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Method development and validation of menthol in cough syrup by gas chromatography

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ABSTRACT

A precise gas chromatographic method has been developed for the determination of menthol content in cough syrup. In this Method DB-1 column with (30m x0.32mm x1.0μm) and nitrogen as a carrier gas at a flow rate of 1.0 ml/min was used. The oven temperature was programmed at 100°C for 2 min and 10°C rise up to 240°C (Hold for 25min). The injector port and detector port temperature is maintained at 240°C and 260°C. Detection was carried out by FID detector. In this method methanol was used as diluent. The results of recovery studies were statistically evaluated for its method precision and accuracy study. The gas chromatography method is less expensive and gives an accurate and precise result.

INTRODUCTION

Menthol is an organic compound naturally occurring in mint plant and also synthetically prepared and itself well known as levorotatory and the racemic forms was described. In the U.S.Pharmacopeia, menthol finds wide application in pharmaceuticals, cosmetic, perfumery, and tobacco products. It acts to stimulate skin cold receptor, which makes the property of cooling effect and as similar to capsaicin chemical found in hot pepper which stimulates the heat receptor. Menthol does not actually change the skin temperature, but merely produces the sensation of temperature change.

It is used in various products to relieve skin irritation, sore throat, or nasal congestion, sunburn, fever, or muscle aches as well. In Asian medicine, it may be prescribed for nausea, diarrhea, indigestion, headache, cold or sore throat. Now a day's many cough and cold syrups contains menthol as one of the active ingredients and a method was developed to determine the percentage of menthol content available in cough and cold syrup by using gas chromatography tech. The literature contains reference to the gas chromatographic analysis of the compounds in the pharmaceutical preparations including camphor and menthol, L. Karuza et al[1], menthol, F. *J.* De Fabrizio[2] and F.Oritz-Boyer, et al[3] menthol and methylsalicylate, Sapio, J.P. et al[4] camphor, menthol and methylsalicylate C.C.J. Douglas[5] and S.V. Sur, et al[6], turpentine, camphor, menthol and methylsalicylate E. Gonzalez-Penas et al[7].

The literature survey shows that some of coworkers worked on menthol content in ointments, gels, balms, sprays, mouthwashes, etc E. Gonzalez-Penas et al[7]. and also some workers studied in essential oil composition of menthol mint Harsono et al.[8] and R.S.Verma et al.[9] Menthol is widely used in a various commercial products and foods, but its clinical pharmacology is not well studied. A.K.Singh et al.[10] reported the menthol content in essential oil composition and chemo arrays of menthol mint cultivars. This paper

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describes a method development and accuracy of menthol content in cough syrup. Till now no published method enables the percentage menthol content in cough syrup and their separation with paracetamol and other ingredients. In this method we had attained well separation with other ingredients and the accuracy level obtained were in the range of 95 to 105%.

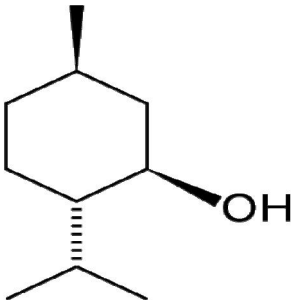

Figure 1 : Menthol structure

EXPERIMENTAL

Chemicals and reagents

Sample of Menthol and its syrup were received from Hindustan mint and agro products pvt ltd, India and HPLC grade methanol were purchased from Merck, Mumbai.

Apparatus and chromatographic conditions

Analysis was performed on Agilent 6890 with auto sampler. Column used was Agilent DB-1 30mx0.32x1.0 µm. We had used different column like DB-5,DB-624, but we could n't obtained a separation with other ingredients, so we had chosen DB-1 column.

The carrier gas was nitrogen and the injector temperature was at 240 °C and the oven temperature was kept 100°C initial for 2minutes, then raise up to 240°C for (25mins) at the rate of 10°C. The carrier gas flow was kept 1ml/min. The carrier flow was kept constant. Hydrogen flow & zero air flow were kept in the ratio of 1:10(i.e. 30:300) and make up flow was 25mL/min and the FID detector temperature was kept 260°C.

Standard solutions

A stock solution was prepared by dissolving 25mg of menthol in diluent (methanol) in10ml volumetric flask.

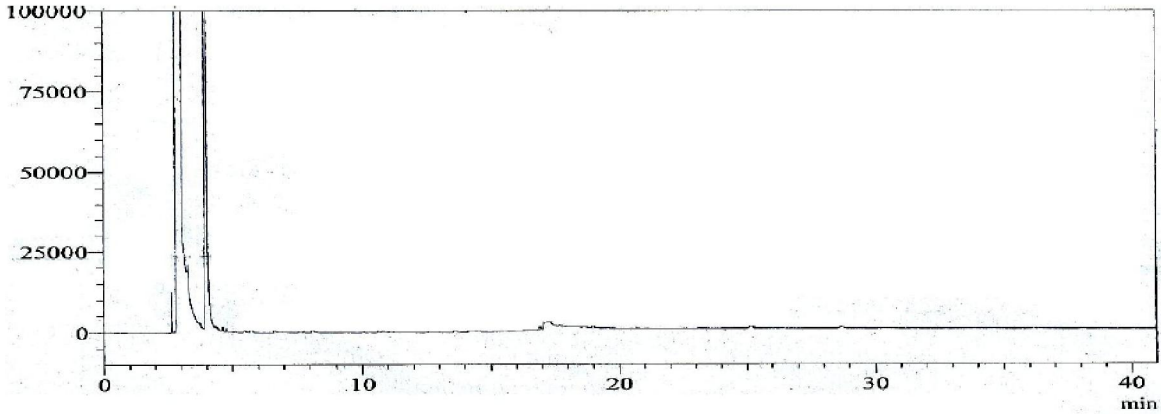

Figure 2 : Blank

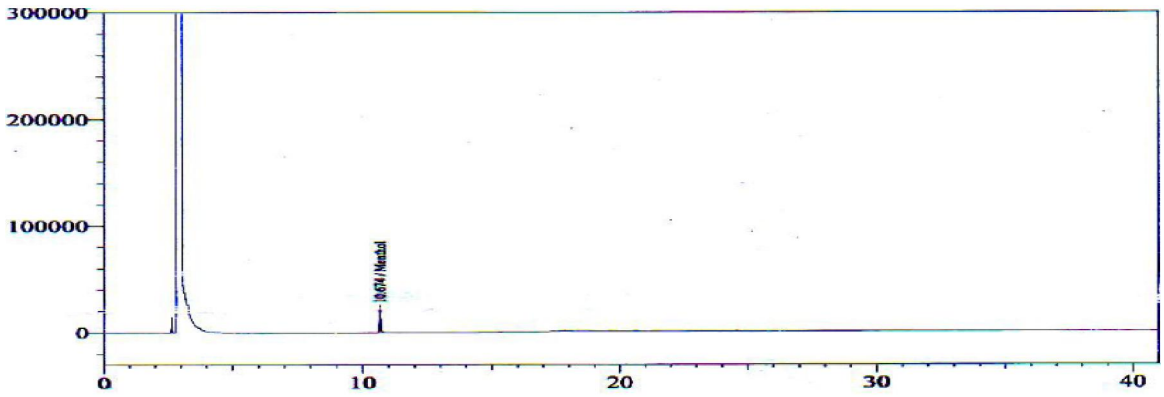

Figure 3 : Standard

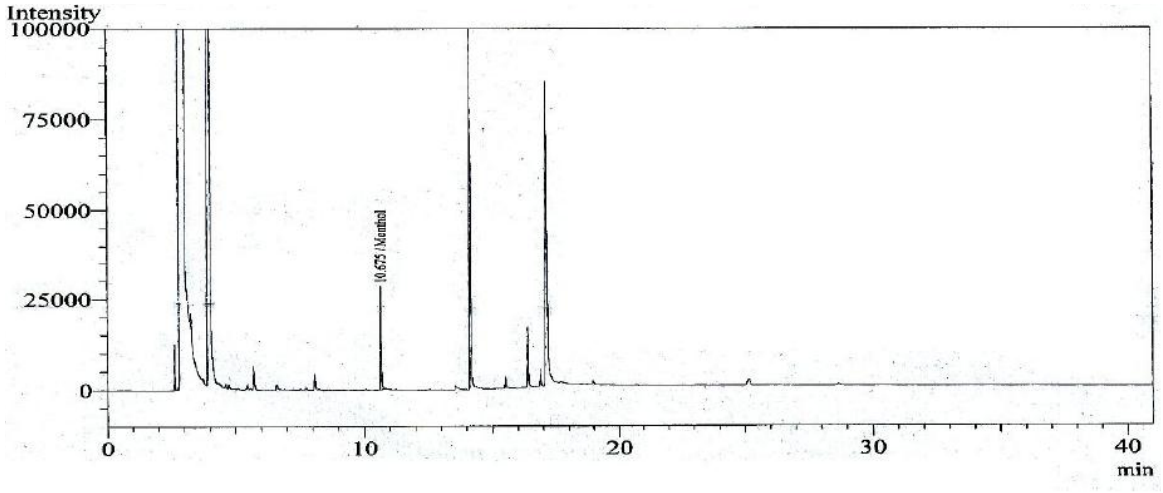

Figure 4 : Menthol content for Label claim 1.0mg per 5ml

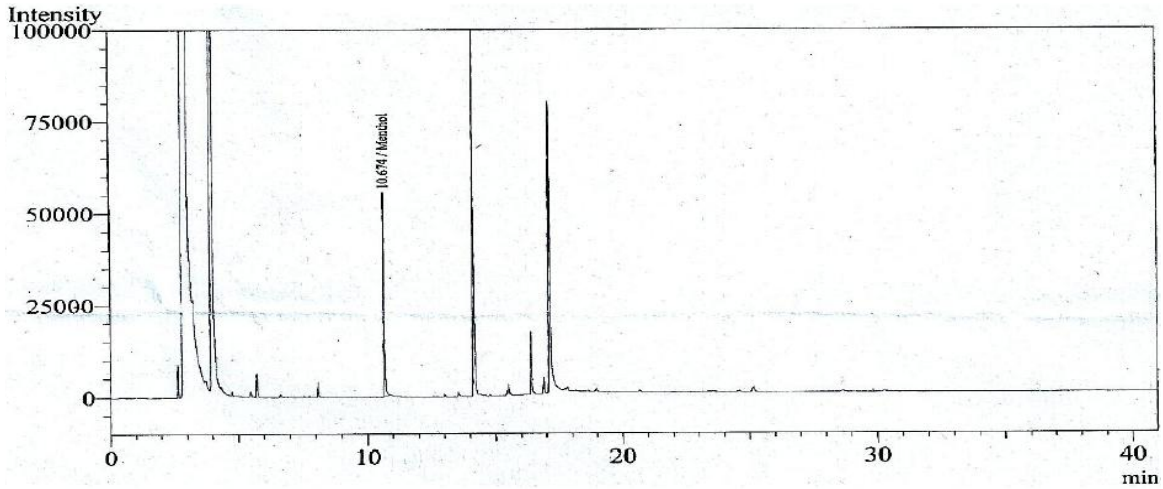

Figure 5 : Menthol content for label claim 2.5mg per 5ml

TABLE 1 : The Relative standard Deviation for menthol content

Relative standard Deviation	
	Area of menthol
%Purity	99.8
Wt(mg)	25.2
1	64.254
2	64.372
3	64.113
4	63.187
5	61.874
6	61.957
Mean	63.293
SD	1.15
%RSD	1.8
Label claim(per5ml)	1.0
Density(gm/ml)	1.19

Further 1ml of the solution in 50ml volumetric flask containing 10ml diluent and then dilute up to the mark with diluent (methanol). This solution was used as final standard.

Sample preparation

Depending upon the percentage of menthol in the sample solution with respect to density, was weighed and dissolved in 50ml volumetric flask containing 20ml diluent and diluted up to the mark with diluent.

Evaluation of system suitability

Injected the blank solution and recorded the chromatogram. Blank*:* Diluent (Methanol)

Injected standard solution (Six replicates) using the

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TABLE 2 : Calculation of menthol content for label claim 1.0mg per 5ml

S.No	Wt of Sample taken	Area of Menthol in Sample	Content of menthol (mg/5ml)	Content of Menthol (%label Claim)	Mean Menthol (mg/5ml)	Mean Menthol (% label claim)
1	14.8758	60.912	0.968	96.8	0.935	93.5
		56.742	0.902	90.2		
2	14.8764	57.619	0.916	91.6	0.917	91.7
		57.780	0.918	91.8		
3	14.8757	57.295	0.911	91.1	0.917	91.7
		58.081	0.923	92.3		
4	14.8759	63.201	1.004	100.4	1.006	100.6
		63.375	1.007	100.7		
5	14.8766	59.789	0.950	95.0	0.949	94.9
		59.695	0.948	94.8		
6	14.8755	64.100	1.019	101.9	1.018	101.8
		63.895	1.016	101.6		
7	14.8762	55.857	0.888	88.8	0.892	89.2
		56.344	0.895	89.5		
8	14.8752	55.924	0.889	88.9	0.885	88.5
		55.352	0.880	88.0		
9	14.8689	59.485	0.946	94.6	0.948	94.8
		59.773	0.950	95.0		

TABLE 3 : Accuracy or Recovery% of menthol content for label claim 1.0mg per 5ml

S.No	Wt of Sample taken	Area of Menthol in Sample	%Recovery of menthol	%Mean Recovery
1	14.7718	120.124	106	105
		118.230	105	
2	14.4764	116.112	102	102
		114.365	101	
3	14.8557	122.112	109	108
		120.212	106	

TABLE 4 : The Relative standard Deviation for menthol content

Relative standard Deviation	
	Area of menthol
%Purity	99.8
Wt(mg)	25.3
1	71.788
2	70.567
3	69.033
4	69.098
5	68.423
6	68.383
Mean	69.549
SD	1.35
%RSD	1.9
Label claim(per5ml)	2.50
Density(gm/ml)	1.23

above GC parameters should comply with the system suitability criteria. The relative standard deviation for the peak area of menthol for six replicates of the standard should not be more than 15%. The retention time of the menthol is ≈ 11.0 min. As Mentioned in the chromatography condition the peak is well resolved. In the quantification of this analysis the principal peak was assumed to be the concentration of Menthol with respective sample concentration.

Calculation:

(Menthol, mg/5ml=AS/As X WS/10 X 1/50 X 50/ Wt X Wml/100 X P X 5 (Menthol label claim)= AS/As X WS/10 X 1/50 X 50/Wt X Wml/LC X P X 5 Where,

AS = Mean area of the peak due to menthol in sample.

As = Mean area of the due to menthol in standard

WS = Weight of menthol working standard taken for standard preparation.

TABLE 5 : Calculation of menthol content for label claim 2.5mg per 5ml

S.No	Wt of Sample taken	Area of Menthol in Sample	Content of menthol (mg/5ml)	Content of Menthol (%label Claim)	Mean Menthol (mg/5ml)	Mean Menthol (%label claim)
1	5.0365	60.297	2.662	106.5	2.632	105.3
		58.916	2.601	104.0		
2	5.0661	61.267	2.689	107.6	2.677	107.1
		60.698	2.665	106.6		
3	5.0144	57.654	2.557	102.3	2.579	103.2
		58.646	2.601	104.0		
4	5.0467	58.658	2.585	103.4	2.590	103.6
		58.892	2.595	103.8		
5	5.0578	61.055	2.662	106.5	2.648	106.0
		59.959	2.634	105.4		
6	5.0835	61.055	2.671	106.8	2.647	105.9
		59.959	2.625	104.9		

Wt = Weight of sample taken for sample preparation in g.

Wml = Weight per ml of syrup in g LC = label claim of menthol in the syrup in mg per 5 ml

P = purity of Menthol working standard.

RESULTS AND CONCLUSION

We had acquired different cough syrups available in the market to analyze the menthol content. The Relative standard deviation (RSD) of the menthol content in standard was 1.8%. We had calculated different cough syrups containing the menthol content with respective to their label claims. The results obtained for the menthol content present were in the range of 91 % to 101 % with respective to 1mg menthol per 5ml and the results for 2.5mg per 5ml menthol were 102 to 107 %. The accuracy results for label claim per 5ml in 1mg menthol recovery were in the range of 105 to 108% shows with in acceptance range of 90 to 120%. The results were shown in the TABLE 1-5 Application of the method*:* The method was developed to separate and determine the percentage content of menthol in cough syrup and also its accuracy studies.

CONCLUSION

A precise gas chromatographic method has been developed for the determination of menthol content in cough syrup. In this method methanol was used as diluent. The results of recovery studies were statistically evaluated for its method precision and accuracy study. The gas chromatography method is less expensive and gives an accurate and precise result. In this method we had attained well separation with other ingredients and the accuracy level obtained were in the range of 95 to 105%.

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