

Analytical Method Development and Validation of Menthol and Methyl Salicylate Content in Topical Cream and Gel by Gas Chromatography

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Abstract

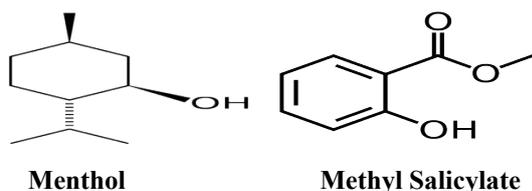
Menthol and Methyl Salicylate are used in topical treatment and high-dose dermal patch, to relieve pain of minor aches and pains of muscle and joints associated with arthritis. Two types of formulations available in the market. In one formulation Menthol and Methyl Salicylate are used as two active ingredients and it is Cream based formulation. In another gel based formulation, only Menthol is added as an active ingredient. Both formulations are used for different therapeutic effects during pain management. The purpose of the present study was to develop accurate and precise single analytical method for determination of Menthol and Methyl Salicylate content in topical cream and gel formulations by gas chromatography. Internal standard technique was used for quantitation of Menthol and Methyl Salicylate by Gas Chromatography in order to reduce sample preparation and injection related errors thereby improving method accuracy. Further developed method was validated to prove its suitability for intended.

Keywords: Menthol; Methyl salicylate; Gas chromatography; Internal standard; Counterirritant; Method validation

Introduction

A counterirritant is a substance which creates irritation or mild inflammation in one location with the goal of lessening discomfort and inflammation in another location. Ingredients such as menthol, methyl salicylate and camphor are called counterirritants because they produce irritation in one part of the body to help relieve irritation in another. They differ from the anaesthetics, analgesics, and antipruritic agents, however upon contact, they create a warming or cooling sensation to dilate blood vessels, re-oxygenate the tissues and relax muscles to ultimately stop the pain messages that are being sent to the brain via nerves in the body, other than the skin areas to which they are applied as for example, in joints, muscles, tendons and certain viscera. The use of these products dates from antiquity [1-5].

Menthol is very soluble in alcohol, chloroform, ether, and hexane and slightly soluble in water. Menthol is an alcohol that is found in mint oils, and similar to peppermint, has a refreshing odour, white crystalline structure and cooling properties. Chemical formula of Menthol is $C_{10}H_{20}O$ and Molecular weight is $156.27 \text{ g}\cdot\text{mol}^{-1}$. Menthol is found in many topical pain relief medications due to its counterirritant and local anaesthetic properties. The mechanism by which menthol is able to impart a cooling sensation when applied topically to the skin is well understood. Menthol in over the counter concentrations has an excellent safety profile [6-10].



Methyl Salicylate is slightly soluble in water; soluble in alcohol and in glacial acetic acid. Methyl salicylate (oil of wintergreen or wintergreen oil) is an organic ester naturally produced by many species of plants,

particularly wintergreens. It is also synthetically produced, used as a fragrance, in foods and beverages, and in liniments. Chemical formula of Methyl Salicylate is $C_8H_8O_3$ and Molecular weight is $152.15 \text{ g}\cdot\text{mol}^{-1}$. When used medicinally, it creates a mild local reaction that provides relief at the area of pain. It may cause analgesic (pain relief) and anti-inflammatory effects by inducing vasodilatation thereby increasing blood flow and temperature to the localized area of tissue.

Chemicals, Instruments and Methods

Chemicals

Menthol Standard, Methyl Salicylate Standard, Anethole (as Internal standard), Isopropyl alcohol (as diluents), Formulation-1 (Menthol 10% w/w and Methyl Salicylate 15% w/w cream) and its placebo (without menthol and methyl salicylate), Formulation-2 (Menthol 10% w/w gel) and its placebo (without Menthol).

Formulation samples

- Formulation-1: Cream contains Menthol 10% w/w and Methyl Salicylate 15% w/w.
- Formulation-2: Gel contains Menthol 2.5% w/w.

Instruments

Gas Chromatograph, Analytical weighing balance, Sonicator.

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Methods

Chromatographic conditions: The chromatographic separation was performed on DB-624 column (30 m, 0.53 mm, 3 μ m). The column oven temperature with gradient method: Initial temperature is 90°C and final temperature is 181°C by increasing rate of 8°C/min. Final temperature hold for 4 min.

Detector temperature: 180°C.

Inlet temperature: 280°C.

Flow rate: 5 mL/min (Helium).

Injection volume: 1 μ L.

Preparation of internal standards, standards and samples

Internal standard solution: Prepare 2.0 mg/mL Anethole in Isopropyl alcohol.

Standard solution: Prepare 2.0 mg/mL Menthol Standard and 3.0 mg/mL Methyl Salicylate Standard in Internal standard solution.

Sample preparation: Prepare separately sample containing Formulation-1 (Cream) equivalent to 2.0 mg/mL Menthol and 3.0 mg/mL Methyl Salicylate in internal standard solution.

Further prepare sample containing Formulation-2 (Gel) equivalent to 2.0 mg/mL Menthol. Sonicate both the samples till it dissolves completely and filter through 0.45 μ filter by discarding first few mL of filtrate.

Method validation

Specificity study: No extraneous peaks observed in the blank and placebo chromatograms at the RT of Menthol and Methyl Salicylate in both formulations.

Linearity: Seven-point linearity plot was constructed in order to accommodate wide range from 1.0 mg/mL to 3.0 mg/mL of Menthol and 1.5 mg/mL to 4.5 mg/mL of Methyl Salicylate, so that Menthol and Methyl Salicylate with its respective formulation can be analyzed easily with $r^2=0.9998$ for the above plot.

Precision: ICH describes precision as closeness of individual measure of analysts when the procedure is applied repeatedly to multiple times. Interday and intraday precision has been established from the proposed method.

Accuracy: It was evaluated at three levels of 50%, 100% and 150% of test concentration by adding known amount of drug to placebo and extracting the sample. Three sets in triplicate were prepared and analyzed separately for both formulations.

Robustness: Varying conditions of flow rate and column oven temperature were carried out as per ICH guidelines to estimate their effects on the method.

Results and Discussion

Method development and optimization

Actual chromatographic conditions were established after number of preliminary experiments for selecting the proper column oven temperature. Different column oven temperatures were tested, and selection of the proper system depended on its ability to give good separation between the Menthol, Methyl salicylate and Anethole peaks. Acceptable separation was achieved on DB-624 column (30 m, 0.53 mm, 3 μ m) using Helium as a carrier gas with a flow rate of 5 mL/min. The

column oven temperature with gradient method: Initial temperature is 90°C and final temperature is 181°C by increasing rate of 8°C/min. Final temperature hold for 4 min. Under these chromatographic conditions, the run time for standard and sample is 16.38 min.

System suitability

System suitability parameters like theoretical plates per meter, tailing factor, percentage relative standard deviation of area ratio and retention time of six injections were carried out and the values are well within the limits as shown in Table 1.

Linearity

A linear calibration plot of Menthol and Methyl salicylate for Formulation-1 (Cream) and of Menthol for Formulation-2 (Gel) was constructed at seven-point concentration levels i.e., 1.0 mg/mL to 3.0 mg/mL of Menthol and 1.5 mg/mL to 4.5 mg/mL of Methyl Salicylate in duplicate. Average peak area ratio of Menthol and Methyl Salicylate was plotted against respective concentration and linear regression analysis was performed. Correlation coefficient was found to be 0.9998 for Menthol and 0.9998 for Methyl Salicylate in Formulation-1 and 0.9990 for Menthol in Formulation-2 indicating proposed GC method is linear (Figures 1-5).

Precision

The precision of the assay method was evaluated for repeatability and intermediate precision. For intra-day precision the percentage relative standard deviation for area ratio of Menthol and Methyl Salicylate against Anethole were found to be 0.39% and 0.54% respectively in Formulation-1 and Menthol against Anethole was found to be 0.61% in Formulation-2. For inter-day precision, the percentage relative standard deviation for area ratio of Menthol and Methyl Salicylate against Anethole found to be 0.51% and 0.43% respectively in Formulation-1 and Menthol against Anethole found to be 1.29% in Formulation-2. These values were well within the acceptable limit of 3.0%, as per USP. Results are given in Table 2.

Accuracy

Known amount of standards were spiked at 50%, 100%, and 150% concentration in placebo and each level performed in triplicate. From the results obtained, percentage recoveries of drugs were calculated for each active content. The accuracy of method was established at three concentration levels at 1, 2 and 3 mg/mL of Menthol standard and 1.5, 3.0 and 4.5 mg/mL of Methyl Salicylate standard in Formulation-1 and 1, 2 and 3 mg/mL of Menthol Standard in Formulation-2. The recovery at three different concentrations found to be within range of 98.0% to 102.0% as per ICH guidelines. Mean % recovery (mean \pm SD) found to be 100.50 \pm 0.58 for Menthol and 101.18 \pm 0.42 for Methyl Salicylate in Formulation-1 and 101.64 \pm 0.55 for Menthol in Formulation-2. The result indicates that the percentage recovery for Menthol and Methyl Salicylate found to be satisfactory at three different concentrations for respective formulations. The recovery results are summarized in Table 3.

Parameter	Acceptance Criteria	Formulation-1		Formulation-2
		Menthol	Methyl Salicylate	Menthol
Tailing factor	NMT 2.0	0.98	0.97	0.99
Theoretical plates	NLT 2000	197768	272298.6	205014.9
% RSD of 6 injection (area ratio)	NMT 3.0	0.02	0.02	0.20
% RSD of 6 injection (retention time)	NMT 3.0	0.01	0.00	0.00

Table 1: System suitability.

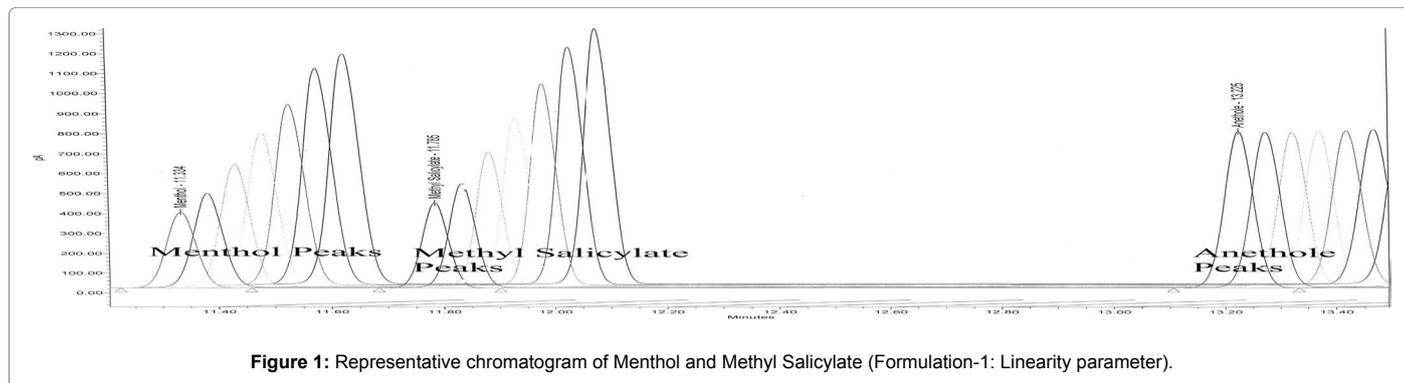


Figure 1: Representative chromatogram of Menthol and Methyl Salicylate (Formulation-1: Linearity parameter).

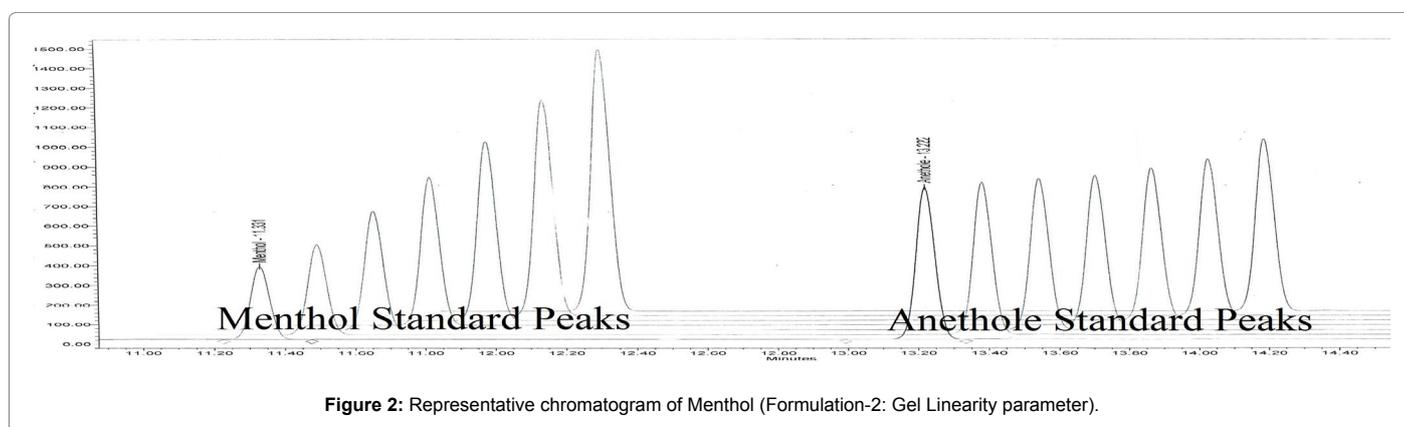


Figure 2: Representative chromatogram of Menthol (Formulation-2: Gel Linearity parameter).

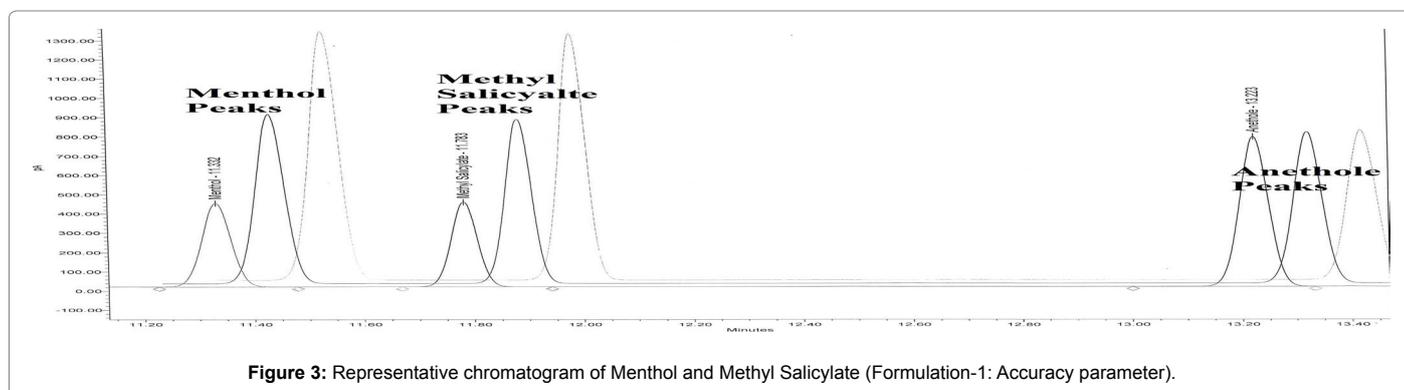


Figure 3: Representative chromatogram of Menthol and Methyl Salicylate (Formulation-1: Accuracy parameter).

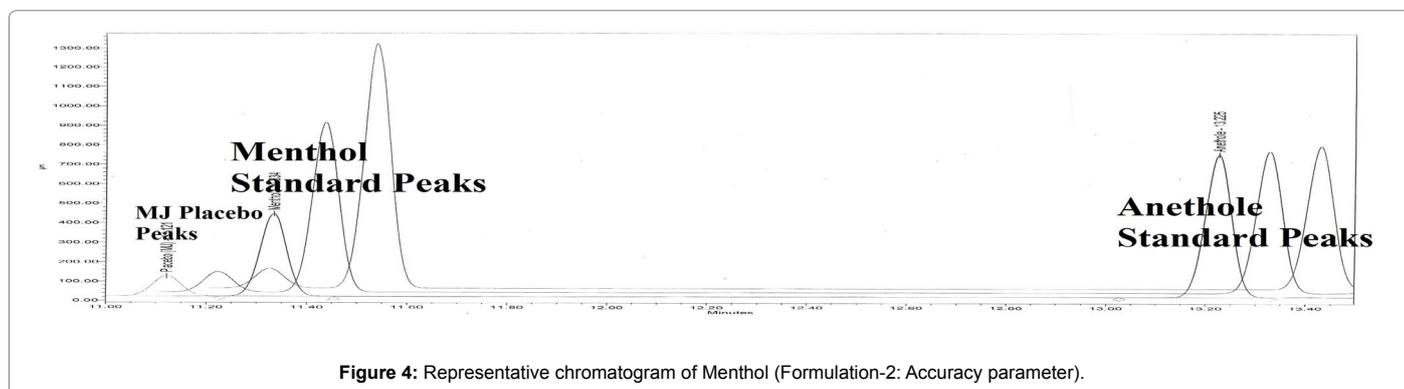


Figure 4: Representative chromatogram of Menthol (Formulation-2: Accuracy parameter).

Robustness

The robustness of assay method was studied by incorporating small

but deliberate changes in analytical method (variation in flow rate and initial column oven temperature). In all the varied chromatographic

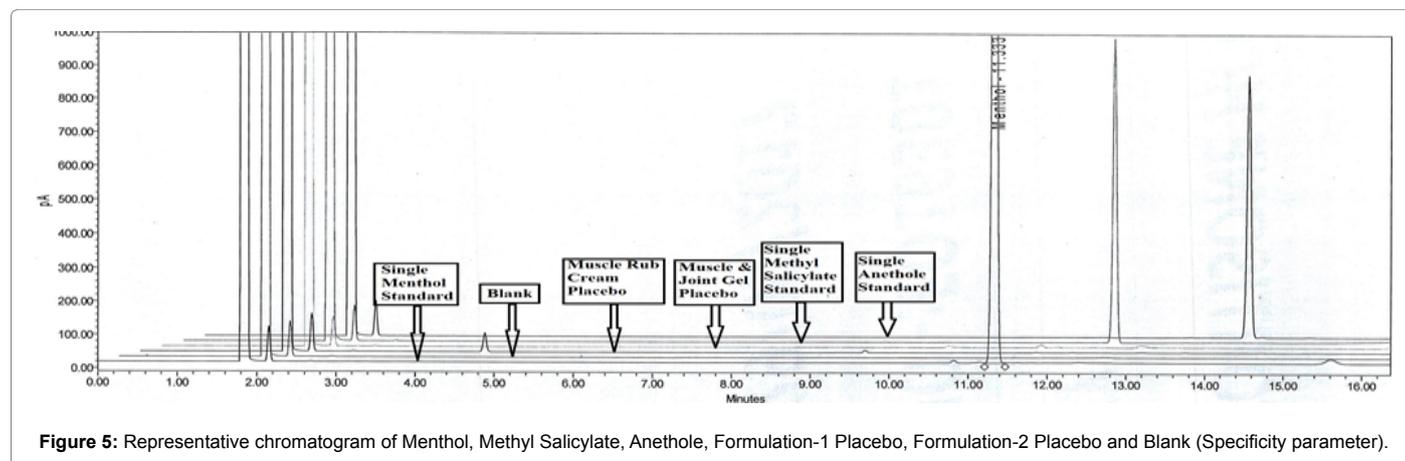


Figure 5: Representative chromatogram of Menthol, Methyl Salicylate, Anethole, Formulation-1 Placebo, Formulation-2 Placebo and Blank (Specificity parameter).

Parameter		% RSD Limit	Formulation-1		Formulation-2
			Menthol	Methyl Salicylate	Menthol
Precision	Repeatability	3.0%	0.39	0.54	0.61
	Intermediate	3.0%	0.51	0.43	1.29
Linearity (r ²)		0.999	0.9998	0.9998	0.9990

Table 2: Precision and Linearity (n=7).

Formulation-1				Formulation-2	
Menthol		Methyl Salicylate		Menthol	
Amount added	% Mean Recovery ± SD	Amount added	% Mean Recovery ± SD	Amount added	% Mean Recovery ± SD
1.0 mg/mL	100.95 ± 0.58	1.5 mg/mL	101.65 ± 0.39	1.0 mg/mL	101.37
2.0 mg/mL	99.76 ± 0.82	3.0 mg/mL	101.69 ± 0.37	2.0 mg/mL	101.77
3.0 mg/mL	100.81 ± 0.33	4.5 mg/mL	100.19 ± 0.51	3.0 mg/mL	101.78

Table 3: Accuracy.

conditions, there was no significant change in system suitability parameters.

Conclusion

Finally, we are successful in developing and validating a new, simple, rapid, precise and accurate internal standard Gas Chromatography method for the simultaneous estimation of Menthol and Methyl Salicylate in topical cream (Formulation-1) and Menthol content gel (Formulation-2).

Proposed method is duly validated and applied for routine estimation of Menthol and Methyl Salicylate content in the both formulations. Both formulations have a lot of advantages for warming or cooling sensation to dilated blood vessels, re-oxygenate the tissues and relax muscles. Till date, no official methods developed for combination

of Menthol and Methyl Salicylate. Hence, this method can be applied for the estimation of Menthol and Methyl Salicylate in drug testing laboratories and pharmaceutical industries.

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