1.0 Purpose

The purpose of this procedure is to define the method for the quantitation and/or identification of Methylsulfonylmethane in raw material and finished product dietary supplements by GC using flame ionization mode.

2.0 Scope

This procedure applies to the quantification and identification of MSM in raw materials and finished products. MSM readily detectable by FID on the GC using the direct inject mode.

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 implement this procedure and 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 **FID** – Flame Ionization Detection

4.2 **DGME** – Di(ethylene glycol) methyl ether

4.3 **MSM** – Methylsulfonylmethane or dimethyl sulfone

4.4 **MeOH** – Methanol

5.0 References

5.1 USP 41 Methylsulfonylmethane Monograph

5.2 MV-LAB-18-069, Protocol, Methylsufonylmethane Determination by GC using Flame Ionization Mode

5.3 D-793, SOP, Cryogenic Grinding of Chewable Gels

6.0 Supplies

6.1 Chemicals: All reagents are GC grade or better.

6.1.1 MeOH

6.1.2 DGME

6.1.3 MSM reference standard

6.2 Glassware

6.2.1 HPLC vials, 12mm x 32mm with screw cap enclosures with septa

6.2.2 Scintillation vials

6.2.3 Mobile phase containers

6.2.4 Volumetric glassware as required by standard and sample preparations

6.3 Disposables

6.3.1 Adjustable pipette tips

6.3.2 Micro-centrifuge tubes

6.3.3 16mL test tubes

6.3.4 Disposable plastic luer lock syringe – 3mL, 6mL, or 10mL

6.3.5 Nylon syringe filters, 0.45µm

6.3.6 Weigh paper

6.4 Equipment

6.4.1 Agilent 7890 GC with headspace

6.4.2 Analytical balance

6.4.3 Ultrasonic bath

6.4.4 Vortex

6.4.5 Stir plate

6.4.6 Eppendorf centrifuge

6.4.7 Adjustable pipettes

7.0 Preparation of Diluent, Samples, and Standards

7.1 Diluent

7.1.1 Scale the Diluent preparation as required.

7.1.2 Transfer 0.60 mL of DGME to a 1-L media bottle.

7.1.3 Add 1000 mL methanol, and mix well.

7.2 Standard Preparation

7.2.1 Accurately weigh and transfer about 30 mg of MSM reference standard into a 100-mL volumetric flask.

7.2.2 Dissolve in and dilute to volume with Diluent

7.3 Sample Preparation

7.3.1 Specific sample testing details are provided in each products profile. If a specific testing details section is not available, then follow preparation procedure as described below, maintaining concentration within the linear range listed below

7.3.2 The linear range for MSM is 0.1 mg/mL – 0.5 mg/mL.

7.3.3 For raw materials: weigh no less than 20 mg into a suitably sized volumetric flask of no less than 25 mL volume to generate an analyte concentration that is within the validated linearity range. Dilute to volume with Diluent, and sonicate for 10 min.

7.3.4 For solid and liquid dose finished products: Combine and homogenize no less than ten dosage units. Based on the label claim and fill weight (capsules), serving size (powders) or tablet weight per dose, weigh no less than 50 mg of the pooled dosages into a suitably sized volumetric flask of no less than 25 mL to generate an analyte concentration that is within the validated linear range. Dilute to volume with Diluent, and sonicate for 10 min.

7.3.5 For chewable gels (gummies), homogenize at least 10 dosage units according to the procedure outlined in D-793 Cryogenic Grinding of Chewable Gels. Quickly weigh a portion of the pooled and homogenized dosages into a beaker. Use several small portions of Diluent to completely transfer the sample into a suitably sized volumetric flask to generate an analyte concentration that is within the validated linear range. Dilute to volume, and sonicate for 10 min.

7.3.6 To manage large volumes, the sample can be initially dissolved in a smaller volume and a portion further diluted using Diluent to bring the analyte

concentration into the linear range of measurement. **Equilibrate the standard solution to room temperature prior to performing further dilution.**

7.3.7 If particulates remain in the final sample preparation, a portion may be centrifuged at 10,000 rpm for 5 min prior to HPLC analysis. Alternatively, the sample may be filtered through a 0.45 µm membrane discarding the first 3 – 4 mL.

8.0 Test Conditions

8.1 Column – Phenomonex ZB-1, 0.53mm x 30m, 5um, GC column, or equivalent.

8.2 Carrier gas– Helium

8.3 Inlet Liner – Restek, 4.0mm ID x 6.3mm OD x 78.5mm length straight liner with wool, or equivalent.

8.4 Flow Rate – 5.0mL/min

8.5 Temperature

8.5.1 Injector – 250°C

8.5.2 Column – 120°C

8.5.3 Detector – 250°C

8.6 Run Time

8.6.1 Blanks and Standards - 7 min

8.6.2 Samples - 30 min (longer may be required if samples exhibit peaks at long time)

8.7 Spit ratio - 2:1

8.8 Injection volume – 1µL

8.9 Septum purge – 3 mL/min

8.10 Air flow – 400 mL/min

8.11 Hydrogen flow – 40 mL/min

8.12 Makeup flow – 30 mL/min (column + makeup = constant)

8.13 Example Sequence

8.13.1 Perform at least one injection of a Blank (Diluent).

8.13.2 Perform five injections of the Working Standard.

8.13.3 Perform a single injection of each Sample Preparation.

8.13.4 Perform a single injection of the Working Standard after every six samples and at the end of the run.

8.14 Retention Times

8.14.1 MSM – about 4.8 min

8.14.2 DGME – about 6.0 min

8.15 System Suitability

8.15.1 The %RSD of the peak area ratio for five injections of the Working Standard is NMT 2.0%.

8.15.2 The %RSD of the peak area ratio for all injections of the Working Standard is NMT 2%.

9.0 Example Calculation

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

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 (decimal)

V_{std} Volume of Working Standard (mL)

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

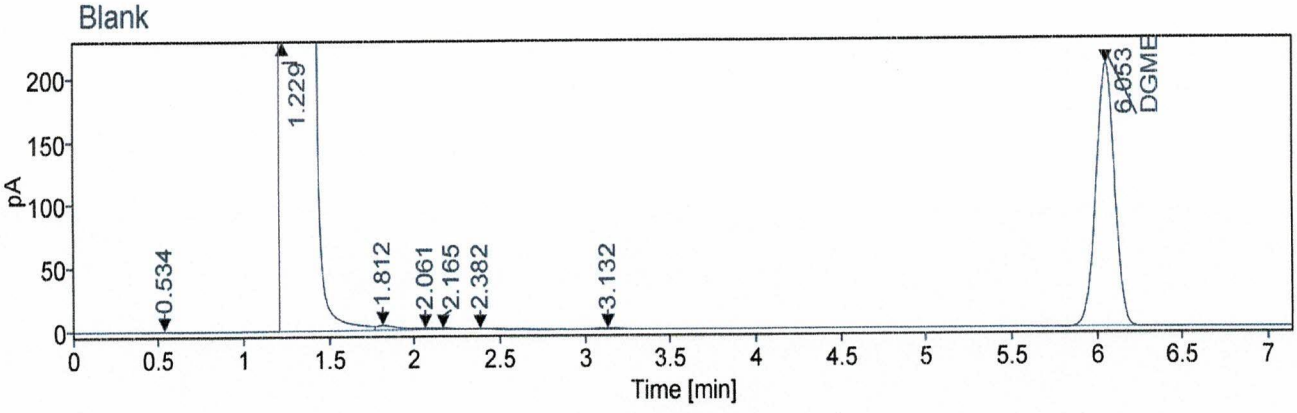
Spl_{wt} Sample weight (mg)

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

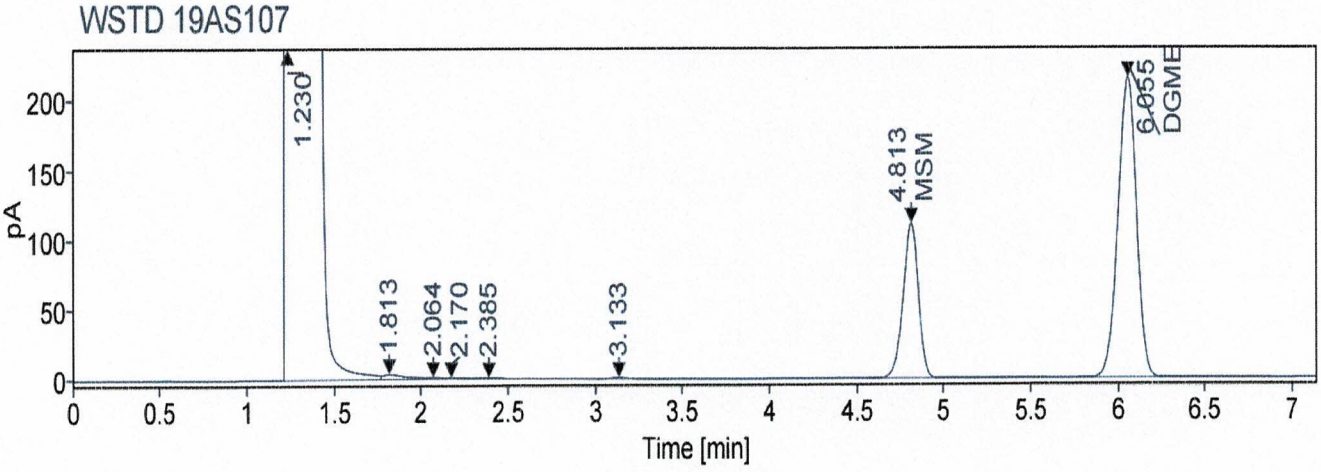
LA Label amount (mg, use 1 for raw materials)

10.0 Example Chromatograms

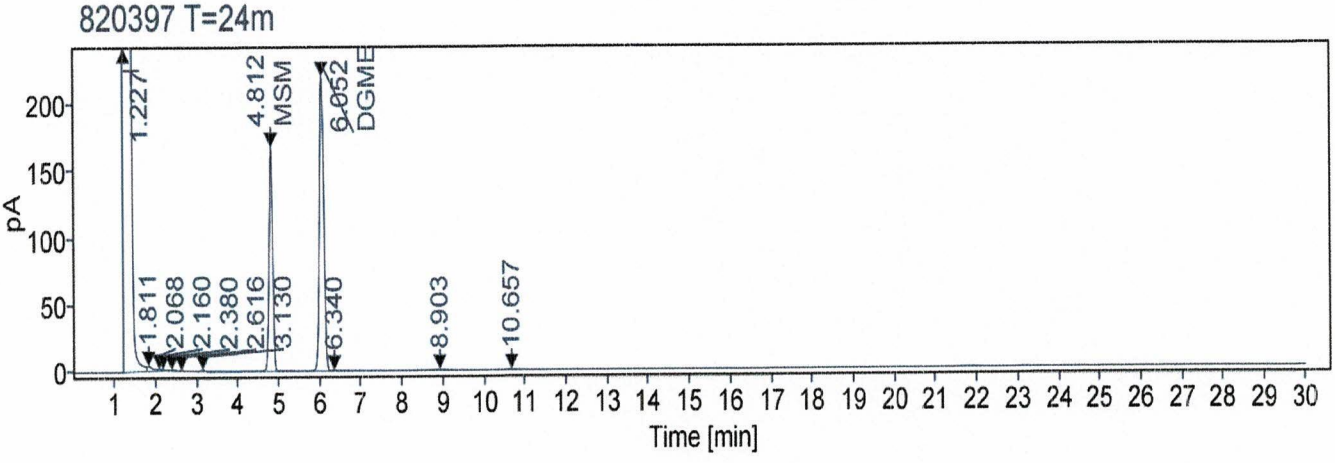
10.1 Blank



10.2 Working Standard



10.3 Sample Preparation



11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	01/02/19	New	N/A	J. Maignan
1	09/07/22	Added reference to validation, include linear range, simplify diluent and standard prep. Added short run time for standards and blanks, add retention times and example chromatograms, update system suitability and calculations. Removed "requirements" section.	CC-22-0365	S. Sassman
2	05/17/23	Removed unnecessary information and align with current SOP format. Added instruction to refer to test details for product specific sample preparation. Added specific sample preparation instruction for different dosage forms, Changed system suitability criteria from NLT 5% to NLT 2%. Changed logo.	CC-23-0230	S. Sassman