Detection of Diethylene Glycol in Glycerin and Propylene Glycol by using high performance thin layer chromatography HPTLC

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Abstract:

We were developed analytical method rapid, sensitive and easy to use to detect Diethylene glycol (DEG) in some of excipients: Glycerin, and propylene glycol by using high-performance thin-layer chromatography HPTLC with plate fluorinated: HPTLC Plate silica gel 60 F 254, and with mobile phase: acetone: toluene: 5 M ammonium hydroxide at rates 85: 5:10, respectively. And Use a scanner with wavelength: 325 nm.

This method allows the detection and assay of toxic Diethylene glycol in excipients: glycerin and propylene glycol in concentrations of not less than 0.1%. This method was verified the validity of it to conduct all the constitutional requirements.

Keywords: Diethylene Glycol (DEG), Propylene Glycol (PG), Glycerin, Ethylene Glycol (EG, High Performance Thin Layer Chromatography (HPTLC).

Introduction:

(DEG) Diethylene Glycol :

DEG: (HO-CH2-CH2-O-CH2-CH2-OH)

Diethylene glycol is Organic solvent has many industrial uses [1,2]. DEG is classified as toxic material, it causes when dealing with multiple systemic disorders until the occurrence of acute kidney failure and death [3,4,5]. Diethylene glycol has physical and chemical properties close to the properties of glycerin and propylene glycol, which is cheaper than both glycerin and propylene glycol, thus forcing some producers and sellers to cheat them with DEG [6,7,2].

Diethylene glycol is synthesized from the reaction of ethylene oxide with Ethylene glycol, in this case Diethylene glycol can contain Ethylene glycol EG also as toxic impurity [9,8,10].

World Health Organization (WHO) has record since 1937 until 2009 in different countries of the world thousands of cases of poisoning with Diethylene glycol, most of these cases from children, and ended in most cases with death. The reason for this poisoning is dealt with oral pharmaceutical preparations such as syrups, suspensions, Elixirs, and toothpastes which contained Diethylene Glycol as excipient.

As a result of that, most of the organizations and agencies concerned with health, particularly the Food and Drug Administration (FDA) Confirmed the necessary of detection of Diethylene glycol in pharmaceutical preparations and to verify the safety of any drug before marketing [7,11,12,13].

USP, British, European pharmacopeias mentioned in monograph of Glycerin to Gas chromatography method for detection of Diethylene Glycol and Ethylene Glycol in Glycerin as raw material [14,15,16]. And there are many reference methods based on high-performance liquid chromatography HPLC [17,18,19,20].

World Health Organization (WHO) and Food and Drug Administration (FDA) had provided a method of thin-layer chromatography TLC (limit method), which were used to detect DEG in Glycerin, Propylene Glycol, and Sorbitol after spray the plate with permanganate solution or iodine.

To be used by institutions and countries which do not have modern equipments which had required to detect DEG as mentioned in pharmacopeias of medicine, this method is an inexpensive, simple and easy to use, but it can not be used in the assay of DEG and determine its quantify in a good accuracy [21,22,23,24].

The study aims to:

Develop analytical High Performance TLC method quick, sensitive, easy to use, economic, with a validity to detect and assay DEG in excipients: glycerin and propylene glycol, from a mobile phase used on the thin layer which is acetone: toluene: ammonium hydroxide 5 M ratio 85: 5:10, respectively. Proposed by the World Health Organization [21,22,23,24].

Standards:

- Standard of Diethylene Glycol DEG: (PROLABO), P (GC): 99%.
- Standard of Ethylene Glycol EG: (England), P (GC): 99%.
- Standard of Propylene Glycol PG: (PROLABO), P: 99%.
- Standard of Glycerin: (England), P (GC): 99 – 100.5%.

Reagents:
- Reagents for HPLC: Methanol (Merck).
- Acetone (Merck).
- Toluene (Merck).
- Ammonia Solution 25% (Merck).

Instruments and tools:
High Performance Thin Layer Chromatography: CAMAG HPTLC :
CAMAG Automatic TLC Sampler
CAMAG TLC Scanner.
Nitrogen gas.
25 HPTLC aluminum sheets (Merck), 20 x 20 cm silica gel 60 F $_{254}$.
CAMAG, HPTLC Immersion Device.
Sartorius Analytic Balance (0.0001 mg).

Preparation of solutions:
Stock standard solution of DEG in Methanol with concentration: 2 mg/ml.
Standard solution of DEG in Methanol with concentration: 0.5 mg/ml.
Sample solution of raw material in Methanol with concentration: 500 mg/ml.
Preparation of Validation solution [14,19,25].

Chromatographic system:
Mobile phase: mixture of Acetone: Toluene: Ammonium Hydroxide 5 M (85:5:10) respectively
HPTLC Plate silica size: 10 x10 cm. and drying plate after chromatography with air.
Spray gas: nitrogen.
Injection volume: 2 μl.
Wavelength: 325 nm.

We calculated the percentage of DEG in the samples accordance with the monograph of glycerine in USP, and British Pharmacopoeia [14,15], and it shouldn't be more than 0.1% from DEG of the weight of samples [14,15].

Results
When we inject each of Diethylene glycol, Ethylene glycol, glycerine, propylene glycol, and Polyethylene glycol 400 on TLC plate in the conditions of the method which we used, and identify the values of Retention Factor RF for each of them (Table 1). The Retention Factor RF of Ethylene glycol is close to The Retention Factor RF of Diethylene glycol, and we cannot distinguish between them if they were together in same sample, so we excluded Ethylene Glycol from this study as shown in Figure 1. The Retention Factor RF of Poly Ethylene glycol 400 is so closed to The Retention Factor RF of Diethylene glycol therefore we cannot use this method to detect DEG in the sample of PEG 400 as in (Figure 2).

Figure 3 shows chromatogram of standard solution DEG (2μl of 0.5 mg/ml DEG). And Table 2 shows areas of peaks resulting from injecting the standard solution for five spots in a row and the value of the relative standard deviation RSD.

Figures 4.5 show chromatograms of Glycerin sample and Propylene Glycol sample respectively (2μl of 500 mg/ml). And Figures 6.7 show chromatograms of Glycerin sample and Propylene Glycol sample respectively that containing standard DEG with concentration (0.5 mg/ml).

Results of Analytical Methods Validation
Accuracy:
The average percentage of recovery is: 106.36, %103.89 for samples solutions of glycerine and PG respectively, with concentrations: (50%, 100%, 120% of DEG standard).

Precision:
The average percentage of recovery is: 103.87%, %100.59 for samples solutions of glycerine and PG respectively, three samples with each concentration: (50%, 100%, 120% of DEG standard), and the value of RSD to these recoveries is 10.81%, 10.81 for samples of glycerine and PG respectively.

Selectivity:

When we inject placebo sample of glycerine or PG didn't contain DEG, there were no response occur in retention time of DEG. And the average of percentage of recovery is: 103.19%, %97.74 for three samples solutions of glycerine or PG with concentrations: 100% of DEG standard respectively.

Linearity & Range:

We recorded the responses of each concentration of DEG Standard (50%, 75%, 100%, 125%, 150%) (Table: 3), the Linear Regression Equation corresponding to these responses (Figure: 8), and the value of the Correlation Factor is: 0.9971.

Detection limit:

Detection limit is equal to 0.033 mg/ml, equivalent to 6.6% of the standard concentration.

Quantification Limit:

Quantification limit is equal to 0.109 mg/ml, equivalent to 21.8% of the standard concentration

Robustness:

The average percentage of recovery for DEG in the samples of Glycerine is: 100.6%, 100.3%, 101.53%, respectively, with the change of the distance which the mobile phase was reached, in the order (80, 90, 100 mm).

As well as for the average percentage of recovery for DEG in the samples of propylene glycol is: 102.7%, 101.19%, 98.71%, respectively, with the same changing in distance as in case of Glycerine.

The Relative retention times of DEG for Glycerine samples is: 1.0, 0.91, 0.98 respectively with the previous changing, and The Relative retention times of DEG for Propylene Glycol samples is: 0.98, 0.88, 0.92 respectively also with the previous changing.

Discussion and Conclusions:

The results of verification tests had shown that the studied method of HPTLC meet the requirements of validation in the Pharmacopoeia [17,18], while the percentages of recovery in tests, accuracy and specificity for Glycerine samples are: 106.4%, 103.19%, respectively, and for Propylene Glycol samples are: 103.8%, 97.74% respectively.

Also the relative standard deviation RSD of values of recovery in tests of repeatability for Glycerine and Propylene Glycol are: 10.8%, 5.1% respectively.

The results also had shown that this method is linear, and the correlation coefficient is close to one: 0.9971 (Figure 8, Table 8).

And the value of detection limit is: 0.033 mg/ml, and the value of Quantification limit is 0.109 mg/ml.

We can by using the applied method of HPTLC, detect and assay DEG as an impurity in glycerin and propylene glycol. And this method is rapid, sensitive and inexpensive and does not require the completion of any analysis of more than small TLC plate dimensions of 10 X 10 cm. And the time required completing of any analysis takes only approximately one hour, .including preparation of solutions and injected and the deportation of the mobile phase on the plate

Appendix of tables and chromatograms:

Table (1): Retention factor of DEG & EG & PG & PEG 400 & Glycerin:

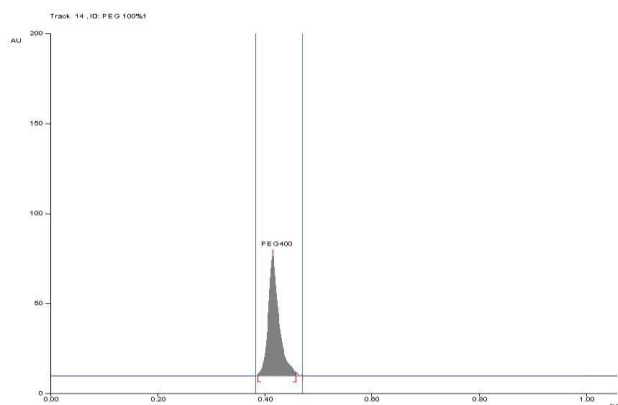| Name	DEG	EG	PG	Glycerin	PEG 400
Retention Factor R_F	0.40	0.44	0.69	0.04	0.43

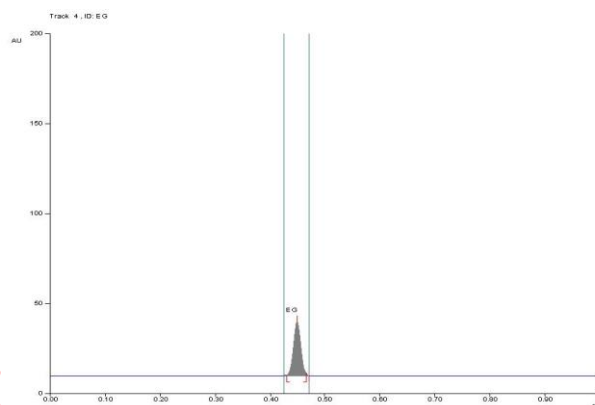**Figure (2):** Chromatogram of sample solution of PEG400

Figure (1): Chromatogram of solution of EG

Table (2): relative standard deviation of area of standard solution

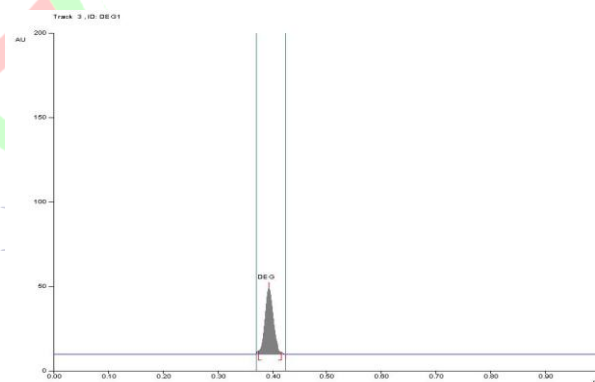| Standard. NO	Area	R_f		
Std - 1	555	0.40		
Std - 2	548	0.39		
Std - 3	544	0.39	Average	560.8
Std - 4	571	0.41	SD	17.5
Std - 5	586	0.39	RSD	3.11

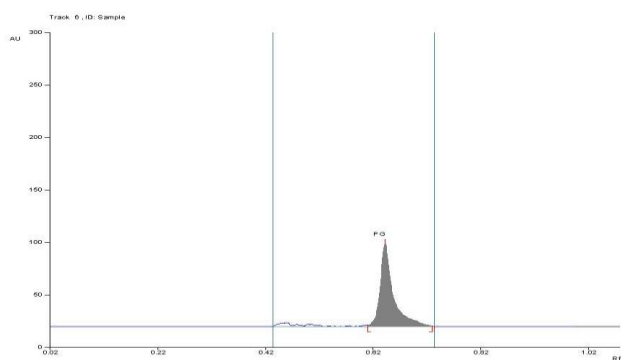**Figure (3):** Chromatogram of standard solution DEG

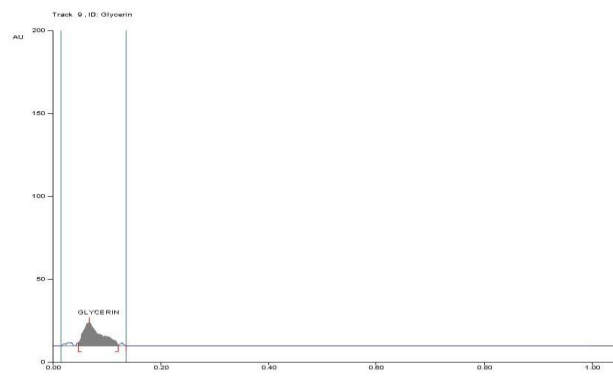**Figure (5):** Chromatogram of sample solution of PG

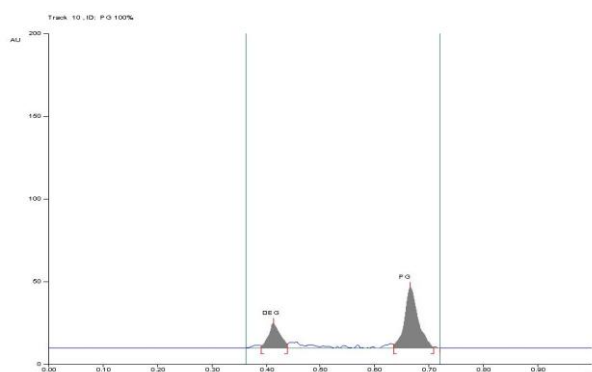**Figure (4):** Chromatogram of sample solution of Glycerin

Figure (7): Chromatogram of sample solution of PG with 100% St Concentration
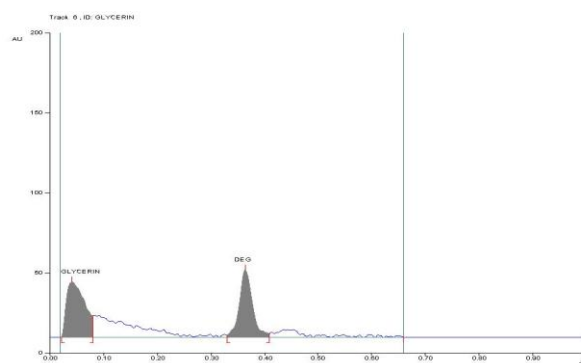

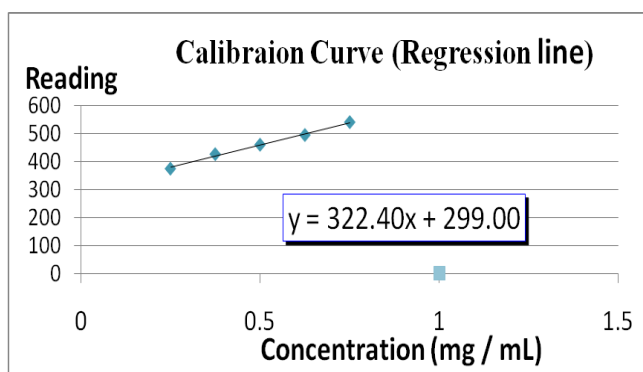**Figure (6):** Chromatogram of sample solution of Glycerin with 100% St Concentration

Figure (8): Chromatogram of Linear Regression Equation

Table (3): result of Linearity of method

St.NO	Concentration		Area
	(%)	(mg/ml)	
1	50	0.25	375
2	75	0.375	427
3	100	0.5	461
4	125	0.625	496
5	150	0.75	542
Correlation Factor		0.9971	
Slope		322.4	

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