5308/R

Methproene
414

Large Scale Collaborative Study for the
Determination of Free (Non-encapsulated) Methoprene in CS formulations

Report to CIPAC by

Shenyang SYRICI Testing Co., Ltd.

Method developed by

Synergetica Changzhou Ltd.

June 2023

Content

1. Participants

Large scale collaborative study for the determination of free methoprene in CS formulations by GC-FID was organized by Shenyang SYRICI Testing Co., Ltd. 10 laboratories responded to participate in the collaborative trials via email. Among them, 8 laboratories completed the experiment and provided test data, which are presented in the following sections. Two laboratories didn't provide results and didn't report any reasons.

Participating labs are listed in random order in the table below whereas lab numbers in the result tables were assigned, chronologically, based upon receipt of results.

No.	Contact	Lab	Country
1	Vanessa	Walloon Agricultural Research Centre (CRA-	Belgium
2	Karasali Helen Petros Tsiantas and Eleftheria Bempelou	Benaki Phytopathological Institute	Greece
3	Wenzhuo Wang	National Center for Pesticide Quality Supervision and Inspection (Beijing) Institute for the Control of Agrochemicals Ministry of Agriculture and Rural Affairs, P. R. China	China
4	Dr. Jim Garvey	Department of Agriculture, Food and The Marine	Ireland
5	Dr. Christian Mink	Analytical Development and Product Chemistry	Switzerland
6	Hou Chunqing	SYRICIT	China
7	Aysel TAKKABULAN	Republic of Turkiye Ministry of Agriculture And Forestry Pesticide Laboratory Quality Control Department Chemical Analysis Unit	Turkey
8	Qu Tingsi	Vaster Testing Technology Co., Ltd.	China

2. Active Ingredient: General Information

CAS Number: 65733-16-6

IUPAC name: isopropyl (E, E)-(S)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate

Chemical name: isopropyl (2E, 4E, 7S)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate

Empirical formula: $C_{19}H_{34}O_3$

RMM: 310.5

B.p.: 279.9 °C at atmospheric pressure (97.2 kPa)

V.p.: 0.623 mPa (20 °C), 1.08 mPa (25 °C) (Knudsen effusion)
S.g./density: 0.924 (20 °C)
Description: a pale yellow liquid, with a fruity odour
Solubility in water 6.85 ppm (20 °C).
Soluble in acetone and hexane >500, methanol >450 (all in g/l, 20±1 °C).
Stability: Stable in water, organic solvents, and in the presence of aqueous acids and alkalis. Sensitive to uv light.

3. Samples

3 batches of test samples and one batch of methoprene analytical standard were sent to the participants:

methoprene CS-1 Batch No. SCL20221220
methoprene CS-2 Batch No. SCL20221221
methoprene CS-2 Batch No. SCL20221222
methoprene reference standard (purity 97.1%)

4. Method

4.1 Scope

Determination of free methoprene in CS formulations.

4.2.Principle

A known quantity of the capsule suspension is transferred to a glass bottle and then subjected to a process of rotary movement extraction with a specified amount of n-hexane containing an internal standard. After mixing/extraction for a specified period, the amount of free methoprene in the n-hexane layer is determined by GC-FID.

4.3 Procedure

Each sample was analyzed using four independent determinations. The samples were analyzed on two different days, each day involving duplicate injections of duplicate weights. Both test and reference solutions were freshly prepared on each day. The four injections of each test solution were bracketed by double injections of the calibration solution. The average response factor, used to calculate the amount of methoprene in the test solution, was calculated using the injection before and after the test injections.

5. Remarks of the Participants

5.1 Analytical Condition

Lab	GC model	Column	Split raio (flow	Temp.(°C)	Inj. VoL. (µl)	Flow rate ml/min

			ml/ min)	Injecto r	Detect or	Oven program.		
1	Aglie nt 7890 B Series	J&W HP-1 GC Column, 30 m, 0.25 mm, 0.25 µm	100:1 (130 ml/ min)	250	300	initial temperatur e: 170 °C program rate: 10 °C/ min final temperatur e: 240 °C (5 min)	1	Nitrogen (30 ml/min) (pressure: 150 kPa)
2	No method deviation reported							
3	Aglie nt 7890 B GC	J&W DB-1 analytical column (30 m ×0.32 mm i.d.×0.25 µm film thickness)	100:1 (140 ml/ min)	250	300	initial temperatur e: 170 °C program rate: 10 °C/ min final temperatur e: 240 °C (5 min)	1	Nitrogen (25 ml/min) (pressure: 150 kPa)
4	No method deviation reported							
5	Helium is used instead of Nitrogen, and no other method deviation reported							
6	No method deviation reported							
7	Agile nt 6890	Agilent HP-1 30 m x 0,32 (i.d), film thickness 0,25 µm	Helium is used instead of Nitrogen, and no other method deviation reported					
8	No method deviation reported							

5.2 Remarks

1	Suggestion for the outline of method : "… and then subjected to a process of rotary evaporating extraction …" should be replaced by "… and then subjected to a process of rotary movement …" in order to indicate that there is no evaporation of the extraction solvent during the process. Comment: accepted.

4	1. Chromatographic Conditions: The method parameters were ambiguous for Split Ratio and Split Flow. As one value determines the value of the other, they cannot be set together as 100:1 and 140mL/min respectively. The parameters we used were Split Ratio at 100:1, which set the Split Flow to 204.53mL/min. We tried setting the Split Flow to 140mL/min, which set the Split Ratio automatically to 68.45 : 1. However this produces different results. This description of the method was unclear. Comment: the analytical condition is identical with the current CIPAC method **Methoprene Technical 413/TC/M**. 2. Linearity Check: There is no acceptability criteria defined for this. We carried out linearity studies at 0.5, 1 and 2 times that of the Calibration solution. This data was not sought but can be provided, if required. Comment: The content is copied from the current CIPAC method **Methoprene Technical 413/TC/M**. 3. Extraction: The extraction method was unusual in the use of a rotary evaporator to mix the solution. We would not normally use a rotary evaporator for this purpose. We would more commonly use magnetic stirrers for this purpose. Comment: If the magnetic stirring is operated at a slower speed, it can avoid damaging the microcapsules. The rotation of the rotary evaporator is a common equipment in a quality control lab and provides smooth and uniform rolling, exerting minimal destructive force. Magnetic stir at high speed is too strong, and it can easily cause damage to the membrane. 4. Error in Excel Spreadsheet: On sheet 'Day 1' cells G36 and H36 do not have correct calculation. Comment: there is no mistake found in the original table. And all the other tables provided by the other labs were checked and no same issue was found. 5. Repeat Analysis: We needed to repeat the analysis of sample CS-1a as the original sample dropped off the rotary evaporator during the extraction. Therefore the result of the repeat sample was taken and so the C7 cell in Excel Spreadsheet page 'Summary' was amended to take cell ='Day 1'!H63, instead of ='Day 1'!H36 Comment: well noted
7	Agilent J&W HP-1 is a general purpose 100% Dimethylpolysiloxane. Agilent HP-1 30 m x 0,32 (i.d), film thickness 0,25 µm was used as capillary column. Helium was used as carrier gas in the Agilent 6890 device. Retention times for eicosane: about 5,36 and for methoprene: about 5,99

6. Evaluation and Discussion

6.1 Data Review

No major method deviations were noted by the participants, which will affect the analytical results significantly.

6.2 Statistical results

The statistical evaluation of the data was accomplished following the “Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods”, according to DIN ISO 5725.

Table 1 Results of CS-1

	Day 1		Day 2		Mean	STD
Lab 1	1.859	2.140	2.111	1.935	2.01	0.1359
Lab 2	1.177	1.212	1.175	1.210	1.19	0.0200
Lab 3	1.070	1.041	1.094	1.055	1.07	0.0227
Lab 4	2.514	2.774	3.351	2.727	2.84	0.0690
Lab 5	0.922	0.924	0.622	0.611	0.77	0.1770
Lab 6	2.267	2.185	2.210	2.044	2.18	0.0945
Lab 7	1.239	1.237	1.509	1.511	1.42	0.1577
Lab 8	1.309	1.309	0.979	0.977	1.14	0.1909

Table 2 Results of CS-2

	Day 1		Day 2		Mean	STD
Lab 1	1.339	1.288	1.279	1.307	1.30	0.0266
Lab 2	0.890	0.917	0.805	0.824	0.86	0.0532
Lab 3	1.737	1.703	1.676	1.577	1.67	0.0690
Lab 4	2.895	3.061	3.43	3.19	3.14	0.0690
Lab 5	0.864	0.818	0.634	0.642	0.74	0.1189
Lab 6	2.104	2.207	2.223	2.321	2.21	0.0079
Lab 7	1.245	1.181	1.071	1.262	1.17	0.0961
Lab 8	1.300	1.301	0.964	0.964	1.13	0.1945

Table3 Results of CS-3

	Day 1		Day 2		Mean	STD
Lab 1	1.245	1.284	1.647	1.366	1.39	0.1815
Lab 2	0.850	0.800	0.886	0.800	0.83	0.0421
Lab 3	1.174	1.201	1.012	1.044	1.11	0.0935

Lab 4	3.211	2.720	2.806	2.810	2.89	0.0690
Lab 5	0.891	0.857	0.663	0.708	0.78	0.1113
Lab 6	2.108	2.093	2.197	2.211	2.15	0.0607
Lab 7	1.564	1.606	1.572	1.438	1.54	0.0889
Lab 8	1.292	1.291	0.924	0.925	1.11	0.2120

Table 4 Mean Values

	Methoprene CS-1	Methoprene CS-2	Methoprene CS-3
Lab 1	2.01	1.30	1.39
Lab 2	1.19	0.86	0.83
Lab 3	1.07	1.67	1.11
Lab 4	2.84	3.14	2.89
Lab 5	0.77	0.74	0.78
Lab 6	2.2	2.21	2.15
Lab 7	1.42	1.17	1.54
Lab 8	1.14	1.13	1.11

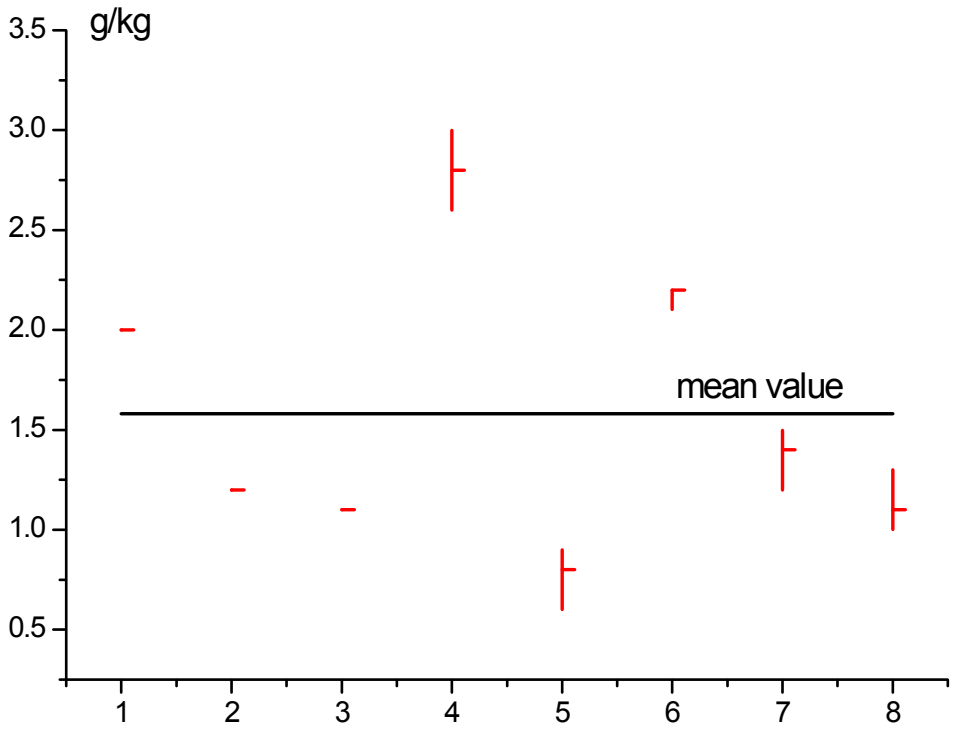
Table 5 Summary of the statistical evaluation

	Methoprene CS-1	Methoprene CS-2	Methoprene CS-3
x_m [g/kg]	1.58	1.53	1.47
x_m [% w/w]	0.158	0.153	0.147
L	8	8	8
S_r	0.1254	0.1013	0.1210
S_R	0.7074	0.8070	0.7275
RSD_R	44.8436	52.7958	49.3489
RSD_R（Hor）	5.2817	5.3063	5.3359
HorRat	8.4903	9.9428	9.2484

x_m = total mean value
L = number of laboratories
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
RSD_R = relative standard deviation of reproducibility
$RSD_{R\ (Hor)}$ = Horwitz Value according to Horwitz equation

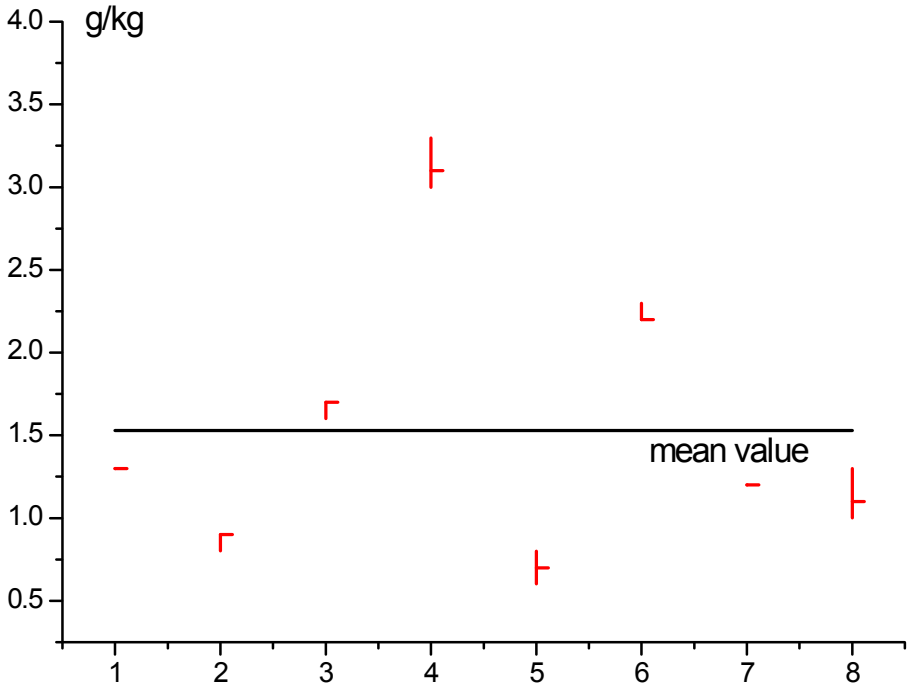
HorRat = Horwitz Ratio (RSD_R / $RSD_{R\,(Hor)}$)

Fig. 1 Methproene CS-1



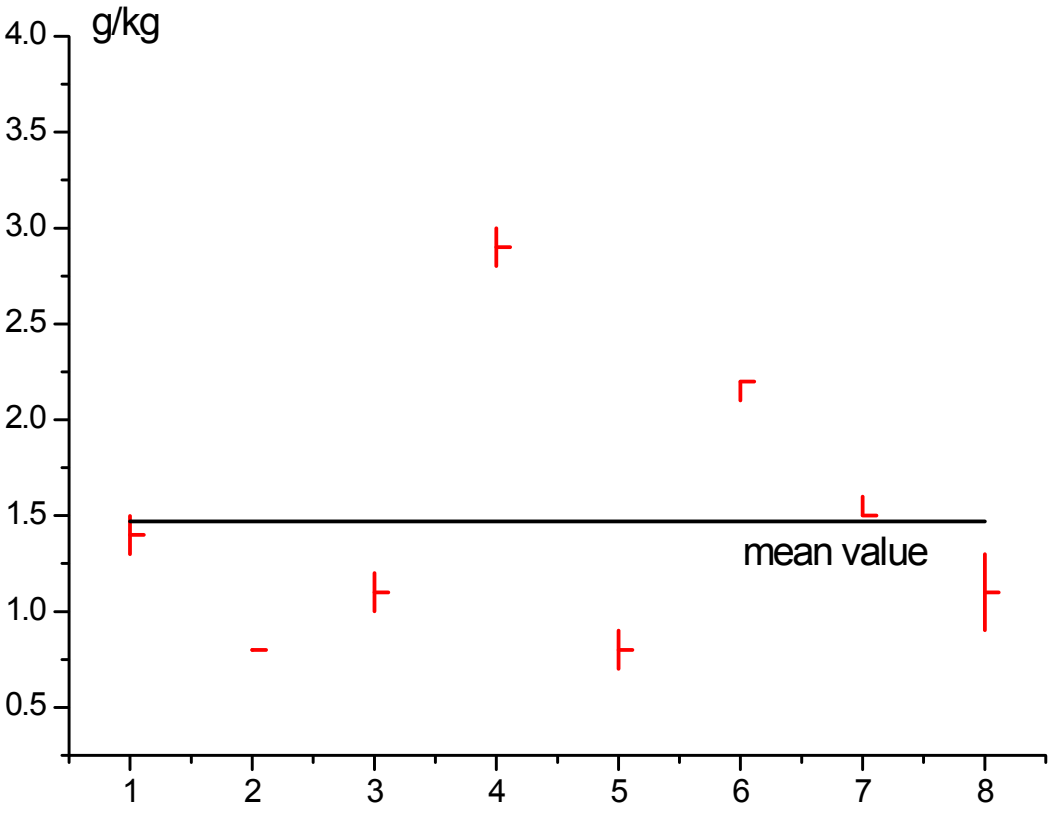
Mean value: 1.58 g/kg
S_r: 0.1254
S_R: 0.7074
RSD_R: 44.8436
$RSD_{R\,(Hor)}$: 5.2817
HorRat: 8.4903

Fig. 2 Methproene CS- 2



Mean value: 1.53 g/kg
S_r: 0.1013
S_R: 0.8070
RSD_R: 52.7598
$RSD_{R\ (Hor)}$: 5.3063
HorRat: 9.9428

Fig. 3 Methproene CS– 3



Mean value: 1.47 g/kg
Sr: 0.1210
SR: 0.7275
RSDR: 49.3489
RSDR（Hor）： 5.3359
HorRat: 9.2484

7. Conclusions

The data presented in the statistical summary showed that this method led to a quite higher RSDR values than expected due to the dynamic equilibrium between the inside and outside of the capsule, and the uncertainties occurred during the extraction process.

Considering the fact that the purpose of the method is to monitor the content of free ai in CS formulation, as indicated in the proposed WHO specification “not greater than 5% of the total AI content”, it is still believed that the method can fulfill its function as specification test.