1 INTRODUCTION

1.1 Scope of the method

For registration of the compound and for monitoring purposes a residue analytical method for the active ingredient BAS 700 F and its metabolites M700F001, M700F002 and M700F007 in water with a limit of quantification (LOQ) of 0.03 µg/L is needed.
The described method no. L0143/01 allows the specific determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007 with the required limit of quantification in water.

This method was developed at BASF SE, Agricultural Center Limburgerhof, Germany.

The purpose of this study was to demonstrate the validity of the method by performing recovery experiments with spiked water samples. Spiked water samples are analysed for BAS 700 F and its metabolites M700F001, M700F002 and M700F007.

The recovery trials were carried out with tap water of the water pipe of Limburgerhof and surface water collected from of a small lake (Kelmetschweiher) near by Schifferstadt (Rhineland-Palatina).

The spiking levels were 0.03 and 0.5 µg/L for water. All fortification levels were analysed in 5 replicates. In addition at least two untreated control samples have been analysed per analytical sample set. The analyses were performed by one person, with the same equipment, in the same laboratory, within a short interval of time.

In the following the design and results of the study are reported.

1.2 Principle of the method

A 50 mL sample aliquot is concentrated on a pre conditioned Strata-X-AW SPE column. After elution with methanol / HCOOH (v+v = 90 + 10) the analytes are determined by HPLC-MS/MS. Residues are dissolved in methanol / water (v+v = 50+50).

Samples at the limit of quantification (LOQ) are measured using the undiluted extract (methanol / water (v+v = 50+50)). Final volume is 1.5 mL.

The method has a limit of quantification (LOQ) of 0.03 µg/L per analyte in water.

1.3 Specificity

The method allows the specific determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007 in water using HPLC/MS-MS detection.

2 MATERIALS AND METHODS

2.1 Test system water

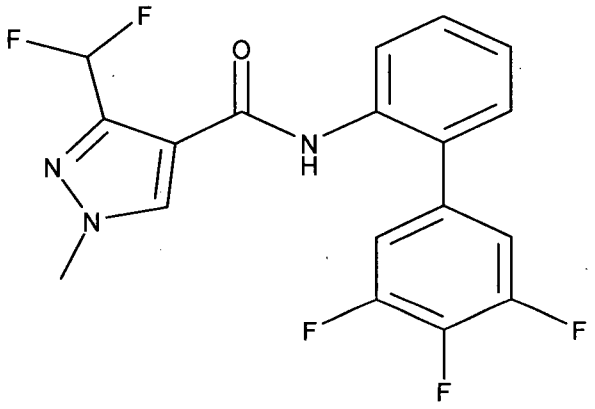
Two different types of water were used: tap water of Limburgerhof water pipe and surface water from a small lake near by Schifferstadt (Rhineland-Palatina). For more details see Appendix 5.1 (page 38).

2.2 Test and reference items

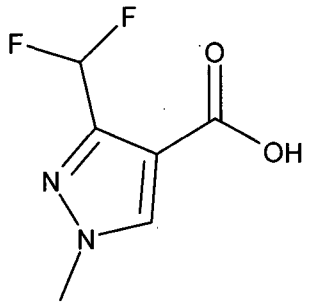
2.2.1 Test items

(used for fortifications)

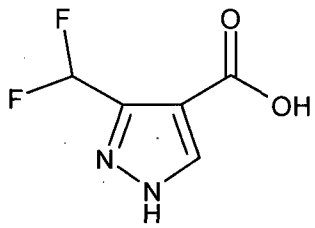
2.2.1.1 BAS 700 F (Reg.no. 5094351)

Reg-No.:	5094351
Chemical name:	3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluoro[1,1'-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide
Structural formula:	
Empirical formula:	$C_{18}H_{12}F_5N_3O$
Molecular weight:	381.3 g/mol
Lot no.:	L80-28
Purity:	99.7% (263302_2)
Stability:	expected to be stable until April 01, 2010, if stored at room temperature (typically + 25°C) or cooler

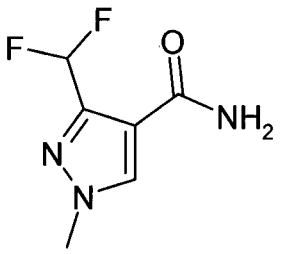
2.2.1.2 M700F001 (Reg.no. 5069089)

Reg-No.:	5069089
Chemical name:	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid
Structural formula:	
Empirical formula:	$C_6H_6F_2N_2O_2$
Molecular weight:	176.1 g/mol
Lot no.:	L80-68
Purity:	99.2% (267640_5)
Stability:	expected to be stable until August 01, 2010, if stored at room temperature (typically + 25°C) or cooler

2.2.1.3 M700F002 (Reg.no. 5435595)

Reg-No.:	5435595
Chemical name:	3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid
Structural formula:	
Empirical formula:	$C_5H_4F_2N_2O_2$
Molecular weight:	162.1 g/mol
Lot no.:	L80-66
Purity:	99.3% (267640_4)
Stability:	expected to be stable until July 01, 2010 if stored in a refrigerator (approxim. + 4 °C) or cooler

2.2.1.4 M700F007 (Reg.no. 5621781)

Reg-No.:	5621781
Chemical name:	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide
Structural formula:	
Empirical formula:	$C_6H_7F_2N_3O$
Molecular weight:	175.1 g/mol
Lot no.:	L81-108
Purity:	99.4% (ASAP09_055)
Stability:	expected to be stable until April 01, 2011 if stored in a refrigerator (approxim. + 25 °C) or cooler

2.2.2 Reference Items
(used for calibration)

Same items as test items, see 2.2.1

2.3 Stability of standard solutions

The stability of standard solutions of BAS 700F, M700F001 and M700F002 has been investigated in study 266245 (BASF DocID 2008/1063799). If stored in a refrigerator standard mix solutions prepared in methanol / water (v+v = 50+50) are stable for at least 28 days. Stability for M700F007 in methanol / water (v+v = 50+50) is tested within this study and resulted in at least 12 days, if stored in a refrigerator (see Table 18).

2.4 Materials and instruments

Materials, instrumentations and instrument methods were used as described in the technical procedure (see Appendix 5.3).

2.5 Analytical procedure

The procedure described in the technical procedure was followed.

2.6 Example of calculation

Calculation of recovery of sample no. ForL0022 (tap water, 0.03 µg/L BAS 700 F)

Analysis Package:	L007 (mass transition: 382 -> 362)	
Calibration curve:	Type	= linear
	Peak area	= slope x concentration + intercept
	Slope	= 28200
	Intercept	= - 350
	Correlation coefficient	= 1.0000
	e.g. sample no. ForL0022:	
	Cone. analyte [ng/mL]	= (Peak area - intercept) / slope
		= (28300 – (-350)) / 28200
		= 1.02

Data required for calculation of residues (control sample no.: ConL0007)

Sample no.:	ConL0007	ForL0022
Sample volume :	50 mL	50 mL
Fortification:	0 mg/kg = untreated	0.03 µg/L BAS 700 F (= 30 pg/mL)
Final volume (V_{end}):	1.5 mL	1.5 mL
Peak area:	0	28300
Cone. of analyte (C_B):	0.0 ng/mL	1.02ng/mL
Aliquot of water extract [%]:	100	100

Equation:

$$R\,[\mu g/L] = \frac{V_{end} \times C_B}{S_M \times Al}$$

R	=	Residue in the water sample
V_{end}	=	End volume of the extract after all dilution steps [mL]
C_B	=	Conc. of analyte in the inj. vol. as read from the calibration curve [ng/mL]
S_M	=	Volume of the water sample extracted [mL]
Al	=	Aliquot of water extract taken for analysis [%]

$$R\,(untreated\ sample) = \frac{1.5 \times 0}{50 \times 100\%} = 0.0\ ng/g$$

$$R\,(fortified\ sample) = \frac{1.5 \times 1.02}{50 \times 100\%} = 0.031 ng/g$$

$$\%\ \text{Recovery} = \frac{R\,(\text{found, fortified}) - R\,(\text{found, untreated})}{R\,(\text{fortified})} \times 100$$

$$= \frac{0.031 - 0}{0.03} \times 100 = 102\%$$

Determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007in water using HPLC/MS-MS

Abstract

BASF method no. L0143/01 allows the determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007in water samples. It is presumed that this method will also allow the determination of other compounds with similar physico -chemical properties. This report includes the analytical procedure of BASF method no. L0143/01.

To date method L0143/01 was successfully tested in water.

Principle of the method

BAS 700 F and its metabolites M700F001, M700F002 and M700F007are extracted from water samples by enrichment on a strata-X SPE cartridge. After washing the SPE column with 1% formic acid in water the analytes are eluted with methanol/HCOOH =90+10(v+v). Final determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007 is performed by HPLC / MS-MS.

BAS 700 F (Reg.no. 5094351)	R_t ~ 10.4 min
BF700M001 (Reg.no. 5069089)	R_t ~ 8.6 min
BF700M002 (Reg.no. 5435595)	R_t ~ 6.9 min
BF700M007 (Reg.no. 5621781)	R_t ~ 6.6 min

Limit of quantification (LOQ):

BAS 700 F (Reg.no. 5094351)	0.03 pg/L
BF700M001 (Reg.no. 5069089)	0.03 pg/L
BF700M002 (Reg.no. 5435595)	0.03 pg/L
BF700M007 (Reg.no. 5621781)	0.03 pg/L

Confirmatory technique:

For determination of BAS 700 F LC/MS-MS uses the protonated molecular ion [M+H]$^+$ at m/z 382 as parent ion, the characteristic daughter ions at m/z 342 and m/z 362 are used for quantification. Mass transitions for all analytes are shown in the following table:

Analyte	Quantifier m/z	Qualifier m/z
BAS 700 F	382/362	382/342
M700F001 (Reg.no. 5069089)	175/ 91	175/111
M700F002 (Reg.no. 5435595)	161/141	161/97
M700F007 (Reg.no. 5621781)	176/156	176/136

LC/MS-MS is thus considered selective and specific and does normally not require an additional confirmatory method. Validation data for both mass transitions of each analyte are given in validation report 314720 (BASF DocID 2009/1069369).

1 *INTRODUCTION*

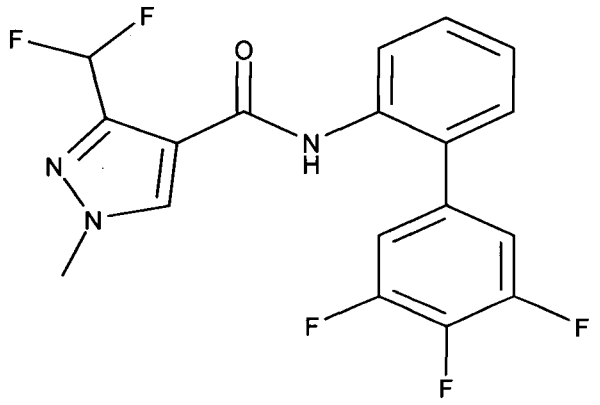
For determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007in water samples an analytical method was needed. The described BASF method no. L0143/01 allows the determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007 with the required limit of determination in water. This method was developed at BASF Agricultural Center Limburgerhof - Crop Protection Division- Ecology and Environmental Analytics- , 67114 Limburgerhof , Germany .

2 *PRINCIPLE OF THE METHOD*

Water samples are extracted by enrichment of the analytes on a strata-x-AW SPE cartridge. After washing the SPE column with Millipore water + 1% formic acid the analytes are eluted with methanol / formic acid = 90 + 10 (v+v).
The determination of BAS 700 F and its metabolites M700F001, M700F002 and M700F007 is performed by LC / MS-MS on a Waters Atlantis T3 column.

3 TEST AND REFERENCE ITEMS

3.1 BAS 700 F, Test and Reference Item

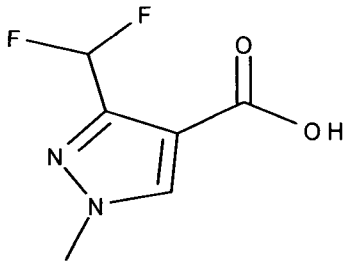
Reg-no.:	5094351
Chemical name:	3-(difluoromethyl)-1-methyl-N-(3',4',5'-trifluoro[1,1'-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide
Structural formula:	
Empirical formula:	$C_{18}H_{12}F_5N_3O$
Molecular weight:	381.3 g/mol
Lot no.:	L80-28
Purity:	99.7% (tolerance ± 1%)
Stability:	expected to be stable until Apr 01, 2010 if stored at room temperature (typically +25°C) or cooler

3.2 M700F001, Reg.no. 5069089, Test and Reference Item

Reg-no.: 5069089

Chemical name: 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid

Structural formula:



Empirical formula: $C_6H_6F_2N_2O_2$

Molecular weight: 176.1 g/mol

Lot no.: L80-68

Purity: 99.2% (tolerance ± 1%)

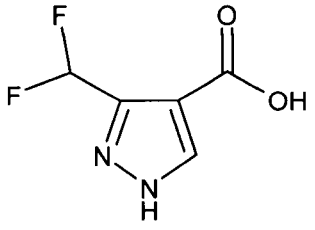
Stability: expected to be stable until Aug 01, 2010 if stored at +25 °C or cooler

3.3 M700F002, Reg.no. 5435595, Test and Reference Item

Reg-no.: 5435595

Chemical name: 3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid

Structural formula:



Empirical formula: $C_5H_4F_2N_2O_2$

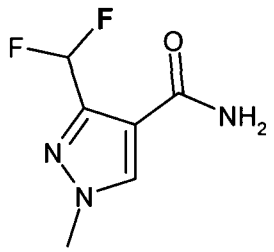
Molecular weight: 162.1 g/mol

Lot no.: L80-66 (267640_1)

Purity: 99.3% (tolerance ± 1%)

Stability: expected to be stable until July 01, 2010 if stored at + 4°C or cooler

3.4 M700F007, Reg.no. 5621781, Test and Reference Item

Reg-no.:	5621781
Chemical name:	3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide
Structural formula:	
Empirical formula:	$C_6H_7F_2N_3O$
Molecular weight:	175.1 g/mol
Lot no.:	L81-108 (ASAP09_055)
Purity:	99.4% (tolerance ± 1%)
Stability:	expected to be stable until April 01, 2011 if stored at room temperature or cooler

4 *MATERIALS AND METHODS*

4.1 Equipment for Enrichment and Sample Work-up

Equipment	Size,Description	Manufacturer/Supplier	Catalog Number
Balance	Analytical , AT 250	Mettler, Giessen (Germany)	
PHENOMENEX strata X SPE columns	6 mL, 500 mg	Phenomenex Zeppelinstr.5 63741 Aschaffenburg	8B-S038-HCH
Reservoirs	50 mL	BAKER	
SPE Vacuum Box	SPE 12-G	J.T Baker	7018-94
Handy step electronic pipet		Brand GmbH + Co KG	705000
Volumetric pipets	various volumes		
Volumetric flasks	various volumes		
Volumetric cylinders	various volumes		
Glass centrifuge tubes with srew cap	10 mL	Schott, Mainz	231751459
Pasteur glass pipettes	length about 150 mm	Fortuna	3.525
HPLC vials	32 * 11.6 mm		
Teflon-lined plastic caps for HPLC vials			

Note: Equivalent equipment from other suppliers may be substituted.

4.2 Reagents

4.2.1 Chemicals

Chemical	Grade	Manufacturer / Supplier	Catalog Number
Formic acid	98/100 %	Bernd Kraft, Duisburg	05314.3010
methanol	LiChrosolv	Merck eurolab, Germany	1.06018
Millipore water	Milli-Q	Millipore , 65760 Eschborn	

4.2.2 Solvent Systems

System	Composition
Solvent System 1 (SS 1)	1 % Formic acid in MILLIPORE water
Solvent System 2 (SS 2)	Methanol + HCOOH = 90 + 10 (v+v)
Solvent System 3 (SS 3)	Methanol + MILLIPORE water = 50 + 50 (v+v)
LC-Solvent System 1 (LCSS 1)	Water + formic acid = 1000+1 (v+v)
LC-Solvent System 2 (LCSS 2)	Methanol + formic acid = 1000+1 (v+v)

Note: Equivalent chemicals from other suppliers may be substituted but all chemicals used must be at least of " analytical grade " or must meet equivalent specifications.

4.3 Standard Solutions

4.3.1 Standard Solution Stability and Storage

Standard solutions containing BAS 700F, M700F001 and M700F002 have been proven to be stable for at least 28 days, if stored in the dark and in a refrigerator (validation report 266245, BASF DocID 2008/1063799).
Stability of M700F007 is given for at least 12 days (validation study 314720, BASF DocID 2009/1069396).

4.3.2 Standard Solutions for Fortification and Calibration

Prepare a 1000 pg / mL stock solution of the respective analyte by weighing an appropriate amount of the respective analyte into a volumetric flask. Dissolve with methanol.

Example:

To prepare a 1 mg / ml stock solution, weigh 10 mg of BAS 700 F into a 10 mL volumetric flask. Dissolve in 10 mL methanol (stock solution A).

To prepare a 1 mg / ml stock solution, weigh 10 mg of M700F001 (Reg.no.5069089) into a 10 mL volumetric flask. Dissolve in 10 mL methanol (stock solution B).

To prepare a 1 mg / ml stock solution, weigh 10 mg of M700F002 (Reg.no.5435595) into a 10 mL volumetric flask. Dissolve in 10 mL methanol (stock solution C).

To prepare a 1 mg / ml stock solution, weigh 10 mg of M700F007 (Reg.no.5621781) into a 10 mL volumetric flask. Dissolve in 10 mL methanol (stock solution D).

Preparation of a standard mix solution containing all analytes

Take solution	Volume (mL)	Dilution with methanol to final volume [mL]	Concentration
Stock solution A	1	10	100 µg / mL mix
Stock solution B	1		
Stock solution C	1		
Stock solution D	1		

Preparation of matrix matched standard solution

1 mL extract of a control sample is evaporated to dryness and the remaining residue is redissolved in 1 mL of the standard solution representing the limit of determination.

Examples for the preparation of standard mix solutions are shown in the following table:

Take solution	Volume (mL)	Dilution with SS 3 to final volume of [mL]	Concentration
100 µg / mL mix	1	10	10 µg / mL mix
10 µg / mL	1	10	1 µg / mL
1 µg / mL	1	10	100 ng / mL
100 ng / mL	1	10	10 ng / mL
100 ng / mL	0.5	10	5 ng / mL
100 ng / mL	0.25	10	2.5 ng / mL
100 ng / mL	0.2	10	2 ng / mL
10 ng / mL	1.5	10	1.5 ng / mL
10 ng / mL	1	10	1 ng / mL
5 ng / mL	1	10	0.5 ng / mL
2 ng / mL	1	10	0.2 ng / mL

Preparation of control samples

For the preparation of control water samples untreated water will be analysed according to analytical method L0143/01(see 5.3).

Preparation of fortified samples

For the preparation of fortified water samples 50 mL of untreated water is acidified with 0.3 mL formic acid and spiked with the fortification solution of the respective analyte mix.
All fortified samples will be analyzed according to method L0143/01 to determine the recovery in the water matrix used.

Example for preparation of fortified samples (based on 100 mL water)

Take mix solutions of (ng/mL)	Volume spiked (µL)	Amount spiked (ng)	Spiking Level (µg / L)
10	150	1.5	0.03
100	250	25	0.5

Note: It is recommended to compare a matrix-matched standard (e.g. 100 % LOQ) with the same standard without matrix to see the instrument recovery in each sample queue.

5 ANALYTICAL PROCEDURE

5.1 Sample Preparation and Storage

Water specimen are stored in a refrigerator (about +4 °C) or frozen until analysis. It is recommended to analyse water specimen immediately after sampling.

5.2 Preparation of the SPE cartridge

Before use the strata-x -AW SPE cartridge is washed with 5 mL of methanol, followed by 5 mL of SS 1. Do not allow to run dry !

5.3 Extraction of the Sample Material

5.3.1 Preparation of fortified water specimen

Note :
Instead of volumetric measurement (mL) water samples can also be weight in for analysis. Therefore 1 g = 1 mL and 1 mL = 1 g for calculation of the residue (by definition: µg/kg = µg/L, water density is 1 kg/L)

5.3.1.1 Fortification with BAS 700 F and its metabolites M700F001, M700F002 and M700F007

To 50 mL of an untreated water sample 0.3 mL of formic acid and the respective amount of the standard mix solution are added. After mixing by shaking the water sample is sucked through a pre conditioned strata-X SPE cartridge with about 1 drop per second.
Do not allow to run dry !

5.3.2 Extraction of unknown water specimen

Add 0.3 mL of formic acid to 50 mL of the unknown water sample and suck it through a pre conditioned strata-X SPE cartridge with about 1 drop per second.
Do not allow to run dry !

Then the cartridge is washed with 5 mL of SS 1.
To remove water air is sucked through the cartridge for about 1 minute.

5.3.3 Elution of the Analytes

Elution of the analytes is performed by sucking 10 mL of SS 2 through the cartridge.
The extract is collected in a glass centrifuge tube and reduced to dryness using an N-EVAP at 50°C water bath temperature. Residue is dissolved in 1.5 mL SS 3.

5.3.4 Preparation of the Final Volume for HPLC/MS-MS Quantitation

The final volume of 1.5 mL is true for the residue in the range of the required determination limit. In case of higher residues dilute with appropriate amounts of SS 3.

Note: Use transfer pipettes (e.g. Pasteur glass pipettes) for solution transfer.

5.4 Quantitation

5.4.1 HPLC / MS-MS Measurement

5.4.1.1 HPLC / MS-MS – Instrumentation

Instrumentation	Autosampler:	CTC PAL
	Mass spectrometer::	Sciex Triple Quadrupole LC/MS/MS
	Type:	API 4000
Manufacturer	PE Sciex Instruments	
Pump	Agilent 1100 LC Binary Pump	
Analytical column	Stationary phase:	Waters Atlantis T3 (3µ)
	Length:	150 mm
	Inner diameter:	3 mm
Mobile Phase A	LCSS 1 (water + HCOOH = 1000 + 1 (v+v))	
Mobile Phase B	LCSS 2 (methanol + HCOOH = 1000 + 1 (v+v))	
Flow rate	0.5 mL/min	
Software	Analyst Version 1.4.1	
Scan type	MRM	
Polarity	positive / negative	
Ion source	Turbo spray	

Quantifier masses

BAS 700 F :	382 -> 362*, 382 -> 342
M700F001 :	175 -> 91*, 175 -> 111
M700F002 :	161 -> 141*, 161 -> 97
M700F007 :	176 -> 156*, 176 -> 136

*most sensitive mass transition (under the described conditions)

Inject volume: 10 pL

Chromatographic conditions:

0	minutes	10 % B
1.5	minutes	10 % B
7.0	minutes	40% B
8.1	minutes	100 % B
11.5	minutes	100 % B
11.6	minutes	10 % B
15.0	minutes	10 % B

Duration of one run: 15 minutes (equilibration time included)

Retention time(s)

BAS 700 F	about 10.4 minutes
M700F001	about 8.6 minutes
M700F002	about 6.9 minutes
M700F007	about 6.4 minutes

Note:

The equipment listed above may be substituted by instruments with similar specifications. LC columns with equivalent stationary phases and similar specification may be available from other manufacturers. If the use of material with specifications other than those stated is intended, applicability of the new equipment for this method must be confirmed.

5.4.2 Calibration Procedure

Calculation of results is based on peak area measurements (peak height could also be used) using calibration curves for the respective analytes. The standard curves are obtained by direct injection of the standard solutions into LC/MS-MS system in the range of 0.2 ng/mL to 2.5 ng/mL.
In a given injection run, the same volume is used for all samples and standards. Typical standard amounts injected (10 pi) range as follows: 0.2, 0.5, 1, 1.5, 2 and 2.5 ng/mL.

The calibration curve is obtained by plotting the peak area (or height) versus the concentration of the analyte. The linear curve in the form y = bx + c is used for the construction of the calibration curve for BAS 700 F and its metabolites M700F001, M700F002 and M700F007. For each injection set, the set should begin and end with standard injections, and each standard level should be injected in duplicate, if less than 5 standard solutions are used.
The average of peak areas (or peak heights) of the same concentration level can be used for obtaining calibration curves.

5.4.3 Limit of Quantification and Limit of Detection

The limit of quantification is defined as the lowest fortification level successfully tested. For 700 F and its metabolites M700F001, M700F002 and M700F007 in water, the limit of quantification is 0.03 pg / L water.
The limit of detection is 2 pg (concentration on the LC column) which is the lowest standard (0.2 ng/mL) injected (10pL) into LC/MS-MS instrument.

6 CALCULATION OF RESULTS

6.1 Principle

Calculation of results is based on peak area (or height) measurements. Residues of
700 F and its metabolites M700F001, M700F002 and M700F007 are calculated using the respective calibration curve. Equation is shown in section 6.2.

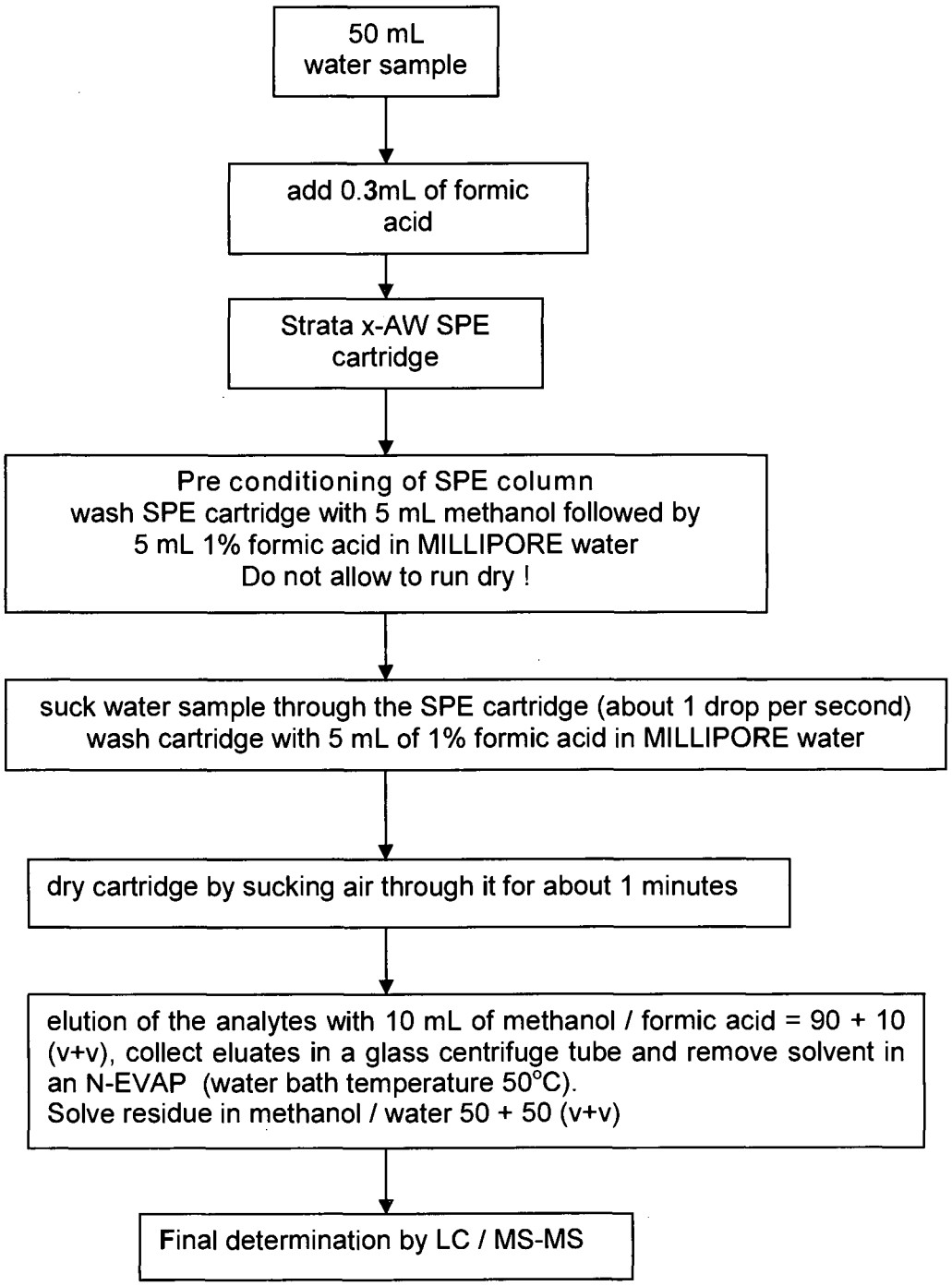
6.2 Calculation of Residues

The residue R (pg/L) of the respective analyte in the collected water sample in pg/L water is calculated as shown in the following equation:

$$R\,[\mu g/L] = \frac{V_{end} \times C_B}{S_M \times Al}$$

R	=	Residue in the water sample
V_{end}	=	End volume of the extract after all dilution steps [mL]
C_B	=	Cone. of analyte in the inj. vol. as read from the calibration curve [ng/mL]
S_M	=	Weight or volume of the water sample extracted [g, mL]
Al	=	Aliquot of water extract taken for analysis [%]

7 FLOW CHART OF METHOD L0143/01



8 METHOD MANAGEMENT AND TIME REQUIREMENT

The analysis of one series of 13 samples (= 10 unknown samples, 2 fortified samples and 1 blank sample) requires about **8** hours of work. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.

9 PROBLEMS, SAFETY AND HEALTH CONSIDERATION

All procedures involving organic solvents should be performed under a well-ventilated hood. Personal protective equipment (gloves, lab coats, safety glasses) should be worn while performing this method. Heed all label statements and precautions.

10 CONFIRMATORY TECHNIQUE

HPLC/MS-MS and UPLC/MS-MS are considered selective and specific and does normally not require an additional confirmatory method, if a second mass transition of the respective analyte is validated (see Validation Report 314720, BASF DocID 2009/1069396).

11 RECOVERIES

During the development of the method the found average recoveries for water were in the range of 70 - 110 % (see Validation Report 314720, BASF DocID 2009/1069396).

11.1 Limit of Quantification, Blank Values

The limit of quantification was defined by the lowest fortification level successfully tested (= 0.03 pg/L of all analytes). The tested untreated blank water samples showed no interfering peaks (or less than 1/3 of the lowest fortification level) at the retention time of the analytes (see Validation Report 314720, BASF DocID 2009/1069396).