Volume 4&6

CAL Study No.: 019-005
RPAC Study No.: EC-98-423

449857-03

STUDY TITLE

Independent Laboratory Validation of Analytical Method:
"Dodine: Method of Analysis for Dodine in Soil Using GC-MSD"

DATA REQUIREMENTS

EPA Guideline Reference No. OPPTS 850.7100

STUDY COMPLETED ON

July 16, 1998

III. INTRODUCTION

The US EPA requires petitioners to furnish results of a successful confirmatory trial of a method by an independent laboratory to ensure its suitability as an enforcement method. This report details the results of the confirmatory trial of the analytical method, "Dodine: Method of Analysis for Dodine in Soil Using GC-MSD".

The validation set consisted of a reagent blank, two control samples, five control samples fortified at the LOQ (10 ppb), and five control samples fortified at 10X the LOQ (100 ppb).

IV. TEST SYSTEM

An untreated California soil sample, from a depth of 0-6", was received at CAL on April 28, 1998 (assigned CAL ID 981898) from Rhône-Poulenc Ag Company. The soil sample was immediately placed in a freezer at $\leq$ -10°C for storage. Sample log-in information can be found in the raw data package associated with this study. Storage records will be kept at Centre Analytical Laboratories, Inc.

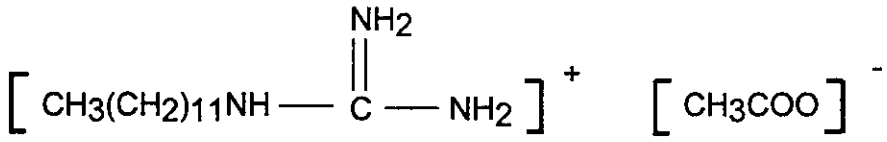
V. STANDARD MATERIALS

Analytical grade Dodine and RPA 410169 (Derivatized Dodine) were received on April 24, 1998 from Rhône-Poulenc Ag Company. Characterization of the standard materials was performed at Rhône-Poulenc Ag Company.

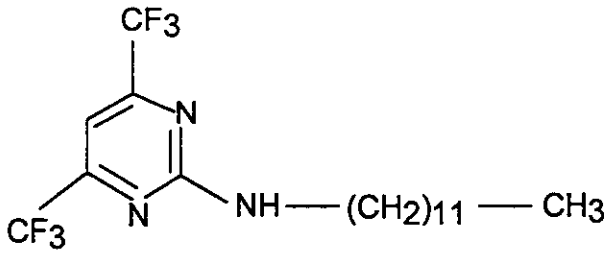
The available information for the standard materials is listed on the next page. The standard materials were stored in a refrigerator (4 ± 2°C) when not in use.

Compound	CAL Number	Reference Number	Batch Number	Purity (%)	Expiration Date
Dodine	98-19-18	AMA2384-AS52932	N/A	100	11/99
Derivatized Dodine (RPA 410169)	98-19-19	AMA2772-AS81059	BESS411	99.0	2/2000

Name or Code: Dodine
Chemical Name : 1-dodecylguanidinium acetate
CAS No.: 2439-10-3
Molecular Weight : 287.45



Name or Code: Derivatized Dodine (RPA 410169)
Chemical Name : 2-dodecylamino-4,6-bis(trifluoromethyl)pyrimidine
CAS No.: N/A
Molecular Weight : 399.4



VI. DESCRIPTION OF ANALYTICAL METHOD

Analytical method entitled "Dodine: Method of Analysis for Dodine in Soil Using GC-MSD", located in Appendix A of this report, was used for this study. Following is a description of the extraction procedure:

Fifty grams of sample were weighed into a 250 milliliter nalgene bottle and fortified (if necessary) using a 500 µl syringe. Approximately 70 milliliters of 0.05 M potassium hydroxide solution in 90:10 methanol:water was added, and the sample was shaken for ~15 minutes, centrifuged for ~5 minutes and filtered. An additional ~70 milliliters of extraction solvent was added to the sample and the extraction was repeated. Approximately 70 milliliters of 1% HCL in methanol was added, and the sample was shaken for ~15 minutes. The sample was filtered and all extracts were combined. The volume was brought up to 250 milliliters with methanol and a 50 milliliter aliqout was taken. This volume was concentrated on a rotary evaporator to ~10 milliliters. The extract was then transferred to a separatory funnel containing 7.90-7.94 g of salt to which 25 milliliters of water was added. The sample was shaken until the salt was dissolved and then it was partitioned three times with Dichloromethane, each time passing the Dichloromethane through sodium sulfate and collecting it. The Dichloromethane was concentrated to dryness on a rotary evaporator. The sample was derivatized with three milliliters of 1-chlorobutane, 100 microliters of methanol and 100 microliters of 1,1,1,5,5,5 Hexafluro-2,4-pentanedione. The sample was heated and stirred for 1 hour, allowed to cool and concentrated to dryness on a rotary evaporator. The sample was diluted to 5 milliliters with methanol. After vortexing and sonicating, the sample was passed through a Gelman 0.45 µm filter and analyzed by gas chromatography using a mass selective detector.

VII. EXPERIMENTAL DESIGN

A. Establishment of the Method

On April 27, 1998, five calibration standards were injected into the GC/MS in order to determine the analyte retention times, the instrument detection limit and the linearity of the instrument response. A control soil sample and a reagent spike were taken through the extraction procedure and injected in order to verify that the matrix was free of interferences at the appropriate retention times. Personnel involved in the study were not familiar with the method and therefore, establishment of the method was necessary. All contact made during establishment of the method was documented and is presented in this report.

B. Validation Set

The validation set consisted of a reagent blank, two control samples, five control samples fortified at the LOQ (10 ppb) and five control samples fortified at 10X the LOQ (100 ppb).

VIII. RESULTS

A. Method and Chromatography Establishment

1. Preparation of Standards and Fortification Solutions

Standard solutions were prepared on April 27, 1998, as specified in Rhône-Poulenc Ag Company analytical method "Dodine: Method of Analysis for Dodine in Soil Using GC-MSD" and also taking into account Protocol Amendment 1 which states that the standard solutions were not combined, as stated in the method. Stock standard solutions of Dodine and Derivatized Dodine were prepared at a concentration of 100 µg/mL by dissolving a known amount of the standard (corrected for purity) in methanol. Fortification solutions of Dodine were prepared at concentrations of 10.0 µg/mL and 1.0 µg/mL by performing ten-fold dilutions of the 100 µg/mL and 10.0 µg/mL standard solutions, respectively, in methanol. Calibration standards of Derivatized Dodine were prepared by first preparing a 1.0 µg/mL intermediate standard by diluting the 100 µg/mL stock solution with methanol. The 1.0 µg/mL standard was diluted with methanol to prepare calibration standards at concentrations of 0.1 µg/mL, 0.05 µg/mL, 0.01 µg/mL, and 0.005 µg/mL. All stock standard, fortification standard, and calibration standard solutions were stored in a refrigerator (4 ± 2°C) when not in use. Documentation of standard preparation can be found in the raw data associated with this report.

2. Chromatography

Quantification of the Derivatized Dodine was accomplished by gas chromatography (GC) with mass selective detection (MSD). The retention time of Derivatized Dodine was ~9.0 minutes. There were no interfering peaks in the control matrix at the analyte retention times.

3. Instrument Sensitivity and Limit of Detection

A two microliter injection of the lowest standard (5 ng/mL) resulted in a greater than 5:1 signal to noise ratio. The instrument proved to be sensitive down to 10 picograms.

The Limit of Detection (LOD), the smallest standard amount injected during the chromatographic run, was 5 ng/mL.

4. Description of Instrument and Operating Conditions

A Hewlett-Packard model 6890 Series Gas Chromatograph/model 5973 mass selective detector was used to analyze the samples. Data aquisition and processing was performed using a Hewlett-Packard Chemstation. Detailed operating conditions are listed below:

Instrument:	Hewlett-Packard model 6890 Series Gas Chromatograph/model 5973 mass selective detector
Column:	Alltech SE-54, 30 m x 0.25 mm ID, 0.25 µm df
Oven Temperature:	Hold at 120°C for 1.0 min., then 20°C/min. to 180°C, hold for 0 min., then 5°C/min to 220°C, hold for 0 min., then 20°C/min. to 300°C, hold for 5.0 min.
Injector Temperature:	250°C
Transfer Line Temperature:	300°C
Carrier Gas:	Helium
GC Head Pressure:	Varies with flow
GC Flow:	2.0 mL/min. for 5 min., decrease from 2.0 mL/min. to 1.0 mL/min.. Hold at 1.0 mL/min. for remainder of run
Injection Mode:	Pulsed Splitless - 25 psi for 1.0 min.
Injection Liner:	4 mm ID Single Gooseneck - Splitless
Injector Purge Delay:	1.0 min.
Purge Flow to Split Vent:	25 mL/min.
Injection Volume:	2 µL
Ionization Potential:	70 eV
Electron Multiplier Voltage:	~1900 V
Dwell Time:	100 msec
Integrator:	Hewlett-Packard Chemstation
Ion(s) Monitored:	m/z 244.0, 245.0, and 399.2
Retention Time:	~9.0 minutes
Total Run Time:	~21 minutes

D. Quantitation and Example Calculation

Two microliters of sample or calibration standard were injected into the GC/MS. The peak area was measured and the standard curve was generated (using linear regression) by Hewlett Packard Chemstation software using four concentrations of standards. The residue concentration was determined from the following equations:

Equation 1. Concentration of analyte in sample in parts per billion (ppb)

$$z = (y - a)/b \times c/d$$

Where:

y = peak area, response of analyte of interest

a = intercept of calibration curve from linear regression

b = slope of calibration curve from linear regression

c = final volume of sample (mL)

d = aliquot sample weight (g)

Where:

final volume = original sample final volume (5 mL) x dilution factor (2) = 10 mL and aliquot sample weight = original sample weight (50 g) x aliquot volume from step C.1. of the method (50 mL)/initial extraction volume from step B.3. of the method (250 mL) =10 g

z = concentration of derivatized analyte in sample (ppb)

Equation 2. Corrected concentration of analyte in sample in ppb

$$Z' = z \times C$$

Where:

Z' = corrected concentration

z = concentration of derivatized analyte in sample (ppb)

C = conversion factor

For the conversion of Derivatized Dodine to Dodine C = 0.72

The percent recovery of fortified samples was calculated using equation 3.

Equation 3. Percent Recovery

$$\% \text{ recovery} = \frac{(\text{ppb found in fortified sample} - \text{ppb found in UTC})}{\text{actual fortification level in ppb}} \times 100$$

An example of a calculation using an actual sample follows:

Soil sample fortified with 10 ppb of Dodine:

Where:

y = 9898
a = 958
b = 724
c = 10 mL
d = 10 g
C = 0.72

From Equation 1.

$z = ((9898 - 958)/724) \times (10 \text{ mL}/10 \text{ g}) = 12.3$ ppb of Derivatized Dodine

From Equation 2.

Z^1 = 12.3 ppb of Derivatized Dodine x 0.72 = 8.9 ppb of Dodine

From Equation 3.

$$\% \text{ Recovery} = \frac{(8.9 \text{ ppb Dodine found} - 0 \text{ ppb Dodine in UTC})}{10 \text{ ppb Dodine added}} \times 100 = 89\%$$

IX. METHOD OBSERVATIONS

A. Problems Encountered

No problems were encountered while performing the method validation.

B. Critical Steps

A critical step in the method is the amount of 1,1,1,5,5,5 Hexafluro-2,4-pentanedione which is needed for derivatizing.

C. Matrix or Solvent Effects

None observed.

D. Signal Enhancement or Suppression

None observed.

E. Stability of Solutions

The analytical phase of the study was completed within one week of standards preparation. No stability problems were observed during this time.

F. Recommended Changes to the Method

The method was successfully validated using the following modifications:

1. Section IV.D.2.:100 µL of 1,1,1,5,5,5 Hexafluro-2,4-pentanedione was added in the derivatization instead of 30 µL.
2. Section III.B.2.:The standard solutions were not combined as indicated in the method.

X. CONCLUSIONS

The method was successfully validated in soil at the limit of quantitation (LOQ) and at 10X the LOQ for Derivatized Dodine. The success of the validation was dependent upon the increase of 1,1,1,5,5,5 Hexafluro-2,4-pentanedione from 30 µl to 100 µl during the derivatization.

XI. TIME REQUIREMENTS

The number of person-hours required to complete one set of thirteen samples was approximately 8 hours. Approximately ten hours were required for the GC/MS analysis, with each injection requiring ~ 21 minutes. A total of two calendar days were required to complete a set of thirteen samples including post-injection data analysis.

APPENDIX I: ANALYTICAL METHOD

"Dodine: Method of Analysis for Dodine in Soil using GC-MSD"

Summary Flowchart of Analytical Method

EXTRACTION

(1). Weigh 50 g of soil into a nalgene bottle.
(2). Spike as needed and wait for 10 min.
(3). Extract with 70 mL of 0.05 M KOH in 90:10 MeOH:H_2O mixture twice.
(4). Extract with 70 mL 1% HCl in methanol once.
(5). Combine all filtered extraction solutions and take final volume to 250 mL.

PARTITION

(1). Take a 50 mL aliquot and rotary evaporate to ~10 mL.
(2). Add 7.9 g salt and 25 mL water to a 125 mL sep-funnel, also transfer 10 mL extract to sep-funnel.
(3). Extract three times with 45 mL dichloromethane. Drain each lower phase through a folded 1 PS filter containing 10 g sodium sulfate.
(4). Rinse the sodium sulfate with 10 mL dichloromethane.
(5). Rotovap to dryness.

DERIVATIZATION

(1). Reconstitute in 6 mL of 1-chlorobutane.
(2). Add 100 µL of methanol, 30 µL of hexafluoroacetylacetone.
(3). Heat and stir at 100 °C for 1 hr .
(4). Dry down the solution under nitrogen at 30 - 35°C.
(5). Dilute to appropriate volume with MeOH and sonicate.
(6). Filter sample through a Gelman nylon filter.

Dodine: Method of Analysis for Dodine in Soil

I. Introduction

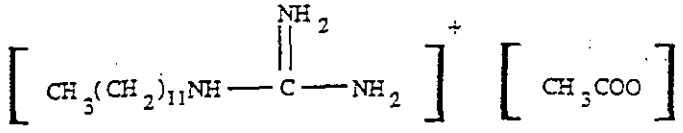
A. Scope

An analytical method is described for the analysis of dodine in soil, as defined in the Pesticide Assessment Guidelines, Subdivision O.

B. Principle

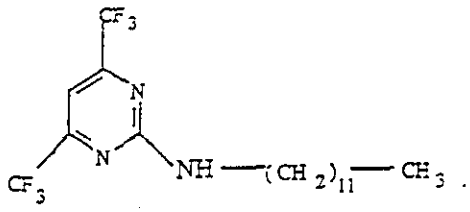
Soil samples are extracted using KOH in methanol/water twice and 1% HCl in methanol once. The extract is filtered and a 50 ml (10 gram equivalent) aliquot is taken. The aliquot is rotary-vap down to ~10 mL, added water and salt and partitioned into Methylene Chloride. A solvent exchange is done and the extract is then derivatized with hexafluoroacetylacetone. Quantification of the derivatized dodine is accomplished by gas chromatography using a mass selective detector.

C. Structures

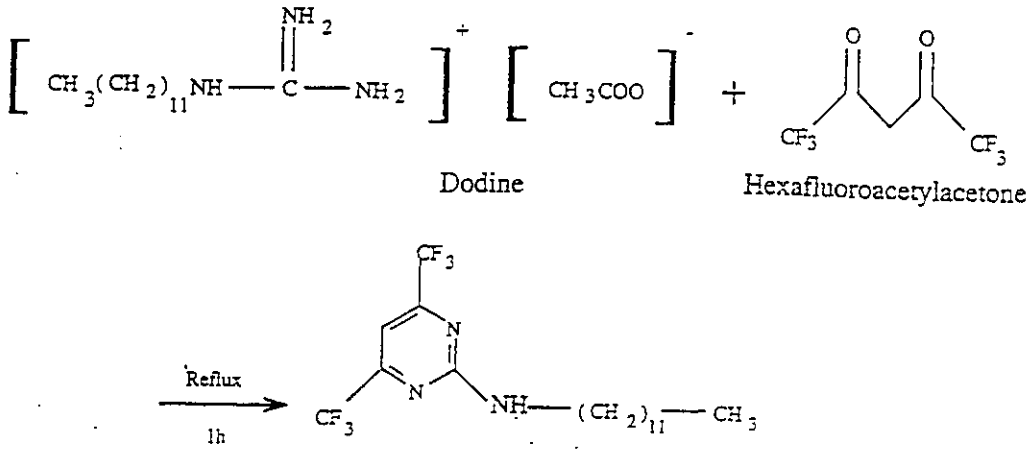


DODINE

DERIVATIZED DODINE



D. Reaction



Dodine

Hexafluoroacetylacetone

II. Materials

Reagents and Solvents were used as received from supplier, unless otherwise noted. Equivalent reagents and equipment may be substituted where appropriate.

A. Reagents

1. Sodium Sulfate, anhydrous, granular, J.T. Baker, Cat. No. 3375-05
2. Sodium Chloride, Reagent Grade, ACS, VWR Cat. No. VW6430-1 or equivalent
3. Potassium Hydroxide, pellets, Fisher Scientific, Cat. No. P251-500 or equivalent
4. Hydrochloric Acid, 36.5% -38%, GR, EM Cat. No. HX0603-13 or equivalent
5. 1,1,1,5,5,5 - Hexafluoro2,4-Pentanedione, 98%, Aldrich Cat. No. 23,830-9

B. Solvents

1. 1-Chlorobutane, 99.5% , Anhydrous, Aldrich Cat. No. 41-425-5
2. Methanol, EM OMNISOLV, VWR Scientific Cat. No EM-MX0484-1 or equivalent
3. Dichloromethane, EM OMNISOLV, VWR Scientific Cat. No EM-DX0831-1 or equivalent
4. Cyclohexane, EM OMNISOLV, VWR Scientific Cat. No EM-CX2286-1 or equivalent
5. Water, EM HPLC Grade, VWR Scientific Cat. No EM-WX0004-1 or equivalent

C. Equipment

1. Aluminum Crimp-Top Seal, 11 mm TFE/RUB Septum, Sun Brokers, Inc., Cat. No. 200 100
2. Analytical Balance
3. Autosampler Vials, 1 ml, clear, Wheaton, Cat. No. 223682
4. Disposable Pasteur Pipettes
5. Polypropylene Copolymer Centrifuge Bottle, 250 ml, Nalgene, Cat. No. 3141-0250
6. Graduated Cylinders, 250 ml with #27 stopper joint
7. Vacuum Adapters, S-1110-Special with #27 inner stopper joint Southeastern Lab Apparatus, Inc
8. Boiling Flasks, 125 ml with 24/40 joint and 250 mL with 24/40 joint
9. Separatory Funnels, 125 ml
10. Sonicator, Model 5200, Branson, Cat. No. B5210DTH
11. Volumetric Flasks, 100 ml, class A

12. Volumetric Pipettes, appropriate sizes, class A

13. Hewlett-Packard 5890 Series II GC equipped with Mass Selective Detector (refer to Section V of this document for details)

14. Capillary Column, DB-5, 30 m X 0.25 mm i.d., 0.25 µm film thickness, J & W Scientific, Cat. No. 122-1232 or equivalent

15. 10 mL Reaction Vial, Pierce Cat. No. 13225

16. Teflon Dics, Pierce Cat. No. 12722

17. Open Top Caps, Pierce Cat. No. 13219

18. Magnetic Stir Bars (spin vanes), VWR Cat. 58949-272 or equivalent

19. Rotary Evaporator, Brinkmann model ROTAVAPOR R110

20. Horizontal Shake with Timer, Thomas or equivalent

21. Buchner funnels, Coors porcelain 9 cm

22. GF/A Filter paper, Whatman Cat No. 1820 090, 9 cm

23. Phase Separator paper, Whatman N0. 1 PS, 11 cm, Cat No. 2200 110

24. Funnel, polypropylene, 66 mm top

25. Pipettes, appropriate sizes, Oxford or equivalent

26. Digital Pipettes, appropriate sizes, Eppendorf or equivalent

27. Reaction-Therm III Heating/Stirring Module, Pierce No. 18935 or equivalent

28. Optional: Dispenserte Bottle-Top, appropriate sizes, Brinkmann

29. Nitrogen evaporation manifold with moisture trap

30. 3 cc Syringe, B-D N0. 309585 or equivalent

31. Nylon Acrodisc filter (13 mm, 0.45 µm), Gelman No. 4426

D. **Analytical Standards**

Analytical Standards available from Rhône-Poulenc Ag Company

1. Dodine: 1-dodecylguanidinium acetate

2. Derivatized dodine: 2-dodecyl-4,6-bis(trifluoromethyl)pyrimidine

III. Standard Solution Preparation

A. **General**

1. The concentrations of standard solutions should be adjusted to account for the purity of the neat solid standards.

2. After preparation, standards should be transferred from the volumetric flasks into screw-capped amber bottles to prevent possible photodegradation.

3. Store standard solutions in the refrigerator at or below 4 °C when not in use.

B. **Fortification and Calibration Standard Solutions**

The following is provided as an example of how standard solutions may be prepared. Other concentrations may be used as appropriate.

1. Weigh 0.1000 g (±0.1 mg) of each analytical standard individually into 100 ml volumetric flasks. Dissolve each analytical standards in methanol and mix well. Dilute to final volume with methanol. Concentration of each standard is 1000 µg / ml.

2. Withdraw a 10.0 ml aliquot from each of the 1000 µg / ml individual standards and add to a 100 ml volumetric flask. Dilute to volume with methanol. The concentration of this standard is 100 µg / ml.

3. By further dilution of the 100 µg / ml standard with methanol, prepare a series of standards to serve as fortification standards or calibration standards.

IV. Methods of Analysis

The tilde symbol (~) indicates 'approximately'.

The "•" symbol indicates an appropriate stopping point. Samples may be stored in freezer(< 0° C) overnight and allowed to come to room temperature before continuing.

A. Sample Preparation

Use samples as received from processor.

B. Extraction

1. Weigh ~50.0 grams of soil into a 250 ml nalgene bottle. Spike at appropriate level and allow to sit for ~10 minutes.

2. Add ~70 mL of 0.05 M KOH in 90:10 MeOH:H_2O and shake on horizontal shaker for ~15 minutes. Centrifuge at ~2500 rpm for ~5 minutes.

3. Attach a 9 cm buchner funnel to a 250 ml mixing cylinder using a vacuum adapter. Decant liquid through a GF/A filter paper with slow vacuum.

4. Repeat step 2 and decant supernate liquid into cylinder.

5. Add ~70 mL of 1 % HCL in methanol to soil and shake for ~15 minutes, transfer everything (including soil) into the buchner funnel. Rinse the nalgene bottle and cap with small amount of methanol and transfer into the buchner funnel.

6.• Rinse buchner funnel tip and adapter with methanol and take volume to 250 mL with methanol. Mix this 250 mL extraction solution well. (Extract A)

C. Partition and solvent exchange

1. Take a 50 mL aliquot of Extract A with a class A Volumetric pipette and transfer to a 125 mL flat bottom flask. Rotary evaporate to ~ 10 mL at 40°C .

2. Add 7.90 - 7.94 g of salt to a 125 ml sep funnel, and transfer the 10 ml of extract from the 125 ml flat bottom flask. Add 25 mL of distilled water to the 125 ml flat bottom flask, swirl and transfer into the 125 ml sep funnel. Shake until all of the salt dissolves.(NOTE-1)

3. Add ~45 mL of Dichloromethane to the 125 ml flat bottom flask and rinse, then transfer to 125 ml sep funnel and shake vigorously for ~1 minute. Remember to vent frequently.

4. Fold PS-1 filter paper into 66 mm plastic funnel and add ~10 grams of sodium sulfate onto the filter paper. Drain the dichloromethane through the funnel and into a 250 ml flat bottom flask.

5. Repeat step 3 two times, draining the dichloromethane through the sodium sulfate and into the 250 ml flask each time. (Extract B) (NOTE-2)

6. Rinse sodium sulfate with ~10 ml of dichloromethane twice combining it with Extract B.

7. Concentrate Extract B to dryness using a rotary evaporator at 30 °C. Blow off any remaining dichloromethane with a gentle stream of nitrogen.(NOTE-3)

D. Derivatization

1. Add 3 mL of 1-chlorobutane and sonicate until residue is dissolved. Transfer the 3 mls to a 10 ml reaction vial, rinse the flask with an additional 3 mls and add that to the reaction vial.

2. Add 100 uL of methanol, 30 uL of 1,1,1,5,5,5 Hexafluro-2,4-pentanedione, and a triangle stir magnet to the reaction vial. Seal tightly with teflon seal and place the reaction vials in the heating block. Heat and stir at 100 °C for 1 hour.

3. Remove from heat and allow to cool for ~5 minutes before opening caps. Rinse cap and stir magnet with methanol, into the reaction vial not exceeding the 10ml volume. Then concentrate to dryness at 30°C-35°C with a gentle stream of N_2. (NOTE-4)

4. Dilute to appropriate volume with methanol and sonicate. (NOTE-5)

5. Filter sample through a Gelman nylon filter to eliminate solids before injecting

E. General Method Notes:

1. The salt solution in sep funnel is a saturated solution, It will be difficult to get all the salt into solution.

2. The 125ml flat bottom flask does not need to be rinsed with dichloromethane after the first time.

3. Nitrogen must have a moisture trap attached. the derivitization that follows is highly sensitive to moisture, any moisture will cause poor recoveries.

4. The heating blocks can be used for this if you allow time for the blocks to cool or replace the hot blocks and allow cool blocks to equilibrate. This is preferable to a water bath, as moisture will give recovery problems.

5. Dilute to no more than 9 mls at first, sonicate and filter, then make further dilutions if necessary from this volume. Use class A pipettes and class A volumetric flasks to do these further dilutions.

V. Gas Chromatography

A. Instrumentation

1.	Gas Chromatograph:	Hewlett-Packard 5890 Series II GC, 7673 Autosampler, 18594B Sampler Controller, Split/Splitless Injector, or an equivalent system
2.	Detector:	Mass Selective Detector, Hewlett-Packard Model 5972 or equivalent
3.	Data Acquisition:	Hewlett-Packard ChemStation
4.	Column:	J & W Scientific DB-5 30 m X 0.25 mm i.d., 0.25 µm film thickness (*or HP-5 same diminsions*)

B. Gas Chromatograph Conditions

1.	Carrier Gas:	Helium, Head Pressure set at 15 PSI with a 1.5 minute 45psi pulse at injection.
2.	Inlet Liner:	4-mm i.d. nominal volume 900 µl, borosilicate glass with single taper on GC end (HP part #5181-3316)
3.	Injector Temperature:	250 °C
4.	Detector Temperature:	325 °C
5.	Oven Temperatures:	Initial: 100 °C, hold 1 minute Ramp 20 °C / min to 195°C, hold 0 minute Ramp 5 °C / min to275 °C, hold 3 minute Ramp 30 °C / min to 300 °C, hold 5 minutes
6.	Injection Volume:	1.0 µL

7. Splitless injection with split vent off for 60 seconds.

C. Mass Selective Detector Parameters

1.	Tune File	Maximum Sensitivity Autotune
2.	Solvent Delay	3.0 or 6.0 minutes
3.	EM voltage	400-600 over tune
4.	Acquisition Mode	SIM
5.	SIM Parameters	GROUP 1 Derivatized dodine High Resolution - Yes Dwell time per ion 100 msec Starting time 8.0 min Quantitation ion 244.00 Qualifier ions 245.00 & 399.20

D. General Chromatography Notes / Potential Problems

1. Several standards should be injected prior to actual analysis using a new column or after the GC has set idle for any considerable length of time to condition and/or to remove any contaminants.

2. A gold plated seal is used at the interface of the glass liner and column. Hewlett-Packard Part No. 18740-20885.

3. The GC parameters are guidelines and can be optimized for the instrument and column actually used. Record the actual GC conditions used for data acquisition and include in report.

4. The detector ion amu values are nominal. Exact values should be determined from a scan run after each tune.

VI. Quantification of Residues

A. Calibration Curves

1. Linear regression should be used to generate a calibration curve for the analyte. At least four different standard concentrations should be run with each set of samples. Standards

should be interspersed with samples to compensate for any minor change in instrument response. Extracts should be diluted such that the peak areas obtained are within the area range between the lowest and highest standards injected.

2. Linear regression coefficients should be calculated from 'peak area' (or 'peak height') versus 'nanogram / ml injected'. Data from the analytical standards should be fit to the linear equation, $y = a + bx$.

where: y = peak area or height
a = calibration line intercept
b = calibration line slope
x = conc of analyte in inj soln

B. Quantification of Residues

1. Derivarized dodine should be quantified by comparison to the standard curves obtained from a linear regression analysis of the data.

2. Equations

2.1 Concentration of analyte in sample in ppb (parts per billion).

$$z = (y - a) / b \times c / d$$

where: y = peak area (or height), response of analyte of interest

a = intercept of calibration line from linear regression (area or height)

b = slope of calibration curve from linear regression (response per ng/ml)

c = final volume of sample (ml)

d = sample weight (g)

z = conc of analyte in sample (ppb)

2.2 Corrected concentration of analyte in sample in ppb.

$$Z' = z \times C$$

where: Z' = corrected concentration

z = concentration found from curve
C = conversion factor

for conversion of

derivatized dodine to dodine C = 0.72

2.3 Percent recovery

$$\% \text{ recovery} = \frac{(\text{ppb found in fort sample} - \text{ppb found in UTC}) \times 100\%}{\text{actual fortification level in ppb}}$$

3. Residues shall be reported as dodine equivalents.