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DEVELOPMENT AND VALIDATION OF SIMPLE COST EFFECTIVE UV AND HPLC METHOD FOR ESTIMATION PREGABALIN AND ETORICOXIB

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ABSTRACT

This study evaluates the performance of ultraviolet (UV) and high-performance liquid chromatography (HPLC) methods for the determination of Pregabalin (PGB) and Etoricoxib (EXB) in pharmaceutical formulations. Both methods were validated for linearity, accuracy, precision, and sensitivity. The UV method demonstrated excellent linearity with correlation coefficients (r^2) of 0.999 for both PGB and EXB. The recovery rates were high, with PGB recovering between 98.21% and 98.57% and EXB between 95.08% and 96.62%. The precision of the UV method was confirmed through low %RSD values in various categories. In comparison, the HPLC method also exhibited high linearity ($r^2 = 0.999$) and accuracy, with recovery percentages ranging from 98.09% to 99.18% for PGB and 98.759% to 99.873% for EXB. The HPLC method showed higher sensitivity with lower LOD and LOQ values compared to UV, making it suitable for detecting trace amounts of the drugs. Both methods were effective in analyzing tablet formulations, with HPLC providing greater sensitivity and precision. This study highlights the strengths of each method and provides guidance on selecting the appropriate technique based on analytical requirements.

Keywords: Pregabalin, Etoricoxib, UV Spectroscopy, High-Performance Liquid Chromatography (HPLC), Linearity, Accuracy, Precision, Sensitivity, Pharmaceutical Analysis, Recovery Studies.

INTRODUCTION

Pregabalin and Etoricoxib are significant pharmaceuticals used in pain management and treatment of inflammatory conditions. Pregabalin, primarily prescribed for neuropathic pain and as an adjunctive therapy for seizures, has a well-established role in clinical practice (Miller *et al.,* 2019). Etoricoxib, a selective COX-2 inhibitor, is widely used to alleviate pain and inflammation associated with osteoarthritis and rheumatoid arthritis (Luo and Zhao, 2014). Accurate, cost-effective analytical methods are essential for the simultaneous estimation of these drugs to ensure quality control and therapeutic efficacy. UV spectroscopy and High-Performance Liquid Chromatography (HPLC) are two widely utilized techniques for this purpose. UV spectroscopy is favored for its simplicity, speed, and affordability, making it suitable for routine analysis (Skoog *et al.,* 2013). On the other hand, HPLC provides high sensitivity and specificity through the separation of compounds based on their interactions with stationary and mobile phases (Iglehart, 2004).

The development and validation of both UV and HPLC methods for Pregabalin and Etoricoxib aim to address the need for reliable analytical techniques that are also cost-

effective. The application of UV spectroscopy in drug analysis has been demonstrated in various studies (Garg and Gupta, 2012), while HPLC methods have been extensively reviewed for their effectiveness in simultaneous drug estimation (Singh & Singh, 2016). This study seeks to provide a comprehensive approach by developing and validating these methods to enhance pharmaceutical quality control and ensure accurate drug analysis.

MATERIALS AND METHODS

Chemicals and Reagents

For method development and validation, it is crucial to use high-quality reference standards of Pregabalin and Etoricoxib to ensure accuracy and reliability. Additionally, HPLC-grade solvents, reagents, and chemicals must be acquired to maintain the purity and consistency of the analytical procedures. These chemicals are essential for preparing standards, calibration curves, and for conducting various validation tests.

Instrumentation

The analysis will be conducted using a UV spectrophotometer for the UV method, which will facilitate the development and validation of the UV-based estimation technique. For the HPLC method, an HPLC system equipped with a UV detector will be employed. This setup is necessary for precise separation, identification, and quantification of Pregabalin and Etoricoxib, ensuring accurate and reliable analytical results.

Methods

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80ml 0.1 N HCl in 100 ml volumetric flask. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 100ml with distilled water to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of PGB and EXB and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with 0.1 N HCl that gave concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution

1) 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with 0.1 N HCl. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for PGB.

2) Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10ml with distilled water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for EXB.

Selection of wavelength for linearity

Solutions of 10 µg/ml of PGB and 10 µg/ml EXB were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of PGB and EXB was observed at 210.0 nm and 234.0 nm, respectively. PGB and EXB showed linearity in the

concentration range of 5-25µg/ml and 5-25µg/ml at their respective maxima.

Method development of Pregabalin (PGB) and Etoricoxib (EXB) using UV Vis. Spectroscopy (Simultaneous equation method)

Study of Overlay Spectra

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of PGB and 10µg/ml of EXB were scanned in the spectrum mode over the range of 200-400 nm against RO Water as blank and the overlain spectra of the two were recorded. PGB showed an absorbance peak at 210.0 nm, whereas EXB at 234.0 nm. The overlain spectra also showed isoabsorptive points at 220.0 nm.

Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method (Kaur *et al.,* 2017).

Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 210.0 nm and 234.0 nm that are λ_{max} of PGB and EXB respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%,\ 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_x = \frac{A_1 ay_2 - A_2 ay_2}{ax_1 ay_2 - ax_2 ay_1} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. (1)}$$

$$C_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_1 ay_2 - ax_2 ay_1} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 260.0 nm and 282.0 nm respectively, ax_1 and ax_2 are absorptivities of PGB at λ_1 (210.0 i.e. λ_{max} of PGB) and λ_2 (234.0 i.e. λ_{max} of EXB) respectively and ay_1 and ay_2 are absorptivities of EXB at λ_1 and λ_2 respectively. C_{EXB} and C_{PGB} are concentrations of PGB and EXB respectively.

Working Linearity

To get the working concentration range, linearity was observed at 260.0 nm and 282.0 nm for PGB and EXB respectively. Standard stock solution of PGB and EXB was prepared in water. The absorbtivity of both drugs were calculated by using equation:

A = abc

Where A=Absorbance
a = Absorbtivity
b = Pathlength
c = Concentration.

Validation of simultaneous equation method

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio (Shirwar *et al.,* 2023).

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of PGB and EXB to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis

was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times (Nagaraju *et al.,* 2014). Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Analysis of tablet sample

Twenty marketed tablets of PGB and EXB were weighed and ground to a fine powder; amount equal to 10 mg of PGB was taken in 10 ml volumetric flask and sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with 0.1 N HCl to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times.

Method development of Pregabalin (PGB) and Etoricoxib (EXB) using RP-HPLC

Selection of mobile phase

Initially to estimate Pregabalin and Etoricoxib in fix dosage form number of mobile phase in different ratio were tried.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min (Sapavadiya *et al.,* 2015).

Preparation of Stock Solution:

Accurately weighed 10 mg API of PGB and EXB was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of PGB and EXB respectively (Stock-B).

Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml, for PGB. In same manner 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of EXB also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml for PGB and 5-25µg/ml for EXB were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 220 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective

concentration and regression equation was derived.

Validation of developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 5 to 25 µg/ ml for PGB) and (5 to 25µg/ ml for (EXB) and areas for each concentration were recorded three times and mean area was calculated. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25µg/ml for PGB and 5, 10, 15, 20 and 25µg/ml for EXB indicates the precision under the same operating condition over short interval time.

Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations (Swartz and Krull, 2005).

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v).

F. Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10 mg of PGB and 10mg of EXB was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 7.5µg/mL PGB and 6µg/mL EXB respectively. The amounts of PGB and EXB in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

RESULTS AND DISCUSSION

Both UV and HPLC methods demonstrate high accuracy and precision in analyzing Pregabalin (PGB) and Etoricoxib (EXB). The linearity of the UV method, as indicated by the correlation coefficients of 0.999 for both drugs (Table 1), reflects a strong correlation between concentration and absorbance. Similarly, the HPLC method shows an identical correlation coefficient of 0.999 (Table 5), underscoring the robustness of both techniques in maintaining linearity within the tested concentration range.

In terms of precision, both methods deliver commendable results. The UV method shows very low %RSD values across all precision categories (repeatability, day-to-day, analyst-to-analyst, and reproducibility) (Table 3). The HPLC method, although similarly precise, has slightly higher %RSD values for robustness, particularly for EXB (Table 7). This suggests that while both methods are reliable, the HPLC method might exhibit a marginally higher sensitivity to changes in experimental conditions, especially for EXB.

Recovery studies for both methods indicate high accuracy. In UV analysis, the recovery percentages for PGB range from 98.21% to 98.57%, and for EXB, they range from 95.08% to 96.62% (Table 2). These results suggest that the UV method is effective in accurately measuring the drugs in formulations. The HPLC method yields similar recovery percentages, with PGB ranging from 98.09% to 99.18% and EXB from 98.759% to 99.873% (Table 6). These results confirm the HPLC method's high accuracy, with recovery values being marginally higher, indicating a slightly better performance in terms of precise measurement.

When comparing sensitivity, the HPLC method significantly outperforms the UV method. The LOD and LOQ values for HPLC are substantially lower than those for UV. For instance, PGB's LOD for HPLC is 0.10 µg/ml compared to 0.50 µg/ml for EXB in UV, and the LOQ for PGB in HPLC is 0.25 µg/ml compared to 1.55 µg/ml for EXB in UV (Table 8). This higher sensitivity in HPLC makes it more suitable for detecting and quantifying lower concentrations of drugs, which is particularly beneficial in scenarios requiring precise detection at trace levels.

Both methods effectively analyze tablet formulations, as evidenced by the % assay values. The UV method shows close correspondence to the label claims for PGB and EXB, with % concentrations consistently near 100% of the label claim (Table 4). Similarly, the HPLC method provides % assay values that are very close to the label claims (PGB: 99.973%, EXB: 99.417%) with very low % RSD values (Table 9). This indicates that both methods are reliable for quality control and quantification in pharmaceutical formulations, although the HPLC method provides an added advantage of greater precision and sensitivity.

The UV method is generally simpler and less costly compared to HPLC. UV spectroscopy requires less sophisticated equipment and less time for sample preparation and analysis. It is a more straightforward approach, suitable for routine analyses where high sensitivity is not critical. On the other hand, HPLC involves more complex instrumentation and requires careful method development, calibration, and maintenance.

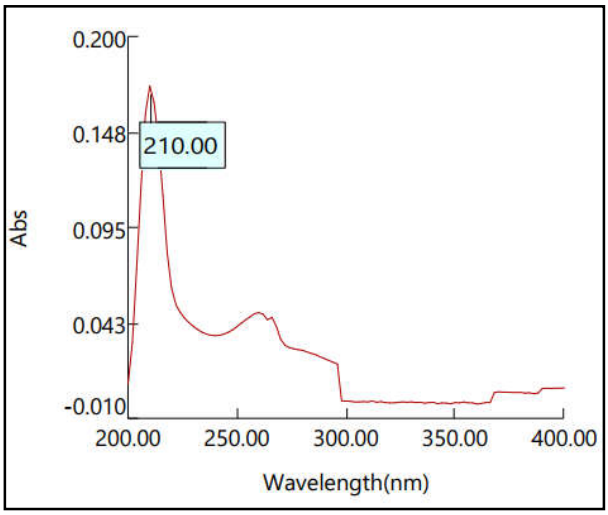

Figure 1: Determination of λ_{max} of Pregabalin

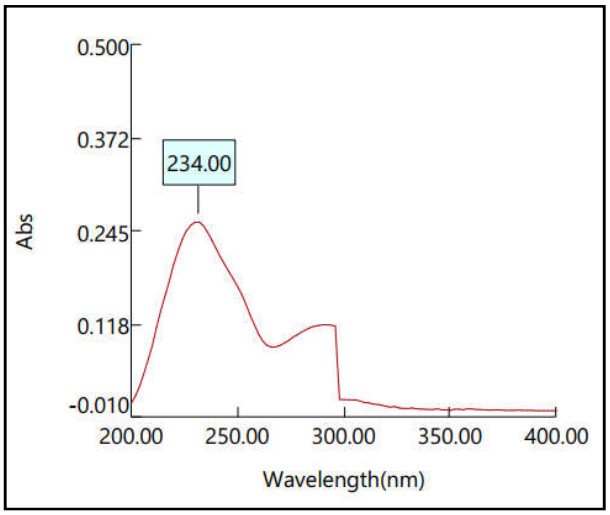

Figure 2: Determination of λ_{max} of Etoricoxib

Table 1: Results of Linearity of Pregabalin and Etoricoxib using UV method

	Results of Linearity	
Parameter	**PGB**	**EXB**
Working λ_{max}	210.0 nm	234.0 nm
Beer's law limit (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	0.018	0.009
Intercept (c)*	0.002	0.000

*Average of five determination

Table 2: Results of recovery studies on marketed formulations using UV method

Recovery Level %	% Recovery (Mean±SD)*	
	PGB	**EXB**
80	98.21±0.855	95.20±0.101
100	98.57±0.317	96.62±0.093
120	98.26±1.583	95.08±0.086

* Value of 3 replicate and 5 concentrations

Table 3: Results of validation using UV method

Parameter (Mean±SD)*			
		PGB	**EXB**
Precision (%R.S.D.)*	Repeatability	99.211±0.033	99.592±0.080
	Day to Day	99.592±0.080	99.530±0.038
	Analyst to Analyst	98.295±0.127	99.450±0.150
	Reproducibility	99.666±0.039	98.492±0.143

*Average of five concentration

Table 4: Analysis of tablet formulation of PGB and EXB using UV method

Conc. present (µg/ml)		**Replicate-1**			
		Conc. found (µg/ml)		**% Conc. found**	
EXB	**PGB**	**EXB**	**PGB**	**EXB**	**PGB**
60	75	58.85	98.083	74.65	99.53
60	75	59.65	99.417	74.65	99.53
60	75	59.78	99.633	74.69	99.59
60	75	59.65	99.417	74.88	99.84
60	75	59.88	99.800	74.66	99.55

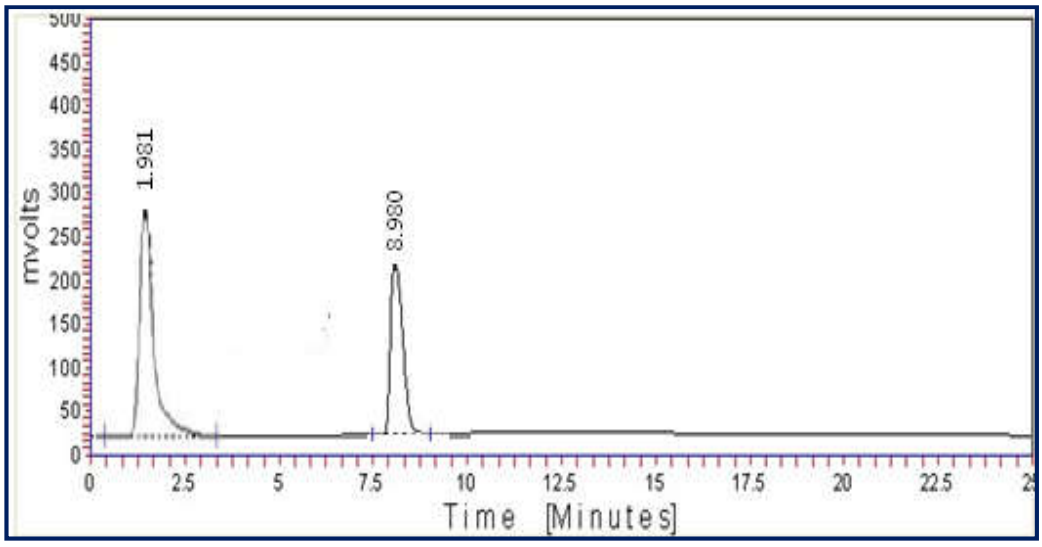

Figure 3: Chromatogram of Both the drug

Table 5: Results of Linearity of Pregabalin and Etoricoxib using HPLC method

	Results of Linearity	
Parameter	**PGB**	**EXB**
Beer's law limit (µg/ml)	5-25	5-25
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	142.7	28.55
Intercept (c)*	3.807	3.807

*Average of five determination

Table 6: Results of recovery studies on marketed formulations using HPLC method

Recovery Level %	% Recovery (Mean±SD)*	
	PGB	**EXB**
80	98.94±0.713	98.759±0.194
100	98.09±1.449	99.644±0.559
120	99.18±0.163	99.873±0.156

***** Value of 3 replicate and 5 concentrations

Table 7: Results of validation using HPLC method

Parameter (Mean±SD)*			
		PGB	**EXB**
Precision (%R.S.D.)*	Repeatability	98.204±0.049	99.584±0.124
	Day to Day	98.667±0.046	99.708±0.046
	Analyst to Analyst	98.811±0.031	99.523±0.047
	Robustness	98.078±0.059	90.376±0.047

*Average of five concentration

Table 8: LOD and LOQ of PGB and EXB

Name	LOD (µg/ml)	LOQ (µg/ml)
PGB	0.10	0.25
EXB	0.50	1.55

Table 9: Analysis of tablet formulation of PGB and EXB using HPLC method

	PGB*	**EXB***
Label Claim (mg)	**75mg**	**60mg**
% Found (mg)	74.98	59.65
% Assay	99.973	99.417
% RSD	0.054	0.064

*Average of three determination

CONCLUSION

In conclusion, while both UV and HPLC methods are highly effective for analyzing Pregabalin and Etoricoxib, the choice between them depends on specific analytical needs. The UV method is cost-effective and sufficient for routine analysis where high sensitivity is not a importantrequirement. However, HPLC, with its superior sensitivity and precision, is more suitable for scenarios demanding detailed analysis and lower detection limits. The decision on which method to use should consider factors such as the required sensitivity, cost, and the complexity of the analysis.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Garg, N. & Gupta, A. (2012) Development and validation of a UV spectrophotometric method for simultaneous estimation of multiple drugs in a formulation. *Journal of Pharmaceutical and Biomedical Analysis*, 71, 82–87.
- Iglehart, J.K. (2004) High-performance liquid chromatography (HPLC) in drug analysis. *Journal of Chromatographic Science*, 42, 245–249.
- Luo, J. & Zhao, S. (2014) Comparative efficacy of Etoricoxib and other non-steroidal anti-inflammatory drugs in osteoarthritis and rheumatoid arthritis: A systematic review and network meta-analysis. *Journal of Rheumatology*, 41, 1374–1380.
- Miller, R.D. & Eriksson, L.I. (2019). *Miller's Anesthesia*, 8th edn. Elsevier: Amsterdam.
- Moffat, A.C., Osselton, M.D. & Widdop, B. (2011). *Clarke's Analysis of Drugs and Poisons*, 4th edn. Pharmaceutical Press.
- Patel, N.M. & Patel, M.R. (2014) Development and validation of an HPLC method for simultaneous estimation of drugs in pharmaceutical formulations. *American Journal of*

Phytomedicine and Clinical Therapeutics, 3, 75–82.

- Skoog, D.A., West, D.M., Holler, F.J. & Crouch, S.R. (2013). *Fundamentals of Analytical Chemistry*, 9th edn. Cengage Learning.
- Kaur, T., Kaur, S. & Kaur, P. (2017) Development and validation of UV-spectrophotometric methods for determination of gemcitabine hydrochloride in bulk and polymeric nanoparticles. *International Journal of Applied Pharmaceutics*, 9, 60–65.
- Nagaraju, P.T., Sreenivasa Rao, M., Ravi Kumar, C., Mabhasha, D., Venu Gopal, K. & Murali Krishna, N.V. (2014) UV-spectrophotometric method development and validation for determination of linezolid in pharmaceutical dosage form. *Research and Reviews: Journal of Pharmaceutical Analysis*, 3, 23–27.
- Sapavadiya, V.K., Sapavadiya, M.V., Shelat, P.K. & Lalwani, A.N. (2015) Development and validation of new RP-HPLC method for the estimation of linezolid in linezolid gel. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7, 250–254.
- Shirwar, M., Birajdar, S., Garad, S. & Kumbhar, S. (2023) Development and validation of novel UV–visible spectrophotometric method for estimation of tepotinib in bulk and in pharmaceutical formulation. *International Journal of Pharmacy and Pharmaceutical Sciences*, 15, 32–36.
- Singh, S. & Singh, R. (2016) Simultaneous estimation of drug combinations by HPLC: A review. *International Journal of Pharmaceutical Sciences and Research*, 7, 1902–1913.
- Swartz, M.E. & Krull, I.S. (2005) Analytical method validation: Accuracy in quantitation. *LC-GC North America*, 23, 46–52.