ABSTRACT

This method validation study was conducted to demonstrate the applicability and repeatability of BASF Analytical Method No. D1505/02 for the determination of residues of afidopyropen (BAS 440 I) and its metabolites M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 in water by LC-MS/MS.

Principle of the method. Residues of afidopyropen in water samples are diluted with acidified acetonitrile, mixed, filtered and then analyzed by direct injection onto a high performance liquid chromatography (HPLC) column with detection by positive ion electrospray ionization tandem mass spectrometry (ESI-MS/MS), for quantitation purposes, the following ion transitions are monitored: m/z 594→148 for parent afidopyropen; m/z 458→148 for M440I001; m/z 526→148 for M440I002; m/z 526→148 for M440I003; m/z 524→148 for M440I005; m/z 542→218 for M440I016; m/z 610→218 for M440I024; and m/z 524→148 for M440I057. Typically for confirmatory purposes, the following alternate ion transitions are monitored, again in the positive ionization mode: m/z 594→202 for parent afidopyropen; m/z 458→106 for M440I001; m/z 526→202 for M440I002; m/z 526→202 for M440I003; m/z 524→80 for M440I005; m/z 542→164 for M440I016; m/z 610→122 for M440I024; and m/z 524→202 for M440I057. The results are calculated by direct comparison of the sample peak responses to those of external standards.

Test conditions. For validation, untreated drinking (well) water and surface (lake) water samples were fortified with each analyte and analyzed according to the established method validation guidelines. The analytical sets for each each water type consisted of a reagent blank, two controls, five replicates fortified with analyte at the method limit of quantitation, 30 ng/L (30 ppt), and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 300 ng/L (300 ppt). For each analyte, the two mass transitions described above were evaluated. In conjunction with the subject study, matrix- and solvent-matched standards were analyzed in a separate experiment to evaluate any potential matrix effects.

Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ was defined by the lowest fortification level successfully tested. The validated LOQ for residues of afidopyropen in water is 30 ng/L (30 ppt), for each analyte. The LOD is set at 6 ng/L (6 ppt) which is 20% of the LOQ during method validation. The LOD is defined as the absolute amount (0.54 pg) of analyte injected into the LC-MS/MS using the lowest standard of the calibration curve.

Selectivity. The method determines residues of afidopyropen in water by LC-MS/MS. No interfering peaks were found at the retention times for these analytes. The multiple reaction monitoring (MRM) transitions used to identify each analyte were determined by product ion spectra. The experiment to evaluate any potential matrix effects showed that the matrix load in the samples from each water type had no significant influence on analysis (matrix effects <20%); therefore, the validation samples were analyzed only using solvent-based calibration standard solutions.

Linearity. Acceptable linearity was observed for the standard range and the two mass transitions tested for each analyte: The method-detector response, for the method validation sets, was linear over the 5.4 to 150 pg/mL range ($r = \geq 0.9967$).

1. INTRODUCTION

1.1 Background and Purpose of Study

The purpose of this study was to validate BASF Analytical Method No. D1505 for the analysis of the BAS 440 I and its metabolites M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 in surface and drinking water using LC-MS/MS

2. MATERIALS AND METHODS

2.1 Test Systems

The water samples used in this study were drinking (well) water and surface (lake) water samples, which were characterized by AGVISE Laboratories. The GLP water characterization reports are provided in Appendix L. The samples were held under refrigeration during the experimental period. Each analysis set was uniquely identified with a Master Sheet Number, which consisted of the study number plus a unique number (e.g., 776699-16). The test system samples were assigned unique numbers and these were recorded in each analytical set or “Master Sheet” (e.g., water fortification sample 776699-16-4, from Master Sheet No. 776699-16). The actual sample numbers used for the analysis were identified in the raw data and in this final report.

2.2 Test and Reference Substances

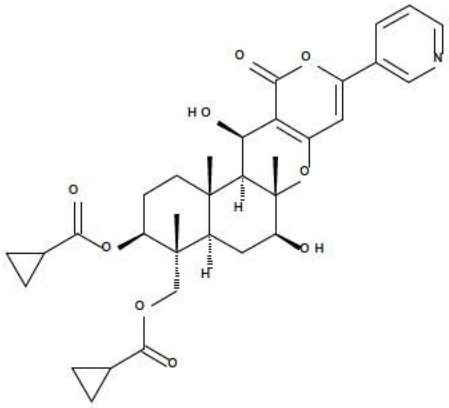
The test/reference standards shown below (see Appendix A for certificates of analysis) were synthesized by BASF Aktiengesellschaft (Limburgerhof, Germany) and used during the analytical portion of this study. The test/reference substances were maintained frozen until use in this study. BASF Aktiengesellschaft determined characterization and purity prior to the substance being used in this study. Details of these determinations are available to BASF and are located at Landwirtschaftliche Versuchsstation der BASF, Limburgerhof, Germany.

The test/reference substances in solution were used in the study to generate data for both instrument and method performance. Quantitation of residues in all samples was achieved using calibration curves calculated by linear regression of instrument responses for the reference substances. The performance of the instrument was evaluated during each injection set.

2.2.1 Afidopyropen

Common Name	Afidopyropen
BAS-Code	BAS 440 I
BASF Reg. No.	5599022
CAS-No.	915972-17-7
Molecular Formula	$C_{33}H_{39}NO_9$
Molecular Weight	593.7 g/mol
IUPAC Name	[(3S, 4R, 4aR, 6S, 6aS, 12R, 12aS, 12bS)-3-(cyclopropanecarbonyloxy)-6, 12-dihydroxy-4, 6a, 12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1, 2, 3, 4, 4a, 5, 6, 6a, 12a, 12b-decahydro-11H, 12H-benzo[f]pyrano[4,3-b]chromen-4-yl]methyl cyclopropanecarboxylate
Lot No.	L82-65
Purity (%)	97.5
Expiration Date	Aug 1, 2015*

Chemical structure:

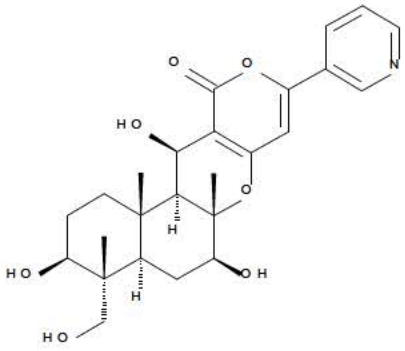


*Re-certified during the study with a purity of 98.1% and expiry date of June 1, 2016.

2.2.2 Metabolite M440I001

Common Name	Not assigned
BAS-Code	M440I001
BASF Reg. No.	5741530
CAS-No.	None assigned
Molecular Formula	$C_{25}H_{31}NO_7$
Molecular Weight	457.5 g/mol
IUPAC Name	(3S, 4R, 4aR, 6S, 6aS, 12R, 12aS, 12bS)-3, 6, 12-trihydroxy-4-(hydroxymethyl)-4, 6a, 12b-trimethyl-9-(pyridin-3-yl)-1, 3, 4, 4a, 5, 6, 6a, 12, 12a, 12b-decahydro-2H, 11H-benzo[f]pyrano [4, 3-b]chromen-11-one
Lot No.	L82-66
Purity (%)	93.9
Expiration Date	February 01, 2016

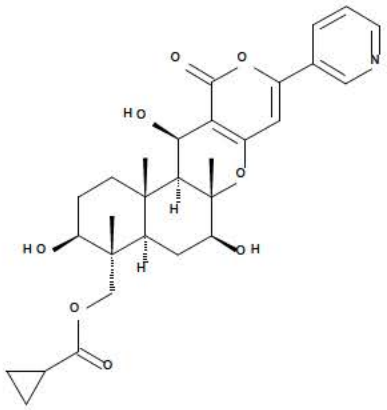
Chemical structure:



2.2.3 Metabolite M440I002

Common Name	Not assigned
BAS-Code	M440I002
BASF Reg. No.	5741532
CAS-No.	None assigned
Molecular Formula	$C_{29}H_{35}NO_8$
Molecular Weight	525.6 g/mol
IUPAC Name	[(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-3,6,12-trihydroxy-4-6a, 12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H, 11H-benzo[f]pyrano[4,3-b]chromen-4yl]methyl cyclopropanecarboxylate
Lot No.	L82-67
Purity (%)	92.5
Expiration Date	February 1, 2016

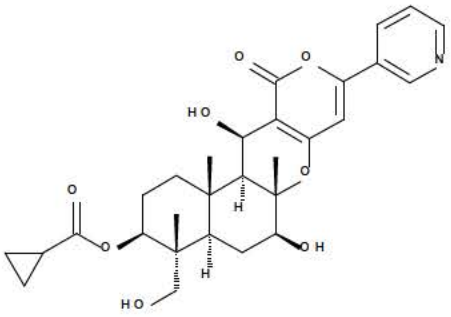
Chemical structure:



2.2.4 Metabolite M440I003

Common Name	Not assigned
BAS-Code	M440I003
BASF Reg. No.	5741533
CAS-No.	None assigned
Molecular Formula	$C_{29}H_{35}NO_8$
Molecular Weight	525.6 g/mol
IUPAC Name	(3S, 4R, 4aR, 6S, 6aS, 12R, 12aS, 12bS)-6, 12-dihydroxy-4-(hydroxymethyl)-4, 6a, 12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1, 3, 4, 4a, 5, 6, 6a, 12, 12a, 12b-decahydro-2H, 11H-benzo[f]pyrano [4,3-b]chromen-3-yl cyclopropanecarboxylate
Lot No.	L82-72
Purity (%)	98.6
Expiration Date	September 1, 2016

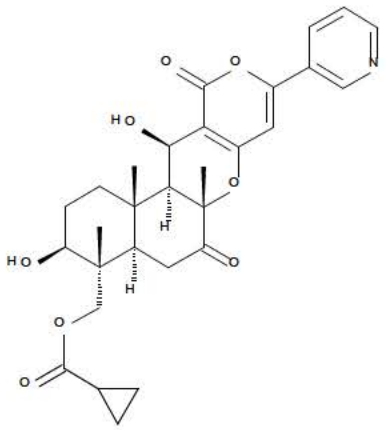
Chemical structure:



2.2.5 Metabolite M440I005

Common Name	Not assigned
BAS-Code	M440I005
BASF Reg. No.	5824382
CAS-No.	None assigned
Molecular Formula	$C_{29}H_{33}NO_8$
Molecular Weight	523.6 g/mol
IUPAC Name	[(3S, 4R, 4aR, 6aS, 12R, 12aS, 12bS)-3,12-dihydroxy-4, 6a, 12b-trimethyl-6, 11-dioxo-9-(pyridin-3-yl)-1, 3, 4, 4a, 5, 6, 6a, 12, 12a, 12b-decahydro-2H, 11H-benzo[f]pyrano[4,3-b]chromen-4-yl] methyl cyclopropanecarboxylate
Lot No.	L82-73
Purity (%)	90.9
Expiration Date	March 1, 2016

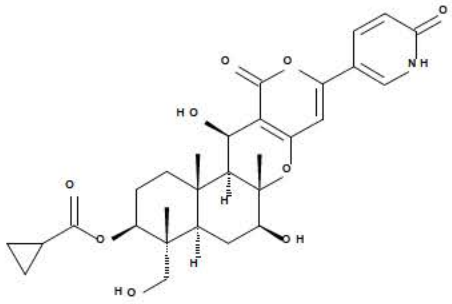
Chemical structure:



2.2.6 Metabolite M440I016

Common Name	Not assigned
BAS-Code	M440I016
BASF Reg. No.	5845597
CAS-No.	None assigned
Molecular Formula	$C_{29}H_{35}NO_9$
Molecular Weight	541.6 g/mol
IUPAC Name	(3S, 4R, 4aR, 6S, 6aS, 12R, 12aS, 12bS)-6, 12-dihyroxy-4-(hydroxymethyl)-4, 6a, 12b-trimethyl-11-oxo-9-(6-oxo-1, 6-dihydropyridin-3-yl)-1, 3, 4, 4a, 5, 6, 6a, 12, 12a, 12b-decahydro-2H, 11H-benzo[f]pyrano[4,3-b]chromen-3-yl cyclopropanecarboxylate
Lot No.	L82-148
Purity (%)	88.9
Expiration Date	May 1, 2016

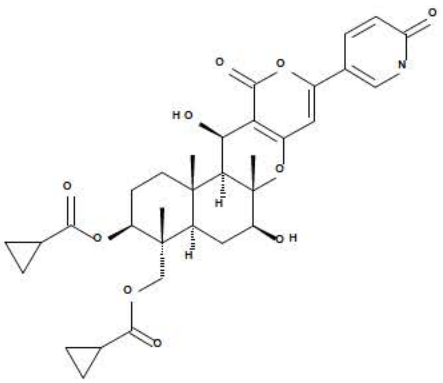
Chemical structure:



2.2.7 Metabolite M440I024

Common Name	Not assigned
BAS-Code	M440I024
BASF Reg. No.	5886215
CAS-No.	None assigned
Molecular Formula	$C_{33}H_{39}NO_{10}$
Molecular Weight	609.7 g/mol
IUPAC Name	[(3S,4a, 4aR, 6S, 6aS, 12R, 12aS, 12bS)-3-[(cyclopropylcarbonyl)oxy]-6, 12-dihydroxy-4, 6a, 12b-trimethyl-11-oxo-9-(6-oxo-1, 6-dihyropyridin-3-yl)-1, 3, 4, 4a, 5, 6, 6a, 12, 12a, 12b-decahydro-2H, 11H-benzo[f]pyrano[4, 3-b]chromen-4-yl]methyl cyclopropanecarboxylate
Lot No.	L82-149
Purity (%)	91.3
Expiration Date	May 1, 2016

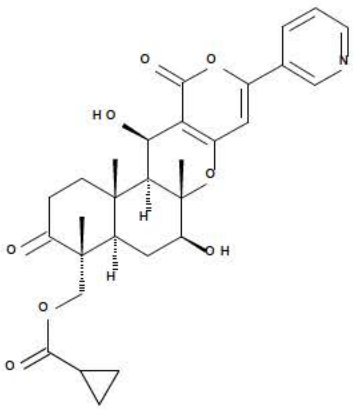
Chemical structure:



2.2.8 Metabolite M440I057

Common Name	Not assigned
BAS-Code	M440I057
BASF Reg. No.	6010129
CAS-No.	None assigned
Molecular Formula	$C_{29}H_{33}NO_8$
Molecular Weight	523.6 g/mol
IUPAC Name	[(4R,4aR,6S,6aS,12R,12aS,12bS)-6, 12-dihydroxy-4,6a, 12b-trimethyl-3, 11-dioxo-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a, 12,12a,12b-decahydro-2H, 11H-benzo(f)pyrano(4,3-b]chromen-4-yl]methyl cyclopropanecarboxylate
Lot No.	L82-164
Purity (%)	97.4%
Expiration Date	January 1, 2017

Chemical structure:



Stock solutions of afidopyropen and the metabolites were prepared in acetone, except for M440I001 and M440I005, which were prepared in methanol:acetone (50:50, v/v). The mixed intermediate/fortification solutions containing each analyte were prepared by combining aliquots of the stock solutions for each analyte and diluting with acetonitrile. The calibration standards were prepared by serial dilution of the intermediate standards using acidified (0.1% formic acid content) water:acetonitrile (90:10, v/v). The stability of the analytes in standard solutions has been determined in conjunction with this study and in a related afidopyropen validation study performed on soil (Reference 1). In this study, to determine the stability of select metabolites in solutions, aged stock, intermediate, and/or calibration standards were analyzed against freshly prepared standard solutions.

During the course of this study, the test/reference substance solutions were stored under refrigeration. Preparation and dilution data forms pertaining to the stock and working solutions are located in the raw data. Example standard dilution and use information, as performed in the subject study, are provided in Appendix K.

2.3 Route of Administration

In this method validation study, the test substances were applied to the test system as analytical standard solutions (in acetonitrile) by micropipette to ensure precise delivery of a small amount of the test substances.

2.4 Analytical Method

2.4.1 Principle of the Method

Using BASF Analytical Method No. D1505/02, residues of afidopyropen in water are quantified using LC-MS/MS. The method procedures validated in this study are provided in Appendix B. A description of the methodology follows.

Briefly, residues of afidopyropen in water samples (10 mL each) are diluted with acidified acetonitrile (1 mL), filtered (0.45 µm nylon syringe), and then analyzed by HPLC/MS/MS.

2.4.2 Specificity/Selectivity

The residues of afidopyropen are determined by HPLC-MS/MS, monitoring in the positive mode for quantitation purposes ion transitions m/z 594→148 for parent afidopyropen; m/z 458→148 for M440I001; m/z 526→148 for M440I002; m/z 526→148 for M440I003; m/z 524→148 for M440I005; m/z 542→218 for M440I016; m/z 610→218 for M440I024; and m/z 524→148 for M440I057. Typically for confirmatory purposes, the following alternate ion transitions are monitored, again in the positive ionization mode: m/z 594→202 for parent afidopyropen; m/z 458→106 for M440I001; m/z 526→202 for M440I002; m/z 526→202 for M440I003; m/z 524→80 for M440I005; m/z 542→164 for M440I016; m/z 610→122 for M440I024; and m/z 524→202 for M440I057. The results are calculated by direct comparison of the sample peak responses to those of external standards.

As HPLC-MS/MS is regarded as a highly-specific detection method when two ion transitions have been validated, an additional confirmatory method or technique is not necessary. The multiple reaction monitoring (MRM) transitions used to identify afidopyropen were determined by product ion scan (see Appendix J).

2.5 Validation of Method

For validation, untreated drinking (well) water and surface (lake) water samples were fortified with each analyte and analyzed according to the established method validation guidelines. To test the repeatability of the method, the analytical sets consisted of a reagent blank, and for each matrix, two controls, five replicates fortified with each analyte at the method limit of quantitation, 30 ppt, and five replicates fortified at a higher level, corresponding to 10X the limit of quantitation, 300 ppt. For each analyte, the two mass transitions described above were evaluated.

2.6 Influence of Matrix Effects on Analysis

In conjunction with the subject study, matrix-matched standards and solvent-based standards were analyzed in a separate experiment to evaluate any potential matrix effects on LC/MS/MS analysis. This involved comparing calibration standards prepared in control matrix against calibration standard solutions prepared with acidified water:acetonitrile (90:10, v/v). The matrix-matched standards were prepared by diluting mixed standards of each analyte with control drinking or surface water to 0.015, 0.03, and 0.06 ng/mL, equivalent to 0.5x, 1x, and 2x the LOQ, respectively. Each set of matrix-matched standards (for each water type) was bracketed by a block of solvent-based calibration standards and included additional single injections of the tested standard levels during the run. All standard injections within that matrix set were used in calculations involving matrix effects.

The data generated were evaluated by comparing the average area response of the standards for three injections of each type (with and without matrix) for each of the three standard concentration levels. Acceptability (i.e., matrices had no significant influence on the analysis) requires a difference in area of <20%, calculated as the "Mean Area Change (%)". For each matrix/ion transition, an overall average "Mean Area Change (%)" across the three tested concentrations was calculated to make a general assessment of acceptability with respect to matrix effects.

2.7 Stability of Extracts

As the method does not consist of a typical “extraction” – the water samples are diluted and analyzed – “extracts” and “final volume” are used interchangeably in this report. The stability of each analyte in stored “extract” solutions was determined in conjunction with the subject method validation study. The stability in the final volume, the solution prepared for LC-MS/MS injection, was established for each matrix by reanalyzing several control and recovery samples which had been stored under refrigeration at the final volume stage. Quantification of the analytes in the stored samples for this experiment was performed for the primary mass transitions.

INTRODUCTION

BAS 440 I is an insecticide used against several insects in various crops.
BASF method no. D1505/02 allows for the determination of BAS 440 I and its metabolites M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 residues in water.
This method was developed at BASF Crop Protection, Research Triangle Park, NC, USA.

The method D1505/02 is successfully tested on surface water and drinking water.

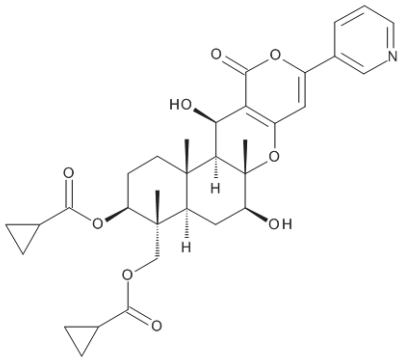
1 MATERIALS

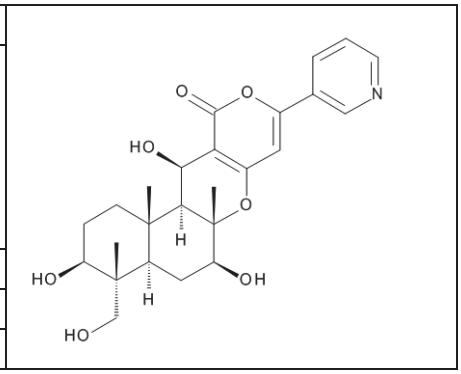
1.1 Safety

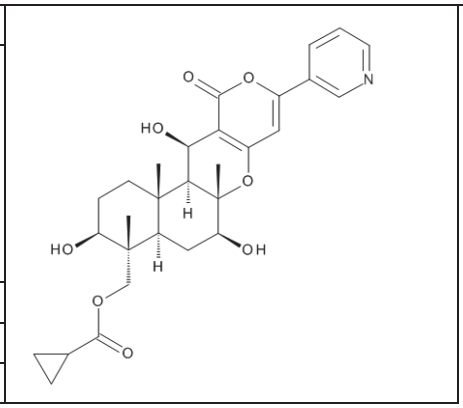
The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Store work clothing separately. Keep away from food, drink and animal feed stuffs. No eating, drinking, smoking or tobacco use at the place of work. Hands and/or face should be washed before breaks and at the end of the shift. Details are given in the Safety Data Sheets (SDS) of the individual substances. All procedures involving organic solvents should be performed in a well-ventilated hood.
Disposal of samples and chemicals must be done in compliance with on-site safety policies and procedures.

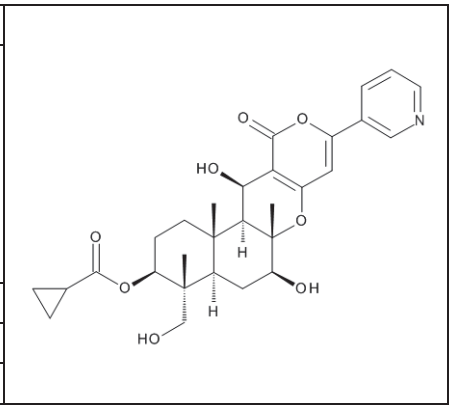
1.2 Test and Reference Items

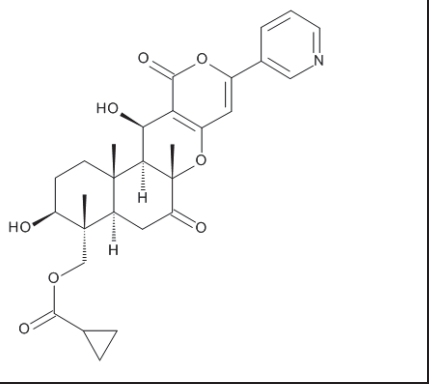
Test and reference items should be stored according to the information provided in the certificate of analysis.

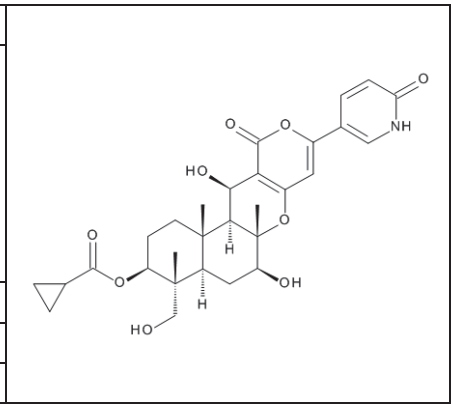
BAS-Code	440 I	
Common Name	Afidopyropen	
IUPAC Name	[(3S, 4R, 4aR, 6S, 6aS, 12R, 12aS, 12bS)-3-(cyclopropanecarbonyloxy)-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-11H,12H-benzo[flpyrano[4,3-b]chromen-4-yl] methylcyclopropanecarboxylate	
BASF Reg. No.	5599022	
Molecular Formula	$C_{33}H_{39}NO_9$	
Molecular Weight	593.7	

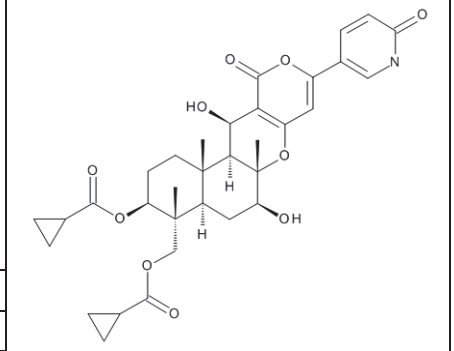
BAS-Code	M440I001	
IUPAC Name	(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-3,6,12-trihydroxy-4-(hydroxymethyl)-4,6a,12b-trimethyl-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f] pyrano[4,3-b]chromen-11-one	
BASF Reg. No.	5741530	
Molecular Formula	$C_{25}H_{31}NO_7$	
Molecular Weight	457.5	

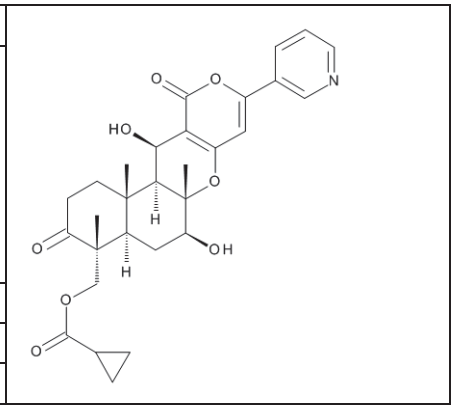
BAS-Code	M440I002	
IUPAC Name	[(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-3,6,12-trihydroxy-4,6a,12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f] pyrano[4,3-b]chromen-4-yl] methylcyclopropanecarboxylate	
BASF Reg. No.	5741532	
Molecular Formula	$C_{29}H_{35}NO_8$	
Molecular Weight	525.6	

BAS-Code	M440I003	
IUPAC Name	(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-6,12-dihydroxy-4-(hydroxymethyl)-4,6a,12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f] pyrano[4,3-b]chromen-3-yl cyclopropanecarboxylate	
BASF Reg. No.	5741533	
Molecular Formula	$C_{29}H_{35}NO_8$	
Molecular Weight	525.6	

BAS-Code	M440I005	
IUPAC Name	[(3S,4R,4aR,6aS,12R,12aS,12bS)-3,12-dihydroxy-4,6a,12b-trimethyl-6,11-dioxo-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f]pyrano[4,3-b]chromen-4-yl] methylcyclopropanecarboxylate	
BASF Reg. No.	5824382	
Molecular Formula	$C_{29}H_{33}NO_8$	
Molecular Weight	523.6	

BAS-Code	M440I016	
IUPAC Name	(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-6,12-dihydroxy-4-(hydroxymethyl)-4,6a,12b-trimethyl-11-oxo-9-(6-oxo-1,6-dihydropyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f]pyrano[4,3-b]chromen-3-yl cyclopropanecarboxylate	
BASF Reg. No.	5845597	
Molecular Formula	$C_{29}H_{35}NO_9$	
Molecular Weight	541.6	

BAS-Code	M440I024	
IUPAC Name	[(3S,4a,4aR,6S,6aS,12R,12aS,12bS)-3-[(cyclopropylcarbonyl)oxy]-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(6-oxo-1,6-dihydropyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f]pyrano[4,3-b]chromen-4-yl]methylcyclopropanecarboxylate	
BASF Reg. No.	5886215	
Molecular Formula	$C_{33}H_{39}NO_{10}$	
Molecular Weight	609.7	

BAS-Code	M440I057	
IUPAC Name	[(4R,4aR,6S,6aS,12R,12aS,12bS)-6,12-dihydroxy-4,6a,12b-trimethyl-3,11-dioxo-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f]pyrano[4,3-b]chromen-4-yl]methyl cyclopropanecarboxylate	
BASF Reg. No.	6010129	
Molecular Formula	$C_{29}H_{33}NO_8$	
Molecular Weight	523.6	

1.3 Equipment

Equipment	Size, Description	Manufacturer	Catalog No.
Balance, Analytical	Model AT100	Mettler	----
Balance, Top Loader	Model PJ3600	Mettler DeltaRange	----
Beakers	Various Sizes	PYREX Brand, VWR Scientific Products	13922-029
Bottle, Amber glass	Qorpak, 2 oz and 4 oz with Teflon®-lined screw cap	VWR Scientific Products Boston Round, Amber	89042-908
Culture tube caps	16 mm	VWR	60828-768
Culture Tubes	Glass, disposable, 16x100mm size	VWR	47729-576
Syringe	1 mL	BD, Fisher Scientific	14-817-25
Syringe Filter	0.45 μm Nylon, 13 mm	Pall Acrodisk, VWR	4426T
Cylinder, Graduated	Various sizes	Various	----
HPLC Column	Acquity UPLC BEH C-18 (2.1 x 50 mm, 1.7 μm)	Waters	186002350
LC Vials	2 mL injection vials	National Scientific	C400-79
LC	Acquity UPLC	Waters	
Mass Spectrometer	API 5500	AB Sciex	
Mechanical shaker	KS501 Digital	IKA Labortechnik	----
MicroMan pipettes	10-1000 µL	Gilson	M-25, M-50, M-250, M-1000
Ultrasonic Bath	Model FS 7652H	Fisher Scientific	
Various Flask, Volumetric	100, 50, 25 ,10 and 5 mL	Various	---
Volumetric, pipettes	Various	Fisher Scientific – Class A	13-650-2A
Vortex mixer	Genie 2	VWR	58816-121

Note: The equipment and instrumentation listed above may be substituted by that of similar specifications. The applicability is confirmed if the recoveries of the fortification experiments are in the expected concentration range.

1.4 Reagents

1.4.1 Chemicals

Chemical	Grade	Manufacturer/Supplier	Catalog No.
Acetonitrile	HPLC Grade	EMD	AX0145P-1
Methanol	HPLC Grade	EMD	MX0475P-1
Acetone	HPLC Grade	EMD	AX0115P-1
Formic acid	≥95%	Sigma-Aldrich	F0507
Water, e.g. Baker® or Millipore®	HPLC Grade	BDH ARISTAR PLUS	87003-652

Note: Equivalent reagents and chemicals from other suppliers may be substituted.

1.4.2 Solutions and Solvent Mixtures

Description	Code	Composition
Solvent 1	**S1**	**Acetonitrile-water, 10/90, v/v with 0.1% formic acid** Add 100 mL of acetonitrile, 900 mL of water, and 1 mL concentrated formic acid into a 1L flask and mix well.
Solvent 2	**S2**	**Acetonitrile with 1% formic acid** Add 1000 mL of acetonitrile and 10 mL of concentrated formic acid into a 1L flask and mix well.
Solvent 3	**S3**	**Acetonitrile with 2% formic acid** Add 100 mL of acetonitrile and 2 mL of concentrated formic acid into a 100 mL flask and mix well.
HPLC mobile phase A	**LC1**	**0.1% Formic Acid in Water** Add 1000 mL of water and 1 mL of concentrated formic acid into a 1L flask and mix well.
HPLC mobile phase B	**LC2**	**0.1% Formic Acid in Acetonitrile** Add 1000 mL of acetonitrile and 1 mL of concentrated formic acid into a 1L flask and mix well.

Note: If necessary, the solutions may also be prepared in different volumes as long as the proportions are not modified.

1.4.3 Standard Solutions

All standard solutions should be stored under refrigerated conditions (approximately +5°C) unless otherwise noted.

Stock Solutions

Prepare individual 1.0 mg/mL stock solutions by weighing an appropriate amount of individual analytes into a flask and add the required volume of acetone (except for M440I001 and M440I005 which require 50/50 methanol/acetone). For example, to prepare 10 mL of 1.0 mg/mL stock solution of an analyte in acetone, weigh 10 mg of the analyte into a 10 mL volumetric flask. Dissolve and dilute to mark with acetone. Ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Independence of standard calibration and fortification solutions should initially be confirmed to show correct preparation of the solutions. This can be achieved for example using one of the following approaches:

- Two stock solutions are independently prepared. One is used for preparation of fortification solutions, the other for calibration standard solutions.
- Fortification and calibration standard solutions should be prepared from one stock solution in separate dilution series.

For subsequent preparations of solutions, freshly prepared solutions can be compared directly to previous standard solutions.

A correction for purity is done if the purity is ≤ 95%. If the purity is > 95 % correction is optional.

Fortification Solutions

Prepare mixed standard solutions for fortification by combining stock solutions of each analyte (see above) in a flask. Dilute volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of mixed Fortification solutions

Take solution (µg/mL)	Volume (mL)	Dilute with acetonitrile to a final volume of (mL)	Concentration (ng/mL)
1,000	0.05	50	1,000
1.0	1.5	50	30
1.0	0.15	50	3.0

Note: A different concentration scheme may be used, if other fortification levels are needed for the analysis. If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

Calibration Standard Solutions

Prepare mixed standard calibration solutions for LC-MS/MS analysis by using the solutions that were prepared in the section "fortification solutions" in flasks. Dilute volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of standard solutions for calibration

Take solution (ng/mL)	Volume (mL)	Dilute with S1 to a final volume of (mL)	Concentration (ng/mL)
30	0.25	50	0.15
0.15	10	25	0.060
0.15	5	25	0.030
0.15	2.5	25	0.015
0.15	0.9	25	0.0054

Note: A different concentration scheme may be used and additional standards may be prepared as needed.
If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

Additional Information:

- Use amber bottles with Teflon-lined screw caps as storage containers for all standard solutions.
- Some BAS 440 I metabolites may be unstable in neutral and alkaline aqueous conditions. The addition of formic acid has a stabilizing effect on BAS 440 I metabolites.

Matrix-Matched Standard Solutions

If significant matrix effects are observed, matrix-matched standards may be utilized. Matrix-matched calibration standards are used for quantitation when signal suppression or enhancement is >20% compared to the response for standards prepared in calibration solution alone. Matrix-matched standards should be prepared in a way that the matrix load is at least 90% of the matrix load in the unknown samples. In addition the matrix load should be the same in all calibration standard solutions. The following procedure may be used to prepare matrix matched standards.

a) Prepare precursor standards for matrix matched calibration standards. Dilute volumetrically with appropriate solvents as exemplified in the table below and ensure a complete homogeneous solution (e.g. by sonication or vortexing).

Preparation of calibration standard precursor solutions for making matrix matched standards

Take solution (ng/mL)	Volume (mL)	Dilute with acetonitrile to a final volume of (mL)	Concentration (ng/mL)
30	5	50	3.00
3	10	25.0	1.20
3	5	25.0	0.60
3	2.5	25.0	0.30
3	0.9	25.0	0.108

Note: A different concentration scheme may be used and additional precursor solutions may be prepared as needed.

If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

b) Prepare the matrix matched standards according to the table below using control matrix and the precursor standards prepared above:

Preparation of matrix matched standards

Take solution (ng/mL)	Take Volume (mL)	Volume of S3 (mL)	Control Matrix (mL)	Concentration (ng/mL)
3.00	0.100	0.100	1.80	0.15
1.20	0.100	0.100	1.80	0.060
0.60	0.100	0.100	1.80	0.030
0.30	0.100	0.100	1.80	0.015
0.108	0.100	0.100	1.80	0.0054

Note: A different concentration scheme may be used and additional standards may be prepared as needed. Final calibration standard solutions should be 10% acetonitrile and 0.1% formic acid. Matrix load must be at least 90% and the matrix load should be the same in all calibration standard solutions.

If necessary, the volume of solution prepared may be changed as long as the proportions are not modified.

1.4.4 Stability of Standard Solutions

The reference item solutions are stable under the specifications described below. If solutions are stored at different conditions or/and for a longer time, the stability of the reference items has to be confirmed. Data from BASF study number 776699 (Reference 1) except where noted.

Analyte	Standard Tested	Solvent / Conditions[1]	Limit of Demonstrated Storage Stability[2]
Afidopyropen	Stock	Acetone	3 months (93 days)
	Fortification	Acetonitrile	1 month (35 days)
	Calibration	Acidified acetonitrile:water (10:90, v/v)	1 month (35 days)
M440I001	Stock	Acetone:methanol (50:50, v/v)	3 months (93 days)
	Fortification	Acetonitrile	1 month (35 days)
	Calibration	Acidified acetonitrile:water (10:90, v/v)	2 weeks (17 days)
M440I002	Stock	Acetone	3 months (93 days)
	Fortification	Acetonitrile	1 month (35 days)
	Calibration	Acidified acetonitrile:water (10:90, v/v)	1 month (35 days)
M440I003	Stock	Acetone	3 months (93 days)
	Fortification	Acetonitrile	1 month (35 days)
	Calibration	Acidified acetonitrile:water (10:90, v/v)	1 month (35 days)
M440I005	Stock	Acetone:methanol (50:50, v/v)	3 months (93 days)
	Fortification	Acetonitrile	1 month (35 days)
	Calibration	Acidified acetonitrile:water (10:90, v/v)	1 month (35 days)
M440I016	Stock	Acetone	3 months (93 days)
	Fortification	Acetonitrile	1 month (35 days)
	Calibration	Acidified acetonitrile:water (10:90, v/v)	2 weeks (17 days)
M440I024	Stock	Acetone	3 months (93 days)
	Fortification	Acetonitrile	1 month (35 days)
	Calibration	Acidified acetonitrile:water (10:90, v/v)	2 weeks (17 days)
M440I057	Stock	Acetone	1 month (33 days)[3]
	Fortification	Acetonitrile	1 month (33 days)[3]
	Calibration	Acidified acetonitrile:water (10:90, v/v)	2 weeks (14 days)

1. Each stored under refrigeration in the dark in amber glass bottles.
2. The stability criteria: average concentration ±20% of nominal, based on LC/MS/MS analysis.
3. Data from BASF Study Number 394795 (Reference 2)

2 ANALYTICAL PROCEDURE

2.1 Sample Preparation

Samples have to be sufficiently mixed beforehand, in order to assure that the aliquot taken for residue analysis is representative for the whole sample.

2.2 Sample Storage

Field sample storage stability will be established in a separate study, if needed.

2.3 Weighing and Fortification

For treated samples and control samples, measure 10 ± 0.1 mL (or weigh 10 ± 0.1 g) of water sample into a culture tube.

For fortified samples, measure 10.0 mL of control water sample into a culture tube and add fortification solutions on the matrix. Vortex mix samples for approximately 30 seconds.

The following scheme may be used:

Sample Type	Sample Volume	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
Control	10 mL	-	-	0 ng/L
Fortification (LOQ)	10 mL	3.0 ng/mL	100 µL	30 ng/L *
Fortification (10xLOQ)	10 mL	30 ng/mL	100 µL	300 ng/L
Treated	10 mL	-	-	-

* limit of quantification

Note: Volume of spiking solution added to generate the fortified sample should not exceed 10% of sample weight or volume.

2.4 Extraction of Sample Material

a) Add a volume of **S2** equal to 10% of sample volume (e.g. add 1.0 mL **S2** to a 10 mL sample aliquot).
Note: For fortification samples, the addition of **S2** should be performed within 5 minutes of spiking the control matrix to prevent analyte interconversion.
b) Vortex mix samples for approximately 30 seconds.

2.5 Preparation for Measurement

a) Filter 1 mL of sample extract through a 0.45 µm nylon syringe filter.
b) For samples with analyte concentrations outside the standard curve, dilute with **S1** as appropriate.

2.6 Influence of matrix effects on analysis

During method development, it was demonstrated that the matrix load tested had no significant influence on the analysis (i.e., matrix effects < 20%).

2.7 Stability of Extracts

The stability in the final volume, the solution prepared for LC/MS/MS injection, was established for drinking and surface waters, according to the table below. Data from BASF study number 776699 (Reference 1).

Analyte	Solution Tested[1]	Limit of Demonstrated Storage Stability (days)
Afidopyropen, M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057	Final volume	Drinking water, 6 days; Surface water, 5 days

1. Samples were stored under refrigeration prior to re-analysis.

3 QUANTIFICATION AND CALCULATION

3.1 Set-up of the analytical run

A sequence for measurement generally consists of:
- o Calibration standards
- o Control samples
- o Procedural recovery samples
- o Unknown samples
- o Instrument recovery sample

Reagent Blanks or blanks can also be injected if necessary. Each injection set should begin and end with an injection of a calibration standard. Standards should be interspersed with samples. Each calibration standard should be at least injected twice. At least 5 calibration levels need to be injected.

3.2 Instrumental analysis

3.2.1 Instrumentation and Conditions

	Parameter		
Chromatographic System	Waters Acquity UPLC		
Analytical-column	Acquity UPLC BEH Phenyl, 100 x 2.1 mm, 1.7 µm particle		
Column Temperature	45°C		
Injection Volume	50 µL (or greater)		
Mobile Phase A Mobile Phase B	Water / formic acid, 1000/1, v/v Acetonitrile / formic acid, 1000/1, v/v		
Flow Rate	600 µL/min		
Gradient (including wash and equilibration)	Time (min)	Phase A	Phase B
	0.00	85	15
	0.05	85	15
	8.50	75	25
	10.25	55	45
	11.00	5	95
	11.95	5	95
	12.00	85	15
	12.50	85	15
Detection System	PE Sciex API 5500 Mass Spectrometer		
Ionisation	Electrospray (ESI)		
Analyte	**Transitions (m/z)**	**Polarity**	**Expected Retention Time**
Period 1: 0.00 to 5.00 min*			
M440I001	458 → 148** 458 → 106 458 → 202***	positive	approx. 1.6 min
M440I002	526 → 148** 526 → 202	positive	approx. 4.2 min
Period 2: 5.00 to 7.00 min*			
M440I005	524 → 148** 524 → 80	positive	approx. 6.4 min
M440I057	524 → 148** 524 → 202	positive	approx. 6.2 min
Period 3: 7.00 to 9.00 min*			
M440I003	526 → 148** 526 → 202	positive	approx. 7.6 min
M440I016	542 → 218** 542 → 164	positive	approx. 7.4 min
Period 3: 9.00 to 12.50 min*			
M440I024	610 → 218** 610 → 122	positive	approx. 10.4 min
BAS 440 I	594 → 148** 594 → 202	positive	approx. 10.7 min

* Transitions between periods should be optimized to actual retention times.

** proposed as quantification transition. Any of these transitions could be used for quantitation in case interference is observed at the same retention time.

*** Secondary transition used during the ILV.

Note:	Instruments with similar specifications may substitute the equipment listed above. The instruments used are applicable for analysis if the recoveries of the fortification experiments are in the acceptable range. In general a divert valve is used to reduce the matrix load on the detection system. Instrument conditions, e.g. injection volumes, columns, gradient steps or mass transitions may be modified, but any changes must be recorded in the raw data. Changes are acceptable, when the recoveries of the fortification experiments are in the acceptable range. Other parameters like gas flows and voltages are depended of the equipment used and therefore not listed. Those parameters may need to be adapted for the used instrument.

3.2.2 Calibration procedures

Calculation of results is based on peak area measurements using a calibration curve. At least 5 calibration levels need to be injected (e.g., required for enforcement). The calibration curve is obtained by direct injection of BAS 440 I, M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 mixed standards for LC-MS/MS in the range of 0.0054 ng/mL to 0.15 ng/mL. In a given injection run, the same injection volume is used for all samples and standards.

Linear calibration functions are preferred for evaluation. If other functions are used (e.g. quadratic), this should be fully justified.

3.2.3 Calculation of Residues and Recoveries

Calculation of results is based on area measurements.
For the procedural recoveries, the sample weight will be considered 10.0 mL in the final calculation of residues [ng/L]. The method requires that the sample weight to be 10 ± 0.1 mL for fortification samples. The recovery is the percentage of the fortified amount (µg or ng), which is recovered through the method and the weights cancels out, as shown in the equation below, during the final calculation step.

The residues of BAS 440 I in mg/kg are calculated as shown in equations **I** and **II**:

I. **Concentration [ng/mL]** $= \dfrac{\text{Response} - \mathit{Intercept}}{\mathit{Slope}} = C_A$

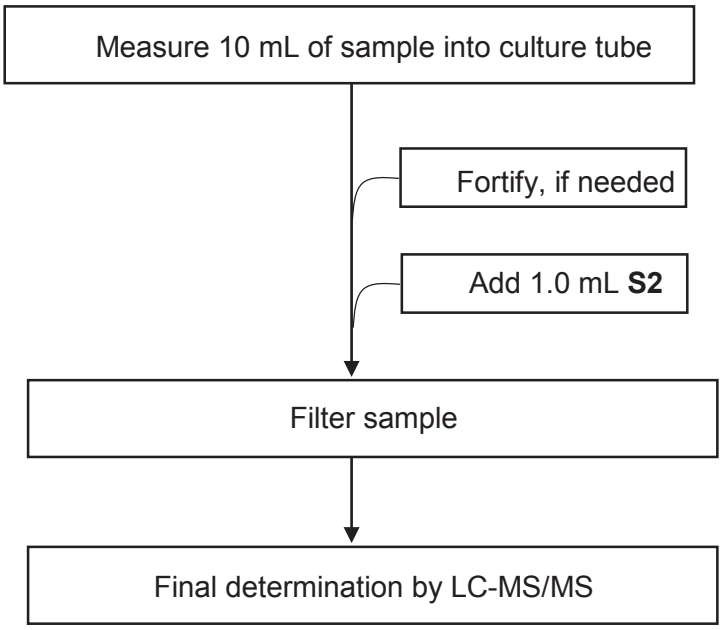
II. **Residue [ng/Lm ppt]** $= \dfrac{V_{end} \times C_A \times 1000}{G \times A_F}$

V_{end}	=	Final volume of the extract after all dilution steps [mL]
C_A	=	Concentration of analyte as read from the calibration curve [ng/mL]
G	=	Weight of the sample extracted [g]
A_F	=	Aliquot factor
1000	=	Factor remaining after all unit conversions

The recoveries of spiked compounds are calculated according to equation **III**:

III. **Recovery %** $\dfrac{(\text{Residue in fortified sample} - \text{Residue in control}) \times 100}{\text{Amount of analyte fortified}}$

4 FLOWCHART



5 METHOD MANAGEMENT AND TIME REQUIREMENTS

The analysis of one series of 13 samples (one reagent blank, two controls, and 10 fortified samples for recovery experiments) requires 0.5 working day (4 hours) per laboratory assistant. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.

6 CONCLUSION AND METHOD CAPABILITIES

Recoveries, Chromatograms, and Calibration Curves
Recovery data will be provided in the validation part of the analytical method D1505/02.

Limit of Quantification (LOQ) and Limit of Detection (LOD)
The limit of quantification is defined as the lowest fortification level successfully tested. The limit of quantification is 30 ng/L (ppt) for all analytes. The limit of detection was set at 20% of the limit of quantification, equivalent to 6 ng/L (ppt) for all analytes. The lowest standard for each analyte in the calibration curve has good detectability (signal to noise ratio greater than 3:1).

Selectivity
The tested untreated water samples showed no significant interferences (< 20 %) at the retention time of all analytes.
Justification of selection of ions will be attached following validation.

Confirmatory Techniques
The LC-MS/MS final determination for BAS 440 I, M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 is a highly selective detection technique. For every compound the quantitation is possible at two different transitions. Therefore, no additional confirmatory technique is required.

Potential Problems
Some BAS 440 I metabolites may be unstable in neutral or alkaline aqueous conditions. The addition of formic acid has a stabilizing effect on these metabolites.

Fortification samples should be acidified in a timely manner. During extraction, fortification samples should be combined with **S2** within 5 minutes of adding fortification solution to prevent analyte interconversion.

If matrix suppression or enhancement is observed, matrix-matched standards should be used.

7 REFERENCES

1. Delinsky, D. (2015) Validation of Method D1505/02: “Method for the Determination of Residues of Afidopyropen (BAS 440 I, Reg No. 5599022) and its Metabolites M440I001 (Reg No. 5741530), M440I002 (Reg No. 5741532), M440I003 (Reg No. 5741533), M440I005 (Reg No. 5824382), M440I016 (Reg No. 5845597), M440I024 (Reg No. 5886215), and M440I057 (Reg No. 6010129) in Surface and Drinking Water by LC-MS/MS” BASF Study Number 776699. BASF Reg. Doc. No. 2015/7003587.

2. Gooding, R. (2015) Validation of Method D1308/02: “Method for the Determination of BAS 440 I (Reg No. 5599022) and its metabolites M440I001 (Reg No. 5741530), M440I002 (Reg No. 5741532), M440I003 (Reg No. 5741533), M440I005 (Reg No. 5824382), M440I016 (Reg No. 5845597), M440I024 (Reg No. 5886215), and M440I057 (Reg No. 6010129) in soil by LC-MS/MS” BASF Study Number 394795. BASF Reg. Doc. No. 2015/7003589.

DEFINITIONS AND ACRONYMS

Sample Set:	A group of samples that are extracted and cleaned up at the same time using the same method represented.
Untreated Sample:	A sample that has not been treated with the test substance.
Control Sample:	Usually an untreated sample used for fortification experiments (can be acquired from same study or from a different source).
Unknown Sample:	The samples with unknown residues.
Treated Sample:	A sample that has been treated with the test substance.
Blank:	Solvent, solution or mobile phase injected together with a sample set.
Reagent Blank:	A complete analysis conducted using solvents and reagents only in absence of any sample. Also known as blank of reagents or procedural blank. This sample is analyzed within the sample set in order to evaluate possible contamination on chemicals/reagents.
Procedural Recovery:	A control sample to which a known amount of analyte has been added before sample work up. This sample is then carried through the method and analyzed with the unknown samples in order to determine the reliability of the method.
Instrument Recovery:	A control sample which is carried through the method and to which a known amount of analyte has been added before injection. This sample is analyzed within the sample set in order to evaluate the matrix effect in the instrument.
Analytical Run:	A group of samples that undergo a determinative measurement on an analytical instrument (such as GC, HPLC, CE, GC/MS, or LC/MS/MS) in a defined and continuous sequence under identical instrumental conditions.
Limit of Quantitation (LOQ):	Lowest tested concentration of the analyte in a sample that can be determined with acceptable accuracy and precision according to the method.
Limit of Detection (LOD):	Concentration of analyte equivalent to a defined percentage of the limit of quantitation of the method (e.g 20% of LOQ). At this concentration, the analyte must be qualitatively detectable in sample matrix (analyte peak height at least 3-5 x baseline noise).

Typical Recovery Calculation for LC/MS/MS Quantitation

Sample No. 776699-19-4. Control surface water sample fortified at the LOQ with afidopyropen (and other analytes), Master Sheet No. 776699-19.

$$\text{Concentration of analyte (ng/mL)} = \frac{\text{peak area - intercept}}{\text{slope}}$$

	Afidopyropen
Peak Area =	42,756
Intercept =	716.7813
Slope =	1617688.8007
Conc. (ng/mL) =	0.0260

The concentration of analyte in ug/kg (ppb) is calculated as shown in equation:

$$\text{Residue [ug/kg]} = \frac{V_{end} \times C_A}{G \times A_F}$$

Where:

V_{end} = Final volume [mL]
C_A = Concentration of analyte as read from the calibration curve [ng/mL]
G = Weight of the sample extracted
A_F = Aliquotation factor

	Afidopyropen
V_{end} =	11.1 mL
A_F =	100%
G =	10.0
Conc. (ng/mL) =	0.0260
Residue (ug/kg) =	0.02885

Net residue (ug/kg of analyte) = Residue (ug/kg of analyte) - Residue in Control (ug/kg)

$$\text{Recovery of analyte (\%)} = \frac{\text{Residue (ug/kg of analyte) - Residue in Control (ug/kg)}}{\text{Amount Fortified (ug/kg)}} \times 100$$

	Afidopyropen
Amount fortified (ug/kg) =	0.0030
Residue (ug/kg) =	0.02885
Residue in control =	0.0000
%Recovery	96%

Use full calculator precision in any intermediate calculations. Round only the final value.