1. INTRODUCTION

Background and Objective:

The objective of this study was to develop and to validate an analytical methods for the determination of pendimethalin and its metabolites M455H001, P48 and Reg. No. 4061757 in ground and surface water with a target limit of quantitation (LOQ) of 0.02 µg/L per analyte.

2. EXPERIMENTAL

2.1 Test System

Ground Water

The ground water was collected on 29-Nov-12 from a local well in Herbrechtingen, located in Southern Germany. The water was characterized for physical and chemical properties by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP):

Total water hardness:	2.65 mmol/L (Deutsche Härtegrade, 14.8°d) German degrees of hardness
TOC (total organic carbon, EN 1484:1997):	3.10 mg/L
DOC (dissolved organic carbon, EN 1484: 1997):	2.5 mg/L
pH (DIN 38 404-C5):	7.69
Silt content (EN 872 Whatman GF 6):	12.5 mg/L
Electrical conductivity at 25°C (EN 27888: 1993):	750 µS/cm

Surface Water

The surface water was collected on 29-Nov-12 from the River Brenz in Herbrechtingen, located in Southern Germany. The water was characterized for physical and chemical properties by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP):

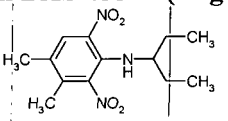
Total water hardness:	2.30 mmol/L (Deutsche Härtegrade, 12.9°d)
TOC (total organic carbon, EN 1484:1997):	3.10 mg/L
DOC (dissolved organic carbon, EN 1484: 1997):	3.0 mg/L
pH (DIN 38 404-C5):	7.72
Silt content (EN 872 Whatman GF 6):	< 0.1 mg/L
Electrical conductivity at 25°C (EN 27888: 1993):	580 µS/cm

Water was stored at room temperature in the dark when not used.

2.2 Analytical Test and Reference Item

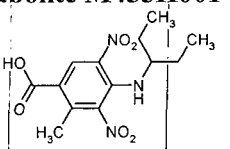
The following standards provided by the Sponsor (see Appendix 1) were used as test / reference items:

Pendimethalin BAS 455 H (Reg. No. 900072)



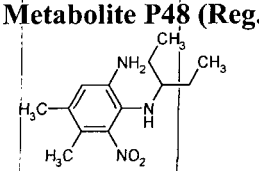
IUPAC name: *N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine
Empirical formula: $C_{13}H_{19}N_3O_4$ Molar mass: 281.3 g/mol

Pendimethalin Metabolite M455H001 (Reg. No. 4108474)



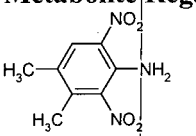
IUPAC name: 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitrobenzoic acid
Empirical formula: $C_{13}H_{17}N_3O_6$ Molar mass: 311.3 g/mol

Pendimethalin Metabolite P48 (Reg. No. 4295966)



IUPAC name: 4,5-dimethyl-3-nitro-N^2-(pentan-3-yl)benzene-1,2-diamine
Empirical formula: $C_{13}H_{21}N_3O_2$ Molar mass: 251.3 g/mol

Pendimethalin Metabolite Reg. No. 4061757



IUPAC name: 2,6-dinitro-3,4-dimethylaniline
Empirical formula: $C_8H_9N_3O_4$ Molar mass: 211.2 g/mol

2.3 Analytical Method

2.3.1 Apparatus

2.3.1.1 Laboratory Equipment

XP205DR balance, Mettler Toledo.
Transsonic 460 bath, Elma Hans Schmidbauer.
Vortex mixer REAX top, Heidolph.
Typical glassware and laboratory equipment.
All glassware was cleaned in a laboratory dishwasher and air-dried before use.

2.3.1.2 LC-MS System

Pendimethalin and its metabolites M455H001 and P48 were quantified using:

Applied Biosystems API 4000 LC/MS system (vacuum solvent degasser, binary LC pump, column oven) and CTC Analytics HTC-Pal autosampler. AB Sciex API 4000 triple quadrupole LC/MS/MS system with Turbolonspray source. Analyst 1.4.2 instrument control and data acquisition software.

2.3.1.3 GC-MS System

Pendimethalin metabolite Reg. No. 4061757 was quantified using:
Thermo TSQ Quantum GC/MS System equipped with TriPlus AS autosampler, Trace Ultra GC gas chromatograph, temperature programmable PTV and split/splitless injector, digital pressure and flow control DPFC.

2.3.2 Solvents and Chemicals

Millipore Water (PTRL Europe)

Acetone, for pesticide residue analysis, Promochem

Acetonitrile, HPLC Grade, Promochem

Methanol, HPLC Grade, Promochem

Toluene, for pesticide residue analysis, Promochem

n-Pentan, for pesticide residue analysis, Promochem

Dichloromethane, for pesticide residue analysis, Promochem

Formic acid (98%-100%) (Sigma Aldrich)

Acetic acid (98%-100%) (Merck)

Ammonia solution (25%) (Merck)

Sodium sulphat anhydrous (p.a. grade 99%) (Merck)

2.3.3 Preparation of Standard Solutions

Stock solutions of pendimethalin and its metabolites M455H001, P48 and Reg. No. 4061757 were prepared, such as described in the following table (e.g.):

Substance name	Weight [mg]	Dissolve in [mL]	Solvent	Obtain [mg/mL] (*)
Pendimethalin (purity 99.2 %)	10.08	10	Acetone	1.0
M455H001 (purity 99.6 %)	10.04	10	Acetone	1.0
P48 (purity 98.0 %)	10.26	20.118	Methanol	0.50
Reg.No.4061757 (purity 96.1 %)	10.53	10.119	Acetone	1.0

(*): Purity taken into account.

Separate fortification solutions for pendimethalin and its three metabolites, with concentrations of 1.0 µg/mL, 0.10 µg/mL and 0.010 µg/mL, were prepared in methanol and acetone (for Reg. No. 4061757) by accurate dilution of the stock solutions.

Calibration solutions containing pendimethalin and M455H001 were prepared by volumetric dilution with acetonitrile/water [50/50 (v/v)] + 0.1% formic acid to obtain concentrations of 10 µg/mL and 0.10 µg/mL (intermediate solutions), and 0.025, 0.050, 0.10, 0.25, 1.0, 2.5 and 10 ng/mL.

Calibration solutions containing P48 were prepared by volumetric dilution with acetonitrile/water [50/50 (v/v)] to obtain concentrations of 1.0 µg/mL (intermediate solution), and 0.010, 0.025, 0.10, 0.50, 1.0, 2.5 and 5.0 ng/mL.

Calibration solutions containing the metabolite Reg. No. 4061757 were prepared by volumetric dilution with toluene to obtain concentrations of 1.0 µg/mL (intermediate solution), and 1.0, 5.0, 10, 50 and 100 ng/mL.

Due to a significant matrix effect matrix-matched standards were prepared for pendimethalin and its metabolites P48 and Reg. No. 4061757 for evaluation of the specimens.

Matrix-matched standards were prepared by adding appropriate volumes of respective calibration solutions in solvent or matrix-matched-standards to final volume of the untreated control specimens. The preparation is exemplified for pendimethalin in the following table:

Solution Number	Use solution of pendimethalin [ng/mL]	Pipette [µL]	Add of final volume from untreated control [µL]	Obtain for matrix-matched standard [ng/mL]
1	100 (calibration solution in solvent)	50	450	10
2	25 (calibration solution in solvent)	50	450	2.5
3	10 (solution 1)	50	450	1.0
4	2.5 (solution 2)	50	450	0.25
5	0.25 (solution 4)	100	400	0.050

Matrix-matched standards were prepared by adding appropriate volumes of respective calibration solutions in solvent or matrix-matched-standards to final volume of the untreated control specimens. The preparation is exemplified for metabolite P48 in the following table:

Solution Number	Use solution of P48 [ng/mL]	Pipette [µL]	Add of final volume from untreated control [µL]	Obtain for matrix-matched standard [ng/mL]
1	1000 (calibration solution in solvent)	10	990	10
2	10 (solution 1)	125	375	2.5
3	10 (solution 1)	50	450	1.0
4	2.5 (solution 2)	50	450	0.25
5	1.0 (solution 3)	25	475	0.050

Matrix-matched standards were prepared by adding appropriate volumes of calibration solutions in solvent or matrix-matched-standards to final volume of the respective untreated control specimens. The preparation is exemplified for metabolite Reg. No. 4061757 in the following table:

Solution Number	Use solution of Reg. No. 4061757 [ng/mL]	Pipette [µL]	Add of final volume from untreated control [µL]	Obtain for matrix-matched standard [ng/mL]
1	1000 (calibration solution in solvent)	40	360	100
2	100 (solution 1)	100	100	50
3	100 (solution 1)	20	180	10
4	100 (solution 1)	10	190	5.0
5	10 (solution 3)	20	180	1.0

All standard solutions were stored refrigerated in amber glass bottles when not in use.

2.3.4 Stability of Standard Solutions and Extracts

Stability of standard solutions in solvent is proven by comparing two calibration solutions in solvent diluted from an old and a freshly prepared stock solution (refrigerated storage; see Table 9). Deviation was < 20 %.

Stability in the final sample volume from both water types was demonstrated by re-injecting selected samples after 4 or 5 days of either refrigerator or freezer storage as shown in Table 10 for pendimethalin and its metabolites M455H001, P48 and Reg. No. 4061757.

After refrigerated or frozen storage, the average recoveries for pendimethalin and its metabolites M455H001, P48 and Reg. No. 4061757 in the final sample volume of both water types were all still within the acceptable range of 70% to 110%. Stability under both storage conditions was considered sufficiently proven. For the metabolite Reg. No. 4061757 the average recoveries in surface water were between 65% and 74%.

2.3.5 Sample Analysis

2.3.5.1 Sample Preparation and Fortification:

For pendimethalin and its metabolite M455H001 and P48 measure 25.0 mL of water sample into 50 mL centrifuge tube.

For pendimethalin metabolite Reg. No. 4061757 measure 250 mL of water sample into 500 mL separatory funnel.

For fortifications, add 50 µL of the appropriate spike solution (0.010 µg/mL, 0.10 µg/mL or 1.0 µg/mL, for example) to the untreated water sample.

2.3.5.2 Sample Extraction for Pendimethalin:

1. Extract the water with 5 mL of pentane, by shaking 2 minutes.
2. Allow phases to separate
3. Pipette the upper phase into a 25 mL tapered flask.

4. Repeat the extraction two more times for a total of three extractions, each time with 5 mL of pentane and combine in the 25 ml tapered flask.
5. Evaporate the combined pentane extract to less than 1.0 mL using a rotary vacuum evaporator with a maximum bath temperature of about 40 °C (not to dryness!). Evaporate with a stream of nitrogen to dryness.
6. Dissolve the residues immediately with 1 mL of acetonitrile. Add 1 mL of water + 0.2% formic acid (V_{End} = 2.0 mL) mix and transfer the final volume into an autosampler vial for LC/MS/MS analysis.
7. Analyse the sample by LC-MS/MS.

The flow chart of the analytical method is outlined in APPENDIX 2.

2.3.5.3 Sample Extraction for M455H001:

1. Acidify the water sample with of 0.50 mL of acetic acid.
2. Extract the water with 5 mL of pentane, by shaking for 2 minutes.
3. Allow the phases to separate.
4. Pipette the upper phase into a 25 mL tapered flask.
5. Repeat the extraction two more times for a total of three extractions, each time with 5 mL of pentane and combine in the 25 ml tapered flask.
6. Evaporate the combined pentane extract to less than 0.5 mL using a rotary vacuum evaporator with a maximum bath temperature of about 40 °C (not to dryness!). Evaporate with a stream of nitrogen to dryness.
7. Dissolve the residues immediately with 1 mL acetonitrile. Add 1 mL water + 0.2% formic acid (V_{End} = 2.0 mL) mix and transfer the final volume into an autosampler vial for LC/MS/MS analysis.
8. Analyse the sample by LC-MS/MS.

The flow chart of the analytical method is outlined in APPENDIX 2.

2.3.5.4 Sample Extraction for P48:

1. Alkalize the water sample with 1 mL of ammonia solution (25%).
2. Extract the water with 5 mL of pentane, by shaking 2 minutes.
3. Allow phases to separate.
4. Pipette the upper phase into a 25 mL tapered flask.
5. Repeat the extraction two more times for a total of three extractions, each time with 5 mL of pentane and combine in the 25 mL tapered flask.
6. Evaporate the combined pentane extract to less than 1.0 mL using a rotary vacuum evaporator with a maximum bath temperature of about 40 °C (not to dryness!). Evaporate with a stream of nitrogen to dryness.

7. Dissolve the residues immediately with 1 mL of acetonitrile. Add 1 mL of water (V_{End} = 2.0 mL) mix and transfer the final volume into an autosampler vial for LC/MS/MS analysis.
8. Analyse the sample by LC-MS/MS.

The flow chart of the analytical method is outlined in APPENDIX 2.

2.3.5.5 Sample Extraction for Reg. No. 4061757:

1. Extract the water with 25 mL of dichloromethane, by shaking 2 minutes.
2. Allow phases to separate.
3. Drain the lower phase into a 150 mL beaker.
4. Repeat the extraction two more times for a total of three extractions, each time with 25 mL of dichloromethane and combine in the 150 mL beaker.
5. Filter the dichloromethane extract into a 100 mL tapered flask, passing the solution through a bed of 25 g anhydrous sodium sulphate (wetted with dichloromethane) supported in a glass funnel equipped with a glass wool plug. It is recommended to test the sodium sulphate for possible adsorption of the Reg. No. 4061757 residues.
6. Wash the sodium sulfate two times with each 10 mL of dichloromethane.
7. Evaporate the combined dichloromethane extract to less than 5 mL using a rotary vacuum evaporator with a maximum bath temperature of about 40 °C.
8. Transfer the residues quantitatively into a 25 mL tapered flask by using 3 x 5 mL dichloromethane.
9. Add about 1 mL of toluene as keeper and concentrate the extract to less than 1.0 mL using a rotary evaporator (water bath temperature about 40°C, not to dryness!).
10. Adjust the final volume in toluene (V_{End} = 1.0 mL) gravimetrically, sonicate and transfer the final volume into an autosampler vial.
11. Analyse the sample by GC-MS/MS.

The flow chart of the analytical method is outlined in APPENDIX 2.

2.4 MS/MS Analysis

2.4.1 LC/MS/MS Analysis

Pendimethalin (BAS 455 H) and metabolite M455H001 (Reg. No. 4108474) were quantified using the following LC/MS/MS system described below:

LC System	Agilent 1200 SL HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler
LC Column	Thermo Betasil C18 column (length: 100 mm, i.d.: 2.1 mm, particle size: 5 µm, oven temp.: 35°C.
LC Injection Volume	50 µL

LC Method	Solvent A: Water containing 0.1% formic acid Solvent B: Methanol containing 0.1% formic acid Program:			
	Time (min)	Flow rate (mL/min)	% A	% B
	0.00	0.60	66	34
	2.00	0.60	26	74
	4.00	0.60	10	90
	6.50	0.60	10	90
	6.60	0.60	0	100
	10.00	0.60	0	100
	10.10	0.60	66	34
	12.00	0.60	66	34
Retention Time	≈ 5.1 minutes for pendimethalin (BAS 455 H) and ≈ 5.0 minutes for metabolite M455H001 (Reg. No. 4108474)			
MS/MS System	Applied Biosystems MDS Sciex API 4000 triple quadrupole LC/MS/MS system with TurboIonspray (ESI) source			
Ion Source Conditions ESI Positive Polarity				
Pendimethalin	Source temperature:	450°C		
	Gas supply (GS 1):	40 (arbitrary units)		
	Gas supply (GS 2).	70 (arbitrary units)		
	Curtain gas:	25 (arbitrary units)		
	CAD gas:	5 (arbitrary units)		
	Entrance potential:	10 V		
	IonSpray voltage:	5200 V		
	Resolution:	Q1: Unit, Q3: Unit		
Ion Source Conditions ESI Negativ Polarity				
M455H001	Source temperature:	450°C		
	Gas supply (GS 1):	40 (arbitrary units)		
	Gas supply (GS 2).	70 (arbitrary units)		
	Curtain gas:	25 (arbitrary units)		
	CAD gas:	5 (arbitrary units)		
	Entrance potential:	-10 V		
	IonSpray voltage:	-4500 V		
	Resolution:	Q1: Unit, Q3: Unit		
MS/MS Conditions				
Pendimethalin	MS/MS transition for quantification:	282 *m/z* > 194 *m/z* (quantification)		
	Collision energy (CE):	25 V		
	Cell exit potential (CXP):	12 V		
	Dwell time:	200 ms		
	Declustering potential (DP):	36 V		
	MS/MS transition for confirmation:	282 *m/z* > 212 *m/z* (confirmation)		
	Collision energy (CE):	15 V		
	Cell exit potential (CXP):	14 V		
	Dwell time:	200 ms		
	Declustering potential (DP):	36 V		
M455H001	MS/MS transition for quantification:	310 *m/z* > 266 *m/z* (quantification)		
	Collision energy (CE):	-14 V		
	Cell exit potential (CXP):	-7 V		
	Dwell time:	200 ms		
	Declustering potential (DP):	-50 V		
	MS/MS transition for confirmation:	310 *m/z* > 236 *m/z* (confirmation)		
	Collision energy (CE):	-20 V		
	Cell exit potential (CXP):	-13 V		
	Dwell time:	200 ms		
	Declustering potential (DP):	-50 V		

Pendimethalin metabolite P48 (Reg. No. 4295966) was quantified using the following LC/MS/MS system described below:

LC System	Agilent 1200 SL HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler			
LC Column	Phenomenex Luna C_{18} column: Length: 50 mm, i.d.: 2 mm, particle size: 5 µm, oven temp.: 35°C.			
LC Injection Volume	50 µL			
LC Method	Solvent A: Water containing 0.1 % formic acid Solvent B: Methanol containing 0.1 % formic acid Program:			
	Time (min)	Flow rate (mL/min)	% A	% B
	0.00	0.60	60	40
	2.00	0.60	60	40
	2.10	0.60	0	100
	6.00	0.60	0	100
	6.10	0.60	60	40
	8.00	0.60	60	40
Retention Time	≈ 3.9 min for pendimethalin metabolite P48			
MS/MS System	Applied Biosystems MDS Sciex API 4000 triple quadrupole LC/MS/MS system with TurboIonspray (ESI) source.			
Ion Source Conditions ESI Positive Polarity				
P48	Source temperature: 450°C Gas supply (GS 1): 40 (arbitrary units) Gas supply (GS 2) 70 (arbitrary units) Curtain gas: 25 (arbitrary units) CAD gas: 5 (arbitrary units) Entrance potential: 10 V IonSpray voltage: 4500 V Resolution: Q1: Unit, Q3: Unit			
MS/MS Conditions				
P48	MS/MS transition for quantification: 252 *m/z* > 119 *m/z* (quantitation) Collision energy (CE): 31 V Cell exit potential (CXP): 10 V Dwell time: 500 ms Declustering potential (DP): 36 V MS/MS transition for confirmation: 252 *m/z* > 146 *m/z* (confirmation) Collision energy (CE): 25 V Cell exit potential (CXP): 12 V Dwell time: 500 ms Declustering potential (DP): 36 V			

See Figure 41 through Figure 43 for the product ion spectra of pendimethalin and its metabolites M455H001 and P48.

The quantitative determination was carried out by external standardization using calibration standards in solvent (only for M455H001) and calibration standards in matrix (for pendimethalin and P48). Calibration functions ranging from 0.050 to 10 ng/mL (≥ 5 levels: 0.050, 0.25, 1.0, 2.50 and 10 ng/mL) were used to evaluate the final sample volume (exemplified in Figure 1 through Figure 6). For evaluation of the stability in the final volume a calibration function ranging from 0.050 ng/mL or 0.10 ng/mL to 10 ng/mL with 5 levels

was used. Linear regression equations were generated with 1/x weighting, resulting in calibration functions with correlation coefficients of $r > 0.99$.

Representative LC-MS/MS ion chromatograms of calibration solutions in solvent and calibration solutions in matrix and of final sample volumes of fortified and control specimens are presented in Figure 9 through Figure 32.

2.4.2 GC/MS/MS Analysis

Pendimethalin metabolite Reg. No. 4061757 was quantified using the following GC/MS/MS method in the negative chemical ionization (NCI) mode:

GC/MS System	Thermo TSQ Quantum GC/MS System equipped with TriPlus AS autosampler, Trace Ultra GC gas chromatograph, temperature programmable PTV and split/splitless injector, digital pressure and flow control DPFC.
Carrier Gas	Helium at 1.5 mL/min (constant flow).
GC Injection Technique	Isothermal injection at 250 °C, splitless.
GC Injection Volume	2.0 µL.
GC Capillary Column	Agilent VF-5MS (30 m length, 0.32 mm inner diameter, 0.25 µm film thickness).
Oven Temperature Program	90°C, 2 min hold, ramp with 50°C/min to 150°C, then with 10°C to 200°C, then with 100°C to 310°C, 4 min hold.
Ion Source Conditions	Emission current: 120 µA Electron energy: -100 eV CI Gas: Methane with constant flow at 2.0 mL/min Negative Chemical Ionisation
MS Conditions	Selected reaction monitoring (SRM) mode, monitoring the following fragment ions: Reg. No. 4061757: 211 m/z -> 193 m/z used for quantification 211 m/z -> 194m/z used for confirmation. (Q2) Collision Gas Pressure: 1.4 mTorr Collision Energy (CE): 15 eV
Retention Time	Reg. No. 4061757: ~ 7.9 minutes

Figure 44 presents the full scan product ion NCI spectrum of pendimethalin metabolite Reg. No. 4061757 showing the selection of the two structurally significant product ions used for the GC/MS/MS quantification/confirmation and the parent ion.

For Reg. No. 4061757, quantification was carried out by external standardization using calibration standards in matrix.

Calibration functions ranging from 1.0 ng/mL to 100 ng/mL (≥ 5 levels: 1.0, 5.0, 10, 50 and 100 ng/mL) were used to quantify Reg. No. 4061757 recoveries (Figure 7 and Figure 8). For evaluation of the stability in the final volume calibration functions ranging from 10 ng/mL to 100 ng/mL with 3 levels injected in duplicates were used. Linear regression equations were generated with 1/x weighting, resulting in calibration functions with regression coefficients of $r^2 > 0.99$.

Representative GC/MS/MS ion chromatograms of calibration solutions in matrix and final sample volumes of fortified and control specimens for Reg. No. 4061757 are presented in Figure 33 through Figure 40.

2.5 Calculations

2.5.1 LC/MS/MS Analysis for Pendimethalin, M455H001 and P48

Recovery results derived from LC-MS/MS analysis and calculations are shown in details in Table 1 through Table 6.

The following equation was used to calculate the individual residues R in µg/L:

$R = c_{End} \times (V_{End}/V_{Sample})$

$= c_{End} \times M$

R: Analyte residue in µg/L.

c_{End}: Concentration of analyte in final sample volume, in ng/mL. (where multiple injections were evaluated: mean).

V_{End}: Final sample volume, in mL: 2.0 mL

V_{Sample}: Sample measured, in mL: 25.0 mL

M: Multiplier

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$Rec. = (R / R_{fortified}) \times 100\ \%$

The pendimethalin metabolite M455H001 calculation is exemplified with the ground water sample P2708-271 fortified at 0.20 µg/L (10 x LOQ). The final sample volume was examined by LC/MS/MS in run file P2708API#366 (Figure 16) to give a final concentration C_{End} of 2.33 ng/mL for 310 *m/z* -> 266 *m/z*. The following calculation is demonstrated for the fragment ion 266 m/z:

$R = c_{End} \times (V_{End}/V_{Sample})$

$= c_{End} \times M$

$R = 2.33\ ng/mL \times (2.0\ mL/25\ mL)$

$= 2.33\ ng/mL \times 0.080$

$= 0.186\ \mu g/L$

$Rec. = (R/R_{fortified}) \times 100\%$

$= (0.186\ \mu g/L / 0.20\ \mu g/L) \times 100\ \% = 93\%$

Calculations were performed with full precision by computer software (Excel). Thus slight discrepancies may arise when using other methods.

2.5.2 GC/MS/MS Analysis for Reg. No. 4061757

Recovery results derived from GC/MS/MS analysis and calculations are shown in detail in Table 7 and Table 8.

The following equation was used to calculate the individual residues R in µg/L:

R: Analyte residue in µg/L.

c_{End}: Concentration of analyte in final sample volume, in ng/mL. (where multiple injections were evaluated: mean).

V_{End}: Final sample volume, in mL: 1.0 mL

V_{Sample}: Sample measured, in mL: 250 mL

M: Multiplier

Recoveries (Rec.) were calculated for the fortified specimens as follows:

Rec. = $(R / R_{fortified}) \times 100\ \%$

The Reg. No. 4061757 calculation is exemplified with the ground water sample P2708-92 fortified at 0.20 µg/L (10 x LOQ). The final sample volume was examined by GC/MS/MS in run file P2708#033 (Figure 35) to give a final concentration C_{End} of 41.8 ng/mL for 211 *m/z* -> 193 *m/z*. The following calculation is demonstrated for the fragment ion 193 m/z:

R = $c_{End} \times (V_{End}/V_{Sample})$

= $c_{End} \times M$

R = 41.8 ng/mL (1.0 mL/250 mL)

= 41.8 ng/mL x 0.004

= 0.167 µg/L

Rec. = $(R/R_{fortified}) \times 100\%$

= (0.167 µg/L/0.20 µg/L) x 100 % = 84%

Calculations were performed with full precision by computer software (Excel). Thus slight discrepancies may arise when using other methods.

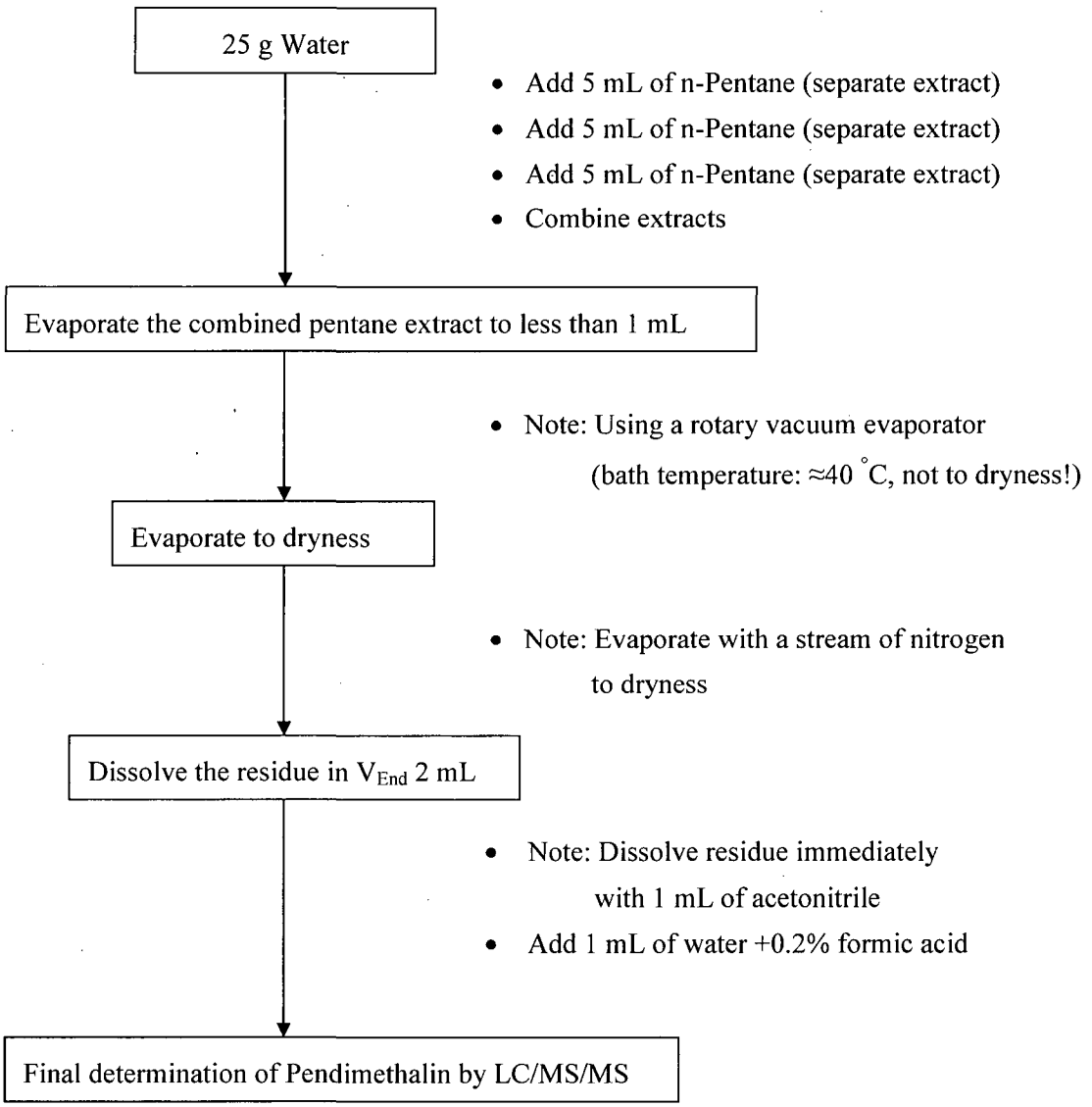
4. CONCLUSION

The analytical method was developed and validated at the 0.02 µg/L (LOQ) level and at the 0.20 µg/L (10xLOQ) level for the determination of pendimethalin and its metabolites M455H001, P48 and Reg. No. 4061757 in ground and surface water.

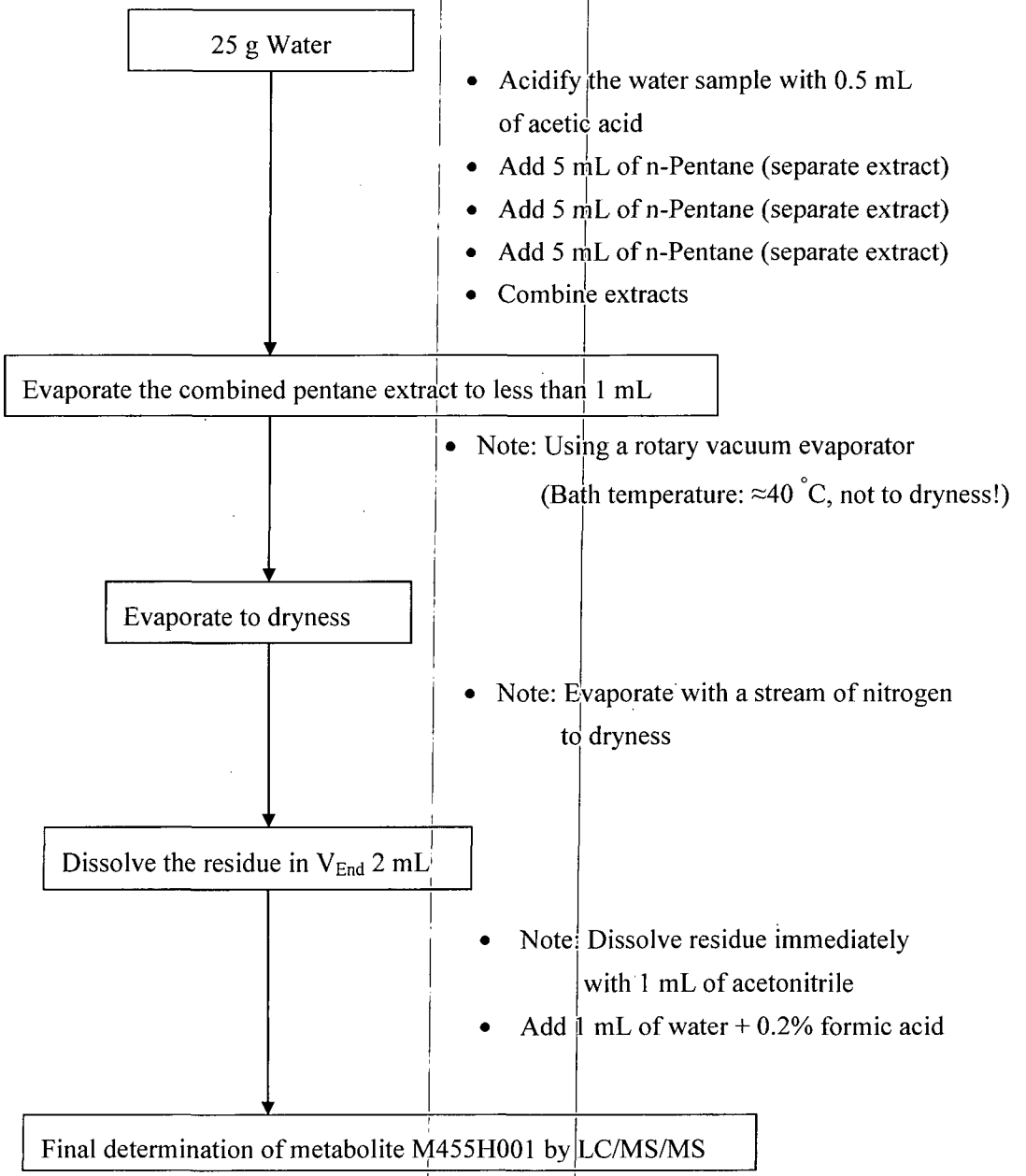
The analytical method fulfills the requirements of the EC Guidance documents on pesticide residue analytical methods (SANCO/825/00 rev. 8.1, 16/11/2010 and SANCO/3029/99 rev. 4, 11-Jul-00).

Appendix 2 Flow Diagram of Analytical Methods

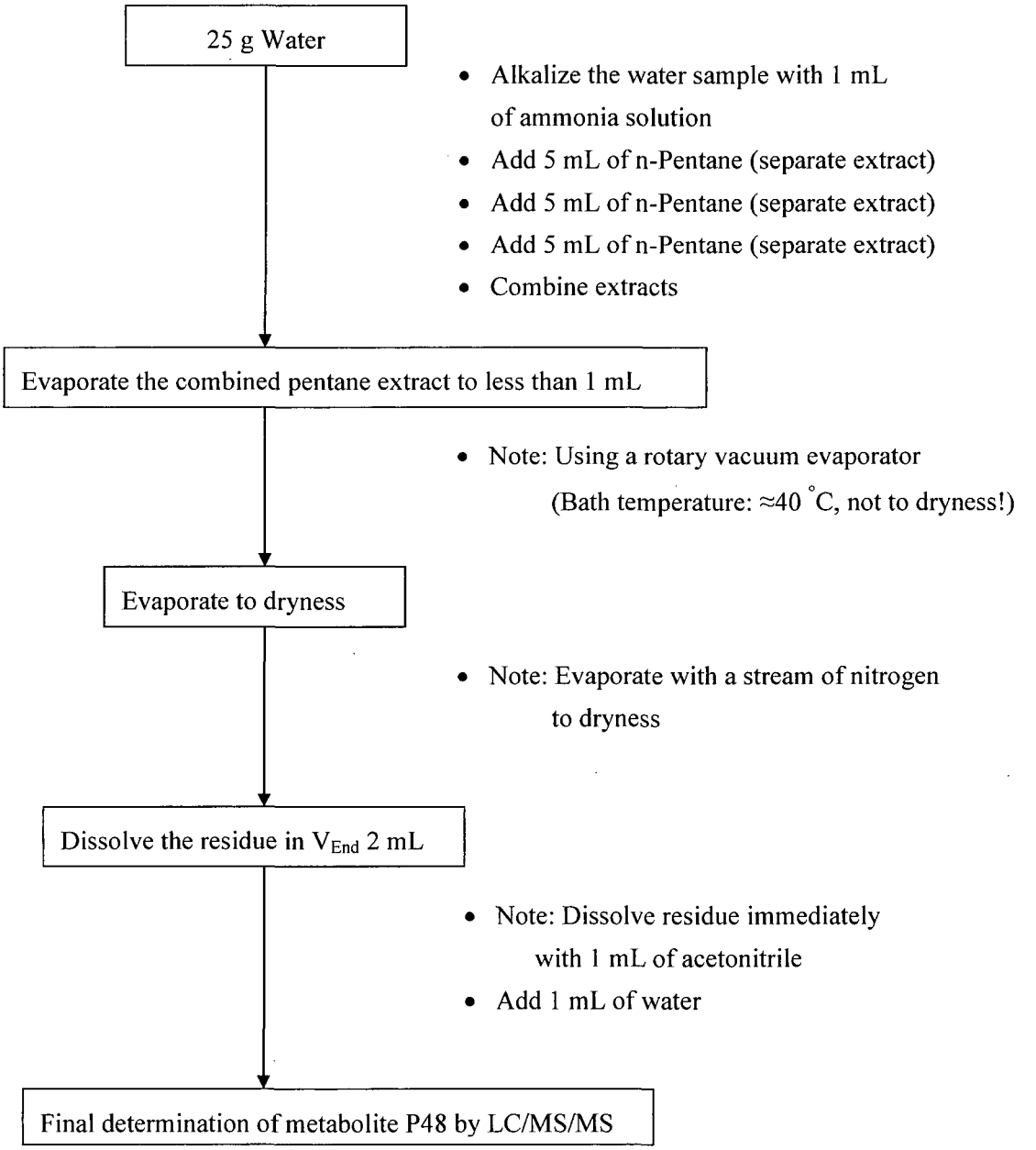
Flow Diagram of Analytical Method for Pendimethalin



Flow Diagram of Analytical Method for Pendimethalin Metabolite M455H001:



Flow Diagram of Analytical Method for Pendimethalin Metabolite P48:



Flow Diagram of Analytical Method for Pendimethalin Metabolite Reg. No. 4061757: