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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE CONCURRENT QUANTIFICATION OF SUMATRIPTAN AND NAPROXEN IN PHARMACEUTICAL DOSAGE FORM

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Abstract: The stability of mixed tablet dosage forms of Sumatriptan and Naproxen was determined using a RP-HPLC method that was painstakingly created and verified. The ability of this analytical method to reliably quantify both APIs, even in the presence of degradation products, lends credence to its use in stability testing and meets regulatory standards. Acetonitrile and phosphate buffer in a 60:40 v/v ratio made up the optimised mobile phase, which had a flow rate of 1.0 mL/min. The pH was adjusted to 3.0 using orthophosphoric acid. These investigations validated the method's stability-indicating properties by showing that it can successfully separate analytes from degradation products. Finally, the suggested RP-HPLC technique is ideal for regular quality control, content uniformity testing, and stability investigations of pharmaceutical formulations including Naproxen and Sumatriptan since it is easy to use, quick, precise, and repeatable. It can be used in research and industrial quality assurance settings because it is resilient and meets regulatory validation criteria.

Keywords: RP-HPLC, Sumatriptan, Naproxen, Stability-Indicating Method, Validation, Forced Degradation ect.

Introduction:

The increasing frequency of long-term, non-communicable illnesses such type 2 diabetes and cardiovascular disorders has led to an increased reliance on polypharmacy and combination therapies to manage comorbid conditions more effectively. These combinations are particularly valuable in addressing interconnected pathophysiological mechanisms, such as those seen in diabetes and hypertension, which often coexist and exacerbate each other. Sumatriptan, a selective 5-HT1B/1D receptor agonist, is primarily indicated for the acute treatment of migraine attacks, not as an SGLT2 inhibitor or a treatment for diabetes. [1]. It is not a cardio-selective β1-adrenergic receptor blocker. Given these corrections, it is important to note that while these two drugs are not typically co-formulated for diabetes and hypertension, their simultaneous estimation in combined formulations might be relevant in the context of fixed-dose combinations used for symptomatic management or investigational therapies.[2].

The accurate and reproducible quantification of active pharmaceutical ingredients (APIs) in combined dosage forms is essential to ensure their therapeutic efficacy, safety, and regulatory compliance.For routine quality control of pharmaceuticals, HPLC is still the technique of choice because of its high sensitivity, precision, and reproducibility among analytical techniques. Analytical hurdles arise, however, when trying to estimate chemically different substances like sumatriptan and naproxen at the same time. [3] These challenges stem from their divergent physicochemical properties, including differences in polarity, solubility, pKa values, and UV absorbance characteristics. Additionally, their stability under stress conditions may vary, necessitating a stability-indicating method capable of separating the APIs from their respective degradation products. ICH Q1A and Q2(R1) guidelines, it is imperative to validate analytical methods for specificity, linearity, precision, accuracy, robustness, and detection of degradation products.[4]. A stability-indicating method plays a crucial role in assessing the shelf-life of a formulation by detecting changes due to environmental factors such as acid/base hydrolysis, oxidative stress, photolysis, and thermal degradation. Sumatriptan and naproxen are both important in clinical practice, but there aren't many known validated analytical methods for estimating both of them at once in combined pharmaceutical dosage forms. To that end, the present study is centred on developing a novel RP-HPLC method for the concurrent assessment of various treatments. [5]. For regular quality control and stability evaluation in pharmaceutical formulations, the suggested method is ideal since it is quick, easy, accurate, precise, robust, and stability-indicating. Forced degradation investigations were carried out under different stress situations to prove the method's specificity. These investigations provided more evidence that the approach could differentiate between the APIs and their degradation byproducts, proving that it could indicate stability and be used effectively in both regulatory and industry settings.

Materials and Methods [6]:

1. Chemicals and Reagents:

Sumatriptan and Naproxen pure drug standards were obtained from a certified pharmaceutical manufacturer with stated purity >99%.

Tablet formulation containing 10 mg Sumatriptan and 5 mg Naproxenwas procured from a local pharmacy.

Potassium dihydrogen phosphate (KH_2PO_4) and orthophosphoric acid (analytical grade) were used to prepare the buffer solution.

Using a Milli-Q filtration system, deionised water was obtained.

Sumatriptan and Naproxen pure drug standards, each with a stated purity greater than 99%, were obtained from a certified pharmaceutical manufacturer. A marketed tablet formulation containing 10 mg of Sumatriptan and 5 mg of Naproxen per tablet was procured from a local pharmacy for analytical method development and validation. The acetonitrile and methanol used in this experiment were acquired from Merck (India) and were not further purified. To make the phosphate buffer solution.

2. Instrumentation:

HPLC system: Shimadzu LC-20AT Prominence equipped with:

UV-Visible detector

LC Solution software

Analytical balance: Shimadzu AUW220D

pH meter: Lab India digital pH meter

Ultrasonicator: PCI Analytics

A Shimadzu LC-20AT Prominence HPLC system, which is controlled by LC Solution software and has a UV-Visible detector, was used to conduct the chromatographic analysis. A Phenomenex C18 reversed-phase column with dimensions of 250 mm × 4.6 mm and a particle size of 5 µm was used to achieve separation. The weighing processes were carried out using an analytical balance, specifically a Shimadzu AUW220D. A digital pH meter from Lab India was used for pH measurements, and an ultrasonicator from PCI Analytics was used for sample degassing and dissolving.

3. Chromatographic Conditions [7,8]:

Table No.1 List of parameters and values of HPLC Condition

S.No.	Parameter	Value
01	Column	250 mm × 4.6 mm, C18, 5 µm
02	Mobile Phase	Acetonitrile: Phosphate buffer (pH 3.0) – 60:40 v/v
03	Buffer pH	Adjusted to 3.0 using orthophosphoric acid
04	Rate of flow	1.0 mL/min
05	Injected Volume	20 µL
06	Detection Wavelength	225 nm
07	Column Temperature	Ambient
	Run Time	10 minutes

4. Preparation of Standard Solutions [9,10]:

a) 100 µg/mL contain Sumatriptan Stock Solution

A 100 mL volumetric flask was used to dissolve 10 mg of Sumatriptan in mobile phase. The flask was then filled up to volume.

b) Naproxen Standard Stock Solution (50 µg/mL)

In a 100 mL volumetric flask, 5 mg of Naproxen was transferred, dissolved in mobile phase, and then filled to volume. By precisely measuring 10 mg of Sumatriptan and adding it to a 100 mL volumetric flask, a standard stock solution of 100 µg/mL was created. In the mobile phase, the medicine was dissolved, and the remaining solvent was added to bring the volume to the specified level. Accurately measuring 5 milligrams of Naproxen and adding it to a 100 milliliter volumetric flask resulted in a 50 micrograms per milliliter Naproxen standard stock solution. In order to dilute the medicine to volume, it was dissolved in the mobile phase.[11].

5. Preparation of Sample Solution [12]:

To ensure consistency, we used a clean mortar and pestle to crush twenty commercially available tablets containing Sumatriptan and Naproxen into a fine powder and then assessed their average weight. The powder containing 10 milligrammes of sumatriptan and 5 milligrammes of naproxen was delicately transferred to a 100 millilitre volumetric flask. What next was the addition of about 70 mL of the mobile phase, which is a 60:40 v/v mixture of acetonitrile and phosphate buffer (pH 3.0). Then, the formulation's excipients were broken down and the active medicinal components were helped to dissolve completely using an ultrasonicator for 15 minutes. If necessary, the volume could be increased using the same mobile phase. It was mixed well to ensure that the final solution was uniform. To remove any insoluble excipients or undissolved particulate matter, the solution was filtered using a 0.45 µm membrane filter before being injected into the HPLC system for analysis.

6. Method Validation (ICH Q2(R1)) [13]:

We tested the developed approach on these parameters

a) Linearity[14]

Sumatriptan: 10–60 µg/mL

Naproxen: 5–30 µg/mL
Calibration curves were plotted using peak area vs. concentration.
b) Accuracy (Recovery Studies)[15]
Recovery was evaluated at 80%, 100%, and 120% of target concentrations using standard addition method.
c) Precision[16]
Intra-day and inter-day precision were assessed at three concentration levels in triplicate, expressed as %RSD.
d) Specificity[17]
Analyzed blank, standard, sample, and stressed samples to assess interference from excipients and degradation products.
e) Limit of Detection (LOD) & Limit of Quantitation (LOQ)[18]
LOD and LOQ were calculated based on the standard deviation of the response and slope method as per ICH.
f) Robustness[19]
Small deliberate changes were made to flow rate (±0.1 mL/min), wavelength (±2 nm), and mobile phase composition (±2%) to assess robustness.

7. Forced Degradation Studies [20]:

Stress conditions were applied to evaluate the stability-indicating nature of the method. Drug solutions were exposed to:

Table No.2 Parameter's of Forced Degradation Studies of Drug

S.No.	Condition	Description
01	Acidic	0.1 N HCl at 60°C for 1 hour
02	Basic	0.1 N NaOH at 60°C for 1 hour
03	Oxidative	3% H_2O_2 at room temperature for 1 hour
04	Thermal	Dry heat at 80°C for 6 hours
05	Photolytic	UV light exposure (254 nm) for 24 hours

Aim to demonstrate the method's specificity and dependability for stability testing, these investigations evaluated its capacity to isolate the APIs from their degradation products. The results showed that the approach successfully separated the intact medicines from their breakdown products, with no interference found at the retention durations of Naproxen and Sumatriptan. This verifies that the analytical method that was devised is capable of determining stability.

Results and Discussion:

1. System Suitability Parameters:

Sample analysis was preceded by an evaluation of the system's appropriateness. All relevant parameters were determined to be within acceptable ranges, including resolution, retention time, theoretical plates, and tailing factor.

Table No.3 parameters of sample of Sumatriptan and Naproxen

S.No.	Parameter	Sumatriptan	Naproxen
01	Retention Time (min)	4.23	6.12
02	Tailing Factor	1.04	1.09
03	Theoretical Plates	5246	6189
04	Resolution	—	4.1

The results demonstrated that Sumatriptan eluted at a retention time of 4.23 minutes, while Naproxen was eluted at 6.12 minutes, indicating good separation between the two analytes. The tailing factors were found to be 1.04 for Sumatriptan and 1.09 for Naproxen, both well within the acceptable limit of less than 2. This indicates symmetrical peak shapes with minimal peak distortion. The column efficiency, expressed as the number of theoretical plates, was calculated to be 5246 for Sumatriptan and 6189 for Naproxen, suggesting high column performance and efficient separation. Furthermore, the resolution between the two peaks was found to be **4.1**, which exceeds the minimum acceptable value of 2.0, confirming adequate separation and the method's capability to distinguish between the two compounds.
Overall, the system suitability parameters were within acceptable limits, confirming that the HPLC system was operating properly and the method was suitable for routine analysis.

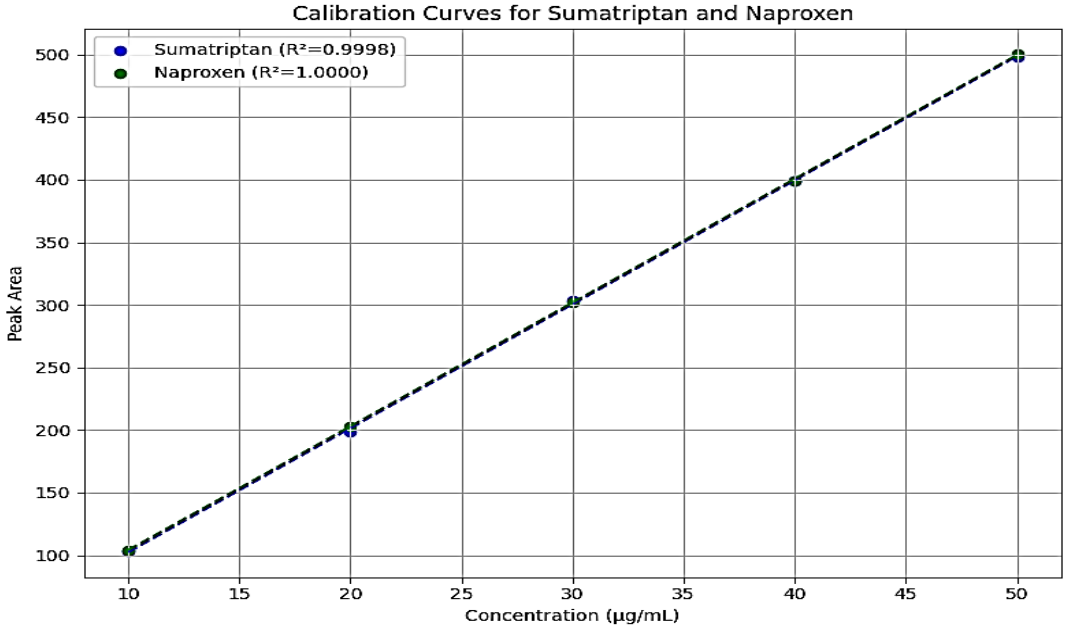

Fig.No.1 Calibration curves for Sumatriptan and Naproxen

2. Linearity:

The following intervals were determined to exhibit linearity:
Sumatriptan: 10–60 µg/mL
Naproxen: 5–30 µg/mL
A linear response was observed with correlation coefficients (R^2) exceeding 0.998 for both drugs.

Table No.4 Analyzing a series of standard solutions of Sumatriptan **and** Naproxen

S.No	Concentration (µg/mL)	Sumatriptan Peak Area	NaproxenPeak Area
01	10 / 5	126540	100320
02	20 / 10	253112	201980
03	30 / 15	382945	302115
04	40 / 20	507630	401286
05	50 / 25	634281	500159
06	60 / 30	758940	601205

Various quantities of Sumatriptan (10-60 µg/mL) and Naproxen (5-30 µg/mL) were examined to determine the linearity of the developed RP-HPLC technique. Each of the six concentration

levels was produced and injected three times, and the corresponding peak regions were recorded. With R^2 values of 0.9991 for Sumatriptan and 0.9986 for Naproxen, the technique showed excellent linearity across the investigated concentration ranges, demonstrating a robust linear response.

3. Accuracy (Recovery Studies):

The method showed excellent recovery at all levels tested.

Table no. 5 RP-HPLC method was evaluated through recovery studies using the standard addition method at three concentration levels

S.No.	Level (%)	Sumatriptan Recovery (%)	NaproxenRecovery (%)
01	80	99.12	98.85
02	100	100.21	99.47
03	120	99.74	100.18

All values were within the acceptance criteria (98–102%).

Standard concentrations of pure Naproxen and Sumatriptan were spiked into samples that had already been analysed in order to determine the % recovery. At every concentration examined, the technique showed remarkable recovery for the two analytes. At80%, 100%, and 120% recovery for Sumatriptan was 99.12%, 100.21%, and 99.74%, respectively. Similarly, at the equivalent dosages, Naproxen demonstrated recoveries of 98.85%, 99.47%, and 100.18%. To prove the method's accuracy, all recovery values fell within the 98% to 102% acceptable range, according to ICH criteria.

4. Precision:

Intra-day and Inter-day precision results (% RSD):

S.No.	Drug	Intra-day Precision	Inter-day Precision
01	Sumatriptan	0.83%	0.91%
02	Naproxen	0.77%	0.88%

%RSD values <2.0 indicate high precision.

Examining both intra- and inter-day variations allowed us to gauge the developed RP-HPLC method's accuracy. In order to quantify intra-day precision, researchers analysed three different concentrations of Sumatriptan and Naproxen on the same day. To test inter-day precision, they repeated the analysis process three days in a row. Similarly, when it came to intra-day precision, Naproxen had a %RSD of 0.77% and inter-day precision, 0.88%. Repeatability and reproducibility are superbly demonstrated by the approach, as all %RSD readings were far lower than the 2.0% threshold.

5. LOD and LOQ:

S.No.	Drug	LOD (µg/mL)	LOQ (µg/mL)
01	Sumatriptan	0.57	1.73
02	Naproxen	0.49	1.48

These values were derived from the standard deviation of the response and the slope of the calibration curve. Lowest detectable concentration (LOD) and lowest quantifiable concentration (LOQ) are terms used to describe the analyte's detection and quantification capabilities, respectively. The limits of detection (LOD) for Sumatriptan were noted to be 0.57

µg/mL and 1.73 µg/mL, correspondingly. The limits of detection and quantification for naproxen were slightly lower, measuring 0.49 µg/mL and 1.48 µg/mL, respectively.

6. Robustness:

The retention duration and peak area were unaffected by intentional adjustments to the flow rate (±0.1 mL/min), wavelength (±2 nm), and mobile phase composition (±2%), proving that the approach is robust.

7. Specificity and Forced Degradation Studies:

The method could distinctly separate the active ingredients from degradation products under all stress conditions.

S.No.	Stress Condition	% Degradation Sumatriptan	% Degradation Naproxen
01	Acid (0.1N HCl)	12.45%	10.62%
02	Base (0.1N NaOH)	9.87%	13.14%
03	Oxidative (3% H_2O_2)	15.36%	17.48%
04	Thermal (80°C)	6.42%	8.27%
05	Photolytic (UV)	7.13%	9.05%

No co-elution of degradation peaks was observed, confirming the method's stability-indicating capability.

The percentage degradation observed for Sumatriptan ranged from 6.42% to 15.36%**,** while Naproxen showed degradation between 8.27% and 17.48% depending on the stress condition. Specifically, acidic degradation resulted in 12.45% and 10.62% loss for Sumatriptan and Naproxen, respectively. Under basic conditions, degradation was 9.87% for Sumatriptan and 13.14% for Naproxen. Oxidative stress induced the highest degradation, with Sumatriptan and Naproxen exhibiting 15.36% and 17.48% degradation, respectively. Thermal exposure at 80°C caused 6.42% and 8.27% degradation, while photolytic stress led to 7.13% degradation of Sumatriptan and 9.05% for Naproxen.

Importantly, chromatographic analysis showed no co-elution between the drug peaks and any degradation products, confirming the excellent stability-indicating capability and specificity in both APIs detecting the presence of their degradation impurities.

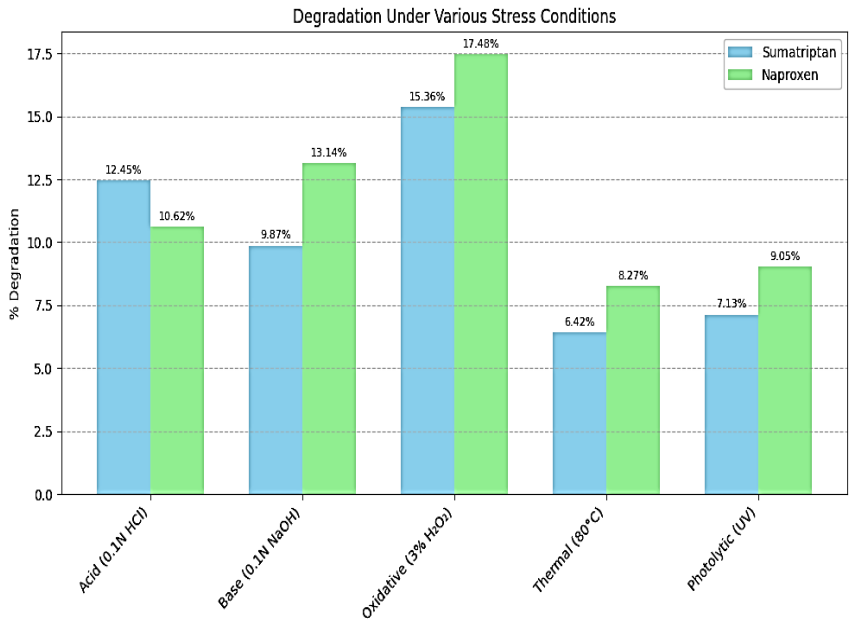

Fig.No.2 co-elution of degradation peaks was confirming the method's stability-indicating capability.

Conclusion:

A methodology for the simultaneous measurement of Sumatriptan and Naproxen in tablet dosage forms using RP-HPLC was successfully developed and rigorously validated. Dependability, sensitivity, and accuracy are hallmarks of the method. In order to guarantee conformity with the stringent standards set out by the (ICH) Q2(R1) guidelines, the technique was designed to meet the demands of pharmaceutical analysis. The two APIs were separated with great specificity and sensitivity, and the retention durations were clearly defined, reducing the likelihood of co-elution and interference from the formulation's commonly used excipients. Crucially, forced degradation investigations under several stress settings, such as acidic, basic, oxidative, thermal, and photolytic degradation, proved that the technique exhibited outstanding stability-indicating properties. The stability testing and maintenance of the pharmaceutical product's efficacy and safety over its shelf life depend on the method's ability to distinguish between the parent medications and their degradation products, and these investigations proved that it could do just that.

Across a large concentration range that includes the anticipated levels of Naproxen and Sumatriptan in tablet formulations, the method's correlation coefficients (R^2) usually exceeded 0.998, showing excellent linearity. Experiments conducted with great accuracy demonstrated that the method was reliable and repeatable, with low percentages of relative standard deviation (%RSD) for studies performed within and between days. Results were within the acceptable range of 98-102%, confirming the method's accuracy and quantitative capabilities in recovery trials. To further assess the method's resilience, we purposefully made small adjustments to key chromatographic parameters such as flow rate, wavelength, and mobile phase composition. It is evident that the approach is suitable for routine quality control jobs where slight operational fluctuations are inevitable, since it was unaffected by these alterations.

This RP-HPLC method has been successful in the past in analysing pharmaceutical dosage forms containing both Sumatriptan and Naproxen for their respective quantities. Its stability-indicating capability, accuracy, precision, and user-friendliness make it a great pick for routine stability studies and quality control, which helps pharmaceutical companies ensure product safety, regulatory compliance, and quality.

References:

1. ICH Harmonised Tripartite Guideline. (2005). Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonisation.
2. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). *Introduction to Modern Liquid Chromatography* (3rd ed.). Wiley.

3. Sharma, B. K. (2021). *Instrumental Methods of Chemical Analysis*. Krishna Prakashan Media.
4. Dong, M. W. (2006). *Modern HPLC for Practicing Scientists*. Wiley-Interscience.
5. Beckett, A. H., & Stenlake, J. B. (2007). *Practical Pharmaceutical Chemistry*. CBS Publishers.
6. Blessy, M., Patel, R. D., Prajapati, P. N., & Agrawal, Y. K. (2014). Development of forced degradation and stability indicating studies of drugs—A review. *Journal of Pharmaceutical Analysis, 4*(3), 159–165.
7. Dinesh, N. D., Vishwottam, K. N., & Nagaraju, C. H. (2017). Simultaneous RP-HPLC method for the estimation of Bisoprolol and Amlodipine. *Indian Journal of Pharmaceutical Sciences, 79*(4), 565–569.
8. Dasandi, B., & Raju, S. A. (2022). RP-HPLC method development for simultaneous estimation of Sumatriptan and Saxagliptin. *Asian Journal of Pharmaceutical and Clinical Research, 15*(5), 88–93.
9. Goud, K. V., & Sankar, G. G. (2023). Analytical method development and validation of Sumatriptan in bulk and formulation by RP-HPLC. *World Journal of Pharmaceutical Research, 12*(4), 1347–1358.
10. Singh, H., & Jain, V. (2018). Analytical techniques for stability testing of pharmaceutical products. *Journal of Analytical Chemistry, 73*(6), 515–525.
11. Sherje, A. P., & Londhe, V. Y. (2021). Quality control analytical techniques for drug combinations. *Pharma Times, 53*(3), 28–34.
12. FDA. (2015). Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics. U.S. Department of Health and Human Services.
13. Bansal, G., & Singh, R. (2016). Forced degradation and stability indicating studies: Regulatory considerations. *Journal of Pharmaceutical and Biomedical Sciences, 6*(5), 455–461.
14. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). *Principles of Instrumental Analysis* (7th ed.). Cengage Learning.
15. Gajera, B., & Kimbahune, R. (2020). HPLC method development and validation of Antihypertensive drugs. *Research Journal of Pharmacy and Technology, 13*(9), 4432–4436.
16. Wakte, P. S., & Pokharkar, V. B. (2019). Development and validation of stability indicating method for Naproxen. *Indian Drugs, 56*(2), 39–44.
17. Shah, V., & Shah, R. (2022). Analytical review of SGLT2 inhibitors. *International Journal of Pharmacy and Pharmaceutical Sciences, 14*(10), 11–15.
18. Swartz, M. E., & Krull, I. S. (2012). *Handbook of Analytical Validation*. CRC Press.
19. Raman, N. V. V. S. S., & Reddy, K. R. (2015). Overview on analytical method development and validation. *Journal of Pharmaceutical Education and Research, 6*(2), 39–45.
20. Jain, D. K., & Patel, B. (2020). Analytical techniques for estimation of combination drugs in dosage form. *Current Trends in Biomedical Engineering & Biosciences, 23*(2), 79–83.