ORIGINAL ARTICLE

Development and Validation of Stability-indicating Gas Chromatography Method for the Quantitative Determination of Ethylhexylglycerin and its Impurities in Bulk and Pharmaceutical Dosage Forms

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Abstract

Aim: A stability-indicating gas chromatographic flame ionization detection (FID) method for the quantitative determination of ethylhexylglycerin (EHG) and its impurities in drug substance and drug product has been developed and validated. The developed method was also applied to related substances identification in the bulk sample. **Materials and Methods**: The chromatographic separation was performed on a fused silica capillary (DB-1, 30 m, 0.32 mm, and 0.25 µm film thickness) column, and with a gradient technique. The column oven was programmed as follows: Initial column oven temperature, 60°C; ramped linearly to 280°C at the rate of 8°C/min; hold for 10 min. The runtime of analysis was 30 min. The injector and detector temperature was kept at 240°C and 260°C, respectively. Nitrogen was used as a carrier gas with a constant flow rate of 2 mL/min. The split ratio was set at 20: **Results and Discussion**: The developed method was validated as per the ICH guidelines. The stability-indicating nature of the method has been proved by establishing peak purity and confirming the mass balance of all samples by subjecting them to stress conditions such as hydrolysis, oxidation, photolysis, and thermal degradation studies. **Conclusion**: The method was successfully applied to analysis of different bulk sample of EHG and its impurities. The proposed gas chromatography coupled with FID method was also found to be specific and selective for the analysis of commercial formulation samples.

Key words: Ethylhexylglycerin, forced degradation studies, ICH guidelines, stability-indicating method, validation and method development

INTRODUCTION

Ethylhexylglycerin (EHG) is an alkyl glyceryl ether having the empirical formula of $C_{11}H_{24}O_3$ and molecular weight of 204.3 g/mol. Chemically it is 3-[(2-ethylhexyl) oxy]-1, 2-propanediol. It is a well-known multifunctional ingredient which is widely used worldwide. It provides excellent antimicrobial efficacy against Gram-positive bacteria.[1] Being an amphiphilic molecule, EHG is surface active and is a globally approved drug marketed as sensiva® SC 50 used in cosmetics.[2-4] EHG and its process-related impurities, namely, alcohol and ether impurity are [Figures 1-3] insensitive to ultraviolet (UV) or visible spectroscopy because of lack of chromophores in its structure. Hence, it is a challenge to the analytical scientist to determine the assay content of EHG and its related substances by a traditional high-performance liquid chromatography (HPLC) method. In the literature, Miralles *et al*., 2016, have developed a method for the determination preservatives in cosmetics by HPLC-UV.[5] This method involves intense sample preparation technique like vortex-assisted liquid-liquid semi-microextraction technique by the use of a derivatizing agent like benzyl chloride as a chromophoric agent which is very time consuming, complex, and tedious process. This derivatization method

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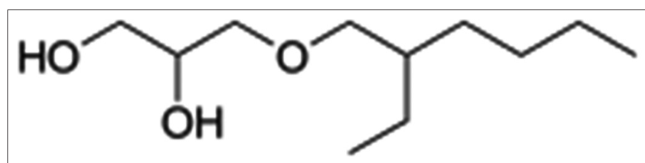

Figure 1: Molecular structure of ethylhexylglycerin

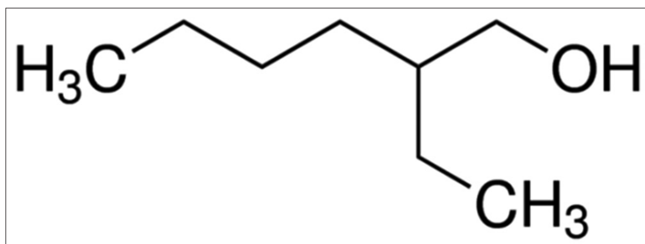

Figure 2: Molecular structure of ethylhexylglycerin-alcohol impurity

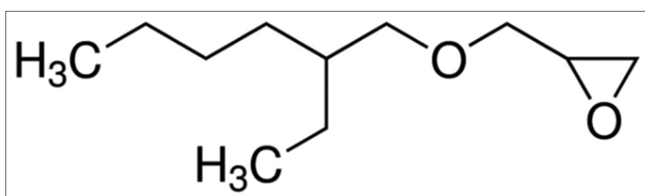

Figure 3: Molecular structure of ethylhexylglycerin-ether impurity

is not suitable in a regular testing mode in any of the laboratory in the cosmetic laboratory. Thus, we felt that there is a need of simple, suitable, accurate, method for stability and related substances identification and quantitation of EHG and its impurities in bulk and pharmaceutical dosage forms. Literature survey indicated that there is no stability-indicating gas chromatography (GC) coupled with flame ionization detector (GC-FID) method available for the determination of EHG and its impurities; thus, we aimed to develop a method in our laboratory.

RP-HPLC is widely used analytical technique for the separation and quantification of impurities in pharmaceutical products.[6] GC is very rarely used for quantitative determination of impurities in pharmaceutical preparation. Developing a stability-indicating method on GC for impurity profiling in active pharmaceutical ingredients or in pharmaceutical formulation is a challenging task. Complexities involved in the development of method are extraction of impurities in the presence of salts, selection of suitable diluents for gas chromatographic analysis, and proving stability-indicating nature of the method. The analytes are typically detected using an FID technique. These FID detectors can be used for almost all pharmaceutical products, as they are able to detect any analyte which contains a C–C or C–H bond. However, to analyze compounds using GC, the compounds must have a high enough vapor pressure to allow them to be volatilized before interaction with the chromatographic column. GC is a highly efficient, sensitive method used to analyze complex mixtures of substances and non-chromophoric impurities. Therefore, in the present study, a gas chromatographic method with FID detection for quantitative determination of impurities, assay content, and related substances in forced degradation studies in a formulation containing EHG has been developed and validated which is precise, specific, accurate, linear, and robust. In our study, a simple stability-indicating GC-FID method was developed and validated for the determination of related substance of EHG in drug substances and drug product. Both the non-chromophoric impurities (IMP-A and IMP-B) are well separated from each other. The method was validated as per ICH guidelines[7] and successfully applied for separation of all compound of interest in the pharmaceutical formulation. The method reported here may find utility both in the pharmaceutical and chemical industries, and it may potentially be applied to the purity/impurity analysis of other pharmaceutical product analysis.

MATERIALS AND METHODS

Chemicals and reagents

The investigated sample of EHG and its potential process-related impurities [Figure 1] were received from synthetic laboratory of Salicylates and Chemicals (P) Ltd, Hyderabad, India. Analytical reagent grades acetone, hydrochloric acid, and sodium hydroxide were purchased from Merck, (Mumbai, India). Highly pure water obtained from Millipore system (Millipore Inc., USA) was used throughout the analysis, and all other chemicals were of 99.9% pure and were of GC grade. 0.22 µm nylon filters were purchased from advanced microdevices, Chandigarh, India, and filter paper of 0.45 µm size was purchased from Millipore.

Method development

The aim of the current study was to develop a simple, robust, and derivatization free method for the analysis of EHG and its impurities in bulk and pharmaceutical dosage forms. As such no analytical method was reported for the determination of EHG and its impurities. Hence, it was significant to develop a method using GC as it is one of the most commonly adopted procedures to determine the presence of volatile components in dosage form. A systematic strategy was utilized to optimize various conditions/parameters for the analysis of EHG and its impurities such as diluent or solvent, temperature at various stages of GC method development.

Instrumentation

GC (analytical) instrumentation and operating conditions

The GC system used for method development and method validation was from Shimadzu Model no: GC-2025 with an autosampler. The detection was performed by means of FID. The output signal was monitored and processed using Ezchrom Elite software version 3.2.1. The DB-1 (30 m length × 0.32 mm ID, 0.25 µm film thickness) column has been procured from Agilent technologies made in the USA and used for the method development and method validation studies. The column oven was programmed as follows: Initial column oven temperature, 60°C; ramped linearly to 280°C at the rate of 8°C/min; hold for 10 min. The runtime of analysis was 30 min. The injector and detector temperature was kept at 240°C and 260°C, respectively. Nitrogen was used as a carrier gas with a constant flow rate of 2 mL/min. The split ratio was set at 20:1 sample was injected by the instrument's autosampler with injection volume of 1.0 µL and acetone as the syringe cleaning solvent between injections.

Preparation of solutions

Preparation of standard solutions and sample solutions

Volumetric method was used for the preparation of all the stock solutions. A solution of EHG was prepared at a concentration of 20 mg/mL and 2 mg/mL in the acetone for related substances determination and assay determination, respectively. The individual stock solutions of each impurity at concentration of about 5 and 2 mg/mL (alcohol impurity and ether impurity) were prepared in acetone and further diluted adequately to study the validation parameters. The resultant final diluted solutions were filtered through 0.22 µm filters. The supernatant liquid was taken for injection. The specification limits used for validation studies were 0.1% for the known impurities (alcohol and ether) and 0.05% for any individual unknown impurities. Hence, the system suitability solution of all impurities and EHG was prepared at specification level by diluting above stock solutions.

Sample solvent selection

Method development was initiated with selection of a sample solvent that dissolves EHG and its impurities. Preliminary injections in GC using methanol and ethanol as sample solvents showed broad peak shapes due to the polarity mismatch between the sample solvents and the stationary phase. The peak shapes of all peaks were significantly improved using acetone as a diluent. Therefore, acetone was selected as a sample solvent (diluent).

Application of stress (forced degradation study)

Forced degradation study was conducted on bulk drug substance to prove the stability-indicating property and selectivity of the established method.[16] Forced degradation of EHG was carried out under hydrolytic (acidic/basic), oxidative, thermolytic, and photolytic stress conditions. The drug substance was exposed with 1 N hydrochloric acid (refluxed for 24 h), aqueous 1 N sodium hydroxide (refluxed for 24 h), and aqueous 30% hydrogen peroxide (kept for 24 h at RT).[8-13] For thermal stress, sample of drug substance was placed in oven with controlled temperature of 60°C for 5 days. For photolytic stress, the sample was exposed to photolytic conditions for 7 days as per ICH guidelines. After the exposure to the above stress conditions, solutions of these samples were prepared by dissolving respective samples of known concentration in acetone and extracted as per extraction procedure and further subjected to analysis using the proposed method. All the stressed samples were quantified for EHG and its impurities. The mass balance (% assay + % of impurities + % of degradation products) was calculated for all of the samples.

Detection of impurities

Laboratory batches of crude EHG were analyzed for their related substances identification using the developed GC-FID method. The synthetic standards of both the impurities were injected separately into the developed method and matched their retention times to confirm their presence in the crude batch.

Method validation

As per ICH guidelines,[7] 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, related substances identification, as well as stability studies.

System suitability

System suitability was evaluated by injecting the standard solution into the optimized method using the GC-FID mode of analysis. Peak areas for all six standard chromatograms are measured. The RSD of peak area ratios was 2.46% for EHG, 0.38% for alcohol impurity, and 0.43% for ether impurity. The resolution of each peak was more than 2.0 and the tailing factor was <2.0. System suitability results are tabulated in Table 1 and the chromatogram is shown in Figure 4.

Specificity

No interfering peaks were found at the retention time of EHG and its impurities while injecting the blank acetone into the system. The retention times of each impurity and EHG were confirmed by comparing their retention times with those obtained from each coinjected individual impurities. All the impurities were adequately separated from each other.

Although the method was only validated for EHG and its impurities, the method can be applied to forced degradation studies as shown in Figure 5.

Linearity and range

The linearity and range samples were serially diluted from each standard solution to obtain six concentrations levels covering LQ to 125% of the assay level. The peak areas at each level of standard were calculated to assess the method linearity. Graphs of each peak area versus each corresponding concentration of standard were plotted. The correlation coefficient of the regression line for each standard ranged from 0.9998 to 0.9992. The detailed results are summarized in Table 2.

Table 1: System suitability results from standard solution

Compound name	Retention time (min)	RRT	Resolution (RS)
2-Ethylhexanol	2.750	0.344	-
2-Ethylhexylglycidyl ether	5.842	0.728	63.554
EHG	8.002	1.000	23.000

EHG: Ethylhexylglycerin

LOD and LOQ

The LOD and LOQ for EHG and its impurities were determined by signal-to-noise ratio (S/N) method. The LOQ was about 0.1% of the individual impurity assay level was injected into GC system to obtain a S/N ratio of more than 10. LOD was estimated based on LOQ to obtain a S/N of more than 3, and the results are summarized in Table 2.

Accuracy/recovery

The recovery experiment was carried out to evaluate the accuracy of the method. The response of three levels (75%, 100%, and 150%) of spiked samples in triplicate yielding a mean recovery of 99.8% with an RSD (n = 9) of 0.5%. Table 3 summarizes the validation data results of recovery of EHG and its impurities. The recovery of all these related substances was found to be in between the predefined acceptance criteria of 85–115%.[13] The results demonstrated that the method has sufficient capability for the accurate quantification of EHG and its impurities in raw materials and also in formulated samples. Table 4 summarizes the accuracy data of related substances at various levels.

Table 2: Results of linearity, LOD, and LOQ

Statistical parameter	Impurity-alcohol	Impurity-ether	EHG
Coefficient correlation (R^2)	0.9995	0.9992	0.9998
Concentration range	0.05–0.5 µg	0.05–0.5 µg	0.1–0.75 µg
Intercept	921×+2420	1236×+346	45276×+94792
Slope	921	1236	452769
% RSD of LOD	4.9	3.8	5.6
% RSD of LOQ	3.2	2.7	5.1

LDO: Limit of detection, LOQ: Limit of quantification

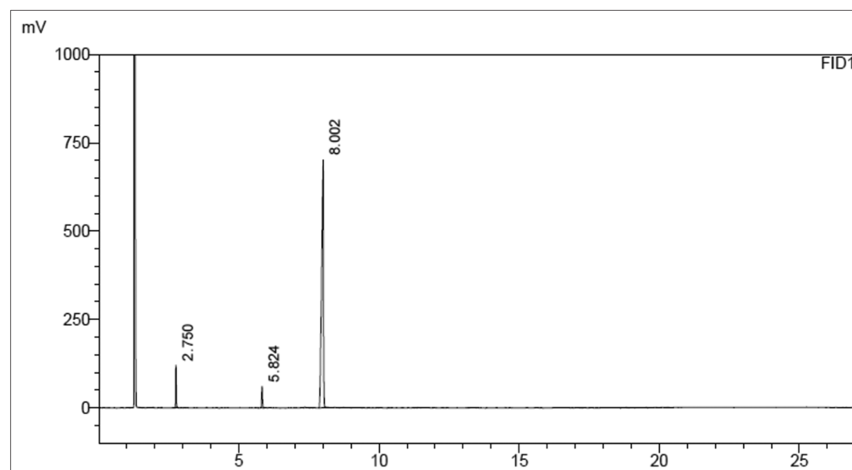

Figure 4: Typical chromatogram of system suitability from standard stock solution

Precision

Precision was studied at two different levels, one is method precision and the other one is intermediate precision. Precision was performed by analyzing the six replicates of a sample at single concentration, and the RSD was obtained results was evaluated and found to be <0.5% for the assay method was evaluated at three different levels: Repeatability intermediate precision and reproducibility. The intraday precision was evaluated using the results of six replicate injections of standard concentrations (X µg/mL), and RSD was found to be <2% system precision for RS determination was verified by system suitability solution. This was also analyzed for 6 times and RSD of EHG and all its impurities were found to be 0.62%. Precision of related substances is given in Table 5.

Robustness

The ruggedness of the analytical procedure was performed by varying various operational parameters each at a time, for example, flow rate, inlet temperature, ramp temperature, oven, and detector temperature. While varying one of the parameters, the remaining parameters kept constant throughout the analytical run. The difference between the quantifying impurities under deliberately modified chromatographic conditions and the quantifying impurities obtained under precision is <10.0% of the absolute value. The low percentage changes reveal that the developed method is robust.[14]

Stability of analyte solution

Stock solution stability studies were carried out using spiked samples of EHG and its impurities on different days at ambient temperature (~20ºC) and refrigerated conditions (2–8°C). The chromatographic profile of aged solution was comparable with that obtained at the initial time point (0 min). The % differences of the individual peaks tested at initial time point versus the stored peak were within ±0.5% of the absolute (initial) value. The lower % values of stability sample changes confirm that the solutions of EHG and its impurities were stable for 7 days when stored at either ambient temperature or refrigerated condition; results are summarized in Table 6.

Table 3: Percentage recovery for EHG assay

% recovery level	Spike amount (ppm)	Amount found (ppm)	% mean recovery
75	75.04	75.678	99.24
	75.02	74.286	
	75.06	73.446	
100	100.04	100.748	100.65
	100.08	102.507	
	100.02	98.831	
125	120.02	120.340	99.81
	120.04	119.295	
	120.06	119.814	

EHG: Ethylhexylglycerin

Table 4: Accuracy data of related substances

Component	LOQ level (%)	50% Specified level[a]	75% Specified level[a]	100% Specified level[a]
Impurity-A	105	99.24	100.65	99.81
Impurity-B	108	99.47	99.98	100.32

[a]0.1% of all related substances. LOQ: Limit of quantification

Table 5: Precision of related substances and assay of EHG

Parameter	Related substances results Precision (mean±RSD)		Assay % of EHG
	Impurity-A	Impurity-B	
Method precision (*n*=6)	0.162±1.149	0.142±1.203	100.42±0.46
Intermediate precision (*n*=6)	0.158±1.271	0.184±1.195	99.91±0.53
Overall results (*n*=12)	0.160±2.186	0.163±1.327	100.16±0.72

EHG: Ethylhexylglycerin

Table 6: Results of stability of analyte solution results

Time period	% Results		
	% Assay-EHG	% Assay-impurity-alcohol	% Assay-impurity-ether
Initial	99.45	98.94	99.05
After 1 days	99.38	98.70	99.03
After 3 days	99.29	98.58	99.08
After 5 days	99.04	98.61	99.99

EHG: Ethylhexylglycerin

Table 7: Impurities identified in formulation and bulk drug batches sample analysis

Sample source	Impurity-A	Impurity-B	MUI	Assay results
Formulation analysis (product)				
Formulation-1	ND	ND	ND	99.8
Formulation-2	ND	ND	ND	99.5
Batch analysis results (%)				
B.NO.EHG/E167/TO/06	ND	ND	0.005	99.7
B.NO.EHG/E167/TO/07	ND	ND	0.007	99.6

ND: Not detected, MUI: Maximum unknown impurity

Table 8: Forced degradation study results

Condition (stressed)	% Assay of EHG	% of degradant	Observation	Mass balance %
Untreated	99.99	-	-	-
Acid hydrolysis (concentration HCL, 36 h, refluxed)	99.98	Nil	No degradation product was formed	99.98
Base hydrolysis (5 M, NaOH, 36 h, refluxed)	99.65	Nil	No degradation product was formed	99.65
Oxidation (30% H2O2, 24 h @RT)	99.53	Nil	No degradation product was formed	99.53
Thermal (60°C for 8 days)	99.82	Nil	No degradation product was formed	99.82
Photolytic	99.71	Nil	No degradation product was formed	99.71

Mass balance: % assay+%impurities+%degradants

Applications

The developed method was successfully applied to the simultaneous estimation of EHG and its impurities in formulation[15,16] and bulk drug samples. Since the percentage of EHG in the selected product was very minimal, so we could not find any impurities in it. The method is capable of quantitative analysis of EHG in dosage forms. The values of impurities detected indicate that the method is capable of detecting known and unknown impurities in routine analysis of bulk and pharmaceutical dosage forms. The results of identified impurities of known, unknown, and related impurities in bulk, dosage form, and forced degraded studies are summarized in Tables 7 and 8, respectively, and its representative chromatogram of acid-stressed condition is given in Figure 6.

CONCLUSION

A GC-FID method was developed for the simultaneous analysis of EHG and its process-related alcohol and ether impurities. The developed sample preparation technique does not require any derivatization methods. Hence, the method was simple and easy to apply in a regular analytical setup. The method is free from interferences from the diluents used in the study, and it demonstrated the specificity for impurities as well. The sensitivity shows that the method is applicable even when it is used as preservative in low concentrations. In addition, the precision for the assay of each impurity is accessible. The method was validated and proved to be specific, precise, and accurate for the analysis of EHG and its related substances. This method can also be used as a quality control method for EHG.

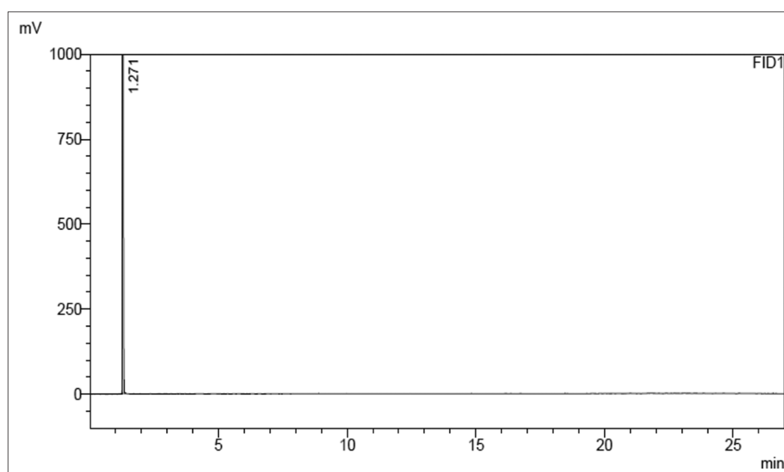

Figure 5: Typical chromatogram of blank (acetone as solvent)

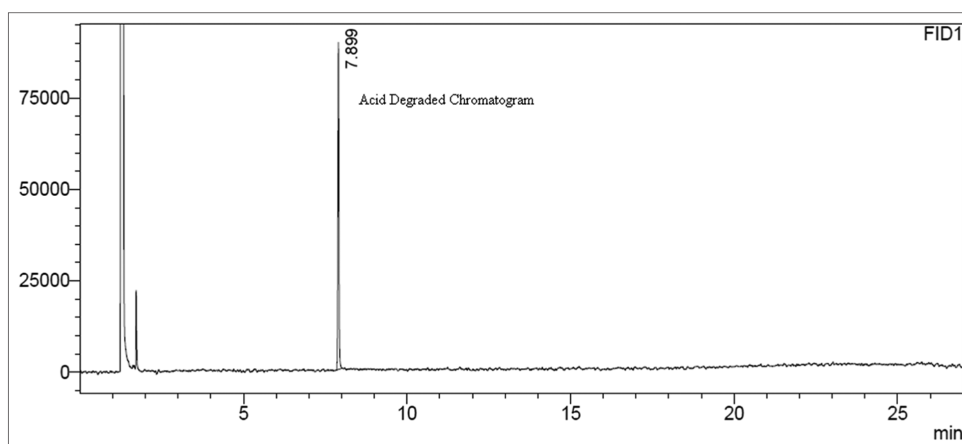

Figure 6: Typical chromatogram of acid refluxed chromatogram of ethylhexylglycerin

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