

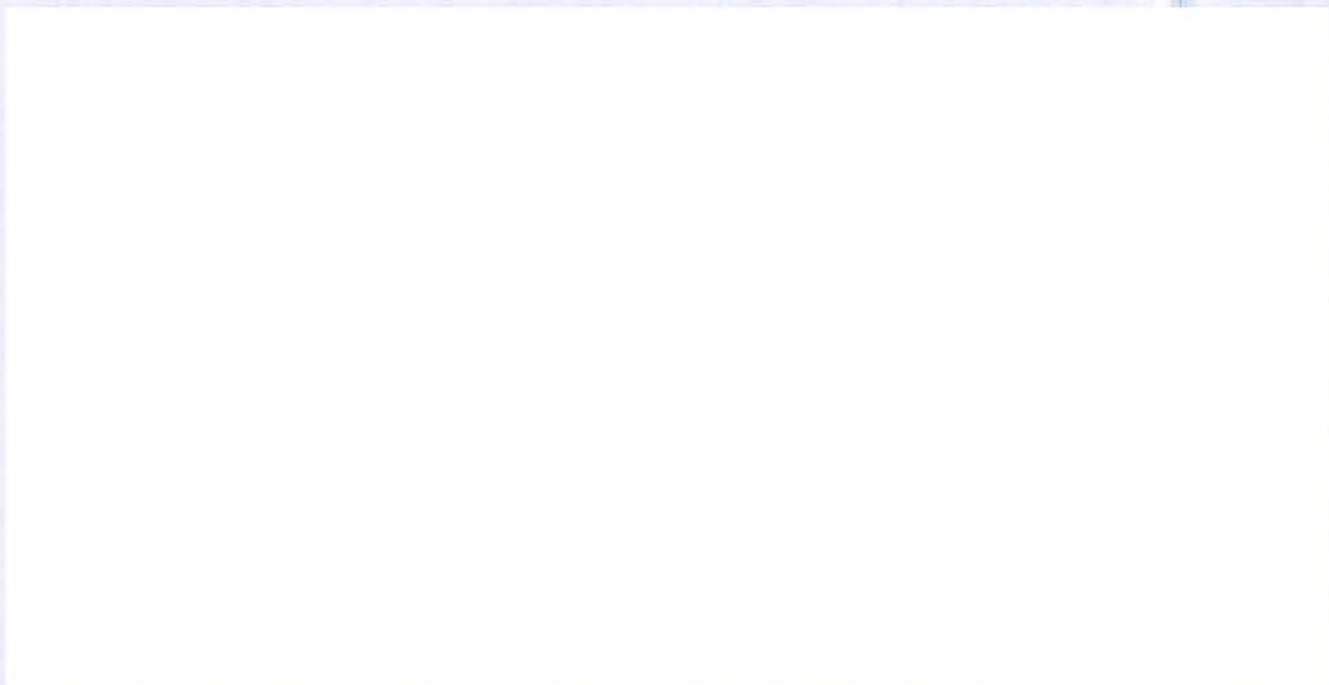
**Final Report**

**Validation of an Analytical Method for Determination of  
Fluometuron and its Metabolites Des-methyl-Fluometuron and  
CGA72903 in Drinking Water and Surface Water**

**Data Requirement**

EC Guidance document on residue analytical methods,  
SANCO/825/00 rev. 8.1 (16/11/2010)

OPPTS Guideline 850.6100



## 1 Summary

A method for the determination of fluometuron, des-methyl-fluometuron and CGA72903 in drinking and surface water has been successfully developed and validated. The analytical methods fulfil all requirements of guideline SANCO/825/00 rev. 8.1 (extraction efficiency has not been tested).

Method principle: Direct analysis of water samples by HPLC-MS/MS

Cleanup: none

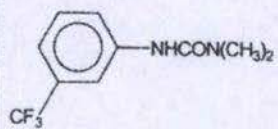
Final determination: HPLC-MS/MS, ESI positive ion mode (two mass transitions per test item)

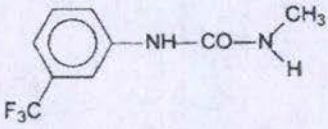
Limit of quantification (LOQ): 0.05 µg/L

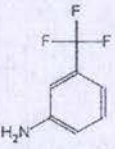
Limit of detection (LOD): below 30 % of LOQ (i.e.  $\leq 0.015$  µg/L)

## 4 Materials and Methods

### 4.1 Test Items

Code:	Fluometuron
Chemical name	N,N-dimethyl-N'-[3-(trifluoromethyl)phenyl]urea
CAS no.:	2164-17-2
Test item code:	2012-000468
Structure:	
Chemical formula:	C <sub>10</sub> H <sub>11</sub> F <sub>3</sub> N <sub>2</sub> O
Molecular weight:	232.2 g/mol
Purity:	99.5 %
Batch no.:	FLMT(3)-1540(V3)
Certificate of analysis:	28 February 2012
Expiry date:	28 February 2016
Supplier:	Agan Chemical Manufacturers Ltd.
Storage conditions:	Ambient temperature

<b>Code:</b>	<b>Des methyl Fluometuron</b>
Chemical name	1-Methyl-1-hydrogen-3-( $\alpha,\alpha,\alpha$ -trifluoro-m-tolyl)urea
CAS no.:	3032-40-4
Test item code:	2012-000467
Structure:	
Chemical formula:	$C_9H_9F_3N_2O$
Molecular weight:	218.2 g/mol
Purity:	99.2 %
Batch no.:	FLMT(2)-BP1-741(V2)
Certificate of analysis:	27 February 2012
Expiry date:	28 February 2016
Supplier:	Agan Chemical Manufacturers Ltd.
Storage conditions:	Ambient temperature

<b>Code:</b>	<b>CGA72903</b>
Chemical name	3-Trifluoromethyl aniline (TFMA)
CAS no.:	98-16-8
Test item code:	2011-002464
Structure:	
Chemical formula:	$C_7H_6F_3N$
Molecular weight:	161.1 g/mol
Purity:	99.5 %
Batch no.:	FLCLDN-BP14-1993 (V1)
Certificate of analysis:	09/2011
Expiry date:	09/2013
Supplier:	Agan Chemical Manufacturers Ltd.
Storage conditions:	Ambient temperature

### **Purity and composition**

All specifications of purity and composition of the test item are provided by the sponsor/supplier.

## **4.2 Standard Solutions**

### **4.2.1 Stock Solutions**

A stock solution containing 1423 µg/mL of fluometuron was prepared by weighing 14.3 mg of the test item into a 10 mL volumetric flask and adjusting the volume to 10 mL with acetonitrile. This stock solution was stored refrigerated (1-10 °C) in the dark.

A stock solution containing 1061 µg/mL of des-methyl-fluometuron was prepared by weighing 10.7 mg of the test item into a 10 mL volumetric flask and adjusting the volume to 10 mL with acetonitrile. This stock solution was stored refrigerated (1-10 °C) in the dark.

A stock solution containing 1301 µg/mL of CGA72903 was prepared by weighing 65.4 mg of the test item into a 50 mL volumetric flask and adjusting the volume to 50 mL with acetonitrile. This stock solution was stored refrigerated (1-10 °C) in the dark.

### **4.2.2 Fortification Solutions**

Combined fortification solutions (1 µg/mL and 0.1 µg/mL final concentration) containing all fluometuron, des-methyl-fluometuron and CGA72903 were prepared in acetonitrile/water (1:1, v/v) from the stock solutions by volumetric dilution with acetonitrile/water (1:1, v/v). All fortification solutions were stored refrigerated (nominally 1-10 °C).

### **4.2.3 Calibration Solutions for HPLC-MS/MS Analysis**

Calibration solutions for HPLC-MS/MS analysis were prepared by volumetric dilution of the 1 µg/mL solution with acetonitrile/water (1:1, v/v) to yield intermediate dilutions of 0.1 µg/mL (100 ng/mL), 0.01 µg/mL (10 ng/mL) and 0.001 µg/mL (1 ng/mL). From these intermediate dilutions, final calibration solutions were prepared as follows:

Final concentration (ng/mL)	Standard solution 100 ng/mL (µL)	Standard solution 10 ng/mL (µL)	Standard solution 1 ng/mL (µL)	HPLC grade water (µL)
10	100	–	–	900
5	50	–	–	950
2	20	–	–	980
1	–	100	–	900
0.5	–	50	–	950
0.2	–	20	–	980
0.1	–	–	100	900
0.05	–	–	50	950
0.02	–	–	20	980
0.01	–	–	10	990

### 4.3 Sample Material

Two different water samples (drinking water and surface water) were used for validation of the analytical method. The drinking water (tap water) of the test facility was used. The surface water was taken of the river “Dendelbach” in Spregelberg / Germany. The water had been stored by ambient temperature until analysis.

Chemical and physico-chemical parameters had been determined at Eurofins Institut Jäger GmbH, Tübingen (non-GLP). Results are reported in Table 4 and Table 5. The data sheets are given in the appendix E in Figure 25 to Figure 26.

Table 4: Results of drinking water

Parameter	Value	Unit	Method of Determination
pH (at 17.5°C)	7.62	–	DIN 38404-5 (C 5)
Specific electric conductivity (at 20°C)	754	µS/cm	DIN EN 27888 (C 8)
Total hardness	4.1	mmol/L	DIN 38409-6 (H 6)
Spectral absorption coefficient (at 436 nm)	< 0.1	m <sup>-1</sup>	DIN EN ISO 7887 (C 1)
Dissolved organic carbon	0.44	mg/L	DIN EN 1484 (H 3)

Table 5: Results of surface water

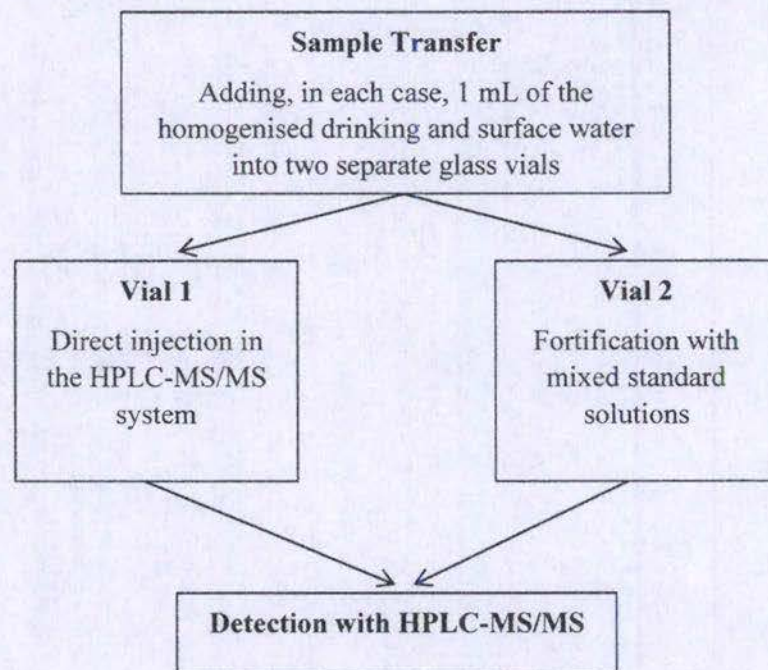
Parameter	Value	Unit	Method of Determination
pH (at 17.8°C)	8.02	–	DIN 38404-5 (C 5)
Specific electric conductivity (at 20°C)	271	µS/cm	DIN EN 27888 (C 8)
Total hardness	1.4	mmol/L	DIN 38409-6 (H 6)
Spectral absorption coefficient (at 436 nm)	0.9	m <sup>-1</sup>	DIN EN ISO 7887 (C 1)
Dissolved organic carbon	4.7	mg/L	DIN EN 1484 (H 3)

#### 4.4 Procedure for Determination of the Test Items

##### 4.4.1 Principle of the Method

Residues of the test items in drinking water and surface water samples were analysed by direct injection of the water samples in the HPLC-MS/MS system and were calculated with standard addition method.

##### 4.4.2 Method Flow Chart



##### 4.4.3 Equipment

Glass bottles (100 mL) with PTFE coated screw caps  
 Analytical balances (Sartorius AC 210S)  
 Volumetric pipettes (Eppendorf): 100-1000  $\mu$ L, 10-100  $\mu$ L  
 Vortex mixer (scientific Industries)  
 Ultrasonic bath USR 18 (Merck)  
 Autosampler glass vials for HPLC analysis  
 HPLC system with MS/MS detector (Agilent HPLC system with API 5000 triple stage quadrupole mass spectrometer)



#### 4.4.4 Reagents

Formic acid	Merck	1.00264
Acetonitrile	Prolabo	83639.320
Water, HPLC grade	Merck	1.15333

#### 4.4.5 Safety

The following chemicals were used, which are classified by the hazardous material regulations. The classification is based on the German guidelines and has to be adapted to the respective national guidelines in case the method is used outside Germany.

Methanol	Highly flammable, toxic, harmful
Formic acid	Corrosive, flammable
Acetonitrile	Highly flammable, irritating

#### 4.4.6 Sample Preparation for Analysis of Fluometuron, Des-methyl-Fluometuron and CGA72903

50 mL aliquot of the homogeneous drinking and surface water sample was transferred into a 100 mL screw-cap glass bottle and fortified with 25 µL of mixed standard solution containing 0.1 µg/mL (in case of LOQ level) and 1 µg/mL (in case of 10\*LOQ level) of the analytes.

From the homogeneous sample, in each case, 1.0 mL aliquots were transferred into two separate glass vials. One of them was analysed directly and the other was fortified with mixed standard solutions (see table below):

Sample	Fortified with mixed standard solution:
Control samples	50 µL of a 1 ng/mL
Recovery samples at LOQ level	50 µL of a 1 ng/mL
Recovery samples at 10*LOQ level	50 µL of a 10 ng/mL

Additionally, 1.0 mL of HPLC grade water (four replicates) was transferred into glass vials and fortified with the same mixed standard solutions as the drinking and surface water samples at the LOQ level and the 10\*LOQ level to verify the fortification procedure and to determine the final concentration of the fortified water samples ( $C_{Std}$ ).

#### 4.4.7 Analysis by HPLC-MS/MS

The sample solutions were used for the HPLC with MS/MS detection, monitoring two characteristic mass transitions for each analyte. For fluometuron one transition ( $m/z$  233  $\rightarrow$  160) was used as quantifier, the other ( $m/z$  233  $\rightarrow$  72) as qualifier. For des-methyl-fluometuron one transition ( $m/z$  219  $\rightarrow$  162) was used as quantifier, the other ( $m/z$  219  $\rightarrow$  142) as qualifier. For CGA72903 one transition ( $m/z$  162  $\rightarrow$  142) was used as quantifier, the other ( $m/z$  162  $\rightarrow$  93) as qualifier. This approach follows the EC Guidance document on residue analytical method SANCO/825/00 rev. 8.1.

#### 4.4.8 Analysis by HPLC-MS/MS

##### HPLC Conditions

HPLC system: Agilent LC-System with autosampler  
 Column: Agilent ZORBAX Eclipse XDB-C18, 150 x 4.6 mm, 5 µm (No. 993967-902) + 4 mm guard col.  
 Mobile phase: A: Water + 0.5 % formic acid  
 B: Acetonitrile + 0.5 % formic acid

Time (min)	% A	% B	Flow rate (mL/min)	Gradient
0.00	50	50	0.5	-
0.50	50	50	0.5	-
5.00	5	95	0.5	linear
7.00	5	95	0.5	-
7.01	50	50	0.5	-
9.00	50	50	0.5	-

Column temperature: 40 °C  
 Injection volume: 84 µL  
 Retention time: Fluometuron: about 5.2 min  
 Des-methyl-Fluometuron: about 4.7 min  
 CGA72903 (TFMA): about 5.5 min

Mass Detector Conditions

Detector:	Sciex API5000
Ionization mode:	ESI
Source polarity:	positive
Curtain gas (CUR):	10 (arbitrary units)
Gas 1 (GS1):	30 (arbitrary units)
Gas 2 (GS2):	50 (arbitrary units)
Temperature (TEM):	400 °C
Interface heater (Ihe):	ON
Collision gas setting (CAD):	5
Ionspray voltage (IS):	5000
Collision cell exit potential (CXP):	18
Entrance potential (EP):	5
Declustering potential (DP):	40

Source and detection parameters for MS/MS experiments:

Compound	Parent m/z	Collision energy (V)	Fragment ions (m/z)	Ion type
Fluometuron	233.0	37	159.9	<u>Quantifier</u>
	233.0	30	71.9	Qualifier
Des-methyl-Fluometuron	219.2	25	162.0	<u>Quantifier</u>
	219.2	38	142.0	Qualifier
CGA72903 (TFMA)	162.0	28	142.0	<u>Quantifier</u>
	162.0	36	92.9	Qualifier

The detector linearity was confirmed over the calibration range of interest by constructing a calibration function of peak area versus concentration within the range of 0.01 µg/L to 10 µg/L in water.

Injections of sample extracts were interspersed with injections of quality control standards after 2-6 samples to verify the detector response and to adjust the calculated concentration as outlined below.

#### 4.4.9 Calculation of Residues

The concentration of the test items was calculated according to the following equation as follows:

$$R = \frac{c_{Std} \cdot \frac{V_{fortified}}{V_{sample}}}{\frac{c_1}{c_2} \cdot c_{sample,fortified} \cdot \frac{V_{fortified}}{V_{sample}} - \frac{c_1}{c_2} \cdot c_{sample}} \cdot \frac{c_1}{c_2} \cdot c_{sample}$$

R	Residue (µg/L)
c <sub>1</sub>	Nominal concentration of bracketed control standards (ng/mL)
c <sub>2</sub>	Average calculated concentration of bracketed control standards, obtained from the calibration function (ng/mL)
c <sub>sample</sub>	Analyzed concentration of final sample, as calculated from the calibration function (ng/mL)
c <sub>Std</sub>	Average calculated concentration of fortified water solution, obtained from the calibration function (ng/mL)
V <sub>fortified</sub>	Final volume of fortified sample (µL)
c <sub>sample, fortified</sub>	Analyzed concentration of fortified sample, as calculated from the calibration function (ng/mL)
V <sub>sample</sub>	Final volume of sample (µL)

Recoveries were calculated by the following equation:

$$Rec = \frac{R_{found}}{R_{fortified}} \cdot 100 \%$$

Rec	Recovery (%)
R <sub>found</sub>	Residue determined (µg/L)
R <sub>fortified</sub>	Fortification level (µg/L)

An example of the calculation for a 0.05 µg/L recovery of fluometuron (quantifier mass transition) from surface water:

$$R = \frac{0.0479 \cdot \frac{1050}{1000}}{\frac{0.1}{0.1055} \cdot 0.105 \cdot \frac{1050}{1000} - \frac{0.1}{0.1055} \cdot 0.052} \cdot \frac{0.1}{0.1055} \cdot 0.052$$

= 0.0449 µg/L (90 % recovery)

c <sub>1</sub>	0.1 ng/mL
c <sub>2</sub>	0.1055 ng/mL (mean of 0.102 ng/mL and 0.109 ng/mL)
c <sub>sample</sub>	0.052 ng/mL
c <sub>Std</sub>	0.0479 ng/mL (mean of 0.0448 ng/mL, 0.0461 ng/mL, 0.0533 ng/mL and 0.0474 ng/mL)
V <sub>fortified</sub>	1050 µL
c <sub>sample, fortified</sub>	0.105 ng/mL
V <sub>sample</sub>	1000 µL

## 5 Deviations from the Study Plan

The study was performed according to the study plan dated 07 March 2012 without any deviation.

## 6.2 Repeatability (Precision)

The relative standard deviation (RSD) at the quantifier and qualifier ion transitions was within the guideline requirements ( $\leq 20\%$  RSD) for each test item, fortification level and matrix.

## 6.3 Blanks

Two untreated control samples were analysed for fluometuron, des-methyl-fluometuron and CGA72903. Residues of the test items were not detectable in these samples (i.e.  $< 30\%$  of LOQ or  $< 0.015\ \mu\text{g/L}$ , see also representative chromatograms in the appendix D).

## 6.4 Limit of Quantification and Limit of Detection

The limit of quantification is defined as the lowest fortification level with mean recoveries ranging between  $70\%$  and  $120\%$  at a relative standard deviation not exceeding  $20\%$  and blanks not exceeding  $30\%$ . These conditions are fulfilled for the  $0.05\ \mu\text{g/L}$  fortification level for each test item. The limit of detection was defined as  $30\%$  of LOQ, i.e.  $0.015\ \mu\text{g/L}$ .

## 6.5 Linearity

For analysis of the test items by HPLC-MS/MS, the detector responses were linear within the range of  $0.01\ \mu\text{g/L}$  to  $10\ \mu\text{g/L}$  with  $r \geq 0.999$  for the quantifier mass transitions of fluometuron, des-methyl-fluometuron and CGA72903 and for the qualifier mass transitions of des-methyl-fluometuron and CGA72903. For the qualifier mass transition of fluometuron the detector response was linear with  $r \geq 0.998$  (see Figure 1 to Figure 6 in appendix C).

## 6.6 Matrix Effects

To compensate the matrix derived possible ion enhancement or suppression effects in HPLC-MS/MS analysis, the standard addition method was used, due to different matrix composition of each sample.

## 6.7 Specificity

The method is highly specific for analysis of the test items (mass transitions from the positively charged molecule ions to two typical fragment ions in MS/MS mode). The retention time of the test items in solvent matched the retention times in fortified samples. No peak interferences occurred at the retention times of the test items.

## **6.8 Storage Stability of the Test Item in Solution**

Since samples were not analyzed within 24 hours after sample preparation, the storage stability in the extract had to be tested.

Sample solutions were stored up to 2 days in the dark. The stability of the test items in the final solution were verified by analysing the recovery samples. The results of this validation indicate that all test items were stable in the solutions over this storage period.

## **6.9 Time Required for Analysis**

For preparation of a set of 24 specimens (two validations with each 10 recoveries and 2 blanks), about 2 laboratory hours are needed. Preparation for HPLC-MS/MS including calibration standards takes an additional 0.5 hour. HPLC-MS/MS analysis can be performed unattended and requires about 10 minutes per run. Thus, a set consisting of 10 calibration standards, 20 recoveries plus 4 control standards without fortification and 20 recoveries plus 4 control standards fortified and injections of standards after 2-6 samples to verify the detector response and retention times requires about 11 hours.

Evaluation of the HPLC-MS/MS results takes about 1 hour. Thus, the time span from initiation of extraction until completion of instrumental analysis is about 14.5 hours.