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DETERMINATION OF OXADIXYL IN WINES BY LC/MS/MS

Scope and Application

This method is designed to determine the concentration of oxadixyl in grape wines by liquid chromatography tandem quadrupole mass spectrometry (LC/MS/MS) using matrix matched external calibration curve and no or minimal sample preparation.

Oxadixyl, a fungicide, is not authorized for use in the US for grape crops. TTB monitors pesticide levels in imported and domestic wines. Unauthorized pesticides, including oxadixyl, have no established maximum residue levels (MRL) and wines are deemed adulterated if any levels of these pesticides are detected.

Levels and Limitations

This method is applicable to all grape wines.

Analyte	Detection Limit	Quantitation Limit	Linear Range	Interferences
Oxadixyl	0.7 ***ppb***	2.0 ***ppb***	2.0 – 20.0 ***ppb***	none

Equipment

Instrumentation:

Waters Acquity UPLC or equivalent
Waters Quattro Premiere XE MS/MS Tandem Quadrupole Mass Spectrometer or equivalent

Instrumentation Parameters (LC):

Column:	Waters BEH C_{18} 1.7 µm, 2.1 x 50 mm (or equivalent) and an appropriate guard column
Mobile Phase:	Solvent A: DI Water with 0.1% v/v formic acid. Solvent B: Acetonitrile with 0.1% formic acid.
Solvent Gradient:	Time (min) Comp A% Comp B% 0 90 10 3.0 10 90 3.1 90 10
Column Remperature	30°C

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Sample Temperature:	10-12°C
Flow rate:	0.400 L/min
Run time:	4 min
Injection Volume:	3-5 µL

Instrumentation Parameters (Mass Spectrometer):

Source voltages	Positive electrospray ionization mode (ESI^+). Capillary: 0.50 kV; Cone: 20V; Extractor: 4V; RF Lens: 0.1V.
SRM (MRM) transitions	Quantifying: 279.20→219.30 Da, Collision Energy (CE) 10 eV; Qualifying: 279.20→132.20 Da, CE 35 eV. Dwell time 0.05 sec.
Gas flow and temperature control	Source temperature: 120°C; Desolvation temperature: 350°C; Cone gas flow: 80 L/Hr; Desolvation gas flow: 600 L/Hr; Collision gas flow: 0.35 mL/min
Mobile phase flow control	Divert the UPLC flow into waste from 0 to 1.3 min and from 1.6 to 4 min. **Note:** Divert valve switch time is column dependent.

Glassware and Supplies:

Class A 25 mL volumetric pipettes
Class A 50 and 100 mL volumetric flasks
50 mL conical polypropylene tubes
LC vials with pre-slit cap septa
25 µL syringe or appropriate single-channel variable volume pipettors

Note: All consumables listed above may potentially introduce contamination and affect test results. Therefore, it is critical that oxadixyl-free blank wines are injected before and after each sequence according the Process Control procedure to insure absence of oxadixyl signal.

Reagent, Standards, and Sample Preparation

Reagents:

DI water, MilliQ (MQ), 18 MegaΩ or better
Acetonitrile, HPLC Grade , Purity ≥99.9%
Formic acid, Mass Spectroscopy Grade, ≥97%

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Oxadixyl (2-methoxy-N-(2-oxo-1,3-oxazolidin-3-yl)-acet-2',6'-xylidide (CAS # 77732-09-3, $C_{14}H_{18}N_2O_4$). Purity ≥ 99%

Preparation of stock and working standards:

1. Oxadixyl 1000±10 mg/L high concentration stock standard is prepared as follows:

   Transfer 0.100±0.001 g of oxadixyl into a 100 mL volumetric flask and bring to the volume with acetonitrile. Stable for 6 months, if stored in the freezer.

2. Oxadixyl 10.0±0.1 mg/L stock standard is prepared as follows:

   Transfer 0.500 mL of 1000 mg/L stock standard solution into a 50 mL volumetric flask and bring to the volume with acetonitrile. Stable for 6 months, if stored in the freezer.

3. Oxadixyl matrix matched working standards are prepared in red wines, if red wines are tested or in white wines, if white wines are tested. Either red or white wines can be used for working standard preparation for rose wine analysis. Prepare the working standards daily as follows:

   Transfer 25.00 mL of white or red oxadixyl-free wine volumes into four separate 50 mL polypropylene conical tubes. Fortify with the appropriate amounts of 10.0 mg/L stock standard to the following concentrations: Level 1 – 2.0 µg/L, Level 2 – 5.0 µg/L, Level 3 – 10.0 µg/L, Level 4 – 20.0 µg/L.

   Note: Matrix matched calibration standards must be prepared in oxadixyl-free wines. All oxadixyl-free wines (usually organic wines) that are used for preparation of the matrix matched standards should be screened first to verify the absence of oxadixyl signal.

Preparation of sample:

1. Most test samples are transferred directly into LC vials and injected neat. Prior filtration, through a 0.45 µm nylon membrane filter, may be necessary if a visible residue is present. In addition, sparkling or carbonated wines must be decarbonated prior to analysis.

Procedures

1. Prime the UPLC system and equilibrate the column to the initial mobile phase conditions of the method until the backpressure becomes stable.

2. Perform LC/MS/MS suitability test according to the Quality Control Section below.

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3. Inject blank samples, standards, test samples, control standards, laboratory control samples in accordance with the Quality Control section.

4. Generate calibration curves using daily prepared matrix matched working standards.

5. Analyze test samples and report the results as described in Calculations and Reporting the Results sections below.

Quality Control

1. Perform LC/MS/MS system suitability test as follows:

   i. Inject a freshly prepared Level 1 (2.0 µg/L**)** calibration standard.

   ii. Verify the presence of oxadixyl peak and correct retention time. The retention time should be within 1.4±0.20 min. Peak S/N ratio should be more than 3. If it is less, inject 4 or 5 µL instead. **Note: Oxadixyl retention time is column dependent.**

   iii. If the retention time or variability fall outside the expected range, troubleshoot the LC/MS/MS system according to the User's Manual.

   Note: The mass spectrometer is calibrated once every year. However, a more frequent calibration may be performed as a part of instrument's troubleshooting. Consult the User's Manual for details.

2. Process control

   i. A blank oxadixyl-free wine sample that was used for preparation of matrix matched calibration standards is injected immediately before and after calibration standards in each running sequence to ensure the absence of oxadixyl signal. The same blank is also injected immediately before and after Level 2 standard check.

   ii. Correlation coefficient of the calibration curve should be no less than 0.993.

   iii. A Level 2 standard check is injected after every seven test samples. The oxadixyl control value should not exceed 5.0 ±1 ppb.

   iv. Two laboratory control samples (LCS) are injected with each sequence. The accuracy of LCSs should not exceed ±1 ppb and the precision is established according to the laboratory practices.

   Note: The LCSs are prepared every three months by spiking wines with oxadixyl in the linear concentration range.

   v. Verify peak area ratios (ion ratio) for both molecular transition of oxadixyl. The ion ratio should be within 2.5±0.8 range.

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Sources of Uncertainty

1. Liquid delivery devices (syringes, pipettors, volumetric flasks, LC Injection needle)
2. Mass Spectrometry
3. Analytical Balances

Calculations

Calibration curves are generated by injecting calibration standards and by plotting peak areas for m/z 279.20→219.30 molecular transition versus concentration of the standards. Second transition (m/z 279.20→132.20) is used for confirmation. Oxadixyl concentrations in the test samples are calculated using QuantLynx feature of Waters' MassLynx software or using other appropriate software packages.

Reporting Results

Report the results as follows in the 2.0-20.0 µg/L concentration range:

Component	Sample Type	Units	Precision	Format
Oxadixyl	Wine	ppb	1 decimal place	***x.X***

Samples x.X < 0.7 µg/L are reported as Not Detected (ND).
Samples 0.7 ≤ x.X < 2.0 µg/L are reported as Below Quantitation Limits (BQL).
Samples x.X > 20.0 µg/L are either reported as "> 20.0 µg/L" or the samples are diluted accordingly with oxadixyl-free red or white wines to yield concentration within the linear range

Safety Notes

Oxadixyl is a possible carcinogen and therefore should be handled with care and according to MSDS. All other solvents and reagents should also be handled according to MSDS.

References

1. Oxadixyl RED Facts (http://www.epa.gov)
2. PAN Pesticides Database (http://www.pesticideinfo.org)

Location of Validation Package.

TM
Quality System Files

Required Training, Certification and Re-certification.

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1. Vendor provided or in house Waters UPLC and Quattro Premiere XE MS/MS Operations Training.

2. Test method training followed by competency demonstration according to laboratory procedures.

Revision History

Revision 1 – Added statement regarding consumables; changed units for LOD, LOQ, and linear range to match reporting units – 3/22/2012

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