SPONSOR

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STUDY TITLE

LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES

DATA REQUIREMENTS

EPA Residue Chemistry Test Guidelines, OPPTS 860.1340,
Residue Analytical Method

AUTHORS:

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STUDY INITIATION DATE:

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November 15, 2002

PERFORMING LABORATORY

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PROJECT ID

Wildlife International, Ltd. 236C-113

Total Pages = 122

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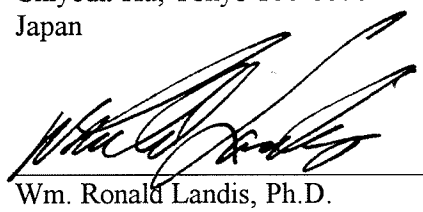

Wm. Ronald Landis, Ph.D.

Date: 5 January, 2002

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and/or 792, August 17, 1989 and OECD Principles of Good Laboratory Practice, (ENV/MC/CHEM (98) 17).

The test substances were not characterized in accordance with Good Laboratory Practice Standards.

The stability of the test substances under storage conditions at the test site were not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

Jon A MacGregor, B.S.
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DATE

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Sponsor's Representative

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TITLE: Laboratory Validation of Method(s) for the Analysis of MTI-446 and Its Metabolites DN and UF in Multiple Crop Substrates

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 236C-113

STUDY COMPLETION DATE: November 15, 2002

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and/or 792, August 17, 1989 and OECD Principles of Good Laboratory Practice, (ENV/MC/CHEM (98) 17). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Matrix Fortification - Tomato	August 2, 2002	August 2, 2002	August 13, 2002
Sample Extraction - Melon	August 14, 2002	August 14, 2002	August 27, 2002
Back Partitioning – Grapes	September 13, 2002	September 13, 2002	September 20, 2002
Sample Processing – Potato Chips	October 8, 2002	October 8, 2002	October 11, 2002
Sample Processing – Potato, Wet Peels	October 9, 2002	October 9, 2002	October 11, 2002
Raw Data and Draft Report	October 16, 18 and 21-25, 2002	October 25, 2002	October 29, 2002
Final Report	November 11, 2002	November 11, 2002	November 15, 2002

Signed: Susan L. Coleman Date 11-15-02
Susan L. Coleman, B.A.
Senior Quality Assurance Representative

REPORT APPROVAL

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WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 236C-113

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Jon A MacGregor, B.S.
Scientist

11-15-02
DATE

MANAGEMENT:

Willard B. Nixon, Ph.D.
Director of Chemistry

11/15/02
DATE

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The following personnel were involved in the conduct or management of this study:

1. Willard B. Nixon, Ph.D., Director of Chemistry
2. Jon A. MacGregor, B.S., Scientist
3. Kenneth Chafey, B.S., Senior Chemist
4 Deborah L. Fischer, M.S., Chemist
5 Raymond L. Van Hoven, Ph.D., Scientist

STUDY IDENTIFICATION

LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES

Wildlife International, Ltd. Project Number:	236C-113
Test Substance:	MTI-446, DN and UF Metabolites of MTI-446
Sponsor:	Mitsui Chemicals, Inc. Agrochemicals Division 3-2-5, Kasumigaseki Chiyoda-Ku, Tokyo 100-6070 Japan
Sponsor's Representative:	William Ronald Landis, Ph.D. Landis International, Inc. 3185 Madison Highway Valdosta, GA 31603-5126 U.S.A.
Study Director	Jon A. MacGregor Wildlife International, Ltd. 8598 Commerce Drive Easton, MD 21601 U.S.A.
Study Timetable:	Experimental Start Date (OECD): July 29, 2002 Experimental Start Date (EPA): August 2, 2002 Experimental Completion Date: October 11, 2002

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ABSTRACT

A study was conducted to provide method validation data for the determination of residues of MTI-446 and its DN and UF metabolites in multiple crop substrates. Representative crops from selected raw agricultural commodity (RAC) groups and representative products from selected processed commodities (PC) were fortified with MTI-446, DN, and UF at 0.0100 (1x LOQ) and 1.00 (100x LOQ) mg/Kg. Samples were processed and analyzed for the determination of MTI-446, DN, and UF residues by high performance liquid chromatography with triple quadrupole mass spectrometric detection (HPLC/MS/MS).

The method was validated in potato and grape RAC groups. In addition, the method was validated using representative crops (indicated in parenthesis) from the following RAC groups: fruiting vegetables (tomato), cucurbits (melon), brassica (broccoli), and leafy vegetables (lettuce). The method was also validated in processed commodities to include tomato paste, tomato puree, potato flakes, potato chips, potato wet peel, raisins and grape juice. The method was demonstrated to be applicable for the determination of residues of MTI-446 and its DN and UF metabolites in multiple crop substrates following foliar and/or soil applications of MTI-446 20%SG at maximum label rates.

INTRODUCTION

Wildlife International, Ltd. performed analytical method(s) validations for the determination of MTI-446 (dinotefuran) and its DN (1-methyl-3-(tetrahydro-3-furylmethyl)guanidine) and UF (1-methyl-3-(tetrahydro-3-furylmethyl)urea) metabolites in multiple crop substrates. The analytical method is based on the methods "Analysis of MTI-446 in Crops (HPLC Method)" (1) and "Method RCC Study Number 841464 MTI-446, DN and UF" (2). A flow chart of the method is presented in Appendix A. The study provided data to validate the method subsequently applied to the analysis of samples harvested for determination of residues of MTI-446 and its DN and UF metabolites in crop substrates (raw and processed) following application of foliar and/or ground applications of MTI-446 20%SG at maximum label rates. The study was performed at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland.

PURPOSE

This study was conducted to validate the analytical method(s) to be used for determination of residues of MTI-446 and its DN and UF metabolites in field-collected samples following foliar and ground applications of MTI-446 20%SG at maximum label rates.

EXPERIMENTAL DESIGN

Selected raw agricultural and processed commodity crop substrates were fortified with each test substance (MTI-446, DN and UF) at two different concentrations and analyzed according to the method to be applied to determination of residues (MTI-446, DN, and UF) in field-harvested samples from residue studies (Appendix B). Reagent and matrix blanks (controls) were analyzed concurrently to evaluate potential analytical interferences.

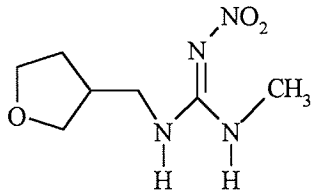
MATERIALS AND METHODS

Test Substances

Test substances of MTI-446, the DN metabolite of MTI-446 and the UF metabolite of MTI-446 were received from Landis International, Inc. on January 4, 2002. These test substances were assigned Wildlife International, Ltd. Identification Numbers 5856, 5857 and 5858, respectively, and transferred to ambient storage in darkness. Certificates of Analysis were received with each test substance (Appendix C) and provided the following information.

MTI-446 (CAS Number 165252-70-0)

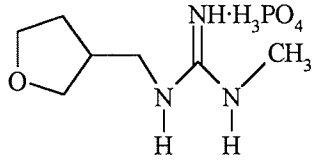
Chemical Name: (*RS*)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine
Structural Formula:



Appearance: white powder
Lot Number: EBI-5-101
Purity: 99.6%
Expiration Date: December 2003

DN

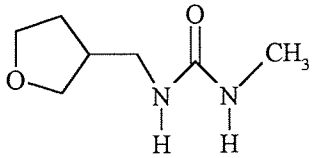
Chemical Name: 1-methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate
Structural Formula:



Appearance: white powder
Lot Number: OFU-1290
Purity: 97.27%
Expiration Date: December 2003

UF

Chemical Name: 1-methyl-3-(tetrahydro-3-furylmethyl)urea
Structural Formula:



Appearance: white powder
Lot Number: OFU-1291

Purity: 99.59%
Expiration Date: December 2003

The standards were stored under ambient conditions and were used to prepare matrix fortification samples and calibration standards.

Test Systems

Submitted field controls and/or locally purchased commodities were employed for preparation of matrix blank and matrix fortification samples in each raw agricultural commodity investigated. Processed commodities were procured from local food markets for preparation of matrix blank and matrix fortification samples in each processed commodity investigated.

Preparation of Stocks and Standards

A primary stock solution of MTI-446 was prepared in a solution of 15% methanol and 85% NANOpure® water (v/v) at a concentration of 1.00 µg /µL. Primary stock solutions of DN and UF were each prepared in NANOpure® water at a concentration of 1.00 µg /µL. A combined secondary stock of MTI-446, DN, and UF was prepared in NANOpure® water from these individual primary stocks at a concentration for each component of 0.100 µg/µL. Additional combined stocks at concentrations for each component of 0.0100 and 0.00100 µg/µL in NANOpure® water (v/v) were prepared by serial dilution from the 0.100-µg/µL combined stock. Working calibration standards in 0.1N HCL, ranging in concentration for MTI-446, DN, and UF from 0.00100 to 0.200 µg/mL, were prepared from the 0.00100-µg/µL (1.00 µg /mL) combined stock for the analysis of the validation samples. All stock solutions were prepared using volumetric flasks, pipets, and/or gas-tight syringes. All stock and calibration standard solutions were stored under refrigerated conditions when not in use.

Fortification of Recovery Samples

MTI-446 and its metabolites DN and UF were fortified into the same control matrix using an appropriate combined stock solution or solutions. For a given fortification level, each matrix was fortified at a concentration of each component of 0.0100 mg/Kg (1x LOQ) and 1.00 mg/Kg (100x the LOQ), as specified in the protocol. Analyses of the control matrix and a reagent blank accompanied each analytical set of fortified samples.

Sample Extraction, Clean-up and Analysis

Sample Extraction - MTI-446, UF and DN Metabolites

Procedural recovery samples for validation of the methodology for determination of MTI-446, and its UF and DN metabolites were prepared for extraction by transfer of 25.0 g of homogenized sample into separate, labeled 500-mL glass bottles with screw caps (wide-mouth French square). Procedural recovery samples were prepared for each analytical set by transfer of 25-g subsamples to glass bottles, followed by fortification with known volumes of the prepared stock solutions of MTI-446, UF and DN. To each sample, 150 mL of acetonitrile:water (80:20, v:v) was added followed by 0.5 mL of concentrated hydrochloric acid. Samples were subsequently blended for approximately

five minutes using a high-speed Ultra Turrax sample homogenizer. Samples were then further extracted by shaking on a shaker table for 30 minutes at 150 revolutions per minute (rpm).

Following extraction, samples were filtered by suction through a pad of Celite 545 (approximately 10 g) into a 1-L round bottom flask. The filter cake was then rinsed with an additional 100 mL of acetonitrile:water (80:20, v:v) and the rinsate combined with the former in the 1-L round bottom flask.

Filtered extracts were transferred to 500-mL separatory funnels. The 1-L flasks were rinsed with 100 mL of hexane and the rinsate also transferred to the separatory funnels. The separatory funnels were shaken for approximately one minute and the phases allowed to separate. The lower acetonitrile:water phases were drained into 1-L round bottom flasks and the hexane portions discarded. The acetonitrile:water phases were transferred back to their respective separatory funnels and partitioned a second time with a 50-mL aliquot of hexane. The acetonitrile:water partitioned phases were returned to their respective 1-L round bottom flasks. Each of the acetonitrile:water phases was rotary evaporated to the aqueous remainders at a water bath temperature of 50 - 60°C. The aqueous remainders were adjusted to approximately pH 8 by drop-wise addition of 0.5M sodium carbonate-sodium hydrogen carbonate buffer (Buffer A). The resulting pH-adjusted aqueous solutions were transferred to 100-mL graduated cylinders and adjusted to volumes of 80.0 mL with NANOpure® water.

Sample Clean-Up - MTI-446 and UF Metabolite

Aliquots of the aqueous extracts, 40-mL each, from the Sample Extraction method phase above, were transferred to 50-mL volumetric flasks containing approximately 15 g of sodium chloride. Volumes were brought to 50-mL with NANOpure® water. The resulting mixtures were transferred to French square bottles, placed on a shaker table and agitated for approximately 60 minutes at 150 rpm.

Aliquots of the aqueous solutions, 3.0 mL, were quantitatively transferred onto Extrelut® NT3 solid-phase extraction columns. The extracts were allowed to absorb for 30 to 60 minutes. Columns were eluted with 120 mL of ethyl acetate while collecting the eluates in 250-mL round bottom flasks. The ethyl acetate eluates were rotary evaporated to dryness at a bath temperature of approximately 40 to 50°C. The dried residues were reconstituted in 3.0-mL aliquots of 0.1N HCl. If particulates were observed in the solution, the extracts were filtered through 0.45-µm Acrodisc filters.

Sample Clean-Up - DN Metabolite

Aliquots, 40.0-mL of the aqueous extracts from the Sample Extraction method phase above, were transferred to 50-mL plastic centrifuge tubes. Aliquots of Buffer A and NANOpure® water, 5 mL each, were added to each and the solutions mixed. If the solutions appeared to contain particulates, at least 5 mL of each were filtered through 0.45-µm Acrodisc filters.

BondElut® CBA solid phase extraction (SPE) columns were prepared by rinsing with 3 mL of methanol followed by 4 mL of 0.05M sodium carbonate-sodium hydrogen carbonate (Buffer B). The columns were not allowed to go to dryness between conditioning rinses nor following the final buffer rinse.

Aliquots, 5.0-mL for each sample, were quantitatively transferred onto the BondElut® CBA SPE columns. The extracts were allowed to pass through the columns at 1 to 2 mL/minute. Each cartridge was rinsed with 10 mL of NANOpure® water followed by 6 mL of methanol. The methanol was drained to just below the surface of the cartridge frit. All eluates were discarded. The column was quantitatively eluted with 4.0 mL of 0.1N hydrochloric acid. The eluate was collected in a 5-mL Class A volumetric flask or equivalent container. Final volumes were adjusted to 5 mL with 0.1N hydrochloric acid. If the final solutions appeared to contain particulates, solutions were filtered through 0.45-µm Acrodisc filters.

Final Solution for MTI-446, UF and DN Quantitation

Equal aliquots of the final 0.1N hydrochloric acid solutions containing MTI-446 and its UF metabolite were combined with the final solutions containing the DN metabolite of MTI-446 for each respective sample. An aliquot of each mixed solution was transferred to an autosampler vial for subsequent analyte separation and quantitation by LC/MS/MS (Table I).

Calculation of Percent Recovery

Standard curves were prepared by plotting the analyte concentration (µg/mL) on the abscissa and the respective peak area response on the ordinate as shown in Figures 1-3. Weighted linear regression or linear regression forced through zero analysis was applied to the data to determine the equation with respect to the abscissa as shown below:

$$PA = mC + b$$

where PA = peak area
m = slope of the line
C = concentration
b = y - axis intercept

Concentrations of analyte in the final solutions of samples were calculated using a rearrangement of the above equation:

$$C = \frac{PA - b}{m}$$

For purposes of this calculation section, the data for MTI-446, only, will be used. The same equations and calculations were applied for the quantitation of the DN and UF metabolites. Using the results from the least squares regression analysis with the data from Figure 1:

$$C = \frac{PA - (6702.1528)}{57168392}$$

The net concentration of MTI-446 in each corresponding recovery sample was determined by substituting the resulting analyte peak area into the above equation and solving for the concentration. Using the data for a 0.0100 mg MTI-446/Kg fortification in tomato (Table II and Appendix D.3,

Sample Number 236C-113-VMAS-1), the concentration in the final sample solution was calculated as:

$$C = \frac{81605 - (6702.1528)}{57168392} = 0.001310 \text{ mg/L}$$

The residue concentrations (mg/Kg) of MTI-446 in the fortified recovery samples were determined as follows:

$$\text{Concentration MTI-446 in mg/Kg} = \frac{(C) \times (V_e) \times (V_{f1}) \times (V_{f2}) \times (V_{f3})}{(W) \times (V_{i1}) \times (V_{i2}) \times (V_{i3})}$$

where C = Concentration (mg/L) as determined above
V_e = Extraction Volume (80.0 mL)
V_{i1} = First Initial Volume (40.0 mL)
V_{i2} = Second Initial Volume (3.00 mL for MTI-446 and UF, 4.00 mL for DN metabolite)
V_{f1} = First Final Volume (50.0 mL)
V_{f2} = Second Final Volume (3.00 mL for MTI-446 and UF, 4.00 mL for DN metabolite)
V_{f3} = Total volume of combined injection solution (2.00 mL)
V_{i3} = Aliquot volume of combined injection solution (1.00 mL)
W = Weight of extracted sub-sample (25.0 g)

Using the data for the 0.0100-mg MTI-446/Kg (236C-113-TOMVMAS-1), the concentration of MTI-446 in tomato was calculated as:

$$\text{Concentration in mg/Kg} = \frac{0.001310 \times 80.0 \times 50.0 \times 3.00 \times 2.00}{25.0 \times 40.0 \times 3.00 \times 1.00}$$

$$\text{Concentration in mg/Kg} = 0.01048 \text{ mg/Kg}$$

The percent recovery was determined by dividing the concentration of the analyte recovered in the fortified sample by the theoretical concentration added as shown below:

$$\text{Recovery} = \frac{\text{mg MTI-446/Kg Found}}{\text{mg MTI-446/Kg Added}} \times 100\%$$

For the above 0.0100 mg MTI-446/Kg sample, the percent recovery of MTI-446 was calculated as:

$$\text{Recovery} = \frac{0.01048 \text{ mg MTI-446/Kg Found}}{0.0100 \text{ mg MTI-446/Kg Added}} \times 100\%$$

$$\text{Recovery} = 105\%$$

Statistical Treatment of Data

Average recoveries for each analyte were calculated by dividing the sum of the percent recoveries by the total number of fortified samples.

Standard deviations for each analyte were also determined. The standard deviation was calculated by summing the squares of the individual deviations from the average recoveries, dividing by the number of degrees of freedom, and extracting the square root of the quotient.

RESULTS AND DISCUSSION

Representative Calibration Curve Data

The coefficient of determination (r^2) of the least squares equation describing the detector response as a function of the concentration of calibration standards injected to establish a standard curve were greater than 0.990 for all analytes, thereby demonstrating the linearity of the detector response to analyte amount. Representative calibration curves of MTI-446, DN and UF are illustrated in Figures 1, 2 and 3, respectively. Representative chromatograms of low and high-level calibration standards for MTI-446, DN, and UF are presented in Figures 4 and 5, respectively.

Analytical Recovery Data

A validation study was conducted on representative crop substrates to demonstrate the robustness and the stated limit of quantitation for the referenced methods (1,2). The results for MTI-446 and metabolites DN and UF in fruiting (tomato), cucurbit (melon), potato, grape, brassica (broccoli), and leafy vegetable (lettuce) RAC groups are summarized in Tables II-VII, respectively. The results for MTI-446 and metabolites DN and UF in tomato (paste and puree), potato (flakes, chips, and wet peel) and grape (raisin and grape juice) processed commodities are summarized in Tables VIII-XIV, respectively. Representative chromatograms of a matrix blank and low- and high-level matrix fortifications for MTI-446 and metabolites DN and UF in each crop substrate are illustrated in Appendix D.

Concurrent with the series of matrix fortification samples, a reagent blank and a matrix blank sample were analyzed in each representative crop substrate to determine possible interferences. No interferences were observed at or above the LOQ during the sample analysis for any of the crop substrates investigated (Tables II-XIV).

The overall mean and range of procedural recoveries obtained for MTI-446 and metabolites DN and UF in each of the RAC groups and processed commodities investigated are summarized as follows:

RAC Group	MTI-446 Mean ± SD Range CV	DN Mean ± SD Range CV	UF Mean ± SD Range CV
Fruiting (Tomato)	110 ± 2.92 105 - 115 2.7%	94.4 ± 4.38 87.5 - 99.2 4.6%	98.5 ± 7.26 89.0 - 106 7.4%
Cucurbit (Melon)	86.4 ± 4.68 82.1 - 97.8 5.4%	101 ± 4.01 95.1 - 108 4.0%	100 ± 6.24 90.9 - 107 6.2%
Potato	107 ± 6.18 97.6 - 115 5.8%	90.0 ± 2.49 86.3 - 93.3 2.8%	104 ± 2.16 99.7 - 107 2.1%
Grape	100 ± 7.20 89.2 - 111 7.2%	87.3 ± 1.58 84.5 – 90.1 1.8%	104 ± 2.80 98.5 - 108 2.7%
Brassica (Broccoli)	99.4 ± 6.39 89.3 - 110 6.4%	89.9 ± 1.57 88.1 – 93.2 1.7%	101 ± 3.37 95.7 - 104 3.3%
Leafy Vegetable (Lettuce)	109 ± 6.48 94.0 - 116 5.9%	108 ± 6.58 99.0 - 118 6.1%	111 ± 3.68 105 - 116 3.3%

Processed Commodity	MTI-446 Mean ± SD Range CV	DN Mean ± SD Range CV	UF Mean ± SD Range CV
Tomato Paste	94.4 ± 7.87 85.6 - 107 8.3%	78.6 ± 6.49 72.3 - 91.1 8.3%	92.5 ± 4.17 85.6 - 99.3 4.5%
Tomato Puree	99.0 ± 8.69 87.6 - 110 8.8%	94.6 ± 4.87 85.5 - 103 5.2%	96.1 ± 4.01 90.9 - 103 4.2%
Potato Flakes	102 ± 9.02 85.7 - 113 8.8%	93.0 ± 2.69 88.7 – 98.0 2.9%	91.8 ± 9.56 74.8 - 104 10%
Potato Chips	95.2 ± 4.70 88.1 - 102 4.9%	90.6 ± 2.77 86.9 – 94.2 3.1%	91.1 ± 3.77 85.7 – 96.6 4.1%
Potato Wet Peel	87.3 ± 12.3 70.6 - 106 14%	104 ± 3.71 98.5 - 111 3.6%	97.0 ± 8.40 85.2 - 107 8.7%
Raisin	92.3 ± 4.61 87.5 - 101 5.0%	72.7 ± 3.05 67.0 – 78.3 4.2%	87.1 ± 5.63 78.2 – 97.4 6.5%
Grape Juice	103 ± 6.51 92.5 - 110 6.3%	88.1 ± 11.5 74.3 - 116 13%	105 ± 3.27 101-111 3.1%

CONCLUSION

Recoveries obtained for MTI-446 and its metabolites DN and UF during method validation in representative raw agricultural and processed commodity matrices are summarized in Tables II –XIV. Based on the data in this report, the analytical methods as presented herein yielded recoveries within the acceptable range of 70 to 120%. Thus, the validation of the method for the analysis of MTI-446 and its metabolites DN and UF in crop substrates was considered successful. The method was demonstrated to be applicable for the determination of residues of MTI-446 and its DN and UF metabolites in field collected samples of multiple crop substrates following foliar and ground applications of MTI-446 20%SG at maximum label rates.

ARCHIVING

The original protocol, raw data and a copy of the final report are archived at the Wildlife International, Ltd. testing facility in Easton, Maryland.

REFERENCES

1. Analysis of MTI-446 in Crops, Mitsui Chemicals, Inc., Life Sciences Laboratory, Crop Protection Section, Dr. Koji Kitajima, June 1999.

2. Method RCC Study Number 841464 MTI-446, DN and UF, RCC Ltd, Environmental Chemistry and Pharmanalytics Division.

3. Residue Analytical Method, U.S. Environmental Protection Agency Series 860 - Residue Chemistry Test Guidelines, OPPTS 860.1340, 1996.

Table I. Analytical Instrumentation and Equipment for the Determination of MTI-446 and Metabolites DN and UF

Instrumentation:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with a PE Sciex API 3000 Triple Quadrupole Mass Spectrometric Detector (LC/MS/MS) and Heated Nebulizer Ion Source
Analytical Column:	Phenomenex Luna C18; 150 x 3 mm, 5µm
Guard Column	Phenomenex Security Guard C18 (4 mm x 3 mm, 5µm)
Mobile Phases:	A1: 5% Methanol: 95% Water: 0.1% IPCC-MS-3 B1: Methanol: 0.1% IPCC-MS-3 Gradient Elution Program: Time / %A1 / %B1 / Flow Rate (µL/min) 0.00 / 100 / 0 / 500 2.00 / 100 / 0 / 500 7.00 / 20 / 80 / 500 10.0 / 20 / 80 / 500 10.1 / 100 / 0 / 800 15.0 / 100 / 0 / 500
Injection Volume:	50 µL
Total Run Time:	16.1 minutes
Mass Spectrometer Conditions:	Ionization Mode: Positive Scan Type: MRM Duration: 16.1 minutes Scan Rate: 1.520 seconds/scan Dwell Time: 500 mseconds/mass range Pause Time: 5 mseconds Mass Range: MTI-446 / DN / UF Q1 Mass (amu): 203.2 / 158.2 / 159.2 Q3 Mass (amu): 128.7 / 101.9 / 101.9 R02: -28 / -26 / -26 R03: -30 / -28 / -28 ST3: -34 / -32 / -32 MS Voltage, Temperature and Gas Settings: NC: +5.0 Temp.: 450°C OR: +21 RNG: +70 QO: -10 IQ1: -11 ST: -15 R01 -11 IQ2: -20 DF: -350 CEM: +2000 or +2200 NEB Gas: 13 or 12; CUR Gas: 9 or 10; CAD Gas: 4
MTI-446 Retention Time:	Approximately 7.2 – 7.4 mins
DN Retention Time:	Approximately 8.0 – 8.2 mins
UF Retention Time:	Approximately 6.1 – 6.5 mins

Table II. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Tomato

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
TOMVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
TOMVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
TOMVMAS-1	0.0100	0.0105	0.00890	0.00875	105	89.0	87.5
TOMVMAS-2	0.0100	0.0108	0.00910	0.00950	108	91.0	95.0
TOMVMAS-3	0.0100	0.0110	0.00921	0.00882	110	92.1	88.2
TOMVMAS-4	0.0100	0.0110	0.00915	0.00906	110	91.5	90.6
TOMVMAS-5	0.0100	0.0113	0.00954	0.00978	113	95.4	97.8
TOMVMAS-6	1.00	1.12	1.04	0.934	112	104	93.4
TOMVMAS-7	1.00	1.11	1.06	0.953	111	106	95.3
TOMVMAS-8	1.00	1.10	1.04	0.992	110	104	99.2
TOMVMAS-9	1.00	1.15	1.06	0.985	115	106	98.5
TOMVMAS-10	1.00	1.07	1.06	0.987	107	106	98.7
				Mean =	110	98.5	94.4
				Standard Deviation =	2.92	7.26	4.38
				Coefficient of Variance =	2.7%	7.4%	4.6%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table III. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Melon

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
MELVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
MELVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
MELVMAS-1	0.0100	0.00847	0.00909	0.00951	84.7	90.9	95.1
MELVMAS-2	0.0100	0.00821	0.00955	0.00963	82.1	95.5	96.3
MELVMAS-3	0.0100	0.00823	0.00953	0.00988	82.3	95.3	98.8
MELVMAS-4	0.0100	0.00978	0.00888	0.0106	88.8	98.7	106
MELVMAS-5	0.0100	0.00830	0.00937	0.0108	83.0	93.7	108
MELVMAS-6	1.00	0.850	1.04	0.985	85.0	104	98.5
MELVMAS-7	1.00	0.886	1.07	1.01	88.6	107	101
MELVMAS-8	1.00	0.893	1.06	1.00	89.2	106	100
MELVMAS-9	1.00	0.865	1.06	1.02	86.5	106	102
MELVMAS-10	1.00	0.848	1.07	1.02	84.8	107	102
				Mean =	85.5	100	101
				Standard Deviation =	2.68	6.24	4.01
				Coefficient of Variance =	3.1%	6.2%	4.0%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table IV. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Potato

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
POTVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
POTVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
POTVMAS-1	0.0100	0.0102	0.0102	0.00874	102	102	87.4
POTVMAS-2	0.0100	0.00997	0.0103	0.00901	99.7	103	90.1
POTVMAS-3	0.0100	0.00976	0.0102	0.00889	97.6	102	88.9
POTVMAS-4	0.0100	0.0101	0.0104	0.00863	101	104	86.3
POTVMAS-5	0.0100	0.0106	0.0104	0.00873	106	104	87.3
POTVMAS-6	1.00	1.08	0.997	0.900	108	99.7	90.0
POTVMAS-7	1.00	1.15	1.07	0.933	115	107	93.3
POTVMAS-8	1.00	1.12	1.05	0.929	112	105	92.9
POTVMAS-9	1.00	1.13	1.06	0.924	113	106	92.4
POTVMAS-10	1.00	1.11	1.05	0.914	111	105	91.4
				Mean =	107	104	90.0
				Standard Deviation =	6.18	2.16	2.49
				Coefficient of Variance =	5.8%	2.1%	2.8%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table V. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Grape

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
GRAVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
GRAVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
GRAVMAS-1	0.0100	0.00892	0.00985	0.00845	89.2	98.5	84.5
GRAVMAS-2	0.0100	0.00937	0.0102	0.00867	93.7	102	86.7
GRAVMAS-3	0.0100	0.00926	0.0103	0.00874	92.6	103	87.4
GRAVMAS-4	0.0100	0.0101	0.0105	0.00875	101	105	87.5
GRAVMAS-5	0.0100	0.00969	0.0103	0.00874	96.9	103	87.4
GRAVMAS-6	1.00	1.00	1.01	0.853	100	101	85.3
GRAVMAS-7	1.00	1.08	1.06	0.879	108	106	87.9
GRAVMAS-8	1.00	1.05	1.05	0.887	105	105	88.7
GRAVMAS-9	1.00	1.06	1.06	0.901	106	106	90.1
GRAVMAS-10	1.00	1.10	1.08	0.876	111	108	87.6
				Mean =	100	104	87.3
				Standard Deviation =	7.20	2.80	1.58
				Coefficient of Variance =	7.2%	2.7%	1.8%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table VI. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Broccoli

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
BROVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
BROVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
BROVMAS-1	0.0100	0.00952	0.0101	0.00890	95.2	101	89.0
BROVMAS-2	0.0100	0.00893	0.00979	0.00881	89.3	97.9	88.1
BROVMAS-3	0.0100	0.00953	0.0104	0.00892	95.3	104	89.2
BROVMAS-4	0.0100	0.00974	0.0104	0.00911	97.4	104	91.1
BROVMAS-5	0.0100	0.00935	0.0102	0.00888	93.5	102	88.8
BROVMAS-6	1.00	1.02	0.957	0.890	102	95.7	89.0
BROVMAS-7	1.00	1.03	0.969	0.909	103	96.9	90.9
BROVMAS-8	1.00	1.01	0.968	0.932	101	96.8	93.2
BROVMAS-9	1.00	1.07	1.03	0.888	107	103	88.8
BROVMAS-10	1.00	1.10	1.04	0.910	110	104	91.0
				Mean =	99.4	101	89.9
				Standard Deviation =	6.39	3.37	1.57
				Coefficient of Variance =	6.4%	3.3%	1.7%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table VII. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Lettuce

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
LETVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
LETVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
LETVMAS-1	0.0100	0.0110	0.0106	0.0111	110	106	111
LETVMAS-2	0.0100	0.0116	0.0116	0.0117	116	116	117
LETVMAS-3	0.0100	0.00940	0.0109	0.0109	94.0	109	109
LETVMAS-4	0.0100	0.0116	0.0113	0.0118	116	113	118
LETVMAS-5	0.0100	0.0114	0.0110	0.0112	114	110	112
LETVMAS-6	1.00	1.04	1.05	0.990	104	105	99.0
LETVMAS-7	1.00	1.09	1.11	1.04	109	111	104
LETVMAS-8	1.00	1.09	1.12	1.03	109	112	103
LETVMAS-9	1.00	1.07	1.12	1.02	107	112	102
LETVMAS-10	1.00	1.09	1.16	1.03	109	116	103
				Mean =	109	111	108
				Standard Deviation =	6.48	3.68	6.58
				Coefficient of Variance =	5.9%	3.3%	6.1%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table VIII. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Tomato Paste (Tomato PC)

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
PASVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
PASVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
PASVMAS-1	0.0100	0.00981	0.00961	0.00911	98.1	96.1	91.1
PASVMAS-2	0.0100	0.0102	0.00856	0.00807	102	85.6	80.7
PASVMAS-3	0.0100	0.0102	0.00881	0.00880	102	88.1	88.0
PASVMAS-4	0.0100	0.0107	0.00891	0.00785	107	89.1	78.5
PASVMAS-5	0.0100	0.00899	0.00899	0.00797	89.9	89.9	79.7
PASVMAS-6	1.00	0.869	0.938	0.736	86.9	93.8	73.6
PASVMAS-7	1.00	0.856	0.935	0.736	85.6	93.5	73.6
PASVMAS-8	1.00	0.980	0.993	0.723	98.0	99.3	72.3
PASVMAS-9	1.00	0.869	0.942	0.760	86.9	94.2	76.0
PASVMAS-10	1.00	0.876	0.951	0.729	87.6	95.1	72.9
				Mean =	94.4	92.5	78.6
				Standard Deviation =	7.87	4.17	6.49
				Coefficient of Variance =	8.3%	4.5%	8.3%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table IX. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Tomato Puree (Tomato PC)

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
PURVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
PURVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
PURVMAS-1	0.0100	0.0102	0.00909	0.00959	102	90.9	95.9
PURVMAS-2	0.0100	0.0109	0.00977	0.00926	109	97.7	92.6
PURVMAS-3	0.0100	0.0109	0.00936	0.0103	109	93.6	103
PURVMAS-4	0.0100	0.0103	0.00934	0.00912	103	93.4	91.2
PURVMAS-5	0.0100	0.0110	0.0100	0.00979	110	100	97.9
PURVMAS-6	1.00	0.901	0.912	0.855	90.1	91.2	85.5
PURVMAS-7	1.00	0.876	0.946	0.954	87.6	94.6	95.4
PURVMAS-8	1.00	0.898	0.976	0.906	89.8	97.6	90.6
PURVMAS-9	1.00	0.957	1.03	0.985	95.7	103	98.5
PURVMAS-10	1.00	0.936	0.993	0.952	93.6	99.3	95.2
				Mean =	99.0	96.1	94.6
				Standard Deviation =	8.69	4.01	4.87
				Coefficient of Variance =	8.8%	4.2%	5.2%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table X. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Potato Flakes (Potato PC)

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
FLAVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
FLAVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
FLAVMAS-1	0.0100	0.0102	0.00894	0.00911	102	89.4	91.1
FLAVMAS-2	0.0100	0.0104	0.00920	0.00909	104	92.0	90.9
FLAVMAS-3	0.0100	0.0113	0.00924	0.00945	113	92.4	94.5
FLAVMAS-4	0.0100	0.0112	0.00764	0.00959	112	76.4	95.9
FLAVMAS-5	0.0100	0.0164	0.00966	0.00980	164[3]	96.6	98.0
FLAVMAS-6	1.00	0.956	0.936	0.887	95.6	93.6	88.7
FLAVMAS-7	1.00	1.10	1.00	0.931	110	100	93.1
FLAVMAS-8	1.00	0.961	1.04	0.938	96.1	104	93.8
FLAVMAS-9	1.00	0.974	0.987	0.919	97.4	98.7	91.8
FLAVMAS-10	1.00	0.857	0.748	0.920	85.7	74.8	92.0
				Mean =	102	91.8	93.0
				Standard Deviation =	9.02	9.56	2.69
				Coefficient of Variance =	8.8%	10%	2.9%
				Number of Measurements =	9	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

[3] Oultier, not used in statistical evaluation of data.

Table XI. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Potato Chips (Potato PC)

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
CHIVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
CHIVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
CHIVMAS-1	0.0100	0.00909	0.00904	0.00869	90.9	90.4	86.9
CHIVMAS-2	0.0100	0.00881	0.00903	0.00926	88.1	90.3	92.6
CHIVMAS-3	0.0100	0.00894	0.00947	0.00922	89.4	94.7	92.2
CHIVMAS-4	0.0100	0.00969	0.00874	0.00872	96.9	87.4	87.2
CHIVMAS-5	0.0100	0.00981	0.00857	0.00869	98.1	85.7	86.9
CHIVMAS-6	1.00	0.931	0.889	0.930	93.1	88.9	93.0
CHIVMAS-7	1.00	0.945	0.926	0.942	94.5	92.6	94.2
CHIVMAS-8	1.00	0.997	0.880	0.907	99.7	88.0	90.7
CHIVMAS-9	1.00	1.02	0.966	0.900	102	96.6	90.0
CHIVMAS-10	1.00	0.989	0.960	0.927	98.9	96.0	92.7
				Mean =	95.2	91.1	90.6
				Standard Deviation =	4.70	3.77	2.77
				Coefficient of Variance =	4.9%	4.1%	3.1%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table XII. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Potato Wet Peel (Potato PC)

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
PEEVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
PEEVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
PEEVMAS-1	0.0100	0.00865	0.00970	0.0102	86.5	97.0	102
PEEVMAS-2	0.0100	0.00888	0.00978	0.0105	88.8	97.8	105
PEEVMAS-3	0.0100	0.00902	0.0105	0.00985	90.2	105	98.5
PEEVMAS-4	0.0100	0.00706	0.00861	0.0107	70.6	86.1	107
PEEVMAS-5	0.0100	0.00720	0.00864	0.0104	72.0	86.4	104
PEEVMAS-6	1.00	0.727	0.852	1.00	72.7	85.2	100
PEEVMAS-7	1.00	0.919	0.973	1.04	91.9	97.3	104
PEEVMAS-8	1.00	0.915	1.05	1.04	91.5	105	104
PEEVMAS-9	1.00	1.06	1.03	1.08	106	103	108
PEEVMAS-10	1.00	1.03	1.07	1.11	103	107	111
				Mean =	87.3	97.0	104
				Standard Deviation =	12.3	8.40	3.71
				Coefficient of Variance =	14.1%	8.7%	3.6%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table XIII. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Raisins (Grape PC)

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
RAIVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
RAIVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
RAIVMAS-1	0.0100	0.00875	0.00782	0.00723	87.5	78.2	72.3
RAIVMAS-2	0.0100	0.00877	0.00839	0.00757	87.7	83.9	75.7
RAIVMAS-3	0.0100	0.00891	0.00793	0.00734	89.1	79.3	73.4
RAIVMAS-4	0.0100	0.00903	0.00869	0.00730	90.3	86.9	73.0
RAIVMAS-5	0.0100	0.00953	0.00898	0.00783	95.3	89.8	78.3
RAIVMAS-6	1.00	0.885	0.888	0.722	88.5	88.8	72.2
RAIVMAS-7	1.00	0.983	0.905	0.733	98.3	90.5	73.3
RAIVMAS-8	1.00	1.01	0.974	0.670	101	97.4	67.0
RAIVMAS-9	1.00	0.918	0.867	0.696	91.8	86.7	69.6
RAIVMAS-10	1.00	0.932	0.897	0.725	93.2	89.7	72.5
				Mean =	92.3	87.1	72.7
				Standard Deviation =	4.65	5.63	3.05
				Coefficient of Variance =	5.0%	6.5%	4.2%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

Table XIV. Method Validation Recoveries of MTI-446 and Metabolites DN and UF in Grape Juice (Grape PC)

Sample Number (236C-113-)	Concentration (mg/Kg)[1,2]				Percent Recovered[2] (%)		
	Fortified	Measured (MTI-446)	Measured (UF)	Measured (DN)	MTI-446	UF	DN
JUIVREB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
JUIVMAB-1	0.00	<LOQ	<LOQ	<LOQ	--	--	--
JUIVMAS-1	0.0100	0.00969	0.0102	0.0116	96.9	102	116
JUIVMAS-2	0.0100	0.00925	0.0101	0.00816	92.5	101	81.6
JUIVMAS-3	0.0100	0.00937	0.0101	0.00813	93.7	101	81.3
JUIVMAS-4	0.0100	0.0104	0.0105	0.00906	104	105	90.6
JUIVMAS-5	0.0100	0.0107	0.0108	0.00743	107	108	74.3
JUIVMAS-6	1.00	1.04	1.05	0.779	104	105	77.9
JUIVMAS-7	1.00	1.10	1.07	0.885	110	107	88.5
JUIVMAS-8	1.00	1.08	1.11	0.884	108	111	88.4
JUIVMAS-9	1.00	1.00	1.03	0.931	100	103	93.1
JUIVMAS-10	1.00	1.10	1.03	0.893	110	103	89.3
				Mean =	103	105	88.1
				Standard Deviation =	6.52	3.27	11.5
				Coefficient of Variance =	6.3%	3.1%	13%
				Number of Measurements =	10	10	10

[1] The limit of quantitation (LOQ) of 0.008 mg/Kg was calculated as the product of the lowest calibration standard concentration (0.00100 mg /L) and the dilution factor of the matrix blank sample (8.00) analyzed in each analysis set.

[2] Results were generated using MacQuan, version 1.6 software. Manual calculations may differ slightly.

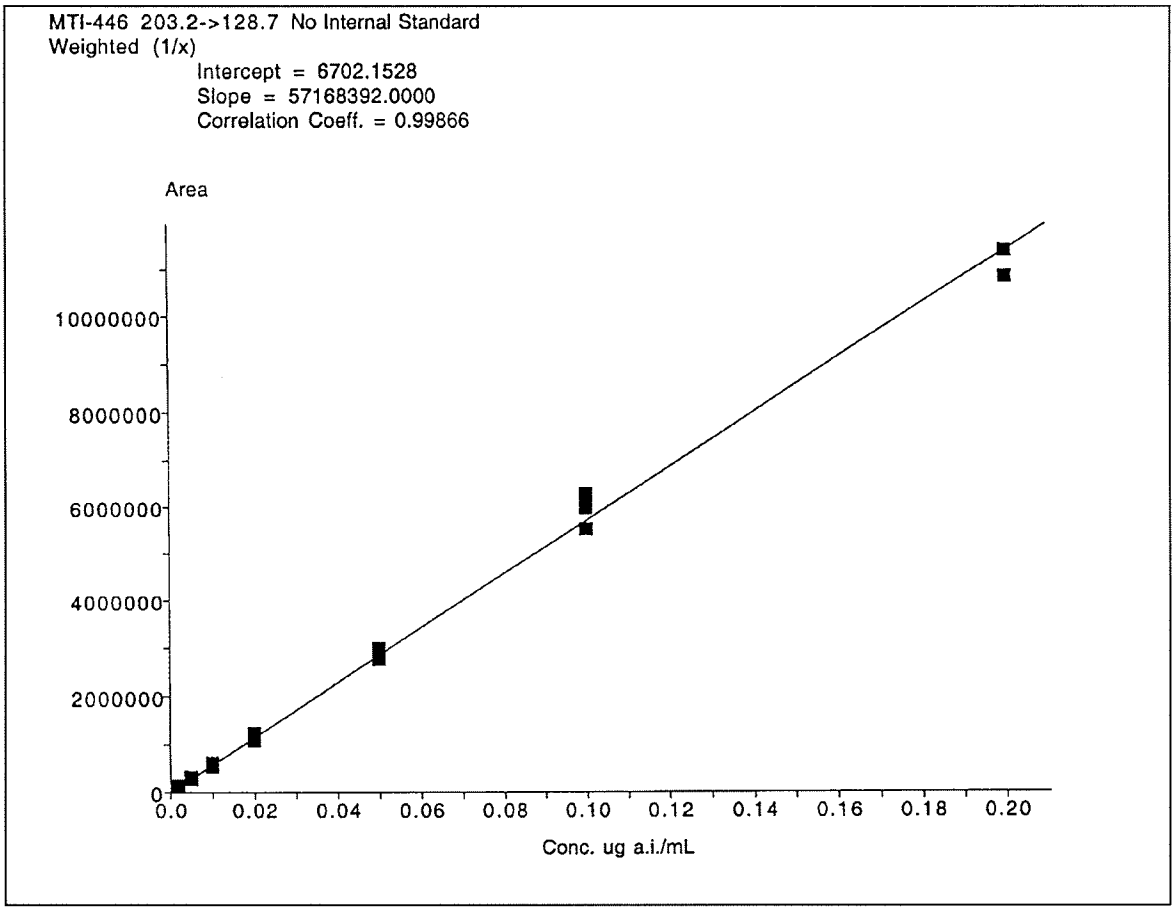


Figure 1. Typical Calibration Curve for the Determination of MTI-446.

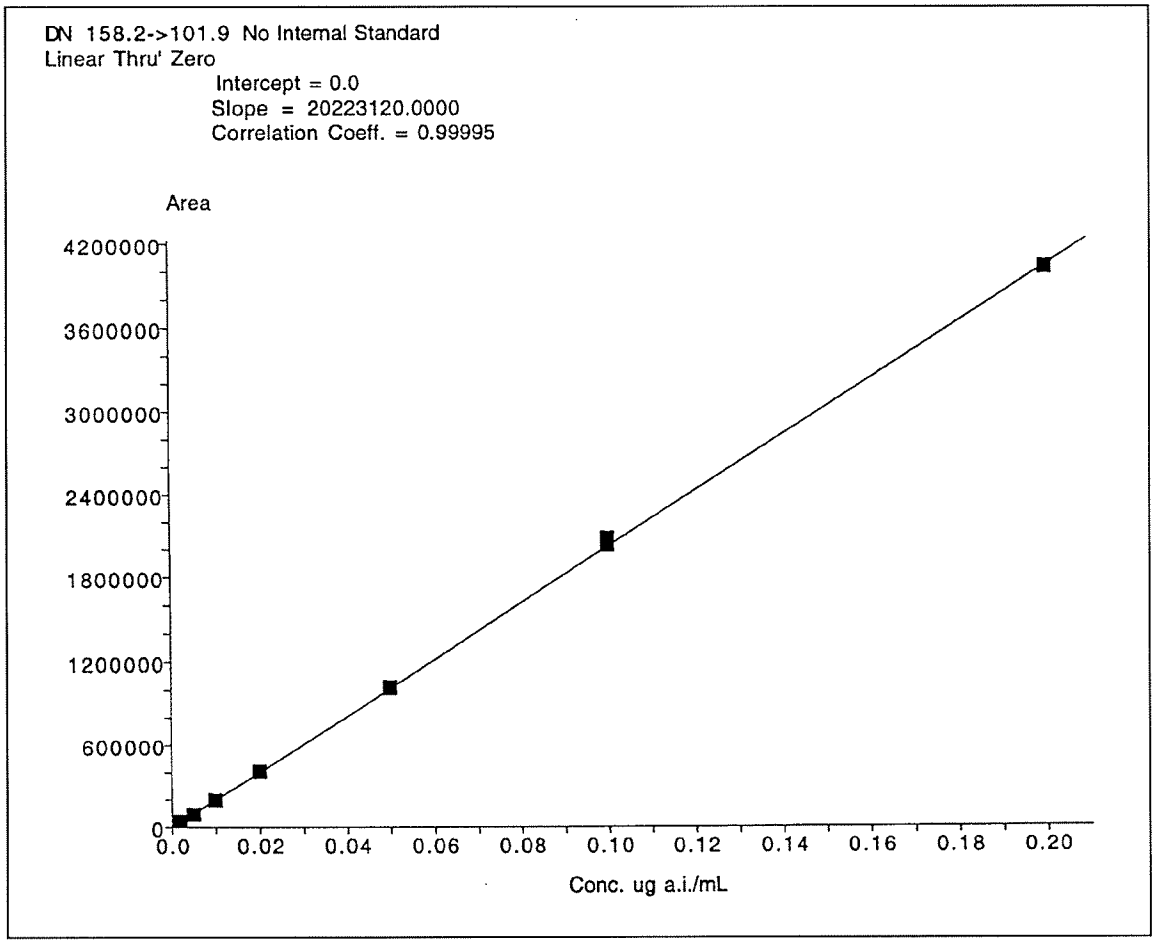


Figure 2. Typical Calibration Curve for the Determination of Metabolite DN.

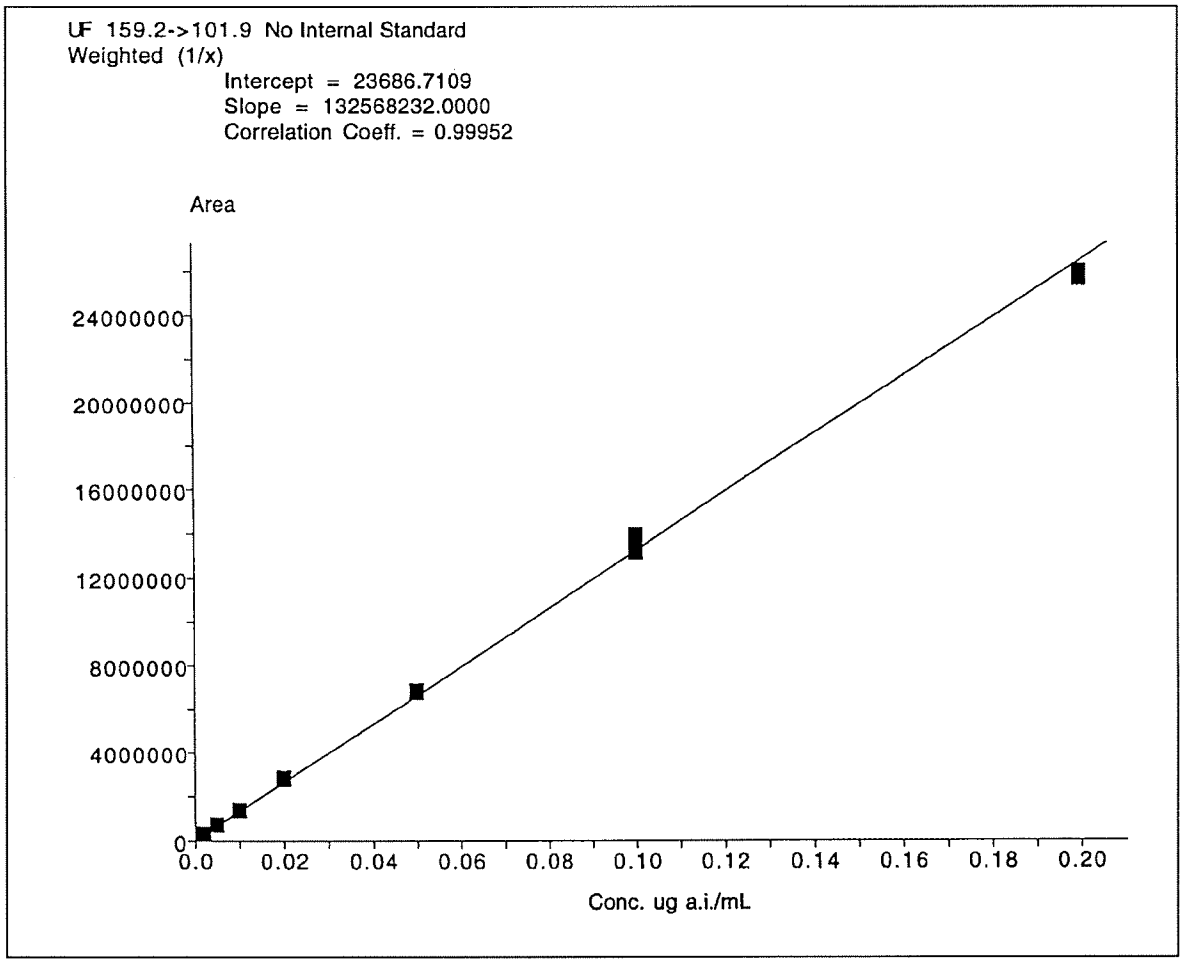


Figure 3. Typical Calibration Curve for the Determination of Metabolite UF.

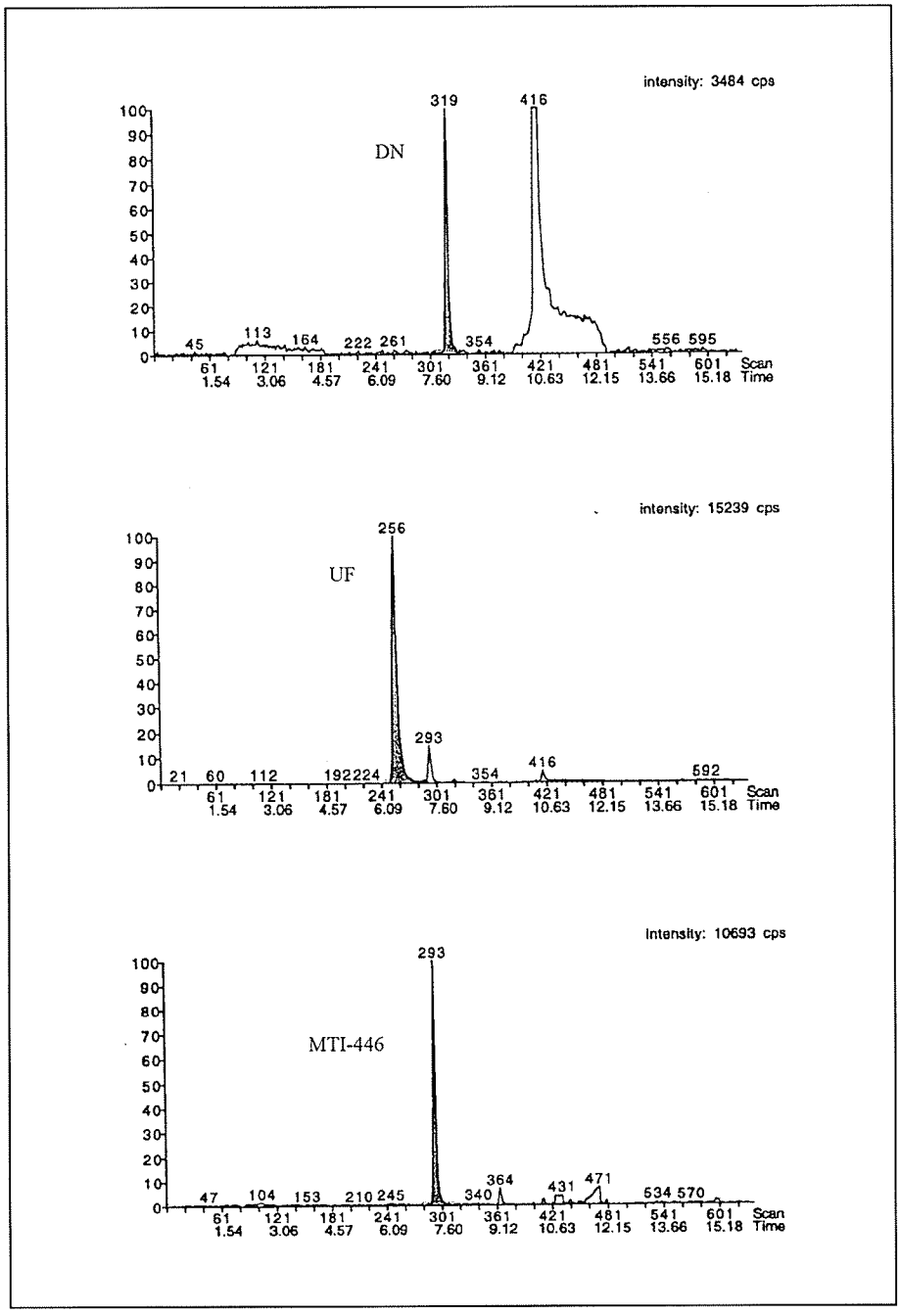


Figure 4. Typical Chromatogram of a Low-Level 0.00100 mg/L Combined Standard of MTI-446 (bottom), DN (top), and UF (middle).

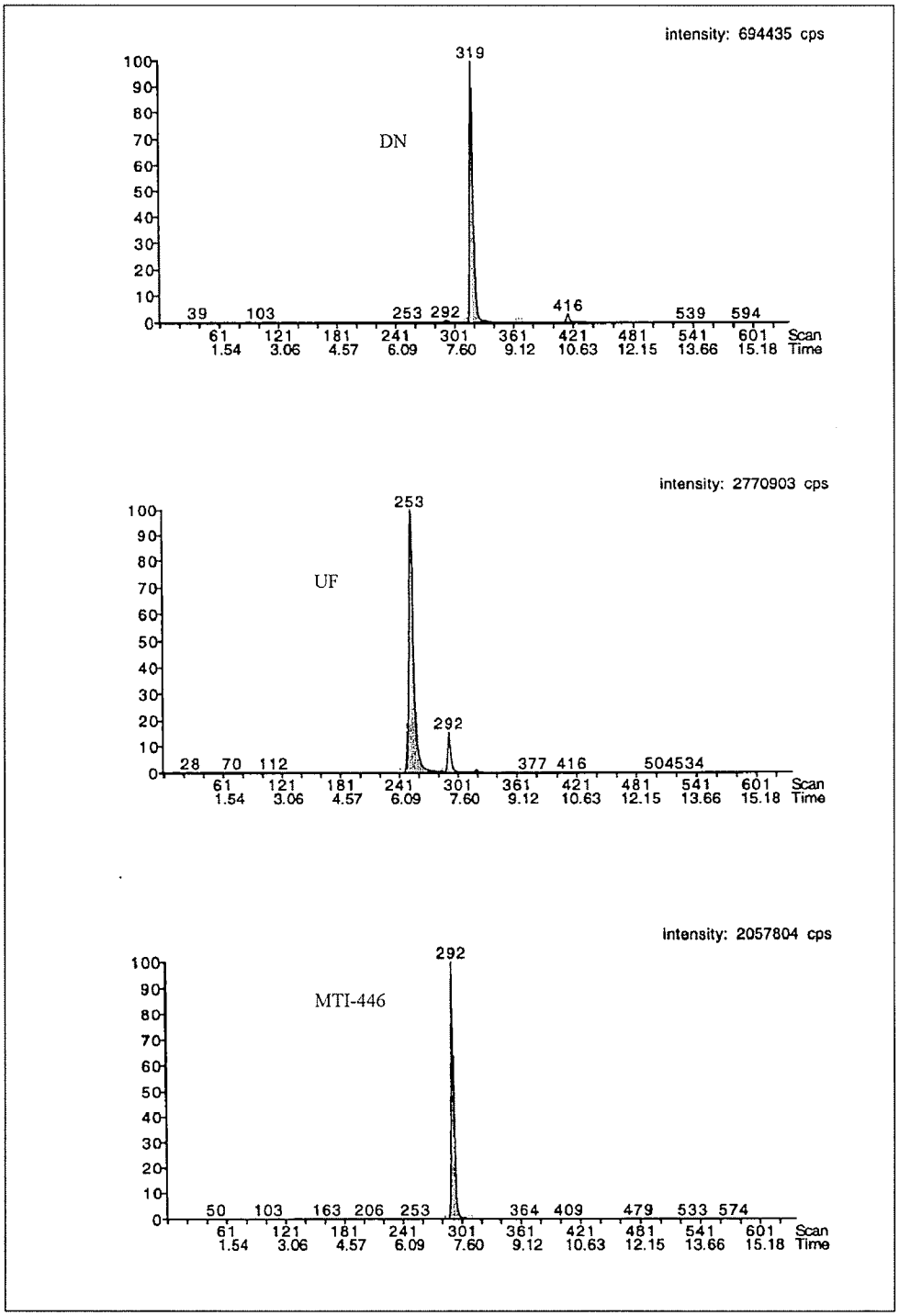


Figure 5. Typical Chromatogram of a High-Level 0.200 mg/L Combined Standard of MTI-446 (bottom), DN (top), and UF (middle).

APPENDIX A

Method for the Analysis of MTI-446 and Metabolites DN and UF in Multiple Crop Substrates

Method Outline for the Analysis of MTI 446 and its Metabolites UF and DN in/on Raw Agricultural and Processed Commodities Following Homogenization and Subsampling

1. **Sample Extraction: MTI-446, UF and DN Metabolites**

Prepare samples and procedural recoveries by weighing 25.0 g of matrix into labeled 500-mL glass screw cap bottles. Fortify procedural recoveries using an appropriate stock solution(s). Unfortified matrix will serve as the matrix blank.

Add 150 mL of 80% CH_3CN: 20% H_2O followed by 0.5 mL of concentrated HCL to each sample. Blend the samples using a high-speed Ultra Turrax (T-25) sample homogenizer for approximately 5 minutes.

Place samples on a shaker table and agitate for 30 minutes at a setting of 150 rpm.

Filter the suspension by suction through a pad of Celite (approximately 10 g) into a 1L-round bottom flask. Rinse the filter cake with an additional 100 mL of 80% CH_3CN: 20% H_2O and combine in its respective round bottom flask.

Transfer the filtrate into a 500-mL separatory funnel. Rinse the round bottom flask with 100 mL of hexane and combine it in its respective separatory funnel. Shake the separatory funnels for approximately 1 minute. Drain the lower CH_3CN/H_2O fraction into a 1L-round bottom flask and discard the hexane. Repeat the hexane- CH_3CN/H_2O partition a second time with 50 mL of hexane and combine the lower CH_3CN/H_2O fraction in its respective round bottom flask.

Rotary evaporate each sample to its aqueous remainder at a bath temperature of approximately 50-60°C.

Adjust the pH of the aqueous remainder to approximately pH 8 by drop-wise addition of buffer solution A (0.5M sodium carbonate - sodium hydrogen carbonate).

Transfer the aqueous solution to a 100-mL graduated cylinder and adjust the final volume to 80.0 mL using NANOpure® H_2O.

2. **Sample Clean Up: MTI-446 and the UF Metabolite (Extrelut NT3)**

Transfer 40 mL of the aqueous solution from Section 1 above into a 50-mL volumetric flask containing 15 g of NaCl and bring to volume with NANOpure® H_2O. Transfer the mixture to a French square bottle, place on a shaker table and agitate for approximately 60 minutes at 150 rpm.

Using a Class A volumetric pipette, remove a 3.0-mL aliquot of the aqueous solution and transfer onto an Extrelut NT3 extraction column. Allow the extract to adsorb into the column for approximately 30-60 minutes.

Elute the analytes into 250-mL round bottom flasks using 120 mL of ethyl acetate.

Rotary evaporate the ethyl acetate extracts to dryness at approximately 40-50°C.

Reconstitute the residues using 3.0 mL of 0.1N HCL. Mix well.

OPTIONAL: If particulates are observed in the reconstituted solution, filter an aliquot of extract through a 0.45-µm Acrodisc filter.

3. Sample Clean-up: DN Metabolite only (Bond Elut CBA)

Measure the remaining 40 mL of aqueous solution from Section 1 and transfer it to a plastic 50-mL centrifuge tube or equivalent. Add 5.0 mL of buffer solution A and 5.0 mL of NANOpure® H_2O. Mix well.

OPTIONAL: If particulates are observed in the reconstituted solution, filter an aliquot of extract through a 0.45-µm Acrodisc filter.

Prepare an appropriate number of Bond Elut CBA SPE columns by rinsing and conditioning with 3 mL of methanol followed by 4 mL of buffer solution B (0.05 M sodium carbonate - sodium hydrogen carbonate). Do not allow the columns to go to dryness.

Using a Class A volumetric pipette, remove a 5.0-mL aliquot of the final aqueous solution from above, transfer it to the prepared CBA cartridge and allow it to pass through at approximately 1-2 mL/minute. Rinse the cartridge with 10 mL of NANOpure® H_2O, followed by 6 mL of methanol. Drain the methanol rinse to just below the surface of the column frit. Discard all eluates.

Elute the analytes with 4.0 mL of 0.1N HCL measured using a Class A volumetric pipette into a 5-mL Class A volumetric flask or equivalent container. Adjust to a 5.0 mL final volume using 0.1N HCL. Mix well.

OPTIONAL: If particulates are observed in the reconstituted solution, filter an aliquot of extract through a 0.45-µm Acrodisc filter

4. Combining of MTI-446/UF and DN Extracts for HPLC/MS/MS Analysis

Volumetrically combine an aliquot of the MTI-446/UF metabolite final aqueous extract from Section 2 with an equal-volume aliquot of the DN metabolite final aqueous extract from Section 3 (1:1, v/v). Mix well. Transfer an aliquot of the final combined extract to an autosampler vial and submit for LC/MS/MS analysis.

APPENDIX B

Protocol and Protocol Amendments

PROTOCOL

LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES

EPA Residue Chemistry Test Guidelines
OPPTS 860.1340
Residue Analytical Method

Submitted to

Mitsui Chemicals Inc.
Agrochemicals Division,
3-2-5, Kasumigaseki,
Chiyoda-Ku,
Tokyo 100-6070, Japan

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

July 23, 2002

Wildlife International, Ltd.

- 2 -

LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES

SPONSOR:	Mitsui Chemicals Inc. Agrochemicals Division, 3-2-5, Kasumigaseki, Chiyoda-Ku, Tokyo 100-6070, Japan
STUDY MONITOR:	Dennis R. Hattermann, Ph.D. Landis International 3185 Madison Highway Valdosta, GA 31603-5126 (229) 247-6472
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601 (410) 822-8600
STUDY DIRECTOR:	Jon A. MacGregor, B.S. Scientist
LABORATORY MANAGEMENT:	Willard B. Nixon, Ph.D. Director of Analytical Chemistry

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: 7/24/02

Experimental Termination Date: 12/15/02

Project No.: 236C-113

PROTOCOL APPROVAL

Jon A MacGregor STUDY DIRECTOR	7/24/02 DATE
Willard B. Nixon LABORATORY MANAGEMENT	7/24/02 DATE
SPONSOR'S REPRESENTATIVE	July 23, 2002 DATE

Wildlife International, Ltd.

INTRODUCTION

Wildlife International, Ltd. will perform analytical method(s) validations for the determination of MTI-446 (dinotefuran) and its DN (1-methyl-3-tetrahydro-3-furylmethyl)guanidine) and UF (1-methyl-3-(tetrahydro-3-furylmethyl)urea) metabolites in multiple crop substrates. The analytical method is based on the methods "Analysis of MTI-446 in Crops (HPLC Method)" (2) and "Method RCC Study Number 841464 MTI-446, DN and UF" (3). A flow chart of the method is presented in Appendix I. This study is being performed to provide data to validate the method(s) to be subsequently applied to the analysis of samples harvested for determination of residues of MTI-446 and its UF and DN metabolites in crop substrates (raw and processed) following application of foliar and ground applications of MTI-446 20%SG at the maximum label rates. The study will be performed at the Wildlife International, Ltd. analytical chemistry facility in Easton, Maryland.

PURPOSE

This study is being conducted to validate the analytical method(s) to be used for determination of residues of MTI-446 and its UF and DN metabolites in field-collected samples following foliar and ground applications of MTI-446 20%SG at maximum label rates.

EXPERIMENTAL DESIGN

Specific raw agricultural and processed commodities (RAC and PC, respectively) will be added by amendment to this protocol. Crop substrates will be fortified with each test substance (MTI-446, DN and UF) at three different concentrations and analyzed according to the method(s) to be applied to determination of residues (MTI-446, DN, and UF) in field-harvested samples from residue studies. Reagent and matrix blanks (controls) will be analyzed concurrently to evaluate potential analytical interferences. Additional concentration levels may be added at the discretion of the Study Director if such are considered to provide additional relevant information on method performance.

MATERIALS AND METHODS

Test Substances

Test substances of MTI-446, the DN metabolite of MTI-446 and the UF metabolite of MTI-446 were received from Landis International, Inc. on January 4, 2002. These test substances

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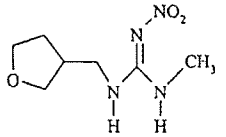
were assigned Wildlife International, Ltd. Identification Numbers 5856, 5857 and 5858, respectively, and transferred to ambient storage in darkness. Certificates of Analysis were received with each test substance and provided the following information:

MTI-446 (CAS Number 165252-70-0)

Chemical Name: (*RS*)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine

Structural Formula:

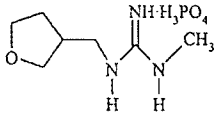


Appearance: white powder Purity: 99.6%
Lot Number: EBI-5-101 Expiration Date: December 2003

DN

Chemical Name: 1-methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate

Structural Formula:

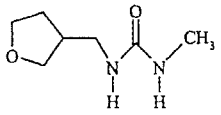


Appearance: white powder Purity: 99.27%
Lot Number: OFU-1290 Expiration Date: December 2003

UF

Chemical Name: 1-methyl-3-(tetrahydro-3-furylmethyl)urea

Structural Formula:



Appearance: white powder Purity: 99.59%
Lot Number: OFU-1291 Expiration Date: December 2003

Test System

The test systems, defined as the crop substrate being fortified, will be added by amendment to this protocol. A table will be presented in each amendment describing the samples to be analyzed as part of the method validation for the specific test system.

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Procedures

Analytical Method

Prior to initiating any of the definitive method validations, Wildlife International, Ltd. will generate acceptable chromatographic data using standards for MTI-446, DN and UF. After demonstration that the chromatographic system is adequate, the methods will be validated. A method outline based on the methodology documented in greater detail in "Method RCC Study Number 841464 MTI-446, DN and UF" is presented in Attachment I. The method outline as presented in Attachment I differs from that presented in the RCC method primarily in the elimination of a second back partition found unnecessary for high-moisture content crop substrates, combining of analyte fractions to analyze for all analytes of interest in a single HPLC/MS/MS run and optimization of the chromatographic conditions for the specific instrumentation available for use at Wildlife International, Ltd. It is anticipated that the generic method as presented in Attachment I may need refinement for specific crop substrates that contain unique interferences and, particularly, high oil content. Where modifications are implemented, these will be described in the final report and noted for the specific crop substrates to which they apply.

Validation Sample Sets

Each sample set will consist of a minimum of one reagent blank, one control and ten fortified samples for a total of twelve samples minimum for each substrate. Fortification levels, depending on the limit of quantitation (LOQ), will be specified in each amendment. Recoveries will be determined by comparison of the recovered amount of analyte (either MTI-446, DN or UF) versus the fortification level. Precision of the method(s), expressed as the standard deviation for each fortification level, will be determined. The following is provided as an example of a validation sequence for a specific raw or processed substrate.

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Summary of Typical Method Validation Data Set for a Crop Substrate

Sample Substrate	Sample Description	MTI-446	DN	UF
Substrate Description if Multipe Substrates	Reagent Blank	1	1	1
	Matrix Blank (Control)	1	1	1
	LOQ Fortification [1]	5	5	5
	100x LOQ Fortification	5	5	5
Total Analytical Recovery Results (10 per analyte)		30		
Total Sample Analyses		12		

Acceptance Criteria for Method Recoveries

The validation will be considered successful for a specific analyte (either MTI-446, UF or DN) if the mean recovery for the analyte at the fortification levels specified above is within the range of 70 to 120% for the crop substrate. Lower or higher recovery ranges may be deemed acceptable upon approval of the Sponsor.

Statistical Analysis of Data

Descriptive statistics to include, but not limited to, means, standard deviations, and regression correlation coefficients will be used to evaluate and present the data.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International, Ltd. will include but not be limited to the following:

1. A copy of the signed protocol, amendments and deviations, if any.
2. Description and source of the test substances and substrates.
3. Dates of initiation and termination of each test.
4. Stock solution calculations and preparation.
5. Dose calculation and preparation procedure for fortified samples.
6. The methods used to analyze each test substance and the results of analytical measurements.
7. Statistical calculations, e.g., means, standard deviations, regression correlation coefficients.
8. A copy of the final report.

Wildlife International, Ltd.

FINAL REPORT

The report will summarize the findings of the validation, the fortification recoveries obtained, and the methods and instrumentation employed. The report will include, but not be limited to the following:

1. Address of the facility performing the study and Study Director.
2. The name of the Study Director and the names of other scientists, professionals and supervisory personnel involved in the study.
3. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.
4. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
5. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates any findings were reported to the Study Director and Management.
6. Objectives and procedures, as stated in the approved protocol, including all changes to the protocol.
7. Description of the test substance including name, code number, source and reported purity.
8. A description of the analytical method(s) used to conduct the study including a description of the instruments used and operating parameters.
9. A description of the preparation of the standards and fortified samples.
10. Recovery and control values.
11. Representative chromatograms for each analyte and substrate.
12. A description of any changes or modifications to the analytical method(s).
13. A description of any circumstances that may have affected the quality or integrity of the data.
14. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analyses.
15. The location where the raw data and the final report are stored at the end of the study or intended storage location.

If corrections or additions to the final report are necessary after finalization, changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly

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identify the part of the final report that is being amended and the reasons for the amendment. The amendment will be signed by the Study Director.

CHANGING OF THE PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and Management of the performing laboratory. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); and OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for certification of compliance with Good Laboratory Practices for procedures performed by other laboratories. Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

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REFERENCES

1. **U.S. Environmental Protection Agency.** 1996. Prevention, Pesticides and Toxic Substances. Residue Chemistry Test Guidelines. OPPTS Number 860.1340, *Residue Analytical Method.*

2. **Analysis of MTI-446 in Crops (HPLC Method),** Mitsui Chemicals, Inc., Life Sciences Laboratory, Crop Protection Section, Dr. Koji Kitajima, June 1999.

3. **Method RCC Study Number 841464 MTI-446, DN and UF,** RCC Ltd, Environmental Chemistry and Pharmanalytics Division.

Wildlife International, Ltd.

ATTACHMENT I

Method Outline for the Analysis of MTI 446 and its Metabolites UF and DN in/on Raw Agricultural Commodities Following Homogenization and Subsampling

1. **Sample Extraction: MTI-446, UF and DN**

   Prepare samples and procedural recoveries by weighing 25.0 g of matrix into labeled 500-mL glass screw cap bottles. Fortify procedural recoveries using an appropriate stock solution(s). Unfortified matrix will serve as the matrix blank.

   Add 150 mL of 80% CH_3CN: 20% H_2O followed by 0.5 mL of concentrated HCL to each sample. Blend the samples using a high-speed Ultra Turrax (T-25) sample homogenizer for approximately 1 minute.

   Place samples on a shaker table and agitate for 30 minutes at a setting of 150 rpm.

   Filter the suspension by suction through a pad of Celite (approximately 10 g) into a 1L-round bottom flask. Rinse the filter cake with an additional 100 mL of 80% CH_3CN: 20% H_2O and combine in its respective round bottom flask.

   Transfer the filtrate into a 500-mL separatory funnel. Rinse the round bottom flask with 100 mL of hexane and combine it in its respective separatory funnel. Shake the separatory funnels for approximately 1 minute. Drain the lower CH_3CN/H_2O fraction into a 1L-round bottom flask and discard the hexane. Repeat the hexane- CH_3CN/H_2O partition a second time with 50 mL of hexane and combine the lower CH_3CN/H_2O fraction in its respective round bottom flask.

   Rotary evaporate each sample to its aqueous remainder at a bath temperature of approximately 50-60°C.

   Adjust the pH of the aqueous remainder to approximately pH 8 by drop-wise addition of buffer solution A (0.5M sodium carbonate - sodium hydrogen carbonate).

   Transfer the aqueous solution to a 100-mL graduated cylinder and adjust the final volume to 80.0 mL using NANOpure® H_2O.

2. **Sample Clean Up: MTI-446 and the UF Metabolite (Extrelut NT3)**

   Transfer 40 mL of the aqueous solution from Section 1 above into a 50-mL volumetric flask containing 15 g of NaCl and bring to volume with NANOpure® H_2O. Transfer the mixture to a French square bottle, place on shaker table and agitate for approximately 60 minutes at approximately 150 rpm.

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Using a Class A volumetric pipette, remove a 3.0-mL aliquot of the aqueous solution and transfer onto an Extrelut NT3 extraction column. Allow the extract to adsorb into the column for approximately 30-60 minutes.

Elute the analytes into 250-mL round bottom flasks using 120 mL of ethyl acetate.

Rotary evaporate the ethyl acetate extracts to dryness at approximately 40-50C°.

Reconstitute the residues using 3.0 mL of NANOpure® H_2O. Mix well by shaking.

OPTIONAL: If particulates are observed in the reconstituted solution, filter an aliquot of extract through a 0.45-µm Acrodisc filter.

3. **Sample Clean-up: DN Metabolite only (Bond Elut CBA)**

Measure the remaining 40 mL of aqueous solution from Section 1 and transfer it to a plastic 50-mL centrifuge tube or equivalent. Add 5.0 mL of buffer solution A and 5.0 mL of NANOpure® H_2O. Mix well by shaking.

OPTIONAL: If particulates are observed in the reconstituted solution, filter an aliquot of extract through a 0.45-µm Acrodisc filter.

Prepare an appropriate number of Bond Elut CBA SPE columns by rinsing and conditioning with 3 mL of methanol followed by 4 mL of buffer solution B (0.05 M sodium carbonate - sodium hydrogen carbonate). Do not allow the columns to go to dryness.

Using a Class A volumetric pipette, remove a 4.0-mL aliquot of the final aqueous solution from above, transfer it to the prepared CBA cartridge and allow it to pass through at approximately 1-2 mL/minute. Rinse the cartridge with 10 mL of NANOpure® H_2O, followed by 6 mL of methanol. Discard all eluates. Dry the cartridge under high vacuum.

Elute the analytes with 4.0 mL of 0.1N HCL measured using a Class A volumetric pipette into a 15-mL graduated tubes. Adjust to a 4.0 mL final volume if necessary using 0.1N HCL. Mix well by shaking.

OPTIONAL: If particulates are observed in the reconstituted solution, filter an aliquot of extract through a 0.45-µm Acrodisc filter

4. **Combining of MTI-446/UF and DN Extracts for HPLC/MS/MS Analysis**

Volumetrically combine an aliquot of the MTI-446/UF metabolite final aqueous extract from Section 2 with an equal-volume aliquot of the DN metabolite final aqueous extract from Section 3 (1:1, v/v). Mix well by shaking. Transfer an aliquot of the final combined extract to an autosampler vial and submit for LC/MS/MS analysis.

PROTOCOL AMENDMENT NO. 1

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of the tomato as a representative raw agricultural commodity (RAC) for validation of the analytical method for analyses of tomatoes and peppers for MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation set for tomatoes are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include tomato as a representative RAC for method validation for residue determination of MTI-446, UF and DN in tomatoes and peppers.

Approved By:

Jon A MacGregor — 7/24/02

STUDY DIRECTOR
Jon A. MacGregor, B.S.

Date

Willard B. Nixon — 7/24/02

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

Date

William R. Landis — 23 July 2002

SPONSOR'S REPRESENTATIVE
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

Date

Attachment I

Control samples of tomatoes, fortified with reference standards of MTI-446, DF and UN, will be analyzed to validate the method prior to analysis of field samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DF and UN in/on tomatoes and peppers if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set - Tomatoes

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 2

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of melon as a representative raw agricultural commodity (RAC) for validation of the analytical method for analyses of cucurbits for MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation set for melons are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include melons as a representative RAC for method validation for residue determination of MTI-446, UF and DN in cucurbits.

Approved By:

Jon A MacGregor — 8/6/02

STUDY DIRECTOR
Jon A. MacGregor, B.S.

Date

Willard B. Nixon — 8/6/02

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

Date

4 Aug 2002

SPONSOR'S REPRESENTATIVE
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

Date

Attachment I

Control samples of melons, fortified with reference standards of MTI-446, DN and UF, will be analyzed to validate the method prior to analysis of field samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DN and UF in/on cucurbits if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set - Melons

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 3

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of potato as a raw agricultural commodity (RAC) for validation of the analytical method for analyses of MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation set for potatoes are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include potato as a RAC for method validation for residue determination of MTI-446, UF and DN.

Approved By:

Jon A MacGregor — 08/27/02
STUDY DIRECTOR — Date
Jon A. MacGregor, B.S.

Willard B. Nixon — 9/13/02
LABORATORY MANAGEMENT — Date
Willard B. Nixon, Ph.D.

William R Landis — 4 August 2002
SPONSOR'S REPRESENTATIVE — Date
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

Attachment I

Control samples of potatoes, fortified with reference standards of MTI-446, DN and UF, will be analyzed to validate the method prior to analysis of field samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DN and UF in/on potatoes if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set - Potatoes

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 4

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of grape as a raw agricultural commodity (RAC) for validation of the analytical method for analyses of MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation set for grapes are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include grape as a RAC for method validation for residue determination of MTI-446, UF and DN.

Approved By:

Jon A MacGregor — 8/27/02

STUDY DIRECTOR
Jon A. MacGregor, B.S.

Date

Willard B. Nixon — 9/13/02

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

Date

4 Aug, 2002

SPONSOR'S REPRESENTATIVE
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

Date

Attachment I

Control samples of grapes, fortified with reference standards of MTI-446, DN and UF, will be analyzed to validate the method prior to analysis of field samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DN and UF in/on grapes if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set - Grapes

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 5

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of lettuce (either leaf or head) as a representative raw agricultural commodity (RAC) for validation of the analytical method for analyses of leafy vegetables for MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation set for lettuce are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include lettuce (either leaf or head) as a representative RAC for method validation for residue determination of MTI-446, UF and DN in leafy vegetables.

Approved By:

STUDY DIRECTOR
Jon A. MacGregor, B.S.

8-15-02
Date

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

8/21/02
Date

SPONSOR'S REPRESENTATIVE
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

4 Aug. 2002
Date

Attachment I

Control samples of lettuce (either leaf or head as available), fortified with reference standards of MTI-446, DN and UF, will be analyzed to validate the method prior to analysis of field samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DN and UF in/on leafy vegetables if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set - Lettuce

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 6

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of tomato paste and puree processed commodities (PC) for validation of the analytical method for analyses of MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation sets are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include tomato paste and puree processed commodities for method validation for residue determination of MTI-446, UF and DN.

Approved By:

Jon A. MacGregor

STUDY DIRECTOR
Jon A. MacGregor, B.S.

8/28/02
Date

Willard B. Nixon

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

9/23/02
Date

SPONSOR'S REPRESENTATIVE
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

8/9/02
Date

Attachment I

Control samples of tomato paste and puree, fortified with reference standards of MTI-446, DN and UF, will be analyzed to validate the method prior to analysis of processed samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DN and UF in paste and puree if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set – Tomato Paste

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

Method Validation Sample Set – Tomato Puree

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 7

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of potato granules/flakes, chips and wet peel processed commodities (PC) for validation of the analytical method for analyses of MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation sets are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include potato granules/flakes, chips and wet peel processed commodities for method validation for residue determination of MTI-446, UF and DN.

Approved By:

STUDY DIRECTOR
Jon A. MacGregor, B.S.

Date

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

Date

SPONSOR'S REPRESENTATIVE
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

Date

Attachment I

Control samples of potato granules/flakes, chips and wet peel, fortified with reference standards of MTI-446, DN and UF, will be analyzed to validate the method prior to analysis of processed samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DN and UF in granules/flakes, chips and wet peel if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set – Potato Granules/Flakes

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

Method Validation Sample Set – Potato Chips

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

Method Validation Sample Set – Potato Wet Peel

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 8

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of raisins and grape juice processed commodities (PC) for validation of the analytical method for analyses of MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation sets are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include raisins and grape juice processed commodities for method validation for residue determination of MTI-446, UF and DN.

Approved By:

Jon A MacGregor — 8/28/02
STUDY DIRECTOR — Date
Jon A. MacGregor, B.S.

Willard B. Nixon — 9/23/02
LABORATORY MANAGEMENT — Date
Willard B. Nixon, Ph.D.

8/9/02
SPONSOR'S REPRESENTATIVE — Date
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

Attachment I

Control samples of raisins and grape juice, fortified with reference standards of MTI-446, DN and UF, will be analyzed to validate the method prior to analysis of processed samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DN and UF in raisins and grape juice if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set – Raisins

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

Method Validation Sample Set – Juice

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 9

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Addition of broccoli as a representative raw agricultural commodity (RAC) for validation of the analytical method for analyses of the brassica crop grouping for MTI-446 and its UF and DN metabolites. Specific samples comprising the method validation set for broccoli are presented in Attachment I.
Reason for Amendment and Effect on the Study:	To include broccoli as a representative RAC for method validation for residue determination of MTI-446, UF and DN in brassica.

Approved By:

Jon A MacGregor — 8/28/02

STUDY DIRECTOR
Jon A. MacGregor, B.S.

Date

Willard B. Nixon — 9/23/02

LABORATORY MANAGEMENT
Willard B. Nixon, Ph.D.

Date

8/9/02

SPONSOR'S REPRESENTATIVE
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

Date

Attachment I

Control samples of broccoli, fortified with reference standards of MTI-446, DN and UF, will be analyzed to validate the method prior to analysis of field samples. The target limit of quantitation will be 0.01 mg/Kg for each analyte (MTI-446, UF and DN). The method will be considered adequate for purposes of analyses of MTI-446, DN and UF in/on brassica if recovery values are within the range of 70 to 120% at all fortification levels with a coefficient of variation ≤20%. A wider range of recovery values and/or a greater coefficient of variation may be approved by the Sponsor's Representative.

Method Validation Sample Set - Broccoli

Sample Description	Fortification Level (mg/Kg) [1]	Number of Replicates
Reagent Blank [2]	-	1
Control [2]		1
MTI-446		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
UF		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
DN		
Fortification 1 [3]	0.010	5
Fortification 2	1.00	5
Total Analytical Recovery Results (10 per analyte)		30
Total Samples		12

[1] The number of fortification levels represents the minimum proposed and may be increased upon agreement between the Study Director and Sponsor's Representative.
[2] The reagent blank and control are common to all fortified samples.
[3] Target LOQ of 0.010 mg/Kg for MTI-446, UF and DN residues.

PROTOCOL AMENDMENT NO. 10

Protocol (Study) Number:	236C-113
Study Title:	LABORATORY VALIDATION OF METHOD(S) FOR THE ANALYSIS OF MTI-446 AND ITS METABOLITES DN AND UF IN MULTIPLE CROP SUBSTRATES
Testing Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Description of Amendment:	Page 4 of protocol; clarification of DN metabolite purity. The purity is incorrectly listed as 99.27% and should be 97.27%.
Reason for Amendment and Effect on the Study:	This was a typographical error and has no effect on the study.

Approved By:

Jon A. MacGregor 10/28/02
STUDY DIRECTOR — Date
Jon A. MacGregor, B.S.

Willard B. Nixon 10/28/02
LABORATORY MANAGEMENT — Date
Willard B. Nixon, Ph.D.

11/8/02
SPONSOR'S REPRESENTATIVE — Date
William R. Landis, Ph.D.
LANDIS INTERNATIONAL, INC.

Reviewed by QA SLC 10-29-02

APPENDIX C

Certificates of Analysis

MITSUI CHEMICALS,INC.
Kasumigaseki Bldg. 3-2-5 Kasumigaseki, Chiyoda-ku
Tokyo 100-6070, Japan

CERTIFICATE OF ANALYSIS OF MTI-446 ANALYTICAL STANDARD

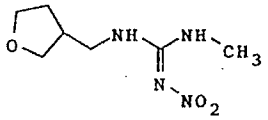
December 7, 2001

CERTIFICATE OF ANALYSIS

We, the manufacturer, hereby certify that the following commodity has been manufactured by our company and that the relative statements below are true and correct.

Active ingredient ;

Identity	:MTI-446
CAS No	:165252-70-0
Chemical name	:(*RS*)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl) guanidine
Structural Formula	:



Appearance	: White powder

Purity of analytical standard (Lot No. EBI-5-101)
Chemical analysis result ;

Purity(Area%)	: 99.6
Method of analysis	: HPLC(internal standard method)
Data analysis	: January 6, 1998
Expiration Date	: December 2003
Storage instruction	: Cool and Dark place

Yasunori Fumoto

Yasunori Fumoto
Agrochemical Group
Life Science Laboratory
Mitsui Chemicals, Inc.

MITSUI CHEMICALS.INC.
Kasumigaseki Bldg. 3-2-5 Kasumigaseki, Chiyoda-ku
Tokyo 100-6070, Japan

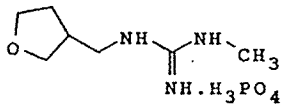
CERTIFICATE OF ANALYSIS OF MTI-446 METABOLITE ANALYTICAL STANDARD

December 7, 2001

CERTIFICATE OF ANALYSIS

We, the manufacturer, hereby certify that the following commodity has been manufactured by our company and that the relative statements below are true and correct.

Active ingredient ;
Identity : DN
Chemical name : 1-methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate
Structural Formula :



Appearance : White powder

Purity of analytical standard (Lot No. OFU-1290)
Chemical analysis result ;
Purity(Area%) : 97.27
Method of analysis : HPLC(UV-210nm)
Data analysis : April 16, 1998
Expiration Date : December 2003
Storage instruction : Cool and Dark place

Yasunori Fumoto

Yasunori Fumoto
Agrochemical Group
Life Science Laboratory
Mitsui Chemicals, Inc.

MITSUI CHEMICALS,INC.
Kasumigaseki Bldg. 3-2-5 Kasumigaseki, Chiyoda-ku
Tokyo 100-6070, Japan

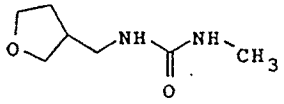
CERTIFICATE OF ANALYSIS OF MTI-446 METABOLITE ANALYTICAL STANDARD

December 7, 2001

CERTIFICATE OF ANALYSIS

We, the manufacturer, hereby certify that the following commodity has been manufactured by our company and that the relative statements below are true and correct.

Active ingredient ;
Identity : UF
Chemical name : 1-methyl-3-(tetrahydro-3-furylmethyl)urea
Structural Formula :



Appearance : White powder

Purity of analytical standard (Lot No. OFU-1291)
Chemical analysis result ;
Purity(Area%) : 99.59
Method of analysis : HPLC(UV-210nm)
Data analysis : April 16, 1998
Expiration Date : December 2003
Storage instruction : Cool and Dark place

Yasunori Fumoto

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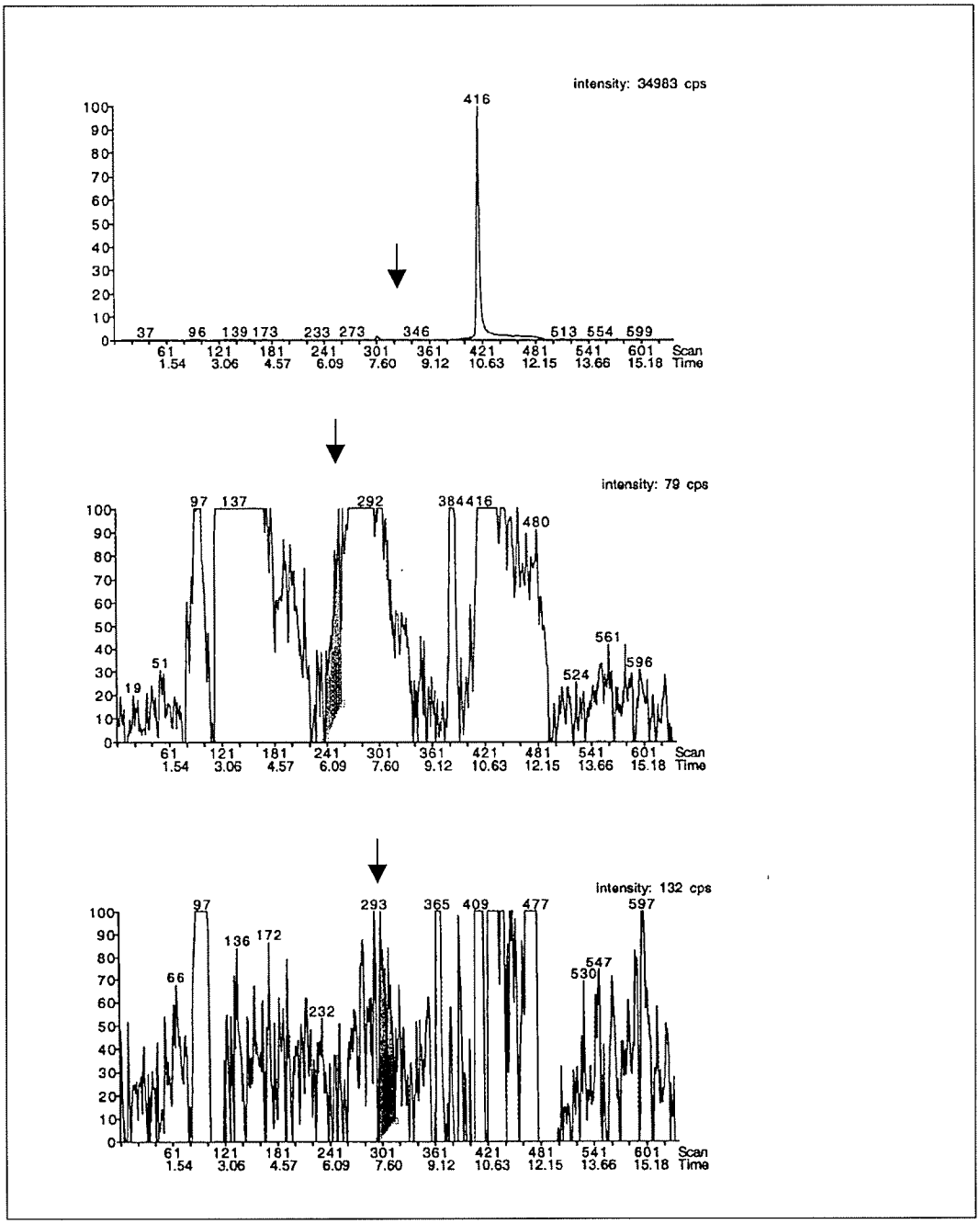
APPENDIX D

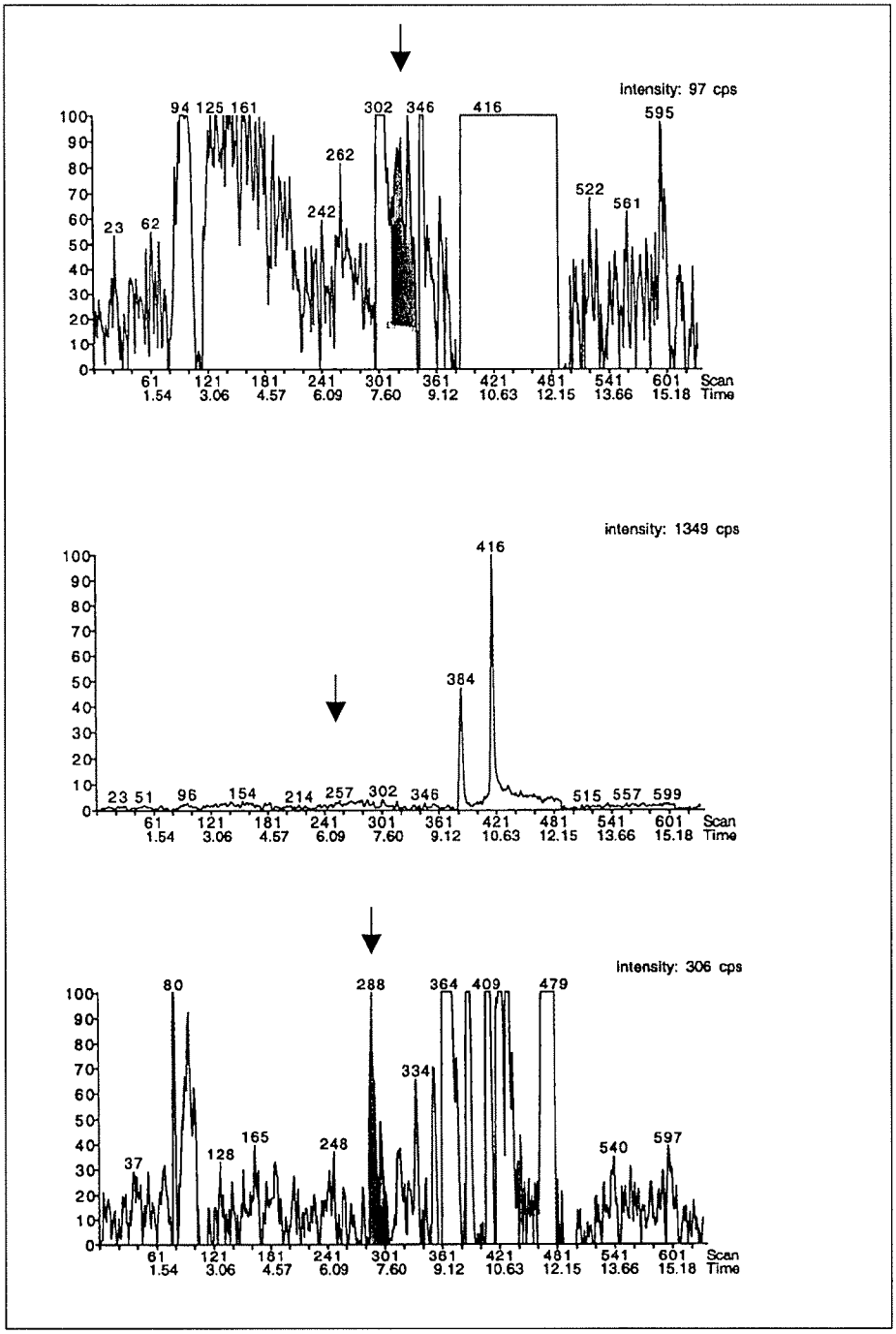
Typical Chromatograms of Multiple Crop Substrate Control Samples and Control Samples Fortified with MTI-446, and Metabolites UF and DN

APPENDIX D.1



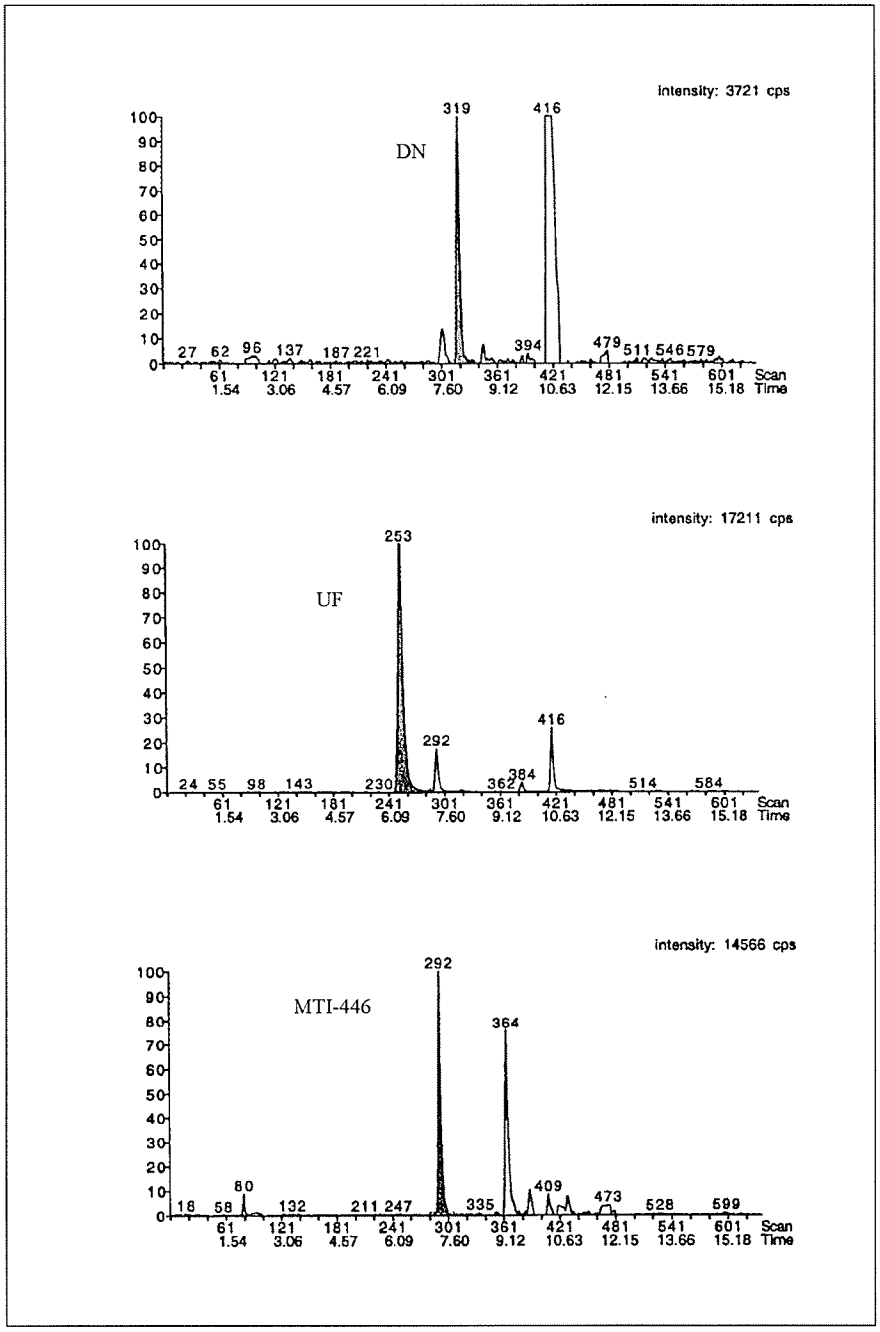
Typical Chromatogram of a Reagent Blank Sample Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-TOMVREB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.2



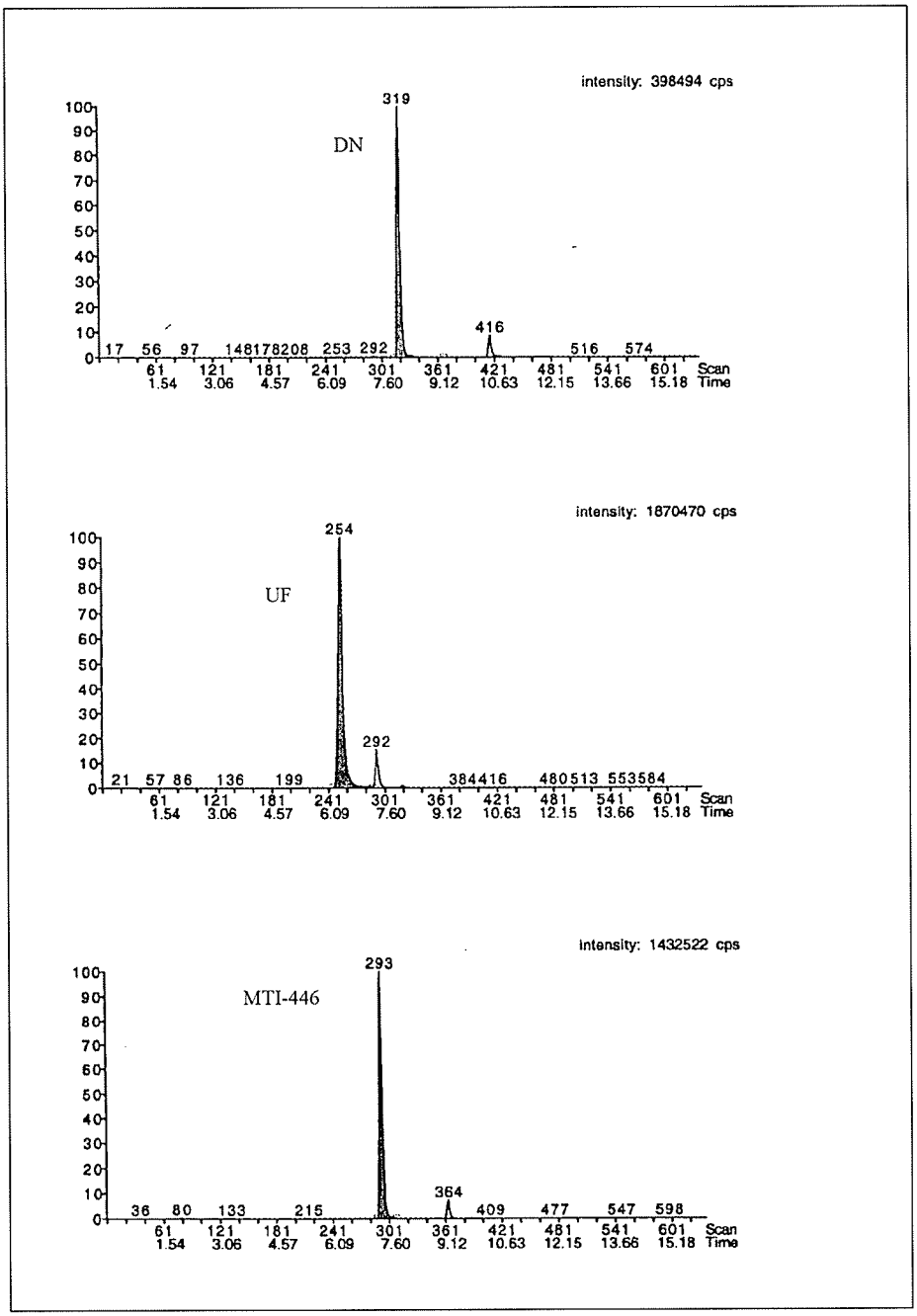
Typical Chromatogram of a Control Sample of Tomato Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-TOMVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.3



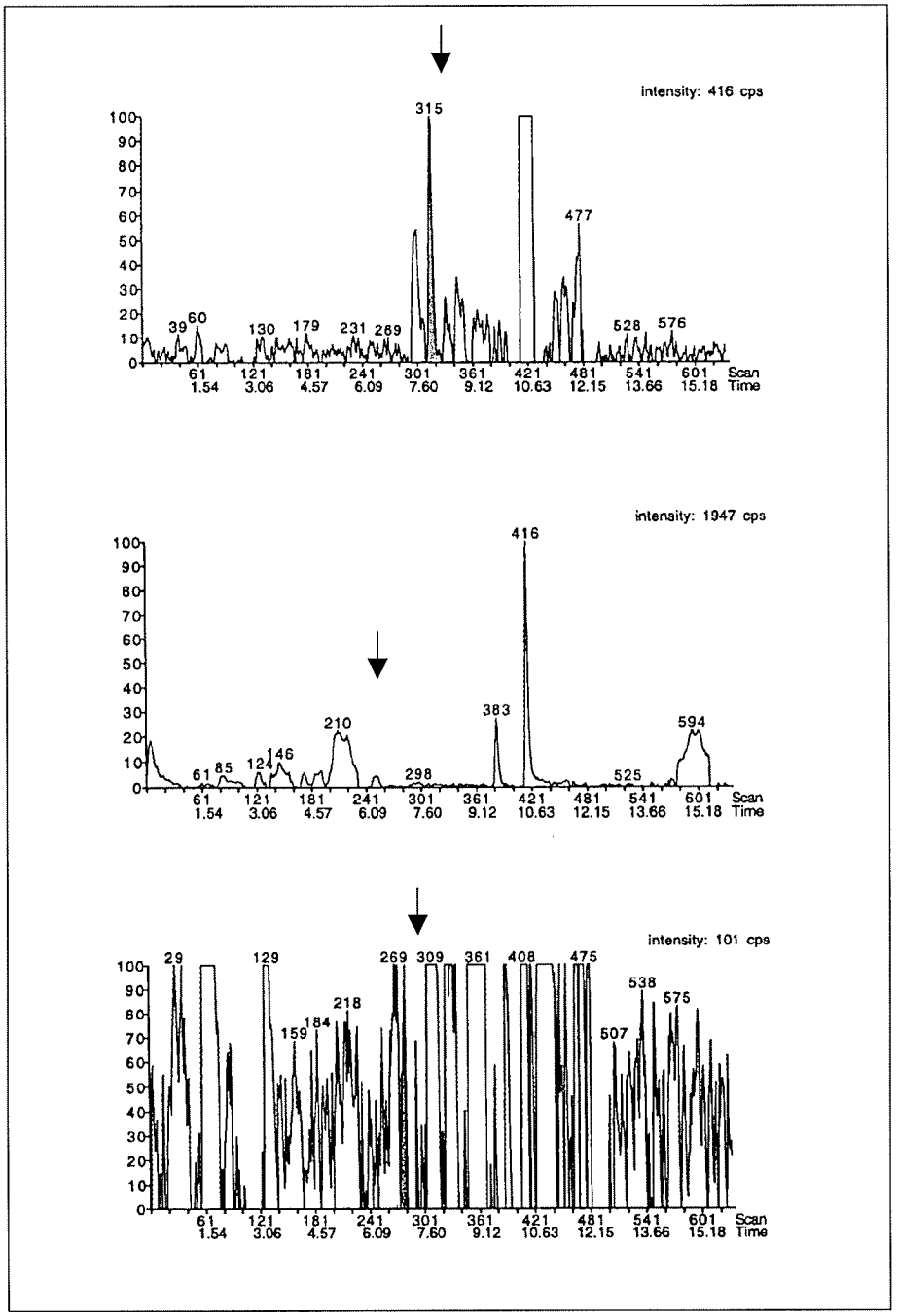
Typical Chromatogram of a Control Sample of Tomato Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-TOMVMAS-1.

APPENDIX D.4



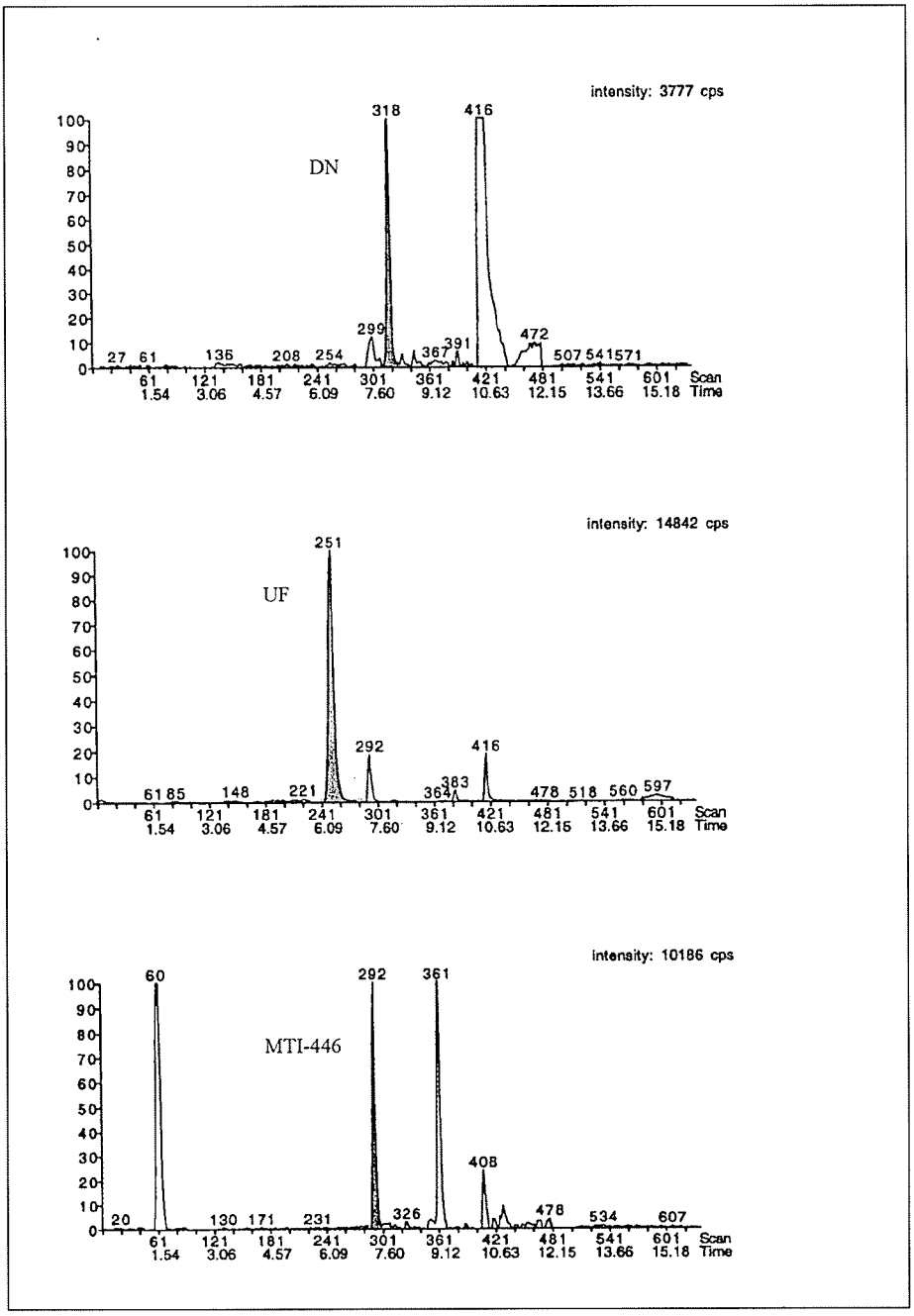
Typical Chromatogram of a Control Sample of Tomato Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100x LOQ), 236C-113-TOMVMAS-6.

APPENDIX D.5



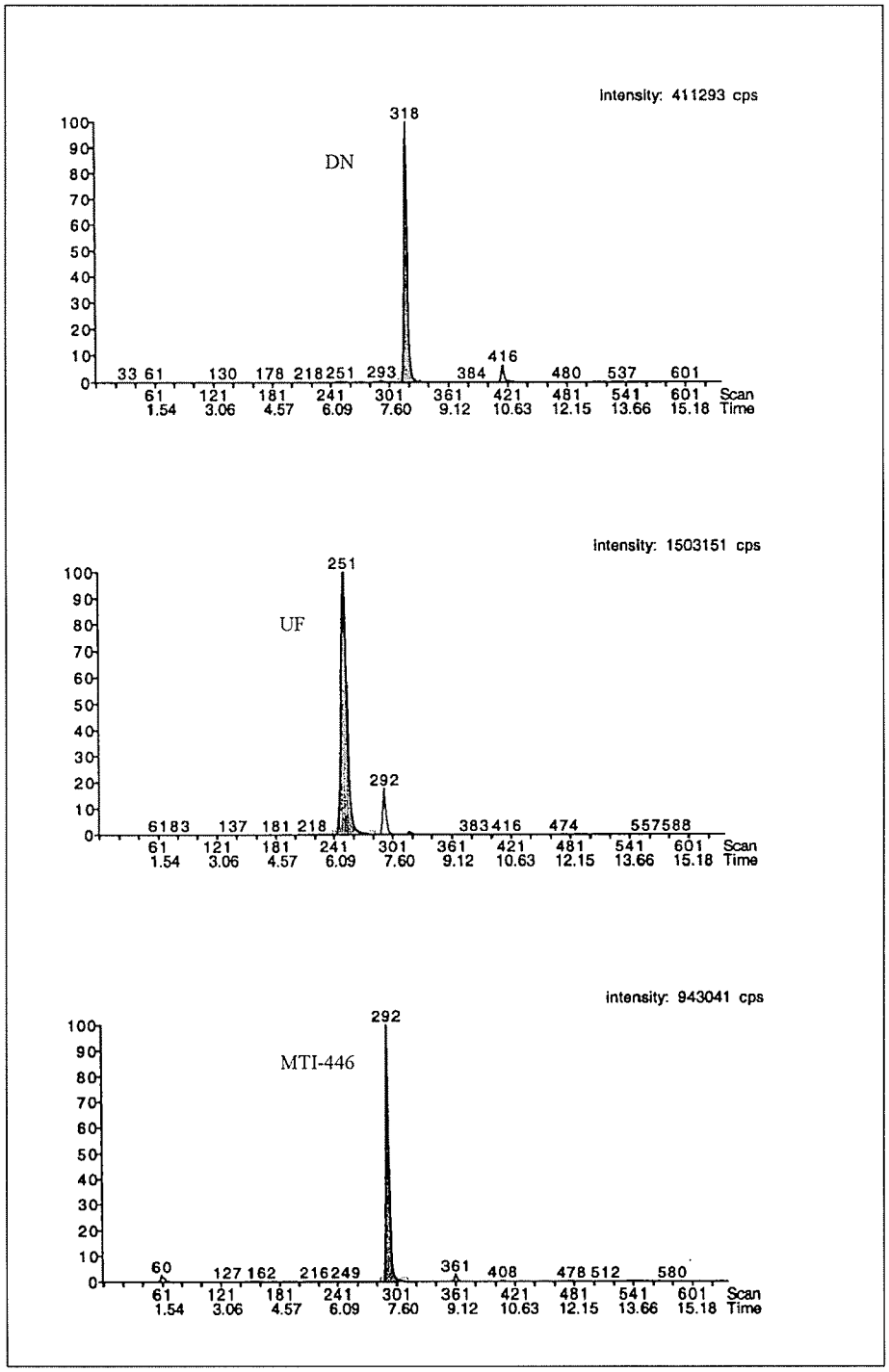
Typical Chromatogram of a Control Sample of Melon Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-MELVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.6



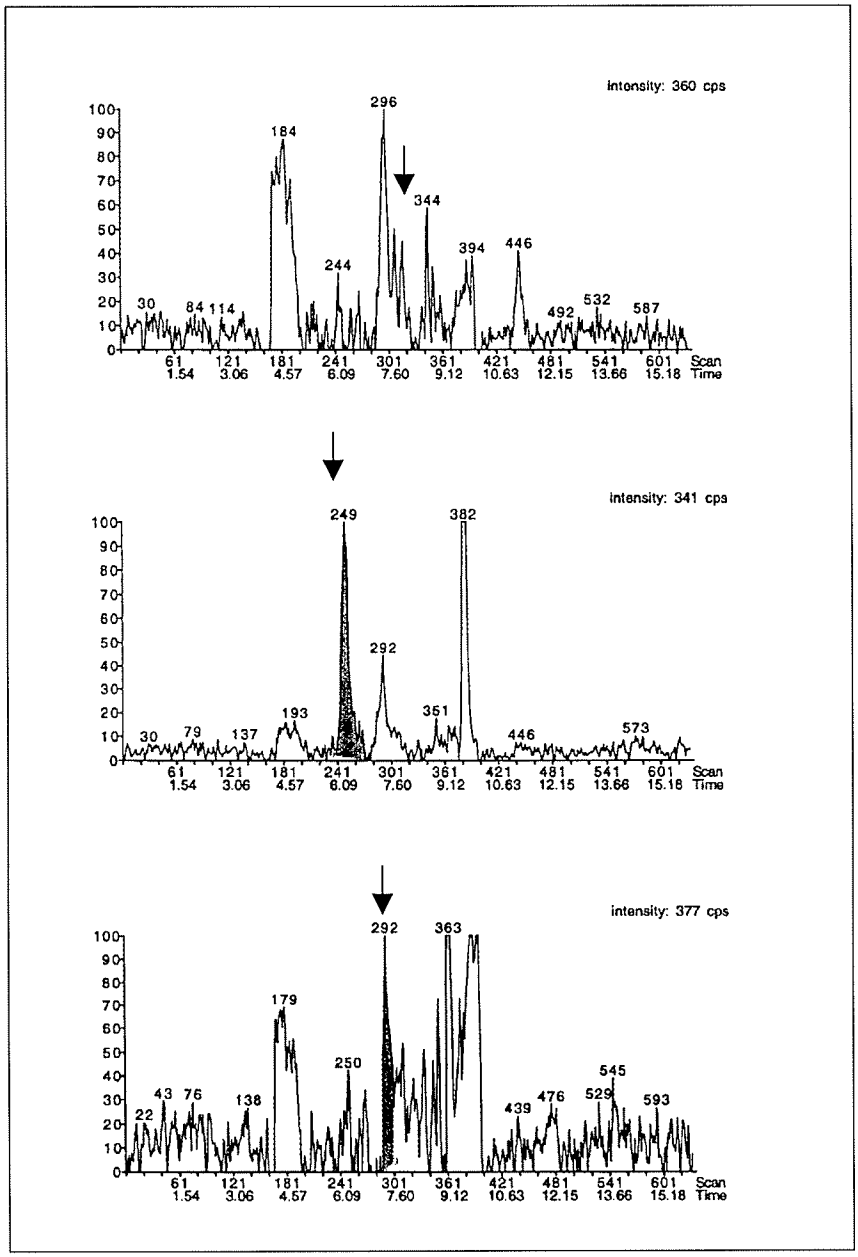
Typical Chromatogram of a Control Sample of Melon Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-MELVMAS-1.

APPENDIX D.7



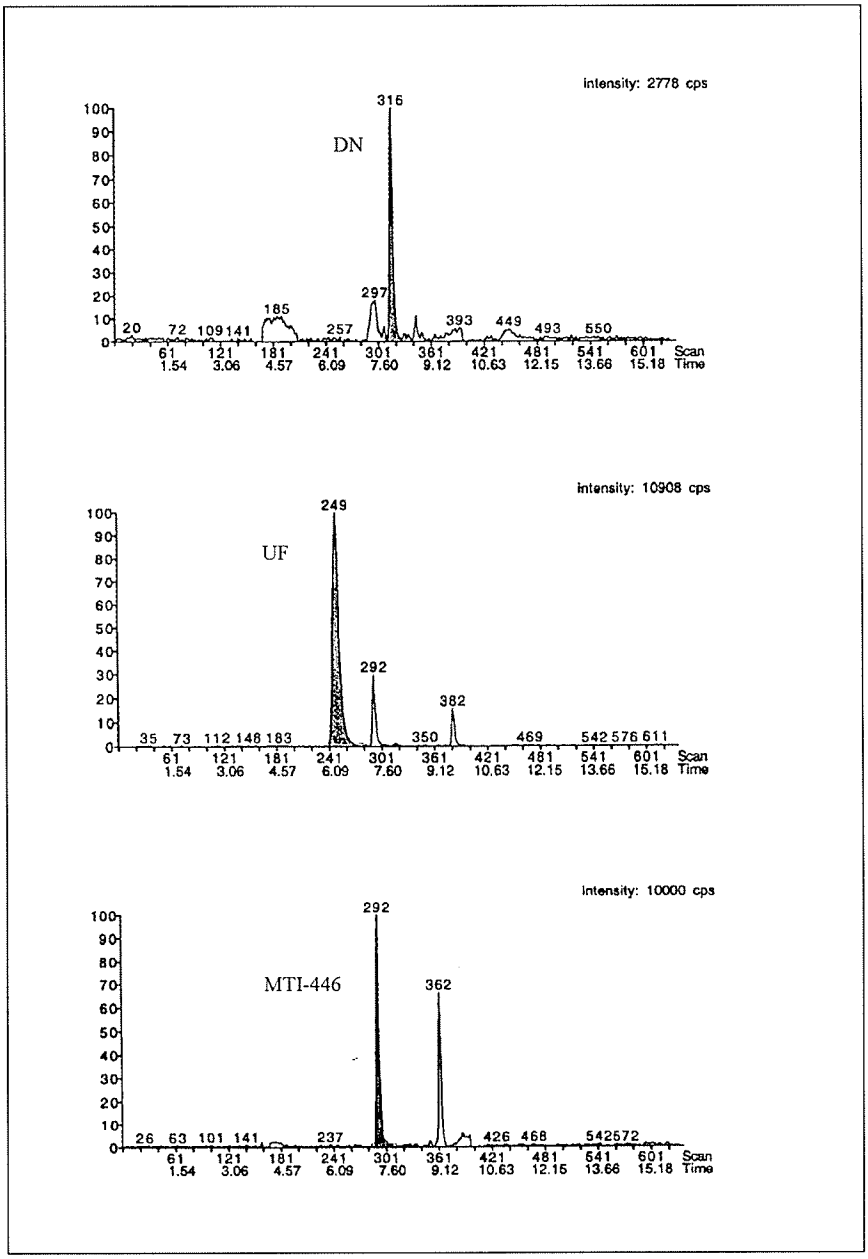
Typical Chromatogram of a Control Sample of Melon Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100x LOQ), 236C-113-MELVMAS-6.

APPENDIX D.8



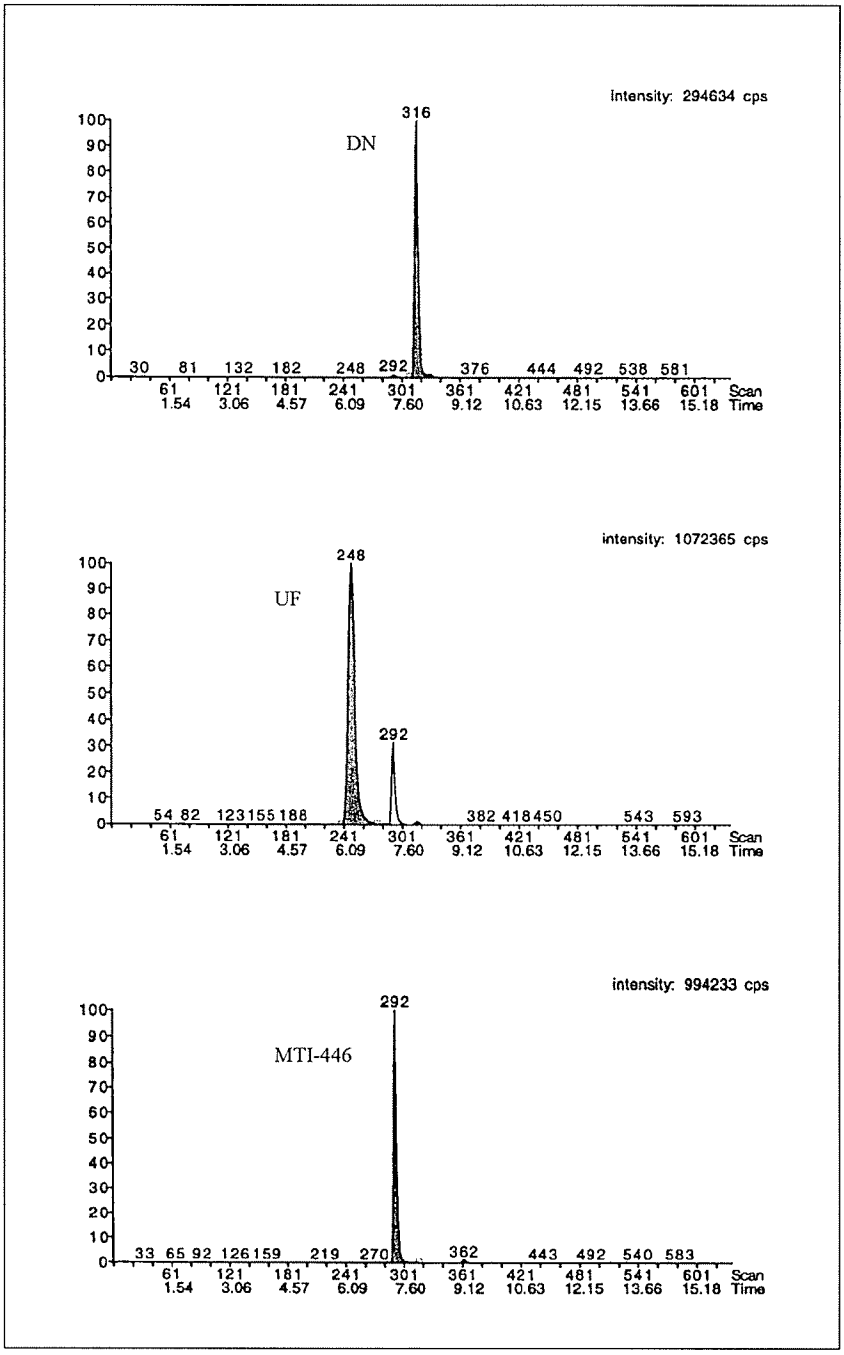
Typical Chromatogram of a Control Sample of Potato Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-POTVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.9



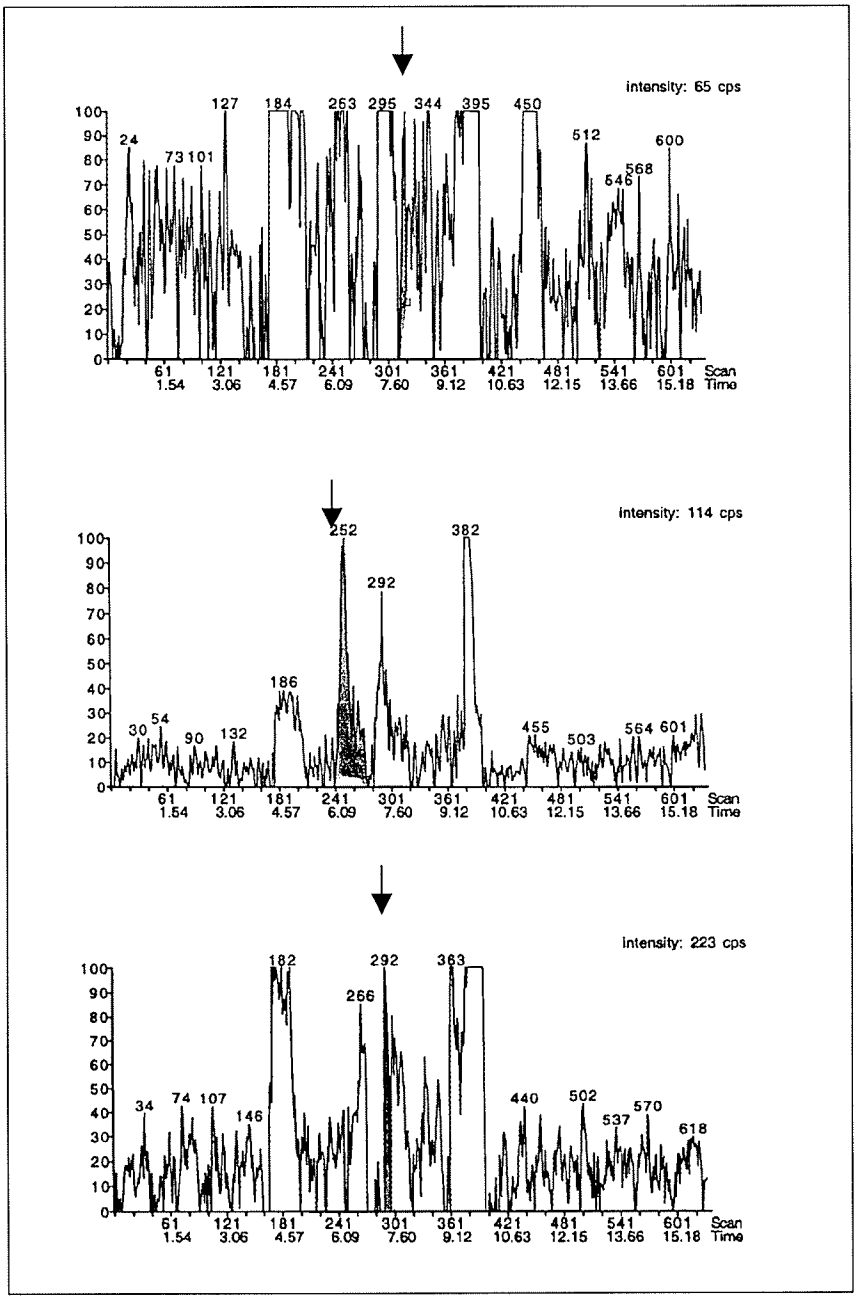
Typical Chromatogram of a Control Sample of Potato Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-POTVMAS-1.

APPENDIX D.10



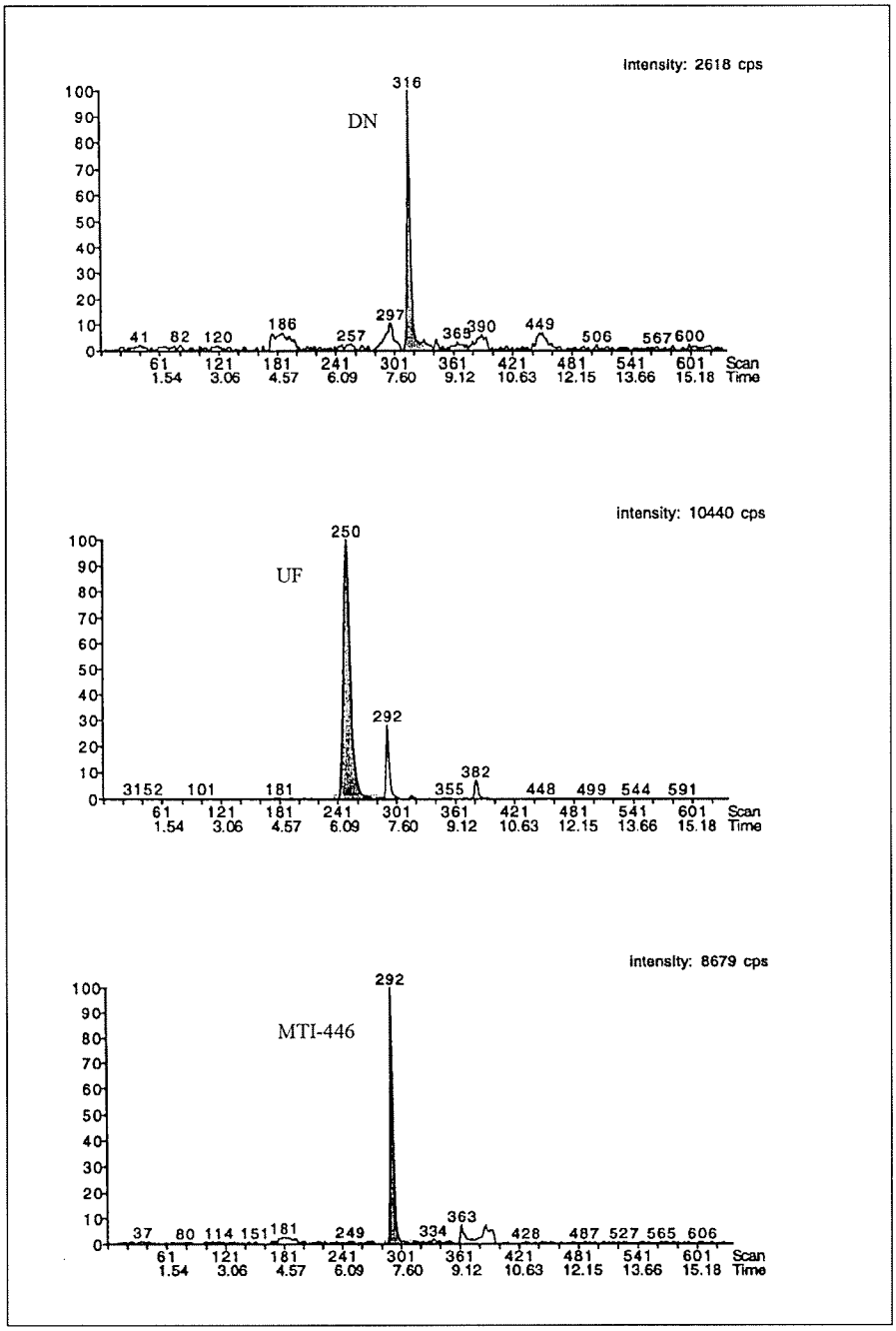
Typical Chromatogram of a Control Sample of Potato Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100x LOQ), 236C-113-POTVMAS-6.

APPENDIX D.11



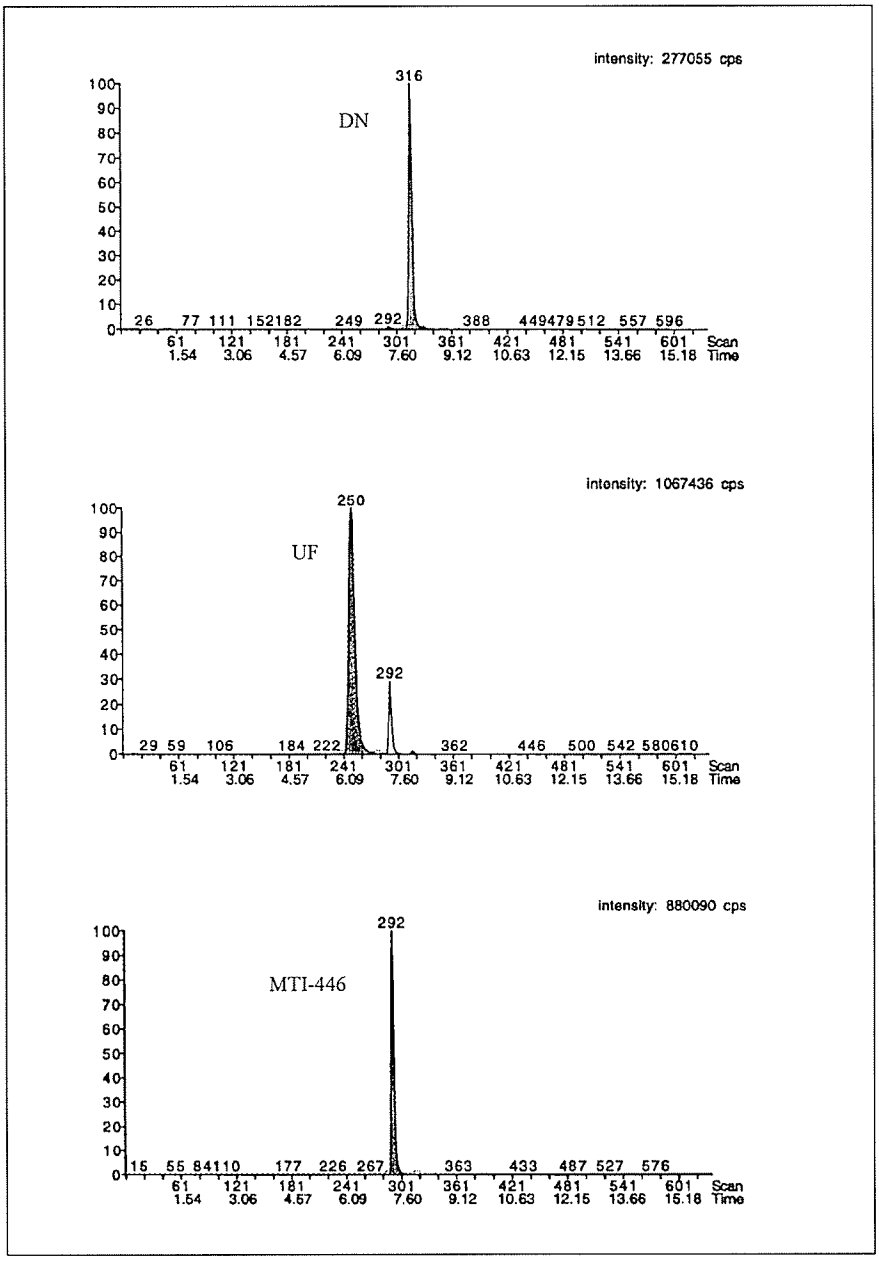
Typical Chromatogram of a Control Sample of Grape Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-GRAVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.12



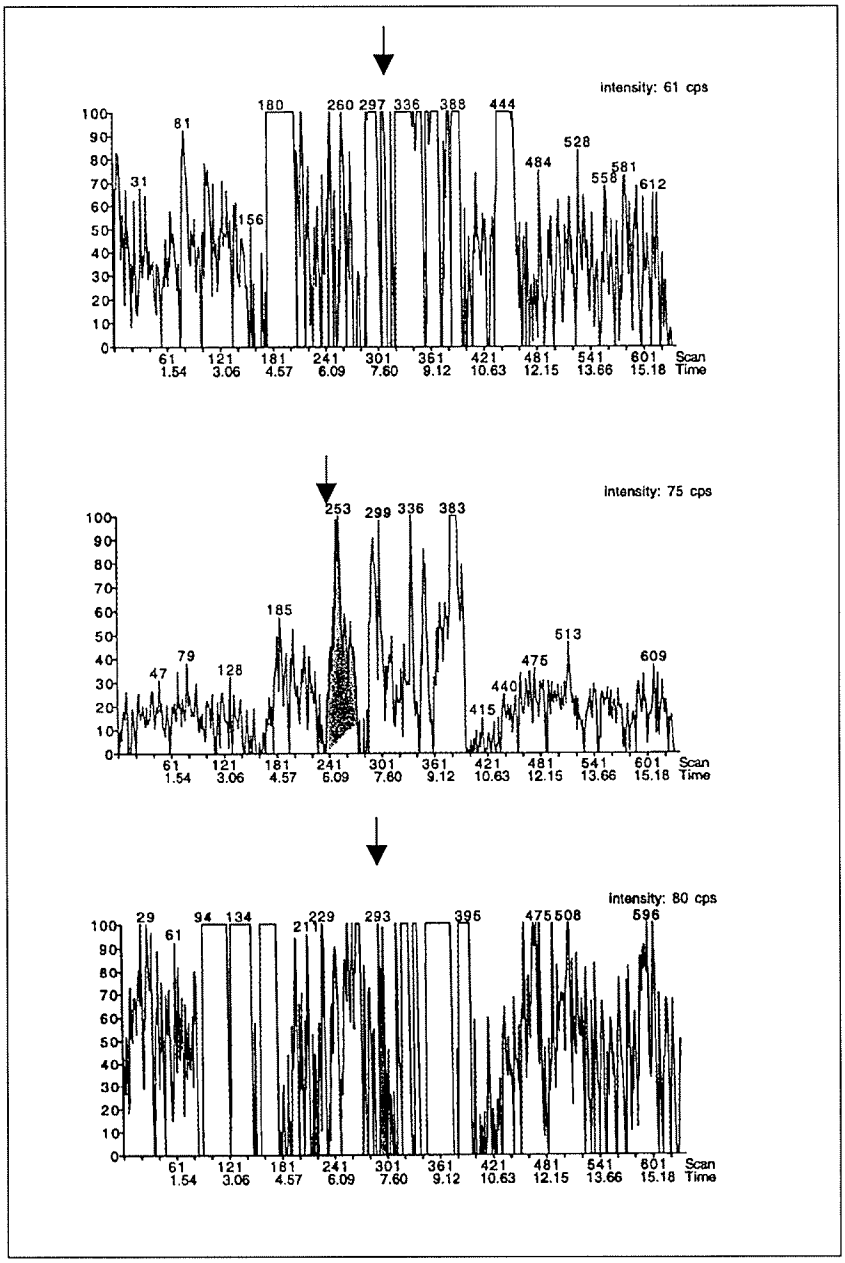
Typical Chromatogram of a Control Sample of Grape Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-GRAVMAS-1.

APPENDIX D.13



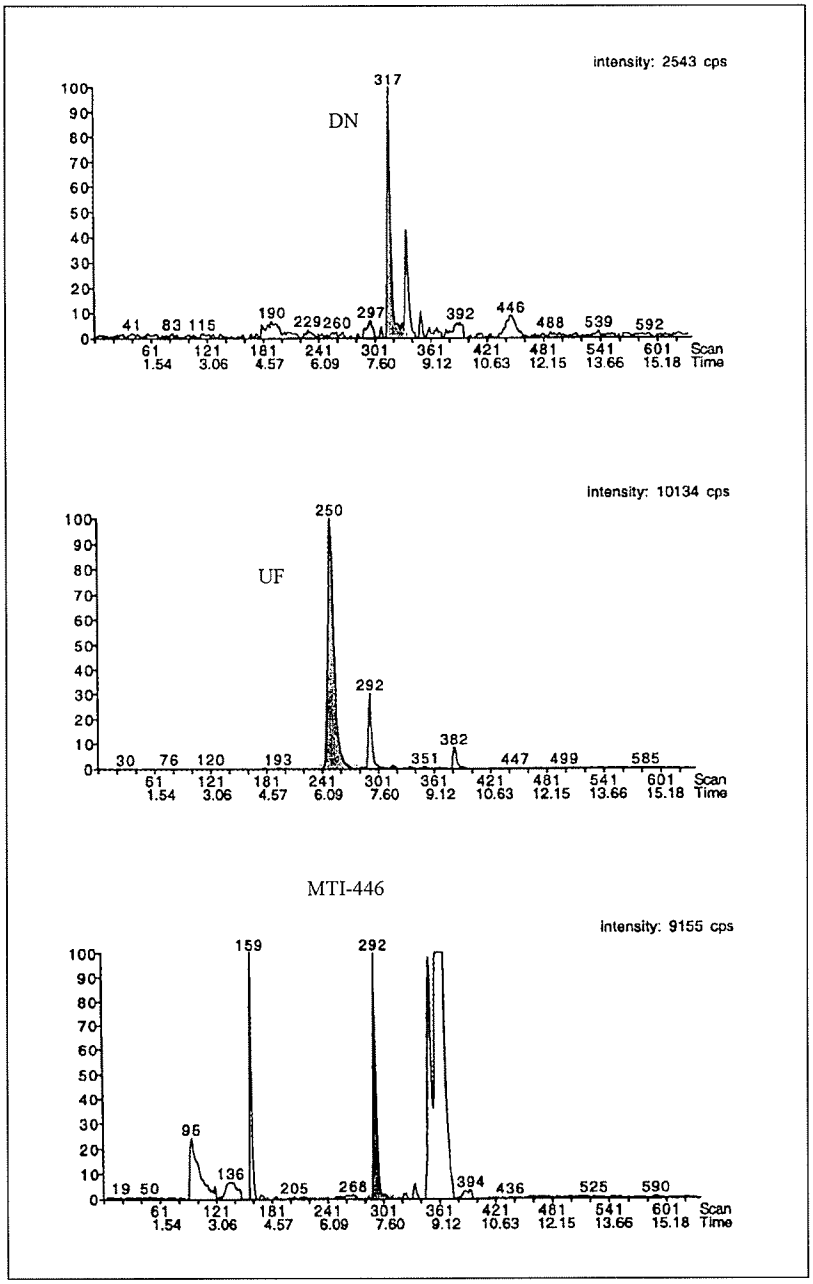
Typical Chromatogram of a Control Sample of Grape Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100x LOQ), 236C-113-GRAVMAS-6.

APPENDIX D.14



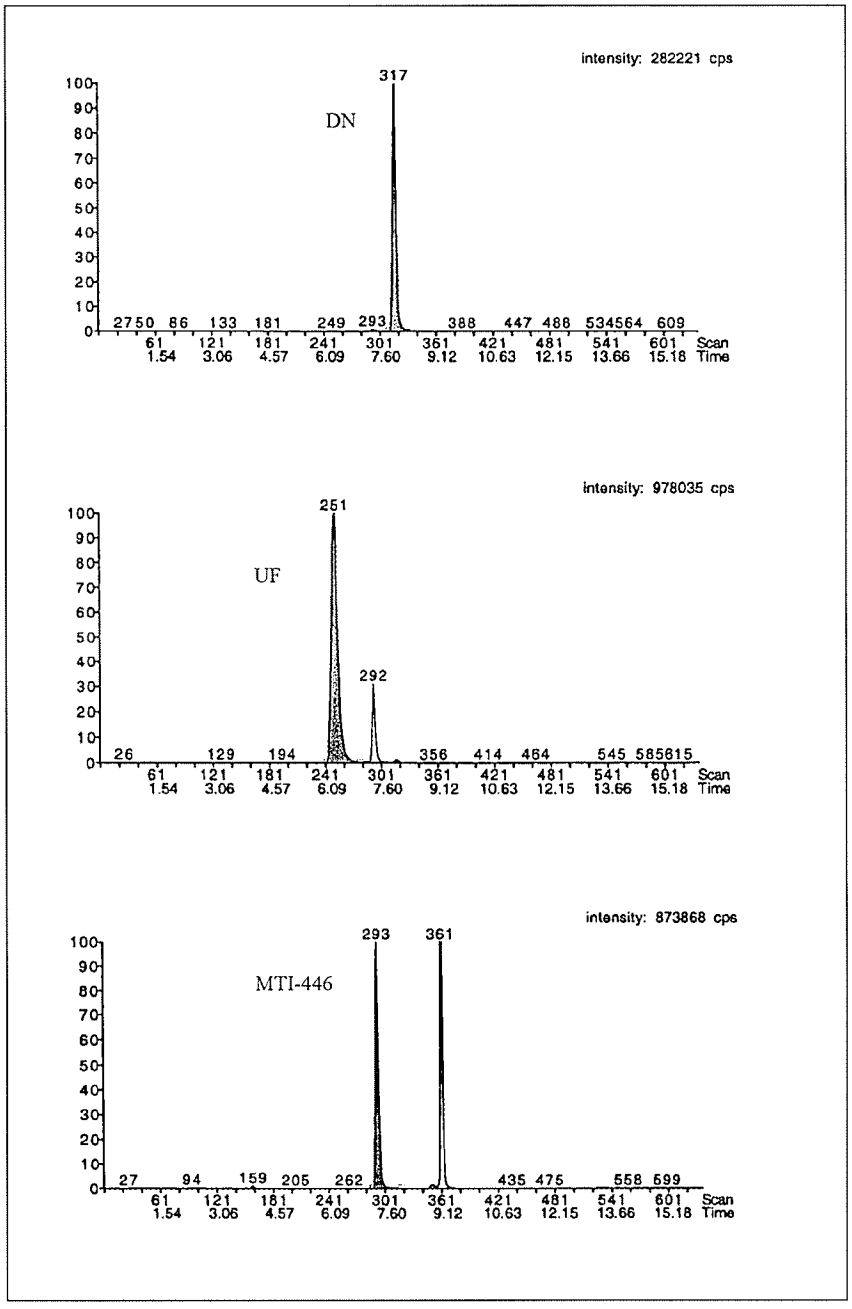
Typical Chromatogram of a Control Sample of Broccoli Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-BROVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.15



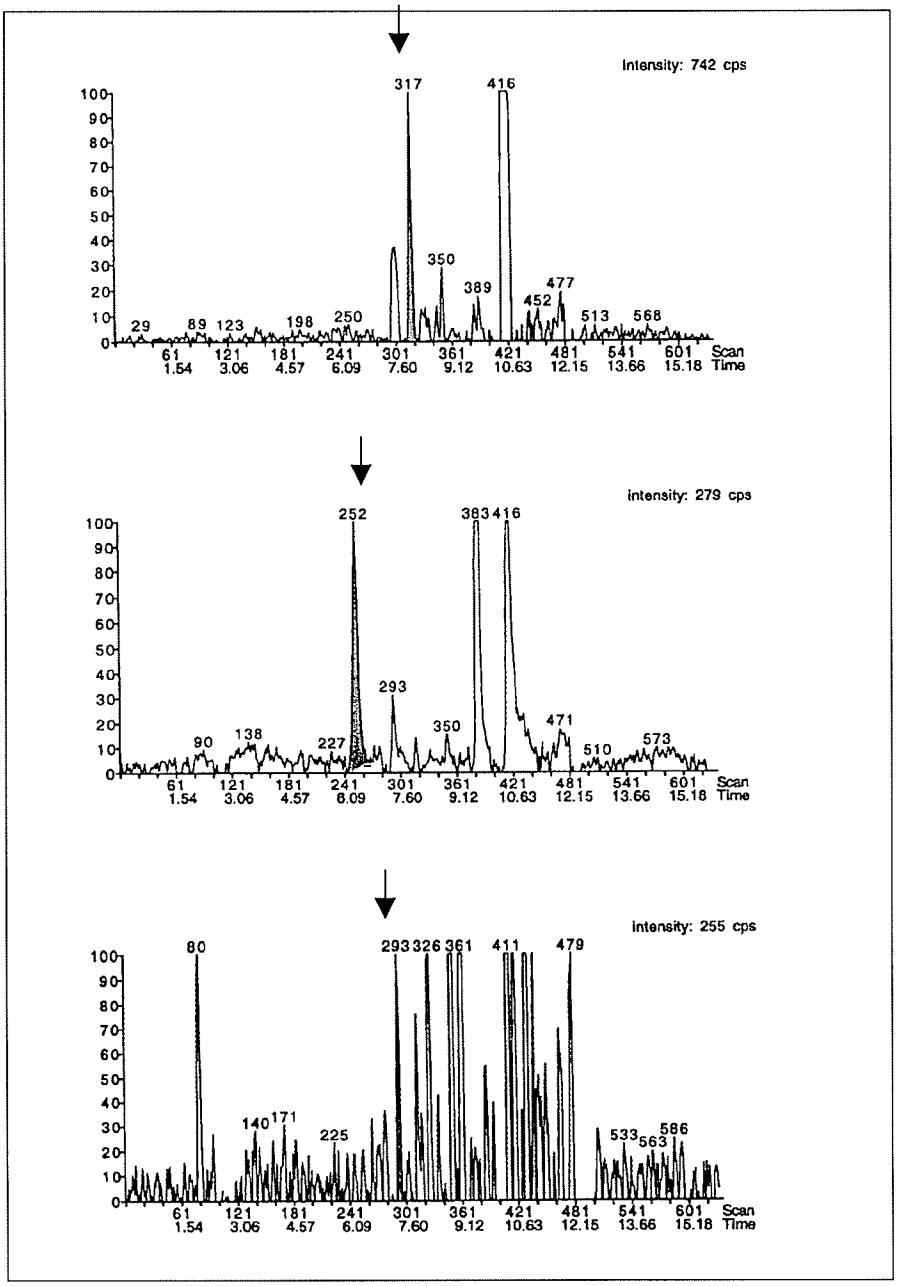
Typical Chromatogram of a Control Sample of Broccoli Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-BROVMAS-1.

APPENDIX D.16



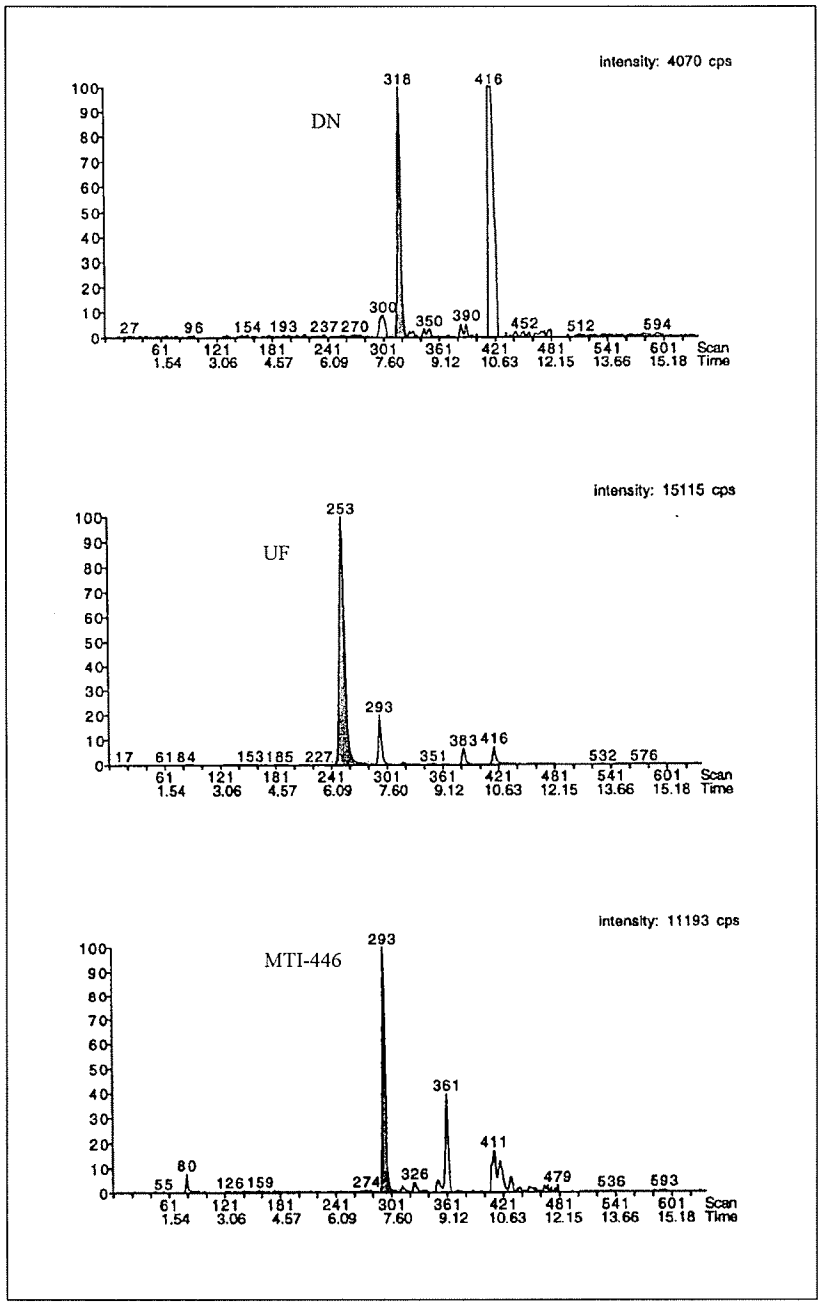
Typical Chromatogram of a Control Sample of Broccoli Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100x LOQ), 236C-113-BROVMAS-6.

APPENDIX D.17



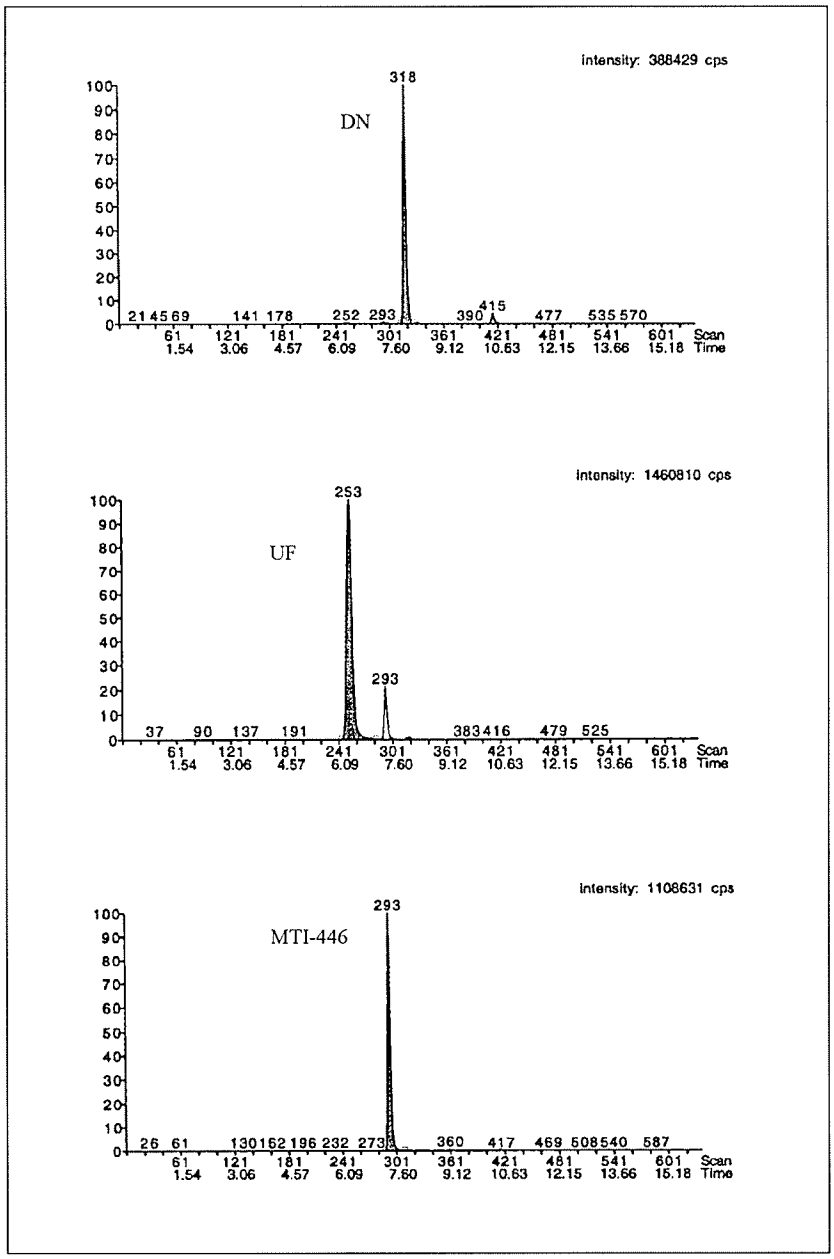
Typical Chromatogram of a Control Sample of Lettuce Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-LETVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.18



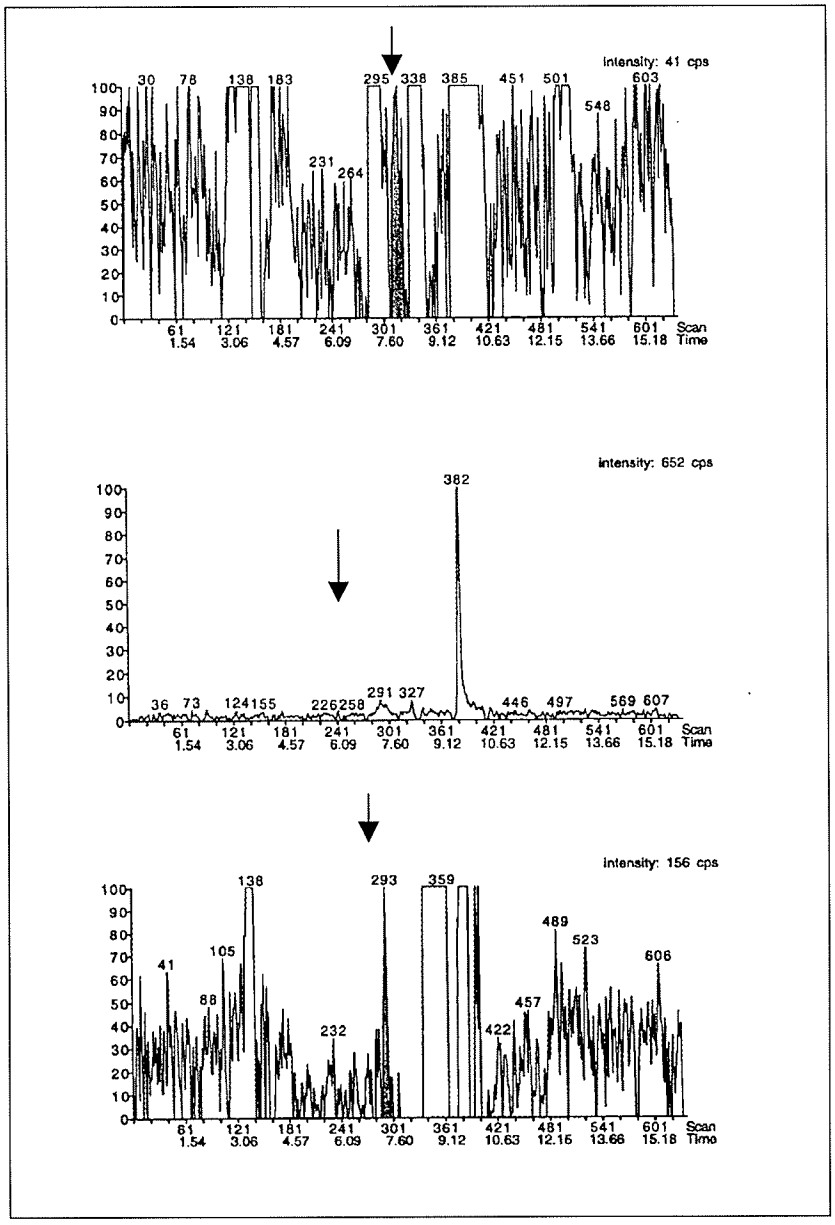
Typical Chromatogram of a Control Sample of Lettuce Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-LETVMAS-1.

APPENDIX D.19



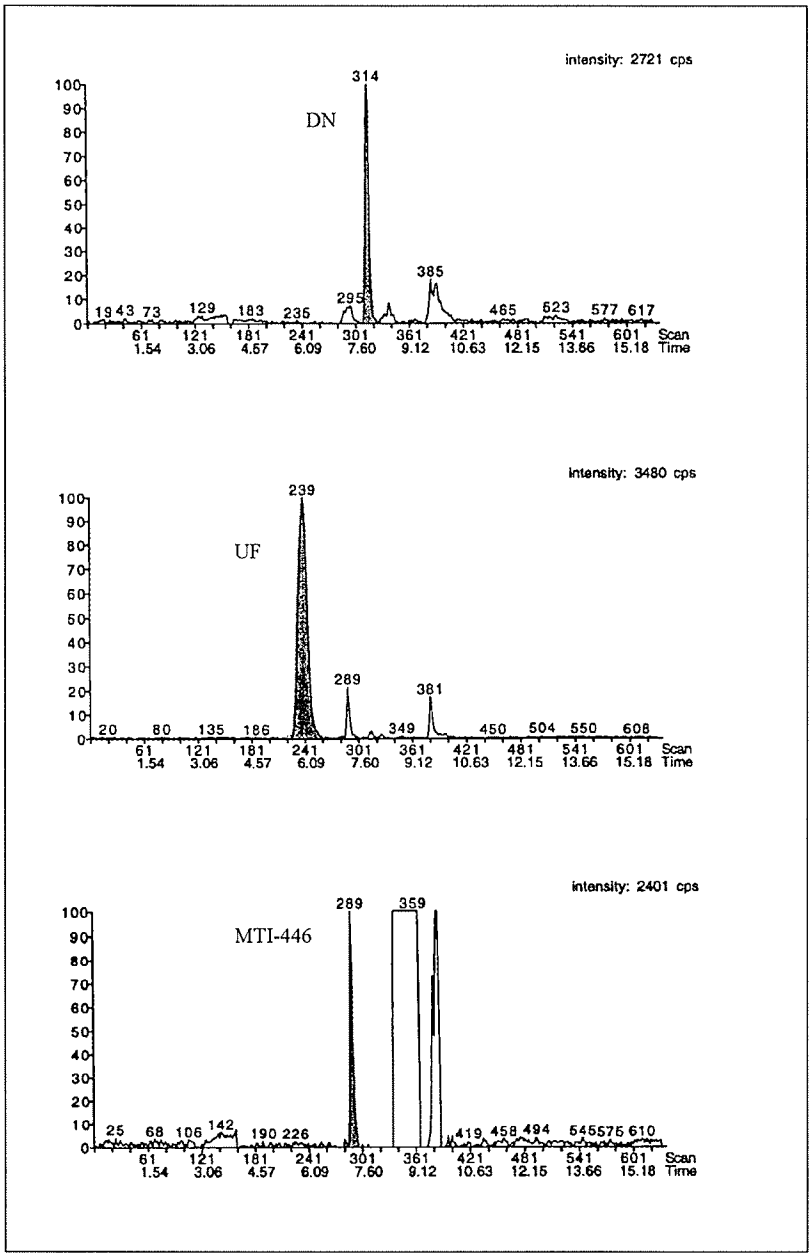
Typical Chromatogram of a Control Sample of Lettuce Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100x LOQ), 236C-113-LETVMAS-6.

APPENDIX D.20



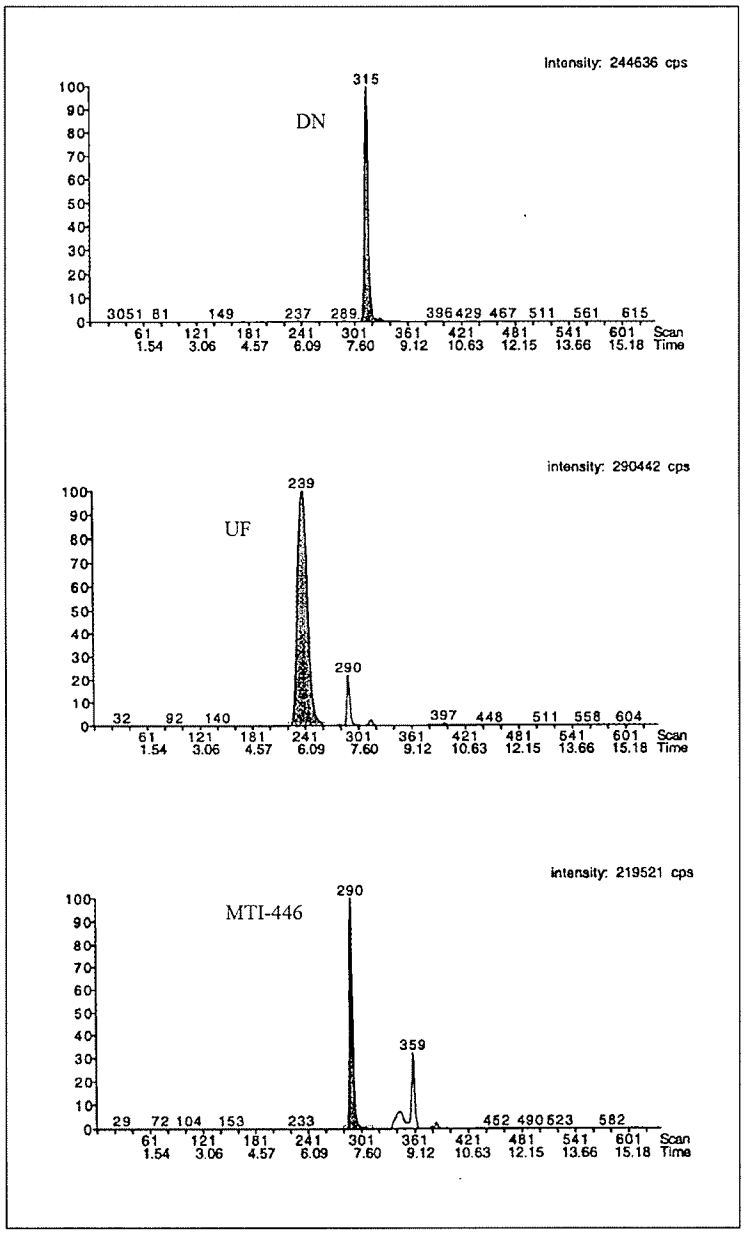
Typical Chromatogram of a Control Sample of Tomato Paste Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-PASVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.21



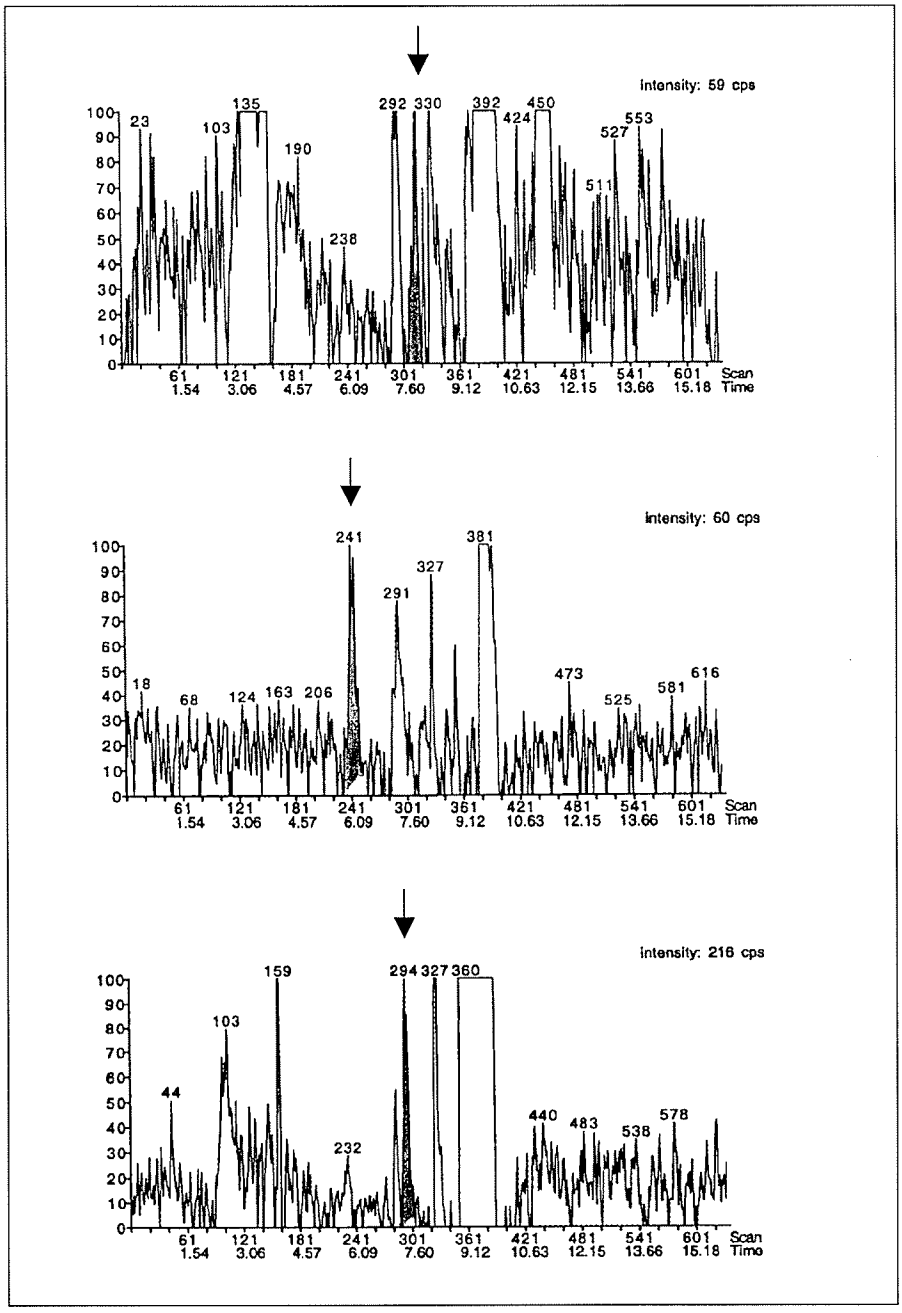
Typical Chromatogram of a Control Sample of Tomato Paste Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-PASVMAS-1.

APPENDIX D.22



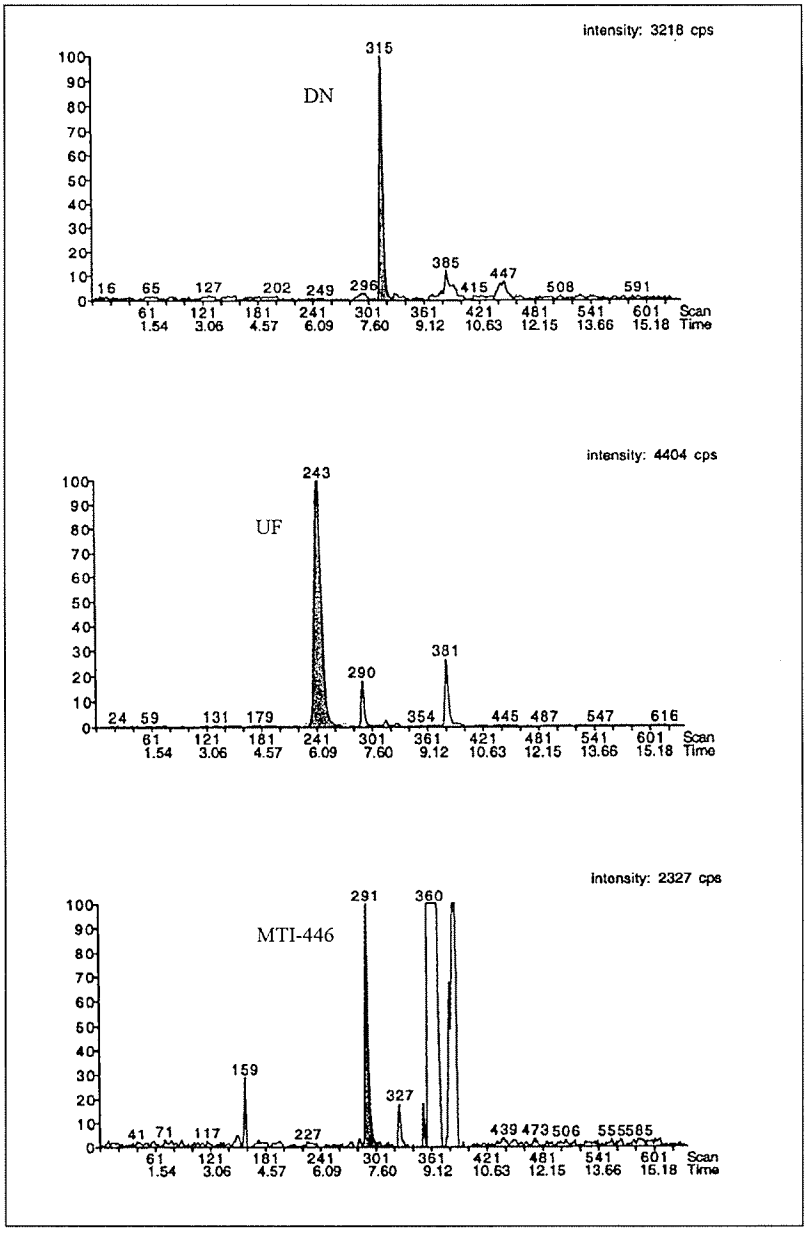
Typical Chromatogram of a Control Sample of Tomato Paste Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100x LOQ), 236C-113-PASVMAS-6.

APPENDIX D.23



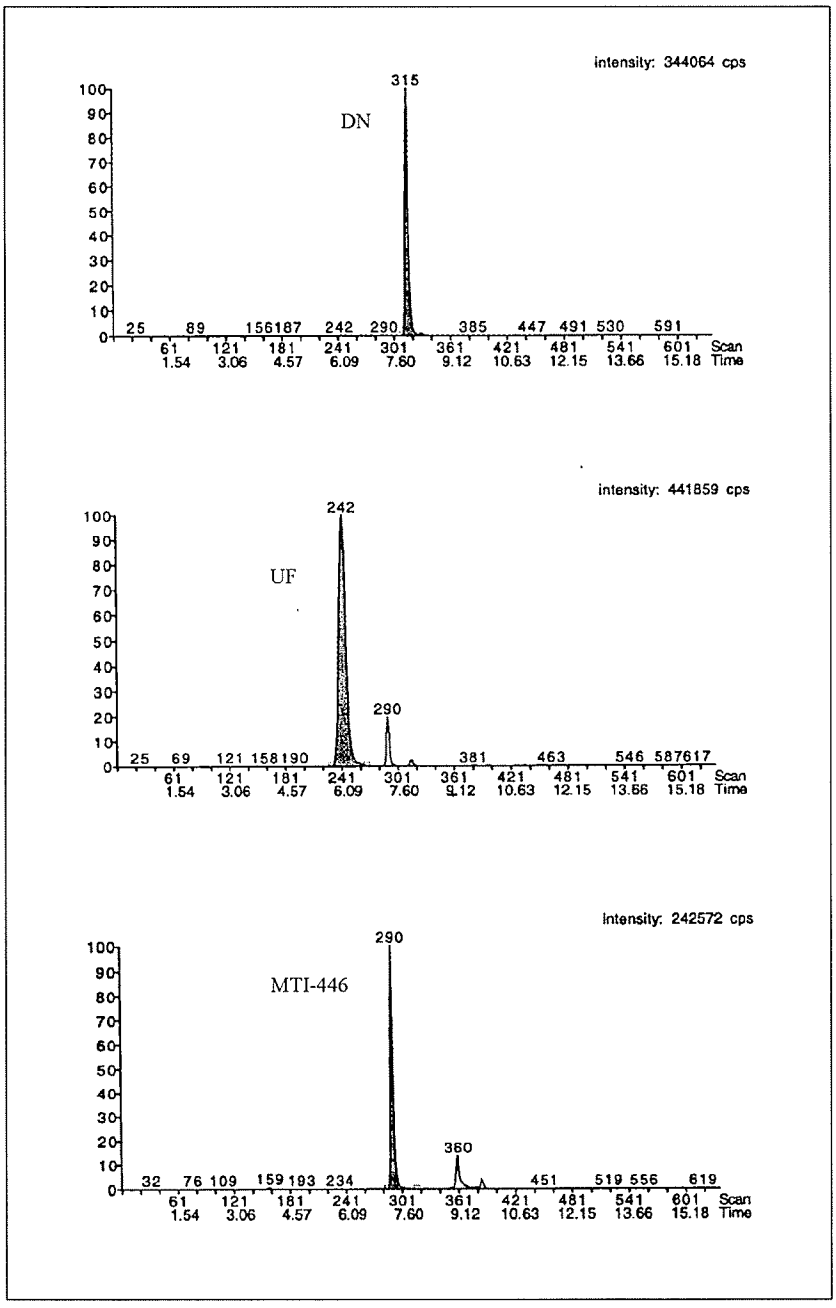
Typical Chromatogram of a Control Sample of Tomato Puree Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-PURVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.24



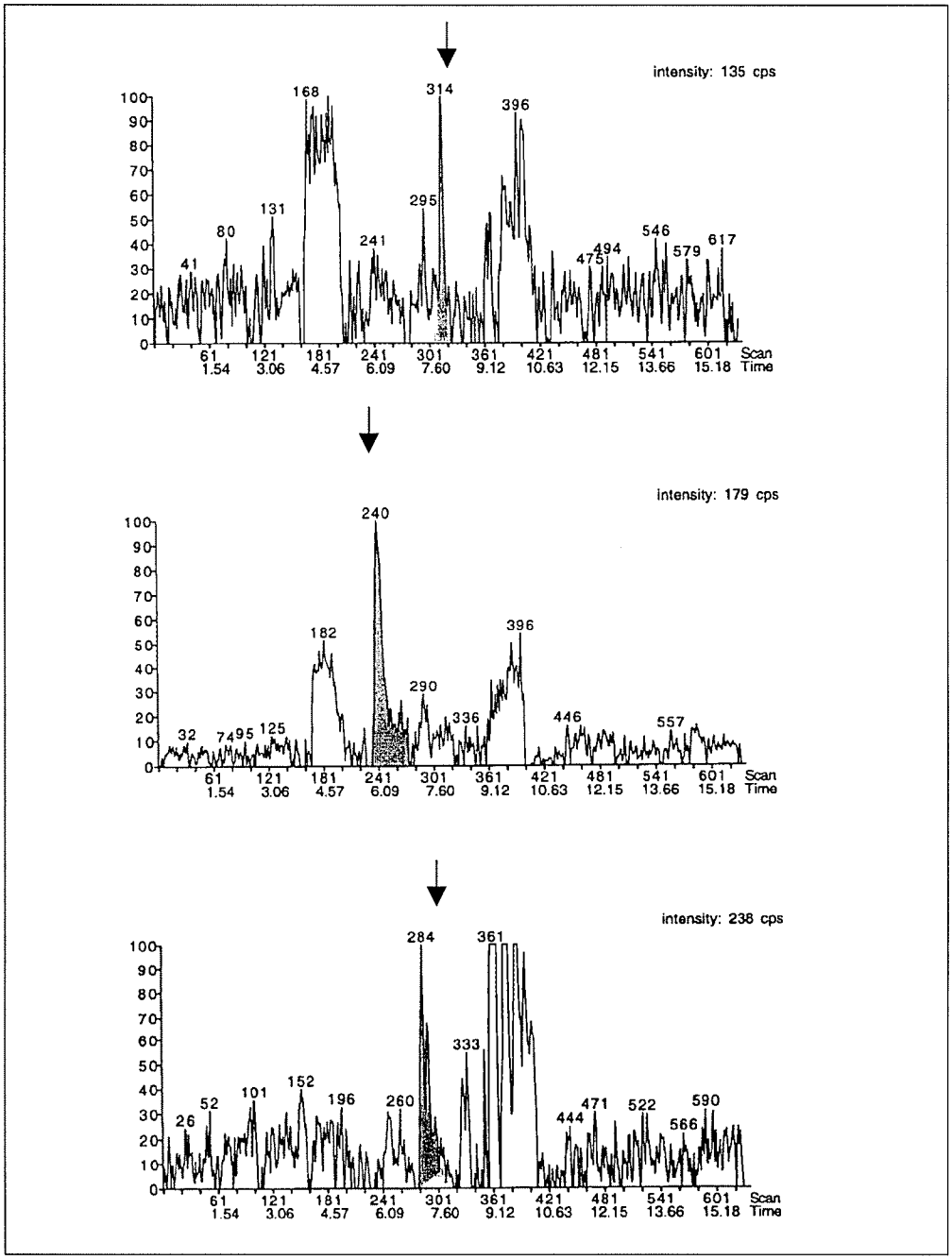
Typical Chromatogram of a Control Sample of Tomato Puree Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-PURVMAS-1.

APPENDIX D.25



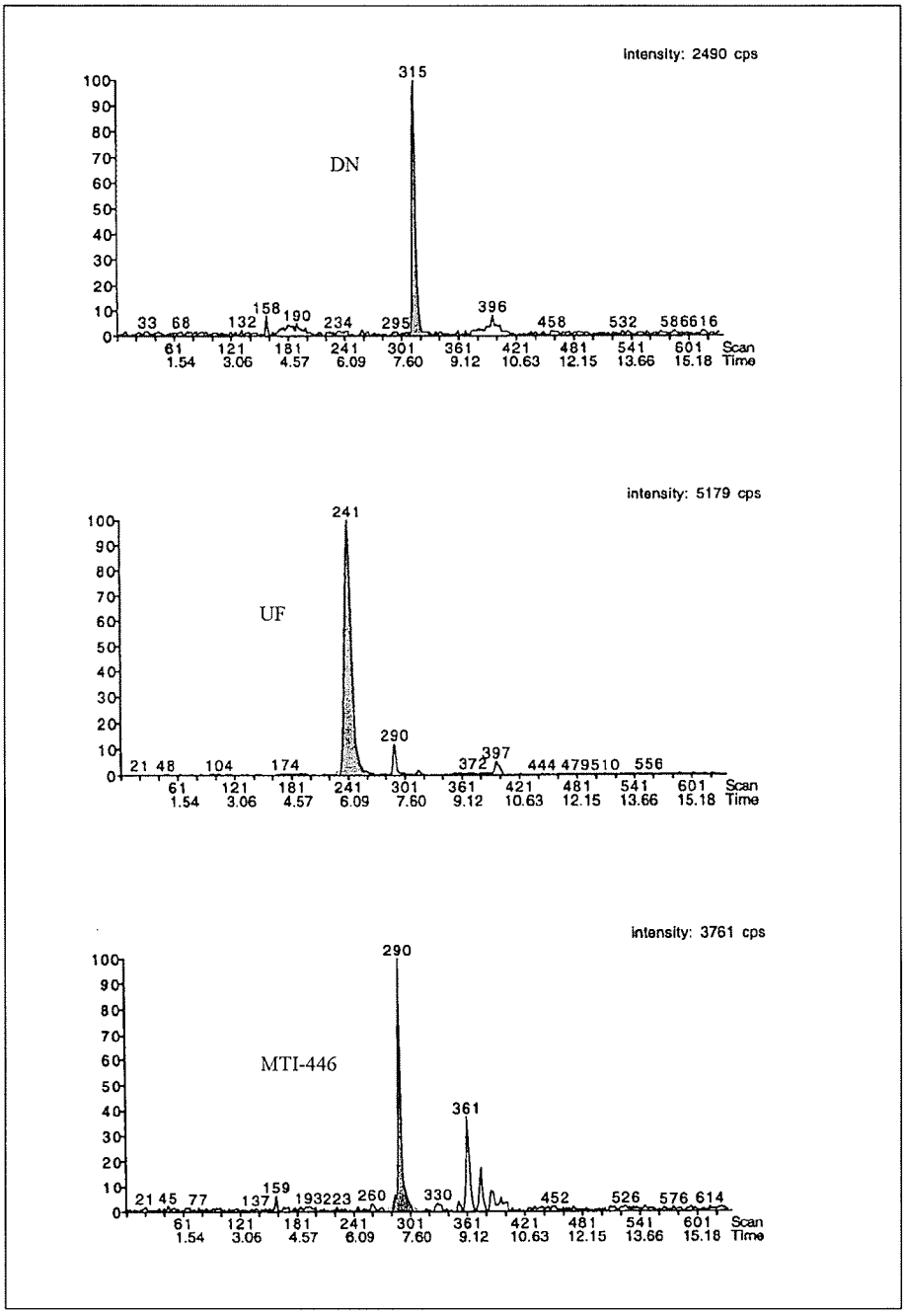
Typical Chromatogram of a Control Sample of Tomato Puree Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100x LOQ), 236C-113-PURVMAS-6.

APPENDIX D.26



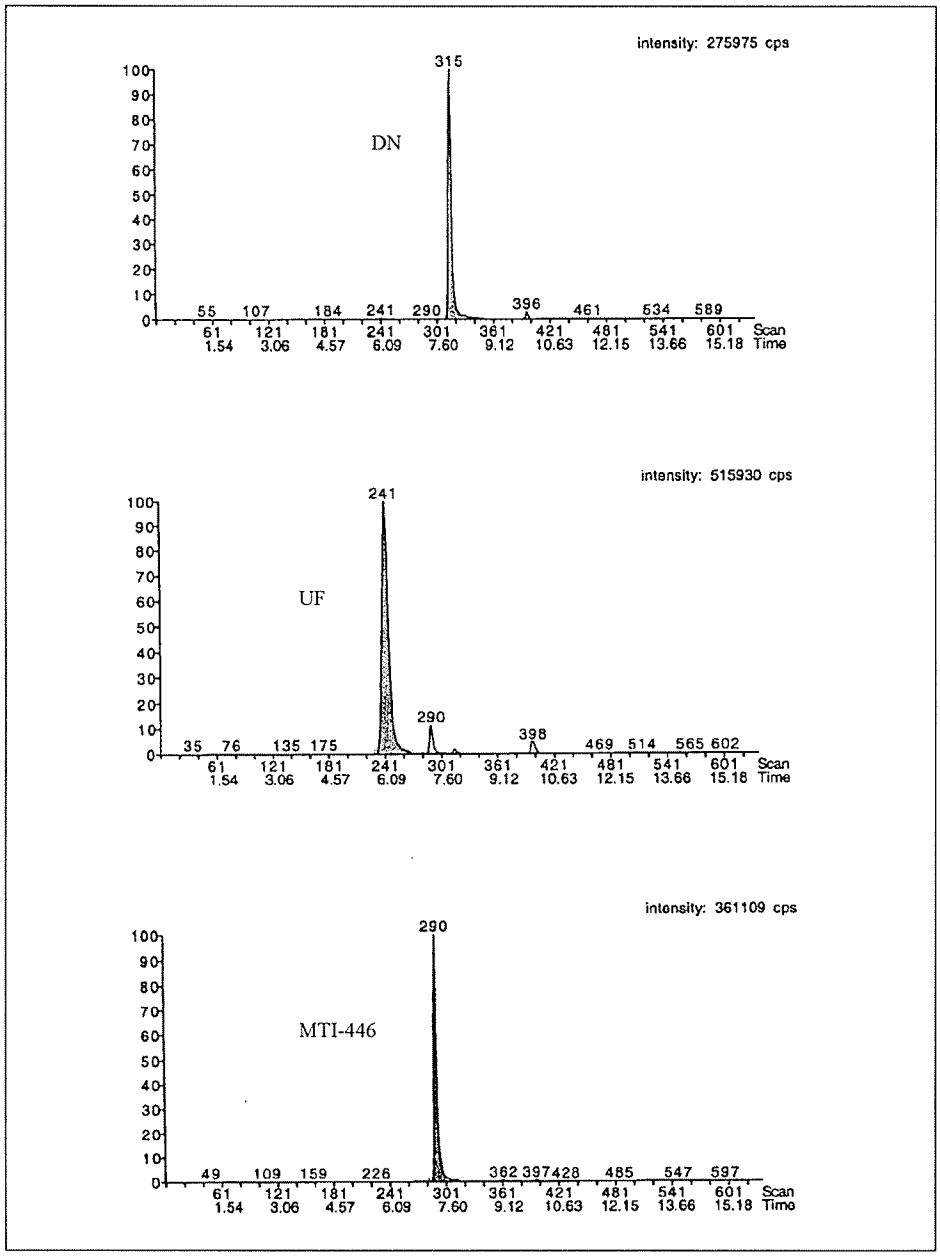
Typical Chromatogram of a Control Sample of Potato Flakes Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-FLAVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.27



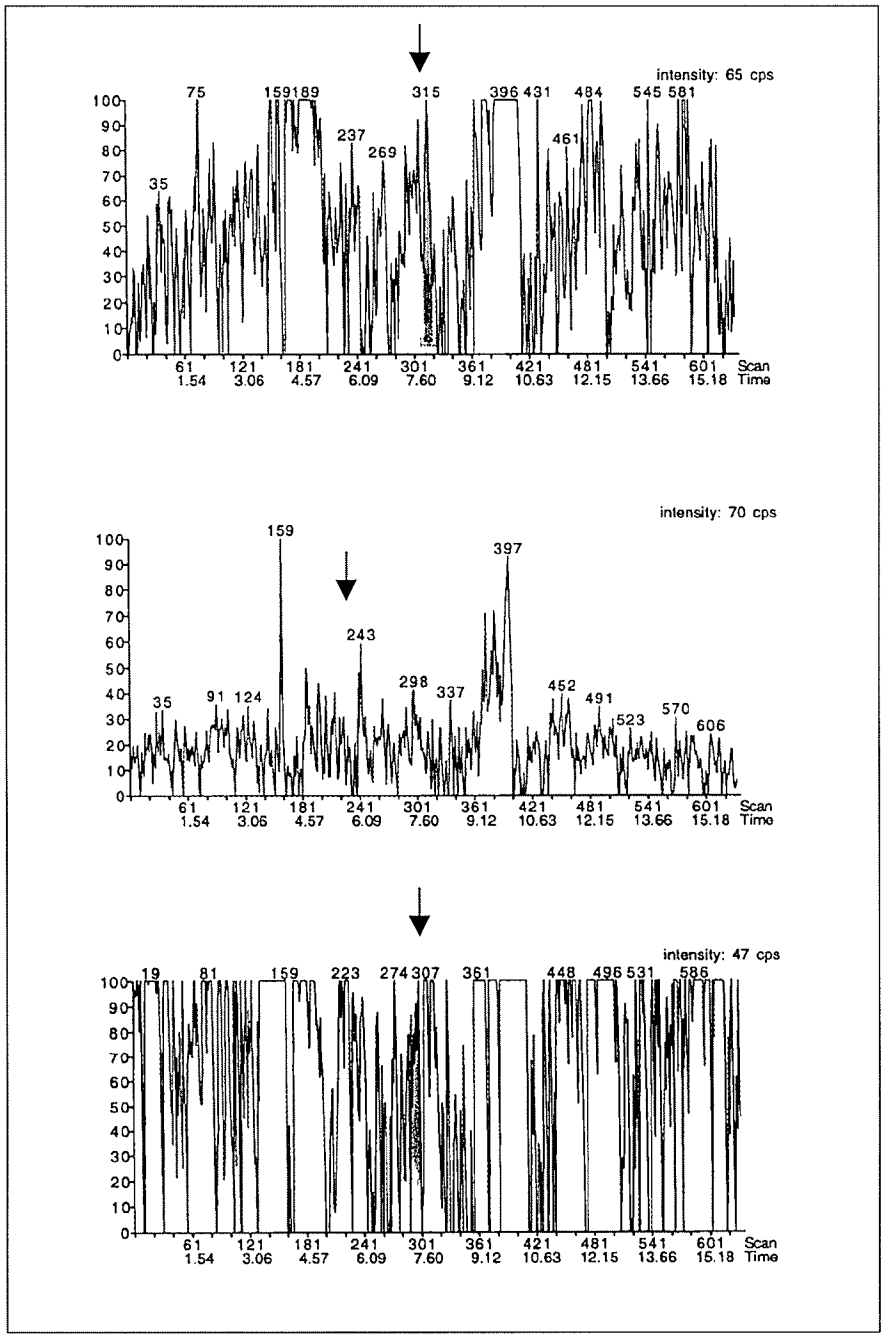
Typical Chromatogram of a Control Sample of Potato Flakes Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-FLAVMAS-1.

APPENDIX D.28



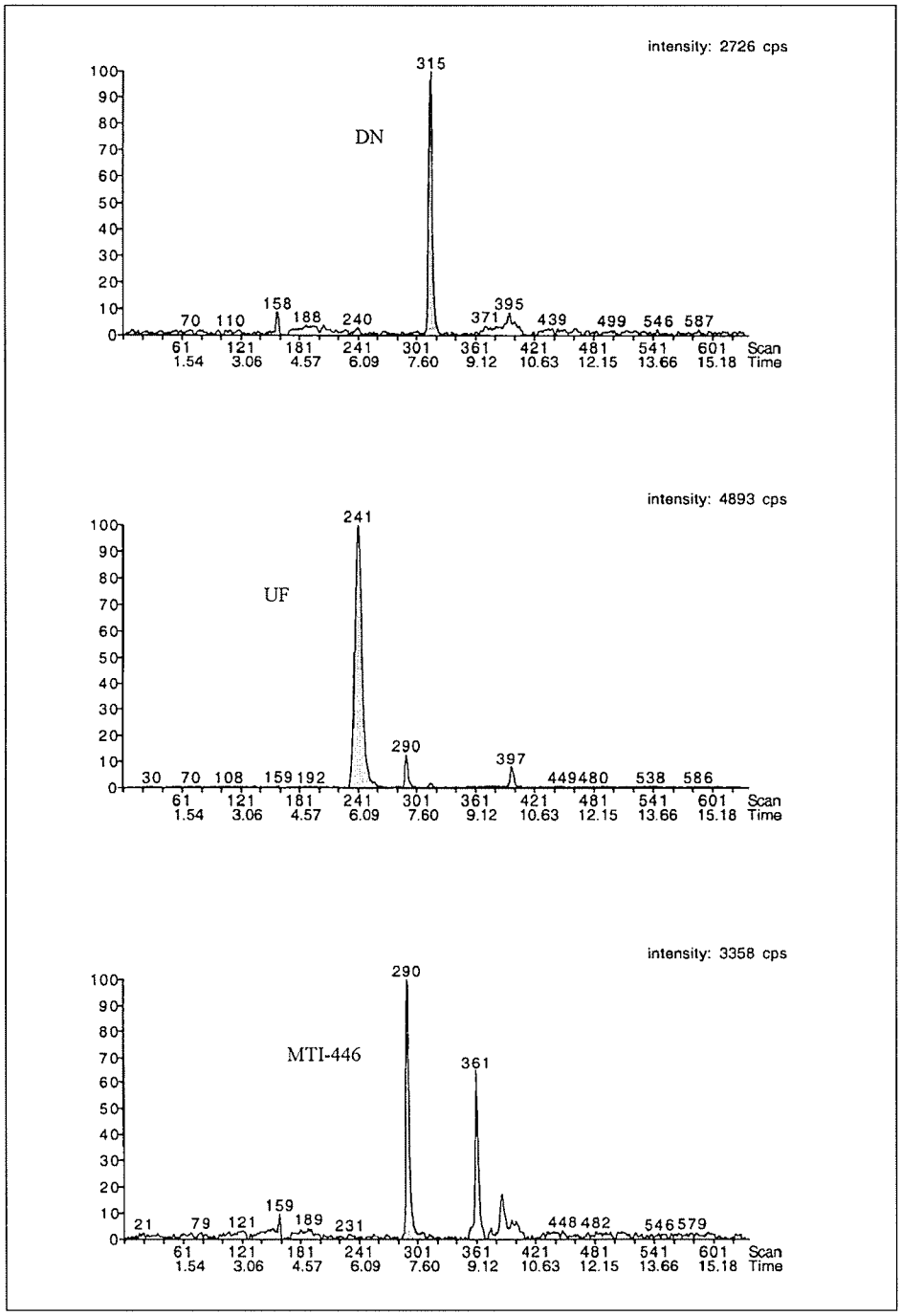
Typical Chromatogram of a Control Sample of Potato Flakes Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100 x LOQ), 236C-113-FLAVMAS-6.

APPENDIX D.29



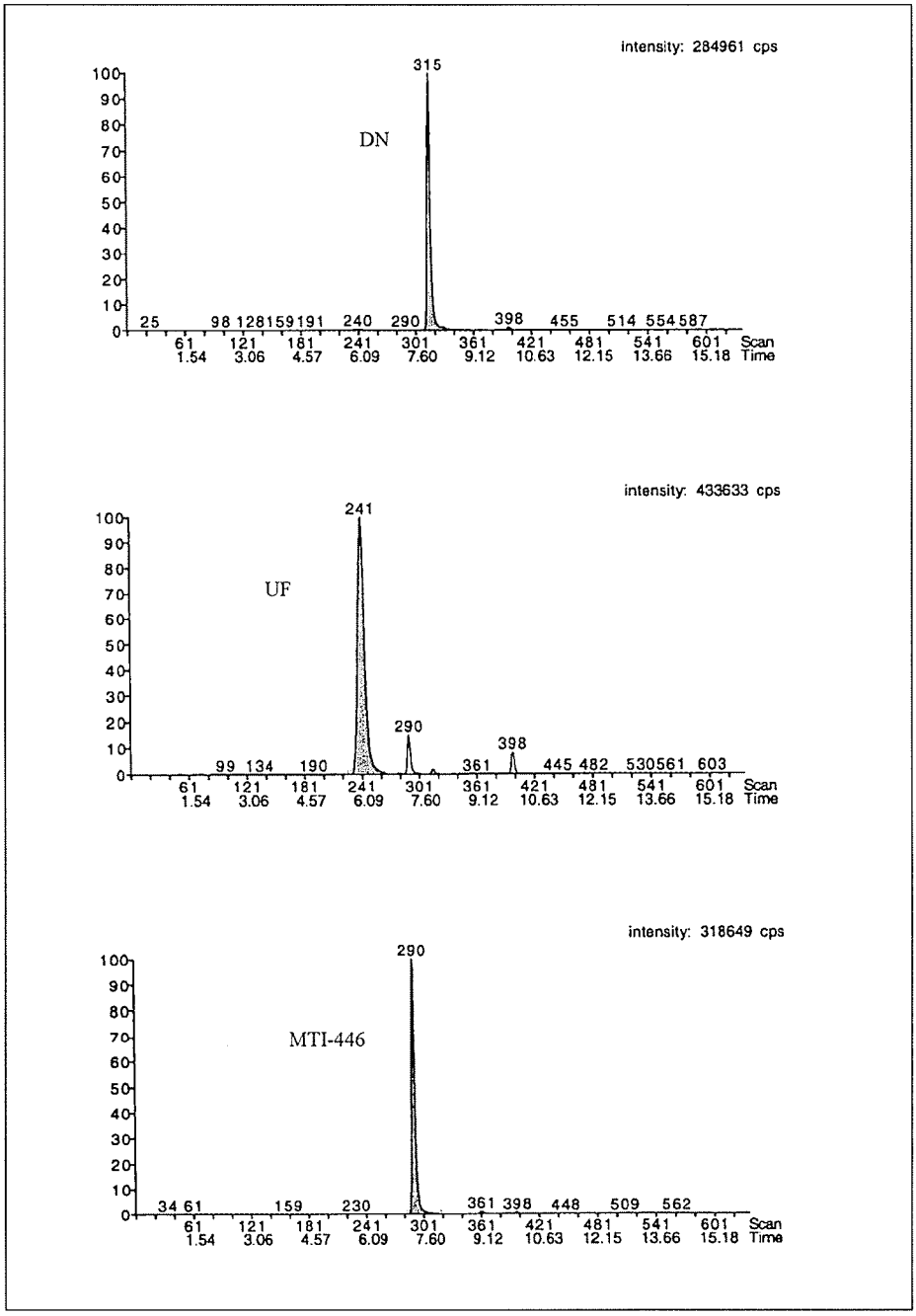
Typical Chromatogram of a Control Sample of Potato Chips Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-CHIVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.30



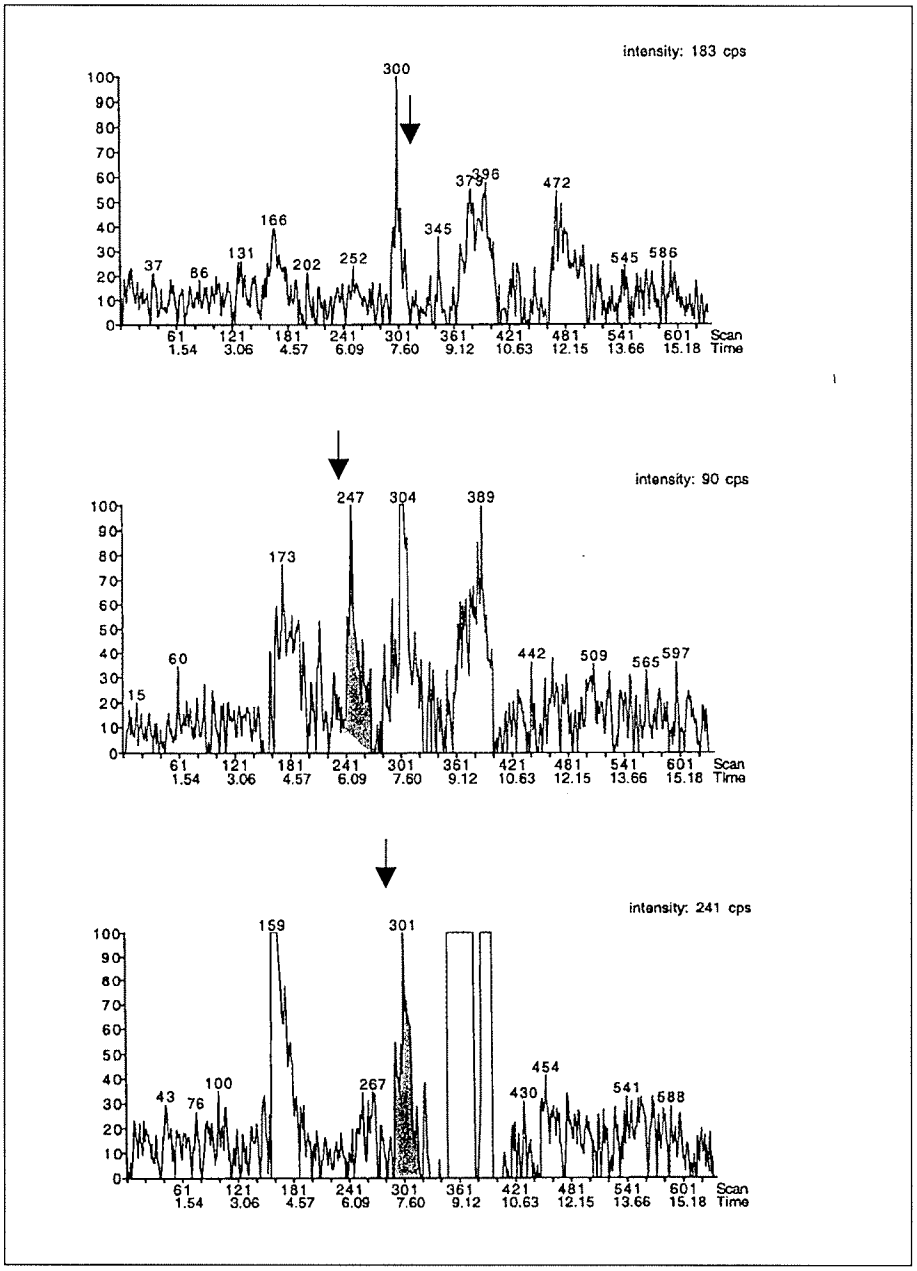
Typical Chromatogram of a Control Sample of Potato Chips Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-CHIVMAS-1.

APPENDIX D.31



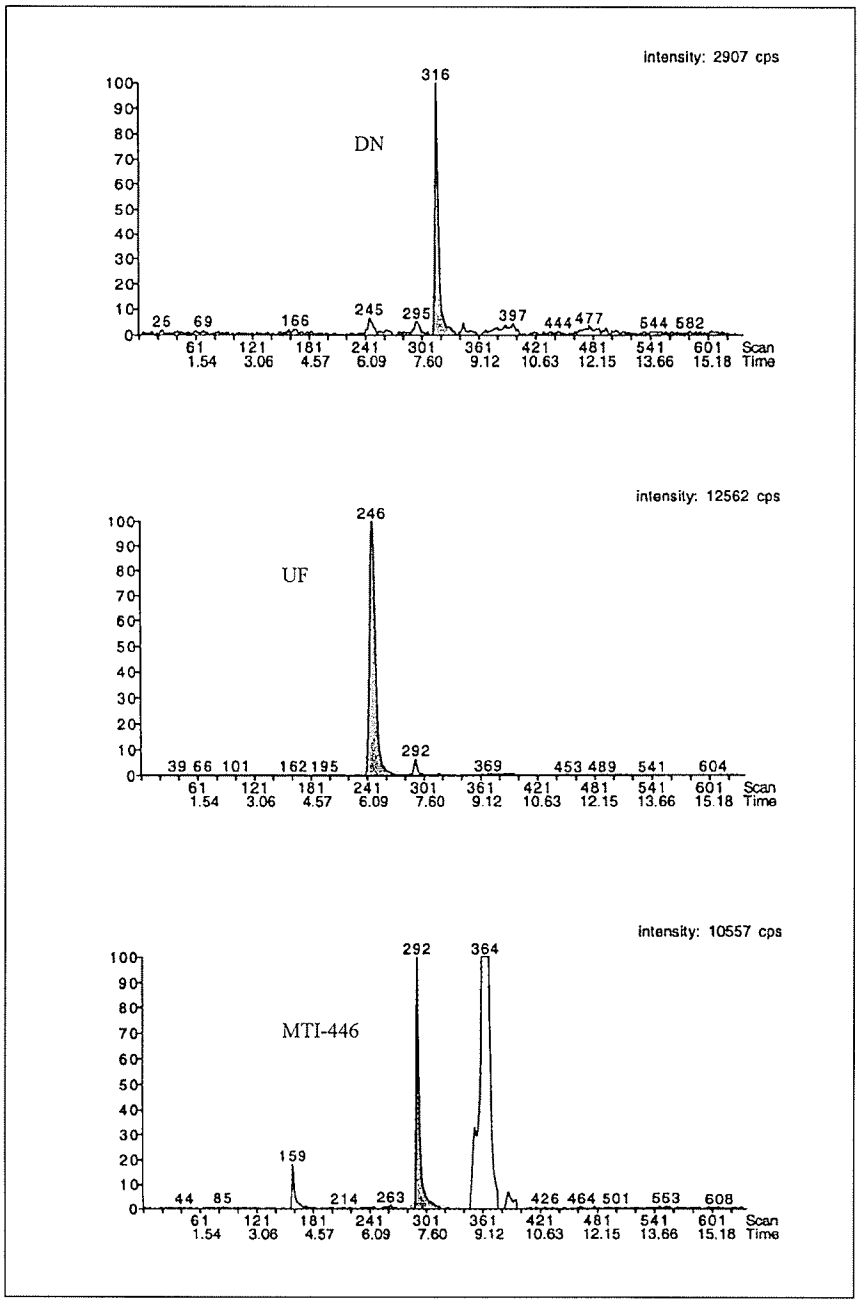
Typical Chromatogram of a Control Sample of Potato Chips Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100 x LOQ), 236C-113-CHIVMAS-6.

APPENDIX D.32



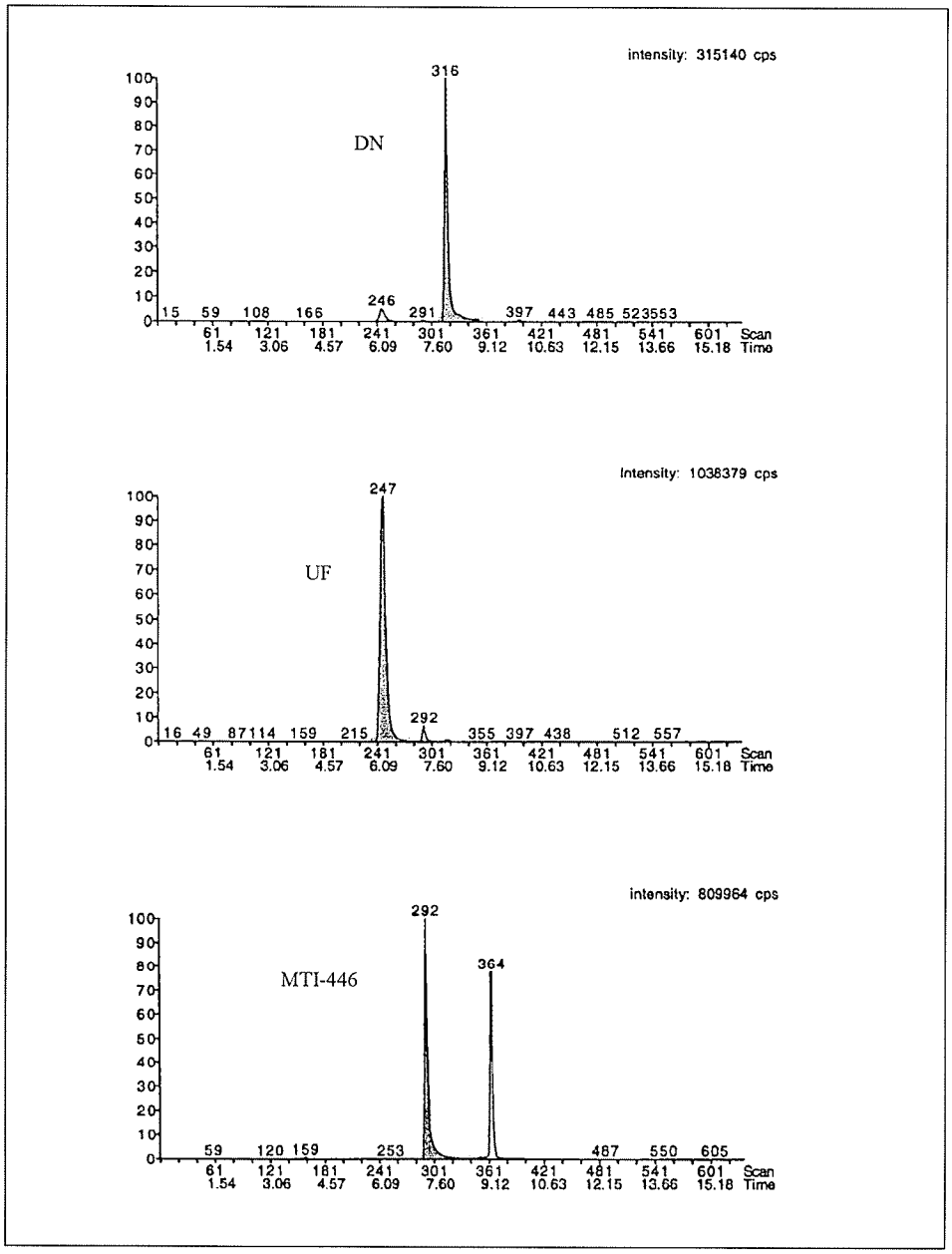
Typical Chromatogram of a Control Sample of Potato Wet Peel Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-PEEVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.33



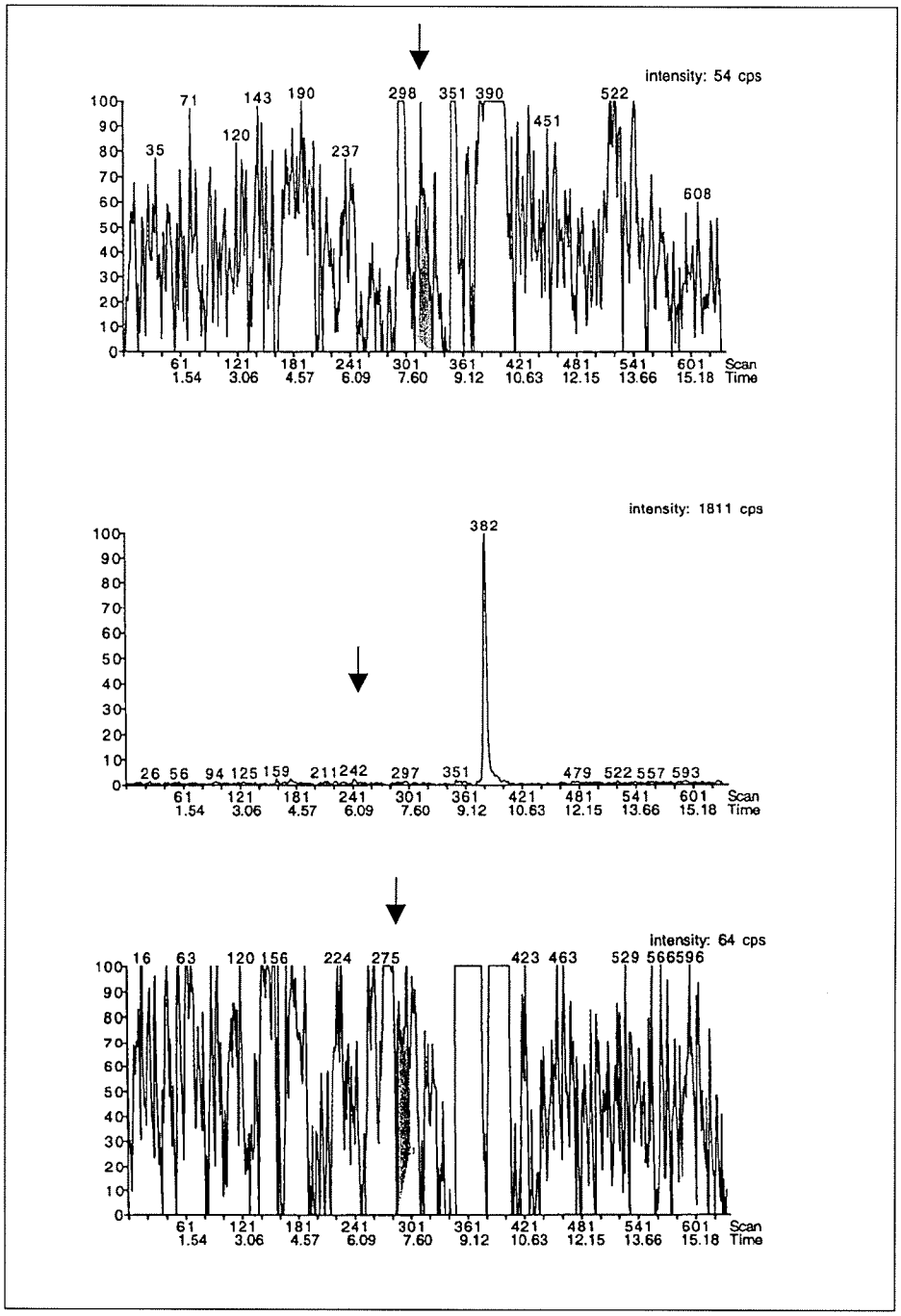
Typical Chromatogram of a Control Sample of Potato Wet Peel Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-PEEVMAS-1.

APPENDIX D.34



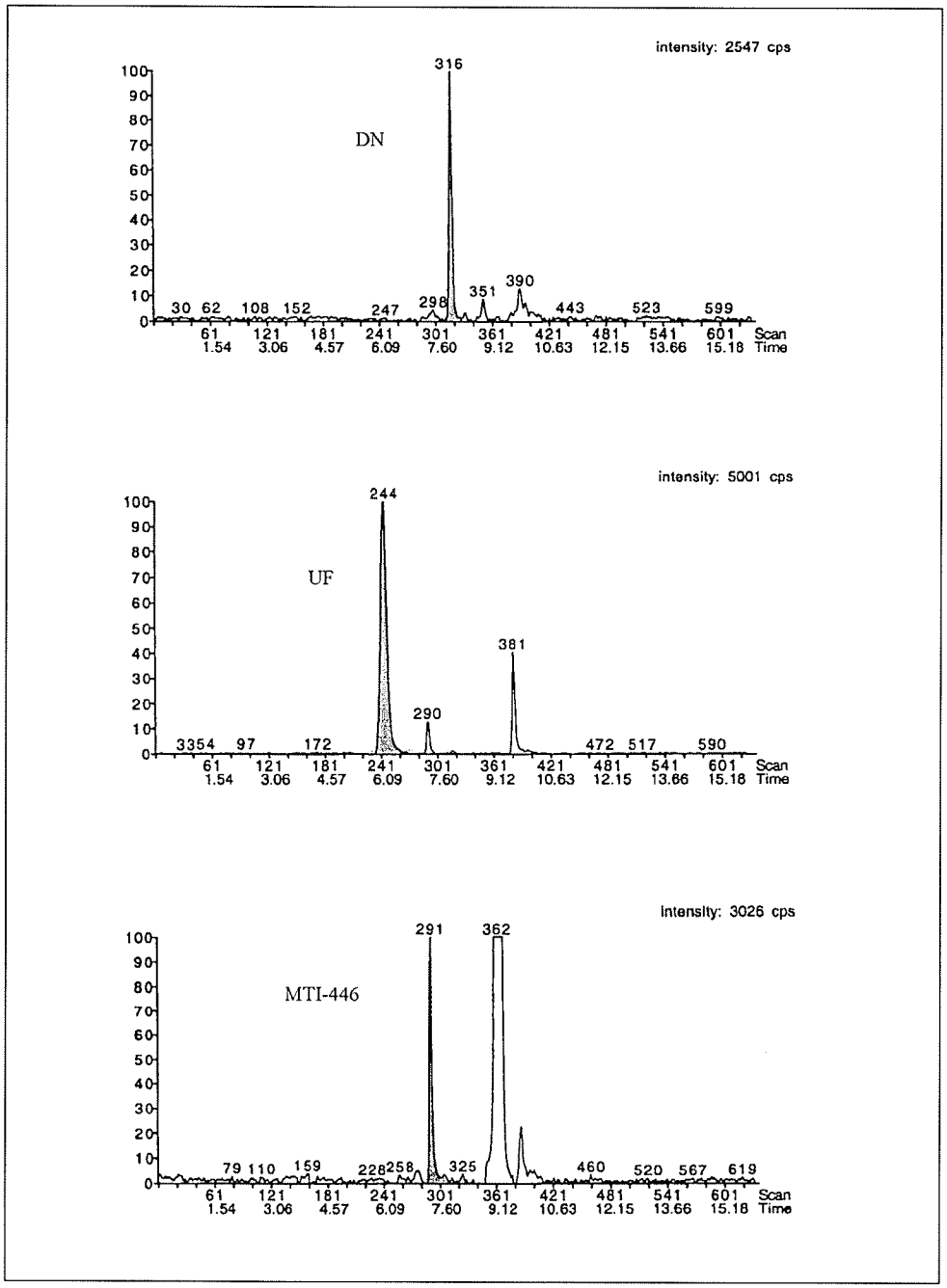
Typical Chromatogram of a Control Sample of Potato Wet Peel Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100 x LOQ), 236C-113-PEEVMAS-6.

APPENDIX D.35



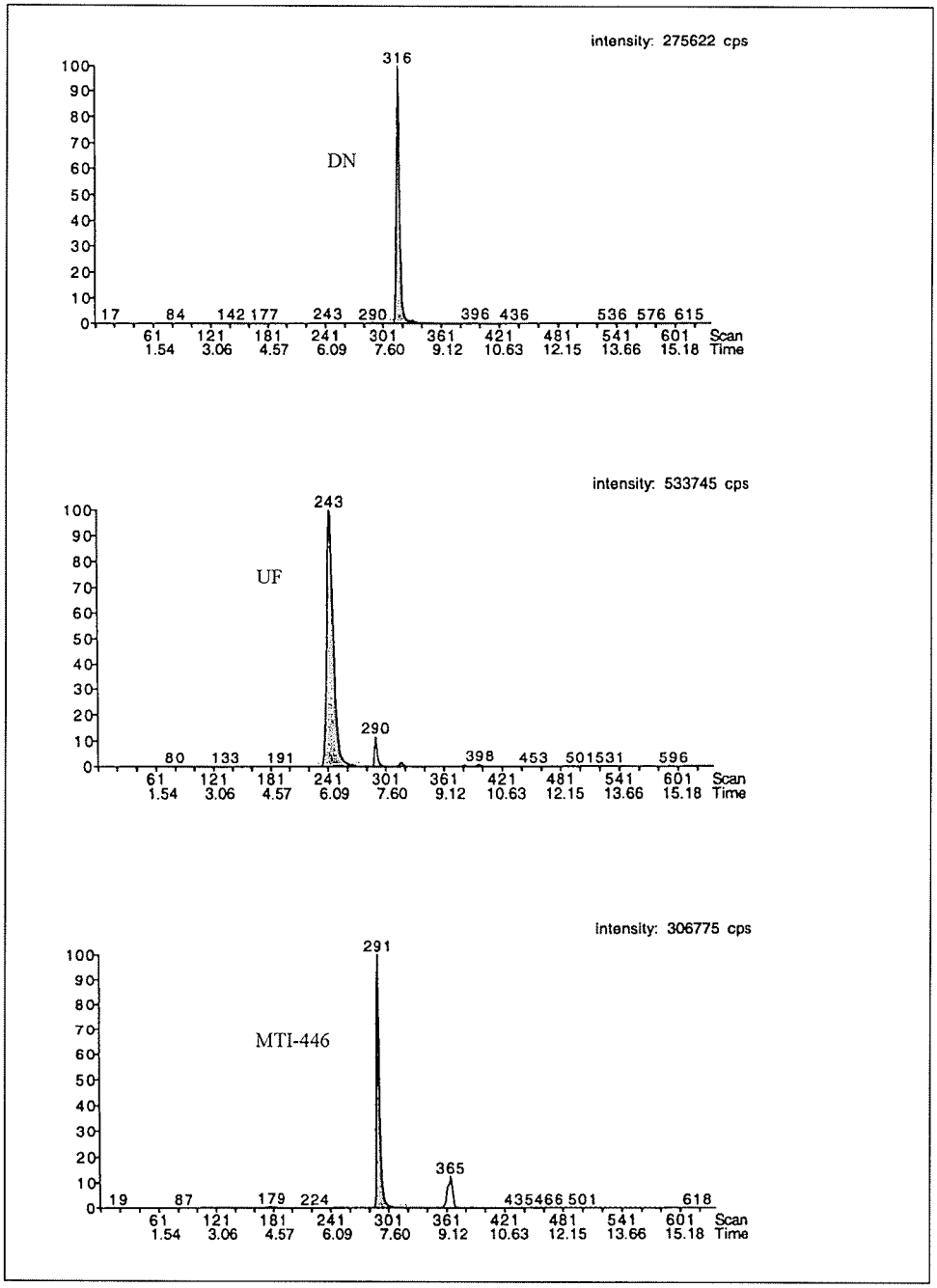
Typical Chromatogram of a Control Sample of Raisins Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-RAIVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.36



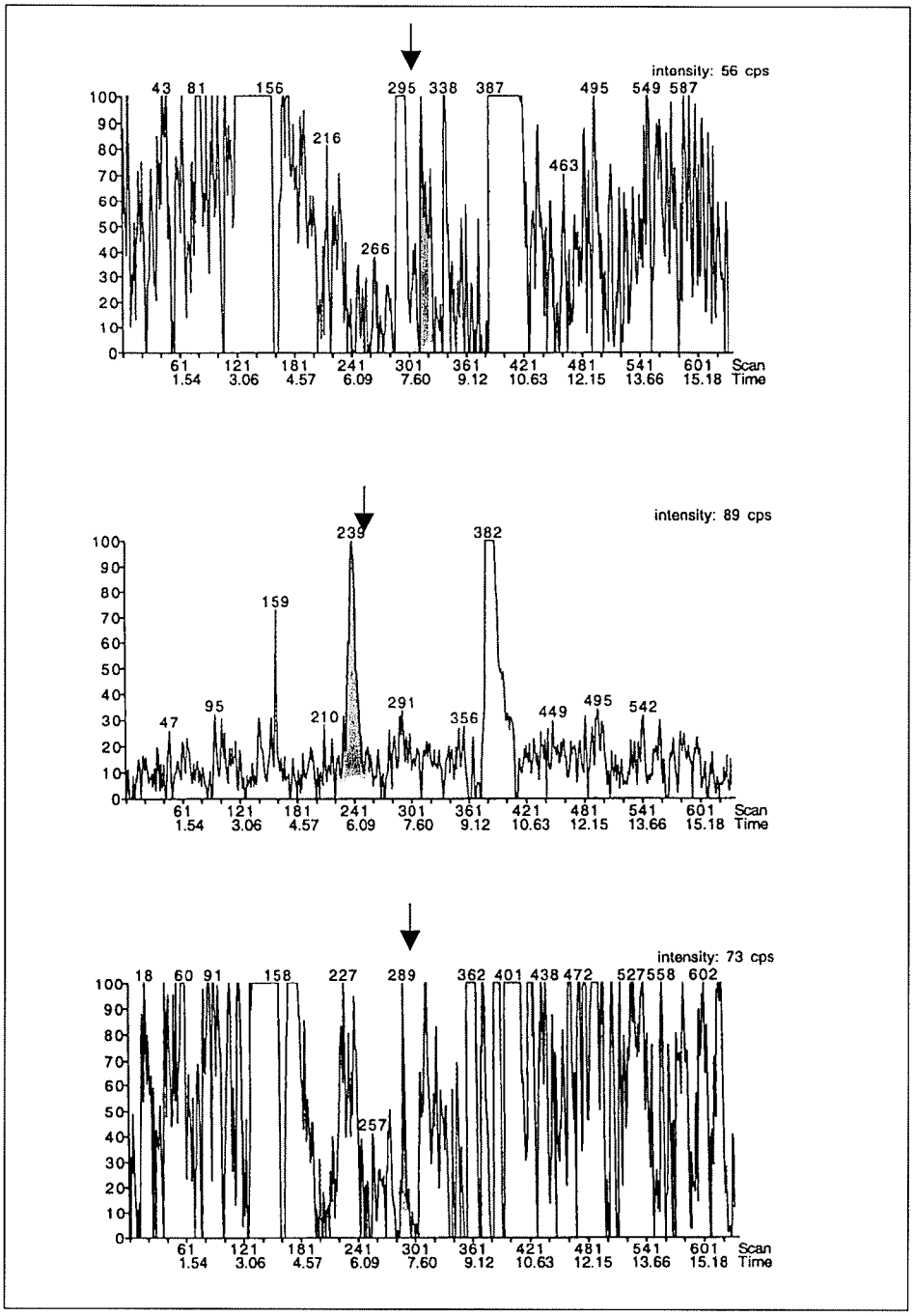
Typical Chromatogram of a Control Sample of Raisins Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-RAIVMAS-1.

APPENDIX D.37



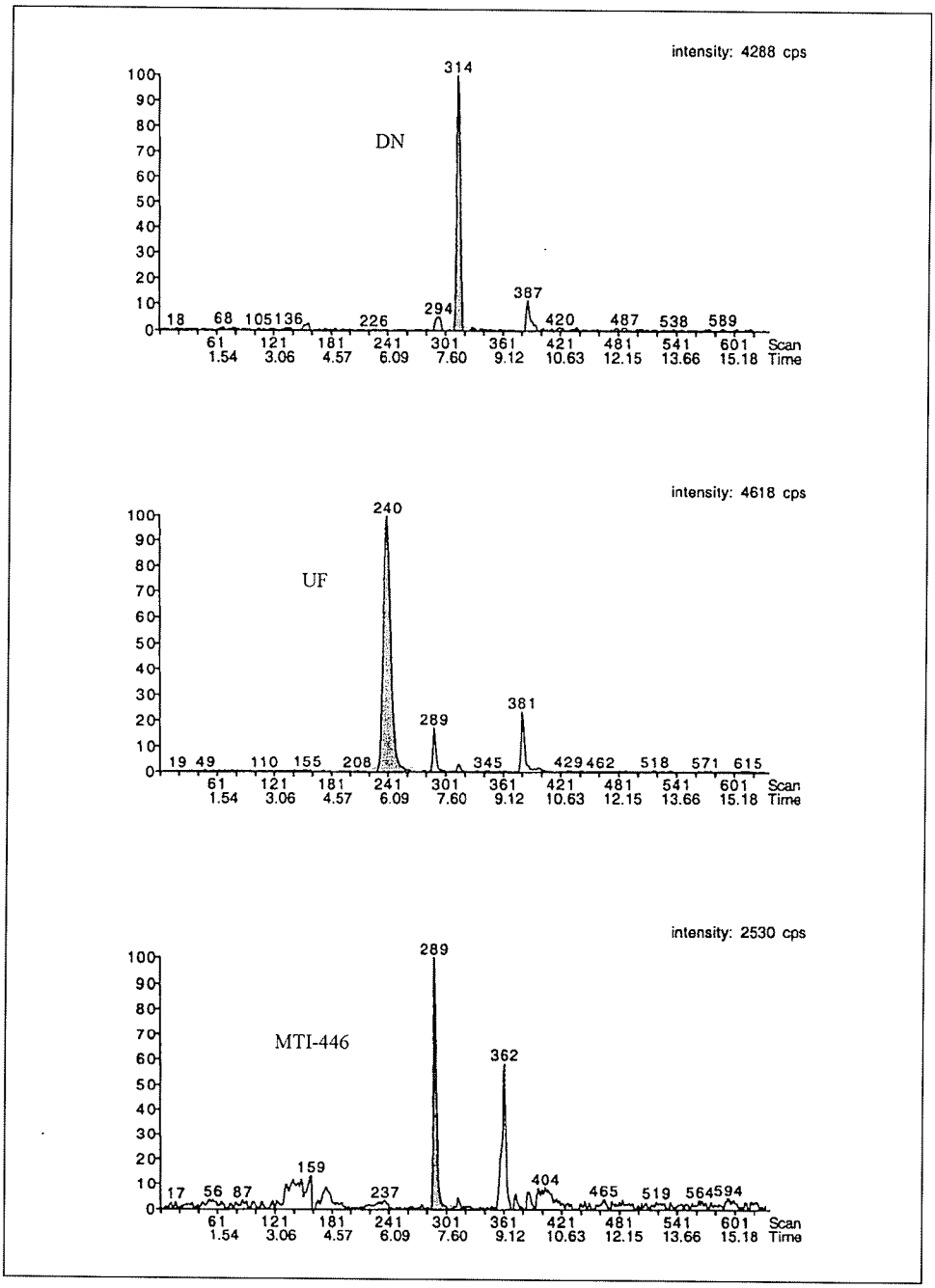
Typical Chromatogram of a Control Sample of Raisins Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100 x LOQ), 236C-113-RAIVMAS-6.

APPENDIX D.38



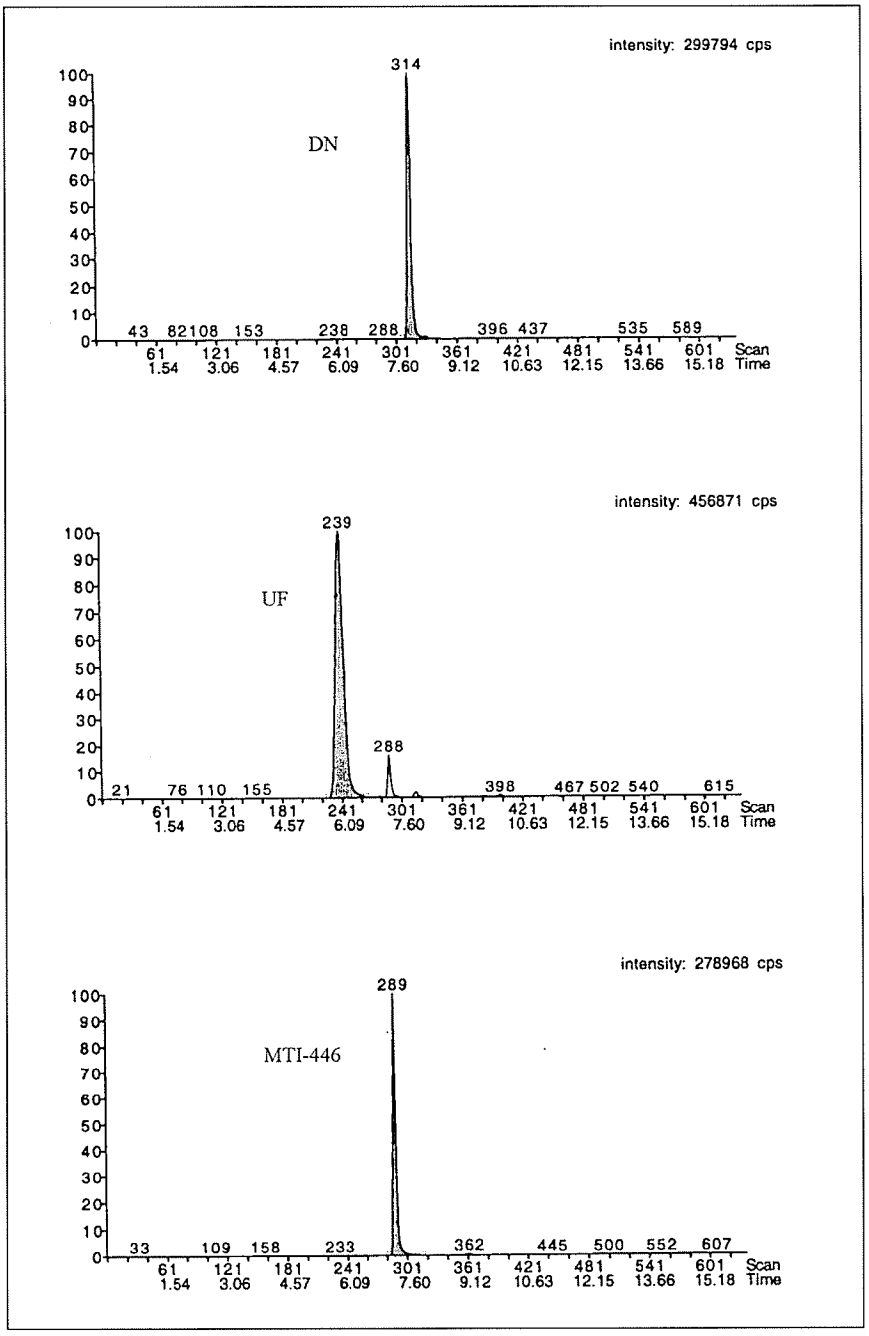
Typical Chromatogram of a Control Sample of Grape Juice Containing No Detectable Residue of DN (top), UF (middle), and MTI-446 (bottom), 236C-113-JUIVMAB-1. The arrows indicate the retention times of DN, UF, and MTI-446, respectively.

APPENDIX D.39



Typical Chromatogram of a Control Sample of Grape Juice Fortified with 0.0100 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (1x LOQ), 236C-113-JUIVMAS-1.

APPENDIX D.40



Typical Chromatogram of a Control Sample of Grape Juice Fortified with 1.00 mg/Kg DN (top), UF (middle), and MTI-446 (bottom), (100 x LOQ), 236C-113-JUIVMAS-6.