## CONDUCT OF STUDY

# 1 INTRODUCTION AND SUMMARY

## 1.1 Scope

This method describes the analysis of soil/sediment samples generated from aquatic dissipation studies in rice paddies for residues of Bentazon, N-Methylbentazon and 8-Chlorobentazon. Soil or sediment samples (20 g) are extracted with 3:1 methanol:methylene chloride by shaking. An aliquot of the extract is concentrated and analysis is performed by thermospray liquid chromatography tandem mass spectrometry (TSP-LC/MS/MS). Bentazon and 8-Chlorobentazon are analyzed by negative ion TSP-LC/MS and N-Methylbentazon is analyzed by positive ion TSP-LC/MS/MS. The extraction and instrumentation methods (Method ID: BASBENS) were developed at ALTA Analytical Laboratory, El Dorado Hills, CA 95762.

# 2 MATERIALS

## 2.1 Equipment

2.1.1 Balance, Analytical, capable of weighing to the nearest 0.0001 g.

2.1.2 Balance, Toploader, capable of weighing to the nearest 0.01 g.

2.1.3 Bottle, amber, appropriate size for storage of standard solutions.

2.1.4 Nalgene plastic bottles, 250 mL (CMS 252-348 or equivalent).

2.1.5 Rotary evaporator, Buchler 421-1409 or equivalent.

2.1.6 Test tubes, 16x125-mm, (Curtin Matheson Scientific (CMS) 251-884 or equivalent).

2.1.7 Graduated centrifuge tubes, 10-mL calibrated "To Contain", readable to 0.1-mL (CMS 253-819 or equivalent).

2.1.8 Positive displacement micropipets, 10/20 uL, 25/50 uL, 50/100 uL, 100/200 uL (Cole Parmer N-07951-10/15/20/25 or equivalent).

2.1.9 Pipets, 0.5-mL (CMS 080-465 or equivalent).

2.1.10 Pipets, 1-mL (CMS 080-507 or equivalent).

2.1.11 Pipets, 2-mL (CMS 080-515 or equivalent).

2.1.12 Pipets, 5-mL (CMS 080-523 or equivalent).

2.1.13 Pipets, 10-mL (CMS 080-531 or equivalent).

2.1.14 Pipets, 25-mL (CMS 368-761 or equivalent).

2.1.15 Pasteur pipettes (CMS 355-123 or equivalent).

2.1.16 Flask, 250-mL round bottom (CMS 096-495 or equivalent).

2.1.17 Flask, volumetric 25-mL (CMS 105-304 or equivalent).

2.1.18 Flask, volumetric 50-mL (CMS 106-138 or equivalent).

2.1.19 Flask, volumetric 100-mL (CMS 105-320 or equivalent).

2.1.20 Autosampler vials, 1-mL (Waters 78514 or equivalent).

2.1.21 Disposable syringe, 5-mL with Luer-Lok (CMS 256-851 or equivalent).

2.1.22 Syringe filter disks, Gelman Acrodisc CR 0.45-um or smaller (CMS 141-226 or equivalent).

2.1.23 Vortex-Genie 2 mixer or equivalent.

2.1.24 Centrifuge (IEC Centra-8 or equivalent).

2.2 Reagents and Standards

2.2.1 Water, EM Omnisolve HPLC grade (CMS MWX004-1 or equivalent).

2.2.2 Sodium sulfate, anhydrous (CMS 426-419 or equivalent).

2.2.3 Formic acid, ~88% (CMS 830-937 or equivalent).

2.2.4 Methylene chloride, EM Omnisolve (CMS DX0831-1 or equivalent).

2.2.5 Ammonium acetate, crystals, (CMS MAX 1220-1 or equivalent).

2.2.6 HPLC mobile phase, 0.2M ammonium acetate/0.1% formic acid.

2.2.7 Methanol, EM Omnisolve HPLC grade (CMS MX0488-1 or equivalent).

2.2.8 Bentazon, N-Methylbentazon and 8-Chlorobentazon analytical standards, BASF Corporation, Agricultural Research Center, P.O. Box 13528, Research Triangle Park, North Carolina, 27709-3528.

2.3 Safety and Health

The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available.

## 3 PRINCIPLES

BASF Analytical Method D9304 (ALTA Analytical Method BASBENS) was developed for the analysis of soil samples generated from aquatic dissipation studies in rice paddies for residues of Bentazon, N-Methylbentazon and 8-Chlorobentazon. Method development and validation was performed with control soil samples from rice paddies fortified with bentazon, 8-chlorobentazon and N-methylbentazon.

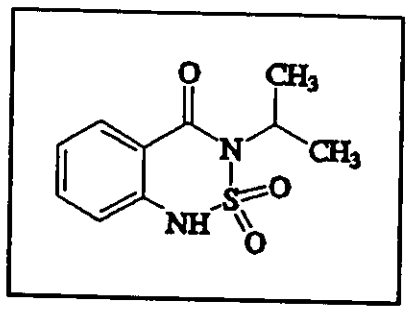
Soil samples (20 g) are combined with approximately 20 g of anhydrous sodium sulfate and 80 mL of 3:1 methanol:methylene chloride and shaken for 3 hours. A 40-mL aliquot of the extract is transferred to a 250-mL round bottom rotary evaporation flask and concentrated to approximately 6 mL. The extract is then quantitatively transferred to a calibrated centrifuge tube and concentrated to approximately 0.5 mL under a stream of nitrogen and brought back to 2 mL with water. No additional clean-up or derivitization is required for this method. The final concentration factor for the method is 5g/1mL.

Standards and sample extracts are injected onto a C-18 reverse phase HPLC system connected to a quadrupole mass spectrometer via a thermospray interface. Bentazon and 8-Chlorobentazon are analyzed by negative ion TSP-LC/MS and N-Methylbentazon is analyzed by positive ion TSP-LC/MS/MS.

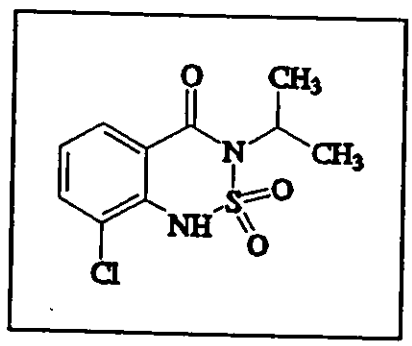
The limit of quantitation for this method was validated at 10 ppb for each compound.

Analytical standards used as the test and reference substances for the method validation were obtained from BASF Corporation, Research Triangle Park, North Carolina. Structures, chemical names, lot numbers, purity, and expiration dates for each can be found below.

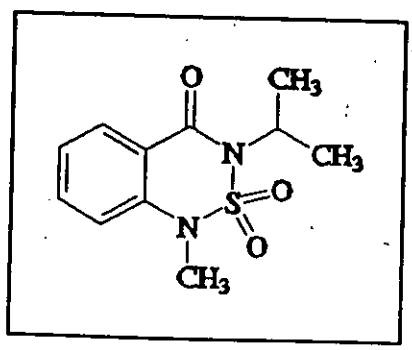
| | |
|---|---|
| Common Name: | Bentazon |
| Chemical Name: | 3-(1-methylethyl)-1H-2,1,3-benzothia-diazin-4(3H)-one-2,2-dioxide |
| ALTA ID: | R921119A |
| Lot Number: | CH40/204-1 |
| Purity: | 99.9% |
| CAS Number: | 25057-89-0 |
| Purity Statement | |
| Valid Until: | 05/1996 |




| | |
|---|---|
| Common Name: | 8-chlorobentazon |
| Chemical Name: | 8-chloro-3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide |
| ALTA ID: | R921119C |
| Lot Number: | L30/259 |
| Purity: | 99.8% |
| Purity Statement | |
| Valid Until: | 05/1996 |




| | |
|---|---|
| Common Name: | N-methylbentazon |
| Chemical Name: | 1-methyl-3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide |
| ALTA ID: | R92119B |
| Lot Number: | L45/197 |
| Purity: | 99.4% |
| Purity Statement | |
| Valid Until: | 05/1994 |



# 4 ANALYTICAL PROCEDURES

## 4.1 Preparation of Standard Solutions

The following standard concentrations are suggestions and may be modified if needed.

4.1.1 A 100-ug/mL stock solution of each analyte is prepared by dissolving 10 mg of pure material in methanol and then diluting to 100 mL with methanol. If the compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Transfer the stock standard solution into an amber bottle and seal with teflon lined caps only. Store the standards in a freezer at -10°C or colder and protect from light when not in use. Stock standard solutions must be replaced after 1 year or sooner if comparison with check standards indicates a problem.

4.1.2 Using the 100-ug/mL stock solutions from 4.1.1, prepare a 10-ug/mL mixed standard containing all analytes in methanol.

4.1.3 Using the 10-ug/mL stock solution from 4.1.2, prepare a 1-ug/mL mixed standard containing all analytes in methanol.

4.1.4 The stock solutions 4.1.2 and 4.1.3 are to be used as fortification standards. Use the appropriate fortification standard such that no more than 1.0 mL is used for fortification.

4.1.5 A three point LC/MS/MS calibration standard curve should be prepared such that one standard is lower than the desired screening. The stock solutions are used for making the calibration standards. Dilutions are made such that the solvent has a composition of 75:25 HPLC water:methanol.

4.1.6 Example Calibration Standards

4.1.6.1 2500-ng/mL standard, dilute 2.5 mL of the 10-ug/mL stock to 10 mL with HPLC water.

4.1.6.2 250-ng/mL standard, dilute 1.0 mL of the 2500-ng/mL calibration standard to 10 mL with 75:25 HPLC water:methanol.

4.1.6.3 25-ng/ml standard, dilute 1.0 mL of the 250-ng/mL calibration standard to 10 mL with 75:25 HPLC water:methanol.

4.2 Extraction/concentration

4.2.1 In a 250 mL Nalgene bottle, combine 20 g of soil and ~20 g anhydrous sodium sulfate.

4.2.2 Add 80 mL of 3:1 methanol:methylene chloride and place on a shaker for 3 hours. Centrifuge for 10 minutes at 4000 rpm.

4.2.3 Transfer 40 mL of extract to a 250 mL round bottom flask using a 25-mL pipet (2-20 mL portions).

4.2.4 Concentrate the extract to ~6 mL by rotary evaporation at 30-35°C.

4.2.5 Transfer the concentrate to a 10-mL centrifuge tube with three 1 mL portions of methanol.

4.2.6 Concentrate the solution to 0.5 mL with nitrogen and water bath at room temperature.

4.2.7 Dilute to 2.0 mL with HPLC water and vortex.

4.2.8 Syringe filter using a 0.45 um or smaller syringe filter disk into an HPLC autosampler vial.

4.3 Dry Weight/Wet Weight determination

4.3.1 Place a moisture tin on the toploader balance and press "TARE" or "ZERO" to zero the balance.

4.3.2 Transfer ~5-6 g of sample (record the actual weight as "Wet Weight") to the tin.

4.3.3 Place the tin/sample in a 107°C oven for at least overnight.

4.3.4 Remove the tin/sample from the oven and cool to room temperature.

4.3.5 Place the tin/sample on the toploader balance and press "TARE" or "ZERO" to zero the balance.

4.3.6 Dump/wipe the residue from the tin and return the tin to the toploader (record the "negative" weight as "Dry Weight").

4.4 Instrumentation

4.4.1 Description of Operating Conditions (See Table II)

4.4.1.1 Standards and extracts are analyzed by LC/MS on a C18 reverse phase column. The analysis is performed with gradient chromatography using methanol as the organic modifier. See Table II for a description of the chromatographic conditions.

4.4.1.2 Bentazon and 8-chlorobentazon are determined by negative ion monitoring of their pseudo molecular ions $[M-H]^-$. The dwell time used for selected ion monitoring is 0.3 seconds for each analyte resulting in a total scan time of approximately 0.8 seconds/scan.

4.4.1.3 For the analysis of N-methylbentazon, the monitored product ion (m/z 213) is formed by collisionally induced fragmentation of a precursor ion in the collision cell of a triple stage quadrupole mass spectrometer (MS/MS). The product ion represents the loss of propyl from the protonated molecular ion (m/z 255). The dwell time used for selected ion monitoring is 1.2 seconds resulting in a total scan time of approximately 1.3 seconds/scan.

4.4.1.4 The analysis is initially performed in the negative ion mode. At 7.5 minutes, the analysis mode is switched to positive ion MS/MS. The approximate retention times for each analyte are listed in Table II. The analysis mode switching time may be changed to allow for approximately the same number of scans to be acquired after 8-chlorobentazon and before N-methylbentazon.

4.4.2 Hardware Tuning and Calibration

4.4.2.1 The successful detection (S/N > 5:1) of the lowest calibration standard and the criteria described in Section 4.8.3 are used to verify that the instrument is tuned and calibrated properly. If these criteria are met, no instrument adjustments are necessary.

4.4.2.2 Generally, no day to day tuning adjustments are required unless major repairs have been made to the mass spectrometer. If tuning or calibration is required, it must be performed by a qualified

instrument operator and recorded in the instrument maintenance logbook.

4.4.2.3 Tuning is based on thermospray reagent ions. For positive ion tuning, reagent ions (e.g. m/z 59, m/z 100) are used for lens optimization. For negative ion, m/z 119 is typically chosen for lens optimization.

4.4.2.4 Mass calibration is based on thermospray reagent and background ions. A low mass calibration point may be set using one of the reagent ions mentioned above. A higher mass calibration point may be set using a background ion (e.g. m/z 269). The instrument will extrapolate all other calibration points. The successful detection of the target analytes at their expected nominal masses is used to verify calibration.

4.5 Potential Interferences

There are no known interferences originating from the sample preparation, extraction, cleanup or concentration procedure.

4.6 Time Required

4.6.1 Approximately 20 samples can be prepared for analysis in an eight hour period.

4.6.2 Each instrumental analysis requires approximately 20 minutes.

4.7 Modifications or Potential Problems

Prior work done by BASF and ALTA suggests that N-methylbentazon is more volatile than bentazon or 8-chlorobentazon. Therefore, N-methylbentazon could be easily lost during the nitrogen concentration steps, especially if the extract goes to complete dryness. The validation data demonstrates that if the method is followed as written, losses of N-methylbentazon can be prevented.

4.8 Methods of Calculations

4.8.1 Quantitation is performed by using the average of a three point curve bracketing the sample in an injection sequence (4.10.2). The low standard should have at least a 5:1 signal/noise ratio for each analyte.

4.8.2 Analytes should be calibrated relative to the average response factor from the two preceding standards and one standard following. Deviation from this guideline should be noted in the raw data packet. The three standards used must be of three different concentrations, encompassing the range of calibration.

4.8.3 If the relative standard deviation (RSD, 4.10.3) for the three standards used for bracketing a sample is less than 20%, then the method is considered to be linear. The calculated RSD for each bracketed sample is documented in the raw data.

4.8.4 If the RSD is greater than 20% then the affected samples must be re-injected after acceptable re-calibration has been established.

4.8.5 No more than three samples may be injected between two standards.

4.8.6 The criteria listed in 4.8.4, does not apply if the results for the associated samples are less than the reporting limit, and the low standard has a signal/noise greater than 5:1.

4.8.7 The sample extract may be diluted with 75:25 HPLC water:methanol if the analyte response exceeds the range of the calibration curve (dilution factor, df = original volume/final volume).

4.8.8 The amount of analyte injected (ng/mL) is determined by dividing the value of the chromatographic peak area by the average response factor. See section 4.10 for a complete description of all calculations.

4.8.9 Deviation from the above guidelines should be noted in the raw data packet.

4.9 Fortified Samples

4.9.1 The method is validated for each set of samples analyzed by including a control sample and one or more samples fortified prior to the extraction procedure with 10 ppb or higher.

4.9.2 Add an appropriate fortification solution (from 4.1.3) to the soil sample, (Example: add 200-uL of a 1 ug/mL fortification solution to a 20 g soil sample to fortify at 10 ppb). Do not exceed 1.0 mL as a fortification volume.

## 4.10 Equations

4.10.1 Calculate the response factor for each standard from the following equation:

$$RF = \frac{Area_{STD}}{Conc_{STD}}$$

Where: $Area_{STD}$ = the area of response for the product ion from an injected standard

$Conc_{STD}$ = concentration of the injected standard (ng/mL)

4.10.2 Quantitation of samples will be performed based on the average response for the standards preceding and following the samples and is defined as follows:

$$RF_{AVG} = \frac{(RF_{STD1}) + (RF_{STD2}) + (RF_{STD3})}{3}$$

Where: $RF_{STD1}$ = the response factor from the first standard

$RF_{STD2}$ = the response factor from the second standard

$RF_{STD3}$ = the response factor from the third standard

Normally, $RF_{STD1}$ and $RF_{STD2}$ will precede the samples and $RF_{STD3}$ will follow.

4.10.3 The relative standard deviation (RSD) is derived from the coefficient of variation (CV):

$$CV = s/RF_{AVG}$$

$$RSD = CV \times 100 \text{ (percent)}$$

Where: CV = coefficient of variation
$RF_{AVG}$ = Average RF (4.10.2)
s = standard deviation (sample) of the $RF_{AVG}$

4.10.4 Sample calculations are done according to the following formula:

$$Amt_{SAMP} = \frac{(Area_{SAMP})\ (FV)}{(Wt_{SAMP})\ (RF_{AVG})\ (Df)\ (Af)}$$

Where: $Amt_{SAMP}$ = Final Sample Amount (ppb),

$Area_{SAMP}$ = Area of response for the product ion from the sample,

$RF_{AVG}$ = Average Response Factor (area/ng/mL),

FV = Final Volume (mL),

$Wt_{SAMP}$ = Sample weight (g),

Df = Dilution factor ($Vol_{init}/Vol_{fin}$).

Af = Aliquot factor ($Vol_{alq}/Vol_{total}$)

Example: For ALTA Lab ID 12179-1-MS5, bentazon;

$$Amt_{SAMP} = \frac{(198756)\ (2.0)}{(20.02)\ (182)\ (0.20)\ (0.5)} = 1091\ ppb$$

4.10.5 If required, residue values are corrected for percent moisture:

$$Amt_{DryWt} = \frac{Amt_{SAMP}\ (ppb)}{(M)}$$

Where: $Amt_{DryWt}$ = Final Sample Amount percent moisture corrected

M = Moisture content correction factor (DryWt/WetWt).

4.10.6 If $Amount_{SAMP}$ is less than the reporting limit then the results are reported as less than this amount.

4.10.7 The results for field samples, control samples, fortified control samples, and percent recoveries are reported to three significant figures.

4.10.8 If the analyte concentration in the control sample used for fortification is greater than half the low calibration standard (1/4 of the reporting limit, 1 ppb), this background will be subtracted from the result for the fortified control before percent recovery is calculated and reported.

## 5 Method Validation

### 5.1 Disposition of Study Materials and Data

Upon completion of the study, copies of all materials associated with this report will be retained in the archives at ALTA Analytical Laboratory, Inc., 5070 Robert J. Mathews Parkway, El Dorado Hills, CA 95762. The originals of the above cited materials will be archived at BASF Corporation, Agricultural Products Group,26 Davis Drive, Research Triangle Park, NC 27706-3528.

### 5.2 Source and Characterization of Control Samples

5.2.1 The control soils used for the method validation were provided as the test system by BASF. The control soils were taken from RCN 92114, Louisiana and RCN 92235, Mississippi.

### 5.3 Preparation of Samples

The control sample (0"-6") from Louisiana arrived frozen in core tubes (4). The soil was removed from the four tubes, mixed with dry ice, homogenized in a grinder/chopper and then sieved.

The control sample from Mississippi did not require any additional preparation prior to aliquoting for extraction.

### 5.4 Fortification

To validate this method, control soils from RCN 92114, Louisiana and RCN 92235, Mississippi were provided by BASF. The soil samples were fortified in duplicate at each of three levels, 10 ppb, 100 ppb, and 1000 ppb.

## 5.5 Sample Management

Soil samples were received at ALTA and handled according to ALTA Standard Operating Procedures, Chapter 10. Samples were received at the laboratory with dry ice and placed into freezers prior to processing. Samples were stored frozen between 0 and -20° C prior to extraction. Freezer temperature records for residue samples were maintained and archived.

## 5.6 Protocol Changes/Deviations

A protocol deviation was generated for the first method validation set that indicated that the curve RSDs for 8-chlorobentazon were greater than 20%, that no QAU audit had been performed on the set and that this set be included in the method validation report.

The proposed Experimental Termination date in Protocol 93057 was June 30, 1993, but the actual termination dates was July 29, 1993. This was addressed in a protocol change.

The chemical name for N-methylbentazon as written in Protocol 93057 was incorrect. A protocol change was issued to provide the correct naming as presented on page 11 of this report.

A change was made to Protocol 93057 that allowed for adding volumes larger than 1-mL when performing fortifications.

Table II
ANALYSIS CONDITIONS FOR THE DETERMINATION
OF BENTAZON, N-METHYLBENTAZON AND 8-CHLOROBENTAZON

**Instrumentation:**

Waters 600-MS HPLC gradient pump (or equivalent)
Waters WISP 712 autosampler (or equivalent)
Finnigan MAT TSQ-700 equipped with a TSP2 thermospray interface (or equivalent).

**HPLC Operating Conditions**

Column: Waters Nova-Pak C18, 3.9 x 150 mm column
Injection Vol. 50-uL (20-100 uL)
Flow Rate 1.2 mL/min

| Time (min.) | Gradient | 0.2M $NH_4OAc$/0.1% Formic Acid | Methanol |
|---|---|---|---|
| Initial | - | 90 | 10 |
| 0-4 | Linear | 40 | 60 |
| 9.5 | Step | 90 | 10 |

Abbreviated LC conditions: When diluted extracts for bentazon only are analyzed, the LC system will be re-equilibrated after the elution of bentazon to facilitate shorter run times.

**Mass Spectrometer Operating Parameters:**

Vaporizer Temperature: 95 degrees C (85 - 130 degrees C)
Source Temperature: 280 degrees C (220 - 320 degrees C)
Repeller Voltage: 0 volts
Collision Gas: Argon
MS/MS Collision Energy: -18eV

| Analyte | MW | Mode | Ion Monitored | Dwell Time (sec.) | Retention Time (approx. min.) |
|---|---|---|---|---|---|
| Bentazon | 240 | neg. ion | m/z 239 | 0.3 | 5.5 |
| 8-Chlorobentazon | 274 | neg. ion | m/z 273 | 0.3 | 6.4 |
| N-Methylbentazon | 254 | positive MS/MS | m/z 255 - m/z 213 (precursor) - (product) | 1.2 | 9.5 |