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STABILITY STUDY OF DINOPROSTONE IN BULK POWDER AND IN PHARMACEUTICAL DOSAGE FORMULATION BY A RAPID VALIDATED STABILITY INDICATING HPLC METHOD

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ABSTRACT

A simple and rapid stability indicating HPLC method was developed and validated for the determination of dinoprostone, naturally occurring prostaglandin E2, (PGE2) in the presence of its induced degradation products. The drug was subjected to stress acidic, alkaline, oxidative and thermal stress conditions, and the stressed samples were analyzed by the proposed method. The developed method utilized Symmetry® C_{18} (75 × 4.6 mm i.d., 5µm) column in an isocratic separation mode. The mobile phase consisted of phosphate buffer (pH 4.4) - acetonitrile (67: 33, v/v) at a flow rate 1mL/min with UV-detection at 200 nm. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines. The method was applied for determination of PGE2 in pure powder and in its pharmaceutical formulation. The proposed method was applied to an accelerated stability study of the compound.

KEYWORDS

Assay, Dinoprostone, RP-HPLC, Stability and Validation.

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INTRODUCTON

Prostaglandins are important signaling molecules that are produced in many tissues and regulate many physiological functions, under both normal and patho physiological conditions[1-3]. They are involved in many nervous system diseases including cancer, inflammation, central nervous system injury, and neuropsychiatric conditions. They are physiologically important in mediating pain, fever, and inflammation[4]. Prostaglandin E2, known in medicine as dinoprostone (DINO), is (Z)-7-[(1R, 2R, 3R)-3-hydroxy-2-[(E)-(3S)-3-hydroxyoct-1-

enyl]-5-oxocyclopentyl] hept-5-enoic acid (PGE2) (Figure No.1). It is an inducer of uterine muscle contraction[5].

In literature survey, some analytical methods including RP-HPLC[6-11] methods have been reported for the estimation of DINO in bulk and pharmaceutical formulations. A number of LC/MS/MS methods were used for determination of DINO in different biological fluids[12-18] in addition to GC methods[11,9]. DINO is assayed in BP[5] but a very long run time is employed, about 36 minutes, which is not economic for routine analysis of pure drug or its pharmaceutical formulations.

In modern analytical laboratory there is always a need for simple and rapid method of analysis. The present work aimed to develop a fast stability-indicating method for determination of DINO. The proposed method is able to selectively, determine DINO in the presence of its impurities, degradation products and placebo components. The developed method was validated with respect to specificity, linearity, limit of detection and quantification, precision, accuracy and robustness. Force degradation studies were performed on the placebo and drug product.

MATERIAL AND METHODS

Instruments

Agilent LC system (Boblingen, Germany) consisted of a quaternary pumping system (Agilent model G1311A), a UV variable wavelength detector (model G1315D, Agilent), and an auto sampler (model G13329A) equipped with a degasser (G1322A, Agilent).Stationary phase consisted of a Symmetry C_{18} analytical column (75 × 4.6mm, i.d, 5µm) (Waters, USA).

Reagents

All chemicals and reagents were of analytical grade.

- Dinoprostone pure powder was kindly supplied by Yon Sung, Fine Chemicals Co., LTD. Korea. Its purity was found to be 99.75%according to the official HPLC method[5]. Pharmaceutical formulation.
- *Dinoglandin E2 Vaginal Tablets* (BN: 01) were obtained from Rotabiogen for Pharmaceutical Investments and Chemicals, Cairo, Egypt). Each vaginal tablet is claimed to contain 3 mg dinoprostone.
- Acetonitrile and Orthophosphoric acid were HPLC grade, Scharlau, Spain. Potassium dihydrogenphosphate was obtained from El-Nasr Company for Chemicals, Cairo, Egypt. De-ionized water: Bidistilled from "Aquatron" Automatic Water Still A4000, Bibby Sterillin Ltd., (Staffordshire, UK).
- Standard solutions of DINO. Primary standard solution of DINO (300µg/mL) was prepared by dissolving 30 mg of DINO in 100mL volumetric flask containing 50mL of mobile phase, and then the volume was completed with the mobile phase. Secondary standard solution of DINO (30 µg/mL) was prepared by diluting 10mL of the primary standard solution to 100mL with the mobile phase.

Procedure

Degradation of DINO

Preparation of DINO acidic degradates

A degraded sample of dinoprostone was prepared by adding 10 mL of 0.1N hydrochloric acid to 30 mg of dinoprostone in a small beaker, and then the solution was left at room temperature (25°C) for 60 minutes. The solution was then neutralized with sodium hydroxide. The contents were quantitatively transferred into 100 mL volumetric flask. The volume was completed with the mobile phase to obtain final concentration (300µg/mL).

Preparation of DINO alkaline degradates

A similar procedure was applied to prepare the alkaline degradates. Complete degradation was achieved using 0.1N sodium hydroxide for 60 minutes at room temperature. The solution was neutralized with hydrochloric acid and then quantitatively, transferred into 100-mL volumetric flask. The volume was completed with the mobile phase to obtain final concentration (300µg/mL).

Preparation of DINO oxidative degradates

An oxidative degradation of dinoprostone was prepared by adding 5 mL of 10% H_2O_2 to 30 mg of dinoprostone in a small beaker, and then the solution was left for 3 hours at room temperature. The contents were quantitatively transferred into

100 mL volumetric flask. The volume was completed with the mobile phase to obtain final concentration (300µg/mL).

Preparation of DINO thermal degradates

A thermal degradation of dinoprostone was done by heating 50mL of dinoprostone solution, (600µg/mL), in a small beaker, at 80°C for 30 minutes, and then allowed to cool. The contents were quantitatively transferred into 100mL volumetric flask. The volume was completed with the mobile phase to obtain final concentration (300µg/mL).

HPLC method

Linearity

Aliquots from DINO primary (300µg/mL) and secondary (30µg/mL) standard solutions were accurately transferred into a series of 10-mL volumetric flasks; the volume was then completed with the mobile phase to obtain a concentration range of 0.3-150µg/mL. Samples were then chromatographed using Symmetry C_{18} column (75 x 4.6mm, i.d., 5µm) as a stationary phase. The mobile phase was formed of potassium dihydrogen orthophosphate (pH 4.4): acetonitrile (67: 33, v/v). The flow rate was 1mL/min., isocratic ally with UV detection at 200 nm. The injection volume was 50µL and column temperature was 35ºC. The samples were filtered also through a 0.45-µm membrane filter. Analysis was usually performed after passing ~50-60mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. Peak areas were plotted against the corresponding concentration of dinoprostone to obtain calibration graph then the regression equation was computed.

The regression equation was used for estimating the concentration of DINO in pure samples, laboratory prepared mixtures with different degradates, unknown samples and in its pharmaceutical formulation.

Accuracy and Precision

The previously mentioned procedures under linearity were repeated for the determination of different concentrations of DINO along its linearity range. The concentrations were calculated from the regression equation. The recovery percent, the mean recovery and SD values were then calculated. Intraday precision was evaluated for DINO by assaying three freshly prepared solutions in triplicate at concentrations (24, 30 and 36 µg/mL) on the same day and mean recovery and RSD were then calculated. Inter-day precision of the proposed method was evaluated by assaying three prepared solutions (24, 30 and 36 µg/mL) in triplicate for 3 days and mean recovery and RSD were calculated.

Analysis of laboratory-prepared mixtures

Portions of DINO stock solution (300µg/mL) were transferred accurately into a series of 10 mL volumetric flasks. Aliquots from its corresponding acidic, alkaline, oxidative or thermal degradates solutions (each, 300µg/mL) were added, separately, to prepare mixtures containing 10-90% of each degradation products. The prepared solutions were chromatographed by the described HPLC method and the concentration of DINO was computed from the regression equation.

Determination of DINO in Dinoglandin E2 Vaginal Tablets

At least 10 vaginal tablets were weighted to determine the average weight of one tablet. A mass equivalent to one vaginal tablet was transferred into a 100- mL volumetric flask containing 50 mL mobile phase, sonicated for 15 minutes then diluted to volume with mobile phase to obtain DINO solution having a concentration of 30µg/mL, and it was passed through a membrane filter (0.45µm porosity). The solution was injected to the LC system and the assay of DINO was done using the regression equation.

The developed method was fully validated according to ICH guidelines. Comparison of the results obtained by the proposed method and the official BP one was done in addition to statistical analysis of data.

RESULTS

Chromatographic separation

Separation of DINO from its degradation products was achieved by applying the chromatographic conditions described in the experimental part. The average retention time of DINO was 5.5 minutes as shown in Figure No.2. Figure No.3 shows

chromatogram of acid hydrolysis degradation. Figure No.4 shows the chromatogram of base hydrolysis degradation. Figure No.5 shows chromatograms of oxidative degradation. Figure No.6 shows the chromatogram of thermal degradation of DINO.

Method validation

The method was linear in the range of 0.3-150µg/mL of DINO. Regression characteristics of the proposed RP-HPLC method are shown in Table No.1. Accuracy was determined by calculating the percentage recovery values which are shown in Table No.2. Repeatability and intermediate precision of the method are also evaluated using two different analysts in different day in same laboratory. Results are shown in Table No.2. System suitability parameters were tested by calculating the capacity factor, tailing factor, the sensitivity factor and resolution. Results of system suitability testing are listed in Table No.2.

*Resolution was calculated with respect to the least resolved peak of the thermal degradates as shown in Figure No.6.

Assay DINO in Dinoglandin E2 vaginal tablets

The chromatogram of the test product (Dinoglandin vaginal tablet) is shown in Figure No.7. The assay results of DINO in Dinoglandin vaginal tablets were estimated. It was found to be 100.22 ±1.615 as shown in Table No.3.

DISCUSSION

Optimization of chromatographic conditions

The aim of this stability-indicating HPLC method is to determine DINO quantitatively in the presence of its expected degradants or excipients by a simple and rapid method. The developed method is valid to determine DINO in its pharmaceutical formulation and in stability studies. The proposed method achieved resolution between DINO and its degradation products. It was applied for short term accelerated stability studies. Resolution between DINO and its degradation products was investigated using different C_{18} and cyano-columns and different mobile phases (containing buffers like phosphate, ammonium acetate, and ammonium formate) with different pH values. Symmetry® C_{18} column showed better performance as compared to other columns. The optimum mobile phase consisted of phosphate buffer (pH 4.4)-acetonitrile (67: 33, v/v) at a flow rate 1 mL/min. in an isocratic mode with UV-detection at 200 nm. The column temperature was maintained at 35°C. The analyte showed symmetrical peak shape, less tailing, and optimum resolution. DINO and its degradation products are well-separated in the optimized conditions. Typical retention time of DINO was about 5.5 minutes as shown in Figure No.2.

Forced degradation study

A target degradation of 20-80% was suggested for the establishing stability-indicating nature of the assay method as even intermediate degradation products should not interfere with any stage of drug analysis[20]. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 20-80%, this could not be achieved in all degradation conditions even after exposure for prolonged duration.

Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 990 indicates a homogeneous peak. The peak purity values indicate homogeneous peaks and thus establishing the specificity of assay method. DINO was subjected to different stress conditions. The stress conditions used were realistic to achieve the expected degradation products that may be formed during storage of DINO on the long run. More drastic conditions were excluded to avoid over stressing of DINO and to avoid formation of unusual and unexpected degradants. The ICH guidelines don't specify certain conditions for accelerated degradation but recommends using 0.1 to 1 N acid or alkali for hydrolysis without specifying the degradation conditions as they differ from drug moiety to other. Figure No.2 shows the chromatogram of intact DINO. Figure No.3 shows chromatogram of acid hydrolysis degradation. Figure No.4 shows the chromatogram of base hydrolysis degradation. Figure No.5 shows chromatograms of oxidative degradation. Figure No.6 shows the chromatogram of thermal degradation of DINO.

Method validation

Linearity of the method was tested by injecting serial concentrations of DINO standard solutions in triplicate. The calibration graph was obtained by plotting peak area against the concentration of the drug

The method was linear in the range of 0.3-150µg/mL of DINO using the following regression equation:

$A = 64.87\ C + 12$, $r = 0.9997$.

Where: A: the peak area,

C: the concentration of DINO (µg/mL) and

R: the correlation coefficient.

The precision of the method was studied by determining the concentration of DINO six times. The results of the precision study indicate that the method is reliable (Table No.2).

The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts in different day in same laboratory. Results are shown in Table No.2.

The peak purity values were carried out using a photodiode array detector. The peak purity was >997 for drug substance and drug products at 200 nm, which shows that the peaks of analytes were pure and that formulation excipients and degradation were not interfering with the analyte peak. The described method was validated with respect to specificity, linearity, system suitability, accuracy, robustness, LOD, LOQ and intermediate precision.

The chromatographic systems described in this work allow complete base line separation of DINO from its degradation products. No other co-eluting peaks were found with the main DINO peak. One sample can be chromatographed in 7 minutes. The chromatographic method described in BP requires a run time of at least 38 minutes to elute DINO and its degradates. The method could be used as a stability indicating assay for determination of DINO in presence of degradation products.

Limit of detection and limit of quantification

The LOD and LOQ for DIC were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The LOD and LOQ were 0.11 and 0.25mg/mL, respectively, for 10-mL injection volume. The LOQ was validated by analyzing a number of samples equal to the quantitation limit with acceptable recoveries and precision.

Assay DINO in Dinoglandin E2 vaginal tablets

The chromatogram of the test product (Dinoglandin vaginal tablet) is shown in Figure No.7. It was similar to that of pure DINO with extra peaks of additives. The assay results of DINO in Dinoglandin vaginal tablets were estimated. It was found to be 100.22 ±1.615 as shown in Table No.3.

The robustness of the HPLC method was examined by the analysis of samples under a variety of experimental conditions such as small changes in the pH (± 0.2units), small changes in proportions of mobile phase, by up to ± 2% mainly of the organic part of the mobile phase. The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified, however the areas and peaks symmetry were conserved.

Stability indication

To assess the stability-indicating efficiency of the proposed method, DINO was mixed with its acidic alkaline, oxidative or thermal degradants, separately, in different ratios and analyzed by the proposed method. DINO could be determined accurately in the presence of up to 90% (w/w) of its corresponding degradants. The suggested method was successfully applied for the determination of DINO in stability studies.

Table No.1: Regression characteristics and validation sheet for determination of Dinoprostone by the proposed method

S.No	Parameter	Dinoprostone
Regression equation		
1	Slope	64.87
2	Intercept	12
3	Correlation coefficient	0.9997
4	Linearity range (µg mL^{-1})	0.3-150
5	Average retention time (min.)	5.5 ± 0.2
Accuracy		
6	Level of addition (%)	
7	80%	98.04%
8	100%	99.39%
9	120%	101.43%
Precision (RSD %)		
10	Repeatability	0.199
11	Intermediate precision	0.596
12	Analyst to analyst	0.990
Day to day		
13	LOD (µg mL^{-1})	0.11
14	LOQ (µg mL^{-1})	0.25

Table No.2: System suitability parameters for determination of Dinoprostone by the proposed HPLC method

S.No	Parameter	Obtained value	Reference value
1	*Resolution	2.88	R > 2
2	Tailing factor	0.96	T=1, for a typical symmetrical peak
3	Selectivity(α)	1.17	>1
4	Column capacity	3.42	1-10 are acceptable
5	Column efficiency	8870	Increases with the efficiency of separation
6	HETP	8.5 µm	The smaller the value, the higher the column efficiency

Table No.3: Assay of Dinoprostone in Dinoglandin E2 Vaginal tablets by the proposed method

S.No	Pharmaceutical formulation	Taken (µg mL^{-1})	Found (µg mL^{-1})	Recovery %
1	Dinoglandin E2 Vaginal tablets (BN: 01)	30	29.85 30.25 30.83 29.36 30.13 29.98	99.50 100.83 102.77 97.87 100.43 99.93
2	Mean ±SD RSD%			100.22 ±1.615 1.611

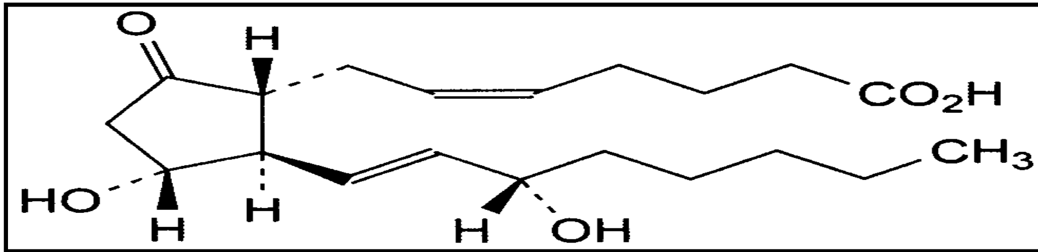

Figure No.1: Structural formula of dinoprostone

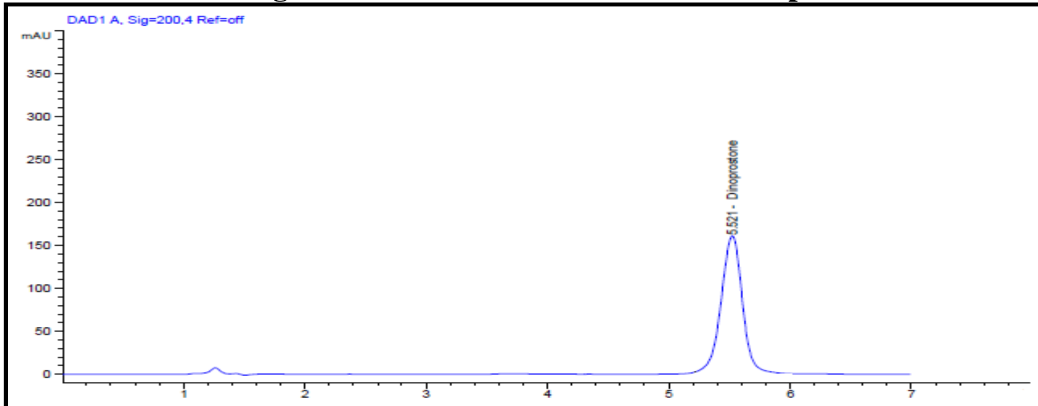

Figure No.2: HPL Chromatogram of Dinoprostone

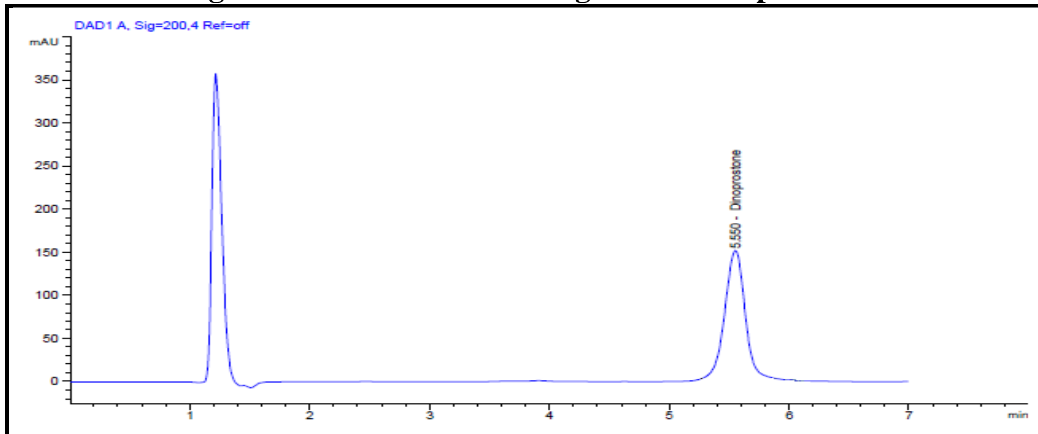

Figure No.3: Representative HPL Chromatogram of Dinoprostone and its acidic degradates

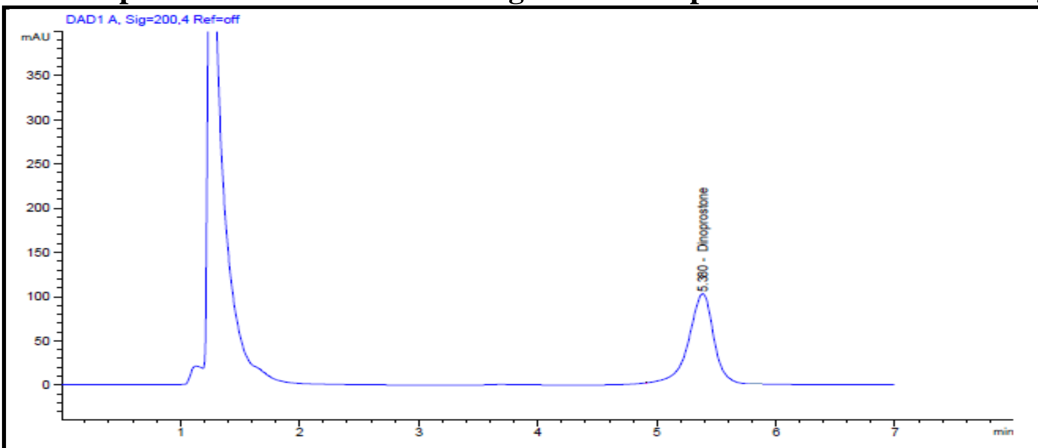

Figure No.4: Representative HPL Chromatogram of Dinoprostone and its alkaline degradates

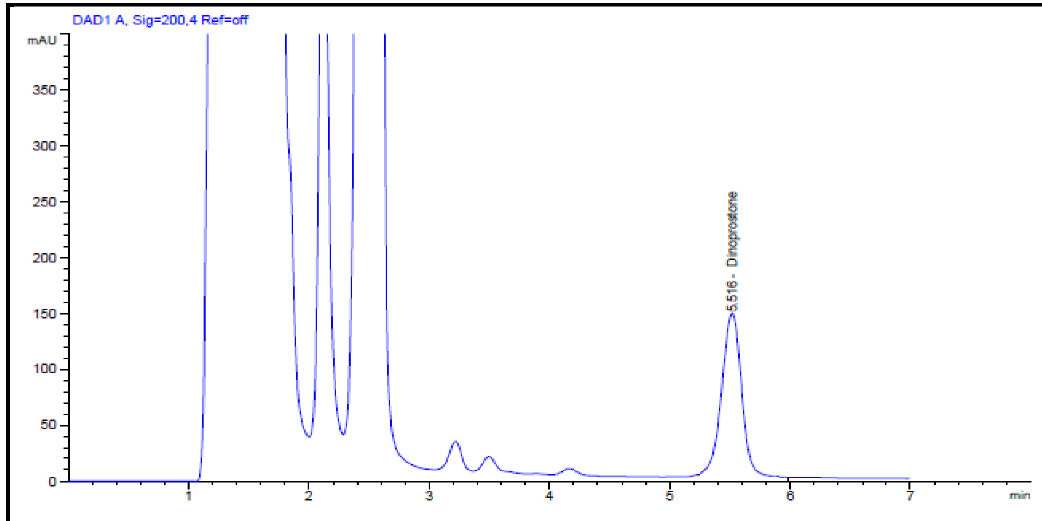

Figure No.5: HPL Chromatogram of Dinoprostone and its oxidative degradates

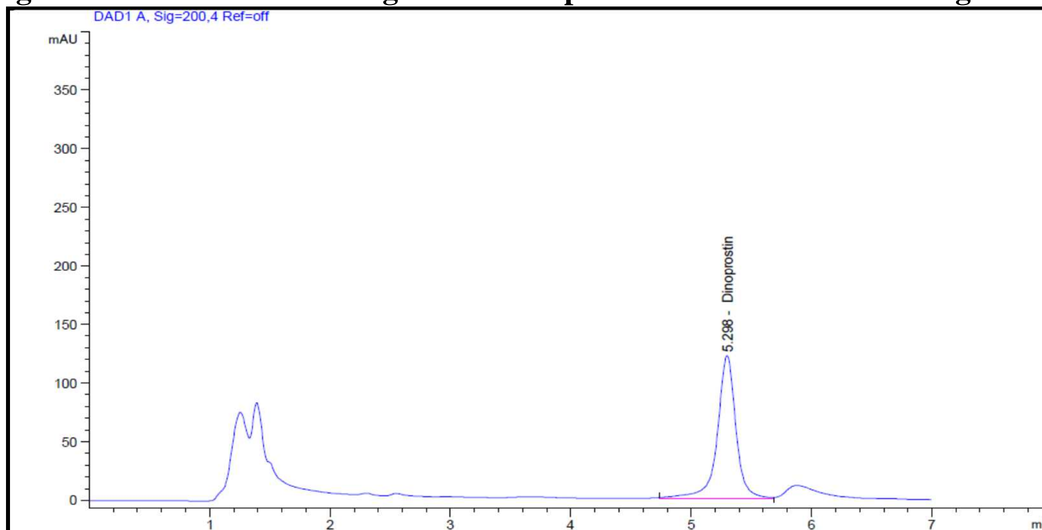

Figure No.6: HPL Chromatogram of Dinoprostone and its thermal degradates

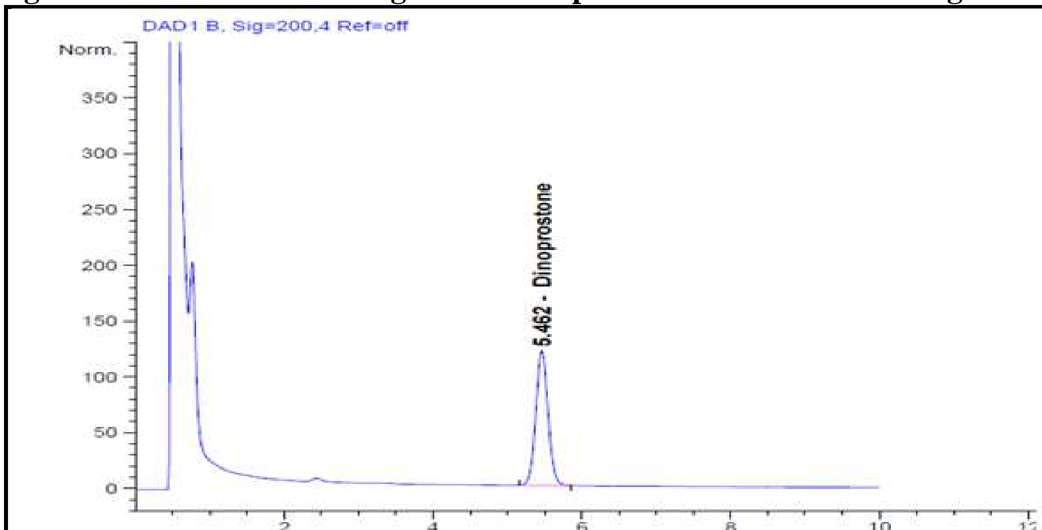

Figure No.7: HPL Chromatogram of Dinoprostone in Dinoglandin E2 Vaginal tablets

CONCLUSION

The suggested method is simple, accurate, selective, and equally sensitive with no significant difference of the precision compared with the official HPLC method of analysis. Application of the proposed method to the analysis of DINO in its pharmaceutical formulation shows that neither the excipient nor the degradation product interferes with the determination. The run time is relatively short which is superior over the official BP method with long run time. This enables rapid determination of many samples in routine and quality control analysis.

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CONFLICT OF INTEREST

There is no any conflict of interest.

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