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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF GLYCERIN CONTENT IN TOPICAL MOISTURIZING GEL OTC FORMULATION BY GAS CHROMATOGRAPHY

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ABSTRACT

Glycerin is widely used in topical treatment of skin and also during oral treatment for dry mouth. Glycerin basically acts as a humectant[8] that means substance that aids in reduction in loss of moisture from skin or from wound. Many types of formulations available in the market that contains Glycerin as principal component mainly used to retain moisture within the skin. Glycerin has many medicinal benefits and it acts as Cleanser, Toner, Moisturizer, treatment of Acne, Dry Skin, and Wrinkles, Skin lightning, treating cracked heels, Scar removal, and can also be used as sunscreen. In sunscreen moisturizing gels, Glycerin is used as main active ingredient with 20% w/w concentration. Estimation of Glycerin in such gel type of topical dosage forms is important as a part of formulation batch release and during stability study. The purpose of the present study was to develop accurate and precise analytical method for determination of Glycerin in topical moisturizing gel by gas chromatography. An internal standard technique is proposed in order to reduce sample preparation and injection related errors thereby improving method accuracy. 2,2,2- Trichloroethanol chemical compound is used as an internal standard purpose while Isopropyl alcohol is used as diluent for standard and sample preparations. Further developed method was validated as per ICH guidelines to prove its suitability for intended purpose.

KEYWORDS: Glycerin, 2,2,2- Trichloroethanol, Isopropyl alcohol, Gas Chromatography, Internal standard, Humectant, Method validation, Topical moisturizing gel, OTC (Over the counter).

INTRODUCTION

A humectant is a substance which retains moisture within the skin. It is used to treat or prevent dry, rough, scaly, itchy skin and minor skin irritations (e.g., diaper rash, skin burns from radiation therapy). Glycerin has several other medical benefits like, it acts as Cleanser, Toner, treatment of Acne, Dry Skin, and Wrinkles, Skin lightning, treating cracked heels, Scar removal, and can also be used as Sunscreen.

Dry skin is caused by a loss of water in the upper layer of the skin. Moisturizers work by forming an oily layer on the top of the skin that traps water in the skin. Glycerin draws water into the outer layer of skin. This helps the dead skin cells fall off, helps the skin keep in more water, and leaves the skin feeling smoother and softer. The use of Glycerin products dates from antiquity.

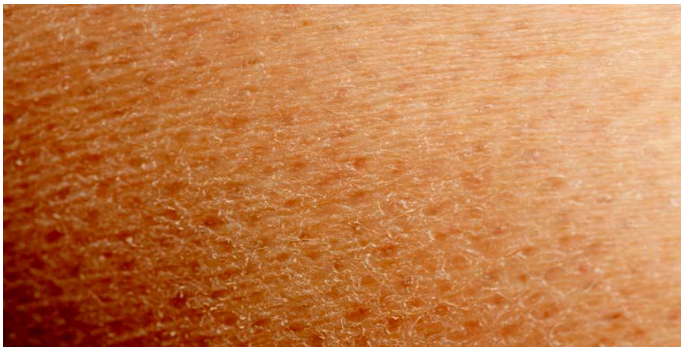
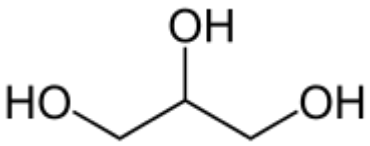
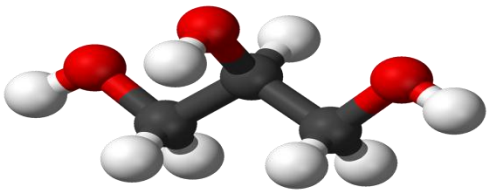


Figure 1: Human skin suffering from dryness and Acne.

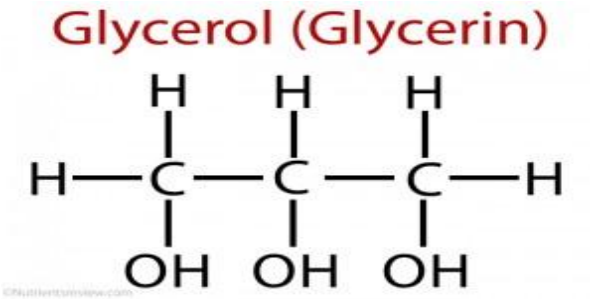
Glycerin is simple polyol compound. It is a colorless, odorless, viscous liquid that is sweet-tasting and non-toxic. The glycerol backbone is found in all lipids known as triglycerides. It is widely used in the food industry as a sweetener & humectant and in pharmaceutical formulations. Glycerol has three hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature.[10] Chemical formula of Glycerin is $C_3H_8O_3$ and Molecular weight is 92.09 $g \cdot mol^{-1}$.



Glycerin



Glycerin 3D model (JSmol)



IUPAC name – 1,2,3-Propanetriol

Table 1: Physicochemical properties of glycerol.

Physicochemical properties of glycerol[7] (at 20°C).
Chemical formula: $C_3H_5(OH)_3$
Molecular mass: 92.09382 g mol^{-1}
Density: 1.261 $g.cm^{-3}$
Viscosity: 1.5 Pa.s
Melting point: 18.2°C
Boiling point: 290°C
Food energy: 4.32 $kcal.g^{-1}$
Flash Point: 160°C (closed cup)
Surface tension: 64.00 mN.m
Temperature coefficient: –0.0598 $mN.(mK)^{-1}$

Chemicals, Instruments and Method

Chemicals

Glycerin Standard, 2,2,2-Trichloroethanol (as Internal standard), Isopropyl alcohol [IPA] (HPLC grade as diluent), Moisturizing Gel 20% w/w formulation product (Purchased from Indian market) and its placebo (without Glycerin).

Formulation Samples

Formulation: Moisturizing gel contains Glycerin 20% w/w.

Instruments required

Gas Chromatograph equipped with FID detector, Analytical precision weighing balance, Sonicator.

Chromatographic Method

Chromatographic conditions

Instrument: GC equipped with FID detector

Column: DB-624 (30m, 0.53mm, 3µm) [Capillary column]

Carrier gas: Helium

Flow rate: 5.0 mL/min

Detector (FID) Temperature: 250°C

Injector Temperature: 220°C

Injection volume: 1.0 µL (through auto-sampler).

Total Run time: 18 min.

Table 2: Column Oven Temperature program.

	Rate (°C/min)	Final Temp (°C)	Hold Time (min)	Total Time (min)
Initial Temp		90	1	--
Ramp - 1	25.00	220	11.80	18.00

Preparation of Internal standard, Standard and Samples

Internal standard solution: 5.0 mg/ml of 2,2,2- Trichloroethanol in IPA.

Standard solution: 3.0 mg/mL of Glycerin in Internal standard solution.

Sample solution: Take Moisturizing Gel sample equivalent to prepare 3.0 mg/mL of Glycerin in Internal standard solution.

Method Validation

Specificity study

No extraneous peaks observed in the blank and placebo chromatograms at the RT of Glycerin and 2,2,2- Trichloroethanol (internal standard) in moisturizing gel formulation. Typical specificity chromatograms are outline as Figure 4.

Linearity

Seven point linearity (from 50% to 150% of standard Glycerin concentration) plot was constructed in order to accommodate wide range from 1.5 mg/ml to 4.5 mg/ml of Glycerin, so that Glycerin can be analyzed easily with $r^2 = 0.999$ for the above plot.

Precision

ICH describes precision as closeness of individual measure of analysts when the procedure is applied repeatedly to multiple times or on multiple sample of homogenous formulation sample. Intraday precision and Interday precision (Ruggedness) has been established for the proposed method with %-RSD for intraday found to be 0.44% and that for inter day 0.42% indicating preciseness of proposed GC method for Glycerin determination in Moisturizing gel formulation.

Accuracy

It was evaluated at three levels of 50%, 100% and 150% of test concentration by adding known amount of Glycerin in to the placebo and estimating the sample on GC. Three sets in triplicate were prepared and analyzed separately for Gel formulation.

Robustness

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

RESULTS AND DISCUSSIONS

Method development and optimization

Actual chromatographic conditions were established after number of preliminary experiments on Gas chromatograph for selecting the proper capillary column stationary phase, column oven temperature and carrier gas flow rate. Different column oven temperatures were tested, and selection of the proper system depended on its ability to give good separation between the Glycerin (Active component) and 2,2,2- Trichloroethanol (Internal standard) peaks. Acceptable separation was achieved on DB-624 (30m, 0.53mm, 3µm) Capillary column using Helium as a carrier gas with a flow rate of 5.0 mL/min. The column oven temperature with temperature gradient method: Initial temperature is 90°C with 1 min hold and achieving final temperature is 220°C by increasing at the rate of 25°C/min. Final temperature hold for 11.8 min. Under these chromatographic conditions, the run time for standard and sample is 18.00 min. Larger run time proposed to avoid carry forward of late eluting peaks from placebo into the next chromatogram.

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 USP limits as shown in Table - 3.

Table 3: System suitability of test method.

Parameter	Acceptance Criteria	Moisturizing gel Glycerin
Tailing factor	NMT 2.0	0.99
Theoretical plates	NLT 2000	205014
% RSD of 6 injection (area ratio)	NMT 3.0%	0.72%
% RSD of 6 injection (retention time)	NMT 1.0%	0.01%

Linearity

A linear calibration plot of Glycerin and 2,2,2- Trichloroethanol was constructed at seven point concentration levels i.e. from 1.5 mg/mL to 4.5 mg/mL of Glycerin against 5.0 mg/mL of 2,2,2-Trichloroethanol (internal standard) and each level injected into GC in duplicate. Average peak area ratio of Glycerin and 2,2,2- Trichloroethanol was plotted against respective concentration and linear regression analysis was performed. Correlation coefficient was found to be 0.9998 for area ratio of Glycerin to 2,2,2-Trichloroethanol indicating proposed GC method is linear within the proposed range. Typical linearity plot is given below as Fig.-2. Further typical chromatograms from 7 linearity concentrations of standard Glycerin (as area ratio against 2,2,2- Trichloroethanol) are outlined as Figure 5.

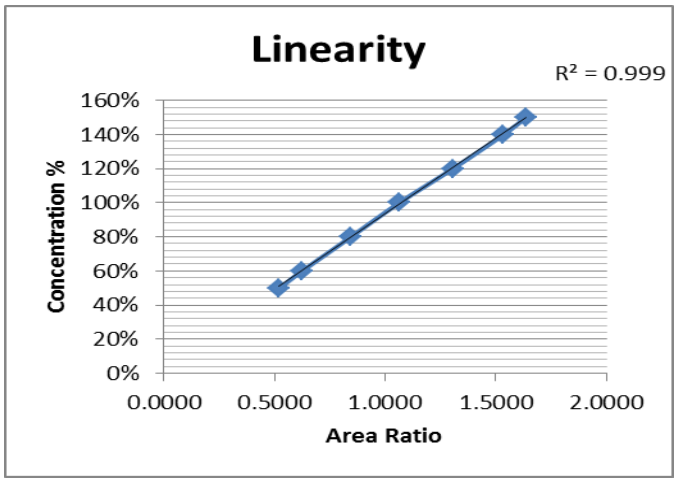


Figure 2: Linearity plot obtained from area ratio between Glycerin and 2,2,2-Trichloroethanol.

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 Glycerin against 2,2,2- Trichloroethanol found to be 0.72% for standard & 0.44% for samples in Moisturizing Gel formulation. For inter-day precision, the percentage relative standard deviation for area ratio of Glycerin against 2,2,2- Trichloroethanol found to be 0.74% for standard & 0.42% for samples in the same formulation. These values were well within the acceptable limit of 3.0%, as per USP. Obtained results are given in Table – 4 with graphical representation as Figure – 3. Further typical chromatograms from 6 replicates of sample determinations for Glycerin content are outlined as Figure 7.

Table – 4: Precision (n = 6) and Linearity (n = 7).

Parameter		Limit for (%- RSD) & r^2	Moisturizing gel Glycerin - % RSD	
			Standard (n=6)	Sample (n=6)
Precision	Repeatability	3.0%	0.72%	0.44%
	Intermediate	3.0%	0.74%	0.42%
Linearity (r^2)	7 - Levels	0.999	0.9998	

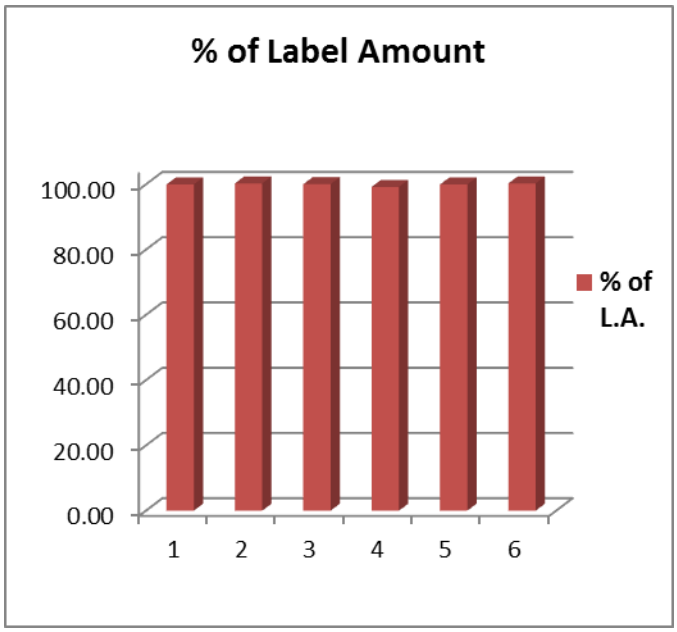


Figure-3: Precision plot obtained from 6 Assay values of Glycerin content in moisturizing gel formulation.

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 Glycerin content. The accuracy of method was established at three concentration levels i.e. at 1.5, 3.0 & 4.5 mg/mL of Glycerin in Moisturizing Gel formulation. The recovery at three different concentrations found to be within range of 95.0% to 105.0% as per ICH guidelines. Mean % recovery (mean ± SD) found to be 99.52% for Glycerin in Moisturizing gel formulation.

The obtained results indicate that the percentage recovery for Glycerin found to be satisfactory at three different concentrations for Moisturizing Gel formulation. The recovery results are summarized in Table – 5. Further typical chromatograms from three different accuracy levels are outlined as Figure 6.

Table 5: Accuracy (%-Recovery).

Moisturizing gel			
Glycerin			
Accuracy levels	**Amount added (Average - mg/mL)**	**Amount recovered (Average - mg/mL)**	**%-Mean Recovery (Limit – 95.0% - 105.0%)**
Level-1 (50%)	1.405	1.396	99.32
Level-2 (100%)	3.083	3.038	98.56
Level-3 (150%)	4.605	4.636	100.68

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 Gas chromatographic conditions, there was no significant change in system suitability parameters indicating proposed method for Glycerin estimation is Robust.

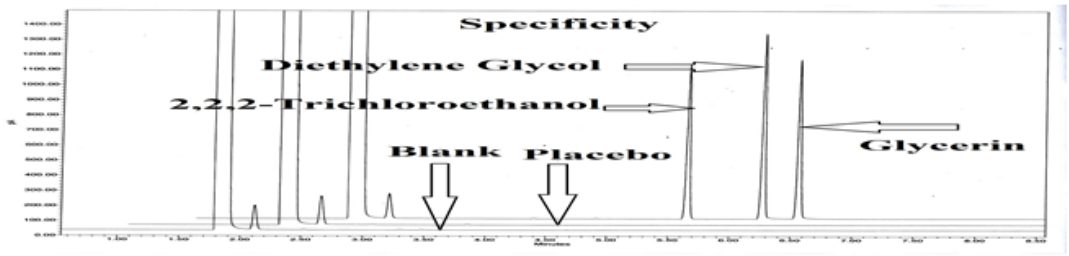


Figure 4: Representative stacked chromatograms of Specificity parameter showing Glycerin, 2,2,2 Trichloroethanol (Internal standard), Di-ethylene Glycol (Impurity) peaks are well separated from each other.

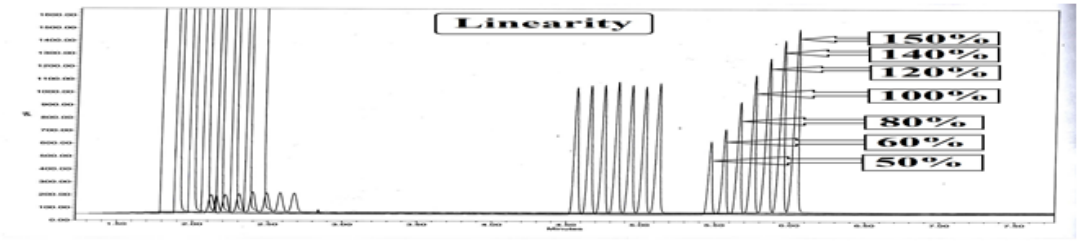


Figure 5: Representative stacked chromatograms of Linearity parameter showing seven levels viz. 50%, 60%, 80%, 100%, 120% 140% and 150% of target Glycerin concentration.

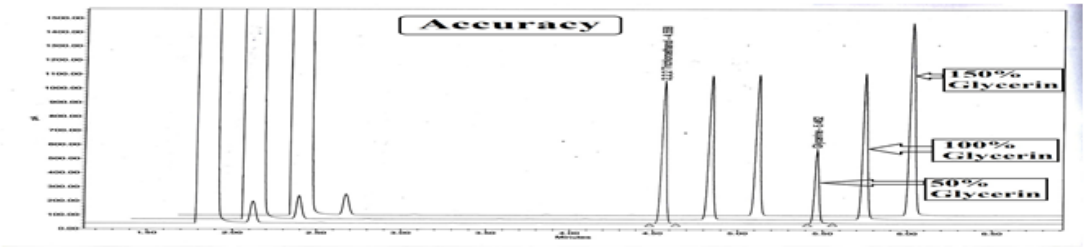


Figure 6: Representative stacked chromatograms of Accuracy parameter showing three levels viz. 50%, 100% and 150% of target Glycerin concentration.

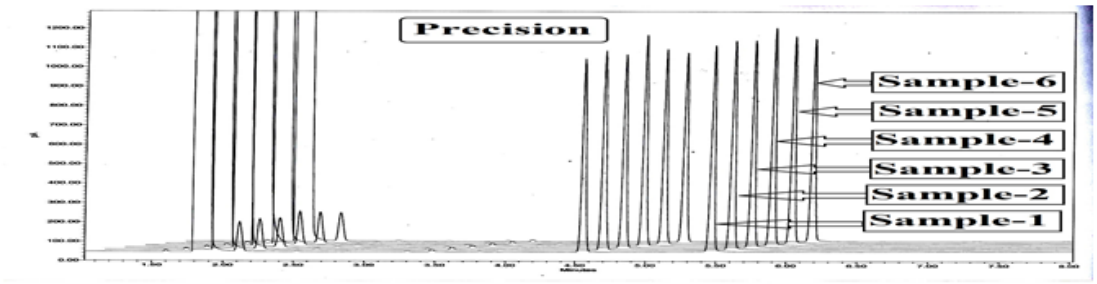


Figure 7: Representative stacked chromatograms of Precision parameter showing 6 replicates of Assay of Moisturizing Gel sample.

CONCLUSION

Finally, based on the summarized data as above, it could be concluded that, we are successful in developing and validating a new, simple, rapid, precise and accurate internal standard based Gas Chromatography method for the estimation of Glycerin in Moisturizing topical gel (OTC) Formulation. This developed method has proved that, high boiling compound like Glycerin can be analyzed by GC using capillary column without using pyrolysis technique.

Proposed method is duly validated and applied for routine estimation of Glycerin in Moisturizing gel formulation. This formulation have a lots of advantages like Cleanser, Toner, Moisturizer, treatment of Acne, Dry Skin, and Wrinkles, Skin lightning, treating cracked heels, Scar removal, and can also be used as sunscreen. Thus, this method can be applied for the estimation Glycerin content in Moisturizing gel as well as similar other several formulations available in the market that contain Glycerin as an active ingredient in OTC products. For other formulations, some modification in sample preparation as well as GC conditions shall be required based types of inactive ingredients present in such formulations.

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