World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF LEUPROLIDE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

Bhagyashri G. Pawar[1], Aejaz Ahmed*[2], G. J. Khan[1] and Patel M. Siddik[1]

[1]Ali-Allana College of Pharmacy, Akkalkuwa- 425415, Nandurbar, Maharashtra, India.
[2]Department of Pharmaceutical Chemistry, Ali-Allana College of Pharmacy, Akkalkuwa- 425415, Nandurbar, Maharashtra, India.

Corresponding Author: Aejaz Ahmed
Department of Pharmaceutical Chemistry, Ali-Allana College of Pharmacy, Akkalkuwa- 425415, Nandurbar, Maharashtra, India.

Article Received on 21/07/2022 **Article Revised on 11/08/2022** **Article Accepted on 01/09/2022**

ABSTRACT

Leuprolide (LPL) was quantified using a DAD detector using a sensitive, accurate, quick, variable, resilient, and economical UV Spectrophotometric and HPLC technique. Using methanol like a solvent, UV spectrophotometric measurement was performed at absorption maxima (max) at 278 nm. In this procedure, LPL was separated using a RP- Agilent C18 (length250mm x 4.6ID, 5 micron) column with something like a mobile phase containing methanol: 0.05 percent OPA (60:40; v/v) with a flow rate of 1 ml/min. 20 L of fluid were injected. LPL's retention time was determined to be 5.56 minutes. The system suitability tests and all essential validation parameters were meticulously completed. Over a large concentration range (5–25 g/ml), the analytic curves was linear (r2 = 1 and 0.999). With a relative standard deviation under 2.0%, the system exhibits sufficient accuracy. With a percent RSD under 2 percent, the approach demonstrated good duplicability and recovery. As a result, the suggested approach was determined to be straightforward, narrowly focused, accurate, linear, and robust. As a result, it may be used to analyse Leuprolide (LPL) in bulk medications.

KEYWORDS: UV Spectroscopy, RP-HPLC, Method development, Validation, Leuprolide.

INTRODUCTION

The science of identifying the elements or compounds contained in materials is known as analytical chemistry. To determine the chemicals that could be present in a product and to determine the precise amounts of the substances that have been found, this science's approach is applied. Analytical chemistry is useful in almost all areas of chemistry. Since they are successful at preserving the material's quality over time, analytical techniques are crucial components of QA and QC.

Analytical method should be

1. Most productive, economical and convenient,
2. As accurate and precise as required,
3. As simple as possible,
4. Most specific

Prior to transmission, it should be completely tuned to verify its properties, such as accuracy, precision, sensitivity, etc.

Cancer, endometriosis, uterine fibroids, precocious puberty, and other sex hormone-related disorders are managed and treated using leuprolide. It belongs to the group of drugs called GnRH agonists. For members of an interprofessional team directing the treatment of patients with prostate cancer and other sex hormone-related illnesses, this exercise discusses the indications, mode of action, contraindications, monitoring procedures, and other critical elements.

Leuprolide: Acetic acid*N*-[1-[[1-[[1-[[1-[[1-[[1-[[5-(diaminomethylideneamino)-1-[2-(ethylcarbamoyl)pyrrolidin-1-yl]-1-oxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-4-methyl-1-oxopentan-2-yl]amino]-3-(4-hydroxyphenyl)-1-oxopropan-2-yl]amino]-3-hydroxy-1-oxopropan-2-yl]amino]-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl]amino]-3-(1*H*-imidazol-5-yl)-1-oxopropan-2-yl]-5-oxopyrrolidine-2-carboxamideits structure is shown in Figure: 1

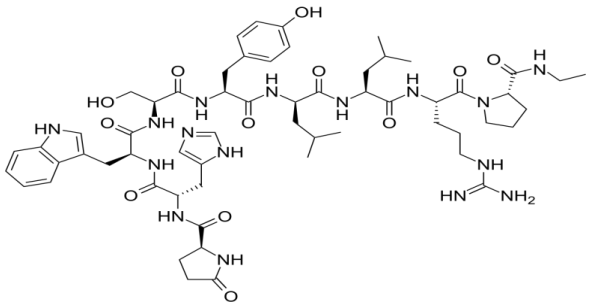

Figure 1: Chemical structure of Leuprolide.

MATERIAL AND METHODS

Reagents and Chemicals: Water, Ortho-phosphoric acid, Methanol were used in the study.

Instrumentation

UV Spectrophotometric is Analytical Technologies Limited with UV analyst software used, for HPLC Analytical Technologies Limited Agilent (1100series) with Auto sampler and DAD detector with Chemstation software were used.

Chromatographic condition

A High performance liquid chromatogram equipped with DAD detector, the purity determination performed on an Agilent C18 (250mm x 4.6ID, Particle size: 5 micron) long filled. C18 column the mobile phase consisting of Methanol: 0.05%OPA (60:40).

Sample preparation for development of UV-spectrophotometric and HPLC method for determination of drug

Preparation of Leuprolide Standard

5 mg of the Leuprolide standard were carefully weighed and transferred into a 10 mL volumetric flask. Next, 5 mL of diluent were added, the volume was shaken to dissolve it, and the remaining volume was brought up to the required level with diluent. (Leuprolide 500 g/ml concentration).

The aforesaid stock solution was further diluted to 0.1 mL in a 10 mL volumetric flask, and the volume was then brought up to the required level with diluent (containing 5 mg/ml of leuprolide concentration).

Preparation of stock solution of Leuprolide

Injection of lupronLuprorin in 4 ml Volumetric flask of 10 ml. To get volumes up to the required level, add 10 ml of diluent, sonicate to thoroughly dissolve it, and then add the remaining diluent. Mix well and run through 0.45 m filter. Pipette 0.3 ml of the aforementioned stock solution into such a 10 ml volumetric flask and add diluents (15 g/ml) to get the desired concentration). The simple chromatogram of test Leuprolide Shown in **figure no.5**

Validation parameter

The goal of validating an analytical method is to show that it is appropriate for the intended use. There is also a tabular summary of the properties relevant to impurity detection, control, and testing processes. Later updates to this paper may take other rational courses of action into account. The following is a list of definative validation attributes that should be taken into account.

Linearity
Accuracy
Precision
Limit of Detection (LOD)
Limit of Quantification (LOQ)
System suitability parameter

Linearity

The analytical method's capacity to produce a reaction that is directly proportional to the concentration (quantity) of analyte in the sample is known as linearity. If the technique is linear, the test findings are directly proportional to the concentration of an analyte in samples within a predetermined range at which the involved response is proportional to the analyte concentration or by a well-defined mathematical transformation.

Accuracy

A measured value's accuracy refers to how closely it matches the real or accepted value. Accuracy refers to the deviation between the actual merit and the communicated merit discovered. Applying the technique to samples that have known quantities of analyte added helps to determine it. To make sure there is no interference, these should be compared to the standard and blank solutions.

Precision

When a technique is applied pragmatically to several samplings of a comparable sample, the amount of agreement among individual test results obtained indicates how accurate the approach is. The reliability of the entire analytical process is gauged by precision.

Limit of Detection (LOD)

The smallest number of analyte that can be detected, but not always specified as an exact value, is the LOD a certain analyte technique. The following formula was used to determine LOD.

$LOD = 3.3\sigma/S$

Where S is the slope resulting from linearity and is the SD determined from the response's correctness.

Limit of Quantification (LOQ)

The smallest quantity of analyte that may be quantitatively identified is known as the LOQ of a certain analytical process. The following formula was used to determine LOQ.

$LOQ = 10.\sigma/S$

Where σ is the standard deviation calculated from accuracy of the response and S is the slope from linearity.

System suitability parameter

The examination of an analytical system's component parts to demonstrate that its performance satisfies the method's performance standards is known as a system suitability parameter. The number of theoretical plates (efficacy), capacity factor, separation (relative retention), resolution, and telling factor relative standard deviation may all be computed experimentally to generate a quantity system suitability test result (precision).

Robustness

A method's robustness is its capacity to withstand minor, intentional changes in the parameters. Small but intentional changes were made to the optimal method parameters to test the suggested technique's resilience. Changes in the mobile phase's composition and flow rate, as well as the impact of wavelength on RT and the drug peak's tailing factor, were investigated.

RESULTS AND DISCUSSION

Determination of λ max

To calculate the absorbance maximum or Lambda max (max), the Ultra Violet (U.V.) spectrum of Leuprolide was first created using an appropriate U.V spectrophotometer. This is crucial since HPLC identification is mostly UV-based, hence the following spectra were obtained using a 10 g/ml concentration of leuprolide in water.

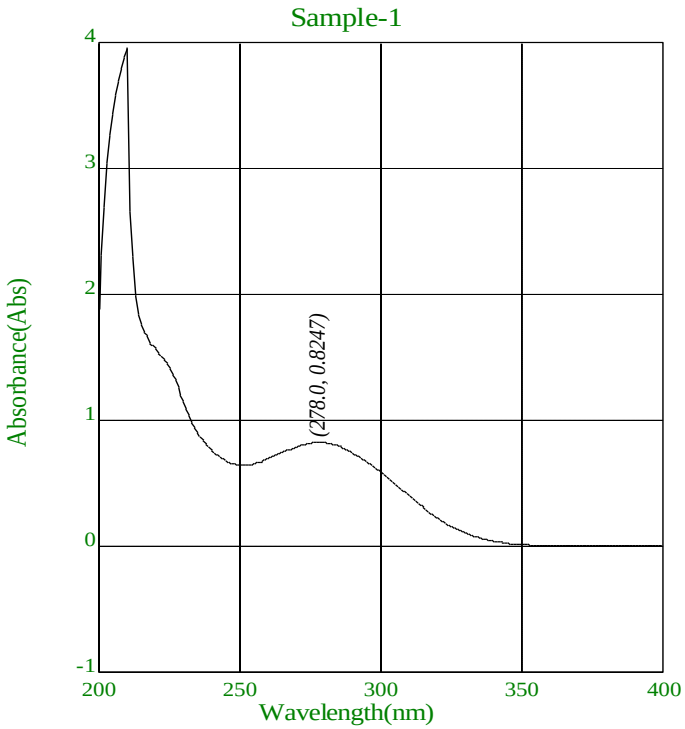

Figure 2: UV spectra of Leuprolide.

Table 1: Chromatographic condition for Leuprolide drug validation.

PARAMETER	CONDITIONS
Stationary Phase (Column)	Agilent C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	Methanol: 0.05%OPA (60:40)
Flow rate	1 ml/min
Injection volume	20 µL
Pump mode	Isocratic
Detector	UV VIS
Wavelength	278nm
Column Temperature	25°C
Run Time	10 min
Retention Time	5.56

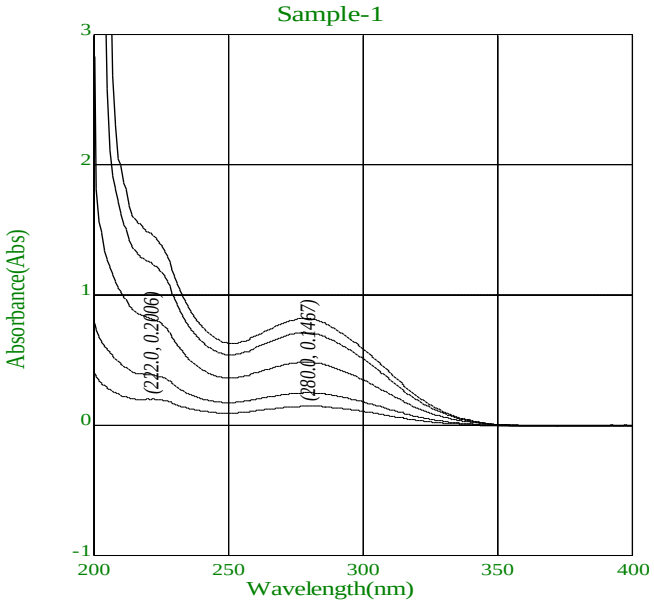

Figure 3: Observed spectrum of Leuprolide for UV.

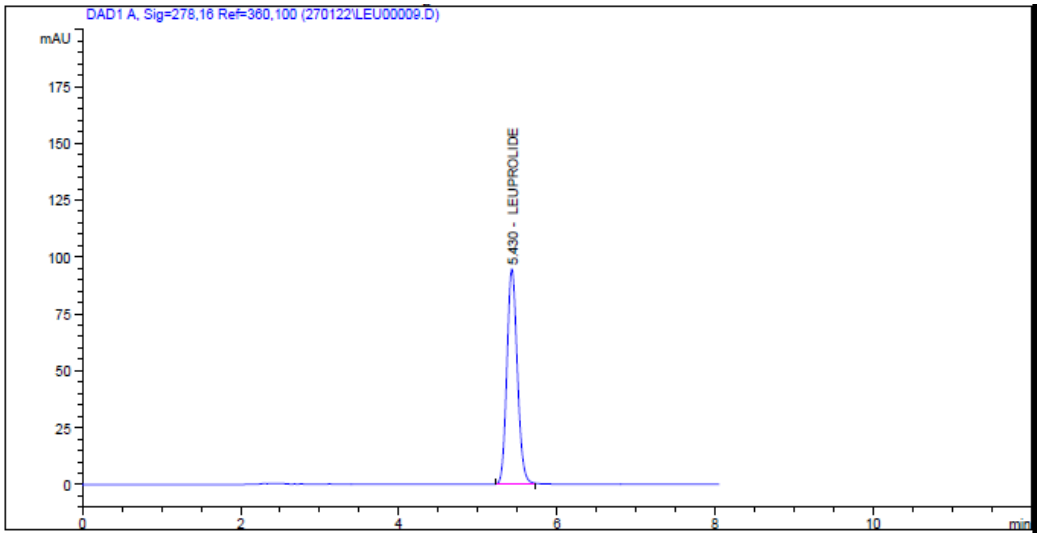

Figure 4: Observed Chromatogram of Leuprolide.

Linearity

Leuprolide standard preparations at concentrations ranging from 5 to 25 g/ml were produced in order to calculate the linearity of the drug. Leuprolide's reaction was discovered to be linear in the concentration range under examination, and its linear regression equation had correlation coefficients of 0.999 for HPLC and 0.20x+0.003 for UV (Figure 3,4).

Table 2: Linearity of Leuprolide.

% Conc. of Standard	Mean Response (Area)	Statistical analysis For HPLC		% Conc. of Standard	Mean Response (Area)	Statistical analysis for UV
5	181.39	**Correlation**	**0.999**	5	0.104	**1**
10	344.5			10	0.203	
15	527.06	**Intercept**	**6.446**	15	0.305	**0.003**
20	706.2			20	0.404	**0.020**
25	864.27	**Slope**	**34.54**	25	0.505	

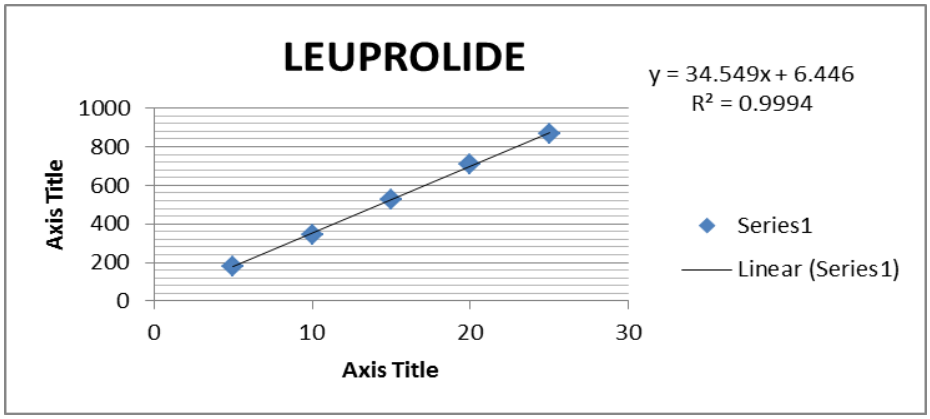

Figure 5: Linearity graph of Leuprolide for HPLC.

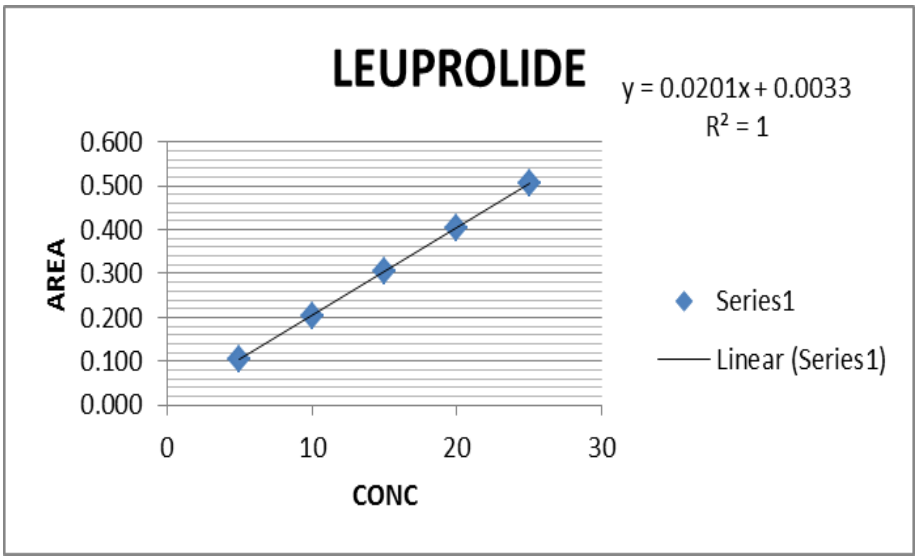

Figure 6: Linearity graph of Leuprolide for UV.

Accuracy

Leuprolide recovery was calculated at three distinct initial concentration (Table 3). The outcome showing the method's accuracy.

Table 3: Accuracy data sheet.

METHOD	Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml	Absorbance/ Area Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
UV Method	LPL	80%	5	4	17.8± 0.07	7.86± 0.07	98.22±0.14
		100%	5	5	19.89± 0.02	9.89 ±0.02	98.90 ±0.21
		120%	5	6	21.72 ±0.03	11.72 ±0.03	97.69±0.21
RP-HPLC Method	LPL	80%	5	4	9.02± 0.006	4.02± 0.006	100.6±0.14
		100%	5	5	10.01± 0.03	5.01 ±0.03	100.2 ±0.75
		120%	5	6	10.99 ±0.02	5.99 ±0.02	99.75±0.44

Precision

For LPL, the intraday and interday precision percent RSD values were 0.29 and 0.261, respectively. The system, method, and intermediate precision studies' percentage RSD was well within the acceptable range (2%), demonstrating the accuracy of the technique. Table 4 displays the precision findings.

Table 4: Result of Intra day and Inter day Precision studies on RP-HPLC and UV method for Leuprolide.

METHOD	Drug	Conc^{n} (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
HPLC METHOD Rp-	LPL	5	178.77 ±0.52	99.78	181.0±1.10	101.07
		15	528.49 ±1.65	100.76	526.03±0.87	100.29
		25	861.67±0.29	99.04	864.48±2.33	99.37
		10	0.2029 ±0.01	99.95	0.2030±0.01	99.96
UV METHOD	LPL	15	0.3090 ±0.08	102.00	0.3091 ±0.09	102.01
		20	0.4006 ±0.02	99.40	0.4007 ±0.03	101.25

System suitability parameters

The aforementioned technique was used to create the standard solution. Two duplicate injections of a 25 L solution were injected after the column had been equilibrated with the mobile phase. Peak response, or peak area, was assessed after the chromatograms were recorded. Table 6 displays the findings of the system suitability parameters.

Robustness

By appropriately altering the optimal condition, the resilience of the approach was created. The chromatographic conditions were purposefully changed, and the resolution between Leuprolide was assessed, in order to gauge the robustness of the established approach. Based on the evaluation of the results, it can be concluded that the approach is not greatly impacted by variations in the changing wavelength or flow rate. The established approach was robust if percent RSD 2 percent. Table 5 displays the robustness findings.

Table 5: Robustness results.

Condition		Leuprolide	
		SD	%RSD
Change in wavelength (278±1 nm)	277nm	1.62	0.19
	279nm	2.46	0.28
Change in flow rate (1.0±0.1 ml/min)	0.9	2.26	0.24
	1.1	1.29	0.26
Change in mobile phase (1.0±0.1 ml/min)	59+41	3.20	0.72
	61+39	0.97	0.24

*Average of three determinations, %RSD: Percentage relative standard deviation

Analysis of Leuprolide from marketed tablets

The percentage assay of marketed formulation was found to be 100.02 for Leuprolide for HPLC and 100.58 for UV respectively.

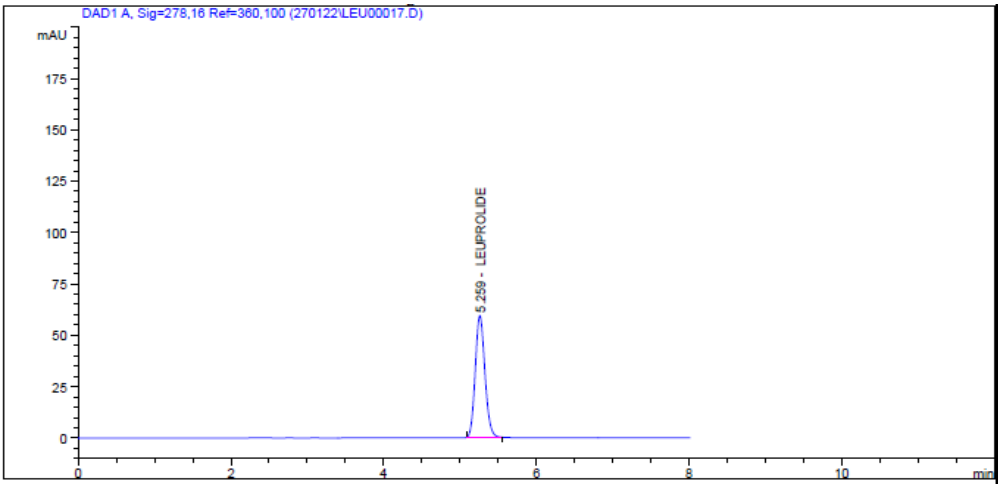

Figure 7: Chromatogram for Marketed Formulation.

Table 6: Summary of validation parameter for UV.

Parameter	LPL
Calibration Range (µg/ml)	5-25
Optimized wavelength (nm)	278nm
Precision (% RSD)	0.52
System suitability (% RSD)	0.57
% Assay	100.58

Table 7: Summary of validation parameter for HPLC.

Parameter	LPL
Calibration Range (µg/ml)	5-25
Optimized wavelength (nm)	278nm
Retention Time	5.56
Precision (% RSD)	0.29-0.61
System suitability (% RSD)	0.09
% Assay	100.02
LOD (µg/ml)	0.2764
LOQ(µg/ml)	0.8375

Average of five determinations, LOD: Limit of detection, LOQ: Limit of quantification.

CONCLUSION

The study as a whole suggests that HPLC is a flexible, repeatable chromatographic approach for medicinal product quantification. It has a wide range of applications in terms of energetic particle approximation, both quantitatively and qualitatively. By adjusting the temperature, gradient slope, flow rate, type, and concentration of mobile-phase modifiers, final optimization may be carried out. According to ICH recommendations, the improved technique is verified using a number of characteristics (such as precision, accuracy, linearity, detection limit, etc.). The present work's utilisation of a C18 column has demonstrated enhanced analyte elution with high resolution and increased plate count. In accordance with ICH Q2 (R2) requirements, the C18 column may be used to analyse Leuprolide with high specificity in a shorter amount of time. The suggested approach for determining and quantifying leuprolide was found to be straightforward, exact, accurate, linear, reliable, and quick. The method for analysing leuprolide in pharmaceutical preparations that was created and verified is incredibly quick, accurate, and exact. Additionally, it has the benefits of a quick runtime and the capacity to analyse a large number of samples, both of which considerably cut down on the analysis time required for each sample.

CONFLICTS OF INTEREST

Authors declare that they have no conflict of interest exists in this investigation.

ACKNOWLEDGMENTS

The authors are thankful for guidance provided by president JIIU Moulana Gulam Mohammad Vastanvi Ali-Allana College of Pharmacy, Akkalkuwa Dist. Nandurbar (Maharashtra) India. We also wish to sincerely thanks to Swapnaroop Drug and pharmaceuticals, Aurangabad (India), for providing gift sample of pure Leuprolide.

REFERENCES

1. Singh, R. HPLC method development and validation-an overview. *Journal of Pharmaceutical Education & Research*, 2013; *4*(1).
2. ICH Harmonised Tripartite Guideline. (October). Validation of analytical procedures: text and methodology Q2 (R1). In *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*, 2005; 1-13.
3. Dhurve, H., Parshuramkar, Y., Umekar, M., & Gupta, K. Development and Validation of UV-Spectrophotometric and HPLC Method determination of Dofetilide in Formulation.
4. Hosseiny Davarani, S. S., Pourahadi, A., & Ghasemzadeh, P. Quantification of controlled release leuprolide and triptorelin in rabbit plasma using electromembrane extraction coupled with HPLC–UV. *Electrophoresis*, 2019; *40*(7): 1074-1081.
5. Sutherland, J. W., & Menon, G. N. HPLC of leuprolide acetate in injectable solutions. *Journal of liquid chromatography*, 1987; *10*(10): 2281-2289.
6. Tan, M. M., Corley, C. A., & Stevenson, C. L. Effect of gelation on the chemical stability and conformation of leuprolide. *Pharmaceutical research*, 1998; *15*(9): 1442-1448.
7. ICH, I. (November). Q2 (R1): Validation of analytical procedures: text and methodology. In *International Conference on Harmonization, Geneva*, 2005.
8. Lokhande, P. Analytical Method Development and Validation of Leuprolide by using RP-HPLC with ICH Guidelines, 2019.

9. Iqbal, J., Vigl, C., Moser, G., Gasteiger, M., Perera, G., & Bernkop-Schnürch, A. Development and in vivo evaluation of a new oral nanoparticulate dosage form for leuprolide based on polyacrylic acid. *Drug Delivery*, 2011; *18*(6): 432-440.
10. Soman, A., Qiu, Y., & Chan Li, Q. HPLC-UV method development and validation for the determination of low level formaldehyde in a drug substance. *Journal of Chromatographic science*, 2008; *46*(6): 461-465.
11. Sarkar, M., Khandavilli, S., & Panchagnula, R. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of antiretroviral drugs in pharmaceutical dosage forms. *Journal of Chromatography B*, 2006; *830*(2): 349-354.