Research Article

DETECTION OF DIETHYLENE GLYCOL IN EXCIPIENTS AND IN PHARMACEUTICAL PRODUCTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH RID DETECTOR

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ABSTRACT

This research was interested in development a new analytical method (suitable, quick, sensitive) by using HPLC with C18 column and RID detector. This method Let us detect toxic material (Diethylene Glycol) in excipients: Glycerin, Propylene Glycol, and Poly Ethylene Glycol 400, and some of finish products which contain them. We make sure of all parameters of validation of this method is compatible with constitutional requirements. We use this method to detect Diethylene glycol in some samples of raw materials and some of finish products which currently used.

Keywords: Diethylene Glycol (DEG), Propylene Glycol (PG), Poly Ethylene Glycol (PEG), Glycerin, Ethylene Glycol (EG), Intoxication Accidents, High Performance Liquid Chromatography (HPLC).

INTRODUCTION

Diethylene glycol is Organic solution 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[3-5]. 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[2,6,7].

Diethylene glycol formed as a byproduct when the synthesis of compounds Polyethylene glycol (PEG) with different molecular weights from condensing ethylene oxide with ethylene glycol. Thus, these compounds may contain rates of DEG impurity in variation amount depending on the preparation and purification methods used in the production[8-10].

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[11-13].

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 contain 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[7,14-16].

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. And there are other methods in these pharmacopeias to detection of Diethylene Glycol in the monograph of Polyethylene glycol compounds based on gas chromatography and colorimetric assay[17-19]. And also there are method based on high-performance liquid chromatography HPLC with RID detector to detect DEG in the excipient Diethylene glycol Stearates[17-19].

The References reported method based on the use of HPLC with RID detector to detect DEG in the juice[20]. And a method for detecting Diethylene Glycol in Propolis Syrup by using HPLC with UV detector, but we should do derivation of the sample before inject it and the value of detection limit is 0.005 mg/ml, and Quantification Limit: 0.05 mg/ml. And there are also other similar methods[21-23].

This study aims to

- Find an analytical method of high performance liquid chromatography HPLC, which is quick, easy, economic, and validity, and used this method to detect DEG in excipients of glycerine, propylene glycol, and Polyethylene glycol 400, And in some pharmaceutical products which are containing these excipients.

- use this analytical method to detect Diethylene glycol in samples of excipients and pharmaceutical products which is traded locally.

We have decided at the beginning of our research to include toxic material Ethylene Glycol in the study also, with Diethylene Glycol to reach a convenient way to detect two materials together in the same experimental conditions in the excipients studied.

- 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%.
- Standard of Poly Ethylene Glycol PEG 400: (England).
- Reagents for HPLC: Water (Merck).

MATERIALS AND METHODS

Samples

We collected the samples from several Syrian pharmaceutical laboratories, and some pharmacies.

1. Raw materials: Include glycerin, propylene glycol (PG) and Polyethylene glycol 400 (PEG400). (Table 1).

2. Pharmaceuticals products: include syrups, oral Drops. (Table 2).

Instruments and tools

HPLC High Performance Liquid Chromatography: Shimadzu

Shimadzu Refractometer Detector RID-10A

Shimadzu Auto Injector SIL – 10AD VP Shimadzu Column Oven CTO-10A C18.) L1) (mm X 250 mm Columns (Merck) RP-18, Purospher Star end capped (5µm), Sartorius Analytic Balance (0.0001 mg).

Preparation of solutions

Stock standard solution of DEG in distilled water with concentration: 1 mg/ml.

Standard solution of DEG in distilled water with concentration: 0.2 mg/ml.

Sample solution of raw material or pharmaceutical preparation in distilled water with concentration: 200 mg/ml.

Preparation of Validation solution

Suitable Chromatographic system

After doing several experiments by changing some chromatographic systems in some pharmacopeia and references methods[17,20], we have been reached to the suitable chromatographic system, which achieved good separation between the studied compounds: glycerine, propylene glycol, Diethylene Glycol, and Polyethylene glycol 400.

Chromatographic system

A high-performance liquid chromatography HPLC, equipped with Refractometer Detector RID, column: C18 (25cm x 4mm), column temperature: 25°C Cell Temperature of the refractometer detector: 30°C Mobile phase: distilled water. Flow rate: 0.5 ml/min. volume of Injection: 20 µl.

We calculated the percentage of DEG in the samples accordance with the monograph of glycerine in USP, and British Pharmacopoeia[17,18], and it shouldn't be more than 0.1% from DEG of the weight of samples[17,18].

RESULTS

Results of Analytical Methods Validation

Accuracy

The average percentage of recovery is: 100.38%, for three samples solutions (PG, glycerine, and PEG 400) with concentrations: (50%, 100%, 120% of DEG standard).

Precision

Repeatability

The average percentage of recovery is: 99.84% for nine samples solutions (PG, glycerine, and PEG 400) with concentrations: (50%, 100%, 120% of DEG standard), and the value of RSD to these recoveries is 1.07%.

Intermediate Precision

The average percentage of recovery is: 101.28% for nine samples solutions (PG, glycerine, and PEG 400) with concentrations: (50%, 100%, 120% of DEG standard), and the value of RSD to these recoveries is 1.40%.

Selectivity

When we inject placebo sample didn't contain DEG, there were no response occur in retention time of DEG. And for three samples solutions (PG, glycerine, and PEG 400) with concentrations: 100% of DEG standard, the average percentage of recovery is: 102.33%.

Linearity and Range

We recorded the responses of each concentration of DEG Standard (50%, 75%, 100%, 125%, 150%) (Table: 6), the Linear Regression Equation corresponding to these responses (Figure: 8), and the value of the Correlation Factor is: 0.9975.

Detection limit

Detection limit is equal to 0.001 mg/ml, equivalent to 0.5% of the standard concentration.

Quantification Limit

Quantification limit is equal to 0.004 mg/ml, equivalent to 2% of the standard concentration.

Robustness

The average percentage of recovery for DEG in the samples is: 101.27%, 100.95%, 100.70%, respectively, with the change of flow rate: 0.4, 0.5, 0.6 ml/min.

Relative retention times of DEG are respectively: 0.975, 0.98, and 0.97 with the previous flow rates.

Results of analysis of samples

(Tables: 3, 4) Shows the Results of analysis of samples for excipients and pharmaceutical products which were studied.

Table 1: Samples of raw materials

Sample No	Raw material	Sample No	Raw material	Sample No	Raw material
1	Glycerin	9	Propylene glycol	17	PEG 400
2	Glycerin	10	PEG 400	18	Glycerin
3	Propylene glycol	11	Glycerin	19	Propylene glycol
4	PEG 400	12	Propylene glycol	20	Glycerin
5	Glycerin	13	PEG 400	21	Propylene glycol
6	Propylene glycol	14	Glycerin	22	PEG 400
7	PEG 400	15	Glycerin		
8	Glycerin	16	Propylene glycol		

Table 2: Samples of pharmaceutical products

Sample No	Finished product	Active ingredient	Percentage %		
			Glycerine	PG	PEG 400
1	Syrup	Loratadine	10	20	
2	Syrup	Pseudoephedrine – Guaifenesin Dextromethorphan	16	10	
3	Syrup	Paracetamol- pseudoephedrine Dextromethorphan – Chlorpheniramine	14.6	14.6	
4	Syrup	Pseudoephedrine – Triprolidine – Guaifenesin	37.47		
5	Drops	Paracetamol	17.8	17.8	
6	Syrup	Zidovudine	10		
7	Drops	Pseudoephedrine – Guaifenesin Dextromethorphan –	14	14	
8	Syrup	Guaifenesin – Oxomemazine	5		
9	Syrup	Paracetamol	16	14	
10	Syrup	Paracetamol – Pseudoephedrine – Chlorpheniramine	16	15	
11	Drops	Vit A – Vit D – Vit C	10	4	
12	Syrup	Cetirizine	9.3	2	
13	Syrup	Metoclopramide	20		
14	Drops	Clonazepam		98	
15	Drops	Haloperidol		14	

Table 3: Results of analysis of testes raw materials

Sample No	(Standard) Average area of DEG	(Sample) Average area of DEG	DEG %
1	28767	0	0
2	52429	0	0
3	45212	0	0
4	52429	5389	0.0106
5	34078	0	0
6	45212	0	0
7	37539	7618	0.0219
8	28767	0	0
9	45212	0	0
10	52429	8119	0.0160
11	28767	5423	0.0187
12	45212	0	0
13	45212	4977	0.0114
14	45212	0	0
15	28767	0	0
16	45212	0	0
17	52429	0	0
18	28767	0	0
19	52429	0	0
20	28767	0	0
21	45212	0	0
22	37539	648	0.0019

Table 5: Pharmacopeia parameters of chromatogram in figure (1):

Name	Retention Time t_R(min)	Resolution (R)	Tailing Factor (T)	Capacity Factor (K`)	Theoretical Plates (N)
Glycerin	5.367	-	1.99	5.71	1883.86
EG	5.833	0.93	0.85	6.29	2107.13
PG	8.508	4.61	1.08	9.64	2713.02
DEG	13.492	6.24	1.10	15.86	3262.59

Table 4: Results of analysis of tested pharmaceutical products

Sample No	(Standard) Average area of DEG	(Sample) Average area of DEG	DEG %	
			Excipients	Syrups
1	29279	*	*	*
2	29279	0	0	0
3	29279	0	0	0
4	29279	*	*	*
5	29279	0	0	0
6	48956	0	0	0
7	29279	*	*	*
8	48956	0	0	0
9	29279	0	0	0
10	48956	0	0	0
11	48956	0	0	0
12	48956	0	0	0
13	29279	*	*	*
14	29279	*	*	*
15	48956	0	0	0

* overlapping peaks of other components compounds of the studied preparations at the retention time of DEG.

Table 6: Pharmacopeia parameters of chromatogram in figure (2):

Name	Retention Time t_R(min)	Resolution (R)	Tailing Factor (T)	Capacity Factor (K`)	Theoretical Plates (N)
Glycerin	5.367	-	1.13	5.68	2036
PG	8.508	5.67	1.08	9.59	2880
DEG	13.492	6.44	1.12	15.79	3494

DISCUSSION AND CONCLUSION

When we inject a mixture of Diethylene glycol, Ethylene glycol, glycerine, propylene glycol, and Polyethylene glycol 400 in the chromatographic system of the method which we used, we had good separation between the peaks of glycerine, propylene glycol, and Diethylene glycol, as soon as the values of Resolution are: 5.67, 6.44 Respectively, and there is no peak appear to Polyethylene glycol 400.

While the retention time of peak of Ethylene Glycol is close to the retention time of peak of glycerine, and the resolution between both:0.93 was not accepted, (Figure 1, Table 5), and therefore we were excluded Ethylene glycol from the study (Figure 2, Table 6).

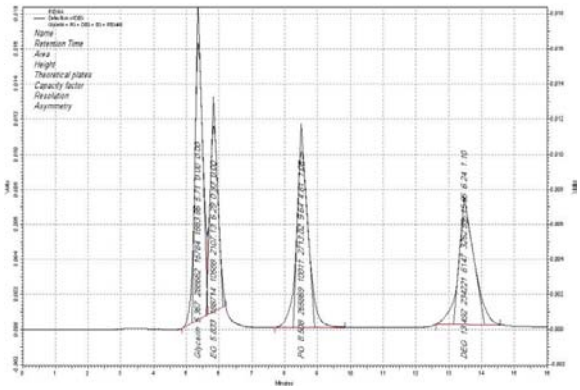

Figure 1: Chromatogram of solution contain: DEG & EG & PG &Glycerin & PEG400

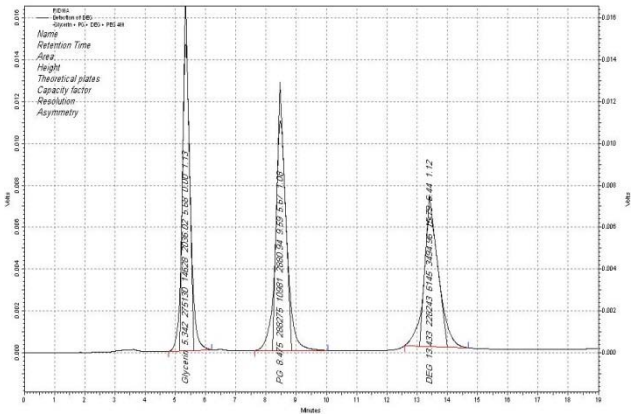

Figure 2: Chromatogram of solution contain: DEG & PG & Glycerin & PEG 400.

We see the chromatogram of standard solution of Diethylene glycol (0.2 mg / ml) in (Figure 3), and (Table 7) shows the areas of peaks resulting from injecting the standard solution for five consecutive times and the value of the relative standard deviation RSD.

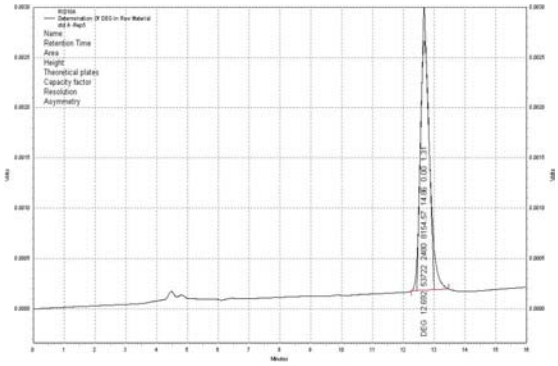

Figure 3: Chromatogram of standard solution of DEG

We see in (figure 4, 5, 6) respectively the chromatograms resulting from injection of 20µl of sample solutions (200 mg/ml) of glycerine, propylene glycol, and Polyethylene glycol 400. And (Figure 7) shows the chromatogram of a mixture of glycerine, propylene glycol, and Polyethylene glycol 400 containing a standard solution of DEG with concentration (0.2 mg / ml).

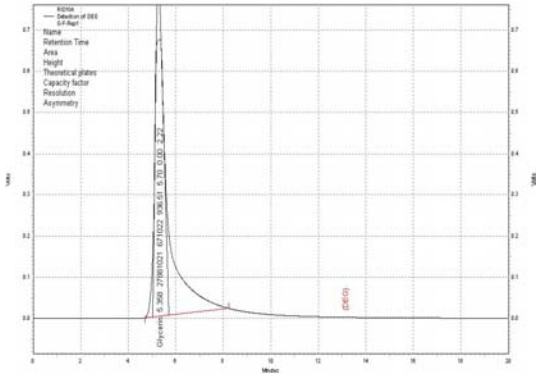

Figure 4: Chromatogram of sample solution of Glycerin

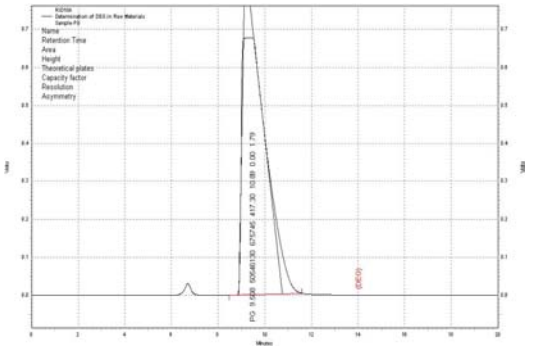

Figure 5: Chromatogram of sample solution of PG

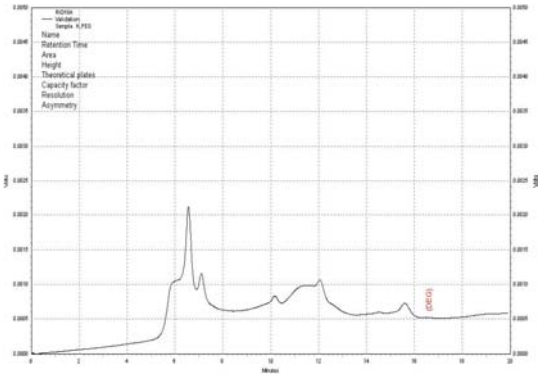

Figure 6: Chromatogram of sample solution of PEG 400

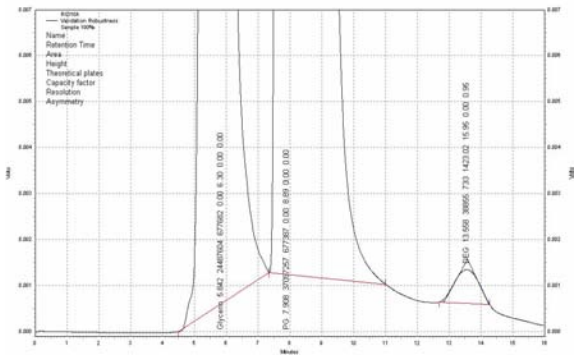

Figure 7: Chromatogram of sample solution of Glycerin & PG & PEG400 with 100% St Concentration

We have found in our research an analytical method HPLC to detect DEG in excipients: glycerine, propylene glycol, and Polyethylene glycol 400. And also in some pharmaceutical preparations which are containing them.

This method is rapid, sensitive, and inexpensive as it requires column C18 and composition of mobile phase and solvent is only distilled water.

First: Results of validation

The results of verification tests had shown that the studied method of HPLC meets the requirements of validation in the Pharmacopoeia[17,18], while the percentages of recovery in both tests, accuracy and specificity are: 100.38%, 102.33%, respectively. Also the relative standard deviation RSD of values of recovery in tests of repeatability and intermediate precision are: 1.07%, 1.4% respectively.

The results also had shown that this method is linear, and the correlation coefficient is close to one: 0.9975 (Figure 8, Table 8).

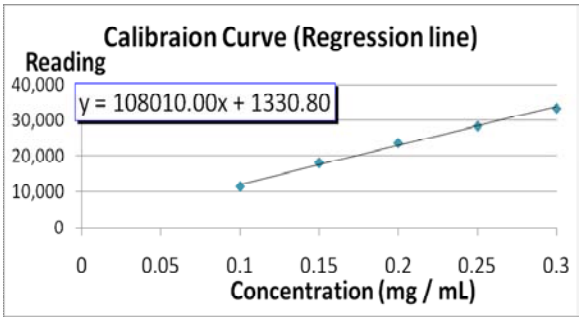

Figure 8: Chromatogram of Linear Regression Equation

Table 7: Relative standard deviation of area of standard solution

Standard No	Area		
Std - 1	46369		
Std - 2	46310		
Std - 3	45444	Average	45756.8
Std - 4	45360	SD	534.8
Std - 5	45301	RSD	1.17

Table 8: Result of Linearity of method

Standard No	Concentration		Area
	(%)	(mg/ml)	
1	50	0.1	11408
2	75	0.15	18038
3	100	0.2	23607
4	125	0.25	28363
5	150	0.3	33248
Correlation Factor		0.9975	
Slope		108010.0	

The value of detection limit is: 0.001 mg/ml, and the value of Quantification limit is 0.004 mg/ml.

These findings which we had in study of validation of HPLC method are similar with the finding of results of validation of Gas chromatography in monograph of

Glycerine[17,18], as well as the values of detection limit 0.001 mg/ml, and Quantification limit 0.004 mg/ml of this used method is smaller than the values of the corresponding reference method for detecting DEG in syrup Propolis, which is for detection limit 0.005 mg/ml, and for Quantification limit 0.05 mg/ml[23].

Second: Results of analysis of samples

The results had shown that the percentage of DEG in all tested excipients (22 samples) is within constitutional limits, and it did not exceed 0.1% of DEG in these samples. Above of that we find that all samples of PEG 400 in addition to one sample of glycerine containing DEG, but at rates below the minimum prescribed by the constitution.

The results of pharmaceutical preparations had shown that the percentage of DEG in all tested preparations (15 samples) is within constitutional limits, and it did not exceed 0.1% of DEG in these samples. It should be noted here that we could not detect DEG in five of 15 tested preparations, due to overlapping peaks of other components compounds of the studied preparations at the retention time of DEG.

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