EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.ejpmr.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DEXAMETHASONE: REVIEW

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ABSTRACT

Analytical method development and validation are the continuous and interdependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product specific acceptance criteria and stability of results. Validations demonstrate that the analytical procedure is suitable for its intended purpose. Literature survey reveals that the analytical methods based on UV spectrometry, RP-HPLC & HPTLC for the determination of dexamethasone individually and in combination with other drugs. The methods were validated according to ICH guideline in terms of accuracy, precision, robustness, and other aspects of analytical validation. The developed methods are simple, sensitive and reproducible and can be used for the routine analysis of dexamethasone in bulk and Tablet dosage form.

KEYWORDS: Dexamethasone, Literature Survey, Method Development, Validation, ICH Guidelines.

INTRODUCTION

Dexamethasone is a type of corticosteroid medication. It is used in the treatment of much condition, including rheumatic problem, a number of skin disease, severe allergies, asthma, chronic obstructive, lung disease, brain swelling, and along with antibiotics in tuberculosis. In adrenocortical insufficiency, it should be used together with a medication that has greater mineralocorticoid effects such as fludrocortisones. In preterm labour, it may used to improve outcomes in the baby. It may be taken by mouth, as an injection into a muscle, or intravenously. The effect of dexamethasone are frequently seen within a day and last for about three day.
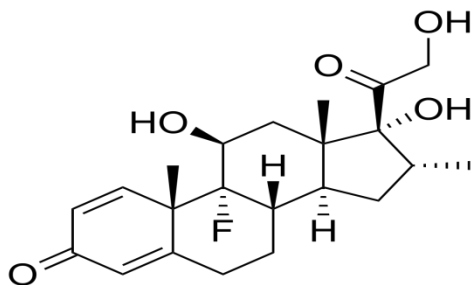
Dexamethasone is chemically (8S,9R,10S,11S,13S,14S,16S,17R)-9-Fluro-11, 17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-3-one, Dexamethasone is a glucocorticoid receptor agonist that will drive the negative feedback system for cortisol secretion in a person with a functional HPA Axis. It has a molecular formula of $C_{22}H_{29}FO_5$ and molecular weight of 392.461g/mol. dexamethasone is freely soluble in ethanol, dimethyl Formamide sparingly soluble in aqueous buffers. Brand name of dexamethasone is Dexone.

REVIEW OF LITERATURE

1. Rúbia Adrieli Sversuta[5] et al., An improved UV spectrophotometric method has been developed and validated for precise, efficient and selective determination of dexamethasone in tablets and capsules. The quantitative analyses were carried out using ethanol/water (2:1 v/v) as background electrolyte and UV detection was carried out at 240 nm. The calibration curve was linear over a concentration range from 4.0 to 40.0 μg/ ml. The average recovery was 97.60 ± 1.06% for tablets and 96.64 ±0.87% for capsules. The limit of detection and limit of quantification were 0.63 and 1.90 μg/ ml, respectively. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met. The results obtained with proposed method confirm improved performance over other methods found in the literature.

2. Karam. R. sireesha[7] et al., A liquid chromatographic method was developed and validated for the simultaneous determination of ofloxacin and

dexamethasone sodium phosphate in bulk and pharmaceutical formulations. Optimum separation was achieved in less than 5 min using a C_{18} 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 4) and acetonitrile (50:50, v/v) was used. Column effluents were monitored at 236 nm at a flow rate of 1ml/min. Retention times of ofloxacin and dexamethasone sodium phosphate were 2.4 and 4 min respectively. The linearity of ofloxacin and dexamethasone sodium phosphate was in the range of 3-18 μg/ml and 1-6 μg/ml respectively. Developed method was economical in terms of the time taken and amount of solvent consumed for each analysis. The method was validated and successfully applied to the simultaneous determination of ofloxacin and dexamethasone sodium phosphate in bulk and pharmaceutical formulations.

3. Maria Cristina Cocenza Urbana1[8], *et al.,* A simple, rapid, accurate and sensitive method was developed for quantitative analysis of dexamethasone acetate in microemulsions using high performance liquid chromatography (HPLC) with UV detection. The chromatography parameters were stainless steel Lichrospher 100 RP-18 column (250 mm x 4 mm i.d., 5 μm particle size), at 30 ± 2 ℃. The isocratic mobile phase was methanol:water (65:35; v/v) at a flow rate of at 1.0 mg/ml. The determinations were performed using UV-Vis detector set at 239 nm. Samples were prepared with methanol and the volume injected was 20ml. The analytical curve was linear (r2 0.9995) over a wide concentration range (2.0-30.0mg/ml). The presence of components of the microemulsion did not interfere in the results of the analysis. The method showed adequate precision, with a relative standard deviation (RSD) smaller than 3%. The accuracy was analyzed by adding a standard drug and good recovery values were obtained for all drug concentrations used. The HPLC method developed in this study showed specificity and selectivity with linearity in the working range and good precision and accuracy, making it very suitable for quantification of dexamethasone in microemulsions. The analytical procedure is reliable and offers advantages in terms of speed and low cost of reagents.

5. Rossana Barcellos Friedrich[8] *et al.,* This work reports the validation of an analytical UV spectrophotometric method to assay dexamethasone in tablets (assay and dissolution studies). The method was linear in the range between 1 and 30mg/ml presenting a good correlation coefficient (r = 0.9998, n = 7). Precision and accuracy analysis showed low relative standard deviation (< 2.00%) and good percentual recoveries (95-105%). The procedure was linear, accurate, precise, and robust. The method is simple, and it has low cost. It does not use polluting reagents and can be applied in dissolution studies, being an adequate alternative to assay dexamethasone in tablets.

6. M.S. Iqbal1, & M.A. Shad[9], *et al.,* 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 (6.0×150mm2). 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 15min. This method is suitable for application to various dosage forms.

7. G.N. Renuka Devi[6] *et al.,* A simple efficient, precise and accurate spectroscopic method has been developed and validated for quantitative estimation of Dexamethasone sodium phosphate in bulk and pharmaceutical dosage form. Dexamethasone sodium phosphate is soluble in distilled water, so it was used as solvent. Dexamethasone sodium phosphate is dissolved in distilled water the resulting solution was then scanned in the UV range (200-400nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The λmax of Dexamethasone sodium phosphate was found to be 242nm. The method obeys Beers law in the concentration range from 5-25 μg/ml. The correlation coefficient was found to be 0.999 (r2= 0.999). The LOD and LOQ were found to be 0.78 and 2.3μg/ ml respectively. The result of estimation of marketed formulation (Demisone) was found to be 94.19%. The accuracy of the method was determined by recovery studies. The percentage recovery was found to be 93.3%. The method was validated statistically as per ICH guidelines. The method showed good reproducibility and recovery with % RSD. less than 2. So, the proposed method was found to be simple, specific, precise, accuracy, linear, and rugged. Hence it can be applied for routine analysis of Dexamethasone sodium phosphate in bulk drug and the Pharmaceutical formulations and selective spectrophotometric methods for the determination of ofloxacin and dexamethasone in their binary mixture were presented. Ofloxacin was determined simply by zero order at its λ_{max} 293.4 nm in a linear range of 1.5–12mg/ml with mean percentage recovery of 100.07 ± 0.66% without any interference.

8. Waropam promsila[7] *et al.,* This article describes a modified QuEchERS extraction for dexamethasone in commercial and herbal medicines, and a determination was performed by HPLC-UV method. A QuEchERS was optimized using solvent extraction with acetronitrile in the presence of MgSO4 (500mg) and Nacl(125mg). An optimal mobile phase was a mixture of (% acetic acid and methanol with ration 40:60) at a flow rate 1.0ml, and UV detection was set at 254nm. The LOD and LOQ were found as 0.3 and 1.0ppm, respectively. A method showed adequate precision with percentage RSO less than 10%. An accurracy was analyzed by adding to a herbal pill, and a recoverces was obtained.

9. Ali Mohammad Akhoundi-Khalafi[11] *et al.*, Dexamethasone is a type of steroidal medications that is prescribed in many cases. In this study, a new reaction system using kinetic spectrophotometric method for quantitative determination of dexamethasone is proposed. The method is based on the catalytic effect of dexamethasone on the oxidation of Orange G by bromate in acidic media. The change in absorbance as a criterion of the oxidation reaction progress was followed spectrophotometrically. To obtain the maximum sensitivity, the effective reaction variables were optimized. Under optimized experimental conditions, calibration graph was linear over the range 0.2–54.0 mg/ml. The calculated detection limit was 0.14 mg/ml for six replicate determinations of blank signal. The interfering effect of various species was also investigated. The present method was successfully applied for the determination of dexamethasone in pharmaceutical and biological samples satisfactorily.

10. A. A. Heda[9] *et al.*, A new simple, selective, rapid, precise and accurate reverse phase HPLC method has been developed for simultaneous estimation of granisetron and dexamethasone. The method was developed using CPS Hypersil CN column (250×4.6mm I.D.) with a mobile phase consisting of acetonitrile: buffer (100 mm Triethylamine adjusted to pH 3.0 with o-phosphoric acid) in ratio of 25:75 at a flow rate of 2 ml/min. Detection was carried out at 242 nm. The developed method was evaluated for various system suitability parameters and validated for linearity, accuracy, precision, LOD, LOQ as per ICH guidelines. It was also evaluated for bench top stability and freeze/thaw stability. The proposed method can be used for the estimation of these drugs in their combined dosage forms.

11. Marianne Alphonse Mahrouse[10] *et al.*, A new, simple, accurate and sensitive UV spectrophotometric method was adopted and validated for the quantitative determination of ciprofloxacin hydrochloride (CIP) and metronidazole (MET), simultaneously, either in pure form or in pharmaceutical dosage form. Second derivative ratio spectrophotometry technique (2DD) was applied, by measuring the amplitude of the maximum at 253nm, using a normalized spectrum of MET as divisor, for the determination of CIP. MET was quantified by measuring the amplitude of the minimum at 301 nm, using the normalized spectrum of CIP as divisor. Linearity was obtained over the concentration range 2 – 16mg/ml, and 4 – 16mg/ml, for CIP and MET, respectively. Mean percentage accuracy was found to be 100.39 ± 0.677 and 100.21 ± 0.982, for CIP and MET, respectively. The proposed procedure was successfully applied for laboratory prepared mixtures without prior separation. The percentages recoveries of CIP and MET in tablet were more than 98%. The method was validated in respect to linearity, accuracy, precision and limit of detection and that of quantification, which prove suitability of the developed method for the routine

estimation of both drugs in bulk powder and solid dosage form.

12. Ankita J. Patel[6] *et al.*, Simple, precise, accurate, and sensitive first order derivative spectroscopic method have been developed for the estimation of Azithromycin and Dexamethasone in market formulation. The wavelength maxima for Azithromycin and Dexamethasone are 215.16 nm and 239.07 nm respectively. For the first order derivative spectroscopic method, the zero crossing points (ZCPs) for Azithromycin and Dexamethasone were obtained at 256.63nm and 214.35nm respectively in 0.1N NaOH using Shimadzu 1800 UV- visible double beam spectrophotometer. The proposed method were successfully utilised for the determination of Azithromycin and Dexamethasone in eye drops, with good linearity, high percentage of recovery and acceptable precision. Different analytical validation parameters like linearity, accuracy, precision, limit of detection and limit of quantification were determined according to International Conference on Harmonization.

13. Kang Ping Xiao[7] *et al.*, Adequate separation is essential for the quantitation of traceamounts of dexamethasone that are typically found betamethasone active pharmaceutical ingredients and vice versa. In paper, three simple and efficient high-performance liquid chromatography methods from which baseline separations between betamethasone and dexamethasone are achieved even concentration ratios between these two epimers are larger than 2000:1. One method is developed on a 5cm ACE C8 column that uses water and acetonitrile as the mobile phase and 20mM cyclodextrin as the mobile phase additive. There solution factor between betamethasone and dexamethasone. The second method is on a 10cm ACE C8 that uses water and acetonitrile as the mobile phase, in which the between epimers. The third method is developed on a 10 cm ACE C8 column using water and tetrahydrofuran as the mobile phase, in which the resolution factor between the epimers. Preliminary validation studies are carried out for the second and third methods.

14. Urvish H. Desai[6] *et al.*, High performance liquid chromatography method was applied to the simultaneous determination of ciprofloxacin and dexamethasone. The chromatographic separation was achieved on reversed-phase C18 column(25 cm × 4.6 mm id, 5 μm) in the isocratic mode using methanol-water-triethylamine (55:45:0.6, v/v/v), pH adjusted to 3.0 ± 0.05 with orthophosphoric acid as the mobile phase at a flow rate 0.8 ml/min. Quantitation was achieved with UV detection at 254 nm. In the HPLC method, quantification was achieved over the concentration range of 3-18 and 16μg/ml, with mean recoveries of 99.94 ±1.51 and 100.28 ± 1.25% for ciprofloxacin and dexamethasone respectively. The proposed methods were successfully applied for the analysis of synthetic mixtures and pharmaceutical formulations of ciprofloxacin and

dexamethasone without any interference from common excipients.

CONCLUSION

Literature survey suggested that various HPLC, UV, and few HPTLC methods were developed and reported. The published methods were validated for various parameters as per ICH guidelines. Statistical analysis proved that the published methods were reproducible and selective. Thus it can be concluded that the reported and published methods can be successfully applied for the estimation of the dexamethasone pure and pharmaceutical dosage form.

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