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PREDNISOLONE: A REVIEW OF ANALYTICAL METHODS

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ABSTRACT
Prednisolone is the most widely used corticosteroid class of drug in the treatment of anti-inflammatory, immunosuppressive and rheumatoid arthritis disease. In addition, the main analytical methods for determining and quantifying the PRD were also reported in this review, such as electro chemical methods, ultraviolet - visible spectrophotometer (UV/VIS), high performance liquid chromatography (HPLC), liquid chromatography tandem mass spectrometry (LCMS/MS), capillary electrophoresis (CE), and high performance thin layer chromatography (HPTLC). On the basis of this review article, important information is included in their clinical research to choose the best method for analysis.

KEYWORDS: Prednisolone, Corticosteroid, Analytical methods, HPLC.

INTRODUCTION
Prednisolone (PRD) chemically is a glucocorticoid and its IUPAC name is 11β, 17α, 21-trihydroxypregna-1, 4-diene-3, 0-dione. It has an empirical formula of $C_{21}H_{28}O_5$ and a molecular weight of 360.4g mol^{-1}(Fig-1).[1] It is mainly used for the treatment of a wide range of inflammatory and auto immune disease such as asthma, multiple sclerosis, rheumatoid arthritis etc.[2] Prednisolone also known as 'disease modifying anti-arthritic drug'[3] because of its anti-inflammatory action by inhibiting gene transcription for COX-2, cytokines, cell adhesion molecules, and inducible NO synthetase.[4,5] Prednisolone derivate is also used in ophthalmology as a content of eye drops used to reduce allergic reaction affecting eyes, such as swelling and itching.[6,7] Due to immune suppressive properties is also widely use after organ transplantation[8] and as a hormone substitution for patients unable to produce proper amount of corticosteroids on its own.[9] Prednisolone is a very slightly water soluble glucocorticoid. So the potential of liquid solid system to improve the dissolution properties of water-insoluble agents was investigated using prednisolone as the model drug in some study.[10] It is administered in orally; slightly soluble in water and methanol; soluble in chloroform and alcohol.[11]

In the present review we have compiled the published analytical methods reported so far in the literature for determination of PRD in pharmaceutical formulations and biological samples. Techniques like potentiometry, spectrophotometry, capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS) and high-performance thin layer chromatography (HPTLC) have been used for analysis, from which HPLC methods are used most extensively.

This review paper focuses the analytical procedure available for the estimation of prednisolone drug, i.e. electrochemical methods, capillaryelectrophoresis (CE), UV/VIS -spectrophotometric methods, HPLC, LC-MS and HPTLC.

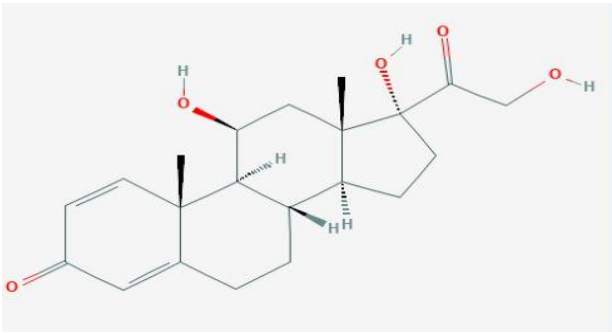

Fig .1 Structure of Prednisolone.

Solubility
Prednisolone is considered Biopharmaceutical Classification System (BCS) class its means a high solubility and high permeability.[12]

Electro chemical methods
Sayed.I.M.Zayed, used equipped with a three electrodes assembly cell consisted of hanging mercury drop electrode as working electrode and 0.04 M Britton

Robinson buffer pH 3.5, an Ag/AgCl in 3 mol/ KCl as a reference electrode and platinum wire as an auxiliary electrode. Under the optimized conditions of a accumulation potential of –0.4 V, scan rate of 50 mVs–1, pulse amplitude of 50 mV and 40s accumulation time, the peak current of the differential pulse cathodic adsorptive stripping voltammograms was found to be linearly related to the PRD concentration in the linear range 7.2–144.2 ng/ml. Limit of detection (LOD) and limit of quantization (LOQ) were found to be 3.95 and 13.17 ng/ml.[13]

Rajendra N. Goyal et al. a new composite electrochemical determination of PRD has been investigated at fullerene-C_{60}-modified gold electrode (C60/AU) and gold nanoparticles modified indium tin oxide electrode (Au/ITO) in phosphate buffer solution of pH 7.2. The oxidation of PRD an anodic peak with peak potential at 570mv and 400mv appeared at nano Au/ITO and C60/AU electrode respectively. The proposed method showed a linear concentration range of 1 µM to 0.10 mM. The limit of detection was obtained at C60/AU electrode was 26 nM and Au/ITO electrode was 90 nM. The authors have applied this method to determine a PRD in human urine and whole blood sample.[14]

In another study Rajendra N. Goyal et al. was applied voltammetric sensor for simultaneous determination of PRD and prednisone in human body fluids. The drug was evaluated by osteryoung square wave voltammetry phosphate buffer medium of pH 7.2. The proposed method showed a good linear response over the concentration range 0.01-100µM and the detection limit of PRD was found to be 0.90×10^{-8}.[15]

Analytical methods

Spectrophotometry

In the literature about 13 methods were reported for the estimation of PRD, using spectrophotometry, of which methods are for determining PRD alone, will the others are for quantifying PRD in combination with other drug substance. Table 1 shows the summary of the reported spectrophotometric methods indicating the basic principle, λmax and solvent, limit of detection (LOD) & limit of quantification (LOQ).

Table 1: Representative of spectrophotometric method for PRD analysis.

Compounds	Method	λ max	Solvent/ procedure	LOD	LOQ	References
PRD and Meslamine	Vireodt's method	246 and 320	DMF (dimethyl formamide) + phosphate buffer solution pH .4(PBS)	-	-	[16]
PRD and OFL	Dual wavelength method	277, 323.40 323.40,	Methanol	0.86	2.63	[17]
PRD and OFL	Simultaneous equation method	243 288	Methanol	0.64	1.957	[18]
PRD and SAL	Area under curve method	249-239 232-222	Methanol	-	-	[19]
PRD and GFN	First order derivative method	263 348	Acetonitrile: water (70:30)	0.16	0.55	[20]
PRD and MSM	Vireodt's method	246 332	DMF(dimethyl formamide) + phosphate buffer solution pH 7.4	0.047	0.14	[21]
PRD and MOX	Simultaneous spectrometric method	247 288	Methanol + water	-	-	[22]
PRD	QBD approach	246	Ethanol+ methanol+ water	0.0538	0.1631	[23]
PRD 5-ASA	Q-absorption ratio method	283 302	DMF(dimethyl formamide) + phosphate buffer solution pH 6.8	-	-	[24]
PRD	Derivative method	242	Ethanol	-	-	[25]
PRD	-	246	Acetonitrile:methanol (30:70)	0.16	0.53	[26]
PRD	-	244	Methanol	-	-	[27]
PRD	-	246	Water (100%)	0.0538	0.1631	[28]

Chromatography

HPLC

Biological samples

Various methods for the determination of PRD in biological samples like plasma, serum and urine are listed in Table 2.

Pharmaceutical samples

Analytical methods for the determination of PRD in pharmaceutical dosages forms using HPLC are shown in Table 3 shows the best HPLC methods for the analysis of PRD.

Table 2: Summary of HPLC methods to determine PRD in biological samples.

Matrix	IS	Sample preparation	Mobile phase	Column	Detection	λ max	Flow rate (mL/min	Retention time (min)	LOD/ LLOQ (ng/mL)	Ref.
Human plasma	Corticosterone	Solid phase extraction	Acetonitrile: 0.08% Trifluoro acetic acid in deionized water (28:72, v/v)	Spherisorb S5 ODS2 (250mm × 4.6mm, 5µm)	UV	254	0.65	19.8	0.5	[29]
Human plasma, whole blood, urine	Betamethasone	Liquid -liquid & solid phase extraction	Methanol: glacial acetic acid: dichloromethane (1.5:8.0:90.5, v/v/v)	Lichrospher Si 60 (250mm × 4.6mm, 5µm)	UV	254	1.8	14	100	[30]
Human Plasma	Betamethasone	Soild phase extraction	16% isopropanol in water containing 0.1% trifluoro acetic acid	Supelcosil LC-18 –DB (150mm × 4.6mm, 5µm)	UV	254	1.2	18	7	[31]
Human plasma	6β-hydroxycortisone	Solid phase extraction	Diethylene dioxide: ethyl acetate: chloroform: n-hexane: pyridine (500:100:100:1400:2, v/v)	Comosil 5SL (250mm × 4.6mm, 5µm)	Fluorimetric	360	1.0	32.4	0.1	[32]
Human plasma	Betamethasone	Liquid – liquid extraction	Methylene chloride: heptanes: glacial acetic acid: ethanol (600:350:10:35, v/v)	Zorbax SIL (250mm × 4.6mm, 5µm)	UV	254	2	21	10	[33]

Table 3: Reported analytical HPLC methods for determination of PRD either alone (or) in combination with other drugs like salbutamol (SAL), ofloxacin (OFX), tertrahydrozoline hydrochloride (THC), moxifloxacin (MFX), phenylephrine hydrochloride(PHE), prednisolone sodium phosphate(PSP), atropine(ATR), homatropine(HOM), mycophenolate mofetil(MMF) and tacrolimus (TAC) in pharmaceutical dosage forms.

Study aim	Mobile phase	Column	Detection	λ max	Flow rate(mL /min	Retention time (min)	LOD/LOQ (ng/mL)	Ref.
Developed and validation for prednisolone in tablet	Methanol: water (58:42, v/v)	Hypersil DS C_{18} (250mm × 4.6mm, 5µm)	UV	254	1	7.029	-	[34]
Estimation of drug and degradation behavior in tablet by RP-HPLC	Water: etrahydrofuran: acetonitrile (15:10:75, v/v)	Teknokromea C18 (150mm × 4.6mm, 5µm)	UV	254	1	9.9	0.0074, 0.025	[35]
Stability indicating method for assay of tablets	Ethanol: water (30:70, v/v)	Phenomenex C18 (150mm × 4.6mm, 5µm)	PDA	254	0.8	10.29	6.93, 16.61	[36]
Pharmaceutical formulation by RP-HPLC method	Acetonitrile: citro phosphate buffer (pH 5) (45:55, v/v)	Bondapak C18 (300mm × 3.9mm, 10µm)	UV	241	1.2	4	-	[37]
Simultaneous	Acetonitrile: 0.025M	Thermo C18	UV	244	1	6.12	-	[38]

estimation with SAL in tablet	potassium dihydrogen orthophosphate buffer (pH 3.5 adjusted with ortho phosphoric acid) (30: 70, v/v)	(250mm × 4.6mm, 5µm)						
Simultaneous estimation with OFX in pharmaceutical dosage form	Phosphate buffer (pH 4): methanol (30:70, v/v)	BDS hypersil C18 (25mm × 0.46mm)	PDA	275	1	5.68	0.0297, 0.090	[39]
Simultaneous estimation with OFX and THC	0.05M Phosphate buffer (pH 2.7 adjusted with ortho phosphoric acid) (65:35, v/v)	Waters Spherisorb ODS (150mm × 4.6mm, 5µm)	UV	254	1.2	9.5	-	[40]
Simultaneous determination with MFX	Phosphate buffer (18mM) containing 0.1% triethylamine (pH 2.8 adjusted with dilute phosphoric acid): methanol (38:62, v/v)	BDS hypersil C18 (250mm × 4.6mm, 5µm)	PDA	254	1.5	7.4	0.175, 0.559	[41]
Simultaneous determination with PHE	Methanol: water: heptane-1-sulfonic acid sodium salt (75:25: 0.1, v/v/w)	XSelect HSS C18(250mm × 4.6mm, 5µm)	UV	230	1	6.5	-	[42]
Simultaneous determination with PSP, ATR and HOM	Acetonitrile: potassium dibasic phosphate buffer (10m mol L^{-1}, pH 6.9) (35:65, v/v)	Phenyl-Hexyl (250mm × 4.6mm, 5µm)	UV	240	1	5.6	0.02	[43]
Simultaneous estimation with MMF and TAC in Immunosuppressant formulation	Acetonitrile: 0.35% triethylamine (pH 4.2 adjusted with orthophosphoric acid) (70:30 v/v)	Kinetex polar C18 (250mm × 4.6mm, 5µm)	UV	254	1.2	2.5	0.44, 1.33	[44]
HPLC method	Water: acetonitrile (55:45)	ODS (460mm × 250mm, 10µm)	UV	254	1	3.28	-	[45]

Capillary electrophoresis

Capillary electrophoresis is an excellent technique which used as the first-choice method for the separation of steroid hormones. The micellar electrokinetic capillary chromatography (MEKC) is a partial filling technique in the class of capillary chromatography. For a few authors, PRD analysis has performed in Capillary electrophoresis as a separation and determination technique. The HPLC and micellar electrokinetic capillary chromatography (MEKC) methods were developed by Jose maria[46] for the determination of PRD and its unsaturated impurity was separated in less than 6.6 min using an uncoated fused – silica capillary (57cm×75 µm i.d.) with extended light path for better (30 kV at 25 ºC) sensitivity and background electrolyte consisting of 5mM phosphate borate buffer adjusted with pH= 8.2 40mM sodium dodecylsulfate (SDS). The sample was introduced into the capillary and detected in 245nm. LOD and LOQ were found to be 0.22µg/mL and 0.73µg/mL respectively.

Lemus gallego J. M.[47] proposed an alternative electrophoretic method for PRD and its metabolite. It separation was carried out on an fused silica capillary using (57cm×75 µm i.d.) with extended light path for better (30 kV at 25 ºC) sensitivity and background electrolyte consisting of 5mM phosphate borate buffer adjusted with pH= 8.2 40mM sodium dodecylsulfate using a followed by a 6s hydrodynamically sample injection. The sample were introduced into the capillary and detected in 247nm. The LOD and LOQ were found to be respicetively 0.22µg/mL and 0.73µg/mL respectively.

Heli siren[48] performing the partial filling miceller electrokinetic chromatography technique for determination of endogenous low – hydrophilic steroids, It separation was carried out on an fused silica capillary using (70 cm×55 µm i.d.) with extended light path for better (30 kV at 35ºC) sensitivity and background electrolyte consisting of (50:50, v/v) methanol - 20mM ammonium acetate at pH 9.68. The hydrodynamic injection was 20s at 2.0 psi. The sample were introduced into the capillary and detected in 247nm. The LOD were found to be 10-100ng/mL.

LC-MS

Various methods have been reported for the identification of prednisolone in human plasma by using LC-MS/MS method due to their high sensitivity and selectivity. Fatemeh Akhlaghi et al. The drug was extracted from human plasma by using Zorbax-SB Phenyl column (150 mm × 2.1mm, 5 µm) with the help of mobile phase containing acetonitrile, water and formic acid (32: 68: 0.1, v/v/v) with a flow rate of 0.140 mL/min. The protein precipitation method was used to sample extraction. The sensitive method was linear in the range of 2-1000ng/mL. The lowest limit of quantification was 2.0ng/mL and the retention time was found to be 6.62min.[49]

LC/MS/MS method was developed by Isabelle Laverdiere[50] the separation was achieved by ACE-3 HL C18 column containing (100 mm × 4.6 mm, 3µm). The mobile phases consisted of water with 3 mM ammonium formate and 0.1% formic acid (solvent A), and methanol with 3 mM ammonium formate and 0.1% formic acid (solvent B) and internal standard was used as Indomethacin. The flow rate was 0.9 mL/min. The extraction of sample was achieved by solid phase method. The linearity was in the range of 2-1000 ng/mL. The retention time was found to be 4.4 min and lowest limit of quantification was 2ng/mL.

Madhusudhana Reddy et al.[51] have discussed simple, rapid (ESI- LC/MS/MS) method was developed for simultaneous screening of doping agents in urine samples. The separation was carried out on an Inertsil ODS-3 (50 mm × 4.6 mm, 3.5µm). The extraction of sample was eluted by using mobile phase acetonitrile and 1% formic acid (50:50, v/v). The internal standard was used as a 17-α-methyl testosterone. Detection was achieved by electron spray ionization mass spectrometry. The lowest limit of detection of the method was 2ng/mL. The linearity range was 15-120 ng/mL and flow rate was 700µl per minute. The retention time of PRD was found to be 5.09 min.

Mei chen et al.[52] LC/MS/MS method developed for the simultaneous quantization of assay of PRD and dipyridamole in human plasma in the internal standard was used as a prednisolone-d_6. Liquid-liquid extraction was used for extracting the analytes from plasma. The samples were separated on a C18 (50 mm × 4.6 mm, 3µm) column using a mixture of methanol and water (50:50, v/v) with a flow rate of 1000 µL/min. The linearity in the range of 0.4-200 ng/mL and the retention time was found to be 1.7 min. The lowest limit of quantification of PRD was 0.4 mg/mL.

Another LC/MS/MS method was developed by Xavier Matabosch et al.[53] detection and characterization of PRD metabolites in human urine. Sample was extracted by liquid-liquid extraction method. The separation was carried out on a BEH C18 column (100 mm × 2.1mm, 1.7µm) at a flow rate of 400µL/min. The samples were separated from using a mixture of mobile phase water and acetonitrile (75:25, v/v). The retention time was found to be 13.5 min and the lowest limit of quantification was 0.1ng/mL.

HPTLC

Astha Metha A. et al.[54] developed a validated HPTLC method for assay of PRD. They carried out separation on aluminium plates precoated silica gel G60 F_{254} using the mixture of chloroform and methanol (95:5, v/v) as the mobile phase. The detection of spot was carried out by densitometry at 250nm. The calibration curve was found to be linear between 2-10µg. The R_f value was found to be 0.53. The LOD and LOQ were found to be 0.2µg,0.6µg/spot respectively.

In the another studies, Ganesh S. Raut et al.[55] proposed HPTLC method for determination of PRD and moxifloxacin hydrochloride the chromatography was performed on silica gel 60 RP- 18F_{254} and the mobile phase was using the methanol:water:triethylamine (7:3:0.3, v/v). The detection was performed densitometry at 274nm. Regression plot revealed good liner relationship in the concentration ranges 600-3600ng. The R_f value was found to be 0.50. The LOD and LOQ were found to be 56.98ng,172.69ng/spot respectively.

Syed Ghulam Mushraf et al.[56] gave an HPLTC method for determination of PRD and chloramphenicol the separation was performed at glass plate precoated silica gel and the mobile phase as using chloroform and methanol (14:1). The detection was carried out by densitometry at 243nm. The proposed method was linear in the range of 200-6000ng. The R_f value was found to be 0.41. The LOD and LOQ were found to be 4.77ng/µL,14.46ng/µL spot respectively.

Sayed M. H. et al.[57] reported a urinary prednisolone was determined by high performance thin layer chromatography method using the mobile phase consisting of dichloromethane:methanol:water (150:10:1, v/v). The method was liner in the concentration range of 0.05-4µg/ml. The R_f value was found to be 0.136. The minimum level of detection was 25ng/ml.

Joanna Nowakowska et al. study reported a simple TLC and HPTLC method for separation of selected the steroid by using silica gel and the mobile phase consisting an mixture of acetonitrile and DMSO (10:90, v/v). The steroids were visualized by spraying the plates with a mixture of concentrated sulphuric acid and methanol 1:4 (v/v). The method was linear range between 70-100µg.[58]

CONCLUSION

This review delineates the reported spectrophotometric and chromatographic methods which are developed and estimated for the drug Prednisolone. In conclusion, a broad range of analytical techniques are available for the analysis of PRD in pharmaceutical formulations and

biological samples. According to the review it can be concluded that different methods of estimation for both single component as well as in combination are available. The analysis of the published data revealed that the HPLC was extensively used for the determination of PRD where methanol, acetonitrile, triethylamine and buffer solutions were used as the major solvents in the review of data drugs like salbutamol, ofloxacin, moxifloxacin, atropine and tacrolimus were used in combination for many estimations. Regarding pharmaceutical formulations, the articles presented in this review show that analytical techniques are most important for the production and research of pharmaceutical products in addition to monitoring the stability of new formulations. This review carried out an overview of the current state of analytical methods for the determination of PRD.

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