449857-02

STUDY TITLE

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

DATA REQUIREMENT

Residue Chemistry Test Guidelines, OPPTS 860.1340

STUDY COMPLETION DATE

March 18, 1998

1.0 INTRODUCTION

1.1 Study Objective

A method for the analysis of dodine residues in soil has been developed at Rhône-Poulenc Ag Company. The objective of this study is to provide validation data for the enforcement method in support of the registration of an end use product in accordance with EPA Residue Chemistry Test Guidelines, OPPTS 860.1340. This study was conducted in compliance with the EPA FIFRA Good Laboratory Practice Standards, Final Rule, 40CFR 160, October 16, 1989.

1.2 Study Protocol

The protocol for study EC-97-384 was approved on June 24, 1997, identifying the following residue method of analysis for use in the study.

The method validation parameters to be determined as set forth in the protocol are: Method Detection Limit (MDL), Limit of Quantitation (LOQ), Precision, Accuracy, Extraction Efficiency, Ruggedness, Time requirements, and Range of Linearity.

The GC-MSD method uses a retention time and three mass ions in a specific ratio for identification during the quantification process. Because of the nature of this technique, no confirmatory methods or specificity testing are required.

The protocol and analytical method are appended to this report in Appendix A.

2.0 MATERIALS AND METHODS

2.1 Test Samples

Soil samples were obtained from untreated areas adjacent to the plots established for the Rhône-Poulenc Ag Company Study US96X10342, a field soil dissipation study conducted in California, Washington, Georgia and New Jersey. The samples were shipped and received frozen at Rhône-Poulenc Ag Company, RTP, North Carolina 27709. The samples were stored frozen in

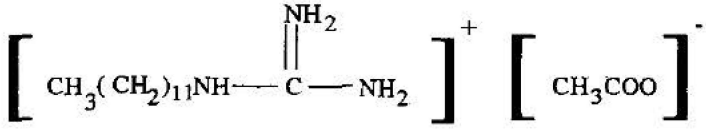
plastic bags when not in use. The dates of collection, shipping and receipt are reported with the above sited study.

2.2 Analytical Standards

The following analytical standard materials were supplied by Rhône-Poulenc Ag Company, Research Triangle Park, NC 27709. Certificates of analysis for the analytical standard materials are included in Appendix E.

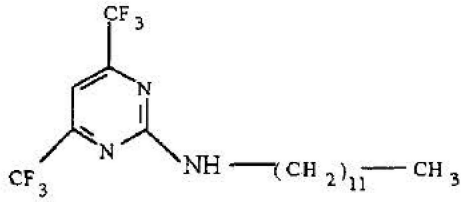
1) Dodine

CAS Number: 2439-10-3
Chemical Name: 1-dodecylguanidinium acetate
Log Number: EA840SD2
Purity: 100.0%
Structure:



2) Derivatized Dodine

Chemical Name: 2-dodecylamino-4,6-bis(trifluoromethyl)pyrimidine
Log Number: EA5242SD1
Purity: 99.4%
Structure:



The analytical standard materials were stored in an airtight container at or below the storage conditions specified on the certificate of analysis. Standard and spiking solutions were stored below -10°C when not in use.

2.3 Standard Solution Stability

Standard solution stability data collected as part of a storage stability study of dodine residues on fruit and their processed fractions (EC-96-357) indicate that solutions of the dodine fortification solutions and of the derivatized dodine calibration solutions are stable for more than six months when stored as indicated above.

2.4 Method of Analysis

The method of analysis used in the study was "Dodine: Method of Analysis for Dodine in Soil using GC-MSD," File Number 45316, approved May 12, 1997. The method is included in Appendix A.

In this method, soil samples are extracted twice using KOH in methanol/water and once using 1% HCL in methanol. The combined extracts are filtered and a 50 mL (10 gram equivalent) aliquot is taken. The aliquot is evaporated to ~10 mL, water and salt is added and the residues are partitioned into methylene chloride. The sample extract is evaporated to dryness and reconstituted with 1-chlorobutane and derivatized with hexafluoroacetyl-acetone. Quantification of the derivatized dodine is accomplished by gas chromatography using a mass selective detector.

2.5 Instrument Parameters

A Hewlett Packard 5890A Gas Chromatograph (S/N 3336A50035) equiped with a Hewlett Packard 5972A Mass Selective Detector (S/N 3329A00660) and a Hewlett Packard 7673 Autosampler (S/N 3338A36260) was used for the analyses. The column used was a 30 m x 0.25 mm, 0.25µm film, J&W DB-5 column. The carrier gas was Helium set at ca. 15 psi with a 1.5 minute 45 psi pulse at injection. The injection volume used was 1.0 µL. The injection technique was splitless for 60 seconds.

The injector temperature was 250°C, the detector temperature was 325°C, the oven temperature program was as follows. The initial oven temperature was 100°C which was held for 1 minute after the injection. This was followed by a 20°C per minute ramp to an oven temperature of 195°C which was followed by a 5°C per minute ramp to an oven temperature of 275°C. This was followed by a 40°C per minute ramp to a final oven temperature of 300°C which was held for 5 minutes.

The Mass Selective Detector parameters are presented in the method of analysis. The nominal quantitation ion for the derivatized dodine was 244.00, the nominal qualifier ions were 245.00 and 399.20. These values are nominal,

the exact values used were determined from a scan run at the beginning of an analytical set.

2.6 Data Collection

A Hewlett Packard ChemStation was used to record data generated by the GC-MSD. Peak areas were collected for the quantitation (or Target) ion and for the two qualifier ions (Q1 and Q2). Subsequent processing of the data for the quantitative ion enabled calculation of analyte concentrations of the samples using standard curves as described below. Ratios of the two qualifier ions with the quantitation ion provided confirmatory information.

2.7 Example Calculations

The concentrations of derivatized dodine was determined using a multi-point calibration curve for the analyte. With each analytical set, a set of calibration standards with a range of concentrations (ng/mL) was used to produce a standard curve. Linear regression coefficients were calculated by regression analysis of peak area vs. concentration. The data from the analytical standards fit the standard linear model to produce a slope and intercept which then were used to determine the concentration in the samples.

The results are converted to dodine using a molecular weight conversion factor. The equation used to determine the concentration of residues is:

$$ppb = \frac{[(y - a)ce]}{(bdf)}$$

where:
- y = peak area or height
- a = intercept (area counts or height)
- b = slope (area counts(or height)/ng/mL)
- c = final dilution volume (mL)
- d = sample weight (g)
- e = extract volume (mL)
- f = volume of aliquot taken for clean-up and derivatization (mL)

The results are converted to dodine as follows:

$$Z' = Z \times 0.72$$

where:
- Z' = dodine concentration
- Z = concentration of derivatized dodine found
- 0.72 = conversion factor (M.W. dodine / M.W. derivatized dodine)

The percent recovery of the freshly fortified UTC samples was calculated as follows:

$$\textbf{Percent Recovery} = \frac{r - u}{s} \times 100$$

where: r = concentration found in fortified sample
u = concentration found in the UTC
s = spiking level

The following example illustrates the determination of dodine in the California soil sample "Spike-1" which is an untreated control sample freshly fortified with 10 ppb of dodine. This example is from the worksheet on page 60 of this report.

Example of Calculations

For this example:

Peak Area for Sample "Spike-1"	= 288319
Intercept of the Standard Curve	= 5614
Slope of the Standard Curve	= 8630
Final Dilution Volume	= 4 mL
Sample Weight	= 50 g
Extract Volume	= 250 mL
Volume of Aliquot Taken	= 50 mL

$$\textbf{ppb} \text{ (derivatized dodine)} = \frac{[(288319 - 5614) \times 4 \times 250]}{(8630 \times 50 \times 50)} = 13.10$$

The result is converted to dodine as follows:

$$\textbf{ppb} \text{ (dodine)} = 13.10 \times 0.72 = 9.43$$

Validation Protocol: Appendix A

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

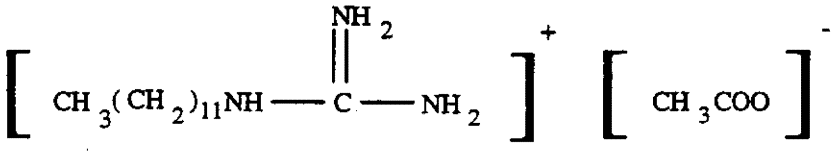
A. Scope

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

B. Principle

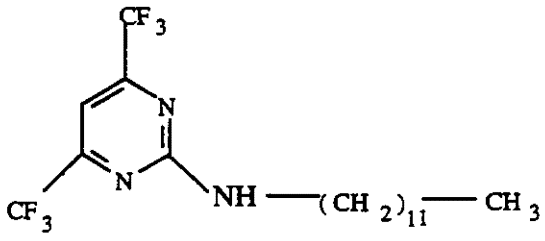
Soil samples are extracted using KOH in methanol/water twice and 1% HCl in methanol once. The extract is filtered and a 50 ml (10 gram equivalent) aliquot is taken. The aliquot is rotary-vap down to ~10 mL, added water and salt and partitioned into Methylene Chloride. A solvent exchange is done and the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

C. Structures

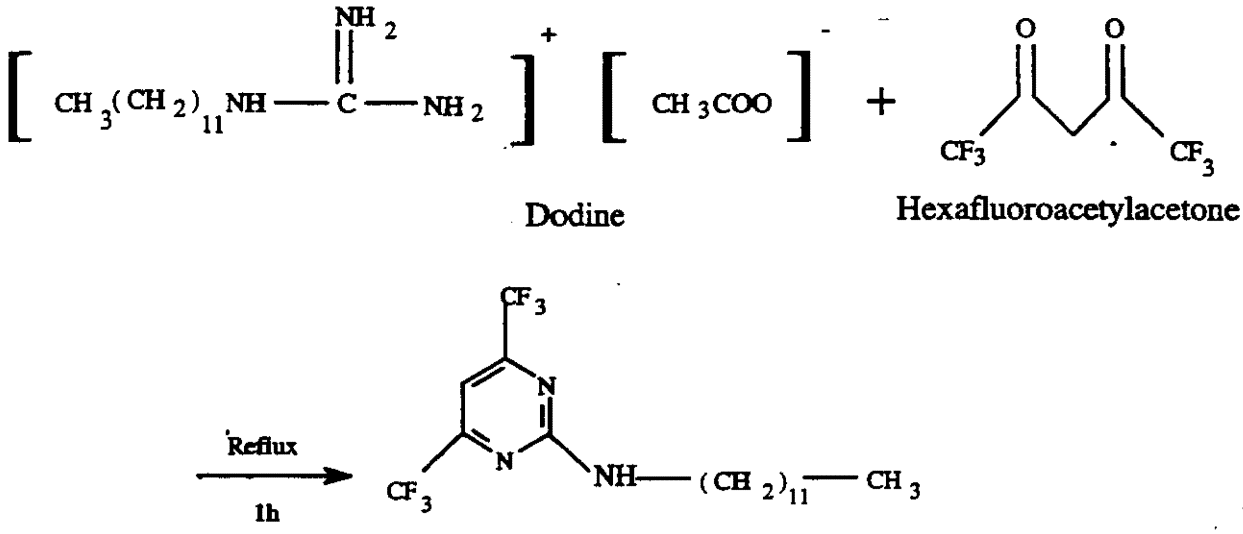


DODINE

DERIVATIZED DODINE



D. Reaction



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: Dispensette 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. Derivatized 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

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

3. Residues shall be reported as dodine equivalents.