(Received-RCB)
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460
Analytical Chemistry Section
Building 402, ARC-East
Beltsville, Maryland 20705

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#4F3148/FAP#4H5443: Bayleton on Milk

FROM: King Zee, Chemist
Analytical Chemistry Section

THRU: Warren R. Bontoyan, Head
Analytical Chemistry Section

THRU: Donald A. Marlow, Chief
Chemical Operations Branch

TO: Charles L. Trichilo, Chief
Residue Chemistry Branch
Hazard Evaluation Division

The Residue Chemistry Branch (RCB) requested ACS to conduct a method trial on the analysis of Bayleton and its three metabolites (KWG-0519, KWG-1323 and KWG-1342) in milk. The method entitled: "Analysis of Bayleton and its Metabolites in Cattle Tissue and Milk by Gas Chromatography/Mass Spectrometry" was developed by Mobay Chemical Corporation. Milk samples are fortified at 0.04 and 0.1 ppm level of the parent compound and metabolites. Recoveries were satisfactory.

Principle of Analysis

Bayleton and its metabolites are extracted from milk using methanol in a Polytron blender. After vacuum filtration and rotary evaporation, the extract is cleaned up by passing it through XAD-4 ion exchange resin. Elute the column with methanol and concentrate by rotary evaporation. The residue is hydrolyzed by refluxing with concentrated hydrochloric acid, which converts the compounds to p-chlorophenol. The resulting phenol is steam distilled and further cleaned-up by acid/base partitioning. An internal standard of 3, 4-dichlorophenol is added before it is derivatized by 2,4-dinitrofluorobenzene. The samples are assayed using gas chromatography/mass spectrometry, monitoring the ions 127 and 161 AMU.

Source of Analytical Standards

Bayleton and metabolites, KWG-0519, 1323, and 1342 were obtained from RTP. The derivatizing reagent, 2,4-dinitrofluorobenzene is a common chemical reagent and is readily available from chemical supply houses.

Derivatization

The sample is calculated by comparing GC/MS responses of the derivatized standard and internal standard. The derivatized standards are not available from the company and RTP. The derivatized standards are prepared each time a sample is analyzed. The analyst should practice the derivatization step until acceptable control responses are achieved.

Gas Chromatography Conditions

Instrument:	HP5985A GC/MS
Software:	SIM (Single ion monitoring) EI - 70 EV
Column:	6' x 2mm id pyrex packed with 3% Dexil 300 on 80/100 Supelcoport
Oven temp:	250 C
Carrier gas:	Helium at 25 ml/min.
Retention times:	DNFB derivative of PCP - 3 min. DNFB derivative of 3,4 PCP - 5 min.

Recoveries

Chemical added	ppm	ppm found	recovery
Bayleton	0.00	<0.01	-
	0.00	<0.01	-
	0.040	0.035	88
	0.040	0.041	103
	0.100	0.088	88
	0.100	0.107	107
KWG 0519	0.040	0.043	108
	0.040	0.028	70
	0.100	0.112	112
	0.100	0.066	66
KWG 1323	0.040	0.037	93
	0.040	0.028	70
	0.100	0.097	97
	0.100	0.076	76
KWG 1342	0.040	0.033	83
	0.040	0.035	88
	0.100	0.079	79
	0.100	0.085	85

Comments:

(1) Method is long; it takes three days to analyze one set of samples.

(2) Method gives satisfactory results and appears to have no safety problems.

(3) Company should provide derivatized standards needed in the GC/MS work. It would reduce the time of analysis and give a direct comparison to the samples.

Method: Residue Analysis Procedure for ®BAYLETON and Metabolites in Barley and Wheat - Report Number 80488 - dated July 27, 1983.

Do not use control values for recovery corrections.

Do not report control values as 0; if less than limit of detection, report as such.

Commodity	Chemical Added	PPM Added	PPM Found	% Recovery
tomatoes	Bayleton	0.00	<.01	
		0.05		80, 80
		0.20		80, 100
tomatoes	KWG-0519	0.00	<.01	
		0.05		80, 80
		0.20		75, 80
tomatoes	KWG-1323	0.00	<.01	
		0.05		80, 80
		0.20		80, 80
tomatoes	KWG-1342	0.00	<.01	
		0.05		80, 80
		0.20		95, 95

Method of Analysis for Bayleton and its Metabolites in Tomatoes

Modifications to method (major or minor): We tried to use Du Pont's HPLC 8000 instrument instead of ABC's gel permeation instrument as specified in the method. HPLC instrument could not do the work. We had to borrow Mobay's gel permeation instrument to complete the trial.

Special precaution to be taken: None.

Source of analytical reference standards: RTP.

If derivatized standard used, give source: None.

Instrumentation for quantification: HP 5730 A: NP detector.

Instrumentation for confirmation: None.

If instrument parameters differ from method given, list parameters used: See Report.

Commercial source for any chemicals or apparatus: Fisher Scientific.

Comments: See Report.

Chromatograms: Included.

Method: Analysis of ®BAYLETON and its Metabolites in Cattle Tissues and Milk by Gas Chromatography/Mass Spectrometry - Report Number 69531 - Dated June 8, 1981.

Do not use control values for recovery corrections.

Do not report control values as 0; if less than limit of detection, report as such.

Commodity	Chemical Added	PPM Added	PPM Found	%Recovery
milk	Bayleton	0.00	<0.01	
		0.04		88, 103
		0.10		88, 107
milk	KWG-0519	0.00	<0.01	
		0.04		108, 70
		0.10		112, 66
milk	KWG-1323	0.00	<0.01	
		0.04		93, 70
		0.10		97, 76
milk	KWG-1342	0.00	<0.01	
		0.04		83, 88
		0.10		79, 85

Method of Analysis for Bayleton and its Metabolites in Milk

Modifications to method (major or minor): None.

Special precautions to be taken: None.

Source of analytical reference standards: RTP.

If derivatized standard use, give source: Derivatized standard not available from Mobay and RTP. We derivatized standards ourselves.

Instrumentation for quantitation: HP 5985 A GC/MS/ SIM.

Instrumentation for confirmation: None.

If instrument parameters differ from method given, list parameters used: EI - 70 EV, Oven Temp. 250°. H_e - 25 ml/min.

Commercial source for any special chemicals or apparatus: Fisher Scientific.

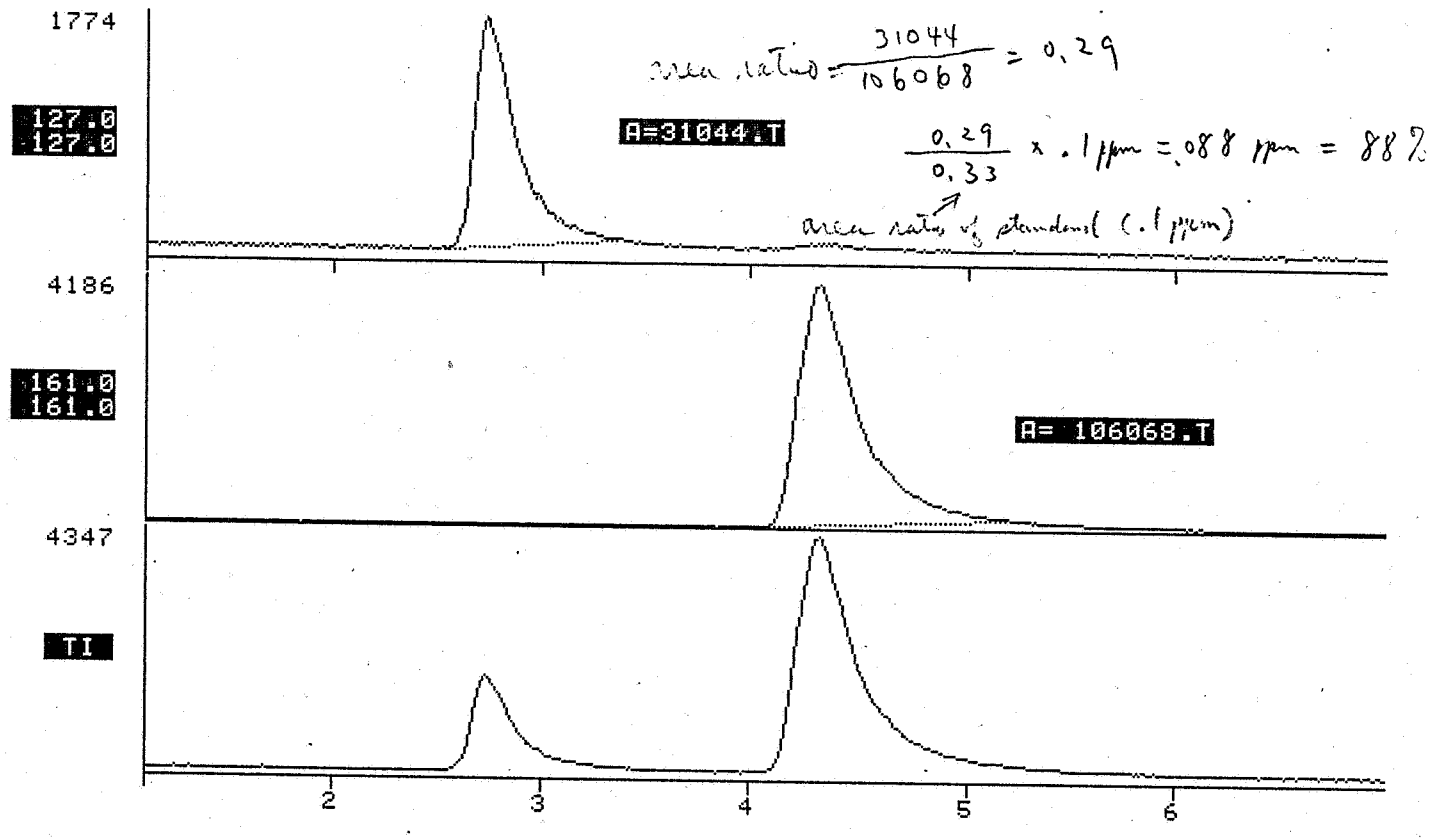
Comments: See Report.

Chromatograms: Included.

milk 0.1 ppm level

NAME A1 0.1PPM 5/
MISC 3%OV3 5/23/85

FRN 7500



area ratio = $\frac{31044}{106068} = 0.29$

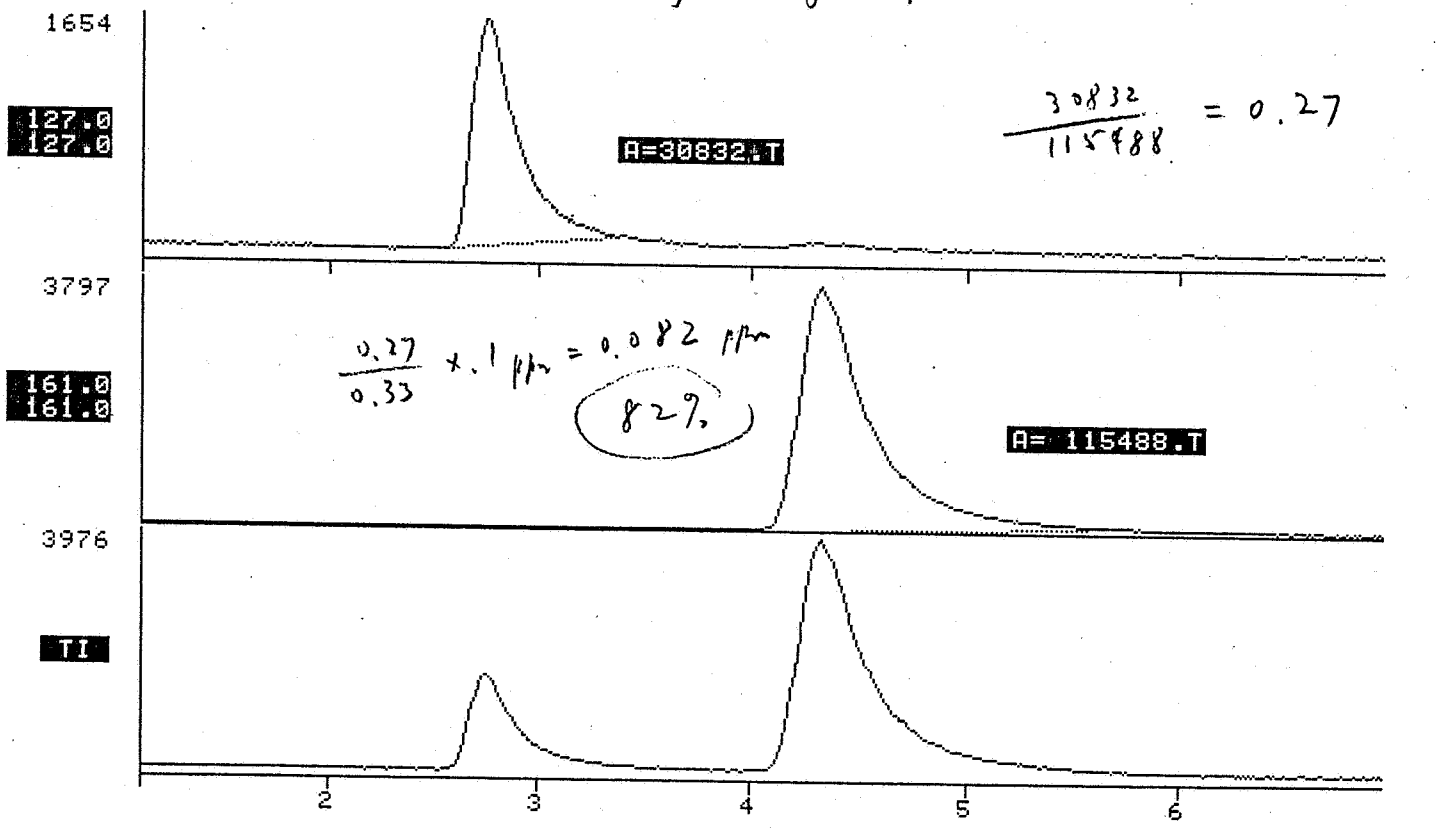
$\frac{0.29}{0.33} \times .1\text{ ppm} = .088\text{ ppm} = 88\%$

area ratio of standard (.1 ppm)

NAME A1 0.1PPM 5/5ML
MISC 3%OV3 5/23/8@$

duplicat injection of sample A1

FRN 7501

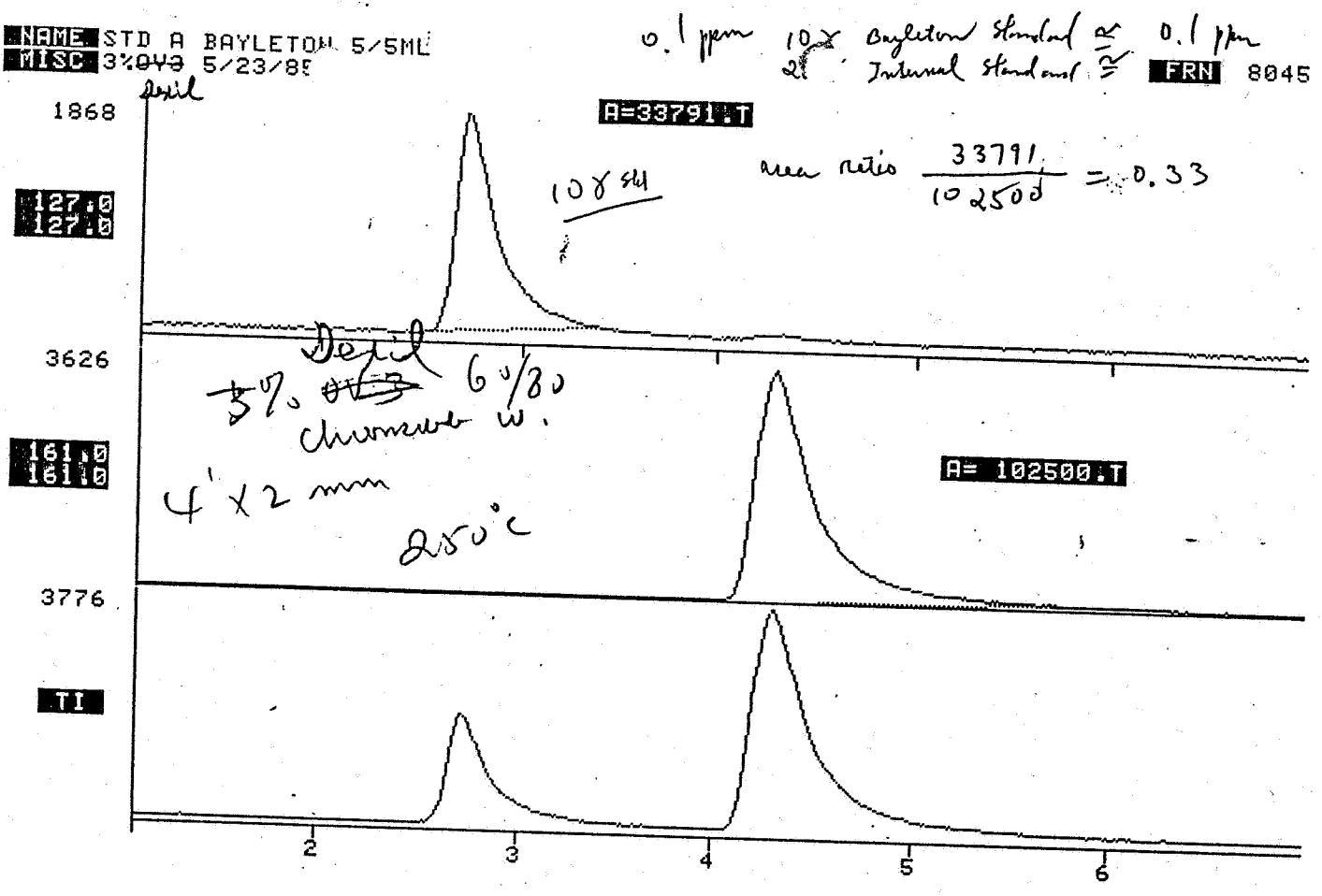


$\frac{30832}{115488} = 0.27$

$\frac{0.27}{0.33} \times .1\text{ ppm} = 0.082\text{ ppm}$

82%

8



0.1 ppm 10x Bayleton Standard ≈ 0.1 ppm
2x Internal Standard ≈

10x std

area ratio $\frac{33791}{102500} = 0.33$

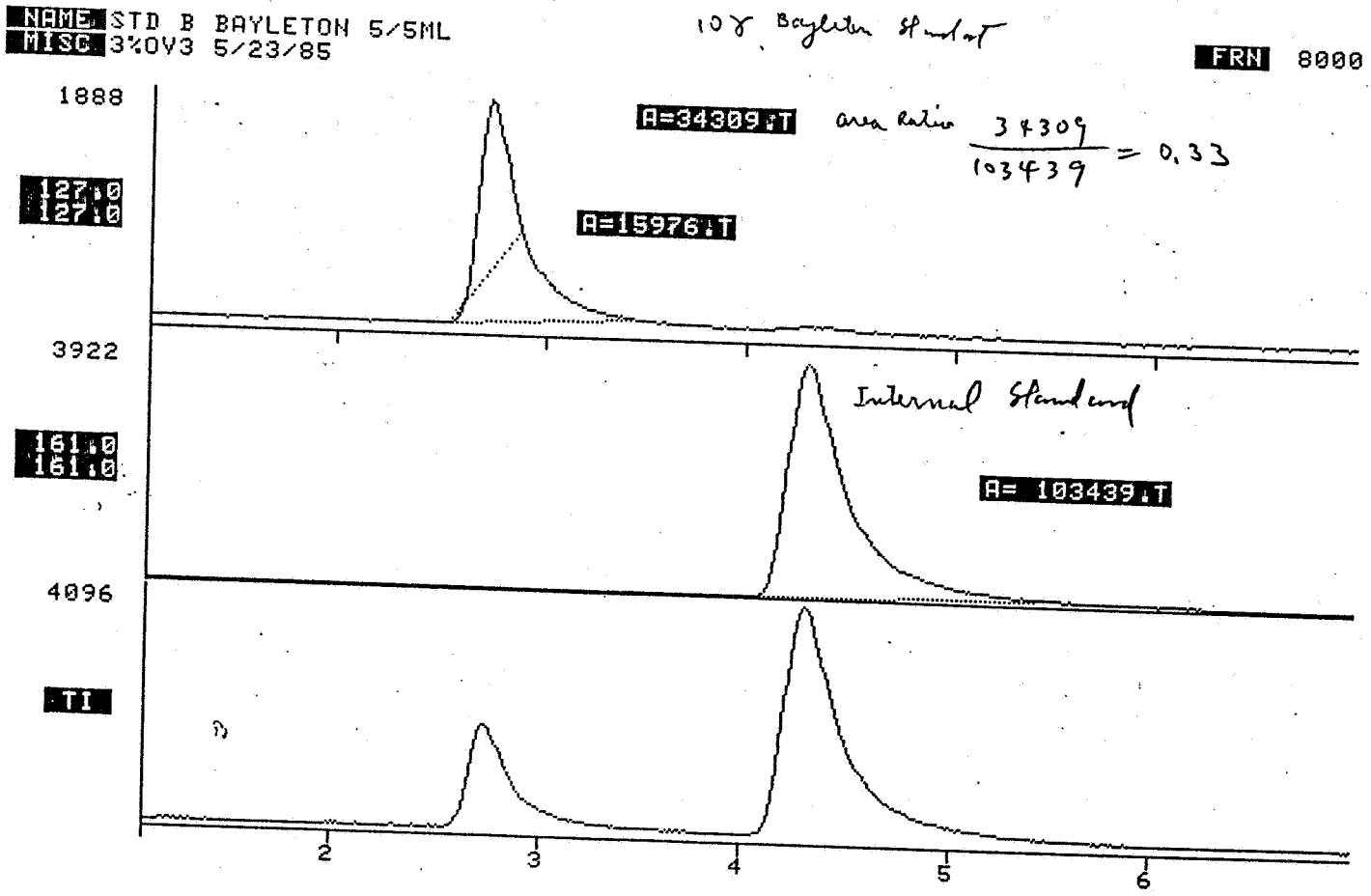
Acid

3% 6'/80

Chromosorb W.

4' x 2 mm

250°C



10x Bayleton Standard

area ratio $\frac{34309}{103439} = 0.33$

Internal Standard

milk control

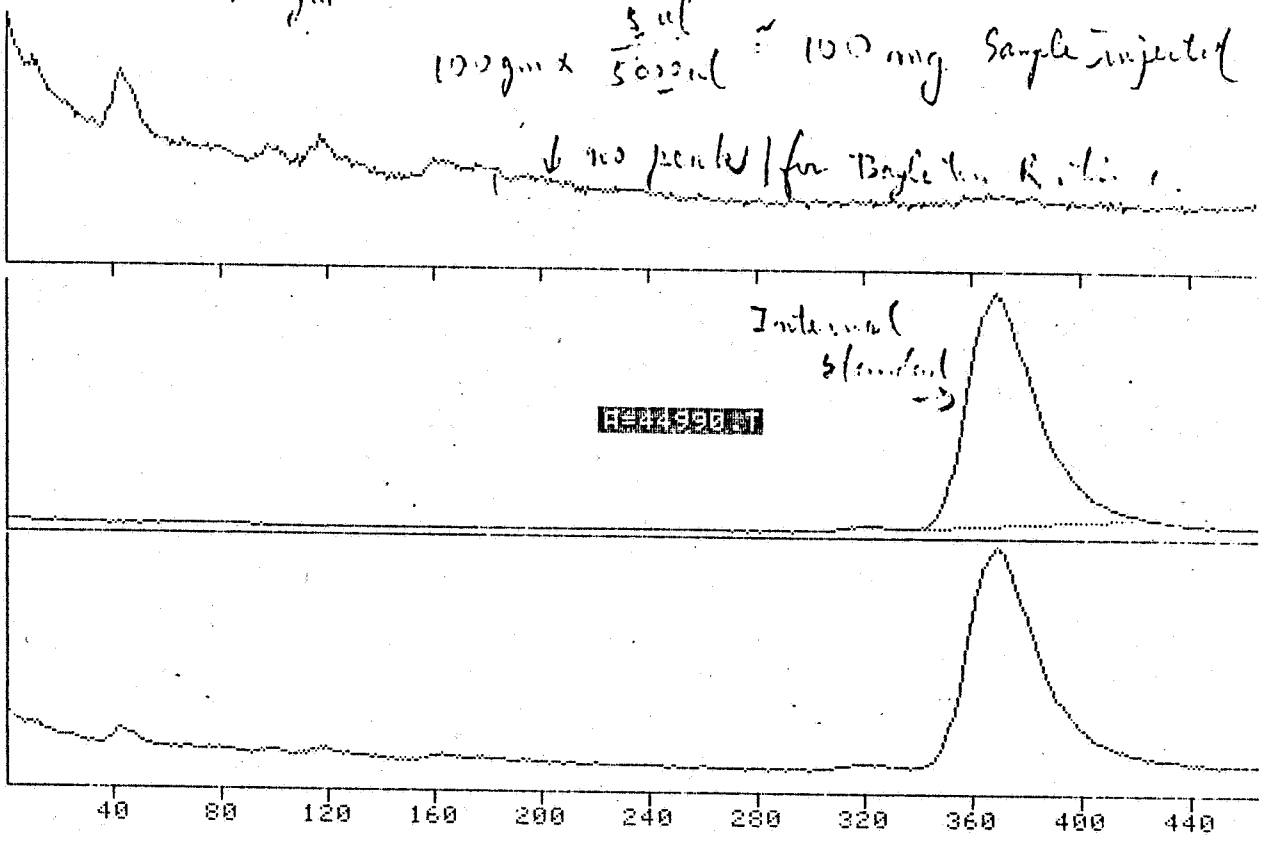
BLANK 1
SUL 5ML

8003

100 gm milk

100 gm x $\frac{5\ ml}{500\ ml}$ = 100 mg Sample injected

↓ no peaks for Bayleton R.T.



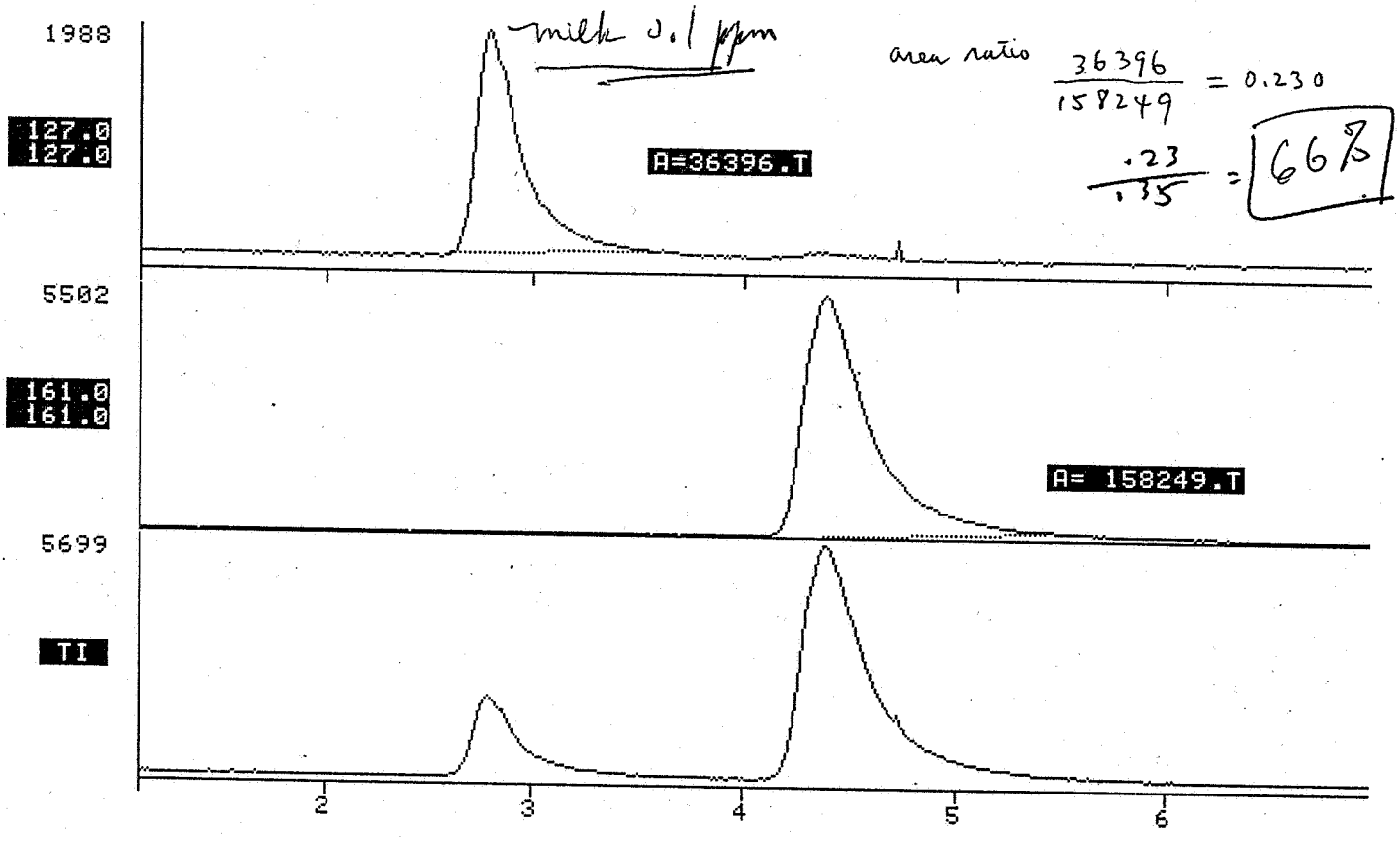
control is ≤ 1 mg

$\therefore \frac{1\ mg}{100\ mg}$ = < 0.01 ppm

B-2 5/5 ml Sample B-2 5/5.0 ml

NAME B25/5ML
MISC 3%OV3 5/23/8[illegible]

FRN 7511



milk 0.1 ppm

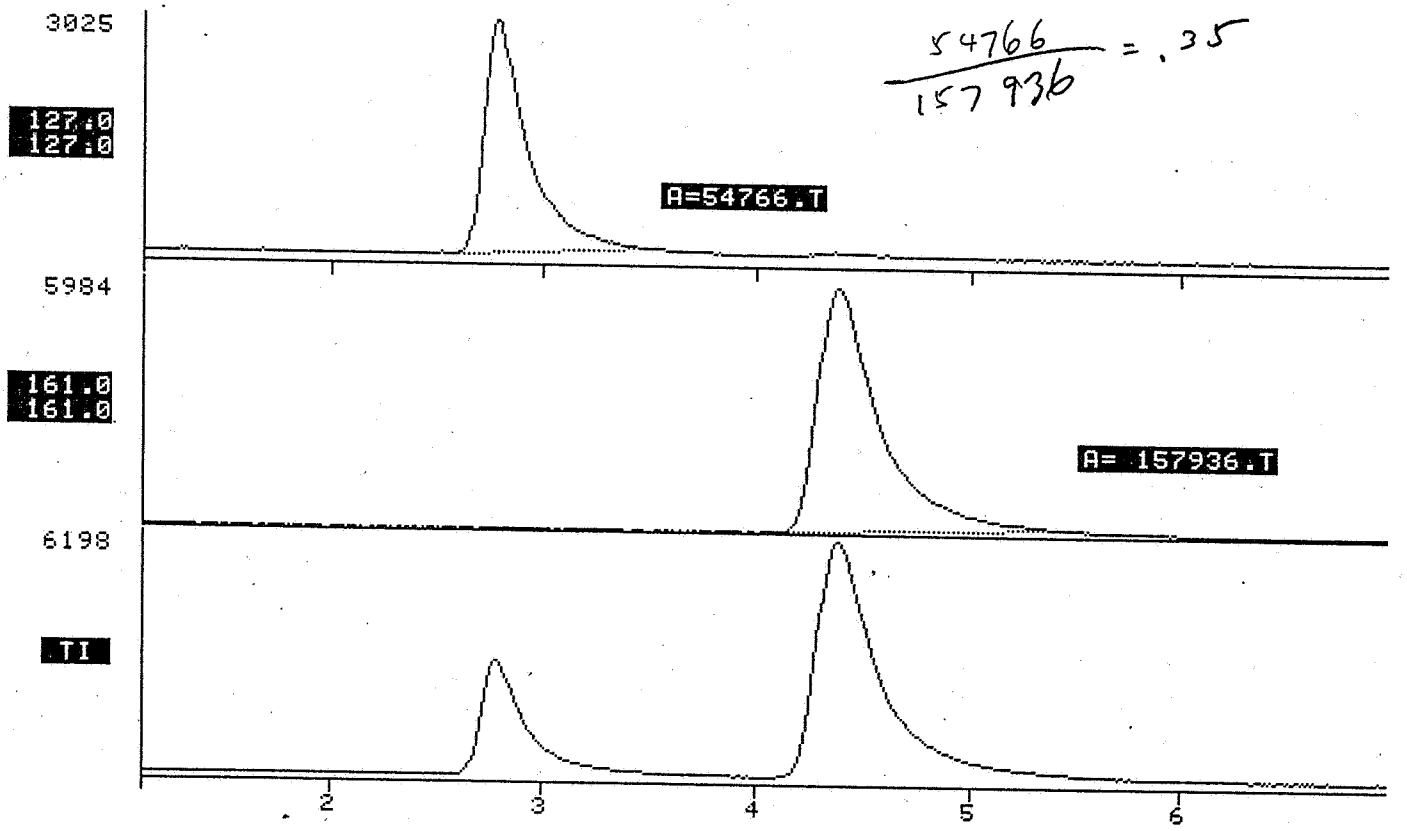
area ratio $\frac{36396}{158249} = 0.230$

$\frac{.23}{.35} = 66\%$

NAME STD B 5/5ML
MISC 3%OV3 5/23/8[illegible]

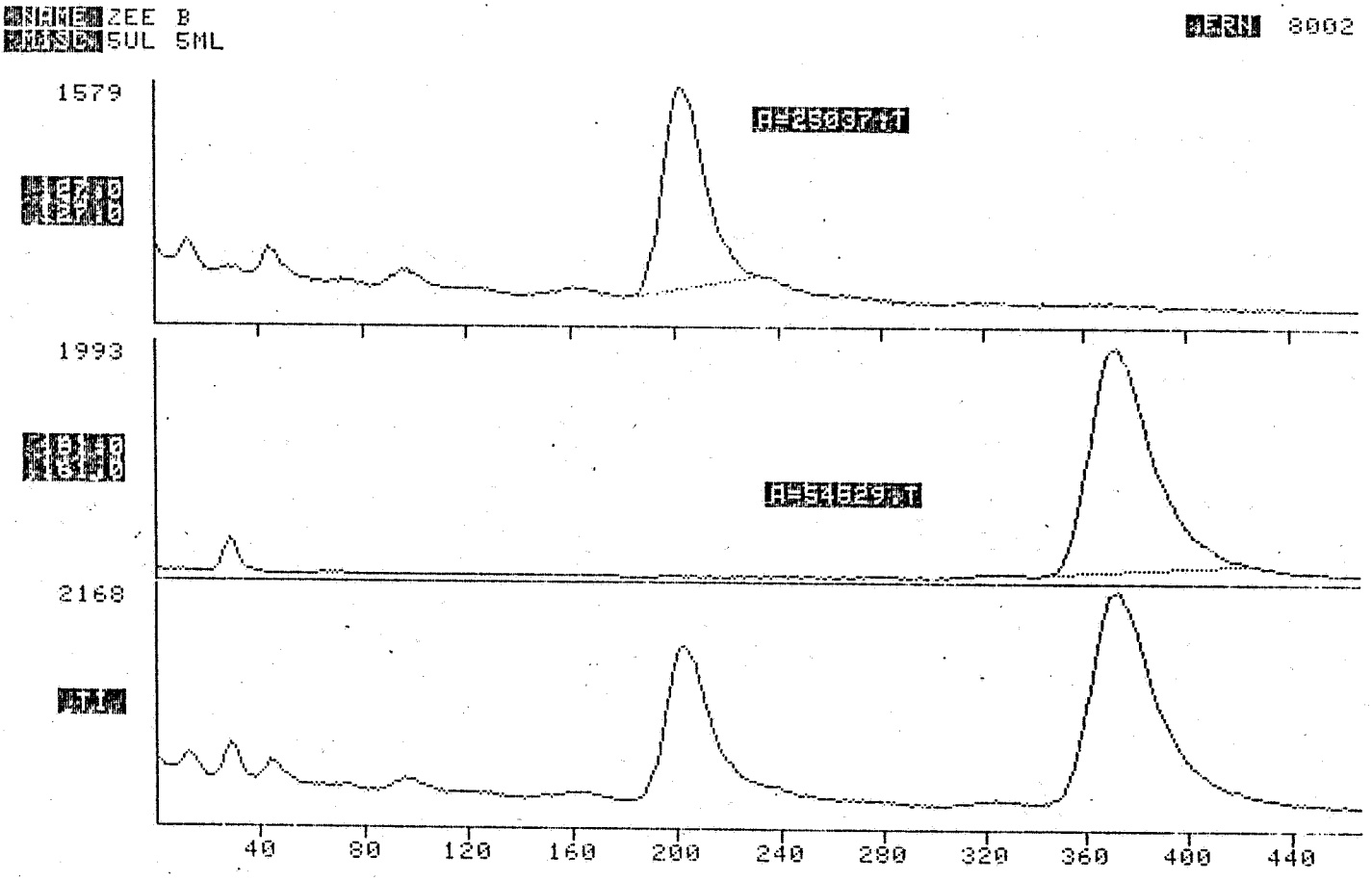
Standard 0519 .1 ppm

FRN 7512



$\frac{54766}{157936} = .35$

$10\gamma \times \frac{5}{5\,ml} \cong 10\,mg$ Standard "Baytril"

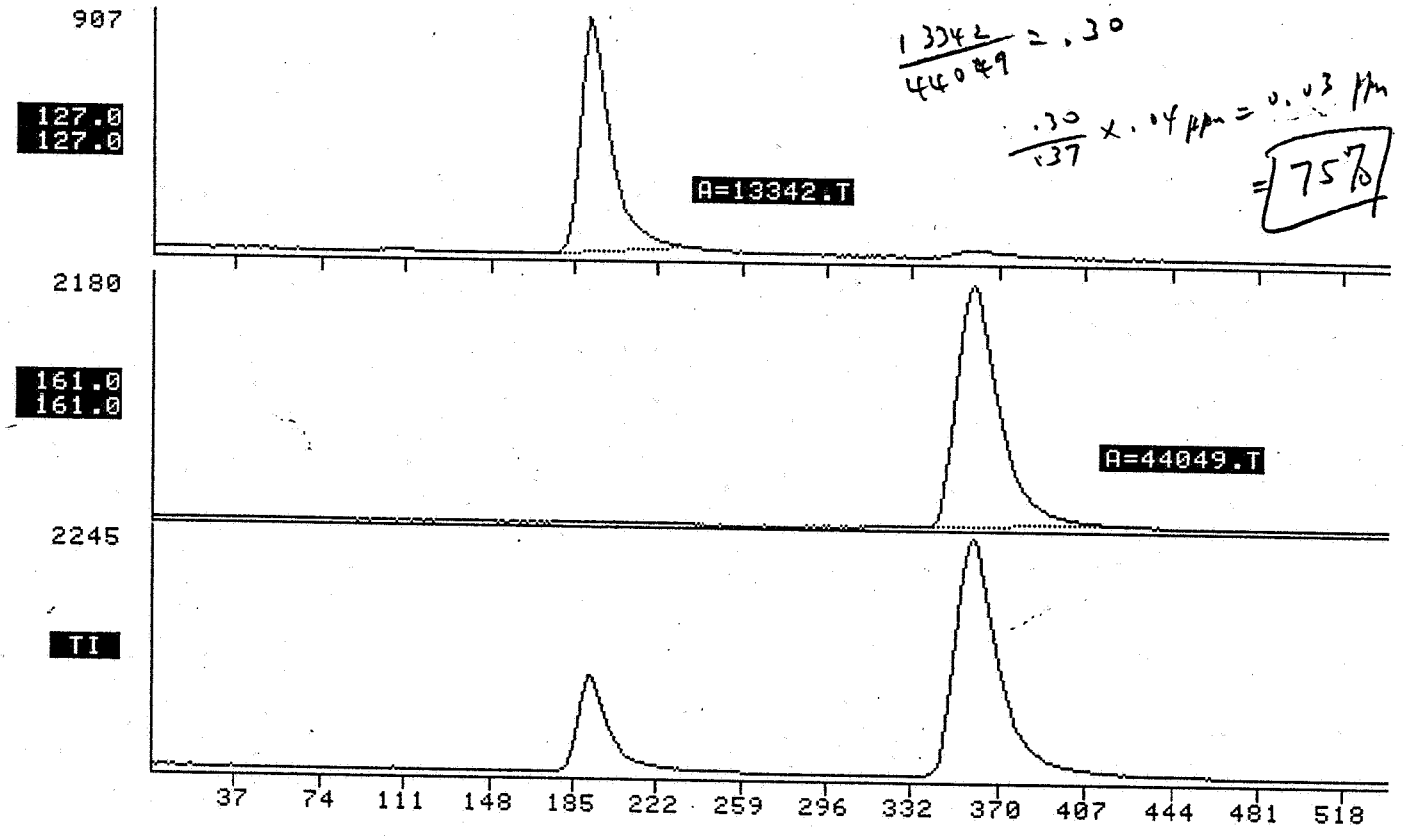


$\text{Area ratio} = \frac{25037}{54529} = 0.46$

Kwe 1342

NAME 0.04 PPM H-1
MISC 40UGX 5/2M@$

FRN 15010



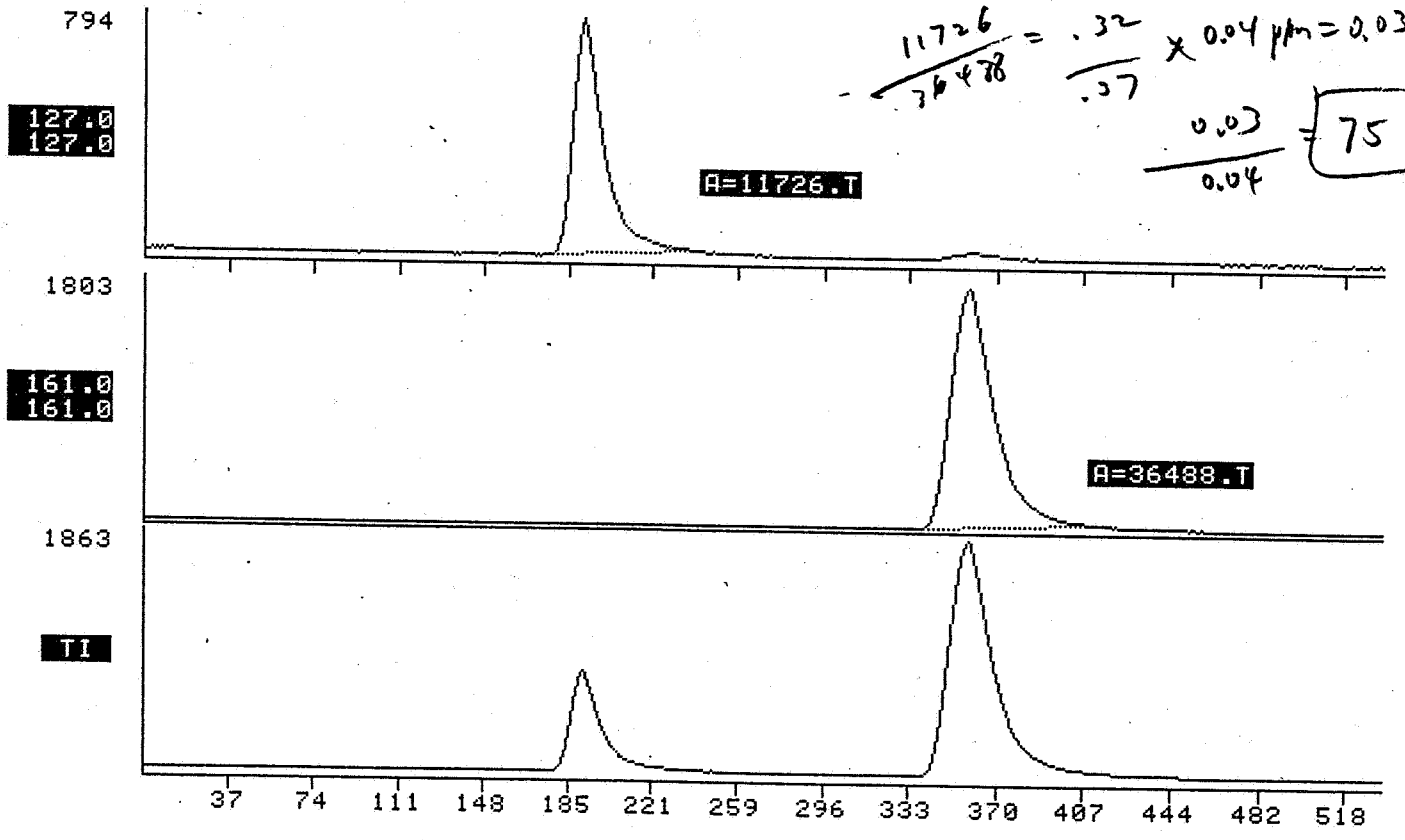
$\frac{13342}{44049} = .30$

$\frac{.30}{.37} \times .04\ \mu m = 0.03\ \mu m$

$= 75\%$

NAME 0.04PPM H-2
MISC 40UGX 5/2M@$

FRN 15011



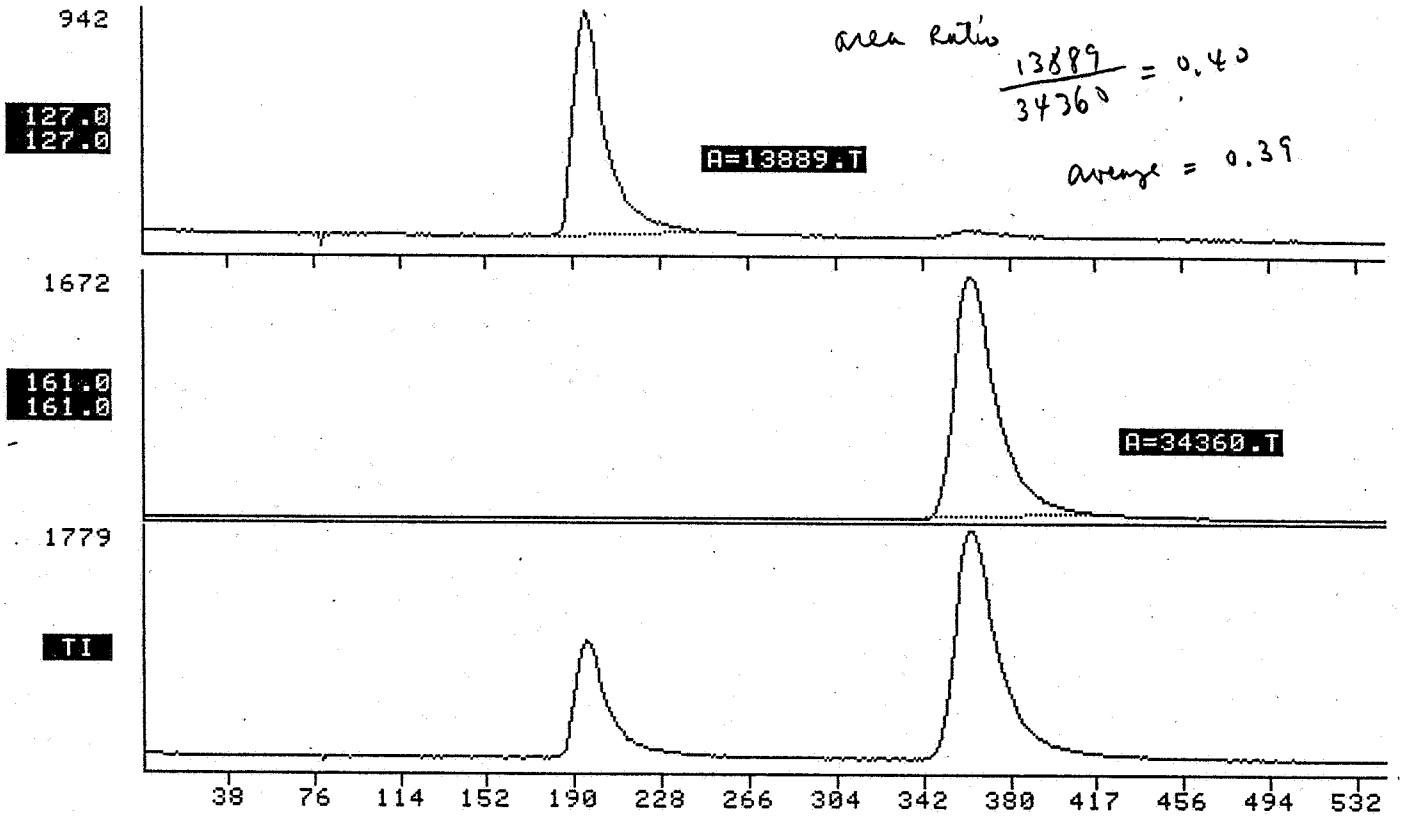
$\frac{11726}{36488} = \frac{.32}{.37} \times 0.04\ \mu m = 0.03$

$\frac{0.03}{0.04} = 75\%$

NAME BAYLETON STD
MISC 40UGX 5/2M@$

Standard 4.0 γ × 5 µl / 2000 µl = 10 γ injected

FRN 15002



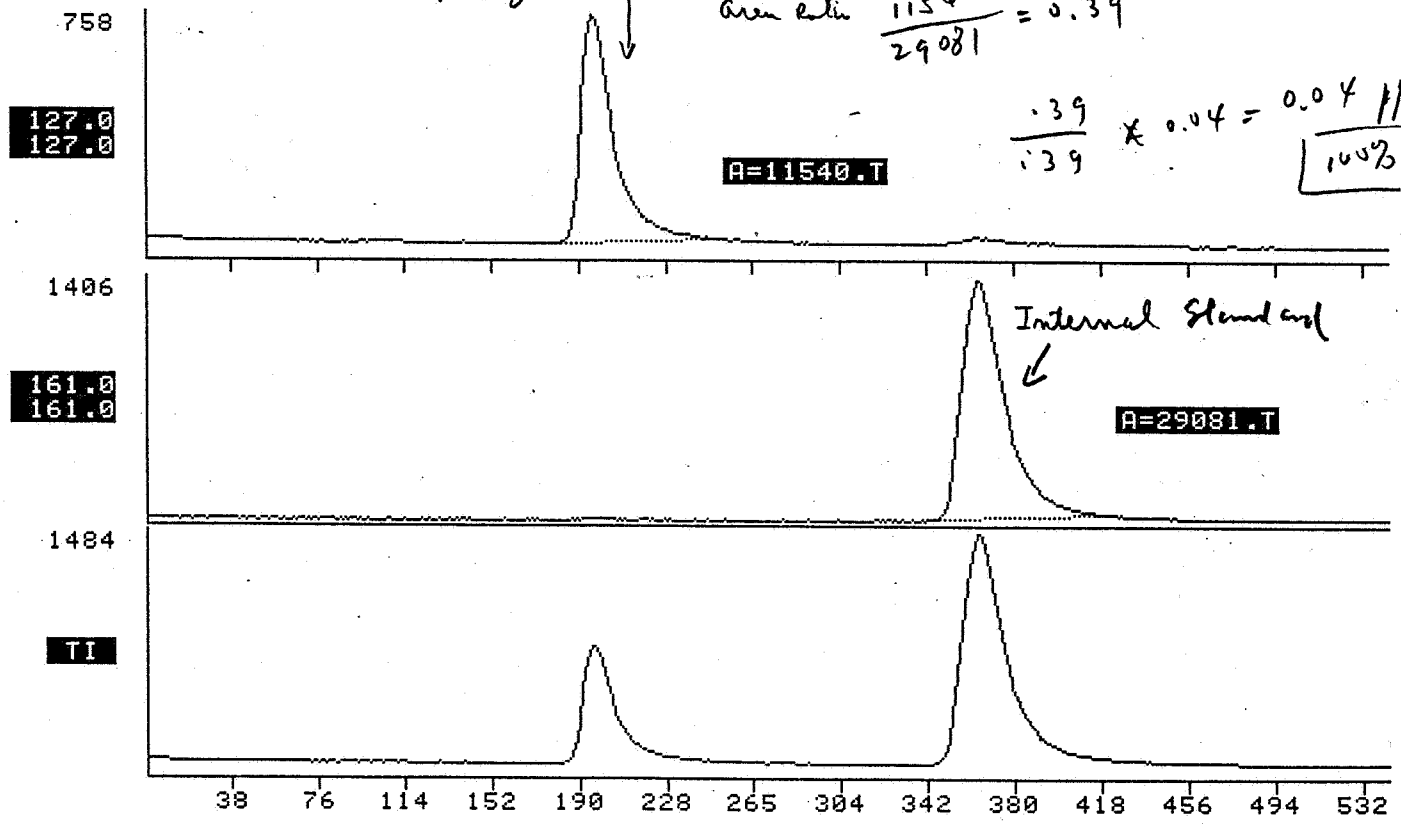
area ratio 13889 / 34360 = 0.40

average = 0.39

NAME .04PPM E-2 5/2ML
MISC 40UGX 5/2M@$

Bayleton

FRN 15003



area ratio 11540 / 29081 = 0.39

Internal Standard

.39 / .39 × 0.04 = 0.04 ppm

100%