**Supporting data**

# Quantitative estimations of Squalene levels in acute respiratory distress syndrome (ARDS) and treated lung tissues by Gas chromatography–Mass spectrometry

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**Table S1:** EI-MS data of squalene and squalane.

| Name of the compound | Quantifier ions (*m/z*) (relative abundance in %) | Other ions (*m/z*) (relative abundance in %) |
|---|---|---|
| squalene | 69(99) | 81(54), 41(26), 95(15), 137(12), 121(12) |
| squalane | 71(78) | 57(99), 85(44), 43(30), 113(28), 127(16) |

**Table S2:** Squalene extraction data (1µg/mL, n=3) obtained from the lung tissue.

| Solvent | Spiked Concentration (µg/mL) | Back- concentration (µg/mL) SD; RSD (%) | Recovery (%) |
|---|---|---|---|
| ethyl acetate | 1 | 0.887386856±0.006;4.2 | 88 |
| hexane | 1 | 1.0661457±0.017;9.7 | 106 |
| methanol | 1 | 0.604511999±0.05;6.2 | 60 |
| chloroform | 1 | 0.703571449±0.02;17.7 | 70 |
| PBS-buffer | 1 | 0.6295603±0.02;2.1 | 62 |

**Table S3:** GC-MS linearity parameters of Squalene.

| S.No | Compound | Linearity equation | $R^{2}$ a) | Linearity range | LOD b) | LOQ c) | Validation accuracy (% RSD) d) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Low (0.5 µg/mL) | Middle (2µg/mL) | High (10 µg/mL) |
| 1 | Squalene | Y = 0.1786x - 0.0114 | 0.996 | 0.5-10 | 0.05 | 0.5 | 115 (3.8) | 95 (4.2) | 94 (3.3) |

*a) Coefficient of Determination, b) LOD – Limit of Detection, c) LOQ – Limit of quantification, d) The values in the parentheses represent %RSD valves (n=3).*

**Table S4:** GC-MS intra-day and inter-day precision studies (n=3) of Squalene obtained from the lung tissue.

| **Concentration (µg/mL)** | **Intra-day Precision** | **Inter-day Precision** |
|---|---|---|
| | **observed concentration (µg/mL) SD; RSD (%)** | **observed concentration (µg/mL) SD; RSD (%)** |
| 0.5 | 0.58±0.011;13.43 | 0.55±0.004;5.45 |
| 2 | 2.01±0.009;2.76 | 2.26±0.051;12.84 |
| 10 | 9.91±0.007;0.39 | 10.44±0.074;3.38 |

**Table S5.** GC-MS robustness study of Squalene.

| **Concentration (µg/ml)** | **Flow variations (SD±%RSD)** | | | **Oven temperature variations ($^{0}$C/min) (SD±%RSD)** | | |
|---|---|---|---|---|---|---|
| | 1.1 mL/min (minus) | 1.2 mL/min (Control) | 1.3 mL/min (plus) | 95 $^{0}$C/min (minus) | 100 $^{0}$C/min (Control) | 105 $^{0}$C/min (plus) |
| 0.5 | 0.004±4.67 | 0.002±2.45 | 0.003±4.01 | 0.006±5.02 | 0.011±11.09 | 0.015±1.81 |
| 2 | 0.024±6.68 | 0.023±6.85 | 0.027±7.28 | 0.051±14.67 | 0.026±7.57 | 0.018±6.10 |
| 10 | 0.037±2.13 | 0.032±1.81 | 0.037±2.13 | 0.048±2.67 | 0.180±9.03 | 0.028±10.27 |

**Table S6:** Recovery data of Squalene (n=3) obtained from the lung tissue.

| **Spiked Concentration (µg/mL)** | **Calculated spiked concentration (µg/mL) SD; RSD (%)** | **Recovery (%)** |
|---|---|---|
| 0.5 | 0.57 ±0.003;3.83 | 115 |
| 2 | 1.91±0.014;4.22 | 95 |
| 10 | 9.41±0.058;3.37 | 94 |

**Table S7:** Observed concentrations of Squalene (n=3) obtained from the lung tissue by linearity graph.

| | **Observed conc. (µg/mL)** | **SD** | **%RSD** |
|---|---|---|---|
| Control | 1.511416206 | 0.011693 | 4.52 |
| | 1.929371287 | 0.015964 | 4.74 |
| | 1.664174188 | 0.041845 | 14.59 |
| | 1.508391472 | 0.025849 | 10.02 |
| | 1.603274766 | 0.030336 | 11.01 |
| | 1.452444932 | 0.013273 | 5.36 |
| | 1.46437065 | 0.017643 | 7.07 |
| | 1.487494785 | 0.006773 | 2.67 |
| Disease | 0.83763504 | 0.004 | 3.01 |
| | 0.796840248 | 0.002944 | 2.35 |
| | 0.963312673 | 0.00686 | 4.38 |
| | 0.780327621 | 0.003989 | 3.26 |

| | | | |
|---|---|---|---|
| | 0.795925217 | 0.004271 | 3.41 |
| | 0.433416462 | 0.003343 | 5.78 |
| | 1.689737809 | 0.016059 | 15.21 |
| | 0.780863288 | 0.003265 | 2.66 |
| Treatment (HAEGS-125 mg/kg) | 0.991814855 | 0.017614 | 10.89 |
| | 0.842386408 | 0.004449 | 3.32 |
| | 0.885240558 | 0.005764 | 4.06 |
| | 0.970826626 | 0.004647 | 2.94 |
| | 0.888033606 | 0.005348 | 3.75 |
| | 0.761918171 | 0.004568 | 3.84 |
| | 1.12412573 | 0.008063 | 4.36 |
| | 0.794598865 | 0.00117157 | 0.95 |
| Treatment (HAEGS-250 mg/kg) | 1.425791013 | 0.015967 | 6.58 |
| | 1.49379667 | 0.010291 | 4.03 |
| | 1.487494785 | 0.006773 | 2.67 |
| | 1.043229402 | 0.01523 | 8.89 |
| | 1.254027438 | 0.012118 | 5.76 |
| | 1.420774808 | 0.00792 | 3.28 |
| | 1.067762695 | 0.022905 | 13.02 |
| | 0.963312673 | 0.00686 | 4.38 |

**Table S8:** Comparison studies of limits of detection (LODs), quantification (LOQs), and recovery (%) found in the previous studies using different extraction techniques in different matrices.

| **S.No.** | **Analyte** | **Matrix** | **Extraction Technique** | **Analysis** | **LOD (**µg/mL**)** | **LOQ (**µg/mL**)** | **Recovery (%)** | Year, Reference |
|---|---|---|---|---|---|---|---|---|
| 1. | SQ | formulations of anthrax vaccine adsorbed | Hexane | HPLC | 0.14 | 0.5 | 88-95 | 2002[1] |
| 2. | SQ | Human hair | Acidic/basic hydrolysis followed by hexane extraction | GC-MS, Raman | 0.1 | 1 | 96.4± 1.46 | 2016[2] |
| 3. | SQ | shark liver oils | Hexane: ethanol (4:1) | IR, Raman, ( | -- | 0.4 | 82-111 | 2016[3] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | (v:v). | MS | | | | |
| 4. | SQ, FAME | fish oil, edible oils | Hexane | GC-FID | 0.4 | 1.3 | 95-105 | 2019[4] |
| 5. | SQ | Olive oil | Hexane: methanol (4:1) (v:v). | GC-MS, FID | 3 | 8 | 98± 3 | 2020[5] |
| 6. | SQ, phytocomponents | Clerodendrum serratum Linn roots | Ethanolic extract | GC-MS, GC-FID | 0.79 | 1.89 | 96-101 | 2021[6] |
| 7. | SQ, SQA | e-cigarette or vaping, products (EVPs) | hexane | GC-MS | 0.25 | -- | 104–113 | 2022[7] |
| 8. | SQ, SQA | Bronchoalveolar Lavage Fluid | hexane | GC-MS | 0.3 | 1 µ | 97–105 | 2022[8] |
| 9. | SQ | edible vegetable oil | Methanol:hexane | GC-MS | 0.013 | 0.04 | 102-105 | 2022[9] |
| 10. | SQ, phytocomponents | coldpressed seed oil of Celastrus paniculatus | Hexane | HPTLC | 13.84 (ng/band) | 41.95 (ng/band) | 90-110 | 2024[10] |
| 11 | squalene, cholesterol and seven COPs | Oxidation Products in Food of Animal Origin | saponification, extraction and silylation | GC-TQ | 0.08 | 0.25 | 89-92 | 2024[11] |

**Abbrivations:** SQ-squalene, SQA-squalane, HPLC- High-Performance Liquid Chromatography, GC-MS- Gas Chromatography-Mass Spectrometry, GC-FID-Gas Chromatography with Flame Ionization Detection, FAME- Fatty acid methyl esters, HPTLC-High-Performance Thin Layer Chromatography, COPs -cholesterol oxidation products.

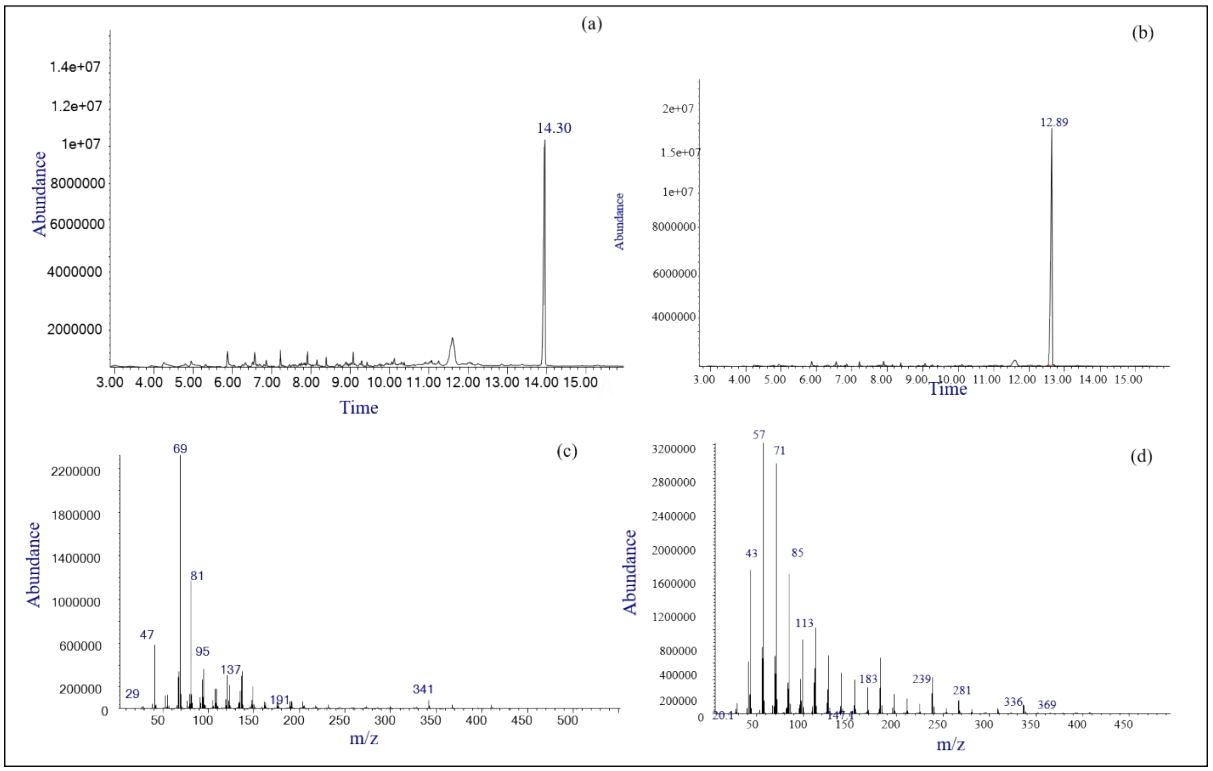


**Figure S1:** a) Total ion chromatogram (TIC) of squalene (Rt=14.30) at 50 µg/mL, b) Total ion chromatogram (TIC) of squalane (Rt= 12.89) at 50 µg/mL, c) EI-MS spectrum of squalene and d) EI-MS spectrum of squalane.

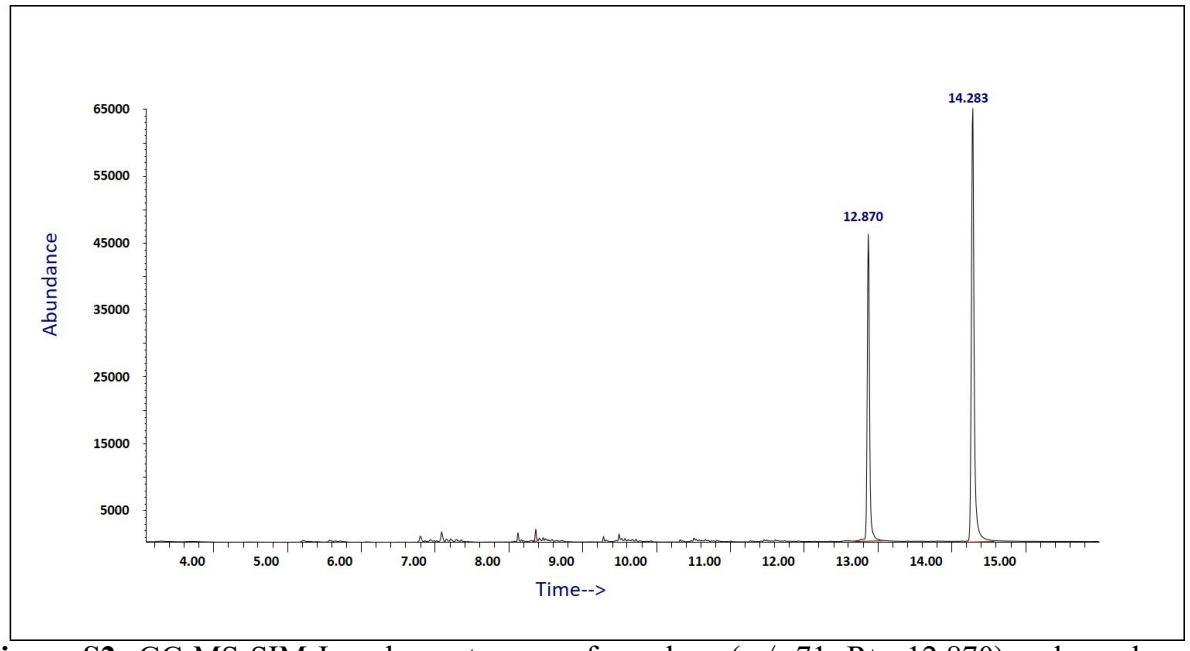


**Figure S2:** GC-MS SIM-Ion chromatogram of squalane (*m/z* 71, Rt= 12.870) and squalene (*m/z* 69, Rt=14.283) at 10 µg/mL.

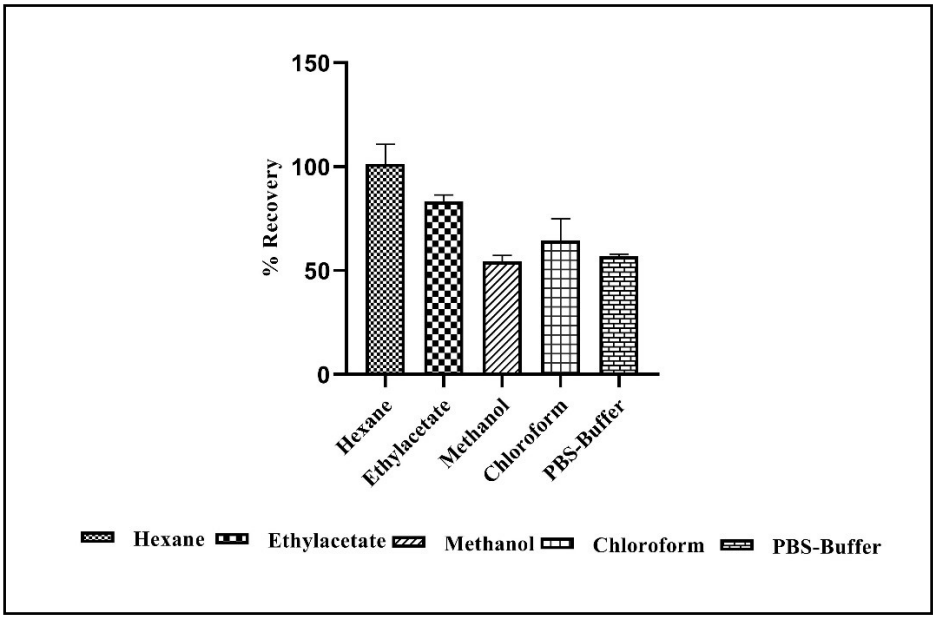


**Figure S3:** Recovery of Squalene at a concentration of 1µg/mL in different solvents.

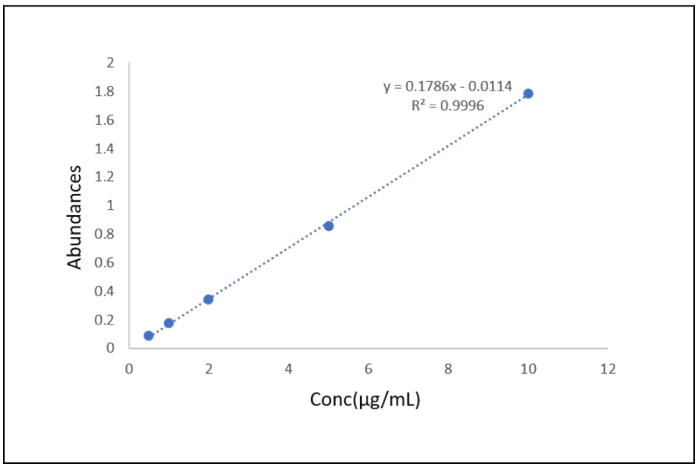


**Figure S4**: Linearity graph of squalene (0.5-10 µg/mL).

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