	Standard Operating Procedure		SOP Number D-1004	Revision 0
	Determination of Squalene by GC-FID		Effective Date 04/25/22	Page 1 of 6
Written by/ Date Step S 02/16/22		Reviewed by/ Date Am Oshper		Approved by/ Date SSD 02/23/22
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1.0 Purpose

The purpose of this procedure is to define the method for the determination of squalene in raw materials and finished products by GC-FID.

2.0 Scope

This procedure applies to the determination of squalene in raw materials and finished products in 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/Analytical Development to keep procedure aligned with current practices.

4.0 Definitions

- 4.1 **QC** – Quality Control
- 4.2 **GC** – Gas Chromatography
- 4.3 **FID** – Flame Ionization Detection
- 4.4 **CoA** – Certificate of Analysis
- 4.5 **TMP** – 2,2,4-trimethylpentane

5.0 References

- 5.1 PRTCL-21-0059, Protocol, Validation of an Analytical Method for the Determination of Squalene by GC-FID

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6.0 Supplies

6.1 Chemicals

- 6.1.1 Squalene reference standard (>95%)
- 6.1.2 Methyl tricosanoate reference standard (>97%)
- 6.1.3 TMP (GC grade or better)

6.2 Compressed Gases (use ultra-high purity gases)

- 6.2.1 Hydrogen
- 6.2.2 Helium
- 6.2.3 Air
- 6.2.4 Nitrogen

6.3 Supplies and Glassware

- 6.3.1 Disposable glass transfer pipet
- 6.3.2 Volumetric glassware as required for standard and sample preparation

6.4 Equipment

- 6.4.1 Agilent 7890 GC with FID detector
- 6.4.2 Analytical Balance

7.0 GC Conditions

- 7.1 Column: Agilent HP-5, 30 m x 0.32 mm x 0.25 μ m 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: 300 °C
- 7.4 Detector Temp: 320 °C
- 7.5 Equilibration Time: 0.5 min
- 7.6 Flow Rate: 1.8 mL/min
- 7.7 Run Time: 16 min

- 7.8 Split ratio: 50:1
- 7.9 Septum purge: 3 mL/min
- 7.10 Air flow: 350 mL/min
- 7.11 Hydrogen flow: 30 mL/min
- 7.12 Makeup flow: 30 mL/min (column + makeup = constant)
- 7.13 Injection Volume 1 µL
- 7.14 Injection Type Standard
- 7.15 Plunger Speed Fast
- 7.16 Wash Solvent TMP
- 7.17 Temperature Ramp (Raw Materials and System Suitability Solution)

Ramp Rate (°C/min)	Temp (°C)	Hold Time (min)
N/A	150	0
10	300	1

8.0 Internal Standard Solution Preparation

- 8.1 Accurately weigh and transfer about 75 mg of methyl tricosanoate into a suitable container.
- 8.2 Add 250 mL of TMP, and mix to dissolve.

9.0 Standard Preparation

- 9.1 **Use glass pipets for transfers and dilutions. Squalene may absorb to plastics, and TMP is not compatible with automatic pipets.**
- 9.2 Place a 20-mL scintillation vial on the balance and press TARE.
- 9.3 Accurately weigh about 30 mg of squalene reference standard into the vial.
- 9.4 Use small portions of *Internal Standard Solution* to dissolve and quantitatively transfer the reference standard into a 100-mL volumetric flask.

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9.5 Dilute to volume with *Internal Standard Solution*.

10.0 Sample Preparation

10.1 The linear range of the method is 0.1 – 0.5 mg/mL. The content of the sample preparation must be within the linear range.

10.2 For finished products, pool at least 10 dosage units. Based on the dosage weight (for powders), fill weight (for capsules) or tablet weight and the label claim, weigh no less than 50 mg of the pooled dosages into a suitably sized volumetric flask to generate an analyte concentration that is within the linear range. For viscous oils, weigh the sample into a vial and use small portions of *Internal Standard Solution* to quantitatively transfer the sample to the volumetric flask. Add *Internal Standard Solution* to about 2/3 of the flask volume and sonicate for 5 minutes, shake for 20 minutes, and dilute to volume with *Internal Standard Solution*.

10.3 For raw materials, based on the expected potency, weigh no less than 25 mg of sample into a suitably sized volumetric flask to generate an analyte concentration that is within the validated linear range. For viscous oils, weigh the sample into a vial and use small portions of *Internal Standard Solution* to quantitatively transfer the sample to the volumetric flask. Add *Internal Standard Solution* to about 2/3 of the flask volume and sonicate for 5 minutes. Shake for 20 minutes, and dilute to volume with *Internal Standard Solution*.

10.4 If particulates remain in sample preparations, remove them by allowing the particulates to settle.

11.0 Recommended Sequence

11.1 Make two injections of *Internal Standard Solution*.

11.2 Make five injections of *Working Standard*.

11.3 Make a single injection of each *Sample Preparation*.

11.4 After every six samples and at the end of the run, make a single injection of *Working Standard*.

12.0 System Suitability Requirements

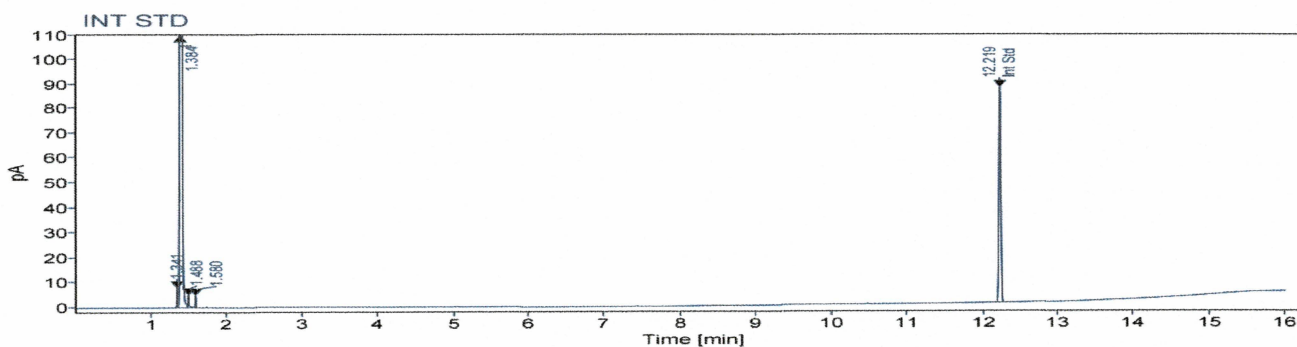
- 12.1 No significant (>0.5%) interfering peaks are present in the blank (Diluent) injection.
- 12.2 The %RSD of the peak area ratio in five consecutive injections of the *Working Standard* is NMT 5.0%.
- 12.3 The %RSD of all injections of the *Working Standard* is NMT 5%.

13.0 Retention Times

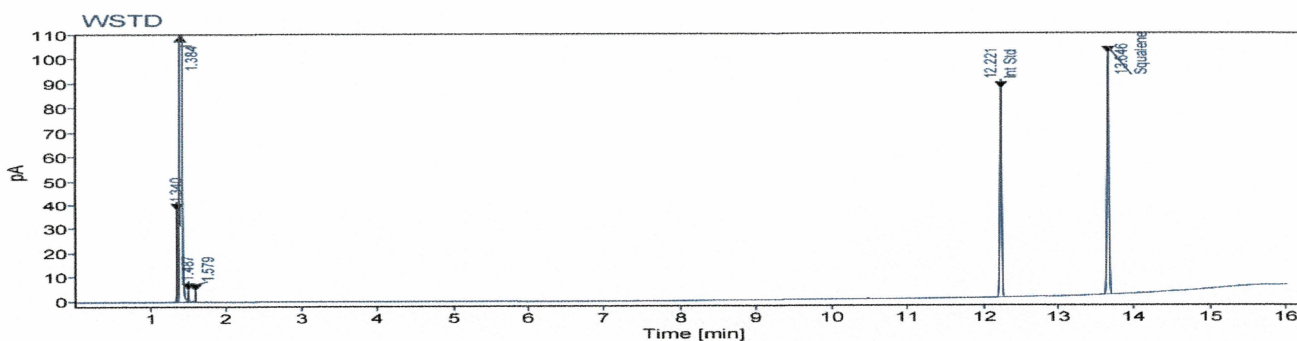
- 13.1 Methyl Tricosanoate (internal standard) \approx 12.2 min
- 13.2 Squalene \approx 13.7 min

14.0 Example Chromatograms

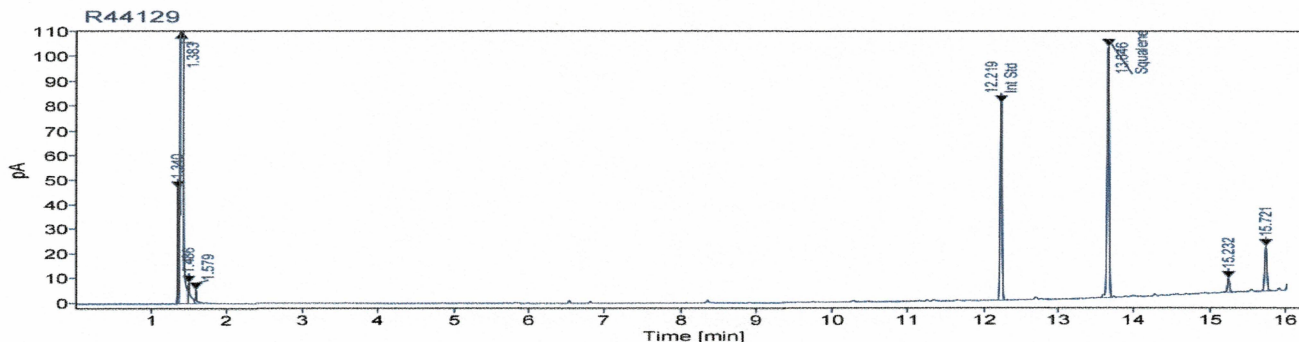
14.1 Blank (Internal Standard Solution)



14.2 Working Standard



14.3 Raw Material Sample



15.0 Example Calculation

$$\% \text{ Label (FP) or } \% \text{ Assay (RM)} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{Spl_{wt}} \times \frac{FW}{LA} \times 100$$

R_u Sample peak area ratio

R_s Mean Working Standard peak area ratio (5 injections)

Wt_{std} Weight of reference standard used to prepare Working Standard (mg)

P Purity of reference standard from the CoA (% w/w)

V_{std} Volume of Working Standard (mL)

V_{spl} Volume of Sample Solution including dilutions (mL)

Spl_{wt} Sample weight (mg)

FW Theoretical fill/tablet weight (mg, use 1 for raw materials)

LA Label amount (mg, use 1 for raw materials)

16.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	02/16/22	New procedure.	N/A	S. Sassman