Development and Validation of an RP-HPLC Method for Estimation of Prednisolone and its Degradation Products in Tablets

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Abstract

A simple, precise, specific, sensitive and accurate stability indicating RP-HPLC method for determination of prednisolone and its degradation products in tablets. The separation was performed on teknokroma, tracer excel C18 column (15cmx 0.46 cm. 5µm) using mobile phase consisting ofwater: tetrahydrofuran: acetonitrile in ratio (75: 15: 10). The flow rate was 1mL/min and detection was set at 254 nm. The column temperature was set at 45^0. The retention times were 7.5, 9.98 and 10.7 for prednisone, prednisolone and hydrocortisone respectively. The calibration curves were found to be linear in the concentration range of 25-75 µg/mL (r^2 =0.9998) and 0.25-7.2 µg/mL (r^2 =0.9999) at assay level and low-level of prednisolone.

The percentage recoveries of prednisolone found to be in the range of 99.3-99.99% at assay level and 98.77-99.75%at low-level respectively at 95% confidence limit. The intraday precision was 0.424% and 0.501% at assay level and low-level, respectively. The intermediate precision was 0.44% and 0.634% at assay level and low-level, respectively.

Keywords: *Prednisolone; HPLC method; repeatability; precision; Hydrogen peroxide*

Introduction

Prednisolone is chemically designated as 11β, 17α, 21-trihydroxypregna-1, 4-diene-3, 20-dione.
Prednisolone is a glucocorticoid and widely used as a potent anti-inflammatory, anti-allergic and immunosuppressive drug.

Estimations of prednisolone have been described by spectrophotometry [1-9]. A HPLC method have been developed for the assay of prednisolone [7,10,17]. The pharmacopeial methods for the determination of prednisolone and its degradation products in tablets are available. In the B.P pharmacopoeia, the mobile phase consisted of methanol: water (58: 42) for the assay. For related substances, the mobile phase composed of the mixture of water and tetrahydrofuran (780: 220) [18].

In USP pharmacopoeia, the mobile phase composed of a mixture of butyl chloride, water-saturated butylchloride, tetrahydrofuran, methanol, glacial acetic acid for assay. For related substances, the mobile phase composed of mixture of water: ACN and the elution are gradient. In the proposed method, the analysis for assay and degradation product was carried out on single method.

Experimental

Instrumentation

An HPLC system from shimadzu corporation consisting of an LC-20 AT pump, SPD-20 A UV/vis-detector, DGU 20A3, a reodyne injector with 20µL loop. Teknokroma, tracer excel. C18 column (15cm x 0.46cm, 5µm) and C18 column (25cm x 0.46cm, 5µm).

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Reference substances, reagents and chemicals

Prednisolone was obtained from KAH.PH and IND.CO. (Egypt). Hydrocortisone was obtained from WHO. Prednisone was obtained from WHO. Methanol HPLC grade, acetonitrile HPLC grade and tetrahydrofuran were obtained from sigma-aldrich (Germany). Water was obtained from arium-sartoriuswater purified system (Germany).

Sample preparation

Twenty tables were weighed, finely powdered and homogenized. An accurately weighed portion of the powder equivalent to 5mg of prednisolone was then taken into 100mL, volumetric flask. About 58mL of mobile phase was added to this volumetric flask and soni-cated in an ultrasonic bath for 10minutes. This solution was then diluted up to the mark with mobile phase and mixed well. It was then filtered through 0.45µm syringe filter and the filtrate was collected after discarding first 10 milliliters and 20µL was injected.

Standard preparation

Standard solution (0.05mg/mL)

The solutions were prepared by dissolving 25mg standard prednisolone with 15mL of the mobile phase and sonicated for 10min-utes with intermittent shaking until a clear solution was obtained. The solution was cooled to room temperature and was diluted up to volume in a 25mL volumetric flask (stock solution). Dilution from this solution was made to obtain a concentration which contains (0.05mg/mL) and 20µL was injected.

Impurity standard solution (0.005mg/mL)

The impurity standard solution were prepared by diluting 0.5mL of the stock standard solution (1mg/mL) with mobile phase to a 100mL volumetric flask to obtain concentration which contains 0.005mg/mL of the impurity standard solution and 20µL was injected.

Impurity sample preparation (0.5mg/mL)

An accurately weighed portion of the powder equivalent to 10mg of prednisolone was then taken in to 20mL volumetric flask and a 10mL of the mobile phase was added and sonicated for 15minutes with intermittent shaking. The solution was cooled to room tem-perature and diluted to 20mL with mobile phase (0.5mg/mL) and mixed well. It was then filtered through 0.45µm syringe filter and the filtrate was collected after discarding first few milliliters and 20µL was injected.

Chromatographic conditions

Column: teknokromea C18 (150mm x 4.6mm,5µm)
Column temperature: 45^0Flow rate: 1mL/min
Wavelength: 254nm
Injection volume: 20µL
Run time: 20min
The mobile phase was composed of tetrahydrofuran 15%: acetonitrile 10%: water 75%.

Determination of linearity for assay of prednisolone

Different aliquots of stock standard solution (1mg/mL) were transferred into a series of volumetric flasks (20mL) and diluted to the mark with mobile phase to obtain final concentrations of 25µg/mL, 35µg/mL, 50µg/mL, 65µg/mL and 75µg/mL. Twenty µL of each of standard solution was injected in triplicate.

Determination of linearity at low-level of prednisolone for (degradation products)

Different aliquots of stock (1mg/mL) and standard solutions (5µg/mL) were transferred into a series of volumetric flasks and diluted to the mark with mobile phase to obtain final concentrations of 0.25µg/mL, 0.5µg/mL, 1.25µg/mL, 2.5µg/mL, 5µg/mL and 7.5µg/mL. Twenty µL of each of standard solution was injected in triplicate.

Determination of accuracy for assay of prednisolone

(Accuracy was performed on 5mg tablet)

Different aliquots of stock solution (1mg/mL) were transferred into a series of volumetric flasks. Placebo (106mg) was then transferred to each flask and diluted to the mark with mobile phase to obtain final concentrations of 25µg/mL, 35µg/mL, 50µg/mL, 65µg/mL and 75µg/mL.

The final solution was filtered through syringe filter (0.45µm) and twenty µL was injected, three replicates were prepared for each level. The percent recovery was determined by an external standard at 100% level.

Determination of accuracy at low-level of prednisolone

Different aliquots of stock solution (1mg/mL) and standard solutions (5µg/mL) were transferred into a series of volumetric flasks, placebo (106mg) was then transferred to each flask and diluted to the mark with mobile phase to obtain final concentrations of 0.25µg/mL, 0.5 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL and 7.5 µg/mL. Three replicates were prepared for each level. The final solution was filtered through syringe filter (0.45µm). The percent recovery was determined by standard solution at 1% (5µg/mL).

Determination of repeatability precision for assay of prednisolone

An accurately measured aliquot (5mL) of stock solution (1mg/mL) was transferred into a 100mL volumetric flask, placebo (108mg) was then transferred into the flask and diluted with mobile phase to the mark to obtain final concentration of 50 µg/mL. The final solution was filtered through syringe filter (0.45µm). Twenty µL was injected. Six replicate spiked placebo solution w prepared (independent preparation) and two injections were injected of each sample preparation

Determination of intermediate precision for assay of prednisolone

Intermediate precision was performed such as repeatability precision procedure on different days and different preparation.

Determination of repeatability precision for degradation products at low-level of prednisolone

An accurately measured aliquot (0.5mL) of stock standard solution (1mg/mL) was transferred into a 100mL volumetric flask. Placebo (106mg) was then transferred into the flask and dilutedto the mark to obtain final concentration of 5µg/mL. The final solution was filtered through syringe filter (0.45µm). Twenty µL was injected. Six replicate sample solutions were prepared and two injections were injected of each sample prepared.

Determination of intermediate precision for degradation products at low-level of prednisolone

Intermediate precision was performed, on different days and different preparation.

Determination of limit of detection and limit of quantitation

Different aliquots of standard solution (5µg/mL) were transferred into a series of volumetric flasks and diluted to the mark with mobile phase to obtain final concentrations of 0.05µg/mL, 0.025 µg/mLand 0.007 µg/mL. Twenty µLwas injected.

Forced degradation

Acid degradation

Two accurately measured aliquots (2X5mL) of stock solution (1mg/mL) were transferred into two separate (100mL) volumetric flask. Placebo (106mg) was then transferred into one (100mL) volumetric flask and 5mL of 0.1N HCl was added to each volumetric flask. The two solutions were stored at room temperature for 2days and then, neutralized with mobile phase. Twenty µL was injected.

Base degradation

Two accurately measured aliquots (2X5mL) of stock solution (1mg/mL) were transferred into two separated (100mL) volumetric flasks. Placebo (106mg) was added into one volumetric flask and 5mL of 0.1N NaOH was added to each flask. The two flasks were stored at room temperature for 1day and then, neutralized with 5mL of 0.1N HCl. The two flasks were diluted to volume with mobile phase. Twenty µL was injected.

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Hydrogen peroxide

Two accurately measured aliquots (2X5mL) of stock solution (1mg/mL) were transferred into two separate (100mL) volumetric flasks. Placebo (106mg) was added to one flask and then 5mL of 3.0% hydrogen peroxide was added to each flask. The two solutions were stored at room temperature for 6 days and then diluted to volume with mobile phase. Twenty µL was injected.

Light degradation

Two accurately measured aliquots (2X5mL) of stock solution (1mg/mL) were transferred into two separate (100mL) volumetric flasks, placebo (106mg) was added to one flask. Two flasks were diluted with mobile phase to volume and exposed to sunlight for 6 hours. Twenty µL was injected.

Heat degradation

Two accurately measured aliquots (2X5mL) of stock solution (1mg/mL) were transferred into two separate (100mL) volumetric flasks, placebo (106mg) was added to one flask. Two flasks
Were stored at 90^0 for 8hours and then diluted with mobile phase to volume. Twenty µL was injected.

Determination of robustness for assay

An assay standard solution was prepared and analyzed as according in the analytical method

> Mobile phase
> Acetonitrile (10%) was varied by ±10%.
> Tetrahydrofuran was varied by ±10%.
> The flow rate (1mL/min) was varied by ±10%.
> The column temperature (45^0) was varied by ±5%.
> The system suitability standard solution was prepared and injected twice.

Determination of solution stability

1. Fresh standard and spiked placebo were prepared and analyzed as per the test method.
2. Time zero value for each were established.
3. An aliquot of each solution was placed in clear glassware and exposed to ambient conditions and aliquot was placed in a refrigerator.
4. The solutions were analyzed every 24 hours for at least 48hours.
5. Two injections were injected of each solution.

Result and Discussion

The mobile phase was chosen after several trials with different solvents in various proportions to reach a suitable mobile phase optimization. These trials include using different mobile phases consisting of methanol in water in different proportions. For 80% v/v, the retention time was 2.82 minutes, entire samples were eluted near t0 (t0=1.5min), no peaks were observed after 15minutes, so a weaker mobile phase was required. Successive reduction in % MeOH by 20% was applied. For 60% methanol (MeOH), the retention time of prednisolone was 7.77 minutes but this ratio of the mobile phase was published by [19].

For 40% methanol, the retention time was 57.7 minute and this ratio was published [2].
Different mobile phase consisting of acetonitrile in water in different ratios were tried. The solvent nomograph was used to estimate the required value of % CAN based on the best % MeOH. For 25% ACNat room temperature, the retention times were 17 and 18.1 min for prednisolone and hydrocortisone, the resolution was 1.4, for 25% ACN at 40^0, the retention times were 12.1, 12.55 and 13.36 minutes for prednisone, prednisolone and hydrocortisone. The resolution between prednisone and prednisolone was 0.9 the resolution was unacceptable, and the resolution between prednisolone and hydrocortisone was 1.66.For 30% ACN at 40^0, the retention times was 8.258 and 8.87 min for prednisolone and hydrocortisone the resolution was 1.6 which was acceptable and the acceptance criterion of ≥ 1.5.

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For 25% ACN: 5% THF: at temperature 45^0 the resolution between prednisone and prednisolone was 2.9 and between prednisolone and hydrocortisone was 1.6. For 15% THF :10% methanol :75% wateran mobile phase at 45^0 the resolution between prednisolone and hydrocortisone was 1.6, when the temperature was increased, the resolution was increased and when the % THF was increase, the resolution was increased and when the % THF was increase, the resolution was increased. Since, the mobile phase which was selected, composed of a mixture of 10% ACN : 15% THF : 75% water the resolution between prednisolone and hydrocortisone was 1.7 as shown in Figure (1).
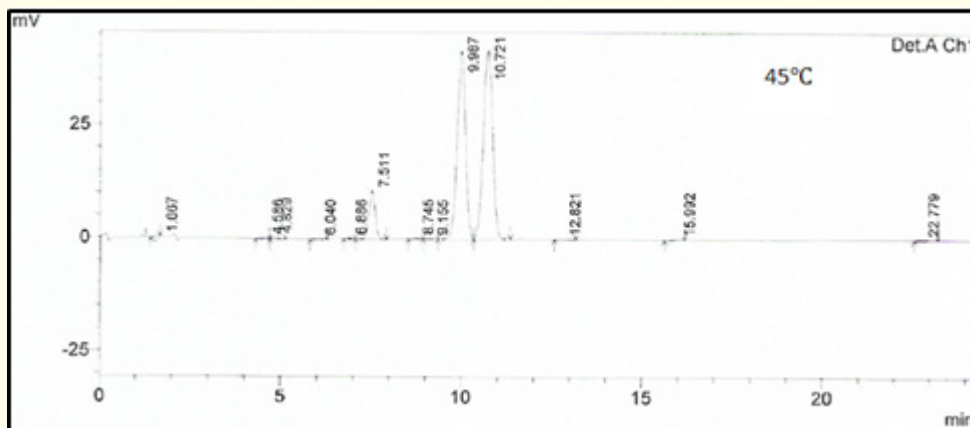

Figure 1: Separation of a prednisolone and its degradation products. Condition: column, $C\_18 \times 15cm \times 0.46cm \times 5\mu m$; flow rate 1mL/minute; 10%v/v/ ACN ; 15%v/v THF as mobile phase at t 40^0 and 45^0

Forced degradation studies were performed to demonstrate specificity of stability-indicating method for prednisolone.

The standard and spiked placebo solutions were exposed to acidic, basic, oxidative, light and heat conditions.

The degradation products that were generated from forced degradation were resolved from prednisolone peak. Prednisolone was found to degrade completely in alkaline condition. Mild degradation was occurred in the acidic, light and thermal condition. Prednisolone disappeared in basic conditions. The loss of prednisolonecontent was corresponding to increase in the level of degradation products as shown in table (1). The results of the forced degradation studies were shown that, the RP-HPLC method was stability indicating for analysis of prednisolone and its degradants

Item	Prednisolone at assay level	Prednisolone at low-level
Retention time	9.9	9.9
Wavelength of detection (nm)	254	254
Range of linearity (µg/mL)	25-75	0.25-7.5
Calibration point	5	6
Regression equation	Y=41958x +11894	Y=42203x +207.9
Regression coefficient(r^2)	0.99978	0.99999
SDa	8845	133.53
SDb	165.98	34.6
95% Confidence limit of the slope	41599-42316	42129-42276

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Item		Prednisolone at assay level	Prednisolone at low-level
95% Confidence limit of the intercept		-7215-31003	-75.2-490.9
Standard error of the estimation		11853	384.7
LOD(μg/mL)		0.7	0.01
LOQ(μg/mL)		2.1	0.031
Intraday %RSD		0.424	0.501
Interday %RSD		0.44	0.634
% Recovery	%Mean	99.65%	99.26%
	95%Confidence limit of mean	99.3-99.99%	98.77-99.75%

Table 1: *results obtained by the proposed RP-HPLC for determination prednisolone and its degradation products.*

Robustness of the method to small but deliberate changes was evaluated with a designed study including two levels (high and low) and three factors: temperature (+/- 5^0) flow rate (±10%), acetonitrile (±10%) and THF (±10%). The parameters were within the system suitable parameters as shown in table (2) and in table (3).

System suitability parameters	prednisolone
N≥	8045
T≤2	1.04
R≥1.5 Resolution between pred- nisolone and hyorocorion	1.55
k≥2	4.7
%RSD≤2.0%	0.58
R.T	8.934

Table 2: *System suitability test for the proposed RP-HPLC method for the determination of prednisolone.*

Parameter change		RT min	Theorectical plate (N)	Tailing factor (T)	Capacity factor	Rs
Method Conditions		9.075	67570	1.019	5.334	1.5
Flow rate	1.1mL/min	8.519	58558	0.997	4.65	1.685
	0.9mL/min	10.367	63915	0.997	6.368	1.758
Column	40°C	10.148	59896	0.994	5.6	1.621
Temperature	50°C	8.673	62024	1.005	4.75	1.8
Mobile phase	-10%ACN	11.317	63052	0.983	6.483	1.738
	+10%ACN	8.895	59691	1.009	4.891	1.685
	- 10% Me OH	12.005	64370	0.989	4.723	1.64
	+10% Me OH	8.615	58883	1.012	4.469	1.73

Table 3: *Result of Robustness for the proposed RP-HPLC method parameter Vanations prednisolone.*

The method was robust. Standard and sample solution were shown to be stable for 48 hours at room temperature

The results in table (1)shown that, the intraday precision were 0.424% and 0.502% at assay and low-level, respectively. The intra-day precision were 0.44% and 0.634% at assay and low-level, respectively.

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The results for the evaluation the linearity of prednisolone at assay level were given in table (1) shown that. The correlation coefficient of 0.99979 which was acceptable –the slope was (41958) and the Y-intercept was (11894.17). The regression equation for the graph was computed and found to be: y=41958x + 11894→[3], where x is concentration in µg/mL. The regression statistics was performed by Excel and SPSS, the Y-intercept was 0.52% compared to the calculated Y-value at 100% standard (250µg/mL) and the acceptance criteria was ≤2.0%.The residual standard deviation was 0.56% (standard error compared to calculated Y-value at 100% level) and acceptance criteria was ≤2.0%.

These results clearly demonstrate a linear relationship between the peak areas and the concentrations of prednisolone in the ranges of 25µg/mL to 75µg/mL.

The results for the evaluation of the linearity of prednisolone at low-level were given in table (1) shown. The correlation coefficient was (0.999989), which was acceptable.The slope was 42203 and intercept was 207.86, the regression equation for the graph was computed and found to be: y=42203x + 207.86 [4], where x is the concentration at low-level. The regression statistics were performed by excel. Y-intercept value was 0.09% and acceptance criteria of≤5% based on 1% level (5µg/mL). The residual standard deviation was 0.182% and the acceptance criterion of 10%.

The difference in slope of upper range and slope of lower range in linearity curves was 0.58% and the acceptance criterion of ≤4.0%. Therefore, the 100% standard or area percent normalization could be used to quantitate the levels of degradation products.

The results in Table (4) show that the percent recovery for assay of prednisolone was inside of 100±2% limit for the average of each level. Each individual sample recovery was inside of 98-102% limit. The recovery of the RP-HPLC method for the assay of prednisolone was demonstrated by a mean of 99.65%.

Name	Treatment	% Degradation	% Prednisolone	R.RT to prednisolone
Acid Degradation for (2days)	API	6.4	93.6	0.138(3.3%), 0.52, 0.55, 0.6, 0.626, 0.85, 0.91, 1.07, 1.365, 1.44, 2.24
	Spiked placebo	6.4	93.6	0.138(2.6%), 0.51, 0.55, 0.623, 0.85, 1.073, 1.29, 1.365, 1.43, 2.24
Base Degradation for (day)	API	100	0	0.144(2.25%), 0.16, 0.19(48%), 0.22(23.3%) , 0.28(3.5%), 0.36(12.2%), 0.16, 0.73, 0.88, 1.11, 1.15, 1.28, 1.33(7.8%), 1.52
	Spiked placebo	100	0	0.14(3.2%), 0.156(8.43%), 0.182(48.8%), 0.216(15.43%), 0.27, 0.357(11.59), 0.46, 0.53, 0.56, 0.61, 0.667, 0.705, 0.725, 0.86, 0.975, 1.33(6.6%), 1.41, 1.51
Peroxide Degradation for (6days)	API	57.56	42.44	0.11(2.1%), 0.172(27.2%), 0.558(7.1%), 1.093, 1.392
	Spiked placebo	56.61	43.39	0.118(2.1%), 0.172(26.3%), 0.558(27.3%), 1.082(0.31%), 1.38

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Light Degradation for (6hr)	API	11.32	88.68	0.34, 0.43(1.7%), 0.55(1.35%), 0.595(2.0%), 0.629(1.84%), 0.66, 0.703, 0.845, 0.936, 1.2(1.15%), 1.16, 1.27, 1.33, 1.36
	Spiked placebo	9	91.4	0.34, 0.436, 0.55, 0.595(1.81%), 0.62(1.75%), 0.66, 0.82, 0.846, 0.923, 1.075(1%), 1.18, 1.276, 1.36
Heated Degradation for (4hr at 90℃)	API	9.2	93.48	0.17(1.49%), 0.27(0.81%), 0.555, 0.63(0.83%), 0.73 0.85, 1.76(0.71%), 1.37(1.399%)
	Spiked placebo	12.71	87.29	0.085(1.8%), 0.142, 0.173, 0.228, 0.28, 0.55(2.2%), 0.68(2.15%), 0.846, 1.074(0.899%), 1.367(3.02%), 2.24

Table 4: *Results of forced degradation for prednisolone.*

The results for the evaluation the linearity of prednisolone at assay level were given in table (1) shown that. The correlation coefficient of 0.99979 which was acceptable –the slope was (41958) and the Y-intercept was (11894.17). The regression equation for the graph was computed and found to be: $y=41958x + 11894\rightarrow[3]$, where x is concentration in μg/mL. The regression statistics was performed by Excel and SPSS, the Y-intercept was 0.52% compared to the calculated Y-value at 100% standard (250μg/mL) and the acceptance criteria was ≤2.0%.The residual standard deviation was 0.56% (standard error compared to calculated Y-value at 100% level) and acceptance criteria was ≤2.0%.

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The results in table (1) show that the percent recovery for assay of prednisolone was inside of 100±2% limit for the average of each level. Each individual sample recovery was inside of 98-102% limit. The recovery of the RP-HPLC method for the assay of prednisolone was demonstrated by a mean of 99.65%.

Theresults in table (1) show that,the recovery ofthe individual and average of each degraded level was within the range of75% - 125% [20] at each concentration level.

The lowest level (LOD) with a signal-to-noise ratio greater than or equal to three was determined to be 0.0015% (0.0074μg/mL) to the concentration of 0.5mg/mL for related substance. The lowest limit of quantitation (LOQ) was determined to be 0.005% (0.025μg/mL) to the concentration of 0.5mg/mL for related substances.

Statistical comparison between the results obtained by the proposed method and reference method (B.P 2007) showed no significant difference concerning accuracy and precision as shown in table (5).

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Statistical terms	Reference method	Proposed method
	96.86	97.09
	97.16	98.46
	98.65	96.42
	96.9	97.39
	96.9	97.09
X⁻	97.294	97.29
± S.D	0.767385	0.744278
% R.S.D	0.788728	0.76501
N	5	5
F test (6.388)	1.063056	
t(2.306)	0.008367	

Table 5: *Test of significance for the proposed HPLC method and the reference method for assay of prednisolone tablet compared with reference method (B.P)*

Statistical comparison between the results of degradants of prednisolone tablets was obtained by the proposed RP-HPLC method and the reference method [18].

"In the chromatogram obtained with test solution (0.5mg/mL), the area of any secondary peak is not greater than the area of the principle peak in the chromatogram obtained with solution (0.005mg/mL)(1%). The sum of the areas of any secondary peak is not greater than three times the area of the principle peak in the chromatogram obtained with solution (0.005mg/mL)(3%). Disregard any peaks with an area less than 0.05 times the area of the principle peak in the chromatogram obtained with solution (0.005mg/mL) and any peak with a retention time of 3 minutes or less '' [18].

The results in Table (6) and Figure (2,3) show that, the level of unknown 1,2,3, prednisone and hydrocortisone (degradants) did not exceed 1% and complied with B.P. 2010 standards. The level of unknown 4 and 5 exceed 1% and did not complied with B.P standards. The sum of the area of any secondary peaks was more than 3% and sample did not complied with B.P 2010 standards. The results obtained by two methods did not complied with B.P standards.

Sample	Name	Reference method (IP)		Proposed method	
		R.R.T	Conc%	R.R.T	Conc%
1	un-known 1	0.49	0.567	0.53	0.541
2			0.583		0.518
3			0.551		0.515
4			0.554		0.523
5			0.567		0.492
X̄=			0.5644		0.5178
%RSD=			2.253732		3.398667

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F test(0.156)		0.522441			
t (2.365)		4.798771			
1	predni-sone	0.83	0.263	0.83	0.26
2			0.263		0.259
3			0.254		0.259
4			0.248		0.561
5			0.247		0.263
X̄ =			0.255		0.3204
%RSD=			3.050265		41.98174
F test(0.1565)		0.003344			
t (2.776)		1.085388			
1	hydro-cortison	1.104	0.305	1.07	0.341
2			0.296		0.338
3			0.297		0.341
4			0.303		0.336
5			0.303		0.344
X̄ =			0.3008		0.34
%RSD=			1.338073		0.906531
F test(6.388)		1.705263			
t (2.365)		17.29038			
1	un-known 2	1.26	0.108	1.37	0.1
2			0.109		0.099
3			0.106		0.103

Table 6: *Tests of significance of the proposed RP-HPLC method for degradation products of prednisolone tablet compared with reference method*

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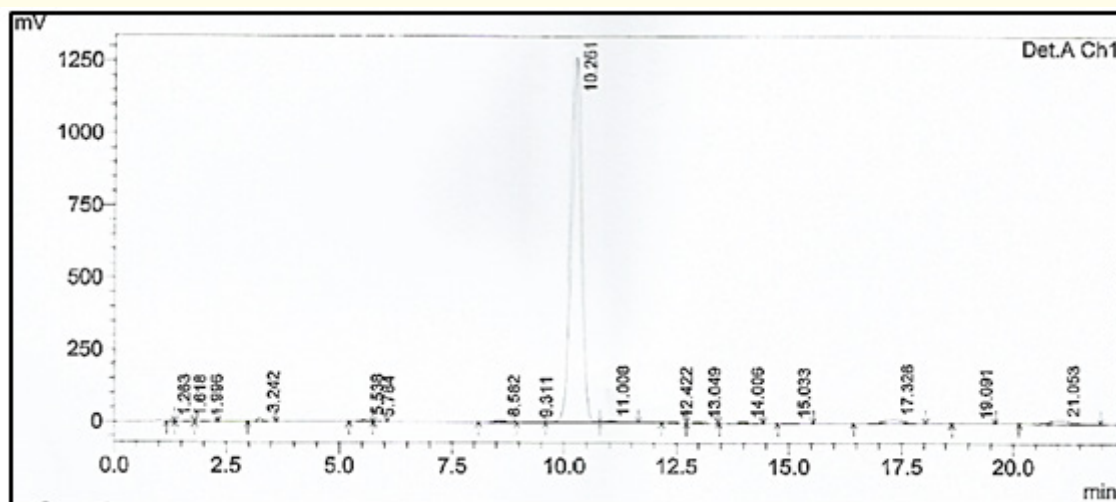
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Figure 2: Chromatogram of degradation products of prednisolone sample analyzed by the proposed method.
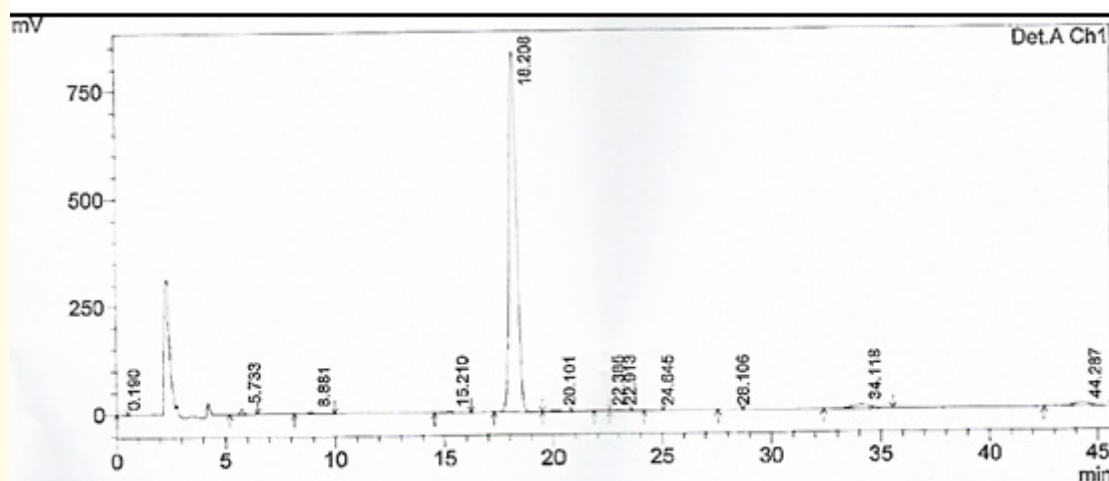

Figure 3: Chromatogram of degradation products of prednisolone sample analyzed B.P method.

Conclusion

1. The proposed method was found to be linear at assay level of prednisolone in the range of 25μ/mL to 75μ/mL and linear at low-level of prednisolone in the range of 0.25μ/mL to 7.5μ/mL.
2. The proposed method was found to be precise, intermediate precise and accurate at assay level and at low-level of prednisolone.
3. All degradation products formed during forced degradation studies were well separated from the prednisolone peak and the degradation products were separated from each other, demonstrating that the new method was specific and stability-indicating.
4. The new method was shown to be robust.

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5. The standard solution and sample solution were stable for 48 hours at room temperature.The 100% standard solution or sample solution could be used to quantitate the levels of degradation products of prednisolone.

6. Test significance for the result obtained by the new method compared to reference method. Thetest ascertained that the new method was precise and accurate as the reference method.

7. Test of significance for the % levels of the degradation products were obtained by the proposed method compared to the reference method were significant difference but practically no significant difference.

8. The LOD and LOQ were found to be 0.0074μg/mL and 0.025μg/mL,respectively.

9. The assay and degradation product were determined by single method.

10. The RP-HPLC proposed method for determination of prednisolone and its degradation products can be used in the routine analysis and quality controls of prednisolone in tablets.

11. The RP_HPLC proposed method for prednisolone can be used for stability studies the prednisolone in tablet.

Bibliography

1. Ashok R., *et al.* "Development and Validation of analytical method for estimation of prednisolone in bulk and tablets using UV-visible spectosiopy". *Journal of Pharmaceutical Sciences* 3.4 (2011): 184-186.

2. Mohamed., *et al.* "Spectrophotometric determination of binary mixtures of prednisolone with some antibiotics". *Thai Journal of Pharmaceutical Sciences* 30 (2006): 63-81.

3. Shareef Nada B. "Direct determination of prednisolone by derivative UV-Spectroscopy". *ISC Journal* 3.19 (2008): 38-46.

4. Kashyap., *et al.* "Development and validation of UV_Spectroscopy method for the estimation of prednisolone in bulk and dosage form". *Journal of chemical and pharmaceutical research* 4.2 (2012): 1090-1096.

5. G Singh., *et al.* "Q-Absorbance Ratio Spectrophotometric Method for the simultaneous estimation of prednisolone and 5-Amino salicylic acid in tablet dosage form". *Journal of applied pharmaceutical science* 2.7 (2012): 222-226.

6. Yazbi L., et al. "National center for biotechnology information U.S. National library of medicine 8600Rockville Pike, Bethesda ND, 20894 USA. J. assoc off and chem. 69.4 (1986): 614-800.

7. Chitlange Sohan S., *et al.* "Development and Validation of spectrophoto metric and HPLC method for the simuitaneous estimation of saibutamol sulphato and prenisolone in tablet dosage form". *Journal of Analytical Bioanalytical Techniques* 2.1 (2011): 1-5.

8. Kwan LC and Schott H. "Colorimetric prednisolone assay and it application to dissolution studies". *Journal of Pharmaceutical Sciences* 73.2 (1984): 157-161.

9. Metha Astha and Thaker Anil. "Validated HPTLC method for assay of prednisolone in tablet and comparison with pharmacopeial methods". *Journal of planar chromatography-Modern TLC* 23.3 (2010): 208-211.

10. Cheng Mary H., et al. "Determination of prednisolone and U metabohtiy inplasmes. Clin. Chem. 34.9 (1988): 1897-1899.

11. Razzaq Syed Naeem., *et al.* "Stability indicating HPLC method for the simultaneous determination of moxifloxacin and prednisolone in pharmaceutical formulation". *Chemistry Central Journal* 6.1 (2012): 1-10.

12. Hashem H and Jira Th. "Chromatographic application on momolithic Columns: Determination of triamcinolone, prednisolone and dexamethasone in pharmaceatical tablet for mutations using solid phase extraction and a monolithic column". *Chromatographia* 61.3 (2005): 133-136.

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13. Rojanarati Theerasak and Thailanid. "ECO-Friendly, operator-SAFE AND cost-Effective RP-HPLC method for stability indicating assay of prednisolone tablets using ethanol :water as mobile phase". *International journal of pharmacy and pharmaceutical science* 4.1 (2012): 444-448.

14. Gai Maria Nella., *et al.* "Determination of prednisolone and prednisone in plasma, whole blood, urine, and bound to-plasma proteins by high-performance liquid chromatography". *Journal of chromatographic science* 43.4 (2005): 201-206.

15. Hartley R. and Brockle bank JT. "Determination of prednisolone in plasma by HPLC". *Chromatography B: Biomedical science and applications* 232.2 (1982): 406-412.

16. Rose James Q and Jusko William J. "Corticosteroid analysis in biological fluids by HPLC". *Journal of chromatography* 162.3 (1979): 273-280.

17. Dekker D and Beijnen JH. "Improved high-performance liquid chromatographic separation of decomposition products of prednisolone by adding sulphite to the mobile phase. Journal of chromatography 193.3 (1980): 480-482.

18. B.P. British Pharmacopoeia. "Published on the Recommendation of the Commission". (2010): 2476-3026.

19. Stuber and Muller, K H. Pharm. Acta Hei 55 (1980) 171.

20. Bliesner David M. "Validating chromatographic methods A practical guide". The United States of America: Wiley-inter science (2006): 304.