https://doi.org/10.46344/JBINO.2026.v15i03.06

PRELIMINARY ANALYSIS AND CHARACTERIZATION OF ALLICIN AND MENTHOL

Ms. Snehal Pawar[1,2], Dr.Minal S. Patil[1*]

[1]DCS's A.R.A. College of Pharmacy, Mumbai-Agra Highway, Nagaon, Dhule- 424005.
Maharashtra India
[2]University Department of Pharmaceutical Sciences,MGM University Chh.Sambhajinagar-431003
Maharashtra India
snehalnpawar@gmail.com[1];minalpatilpharma@gmai.com[2]

ABSTRACT

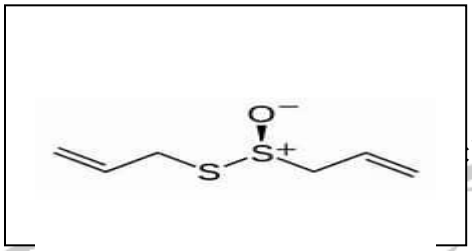
Menthol and allicin are naturally occurring bioactive substances that have been extensively researched for their therapeutic, medicinal, and antibacterial qualities. While menthol, a monoterpene alcohol derived mostly from mint species (Mentha spp.), is highly regarded for its cooling properties and therapeutic uses, allicin, a sulfur-containing component derived from garlic (Allium sativum), is recognized for its antibacterial and antioxidant properties. The extraction, qualitative analysis, and physicochemical characterisation of menthol and allicin using conventional analytical methods are the main objectives of this pilot study. Solubility tests, melting point measurements, UV-visible spectroscopy, and Fourier Transform Infrared (FTIR) spectroscopy were used for characterization. The findings support the existence of distinctive functional groupings and physicochemical characteristics that are in line with published research. For more quantitative analysis and formulation-based research involving these bioactive chemicals, this study offers baseline data.

Keywords: Allicin; Menthol; UV; FT-IR; HPLC; GC-MS; DSC

1. Introduction

1.1 Allicin

Allicin (diallyl thiosulfinate**)** is a sulfur-containing compound produced enzymatically from alliin when garlic (*Allium sativum*) tissues are crushed or damaged. It is primarily responsible for the characteristic odor of garlic and exhibits a wide range of biological activities, including antimicrobial, antifungal, antioxidant, anti-inflammatory, and cardio protective effects [Patel D et.al]. Despite its potent therapeutic potential, allicin is chemically unstable and highly reactive, which limits its direct pharmaceutical application. Therefore, preliminary studies focusing on its identification, stability, solubility, and analytical characterization are crucial before further formulation development [Fujisawa H et.al]. Allicin is a biologically active sulfur-containing compound responsible for the characteristic odor and pharmacological properties of garlic (*Allium sativum* L.)[Salehi B 2022, Prausnitz MR et.al 2021].



in

- Molecular formula: $C_6H_{10}OS_2$
- IUPAC name: 2-propenesulfinothioic acid S-2-propenyl ester

Fig: 2 Allicin

1.2 Menthol

Menthol, a cyclic monoterpene alcohol obtained mainly from peppermint oil (*Mentha piperita*), is widely used in pharmaceutical, cosmetic, and food industries. It is known for its cooling sensation, analgesic, antipruritic, antimicrobial, and penetration-enhancing properties [Prajapati ST et.al 2022]. In drug delivery research, menthol has been extensively explored as a permeation enhancer**,** particularly in transdermal and topical formulations [Zhang Y et.al]. Its

favorable physicochemical characteristics and Generally Recognized as Safe (GRAS) status make it an attractive candidate for combination with other bioactive compounds.Menthol is a naturally occurring monoterpenoid alcohol commonly obtained from peppermint (*Mentha piperita* L.) [Kasting GB et.al 2020].

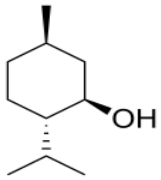


Fig: 3 Structure of Menthol

- Molecular formula: $C_{10}H_{20}O$
- Molar mass: 156.27 g/mol

Fig: 4Menthol

2. Materials and Methods

2.1 Materials

Allicin and menthol were purchased from Yucca Enterprises, Mumbai. The received sample of drug Allicin & Menthol was confirmed by following tests:

2.2 Methods

2.2.1 Organoleptic Evaluation

1. The Allicin & Menthol sample were evaluated visually for appearance, color, odor and taste. Physical evaluation like color, odor and determination of melting point performed to confirm identity. Melting point determination of the pure drug sample was done, as it is a first indication of purity of the sample. It was determined by melting point determination apparatus [Skoog DA 2011, Borlinghaus J et.al 2014].

2.2.2 Solubility Studies

Solubility of Allicin and Menthol was determined in various solvent like Ether, chloroform, ethanol, methanol, water [Silverstein RM et.al 2014].

2.2.3 UV–Visible Spectrophotometric Analysis

UV–visible spectrophotometric analysis was performed using a UV–Visible spectrophotometer (Model 1800). Drug solutions (10 µg/mL) prepared in methanol were scanned between 200–400 nm.

2.2.4 High Performance Chromatography (HPLC)

Chromatographic separations were achieved using a Column: Phenomex ProdigyTM ODS (3), 5 µm, 100 Å, 4.6 mm × 250 mm,analytical column. The mobile phase consisting of methanol:water (50:50),. The flow rate was maintained at 1.0 mL/min and the measurements were made at 240 nm. The column and the HPLC system were kept in 28°C temperature &run time: 12.5 minutes [Ankri S, Eccles R et.al 1999].

2.2.5 Gas Chromatography-Mass Spectrometry (GC-MS)

GC analysis was performed using a capillary column (ZB-1MS or HP-5, 15–30 m × 0.25 mm × 0.25–1 µm) with helium as the carrier gas at 1 mL/min. Samples were injected in splitless mode at 250 °C. The oven temperature was programmed from 50 °C (1 min hold) to 135 °C at 10 °C/min, and then increased to 250–260 °C at 15–40 °C/min, with a total run time of about 17 minutes. MS detection was carried out using electron impact ionization at 70 eV in SIM model. Menthol was quantified at m/z 138 using menthol-d4 as the internal standard (m/z 142), with confirmation ions atm/z 123 and 95 [Snyder LR et al 2010].

2.2.6 Fourier Transform Infrared (FTIR) spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was performed to identify the functional groups and evaluate the compatibility of the samples. FTIR analysis of Allicin and Menthol was carried out using an FTIR spectrophotometer in the range of **4000–400 cm^{-1}**. The spectra were examined for characteristic peaks corresponding to functional groups and to evaluate.

2.2.7 Differential Scanning Calorimetry (DSC) of Menthol

Differential Scanning Calorimetry (DSC) is a thermal analytical technique used to study the melting behavior, phase transitions, and thermal stability of pharmaceutical compounds [Vyas SP et.al 2002]. In the present study, DSC analysis was carried out to evaluate the thermal characteristics and purity of menthol.About 3–5 mg of the sample was sealed in an aluminum pan and heated from 25–80 °C at a rate of 10 °C/min under a nitrogen atmosphere, using an empty pan as reference [Poole CF et.al 2021]. The thermogram was analyzed for melting temperature and enthalpy change.

3. Results

3.1 Material

The received samples of Allicin and Menthol were evaluated for their organoleptic and identification characteristics. The observed results were found to be in accordance with the standard reported specifications, confirming the authenticity and purity of the obtained materials.

3.2.1 Organoleptic Evaluation

Allicin appeared as a slightly yellow liquid with a pungent odor, whereas menthol appeared as a white crystalline solid with a characteristic minty odor. Both compounds were soluble in organic solvents and sparingly soluble in water

Sr. No	Parameter	Observation **Allicin**	Observation **Menthol**
1	Color	Slightly yellow liquid	Whitish
2	Odor	Ammonia like	mint-licorice
3	Taste	Pungent	Strong & Minty
4	Appearance	Liquid	White & Crystalline Solid

Table: 1

Organoleptic Evaluation of Allicin & Menthol

3.2.2 Solubility Studies

Solubility of allicin and menthol was determined in methanol, ethanol, chloroform, ether, and distilled water (Tables 2).

Sr.no		**Allicin**	**Menthol**
	Methanol	Soluble	Soluble
	Ethanol	Soluble	Soluble
	Chloroform	Soluble	Soluble
	Ether	Soluble	Soluble
	Distilled water	Sparingly soluble	Sparingly soluble

Table: 2 Solubility studies of Allicin & Menthol

3.2.3 UV Studies

Allicin exhibited a maximum absorbance (λmax) at 240 nm, while menthol showed λmax at 218 nm, confirming their identity (Figures 5 and 6).

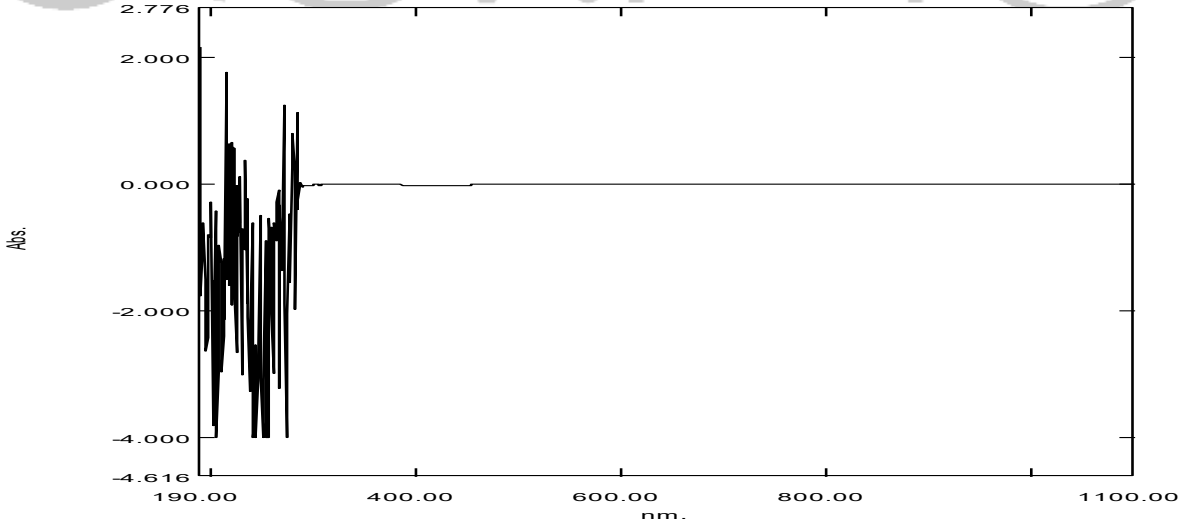


Fig: 5 UV of Menthol

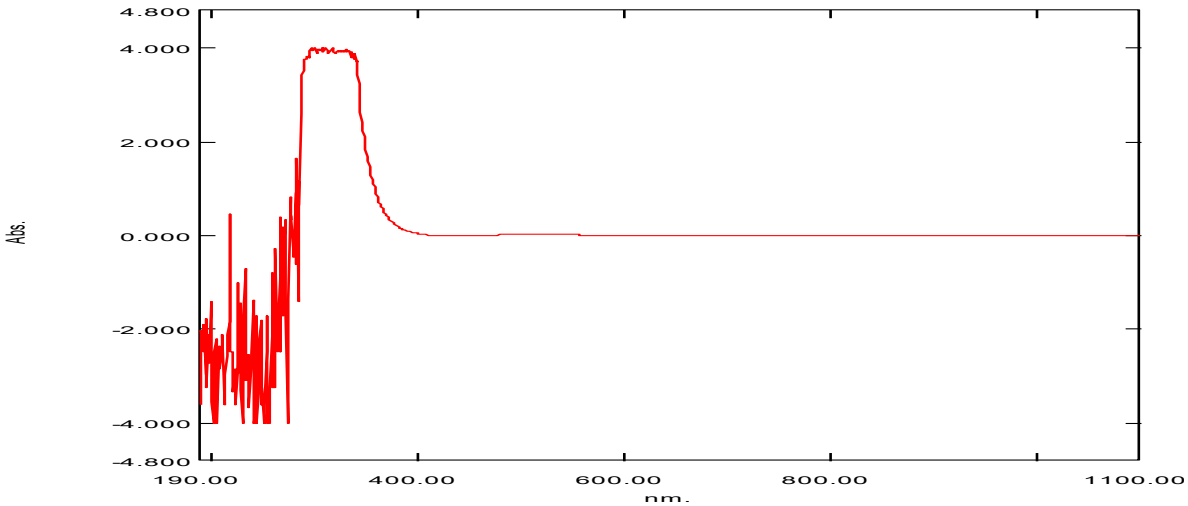


Fig: 6 UV of Allicin

3.2.4 HPLC Analysis of Allicin

HPLC analysis of allicin showed a retention time of 6.63 min, indicating purity (Figure 3). GC-MS analysis of menthol showed a single major peak at 13.08 min with a molecular ion peak at m/z 156, corresponding to its molecular weight (Figure 7).

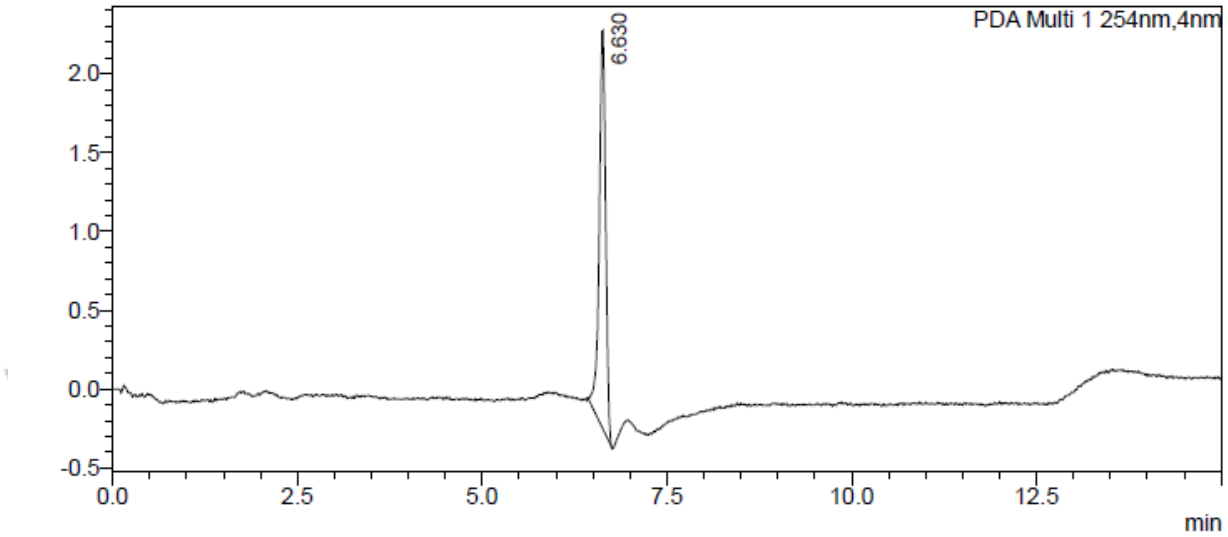


Fig: 7 HPLC of Allicin

3.2.5 GC-MS Analysis of Menthol

GC-MS analysis of menthol was carried out to determine purity and molecular identity based on retention time and molecular ion peak.This GC chromatogram shows a sharp, well-resolved peak at a retention time of ~13.2 min, which corresponds to menthol under the given GC–MS conditions. The peak is symmetrical, indicating good column efficiency and minimal tailing. No significant interfering peaks are observed around the menthol retention time, suggesting good specificity and purity of the analyte. The early small peak near the solvent front represents the solvent or volatile impurities, while the absence of late-eluting peaks indicates complete elution within the programmed run time. Overall, the chromatogram confirms successful separation and reliable detection of menthol.

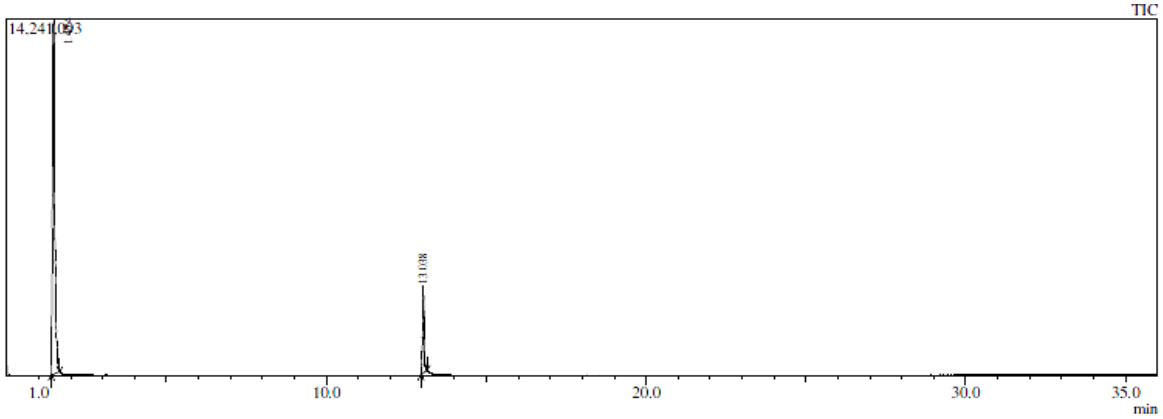


Fig: 8 GC-MS of Menthol

3.2.6 FT-IR Analysis

FTIR spectra of allicin and menthol confirmed the presence of their characteristic functional groups. Allicin exhibited prominent peaks corresponding to thiosulfinate (S=O, S–O, and C–S) groups, while menthol showed a broad O–H stretching peak and C–O vibrations confirming its alcoholic nature. The spectra indicated no structural alteration, confirming the identity and purity of both compounds and their suitability for formulation studies.

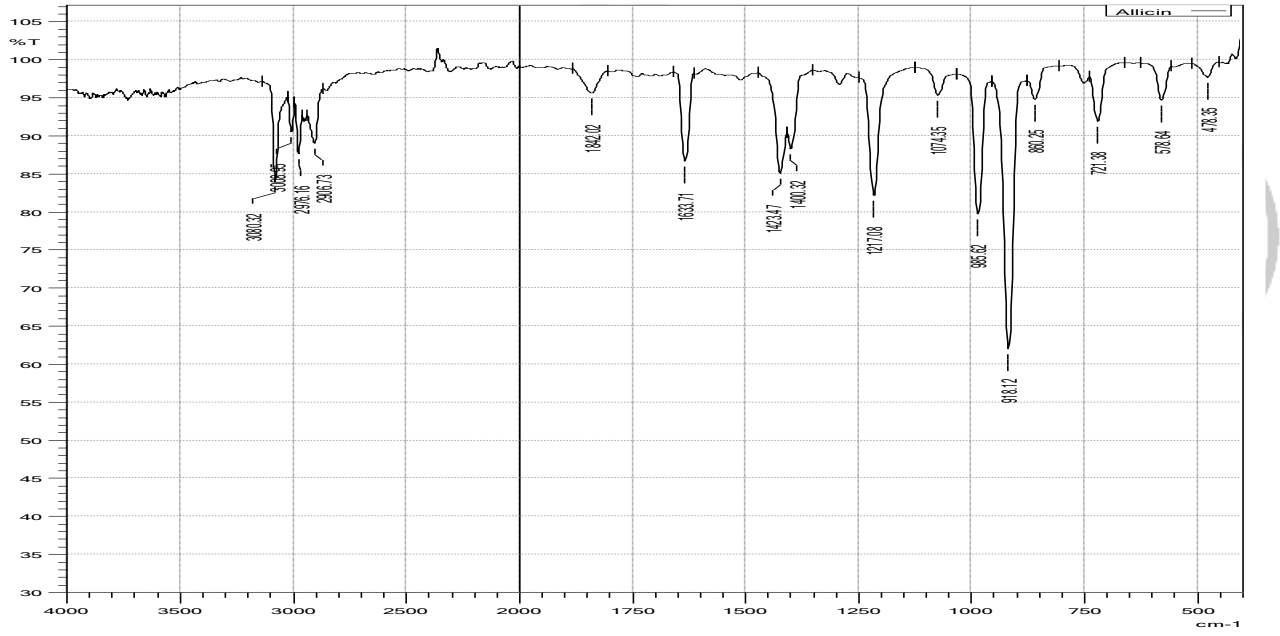


Fig: 9 FT-IR of Allicin

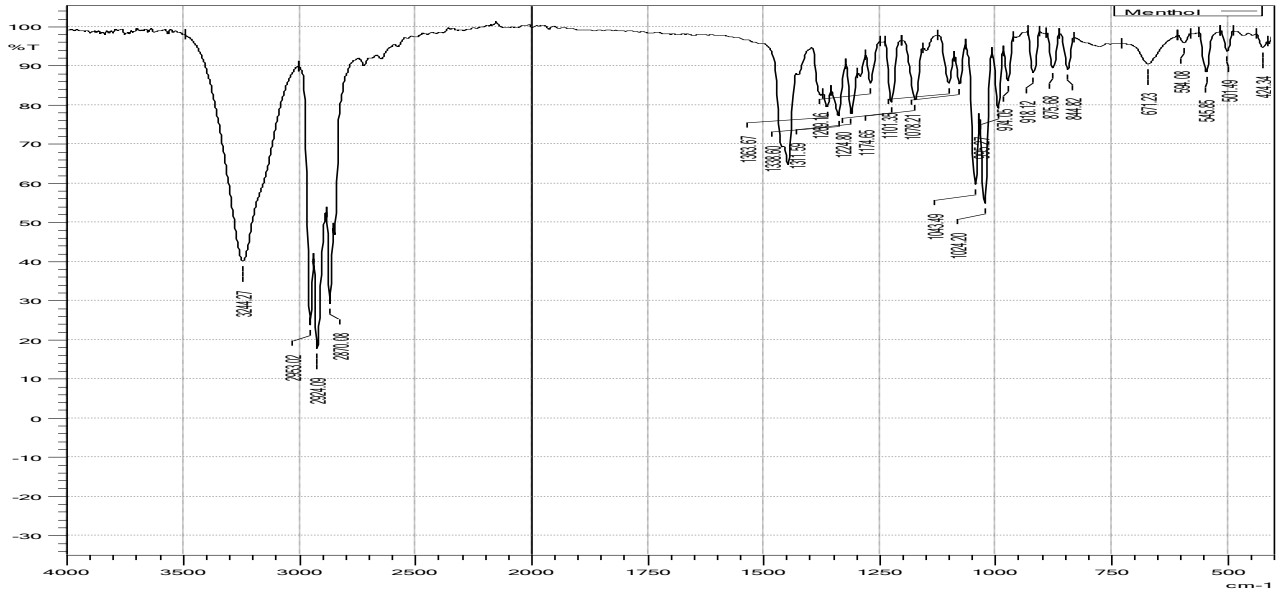


Fig: 10 FT-IR Menthol

3.2.7 DSC Analysis

As a part of preliminary studies, Differential Scanning Calorimetry (DSC) was carried out to evaluate the thermal behavior, purity, and stability of menthol prior to formulation development.

The DSC thermogram of menthol showed a sharp endothermic peak at 42.10 °C, with an onset at 40.93 °C and endset at 43.31 °C, corresponding to the melting point of menthol. The narrow melting range indicates the crystalline nature and high purity of menthol.The observed endothermic heat flow (ΔH = –154.17 mJ; –77.08 J/g) represents the energy required for the phase transition from solid to liquid. No additional endothermic or exothermic peaks were observed, suggesting the absence of thermal degradation or polymorphic transitions within the studied temperature range.

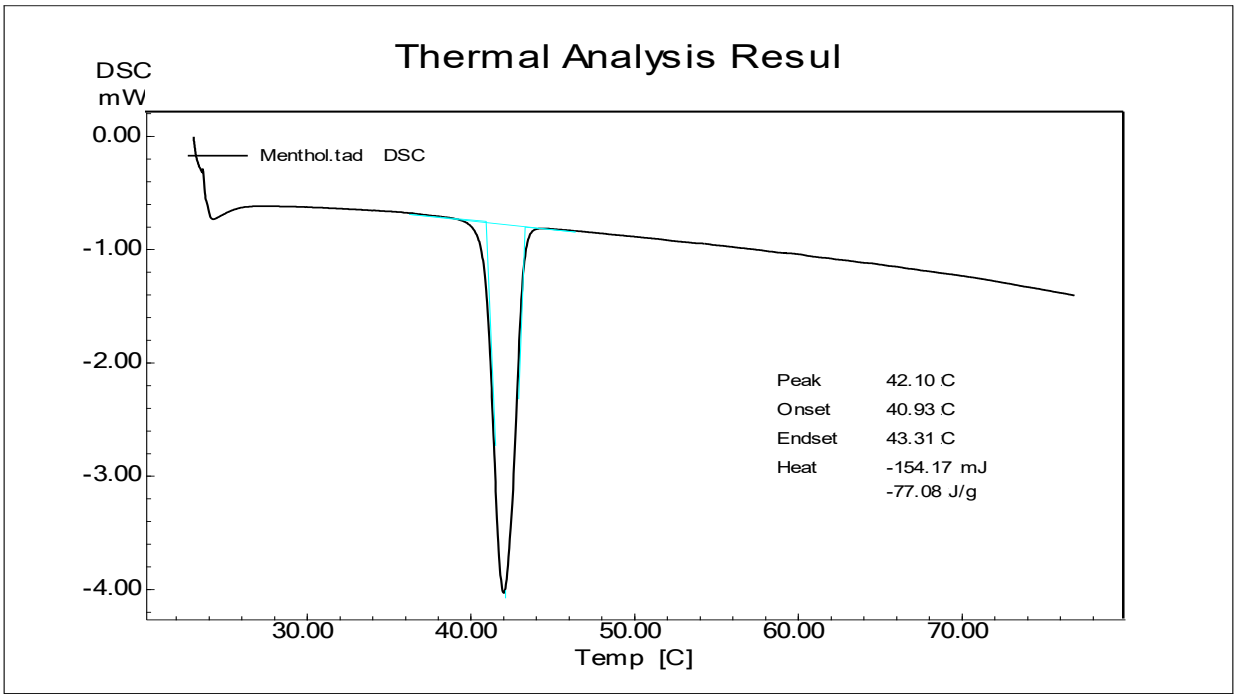


Fig: 11 DSC of Menthol

4. Discussion

The preliminary characterization demonstrated that allicin and menthol possess suitable physicochemical properties for transdermal delivery. The absence of drug–excipient interactions suggests formulation stability, while the purity confirmed by chromatographic techniques supports further formulation development.

5. Conclusion

The study concludes that allicin and menthol are suitable candidates for incorporation into transdermal drug delivery systems. Their confirmed identity, purity, solubility, and compatibility with selected polymers support further formulation and in-vitro/in-vivo evaluation..

6. References

1. Ankri, S., & Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. Microbes and Infection, 1(2), 125–129.
2. Borlinghaus, J., Albrecht, F., Gruhlke, M. C. H., & Slusarenko, A. J. (2014). Allicin: Chemistry and biological properties. Molecules, 19(8), 12591–12618.
3. Borlinghaus, J., Albrecht, F., Gruhlke, M. C. H., & Slusarenko, A. J. (2021). Allicin: Chemistry, biological properties and therapeutic potential. Molecules, 26(3), 713.
4. Coates, J. (2020). Interpretation of infrared spectra: A practical approach. In Encyclopedia of Analytical Chemistry.
5. Eccles, R. (1994). Menthol and related cooling compounds. Journal of Pharmacy and Pharmacology, 46(8), 618–630.
6. Fujisawa, H., Suma, K., Origuchi, K., Kumagai, H., Seki, T., & Ariga, T. (2020). Stabilization and pharmaceutical applications of allicin and garlic-derived sulfur compounds. Journal of Functional Foods, 67, 103853.
7. Giroux, H. J., & Britten, M. (2021). Differential scanning calorimetry as a tool for purity and compatibility studies of pharmaceutical compounds. Journal of

Thermal Analysis and Calorimetry, 144, 163–172.

8. Kasting, G. B., & Miller, M. A. (2020). Effects of menthol on skin permeation and barrier properties. International Journal of Pharmaceutics, 586, 119570.
9. Lawson, L. D., & Wang, Z. J. (2005). Allicin and allicin-derived garlic compounds increase breath acetone through allyl methyl sulfide. The Journal of Nutrition, 135(3), 580–586.
10. Patel, D., Chaudhary, S. A., Parmar, B., et al. (2023). Advances in transdermal drug delivery systems: A review. International Journal of Pharmaceutics, 640, 122973.
11. Poole, C. F. (2021). Gas chromatography–mass spectrometry in pharmaceutical analysis. Journal of Chromatography A, 1658, 462580.
12. Prajapati, S. T., Patel, C. G., & Patel, C. N. (2022). Role of terpenes as skin permeation enhancers in transdermal drug delivery systems. Journal of Drug Delivery Science and Technology, 68, 103047.
13. Prausnitz, M. R., & Langer, R. (2021). Transdermal drug delivery. Nature Biotechnology, 39, 6–19.
14. Salehi, B., Zucca, P., Sharifi-Rad, M., et al. (2022). Therapeutic potential of allicin and related organosulfur compounds. Phytotherapy Research, 36(1), 110–135.
15. Silverstein, R. M., Webster, F. X., & Kiemle, D. J. (2014). Spectrometric identification of organic compounds (8th ed.). John Wiley & Sons.
16. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2014). Principles of instrumental analysis (6th ed.). Cengage Learning.
17. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). Introduction to modern liquid chromatography (3rd ed.). Wiley.
18. Vyas, S. P., & Khar, R. K. (2002). Targeted and controlled drug delivery: Novel carrier systems. CBS Publishers.
19. Watson, D. G. (2005). Pharmaceutical analysis: A textbook for pharmacy students and pharmaceutical chemists (2nd ed.). Elsevier.
20. Zhang, Y., Chen, X., Wang, J., et al. (2021). Menthol as a penetration enhancer in transdermal drug delivery systems. Drug Development and Industrial Pharmacy, 47(6), 911–920.