Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Chlorsulfuron

MRID #: 434067-01

Matrix: Water

Analysis: HPLC/UV

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DETERMINATION OF CHLORSULFURON, DPX-A7881, DPX-F5384, DPX-L5300, DPX-M6316, AND METSULFURON METHYL IN GROUNDWATER

1. SCOPE AND APPLICATION. This procedure describes a high performance thin-layer chromatography (HPTLC) screening method and a more specific and sensitive high performance liquid chromatography (HPLC) confirmation method applicable to the determination of chlorsulfuron [2-chloro-N-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino)carbonyl)benzene-sulfonamide], DPX-A7881 [2-(((((4-methylamino-6-ethoxy-1,3,5-triazin-2-yl)amino)carbonyl)amino)sulfonyl)benzoic acid, methyl ester], DPX-F5384 [2-(((((((4,6-dimethoxy-2-pyrimidinyl)amino)carbonyl)amino)sulfonyl)-methyl)benzoic acid, methyl ester], DPX-L5300 [2-(((((4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino)carbonyl)amino)sulfonyl)benzoic acid, methyl ester], DPX-M6316 [3-(((((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino)carbonyl)amino)sulfonyl)-2-thiophenecarboxylic acid, methyl ester], and metsulfuron methyl [2-(((((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino)carbonyl)amino)-sulfonyl)benzoic acid, methyl ester] in groundwater (structures shown in Figure 1). Both procedures allow determination of these analytes at 0.1 µg/L or lower levels as specified in EEC directive 80/778/EEC in water.

Single-laboratory method accuracy and precision data have been determined for these pesticides:

Analyte	Molecular Formula	Chemical Abstracts Service Registry Number (CASRN)
Chlorsulfuron	$C_{12}H_{12}ClN_5O_4S$	64902-72-3
DPX-A7881	$C_{15}H_{18}N_6O_6S$	–
DPX-F5384	$C_{16}H_{18}N_4O_7S$	83055-99-6
DPX-L5300	$C_{15}H_{17}N_5O_6S$	101200-48-0
DPX-M6316	$C_{12}H_{13}N_5O_6S_2$	79277-27-3
Metsulfuron methyl	$C_{14}H_{15}N_5O_6S$	74223-64-6

2. SUMMARY OF METHOD -- Pesticides are extracted from water by solid phase extraction (SPE). Two methods are available for detection and quantification of analytes: a multiresidue screening procedure and a more specific and sensitive confirmation method to determine individual analytes.

2.1 SOLID PHASE EXTRACTION -- A measured volume of groundwater of approximately 1 L is passed through a cartridge containing 5 g of C-18 adsorbent. The cartridge is dried, and the pesticides are eluted with acetonitrile. The acetonitrile extract is concentrated to a final volume of 100 to 300 µL.

2.2 SCREENING METHOD -- The screening method is based on HPTLC using automated multiple development (AMD) to enhance the separation of compounds on the plate(1). Half of the sample extract is applied to the plate as a narrow line using a sample application apparatus. The developed plate is analyzed by ultraviolet (UV) reflectance detection at different wavelengths.

2.3 CONFIRMATION METHOD -- Reverse phase HPLC conditions are provided for confirmation of analytes in the sample extract. Analytes are separated by elution chromatography and detected using an ultraviolet (UV) detector.

3. APPARATUS AND EQUIPMENT -- All specifications are suggested. Catalog numbers are included for illustration only.

3.1 GENERAL LABORATORY EQUIPMENT

3.1.1 Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

3.1.2 Volumetric flasks -- glass, different sizes from 10-mL to 1000-mL.

3.1.3 Sample storage bottles -- 1-L, amber glass, with ground glass stoppers.

3.1.4 Solution storage bottles -- glass, various sizes with PTFE-fluorocarbon-lined screw caps.

3.1.5 Microsyringes -- various sizes.

3.1.6 pH meter -- electronic.

3.1.7 Chromatographic chambers -- glass, approximately 200 mm x 100 mm x 200 mm.

3.1.8 Oven -- capable of heating to 120 ± 5°C.

3.1.9 Cover plates -- glass, 100 mm x 200 mm.

3.1.10 Jar -- glass, approximately 500 mm diameter or larger, for short-term storage of cleaned TLC plates.

3.1.11 Centrifuge tubes -- 10-mL glass, conical, graduated in 0.1-mL units.

3.1.12 Crucible -- porcelain, approximately 100-mL.

3.1.13 Desiccator -- approximately 500 mm diameter or larger capable of receiving up to 14 crucibles.

3.1.14 ReactiVial -- 3-mL or equivalent.

3.1.15 Evaporation apparatus -- for evaporation of organic extracts under a nitrogen stream (Pierce Silli-Vap Evaporator or equivalent).

3.1.16 Oven -- muffle, capable of heating to approximately 500°C.

3.1.17 Magnetic stirrer with stirring bar.

3.2 SOLID PHASE EXTRACTION -- equipment consisting of a sample reservoir, tubing, cartridge filled with SPE material, peristaltic pump, and waste container (Figure 2). For conditioning the cartridges, additional conventional SPE manifolds are suggested.

3.2.1 Sample reservoir -- 1-L, amber glass with screw cap which has at least two passages for tubing (MERCK solvent bottles with MERCK adapter S 40 for direct withdrawal of solvents for HPLC, Part No. 9996 or equivalent). One passage is fitted to the LUER cone of a 5-mL hypodermic syringe body filled with granulated charcoal (MERCK, Part No. 2514 or equivalent).

3.2.2 Tubing -- PTFE, 2.4 mm I.D. x 3.2 mm O.D.. The tube must fit tightly in the other passage of the sample reservoir screw cap.

3.2.3 Tubing connections -- leak-tight connector system and LUER adapters, female and male [Omnifit system (Macherey & Nagel) or Latek's system (Latek) or equivalent].

3.2.4 Cartridge adapter -- PTFE, outer diameter fitting into the top of the glass cartridge, axial boring with LUER female dimensions (custom manufactured -- shown in Figure 3).

3.2.5 Cartridge for SPE material -- glass hypodermic syringe body, 10-mL volume, 14 mm I.D. with concentric LUER cone. The cone may be constructed of either glass or metal (Fortuna Type Optima or equivalent -- shown in Figure 3). To prevent SPE material from pouring out of the cartridge, the material is covered at both ends with 14 mm diameter glass fiber filters (Macherey and Nagel Type MN 85/200).

3.2.6 Peristaltic pump -- multichannel, connected to the eluate

side of the SPE cartridge(s). Each channel is adjusted to a flow rate of approximately 1 mL/min (Ismatec Type IPN-16 or equivalent).

3.2.7 Waste container -- drain or plastic barrel of suitable size.

3.2.8 Cartridge conditioning -- SPE manifold with capacity for several commercially-available SPE cartridges (Baker, Supelco, or equivalent).

3.2.9 Drying apparatus for SPE cartridges -- device to divide a stream of nitrogen into different channels leading into the top of loaded SPE cartridges to blow off water remaining after sample application. For this purpose, an apparatus was manufactured from 30 mm diameter glass tubing, six 5-mm to 3-mm cones, and a Quickfit tube adapter. The adapter is connected to a nitrogen tank with 7 mm O.D. PTFE tubing. The cones connected to the glass tubing are attached to flexible 250 mm long x 2.4 mm I.D. PTFE tubes. The other end of the PTFE tubing is attached to LUER cone adapters. The drying apparatus is shown in Figure 4.

3.3 HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY APPARATUS -- HPTLC equipment consisting of a semiautomated apparatus to apply the sample on the TLC plate, an AMD apparatus, and a computerized UV/VIS scanner (Figure 5).

3.3.1 Sample applicator -- to apply a predetermined volume of sample solution on a TLC plate. The sample must be applied as a narrow line a few millimeters in length defined as the "starting zone". The length of the starting zone and its position on the plate must be adjustable. Solvent must be blown off during the application procedure (Camag Linomat IV or equivalent).

3.3.2 AMD apparatus -- capable of multistep development of TLC plates and capable of changing the polarity of the mobile phase automatically during each step of the multistep development (Camag AMD System or equivalent).

3.3.3 UV/VIS scanner -- for detecting analyte bands on the developed TLC plate at different wavelengths. The scanner must automatically scan a given number of tracks on the TLC plate (Camag TLC Scanner II or equivalent).

3.3.4 Data system -- capable of collecting raw data from the UV/VIS scanner, calculating resultant peak sizes, overlaying a selected number of scans at different wavelengths, and plotting the chromatograms as multicolor

diagrams [Hewlett Packard HP 300 Workstation with HP 9122 disk unit, HP 7474A plotter and Camag Evaluation Software 86 (HP 310) revision 7.04 or equivalent].

3.4 HPLC APPARATUS

3.4.1 High Performance Liquid Chromatograph (HPLC) -- system capable of injecting 50-µL aliquots and maintaining a constant flow rate.

3.4.1.1 Column -- 250 mm x 4.6 mm I.D. stainless steel packed with 5 µm DuPont Zorbax ODS or equivalent.

3.4.2 Detector -- ultraviolet absorbance at 225 nm (Kratos Spectraflow 757 or equivalent).

3.4.3 Data system -- capable of collecting raw data from the HPLC instrumentation and calculating resultant peak areas or peak heights.

3.4.4 Filtration apparatus -- to filter samples prior to HPLC analysis.

3.4.4.1 Filter holder -- 13 mm (Millipore stainless steel XX300/200 or equivalent).

3.4.4.2 Filters -- 13 mm diameter 0.2 µm polyester (Nuclepore 180406 or equivalent).

3.4.4.3 Hypodermic syringe -- 10-mL glass, with Luer-Lock tip.

4. REAGENTS AND CONSUMABLE MATERIALS

4.1 REAGENT WATER -- Reagent water used to generate the data in this method was generated using an apparatus which automatically applies different water purifying processes such as reverse osmosis, ion exchange, microfiltration, and adsorption of organic material (Hewlett Packard HP 661A Water Purifier or equivalent).

4.2 SODIUM CHLORIDE (NaCl), CRYSTAL -- Heat treat 100-g portions contained in a crucible in a 500°C oven for a minimum of six hours (ideally overnight) to remove interfering organic substances.

4.3 SODIUM HYDROXIDE (NaOH) – 1 M - Prepare by dissolving 40 g solid NaOH in 1 L of reagent water.

4.4 HYDROCHLORIC ACID (HCl) -- 1 M and 0.1 M - Prepare 0.1 M solution by diluting 100 mL of 1 M HCl (Merck Titrisol) to 1 L with reagent water.

4.5 ACETONITRILE, METHANOL, DICHLOROMETHANE, TRICHLOROMETHANE, ETHYLACETATE AND ISOPROPANOL -- distilled-in-glass quality [Promochem (Mallinckrodt) Nanograde or equivalent].

4.6 FORMIC ACID -- p.a. quality 98-100 percent [Merck, Part No. 264].

4.7 AMMONIA SOLUTION -- 25 percent [Merck, Part No. 5432].

4.8 STOCK STANDARD SOLUTIONS (1.0 mg/mL) -- Stock standard solutions are prepared from pure standard materials using the following procedure. These stock standard solutions can be used for both the screening method and the confirmation method.

4.8.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material (compound purity is certified at ≥96%). Dissolve the material in a suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. Larger volumes must be used if standard material cannot be dissolved in 10 mL of solvent.

For instance the stock solutions used to generate the data related to the screening method of this report were prepared as follows:

Standard Material	Solvent	mg/mL
Chlorosulfuron	Trichloromethane	10/10
A 7881	Methanol	10/25
L 5300	Ethylacetate	10/10
Metsulfuron Methyl	Ethylacetate	10/10
F 5384 (Londax)	Ethylacetate	10/10
M 6316	Ethylacetate	10/10

4.8.2 Transfer the stock standard solutions into PTFE-fluorocarbon-sealed screw cap vials, label, and store at -20°C protected from light.

4.9 FORTIFICATION SOLUTION (1 µg/mL) -- The fortification solution is used to spike reagent or groundwater samples prior to extraction and analysis for evaluation of analyte recoveries. Prepare the fortification solution by measuring 1 mL each of the sulfonylurea compound stock solutions (1 mg/mL) into a 10-mL

volumetric flask and diluting to volume with methanol. Thoroughly mix the solution. Repeat the 10-fold dilution procedure twice, using the newly-prepared dilutions, to prepare a 1 µg/mL solution. Transfer the fortification solution into a vial with a PTFE fluorocarbon-sealed crimp-on cap and label. Store at -20°C and protect from light. The vial should be recapped whenever the fortification standard is used.

4.10 SOLID-PHASE EXTRACTION -- Octadecyl (C-18) modified silica gel, 40 µm. The data in this method were generated using Analytichem Bondesil (Part No. 11073).

4.11 HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

4.11.1 TLC Plates -- glass plates precoated with silica gel containing an inorganic fluorescence indicator, 100 mm x 200 mm, specified for HPTLC. The data in this method were generated using Merck HPTLC Precoated Plates Silica Gel 60 F 254 (Part No. 5642). The plates must be purified before use.

4.11.1.1 Plate preparation -- Completely immerse the plate in isopropanol contained in a chromatographic chamber (chamber 1) for at least four hours. Immerse the plate in fresh isopropanol contained in a second chromatographic chamber (chamber 2) for another four hours or longer. Chamber 2 may be substituted for chamber 1 for the next series of plates; chamber 1 must be refilled with fresh isopropanol and may serve as chamber 2 for preparation of the next series of plates.

4.11.1.2 Plate conditioning -- Remove the plates from chamber 2 using tweezers and shake them slightly to remove excess isopropanol. Immediately place the washed plates into a clean 120°C oven for 30 minutes. The oven should be ventilated to avoid buildup of potentially dangerous solvent vapors. Remove the plates from the oven, cover the TLC plate with a clean glass cover plate, and store them in a glass jar until they are cooled to room temperature (approximately 30 minutes). Immediately apply samples to the plate for HPTLC analysis. **Note:** Care should be taken to avoid exposure of the plates to the laboratory atmosphere. Plates will adsorb contaminants and/or moisture.

4.12 Calibration Solution (10 µg/mL) – Prepare the calibration

solution by measuring 1 mL each of the analyte stock solutions (1 mg/mL) into a 10-mL volumetric flask and diluting to volume with methanol. Thoroughly mix the solution. Repeat the 10-fold dilution procedure once, using the newly-prepared dilution, to prepare a 10 µg/mL solution. Transfer the calibration into a vial with a PTFE fluorocarbon-sealed crimp-on aluminum cap and label. Store at -20°C and protect from light. The vial should be recapped whenever the calibration standard is used.

4.12 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

4.12.1 Helium, for degassing dissolved oxygen.

4.12.2 Hydrocortisone (HC) -- >98 percent purity, for use as internal standard for HPLC analyses (available from Sigma Chemical Co.).

4.12.3 Mobile Phase

4.12.3.1 Water, containing 0.01 percent (v/v) acetic acid--Add 100 µL glacial acetic acid to 1 L of reagent water. Filter and degas before use.

4.12.3.2 Acetonitrile, containing 0.01 percent (v/v) acetic acid--Add 100 µL of glacial acetic acid to 1 L of HPLC grade acetonitrile. Filter and degas before use.

4.12.4 Internal Standard Solutions

4.12.4.1 Internal standard stock solution (1.0 mg/mL) -- Prepare an internal standard stock solution by accurately weighing 0.0100 g of pure HC. Dissolve the HC in acetonitrile and dilute to volume in a 10-mL volumetric flask. Mix thoroughly.

4.12.4.2 Intermediate internal standard solution (100 µg/mL) -- Prepare an internal standard stock solution by adding 5.0 mL of the internal standard stock solution (Section 4.12.4.1) to approximately 50 mL of acetonitrile in a 50-mL volumetric flask. Dilute to volume with acetonitrile. Mix thoroughly.

4.12.4.3 Internal standard spiking solution (0.8 µg/mL) -- Prepare an internal standard spiking solution by adding 800 µL of intermediate internal standard solution (Section 4.12.4.2) to approximately 50 mL

of mobile phase in a 100-mL volumetric flask and diluting to volume with mobile phase (1:1 mixture of solutions described in 4.12.3). Mix thoroughly.

4.12.4.4 Transfer the internal standard solutions into vials with PTFE fluorocarbon-sealed crimp-on aluminum caps and labels. Store at -20°C and protect from light. These solutions should be recapped whenever they are used.

4.12.5 Calibration Solutions

4.12.5.1 Intermediate stock solutions (100 µg/mL) -- Prepare each individual stock solution by adding 1 mL of the stock standard solution (Section 4.8) to approximately 5 mL of acetonitrile in a 10 mL volumetric flask, diluting to volume with acetonitrile, and thoroughly mixing the contents of the flask.

4.12.5.2 Intermediate stock solutions (10 µg/mL) -- Prepare each individual intermediate stock solution by adding 1 mL of the 100 µg/mL intermediate stock solution (Section 4.12.5.1) to approximately 5 mL of acetonitrile in a 10-mL volumetric flask, diluting to volume with acetonitrile, and thoroughly mixing the contents of the flask.

4.12.5.3 HPLC calibration solutions -- Prepare HPLC calibration solutions by adding 500, 200, 100, 50, or 20 µL of each 10 µg/mL intermediate stock solution (Section 4.12.5.2) and 80 µL of the intermediate internal standard solution (Section 4.12.4.2) to approximately 5 mL of acetonitrile in a 10-mL volumetric flask and diluting to volume with acetonitrile. Resulting analyte concentrations are 0.5, 0.2, 0.1, 0.05 and 0.02 µg/mL and internal standard is present in all the calibration solutions at 0.8 µg/mL.

4.12.5.4 Transfer the intermediate stock solutions and calibration solutions into vials with PTFE fluorocarbon-sealed crimp-on aluminum caps and labels. Store at -20°C and protect from light These solutions should be recapped whenever they are used.

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 SAMPLE COLLECTION -- Collect samples in glass containers. Conventional sampling practices (2-4) should be followed.

5.2 SAMPLE PRESERVATION -- Samples must be iced or refrigerated at 4°C from the time of collection until extraction. Sample extracts must be stored at -20°C protected from light from the time of generation until analysis.

In a single laboratory, analyte recoveries from reagent water were determined 0, 7, and 14 days after sample preparation using the required storage conditions. Conditions used for stability studies were not compatible with DPX-L5300 storage requirements; stability results for this analyte are not reported. Remaining analyte recoveries are given in Table 2; these results indicate that the method analytes examined are stable in water samples for at least 14 days and in sample extracts for at least 28 days when these storage conditions are used. **Note:** Analyte stability may be affected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the samples being studied.

6. ANALYSIS PROCEDURE

6.1 pH ADJUSTMENT -- Adjust the pH of the 1-L sample to pH 6 ± 0.2 by adding dilute NaOH (Section 4.3) or dilute HCl (Section 4.4) to each sample. Use an electronic pH meter to measure sample pH, and stir the sample using a magnetic stirrer.

Note: Sample must be adjusted to pH 6 immediately before solid phase extraction. Avoid pH < 6 because sulfonyl urea compounds may decompose rapidly when the sample is acidified.

6.2 SOLID PHASE EXTRACTION -- SPE cartridges are prepared and conditioned. The sample is applied to the cartridges, remaining water is removed from the cartridge, and the analytes are eluted from the cartridge with acetonitrile (Figure 6).

6.2.1 Preparation and Conditioning of SPE Cartridges

6.2.1.1 Place 5 ± 0.1 g C-18 SPE material (dry) into a glass syringe body which is closed at the bottom with a glass fiber filter. Place another glass fiber filter over the SPE material to hold the material in the syringe body.

6.2.2.2 Place the filled cartridge on a SPE manifold and apply vacuum. Rinse the cartridge with 30 mL of

acetonitrile and 50 mL of reagent water, respectively. Do not let the SPE material go dry during or after this procedure. Close the prepared cartridge with a PTFE adapter. Prepare and condition cartridges just before use.

6.2.2 Sample Adsorption

6.2.2.1 Add 100 g of purified NaCl to the 1-L water sample before pH adjustment (Section 6.1). In order to prepare recovery samples, add analytes to the sample by injecting a suitable microliter volume of fortification solution using a syringe. Stir the sample at room temperature until NaCl is dissolved completely.

6.2.2.2 Connect the sample bottle as a reservoir to the tubing (see Section 3.2). Fill the tube leading from the reservoir to the cartridge with sample water using a hypodermic glass syringe equipped with a Luer-Lok fitting. Completely fill the cartridge with reagent water before the adapter is placed on the body. The tube and the cartridge are connected such that air bubbles in the system are avoided.

6.2.2.3 Adjust the flow of the peristaltic pump to 1 mL/min. When the entire sample has passed through the cartridge (approximately 16 to 17 hours), disconnect the column. **Note:** The cartridge can go dry at this point for a short while.

6.2.2.4 Place the cartridge on a SPE manifold and wash the SPE material with 20 mL of reagent water while applying vacuum.

6.2.3 Cartridge Drying -- Careful drying of the SPE material in the cartridge is essential for acceptable analyte recoveries.

6.2.3.1 Reseal the top of the cartridge with the PTFE adapter and connect the adapter to the flexible tubes of the nitrogen drying apparatus (see Section 3.2.9).

6.2.3.2 Adjust the nitrogen flow to approximately 50 to 100 mL/min. Dry the cartridges at room temperature until the material is completely dry (approximately 24 hours).

6.2.4 Cartridge Elution – When the SPE material is completely dry, place the cartridge on a SPE manifold. Do not use the same manifold that was used for cartridge conditioning and washing.

6.2.4.1 Elute the cartridge with 10 mL of acetonitrile. Let the solvent pass through the cartridge without applying vacuum. Elute with two 5-mL portions. Apply slight vacuum at the end of the elution process.

6.2.4.2 Evaporate the extract under a stream of nitrogen at room temperature to a suitable final volume of 200 to 300 µL.

6.3 HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHIC ANALYSIS -- HPTLC analysis includes application of the concentrated sample extract in acetonitrile on the TLC plate, development of the plate by AMD, and analysis using UV detection.

6.3.1 Table 1 summarizes the operating conditions as used to validate this method. Included in Table 1 are retention times observed using this method. Examples of the separations achieved using these conditions are shown in Figures 7-21.

6.3.2 Calibration – The sizes of the peaks detected by the UV detector are measured by the computer system in terms of peak height. The corresponding amount of the analyte is computed by comparison of the measured peak height to a calibration curve generated from standard solutions. Establish HPTLC operating conditions equivalent to those indicated in Table 1. Calibrate the HPTLC system as follows:

6.3.2.1 Reserve up to five tracks on each TLC plate for calibration. Apply appropriate volumes of the calibration solution to each reserved track. Calibration levels can range from 20 to 300 ng. The corresponding required volumes of calibration solution are for instance 20 µL of 1 µg/mL calibration solution and 5, 8, 10 and 30 µL, respectively of 10 µg/mL calibration solution.

6.3.2.2 Generate a calibration curve from the peak height data obtained from analysis of the calibration standards using one of the following equations (1) or (2):

(1) $y = ax^2 + bx + c$

(2) $y = (a \cdot x) \cdot (b + x)^{-1}$

where: y = the concentration of the analyte in ng per spot,
x = the analyte peak height,
a, b, c = constants calculated by the computer system.

6.3.3 **Note:** Move the cover plate to expose approximately 1.5 cm of the bottom of the plate (starting zone position) to allow application of the sample without exposing the rest of the plate to the laboratory atmosphere.

6.3.4 Apply half of the acetonitrile sample extract to one track of the TLC plate; record the volume applied and the total volume of the extract. Repeat the application procedure for the remaining samples and tracks using the procedure outlined in Table 1. After development and analysis of the plate, record resulting peak sizes preferably in height units.

6.3.5 Calculate analyte concentrations in the sample from the peak height of the analyte using one of the equations described in Section 6.3.2.2. The equation which better fits the data points in the calibration curve should be preferred.

6.4 HPLC ANALYSIS

6.4.1 Evaporate the SPE extract (Section 6.2.4.2) to dryness and add 200 µL of HPLC internal standard spiking solution (Section 4.12.4.3).

6.4.2 Table 1 summarizes the recommended HPLC operating conditions. Included in Table 1 are retention times observed using this method. Example of the separations achieved using these conditions are shown in Figures 22-31.

6.4.3 Calibration – Establish HPLC operating conditions equivalent to those indicated in Table 1. Calibrate the HPLC system using the internal standard technique.

6.4.3.1 Inject 50 µL of each calibration standard and tabulate the relative response for each analyte (RR_a) to the internal standard using the equation:

$$RR_a = H_a/H_{is}$$

where: H_a = the peak area of the analyte, and
H_{is} = the peak area of the internal standard.

Generate a calibration curve of analyte RR_a versus comparable analyte concentration in the sample in µg/L.

6.4.4 Inject 50 µL of the sample. Record the volume injected and the resulting peak size in area units.

6.4.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

6.4.6 Calculate analyte concentrations in the sample from the relative response for the analyte (RR_a) to the internal standard using the equation the calibration curves described in Section 6.4.3.1.

6.4.7 If the response for the peak exceeds the working range of the system, dilute the sample with additional internal standard spiking solution and reanalyze. Make the appropriate correction for the volume in the calculation of the analyte concentration in the original sample.

7. CALCULATIONS

7.1 Calculate the analyte percent recovery (R_i) using the equation:

$$R_i = \frac{100 \cdot (C_c - C_b)}{C_s}$$

where C_c = the calculated analyte concentration in the spiked sample,
C_b = the average calculated pesticide concentration in the unspiked samples, and
C_s = the original pesticide spiking concentration.

7.2 Calculate the average percent recovery (R_a) of each analyte using the equation:

$$R_a = \sum_{i=1}^{n} R_i/n$$

where n = the number of measurements.

7.3 Calculate the standard deviation of the percent recovery measurements (s) using the equation:

$$s = \left[\frac{\sum_{i=1}^{n} (R_i - R_a)^2}{n-1} \right]^{1/2}$$

7.4 Calculate the percent relative standard deviation of the percent recovery measurements (RSD) using the equation:

$$RSD = \frac{100 \cdot s}{R_a}$$

8. EVALUATION OF RESULTS (5,6)

8.1 ANALYTE RECOVERY

8.1.1 Accuracy -- The average percent recovery is an indication of the accuracy of the method at the specified analyte concentration level. In general, a percent recovery between 70 percent and 120 percent is acceptable.

8.1.2 Precision -- The percent relative standard deviation is an indication of the precision of the method at the specified analyte concentration level. In general, a percent relative standard deviation ≤ 20 is acceptable.

8.1.3 Note: The acceptable range of accuracy and precision is most valid for the confirmation method. The recommended HPTLC screening method is more influenced by comigrating compounds and it is not unlikely the observed accuracy and precision will be outside the limits suggested in Sections 8.1.1 and 8.1.2. The screening method has been validated to show

that the absence of a signal or a signal lower than 0.1 µg/L can be reliably detected. Therefore, any positive identification above 0.1 µg/L made by HPTLC must be validated using the confirmation method described in this method.

8.1.4 Table 3 provides single laboratory recovery and precision data obtained for the analytes spiked into reagent water at 0.05, 0.1, 0.2, and 0.5 µg/L as determined by HPTLC analytical conditions. Similar results from spiked reagent water should be expected by any experienced laboratory. Analytes were separated into two spiking mixtures to avoid coelutions. Sample HPTLC chromatograms obtained from calibration standards, reagent water spiked with the analytes at the 0.1 µg/L level, and reagent water blanks are shown in Figures 7-10 and 15-17.

8.1.5 Table 4 provides single laboratory recovery and precision data obtained for the analytes spiked into reagent water at 0.05, 0.1, 0.2, and 0.5 µg/L as determined by HPLC analytical conditions. Similar results from spiked reagent water should be expected by any experienced laboratory. Sample HPLC chromatograms obtained from a calibration standard, reagent water spiked with the analytes at the 0.1 µg/L level, and reagent water blanks are shown in Figures 22-25.

8.1.6 In a single laboratory, recoveries were determined for the analytes spiked into two groundwaters (Type 1 groundwater and Type 2 groundwater) at 0.05, 0.1, and 0.15 µg/L. Recoveries determined by HPTLC and HPLC from the two groundwaters are given in Tables 5 and 6, respectively. Characterization results from the two groundwater samples are given in Table 7.

Sample HPTLC chromatograms obtained from Type 1 groundwater spiked with the analytes at the 0.1 µg/L level, a Type 1 groundwater blank, Type 2 groundwater spiked with the analytes at the 0.1 µg/L level, and a Type 2 groundwater blank are shown in Figures 11-14 and 18-21. Sample HPLC chromatograms obtained from Type 1 groundwater spiked with the analytes at the 0.1 µg/L level, a Type 1 groundwater blank, Type 2 groundwater spiked with the analytes at the 0.1 µg/L level, and a Type 2 groundwater blank are shown in Figures 26-31, respectively.

8.2 MINIMUM QUANTIFICATION LIMIT (MQL) – The lowest pesticide concentration level for which acceptable accuracy and precision results are demonstrated. Quantification results for pesticides detected above the MQL can be reported with an acceptable degree of confidence.

8.2.1 In a single laboratory, acceptable accuracy and precision results were demonstrated for the analytes spiked into reagent water at 0.05 µg/L (see Tables 3 and 4). The MQL for analytes using this method is 0.05 µg/L.

The procedure proposed by BBA (lit. /6/) was used for determining MQL. BBA considers MQL confirmed if:

- MQL is ≥ limit of detection,
- Recovery of samples spiked at MQL must be ≥ 70 percent, and
- Relative standard deviation must ≤ 20 percent.

8.2.2 **Note:** Observed MQLs may vary between groundwaters depending upon the nature of interferences in the sample matrices and the specific instrumentation used. The extent of matrix interferences will vary considerably from source to source, depending upon the groundwater sampled. Cleanup of sample extracts may be necessary to demonstrate MQLs of 0.05 µg/L in some groundwater.

9. REFERENCES

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(2) ASTM Annual Book of Standards, Part 11, Volume 11.01, D3370-82, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 130, 1986.

(3) Weil, L.: Entnahme und Vorbereitung von Wasserproben in Rückstandsanalytik von Pflanzenschutzmitteln, Mitteilung VI der Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Voratsschutzmittel, Methodensammlung der Arbeitsgruppe "Analytik", Deutsche Forschungsgemeinschaft, 1. bis 9. Lieferung (1987).

(4) DIN 38 402-A14 in Deutsche Einheitsverfahren zur Wasseruntersuchung, herausgegeben von der Fachgruppe Wasserchemie der Gesellschaft Deutscher Chemiker, Verlag Chemie, Weinheim, 3. Auflage, letzte Ergänzung durch Lieferung 18 (1987).

(5) Rückstandsanalytik von Pflanzenschutzmitteln, Mitteilung VI der Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Voratsschutzmittel, Methodensammlung der Arbeitsgruppe "Analytik", Deutsche Forschungsgemeinschaft, 1. bis 9. Liebferung 1987.

(6) Weinmann, W., H.-G. Nolting, und J. Siebers; Biologische Bundesanstalt für Land- und Forstwirtschaft, Merkblatt Nr. 58, I. Auflage, Rückstanduntersuchungen Richtlinie zur Durchführung der Analysen, Bundesrepublik Deutschland, 1983.

TABLE 1.
RETENTION CHARACTERISTICS AND CHROMATOGRAPHIC CONDITIONS

Analyte	HPTLC Migration, mm(a)	HPLC Retention Time, min(b)
Chlorsulfuron	27.7 (39.3)	9.4
DPX-A 7881	20.2 (32.0)	10.9
DPX-F 5384	25.7 (37.7)	17.3
DPX-L 5300	36.4 (44.0)	(c)
DPX-M 6316	20.3 (35.5)	7.6
Metsulfuron methyl	24.3 (35.6)	8.2
Hydrocortisone(d)	--	5.1

(a) Migration distances using alternative AMD conditions are given in parenthesis. Migration position of start is 10 mm using the following HPTLC conditions:

Sample Application (Camag Linomat IV):

Plate Width:	200 mm
X Start Position:	20 mm
Y Start Position:	10 mm
Band Lengths:	4 mm
Space Between Bands:	6 mm
Application Speed:	8 sec/µL
Volume Applied:	50% volume of sample extract
Calibration:	20 ng to 300 ng Applied in 20 µL of standard calibration solution (1 µg/mL) and 5 µL to 30 µL of standard calibration solution (10 µg/mL)

Chromatographic Development (Camag AMD - System):

Gradient:	30-step
Reservoir 1:	Methanol/dichloromethane (10:90), 0.01% NH_3
Reservoir 2:	Methanol/dichloromethane/HCOOH (5:95:0.1%)
Reservoir 3:	Dichloromethane/HCOOH (100:0.1%)
Reservoir 4:	Dichloromethane/HCOOH (100:0.1%)
Reservoir 5:	Dichloromethane/HCOOH (100:0.1%)
Reservoir 6:	Dichloromethane (100)
Drying Step:	3 min/step
Development Time:	Approximately 5.5 hours

TABLE 1. RETENTION TIME AND CHROMATOGRAPHIC CONDITIONS (Continued)

Alternative AMD Gradient:
Chromatographic Development (Camag AMD - System):

Gradient:	30-step
Reservoir 1:	Methanol/dichloromethane (10:90), 0.01% NH_3
Reservoir 2:	Methanol/dichloromethane/HCOOH (10:90:0.1%)
Reservoir 3:	Methanol/dichloromethane/HCOOH (5:95:0.1%)
Reservoir 4:	Dichloromethane/HCOOH (100:0.1%)
Reservoir 5:	Dichloromethane/HCOOH (100:0.1%)
Reservoir 6:	Dichloromethane (100)
Drying Step:	3 min/step
Development Time:	Approximately 5.5 hours

Note: The conditions below were used to generate the data provided in this report. The parameters are typical but may vary slightly depending on the sensitivity of the scanner which may vary from instrument to instrument.

Scanning (Camag TLC Scanner II):

No. of tracks		17(1)
Y - Position Zero Adjust	[mm]	7
Y - Position Start	[mm]	8
Y - Position End	[mm]	80
X - Start	[mm]	21(1)
X - Trackspace	[mm]	10
Peak Optimization Mode		0
No. of Wave Lengths		6
Y - Step	[µm]	100
SENS		AUTO
Monochromator Bandwidth	[nm]	10
Slit Width	[mm]	4
Slit Length	[mm]	5
No. of Smoothing Points		3
Peak Threshold		10
Baseline Correction		1
Light Source		D2-lamp
Wavelengths:		190, 220, 240, 260, 280, 300 nm

(1)These conditions might vary from plate to plate.

TABLE 1. RETENTION TIME AND CHROMATOGRAPHIC CONDITIONS (Continued)

(b) HPLC Conditions:

Column:	250 mm x 4.6 mm I.D. DuPont Zorbax ODS (5 µm)
Mobile Phase:	Linear gradient from 30:70 acetonitrile:water to 60:40 acetonitrile:water in 20 minutes. Hold at 60:40 acetonitrile:water for 20 minutes. Note: acetonitrile and water both contain 0.01 percent (v/v) acetic acid.
Flow Rate:	0.5 mL/min
Injection Volume:	50 µL
Detector:	Absorbance at 225 nm

(c) HPLC conditions used for determination of these analytes cause rapid decomposition of DPX-L5300. A decomposition product of DPX-L5300 which elutes earlier than chlorsulfuron is observed.

(d) HPLC internal standard.

TABLE 2. RESULTS FROM ANALYTE STABILITY STUDIES(a)

Sample Description and Storage Conditions	Chlorsulfuron		DPX-A7881		DPX-F5384		DPX-M6316		Metsulfuron Methyl	
	R_a(b)	RSD(c)	R_a	RSD	R_a	RSD	R_a	RSD	R_a	RSD
Reagent water analyzed immediately after spiking	106	9.8	93	3.2	98	18	97	8.7	98	10
Reagent water spiked then stored for 7 days at 4°C protected from light	104	15	74	5.7	91	4.5	93	6.5	89	6.4
Reagent water spiked then stored for 14 days at 4°C protected from light	102	ND(d)	80	ND	82	ND	84	ND	89	ND
Acetonitrile extract stored 7 days after preparation at -20°C protected from light	113	20	77	15	102	19	104	16	99	17
Acetonitrile extract stored 14 days after preparation at -20°C protected from light	121	11	95	6.0	106	11	125	20	101	7.0
Acetonitrile extract stored 28 days after preparation at -20°C protected from light	116	20	84	13	87	7.0	92	10	99	9.0

(a) Stability studies were conducted using conditions not compatible with DPX-L5300 stability; results are not reported.
(b) R_a = average percent recovery of three measurements.
(c) RSD = percent relative standard deviation of three measurments.
(d) ND = single recovery measurement; RSD not calculated.

TABLE 3. RECOVERY OF ANALYTES FROM REAGENT WATER AND MQLs DETERMINED BY HPTLC

Analyte	Spiking Level, µg/L	n(a)	R_a(b)SD(c)		MQL, µg/L(d)
Chlorsulfuron	0	3	ND(e)	--	--
	0.1	6	89.2	30.2	--
	0.2	8	62.8	12.4	0.2
	0.5	7	74.3	6.7	--
	Total	21	74.1	20.4	--
DPX-A7881	0	3	ND	--	--
	0.1	6	78.0	6.3	0.1
	0.2	8	64.5	15.8	--
	0.5	7	83.4	9.7	--
	Total	21	74.7	14.1	--
DPX-F5384	0	3	ND	--	--
	0.1	7	97.1	12.5	0.1
	0.2	7	107	18.0	--
	0.5	7	89.3	8.3	--
	Total	21	97.7	15.4	--
DPX-L5300	0	3	ND	--	--
	0.1	7	100	15.3	0.1
	0.2	7	116	16.2	--
	0.5	7	89.6	5.2	--
	Total	21	102	17.5	--
DPX-M6316	0	3	ND	--	--
	0.05	7	80.0	34.0	--
	0.1	7	97.4	14.8	0.1
	0.2	7	95.0	15.4	--
	0.5	7	87.1	6.2	--
	Total	28	89.9	19.7	--
Metsulfuron methyl	0	3	ND	--	--
	0.1	7	58.7	13.1	0.1
	0.2	8	51.6	12.0	--
	0.5	7	77.1	10.8	--
	Total	21	62.1	16.0	--

(a) n = number of recovery data points.
(b) R_a = average percent recovery.
(c) SD = standard deviation.
(d) MQL = the minimum quantification limit, defined as the lowest pesticide concentration level for which acceptable accuracy and precision results were demonstrated. Quantification results for pesticides detected above the MQL can be reported with an acceptable degree of confidence.
(e) ND = interference not detected in blank.

TABLE 4. RECOVERY OF ANALYTES FROM REAGENT WATER AND MQLs DETERMINED BY HPLC

Analyte	Spiking Level, µg/L	n(a)	R_a(b)SD(c)		MQL, µg/L(d)
Chlorsulfuron	0	3	ND(e)	--	--
	0.05	7	45.9	6.4	--
	0.1	7	78.1	6.5	0.1
	0.2	7	88.6	5.2	--
	0.5	7	90.4	11.7	--
	Total	28	75.8	19.7	--
DPX-A7881	0	3	ND	--	--
	0.05	7	97.3	10.7	0.05
	0.1	7	117	11.0	--
	0.2	7	120	6.1	--
	0.5	7	118	9.8	--
	Total	28	117	13.1	--
DPX-F5384	0	3	ND	--	--
	0.05	7	112	40.6	--
	0.1	7	109	10.8	0.1
	0.2	7	124	12.9	--
	0.5	7	122	11.8	--
	Total	28	117	22.5	--
DPX-M6316	0	3	ND	--	--
	0.05	7	87.4	23.4	--
	0.1	7	106	10.0	0.1
	0.2	7	110	7.5	--
	0.5	7	118	10.5	--
	Total	28	105	17.6	--
Metsulfuron methyl	0	3	ND	--	--
	0.05	7	41.9	16.9	--
	0.1	7	80.1	10.2	0.1
	0.2	7	88.4	5.7	--
	0.5	7	89.7	11.2	--
	Total	28	75.0	22.7	--

(a) n = number of recovery data points.
(b) R_a = average percent recovery.
(c) SD = standard deviation.
(d) MQL = the minimum quantification limit, defined as the lowest pesticide concentration level for which acceptable accuracy and precision results were demonstrated. Quantification results for pesticides detected above the MQL can be reported with an acceptable degree of confidence.
(e) ND = interference not detected in blank.

TABLE 5. RECOVERY OF ANALYTES FROM GROUNDWATER #1 DETERMINED BY HPTLC AND HPLC(a)

Analyte	Spiking Level, µg/L	HPTLC n(b)	HPTLC R_a(c)	HPTLC SD(d)	HPLC n	HPLC R_a	HPLC SD
Chlorsulfuron	0	3	ND(e)	--	3	ND	--
	0.05	2	72	--	6	100	10.3
	0.1	5	78.0	21.2	7	90.1	4.2
	0.15	7	93.6	11.9	7	88.6	5.4
	Total	14	85.1	17.5	20	92.6	8.3
DPX-A7881	0	3	ND	--	3	ND	--
	0.05	--	--	--	7	77.6	22.6
	0.1	5	52.8	14.5	7	60.4	24.4
	0.15	7	66.4	14.9	7	79.9	9.1
	Total	12	60.8	15.7	21	72.6	20.9
DPX-F5384	0	3	ND	--	3	ND	--
	0.05	2	93	--	7	82.1	18.1
	0.1	5	78.2	12.5	7	85.9	20.1
	0.15	7	69.3	20.4	7	82.7	8.0
	Total	14	75.9	17.8	21	83.6	15.5
DPX-L5300	0	3	ND	--	--	ND	--
	0.05	2	107	--	--	--	--
	0.1	7	92.9	19.7	--	--	--
	0.15	7	97.1	21.2	--	--	--
	Total	16	96.5	19.8	--	--	--
DPX-M6316	0	3	ND	--	3	ND	--
	0.05	7	65.2	10.0	7	67.0	16.8
	0.1	7	59.3	8.8	7	81.4	27.1
	0.15	7	42.9	10.3	7	74.7	15.6
	Total	21	54.8	13.3	21	74.4	20.4
Metsulfuron methyl	0	3	ND	--	3	ND	--
	0.05	2	70.0	--	7	107	4.5
	0.1	5	63.6	7.8	7	91.6	4.5
	0.15	7	90.0	11.3	7	87.9	5.1
	Total	14	77.7	16.5	21	95.6	10.1

(a) Artifically enhanced groundwater (see Table 7 for characterization).
(b) n = number of recovery data points.
(c) R_a = average percent recovery.
(d) SD = standard deviation.
(e) ND = interference not detected in blank.

TABLE 6. RECOVERY OF ANALYTES FROM GROUNDWATER #2 DETERMINED BY HPTLC AND HPLC(a)

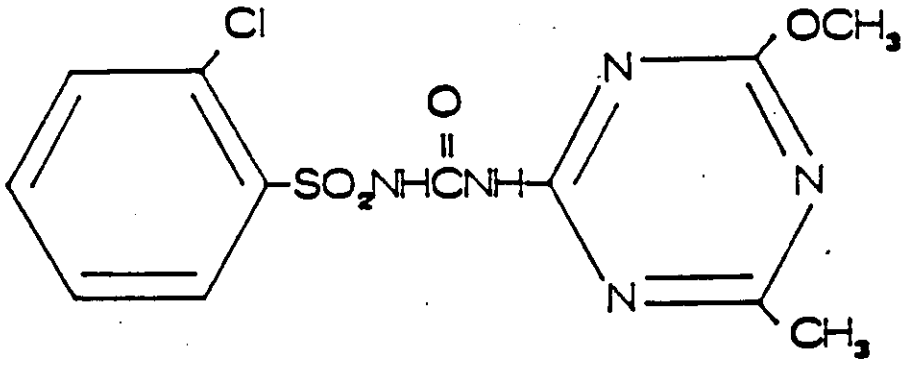
Analyte	Spiking Level, µg/L	HPTLC n(b)	HPTLC R_a(c)	HPTLC SD(d)	HPLC n	HPLC R_a	HPLC SD
Chlorsulfuron	0	3	ND(e)	--	3	ND	--
	0.05	6	82.7	19.1	7	94.4	11.7
	0.1	7	71.9	12.4	6	67.7	8.3
	0.15	7	74.7	8.5	7	76.4	5.5
	Total	20	76.1	12.5	20	80.1	14.1
DPX-A7881	0	3	ND	--	3	ND	--
	0.05	4	55.2	10.0	7	61.7	33.2
	0.1	6	34.2	11.6	6	37.0	17.9
	0.15	7	38.7	54.0	7	60.7	19.6
	Total	17	41.0	10.3	20	54.0	26.1
DPX-F5384	0	3	ND	--	3	ND	--
	0.05	5	78.0	17.7	7	43.4	8.6
	0.1	7	75.6	22.7	7	64.3	12.5
	0.15	7	77.3	11.5	7	75.9	12.7
	Total	19	76.8	14.0	21	61.2	17.5
DPX-L5300	0	3	ND	--	--	--	--
	0.05	7	138	13.9	--	--	--
	0.1	7	129	8.2	--	--	--
	0.15	7	111	10.6	--	--	--
	Total	21	123	14.6	--	--	--
DPX-M6316	0	3	ND	--	3	ND	--
	0.05	7	81.1	22.2	7	107	9.6
	0.1	7	68.0	18.5	7	69.6	7.2
	0.15	7	71.4	19.2	7	58.9	7.3
	Total	21	73.5	20.8	21	78.6	22.7
Metsulfuron methyl	0	3	ND	--	3	ND	--
	0.05	7	89.0	14.0	7	101	9.5
	0.1	7	72.3	11.4	6	63.5	8.2
	0.15	7	76.7	13.2	7	73.9	5.9
	Total	21	78.3	12.1	20	80.2	17.8

(a) Mountain groundwater (see Table 7 for characterization).
(b) n = number of recovery data points.
(c) R_a = average percent recovery.
(d) SD = standard deviation.
(e) ND = interference not detected in blank.

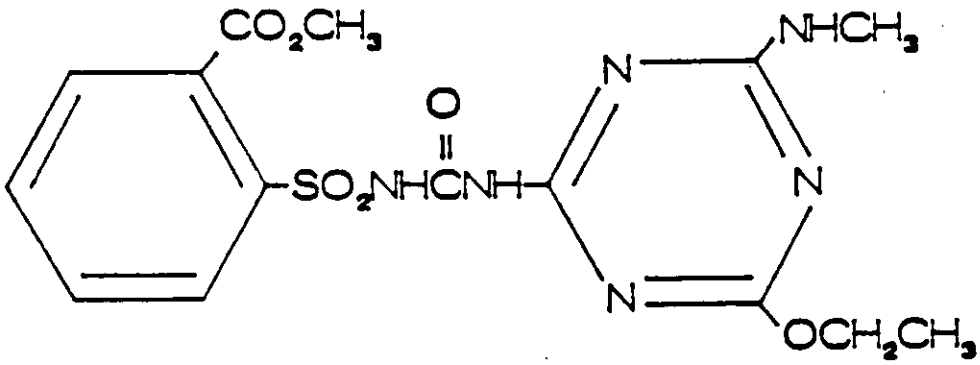
TABLE 7. CHARACTERIZATION OF GROUNDWATER SAMPLES

Characterization Parameter	Type 1 Groundwater(a)	Type 2 Groundwater(b)
Temperature, °C	11.5	10.8
pH	7.30	6.28
Conductivity, mS/m	79.8	8.6
AOX, g/L	6	(c)
Nitrate, mol/m^3	0.080	0.058
Chloride, mol/m^3	3.33	0.21
Monophosphate, $mmol/m^3$	(c)	(c)
Calcium, mol/m^3	2.23	0.24
Magnesium, mol/m^3	0.53	0.14
Organic carbon (DOC), g/m^3	1.29	0.27

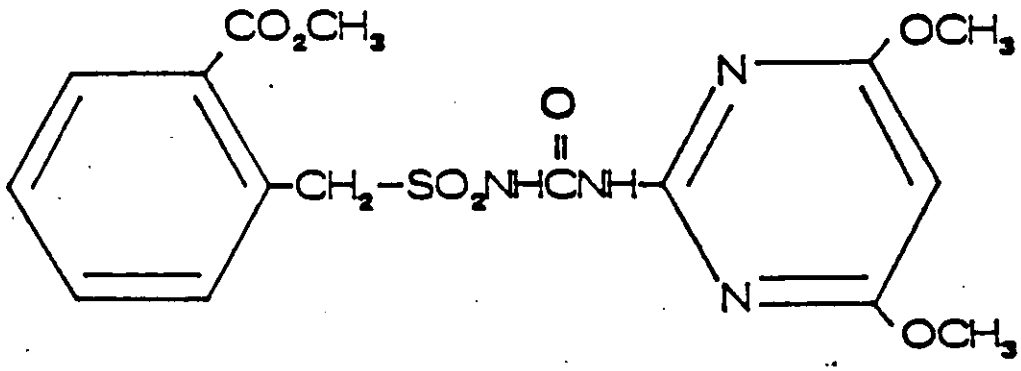
(a) Artificial recharged groundwater (water catchment beside a large river). The groundwater consists of natural groundwater, infiltration water from the river, and purified surface water which is pumped back into the aquifer. High anthroprogenic influence is expected. Data are mean values from June, 1988.
(b) Groundwater from clefts in the mountains (water catchment mostly covered with forests). Low anthropogenic influence is expected. Data are mean values from 1987.
(c) Not determined.



Chlorsulfuron

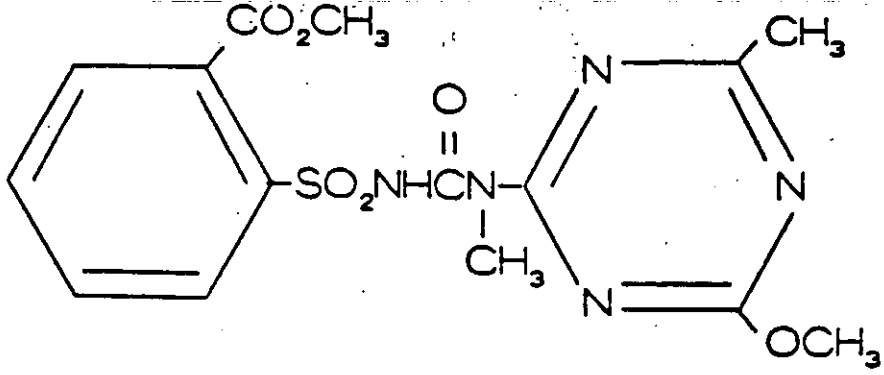


DPX-A7881

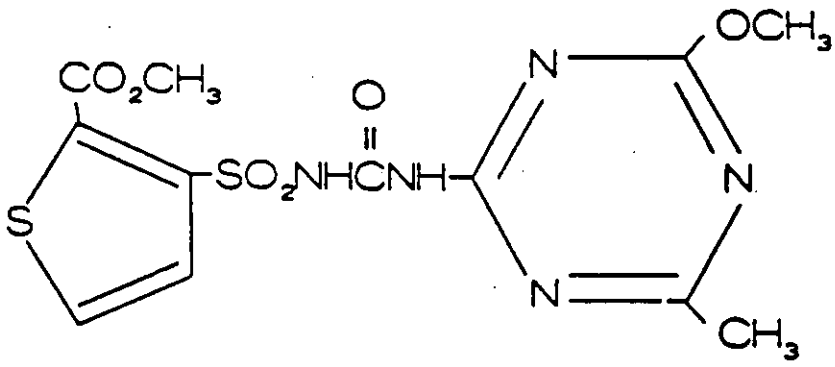


DPX-F5384

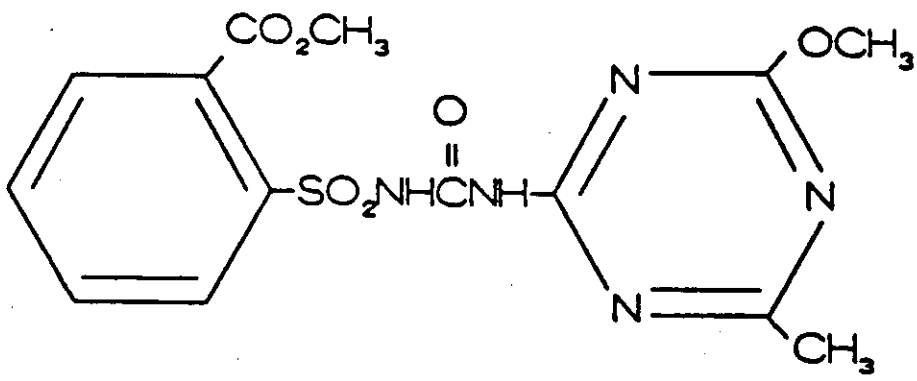
FIGURE 1. ANALYTE STRUCTURES



DPX-L5300



DPX-M6316



Metsulfuron Methyl

FIGURE 1. (Continued)

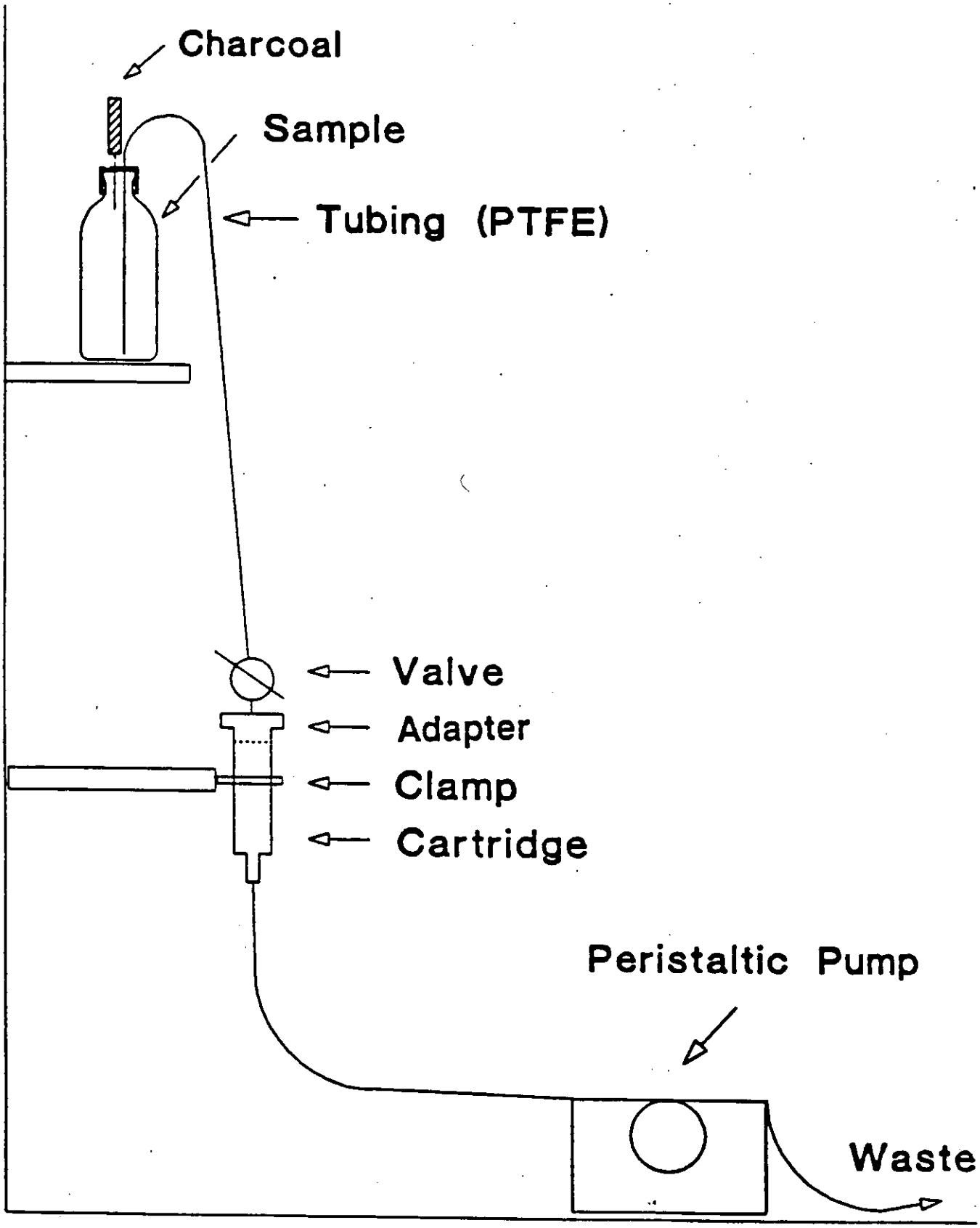


FIGURE 2. SPE EQUIPMENT

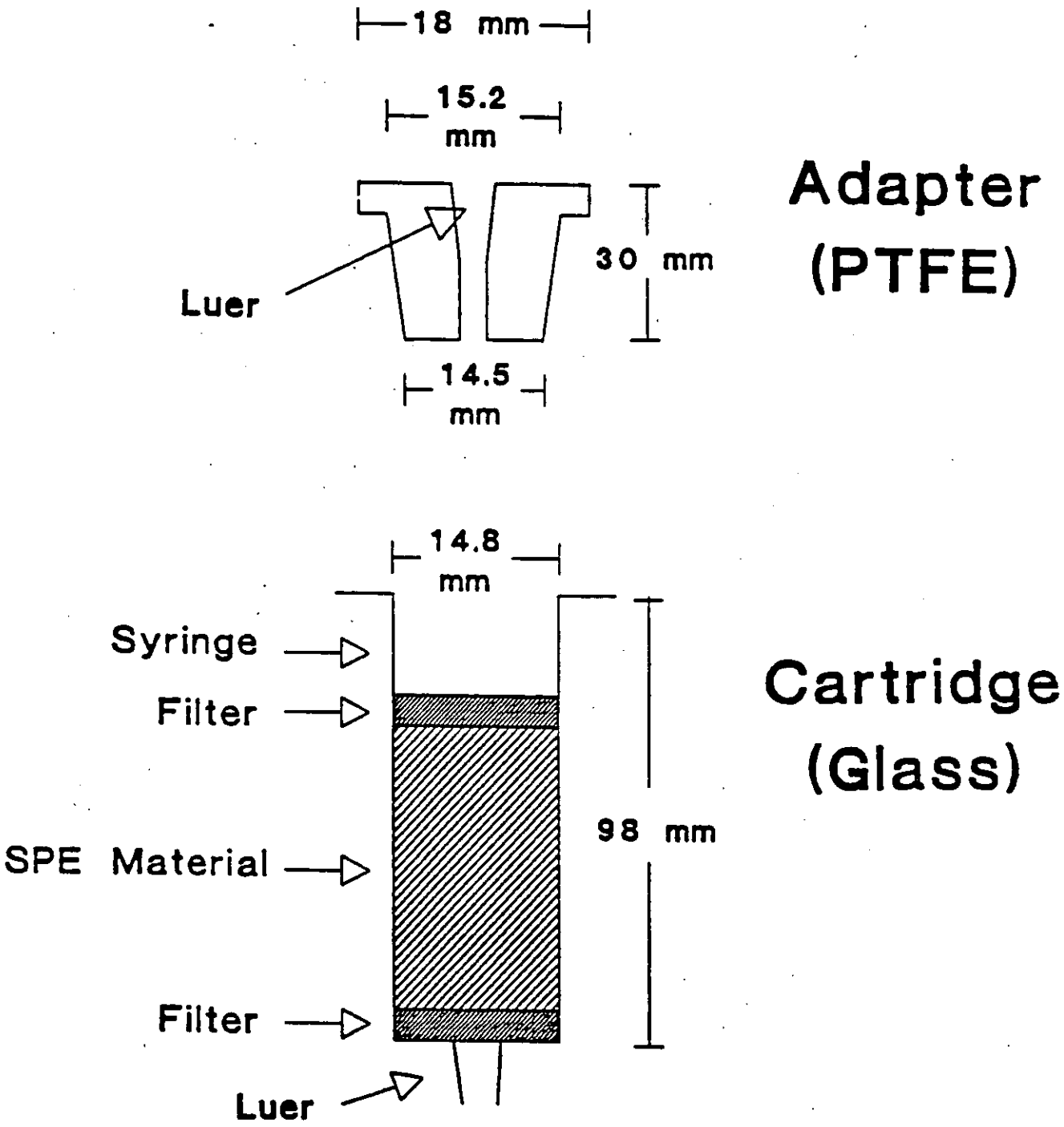


FIGURE 3. SPE CARTRIDGE ADAPTER AND CARTRIDGE

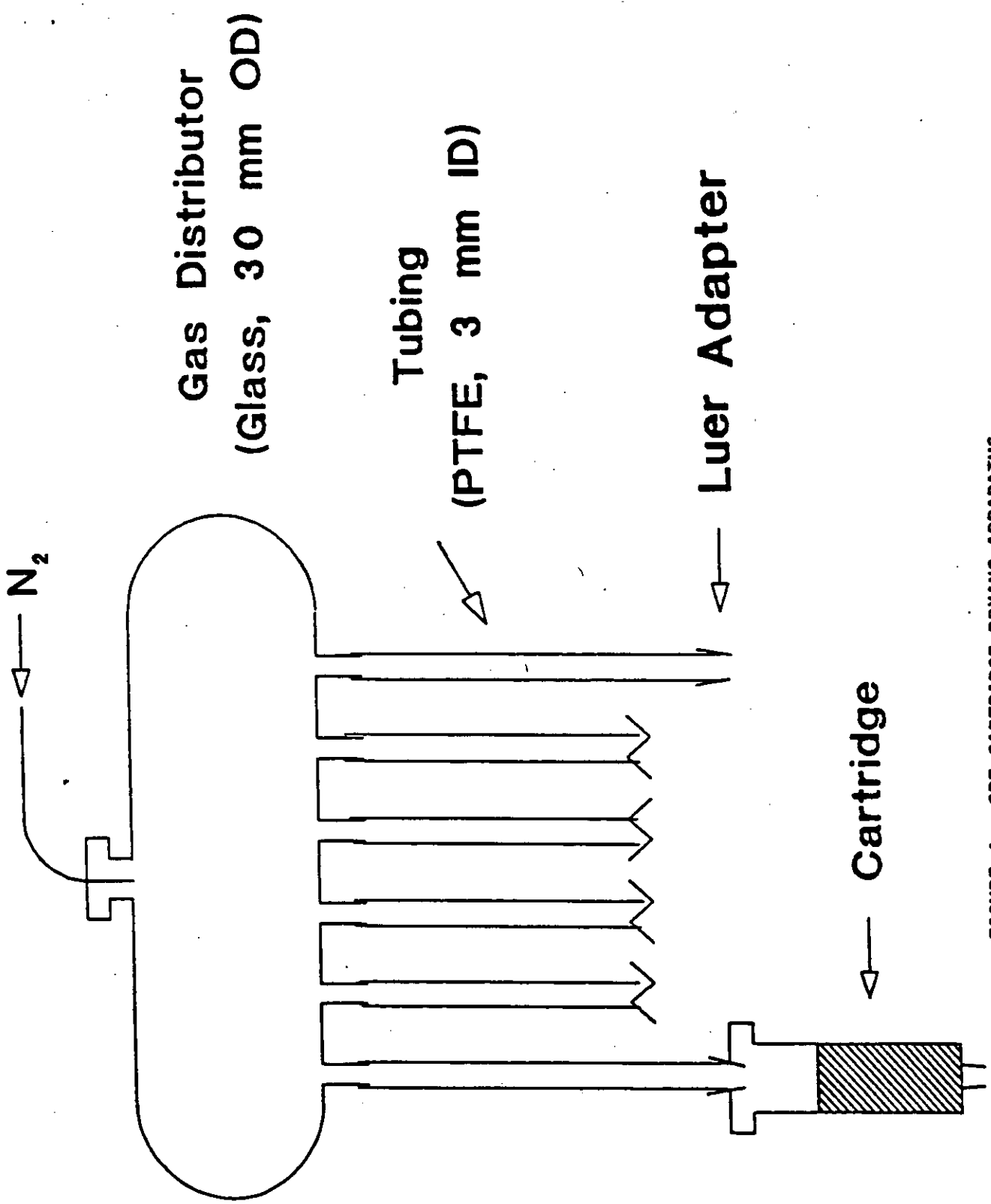


FIGURE 4. SPE CARTRIDGE DRYING APPARATUS

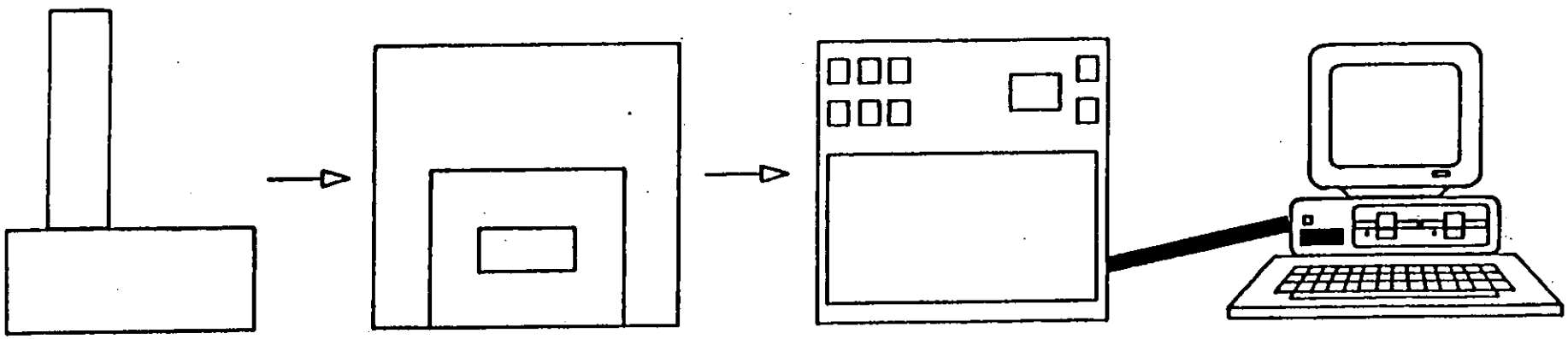


FIGURE 5. HPTLC APPARATUS

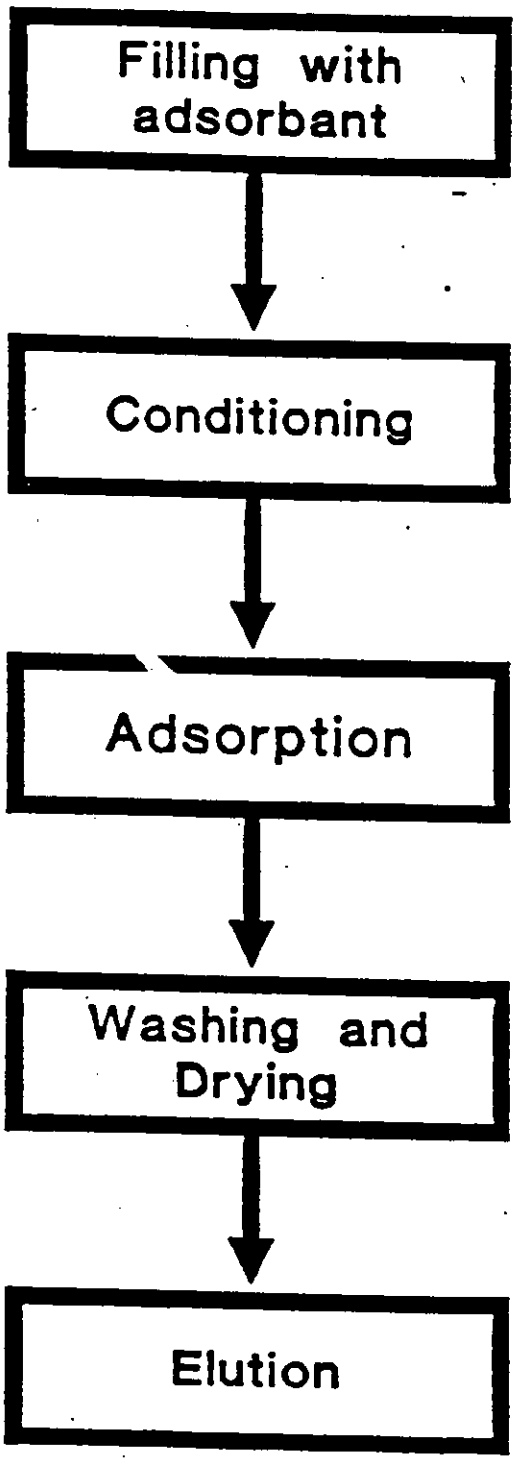


FIGURE 6. SPE PROCEDURE

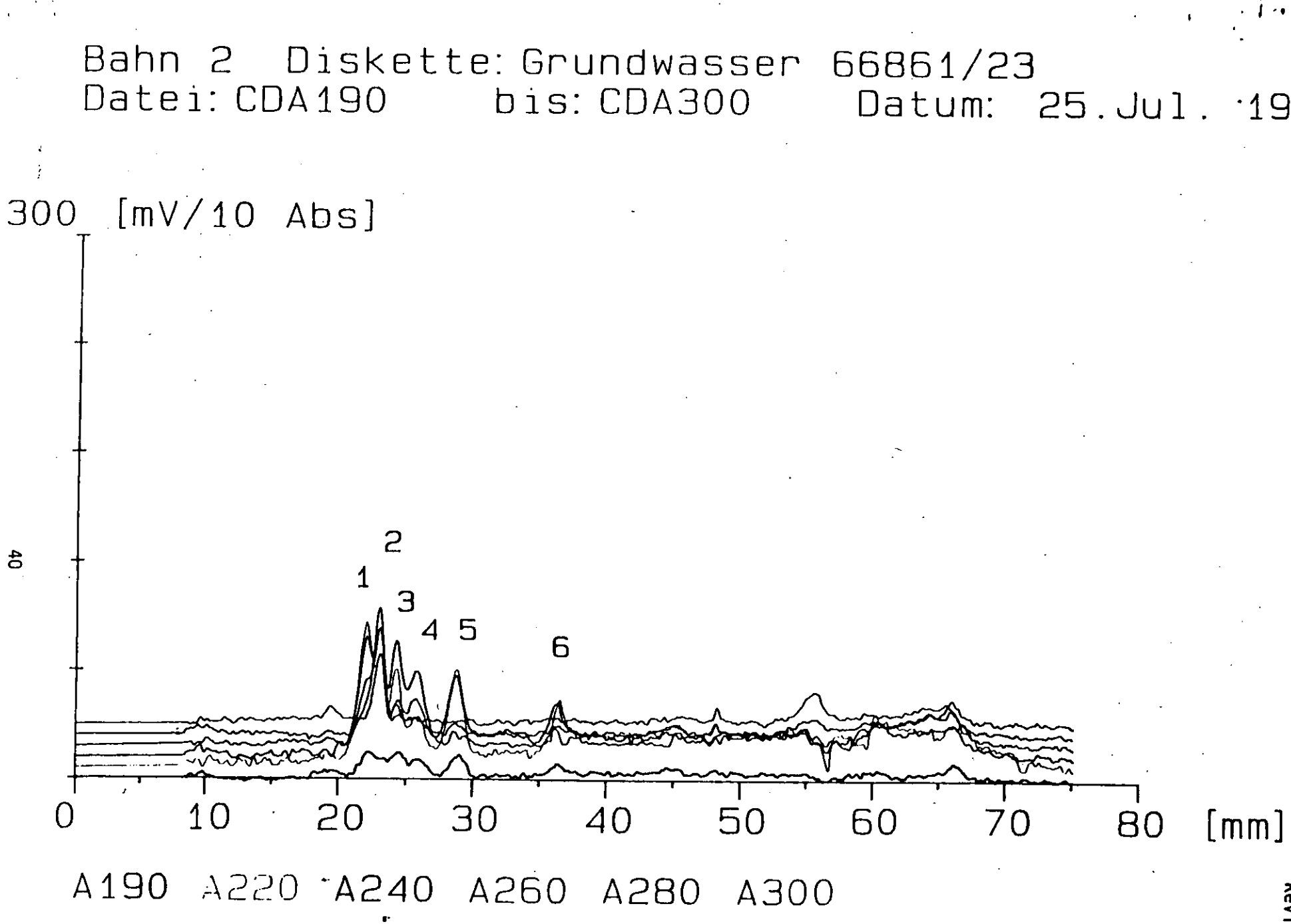


FIGURE 7. HPTLC CHROMATOGRAM OF DPX-A7881 (1), DPX-M6316 (2), METSULFURON METHYL (3), DPX-F5384 (4), CHLORSULFURON (5), AND DPX-L5300 (6) CALIBRATION STANDARD. Each peak represents 50 ng corresponding to 0.1 µg/L.

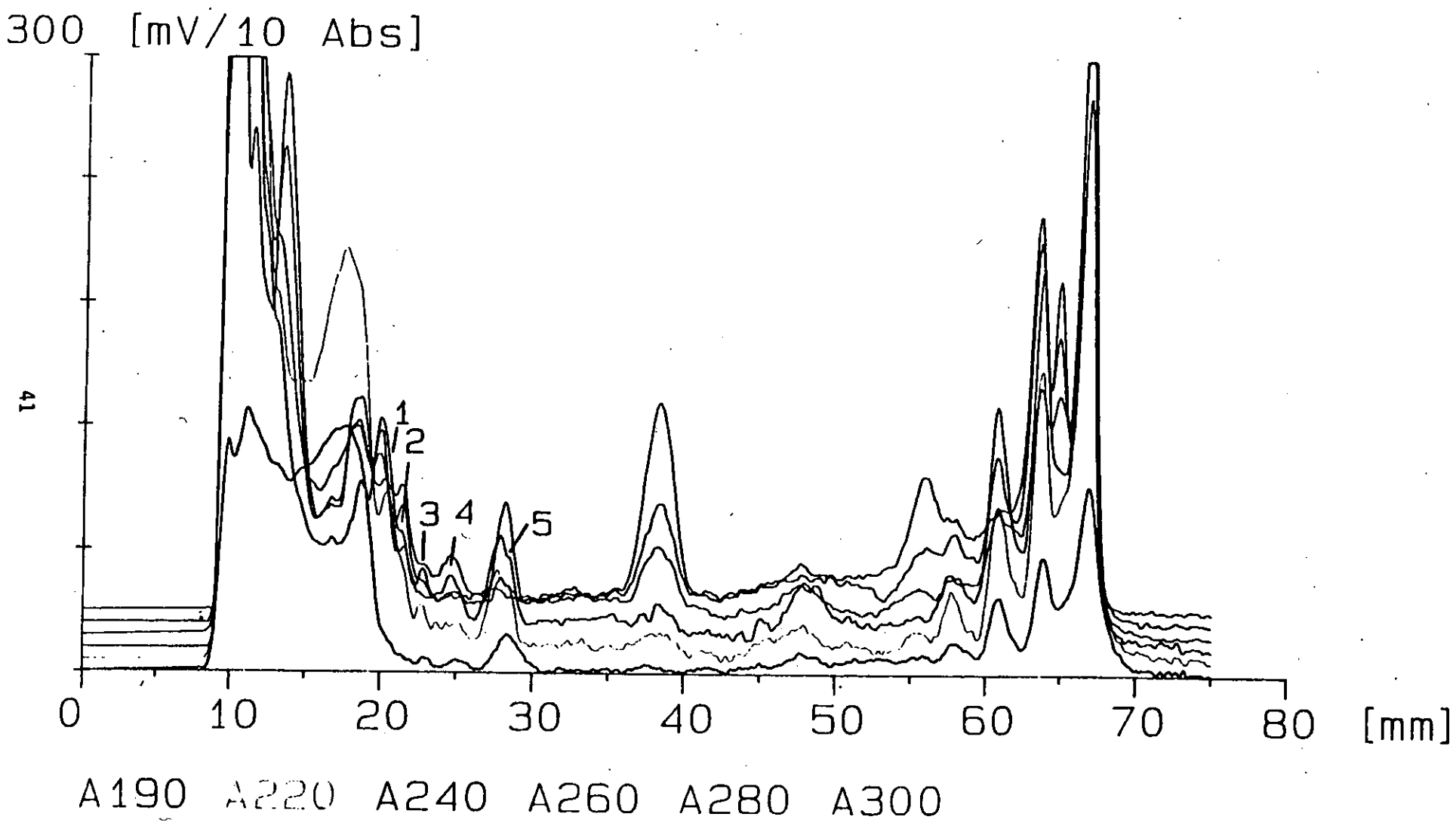


FIGURE 8. HPTLC CHROMATOGRAM OF REAGENT WATER SPIKED WITH DPX-A7881 (1), DPX-M6316 (2), METSULFURON METHYL (3), DPX-F5384 (4), CHLORSULFURON (5), AND DPX-L5300 (6) AT 0.1 µG/L

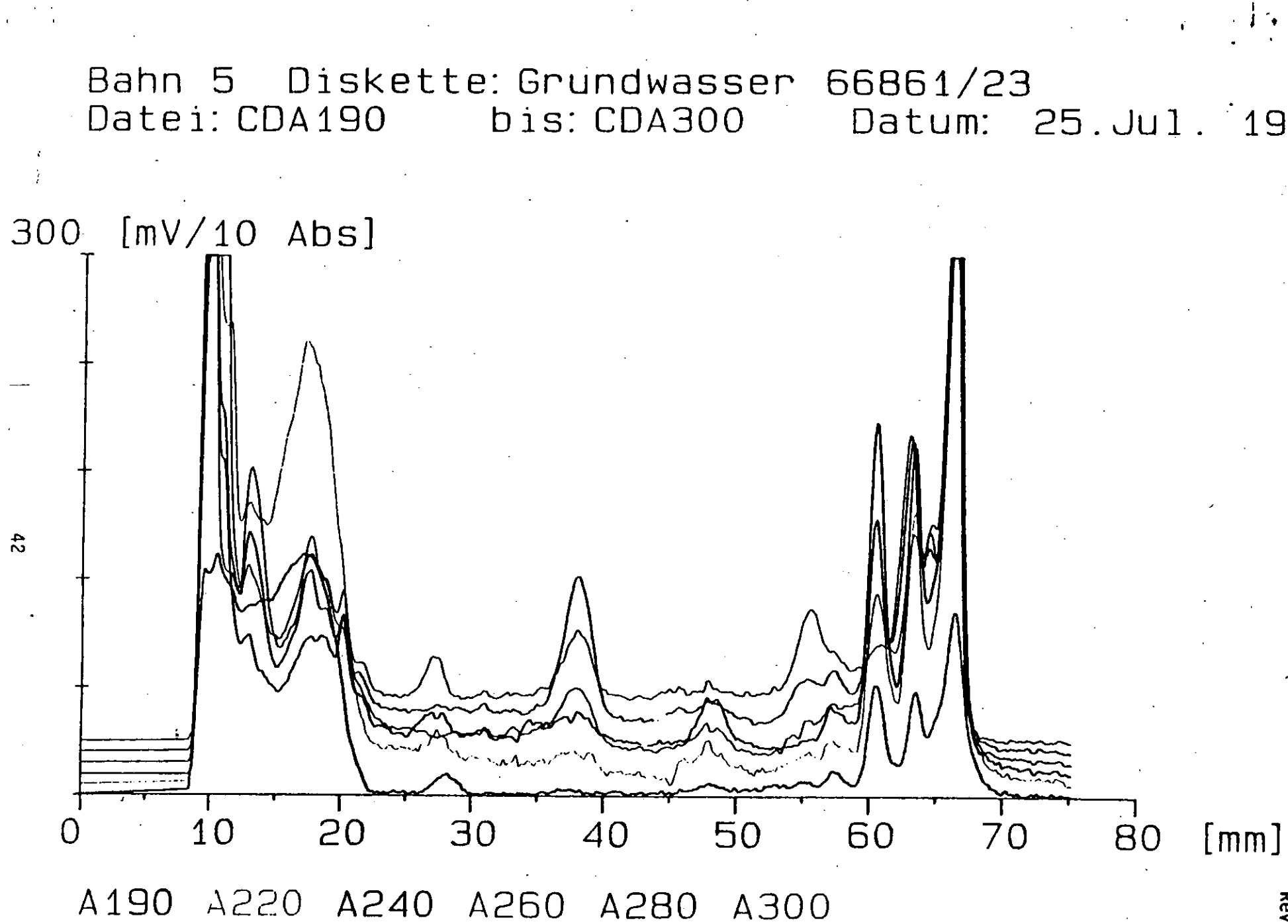


FIGURE 9. HPTLC CHROMATOGRAM OF REAGENT WATER BLANK

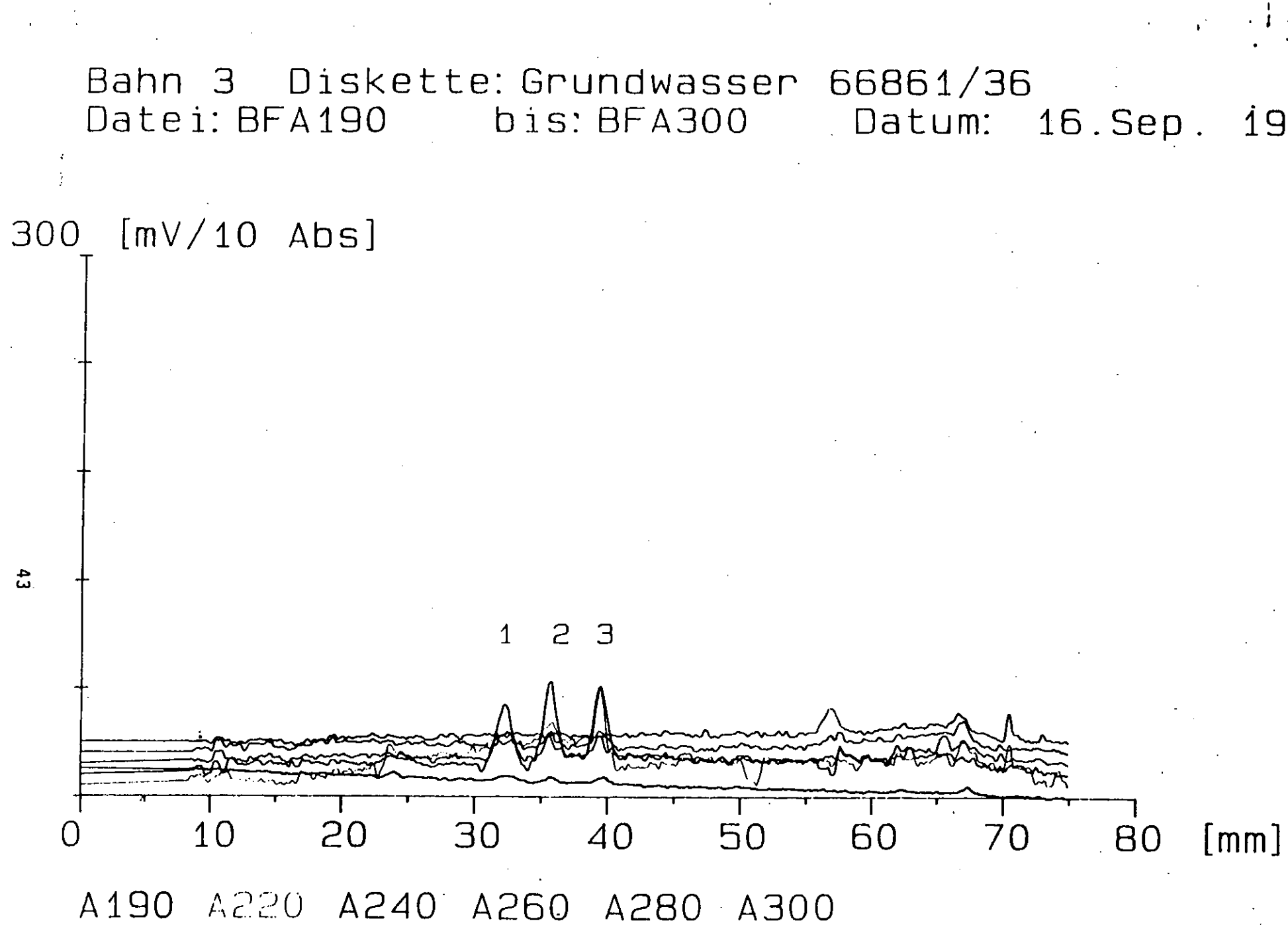


FIGURE 10. HPTLC CHROMATOGRAM OF DPX-A7881 (1), METSULFURON METHYL (2), AND CHLORSULFURON (3) CALIBRATION STANDARD USING ALTERNATIVE AMD CONDITIONS. Each peak represents 50 ng corresponding to 0.1 µg/L.

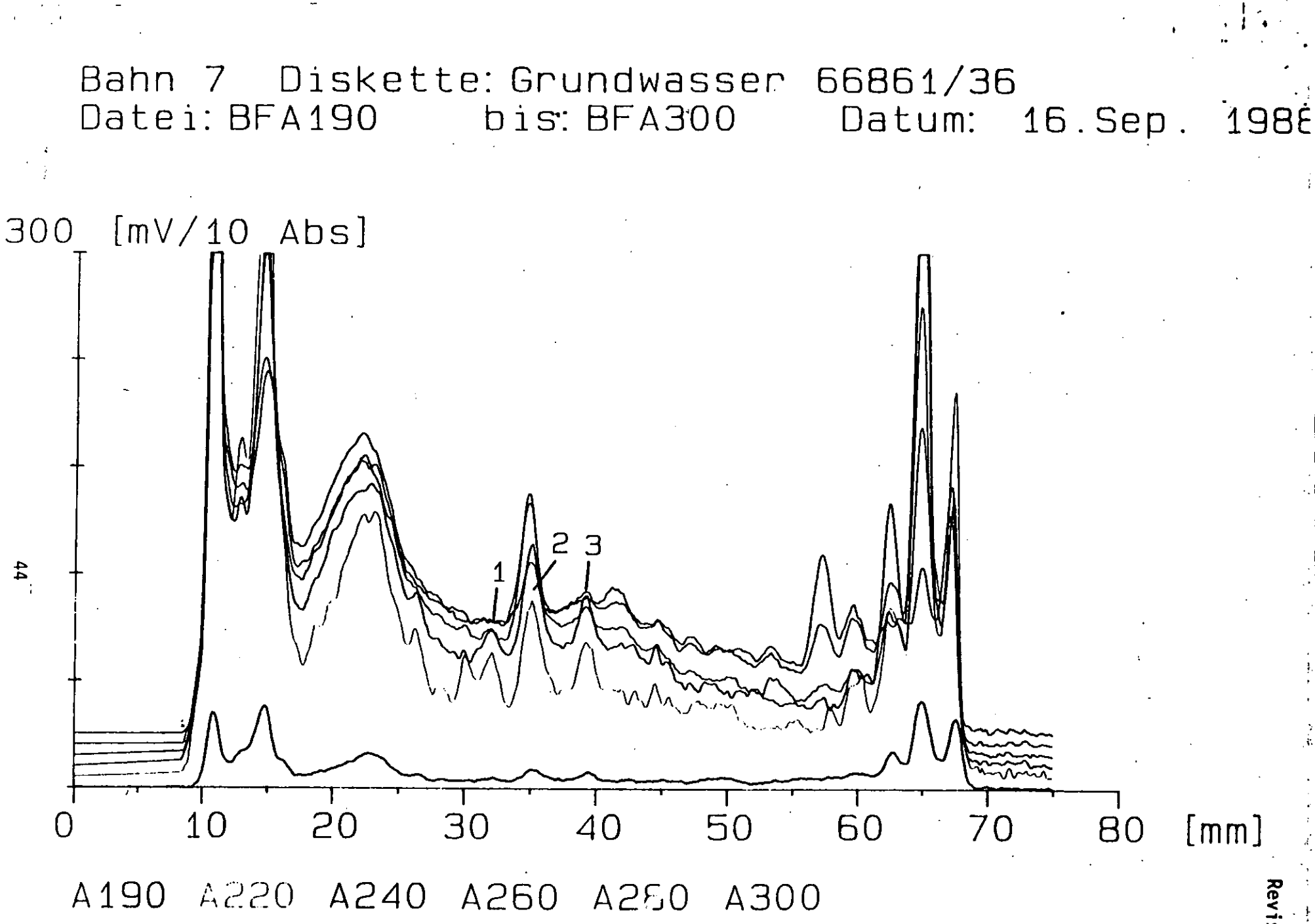


FIGURE 11. HPTLC CHROMATOGRAM OF TYPE 1 GROUNDWATER SPIKED WITH DPX-A7881 (1), METSULFURON METHYL (2), AND CHLORSULFURON (3) AT 0.1 µG/L USING ALTERNATIVE AMD CONDITIONS

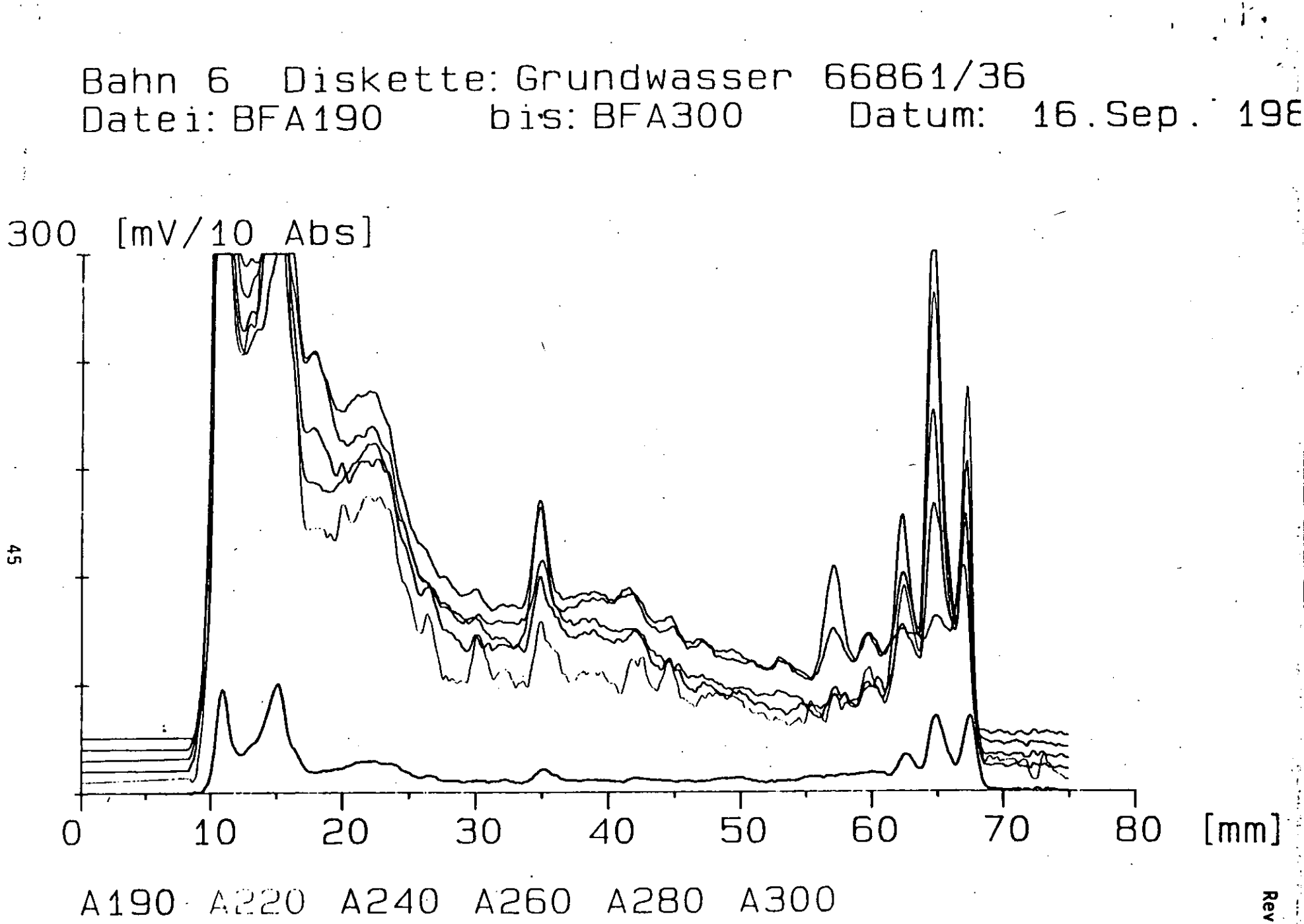


FIGURE 12. HPTLC CHROMATOGRAM OF TYPE 1 GROUNDWATER BLANK USING ALTERNATIVE AMD CONDITIONS

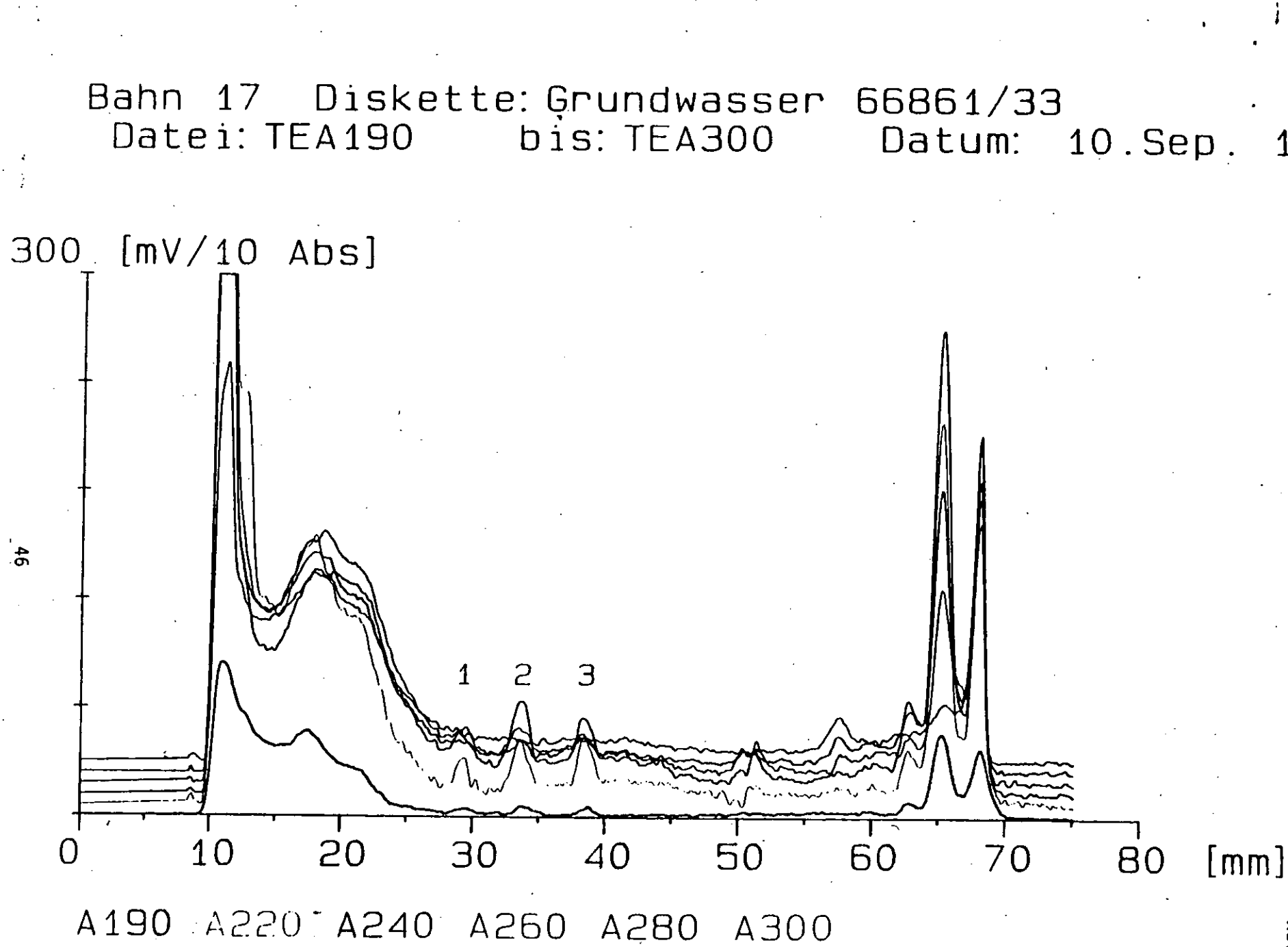


FIGURE 13. HPTLC CHROMATOGRAM OF TYPE 2 GROUNDWATER SPIKED WITH DPX-A7881 (1), METSULFURON METHYL (2), AND CHLORSULFURON (3) AT 0.1 µG/L USING ALTERNATIVE AMD CONDITIONS

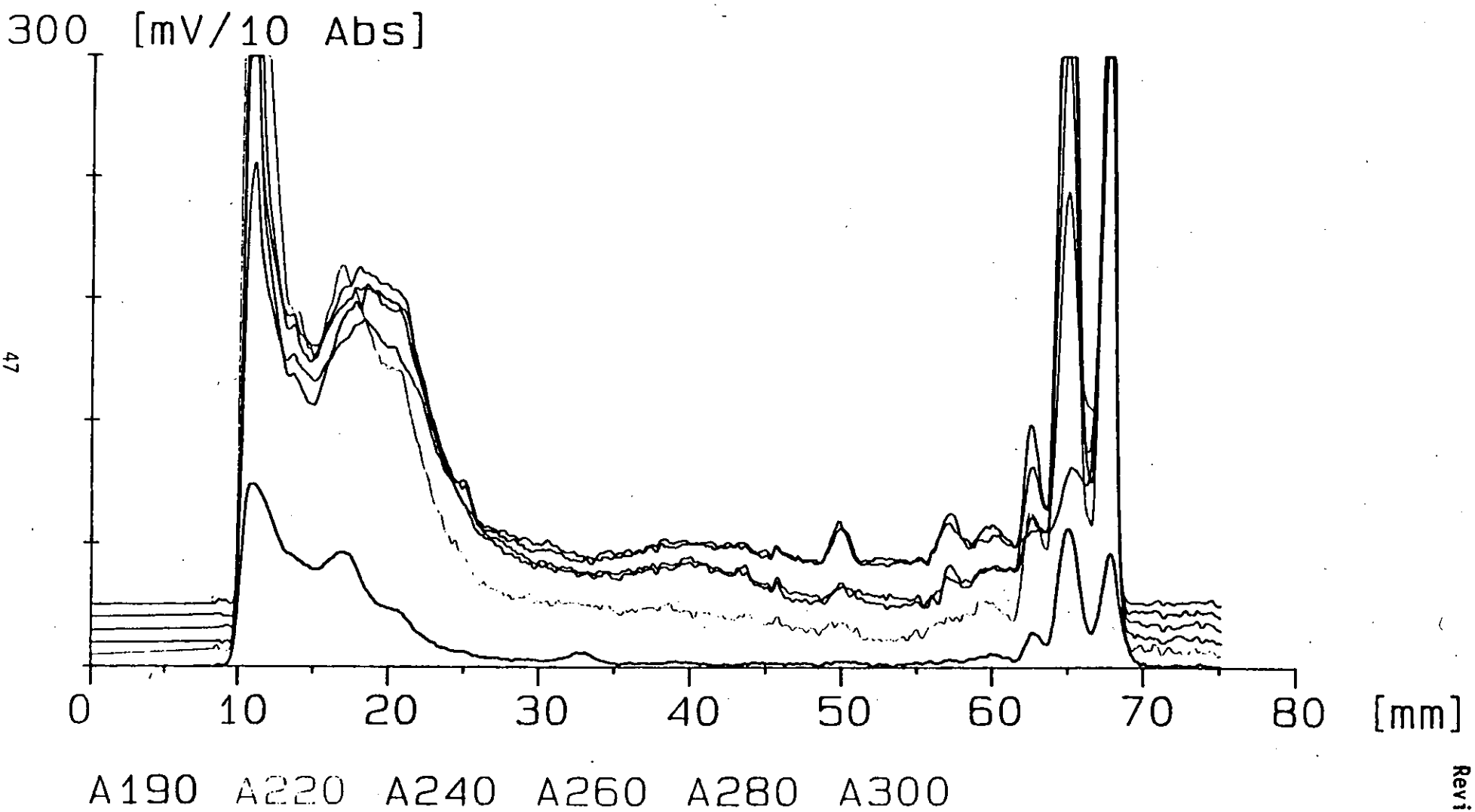


FIGURE 14. HPTLC CHROMATOGRAM OF TYPE 2 GROUNDWATER BLANK USING ALTERNATIVE AMD CONDITIONS

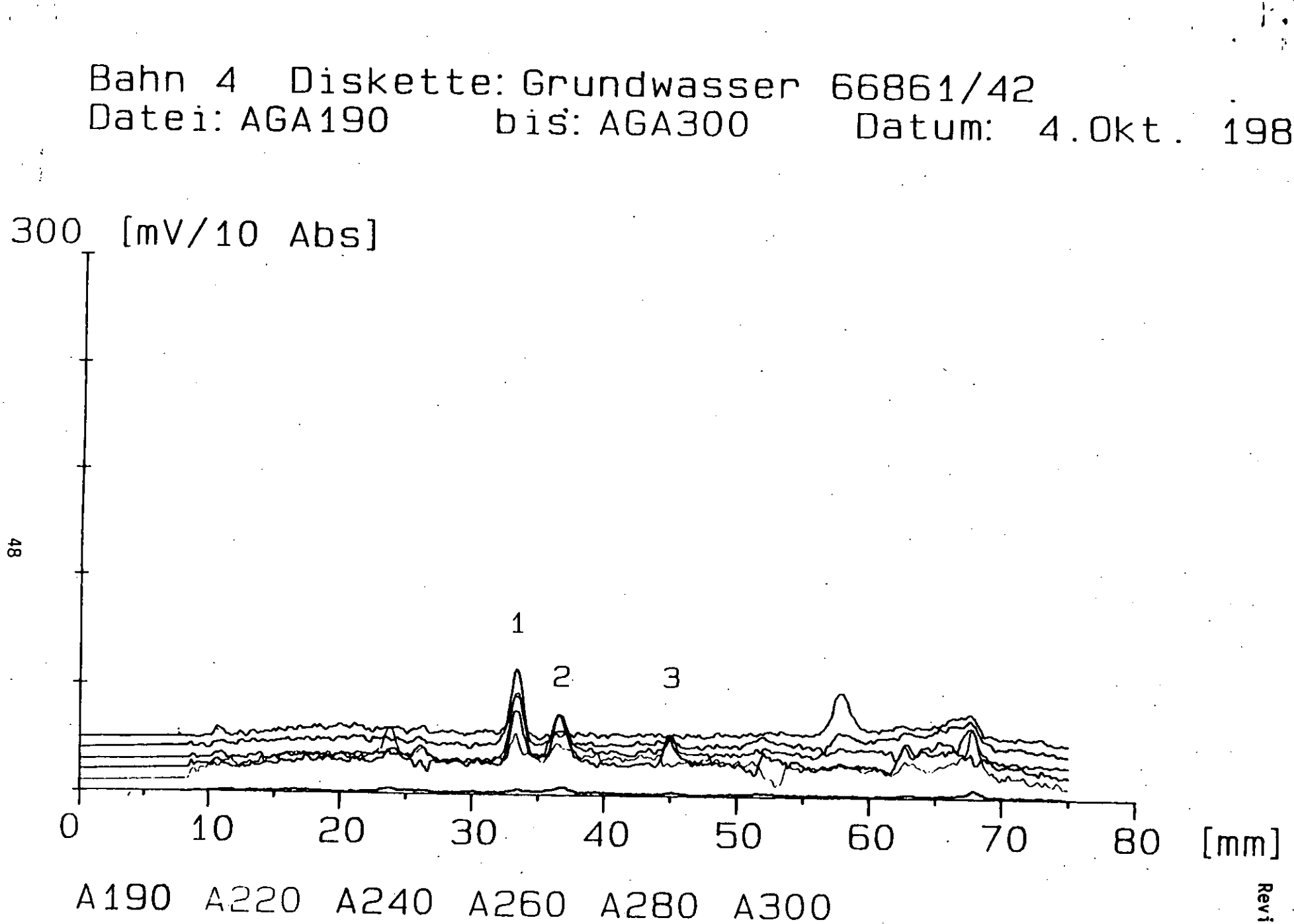


FIGURE 15. HPTLC CHROMATOGRAM OF DPX-M6316 (1), DPX-F5384 (2), AND DPX-L5300 (3) CALIBRATION STANDARD USING ALTERNATIVE AMD CONDITIONS. Each peak represents 50 ng corresponding to 0.1 µg/L.

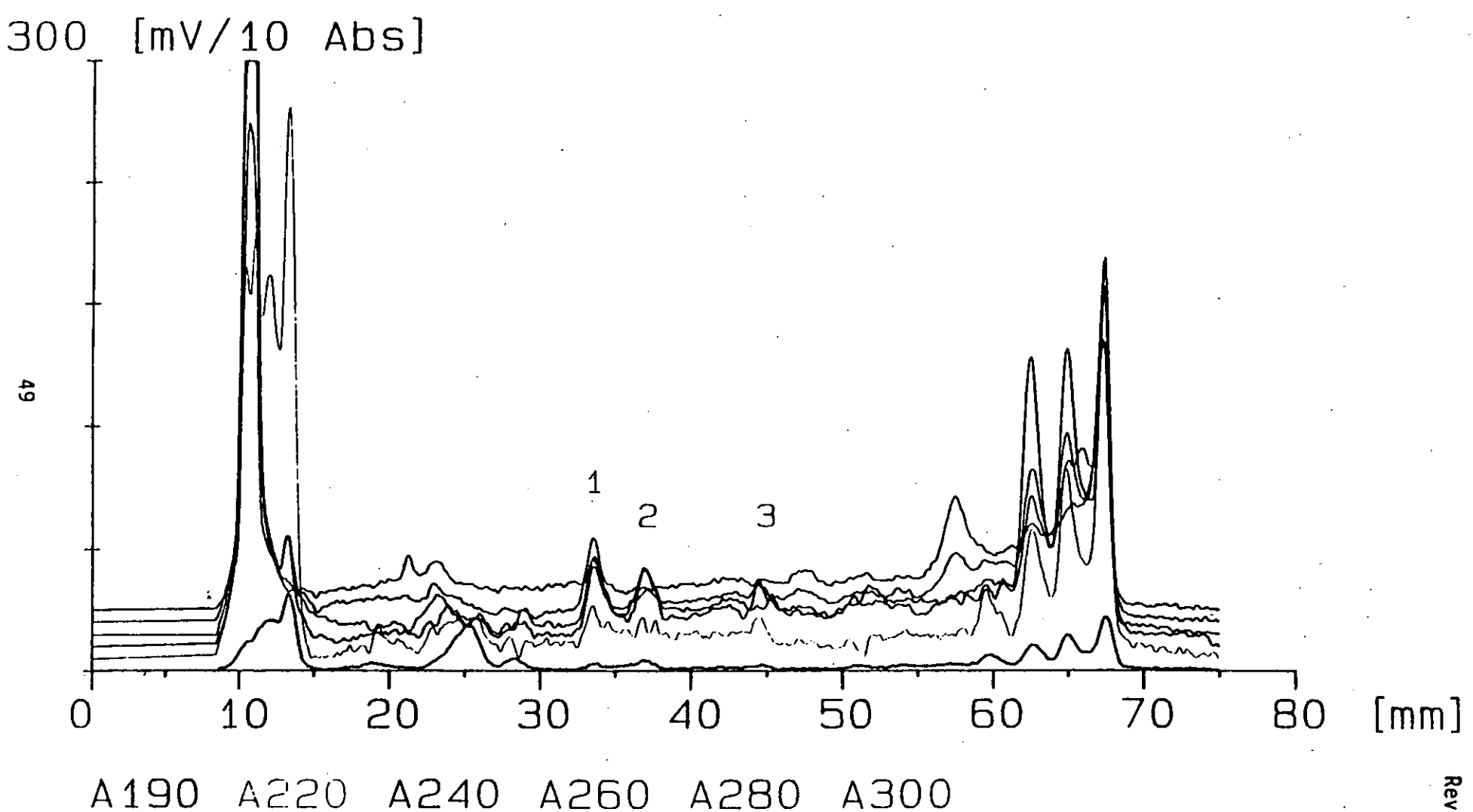


FIGURE 16. HPTLC CHROMATOGRAM OF REAGENT WATER SPIKED WITH DPX-M6316 (1), DPX-F5384 (2), AND DPX-L5300 (3) AT 0.1 µG/L USING ALTERNATIVE AMD CONDITIONS

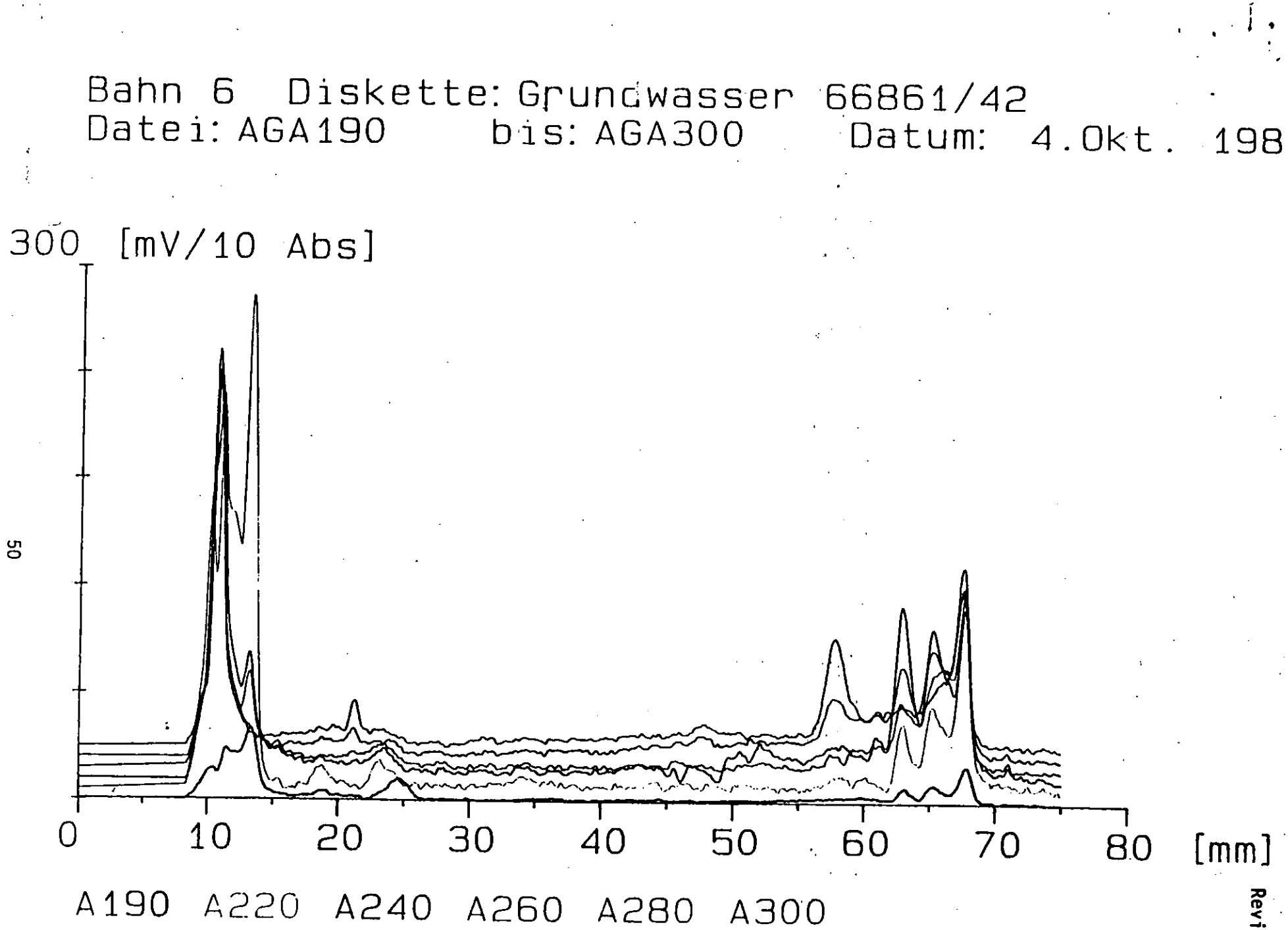


FIGURE 17. HPTLC CHROMATOGRAM OF REAGENT WATER BLANK USING ALTERNATIVE AMD CONDITIONS

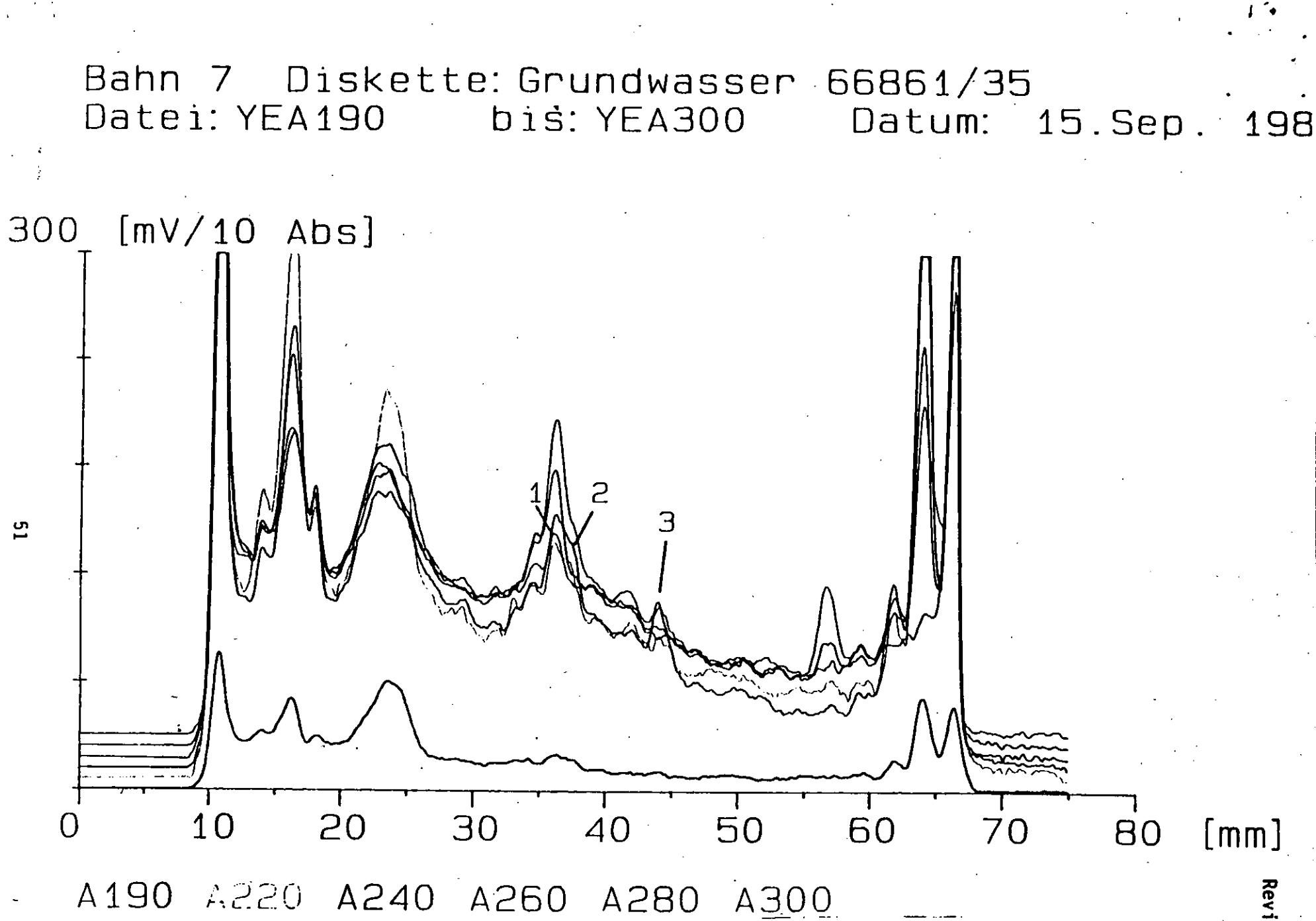


FIGURE 18. HPTLC CHROMATOGRAM OF TYPE 1 GROUNDWATER SPIKED WITH DPX-M6313 (1), DPX-F5384 (2), AND DPX-L5300 (3) AT 0.1 μG/L USING ALTERNATIVE AMD CONDITIONS

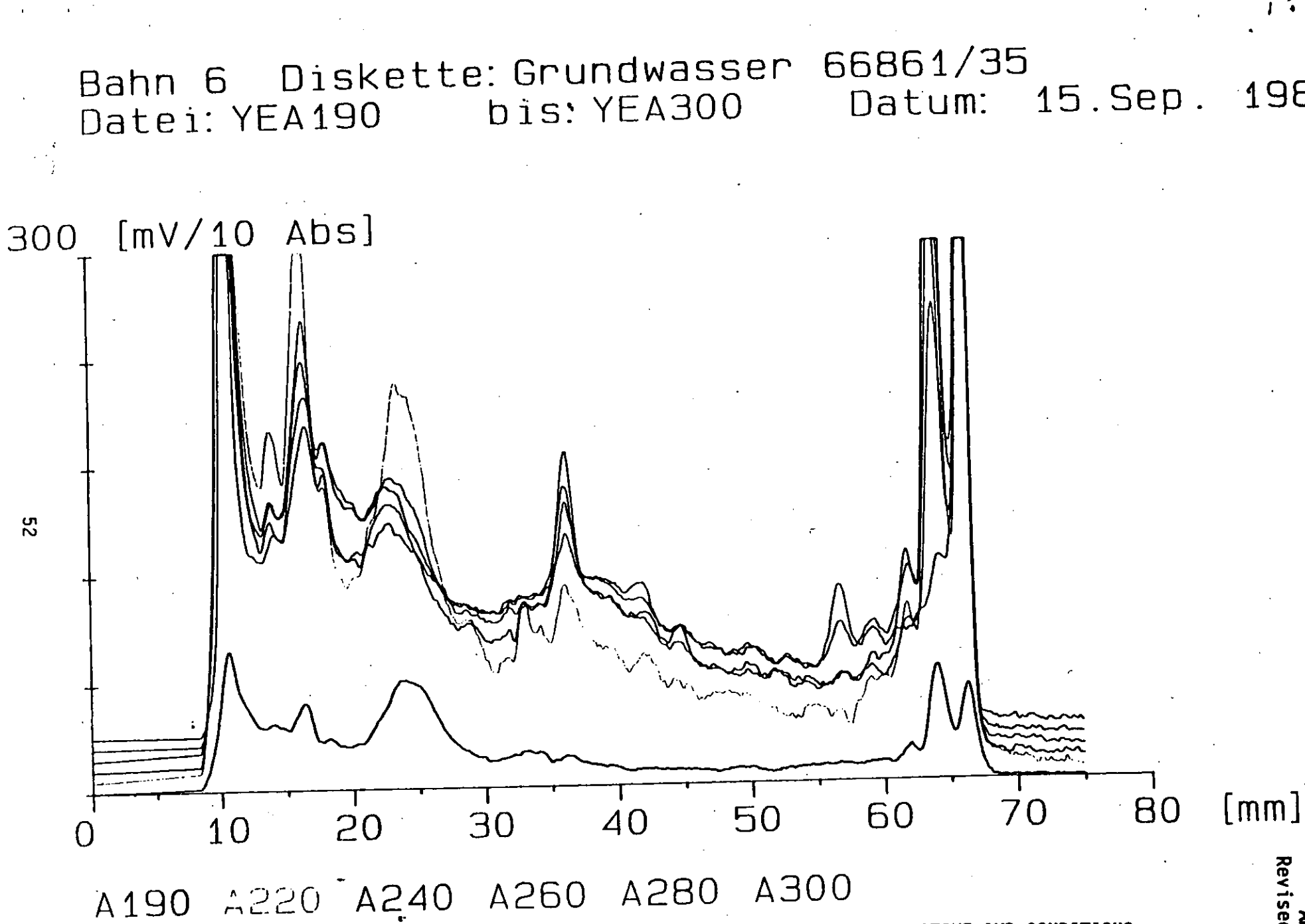


FIGURE 19. HPTLC CHROMATOGRAM OF TYPE 1 GROUNDWATER BLANK USING ALTERNATIVE AMD CONDITIONS

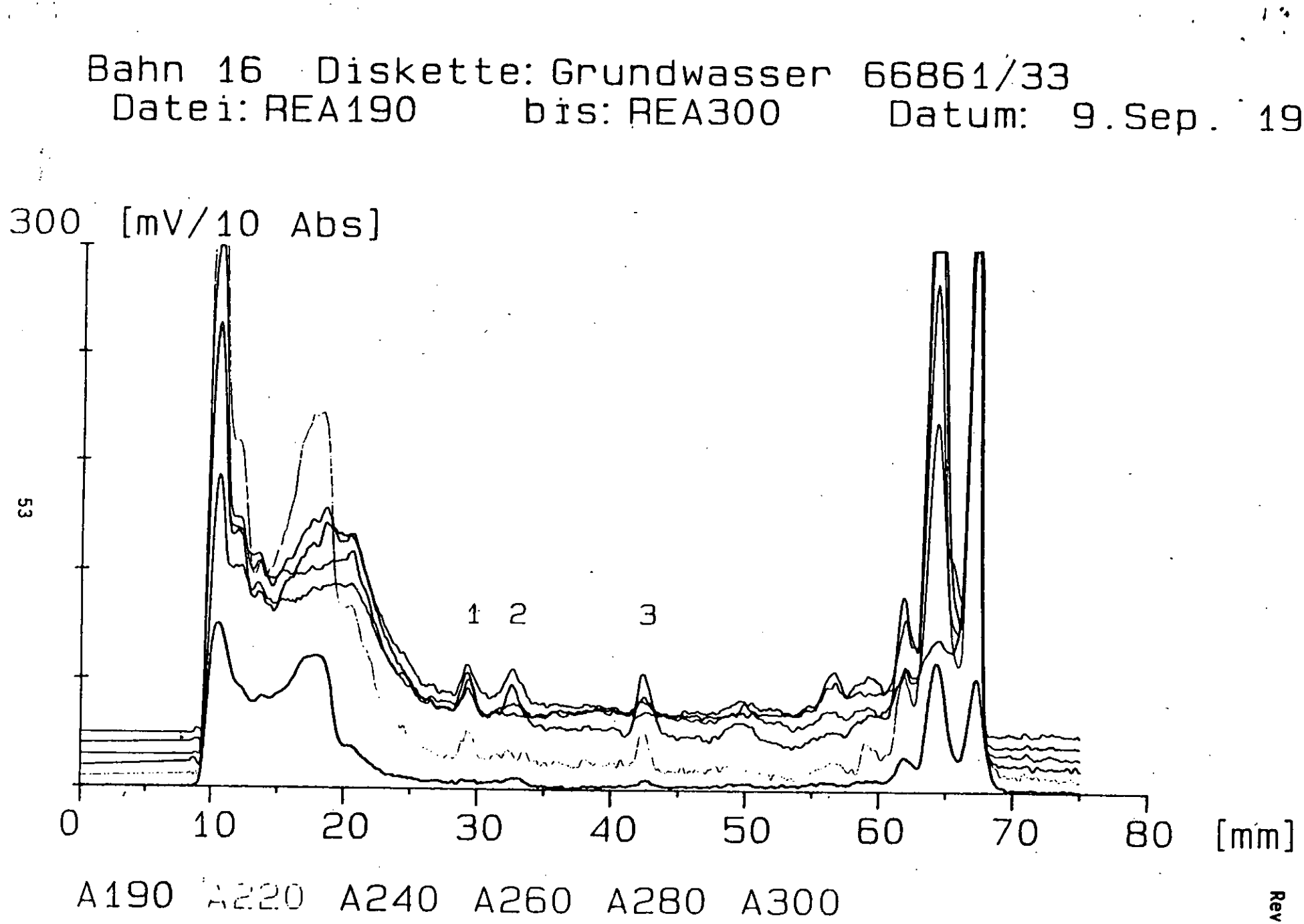


FIGURE 20. HPTLC CHROMATOGRAM OF TYPE 2 GROUNDWATER SPIKED WITH DPX-M6313 (1), DPX-F5384 (2), AND DPX-L5300 (3) AT 0.1 μG/L USING ALTERNATIVE AMD CONDITIONS

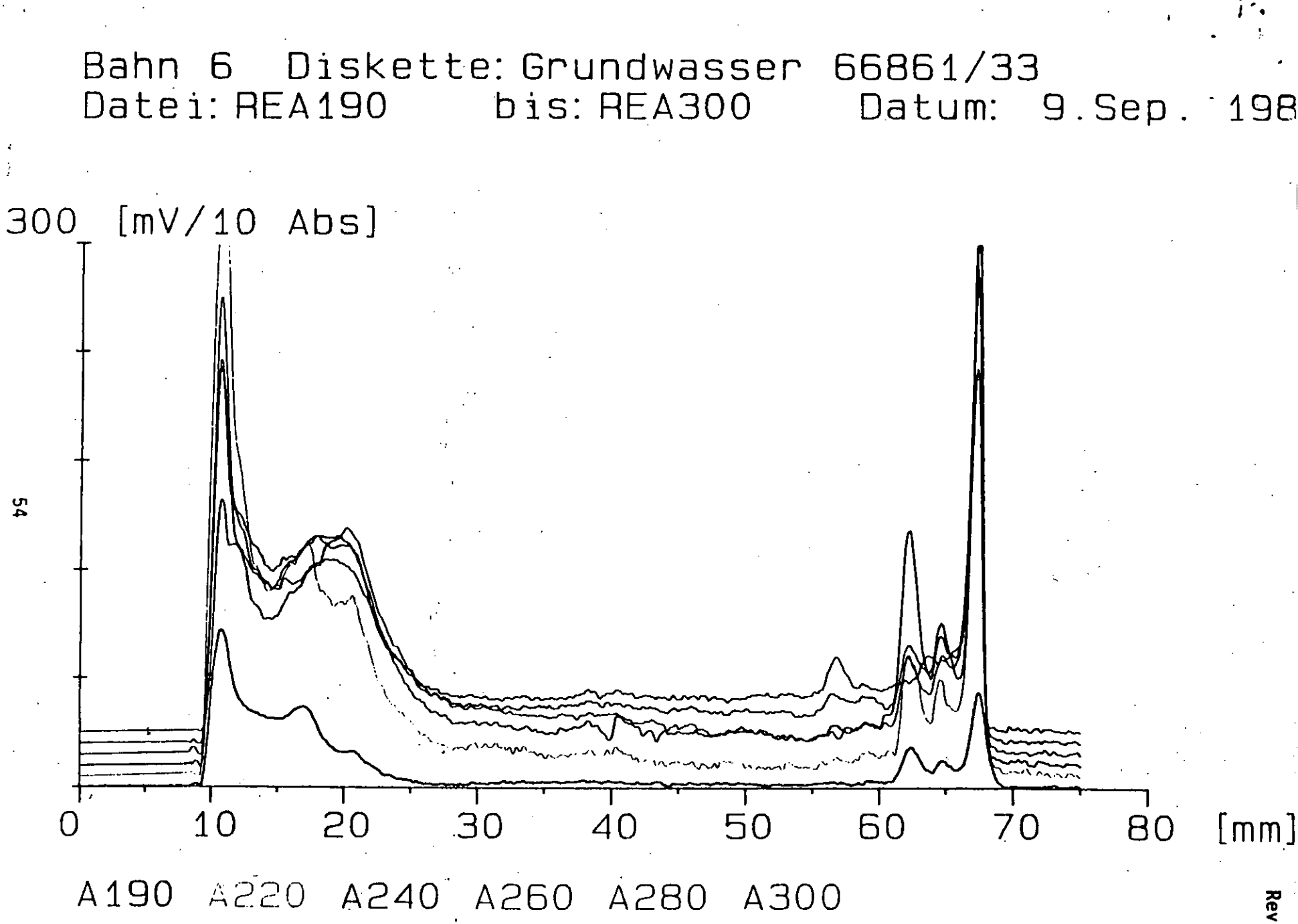


FIGURE 21. HPTLC CHROMATOGRAM OF TYPE 2 GROUNDWATER BLANK USING ALTERNATIVE AMD CONDITIONS

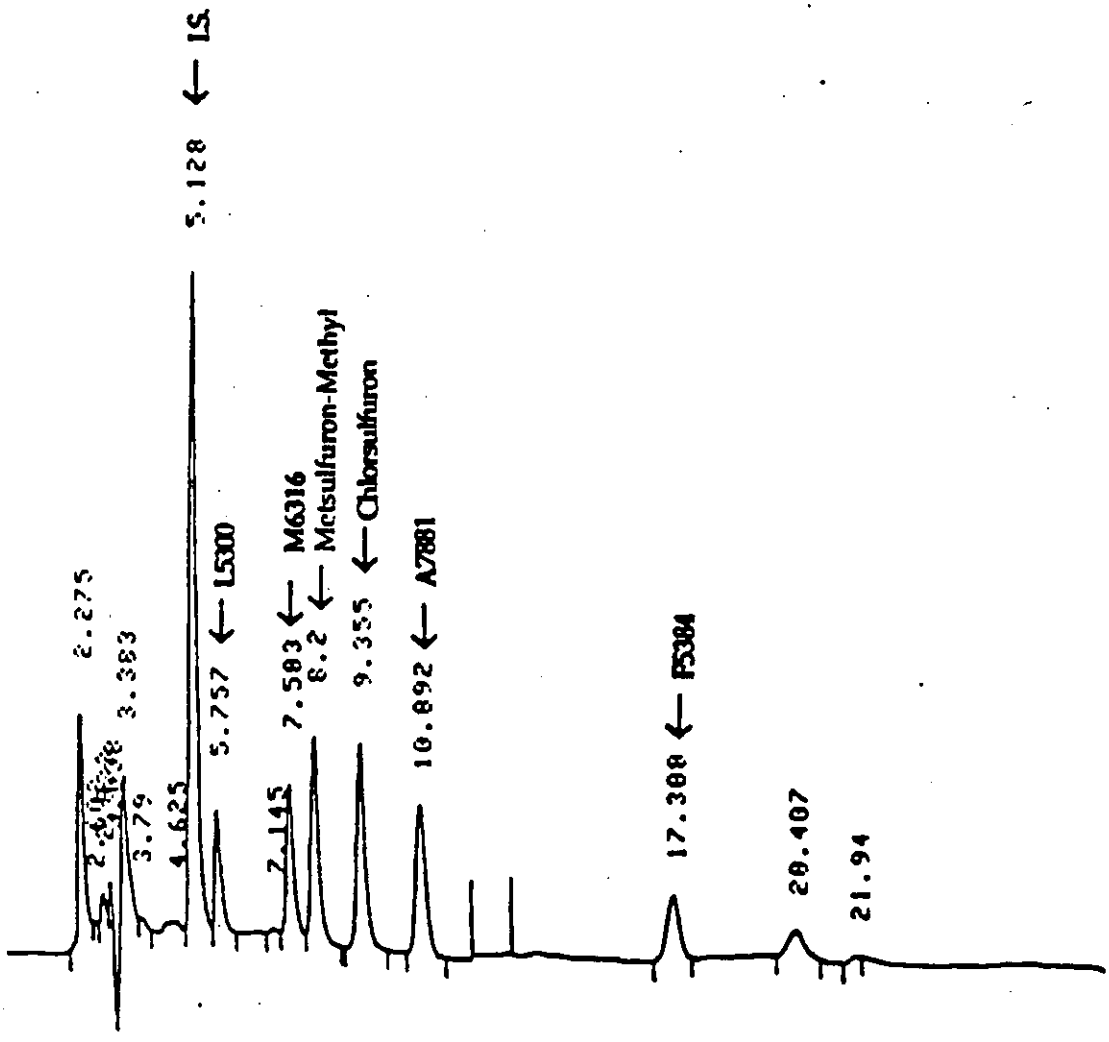


FIGURE 22. HPLC CHROMATOGRAM OF CALIBRATION STANDARD REPRESENTING 0.1 µG/L

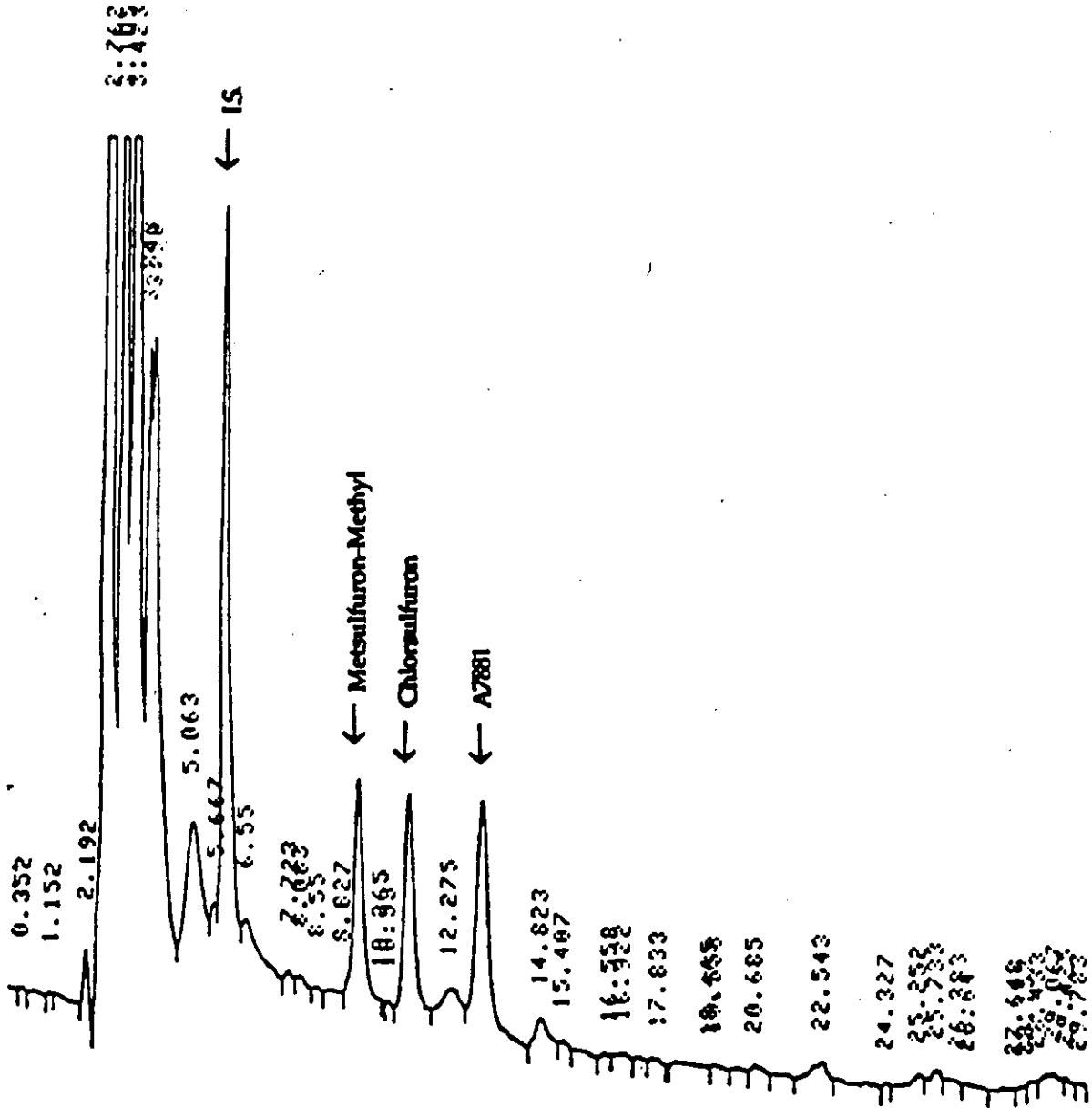


FIGURE 23. HPLC CHROMATOGRAM OF REAGENT WATER SPIKED WITH METSULFURON METHYL, CHLORSULFURON, AND DPX-A7881 AT 0.1 µG/L

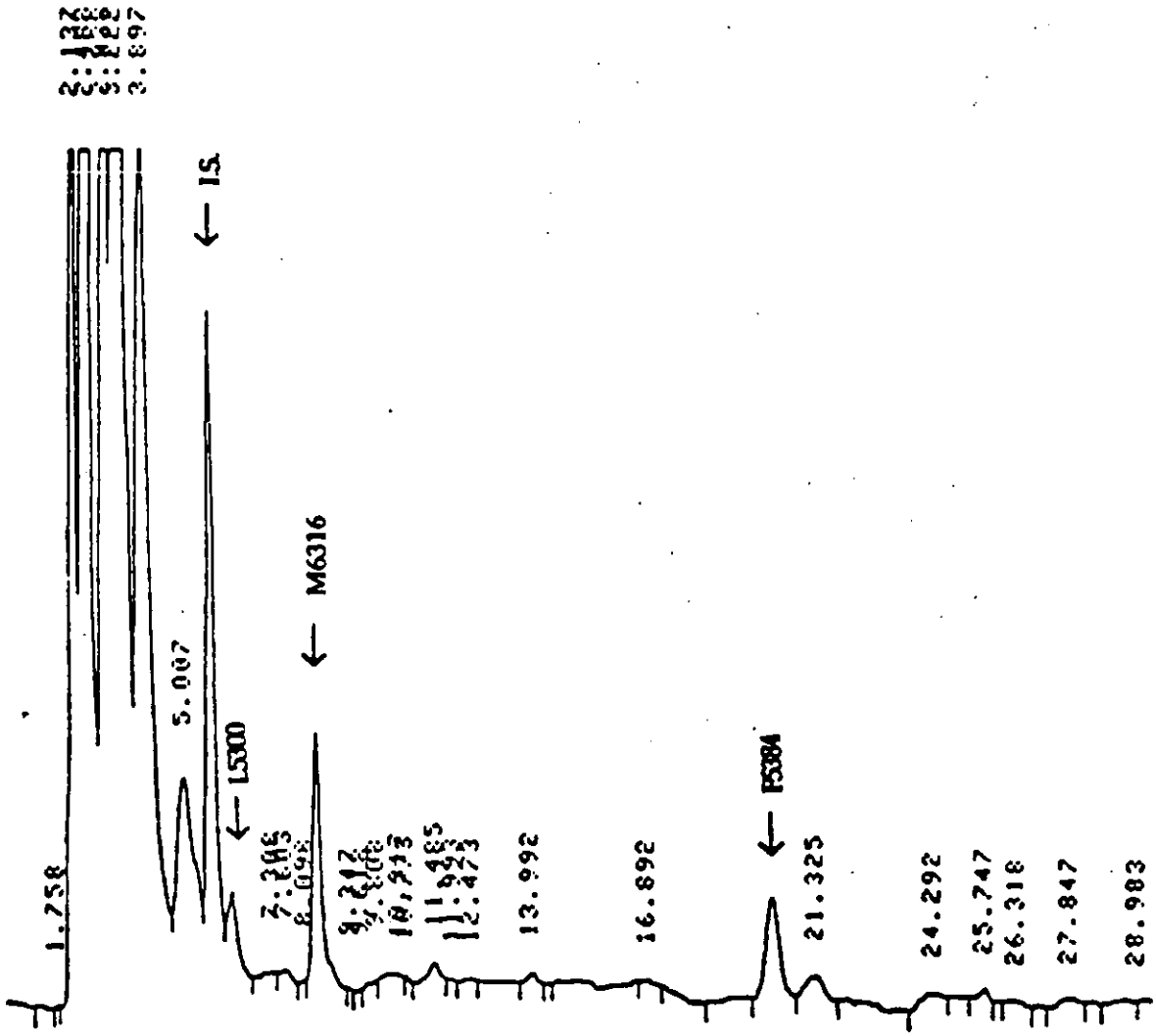


FIGURE 24. HPLC CHROMATOGRAM OF REAGENT WATER SPIKED WITH DPX-L5300, DPX-M6316, AND DPX-F5384 AT 0.1 µG/L

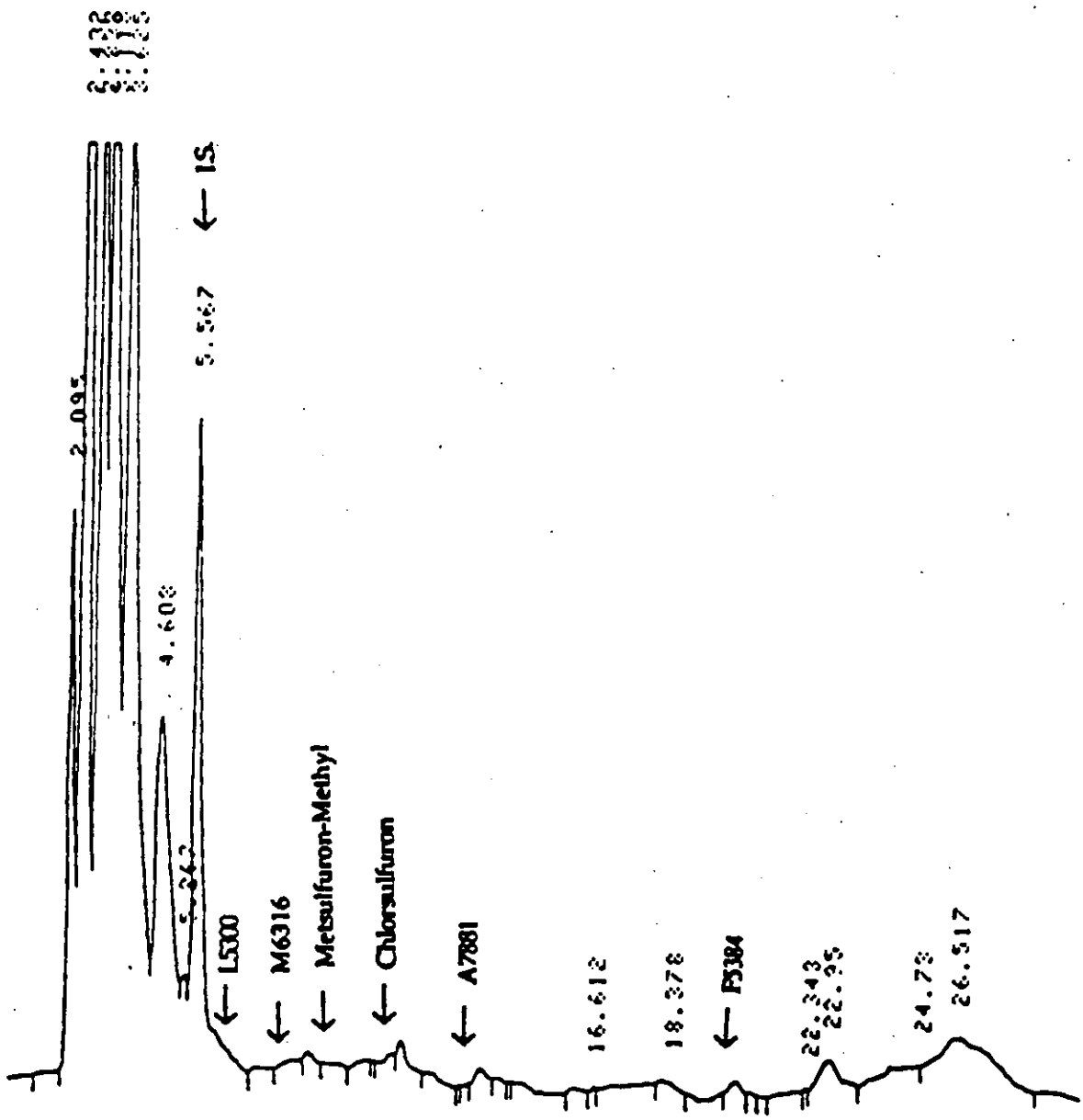


FIGURE 25. HPLC CHROMATOGRAM OF REAGENT WATER BLANK

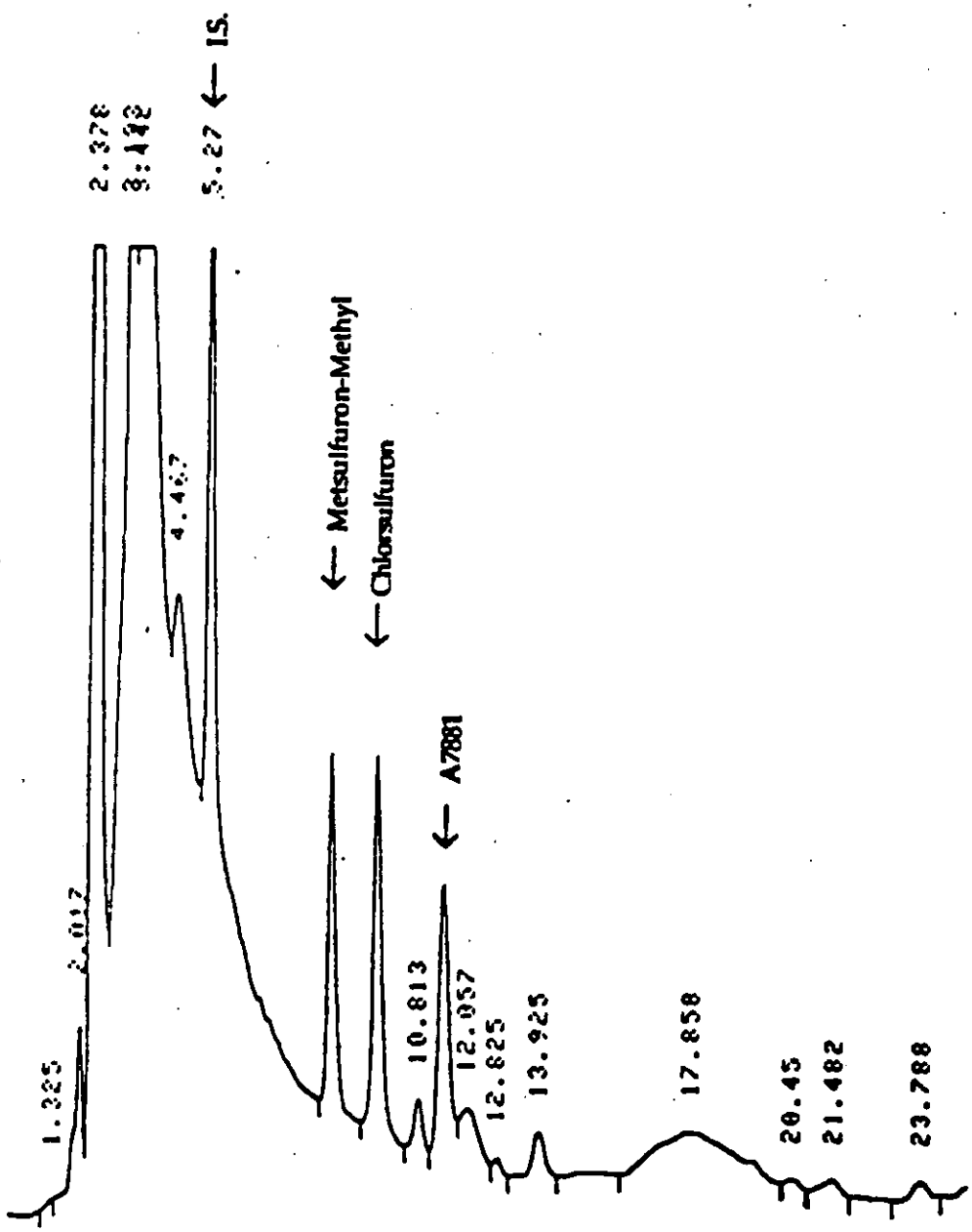


FIGURE 26. HPLC CHROMATOGRAM OF TYPE 1 GROUNDWATER SPIKED WITH METSULFURON METHYL, CHLORSULFURON, AND DPX-A7881 AT 0.1 µG/L

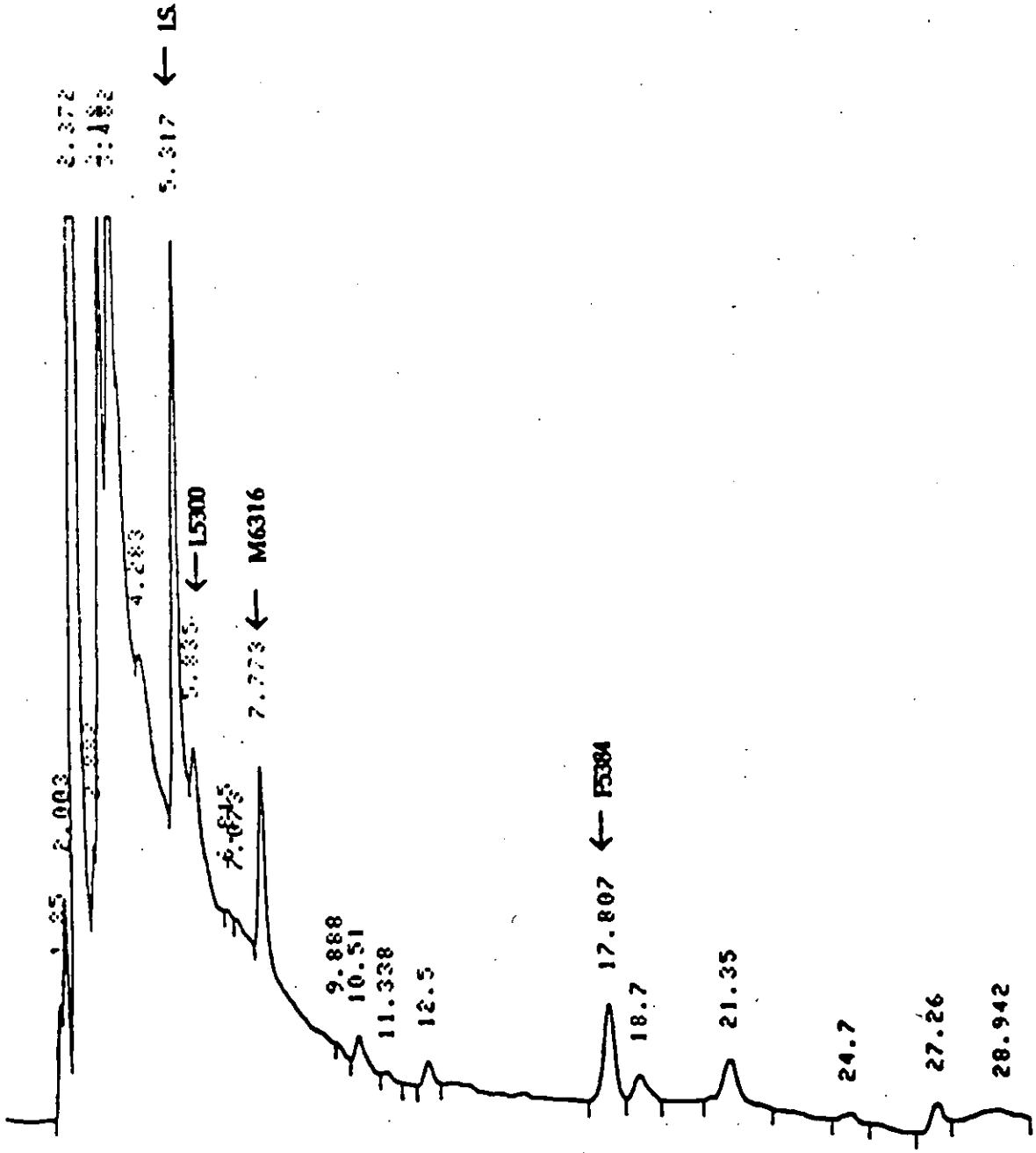


FIGURE 27. HPLC CHROMATOGRAM OF TYPE 1 GROUNDWATER SPIKED WITH DPX-L5300, DPX-M6316, AND DPX-F5384 AT 0.1 µG/L

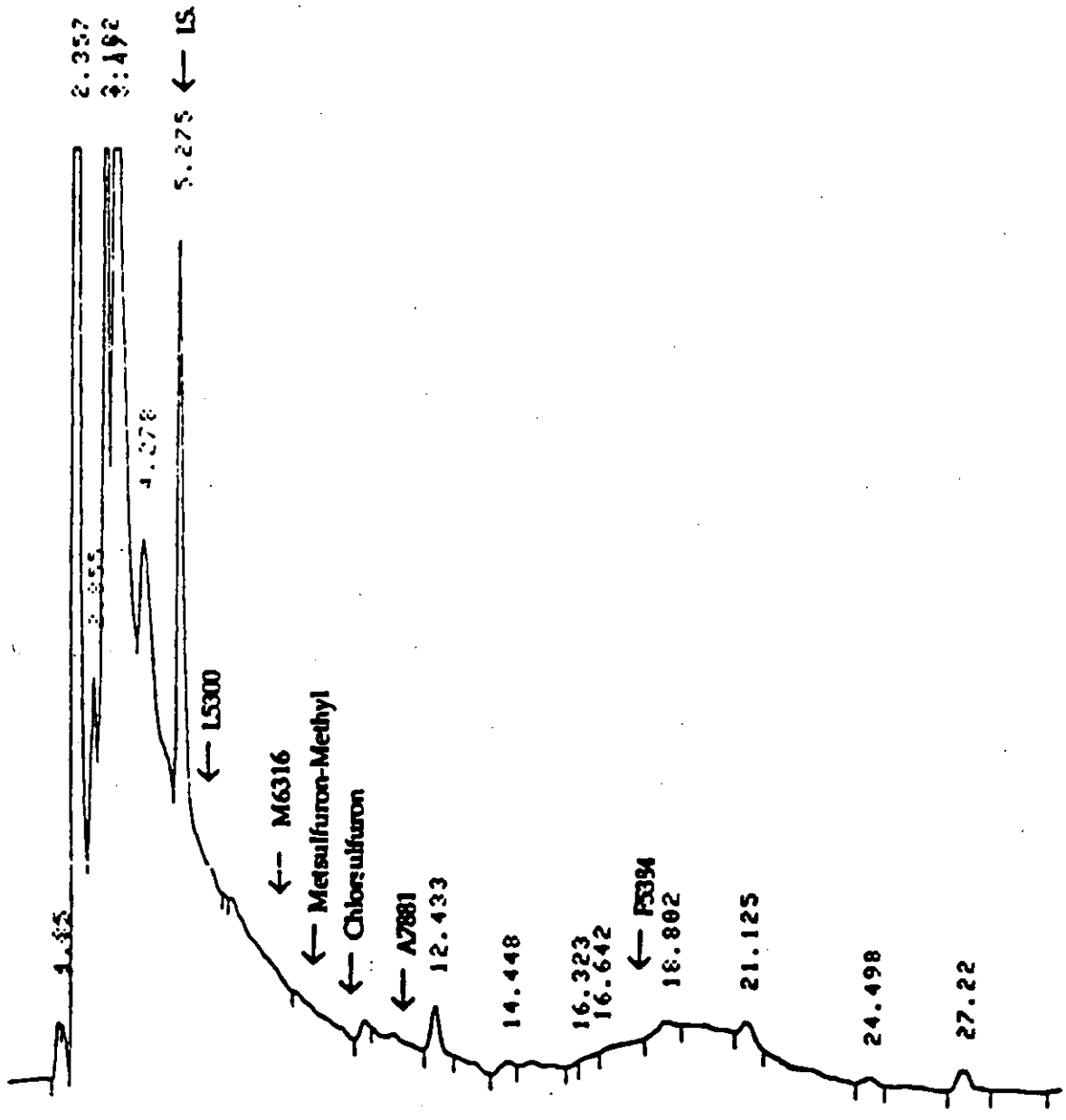


FIGURE 28. HPLC CHROMATOGRAM OF TYPE 1 GROUNDWATER BLANK

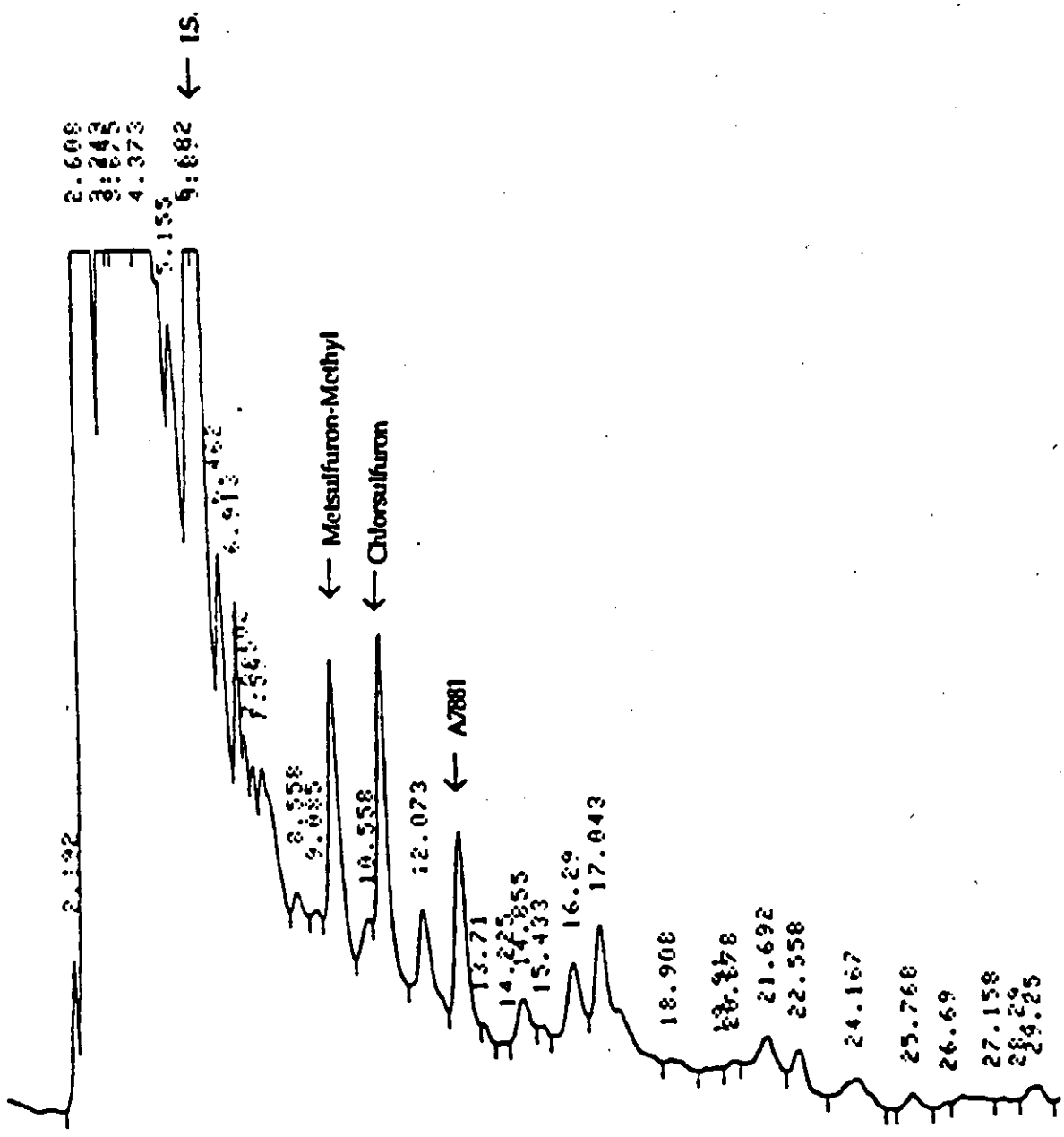


FIGURE 29. HPLC CHROMATOGRAM OF TYPE 2 GROUNDWATER SPIKED WITH METSULFURON METHYL, CHLORSULFURON, AND DPX-A7881 AT 0.1 µG/L

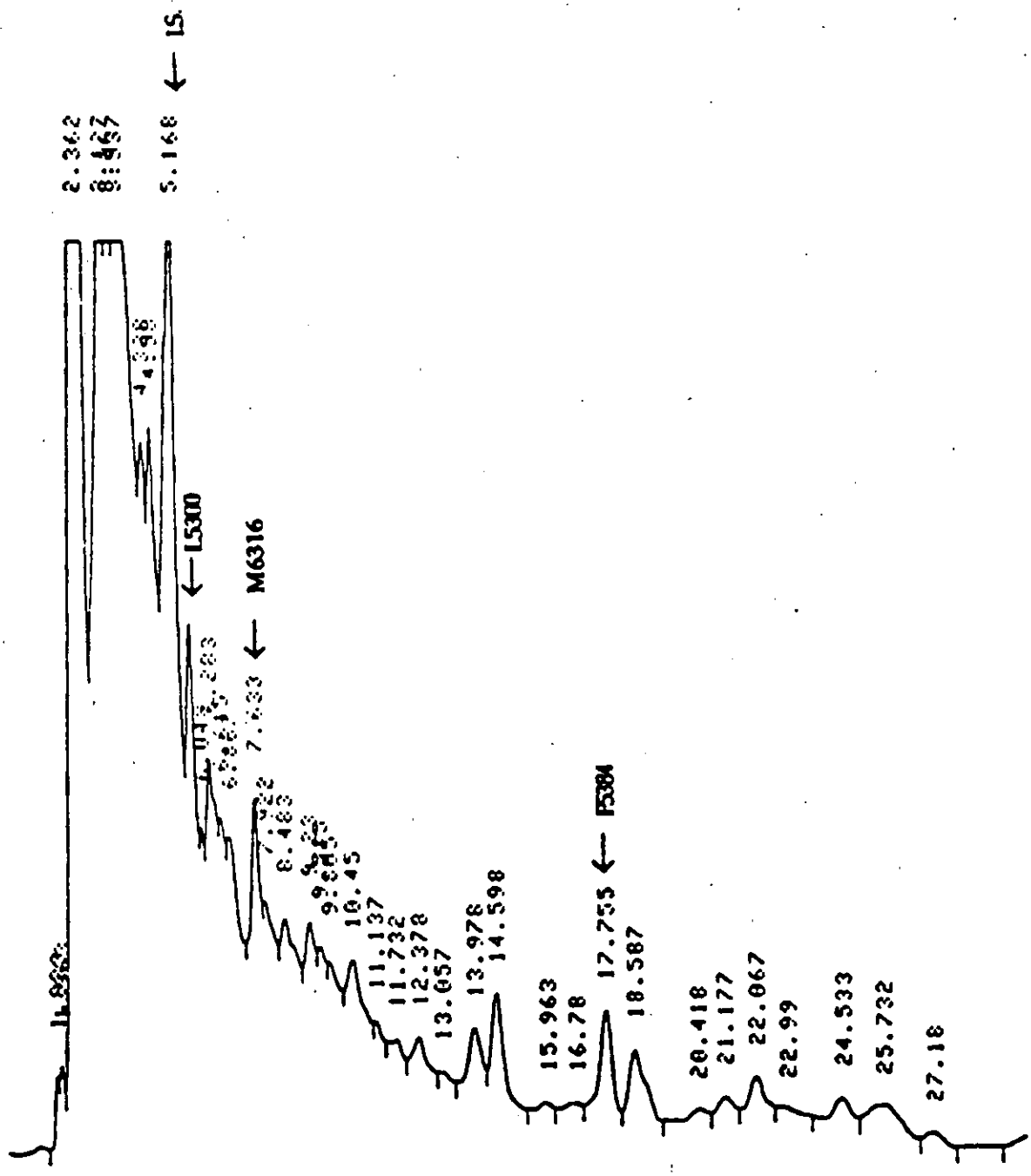


FIGURE 30. HPLC CHROMATOGRAM OF TYPE 2 GROUNDWATER SPIKED WITH DPX-L5300, DPX-M6316, AND DPX-F5384 AT 0.1 µG/L

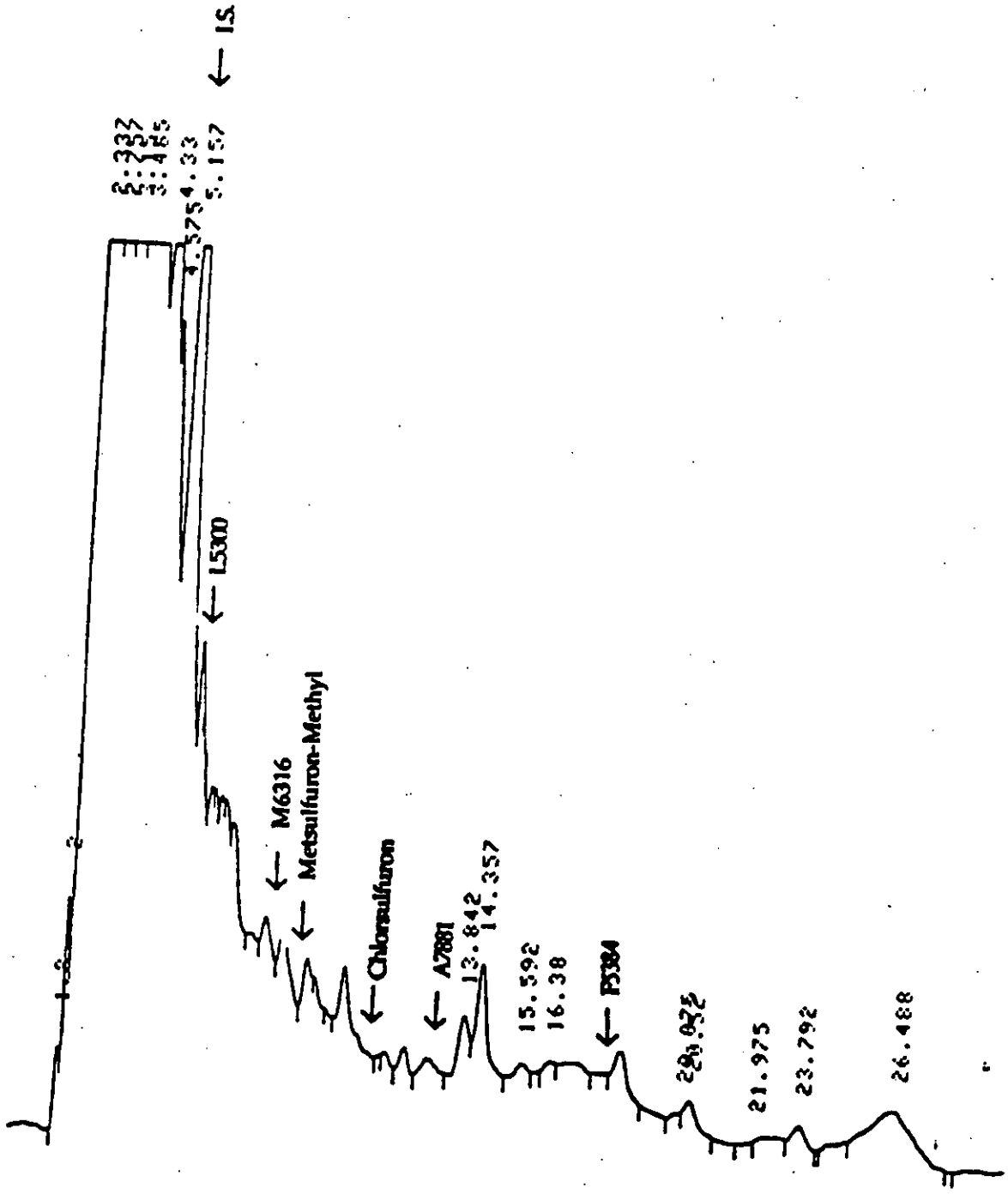


FIGURE 31. HPLC CHROMATOGRAM OF TYPE 2 GROUNDWATER BLANK