



Determination Of Benzidines and Nitrogen-Containing Pesticides in Water Liquid-Solid Extraction And Reverse Phase High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry

EPA Method 553 Revision 1.1

UCT Products: **ECDVB156** (6 mL cartridge with 500 mg PSDVB)
ECUNIDVB500 (Universal cartridge with 500 mg PSDVB)

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Background

This is a general purpose method for the determination of benzidines and nitrogen-containing pesticides in drinking water. These compounds are efficiently extracted using a solid-phase cartridge containing styrene divinylbenzene and are amenable to separation on a reverse phase liquid chromatography column and transferable to the mass spectrometer with a particle beam interface. The compounds listed below are potential method analytes and single-laboratory accuracy and precision data have been determined for the compounds.*

Table of Analytes Determined by this Method

Analyte	Abbreviation	MW	CASRN
Benzidines	BZ	184	92-87-5
Benzoylprop ethyl	BP	365	33878-50-1
Caffeine	CF	194	58-08-2
Carbaryl	CL	201	63-25-2
o-chlorophenyl thiourea	PT	186	5344-82-1
3,3' dichlorobenzidine	DB	252	91-94-1
3,3' dimethoxybenzidine	MB	244	119-90-4
3,3' dimethylbezidine	LB	212	612-82-8
Diuron	DI	232	330-54-1
Ethylene thiourea	ET	102	96-45-7
Linuron (Lorox)	LI	248	330-55-2
Monuron	MO	198	150-68-5
Rotenone	RO	394	83-79-4
Siduron	SI	232	1982-49-6

Method Summary

Organic compound analytes and surrogates are extracted from 1 L of water sample by passing a water sample through a cartridge containing a neutral polystyrene/divinylbenzene polymer. Analytes are eluted from the LSE cartridge using a small quantity of methanol, and then concentrated by evaporation of the solvent. The sample components are identified, and measured by injecting an aliquot of the methanol solution into an HPLC containing a reverse phase HPLC column interfaced to a mass spectrometer (MS) with a particle beam (PB) interface.

Safety

- The toxicity or carcinogenicity of chemicals used in this method have not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized
- Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens

Sample Collection, Preservation

- When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized
- Adjust the flow to about 500 mL/min and collect samples from the flowing stream. Keep samples sealed from collection time until analysis
- When sampling from an open body of water, fill the sample container with water from a representative area
- Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach analytes into water. Automatic samplers that composite samples over time must use refrigerated glass sample containers

Preservation Reagents

- All samples should be iced or refrigerated at 4°C from the time of collection until extraction
- Residual chlorine should be reduced at the sampling site by addition of a reducing agent such as 40-50 mg of sodium sulfite or sodium thiosulfate. This can be added in solid form with stirring until dissolved to each liter of water

Sample Shipment, Storage and Extract Holding Times

- All samples should be iced or refrigerated at 4°C from the time of collection until extraction
- Samples must be extracted within seven days and extracts analyzed within 30 days of sample collection
- Extracts should be stored in an amber vial in a freezer <0°C

Interferences

- All glassware must be meticulously cleaned by washing with detergent and water, rinsed with reagent water or solvents and heated in an oven. Do not heat volumetric glassware
- The degree of signal enhancement by co-elution is compound dependent
- When two compounds co-elute, the transport efficiency of both compounds through the particle beam interface generally improves and enhanced ion abundances are observed in the mass spectrometer
- The co-elution effect invalidates the external calibration curve and, if not recognized, will result in incorrect concentration measurements. Procedures given in this method to check for coeluting compounds must be followed to preclude inaccurate measurements
- An optional isotope dilution calibration procedure has been included for use when interfering matrix or coeluting compounds are present
- During analysis, major contaminant sources are reagents, chromatography columns
- Analyses of field and laboratory reagent blanks provide information about the presence of contaminants
- Analysis of samples of high concentration must be followed by a reagent blank
- Syringes, injectors, and other equipment must be thoroughly clean

Stock Standard Solutions (SSS)

- Prepare by adding 10 mg (weighed to 0.1 mg) of the pure material to 1.9 mL of methanol or acetonitrile in a 2 mL volumetric flask then transfer to an amber glass vial
- Certain analytes, such as 3,3'-dimethoxybenzidine, may require dilution in 50% v/v acetonitrile or methanol: water solution
- Benzidines as the free base or as acid chlorides may be used for calibration. The concentration of the standard must be calculated as the free base

Primary Dilution Standard PDS (500 µg/mL)

The stock standard solutions are used to prepare a primary dilution standard solution containing multiple analytes

- The recommended solvent for this dilution is a 50% v/v acetonitrile:water mixture
- Store the primary dilution standard solution in an amber vial in a freezer at <math><0^{\circ}\text{C}</math>

Fortification Solution of Surrogates

Recommended surrogates for the analysis of benzidines and nitrogen-containing pesticides:

- benzidine-D₈ (DBZ)
- caffeine- ¹⁵N₂ (NCF)
- 3,3'-dichlorobenzidine-D₆ (DCB)
- bis-(perfluorophenyl)-phenylphosphine oxide (OD)
- Prepare a solution of the surrogates in methanol or acetonitrile at a concentration of 5 mg/mL each
- Store the surrogate fortifying solution in an amber vial in a freezer at <0° C

Extraction Procedure

- A 1 liter sample is recommended
- Using good mixing, adjust sample pH to 7.0 by dropwise addition of HCl or 1N sodium hydroxide
- Add a 10 µL aliquot of the fortification solution for surrogates and mix

1. Cartridge Preparation

- a. Assemble a vacuum manifold system. Automated extraction equipment may also be used
- b. Place a **ECDVB156** or **ECUNIDVB500** cartridge(s) on the manifold
- c. Rinse the cartridge(s) with a 10 mL aliquot of methanol
- d. Slowly draw methanol to the top of the frit
- e. Add a second 10 mL aliquot of methanol and draw through to top of frit

Note: Do not let the cartridge go dry after addition of methanol otherwise repeat

- f. Add 10 mL of reagent water to the cartridge and draw through to surface of frit

2. Sample Extraction

- a. Add water sample to the cartridge and draw through at 20 mL/minute
- b. Rinse the sample container with 10 mL of reagent water and add to the cartridge
- c. Place a clean collection vial in the vacuum manifold

3. Cartridge Elution

- a. Add 15 mL of methanol to the sample container, swirl then add to the cartridge
- b. Elute the cartridge with 2 x 7.5 mL aliquots of methanol

4. Extract Concentration

- a. Concentrate the extract under a gentle stream of N₂
- b. Bring to a known volume after concentration step

5. HPLC Analysis

- HPLC Mobile Phase – 0.3 mL/min flow

Analyte Retention Times

Analyte	Retention Time min/sec		Quantitation Ion
	A	B	m/z
Benzidines	4.3	4.9	184
Benzoylprop ethyl	24.8	31.3	105
Caffeine	1.4	1.6	194
Carbaryl	10.1	14.47	144
o-chlorophenyl thiourea	2.7	3.0	151
3,3' dichlorobenzidine	16.6	22.7	252
3,3' dimethoxybenzidine	8.1	11.5	244
3,3' dimethylbezidine	8.5	12.4	212
Diuron	11.0	16.1	72
Ethylene thiourea	1.2	1.4	102
Linuron (Lorox)	16.0	21.9	161
Rotenone	21.1	27.7	192
Siduron	14.8	20.6	93
Surrogates			
Benzidines-D₈	4.2	4.8	192
Caffeine- ¹⁵N₂	1.3	1.6	196
3, 3'-dichlorobenzidine-D₆	16.5	22.6	258
bis(perfluorophenyl)-phenylphosphine oxide	22.0	28.9	271

A These retention times were obtained on a Hewlett-Packard 1090 liquid chromatograph with a Waters C18 Novapak 15 cm x 2 mm column using gradient conditions Solvent A: 75:25 v/v water/acetonitrile solution hold 1 minute then to 70% acetonitrile in 29 minutes

B These retention times were obtained on a Water's 600 liquid chromatograph with a Waters C18 Novapak 15 cm x 2 mm column using gradient conditions Solvent A: 75:25 v/v water/acetonitrile solution hold 1 minute then to 70% acetonitrile in 29 minutes

*The analyst should refer to EPA Method 553 "Determination Of Benzidines and Nitrogen-Containing Pesticides in Water By Liquid-Liquid Extraction or Liquid-Solid Extraction and Reverse Phase High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry", Revision 1.1 Issued August 1992, by Thomas D. Behymer, Thomas A. Bellar, James S. Ho, William L. Budde, US EPA, Office of Ground Water and Drinking Water, EPA, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio 45268

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