MENTHOL IN SLEEVE SILVERPRO

Report # 42605

This study is a quantification of Menthol in Sleeve SilverPro using the method ACS-GC-002 “Camphor, Menthol and Methyl Salicylate by Gas Chromatography-Flame Ionization Detector (GC-FID)" Rev. 6.

Method: ACS-GC-002 “Camphor, Menthol and Methyl Salicylate by Gas Chromatography-Flame Ionization Detector (GC-FID)” Rev. 6

APPLIED CONSUMER SERVICES INC.

Table of Content

REPORT
N° 42605

1. GENERAL INFORMATION

1.1 Purpose

Quantification of Menthol in Sleeve SilverPro after washing and drying/squeezing using the method ACS-GC-002 “Camphor, Menthol and Methyl Salicylate by Gas Chromatography-Flame Ionization Detector (GC-FID)" Rev. 6.

1.2 Scope

This study is applicable for the determination of Menthol in the range 0.2 to 30% in Sleeve SilverPro matrix (Counterirritant external analgesic)

1.3 Reference Method

- ACS-GC-002 “Camphor, Menthol and Methyl Salicylate by Gas Chromatography-Flame Ionization Detector (GC-FID)" Rev. 6

1.4 Matrix

- ✓ Sleeve SilverPro (Counterirritant external analgesic)

1.5 Specification

Menthol content in Sleeve SilverPro: 1.25 to 16%

1.6 Definitions

1.6.1 **Counterirritant:** A topically (externally) applied drug that causes irritation or mild inflammation of the skin for the purpose of relieving pain in muscles, joints, or viscera distal to the site of application by stimulating cutaneous sensory receptors.

1.6.2 **External analgesic:** A topically (externally) applied drug that has a topical analgesic, anesthetic, or antipruritic effect by depressing cutaneous sensory receptors, or that has a topical counterirritant effect by stimulating cutaneous sensory receptors.

1.6.3 **Over the counter (OTC):** Is an external analgesic drug product in a form suitable for topical administration is generally recognized as safe and effective.

2 STUDY CRITERIA

The study was developed following the method ACS-GC-002 “Camphor, Menthol and Methyl Salicylate by Gas Chromatography-Flame Ionization Detector (GC-FID)" Rev. 6

2.1 Equipment

2.1.1 Gas Chromatography Auto System Perkin Elmer or Equivalent.
2.1.2 Analytical Balance with 0.1mg resolution.
2.1.3 Dehydrator (oven)
2.1.4 Shaker

2.2 Materials

2.2.1 Agilent DB5 Capillary column 30m x 0.53mm x 0.5µm film thickness or equivalent.
2.2.2 Magnetic stirring plate.
2.2.3 Magnetic stirring bar.
2.2.4 Iso-Disc nylon syringe filters 0.45µm x 13mm, or equivalent.
2.2.5 1mL disposable tuberculin syringe.
2.2.6 5mL disposable transfer pipettes.
2.2.7 2mL autosampler vials.
2.2.8 40ml glass vial.

2.3 Reagents

2.3.1 Helium, grade 5.
2.3.2 Ultra-high purity Air, compressed.
2.3.3 Ultra-high purity hydrogen.
2.3.4 Isopropyl Alcohol IPA), GC or HPLC grade.
2.3.5 Menthol (for calibration standard preparation), USP reference standard or equivalent.
2.3.6 Menthol (for control standard preparation), USP reference standard or equivalent.

Note 1: The reference standard for the calibration standard preparation and control standard preparation must be from different brands or lots.

2.4 Procedure

2.4.1 Calibration and Control Standards Preparation.

2.4.1.1 Accurately weigh into a tared 40mL glass vial about 10.0 mg of Menthol standard.
2.4.1.2 Dilute with Isopropyl Alcohol to 20.0g, cap and mix. This solution has a final concentration of 0.5mg/g of Menthol.

2.4.2 **Sample Preparation.**

2.4.2.1 Ten samples of Sleeves SilverPro were chosen for the experiment.
2.4.2.2 Samples of Sleeve Silver Pro were washed and squeezed under running tepid water with no detergent for five seconds.
2.4.2.3 Each sample was wrung dry after washing.
2.4.2.4 At the end of twenty-five wash/wring-dry cycles, two samples taken and placed in a 95°F oven for two hours to remove extra moisture to prepare for menthol testing by GC-FID.
2.4.2.5 The procedure 2.4.2.2 to 2.4.2.4 were repeated and samples were taken at the 50th, 75th, 100th, and 125th wash/wring dry cycles for menthol testing by gas chromatography.
2.4.2.6 Wash/Wring Dried samples including positive and negative controls, were cut into small pieces.
2.4.2.7 About 0.3g of cut sample was weighed into a 40mL vial with cap,
2.4.2.8 The sample was diluted with Isopropyl Alcohol (IPA) to about 20 g, closed, mixed, and extracted in a shaker for 20 minutes.
2.4.2.9 Extracted sample was filtered through an Iso-Disc™ nylon filter using a disposable syringe into a 2.0mL autosampler vials for analysis.

Note 2: Negative control is one sample of SilverPro without Menthol and positive control is one SilverPro sample with Menthol. Positive and negative controls were washed, squeezed, and dried.

Note3: The samples, control standard, and standard were prepared at the same time as part of quality assurance.

2.4.5 **Determination.**

2.4.5.1 Following parameters described below:

Column:	Agilent DB5 Capillary column 30m x 0.53mm x 0.5µm
Oven temperature program:	40 ºC-2min-r 10 ºC/min- 300ºC- 5min
Injection volume:	1 µL
Detector:	300 ºC
Injector:	250 ºC
Carrier:	4.0 mL/min
Split control:	20.0 mL/min
Run time:	33 min
Retention Time:	~ 10.9 min for Menthol

2.4.6 **System Suitability.**

2.4.6.1 System Suitability Specifications:

Inject 1 µL of the standard preparation into the chromatographic system 5 times.

- The relative standard deviation for menthol is not more than 2.0%.

2.4.7 **Menthol Quantification**

2.4.7.1 Calculate the concentration of menthol in mg/Sleeve using the following equation:

$$Menthol, g/sleeve = \frac{R_U}{R_S} x \frac{C_S x W_f x A_w}{W_U} x100$$

R_U	=	Peak response of menthol to from the Sample solution
R_S	=	Peak response of menthol to from the Standard solution
C_S	=	Concentration of Menthol in the Standard solution (mg/g)
W_U	=	Sample weight (mg)
W_f	=	Weight of IPA in diluted sample in g
A_w	=	Sleeve SilverPro Average Weight in g

3 EXPERIMENTAL RESULT

Table # 1: Quality Control Results

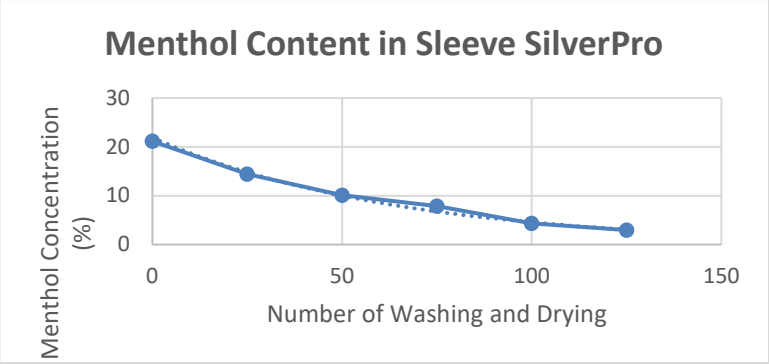
Attributes	ACS Specification	Result	Conformity
Relative Standard Deviation	≤ 2%	1.69%	Pass
Accuracy	95 – 105%	100.96 – 102.83	Pass
Relative Percent Difference (RPD)	0 – 10%	7.6%	Pass

- Specification: Is established in the ACS-GC-002 "Camphor, Menthol and Methyl Salicylate by Gas Chromatography-Flame Ionization Detector (GC-FID)" Rev. 6

Table # 2: Experimental Results

Lab Number	Sample Description	Menthol		Average Weight
		(%)	(g/Sleeve)	(g/Sleeve)
42605/1	Sleeve SilverPro - Negative Control (0 Washing/drying)	ND	ND	9.37
42605/2	Sleeve SilverPro - Positive Control (0 Washing/drying)	21.15	3.04	14.35
42605/3	Sleeve SilverPro with 25 times washing/drying	14.44	1.93	13.35
42605/4	Sleeve SilverPro with 50 times washing/drying	10.12	1.35	13.35
42605/5	Sleeve SilverPro with 75 times washing/drying	7.85	0.98	12.43
42605/6	Sleeve SilverPro with 100 times washing/drying	4.32	0.51	11.82
42605/7	Sleeve SilverPro with 125 times washing/drying	2.97	0.33	11.16

Note:
ACS: Applied Consumer Services, Inc.
ND: Not Detected



4 ANALYST

Joan Araluce Analyst

5 REFERENCE DOCUMENT

5.1 ACS-GC-002 "Camphor, Menthol and Methyl Salicylate by Gas Chromatography-Flame Ionization Detector (GC-FID)" Rev. 6.
5.2 Over the Counter (OTC) Monograph M017: External Analgesic Drug Products for Over-the-Counter Human Use (Posted May 2,2023)

6. CONCLUSION

Washing and wring-dry cycles 125 times and sampling every 25 cycles resulted with a menthol content of 2.97% at the end of the 125th wash/dry cycle. In addition, even after 125 washes, the sleeve that has the relatively low concentration of Menthol, keep providing a cooling sensation.

7. EFFECTIVE DATE

This experiment was finished on 9/11/2023.

Joan Araluce
Analyst
September 11, 2023

Ernesto Melgarejo
Quality Manager
September 11, 2023