ABSTRACT

The purpose of this study was to demonstrate that BASF Analytical 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”, could be performed successfully at an outside facility with no prior experience with the method (Reference 1).

Principle of the method. The residues of BAS 440 I and its metabolites M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 are extracted from 10-mL of water by adding 1-mL of acetonitrile with 1% formic acid. An aliquot of the resulting extract is then directly used for LC-MS/MS determination.

Test conditions. For validation, untreated water samples were fortified with BAS 440 I and its metabolites and analyzed according to the established method validation guidelines. The analytical sets for each matrix typically consisted of a reagent blank, two controls, five replicates fortified with each analyte at the method limit of quantitation (LOQ) and five replicates fortified at a higher level, corresponding to 10× the LOQ. The mass transitions evaluated are listed below.

	Quantitation (*m/z*)	**Confirmation (*m/z*)**
BAS 440 I	*m/z* 594.2 → 202.3	*m/z* 594.2 → 148.2
M440I001	*m/z* 458.2 → 148.2	*m/z* 458.2 → 106.1 (**Tertiary Transition Ion:** *m/z* 458.2 → 202.1)
M440I002	*m/z* 526.2 → 148.1	*m/z* 526.2 → 202.1
M440I003	*m/z* 526.2 → 148.0	*m/z* 526.2 → 202.2
M440I005	*m/z* 524.2 → 148.3	*m/z* 524.2 → 80.0
M440I016	*m/z* 542.2 → 218.1	*m/z* 542.2 → 164.0
M440I024	*m/z* 610.5 → 218.1	*m/z* 610.5 → 122.0
M440I057	*m/z* 524.1 → 148.2	*m/z* 524.1 → 202.2

Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ was defined as the lowest fortification level tested. The LOQ for BAS 440 I and its metabolites in water matrices was 30 ppt. The LOD for BAS 440 I and its metabolites in water matrices was set at 6 ppt, which was 20% of the defined LOQ.

Selectivity. The method determines BAS 440 I, M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 residues in water matrices by LC-MS/MS. No interfering peaks were found at the retention times for BAS 440 I, M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057. The experiment to evaluate any potential matrix effects showed that the matrix load in the samples from each commodity had no significant influence on analysis (matrix effects < 20%); therefore, the validation samples were analyzed only using solvent-based calibration standard solutions (Reference 1).

Linearity. Acceptable linearity was observed for the standard range and the two mass transitions tested: The method-detector response was linear over the 0.0054–0.15 ng/mL range (r = ≥0.990), for all water matrix analyses.

1. INTRODUCTION

1.1 Scope of the Method

BASF Analytical Method No. D1505/02 was developed to determine the residues of BAS 440 I and its metabolites M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 in water matrices using LC-MS/MS at BASF Crop Protection in Research Triangle Park, North Carolina. This method was independently validated at ADPEN Laboratories, Inc.

The independent lab validation was conducted using two fortification levels at the limit of quantitation (30 ppt) and ten times of limit of quantitation (300 ppt) for all water matrices. For each fortification level and matrix, five replicates were analyzed. Additionally, one reagent blank and two replicates of unfortified samples were examined.

1.2 Principle of the Method

The water samples (10 mL) were fortified with acetonitrile with 1% formic acid and thoroughly mixed. An aliquot of resulting solution was analyzed to determine the residues of BAS 440 I and its metabolites using LC-MS/MS. The transitions for BAS 440 I and it metabolites were monitored in positive mode for primary and secondary quantification.

1.3 Specificity

To demonstrate the specificity of the analytical method, one additional mass transition was monitored simultaneous to the primary quantitation transition for analysis of all analytes. Primary, secondary and tertiary (M440I001) transitions for each analyte are listed below:

Analyte	Primary Transition Ion	Secondary Transition Ion
BAS 440 I	*m/z* 594.2 → 202.3	*m/z* 594.2 → 148.2
M440I001	*m/z* 458.2 → 148.2	*m/z* 458.2 → 106.1 (**Tertiary Transition Ion:** *m/z* 458.2 → 202.1)
M440I002	*m/z* 526.2 → 148.1	*m/z* 526.2 → 202.1
M440I003	*m/z* 526.2 → 148.0	*m/z* 526.2 → 202.2
M440I005	*m/z* 524.2 → 148.3	*m/z* 524.2 → 80.0
M440I016	*m/z* 542.2 → 218.1	*m/z* 542.2 → 164.0
M440I024	*m/z* 610.5 → 218.1	*m/z* 610.5 → 122.0
M440I057	*m/z* 524.1 → 148.2	*m/z* 524.1 → 202.2

The method was able to accurately determine residues of BAS 440 I and its metabolites and no interference was observed at the retention time of the analyte peaks. No matrix suppression or enhancement was found to affect the analytes in water.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems

The test systems considered in this study were surface and drinking water.

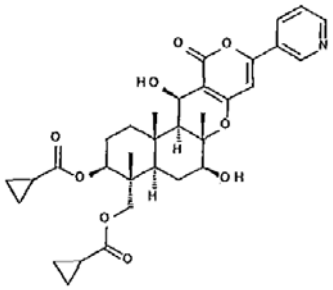
The control samples were provided by BASF. The water samples were received on December 4, 2015. Upon arrival at the laboratory, the samples were opened, inspected, and checked

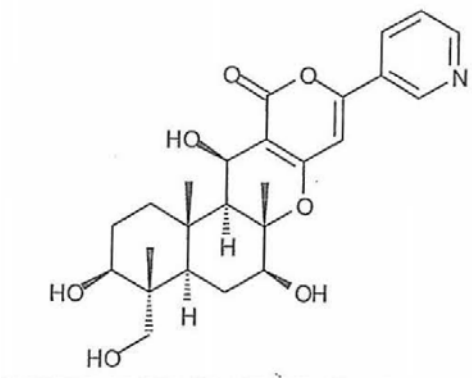
against enclosed shipping forms. The test systems were received frozen and stored under frozen conditions at all times, unless necessary for laboratory analysis. The test systems were characterized at AGVISE Laboratories (604 Highway 15 West, Northwood, ND 58267). A copy of the characterization data for the sample is provided in Appendix E.

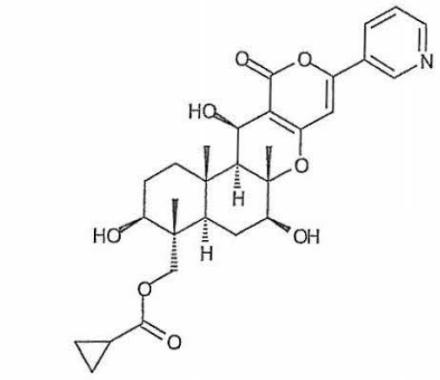
2.2 Test and Reference Substances

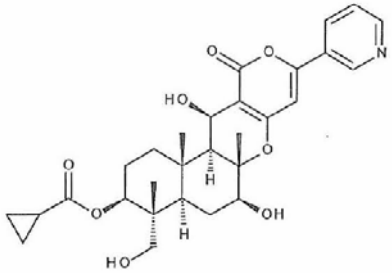
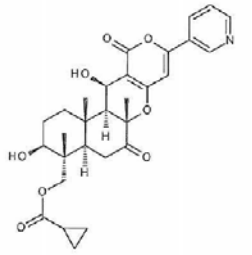
The standard substances were stored in a freezer (≤ -5°C) until use. BASF has retained a reserve sample of this chemical, and has documentation specifying the location of the synthesis and characterization information available at BASF Crop Protection, Research Triangle Park, North Carolina.

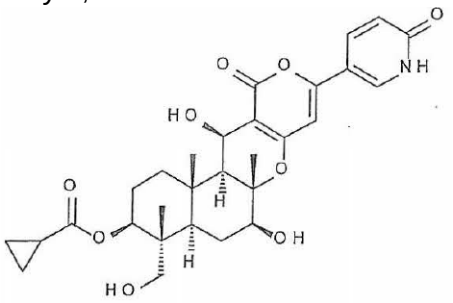
The BAS 440 I, M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 reference substances were provided by the sponsor and received on August 12, 2015 and September 23, 2015. The certificate of analysis for all substances is presented in Appendix B. A detailed summary of the reference substances is presented below.

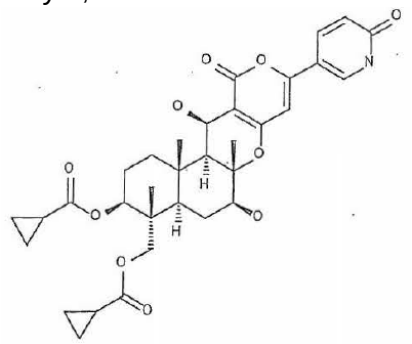
BASF Code Name:	BAS 440 I
Common Name:	Afidopyropen
Batch Number:	COD-002022
BASF Registry Number:	5599022
CAS Number:	915972-17-7
IUPAC Name:	[(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-3 (cyclopropanecarbonyloxy)-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1,2,3,4,4a,5,6,6a, 12a,12b-decahydro 11H,12H-benzo[f]pyrano[4,3-b]chromen-4-yl]methyl cyclopropanecarboxylate
Molecular Formula:	$C_{33}H_{39}NO_9$
Molecular Weight:	593.7 g/mol
Purity:	94.4%
Expiration Date:	November 30, 2016
Chemical Structure:	

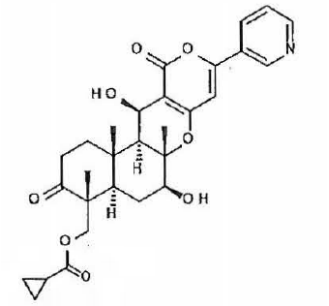
BASF Code Name:	M440I001
Batch Number:	L82-66
BASF Registry Number:	5741530
IUPAC Name:	(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-3,6,12-trihydroxy-4-(hydroxymethyl)-4,6a,12b-trimethyl-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f]pyrano[4,3-b]chromen-11-one
Molecular Formula:	$C_{25}H_{31}NO_7$
Molecular Weight:	457.5 g/mol
Purity:	93.9%
Expiration Date:	February 1, 2016
Structural Formula:	

BASF Code Name:	M440I002
Batch Number:	L82-67
BASF Registry Number:	5741532
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]methyl cyclopropanecarboxylate
Molecular Formula:	$C_{29}H_{35}NO_8$
Molecular Weight:	525.6 g/mol
Purity:	92.5%
Expiration Date:	February 1, 2016
Structural Formula:	

BASF Code Name:	M440I003
Batch Number:	L82-72
BASF Registry Number:	5741533
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
Molecular Formula:	$C_{29}H_{35}NO_8$
Molecular Weight:	525.6 g/mol
Purity:	98.6%
Expiration Date:	September 1, 2016
Structural Formula:	
BASF Code Name:	M440I005
Batch Number:	L82-73
BASF Registry Number:	5824382
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
Molecular Formula:	$C_{29}H_{33}NO_8$
Molecular Weight:	523.6 g/mol
Purity:	90.9%
Expiration Date:	March 1, 2016
Structural Formula:	

BASF Code Name:	M440I016
Batch Number:	L82-148
BASF Registry Number:	5845597
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
Molecular Formula:	$C_{29}H_{35}NO_9$
Molecular Weight:	541.6 g/mol
Purity:	88.9%
Expiration Date:	May 1, 2016
Structural Formula:	

BASF Code Name:	M440I024
Batch Number:	L82-149
BASF Registry Number:	5886215
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]methyl cyclopropanecarboxylate
Molecular Formula:	$C_{33}H_{39}NO_{10}$
Molecular Weight:	609.7 g/mol
Purity:	91.3%
Expiration Date:	May 1, 2016
Structural Formula:	

BASF Code Name:	M440I057
Batch Number:	L82-164
BASF Registry Number:	6010129
IUPAC Name:	[(4R,4aR,6S,6aS,12R,12aS,12bS)-6,12-dlhydroxy-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
Molecular Formula:	$C_{29}H_{33}NO_8$
Molecular Weight:	523.6 g/mol
Purity:	97.4%
Expiration Date:	January 1, 2017
Structural Formula:	

2.3 Test System

Surface and drinking water samples were provided and homogenized by BASF. The water samples were sent from BASF Crop Protection, Inc. on December 3, 2015 and received by ADPEN Laboratories, Inc. on December 4, 2015.

The Laboratory Information Management System (LIMS) provided a unique laboratory analysis code (e.g., 151207003-001) for each sample and is cross-referenced on the detailed reports to the assigned unique sample number.

3. ANALYTICAL METHOD

BASF Analytical 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" was used for the analysis of the samples.

The residues of BAS 440 I and its metabolites M440I001, M440I002, M440I003, M440I005, M440I016, M440I024, and M440I057 are extracted from 10-mL of water by adding 1 mL of acetonitrile with 1% formic acid. An aliquot is taken from the resulting extract and directly used for LC-MS/MS determination. Any necessary dilutions were prepared using water:acetonitrile with 0.1% formic acid (90:10, v/v). Instrument parameters are described in Table 36.
The primary (quantitative) and secondary (confirmatory) transition ions monitored are presented below:

Table 36 Instrument Conditions and Parameters

HPLC Conditions			
Chromatographic System:	Agilent 1290 UPLC		
Column:	Acquity UPLC BEH Phenyl; 1.7 µm, 2.1 × 100 mm; S/N 01403526518380		
Temperature:	45 °C		
Flow rate (µL/min)	600		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.00	85.0	15.0
	0.05	85.0	15.0
	8.50	75.0	25.0
	10.25	55.0	45.0
	11.00	5.0	95.0
	11.95	5.0	95.0
	12.00	85.0	15.0
	12.50	85.0	15.0
Mobile Phase A:	0.1% formic acid in omni water		
Mobile Phase B:	0.1% formic acid in acetonitrile		
Injection Volume:	100 µL		

MS/MS Conditions						
Detection System:	AB SCIEX 6500 QT					
Ionization:	Turbo Spray					
Polarity:	Positive					
Curtain gas (CUR):	10.00					
Temperature (TEM):	500 °C					
Collision gas setting (CAD):	8.00					
GS1:	30.00					
GS2:	30.00					
Entrance potential (EP):	7.00					
Scan type:	MRM					
MRM Conditions	Transition (m/z)	Dwell (msec)	DP	CE	CXP	Retention Time (min)
BAS 440 I Reg. No. 5599022	594.200 → 202.300	20	116.80	46.20	6.20	11.0
	594.200 → 148.200			85.90	13.20	
M440I001 Reg. No. 5741530	458.200 → 148.200	100	106.60	45.00	7.10	1.5
	458.200 → 106.100			70.00	17.50	
	458.200 → 202.100			38.60	15.90	
M440I002 Reg. No. 5741532	526.200 → 148.100	100	17.90	50.30	8.80	4.7
	526.200 → 202.100	50		41.00	13.70	
M440I003 Reg. No. 5741533	526.210 → 148.000	20	81.10	48.90	8.90	8.5
	526.210 → 202.200			43.80	10.40	
M440I005 Reg. No. 5824382	524.200 → 148.300	50	71.50	55.10	6.90	7.2
	524.200 → 80.000	20		120.20	14.00	

Table 36 Instrument Conditions and Parameters (continued)

MS/MS Conditions (continued)						
MRM Conditions	Transition (m/z)	Dwell (msec)	DP	CE	CXP	Retention Time (min)
M440I016 Reg. No. 5845597	542.200 → 218.100	200	86.90	44.60	11.90	8.2
	542.200 → 164.000			54.90	10.30	
M440I024 Reg. No. 5886215	610.500 → 218.100	200	80.00	43.80	13.10	10.8
	610.500 → 122.000			94.70	19.30	
M440I057 Reg. No. 6010129	524.100 → 148.200	50	112.20	49.30	9.10	6.9
	524.100 → 202.200	20		42.10	11.80	

Appendix A. Recommendations for BASF Analytical Method D1505/02

The following recommendations should be incorporated into the technical procedure:

1. Section 3.3: Weighing and Fortification

   It is suggested that all non-fortification samples have 100 µL of acetonitrile added so that both samples and fortifications have a final volume of 11.1 mL and the sample solvent composition is the same.

2. Section 4.2.1: Instrumentation and Conditions

   The instrument method lists *m/z* 458.2 → 106.1 as the confirmation ion for M440I001. It is suggested that the mass transition used for confirmatory purposes be *m/z* 458.2 → 202.1 since it has approximately five times better response than the listed transition.

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).

1 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.

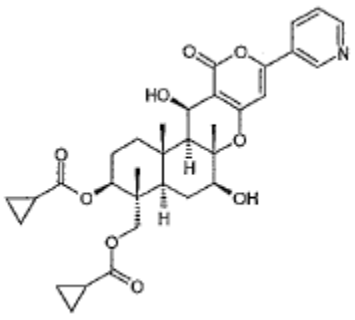
2 MATERIALS

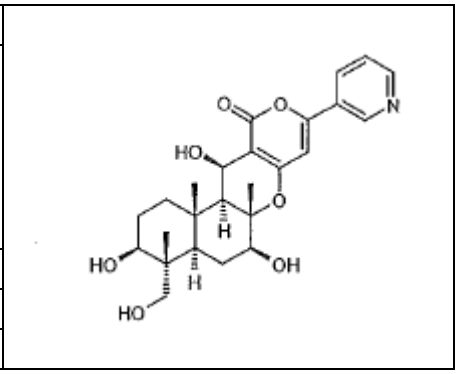
2.1 Safety

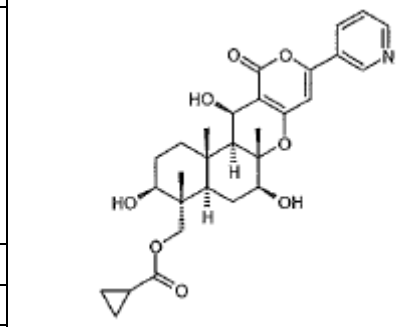
The test and reference items, as well as the chemicals required for this analysis, should be handled in accordance with good industrial hygiene and safety practice. Avoid contact with the skin, eyes and clothing. Wearing of closed work clothing is recommended. Remove contaminated clothing. Store work 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.

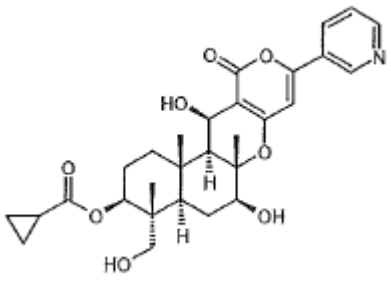
2.2 Test and Reference Items

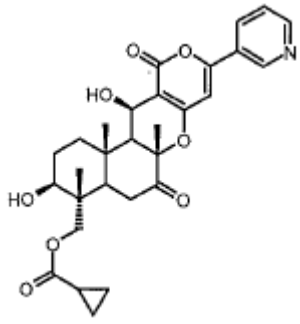
Test and reference items should be stored according to the information provided in the certificate of analysis.

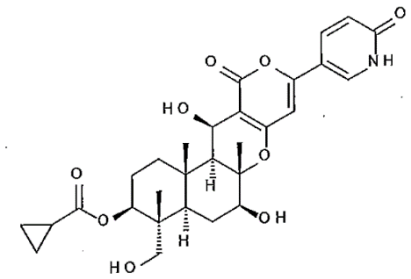
BAS-Code	440 I	
Common Name	Afidopyropen	
IUPAC Name	[(3S, 4R, 4aR, 6S, 6aS, 12R, 12aS, 12bS)-3-(cyclopropanecarbonyloxy)-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-11H,12H-benzo[flpyrano[4,3-b]chromen-4-yl] methylcyclopropanecarboxylate	
BASF Reg. No.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

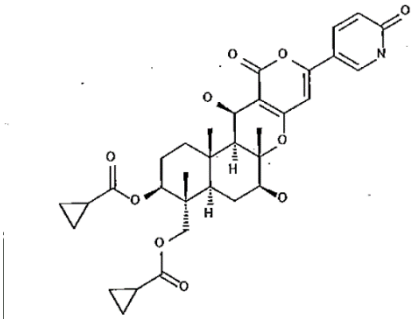
BAS-Code	M440I001	
IUPAC Name	(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-3,6,12-trihydroxy-4-(hydroxymethyl)-4,6a,12b-trimethyl-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f] pyrano[4,3-b]chromen-11-one	
BASF Reg. No.	5741530	
Molecular Formula	$C_{25}H_{31}NO_7$	
Molecular Weight	457.5	

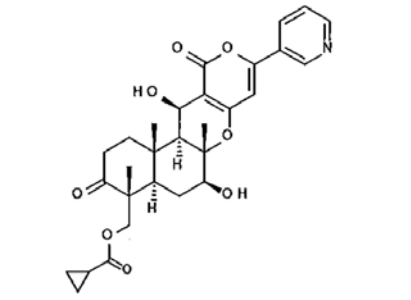
BAS-Code	M440I002	
IUPAC Name	[(3S,4R,4aR,6S,6aS,12R,12aS,12bS)-3,6,12-trihydroxy-4,6a,12b-trimethyl-11-oxo-9-(pyridin-3-yl)-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-2H,11H-benzo[f] pyrano[4,3-b]chromen-4-yl] methylcyclopropanecarboxylate	
BASF Reg. No.	5741532	
Molecular Formula	$C_{29}H_{35}NO_8$	
Molecular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

2.3 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.

2.4 Reagents

2.4.1 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.

2.4.2 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.

2.4.3 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.

2.4.4 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 (15 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)

3 ANALYTICAL PROCEDURE

3.1 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.

3.2 Sample Storage

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

3.3 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.

3.4 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.

3.5 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.

3.6 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%).

3.7 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.

4 QUANTIFICATION AND CALCULATION

4.1 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.

4.2 Instrumental analysis

4.2.1 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	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. 10.7 min
BAS 440 I	594 → 148** 594 → 202	positive	approx. 10.4 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.

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.

4.2.2 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.

4.2.3 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]** $= \dfrac{\text{Response} - \mathit{Intercept}}{\mathit{Slope}} = C_A$

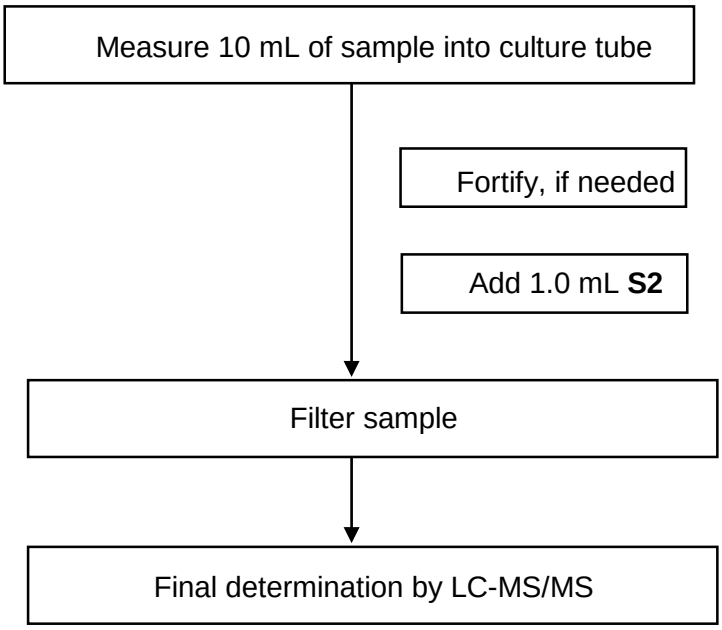
II. **Residue [ng/Lm ppt]** $= \dfrac{V_{end} \times C_A \times 1000}{G \times A_F}$

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

5 FLOWCHART



6 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.

7 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.

8 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.

9 APPENDIX

9.1 Example of Calculation

Example: BAS 440 I, 394 → 148; surface water sample fortified at 30 ng/L:

Concentration in the final volume [ng/mL]

$$\textbf{Concentration [ng/mL]} = \frac{\text{Response} - \textit{Intercept}}{\textit{Slope}} = C_A$$

Residue in the sample [ng/L]

$$\textbf{Residue [ng/L, ppt]} = \frac{V_{end} \times C_A \times 1000}{G \times A_F}$$

$$\textbf{Recovery \%} = \frac{\text{Residue in fortified sample} - \text{Residue in control} \times 100}{\text{Amount of analyte fortified}}$$

The following values were used in this calculation:

Response of fortified sample	16036
Response of control sample	0
Slope:	587000
Intercept:	-290
Sample Weight (G):	10 g (10 mL)
Final Volume (V_{end}):	11.1 mL
Aliquotation factor A_F:	1.0 (= 100%)
Conversion factor mL → L:	1000
D_{H2O} (Density of Water)	1 g/mL

$$\textbf{Concentration (ng/mL)} = \frac{16036 + 290}{587000} = 0.0278\ ng/mL$$

Residue (ng/L)

$$= \frac{11.1 ml \times 0.0278\ ng/mL \times 1000}{10\ mL \times 1} = 30.86\ ng/L$$

$$\textbf{Recovery \%} = \frac{(30.86\ ng/L - 0.00000\ ng/L) \times 100}{30.0\ ng/L} = 102.9\%$$