JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

A Review on Analytical Method for the Estimation of Dexamethasone in Bulk, Biological Fluids and Pharmaceutical Dosage Form

Aayushi S. Mahyavanshi*, Jasmina S. Surati, Ashok H. Akbari, Sagarkumar P. Patel, Dhiren P. Shah

Department of Pharmaceutical Quality Assurance, Shree Naranjibhai Lalbhai Patel College of Pharmacy, Bardoli, Gujarat, India

Article History:

Received 13 Aug 2021
Accepted 29 Nov 2021
Available online 20 Dec 2021

Citation:
Mahyavanshi A. S., Surati J. S., Akbari A. H., Patel S. P., Shah D. P., Evaluation of Knowledge Teachers for First aid in Schools **J Pharm Sci Bioscientific Res**. 2021. 11(1): 39-50

For Correspondence:

Aayushi S. Mahyavanshi

Department of Pharmaceutical Quality Assurance,

Shree Naranjibhai Lalbhai Patel College of Pharmacy, Bardoli, Gujarat, India.

(www.jpsbr.org)

ABSTRACT:

Dexamethasone is a type of corticosteroid are released by the adrenal cortex, which includes glucocorticoid and mineralocorticoids. Glucocorticoids have important role in the effective anti-inflammatory and immunosuppressive activity. Due to their intense immunosuppressant and anti-inflammatory activity, glucocorticoids are used in the treatment of endocrine, rheumatic, collagen, dermatology, allergic, ophthalmic, gastrointestinal, respiratory, hematologic, neoplastic, oedematous. On 16 June 2020 the recovery trial announced preliminary result starting that Dexamethasone improves survival rate of COVID-19 patients. The objective of this review is to provide an overview of the analytical methods in bulk, biological fluids and pharmaceutical dosage form. Various analytical methods have been reported such as U.V, HPLC, HPTLC, LC/MS, GC/MS, Capillary chromatography, XRDS and DSC.

KEY WORDS: Dexamethasone, COVID-19, Analytical methods, Bulk, Biological fluids, pharmaceutical dosage form.

INTRODUCTION

Dexamethasone is a type of corticosteroid medication. Corticosteroid fluorinated at position 9 used for treatment of endocrine, rheumatic, collagen, dermatologic, allergic, ophthalmic, gastrointestinal, respiratory, hematologic, neoplastic, edematous. It is a potent anti-inflammatory and immunosuppressant drug and also use for autoimmune disorders and also for chemotherapy.

Dexamethasone was first made in 1957 by Philp Showalter Hench. On 30 October 1958 approved by FDA for medical use and on 2nd November 2004 central drug standard control organization (CDSCO) also approve in India. The DECADRON® (Dexamethasone tablets, USP) for oral administration, are supplied in tow potencies, 0.5mg and 0.75mg. Inactive ingredients are calcium phosphate, lactose, magnesium stearate, and starch. Tablet DECARDON 0.5mg also contain D&C Yellow 10 and FD&C Yellow 6. tablets DECARDON 0.75mg also contain FD&C Blue 1. Dexamethasone which is sometime called as “Dex” is also known by the brand names Decadron®, Diodex®, Hexadrol®, hexadrol®, and maxidex®. It is one of the most frequently used medications in the treatment of multiple myeloma.

On 16 June 2020, the recovery trial announced preliminary result stating that Dexamethasone improves survival rate of COVID-19 patients. Dexamethasone decrease death by approximately one third in patient requiring ventilation and by one fifth in those requiring oxygen. The world health organization (WHO) states that Dexamethasone should be receiving COVID-19 treatment. In July 2020 the European Medicines Agency (EMA) started reviewing result from the RECOVERY study for COVID-19 patients.

Patents For Dexamethasone: [5] CN1065255842- Quick detection method of Dexamethasone-This invention for the technical filed of analytical chemical in particular to a quick detection of Dexamethasone. CN104749270A- This method for simultaneously detecting tow glucocorticoid

isometries in animal- the method separates two enantiomers of Dexamethasone by adopting a conventional chromatographic column and detect residual amount of Dexamethasone in animal derived food and confirm. A C18 chromatic column is using for liquid chromatogram.CN-104458647A- The method for detection of compound Dexamethasone acetate emulsifiable paste online by virtue of near infrared spectroscopy.

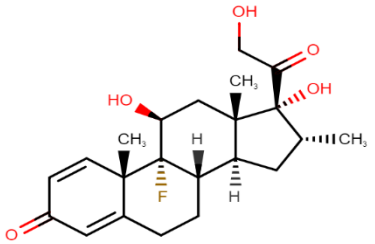
Dexamethasone use for Covid-19: [6, 7] The excessive inflammatory response is a cause of deterioration of coronavirus patients in hospital. Accordingly, it was considered was as a possible treatment for particularly sick COVID-19 particularly sick COVID-19 patients. March 2020, the Randomized Evaluation of COVID-19 therapy trial was established at Oxford University as a randomized clinical trial to test a range of potential treatment for COVID-19, including Dexamethasone. Proffecer Peter Horby, one of the chief investigators, commented that: "Dexamethasone is the first drug to be shown to improve survival in COVID-19. The survival benefit is clear and large in those COVID-19 patients who are sick enough to require oxygen treatment.

The severe acute respiratory syndrome corona virus 2 is a new and recognized infectious disease of the respiratory tract and its outbreak deemed a pandemic in March 2020. Estimates show around 5% of all patients develops ARDS. This Study indicates that inflammation and cytokine storm might be involved in the pathophysiological pathway to ARDS in these patients. The corticosteroids have been tested in deferent scenarios of ADRS, including pneumonia, and the early use of Dexamethasone is safe and appears to reduce the duration of mechanical ventilation in ADRS Patients.

Mechanism Of Action in Covid-19: [8]

The sickest patients with COVID-19 suffer a hyperinflammatory state – a cytokine storm-that has features in common with a rare hematological condition called hemophagocytic lymph histiocytosis. The Immune suppression should help to patients by contrast immune suppression during the early phase of the viral infection might allow increased viral replication and aggravate the disease. The 3C-like proteinase on severe acute respiratory syndrome coronavirus 2 inhibits HDAC2 transport into the nucleus and so impairs the way in which it mediates inflammation and cytokine responses, so activation of histone deacetylase by dexamethasone may directly oppose the action of severe acute respiratory syndrome coronavirus 2.

Table 1 DRUG PROFILE: [1, 9]

PROPERTIES	DETAIL
Product name	Dexamethasone sodium phosphate
CAS NO.	2392-39-4
Structure	
Chemical formula	$C_{22}H_{29}FO_5$
IUPAC Name	9α-fluoro-11β,17,21 -trihydroxy-16α-methylpregna-1,4-diene-3,20-dione
Molecular weight	392.5 g/mol
Characteristics	Dexamethasone is an ordourless white to off-white crystalline powder with a slightly bitter taste
Solubility	Soluble in DMSO ()25mg/ml), 100% ethanol(25/1.6ml) or methanol, and water
Melting point	262°C (504°F)
Log P	1.183/1.56
pKa (strongest acidic & basic)	1.18, -3.4

Analytical Methods:[10]

Analytical method could spectral, chromatographic, electrochemical, hyphenated or miscellaneous. Analytical method development is the process of selecting an accurate assay procedure to determine the process of selecting an accurate assay procedure to determine the composition of a formulation. The need of validation of the analytical method development and validation emerged due to international competition, maintaining the standard of products in high commercial and market value and ethical reasons.

Reported Analytical Methods:

UV-Methods: [11, 12]

1. Behera S.et al was developed estimation of Dexamethasone sodium phosphate (DEX) in bulk and pharmaceutical dosage form by UV-Spectrophotometric method. Method was developed by using UV-Visible

double beam spectrometer (Thermo scientific, evolution 201). Absorbance of DEX was taken at maximum wavelength 242nm by using distilled water as a solvent. The linearity range is 5-25µg/ml with correlation coefficient 0.999. The recovery range found to be 90.5 % - 98.99% and relative standard deviation for precision 0.748% (Intraday) and 0.801% (Inter day). Limit of detection found to be 2.30µg/ml and limit of quantitation was found to be 0.78µg/ml. The % assay was found to be 94.19% for pharmaceutical dosage form. [11]

2. UV- spectrophotometric method developed for determination of Dexamethasone assay in tablets. The maximum absorbance observed at 241nm by using methanol. The linearity range was 1.0-30.0µg/ml. Developed method was validated as per ICH guideline. [12]

3. A simple and rapid UV spectrophotometric method for Dexamethasone assay in tablet. Linear relationship was found between the absorbance at 241 nm and the concentration in the range of 1.0 to 30.0µg/ml by using methanol:water 1:2 v/v as a solvent. Correlation coefficient was 0.9998 indicating good linearity (r > 0.999). The linear equation was y = 0.0390x + 0.0019, calculated by the least square's method. The LOQ and LOD were found as 1.56µg/ml and 0.52µg/ml. The developed method was validated as per ICH guideline. [13]

High Performances Liquid Chromatography (HPLC): [14-23]

1. Katakam at al developed stability indicating HPLC method for simultaneous estimation of Dexamethasone sodium phosphate (DEX) in bulk and formulation. The method was carrying out by using isocratic mode. The chromatographic separation was done by using a C18 column (250 mm x4.6mm i.d, 5µ particle size) and shimadzu UV-visible detector (SPD-10AT VP). The mobile phase consisting of phosphate buffer (pH 6.8) and acetonitrile (70:30v/v) was used. Detection wavelength at 254nm and flow rate is 1ml/min. Retention time of dexamethasone sodium phosphate were 2.3min. The linearity of Dexamethasone was in the range of 1-6 µg/ml. Dexamethasone were subjected to forced degradation by acid (0.1 N HCL: 19.44), alkali (Base 0.1 NaoH: 23.17), chemical oxidation (3% H2O2:0) and heat (30.08). Developed method was economical in terms of the time and amount of solvent used for analysis. The method was validated as per ICH guideline and successfully applied to the simultaneous determination of DEX in bulk and pharmaceutical formulations. [14]

2. Determination of Dexamethasone (DEX) related substances on DEX coated drug-eluting stents by stability indicating HPLC method. The separation of DEX from its impurities and degradation products was achieved by using Zorbax Eclipse XDB C8 column using gradient elution and UV detection at 239nm. The method was determined to be linear in the range of 0.01-0.30µg/ml. The accuracy was assessed by spiking Dexamethasone acetate at three levels in range of 0.025-0.175µg/ml. The recovery range for Dexamethasone acetate 89.6 to 105.8%. The precision over the three levels and nine determinants of DEX with relative standard deviation values of 1.6-4.1% respectively. The method was found to be sensitive with a Limit of detection 0.008µg/ml and Limit of quantitation 0.025µg/ml. Finally, the method was robust, small variations of chromatographic conditions like pH, mobile phase organic/aqueous composition and column temperature. The Identification of unknown DEX degradation products in DEX -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 structures of four major DEX degradants. The developed method was validated according to ICH guideline. [15]

3. A simple, sensitive HPLC-UV method was developed and validated for the determination of Sparfloxacin(SFC) and Dexamethasone (DEX) in bulk and pharmaceutical formulations. The separation was achieved by using a C18 column (250 mmx4.6 mm i.d, 5µ particle size) by isocratic elution. The mobile phase consisting of a mixture of mixed phosphate buffer (pH 6.8) and acetonitrile (50:50, v/v) was used. The chromatogram was monitored at 224nm at a flow rate of 1ml/min. Retention times of SFC and DEX were 3.01 and 6.47min respectively. The linearity of SFC and DEX was in the range of 3-18µg/ml and 1-6µg/ml respectively. The developed method was economical because, the time taken, and amount of solvent used for each analysis was less. The method was validated and was applied to the simultaneous determination of both drugs in bulk and pharmaceutical formulations. [16]

4. Validate a HPLC method for the quantitative determination of Dexamethasone acetate (DEX) contained in cream preparation. The method was achieved by using shimadzu-10 AT VP instrument and chromosil C18 column

(250 mm×4.6 mm, 5µ particle size) column. Mobile phases contain phosphate buffer pH-4 and acetonitrile (ACN) in the ratio of 50:50v/v, Flow rate 1.0 ml/min and an UV detector (SPD 10 AT VP) set at 224nm were used to evaluate the parameters such as linearity, precision, accuracy, specificity, and LOD, LOQ. The calibration curve showed a good linearity in range of 1-6 µg/ml with correlation coefficient of 0.9999. The precision was demonstrated by RSD for Intraday: 0.49% and Inter day: 0.68%. The accuracy result in rage of 99.85-100.77%. The LOD and LOQ determined were 0.069µg/ml and 0.024µg/ml respectively. The specificity test showed no interference in the drug peak. [17]

5. Dexamethasone (DEX) in drug substance and drug product analyses and the assay of preservatives in drug product by validated, stability indicating HPLC method A new HPLC procedure for the determination of DEX, impurities, degradation products and product preservatives is described. Method was achieved by using HPLC instrument (series 1100 and waters alliance) and diode array UV detector. The column is G1316A column and mobile phase contain acetonitrile: water: phosphoric acid (900:100:0.5v/v/v) with flow rate 1.4ml/min**.** A three-stage, linear gradient with UV detection at 240 nm allows the analysis of DEX drug substance and DEX in two formulated products, using the same chromatographic system. The LOQ of DEX impurities in drug substance is 0.05%, and 0.1% for DEX degradation products in formulated products. The developed method is linear, precise, accurate and robust. The several degradation products of stressed DEX have been identified.[18]

6. Determination of Dexamethasone sodium phosphate in eye drops by HPLC and absorbance correction method**.**

METHOD: A HPLC method was carrying out by using RP-HPLC, Shimadzu, and Kyoto, Japan. The reversed phase c18 column (Grace Discovery and division 5 µm 25cm × 4.6 mm) and isocratic elution was used for development of method. The UV detector set at 244.2nm. Mobile phases consist of methanol -water- triethylamine (60:40:0.75v/v/v pH: 3.25 ± 0.05 with orthophosphoric acid) and flow rate is 0.8 ml/min. The calibration curve shows good linearity in range of 1-6 µg/ml with correlation coefficient 0.9998. Accuracy result is 101.50% with standard deviation ±1.64%. Precision result for Intraday: 0.51-1.37 % and Interday: 0.90-2.39 % LOD and LOQ was found to be 0.13 µg/ml and 0.40µg/ml respectively.

METHOD B**:** Absorbance correction method. Linearity in the range of 2-10 µg/ml and detection wavelength is 244.2nm. [19]

7. Estimation of DEX. in eye/ear drops by RP-HPLC method. The chromatographic separation was achieved on grace smart reversed-phase C18 column (Grace discovery and division 5 µm, 25cm ×4.6mm in the isocratic elution using methanol-water-triethylamine (55:45:0.6, v/v/v), pH adjusted to 3.0 ± 0.05 with orthophosphoric acid(OPA) as the mobile phase at a flow rate 0.8 ml/min. The SPD-10A UV -Visible detector used for detection and detection wavelength at 254 nm. In this method, quantification was achieved over the concentration range of 3-18 and 1-6µg/ml. Retention time is 9.688±0.049 min for DEX. Recovery results in range of 99-101.53%. The proposed developed methods were successfully applied for the analysis of synthetic mixtures and pharmaceutical formulations of DEX without any interference of excipients. [20]

8. Simple, precise, accurate and reproducible UV-spectrophotometric and stability indicating RP-HPLC methods for simultaneous estimation of Gemifloxacin (GAT) and Dexamethasone (DEX) in ophthalmic dosage form.

Method A: Dual wavelength spectrophotometric method. The measurement of maximum absorbances at 279nm and 239nm, which are the λmax of GAT and DEX respectively. Linearity range was found to be 6-18µg/ml and 2-6µg/ml for GAT and DEX, respectively. The precision was demonstrated by RSD for Intraday: 0.33%and Inter day: 0.26%. LOD and LOQ for Dexamethasone were obtained 0.169µg/ml and 0.512µg/ml respectively.

Method B: The reverse phase high performance liquid chromatography method is carried out on Shiseido C18 column (250 mm × 4.6 mm I. D.), using 0.1% orthophosphoric acid (OPA) in water and ACN in the ratio of (50:50 v/v) as the mobile phase with a flow rate of 1.0 ml/min. The UV-detector (LC-20AD) was used, and detection wavelength is 241 nm. Retention times were found to be 2.124 ± 0.5min and 4.578 ± 0.5 min for Gatifloxacin and Dexamethasone respectively. The linearity range was found to be 15-75µg/ml and 5-25µg/ml for GAT and DEX respectively. The recoveries of both the drugs GAT and DEX from the ophthalmic form were 99.46% and 98.71%, respectively by UV method and 99.65% and 99.16%, respectively by HPLC method. The correlation

coefficients of both the drugs were found to be more than 0.999 by two methods indicate that good linearity. Other parameters such as ruggedness, robustness was well within the acceptance criteria. The UV-spectrophotometric and stability indicating RP-HPLC methods were found to be accurate, rapid, precise, and simple. These developed methods can be used for the simultaneous estimation of GAT and DEX in bulk and in ophthalmic dosage form. [21]

9. Xiong, Yuan & Xiao et al developed and validated stability-indicating RP HPLC method to separate low levels of Dexamethasone (DEX).and Betamethasone (BM). BM is an API or an intermediate which is used to manufacture various finished pharmaceutical products. BM is also used as a starting material to manufacture other active pharmaceutical ingredients that are related to this steroid family. It is quite difficult to separate DEX peak (the alpha epimer) and other structurally related compounds from Betamethasone. A stability-indicating RP-HPLC method has been developed which can separate and accurately quantitate low levels of Dexamethasone and other related compounds from Betamethasone and also from each other. A gradient mobile phase system consisting of (A) water:acetonitrile (90:10, v/v) and (B) acetonitrile:isopropanol (80:20, v/v) was used. Method was achieved by using ACE Phenyl column (10 cm × 4.6 mm, 3µm particles, 100 Å pore size) and UV-detector (LC-20AD) for detection at 240 nm. This developed method was successfully validated for the purpose of conducting stability studies of BM in QC laboratories. The stability-indicating capability of this method was demonstrated by adequate separation of DEX and all the degradation product peaks from BM peak and also from each other in aged stability samples of Betamethasone. [22]

10. Simultaneous determination of Dexamethasone (DEX) drug in water sample by UHPLC. The method was achieved by using C18 monolithic column (50 × 2 mm) hypersil GOLD™ column (50 × 2,1mm, 1.9 µm) and acetonitrile: 0.05% TFA in water,0.1%acetic acid, 0.5% acetic acid or 0.1 % formic acid(50:50v/v) as mobile phase with flow rate: 2.0 ml/min. The UV detection set at 241nm.Retention time at 1.681min for DEX. The Linearity rang 0.25-40µg/ml with Correlation coefficient 0.9981 indicate that good linearity. LOD and LOQ were obtained 0.09µg/ml and 0.26µg/ml respectively. [23]

LIQUID CHROMATOGRAPHY/MASS SPECTROSCOPY (LC/MS): [24- 32]

1. Separation and estimation of Dexamethasone sodium phosphate (DexP) in cochlear perilymph fluid (CPF) by liquid chromatography with ultraviolet monitoring and electrospray ionization mass spectrometry characterization. The method for separation and determination of DexP in CPF of cavy was developed using HPLC with UV monitoring and ESI/MS identification. The quantitative determination achieved by HPLC with UV detection at 245 nm. The separation was carried out by using a Phenomenex ODS (3) column (250 mm x 4.6 mm i.d., 5 microm) with the mobile phase of acetonitrile-5mmol/l ammonium acetate (23:77 (v/v)) with flow rate of 1.0 ml/min. The linearity ranged from 0.5- 50µg/ml with correlation coefficient: 0.9991 indicate that good linearity. The LOD was 0.10µg/ml. The accuracy ranged from 98.5 - 100.8% and R.S.D.s of intra- and inter-day peak area were between 0.7-1.3 and 1.2-3.5%, respectively. Both full scan MS and MS2 of Dexamethasone sodium phosphate with positive and negative polarity were obtained such as Precursor ion: m/z 471, Specification ion: [M+H-2Na] at m/z 471 [M+H-2Na-CHO]-m/z 441 and elucidated. The specific ions were chosen to characterize Dexamethasone sodium phosphate in the CPF sample. Using the proposed HPLC with UV monitoring and ESI/MS method, the concentration of Dexamethasone sodium phosphate in cochlear perilymph fluid samples after both vein and middle ear injections were determined, and the relationships between concentration and time were obtained. This method offered reference data for clinical investigation of Dexamethasone sodium phosphate to cure ear diseases. [24]

2. The determination of Dexamethasone was performed by LC (Ultimate 3000 Thermo Scientific, Waltham, MA, USA) coupled to a quadrupole-orbitrap hybrid mass analyzer (Q-Exactive, Thermo Scientific) for urine and serum samples. The chromatographic separation was achieved by using a Kinetex XB - C18 analytical column (100 × 3.0 mm, 2.6 µm, Phenomenex, Torrance, CA, USA) connected to a guard cartridge (Gemini C18, 4 × 2.0 mm, Phenomenex). The mobile phase contains (A) water with 0.1% acetic acid and (B) ACN with 0.1% acetic acid with gradient elution. Flow rate and the injection volume were 0.4 ml/min and 10 µl respectively. Acquisition was carried out in positive ionization mode (ESI +). Precursor ion ([M + H] ±) was m/z 393.2066 and the fragment ions m/z 373.2000 and m/z 355.1896. Both procedures were successfully validated according to ICH guideline. [25]

3. Chiesa, Luca & Nobile et al developed LC -MS/MS method for suitability of bovine bile compared to urine for detection of Dexamethasone (DEX). The method was carried out by using reverse-phase LC column synergy hydro RP 150 2mm, 4lm with C18 43mm guard column and mobile phase A:0.1% aqueous formic acid, B: methanol. The flow rate is 2ml/min and gradient elution. Precursor ion [MH] OR [MH] + m/z: 437 and Product ions CE m/z: 307_{33}, 361_{20}, 391_{14}.[26]

4. Quantitative measurement of Dexamethasone (DEX) in rabbit ocular matrices by liquid chromatography tandem mass spectrometry (LC-MS/MS) The method was developed by using reversed phase C18 Gemini column (50mm ×4.6mm) and 30% acetonitrile in water containing 0.1% of formic acid as a mobile phase. Flow rate 0.2 ml/min and elution mode is isocratic **.** The linearity in range of 2.7-617.6 ng/ml with correlation coefficient 0.9989 shows that good linearity. Retention time of DEX is 4.27min and m/z is 393.20 →355.30. The developed method was validated with acceptance criteria. [27]

5. Patel, Parul & Tanna et al developed and validated LC-MS method for the determination of Dexamethasone (DEX) in dried blood spot (DBS) samples. The preparation of DBS samples whole blood spiked with analyte was used to produce 30µl blood spots on specimen collection cards. An 8mm disc was cut from the dried blood spot sample and extracted with combination of methanol: water (70:30, v/v) containing the internal standard, triamcinolone acetonide. The extracts were centrifuged, and chromatographic separation was carried out by using a Zorbax Eclipse Plus C18 column using gradient elution with acetonitrile and water with formic acid as a mobile phase at a flow rate of 0.2ml/min. The LC-MS detection was conducted with single ion monitoring using target ions at m/z 393.1 for DEX and 435.1 for the internal standard. Method was linear within calibration range of 15-800ng/ml. The recovery of DEX was 99.3% (94.3-105.7%). [28]

6. Determination of Dexamethasone (DEX) urinary excretion profile in cattle by LC-MS/MS. The method was achieved by using Agilent 110 series liquid chromatography instrument. For estimation of DEX 4u fusion (150 x 2.0 µm, Phenomenex, Torrance, CA 4000 triple quadrupole mass spectrometers with mobile phase water and acetonitrile at Flow rate**:**200 µl/min and gradient elution system were used. Retention time for DEX is 23.11 min. Linearity in range 0.5ng/ml -20ng/ml with Correlation coefficient 0.9966. LC-MS detection was conducted with single ion monitoring using target ions at m/z 393.3. [29]

7. A highly sensitive LC-MS/MS method Development and validation for the determination of Dexamethasone (DEX) in nude mice plasma and its application to a pharmacokinetic study. The method used isocratic reverse phase separation over a Dionex C18 column with a mobile phase composed of acetonitrile–water (40:60,v/v) and analyte was detected by a triple quadrupole tandem mass spectrometer via electrospray. The multiple reaction monitoring was employed to select both DEX at m/z 393.0/147.1 and testosterone at m/z 289.5/97.3 in the positive ion mode. The calibration curves were linear correlation coefficient 0.999 ranging from 2.5- 500ng/ml. The LOQ of DEX obtained 2.5ng/ml. This developed method was successfully applied to a preclinical pharmacokinetic study of DEX and its pharmacokinetics was characterized by a two-compartment model with first-order absorption in female nude mice. [30]

8. Estimation of Dexamethasone (DEX) and Dexamethasone sodium phosphate (DEXP) in human plasma and cochlear perilymph by LC-MS/MS. Method was obtained by using LC-MS/MS (Shimadzu corporation, Kyoto, Japan) and C18 4mm × 2mm internal diameter guard column. The mobile phase A**:**5Mm ammonium acetate, and Mobile phase B: methanol at flow rate 0.3 ml/min with gradient elution. Retention time of DEX is 2.79min .Linearity in range of 0.5-500g/l with correlation coefficient 0.99. LOQ of DEX obtained 0.5g/l. m/z 393→373 for DEX in the positive ion mode. [31]

9. Simultaneous estimation of Dexamethasone (DEX) and Lenalidomide (LDM) in rat plasma by solid phase extraction and UPLC-MS/MS. The sample preparation was performed using solid phase extraction methodology (SPE) followed by chromatographic analysis on a C 18 column (100 × 1.0 mm, i.d., 1.7 µm particle size) and a mobile phase composed of 0.1% formic acid in water:0.1% formic acid in acetonitrile (20:80, v/v) at the flow rate of 0.2 ml/min. The positive ionization mode using multiple reactions monitoring was applied to detect the transitions of DEX at m/z 393 > 147, LDM at m/z 260 > 149.The concentration range of 0.01-5 ng/ml with a LOD obtained 0.01ng/ml for both LND and DEX indicate that method was highly sensitive. The recovery range of analytes from plasma samples ranged from 86-106%. The intra-day and inter-day precision was evaluated, and RSD values were within the acceptance values (<15%). Applicability of the developed

method was extended to the determination of the pharmacokinetics of both LDM and DEX, following their oral administration to rats, either alone or in combination, suggesting the applicability of the method in further clinical studies. [32]

High Performance Thin Layer Chromatography (HPTLC):

1. Simultaneous determination of Dexamethasone sodium phosphate (DEX) in eye drop by HPTLC method**.** The method was obtained by using aluminum-backed layer of silica gel 60F254 as stationary phase and mobile phase acetonitrile: water: ammonia (10:3:0.5v/v/v). Detection wavelength is 262nm.Linearity in range of 80-300ng with correlation coefficient 0.9995 shows that good linearity. Recovery for DEX this method was found in the range 98.86-101.05%. Precision was evaluated and RSD values were within the acceptance values 0.05. Rf value obtained for DEX 0.38±0.02. [33]

2. Bonthu, Mohan et.al Developed and validated HPTLC Method for the Simultaneous Estimation of Moxifloxacin and Dexamethasone in Bulk and Ophthalmic Dosage. The separation was achieved on HPTLC aluminum plates using acetonitrile: water: ammonia (8:1:0.5 v/v/v) as mobile phase and developed plates were detected at 266 nm. Both drugs were resolved satisfactorily with Rf values of 0.09±0.01 and 0.74±0.01 for MOX and DEX, respectively. [34]

Gas Chromatography Mass Spectrometry (GC/MS): [35-39]

1**.** Determination of Dexamethasone (DEX) in mixture by using GC-MS The Hew lettpackard model 5989A MS engine and 5890 series ‖ gas chromatographs use for method. For separation Hewlett-Packard HP-5 MS fused-silica capillary use as stationary phase and helium as a carrier gas at flow rate 0.8 ml/min. The Pressure and temperature were maintained at 133.3pa and 1508 °C. Ionization Current and voltage were 300mA, 230eV respectively. The most intense ion at m/z 310 [M-HF], while the second at m/z 330. [35]

2. Estimation of Dexamethasone (DEX) in urine by gas chromatography with negative chemical ionization mass spectrometry (GC/M). The GC/MS method was performed on gas chromatography (5890A) with mass spectrometer (HP 5988A) in NCI mode (Hewlett-Packard). The analysis achieved by using SPB-5 column use as stationary phase and helium as a carrier gas at flow rate 1ml/min. The quantification was carried out by relating the ratio between the abundance of m/z 310 (most abundant fragment of DEX). [36]

3. Kasuya, Yasuji & Althaus et al developed GC/MS method for quantitative determination of Dexamethasone (DEX) in human plasma. DEX was isolated from human plasma using a CIS-bonded reverse-phase cartridge, purified by subsequent normal-phase High performance liquid chromatography, and the corresponding trimethylsilyl derivative analysed by GC-MS. Method was perform on glass column (60.96 2mm) packed with 3% sp-2100s and Methane was used as the GC carrier gas and as the chemical-ionization reagent gas at flow rate 5ml/min. Quantitation by isotope-dilution MS was carried out by selected-ion monitoring on the (M t I)+ ion of the trimethylsilyl derivative of DEX and its stable isotopically labelled diluent, ['3Cs,2H3] DEX (681 and 690 m/z, respectively). [37]

4. Estimation of plasma Dexamethasone (DEX) by chemical oxidation and electro capture negative ionization mass spectrometry (GC/EI/MS). GC/EI/MS analyses were performed on a Hewlett Packard model 5970 mass-selective detector and a model 5890 gas chromatograph. The DB-1 fused silica capillary column (15 m x 0.25 mm x 0.1 pm film thickness) from J&W Scientific, and helium was used as the carrier gas for the analyses. The peak height ratio of m/z 310 to m/z 319 of the first epimer was used to calculate the DEX concentrations of the ten DST plasma samples that were analysed by this method. [38]

5. Estimation of Dexamethasone (DEX) in human plasma and urine by electron -impact mass spectrometry (GC-MS-SIM). The GC-MS-SIM instruments were made with a Shimadzu QPlOOO gas chromatograph-mass spectrometer system equipped with a data processing system. GC-MS method was performed on a glass column (58 cm X 3.0 mm I.D.) packed with about a 20-cm length of 1.5% SP-2100 on Supelcoport (W-100 mesh) and helium was used as the carrier gas at a flowrate 40 ml/min. Column temperature was 268°C, the injector temperature 275°C and the ion source temperature 280°C. Electron energy was set at 20eV. Multiple-ion detector was focused on the molecular ions at m/z 680 for the TMS derivative of non-labelled DEX and at m/z 689 for the TMS tetra (trimethylsilyl) derivative of DEX. [39]

Capillary Chromatography: [40-44]

1. Estimation of Dexamethasone (DEX) in tears by capillary electrophoresis (capillary zone electrophoresis) (CZE) $HP^{3D}CE$ system. Method was carried out on a HP CE system (Hewlett-Packard, Wilmington, DE, USA). System consisted

of a capillary electrophoresis unit equipped with a diode array detector (DAD) and sodium tetraborate buffer used for separation pH was 9.2. The electrophoresis was performed at constant voltage of 25 kV (388 V cm). The temperature maintained at 258°C and detection wavelength at 242nm. Dex. with migration times of 12.26 min and 15.46 min, respectively. LOD and LOQ were obtained 0.5 mg/ml (signal to noise ratio 3:1) and 2mg/ ml (signal to noise ratio 10:1). Linearity of the response was demonstrated from 2 to 100 mg ml with correlation coefficient 0.9997. [40]

2. The estimation of Dexamethasone (DEX) by micellar electro kinetic capillary chromatography. The capillary electrophoresis was performed with a Beckman (Fullerton, CA, USA) P/ACE 5510 system equipped with a diode-array detector and controlled by a Dell Dimension P133 V computer running P/ACE Station software. The compounds were separated in a 57 cm (50 cm to the detector) × 75 µm i.d. fused silica capillary and optimized solvent (10 mmol/l borate-phosphate buffer adjusted to pH 8. The separations were performed at 30 kV for 10 min at 25 °C. The linearity in range of 2.8-127.8mg/ml with correlation coefficient 0.9983. Migration time is 4.27min and. detection wavelength is 242nm. LOD and LOQ were obtained 0.71 mg/l and 2.36mg/l respectively. [41]

3. The estimation of Dexamethasone (DEX) in pharmaceutical formulation by capillary electrophoresis. Method was carried out by using fused silica capillary 645 (560) × 0.05mm with extended light path (0.15mm) and 20ml ammonium acetate in methanol with 1%acetic acid as a solvent. The detection wavelength at 242nm. The separation was performed at 250 °C temperature and voltage +30kV 16pA. The LOD was obtained 1.6 µg/ml and correlation coefficient 0.995 for dexamethasone. [42]

4. A simple and reliable MEKC method has been presented for the simultaneous estimation of Betamethasone (BTS) and its epimer Dexamethasone (DEX) in human urine and serum. The Rapid and baseline separation of BTS and DEX was obtained by using unfused-silica capillary with an effective length of 30cm and within 7 min with the optimum conditions of 30mM borax buffer, 30mM sodium dodecyl sulphate at pH 10, separation voltage at 18 kV, injection time 15 s at a height of 10 cm, using sodium sorbate as internal standard. Good relationship between peak area ratio and analyte concentration was linear over 30–1,000 µg/ml for BTS and DEX with correlation coefficients 0.9993. The RSD of the method were all less than 4.50% in the intra-day and inter-day analysis. The recoveries in the range of 97.5–100.5%. The developed method was validated with respect to stability, precision, linearity and accuracy. [43]

5. The study of multiple binding constant of Dexamethasone (DEX) with human serum albumin by capillary electrophoresis frontal analysis and multivariate regression (AP/ACE system 5010). The method was achieved by using fused-silica capillaries and 1 M NaOH, deionized water and 67 mM phosphate buffer (pH7) as a solvent. The separation was performed at UV detection 214nm and 8kV voltage. The linearity in range of 50-800 µmol/l with correlation coefficient 0.9919. [44]

X-Ray Diffraction Technique (XRD): [45-48]

1. Removal of the hormones Dexamethasone (DEX) by Ag doped on TIO2 photolysis XRD. The XRD patterns of the photocatalysts were recorded using a Shimadzu-600 X-ray diffractometer with Cu K radiation over the range of 2 angles from 20 to 80.In this method morphology of the nanoparticles were analysed by scanning electron microscope (SEM, Hitachi- SU3500) and to determine elemental composition of samples using Energy Dispersion X-ray Spectroscopy (Tescan, Czech).The scanning range is 20°– 80°.The modified samples represented a new peak at 44.4°showing the good dispersion of Ag within the crystal matrix of TiO2. [45]

2. XRD method developed for determination of Dexamethasone (DEX) into biodegradable polymeric nanoparticles. XRD patterns were measured on a Siemens D5000 diffractometer in Bragg–Brentano geometry using Ni-filtered Cu K radiation. The data were collected over an angular range comprised between 2 and 55◦ (2θ) with a step size of 0.1◦ and a counting time comprised between 150 and 500 s per step. [46]

3. The X-ray Spectroscopy developed for determination of pharmaceutical compatibility of Dexamethasone (DEX) with excipients commonly used in solid oral dosage form. The X-ray Spectroscopy patterns were recorded by using a XRD 6000 diffractometer (Shimadzu), radiation source of Cu Kα. The scanned range was 5°–45° (2θ) at a digitalization speed of 2° min−1. This equipment was maintained at voltage of 40.0 kV and current of 30.0 mA. The Dexamethasone diffractogram showed two intense peaks at 14.24 and 16.92° 2θ and secondary peaks at 7.56°, 10.72°, 12.60°, 13.72°, 15.18°, 15.70°, 17.80°, 18.58°, 22.84°, 23.56°, 26.78°, 28.02. [47]

4. X-ray diffractometer method developed for determination of ocular permeation and sustained anti-inflammatory activity of Dexamethasone (DEX) from kaolin nano dispersion hydrogel system .X-ray diffractometer patterns of pure drug, kaolin, and formulations were obtained by using X-ray diffractometer (D8 Discover, Bruker). The detector with an anode voltage of 40 kv and current of 15 mA was employed to detect the data of scattered radiation. X-ray source was anode material Cu, κ-Alpha (radiation 1.5406 Å). The measurements were carried out from the scanning angle ranging from 5 to 70° 2θ at a scan speed of 1° min^{-1}. [48]

Differential Scanning Calorimetry (DSC): [49-51]

1. The physicochemical properties and dissolution studies of Dexamethasone (DEX) acetate-β-cyclodextrin inclusion complexes produced by DSC. The differential scanning calorimetry curves were obtained on a Shimadzu DSC-60 cell using aluminium crucibles with about 2mg of samples, under dynamic nitrogen flow rate 50ml/min and heating rate of 10°C min−1 under a temperature range from 25 to 550 °C. The differential scanning calorimetry cell was calibrated with indium (mp 156.6 °C; ΔHfus=28.54 J g−1), and zinc (mp 419.6 °C). Differential scanning calorimetry curve of DEX was typical of a crystalline anhydrous substance with a sharp melting endotherm (Tonset=227.35 °C and ΔH fusion=129.1 mJ). [49]

2. Detection of polymorphism in pharmaceutical anhydrous Dexamethasone (DEX) acetate by DSC. The differential scanning calorimetry curves were obtained on TA instruments Q-series TM Q1000 DSC. The heating rate 10-150 °C/min, under nitrogen flow rate 50 ml/min. Differential scanning calorimetry curve of DEX was typical of a crystalline anhydrous substance with a sharp melting endotherm at 210 °C. [50]

3. Ocular permeation and sustained anti-inflammatory activity of DEX from kaolin nano dispersion hydrogel system by differential scanning calorimetry. The formulations were analysed by using aluminium crucibles (40 µL) on a differential scanning calorimeter (Mettler Toledo DSC 1, Switzerland). The differential scanning calorimetry runs were conducted under dynamic nitrogen atmosphere 50ml/min and heating rate of 10°C per min under a temperature range from 30 to 280°C. Indium (mp 156.6°C; ΔH fus = 28.54 J g−1) was used for calibration of the DSC cell. Differential scanning calorimetry curve of DEX was sharp melting point at 229.50 °C. [51]

CONCLUSION

In this study, the predominance of chromatography methods for the analysis of Dexamethasone from bulk, pharmaceutical dosage form is evident for both as well as for biological fluid. UV detectors are most commonly used, through the detection wavelength can vary for the Dexamethasone. Methods such as spectrophotometry are also used but given advantages of chromatography methods such as better selectivity and sensitivity, they have become the choice for analysis of this drug. This is because the pharmaceutical matrix can be a simple or a complex solution, such as cream, ointments, and tablets as well as the DEX that are found in very small concentration in the formulations (in the range of micrograms), therefore in these cases, the application of a more sensitive method makes it more useful. There are well-defined validated methods that meet the needs for identification and quantification of corticosteroids, as well as those applicable to the quality control analysis routines of pharmaceutical dosage forms, bulk and biological fluids.

REFERENCES

1. Drug bank dexamethasone (https://go.drugbank.com/drugs/DB01234). Accessed on 7 June 2020.
2. wikipedia dexamethasone (https://en.wikipedia.org/wiki/Dexamethasone). Accessed on 7 June 2020
3. CDSCO (https://cdscoonline.gov.in/CDSCO/Drugs). Accessed on 7 June 2020.
4. Dailymed(https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=f8d52954-be1d-45d3-9245-ff2e3e81a8ac&type=display). Accessed on 8 June 2020.
5. PATENT : CN1065255842- Quick detection method of dexamethasone, CN104749270A-Method for simultaneously detecting tow glucocorticoid isometries in animal, CN104458647A-Method for detecting compound dexamethasone acetate emulsifiable paste online by virtue of near infrared spectroscopy
6. DEXAMETHASONE:the first lifesaving drug for very sick Covid-19 patient (https://www.rms.com/blog/2020/07/06/dexamethasone-the-first-life-saving-drug-for-very-sick-covid-19-patients) . Accessed on 8 June 2020.

7. Clinical trial (https://clinicaltrials.gov/ct2/show/NCT04327401). Accessed on 8 June 2020.
8. CEBM: DEXAMETHASON (https://www.cebm.net/covid-19/dexamethasone/). Accessed on 8 June 2020.
9. Pub chem. (https://pubchem.ncbi.nlm.nih.gov/compound/Dexamethasone#section=Fire-Fighting). Accessed on 8 June.
10. Chauhan A. Analytical Method Development and Validation: A Concise Review, Anal Bioanal Tech, 2015; 233(6):155-172.
11. Behera S. UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation, J Anal Bioanal Techniques, 2012; 151(3):417-498.
12. Shaik, Abdul. Development and validation of UV spectrophotometric method for the estimation of Dexamethasone sodium phosphate in bulk and Pharmaceutical dosage form.Indo .American Journal of Pharmaceutical Research,2013; 122(4):145-156.
13. Friedrich, Rossana Barcellos.Validation of a simple and rapid UV spectrophotometric method for dexamethasone assay in tablet, Química Nova, 2009; 32(4):1052-1054.
14. Katakam.Stability indicating HPLC method for simultaneous determination of dexamethasone sodium phosphate and chloramphenicol in bulk and formulations, International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 67(4):505-510.
15. Chen, Quan & Zielinski. A validated stability-indicating HPLC method for the determination of dexamethasone related substances on dexamethasone-coated drug-eluting stents, Journal of pharmaceutical and biomedical analysis, 2008; 48(2):732-738.
16. Prakash, Katakam&Sireesha, Karanam. HPLC-UV method for simultaneous determination of sparfloxacin and dexamethasone sodium phosphate in eye drops, Pakistan journal of pharmaceutical sciences, 2019; 32(4):1057-1061.
17. Garcia, C &Breier. Determination of dexamethasone acetate in cream by HPLC, Journal of pharmaceutical and biomedical analysis, 2003; 31(2):597-600.
18. Spangler, M. &Mularz. 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, 2001; 54(4):329-334.
19. Suhagia, Bhanubhai&M.J.Dabhi. Simultaneous Determination of Moxifloxacin Hydrochloride and Dexamethasone Sodium Phosphate in eye Drops by HPLC and Absorbance Correction Method, journal of chemistry and pharmaceutical research, 2012; 34(5):4462-4467.
20. Suhagia, Bhanubhai&H.Desai, Urvish& Patwari. RP-HP LCMethod for Simultaneous Estimation of Ciprofloxacin and Dexamethasone in Eye/Ear Drops, International Journal of Pharmaceutical Science and Drug research, 2013; 47(5):62-66.
21. Bonthu, Mohan &Atmakuri. Validated spectrophotometric and stability indicating RP-HPLC methods for the simultaneous estimation of gatifloxacin and dexamethasone in ophthalmic dosage form, International journal of pharmaceutical scince and Research, 2016; 14(2):617-634.
22. Xiong, Yuan & Xiao. Development and validation of a stability-indicating RP-HPLC method to separate low levels of dexamethasone and other related compounds from betamethasone, Journal of Pharmaceutical and Biomedical Analysis, 2009; 49(3):646-654.
23. Baranowska, Irena & Kowalski, Bartosz. The Development of SPE Procedures and an UHPLC Method for the Simultaneous Determination of Ten Drugs in Water Samples. Water, Air, & Soil Pollution, 2010; 211(6):417-425.
24. Liu, Hongxia& Chen. Separation and determination of dexamethasone sodium phosphate in cochlear perilymph fluid by liquid chromatography with ultraviolet monitoring and electrospray ionization mass spectrometry characterization,Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 2004; 805(2):255-260
25. Sebastianelli, Martina & Forte. LC-MS/MS analyses of bile and histological analyses of thymus as diagnostic tools to detect low dose dexamethasone illicit treatment in beef cattle at slaughterhouse, Steroids, 2020; 160(3):108671-108675.

26. Chiesa, Luca & Nobile. Suitability of bovine bile compared to urine for detection of free, sulfate andglucuronateboldenone,androstadienedione, cortisol, cortisone, prednisolone, prednisone and dexamethasone by LC–MS/MS, Food Chemistry, 2015; 188(4):131-134.

27. Earla, Ravinder &Boddu. Development and validation of a fast and sensitive bioanalytical method for the quantitative determination of glucocorticoids-Quantitative measurement of dexamethasone in rabbit ocular matrices by liquid chromatography tandem mass spectrometry, Journal of pharmaceutical and biomedical analysis, 2010; 52(1):525-533

28. Patel, Parul&Tanna. Dexamethasone quantification in dried blood spot samples using LC-MS: The potential for application to neonatal pharmacokinetic studies. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences. 2010, 878(9):3277-3282.

29. Vincenti, Marco &Girolami. Study of Dexamethasone Urinary Excretion Profile in Cattle by LC-MS/MS: Comparison between Therapeutic and Growth-Promoting Administration, Journal of agricultural and food chemistry, 2009; 57(3):1299-1306.

30. Yuan, Yin & Zhou. Development and validation of a highly sensitive LC-MS/MS method for the determination of dexamethasone in nude mice plasma and its application to a pharmacokinetic study, Biomedical Chromatography, 2014; 29(3):3311-3316.

31. Zhang, Mei & Moore. Determination of dexamethasone and dexamethasone sodium phosphate in human plasma and cochlear perilymph by liquid chromatography/tandem mass spectrometry, Journal of chromatography, 2011; 879(9):17-24.

32. Maher, Hadir&Alzoman. Simultaneous determination of dexamethasone and lenalidomide in rat plasma by solid phase extraction and ultra-performance liquid chromatography-tandem mass spectrometry: Application to pharmacokinetic studies. RSC Adv, 2015; 65(5):1039-1056.

33. Seid, Y. &Hymete, Ariaya&Bekhit, Adnan. Application of a stability-indicating HPTLC method for simultaneous determination of chloramphenicol and dexamethasone sodium phosphate in eye drop,Thai Journal of Pharmaceutical Sciences, 2012; 36(2):94-107.

34. Abdelwahab, Nada & Ali. Simultaneous Determination of Thalidomide and Dexamethasone in Rat Plasma by Validated HPLC and HPTLC With Pharmacokinetic Study, Journal of chromatographic science, 2018; 57(3):1093-1094.

35. de wasch, Katia & De Brabander. Differentiation between dexamethasone and betamethasone in a mixture using multiple mass spectrometry, Journal of chromatography, 2001; 926(7):79-86.

36. Huetos, Olga & López. Determination of dexamethasone in urine by gas chromatography with negative chemical ionization mass spectrometry, Journal of chromatography, 2003; 788(3):137-146.

37. Kasuya, Yasuji & Althaus. Quantitative determination of dexamethasone in human plasma by stable isotope dilution mass spectrometry, Journal of pharmaceutical sciences, 1984; 73(2):446-51.

38. Kayganich, Kathleen & Watson. Determination of plasma dexamethasone by chemical oxidation and electron capture negative ionization mass spectrometry. Biomedical & environmental mass spectrometry, 1990; 19(3):341-347.

39. Minagawa, Kazuo &Kasuya. Determination of dexamethasone in human plasma and urine by electron-impact mass spectrometry, Journal of chromatography, 1985; 343(7):231-237.

40. Baeyens, Vincent &Varesio. Determination of dexamethasone in tears by capillary electrophoresis, Journal of chromatography, 1997, 692(3):222-6.

41. Berzas, J. &Castañeda, G. & Pinilla, M. Micellar Electrokinetic Capillary Chromatography as an Alternative Method for the Determination of Estrogens. Analytical Letters - ANAL LETT, 1999; 32(3), 2453-2469.

42. Raith, Klaus &Althoff. Two examples of rapid and simple drug analysis in pharmaceutical formulations using capillary electrophoresis: Naphazoline, dexamethasone and benzalkonium in nose drops and nystatin in an oily suspension, Electrophoresis, 1998; 19(3):2907-2911.

43. Song, Lihua& Bai, Jianguo& Zhou, Weihong. Determination of Betamethasone and Dexamethasone in Human Urine3 and Serum by MEKC

After an Experimental Design, Chromatographia, 2008; 68(5):287-293.

44. Zhao, Peng & Zhu, Guijie& Zhang. Study of multiple binding constants of dexamethasone with human serum albumin by capillary electrophoresis–frontal analysis and multivariate regression, Analytical and bioanalytical chemistry, 2008; 393(5):257-261.

45. Pazoki, Maryam &Parsa, Mehran &Farhadpour, Reza. Removal of the hormones dexamethasone (DXM) by Ag doped on TiO 2 photocatalysis, Journal of Environmental Chemical Engineering, 2016; 34(4):1016-1034.

46. Gómez-Gaete, Carolina &Tsapis, Nicolas &Besnard. Encapsulation of dexamethasone into biodegradable polymeric nanoparticles, International journal of pharmaceutics, 2007; 331(11):153-159.

47. Santos, Widson&Nóbrega. Pharmaceutical compatibility of dexamethasone with excipients commonly used in solid oral dosage forms, Journal of Thermal Analysis and Calorimetry, 2020; 10(2):1007-1009.

48. Pramanik, Arunima & Sahoo, Rudra & Nanda. Ocular Permeation and Sustained Anti-inflammatory Activity of Dexamethasone from Kaolin Nanodispersion Hydrogel System. Current Eye Research, 2018; 43(3):1-11.

49. Doile, Mayara& Fortunato. Physicochemical Properties and Dissolution Studies of Dexamethasone Acetate-β-Cyclodextrin Inclusion Complexes Produced by Different Methods, AAPS PharmSciTech, 2008; 22(9):314-321.

50. Lawandy, S.N. & Hepburn, C. DSC Detection of radiation damage in thermoplastic polyurethane, flastomerics, 1980; 112(2):42-45.

51. Pramanik, Arunima & Sahoo. Ocular Permeation and Sustained Anti-inflammatory Activity of Dexamethasone from Kaolin Nanodispersion Hydrogel System. Current Eye Research. 2018; 43(4):1-11.