

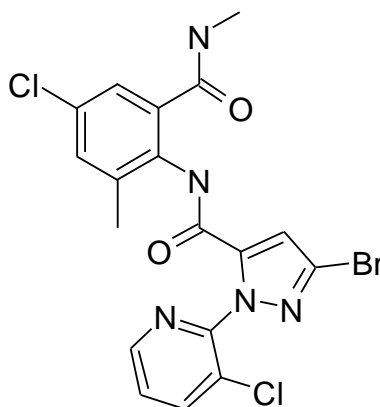
E.I. DUPONT DE NEMOURS & COMPANY

794. Chlorantraniliprole CIPAC Assay Method

5008/m

Mary Ellen P. McNally, Ph.D.

6/5/2015

CHLORANTRANILIPROLE**794**

<i>ISO Common name</i>	Chlorantraniliprole
<i>Chemical name</i>	3-bromo-4'-chloro-1-(3-chloro-2-pyridyl)-2'-methyl-6'-(methylcarbamoyl)pyrazole-5-carboxanilide (IUPAC) 3-Bromo-N-[4-chloro-2-methyl-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide (CAS 500008-45-7)
<i>Empirical formula</i>	C ₁₈ H ₁₄ BrCl ₂ N ₅ O ₂
<i>RMM</i>	483.15
<i>m.p.</i>	208-210°C
<i>Solubility in various solvents at 20°C</i>	1.0 µg/mL in water 124 mg/mL in dimethylformamide 3.4 mg/mL in acetone 2.5 mg/mL in dichloromethane 1.7 mg/mL in methanol 1.1 mg/mL in ethyl acetate 0.71 mg/mL in acetonitrile 0.39 mg/mL in n-octanol 0.16 mg/mL in o-xylene <0.1 µg/mL in n-hexane
<i>Description</i>	Fine crystalline off-white powder
<i>Stability</i>	Aqueous hydrolysis: Chlorantraniliprole is stable at pH 4 and 7; at pH 9, chlorantraniliprole hydrolysed with a half-life of approximately 10 days.

Formulations

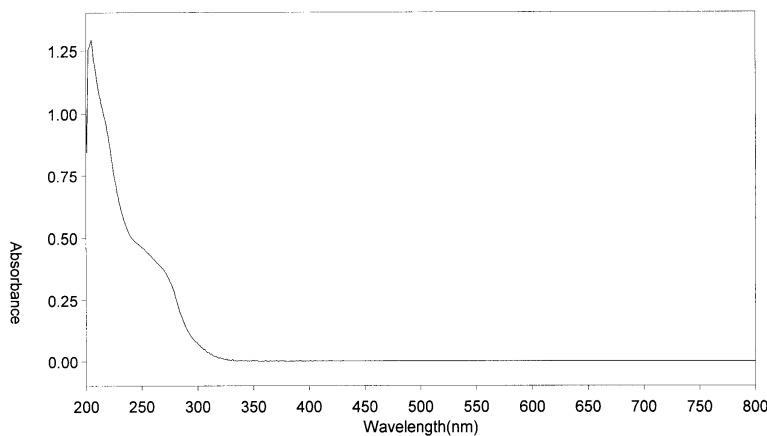
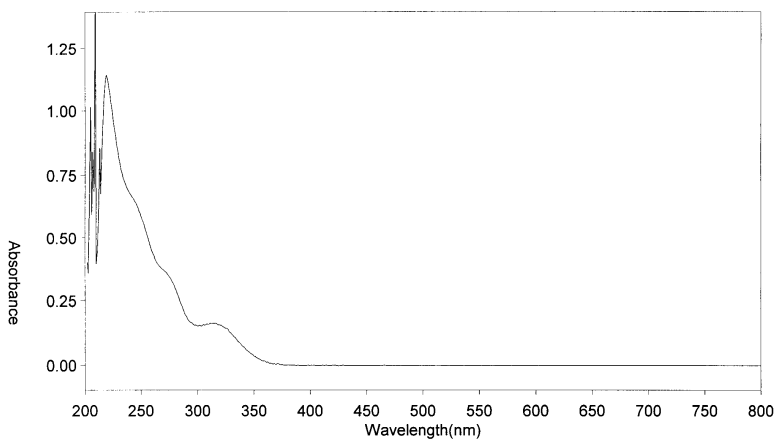
625 FS, Flowable Concentrate for Seed Treatment
35 WG, Wettable Granule
200 SC, Suspension Concentrate
50 SC, Suspension Concentrate

1. Sampling.

As received.

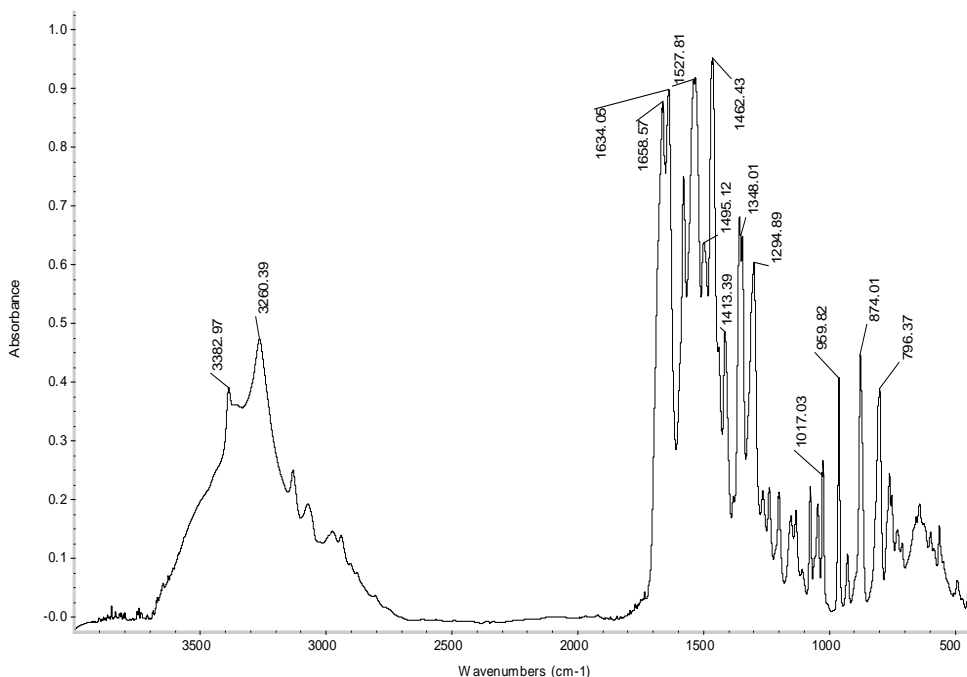
2. Identity Tests.

- 2.1 **HPLC.** Use the HPLC method below. The relative retention time of the chlorantraniliprole peak in the sample solution should not deviate by more than 5% from that of the calibration solution and the UV spectrum measured from this peak should match that obtained from the calibration substance.

UV/Vis spectra in neutral, acidic and basic solutions of Technical Sample of Chlorantraniliprole.**A. Neutral and acidic solutions****B. Basic solution**

- 2.2 **Infrared (IR).** Prepare potassium bromide discs for the technical sample and chlorantraniliprole reference substance. Formulated samples: mix 100 mg of formulation with 5-10 mL of acetonitrile, mix well, and filter. Evaporate the filtrate to dryness and prepare potassium bromide discs from the residues. The spectra from the samples should not differ significantly from that of the reference substance.

Infrared Spectrum of Technical Sample of Chlorantraniliprole



3. Chlorantraniliprole

OUTLINE OF METHOD

The sample is dissolved in tetrahydrofuran and subsequently diluted with the mobile phase, a mixture of acetonitrile and a buffer of 0.005M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ adjusted to pH 3.0 with H_3PO_4 . The analysis is conducted via reversed-phase liquid chromatography using a 3.0- μm particle, 4.6 x 150 mm Ace C18[®] column (No substitutions.) and UV detection at 275 nm. The weight percent of chlorantraniliprole in each sample is determined by comparison of peak area or peak area ratio if an internal standard is used versus a calibration curve prepared from the analysis of standard solutions.

REAGENTS

Acetonitrile, HPLC grade

Tetrahydrofuran, Spectroscopic grade (un-stabilized – without BHT)

Comment: Un-stabilized THF is required due to a potential chromatographic interference. The THF should not be stored longer than 6 months to avoid the formation of peroxides. Peroxides are highly-explosive on storage in air. To minimize this problem, commercial samples of THF are often inhibited with BHT. Since BHT is not present in the unstabilized THF, the THF used for this analysis should be labeled with the date the container is opened and discarded within 6 months of that date.

Water, Deionized, HPLC grade

HPLC Internal Standard, Phenyl sulfone, 97% purity.

pH 1.68, 4.0 and/or 7.0 standard buffers (phthalate-free buffers) for pH calibration

Phosphoric acid, 85% H₃PO₄

Sodium phosphate monobasic, monohydrate NaH₂PO₄•H₂O

Mobile phase

The mobile phase is a gradient mixture of acetonitrile and pH 3.0 buffer. The buffer is prepared by dissolving 1.38 g NaH₂PO₄•H₂O in a 2000 mL (0.64 g per 1000 mL) in a 2000 mL volumetric flask filled with approximately 500 mL of water. Dilute to the mark with water and measure the pH of this solution, which should nominally be pH 4.8. Adjust the pH to 3.0 +/- 0.001 with H₃PO₄ added drop wise.

Internal Standard Solution

The internal standard solution is a preparation of a 10 mg/mL Phenyl sulfone solution in tetrahydrofuran. The internal standard is made by transferring 10 g (± 0.1g) of Phenyl sulfone into a 1000-mL volumetric flask. Add approximately 500 mL of HPLC grade tetrahydrofuran and ultrasonicate until all the phenyl sulfone has dissolved. Equilibrate the flask to room temperature and dilute to volume with THF.

Calibration solutions

Accurately weigh (to at least 0.1 mg precision) 7.5, 12.5, 25, 37.5, 50, 62.5, and 72.5 mg (± 5.0 mg) of chlorantraniliprole analytical standard into seven separate 50-mL volumetric flasks and record the weights. Add 5 mL of aqueous mobile phase and ultrasonicate for 2 minutes to aid the dissolution of the formulated products. Using a pipet or Repipet®, add 25 mL of (10 mg/mL) phenyl sulfone internal standard solution to each of the volumetric flasks. Weigh (± 0.1g) the aliquot of internal standard solution delivered to each flask and record. Ultrasonicate for an additional 5 minutes, swirling occasionally. Dilute to volume with 20 mL 1:1 acetonitrile/aqueous mobile phase buffer and ultrasonicate again for an additional 5 minutes. Equilibrate each flask to room temperature. Filter a portion of the sample

solution through a 0.45- μm Acrodisc[®] into an LC vial. Inject in duplicate. In this solvent, the standard solutions are stable for at least 90 days at room temperature.

APPARATUS

High performance liquid chromatograph A high-performance liquid chromatograph, gradient capable, equipped with a constant-temperature column compartment, an automated sample injector capable of injecting 5- μL aliquots, a variable wavelength UV detector and digital integrator or other data handling system.

HPLC column: 15 cm x 4.6 mm i.d. Ace[®] 3 C18 column, 3- μm particle size MacMod Analytical Inc. Chadds Ford, PA, USA 19317(Part No. ACE-111-1546).

No substitutions.

Filtering apparatus for sample and standard solutions: 3 mL disposable plastic syringes (or equivalent) fitted with 0.45- μm Acrodisc[®] PTFE filters.

Ultrasonic bath

Analytical Balance: capable of measuring ± 0.1 mg.

pH meter: capable of measuring ± 0.01 pH units.

PROCEDURE

(a) *Operating Conditions*

Column 15 cm x 4.6 mm i.d. Ace[®] 3 C18 column, 3- μm particle size

Mobile Phase

A = 0.005M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, pH adjusted to 3.0 with H_3PO_4

B = Acetonitrile, CH_3CN

LC Gradient Conditions

<u>Time</u>	<u>% CH_3CN</u>	<u>% pH 3.0 H_2O</u>
0.0	40	60
3.0	44	56
5.0	95	5
7.0	95	5
7.5	40	60
8.0 Stop Time	Post Time 2.0 minutes	

A 15 cm Ace[®] 3 C18 column was used in the development of this method.

No column substitutions may be made. We recommend that the column be rinsed with 50% Acetonitrile/Milli-Q[®] H_2O , followed by 100% acetonitrile, at the end of each day. This will ensure a stable baseline and extend the column lifetime.

<i>Eluent flow rate</i>	2.0 mL/min	
<i>Column temperature</i>	50°C	
<i>Injection volume</i>	5 µL	
<i>Detection wavelength</i>	275 nm (bandwidth 4 nm)	
<i>Reference wavelength</i>	450 nm (bandwidth 80 nm)	
<i>Retention Times</i>	Impurity 1	2.36
	Phenylsulfone	3.60
	Impurity 2	4.08
	DPX-E2Y45	4.23
	Impurity 3	5.29

The retention times of Impurity 2 and chlorantraniliprole peaks may vary slightly (within $\pm 10\%$), depending on the packing lot and the age of the column. However, baseline resolution and integration of Impurity 2 and chlorantraniliprole must be maintained.

Analysis Stop Time 10 minutes

(b) *Sample preparation.*

- i.) Prior to weighing, mill or grind 35WG samples thoroughly until the material is a uniform size and granules are no longer visible.
- ii.) Before sub-sampling liquid samples, mix thoroughly by swirling for approximately 1 to 2 minutes.
- iii.) Refer to the table below and accurately weigh (to at least 0.1 mg precision) the amount of sample indicated into a 50 mL volumetric flask.

<u>Sample Type</u>	<u>Amount (mg)</u>
Chlorantraniliprole Technical	50 \pm 5
Chlorantraniliprole 625 FS	100 \pm 10
Chlorantraniliprole 35 WG	143 \pm 10
Chlorantraniliprole 200 g/L Suspension Concentrate (SC)	270 \pm 20
Chlorantraniliprole 50 g/L Suspension Concentrate (SC)	1000 \pm 30

After the sample has been weighed with the amounts above, and the weight recorded, add approximately 5 mL of the aqueous mobile phase buffer (i.e., 0.005M NaH₂PO₄•H₂O, pH adjusted to 3.0 with H₃PO₄) into each flask and swirl. Place on balance and tare balance. Then, using a pipet or Repipet®, add 25 mL of (10 mg/mL) Phenyl sulfone internal standard solution to each of the volumetric flasks. Weigh (± 0.1 g) the aliquot of internal standard solution delivered to each flask and record. Dilute to volume with approximately 20 mL of a 1:1 acetonitrile: aqueous mobile phase buffer mixture and ultrasonicate again for an additional 5 minutes. Equilibrate each flask to room temperature and filter a portion of the sample solution through a 0.45- μ m Acrodisc® PTFE filter into a sample vial. Inject in duplicate. The sample solutions are stable for 90 days at room temperature in the solvent system.

Not all formulation inert ingredients will completely dissolve, especially for the chlorantraniliprole 35 WG formulation. Swirling during the final 5 minutes of ultrasonication, aids the dissolution.

- (c.) *Determination.* Equilibrate the column with mobile phase until a stable baseline is obtained. Inject, in duplicate, 5 μ L each of a solvent blank (50:25:25 THF: acetonitrile: aqueous mobile phase buffer mixture), standards, and samples, bracketing the samples with calibration standards.
- (d) *Calculation.* Prepare a calibration curve for chlorantraniliprole by plotting the peak areas versus the weights of the standards in milligrams, corrected for purity of the standard. Using the method of least squares, calculate the equation for the straight line which best fits the experimental calibration data. Typically, the correlation coefficient for such least square calculations is 0.999 or better.

Determine the weight % of chlorantraniliprole for each sample injection using the following equation:

$$Wt\%_{chlorantraniliprole} = \left(\frac{Peak\ Area - b}{m} \right) \div mg_{sample} \times 100\%$$

Where, m = slope of least-squares line

b = y-intercept

mg_{sample} = weight of sample in milligrams

When using internal standard, calculate the peak area ratio and weight ratio of each calibration standard using the following equations.

- a. Peak Area Ratio = $\frac{\text{Chlorantraniliprole Peak Area}}{\text{Internal Standard Peak Area}}$
- b. Weight Ratio = $\frac{\text{Weight of Chlorantraniliprole Standard}^*, \text{ mg}}{\text{Weight of Internal Standard Solution, mg}}$

*Corrected for purity of standard.

Prepare a calibration curve for chlorantraniliprole by plotting the peak area ratio versus the weight ratio of the standards. Using the method of least squares, calculate the equation for the straight line which best fits the experimental calibration data. Typically, the correlation coefficient for such least square calculations is 0.999 or better.

Calculate the peak area ratio for each sample solution using the above equation (see Equation a, above).

Calculate the % DPX-E2Y45, w/w, in each sample using the following equation:

$$\% \text{ DPX-E2Y45} = \left(\left(\left(\frac{\text{Peak Area Ratio} - b}{m} \right) \times \text{Wt of Internal Standard aliquot, mg} \right) \div \text{Sample Wt, mg} \right) \times 100$$

4. Suspensibility

Reagents and Apparatus as for MT 168 except:

Calibration solutions: Same as above. Prepare in the same way standard solutions at other concentrations, if needed. Filter a small portion of each solution through a 0.45- μm PTFE filter prior to analysis.

PROCEDURE

- (a) *Preparation of suspension and determination of sedimentation. MT 168.*
- (b) *Assay starting material.* Determine c, mass of chlorantraniliprole actually taken, according to above assay procedure
- (c) *Determination of chlorantraniliprole in the bottom 25 mL of suspension.*
After removal of the top 225 mL of suspension, add 125 mL of THF to the remaining 25 mL of suspension (Total volume: 150 mL). Place the cylinder in an ultrasonic bath for 5 minutes. Bring the total volume to 250 mL with a mixture of 50:50 acetonitrile: aqueous mobile phase. Place in an ultrasonic bath again for 5 minutes. Allow to cool to room temperature, mix well by inverting the cylinder several times, then take a suitable aliquot of the solution for the determination of the mass of chlorantraniliprole (*Q* g). Dilution of this solution may be necessary to fall within the calibration of the method.

- (d) *Calculation*

$$\text{Suspensibility} = \frac{111(c-Q)}{c} \%$$

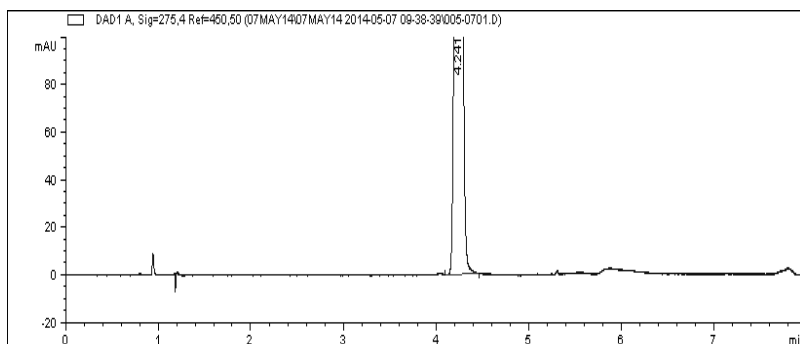
where, c = mass of chlorantraniliprole actually taken

Q = mass of chlorantraniliprole in the 25 mL remaining in the cylinder

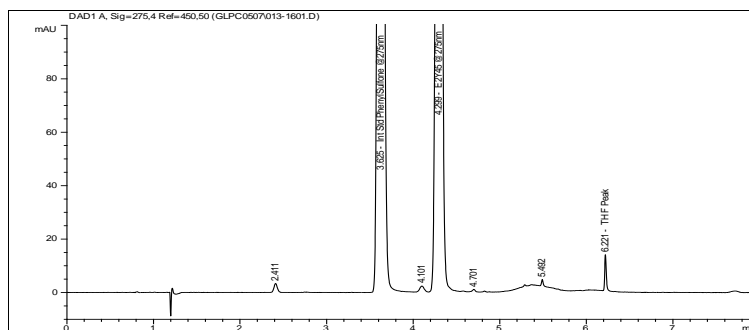
EXAMPLE CHROMATOGRAMS:

Standard:

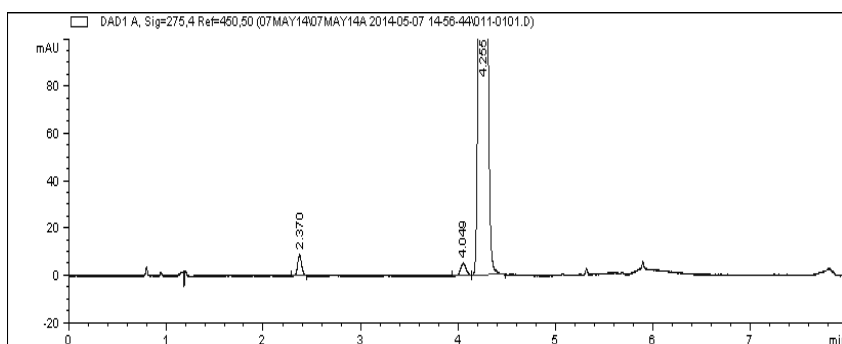
- a) Standard Solution Containing 1.012952 mg/mL of Chlorantraniliprole.



- b) Standard solution containing 1.001 mg/mL of standard and a nominally 5mg/mL of phenyl-sulfone, internal standard.

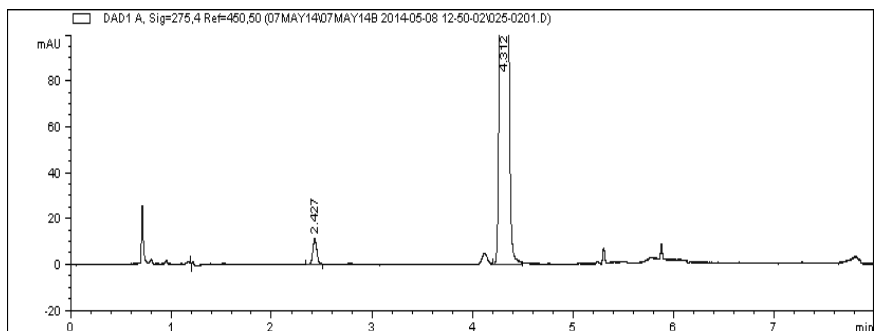


1. DPX-E2Y45-379; Chlorantraniliprole Technical Sample Solution at 1.034 mg/mL.

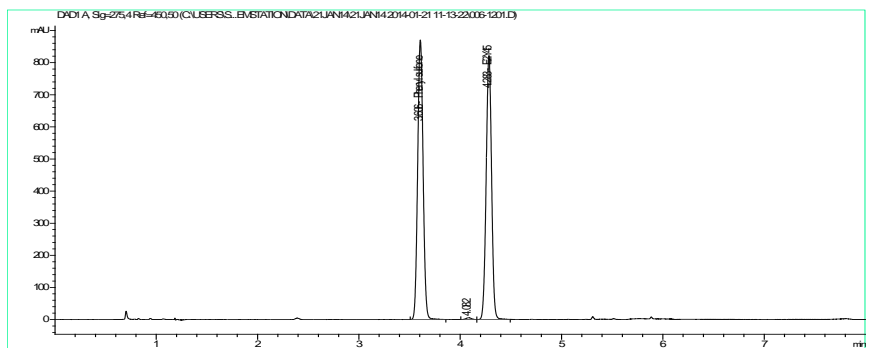


2.

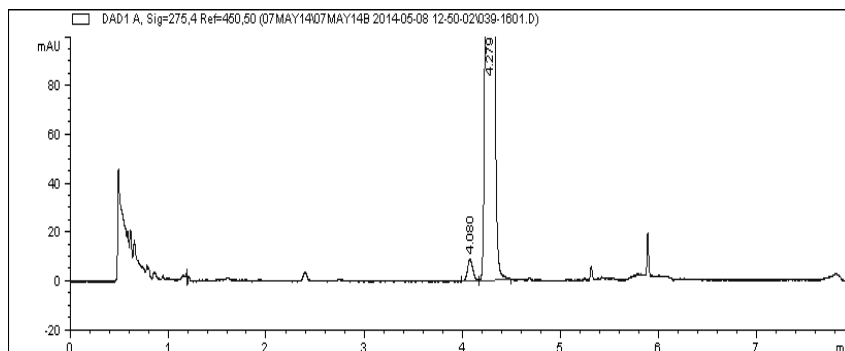
- a) DPX-E2Y45-595; Chlorantraniliprole 625 FS Sample Solution at 1.852 mg/mL.



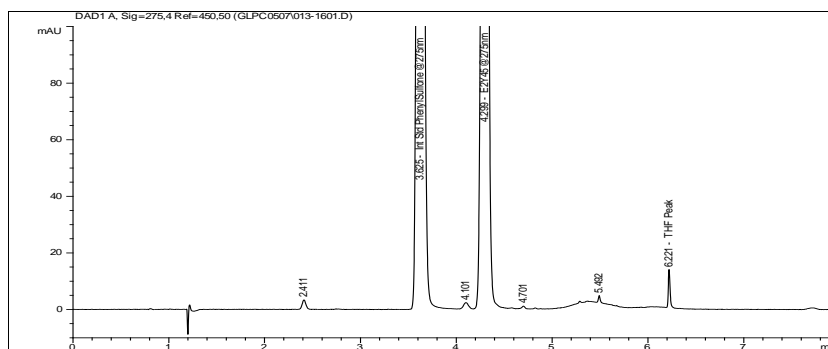
- b) DPX-E2Y45-561; Chlorantraniliprole 625 FS Sample Solution at 1.934 mg/mL and nominally 5 mg/mL phenyl sulfone internal standard.



3.
a) DPX-E2Y45-516; Chlorantraniliprole 35 WG Sample Solution at 2.854 mg/mL.

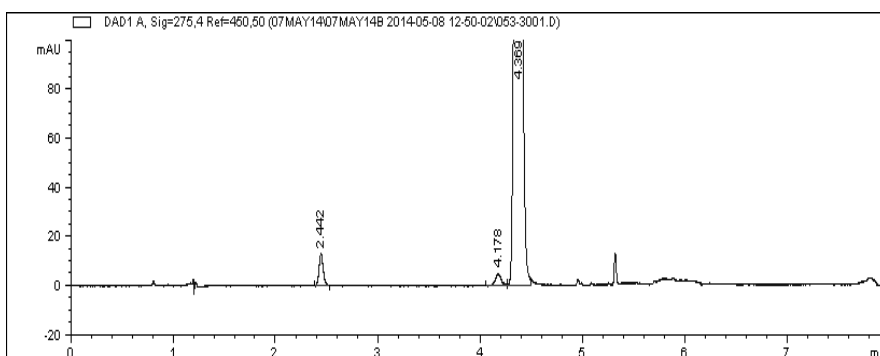


- b) E2Y45-215; Chlorantraniliprole 35WG sample solution at 3.277 mg/mL of sample and nominally 5 mg/mL of phenyl sulfone, internal standard.

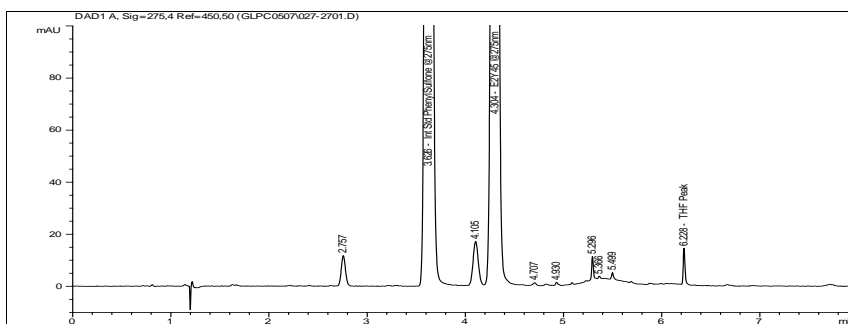


4.

- a) DPX-E2Y45-570; Chlorantraniliprole 200 g/L SC Sample Solution at 5.684 mg/mL.

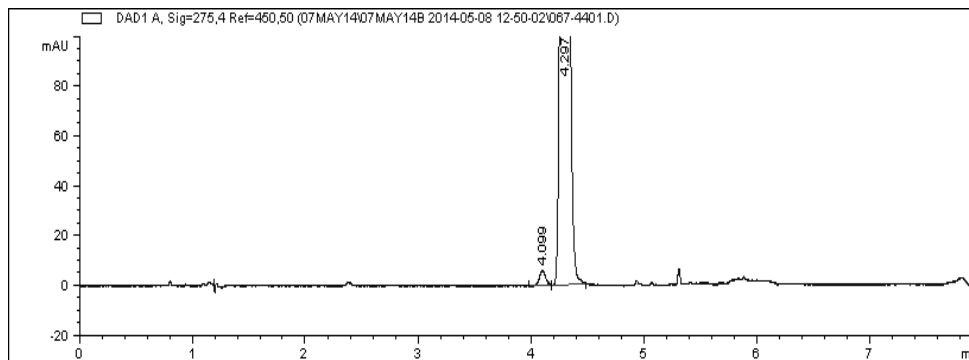


- b) E2Y45-239; Chlorantraniliprole 200 g/L SC sample solution at 5.818 mg/mL of sample and nominally 5 mg/mL of phenyl sulfone, internal standard.



5.

- a) DPX-E2Y45-599; Chlorantraniliprole 50 g/L SC Sample Solution at 19.448 mg/mL.



- b) DPX-E2Y45-262; Chlorantraniliprole 5SC sample solution at 19.966 mg/mL of sample and nominally 5 mg/mL of phenyl sulfone.

