



زانكۆی سه لاهه دین-ههولير

Salahaddin University-Erbil

# **Determination of Dexamethasone in Drugs By High Performance Liquid Chromatography (HPLC)**

Graduation Research Project

Submitted to the Department of Chemistry in Partial  
Fulfillment of the Requirements for the Degree of  
Bachelor of Science in Chemistry.

**Prepared by:**

**Zaynab Mutalib Aula**

**Supervised by:**

**Ms. Sara Hadi Assaf**

2023-2024

| <b>Section</b> | <b>Titles</b>           | <b>Pages</b> |
|----------------|-------------------------|--------------|
|                | <b>Table Of Content</b> | <b>i.</b>    |
|                | <b>Abstract</b>         | <b>ii.</b>   |

## **Chapter One: Introduction**

|              |   |           |
|--------------|---|-----------|
| <b>1.1</b>   | <b>Drug Analysis</b>  | <b>1</b>  |
| <b>1.2</b>   | <b>Dexamethasone</b>  | <b>2</b>  |
| <b>1.3</b>   | <b>Roles Of Dexamethasone</b>   | <b>5</b>  |
| <b>1.3.1</b> | <b>Dexamethasone in cancer Treatment</b>  | <b>5</b>  |
| <b>1.3.2</b> | <b>Dexamethasone in Pregnancy</b>   | <b>5</b>  |
| <b>1.3.3</b> | <b>Dexamethasone In Surgery</b>   | <b>6</b>  |
| <b>1.3.4</b> | <b>Dexamethasone In Covid -19 Treatment</b>   | <b>7</b>  |
| <b>1.4</b>   | <b>Adminstration of Dexamethasone</b>   | <b>7</b>  |
| <b>1.5</b>   | <b>Side effect of Dexamethasone</b>   | <b>8</b>  |
| <b>1.6</b>   | <b>Contraindication of dexamethasone</b>  | <b>8</b>  |
| <b>1.7</b>   | <b>HPLC method</b>  | <b>9</b>  |
| <b>1.7.1</b> | <b>Basic Principle of High-Performance Liquid Chromatography</b>  | <b>11</b> |
| <b>1.7.2</b> | <b>Depending on the nature of the stationary phase, the separation process can be of five different modes</b> | <b>12</b> |
|              |   |           |

| <b>Section</b>   | <b>Title</b>   | <b>Page</b> |
|--|--|-------------|
| <b>Chapter Two: Literature Review on The Determination of Dexamethasone in Drugs by (HPLC)</b> |  |             |
| <b>2.1</b>   | <b>A Novel Sensor for Determination of Dexamethasone Disodium Phosphate in Different Pharmaceutical Formulations</b>   | <b>15</b>   |
| <b>2.2</b>   | <b>Determination of dexamethasone acetate in cream by HPLC</b>   | <b>16</b>   |
| <b>2.3</b>   | <b>Determination of dexamethasone sodium phosphate in the vitreous by high performance liquid chromatography</b>   | <b>16</b>   |
| <b>2.4</b>   | <b>A validated, stability-indicating HPLC method for the determination of dexamethasone related substances on dexamethasone-coated drug-eluting stents</b>   | <b>17</b>   |
| <b>2.5</b>   | <b>A Validated, Stability-Indicating Method for the Assay of Dexamethasone in Drug Substance</b>   | <b>18</b>   |
| <b>2.6</b>   | <b>Development and Validation of an HPLC Method for the Determination of Dexamethasone, Dexamethasone Sodium Phosphate and Chloramphenicol in Presence of Each Other in Pharmaceutical Preparations.</b> | <b>19</b>   |

|  |                   |           |
|--|-------------------|-----------|
|  | <b>Conclusion</b> | <b>20</b> |
|  | <b>References</b> | <b>21</b> |

## **Abstract:**

The Dexamethasone is a type of medicine called a steroid (or corticosteroid), is a potent anti-inflammatory and immunosuppressive properties in various medical conditions including allergic reactions, respiratory issues, cancer treatment, cerebral edema, inflammatory bowel disease, skin disorders, eye conditions, organ transplantation. Dexamethasone can cause a range of side effects like Mood Changes, Elevated Blood Sugar Level, Gastrointestinal Effects. This research project focuses on the determination of Dexamethasone in Drugs By using High Performance Liquid Chromatography (HPLC) as the analytical technique. High-performance liquid chromatography (HPLC) has emerged as a powerful analytical technique for the quantitative analysis of dexamethasone due to its high sensitivity, selectivity, and reproducibility. this review provides valuable insights into the analytical methodologies and applications of HPLC for the determination of dexamethasone in pharmaceutical formulations, contributing to the advancement of drug quality assessment, therapeutic monitoring, and pharmaceutical research.

**Keywords:** Dexamethasone, Pharmaceutical formulations, HPLC.



**Chapter One**  
**Introduction**

# **1. Introduction**

## **1.1 Drug Analysis**

Pharmaceutical analysis is a broader term and there are many ways to define it. It is the process or series of processes that can be used for the identification, determination, separation, purification, and structure elucidation of the given compound used in the formulation of pharmaceutical products. Drug analysis encompasses tests on raw materials (purity criteria), pharmaceutical or veterinary formulations, and a number of other, more complex matrices such as foods of animal origin, drinks, and foodstuffs that are conducted for clinical, forensic (Calatayud and Zamora 2013). The components to which the pharmaceutical analysis is done, are normally active pharmaceutical ingredients (APIs), pharmaceutical excipients (disintegrants, binders, surfactants, suspending agents, viscosity increasing agents, polymers, adhesives, lubricants, etc.), contaminants present in pharmaceutical products, or drug metabolites. In pharmaceutical analysis, the samples are typically finished pharmaceutical products (tablets, capsules, syrups, creams, lotions, ointments, injections, etc.), biological samples (blood and/or urine, tissue samples that contain one or more ingredients), impurities, contaminants, and pharmaceutical raw materials. Pharmaceutical analysis can be done using various analytical techniques. The analysis of pharmaceutical drugs is carried out through various analytical techniques and methodologies, each tailored to specific purposes and requirements. These techniques include spectroscopic methods (such as UV-Vis spectroscopy, infrared spectroscopy, and nuclear magnetic resonance spectroscopy), chromatographic techniques (such as high-performance liquid chromatography, gas chromatography, and thin-layer chromatography), and other advanced analytical methods like mass spectrometry, titrimetry, and dissolution testing (Kar 2005).

## 1.2 Dexamethasone

The Dexamethasone is a type of medicine called a steroid , and was first synthesized in 1957. characterized by a huge anti-inflammatory and immunosuppressive effect, Dexamethasone provides relief for inflamed areas of the body. It is used to treat a number of different conditions, such as inflammation (swelling), It is used for the treatment of various cancers, viral infections, respiratory diseases, and immune system disorders, liver disorders, gastrointestinal diseases, skin disorders and nervous system abnormalities severe allergies, adrenal problems, arthritis, blood or bone marrow problems, kidney problems, skin conditions, and it is on the World Health Organization's List of Essential Medicines In June 2020 (Ohannesian and Streeter 2002). dexamethasone has a rather long biological half-life of 36–72 h (Polderman, Farhang-Razi et al. 2019). Is a potent glucocorticoid, Glucocorticoids (GCs) As a class of steroid hormones secreted by the adrenal gland and circulating in the blood, glucocorticoids spread almost all over the essential organs of the body and which acts as an anti-inflammatory and immunosuppressant (Liu, X., et al. 2004).Dexamethasone is available as IV, IM, oral, nasal, ophthalmic, eyedrop, and typical cream formulation for treatment of various diseases (De Oliveira Jr, et al. 2013). and Dexamethasone sometimes combined with other drugs for various reasons, depending on the specific medical condition dexamethasone is often used in combination with chemotherapy drugs to improve their effectiveness in killing cancer cells on being treated, dexamethasone might be combined with other drugs, in cancer treatment and can help mitigate its side effects is sometimes used to reduce inflammation and swelling caused by other chemotherapy drugs. Dexamethasone can have several potential side effects, particularly when used for an extended period or in high doses. Some common side effects such as Increased appetite, Fluid retention, Dexamethasone can cause stomach irritation, Increasing blood glucose



which may be problematic for individuals with diabetes, Skin changes can cause thinning of the skin (Polderman, J., et al. 2019). Dexamethasone has several contraindications such as Dexamethasone use is contraindicated if patients have systemic fungal infections, hypersensitivity to dexamethasone, or cerebral malaria. Another contraindication is to administer live or live-attenuated vaccines during dexamethasone use. The immune system will be suppressed, placing the patient at risk of infection. It is still permissible to administer killed or inactivated vaccines. However, it bears mentioning that corticosteroids may attenuate immune response, and it is unpredictable if immunity develops, Recommendations include using dexamethasone cautiously during pregnancy as there is an increased risk of oral cleft formation (Kocienski 2020). The dosing of dexamethasone can vary widely depending on the specific condition being treated, the severity of the condition, the patient's age, weight, and individual response to the medication (Lim-Fat, M.J., et al. 2019). They are including spectrophotometry liquid chromatography, liquid chromatography-mass spectrometry, and electrochemical methods, High-Performance Liquid Chromatography (HPLC), The methods have limitations such as high cost and hard operation and low repeatability. One commonly used technique is High-Performance Liquid Chromatography (HPLC), which separates the components of a mixture based on their interaction with a stationary phase and a mobile phase. In HPLC analysis of dexamethasone, a sample is injected into the chromatographic system, where it is carried by the mobile phase through a column. The dexamethasone molecules interact with the stationary phase at different rates, allowing for their separation and detection using a UV detector. Another widely utilized method is Spectrophotometry, which measures the absorbance of light by dexamethasone molecules in a sample solution. By comparing the absorbance of the sample to that of known standards, the concentration of dexamethasone can be determined. Additionally, techniques such as Mass Spectrometry (MS) and Nuclear

Magnetic Resonance (NMR) spectroscopy are employed for the identification and quantification of dexamethasone in drug samples. These methods offer high sensitivity and specificity, allowing for accurate analysis even at low concentrations. Overall, the application of various analytical methods ensures the reliable determination of dexamethasone content in pharmaceutical drugs, contributing to their quality control and regulatory compliance (Akhoundi-Khalafi and Shishehbore 2015).

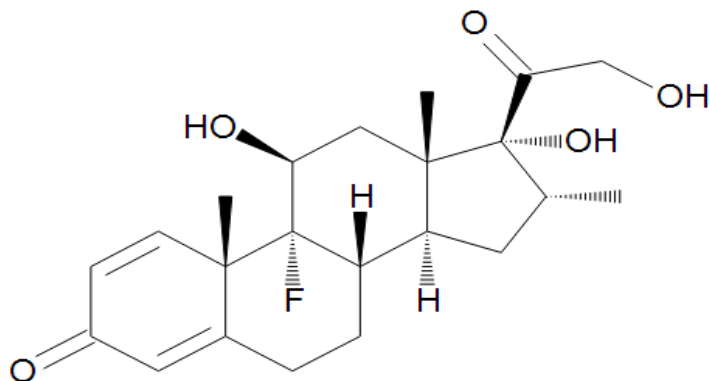
Molecular Weight: 392.5 g/mol

Molecular Formula: C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>

Solubility: Insoluble in water

Synonym: 9 $\alpha$ -fluoro-11 $\beta$ ,17 $\alpha$ ,21-trihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione

Brand name: Decadron, Dexasone



**Figure 1:** Structure of Dexamethasone

## **1.3 Roles of Dexamethasone**

### **1.3.1. Dexamethasone in cancer Treatment**

Dexamethasone is commonly used to reduce inflammation and suppress the immune response. In cancer patients, it can help alleviate symptoms related to inflammation caused by the tumor itself or as a side effect of certain cancer treatments such as chemotherapy or radiation therapy. Dexamethasone was commonly administered to prevent or treat postoperative nausea and vomiting and allergic reaction to drugs and blood transfusion to help prevent or reduce chemotherapy-induced nausea and vomiting (Yu, H., et al. 2015) and Cancer patients, especially those with brain tumors, may experience edema (swelling) in the brain. Dexamethasone can help reduce this swelling and relieve associated symptoms such as headaches and neurological deficits (Dietrich, J., et al. 2011).

### **1.3.2. Dexamethasone in Pregnancy**

Pregnant women receiving Dexamethasone therapy, suffer the same side effects and benefits as do treated women who are not pregnant. Because both pregnancy and dexamethasone therapy induce diabetes and sugar rise more easily to abnormal levels in pregnant than in non-pregnant women. Dexamethasone nasal has been assigned to pregnancy category C ( Lockshin and Sammaritano 1998) can be given to pregnant women who are at risk of delivering their babies early to help improve either baby's health. Giving dexamethasone to the mother while she is still pregnant in order to improve the baby's maturity are usually administered 12 mg for 2 doses 12 hours apart or 6 mg for 4 doses 12 hours apart in contemporary practice. Both regimens are considered effective in helping the baby.

However, this medication can cause the mother's blood sugar level to go up, an effect which may last up to 5 days. High maternal blood sugar level can slow the baby's lung maturation undermining the positive impact the medication can have on the baby (Bakker, J.M., et al. 1995).

### **1.3.3. Dexamethasone In Surgery**

Dexamethasone is often administered before surgery to prevent postoperative nausea and vomiting (PONV), Taking Dexamethasone A number of factors, including age, obesity, surgical procedure, anesthetic technique, the effects of dexamethasone plus either droperidol and metoclopramide with each antiemetic alone for preventing postoperative nausea and vomiting (Collaborators and Collaborative 2017). Reduction of Inflammation In some surgeries, especially those involving significant tissue trauma or inflammation (Weber and Griffin 1994) may be used as part of the treatment for severe allergic reactions or anaphylaxis that can occur during surgery or as a reaction to medications administered during the procedure, and However, concerns about potential adverse effects, including inadequate serum glucose control, infectious complications, poor wound healing, and gastrointestinal bleeding (Dieleman, J.M., et al. 2012).

### **1.3.4. Dexamethasone in Covid -19 Treatment**

Dexamethasone is the first and an important therapeutic to significantly reduce the risk of death in COVID-19 patients with severe disease. Due to powerful anti-inflammatory and immunosuppressive effects (Chen, F., et al. 2021) and has been reported as the first effective treatment for the sickest patients with COVID-19, and, given its low cost, known safety profile, and widespread availability, it is a likely candidate for immediate worldwide use, For COVID-19 patients, Dexamethasone has been demonstrated to prolong the analgesic effect as the disease causes diffuse lung damage mediated through pro-inflammatory cytokines. Hence, DEXA is now used to delay respiratory failure and ultimate death in COVID-19 patients by minimizing the damaging effect of cytokines (Musee, N., et al. 2021)with their treatment use accounting for up to 50% they have been found to aid the recovery of patients on ventilators and to improve the condition of those receiving oxygen but not on ventilators, with a concomitant 20% death reduction (Chappell, L., et al. 2020).

### **1.3.5. Administration of Dexamethasone**

- a. Oral Administration: Dexamethasone is available in tablet This route is often used for chronic conditions such as certain autoimmune diseases, allergic reactions, and inflammation. Oral administration allows for convenient dosing and gradual absorption of the medication through the digestive system. the bioavailability of oral dexamethasone is regarded to be between 70–78% in healthy individuals (Weijtens, O., et al. 1998).

- b. Intravenous (IV) Administration: Dexamethasone can be administered intravenously, typically in emergency situations or when rapid onset of action is required. IV administration allows for the medication to be delivered directly into the bloodstream, leading to faster effects compared to oral administration. bioavailability of dexamethasone after IM administration was 100% (Chandrasekhar, S.S., et al. 2000).
  
- c. Intramuscular (IM) Administration: Dexamethasone can also be administered via intramuscular injection. This route may be used when oral administration is not feasible or when a rapid effect is desired but intravenous access is not readily available. Intramuscular injections are often used in emergency situations or for certain acute conditions. was absorbed rapidly after intramuscular route with 86% bioavailability (Samtani and Jusko 2005).
  
- d. Topical Administration: Dexamethasone is available in the form of creams, ointments, lotions, and eye drops for topical application. This route is primarily used for treating skin conditions such as eczema, dermatitis, and psoriasis, as well as certain eye conditions like inflammation or allergic condition (Short, C., et al. 1966).
  
- e. Nasal Administration: Dexamethasone can be administered intranasally as a nasal spray. This route is often used to treat allergic rhinitis, nasal congestion, and inflammation of the nasal passages. Nasal administration allows for localized treatment of nasal symptoms while minimizing systemic effects (Passalacqua, G., et al. 2000).

### **1.3.6. Side effect of Dexamethasone**

Dexamethasone, like other corticosteroids, can have a range of potential side effects, especially when used for prolonged periods or at high doses, they are well known to have adverse effects, which are dose dependent and related to the half-life, frequency, and time of day administered and the route of administration of the drugs. Short-term adverse effects include insomnia, constipation, bloating, mood changes, and hyperglycemia, whereas long-term treatment is associated with osteoporosis, bone necrosis, arterial hypertension, diabetes, hypothalamic-pituitary-adrenal axis suppression, Cushing syndrome, cataracts, glaucoma, skin thinning, easy bruising, and muscle atrophy, stomach irritation, ulcers, or gastrointestinal bleeding, particularly with high doses or prolonged use (Berthon, B.S., et al. 2014).

### **1.3.7. Contraindications of Dexamethasone**

Contraindications for the use of dexamethasone are a history of hypersensitivity to any of the components of the preparation, fungal diseases of the skin, untreated bacterial diseases, tuberculosis of the skin, or certain types of viral disease, e.g., herpes simplex, vaccinia, and varicella. Precautions include infancy, most facial dermatoses, widespread inflammatory skin disease, hepatic failure, and infected dermatoses, unless accompanied by the use of an appropriate antimicrobial agent (Hannon, Croxtall et al. 2003). In patients with cirrhosis, diverticulitis, myasthenia gravis, renal insufficiency, or ulcerative diseases such as peptic ulcer disease or ulcerative colitis, it is important to use caution when prescribing dexamethasone (Messer and Keller 1975).

## 1.4 HPLC method

High-Performance Liquid Chromatography (HPLC) was first proposed by Kirkland and Huber, developed in the late 1960s and early 1970s (Ali 2022). was derived from the classical column chromatography and is one of the most important tools of analytical chemistry today In the modern pharmaceutical industry, biotechnology, environmental, polymer and food industries and applied in all stages of drug discovery, development, and production and quality control (Snyder, L.R., et al. 2012). It is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale-up, evaluating new formulations, and carrying out quality control/assurance of the final drug products (Narula and Pal 2021).HPLC It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid and is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability (Vidushi et al., 2017). HPLC is accomplished by injection of a small amount of liquid sample into a moving stream of liquid (called the mobile phase) that passes through a column packed with particles of the stationary phase. The separation of a mixture into its components depends on different degrees of retention of each component in the column. The extent to which a component is retained in the column is determined by its partitioning between the liquid mobile phase and the stationary phase. In HPLC this partitioning is affected by the relative solute/stationary phase and solute/mobile phase interactions. Thus, unlike GC, changes in mobile phase composition can have an enormous impact on your separation. Since the compounds have different mobilities, they exit the column at different times; i.e., they have different retention times, The retention time is the time between injection and detection. Thus HPLC is most often used when one is performing a target compound



analysis, where one has a good idea of the compounds present in a mixture so reference standards can be used for determining retention times (Ali 2022).

### 1.4.1 Basic Principle HPLC principle

is that solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase. HPLC is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially (Belanger, J.M., et al. 1997).

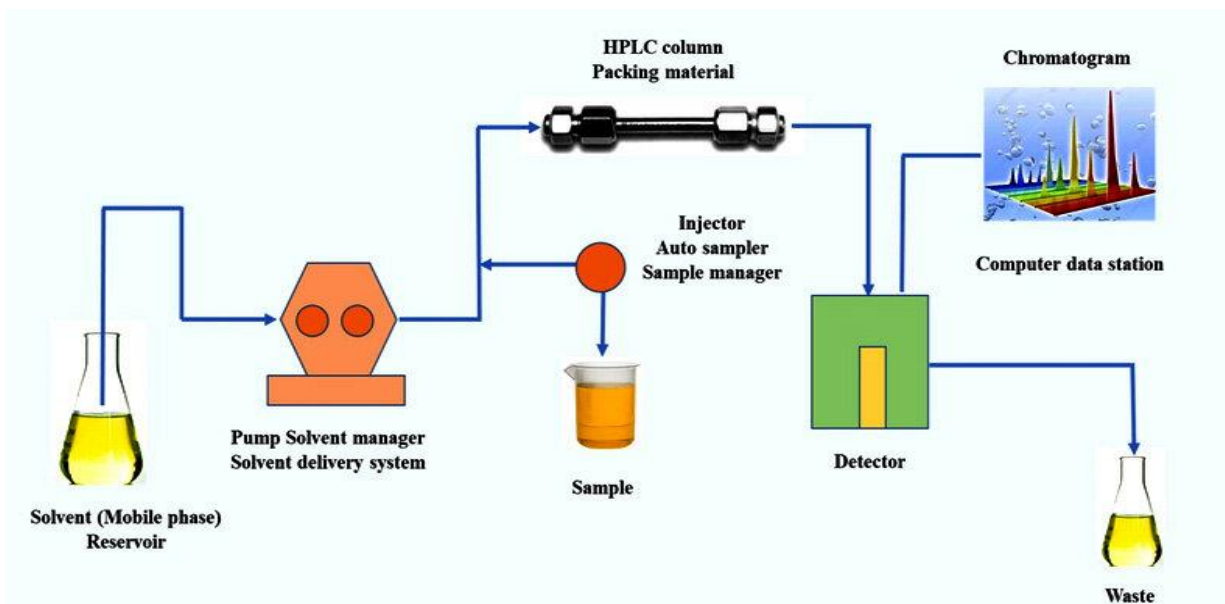


Figure 3. shows a basic overview of the HPLC process.

### **1.4.2 Depending on the nature of the stationary phase, the separation process can be of five different modes**

**Normal Phase Chromatography In (NP-HPLC)** the nature of stationary phase is polar and mobile phase is non-polar. In a mixture of components to be separated, those analytes which are relatively more polar will be retained by the polar stationary phase longer than those which are relatively less polar. Therefore the least polar component will elute first. The attractive forces which exist are mostly dipole-dipole and hydrogen bonding interaction. It is first choice for mixtures of isomers and for preparative scale HPLC and second choice for lipophilic samples that cannot dissolve well in water-organic mixtures.

**Reversed Phase Chromatography (RP-HPLC)** has a non-polar stationary phase and polar or moderately polar mobile phase. RP-HPLC is based on the principle of hydrophobic interaction. In a mixture of components, those analytes which are relatively less polar will be retained by the non-polar stationary phase longer than those which are relatively more polar. Therefore the most polar component will elute first. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution.

**Size Exclusion Chromatography (SEC)**, also called as gel permeation chromatography or gel filtration chromatography mainly separates particles on the basis of size. The column is filled with material having precisely controlled pore sizes, and the sample is simply screened or filtered. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later. This technique is widely used for the molecular weight determination of polysaccharides.

**Ion Exchange Chromatography** In Ion-exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute.

**Bio-Affinity Chromatography** Separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands (Chawla and Chaudhary 2019).

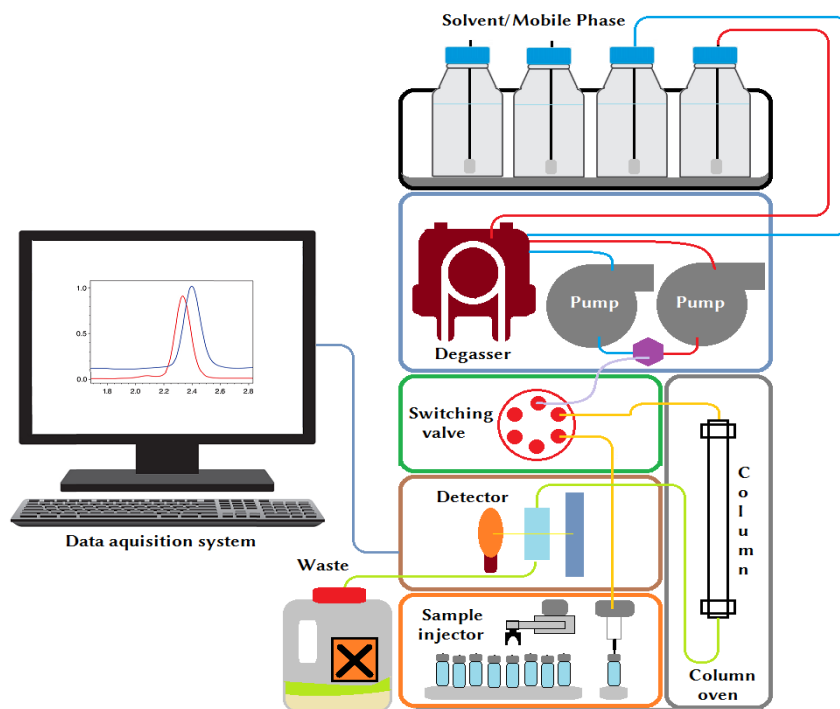


Figure 1. Components of an HPLC instrument

## **Chapter Two**

### **Literature Review on The Determination of Dexamethasone In Drugs By High Performance Liquid Chromatography (HPLC)**

## **2. Literature Review on The Determination of Dexamethasone In Drugs By High Performance Liquid Chromatography (HPLC)**

### **2.1 A Novel Sensor for Determination of Dexamethasone in Different Pharmaceutical Formulations**

A novel electrode was developed for potentiometric determination of dexamethasone using tetra heptyl ammonium bromide (THB) as an anionic exchanger in polyvinyl chloride (PVC) matrix and 2-nitrophenyl octyl ether (2-NPOE) as a plasticizer. Linear responses of  $1 \times 10^{-5}$  to  $1 \times 10^{-2}$  M with slope of  $-26.50 \pm 0.39$  mV/decade within working pH range 8-12 were achieved. The percentage recovery of determination of DSP by the proposed dexamethasone selective electrode was  $99.96 \pm 0.95$ . Determination of dexamethasone in its pharmaceutical formulations by the proposed electrode revealed its applicability for determination. Moreover, the electrode exhibits good selectivity for dexamethasone with respect to a large number of interfering substances and co-formulated drugs. The fabricated sensor was validated according to ICH guidelines and successfully applied for determination of the studied drug in pure form and pharmaceutical formulations without any interference from additives either labeled or non-labeled. The obtained results have been statistically compared to that of an official spectrophotometric method to give a conclusion that there is no significant difference between the proposed methods and the official one with respect to accuracy and precision (Duarah, S., et al. 2021).

## **2.2 Determination of dexamethasone in cream by HPLC**

The aim of this research was to validate a high performance liquid chromatographic method for the quantitative determination of dexamethasone contained in cream preparation. A Meta Sil octadecyl silane (250/4.6 mm, 5mm) column, a methanol: water (65:35; v/v) mobile phase (1.0 ml min<sup>-1</sup>) and an UV detector (set at 254 nm) were used to evaluate the parameters: linearity, precision, accuracy, specificity, as well as, quantitation and detection limits. The calibration curve showed a correlation coefficient of 0.9999. The precision was demonstrated by the relative standard deviation (RSD) of 0.53. The recovery test resulted in an average of 97.85%, what confirmed the accuracy of the method. The quantitation and detection limits determined were 1.41 and 0.47 mg ml<sup>-1</sup>, respectively. The specificity test showed there was no interference in the drug peak (Garcia, C.V., et al. 2003).

## **2.3 Determination of dexamethasone sodium phosphate in the vitreous by high performance liquid chromatography**

Dexamethasone sodium phosphate (DSP) is increasingly used in the treatment of ocular inflammatory diseases by systemic, periocular, and recently, intravitreal injection. We have developed a method for the determination of vitreous levels of DSP by reverse phase HPLC. In this method, co-elution of vitreous proteins with DSP is resolved by a prior sample clean-up procedure using Waters Sep-Pak C18 cartridge; the protein is separated and eluted with water while DSP, paraben and prednisone are eluted with methanol. DSP in the resulting sample is then separated by reverse phase HPLC and quantified by UV absorption at 254 nm. The recovery of DSP through the sample clean-up is 68.9 +/- 3.0%. DSP quantitation is linear from 0.1 mg to 1.0 mg per 1.0 ml vitreous. This method provides a simple, sensitive and reliable technique for determining the vitreous levels of DSP (Kwak, H.W. et al. 1995).

## **2.4 A validated, stability-indicating HPLC method for the determination of dexamethasone related substances on dexamethasone-coated drug-eluting stents**

An HPLC method was developed and validated to determine trace amounts of dexamethasone related substances on dexamethasone-coated drug-eluting stents. Separation of dexamethasone from its major process impurities and degradation products was achieved on a Zorbax Eclipse XDB C8 column using gradient elution and UV detection at 239 nm. The method was validated according to ICH guideline requirements. In addition, stent extraction efficiency, solution stability and method robustness were evaluated. The method was determined to be linear in the range of 0.01–0.30 g ml<sup>-1</sup> for the quantitation of major dexamethasone related substances. Method accuracy was assessed by spiking dexamethasone acetate at three levels over the range of 0.025–0.175 g ml<sup>-1</sup>. The dexamethasone acetate recovery ranged from 89.6 to 105.8%. The intermediate precision over the three levels was less than 6% (n = 9). The method was also shown to be repeatable at concentration levels of 0.025, 0.125 and 0.175 g ml<sup>-1</sup> dexamethasone with relative standard deviation values of 4.1, 1.7 and 1.6%, respectively. The method was found to be specific, stability-indicating, and sensitive with a detection limit of 0.008 g ml<sup>-1</sup> and a quantitation limit of 0.025 g ml<sup>-1</sup> dexamethasone. Finally, the method was demonstrated to be robust, resistant to small variations of chromatographic variables such as pH, mobile phase organic/aqueous composition and column temperature. Identifying unknown dexamethasone degradation products in dexamethasone-coated drug-eluting stents was of critical interest to ensure product quality, since degradants have a significant impact on safety, efficacy, and product storage and handling. The developed chromatographic method was designed to be compatible with mass spectrometric detection. This paper also discusses using this chromatographic method coupled to

an ion-trap LCQ mass spectrometer to elucidate proposed structures for four major dexamethasone degradants (Duarah, S., et al. 2021).

## **2.5 A Validated, Stability-Indicating Method for the Assay of Dexamethasone in Drug Substance**

A new high-performance liquid chromatographic (HPLC) procedure for the determination of dexamethasone, impurities, degradation products and product preservatives is described. A three-stage, linear gradient with UV detection at 240 nm allows the analysis of dexamethasone drug substance and dexamethasone in two formulated products, using the same chromatographic system. The Limit of Quantitation (LOQ) of dexamethasone impurities in drug substance is 0.05%, and 0.1% for dexamethasone degradation products in formulated products. The method is linear, precise, accurate and robust. Sample preparations are simple, and are accomplished without the use of an internal standard. Several degradation products of stressed dexamethasone have been identified (Spangler, M. and Mularz. 2001).



## **2.6 Development and Validation of an HPLC Method for the Determination of Dexamethasone, Dexamethasone Sodium Phosphate and Chloramphenicol in Presence of Each Other in Pharmaceutical Preparations.**

An HPLC method for the determination of dexamethasone, dexamethasone sodium phosphate and chloramphenicol in presence of each other in pharmaceutical preparations has been developed using a Shim-Pack CLC-ODS column (6.0 · 150 mm<sup>2</sup>). These analytes were separated under isocratic conditions. Various chromatographic parameters including linearity, precision and accuracy have been evaluated. The method was found to be suitable for analysis of these drug substances in presence of each other. The run time was less than 15 min. This method is suitable for application to various dosage forms (Iqbal, M.S. et al.2006).

## **Conclusion**

The determination of dexamethasone in pharmaceutical formulations using the High-Performance Liquid Chromatography (HPLC) technique offers a robust and reliable method for ensuring drug quality, efficacy, and patient safety. Through meticulous method development, optimization of chromatographic parameters, and validation procedures, HPLC has demonstrated its efficacy in accurately quantifying dexamethasone in various drug formulations. The utilization of HPLC for the determination of dexamethasone in drugs represents a cornerstone in pharmaceutical analysis, providing a robust analytical method essential for ensuring the quality, safety, and efficacy of dexamethasone-containing pharmaceutical products. Continued research and innovation in HPLC methodology will further advance the field, facilitating improved drug development, regulatory compliance, and patient care.

## Reference

Akhoundi-Khalafi, A. M. and M. R. Shishehbore (2015). "A new technique for quantitative determination of dexamethasone in pharmaceutical and biological samples using kinetic spectrophotometric method." International Journal of Analytical Chemistry **2015**.

Ali, A. H. (2022). "High-performance liquid chromatography (HPLC): a review." Ann. Adv. Chem **6**: 010-020.

Bakker, J.M., Schmidt, E.D., Kroes, H., Kavelaars, A., Heijnen, C.J., Tilders, F.J. and van Rees, E.P., 1995. Effects of short-term dexamethasone treatment during pregnancy on the development of the immune system and the hypothalamo-pituitary adrenal axis in the rat. *Journal of neuroimmunology*, 63(2), pp.183-191.

Belanger, J.M., Paré, J.J. and Sigouin, M., 1997. High performance liquid chromatography (HPLC): principles and applications. In *Techniques and instrumentation in analytical chemistry* (Vol. 18, pp. 37-59). Elsevier.

Berthon, B.S., MacDonald-Wicks, L.K. and Wood, L.G., 2014. A systematic review of the effect of oral glucocorticoids on energy intake, appetite, and body weight in humans. *Nutrition Research*, 34(3), pp.179-190.

Calatayud, J. M. and L. L. Zamora (2013). "Spectrophotometry| pharmaceutical applications."

Chandrasekhar, S. S., et al. (2000). "Dexamethasone pharmacokinetics in the inner ear: comparison of route of administration and use of facilitating agents." Otolaryngology—Head and Neck Surgery **122**(4): 521-528.

Chappell, L., Horby, P., Lim, W.S., Emberson, J.R., Mafham, M., Bell, J.L., Linsell, L., Staplin, N., Brightling, C., Ustianowski, A. and Elmahi, E., 2020. Dexamethasone in hospitalized patients with Covid-19-preliminary report. The New England journal of medicine.

Chawla, G. and K. K. Chaudhary (2019). "A review of HPLC technique covering its pharmaceutical, environmental, forensic, clinical and other applications." Int J Pharm Chem Anal **6**(2): 27-39.

Chen, F., Hao, L., Zhu, S., Yang, X., Shi, W., Zheng, K., Wang, T. and Chen, H., 2021. Potential adverse effects of dexamethasone therapy on COVID-19 patients: review and recommendations. *Infectious diseases and therapy*, 10, pp.1907-1931.

Collaborators, D. T. and W. M. R. Collaborative (2017). "Dexamethasone versus standard treatment for postoperative nausea and vomiting in gastrointestinal surgery: randomised controlled trial (DREAMS Trial)." bmj **357**.

De Oliveira Jr, G.S., Castro-Alves, L.J.S., Ahmad, S., Kendall, M.C. and McCarthy, R.J., 2013. Dexamethasone to prevent postoperative nausea and vomiting: an updated meta-analysis of randomized controlled trials. *Anesthesia & Analgesia*, 116(1), pp.58-74.

Dieleman, J.M., Nierich, A.P., Rosseel, P.M., van der Maaten, J.M., Hofland, J., Diephuis, J.C., Schepp, R.M., Boer, C., Moons, K.G., van Herwerden, L.A. and Tijssen, J.G., 2012. Intraoperative high-dose dexamethasone for cardiac surgery: a randomized controlled trial. *Jama*, 308(17), pp.1761-1767.

Dietrich, J., Rao, K., Pastorino, S. and Kesari, S., 2011. Corticosteroids in brain cancer patients: benefits and pitfalls. *Expert review of clinical pharmacology*, 4(2), pp.233-242.

Duarah, S., Sharma, M. and Wen, J., 2021. Rapid and simultaneous determination of dexamethasone and dexamethasone sodium phosphate using HPLC-UV: Application in microneedle-assisted skin permeation and deposition studies. *Journal of Chromatography B*, 1170, p.122609.

Garcia, C.V., Breier, A.R., Steppe, M., Schapoval, E.E.S. and Oppe, T.P., 2003. Determination of dexamethasone acetate in cream by HPLC. *Journal of pharmaceutical and biomedical analysis*, 31(3), pp.597-600.

Hannon, R., Croxtall, J.D., Getting, S.J., Roviezzo, F., Yona, S., Paul-Clark, M.J., Gavins, F.N., Perretti, M., Morris, J.F., Buckingham, J.C. and Flower, R.J., 2003. Aberrant inflammation and resistance to glucocorticoids in annexin 1<sup>-/-</sup> mouse. *The FASEB Journal*, 17(2), pp.253-255.

Iqbal, M.S., Shad, M.A., Ashraf, M.W., Bilal, M. and Saeed, M., 2006. Development and validation of an HPLC method for the determination of dexamethasone, dexamethasone sodium phosphate and chloramphenicol in presence of each other in pharmaceutical preparations. *Chromatographia*, 64, pp.219-222.

Kar, A. (2005). Pharmaceutical drug analysis, New Age International.

Kocienski, P. (2020). "Synthesis of Dexamethasone." Synfacts 16(09): 1027.

Kwak, H.W. and D'amico, D.J., 1995. Determination of dexamethasone sodium phosphate in the vitreous by high performance liquid chromatography. *Korean Journal of Ophthalmology*, 9(2), pp.79-83.

Lim-Fat, M.J., Bi, W.L., Lo, J., Lee, E.Q., Ahluwalia, M.S., Batchelor, T.T., Chang, S.M., Chiocca, E.A., Chukwueke, U., Cloughesy, T.F. and Colman, H., 2019. when less is more: dexamethasone dosing for brain tumors. *Neurosurgery*, 85(3), pp.E607-E608.

Liu, X., De Scheerder, I. and Desmet, W., 2004. Dexamethasone-eluting stent: an anti-inflammatory approach to inhibit coronary restenosis. *Expert review of cardiovascular therapy*, 2(5), pp.653-660.

Lockshin, M. and L. Sammaritano (1998). "Corticosteroids during pregnancy." *Scandinavian Journal of Rheumatology* 27(sup107): 136-138.

Messer, E. J. and J. J. Keller (1975). "The use of intraoral dexamethasone after extraction of mandibular third molars." *Oral surgery, oral medicine, oral pathology* 40(5): 594-598.

Musee, N., Kebaabetswe, L.P., Tichapondwa, S., Tubatsi, G., Mahaye, N., Leareng, S.K. and Nomngongo, P.N., 2021. Occurrence, fate, effects, and risks of dexamethasone: Ecological implications post-COVID-19. *International journal of environmental research and public health*, 18(21), p.11291.

Narula, P. and B. Pal (2021). "A comprehensive review of method development by HPLC." *World Journal of Pharmaceutical Research* 10(6): 1839-1858.

Ohannesian, L. and A. J. Streeter (2002). *Handbook of pharmaceutical analysis*, Marcel dekker New York.

Passalacqua, G., Albano, M., Canonica, G.W., Bachert, C., Van Cauwenberge, P., Davies, R.J., Durham, S.R., Kontou-Fili, K., Horak, F. and Malling, H.J., 2000. Inhaled and nasal corticosteroids: safety aspects. *Allergy*, 55(1), pp.16-33.

Polderman, J.A., Farhang-Razi, V., Van Dieren, S., Kranke, P., DeVries, J.H., Hollmann, M.W., Preckel, B. and Hermanides, J., 2018. Adverse side effects of dexamethasone in surgical patients. *Cochrane Database of Systematic Reviews*, (11).

Qu, L. and B. Jiao (2023). "The interplay between immune and metabolic pathways in kidney disease." *Cells* 12(12): 1584.

Samtani, M. N. and W. J. Jusko (2005). "Comparison of dexamethasone pharmacokinetics in female rats after intravenous and intramuscular administration." *Biopharmaceutics & drug disposition* 26(3): 85-91.

Short, C., Keates, R.H., Donovan, E.F., Wyman, M. and Murdick, P.W., 1966. Ocular penetration studies: I. Topical administration of dexamethasone. *Archives of Ophthalmology*, 75(5), pp.689-692.



Snyder, L.R., Kirkland, J.J. and Glajch, J.L., 2012. Practical HPLC method development. John Wiley & Sons.

Spangler, M. and Mularz, E., 2001. A validated, stability-indicating method for the assay of dexamethasone in drug substance and drug product analyses, and the assay of preservatives in drug product. *Chromatographia*, 54, pp.329-334.

Weber, C. R. and J. M. Griffin (1994). "Evaluation of dexamethasone for reducing postoperative edema and inflammatory response after orthognathic surgery." *Journal of oral and maxillofacial surgery* **52**(1): 35-39.

Weijtens, O., Schoemaker, R.C., Cohen, A.F., Romijn, F.P., Lentjes, E.G., van Rooij, J. and van Meurs, J.C., 1998. Dexamethasone concentration in vitreous and serum after oral administration. *American journal of ophthalmology*, 125(5), pp.673-679.

Yu, H.C., Luo, Y.X., Peng, H., Kang, L., Huang, M.J. and Wang, J.P., 2015. Avoiding perioperative dexamethasone may improve the outcome of patients with rectal cancer. *European Journal of Surgical Oncology (EJSO)*, 41(5), pp.667-673.



زانکۆی سه‌لاحه‌دین-ههولیر

Salahaddin University-Erbil

## دیاریکردنی دیکسامیثاسۆن له‌ناو دهرمان به‌پریگای

### HPLC

پروژه‌ی دهرچوونه پیشکەشه به بهشی کیمیایی کۆلیژی زانست زانکۆی سه‌لاحه‌دین-ههولیر  
وهک به‌شیک له پێداویستییه‌کانی به‌دهسته‌ینانی بروانامه‌ی به‌کالۆریۆس له زانستی کیمیادا

ناماده‌کردنی:

زینب مطلب عولا

به‌سه‌ر په‌رشتی:

م.سارا هادی عساف

٢٠٢٣-٢٠٢٤