Quantitative Determination of A Steroidal Drug Using Reversed-Phase High- Performance Liquid Chromatography (RP-HPLC)

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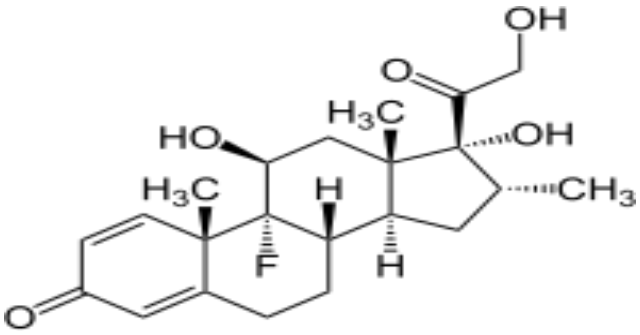
Abstract
Dexamethasone was quantified in a fast, accurate and sensitive way using Reversed-phase high-performance liquid chromatography (RP-HPLC) method.
In this method, a separation column of porous silica particles L3 (250 × 4.6mm) was used, the flow rate is 0.5 ml/min, the column temperature is 25 °C, the Reagent wavelength is 250 nm, and the injection volume is 20 µl. As for the mobile phase, it consisted of acetonitrile and buffer solution dipotassium phosphate and monopotassium phosphate (50:50 v/v) and the pH was 8. The method was validated by calculating the recoverability that ranged between (103.39-104.15%), and the correlation coefficient (0.9959-0.9964) and linearity (0.1-100 µg/ml) the method was successfully applied for the quantitative determination of the mentioned drug.
Keywords: dexamethasone, dexone, and Reversed-phase high-performance liquid chromatography (RP-HPLC).

1. Introduction



Dexamethasone is one of the steroidal drugs that has anti-inflammatory and immunosuppressive effects, and when compared with cortisol, it has a stronger effect in terms of its functional effect. According to the World Health Organization's List of Essential Medicines, dexamethasone is one of the most important drugs required in the basic health system [1]. It is used to treat many inflammatory and autoimmune diseases, such as rheumatoid arthritis and bronchospasm [2, 3].
In addition, it is given in small amounts in some dental surgery and is useful to counteract anaphylactic shock if given in high doses. It is also found in some eye drops, nasal sprays, and certain ear drops [4, 5]. Figure 1 shows the chemical composition of the drug, which is an inorganic ester and is associated with chemical treatments [6].
Figure 1: The chemical structure of dexamethasone.
By reviewing the literature, we note the interest of many researchers in estimating this drug for its medical importance by different analytical methods, such as spectroscopic methods using the relative derivative [7], as well as using the first and third derivatives [8]. Ultraviolet rays were also used to estimate the drug in some pharmaceutical preparations [9]. The colorimetric methods were used in the quantitative estimation of the drug under study [10] and also the estimation of Dexamethasone using high-performance liquid chromatography technique [11], as well as the quantification of the drug by electrical methods [12, 13].

Practical Part Materials and reagents

The drug dexamethasone used as a standard solution is of Indian origin, acetonitrile is supplied by Supelco (USA), and distilled water is used by the Dubai Company, and for the two substances mono and dipotassium phosphate, they were
Prepared by Sigma-Aldrich (Germany) company, and the same substances mentioned throughout the experiment was used after they were purchased from the local Iraqi market. As for the pharmaceutical preparations, they contained dexamethasone 0.5 mg; it was obtained from the State Company for the Pharmaceutical Industry and Supplies in Samarra SDI.

Chromatographic Conditions

An HPLC device was used, type Shimadzu, Japan, Kyoto, which consists of a pump type LC-20AD and a reagent SPD-20A, and used a separation column type Porous silica particles (250mm), and the working conditions were: flow rate 0.5 ml/min; column temperature 25°C; The wavelength of the reagent is 250 nm; The injection volume was 20µl, the mobile phase was made of acetonitrile and buffer solution dipotassium phosphate and monopotassium phosphate, and the pH was adjusted at 8 with potassium hydroxide solution of (0.1 molar). Then also used Whatman filter paper No. 0.45 to remove impurities so that the prepared and used solutions were clear and measurable.

Solutions

The solvents used in this work are HPLC grade.

Standard Solutions

The standard solution was prepared by dissolving the drug in the mobile phase solution, and then it was diluted to the required concentration. The mobile phase was prepared from acetonitrile: the buffer solution (the buffer solution is dipotassium phosphate and monopotassium phosphate (50:50 v/v) and the PH was adjusted at 8 with a solution of

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potassium hydroxide (0.1 molarity)).

Preparation of Standard Solutions

Prepare a dexamethasone solution (1000 µg/ml) by dissolving 0.1 g of the drug in an appropriate volume of solvent, then fill the volume up to 100 ml of the same solvent in a suitable volumetric flask, then transfer 2.5 ml standard stock solutions with a concentration of 1000 µg/ml of the drug into 50 volumetric flask. The volume was supplemented with the mobile phase to obtain a concentration of 50 µg/ml of the drug.

Preparation of Drug Sample Solution

Ten tablets containing the drug were weighed and then finely crushed, then an amount equivalent to one tablet containing 0.5 mg of dexamethasone was transferred to a volumetric flask of 50 ml and dissolved in the mobile phase (acetonitrile: buffer) and mixed for 10 minutes to dissolve the drug to obtain 10 µg/ml As for the syrup, 5ml containing 0.5mg of the active substance was transferred to a volumetric flask of 10ml capacity, then completed the volume in the mobile phase to the mark to obtain 50 µg/ml, then the solutions were filtered using Whatman No. 0.45 filter paper, To become clear and measurable.

2. Results and Discussion

The Optimal Conditions for the Experiment

The optimum chromatographic conditions were improved by changing the wavelength, as it was found that the best wavelength gives the highest value of the theoretical platen number was 250 nm, Figure 2, Table 1.

Table (1): The wavelengths effect.		
Wavelength(nm)	**tR**	**Theoretical plate number**
230	2.907	4481.417
235	2.903	4458.701
241	2.905	4403.997
245	3.146	4892.661
250	3.076	5168.931

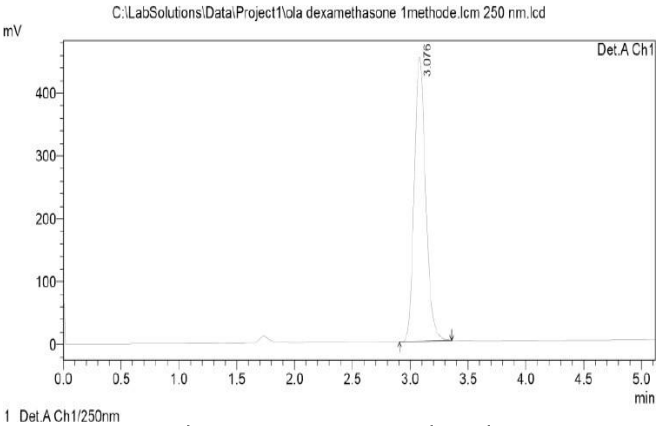


Figure 2: Chromatogram at wavelength 250 nm.

It was also found that the best mixture ratio for the mobile phase was (50:50), as the number of plates for this ratio was 5614.526, Figure 3, and Table 2.

Table (2): Platelet count values for each mix ratio.		
Mobile phase (acetonitrile: Buffer)	**tR**	**Theoretical plate number**
90:10	1.981	3051.516
80:20	2.023	2933.714
70:30	2.170	3070.190
60:40	2.486	3797.400
50:50	3.005	5614.526
40:60	2.098	2546.224
30:70	2.107	2033.652
20:80	2.169	3924.014
10:90	5.284	5767.192

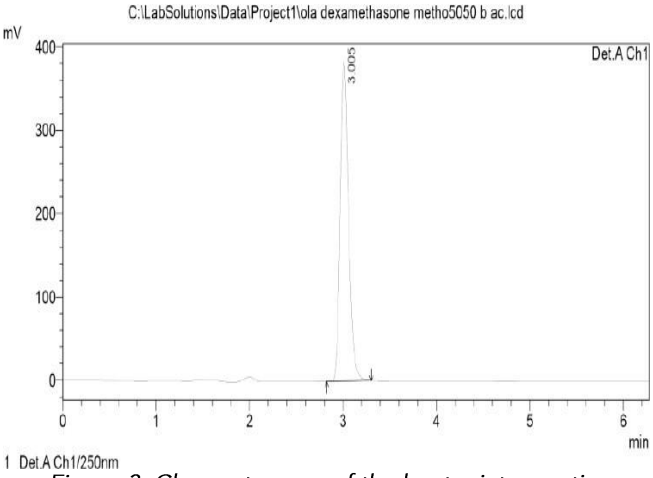
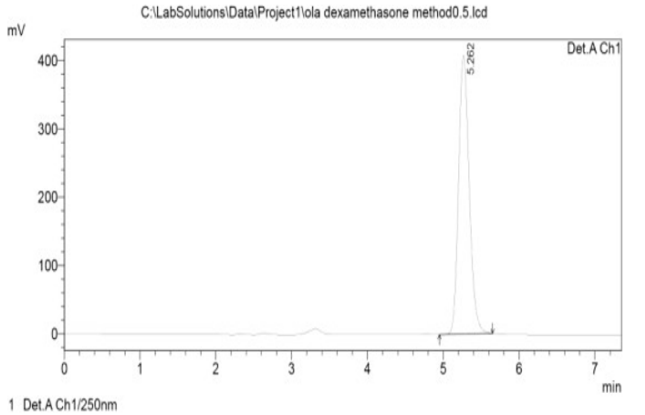


Figure 3: Chromatogram of the best mixture ratio.

The flow rate average was studied, and depending on the optimal conditions above and Figure 4, and Table 3 it was found that the best flow velocity is 0.5 ml/min.

Table (3): Platelet count values for each flow rate.		
Flow rate (ml/min)	**tR**	**Theoretical plate number**
0.5	5.262	6411.408
0.8	3.257	4889.487
1	2.660	4412.520
1.2	2.204	3725.449
1.4	1.824	3189.510



The column used for the separation when choosing the best conditions above was of the type L1, and then the best column for the separation was studied and it was of the type L3, which gave the best results, Figure 5 and Table 4.

Table (4): Type of column used for separation		
Column type	**tR**	**Theoretical plate number**
L3	8.657	9611.900
L1	4.809	6033.589
L7	1.801	338.648

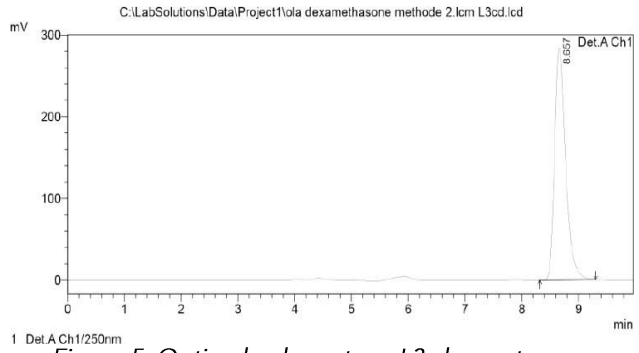


Figure 5: Optimal column type L3 chromatogram

The pH had a significant effect on the shape of the chromatogram as well as on the number of theoretical plates, as it was found that the pH was 8 gives the best results as in Figure 6 and Table 5.

Table (5): Platelet count values for each pH.		
pH	**tR**	**Theoretical plate number**
4.5	8.558	10476.936
6	8.610	10326.677
8	8.802	10772.102
9	8.637	9647.423

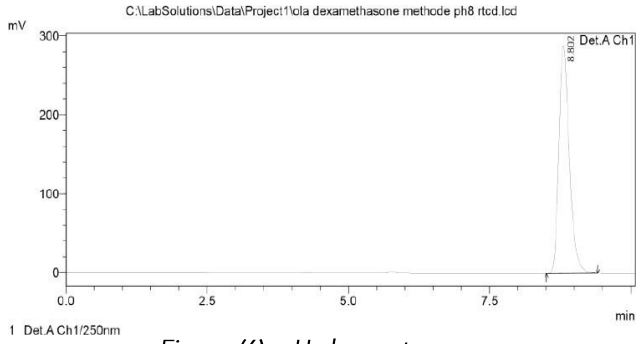


Figure (6): pH chromatogram.

Calibration Curve

The calibration curve of dexamethasone was built according to the optimal conditions above and it was found that the range of concentrations was (0.1-100 µg/ml). Table 6 shows the linearity and correlation coefficient of the drug, and also shows that the method has good agreement through the RSD % study.

Table (6): Results of calibration curves of dexamethasone					
Drugs	**Type**	**LO D (µg/mL)**	**Precision (%RSD)**	**Linearity (µg/mL)**	**Linear regression equation with coefficient of correlation**
Dexamethasone	Peak area	5.87×10-9	1.11×10-5	0.1- 100	Y = 83391x - 29273, 0.9987
	Peak high	1.90×10-6	1.00×10-4	0.1-100	Y = 6011.2x – 2350.5, 0.9987
*n = 4 %RSD = percent relative standard deviation LOD = limit of detection					

3. Method Accuracy

The recoverability was calculated to show the accuracy of the drug estimation method. Table 7 shows that the proposed method for instantaneous drug estimation
Has high accuracy, as the recoverability of dexamethasone reached (103.39-104.85), and therefore the proposed method can be adopted for quantitative estimation.

Table 7. Results of the Recovery Tests for the Drugs*			
Drugs	**Conc taken. (µg/mL)**	**Conc found. (µg/mL)**	**(%) Recovery**
Dexamethasone	10	10.485	104.85
	50	51.699	103.39
*n = 3			

Method Application

The proposed method was applied to some pharmaceutical forms available in the local market, where it was found that the method is effective, as the Recovery percentage (103.39-104.15 %), Table 8.

Table (8): Application of the method to some pharmaceutical forms containing dexamethasone.			
Sample	**Conc. taken (µg/mL) of drug**	**Weight* found (mg/dosage)**	**Recovery %**
Dexon/ syrup (0.5 mg\5ml)	50	0.5169	103.39
Dexon/ tablet (0.5 mg)	10	0.5207	104.15
*n = 3			

4. Conclusion

This method can be used for the quantitative determination of dexamethasone in pharmaceutical forms. The method was validated and found to have high accuracy and acceptable agreement. This method can also be used in the quality control department to test the pharmaceutical forms that contain dexamethasone.

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