Original Article

Spectrophotometric Determination of Prednisolone Drug Using Cloud Point Extraction and pharmaceutical application

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DOI: 10.47750/pnr.2022.13.S01.23

Abstract

In this paper, describes a separation and Preconcentration method for the determination of Prednisolone. extract Prednisolone (Pred) in the form of an ionic bonding complex, and the complexing agent FeCl3.6H2O was used in this compound UV-VIS wavelength spectrum to confirm its structural formula on its greatest wave 390.5 nm Optimum conditions for extraction and spectroscopic determination of Pred were found: Pred concentration and reagent (100 µg.ml-1) (1×10-3M) respectively, Triton-114 V/V (10%) . and heating (10min), centrifugal speed of 4000 r/min , the linear concentrations range that obeyed Beer's is (2-18 µg.ml-1) and value correlation coefficient R2 = 0.995 , limit of detection LOD (0.0293 µg · ml-1), limit of quantification LOQ (0.0888 µg · ml-1), and mole ratio were studied in order to optimize the reaction conditions. The mole ratio for the composition of product is (1:3). molar absorption coefficient (21798 l mol-1. cm) , Sandel sensitivity (0.0222 µg.cm-2).

Keywords: Prednisolone, ionic bonding complex, UV-VIS wavelength spectrum.

INTRODUCTION

There are several techniques used in the preparation of the sample, but the most popular ones are liquid–liquid extraction (LLE) and solid-phase extraction (SPE). Classical LLE consumes large quantities of organic solvents and SPE is time-consuming and relatively expensive. Therefore, there is an urgent need for a new technique that can be fast, reliable and environment-friendly [1]. Modern analytical techniques should be simple, ecologically safe, sensitive and selective [2].Cloud point extraction(CPE), a promising extraction method [3] . The advantages and disadvantages of the (CPE) advantages (Low use of organic solvents, large selection of sorbents, universal, possibilities of process automatization and miniaturization, possibilities to analyze complex matrix) disadvantages (Poor reproducibility) [4][5].

Prednisolone (11β,17,21-trihydroxypregna-1,4-diene-3,20-dione) is a synthetic glucocorticoid, a class of steroid hormones, which is produced by the adrenal gland and is known for its anti-inflammatory and immunosuppressive actions [6]. prednisolone are widely used synthetic corticosteroids in inflammatory conditions, neoplastic diseases, asthma, and as immunosuppressive agents. Prednisone is both a prodrug and a metabolite of the active drug prednisolone[7]. molecular weight =360.4 , molecular formula C21H28O5 , describes the chemical composition of the Prednisolone fig .1 [8]

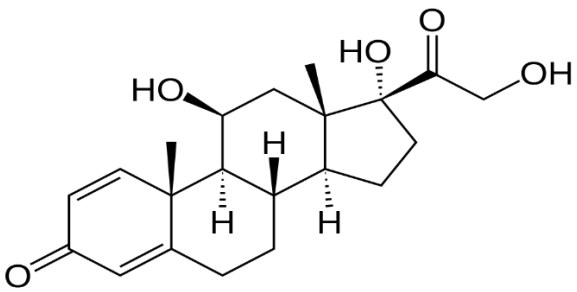

Figure 1. Chemical Structure of Prednisolone

Material and Methods:

UV-Vis spectrophotometer: SHIMADZU, Double beam UV-Vis, model UV-1800 made in Japan, The range of wavelength (190-1100) nm, quartz cell with 1cm path. Water Bath: A thermostat water bath, made in Germany. Electric Balance: Sartorius (0.0000), made in Germany. Centrifuge Typ 2002 Made in Gamany 6000 U/min & PH meter: 3310 , JENWAY

Preparation Standard Solutions:

All materials and reagent used in this work were of high purity materials. Distilled water was used to dissolve and prepare solutions. Stock solution of Prednisolone (Pred) 1000µg. ml-1 was prepared by dissolving 0.1gm in a small amount of distilled water then completed to 100ml by distilled water. Stock solution of reagent FeCl3.6H2O 1000µg. ml-1 was prepared by dissolving 0.1gm in a small amount of distilled water then completed to 100ml by distilled water. (10% V/V) Different surfactants[TritonX-114] were prepared by diluting 10 ml with distilled to 100 ml volumetric flask.1000 µg.ml-1 stock solution of interferences were prepared by dissolving the organic compound 0.1gm from the following [Starch, Aersiol , Talc] in distilled water and diluting to the mark in 100 ml volumetric flask . NaOH 0.1M was prepared by dissolving 0.4 gm in a small amount of distilled water then completed to 100ml by distilled water.

General procedure for CPE:

A typical experiment of cloud point includes the following steps: taking the volumetric flask (10ml) and use the optimum condition for complex formation. Add 0.4 ml for surfactant (10%) and complete the volume by ethanol . The content of volumetric flask is transferred to centrifuge test tube then the mixture is placed in a water bath for 10 minutes at 50°C and separated by centrifugation for 10 minutes at 4000 rpm. Test tube is taken in ice bath to increased viscosity micelles layer for 1minutes, then become easily separated. The separated sediments dissolved by 5ml of distilled water and it's the complex absorption spectrum are recorded in Fig. 2.

Reagent absorption spectrum spectrum are recorded in Fig.3.

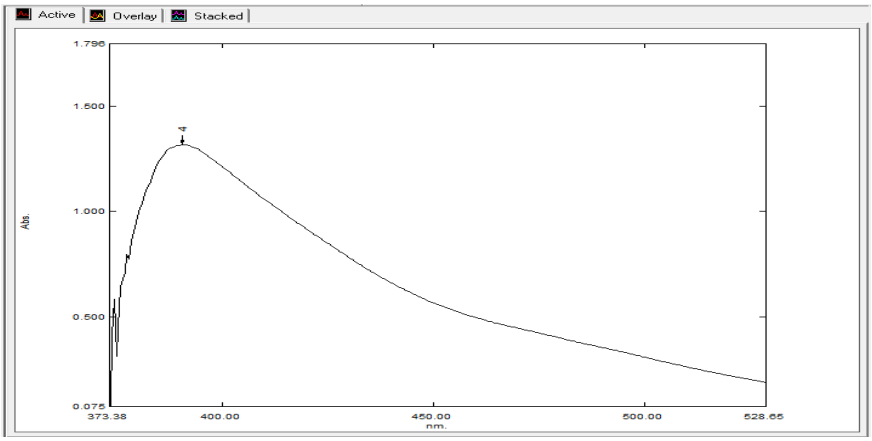

Figure 2. Absorbance spectra of the complex.

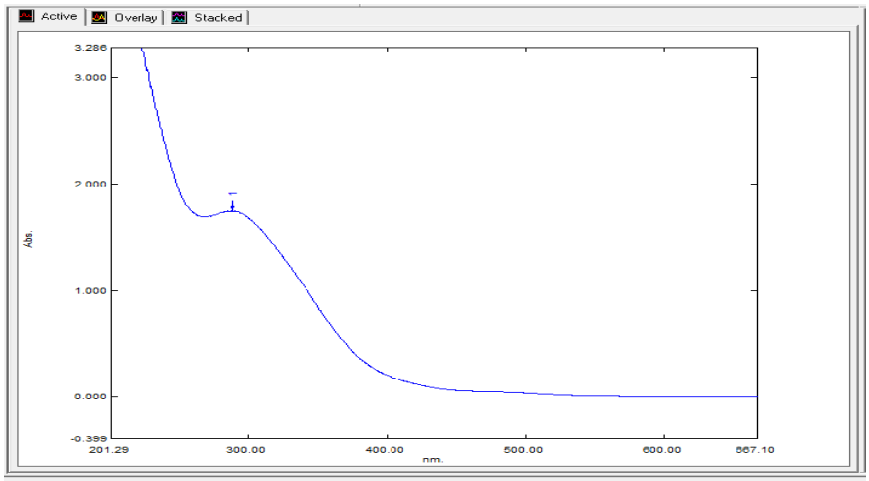

Figure3. Absorbance spectra of reagent FeCl3.6H2O

Results and Discussion:

Spectrophotometric Determination Prednisolone (Pred) of by Coupling with Reagent. Optimization Parameters for Reaction. All of the factors that affect the absorbance of complex formed are optimized to improve the sensitivity and detection limit for the determination of the drug. All optimization work under wavelength at 390.5nm.

Choosing the appropriate wavelength for the complex formed for drug Pred with regent:

The maximum wavelength was determined after conducting a series of experiments. The method that gave the highest wavelength was chosen.

This is by mixing 0.5 ml of a Pred solution of a concentration of 1 ×10-3 M with a reagent solution of 0.3 ml of a concentration of 1×10-3 M with a solution of a NaOH 0.5 ml concentration of 0.1 M, and then we add TritonX-114 0.5 ml of a concentration of 10% . The resulting colored spectrum was measured, as it gave the highest absorption at the wavelength 390.5nm.

1-Effect of Optimum Volume of (0.1M) NaOH.

The same addition for Pred is [0.5 ml drug, 0.3 ml FeCl3 ,0.5ml TritonX-114, and (0.1-0.9) ml of NaOH in 10 ml volumetric flask and the volume is completed by ethanol .Then the absorbance and the optimum volume for higher absorbance that fixed at 0.3ml for sequence experiment are measured as shown Fig. 4.

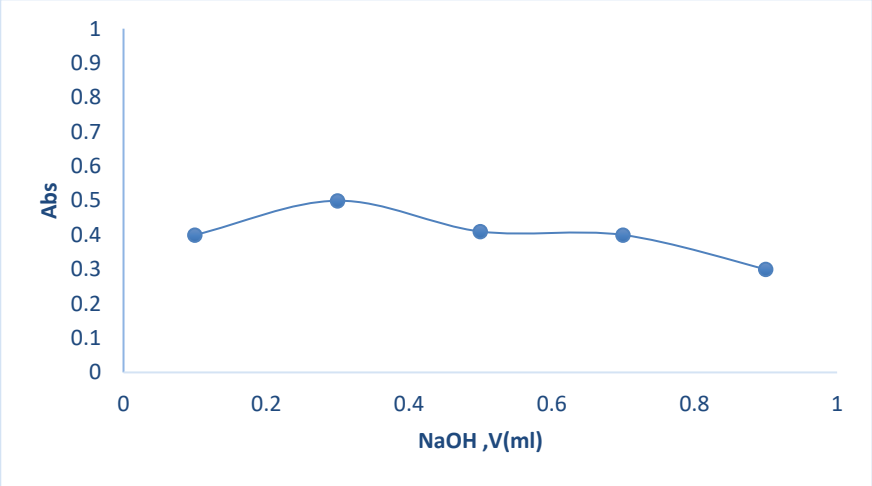

Figure 4. Different Volume of NaOH.

2-Effect of Optimum Volume of Reagent.

The same additions [0.5ml for pred , 0.5ml TritonX-114, with varying volume from (0.1-0.9) ml FeCl3 and 0.3 ml NaOH are put in volumetric flask 10 ml and the higher absorbance of optimum volume 0.5 ml at maximum wavelength is fixed for sequence experiment as shown in Fig.5.

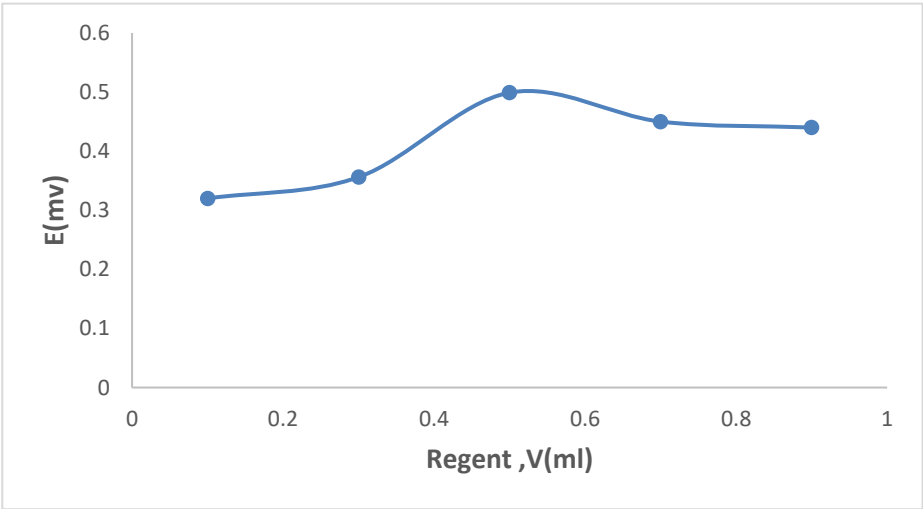

Figure 5. volume of reagent

3-Effect of Optimum Volume of TritonX-114 .

The same additions [0.5ml for Pred , with varying volume from (0.1-0.6) ml TritonX-114 and 0.5ml regent and 0.5 ml NaOH are put in volumetric flask 10 ml and the higher absorbance of optimum volume 0.4 ml at maximum wavelength is fixed for sequence experiment as shown in Fig.6.

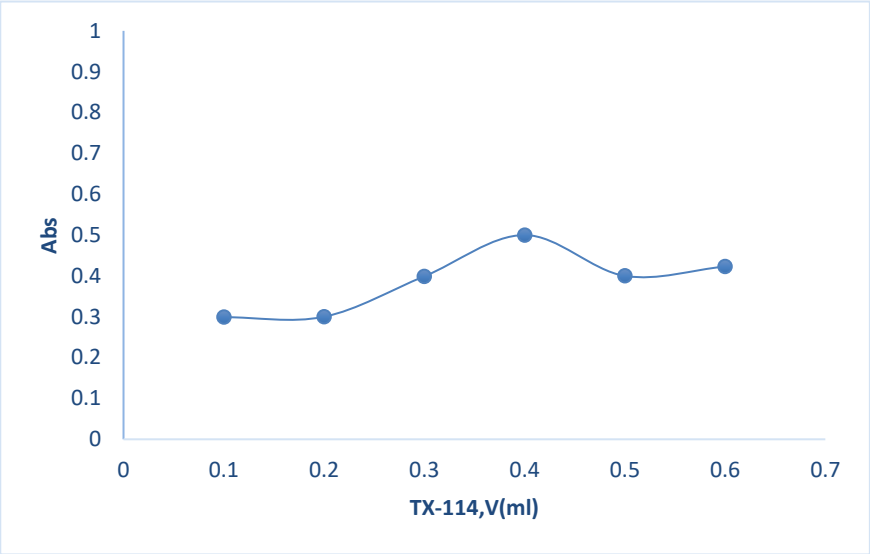

Figure 6. Different Volume of TritonX-114

4-Effect of Temperature

The rest additions are optimal conditions then diluted with ethanol in volumetric flask in 10 ml ,then the effect of different temperature on the color product have been (30-70) ℃. It is evident the best temperature is 50℃ because is give the best absorbance shown in Fig.7.

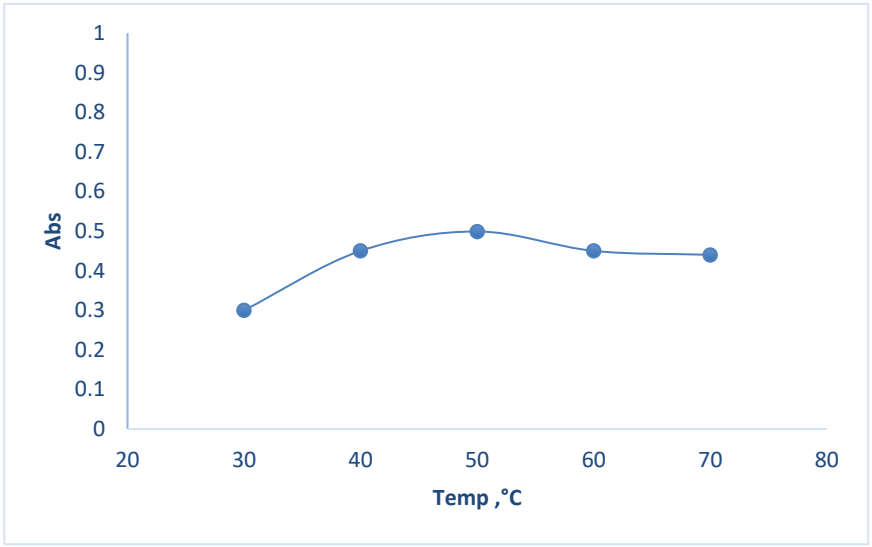

Figure 7. Effect of Temperature

5-Effect of Incubation Time

The solution [0.5ml Pred , 0.3ml NaOH ,0.5 ml FeCl3 ,0.4 ml Triton X-114] is prepared in volumetric flask 10 ml and the volume is complete by ethanol at temperature 50 ℃ and the incubation time for (5-25minutes) to form cloud point and separated by centrifugation at 4000rpm for 15 minutes, 5ml ethanol will be added and measured by UV-VIS at λmax=390.5 nm. The best of incubation time is 10 minutes as shown in the Fig.8.

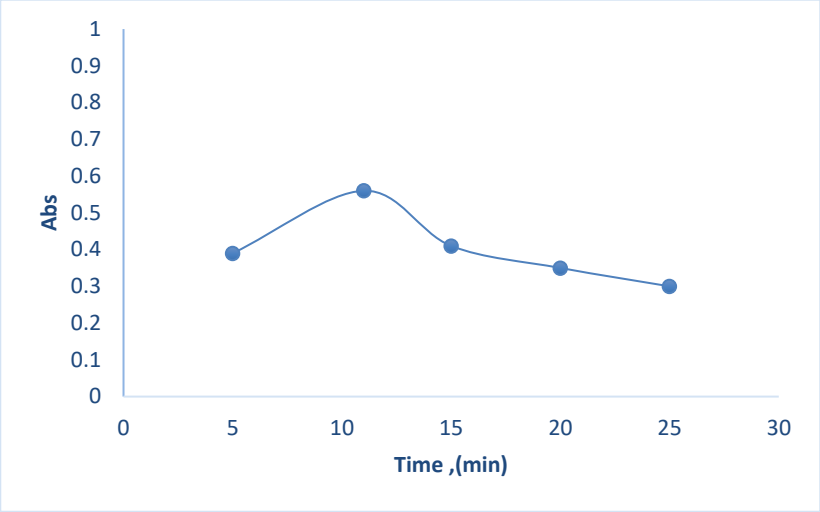

Figure 8. Absorbance of different incubation time.

6-The effect of centrifugation time:

The above general method was followed. by preparing a series of 10 ml volumetric flask, Then the solutions are placed in a centrifuge at different times (5-25)min and with a number of cycles 4000 cycl/min and complete other analytical steps. Then measure its absorbance at the specified wavelength in Fig.9.

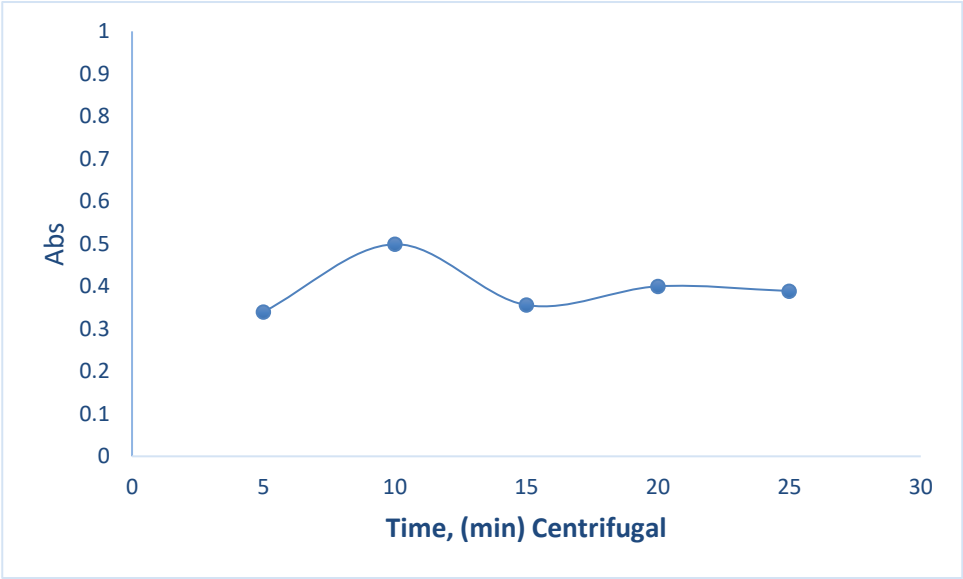

Figure 9. effect of centrifugation time with Pred

7- Effect of the Order of Addition

In this study, the effect of sequence of addition with optimum volumes depends on the same procedure is applied. The absorbance is measured and recorded in Table1. R: FeCl3 .6H2O , D: Drug (Pred), B: Base (NaOH). It is evident that the best addition is the order two because it gives the upper absorbance as shown in Fig. 9

Table1. Data of different addition of Pred

Addtion	Abs
D+R+T+B	0.35
B+D+T+R	0.5
D+R+B+T	0.42
R+D+T+B	0.451

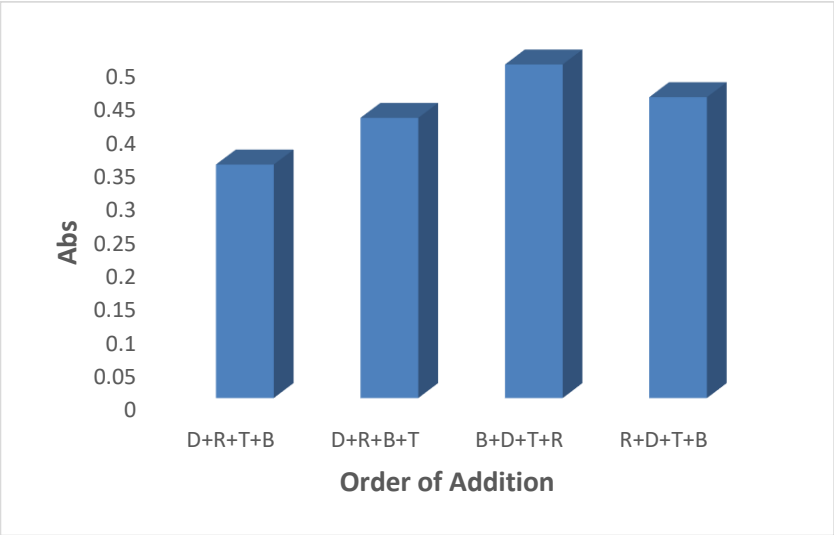

Figure (10): Different addition with Pred

Preparation of Calibration Curve

in CPE The prepared solution prepared increasing concentration(2-18 µg mL-1) by taking [0.3 ml NaOH , 0.5 ml FeCl3 ,0.4 ml Triton X114],and in volumetric flask 10 ml the volume is completed by ethanol at temperature 50°C and the incubation time for(10 minutes) to form cloud point and separated by centrifugation at 4000 rpm for 10 minutes, 5ml distilled water will be added and measured by UV-VIS at λmax = 390.5 nm and show the result in Fig (13).

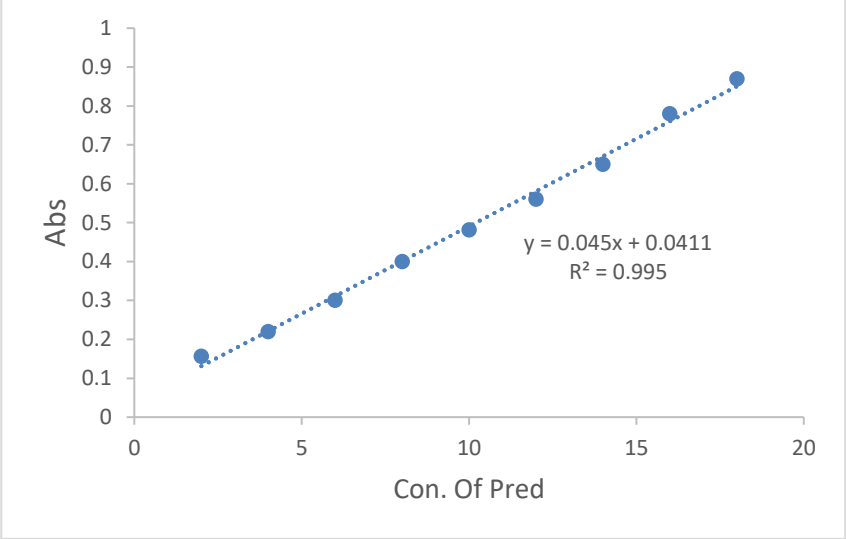

Figure 11. Calibration Curve of Pred

Stoichiometric Determination of Product Continuous Variation Method

A series of different volumes of reagent and drug are prepared (0.1-0.9) ml, with concentration (1x10-3 M) in volumetric flask 10 ml .The additions are optimal condition and the volume is complete by ethanol (10). Then absorbance is measured by UV-VIS at λmax =390.5 nm. The stoichiometric ratio between reagent [R] and drug [D] result 1:3 as shown in Table 2 and Fig .10

Table 2. Data of absorbance for Continuous Variation Method Result for Pred

Volume of Drug/ml	Volume of Reagent/ ml	Abs.
0.1	0.9	0.2
0.2	0.8	0.35
0.3	0.7	0.55
0.4	0.6	0.45
0.5	0.5	0.35
0.6	0.4	0.25

0.7	0.3	0.22
0.8	0.2	0.2
0.9	0.1	0.17

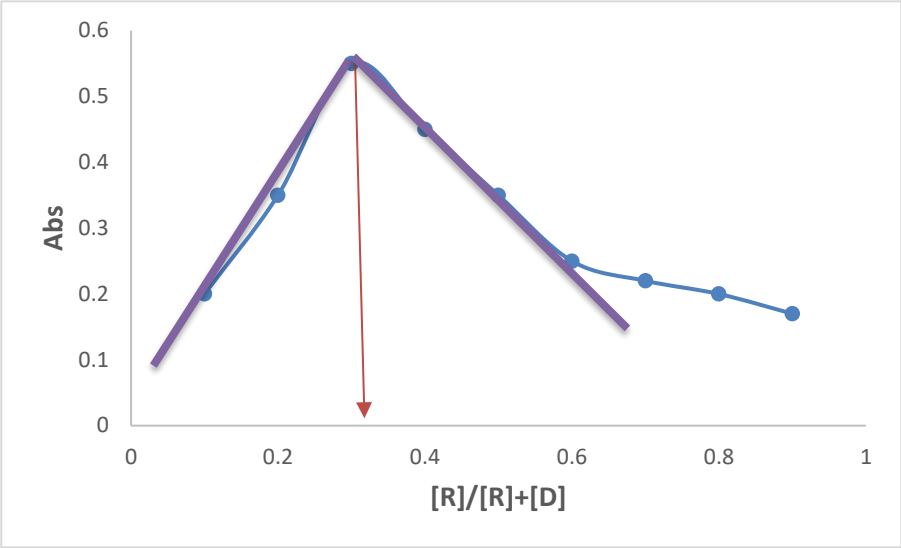

Figure 12. Continuous Variation method of Pred

Mole Ratio Method

In this method the volume of drug is fixed at I ml with concentration (1x10-3M) and the volume of reagent changes (0.2 -2 ml). The optimum of addition is completed by ethanol in volumetric flask 10 ml and the absorbance is measured by UV-VIS at λmax =390.5 nm. The stoichiometric ratio between reagent[R] and drug [D] result 1:3 as shown in Table 3 and Fig 11

Table 3. Data of Absorbance Mole Ratio Method.

Volume of Reagent/ ml	**Abs**
0.25	0.07
0.33	0.09
0.5	0.15
0.6	0.17
0.7	0.22
1.6	0.47
2.5	0.48
5	0.52

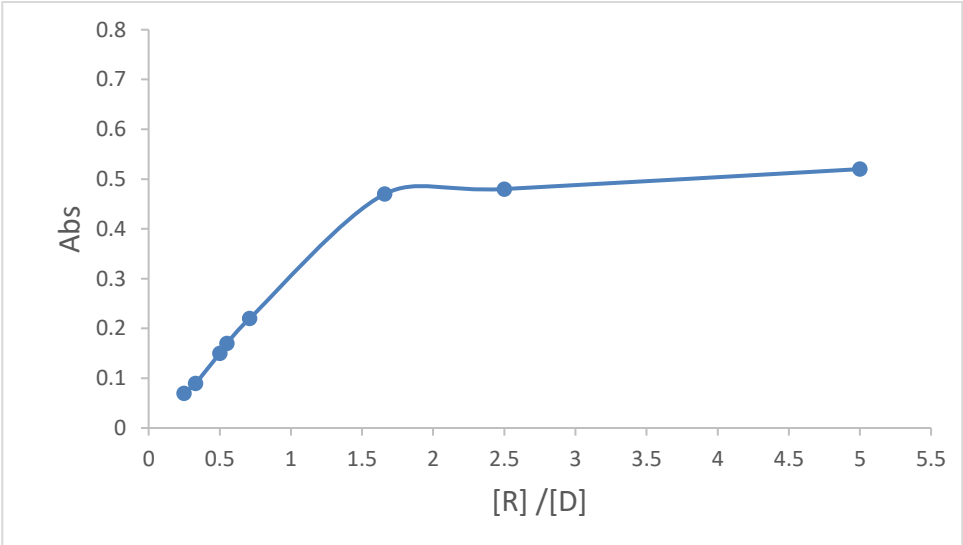

Figure 13. Mole Ratio Method of Pred.

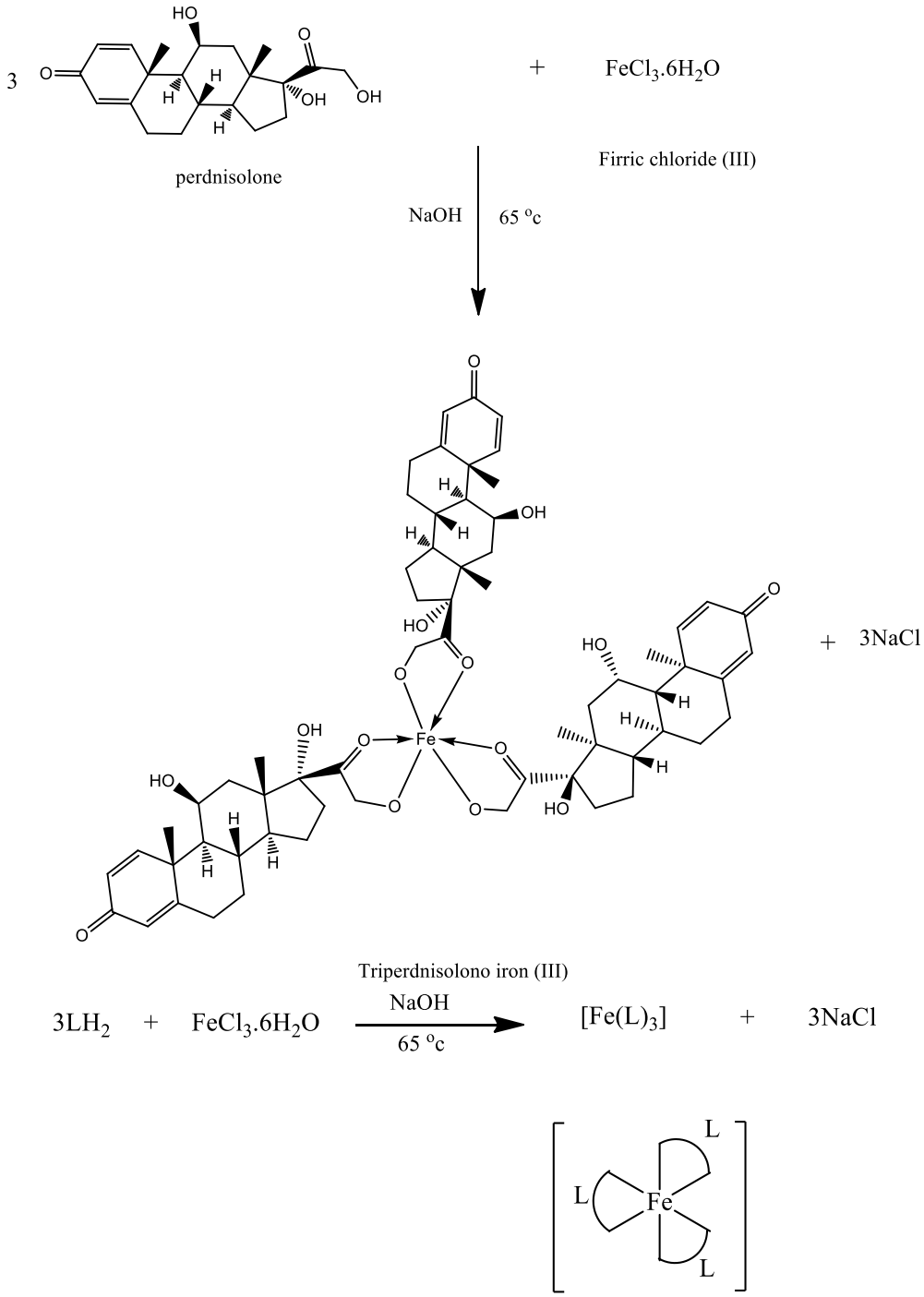

Fig.14 method composition Complex (Pred+FeCl3.6H2O) to extract (Pred).

Effect of interference

In this study effect of interference expected are present with Pred by adding 1ml (1000 ppm), and the rest of optimum addition in volumetric flask 10 ml and complete by distilled water. Then the absorbance is measured by UV-VIS.

Table 4. Data of Absorbance of interference

This result showed in Table indicate there is no interaction between interference and Pred .

Foreign compound	**% Recovery of 18 ppm of Pred ppm Foreign compound added**		
Amount in ppm	30	60	90
Aerosil	100.2	99.9	99.5
Maize Starch	99.7	100.4	98.32
Talc powder	100.8	97.9	99.8
Magnesiume Stearate	99.7	98.6	99.5

*= Average for five determination.

Application:

The proposed method is applied on (PRISOLONE 5 mg) the result is good and summarized in Table.6.

Table 6. Data of Accuracy and Precision Test

Preparative drug	**Taken µg/mL**	***Found µg/mL**	**Recovery %**	**RSD %**
PRISOLONE 5 mg	4	3.98	99.5	0.37
	10	9.99	99.9	0.85
	16	16.08	100.5	0.53

*= Average for six determination

REFERENCES

1. G. Kojro and P. Wroczyński, "Cloud point extraction in the determination of drugs in biological matrices," J. Chromatogr. Sci., vol. 58, no. 2, pp. 151–162, 2020.
2. K. Pytlakowska, V. Kozik, and M. Dabioch, "Complex-forming organic ligands in cloud-point extraction of metal ions: a review," Talanta, vol. 110, pp. 202–228, 2013.
3. J. I. Cacho, N. Campillo, P. Viñas, and M. Hernández-Córdoba, "Cloud point extraction and gas chromatography with direct microvial insert thermal desorption for the determination of haloanisoles in alcoholic beverages," Talanta, vol. 160, pp. 282–288, 2016.
4. M. Saraji and M. K. Boroujeni, "Recent developments in dispersive liquid–liquid microextraction," Anal. Bioanal. Chem., vol. 406, no. 8, pp. 2027–2066, 2014.
5. M. M. Moein, R. Said, F. Bassyouni, and M. Abdel-Rehim, "Solid phase microextraction and related techniques for drugs in biological samples," J. Anal. Methods Chem., vol. 2014, 2014.
6. M. Finšgar, A. Perva-Uzunalić, H. Behr, N. Ledinek, Z. Knez, and Z. Novak, "An improved reversed-phase high-performance liquid chromatography method for the analysis of related substances of prednisolone in active ingredient," ACS omega, vol. 5, no. 14, pp. 7987–8000, 2020.
7. M. N. Gai, E. Pinilla, C. Paulos, J. Chávez, V. Puelles, and A. Arancibia, "Determination of prednisolone and prednisone in plasma, whole blood, urine, and bound-to-plasma proteins by high-performance liquid chromatography," J. Chromatogr. Sci., vol. 43, no. 4, pp. 201–206, 2005.
8. N. B Shareef, "Direct determination of prednisolone by derivative UV spectroscopy," Rafidain J. Sci., vol. 19, no. 5, pp. 38–46, 2008.