ION LABS	Standard Operating Procedure **Determination of β-Caryophyllene by GC/FID**	**SOP Number** **D-779**	**Revision** **1**
		Effective Date 07/21/22	**Page** **Page 1 of 5**

Written by/ Date	**Reviewed by/ Date**	**Approved by/ Date**
07/21/22	SAS 07/21/22	07/21/22
Title: Quality Systems Manager	**Title: Analytical Development Scientist**	**Title: QC Laboratory Director**

1.0 Purpose

The purpose of this procedure is to define the method for the quantification and/or identification of β-Caryophyllene in raw materials and finished products by GC using flame ionization detection.

2.0 Scope

This procedure applies to the quantification and identification of β-Caryophyllene in raw materials and finished products by the QC laboratory at ION Labs.

3.0 Responsibility

3.1 It is the responsibility of QC Chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to ensure that this procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **GC** – Gas Chromatography

4.2 **FID** – Flame Ionization Detection

4.3 **CofA** – Certificate of Analysis

4.4 **BCP** – β-Caryophyllene

4.5 **IPA** – Isopropanol

5.0 References

5.1 MV-LAB-19-042, Protocol, Validation of a Method for the Determination of β-Caryophyllene and α-Humulene by GC-FID

6.0 Reagents, Supplies Glassware and Equipment

6.1 Chemicals: All reagents are GC grade or better.

6.1.1 BCP

6.1.2 IPA

6.2 Compressed Gases

6.2.1 Hydrogen

6.2.2 Helium

6.2.3 Air

6.2.4 Nitrogen

6.3 Glassware

6.3.1 Volumetric glassware as required by standard and sample preparations

6.4 Equipment

6.4.1 Agilent 7890 GC

6.4.2 Analytical Balance

7.0 GC Conditions

7.1 Column: Agilent HP-5, 30 m x 0.32 mm x 0.25 µm or equivalent

7.2 Inlet Liner: Restek, 4.0 mm ID x 6.3 mm OD x 78.5 mm length straight liner with glass wool or equivalent

7.3 Injector Temp: 160 °C

7.4 Detector Temp: 260 °C

7.5 Equil Time: 0.5 min

7.6 Flow Rate: 1.2 mL/min

7.7 Run Time: 13.4 min

7.8 Split ratio: 20:1

7.9 Septum purge: 2 mL/min

7.10 Air flow: 400 mL/min

7.11 Hydrogen flow: 40 mL/min

7.12 Makeup flow: 25 mL/min (column + makeup = constant)

7.13 Temperature Ramp:

Ramp Rate (°C/min)	Temp (°C)	Hold Time (min)
N/A	60	0.3
50	130	0
1.5	143	0
50	245	1

8.0 Standard Preparation

8.1 Use the actual purity from the CofA or the standard certification for the reference material in calculations.

8.2 Stock Standard: Use a pipet to transfer about 250 mg of BCP directly into a 100-mL volumetric flask. Dilute to volume with IPA and mix well.

8.3 Working Standard: Transfer 2.0-mL of Stock Standard to a 100-mL volumetric flask. Dilute to volume with IPA and mix well.

8.4 Alternatively, a commercially pre-prepared Stock Standard may be used. Alternative dilution schemes may be used.

9.0 Sample Preparation

9.1 The validated linear range of the method is 2 µg/mL – 200 µg/mL. The BCP content of the sample preparation must be within the linear range.

9.2 Place a suitably sized volumetric flask of no less than 25 mL on the balance and press Tare. Based on the label claim, weigh no less than 250 mg of sample directly into the volumetric flask to generate a concentration of BCP that is within the linear range of the method. Record the sample weight. Dilute to volume with IPA and mix thoroughly. Perform further dilutions as required using IPA.

9.3 For raw materials or finished products being analyzed for the first time using this method, in-process verification is required to demonstrate specificity and extraction efficiency before the method can be implemented.

10.0 Recommended Sequence

10.1 Make 2 injections of the Blank (IPA).

10.2 Make five (5) injections of the Working Standard.

10.3 Make a single injection of each Sample Preparation.

10.4 Make a single injection of the Working Standard after every ten (10) sample injections and at the end of a run.

11.0 System Suitability Requirements

11.1 The %RSD of the first five (5) standard injections is NMT 3.0%.

11.2 The %RSD of all standard injections is NMT 3%.

11.3 No significant (>0.5%) interfering peaks are present in the blank (IPA) injection.

12.0 Retention Times

12.1 The retention time of BCP is about 9.7 min.

13.0 Calculations

13.1 Calculation for determining finished product % label or raw material % purity

13.1.1 $$\%\ \text{assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{Spl_{wt}} \times \frac{100}{LA}$$

R_u Sample peak area

R_s Mean working standard peak area

Wt_{std}	Weight of reference standard in mg (correct for moisture if required)
V_{std}	Volume of the standard preparation accounting for dilutions in mL
P	Purity of the reference standard in percent
Spl_{wt}	Sample weight in mg
V_{spl}	Volume of the sample preparation accounting for dilutions in mL
LA	Label amount in percent (use 100 for raw materials)

14.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	06/26/19	New procedure.	N/A	S. Sassman
1	07/21/22	Scheduled review: updated logo and format.	CC-22-0292	K. Burris