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QUANTITATIVE ESTIMATION OF DEXAMETHASONE DRUG BY USING UV VISIBLE SPECTROSCOPY

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ABSTRACT

In this study, it was concluded that the content of Dexamethasone in the marketed tablet formulation was found to be within the specified limits as claimed by the manufacturer. The analytical data obtained confirmed that the developed UV-spectrophotometric method provides accurate and reproducible results, fulfilling the acceptance criteria set for assay determination. The precision and reliability of the method ensure its suitability for routine pharmaceutical analysis. The content of Dexamethasone was found to be 99% (0.495mg) at wavelength of 230nm. Std. show absorbance 0.753 at 230nm at 230nm wavelength and sample shows absorbance 0.782 at 230nm, which indicates accuracy in result. This study highlights the efficiency of UV-spectroscopy in both qualitative and quantitative determination of Dexamethasone in tablet dosage forms. It can be used effectively to monitor the quality and stability of the drug in various formulations. Furthermore, this method minimizes analytical errors, reduces solvent consumption, and supports environmentally friendly analytical practices. Overall, UV-spectrophotometric analysis stands as a powerful and dependable analytical tool for assessing the purity, potency, and efficacy of Dexamethasone in pharmaceutical preparations. The findings confirm that the developed method can serve as a valuable technique for regular quality control analysis in academic, industrial, and research laboratories.

KEYWORDS: Absorption, Dilution, Spectrum, Precision, Inflammation.

INTRODUCTION

Dexamethasone has a wide variety of uses in the medical field. As a treatment, dexamethasone has been useful in treating acute exacerbation of multiple sclerosis, allergies, cerebral edema, inflammation, and shock. Patients with COVID-19, asthma, atopic and contact dermatitis, and drug hypersensitivity reactions have benefited from dexamethasone. Clinicians use it as a diagnostic agent for Cushing disease. This activity will highlight the mechanism of action, adverse event profile, FDA-approved and off-label uses, administration, dosing, contraindications, pharmacodynamics, pharmacokinetics, monitoring parameters, and relevant interactions of dexamethasone pertinent for inter professional team members using dexamethasone for any of its intended indications. 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]

Many analytical methods such as UV-spectrophotometry, differential UV spectrophotometry and reverse-phase HPLC and TLC have been utilized to assay dexamethasone. Spectral analysis of mixtures containing multi-component is one of the main challenges of analytical chemistry. On the other hand, some of the other spectral analysis problems are the correction of spectral baseline and the elimination of noise. In addition, in complex samples the main signal of analyte can interfere with a noise peak. Spectroscopy is the branch of science which deals with the study of the

interaction of electromagnetic radiation with matter. In such type of interaction, energy is absorbed or emitted by the matter in discrete amounts known as quanta. The absorption or emission processes take place ranging from the gamma region to the radio region throughout the electromagnetic spectrum. The data that are obtained from this technique are called a spectrum.

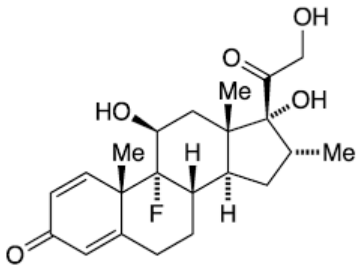
A UV–Vis spectrophotometer is an analytical instrument that measures the amount of ultraviolet (UV) and visible light that is absorbed by a sample. It is a widely used technique in chemistry, biochemistry, and other fields, to identify and quantify compounds in a variety of samples. UV–Vis spectrophotometers work by passing a beam of light through the sample and measuring the amount of light that is absorbed at each wavelength. The amount of light absorbed is proportional to the concentration of the absorbing compound in the sample. The method is most often used in a quantitative way to determine concentrations of an absorbing species in solution, using the Beer–Lambert law:

$$A = \log_{10} I_0/I = \varepsilon cL$$

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. Chemically, DSP is a pregna-1,4-diene-3,20-dione,9-fluoro-11,17-dihydroxy-1 6-methyl-21-(phosphonoooxy)-, disodium salt, (11 β, 16 α) with the chemical formula of C22H28FO8 PNa2. 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. DSP penetrates the central nervous system and is metabolized in the liver, being mainly eliminated in the urine. Unbound dexamethasone crosses cell membranes and binds with a great affinity to specific cytoplasmic glucocorticoid receptors.

DRUG PROFILE

Dexamethasone is a synthetic fluorinated glucocorticoid used to manage a wide variety of medical conditions. These include rheumatic disorders, various skin conditions, severe allergic reactions, asthma, and chronic obstructive pulmonary disease (COPD). It is also effective in treating croup, cerebral edema (swelling in the brain), eye discomfort following ocular surgery, and superior vena cava syndrome — a complication that can arise from certain cancers. Additionally, it is used as an adjunct therapy with antibiotics in the treatment of tuberculosis. For individuals with adrenocortical insufficiency, dexamethasone is often combined with a mineralocorticoid like fludrocortisone to ensure adequate hormonal replacement. In cases of preterm labor, the drug is administered to enhance fetal outcomes, particularly lung development.



DEXAMETHANSONE

The chemical formula of dexamethasone is $C_{22}H_{29}FO_5$, and it has a molecular weight of 392.46g/mol. It is built on the classic four-ring steroid nucleus known as cyclopenta [a] phenanthrene, composed of three six-membered rings and one five-membered ring fused together. The molecule contains eight chiral centers, giving it a very specific three-dimensional shape critical for receptor binding. Key structural features include a fluorine atom at the 9α-position, a methyl group at the 16α-position, a hydroxyl group at the 11β-position, and a double bond between carbon atoms 1 and 2. These modifications greatly enhance its glucocorticoid receptor affinity, reduce mineralocorticoid receptor activity, and increase its metabolic stability.

Mechanism of Action

It effects primarily by modulating gene expression through intracellular glucocorticoid receptors (GRs). Being lipophilic, dexamethasone diffuses across the cell membrane and binds to cytoplasmic glucocorticoid receptors, which are normally bound to inhibitory chaperone proteins like heat shock proteins (HSPs). Upon binding, dexamethasone induces a conformational change in the receptor, causing dissociation of the chaperone proteins and activation of the receptor. The activated receptor-dexamethasone complex then translocate into the nucleus, where it interacts with glucocorticoid response elements (GREs) on DNA. This interaction leads to transcriptional regulation:

1. Regulation of anti-inflammatory proteins: Dexamethasone stimulates the synthesis of proteins such as annexin-1 (lipocortin-1), which inhibits phospholipase A2, thereby decreasing the production of arachidonic acid and downstream inflammatory mediators like prostaglandins and leukotriene's.
2. Suppression of pro-inflammatory genes: The receptor complex also inhibits transcription factors such as NF-κB and AP-1, reducing the expression of cytokines (e.g., IL-1, IL-6, TNF-α), chemokine's, adhesion molecules, and enzymes like cyclooxygenase-2.

Through these actions, dexamethasone produces powerful anti-inflammatory, immunosuppressive, and anti-allergic effects. Additionally, glucocorticoids can exert non-genomic effects by interacting with cell membranes or cytoplasmic signaling pathways, leading

to rapid modulation of cell function, particularly in immune cells.

Pharmacodynamics

Dexamethasone exerts its primary pharmacodynamic action at the genetic level by influencing protein synthesis and cellular function, particularly within the immune and inflammatory pathways. Being a synthetic glucocorticoid, dexamethasone easily enters cells due to its lipophilic nature. Once inside the cytoplasm, it binds with high affinity to the intracellular glucocorticoid receptor (GR), which is normally associated with chaperone proteins like heat shock proteins (HSPs). Upon binding, the receptor undergoes a conformational change that causes dissociation from these chaperones, leading to activation of the receptor.

They produces strong anti-inflammatory and immunosuppressive effects by decreasing the production of inflammatory mediators. It achieves this by inhibiting the transcription of genes responsible for producing key pro-inflammatory substances. These include cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), as well as other mediators like prostaglandins and leukotrienes. By suppressing these molecules, dexamethasone helps reduce inflammation, tissue damage, and immune system overactivity in various conditions. It also has significant metabolic effects, primarily by increasing gluconeogenesis in the liver. It stimulates the production of glucose from non-carbohydrate sources such as amino acids, which can lead to elevated blood glucose levels, a condition known as hyperglycemia. This effect is especially important to monitor in patients with diabetes or those at risk of developing glucose intolerance, as prolonged use of dexamethasone can impair glucose regulation and contribute to insulin resistance.

Pharmacokinetics

Absorption- Dexamethasone is **well absorbed** from the gastrointestinal (GI) tract after oral administration. It has **high oral bioavailability**, typically around **80–90%**, meaning most of the drug reaches systemic circulation. Peak plasma concentrations are usually achieved within **1 to 2 hours** after ingestion.

Distribution- Once in the bloodstream, dexamethasone is widely distributed throughout body tissues. It is **highly lipophilic**, allowing it to cross cell membranes easily and enter cells. It also crosses the **blood-brain barrier** and **placenta**. Around **70%** of dexamethasone in the blood is **bound to plasma proteins**, mainly albumin. It has a **volume of distribution (Vd)** of approximately **0.5–1 L/kg**, indicating widespread tissue penetration.

Metabolism- Dexamethasone is primarily metabolized in the **liver** by the enzyme **CYP3A4**, part of the cytochrome P450 system. The metabolism results in **inactive metabolites**. Liver function can influence how quickly dexamethasone is broken down, and drug interactions with CYP3A4 inhibitors or inducers can alter its metabolism.

Excretion- Dexamethasone and its metabolites are mostly excreted in the **urine**. A small portion may be excreted unchanged. The **elimination half-life** is around **3 to 5 hours**, but its **biological effects last much longer** - up to **36 to 54 hours** - due to gene-level actions.

Adverse Effects- It is a potent drug that can cause a variety of adverse effects, especially when used at high doses or for extended periods. The risk of side effects increases with longer duration of treatment, and abrupt discontinuation can lead to serious withdrawal symptoms due to adrenal suppression.

Side effects

- One of the common side effects is insomnia along with mood changes such as anxiety, irritability, agitation, or even depression. In rare cases, severe psychiatric reactions like psychosis can occur.
- Dexamethasone often leads to increased appetite and weight gain. This is due to its impact on metabolism and fluid balance, which may cause patients to eat more and retain fat and fluids.
- Gastrointestinal discomfort is another frequent issue. Patients may experience nausea, vomiting, indigestion, or stomach upset. These symptoms are typically less severe when the medication is taken with food.
- Fluid retention is also common and may present as swelling in the hands, ankles, or feet. This can contribute to bloating and sometimes elevated blood pressure.

Contraindications

- Uncontrolled infections
- Known hypersensitivity to dexamethasone
- Cerebral malaria
- Systemic fungal infection
- Concurrent treatment with live virus vaccines (including smallpox vaccine)

Medical uses

1) Anti-inflammatory

Dexamethasone is used to treat many inflammatory and autoimmune disorders, such as rheumatoid arthritis and bronchospasm Idiopathic thrombocytopenic purpura, a decrease in numbers of platelets due to an immune problem, responds to 40 mg daily for four days; it may be administered in 14-day cycles. It is unclear whether dexamethasone in this condition is significantly better than other glucocorticoids.

2) Cancer

People with cancer undergoing chemotherapy are often given dexamethasone to counteract certain side effects of their antitumor treatments. Dexamethasone can increase the antiemetic effect of 5-HT_3 receptor antagonists, such

as ondansetron. The exact mechanism of this interaction is not well-defined, but it has been theorized that this effect may be due to, among many other causes, inhibition of prostaglandin synthesis, anti-inflammatory effects, immune suppressive effects, decreased release of endogenous opioids, or a combination mentioned.

3) COVID-19

Dexamethasone is recommended by the National Health Service in the UK and the National Institutes of Health (NIH) in the US for people with COVID-19 who need either mechanical ventilation or supplemental oxygen (without ventilation).

4) Surgery

Dexamethasone is used fairly, regularly, often as a single intravenous dose, during surgery to prevent postoperative nausea and vomiting, manage pain, potentially reduce the amount of pain, medication required, and help reduce post-surgery hospitalization time.

5) Pregnancy

Dexamethasone may be given to women at risk of delivering prematurely to promote maturation of the foetus's lungs. This administration, given from one day to one week before delivery, has been associated with low birth weight, although not with increased rates of neonatal death.

6) Endocrine

Dexamethasone is the treatment for the very rare disorder of glucocorticoid resistance. In adrenal insufficiency and Addison's disease, dexamethasone is prescribed when the patient does not respond well to prednisone or methyl prednisolone.

EXPERIMENTAL WORK

To determine the quantity of dexamethasone using UV-Visible spectroscopy, a calibration graph is first prepared using standard solutions of known concentrations. The absorbance of the sample with unknown concentration is then measured, and its concentration is calculated from the graph according to the Beer-Lambert law. This method can be applied for the analysis of either the pure drug or its pharmaceutical dosage forms.

Materials: Dexamethasone used as a reference substance was obtained from Henrifarma (São Paulo, Brazil). The tablets were purchased locally and were demanded to contain 4.0 mg dexamethasone. Methanol was of analytical grade and used as received.

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An ultraviolet (UV)-visible double-beam spectrophotometer UV-1900, 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.

Materials

- **The tablets were purchased locally and were demanded to contain 0.5mg dexamethasone.**
- **Methanol was of analytical grade and received from the laboratories and from clorofiltind.**
- Dexamethasone's analysis of quantitative estimation (Quality Control) method begins with a study of physiochemical properties of drug substances.

Study of Physiochemical properties

Dexamethasone Tablet: Drug Profile and Physicochemical Properties

Property	**Details**
Drug Name	Dexamethasone
Reference Standard Source	Cadila (Gujrat, India)
Dosage Form	Tablet
Brand/Product Source	Zydus (local pharmacy)
Label Claim	0.5 mg per tablet
Appearance	White to off-white crystalline powder
Molecular Formula	$C_{22}H_{29}FO_5$
Molecular Weight	392.5 g/mol
Melting Point	262–264 °C
Solubility	Poorly soluble in water (<1 mg/mL); soluble in methanol, ethanol, and acetone
Bioavailability	Approximately 80% orally
Derivatives for Injection	Dexamethasone sodium phosphate (water-soluble)
Stability	Stable under normal conditions; sensitive to light and heat
Analytical Use	Absorbs UV light (λmax ≈ 230 nm in methanol), suitable for UV-Visible spectroscopy estimation

Preparation of Stock Standard

1) Dexamethasone reference standard (10 mg) was accurately weighed and transferred into a 100 mL volumetric flask. Methanol of analytical grade was used as the solvent.
2) The drug was dissolved in methanol, and the volume was made up to the mark to obtain a stock solution of 100µg/mL**.** The solution was mixed thoroughly to ensure complete dissolution.
3) The stock solution was stored in a cool, dark place to maintain stability. It was further diluted with methanol to prepare working solutions of desired concentrations for calibration and quantitative estimation.

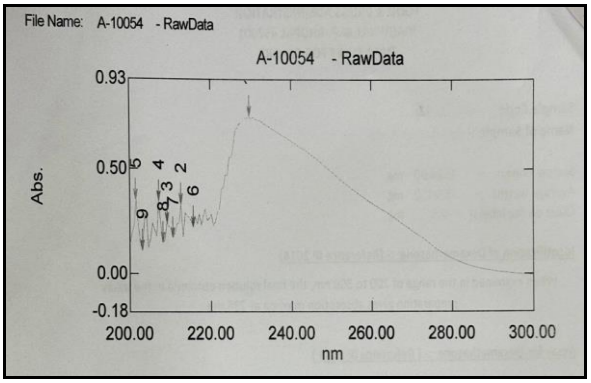
Preparation of Sample Solutions

I. Twenty dexamethasone tablets (Zydus Cadila, 0.5 mg) were accurately weighed, and the average weight of a single tablet was calculated to ensure uniformity. The tablets were then crushed into a fine powder using a mortar and pestle to facilitate complete extraction of the drug.
II. An amount of powdered tablet equivalent to 2.0 mg of dexamethasone was transferred into a 25 mL volumetric flask. 15 mL of diluted methanol (methanol: water, 1:2 v/v) was added, and the flask was subjected to ultrasonic shaking for 15 minutes to enhance dissolution of the active ingredient. The volume was then made up to the mark with the same diluted methanol.
III. The solution was filtered through quantitative filter paper (Sartorius, Germany) to remove insoluble excipients and ensure clarity. Any un-dissolved particles were discarded to avoid interference in spectrophotometric measurement.
IV. Subsamples of the filtered solution were further diluted with distilled water to obtain a final concentration of 10µg/mL, suitable for UV-Visible analysis.
V. The procedure was carried out under ambient temperature to prevent degradation of dexamethasone. All glassware was clean and dry, and analytical grade solvents were used to avoid contamination. The prepared sample solutions were protected from light to maintain stability until analysis. Each step was performed in triplicate to ensure reproducibility and accuracy of the results.

UV–Visible Spectroscopy of Standard and Sample Solutions

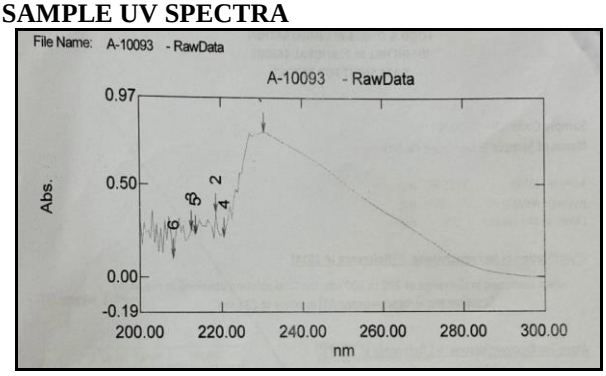
The quantitative estimation of dexamethasone was performed using a UV–Visible spectrophotometer, which measures the amount of light absorbed by the drug at a specific wavelength. The instrument was first switched on and allowed to warm up, and calibration was performed using methanol as the blank to eliminate any interference from the solvent. For the sample solutions, aliquots of the filtered and diluted tablet solution were prepared to match the concentration range of the calibration standards. The absorbance was measured at the same wavelength (242 nm), and the concentration of dexamethasone in the sample was calculated using the previously prepared calibration curve.

STANDARD DATA



S. No.	Concentration	Absorbance	Wave Length
Std. 1	9.6µg/ml	0.753	230nm

SAMPLE UV SPECTRA



S No.	Concentration	Absorbance	Wave Length
Sample	10µg/ml	0.782	230nm

CALCULATION

Powder weight calculations
Label claim of tablet = 0.5mg Dexamethasone
Wt. of tablets = 1511.8mg
Avg. wt. of each tablet = 75.59mg
X gm of powder contain = 0.0005gm Dexamethasone
Y gm of powder = 0.0004gm Dexamethasone
Y = X ×0.0004 / 0.0005 = 0.00048 gm of powder equivalent to Dexamethasone

Formula

$$\textbf{C test} = \frac{Atest \times C\ std.}{A\ std.}$$

$$\textbf{C test} = \frac{0.782 \times 9.6}{0.753}$$

$$\textbf{C test} = \frac{7.5072}{0.753}$$

C test = 9.9 µg/mL

$$\% \text{ Content} = \frac{C\ test\ x\ 100}{10}$$

$$= \frac{9.9\ x\ 100}{10}$$

$$= 99\%$$

$$\text{Drug in mg} = \frac{99\ x\ 0.5}{100}$$

$$= 0.495\text{mg}$$

RESULT AND DISCUSSION

The content of Dexamethasone was found to be 99% (0.495mg) at wavelength of 230nm. Std. show absorbance 0.753 at 230nm at 230nm wavelength and sample shows absorbance 0.782 at 230nm, which indicates accuracy in result.

SUMMARY AND CONCLUSION

In this study, it was concluded that the content of Dexamethasone in the marketed tablet formulation was found to be within the specified limits as claimed by the manufacturer. The analytical data obtained confirmed that the developed UV-spectrophotometric method provides accurate and reproducible results, fulfilling the acceptance criteria set for assay determination. The precision and reliability of the method ensure its suitability for routine pharmaceutical analysis. The proposed UV-Visible spectrophotometric method for the estimation of Dexamethasone is simple, sensitive, rapid, cost-effective, and does not require expensive instrumentation or complex sample preparation. The results obtained were found to be highly consistent, demonstrating excellent, accuracy, and precision within the prescribed limits. This makes the method especially beneficial for laboratories and pharmaceutical industries with limited resources.

This study highlights the efficiency of UV-spectroscopy in both qualitative and quantitative determination of Dexamethasone in tablet dosage forms. It can be used effectively to monitor the quality and stability of the drug in various formulations. Furthermore, this method minimizes analytical errors, reduces solvent consumption, and supports environmentally friendly analytical practices. Overall, UV-spectrophotometric analysis stands as a powerful and dependable analytical tool for assessing the purity, potency, and efficacy of Dexamethasone in pharmaceutical preparations. The findings confirm that the developed method can serve as a valuable technique for regular quality control analysis in academic, industrial, and research laboratories.

Hence, it can be concluded that this validated spectrophotometric method provides an efficient, economical, and reliable approach for the estimation of Dexamethasone, ensuring its therapeutic effectiveness and safety in pharmaceutical applications.

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