Isocycloseram

Collaborative study

Ful scale collaborative study for the determination of

ISOCYCLOSERAM

in TC and WP formulation by LC

Report to CIPAC

By

Syngenta Crop Protection
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1. Participants

Participating Laboratories are listed in alphabetical order in the table below. Laboratory numbers in the result tables were assigned, chronologically, based upon receipt of results.

Company / Lab	Contact	Country
Agence Fédérale pour la Sécurité de la chaine Alimentaire	Isabelle Monisse, Xavier Buol	Belgium
Bayer AG	Peter Wagener	Germany
Benaki Phytopathological Institute	Elen Karassali, Eleftheria Bebelou	Greece
Bundesamt für Verbraucherschutz	Claudia Vinke	Germany
Centre wallon de Recherches agronomiques	Marie Baes	Belgium
Currenta GmbH & Co OHG	Michael Haustein	Germany
Deccan Fine Chemicals (India) Pvt L	Navin Raj Abraham	India
Department of Agriculture	Jim Garvey	Ireland
Mérieux NutriSciences	Erica Sbrissa	Italia
Ministry of Agriculture and Rural Affairs	Wenzhuo Wang	PR China
Ministry Of Agriculture And Forestry	Aysel Takkabulan	Turkey
Istituto Superiore di Sanità, Ministry of Agriculture, Fisheries and Food	Javier García-Hierro Navas	Spain
National Institute of Health Italy	Angela Santilio	Italy
PT. Agriculture Construction	Mr. Suswianto	Indonesia
Syngenta Crop Protection AG	Peter Stäuble, Christian Mink	Switzerland
UKZUZ Cent. Inst. Superv. Test. Agric	Olga Nováková	Czech Republic

2. General Information

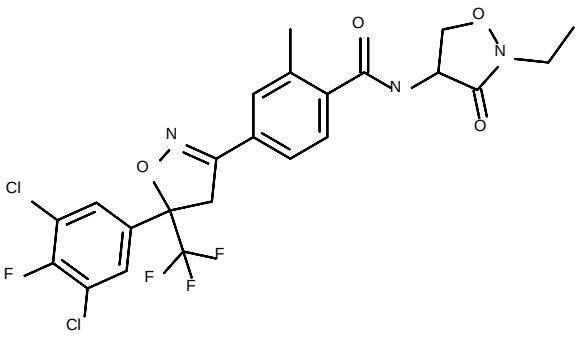
ISO common name: Isocycloseram

IUPAC name: 4-(5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl)-N-(2-ethyl-3-oxo-1,2-oxazolidin-4-yl)-2-methylbenzamide

Molecular mass: 548.3g mol^{-1}

Empirical formula: $C_{23}H_{19}Cl_2F_4N_3O_4$

Structure:



3. Samples

In total five samples, two TC samples and three WP formulated samples have been shipped together with reference standard.

- Isocycloseram TC– sample A
- Isocycloseram TC– sample B
- Isocycloseram WP– sample C
- Isocycloseram WP – sample D
- Isocycloseram WP – sample E
- Isocycloseram reference standard (purity 98.2 %w/w)

4. Method scope

The method is set up to determine the content of Isocycloseram. The sample is dissolved in acetonitrile and quantification is done against external standard, by liquid chromatography using UV detection.

5. Procedure

Each sample was analyzed using four independent determinations: Two sample preparations double injected, analyzed on two different days.

6. Remarks

In table 1 the instruments, columns and chromatographic conditions noted by the participating laboratories are given.

Table 1: Chromatographic conditions used by the participants.

Lab	Instrument	Stationary phase (particle size, type)	length, diameter [mm]	Flow rate [mL/min]	Injection volume [µL]
1	Agilent 1260 MWD	Kinetex C18 (2.6 µm)	100 * 4.6	1.0	5
2	Alliance 2695 + PDA 2996	Kinetex C18 (2.6 µm)	100 * 4.6	1.0	5
3	Agilent 1260	Kinetex C18 (2.6 µm)	100 * 4.6	1.0	5
4	Perkin Elmer Flexar	ZORBAX Eclipse Plus -C18 (3.5 µm)	100 * 4.6	1.0	5
5	Agilent Infinity 1290	Kinetex C18 (2.6 µm)	100 * 4.6	1.0	5
6	Waters H-class	Phenomenex-biphenyl (2.6 µm)	105 * 2.1	0.3	1
7	AGILENT 1260 INFINITY II	CORTECS C18+ , 2.7 µm	100 * 4.6	1.0	5
8	Agilent 1260 Infinity II	Poroshell EC-C18 (2.7 µm)	100 * 4.6	1.0	5
9	SHIMADZU LC-20AB	Kinetex C18 (2.6 µm)	100 * 4.6	1.0	5
10	Agilent 1260 (1290 column compartment)	Kinetex C18 (2.6 µm)	100 * 4.6	1.0	5
11	Shimadzu CTO-20AC with SPD-M20A Diode Array Detector	Kinetex C18 (2.6 µm)	100 * 4.6	1.0	5
12		Kinetex C18 (2.6 µm)	100 * 4.6	1.0	5
13	Perkin Elmer Flexar	ZORBAX Eclipse Plus -C18 (3.5 µm)	100 * 4.6	1.0	5
14	AGILENT 1100	ZORBAX Eclipse Plus -C18 (3.5 µm)	100 * 4.6	1.0	5
15	Shimadzu LC-20 AT	Thermo hypersyl C18 5 µm	150 * 4.6	1.0	5
16	Agilent 1200	ZORBAX Eclipse Plus -C18 (3.5 µm)	151 * 4.6	1.0	5

*performed for all of the solutions: about 25 mg to 50 ml, then 2 ml diluted to 10 ml.

7. Evaluation and Discussion

Data Review

In a first approach all deviations noted by the participating laboratories were deemed not to affect the analytical results. Therefore, all data sets were included within the statistical assessment. In a second attempt only the laboratories using the conditions outlined in the method were considered and in a third approach a statistical straggler has been excluded.

Statistical results

In the tables 2 to 6 and the figures 1 to 7 the full set of analytical results of all participating laboratories is shown.

Table 2: Results of the different laboratories for Sample A (TC).

	Isocycloseram SAMPLE A		
	Day1	**Day2**	**mean**
Laboratory 1	992.1	989.2	990.7
Laboratory 2	985.1	996.7	990.9
Laboratory 3	991.1	992.0	991.6
Laboratory 4	1000.5	976.7	988.6
Laboratory 5	988.7	992.5	990.6
Laboratory 6	990.7	986.0	988.4
Laboratory 7	981.7	983.1	982.4
Laboratory 8	983.6	1001.0	992.3
Laboratory 9	992.2	993.8	993.0
Laboratory 10	991.0	990.6	990.8
Laboratory 11	987.1	990.0	988.6
Laboratory 12	985.4	982.2	983.8
Laboratory 13	988.7	988.3	988.5
Laboratory 14	991.8	985.6	988.7
Laboratory 15	977.4	977.9	977.7
Laboratory 16	989.4	988.5	989.0

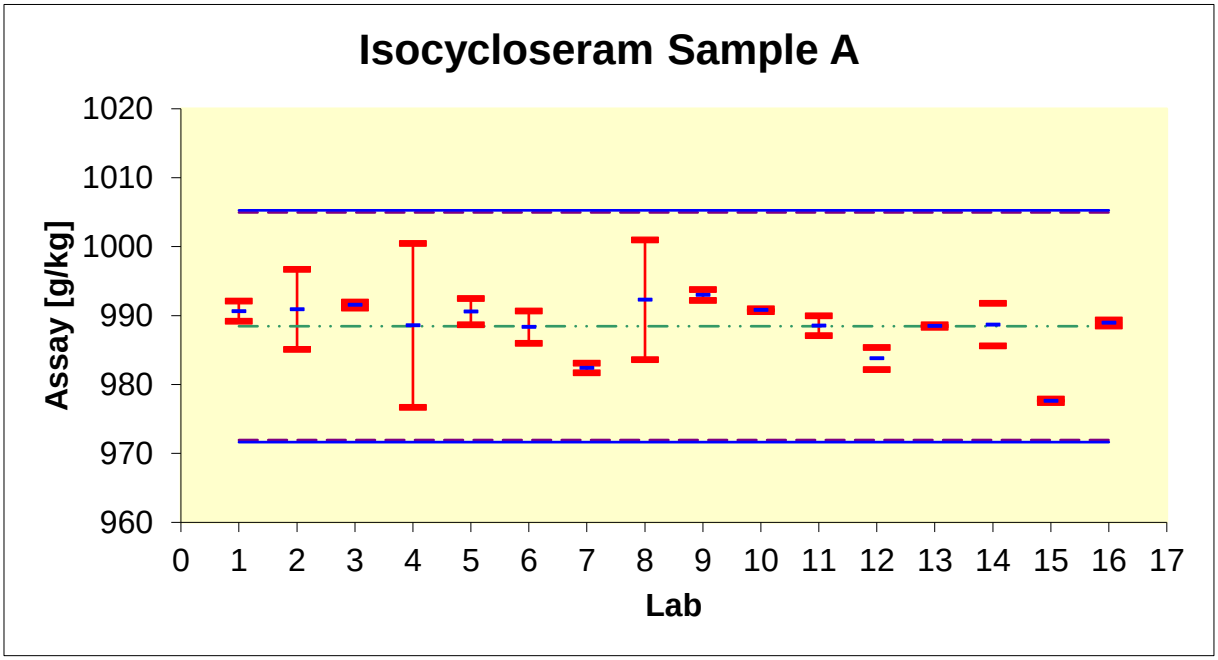


Figure 1: Graphical presentation of the results of the different laboratories for Sample A (TC). For each laboratory (laboratories 1 to 16) the red bars represent day 1 and day 2. The blue bar represents the average.

Table 3: Results of the different laboratories for Sample B (TC).

	Isocycloseram SAMPLE B		
	Day1	**Day2**	**mean**
Laboratory 1	982.1	983.7	982.9
Laboratory 2	982.0	995.2	988.6
Laboratory 3	987.4	989.9	988.7
Laboratory 4	969.3	1000.3	984.8
Laboratory 5	978.0	989.7	983.9
Laboratory 6	983.8	979.2	981.5
Laboratory 7	978.8	977.8	978.3
Laboratory 8	1015.0	1009.5	1012.3
Laboratory 9	992.6	999.2	995.9
Laboratory 10	982.0	985.1	983.6
Laboratory 11	994.4	987.0	990.7
Laboratory 12	982.8	978.3	980.6
Laboratory 13	977.1	982.6	979.9
Laboratory 14	997.0	985.3	991.2
Laboratory 15	971.6	973.5	972.6
Laboratory 16	982.2	978.0	980.1

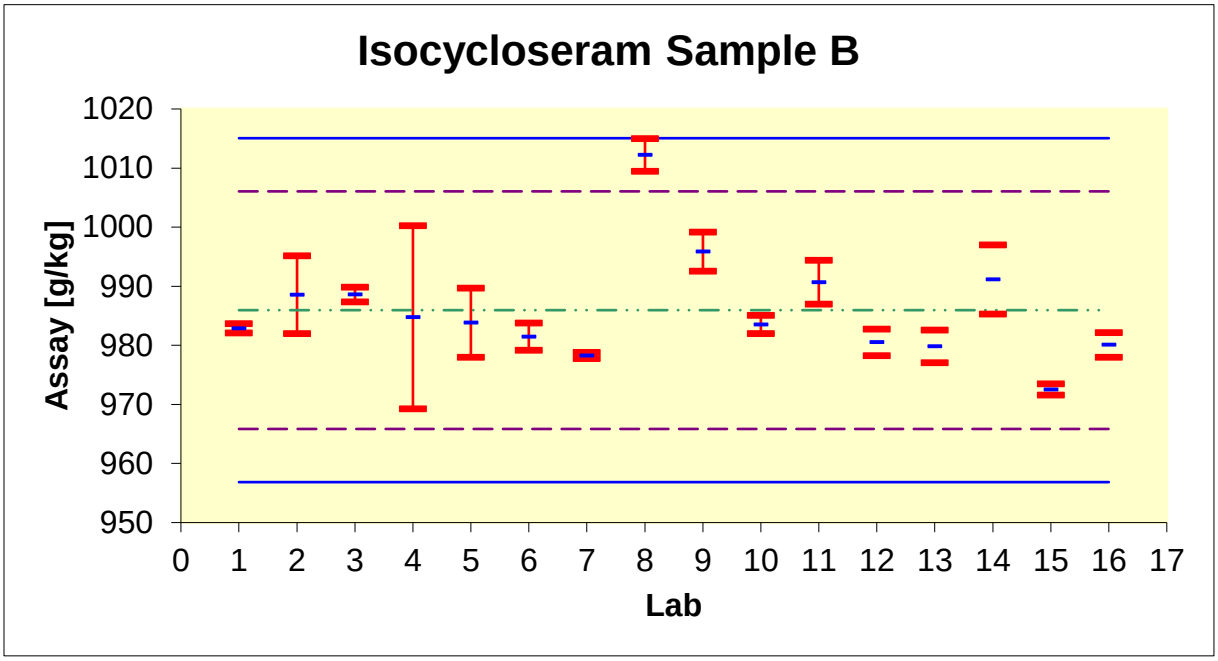


Figure 2: Graphical presentation of the results of the different laboratories for Sample B (TC). For each laboratory (Laboratories 1 to 16) the red bars represent day 1 and day 2. The blue bar represents the average. Laboratory 8 is a straggler.

Table 4: Results of the different laboratories for Sample C (WP 15).

	Isocycloseram SAMPLE C		
	Day1	**Day2**	**mean**
Laboratory 1	147.7	146.0	146.9
Laboratory 2	146.5	148.1	147.3
Laboratory 3	146.3	150.3	148.3
Laboratory 4	142.5	143.8	143.2
Laboratory 5	145.9	147.1	146.5
Laboratory 6	146.0	147.0	146.5
Laboratory 7	144.7	146.2	145.5
Laboratory 8	144.2	151.9	148.1
Laboratory 9	151.3	149.2	150.3
Laboratory 10	149.0	146.5	147.8
Laboratory 11	146.8	145.6	146.2
Laboratory 12	145.7	146.6	146.2
Laboratory 13	145.5	145.9	145.7
Laboratory 14	151.1	150.4	150.8
Laboratory 15	146.2	146.2	146.2
Laboratory 16	145.3	142.0	143.7

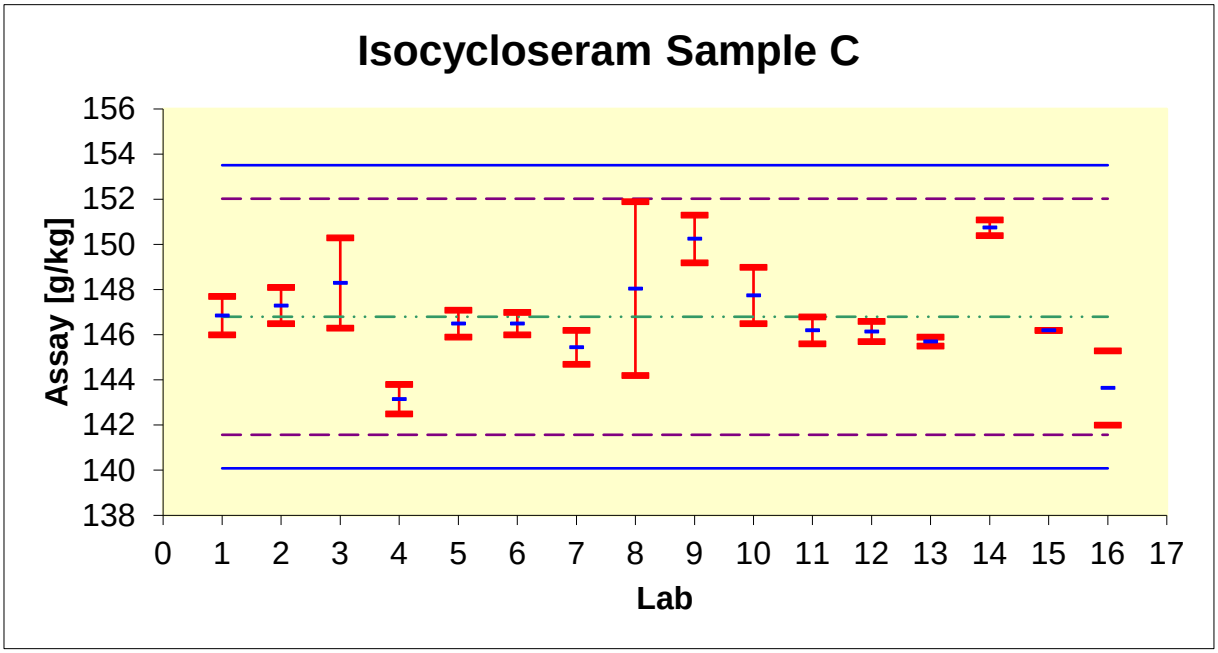


Figure 3: Graphical presentation of the results of the different laboratories for Sample C (WP). For each laboratory (laboratories 1 to 16) the red bars represent day 1 and day 2. The blue bar represents the average.

Table 5: Results of the different laboratories for Sample D (WP 15).

	Isocycloseram SAMPLE C		
	Day1	**Day2**	**mean**
Laboratory 1	151.1	150.8	151.0
Laboratory 2	150.3	152.3	151.3
Laboratory 3	152.1	154.7	153.4
Laboratory 4	148.4	149.4	148.9
Laboratory 5	151.2	151.1	151.2
Laboratory 6	151.4	154.7	153.1
Laboratory 7	152.2	150.8	151.5
Laboratory 8	151.7	157.1	154.4
Laboratory 9	154.6	155.6	155.1
Laboratory 10	151.1	151.5	151.3
Laboratory 11	151.4	150.1	150.8
Laboratory 12	150.9	150.6	150.8
Laboratory 13	149.8	151.2	150.5
Laboratory 14	153.5	153.9	153.7
Laboratory 15	151.7	149.5	150.6
Laboratory 16	148.5	148.1	148.3

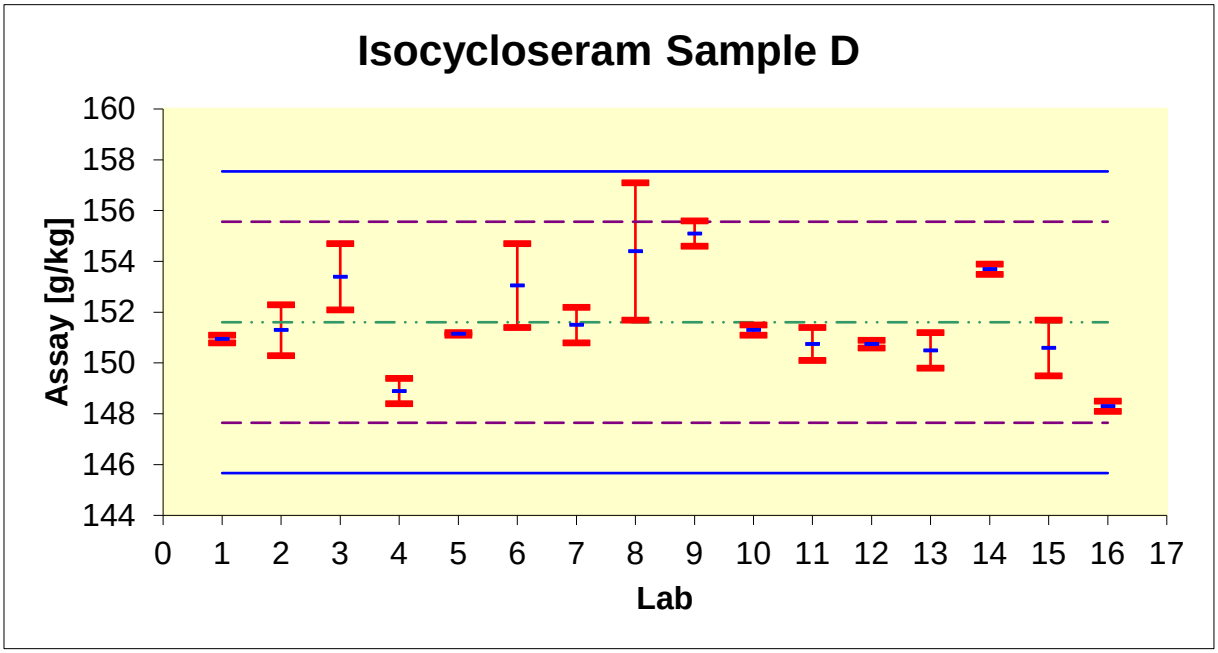


Figure 4: Graphical presentation of the results of the different laboratories for Sample D (WP15). For each laboratory (laboratories 1 to 16) the red bars represent day 1 and day 2. The blue bar represents the average.

Table 6: Results of the different laboratories for Sample E (WP 15).

	Isocycloseram SAMPLE C		
	Day1	**Day2**	**mean**
Laboratory 1	150.4	150.2	150.3
Laboratory 2	150.2	152.7	151.5
Laboratory 3	152.4	152.0	152.2
Laboratory 4	150.0	150.2	150.1
Laboratory 5	151.3	151.6	151.5
Laboratory 6	152.2	153.6	152.9
Laboratory 7	149.9	151.1	150.5
Laboratory 8	150.9	155.1	153.0
Laboratory 9	153.7	153.5	153.6
Laboratory 10	151.5	151.1	151.3
Laboratory 11	150.5	150.7	150.6
Laboratory 12	150.7	150.5	150.6
Laboratory 13	150.2	151.3	150.8
Laboratory 14	155.5	151.2	153.4
Laboratory 15	148.7	149.7	149.2
Laboratory 16	150.1	148.4	149.3

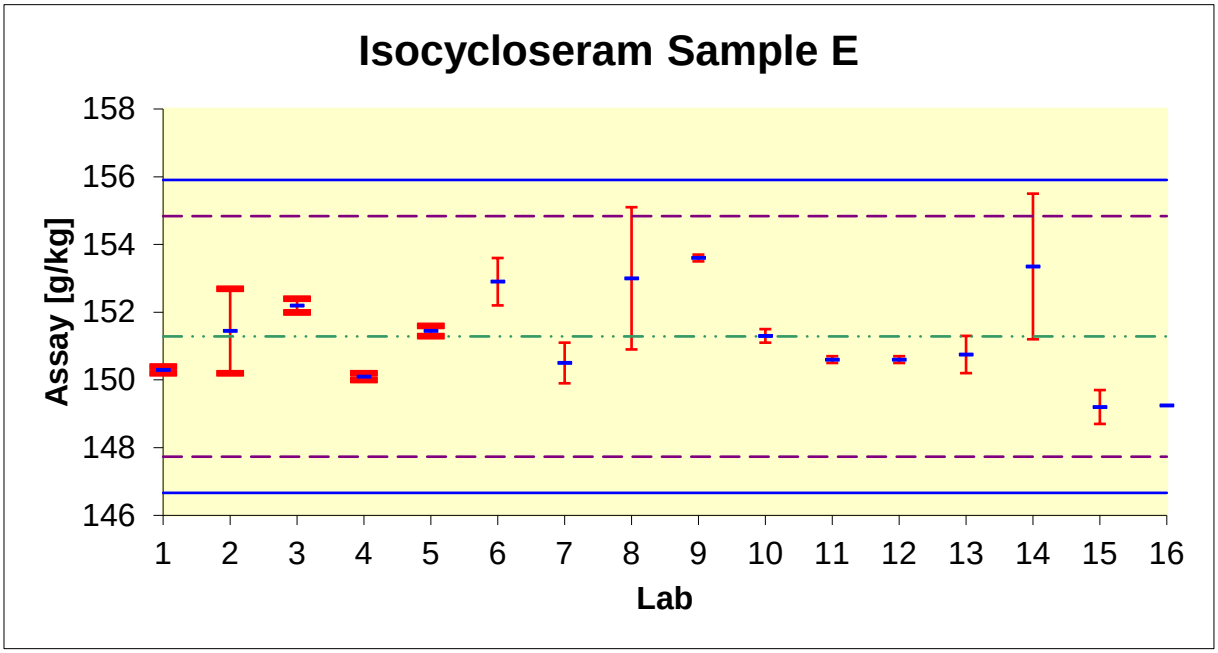


Figure 5: Graphical presentation of the results of the different laboratories for Sample E (WP 15). For each laboratory (laboratories 1 to 16 the red bars represent day 1 and day 2. The blue bar represents the average.

Table 7: Overall statistics on all submitted results:

	sample A	sample B	sample C	sample D	sample E
Xm	988.5	986.0	146.8	151.6	151.3
L	16	16	16	16	16
Sr	5.9	7.2	1.9	1.4	1.3
SL	1.1	7.5	1.5	1.6	1.1
SR	6.0	10.4	2.4	2.1	1.6
r	16.5	20.1	5.2	4.0	3.6
R	16.8	29.1	6.7	5.9	4.6
RSDr	0.6	0.7	1.3	0.9	0.8
RSDR	0.6	1.1	1.6	1.4	1.1
RSDR(Hor)	2.0	2.0	2.7	2.7	2.7
Horrat	0.3	0.5	0.6	0.5	0.4

Even without elimination of outliers or stragglers, the between laboratory experimental Relative Reproducibility Standard Deviation (RSDR) is below the acceptance limit based on the Horwitz curve calculation (RSDR(Hor)) for all samples.

Table 8: Statistics after elimination of laboratory 6 using a different column:

	sample A	sample B	sample C	sample D	sample E
Xm	988.5	986.2	146.8	151.5	151.5
L	15	15	15	15	15
Sr	6.0	7.4	1.9	1.3	1.3
SL	1.0	7.7	1.6	1.6	1.6
SR	6.1	10.7	2.5	2.1	2.1
R	16.9	20.6	5.4	3.7	3.7
R	17.1	29.9	6.9	5.9	5.9
RSDr	0.6	0.7	1.3	0.9	0.9
RSDR	0.6	1.1	1.7	1.4	1.4
RSDR(Hor)	2.0	2.0	2.7	2.7	2.7
Horrat	0.3	0.5	0.6	0.5	0.5

In a second approach only the laboratories applying the method as outlined were considered for statistical evaluation (see table 8).

Table 9: Statistical evaluation of all laboratories using the described C-18 column and after removal of the straggler result.

	sample A	sample B	sample C	sample D	sample E
Xm	988.5	984.4	146.8	151.5	151.5
L	15	14	15	15	15
Sr	6.0	7.56	1.9	1.3	1.3
SL	1.0	3.00	1.6	1.6	1.6
SR	6.1	8.13	2.5	2.1	2.1
r	16.9	21.16	5.4	3.7	3.7
R	17.1	22.77	6.9	5.9	5.9
RSDr	0.6	0.77	1.3	0.9	0.9
RSDR	0.6	0.83	1.7	1.4	1.4
RSDR(Hor)	2.0	2.00	2.7	2.7	2.7
Horrat	0.3	0.4	0.6	0.5	0.5

Table 9 summarizes the results after eliminating the straggler result for sample B.

8. Summary and Conclusion

A total of 16 laboratories from Asia and Europe participated in the trial, came back in time and provided results. The data sets from all these laboratories have been considered for the statistical evaluation (Figure 1 to 5 and Tables 2 to 7). In Table 8 all 15 labs using the chromatographic conditions as outlined in the method have been shown. Laboratory 6 has been excluded here as the stationary phase and the flow where significantly different. As for sample B the result of Laboratory 8 was a straggler, this result was excluded in a 3rd approach. Table 9 summarizes the statistical evaluation for the remaining 14 laboratories in Sample B together with all other results. In all cases shown in Tables 7, 8 and 9 the Horrat is well below 1.

Syngenta considers this method to be suitable for the intended purpose and recommends accepting it as a provisional CIPAC method for the determination of Isocycloseram in TC as well as WP formulated material.