AI

Collaborative Study

Full Scale Collaborative Study
for the
Determination of AI content of
Eucalyptus citriodora oil hydrated,
cyclized by GC-FID

Report to CIPAC
by
Citrefine International Ltd.

May 2025

1. Participants

In November 2024, Information Sheet No. 350 was sent out by the CIPAC Secretary, inviting members to participate in a collaborative trial on a method for Eucalyptus citriodora oil, hydrated, cyclized – 1027.
The results of all 8 participants were evaluated.

The participating 8 laboratories are listed in alphabetical order, whereas lab numbers in the result tables were assigned randomly.

Agro Est Muntenia Lalelelor Street, No. 1B, Cioranca, Buzau County, Zip code 127381 Romania
Anthem Biosciences No. 49, F1 & F2, Canara Bank Road, Bommasandra Industrial Area – Phase 1 Bommasandra, Bengaluru – 560 099, Karnataka, India
Laboratory of Chemical Control of Pesticides Benaki Phytopathological Institute 8 Stefanou Delta Street, Kifissia, 14561 Athens Greece
Federal Office of Consumer Protection and Food Safety (BVL) Unit 214/ Dept. Plant Protection Products Federal Office of Consumer Protection and Food Safety Bundesallee 51; D-38116 Braunschweig Germany
National Phytosanitari Authority AUTORITATEA NATIONALA FITOSANITARA, Blvd. Voluntari 11, 077190, VOLUNTARI, ILFOV, ROMANIA

Official Control Labs of the Andalusian Net of AgroFood Labs Dpto. de Química analítica Laboratorio de Control Oficial Agroalimentario y Agroganadero de Sevilla. Agencia de Control Agraria y Pesquera de Andalucía. Carretera de Utrera km.1, nº 3. 41013 Sevilla, España
Syngenta 3210.E44 (office), 3120.E36 and E72 (Labs) Breitenloh 5, 4333 Münchwilen, Switzerland
Ukzuz ÚSTŘEDNÍ KONTROLNÍ A ZKUŠEBNÍ ÚSTAV ZEMĚDĚLSKÝ Zemědělská 1A, Brno, PSČ 613 00, Česká Republika

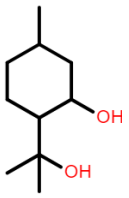
2. Active Ingredient: General Information

Chemical name: IUPAC Eucalyptus citriodora oil, hydrated, cyclized

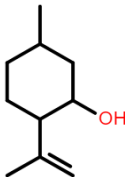
CAS 1245629-80-4

ISO common name: N/A

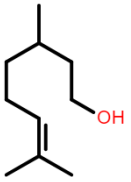
Structure: As a UVCB there is no single structure, the analyte structures are below



p-Menthane-3,8-diol



Isopulegol



Citronellol

Molecular formula:	UVCB
Molecular mass:	$C_{10}H_{20}O_2$ (PMD value used as majority component) 172.26g/mol (PMD value used as majority component)
Activity:	Insect Repellent for human application

3. Samples

Five test samples and three analytical standards were sent to the participants:

1. Eucalyptus citriodora oil, hydrated, cyclized, batch 033926/00524 – Sample A
2. Eucalyptus citriodora oil, hydrated, cyclized, batch 034212/00541 – Sample B
3. Eucalyptus citriodora oil, hydrated, cyclized, batch 036977/00589 – Sample C
4. Eucalyptus citriodora oil, hydrated, cyclized, batch 039167/00599 – Sample D
5. Eucalyptus citriodora oil, hydrated, cyclized, batch 039355/00605 Sample E
6. (+/-)-β-Citronellol, batch BCCH6338 99.9% purity – analytical standard
7. (-)-Isopulegol, batch BCCJ9812 100% purity – analytical standard
8. cis p-Menthane-3,8-diol, batch CIL15012501 99.1% purity – analytical standard
9. n-Tridecane, batch MKCS4909 99.1% purity – internal standard

4. Method

4.1. Scope

The determination of active ingredient content contained within a technical sample (TC).

4.2. Principle

The content of AI (g/kg) of isopulegol, citronellol and p-menthane-3,8-diol present within a sample of Eucalyptus citriodora oil, hydrated, cyclized is determined by capillary gas chromatography using a (5%-phenyl)-methylpolysiloxane non-polar fused silica column (such as an Agilent HP-5, Restek Rxi-5ms, Perkin Elmer Elite-5ms or equivalent, with dimensions of 30m length x 0.25mm I.D. x 1.0µm film thickness), using hydrogen as the carrier gas and flame ionisation detection. Quantitation is by comparison to internal and external standards.

4.3. Procedure

Each sample was analysed at each laboratory using two independent analyses performed across two days.

5. Remarks of the Participants

Several participants provided comments about the method performance and made notes of deviations from the method:

Laboratory 1	GC System (Make/Model): Column: Flow rate: Injection volume: Remarks:	Agilent 7890 Agilent, DB-5, 30 m x 0.25 (i.d.) mm, 1 µm film thickness 2 mL/min Hydrogen 1.0 µL “In the "results" sheet the order of the samples is different compared to your template”
Laboratory 2	GC System (Make/Model): Column: Flow rate: Injection volume: Remarks:	Agilent 8890 DB-5MS, 30 m x 0.25 mm x 0.25 um 2.0 mL/min 1.0 µL “Purity of tridecane is missing”
Laboratory 3	GC System (Make/Model): Column: Flow rate: Injection volume: Remarks:	Trace 1610, SSL injector, FID detector Agilent, DB-5, 30 m x 0.25 (i.d.) mm, 1µm film thickness 100:1 1.0 µL “In our case the carrier gas is Nitrogen”
Laboratory 4	GC System (Make/model): Column: Flow rate: Injection volume: Remarks:	Agilent 6890N RX-5Sil MS; 30 m x 0.25 mm x 1µm 2 mL/min 1.0 µL None
Laboratory 5	GC System (Make/Model): Column: Flow rate: Injection volume: Remarks:	Shimadzu, Japan: GC-2010 Plus Shimadzu, RX-5 Sil, 30 m length x 0.25 mm ID x 0.25 µm film thickness 0.60 mL/min Helium 1.0 µL Column used is different from specified. Helium used as a carrier gas.
Laboratory 6	GC System (Make/Model): Column: Flow rate: Injection volume: Remarks:	Thermo Trace 1610, Thermo AI 1610 HP Ultra 2 25m x 0.32mm x 0.52µm film thickness 0.5ml/min 1.0 µL Modified the eluent flow rate to obtain the retention times specified in the method
Laboratory 7	GC System (Make/model): Column: Flow rate: Injection volume:	Agilent 8890 HP 5, 30 m x 0.32 mm x 0.25 µm) 2 mL/min 1 µL

	Remarks:	Helium used as a carrier gas
Laboratory 8	GC System (Make/model): Column: Flow rate: Injection volume: Remarks:	Shimadzu GC-2010 Plus DB-5, 30 m x 0.53 mm x 1.5 µm Flow rate: 8.1 mL/min 1 µL None

6. Evaluation and Discussion

6.1. Data Review

The data obtained from each laboratory was visually reviewed to determine if there were any significant chromatography differences from what was expected, which might affect the analytical results. There were no significant differences from the expected chromatography and only one anomaly in which one laboratory reported a higher value for one of the PMD isomers. This is unexplained, however, the total results were in line with expected values. It was theorized that it could be due to the helium gas used, however, another laboratory also used helium and obtained an expected isomer ratio.

In summary it can be stated that the method deviations, noted by the participating laboratories, were not of significance. The laboratories in total used three carrier gases: hydrogen, helium and nitrogen, but there were no significant differences in analysis results. There were some deviations from the column specified in that some used a 0.25µm film thickness, but this had no noticeable effect on the analysis results and was used by more than one laboratory. The purity of tridecane comment by one laboratory is noted but will have no effect on the overall calculation as it will apply to both sample and standard and in effect cancel out.

6.2. Determination of AI

The statistical evaluation of the data was accomplished following the new CIPAC Guideline, according to DIN ISO 5725.
The testing for outliers / stragglers of the laboratory mean values were performed according to Grubbs test on a 1 % / 5 % significance level, respectively.
All results reported by the 8 laboratories are shown and the statistical evaluation of these are listed in Tables 1-10 and displayed in Figures 1-19. These results are reported without any exclusion of outliers and/or stragglers.
In addition, separate evaluations, following on from the statistical evaluations in tables 2,6 and 9, display the results with the exclusion of outliers.

Determination of p-Menthane,3,8-diol – Full set of 8 participants

Table 1 – p-Menthane-3,8-diol Results (content in g/kg)

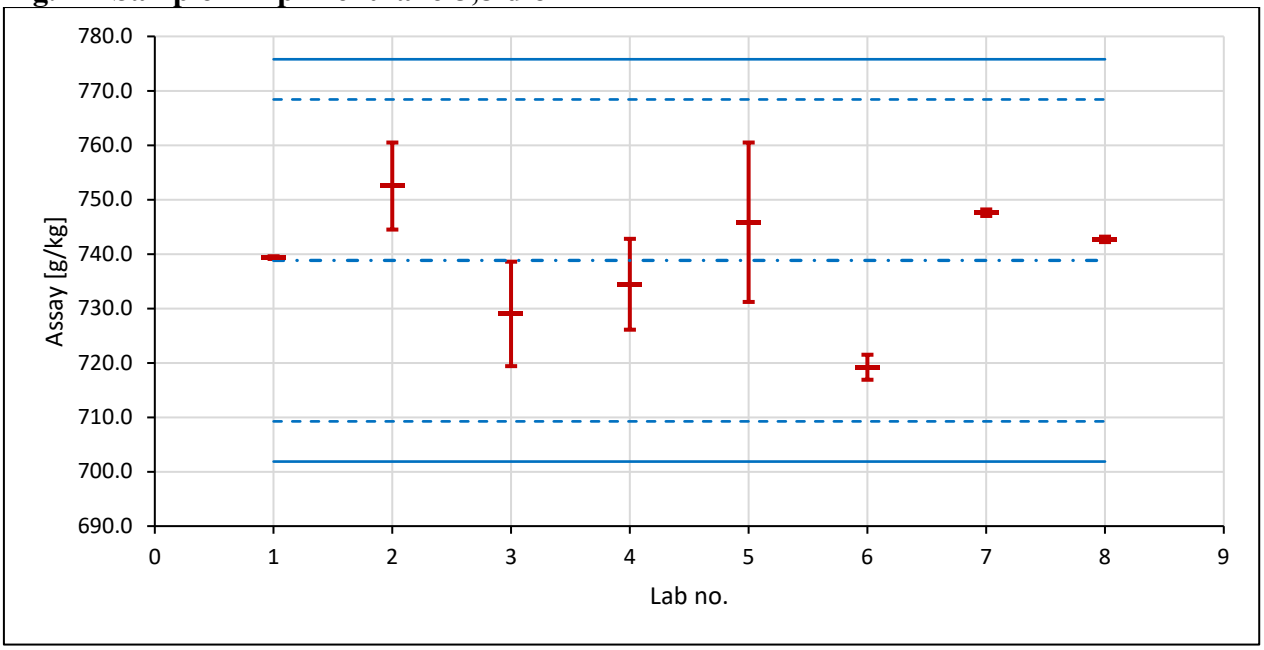
Laboratory	Day	Sample A 033926/00524		Sample B 034212/00541		Sample C 036977/00589		Sample D 039167/00599		Sample E 039355/00605	
1	1	739.6	739.4	721.3	720.3	709.9	708.7	700.9	701.6	726.7	726.1
	2	739.1		719.3		707.5		702.3		725.5	
2	1	744.5	753.5	721.0	729.7	712.3	721.3	706.2	713.9	728.4	727.1
	2	760.5		738.3		730.2		721.5		725.8	
3	1	719.4	729.0	705.6	713.6	702.4	698.1	700.6	699.4	627.3	625.4
	2	738.6		721.6		693.7		698.1		623.5	
4	1	742.8	734.5	718.0	712.5	698.4	700.2	698.2	697.6	727.5	728.5
	2	726.1		707.0		702.0		697.0		729.5	
5	1	760.5	745.9	730.0	720.6	717.6	711.0	683.6	671.3	731.1	721.5
	2	731.2		711.2		704.3		659.0		711.8	
6	1	716.9	719.2	707.2	698.4	657.5	671.2	688.6	673.3	736.2	721.7
	2	721.5		689.5		684.8		658.0		707.2	
7	1	748.2	747.6	721.4	725.9	721.5	727.4	719.8	718.4	765.3	746.5
	2	747.0		730.4		733.2		717.0		727.7	
8	1	743.2	742.7	690.8	696.0	696.0	699.1	693.8	689.4	704.7	700.1
	2	742.2		701.1		702.2		684.9		695.4	

Table 2 – Summary of the statistical evaluation

	Sample A 033926/00524	Sample B 034212/00541	Sample C 036977/00589	Sample D 039167/00599	Sample E 039355/00605
x_m [g/kg]	738.8	714.6	704.6	695.6	712.1
x_m [% w/w]	73.88	71.46	70.46	69.56	71.21
n	8	8	8	8	8
s_r	10.56	9.79	9.72	10.82	13.09
s_R	13.20	14.02	18.49	18.68	38.39
r	29.58	27.42	27.22	30.29	36.64
R	36.97	39.25	51.78	52.51	107.48
RSD_R [%]	1.79	1.96	2.62	2.69	5.39
$RSD_{R\ (Hor)}$ [%]	2.09	2.10	2.11	2.11	2.10
HorRat	0.85	0.93	1.25	1.27	2.56

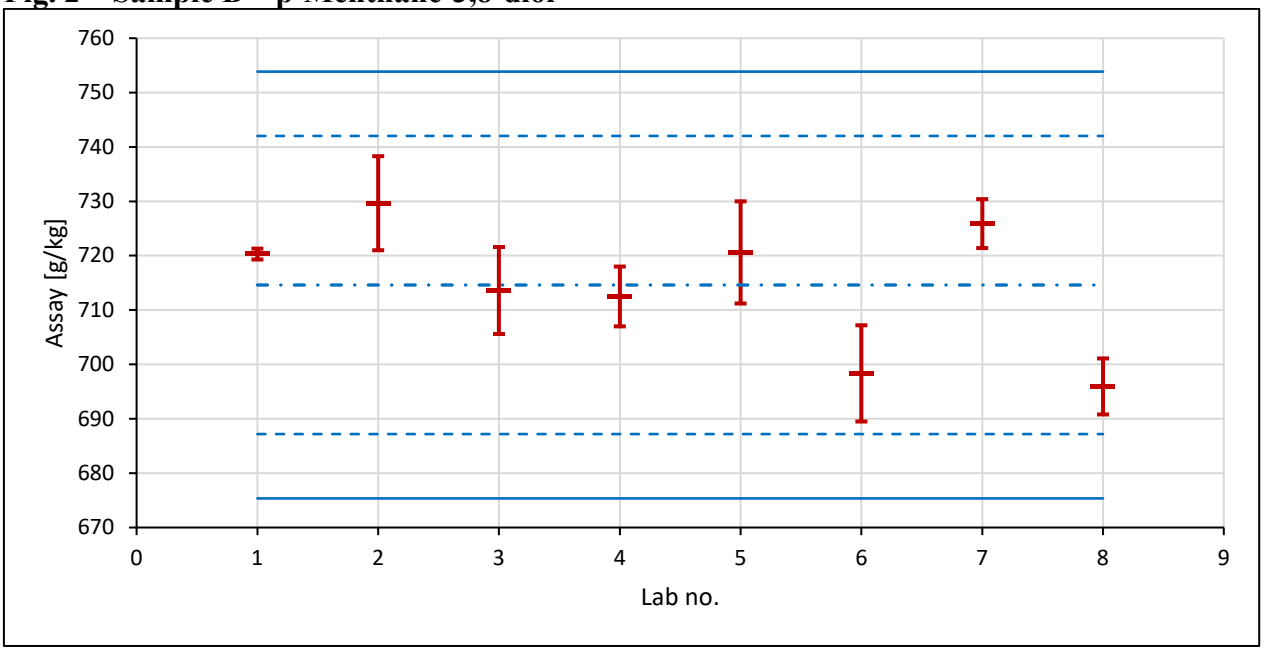
x_m = overall sample mean
n = number of laboratories
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
r = repeatability limit
R = reproducibility limit
RSD_r = relative repeatability standard deviation [%]
RSD_R = relative reproducibility standard deviation [%]
HorRat = $RSD_R/RSD_{R\ (Hor)}$ (Horwitz Ratio)

Fig. 1 – Sample A – p-Menthane-3,8-diol



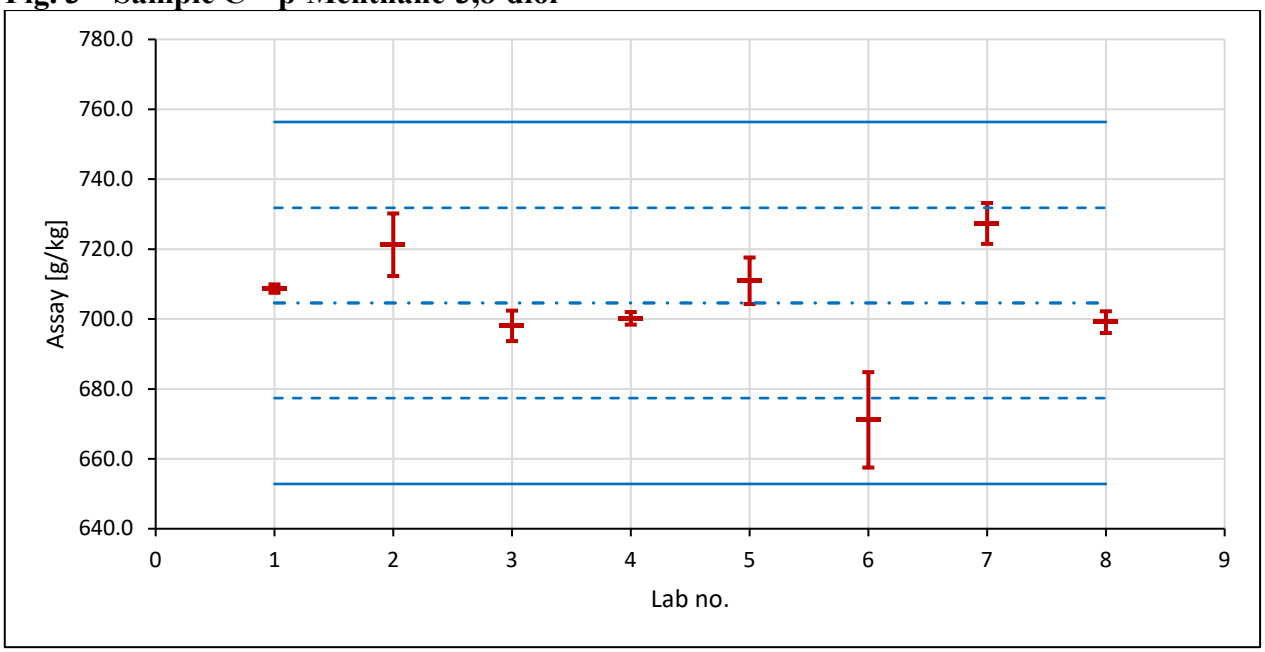
Mean value	738.83	RSD_R	1.79 %
s_r	10.56	$RSD_{R\ (Hor)}$	2.09 %
s_R	13.20	HorRat	0.85
Outlier (Grubbs)	none		

Fig. 2 – Sample B – p-Menthane-3,8-diol



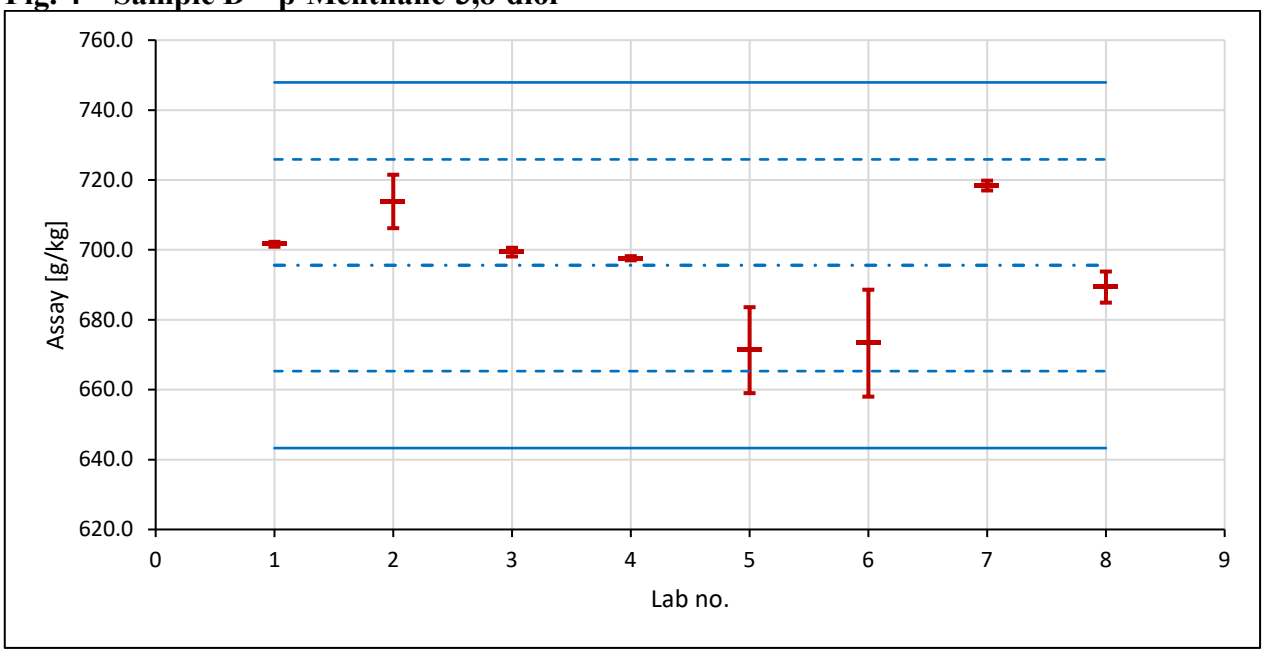
Mean value	714.61	RSD_R	1.96 %
s_r	9.79	$RSD_{R\ (Hor)}$	2.10 %
s_R	14.02	HorRat	0.93
Outlier (Grubbs)	none		

Fig. 3 – Sample C – p-Menthane-3,8-diol



Mean value	704.59	RSD_R	2.62 %
s_r	9.72	$RSD_{R\ (Hor)}$	2.11 %
s_R	18.49	HorRat	1.25
Outlier (Grubbs)	none		

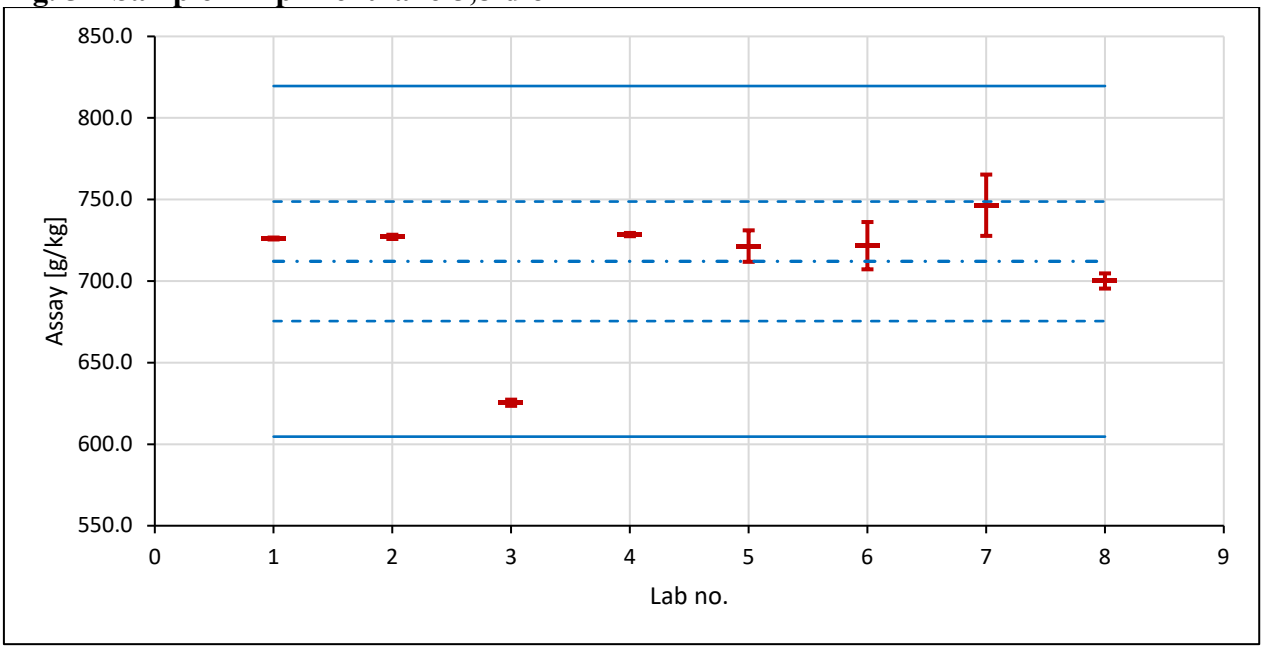
Fig. 4 – Sample D – p-Menthane-3,8-diol



Mean value	695.59	RSD_R	2.69 %
s_r	10.82	$RSD_{R\ (Hor)}$	2.11 %
s_R	18.68	HorRat	1.27

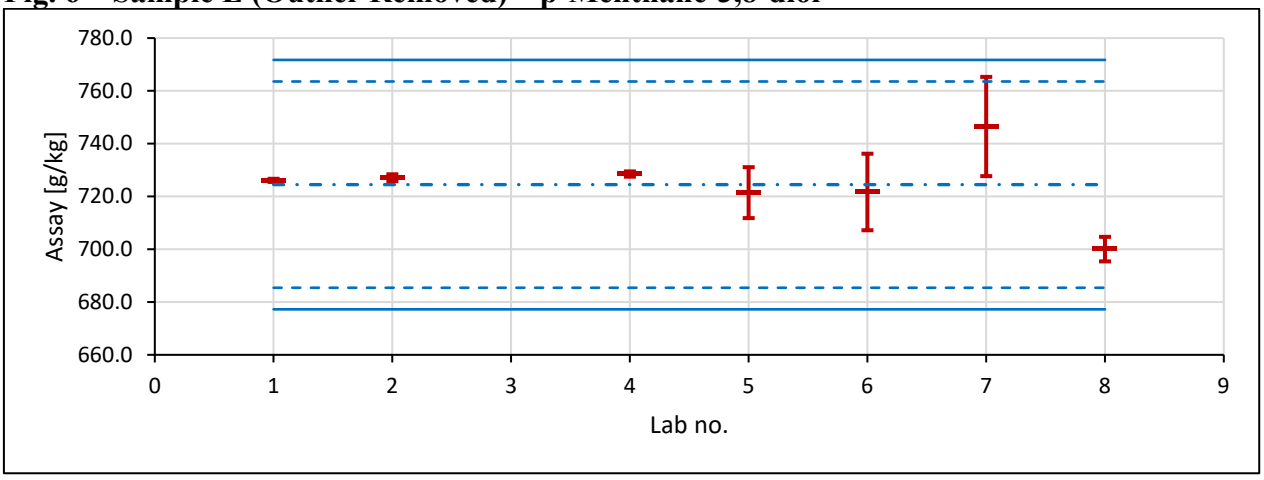
Outlier (Grubbs) none

Fig. 5 – Sample E – p-Menthane-3,8-diol



Mean value	712.10	RSD_R	5.39 %
s_r	13.09	$RSD_{R\ (Hor)}$	2.10 %
s_R	38.39	HorRat	2.56
Outlier (Grubbs)	Laboratory 3 – 625.40		

Fig. 6 – Sample E (Outlier Removed) – p-Menthane-3,8-diol



Mean value	724.49	RSD_R	2.33 %
s_r	13.95	$RSD_{R\ (Hor)}$	2.10 %
s_R	16.87	HorRat	1.11
Outlier (Grubbs)	removed		

Determination of p-Menthane-3,8-diol – Elimination of outliers

Table 3 – Summary of the statistical evaluation (without outliers)

	Sample A 033926/00524	Sample B 034212/00541	Sample C 036977/00589	Sample D 039167/00599	Sample E 039355/00605 (1 outlier removed)
x_m [g/kg]	738.8	714.6	704.6	695.6	724.49
x_m [% w/w]	73.88	71.46	70.46	69.56	72.45
n	8	8	8	8	7
s_r	10.56	9.79	9.72	10.82	13.95
s_R	13.20	14.02	18.49	18.68	16.87
r	29.58	27.42	27.22	30.29	39.07
R	36.97	39.25	51.78	52.51	47.25
RSD_R [%]	1.79	1.96	2.62	2.69	2.33
$RSD_{R\ (Hor)}$ [%]	2.09	2.10	2.11	2.11	2.10
HorRat	0.85	0.93	1.25	1.27	1.11

x_m = overall sample mean
n = number of laboratories
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
r = repeatability limit
R = reproducibility limit
RSD_r = relative repeatability standard deviation [%]
RSD_R = relative reproducibility standard deviation [%]
HorRat = $RSD_R/RSD_{R\ (Hor)}$ (Horwitz Ratio)

Table 4 – Summary of the p-menthane-3,8-diol isomers

	Proportion cis-PMD (%)	Proportion trans-PMD (%)	Proportion Other PMD (%)
Sample A	66.2	31.7	2.1
Sample B	64.2	30.5	2.2
Sample C	63.5	29.9	2.1

Sample D	62.7	29.5	2.1
Sample E	62.5	31.8	2.3

Determination of Citronellol – Full set of 8 participants

Table 5 – Citronellol Results (content in g/kg)

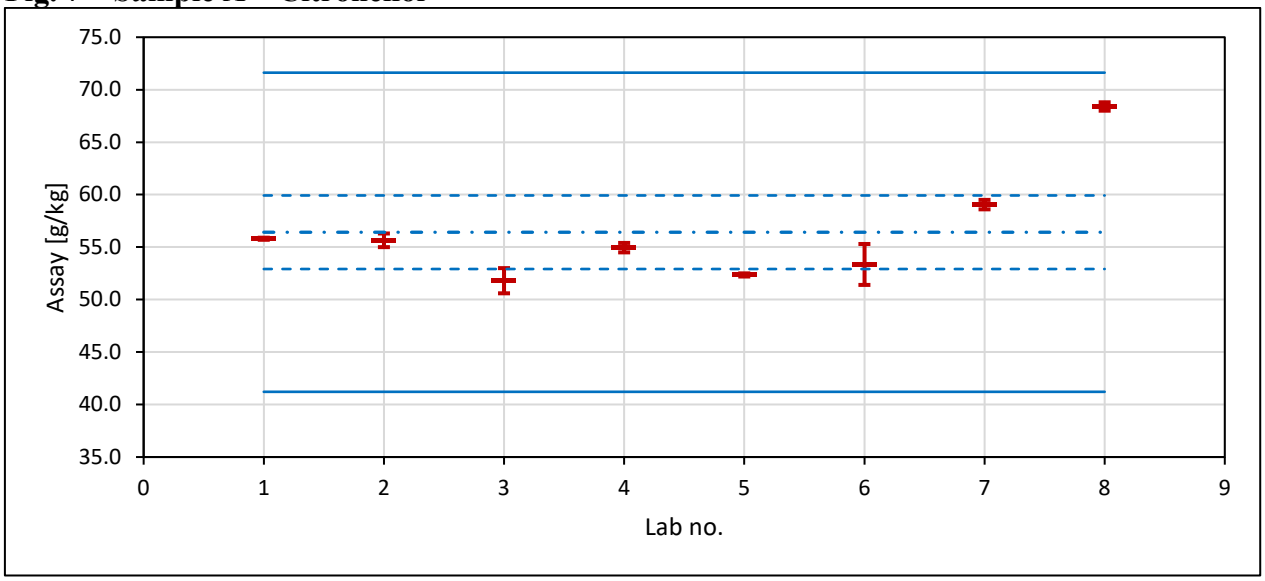
Laboratory	Day	Sample A 033926/00524		Sample B 034212/00541		Sample C 036977/00589		Sample D 039167/00599		Sample E 039355/00605	
1	1	55.7	55.8	63.0	63.0	75.0	75.0	62.1	62.1	66.2	66.3
	2	55.9		63.0		74.9		62.1		66.4	
2	1	55.0	55.7	61.4	62.3	72.9	73.9	60.7	61.5	64.6	64.6
	2	56.3		63.1		74.8		62.2		64.5	
3	1	50.6	51.8	58.9	60.2	70.0	70.9	57.6	58.6	82.8	84.3
	2	53.0		61.5		71.8		59.6		85.7	
4	1	55.4	55.0	61.5	61.3	72	72.4	62.1	61.7	65.2	65.4
	2	54.5		61.1		72.7		61.3		65.6	
5	1	52.2	52.4	60.6	59.7	71.8	71.65	57.0	56.5	61.5	62.1
	2	52.5		58.8		71.5		55.9		62.6	
6	1	55.3	53.4	64.1	60.9	76.4	73.0	64.8	60.4	69.9	66.3
	2	51.4		57.6		69.5		55.9		62.6	
7	1	59.5	59.1	66.3	66.3	79.3	79.6	66.5	66.2	72.9	70.9
	2	58.6		66.2		79.8		65.8		68.8	
8	1	68.8	68.4	70.0	68.9	79.3	80.1	66.8	66.7	70.0	70.1
	2	68.0		67.7		80.5		66.7		70.2	

Table 6 – Summary of the statistical evaluation

	Sample A 033926/00524	Sample B 034212/00541	Sample C 036977/00589	Sample D 039167/00599	Sample E 039355/00605
x_m [g/kg]	56.42	62.80	74.53	61.69	68.72
x_m [% w/w]	5.64	6.28	7.45	6.17	68.7
n	8	8	8	8	8
s_r	1.25	1.95	1.87	2.34	2.24
s_R	5.43	3.47	3.73	3.04	7.07
r	3.50	5.45	5.24	6.56	6.26
R	15.20	9.73	10.44	10.75	19.80
RSD_R [%]	9.62	5.53	5.00	6.23	10.29
$RSD_{R\ (Hor)}$ [%]	3.08	3.03	2.96	3.04	2.99
HorRat	3.12	1.82	1.69	2.05	3.44

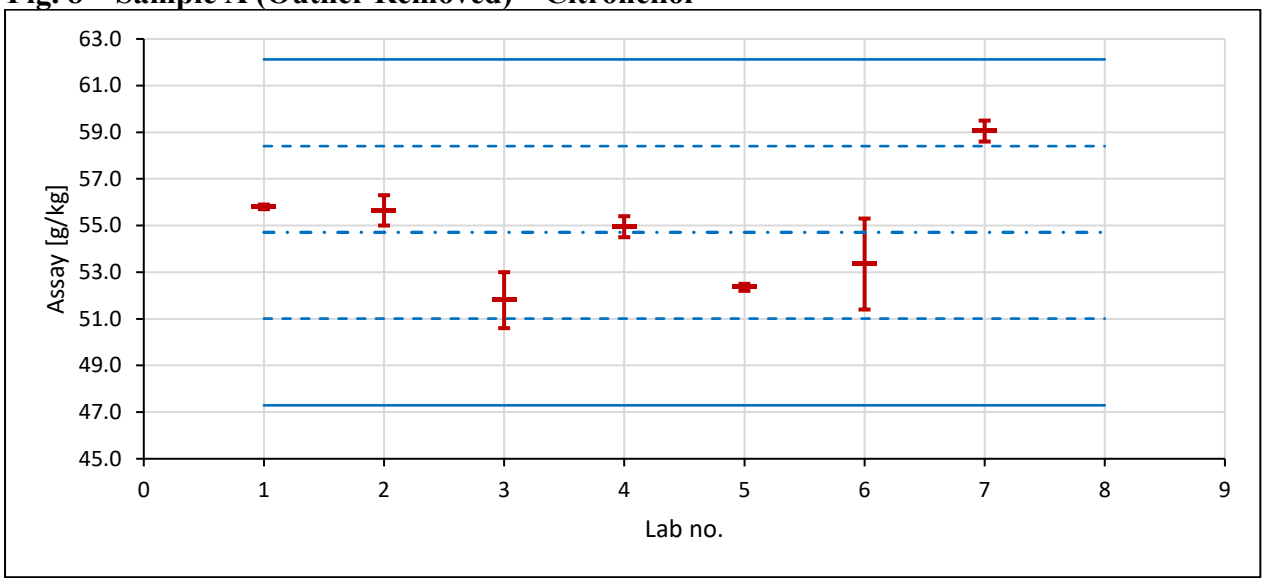
x_m = overall sample mean
n = number of laboratories
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
r = repeatability limit
R = reproducibility limit
RSD_r = relative repeatability standard deviation [%]
RSD_R = relative reproducibility standard deviation [%]
HorRat = $RSD_R/RSD_{R\ (Hor)}$ (Horwitz Ratio)

Fig. 7 – Sample A – Citronellol



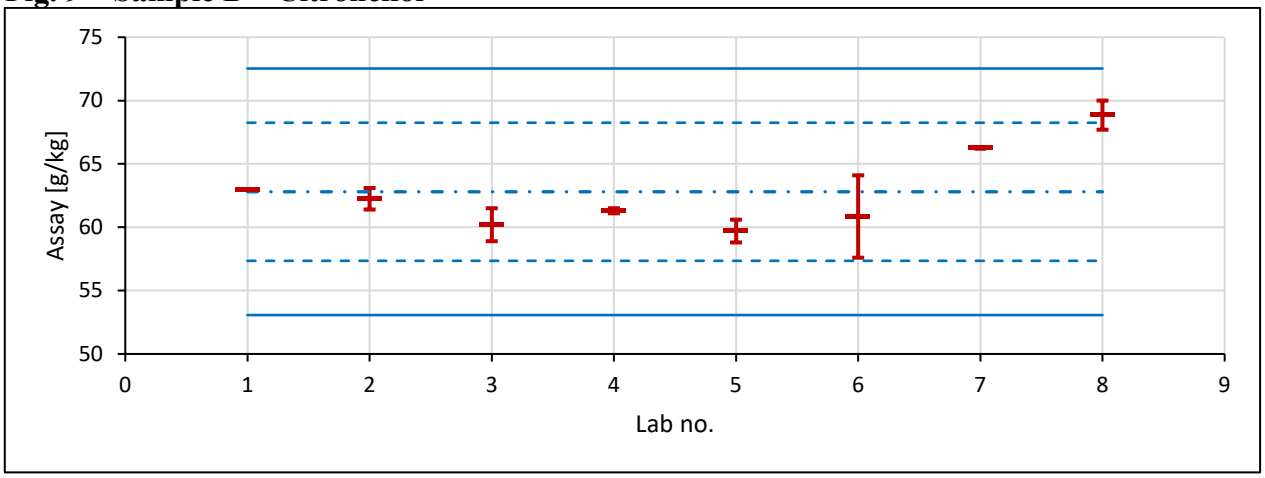
Mean value	56.42	RSD_R	9.62 %
s_r	1.25	$RSD_{R\ (Hor)}$	3.08 %
s_R	5.43	HorRat	3.12
Outlier (Grubbs)	Laboratory 8 – 68.40		

Fig. 8 – Sample A (Outlier Removed) – Citronellol



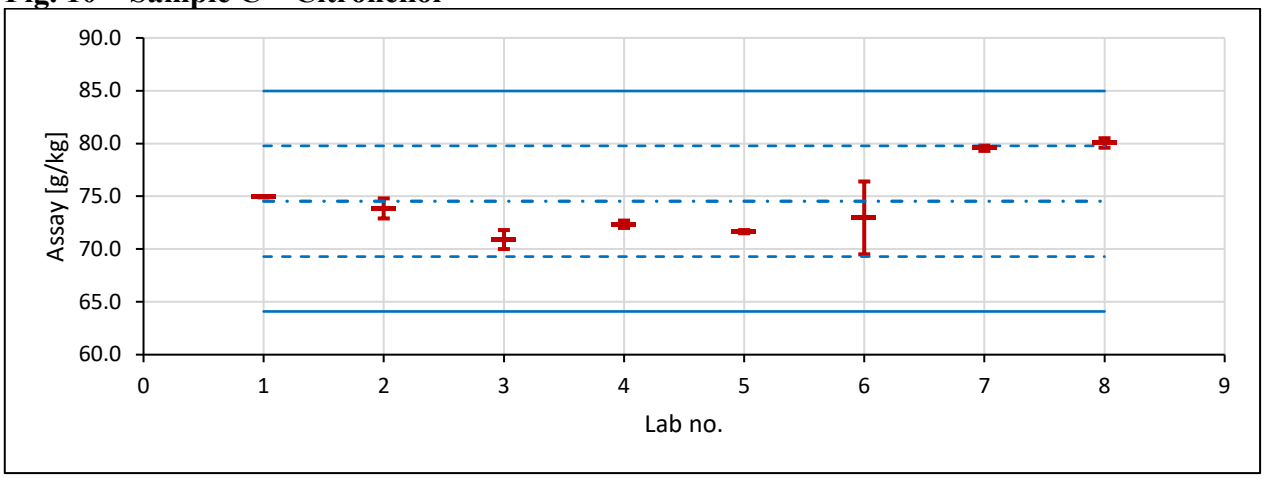
Mean value	54.71	RSD_R	4.84 %
s_r	1.32	$RSD_{R\ (Hor)}$	3.10 %
s_R	2.65	HorRat	1.56
Outlier (Grubbs)	removed		

Fig. 9 – Sample B – Citronellol



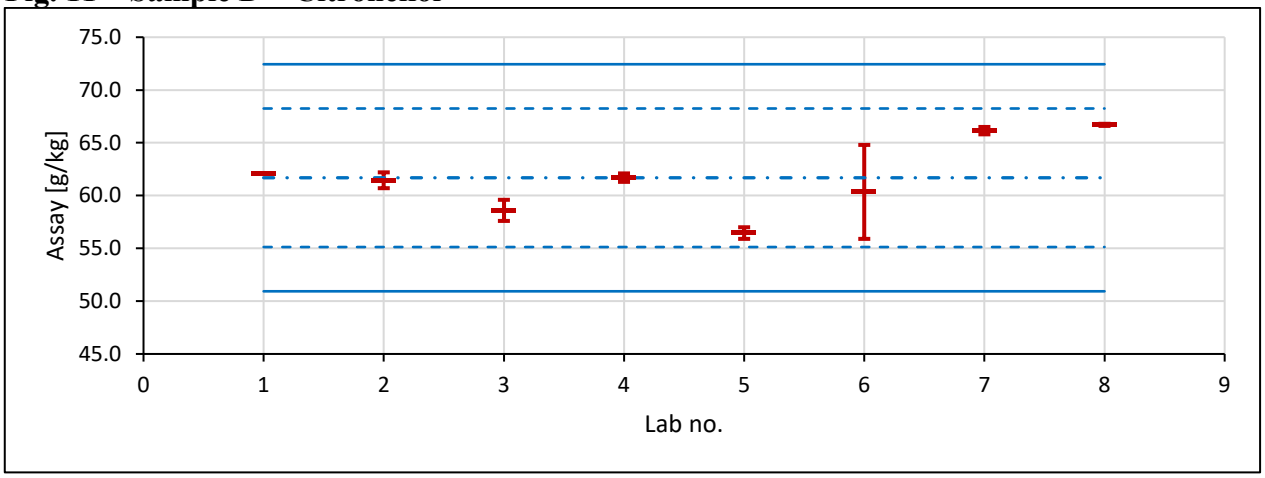
Mean value	62.80	RSD_R	5.53 %
s_r	1.95	$RSD_{R\ (Hor)}$	3.03 %
s_R	3.47	HorRat	1.82
Outlier (Grubbs)	none		

Fig. 10 – Sample C – Citronellol



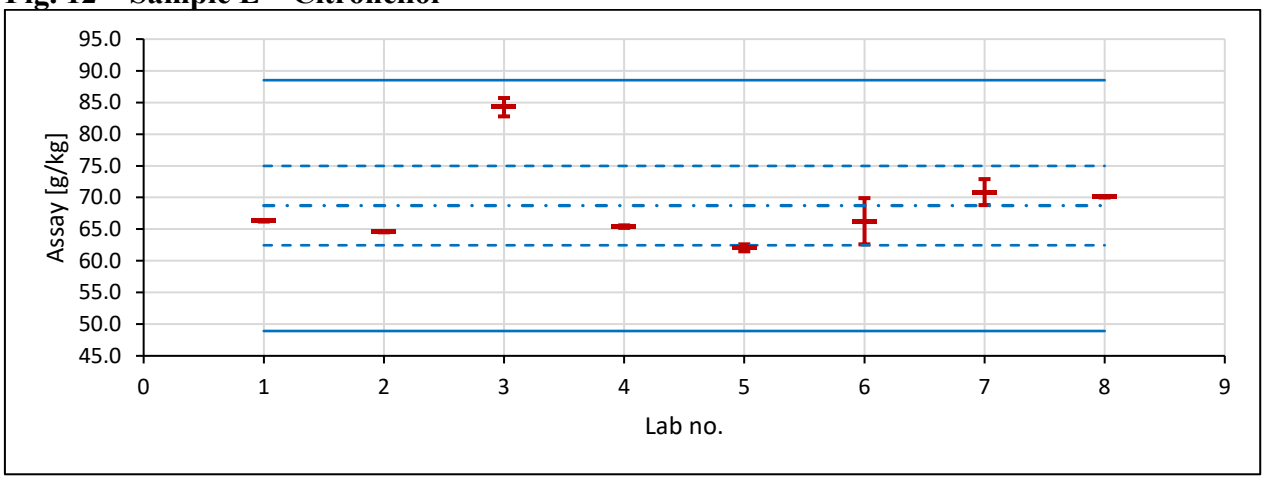
Mean value	74.53	RSD_R	5.00 %
s_r	1.87	$RSD_{R\ (Hor)}$	2.96 %
s_R	3.73	HorRat	1.69
Outlier (Grubbs)	none		

Fig. 11 – Sample D – Citronellol



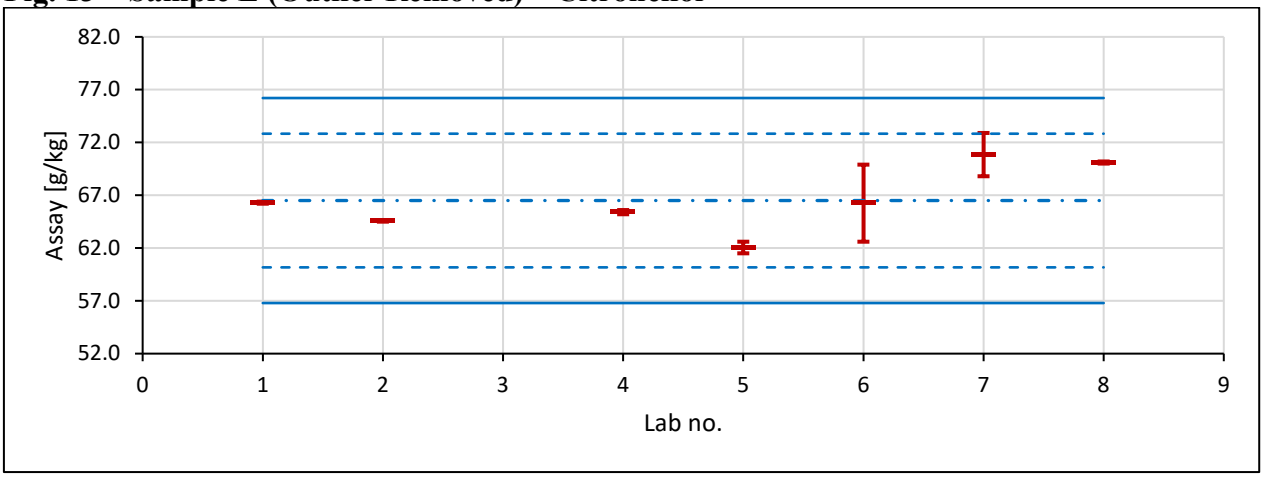
Mean value	61.69	RSD_R	6.23 %
s_r	2.34	$RSD_{R\ (Hor)}$	3.04 %
s_R	3.84	HorRat	2.05
Outlier (Grubbs)	none		

Fig. 12 – Sample E – Citronellol



Mean value	68.72	RSD_R	5.39 %
s_r	2.24	$RSD_{R\ (Hor)}$	2.10 %
s_R	38.39	HorRat	2.56
Outlier (Grubbs)	Laboratory 3 – 84.25		

Fig. 13 – Sample E (Outlier Removed) – Citronellol



Mean value	66.5	RSD_R	5.21 %
s_r	2.26	$RSD_{R\ (Hor)}$	3.01 %
s_R	3.47	HorRat	1.73
Outlier (Grubbs)	removed		

Determination of AI – Elimination of outliers

Table 7 – Summary of the statistical evaluation (without outliers)

	Sample A 033926/00524 (1 outlier removed)	Sample B 034212/00541	Sample C 036977/00589	Sample D 039167/00599	Sample E 039355/00605 (1 outlier removed)
x_m [g/kg]	54.71	62.80	74.53	61.69	66.50
x_m [% w/w]	5.47	6.28	7.45	6.17	66.5
n	7	8	8	8	7
s_r	1.32	1.95	1.87	2.34	2.26
s_R	2.65	3.47	3.73	3.04	3.47
r	3.70	5.45	5.24	6.56	6.33
R	7.42	9.73	10.44	10.75	9.71
RSD_R [%]	4.84	5.53	5.00	6.23	5.21
$RSD_{R\ (Hor)}$ [%]	3.10	3.03	2.96	3.04	3.01

HorRat	1.56	1.82	1.69	2.05	1.73

x_m = overall sample mean
n = number of laboratories
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
r = repeatability limit
R = reproducibility limit
RSD_r = relative repeatability standard deviation [%]
RSD_R = relative reproducibility standard deviation [%]
HorRat = $RSD_R/RSD_{R\ (Hor)}$ (Horwitz Ratio)

Determination of Isopulegol – Full set of 8 participants

Table 8 – Isopulegol Results (content in g/kg)

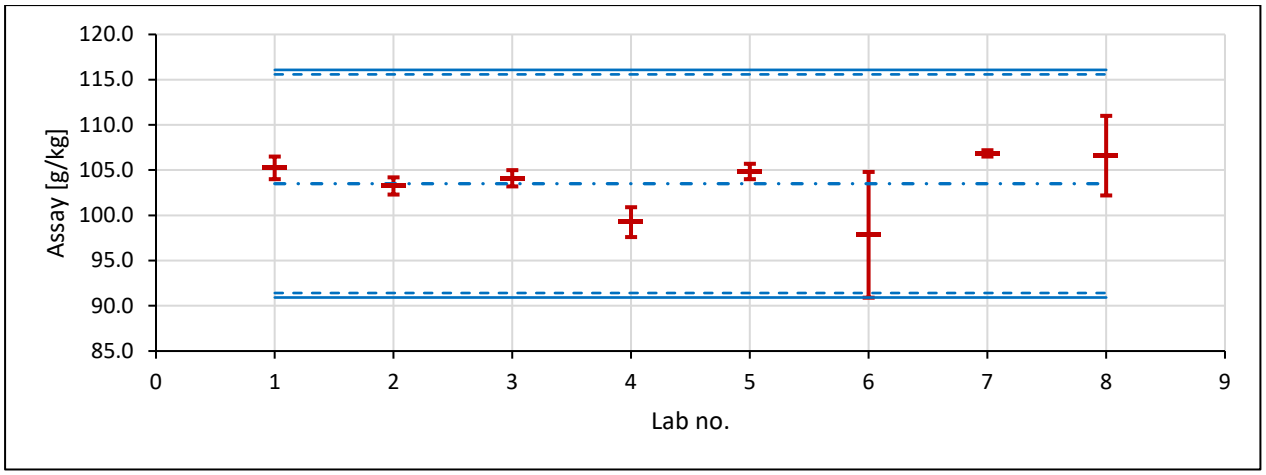
Laboratory	**Day**	**Sample A 033926/00524**		**Sample B 034212/00541**		**Sample C 036977/00589**		**Sample D 039167/00599**		**Sample E 039355/00605**	
1	1	104.0	105.3	103.6	104.9	108.9	109.7	117.1	117.8	108.0	109.0
	2	106.5		106.1		110.4		118.4		110.0	
2	1	102.3	103.3	101.0	102.1	106.2	107.3	113.8	115.0	105.6	105.2
	2	104.2		103.1		108.4		116.1		104.8	
3	1	103.2	104.1	103.1	105.0	109.5	109.5	117.6	118.2	138.0	140.5
	2	105.0		106.8		109.5		118.7		142.9	
4	1	100.9	99.3	99.3	97.9	103.0	102.5	114.2	112.4	105.5	104.0
	2	97.6		96.4		101.9		110.6		102.4	
5	1	104.0	104.9	105.0	105.0	107.3	108.1	113.2	114.0	106.5	107.7
	2	105.7		105.0		108.9		114.7		108.8	
6	1	90.9	97.9	91.4	98.1	95.3	102.7	103.7	110.6	94.5	101.6
	2	104.8		104.8		110.1		117.5		108.6	
7	1	107.2	106.9	105.7	105.7	113.2	113.1	120.5	120.3	114.7	111.9
	2	106.5		105.7		113.0		120.0		109.1	
8	1	111.0	106.6	101.4	101.1	109.2	107.7	117.0	115.4	108.3	106.3
	2	102.2		100.8		106.1		113.8		104.3	

Table 9 – Summary of the statistical evaluation

	Sample A 033926/00524	Sample B 034212/00541	Sample C 036977/00589	Sample D 039167/00599	Sample E 039355/00605
x_m [g/kg]	103.50	102.44	107.56	115.43	110.75
x_m [% w/w]	10.35	10.24	10.76	11.54	11.08
n	8	8	8	8	8
s_r	4.32	3.65	3.87	3.74	4.26
s_R	4.49	4.09	4.49	4.14	12.77
r	12.08	10.21	10.83	10.48	11.91
R	12.58	11.45	12.58	11.59	35.75
RSD_R [%]	4.34	3.99	4.18	3.59	11.53
$RSD_{R\ (Hor)}$ [%]	2.81	2.82	2.8	2.77	2.79
HorRat	1.54	1.42	1.49	1.30	4.14

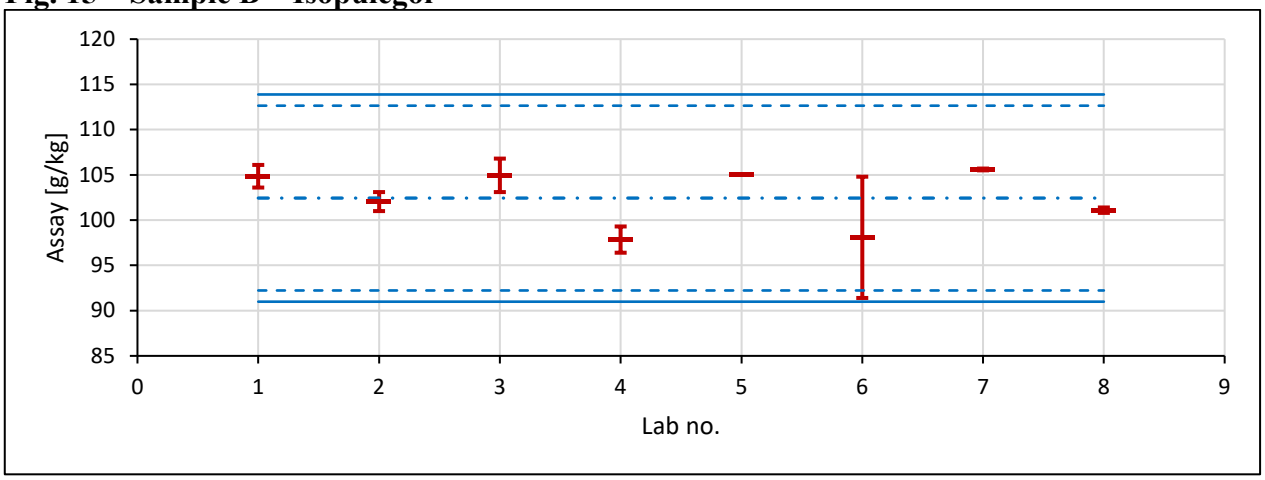
x_m = overall sample mean
n = number of laboratories
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
r = repeatability limit
R = reproducibility limit
RSD_r = relative repeatability standard deviation [%]
RSD_R = relative reproducibility standard deviation [%]
HorRat = $RSD_R/RSD_{R\ (Hor)}$ (Horwitz Ratio)

Fig. 14 – Sample A – Isopulegol



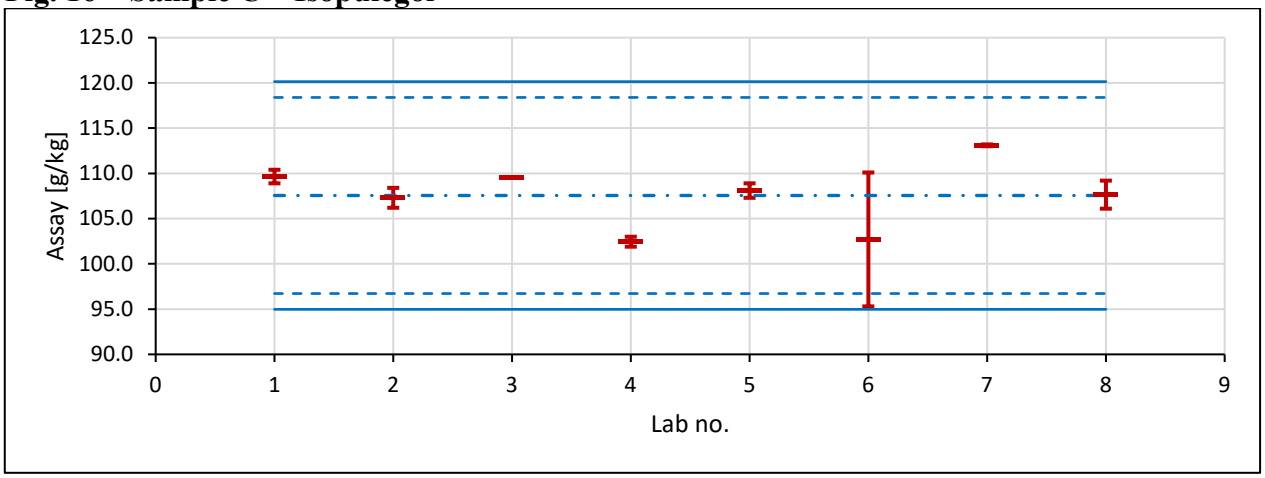
Mean value	103.50	RSD_R	4.34 %
s_r	4.32	$RSD_{R\ (Hor)}$	2.81 %
s_R	4.49	HorRat	1.54
Outlier (Grubbs)	none		

Fig. 15 – Sample B – Isopulegol



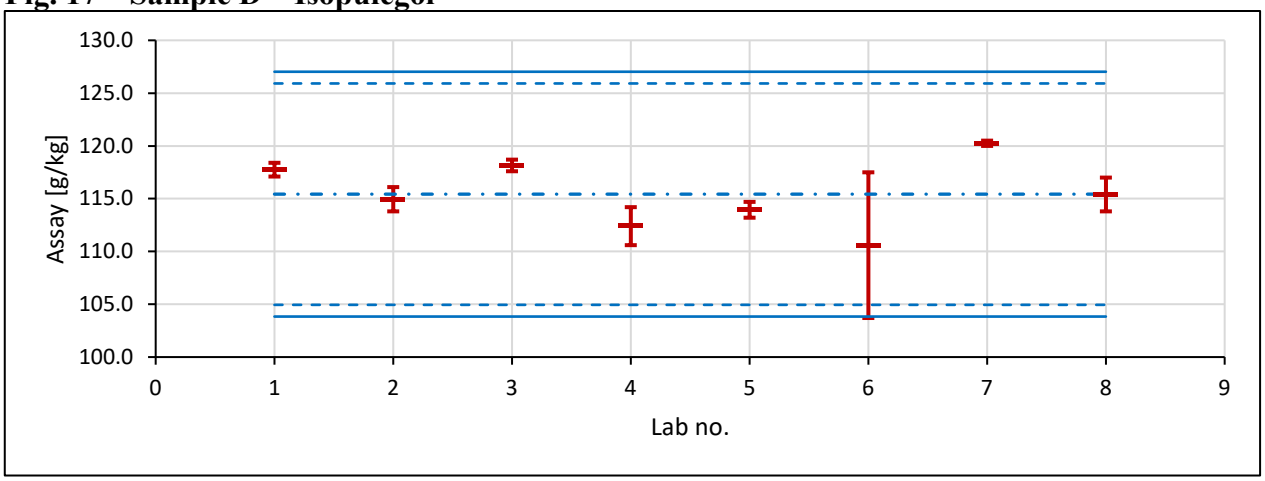
Mean value	102.44	RSD_R	3.99 %
s_r	3.65	$RSD_{R\ (Hor)}$	2.82 %
s_R	4.09	HorRat	1.42
Outlier (Grubbs)	none		

Fig. 16 – Sample C – Isopulegol



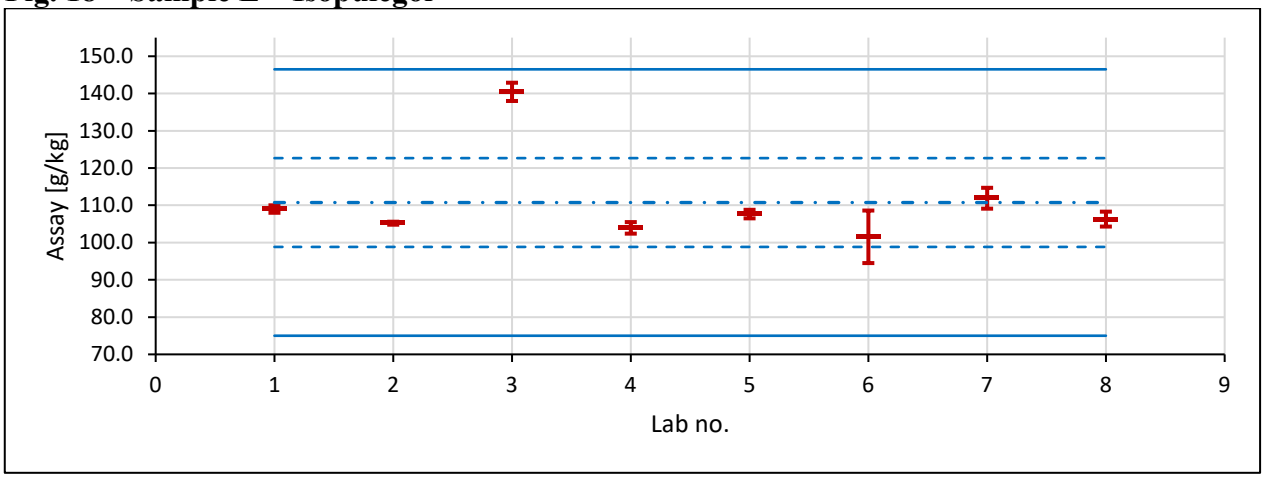
Mean value	107.6	RSD_R	4.18 %
s_r	3.87	$RSD_{R\ (Hor)}$	2.80 %
s_R	4.49	HorRat	1.49
Outlier (Grubbs)	none		

Fig. 17 – Sample D – Isopulegol



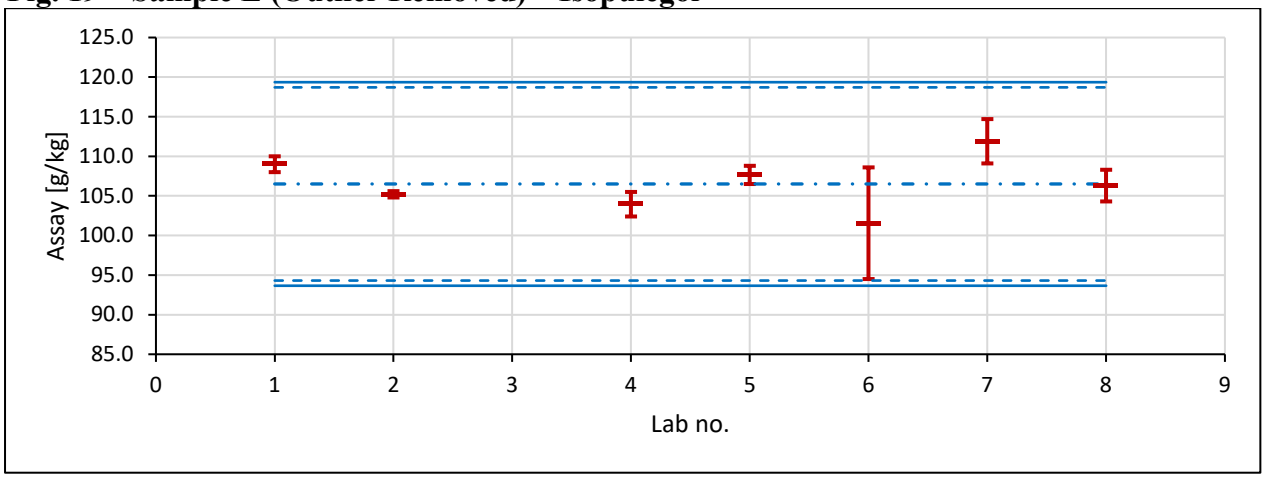
Mean value	115.43	RSD_R	3.59 %
s_r	3.74	$RSD_{R\ (Hor)}$	2.77 %
s_R	4.14	HorRat	1.30
Outlier (Grubbs)	none		

Fig. 18 – Sample E – Isopulegol



Mean value	110.75	RSD_R	11.53 %
s_r	4.26	$RSD_{R\ (Hor)}$	2.79 %
s_R	12.77	HorRat	4.14
Outlier (Grubbs)	Laboratory 3 – 140.45		

Fig. 19 – Sample E (Outlier Removed) – Isopulegol



Mean value	106.51	RSD_R	4.31 %
s_r	4.36	$RSD_{R\ (Hor)}$	2.80 %
s_R	4.59	HorRat	1.54
Outlier (Grubbs)	removed		

Determination of AI – Elimination of outliers

Table 10 – Summary of the statistical evaluation (without outliers)

	Sample A 033926/00524	Sample B 034212/00541	Sample C 036977/00589	Sample D 039167/00599	Sample E 039355/00605 (1 outlier removed)
x_m [g/kg]	103.50	102.44	107.56	115.43	106.51
x_m [% w/w]	10.35	10.24	10.76	11.54	10.65
n	8	8	8	8	7
s_r	4.32	3.65	3.87	3.74	4.36
s_R	4.49	4.09	4.49	4.14	4.59
r	12.08	10.21	10.83	10.48	12.20
R	12.58	11.45	12.58	11.59	12.85
RSD_R [%]	4.34	3.99	4.18	3.59	4.31
$RSD_{R\ (Hor)}$ [%]	2.81	2.82	2.8	2.77	2.80
HorRat	1.54	1.42	1.49	1.30	1.54

x_m = overall sample mean
n = number of laboratories
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
r = repeatability limit
R = reproducibility limit
RSD_r = relative repeatability standard deviation [%]
RSD_R = relative reproducibility standard deviation [%]
HorRat = $RSD_R/RSD_{R\ (Hor)}$ (Horwitz Ratio)

7. Conclusions

In total, 8 laboratories across Asia and Europe participated in the collaborative study, all 8 laboratories came back in time and provided results. The data sets from all these laboratories have been considered for the statistical evaluation (Figures 1 to 19 and Tables 1 to 10).

For p-menthane-3,8-diol and isopulegol the reported values contained in this document have been summed and reflect the total content including all isomers. The individual results have been calculated and will be referred to for isomer content discussions but in the interest of clarity have not been included in this report.

p-Menthane-3,8-diol (PMD)

For Sample E one laboratory was eliminated as an outlier. This result contained a comparatively low amount of PMD at 625g/kg compared to the average value of 724g/kg. Looking at the individual PMD isomer content of this batch, the cis and trans PMD isomers are roughly equal in content. This is typical of EC Oil (H/C) that has not been sufficiently homogenized. cis-PMD crystallizes preferentially and should be approximately twice the content of the trans-PMD, a low ratio usually means solid cis-PMD is present, and the sample has not fully homogenized. This has two effects, a reduced PMD content and the other constituents are overstated. This is reflected in the isopulegol and citronellol results for sample E. These have also been identified as outliers and should be removed from the final results.

Post removal of the outlier value for sample E, the HorRat values for all samples spans the range of 0.85-1.27 (Table 3). While some results are above the ideal value of <1, sample analysis of EC Oil (H/C) is difficult for several reasons. It is a UVCB with a natural origin and as such a difficult matrix, there is inherent variability and potential for interference. In addition to the matrix difficulties, the total value of PMD is the summation of four separate isomers calculated independently and one isomer of PMD contained within EC Oil (H/C) will crystallize, leading potentially to inhomogeneity.

Regarding the isomer ratios all values found were within the proposed specification with an average of 64% cis-PMD, 31% trans-PMD and the remaining isomers making up 2%, see table 4. It has been noted that for one laboratory the isomer ratio was increased and more cis-PMD was detected with a corresponding reduction in the trans-PMD isomer. However, the total PMD was within expected ranges and no cause for the possible epimerization could be found. It was theorized that this may be due to the difference in carrier gas or potential pH changes however another laboratory used the same carrier gas with an expected result and the EC Oil (H/C) is not particularly pH sensitive or prone to dissociation.

Isopulegol

For Sample E one laboratory was eliminated as an outlier due to inhomogeneity as discussed in the PMD section.

Post removal of the outlier value for sample E, the HorRat values for all samples spans the range of 1.30-1.54 (Table 6). Similar to PMD, the results are above ideal values but within the acceptable range considering the complexity of analysing naturally derived materials. As with PMD this figure is a sum of three separate isomers.

Citronellol

For Samples A & E two laboratories were eliminated as outliers. One laboratory was eliminated due to the homogeneity issue as discussed in the PMD section; the other laboratory reported a much higher value than expected.

Post removal of the outlier values, the HorRat values for all samples spans the range of 1.56-2.05. Similar to PMD, the results are above ideal values but within the acceptable range considering the complexity of analysing naturally derived materials, with the exception of one value for Sample D at 2.05. This is above the average value of 1.8 and is unexpected as no outliers were found in this batch. There is no obvious reason for the elevated ratio and while variation was seen between the different days analysis, it is not sufficient to disregard any results. Citronellol is present at the lowest level of the three main constituents and is present at levels between 20g/kg and 110g/kg. For Sample D recorded results were between 56.5/kg and 66.7g/kg, a variation of approximately 10g/kg, this content will further be reduced upon dilution to usage levels of EC Oil (H/C). While in this case the value has fallen slightly outside of the acceptable range, this method is derived from effectively identical methods that have been validated according to SANCO 3099, used to support GLP studies for submission to regulatory authorities including ECHA and as analytical enforcement methods by the EPA. For this reason, we believe that despite the anomalous result in one sample for a single constituent the method should be considered robust.

The data presented in the statistical summary show that the method is suitable to obtain acceptable and reproducible results for all samples tested and is therefore regarded to be robust. The requirement of the CIPAC guideline that accepted results were provided by not less than 8 laboratories coming from at least 5 countries of 2 continents is fulfilled.

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Citrefine International Ltd. considers this method to be suitable for the intended purpose, without further changes, and recommends accepting it as a **provisional CIPAC method** for the determination of AI in technical material.