INTRODUCTION

The objective of this study was to independently validate the Dow AgroSciences residue method, study number 110587, for the determination of propyzamide and five metabolites (RH-24644, RH-24655, RH-24580, RH-26059, and UK1) in water. The independent validation (ILV) was conducted using untreated control samples of representative water (surface (pond), ground (well) and drinking (tap)). These matrices are considered representative for the intent of the method.

The method was found to be suitable for the determination of propyzamide and five metabolites in water over the concentration range 0.05 µg/L to 1.0 µg/L, with a validated limit of quantitation (LOQ) of 0.05 µg/L.

This independent laboratory validation was conducted to satisfy the requirements of the European Council Directive 91/414/EEC, as amended by European Commission Directive 96/46/EC, and the European Commission Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 8.1 (2, 3, 4). The study was also conducted to satisfy the requirements of U.S. EPA Guideline OPPTS 850.7100 (5), and PR Notice 96-1 (6). This validation report presents the results of the independent laboratory validation for propyzamide and five metabolites in water.

The independent laboratory, the Study Director, and the analyst chosen to conduct the ILV were unfamiliar with the method, both in its development and subsequent use in analyzing the water samples. The independent laboratory has no organizational ties to Dow AgroSciences, and used all of its own equipment and supplies, so that there was no common link between Dow AgroSciences LLC and the Study Director or analyst. 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.

This report was amended to include information regarding the confirmation ions, which were monitored but not included in the original report. Recovery tables, statistics, data spreadsheets, and chromatograms for the confirmation ions were added, as well as a protocol amendment changing the study director. In addition, descriptions were added to the existing recovery tables, statistics, data spreadsheets, and chromatograms to differentiate between quantitation ion data and confirmation ion data. Administrative changes, such as updating study personnel, tables of contents, and page numbering, were also made. No changes to existing quantitation ion data were made.

ANALYTICAL

Sample Receipt, Labeling and Storage

The control matrices were acquired from the Sponsor. The control samples were assigned unique master logbook (MLB) number 23407564 and stored refrigerated (approximately 2–8°C).

Preparation of Solutions and Standards

The analytical reference standards/test substances utilized during the independent laboratory method validation are summarized below. The reference standards were received from the Sponsor and assigned unique MLB numbers. All standards were stored at ambient conditions and protected from light, except for RH-24644, which was stored refrigerated (2-8°C). The Certificates of Analysis are included in Appendix A.

Standard	TSN No.	Percent Purity	Recertification Date	Lot Number
Propyzamide (RH-23315)	TSN105825	98.2%	23Jul2013	F1245-77
RH-24644	TSN029409-0001	99.0%	01Sep2012	V43-037424-64
RH-24580	TSN103029	99.3%	01Sep2014	WDW60:19A
RH-24655	TSN103034	95.41%	30Sep2013	DGW45:59
RH-26059	TSN103038	99%	01Sep2013	TAM33:61
UK1	TSN103018	99%	01Sep2014	VM0413

Standard solutions and calibration standard solutions were prepared as described below and stored refrigerated (2–8°C) when not in use.

The following stock solutions were prepared in acetonitrile to obtain nominal concentrations of 1.00 mg/mL:

Analyte	Solution Type	Solution Lot Number	Weight [mg]	Dissolve In [mL]	Obtain [mg/mL]*
Propyzamide	Standard	N740P1-1	25.2	25.0	0.990
RH-24644	Standard	N740P1-2	29.3	25.0	1.16
RH-24580	Standard	N740P2-2	25.5	25.0	1.01
RH-24655	Standard	N740P2-1	26.3	25.0	1.00
RH-26059	Standard	N740P3-1	28.1	25.0	1.11
UK1	Standard	N740P3-2	28.0	25.0	1.11

*Resulting concentrations after correcting for purity

Mixed intermediate solutions were prepared in acetonitrile:

From Solution Lot Number	Concentration [mg/mL]	Pipette [µL]	Dilute To [mL]	Obtain Total [µg/mL]	Final Solution Lot Number
N740P1-1	0.990	252.5	25.0	10.00	N740P4-1
N740P1-2	1.16	215.5			
N740P2-2	1.01	247.5			
N740P2-1	1.00	250.0			
N740P3-1	1.11	225.2			
N740P3-2	1.11	225.2			

Mixed fortification solutions were prepared in acetonitrile:

From Solution Lot Number	Concentration [µg/mL]	Pipette [µL]	Dilute To [mL]	Obtain Total [ng/mL]	Final Solution Lot Number
N740P4-1	10.00	100	10	100	N740P5-1
N740P5-1	0.100	500	10	5.0	N740P5-2
N740P5-1	0.100	150	10	1.5	N740P6-1

Mixed calibration standards were prepared fresh daily in 80/20 water/acetonitrile (v/v) using gas tight glass syringes and glass volumetric pipettes:

From Solution Lot Number	Concentration [ng/mL]	Aliquot of Stock Solution [µL]	Final Solution Volume [µL]	Calibration Solution Final Concentration [ng/mL]	Equivalent Sample Concentration [µg/L]*
N740P5-1	100	80	5000	1.6	2.0
N/A	1.6	1000	1500	1.07	1.333
N/A	1.6	1000	2000	0.8	1.0
N/A	0.8	1000	2000	0.4	0.5
N/A	0.4	1000	2000	0.2	0.25
N/A	0.2	1000	2000	0.1	0.125
N740P6-1	1.5	80	3000	0.04	0.05
N/A	0.04	1000	3333	0.012	0.015

*Equivalent sample concentration is based on a dilution factor of 0.8

Fortification of Recovery Samples

The ILV trial of the method was performed for propyzamide and five metabolites in each water type. The trial was comprised of one batch, which consisted of the following samples in each matrix:

1 (one) reagent blank (containing no matrix or analyte)

1 (one) unfortified control sample

1 (one) control sample fortified at 0.015 µg/L, the proposed limit of detection

5 (five) control samples fortified at 0.05 µg/L, the LOQ of the method

5 (five) control samples fortified at 1.0 µg/L, or 20×LOQ

For preparation of recovery control specimens, appropriate volumes of the fortification standards were added as indicated below using gas tight glass syringes:

Specimen Portion	Nominal Target Fortification Level [µg/L]	Aliquot of Fortification Solution [µL]	Fortification Solution Concentration [µg/L]
4.00 mL	0.05	40.0	5.00
	1.0	40.0	100

Sample Analysis

The ILV trial was conducted as described in the Dow AgroSciences residue method, study number 110587 (1).

The sample extracts for the analysis of propyzamide and five metabolites were analyzed by HPLC with positive-ion electrospray tandem mass spectrometry (LC/MS/MS) for propyzamide, RH-24644, RH-24655, RH-24580, and UK1, and negative-ion electrospray LC/MS/MS for RH-26059.

Confirmation ions were also monitored.

For more specific details, refer to the analytical method (1).

Analytical Instrumentation and Equipment

Prior to initiation of the first ILV trial, the independent laboratory conducted preliminary studies necessary for establishing acceptable performance of the extraction and chromatographic instrumentation supplied by the method. These preliminary studies established that adequate retention times of the analytes and detector sensitivity could be achieved. The mixed fortification solutions that were used were also used throughout the remainder of the study. The following instruments and equipment were utilized in the conduct of the independent laboratory validation of the residue analytical method:

Instrumentation

Typical HPLC Conditions

Instrument:	Spark Holland Symbiosis (System 13)
Column:	Zorbax SB-C8, 3.5 µm, 75 × 4.6 mm
Temperature:	Ambient
Injection Volume:	30 µL
Run Time:	7 minutes
Mobile Phase:	A: 0.1% formic acid in water
	B: 0.1% formic acid in acetonitrile
Total Flow Rate:	0.8 mL/min (approximately 300 µL/min split to source

Gradient:

Time, min	% A	% B
0.00	90	10
3.00	0	100
5.00	0	100
5.15	90	10
7.00	90	10

Typical MS Conditions

Mass Spectrometer: Applied Biosystems API 5000 LC/MS/MS (System 15)

Positive Acquisition Mode for Propyzamide, RH-24644, RH-24655, RH-24580 and UK1

Detector Mode: Positive-ion electrospray
Source Temperature: 500°C
Ions Monitored:

	Transition	Declustering Potential V	Collision Energy eV	Dwell Time ms	Retention Time (+/- 0.3 min.)
Propyzamide	256 → 190 258 → 192	46	21	50	3.74
RH-24644	256 → 173 256 → 109	80 80	33 73	50	4.35
RH-24655	258 → 190 258 → 173	60	17 31	50	3.91
RH-24580	274 → 173 274 → 109	58	31 75	50	3.61
UK1	222 → 156 222 → 139	50	19 29	50	3.47

Negative Acquisition Mode for RH-26059

Detector Mode: Negative-ion electrospray
Source Temperature: 500°C
Ions Monitored:

	Transition	Declustering Potential V	Collision Energy eV	Dwell Time ms	Retention Time (+/- 0.3 min.)
RH-26059	288 → 188 290 → 190	-90	-22	50	3.37

Equipment

Analytical Balance, Sartorius, model number AC 120S, serial number 20103137 (EQ37)

Pico Centrifuge, Sorvall Biofuge, serial number 40219357 (EQ56)

Materials

Glass volumetric flasks, various sizes

Glass displacement pipettes, various sizes

Gilson positive displacement pipettes, various sizes

Gas tight glass syringes, various sizes

Disposable culture tubes, 100 × 16 mm

1.8 mL clear glass HPLC vials

Chemicals

Acetonitrile, HPLC grade, lot numbers DE419, DE645, VWR

Formic acid, HPLC grade, lot numbers SZBA3280V, VWR

Purified reagent water, HPLC Grade, lot numbers DE459-B, 02911-02, VWR

Calculations

Calculations were not modified from the original analytical method. Using the calibration curve calculated by power fit regression, the calculated analyte concentration in the sample extracts in ng/mL (µg/L) was calculated using Equation 1:

$$y = mx^b \quad (1)$$

Where:

y = the analyte peak area
m = constant
x = the analyte concentration found in final extract (µg/L)
b = exponent

Equation 1 was rearranged as Equation 2 to solve for the analyte concentration.

$$x = \left(\frac{y}{m}\right)^{1/b} \quad (2)$$

The percent recovery of the fortified samples was calculated using Equation 3:

$$\% \text{ Recovery} = \frac{AC}{FC} \times 100 \qquad (3)$$

Where:

AC = Analyte concentration in µg/L
FC = Concentration fortified (µg/L)

As an example, the LOQ 1 quality control ground water sample (Table 43, Figure 67) resulted in a propyzamide recovery of 100%. The calculations for this sample are demonstrated below as a representative example of how all the sample results were calculated for this study.

The power regression analysis of the calibration curve used in the analysis of propyzamide residues in ground water samples from Trial 2 was determined to have the following regression coefficients: m = 5.54E+04 and b = 0.981 (Figure 7). The analyte peak area (y) was 2.94E+03; therefore the concentration of propyzamide in the final extract of this sample was calculated using Equation 2:

$$x = \left(\frac{2.94E+03}{5.54E+04}\right)^{1/0.981} = 0.0502 \text{ µg/L} \qquad (2)$$

The percent recovery of the sample was calculated using Equation 3:

$$\% \text{ Recovery} = \frac{0.0502 \text{ µg/L}}{0.0500 \text{ µg/L}} \times 100 = 100\% \qquad (3)$$

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the "AVERAGE" 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 recoveries for a sample was calculated using the "STDEV" 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.

Method Modifications

Due to possible stability issues with the analytes in Trial 1, the autosampler temperature was reduced to approximately 15°C in Trial 2. Additionally, a linear regression with 1/x weighting was used in Trial 1; after clarification was received from the sponsor, a power regression was used in Trial 2 as per the method.

Critical Steps

Based on the variability of the results obtained in Trial 1, and the inconsistent accuracy of the mixed calibration standards, it should be emphasized that standards should be prepared fresh daily and that the autosampler temperature is critical. Additionally, the possibility that all analytes may adhere to plastic should be mentioned. Therefore, to reduce contact with plastic, the use of syringes and glass pipettes to fortify samples is critical.

Sample Analysis Time Requirements

One batch of 38 samples required approximately four person hours over one calendar day to complete the preparation. Analysis of water samples on the instrument was performed over a three-hour time period. Data manipulation required an additional one to two person hours. Initial solution preparation required approximately three person hours.

Communications

All contacts between the Study Director at the independent laboratory and the Study Monitor at Dow AgroSciences LLC, the method developers, or others familiar with the method are documented in Appendix C.