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*Full Paper*

# Improved UV Spectrophotometric Method for Precise, Efficient and Selective Determination of Dexamethasone in Pharmaceutical Dosage Forms

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**Abstract:** An improved UV spectrophotometric method has been developed and validated for precise, efficient and selective determination of dexamethasone in tablets and capsules. The quantitative analyses were carried out using ethanol/water (2:1 v/v) as background electrolyte and UV detection was carried out at 240 nm. The calibration curve was linear over a concentration range from 4.0 to 40.0 μg $mL^{-1}$. The average recovery was 97.60 ± 1.06% for tablets and 96.64 ±0.87% for capsules. The limit of detection and limit of quantification were 0.63 and 1.90 μg $mL^{-1}$, respectively. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met. The results obtained with proposed method confirm improved performance over other methods found in the literature.

**Keywords:** glucocorticoid; quantification; drug; pharmaceutical preparation

## 1. INTRODUCTION

Dexamethasone (DEX; Figure 1), 9α-fluoro-16α-methyl-11β, 17α, 21-trihydroxy-1,4-pregnadiene-3,20-dione, is a synthetic glucocorticoid class of steroid drugs with anti-inflammatory and immunosuppressive activity [1]. It is widely used in clinical practice in the treatment of rheumatoid arthritis, asthma, ocular diseases, especially related to connective tissue. It is also often used in immune compromised transplant patients to suppress immunological reactions[2].

Several analytical methods have been reported in scientific literature for the analysis of DEX in biological fluids such as saliva [3], tears [4], hair [5], urine [6-10], plasma [8, 11-13], as well as in pharmaceutical formulations [8, 14-20].

The methods applied to the determination of DEX in pharmaceutical formulations include micellar liquid chromatography[14], high performance liquid chromatography with mass spectrometry (HPLC-MS) [8], high performance liquid chromatography with UV detection (HPLC-UV) [14-19], and UV-spectrophotometric and multivariate calibrations [18]. An UV spectrophotometric method has also been reported for the determination of DEX in a tablet dosage form [20].

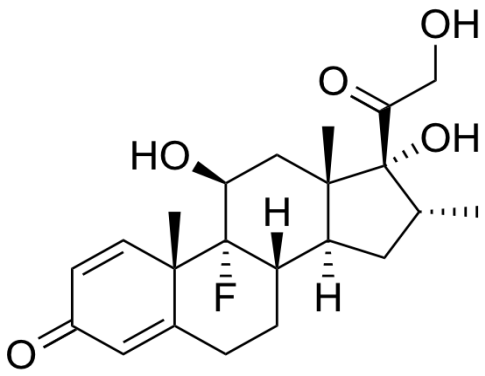


**Figure 1.** Chemical structure of dexamethasone.

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Amongst official compendia methods, the United States Pharmacopeia describes HPLC method for quantitative determination of DEX in elixirs, injectables, ophthalmic suspensions, and tablets [21]. The British Pharmacopoeia describes a HPLC method for quantitative determination of DEX in tablets and an UV spectrophotometric method for active pharmaceutical ingredient (API) [22].

To avoid the obvious disadvantages of several sophisticated and expensive methods, and to help the analysts to choose the most applicable method for a given laboratory routine, the aim of the present study was to develop and validate an improved and alternative UV spectrophotometric method for precision, specificity, and efficient quantification of DEX in tablets and capsules.

## 2. MATERIAL AND METHODS

### Material

The DEX reference substance (assigned purity 100.0%) was kindly donated by Tianjin Yuanlong Chemical Industry Co., Ltd, China (batch: M116023-1). and was used as reference standard without further purification. The commercial DEX (free base) dosage forms were tablets and capsules containing 4 mg of API (declared content), were obtained from local compounding pharmacy and drugstore. The DEX reference substance, as well as the tablets and capsules, were kept protected from light throughout the study. Analytical grade ethanol and freshly distilled water was used in all solution preparation. All solutions were filtered through hydrophilic membrane with 0.45 µm pore size.

### Selection of Solvent

From the standard solution (20.0 µg $mL^{-1}$) approximately 3.0 mL was taken and scanned from 200 to 400 nm. Based on these criteria's a mixture of ethanol/water (2:1 v/v) was selected and absorption spectra were taken at 240 nm.

### Instrumentation and conditions

A Evolution 60® (Thermo Scientific, USA) UV-VIS spectrophotometer was used for measurements. UV spectra absorbance of reference and sample solutions was recorded in 10 mm quartz cells.

### Methods

#### *Preparation of standard solutions*

The DEX reference standard solution (100.0 µg $mL^{-1}$) was prepared by accurately weighing 10.0 mg of DEX reference in a 100.0 mL volumetric flask. The volume was completed with ethanol/water (2:1 v/v) solution. This flask was sonicated for 12 minutes. The above solution was diluted in a 25 mL volumetric flask with ethanol/water (2:1 v/v) solution to obtain a final solution containing 20.0 µg $mL^{-1}$ of DEX.

#### *Calibration curve*

The calibration curve for DEX was constructed by analyzing a series of different concentrations of standard solutions, prepared on the same day. Accurately weighed 10.0 mg of DEX was transferred to a 250 mL volumetric flask and dissolved with ethanol/water (2:1 v/v). The concentration range varied from 4.0 to 40.0 µg $mL^{-1}$. All determinations were made in triplicate at 240 nm, using ethanol/water (2:1 v/v) as blank. The calibration curve was constructed by plotting mean response versus respective DEX concentration.

#### *Sample preparation*

For the assay of DEX in tablets, twenty tablets of each sample were individually weighed and triturated to obtain homogeneous mixture. An amount of powder equivalent to 2.0 mg of free base was transferred to 100.0 mL volumetric flask. The volume was completed with ethanol/water (2:1 v/v) solution. The resulting solution was sonicated during 12 minutes to facilitate proper solubilization. Aliquots of this solution were accordingly diluted with ethanol/water (2:1 v/v) solution, in order to obtain a solution with final concentration of 20.0 µg $mL^{-1}$. All sample and standard solution were filtered through hydrophilic membrane of 0.45 µm pore size, Millipore® Millex-HV filter units. All determinations were made in triplicate.

Similar procedure was used in the assay of DEX capsules. The API of 20 capsules was pooled in a beaker and was homogenized before analytical procedures.

### Method validation

#### *Linearity*

The linearity was determined by plotting concentration against corresponding absorbance. The calibration curve was defined in the concentration interval in which the intensity of the spectrophotometer response was linearly proportional to the concentration of the analyzed substance:

$$A = a.C + b \quad (1)$$

where $A$ is the absorbance; $C$ is concentration; $a$ is slope of the curve; and $b$ is intercept of the curve on y axes.

The linearity was evaluated by linear regression analysis, which was calculated by the least mean square regression method with triplicate determinations at each concentration level.

***Precision***

The method precision was evaluated by inter- and intra-day repeatability. The intra-day repeatability was done by analyzing a single concentration (20.0 µg $mL^{-1}$) of samples in replicate (n = 10). The inter-day repeatability was determined by analyzing sample solutions prepared fresh by weighing sample equivalent to 2.0 mg of drug in free base form , on three consecutive days. The sample solutions were prepared fresh at the same concentration level and the responses were determined in replicate (n = 10). The procedure was determined on three consecutive days by two analysts.

***Accuracy***

The accuracy of the method was evaluated through the recovery test. These tests were performed by adding known amounts of standard solutions to samples followed by analyses using the proposed method. Aliquots of standard and sample solutions were transferred to a 25 mL volumetric flask and final volumes were completed with ethanol/water (2:1 v/v). The percentage of recovery (R) was calculated as indicated by Association of Official Analytical Chemists International [25]:

$$R = [\ (C_F - C_U)\ /\ C_A\ ]\ x\ 100, \quad (2)$$

where $C_F$ represents the concentration of analyte measure in fortified test sample; $C_U$ , the concentration of analyte measure in unfortified test sample; and, $C_A$ , the concentration of analyte added to fortify the test sample.

***Specificity***

The specificity of the proposed method was evaluated through the analysis of a placebo solution. The placebo sample solutions were prepared by mixing excipients such as corn starch, magnesium stearate, mannitol and povidone in their usual concentration, as employed in tablets or capsules. These solutions were analyzed by proposed method in order to check if any of these components interfere in the analysis.

***Detection Limit and quantitation limit***

The LOD and LOQ were calculated according to International Conference on Harmonization guidelines [24]:

$$LOD = 3.3 \cdot SD_b / a, \quad (3)$$

$$LOQ = 10.0 \cdot SD_b / a, \quad (4)$$

where $SD_b$ represents the standard deviation of y-intercept and $a$ is the slope of calibration curve.

***Robustness***

Robustness of the proposed method was evaluated by varying the ratio of solvents (±5 %) and changing the brand of ethanol.

## 3. RESULTS AND DISCUSSION

DEX was analyzed by proposed UV spectrophotometric method in tablets and capsules. The calibration curve showed linearity over a concentration range from 4.0 to 40.0 µg $mL^{-1}$. The linearity can be defined by following equation A = 0.0446C + 0.0177 (Figure 2), where A and C are DEX absorbance and concentration, respectively. The correlation coefficient of the curve obtained with linear regression method was 0.999, indicating good linearity.

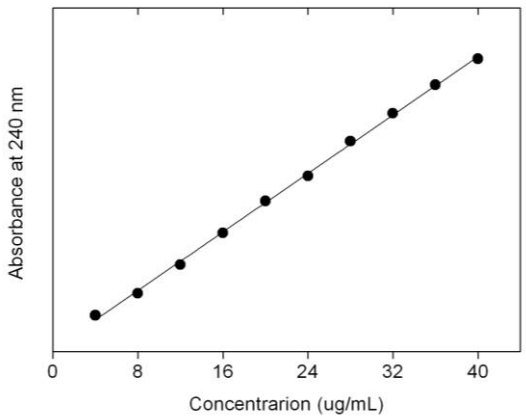


**Figure 2.** Calibration curve of dexamethasone from standard solutions in the concentration range of 4.0 to 40.0 µg $mL^{-1}$. A = 0.0444C + 0.0177.

DEX is insoluble in water and sparingly soluble in methanol and soluble 1 in 42 of ethanol.[23] Different solvent mixtures were tested to establish good solubility and better absorption maxima of DEX (Figure 3). Based on these criteria's a mixture of ethanol/water (2:1 v/v) was selected and

absorption spectra were taken at 240 nm. This solution offers additional advantage because ethanol is affordable and presents low human and environmental toxicity compared to methanol.

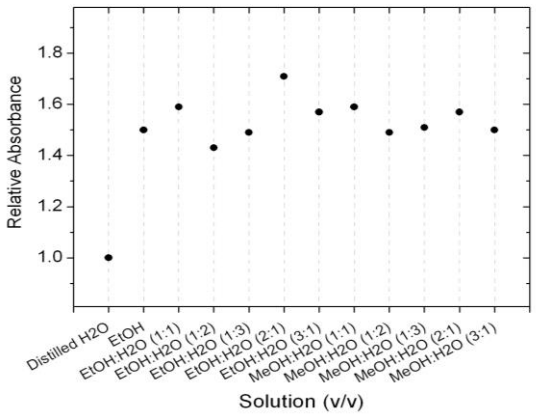


**Figure 3.** The comparative data on relative absorbance of DEX in different solvents mixtures.

The Table 1 shows results for the precision parameter expressed as repeatability (intra-day) and intermediate precision (inter-day). The RSD% for precision intra- and inter-day of the samples were <1.1%, indicating that the proposed method has good precision in the analysis of DEX. The mean contents percentages of DEX were 102.66 ± 1.06% and 101.03 ± 0.78% for tablets and capsules, respectively (Table 1).

The validation data shows that the excipients present in pharmaceutical dosage form did not interfere in the analysis. Thus, the proposed method is specific for unequivocal determination of DEX in the presence of matrix compounds (excipients).

The percentage of recovery values obtained were 97.60 ± 1.06% and 96.64 ± 0.87% for tablets and capsules forms, respectively (Table 2). These results confirm good accuracy of the proposed method.

The Detection Limit (LOD) and Quantitation Limit (LOQ) of DEX by proposed method were determined using Eq. 2 and 3, respectively. The LOD was found as 0.63 μg $mL^{-1}$, while the LOQ was 1.90 μg $mL^{-1}$. The obtained values were confirmed by actual analysis of these concentrations. The RSD% values were within acceptable limits at LOQ (less than 1.0%).

**Table 1.** Precision results and statistical data of DEX determination in pharmaceutical preparation. Theoretical concentration is 20.00 μg $mL^{-1}$.

| | Intra-Day[a] | | | Inter-Day [b] | Content Found (%) |
|---|---|---|---|---|---|
| | **Day 1** | **Day 2** | **Day 3** | | |
| **Tablets (μg $mL^{-1}$)** | 20.00 ± 0.07 | 20.03 ± 0.12 | 20.39 ± 0.08 | 20.14 ± 0.22 | 102.66 ± 1.06 |
| **Capsules (μg $mL^{-1}$)** | 20.04 ± 0.04 | 20.24 ± 0.08 | 20.35 ± 0.03 | 20.21 ± 0.16 | 101.03 ± 0.78 |

[a] Mean of 10 determinations. [b] Mean determinations of three days.

**Table 2.** Recovery data of standard solutions added to the samples analyzed by proposed UV spectrophotometric method.

| Pharmaceutical Dosage Form | Fortified theoretical concentration (μg $mL^{-1}$) | Found experimental concentration (μg $mL^{-1}$)* | Recovery (%) | |
|---|---|---|---|---|
| | | | | Average ± RSD% |
| **Tablet (4 mg)** | 16.00 | 15.50 | 96.60 | 97.60 ± 1.06 |
| | 24.00 | 23.37 | 97.49 | |
| | 32.00 | 31.46 | 98.72 | |
| **Capsule (4 mg)** | 16.00 | 15.73 | 95.65 | 96.64 ± 0.87 |
| | 24.00 | 23.64 | 97.30 | |
| | 32.00 | 31.35 | 96.97 | |

*Average of 10 determinations.

In order to test the robustness of method, deliberate changes were made in the ratio of solvent (±5%) and changed the brand of ethanol. In all tests, the found concentration of DEX was between 90 and 110% of nominal value, as recommended by the United States Pharmacopoeia [22].

The good results for accuracy, LOD, LOQ, and robustness indicate that our method is efficient. In addition, ethanol and water were used as solvents because they are affordable and present low toxicity to human health and to the environment.

The proposed method for determination of DEX in pharmaceutical formulations was compared with those found in the literature, one can observe that: two HPLC methods [16, 19] showed linearity range higher than the proposed method. All literature methods presented similar accuracy. The reported methods in the literature showed precision of about 1.5% [19, 20], 4% [17], and 5% [14]. However, the proposed method showed precision below 1.1% as recommended by International Conference on Harmonization Guidelines [24]. The LOD and LOQ presented in the literature are higher as compared to the proposed method except HPLC-UV method [14] and UV spectrophotometric method[20].

## 4. CONCLUSION

Thus, we conclude that our method proposed here is specific, precise, and efficient for quantification of DEX in pharmaceutical formulations. Thereby, our method may be used as alternative to assess the quality of commercially available DEX drug products.

## 5. ACKNOWLEDMENTS

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