Determination of glycerin in pharmaceutical formulations by liquid chromatography

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ABSTRACT: **Glycerin is used in different concentrations according to the intended use such as an antimicrobial preservative, emollient, aqueous and non-aqueous gel vehicle, humectant, and plasticizer in tablet film coating and sweetening agent in alcoholic elixirs. A precise, accurate, and reversed-phase high-performance liquid chromatographic (HPLC) method was developed for the determination of glycerin in different pharmaceutical preparations. Analysis was performed on Microsorb-MV 100-5 NH2 (150 x 4.6 mm) or equivalent column with an isocratic mobile phase consisting of hexane buffer 0.005M (pH 3), Acetonitrile, and Methanol (12:78:10 v/v/v) at a flow rate of 1.0 mL/min and ambient temperature using photodiode array (PDA) detector at 202 nm. The retention time of the drug was found to be 3.011 ± 0.007 min. The injection volume was 20 µL. The mobile phase was used as a diluent during the standard and test sample preparation. The developed method was found to be linear over the range of 3.75-22.5 mg/mL for glycerin. The currently developed method can be easily performed in quality control laboratories for simultaneous determination of glycerin, where no expensive tools are available.**

Keywords: Glycerin; Reversed phase-HPLC; Method validation; Pharmaceutical preparations.

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I.   INTRODUCTION

Glycerin is chemically designated as 1,2,3-Propanetriol (Figure 1). Glycerin is used in a wide variety of pharmaceutical formulations including oral, otic, ophthalmic, topical, and parenteral preparations [1]. It is used in different concentrations according to the intended use such as an antimicrobial preservative, emollient, aqueous and non-aqueous gel vehicle, humectant, and plasticizer in tablet film coating, and sweetening agent in alcoholic elixirs. In the official monograph of glycerin in USP 43, an assay of glycerin is prepared by titrimetric oxidation-reduction reaction [1] as defined under glycerin ophthalmic solution and glycerin oral solution. It is used as a demulcent in cough preparations. It is readily absorbed from the gastrointestinal tract and undergoes extensive metabolism, mainly in the liver. It may be used in the synthesis of lipids, metabolized to glucose or glycogen, or oxidized to carbon dioxide and water. It may also be excreted in the urine unchanged [2].
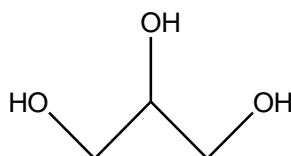

Figure 1. The chemical structure of glycerin

Different analytical methods have been published for glycerin in the literature. Glycerin has been widely studied by different methods such as chromatography [3-9], spectrophotometry [10-13], and electrochemical [14] methods.

This work aimed to develop and validate a simple, precise, accurate, isocratic, and reversed-phase HPLC method for the determination of glycerin in raw material and in dosage forms.

. II.Materials and Methods

2.1. Instrumentation:

Agilent 1200 series HPLC system with quaternary pump, UV-detector, DAD system, and Chemstation software (Tokyo, Japan). The pH measurements were taken using a Hanna pH meter from Portugal that included a combination glass-calomel electrode (HI: 9321).

2.2. Chemicals and reagents

The glycerin reference standard with a purity of 99.89±0.90% was kindly provided by Uniswab pharmaceutical industries, Egypt by applying the official method [1].

Swabirase oral syrup, labeled to contain 0.75 ml Glycerin per 5ml Syrup, a product of uniswab pharmaceutical industries, Egypt was purchased from local pharmacies and Benylin® infant's cough syrup, UK.

Acetonitrile and methanol HPLC grade were purchased from LAB-SCAN, Analytical Sciences (Gliwice, UL, Sowinskiego, Poland). Phosphoric acid, Hexane sulphonic acid sodium salt, and sodium hydroxide were purchased from Sigma–Aldrich (St. Louis, MO, USA). Double distilled water was applied during the entire analysis. 0.45-mm nylon filters (Millipore, USA) were used for mobile phase filtering.

2.3. Preparing standard solutions

A stock solution containing 15 mg/mL of an analyte, 50 mL of water, and 15 g of glycerin was combined in 100 mL volumetric flasks, shaken, and sonicated for 10 minutes, and then the solution was diluted to volume using the same solvent. The stock solution was further diluted while using the mobile phase as a solvent to prepare the working standard solutions.

2.4. Constructing calibration curves

A series of 10 mL volumetric flasks were used to prepare aliquots of the standard solution, ranging from 3.75-22.5 mg/mL, and 20 μL were injected into the instrument. Detection was used at wavelength 202 nm. The peak area at wavelength 202 nm was plotted against the appropriate injection concentrations to construct the calibration graph.

2.7. Pharmaceutical formulations procedure

Evacuate Swabirase Oral Syrup and Benylin® infant's cough syrup, UK. (Containing 0.75 ml Glycerin per 5ml Syrup) was weighed. A quantity containing (50, 100, and 150%) glycerin was obtained and handled as described under the procedure for pharmaceutical formulations, and the 3 prepared solutions were examined as described before.

2.8. Validation of the procedure

The procedure was validated in accordance with the International Conference on Harmonization (ICH) requirements [15] which included linearity, Limit of detection (LOD), the limit of quantitation (LOQ), accuracy, precision, robustness, ruggedness, and stability.

2.8.1. Linearity

Peak areas in the glycerin concentration range of 3.7–22.5 mg/mL were used to create calibration curves. The analysis of each solution was performed three times.

2.8.2. Precision

The repeatability, inter-day, and intraday precision were used to verify the method precision. different analysts were selected and analyzed three concentration levels as previously specified three times on the same day (intra-day precision), and on successive three days (inter-day precision). The relative standard deviation (RSD %) was used to determine the method's precision.

2.8.3. Accuracy

Recovery experiments of analysis of three different concentrations of prepared tablets and pharmaceutical formulations (50, 100, and 150%) were used to verify the accuracy of the method as mentioned in sections 2.7 and 2.8.

2.8.3. Robustness

Small changes in the optimum conditions were made, and the standard solution was injected, to determine the robustness of the developed method. Differences in parameters were studied including injection volume, detection wavelength, mobile phase flow rate, and column temperature.

2.8.4. Ruggedness

Two analysts independently examined a standard glycerin solution under identical operational and environmental parameters. The relative standard deviation percentage of the peak area was determined.

2.8.5. Limits of detection and quantitation

Based on the calibration curves, the LOD and LOQ were separately calculated. The standard deviation of the slope and the intercept of the regression line were used.

Using the formulas, The LOD and LOQ were obtained.

$$LOD = 3.3 \times S.D./ S$$
$$LOQ = 10 \times S.D./ S$$

Where,

S = Regression line slope.

S.D. = Standard deviation of the regression line y-intercept.

2.8.6. Stability

By storing these solutions for three days and comparing the changes in peak area at the corresponding retention times of each analyte with that of freshly prepared solution, it was found that glycerin is affected by reflux with HCl, NaOH, and H2O2, and exposure to UV radiation leads to their degradation.

III.RESLUTS AND DISCUSSIONS

3.1. The optimum chromatographic conditions

In high-performance liquid chromatography reversed-phase (HPLC). The method was detected for the determination of glycerin in different pharmaceutical preparations. Analysis was conducted on Microsorb-MV 100-5 NH2 (150 x 4.6 mm) or equivalent column with an isocratic mobile phase consisting of hexane buffer 0.005M (pH 3), acetonitrile, and methanol (12:78:10 v/v/v) using photodiode array (PDA) detector at 202 nm at a flow rate of 1.0 mL/min and ambient temperature. The drug retention time was determined at 3.011 ± 0.007 min. The volume of injection was 20 µL.

3.2. Optimization of HPLC conditions:

There are many factors affecting the different choices for the separation of glycerin.

3.2.1. Column selection:

Different columns were used for performance investigations, including Microsorb-MV 100 NH2; 5µm 4.6×150 mm, Inertsil ODS-3V 5µm 4.6×150 mm, and Eclipse XDB-C18; 5 µm 4.6×150 mm. The experimental studies revealed that the first column that contained the amino group was the most suitable one since a good separation of the peak was obtained. The other columns did not successfully elute the drug peak, so they were disregarded.

3.2.2. Detecting wavelength selection:

The maximum ultraviolet absorption spectrum of glycerin in water was examined from 195 to 206 nm. Therefore, the glycerin peak was detected at different wavelengths. The most efficient wavelength was 202 nm which had the best sensitivity with a reasonable response and not interfered with the solvent.

3.2.3. Mobile phase composition:

Various changing in the mobile phase components were done. These changes include different proportions of hexane buffer, acetonitrile, and methanol, and variables in the pH and concentration of hexane buffer. The optimum chromatographic performances were obtained when using a mobile phase composed of 0.005M hexane buffer adjusted to pH 3, acetonitrile, and methanol [12:78:10]. The optimization composition of the mobile phase can be obtained as shown in Figure 2.
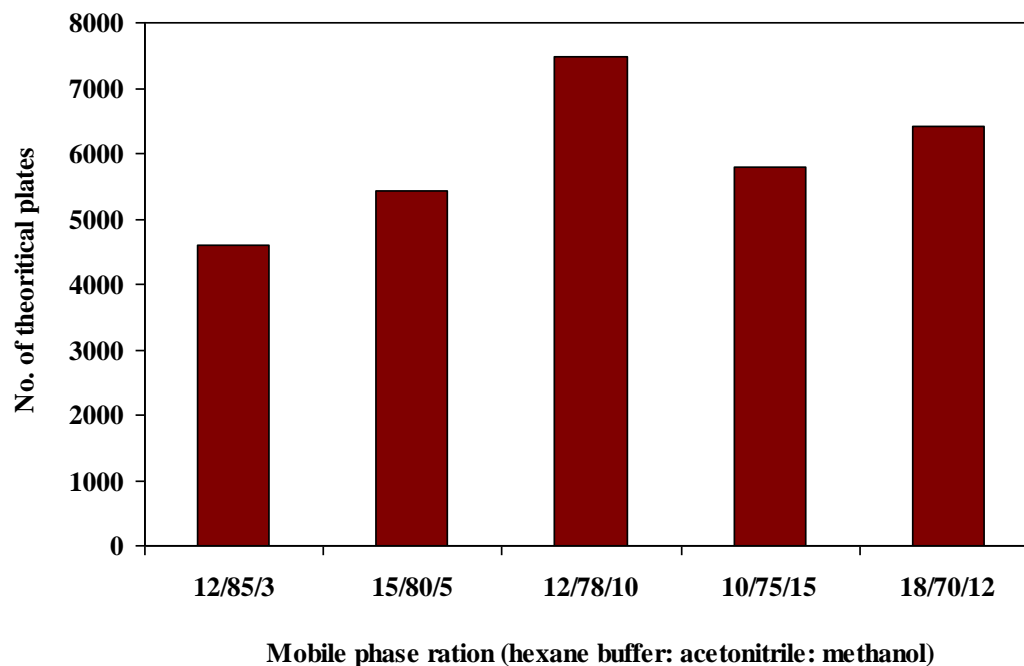

Figure 2: Effect of mobile phase ratio on the chromatographic performance of glycerin.
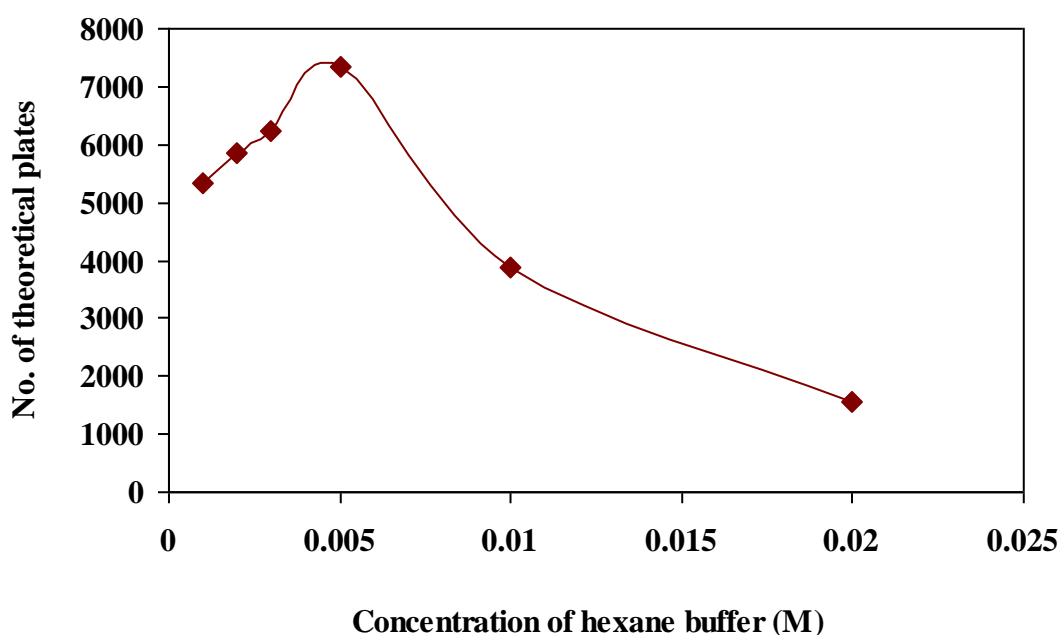
3.2.3.1. Hexane buffer pH:

Adjusting the pH of the phosphate buffer between the ranges of 2.2 and 5 was done by using phosphoric acid or sodium hydroxide. The shape of the peak above pH 5 was unsuitable for measurements. The study showed that the measured hexane buffer pH range, the hexane buffer pH dependence was not useful. In considering the various chromatographic parameters, the optimum pH was determined to be 3. As shown in Figure 3.
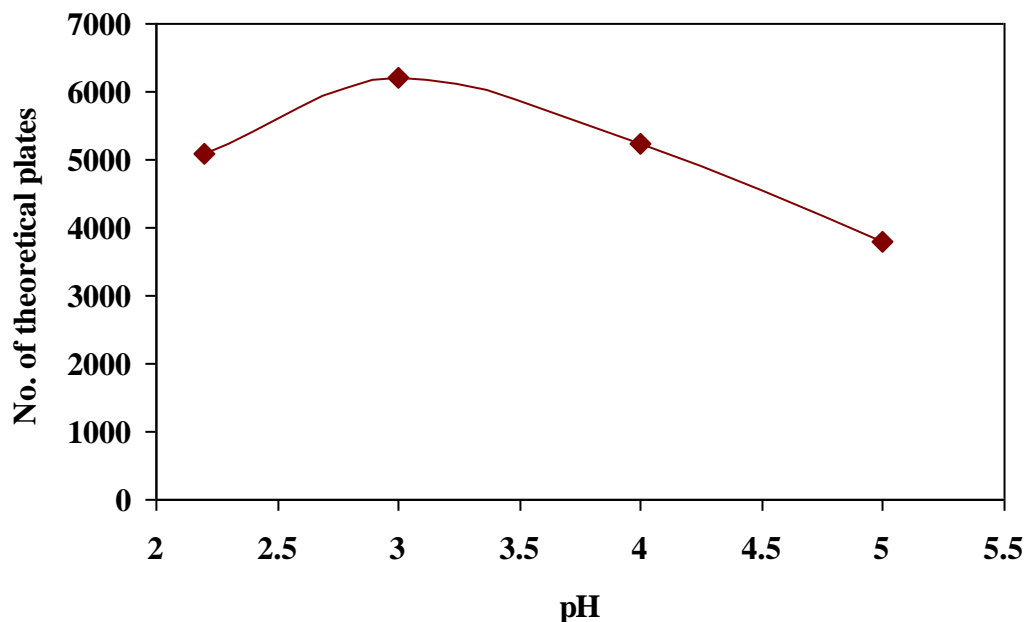
Figure 3: The effect of pH on the chromatographic performance of glycerin.

3.2.3.2. Hexane buffer concentration:

Experimental studies were done to determine glycerin chromatographic performance on hexane buffer concentration was changed. Different concentrations between 0.001 and 0.02 M were studied. According to the study, using 0.005 M hexane buffer resulted in the optimum chromatographic performance. It provided the maximum number of theoretical plates, the best resolution values, high reproducibility of observed retention times, and reasonably good tailing factor values (Figure 4).

Figure 4: The effect of the concentration of hexane buffer on the chromatographic performance of glycerin.

3.2.3.3. Acetonitrile and methanol changing:

With an increase in acetonitrile ratio, the peak of glycerin interfered with the peak of solvent, and it is difficult for detecting it. By the time decrease in acetonitrile percentage, glycerin peak was tailed and low theoretical plates. By increasing and decreasing in methanol percentage in the mobile phase, the peak of glycerin interfered with the peak of solvent and bad values in tailing factors. The ratio of [78:10] of acetonitrile and methanol respectively in the mobile phase is the optimum percent that gave good theoretical plates and the highest resolution values, very good reproducibility of retention times observed, and the relatively good values of the tailing factors.

3.2.4. Choice of flow rate:

To optimize the developed method's chromatographic performance and improve the resolution of the eluted peaks, the effect of flow rate was examined. One mL/min was found to be the ideal flow rate for achieving excellent separation on a regular basis after adjusting the flow rate between 0.5 and 1.5 mL/min.

3.3. Method validation

3.3.1. System suitability

The HPLC chromatographic performance parameters, including the RSD of the retention time of the evaluated drug, capacity factor (k'), and the USP theoretical plates (N), were determined to be within the acceptable ranges, as shown in Table 1.

Table 1. System suitability and regression data.

| Parameters	Glycerin
System suitability	
$t_R \pm$ SD (min)	3.011 ± 0.007
N	7496
k'	1.464
Linearity and regression data	
Linearity range (mg/mL)	3.75-22.5
Detection limit (mg/mL)	0.449
Quantitation limit (mg/mL)	1.360
Slope (b)	10.567
Intercept (a)	0.138
Determination Coefficient (R2)	0.9998

3.3.2. Selectivity

Analyzing prepared Syrup showed Method selectivity. The excipients in the formulation did not interfere with the detection of glycerin, as determined by a comparison of the chromatograms of the raw and extracted glycerin from the syrup. (Figures 5-9).
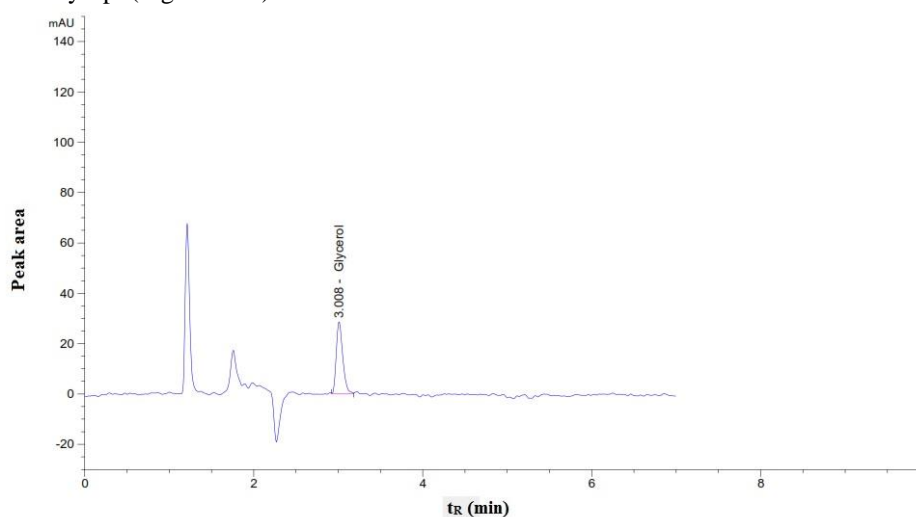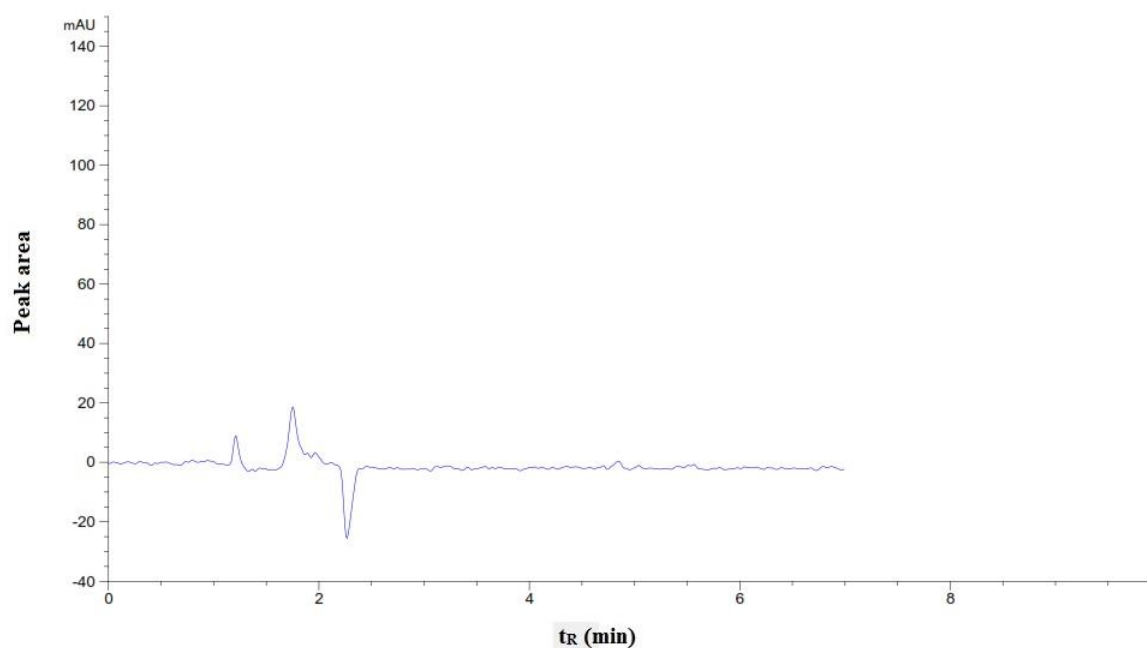

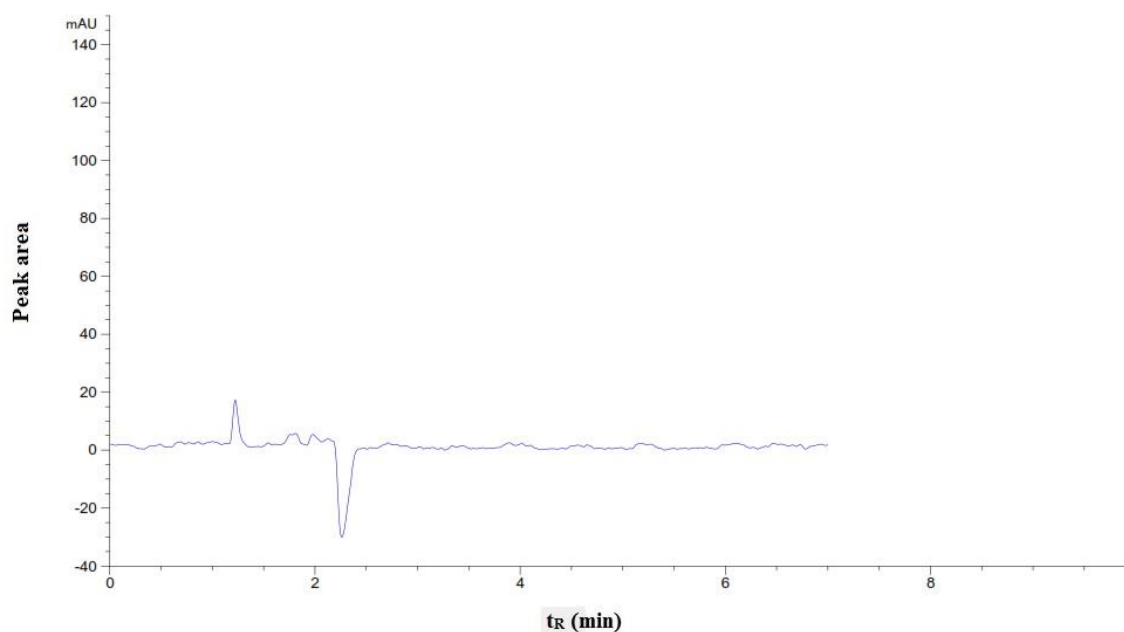**Figure 5.** Chromatograms of (15 mg/mL) glycerin from raw material
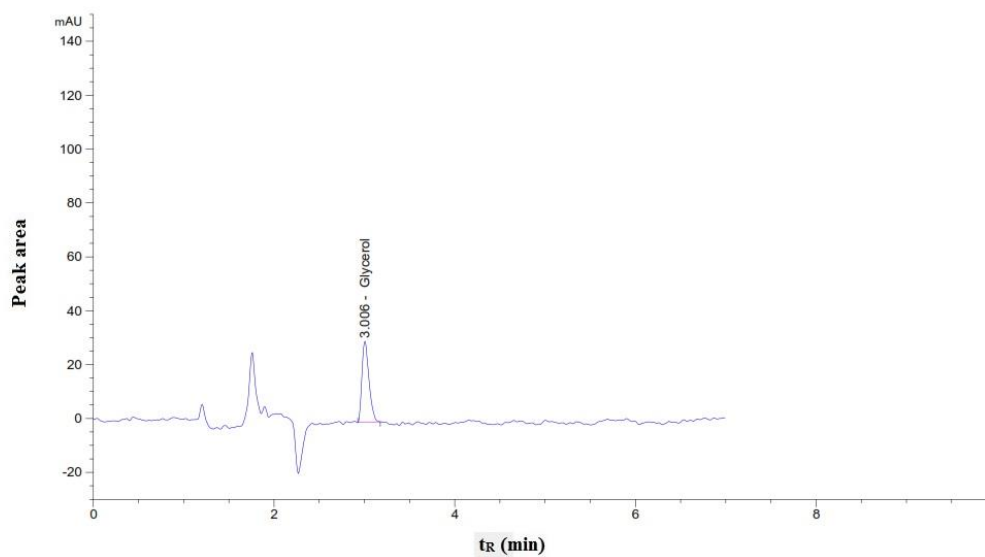
Figure 6. Chromatograms of (15 mg/mL) glycerin from Swabirase oral syrup
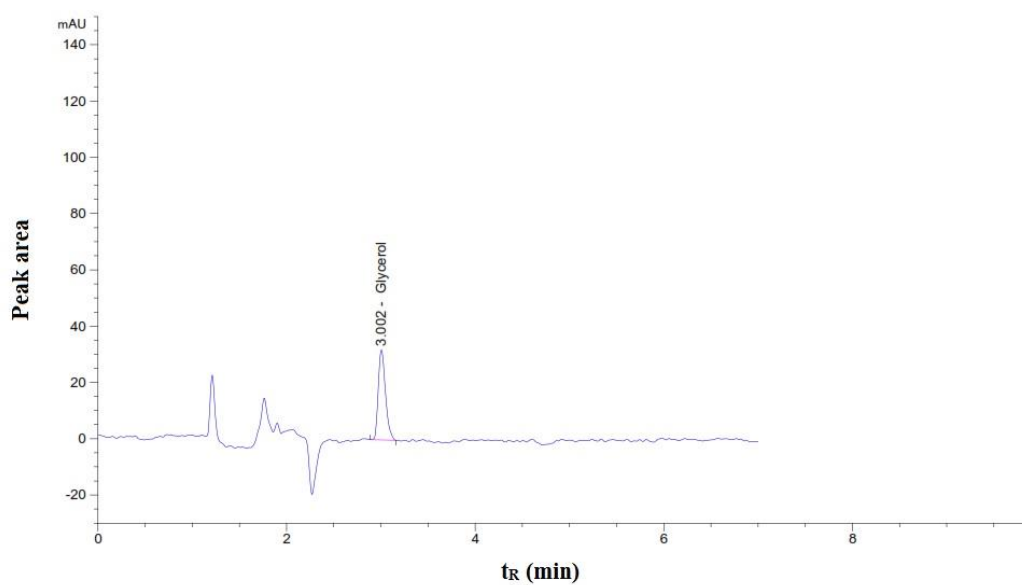
Figure 7. Chromatograms of (15 mg/mL) glycerin from Benylin® infant's cough syrup

Figure 8. Chromatograms of placebo

Figure 9. Chromatograms of solvent

3.3.3. Linearity range

Regression analysis proved linearity in the range of 3.75-22.5 mg/mL for glycerin with high determination coefficients, Table 1 and Figure 10.
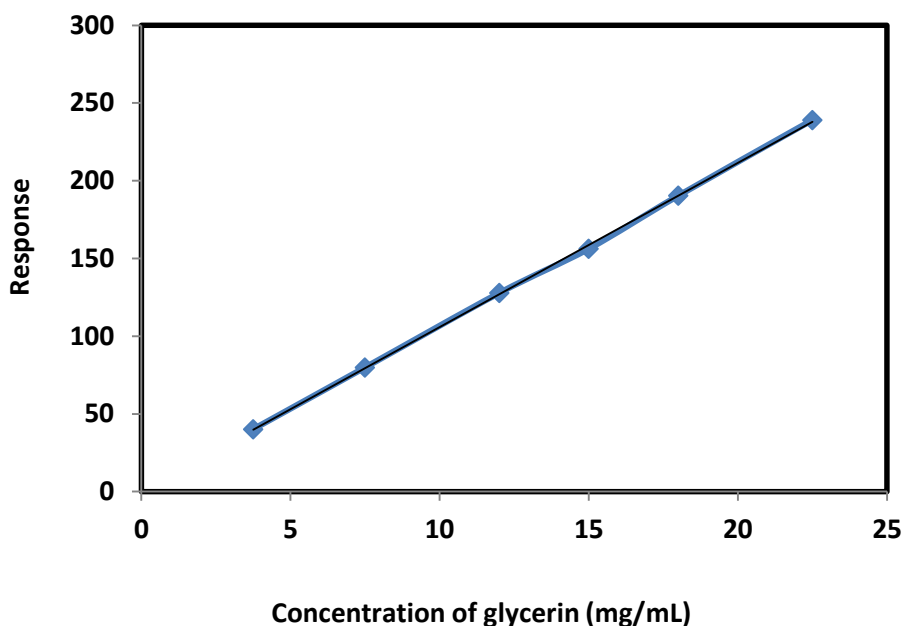
Concentration of glycerin (mg/mL)

Figure 10. Calibration curve for glycerin.

3.3.4. Accuracy and precision

Results of intra-and inter-day precisions observed that RSD% was within the range 0.01-0.12% and recovery values ranged from 98.47-100.53%, thus confirming the good precision and accuracy of the proposed method (Table 2).

Table 2. Accuracy and precision results for determination of glycerin in pure form.

Taken (mg/mL)	Intra-day (n=5)			Inter-day (n=5)		
	Found ± S.D.	RSD %	Recovery%	Found ± S.D.	RSD %	Recovery%
7.5	7.54 ± 0.006	0.08	100.53	7.51 ± 0.009	0.12	100.13
15	14.77± 0.007	0.05	98.47	14.91± 0.006	0.04	99.40
22.5	22.61± 0.003	0.01	100.49	22.56 ± 0.02	0.09	100.27

3.3.5. Robustness of the method

Temperature, flow rate, detecting wavelength, and the injection volume were used to evaluate the robustness of the developed method (Table 3). The peak shape was not affected by any small variations in the examined factors. The determined relative standard deviation percentage of peak area and retention time of the analytes was within 0.48 – 1.76%.

Table 3. Robustness and ruggedness of the proposed method for glycerin

Factor	Column temp. (°C)		Flow rate (mL/min)		Detection Wavelength (nm)		Injected volume (µL)		Analyst to analyst	
Changes	23, 25 and 27		0.95, 1.0 and 1.05		200, 202 and 204		19.9, 20 and 20.1			
Tested parameter	Peak area	t_R	Peak area	t_R	Peak area	t_R	Peak area	t_R	Peak area	t_R
RSD %	0.48	0.39	1.15	0.95	1.76	0.83	1.52	0.74	0.84	0.46

3.3.6. Ruggedness

Two different analysts examined the developed method's ruggedness under the same operational and environmental conditions (Table 5). The relative standard deviation percentage of the peak area was determined within 0.84%.

3.3.7. Stability

According to the stress degradation studies (Figures 11 to 15), glycerin is affected by reflux at 45°C with 1N HCl, 1N NaOH, and 30% H_2O_2 and is degraded by exposure to UV light for 30 minutes. The intact peak drug does not undergo any interference that the method is stability indicating. By injection of the prepared solution at periodic intervals into the chromatographic system for up to about 3.0 days, the stability of the standard solution was studied. The RSD% of peak area was determined to be within 0.64%.for glycerin.
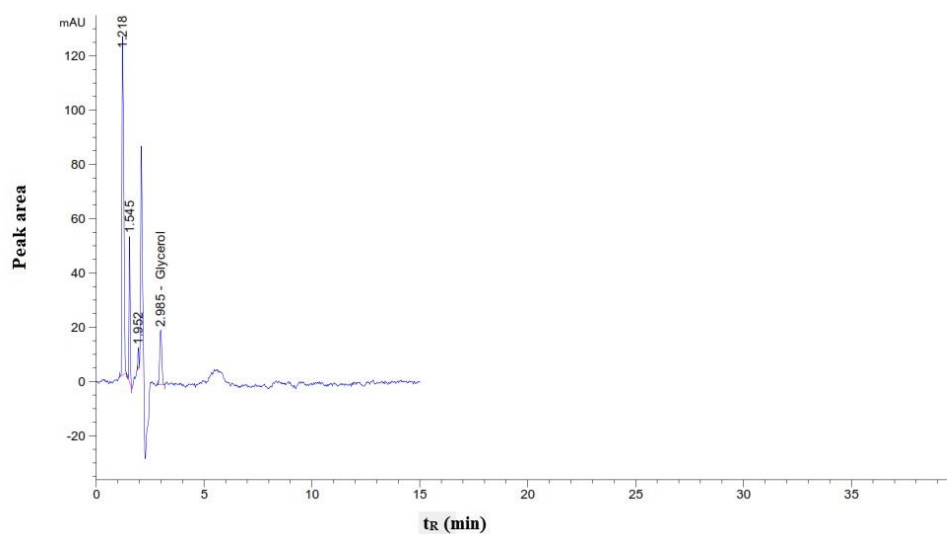
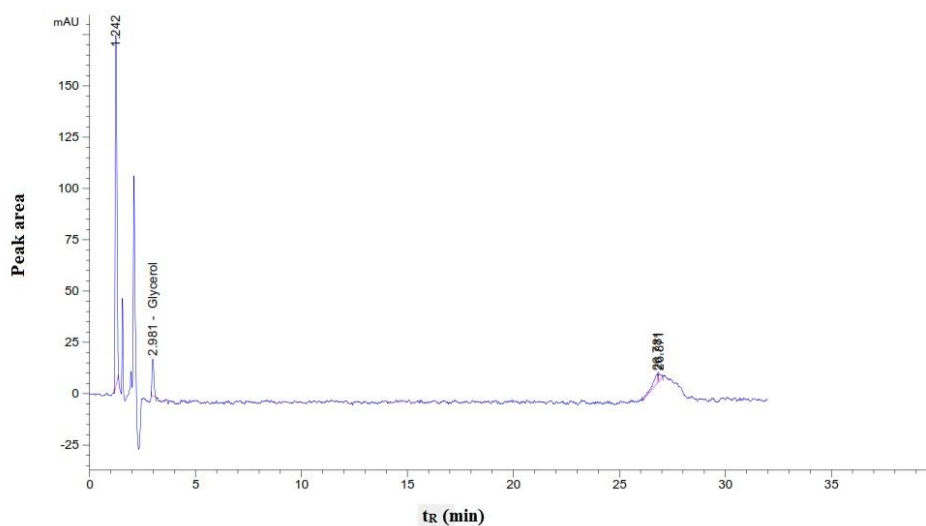**Figure 11.** Chromatograms of (15 mg/mL) glycerin degradation with acid

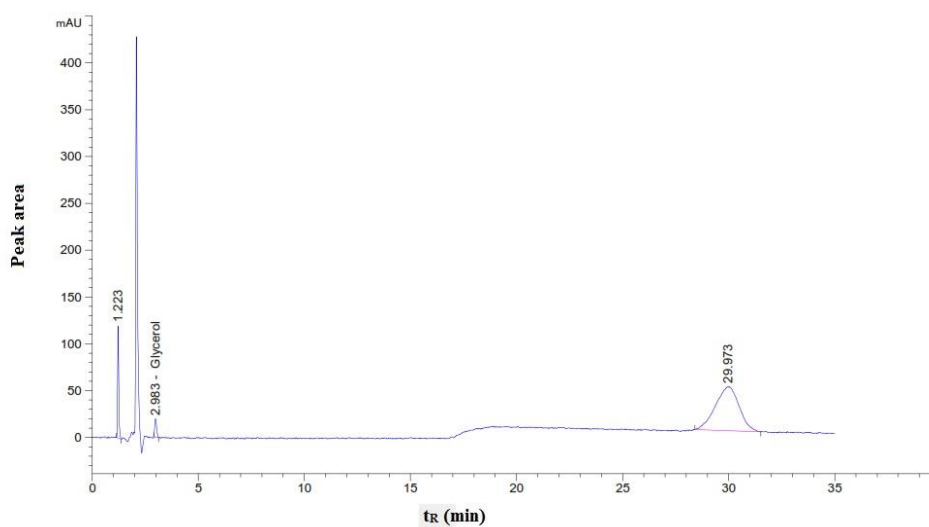**Figure 12.** Chromatograms of (15 mg/mL) glycerin degradation with base

Figure 13. Chromatograms of (15 mg/mL) glycerin degradation with oxidative
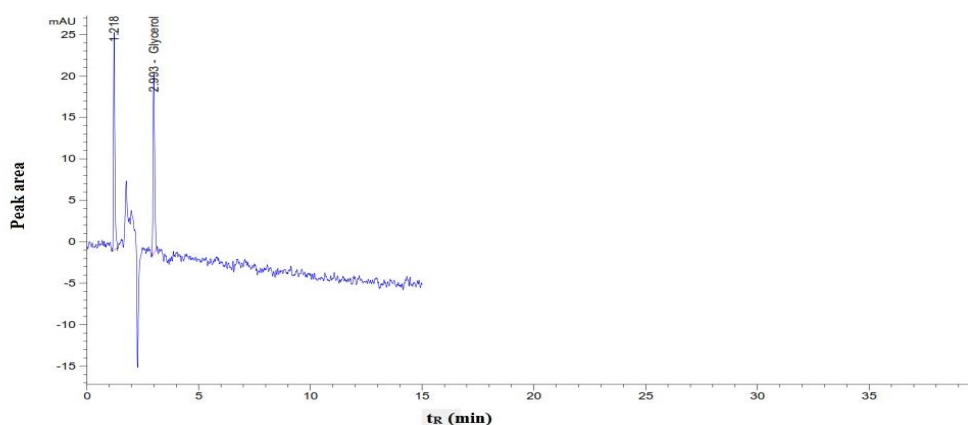
Figure 14. Chromatograms of (15 mg/mL) glycerin degradation with heat
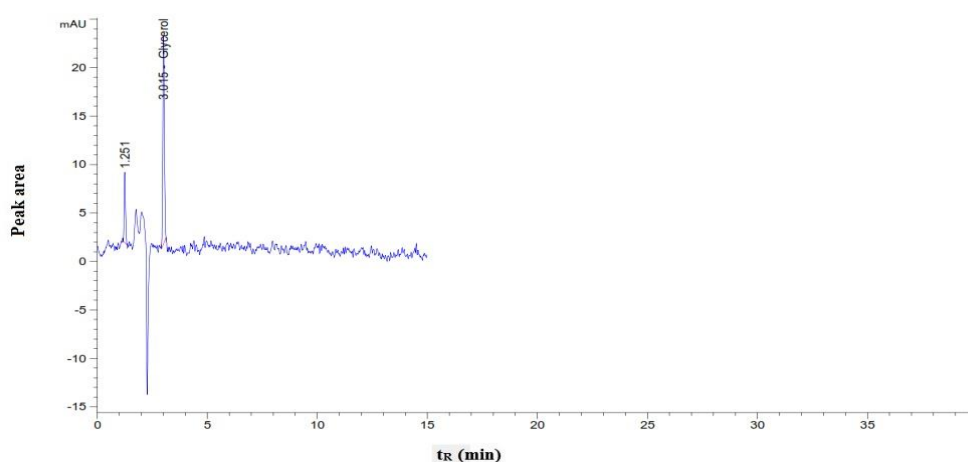

Figure 15. Chromatograms of (15 mg/mL) glycerin degradation with UV.

3.4. Application

The determined mean recovery percent for glycerin of analysis of swabirase oral syrup, Benylin® infant's cough syrup was achieved by the proposed method applied on syrup compared with a reported method [3]. It was found that the determined t and F values are less than the tabulated ones which means that no significant difference between the proposed method and the reported one (Table 3).

Table 3. Statistical analysis of results obtained by the proposed method applied on syrup compared with a reported method.

Parameters	Proposed method		Reported method [3]
	Swabirase oral syrup	**Benylin® infant's cough syrup**	
Mean recovery% [a]	99.98	99.88	99.50
± SD	0.57	0.37	0.65
± R.S.D%	0.57	0.37	0.65
Variance	0.33	0.13	0.42
S.E	0.26	0.16	0.29
t-value [b]	1.28	1.36	
F-value [b]	1.27	3.23	

[a] Average of five determinations (n = 5).

[b] Tabulated values for t (2.571) and f (5.05) at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05)

IV. Conclusion

Glycerin is used in a wide variety of pharmaceutical formulations including oral, otic, ophthalmic, topical, and parenteral preparations. It is used in different concentrations according to the intended use such as an antimicrobial preservative, emollient, aqueous and non-aqueous gel vehicle, humectant, and plasticizer in tablet film coating and sweetening agent in alcoholic elixirs. Many methods were used for the determination of glycerin in pure and in different pharmaceutical dosage forms this method is an accurate, precise, and reversed-phase high-performance liquid chromatographic (HPLC). The method was developed for the determination of glycerin in different pharmaceutical preparations.

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