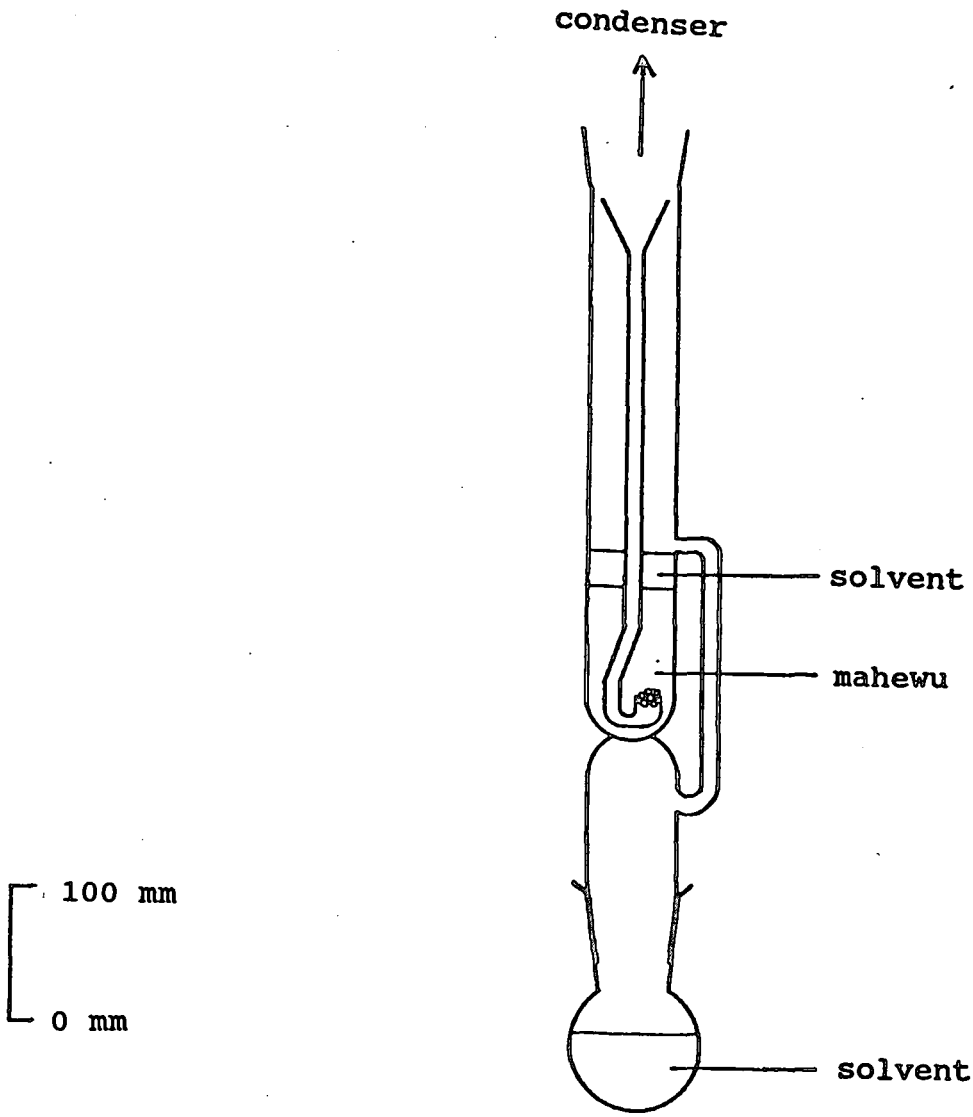
After seeing a paper by Dupuy et al[10] on the use of vegetable oil to extract flavour components this basic idea was used on mahewu.

3.2 SOLVENT EXTRACTION METHODS

3.2.1 With solvents less dense than mahewu.

The apparatus shown in FIGURE 1 was used.

FIGURE 1



Here the solvent in the round-bottomed flask was heated with a heating mantle, the vapour condensed in the condenser collected in the funnel, dropped down the tube and rose through the sintered glass disc thence through the mahewu sample. Excess solvent that contained extracted components dripped back into the heated vessel, this all in a continuous cycle.

50 cm^3 mahewu was extracted for 3 hours with 300 cm^3 hexane. A 60 cm^3 aliquot of the hexane portion was concentrated to 10 cm^3 on a rotary evaporator at 100 rpm, i.e. a concentration of 6 times. 0.5 μl of this concentrate was injected into the GC and CHROMATOGRAM 13 obtained using split injection and the SE-52 capillary column.

0.5 μl of the neat hexane, concentrated in the same way, was injected and CHROMATOGRAM 14 obtained. This was done to ensure that any peaks in the chromatogram were not confused with peaks due to impurities in the hexane. It was seen that the hexane contained very few impurities. This was the case with all solvents used.

The same extraction was repeated for 8 hours and the resulting CHROMATOGRAM 15 obtained.

These chromatograms showed that the longer the extraction the greater the concentration of the extracted components in the extracting solvent as shown by the greater height of the peaks. It was therefore decided to perform 8 hour extractions.

An 8 hour extraction using hexane, concentrated 20 times produced CHROMATOGRAM 16. Comparing CHROMATOGRAMS 15 & 16 shows that the greater the concentration the greater the size of the peaks and that the rotary evaporation method of concentration is satisfactory.

Similar extractions were performed with MEK, using the same procedure. With the PEG-20M column, the following chromatograms were obtained:

CHROMATOGRAM 17	hexane
CHROMATOGRAM 18	hexane, 8 hour extract, concentrated 20 times
CHROMATOGRAM 19	MEK
CHROMATOGRAM 20	MEK, 8 hour extract, concentrated 20 times

In the run for chromatogram 18, after 4 min. the attenuation was changed to a more sensitive value to see if there were any other components in small quantities eluting after that time; some peaks are seen.

Using the GC/MS and the BP-1 column, the following chromatograms were obtained with 2 μl injections:

CHROMATOGRAM 21	hexane, 8 hour extract, concentrated 20 times (Filed as RDG1)
CHROMATOGRAM 22	hexane, 8 hour extract, unconcentrated (Filed as RDGE1)
CHROMATOGRAM 23	hexane, 8 hour extract, concentrated 20 times (Filed as RDGE2)
CHROMATOGRAM 24	MEK, 8 hour extract, concentrated 20 times (Filed as RDG2)

An ether extract was performed by using 400 cm^3 mahewu with 40 cm^3 ether and 250 cm^3 water in the boiling flask. The continuous extraction was performed for 8 hours.
An ether blank was obtained by evaporating to dryness a 15 cm^3 aliquot of ether by blowing a stream of nitrogen over the surface of the liquid. 100 μl of ether was added and swirled around the inside of the vessel, i.e. concentrating the extract 150 times. 0.5 μl of this was injected, producing CHROMATOGRAM 25.

The same concentration procedure was used for the ether extract, producing CHROMATOGRAM 26. Because the peaks after the initial large solvent peak were so small another injection was made using the splitless mode, resulting in CHROMATOGRAM 27, which shows that this is a much better technique, but still very disappointing because they are not well resolved, still relatively small and very close to the very large solvent peak at the beginning of the chromatogram.

This concentration procedure was used for a new hexane extract in which 400 cm^3 mahewu and 200 cm^3 water was extracted with 50 cm^3 hexane for 8 hours. A blank was obtained, CHROMATOGRAM 28 (File HEXANE01) by evaporating 15 cm^3 of the hexane to dryness and adding 100 μl hexane. 4 μl was injected using splitless mode for 30 s and the following temperature programme:

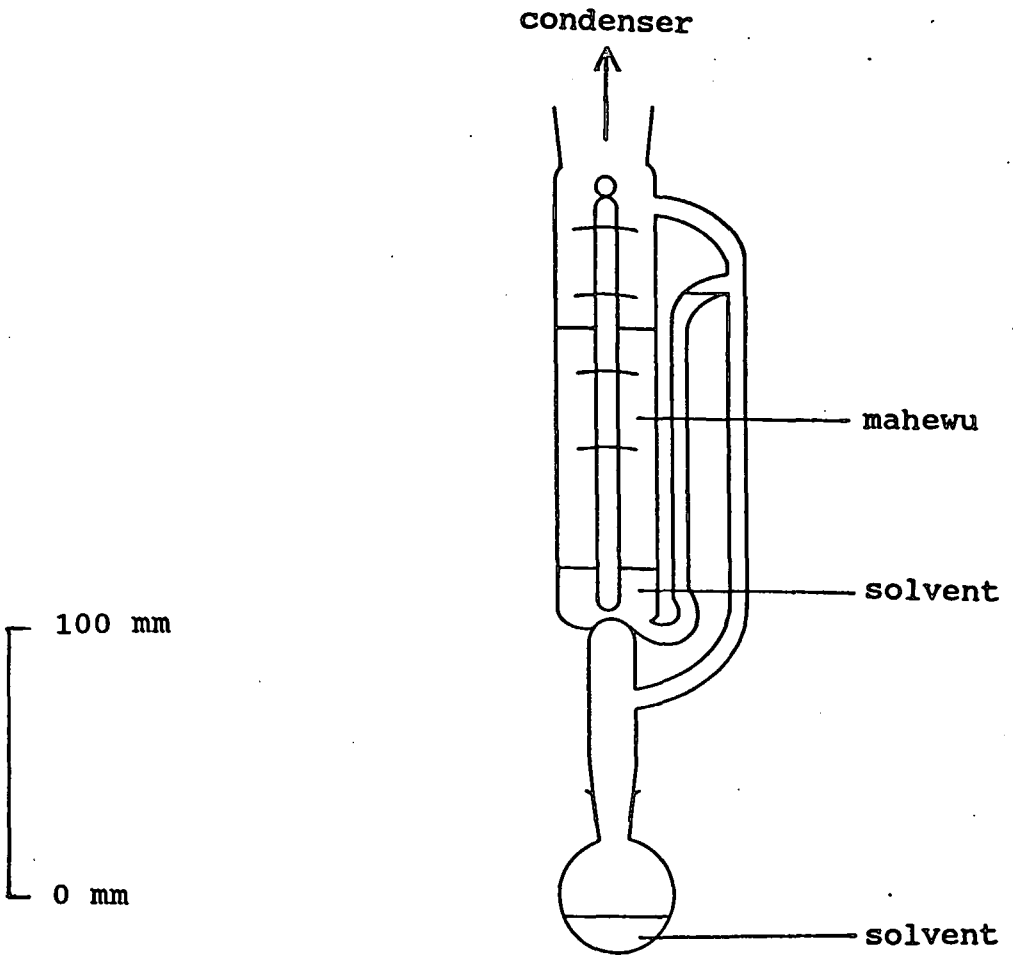
Initial temperature	60 °C
Final temperature	230 °C
Initial time	5 min.
Ramp rate	5 °C/min.
Final time	5 min.

The same concentration procedure was used for the hexane extract to obtain CHROMATOGRAM 29 (File HEXANE03). The evaporated extract had a strong odour so the odoriferous components must have come out with the solvent peak. In order to confirm this, another aliquot of the hexane extract was evaporated and an injection made with the residue to obtain CHROMATOGRAM 30 (File HEXANE07). The two peaks are due to odoriferous components. Their quantities are extremely small as shown by the ion count.

3.2.2 With solvents more dense than Mahewu.

The apparatus shown in FIGURE 2 was used.

FIGURE 2



The solvent was boiled using a heating mantle. The vapours went up to the condenser, condensed and dropped through the mahewu. Excess solvent that contained extracted material overflowed back into the boiling flask, the process thus cycling continuously during the course of extraction.

In this way, 30 cm^3 mahewu was extracted for 4 hours using 200 cm^3 dichloromethane. 125 cm^3 of the dichloromethane extract was concentrated on a rotary evaporator to 5 cm^3, i.e. 25 times. 0.5 μl dichloromethane was injected and CHROMATOGRAM 31 obtained. Likewise, CHROMATOGRAM 32 was obtained for the extract.
A similar extraction was carried out for 8 hours, the extract concentrated 30 times, yielding CHROMATOGRAM 33. With this method of concentration it was difficult to stop the evaporation at the same point each time. However, this could have been improved by adding a little solvent to make it up to the same volume each time.

3.2.3 Oil extracts

The vegetable oil used for these extracts was Helios Cooking Oil, a sunflowerseed oil.

The sample heating unit on the Tekmar was operational for these analyses.

A blank was obtained, CHROMATOGRAM 34 (File 1), by placing 0.2 cm^3 oil on glass wool in a clean sample tube, connected it to the Tekmar and purged at 100°C for 10 min. before desorbing on to the GC/MS using split mode and the following heating programme:

Initial temperature	40 °C
Final temperature	170 °C
Initial time	4 min.
Ramp rate	10 °C/min.
Final time	10 min.

The oil extraction was effected by stirring together 10 cm^3 oil and 100 cm^3 mahewu on a magnetic stirrer for 1 hour. The resulting mixture was centrifuged and separated. 0.2 cm^3 of the oil extract was placed on glass wool in a clean sample tube, heated to 100°C and purged. The trapped components were forwarded to the GC/MS to produce CHROMATOGRAM 35 (File 2).

Another 0.2 cm^3 of the oil extract was placed on glass wool in a clean sample tube, heated to 40°C, purged and chromatographed, giving CHROMATOGRAM 36 (File 3). The oil extract was left on the glass wool in the sample tube and the same procedure repeated at 50 °C, 60 °C and 70 °C to see if any other peaks appeared. The following were obtained:

50 °C:	CHROMATOGRAM 37, File 4
60 °C:	CHROMATOGRAM 38, File 5
70 °C:	CHROMATOGRAM 39, File 6

Comparing the above chromatograms it seems that 60 °C would be a suitable temperature to use and that only the component giving the peak at about scan 300 could be studied.

Another extraction was performed using 20 cm^3 oil and 200 cm^3 mahewu.

An oil blank was run using 1 cm^3 of the oil on glass wool at 40 °C (CHROMATOGRAM 40, File 11).

1 cm^3 oil extract on glass wool was run at 40 °C (CHROMATOGRAM 41, File 12).

Comparing Chromatograms 36 & 38 it is seen that the peaks of the latter are larger. The larger volume of oil extract on the glass

wool is thus recommended.

Fresh samples of the second oil extract were placed on glass wool at different temperatures and the data filed as:

50 °C:	File 13
60 °C:	File 114
70 °C:	File 17
80°C:	File 20

It is concluded that the solvent extraction method using oil is not very useful because only one peak appears consistently in all these chromatograms, namely that at about scan 300.

3.2.4 Microextractions

Simpler extractions on a much smaller scale were performed. This technique was attempted because if successful, it would be a very convenient method of extraction requiring little time and effort. Furthermore, it would not involve the attrition of the more volatile components which occurs in the concentration steps used above.

A blank was run by injecting 5 μl DCM using the following temperature programme:

Initial temperature	40 °C
Final temperature	200 °C
Initial time	5 min.
Ramp rate	10 °C/min.
Final time	2 min.

Using the split mode CHROMATOGRAM 42 (File 100) was obtained.
Using the splitless mode for 25 s CHROMATOGRAM 43 (File DCM101) was obtained.

A mixture of 3 cm^3 mahewu and 200 μl DCM was shaken in a 5 cm^3 syringe, allowed to separate and the DCM portion carefully removed using a narrow J-tube connected to the syringe. 5 μl of the DCM extract was injected.
Using the split mode (File DCM102) was obtained.
Using the splitless mode for 25 s CHROMATOGRAM 44 (File DCM103) was obtained.

There is a difference between chromatograms 42 and 43 because with the split, only 1/15th of the sample went on to the column. Consequently, with the injection being splitless for a short while, the whole sample is allowed to enter the column, the split valve is opened and the sample chromatographed with a smaller mass flow rate of carrier gas, seeing that most of the gas is being split off. So the injection being splitless for 25 s improves the situation.
Comparing chromatograms 43 and 44, an extra peak appears at scan 254 on the sample chromatogram which is not on the blank, so this micro-extraction method with a splitless mode for 25 s could be used for the component producing this peak, but overall the technique at present does not appear to be promising, mainly because it appears to be so specific.

CHAPTER 4

ANALYSIS

4.1 IDENTIFICATION OF SOME COMPONENTS

The Library Search facility on the computer of the GC/MS was used on all the files that were generated by the various runs mentioned previously. A printout was obtained for each significant peak. Each printout gave, *inter alia*, the three compounds in its library whose mass spectra best fitted those of the sample spectrum. From these were extracted those compounds mentioned which appeared often. It was borne in mind that the computer's library was limited. A list of these compounds appears in Table 1, with the names of the files in which they were found, the scan number of the peak, the rank given by the computer, the purity of the match (which is an indication of the closeness of the match) and the reconstructed ion chromatogram count (RIC), which gave an indication of the quantity of the compound present. The names shown are those used by the computer.

TABLE 4.1

SUBSTANCE	CHROMAT	SCAN	RANK	PURITY	RIC
ACETIC ACID,ANHYDRIDE	ETHER01	131	3	736	9759
	LPURGE3	320	1	836	3155
ACETIC ACID,HYDRAZIDE	ETHER01B	277	2	830	7439
	ETHER07B	391	5	594	88
ACETIC ACID,BUTYLESTER	LPURGE1A	1152	3	645	4015
	LPURGE2	542	2	938	5695
ACETIC ACID,ETHYLESTER	ETHER01	131	1	980	9759
	ETHER01B	277	1	920	7439
	ETHER07A	269	1	975	177407
	ETHER07B	273	1	919	5639
	LPURGE1	194	1	978	
	LPURGE2	202	1	989	83199
	LPURGE3	320	2	932	3155
	DEGOEDE5	97	1	979	202239
ACETIC ACID,ETHENYL	ETHER01B	277	5	776	7439
ESTER	ETHER07B	273	5	879	5639
	20	173	3	868	9663
ACETIC ACID,HEXYLESTER	LPURGE1A	1152	1	750	4015
ACETIC ACID,PENTYLESTER	DEGOEDE43	554	1	940	46591
	LPURGE1	798	1	930	29471
	LPURGE1A	796	1	937	122239
	LPURGE2	822	1	937	135679
	LPURGE3	1134	2	932	1831
	LPURGE3	1146	1	928	27487
	LPURGE3	1167	2	951	11167

ACETIC ACID,PROPYLESTER	ETHER01 ETHER01B ETHER07A ETHER07B	131 220 269 273	2 4 3 3	788 793 741 812	9759 7439 177407 5639
ACETIC ACID,TRIFLUORO-1,5-PENTANEDIYLESTER	DEGOEDE43	415	2	807	1173
ACETIC ACID,(1-METHYLETHOXY)-,ETHYLESTER	ETHER07A DEGOEDE5	269 97	2 2	768 769	177407 202239
ACETIC ACID,2-METHYLPROPYLESTER	LPURGE2	542	1	951	5695
ACETIC ACID,2-PHENYLETHYLESTER	HEXANE03	1751	1	930	1061
BENZENE,1,4-DICHLORO	LPURGE1A	1136	1	790	450
BENZENEETHANAMINE,N-(2-PHENYLETHYL)-,HYDROCHLORIDE	DEGOEDE43	826	1	863	741
BENZENEETHANOL	ETHER07A DEGOEDE43	1614 890	2 1	864 872	1022 4223

BENZENE,ETHYL-	HEXANE01	836	2	584	216
	DEGOEDE43	517	1	766	1327
	DEGOEDE43	532	3	943	18079
	DEGOEDE43	573	3	939	23327
	114	509	3	584	138
	LPURGE1	757	1	932	1091
	LPURGE1A	753	1	972	3251
	LPURGE1A	778	3	914	9871
	LPURGE1A	838	1	926	1811
	LPURGE2	776	1	809	618
	LPURGE2	801	1	934	1305
	DEGOEDE5	438	1	998	1967
BENZENE,ETHENYL-	LPURGE1	828	2	838	556
	LPURGE1A	826	3	971	4231
	LPURGE2	849	2	903	1169
	LPURGE3	1189	1	781	305
BENZENE,1,2-DIETHYL	LPURGE1	1251	2	919	4599
	LPURGE1A	1257	2	868	158975
BENZENE,1,3-DIETHYL-	LPURGE1	1251	3	917	4599
	LPURGE1A	1257	3	864	158975
BENZENE,1,4-DIETHYL-	LPURGE1	1251	1	937	4599
	LPURGE1A	1257	1	894	158975
BENZENE,1-ETHENYL-2-METHYL-	DEGOEDE43	785	3	899	1711
BENZENE,ETHYLMETHYL-	DEGOEDE43	785	5	882	1711
BENZENE,METHYL	HEXANE01	556	5	699	178
	HEXANE04	539	4	767	304
	DEGOEDE43	890	4	751	4223
	LPURGE2	521	1	971	5663
	DEGOEDE5	228	1	970	4032

BENZENE,1,2-DIMETHYL	HEXANE01	836	3	559	216
	DEGOEDE43	517	2	688	1327
	DEGOEDE43	532	1	957	18079
	DEGOEDE43	573	1	947	23327
	114	509	2	591	138
	LPURGE1	757	3	932	1091
	LPURGE1A	753	2	892	3251
	LPURGE1A	778	1	926	9871
	LPURGE1A	838	2	916	949
	LPURGE2	801	2	934	1305
	DEGOEDE5	438	2	964	1967
BENZENE,(1-METHYLETHYL)-	DEGOEDE43	625	1	923	2767
	DEGOEDE43	682	4	904	42047
	DEGOEDE43	708	1	925	14975
	LPURGE1A	1027	1	930	1315
	LPURGE1A	1071	1	793	439
BENZENE,1-METHYL-2-	DEGOEDE43	747	3	833	584
PROPYL-	DEGOEDE43	806	2	941	3371
	DEGOEDE43	826	2	841	741
BENZENE,1-METHYL-3-	DEGOEDE43	806	3	933	3371
PROPYL-	DEGOEDE43	826	5	806	741
BENZENE,1-METHYL-4-	DEGOEDE43	747	4	832	584
PROPYL-	DEGOEDE43	806	1	955	3371
	DEGOEDE43	826	4	837	741
BENZENE,(1-	DEGOEDE43	747	1	837	584
METHYLPROPYL)-	DEGOEDE43	806	4	931	3371
	DEGOEDE43	826	3	840	741
BENZENE,PROPYL-	DEGOEDE43	669	1	966	10303
	LPURGE1A	1008	1	914	783
BENZENE,1-PROPENYL-	DEGOEDE43	785	4	888	1711

BENZENE,2-PROPENYL-	DEGOEDE43	785	2	905	1711
BUTANOIC ACID,ETHYLESTER	LPURGE2	612	1	914	1331
	DEGOEDE5	306	1	983	3983
	DEGOEDE5	741	5	833	2379
BUTANOIC ACID,2-ETHYL-1,2,3-PROPANETRIYLESTER	DEGOEDE5	741	3	839	2379
BUTANOIC ACID,3-METHYLBUTYLESTER	DEGOEDE5	849	4	893	2033
	DEGOEDE5	911	2	881	4615
BUTANOIC ACID,1-METHYLETHYLESTER	DEGOEDE5	306	5	846	3983
BUTANOIC ACID,2-METHYL-2-METHYLBUTYLESTER	LPURGE1	1395	1	778	944
2-BUTANOL,3-METHYL-	DEGOEDE5	195	1	951	16383
1-BUTANOL,2-METHYL-,ACETATE	LPURGE2	827	1	956	15935
	LPURGE3	1167	3	881	11167
1-BUTANOL,3-METHYL-,ACETATE	DEGOEDE43	554	2	928	46591
	LPURGE1	798	2	908	29471
	LPURGE1	796	2	921	122239
	LPURGE1	892	1	904	8879
	LPURGE2	822	2	923	135679
	LPURGE2	827	2	906	15935
	LPURGE3	1134	1	933	1831
	LPURGE3	1146	2	915	27487
	LPURGE3	1167	1	954	11167
CYCLOBUTANOL,2-ETHYL-	12	388	1	899	40191
	20	355	1	903	273919

1,3,5-CYCLOHEPTATRIENE	ETHER07A	1614	5	821	1022
	HEXANE01	556	2	740	178
	HEXANE04	539	3	758	304
	LPURGE2	521	3	948	5663
	DEGOEDE5	228	3	972	4023
1,3,5,7-CYCLOOCTATETRAENE	LPURGE1	828	1	849	556
	LPURGE1A	826	1	989	4231
	LPURGE2	849	1	906	1169
	LPURGE3	1189	2	779	811
CYCLOTRISILOXANE,HEXAMETHYL-	11	456	2	807	366
FURAN,2,5-DIHYDRO-	ETHER01	154	3	845	773
	ETHER01B	541	4	857	1129
	ETHER07A	306	4	809	16079
FURAN,2,3-DIHYDRO-4-METHYL-	ETHER07A	566	5	696	337
FURAN,4-METHYL-2-PROPYL-	12	732	2	696	495
FURAN,2-PENTYL-	12	680	1	909	1789
	20	672	1	631	2699
	LPURGE1A	1110	1	756	9343
	DEGOEDE5	729	1	996	2099
1-HEPTANOL,6-METHYL-	2	646	5	798	526
2-HEPTANONE	20	522	1	944	41663
	LPURGE1A	817	1	923	5311
2-HEPTANONE,4-METHYL-	LPURGE1	1086	2	881	1759
4-HEPTANONE,3-METHYL-	ETHER07B	1833	4	789	475
	HEXANE03	2159	2	812	785
HEXANOIC ACID	DEGOEDE43	972	3	662	1943

HEXANOIC ACID,ETHYLESTER	LPURGE1A	1118	1	790	770
	LPURGE2	1140	1	921	1041
	DEGOEDE5	741	1	987	2379
HEXANOIC ACID,2-PROPENYLESTER	LPURGE1A	1318	1	870	1335
	DEGOEDE5	887	1	961	3975
HEXANOIC ACID,2-METHYL-3-OXO-,ETHYLESTER	DEGOEDE5	146	4	892	8415
HEXANEDIOIC ACID,2,4-DIMETHYL-,METHYLESTER	DEGOEDE5	1102	2	868	4951
3-HEXANETHIOL,3-ETHYL-	4	1075	5	625	654
1-PENTANOL	2	296	3	853	1167
	LPURGE1A	424	2	902	35071
	DEGOEDE5	195	2	883	16383
	LPURGE3	651	2	839	3875
1-PENTANOL,3-METHYL-	1	703	2	776	1605
	12	695	4	703	1909
	20	687	2	715	11007
2-PENTANONE	20	173	1	989	9663
2-PENTANONE,5-PHENYL-	HEXANE03	1751	3	770	1061
4-PENTENAL,2-ETHYL	2	646	1	832	532
	20	623	1	873	5671
1-PENTENE	ETHER07B	574	3	815	695
	HEXANE03	293	3	872	287231
	4	300	3	846	14479
2-PENTENE,5-(PENTYLOXY),(E)-	DEGOEDE	887	3	884	3975
2-PENTEN-1-OL,2-METHYL-	20	544	4	787	13663

4-PENTEN-2-OL	ETHER07B	75	3	746	227
3-PENTENOIC ACID,4-METHYL-	4	99	4	835	30143
PHENOL,2,6-BIS(1,1-DIMETHYLETHYL)-4-METHYL-	20	1264	1	879	3179
	12	1252	1	833	1109
	2	1216	1	814	1267
	2	1276	1	727	597
	4	1283	1	774	1063
PHENOL,2,6-BIS(1,1-DIMETHYLETHYL)-4-METHYLCARBAMATE	2	1216	2	680	1267
	2	1276	3	536	597
	4	1283	2	590	1063
	12	1252	2	784	1109
	20	1264	2	839	3179
PHENETHYLAMINE,N-BENZYL,P-CHLORO-HYDROCHLORIDE	DEGOEDE43	669	4	838	10303
1-PROPANOL,2-METHYL	ETHER07B	1764	1	741	57
2-PROPANOL	ETHER01	62	4	841	47039
	ETHER07B	75	2	802	227
2-PROPANONE	HEXANE01	93	1	911	1845
	4	99	1	968	30143
	11	30	2	855	906
	20	75	2	913	212735
	20	522	4	811	41663
PROPANOIC ACID,ANHYDRIDE	DEGOEDE43	246	2	727	1393
PROPANOIC ACID,ETHYLESTER	DEGOEDE43	246	1	896	1393
	LPURGE2	384	1	807	1169
2-PROPANONE,1-FLUORO-	LPURGE3	320	4	804	3155

PROPANOIC ACID,2-HYDROXY-,ETHYLESTER	ETHER01	62	5	832	47039
	ETHER01B	76	4	671	465
	1	93	3	856	33791
	12	86	3	854	55167
PROPANOIC ACID,2-HYDROXY-2-METHYL-	ETHER01	125	5	731	1349
	ETHER07A	252	2	803	81535
	ETHER07B	257	2	747	1625
PROPANOIC ACID,2-METHYL	ETHER01B	220	4	784	4151
PROPANOIC ACID,2-METHYL-,ANHYDRIDE	LPURGE2	612	2	782	1331
PROPANOIC ACID,2-METHYL-,ETHYLESTER	LPURGE2	612	4	769	1331
	DEGOEDE5	306	2	933	3983
PROPANOIC ACID,2-METHYL-,PENTYLESTER	LPURGE1A	1354	1	802	873
	DEGOEDE5	911	1	978	4615
PROPANOIC ACID,2-METHYL-,2-PHENYLETHYLESTER	HEXANE03	1751	2	829	1061

From the above compounds were selected those with a rank of 1 and a reasonable RIC count. These appear in Table 4.2, together with some physical properties[11].

TABLE 4.2

SUBSTANCE	MELTING POINT/^{o}C	BOILING POINT/^{o}C	SOLUBILITY
ACETIC ACID,ETHYLESTER	-84	77	soluble
ACETIC ACID,PENTYLESTER	-71	149	al, eth

BENZENE,ETHYL-	-75	136	al, eth
BENZENE,1,4-DIETHYL-	-43	184	al, eth, bz, ac
BENZENE,METHYL-	-95	111	al, eth, ac, bz
BENZENE,(1-METHYLETHYL)-	-96	152	al, eth, ac, bz
BENZENE,PROPYL-	-100	159	al, eth, ac, bz
BUTANOIC ACID,ETHYLESTER	-101	121	al, eth
1,3,5,7-CYCLOTETRAENE	-5	141	al, eth, ac, bz
2-HEPTANONE	-36	151	al, eth
PHENOL,2,6-BIS(1,1-DIMETHYLETHYL)-4-METHYL-	71	265	al, eth, bz, chl
PROPANOIC ACID,ETHYLESTER	-74	99	al, eth, ac

Considering the boiling points of the compounds, the properties of the compounds of only those in the following table were obtained.

TABLE 4.3

SUBSTANCE	PROPERTIES[12]
ACETIC ACID,ETHYLESTER	Fruity odour; in artificial fruit essences.
ACETIC ACID,PENTYLESTER	
BENZENE,ETHYL-	Irritant TLV 435
BENZENE,1,4-DIETHYL-	
BENZENE,METHYL-	Moderately toxic vapour.
BENZENE,(1-METHYLETHYL)-	Toxic, irritant TLV 375
BENZENE,PROPYL-	Moderately toxic
BUTANOIC ACID,ETHYLESTER	
1,3,5,7-CYCLOTETRAENE	Highly flammable
FURAN,2-PENTYL	Occurs in many foods including coffee, potatoes, tomatoes, soya bean oil - aroma. Probably results from oxidation of unsaturated fatty acids
2-HEPTANONE	Moderately toxic TLV 465 Alarm substance of ants.Flavour ingredient. Light yellow oil.
PHENOL,2,6-BIS(1,1-DIMETHYLETHYL)-4-METHYL-	Antioxidant. Moderately toxic.
PROPANOIC ACID,ETHYLESTER	Used as flavouring agent.

Considering the boiling points, other properties and availability of the compounds, the following (with the names on the bottles in parentheses) were purchased in order to confirm, or otherwise, the presence of the substances in mahewu.

acetic acid,ethylester (ethyl acetate)

acetic acid,pentylester (pentyl acetate)

benzene,ethyl (ethyl benzene)

benzene,propyl (propyl benzene)

2-heptanone (pentyl methyl ketone)

propanoic acid,ethylester (ethyl propionate)

4.2 CONFIRMATION OF SUBSTANCES PRESENT IN THE HEADSPACE OF MAHEWU

This was performed on the GC.

To confirm whether the suspected substances were present, 5.0 cm^3 of each substance in turn was pipetted into a clean sample tube, purged for 2 min. at room temperature and introduced into the GC, using the following temperature programme.

Initial temperature	50 °C
Final temperature	170 °C
Initial time	2 min.
Ramp rate	5 °C/min.
final time	2 min.

The number of the chromatogram obtained for each substance is given in Table 4.4 together with the retention times for the main peaks.

TABLE 4.4

SUBSTANCE	CHROMATOGRAM NUMBER	RETENTION TIME/min.
ethyl acetate	CHROMATOGRAM 45	2.4
ethyl propionate	CHROMATOGRAM 46	3.0
ethyl benzene	CHROMATOGRAM 47	5.4
pentyl methyl ketone	CHROMATOGRAM 48	6.2
propyl acetate	CHROMATOGRAM 49	6.7 & 2.3
propyl benzene	CHROMATOGRAM 50	7.6

A 5.0 cm^3 sample of mahewu was purged for 10 min. and chromatographed, producing CHROMATOGRAM 51.

This chromatogram had peaks corresponding with those from Table 4.4 at 2.4 min., 3.8 min. and 7.6 min. This indicates that ethyl acetate, ethyl propionate and propyl benzene were present in the headspace of mahewu. Peaks did not appear at 5.4 min., 6.2 min. and 6.7 min. which suggests that ethyl benzene, pentyl methyl ketone and propyl acetate were not present.

The chromatograms of the headspace of mahewu show a large number of peaks. What has been established here is the identity of only three of these components of the headspace. The mass spectra of the remainder were such that the computer could not give satisfactory matches with those in its library. Printouts of a peak for each component identified are shown in the appendix as **MASS SPECTRA 1, 2 & 3.** In each case the result of the library search on the mass spectrometer at the University of Natal, Durban, is shown together with the mass spectrum of the sample peak above, followed by the mass spectra of the three compounds in the library whose mass spectra best match that of the sample. Here propanoic acid, ethyl ester is ranked 2 whereas the original rank of 1 was obtained on the mass spectrometer at Technikon Natal.

4.3 QUANTITATION

This was performed on the GC.

After a few investigative runs with different concentrations of standards in the mahewu the following solutions were prepared to determine the concentration of each of the suspected substances in a mahewu sample using the method of standard addition. Because the volumes added were small, no adjustment was made for the volumes of solution.

100 μl of each of the six substances in 100.0 cm^3 mahewu giving a mahewu solution 0.10% in each standard.

75 μl of each of the six substances in 100.0 cm^3 mahewu giving a mahewu solution 0.075% in each standard.

50 μl of each of the six substances in 100.0 cm^3 mahewu giving a mahewu solution 0.050% in each standard.

25 μl of each of the six substances in 100.0 cm^3 mahewu giving a mahewu solution 0.025% in each standard.

For each solution, 5.0 cm^3 was purged for 10 min. and chromatographed, giving the following:

0.010% solution CHROMATOGRAM 52

0.075% solution CHROMATOGRAM 53

0.050% solution CHROMATOGRAM 54

0.025% solution CHROMATOGRAM 55

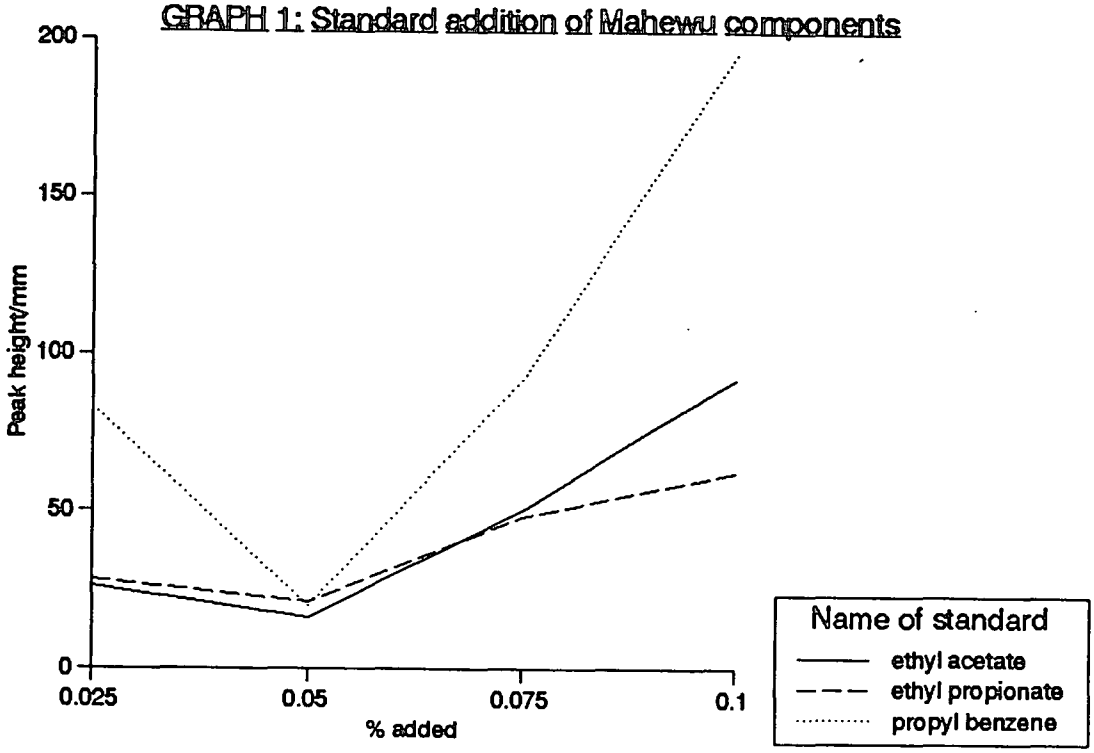
The heights of the peaks, measured from the interpolated baselines for each standard substance is shown in Table 4.5.

TABLE 4.5

PEAK HEIGHT/mm

SUBSTANCE	RETENTION TIME/min.	% ADDED			
		0.025	0.050	0.075	0.10
ethyl acetate	2.4	26	16	50	92
ethyl propionate	3.8	28	21	48	62
ethyl benzene	5.4	-	-	-	-
propyl methyl ketone	6.2	-	-	-	-
propyl acetate	6.7	-	-	-	-
propyl benzene	7.6	83	20	92	195

GRAPH 1: Standard addition of Mahewu components



These graphs show that this method of standard addition is not satisfactory for the determination of concentrations of these substances at these low concentrations. The concentrations of those which give peaks could be said to be about 0.03%. The only inference which can be made about the propyl benzene is that its concentration is less than 0.1%.

CHAPTER 5

DISCUSSION

The flavour component of foods total no more than 0.1%. When analyzing the olfactory components there are a host of problems associated with the analysis. There are usually many components in varying concentrations and the components vary greatly in their odoriferous thresholds. Also the response of the human nose is completely different to that of a detector used in chromatography. Often a component present in extremely small concentration, thus producing a very small peak, has a profound effect on the overall quality of the smell. The interaction of olfactory components cannot be ignored and the aromas we experience depend on the interaction of aromas of components present.

With regard to sampling, cognisance must be taken of the fact that the packaging can have an effect on the aroma. Often a component is leached out of the material with which the food is in contact. In the case of mahewu this is a carton. Sampling the headspace above the manufacturers' tank obviates this problem. This method of obtaining a sample does show possibilities as shown by chromatogram 12. However, a suitable method of removing the water from the headspace without removing other constituents would have to be found. This would be the

best place to sample the headspace in order to detect at an early stage any problems that arise in the production process. The purge-and-trap method is suitable, both on the large tanks and in the laboratory.

Whereas the addition of sodium chloride was used to attempt to drive the volatiles out of the mahewu without much success, various salts could be tried because they have different effects.[13]

Considering the solvent extraction techniques used here, none seemed to be completely satisfactory for the purpose of investigating the whole range of components which might be extracted. However, they may be used for specific components.

Regarding chromatograph 21, it would be very difficult to resolve any peak which is very close to the large solvent peak around scan 100. These peaks would be due to components with low boiling points, not being retained greatly by the column. One of these components may well contribute greatly to the odour, so another column or temperature programme or solvent would have to be found which would separate this component's peak from the solvent peak. After scan 500 there appears a large number of peaks which are not small judging by the total ion count of 6553590. These peaks could be resolved to a greater extent than has already been done. These components must be of higher boiling points, thus being retained longer on the column. These

compounds need careful consideration when assessing their contribution to the aroma. The fact that a compound has a high boiling point and low vapour pressure at room temperature does not mean that it does not contribute greatly to the aroma. If certain of these compounds have a low threshold of detection by olfactory receptors then they will have a profound effect on the overall smell of mahewu.

Of the solvents used for extraction only ether and the sunflowerseed oil seemed to extract very little in the way of volatile components. The others seemed to be effective. The technique used to concentrate these extracts has caused the loss of the more volatile components, especially the blow dry method. This is shown by chromatograms 21 and 29, except that with the blow dry method nothing appeared after the solvent peak. It would be better to blow it not completely to dryness but only to a certain volume. This may be difficult to reproduce as was the case with using the rotary evaporator but the volumes can always be made up with solvent. The rotary evaporation technique of concentration does mitigate mainly against the low boilers because they would have high vapour pressures at ordinary temperatures and greater rates of evaporation, under the vacuum conditions.

Chromatogram 22, of hexane extract that had not been concentrated, showed peaks of reasonable size, nowhere near the solvent peak. For analysis of these components a concentration technique is not required. This is highly desirable because of possible losses as mentioned above.

On the qualitative analysis side a great deal of work was done but what was confirmed is very limited. It was established that three substances, *viz*. ethyl acetate, ethyl propionate and propyl benzene are constituents of the headspace of mahewu. It is surprising that the butyl esters do not appear. It is also noteworthy that the established components have relatively low boiling points. This is a little surprising considering that the later peaks in the chromatograms of mahewu are well resolved. It does, however, depend on the mass spectrum obtained by the MS.

Quantitatively, what has been achieved is very limited. A rough estimation of the concentrations of ethyl acetate and ethyl propionate in mahewu is of the order of 0.03%, while it can only be said that that of propyl benzene is less than 0.1%. Headspace analysis by purge-and-trap is a way of analyzing qualitatively for them but not quantitatively. This technique does not give consistent enough results for it to be satisfactory. If the MS is to be used as detector, long purges for 48 hours are necessary for detection. The more sensitive flame ionization detector can be used for purge times of only 10 minutes, but the electrometer has to be set at a very sensitive value which mitigates against accuracy when considering quantitation.

In future work the nose could be introduced as a detector for identification of odoriferous components of the effluent from the column.[14] This approach enables the analyst to assess the level of sensory importance attributable to given regions of the chromatogram. The "sniff port" can either be used after a non-destructive detector, by a split-effluent technique or by sequential separations. None of these provides the ideal solution: the non-destructive detectors available cannot really be used with high-resolution columns (i.e. not with capillary columns); temperature variations affect the split ratio of a stream splitter; sequential separations involve changing the outlet of the capillary for successive runs, resulting in variation of the retention time of a particular component. Also capillary columns, which resolve mixtures well, are not suitable for sniffing because of the small quantities of mixture injected.[15]

Work is being done on an "artificial olfactory sensor".[16] This sensor consists of a set of piezoelectric sensors which incorporate different hydrophobic polymer materials. This type of sensor would obviate the need to rely on a subjective human nose to perform the detection because it, like the nose, does not separate compounds before responding to them. It therefore offers great possibilities.

It is clear from the present study, that mahewu, as with most other food product, presents great challenges when it comes to the analysis of volatile compounds present. Not only is the dynamic range of compound concentration very great: extraction

is a problematic area, and detection/assessment of aroma importance is still a major area in which progress has to be made, before complete characterisation of the profiles of volatiles of mahewu, and other foods, can be put on a scientific footing which stands separate from the territory of the subjective methods currently used in industry.

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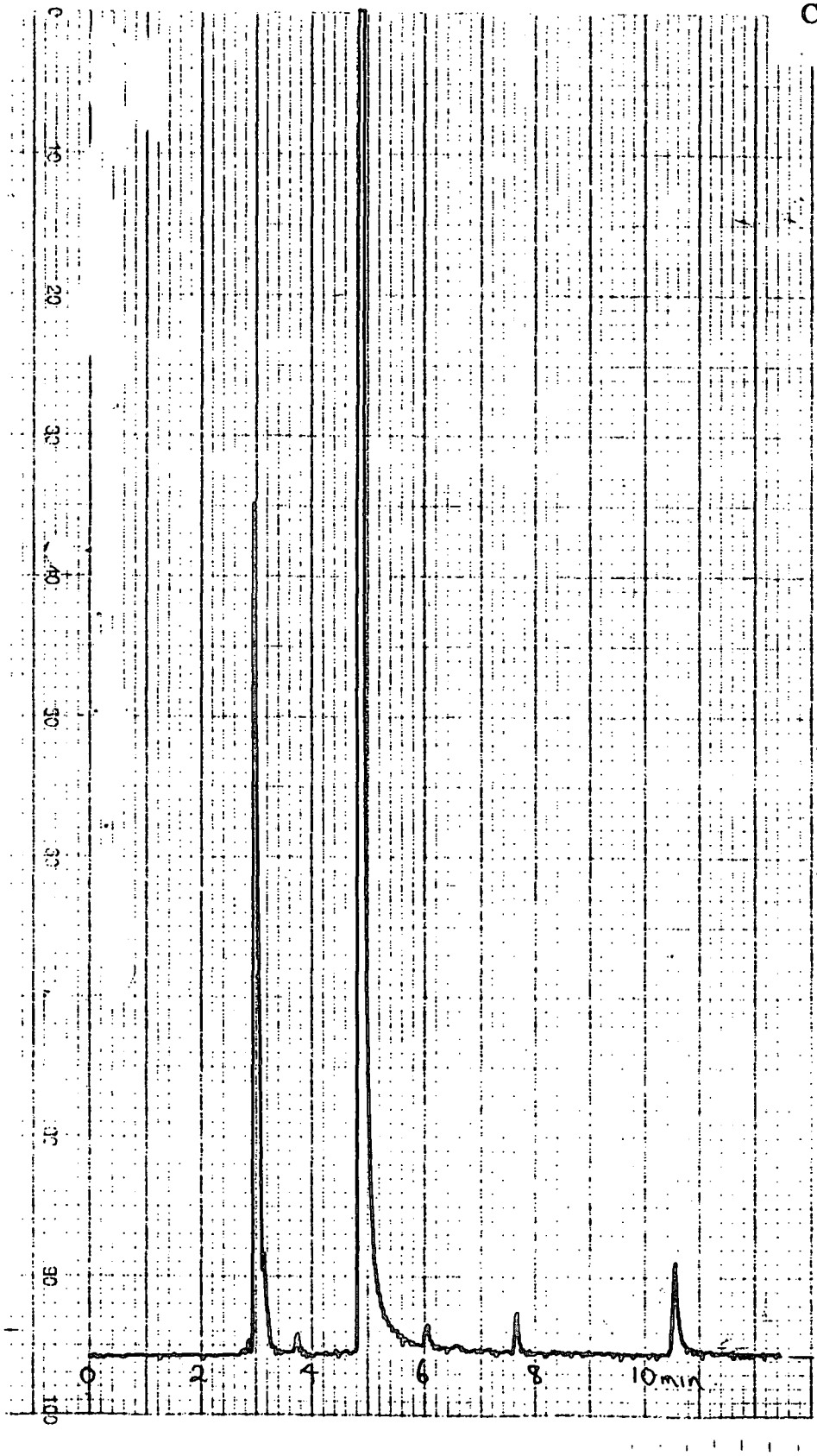
APPENDIX

CHROMATOGRAM 1 to 55 and MASS SPECTRUM 1, 2 & 3 (all reduced to 85% of their original size) follow:

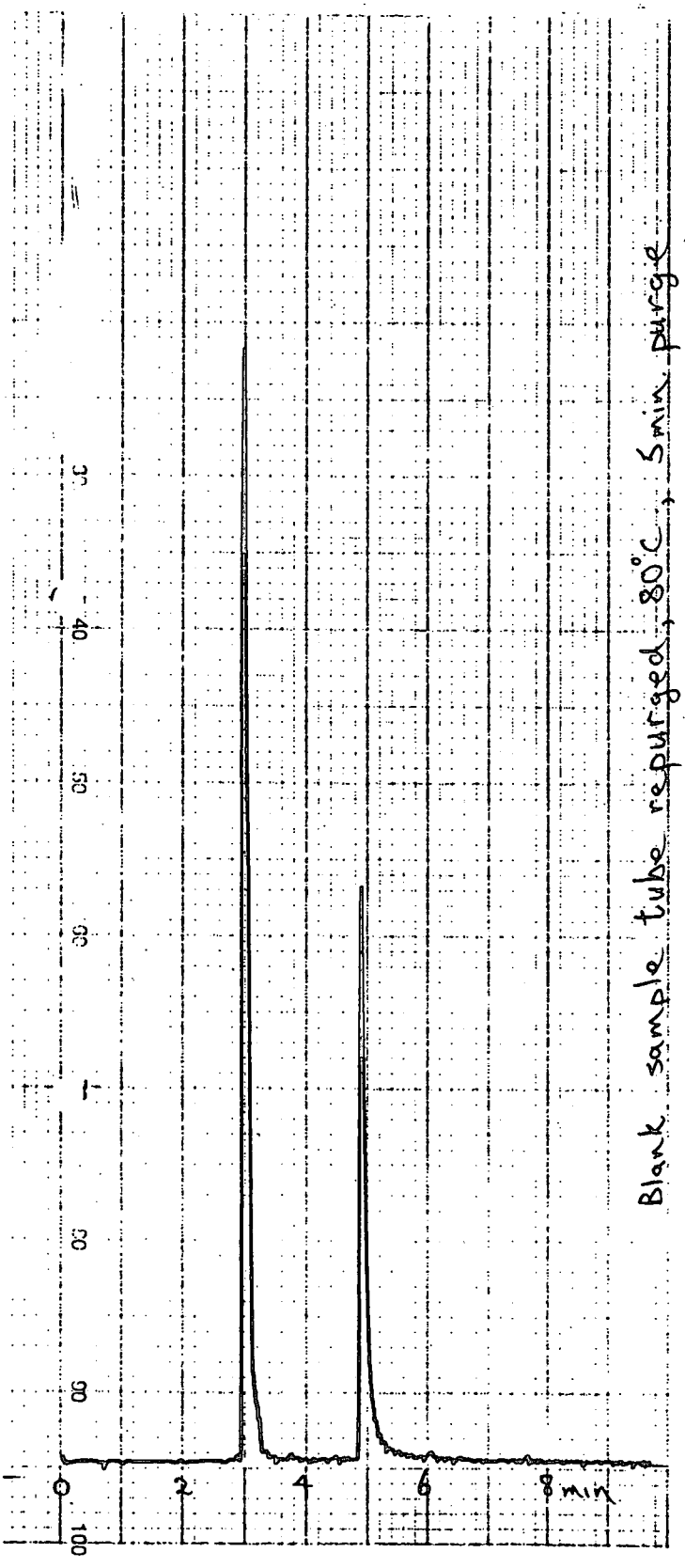
RK09270150I W+W 1043

CHROMATOGRAM 1



Blank sample tube, 80°C, 5min purge

CHROMATOGRAM 2



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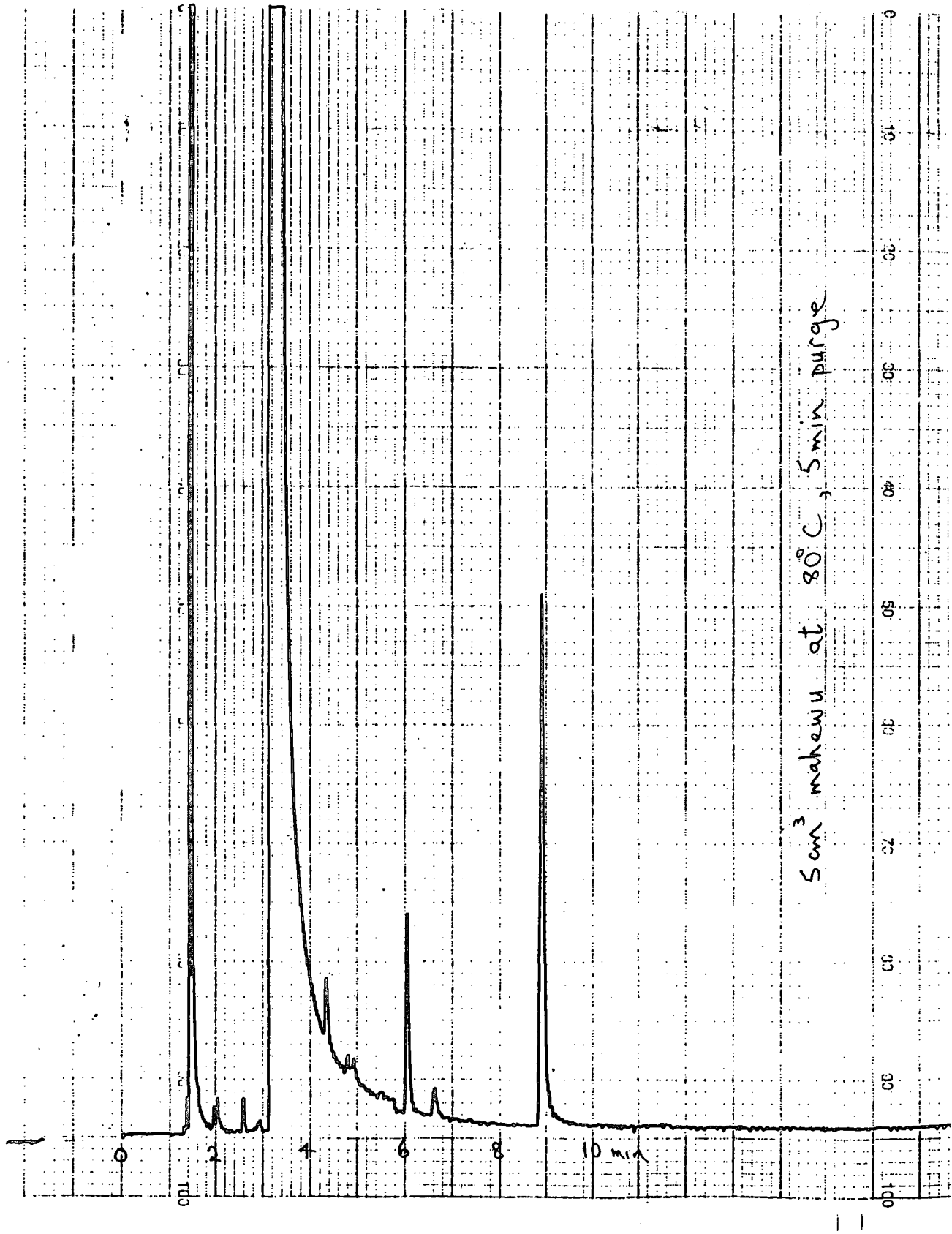

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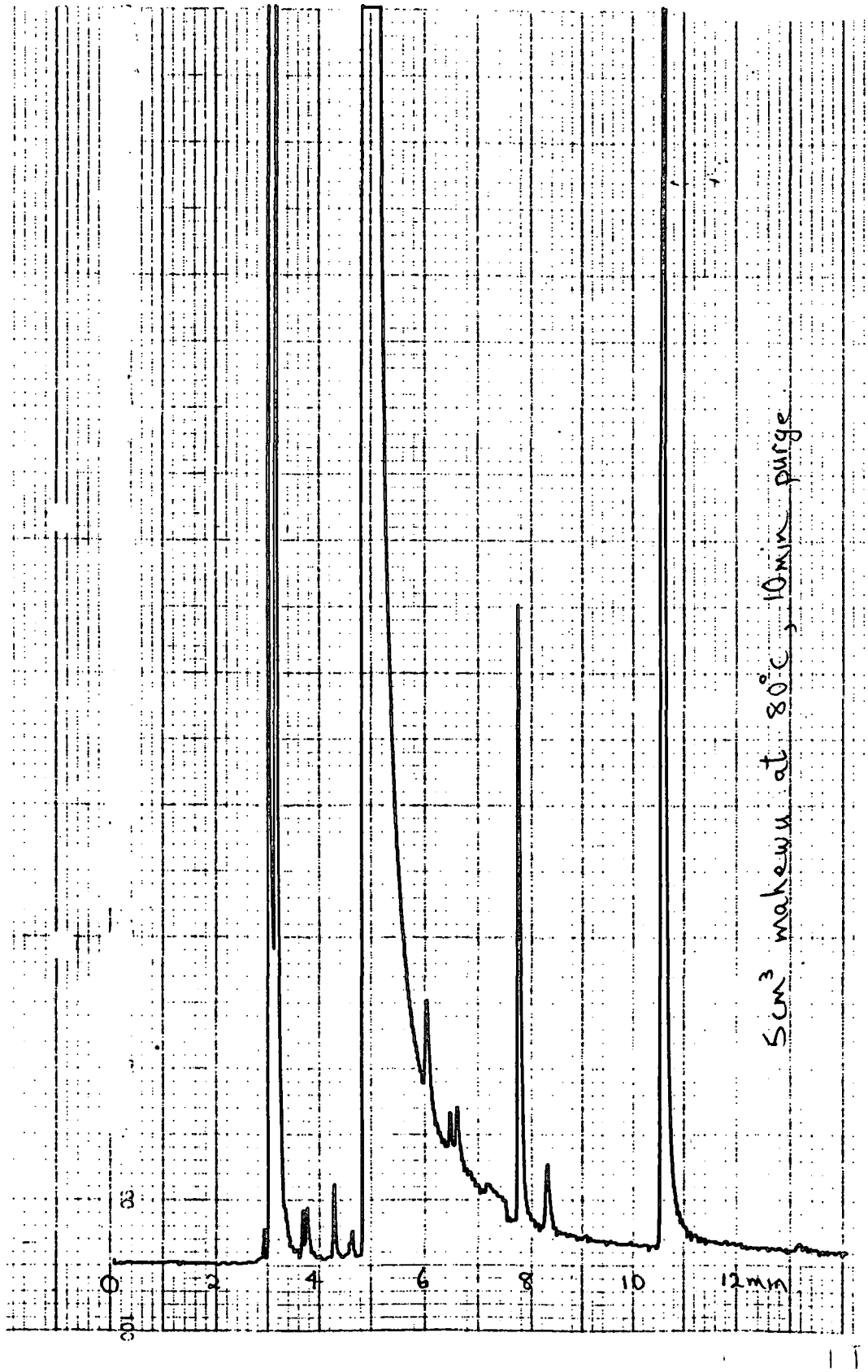

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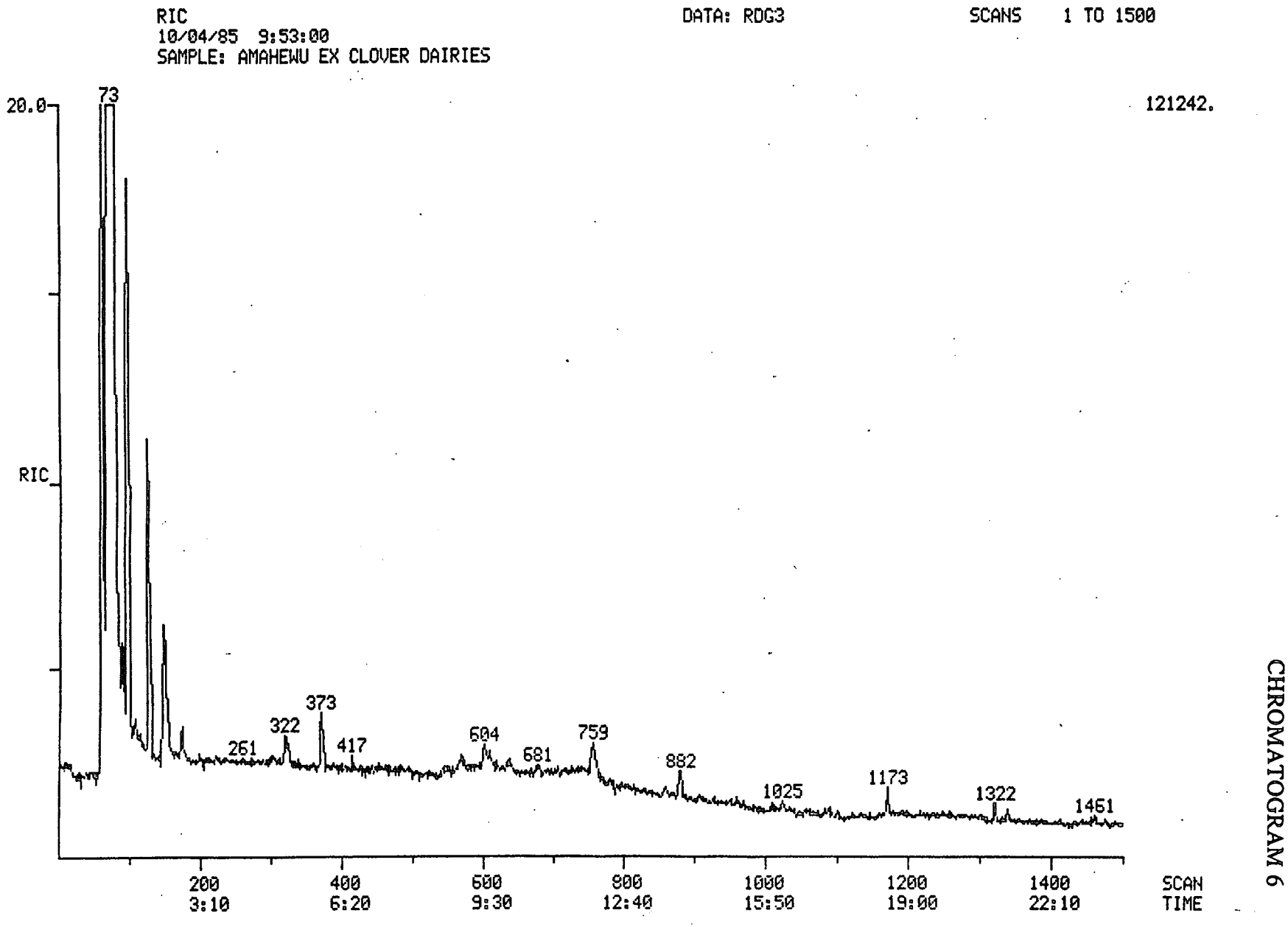

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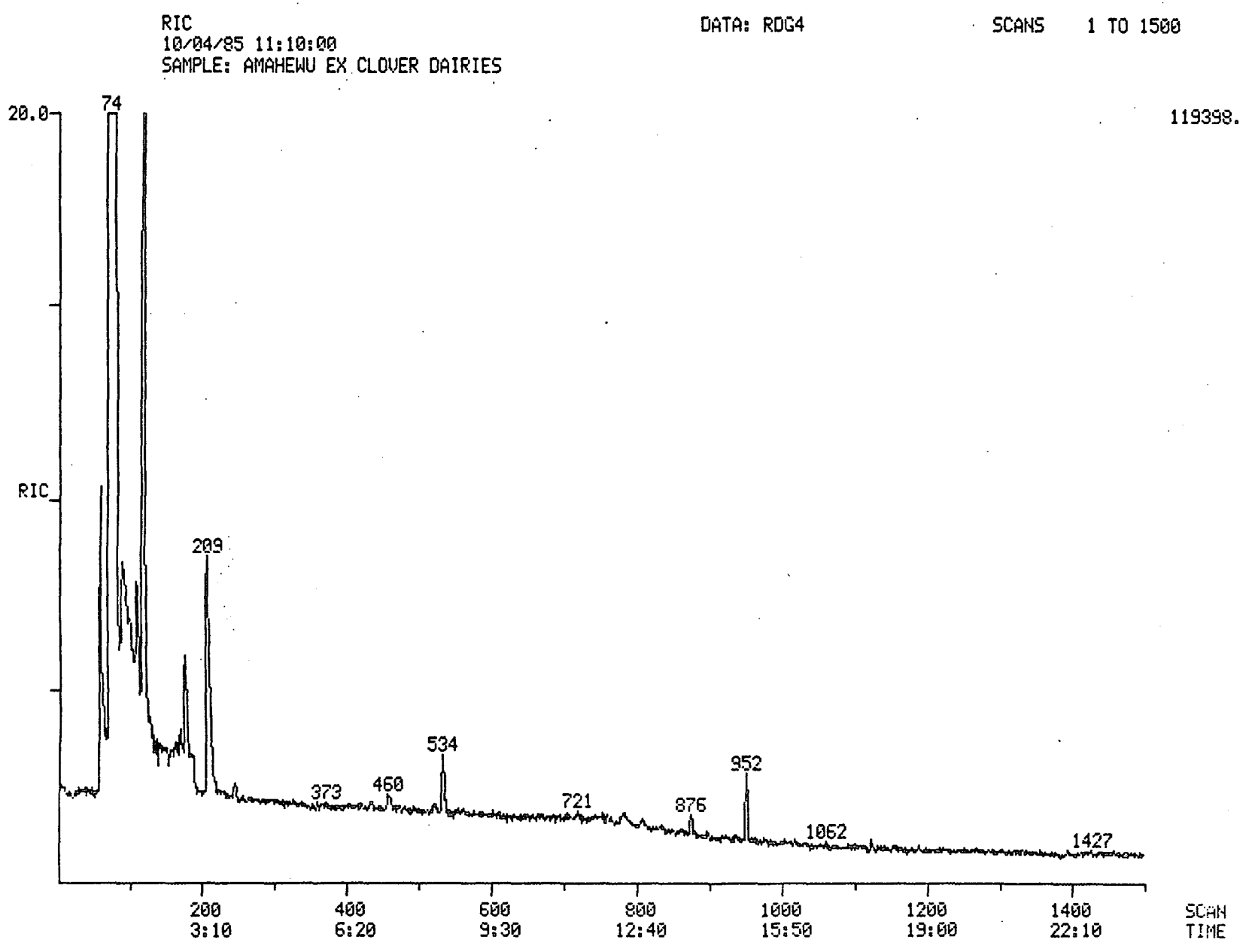

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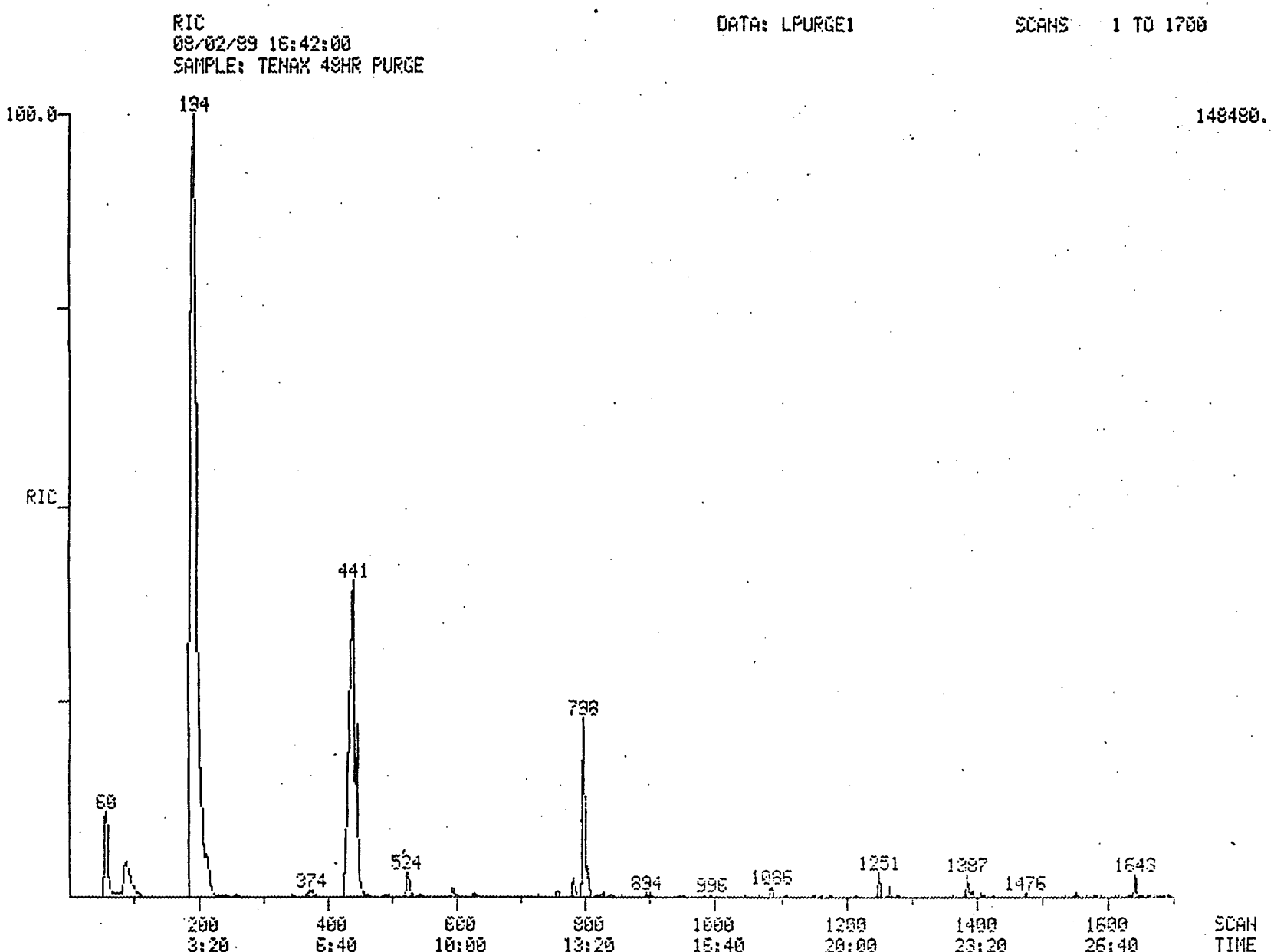

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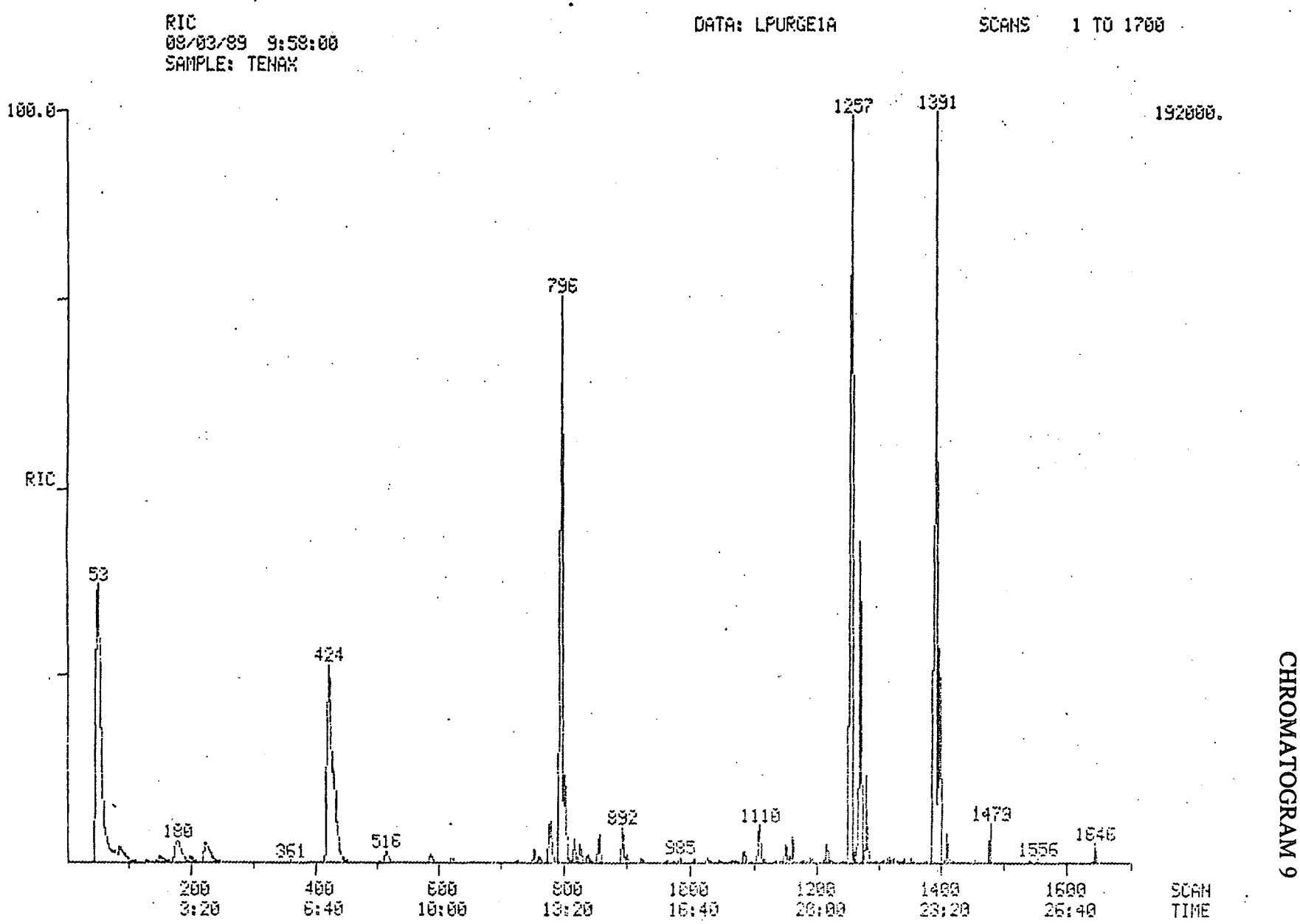

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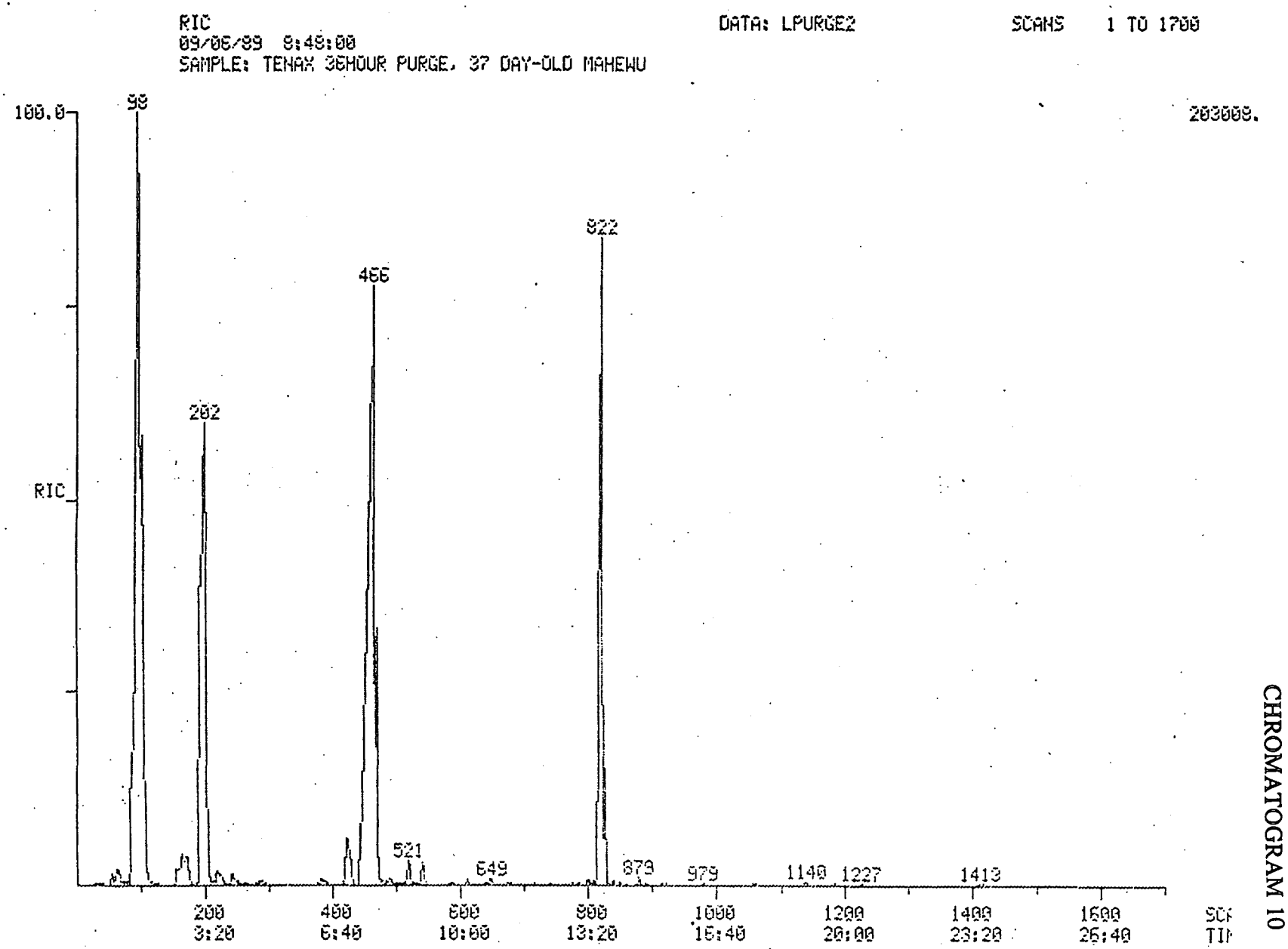

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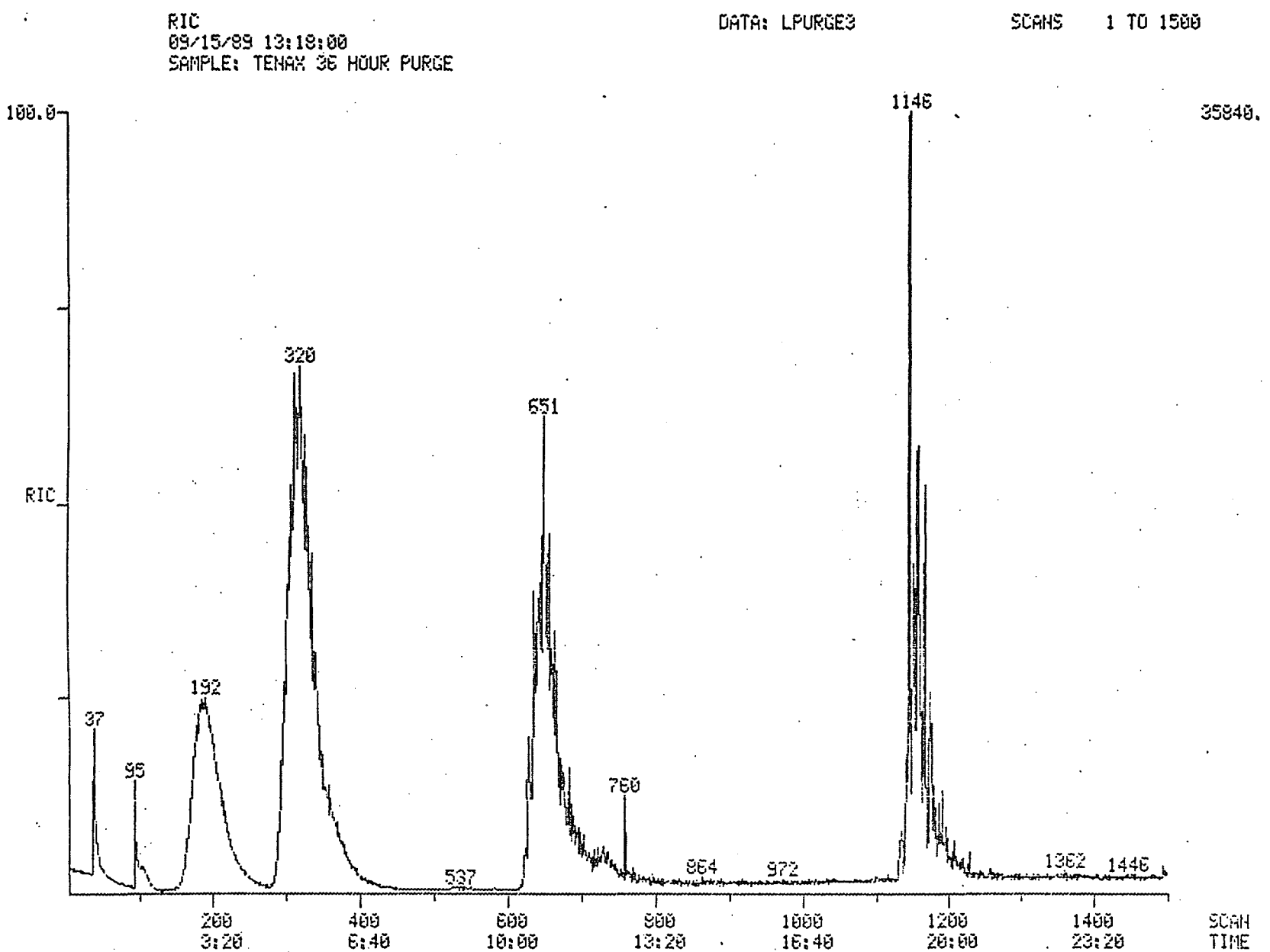

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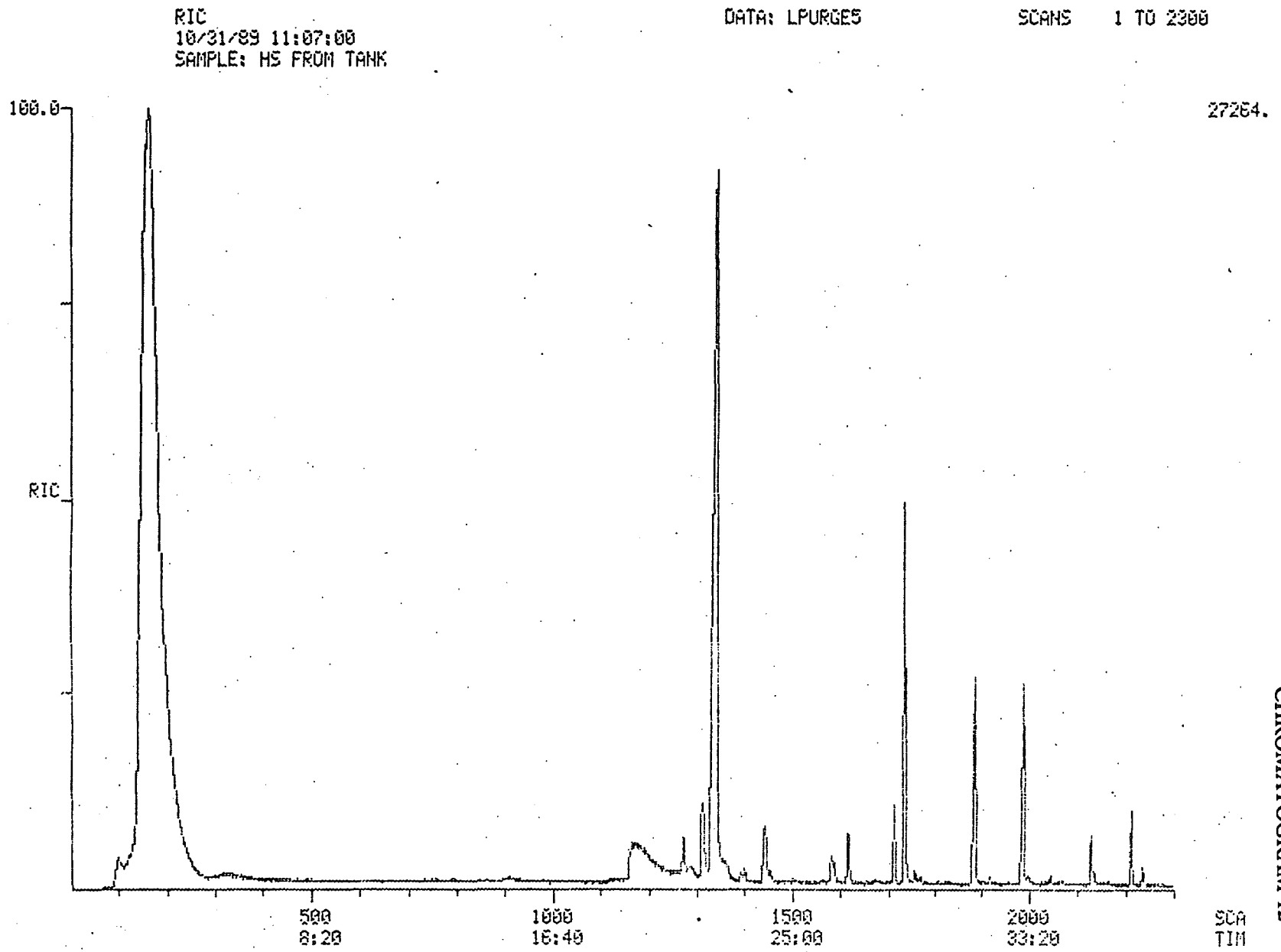
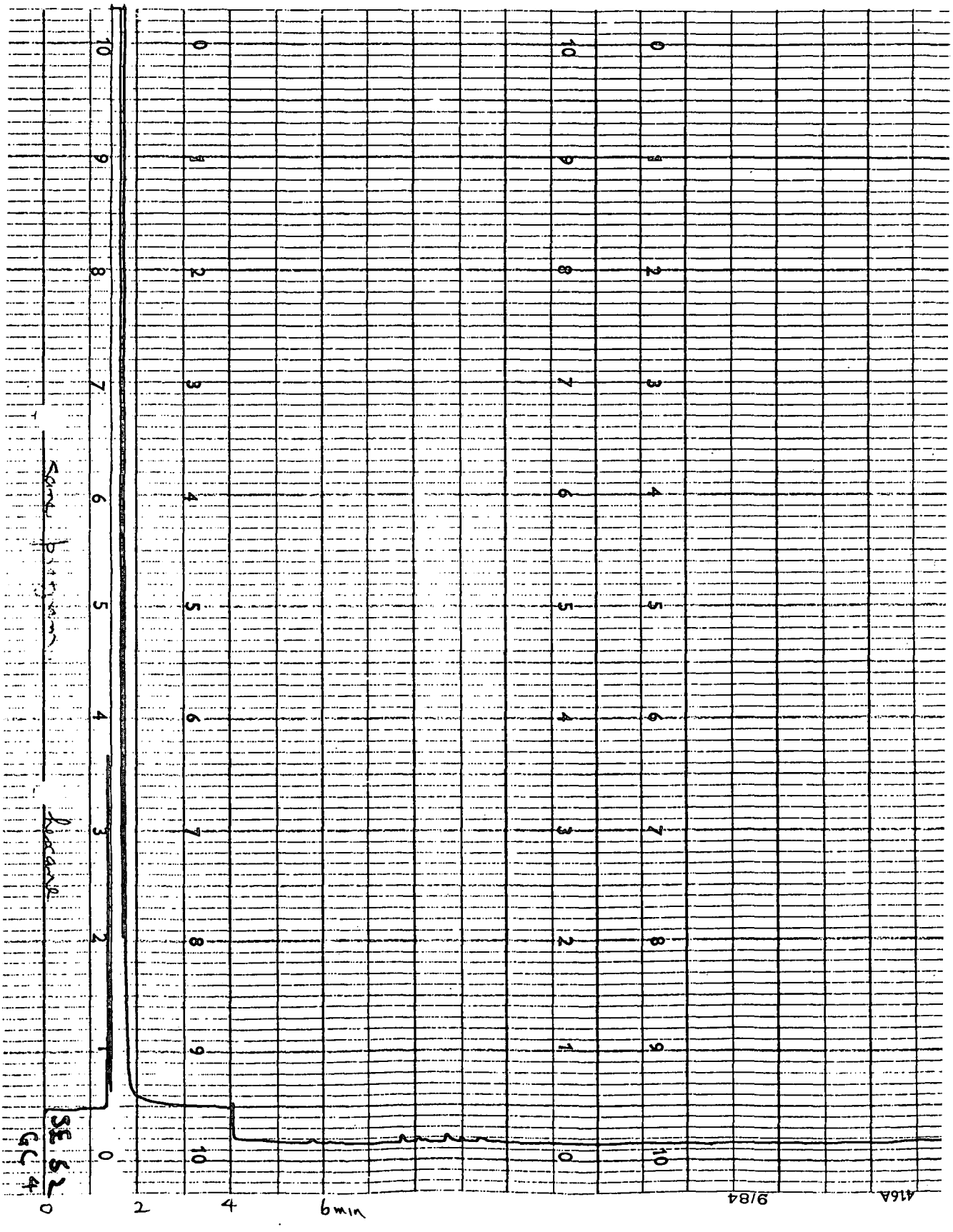


CHROMATOGRAM 12

CHROMATOGRAM 13

CHROMATOGRAM 14



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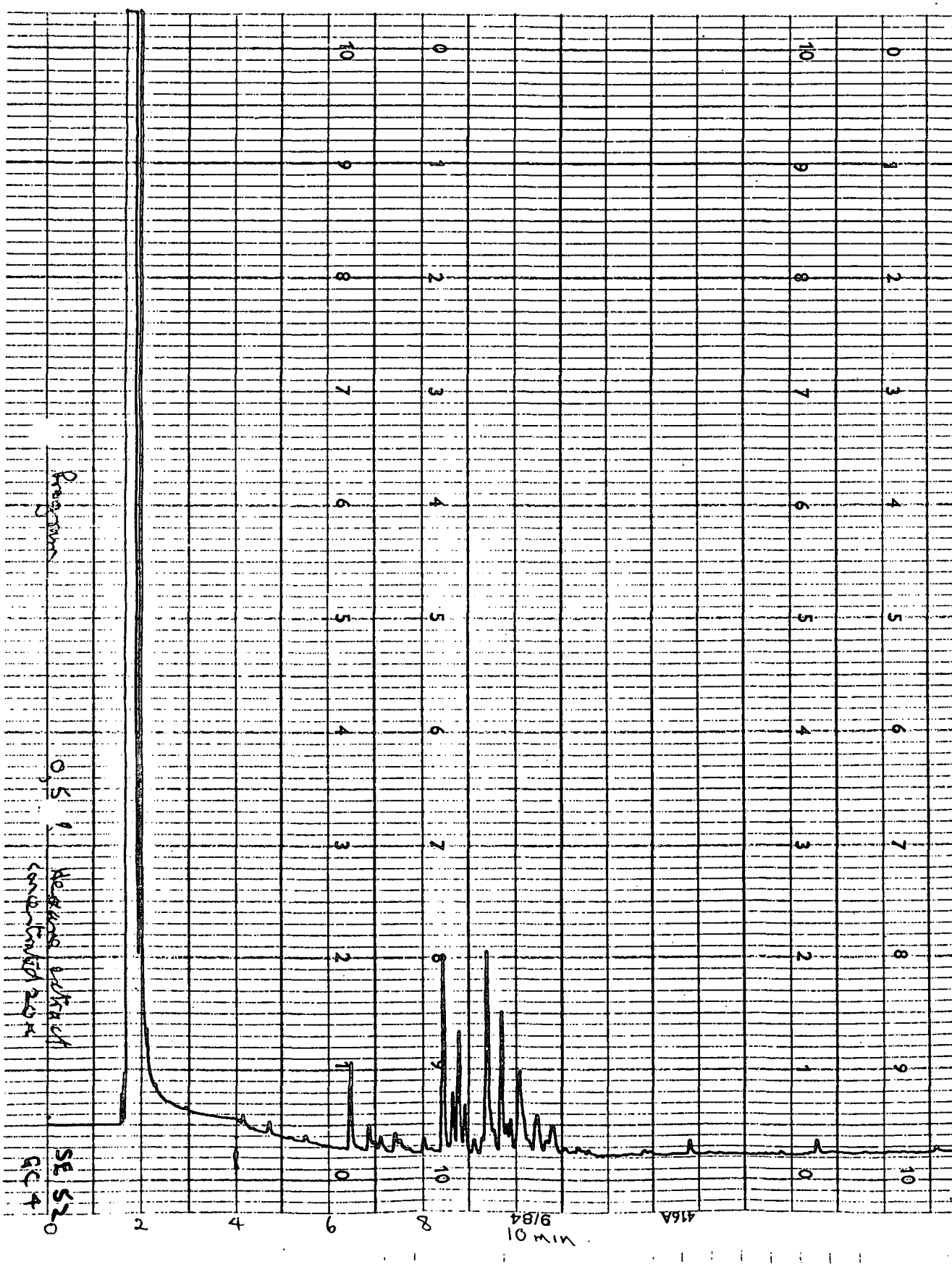

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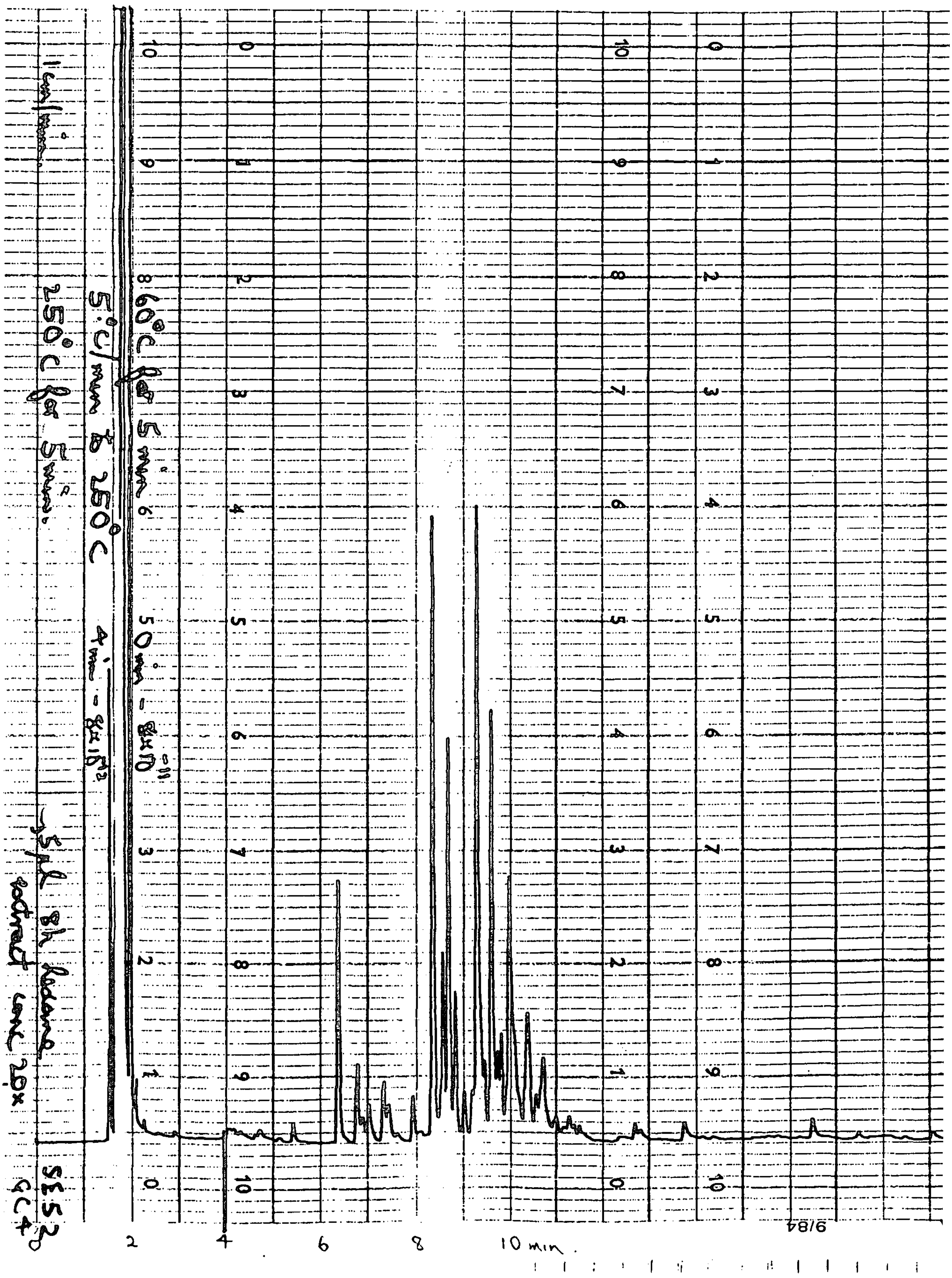
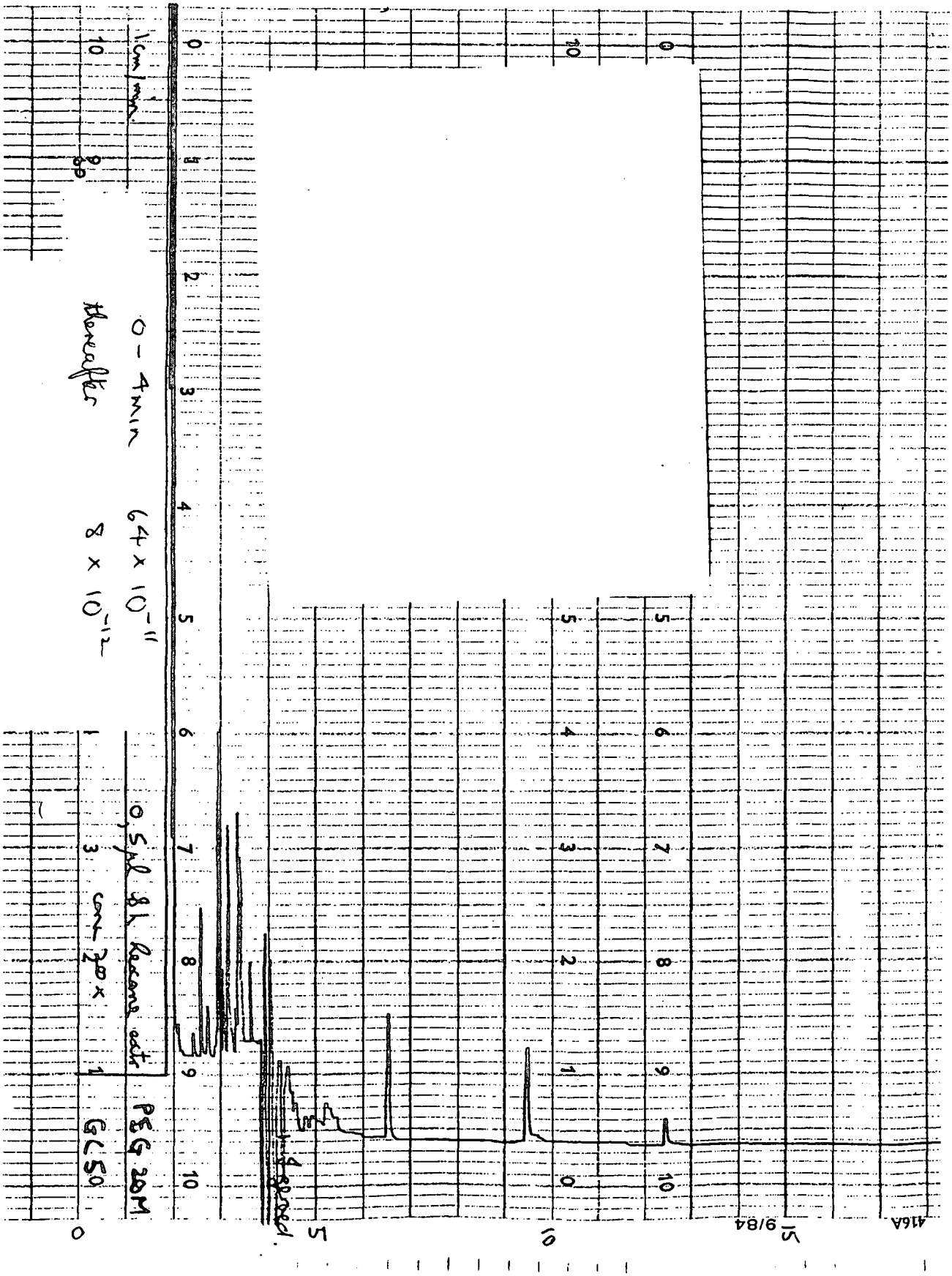


CHROMATOGRAM 17

CHROMATOGRAM 18



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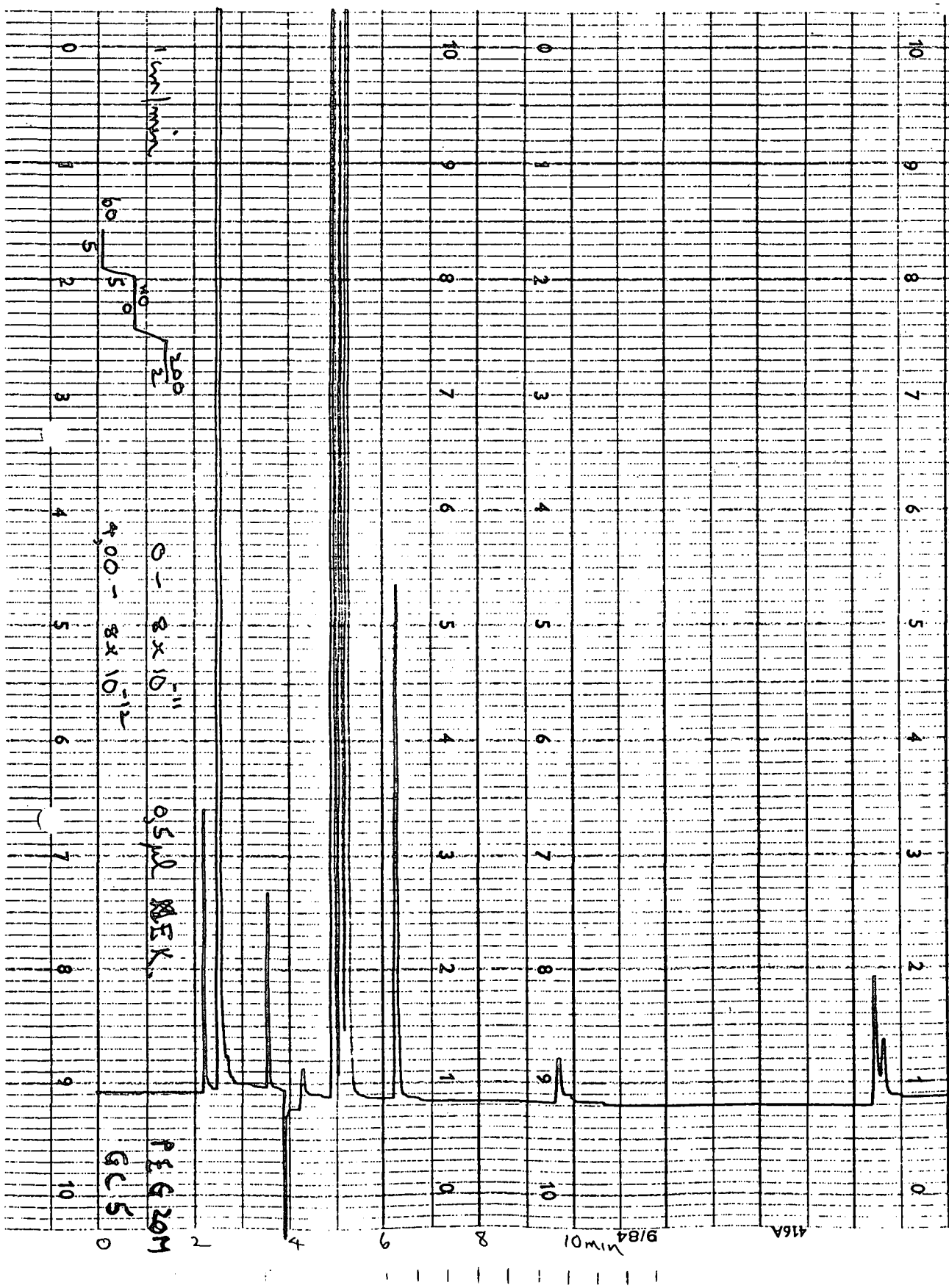

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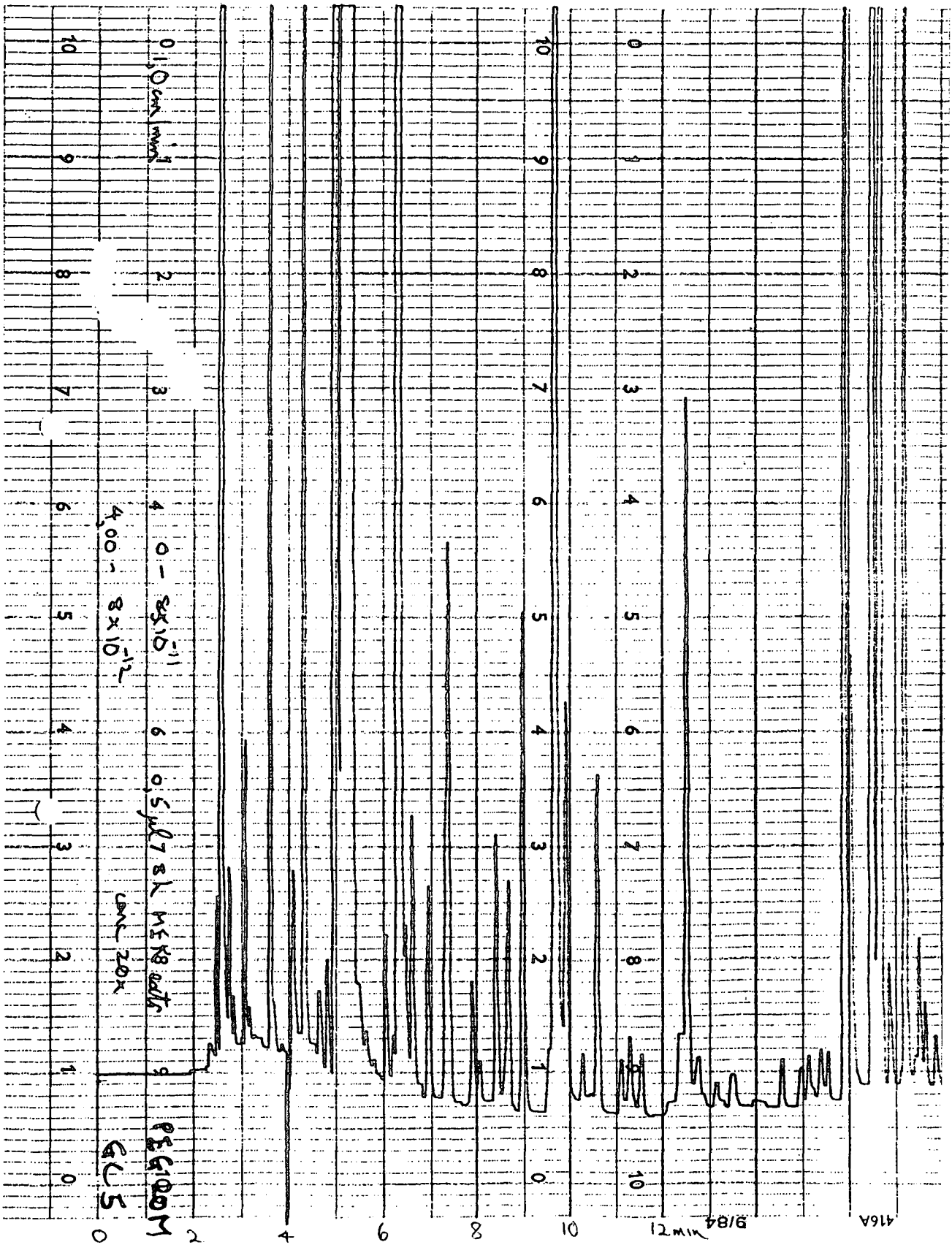

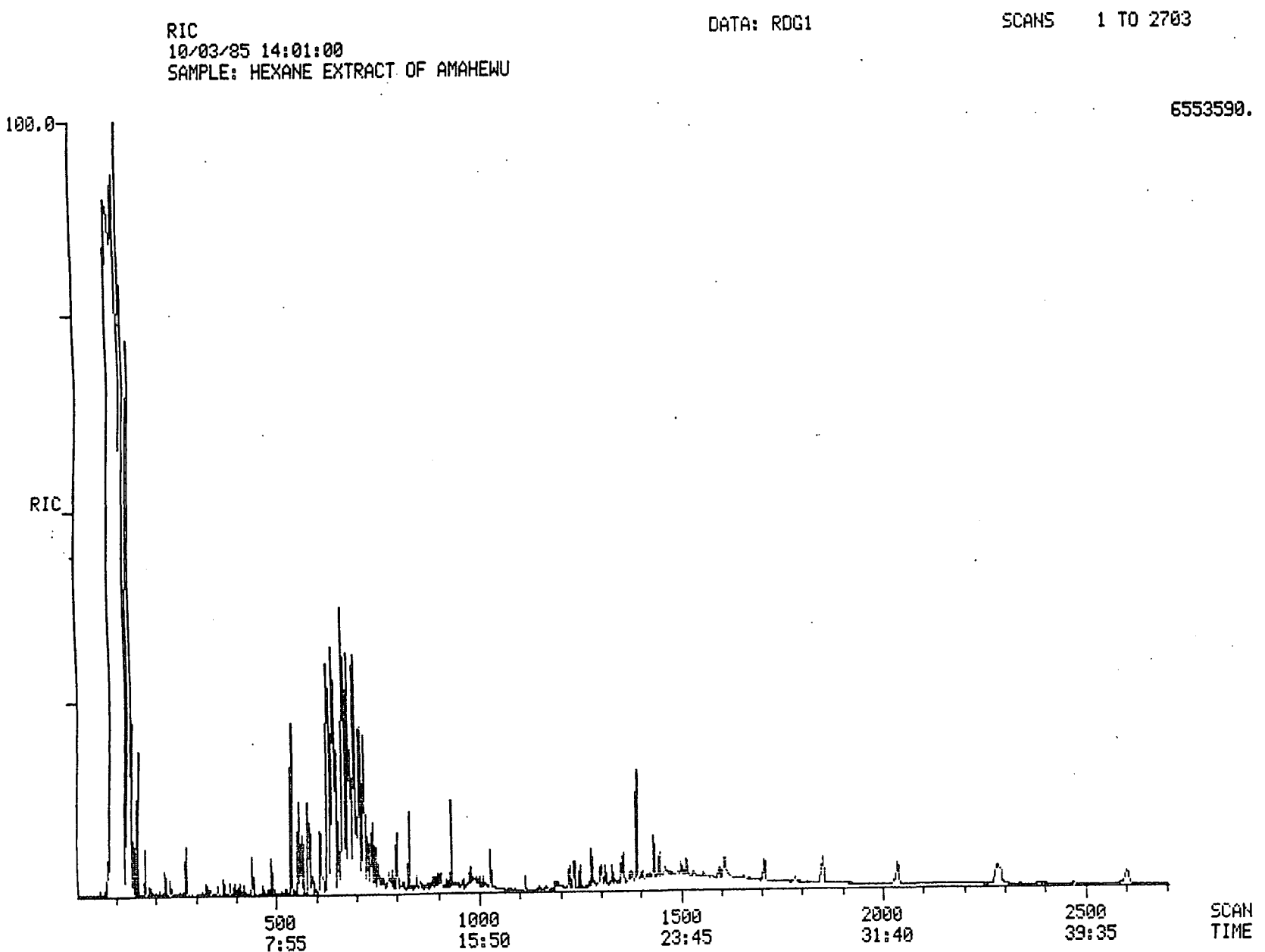

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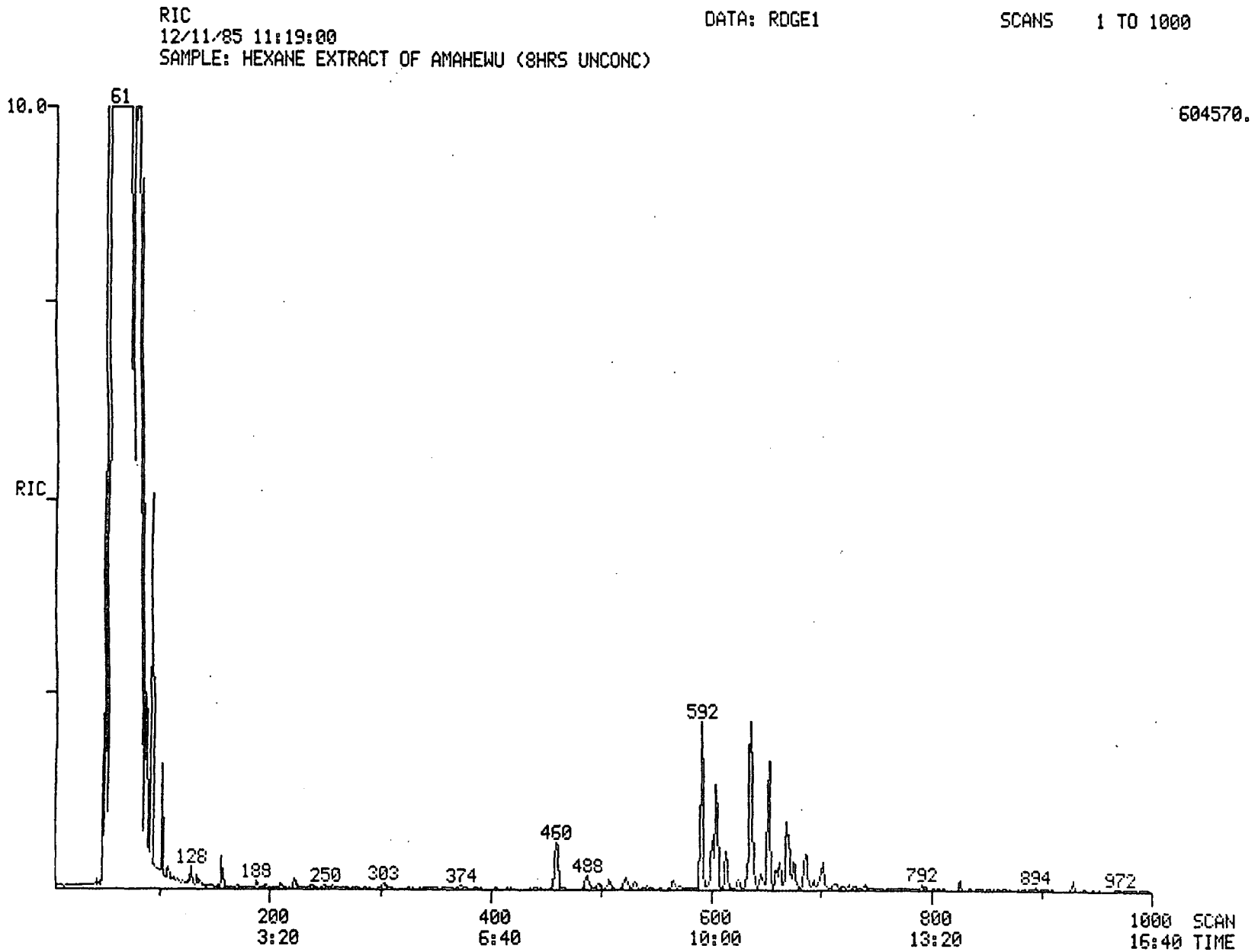

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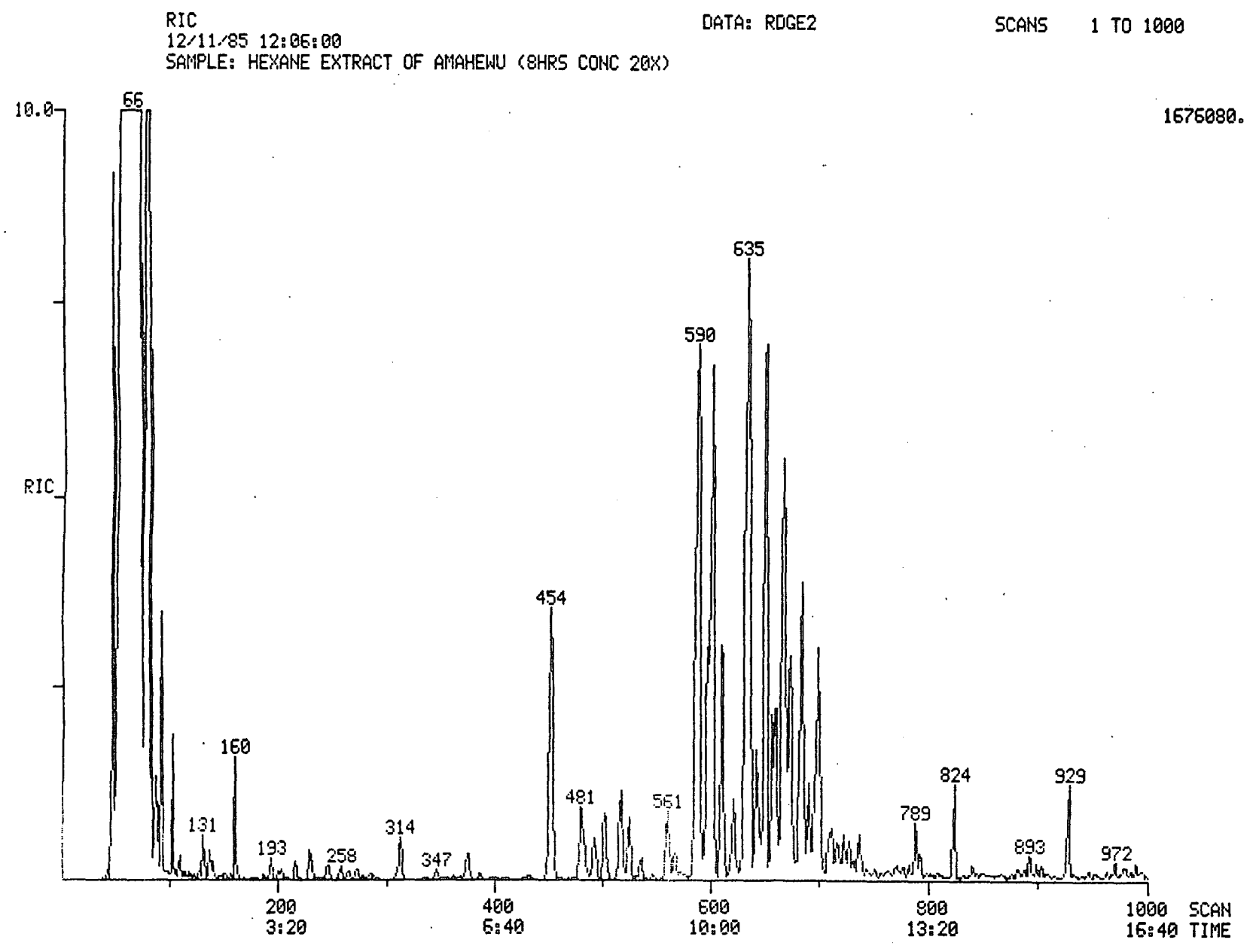

CHROMATOGRAM 23

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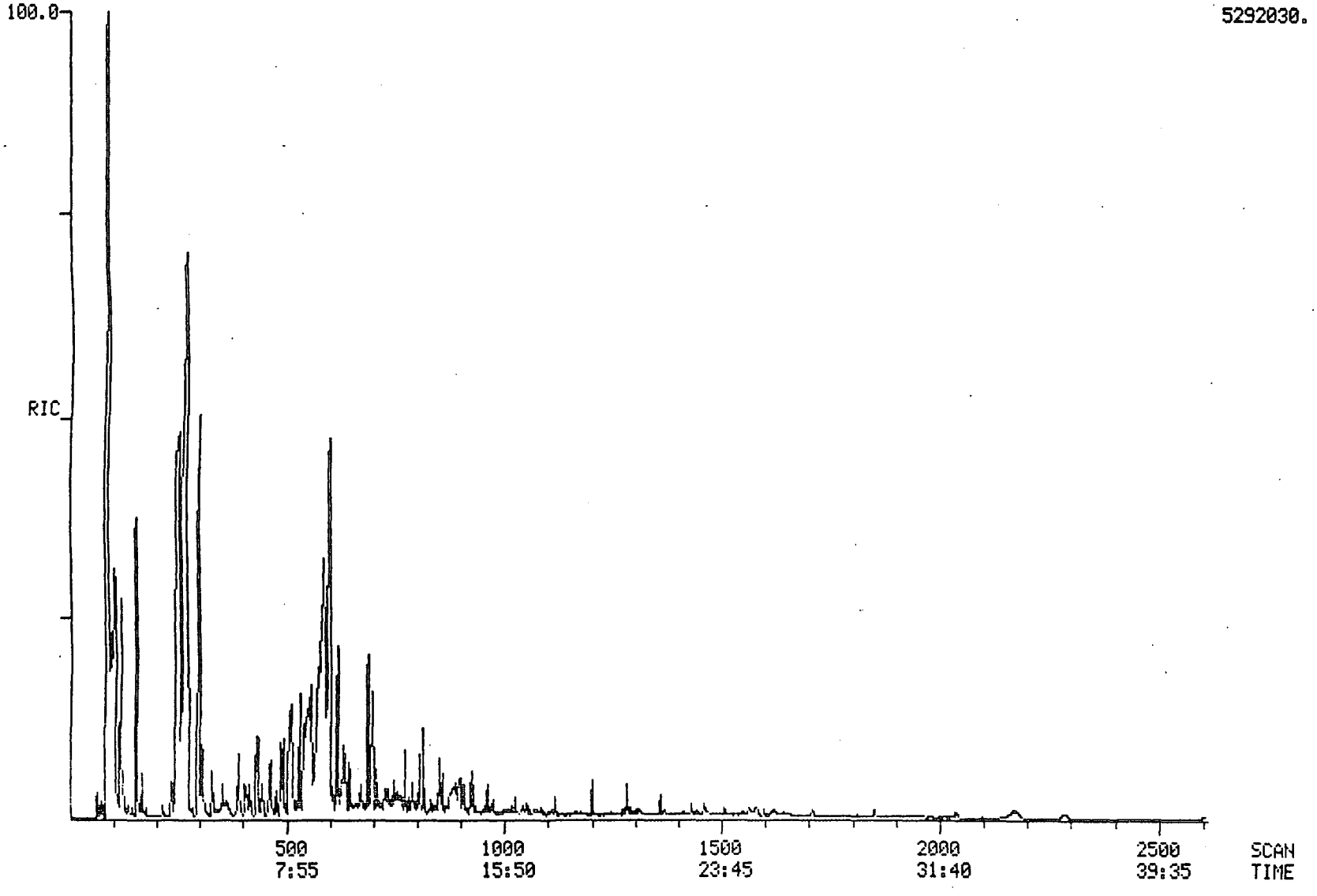
10/03/85 14:59:00
SAMPLE: MEK EXTRACT OF AMAHEWU

DATA: RDG2

SCANS 1 TO 2609



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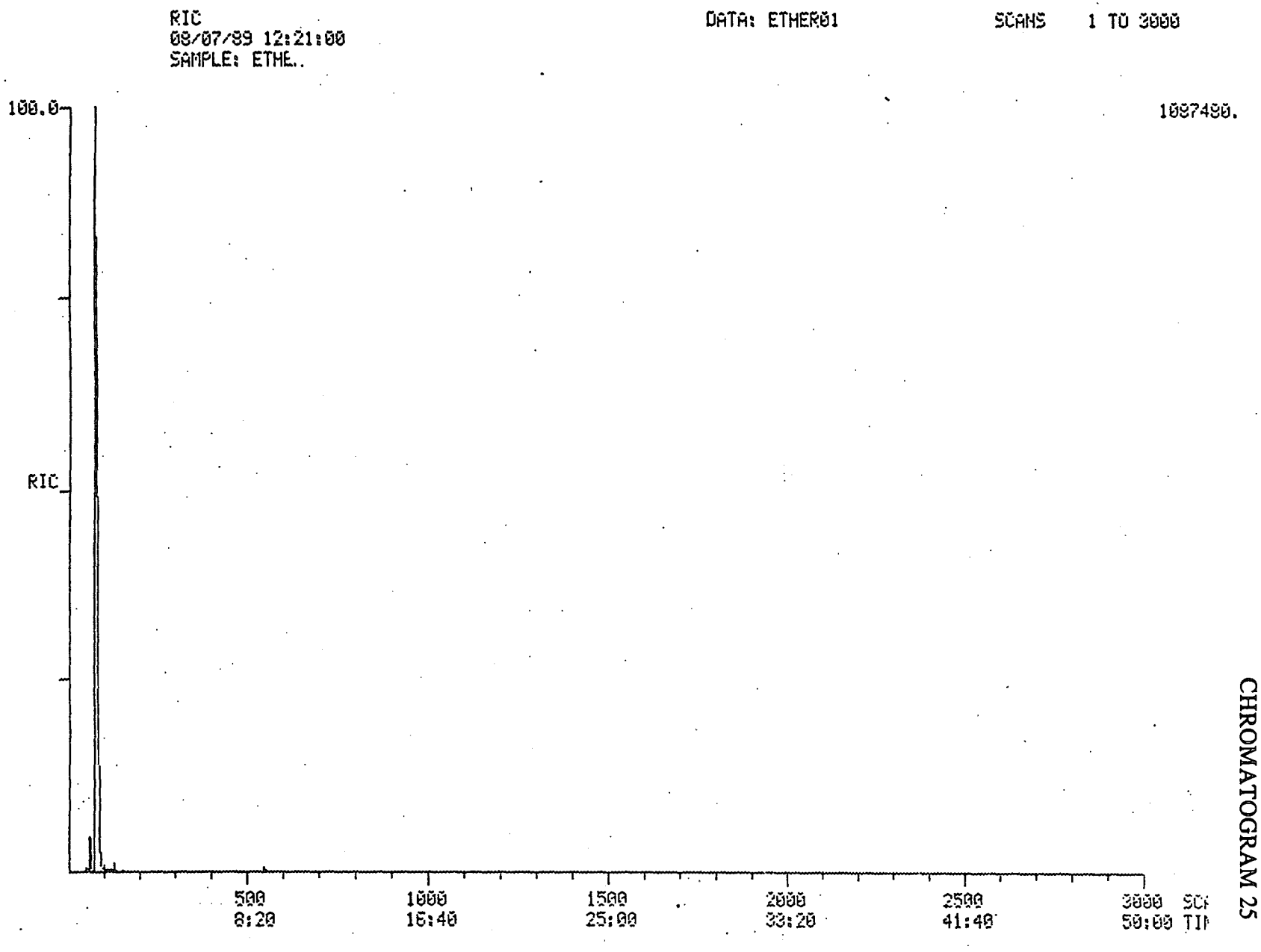

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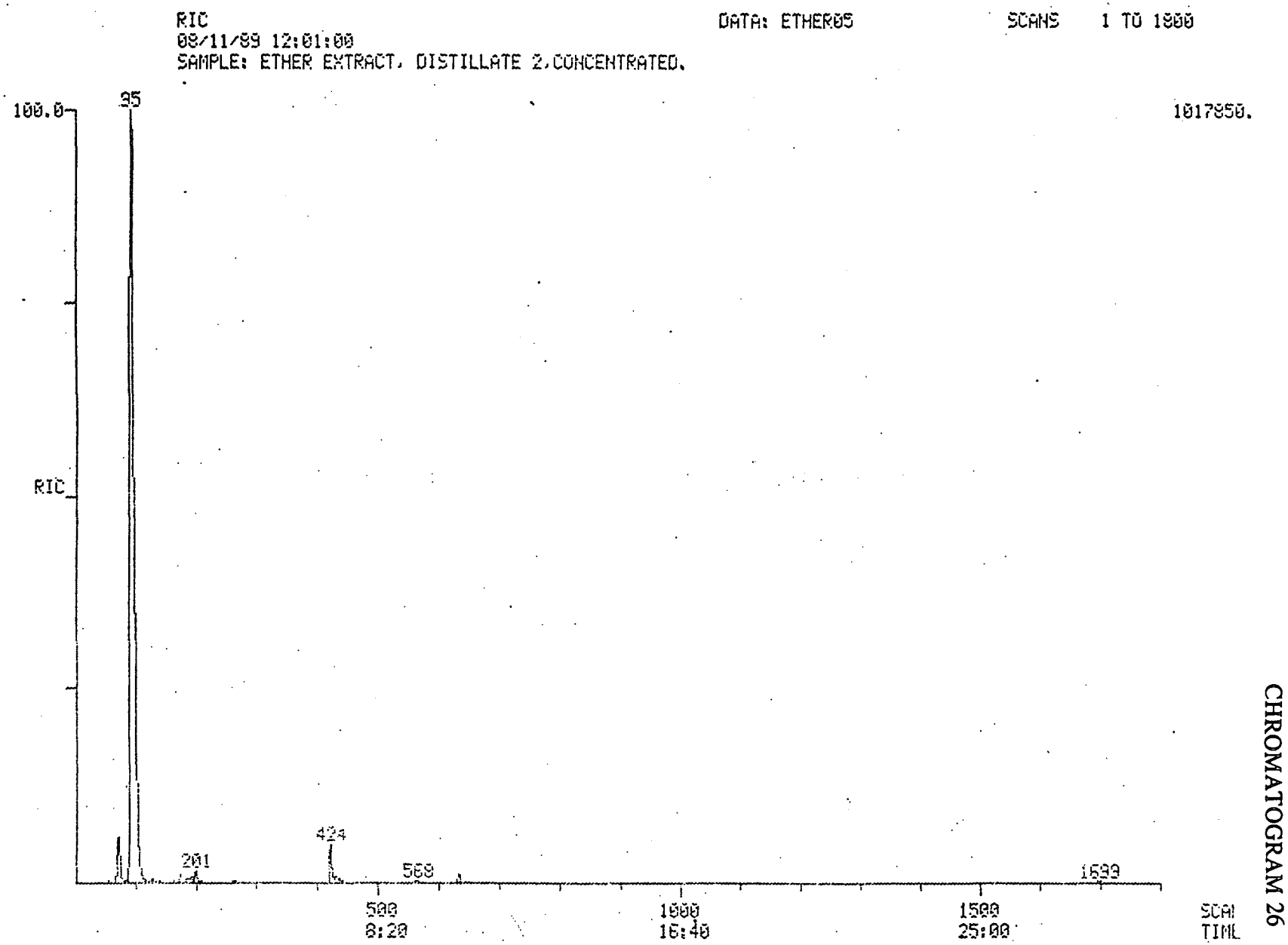

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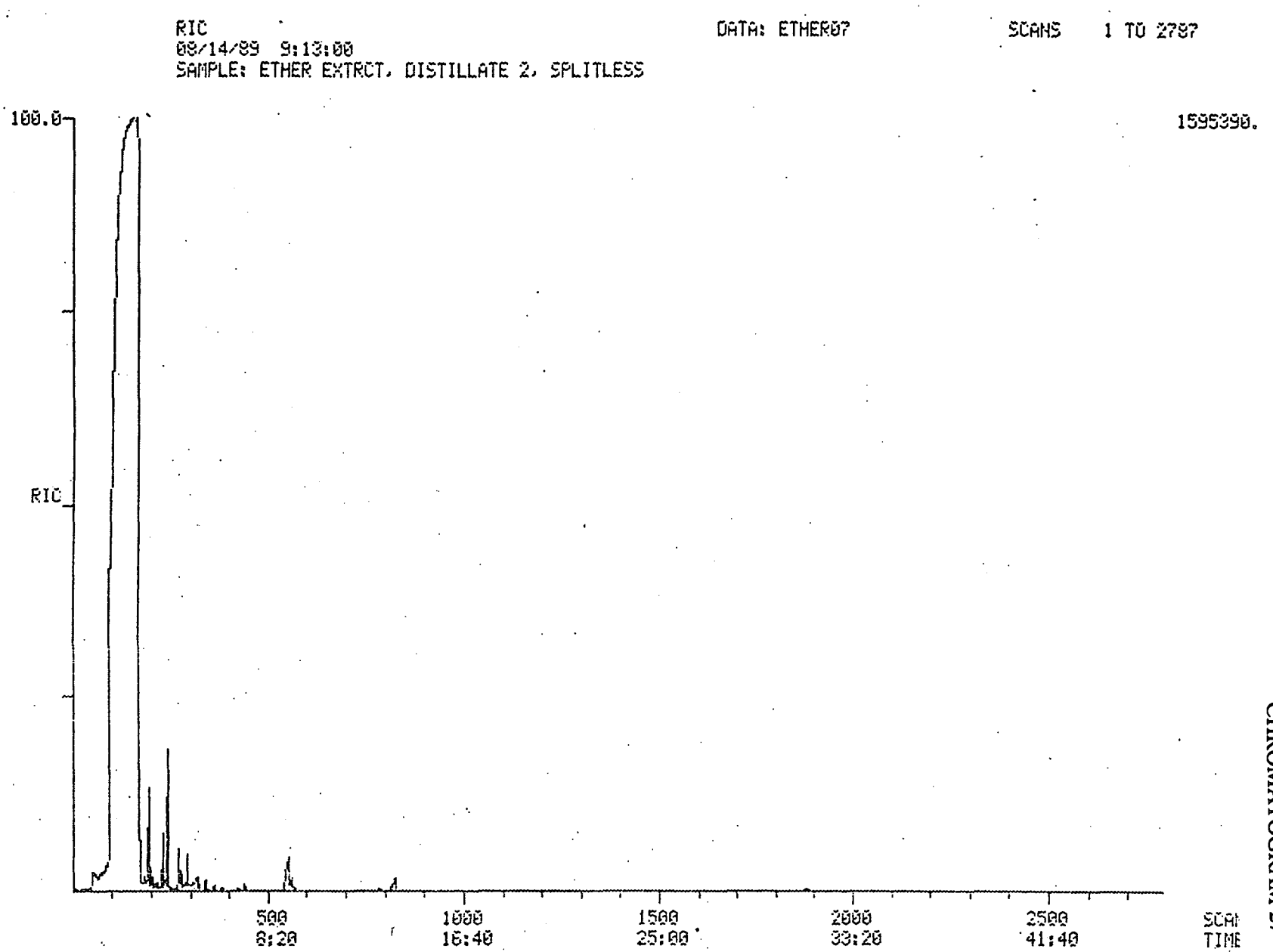

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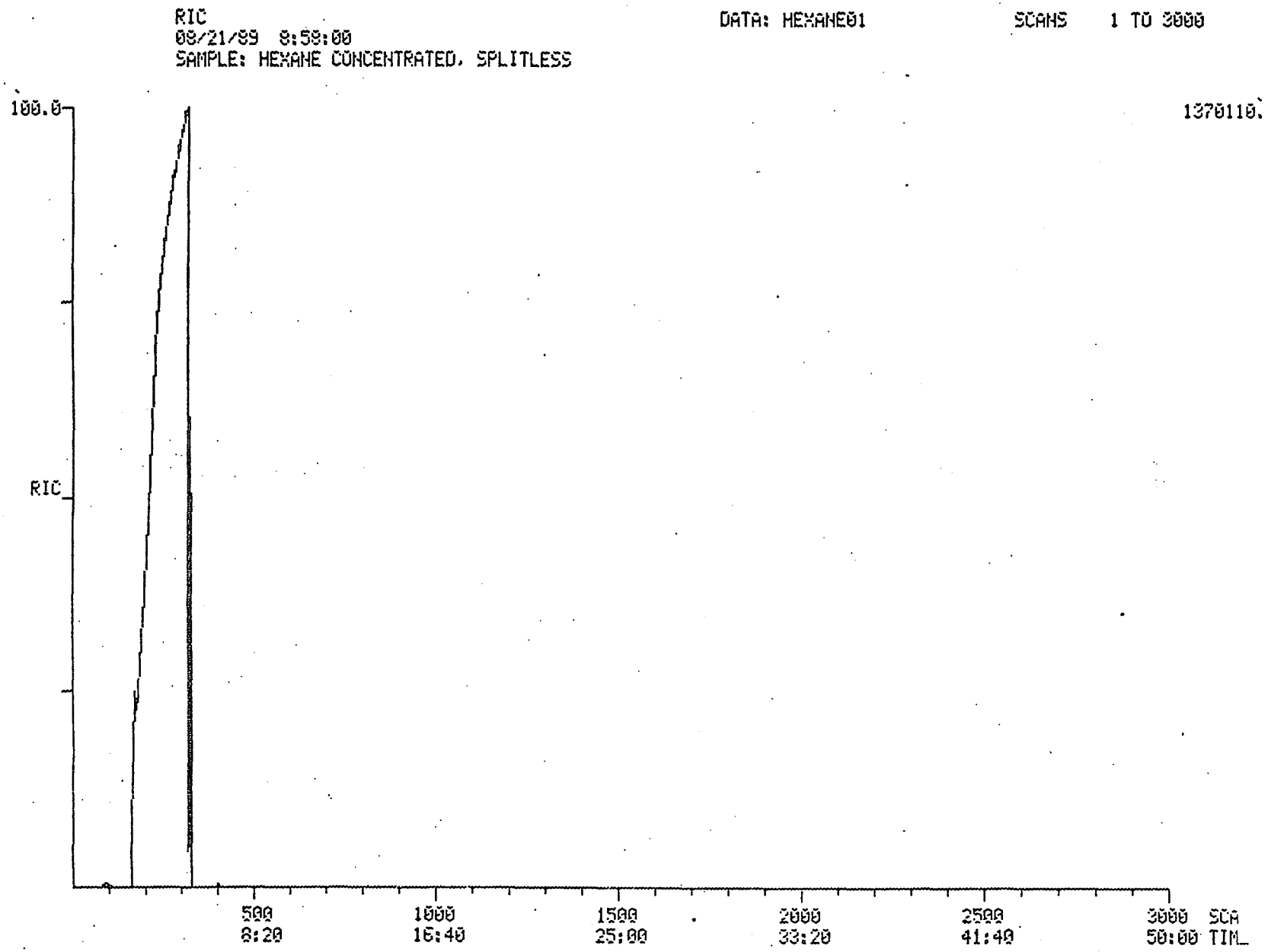

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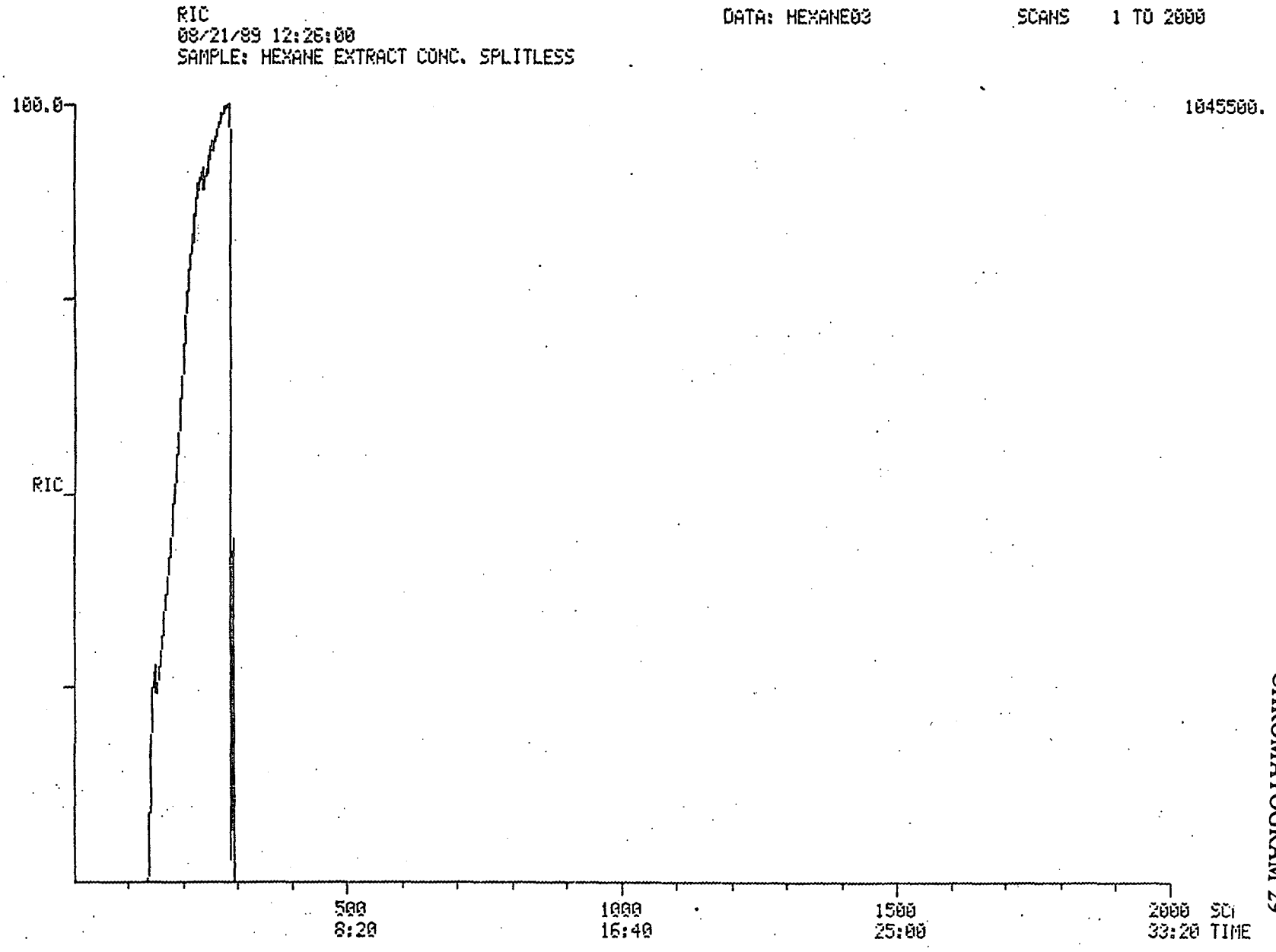

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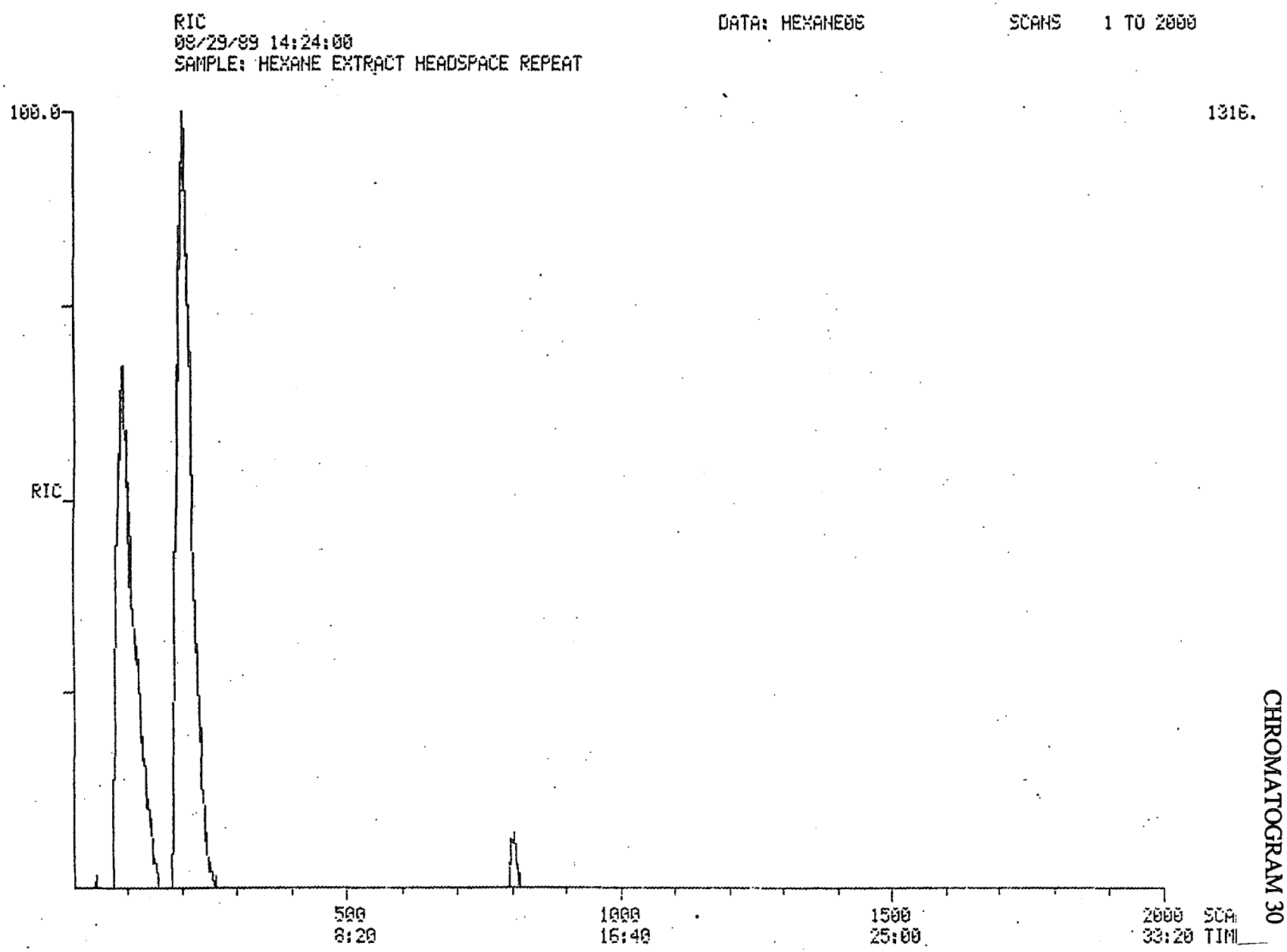
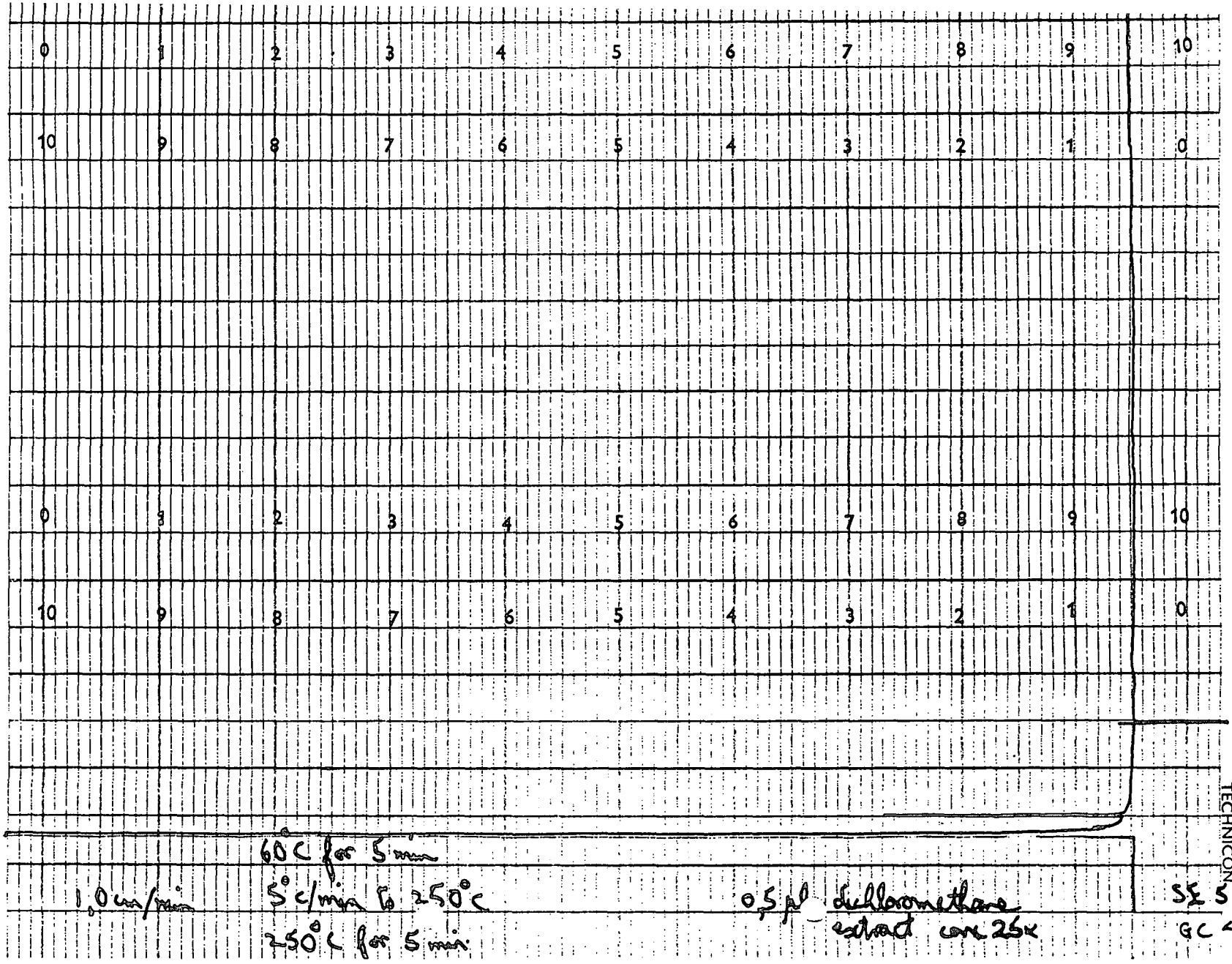


CHROMATOGRAM 30

0,5 µl dichloromethane
SE 52
GC 4
9/84
416A

CHROMATOGRAM 32



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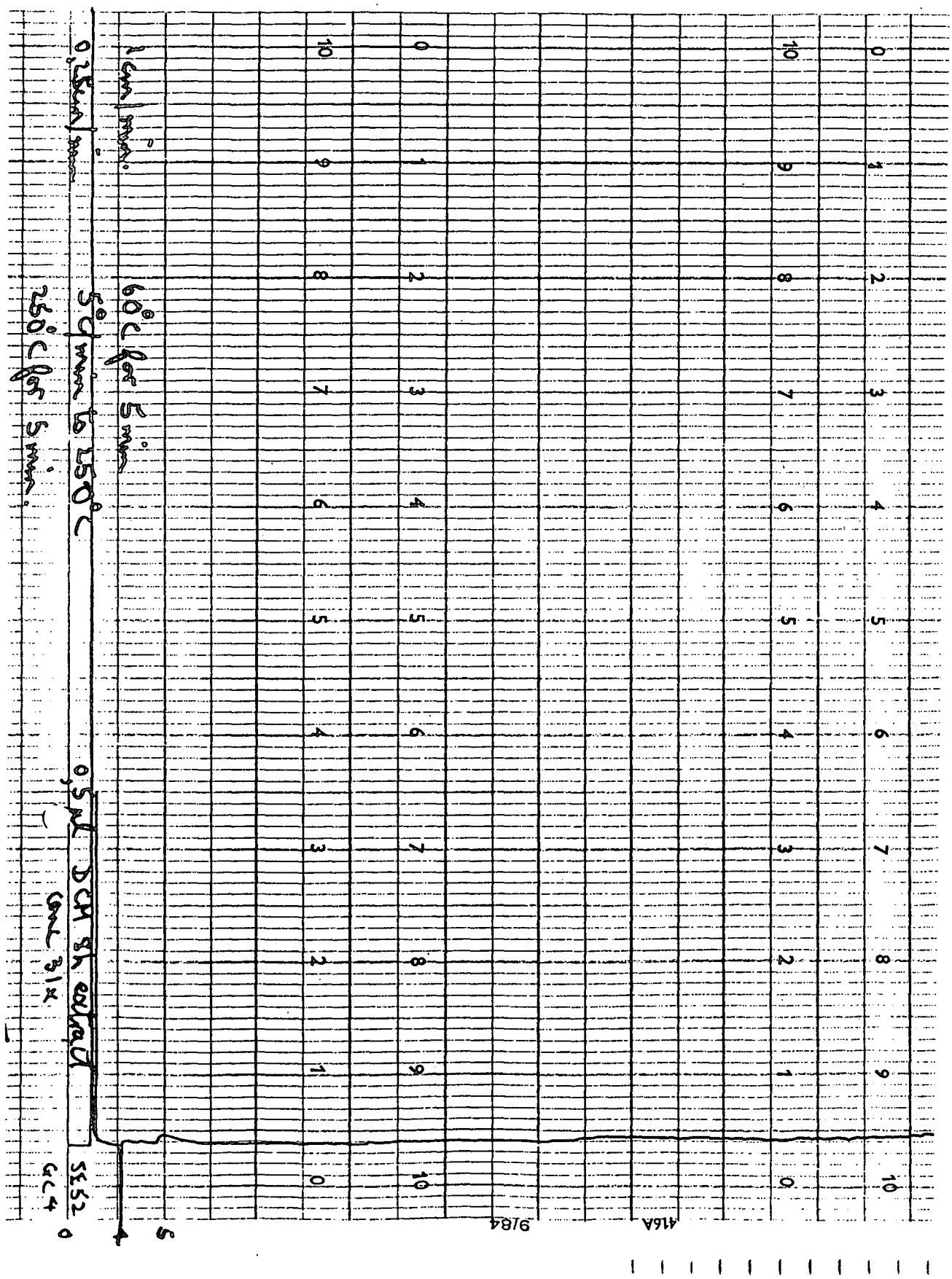

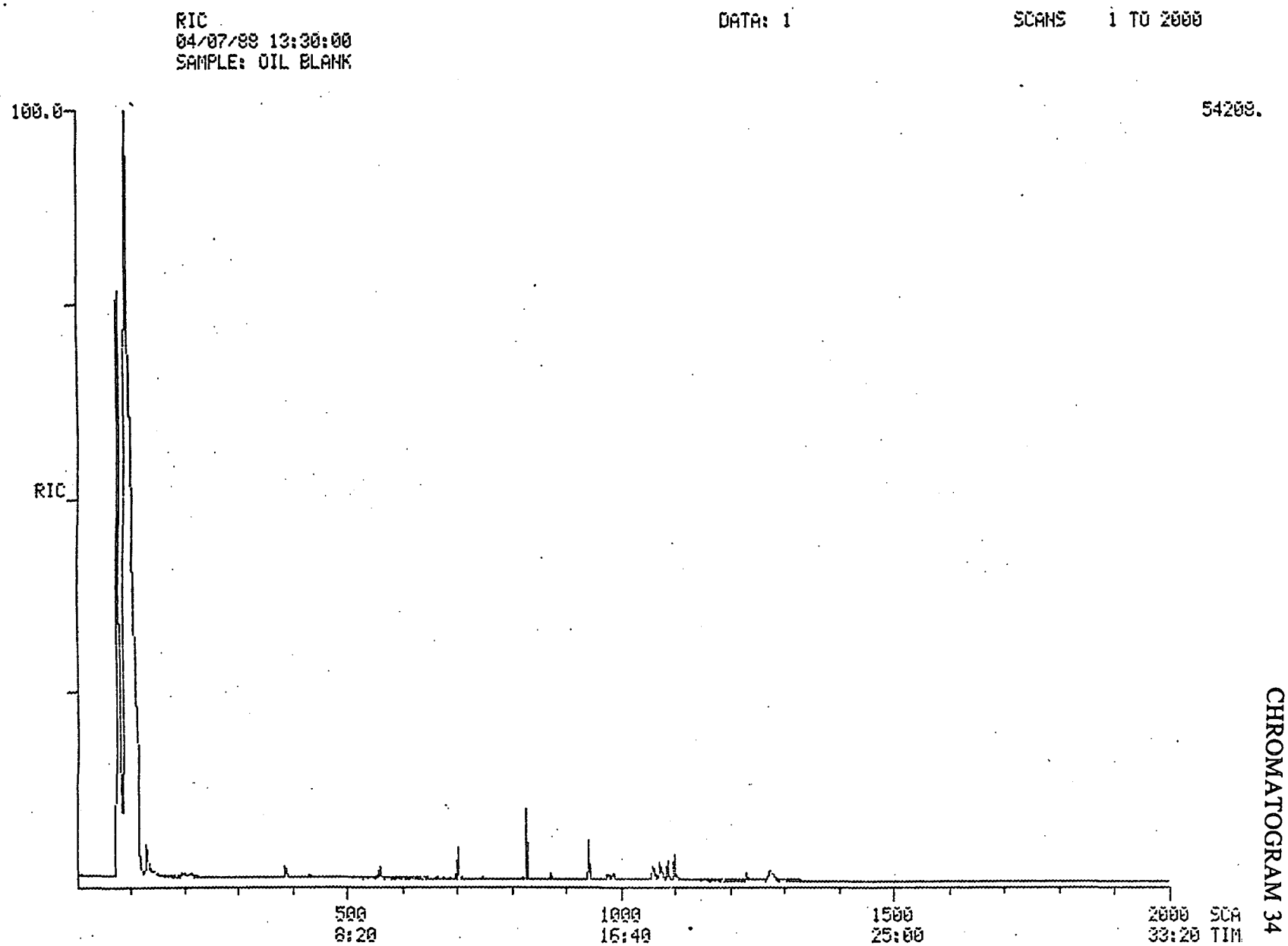

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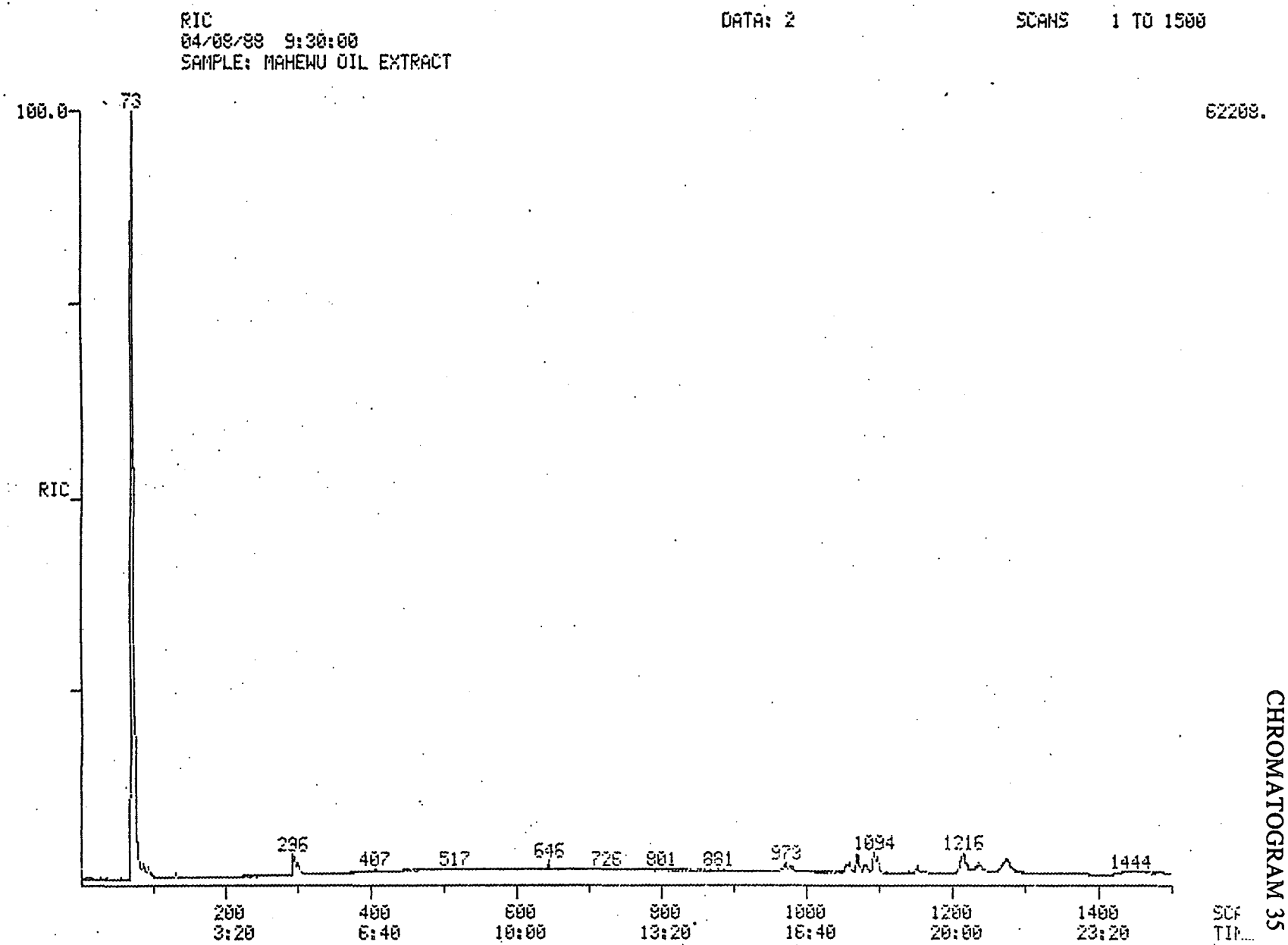

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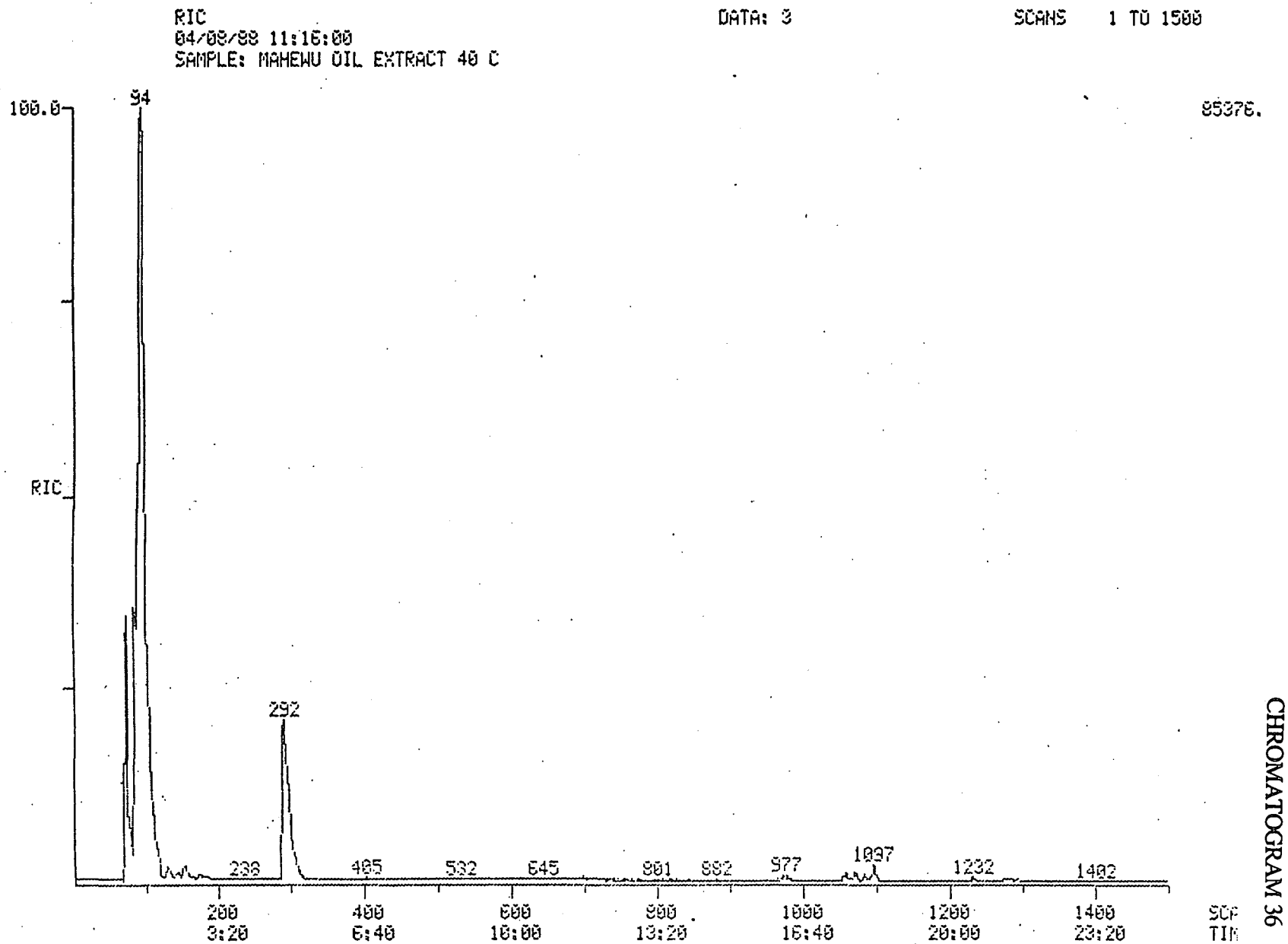

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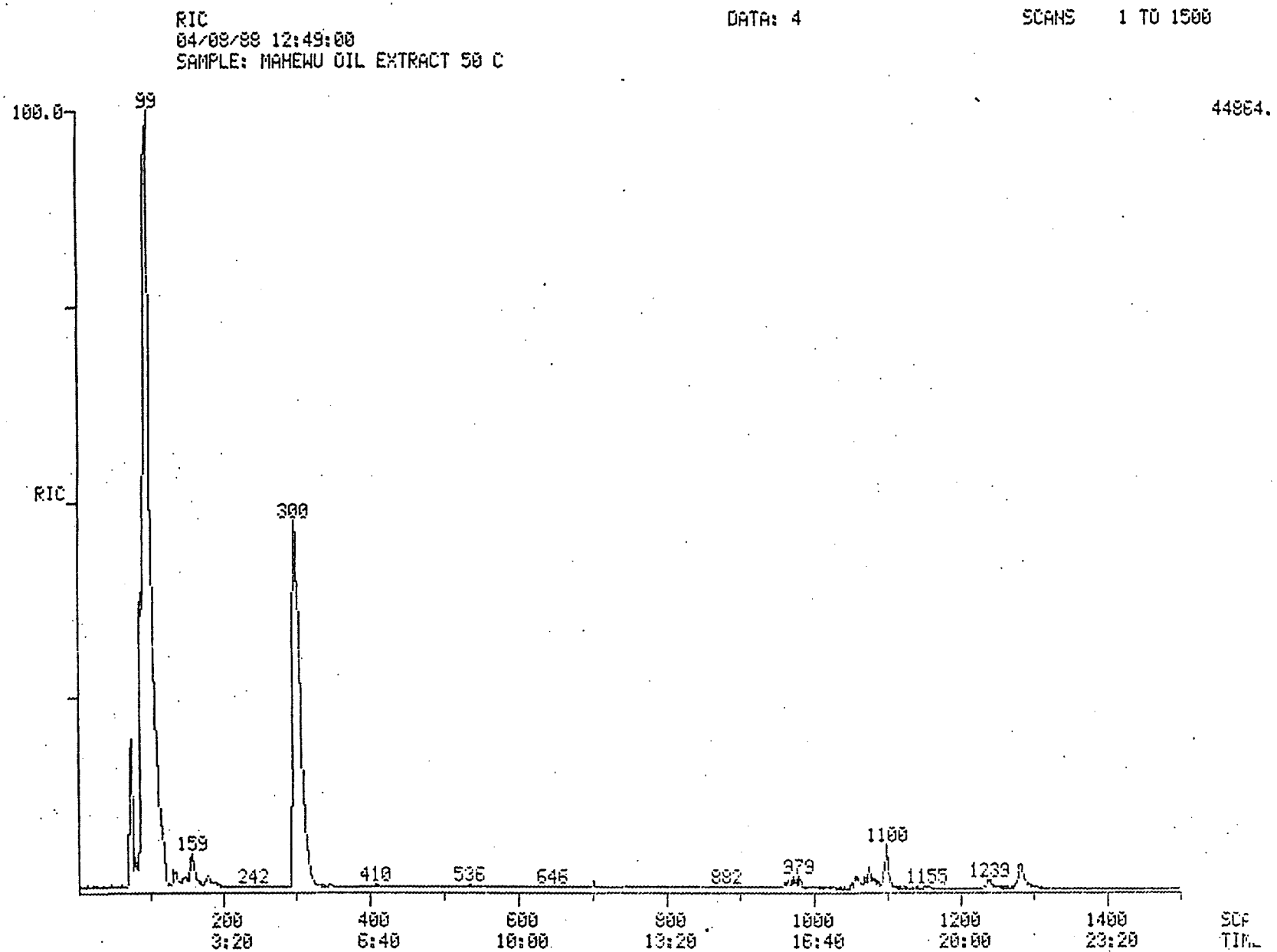

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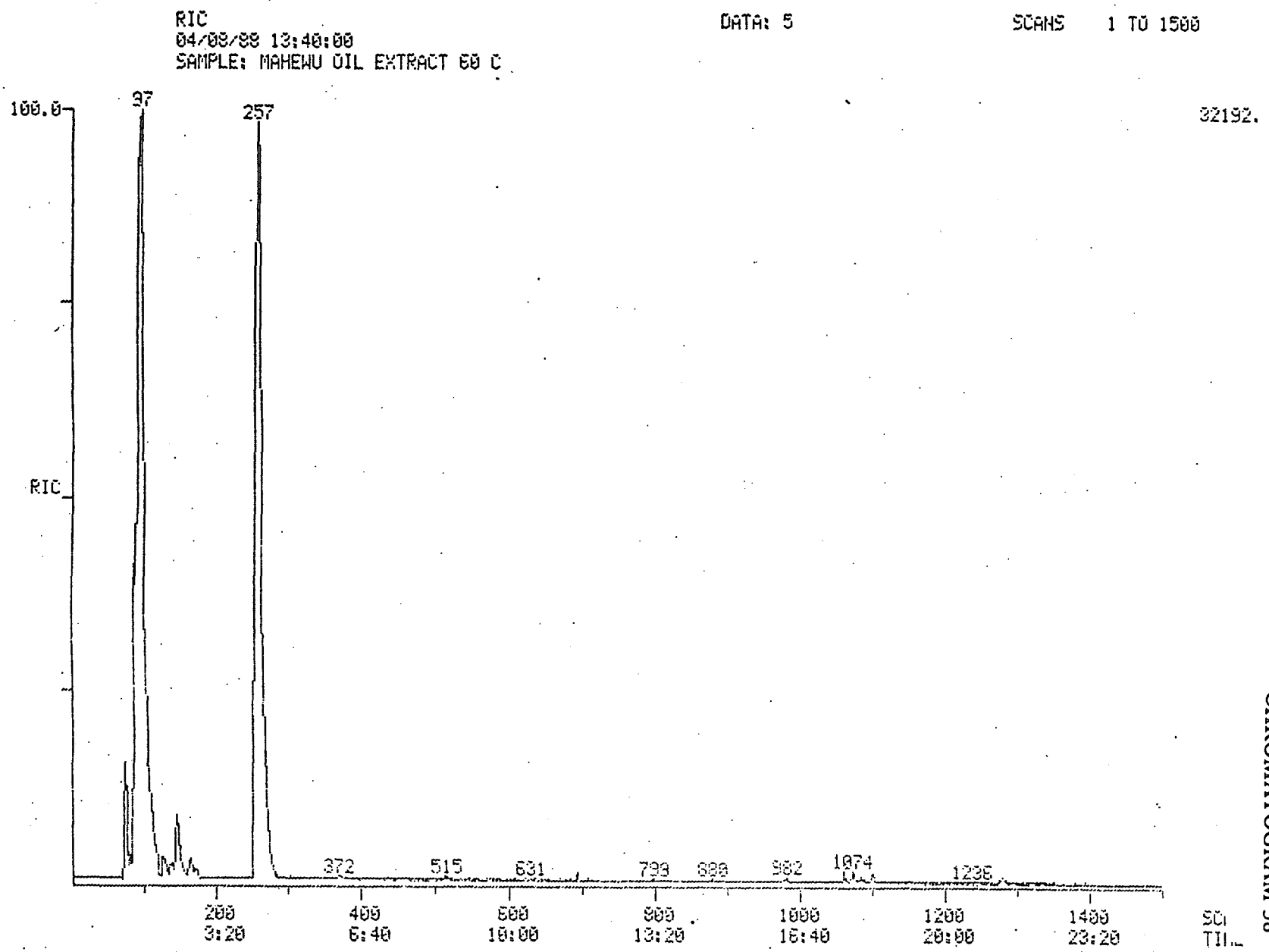

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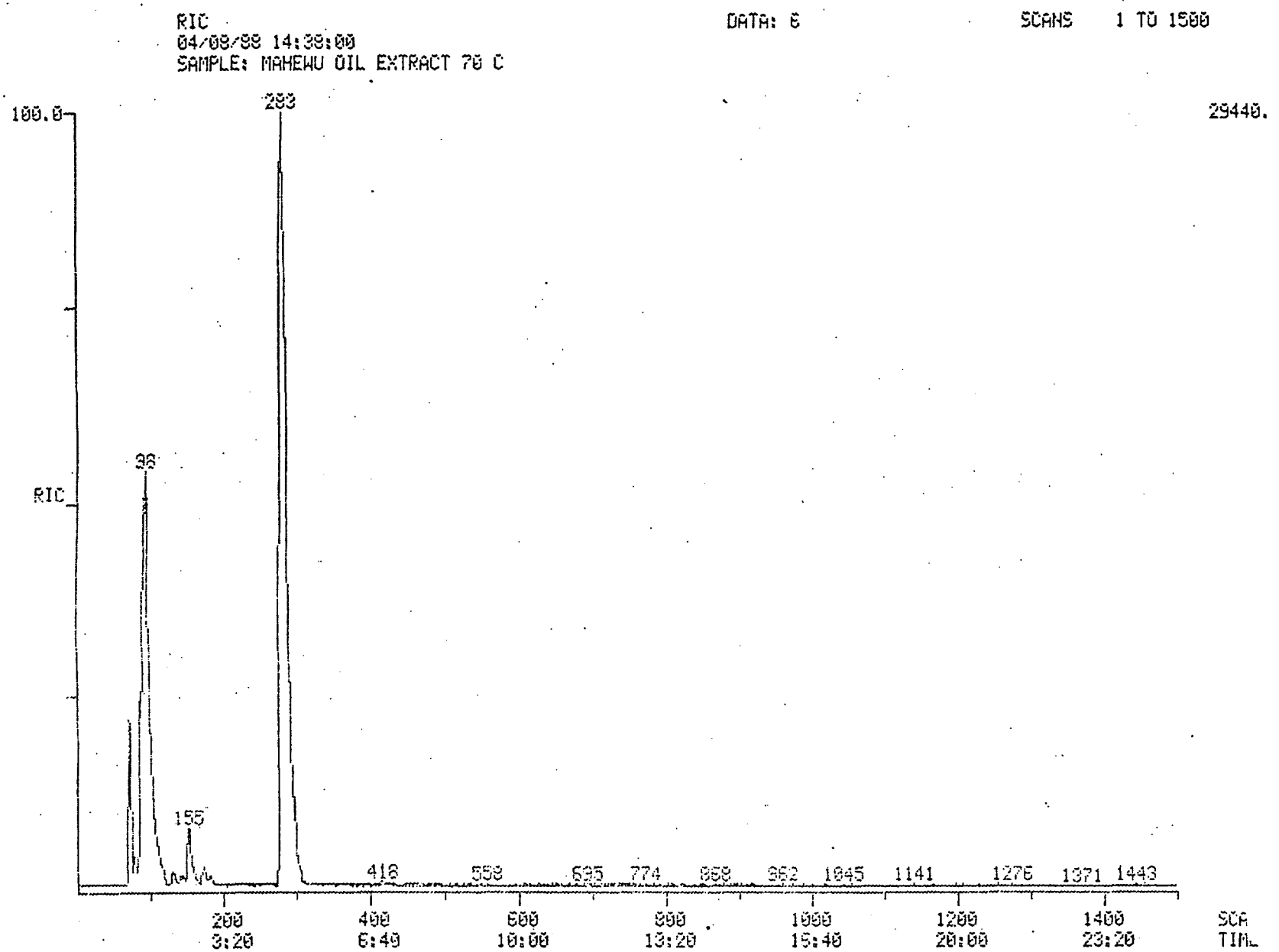

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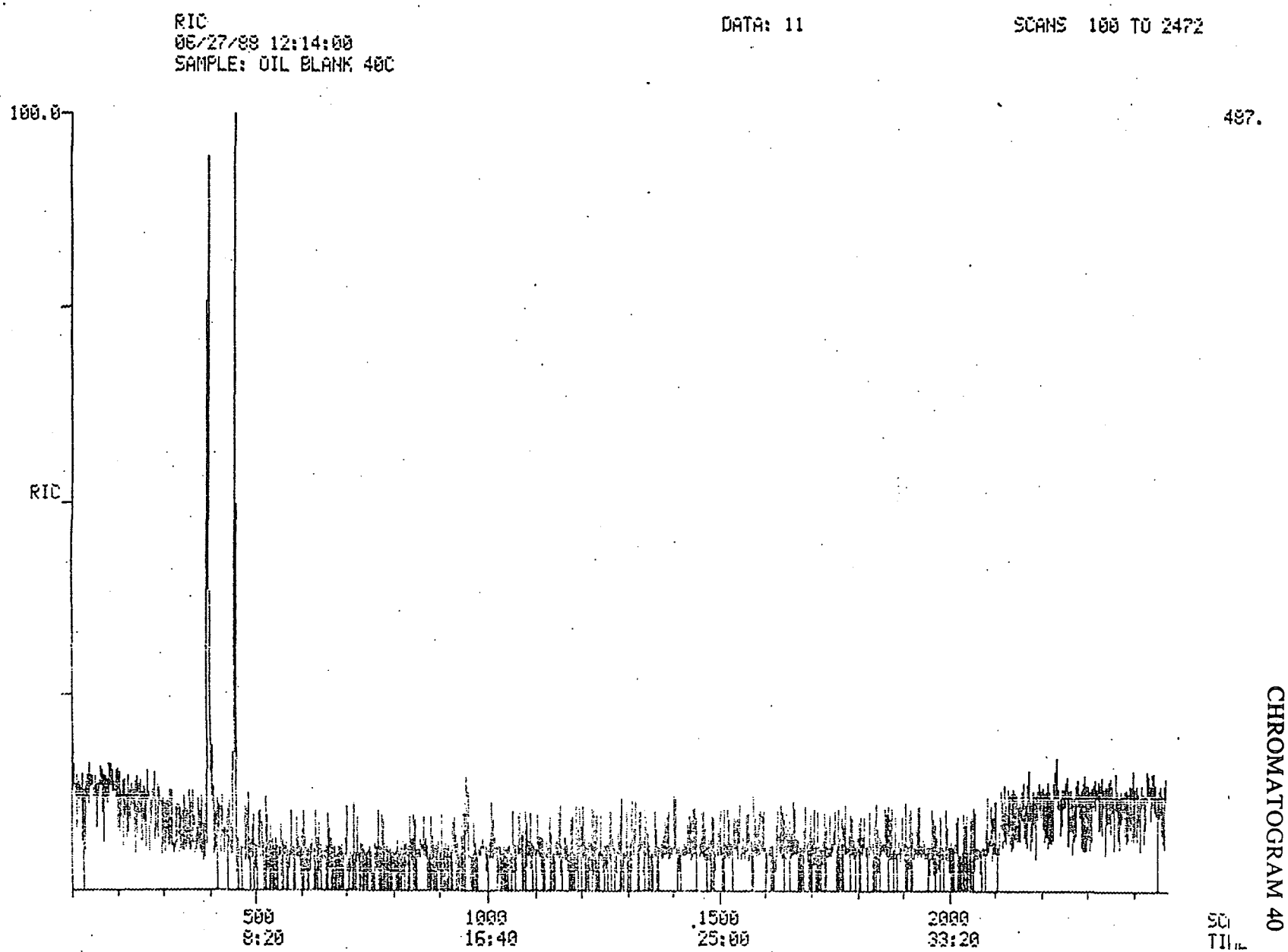
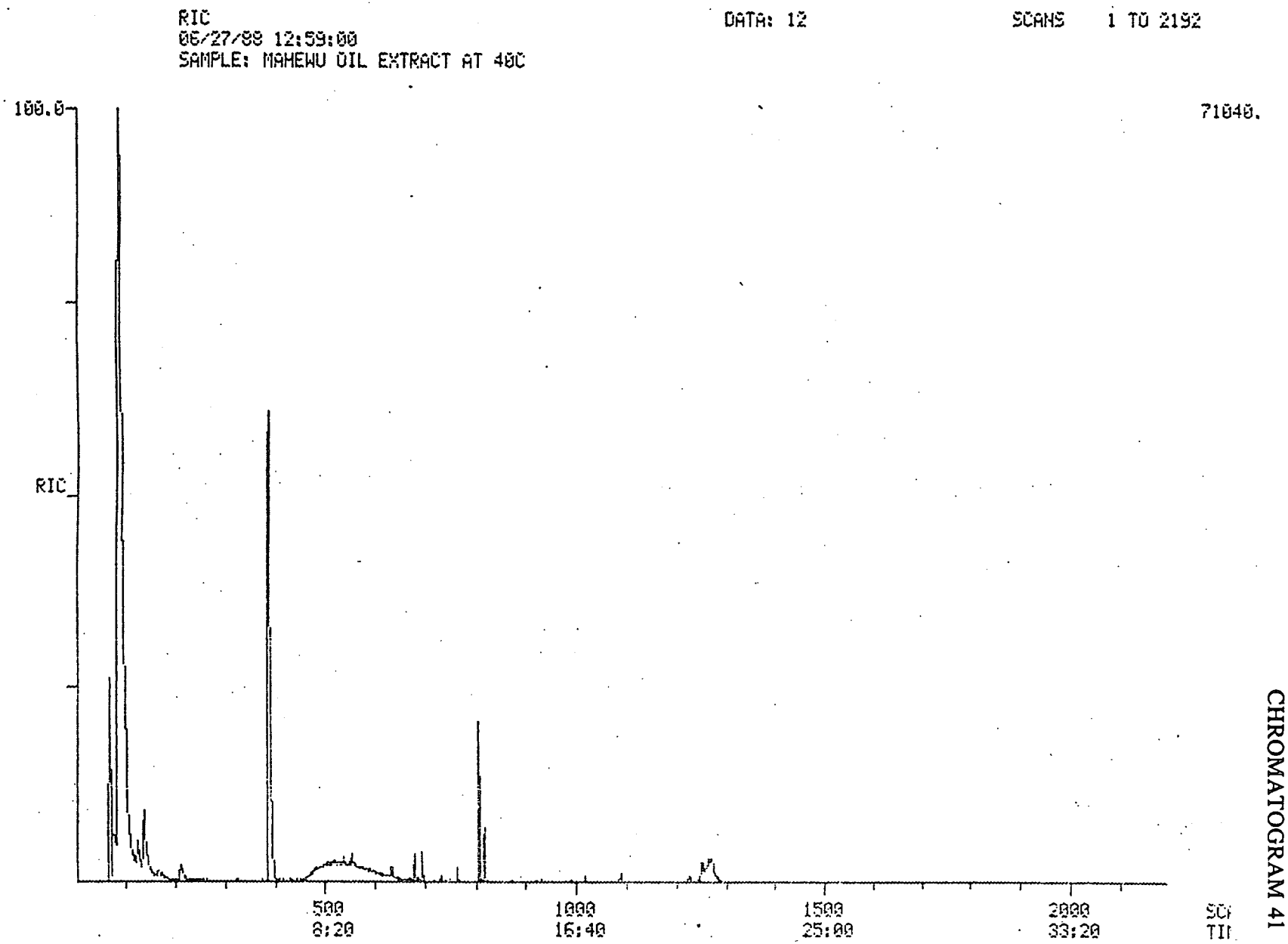


CHROMATOGRAM 40



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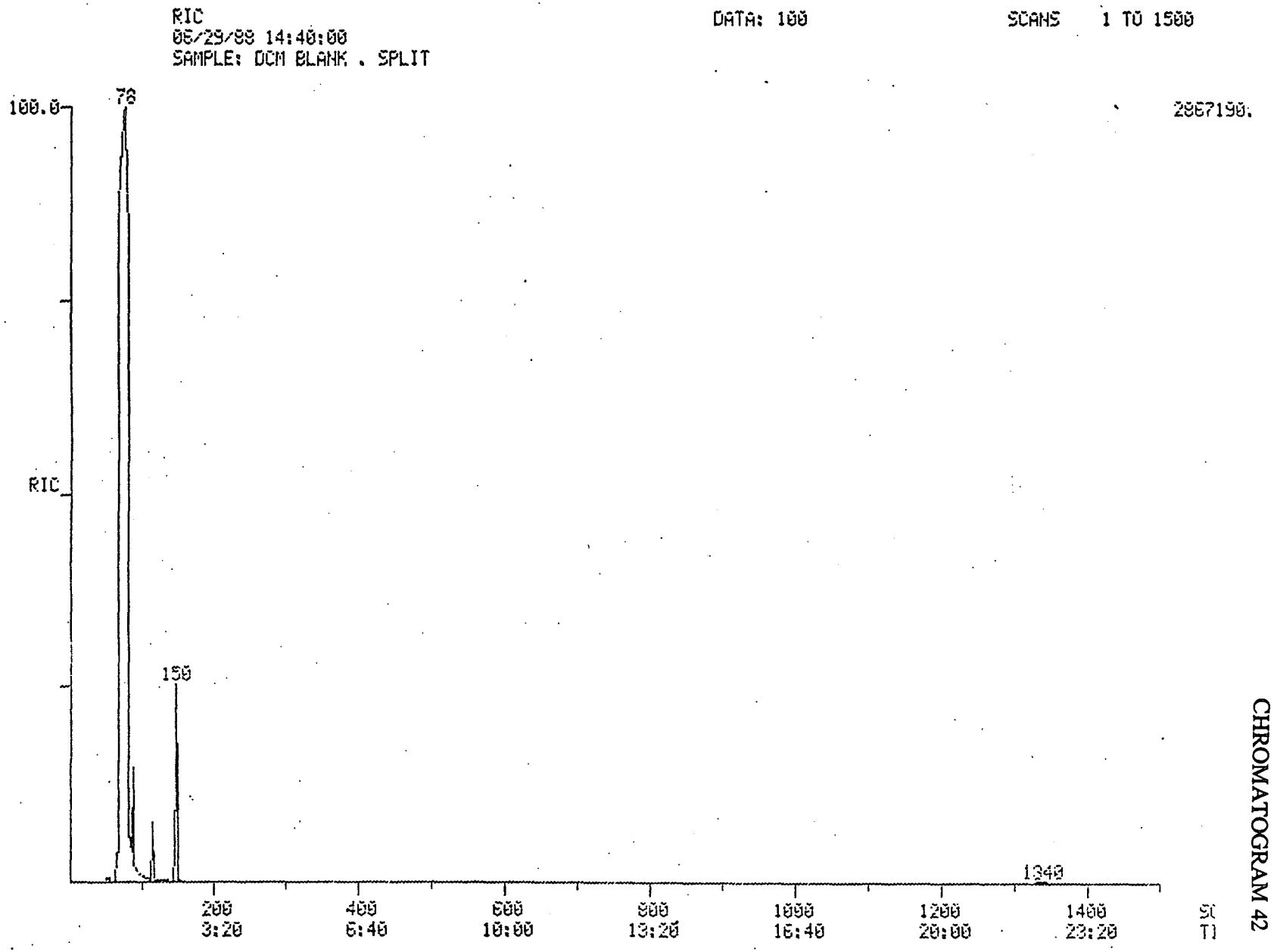
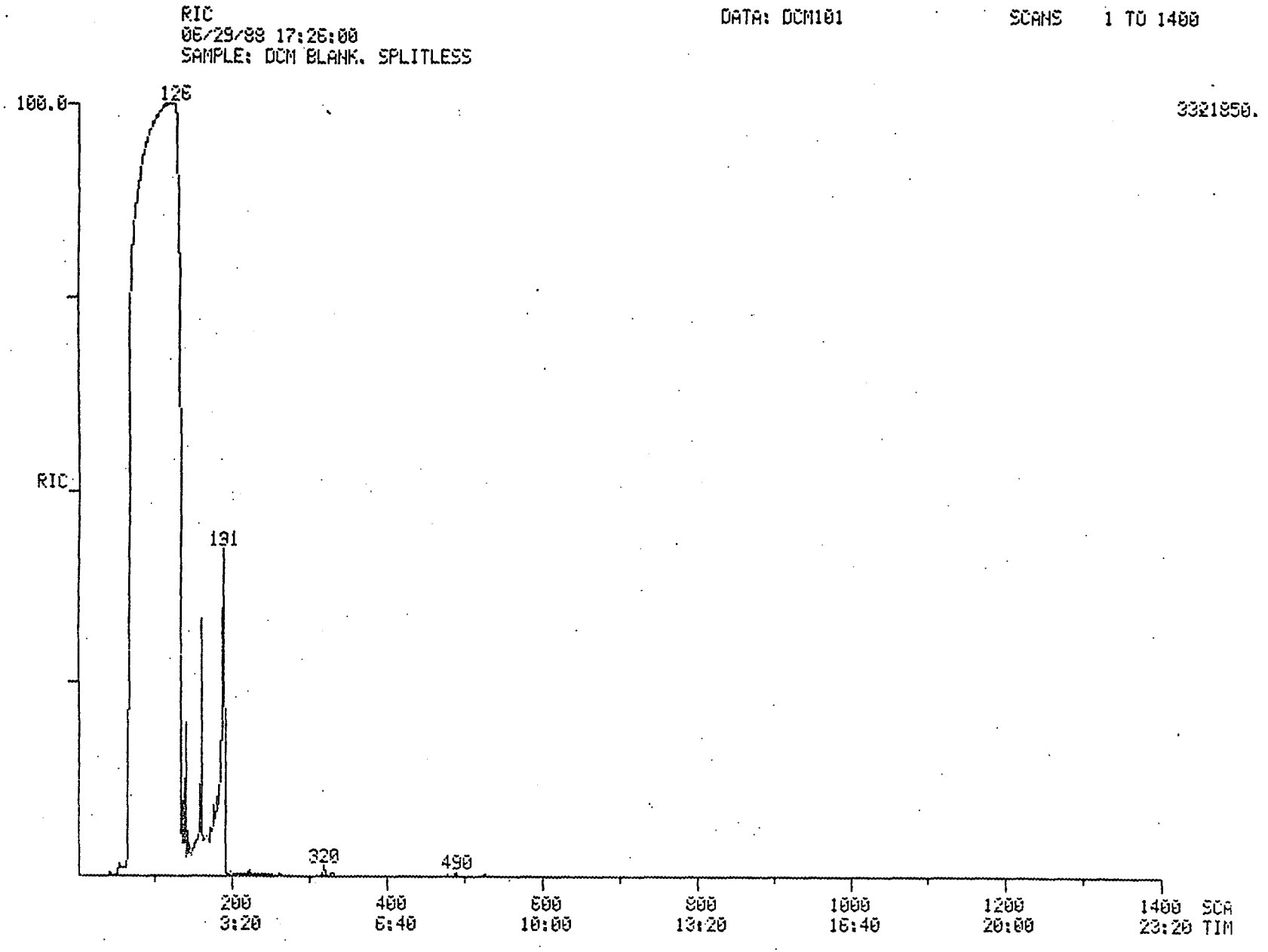


CHROMATOGRAM 42



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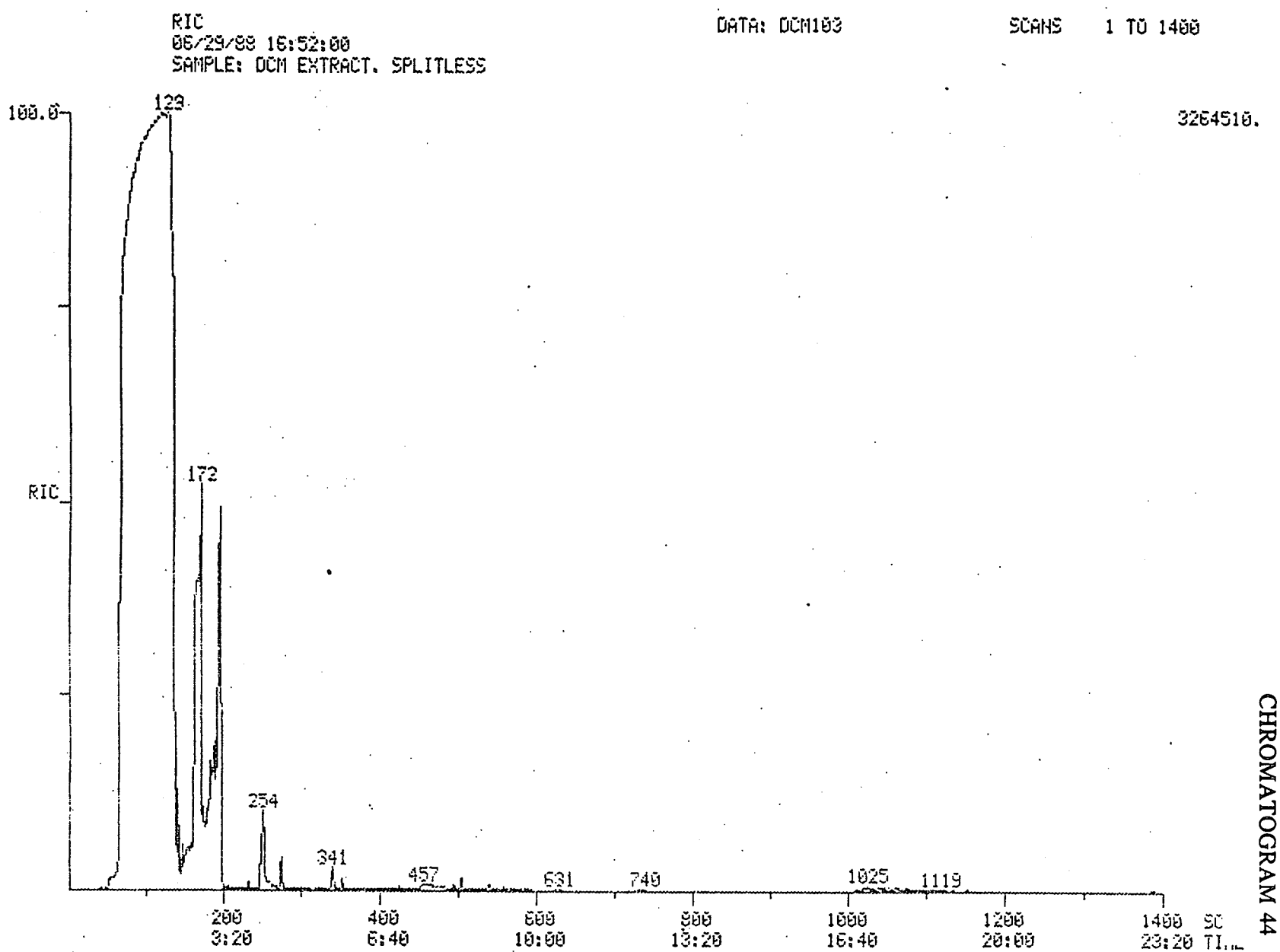
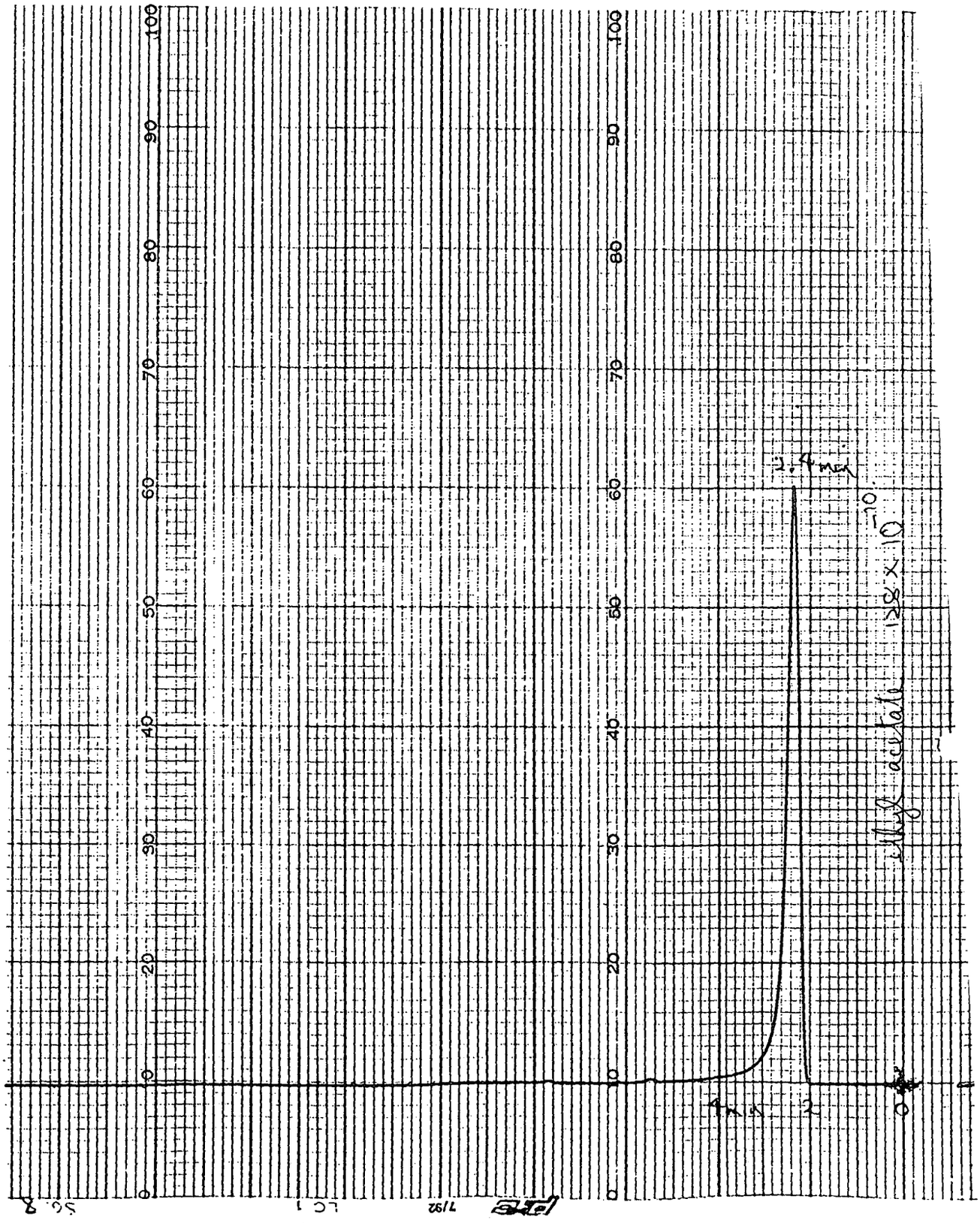
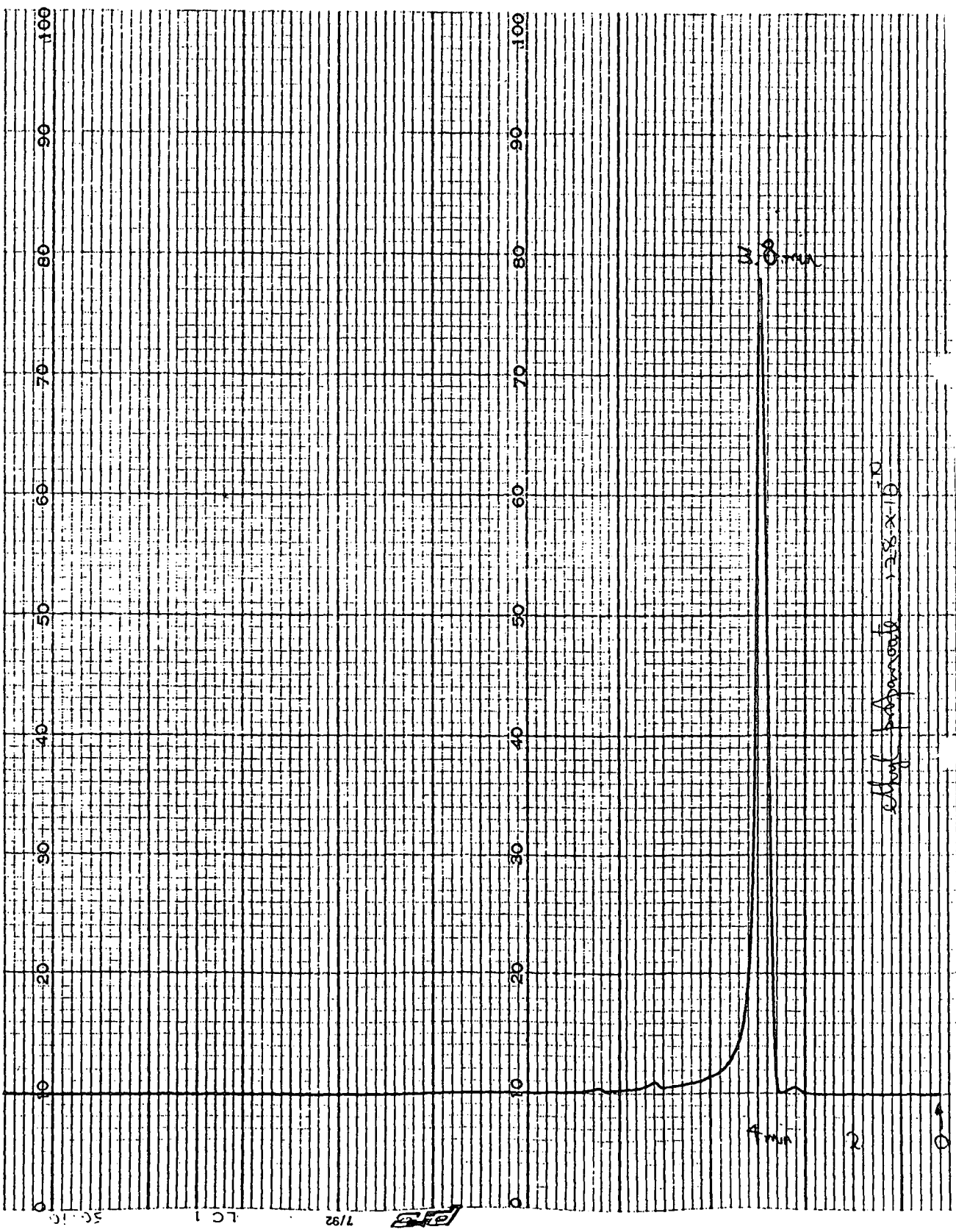


CHROMATOGRAM 44

CHROMATOGRAM 45



CHROMATOGRAM 46



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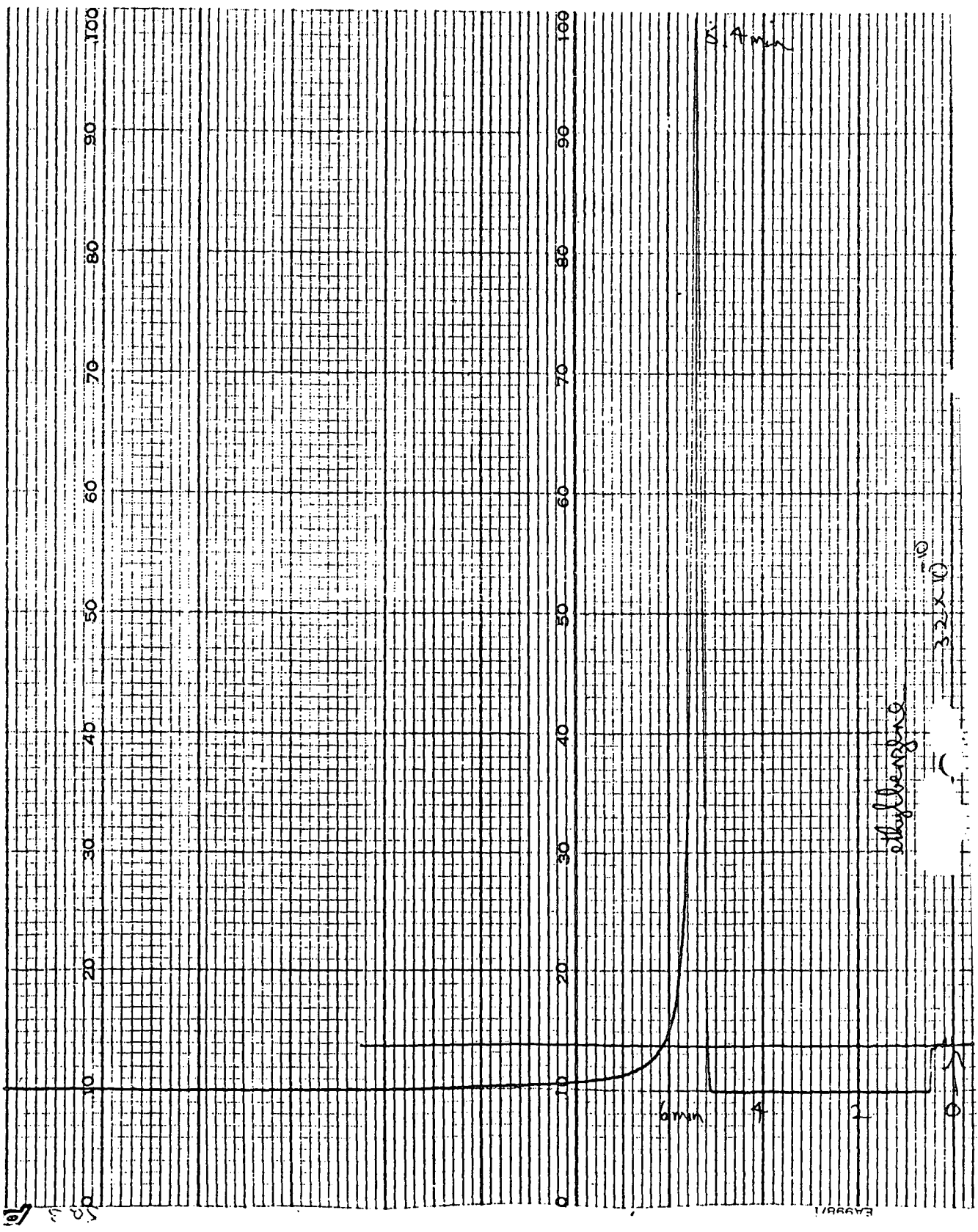

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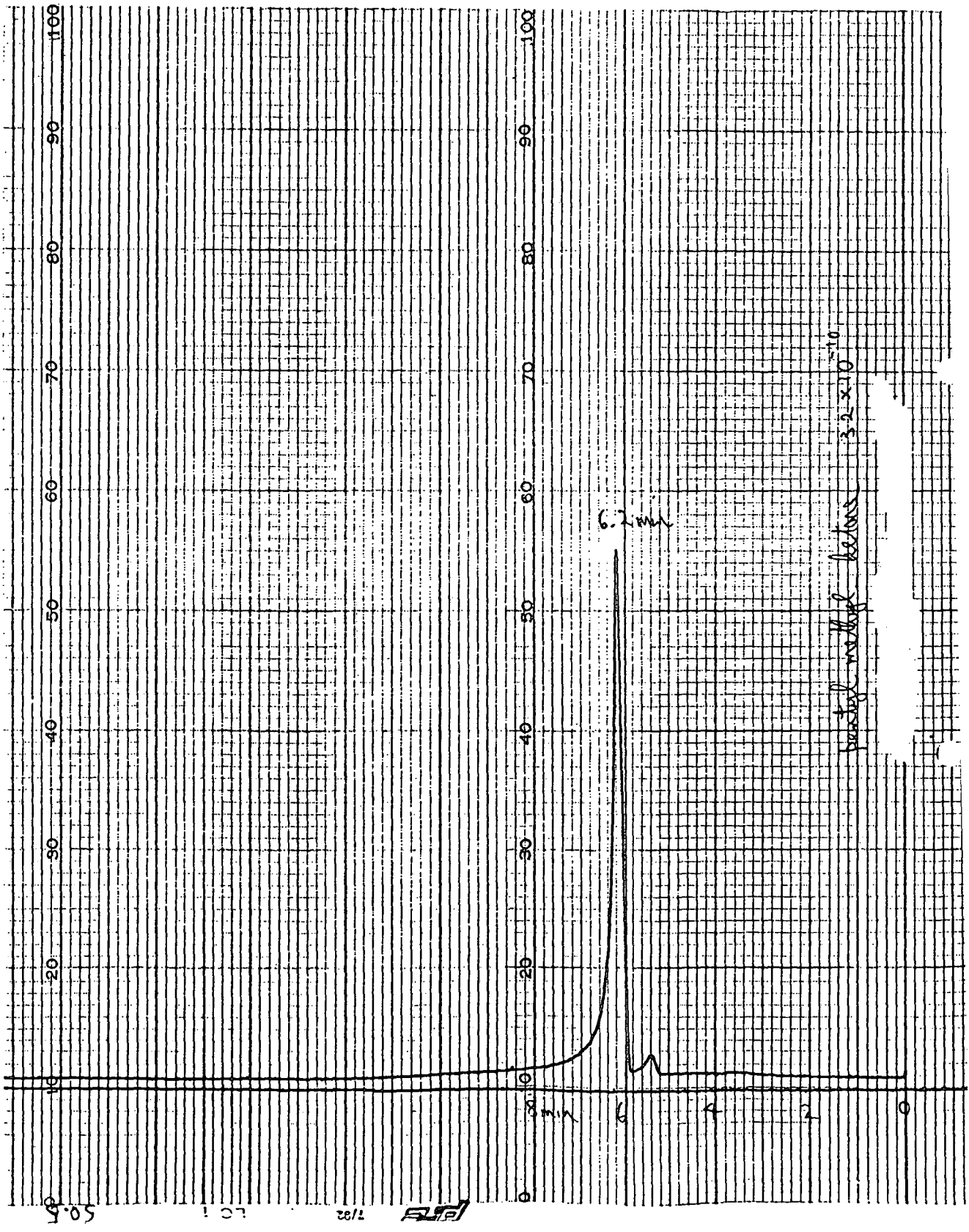
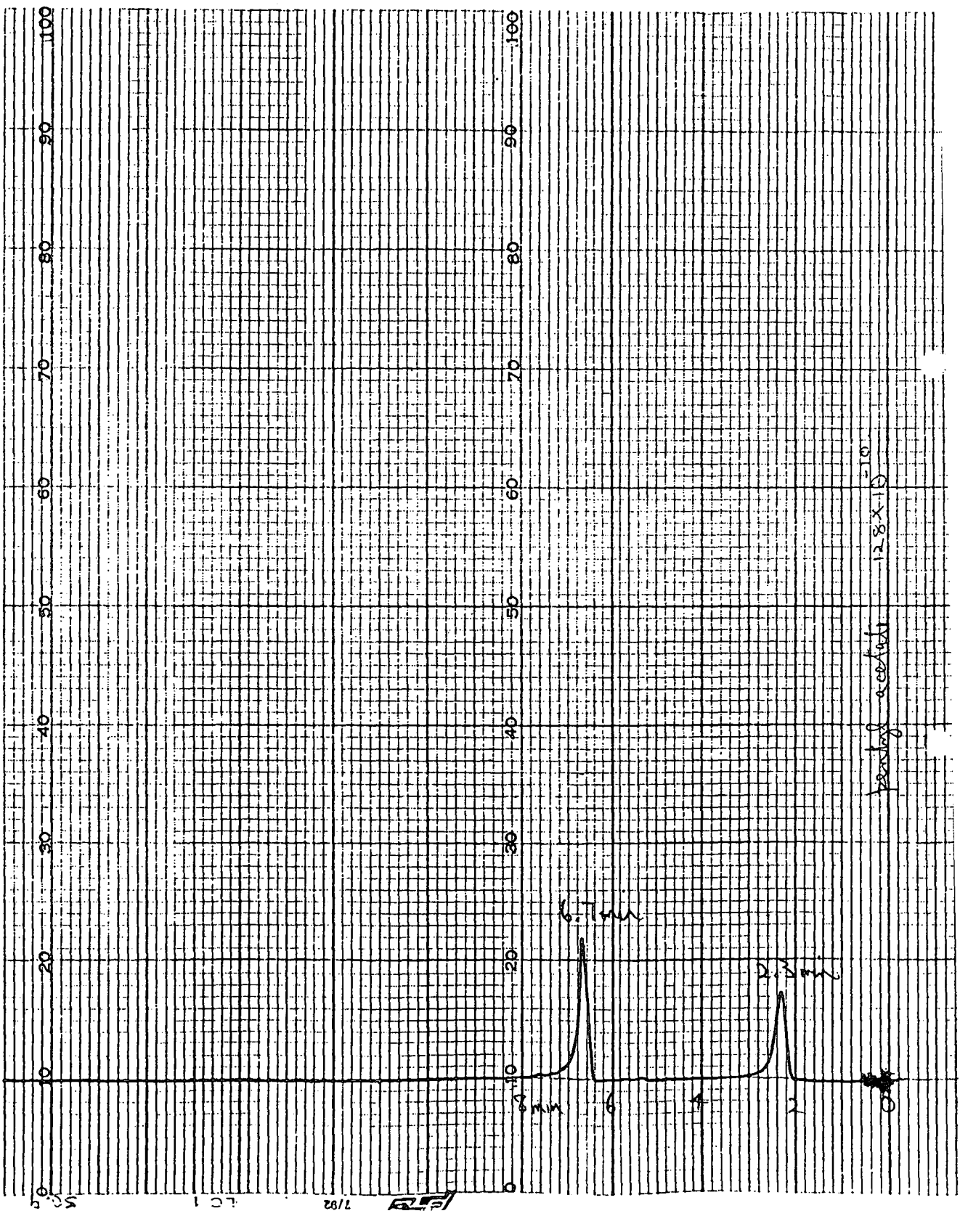
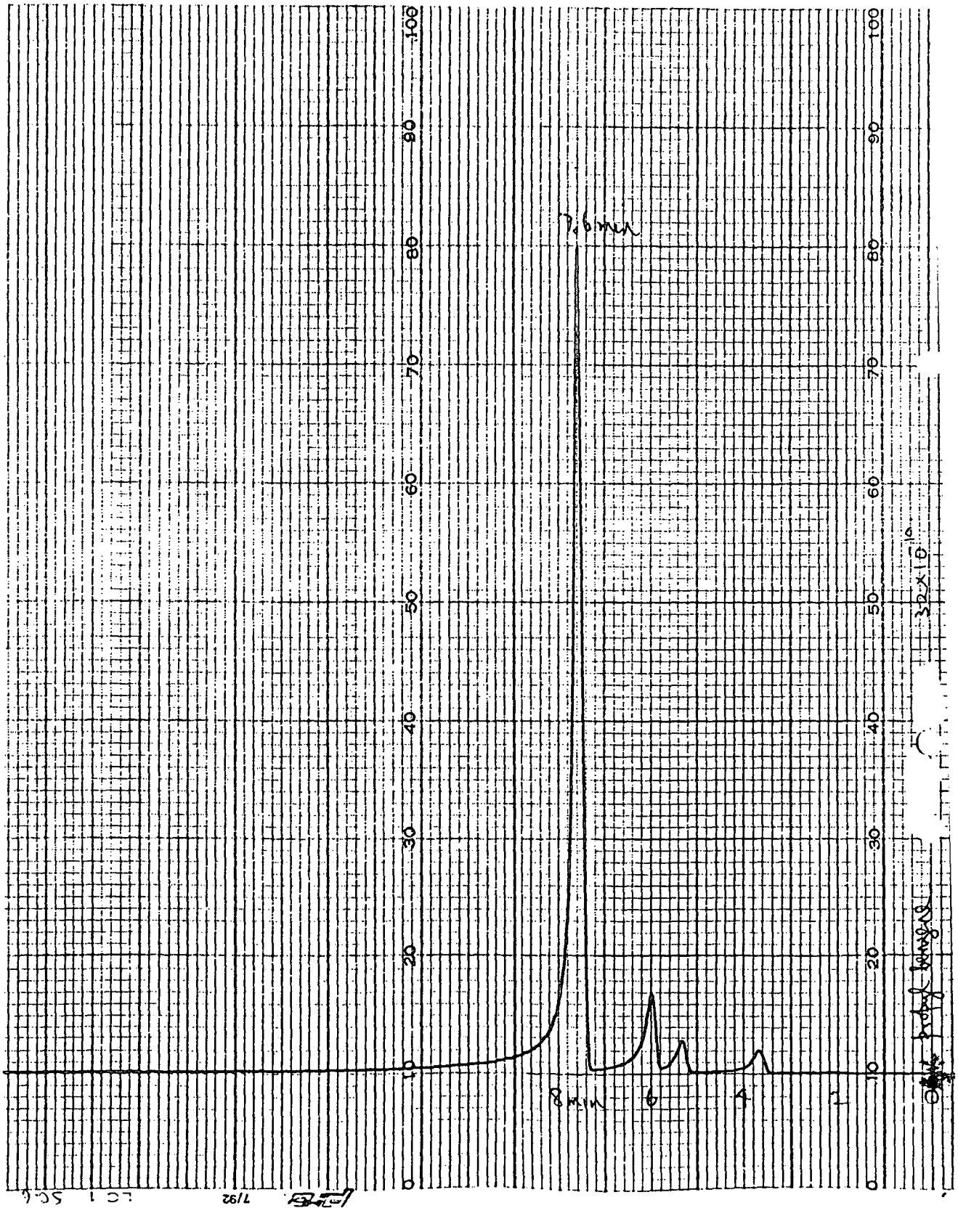


CHROMATOGRAM 49



CHROMATOGRAM 50



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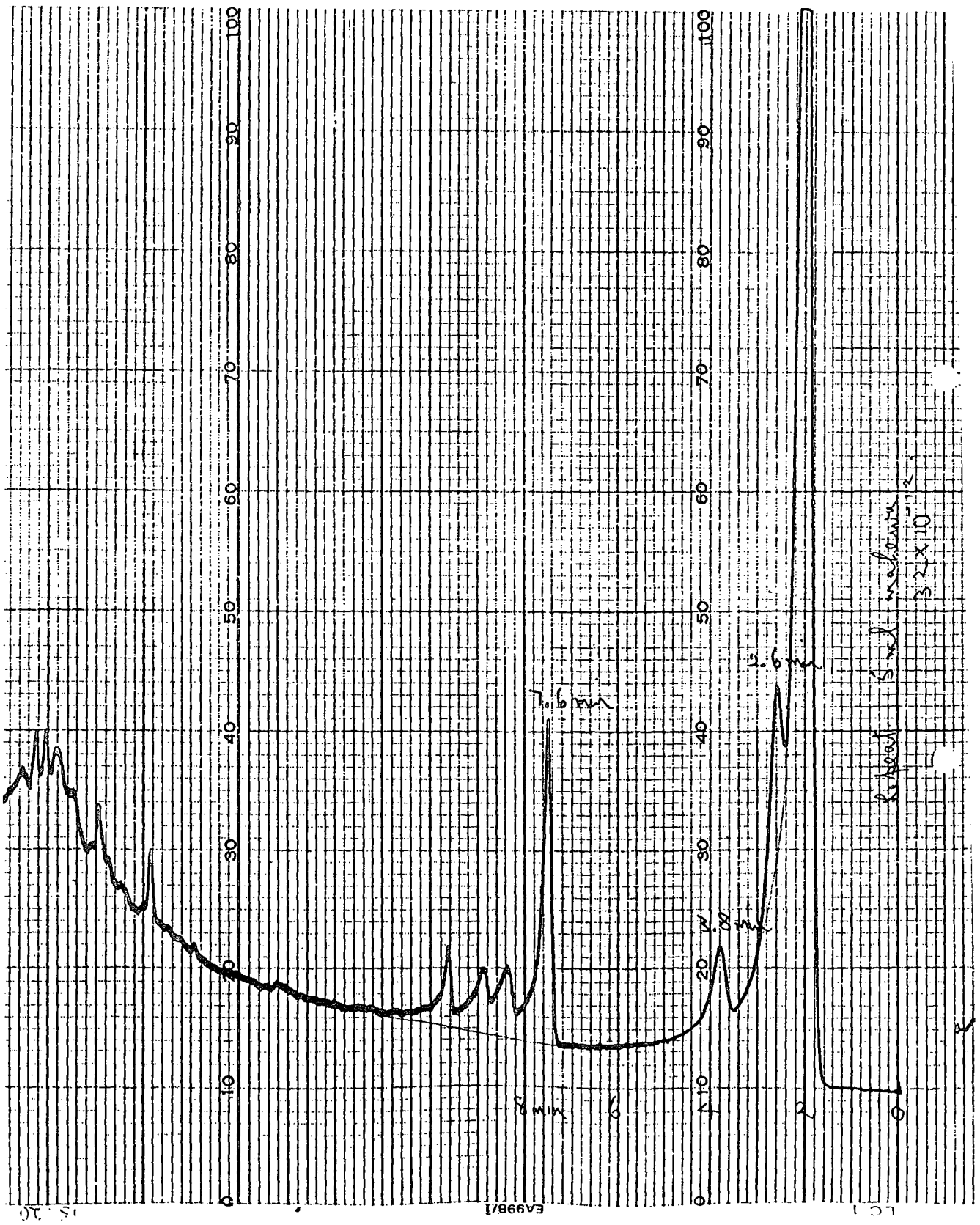

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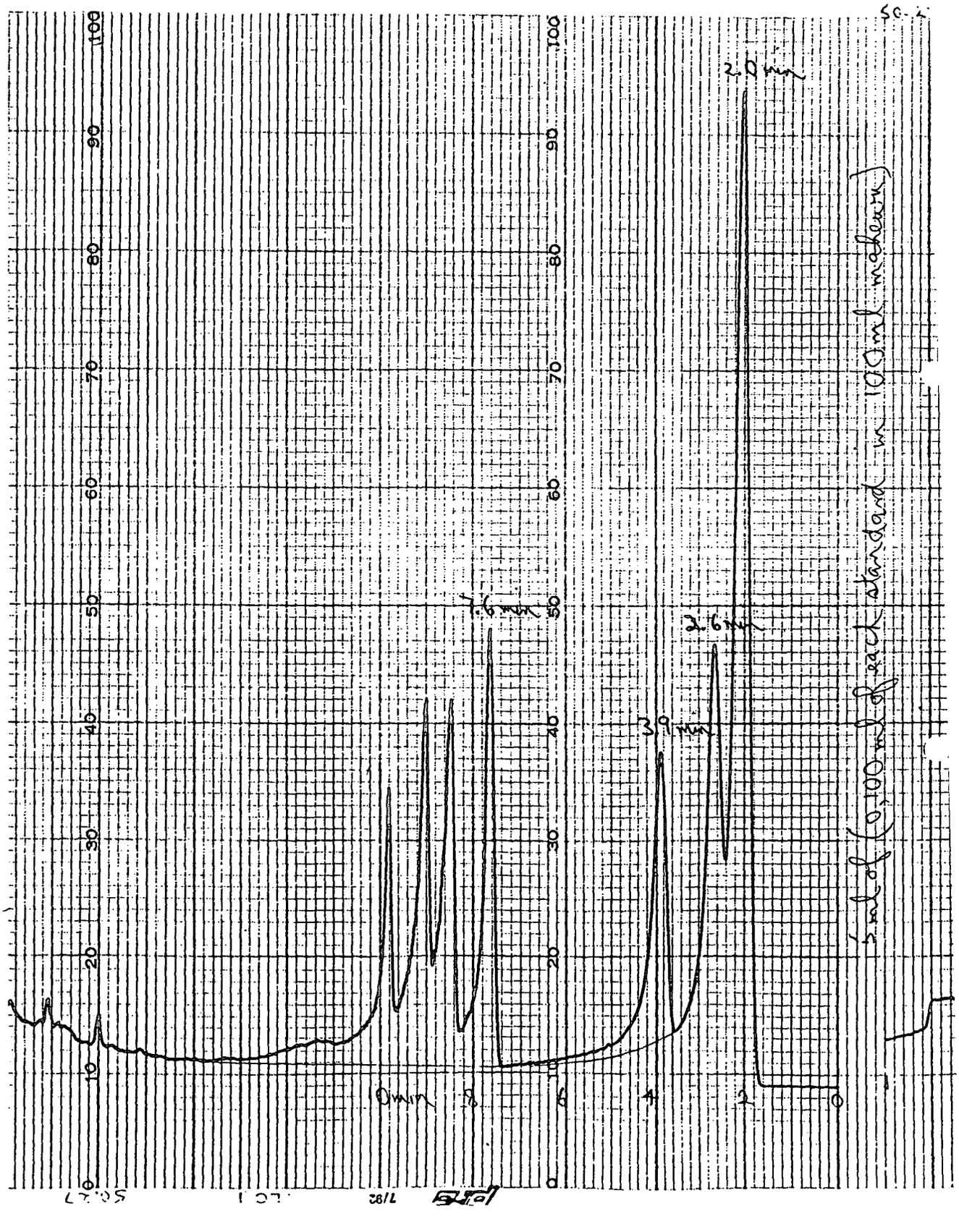

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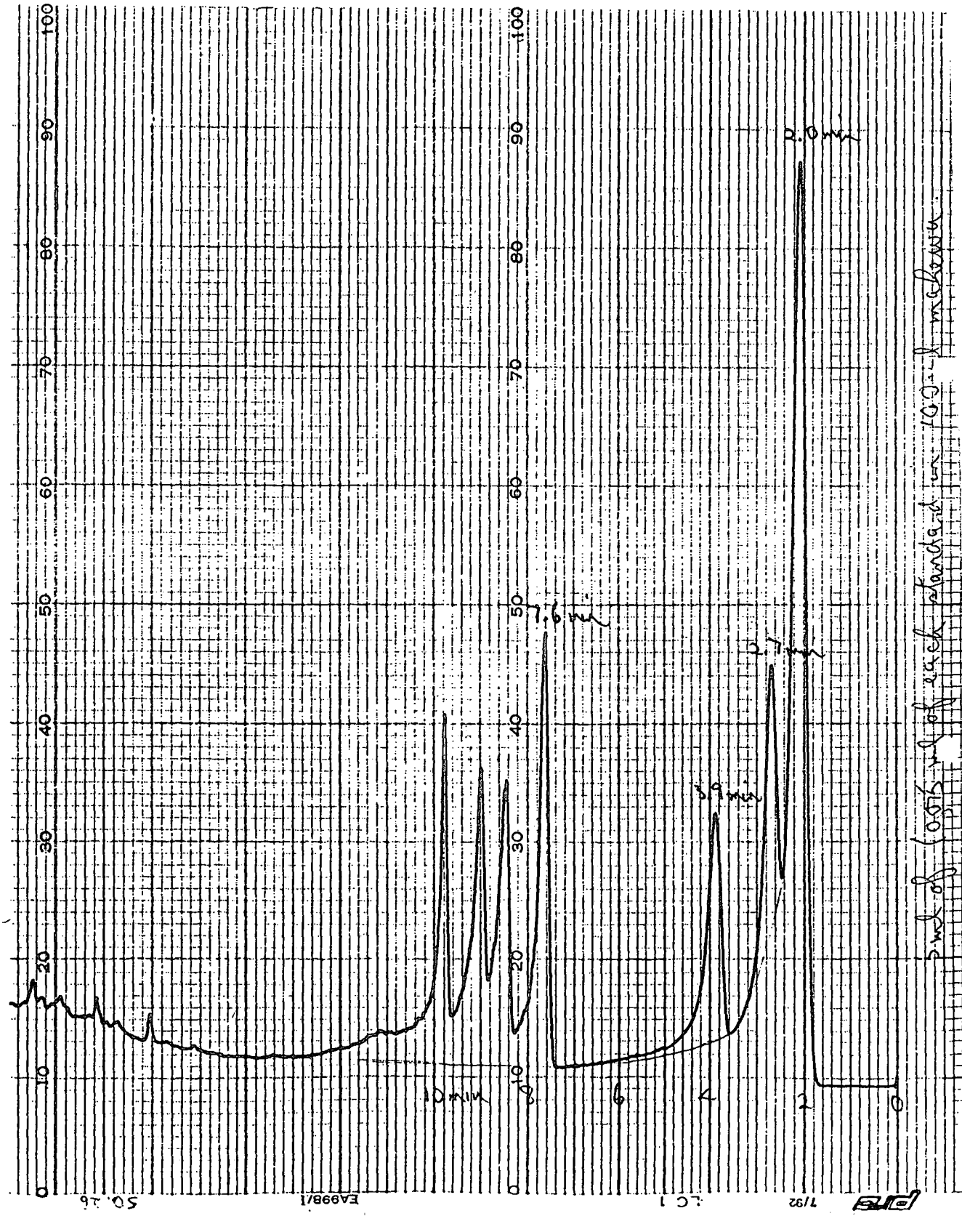
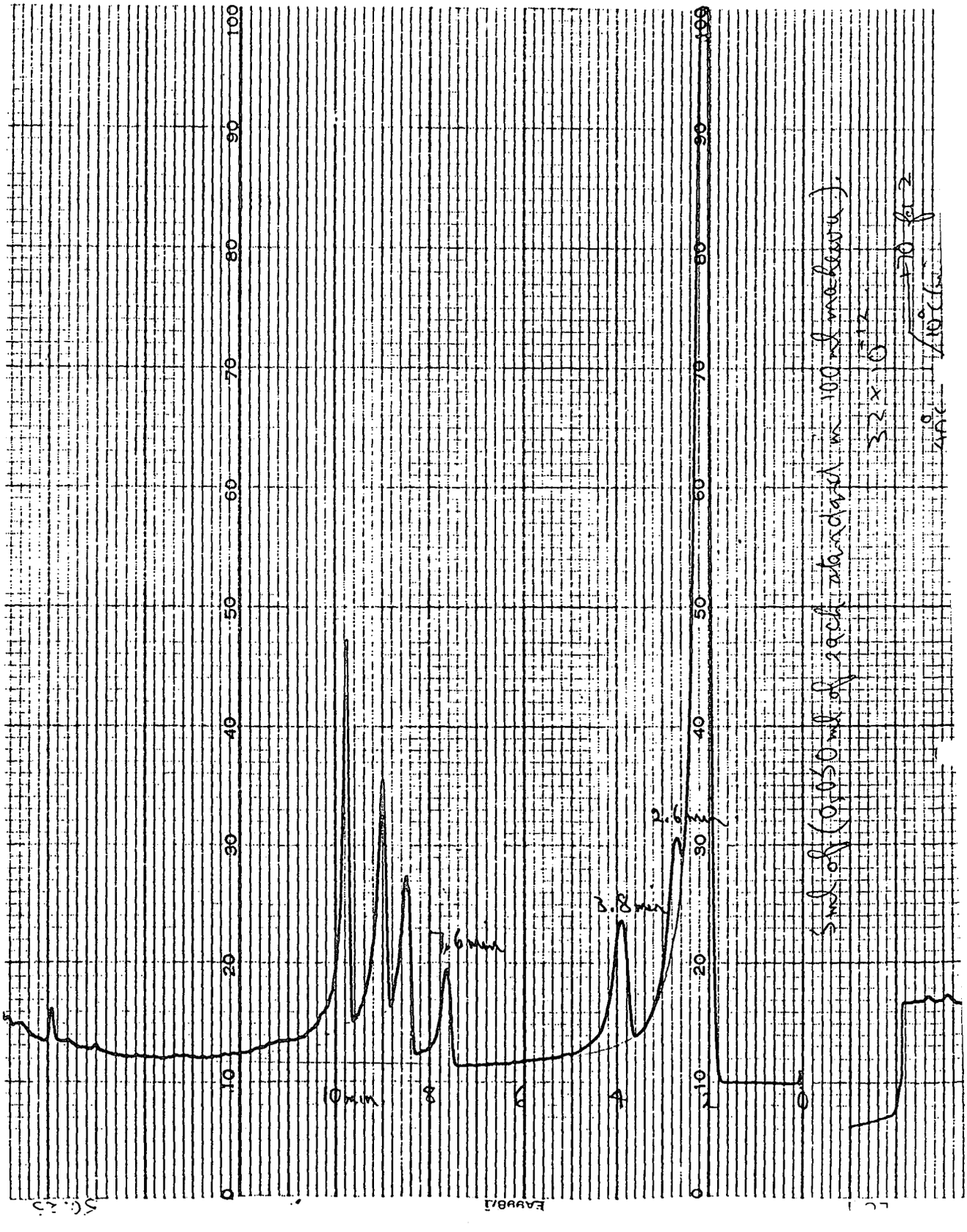
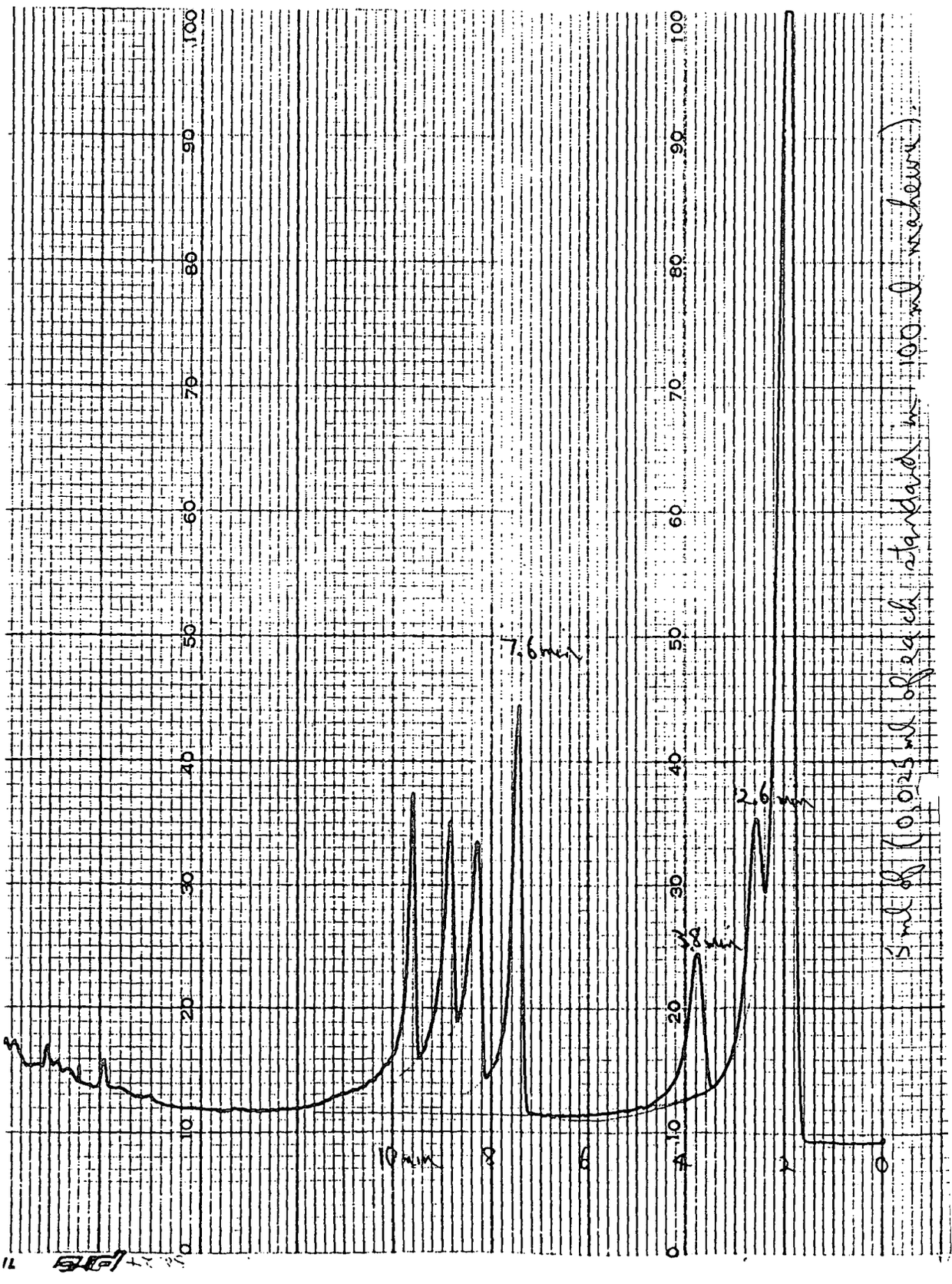
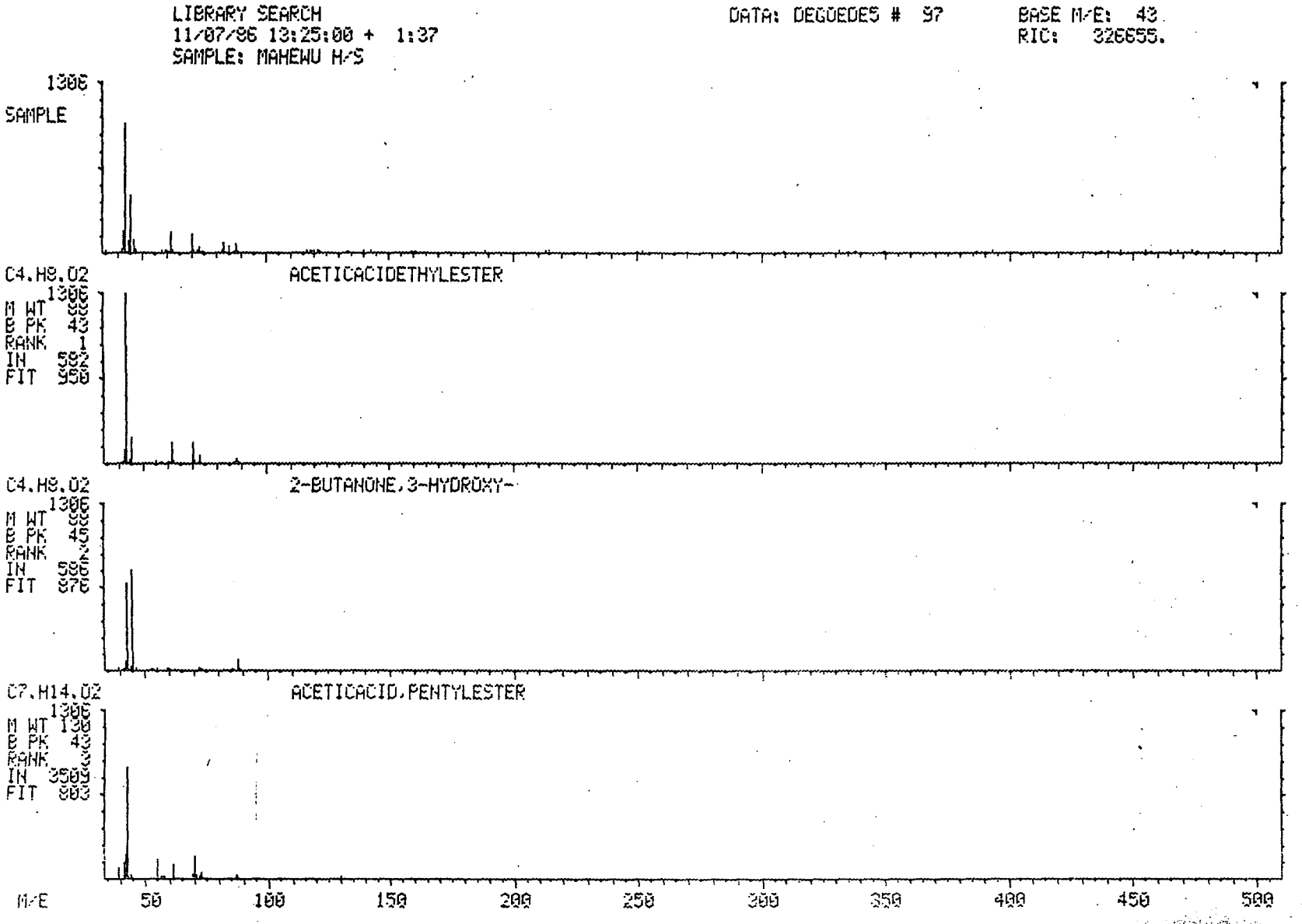


CHROMATOGRAM 54

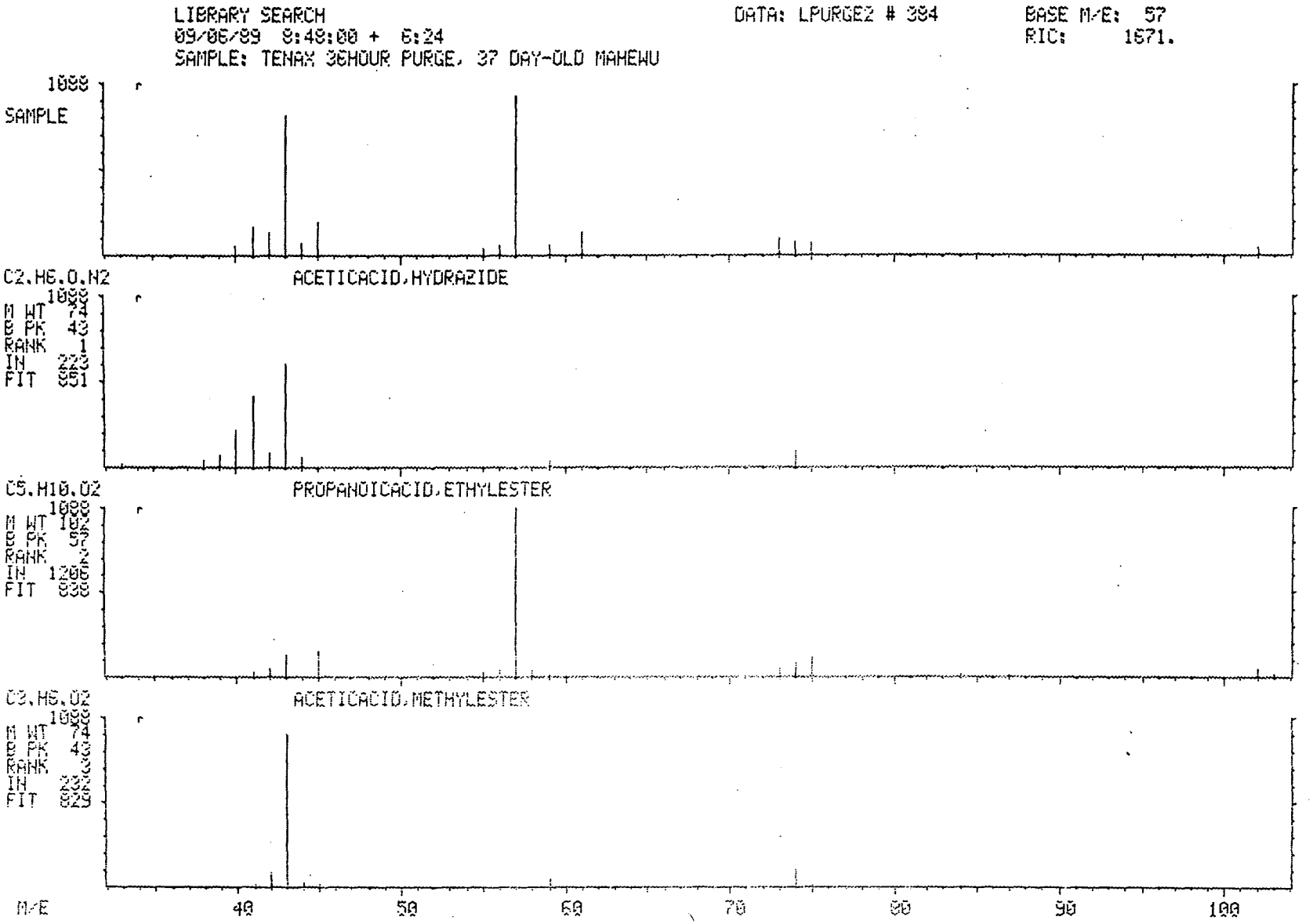


CHROMATOGRAM 55

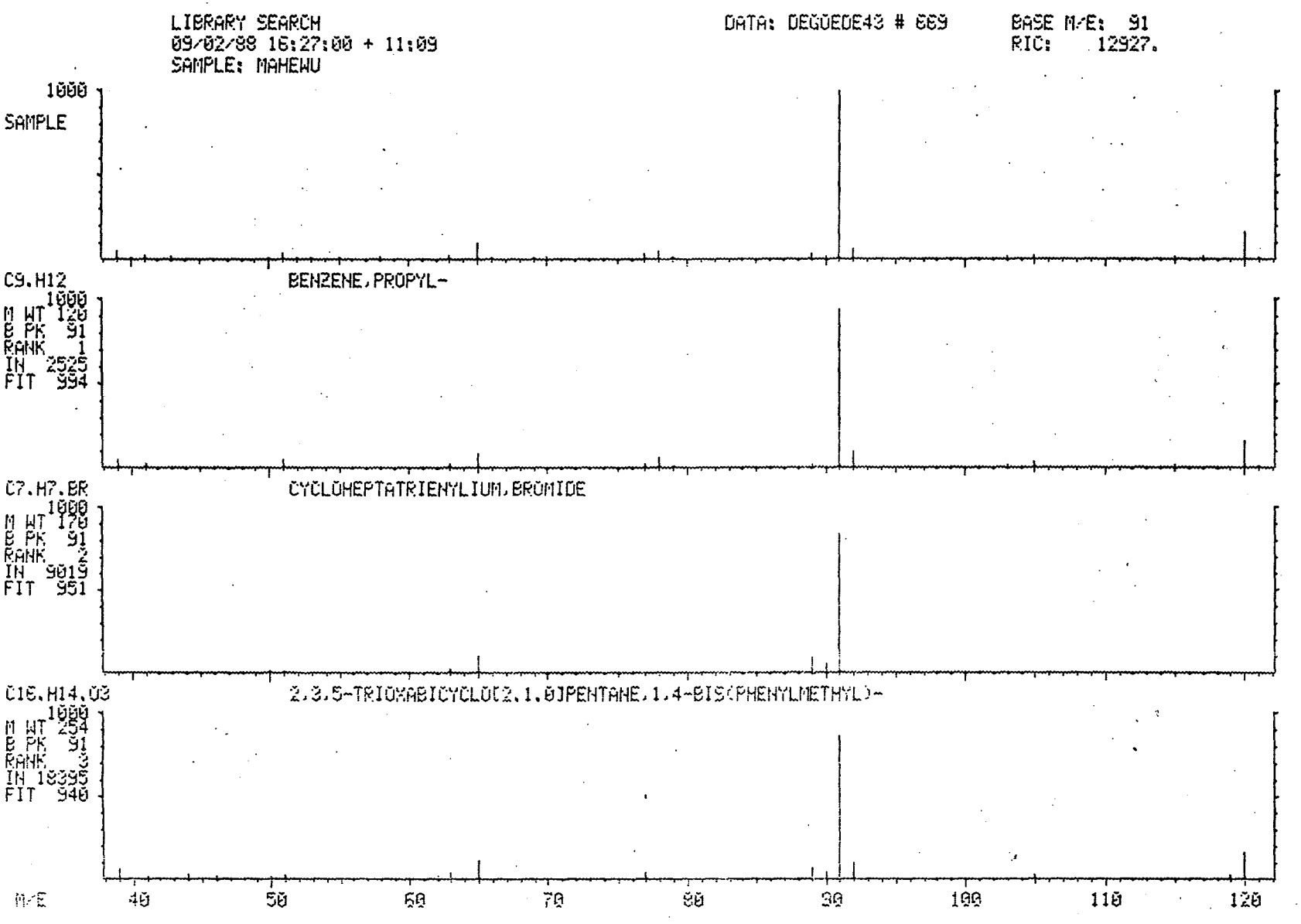




MASS SPECTRUM 1



MASS SPECTRUM 2



MASS SPECTRUM 3