Available Online at

http://www.ijcpa.in

April-June 2016

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-3 | Issue-3 | Article ID: 973

ANALYTICAL METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF METHYL SALICYLATE, MENTHOL, THYMOL AND CAMPHOR IN AN OINTMENT AND ITS VALIDATION BY GAS CHROMATOGRAPHY

Madhusudan T. Bachute[1], S.V.Shanbhag[2]

[1]KarmaveerBhauraoPatilMahavidyalaya,Pandharpur,Solapur University, Solapur-413304, India.

[2]Wallace Pharmaceuticals Pvt. Ltd, R & D Department, Ponda, Goa- 403409, India.

***Corresponding Author:** svshanbag@gmail.com*

Received: 5 March 2016 / Revised: 14 April 2016 / Accepted: 17 April 2016 / Available online : 30 June 2016

ABSTRACT

A simple, precise and fast Gas Chromatographic method has been developed first time for the simultaneous determination of Methyl Salicylate, Menthol, Thymol and Camphor in an ointment formulation. Chromatographic separations of the four drugs were performed on fused silica column of length 30meters, diameter 0.53mmwith ethylene glycol film thickness 1µm as stationary phase. Nitrogen was flown through the column as carrier gas at a flow rate of 5.0mL/min. The initial oven temperature was 120°C (5 min) and raised at the rate of 15°C per minute to final temperature 200°C(3min.). Detector and injection temperatures were fixed at 270°C and 230°C respectively. The injection volume was 1 µl with run time 20 minutes .The linearity of Methyl Salicylate, Menthol, Thymol and Camphor were in the range of 0.1-0.15 mg/ml,0.1-0.15 mg/ml, 0.02-0.03 mg/ml and 0.2-0.3 mg/ml respectively. The recovery was calculated by standard addition method and the average recovery was found to be 100.629%, 100.417%, 100.086% and 100.045% for Methyl Salicylate, Menthol, Thymol and Camphor respectively. The proposed method is simple, accurate, precise and rapid for the simultaneous estimation of these four actives.

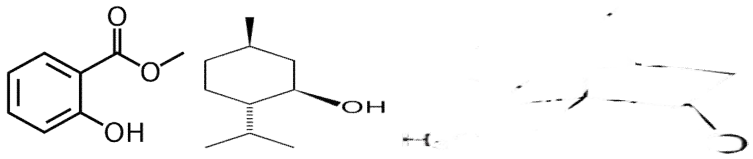
Keywords *–Gas Chromatography, Methyl Salicylate, Menthol, Thymol and Camphor, Silica column.*

1. INTRODUCTION

Methyl Salicylate, Menthol, Thymol and Camphor are the active principles in many topical formulations commonly used for the treatment of rheumatic diseases due to their analgesic and anti- inflammatory properties. It is observed that these four are mostly used in ointments and certain gel preparations with hydrocarbon base (white soft paraffin) or ester bases. It is very difficult to estimate these actives due to their similar physical and chemical properties, such as volatility and solubility. Another difficulty is a larger disproportion in label claim combinations. So Chromatographic separation methods are recommendedfor the analysis of these constituents. From literature search, it is found that Gas Chromatography (GC) would be a preferred method for simultaneous analysis. The GC method is useful for determining Menthol and Methyl Salicylate in solid and liquid formulations, natural products and biological materials. After reviewing various articles on GC it was concluded that capillary column could be suitable for simultaneous identification of active substances as well as purity evaluation in the presence of matrix constituents. To confirm suitability of method

for routine analysis method has been validated as perICH guidelines. All validation parameters meet requirements laid down in ICH guidelines. This method can be easily adopted for routine quality analysis for simultaneous determination of Methyl Salicylate, Menthol, Thymol and Camphor.

The structural formulae of these four actives are given in Fig.1.



Methyl Salicylate Menthol Thymol Camphor

Fig 1 : Structural formulae of Methyl Salicylate, Menthol, Thymol and Camphor.

In this paper, we report a GC method for simultaneous estimation of Methyl Salicylate (5%), Menthol (5%), Thymol (1%) and Camphor (10%) in a commercial ointment formulation. The method described here is found to be simple, precise and accurate for simultaneous determination of these four components.

2. MATERIALS AND METHODS

Gas Chromatographic method was used to estimate four actives simultaneously. Gas Chromatograph of Perkin Elmer make equipped with FID detector was used with following parameters .

2.1 Chromatographic parameters:

Column used: 30-m x 0.53-mm fused silica column coated with 1-µm layer of polyethylene glycol, modified with nitro terephthalate, as stationary phase. DB-FFAP or equivalent column was used, which met the experimental requirements "Evaluation of system suitability".

Oven temp.: Initial 120°C for 5 minutes, Final 200°C for 3 minutes

Temperature rise rate 15°C per minute

Time of run: Initial 5 minutes, final 3 minutes.

Injector temp. 250°C

Detector temp. 270°C

Carrier Gas (Nitrogen) flow 5 ± 0.05 ml/min.

Auxiliary Gas (Nitrogen) flow 20 ±1 ml/min

Injection Volume 1 µl

The pure N,Ndimethylformamide, Methyl Salicylate, Menthol, Thymol and Camphor from Aldrich were used. All the solutions were freshly prepared and used.

2.2 Preparation of diluent

Analytical grade N,N-dimethylformamide was used as diluent.

2.3 Preparation of Internal standard solution

Accurately weighed 0.50 gm of 2-phenyl-2-propanol wasdissolved in diluents in a 25 ml volumetric flask and diluted up to the mark with the diluent.

2.4 Preparation of Sample solution

Accurately weighed 5.00 g of ointment sample was transferred to a 100 ml volumetric flask containing about 20 ml of n,n-dimethylformamide. The ointment was dissolved by warming and shaking the flask. The total volume of 100 ml was made by adding the diluent.5.0 ml of the resulting solution and 1.0 ml of internal standard solution was transferred to a 100 ml volumetric flask and diluted to 100 ml with the diluent.

2.5 Preparation of standard solution

Accurately weighed 0.250 gm of Menthol, 0.500 gm of Camphor, 0.050 gm of Thymol, 0.250 gm of Methyl Salicylate were dissolved in 20 ml diluent in a 100mL volumetric flask and diluted up to mark with the diluent.5.0 ml of the resulting solution and l.0 ml of internal standard solution were transferred to a 100 ml volumetric flask and diluted 100 ml with the diluent.

2.5 Procedure

Evaluation of system suitability: The system suitability solution (standard) was injected in the chromatograph and the chromatograms were recorded.

The system was suitable for analysis, since the resolution between 2-phenyl-2-propanol and methyl salicylate peaks was not less than 1.5.

The standard and sample solutions were injected in duplicate into theequilibratedgas chromatograph and chromatograms were recorded. The retention time of menthol peak was about 5 minutes.

The relative retention times (RRT's) of the major peaks were determined by separately injecting standard solutions of each item (prepare individually) in the chromatograph.

2.6 Calculations

The content of the active ingredient in the sample solution was calculated by using following formula

$$X_i = \frac{AT \times WS \times 5X100 \; X100 \; XPX \; 100}{AS \times 100 \times 100 \; XWTX \; 5X100},$$

Where:

X : Content of active ingredient (% w/w)

AT : Average ratio of peak area counts of active ingredient to that of 2- phenyl-2-propanol in the Sample solution

AS : Average ratio of peak area counts of active ingredient to that of of 2-phenyl-2-propanol in the standard solution

WS : Weight of working standard in gram

WT : Weight of ointment in gram

P : Purity of working standard in (%)

2.7 Standards used:

a) Menthol (Mfg. By: Ruchi Aromatics Chemicals)

Batch No: 32 ;Purity: 99.7%

b) Thymol (Mfg.By :Ruchi Aromatics Chemicals)

Batch No: 20; Purity: 99.5%

c) Methyl Salicylate (Mfg. By: Salicylates & Chemicals Pvt. Ltd.)

Batch No: E350; Purity: 99.95%

d) Camphor (Mfg. By: Camphor & Allied Products Ltd.)

Batch No: 013; Purity: 100.0%

2.8 Method Validation

2.8.1 Precision

The precision of the analytical method was carried out by performing the assay six times for six different aliquots of a homogeneous sample by a single analyst within the laboratory over a short period of time and using identical reagents and equipment using the method in 5.0. Calculation equation.

Recorded the weights, readings and the values in the format of PRECISION.

Calculated the mean & Relative standard deviation, % RSD.

2.8.2 Linearity

Linearity of the analytical method for the test of assay was studied using the Standard in the required proportions. Series of solutions of standard ranging from 80% to 120% of the final test concentration. (i.e. 80%, 90%, 100%, 110%,120%) were prepared.

Table – 1 : Data of linearity study

Component	Weight of component gm	Percentconcentration	Total volume by adding diluent , mL
Menthol	0.200	80	100
Camphor	0.400		
Thymol	0.040		
Methyl salicylate	0.400		
Menthol	0.225	90	100
Camphor	0.450		
Thymol	0.045		
Methyl salicylate	0.225		
Menthol	0.250	100	100
Camphor	0.500		
Thymol	0.050		
Methyl salicylate	0.250		
Menthol	0.275	110	100
Camphor	0.550		
Thymol	0.055		
Methyl salicylate	0.275		
Menthol	0.300	120	100
Camphor	0.600		
Thymol	0.060		
Methyl salicylate	0.300		

55.0 ml of the resulting solution and l.0 ml of internal standard solution were transferred to a 100 ml volumetric flask and diluted with additional diluent to make total volume 100mL. These solutions were used for linearity study.

The absorbance/areas of the solutions were determined as per the assay procedure. The values were recorded in the format of the linearity study. The graph of Concentration in mg/ml v/s Absorbance was plotted.

From the slope, coefficient of correlation of the regression was calculated.

Table-2 : Data of linearity study of Menthol

Conc.(mg/mL)	Abs/ Area
0.1000	0.4753
0.1125	0.5387
0.1250	0.5984
0.1375	0.6641
0.1500	0.7251

Correction Factor: 1.000

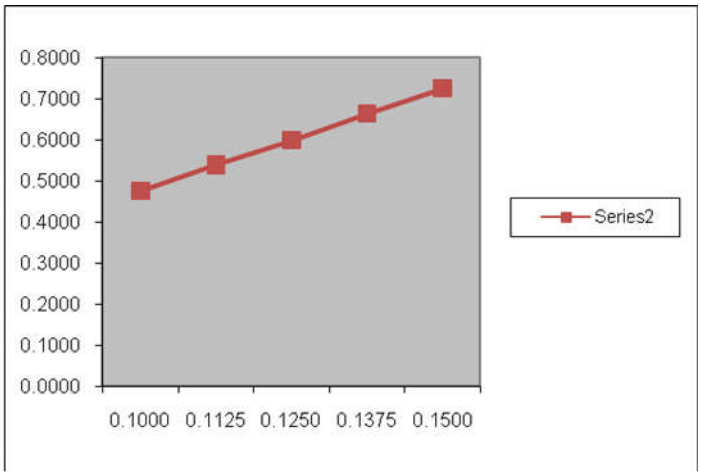


Fig 2: Linearity curve of Menthol

Table-3 : Data of linearity study of Thymol

Conc.(mg/mL)	Abs/ Area
0.0200	0.0895
0.0225	0.1030
0.0250	0.1154
0.0275	0.1282
0.0300	0.1395

Correction. Factor 1.000

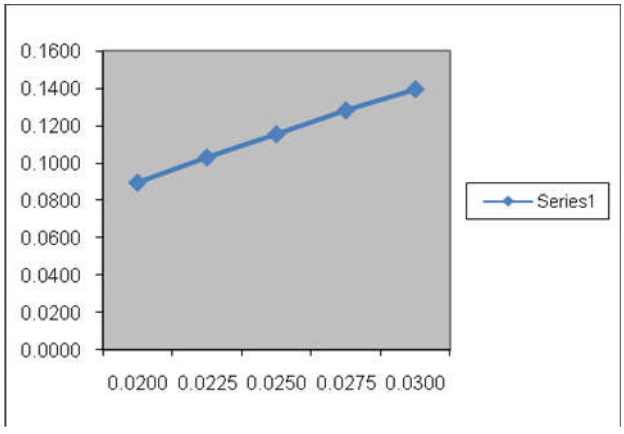


Fig 3: Linearity curve of Thymol

Table-4 : Data of linearity study of Methyl salicylate

Conc.(mg/mL)	Abs/ Area
0.1000	0.2900
0.1120	0.3326
0.1250	0.3640
0.1375	0.4082
0.1500	0.4488

Correction Factor :0.999

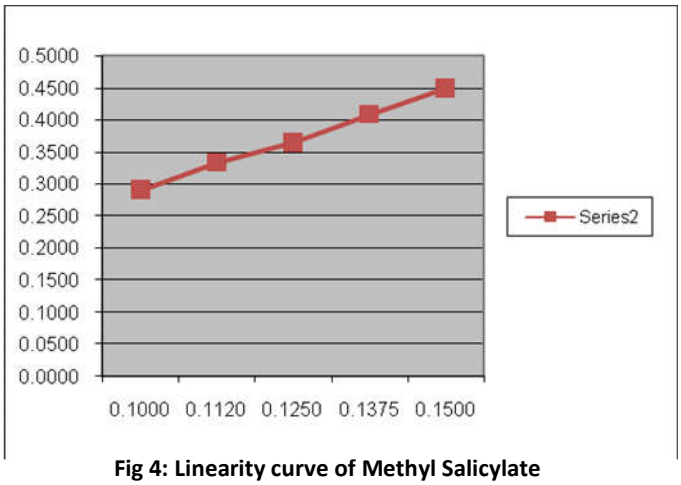


Fig 4: Linearity curve of Methyl Salicylate

Table-5 : Data of linearity study of Camphor

Conc.(mg/mL)	Abs/ Area
0.200	0.9204
0.225	1.0354
0.250	1.1872
0.275	1.2742
0.300	1.4037

Correction Factor: 0.99

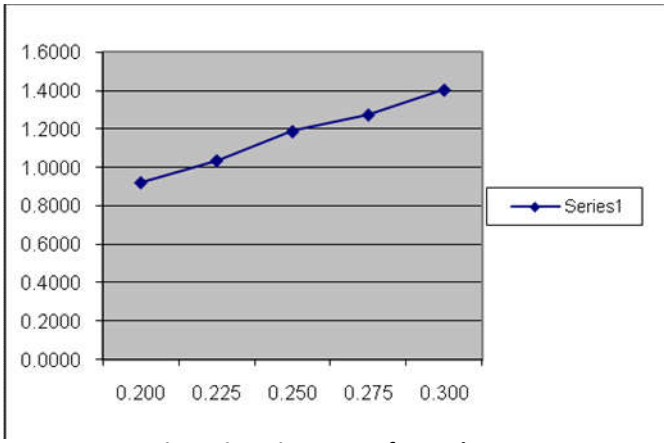


Fig 5: Linearity curve of Camphor

2.8.3 Accuracy

Accuracy is determined by using a minimum of nine determinations over a minimum of three concentration levels in the range 80% to 120% of the test Concentration and three replicates of each concentration. A known quantity of standard (of known purity) was added to the drug product (Placebo) within the required range. Nine determinations, three in each level i.e. 80%, 100% and 120% were prepared and the assay of the nine determinations was carried out as in linearity. Nine determinations, three in each level i.e. 80%, 100% and 120% were prepared.

Table – 6 : Data of Accuracy study

Component	Weight of component gm	Level (Percen	Total volume by adding diluent , mL
Placebo	5.000	80	100
Menthol	0.200		
Camphor	0.400		
Thymol	0.040		
Methyl salicylate	0.200		
Placebo	5.000	100	100
Menthol	0.250		
Camphor	0.500		
Thymol	0.050		
Methyl salicylate	0.250		
Placebo	5.000	120	100
Menthol	0.300		
Camphor	0.600		
Thymol	0.060		
Methyl salicylate	0.300		

5.0 ml of the resulting solution and l.0 ml of internal standard solution were transferred to a 100 ml volumetric flask and diluted with additional diluent to make total volume 100mL. These solutions were used for accuracy study

From the standard weights, amount added and amount found from ACCURACY STUDY, the mean percentage recovery and the RSD for all the four actives were obtained as shown in the following tables.

Table-7 : Results of recovery study for methyl salicylate

Sr. No	Standard Weight	Amount added (mg/gm)	Amount found (mg/gm)	Recovery	RSD	Level
1	252.9000	199.8000	199.30000	99.7497		
2	252.9000	203.6000	203.60000	100.7422	0.6197	Level -1
3	252.9000	202.4000	202.40000	100.8973		
1	252.9000	253.6000	253.60000	101.1971		
2	252.9000	254.4000	254.40000	100.4739	0.5292	Level -2
3	252.9000	252.6000	252.60000	100.1586		
1	252.9000	308.3000	308.30000	100.4562		
2	252.9000	311.6000	311.60000	101.0376	0.3100	Level -3
3	252.9000	309.6000	309.60000	100.9455		

Mean Recovery: 100. 6287 %, RSD: 0.4863 (NMT2.0%)

Table-8 : Results of recovery study for menthol

Sr. No	Standard Weight	Amount added (mg/gm)	Amount found (mg/gm)	Recovery	RSD	Level
01	0.2498	0.2029	0.205800	101.4293		
02	0.2498	0.2058	0.207100	100.6317	0.5369	Level -1
03	0.2498	0.2017	0.202500	100.3966		
01	0.2498	0.2534	0.253300	99.9605		
02	0.2498	0.2489	0.248500	99.8393	0.3567	Level -2
03	0.2498	0.2552	0.256500	100.5094		
01	0.2498	0.3052	0.306700	100.4915		
02	0.2498	0.3035	0.304300	100.2636	0.1409	Level-3
03	0.2498	0.3008	0.301500	100.2327		

Mean Recovery: 100.4172 % ; RSD: 0.3448 (NMT 2.0 %)

Table-9 : Results of recovery study for thymol

Sr. No	Standard Weight	Amount added (mn/gm)	Amount found (mg/gm)	Recovery	RSD	Level
01	50.1000	38.8000	39.000000	100.5155		
02	50.1000	39.8000	39.500000	99.2462	0.7310	Level -1
03	50.1000	39.1000	39.300000	100.5115		
01	50.1000	49.8000	49.400000	99.1986		
02	50.1000	48.8000	49.000000	100.4098	0.6518	Level-2
03	50.1000	50.0000	49.700000	99.400		
01	50.1000	60.3000	60.30000	100.0000		
02	50.1000	60.1000	60.80000	101.1647	0.5969	Level-3
03	50.1000	60.0000	60.20000	100.3333		

Mean Recovery: 100.0864 %; RSD: 0.6599 (NMT 2.0%)

Table-10 : Results of recovery study for Camphor

Sr. No	Standard Weight	Amount added (mn/gm)	Amount found (mg/gm)	Recovery	RSD	Level
01	0.5002	0.4002	0.400800	100.1499		
02	0.5002	0.4001	0.401200	100.2749	0.4238	Level -1
03	0.5002	0.4035	0.407300	100.9418		
01	0.5002	0.5013	0.500200	99.7806		
02	0.5002	0.5003	0.497600	99.4603	0.4349	Level -2
03	0.5002	0.5007	0.502300	100.3196		
01	0.5002	0.5998	0.595500	99.2831		
02	0.5002	0.6027	0.603500	100.1327	0.4734	Level -3
03	0.5002	0.5999	0.600300	100.0667		

Mean Recovery: 100.455% ; RSD: 0.4440 (NMT 2.0%)

2.8.4 Ruggedness

The ruggedness of the analytical method for test for assay was studied by analyzing same sample by two different analysts using the same instrument. Each analyst analysed the same sample twice. The results of analysis were entered in the format of RUGGEDNESS STUDY. Calculated the RSD of the results.

2.8.5 Specificity

An appropriate quantity of the placebo of the formulation was taken and subjected to the entire procedure of the determination of assay of the method. Similarly the assay was carried out for the standard. Specificity was calculated as percentage interference of the placebo. Results of the test were complied in Specificity (Placebo study).

Elution order remains as Camphor ,Menthol ; Internal Standard ; Methyl Salicylate ; Thymol

Table-11: Typical Elution order shown as follows

Sr. No	Name of Component	Retention time
1	Camphor	About 6.0 min
2	Menthol	About 7.4 min
3	Internal Standard	About 8.9 min
4	Methyl Salicylate	About 9.3 min
5	Thymol	About 13.7 min

Retention time of Camphor ,Menthol ; Internal Standard ; Methyl Salicylate ; Thymol is purely dependent on the presence of Camphor ,Menthol ; Internal Standard ; Methyl Salicylate ; Thymol respectively, and not due to of other ingredient from the formulation placebo .Resolution between the peaks is also not less than 1.5 so there is no any interference from any ingredient of the formulation. Hence identification test found specific, pure for Camphor, Menthol; Internal Standard; Methyl Salicylate; Thymol respectively and it is found without any interference from formulation.

2.8.6 Robustness

The assay of the sample under normal conditions of the method parameters was determined. Assay of the same sample was determined again by a slight variation of one method parameter, such as flow rate, mobile phase, concentration, pH, strength of solutions, temperature, reaction time, etc. The percentage deviation of the assay from the original assay wasfound out and recorded .The Precision assay may also be considered to compare with the assay with change in parameters. Another assay was performed by slight variation of another method parameter and deviation was found out. Results of the test were recorded.

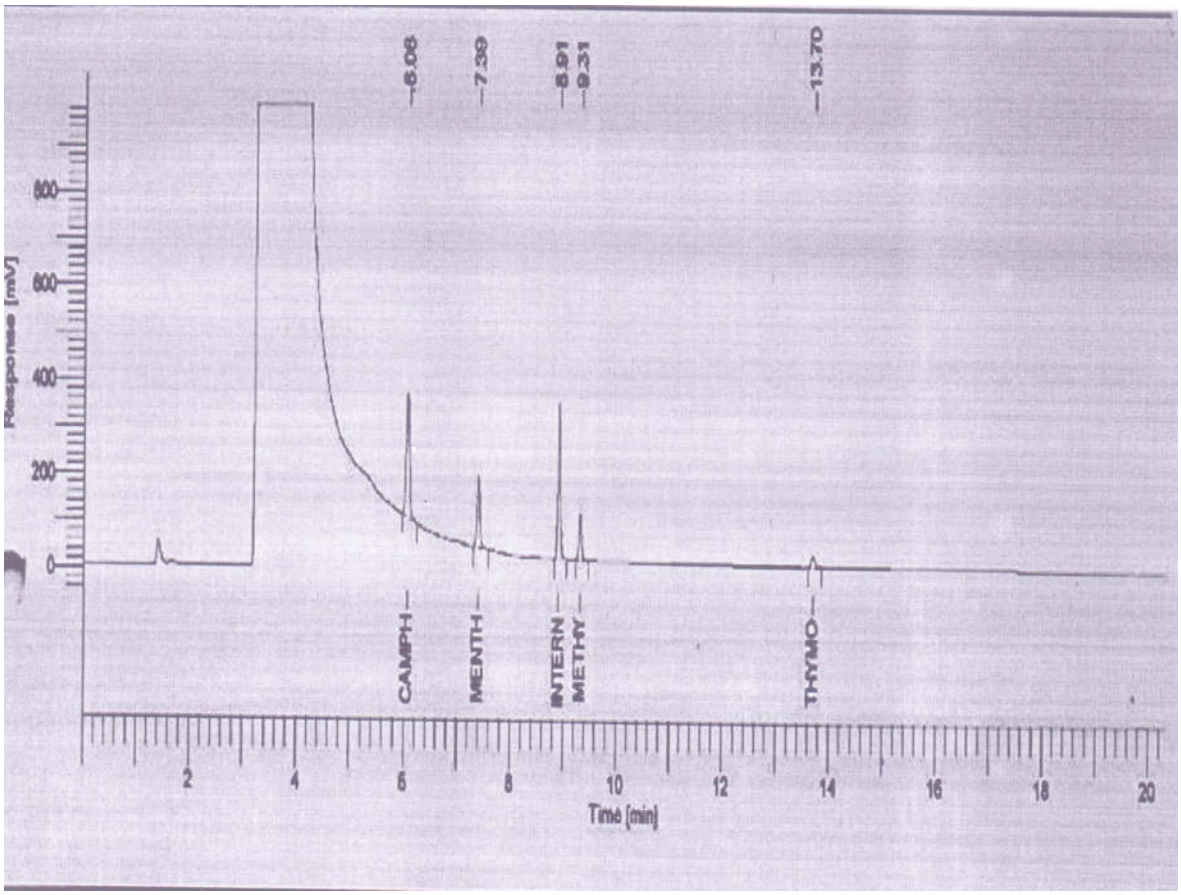


Fig 6: Typical Gas Chromatograph
Camphor (6.06); Menthol (7.39); Internal Standard (8.91); Methyl Salicylate (9.31); Thymol (13.70)

Table-12 : Acceptance Criteria

Sr.no	Parameters	Acceptance limits(assay)
1.	Precision	R.S.D NMT 2.00 %
2.	Linearity & Range	r>0.997
3.	Accuracy (% Recovery)	98-102%
4.	Ruggedness	R.S.D NMT 2.0%
5.	Specificity Study (Placebo study)	NMT 1.0%
6.	Robustness	+/-2.0% R.S.D NMT 2.00%

r :Correlation Coefficient ,% RSD: % Relative Standard Deviation

2.9 Summary

Summary of the results of completed analytical method validation study are given in the table 13.

Table-13 : Summary report

Sr.No	Performa Criteria	Active Ingredient	Assay Content (% /mg /gm) (%)	R SD (%)	Recovery (%)	Slope of Line (%)	Correction Coefficient	% of Placebo Interfere-nce	Assay on (%/mg/ gm)	Deviation From Orig. Assay NMT 2.0%)
01	Precision	Camphor	10.3250	1.4492						
01	Precision	Menthol	5.4283	0.4105						
01	Precision	Methyl Salicylate	5.2500	1.7122						
01	Precision	Thymol	1.0300	1.8421						
02	Linearity	Camphor				4.7173	1.0000			
02	Linearity	Menthol				5.0347	1.0000			
02	Linearity	Methyl Salicylate				3.1520	1.0000			
02	Linearity	Thymol				5.1600	1.0000			
03	Accuracy	Camphor		0.4440	100.0455					
03	Accuracy	Menthol		0.3448	100.4172					
03	Accuracy	Methyl Salicylate		0.4863	100.6287					
03	Accuracy	Thymol		0.6599	100.0864					
04	Ruggedness	Camphor		10.3325	1.3854					
04	Ruggedness	Menthol		5.3850	0.9285					
04	Ruggedness	Methyl Salicylate		5.2400	1.2368					
04	Ruggedness	Thymol		1.0350	0.5578					
05	Specificity	Camphor						NIL		
05	Specificity	Menthol						NIL		
05	Specificity	Methyl Salicylate						NIL		
05	Specificity	Thymol						NIL		
06	Robustness	Camphor	10.3300						10.3050	-0.2420
06	Robustness	Menthol	5.4300						5.3350	-1.7496
06	Robustness	Methyl Salicylate	5.2500						5.1900	-1.1429
06	Robustness	Thymol	1.0300						1.0350	0.4854

3. CONCLUSION

Extensive literature search revealed that no method is reported for simultaneous determination of these four compounds in an Ointment or Gel formulation. The proposed method would be the only method for simultaneous determination of Menthol, Methyl Salicylate ,Thymol and Camphor from a topical formulation. The method validation parameters meet the recommended requirements of ICH guidelines. This method can be adopted easily for routine analysis in pharmaceutical industries.

4. ACKNOWLEDGEMENT

Authors would like to thank M/s Wallace Pharmaceuticals Private Ltd, Goa for providing resources for formulation and subsequent analysis.

5. REFERENCES

1. Sharma, B. K. *Instrumental Methods of Chemical Analysis.* 24th Edition, Goel Publishing House, Meerut. 2005, -3-8.
2. Braun, R. D. *Introduction to Instrumental Analysis.* 1st Edition, Pharma Book Syndicate, Hyderabad. 2006, 839-865.
3. Chatwal, G. R.; Anand, S. K. *Instrumental Methods of Chemical Analysis.* 5th Edition, Himalaya Publishing House, New Delhi. 2007,2.624-2.629.
4. Panda S.K., Sharma A.K., and Sahu L.K., *Indian Journal of Pharmaceutical sciences*, (2002)64(6), 540-544.
5. Jain S.K., Jain Deepti,TiwariM.andChaturvedi S.C., *Indian Journal of Pharmaceutical sciences*, (2002), 64(3), 267-270.
6. Singhvi I., Chaturvedi S.C., *Indian drugs*, (1998), 35(4), 234-238.
7. Sethi P.D., Qualitative Analysis of drugs in Pharmaceutical Formulations, (1997), 3rd edition, 182-184.
8. Willard Hobart. H., Merritt L.L., Dean John.A., Instrumental Methods of Analysis, 7th edition, CBS Publishers, 580-610.
9. Shankar M.B., Mehta F.A., Bhatt K.K., Mehta R.S., and Geetha M, *Indian Journal of pharmaceutical sciences*, 65(2), 167-170.
10. Jeffery, G. H.; Bassett, J.; Mendham, J.; Denney, R. C. *Vogel's Textbook of Quantitative Chemical Analysis*. 5th Edition, Addison Wesley Longman Inc, Singapore. 2001, 4-5. 11. Krzek j.: Actapol.42.479(1986).
11. Jan Krzek, Januszslawomirczekaj and wlodzimierzrzeszutko, ActaPoloniaePharmaceutica- Drug Research Vol 60 No.5 pp.343-349, 2003.
12. Podelwski J. k, Chwalibogowska-podelwska a.: Lekiwspolczesnejterapii, Split trading Sp.Z.o.o Warsaw,1994.