INTRODUCTION

Analytical method GRM 05.19, “Determination of Residues of XDE-742 and Its Metabolites in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometry” (Appendix A), was developed and validated at Dow AgroSciences LLC. The method was found to be suitable for the determination of residues of XDE-742 in water over the concentration range of 0.05-50 µg/L. The validated limit of quantitation was 0.05 µg/L. An independent laboratory validation of method GRM 05.19 was conducted on drinking water and surface water to satisfy the requirements of the Subdivison N (Environmental Fate), Series 164-1; Publication of Addenda for Data Reporting E, K, and N Requirements for Pesticide Assessment Guidelines; Guideline OPPTS 850.7100 "Public Draft"; PR Notices 86-5 and 96-1, EU Council Directive 91/414/EEC and SANCO/825/00 rev. 7, 17-Mar-2004 (see References).

The independent laboratory, the Study Director, and the analysts chosen to conduct the ILV were unfamiliar with the method, both in its development and subsequent use in analyzing samples. The independent laboratory used all of its own equipment and supplies, so that there was no common link between Dow AgroSciences and the ILV analysts. Throughout the conduct of the study, any communications between Dow AgroSciences and the Study Director and/or the analyst were logged for inclusion in the report. No one from Dow AgroSciences was allowed to visit the independent laboratory during the ILV trial to observe, offer help, or assist the chemists or technicians. These steps successfully maintained the integrity of the ILV study.

ANALYTICAL

Preparation and Storage of Samples

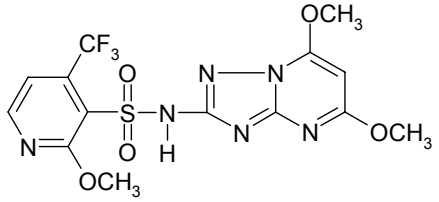
Drinking water was collected on 03-Jan-06 in the morning from a tap at PTRL Europe. Surface water was collected on 07-Jan-06 in the morning from a pond in Ulm, located in Southern Germany. The appearance of both types of water was colourless without any smell. The waters were characterized for physical and chemical properties as follow: Drinking water: pH 7.5, total water hardness: 16° d (Deutsche Härtegrade, 2.9 mmol/L), dissolved organic carbon (DOC): 0.50 mg/L, turbidity: 0.48 NTU and filterable compounds: < 1.0 mg/L. Surface water: pH 7.5, total water hardness: 18° d (Deutsche Härtegrade, 3.2 mmol/L), dissolved organic carbon (DOC): 1.4 mg/L, turbidity: 3.18 NTU and filterable compounds: 3.9 mg/L.

Preparation of Solutions and Standards

Reagents (obtained from Fluka, Merck, Promochem and Kordon) used were of equivalent specifications as described in Section 6.1 of method GRM 05.19. Solutions were prepared as described in Section 6.3 of method GRM 05.19.

The following analytical reference standard/test substance (obtained from the Sponsor) was utilized during the independent laboratory method validation:

XDE-742

Common Name of Compound	Structural Formula and Chemical Name (IUPAC)
XDE-742 (parent) Molecular Formula: $C_{14}H_{13}F_3N_6O_5S$ Formula Weight: 434.4 g/mole Nominal Mass: 434 CAS Number 422556-08-9	 *N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide

Test Substance/ Analytical Standard(s)	AGR/TSN No.	Percent Purity	Certification Date	Reference
XDE-742 (parent)	TSN102482	100	04-May-2005	05-729-L

Standard solutions and calibration standard solutions were prepared as described in Section 7 of method GRM 05.19.
Stability of solutions was not tested specifically, but was considered to be stable due to consistent LC/MS/MS response during the study.

Fortification of Recovery Samples

One ILV trial of the method was run for each water type and consisted of the following:

1 reagent blank (containing no matrix or analyte)
2 unfortified control samples
5 control samples fortified at 0.05 µg/L with XDE-742 (the LOQ of the method)
5 control samples fortified at 0.5 µg/L with XDE-742 (10 x LOQ).

Fortification solutions were prepared as described in Section 7.1 of the residue analytical method GRM 05.19.

Sample Extraction, Purification and Analysis

The ILV trial was conducted as described in Section 9.3 of method GRM 05.19, with negligible variations due to slightly different laboratory equipment and practices.

Analytical Instrumentation and Equipment

Analytical Equipment used was of equivalent specifications as described in Section 4.1 of method GRM 05.19.
Prior to initiation of the first ILV trial, the independent laboratory conducted preliminary studies necessary for establishing acceptable performance of the chromatographic instrumentation to be used. These preliminary studies included establishing that adequate HPLC retention times of the

analytes and MS/MS detector sensitivity could be achieved. Verification of a lack of XDE-742 contamination in the control sample matrices was not conducted prior to the method trial.

The instrumental conditions used during the ILV trial were conducted as described in Section 8 of method GRM 05.19, with minor adaptations as given below:

LC and MS parameters for the determination of XDE-742

Liquid Chromatography Operating Conditions

Instrumentation:	CTC Analytics HTC PAL Autosampler Agilent Model 1100 binary pump Agilent Model 1100 degasser
Column:	Phenomenex Synergi Polar-RP 75 x 4.6 mm i.d., 4 µm particle size Securityguard: Phenomenex Polar-RP, 4 x 3 mm, 4 µm particle size
Column Temperature:	35 °C
Injection Volume:	100 µL
Run Time:	11 minutes
Mobile Phase:	A – 0.01% formic acid in water B – 0.01% formic acid in acetonitrile
Flow Rate:	300 µL/min

Gradient:

Time, min	A, %	B, %
0.00	95	5
3.00	5	95
8.00	5	95
8.10	95	5
11.00	95	5

Mass Spectrometry Operating Conditions

Instrumentation:	Applied Biosystems API 3000 LC/MS/MS System Applied Biosystems Analyst 1.3.1 data system
Interface:	TurboIonSpray
Scan Type:	MRM
Resolution:	Q1 – Unit, Q3 – Unit
Curtain Gas (CUR):	12
Collision Gas (CAD):	4
Temperature (TEM):	450 °C
Nebulizer Gas (NEB):	14
Run time:	11 minutes
Polarity:	Positive
IonSpray Voltage (IS:)	5000 V

Compound:	Ion, m/z		Dwell Time, ms	Collision Energy, V	CXP, V
	Q1	Q3			
XDE-742 (quantitation)	435	195	75	37	16
XDE-742 (confirmation)	435	82	100	87	6

Calculations

Linear regression equations using external standards were generated for XDE-742 by injecting calibration standards. Regression calculation was performed by the Analyst software, with 1/x weighting, using the concentration in ng/mL, for the X-axis, versus the peak area for the Y-axis (see Figure 1).

Calibration standards (see Figure 2 to Figure 4 for examples) with 0.015, 0.05, 0.25, 0.5, and 1.0 ng/mL of the analyte were prepared in water/acetonitrile with 0.03 % formic acid (9/1, v/v, see GRM 05.19, Section 7.2.1.).

Concentrations of the analyte in the final extracts were determined by substituting the peak area into the linear regression equation as shown below:

$Y = aX + b$

Y: Analyte peak area

X: Analyte concentration c_{End}

Thus:

$c_{End} = (Y - b) / a$

$c_{End} = (\text{Analyte peak area} - b) / a$

The analyte concentration is thus obtained as residue R (in µg/L) by the following calculation:

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

$= c_{End} \times \text{Multiplier M}$

where:

c_{End}: = Concentration of final extracts in ng/mL

V_{End} = Extraction volume = Volume of final extracts (10 mL)

W: = Sample volume (9.0 mL)

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

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

Example for Calculation of XDE-742:

The calculation is exemplified with the drinking water specimen PTRL-ID P1001-30.

9.0 mL water was fortified at 0.05 µg/L (LOQ) by dosing 90 µL of the 5 ng/mL XDE-742 fortification solution.

After addition of 1.0 mL of acetonitrile, the final extract was examined by LC/MS/MS in run file P1001-287 (Figure 6), resulting in a XDE-742 peak area of 38030 counts for the m/z Q1/Q3 435/195.

The Analyst software used the calibration function

Y = 955000 x X + 2350 (Figure 1, top)

which was established by injecting calibration solutions interspersed with final extracts, whereby:

Y: Analyte peak area

X: Analyte concentration

Including the intercept b with 2350, the linear calibration function becomes:

c_{End} = $(Y - b) / a$

= (Analyte peak area - 2350) / 955000

= (38030 counts - 2350) / 955000

= 0.037 ng/mL

The analyte concentration is thus obtained as residue R (in µg/L) by the following calculation:

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

= 0.037 ng/mL x (10 mL / 9 mL)

= 0.037 ng/mL x 1.11

= 0.041 ng/mL (µg/L)

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

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

= (0.041 µg/L / 0.050µg/L) x 100 %

= 82 %

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the "AVERAGE (MITTELWERT)" function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recovery for a sample was calculated using the "STDEV (STABW)" function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. Percent relative standard deviation, % RSD, was calculated by dividing the standard deviation by the mean, and then multiplying by 100.

Confirmatory Evaluation

For confirmation of residues a second MRM was monitored.

Problems Encountered, Changes or Modifications Made, and Critical Steps

No problems were encountered with the methodology for analysis of XDE-742.

Sample Analysis Time Requirements

One set of 13 samples required approximately 3 person-hours or approximately a half work day to complete in the laboratory, followed by unattended over-night LC/MS/MS analysis, and by approximately 2 hours of evaluation and data transcription. Thus, such a set can be completed in approximately a half calendar day.

Communications

No contacts between the Study Director at the independent laboratory and the method developers, or others familiar with the method were necessary.

CONCLUSION

Dow AgroSciences LLC Method GRM 05.19 has been successfully validated by an independent laboratory analyst who had no prior experience with the method and no prior knowledge of the residue analytical methodology.

The LOQ of the method was confirmed as 0.05 µg/L for XDE-742 in water.

ARCHIVING

At the conclusion of the study, the raw data, the original study plan, amendments, deviations, and the original version of the final report will be archived at Datacare Business Systems Limited, 3012 Heyford Park, Heyford Park, Upper Heyford, Oxon, OX25 5HF, United Kingdom.

REFERENCES

1. Subdivision N (Environmental Fate) of the Pesticide Assessment Guidelines, prepared by OPPTS/EPA; Washington, DC, 1982).

2. Publication of Addenda for Data Reporting E, K, and N Requirements for Pesticide Assessment Guidelines. Federal Register Notice and Addenda. U.S. Environmental Protection Agency). U.S. Government Printing Office: Washington, DC, 1995, Vol. 60 No. 75.

3. *Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods*; "Public Draft." U.S. Environmental Protection Agency. Office of Prevention, Pesticides, and Toxic Substances (7101). U.S. Government Printing Office: Washington, DC, 1996, EPA-712-C-96-348.

4. *Pesticide Regulation Notice 86-5,* U.S. Environmental Protection Agency, Office of Pesticide Programs: Washington, DC, 1986.

5. *Pesticide Regulation Notice 96-1,* U.S. Environmental Protection Agency, Office of Pesticide Programs: Washington, DC, 1996.

6. *EU Council Directive, 91/414/EEC, Section 2 of European Commission Guidance Document-SANCO/825/00 rev.7,* Directorate General Health and Consumer Protection, 17-Mar-2004.