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Quantitative determination and validation of ethylhexylglycerin using Gas chromatography method

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ABSTRACT

Ethylhexylglycerin, an alkyl glyceryl ether, used in various cosmetics and deodorants is also known to have anti-microbial activity and hence used as an adjuvant along with other preservatives to produce synergistic effect. In the present study, a gas chromatographic method has been employed and validated to determine the presence of impurities along with ethylhexylglycerin. A column having the dimension of DB-1 30m x 0.32mm; 0.25µm with acetone as solvent was found to be optimal for the ideal separation of ethylhexylglycerin from its impurities. Injection volume was set to 1 µl and temperature was maintained at 240°C. A clear peak was observed with the retention time of 8.002 minutes. As per ICH guidelines, the developed method was validated with respect to specificity, sensitivity, Limit of detection, Limit of quantification, linearity, precision, and also stability studies. As per the method, it shows a good correlation co-efficient (R^2) value of 0.999 within the concentration range of 0.1- 0.75µg. Therefore, a gas chromatographic method was proposed which can be precise, specific and can be employed for the determination of ethylhexylglycerin in various pharmaceutical formulations.

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INTRODUCTION

Ethylhexylglycerin is chemically 3-[(2-Ethylhexyl) oxy]-1,2-propanediol. It is an alkyl glyceryl ether having the empirical formula of $C_{11}H_{24}O_3$ and molecular weight of 204.3g·mol−1 and is a globally approved drug marketed as sensiva® SC 50 used in cosmetics. It is known to be used as a good emollient and mild humectants as well as effective deodorant (Beilfuss W *et al*; Leschke M *et al., 2006*) due to its ability to inhibit the growth and multiplication of odor causing bacteria without affecting the beneficial flora of the skin. It has also been used as an additive since it shows synergistic effect with other preservatives (Beilfuss W *et al*; Leschke M *et al;* Langsrud S *et al., 2016;* Gaonkar TA *et al., 2006).* Despite several uses, there has also been reported the allergic reactions such as contact dermatitis (Linsen G *et al., 2002*; Sasseville D *et al., 2014*; Stausbol-Gron B *et al., 2007*) caused by it which is very rare (Aerts O *et al., 2007*). However, it is the most commonly used drug in cosmetics (Leschke M *et al., 2010*). Literature review has shown no methods for the quantitative determination of ethylhexylglycerin. Therefore, in the present study, a gas chromatographic method with FID detection for quantitative determination of impurities and assay content in a formulation containing

Ethylhexylglycerin has been developed and validated which is precise, specific, accurate, linear and robust.

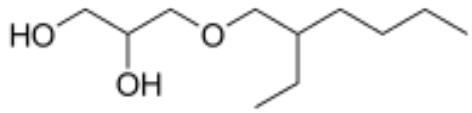

Figure 1: Molecular structure of Ethylhexylglycerin

EXPERIMENTAL

Chemicals and reagents

Acetone which was used as mobile phase was obtained from SDFCL, Mumbai, India. Ethylhexylglycerin was gifted by Salicylates and Chemicals Pvt LTD, 2-Ethylhexanol and Epichlorohydrin which were purchased from MERCK and TCI, India respectively and all the chemicals were of 99.9% pure and were of GC grade. 0.22µ nylon filters were purchased from advanced micro devices, Chandigarh, India and filter paper of 0.45µ size was purchased from Millipore, India.

Instrumentation

Gas chromatographic system (SHIMADZU) Model no: GC-2025, was equipped with an Auto sampler, and FDI detector and was used in the study for the determination of ethylhexyl glycerin. The column used was DB-1 with the dimensions of 30m x 0.32mm; 0.25µm obtained from J&K Scientific with Nitrogen as the carrier gas with a flow rate of 2ml/min. The injector temperature was 240°C and that of detector was 260°C.

METHOD DEVELOPMENT

Literature search reveals there was no analytical method was reported for the determination of Ethylhexylglycerin. Hence it was significant to start the method development using gas chromatography as it is one of the most commonly adopted procedures to determine the presence of Volatile components. The column DB-1 30m x 0.32mm; 0.25µm was used and acetone was used as a solvent because of its evaporative nature. The injection volume of 1µl and the port temperature at 240°C was maintained. The carrier gas used was Nitrogen with a flow rate of 2ml/min. Optimization was obtained based on various parameters such as retention time, number of theoretical plates and resolution.

Preparation of solutions

Preparation of 2-Ethylhexanol stock solution

500mg of 2-Ethylhexanol was added to 100ml volumetric flask and mixed and then diluted it with 100ml of acetone.

Preparation of 2-Ethylglycedial solution

200mg of 2-Ethylglycedial ether was transferred to 100ml volumetric flask and diluted to volume with acetone and further dilution was made by dissolving the 10ml of the solution to 100ml with acetone.

Preparation of standard solution

Standard solution was prepared my mixing 200mg of Ethylhexylglycerin WS and 1.0ml each of 2-Ethylhexanol stock solution and 2-Ethylhexylglycedial ether stock solution in a 10ml volumetric flask and made up the volume with acetone.

Preparation of sample solution

Quantity of the drug equivalent to 200mg of Ethylhexylglycerin sample was transferred and diluted with acetone in a 10ml volumetric flask.

Method Development and optimization

Acetone of GC grade was found to be the most suitable solvent for ideal separation of Ethylhexylglycerin since there was no interference found with it. The sample was injected with an injection volume of 1 µl and the injector port temperature of 240°C was maintained without variation and the carrier gas with the flow rate of 2ml/min was used. The column was saturated by pumping acetone through it for at least 30 minutes prior to the injection of drug solution. 20µl of standard and sample solutions were injected in to the chromatographic system and the area for the peak were measured. The run time was set as 27 min. Under these optimized conditions, the retention time for the drug was observed to be 8.002 minutes.

Method validation

As per ICH guidelines (2005), the developed and optimized method were subjected to various validation parameters such as specificity, sensitivity, Limit of detection (LOD), Limit of quantification (LOQ), linearity, precision, accuracy and robustness as well as stability studies.

System Suitability

System suitability was evaluated by injecting the Standard solution in to the optimized method using the GC mode of analysis. Peak areas for all six standard chromatograms are measured. System suitability results are tabulated in Table 1 & 2 and the chromatogram is shown in the figure no: 1.

Specificity

The specificity of the analytical method was determined by injecting blank (Acetone) solution alone and looked for any interferences present in it and were compared with LLOQ samples. It was required that no significant interference be seen in the chromatograms at the retention times of

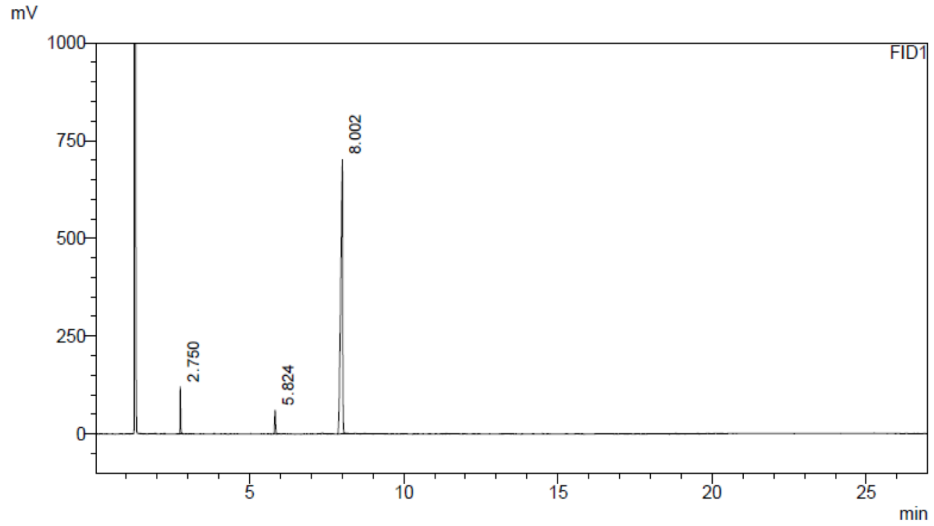

Figure 2: Typical Chromatogram of System suitability from Standard stock solution

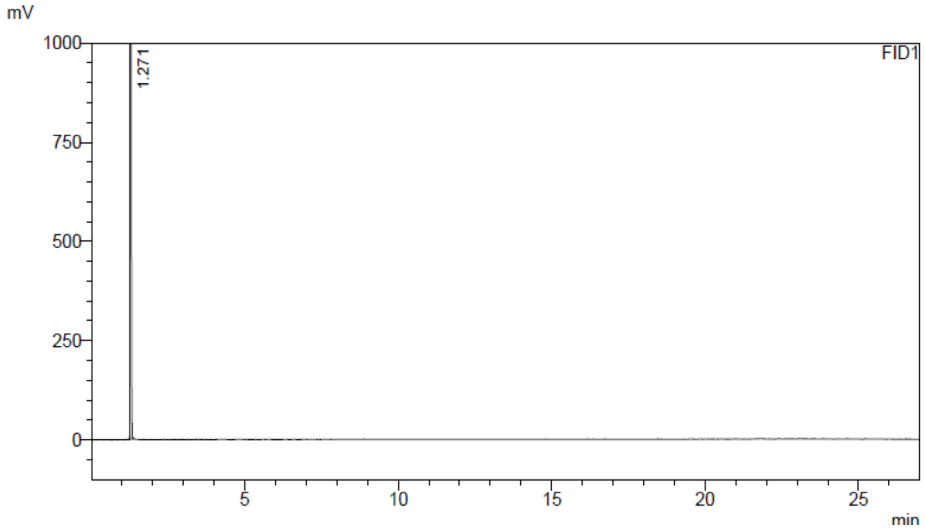

Figure 3: Typical chromatogram of Blank (Acetone as solvent)

Ethylhexylglycerin. This indicates that the solvent does not interfere in the quantification of impurity peak and shows a good resolution. The graphs are shown in the Figure no: 2 and Figure no: 3 and their results are shown in table no: 3 & 4 respectively.

Linearity &Range

The linearity was evaluated by measuring area response for each impurity over the range of Quantitation limit to 0.75% to Specification of impurity. Seven concentrations were prepared across the range and injected then peak area of each solvent was plotted against the concentration. The Correlation coefficient of determination (r^2) obtained for each impurity are listed in Table no: 5.

Limit of detection (LOD) and Limit of quantification (LOQ)

The Limit of detection (LOD) and Limit of quantification (LOQ) for Ethylhexylglycerine were determined by signal-to-noise ratio (S/N) method. The minimum concentration (ppm) at 3:1 S/N (for LOD) and the concentration at 10:1 S/N (for LOQ) are listed in Table 5. Solution containing each solvent was prepared around its LOQ concentration and injected in six replicates. The %RSD value obtained for the area of each solvent was less than 10% at LOQ. Chromatogram of LOQ and LOD are shown in Figure no: 4 & 5 respectively.

Accuracy

Accuracy of the method was validated through recovery experiments by spiking known amount of each impurity at 75%, 100%, and 125% with respect to the sample weight 500mg. Each preparation was analyzed in triplicate (n=3) and percent recovery was calculated. The recovery was found to be between 98% and 102% results are summarized in Table no: 6.

Precision

Repeatability study was carried out by preparing test solution with respect to Ethylhexylglycerin. Peak response for each peak was measured and %

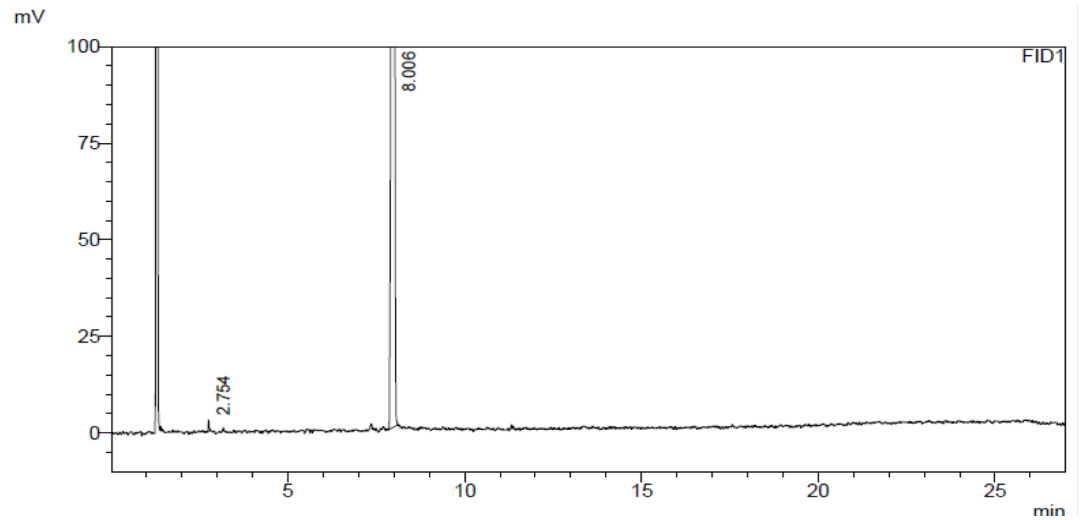

Figure 4: Typical chromatogram of Ethylhexylglycerin

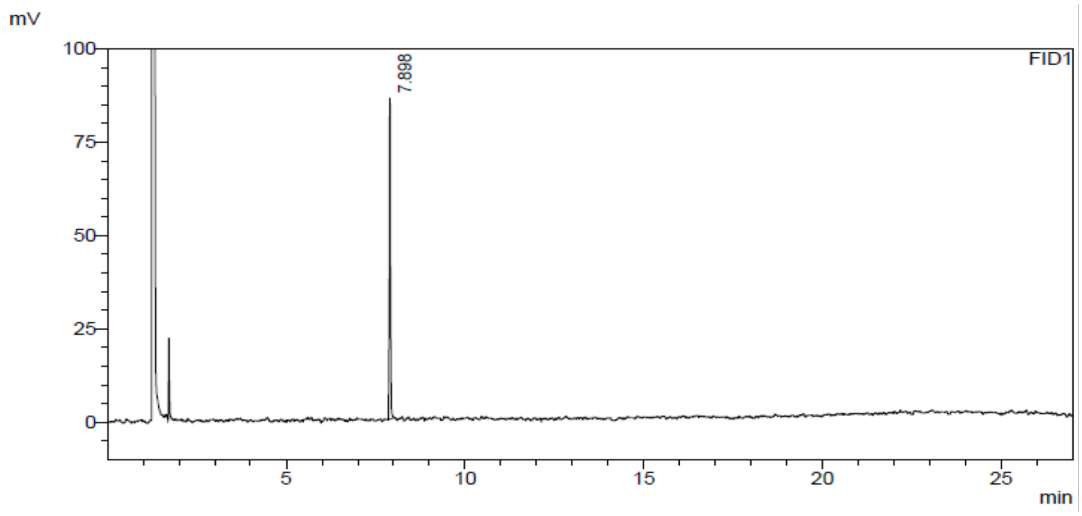

Figure 5: Typical Chromatogram from LOQ solution

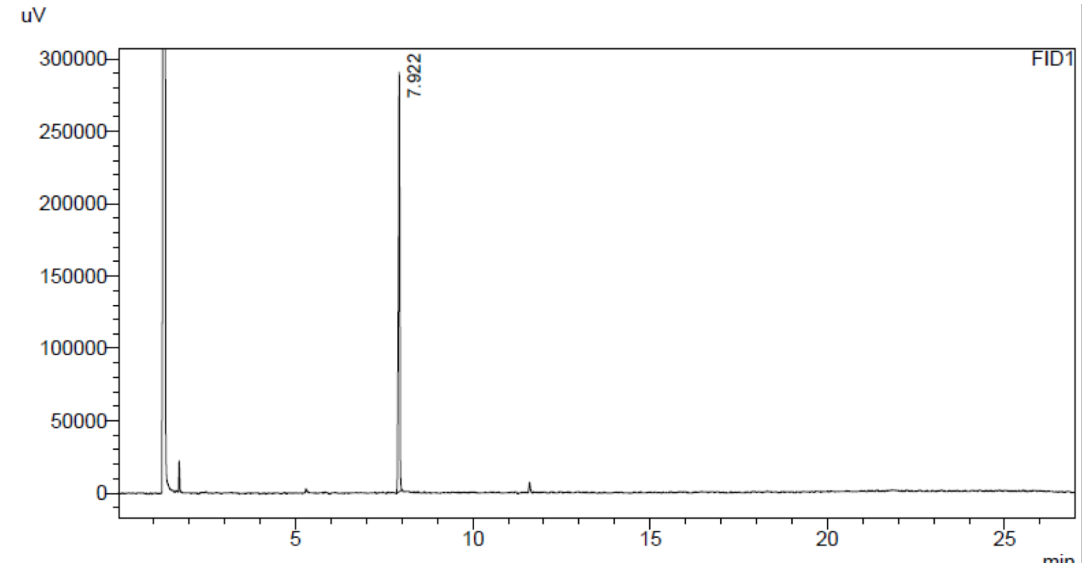

Figure 6: Typical Chromatogram from LOD solution

relative standard deviation was calculated. The results of repeatability and intermediate precision are summarized in the Table no: 7 and Table no: 8.

Robustness

The robustness of the analytical procedure is a measure of its capacity to remain unaffected by

Table 1: System suitability results from standard solution

Injection No	Area	Resolution
1	3155169	23.00
2	3271659	22.84
3	3135884	23.21
4	3124429	22.93
5	3267433	23.01
Mean	3190914.8	
Std. Dev.	72631.2	
%RSD	2.3	

Table 2: Results of system suitability of standard stock solution

Peak	Name	RT	Height	Area%	RRT	Resolution (USP)
1.	2-Ethyl hexanol	2.750	121515	5.387	0.344	-
2.	2-Ethylhexyl glycidyl	5.824	61225	3.763	0.728	63.554
3.	Ethyl hexyl glycerin	8.002	691780	90.850	1.000	23.000
Total			874519	100.00		

Table 3: Result of the Blank Chromatogram

Peak	Name	Retention time	Heights	Area %	RRT	Resolution
1.	Acetone	1.271	34984812	100.00	0.159	-
Total			34984812	100.00		

Table 4: Result of specificity with Ethylhexylglycerin

Peak	Name	Retention time	Heights	Area %	RRT	Resolution
1.	2-Ethylhexanol	2.754	2880	0.161	0.344	-
2.	Ethylhexylglycerine	8.002	724612	99.839	1.001	60.897
Total			72.7492	100.000		

Table 5: Results of linearity, LOD and LOQ

Statistical parameter	Results
Co-efficient correlation (R^2)	0.999
Concentration range	0.1-0.75µg
Intercept	45276x + 94792
Slope	452769
% R.S.D of LOD	5.6
% R.S.D of LOQ	5.1

Table 6: % Recovery results for Ethylhexylglycerin assay

S. No	% Recovery level	Spike Amount (ppm)	Amount found (ppm)	% Mean Recovery
1		75.04	75.678	
2	75%	75.02	74.286	99.24
3		75.06	73.446	
1		100.04	100.748	
2	100%	100.08	102.507	100.65
3		100.02	98.831	
1		120.02	120.340	
2	125%	120.04	119.295	99.81
3		120.06	119.814	

small but deliberate changes to flow rate and temperature. Robustness provides an indication to the reliability during routine use. Determine by quantifying the impurities in paraben samples from the same homogenous batch used in the precision studies. The difference between the quantifying impurities under deliberately modified chromatographic conditions and the quantifying impurities obtained under Precision is less than 10.0% of the absolute value. Robust study of system suitability and sample are shown in the table no: 9 & 10.

Stability of analyte solution

Five replicate injections of 1µl of standard solution, blank preparation and Sample solution were injected into the chromatograph and were recorded. Peak responses for major peaks for all solutions were measured and it was continued with the periodic injections for Standard, Blank & Sample

Table 7: Repeatability Results

Preparation No.	Results %			
	% Assay	% Purity	% Unspecified	% Total impurities
1	98.94	99.047	0.337	0.953
2	99.27	99.053	0.338	0.947
3	99.06	99.05	0.341	0.95
4	99.18	99.022	0.341	0.978
5	98.93	99.047	0.343	0.953
6	98.64	98.999	0.342	1.001
Mean	99.00	99.04	0.34	0.96
%RSD	0.22	0.02	0.69	2.22

Table 8: Intermediate Precision Results

Prep. No	% Assay	% Difference	% Purity	% Difference
1	101.59	2.65	99.071	0.024
2	99.84	0.56	99.057	0.004
3	99.78	0.72	99.057	0.007
4	99.48	0.31	99.071	0.049
5	99.95	1.03	99.072	0.025
6	100.13	1.49	99.065	0.066
Mean	100.13	-	99.07	-
% RSD	0.75	-	0.01	-

Table 9: Robustness study system suitability results

Robust Condition	System suitability Results	Resolution
	RT	
Normal	7.927	23.00
Carrier flow – 1.8ml/min	8.055	24.99
Carrier flow – 2.2ml/min	8.036	22.33
Oven temp – (-5%)	8.011	21.99
Oven temp – (+5%)	7.97	22.54
Mean	8.000	
Std. Dev.	0.052	
%RSD	0.646	

Table 10: Robust study sample results

Robust Condition	%Results			
	% Assay	% Diff.	% Purity	% Diff.
Normal	98.94	-	99.047	-
Carrier flow – 1.8ml/min	99.44	0.500	99.115	0.068
Carrier flow – 2.2ml/min	99.62	0.680	99.099	0.052
Oven temp – (-5%)	99.43	0.490	98.988	0.059
Oven temp – (+5%)	99.42	0.480	99.075	0.028

Table 11: Results of stability of analyte solution results

Time period	% Results			
	% Assay	% Difference	% Purity	% Difference
Initial	98.94	-	99.047	-
After 4 hours	98.70	0.25	-	-
After 12 Hours	98.58	0.37	99.077	0.030
After 36 Hours	99.04	0.09	98.985	0.062

preparations in the interval of 4 hrs; 12hrs or suitable interval depending on the instrument utilization and sequence of injections over a period of 36 hours and the results are as shown in table no: 11.

CONCLUSION

A GC-FID method for the determination of Ethylhexylglycerin and its impurities in the pharmaceutical preparations was developed and validated. This method met the regulatory requuirements for specificity, selectivity, Sensitivity, Precision, Accuracy and Stability. Since validation the

procedure was also found to be robust since the method remained unaffected by deliberate change in flow rate and temperatures. Hence, the method can be employed for determining ethylhexylglycerin in bulk, stability studies and as also in various pharmaceutical formulations.

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