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Determination of Residues of Oxyfluorfen in Agricultural Commodities by Gas Chromatography with Negative-Ion Chemical Ionization Mass Spectrometry

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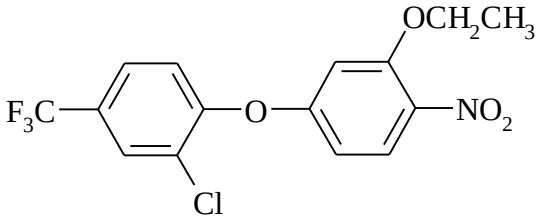
1. SCOPE

This method is applicable for the quantitative determination of residues of oxyfluorfen in agricultural commodities. The method was validated over the concentration range of 0.010-0.50 µg/g with a validated limit of quantitation of 0.01 µg/g.



Oxyfluorfen
CAS No. 42874-03-3

Common and chemical names for the above structure and related compounds are given in Table 1.

3. SAFETY PRECAUTIONS

3.1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non Dow AgroSciences LLC products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

3.2. Acetone, methanol, and toluene are flammable and should be used in well-ventilated areas away from ignition sources.

8. INSTRUMENTAL CONDITIONS

8.1. Column

Install the splitless column inlet sleeve and the capillary column in the split/splitless injection port of the gas chromatograph following the manufacturer's recommended procedures.

8.2. Typical Gas Chromatography Operating Conditions

Instrumentation:	Agilent Model 6890A gas chromatograph Agilent Model 7683 autoinjector Agilent Model 5973N mass spectrometer Agilent Model G1701DA data system
Column:	J & W fused silica capillary Durabond-5MS liquid phase 30 m x 0.25 mm i.d. 0.25-µm film thickness
Oven Method:	
Column	110 °C for 2.0 min 110 °C to 320 °C at 20 °C/min 320 °C for 2.5 min
Transfer Line	300 °C
Carrier Gas Method:	helium
Constant Flow	1.0 mL/min
Vacuum Compensation	on
Initial Head Pressure	~79 kPa
Linear Velocity	~approximately 37 cm/s

Injection Method:	splitless
Injector Temperature	280 °C
Purge Delay	1.9 min
Splitter Flow	50 mL/min
Septum Purge	on
Injection Volume	2 µL

8.3. Typical Mass Spectrometry Operating Conditions

Detector Mode:	negative-ion chemical ionization
Source Temperature	150 °C
Quad Temperature	106 °C
Reagent Gas	methane
Flow Setting	40%
Pressure	2.0×10^{-4} torr
Calibration Program	negative-ion chemical ionization autotune
Electron Multiplier	1906 volts (~300 volts above autotune)
SIM Resolution	high
Dwell Time	50 msec

Ions Monitored:

oxyfluorfen	
quantitation	*m/z* 361
confirmation (primary)	*m/z* 363
confirmation (secondary)	*m/z* 296
D_5-oxyfluorfen	
quantitation	*m/z* 366
confirmation (primary)	*m/z* 368

8.4. Mass Spectra

Full-scan methane negative-ion chemical ionization mass spectra of oxyfluorfen and D_5-oxyfluorfen (internal standard) are shown in Figure 1 (not yet provided).

8.4. Typical Calibration Curve

A typical calibration curve for the determination of oxyfluorfen in agricultural commodities is shown in Figure 2.

8.5. Typical Chromatograms

Typical chromatograms of a standard, a control sample, and a 0.01-µg/g (LOQ)

recovery sample for the determination of oxyfluorfen in blueberries are illustrated in Figure 3.

9. DETERMINATION OF RECOVERY OF OXYFLUORFEN FROM AGRICULTURAL COMMODITIES

9.1. Method Validation

Validate the analytical procedure given in Section 9.3 by analyzing the following with each sample set:

At least one reagent blank.
At least two unfortified controls.
At least two controls fortified at the limit of quantitation.
At least two controls fortified at a level exceeding the expected residue concentration in the samples.

9.2. Sample Preparation

Prepare the samples for analysis by freezing with dry ice or liquid nitrogen and then grinding or chopping using a hammer mill with a 1/8- to 1/4-inch screen size. Prepared samples should be stored frozen at approximately -10 to -20 °C until analysis.

9.3. Sample Analysis

9.3.1. Weigh 5.0 ± 0.05-gram portions of the prepared sample into a series of 8-ounce HDPE bottles.

9.3.2. For preparing fortified samples, add 1.0-mL aliquots of the appropriate spiking solutions to encompass the necessary concentration range.

9.3.3. Add 100.0 mL of the methanol/water (80:20 v/v) extraction solution to the sample bottle.

9.3.4. Homogenize the sample for approximately 60 seconds at 10000 rpm using an Omni-Mixer homogenizer fitted with a 20-mm probe.

9.3.5. Cap the sample bottle and shake the sample for a minimum of 30 minutes on a reciprocating shaker at approximately 180 excursions/minute.

9.3.6. Centrifuge the sample bottle for 5 minutes at 2000 rpm.

9.3.7. Pipet 1.0 mL of the sample solution into a 7-mL vial and then add 3.0 mL of water.

9.3.8. Cap the sample vial with a PTFE-lined cap and then vortex mix the sample for 1-2 seconds.

9.3.9. Purify the sample using the following SPE procedure:

a. Place a Phenomenex Strata-X (30-mg) SPE column on the vacuum manifold.

b. Condition the SPE column with 1 mL of methanol followed by 1 mL of water. (Dry the SPE column under full vacuum for 5 seconds between solvents.)

c. Transfer 1.0 mL of the sample solution from Step 9.2.8 to the SPE column. Draw the sample through the column at a flow rate of approximately 1.0 mL/min, discarding the eluate.

d. Wash the SPE column with three 1000-µL aliquots of a methanol/water (80:20 v/v) solution. Draw the solvent through the column at a flow rate of approximately 1 mL/min, discarding the eluate. (Dry the SPE column under full vacuum for 5 seconds between aliquots.)

e. Dry the SPE column under full vacuum (≈ -10 in Hg) for 5 minutes.

f. Elute the oxyfluorfen from the SPE column with two 750-µL aliquots of dichloromethane, collecting the eluate in a 7-mL vial.

9.3.10. Evaporate the sample eluate to dryness using an N-Evap evaporator set at 35 °C and a nitrogen flow rate of approximately 500 mL/min. (NOTE: At elevated water bath temperatures and/or flow rates, the oxyfluorfen will volatilize, thereby reducing recoveries.)

9.3.11. For quantitation of residues of oxyfluorfen with single-ion confirmation, reconstitute the sample with 500 µL of a toluene/acetone (80:20 v/v) solution containing 5.0 ng/mL of D_5-oxyfluorfen internal standard.

9.3.12. Cap the sample vial and firmly seal with a PTFE-lined cap. Vortex mix the sample for 1-2 seconds, and then sonicate the sample for 1-2 seconds.

9.3.13. Transfer a portion of the sample to a 2-mL autosampler vial containing a limited-volume insert and seal the vial with a cap.

9.3.14. Analyze the crossover standards, calibration standards and samples by gas chromatography with negative-ion chemical ionization mass spectrometry detection.

9.3.15. Analyze the crossover standards, calibration standards, and samples by capillary gas chromatography with negative-ion chemical ionization mass spectrometry as described in Section 8.2. Determine the suitability of the chromatographic system using the following performance criteria:

a. Standard curve linearity: Determine that the correlation coefficient equals or exceeds 0.995 for the least squares equation which describes the detector response as a function of standard curve concentration. If power regression is used, the power exponent should be between 0.90-1.10.

b. Peak resolution: Visually determine that sufficient resolution has been achieved for the analyte and internal standard relative to background interferences.

c. Appearance of chromatograms: Visually determine that the chromatograms resemble those shown in Figure 3 with respect to peak response, baseline noise, and background interference. Visually determine that a minimum signal-to-noise ratio of 10:1 has been attained for the analyte in the 0.25-ng/mL calibration standard.

13. REFERENCES

13.1. Freund, J. E.; Williams, F. J. *Dictionary/Outline of Basic Statistics*; Dover: New York, 1991; p 170.

13.2. Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. *Anal. Chem.* **1983**, *55*, 2210-2218.

13.3. Baldwin, R.; Bethem, R. A.; Boyd, R. K.; Budde, W. L.; Cairns, T.; Gibbons, R. D.; Henion, J. D.; Kaiser, M. A.; Lewis, D. L.; Matusik, J. E.; Sphon, J. A.; Stephany, R. W.; Trubey, R. K.; *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 1180-1190.

Table 1. Identity and Structures of Oxyfluorfen and Internal Standard

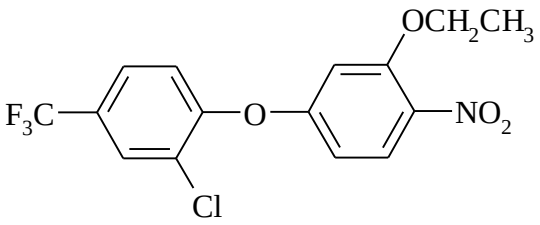
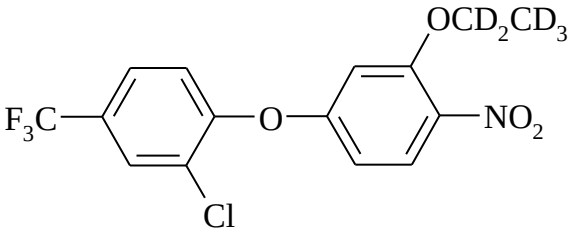
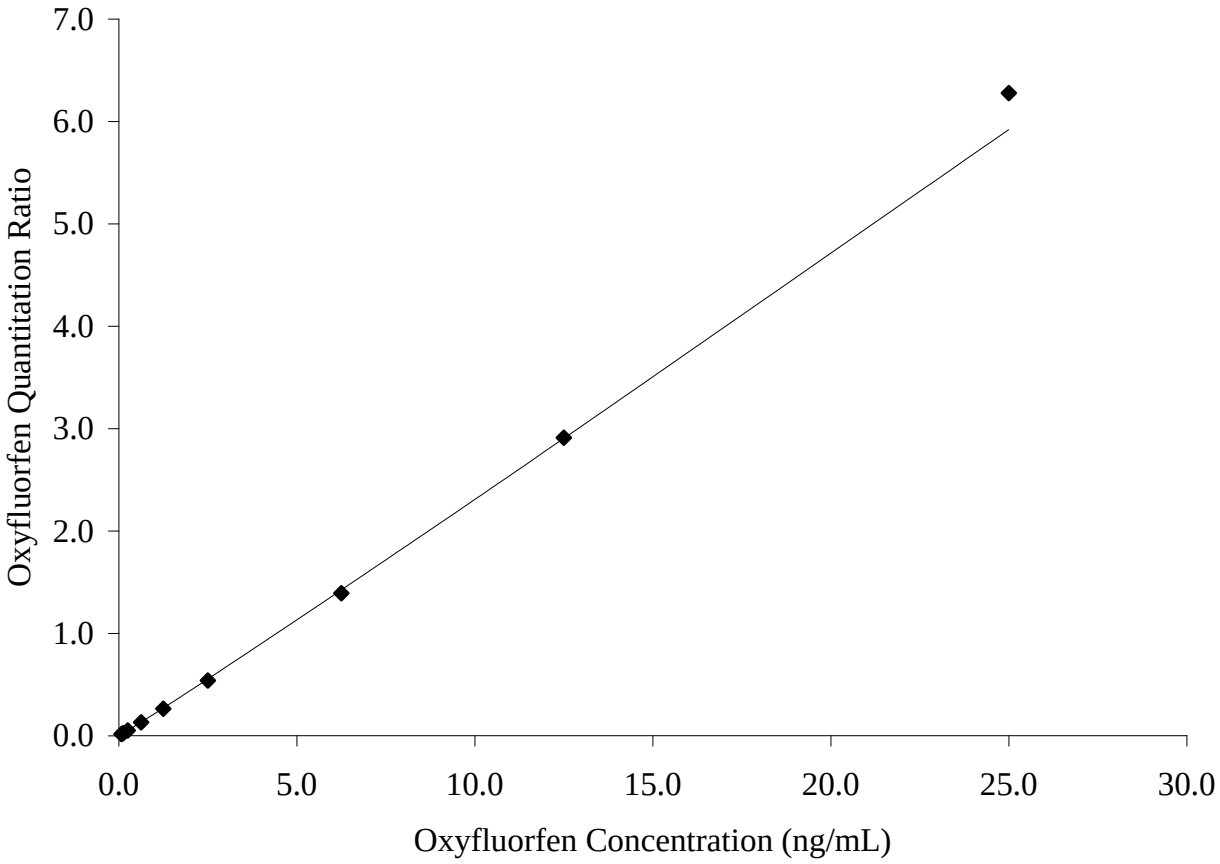
Common Name of Compound	Structure and CAS Name
Oxyfluorfen Molecular Formula: $C_{15}H_{11}ClF_3NO_4$ Formula Weight: 361.70 Nominal Mass: 361 CAS Number: 42874-03-3	 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene
D_5-Oxyfluorfen Molecular Formula: $C_{15}D_5H_6ClF_3NO_4$ Formula Weight: 366.73 Nominal Mass: 366 CAS Number: not available	 2-chloro-1-(3-[D_5]ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene

Table 2. Recovery of Oxyfluorfen from Agricultural Commodities

Sample Number	Sample Matrix	Date of Analysis[a]	Oxyfluorfen, µg/g		Percent Recovery[b]
			Added	Found[b]	
004-0001	blueberry	04-Apr-2007	0.000	ND	--
004-0001	blueberry	04-Apr-2007	0.003	0.0029	NA
004-0001	blueberry	04-Apr-2007	0.010	0.0086	86
004-0001	blueberry	04-Apr-2007	0.010	0.0081	81
004-0001	blueberry	04-Apr-2007	0.010	0.0085	85
004-0001	blueberry	04-Apr-2007	0.100	0.0849	85
004-0001	blueberry	04-Apr-2007	0.100	0.0768	77
004-0001	blueberry	04-Apr-2007	0.100	0.0765	77
004-0001	blueberry	04-Apr-2007	0.500	0.4492	90
004-0001	blueberry	04-Apr-2007	0.50	0.4588	92
004-0001	blueberry	04-Apr-2007	0.50	0.4481	90

[a] The 'Date of Analysis' indicates the date that the samples were extracted.
[b] All calculations were done using Microsoft Excel 2003 with full precision.
[c] ND = not detected. The residue was below the 0.003-µg/g limit of detection.
[d] Samples fortified at the method's limit of detection of 0.003 µg/g are reported with a lower degree of confidence than values above the limit of quantitation.
[e] NA = not applicable. The residue was below the 0.010-µg/g limit of quantitation.

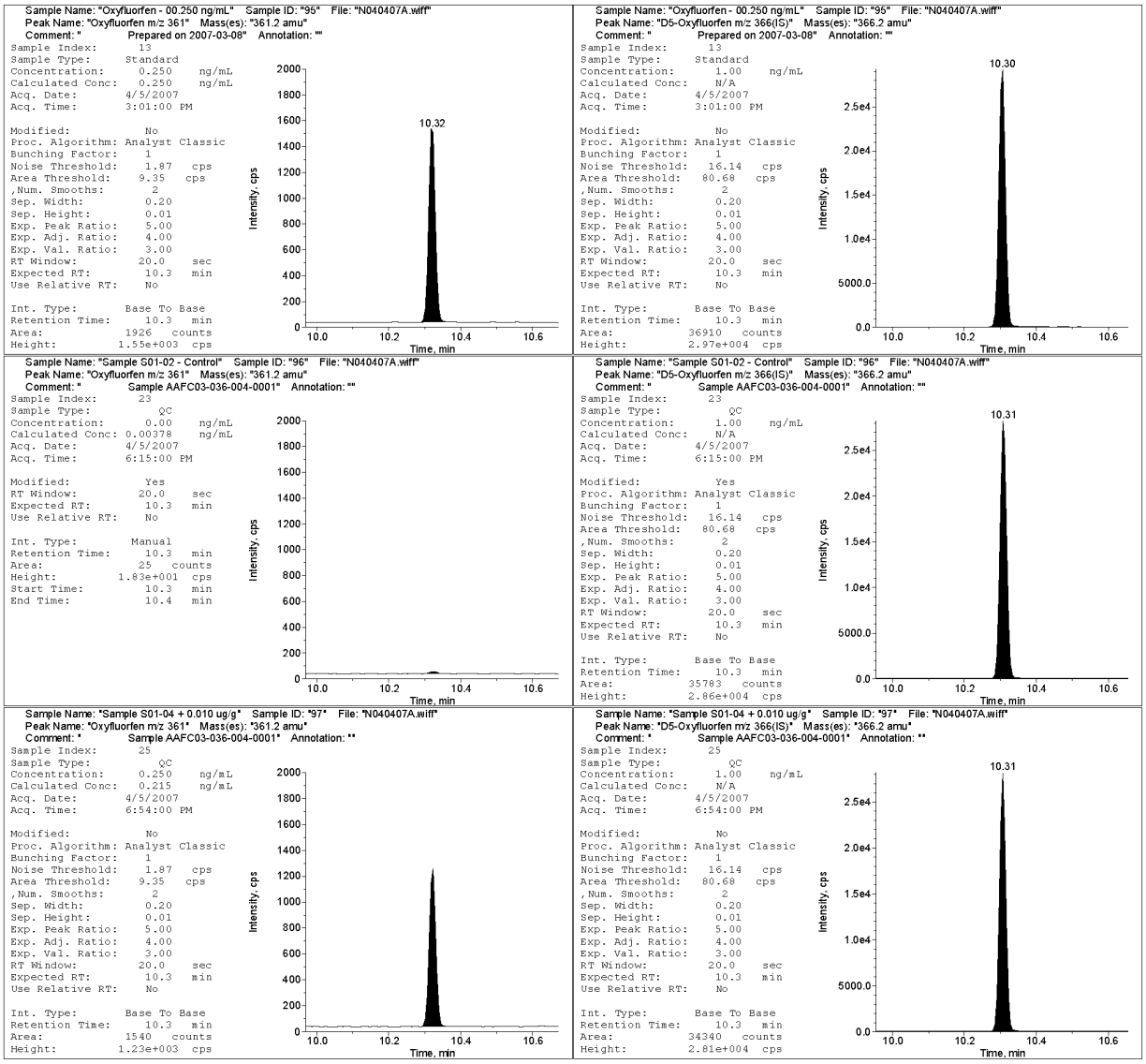
Figure 1. Methane Negative-Ion Chemical Ionization Mass Spectra of Oxyfluorfen and D_5-Oxyfluorfen (Internal Standard)



Oxyfluorfen Concentration	Equivalent Sample Conc.	Oxyfluorfen Quantitation Ratio
ng/mL	µg/g	*m/z* 361 / *m/z* 366
0.075	0.003	0.01580
0.125	0.005	0.02594
0.250	0.010	0.05218
0.625	0.025	0.13057
1.250	0.050	0.26480
2.500	0.100	0.54039
6.250	0.250	1.39178
12.50	0.500	2.91141
25.00	1.000	6.27665

Power Regression Equation: X = (Y/8.0165)^(1/1.0198)
Correlation Coefficient (r^2): 0.9999

Figure 2. Typical Calibration Curve for the Determination of Oxyfluorfen in Agricultural Commodities



a) Typical Chromatogram of a 0.25-ng/mL Standard Equivalent to 0.010 µg/g of Oxyfluorfen

b) Typical Chromatogram of a Control Blueberry Sample Containing No Detectable Residue of Oxyfluorfen

c) Typical Chromatogram of a Control Blueberry Sample Fortified with 0.010 µg/g of Oxyfluorfen (86% Recovery)

Figure 3. Typical Chromatograms for the Determination of Oxyfluorfen in Blueberry