INTRODUCTION

This study was conducted following procedure described in the EPA Ecological Effects Test Guidelines, OPPTS 850.7100: Data Reporting for Environmental Chemistry Methods, (d) Independent Laboratory Validation. This study was initiated on November 15, 2010, the day the Study Director signed the protocol (Appendix 1). The laboratory work was conducted on November 15 – December 14, 2010. The study was conducted at Valent Technical Center, located in Dublin, California, U.S.A.

Quality assurance measures taken during the experimental portion of this study included, but were not limited to, the following:

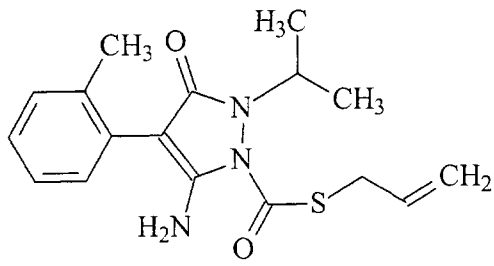
- All measuring equipment used in this study was calibrated in accordance with the proper Valent Standard Operating Procedures.
- The original protocol, all raw data, and the final report for this study are stored in the Valent archive located at the Valent Technical Center, Dublin, California.
- An in-life audit and audit of the data and the report were performed by the Valent QA unit.
- A subsample of the test and reference substances used in this study was archived at the Valent Technical Center, Dublin, California.

MATERIALS

Test substance:

The test substance was supplied by the Valent Technical Center in Dublin, CA. A copy of the certificate of quality is included in Appendix 2.

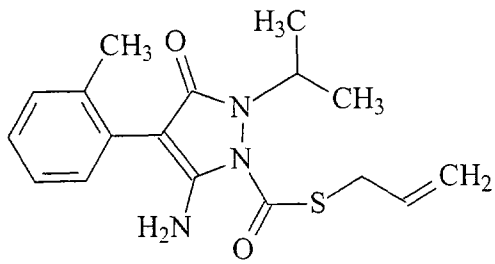
Common Name:	Fenpyrazamine
Code Name:	V-10135, S-2188
Chemical Name (CA):	1*H*-Pyrazole-1-carbothioic acid, 5-amino-2,3-dihydro-2-(1-methylethyl)-4-(2-methylphenyl)-3-oxo-, S-2-propenyl ester
CAS Number:	473798-59-3
Molecular Weight:	331.4
Molecular Formula:	$C_{17}H_{21}N_3O_2S$
Lot Number:	AS 2177c
Chemical Purity:	98.8%
Expiration Date:	7/14/2011
Chemical Structure:	



Reference Substance:

The reference substance was supplied by the Valent Technical Center in Dublin, CA. A copy of the certificate of quality is included in Appendix 2.

Common Name:	Fenpyrazamine
Code Name:	V-10135, S-2188
Chemical Name (CA):	1*H*-Pyrazole-1-carbothioic acid, 5-amino-2,3-dihydro-2-(1-methylethyl)-4-(2-methylphenyl)-3-oxo-, S-2-propenyl ester
CAS Number:	473798-59-3
Molecular Weight:	331.4
Molecular Formula:	$C_{17}H_{21}N_3O_2S$
Lot Number:	AS 2277b
Chemical Purity:	99.8%
Expiration Date:	11/09/2011
Chemical Structure:	



Test system:

The test system was freshwater prepared by fortifying Dublin, CA city tap water with salts (147 mg L^{-1} $NaHCO_3$, 92 mg L^{-1} $CaSO_4 \cdot 2H_2O$, 92 mg L^{-1} $MgSO_4$ and 6.0 mg L^{-1} KCl) and the test substance.

Chemicals and Reagents:

Methanol suitable for GC, HPLC, and Spectrophotometry (EMD Chemicals, Gibbstown, NJ, U.S.A.);

Water suitable for HPLC, Spectrophotometry, Gas Chromatography, and Gradient Analysis (EMD Chemicals, Gibbstown, NJ, U.S.A.);

Acetonitrile suitable for GC, HPLC, and Spectrophotometry (EMD Chemicals, Gibbstown, NJ, U.S.A.);

Acetic Acid, glacial, reagent grade (Mallinckrodt Baker, Inc., Phillipsburg, NJ, U.S.A.);

Sodium Bicarbonate, 100% (Fisher Scientific, Pittsburgh, PA, U.S.A.);

Calcium Sulfate, 98% (Sigma-Aldrich, St. Louis, MO, U.S.A);

Magnesium Sulfate, 99.5% (Sigma-Aldrich, St. Louis, MO, U.S.A);

Potassium Chloride, 99% (Sigma-Aldrich, St. Louis, MO, U.S.A).

Reagent solutions:

HPLC-grade Water acidified with 0.05% acetic acid;

Acetonitrile/HPLC-grade Water (50:50, v/v) acidified with 0.05% acetic acid.

Glassware:

A list of glassware used in the method validation is shown below. Similar glassware from other suppliers may also be used.

100, 50, 25, and 10 mL volumetric flasks (Fisher Scientific, Pittsburgh, PA, U.S.A.);

Volumetric pipettes, variable volumes (Fisher Scientific, Pittsburgh, PA, U.S.A.);

Disposable Pasteur pipettes, 150-mm long, 5 mm I.D. (VWR, Radnor, PA, U.S.A.);

Conical-bottom, graduated glass tubes, screw-PTFE lined cap, 15 mL (VWR, Radnor, PA, U.S.A.);

Glass vials, screw-cap, 20 and 50 mL (Fisher Scientific, Pittsburgh, PA, U.S.A.);

Autosampler vials, 1.8 mL, with Teflon®-lined septum caps.

Equipment and supplies:

A list of equipment used in the method validation is shown below. Similar equipment from other suppliers may also be used.

Balance Shimadzu AUX220 (Shimadzu Corporation, Columbia, MD, U.S.A.);

Hamilton microsyringes, 100 µL, 500 µL (Fisher Scientific, Pittsburgh, PA, U.S.A.);

Vacuum manifold equipped with vacuum gauge, 12 ports, J.T. Baker (VWR, Radnor, PA,

U.S.A.);

Visiprep large volume transfer tubes (Supelco, Bellefonte, PA, U.S.A);

Vacuum trap made from 1-L sidearm flask fitted with single-hole rubber stopper;

Vacuum source, capable of maintaining 25 inches Hg;

Oasis® HLB SPE cartridges, 500mg, 6cc, 60 µm (Waters, Milford, MA, U.S.A.);

YMC Pack ODS-AQ column, S-3 µm, 12 nm, 2.0×50 mm (YMC America Inc., Allentown, PA, U.S.A.);

Agilent 1200 HPLC system equipped with a column thermostat compartment, a binary pump, a degasser, and an autosampler (Agilent Technologies Inc., Palo Alto, CA, U.S.A.);

Applied Bioscience API 4000 triple quadrupole mass spectrometer operating in positive electron spray ionization mode (ESI) (Applied Biosystems, Foster City, CA);

Analyst® software (version 1.4.2) (Applied Biosystems, Foster City, CA).

ANALYTICAL METHOD

Validation sample sets:

Three trials were conducted with a validation set consisted at least of instrument calibration standards, 1 reagent blank, 2 unspiked control samples, 5 water samples fortified with analyte at 1.00 µg L^{-1} (LOQ), and 5 water samples fortified with analyte at 10.0 µg L^{-1} (10XLOQ).

Preparation of standard stock solutions:

Stock solution of the test substance (1.00 mg mL^{-1}). A V-10135/S-2188 stock solution (1.00 mg mL^{-1}) was prepared by accurately weighing 25.3 mg (25.0 mg a.i.) of the test substance into a 25 mL volumetric flask and adding acetonitrile to the mark. The concentration of the standard was corrected for the purity of test substance.

Stock solution of the test substance (10.0 µg mL^{-1}). A solution of the test substance (10.0 µg mL^{-1}) was prepared by diluting the stock solution of the test substance in acetonitrile. For that, 500 µL of the stock solution of the test substance (1.00 mg mL^{-1}) was transferred to a 50 mL volumetric flask and diluted to the mark with acetonitrile. This solution was used to fortify water samples at the 10.0 ng mL^{-1} level.

Stock solution of the test substance (1.00 µg mL^{-1}). A solution of the test substance (1.00 µg mL^{-1}) was prepared by diluting the stock solution of the test substance in acetonitrile. For that, 5 mL of the stock solution of the test substance (10.0 µg mL^{-1}) was transferred to a 50 mL volumetric flask and diluted to the mark with acetonitrile. This solution was used to fortify water samples at the 1.00 ng mL^{-1} level.

Stock solution of the reference substance (1.00 mg mL^{-1}). A S-2188 stock solution (1.00 mg mL^{-1}) was prepared by accurately weighing 25.0 mg of the reference substance into a 25 mL volumetric flask and adding acetonitrile to the mark. The concentration of the standard was corrected for the purity of reference substance.

Stock solution of the reference substance (10.0 µg mL^{-1}). A solution of the reference substance (10.0 µg mL^{-1}) was prepared by diluting the stock solution of the reference substance in acetonitrile. For that, 500 µL of the stock solution of the reference substance (1.00 mg mL^{-1}) was transferred to a 50 mL volumetric flask and diluted to the mark with acetonitrile.

Stock solution of the reference substance (1.00 µg mL^{-1}). A solution of the reference substance (1.00 µg mL^{-1}) was prepared by diluting the stock solution of the reference substance in acetonitrile. For that, 5 mL of the stock solution of the reference substance (10.0 µg mL^{-1}) was transferred to a 50 mL volumetric flask and diluted to the mark with acetonitrile. This solution was used to prepare instrument calibration standards.

All stock solutions of the reference and test substances were stored in a refrigerator (~4 °C) in glass vials with screw caps.

Preparation of instrument calibration standard solutions:

Five levels of instrument calibration standards at 1.00, 5.00, 10.0, 25.0, and 50.0 ng mL^{-1} (in acetonitrile/HPLC-grade water acidified with 0.05% acetic acid) were prepared by serial dilution of the 1.0 µg mL^{-1} of standard solution of the reference substance. The instrument calibration standards were prepared just prior to the analysis of each sample set.

Preparation of fortification samples:

LOQ fortification samples at 1.00 µg L^{-1} (1.00 ng mL^{-1}; or 1.00 ppb) were prepared by adding 100 µL of the 1.00 µg mL^{-1} stock standard solution of the test substance into 100 mL of freshwater using a 100 µL Hamilton microsyringe.

10XLOQ fortification samples at 10.0 µg L^{-1} (10.0 ng mL^{-1}; or 10.0 ppb) were prepared by adding 100 µL of the 10.0 µg mL^{-1} stock standard solution of the test substance into 100 mL of freshwater using a 100 µL Hamilton microsyringe.

Extraction procedure:

Trial 1. Water samples were extracted in two batches of seven samples (1 reagent blank, 2 unspiked control samples, 5 water samples fortified with the analyte at 1.00 µg L^{-1} or 10.0 µg L^{-1}) using 6 mL, 500 mg (60µm) Oasis® hydrophilic-lipophilic balance (HLB) cartridges and a vacuum manifold SPE extraction system. Before extraction, the SPE cartridges were placed on the vacuum manifold and sequentially precondition with 6 mL of methanol and 6 mL of HPLC-grade water under gravity. The water was allowed to drain to the top of the frit, and 60 mL reservoirs were connected to the cartridges. The water samples were then loaded onto the cartridges at 15-20 mL min^{-1} (~7 inches Hg), after which the water flasks were rinsed with 10 mL of HPLC-grade water, and the water rinsates were transferred onto the cartridges. During the loading of the samples onto the SPE cartridges, 1 sample in the LOQ fortified batch and 1 sample in the 10XLOQ fortified batch leaked between the reservoir and the SPE cartridge.

Therefore, the initial set (Trial 1) was rejected and not used for the further validation.

Trial 2. Water samples were extracted in two batches of seven samples (1 reagent blank, 2 unspiked control samples, 5 water samples fortified with the analyte at 1.00 μg L^{-1} or 10.0 μg L^{-1}) using 6 mL, 500 mg (60μm) Oasis® hydrophilic-lipophilic balance (HLB) cartridges and a vacuum manifold SPE extraction system. Before extraction, the SPE cartridges were placed on the vacuum manifold and sequentially precondition with 6 mL of methanol and 6 mL of HPLC-grade water under gravity. The water was allowed to drain to the level about 2 cm above top of the frit, and Visiprep large volume transfer tubes were connected to the cartridges. The water samples were then loaded onto the cartridges at about 10 mL min^{-1} (~7 inches Hg) using the Visiprep large volume transfer tubes, after which the water flasks were rinsed with 10 mL of HPLC-grade water, and the water rinsates were transferred onto the cartridges. After all of the samples have passed through the SPE cartridges, the cartridges were dried about 40 min at high vacuum (16 inches Hg). Next, the cartridges were eluted with 5 mL of acetonitrile into 15mL calibrated centrifuge tubes under gravity flow (~1 mL min^{-1}). The extracts were diluted with acetonitrile to a volume of 5 mL. Further 1:1 dilution with HPLC-grade water acidified with 0.05% acetic acid was made for the 1.00 ng mL^{-1} fortified water samples, and 1:10 dilution with HPLC-grade water/acetonitrile (50:50, v/v) acidified with 0.05% acetic acid was made for the 10.0 ng mL^{-1} fortified water samples. The final volume of the extracts was 10 mL. The prepared extracts were transferred into autosampler vials and analyzed by a liquid chromatography/mass spectrometry (LC/MS-MS).

Trial 3. Water samples were extracted in two batches of seven samples (1 reagent blank, 2 unspiked control samples, 5 water samples fortified with the analyte at 1.00 μg L^{-1} or 10.0 μg L^{-1}) using 6 mL, 500 mg (60μm) Oasis® hydrophilic-lipophilic balance (HLB) cartridges and a vacuum manifold SPE extraction system. Before extraction, the SPE cartridges were placed on the vacuum manifold and sequentially precondition with 6 mL of methanol and 6 mL of HPLC-grade water under gravity. The water was allowed to drain to the level about 2 cm above top of the frit, and Visiprep large volume transfer tubes were connected to the cartridges. The water samples were then loaded onto the cartridges at about 20-25 mL min^{-1} (~7 inches Hg) using the Visiprep large volume transfer tubes, after which the water flasks were rinsed with 10 mL of HPLC-grade water, and the water rinsates were transferred onto the cartridges. After all of the samples passed through the SPE cartridges, the cartridges were dried about 35 min at high vacuum (15-18 inches Hg). Next, the cartridges were eluted with 5 mL of acetonitrile into 15 mL calibrated centrifuge tubes under vacuum. The flow rate was 2-3 mL min^{-1}. The extracts were diluted with acetonitrile to a volume of 5 mL. Further 1:1 dilution with HPLC-grade water acidified with 0.05% acetic acid was made for the 1.00 ng mL^{-1} fortified water samples, and 1:10 dilution with HPLC-grade water/acetonitrile (50:50, v/v) acidified with 0.05% acetic acid was made for the 10.0 ng mL^{-1} fortified water samples. The final volume of the extracts was 10 mL. The prepared extracts were transferred into autosampler vials and analyzed by a liquid chromatography/mass spectrometry (LC/MS-MS).

Trial 4. Water samples were extracted in batch of 14 samples (2 reagent blanks, 2 unspiked control samples, 5 water samples fortified with the analyte at 1.00 μg L^{-1}, and 5 water samples fortified with the analyte at 10.0 μg L^{-1}) using 6 mL, 500 mg (60μm) Oasis® hydrophilic-lipophilic balance (HLB) cartridges and a vacuum manifold SPE extraction system. Before extraction, the SPE cartridges were placed on the vacuum manifold and sequentially precondition with 6 mL of methanol and 6 mL of HPLC-grade water under gravity. The water was allowed to drain to the level about 2 cm above top of the frit, and Visiprep large volume transfer tubes were connected to the cartridges. The samples were then loaded onto the cartridges at 10-15 mL min^{-1} (~7

inches Hg) using the Visiprep large volume transfer tubes, after which the water flasks were rinsed with 10 mL of HPLC-grade water, and the water rinsates were transferred onto the cartridges. After all of the samples passed through the SPE cartridges, the cartridges were dried for 10 min at high vacuum (15-18 inches Hg). Next, the cartridges were eluted with 5 mL of methanol followed by 5 mL of acetonitrile into 15 mL calibrated centrifuge tubes under gravity flow. The extracts were diluted with acetonitrile to a volume of 10 mL. Further 1:1 dilution with HPLC-grade water acidified with 0.05% acetic acid was made for the 1.00 ng mL^{-1} fortified water samples, and 1:10 dilution with HPLC-grade water/acetonitrile (50:50, v/v) acidified with 0.05% acetic acid was made for the 10.0 ng mL^{-1} fortified water samples. The final volume of the extracts was 10 mL. The prepared extracts were transferred into autosampler vials and analyzed by a liquid chromatography/mass spectrometry (LC/MS-MS).

LC/MS-MS operation parameters:

The analyte was analyzed using an YMC-Pack ODS-AQ, 50×2.0 mm I.D. column with 3μm particle size. An isocratic gradient consisting of HPLC-grade water and acetonitrile (50:50, v/v) acidified with 0.05 % acetic acid at a flow rate of 200 μL min^{-1} was used. The temperature of the analytical column was maintained at 20±1°C. A 10 min equilibration step with mobile phase was used at the beginning of each run. The total run time was 10 min, and injection volume for all LC/MS-MS runs was 5 μL. The retention time of V-10135/S-2188 was approximately 4.62 min.

The mass spectrometer parameters were optimized for the V-10135/S-2188 analyte prior to the sample analysis. Operating parameters used during the method validation were:

Scan Type:	MRM
Polarity:	Positive
Ion Source:	TurboSpray® electro spray interface
Dwell time:	150 msec
Q1/Q3 mass:	332.1/272.0 amu
Q1/Q3 mass:	332.1/230.3 amu
Declustering potential:	75 V
Entrance potential:	8 eV
Collision cell exit potential:	16 V
Collision activated dissociation gas:	12 psig
Curtain gas:	20 psig
Ion source gas 1, nebulizer gas:	35 psig
Ion source gas 2, heater gas:	25 psig
Ion spray voltage:	5000 V
Temperature:	550 °C

Analytical Sequence Setup:

For each set of analyses, the LC/MS-MS instrument was conditioned with several injections of a mid-level instrument calibration standard before the injection of the first calibration set. Two sets of instrument calibration standards were analyzed with each LOQ and 10XLOQ sample set. The analytical sequence began with the first instrument calibration set, then the LOQ sample set followed by the second instrument calibration standard set, etc. A single injection was performed for each standard solution and sample.

Data Integration:

The Analyst software associated with the instrument was used to integrate the peaks of interest. The integration was based on the Extracted Ion Chromatogram (XIC for the analyte).

Calculations:

Calibration procedures. The V-10135/S-2188 analyte was calibrated externally using a 1st order polynomial regression with 1/concentration weighting. The area peak (detector response) data provided by the Analyst software was entered into a GraphPad Prism 4.03 Software (GraphPad Software Inc.). A 1st order polynomial fit curve (y=mx+b) with 1/concentration weighting was generated for the analyte with each set of analyses, and the curve constants (intercept, b, and slope, m) were determined. The curve was used to calibrate the instrument, determine the acceptability of the standard injections and to calculate the sample residues. The curve was generated by plotting the instrument calibration standard peak area (detector responses) versus the instrument calibration standard concentration.

Calculation of analyte concentrations and sample residues. Analyte concentrations for the standards and the samples were calculated by the Excel spreadsheet using the equation:

$$x = ((y - b)/m) \times (FSV) \times (DF)/(SV)$$

where x - concentration of analyte, ng mL^{-1} ($\mu g\ mL^{-1}$, ppb),
y - peak area (detector response),
m - calibration curve coefficient (slope),
b - calibration curve coefficient (intercept),
FSV - final sample volume, mL,
DF – dilution factor,
SV – sample volume, mL.

Example calculation. Concentration of V-10135/S-2188 in the sample LOQ A, 1.00 ng mL^{-1}, extracted 13-Dec-10 and analyzed on 13-Dec-10:
y = 241870,
m = 45287,
b = 6584,
Final Sample Volume = 10 mL,
Dilution factor = 2
Sample Volume = 100 mL

$$x = [(241870-6584)/45287] \times 10 \times 2 \div 100 = 1.04 \text{ ng } mL^{-1} (\mu g\ L^{-1})$$

Calculation of fortification sample percent recovery. To calculate the percent recoveries for the fortified water samples, ng mL^{-1} (ppb) residues found in the control samples (if any) was subtracted from the ng mL^{-1} (ppb) residues found in the fortified samples, and then dividing the result by the fortification level.

Example calculation. Percent recovery for sample LOQ A extracted and analyzed on 13-Dec-10:

1.04 ng mL^{-1} in fortified – 0.000 ng mL^{-1} in unspiked ÷ 1.00 ng mL^{-1} fortification level x 100% =

= 1.04 ÷ 1.00 x 100% = 104%

Note: these calculations, when done by hand, may differ slightly from the results reported by the Excel spreadsheet due to rounding differences.

Calculation of standard deviation and coefficient of variation. Standard deviations (SD) and or coefficient of variations (CV) were used to evaluate the data for the fortification samples. The standard deviations (SD) were calculated from the mean values of the samples being

considered, and expressed as an absolute percent value (coefficient of variation, CV) using the mean values:

$$\text{Coefficient of Variation, \%} = \frac{\text{standard deviation} \times 100}{\text{mean}}$$

Example calculation. Using the five replicates fortified at 1.00 ng mL^{-1} (LOQ) in the fourth analytical set, V-10135/ S-2188 fortification recoveries, where the mean recovery was 103% and the standard deviation was 2.68%:

$$\text{CV, \%} = \frac{2.68\% \times 100\%}{103\%}$$

$$= 2.60\%$$

Note: these calculations, when done by hand, may differ slightly from the results reported by the Excel spreadsheet due to rounding differences.

Acceptance Criteria. Criteria for the acceptance of the calibration curve was not specified in the method, however, the following criteria was applied: the coefficient of determination (r^2) of the standard regression curves were required to be ≥0.99. The apparent concentration of each calibration standard calculated using the equation for the calibration curve were required to be within 10% of the actual concentration.

Threshold area counts. Using a 5 µL injection, the signal-to-noise ratio for V-10135/S-2188 for the smallest standard (1 ng mL^{-1}) was about 150:1. Minor responses were observed in the unspiked control samples for V-10135/S-2188, however the responses were less than 10% of the smallest standard and therefore were not considered to be V-10135/S-2188, and were reported as "0 ng mL^{-1}". Fortification recoveries were not adjusted for these responses in these control samples.

Statistics statement:

The average percent recoveries, standard deviations, and coefficients of variation were calculated using Excel spreadsheets. The GraphPad Prism 4.03 Software calculated the 1st order polynomial fit curve (y=mx+b) and the coefficient of determination (r^2) for each of the standard calibration curves.

Deviations:

A 5 µL injection was used instead of 10 µL.

A 5 mL of methanol followed by 5 mL of acetonitrile was used to elute the analyte from the Oasis® HLB cartridge.

A 10 mL of HPLC-grade water was used to rinse the water flasks.

A 1st order polynomial fit curve (y=mx+b) was used to obtain standard calibration curves.

These deviations did not have any impact on the outcome of the study.

Validation and contact with sponsor:

The initial set of samples (Trial 1) was not used in the independent validation study due to leakage and loss of sample between the reservoirs and SPE cartridges while loading the water samples onto the SPE cartridges. This experimental difficulty was avoided by changing the reservoirs to Visiprep large volume transfer tubes that avoid any loss of water samples during the loading procedure. Thus, Trial 1 was not considered a validation attempt.

The performance data for the first set of samples (Trial 2) was unsuccessful because the recoveries of V-10135/S-2188 were low. Average percent recovery for V-10135/S-2188 was 52.4 % (Table 1). The method's developer, Marjiorie Dix, was contacted on 12/2/2010 to clarify the flow rate of the loading and elution steps during the SPE extraction procedure. According M. Dix, the flow rate of the loading step should be ~25 mL min^{-1}, and elution of the analyte has to be done under vacuum with flow rate more than 1 mL min^{-1}. These conditions were applied in the second set of samples (Trial 3). However, the recoveries of V-10135/S-2188 were still low, with average percent recovery of 48.9% (Table 2). The recoveries of V-10135/S-2188 from the water samples were not acceptable in the first and second sets of samples, and therefore these sets were rejected. The results for these sets are not included in the following discussion.

An Oasis® HLB SPE (500 mg, 60 μM, 6cc) cartridge performance check was conducted using 5 mL of acetonitrile, 10 mL of acetonitrile, 14 mL of acetonitrile, and 5 mL of methanol followed my 5 mL of acetonitrile. The results showed that sufficient recovery of V-10135/S-2188 from an Oasis® HLB SPE cartridge (500 mg, 60 μM, 6cc) was obtained using 5 mL of methanol followed by 5 mL of acetonitrile, while using 5mL, 10 mL, or 14 mL of acetonitrile was not sufficient to completely elute the analyte from the SPE cartridge. Therefore, the method elution step was modified, and elution with 5 mL of methanol followed 5 mL of acetonitrile was used in the third set of samples (Trial 4). In the third set of samples (Trial 4), recoveries of V-10135/S-2188 from water samples met the criteria for the independent lab validation. The results for the first and second sets are not included in the following discussion. The independent laboratory validation was successful on the third full attempt – the method performed as written in Springborn Smithers Laboratories method, "S-2188 – Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions Following OPPTS 860.1340, SANCO/3029/99 rev.4 and SANCO/825/00 rev.7" with a modification of elution solvent.

Suggested method edits:

In the Test System Section:

Change "$CaSO_4 \cdot H_2O$" to "$CaSO_4 \cdot 2H_2O$".

In the Equipment Section:

Include "vacuum manifold equipped with vacuum gauge, 12 ports, J.T. Baker (VWR, Radnor, PA, U.S.A.); Visiprep large volume transfer tubes (Supelco, Bellefonte, PA, U.S.A); vacuum trap made from 1-L sidearm flask fitted with single-hole rubber stopper; Vacuum source, capable of maintaining 25 inches Hg".

Include the particle size for Oasis HLB sorbent.

In the Solid phase Extraction section:

Include a SPE cartridge performance check section. The section suggested to be "In order to be used for extraction of aqueous samples, the performance of the Oasis® HLB SPE cartridges must be checked at least once for each manufacturer's lot of cartridges. This performance check is accomplished by processing a spiked reagent water sample through the extraction procedure and analyzing the extract. Cartridge performance is acceptable if the recoveries of the analyte are within 70-120%".

Consider specifying how the samples were loaded on the SPE cartridges.

Include the flow rate of loading the sample on the cartridges (10 - 15 mL min^{-1}).

Include the flow rate of elution of the analyte from the cartridges (gravity flow, 1 - 2 mL min^{-1}).

Suggest using 10 mL of HPLC-grade water to rinse a water flask.

In the Analysis Section:

Add second transition masses for the analyte, 332.1/272.0.

In the Calculations Section:

Specify that the coefficient of determination (r^2) must be greater than 0.99 (or similar) for acceptance of the calibration curve.

Consider to use weighted linear fit or polynomial fit calibration curves that will produce the percent differences between actual calibration standard concentration and standard concentration calculated from the standard peak response ≤10%.