SUPERCRITICAL FLUID FRACTIONATION OF FISH OILS— CONCENTRATION OF EICOSAPENTAENOIC ACID

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RESEARCH REPORT

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SUPERCRITICAL FLUID FRACTIONATION OF FISH OILS - CONCENTRATION OF EICOSAPENTAENOIC ACID

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Eicosapentaenoic acid (EPA) is currently being studied because of increasing evidence of its therapeutic value, specifically that in the body it is a precursor to prostaglandins; many laboratories are carrying out development directed to increasing this active species in various formulations. Supercritical fluid solvents offer the ability to separate low vapor pressure, heat-labile compounds, and several fish oils were tested for their response to supercritical fluid fractionation.

In brief summary of the results (as of November 1983), fish oils in triglyceride form are difficult to fractionate using solely carbon dioxide, although some benefication of EPA from a base concentration of 18% to 22% is achievable. However, the same oil in methyl ester form can be upgraded substantially more, to a concentration of 29%. Work is continuing with the methyl esters, and the then-current results will be presented.

SUPERCRITICAL FLUID FRACTIONATION OF FISH OILS— CONCENTRATION OF EICOSAPENTAENOIC ACID

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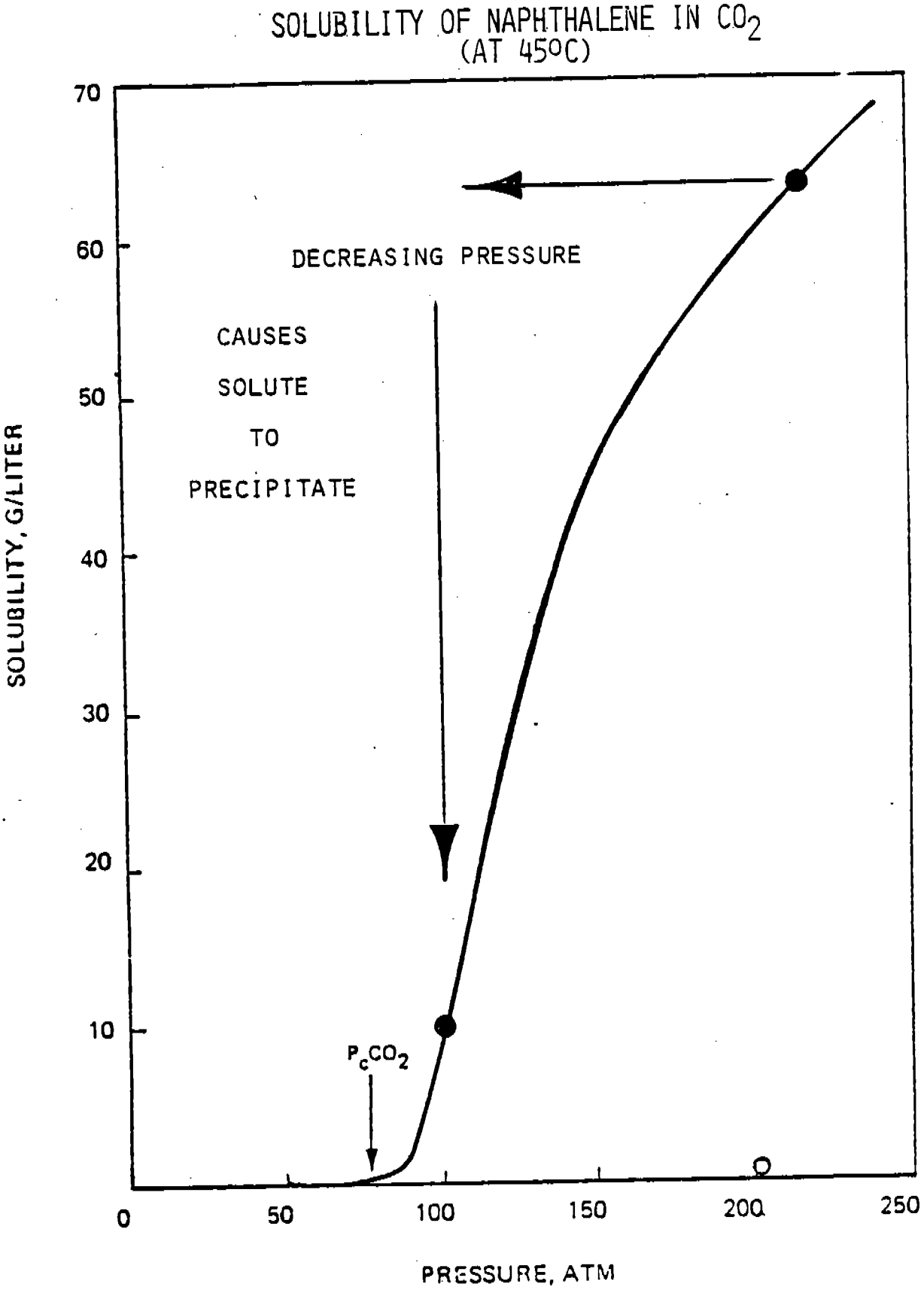
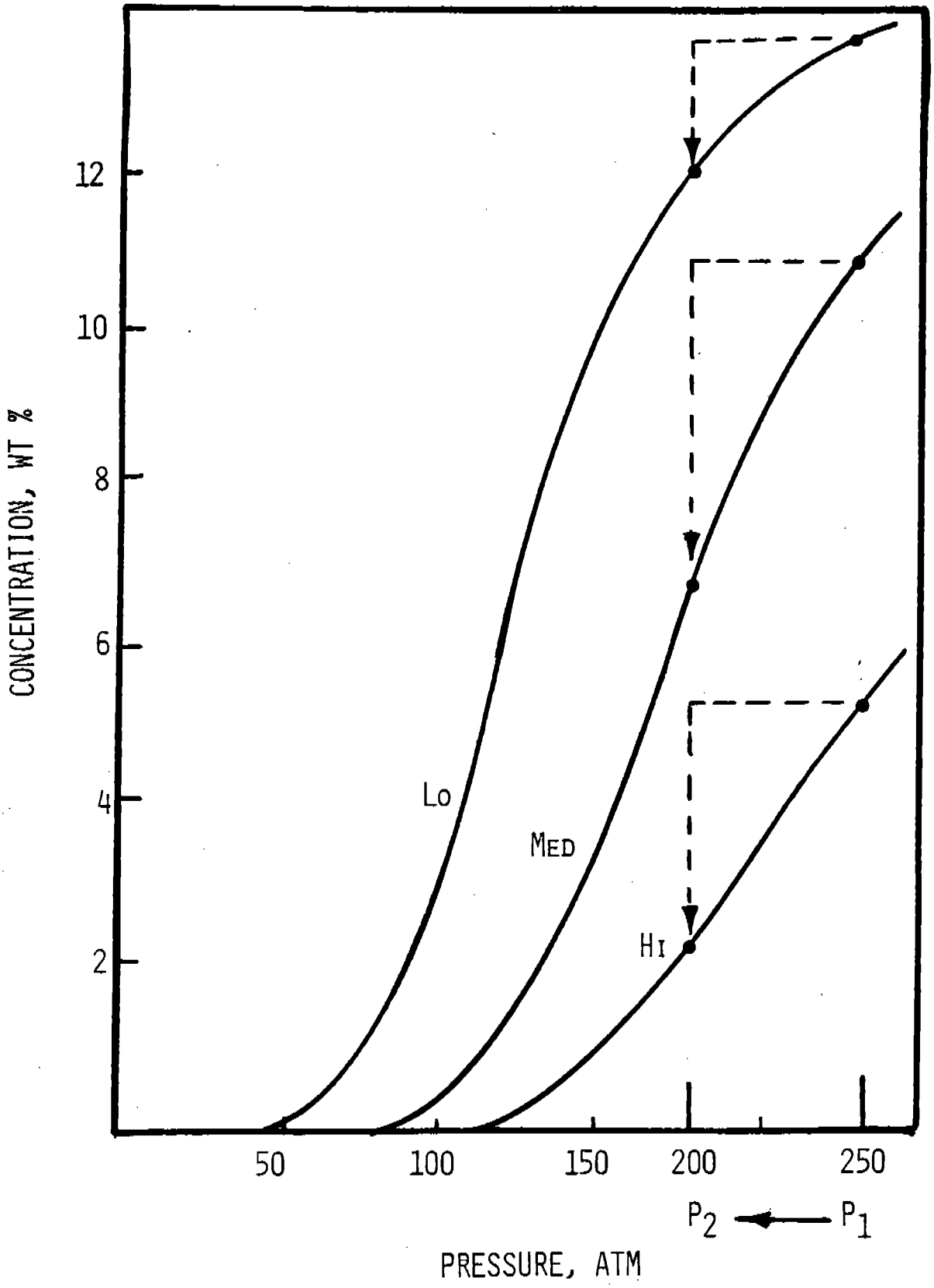
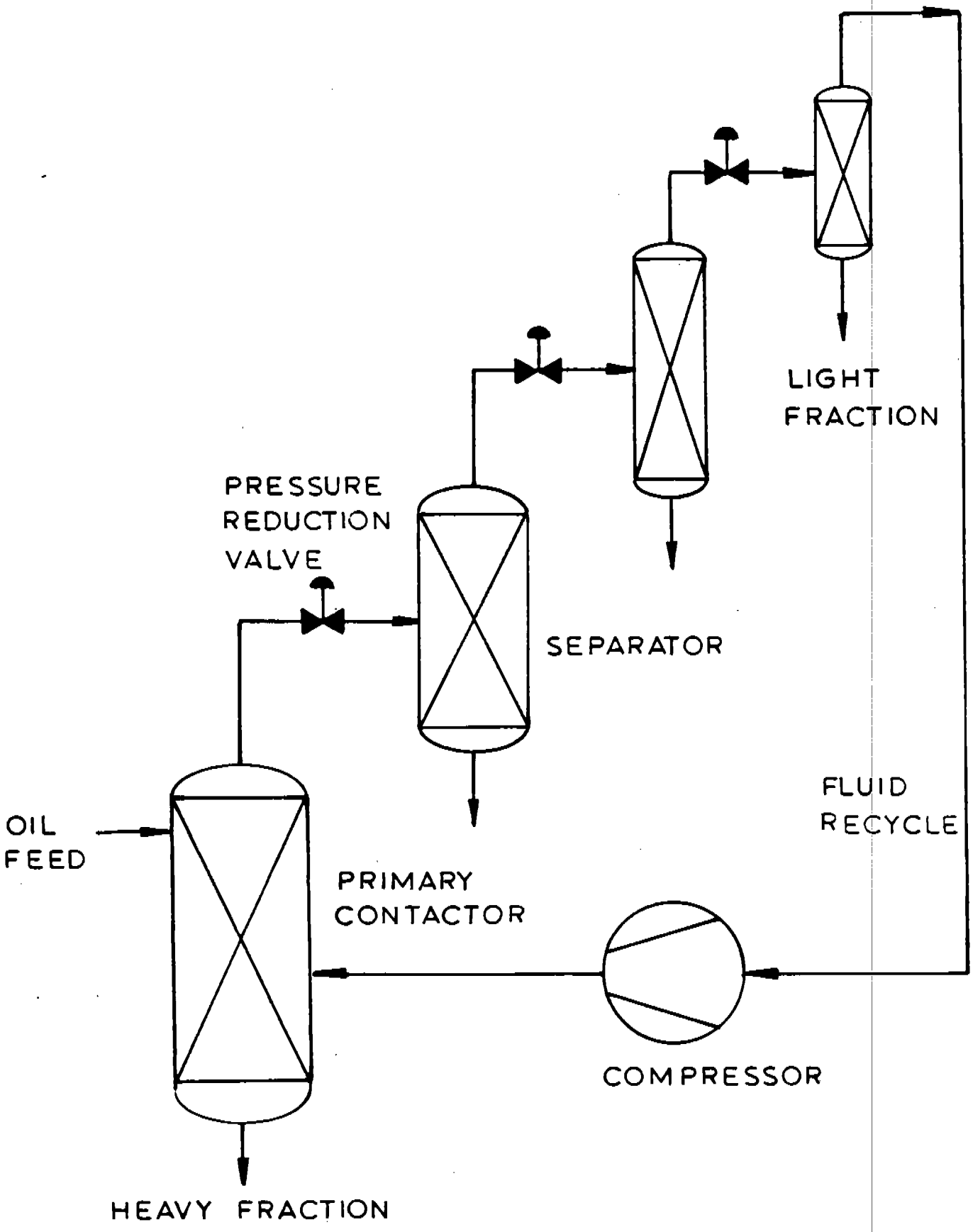
- Background on SCF Fractionation
- Experimental—Analytical
- Fractionation of Triglycerides
- Fractionation of Methyl Esters


SOLUBILITY OF NAPHTHALENE IN CO_2
(AT 45°C)
70
60
50
40
30
20
10
SOLUBILITY, G/LITER
DECREASING PRESSURE
CAUSES
SOLUTE
TO
PRECIPITATE
$P_c CO_2$
0
50
100
150
200
250
PRESSURE, ATM

SOLUBILITY OF HOMOLOGOUS SERIES MEMBERS



SUPERCRITICAL FLUID FRACTIONATION



EXPERIMENTAL

* * * *

- CO_2 AS SOLVENT
- PRESSURE RANGE, 1800—5500 PSI
- TEMPERATURE, 40°C & 80°C
- MOST TESTS AT INCREASING PRESSURE:
  ONE WITH STAGEWISE REDUCTION
- SOME TESTS WITH TRIGLYCERIDES
  - * MENHADEN
  - * HERRING
  - * ANCHOVY
- SOME TESTS WITH METHYL ESTERS OF SOME OILS

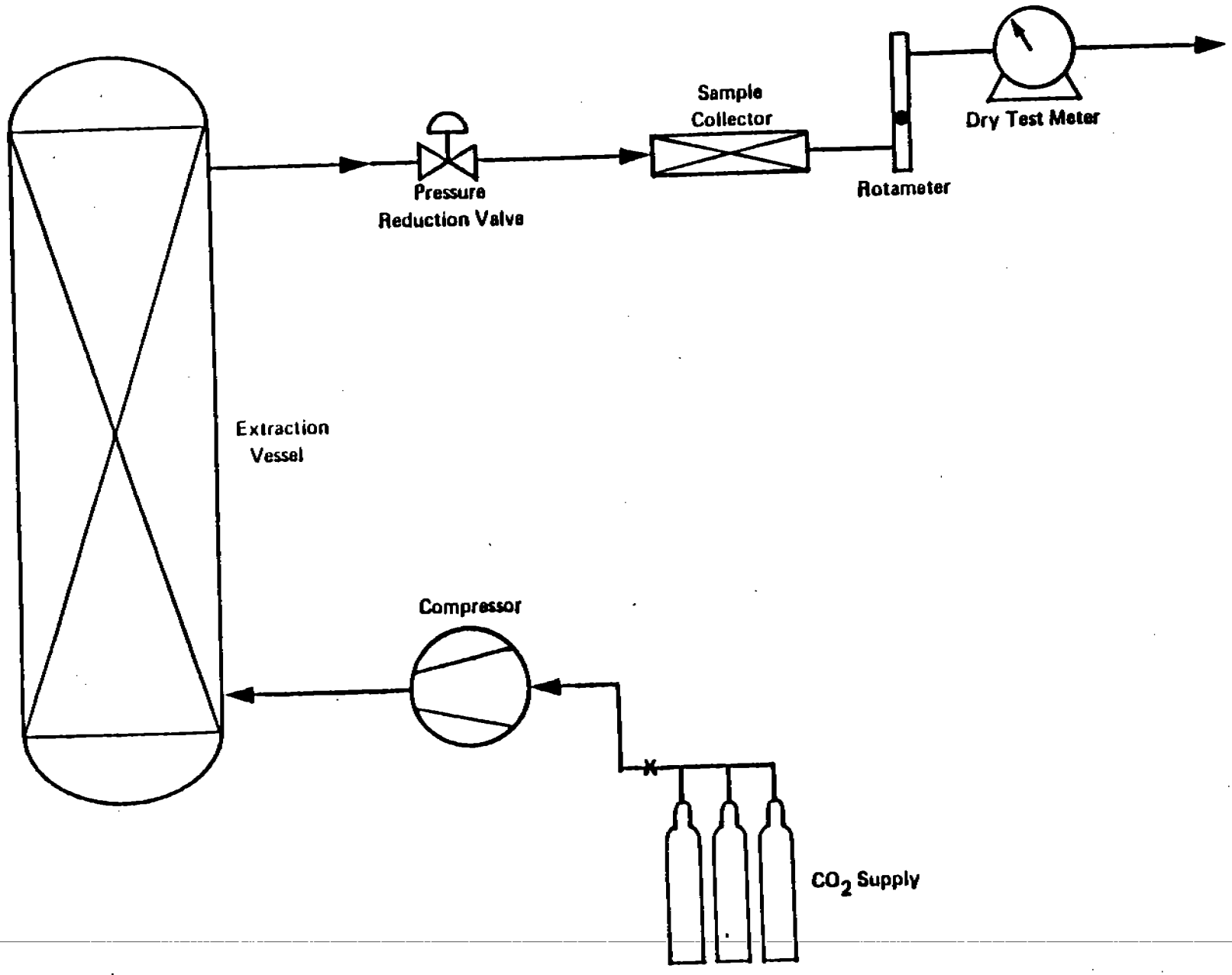
ANALYTICAL

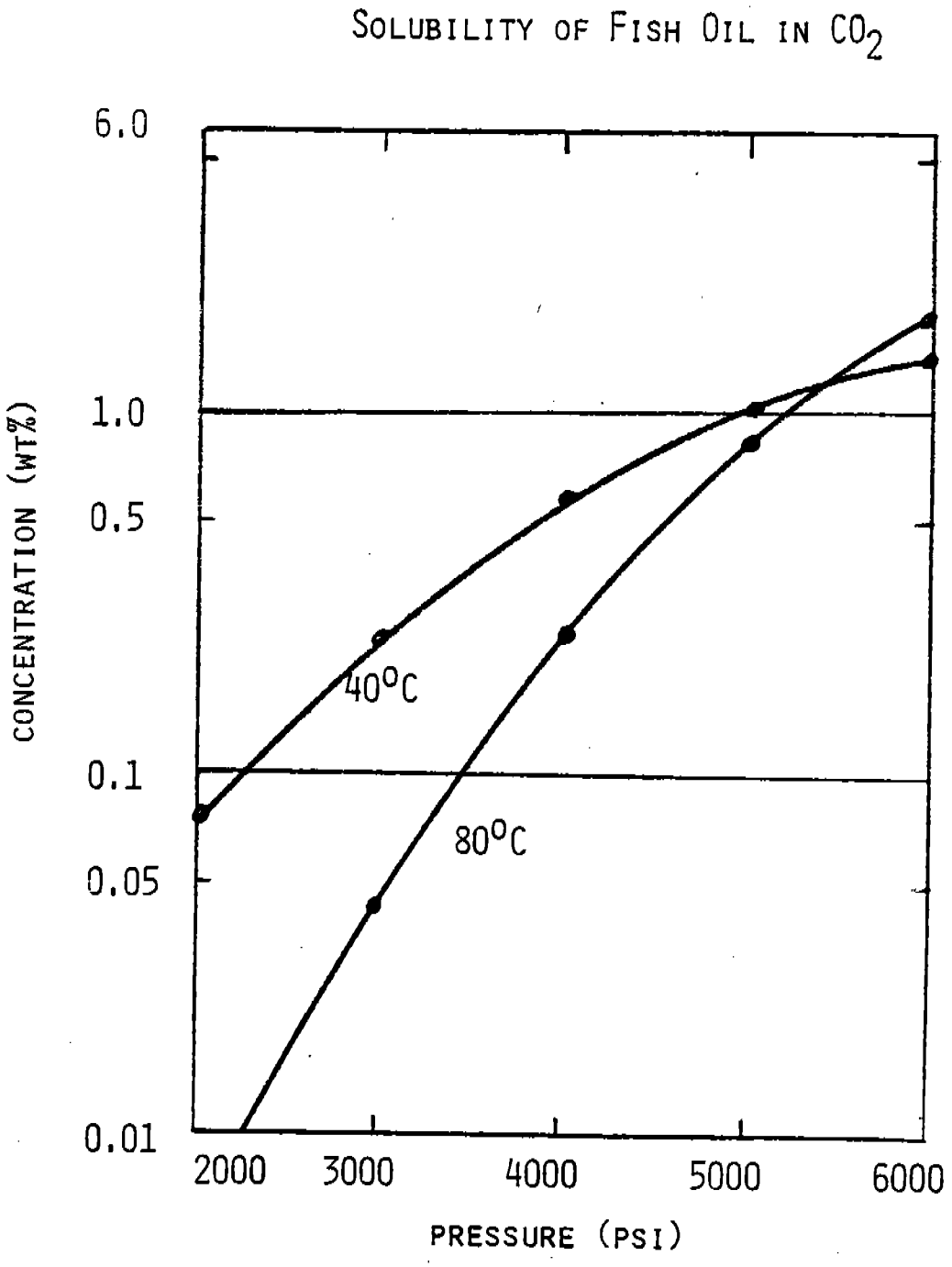
* * * * *

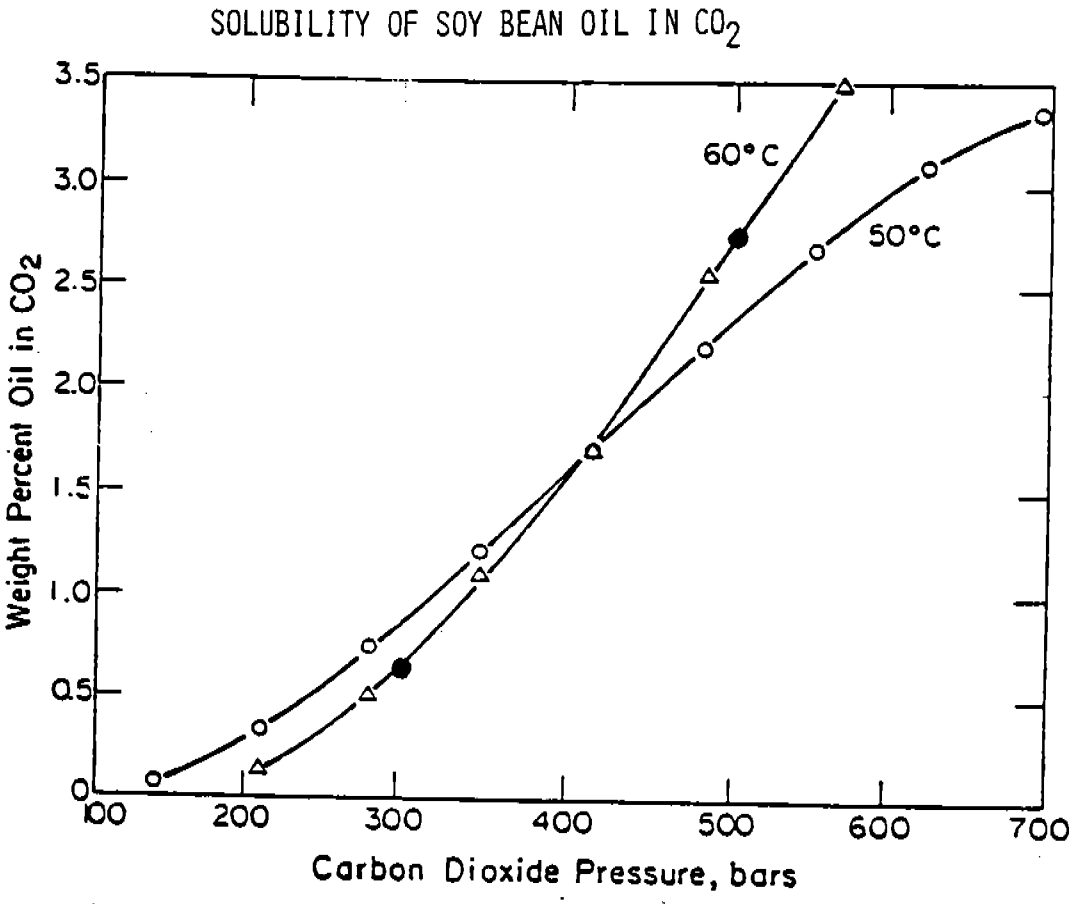
- GRAVIMETRIC FOR SOLUBILITY DETERMINATIONS

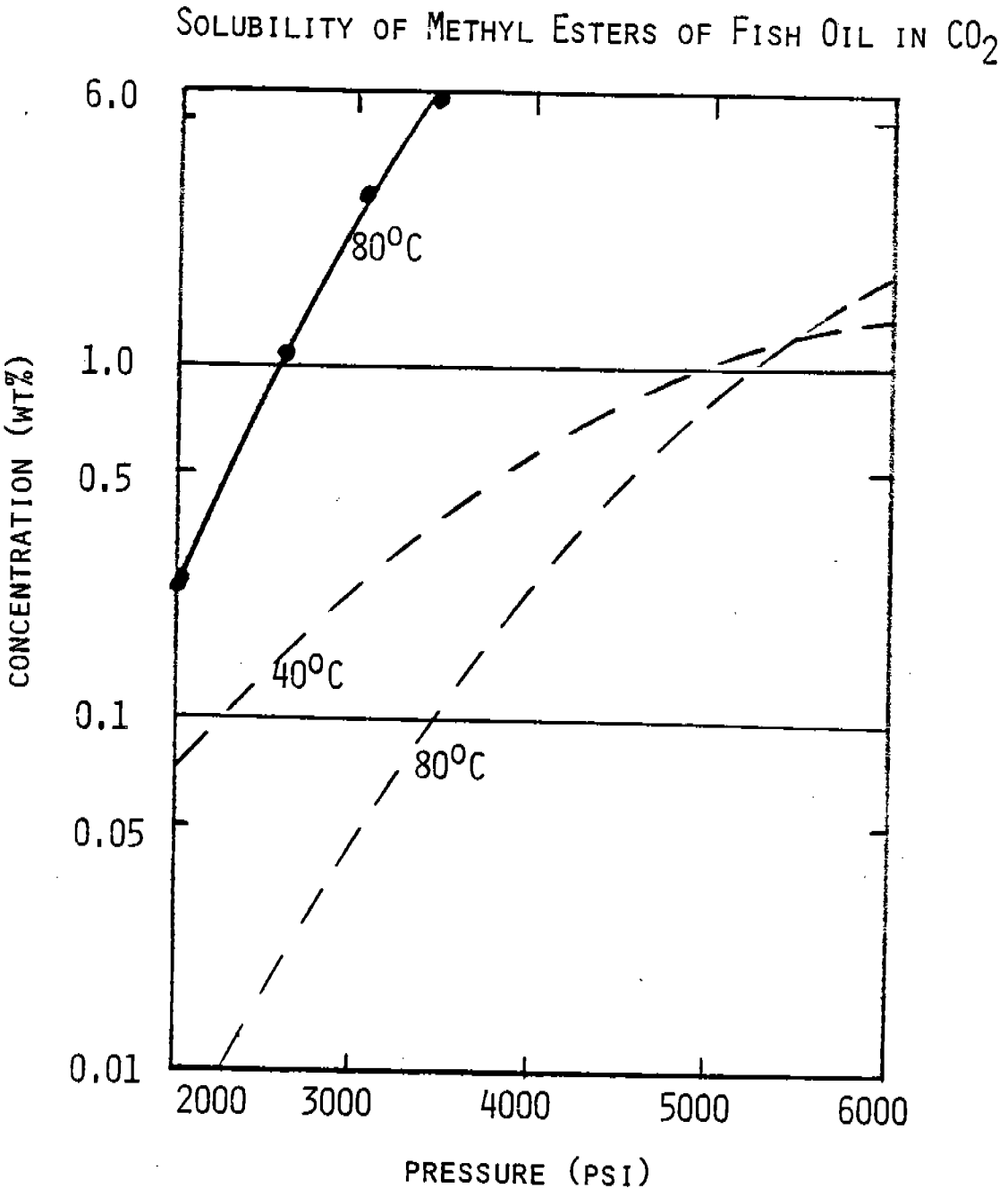
- GC ANALYSIS FOR COMPOSITION DETERMINATION
  - * Glass Capillary Column
  - * For Triglyceride Tests - Derivitized to Methyl Esters
  - * For Methyl Ester Tests - Injected Directly

EXPERIMENTAL APPARATUS FOR SUPERCRITICAL FLUID EXTRACTION




SOLUBILITY OF FISH OIL IN CO_2
6.0
1.0
0.5
0.1
0.05
0.01
CONCENTRATION (WT%)
40°C
80°C
2000
3000
4000
5000
6000
PRESSURE (PSI)


SOLUBILITY OF SOY BEAN OIL IN CO_2
Weight Percent Oil in CO_2
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
60° C
50° C
100
200
300
400
500
600
700
Carbon Dioxide Pressure, bars


SOLUBILITY OF METHYL ESTERS OF FISH OIL IN CO_2
6.0
1.0
0.5
0.1
0.05
0.01
80°C
40°C
80°C
CONCENTRATION (WT%)
2000
3000
4000
5000
6000
PRESSURE (PSI)

#	NAME	TIME		CONC	MK	AREA
0		1.21		0.8497		1932
0		1.77		0.0217		845
0		2.01	A	5.5158	V	214264
0		2.65		0.354	T	13751
0		3.11		0.0766	T	2978
0		3.53	C	15.5613	V	604484
0		4.11	D	9.9035	V	384707
0		4.81		1.8307	V	71117
0		5.39		1.4233	V	55289
0		6.41	H	3.9116	V	151948
0		7.33	I	11.5095	V	447089
0		8.47		1.2436	V	48309
0		9.15	L	2.367	V	91947
0		10.83		0.3978	V	15452
0		12.19	N	1.8957	V	73642
0		13.39		1.3242	V	51439
0		14.59	O	4.6608	V	181049
0		16.87		0.1186	T	4609
0		22.31	R	1.222		47471
0		26.07	S	1.4698		57096
0		29.99	T	13.6544		530410
0		41.75		0.7817		30366
0		47.51		0.6539		25401
0		55.35	W	2.1618		83977
0		63.99	X	17.8899	V	694938
	TOTAL			99.9999		3884522

COMPOSITION OF FEED & SCF FRACTIONS

("JAPANESE" TRIGLYCERIDE FEED)

* * *

COMPONENT	16:0	16:1	18:1	20:1	20:5	22:6	MB
CONTROL (%)	16.4	7.8	14.1	6.4	19.3	9.8	100%
-1	27.0	11.8	16.2	5.8	5.7	0.4	4.2
-2	30.2	11.7	16.6	6.7	5.1	0.5	4.9
-3	30.1	11.1	16.4	8.0	5.8	0.5	8.8
-4	23.2	10.7	14.0	8.0	6.3	0.6	10.6
-5	19.7	9.1	13.1	9.9	11.5	1.9	12.4
-6	12.7	6.4	12.8	11.0	13.5	4.3	15.2
-7	8.3	5.8	12.1	12.8	18.0	8.7	39.6

COMPOSITION OF FEED & SCF FRACTIONS

("JAPANESE" FATTY ACID FEED)

* * * *

COMPONENT	16:0	16:1	18:1	20:1	20:5	22:6	MB
CONTROL (%)	17.6	8.5	11.5	5.8	12.5	13.4	100%
FRACTION-1	36.7	14.7	14.0	4.5	1.1	0.4	12.4
-2	19.3	11.5	15.8	12.7	1.4	0.7	18.9
-3	16.2	5.6	16.1	14.	4.1	3.8	66.4

COMMENTS ON FATTY ACID & TRIGLYCERIDE RESULTS

* * * *

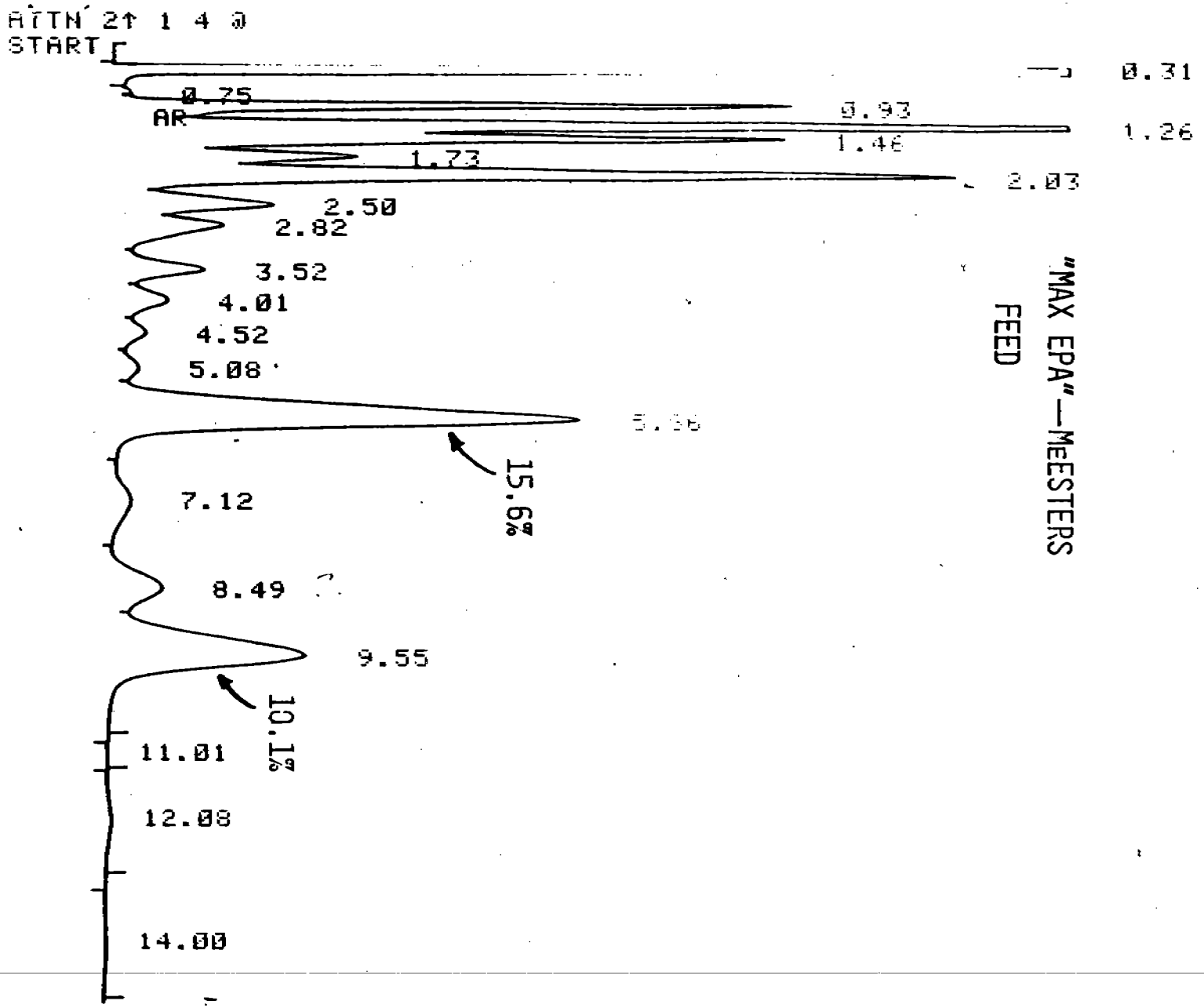
- MATERIAL BALANCE ON LOW UNSATURATES OK.
- 20:5 & 22:6 DISAPPEAR
- PARAMETERS WRONG?
    - * TEMPERATURE TOO HOT?
    - * NO, $40^{o}C$ GAVE SAME RESULTS

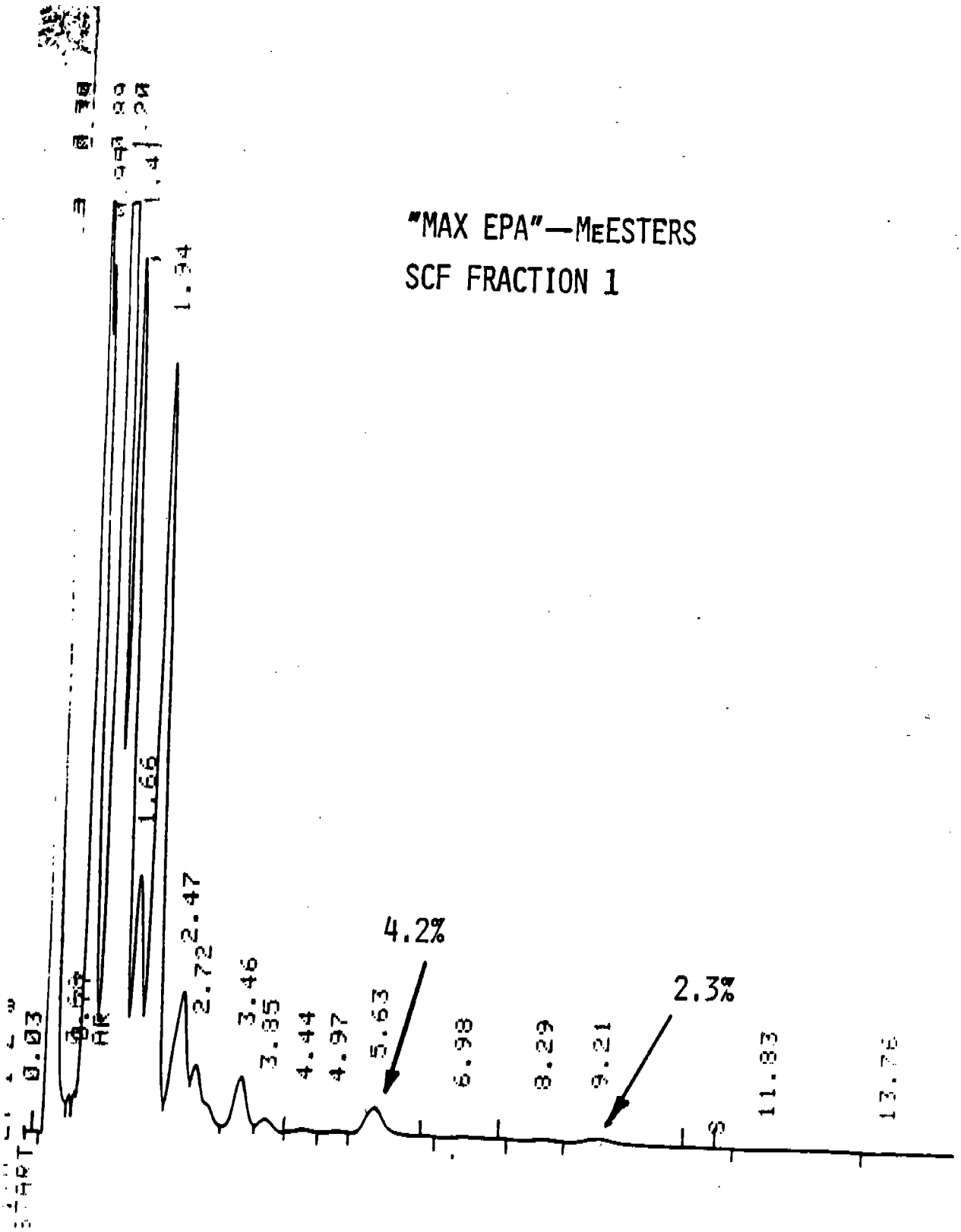
COMPOSITION OF FEED & SCF FRACTIONS

("JAPANESE" METHYL ESTERS)

* * * *

COMPONENT	16:0	16:1	18:1	20:1	20:5	22:6	MB
CONTROL %	17.9	9.3	13.4	6.1	14.6	9.8	100%
FRACTION-1	31.7	19.2	12.4	2.1	5.2	0.9	15.1
-2	19.8	9.3	27.7	8.8	11.4	2.8	33.1
-3	1.3	0.5	8.1	11.7	21.2	22.1	45.4

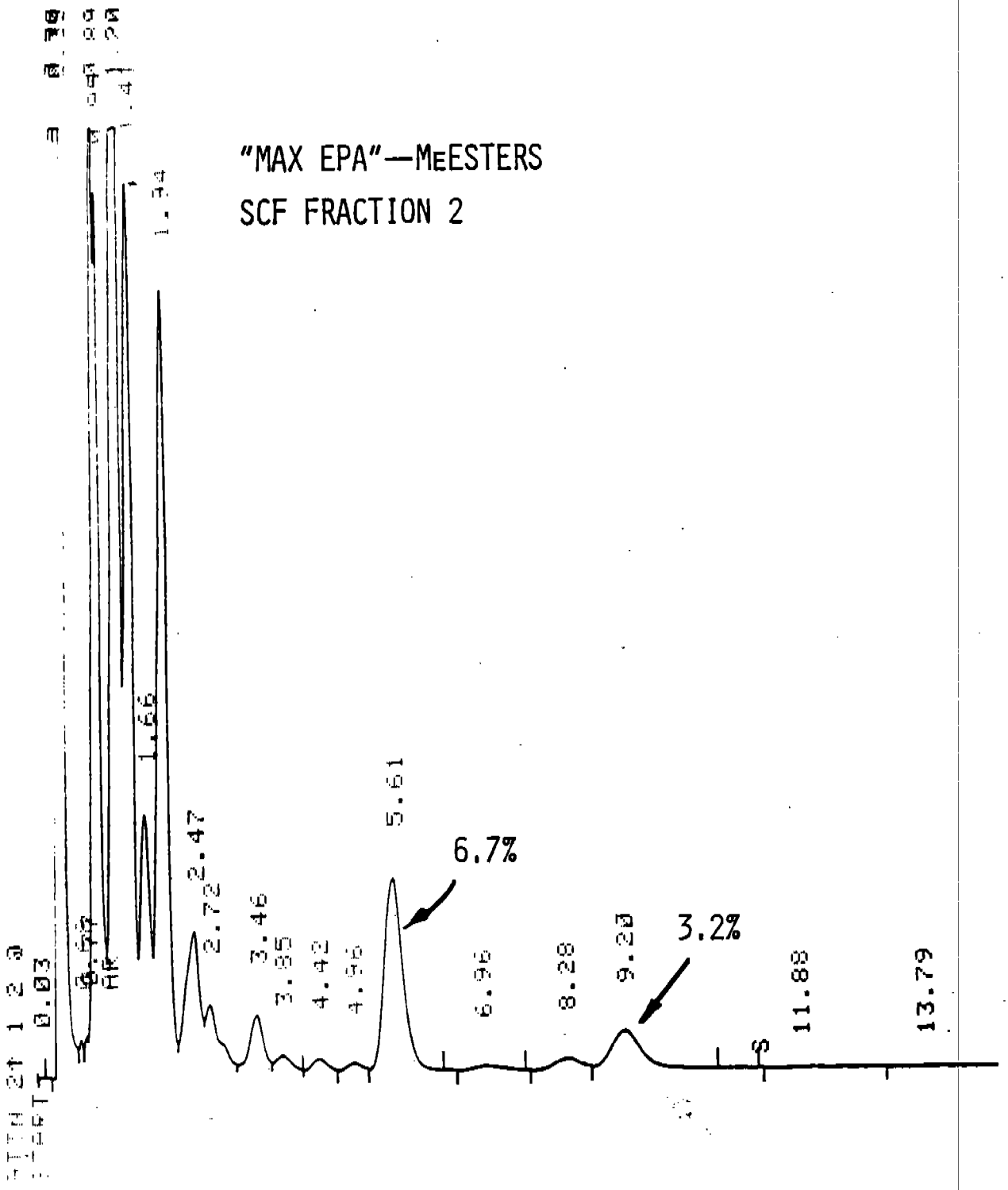

ATTN 2↑ 1 4 Ø
START
0.31
0.75
AR
0.93
1.26
1.46
1.73
2.03
2.50
2.82
3.52
4.01
4.52
5.08
5.36
15.6%
7.12
8.49
9.55
10.1%
11.01
12.08
14.00
"MAX EPA"—MEESTERS
FEED


"MAX EPA"—MeESTERS
SCF FRACTION 1
1.34
1.66
2.72
2.47
3.46
3.85
4.44
4.97
5.63
4.2%
6.98
8.29
9.21
2.3%
11.83
13.75

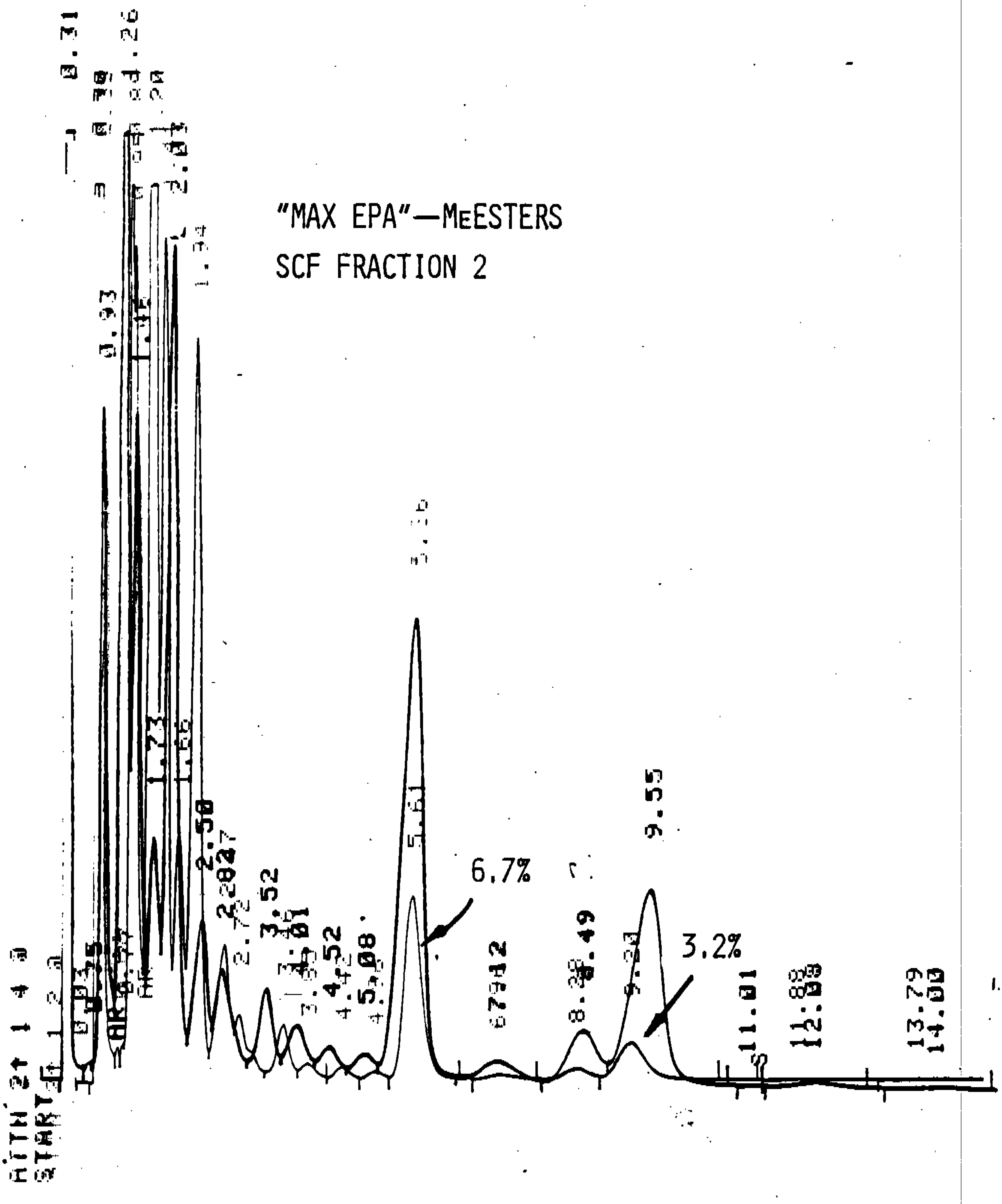
"MAX EPA"—MEESTERS

SCF FRACTION 1

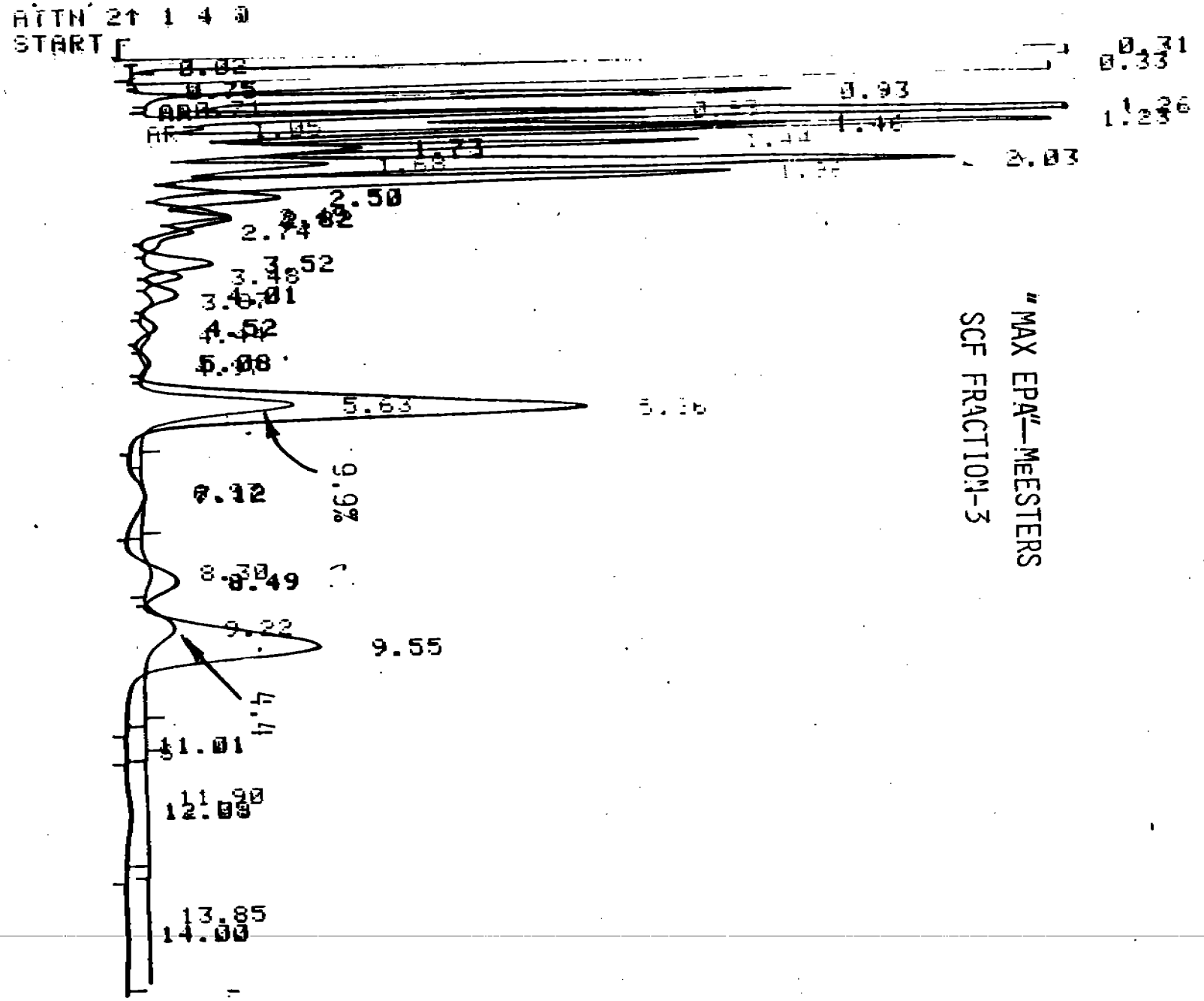
027


"MAX EPA"—MEESTERS
SCF FRACTION 2
6.7%
3.2%
0.03
1.41
1.44
1.66
2.47
2.72
3.46
3.85
4.42
4.56
5.61
6.96
8.28
9.28
11.88
13.79

027

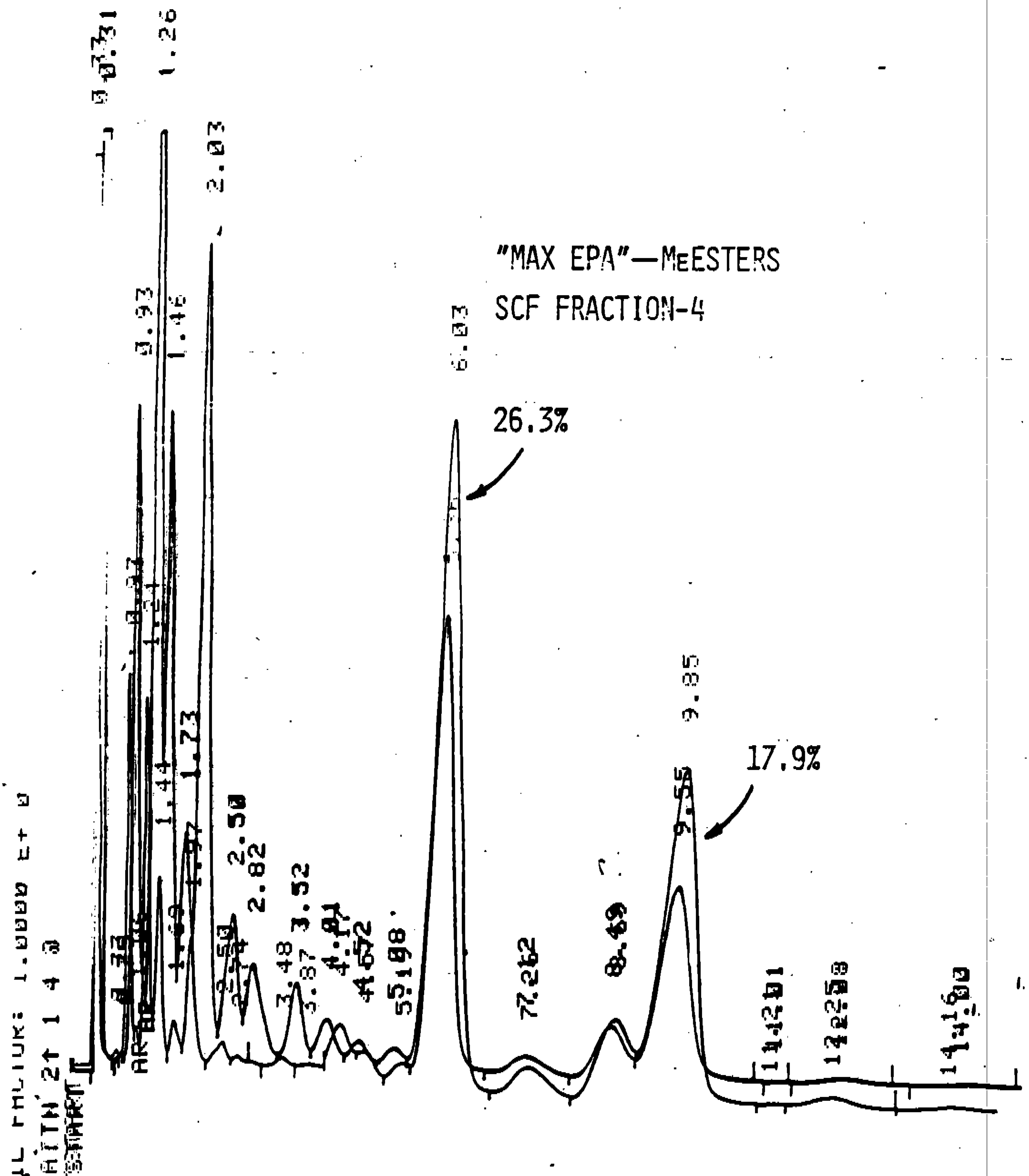


"MAX EPA"—MEESTERS
SCF FRACTION-3


ATTN 21 1 4 0
START
0.02
0.75
0.93
1.23
2.03
2.50
2.74
3.52
4.52
5.63
7.12
9.55
11.01
13.85
14.00
"MAX EPA"—MEESTERS
SCF FRACTION-3

"MAX EPA"—MeESTERS

SCF FRACTION-4

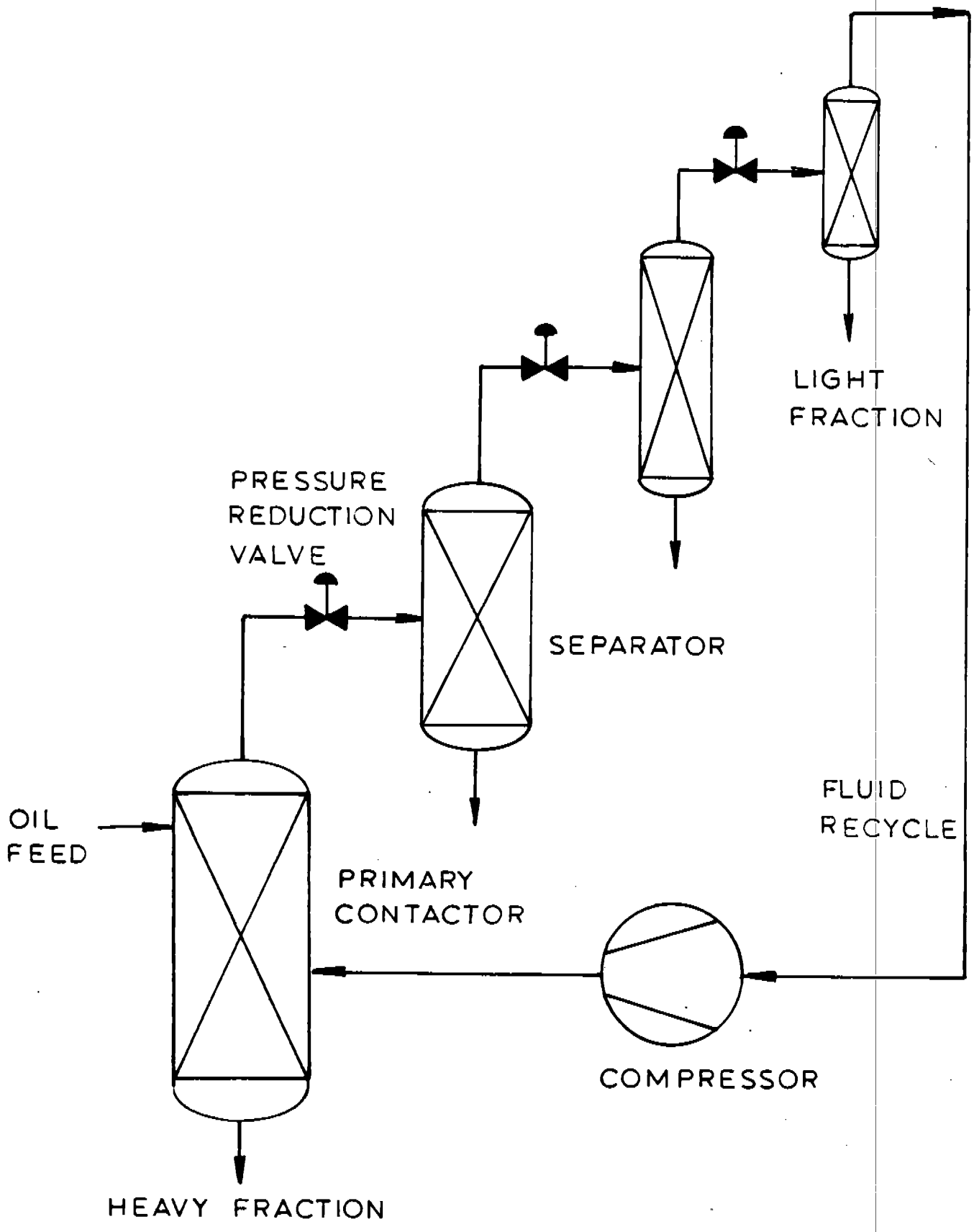

"MAX EPA"—MeESTERS
SCF FRACTION-4
26.3%
17.9%

COMMENTS ON METHYL ESTER RESULTS

* * * *

- MATERIAL BALANCE ON ALL COMPONENTS VERY GOOD
- IMPLIES METHYL ESTERS OF 20:5 & 20:6 ARE LESS LABILE
- OTHER TESTS SUGGESTED
    - * CLOSER ANALYSIS OF TRIGLYCERIDE FRACTIONS TO SEARCH FOR DEGRADATION, POLYMERIZATION
    - * RAPID ANALYSIS OF TRIGLYCERIDE FRACTIONS AFTER EXTRACTION

SUPERCRITICAL FLUID FRACTIONATION



COMPOSITION OF FEED & SCF FRACTIONS

("MAX-EPA" TRIGLYCERIDES—
EXTRACTION FOLLOWED BY IMMEDIATE ANALYSIS)

COMPONENT	20:5	22:6	MB
CONTROL (%)	17.1	11.4	100%
FRACTION-1	15.5	9.6	6.5
-2	15.6	10.4	8.4
-3	17.8	13.5	81.1

- MATERIAL BALANCE ON 20:5, 22:6 VERY GOOD

COMPOSITION OF FEED & SCF FRACTIONS

(METHYL ESTERS OF ANCHOVY OIL)

* * * *

COMPONENT	14:0	16:0	18:1	20:5	22:6	MB
CONTROL (%)	8.8	21.3	13.0	15.4	10.1	100%
-1	21.0	32.8	12.9	5.1	1.7	14.1
-2	10.9	29.9	17.4	6.1	1.9	34.3
-3	3.5	14.3	14.6	22.4	11.7	29.3
-4	2.5	6.1	3.4	29.5	45.8	18.3

SUMMARY—CONCLUSIONS

- SCF FRACTIONATION OF FISH OILS CAN ENHANCE 20:5 & 22:6
- METHYL ESTERS ARE BENEFICIATED TO A HIGHER DEGREE THAN TRIGLYCERIDES ARE
- TRIGLYCERIDES APPEAR TO BE MORE LABILE
- IMMEDIATE ANALYSIS OF FRACTIONS SHOWED THAT 20:5 &22:6 ARE PRESERVED.