SUMMARY

Diflubenzuron is extracted with ethyl acetate from filtered water acidified with formic acid. After drying and concentration of the organic extract, the extract is analyzed for diflubenzuron by High Performance Liquid Chromatography (HPLC) with UV detection at 254 nm. The Limit of Quantitation (LOQ) is 1.0 µg/L for diflubenzuron.

4-Chlorophenylurea (CPU) and 2,6-difluorobenzoic acid (DFBA) are extracted from water by partitioning an acidified water sample with ethyl acetate. The ethyl acetate extract is dried with Na_2SO_4, concentrated to dryness and taken up in methanol:water. Analysis for CPU is performed by HPLC with UV detection at 254 nm. This method has demonstrated a Limit of Quantitation (LOQ) of 1 µg/L for CPU in water. Analysis for DFBA is performed by HPLC/MS. This method has demonstrated an LOQ of 5 µg/L for DFBA in water.

The confirmatory method presented in this report for diflubenzuron may be used to confirm the presence of diflubenzuron when determined with Uniroyal Method AC-7006, where the LOQ is 0.1 µg/L.

I. ANALYTICAL METHOD - DIFLUBENZURON

I-A. MATERIALS

Solvents, reagents and instruments mentioned in this report are those used to perform the work discussed. Equivalent materials may be substituted as required.

I-A.1. Equipment

Glassware and Miscellaneous Equipment

- Balance
- Filter paper, Whatman #4 or Falcon 0.22 µM cellulose acetate
- Filter flask
- Flasks, round bottom, 50 mL, 250 mL
- Funnels, Büchner
- Graduated cylinders, various sizes
- Pasteur pipettes
- pH paper
- Separatory funnels, 500 mL
- Syringes, microliter, 500 µL, 100 µL
- Thermometer, mercury

- Vacuum evaporator, Büchi Model RE111 with temperature controlled bath, Brinkmann Instruments, Burlingame, CA
- Vials, glass with teflon-lined cap
- Volumetric flasks, various sizes
- Volumetric pipettes, various sizes

I-A.2. Reagents/Supplies

Solvents/Reagents - All solvents are HPLC grade unless noted otherwise.

- Acetonitrile
- Ethyl acetate
- Formic Acid
- 1,4-Dioxane
- Water
- Sodium sulfate, anhydrous, ACS Grade, Fisher Scientific

I-A.3. Analytical Standards

Analytical standards of diflubenzuron are available from Uniroyal Chemical Company, Inc. division of Crompton Corporation. Standards are kept frozen. A typical Certificate of Analysis (COA) for the standard is shown in Appendix 1. The COA shows the structure of the standard and a typical purity. Appendix 1 also contains an MSDS for the standard. One should obtain an MSDS for the solvents directly from their suppliers.

I-B. SAFETY AND HEALTH

This method should be performed by trained chemical personnel. Hazards associated with the chemicals analyzed by this analytical method are shown in the MSDS in Appendix 1.

I-C. METHODOLOGY - DIFLUBENZURON

I-C.1. Principle of the Method

A water sample is filtered through 0.22 micron cellulose acetate to remove particulate material. The pH of the water is adjusted to ~3 with neat formic acid, and the solution is partitioned with ethyl acetate. The organic fraction is then dried with Na_2SO_4, filtered, concentrated to dryness, taken up in acetonitrile/water/1,4-dioxane and analyzed by High Performance Liquid Chromatography (HPLC). Detection of the diflubenzuron analyte is by ultraviolet (UV) at 254 nm.

I-C.2. Types of Water Matrix

This method is predicted to be applicable to most water types. The results described in this report were obtained using water from pond or rice paddy sources. The analytical method worked well on all these types and is expected to perform equally well on any type of water including ground water, surface water, etc.

I-C.3. Sample Processing

All samples are received from the field frozen and are stored frozen at approximately -20 °C. They must remain frozen until used for fortification and/or analysis. Thawed samples are stored at refrigerator temperatures (4-10 °C) when not in use. Water samples are filtered through a Falcon filter system or a Buchner funnel fitted with a Whatman #4 filter paper into a 1000 mL flask prior to extraction.

I-C.4. Extraction Method

Fortify appropriate water samples at this point.

1. Thaw and filter water (Falcon 0.22 micron cellulose acetate, or Whatman #4).
2. Adjust to pH ~3 with neat formic acid .
3. Partition 200 mL aliquot of acidified, filtered water against ethyl acetate, 3 x 40 mL.
4. Dry combined EtOAc extract through a bed of Na_2SO_4.
5. Rinse Na_2SO_4 and separatory funnel with 15 mL EtOAc. Combine rinse with dried extract.
6. Concentrate dried EtOAc extract by rotary evaporation at 35 °C to approximately 2-3 mL.
7. Quantitatively transfer concentrate to smaller, secondary flask with approximately 2.5 mL EtOAc rinse.
8. Continue concentrating to dryness by rotary evaporation at 35 °C.
9. Re-dissolve residue in 3.0 mL acetonitrile/water/1,4-dioxane (45:45:10, v:v:v).

10. Analyze by HPLC/UV.

I-C.5. Fortifications

Preparation of Standards

A 0.5 mg/mL stock solution of the diflubenzuron (DFB) reference standard is prepared in acetonitrile as described under the "Method of Calculations" (section I-H.1). Working solutions are made by diluting the stock standard to prepare fortification and calibration standard solutions, as described below. Microliter syringes, volumetric pipettes and volumetric flasks are used throughout.

Final Concentration (µg/mL)	Volume of DFB Solution (mL)	Volume acetonitrile (mL)
10	2.0 mL of the 0.5 mg/mL Stock Solution	100.0
1	5.0 mL of the above 10 µg/mL Stock Solution	50.0

Fortification Procedure

Fortify portions of water (100 mL) at 5 µg/L concurrent with sample analysis. The untreated water was filtered and fortified at 1,5, and 10 µg/L then acidified to pH ~3 with concentrated formic acid with sample analysis as follows:

Fortification Level (µg/L)	Volume of Standard in acetonitrile	Water Volume (mL)
1	100 µL of a 1.0 µg/mL standard	100
5	50 µL of a 10.0 µg/mL standard	100
10	100 µL of a 10.0 µg/mL standard	100

I-C.5.1. Preparation of Linearity Standards

Linearity standards for DFB in water are prepared by dilution of the 10 µg/mL acetonitrile stock solution. For the calibration curve, the final concentrations are 0.025, 0.05, 0.1, 0.2, 0.4, and 0.6 µg/mL of DFB, where all dilutions are made with ACN:H_2O:dioxane (45:45:10, v:v:v). A standard preparation is provided below. A calibration curve is generated with each sample set to determine linearity and to quantitate DFB (see "Methods of Calculation" in Section I-H.1. for an example). At least five concentrations should be used to construct the calibration curve.

DFB Standard	Dilution (in acetonitrile: water:1,4-dioxane (45:45:10))
0.6 µg/mL	6 mL of 10 µg/mL ACN stock diluted to 100 mL
0.4 µg/mL	4 mL of 10 µg/mL ACN stock diluted to 100 mL
0.2 µg/mL	2 mL of 10 µg/mL ACN stock diluted to 100 mL
0.1 µg/mL	1 mL of 10 µg/mL ACN stock diluted to 100 mL
0.05 µg/mL	0.5 mL of 10 µg/mL ACN stock diluted to 100 mL
0.025 µg/mL	0.25 mL of 10 µg/mL ACN stock diluted to 100 mL

A minimum of two fortification spikes are run for each sample set. This is approximately one spike for every three treated samples.

I-C.6. Clean-up

A clean-up procedure has not been required to obtain satisfactory analysis of diflubenzuron. However if an extremely dirty water sample is encountered, an additional clean-up may be carried out by C-18 SPE column chromatography as described below:

1. Reconstitute the organic fraction in 5 mL ACN:H_2O:dioxane (45:45:10) with the aid of sonication.

2. Set up appropriate number of 6cc/1-g Varian C18 SPE cartridges. Pre-condition each with one cartridge volume (6 mL) of MeOH, followed by 1 cartridge volume of HPLC grade H_2O and 1 cartridge volume of ACN:H_2O:1,4-dioxane (45:45:10, v:v:v).

3. Transfer 2 mL of sample to the pre-conditioned column.

4. Elute analytes as follows :

ACN:H_2O	Elution vol. (mL)	Disposition
(40:60)	6	discard
(60:40)	3	discard
(60:40)	3	collect as fraction

A preliminary trial may be required to determine the precise volumes and ratios of ACN:H_2O for a particular interference.

I-C.7. Derivatization

No derivatization is required for the method used for diflubenzuron described in this report.

I-D. INSTRUMENTATION

HPLC Components:

LC Pump	HP 1050
Detector	HP Series Variable Wavelength UV/Vis
Integrator	HP 2296 Series II or Dionex/Peaknet Chromatography System
Autosampler	HP 1050 Autosampler or Biorad or Cygnet ISCO fraction collector
Software	Dionex/Peaknet Chromatography System

HPLC Method for Water Sample Analysis

Column:	C8 Zorbax (4.6mm ID x 25cm)
Solvent A:	Acetonitrile(ACN):Water(H_2O):dioxane (45:45:10)
Solvent B:	Acetonitrile:Water:dioxane (85:5:10)
Injection volume:	20 µL
Flow Rate:	1.5 mL/minute
Detection:	UV 254 nm

Step	Time (min)	% A	% B
0	0	100	0
1	15.5	100	0
2	20	0	100
3	20.5	0	100
4	30	100	0

I-E. CONFIRMATORY TECHNIQUE

A confirmatory method involving liquid chromatography and mass spectrometry (LC/MS) is used to authenticate diflubenzuron as the material giving rise to the HPLC peak at the expected retention time. Diflubenzuron is confirmed by negative ion Atmospheric Pressure Chemical Ionization (NI/APCI) mass spectrometry. The chlorine ^{37}Cl isotope mass occurring at m/e [M+2] is also diagnostic for diflubenzuron.

Material	Mode	Masses scanned	Ions monitored
Diflubenzuron	Negative ion	270.0-370.0	289 (291) [M-H]-HF 309 (311) M-H

An example of confirmation of diflubenzuron from a water sample by MS is presented in Appendix 3.

I-F. TIME REQUIRED FOR ANALYSIS

Analysis of water for diflubenzuron
- per set, including 2 controls, 2 fortifications and six treated samples

Extraction time -- approximately 6 hours
Analysis time -- approximately 24 hours

Assuming an automated data analysis and capture system, total time for one set is approximately two working days.

I-G. MODIFICATIONS OR POTENTIAL PROBLEMS

If interferences are encountered at the retention time of diflubenzuron, a clean-up procedure as described in section I-C.6 may be used.

I-H. METHODS OF CALCULATION

The residue data included the following statistical calculations: averages, standard deviations and linear regression analysis.

I-H.1. Calculation for Diflubenzuron Standard

Preparation of Stock Solution of Standard

$$\text{Volume of solvent (mL)} = \frac{(W) \times (P)}{(FC)}$$

where, W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (mg/mL)

I-H.2. Calculation of Recoveries

Recovery - Diflubenzuron

Linear regression formula for diflubenzuron peak area, calibration curve,

$$y = mx + b$$

where y = peak area
x = µg/mL DFB injected
m = slope
b = calibration intercept

The DFB concentration in fortified water was calculated as follows:

$$\text{Theoretical DFB } (\mu g/mL) = \frac{\text{Fort. level (ppb)}}{\text{Final vol. (mL)}} \text{ x Sample vol (mL) x 1 } \mu g/1000ng$$

$$\% \text{ Recovery} = \frac{\mu g/mL \text{ DFB injected}}{\text{Theoretical DFB } (\mu g/mL)} \times 100$$

where control residues are subtracted from fortified residues, where applicable.

I-H.3. Residue Levels

Validity of the DFB analytical method in water was established by acceptable recovery (70-110%) from fortified untreated control samples, where any apparent interference in the control was subtracted from residues in the fortified samples. Residues of diflubenzuron (µg/L diflubenzuron) in treated samples are calculated as for the fortified samples, without subtraction from the controls.

Diflubenzuron linearity standards are prepared in acetonitrile:water:1,4-dioxane (45:45:10) (I-C.5.1 - Preparation of Linearity Standards). These standards range in concentration from 0.025 µg/mL to 0.6 µg/mL. A calibration curve is generated with each sample set. The equation of the line based on the peak area of the standard versus the µg/mL is generated by least squares linear regression calculated by the computer program, Excel (Microsoft Corporation).

An example calculation for the recovery of DFB (3.41 µg/L fortification) from water (Uniroyal Study no. 98012, PTRL sample no. 734W-273, ref. *1*) is shown below:

Linear regression analysis of the diflubenzuron standards gave a curve with the equation $x = (y + 183370.83) \div 8517557.90$ ($r^2 = 0.9953$). The µg/mL diflubenzuron injected determined by this curve was:

µg/mL of DFB injected = (1897715+183370.83) ÷ 8517557.90 = 0.244 µg/mL

Theoretical DFB concentration, µg/mL =

$$\frac{3.41\,ppb}{3\,mL} \times 200\,mL \times 1\,\mu g/1000\,ng = 0.227\,\mu g/mL$$

$$\text{Percent DFB recovery} = \frac{0.244\,\mu g/mL}{0.227\,\mu g/mL} \times 100\% = 107.5\%$$

II. ANALYTICAL METHOD - 4-CHLOROPHENYLUREA AND 2,6-DIFLUOROBENZOIC ACID

II-A. MATERIALS

Solvents, reagents and instruments mentioned in this report are those used to perform the work discussed. Equivalent materials may be substituted as required.

II-A.1. Equipment

- Balance
- Bottles, centrifuge
- Büchner funnels
- Filter paper, Whatman #4 or Falcon 0.22 µM cellulose acetate
- Flasks, round bottom, various sizes
- Graduated cylinders, various sizes
- Pasteur pipettes
- pH paper or pH meter

- Pipettman, Gilson 1000
- Separatory funnels, 500 mL
- Suction flasks
- Syringes, microliter, various sizes
- Thermometer
- Vacuum evaporator, Büchi Model RE111 with temperature controlled bath, Brinkmann Instruments, Burlingame, CA
- Vials, glass with teflon®-lined cap
- Volumetric flasks, various sizes
- Volumetric pipettes, various sizes

II-A.2. Reagents/Supplies

Solvents/Reagents - All solvents were HPLC grade unless noted otherwise.

- Acetonitrile
- 1,4-Dioxane
- Ethyl Acetate
- Formic Acid
- Methanol
- Water
- Sodium Sulfate, Anhydrous

II-A.3. Analytical Standards

Analytical standards of CPU and DFBA are available from Uniroyal Chemical Company, Inc., a division of Crompton Corporation. Standards are kept frozen. A typical Certificate of Analysis (COA) for the standard is shown in Appendix 1. The COA shows the structures of the standard and a typical purity. Appendix 1 also contains an MSDS for the standard. One should obtain an MSDS for the solvents directly from their suppliers.

II-B. SAFETY AND HEALTH

This method should be performed by trained chemical personnel. Hazards associated with the chemicals analyzed by this analytical method are shown in the MSDS in Appendix 1.

II-C. METHODOLOGY - 4-CHLOROPHENYLUREA AND 2,6-DIFLUOROBENZOIC ACID

II-C.1. Principle of the Method

CPU and DFBA are extracted from filtered and acidified water by partitioning into ethyl acetate. After drying (Na_2SO_4) and concentrating the ethyl acetate

extracts, the residue is taken up in ACN:H_2O:1,4-dioxane (45:45:10). CPU is analyzed by HPLC/UV. DFBA is analyzed by HPLC/MS.

II-C.2. Types of Water Matrix

This method is predicted to be applicable to most water types. The results described in this report were obtained using water from pond or rice paddy sources. The analytical method worked well on all these types and is expected to perform equally well on any type of water including ground water, surface water, etc.

II-C.3. Sample Processing

All samples are received from the field frozen and are stored frozen at approximately -20 °C. They must remain frozen until used for fortification and/or analysis. Thawed samples are stored refrigerated (4-10 °C) when not in use. Water samples are filtered through a Falcon filter system or a Buchner funnel fitted with a Whatman #4 filter paper into a 1000 mL flask prior to extraction.

II-C.4. Extraction Method

1. Thaw and filter raw water (Falcon 0.22 micron cellulose acetate, or Whatman #4). Use a sample aliquot of 100 or 200 mL. Fortify as necessary.

2. Adjust to pH ~3 with neat formic acid (50µL). Place in a 500 mL separatory funnel.

3. Partition each aliquot of acidified, filtered water against ethyl acetate, 3 x 40 mL.

4. Dry combined EtOAc extracts through a bed of Na_2SO_4. Rinse separatory funnel with 15 mL EtOAc and pass through Na_2SO_4 bed.

5. Roto-evaporate in 250 mL round bottom flask to 2-3 mL at 35 °C.

6. Transfer to 50 mL round bottom flask with ~3 mL EtOAc and roto-evaporate to dryness.

7. Re-dissolve residue in 3.0 mL acetonitrile:water:1,4-dioxane (45:45:10).

II-C.5. Fortifications

Preparation of Standards

A stock solution of DFBA is prepared at 1.0 mg/mL in methanol:water (1:1, v/v). A stock solution of CPU is prepared at 1.0 mg/mL in methanol:water (1:1, v/v). Both solutions are prepared using the formula described in the "Methods of Calculation" section (II-G.1). Separate dilutions of the DFBA and CPU stocks are prepared for the 1, 10 and 50 µg/mL stock solutions, as described below for fortifications and linearity standards (II-C.5.1.). The 50 µg/mL DFBA and CPU standards are prepared by dilution of 500 µL of the 1 mg/mL solutions to 10 mL with methanol:water (1:1, v/v). Reference standards are considered stable throughout the conduct of the study based on comparisons of chromatograms of the first and last analysis. Microliter syringes, volumetric pipettes and volumetric flasks are used throughout.

Fortification Procedure

Water is acidified with concentrated formic acid to pH 3.4, then fortified at 1, 5 and 10 µg/L to validate the method and at 5 µg/L for concurrent method spikes with sample analysis as follows:

Fortification Level (µg/L)	Volume of Water (mL)	Volume of DFBA + CPU stock standards (mL)
1	100	100 µL of 1.0 µg/mL each
5	100	50 µL of 10 µg/mL each
10	100	100 µL of 10 µg/mL each

II-C.5.1. Preparation of Linearity Standards

The linearity standards are prepared from the stock solutions described above. For ease of analysis, mixed standards of DFB, DFBA and CPU may be prepared in a single solution for analysis of water samples in concentrations ranging from 0.0125 to 2.0 µg/mL (methanol:water, 1:1, v:v) for method validation. Linearity standards utilized for the sample analyses were mixed DFB/DFBA/CPU standards, as shown below. Linearity standards are prepared using volumetric flasks, pipettes and microliter syringes. If DFB is not an analyte, it should be omitted from the mixed standard.

The mixed standard preparation may be prepared as provided below, where diflubenzuron, DFBA and CPU together make a set of general linearity standards (see "Methods of Calculation" in Section II-G for an example). At least five concentrations should be used to construct the calibration curve. A calibration curve is generated with each sample set to determine linearity and to quantitate DFBA residues and CPU residues separately.

Mixed Standard DFB/CPU/DFBA (µg/mL)	Dilution (with Acetonitrile:H_2O:1,4-Dioxane (45:45:10))
0.6	6 mL of 10 µg/mL DFB, 6 mL of 10 µg/mL CPU and 6 mL of 10 µg/mL DFBA diluted to 100 mL
0.4	4 mL of 10 µg/mL DFB, 4 mL of 10 µg/mL CPU and 4 mL of 10 µg/mL DFBA diluted to 100 mL
0.2	2 mL of 10 µg/mL DFB, 2 mL of 10 µg/mL CPU and 2 mL of 10 µg/mL DFBA diluted to 100 mL
0.1	1 mL of 10 µg/mL DFB, 1 mL of 10 µg/mL CPU and 1 mL of 10 µg/mL DFBA diluted to 100 mL
0.05	500 µL of 10 µg/mL DFB, 500 µL of 10 µg/mL CPU and 500 µL of 10 µg/mL DFBA diluted to 100 mL
0.025	250 µL of 10 µg/mL DFB, 250 µL of 10 µg/mL CPU and 250 µL of 10 µg/mL DFBA diluted to 100 mL.

Fortifications are carried out by adding the appropriate standard solution directly into a measured volume of an untreated sample check (control) in the extraction container. During an analysis, checks (control) and fortified (spiked) samples are extracted along with each set of treated samples.

A minimum of two fortification spikes are run for each sample set. This is approximately one spike for every three treated samples.

II-C.6. Clean-up

Clean-up of the water sample is genarally not required.

II-C.7. Derivatization

Derivatization is not required for the anaysis of CPU and DFBA in water.

II-D. INSTRUMENTATION

II-D.1. CPU HPLC/UV Method

Instrumentation

Liquid chromatograph	HP 1050
Detector	HP Series Variable Wavelength UV/Vis
Integrator	Dionex/Peaknet Chromatography System
Autosampler	Biorad or Cygnet ISCO fraction collector
Column	Zorbax C8 (4.6 mm ID x 25 cm)
Flow rate	1.5 mL/minute
Detection	UV 254 nm
Injection Volume	100 µL
Solvent A	Acetonitrile(ACN):Water(H_2O):1,4-Dioxane (25:65:10)
Solvent B	Acetonitrile(ACN):Water(H_2O):1,4-Dioxane (85:5:10)

Program:

Step	Time (min)	% A	% B
0	0	100	0
1	5	100	0
2	15	25	75
3	16	25	75
4	25	100	0

ThermoSeparation Product Spectra P4000

II-D.2. DFBA LC/MS Method

Instrumentation

Liquid chromatograph	ThermoSeparation Product Spectra P4000
Detector	Finnigan MAT LCQ Mass Spectrometer
Integrator	3396B Series II
Autosampler	ThermoSeparation Product Spectra Autosampler AS3000
Column	Rainin Microsorb MV C18 (4.6 mm ID x 25 cm)
Injection Volume	250 µL
Solvent A	0.05% Formic acid in water
Solvent B	Acetonitrile

Short HPLC/MS method, used when samples are relatively free from any interferences:

Flow rate 0.8 mL/minute

Step	Time (min)	% A	% B
1	0.0	95	5
2	17.0	68	32
3	18.0	0	100
4	23.0	0	100
5	27.0	95	5
6	33.0	95	5

HPLC flow is diverted from waste to detector between 0.0 and 12.0 minutes.

Segments	1	2
Duration (min)	12.0	5.0
Event	Negative	Negative
m/z scanned	100-220	100-220

Long HPLC/MS program, used for most water samples:

Flow rate 0.4 mL/minute

Step	Time (min)	% A	% B
1	0.0	95	5
2	30.0	75	25
3	31.0	0	100
4	35.0	0	100
5	38.0	95	5
6	43.0	95	5

Flow is diverted from waste to detector at 7.0 minutes.

Segments	1	2	3	4
Duration (min)	7.0	5.1	9.0	9.9
Event	Negative	Negative	Positive	Negative
m/z scanned	100-220	100-220	120-250	270-400

MS detector settings for both Long and Short methods:

Default charge state:	2
Isolation width:	2
Collision energy	35
Minimum signal required	100000

II-E. CONFIRMATORY TECHNIQUE

A confirmatory method involving liquid chromatography and mass spectrometry (LC/MS) is used to authenticate CPU as the material giving rise to the HPLC peak at the expected retention time. CPU is confirmed with positive ion Atmospheric Pressure Chemical Ionization (PI/APCI). Increased sensitivity may be obtained using ElectroSpray Ionization (ESI) for confirmation of CPU. However for convenience, if both diflubenzuron and CPU are present in the analyte solution, they can be detected in a single MS analysis using APCI with pulsing between positive and negative modes. The chlorine ^{37}Cl isotope mass occurring at m/e [M+2] is also diagnostic for both diflubenzuron and CPU.

Material	Mode	Masses scanned	Ions monitored
Diflubenzuron	Negative ion	270.0-400.0	289 (291) [M-H]-HF 309 (311) M-H
CPU	Positive ion	120.0-250.0	171 (173) M+H 212 (214) [M+H]+ACN

Examples of confirmation of CPU in water by MS are presented in Appendix 3.

The analysis for DFBA is performed using LC/MS. Use of LC to separate the analyte coupled with mass spectrometry as the detection method allows simultaneous confirmation of the presence or absence of the analyte at its predetermined retention time. Specific ions at m/e 157 and 203 are monitored.

II-F. TIME REQUIRED FOR ANALYSIS

Analysis of water for DFBA/CPU
- per set, including 2 controls, 2 fortifications and six treated samples

Extraction time = 6 hours
Analysis time DFBA (LC/MS) = 12 hours
Analysis time if CPU (HPLC/UV) is included = 12 hours
TOTAL time equals 30 hours

Assuming an automated data analysis and capture system, total time for one set, including all analytes, is approximately two working days.

II-G. METHODS OF CALCULATION

The residue data included the following statistical calculations: means, averages, standard deviations and linear regression analysis.

II-G.1. Calculations for DFBA Standard

Preparation of Stock Solutions of Standard

$$\text{Volume of solvent} = \frac{(W) \times (P)}{(FC)}$$

where, W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (mg/mL)

II-G.2. Calculation of Recoveries

II-G.2.1. Recovery - DFBA

The recoveries of DFBA from fortified water samples were calculated as follows:

Linear regression formula from calibration curve, $y = mx + b$

$$\text{DFBA (ng on column)} = \frac{y - b}{m}$$

where y = sample peak area
b = calibration intercept
m = slope

If x is the concentration, the peak area will similarly calculate the sample concentration.

$$\text{Theor. ng DFBA (on column)} = \frac{\text{DFBA ng/mL x Sample vol (mL)}}{\text{Final vol. (mL)}} \text{ x injection vol. (mL)}$$

$$\text{Theoretical DFBA (ng/mL)} = \frac{\text{DFBA ng/mL x Sample vol. (mL)}}{\text{Final vol. (mL)}}$$

$$\text{\% Recovery} = \frac{\text{ng DFBA Fortified Sample - ng of DFBA Control}}{\text{Theoretical ng DFBA}} \text{ x 100\%,}$$

or,

$$= \frac{\text{DFBA Concentration Fort. Sample - DFBA Concentration Control}}{\text{Theoretical DFBA Concentration}} \text{ x 100\%}$$

Residues of DFBA (µg/L) =

$$\text{Calculated DFBA Concentration (ng/mL)} \times \frac{\text{Final Vol (mL)}}{\text{Sample Vol. (mL)}} \times 1000 \text{ ng/µg}$$

II-G.2.2. Recovery - CPU

CPU residues and recoveries were similarly calculated. The calculations for CPU residues were modified for treated sample analysis. In analysis sets, the linearity was plotted with peak area vs. analyte concentration (µg/mL). Therefore, the recoveries were based on theoretical concentrations relative to calculated concentrations.

II-G.3. Residue Levels

Validity of the analytical method for acceptable recovery (70-120%) of the DFBA or CPU is demonstrated by fortifying a control sample for each set of residue samples, as described above. Residues of DFBA (µg/L) or CPU (µg/L) in treated samples is calculated as shown above, with no control residues subtracted.

After spiking the samples, they are extracted and analyzed as previously described. DFBA/CPU mixed standards are prepared in the mobile phase of the appropriate LC method (see Preparation of Linearity Standards, II-C.5.1). A calibration curve is generated with each sample set. The equation of the line based on the peak area of the standard versus the concentration (µg/mL) injected is generated by least squares linear regression.

An example calculation of DFBA (or CPU) analysis in water (Uniroyal Sample No. 1414, (PTRL Sample No. 734W-317), 5.0 µg/L) is shown below:

Linear regression analysis of DFBA standards gave a curve with the equation $y = -111373 + 7.31104e^6 x$ ($r^2 = 0.9995$). The µg/mL injected determined by this equation was

$$\text{µg/mL DFBA} = [2658150.86 + 111373] \div 7.31104\ e^6 = 0.379 \text{ µg/mL}$$

$$\text{Theoretical DFBA concentration} = \frac{5.0 \text{ ppb} \times 200 \text{ mL}}{3 \text{ mL}} = 0.333 \text{ µg/mL}$$

$$\text{Percent Recovery} = \frac{0.379 \text{ µg/mL}}{0.333 \text{ µg/mL}} \times 100 = 113.8\%$$

Calculation of CPU analysis in water is performed in a similar manner.