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SIMULTANEOUS DETERMINATION OF PREDNISOLONE AND ASPIRIN IN SYNTHETIC MIXTURE BY VIERORDT´s METHOD

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Keywords:

Prednisolone (PRE), Aspirin (ASP), Simultaneous Equation Method (Vierordt´s Method), ICH (International Council for Harmonization), Analytical Method Validation

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ABSTRACT: Prednisolone (10mg/day) plus low dose Aspirin (80mg/day) improve the implantation rate in women with autoimmune condition who are undergoing *in-vitro* fertilization. A new, simple, precise, accurate, and validated simultaneous equation method (Vierordt´s Method) has been developed for simultaneous determination of Prednisolone and Aspirin in the synthetic mixture. The method was based on the measurement of absorbance of both components at their λ_{max} 243 nm and 226 nm of Prednisolone and Aspirin in methanol as solvent correspondingly. Linearity was obtained over a range of 2-6 µg/mL for Prednisone and 16-48 µg/mL for Aspirin. The percentage recovery obtained for Prednisolone and Aspirin was found to be in the range 98.85% to 99.95% and 99.41% to 99.74%, respectively. The results of the proposed method were validated for linearity, precision, accuracy, robustness, ruggedness according to ICH guideline Q2(R1). The developed method can be successfully be applied for simultaneous estimation of drugs in all commercial products.

INTRODUCTION: Prednisolone is chemically 11β, 17, 21-Trihydroxypregna-1,4-diene-3,20-dione is well known Glucocorticoid. It is official in British Pharmacopoeia (BP) and Indian Pharmacopeia (IP). PRE is estimated by a spectrophotometric method as per IP and BP [1-2]. It is indicated for the treatment of a wide range of inflammatory and auto-immune diseases such as asthma, multiple sclerosis, rheumatoid arthritis, autoimmune hepatitis, *etc*. [3-5] On an extensive survey of the literature, several analytical methods such as UV spectroscopy [6-12], RP-HPLC method [12-15], GLS and chemical ionization mass spectrometry [16], LC-MS/MS [17] have been reported for estimation of Prednisolone in bulk and pharmaceutical dosage form and also in synthetic mixture.

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Aspirin is chemically 2-(Acetyloxy) benzoic acid and it indicated as antipyretic, analgesic, anti-inflammatory. It is official in British Pharmacopeia, Indian Pharmacopeia and United State Pharmacopeia (USP). Official method describes Titration method for its determination [1, 2, 18]. Literature survey reveals UV [19-22] and HPLC [23-26] method for determination of Aspirin in bulk and pharmaceutical dosage form and also in synthetic mixture. Prednisolone (10mg/day) plus low dose Aspirin (80mg/day) improve the implantation rate in women with autoimmune condition who are undergoing *in-vitro* fertilization [27-29].

The combination of these two drugs is not official in any pharmacopeia; hence no official method available for the simultaneous estimation of Prednisolone and Aspirin in their combined dosage forms. Literature survey does not reveal any spectrophotomertic method for simultaneous estimation of Prednisolone and Aspirin in synthetic mixture or dosage forms. Based on above-mentioned fact, it was decided to develop and

validate a simple, new, precise, accurate for Vierordt´s method for quantification of PRE and ASP in a synthetic mixture.

MATERIALS AND METHODS:

Material: PRE and ASP were obtained as a sample for research purposes from Reliance formulation Pvt. Ltd, Ahmadabad, Gujarat. Methanol (AR-Grade) was purchased from RANKEM chemicals. Whatman filter paper no.41 (Millipore, USA) was used in the study.

Instrument and Apparatus: Analytical balance METTLER TOLEDO was used for weighing purposes. For sonication purposes, ELECTROQUIPE ultrasonic cleaner was used. SHIMADZU-1800 double beam spectrophotometer was used in the present study equipped with UV-Probe 2.42 as system software.

Preparation of standard stock solutions (100 µg/mL): An accurately weighed quantity of standard PRE (10mg) and ASP (10mg) powder were weighed and transferred to 100 ml separate volumetric flask and dissolved in methanol. The flask was shaken, and volume was made up to mark with methanol to give a solution containing 100µg/mL each of PRE and ASP.

Preparation of Sample Solution (5+40 µg/mL of PRE and ASP): As the proposed synthetic mixture is having a dose of 10 mg of PRE and 80 mg of ASP, 10 mg of PRE and 80 mg of ASP was mixed and diluted appropriately to give a mixture containing 5 µg/mL of PRE and 40 µg/mL of ASP.

Methodology: Vierordt´s method uses absorbance at two selected wavelengths, which is their λ_{max} of both components. From the overlay spectra of two drugs, it is evident that PRE and ASP λ_{max} is 243 nm and 226 nm, respectively. Five standard working solutions having concentration 2-6 µg/mL for PRE and 16-48 µg/mL for ASP were prepared in methanol and absorbance at 243 nm(λ_{max} of PRE) and 226 nm (λ_{max} of ASP) were measured and absorptivity coefficient were calculated using calibration curve. Finally, Absorbance of the mixture (sample solution) was measured at 243 nm and 226 nm, respectively.

The concentration of two drugs in the mixture can be calculated using the following equation.

$$C_x = [A_2a_{y1} - A_2a_{y2}] / [a_{x2}a_{y1} - a_{x1}a_{y2}]$$
$$C_y = [A_1a_2 - A_2a_{x1}] / [a_{x2}a_{y1} - a_{x1}a_{y2}]$$

Where A_1 and A_2 are absorbance of mixture at 243 nm and 226 nm; a_{x1} and a_{y1} are absorptivities of PRE and ASP at 243 nm; a_{x2} and a_{y2} are absorptivities of PRE and ASP at 226 nm, respectively.

Validation of the Proposed Method: The proposed method validation according to the International Conference on Harmonization (ICH) guideline [30].

Linearity (Regression Method): The calibration curves were plotted over a concentration range of 2-6 µg/mL for PRE and 16-48 µg/mL for ASP. Accurately measured aliquots of PRE (0.2, 0.3, 0.4, 0.5 and 0.6 ml) and ASP (1.6, 2.4, 3.2, 4.0 and 4.8 ml) were transferred to a series of 10 ml of volumetric flask and diluted to the mark with methanol. The absorbance's of solutions were measured at 243 and 226 nm against methanol as blank. The calibration curves were constructed by plotting absorbance versus concentration and the regression coefficient was monitored.

Method Precision (Repeatability): The precision of instrument was checked by repeated scanning and measurement of absorbance of solutions (n=6) for PRE (2-6 µg/mL) and ASP (16-48 µg/mL) without changing the parameter of the proposed spectrophotometry method.

Intermediate Precision: Intermediate precision was determined by performing intraday and interday precision. PRE that represents the overall range (2, 4, and 6 µg/mL) were analyzed on the same day at different time intervals for intraday precision and different days for interday precision. ASP that represents the overall range (16, 32, and 48 µg/mL) were analyzed on the same day at different time intervals for intraday precision and different days for interday precision.

Accuracy Study: The accuracy of the analytical method was adjudged by spiking of a blank with a standard solution. Methanol was selected as blank and was spiked at 50, 100, and 150% of target concentration (4+32 µg/mL of PRE and ASP) **Table 1**. Each spiked concentration was analyzed three times, and mean % recovery was observed at each spiked level.

TABLE 1: PREPARATION OF SOLUTION FOR ACCURACY STUDY

Level of Spiking	Quantity of Placebo (mg)	Volume of standard solution (mL)	Final dilution in 10 mL volumetric flask	Final Concentration (µg/mL)	
				PRE	ASP
Unspiked	160	0	volume make up was done	-	-
50 %	160	0.2	with methanol	2	16
100 %	160	0.4		4	32
150 %	160	0.6		6	48

Standard solution: 10 mg PRE and 80 mg ASP dissolved in 50 mL methanol, 100 µg/mL and 800 µg/mL of PRE and ASP, respectively.

Solvent Stability: Solvent stability was determined by scanning the same solution prepared in the selected solvent (methanol) at 3 different time intervals that is at 0 h, 6 h, and 24 h. Mixtures of 5 ± 40 µg/ml solutions of PRE and ASP in methanol were scanned at a selected time interval, and characteristics of spectra were compared (λ_{max}).

Assay: As the proposed synthetic mixture is having a dose of 10 mg of PRE and 80 mg of ASP, 10 mg of PRE and 80 mg of ASP was mixed and diluted appropriately to give a mixture containing 5 µg/mL of PRE and 40 µg/mL of ASP. This mixture was scanned between 200-400 nm. Absorbance was measured at selected wavelengths and was transformed to concentration with the help of linear regression equation. This mixture was analyzed for three times, and mean % assay was drawn.

RESULTS AND DISCUSSION:

Selection of Analytical Wavelength: Proper wavelength selection for estimation of both drugs depends on the nature of the drug and their solubility. For the selection of analytical wavelength solution containing 10 µg/mL of PRE and ASP were scanned individually and overlapped **Fig. 1**. The method employs solving equation based on measurement of absorbance at 243nm and 226 nm, which were selected as λ_1 and λ_2, respectively.

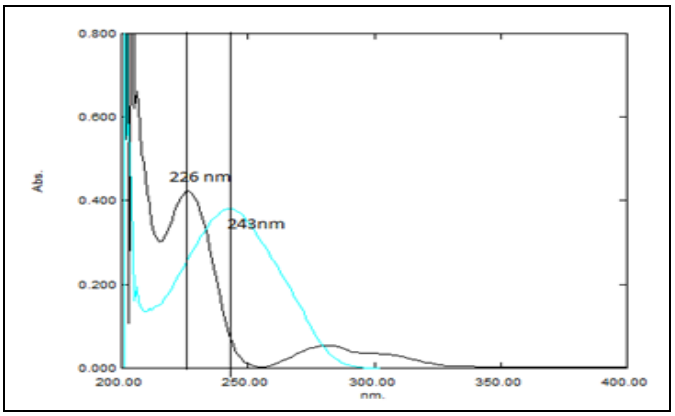

FIG. 1: SELECTION OF λ_1 (243 nm) AND λ_2 (226 nm) FOR THE VIERORDT´S METHOD

Analytical Method Validation:

Linearity and Range: The calibration curve was constructed between absorbance and concentration in the range of 2-6 µg/mL of PRE and 16-48 µg/mL of ASP **Fig. 2-4**. When the calibration curve was plotted for given concentration range **Fig. 5-8**, the value of linear regression coefficient was found to be 0.999 and 0.997 for PRE and 0.995 and 0.999 for ASP at 243 nm and 226nm, respectively.

Regression equation was found to be y = 0.034x - 0.0001 and y = 0.022x + 0.0003 for PRE and y = 0.009x + 0.0006 and y = 0.041x - 0.014 for ASP at 243 nm and 226 nm respectively **Table 2 -6**.

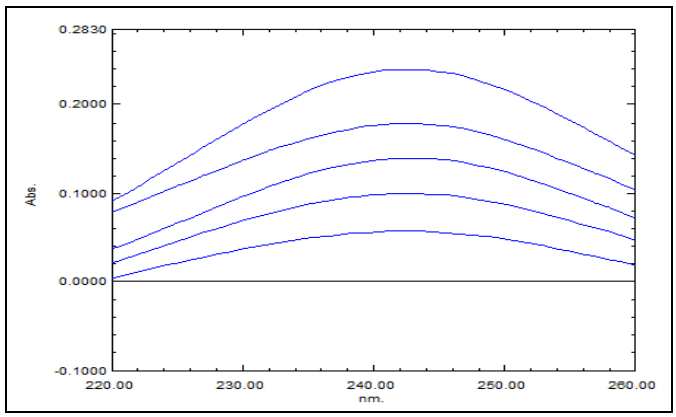

FIG. 2: OVERLAY SPECTRA OF LINEARITY OF PREDNISONE (2-6 µg/mL)

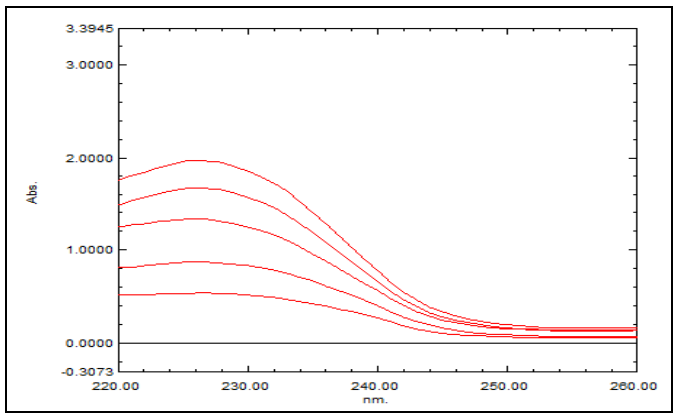

FIG. 3: OVERLAY SPECTRA OF LINEARITY OF ASPIRIN (16-48 µg/mL)

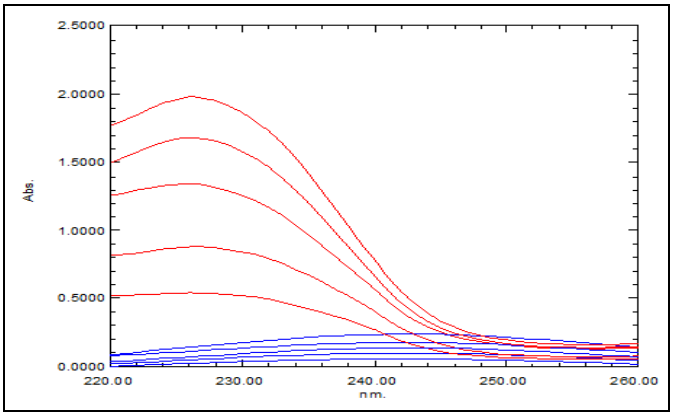

FIG. 4: OVERLAY SPECTRA OF LINEARITY OF PREDNISOLONE (2-6 µg/mL) AND ASPIRIN (16-48 µg/mL)

TABLE 2: LINEARITY DATA OF PRE AT 226 nm

S. no.	Concentration (µg/mL)	Mean ± SD	Absorptivity
1	2	0.0442 ± 0.0003	221
2	3	0.0662 ± 0.0004	220.66
3	4	0.0923 ± 0.0004	230.75
4	5	0.1114 ± 0.0003	222.8
5	6	0.1322 ± 0.0003	220.33
-	-	Mean of Absorptivity	223.11

TABLE 3: LINEARITY DATA OF PRE AT 243 nm

S. no.	Concentration (µg/mL)	Mean ± SD	Absorptivity
1	2	0.0681 ± 0.00046	340.50
2	3	0.1037 ± 0.00043	345.66
3	4	0.1393 ± 0.00042	348.25
4	5	0.1742 ± 0.00034	348.40
5	6	0.2066 ± 0.00030	344.33
-	-	Mean of Absorptivity	345.43

TABLE 4: LINEARITY DATA OF ASP AT 226 nm

S. no.	Concentration (µg/mL)	Mean ± SD	Absorptivity
1	16	0.651 ± 0.00079	406.87
2	24	0.978 ± 0.00080	407.50
3	32	1.321 ± 0.00085	412.81
4	40	1.674 ± 0.00033	418.50
5	48	1.970 ± 0.00030	410.41
-	-	Mean of Absorptivity	411.22

TABLE 4: LINEARITY DATA OF ASP AT 243 nm

S. no.	Concentration (µg/mL)	Mean ± SD	Absorptivity
1	16	0.158 ± 0.00024	98.75
2	24	0.236 ± 0.00030	98.33
3	32	0.332 ± 0.00039	103.75
4	40	0.397 ± 0.00040	99.25
5	48	0.467 ± 0.00032	97.29
-	-	Mean of Absorptivity	99.47

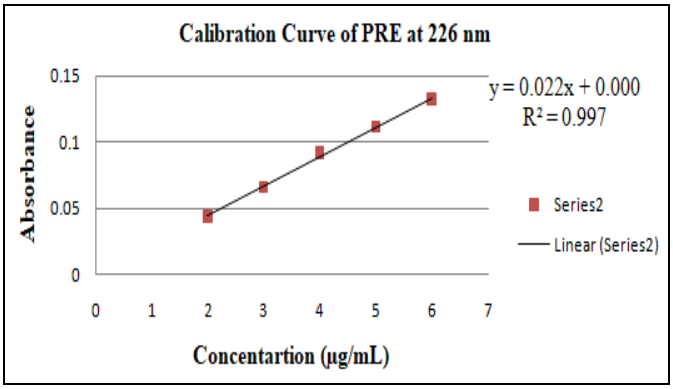

FIG. 5: CALIBRATION CURVE OF PRE (2 - 6 µg/mL) AT 226 nm

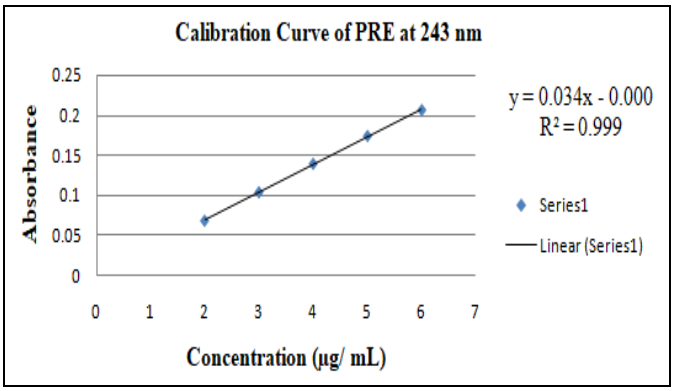

FIG. 6: CALIBRATION CURVE OF PRE (2 - 6 µg/mL) AT 243 nm

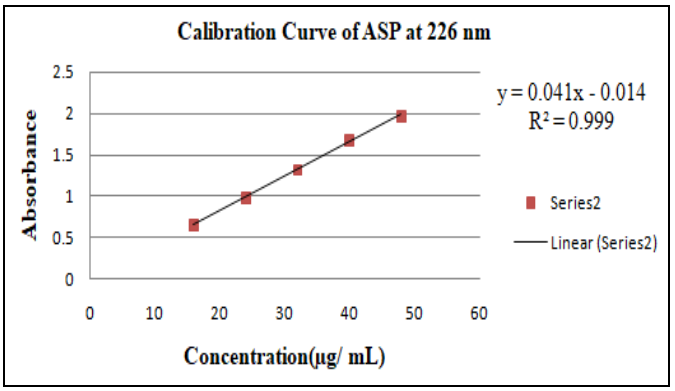

FIG. 7: CALIBRATION CURVE OF ASP (16 - 48 µg/mL) AT 226 nm

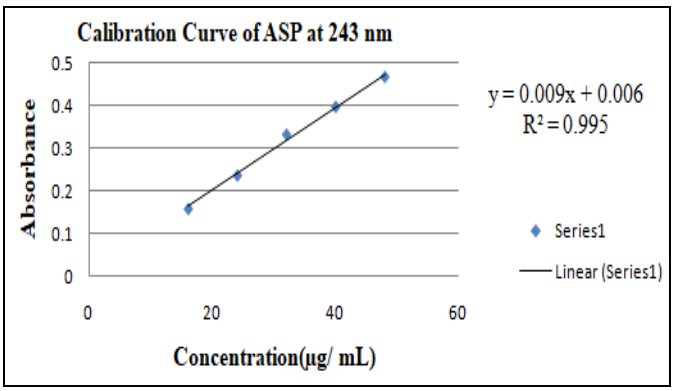

FIG. 8: CALIBRATION CURVE OF ASP (16 - 48 µg/mL) AT 243 nm

TABLE 6: LINEARITY DATA OF ASP AND PRE

Parameter	Prednisolone		Aspirin	
	At 243 nm	At 226 nm	At 226 nm	At 243 nm
Linearity range	2-6 µg/mL	2-6 µg/mL	16-48 µg/mL	16-48 µg/mL
Regression equation	Y=0.034x-0.0001	Y=0.022x+ 0.0003	Y=0.041x -0.014	Y=0.009x + 0.006
Correlation coeffient (r^2)	0.999	0.997	0.999	0.995
Intercept	0.0001	0.0003	0.014	0.006
Slope	0.034	0.022	0.041	0.009

Method Precision (Repeatability): When all PRE and ASP were analyzed at all concentration, calculated relative standard deviation at each level was found to be less than 2, so that method was found to be repeatable over the range of 2-6 µg/mL for PRE and 16-48 µg/mL for ASP **Table 7-10**.

TABLE 7: REPEATABILITY DATA OF PRE AT 243 nm

S. no.	Concentration (µg/mL)				
	2	3	4	5	6
1	0.0678	0.1034	0.1389	0.1736	0.2071
2	0.0681	0.1045	0.1396	0.1742	0.2068
3	0.0689	0.1037	0.1397	0.1744	0.2062
4	0.0683	0.1034	0.1394	0.1746	0.2063
5	0.0676	0.1036	0.1388	0.1739	0.2069
6	0.0684	0.1041	0.1398	0.1746	0.2064
MEAN	0.0681	0.1037	0.1393	0.1742	0.2066
SD	0.00046	0.00043	0.00042	0.00040	0.00036
RSD	0.677	0.419	0.303	0.230	0.176

(n = 6 determinations)

TABLE 8: REPEATABILITY DATA OF PRE AT 226 nm

S. no.	Concentration (µg/mL)				
	2	3	4	5	6
1	0.0438	0.0656	0.0919	0.1109	0.1318
2	0.0444	0.0663	0.0926	0.1112	0.1326
3	0.0441	0.0667	0.0923	0.1116	0.1319
4	0.0446	0.0664	0.0918	0.1115	0.1323
5	0.0439	0.0659	0.0924	0.1114	0.1324
6	0.0445	0.0666	0.0929	0.1119	0.1323
MEAN	0.0442	0.0662	0.0923	0.1114	0.1322
SD	0.00033	0.00042	0.00041	0.00034	0.00030
RSD	0.748	0.638	0.451	0.307	0.231

(n = 6 determinations)

TABLE 9: REPEATABILITY DATA OF ASP AT 243 nm

S. no.	Concentration (µg/mL)				
	16	24	32	40	48
1	0.1584	0.2361	0.3323	0.3979	0.4671
2	0.1581	0.2366	0.3331	0.3981	0.4673
3	0.1586	0.2363	0.3321	0.3971	0.4679
4	0.1582	0.2364	0.3327	0.3973	0.4678
5	0.1579	0.2366	0.3326	0.3974	0.4674
6	0.1582	0.237	0.3321	0.3972	0.4672
MEAN	0.1582	0.2365	0.3324	0.3975	0.4674
SD	0.00024	0.00030	0.00039	0.00040	0.00032
RSD	0.153	0.131	0.117	0.101	0.069

(n = 6 determinations)

TABLE 10: REPEATABILITY DATA OF ASP AT 226 nm

S. no.	Concentration (µg/mL)				
	16	24	32	40	48
1	0.6498	0.9772	1.3199	1.6736	1.9704
2	0.6514	0.9779	1.3212	1.6744	1.9703
3	0.6509	0.9784	1.322	1.6741	1.9698
4	0.6519	0.9774	1.3224	1.6743	1.9706

5	0.6517	0.9789	1.3214	1.6739	1.9699
6	0.6518	0.9792	1.3212	1.6745	1.9701
MEAN	0.6512	0.9781	1.3213	1.6741	1.9701
SD	0.00079	0.00080	0.00085	0.00033	0.00030
RSD	0.122	0.082	0.064	0.020	0.015

(n = 6 determinations)

Intermediate Precision: For determining interday and intraday precision, RSD was monitored at the selected concentration level, which was found to be less than 2, so the method was found to be precise for estimation of PRE and ASP **Table 11-14**.

TABLE 11: INTRADAY AND INTER - DAY PRECISION DATA OF PRE AT 243 nm

Concentration (µg/ml)	Intraday Mean ± SD	RSD	Inter-Day Mean ± SD	RSD
2	0.0682 ± 0.0005	0.83	0.0679 ± 0.0008	1.18
4	0.1393 ± 0.0006	0.48	0.1392 ± 0.0010	0.74
6	0.2067± 0.0004	0.22	0.2066 ± 0.0007	0.36

(n = 3 determinations)

TABLE 12: INTRADAY AND INTER - DAY PRECISION DATA OF PRE AT 226 nm

Concentration (µg/ml)	Intraday Mean ± SD	RSD	Inter-Day Mean ± SD	RSD
2	0.0441± 0.0003	0.68	0.0441 ± 0.0003	0.85
4	0.0922 ± 0.0003	0.38	0.0922 ± 0.0004	0.49
6	0.1321± 0.0004	0.32	0.1319± 0.0005	0.41

(n = 3 determinations)

TABLE 13: INTRADAY AND INTER - DAY PRECISION DATA OF ASP AT 243 nm

Concentration (µg/ml)	Intraday Mean ± SD	RSD	Inter-Day Mean ± SD	RSD
16	0.1583 ± 0.0002	0.15	0.1584 ± 0.0005	0.36
32	0.3326 ± 0.0004	0.12	0.3323 ± 0.0006	0.20
48	0.04674± 0.0004	0.08	0.4675 ± 0.007	0.14

(n = 3 determinations)

TABLE 14: INTRADAY AND INTER - DAY PRECISION DATA OF ASP AT 226 nm

Concentration (µg/ml)	Intraday Mean ± SD	RSD	Inter-Day Mean ± SD	RSD
16	0.6505 ± 0.0011	0.16	0.6503 ± 0.0013	0.21
32	1.321 ± 0.0013	0.10	1.3211 ± 0.0018	0.14
48	1.9698± 0.0015	0.08	1.9699 ± 0.002	0.11

(n = 3 determinations)

Accuracy Study: Spiked blank with standard solution at 50, 100, and 150% level was analyzed for % recovery which was found within 98 to 102, so the method was found to be accurate **Table 15**.

TABLE 15: ACCURACY DATA OF PRE AND ASP AT 50, 100 AND 150% OF TARGET CONCENTRATION

Level of spiking	Quantity of placebo (mg)	Amount of std. drug added (µg/mL)		Amount of drug recovered (µg/mL)		% Recovery	
		PRE	ASP	PRE	ASP	PRE	ASP
Unspiked	160	-	-	-	-	-	-
50 %	160	2	16	1.977±0.0052	15.906±0.048	98.85±0.26	99.41±0.30
100 %	160	4	32	3.981±0.0068	31.895±0.039	99.53±0.17	99.67±0.12
150 %	160	6	48	5.973±0.0017	47.870±0.061	99.55±0.28	99.74±0.12

(n = 3 determination for each set)

Solvent Stability: As the $\lambda_{max\ was}$ stable over a period of 24 hrs, the solvent was found to be suitable, and the drug was found to be stable.

Assay: When the prepared synthetic mixture was analyzed by a developed and validated method, % assay was found to be 99.26 ± 1.17 for PRE and for 99.33 ± 0.32 ASP **Table 16**.

TABLE 16: DETERMINATION OF PRE AND ASP FROM SYNTHETIC MIXTURE

Drug	Amount taken (µg/mL)	Amount found (µg/mL)	% Assay
PRE	5	4.91 ± 0.11	99.26 ± 1.17
ASP	40	39.71 ± 0.19	99.33 ± 0.32

(n = 3 determinations)

SUMMARY AND CONCLUSION: Vierordt´s method was developed and validated as per ICH Q2 R1 guidelines and was successfully applied for the determination of PRE and ASP from its synthetic mixture. The current developed and validated method was found to be simple, new, precise, and accurate.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

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