

**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)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:

<u>Analyte</u>	<u>Molecular Formula</u>	<u>Chemical Abstracts Service Registry Number (CASRN)</u>
Chlorsulfuron	C <sub>12</sub> H <sub>12</sub> ClN <sub>5</sub> O <sub>4</sub> S	64902-72-3
DPX-A7881	C <sub>15</sub> H <sub>18</sub> N <sub>6</sub> O <sub>6</sub> S	-
DPX-F5384	C <sub>16</sub> H <sub>18</sub> N <sub>4</sub> O <sub>7</sub> S	83055-99-6
DPX-L5300	C <sub>15</sub> H <sub>17</sub> N <sub>5</sub> O <sub>6</sub> S	101200-48-0
DPX-M6316	C <sub>12</sub> H <sub>13</sub> N <sub>5</sub> O <sub>6</sub> S <sub>2</sub>	79277-27-3
Metsulfuron methyl	C <sub>14</sub> H <sub>15</sub> N <sub>5</sub> O <sub>6</sub> S	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  $120 \pm 5^{\circ}\text{C}$ .
- 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- $\mu$ L 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  $\mu$ m 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  $\mu$ m 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  $\geq 96\%$ ). 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:

<u>Standard Material</u>	<u>Solvent</u>	<u>mg/mL</u>
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  $-20^{\circ}\text{C}$  protected from light.
- 4.9 FORTIFICATION SOLUTION (1  $\mu\text{g/mL}$ ) -- 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  $\text{pH } 6 \pm 0.2$  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  $\text{pH} < 6$  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  $5 \pm 0.1$  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  $\mu$ L.
- 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  $\mu$ L of 1  $\mu$ g/mL calibration solution and 5, 8, 10 and 30  $\mu$ L, respectively of 10  $\mu$ g/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):

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

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  $\mu$ L 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  $\mu$ L 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  $\mu\text{g/L}$ .

- 6.4.4 Inject 50  $\mu\text{L}$  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 ( $R_1$ ) using the equation:

$$R_1 = \frac{100 \cdot (C_c - C_b)}{C_s}$$

- 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:

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

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:

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

**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/ $\mu$ L  
 Volume Applied: 50% volume of sample extract  
 Calibration: 20 ng to 300 ng  
 Applied in 20  $\mu$ L of standard calibration solution (1  $\mu$ g/mL) and 5  $\mu$ L to 30  $\mu$ L of standard calibration solution (10  $\mu$ g/mL)

**Chromatographic Development (Camag AMD - System):**

Gradient: 30-step  
 Reservoir 1: Methanol/dichloromethane (10:90), 0.01% NH<sub>3</sub>  
 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 <sub>3</sub>
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.

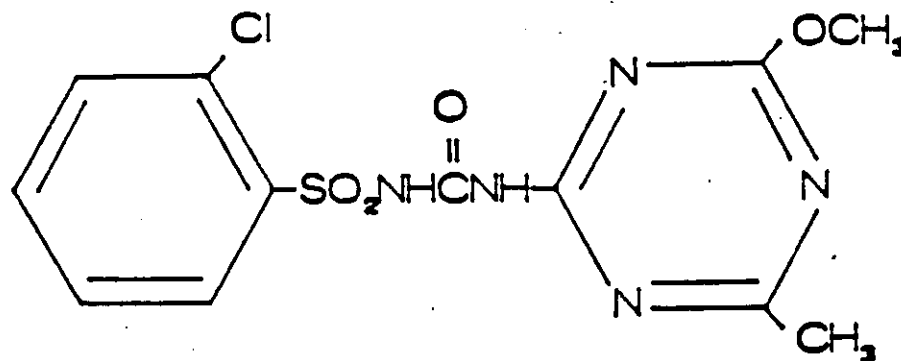
**TABLE 1. RETENTION TIME AND CHROMATOGRAPHIC CONDITIONS  
(Continued)**

(b) HPLC Conditions:

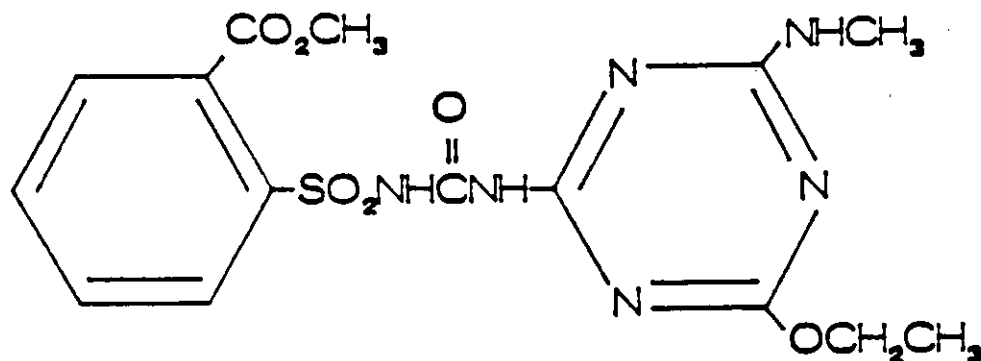
Column:	250 mm x 4.6 mm I.D. DuPont Zorbax ODS (5 $\mu$ m)
Mobile Phase:	Linear gradient from 30:70 acetonitrile:water to 60:40 acetonitrile:water in 20 minutes. Hold at 60:40 acetonitrile:water for 20 minutes. <u>Note:</u> acetonitrile and water both contain 0.01 percent (v/v) acetic acid.
Flow Rate:	0.5 mL/min
Injection Volume:	50 $\mu$ L
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.

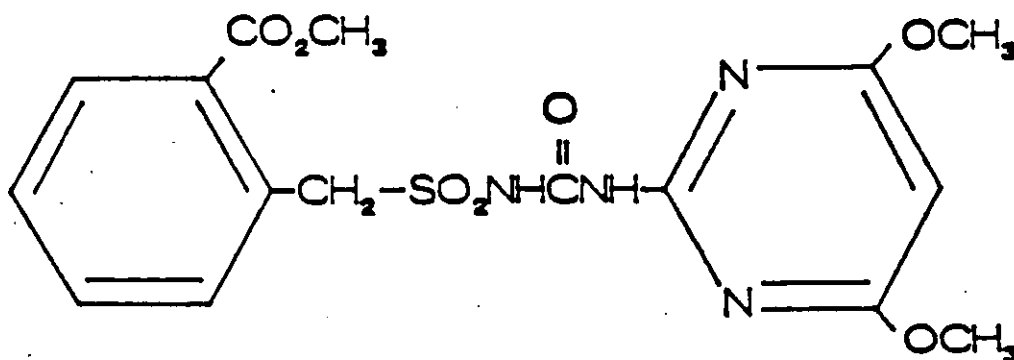
(d) HPLC internal standard.



**Chlorsulfuron**

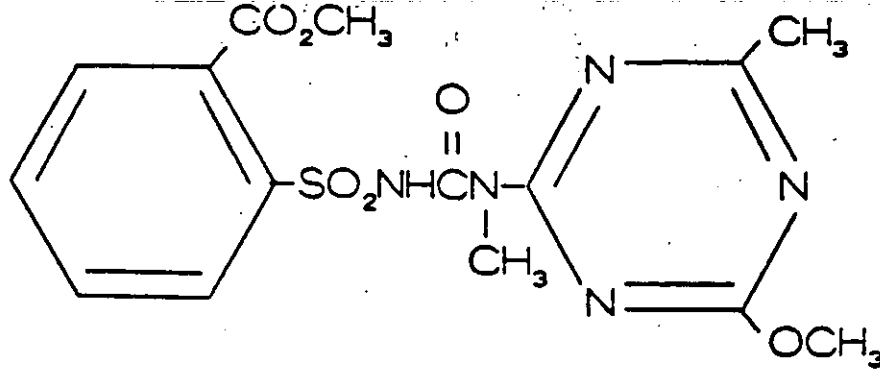


**DPX-A7881**

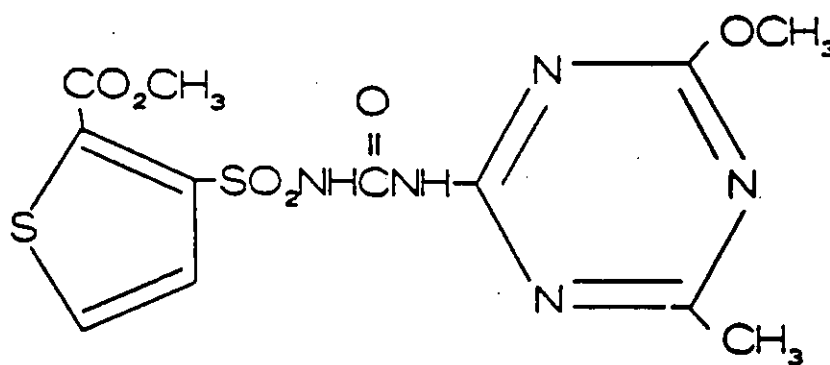


**DPX-F5384**

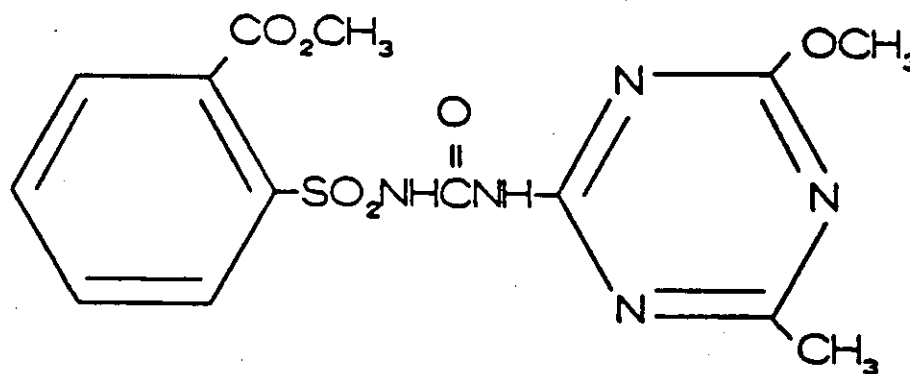
FIGURE 1. ANALYTE STRUCTURES



**DPX-L5300**



**DPX-M6316**



**Metsulfuron Methyl**

FIGURE 1. (Continued)

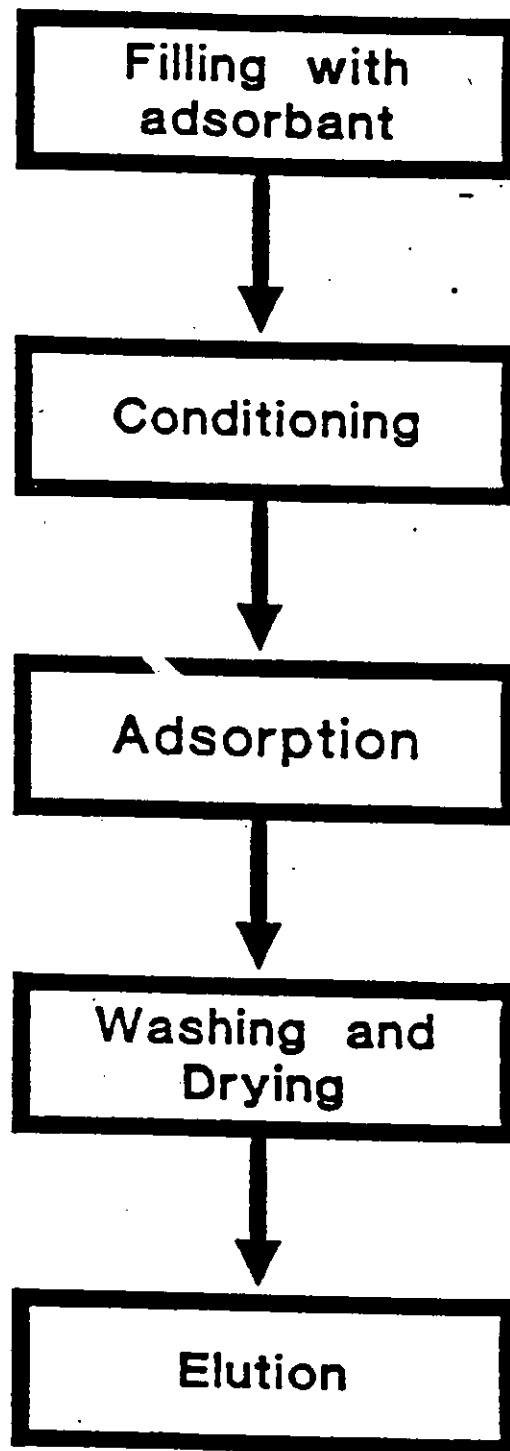


FIGURE 6. SPE PROCEDURE