Quantitative determination of dexamethasone sodium phosphate in bulk and pharmaceuticals at suitable pH values using the spectrophotometric method

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Abstract

Dexamethasone sodium phosphate (DSP) is an ester of dexamethasone with anti-inflammatory action. This study provides new insights to develop a simple, precise, and accurate spectrophotometric method for the quantitative determination of DSP in bulk and pharmaceuticals. The method was validated before being applied to determine the DSP in six pharmaceutical injection forms from different companies. DSP is soluble in phosphate buffer, so it was used as a solvent, and a pH of 6 was found to be suitable for determination purposes. The DSP solution was scanned in the ultraviolet range (200–400 nm) using a double-beam spectrophotometer with a 1-cm quartz cell. The wavelength (λ max) of DSP was set at 242.5 nm, following the Beer–Lambert law for concentrations from 2 to 50 μg/ml. Dexa AIWA (Germany) showed the best results, being very close to the bulk value with no significant variation. Similarly, Dexamed (Cyprus) and HEMAZON (Syria) showed no significant differences from the bulk; however, the three remaining injections, DEXAKAL (India), DEXABRU (India), and DEXARON (China), showed significant variations from the bulk. Estimated limit of detection and limit of quantitation values for DSP were 0.83 and 2.5 μg/ml, respectively, with a regression coefficient of 0.999. Recovery studies were then used to determine the accuracy of the suggested method. The percentage of recovery was found to be 98.58%–102.52%. All results are suggesting a pivotal method for the routine analysis of DSP both in pure form and the commercially pharmaceutical forms.

Key words: Dexamethasone sodium phosphate, phosphate buffer, ultraviolet spectrophotometry, validation

INTRODUCTION

Dexamethasone sodium phosphate (DSP) is an inorganic ester of dexamethasone that is used to treat inflammatory, allergy, endocrine, rheumatic, dermatologic, and others. It is also used in a majority of chemotherapy patients.[1]

Chemically, DSP is a pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonooxy),

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disodium salt, \((11\beta, 16\alpha)\) with the chemical formula of \(C_{22}H_{26}FO_4PNa_2\) [Figure 1]. DSP generally appears as a white-to-creamy white powder with a molecular weight of 516.41 g/mol. It is excessively hygroscopic, with a water solubility of 1.52 mg/ml, and its solutions have pH values between 7 and 8.5 with pKa of 1.89.\[3\]

DSP penetrates the central nervous system and is metabolized in the liver, being mainly eliminated in the urine.\[3\] Unbound dexamethasone crosses cell membranes and binds with a great affinity to specific cytoplasmic glucocorticoid receptors. The forming complex crosses the nuclear membrane and modulates the gene-mediated protein production. Dexamethasone's anti-inflammatory effects are assumed to be due to phospholipase A2 inhibitory proteins called lipocortins, which regulate the manufacture of potent inflammatory mediators such prostaglandins and leukotrienes.\[14\]

Literature shows various methods have been developed to estimate DSP. These methods include spectrophotometry,\[5,6\] kinetic spectrophotometry,\[7\] liquid chromatography,\[8-10\] high-performance liquid chromatography (HPLC),\[11,12\] HPLC with mass spectrometry (HPLC/MS),\[13,14\] reversed-phase HPLC in combination with other drugs,\[15,16\] and electrochemical methods.\[17\]

This study used a mixed-methods approach based on ICH guidelines for assessing DSP in the injection dosages and then to be checked afterward. The approach proposes a new methodology for assessing DSP uses well-known generic products to develop a cheap, sensitive, and effective method for the quantitative determination of DSP in pure and pharmaceutical in a suitable buffer as a solvent.

**MATERIALS AND METHODS**

**Instrumentation**

An ultraviolet (UV)-visible double-beam spectrophotometer UV-1800, with two 1-cm quartz cells (Shimadzu UV spectrophotometer, Japan), a pH meter (Hanna, Romania), pipettes of various volumes, and a digital electronic balance (Denver, Germany) were used in this study.

![Figure 1: Chemical structure of dexamethasone sodium phosphate](image)

**Materials**

DSP (100% purity) was obtained from Samarra Drug Industry; this was used as a reference standard. Monosodium phosphate (NaH\(_2\)PO\(_4\), 2H\(_2\)O) (99% purity) was provided by the Gainland Chemical Company, UK, while HiMedia Laboratories, India, provided disodium phosphate (Na\(_2\)HPO\(_4\)) and sodium hydroxide (NaOH) (each of 99% purity). The commercial dosage forms of DSP from six different companies were all injections of 8 mg/2 mL. These dosages were bought from the local market after checking both manufacturing and expire dates, and took the form Dexa AIWA® (T and D Pharma GmbH, Germany), DEXABRU (Brawn Laboratories Limited, India), DEXAKAL (Khandelwal Laboratories Pvt. Ltd., India), Dexamed (Medochemie Ltd., Limassol, Cyprus), DEXARON® (Shanghai Pharm. Co., Ltd., China), and HEMAZON (Ibn Hayyan Pharm., Syria).

**Preparation of stock solutions**

*Dexamethasone sodium phosphate solution (100 μg/mL)*

The working solution was prepared by taking 0.01 g of DSP and dissolving it in 10 mL of distilled water (DW); then, the solution was diluted with DW to 100 mL.

*Sodium dihydrogen phosphate and disodium hydrogen phosphate solutions*

A 7.8 g of sodium dihydrogen phosphate (NaH\(_2\)PO\(_4\)) and 7.10 g of Na\(_2\)HPO\(_4\) were accurately weighed and transferred into a 250-ml separate graduated volumetric flask and solubilized in 50 and 100 mL of DW, respectively. The solutions were then made up with DW to achieve a solution of 0.2 M each of NaH\(_2\)PO\(_4\) and Na\(_2\)HPO\(_4\).

**Preparation of buffer solutions**

Buffers with various pH values (2, 3, 4, 6, 6.4, 7, and 8) were prepared using NaH\(_2\)PO\(_4\) solution and Na\(_2\)HPO\(_4\) solution in different proportions, as shown in Table 1; then, 0.1 M HCl and 0.1 M NaOH solution were used to adjust the pH of solutions and measured using the pH meter device.

**Determination of dexamethasone spectrum**

After dilution of standard drug solutions with different proportions, as shown in Table 1; then, 0.1 M HCl and 0.1 M NaOH solution were used to adjust the pH of solutions and measured using the pH meter device.

**Table 1: Solution amounts used to prepare various pH values**

| pH value | NaH\(_2\)PO\(_4\) solutions (0.2 M) (mL) | Na\(_2\)HPO\(_4\) solutions (0.2 M) (mL) |
|----------|-------------------------------------|-------------------------------------|
| 2        | 0.66                                | 11.8                                |
| 3        | 21.9                                | 3.1                                 |
| 4        | 1.32                                | 23.67                               |
| 6        | 43.85                               | 6.15                                |
| 6.4      | 36.75                               | 13.25                               |
| 7        | 19.5                                | 30.5                                |
| 8        | 2.65                                | 47.35                               |

NaH\(_2\)PO\(_4\): Sodium dihydrogen phosphate, Na\(_2\)HPO\(_4\): Disodium hydrogen phosphate
scanned from 200 to 400 nm to select the maximum wavelength ($\lambda_{\text{max}}$). The solution shows maximum absorption at 242.5 nm.

**Selection of suitable pH**

A 10 $\mu$g/ml standard solution of DSP was prepared using 1 mL of 100 $\mu$g/ml stock solution; this was then transferred into a series of 10-ml graduated volumetric flasks. Then, the volume was made up to 10 mL with one of the buffers; for each buffer value, two samples were prepared along with a control flask. The samples were scanned using the spectrophotometer to measure the absorbance of DSP at the $\lambda_{\text{max}}$ (242.5 nm). The highest absorbance appeared at pH 6, and this pH was selected for the preparation of the calibration curve. The data are summarized in Table 2.

**Procedure for sample preparation**

Each injection solution (8mg/mL) was transferred to a 100 mL volumetric flask and diluted using a previously prepared buffer of pH 6; in each case, the flask was standardized by adding the buffer first. After that, for each product, three different concentrations of the drug (5, 10, and 15 $\mu$g/ml) were prepared to estimate absorbance accurately, with a control sample consisting of just the buffer solution at pH 6.

**RESULTS AND DISCUSSION**

**Selection of suitable pH**

The highest and lowest levels of pH were determined based on their pKa values (1.8 and 6.4). Consequently, at lower pH (<3), DSP becomes uncharged, while when the pH increases, it takes on anionic forms as monoanionic and dianionic dexamethasone phosphate (DSP$^-$, DSP$^{2-}$). This means the reaction is dependent on pH value. In terms of spectroscopy, this is reflected in the transfer of electrons between different energy levels, such as the move from the nonbonding orbital sigma ($\sigma$) to the sigma ($\sigma^*$) antibonding orbital, which reverses higher pH values. This could explain the higher absorbance value at pH = 6 [Table 2].$^{[18,19]}$

**Selection of wavelength**

With the reference solution, a UV spectroscopic scanning run between 200 and 400 nm that was performed to determine the optimal UV wavelength (maximum) for detection of DSP. Therefore, 242.5 nm was selected as the working wavelength for DSP, as shown in Figure 2.

**Validation**

**Linearity and calibration curve**

The stock solution was diluted with a buffer (pH = 6) to

| pH value | Absorbance Mean |
|----------|-----------------|
| Sample number 1 | Sample number 2 |
| 2 | 0.248 | 0.266 | 0.257 |
| 3 | 0.259 | 0.261 | 0.260 |
| 4 | 0.268 | 0.265 | 0.267 |
| 6 | 0.261 | 0.276 | 0.268 |
| 6.4 | 0.250 | 0.256 | 0.253 |
| 7 | 0.255 | 0.266 | 0.260 |
| 8 | 0.250 | 0.259 | 0.254 |

**Table 3: Calibration data for dexamethasone sodium phosphate**

| Concentration ($\mu$g/ml) | Absorbance |
|--------------------------|------------|
| 2 | 0.051 |
| 4 | 0.101 |
| 6 | 0.150 |
| 8 | 0.224 |
| 10 | 0.266 |
| 15 | 0.398 |
| 20 | 0.526 |
| 25 | 0.654 |
| 30 | 0.784 |
| 35 | 0.875 |
| 40 | 0.999 |
| 45 | 1.135 |
| 50 | 1.260 |

**Table 4: Accuracy and precision in standard solution**

| Concentrations taken ($\mu$g/ml)* | Concentrations found ($\mu$g/ml)* | RE (%)* | Recovery (%)* | SD* | RSD (%)* |
|-------------------------------|-------------------------------|---------|---------------|-----|----------|
| 15 | 15.084 | 0.56 | 100.65 | 0.380 | 2.52 |
| 30 | 30.802 | 2.67 | 102.67 | 0.141 | 0.46 |
| 45 | 44.902 | 0.21 | 99.7 | 0.040 | 0.091 |

*Mean of four replicates. RE: Relative error, SD: Standard deviation, RSD: Relative SD

*Figure 2: Spectrum of dexamethasone sodium phosphate (40 $\mu$g/ml) at pH = 6*
make a series of DSP standard solution concentrations ranging from 2 to 50 μg/ml. Absorbances were determined. By plotting the absorbance versus concentrations, the calibration curve was constructed and regression equation was intended. Regarding the curve shown in Figure 3, the linear equation was $y = 0.025x + 0.0132$ and the correlation coefficient ($r^2$) was 0.9999 which is indicated a good linearity. The calibration data are shown in Table 3.

**Accuracy and precision**
The accuracy of the method was represented by percent relative error (RE%) while the precision was represented by the relative standard deviation (RSD%). The accuracy (recovery) and the precision were thus estimated for a series of four replicates of three concentration levels of the standard solution (15, 30, and 45 μg/ml). The percentage of recovery, RE%, and RSD% were estimated for each sample. The mean RE% and the mean recovery were found to be 1.15% and 101.0%, respectively, while the mean RSD% was 1.024%. The results summarized in Table 4 confirmed that the method used was accurate and precise.

**Detection limit and quantification limit**
Following ICH guidelines, limit of quantitation (LOQ) and limit of detection (LOD) were estimated as 3 SD/slope and 10 SD/slope, respectively, where SD is the standard deviation of the intercept. The LOD was 0.6371 μg/ml and the LOQ was 1.930 μg/ml in DSP for five replicate determinations. A summary of the validation parameters is shown in Table 5.

**Determination of active dexamethasone sodium phosphate in injection dosage forms in the Iraqi market**
The summarized analysis results, shown in Table 6, indicated a high percentage of recovery with low RSD%, and indicated that the method is applicable for routine analysis of pharmaceutical forms.

### Table 5: Validation parameters for dexamethasone sodium phosphate using the proposed method

| Parameters                  | DSP            |
|-----------------------------|----------------|
| Linearity (μg/mL)           | 2-50           |
| Wavelength (nm)             | 245.5          |
| Slope (m)                   | 0.025          |
| Intercept                   | 0.0132         |
| Correlation coefficient ($r^2$) | 0.999         |
| LOD (μg/mL)                 | 0.6371         |
| LOQ (μg/mL)                 | 1.930          |
| Linear equation             | $y = 0.025x + 0.0132$ |
| RSD (%)                     | 1.024          |

DSP: Dexamethasone sodium phosphate, RSD: Relative standard deviation, LOD: Limits of detection, LOQ: Limit of quantification

### Table 6: Accuracy and precision of dexamethasone sodium phosphate determination in injections

| Trade name/company | Taken (µg/mL)* | Found (µg/mL)* | RE (%)* | Recovery (%)* | SD* | RSD (%)* |
|--------------------|----------------|---------------|---------|---------------|-----|----------|
| DEXARON (China)    | 5              | 4.818         | 0.017   | 95.8          | 0.082 | 1.72     |
|                    | 10             | 9.512         | 0       | 95.12         | 0.422 | 4.447    |
|                    | 15             | 14.298        | 0.005   | 95.32         | 0.240 | 1.685    |
| Dexa AIWA (Germany)| 5              | 4.912         | -0.0033 | 98.24         | 0.104 | 2.137    |
|                    | 10             | 9.685         | 0.0033  | 96.85         | 0.146 | 1.517    |
|                    | 15             | 14.992        | 0.00667 | 99.94         | 0.078 | 0.526    |
| DEXA KAL (India)   | 5              | 4.7           | 0.018   | 95.30         | 0.099 | 2.112    |
|                    | 10             | 9.352         | 0.004   | 93.52         | 0.069 | 0.740    |
|                    | 15             | 13.77         | 0.014   | 91.85         | 0.100 | 0.7305   |
| HEMAZON (Syria)    | 5              | 5.058         | 0.044   | 101.16        | 0.121 | 2.41     |
|                    | 10             | 9.352         | 0       | 93.52         | 0.119 | 1.283    |
|                    | 15             | 14.445        | 0.006   | 96.3          | 0.160 | 1.114    |
| DEXABRU (India)    | 5              | 4.698         | 0.046   | 93.96         | 0.099 | 2.124    |
|                    | 10             | 9.298         | 0.021   | 92.98         | 0.023 | 0.248    |
|                    | 15             | 13.618        | 0.015   | 90.78         | 0.151 | 1.111    |
| Dexam (Cyprus)     | 5              | 5.365         | 0.14    | 107.26        | 0.100 | 1.877    |
|                    | 10             | 9.978         | 0.02    | 99.78         | 0.122 | 1.224    |
|                    | 15             | 14.832        | -0.0123 | 98.88         | 0.211 | 1.426    |

*Mean of four replicates. SD: Standard deviation, RSD: Relative SD
Table 7: t-test data

| Companies               | Unpaired t-test  |
|-------------------------|------------------|
|                         | P                |
|                         | summary          |
| Bulk versus DEXAKAL (India) | 0.0056           |
| Bulk versus DEXARON® (China) | 0.0008           |
| Bulk versus Dexa AIWA® (Germany) | 0.3611 | NS     |
| Bulk versus Dexam (Cyprus) | 0.3828           |
| Bulk versus HEMAZON (Syria) | 0.3579           |
| Bulk versus DEXABRU (India) | 0.0026           |
| DEXAKAL (India) versus DEXARON® (China) | 0.1417 | NS     |
| DEXAKAL (India) versus Dexa AIWA® (Germany) | 0.0329 | *      |
| Dexam (Cyprus) versus DEXARON® (China) | 0.0413 | *      |
| Dexam (Cyprus) versus DEXAKAL (India) | 0.2325 | NS     |
| DEXARON® (China) versus HEMAZON (Syria) | 0.5125 | NS     |
| DEXAKAL (India) versus DEXABRU (India) | 0.0418 | *      |
| DEXARON® (China) versus DEXAKAL (India) | 0.5198 | NS     |
| DEXARON® (China) versus DEXABRU (India) | 0.2649 | NS     |
| HEMAZON (Syria) versus DEXA KAL (India) | 0.6045 | NS     |
| DEXARON® (China) versus Dexamed (Cyprus) | 0.0011 | *      |
| Dexamed (Cyprus) versus HEMAZON (Syria) | 0.2245 | NS     |
| DEXAKAL (India) versus DEXABRU (India) | 0.0289 | *      |
| Dexam (Cyprus) versus HEMAZON (Syria) | 0.1421 | NS     |

*Signifies P<0.05, **Signify P<0.01, ***Signify P<0.001. NS signifies P>0.05. NS: No significance.

Figure 4: Relative content of dexamethasone sodium phosphate in comparison to the bulk

| Companies | % Drug Content |
|-----------|---------------|
| Bulk      | 0.3828        |
| DEXAKAL (India) | 0.3611 |
| DEXARON® (China) | 0.3579 |
| DEXABRU (India) | 0.0026 |
| DEXA KAL (India) | 0.2325 |
| Dexamed (Cyprus) | 0.5125 |
| HEMAZON (Syria) | 0.0418 |
| Dexa AIWA® (Germany) | 0.5198 |
| DEXAKAL (India) | 0.2649 |
| DEXARON® (China) | 0.0329 |
| Dexamed (Cyprus) | 0.0413 |
| DEXAKAL (India) | 0.0056 |
| DEXARON® (China) | 0.0008 |
| HEMAZON (Syria) | 0.3611 |
| DEXA KAL (India) | 0.3828 |
| Dexamed (Cyprus) | 0.3828 |
| HEMAZON (Syria) | 0.3828 |
| Dexa AIWA® (Germany) | 0.3828 |

Statistical analysis of the results of dexamethasone sodium phosphate commercial dosages

Statistical analysis using a t-test showed a set of significant differences between the products. Dexa AIWA offered the best results, being very close to the bulk with no significant variations. This was followed by Dexamed and HEMAZON, which also showed no significant differences in comparison with the bulk.

However, DEXA KAL and DEXABRU showed significant differences from the bulk. Thus, these forms showed a significant variation at the two-star level (**), which represents P < 0.01, suggesting that the drug content of these Indian brands was lower than that of the bulk, while DEXARON showed a very significant variation at the three-star (***)) level, which represents P < 0.001, resulting in the injection’s drug content differing from that of the bulk chemical.

Comparing the company products with each of the others shows that DEXA KAL and DEXARON are closely matched, with no significant differences between them. In the similar manner, there are no significant differences and approximately equivalent drug content among the following products: DEXAKAL and HEMAZON; DEXAKAL and DEXABRU; DEXARON and DEXABRU; DEXA KAL and DEXARON; and; Dexa AIWA and Dexamed; Dexa AIWA and DEXA; Dexamed and HEMAZON; and HEMAZON and DEXABRU. However, significant differences could observe between other companies products such as between DEXA KAL and Dexa AIWA, where a comparison generates a one star (*) significant, representing P<0.05, which indicates the differences in the drug content of the injections produced by these companies. Similar differences can also be seen between DEXAKAL and DEXA; DEXARON and DEXABRU; Dexa AIWA and DEXA; and; Dexamed and HEMAZON; and HEMAZON and DEXABRU. Moreover, significant differences could be observed among the DEXA KAL and DEXARON, and DEXA KAL and DEXABRU, with no significant differences between them.

CONCLUSION

The findings of this study show that the UV approach can be utilized for routine analysis of DSP in bulk formulations, as well as for the analysis of marketing injections. It is ideal for the intended application, especially in forensic science laboratories and other pharmaceutical analysis laboratories. By comparing a series of the pharmaceutical preparations from different companies with the bulk, Dexa AIWA® (Germany), Dexamed (Cyprus), and HEMAZON (Syria) were found to be close to the bulk, offering reliable drug content levels. However, DEXAKAL (India), DEXABRU (India), and DEXARON® (China) were found to have significant differences in terms of variation in drug content as compared with the bulk.
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Conflicts of interest
There are no conflicts of interest.

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