International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

Development and Validation of Analytical RP-HPLC Method for Determination of Progesterone in Bulk and Formulation.

A. R. Shirode*, R. V. Kolekar, V. J. Kadam
Department of Quality Assurance, Bharati Vidyapeeth's College of Pharmacy, Sector 8, C.B.D. Belapur, Navi Mumbai - 400614, India.

Submitted: 05-02-2023 Accepted: 20-02-2023

ABSTRACT:
A New Analyticalreversed-phase high-performance liquid chromatographic (RP- HPLC) method has been developed for the drug Progesterone. The goal of this method development was to provide a precise, dependable, and robust analytical RP HPLC method for quantitatively estimating progesterone in bulk and formulations.The proposed method is based on the estimation of the Progesterone drug in reversed-phase mode using BDS HYPERSIL C18 (4.6mm×250mm) analytical column. The optimized mobile phase consisted of Methanol: Acetonitrile in the ratio of 90:10 V/V. A flow rate of 1.5 ml/min was used, with UV detection at 241 nm.The drug waswell resolved and retained at 2.89minutes. The total run time was 10min. This newly developed analytical RP-HPLC method was validated as per the recommendations of ICH Revised Q2 (R1) guidelines of analytical method validation, to prove that the new analytical method, meets the reliability characteristics. The method characteristics showed the capacity of an analytical method to keep, all over time, the basic standards for validation: selectivity, linearity, precision, accuracy, and sensitivity. The calibration plot gave a linear relationship over the concentration range8 to 20 µg/ml for the drug Progesterone. The LOD and LOQ were 0.95287 µg/ml and 2.88751 µg/ml for PGST. The repeatability testing for the drug showed that the method is precise within the acceptable limit. The % RSD of the robustness study results was discovered to be within acceptable bounds. The validated method was successfully used for content uniformity and Quality control analysis of marketed formulations of Progesterone.

KEYWORDS: RP-HPLC, Progesterone, Hormone Replacement Therapy.

I. INTRODUCTION:

Analytical method development and validation is an important aspect for the discovery, development, and manufacture of pharmaceuticals to ensure the identity, purity, potency, and performance of the drug products. To study physicochemical properties of the drug, to selectand set up chromatographic conditions such as column, mobile phase system, detection wavelength, flowrate conditions, sample preparation, method optimization. The main goalbehind development of the RP-HPLC method is to have a reliable analytical method to separate and quantify the drug from its bulk and formulation, so that it can be used for Quality Control analysis.

During menopause, your estrogen level falls. Some women experience undesirable symptoms including vaginal dryness and hot flashes. The most effective treatment for menopause symptoms is HRT, commonly referred to as hormone therapy, menopausal hormone therapy, and estrogen replacement therapy. Hormone therapy with estrogen, progesterone, and progestin the main pregestational steroid produced mainly by the placenta and corpus luteum. The uterus, mammary glands, and the brain are all affected by progesterone. It is necessary for the development of mammary tissue for milk production, pregnancy maintenance, and embryo implantation. Pregnenolone is transformed to progesterone, which also functions as an intermediary in the manufacture of an adrenal corticosteroid and gonadal steroid hormones. A C-21 steroid hormone called progesterone is important in the female menstrual cycle, pregnancy (it aids in gestation), and human and other species' embryogenesis. The main progestogen found in humans naturally is progesterone, which is a member of the progestogen hormone class. Progesterone seems to lessen the maternal immune response during implantation and gestation to enable acceptance of the pregnancy. The uterine smooth muscle's ability to contract is reduced by progesterone. The foetus breaks down placental progesterone to produce the mineralo- and glucosteroids found in the adrenal glands. One process that can help labour start is a decline in progesterone levels. Progesterone also prevents lactation throughout pregnancy. One of the factors

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

that stimulates the production of milk is the drop in progesterone levels after birth. Pregnene hydroxylation deficit, an inherited metabolic mistake, has been linked to progesterone.Due to the fact that it contains both progestin, a synthetic version of progesterone, and oestrogen, this is frequently referred to as combination therapy. It is intended for females who still possess their uteri. Compared to oestrogen alone, taking oestrogen and progesterone lowers your risk of developing endometrial cancer, which is the lining of the uterus.Progesterone can assist alleviate a variety of menopausal symptoms, such as hot flashes, despite being typically used as a birth control method.

- Oral progesterone acetate (Provera) and synthetic progestin tablets are two examples of progestin medicines taken orally (norethindrone, norgestrel). Many specialists now use natural progesterone instead of synthetic progestins to treat the bulk of their menopausal patients.Natural progesterone may not negatively affect lipids, according to research, and is a suitable option for women with high cholesterol levels. In comparison to medroxyprogesterone acetate, natural progesterone may also have other benefits.
- Intrauterine progestin - Liletta, Kyleena, Mirena, and Skyla are brand names for low-dose intrauterine devices (IUDs) that contain levonorgestrel. These are sometimes used in conjunction with oestrogen "off-label" and are approved in the United States for the prevention of pregnancy and the control of bleeding. Your doctor could advise you to maintain one of these IUDs in place until perimenopause is over if you have one of them when you start to experience irregular periods.

II. MATERIALS:

Working standards of pharmaceutical-grade progesterone were obtained as a generous gift sample from a local API manufacturing unit. Marketed formulation of Sustained Release tablets containing 200mg was procured from a local pharmacy shop. Acetonitrile and methanol werepurchased from S.D. Fine Chemicals, Mumbai, India.

DRUG PROFILE:

PROGESTERONE (PGST):

Progesterone is a C21-steroid hormone in which a pregnane skeleton carries oxo substituents at positions 3 and 20 and is unsaturated at C(4)-C(5). As a hormone, it is involved in the female menstrual cycle, pregnancy, and embryogenesis of humans and other species. It has a role as a contraceptive drug, a progestin, a progesterone receptor agonist, a human metabolite, and a mouse metabolite. It is a 20-oxo steroid, a 3-oxo-Delta(4) steroid, and a C21-steroid hormone. It derives from a hydride of a pregnane.

Therapeutic Progesterone is the therapeutic form of the naturally occurring hormone progesterone. Progesterone binds to the progesterone receptor, resulting in dissociation of heat shock proteins, receptor phosphorylation, and transcription activation through direct or indirect interaction with transcription factors. This agent exerts inhibitory effects on estrogen by decreasing the number of estrogen receptors and increasing its metabolism to inactive metabolites. Progesterone induces secretory changes in the endometrium, decreases uterine contractility during pregnancy, and maintains pregnancy.

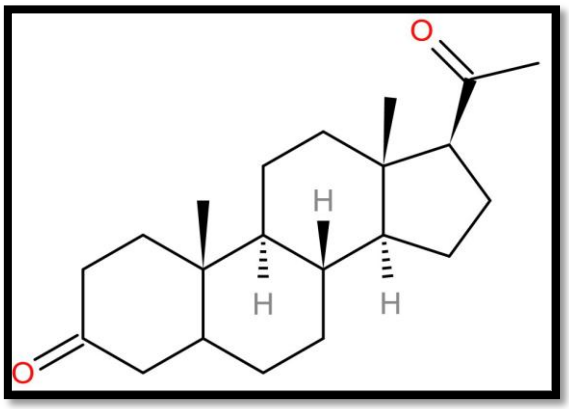

Figure 1: Chemical structure of Progesterone (PGST)

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

Table 1: Physical properties of Progesterone (PGST)

PROPERTIES	PROGESTERONE(PGST)
Molecular formula	$C_{21}H_{30}O_2$
Route of administration	Oral, Intramuscular, Vaginal inserts, gel.
BCS Class	II
Bioavailability	<2.4% -Oral 4-8% -Vaginal
Protein binding	88-90%
Molecular Weight	314.46
Category	Steroidal Hormone
Route of Elimination	Kidney
Melting point	121-130^{o}C
Half-life	5-10 hours.
Solubility	Soluble in methanol, ethanol, dioxanes, acetonitrile, sulfuric acid, Insoluble in water.
Ph	7.780 ± 0.123
PKa	Strongest acid: 18.92 Strongest basic: -4.8
LogP	3.87
Dose	200mg, 300, 400 mg (Tablet and Capsule form) 200 mg (twice a day) 400 mg (once a day)
Lambda max (λ_{max})	241

The objective of the proposed work was to develop a reliable analytical method and validate the same as per the recommendations of ICH Revised Q2 (R1) guidelines of analytical validation. The purpose behind this method development was to have a robust analytical HPLC method for the estimation of progesterone.

III. METHOD:

Reversed-Phase High-Performance Liquid Chromatographic (RP- HPLC) method.

INSTRUMENTS USED:

The HPLC system; make Shimadzu UFLC series employed with LAB SOLUTION software (Version 6.72 SP1).

EXPERIMENTAL STUDIES:

➢ **ANALYTICAL METHOD DEVELOPMENT:**

- **Preparation of standard stock and working solution for RP-HPLC method development studies:**

10mg quantity of PGST weighed accurately and transferred into 10ml volumetric flask labelled as

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

'Standard/Stock solution A' and volumes was made up with methanol.

Working solution (Solution B) to be used for method development for estimation of PGST (Solution B: a solution containing 100µg/ml of a drug) was prepared from standard solution,1 ml from stock solution were pipetted out and transfer to 10 ml capacity volumetric flask and volume was made up to mark with mobile phase. Solution B was diluted 10 times with mobile phase was used for sample injection during experimental trials of method development studies.

- **Selection of detection wavelength:**

UV spectra of 10 ppm solution of PGST was generated scanning over the range of 200-400 nm using Double Beam Spectrophotometer (Shimadzu UV1801). The wavelength corresponding to maximum absorbance was selected as detection wavelength for estimation of PGST by UV detector in HPLC system (Shimadzu UFLC series).

- **Optimisation of chromatographic conditions:**

Many preliminary trials and replicate analysis were carried out for selection and optimisation of stationary phase, mobile phase, flow rate, injection volume and column temperature.

- **Analytical Method Validation:**

Performance characteristics of newly developed analytical HPLC method were statistically validated as per Method Validation protocol prepared using ICH Q2(R1) guideline for analytical method validation.

Table 2: Protocol of analytical method validation parameters with its method to be followed according to ICH revised Q2 (R1) guideline. (ICH Q2(R1))

Parameter	**Purpose**	**Recommendation as per ICH revised Q2(R1) guideline**		**Acceptable criteria**
Accuracy	Assay (Content/potency) : Recovery studies	Accuracy was established across the specified range of analytical procedure by adding known added quantities of analyte to the synthetic mixture of drug product components and to the combined dosage form. As per ICH, Accuracy should be assessed using a minimum of 9 determinations over a minimum of three concentration levels covering the specified range i.e. 3 concentration levels in triplicate. (e.g. 3 Concentration/3 replicates each). Accuracy of the method is reported as percent recovery of known added amount of analyte in sample.		98-102 % Recovery of known added amount of analyte.
Precision	Assay (Content/potency) : Repeatability and Reproducibility	**Repeatability**	**Intermediate Precision**	
		Repeatability was assessed by using minimum of 9 determination covering the specified range for the procedure (e.g. 3 concentration/3 replicates each)	Intermediate precision was established to study the effects of random events i.e. days, on the precision of the analytical procedure. Intraday and	RSD≤ 2%

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

			interday precision studies were performed by taking 9 determinations of 3 concentration/3 replicates each, at 3 times in a same day and on 3 different days, respectively.	
		Precision is reported as relative standard deviation (coefficient of variation) for each type of precision investigation.		
Specificity	Identification, Testing for impurities and Assay (Content/potency)	As per ICH revised Q2 (R1), Specificity should be carried out ensure identification tests, the determination impurity and assay.		No interference In analyte determination
Detection Limit and Quantification Limit.	Testing for impurities Sensitivity of analytical method and Assay: Determination of minimum detectable and quantifiable concentration of analyte solution.	Detection limit and quantification limit is determined based on the standard deviation of the response and the slope. **LOD**=3.3×σ/S **LOQ**=10×σ/S σ = Standard deviation of response estimated based on the calibration curve. S = Slope of the calibration curve.		NA
Linearity	Testing for impurities and Assay (Content/potency) : To check linear relationship of performed concentration.	As per ICH, for the establishment of linearity, a minimum of 5 concentrations are approved. Linearity is reported by the value of the correlation coefficient, y-intercept, and slope of the regression line along with a plot of the data.		$R^2 \geq 0.99$
Robustness	To establish reliability of analytical method.	Robustness was evaluated for proving the reliability of an analytical method with respect to deliberate variations in method parameters. To establish robustness of analytical method following factors were studied: ➢ Influence of variations in pH of a mobile phase. ➢ Influence of variations in mobile phase composition ➢ Flow rate ➢ Detection wavelength ➢ Injection volume ➢ Change in column temperature.		Pooled RSD≤3% in every change item

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

LAB STUDIES FOR THE ESTABLISHMENT OF ANALYTICAL METHOD VALIDATION PARAMETERS:

- **Accuracy:**

Accuracy experiments were performed as per method validation protocol (Table 2) by conducting recovery studies of the known added amount of PGST over three concentration levels viz. 80%, 100% and 120%. Recovery studies were also performed using marketed formulations.

- **Precision:**

Experimental determinations for the establishment of repeatability and reproducibility of the analytical method were carried out as per method validation protocol (Table 2).

- **Intra-day precision:**

Three replicate analysis was performed at three different concentration mixture levels low [LQC: 1 ppm], mid [MQC:3 ppm], and high [HQC:5 ppm] for PGST respectively within the same day at three different times (Session 1, 2, 3).

- **Specificity:**

To determine specificity chromatograms were obtained for blank (mobile phase), PGST, placebo, marketed formulations, in-house formulations. All chromatograms were analysed evaluated for any interference with the analyte of interest.

- **Linearity and Range:**

Experimental determinations were carried out on seven serial dilutions of working solution (Solution B) prepared using mobile phase as diluting solvent. As per the method validation protocol (Table 2), the linear relationship was checked by plotting average peak areas against sample concentrations. It was evaluated across the range of 8-20 ppm for PGST.

- **Robustness:**

To evaluate and check the reliability of the newly developed analytical RP-HPLC method deliberate changes were made in critical method parameters as listed in table 3.

Table 3: Robustness studies- variations in method parameters and levels of variation.

Method Parameters and Variations	**Levels of Variation**	**Actual values of method parameters after changes**
Injection volume (20µl ± 10 µl)	-10µl	10µl
	+10µl	30µl
	+20 µl	40µl
	+30 µl	50µl
Flow Rate (1.5 ± 0.5 ml/min)	-0.5ml/min	1.0ml/min
	+0.5ml/min	2.0ml/min
Wavelength (241± 10nm)	-10nm	231nm
	+10nm	251nm
Mobile Phase Organic/ Aqueous (90:10V/V)	∓10(V/V)	80:20(V/V)
	∓20(V/V)	70:30(V/V)

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

- **Application Of Newly Developed And Validated Rp-Hplc Method For Routine Sample Analysis Of Marketed Formulations:**

CONTENT UNIFORMITY ASSAY:

For the analyte assay, 10 drug tablets were precisely weighed and powdered. The weight of powder equivalent to label claim of PGST was transferred into individual 100 ml volumetric flask and dissolved completely in methanol with the aid of sonication for 10 mins. The solutions were filtered through 0.45µm filter paper. Further dilutions were made up using the mobile phase. Then these solutions were filtered througha 0.45µ syringe filter and 20µL of this filtered solution was were aspected into theHPLC column and corresponding chromatograms were recorded. The data were statistically processed for calculation of the percent drug content of the stated amount.

IV. RESULT AND DISCUSSION

Analytical Method Development:

➢ **Detection of wavelength:**

UV absorbance spectra for 10 ppm solution of PGST wereanalysed (Figure 2) and 241 nm was selected as a detection wavelength for estimation of chromatographic determination of PGST.

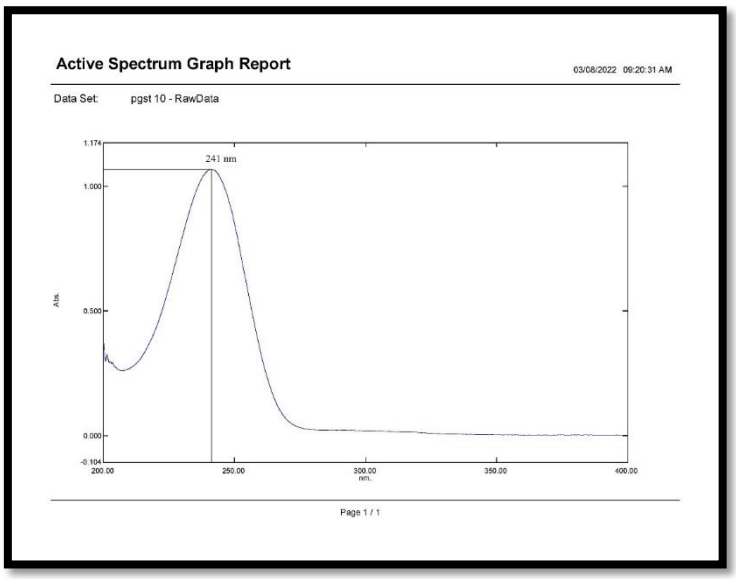

Figure 2: UV spectra of PGST

➢ **Optimization of chromatographic condition:**

According to the referred scientific-analytical literature, it is found that the drug when estimated or analysed HPLC from the single-component formulation or bulk this drug get separated and retained on Octadecyl silane (ODS) C-18 HPLC columns. Thus, to get optimum resolution during chromatographic estimation C18 column [BDS HYPERSIL C18 (4.6mm×250mm) analytical column] was used. Experiments were designed and experimental trials were carried out for a selection of the mobile phase; some of these are tabulated in table 3.

Table 3: Trials for optimization of mobile phase composition for estimation of PGST.

Mobile phase components	Compositions
MeOH: ACN	70:30
MeOH: Water	80:20
MeOH: ACN	80:20
MeOH: ACN: Water	25:35:40
Formic acid: ACN	5:95
ACN: Water	90:10
ACN: Water	50:50
Water: MeOH	30:70

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

During experimental trials of RP-HPLC method development, different flow rates varying from 0.5 to 2.0 ml/min as well as variable injection volumes in the range of 10 µl to 50 µl were tried. At the end of all experimental trials, based onresults and experimental observations with respect to response, resolution, peak sharpness, peak symmetry, etc. chromatographic condition was finalized. (Table 4)

Table 4: Optimized chromatographic condition.

Mobile Phase	MeOH:ACN (90:10)
Stationary Phase	BDS HYPERSIL C18 (4.6mm×250mm) analytical column
Flow rate	1.5 ml/min
Detection wavelength	241nm
Injection volume	20µl

Chromatograms obtained using this optimized chromatographic condition showed that the drug namely PGST was well resolved and retained at 2.899min respectively. A representative chromatogram of PGST is shown in figure 3.

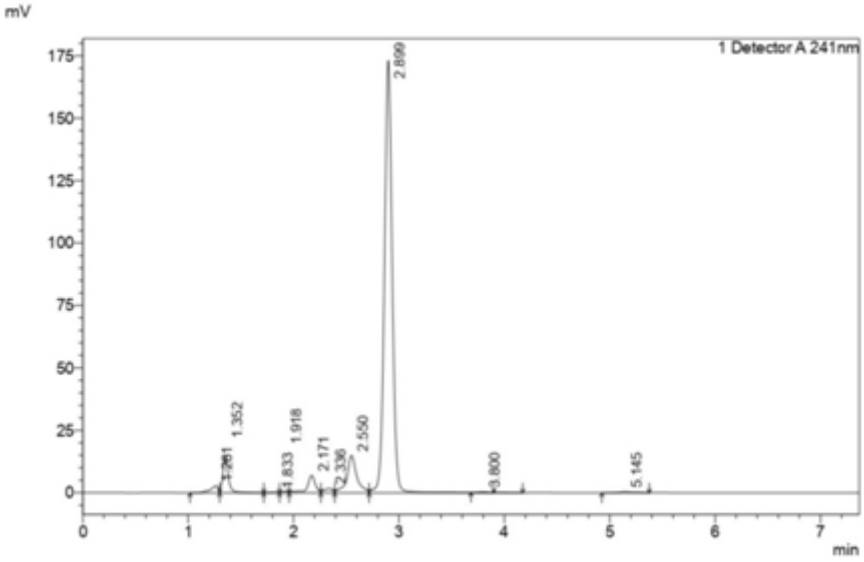

Figure :3 Representative chromatogram of PGST.

Analytical Method Validation:

LAB STUDIES FOR THE ESTABLISHMENT OF ANALYTICAL METHOD VALIDATION PARAMETERS:

➢ **Accuracy**:

Accuracy of the method is reported as percent recovery of the known added amount of analyte in the sample. Experimental observations and results are tabulated in Table 5.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

Table 5: Accuracy: Recovery studies on bulk drugs.

Observation						
Drug	**% Level**	**Concentration before spiking (µg/ml)**	**Total concentration after spiking (µg/ml)**	**Amount Recovered (µg/ml)**	**% Recovery**	**Inference**
PGST	80	14	25.2	24.9681	99.08	**Acceptable recovery hence accurate**
	100	14	28	28.2856	101.02	
	120	14	30.8	31.9088	103.6	

Recovery studies were also performed on tablets containing PGST. The marketed tablets of PGST were triturated and a sample solution was prepared which yield a concentration of 14 µg/ml PGST. To this solution,a known amount of PGSTwas added at three concentration levels viz. 80%, 100%, 120%, and dilutions were carried out with mobile phase and injected for RP-HPLC analysis. Experimental observations and results are tabulated in Table 6.

Table 6: Accuracy: Recovery studies on tablet formulation.

Observation						
Drug	**% Level**	**Concentration before spiking (µg/ml)**	**Total concentration after spiking (µg/ml)**	**Amount Recovered (µg/ml)**	**% Recovery**	**Inference**
PGST	80	14	25.2	24.9681	96.16	**Acceptable recovery hence accurate**
	100	14	28	28.2856	98.29	
	120	14	30.8	31.9088	102.24	

➢ **Precision:**

The results of intraday and inter-day precision studied are tabulated in table 7 and 8 respectively. Percent RSD values for both intraday and inter-day precision were found within the acceptable limit.

Table 7: Intra-day Precision Results

		Observation			Inference
		PGST			**Acceptable %RSD, hence precise**
Levels		**LQC**	**MQC**	**HQC**	
Amount (µg/ml)		8	14	20	
Peak Area	**1**	864508	1213378	1584837	
	2	860139	1216991	1588162	
	3	873204	1216991	1583732	
Average Peak Area		865954	1213703	1585577	
S.D.		6649.552	3138.619	2305.846	
% RSD		0.767887	0.258986	0.145426	

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

Table 8: Inter-day Precision Results

		Observation			Inference
		PGST			
Levels		LQC	MQC	HQC	
Amount (µg/ml)		8	14	20	
Peak Area	1	854508	1213378	1584837	
	2	843484	1187671	1549598	
	3	834552	1173582	1528743	
Average Peak Area		847518	1191543	1554393	Acceptable %RSD, hence precise
S.D.		15385.89	20178.52	28352.37	
% RSD		1.815401	1.693477	1.824016	

➢ **Specificity:**

Separate chromatograms were obtained for blank (mobile phase) and PGSTto ensure the method is found to be selective and specific for the analyte (PGST). Overlayed chromatogram of mobile phase and PGSThave shown in **figure 4**.

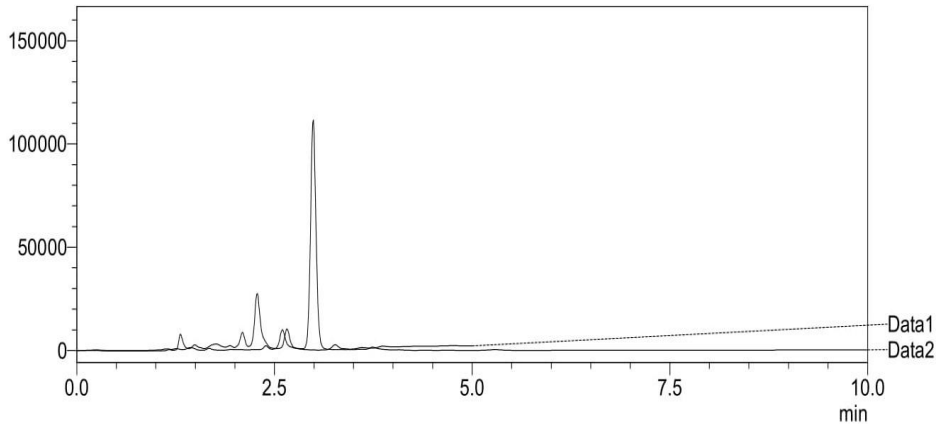

Figure 4: Overlain Chromatogram of blank (mobile phase) and PGST

Linearity:

Seven serial dilutions of PGST were prepared using a standard stock solution and dilutions were made with the mobile phase. Replicate analysis was performed in triplicate and the average peak areas were plotted againstconcentrationstoobtainthe calibration curve. PGSTwas found linear across the range of 8-20 ppm. The linearity plot of PGST is given in figure 5. The values of the correlation coefficient, y-intercept, and slope of the regression line are shown in table 9.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

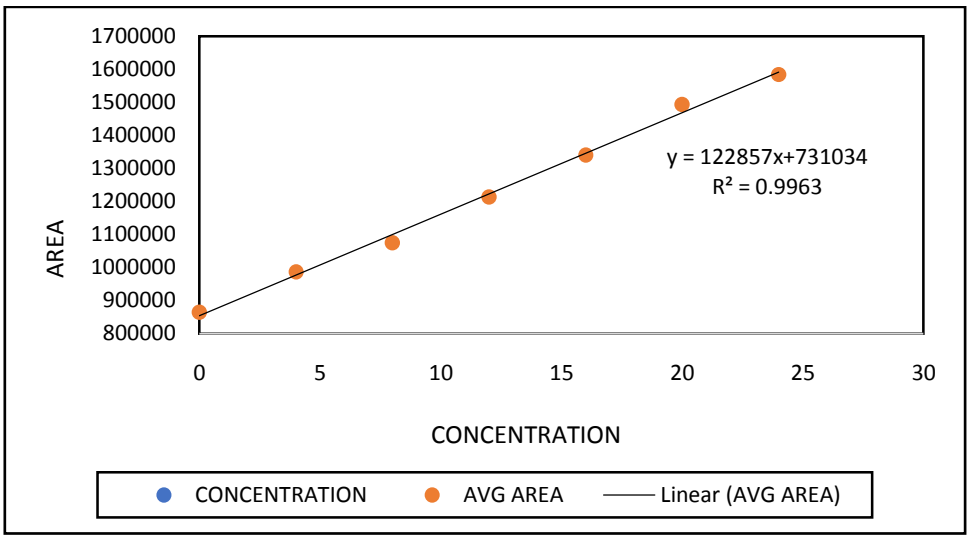

Figure 5: Linearity: Calibration plot for PGST

Table 9: Linear regression data of calibration plot.

Drug	Range	R^2	y-intercept	Slope
PGST	8-20 µg/mL	0.9963	731034	122857

➢ **Limit of Detection and Limit of Quantification**

Values for detection limit and quantification limit were determined based on the standard deviation of the response and the slope of the regression line. The calculated values of the limit of detection (LOD) and limit of quantitation (LOQ) for PGSTare shown in table 10.

Table 10: The calculated value of LOD and LOQ.

	PGST
LOD	4.7287 µg/ml
LOQ	14.3751 µg/ml

➢ **Robustness**

To determine the robustness of the analytical HPLC method changes observed in retention time and response were recorded. The method was found to be reliable and robust as method performance (retentiontimeandresponse) is not much affected by deliberate variations in mobile phase composition, column temperature, Injection volume, and flow rate. The results obtained are tabulated in table 11.

Table 11: Robustness: Effect on retention time and response by variation in mobile phase composition flow rate and injection volume.

Method Parameters and Variations	Level of Variation	Actual values of method parameters after changes	PGST	
			%RSD of recorded response (Area)	Retention time (Min)
Injection volume (20µl ± 10 µl)	-10	10 µl	0.145207	2.702
	+10	30 µl	0.174118	2.875
	+20	40 µl	0.012831	2.870
	+30	50 µl	0.193521	2.873
Flow Rate (1± 0.5 ml/min)	-0.5	0.5 ml/min	0.303161	4.265
	+0.5	1.5 ml/min	0.269867	2.160
Wavelength (222±	-10	212 nm	0.138893	2.845

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

10nm)	+10	232 nm	0.033653	2.848
Mobile phase Organic/ Aqueous (90:10 V/V)	∓20(V/V)	70:30	0.470981	2.837
	∓10(V/V)	80:20	0.334136	2.865

APPLICATION OF NEWLY DEVELOPED AND VALIDATED RP-HPLC METHOD FOR ROUTINE SAMPLE ANALYSIS OF MARKETED FORMULATIONS:
CONTENT UNIFORMITY ASSAY:

The method was conveniently adopted for stability analysis, content uniformity assay for formulation containing Progesterone. So, we proposed that the method will be useful for quality control (QC) analysis of single-component formulation of the drug.

Table 13: Calculated values of drug content uniformity assay for the marketed formulation of PGST.

Drug	Formulation	Label claim (mg)	Amount of drug (mg)	% Drug content of the label claim
PGST	Marketed	200 mg/tab	197.33mg/tab	98.66%

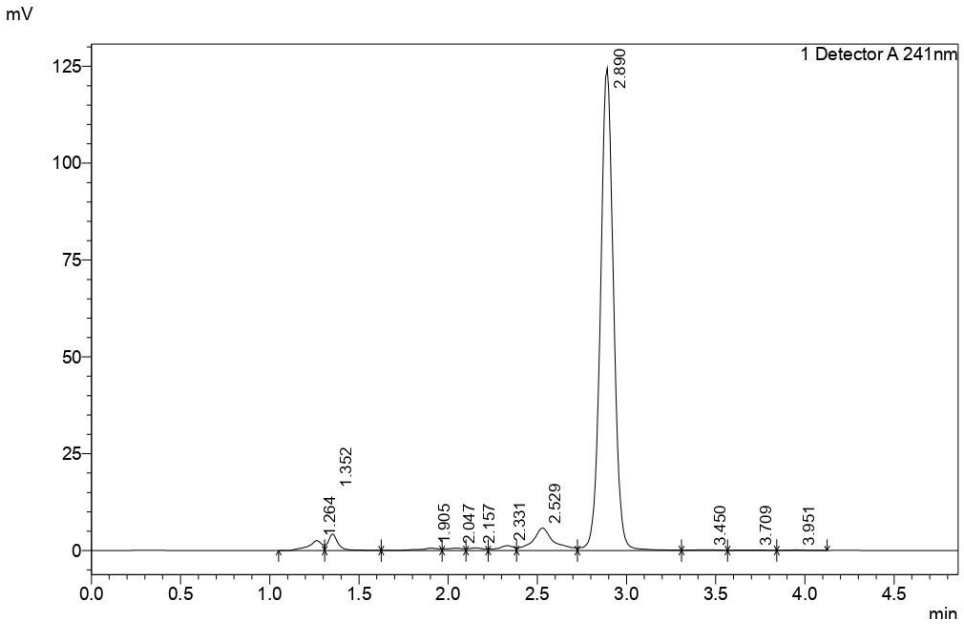

Figure 6: Representative chromatogram of Marketed PGST formulation.

V. CONCLUSION:

An analytical RP-HPLC method for quantitative estimation and content uniformity assay of Progesterone (PGST) from their bulk, the single componentwas successfullydeveloped and statistically validated.

Validation studies were performed as per the validation protocol developed following the recommendations of ICH revised Q2 (R1) guidelines in order to prove that the new analytical method, meets the reliability characteristics. Validation studies assured that the newly developed RP-HPLC method is specific, accurate, precise, and robust.

The newly developed and validated RP-HPLC method was successfully applied for quantitative estimation of PGST from the marketed formulation.

Results and corresponding data obtained from all experimental studies indicated that the proposed method is suitable for the estimation of PGST in bulk and in pharmaceutical formulation.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 1857-1869www.ijprajournal.com ISSN: 2249-7781

ABBREVIATIONS:

RP-HPLC: Reverse phase liquid chromatography, ICH: International conference of harmonization, PGST: Progesterone drug, LOD: Limit of detection, LOQ: Limit of quantification, SD: Standard deviation, RSD: Relative standard deviation, NA: Not applied, QC: Quality control.

ACKNOWLEDGMENTS

The authors would like to thank Bharati Vidyapeeth's College of Pharmacy, C.B.D. Belapur, Navi Mumbai, for providing the necessary facilities to perform this study.

REFERENCE:

[1]. Inna S Levina , Andrey K Nazarov , Georgy V Nazarov , Lidia E Kulikova , Yury V Kuznetsov, Andrey S Dmitrenok , Dmitry V Minin , Aleksey V Aksenov , Igor V Zavarzin. In vivo study on biotransformation of pentacyclic analogs of progesterone: Identification of their metabolites by HPLC-MS method. Doi: 10.1016/j.jsbmb.2019.105436

[2]. Luke J Ney , Kim L Felmingham , David Nichols. Reproducibility of saliva progesterone measured by immunoassay compared to liquid chromatography mass spectrometry. Doi:10.1016/j.ab.2020.113984

[3]. Casey L Sayre , Christopher Cox , Gabriel Velazquez , Minh Thi Le , Amy Hong Nguyen , Marjorie Ramos , Anna Burrows , Venkata Kashyap Yellepeddi.Chemical Stability of Progesterone in Compounded Oral Rapid-dissolving Tablets.

[4]. Ying Huang , Hui Xiao , Yaxiong Liu , Jiaming Gan , Quanhong Yan Column selection approach for related substances determination of progesterone by high-performance liquid chromatography. DOI: 10.1111/cbdd.13375

[5]. Dongke Liu [1], Rusong Zhao [2], Shigang Zhao [3], Zhaobao Wang [4], Ruichen Liu [5], Fengshan Wang [6], Yanhui Gao. A developed HPLC-MS/MS method to quantitate 5 steriod hormones in clinical human serum by using PBS as the surrogate matrix. DOI: 10.1016/j.jchromb.2021.123002

[6]. Roger A Lobo. Hormone replacement therapy: Current Thinking. Doi: 10.1038/nrendo.2016.164

[7]. E Anne MacGregor . Migraine, menopause and hormone replacement therapy. DOI: 10.1177/2053369117731172

[8]. V W Henderson . Progesterone and human cognition. DOI: 10.1080/13697137.2018.1476484

[9]. https://pubchem.ncbi.nlm.nih.gov/compound/Progesterone

[10]. ICH Expert working group, ICH guideline Q2(R1) Validation of analytical methods definitions and terminology. International conference on Harmonisation. https://www.ich.org/products/guidelines/quality/article/quality-guidelines.html.

[11]. Indian pharmacopoeia commission (2018), Ministry of health and family welfare, govt. of India. https://en.wikipedia.org/wiki/Indian_Pharmacopoeia_Commission,

[12]. Adrian M Alambiaga-Caravaca , Iris M Domenech-Monsell , o , María Miranda , Cristina Balaguer-Fernandez, Aracely Calatayud-Pascual , VicentRodilla , Alicia Lopez-Castellano HPLC-UV analytical validation of a method for quantification of progesterone in ex vivo trans-corneal and trans-scleral diffusion studies. Doi: 10.1016/j.jpba.2020.113749