APPLICATION FOR METHOD EXTENTION TO DELTAMETHRIN LONG-LASTING (COATED ONTO FILAMENTS) INSECTICIDAL NETTING - 333/LN/1

-- FONYI ® LN by method of *DETERMINATION OF deltamethrin in PermaNet ® BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY*

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1. INTRODUCTION

This document is intended to give detailed information and data of Fonyi Long-Lasting Insecticidal Mosquito Nets (LN) concerning method validation of deltamethrin determination, according to as demanded in *WHOPES TESTING AND EVALUATION OF LONG-LASTING INSECTICIDAL MOSQUITO NETS (LNs) -Guidance for submission of application package*, for WHOPES LN evaluation and corresponding CIPAC method evaluation.

Fonyi LN is independent developed by Hangzhou Blossom Trading Co., Ltd through years of research, which is made of 100 denier multi-filament polyester yarn with deltamethrin technically coated onto the warp knitted netting after the pre-treatment.

As the technical route of insecticidal treatment, which is coating of deltamethrin onto polyester netting, and targeted dose of deltamethrin on the netting are similar with PermaNet ®, the method *DETERMINATION OF deltamethrin in PermaNet ® BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY* available at CIPAC official website was adopted. During testing, the sampling protocol and Deltamethrin content analysis were strictly followed as described in the method.

During the method validation, documented evidence were collected and listed in the file. The main HPLC method validation characteristics are carried out and the results are discussed in this document, including accuracy, precision, linearity.

As the technical route is coating on polyester netting, the *WHO SPECIFICATIONS AND EVALUATIONS FOR PUBLIC HEALTH PESTICIDES—DELTAMETHRIN LONG-LASTING (COATED ONTO FILAMENTS) INSECTICIDAL NET* is adopted as the targeted specification and criteria to meet for Fonyi LN.

2. Validation protocol

Outline of the validation protocol

This protocol is used for validation of HPLC method *DETERMINATION OF deltamethrin in PermaNet ® BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY* (Appendix 1) for Fonyi LN to in terms of linearity, precision and accuracy.

The sample is extracted in a mixture of iso-octane and 1,4-dioxane. The Deltamethrin content is determined by normal phase high performance liquid chromatography using dipropyl phthalate as internal standard and detection at 236

nm.

REAGENTS

- Iso Octane, HPLC grade <Fisher Scientific, USA>
- 1,4 Dioxan, HPLC grade. <Fisher Scientific, USA>
- Deltamethrin, neat standard, Dr. Ehrenstorfer Gmbh
- Dipropyl Phthalate, Dr. Ehrenstorfer Gmbh
- Water, three distilled water
- Extraction solvent (ES): iso octan + 1,4 dioxane = 95+5
- Mobile Phase (MP): iso octan + 1,4 dioxane = 95+5
- Internal Standard solution (IS): 0.5 mg/ml of dipropyl phthalate in extraction solvent.

APPARATUS

- Shaker
- Ultrasonic bath
- HPLC, equipped with pump, auto-injector, column oven and UV detector. Analytical Column SinoChrom Si60, 5um, 150x4.6

Operating conditions

- Mobile Phase: iso octan + 1,4 dioxane = 95+5
- Flow rate : 1.3ml/min, isocratic
- Analytical Column: SinoChrom Si60, 5um, 150x4.6
- Column temperature: 40℃
- Inject Volume: 20ul
- Wavelength 236nm.
- Run time: 15minute.

Deltamethrin standard solution 0.6mg/ml (DS)

Weight 0.03 g (to the nearest of 0.01 mg) of Deltamethrin neat standard, quantitatively transfer to 50 volumetric flask, dissolve completely with extraction solvent, keep regulated by water bath at 20oC, fill up to mark with extraction solvent.

Preparation of calibration curve

Into a series of clean 20ml PTFE liner screw cap vials, add accordingly as table below (See next page).

Code	IS(ml)	DS(ml)	ES(ml)	Delta(mg)	Total Vol(ml)
C1	1	0.5	13.5	0.30	15
C2	1	0.7	13.3	0.42	15
C3	1	0.9	13.1	0.54	15
C4	1	1.1	12.9	0.66	15
C5	1	1.3	12.7	0.78	15

Preparation of sample

Weigh (to the nearest 0.1 mg) into an Erlenmeyer or a screw cap neutral glass bottle (50ml) sufficient sample to contain about 0.5 mg of Deltamethrin. For 75D, 100D and 150D netting sample, suitable weight (w g) is 0.3g, 0.4 and 0.6 g respectively. Add by pipette 1.0 ml internal standard solution. Add 14 ml extract solvent. Replace the cap closely.

Put the bottles into the ultrasonic bath, setting temperature 80oC, running time 15min. Vigorously shake the bottle using the shaker in 30 min at room temperature, speed of shaking is at level of 150-200 beats per minute.

Using on syringe membrane filter with pore size of 0.45um or finer, filter c.a. 1ml of extract solution into clean amber. Sample shall be injected within 24 hours since extraction, for longer waiting time the vial should be kept in a refrigerator.

Determination

Inject in sample of 20ul portion of each sample to HPLC instrument and measure the relevant peak areas.

3. Linearity

The linearity is determined by testing the five concentrations described in the adopted methods, shown in below table, and calculating the regression line using a mathematical treatment of the results (ie. least mean squares) vs analyte concentration. The correlation coefficient of the calibration line is 0.999.

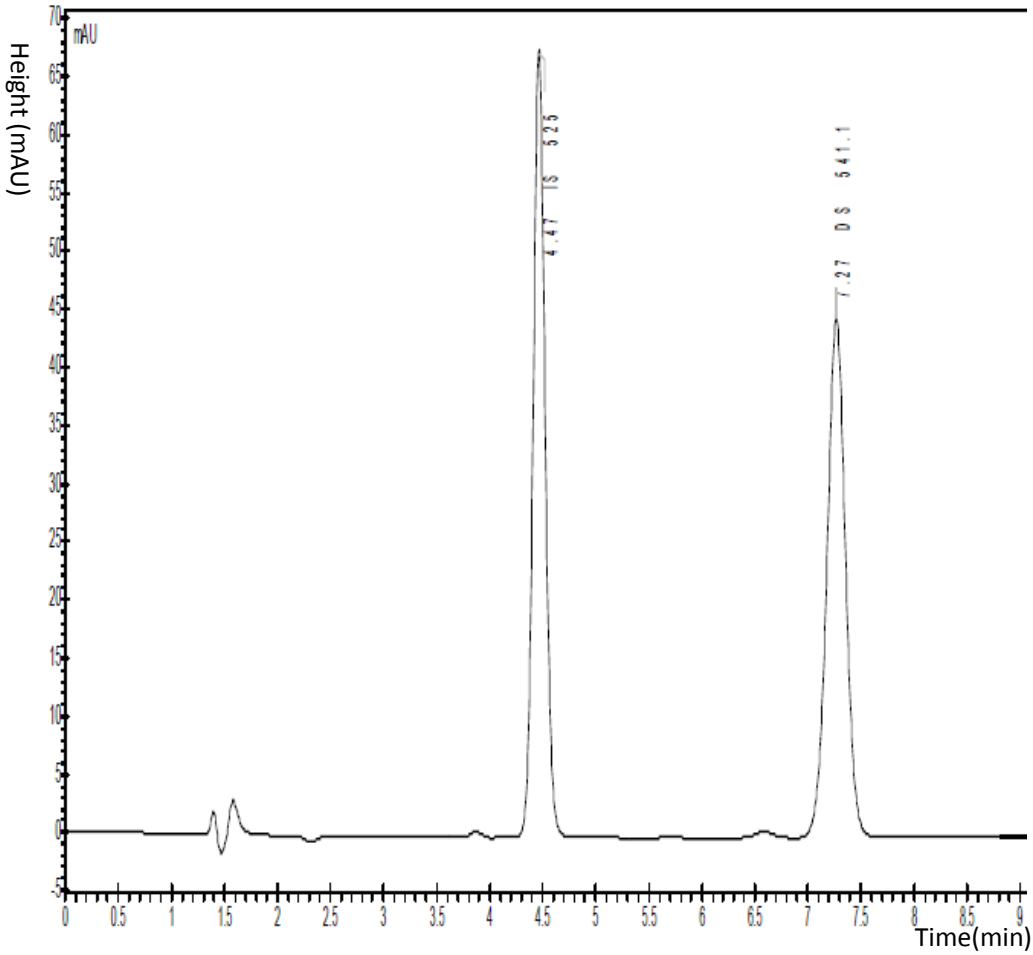

Figure 1: Chromatogram Of Sample C1

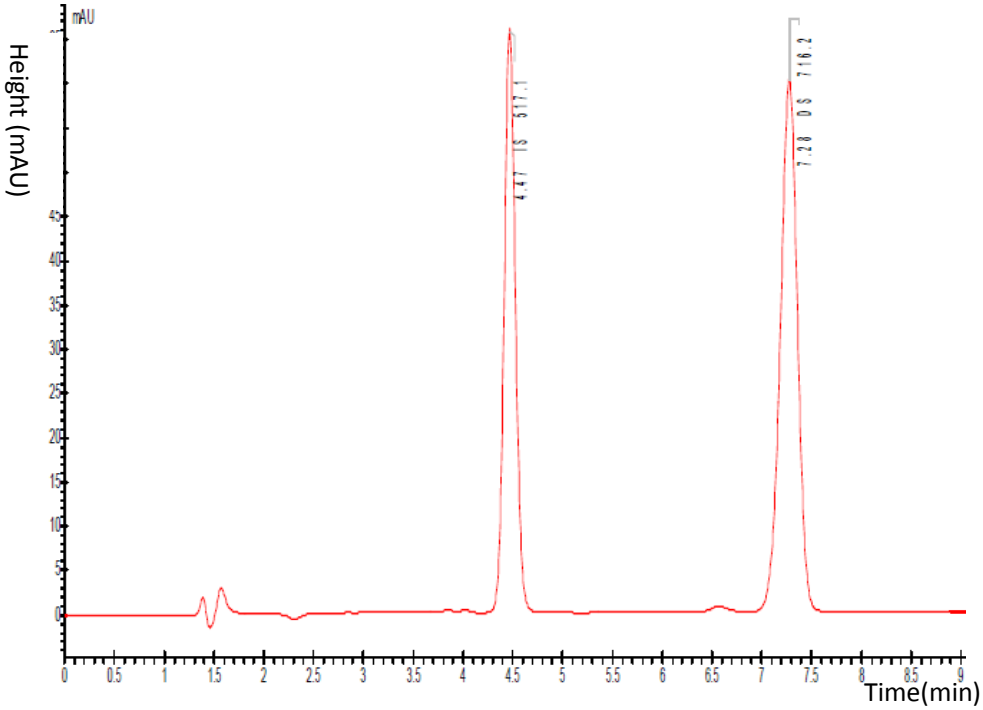

Figure 2: Chromatogram Of Sample C2

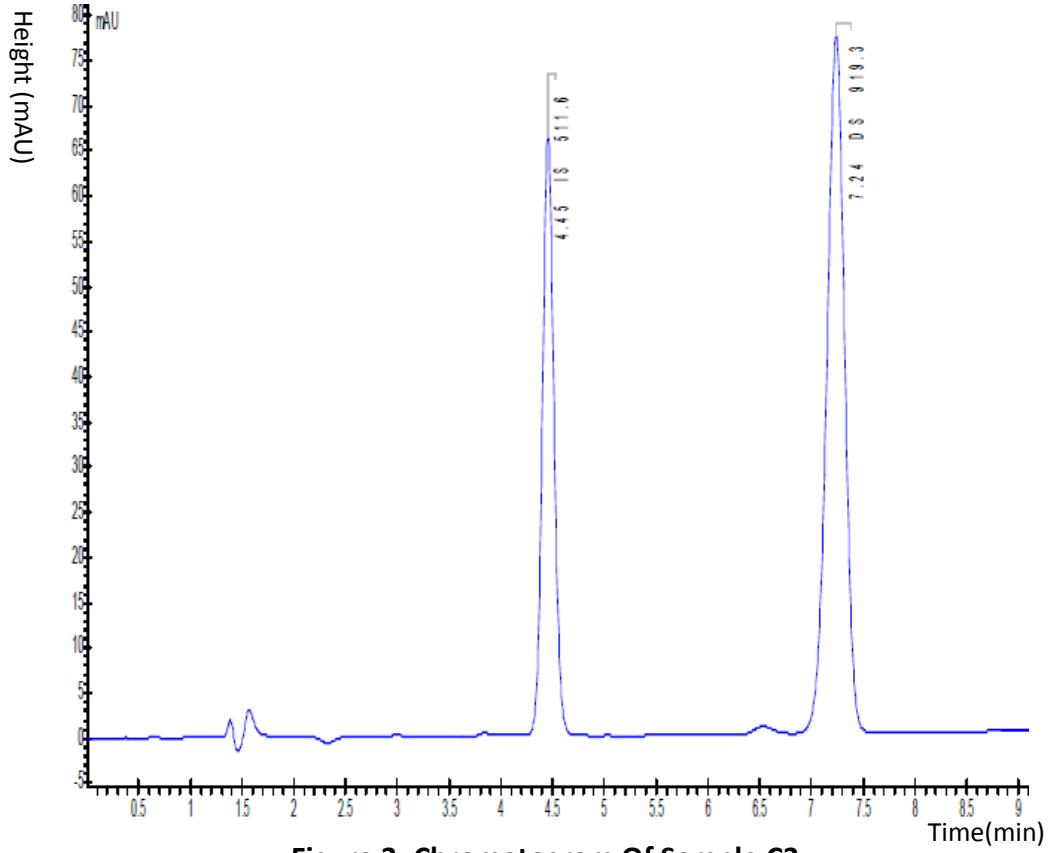

Figure 3: Chromatogram Of Sample C3

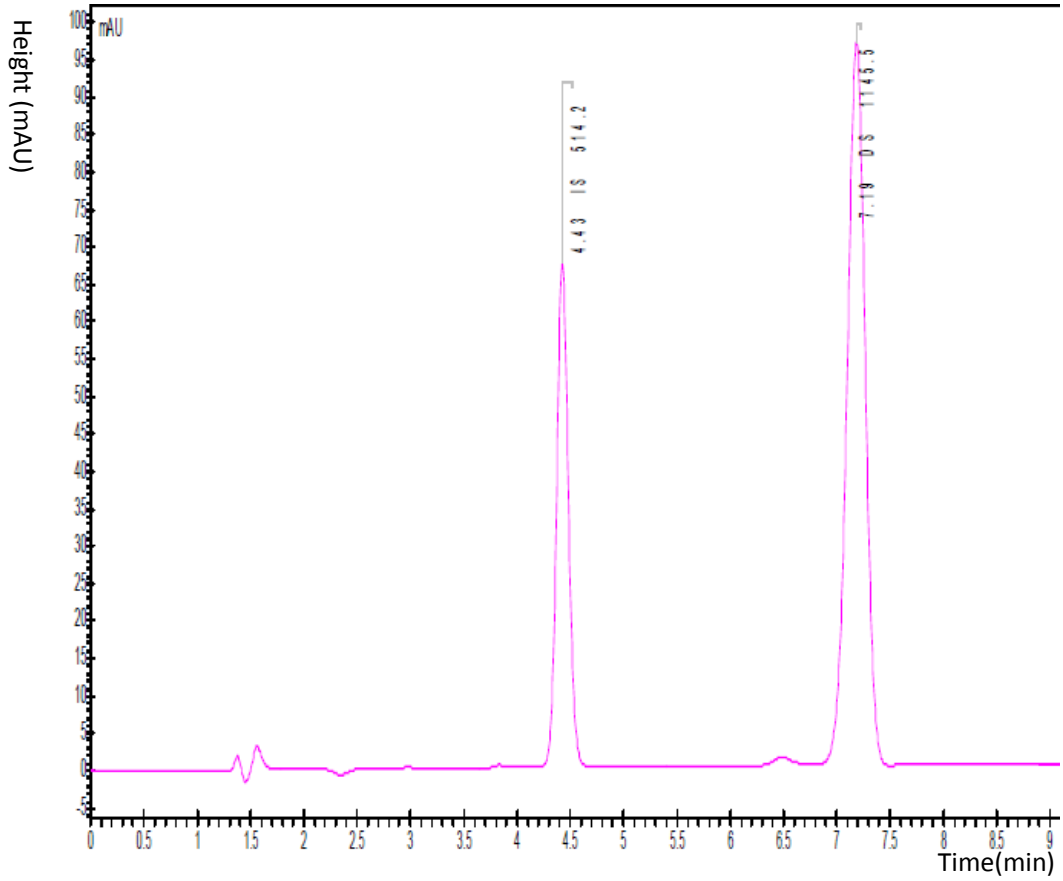

Figure 4: Chromatogram Of Sample C4

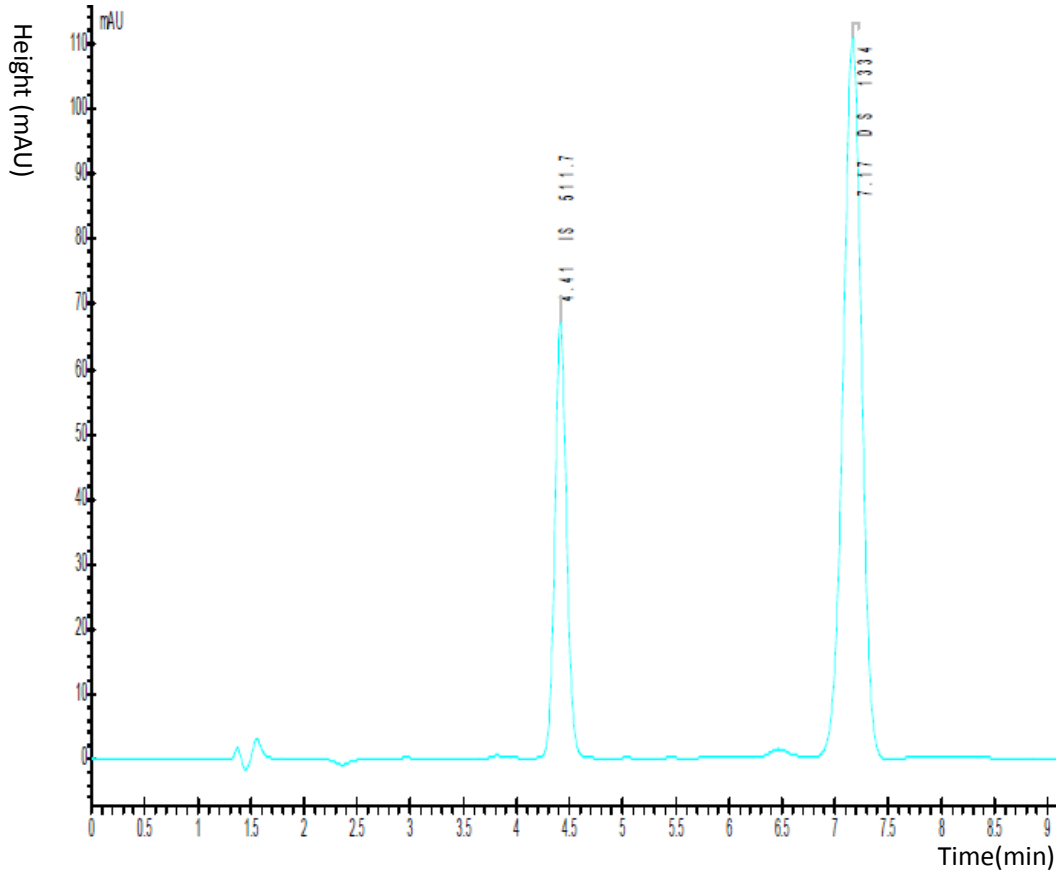

Figure 5: Chromatogram Of Sample C5

Table 1： Chromatogram report

Code	Name	Time (min)	Quantity (% Area)	Height (mAU)	Area (mAU·sec)	Area% (%)
C1	IS	4.47	49.23	67.7	525.0	49.234
	DS	7.27	50.77	44.8	541.4	50.766
C2	IS	4.47	41.93	66.1	517.1	41.927
	DS	7.28	58.07	60.2	716.2	58.073
C3	IS	4.45	35.75	66.4	511.6	35.754
	DS	7.24	64.25	77.3	919.3	64.246
C4	IS	4.43	30.98	67.6	514.2	30.984
	DS	7.19	69.02	96.6	1145.5	69.016
C5	IS	4.41	27.72	67	511.7	27.722
	DS	7.17	72.28	111.1	1334.0	72.278

The five chromatogram data is shown in Table 1, and the calculating of the linearity shown in Table 2. And figure 6 is the linearity line.

Table 2： linearity data

Code	C1	C2	C3	C4	C5
Concentration(μg/ml)	20	28	36	44	52
Time	4.47	4.47	4.45	4.43	4.41
Height	67.7	66.1	66.4	67.6	67.0
$Area_{IS}(mAU \bullet sec)$	525.0	517.1	511.6	514.2	511.7
Time	7.27	7.28	7.24	7.19	7.17
Height	44.8	60.2	77.3	96.6	111.1
$Area_{DS}(mAU \bullet sec)$	541.4	716.2	919.3	1145.5	1334.0
Area Ratio ($\frac{Area_{IS}}{Area_{DS}}$)	1.03	1.38	1.80	2.23	2.61

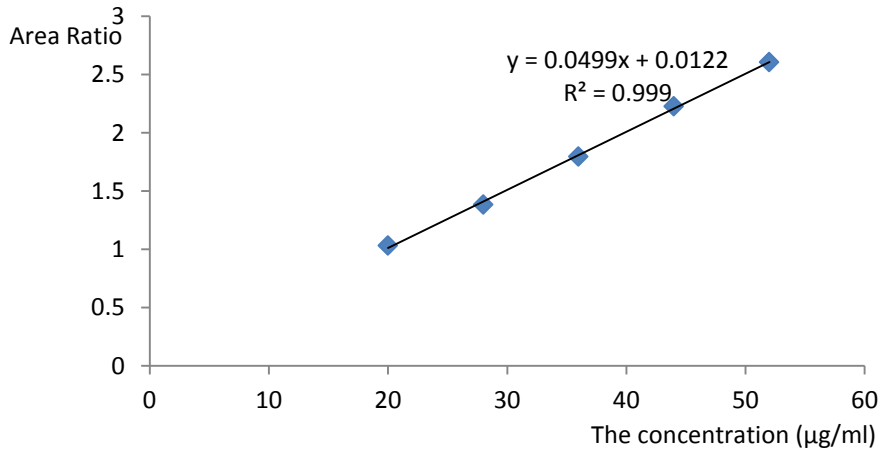

Figure 6: linearity line

In the Figure 6, the correlation coefficient, y-intercept, slop of the regression line and residual sum of squares are shown next to the plot of the linearity line. All the Chromatogram is shown in Appendix 2.

4. Accuracy

Accuracy was determined by applying the method to samples to which known amounts of analyte with content of 0.12mg, 0.24mg, 0.36mg have been added and then analyzed, with three replicates per level, against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay.

Table 3: Accuracy validation data

Test No.	1				2				3			
	Background	Recruitment(mg)			Background	Recruitment(mg)			Background	Recruitment(mg)		
		0.12	0.24	0.36		0.12	0.24	0.36		0.12	0.24	0.36
Time(min)	3.21	4.79	4.79	4.85	4.83	4.85	4.84	4.86	4.83	4.86	4.85	4.86
Height(mAU)	61.2	60.2	59.4	59.3	61.3	61.1	59.6	59.4	61.1	60.6	60.1	59.4
$Area_{IS}$ (mAU • sec)	513.7	519.7	498.2	494.6	510.4	508.3	495.2	494.9	518.7	500.9	497.3	499.4
Time(min)	5.82	7.38	7.37	7.46	7.41	7.41	7.41	7.45	7.39	7.41	7.44	7.47
Height(mAU)	64.9	80.7	97.4	114.2	65.3	82.9	97.8	114.4	65.7	82.3	98.3	113.8
$Area_{DS}$ (mAU • sec)	797.3	987.4	1191.3	1396	790.5	990.1	1193.7	1387.6	808	988.6	1189.5	1400.5
Area Ratio(y)	1.55	1.90	2.39	2.82	1.55	1.95	2.41	2.80	1.56	1.97	2.39	2.80
Concentration(μ g/ml) (y-0.0122)/0.0499	30.86	37.83	47.68	56.32	30.79	38.79	48.06	55.94	30.97	39.31	47.69	55.96
Absolute content (mg/15ml)	0.46	0.57	0.72	0.84	0.46	0.58	0.72	0.84	0.46	0.59	0.72	0.84
Recovery rate (%)		87.14	105.10	106.08		99.97	107.94	104.79		104.19	104.48	104.09
Average recovery rate	99.44				104.23				104.25			
conclusion	102.64%											

According to the results shown in Table 3, the recovery rate indicates the deviation between the mean value found and the true value. The Chromatogram is shown in appendix 3.

5. Precision

"The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample - (6) Precision is a measure of the reproducibility of the whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances.

Repeatability

Repeatability is determined by using the method to assay samples, with content of0.42mg/15ml, 0.54mg/15ml, 0.66 mg/15ml for a 6 times respectively to obtain statistically valid results. The acceptance criteria set for RSD on the average recovery. For the assay range (80.0-120.0%), this may be $\leqslant$1.5 for the DS method.

The precision is then expressed as the relative standard deviation:

$$\%RSD = \frac{STDEV \times 100\%}{mean}$$

Table 5: Repeatability validation data

Content (mg/15ml)	STDEV	Mean	Individual RSD(%) $\%\text{RSD} = \frac{\text{STDEV} * 100\%}{Mean}$	Average RSD(%)
0.42	0.004	0.414	1.068	
0.54	0.003	0.52	0.6	0.704
0.66	0.003	0.651	0.443	

According to the results shown in Table 5, the recovery rate indicates the deviation between the mean value found and the true value. The Chromatogram is shown in appendix 4.

Table 4: Repeatability testing data

Content (mg/15ml)	Code	Time (min)	Height (mAU)	Area (mAU • sec)	Time (min)	Height (mAU)	Area (mAU • sec)	Ratio	Concentration (μ g/ml)	Concentration (mg/15ml)
0.42	C2-1	4.81	72.3	568.8	7.38	65.6	803.1	1.41	28.05	0.42
	C2-2	4.79	72.7	574.5	7.36	65.7	803.1	1.40	27.77	0.42
	C2-3	4.75	72.8	571.5	7.33	66.3	795	1.39	27.63	0.41
	C2-4	4.73	71.9	571.1	7.31	65.3	787.1	1.38	27.38	0.41
	C2-5	4.69	73.1	570.7	7.29	65.6	789.7	1.38	27.49	0.41
	C2-6	4.65	73.5	581.3	7.27	66.2	796.9	1.37	27.23	0.41
0.54	C3-1	4.69	76.1	610.5	7.32	85.9	1060.8	1.74	34.58	0.52
	C3-2	4.69	77.7	632.9	7.34	91	1111.6	1.76	34.95	0.52
	C3-3	4.68	78.0	653.7	7.33	91.3	1131.8	1.73	34.45	0.52
	C3-4	4.67	77.6	648.5	7.32	91.4	1122.9	1.73	34.46	0.52
	C3-5	4.69	78.0	636.5	7.34	91.3	1110	1.74	34.70	0.52
	C3-6	4.67	77.9	629.7	7.32	90.5	1102.8	1.75	34.85	0.52
0.66	C4-1	4.67	77.9	615	7.28	110.8	1337.9	2.18	43.35	0.65
	C4-2	4.67	77.4	617.8	7.32	108.8	1340.6	2.17	43.24	0.65
	C4-3	4.66	78.1	618.7	7.30	111.3	1345.4	2.17	43.33	0.65
	C4-4	4.67	78.3	619.2	7.31	111.4	1346.8	2.18	43.34	0.65
	C4-5	4.69	78	620.4	7.33	111.5	1352.6	2.18	43.45	0.65
	C4-6	4.69	78.4	618.7	7.32	112.1	1359.4	2.20	43.79	0.66

6. CONCLUSION

Only minor modification were made to the adopted HPLC detection method of deltamethrine coated LN concerning Mobile Phase (MP): iso octan + 1,4 dioxane = 95+5 and the injection volume of 20ul.

The method is considered appreciated for the determination of deltamethrin in FONYI LN.