STATUS REPORT

COLLABORATIVE STUDY OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF INDOXACARB TECHNICAL MATERIAL AND FORMULATED PRODUCTS

Report to CIPAC on Method Validation
Conducted by the DuPont Company

by

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TABLE OF CONTENTS

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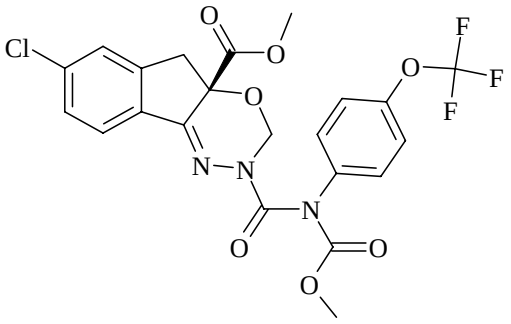
II. INTRODUCTION

INDOXACARB
612



ISO Common name	Indoxacarb
Chemical name	(S)-7-chloro-3-[methoxycarbonyl-(4-trifluoromethoxy-phenyl)-carbamoyl]-2,5-dihydro-indeno[1,2-e][1,3,4]oxadiazine-4a(3*H*)-carboxylic acid methyl ester (IUPAC) (S)-methyl 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)= phenyl]amino]carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3*H*)-carboxylate (CAS 173584-44-6)
Empirical formula	C_{22} H_{17} Cl F_3 N_3 O_7
RMM	527.837
m.p.	88.1°C
Solubility	In water 0.20mg/L (25°C). In n-hexane 1.307 g/L, methanol 109.9 g/L, n-octanol 11.31 g/L, acetone >250 g/L, acetonitrile >250 g/L, ethyl acetate >250 g/L, dichloromethane >250 g/L, dimethylformamide >250 g/L, o-xylene >250 g/L (all at 20°C)
Description	Off-white crystalline solid
Stability	Aqueous hydrolysis: DT_{50} >30 d (pH 5), 38 d (pH 7), 1 d (pH 9).
Formulations	150 SC: Suspension concentrate (SC) 30 WG: Water dispersible granules (WG), Dry Flowable 150 EC: Emulsifiable concentrate (EC)

In November 2007, Information Sheet No. 277 was sent out by the CIPAC Secretary inviting members to participate in a collaborative study to validate the high performance liquid chromatographic assay method for indoxacarb in technical material and formulated products. A copy of the analytical method, protocol for the performance of the study, analysis report forms, samples and standards required for the analysis were sent to the respondents. The thirteen respondents who completed the study are listed in Section I.

III. SAMPLES

Five product test samples and analytical standard were sent to the participants.

Test Sample	Identification Code
DPX-KN128 Technical	DPX-KN128-192
DPX-MP062 MUP	JAN06MB10B
Indoxacarb 30 WG	DPX-MP062-538
Indoxacarb 150 EC	DPX-KN128-206
Indoxacarb 150 SC	DPX-MP062-455

Indoxacarb analytical standard: JW062-181, 99.6% purity. Note: DPX-JW062 is a racemic mixture (exactly 50:50 ratio of DPX-KN128 and IN-KN127). The label purity of the standard is 99.6% which means that it contains 49.8% DPX-KN128 (active ingredient enantiomer) and 49.8% IN-KN127 (inactive enantiomer (R-enantiomer)).

IV. ANALYTICAL METHOD

1. Scope

This method is applicable to determination of indoxacarb in technical material, and formulations including 30 WG (water-dispersible granule), 150 SC (a 150 g/L suspension concentrate), and 150 EC (a 150 g/L emulsifiable concentrate).

2. Outline of method

A solution of the sample is analyzed by chiral normal-phase high performance liquid chromatography using a 4.6 mm x 25 cm Chiralcel OD column with 10-micron particles. The mobile phase is composed of 75%(v/v) hexane/25%(v/v) 2-propanol. Indoxacarb is detected with a UV detector at 310 nm. The weight percent of indoxacarb in each sample is determined by comparing its peak area against a calibration curve prepared from the analysis of standard solutions.

3. Procedure

Each sample was analyzed by four independent determinations. The five samples were analyzed in a first run by duplicate injections of two weights for each sample. The sequence was repeated on a second date with two other weights of each sample and a freshly-prepared set of analytical standard solutions. The weight percent of indoxacarb for each injection was calculated from the calibration curve based on linear regression analysis of the calibration standards. For the calculation of the content of a sample, the mean value of the duplicate injections was used.

V. REMARKS OF PARTICIPANTS

Several of the labs made comments about the performance of the method and noted deviations from the method that occurred:

Lab 1 No comment.

Lab 2 Samples were filtered using 0.45-um filters instead of 0.2-um as stated in the method. We found the method very straightforward.

Lab 3 No comment.

Lab 4 The method is easy to use and straightforward. We found the injection volume of 5µl rather low. We wonder whether autosamplers with less reliable injection volume can really handle this volume in a reproducible way. We used heptane instead of hexane for (toxicological reasons, further advantage is reduced vapor pressure of heptane). We refer to the outline of method on page 3: "The weight percent of indoxacarb is detected with a UV by comparing its peak area against a calibration curve prepared from the analysis of standard solutions." However, in the determination part (page 6) of the method we read: …."bracketing the samples with calibration standards". We feel that these two statements disagree and should be clarified (to be described as the actual results are calculated). We realized that precision of weighing on day 1 is different of that on day 2 in the provided excel sheet. We assume that the rounding is handled in the same way at two days (to significant digits after the point. This should certainly be checked when the statistic is made.

Lab 5 During the analyses of reproducibility of standard injections responses of the peaks of standards varied, that might caused by the staying of the rests of samples in the injection block. It is evident also from the chromatograms of the blank injections of the mobile phase and the ethyl acetate. That's why we placed blank injection (mobile phase) for 5 minutes into sequence between every injection of the samples and standards. In the 3rd day, we performed analyses of 3 weights of the 150 EC formulations. In Excel results worksheets, B and C weights are reported. Results of the A weight (percentage) is reported at the summary percentage report ("Amount_Percentage_Report_Day_3"). All solvents we used were HPLC quality and were lately purchased only for these analyses. They were not dried under the molecular sieves, because analyses were performed during the 3 days. On the 2nd day retention times of the peaks moved during the analyses, this might caused by the incomplete mixture of the mobile phase, then we repeated all the analyses in the 3rd day. These results are listed in the Excel results worksheet for the 2nd day. Reports in PDF format ("Amount_Percentage_Reports") contains assay results generated by the Empower software. These results are calculated on the standard of concentration 100 mg/100mL of Indoxacarb, which was analyzed in sequence and slightly differ from the results calculated on the calibration curve.

Lab 6 We did not control the column temperature, but analyzed the samples at room temperature.

Lab 7 The description of the sample analysis sequence is not clear. Should the duplicate injections of the standard solutions be done before the sample

solutions are measured or should the standard solutions also be measured between and after measurement of the samples? Two variation were done, the variation with measurement of the standard solutions just before the measurement of the sample solutions is reported, sequence with every measurement twice: solvent / 1. standard / 2. standard / 3. standard / KN128-192-1 / KN128-192-2 / MP062-1 / MP062-2 / MP062-538-1 / MP062-538-2 / KN128-206-1 / KN128-206-2 / MP062-455-1 / MP062-455-2.

Following sequence was also measured (each twice): solvent / 1. standard / 2. standard / 3. standard / KN128-192-1 / KN128-192-2/ 1. standard / 2. standard / 3. standard / MP062-1 / MP062-2 / 1. standard / 2. standard / 3. standard / MP062-538-1 / MP062-538-2 / 1. standard / 2. standard / 3. standard / KN128-206-1 / KN128-206-2 / 1. standard / 2. standard / 3. standard / MP062-455-1 / MP062-455-2 / 1. standard / 2. standard / 3. standard.

The calculations were done by using the values from the standard solution measurements just before and after the measurement of the samples concerned. The results are not reported in detail. If necessary also the chromatograms and peak areas of this measurement can be provided..

Lab 8 No comment.

Lab 9 MP062-433 150 SC – sample contains sediments that are difficult to homogenize.

Lab 10 For WG formulation extraction was performed direct in ethyl acetate without water dispersing..

Lab 11 We didn't grind WG samples prior to weighing because, when we did, we found fine particles adhered to the side of the flask. From the instruction, column Chiralcel OD must only be exposed to hexane and 2-propanol. Water or even acetonitrile would ruin the column. But from this method, water was added to WG samples. We suspected that the water will ruin the column or not.

Lab 12 The volumetric flasks are filled up to volume at 20°C ± 1°C instead of at room temperature.

Lab 13 We followed the method such as you described. It was a good experience for us, because we had problem to separate both enantiomers with inspection control samples. The retention time was DPX-KN127 (inactive enantiomer): 6.7 min and DPX-KN128 (active ingredient Indoxacarb): 8.4 min.

VI. RESULTS AND DISCUSSION

1. Screening for valid data

The data from each of the laboratories were reviewed to determine if there were any problems with analysis procedure used, chromatography, or reporting of results, which might affect the analysis results. The results reported by the participants were used as received in the spreadsheets.

2. Statistical Evaluation

The assay results reported by the laboratories are listed in Tables 2 through 6, and are displayed in Figures 1 through 5.

Statistical evaluation of the data was done following "Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods." The data were examined for outliers and stragglers using Cochran's test on the within-lab variability, followed by Grubb's test on the lab means, and iterating where necessary. The tests were performed at an alpha level of 0.01 for outlier, and 0.05 for straggler. Based on this procedure, the Cochran and Grubb tests identified the following outlier labs for the samples. Stragglers were also identified by the tests (see notes in the tables).

Test Sample	**Identification Code**	**Cochran Outlier**	**Grubbs Outlier**
DPX-KN128 Technical (TC)	DPX-KN128-192	None	None
DPX-MP062 MUP (TK)	JAN06MB10B	Lab 5	None
Indoxacarb 30 WG	DPX-MP062-538	None	None
Indoxacarb 150 EC	DPX-KN128-206	Labs 5, 6	Lab 5
Indoxacarb 150 SC	DPX-MP062-455	None	Lab 5

No outlier lab data were removed from the data sets. Analysis of variance using a nested ANOVA procedure was done on the data sets to determine the within-lab and between-labs variance components. Since duplicate sample preparations were analyzed for each sample for each run, it was possible to obtain three components of variance, and the corresponding repeatability and reproducibility values, for the data:

$s_r = (s^2_{\text{test portion}} + s^2_{\text{Run}})^{1/2}$ $\qquad r = 2.8\ s_r$

$s_L = s_{\text{Lab}}$ $\qquad R_L = 2.8\ s_L$

$s_R = s_{\text{total}} = (s^2_{\text{test portion}} + s^2_{\text{Run}} + s^2_{\text{Lab}})^{1/2}$ $\qquad R = 2.8\ s_R$

$\%RSD_R = (R \times 100)/ (2.8 \times \text{Mean}) = (s_R \times 100)/ \text{Mean}$

where:

r = repeatability (s_r*2.8)

R_L = between laboratory reproducibility

R = Reproducibility (s_R*2.8)

$\%RSD_R$ = Reproducibility Relative Standard Deviation (between labs)

A summary of the statistical evaluation for the labs is given in Table 1, which includes the above repeatability and reproducibility values, as well as the between-lab experimental Reproducibility Relative Standard Deviation, $\%RSD_R$, and the calculated acceptable value, $\%RSD_{R(Hor)}$, based on the Horwitz curve calculation,

$\%RSD_{R(Hor)} = 2^{(1 - 0.5\log c)}$,

where c is the concentration of the analyte as a decimal fraction (e.g. for 100% concentration c = 1).

VII. CONCLUSIONS

The statistical analysis of the study results shows the between-lab Reproducibility Relative Standard Deviation ($\%RSD_R$) for all samples to be well below the limits calculated ($\%RSD_{R(Hor)}$) using the Horwitz equation, therefore meeting the acceptance criterion. The acceptance of this method as an approved CIPAC method for assay of indoxacarb insecticide formulations and technical samples is recommended.

ACKNOWLEDGEMENTS

The author wishes to thank all the laboratories and their staff who participated in this study.

TABLES (1-6)

Table 1A
Summary Of The Statistical Evaluation Of The Collaborative Study Data
No Test Results Eliminated

	KN128 Tech	MP062 MUP	30 WG	150 EC	150 SC
No. of Labs	13	13	13	13	13
No. of Stragglers	2	0	1	0	0
No. of Outliers	0	1	0	2	1
No. of Labs Retained	13	13	13	13	13
No. of Results	52	52	52	52	52
Total Mean, X (wt%)	96.00	55.58	29.95	16.10	14.12
s_r	0.85	0.59	0.29	0.21	0.12
s_L	0.17	0.53	0.27	0.24	0.20
s_R	0.87	0.79	0.40	0.32	0.23
r	2.40	1.67	0.81	0.59	0.33
R_L	0.49	1.49	0.77	0.69	0.55
R	2.45	2.23	1.12	0.90	0.64
RSD_R	0.90	1.42	1.32	1.98	1.61
$RSD_{R(Hor)}$	2.01	2.18	2.40	2.63	2.69

Table 1B
CIPAC Format for the Presentation of the Summary of the Results of the Indoxacarb Collaborative Study

	KN128 Tech	MP062 MUP	30 WG	150 EC	150 SC
x (g/kg)	960.0	555.8	299.5	161.0	141.2
L	13	13	13	13	13
s_r	8.48	5.90	2.87	2.08	1.16
s_L	1.73	5.25	2.72	2.43	1.95
s_R	8.65	7.90	3.95	3.20	2.27
RSD_r	0.88	1.06	0.96	1.29	0.82
RSD_R	0.90	1.42	1.32	1.98	1.61
r	24.0	16.7	8.1	5.9	3.29
R	24.5	22.3	11.2	9.0	6.43
$RSD_{R(Hor)}$	2.01	2.18	2.40	2.63	2.69

Where:

x = average
L = number of laboratories
s_r = repeatability standard deviation
s_L = "pure" between laboratory standard deviation
s_R = reproducibility standard deviation = $(s_r^2 + s_L^2)^{0.5}$
RSD_r = repeatability relative standard deviation ($s_r/x*100$)
RSD_R = reproducibility relative standard deviation ($s_R/x*100$)
r = repeatability ($s_r*2.8$)
R = reproducibility ($s_R*2.8$)
$RSD_{R(Hor)}$ = Horwitz value calculated from $2^{(1-0.5\log c)}$
where c = the concentration of the analyte as a decimal fraction

NB Where appropriate values should be given in units of g/kg

Table 2
DPX-KN128 Technical Assay Results Summary By Laboratory

Tech-1 DPX-KN128-192 Assay Results Summary by Lab

	RUN 1		RUN 2				
LAB	A	B	A	B	Mean	Std. Dev.	Notes
1	95.86	96.05	96.07	96.01	96.00	0.09	
2	94.24	94.39	94.79	97.72	95.29	1.64	c
3	95.73	96.46	94.34	94.18	95.18	1.10	
4	96.45	95.50	95.68	95.66	95.82	0.43	
5	97.26	98.09	95.93	97.44	97.18	0.91	
6	96.25	95.99	95.85	96.67	96.19	0.36	
7	96.44	96.14	95.86	96.11	96.14	0.23	
8	96.00	96.11	96.66	96.73	96.37	0.38	
9	96.44	97.06	95.59	96.04	96.28	0.62	
10	95.05	95.66	96.32	96.14	95.79	0.57	
11	96.20	96.54	96.46	96.36	96.39	0.15	
12	96.43	95.36	96.02	96.14	95.99	0.45	
13	95.77	96.72	95.28	93.59	95.34	1.31	c

c - Cochran straggler, **C** - Cochran outlier, g - Grubb straggler, **G** - Grubb outlier

Table 3
DPX-MP062 MUP Assay Results Summary By Laboratory

MP062 MUP

	RUN 1		RUN 2				
LAB	A	B	A	B	Mean	Std. Dev.	Notes
1	55.56	55.69	55.78	55.64	55.67	0.09	
2	54.68	54.49	55.20	55.63	55.00	0.52	
3	55.62	55.82	55.24	54.19	55.22	0.73	
4	55.40	55.31	55.93	56.14	55.70	0.40	
5	58.19	58.55	55.44	57.75	57.48	1.40	**C**
6	55.36	55.18	55.91	55.97	55.61	0.40	
7	55.55	55.37	55.43	55.19	55.38	0.15	
8	54.87	55.07	55.67	55.40	55.25	0.35	
9	56.09	56.20	55.62	55.52	55.86	0.34	
10	55.29	55.17	54.93	54.76	55.04	0.24	
11	54.97	55.17	55.64	55.56	55.34	0.32	
12	56.04	56.05	55.49	55.77	55.84	0.27	
13	55.50	54.93	55.30	54.82	55.14	0.32	

c - Cochran straggler, **C** - Cochran outlier, g - Grubb straggler, **G** - Grubb outlier

Table 4
Indoxacarb 30 WG
Assay Results Summary By Laboratory

MP062 30 WG

	RUN 1		RUN 2				
LAB	**A**	**B**	**A**	**B**	**Mean**	**Std. Dev.**	**Notes**
1	30.07	29.93	30.10	30.11	30.05	0.08	
2	29.89	29.95	29.96	29.82	29.91	0.06	
3	30.06	29.97	29.55	29.66	29.81	0.24	
4	30.28	29.96	30.46	30.39	30.27	0.22	
5	30.61	31.28	30.21	30.20	30.58	0.51	
6	29.88	29.40	30.44	30.57	30.07	0.54	c
7	30.01	30.07	30.02	30.06	30.04	0.03	
8	29.96	29.80	30.11	30.18	30.01	0.17	
9	29.87	29.84	29.50	29.63	29.71	0.18	
10	29.38	29.23	29.16	28.82	29.15	0.24	
11	29.78	29.75	29.78	29.96	29.82	0.10	
12	30.17	30.16	29.96	30.08	30.09	0.10	
13	29.76	29.79	29.73	29.90	29.79	0.07	

c - Cochran straggler, **C** - Cochran outlier, g - Grubb straggler, **G** - Grubb outlier

Table 5
Indoxacarb 150 EC
Assay Results Summary By Laboratory

KN128 150 EC

	RUN 1		RUN 2				
LAB	**A**	**B**	**A**	**B**	**Mean**	**Std. Dev.**	**Notes**
1	16.03	16.07	16.00	16.03	16.03	0.03	
2	15.88	15.87	16.03	16.02	15.95	0.09	
3	15.91	16.05	15.70	15.73	15.85	0.16	
4	16.32	16.36	16.32	16.35	16.34	0.02	
5	17.64	16.63	16.81	16.44	16.88	0.53	**C, G**
6	15.77	15.95	16.49	16.29	16.12	0.32	**C**
7	15.97	15.99	16.08	15.97	16.01	0.05	
8	16.00	15.96	16.17	16.16	16.07	0.11	
9	16.29	16.25	16.17	16.18	16.22	0.06	
10	16.00	15.96	15.64	15.68	15.82	0.18	
11	15.97	15.95	16.08	16.09	16.02	0.07	
12	16.19	16.01	16.08	16.02	16.08	0.08	
13	15.90	15.95	15.92	15.99	15.94	0.04	

c - Cochran straggler, **C** - Cochran outlier, g - Grubb straggler, **G** - Grubb outlier

Table 6
Indoxacarb 150 SC
Assay Results Summary By Laboratory

MP062 150 SC

	RUN 1		RUN 2				
LAB	**A**	**B**	**A**	**B**	**Mean**	**Std. Dev.**	**Notes**
1	14.04	14.09	14.10	14.05	14.07	0.03	
2	14.10	14.11	14.05	13.99	14.06	0.06	
3	13.79	14.21	13.93	13.96	13.97	0.17	
4	14.30	14.36	14.39	14.42	14.37	0.05	
5	14.62	14.97	14.51	14.66	14.69	0.20	**G**
6	14.02	14.05	14.30	14.16	14.13	0.13	
7	14.03	13.94	14.07	13.99	14.01	0.06	
8	14.02	13.88	14.08	14.10	14.02	0.10	
9	14.23	14.32	13.89	14.15	14.15	0.19	
10	14.02	13.99	13.89	13.83	13.93	0.09	
11	14.00	14.00	14.10	14.13	14.06	0.07	
12	14.16	14.18	14.05	14.09	14.12	0.06	
13	13.97	13.92	13.90	13.98	13.94	0.04	

c - Cochran straggler, **C** - Cochran outlier, g - Grubb straggler, **G** - Grubb outlier

FIGURES (1-5)

Figure 1
DPX-KN128 Technical

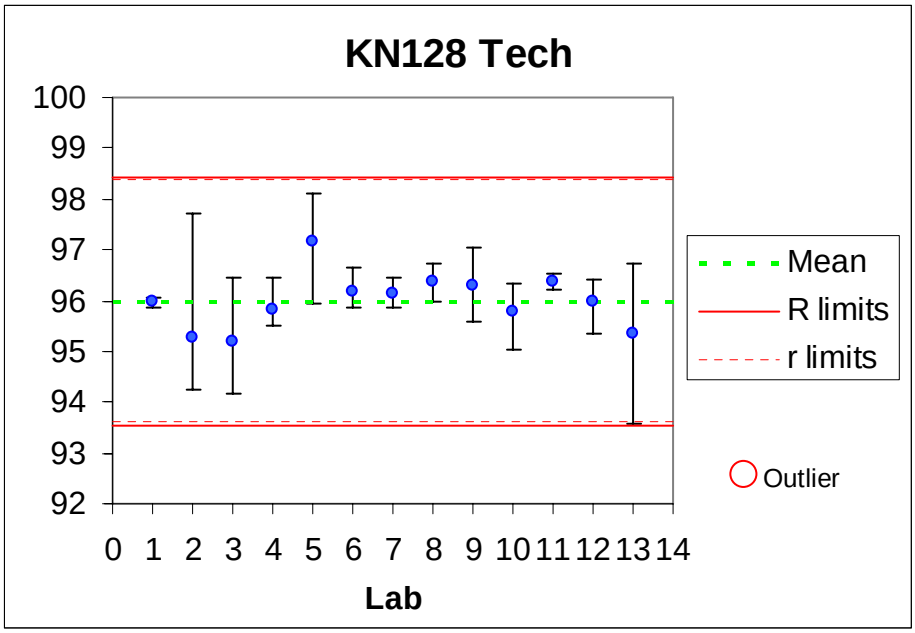


Figure 2
DPX-MP062 MUP

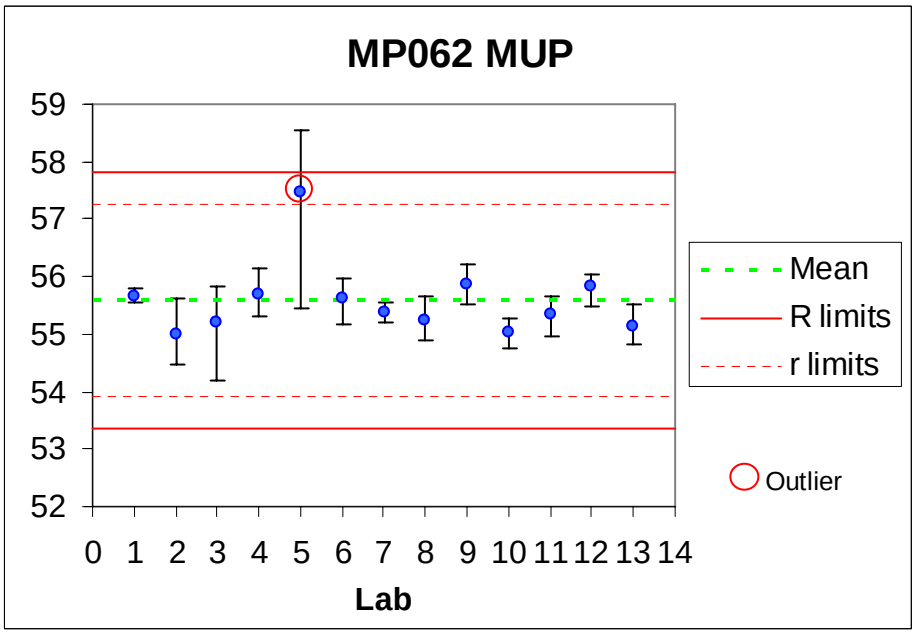


Figure 3
Indoxacarb 30 WG

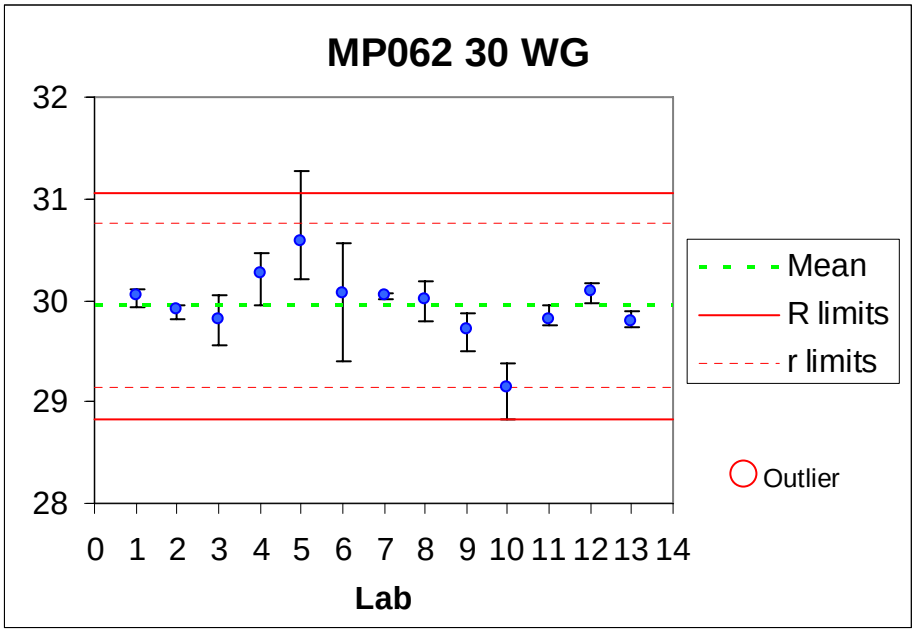


Figure 4
Indoxacarb 150 EC

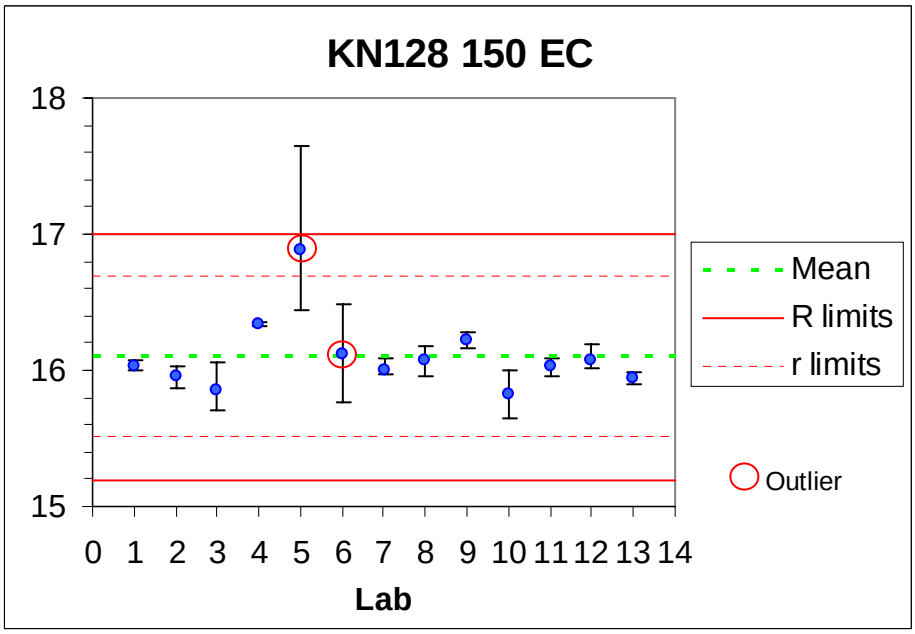


Figure 5
Indoxacarb 150 SC