

1/23/40

MEMORANDUM

SUBJECT: Method Validation Report for Imazaquin (SCEPTER) in Soil
Environmental Chemistry Method ECM0089S1

TO: Robert Taylor, PM 25, and Edward Allen, PM Team Reviewer
Registration Division (7505C)

FROM: Alex T. Clem, Environmental Scientist *Alex Clem*
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THRU: Akiva D. Abramovitch, Ph.D, Head *Akiva Abramovitch*
Environmental Chemistry, Review Section 3, EFGWB

Henry Jacoby, Chief
EFGWB *Henry Jacoby*

The Environmental Chemistry Laboratory of BEAD/ACB has determined that a *soil method of analysis (ECM0089S1) for the herbicide imazaquin (CL 252,214, SCEPTER) is acceptable*. American Cyanamid designates the method as M-1854, and gives their procedure in Report #C3118.01 (EPA MRID 430198-01). The official EPA lab memorandum and full report with comments is attached for release and general use.

Part I, page 3 of the EPA lab report (the Summary and Conclusions section) succinctly describes the analytical procedure and gives the method range and limitations. Although the EPA analyst obtained a limit of quantitation (LOQ) of only 12.5 ppb, rather than an LOQ of 5 ppb as indicated by Cyanamid, the analyst "believes that the sensitivity was limited by the instrumentation, not by the methodology itself" (comment 2, page 7 of report).

Even though it is standard lab practice, EFGWB cautions that individual laboratories must run appropriate sets of soil samples and calibration standards to determine actual, specific experimental limits (recovery, minimum detection limit, limit of quantitation). A good analyst should be able to obtain results similar to those of the EPA lab or American Cyanamid lab with proper refinement and good technique.



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JUL 23 1996

MEMORANDUM

SUBJECT: Environmental Chemistry Method Lab Evaluation Report,
ECM 0089S1 Imazaquin in Soil (MRID 430198-01)

FROM: Aubry E. Dupuy, Jr., Section Chief *Aubry E. Dupuy, Jr.*
BEAD/ACB/Environmental Chemistry Section

THRU: Donald A. Marlow, Branch Chief *DM*
BEAD/Analytical Chemistry Branch

TO: Henry M. Jacoby, Branch Chief
EFED, Environmental Fate and Groundwater Branch (7507C)

The Environmental Chemistry Section has completed an Environmental Chemistry Method Lab Evaluation on Imazaquin (CL252,214, Scepter) in soil. The method is to listed in a report submitted by American Cyanamid Company, Report #C3118.01 under a title "Scepter (CL 252,214): Validation of HPLC method M-1854 for the determination of CL 252,214 Residues in Soil," MRID #430198 - 01, submitted 11/16/93.

Soil samples were fortified at three levels of 10, 20, and 100 parts-per-billion (ppb). Four replicate analyses were performed for each fortification level and sample blank. The attached analysis report contains a summary, analysis results and experimental details, including representative chromatograms, calibration curve and example of calculation.

If you have any questions concerning this report, please contact Han Tai (601-688-3252) or myself at (601-688-3212).

cc: Christian Byrne, QA Officer, ECS
Han Tai

ENVIRONMENTAL CHEMISTRY METHOD VALIDATION REPORT

IMAZAQUIN IN SOIL

ECM 0089S1

ENVIRONMENTAL CHEMISTRY SECTION

ANALYTICAL CHEMISTRY BRANCH

BIOLOGICAL AND ECONOMIC ANALYSIS DIVISION

July 2, 1996

Prepared By: Han Tai

Han Tai

ECS Chemist signature

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PART I

Summary and Conclusions

An Environmental Chemistry Method Lab Evaluation has been performed on the analysis of Imazaquin (CL 252,214, Scepter) in soil. The analytical method is described in American Cyanamid Company Report C3118.01 (EPA MRID Number 430198-01), "Scepter (CL 252,214): Validation of HPLC Method M1854 for the determination of CL 252,214 Residues in Soil," submitted on 11/16/93.

Imazaquin residues are extracted from soil with 0.5N sodium hydroxide in 30/70 methanol/water. The extract is acidified and cleaned-up by solid phase extraction with C-18 and cationic SCX columns, followed by solvent partitioning into methylene chloride. The organic solvent is then evaporated, and the final extract is brought to an exact volume with pH 6.5 phosphate buffer solution. Imazaquin in the extract is measured by high performance liquid chromatography (HPLC) with UV detection at 240nm.

The soil samples were fortified with Imazaquin at three levels of 10, 50, and 100 parts per billion (ppb). Four replicate analyses have been done for each fortification level. The mean of percent recovery and standard deviation are, respectively, as follows: 10 ppb - 84.0%, 5.4%; 20 ppb - 82.7%, 4.3%; 100 ppb - 87.0%, 4.2%. The % recovery values were all within the range between 70 and 120%. Therefore, we consider that the analysis results are acceptable. The present HPLC instrumentation gives a minimum detection limit (MDL) of 3.8 ppb, and a limit of quantitation (LOQ) of 12.5 ppb.

Part II
 Analysis Results

Method: "Scepter (CL 252,214): Validation of HPLC method M-1854 for Determination of CL 252,214 Residues in Soil," by G. Picard, June 3, 1993, American Cyanamid Company Report C3118.01. EPA MRID #430198-01, submitted 11/16/93.

Results:

ppb added	(1)		(2)		
	ppb Found	% Recovery	X	SD	%RSD
100	92.73	92.7			
100	87.27	87.3			
100	82.72	82.7			
100	85.45	85.4	87.0	4.2	4.8
20	16.82	84.1			
20	15.46	77.3			
20	16.36	81.8			
20	17.50	87.5	82.7	4.3	5.2
10	8.48	84.8			
10	9.13	91.3			
10	8.11	81.1			
10	7.89	78.9	84.0	5.4	6.4
		(3)			
0	ND	---			
0	ND	---			
0	ND	---			
0	ND	---			

Notes: (1) Values are results of four replicates, which include extraction, column clean-up, solvent partitioning and HPLC.

(2) x = mean; SD = Standard Deviation;
 %RSD = %Relative Standard Deviation (100 SD/x).

(3) ND = Not Detected

For present procedure with sample size of 50 gm, final extract volume of 5.0 ml and median noise level of 5.5 nm:

Minimum Detection Limit (MDL, 3 x noise) - 3.8 ppb.
 Limit of Quantitation (LOQ, 10 x noise) - 12.5 ppb.

Part III
Experimental

General Description of Method:

A soil sample of 50 gm is extracted with 150 ml of 0.5N sodium hydroxide in 30% methanol-water on a reciprocating shaker for an hour, mixed with 15 gm Celite and filtered. The filter cake is washed with 50 ml of the same extracting solution. The combined filtrate and wash are adjusted to a volume of 200 ml, of which an aliquot of 80 ml (equivalent to 20 gm soil) is used for further processing. The aliquot is neutralized to pH 2 by 6 N hydrochloric acid and passed through a C18 solid phase extraction cartridge. The C-18 cartridge is washed with water, then connected in tandem with an SCX cartridge, and eluted with 50/50 methanol/water. The analyte on the SCX cartridge is then eluted with pH 6.5 phosphate buffer solution. The solution is neutralized to pH 2, and then partitioned with methylene chloride. The organic solution is evaporated to dryness. The dried solids, containing the analyte, are dissolved in an exact volume of pH 6.5 buffer, usually 5.0 ml. The amount of Imazaquin in the final extract is determined by HPLC.

HPLC Instrument Parameters:

Column: Supelcosil LC-8-DB, deactivated, 5um, 150 x 4.6 mm. Part #5-8347, Lot #089064AE

Mobile Phase: Methanol/water/acetic acid, 50/50/4, v/v/v; flow rate 0.75 ml/min, isocratic.

Pump: Waters Model 590 Programmable HPLC Pump.

Injector: Water Model U6K Manual Injector.

Detector: Waters Model 490 Multi-Wavelength Programmable Detector-UV 254 nm; absorbance mode; absorbance unit full scale (AUFS) 0.05; time constant 5.0.

Temperature: Waters Temperature Control System, set at 25.0°C.

Recorder: Kipp-Zonen Model BD-41- Full scale 10 mv; chart speed 5 mm/min.

Injection Volume: 500 ul.

Retention Time: Approximately 8.3 minutes.

Example of Calculation:

1. Peak Intensity:

The peak intensity is expressed in terms of peak height. The peak height, in millimeters (mm), is the distance measured manually from the apex of the analytical peak to the base-line drawn across the peak. Injection volume for all standards and samples was 500 ul, therefore, no volume adjustment is necessary in calculation.

2. Calculation Formulas:

a. ppb Found, Imazaquin = $(B/A)(S)(V)(F)/(W)$

A - Average peak height of two bracketing standards, mm
B - Peak height of sample peak, mm
S - Concentration of standard, ng/ml
V - Volume of final extract, ml
F - Dilution factor
W - Weight of sample aliquot, gm.

b. % - Recovery = $(\text{ppb found})(100)/(\text{ppb Added})$

3. Example:

Sample fortified at 100 ppb - chromatogram B-3

a. Data:

$$A = (110 + 110)/(2) = 110; B = 94; S = 100 \text{ ng/ml.}$$
$$V = 5.0 \text{ ml}; F = 4; W = 20.0 \text{ gm.}$$

b. ppb Found = $(94/110)(100)(5)(4)/(20) = 85.45$

c. % Recovery = $(85.45)(100)/(100) = 85.4$

Source of Analytical Reference Standard:

Scepter, F-003823-01 (F823), Lot FWS-1, 27 June, 95,
Purity 96.0%

Received from Pesticides Repository, USEPA, (MD-8)
c/o Man Tech Environmental Technology, Inc
2 Triangle Drive, RTP, NC 27709

Source of Sample Matrix:

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ECS file soil, which has been stored in freezer.
Characterization of this soil is listed in Appendix 1.

Comments:

1. Procedure Step 7 (p. 12) - After hydrochloric acid neutralization, some gelatinous precipitate was formed. The precipitate was filtered with a coarse filter (Fisher Scientific 09790-14E) and washed twice with 5 ml pH 2 water. The wash was combined with the extract for the C-18 cartridge.

The neutralized solution was clear with the addition of up to 10 gm of sodium chloride. When 20 gm was added, an appreciable amount of solids remained undissolved and additional gelatinous precipitate was formed. The mixture appeared to be colloidal and was almost impossible to be filtered.

2. HPLC detector range (p.11)- The procedure lists a range at 0.001 AUFS. At this setting, the present Waters Model 490 Detector showed excessive noise of more than 10% deflection. The detector was set at 0.05 AUFS, time constant 5.0. At this range, the 50 ppb working standard, 500 ul, gave about 30% deflection which fits the condition as required in Section F-1 (p.11).

It should also be noted that, at the present instrument settings, the noise level ranged from 4 mm to 7 mm. With 5.5 mm as a median value, the minimum detection limit (MDL, 3 x noise) is calculated to be 3.8 ppb, and the limit of quantitation (LOQ, 10 x noise), 12.5 ppb. The HPLC system presently used for this study was not sensitive to the LOQ of 5 ppb (as indicated in the method) due the noise level. However, the analyst believes that the sensitivity was limited by the instrumentation, but not by methodology itself.

3. With several manual operating steps, an operator can work on two samples at the same time and complete processing in about 4 hours, or a group of 4 samples in one 8-hr working day. HPLC for each takes about 20 minutes. A group of 8 samples, 5 bracketing standards and 3 calibrating standards needs about one 8-hr working day for HPLC.

Chromatograms:

A. Imazaquin Standards:

A-1 0.020 u/ml (20 ppb)
A-2 0.050 u/ml (50 ppb)
A-3 0.100 u/ml (100 ppb)
A-4 Calibration Curve

B. Sample - Soil:

B-1 Fortified 10 ppb
B-2 Fortified 20 ppb
B-3 Fortified 100 ppb

C. Blanks :

C-1 Sample (soil) blank
C-2 Method blank
C-3 Solvent - pH 6.5 phosphate buffer

- Notes: 1. Injection volume for all standards and samples is 500 ul.
2. An arrow indicates the location of the retention time of the analytical peak.

A) IMAZARON STANDARDS

Inj. Vol - 500 μ l

A-1 0.020 μ g/ml (20 ppb)

A-2 0.050 μ g/ml (50 ppb)

A-3 0.100 μ g/ml (100)

8.33' (111 mm)

8.33' (56 mm)

8.30' (26 mm)

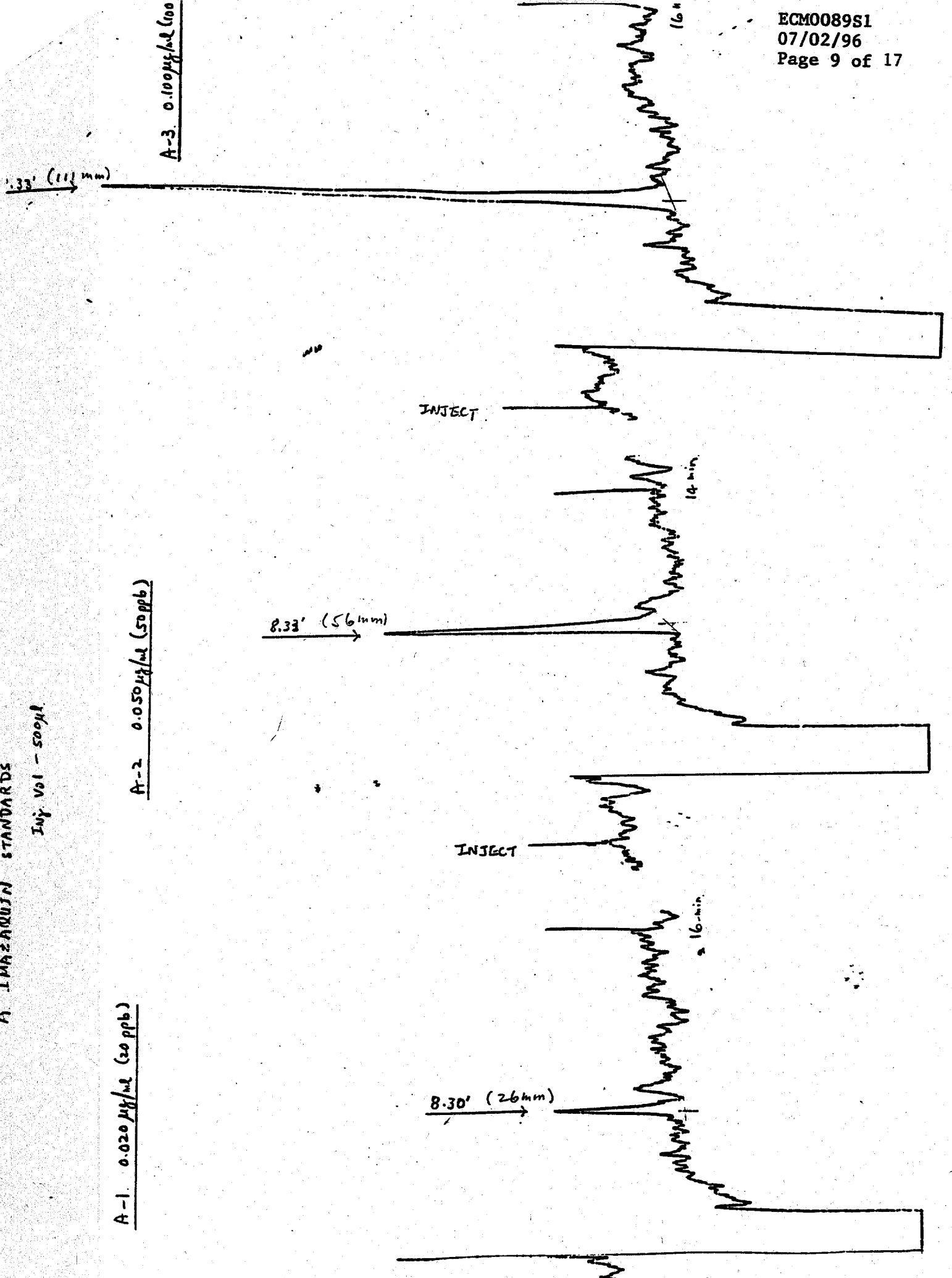
INJECT

INJECT

16 min

14 min

16 min



A) IMAZARUIN STANDARDS
Inj Vol - 500 μ l

A-1. 0.020 μ g/ml (20 ppb)

A-2. 0.050 μ g/ml (50 ppb)

A-3. 0.100 μ g/ml (100)

8.33' (26mm)

8.33' (56mm)

8.30' (26mm)

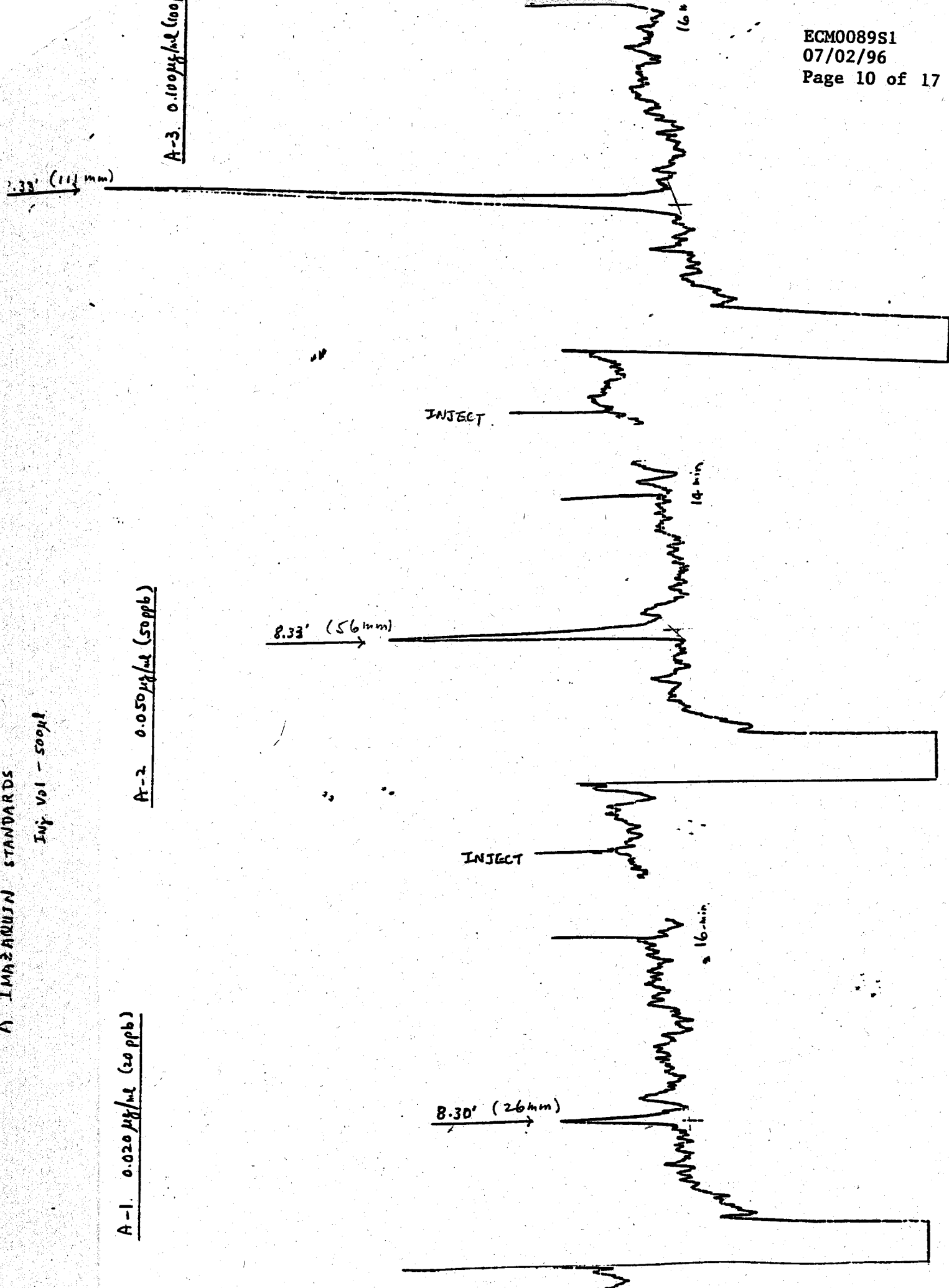
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INJECT

16 min

14 min

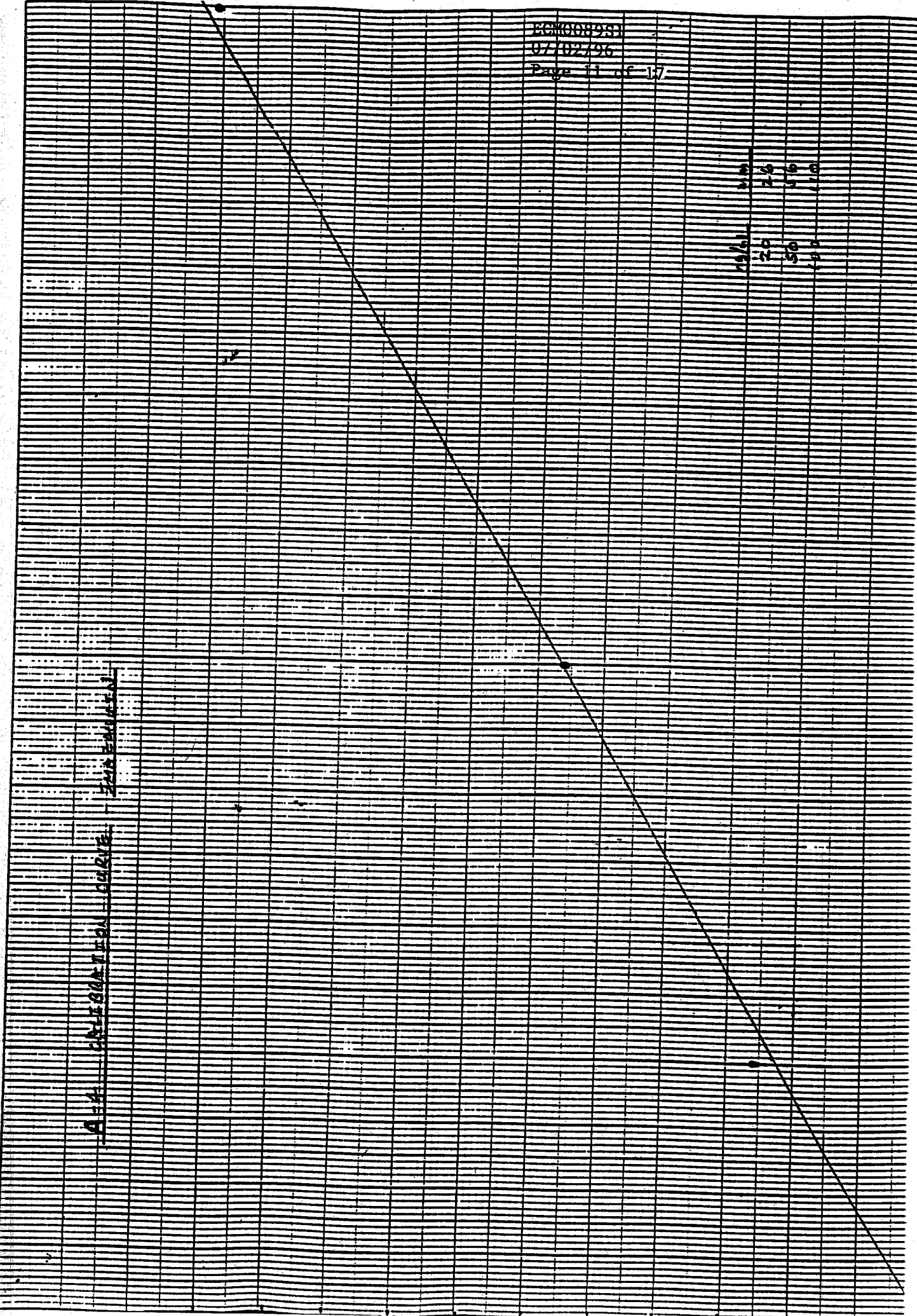
16 min



DATA CR-1000 IN PA CR-1000 DATA

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15/11
20
50
100



B SAMPLE - SOIL
Inj. vol. 500µl

B-3 FORTIFIED 100ppb - Final

8.32' (94mm)

B-2 FORTIFIED 20ppb
Final extract vol - 5ul

8.32' (74mm)

B-1 FORTIFIED 10ppb

Final extract vol. - 5ul

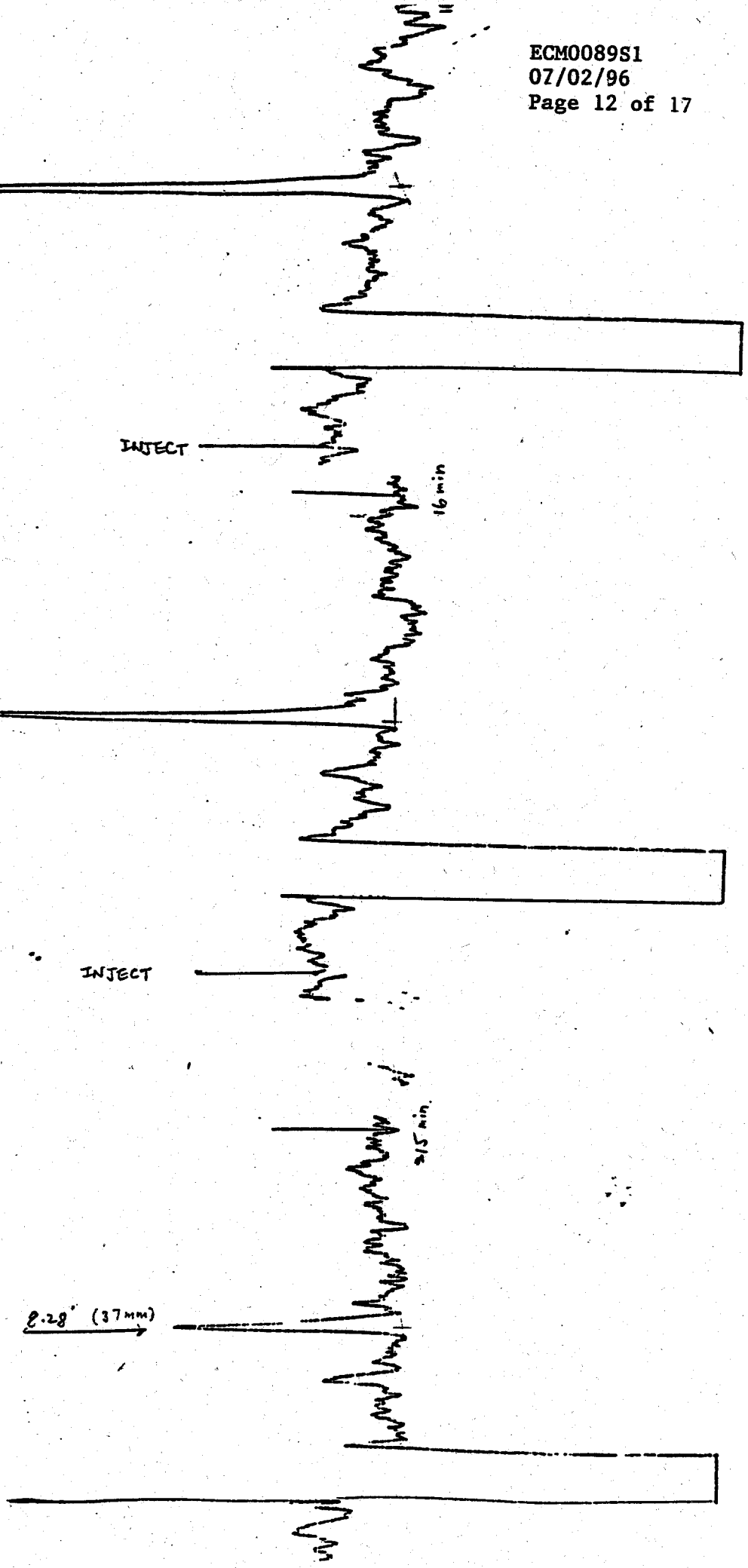
2.28' (37mm)

INJECT

INJECT

16 min

215 min



B SAMPLE - SOIL

Inj vol. 500µl

B-1 FORTIFIED 10 PPB

Final extract vol. - 5ml

8.28' (27mm)

15 min

B-2 FORTIFIED 20 PPB

Final extract vol - 5ml

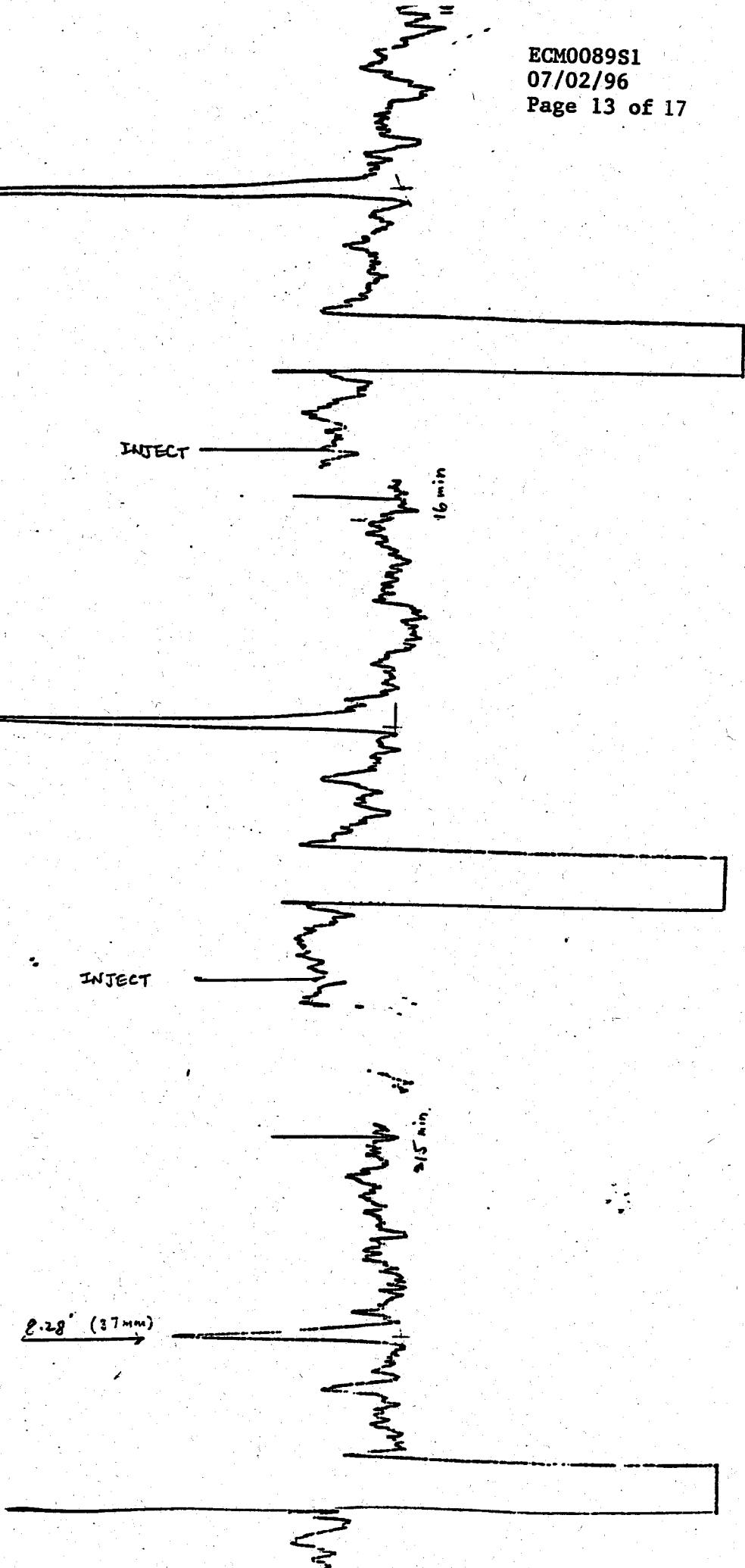
8.32' (74mm)

16 min

INJECT

B-3 FORTIFIED 100 PPB - Final

8.32' (94mm)



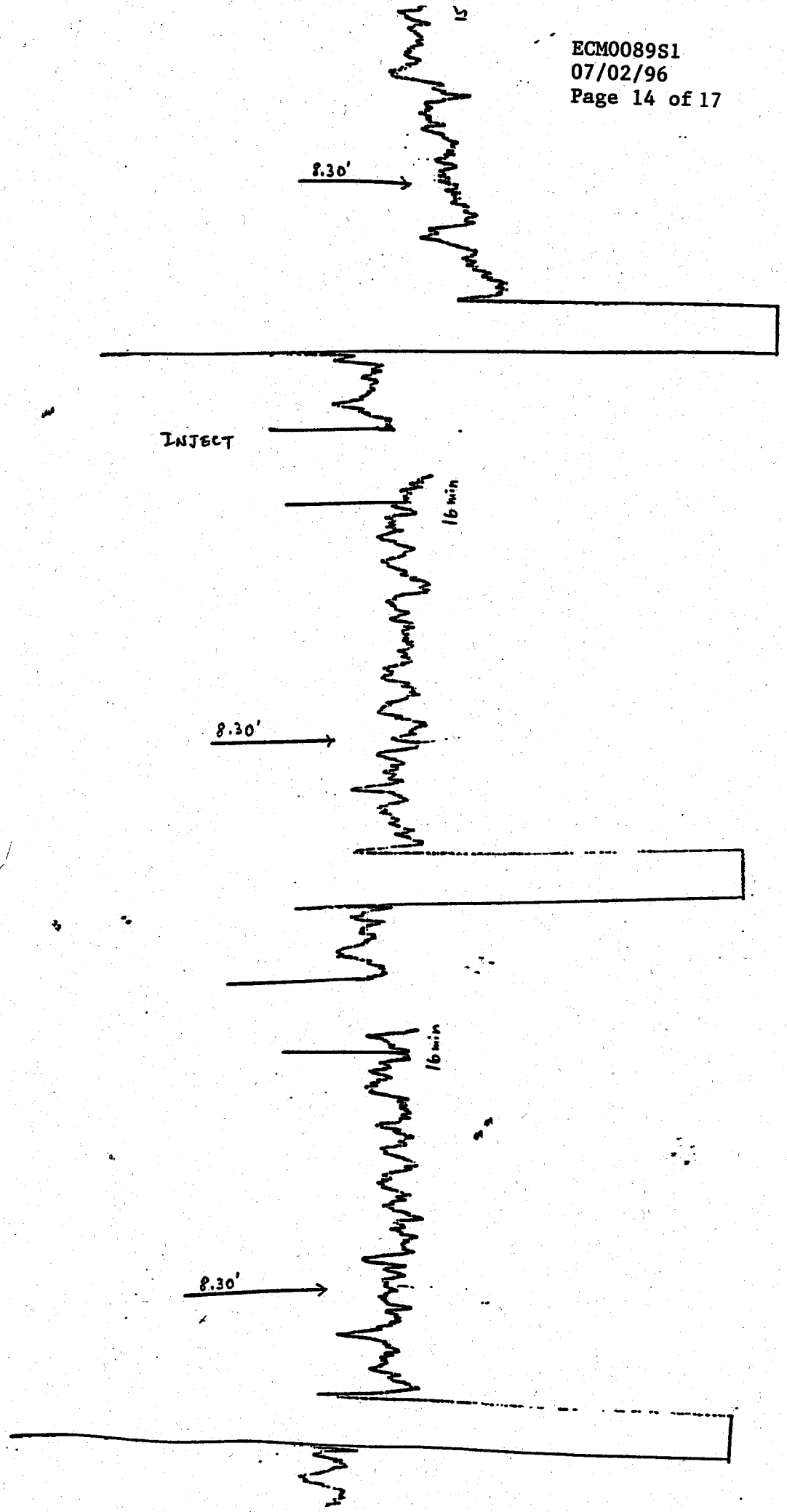
C. BLANKS

Inj. vol - 500µl

C-1. SAMPLE - SOIL

C-2. METHOD BLANK

C-3. SOLVENT - Phosphate Buffer, pH 6.5



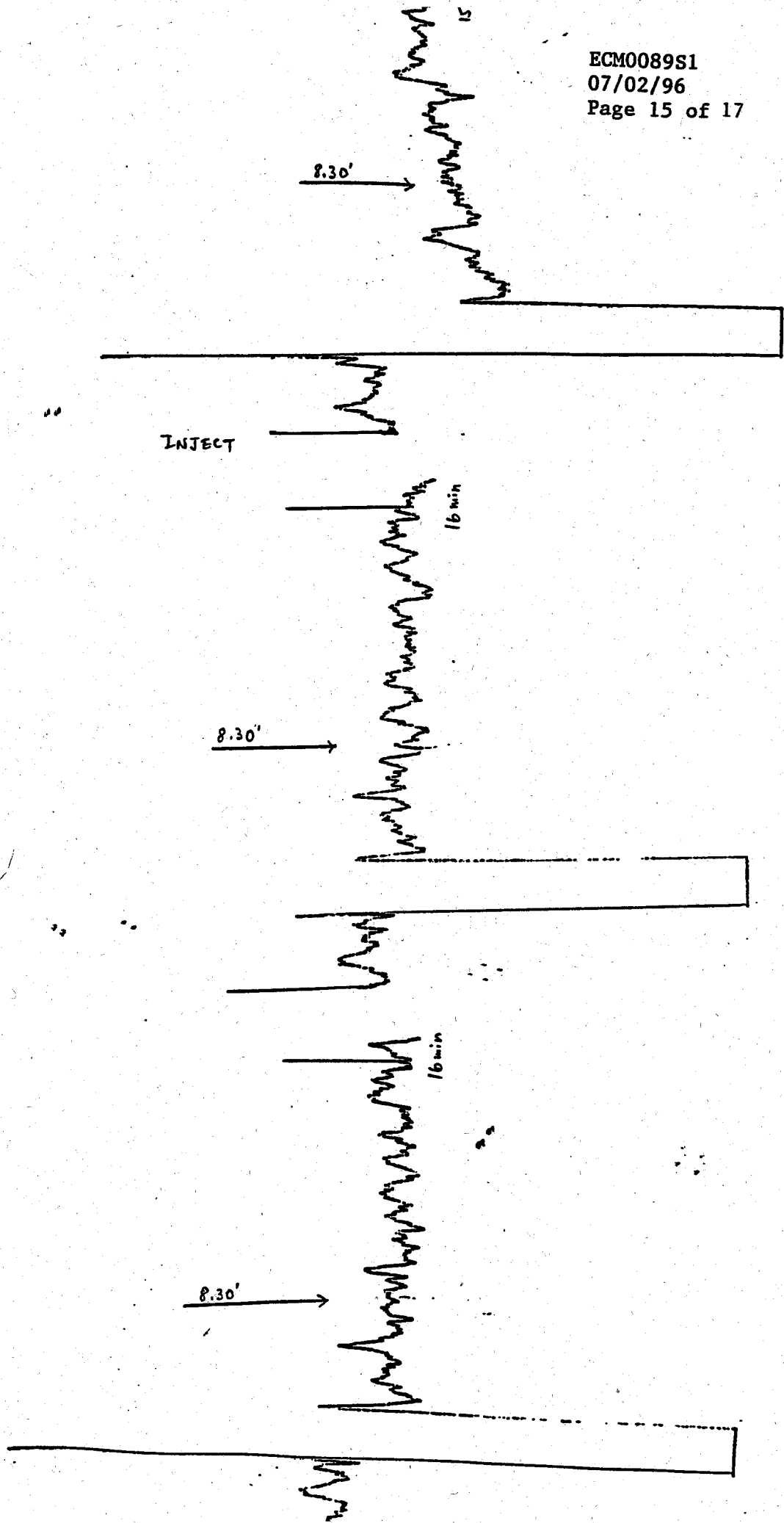
C. BLANKS

Inj. Vol - 500µL

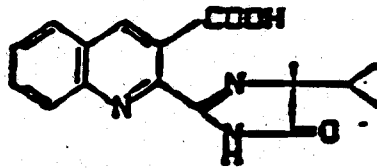
C-1 SAMPLE - SOIL

C-2 METHOD BLANK

C-3 SOLVENT - Phosphate Buffer, pH 6.5



Attachment 1: Structure of Imazaquin
(CL 252,214)



CL 252,214 [3-quinolinecarboxylic acid, 2-[4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-]

MECHANICAL (TEXTURE) ANALYSIS
Mississippi Cooperative Extension Service, Soil Testing Lab
P.O. Box 9610
Mississippi State, MS 39762

ECM0089S1
 07/02/96
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U.S. EPA / E. Flynt

NAME:

Building 1105, Stennis Space, MS 39529

ADDRESS:

November 15, 1993

DATE:

UNCL

COUNTY:

<u>LAB#</u>	<u>FIELD#</u>	<u>%CLAY</u>	<u>%SILT</u>	<u>%SAND</u>	<u>TEXTURE</u>
<u>EPA1</u>	<u>1</u>	<u>15.5</u>	<u>20.3</u>	<u>64.2</u>	<u>SILT LOAM</u>
<u>EPA2</u>	<u>1</u>	<u>14.8</u>	<u>22.0</u>	<u>63.2</u>	<u>SILT LOAM</u>
<u>(Average)</u>	<u>1</u>	<u>15.2</u>	<u>21.2</u>	<u>63.7</u>	<u>SILT LOAM</u>

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