NCASI METHOD HIMS-W106.01

HEXAZINONE, IMAZAPYR, METSULFURON METHYL, AND SULFOMETURON METHYL IN WATER BY SOLID PHASE EXTRACTION AND HPLC/UV

NCASI
West Coast Regional Center
Organic Analytical Program
February 2007

Acknowledgements

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National Council for Air and Stream Improvement, Inc. (NCASI). 2007. Hexazinone, Imazapyr, Metsulfuron Methyl, and Sulfometuron Methyl in Water by Solid Phase Extraction and HPLC/UV. *NCASI Methods Manual*. Research Triangle Park, NC: National Council for Air and Stream Improvement, Inc.

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NCASI METHOD HIMS-W106.01

HEXAZINONE, IMAZAPYR, METSULFURON METHYL, AND SULFOMETURON METHYL IN WATER BY SOLID PHASE EXTRACTION AND HPLC/UV

1.0 Scope and Application

1.1 Method HIMS-W106.01 is applicable for the determination of dissolved concentrations of hexazinone (the active ingredient in Velpar™), imazapyr (the active ingredient in Arsenal™, Chopper™, and Assault™), metsulfuron methyl (the active ingredient in Escort™ and Ally™), and sulfometuron methyl (the active ingredient in Oust™ and Oustar™) in ambient waters. The general analytical approach used in this method is based on multiple literature reports (1,2,3).

1.2 During routine analysis, quantification of the targeted analytes is performed using high-performance liquid chromatography (HPLC) employing ultraviolet (UV) detection. Sample extracts prepared using this method can also be analyzed using HPLC with mass spectrometric detection (LC/MS-MS) without any additional manipulations except (potentially) dilution.

1.3 The analytical working range for this method is from 1.0 ppb (ng/mL) to 50 ppb when 200-mL volumes of samples are extracted and analyzed by HPLC/UV. The higher end of the working range could be extended to an unknown level by adding higher level calibration standards, diluting samples at extraction, diluting sample extracts prior to analysis, or any combination of these. The only sure means of extending the lower end of this range is to analyze extracts by LC/MS-MS, which can provide quantifications to 0.1 ppb or less.

1.4 The low end of the analytical working range (1 ppb) is supported by experimentally determined method detection limits (MDLs) and minimum levels (MLs) in reagent water and field samples (Table 1, Section 17).

1.5 Method HIMS-W106.01 has been used for determining dissolved concentrations of hexazinone, imazapyr, and sulfometuron methyl in forest stream waters, including runoff waters resulting from precipitation events. As part of these analyses, metsulfuron methyl was spiked as a recovery surrogate. Imazapyr and hexazinone results from these analyses have been confirmed by independent analyses performed by LC/MS-MS (Figures 1 and 2, Section 17).

1.6 This method is restricted to use by, or under the supervision of, analysts experienced in the use of HPLC/UV chromatography. Each analyst must demonstrate the ability to generate acceptable results with this method (Section 9.2).

2.0 Summary of Method

2.1 Samples are collected in high-density polyethylene (HDPE) bottles, buffered to pH 7 at the time of collection, and frozen for long-term storage.

2.2 After thawing, samples are filtered through a 0.45-µm nylon membrane filter and the filtrate is adjusted to pH ≤2.3 by addition of dilute phosphoric acid. Immediately following acidification, the targeted herbicides are extracted by pulling the sample through a reverse-phase solid phase extraction (SPE) cartridge. The SPE cartridge is rinsed with water to reduce residual acidity within the cartridge, and then dried to remove any residual water. The dried SPE cartridge is stacked on top of a strong anion exchange (SAX) cartridge, and the analytes are eluted off the SPE cartridge and through the SAX cartridge using 50 mL of methanol. The resulting eluate is evaporated to dryness and the residue is redissolved in exactly 1.0 mL of water:acetonitrile (80:20, v/v). This final extract is fortified with a retention time standard (an "internal standard" used as a retention time marker only), filtered through a 0.45-µm membrane filter, and submitted for analysis.

2.3 A 25-µL volume of the sample extract is injected onto an HPLC/UV and the analytes are detected by monitoring absorbance at 235 nm. Analytes are identified based on relative retention times determined vs. the retention time standard, and an assessment of "peak purity" is made on chromatographic peaks tentatively identified as analytes using absorbance ratios. Quantifications are made vs. analyte-specific external calibrations using average response factors.

3.0 Definitions

3.1 The definitions below are specific to this method, but conform to common usage as much as possible.

3.1.1 Dissolved Concentration–the concentration determined in filtrates of aqueous samples after filtration at 0.45 µm

3.1.2 May–this action, activity, or procedural step is neither required nor prohibited

3.1.3 Method Detection Limit (MDL)–As defined by EPA (4), the MDL is the lowest concentration that can be identified at the 99% confidence level as being greater than background.

3.1.4 Minimum Level (ML)–The lowest concentration at which the entire analytical system gives a recognizable signal and acceptable calibration point. As defined by EPA (5), the ML is calculated by multiplying the MDL (Section 3.1.3) by 3.182. This is equivalent to Keith's (6) Limit of Quantification (LOQ).

3.1.5 Must not–this action, activity, or procedural step is prohibited

3.1.6 Must–this action, activity, or procedural step is required

3.1.7 Should–this action, activity, or procedural step is suggested, but not required

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample processing equipment may contribute contamination to samples that manifests as interference at the measurement step of the analysis. Method blanks (MB) must be analyzed with each sample set to document the level of this potential interference, and when MB results show unacceptable levels of contamination analyses must be halted until the source of the contamination is eliminated.

4.2 UV absorbance is a fairly nonspecific mode for detecting analytes. Thus the presence of nontarget chemicals may impart bias to measured concentrations when they co-elute with analytes. To monitor for this potential, absorbance at multiple wavelengths must be monitored and "peak purity" evaluated by comparing absorbance ratios (e.g., 235 nm/195 nm) obtained from samples to those obtained from analysis of standards. In cases where these ratios do not match, results should be considered suspect (Section 12.2.5).

4.3 Interference associated with general laboratory (blank) contamination from glassware and the SPE/SAX cartridges limits the lower end of the analytical working range in standards or laboratory control samples, while interference resulting from chromatographic co-elution of sample-specific co-extractives generally limits detectability in field samples. Because the lower end of the working range in samples is limited by sample-specific co-extractives, extracting larger sample masses (or volumes) and/or injecting larger volumes of sample extracts will usually not be effective approaches to reducing the low end of the analytical working range (1 ppb).

4.4 The significance of co-extractive interferents varies considerably from sample to sample, and NCASI has seen instances in which interference manifested in only a few of many samples collected from a single site over time; i.e., the interference manifested as a temporal event over the course of a sampling episode.

4.5 In cases where absorbance ratios indicate an unacceptable level of co-elution, the HPLC conditions may be modified to effect a different chromatographic separation.

4.6 In cases were severe interferences prevent confident identification and quantification of an analyte by HPLC/UV, sample extracts prepared using the procedures provided in this method may be analyzed using LC/MS-MS. Analysis of extracts by LC/MS-MS can also provide reliable quantifications of the targeted herbicides down to approximately 0.1 ppb.

4.7 Carryover may occur on the HPLC when samples containing high concentrations of the target analytes or interfering chemicals are analyzed in sequence with low-concentration samples. Whenever unusually concentrated samples are encountered, they should be followed by analysis of injection (solvent) blanks to check for carryover.

5.0 Safety

5.1 All chemicals should be treated as potential health hazards. It is recommended that prudent practices for handling chemicals in the laboratory be employed (7).

5.2 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness of OSHA regulations regarding safe handling of chemicals used in this method. Material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies

***Note**: Brand names and suppliers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and material other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sampling equipment

6.1.1 Samples must be collected in HDPE plastic bottles. It is recommended that certified pre-cleaned bottles be used; e.g., Eagle Pitcher "Level 1" bottles (1-L bottles, Cat. #EP150-01WMN, VWR, West Chester, PA).

6.1.2 Automatic sampling equipment may be used as long as clean HDPE containers are used and samples are preserved by buffering at pH 7 at the time of sample collection (this can be accomplished by adding the appropriate volume of buffer to the sample container prior to sample collection).

6.2 Solid phase extraction (SPE) equipment

6.2.1 Vacuum manifold for solid phase extraction (Visiprep™-DL solid phase extraction vacuum manifold, Cat. #57044, Sigma-Aldrich/Supelco, Bellefonte, PA)

6.2.2 Vacuum pump (GAST Model #DOA P161-AA, Benton Harbor, MI)

6.2.3 Flow control valve liners for the Visiprep™-DL (Cat. #57059, Sigma-Aldrich/Supelco, Bellefonte, PA)

6.2.4 Plastic stopcock with Luer connection (e.g., polycarbonate with HDPE handle, Cat. #C-30600-01, Cole Parmer, Vernon Hills, IL)

6.2.5 SPE cartridge–ENVI™-Chrom P SPE tubes, 6-mL/0.5-g (Cat. #57226, Sigma-Aldrich/Supelco, Bellefonte, PA)

6.2.6 SAX cleanup cartridge–Mega-BE-SAX, 1-g/6-mL (Cat. #12256013, Varian Sample Preparation Products, Harbor City, CA)

6.2.7 Adapter caps (Cat. #57020-U, Sigma-Aldrich/Supelco, Bellefonte, PA)

6.2.8 60-mL reservoirs (Cat. #12131012, Varian Sample Preparation Products, Harbor City, CA)

6.2.9 VisiDry™ attachment for Visiprep™-DL solid phase extraction vacuum manifold for solvent rinsing/drying syringe filters or SPE cartridges (Cat. #57100-U, Sigma-Aldrich/Supelco, Bellefonte, PA)

6.3 Miscellaneous glassware and equipment

6.3.1 Pear-shaped 100-mL evaporation flask, 24/40 joint (KONTES brand, Cat. #K608700-0224, Fisher Scientific, Pittsburgh, PA)

***Note**: To minimize adsorptive losses to active sites during extract evaporation and concentration, glass pear-shaped flasks must either (1) never have been scratched, or (2) be silylated (siliconized) prior to use. These flasks must be solvent rinsed between each use, and should be cleaned periodically using a cleaning agent such as Chem-Solv®.*

6.3.2 Cork ring support for 100-mL evaporating flask (Cat. #56250-024, VWR, West Chester, PA)

6.3.3 250-mL polypropylene beakers (Cat. #13890-080, VWR, West Chester, PA)

6.3.4 Balances–Analytical balance capable of weighing to ±0.1 mg; top-loading balance capable of weighing to ±0.01 g

6.3.5 Graduated cylinders–Glass, 25-, 50-, 250-, and 1000-mL

6.3.6 Glass syringes with Teflon™ tipped plungers– 25-, 50-, 100-, 250-, 500-, and 1000-µL (Cat. #60376-230, #60376-241, #60376-252, #60376-263, #60376-274, and #60376-285, VWR, West Chester, PA.)

6.3.7 Adjustable volume pipettors– 1000- and 5000-µL (Cat. #53513-410 and #53513-412, VWR, West Chester, PA.)

6.3.8 Filtration apparatus–Polysulfone 300-mL funnel, to hold 47-mm filters (Cat. #28143-550, VWR, West Chester, PA**)**

6.3.9 47-mm, 0.45-µm nylon membrane filters (Cat. #28159-746, VWR, West Chester, PA**)**

6.3.10 Autosampler vials suitable for 1-mL extracts and instrument to be used, preferably certified for cleanliness (particulate-free)

6.3.11 Amber VOA vials with Teflon™-lined caps, certified clean or solvent rinsed and dried– 7-, 15-, and 40-mL (Cat. #27341, #27342, and #23188, Sigma-Aldrich/Supelco, Bellefonte, PA)

6.3.12 Volumetric flasks, class A glass, ***never scratched***– 1-, 2-, 10-, 25-, 50-, 100-, 250-, and 1000-mL

6.3.13 pH meter, capable of 2-point calibration

6.3.14 Pasteur pipettes–Glass, 9-inch and 5¾-inch, disposable (Cat. #14672-380 and #14672-200, VWR, West Chester, PA)

6.3.15 Nitrogen evaporation apparatus

6.4 Evaporator/concentration equipment

6.4.1 Rotary evaporator (Buchi Rotavapor RE 111/Buchi 461 water bath)

6.4.2 Safety trap for rotary evaporator with 24/40 lower join (Cat. #27582-166, VWR, West Chester, PA)

6.4.3 High vacuum pump–Edwards Model E2M2.

6.4.4 Solvent vapor vacuum trap, suitable for dry ice in isopropanol bath (e.g., Cat. #4519-01, Chemglass, and Cat. #80063-072, VWR, West Chester, PA)

6.4.5 4-mm, 0.45-µm nylon membrane syringe filters (Cat. #AFO-0420, Phenomenex, Torrance, CA). These filters must be solvent rinsed and dried prior to use by passing at least 2 mL acetonitrile followed by at least 2 mL of methanol through each filter, then drying. These operations can be performed on the SPE manifold (Section 6.2.1). Use the VisiDry™ attachment (Section 6.2.9) with clean, dry nitrogen at nominally 20 psi to push the solvents through the filters, and then dry the filters by passing nitrogen through them for about 30 minutes.

Note*: The polycarbonate stopcocks are not acetonitrile resistant, and therefore must not be in-line while solvent rinsing the syringe filters.*

6.4.6 Plastic transfer pipettes (Cat. #336 B/B-PET, Samco Scientific Corp., San Fernando, CA, or Cat. #14670-001, VWR, West Chester, PA)

6.4.7 Plastic Luer-lok™ syringes (Cat.# 309585, Becton Dickinson, Franklin Lakes, NJ, or Cat. #BD309585, VWR, West Chester, PA)

6.5 Analytical instrumentation

6.5.1 HPLC/UV system–An HPLC/UV system capable of gradient elutions, including: a thermostated autosampler for maintaining sample extracts at 20°C and configured to make 25-µL injections; a thermostated column oven for maintaining the HPLC column at 35°C; a UV detector capable of monitoring absorbance at a minimum of two wavelengths; and data system for acquiring all chromatographic data and (ideally) calculating analytical results.

6.5.2 HPLC column– 5-µm, 17.5%, 250 x 4.6-mm Phenomenex Luna phenyl-hexyl (Cat. #00G-4257-EO, Phenomenex, Torrance, CA)

6.5.3 Guard column–Phenomenex Security Guard™ system equipped with two 4-mm x 3-mm Phenomenex Phenyl (phenylpropyl) cartridges (kit including holder and 3.0-mm ID cartridges is Cat. #KJO-4282; refill cartridges are Cat. #AJO-4351, Phenomenex, Torrance, CA)

7.0 Reagents and Standards

7.1 Reagents

7.1.1 Reagent water–Organic-free, deionized, and submicron (0.2-µm) filtered water such as produced by a Sybron/Barnstead Model D2798 Nanopure-A water purification system and blank checked to verify the absence of any target analytes. Bottled HPLC grade water can also be used if blank checked.

7.1.2 85% phosphoric acid–ACS HPLC grade (EM Science, Cat. #PX0996-6)

7.1.3 25.5% phosphoric acid–Add reagent water (Section 7.1.1) to a 100-mL class A volumetric flask. Use a graduated cylinder to add 30.0 mL of 85% phosphoric acid, rinsing the cylinder three times with reagent water into the volumetric flask. Mix and allow to cool before adjusting the volume to exactly 100 mL with reagent water.

7.1.4 0.1275% phosphoric acid–Add reagent water (Section 7.1.1) to a 1-L class A volumetric flask. Add 5.00 mL of 25.5% phosphoric acid. Adjust the volume to exactly 1 L with reagent water.

7.1.5 2-M phosphate buffer solution–Weigh 13.6 grams of potassium phosphate monobasic and 17.4 grams of potassium phosphate dibasic (J.T. Baker ultrapure bioreagent, Cat. #4008-01 and #4012-01) into a 100-mL beaker. Transfer with reagent water (Section 7.1.1) rinses into a 100-mL class A volumetric flask. Adjust the volume to exactly 100 mL with reagent water and sonicate as necessary to completely dissolve the solids. Transfer to multiple smaller containers for storage and use (e.g., three 40-mL amber VOA vials). When not in use refrigerate and protect from light.

7.1.7 Methanol–HPLC-grade (Burdick & Jackson, Cat. #230-4)

7.1.8 Acetonitrile–HPLC-grade (Mallinckrodt Chrom AR®, Cat. #2856)

7.1.9 80:20 water:acetonitrile–Measure 1000 mL of reagent water (Section 7.1.1) and 250 mL of acetonitrile (Section 7.1.8). Pour both into a single large glass container (e.g., a 2- to 3-L beaker or Erlenmeyer flask) and mix thoroughly. Transfer to multiple smaller wide-mouth glass containers suitable for use as pipettor reservoirs and top with Teflon™-lined caps.

7.1.10 Chem-SOLV® glassware cleaner (Cat. # MK215704, VWR, West Chester, PA)

7.1.11 10% Chem-SOLV®–Mix 100 mL of Chem-SOLV® (Section 7.1.10) with 900 mL of tap water

7.1.12 Buffers for calibrating pH meter–pH 7 and pH 2 (e.g., Cat. #34175-242 and #34175-027, VWR, West Chester, PA). To prepare either buffer, open one capsule and completely dissolve the contents in 100 mL of reagent water (Section 7.1.1). Discard the capsule.

7.2 Primary standard solutions

7.2.1 Prepare primary standard solutions from standards with the highest purity available. Correct for purity <98% by adjusting the concentration of the initial primary standard according to the stated purity.

7.2.1.1 Imazapyr (Cat. #PS-2016, Chem Service)

7.2.1.2 Imazethapyr (Cat. #PS-2039, Chem Service)

7.2.1.3 Hexazinone (Cat. #PS-416, Chem Service)

7.2.1.4 Metsulfuron methyl (Cat. #PS-1078, Chem Service)

7.2.1.5 Sulfometuron methyl (Cat. #PS-1074, Chem Service)

7.2.2 Imazapyr, hexazinone, metsulfuron methyl–Accurately weigh and quantitatively transfer nominally 10 mg (recorded to 0.1 mg) of pure analytical standard to a 10-mL class A volumetric flask. Bring to volume with HPLC grade acetonitrile to make an individual primary stock standard solution with a concentration of nominally 1 mg/mL. Mix well and split into smaller amber containers (e.g., two 7-mL amber vials).

7.2.3 Imazethapyr–Accurately weigh and quantitatively transfer nominally 25 mg (recorded to 0.1 mg) of pure analytical standard to a 25-mL class A volumetric flask. Bring to volume in HPLC grade acetonitrile to make an individual primary stock standard solution with a concentration of nominally 1 mg/mL. Mix well and split into smaller amber containers (e.g., two 15-mL amber vials).

7.2.4 Sulfometuron methyl–Accurately weigh and quantitatively transfer nominally 10 mg (recorded to 0.1 mg) of pure analytical standard to a 50-mL class A volumetric flask. Bring to volume with HPLC grade acetonitrile to make an individual primary stock standard solution with a concentration of nominally 0.20 mg/mL. Mix well and split into smaller amber containers (e.g., four 15-mL amber vials).

***Note**: Sulfometuron methyl has reduced solubility in acetonitrile and tends to drop out of solution at higher concentrations, so this primary standard is prepared at a higher dilution.*

7.2.5 Store all standards in a freezer (-8 to -22°C) in the dark.

7.3 Combined standard solution

7.3.1 Prepare a combined standard solution containing hexazinone, imazapyr, metsulfuron methyl, and sulfometuron methyl by adding 500 µL each of hexazinone, imazapyr, and metsulfuron methyl primary stock solution (Section 7.2.2) and 2.5 mL of sulfometuron methyl primary stock solution (Section 7.2.4) to a 10-mL volumetric flask. Bring to volume with acetonitrile to obtain a combined calibration standard containing nominally 50 µg/mL of each chemical. Mix well, split into smaller amber containers (e.g., two 7-mL amber vials), and store at -8 to -22°C.

7.4 Working surrogate (metsulfuron methyl) spiking solution (optional)

7.4.1 Transfer 2.5 mL of 1-mg/mL metsulfuron methyl primary stock (Section 7.2.2) into a 100-mL class A volumetric flask. Bring to volume with HPLC-grade acetonitrile to obtain a nominal 25-µg/mL working surrogate spiking solution. Mix well, split into smaller amber containers (e.g., seven 15-mL amber vials), and store at -8 to -22°C.

***Note**: As written the method assumes that metsulfuron methyl will be used as a recovery surrogate. However, the use of a surrogate is not required.*

7.5 Working retention time standard (imazethapyr) spiking solution

7.5.1 Transfer 10 mL of 1-mg/mL imazethapyr (retention time standard) primary stock (Section 7.2.3) into a 100-mL class A volumetric flask. Bring to volume with HPLC-grade acetonitrile to obtain a nominal 0.10-mg/mL working imazethapyr (retention time standard) spiking solution. Mix well, split into smaller amber containers (e.g., seven 15-mL amber vials), and store at -8 to -22°C.

7.6 Working combined matrix spike solution (hexazinone, imazapyr, sulfometuron methyl)

7.6.1 Add 1.0 mL of 0.2-mg/mL sulfometuron methyl primary stock solution (Section 7.2.4) and 200 µL each of imazapyr and hexazinone primary stock solution (Section 7.2.2) to a single 10-mL volumetric flask. Bring to volume with acetonitrile to obtain a nominal 20-µg/mL combined matrix spike solution. Mix well, split into smaller amber containers (e.g., two 7-mL amber vials), and store at -8 to -22°C.

***Note**: If metsulfuron methyl is a targeted analyte it must be included in the working combined matrix spike solution at the same level as the other analytes (nominally 20 µg/mL).*

7.7 Working calibration standard solutions

7.7.1 Prepare the following calibration standards in 80:20 water:acetonitrile no earlier than a day before they will be analyzed.

7.7.1.1 10.0 µg/mL–Spike 200 µL of 50-µg/mL combined standard solution (Section 7.3.1) into reagent water in a 1-mL volumetric flask (do not add any additional acetonitrile). Bring to volume with reagent water to obtain nominally 10 µg/mL in 80:20 water:acetonitrile combined calibration solution (10 µg/mL corresponds to 50.0 ng/mL in the analysis of a 200-mL sample). Spike with 25 µL of working imazethapyr (retention time standard) spiking solution (Section 7.5.1). Mix well and transfer to an autosampler vial.

7.7.1.2 5.0 µg/mL–Spike 100 µL of acetonitrile and 100 µL of 50-µg/mL combined standard solution (Section 7.3.1) into reagent water in a 1-mL volumetric flask. Bring to volume with reagent water to obtain 5 µg/mL in 80:20 water:acetonitrile combined calibration solution (5 µg/mL corresponds to 25.0 ng/mL in the analysis of a 200-mL sample). Spike with 25 µL of working imazethapyr (retention time standard) spiking solution (Section 7.5.1). Mix well and transfer to an autosampler vial.

7.7.1.3 2.5 µg/mL–Spike 150 µL of acetonitrile and 50 µL of 50-µg/mL combined standard solution (Section 7.3.1) into reagent water in a 1-mL volumetric flask. Bring to volume with reagent water to obtain 2.5 µg/mL in 80:20 water:acetonitrile combined calibration solution (2.5 µg/mL corresponds to 12.5 ng/mL in the analysis of a 200-mL sample). Spike with 25 µL of working imazethapyr (retention time standard) spiking solution (Section 7.5.1). Mix well and transfer to an autosampler vial.

7.7.1.4 1.25 µg/mL–Spike 175 µL of acetonitrile and 25 µL of 50-µg/mL combined standard solution (Section 7.3.1) into reagent water in a 1-mL volumetric flask. Bring to volume with reagent water to obtain 1.25 µg/mL in 80:20 water:acetonitrile combined calibration solution (1.25 µg/mL corresponds to 6.25 ng/mL in the analysis of a 200-mL sample). Spike with 25 µL of working imazethapyr (retention time standard) spiking solution (Section 7.5.1). Mix well and transfer to an autosampler vial.

7.7.1.5 0.625 µg/mL–Spike 375 µL of acetonitrile and 25 µL of 50-µg/mL combined standard solution (Section 7.3.1) into reagent water in a 2-mL volumetric flask. Bring to volume with reagent water to obtain 0.625 µg/mL in 80:20 water:acetonitrile combined calibration solution

(0.625 µg/mL corresponds to 3.12 ng/mL in the analysis of a 200-mL sample). Mix well and transfer a 1.00-mL aliquot to an autosampler vial. Spike the aliquot with 25 µL of working imazethapyr (retention time standard) spiking solution (Section 7.5.1). Mix well.

7.7.1.6 0.125 µg/mL–Transfer 1975 µL of acetonitrile into reagent water in a 10-mL volumetric flask. Spike with 25 µL of 50-µg/mL combined standard solution (Section 7.3.1). Mix well and bring to volume with reagent water to obtain 0.125 µg/mL in 80:20 water:acetonitrile combined calibration solution (0.125 µg/mL corresponds to 0.625 ng/mL in the analysis of a 200-mL sample). Mix well and transfer a 1.00-mL aliquot to an autosampler vial. Spike the aliquot with 25 µL of the working imazethapyr (retention time standard) spiking solution (Section 7.5.1). Mix well.

7.7.1.7 Calibration blank–Spike 200 µL of acetonitrile into reagent water in a 1-mL volumetric flask. Bring to volume with reagent water to obtain an 80:20 water:acetonitrile calibration blank. Spike with 25 µL of working imazethapyr (retention time standard) spiking solution (Section 7.5.1). Mix well and transfer to an autosampler vial.

7.8 Storage stability

7.8.1 Standards prepared in 100% acetonitrile and stored at -8 to -22°C in the dark have been shown to be stable for an indefinite period (up to two years). In general, standards in 100% acetonitrile (Sections 7.2, 7.3, 7.4, 7.5, and 7.6) should be replaced when continuing calibration verification (CCV) analyses fail to meet recovery criteria (Section 9.3; Table 3, Section 17).

7.8.2 Standards prepared in 80:20 water:acetonitrile and stored at -8 to -22°C in the dark have been shown to be stable for at least 5 days. After 25 days of storage, approximate 10% losses of metsulfuron methyl and sulfometuron methyl manifested, but no losses of imazapyr or hexazinone were observed.

8.0 Sample Collection, Preservation, and Storage

8.1 Collection

8.1.1 Grab samples must be collected in clean HDPE bottles. These bottles should be filled to no greater than 75% capacity to avoid splitting upon freezing (Section 8.2.2).

8.1.2 Automatic sampling equipment can be used to collect individual samples in clean HDPE containers. However, each container must contain the appropriate amount of buffer (Section 8.2.1) to effect pH preservation at the time of collection.

8.2 Preservation

8.2.1 Samples must be preserved at pH 7 at the time of collection. This is achieved by adding sufficient 2-M phosphate buffer (Section 7.1.5) to give nominally 0.01 M of the buffer in the final sample volume; e.g., 5 mL per liter of sample.

8.2.2 Samples must be frozen as soon as possible after collection. To avoid splitting the bottles upon freezing, sample bottles should be filled to no greater than 75% capacity and should be frozen on their sides.

8.3 Storage

8.3.1 All samples must be kept frozen until thawed for analysis.

8.3.2 Once frozen, samples are stable for an indefinite period of time (on the order of years).

9.0 Laboratory Quality Control/Quality Assurance (QA/QC)

9.1 Formal QA/QC program

9.1.1 Each laboratory using this method is required to operate a formal QA/QC program. The minimum requirements for this program include an initial demonstration of laboratory capability (Section 9.2), ongoing analyses of standards (Section 9.3) and blanks (Sections 9.4 and 9.5) to verify continued performance, and ongoing analyses of sample matrix spikes (MS/MSD) to characterize precision and bias in real samples (Section 9.6). Under some conditions, MS/MSD analyses can be supplanted by analysis of ongoing precision and recovery (OPR) samples (Section 9.7).

9.1.2 Initially, laboratory performance is assessed vs. the established performance criteria given in Section 17. Subsequently, laboratories may develop in-house criteria (Section 9.8).

9.1.3 Depending on the herbicides targeted for analysis, one of the analyte herbicides can be used as a recovery surrogate to provide an additional measure of precision and bias in real samples (e.g., this method describes the use of metsulfuron methyl for this purpose).

9.2 Initial demonstration of laboratory/analyst capability

9.2.1 Initial calibration (ICAL)–Each laboratory/analyst must demonstrate an ability to generate acceptable calibration data (Section 10).

9.2.2 Laboratory-specific reagent water method detection limits (MDLs) and minimum levels (MLs)–After development of a valid calibration, the MDL for each analyte must be determined according to the procedure at 40 CFR 136, Appendix B (4).

Thus, seven replicate 200-g aliquots of reagent water are fortified with phosphate buffer (Section 11.2.2) and a low-level herbicide spike (e.g., 10 µL of a 1:4 dilution of the 20-µg/mL working combined matrix spike solution from Section 7.6), and are analyzed using all the same apparatus, reagents, and standards that will be used in the analysis of samples (Section 11). The resulting analyte-specific MLs (3.182 x MDL) must fall within the ranges given in Table 1, Section 17.

***Note**: Reagent water MDLs and MLs are used to demonstrate laboratory/analyst capability only. They do not validate the ability to achieve the same MDLs in real samples.*

9.3 Continuing calibration verification (CCV)

9.3.1 Prior to initiating analysis of samples, the validity of the initial calibration must be verified by analysis of a freshly prepared calibration standard (Section 7.7) at an appropriate concentration level. This analysis must be performed using the same HPLC elution conditions (Sections 10.1 and 10.2) used in developing the initial calibration and analyzing samples.

9.3.2 Confirm that all absolute and relative retention time criteria are met (Section 12.1). If the analysis fails any of these criteria repeat the analysis. If the second analysis also fails these criteria examine the HPLC system to determine the cause of the retention time drift. After taking appropriate corrective action reanalyze the CCV standard. If this test also fails the retention time criteria, the analytical system is out of control and samples must not be analyzed.

9.3.3 Quantify all analyte (including any surrogate) peaks vs. the initial calibration (Section 12.2) and calculate a percent recovery for each analyte using Equation 1.

Equation 1

% Recovery =100 x [*CCV ppb*/*ppb spiked*]
where: CCV ppb = the concentration found in the CCV analysis

9.3.4 Compare the analyte-specific recoveries to the criteria presented in Table 3, Section 17 (or alternate laboratory-specific criteria). If the CCV analysis fails any of the recovery criteria the analysis can be repeated using the same standard. If the second analysis also fails the recovery criteria, prepare a new standard at the same concentration level and repeat the test. If this test also fails the recovery criteria, the analytical system is out of control and samples must not be analyzed.

9.3.5 When all CCV criteria are met, analysis of samples can begin (Section 11.5.4). Each analytical set must include, at a minimum, re-analysis of the same CCV standard after analysis of all sample extracts (up to a maximum of 12 extracts).

9.3.6 After completion of an analytical set, confirm that all absolute and relative retention time criteria are met (Section 12.1) in each CCV analysis. Any sample analysis not bracketed by CCV analyses that meet these retention time criteria must be discarded and the instrumental analysis repeated.

9.3.7 After completion of an analytical set, calculate the percent recovery (Section 9.3.3) for all analytes in each CCV analysis and evaluate the percent recoveries against the recovery criteria given in Table 3, Section 17 (or alternate laboratory-specific criteria). Any sample analysis not bracketed by CCV analyses that meet these recovery criteria must be discarded and the instrumental analysis repeated.

9.3.8 All CCV results should be entered into a database for development of laboratory-specific acceptance criteria (Section 9.8.3).

9.4 Injection blank

9.4.1 To characterize the potential for background interference from the instrumental analysis, an injection blank (25 µL of 80:20 water:acetonitrile, Section 7.1.9) should be analyzed after each CCV.

9.4.2 If excessive background is observed within the approximate retention time window of any analyte, the associated sample results should be qualified and reanalysis of the extracts considered.

9.5 Method blanks (MB)

9.5.1 Every sample set must include at least one method blank generated according to the procedures given in Section 11.

9.5.2 Inject all method blanks immediately after the initial CCV analysis (Section 9.3) or injection blank analysis (Section 9.4).

9.5.3 All chromatographic peaks meeting analyte-specific relative retention time criteria (Section 12.1) must be quantified vs. the initial calibration (Section 12.2).

9.5.4 If a peak identified as an analyte gives a quantification exceeding the criterion given in Table 4, Section 17 (or an alternate laboratory-specific criterion) or is greater than one-fifth of the lowest concentration found in a sample extract, whichever is greater, any associated sample results must not be reported and analysis of samples must be halted until the source of the blank contamination is eliminated.

9.5.5 All MB results should be entered into a database for development of laboratory-specific acceptance criteria (Section 9.8.4).

9.6 Matrix spike and matrix spike duplicate (MS/MSD) analyses

9.6.1 Every sample set must include either an ongoing precision and recovery (OPR) analysis (Section 9.7) or a set of MS/MSD analyses prepared according to the procedures in Section 11. The MS/MSD experiment must be performed when a laboratory is analyzing samples with which it is not familiar. When a laboratory has sufficient (historic) MS/MSD data relevant to the samples in a specific analytical batch, a single OPR analysis can be performed in place of the MS/MSD experiment.

9.6.2 After quantifying MS and MSD results (Section 12.2), calculate the percent recovery of each analyte from the MS and MSD analyses using Equation 2.

Equation 2

$$\%\ Recovery = 100 \times [(MS\ or\ MSD\ ppb - unspiked\ sample\ ppb)/ppb\ spiked]$$

9.6.3 Using the MS and MSD percent recoveries, calculate an average recovery for each analyte. Compare the average recoveries to the criteria in Table 5, Section 17 (or alternate laboratory-specific criteria). If the mean recoveries meet the criteria, the bias in the associated sample results may be characterized using the overall mean from historic MS/MSD analyses (e.g., Table 5 data). If any mean recovery falls outside the criteria, the bias in the associated samples must be characterized using the result from the associated MS/MSD experiment only, and the sample results must be flagged to indicate failure of the MS/MSD recovery criteria.

9.6.4 Calculate the analyte-specific relative percent differences (RPD) between the MS and MSD analyses using Equation 3.

Equation 3

$$RPD = 200 \times [(|MS\ ppb - MSD\ ppb|)/(MS\ ppb + MSD\ ppb)]$$

9.6.5 Compare the MS/MSD RPD results to the criteria in Table 5, Section 17 (or alternate laboratory-specific criteria). If an experimental RPD meets the criteria given in Table 5, the precision in the analytical results may be characterized using the overall mean from historic MS/MSD analyses. If an experimental RPD falls outside the criteria given in Table 5, the precision in the associated sample results must be characterized using the RPD result from the associated MS/MSD experiment only, and the sample results must be flagged to indicate failure of the MS/MSD RPD criteria.

9.6.6 All MS/MSD results should be entered into a database for development of laboratory- or matrix-specific acceptance criteria (Section 9.8.5).

9.7 Ongoing precision and recovery (OPR)

9.7.1 Every sample set must include either an ongoing precision and recovery (OPR) analysis or a set of MS/MSD analyses (Section 9.6) prepared according to the procedures given in Section 11. The MS/MSD experiment must be performed when a laboratory is analyzing samples with which it is not familiar. When a laboratory has sufficient (historic) MS/MSD data relevant to the samples in a specific analytical batch, a single OPR analysis can be performed in place of the MS/MSD experiment.

9.7.2 After quantifying the OPR results (Section 12.2), calculate the percent recovery of each analyte using Equation 4.

Equation 4

% Recovery = 100 x [*OPR ppb*/*ppb spiked*]
where: OPR ppb = the concentration found in the OPR analysis

9.7.3 Compare the percent recoveries obtained from the OPR analysis to the criteria listed in Table 4, Section 17 (or alternate laboratory-specific criteria). If the OPR fails these criteria for any analyte, the associated sample results for that analyte should be considered suspect and flagged as potentially biased (according to the OPR result), and it may be appropriate to repeat the complete analysis.

9.7.4 All OPR percent recovery results should be entered into a database for developing laboratory-specific acceptance criteria (Section 9.8.6).

9.8 Laboratory-specific QA/QC criteria

9.8.1 Every laboratory utilizing this method must apply either the QA/QC criteria given in Section 17 or laboratory-specific QA/QC criteria to assess all analytical results.

9.8.2 To begin development of laboratory-specific QA/QC criteria, use the results from a good calibration (Section 10) to populate a database. The metrics that must be controlled on an ongoing basis are: (1) the absolute retention time of the retention time standard; (2) the relative retention time of each analyte; (3) the percent recovery for each analyte; and (4) the absorbance ratio for each analyte.

9.8.3 To develop laboratory-specific QA/QC criteria, results from every CCV analysis (Section 9.3) used to support sample results must be entered into a database initially populated with ICAL results (Section 9.8.2). The metrics that must be controlled on an ongoing basis are: (1) the absolute retention time of the retention time standard; (2) the relative retention time of each analyte; (3) the percent recovery for each analyte; and (4) the absorbance ratio for each analyte.

9.8.4 To develop laboratory-specific QA/QC criteria, results from every method blank used to support sample analyses must be entered into a database. For method

blanks the only metric that must be controlled on an ongoing basis is analyte concentration.

9.8.5 To develop laboratory-specific QA/QC criteria, results from every set of MS/MSD analyses must be entered into a database. For MS/MSD analyses, the metrics that must be monitored on an ongoing basis are: (1) spike recovery; and (2) relative percent difference.

9.8.6 To develop laboratory-specific QA/QC criteria, results from every OPR analysis used to support sample analyses must be entered into a database. For OPR analyses, the only metric that must be controlled on an ongoing basis is percent recovery.

9.8.7 Laboratory-specific acceptance criteria can be calculated as the mean ±3 standard deviations, or according to whatever convention is deemed acceptable by an individual laboratory (6,8). Means (and thus standard deviations) can be calculated using all the data in a database or as rolling averages using whatever window is deemed appropriate by the laboratory (6,8). However, each laboratory is responsible for clearly stating exactly how laboratory-specific criteria are derived.

10.0 Calibration and Standardization

10.1 HPLC/UV operating conditions

***Note**: Depending on the nature and severity of any sample-specific interferences encountered, the HPLC elution conditions may need to be modified to effect different separations. This method includes one set of conditions (Elution B) for determination of imazapyr, hexazinone, metsulfuron methyl, and sulfometuron methyl, and a second (Elution C) optimized for the determination of imazapyr only. Section 17 provides performance data generated in a single laboratory using both HPLC elutions. As illustrated by the results presented in Figures 1 and 2, Section 17, when imazapyr and hexazinone peaks meet the associated absorbance ratio criteria (Section 10.5), the resulting quantifications are not subject to bias resulting from chromatographic interference.*

10.1.1 HPLC elution conditions–Chromatographic separations are performed on a Phenomenex Luna phenyl-hexyl column (Section 6.5.2) held at 35°C using mixtures of 0.024 M phosphoric acid and methanol (always at a flow rate of 1 mL/min). Table 6, Section 17, gives the two elutions that have proven to be the most useful in the analysis of field samples to date (additional elution conditions were used in method development and for a limited number of sample analyses), and Section 17 gives performance data generated using each elution.

10.1.2 Extract temperature control–To maintain stability of the sample extracts, the autosampler should be set to hold extracts at 20°C at all times during analysis.

Ideally, the autosampler temperature would be dropped to 4°C after analysis of an analytical batch (to facilitate preservation of sample extracts).

10.1.3 UV detector–All chromatograms are acquired by monitoring UV absorption at 235 nm and 195 nm (at a minimum).

10.1.4 Injection volume–The standard injection volume for all calibration standards and samples is 25 µL.

10.2 Initial multipoint calibration (ICAL)

***Note**: Although the response factor (Rf) data presented in Section 17 show that Rfs developed using the two different elution conditions given in this method are equivalent, separate ICAL injections using each set of conditions should be performed and separate calibrations should be generated (HPLC data systems may, in fact, require this).*

10.2.1 Inject 25 µL of each of the calibration standards (Section 7.7) under the conditions specified in Section 10.1 and obtain the peak areas and retention times for each analyte, surrogate, and the retention time standard in each calibration standard.

10.3 Initial multipoint calibration retention times

10.3.1 Using the results from all the calibration injections, calculate the mean (Rt_{ave}), standard deviation (SD), and relative standard deviation (RSD = 100 x SD/Rt_{ave}) of the retention time (minutes) of the retention time standard (imazethapyr). The RSD should be <0.5%, and must be <1% to proceed with developing the calibration. If the RSD is >1% the instrument system is out of control and the calibration data cannot be used. The calibration analyses must be repeated after any instrument maintenance.

10.3.2 For each calibration injection, compare the retention time of the retention time standard (imazethapyr) to the criteria listed in Table 2, Section 17 (or alternate laboratory-specific criteria). If any injection fails these criteria, trouble shoot the instrumental system to determine the cause of the retention time shift and repeat the calibration analyses after performing instrument maintenance. Alternatively, the laboratory can use the original calibration injections to establish new retention time criteria (Section 9.8.2; this will require developing new RRt criteria for all analytes as well).

10.3.3 Calculate the relative retention time for each analyte or surrogate in each calibration analysis using Equation 5.

Equation 5

$RRt_a = (Rt_a/Rt_{rt}) \times 1000$

where: RRt_a = *the injection-analyte-specific relative retention time (unitless)*
Rt_a = *the injection-specific retention time (minutes) of the analyte (or surrogate) peak*
Rt_{rt} = *the injection-specific retention time (minutes) of the retention time standard*

10.3.4 Compare the analyte- and injection-specific RRts to the criteria given in Table 2, Section 17 (or alternate laboratory-specific criteria). If any injection fails these criteria, trouble shoot the instrument system to determine the cause of the relative retention time shift and repeat the calibration analyses after performing instrument maintenance.

10.4 Initial multipoint calibration response factors

10.4.1 Calculate the response factor for each analyte or surrogate in each calibration standard using Equation 6.

Equation 6

$Rf = A/C$

where: Rf = *the injection- and chemical-specific response factor*
A = *peak area (235 nm) obtained for each analyte (or surrogate)*
C = *exact concentration of analyte or surrogate in standard (µg/mL)*

10.4.2 Calculate the average response factor for each analyte or surrogate over the calibration range using Equation 7.

Equation 7

$$Rf_{ave} = \left(\sum_{i=1}^{n} Rf_i\right) \div n$$

where: Rf_{ave} = *the chemical-specific average response factor*
Rf_i = *the chemical-specific response factor from calibration point i*
n = *number of calibration standards (calibration points)*

10.4.3 Calculate the standard deviation (SD) and relative standard deviation (RSD = 100 x SD/Rf_{ave}) of the average response factor for each analyte or surrogate and compare both the average Rf and the Rf RSD to the criteria given in Table 7, Section 17 (or alternate laboratory-specific criteria). If either criterion is failed, the calibration for the specific analyte is invalid and a new calibration must be developed.

10.4.4 If the average response factor result meets the criteria, use the average response factor to calculate the concentration found in the lowest level calibration standard,

then calculate the percent recovery. Compare this percent recovery to the criteria given in Table 7, Section 17 (or alternate laboratory-specific criteria). If the analyte-specific criterion is met, the calibration is acceptable and the average response factor can be used to quantify sample results. If the percent recovery fails the criterion, the analyte-specific calibration is invalid and a new calibration must be prepared.

10.5 Absorbance ratios

***Note**: The exact absorbance ratio for each analyte may vary with standard purity (i.e., with the level and nature of any contaminants in the original standard), instrumentation, and/or HPLC elution conditions. Table 8, Section 17, summarizes 235 nm/195 nm results obtained from analysis of ICALs and CCVs in a single laboratory using the two elution conditions given in this method and standards prepared over time using three different lots of Chem Service solids (Section 7.2.1).*

10.5.1 Absorbance ratios must only be calculated if the ICAL meets all retention time and response factor criteria (Sections 10.3 and 10.4; Tables 7 and 8, Section 17).

10.5.2 Calculate the 235-nm/195-nm absorbance ratio for each herbicide peak in all the calibration standards except the 0.125-µg/mL standard (Section 7.7), which often gives a degraded ratio.

10.6 Laboratory-specific QA/QC criteria

10.6.1 Results from a calibration meeting all acceptance criteria can be added to an existing database for ongoing calculation of laboratory-specific QA/QC criteria (Section 9.8). Alternately, these same results can be used to populate a new database (Section 9.8.2).

11.0 Procedure

11.1 Thawing frozen samples

11.1.1 Samples can be thawed at room temperature or in a refrigerator over an extended period (nominally 48 hours for 600-mL samples in a 4°C refrigerator).

11.1.2 Samples should be extracted as soon as they are fully thawed and brought to room temperature.

11.2 Sample fortification and filtration

***Note**: NCASI uses the SPE vacuum manifold (Section 6.2.1) with a modified cover to perform all filtrations.*

11.2.1 Assemble a clean filtration funnel and pre-rinse a 0.45-µm nylon membrane filter and the inside of the filter holder using a minimum of 15 mL of water. Discard the rinsate.

***Note**: Nylon membrane filters may be precleaned and stored dry. If this is done a solvent rinse can also be used; e.g., filters can be rinsed three times with reagent water followed by three rinses with methanol. After the methanol rinses, the filter must be dried fully prior to storage.*

11.2.2 For MBs (Section 9.5) and OPRs (Section 9.7) weigh 200 ±5 g of laboratory reagent water into a 250-mL plastic beaker and add 1 mL of 2-M phosphate buffer (Section 7.1.5). Record the exact mass of sample to ±0.01 g or perform all quantifications assuming a uniform 200-g sample size.

11.2.3 For samples, shake well to mix solids and weigh 200 ±5 g into a 250-mL plastic beaker. Record the exact mass of sample to ±0.01 g.

11.2.4 After allowing the solution to reach room temperature, fortify each sample (Sections 11.2.2 and 11.2.3) with 25 µL of surrogate spiking solution (Section 7.4). Swirl to mix.

11.2.5 After allowing the solution to reach room temperature, spike the specific samples identified as matrix spike, matrix spike duplicate, and/or OPR aliquots with the appropriate combined matrix spike solution (Section 7.6). For OPRs or samples assumed to have herbicide concentrations <1 ppb, the standard spike is 20 µL of the nominal 20-µg/mL combined matrix spike solution (Section 7.6), which results in fortification at nominally 2 ng/mL (ppb) in the sample (or OPR).

11.2.6 Filter the sample using prerinsed filtration equipment (Section 11.2.1) and collect the filtrate in a 250-mL plastic beaker. If the filter clogs it may be necessary to use multiple filters for the same sample, and each filter must be rinsed (Section 11.2.8) prior to replacing it.

11.2.7 After all the sample has been added to the filtration apparatus, rinse the original beaker with three nominal 2-mL volumes of reagent water (Section 7.1.1) and filter each rinse. Collect all rinses with the sample filtrate.

11.2.8 After all the sample has been filtered, rinse the solids on each membrane filter and the inside surfaces of the filtration apparatus using three nominal 5-mL volumes of reagent water (Section 7.1.1). Collect all rinses with the sample filtrate.

11.2.9 If multiple filters have been used for any of the samples in a set, the method blank should be passed through the maximum number of filters used for an individual sample within the set (thus the MB should be filtered last).

11.3 Sample extraction

Note: *Both the SPE and SAX cartridges should be tested prior to use. To test the SPE cartridges, fortify 200 mL of reagent water with nominally 5 µg of the herbicides and extract according to Sections 11.3.1 through 11.3.6. Then elute the dried cartridge with 50 mL of methanol, collecting all the methanol in a pear-shaped 100-mL evaporation flask. Complete the preparation according to Sections 11.4.4 through 11.4.10. To test the SAX cartridges, spike 50 mL of methanol with 5 µg of the herbicides, pass it through a conditioned SAX cartridge (Section 11.4.1), and obtain a final extract according to Sections 11.4.4 through 11.4.10. Analyze the resulting extracts and determine percent recoveries of the spikes. Recoveries should be >90%. Blank extracts should be generated in parallel with the spiked extracts, and the chromatograms from analyses of these blank extracts should be examined for unacceptable background.*

11.3.1 Set the vacuum on the SPE vacuum manifold to produce a flow rate sufficient to pull 200 mL of sample through the SPE cartridge in approximately 5 minutes. Use the same vacuum settings to condition, load, and elute all cartridges.

Note: *The exact flow rate at which sample is pulled through the SPE and/or SAX cartridges has not proven critical over the range from approximately 10 to 60 mL/min.*

11.3.2 Condition a 6-mL/0.5-g ENVI™-Chrom P SPE cartridge by passing 3 x 6 mL of methanol followed by 3 x 6 mL of 0.1275% phosphoric acid (Section 7.1.4) through it. Do not allow the cartridge to become dry after conditioning (stop elution when conditioning solvent reaches ~1 cm above the frit). Discard eluate. Attach a 60-mL reservoir to the SPE cartridge using an adapter cap.

Note*: The sulfonylurea herbicides (especially metsulfuron methyl) are susceptible to hydrolysis under acidic conditions. Thus, the time between acidification of the sample and completion of the initial (SPE) extraction step must be kept to an absolute minimum.*

11.3.3 Working one (fortified and filtered) sample at a time, add 1.0 mL of 25.5% phosphoric acid (Section 7.1.3) to a sample while monitoring sample pH using a pH probe. Add additional acid as necessary to achieve a sample pH ≤2.3, and then ***immediately*** pass the entire sample through the SPE cartridge. Rinse the sample container (plastic beaker) 2 to 3 times with small portions of 0.1275% phosphoric acid (Section 7.1.4; do not exceed 10-mL total additional volume) and add the rinses to the 60-mL reservoir.

11.3.4 Continue to apply vacuum until elution stops.

11.3.5 Remove the reservoir and adapter cap and rinse the SPE cartridge with two 5-mL rinses of reagent water (Section 7.1.1), eluting each rinse to dryness.

11.3.6 Dry the SPE cartridge by allowing it to remain under vacuum. If not already at maximum, the vacuum should be increased (within safety limits). Continue to apply vacuum until the solids in the cartridge flow freely (1 to 1.5 hours, depending upon conditions). Discard all eluate.

11.4 Sample elution/cleanup, final concentration, and fortification with retention time standard

11.4.1 While the ENVI™-Chrom P extraction cartridge is drying (Section 11.3.6), condition a 6-mL/1-g SAX cleanup cartridge with 3 x 6 mL of methanol. Discard all washes, allowing the third methanol wash to elute just halfway through the column reservoir and then stopping.

11.4.2 Use a clean, dry adapter cap to connect the dried ENVI™-Chrom P SPE cartridge from Section 11.3.6 to the top of the conditioned SAX cartridge. Attach a clean, dry 60-mL reservoir to the ENVI™-Chrom P cartridge, using a clean, dry adapter cap.

11.4.3 Elute the SPE extraction cartridge through the SAX cartridge using 50 mL of methanol and collect the eluate in a pear-shaped 100-mL evaporation flask.

11.4.4 Add 5 mL of acetonitrile to the flask.

***Note**: At this point samples can be stored at 4°C in the dark for up to four days prior to completing sample preparation.*

11.4.5 Concentrate the eluate to between 0.2 and 0.5 mL using a rotary evaporator over a 35°C water bath.

11.4.6 Remove the flask from the evaporator, add ~5 mL of acetonitrile, return to the evaporator, and concentrate to between 0.2 and 0.5 mL.

***Note**: If there is any water remaining in the extract at this point repeat step 11.4.6. The extract must be free of water prior to proceeding to 11.4.7.*

11.4.7 Manually blow the extract to dryness in the flask using a gentle stream of nitrogen. Immediately dissolve the residue in exactly 1.0 mL of 80:20 water:acetonitrile (Section 7.1.9). Swirl and rotate the flask for 3 minutes to ensure all residue is dissolved.

11.4.8 Spike the final extract with 25 µL of the retention time standard spiking solution (Section 7.5).

11.4.9 With a disposable plastic pipette, transfer the extract to a Hamilton Teflon™ or a disposable plastic Luer-lok™ syringe (Section 6.4.7) fitted with a solvent rinsed and dried syringe filter (Section 6.4.5).

11.4.10 Slowly push the extract through the membrane filter into an HPLC autosampler vial and cap the vial.

11.4.11 Store sample extracts at -8 to -22°C in the dark at all times when not undergoing HPLC analysis.

11.4.12 Extracts should be analyzed as soon as possible. Although no formal stability study has been performed with sample extracts, NCASI has observed no loss of imazapyr or hexazinone to 20 days of storage in any sample extract, but approximate 5% losses of sulfometuron methyl and 10% losses of metsulfuron methyl in some sample extracts after initial analysis and 10 days of storage.

11.5 Sample analysis

***Note**: Depending on the levels of chromatographic interference encountered when using HPLC Elution B (Table 6, Section 17), it may be necessary to determine imazapyr using HPLC Elution C (Table 6, Section 17).*

11.5.1 The HPLC/UV conditions must be the same as those used in generating the calibration (Section 10.1).

11.5.2 Perform a CCV analysis and evaluate the results (Section 9.3).

11.5.3 (Optional) Following the CCV analysis inject an injection blank (Section 9.4). If this blank shows evidence of carryover, samples should not be analyzed until the reason for the carryover has been determined and corrected.

11.5.4 Begin analysis of each sample set by injecting all method blanks followed by all sample extracts. At a minimum, the CCV analysis (Section 9.3) must be repeated after analysis of all samples.

11.5.5 Following injection of the last sample of an analytical batch, the chromatographic system should be flushed and the autosampler temperature reduced to 4°C.

12.0 Data Analysis and Calculations

12.1 Identification of compounds

12.1.1 The absolute retention time of the retention time standard must fall within the window given in Table 2, Section 17 (or alternate laboratory-specific criteria). If this criterion is not met sample results must not be reported.

12.1.2 Analytes are identified in sample extracts by relative retention time calculated (Section 10.3.3) vs. the retention time standard according to the criteria given in Table 2, Section 17 (or alternate laboratory-specific criteria).

12.1.3 Any chromatographic peak meeting the relative retention time criterion for a specific herbicide must be reported as the analyte.

12.2 Quantification of compounds and reporting results

12.2.1 Chromatographic peaks satisfying the retention time criteria (Section 12.1) are quantified using the appropriate average response factor (Equation 8).

Equation 8

$C_s = (A/Rf_{ave})1000/V_s$

where: C_s = *analyte sample concentration in ng/mL (ppb)*
A = *peak area (235 nm) obtained for specific analyte*
Rf_{ave} = *analyte-specific average response factor (Section 10.4)*
V_s = *sample-specific analytical volume (mL) or mass (g)*

12.2.2 If the calculated concentration of any analyte is greater than the highest calibration point, the extract must be diluted and reanalyzed, a smaller aliquot of the sample extracted (dilute the sample prior to extraction) and analyzed, or a higher-level calibration standard prepared and calibration linearity at the appropriate concentration demonstrated.

12.2.3 If the calculated concentration is less than 1 ppb or the laboratory-specific and/or matrix-specific ML (Section 9.2.2; Table 1, Section 17), the result should be reported as <1 ppb or <ML.

12.2.4 The absorbance ratio (235 nm/195 nm) of any chromatographic peak with a reportable quantification (Sections 12.2.2. and 12.2.3) must be evaluated against the absorbance ratio criteria given in Table 8, Section 17 (or alternate laboratory-specific criteria). If the analysis-specific ratio meets the appropriate criteria, the associated quantification is reported without qualification.

12.2.5 If the analysis-specific absorbance ratio fails the appropriate criteria, the sample result must be qualified (failure of the absorbance ratio criteria indicates bias in the quantification and/or a false positive). Under these conditions, an alternate HPLC elution could be used in an attempt to obtain a "cleaner" peak. If this fails, consideration should be given to submitting the sample for analysis by LC/MS-MS (this will require submission of the original sample for re-extraction if LC/MS-MS instrumentation is not immediately available).

13.0 Method Performance

13.1 Single laboratory performance metrics for this method are presented in Tables 1, 2, 3, 4, 5, 7, and 8, Section 17.

13.2 Figures 1 and 2, Section 17, show plots comparing imazapyr (Figure 1) and hexazinone (Figure 2) results from HPLC/UV and LC/MS-MS analyses of split samples performed

by two independent laboratories. The slopes of both plots are not significantly different than 1 (95% confidence level) and the intercepts are not significantly different than 0 (95% confidence level), indicating that there was no systematic bias in the HPLC/UV results relative to the LC/MS-MS results.

14.0 Pollution Prevention

14.1 The laboratory should check state and local requirements to determine if pollution prevention equipment is required or recommended in its area.

15.0 Waste Management

15.1 It is the responsibility of the laboratory to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and lands by minimizing releases into the environment. Compliance with all sewage discharge permits and regulations is also required.

16.0 References

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17.0 Tables and Figures

Table 1. Single Laboratory Method Detection Limits (MDLs) and Minimum Levels (MLs) in Reagent Water and Stream Water[a]

Compound	CAS Registry Number	HPLC Elution	Reagent Water		Stream Water[b]	
			MDL (ppb)[c]	ML (ppb)[c]	MDL (ppb)[c]	ML (ppb)[c]
Imazapyr	081334-34-1	B	0.12, 0.12	0.40, 0.38	NA[d]	NA[d]
Imazapyr		C	0.05, 0.02	0.16, 0.05	0.21, 0.30	0.66, 0.97
Hexazinone	051235-04-2	B	0.03, 0.02	0.08, 0.05	0.19, 0.30	0.62, 0.96
Sulfometuron Methyl	074223-56-6	B	0.02, 0.02	0.06, 0.06	0.14, 0.17	0.45, 0.55
Metsulfuron Methyl	074223-64-6	B	0.20, 0.12	0.63, 0.38	0.13, 0.18	0.42, 0.56

[a] MDL experiments performed at 0.25 ppb in reagent water and 1 ppb in stream water according to Section 9.2.2

[b] storm water runoff from forestry land following harvest and replanting

[c] results from two separate MDL experiments

[d] because of chromatographic interference, elution B not applicable for the determination of imazapyr in specific samples tested

Table 2. Single Laboratory Retention Time Statistics for HPLC Elutions B and C[a]

	RRT[b]							
	Elution B				Elution C			
	Mean	SD	n[c]	Acceptance Window[d]	Mean	SD	n[c]	Acceptance Window[d]
Imazapyr	651.0	2.7	284	642.8 -- 659.1	469.3	2.0	290	463.4 -- 475.3
Hexazinone	1497	2.3	284	1490 -- 1504	NA[e]			
Sulfometuron methyl	1629	5.1	284	1614 -- 1644	NA[e]			
Metsulfuron methyl	1729	5.6	284	1712 -- 1746	NA[e]			
	RT (minutes)							
	Elution B				Elution C			
	Mean	SD	n[c]	Acceptance Window[d]	Mean	SD	n[c]	Acceptance Window[d]
Imazethapyr	6.653	0.034	284	6.552 -- 6.754	20.5815	0.076	290	20.354 -- 20.809

[a] statistics calculated from pooled initial calibration and CCV analyses

[b] RRT is relative retention time calculated as [(RT analyte/RT imazethapyr) x 1000]

[c] number of discrete injections included in the pooled initial calibration and CCV dataset

[d] calculated as [mean ± (3 x SD)]

[e] NA is not applicable (elution C for determination of imazapyr only)

Table 3. Single Laboratory Continuing Calibration Verification (CCV) Statistics for HPLC Elutions B and C

Compound	HPLC Elution	Percent Recovery			
		Mean	SD	Acceptance Window[a]	n
Imazapyr	C[b]	100.21	2.00	94.22 -- 106.20	278
	B[c]	100.26	2.59	92.49 -- 108.02	266
Hexazinone	B[c]	99.60	2.01	93.56 -- 105.64	266
Sulfometuron methyl	B[c]	99.76	2.01	93.74 -- 105.78	266
Metsulfuron methyl	B[c]	99.49	2.17	92.99 -- 105.99	266

[a] calculated as [mean ± (3 x SD)]

[b] all CCV analyses at 1.25 µg/mL in the standard (equivalent to 6.25 ppb in samples)

[c] CCV analyses performed at 0.625 µg/mL and 1.25 µg/mL in the standard (equivalent to 3.125 ppb and 6.25 ppb in samples)

Table 4. Single Laboratory Precision and Bias in Reagent Water

OPR Results[a]	Percent Recovery			
	Mean	SD	n	acceptance window[d]
Imazapyr[b]	92.9	5.7	68	75.8 -- 110.0
Hexazinone[c]	104.6	6.1	71	86.2 -- 123.0
Sulfometuron methyl[c]	103.3	8.0	71	79.4 -- 127.2
Metsulfuron methyl (surrogate)[c]	99.7	5.3	177	83.8 -- 115.7

Method Blank Results	Sample Concentration (ppb)[e]			
	Mean	SD	n	control limit[f]
Imazapyr[b]	0.002	0.015	68	0.047
Hexazinone[c]	0.039	0.057	74	0.210
Sulfometuron methyl[c]	0.045	0.048	74	0.189
Metsulfuron methyl (surrogate)[c]	NA[g]	-	-	-

[a] imazapyr, hexazinone, and sulfometuron methyl spiked at nominally 2ppb; metsulfuron methyl spiked at nominally 3ppb; all recoveries calculated without blank subtraction

[b] results from elution C only

[c] results from elution B only

[d] calculated as [mean ± (3 x SD)]

[e] uncensored results with true non-detects (no chromatographic peak) included as 0

[f] calculated as [mean + (3 x SD)]

[g] not applicable as all method blanks were spiked with metsulfuron methyl as a recovery surrogate

Table 5. Single Laboratory Method Performance in Sample Matrices (Single Laboratory MS/MSD and Surrogate Recoveries)

	MS/MSD % Recovery			MS/MSD Precision			
	Mean (%)	SD (%)	acceptance window[a]	Mean RPD[b] (%)	SD (%)	control limit[c]	n
Imazapyr[d]	95.0 [e]	4.8	80.6 -- 109.4	2.3	1.8	7.7	39[f]
Hexazinone[g]	90.4 [e]	9.2	62.8 -- 118.0	2.2	2.9	10.9	45[f]
Sulfometuron methyl[g]	88.8 [e]	4.8	74.4 --103.2	2.7	2.4	9.9	45[f]
Metsulfuron methyl[h]	96.9 [g]	11.4	62.7 -- 131.1				480[i]
Metsulfuron methyl[h]				2.9	2.5	10.4	45[f]

[a] calculated as [mean ± (3 x SD)]

[b] relative percent difference (RPD) between matrix spike (MS) and matrix spike duplicate (MSD) results (Section 9.6.4)

[c] calculated as [mean + (3 x SD)]

[d] results from elution C only; nominal spike levels from 2 to 50 ppb

[e] mean of average percent recoveries from n MS/MSD pairs

[f] number of matched MS/MSD pairs (equal to number of different samples)

[g] results from elution B only; nominal spike levels from 2 to 35 ppb

[h] results from elution B only; nominal 3 ppb (surrogate) spike level in all samples; approximately 6% of original results showed evidence of co-eluting background (per peak area ratio criteria, Table 8); those 6% (n=33) were deleted prior to precision and bias calculations

[i] mean from 480 independent surrogate spike experiments in 435 different samples (includes 45 MS/MSD pairs)

Table 6. HPLC Elution Conditions for NCASI Method HIMS-W105.01

Column:	Phenomenex Luna® phenyl-hexyl 5 µm, 17.5%, 25.0 x 4.6 mm			
Guard Cartridge:	Phenomenex Security Guard® System 2 Phenomenex Phenyl(phenylpropy) cartridges (4 mm L x 3 mm ID)			
Column Temperature:	35 ± 1°C			
Injection Volume:	25 µL			
Autosampler Temperature:	20 ±1°C[a]			
Detector:	UV/VIS, 235 nm and 195 nm			
Flow Rate:	1 mL/min			
Gradient Transitions:	Linear			
Mobile Phase Gradient	Time (min)	0.024 M H_3PO_4 (%)	Methanol (%)	Comment
Elution B	0.0	41.4	58.6	Data Acquisition
	15.0	41.4	58.6	
	17.0	6.1	93.9	Flush
	27.0	6.1	93.9	
	29.0	41.4	58.6	Re-equilibration
	34.0	41.4	58.6	
Elution C	0.0	65.0	35.0	Data Acquisition
	9.5	65.0	35.0	
	20.5	40.0	60.0	
	22.5	6.1	93.9	Flush
	32.5	6.1	93.9	
	34.5	65.0	35.0	Re-equilibration
	39.5	65.0	35.0	

[a] if extracts will sit in the autosampler for any extended period after completion of all analyses, reducing autosampler temperature to 4 ±1ºC after the last injection is recommended

Table 7. Single Laboratory Initial Calibration (ICAL) Statistics[a]

	Average Response Factor (Rf)			
	Range[b]	Mean[c]	SD[c]	Acceptance Window[d]
Imazapyr	49955 -- 54210	52574	1682	47529 -- 57619
Hexazinone	55985 -- 63137	60448	2677	52416 -- 68480
Sulfometuron methyl	92074 -- 96572	94286	1619	89429 -- 99144
Metsulfuron methyl	75581 -- 84272	78184	3553	67525 -- 88842
	Average Relative Standard Deviation (RSD)			
	Range (%)[e]	Mean (%)[f]	SD (%)[f]	Control Limit (%)[g]
Imazapyr	0.987 -- 4.030	2.0	1.25	5.7
Hexazinone	0.951 -- 1.999	1.3	0.42	2.5
Sulfometuron methyl	0.843 -- 1.680	1.3	0.36	2.4
Metsulfuron methyl	0.808 -- 2.213	1.3	0.57	3.1
	Percent Recovery at the LCL[h]			
	Range (%)	Mean (%)	SD (%)	Acceptance Window (%)[d]
Imazapyr	98.3 -- 104.6	101.4	2.29	94.6 -- 108.3
Hexazinone	98.5 -- 103.8	100.5	2.37	93.3 -- 107.6
Sulfometuron methyl	99.1 -- 102.8	101.1	1.36	97.0 -- 105.2
Metsulfuron methyl	98.1 -- 104.0	100.6	2.18	94.0 -- 107.1

[a] ICAL statistics calculated using pooled results from five multipoint calibrations developed using three unique HPLC elutions (including HPLC elutions B and C) for imazapyr and two unique HPLC elutions (including elution B) for hexazinone, sulfometuron methyl, and metsulfuron methyl

[b] range of n=5 ICAL-specific average response factors (i.e., range of intra-ICAL average Rfs)

[c] mean and SD of n=5 ICAL-specific average response factors (i.e., inter-ICAL mean and SD of n=5 intra-ICAL average response factors)

[d] calculated as [mean ± (3 x SD)]

[e] range of n=5 ICAL-specific average response factor RSDs

[f] mean and SD of n=5 ICAL-specific average Rf RSDs (i.e., inter-ICAL mean and SD of n=5 intra-ICAL average RF RSDs)

[g] calculated as [mean + (3 x SD)]

[h] percent recovery of the lowest calibration level (LCL) standard

Table 8. Single Laboratory Absorbance Ratio ("Peak Purity") Statistics for HPLC Elutions B and C

		Absorbance Ratio[a]			
	HPLC Elution	Mean	SD	Acceptance Window[b]	n[c]
Imazapyr	C[d]	0.4028	0.0108	0.3703 -- 0.4352	288
	B[e]	0.4101	0.0128	0.3716 -- 0.4486	281
Hexazinone	B[e]	0.8085	0.0397	0.6895 -- 0.9275	281
Sulfometuron methyl	B[e]	0.6371	0.0349	0.5324 -- 0.7418	200
Metsulfuron methyl	B[e]	0.4280	0.0293	0.3400 -- 0.5159	200

[a] peak area ratios calculated as (235nm/195nm); statistics from pooled initial calibration and CCV analyses

[b] calculated as [mean ± (3 x SD)]

[c] ratios for sulfometuron methyl and metsulfuron methyl not initially monitored (so reduced n)

[d] spike level nominally 6 ppb for CCV analyses and 3 to 50 ppb for ICAL analyses

[e] spike level nominally 3 or 6 ppb for CCV analyses and 3 to 50 ppb for ICAL analyses

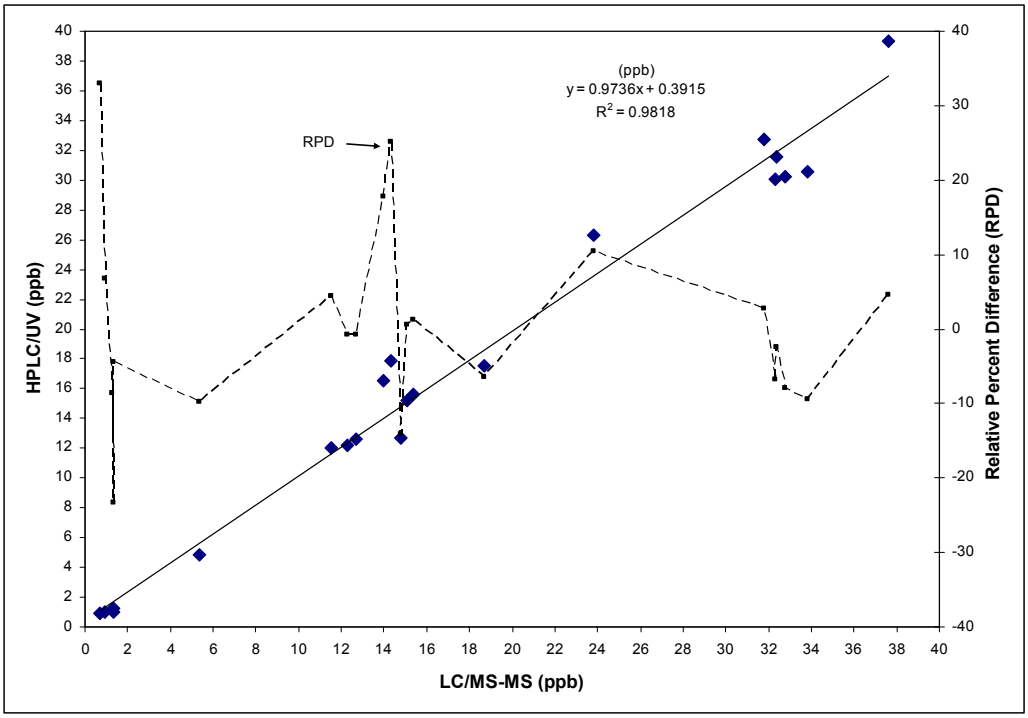

Figure 1. Comparison of Imazapyr Quantifications from Analysis of Split Samples Performed by Two Independent Laboratories; one using HPLC/UV and one using LC/MS-MS

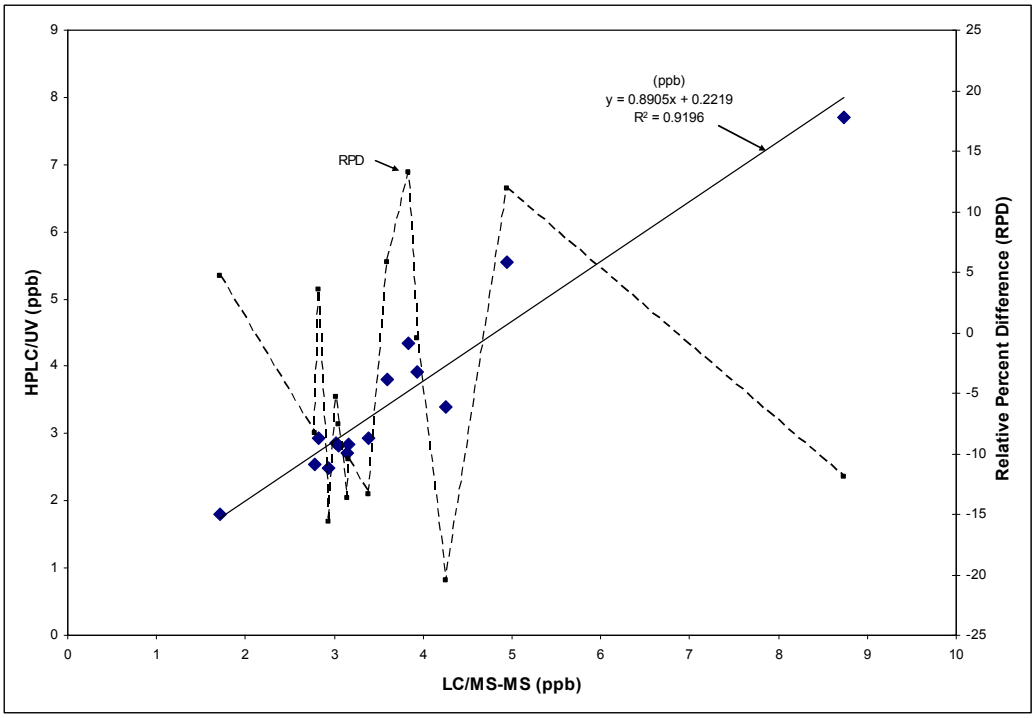

Figure 2. Comparison of Hexazinone Quantifications from Analysis of Split Samples Performed by Two Independent Laboratories; one using HPLC/UV and one using LC/MS-MS